Research Article

Substituted-Amidine Functionalized Monocyclic β-Lactams: Synthesis and In Vitro Antibacterial Profile

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Background. Owing to the intrinsic stability against common β-lactamases and metallo-lactamases, monobactams gathered special attention in antibiotic drug development. However, so far, aztreonam is the only monobactam approved by FDA for clinical use. We designed new derivatives of aztreonam to enhance its antibacterial efficacy.

Methods. We synthesized a series of monocyclic β-lactams by modifying mainly at the C3 position of azetidinone ring. NH₂ group at C3 of azetidinone was attached to thiazole and thiadiazole which in turn was linked to nitrogenous heterocyclic rings via amidine moieties. We then investigated the in vitro antibacterial activities of synthesized compounds against ten bacterial strains of clinical interest in comparison to aztreonam and ceftazidime.

Results. All compounds showed improved antibacterial activities against tested strains compared to reference drugs. Compounds 14d and 14e were most potent and showed the highest potency against all bacterial strains, with MIC values ranging from 0.25 µg/mL to 8 µg/mL, as compared to aztreonam (MIC >64 µg/mL) and ceftazidime (MIC >64 µg/mL). These compounds (14d and 14e) may be valuable lead targets against multidrug-resistant Gram-negative bacteria.

1. Introduction

Continuous increase of β-lactamases [1], in association with variable resistance mechanisms [2, 3], has managed the generation of multidrug-resistant (MDR) bacteria [4] over the years. Of these lactamases, extended-spectrum β-lactamases (ESBLs) are the prime cause of resistance in MDR bacteria that compromises the effectiveness of antibiotic therapy. The fact that present antibiotic drugs may be ineffective in the future requires continuous development of new antibiotics capable of combating bacterial resistance over time. To this end, different classes of β-lactams [5] are still growing areas of research despite the increasing number (more than 1000) [6] of β-lactamases.

β-Lactams are the broad spectrum and most widely used antibiotics for the treatment of serious bacterial infections. Among various classes of β-lactams including cephalosporins, carbapenems, and penicillins [5], monocyclic-β-lactams [7, 8] are inherently resistant to common β-lactamases and metallo-lactamases (MBLs). Since the discovery of nocardicins and other monobactams from Nocardia uniformis and Pseudomonas strains, fine-tuning of the azetidinone ring is the common phenomenon to acquire the optimal potency. As a result, a vast variety of synthetic monobactam derivatives have been evaluated for antibacterial activity. The data gathered around the years revealed that the sulfonic acid group on the N1 position of the lactam ring is essential for the activation of the carbonyl group, whereas substitution at C3 and C4 maintains the stability of the molecule and plays part toward the antibacterial activity [8].

Nonetheless, despite enormous efforts, only one monobactam has been approved for clinical applications so far. Aztreonam (Figure 1) as the single FDA-approved monobactam shows a broad spectrum of activities against aerobic
and anaerobic Gram-negative bacteria [9]. Although resistant to MBLs, aztreonam is susceptible to most of the ESBLs, AmpCs, and KPCs coproduced by the MBL producing Enterobacteriaceae strains [10–12]. Therefore, many researchers are trying to improve the efficacy of aztreonam by introducing variable substituents at the C3 and C4 positions of the azetidinone ring. Recently, Reck et al. synthesized a number of compounds with variable substituents at C3 and C4 positions of azetidinone and identified a compound (LYS228) (Figure 1) that is resistant to all classes of β-lactamases. It also showed potent activity against carbapenem-resistant Enterobacteriaceae (CRE) [13]. BAL30072 (Figure 1), another monobactam in phase-I clinical trials in Switzerland, demonstrated stability against MBLs, class C, and some of the class A and D β-lactamases [14].

As part of our ongoing efforts on the synthesis of monocyclic β-lactams, we previously reported the synthesis and in vitro antibacterial activity of a series of monobactams containing urea moiety. Although a few of the compounds exhibited improved antibacterial activity, none of them could achieve the acceptable MIC value against all tested strains [15]. We, therefore, decided to replace the urea moiety with amidine based on our own experience, and literature report on the previous amidine substituted monocyclic β-lactams [16]. Inspired from the literature and our previous data, the following substitutions were emphasized in the designing of final target molecules: (i) N1 position of the azetidinone ring was substituted with OSO$_3$H or SO$_3$H, (ii) NH$_2$ at C3 of the azetidinone was attached to thiazole or thiadiazole moiety which was further linked to heterocyclic (four to six membered) nitrogenous ring via a linker containing amidine and (iii) C4 of the azetidinone was attached to thiazole or thiadiazole moiety. Recently, Reck et al. synthesized a number of compounds with variable substituents at C3 and C4 positions of azetidinone and identified a compound (LYS228) (Figure 1) that is resistant to all classes of β-lactamases. It also showed potent activity against carbapenem-resistant Enterobacteriaceae (CRE) [13]. BAL30072 (Figure 1), another monobactam in phase-I clinical trials in Switzerland, demonstrated stability against MBLs, class C, and some of the class A and D β-lactamases [14].

