

Original Article

New heterocyclic benzoxazole-pyrrolidin-2-one derivatives: Synthesis, biological assessment, DFT and docking investigations

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ABSTRACT

In this study, we synthesized new heterocyclic derivatives of pyrrolidinone linked to benzoxazole as part of an ongoing effort to combat the growing global threat of bacterial resistance. Antibiotic resistance, particularly against common pathogenic bacteria, has become a major challenge in public health, highlighting the urgent need for novel antimicrobial agents. The synthesized derivatives were obtained from 2-aminophenol and substituted amines. The structure of compounds **5-8** was confirmed using various analytical techniques, including ^1H , ^{13}C , MS, and fourier transform infrared spectroscopy (FTIR) spectroscopy, which were consistent with the proposed structures. A brief density functional theory (DFT) theoretical study was conducted to explain the observed stability of derivatives **5-8**, followed by a comparative analysis of theoretical and experimental NMR data. The antibacterial activity of the compounds was tested against four pathogenic bacterial strains, addressing the critical need for new agents with broad-spectrum efficacy. Finally, molecular docking studies were performed to explore the binding interactions and identify the most significant binding sites of the prepared ligands with biological targets. The calculated binding energy for derivative **8** is -8.54 kcal/mol, making it the best derivative due to its more significant interactions.

1. Introduction

The human organism is constantly under threat from a wide array of pathogens, including bacteria, viruses, and fungi. Among these, bacteria are particularly concerning due to their capacity to invade tissues and cause life-threatening infections. Gram-negative bacteria, such as *E. coli* [1], *K. pneumoniae* [2], *P. aeruginosa* [3], and *A. baumannii* [4], are especially problematic because they are implicated in severe health conditions, including pneumonia, urinary tract infections, meningitis, and sepsis. Similarly, Gram-positive bacteria like *S. aureus* and *B. cereus* are responsible for a variety of infectious diseases. *S. aureus*, which was associated with over a million deaths in 2019 [5,6], is known to cause conditions such as pneumonia, surgical site infections, neonatal mortality, and sepsis [7]. *B. cereus*, traditionally regarded as a pathogen in food and soil, has emerged as a cause of more severe infections, including endocarditis, respiratory diseases, and skin infections [8,9]. The growing issue of antibiotic resistance further complicates the treatment landscape. While antibiotics have been the primary method for managing bacterial infections, the rapid development of resistance, especially to third-generation antibiotics like carbapenems, has rendered many treatments ineffective. The emergence of these resistant strains presents a serious public health threat, leading the World Health Organization to prioritize research into combating antibiotic-resistant bacteria and to advocate for the development of novel treatment approaches [10,11].

Pyrrolidinones scaffold is found in diverse active natural compounds. They have attracted more importance because of their applications in medicinal chemistry [12]. This molecule has shown a variety of therapeutic activities such as antibacterial [13], antimicrobial [14,15], analgesic [16], anticonvulsant [17], HIV-1 inhibitors [18], and anticancer [19]. Therefore, diifent synthetic paths have been used for access to pyrrolidinones molecules [20-23].

On the other hand, benzoxazole moieties have been attracted more attention of the scientist due to their therapeutic uses [24,25]. Actually, they have been known to show a prominent anti-cancer [26], anti-tumor [27], antimicrobial [28,29] and HIV-1 inhibitors [30] activities.

Small-molecule inhibitors targeting the essential prokaryotic cell division protein FtsZ have emerged as promising candidates for therapies, especially in the context of antibiotic-resistant infections [31]. However, no FtsZ inhibitors have yet advanced to clinical stages. Recently, a new series of substituted derivatives, specifically compounds **A** and **B** (Figure 1), were reported. These isoxazole derivatives showed significant improvements in both in vitro and in vivo models. Notably, the most remarkable enhancement was observed in their activity against the FtsZ G196A mutation, a common alteration associated with resistance to certain antibacterial agents, which makes S less effective [32].

Recently, there has been significant interest in the development of heterocyclic scaffolds as potential antibacterial agents [33,34]. Among these, nitrogen-containing heterocyclic compounds have

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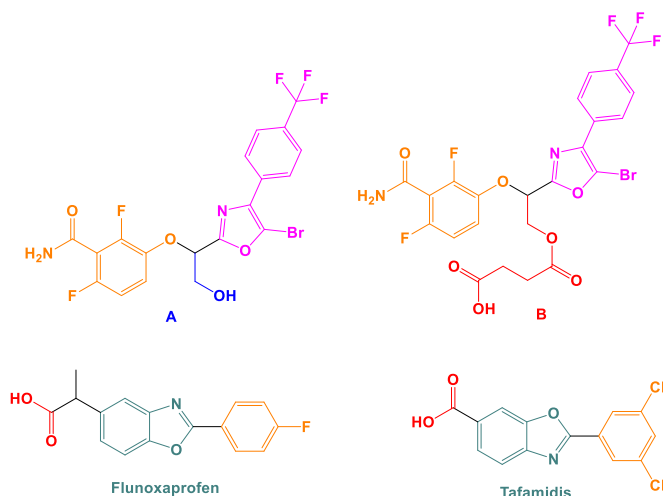


