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. NH_2 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 $\mu\text{g/mL}$ to 8 $\mu\text{g/mL}$, as compared to aztreonam (MIC 16 $\mu\text{g/mL}$ to >64 $\mu\text{g/mL}$) and ceftazidime (MIC >64 $\mu\text{g/mL}$). 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 antibacterial 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

FIGURE 1: Structures of currently approved aztreonam and other monobactams in clinical trials.

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_3H or SO_3H , (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 ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE NEO 400 NMR operating at 400 MHz for

^1H 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_3OD , or $\text{DMSO}-d_6$ containing tetramethylsilane (TMS) an internal standard. Chemical shift (δ) 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. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 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 $[\text{M}+\text{H}]^+$ m/z 222.1 (calcd for $\text{C}_{11}\text{H}_{11}\text{NO}_4$, 221.07).

FIGURE 2: General structures of the target compounds.

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) as a light green solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ 3.66 (s, 3 H), 4.24 (s, 3 H), 4.27–4.31 (m, 2 H), 4.48 (t, *J* = 4.4 Hz, 1 H), 7.16 (d, *J* = 9.1 Hz, 2 H), 8.09 (d, *J* = 9.1 Hz, 2 H). LC-MS [*M*+H]⁺ *m/z* 254.1.1 (calcd for C₁₂H₁₅NO₅, 253.10).

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. ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.38 (s, 9H), 1.54–1.43 (m, 2H), 1.82–1.93 (m, 2H), 2.60–2.90 (m, 2H), 3.64 (s,

3H), 3.90–4.00 (m, 3H), 4.12–4.28 (m, 2 H), 4.44–4.50 (m, 1H), 5.98 (d, $J = 6.1$ Hz, 1H), 7.22 (d, $J = 8.8$ Hz, 2H), 7.69 (d, $J = 8.8$ Hz, 2H), 9.43 (s, 1H). LC-MS $[M+H]^+$ m/z 422.2 (calcd for $C_{21}H_{31}N_3O_6$, 421.22).

6a: to the solution of compound **5a** (1.0 g, 2.37 mmol) in THF (2 mL), a solution of sodium hydroxide (0.2 g, 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 aqueous 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 white solid. The crude product was directly used for the next step without purification. 1H NMR (400 MHz, DMSO- d_6): δ 1.41 (s, 9H), 1.49 (br s, 2H), 1.92 (br s, 2H), 2.84 (br s, 2H), 3.86 (br s, 1H), 4.00 (br s, 4H), 4.26 (d, $J = 9.8$ Hz, 1H), 7.12 (s, 2H), 7.74 (s, 2H). LC-MS $[M+H]^+$ m/z 408.2 (calcd for $C_{20}H_{29}N_3O_6$, 407.21).

7a: to a solution of compound **6a** (0.97 g, 2.37 mmol) in MeOH (2 mL), a solution of diazo(diphenyl)methane (Ph_2CN_2 , 0.42 g, 3.56 mmol) in CH_2Cl_2 (1.5 mL) was slowly added. The resulting mixture was stirred at rt overnight and the solvent was concentrated to dryness. The crude product was purified by column chromatography to give product **7a** (1.1 g, 80% yield in two steps) as a white solid. 1H NMR (400 MHz, DMSO- d_6): δ 1.39 (s, 9H), 1.42–1.52 (m, 2H), 1.87–1.92 (m, 2H), 2.82 (br s, 2H), 3.90–4.00 (m, 3H), 4.30–4.42 (m, 2H), 4.62–4.65 (m, 1H), 6.48 (d, $J = 7.6$ Hz, 1H), 6.84 (s, 1H), 7.09 (d, $J = 8.8$ Hz, 2H), 7.22–7.41 (m, 10H), 7.69 (d, $J = 8.8$ Hz, 2H), 9.10 (br s, 1H), 9.38 (br s, 1H). LC-MS $[M+H]^+$ m/z 574.1 (calcd for $C_{33}H_{39}N_3O_6$, 573.28).

