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SYNTHESIS, MOLECULAR DOCKING AND ANTIBACTERIAL ACTIVITY OF NEW BENZIMIDAZOLE COMPOUNDS DERIVED FROM 3,4-DIAMINOBENZOPHENONE CONTAINING 1,3,4-THIADIAZOLE RING

Ziad T. I. Alkayar[1], Maha Salih Hussein[2], Mohammed Khalis Mohammed[1] and Muayad Ahmed Rdaiaan[1*]

[1]Department of Chemistry, College of Education for Pure Science, University of Diyala, Diyala, Iraq
[2]Department of Chemistry, College of Education, University of Samarra, Samarra, Iraq

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ABSTRACT. The aim of this present study was to synthesize new benzimidazole compounds derived from 3,4-diaminobenzophenone, which include a 1,3,4-thiadiazole ring. The final compounds **4**(**A-E**) were synthesized by a multistep procedure: First, 2-mercapto-5-benzoylbenzimidazole was prepared by reaction of 3,4-diaminobenzophenone with CS_2 in C_2H_5OH. Then various 2-amino-5-substituted-1,3,4-thiadiazole were prepared through reacting thiosemicarbazide with various benzoic acids using $POCl_3$. These compounds were reaction with chloroacetylchloride to yielded 2-chloroacetamide derivatives. Finally, nucleophilic substitution reaction between these intermediates and compound (**1**) in the presence of K_2CO_3/DMF, reflux, yielded the target compounds. The structural authenticity of the targeted compounds was confirmed using spectral data obtained from FT-IR and ^{1}H and ^{13}C-NMR. Subsequently the antibacterial activity of the final target compounds **4**(**A-E**) was examined *in vitro* against four types of bacterial isolates: two gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*). Compounds **4**(**B**, **C**, **D**, and **E**) demonstrated promising pharmacological activity *in vitro* when compared to the standard antibacterial agent, azithromycin. The synthesized molecules underwent molecular docking studies specifically with DNA gyrase subunit b (PDB ID:1KZN).The outcomes of these molecular docking studies offer support for the antibacterial activity of the compounds, demonstrating notable inhibition constants and binding energies.

KEY WORDS: Benzimidazole, 1,3,4-Thiadiazole, Molecular docking, Antibacterial activity, 3,4-Diaminobenzophenone

INTRODUCTION

Because of their numerous therapeutic applications and effective reported druggability, heterocyclic templates have gained respect and garnered much interest among medicinal chemists over time [1] Benzimidazole, an organic compound with a heterocyclic aromatic structure, has significantly contributed to the advancement of heterocyclic chemistry theory and organic synthesis [2].

The parasitic and antiviral activities of benzimidazole and its derivatives have received considerable attention due to their extensive applications in the pharmaceutical industry. The benzimidazole ring system is essential for exhibiting chemical functionality in several biologically active compounds. Some of its derivatives demonstrate potent biological activities as antivirals [3–6], anthelmintic [7], antihypertensive [8], analgesic [9, 10], antitumor [11–13], anticancer [14] agents. Commercial benzimidazole-based drugs such as thiabendazole and albendazole are used as antibacterial agents against both gram-positive and gram-negative microbes [15, 16]. Benzimidazole derivatives serve as bioisosteres of purine, a crucial component for bacterial cell wall, nucleic acid, and protein biosynthesis [17]. Benzimidazole compounds have been extensively studied in industrial applications as agents that inhibit corrosion on metal and alloy surfaces [18]. Additionally, benzimidazoles find applications in liquid crystals [19], OLED's [20].

*Corresponding authors. E-mail: muayad.rdaiaan@uodiyala.edu.iq
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Due to the presence of the N=C-S moiety, the 1,3,4-thiadiazole possesses a privileged five-membered ring structure that has gained significant recognition for exploring a wide range of biological activities. Extensive literature reviews reveal that 1,3,4-thiadiazole derivatives exhibit a broad spectrum of pharmacological properties, including antimicrobial, anti-hepatitis B viral, antitubercular, antileishmanial, analgesic, anti-inflammatory, anticancer, anticonvulsant, central nervous system (CNS) depressant, antioxidant, molluscicidal, antidiabetic, diuretic, and antihypertensive activities [20–23]. The aim of this research was to synthesize a new series of compounds combining benzimidazole and 1,3,4-thiadiazole moieties, and to evaluate their potential as antibacterial agents. These heterocycles were selected based on their known pharmacological profiles, and the study aimed to explore how structural modification influence biological activity.

