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Substituted-amidine derivatives of diazabicyclooctane as prospective β-lactamase inhibitors

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Abstract

Discovery of β-lactamase inhibitors is a continuous process due to inherent capability of resistance in bacteria against existing inhibitors. Diazabicyclooctane ring is a non-β-lactam motif capable of inhibiting the β-lactamases. As part of our efforts, we synthesized a series of diazabicyclooctane derivatives where C2 position of the bicyclic ring is linked with various substituted-amidine moieties. The newly synthesized compounds, alone and in combination with meropenem, were tested against ten bacterial strains for their antibacterial activity in vitro. All compounds did not show antibacterial tendency when tested alone (MIC > 64 mg/dm^3) however, showed antibacterial activity in combination with meropenem. All compounds enhanced the potency of meropenem (MIC 2–4 mg/dm^3) with MIC values ranging from < 0.125 to 1 mg/dm^3 indicating their prospective β-lactamase inhibition capability. One derivative proved to be the most potent among all, and is comparable to avibactam, against eight out of ten bacterial strains.

Graphical abstract
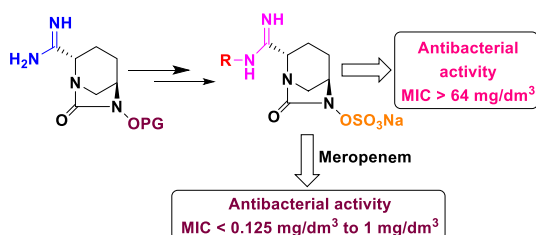
Yuanbai Liu, Jingwen Ji and Jian Sun contributed equally for this work.

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Introduction

In addition to other mechanisms, emergence of new β-lactamases is the major cause of antibiotic resistance in Gram-negative bacterial strains. β-Lactamase inhibition is an alternative strategy to cope with the antibiotic resistance where the inhibitor is employed in combination with existing antibiotic. With the prescription of multidrug-therapy a number of traditional β-lactam (BL) antibiotics restored their activity in combination with suitable β-lactamase inhibitors (BLI) such as clavulanic acid [1, 2], sulbactam, and tazobactam [3]. Clavulanic acid/amoxicillin (augmentin) combination successfully inactivated class A β-lactamases and extended spectrum β-lactamases (ESBLs) [4] for around

40 years of its first clinical application. Following, sulbactam and tazobactam combinations proved effective against class A, class C, as well as a few class D β-lactamases [5].

Since diazabicyclooctane (DBO) has been recognized as a synthetic alternative to BL ring [6], a series of second-generation inhibitors based on DBO functionalization have been prepared [7] and evaluated against various β-lactamases [8]. Worth mentioning are avibactam and relebactam which are potent inhibitors of class A carbapenemases (KPCs), AmpCs (class C), and some of class D lactamases [9]. These DBO based BLIs have been approved for clinical use against multiple bacterial indications in combination with existing BLs [10]. These multi-drugs are currently first line defence against extended spectrum β-lactamases (ESBLs), carbapenemases, MDR [11], *Enterobacteriaceae* as well as *Pseudomonas aeruginosa* [12–14]. However, these combinations are not effective against class B and most of class D β-lactamases. Therefore, several other DBO based BLIs (Fig. 1) [6, 15–17] are passing through phase I or phase III clinical trials [10, 18] in combination with different types of β-lactams (such as meropenem, cefepime, cefpodoxime, etc.).

Recently we reported the synthesis and β-lactamase inhibition tendency of a number of amidine substituted DBOs [19–22], where NH₂ group of amidine attached to the C2 of DBO ring was functionalized with numerous substituents ranging from alkyl to aryl and N-heterocyclics to N,S-heterocycles. Although all compounds showed variable lactamase inhibition capability, however, all compounds could not compete with avibactam. After careful examination of our previously generated data, we chose N-containing substituents to be explored further and here we report the synthesis and antibacterial activities of new compounds alone, as well as a combination with existing β-lactam, meropenem (MER).

Results and discussion

Synthesis of compounds **4a–4e** is shown in Scheme 1. Synthesis of amidine compound **1** was accomplished by our previously reported method [19, 20] and it was obtained as diastereomeric mixture of *R*- and *S*-configuration. The *S*-isomer was found to be antibacterial as compared to its counterpart [19, 20, 23].

The phenomenon of racemization at this stage was surveyed in literature and it was found that the racemization at C2 of DBO has been observed while reacting similar substrate with ammonia [23]. Therefore, based on our own observations and literature reports, we propose the following mechanism for this epimerization (Scheme 2). We have observed the evolution of a gas during the addition of AlMe₃ into NH₄Cl suspension, indicating the formation of the methyl aluminum amide [24, 25] which acts as source of ammonia and a mild base. Hydrogen abstraction at C2 of the compound **A** generates the enimine intermediate **B**, which upon nucleophilic addition of ammonia converts to regioisomers through the formation of intermediate **C** (Scheme 2).