Figure 1. Chemical structures of isoxazole and benzoxazole derivatives.

gained considerable attention from medicinal chemists. Benzimidazole derivatives are prominent within this group of potential therapeutic agents. They are recognized as a promising class of bioactive molecules, exhibiting a broad spectrum of activities, including antiprotazoal, anti-inflammatory, antihelminthic, antimalarial, antimicrobial, antiviral, antiparasitic, and antimycobacterial effects [35,36]. Additionally, benzoxazoles have garnered attention as key pharmacophores in synthetic and medicinal chemistry due to their significant pharmacological properties. This diverse family of compounds demonstrates a wide array of biological effects, such as anti-diabetic, anti-inflammatory, anticonvulsant, anticancer, antiviral, antibacterial, antituberculosis, antioxidant, and enzyme-inhibiting activities [37,38]. Figure 1 illustrates some commercially available drugs containing Benzoxazole derivatives, including examples like Flunoxaprofen and Tafamidis.

In continuation of our ongoing research projects focused on the development of biologically active heterocycles [39-42], this study aims to design and synthesize novel benzoxazole derivatives linked to 2-pyrrolidinones. These new compounds were carefully characterized and evaluated for their antibacterial activity, specifically against a range of pathogenic bacterial strains. In addition to the experimental antibacterial testing, computational studies, including Density Functional Theory (DFT) and molecular docking, were conducted to provide a deeper understanding of the interactions between the synthesized derivatives and potential bacterial targets. By combining experimental and computational approaches, we seek to identify promising candidates with enhanced antibacterial properties and offer insights into their mechanism of action, contributing to the development of new therapeutic agents in the fight against antibiotic-resistant bacterial infections.

2. Materials and Methods

2.1. Chemistry

The NMR spectra were recorded using a Bruker 300 MHz spectrometer with DMSO- d_6 as the solvent. The molecular mass of the products was determined by UPLC-MS (Q-TOF-ESI). The IR spectrum was obtained using a Shimadzu FT-IR spectrometer with KBr pellets.

2.1.1. Synthesis of derivatives 1-4

An equimolar mixture of the selected amines (45 mmol) and itaconic acid was dissolved in 30 mL of triple-distilled water and heated under reflux for approximately 45 mins. After the reaction mixture had cooled, it was filtered and washed with cold water. The obtained solid was then dissolved in a small amount of 10% aqueous NaOH then filter.

1-Methyl-5-oxopyrrolidine-3-carboxylic acid 1. Solid, yield 64 %, m.p. 151-153 °C. IR (KBr, cm^{-1}): 1672 (N-C=O), 1713 (HO-C=O),

2926 (Csp³-H), 3250 (COO-H). ¹H-NMR (δ , ppm): 2.59-2.78 (m, 2H, COCH₂), 3.61 (s, 3H, CH₃), 3.91-4.05 (m, 3H, NCH₂ & CH_{pyrr}), 11.32 (s, 1H, H_{acid}); MS (m/z): 143.09 (M⁺). calcd: C, 50.35; H, 6.34; N, 9.79. Found: C, 50.05; H, 6.40; N, 9.91.

1-Ethyl-5-oxopyrrolidine-3-carboxylic acid 2. Solid, yield 66 %, m.p. 156-158 °C. IR (KBr, cm^{-1}): 1665 (N-C=O), 1721 (HO-C=O), 2935 (Csp³-H), 3218 (COO-H). ¹H-NMR (δ , ppm): 1.40-1.44 (t, 3H, CH₃, $J = 6$ Hz); 2.59-2.79 (m, 2H, COCH₂), 3.57-3.72 (m, 2H, H₂C), 3.97-4.13 (m, 3H, NCH₂ & CH_{pyrr}), 11.39 (s, 1H, H_{acid}); MS (m/z): 157.10 (M⁺). calcd: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.55; H, 7.12; N, 8.87.

1-Isopropyl-5-oxopyrrolidine-3-carboxylic acid 3. Solid, yield 68 %, m.p. 160-162 °C. IR (KBr, cm^{-1}): 1652 (N-C=O), 1718 (HO-C=O), 2941 (Csp³-H), 3225 (COO-H). ¹H-NMR (δ , ppm): 1.29-1.33 (d, 6H, H₃C, $J = 6.3$ Hz), 2.60-2.82 (m, 2H, COCH₂), 3.56-3.75 (m, 1H, CH), 3.98-4.14 (m, 3H, H₂C-N & CH_p), 11.34 (s, 1H, H_{acid}); ESI-MS (m/z): 171.11 (M⁺) (C₈H₁₃NO₃). calcd: C, 56.13; H, 7.65; N, 8.18. Found: C, 56.32; H, 7.42; N, 8.25.