RESULTS AND DISCUSSION

Synthesis

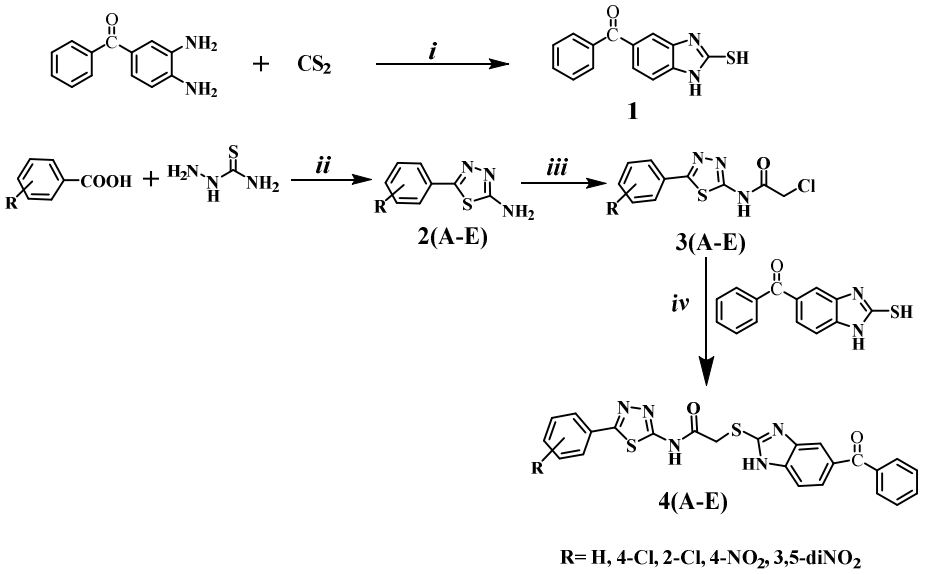
The synthetic pathway of compounds **4**(**A-E**) is outlined in Scheme 1. Compound (**1**) was obtained by cyclizing 3,4-diaminobenzophenone with CS_2 in the presence of potassium hydroxide using ethanol as the medium. This synthesized compound was then characterized using FT-IR and ^{1}H NMR. In the IR spectrum of compound (**1**), stretching bands corresponding to (N-H), (S-H), (C=O), and (C=N) were observed at 3134 cm^{-1}, 2569 cm^{-1}, 1641 cm^{-1}, and 1618 cm^{-1}, respectively. The ^{1}H NMR spectrum of compound (**1**) displayed a doublet NH signal of benzimidazole at δ (12.15 and 13.15) ppm, resulting from a tautomerism mechanism between the thiol group and NH group on the imidazole ring. Additionally, a singlet SH signal of (S-H) appeared at δ (2.59) ppm.

2-Amino-5-(substituted)-1,3,4-thiadiazole **2**(**A-E**) was synthesized by reacting various derivatives of carboxylic acid with thiosemicarbazide in the presence of phosphorousoxy chloride. The FT-IR spectra of compounds **2**(**A-E**) showed the presence of a C=N group at (1635-1624 cm^{-1}) and two bands at (3282-3259 cm^{-1}) and (3116-3105 cm^{-1}), which could be attributed to the asymmetric and symmetric stretching vibrations of the NH_2 group. The ^{1}H NMR spectra of these compounds displayed a singlet at δ (6.9-7.7) ppm due to the NH_2 protons.

Compounds **3**(**A-E**) were synthesized through nucleophilic substitution reactions of compounds **2**(**A-E**) with chloroacetyl chloride in the presence of TEA in DMF medium, yielding compounds **3**(**A-E**). The chemical structures of these compounds were elucidated via FT-IR and ^{1}H NMR. In the IR spectra of all compounds, the presence of amide group (N-H) bonds was confirmed by bands in the region of (3182-3163 cm^{-1}). The presence of a carbonyl group (C=O) in the structure was proven by the presence of a sharp peak at (1712-1705 cm^{-1}). Additionally, the presence of C=N in the 1,3,4-thiadazole nucleus was confirmed by a sharp absorption band at (1631-1624 cm^{-1}). The 1H NMR spectra of these compounds displayed a singlet at δ (6.9-7.7) ppm due to the NH (amide) protons. Also, the methylene protons resonated at δ (4.46-4.52) ppm, and this downfield value can be attributed to the inductive effect of the carbonyl group and chlorine atom.

In the final step, compound (**1**) was reacted with compounds **3**(**A-E**) using anhydrous K_2CO_3 in DMF medium to yield the target compounds **4**(**A-E**). The chemical structures of all target compounds were established by FT-IR, ^{1}H NMR, and ^{13}C NMR spectra. The IR data obtained for the final compounds **4**(**A-E**) were instrumental in confirming their formation. Upon observing the data for all synthesized compounds, absorption peaks at (3278-3325 cm^{-1}), (3132-3167 cm^{-1}), (1670-1668 cm^{-1}), and (1643-1647 cm^{-1}) confirmed the presence of N-H amide, N-H benzimidazole, and (C=O, amide) (C=O, ketone) groups, respectively. A sharp peak at (2951-2808 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 (1608-1616 cm^{-1}).