Next step was the coupling of the organic acids with amidine **1** to form the corresponding derivatives **2a–2e**. Classical coupling reagent such as HATU [26] (*O*-(7-aza-1*H*-benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate) was used in DMF (dimethylformamide) or CH₂Cl₂ in the presence of DIPEA (*N,N*-diisopropylethylamine) as a base. For the preparation of **2c**, (*S*)-1-methylpiperidine-3-carboxylic acid was required which was prepared from (*S*)-piperidine-3-carboxylic acid by slight modification to a known procedure [27]. Thus, (*S*)-piperidine-3-carboxylic acid dissolved in methanol was reacted with paraformaldehyde in the presence of
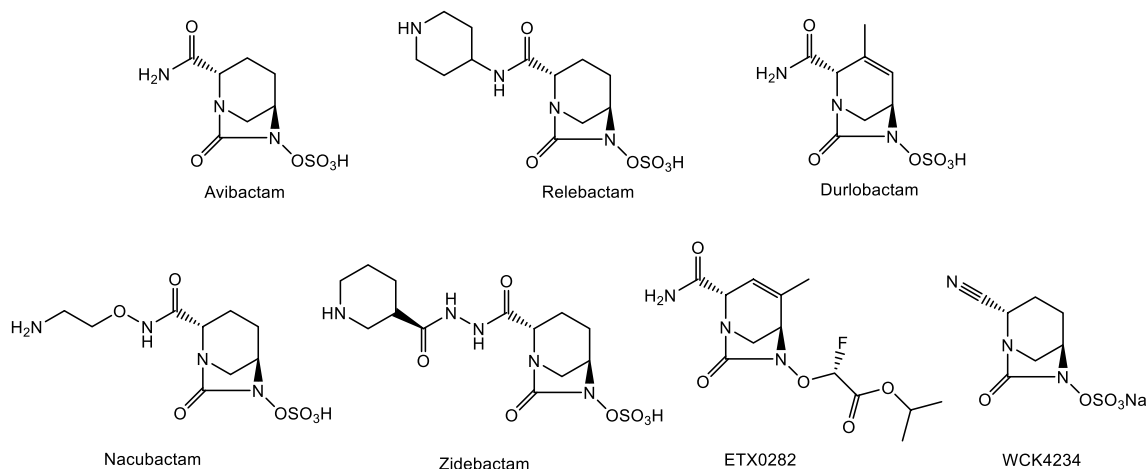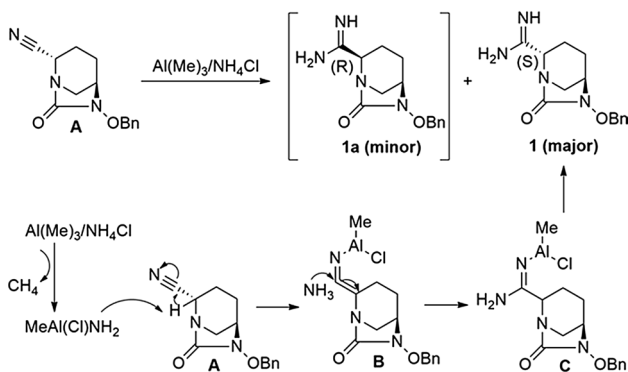