1-Cyclohexyl-5-oxopyrrolidine-3-carboxylic acid 4. Solid, yield 72 %, m.p. 185-187 °C. IR (KBr, cm^{-1}): 1638 (N-C=O), 1741 (HO-C=O), 2945 (Csp³-H), 3235 (COO-H). ¹H-NMR (δ , ppm): 1.05-1.75 (m, 10H, H₂C), 2.62-2.77 (m, 2H, COCH₂), 3.61-3.77 (m, 1H, H-C), 4.05-4.19 (m, 3H, H₂C-N & CH_{pyrr}), 11.30 (s, 1H, H_{acid}); MS (m/z): 211.14 (M⁺) (C₁₁H₁₇NO₃). calcd: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.42; H, 7.98; N, 6.48.

2.1.2. Synthesis of derivatives 5-8

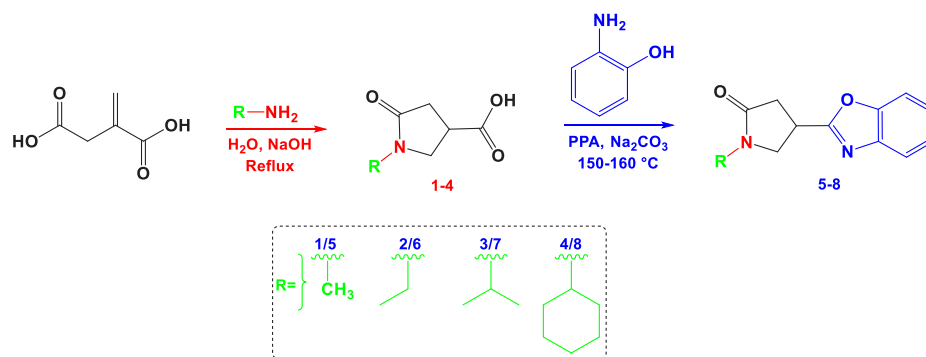
A mixture of the appropriate 1-(methyl/ethyl/isopropyl/cyclohexyl-substituted)-5-oxopyrrolidine-3-carboxylic acids (15 mmol), polyphosphoric acid (15 g), and 2-aminophenol (15 mmol) was heated to 165 °C and stirred for 4h. A 25 mL solution of 5-10% Na₂CO₃ was then added, and the mixture was heated for an additional 10 mins. After this period, 100 mL of water was added, and the mixture was stirred at 25 °C for 15 mins. The obtained mixture was purified by column chromatography using a hexane/ethyl acetate eluent.

4-(Benzo [d] oxazol-2-yl)-1-methylpyrrolidin-2-one 5. Solid, yield 45 %, m.p. 87-89 °C. IR (KBr, cm^{-1}): 1390 (C-N), 1558 (C=C), 1622 (CN), 1702 (CO), 3081 (CH_{Ar}). ¹H-NMR (δ , ppm): 2.89-3.09 (m, 2H, H₂C-CO), 3.76 (s, 3H, H₃C), 4.00-4.07 (m, 1H, CH_{pyrr}), 4.16-4.32 (m, 2H, NCH₂), 7.55-7.58 (d, 2H, H-4 & -7_{oxazole}), 8.10-8.13 (t, 2H, H-5 & -6_{oxazole}, $J = 7.2$ Hz); ¹³C-NMR (δ , ppm): 172.82, 168.12, 151.21, 142.52, 125.72, 124.44, 120.02, 111.36, 50.53, 41.15, 35.51, 29.16; MS (m/z): 216.11 (M⁺). Calcd: C, 66.65; H, 5.59; N, 12.96. Found: C, 66.82; H, 5.48; N, 13.15.

4-(Benzo [d] oxazol-2-yl)-1-ethylpyrrolidin-2-one 6. Solid, yield 48 %, m.p. 99-101 °C. IR (KBr, cm^{-1}): 1392 (C-N), 1556 (C=C), 1620 (CN), 1705 (CO), 3088 (CH_{Ar}). ¹H-NMR (δ , ppm): 1.85 (d, 3H, H₃C), 2.87-3.19 (m, 2H, COCH₂), 4.14-4.19 (m, 2H, H₂C), 4.22-4.37 (m, 3H, NCH₂ & CH_{pyrr}), 7.79-7.82 (d, 2H, H₄ & 7_{of oxazole}), 7.94-7.99 (t, 2H, H-5/-6_{of oxazole}, $J = 7.5$ Hz); ¹³C-NMR (δ , ppm): 172.55, 168.46, 151.12, 142.65, 125.37, 124.60, 120.09, 111.15, 49.24, 43.74, 41.47, 29.44, 19.52; MS (m/z): 230.12 (M⁺); Anal. calcd: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.95; H, 6.27; N, 12.36.