8a: to a solution of compound **7a** (1.2 g, 2.1 mmol), *N*-hydroxyphthalimide (1.03 g, 6.3 mmol), and triphenylphosphine (1.7 g, 6.3 mmol) in anhydrous THF (5 mL), a solution of diethyl azodicarboxylate (1.09 g, 6.3 mmol) was added dropwise at 0°C. The reaction mixture was stirred at rt overnight followed by the evaporation of THF to furnish the crude product, which was purified by column chromatography to afford compound **8a** (1.0 g, 66.7% yield) as white solid. 1H NMR (400 MHz, DMSO- d_6): δ 1.42 (s, 9H), 1.44–1.53 (m, 2H), 1.92–1.96 (m, 2H), 2.82 (br s, 2H), 3.92–4.16 (m, 3H), 4.68 (br s, 2H), 5.48 (br s, 1H), 6.98 (s, 1H), 7.11 (d, $J = 8.6$ Hz, 2H), 7.26–7.48 (m, 10H), 7.75 (d, $J = 8.6$ Hz, 2H), 7.86 (s, 4H), 9.24 (br s, 1H), 9.40 (br s, 1H). LC-MS $[M+H]^+$ m/z 719.3 (calcd for $C_{41}H_{42}N_4O_8$, 718.30).

9a: to the solution of compound **8a** (0.33 g, 0.45 mmol) in anhydrous ethanol (5 mL), hydrazine monohydrate (23 μ L, 0.52 mmol) was added at 0°C. The resulting mixture was stirred at rt for 3.5 hours, filtered off and washed with ethanol (2 $\times$ 5 mL). The filtrate was evaporated to dryness and the residue was suspended in CH_2Cl_2 (10 mL), filtered off and rinsed with CH_2Cl_2 (2 $\times$ 3 mL). The filtrate was concentrated to give compound **9a** (0.26 g, quantitative yield) as a pale yellow foam. The crude product was used for the next step

without purification. 1H NMR (400 MHz, DMSO- d_6): 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 (calcd for $C_{33}H_{40}N_4O_6$, 588.29).

9b: 1H NMR (400 MHz, DMSO- d_6): 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 (calcd for $C_{33}H_{40}N_4O_6$, 588.29).

9c: 1H NMR (400 MHz, DMSO- d_6): 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 (calcd for $C_{33}H_{40}N_4O_6$, 588.29).

9d: 1H NMR (400 MHz, DMSO- d_6): 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 (calcd for $C_{32}H_{38}N_4O_6$, 574.28).

9e: 1H NMR (400 MHz, DMSO- d_6): 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), 6.45 (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), 7.41–7.46 (m, 2H), 7.73 (d, $J = 8.8$ Hz, 2H). LC-MS $[M+H]^+$ m/z 575.3 (calcd for $C_{32}H_{38}N_4O_6$, 574.28).

9f: 1H NMR (400 MHz, DMSO- d_6): δ 1.39 (s, 9H), 3.41–3.46 (m, 3H), 3.85–3.92 (m, 2H), 4.18–4.26 (m, 1H), 4.34–4.38 (m, 2H), 4.01–4.45 (m, 1H), 4.61–4.64 (m, 1H), 6.44 (s, 2H), 6.90 (s, 1H), 7.04 (d, $J = 8.8$ Hz, 2H), 7.22–7.31 (m, 4H), 7.33–7.40 (m, 4H), 7.41–7.45 (m, 2H), 7.75 (d, $J = 8.8$ Hz, 2H). LC-MS $[M+H]^+$ m/z 561.2. (calcd for $C_{31}H_{36}N_4O_6$, 560.26).

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-oxoacetic 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) H_2 (gas), dry MeOH, 0°C – rt, 82%; (c) triethylamine, $\text{H}_2\text{N-R}_{\text{a-f}}$, MeOH, 0°C – rt, 57–95%; (d) NaOH, THF-water, 0°C, 75–99%; (e) Ph_2CN_2 , CH_2Cl_2 , rt, 31–84%; (f) PhthN-OH, DEAD, PPh_3 , THF, rt, 33–66%; (g) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$, EtOH, rt, quantitative.

CH_2Cl_2) to give compound **11a** (0.31 g, 71% yield) as a pale yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 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, 4H), 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 $[\text{M}+\text{H}]^+$ m/z 877.1 and $[\text{M}+\text{H}+2]^+$ m/z 879.1 (calcd for $\text{C}_{43}\text{H}_{49}\text{ClN}_6\text{O}_{10}\text{S}$, 876.29).