As part of our ongoing efforts on the synthesis of monocyclic β-lactams, we previously reported the synthesis and in vitro antibacterial activity of a series of monobactams containing urea moiety. Although a few of the compounds exhibited improved antibacterial activity, none of them could achieve the acceptable MIC value against all tested strains [15]. We, therefore, decided to replace the urea moiety with amidine based on our own experience, and literature report on the previous amidine substituted monocyclic β-lactams [16]. Inspired from the literature and our previous data, the following substitutions were emphasized in the designing of final target molecules: (i) N1 position of the azetidinone ring was substituted with OSO$_3$H or SO$_3$H, (ii) NH$_2$ at C3 of the azetidinone was attached to thiazole or thiadiazole moiety which was further linked to heterocyclic (four to six membered) nitrogenous ring via a linker containing amidine and carboxylic groups, and (iii) C4 of the azetidinone ring was substituted with methyl or geminal dimethyl groups. Thus, designed molecules 14a–e, 14g, 18a, 18b, and 18d–e (Figure 2) were recognized as our synthetic targets. Herein, we describe their synthesis and antimicrobial activities in vitro against ten bacterial strains containing variable β-lactamases.

2. Materials and Methods

All $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AVANCE NEO 400 NMR operating at 400 MHz for $^1$H and 100 MHz for $^{13}$C, respectively, and signals for NMR data are described as chemical shifts. All NMR spectra were recorded in deuterated solvents such as CDCl$_3$, CD$_3$OD, or DMSO-d$_6$ containing tetramethylsilane (TMS) an internal standard. Chemical shift ($\delta$) values are denoted in parts per million (ppm), whereas coupling constant ($J$) values are provided in Hertz (Hz). Signal multiplicities are reported as follows: s, for singlet; br s, for broad singlet; d, for doublet; t, for triplet; and m, for multiplet. Final compounds were purified by preparative HPLC using Agilent 1260 Infinity II System equipped with Agilent 10 prep-C18 250 × 21.2 mm column. Acetonitrile/water containing 0.1% trifluoroacetic acid or acetonitrile/water containing 0.1% formic acid was used as a solvent system for gradient elution at 22°C. LC-MS spectra were recorded on Agilent 1260 Infinity II System using either negative (ES$^-$) or positive (ES$^+$) ionization modes. HRMS spectra were performed on a Waters Xevo G2-XS QTof using either ES$^-$ or ES$^+$ ionization modes. Column chromatographic separation and purification were performed using glass columns filled with Qingdao Inc. Silica Gel: CC Grade (230–400 Mesh). Commercially available dry solvents were used in all synthesis experiments whereas commercial reagents were purchased from suppliers and used without purification.

2.1. Chemistry

2.1.1. Synthesis of Compound 2. A mixture of a Co(III)-catalyst (7.0 g, 8.4 mmol) and 4 Å molecular sieves (10 g) in tert-butyl methyl ether (50 mL) was treated with methyl (R)-oxirane-2-carboxylate (45.0 g, 441 mmol) and 4-hydroxybenzonitrile (1) (26.3 g, 220 mmol). The resulting mixture was stirred at room temperature for 48 hours, and the precipitates formed were filtered through celite pad. The filter cake was washed with ether, and the filtrate was evaporated under reduced pressure to afford a dark brown residue. The crude product was purified by column chromatography to yield the title compound 2 (79.0 g, 81.1% yield) as a brown oil. $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 3.68 (s, 3H), 4.26 (m, 2H), 4.49 (m, 1H), 5.96 (s, 1H), 7.11 (d, $J$ = 8.96 Hz, 2H), 7.77 (d, $J$ = 9.04 Hz, 2H). LC-MS [M+H]$^+$ m/z 222.1 (calcd for C$_{11}$H$_{11}$NO$_4$, 221.07).
2.1.2. Synthesis of Compound 3. A mixture of compound 2 (36.6 g, 165 mmol) in anhydrous methanol (160 mL) was cooled to 0°C in a sealed vessel. Anhydrous HCl gas was bubbled through the reaction solution until saturation. The flask was sealed and stirred at 0°C to rt for 18 hours resulting in the formation of suspension. The solid was filtered and washed with diethyl ether to yield the desired compound 3 (29 g) as an HCl salt. The mother liquor was concentrated to precipitate another crop of solid which was collected and washed with ether to afford the additional desired compound (9.89 g) (totally 38.9 g of the desired compound obtained, 81.5% yield).