4-(Benzo [d] oxazol-2-yl)-1-isopropylpyrrolidin-2-one 7. Solid, yield 48 %, m.p. 94-96 °C. IR (KBr, cm^{-1}): 1385 (C-N), 1562 (C=C), 1618 (CN), 1704 (CO), 3078 (CH_{Ar}). ¹H-NMR (δ , ppm): 2.19 (d, 6H, H₃C), 2.91-3.10 (m, 2H, COCH₂), 3.97-4.07 (m, 1H, HC), 4.19-4.31 (m, 3H, NCH₂ & CH_{pyrr}), 7.80-7.79 (d, 2H, H-4/-7_{of oxazole}, $J = 9$ Hz), 8.12-8.17 (t, 2H, H-5/-6_{of oxazole}, $J = 7.5$ Hz). ¹³C-NMR (δ , ppm): 172.55, 168.46, 151.37, 142.95, 125.33, 124.58, 120.07, 111.26, 53.28, 47.14, 41.93, 29.81, 20.74; MS (m/z): 244.14 (M⁺); Calcd: C, 68.83; H, 6.60; N, 11.47. Found: C, 69.11; H, 6.83; N, 11.68.

4-(Benzo [d] oxazol-2)cyclohexylpyrrolidinone 8. Solid, yield 55 %, m.p. 129-131 °C. IR (KBr, cm^{-1}): 1378 (C-N), 1570 (C=C), 1625 (CN), 1700 (CO), 3097 (CH_{Ar}). ¹H-NMR (δ , ppm): 1.08-1.76 (m, 10H, Cyclohexane), 2.71-2.81 (m, 2H, H₂C), 3.65-3.84 (m, 3H, NCH₂ & CH_{pyrr}), 3.96-3.95 (m, 1H, HC), 7.35-7.38 (m, 2H, H-4/-7_{of oxazole}), 7.59-7.73 (m, 2H, H-5/-6_{of oxazole}). ¹³C-NMR (δ , ppm): 172.44, 168.39, 151.59, 142.91, 125.88, 124.73, 120.51, 111.48, 57.65, 48.38, 41.93, 29.87, 28.53, 26.18, 23.48; MS (m/z): 284.16 (M⁺). Calcd: C, 71.81; H, 7.09; N, 9.85. Found: C, 72.10; H, 7.25; N, 9.90.



Scheme 1. Scheme for intermediates 1-4 and target compounds 5-8 synthesis.

2.2. Theoretical details

The ground-state geometries of compounds **5-8** were optimized using the DFT method at the B3LYP/6-311++G(d,p) level. Frequency analyses were conducted to confirm that the optimized structures of **5-8** represent true minima [43]. These calculations were carried out in a solvent, employing the Polarizable Continuum Model (PCM) to account for solvent effects.

2.3. Docking studies

The docking of the synthesized compounds into the active site of tyrosyl-tRNA synthetase was performed using the PDB entry 1JJJ [44]. The docking simulations were carried out using the AutoDock software suite [45]. A detailed description of the molecular docking procedure can be found in the literature [46].

3. Result and Discussion

3.1. Chemistry

The intermediate compounds **1-4**, namely 1-(methyl/ethyl/isopropyl/cyclohexyl)-pyrrolidin-2-one-3-carboxylic acids, were synthesized by the fusion of appropriate amines with methylidenesuccinic acid in water. The subsequent reaction of these carboxylic acids **1-4** with 2-aminophenol was carried out using polyphosphoric acid, resulting in improved yields and purity of the benzoxazole derivatives **5-8** (Scheme 1).

The prototype intermediate derivative **1** exhibited characteristic absorption peaks in its IR spectrum, with bands at 1672 cm^{-1} (CO of the acid), 1714 cm^{-1} (CO), and 3250 cm^{-1} (O-H). In the $^1\text{H-NMR}$ spectrum of derivative **1**, the methyl protons appeared as a singlet at δ 3.59. The multiplets between δ 2.61 and 2.78 corresponded to two COCH_2 protons, while the range δ 3.91-4.05 represented two $\text{H}_2\text{C-N}$ protons and one CH proton from the pyrrolidine ring. A singlet at δ 11.32 was assigned to the acid proton, which was confirmed to be exchangeable with D_2O . The final prototype compound **5** displayed specific IR absorption bands at 1622 cm^{-1} (CN) and 1702 cm^{-1} (CO). In the $^1\text{H-NMR}$ spectrum, the methyl protons appeared as a singlet at δ 3.77. The two COCH_2 protons from the pyrrolidine ring were identified as a multiplet in the range δ 2.93-3.09. Multiplets at δ 3.99-4.09 and δ 4.16-4.32 were assigned to one H-C proton and two $\text{H}_2\text{C-N}$ protons of the pyrrolidine ring, respectively. The doublet at δ 7.54-7.57 was attributed to the H-4 and H-7 protons of the benzoxazole ring ($J = 8.1$). The other two protons of the benzoxazole ring were observed as a triplet at δ 8.08-8.12 ($J = 7.2$). The molecular ion (M^+) peak of derivative **5** was detected at m/z 216.11, confirming its successful synthesis.