13a: to the solution of compound **11a** (0.305 g, 0.350 mmol) in anhydrous DMF (3.5 mL), DCC (0.11 g, 0.52 mmol) and HOBT (70.0 mg, 0.52 mmol) were added. The reaction mixture was stirred at rt for 45 minutes, and then (*S*)-3-amino-2,2-dimethyl-4-oxazetidin-1-yl-hydrogen sulfate **12a** (0.11 g, 0.52 mmol) was added followed by NaHCO_3 (88.0 mg, 1.05 mmol) addition. The reaction mixture was further stirred at rt for 16 hours. After the reaction completion, the solvent was removed under reduced pressure to afford a residue which was purified by flash chromatography on silica gel eluting by 5–10% MeOH in CH_2Cl_2 to give the title compound **13a** (0.32 g, 86% yield) as a pale yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 1.11 (s, 3H), 1.37 (s, 3H), 1.41 (s, 9H), 1.46 (s, 9H), 1.44–1.51 (m, 2H), 1.87–1.97 (m, 2H), 2.72–2.89 (m, 2H), 3.78–3.88 (m, 1H), 3.94–4.04 (m, 2H), 4.45–4.52 (m, 1H), 4.57 (d, $J = 7.8$ Hz, 1H), 4.58–4.65 (m, 1H), 5.33–5.38 (m, 1H), 6.92 (s, 1H), 7.07 (d, $J = 8.8$ Hz, 2H), 7.17–7.50 (m, 10H), 7.70 (d, $J = 8.8$ Hz, 2H), 8.98 (s, 1H), 9.30 (s, 1H), 9.38 (d, $J = 7.8$ Hz, 1H), 9.63 (d, $J = 7.8$ Hz, 1H), 12.1 (s,

1H). LC-MS $[\text{M}-\text{H}]^-$ m/z 1067.3 and $[\text{M}+2-\text{H}]^-$ m/z 1069.3 (calcd for $\text{C}_{48}\text{H}_{57}\text{ClN}_8\text{O}_{14}\text{S}_2$, 1068.31)

14a: to the solution of compound **13a** (0.32 g, 0.30 mmol) in anhydrous CH_2Cl_2 (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 (0.28 g) as a pale yellow powder, which was further purified by preparative HPLC on a Agilent 10 prep-C18 250 × 21.2 mm column and lyophilized to give the target **14a** (16 mg, 7.6% yield) as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 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). ^{13}C NMR (100 MHz, D_2O): 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 $[\text{M}-\text{H}]^-$ m/z 701.1 and $[\text{M}+2-\text{H}]^-$ m/z 703.2 (calcd for $\text{C}_{25}\text{H}_{31}\text{ClN}_8\text{O}_{10}\text{S}_2$, 702.13). HREIMS m/z calcd for $\text{C}_{25}\text{H}_{31}\text{ClN}_8\text{O}_{10}\text{S}_2$ $[\text{M}-\text{H}]^-$, 701.1215; found 701.1218.

14b: ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 1.27 (s, 3H), 1.41 (s, 3H), 1.62–1.74 (m, 2H), 1.87–2.02 (m, 2H), 2.82–2.92 (m, 2H), 3.04–3.12 (m, 2H), 3.82–3.93 (m, 1H), 4.18–4.26 (m, 1H), 4.39–4.46 (m, 1H), 4.69 (d,

$J = 8.7$ Hz, 1H), 4.74 (d, $J = 5.5$ Hz, 1H), 7.08 (d, $J = 8.2$ Hz, 2H), 7.36 (br s, 2H), 7.62 (d, $J = 8.2$ Hz, 2H), 9.29 (br s, 2H), 10.63 (br s, 1H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 20.6 (s), 24.5 (s), 26.9 (s), 29.1 (s), 44.7 (s), 48.4 (s), 49.7 (s), 60.7 (s), 67.3 (s), 68.7 (s), 83.6 (s), 111.0 (s), 115.2 (s), 121.4 (s), 130.0 (s), 130.8 (s), 138.4 (s), 150.9 (s), 162.3 (s), 162.8 (s), 163.1 (s), 164.8 (s), 171.7 (s). LC-MS $[\text{M}-\text{H}]^-$ m/z 700.9 and $[\text{M}+2-\text{H}]^-$ m/z 702.8 (calcd for $\text{C}_{25}\text{H}_{31}\text{ClN}_8\text{O}_{10}\text{S}_2$, 702.13). HREIMS m/z calcd for $\text{C}_{25}\text{H}_{31}\text{ClN}_8\text{O}_{10}\text{S}_2$ $[\text{M}-\text{H}]^-$, 701.1215; found 701.1218.