In the ^{1}H NMR spectra of all compounds, the methylene protons resonated at δ (4.46-4.52) 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 (12.15-13.15) in all compounds, while the singlet NH signal of (N-H amide) appeared at δ (8.59-8.65) in all compounds. The signals belonging to the aromatic region were observed at δ (7.91-6.93) ppm. In the ^{13}C NMR spectra, the methylene carbon between the sulfur atom and carbonyl group was observed at δ (35.24-36.08) ppm. Two carbons on the thiadiazole ring were observed at δ (163.85-157.45) ppm. The carbonyl carbon of the amide group was observed at δ (167.39-170.75) ppm, and the carbonyl carbon of the ketone group was observed at δ (195.38-195.95) ppm. All other aromatic carbons were recorded between δ (139.97) and δ (111.28).



Reagents and condition: (i) KOH, EtOH, reflux 3h; (ii) $POCl_3$, reflux 3h; H_2O, reflux 4 h; KOH; (iii) $ClCH_2COCl$, TEA, DMF, reflux 2h; (iv) K_2CO_3, DMF, reflux 10 h

Scheme 1. Synthetic route for preparation of compounds **4**(**A-E**).

Table 1. Physical properties of the synthesized compounds.

Comp. No.	R	Molecular formula	M.Wt.	Color	M.P. (°C)	Yield %	R_f
1	-	$C_{14}H_{10}N_2OS$	254.31	Pale yellow	258-260	85	0.56
2(**A**)	H	$C_8H_7N_3S$	177.23	Pale yellow	224-227	76	0.46
2(**B**)	4-Cl	$C_8H_6ClN_3S$	211.67	Yellow	226-229	80	0.51
2(**C**)	2-Cl	$C_8H_6ClN_3S$	211.67	Yellow	214-216	70	0.58
2(**D**)	4-NO_2	$C_8H_6N_4O_2S$	222.22	Dark yellow	244-247	78	0.45
2(**E**)	3,5-di-NO_2	$C_8H_5N_5O_4S$	267.22	Dark yellow	265-268	75	0.62
3(**A**)	H	$C_{10}H_8ClN_3OS$	253.70	Pale yellow	233-235	82	0.48
3(**B**)	4-Cl	$C_{10}H_7Cl_2N_3OS$	288.15	Beige	254-256	85	0.54
3(**C**)	2-Cl	$C_{10}H_7Cl_2N_3OS$	288.15	Beige	230-232	84	0.48

3(**D**)	4-NO_2	$C_{10}H_7ClN_4O_3S$	298.70	Dark beige	250-252	80	0.47
3(**E**)	3,5-di-NO_2	$C_{10}H_6ClN_5O_5S$	343.70	Pale brown	240-243	86	0.53
4(**A**)	H	$C_{24}H_{17}N_5O_2S_2$	471.55	Dark brown	210-212	74	0.38
4(**B**)	4-Cl	$C_{24}H_{16}ClN_5O_2S_2$	506.00	Bronze	224-226	67	0.43
4(**C**)	2-Cl	$C_{24}H_{16}ClN_5O_2S_2$	506.00	Orange	204-207	70	0.41
4(**D**)	4-NO_2	$C_{24}H_{16}N_6O_4S_2$	516.55	Dark brown	199-202	71	0.39
4(**E**)	3,5-di-NO_2	$C_{24}H_{15}N_7O_6S_2$	561.55	Dark brown	193-196	76	0.42

Biological evaluation

The antibacterial activities of all the synthesized compounds **4**(**A-E**) were evaluated using the cup-plate agar diffusion method [28]. The antibacterial activity was assessed by measuring the inhibition zone in millimeters. Azithromycin, at a concentration of 300 µg/mL, served as the standard drug for comparison. The compounds were tested for their antibacterial activity against four types of bacterial isolates: two gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) on Muller Hinton agar. The agar media were sterilized, poured into Petri dishes, and allowed to solidify. Microbial suspensions were then spread on the surface of the media using a sterilized triangular loop. Cavities were created using pre-sterilized stainless steel cylinders with a 12 mm diameter.

All the synthesized compounds (300 µg/mL) were sequentially placed into the cavities using a micropipette and allowed to diffuse for one hour. DMSO served as the solvent for all compounds (as a stock), and then a concentration of 300 µg/mL was prepared using sterile distilled water. After incubating the plates at 37 °C for 48 hours, the zone of inhibition surrounding the cavities was measured, and the percentage inhibition of the compounds was calculated. The findings are detailed in Table 2. When we examine the data for the inhibition zone percentages of all compounds **4**(**A-E**) in Table 2, some important results emerge. Firstly, compounds **4**(**D**) and **4**(**E**) exhibit notable activity against *E. coli* and *S. aureus*. Upon analyzing the percentage of inhibition zone for compounds **4**(**D**) and **4**(**E**) against *E. coli* and *S. aureus*, it becomes evident that the antibacterial activity of these compounds increases with the number of (NO_2) groups on the benzene ring of the molecules.