3.2. NMR elucidation

NMR prediction consists a power tool to validate the observed chemical shifts. The experimental chemical shifts of **5-8** along with their predicted ones are displayed in Table 1. Figures 2 and 3 represent the optimized structures of **5-8** and the correlation curves obtained

for compound **1** between the experimental and the predicted values. The correlations curves between the predicted NMR chemical shifts of **5-6** and their corresponding experimental ones are relatively well reproduced with correlation coefficients higher than 98%. For ^1H , among **5-8**, a minimal deviation is of 0.03 ppm obtained for **6**, while a maximal deviation of 0.76 is obtained for **3** (Table 1). For $^{13}\text{C-NMR}$, among **5-8**, a minimal deviation of 2.54 is obtained for **7**, while a maximal deviation of 17 ppm is obtained for **8** (Table 1).

3.3. Antibacterial activity

The synthesized derivatives were subjected to antibacterial testing against several bacterial strains, which included two Gram-positive (GP) organisms (Methicillin-resistant *Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (GN) species (*Pseudomonas aeruginosa* and *Escherichia coli*). The assessment was carried out according to a standardized protocol outlined in reference. The outcomes of the antibacterial tests are summarized in Table 2. Among the compounds investigated, derivatives **5-8** exhibited notable antimicrobial efficacy against all the bacterial strains, except for *P. aeruginosa*, where they showed no significant inhibitory effect. This indicates that these derivatives have promising antibacterial properties, especially against GP bacteria, but their activity against GN pathogens like *P. aeruginosa* may require further refinement to improve efficacy.

3.4. Molecular docking

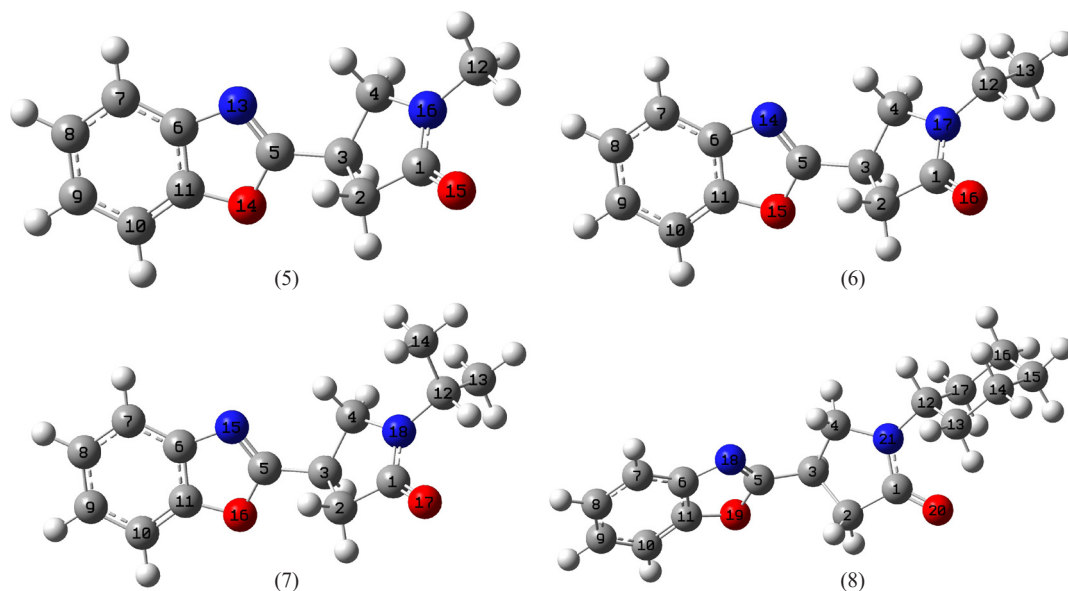
The antibacterial efficiency of prepared compounds **5-8** against methicillin-resistant staphylococcus aureus (MRSA) is displayed in Table 3. Compounds **5-7** showed moderate antibacterial activity, while **8** show potent activity. **5-7** differ slightly, in which the amine is substituted with methyl, ethyl and isopropyl alkyls, while in **4**, the amine is substituted with cyclohexane. Then, the higher activity of **8** may relate to the cyclohexyl moiety.

