

Synthesis, Structural Characterization and Evaluation of Antibacterial Activity of New 2-methyl-1*H*-benzimidazole Derivatives

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ABSTRACT

A new series of benzimidazole derivatives compounds containing 1,3,4-thiadiazole ring and amide group were synthesized by reaction of 2-methyl-1*H*-benzimidazole with 2-chloro-*N*-(5-(substitutedphenyl)-1,3,4-thiadiazol-2-yl)acetamide. The resulting compounds were characterized by FTIR and ¹H-NMR spectral analysis, and evaluated their antibacterial activity *in vitro* against four types of bacteria isolates two gram-negative (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two gram-positive (*Escherichia coli* and *Pseudomonas aeruginosa*). Some of the synthesized compounds showed moderate to good activity against these types of bacteria.

Keywords: 1,3,4-thiadiazole, Acetamide, Antibacterial, Benzimidazole.

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INTRODUCTION

Benzimidazole is a heterocyclic aromatic organic compound containing nitrogen.¹ The promising pharmacophore benzimidazole with a historical, biological response and a privileged structure is profusely used in medicine production.² This nucleus, in particular, is a component of vitamin B₁₂.³ Benzimidazole derivatives have received a significant concern over the last few decades because of their pharmacological properties,⁴ such as anticancer,⁵ antimicrobial,^{6,7} analgesic,⁸ antioxidant,⁹ anti-inflammatory,¹⁰ antiviral,¹¹ enzyme inhibitor,¹² antidiabetic,¹³ and corrosion inhibitor.¹⁴ The 1,3,4-thiadiazole nucleus has been integrated into a wide range of therapeutically relevant drugs, mostly to give antibacterial activities. Looking at the importance and relevance of the benzimidazole and 1,3,4-thiadiazol nucleus, we thought that we designed and synthesized some novel benzimidazole derivatives containing the 1,3,4-thiadiazole moiety and screening them for possible antibacterial activity would be useful.

EXPERIMENTAL

Material and Methods

Pure starting materials from sigma were used for the preparation of synthesized compounds, all chemicals, solvents, and reagents were of synthetic grade and were buy it commercially, Stuart-SMP3 electronic system was used to measure melting points, the fourier transform infrared spectroscopy (FTIR) spectrum was recorded by SHIMADZU (FTIR 8400S), while Bruker (400MHz) used for recorded ¹H-NMR spectrums,

Dimethyl sulfoxide-d₆ (DMSO-d₆) as a solvent and TMS as a reference.

Synthesis of 2-methyl-1*H*-benzimidazole (a)

A mixture of 15 mmol of *o*-phenylenediamine and 15 mmol of acetic acid was dissolved in 20–30 mL of 4N HCl by heating, the reaction mixture was refluxed for 8 hours. The mixture cooled down and neutralized with 32% ammonia solution and the obtained precipitate was filtered, and washed several times, and dried. The obtained product was recrystallized from ethanol.

Beige, yield 80%, mp of 160–161°C, FTIR (cm⁻¹) 3103 (N-H), 3061 (C-H, Ar.), 2991-2848 (C-H, aliph.), 1624 (C=N), 1550-1450 (C=C, Ar.).

Synthesis of Compounds (1-5) b

A 10 mmol, 5mL of POCl₃ was added thoroughly and carefully to a mixture of (10 mmol) from benzoic acid derivatives and 0.91 g, 10 mmol from thiosemicarbazide. After 3 hours reflux, the mixture was cooled down and (25 mL) of distilled water was added dropwise and carefully with stirring and refluxed for 4 hours. The mixture cooled down and then neutralized with potassium hydroxide solution, and the obtained precipitate was filtered, washed with distilled water several times, and dried. The product was recrystallized from ethanol.

5-phenyl-1,3,4-thiadiazol-2-amine (1b)

Pale yellow, yield 86%, mp 223–225°C, FTIR (cm⁻¹) 3391 and 3270 (NH₂), 3098(C-H, Ar.), 1630(C=N), 1586 and 1455 (C=C, Ar.).

5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine(2b)

Pale yellow, yield 81%, mp 174–76°C, FTIR (cm⁻¹) 3272 and 3197 (NH₂), 3089(C-H, Ar.), 1639(C=N), 1581 and 1475 (C=C, Ar.).

5-(2-chlorophenyl)-1,3,4-thiadiazol-2-amine (3b)

Pale yellow, yield 80%, mp 213–215°C, FTIR (cm⁻¹) 3290 and 3203 (N-H₂), 3086 (C-H, Ar.), 1639 (C=N), 1596 and 1458 (C=C, Ar.).