Table 1. Antibacterial activities of compounds **4**(**A-E**).

Comp. No.	Gram-positive				Gram-negative			
	S. aureus		*S. epidermidis*		*P. aeruginosa*		*E. coli*	
	Zone of inhibition (mm)	% inhibition	Zone of inhibition (mm)	% inhibition	Zone of inhibition (mm)	% inhibition	Zone of inhibition (mm)	% inhibition
4(**A**)	14	46.67	0	0	0	0	10	50.00
4(**B**)	35	116.67	0	0	0	0	30	150.00
4(**C**)	34	113.33	0	0	0	0	27	135.00
4(**D**)	35	116.67	0	0	0	0	20	100.00
4(**E**)	36	120.00	0	0	0	0	25	125.00
Azithromycin	30	100	28	100	22	100	20	100
DMSO solvent	-		-		-		-	

Furthermore, compounds **4**(**B**) and **4**(**C**) also display significant activity against *S. aureus* and *E. coli*. Upon evaluating the percentage of inhibition zone for compounds **4**(**B**) and **4**(**C**) against *E. coli* and *S. aureus*, it becomes evident that the strong antibacterial activity of these compounds is attributed to the presence of a chlorine atom on the benzene ring of the molecules.

Additionally, we observed that compound **4**(**A**) displayed moderate activity against *S. aureus* and *E. coli* but lacked antibacterial effects against *P. aeruginosa* and *S. epidermidis*. Similarly, all compounds **4**(**A-E**) showed no antibacterial activity against *P. aeruginosa* and *S. epidermidis* at a concentration of 300 µg/mL. Overall, the screening results indicate that the majority of 1,3,4-thiadiazole derivatives **4**(**A-E**) exhibit superior antibacterial activity compared to the standard (azithromycin) against *E. coli* and *S. aureus*, while they do not exert any antibacterial effects against *P. aeruginosa* and *S. epidermidis* at the tested concentration.

Molecular docking

The Auto Dock 4.2 (MGL tools-1.5.6) software was employed to conduct all docking experiments. A new series of 1,3,4-thiadiazoles that have a benzimidazole ring, were docked with the DNA gyrase subunit B (PDB ID:1KZN). From the protein data bank (RCSB) (http://www.rcsb.org/pdb). The enzyme that topoisomerizes DNA was located. To conduct in silico research, the 2D structures of the manufactured ligands **4**(**A-E**) were created and then converted to energy-minimized 3D structures in the pdb file format using Marvin Sketch (Chem Axon). By deleting the heteroatoms, the water molecule and other associated compounds, the goal of the mission was to have the target protein's file prepared by leaving the associated residue with the protein using Auto Dock 4.2 (MGL tools- 1.5.6). The process of preparing the target protein's file using Auto Dock 4.2 (MGL tools-1.5.6) has been accomplished; this involves the assignment of Gasteiger charges to all of the atoms in the molecule that are converted into AD4 type. The simulation of docking for the compounds **4**(**A-E**) was performed against the active site of DNA gyrase subunit b, the final step was the use of Discovery Studio 2021 to visualize the results of the docking. These results are listed in Table 2.

Table 2. Molecular docking reports for compounds **4**(**A-E**) against protein 1KZN.

Comp. No.	Binding energy (kcal/mol)	Inhibition constant (Ki)	Residue involved H-bond
4(**A**)	-8.77	369.84	-
4(**B**)	-9.24	170.00	THR165, GLU50
4(**C**)	-8.13	1.10	-
4(**D**)	-8.74	392.07	THR165, VAL120,ASN46 ,GLY117, HIS116
4(**E**)	-7.97	74.34	ARG136, THR156, ARG76