Table 3 presents an overview of the computed binding energies (BE) for the stable ligand-receptor complexes, along with the count of conventional intermolecular hydrogen bonds (HBs) formed between the docked compounds and the active site residues of the target enzyme. It also lists the specific amino acids involved in these interactions with the docked derivatives.

The interaction of the docked compounds **5-6** with the amino acids at the binding site of tyrosyl-tRNA synthetase results in negative bending energies, which could account for their significant antibacterial efficacy, as presented in Table 3. The molecular interactions between derivatives **5-8** and the tyrosyl-tRNA synthetase binding site are illustrated in Figure 4. In relation to the observed antibacterial potency, the superior activity of derivative **4** over derivatives **5-7** may be attributed to the enhanced stability of the complex between product **8** and tyrosyl-tRNA synthetase compared to the complexes formed with derivatives **5-7**. A more negative binding energy indicates greater stability of the complex, which correlates with stronger binding affinity of the docked derivative within the active site of tyrosyl-tRNA synthetase (Table 3). The lone pairs on the nitrogen and oxygen atoms of benzo[d]oxazole

Table 1. Experimental and predicted (^1H & ^{13}C)-NMR chemical shifts (ppm) for final products 5-8.

	5		$\Delta\delta$	6		$\Delta\delta$	7		$\Delta\delta$	8		$\Delta\delta$
	δ_{Pred}	δ_{Exp}		δ_{Pred}	δ_{Exp}		δ_{Pred}	δ_{Exp}		δ_{Pred}	δ_{Exp}	
^1H-NMR												
H2	2.96	3.01	0.05	3.11	3.03	0.08	3.20	3.00	0.20	2.66	2.7	0.04
H3	4.42	4.04	0.38	4.42	4.29	0.13	4.20	4.26	0.06	3.70	3.75	0.05
H4	3.80	4.24	0.44	4.15	4.29	0.14	4.52	4.26	0.26	3.55	3.75	0.20
H7	8.00	8.1	0.10	8.05	7.97	0.08	8.03	8.15	0.12	7.74	7.68	0.06
H8	7.70	7.56	0.14	7.76	7.8	0.04	7.74	7.81	0.07	7.44	7.37	0.07
H9	7.70	7.56	0.14	7.75	7.8	0.05	7.74	7.81	0.07	7.43	7.37	0.06
H10	7.86	8.1	0.24	7.92	7.97	0.05	7.90	8.15	0.25	7.60	7.68	0.08
H12	3.94	3.77	0.17	4.27	4.17	0.10	4.79	4.03	0.76	3.91	3.95	0.04
H13	-	-	-	1.75	1.85	0.10	1.89	2.20	0.31	1.39	1.3	0.09
H14	-	-	-	-	-	-	1.85	2.20	0.35	1.37	1.27	0.10
H15	-	-	-	-	-	-	-	-	-	1.50	1.53	0.03
H16	-	-	-	-	-	-	-	-	-	1.35	1.08	0.27
H17	-	-	-	-	-	-	-	-	-	1.50	1.74	0.24
^{13}C-NMR												
1	181.9	172.82	9.05	179.62	172.55	7.07	180.3	172.55	7.74	185.9	172.44	13.49
2	50.2	41.15	9.01	49.93	43.74	6.19	50.5	41.93	8.60	52.1	41.93	10.14
3	45.4	35.51	9.89	44.95	29.44	15.51	45.7	29.81	15.87	47.3	29.87	17.47
4	62.4	50.53	11.91	59.01	49.24	9.77	55.0	47.14	7.82	57.7	48.38	9.35
5	177.9	168.12	9.75	175.97	168.64	7.33	177.0	168.46	8.53	182.3	168.39	13.93
6	152.3	142.52	9.83	150.64	142.65	7.99	151.5	142.95	8.51	156.0	142.91	13.12
7	130.5	120.02	10.48	128.99	120.09	8.90	129.7	120.07	9.62	133.6	120.51	13.09
8	135.4	124.44	10.91	133.80	124.60	9.20	134.5	124.58	9.95	138.6	124.73	13.85
9	135.9	125.72	10.17	134.31	125.37	8.94	135.0	125.33	9.70	139.1	125.88	13.22
10	121.1	111.36	9.76	119.74	111.15	8.59	120.4	111.26	9.12	124.0	111.48	12.54
11	162.4	151.21	11.19	160.48	151.12	9.36	161.4	151.37	9.99	166.2	151.59	14.60
12	38.8	29.16	9.60	48.50	41.47	7.03	55.8	53.28	2.54	64.5	57.65	6.87
13	-	-	-	23.27	19.52	3.75	29.4	20.74	8.65	43.1	28.53	14.59
14	-	-	-	-	-	-	29.6	20.74	8.84	39.8	23.48	16.35
15	-	-	-	-	-	-	-	-	-	39.2	23.48	15.69
16	-	-	-	-	-	-	-	-	-	40.0	26.18	13.86
17	-	-	-	-	-	-	-	-	-	43.6	29.87	13.78