5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine (4b)

Yellow, yield 82%, mp 244–246°C, FTIR (cm⁻¹) 3377 and 3294 (NH₂), 3069(C-H, Ar.), 1620(C=N), 1604 and 1440, 1508 and 1342 (NO₂), (C=C, Ar.).

5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-amine (5b)

Dark yellow, yield 85%, mp 265–267°C, FTIR (cm⁻¹) 3360 and 3251 (NH₂), 3097 (C-H, Ar.), 1627(C=N), 1535 and 1350 (NO₂), 1593 and 1435 (C=C, Ar.).

Synthesis of 2-chloro-*N*-(5-substitutedphenyl)-1,3,4-thiadiazol-2-yl)acetamide derivatives (1-5) c:

(5 mmol) (1-5) b and (5 mmol) TEA were dissolved in DMF, then (10 mmol) chloroacetyl chloride was added slowly to the mixture and refluxed for 2 hours. After that, the solution was poured onto crushed ice, filtered, washed, dried, and recrystallized from C₂H₅OH.

2-chloro-*N*-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide (1c)

Pale yellow, yield 82%, mp 233–235°C, FTIR (cm⁻¹) 3184 (N-H), 3037(C-H, Ar.), 2939 and 2839(C-H, alip.), (C=O) 1708, (C=N), 1573 and 1440 (C=C, Ar.).

2-chloro-*N*-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)acetamide (2c)

Beige, yield 80%, mp 171–173°C, FTIR (cm⁻¹) 3176 (N-H), 3066(C-H, Ar.), 2943and2881, (C-H, alip.), 1708 (C=O) 1639,(C=N), 1585 and 1494 (C=C, Ar.).

2-chloro-*N*-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)acetamide (3c)

Beige, yield 84%, mp 230–232°C, FTIR (cm⁻¹) 3186 (N-H), 3035(C-H, Ar.), 2939 and 2881 (C-H, alip.), 1708 (C=O), 1662(C=N), 1585 and 1438 (C=C, Ar.).

2-chloro-*N*-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide (4c)

Dark beige, yield 80%, mp 250–252°C, FTIR (cm⁻¹) 3170 (N-H), 3089(C-H, Ar.), 2947and 2839(C-H, alip.), 1705 (C=O) 1618(C=N), 1585 and 1494 (C=C, Ar.).

2-chloro-*N*-(5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide (5c)

Pale brown, yield 86%, mp 240–243°C, FTIR (cm⁻¹) 3278 (N-H), 3097(C-H, Ar.), 2939 and 2862(C-H, alip.), 1685 (C=O), 1620(C=N), 1585 and 1494 (C=C, Ar.).

Synthesis of *N*-(5-(2-substitutedphenyl)-1,3,4-thiadiazol-2-yl)-2-(2-methyl-1*H*-benzo[d]imidazol-1-yl)acetamide (1-5) d

A mixture of 5 mmol of compound (a), 5 mmol of 1,3,4-thiadiazol-2-amine derivatives (1-5) c and 5 mmol K₂CO₃ were

dissolved in 25 mL acetone. After 5 hours of refluxing, the mixture cooled down, poured onto crushed ice. The precipitate was filtered, washed several times, dried and recrystallized from ethanol.

2-(2-methyl-1*H*-benzo[d]imidazol-1-yl)-*N*-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide (1d)

Beige, yield 76%, mp 159–161°C, FTIR (cm⁻¹) 3197 (N-H), 3068(C-H, Ar.),2990 and 2800 (C-H, alip.),1708, (C=O) 1612(C=N), 1531 and 1427 (C=C, Ar.); ¹H-NMR(DMSO-d₆); 3.46 ppm (CH₃), 4.46 ppm (CH₂), (7.06-8.45) ppm (H-Ar.), 10.72 ppm (NH, amide).

***N*-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)-2-(2-methyl-1*H*-benzo[d]imidazol-1-yl)acetamide(2d)**

Pale beige, yield 71%, mp 205–207°C, FTIR (cm⁻¹) 3165 (N-H), 3062(C-H, Ar.), 2978 and 2810(C-H, alip.), 1705 (C=O) 1633(C=N), 1591 and 1477 (C=C, Ar.).

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

Dark beige, yield 74%, mp 204–207°C, FTIR (cm⁻¹) 3387 (N-H), 3066(C-H, Ar.), 2939 and 2827(C-H, alip.),1708 (C=O) 1627(C=N), 1585 and 1438 (C=C, Ar.); ¹H-NMR(DMSO-d₆); 2.91 ppm (CH₃), 3.50 ppm (CH₂), (7.14-8.19) ppm (H-Ar.), 10.55 ppm (NH, amide).