14c: ^1H NMR (400 MHz, DMSO- d_6): δ 1.26 (s, 3H), 1.41 (s, 3H), 1.54–1.79 (m, 3H), 1.92–2.02 (m, 1H), 2.73–2.81 (m, 1H), 2.87–2.98 (m, 3H), 3.84–3.91 (m, 1H), 4.10–4.16 (m, 1H), 4.35 (t, $J = 9.5$ Hz, 1H), 4.67 (d, $J = 8.8$ Hz, 1H), 4.70 (d, $J = 8.5$ Hz, 1H), 7.13 (d, $J = 8.5$ Hz, 2H), 7.33 (br s, 2H), 7.72 (d, $J = 8.5$ Hz, 2H), 9.21 (br s, 3H), 11.08 (br s, 1H). LC-MS $[\text{M}-\text{H}]^-$ m/z 701.1 and $[\text{M}+2-\text{H}]^-$ m/z 703.1 (calcd for $\text{C}_{25}\text{H}_{31}\text{ClN}_8\text{O}_{10}\text{S}_2$, 702.13). HREIMS m/z calcd for $\text{C}_{25}\text{H}_{31}\text{ClN}_8\text{O}_{10}\text{S}_2$ $[\text{M}-\text{H}]^-$, 701.1215; found 701.1219.

14d: ^1H NMR (400 MHz, DMSO- d_6): δ 1.27 (s, 3H), 1.41 (s, 3H), 2.00–2.09 (m, 1H), 2.17–2.26 (m, 1H), 3.07–3.15 (m, 1H), 3.21–3.36 (m, 3H), 4.22–4.27 (m, 1H), 4.36–4.44 (m, 2H), 4.65 (t, $J = 8.6$ Hz, 1H), 4.72 (d, $J = 8.5$ Hz, 1H), 7.13 (d, $J = 8.5$ Hz, 2H), 7.35 (br s, 2H), 7.74 (d, $J = 8.5$ Hz, 2H), 8.30 (br s, 3H), 10.35 (d, $J = 8.6$ Hz, 1H). LC-MS $[\text{M}-\text{H}]^-$ m/z 687.1 and $[\text{M}+2-\text{H}]^-$ m/z 689.1 (calcd for $\text{C}_{24}\text{H}_{29}\text{ClN}_8\text{O}_{10}\text{S}_2$, 688.1). HREIMS m/z calcd for $\text{C}_{24}\text{H}_{29}\text{ClN}_8\text{O}_{10}\text{S}_2$ $[\text{M}-\text{H}]^-$, 687.1058; found 687.1051.688.11.

14e: ^1H NMR (400 MHz, DMSO- d_6): δ 1.27 (s, 3H), 1.42 (s, 3H), 2.14–2.27 (m, 2H), 3.25–3.45 (m, 3H), 3.57–3.64 (m, 1H), 4.16 (d, $J = 9.7$ Hz, 1H), 4.40 (t, $J = 9.7$ Hz, 1H), 4.45–3.51 (m, 1H), 4.63 (d, $J = 8.4$ Hz, 1H), 4.77 (d, $J = 8.7$ Hz, 1H), 7.09 (d, $J = 8.3$ Hz, 2H), 7.36 (br s, 2H), 7.75 (d, $J = 8.3$ Hz, 2H), 9.04 (br s, 2H), 10.35 (br s, 1H). ^{13}C NMR (100 MHz, D_2O): δ 19.6 (s), 22.1 (s), 29.6 (s), 44.4 (s), 48.9 (s), 51.7 (s), 61.0 (s), 67.9 (s), 70.8 (s), 85.3 (s), 115.2 (s), 120.5 (s), 130.1 (s), 146.5 (s), 159.9 (s), 161.2 (s), 161.7 (s), 163.6 (s), 164.1 (s), 167.2 (s), 174.4 (s), 183.9 (s). LC-MS $[\text{M}-\text{H}]^-$ m/z 686.9 and $[\text{M}+2-\text{H}]^-$ m/z 688.9 (calcd for $\text{C}_{24}\text{H}_{29}\text{ClN}_8\text{O}_{10}\text{S}_2$, 688.11). HREIMS m/z calcd for $\text{C}_{24}\text{H}_{29}\text{ClN}_8\text{O}_{10}\text{S}_2$ $[\text{M}-\text{H}]^-$, 687.1058; found 687.1049.