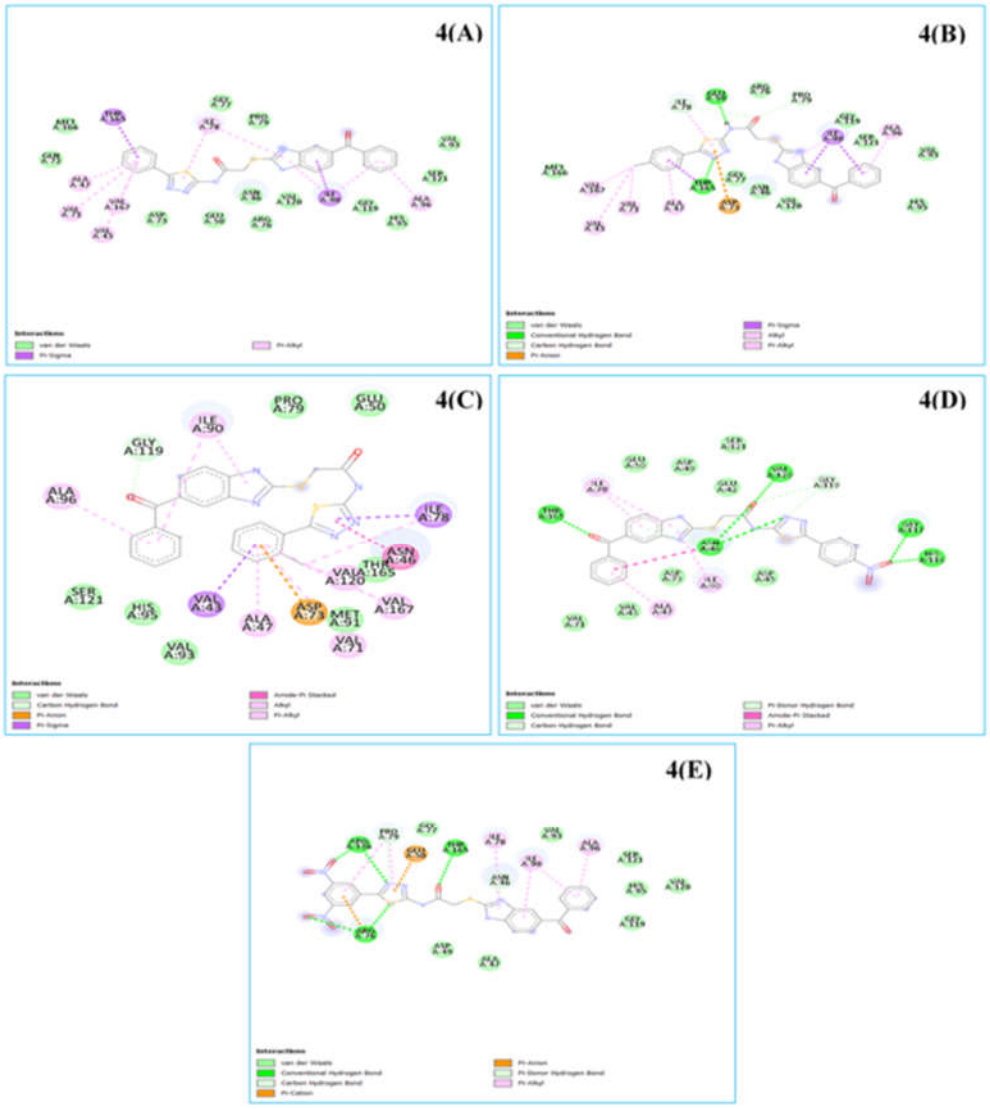


Figure 1. Ligand–protein 2D interaction on the active site of PDB 1KZN.

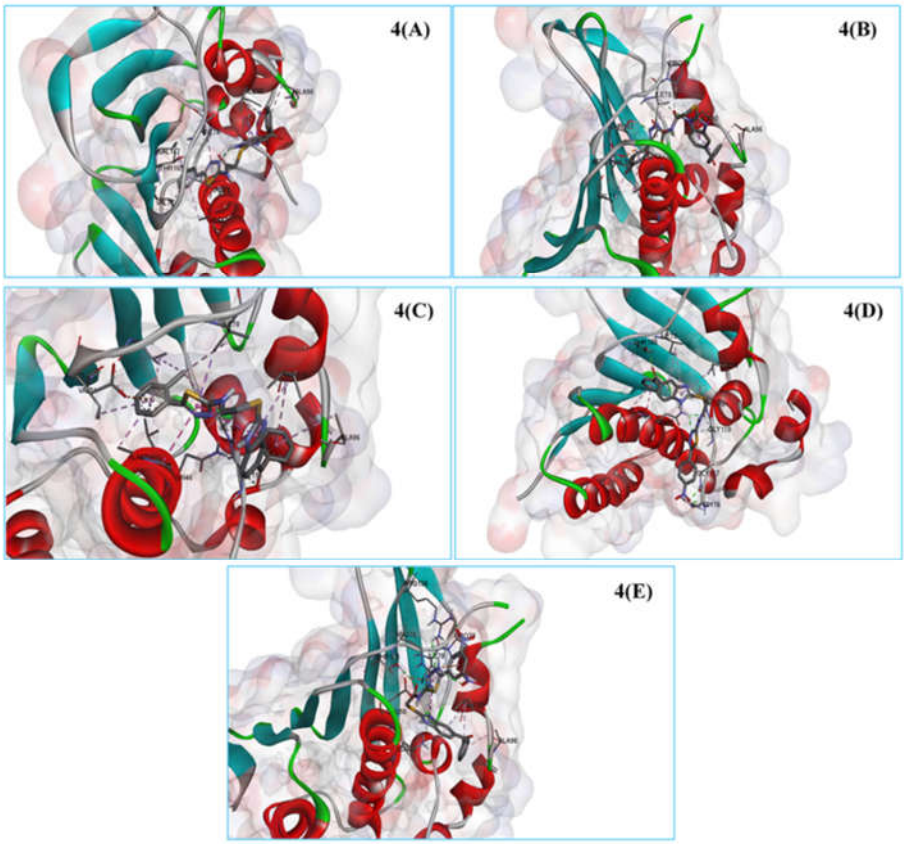


Figure 2. Ligand–protein 2D interaction on the active site of PDB 1KZN.