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

Dark brown, yield 68%, mp 246–249°C, FTIR (cm⁻¹) 3124 (N-H), 3064(C-H, Ar.), 2978 and 2792(C-H, alip.), 1701 (C=O) 1629(C=N), 1597 and 1419 (C=C, Ar.).

***N*-(5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl)-2-(2-methyl-1*H*-benzo[d]imidazol-1-yl)acetamide(5d)**

Dark brown, yield 67%, mp 193–196°C, FTIR (cm⁻¹) 3174 (N-H), 3101(C-H, Ar.), 2966 and 2781(C-H, alip.),1685 (C=O) 1627(C=N), 1543 and 1446 (C=C, Ar.); ¹H-NMR(DMSO-d₆); 3.45 ppm (CH₃), 4.44 ppm (CH₂), (7.08-8.13) ppm (H-Ar.), 10.82 ppm (NH, amide).

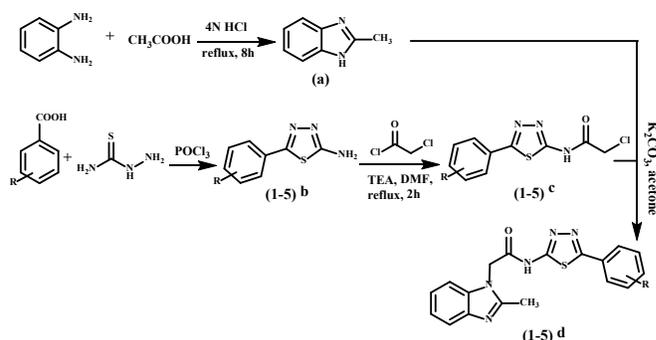
RESULTS AND DISCUSSION**Synthesis**

The synthetic path of title compounds (1-5) d showed in Scheme 1. Cyclization of acetic acid with *o*-phenylenediamine in the presence of 4 HCl gave compound (a).^{15,16} FTIR diagnosed this synthesized compound. In the FTIR spectrum for this compound, a stretching band belonging to (N-H) and (C=N) were observed at 3103 and 1624 cm⁻¹, respectively.

2-amino-5-(substituted)-1,3,4-thiadiazole (1-5) b were synthesized by reacting the various derivatives of the carboxylic acid with thiosemicarbazide in the presence of phosphorousoxy chloride.¹⁷⁻¹⁹ The FTIR spectra of compounds (1-5) b showed the presence of a C=N group at 1639 to 1620 cm⁻¹, 3282 to 3259 cm⁻¹, and 3116 to 3105 cm⁻¹, which could be attributed to asymmetric and symmetric stretching vibrations of NH₂ group.

Compounds 1 to 5 c were prepared by the reaction of 1 to 5 b with chloroacetyl chloride in the presence of TEA as a base in the DMF medium.²⁰ FTIR spectra for all compounds showed stretching (N-H) bonds of amide group were confirmed through bands in the region of (3182–3163 cm⁻¹). The presence of the carbonyl group (C=O) in the structure is proved due to the presence of a sharp peak at 1712–1705 cm⁻¹. The presence of C=N in the 1,3,4-thiadiazole nucleus was also confirmed by the presence of a sharp absorption band at 1631 to 1624 cm⁻¹.

In the final step, Compound (a) was reacted with compounds (1-5) c in the presence of anhydrous K₂CO₃ in DMF to give the target compounds (1-5) d.²¹ The chemical structures of all



Scheme 1: Synthesis of benzimidazole derivatives

Table 1: Physical characteristics of synthesized compounds

No.	R	Formula	M.wt	Color	M.P (°C)	Yield%
a	H	C ₈ H ₇ BrN ₂	211.06	Beige	160–161	80
1b	H	C ₈ H ₇ N ₃ S	177.23	Pale yellow	223–225	86
2b	4-Br	C ₈ H ₆ BrN ₃ S	256.13	Pale yellow	174–178	81
3b	2-Cl	C ₈ H ₆ ClN ₃ S	211.68	Pale yellow	213–215	80
4b	3-NO ₂	C ₈ H ₆ N ₄ O ₂ S	222.23	Yellow	234–236	89
5b	3,5-di NO ₂	C ₈ H ₅ N ₅ O ₄ S	267.23	Dark yellow	265–267	85
1c	H	C ₁₀ H ₈ ClN ₃ OS	253.70	Pale yellow	233–235	82
2c	4-Br	C ₁₀ H ₇ BrClN ₃ OS	332.60	Beige	171–173	80
3c	2-Cl	C ₁₀ H ₇ C ₁₂ N ₃ OS	288.15	Beige	230–232	84
4c	3-NO ₂	C ₁₀ H ₇ ClN ₄ O ₃ S	298.70	Dark beige	157–159	82
5c	3,5-di NO ₂	C ₁₀ H ₆ ClN ₅ O ₅ S	343.70	Pale brown	240–243	86
1d	H	C ₁₈ H ₁₅ N ₅ OS	349.41	Beige	159–161	76
2d	4-Br	C ₁₈ H ₁₄ BrN ₅ OS	428.31	Pale beige	204–207	71
3d	2-Cl	C ₁₈ H ₁₄ ClN ₅ OS	383.85	Dark beige	204–207	74
4d	3-NO ₂	C ₁₈ H ₁₄ N ₆ O ₃ S	394.41	Brown	210–212	58
5d	3,5-di NO ₂	C ₁₈ H ₁₄ BrN ₅ OS	428.31	Dark brown	193–196	67