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} \\
\text{O} & \quad \text{OSO}_3\text{H}
\end{align*}
\]

14a-e

2.1.3. Synthesis of Compounds 9a–f. Compounds 9a–f were synthesized according to Scheme 1 using general procedures described for a representative compound for each step as follows:

5a: to a solution of compound 3 (1.0 g, 3.45 mmol) in anhydrous methanol (3 mL), triethylamine (0.38 g, 3.8 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (0.69 g, 3.45 mmol) were added at 0°C. The resulting mixture was stirred at room temperature overnight followed by the evaporation of solvent till dryness to afford a residue. The residue was purified by column chromatography to furnish the desired compound 5a (1.0 g, 69% yield) as a white solid.

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} \\
\text{O} & \quad \text{OSO}_3\text{H}
\end{align*}
\]

18a,b and 18d-f

Figure 2: General structures of the target compounds.
4.74 mmol) in water (1 mL) was added at 0°C and then stirred at rt for 5 hours. THF was removed, and the aqeous phase was neutralized to pH 5 by 1 N aqueous HCl. The resulting mixture was lyophilized to afford crude compound 6a (0.97 g, containing NaCl salt) as a pale yellow foam. The crude product was used for the next step without purification. 1H NMR (400 MHz, DMSO-d6): δ 1.42 (s, 9H), 1.42–1.54 (m, 2H), 1.87–1.97 (m, 2H), 2.72–2.94 (m, 2H), 3.82–4.06 (m, 3H), 4.30–4.49 (m, 2H), 4.60–4.68 (m, 1H), 6.91 (s, 1H), 7.10 (d, J = 8.8 Hz, 2H), 7.25–7.47 (m, 10H), 7.71 (d, J = 8.8 Hz, 2H), 9.15 (br s, 4H). LC-MS [M+H]+ m/z 589.3 (calcld for C33H46NO10, 588.29).

9b: 1H NMR (400 MHz, DMSO-d6): δ 1.38 (s, 9H), 1.43–1.49 (m, 1H), 1.52–1.60 (m, 1H), 1.72–1.82 (m, 1H), 1.96–2.03 (m, 1H), 2.91–3.04 (m, 1H), 3.33–3.39 (m, 3H), 3.75–3.82 (m, 1H), 4.36–4.47 (m, 2H), 4.62–4.65 (m, 1H), 6.45 (s, 2H), 6.90 (s, 1H), 7.07 (d, J = 8.9 Hz, 2H), 7.23–7.32 (m, 4H), 7.33–7.40 (m, 4H), 7.42–7.45 (m, 2H), 7.69 (d, J = 8.9 Hz, 2H). LC-MS [M+H]+ m/z 589.3 (calcld for C33H46NO10, 588.29).

9c: 1H NMR (400 MHz, DMSO-d6): δ 1.39 (s, 9H), 1.43–1.49 (m, 1H), 1.51–1.61 (m, 1H), 1.74–1.82 (m, 1H), 1.96–2.04 (m, 1H), 2.92–3.04 (m, 1H), 3.40–3.47 (m, 3H), 3.74–3.83 (m, 1H), 4.37–4.44 (m, 2H), 4.64 (t, J = 3.9 Hz, 1H), 6.46 (s, 2H), 6.90 (s, 1H), 7.09 (d, J = 8.7 Hz, 2H), 7.22–7.32 (m, 4H), 7.33–7.40 (m, 4H), 7.41–7.47 (m, 2H), 7.69 (d, J = 8.7 Hz, 2H). LC-MS [M+H]+ m/z 589.3 (calcld for C33H46NO10, 588.29).

9d: 1H NMR (400 MHz, DMSO-d6): δ 1.34 (s, 9H), 1.80–1.99 (m, 1H), 2.11–2.18 (m, 1H), 3.33–3.39 (m, 3H), 3.52–3.61 (m, 1H), 4.27–4.39 (m, 3H), 4.54–4.60 (m, 1H), 6.38 (s, 2H), 6.82 (s, 1H), 7.02 (d, J = 7.8 Hz, 2H), 7.17–7.24 (m, 4H), 7.26–7.33 (m, 4H), 7.34–7.38 (m, 2H), 7.63 (d, J = 7.8 Hz, 2H). LC-MS [M+H]+ m/z 575.3 (calcld for C33H39NO10, 574.28).

9e: 1H NMR (400 MHz, DMSO-d6): δ 1.41 (s, 9H), 1.97–2.07 (m, 1H), 2.17–2.27 (m, 1H), 3.40–3.47 (m, 3H), 3.60–3.68 (m, 1H), 4.35–4.47 (m, 3H), 4.62–4.65 (m, 1H), 4.65 (s, 2H), 6.90 (s, 1H), 7.07 (d, J = 8.8 Hz, 2H), 7.22–7.32 (m, 4H), 7.33–7.40 (m, 4H), 7.41–7.47 (m, 2H), 7.73 (d, J = 8.8 Hz, 2H). LC-MS [M+H]+ m/z 575.3 (calcld for C33H39NO10, 574.28).