2.1.5. Synthesis of Compound 14g. Synthesis of compound **14g** was carried out according to Scheme 3. Compounds **11c** and **12b** were coupled according to the procedure described for **13a** from the compound **13g**. Boc deprotection in **13g** was accomplished using analogous procedure described for compound **14a** to afford the final compound **14g** in 11% yield. ^1H NMR (400 MHz, DMSO- d_6): δ 1.35 (d, $J = 6.4$ Hz, 3H), 1.42–1.49 (m, 1H), 1.63–1.71 (m, 1H), 1.79–1.87 (m, 1H), 1.95–2.02 (m, 1H), 2.86–2.95 (m, 1H), 2.98–3.08 (m, 3H), 3.60–3.65 (m, 1H), 3.91–3.97 (m, 1H), 4.13–4.19 (m, 1H), 4.30 (t, $J = 8.6$ Hz, 1H), 4.42 (dd, $J = 7.9, 2.6$ Hz, 1H),

4.72 (d, $J = 7.4$ Hz, 1H), 6.65 (br s, 1H), 7.14 (d, $J = 8.7$ Hz, 2H), 7.19 (br s, 2H), 7.72 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR (100 MHz, D_2O): δ 16.6 (s), 23.7 (s), 24.9 (s), 42.5 (s), 45.9 (s), 49.7 (s), 61.0 (s), 63.1 (s), 66.3 (s), 79.3 (s), 100.7 (s), 114.6 (s), 120.3 (s), 119.0 (s), 130.1 (s), 144.0 (s), 158.1 (s), 160.1 (s), 163.5 (s), 172.0 (s), 175.0 (s), 185.2 (s). LC-MS $[\text{M}-\text{H}]^-$ m/z 671.1 and $[\text{M}+2-\text{H}]^-$ m/z 673.1 (calcd for $\text{C}_{24}\text{H}_{29}\text{ClN}_8\text{O}_{10}\text{S}_2$, 672.12). HREIMS m/z calcd for $\text{C}_{24}\text{H}_{29}\text{ClN}_8\text{O}_{10}\text{S}_2$ $[\text{M}-\text{H}]^-$, 672.1187; found 672.1181.

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-(5-((*tert*-butoxycarbonyl)amino)-1,2,4-thiadiazol-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 vacuo and purification of the crude product by flash column chromatography using 10–20% MeOH in CH_2Cl_2 as solvent. The title compound **16a** (88 mg, 26% yield) was obtained as pale yellow solid. ^1H NMR (400 MHz, DMSO- d_6): δ 1.40 (s, 9H), 1.49 (s, 9H), 1.82–1.98 (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 $[\text{M}+\text{H}]^+$ m/z 844.3 (calcd for $\text{C}_{42}\text{H}_{49}\text{N}_7\text{O}_{10}\text{S}$, 843.33).

17a: to the solution of compound **16a** (0.179 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-oxoazetidin-1-ylhydrogen sulfate **12a** (69 mg, 0.33 mmol) and NaHCO_3 (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 CH_2Cl_2 as solvent. The desired compound **17a** (98 mg, 44%) was obtained as pale yellow solid. ^1H NMR (400 MHz, DMSO- d_6): 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 $[\text{M}-\text{H}]^-$ m/z 1034.3 (calcd for $\text{C}_{47}\text{H}_{57}\text{N}_9\text{O}_{14}\text{S}_2$, 1035.35).

18a: a solution of compound **17a** (80 mg, 0.077 mmol) in anhydrous CH_2Cl_2 (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

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%.

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-*d*₆): 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-*d*₆): δ 1.21 (s, 3H), 1.40 (s, 3H), 1.69–1.79 (m, 2H), 1.94–2.04 (m, 2H), 2.87–3.01 (m, 2H), 3.11–3.19 (m, 2H), 3.84–3.92 (m, 1H), 4.16–4.23 (m, 1H), 4.41–4.47 (m, 1H), 4.66 (d, *J* = 8.1 Hz, 1H), 4.78–4.82 (m, 1H), 7.02 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.6 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), 163.6 (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.

18d: ¹H NMR (400 MHz, DMSO-*d*₆): 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%.

(br s, 1H). ¹³C NMR (100 MHz, D₂O): δ 19.6 (s), 22.1 (s), 29.6 (s), 44.3 (s), 49.0 (s), 51.7 (s), 60.9 (s), 68.0 (s), 70.9 (s), 84.2 (s), 115.3 (s), 118.2 (s), 129.9 (s), 152.8 (s), 162.6 (s), 162.9 (s), 163.8 (s), 164.0 (s), 169.0 (s), 171.1 (s), 184.4 (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 654.1409.