**Figure 2.** The optimized geometries of derivatives 5-8.

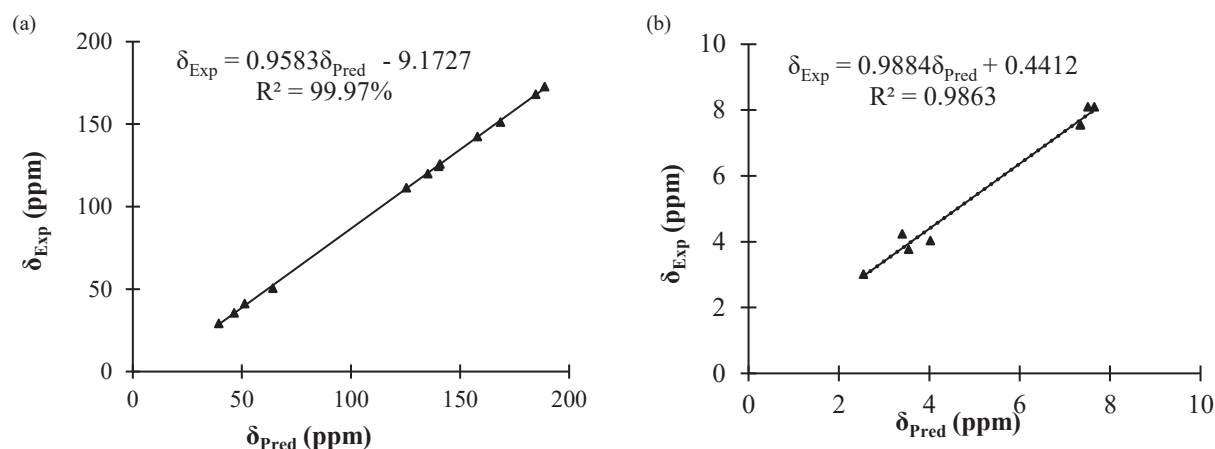


Figure 3. Predicted and experimental chemical shifts (a) ^{13}C - and (b) ^1H -NMR of product 5.

Table 2. Antibacterial activity of the derivatives 5-8.

			Bacterial Strains			
			Methicillin-resistant <i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>P. aeruginosa</i>	<i>Escherichia coli</i>
Derivatives 5-8	5	MIC ($\mu\text{g/ml}$)	50	37.5	-	25
	6		50	37.5	-	25
	7		37.5	37.5	-	18.75
	8		10	11	-	13
	5	MBC ($\mu\text{g/ml}$)	37.5	25	-	18.75
	6		37.5	25	-	18.75
	7		25	25	-	12.5
	8		12.5	12.5	-	9.5
	5	Inhibition zone in mm (100 $\mu\text{g/ml}$)	7	9	-	12
	6		8	10	-	12
	7		10	11	-	13
	8		16	15	-	22
	5	IC_{50} (μM)	231.48	173.61	-	115.74
	6		217.39	163.04	-	108.69
	7		153.68	153.68	-	76.84
	8		16	15	-	22
Ciprofloxacin		MIC ($\mu\text{g/ml}$)	18.75	12.5	12.5	12.5

Table 3. Docking of BEs, HBs and IC_{50} values against MRSA.

Product	BEs (kcal/mol)	HBs	Residues interacted with ligand in the active site	IC_{50} (μM) against MRSA
5	-6.02	1	PRO A53, CYS A37, GLY A38, HIS A50	231.48
6	-6.40	1	PRO A53, CYS A37, GLY A38, PHE A54, HIS A54	217.39
7	-6.82	1	PRO A53, CYS A37, GLY A193, GLY A38, TYR A36	153.68
8	-8.54	1	PRO A53, CYS A37, GLY A193, GLY A38, LEU A70	44.01

in compounds 5-8 form hydrogen bonds with the CYS A37 residue of tyrosyl-tRNA synthetase at distances of 3.23, 3.00, 3.00, and 3.30 Å, respectively (Figure 4).