EXPERIMENTAL

Materials and methods

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, ^{1}H and ^{13}C-NMR spectra were recorded on Bruker model Ultra shield 500 MHz spectrophotometer using DMSO-d_6 as a solvent and TMS as an internal reference. Direct insertion probe (EI-DIP-MS) spectroscopy from Agilent Technologies was used to produce mass spectra for compounds **4**(**A-E**). The spectra were recorded at the University of Isfahan in Islamic Republic of Iran. The compounds were evaluated for their purity on silica gel TLC plates and the visualization of spots achieved by UV light.

*Synthesis of 2-mercapto-5-benzoylbenzimidazole (**1**).* A mixture of (20 mmol) of *3,4-diaminobenzophenone*, (20 mmol) of carbon disulfide, (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 min, 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)).

Pale yellow powder, yield (85%), m.p. 258-260 °C, R_f = 0.56; FT-IR (KBr disk, cm^{-1}) 3134 (N-H), 3041 (C-H, aromatic), 2569 (S-H), 1641 (C=O), 1618 (C=N), 1595 and 1473 (C=C, aromatic). ^{1}H NMR (DMSO-*d6*, 500 MHz, *δ*) 12.95 and 12.85 (s, 2H, benzimidazole-NH, tautomeric), 3.33 (s, 1H, SH). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 201.9 (C=O), 170.2 (C=N), 115.12-149.20 (aromatic rings).

General procedure for the synthesis of 2-amino-5-(substituted)-1,3,4-thiadiazole ***2(A-E)***

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)).

2-Amino-5-(phenyl)-1,3,4-thiadiazole ***2(A)***. Pale yellow powder, yield (76%), m.p. 224-227 °C, R_f = 0.46; FT-IR (KBr disk, cm^{-1}) 3275 and 3113 (NH_2), 3043 (C-H, aromatic), 1631 (C=N), 1581 and 1469 (C=C, aromatic). ^{1}H NMR (DMSO-*d6*, 500 MHz, *δ*) 7.75–7.49 (m, 5H, Ar–H), 7.33 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 180.8, 165.4 (C=N), 135.1-125.2 (aromatic ring).

2-Amino-5-(4-chlorophenyl)-1,3,4-thiadiazole ***2(B)***. Yellow powder, yield (80%), m.p. 226-229 °C, R_f = 0.51; FT-IR (KBr disk, cm^{-1}) 3271 and 3105 (NH_2), 3047 (C-H, aromatic), 1631 (C=N), 1597 and 1462 (C=C, aromatic). ^{1}H NMR (DMSO-*d6*, 500 MHz, *δ*) 7.85–7.451 (m, 4H, Ar–H), 7.43 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 179.5, 163.7 (C=N), 136.1-124.2 (aromatic ring).

2-Amino-5-(2-chlorophenyl)-1,3,4-thiadiazole ***2(C)***. Yellow powder, yield (70%), m.p. 214-216 °C, R_f = 0.58; FT-IR (KBr disk, cm^{-1}) 3282 and 3105 (NH_2), 3028 (C-H, aromatic), 1635 (C=N), 1597 and 1458 (C=C, aromatic). ^{1}H NMR (DMSO-*d6*, 500 MHz, *δ*) 7.88–7.69 (m, 4H, Ar–H), 7.13 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 178.3, 164.5 (C=N), 137.3-128.2 (aromatic ring).

2-Amino-5-(4-nitrophenyl)-1,3,4-thiadiazole ***2(D)***. Dark yellow powder, yield (78%), m.p. 244-247 °C, R_f = 0.45; FT-IR (KBr disk, cm^{-1}) 3259 and 3113 (NH_2), 3066 (C-H, aromatic), 1627 (C=N), 1597 and 1458 (C=C, aromatic), 1508 and 1346 (NO_2). ^{1}H NMR (DMSO-*d6*, 500 MHz, *δ*) 7.95–7.69 (m, 4H, Ar–H), 7.43 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 179.5, 163.2 (C=N), 150.3-125.7 (aromatic ring).

2-Amino-5-(3,5-dinitrophenyl)-1,3,4-thiadiazole ***2(E)***. Dark yellow powder, yield (75%), m.p. 265-268 °C, R_f = 0.62; FT-IR (KBr disk, cm^{-1}) 3275 and 3116 (NH_2), 3055 (C-H, aromatic), 1624 (C=N), 1593 and 1419 (C=C, aromatic), 1539 and 1350 (NO_2). ^{1}H NMR (DMSO-*d6*, 500 MHz, *δ*) 8.15–7.69 (m, 3H, Ar–H), 7.53 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 180.5, 163.8 (C=N), 153.3-127.7 (aromatic ring).