2.1.4. Synthesis of Compounds 14a–e. Compounds 14a–e were synthesized according to Scheme 2 using general procedures described for a representative compound for each step as follows:

11a: to the solution of compound 9a (0.294 g, 0.50 mmol) in ethanol (3 mL) and chloroform (3 mL), 2-(2-((tert-butoxycarbonyl)amino)-5-chlorothiazol-4-yl)-2-oxoactic acid 10 (0.151 g, 0.490 mmol) [17] was added. The reaction mixture was stirred at rt for 18 hours, concentrated under vacuo, and purified by flash column chromatography (10–20% MeOH in
Scheme 1: Synthesis of compounds 9a-f. Reagents and conditions: (a) methyl (R)-oxirane-2-carboxylate, (salen)Co(III) complex, 4 Å molecular sieves, MTBE, rt, 81%; (b) H₂(g), dry MeOH, 0°C – rt, 82%; (c) triethylamine, H₂N-Raf, MeOH, 0°C – rt, 57–95%; (d) NaOH, THF-water, 0°C, 75–99%; (e) Ph₂CN₂, CH₂Cl₂, rt, 31–84%; (f) PhthN-OH, DEAD, PPh₃, THF, rt, 33–66%; (g) NH₂NH₂ quantitative.

13a: to the solution of compound 11a (0.32g, 0.30 mmol) in anhydrous CH₂Cl₂ (4 mL), TFA (3 mL) at 0°C was added. After stirring for 1 hour at 0°C, the resulting mixture was warmed to rt and stirred for additional 2 hours. After completion of the reaction, the solvent was evaporated to give a residue which was dissolved in water (20 mL) and washed with petroleum ether/EtOAc(2:1, 40 mL). The aqueous layer was separated and freeze-dried to furnish the crude title compound (0.28g) as a pale yellow powder, which was further purified by preparative HPLC on a Agilent 10 prep-C18 250 x 21.2 mm column and lyophilized to give the target 14a (16mg, 7.6% yield) as a white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 1.42 (s, 9H), 1.46 (s, 9H), 1.54–1.43 (m, 2H), 1.86–1.96 (m, 2H), 2.71–2.91 (m, 2H), 3.84–4.03 (m, 3H), 4.43–4.57 (m, 2H), 5.05–5.12 (m, 1H), 6.87 (s, 1H), 7.09 (d, J = 8.8 Hz, 2H), 7.20–7.30 (m, 6H), 7.41–7.50 (m, 7H), 7.68 (d, J = 8.8 Hz, 2H), 9.20 (s, 1H), 9.38 (s, 1H), 9.47 (s, 1H), 11.9 (s, 1H). LC-MS [M+H]+ m/z 877.1 and [M+H+2]+ m/z 879.1 (calcd for C₄₃H₄₉ClN₆O₁₀S, 876.29).

14a: to the solution of compound 13a (0.32g, 0.30 mmol) in anhydrous CH₂Cl₂ (4 mL), TFA (3 mL) at 0°C was added. After stirring for 1 hour at 0°C, the resulting mixture was warmed to rt and stirred for additional 2 hours. After completion of the reaction, the solvent was evaporated to give a residue which was dissolved in water (20 mL) and washed with petroleum ether/EtOAc(2:1, 40 mL). The aqueous layer was separated and freeze-dried to furnish the crude title compound (0.28g) as a pale yellow powder, which was further purified by preparative HPLC on a Agilent 10 prep-C18 250 x 21.2 mm column and lyophilized to give the target 14a (16mg, 7.6% yield) as a white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 1.29 (s, 3H), 1.42 (s, 3H), 1.71–1.87 (m, 2H), 1.98–2.12 (m, 2H), 2.81–2.96 (m, 3H), 3.80–3.91 (m, 2H), 4.37–4.53 (m, 2H), 4.69 (d, J = 8.8 Hz, 1H), 4.73 (d, J = 7.8 Hz, 1H), 7.20 (d, J = 8.2 Hz, 2H), 7.36 (br s, 2H), 7.69 (d, J = 8.2 Hz, 2H), 8.99 (br s, 1H), 9.45 (br s, 1H), 9.63 (br s, 1H), 11.6 (d, J = 6.84 Hz, 1H). ¹³C NMR (100 MHz, D₂O): δ 19.6 (s), 22.1 (s), 26.9 (s), 42.5 (s), 47.5 (s), 61.0 (s), 68.0 (s), 70.9 (s), 83.8 (s), 115.3 (s), 121.0 (s), 129.9 (s), 131.5 (s), 146.6 (s), 162.4 (s), 163.7 (s), 165.8 (s), 166.9 (s), 174.3 (s), 179.9 (s), 183.9 (s). LC-MS [M+H]+ m/z 670.1 and [M+2+H]+ m/z 703.2 (calcd for C₂₅H₂₅ClN₈O₁₀S₂, 702.13). HREIMS m/z 670.1215; found 670.1218.
2.1.6. Synthesis of Compounds 18a, 18b, and 18d-f