Table 2: Antibacterial activity of synthesized compounds

Comp. No.	Concentration mg/mL	Zone of inhibition (in mm)			
		Gram-positive		Gram-negative	
		<i>S. aureus</i>	<i>S. epidermidis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
1d	100	14	12	17	15
	50	21	21	0	0
	25	21	19	0	0
	12.5	0	0	0	0
2d	100	13	12	14	15
	50	13	12	0	0
	25	11	12	0	0
	12.5	0	0	0	0
3d	100	11	13	20	16
	50	14	0	0	0
	25	0	0	11	0
	12.5	0	0	0	0
4d	100	14	0	11	11
	50	15	0	0	0

Comp. No.	Concentration mg/mL	Zone of inhibition (in mm)			
		Gram-positive		Gram-negative	
		<i>S. aureus</i>	<i>S. epidermidis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
5d	25	13	0	0	0
	12.5	0	0	0	0
	100	21	11	27	18
	50	22	20	0	11
	25	16	11	23	0
	12.5	14	0	0	0
Clarithromycin	15	12	13	-	-
Ceftazidime	30	-	-	17	-
Ceftriaxone	30	-	-	-	12
Amikacin	30	18	21	-	-
Azetronam	30	-	-	30	-
Chloramphenicol	30	-	-	-	21
DMSO	-	-	-	-	-

target compounds were established by FTIR and ¹H-NMR (for compounds (1, 3 and 5) d. FTIR spectra for these final compounds help us to a great extent to confirm their formation. Observing the data for all target compounds and absorption bands at 3348 to 3282 cm⁻¹ and 1685 and 1662 cm⁻¹ have helped confirm the presence of N-H amide and C=O groups, respectively. A sharp peak at 2981 to 2808 cm⁻¹ helped to assign the presence of C-H bond in the final products. A sharp band confirmed the presence of C=N at 1631–1616 cm⁻¹. In the ¹H-NMR spectra of compounds (1,3 and 5) d, the CH₂ protons have been resonated at δ (4.46,3.50 and 4.44) ppm, respectively. In addition of ¹H-NMR was showed singlet peak at δ (3.46, 2.91 and 3.45) ppm respectively, accounting for three protons of CH₃ group. Singlet peak between δ (7.06–8.45) ppm referred to aromatic protons. Also, it showed singlet peak at δ (10.72, 10.55 and 10.82) ppm, respectively, related to one proton of NH amide.

Antibacterial Activity

The listed results in Table 2 showed the study of the antibacterial activity of some synthesized compounds in four concentrations (100, 50, 25, 12.5) mg/mL. They showed moderate to good activity as the antibacterial agents compared with standard antibiotic ceftazidime, ceftriaxone, clarithromycin, amikacin, chloramphenicol, azetronam. The antibacterial activity of the synthesized compounds was tested against two types of Ge^{+ve} bacteria isolates (*S. aureus*, and *S. epidermidis*) and two types of Ge^{-ve} bacteria isolates (*P. aeruginosa* and *E. coli*). According to results in table 2 all synthesized compounds showed good activity against (*S. aureus*, and *S. epidermidis*), and the most active compound was 5d. While against *P. aeruginosa* all analogs showed poor activity and in some concentrations did not show any activity due to position and type of substituted group, and the most active compound was 5d.

CONCLUSION

In this study, seven target derivatives of benzimidazole have been synthesized. All analogs were characterized by FTIR

and ¹H-NMR. All final compounds have been evaluated for antibacterial activity against four types of bacteria and showed moderate to good activity against (*S. aureus*, and *S. epidermidis*) and (*P. aeruginosa*, *E. coli*).

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