*General procedure for the synthesis of 2-chloro-N-(5-((un)substituted phenyl)-1,3,4-thiadiazol-2-yl) acetamides **3(A-E)***

*Compounds **2(A-E)***. (5 mmol) were dissolved in DMF and TEA (5 mmol) was added to the reaction mixtures. Chloroacetyl chloride (10 mmol) was slowly added to the reaction mixtures. The reaction mixtures were heated for 2 h under reflux. The solution was then added into crushed ice and the obtained product was filtered, washed with water, 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)).

*2-Chloro-N-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide **3(A)***. Pale yellow powder, yield (82%), m.p. 233-235 °C, R_f = 0.48; FT-IR (KBr disk, cm^{-1}) 3182 (N-H), 3043 (C-H, aromatic), 2947, 2835 (C-H, aliphatic), 1705 (C=O), 1627 (C=N), 1573 and 1438 (C=C, aromatic). ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.60 (s, 2H, CH_2), 7.43-7.81 (m, 5H, Ar-H), 8.65 (s, 1H, NH, amide). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 179.5, 153.8 (C=N), 168(C=O),135.3-128.7 (aromatic ring), 45.3(C-Cl).

*2-Chloro-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)acetamide **(3(B)***. Beige powder, yield (85%), m.p. 254-256 °C, R_f = 0.54; FT-IR (KBr disk, cm^{-1}) 3170 (N-H), 3043 (C-H, aromatic), 2943, 2835 (C-H, aliphatic), 1705 (C=O), 1631 (C=N), 1570 and 1442 (C=C, aromatic). ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.63 (s, 2H, CH_2), 7.47-7.84 (m, 4H, Ar-H), 8.55 (s, 1H, NH, amide). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 178.3, 155.9 (C=N), 167 (C=O), 137.3-126.5 (aromatic ring), 45.7 (C-Cl).

*2-Chloro-N-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)acetamide **3(C)***. Beige powder, yield (84%), m.p. 230-232 °C, R_f = 0.48; FT-IR (KBr disk, cm^{-1}) 3174 (N-H), 3032 (C-H, aromatic), 2939, 2831 (C-H, aliphatic), 1708 (C=O), 1624 (C=N), 1581 and 1438 (C=C, aromatic). ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.58 (s, 2H, CH_2), 7.53-7.84 (m, 4H, Ar-H), 8.63 (s, 1H, NH, amide). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 178.5, 155.4 (C=N). 166.5 (C=O), 136.5-125.5 (aromatic ring), 45.5 (C-Cl).

*2-Chloro-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide **3(D)***. Dark beige powder, yield (80%), m.p. 250-252 °C, R_f = 0.47; FT-IR (KBr disk, cm^{-1}) 3163 (N-H), 3032 (C-H, aromatic), 2943, 2839 (C-H, aliphatic), 1708 (C=O), 1627 (C=N), 1593 and 1438 (C=C, aromatic), 1519 and 1346 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.71 (s, 2H, CH_2), 7.83-8.14 (m, 4H, Ar-H), 8.75 (s, 1H, NH, amide). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 178.9, 154.5 (C=N), 166 (C=O), 147.3-125.5 (aromatic ring), 44.7 (C-Cl).

*2-Chloro-N-(5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide **3(E)***. Pale brown powder, yield (86%), m.p 240-243 °C, R_f = 0.62; FT-IR (KBr disk, cm^{-1}) 3182 (N-H), 3035 (C-H, aromatic), 2943, 2877 (C-H, aliphatic), 1712 (C=O), 1624 (C=N), 1593 and 1438 (C=C, aromatic), 1543 and 1350 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.68 (s, 2H, CH_2), 7.88-8.21 (m, 3H, Ar-H), 8.69 (s, 1H, NH, amide). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 180.1, 157.5 (C=N). 168.3 (C=O), 150.3-128.5 (aromatic ring), 44.3 (C-Cl).

*General procedure for the synthesis of the compounds **4(A-E)***

A mixture of 2-chloro-N-(5-((un)substituted phenyl)-1,3,4 thiadiazole-2-yl) acetamide (5 mmol), 2-mercapto-5-benzoylbenzimidazole (5 mmol) and K_2CO_3 (5 mmol) in DMF (25 mL) was refluxed for 10 h. The solution was then added into crushed ice and the obtained product 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 (2:3)).