Compounds 18a, 18b, and 18d-f were synthesized according to Scheme 4 using general procedures described for a representative compound for each step as follows:

16a: 2-(tert-butoxycarbonyl)amino)-1,2,4-thiazidol-3-yl)-2-oxoacetic acid (15 (0.27 g, 1.0 mmol) [17] was added to the solution of compound 9a (0.235 g, 0.4 mmol) in ethanol (3 mL) and chloroform (3 mL). Afterward, the reaction mixture was stirred at rt for 18 hours followed by the concentration of the solvent under vacuum and purification of the crude product by flash column chromatography using 10–20% MeOH in CH2Cl2 as solvent. The title compound 16a (88 mg, 26% yield) was obtained as pale yellow solid. 1H NMR (400 MHz, DMSO-d6): δ 1.40 (s, 9H), 1.49 (s, 9H), 1.88–1.98 (m, 3H), 2.81 (s, 2H), 3.88 (s, 2H), 3.98 (s, 2H), 4.46–4.58 (m, 2H), 5.16 (s, 1H), 6.88 (s, 1H), 7.10 (d, J = 8.7 Hz, 2H), 7.14–7.45 (m, 10H), 7.49 (d, J = 7.3 Hz, 2H), 7.68 (d, J = 8.7 Hz, 2H), 9.12 (s, 1H), 9.38 (s, 1H), 9.45 (s, 1H). LC-MS [M+] m/z 2844.3 (calcd for C42H57N9O15S4, 843.33).

17a: to the solution of compound 16a (0.079 g, 0.22 mmol) in anhydrous DMF (3.5 mL), DCC (68 mg, 0.33 mmol) and HOBT (0.045 g, 0.33 mmol) were added. The reaction mixture was stirred at rt for 45 minutes. (S)-3-amino-2,2-dimethyl-4-oxazolidin-1-ylhydroxysulfate (12a (69 mg, 0.33 mmol) and NaHCO3 (55 mg, 0.66 mmol) were then added, and the reaction mixture was stirred at rt for 16 h. After completion, the reaction mixture was concentrated in vacuo to give crude product which was purified by flash chromatography on silica gel using 5–10% MeOH in CH2Cl2 as solvent. The desired compound 17a (98 mg, 44%) was obtained as pale yellow solid. 1H NMR (400 MHz, DMSO-d6): δ 1.09 (s, 3H), 1.23 (s, 3H), 1.39 (s, 9H), 1.50 (s, 9H), 4.01 (s, 2H), 4.12 (s, 2H), 4.22 (s, 2H), 4.50 (s, 1H), 4.62 (s, 1H), 5.49 (s, 1H), 6.92 (s, 1H), 7.08 (d, J = 8.5 Hz, 2H), 7.13–7.38 (m, 10H), 7.48 (d, J = 7.1 Hz, 2H), 7.63 (s, 1H), 7.76 (d, J = 8.5 Hz, 2H), 7.90 (s, 1H), 8.91 (s, 1H), 9.45 (s, 1H), 9.67 (s, 1H). LC-MS [M+] m/z 1034.3 (calcd for C42H57N9O15S4, 1035.35).

18a: a solution of compound 17a (80 mg, 0.077 mmol) in anhydrous CH2Cl2 (2 mL) was added with TFA (2 mL) at 0°C. After stirring for 1 hour at 0°C, the reaction mixture was warmed to rt slowly and stirred for additional 3 hours. Subsequently, the reaction mixture was concentrated, and the residue was...
dissolved in water (10 mL) and washed with petroleum ether/EtOAc (2:1, 30 mL). The aqueous layer was freeze-dried to give the crude title compound (70 mg) as a pale yellow powder, which was purified by preparative HPLC on Agilent 10 prep-C18 250 × 21.2 mm column and lyophilized to give the title compound 18a (15 mg, 29.1% yield) as a white solid. 1H NMR (400 MHz, DMSO-d6): δ 1.23 (s, 3H), 1.41 (s, 3H), 1.71–1.85 (m, 2H), 2.01–2.09 (m, 2H), 2.81–2.93 (m, 3H), 3.79–3.88 (m, 2H), 4.36–4.50 (m, 2H), 4.67 (d, J = 8.4 Hz, 1H), 4.76 (d, J = 6.9 Hz, 1H), 7.21 (d, J = 7.1 Hz, 2H), 7.70 (d, J = 7.1 Hz, 2H), 8.14 (br s, 1H), 8.17 (br s, 1H), 8.99 (br s, 1H), 9.43 (br s, 1H), 9.67 (br s, 1H), 11.02 (br s, 1H). 13C NMR (100 MHz, D2O): δ 19.6 (s), 22.1 (s), 26.9 (s), 42.5 (s), 47.6 (s), 60.9 (s), 67.9 (s), 70.9 (s), 83.9 (s), 115.3 (s), 121.0 (s), 129.9 (s), 146.9 (s), 160.8 (s), 162.4 (s), 163.4 (s), 163.5 (s), 163.6 (s), 174.6 (s), 184.4 (s). LC-MS [M–H]− m/z 667.9 (calcd for C24H31N9O10S2, 669.16). HREIMS m/z calcd for C24H31N9O10S2 [M–H]−, 668.1557; found 668.1550.