4. Conclusions

In Summary, the synthesis of novel benzoxale moiety linked to 2-pyrrolidinone derivatives was described using different available

reactants. The final products 5-8 were tested for their antibacterial efficiency. All prepared compounds were found active against tested bacterial strains except *Pseudomonas aeruginosa*. In addition, molecular docking simulations were employed to investigate the binding interactions between the synthesized derivative and the target protein, tyrosyl-tRNA synthetase. The calculated binding energy for derivative 8 is -8.54 kcal/mol, making it the best derivative due to its more significant interactions. Moreover, a brief DFT theoretical study

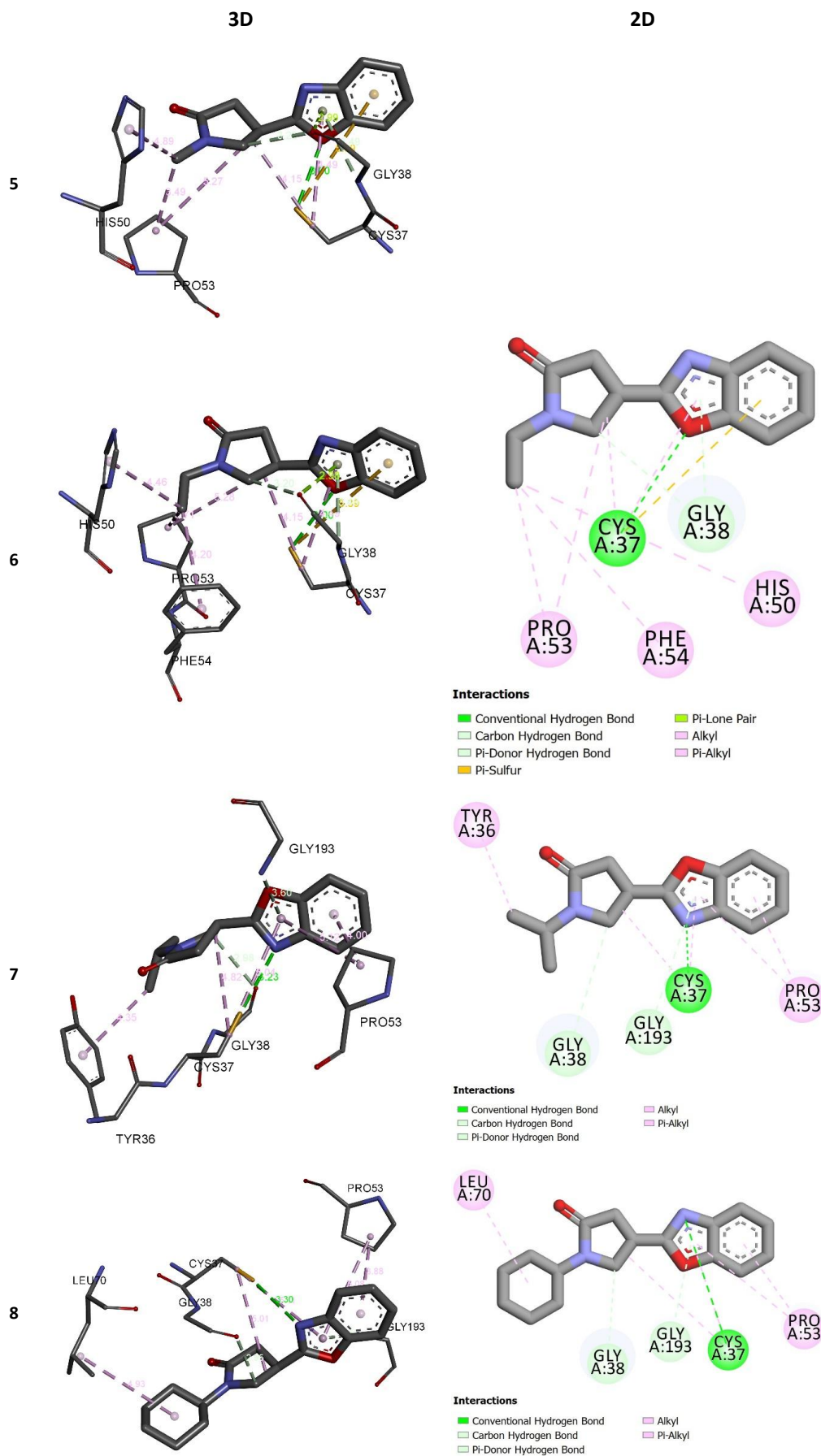


Figure 4. Interactions (2D & 3D) of derivatives 5-8 with tyrosyl-tRNA synthetase.

was carried out to explain the stability observed experimentally for derivatives 5-8, followed by a comparative analysis of theoretical and experimental NMR data which shows a significant correlation between the two studies.

CRedit authorship contribution statement

Abdulmalik S. A. Altamimi: Conceptualization, Data curation, Methodology, Investigation, Methodology, Formal analysis, Validation, Writing - Original Draft. Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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Supplementary data

Supplementary material to this article can be found online at https://dx.doi.org/10.25259/AJC_183_2025.

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