2-((5-Benzoylbenzimidazol-2-yl)thio)-N-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide ***4(A)***. Dark brown powder, yield (74%), m.p. 210-212 °C, R_f = 0.38; FT-IR (KBr disk, cm^{-1}) 3282 (N-H, amide), 3140 (N-H, benzimidazole), 3055 (C-H, aromatic), 2947, 2877 (C-H, aliphatic), 1670 (C=O), 1647 (C=O, ketone), 1608 (C=N), 1562 and 1431 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.50 (s, 2H, CH_2), 7.42-7.80 (m, 13H, Ar-H), 8.60 (s, 1H, NH, amide), 12.95 (s, 1H, benzimidazole-NH); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 35.52, 117.08, 122.34, 126.22, 128.12, 129.20, 130.84, 131.12, 131.25, 131.84, 132.38, 132.76, 137.94, 149.39, 159.46, 161.44, 168.22, 195.77, MS (m/z): 471.18 (M+).

2-((5-Benzoyl benzimidazol-2-yl)thio)-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)acetamide ***4(B)***. Bronze powder, yield (67%), m.p. 224-226 °C, R_f = 0.43; FT-IR (KBr disk, cm^{-1}) 3325 (N-H, amide), 3151 (N-H, benzimidazole), 3051 (C-H, aromatic), 2951, 2885 (C-H, aliphatic), 1678 (C=O), 1643 (C=O, ketone), 1612 (C=N), 1593 and 1427 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.46 (s, 2H, CH_2), 7.25-7.77 (m, 12H, Ar-H), 8.62 (s, 1H, NH, amide), 12.88 (s, 1H, benzimidazole-NH); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 36.08, 120.22, 122.39, 125.30, 128.82, 129.77, 130.84, 131.55, 131.86, 132.18, 132.47, 137.44, 149.09, 160.22, 162.12, 168.46, 195.83, MS (m/z): 505.12 (M+).

2-((5-Benzoyl benzimidazol-2-yl)thio)-N-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)acetamide ***4(C)***. Orange powder, yield (70%), m.p. 204-207 °C, R_f = 0.41; FT-IR (KBr disk, cm^{-1}) 3305 (N-H, amide), 3167 (N-H, benzimidazole), 3055 (C-H, aromatic), 2904, 2835 (C-H, aliphatic), 1674 (C=O), 1647 (C=O, ketone), 1612 (C=N), 1573 and 1431 (C=C, aromatic); ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.52 (s, 2H, CH_2), 7.50-7.79 (m, 12H, Ar-H), 8.62 (s, 1H, NH, amide), 13.15 (s, 1H, benzimidazole-NH); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 35.24, 119.22, 124.34, 128.30, 128.82, 129.27, 129.84, 131.01, 131.25, 131.49, 132.28, 132.47, 138.50, 148.09, 158.39, 160.44, 167.39, 195.95, MS (m/z): 505.08 (M+).

2-((5-Benzoyl benzimidazol-2-yl)thio)-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide ***4(D)***. Dark brown powder, yield (71%), m.p. 199-202 °C, R_f = 0.43; FT-IR (KBr disk, cm^{-1}) 3278 (N-H, amide), 3132 (N-H, benzimidazole), 3055 (C-H, aromatic), 2939, 2873 (C-H, aliphatic), 1685 (C=O), 1643 (C=O, ketone), 1612 (C=N), 1597 and 1442 (C=C, aromatic), 1519, 1346 (NO_2); ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.52 (s, 2H, CH_2), 7.25-7.77 (m, 12H, Ar-H), 8.59 (s, 1H, NH, amide), 12.92 (s, 1H, benzimidazole-NH); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 35.32, 111.28, 119.56, 124.88, 125.79, 128.40, 128.80, 128.87, 129.78, 131.42, 132.60, 132.65,136.26, 138.20, 148.59, 160.42, 163.85, 170.75, 195.38. MS (m/z): 516.1 (M+).

2-((5-Benzoyl benzimidazol-2-yl)thio)-N-(5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide ***4(E)***. Dark brown powder, yield (76%), m.p. 193-196 °C, R_f = 0.42; FT-IR (KBr disk, cm^{-1}) 3360 (N-H, amide), 3178 (N-H, benzimidazole), 3093 (C-H, aromatic), 2939, 2877 (C-H, aliphatic), 1678 (C=O), 1654 (C=O, ketone), 1616 (C=N), 1593 and 1423 (C=C, aromatic), 1543 and 1346 (NO_2); ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 4.49 (s, 2H, CH_2), 7.50-7.79 (m, 11H, Ar-H), 8.65 (s, 1H, NH, amide), 13.12 (s, 1H, benzimidazole-NH); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 35.33, 118.12, 125.30, 128.30, 128.52, 129.42, 129.82, 130.11, 131.25, 131.88, 132.28, 134.57, 137.50, 149.16, 159.19, 160.68, 168.39, 195.75. MS (m/z): 561.02 (M+).

CONCLUSION

Benzimidazole compounds containing 1,3,4-thiadazole moiety were prepared and structurally characterized using spectroscopic techniques. The pharmacological study was performed to study the substituent effects on the antibacterial activity, some of the derivatives showed good to moderate activity against bacteria.

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