18b: 1H NMR (400 MHz, DMSO-d6): δ 1.23 (s, 3H), 1.40 (s, 3H), 1.69–1.79 (m, 2H), 2.01–2.09 (m, 2H), 2.81–2.93 (m, 2H), 3.79–3.88 (m, 2H), 4.36–4.50 (m, 2H), 4.67 (d, J = 8.4 Hz, 1H), 4.76 (d, J = 6.9 Hz, 1H), 7.21 (d, J = 7.1 Hz, 2H), 7.70 (d, J = 7.1 Hz, 2H), 8.14 (br s, 2H), 9.02 (br s, 1H), 9.23 (br s, 1H), 11.04 (br s, 1H). 13C NMR (100 MHz, D2O): δ 19.6 (s), 20.1 (s), 22.1 (s), 26.9 (s), 43.4 (s), 45.1 (s), 46.9 (s), 60.9 (s), 67.9 (s), 70.9 (s), 83.9 (s), 115.3 (s), 120.7 (s), 130.0 (s), 146.9 (s), 160.7 (s), 162.5 (s), 163.5 (s), 163.8 (s), 167.2 (s), 174.6 (s), 184.4 (s). LC-MS [M–H]− m/z 667.9 (calcd for C24H31N9O10S2, 669.16). HREIMS m/z calcd for C24H31N9O10S2 [M–H]−, 668.1557; found 668.1563.

Scheme 2: Synthesis of compounds 14a–e. Reagents and conditions: (a) 10, EtOH-CHCl3, rt, 71–87%; (b) 12a, HOBT, DCC, NaHCO3, DMF, 0°C – rt, 41–88%; (c) TFA, CH2Cl2, 0°C – rt, 7–15%.

Scheme 3: Synthesis of compound 14g. Reagents and conditions: (a) 12a, HOBT, DCC, NaHCO3, DMF, 0°C – rt, 69%; (b) TFA, CH2Cl2, 0°C – rt, 11%.

18d: 1H NMR (400 MHz, DMSO-d6): δ 1.26 (s, 3H), 1.43 (s, 3H), 1.98–2.03 (m, 1H), 2.07–2.15 (m, 1H), 3.27–3.34 (m, 1H), 3.35–3.45 (m, 2H), 3.46–3.54 (m, 1H), 4.16–4.21 (m, 1H), 4.40–4.45 (m, 2H), 4.69 (d, J = 8.7 Hz, 1H), 4.72 (t, J = 4.9 Hz, 1H), 7.18 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 8.12 (br s, 2H), 8.41 (br s, 5H), 11.25
Scheme 4: Synthesis of compounds 18a,b and 18d-f. Reagents and conditions: (a) 15, EtOH-CHCl₃, rt, 26–60%; (b) 12a, HOBT, DCC, NaHCO₃, DMF, 0°C – rt, 41–84%; (c) TFA, CH₂Cl₂, 0°C – rt, 12–29%.

(1R,3R)-N-(1-[(tert-Butoxycarbonyl)amino]-2,3-dihydrobenzo[d][1,3]dioxole-6-carboxamido)pyrrolidine-3-carboxamide (18a): 1H NMR (400 MHz, DMSO-d₆): δ 1.15 (s, 3H), 655.15. HREIMS m/z calcd for C₂₃H₂₉N₉O₁₀S₂ [M-H]−, 654.1401; found 654.1407.

(1R,3R)-N-(1-[(tert-Butoxycarbonyl)amino]-2,3-dihydrobenzo[d][1,3]dioxole-6-carboxamido)pyrrolidine-3-carboxylic acid (18e): 1H NMR (400 MHz, DMSO-d₆): δ 1.19 (s, 3H), 1.40 (s, 3H), 2.04–2.23 (m, 2H), 3.37–3.42 (m, 3H), 3.57–3.63 (m, 1H), 4.19–4.26 (m, 1H), 4.30–4.44 (m, 2H), 4.63 (d, J = 8.4 Hz, 1H), 4.76 (d, J = 7.8 Hz, 1H), 7.11 (d, J = 7.8 Hz, 2H), 7.74 (d, J = 7.8 Hz, 2H), 8.23 (br s, 2H), 10.35 (d, J = 8.4 Hz, 1H). 13C NMR (100 MHz, D₂O): δ 19.5 (s), 22.2 (s), 29.6 (s), 44.4 (s), 48.9 (s), 51.7 (s), 60.9 (s), 68.0 (s), 70.9 (s), 84.2 (s), 115.3 (s), 120.6 (s), 130.1 (s), 146.8 (s), 162.6 (s), 162.7 (s), 163.0 (s), 163.6 (s), 168.9 (s), 175.0 (s), 184.5 (s). LC-MS [M-H]− m/z 654.2 (calcd for C₂₃H₂₉N₉O₁₀S₂, 655.15). HREIMS m/z calcd for C₂₃H₂₉N₉O₁₀S₂ [M-H]−, 654.1401; found 654.1407.