18e: ¹H NMR (400 MHz, DMSO-*d*₆): 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.

18f: ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.15 (s, 3H), 1.37 (s, 3H), 4.01–4.09 (m, 2H), 4.16–4.26 (m, 2H), 4.29–4.39 (m, 2H), 4.55–4.64 (m, 2H), 4.78–4.86 (m, 1H), 7.04 (d, *J* = 8.6 Hz, 2H), 7.77 (d, *J* = 8.6 Hz, 2H), 8.14 (br s, 2H), 10.20 (m, 1H). ¹³C NMR (100 MHz, D₂O): δ 19.9 (s), 22.2 (s), 46.6 (s), 51.7 (s), 60.9 (s), 71.0 (s), 72.3 (s), 84.2 (s), 115.3 (s), 120.2 (s), 130.2 (s), 148.2 (s), 160.7 (s), 158.7 (s), 161.7 (s), 163.7 (s), 169.9 (s), 175.0 (s), 183.0 (s). LC-MS [M-H][−] *m/z* 640.7 (calcd for C₂₂H₂₇N₉O₁₀S₂, 641.13). HREIMS *m/z* calcd for C₂₂H₂₇N₉O₁₀S₂ [M-H][−], 640.1244; found 640.1251.

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-lactamase/s (indicated in parenthesis) were purchased from China Pharmaceutical Culture Collection (CPCC), 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 (**1**) catalyzed by (salen)Co(III) complex [19, 20] in *tert*-butylmethylether (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 (H_2N-R_{a-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 (Ph_2CN_2) to afford the corresponding derivatives **7a–f**. Phthalimido derivatives **8a–f** were obtained by reacting hydroxyl groups in compounds **7a–f** under Mitsunobu conditions using *N*-hydroxyphthalimide (PhthN-OH) in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (PPh₃) at room temperature. The conversion of phthalimido derivatives into the corresponding aminoxy 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*-hydroxybenzotriazole (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 diphenylmethyl groups in **13a–e** was accomplished in one step by trifluoroacetic acid (TFA) in CH_2Cl_2 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 μ g/mL to >64 μ g/mL in contrast to ceftazidime which did not prove to be potent against these strains showing MIC value >64 μ g/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 R^2 -groups, showed MIC values ranging from 0.25 to 64 μ g/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 μ g/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 μ g/mL. Furthermore, it can be ascertained that *A. baumannii* clinical isolate exhibited the highest resistance (MIC 8 μ g/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 μ g/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 OSO_3H instead of SO_3H 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 μ g/mL to 64 μ g/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

TABLE 1: *In vitro* antibacterial activities of monobactam derivatives **14** and **18** against selected Gram-negative bacteria.

Sample	MIC ($\mu\text{g/mL}$)									
	E. coli ^a (TEM-1)	E. coli ^b (CTX-M15)	K. p ^c (SHV-1)	K. p ^d (KPC-3, TEM-1)	E. c ^e (P99)	E. c ^f (AmpC)	A. b ^g (OXA- 23/40)	A. b ^h (OXA-24)	P. a ⁱ (KPC-2)	P. a ^j (AmpC)
Aztreonam	>64	16	64	64	>64	>64	>64	32	>64	16
Ceftazidime	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
14a	8	1	4	8	8	8	16	8	2	2
14b	2	0.5	2	8	8	8	16	8	4	1
14c	0.5	0.5	32	64	32	64	16	32	4	2
14d	1	0.25	1	1	2	4	8	8	2	2
14e	0.25	0.25	1	4	2	4	8	4	1	0.5
14g	1	0.5	32	64	64	32	32	16	16	1
18a	0.5	0.125	16	64	8	64	8	32	1	1
18b	0.25	0.25	8	64	16	64	8	16	2	1
18d	8	1	4	8	8	8	16	8	2	2
18e	0.5	0.125	1	64	4	32	4	16	2	0.5
18f	0.125	0.25	2	32	8	16	4	8	0.5	0.5

^aE. coli clinical isolate; ^bE. coli 8739; ^cK. pneumoniae clinical isolate; ^dK. pneumoniae 700603; ^eE. cloacae clinical isolate; ^fE. cloacae 700323; ^gA. baumannii clinical isolate; ^hA. baumannii 19606; ⁱP. aeruginosa clinical isolate; ^jP. aeruginosa 9027.

MIC values ranging from 1 to 16 $\mu\text{g/mL}$, 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**, and **18d–f**) 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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