2.2. Antibacterial Studies

2.2.1. Acquisition of Bacterial Strains. Five type bacterial strains, namely, *E. coli* 8739 (CTX-M15), *K. pneumoniae* 700603 (KPC-3, TEM-1), *E. cloacae* 700323 (AmpC), *A. baumannii* 19606 (OXA-24), and *P. aeruginosa* 9027 (AmpC), containing different β-lactamases (indicated in parenthesis) were purchased from China Pharmaceutical Culture Collection (CPC), whereas five species of clinical isolates such as *E. coli* clinical isolate (TEM-1), *K. pneumoniae* clinical isolate (SHV-1), *E. cloacae* clinical isolate (P99), *A. baumannii* clinical isolate (OXA-23/40), and *P. aeruginosa* clinical isolate (KPC-2) containing different β-lactamases were indigenously isolated and cultured from patients at Ningxia Medical University Hospital, P.R. China.

2.2.2. Antibacterial Assay. Synthesized compounds 14 and 18 were analyzed for their antibacterial activity using the broth microdilution method, and the results are presented as minimum inhibitory concentrations (MICs, in µg/mL) for each compound and references. The MIC values were measured according to the guidelines of the Clinical Laboratories and Standards Institute [18]. As a typical example, 14a was dissolved in DMSO and diluted, twofold in serial, with microbial growth medium (Mueller Hinton Broth II, cation adjusted) to reach the final concentration in a range of 0.063–64 µg/mL. The final DMSO concentration was kept at less than 0.5% in each sample. At this stage, bacteria (5 × 10⁵ colony-forming units/mL (CFU/mL)) were added to each...
concentration of the test compound in a 96-well plate. The microtiter well plate was then incubated at 37°C for 18–24 hours followed by their observation of MIC values by the naked eye (visually). Aztreonam and ceftazidime were used as the reference compounds in the same range of concentrations as the synthesized monobactams 14 and 18. Each compound and reference compounds were tested in triplicate for each concentration.

3. Results and Discussion

3.1. Synthesis of Intermediates 9a–f. The synthesis of compounds 9a–f (Scheme 1) started from ring opening of methyl (R)-oxirane-2-carboxylate by commercially available 4-cyanophenol (I) catalyzed by (salen)Co(III) complex [19, 20] in tert-butylmethyl ether (TBME) at room temperature to form α-hydroxyester 2 in 81% yield following the procedure described in the literature [21]. Later, the cyano group in compound 2 was reacted with methanolic HCl to generate the intermediate 3 as HCl salt in excellent yield. Compound 3 was then converted to amidines 5a–f through nucleophilic substitution with appropriate amines (H₂N–Ra–f) in the presence of trimethylamine. Base catalyzed hydrolysis of ester group in compounds 5a–f afforded the respective α-hydroxy acids 6a–f. The carboxyl group in compounds 6a–f was then protected by reacting with diazo(diphenyl)methane (Ph₂CN₂) to afford the corresponding derivatives 8a–f. Phthalimido derivatives 8a–f were obtained by reacting hydroxyl groups in compounds 7a–f under Mitsunobu conditions using N-hydroxypthalimide (PhthN-OH) in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (PPh₃) at room temperature. The conversion of phthalimido derivatives into the corresponding aminooxy compounds 9a–f was accomplished by the reaction of 8a–f with hydrazine hydrate in good to excellent yields.

3.2. Synthesis of Final Target Compounds. Compounds 9a–e prepared in Scheme 1 were coupled with compound 10 [22] in a solvent mixture of ethanol and chloroform at room temperature to furnish intermediates 11a–e. The compounds 11a–e were then reacted individually with compound 12a [23] to yield the corresponding compounds 13a–e. This coupling was achieved by N-hydroxybenzotriazol (HOBT) mediated activation of carboxyl group in compounds 13a–e followed by the removal of water by dicyclohexylcarbodiimide (DCC).

Subsequently, deprotection of the Boc and the diphenylnmethyl groups in 13a–e was accomplished in one step by trifluoroacetic acid (TFA) in CH₂Cl₂ at room temperature in 2 hours. The resulting compounds 14a–e were purified by preparative HPLC followed by the lyophilization to afford the final compounds as solids.

Compound 14g was prepared to start from 11c and coupling it with 12b [23] under HOBt/DCC activation to afford 13g in 69.3% yield after purification. 13g was then deprotected using the aforementioned reaction conditions to get the final compound 14g in 11.1% yield (Scheme 3). Following Scheme 4, compounds 9a,b and 9d–f were also reacted with thiadiazole 15 [22, 23] to afford the compounds 16a,b and 16d–f, which were converted into final compounds 18a,b and 18d–f following the procedures described for 14a–e.

3.3. In Vitro Antimicrobial Activity. All the synthesized compounds 14a–e, 14g, 18a,b, and 18d–f were tested in vitro for their antibacterial activities against ten bacterial species, i.e., E. coli clinical isolate, E. coli 8739, K. pneumoniae clinical isolate, K. pneumoniae 700603, E. cloacae clinical isolate, E. cloacae 700323, A. baumannii clinical isolate, A. baumannii 19606, P. aeruginosa clinical isolate, and P. aeruginosa 9027, using broth microdilution method according to the guidelines of Clinical Laboratories and Standards Institute [18]. Minimum inhibitory concentration (MIC) for each compound was determined compared to aztreonam and ceftazidime as two reference antibiotics and is noted in Table 1. Aztreonam showed variable potencies against different strains with MIC values ranging from 16 μg/mL to >64 μg/mL in contrast to ceftazidime which did not prove to be potent against these strains showing MIC value >64 μg/mL for all tested strains.

As evident from the data reported in Table 1, all newly synthesized compounds showed variable degrees of potency against all tested bacterial strains. Compounds 14a–e, having variable R² groups, showed MIC values ranging from 0.25 to 64 μg/mL. Observing individual data of compounds 14, it can be observed that the compounds 14d and 14e showed the highest potency against all tested strains with MIC values ranging from 0.25 to 8 μg/mL. E. coli 8739 was the most susceptible strain toward compound 14d, whereas 14e showed the highest potency against both E. coli strains with a MIC value of 0.25 μg/mL. Furthermore, it can be ascertained that A. baumannii clinical isolate exhibited the highest resistance (MIC 8 μg/mL) against 14e, whereas both strains of A. baumannii showed the highest level of resistance against the compound 14d.

Compound 14g exhibited antibiotic potency with MIC values ranging from 0.5 to 64 μg/mL. Compounds 14c and 14g are structurally similar with a slight difference; C4 of the azetidinone ring of 14c is substituted with dimethyl instead of monomethyl and N1 position is linked with OSO₂H instead of SO₂H group. Comparing the activities of these two compounds, it is evident that the antibacterial efficacy of 14g is slightly compromised against most of the strains. This may be attributed to the structural variation of these two compounds at N1 and C4 positions resulting in the geometric orientation of 14c in a way that makes it more stable and hence better interaction and activity.

The antibacterial potency of compounds 18, which possess thiadiazole moiety instead of thiazole in contrast to its counterparts 14, varies from MIC values of 0.125 μg/mL to 64 μg/mL. Among these, compounds 18e and 18f showed the highest potency against E. Coli 8739 and E. Coli clinical isolate strains, respectively. However, overall the compound 18d proved to be the most potent against all strains with...
MIC values ranging from 1 to 16 µg/mL, exhibiting the highest potency against *E. Coli* 8739 and lowest against *A. baumannii* clinical isolate.

### 4. Conclusion

We successfully synthesized eleven new monobactams with diverse structural variations at N1, C3, and C4 positions of the azetidinone ring. Thus, obtained final targets (14a–e, 14g, 18a–b) were evaluated for their antibacterial strength *in vitro* using ten different bacterial strains, and the results were compared with two reference antibiotics, aztreonam and ceftazidime. All compounds showed variable antibacterial activity against all tested bacteria; however, both strains of *E. coli* were most susceptible to the newly synthesized derivatives. Comparing the overall activity of the compounds, it can be visualized that the compounds 14d and 14e are more active *in vitro* than the reference antibiotics, aztreonam and ceftazidime, against all tested bacteria. Antimicrobial activities of the compounds demonstrated that changes made in the lactam ring structure are beneficial, for example, thiazole moiety proved to be better as compared to thiadiazole. In addition, these results clearly indicate that monobactams 14d and 14e are valuable lead compounds for the development of new potent antibiotics; most importantly, they may lead to antibacterial candidates against MDR Gram-negative bacteria.

### Data Availability

The data used to support the findings of this study are included within the Supplementary Materials.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

### Authors’ Contributions

Lili He and Lijuan Zhai are equal contributors to this work.

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### Supplementary Materials

NMR spectra of key intermediates and final compounds. (Supplementary Materials)

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