Fig. 1 Structures of diazabicyclooctane derivatives currently approved or in clinical trials
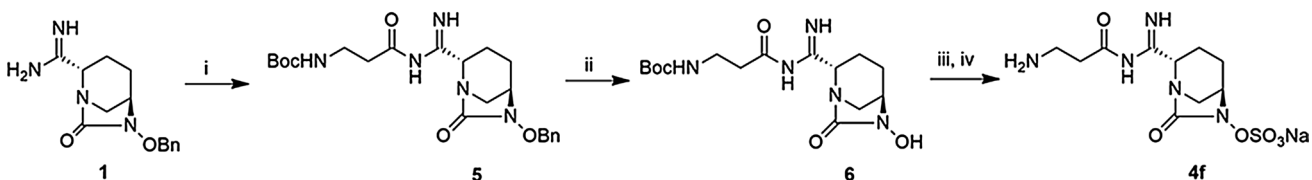
Scheme 1
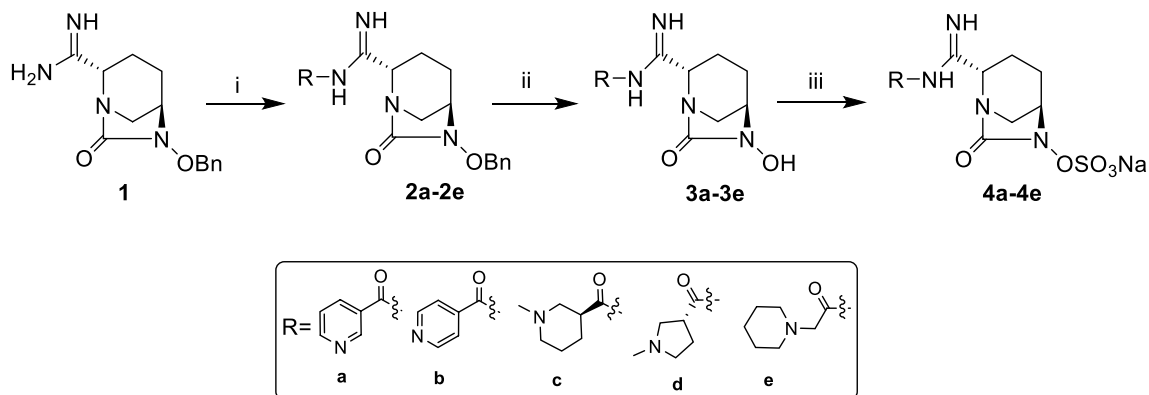
Reagents and conditions: (i) HO-R$_{a-e}$, HATU, DIPEA, DMF/CH$_2$Cl$_2$, RT, 16 h, 44-94%; (ii) Pd/C (wet), THF/TEA, H$_2$, RT, 16 h, 36%-quantitative; (iii) SO$_3$-pyridine or SO$_3$-NMe$_3$, pyridine or TEA, RT, 16 h, Dowex-50wx Na$^+$, 2-70%
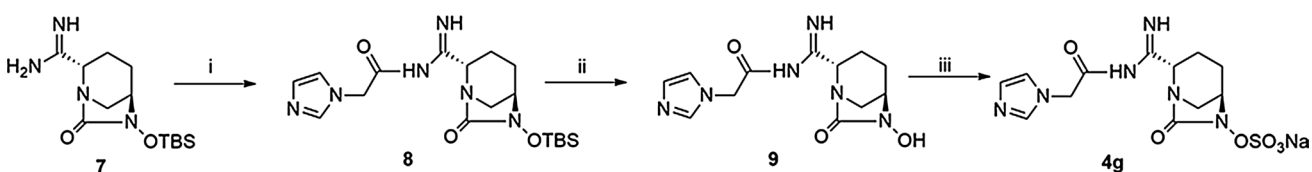
Scheme 1

palladium catalyst under hydrogen atmosphere at 50 °C for 16 h. Similarly (R)-1-methylpyrrolidine-3-carboxylic acid was prepared [27] from (R)-pyrrolidine-3-carboxylic acid before its condensation with compound **1** to yield the compound **2d**. Palladium catalysed hydrogenation of compounds **2a–2e** in THF led to the debenzylation affording hydroxy derivatives **3a–3e**. Compounds **3a–3e** were then reacted with SO$_3$-pyridine complex or SO$_3$-NMe$_3$ to form sulfonic acid derivatives **4a–4e** after purification by preparative HPLC. Sodium salts of these compounds were obtained by ion exchange using column filled with Dowex-50wx Na$^+$ resin. Water was used as an eluant which was

Scheme 3

Reagents and conditions: (i) 3-[(tert-butoxycarbonyl)amino]propanoic acid, DCC, DMAP, THF, RT, 16 h, 90%; (ii) Pd/C (wet), EtOAc/TEA, H$_2$, RT, 16 h, 69%; (iii) SO$_3$-pyridine, pyridine, RT, 16 h, 94% (crude); (iv) TFA, CH$_2$Cl$_2$, 0 °C, 3.5 h, Dowex-50wx Na$^+$, 10%

Scheme 4

Reagents and conditions: (i) 2-(1H-imidazol-1-yl)acetic acid, HATU, DIPEA, DMF/CH$_2$Cl$_2$, RT, 24 h, 57%; (ii) TBAF, THF, 0 °C, 1.5 h, 98%; (iii) SO$_3$-NMe$_3$, TEA, THF/H$_2$O, Dowex-50wx Na$^+$, 44%

removed by lyophilization to obtain the sodium salts of desired compounds (Scheme 1).

Synthesis of compound **4f** was accomplished following the Scheme 3. Amidine **1** was condensed with 3-[(*tert*-butoxycarbonyl)amino]propanoic acid using DCC (*N,N′*-dicyclohexylcarbodiimide) and DMAP (4-dimethylaminopyridine) as the coupling reagents to yield 90% of compound **5**. Benzyl deprotection of **5** was achieved by Pd/C catalysed hydrogenation in EtOAc. It has been observed that a catalytic amount of triethylamine in EtOAc enhances the rate of hydrogenolysis of benzyl ether derivatives [28]. Final compound **4f** was obtained by sulfonation of **6** followed by deprotection of Boc group by reacting the intermediate sulfonic acid derivative with TFA (trifluoroacetic acid) in CH_2Cl_2 at low temperature and subsequent ion exchange by Dowex-50wx Na^+ resin.

Synthesis of analogous compound **4g** was accomplished following the procedures elaborated in Scheme 4 because the benzyl deprotection was unsuccessful in this case while following the Scheme 3. Therefore, we prepared TBS (*tert*-butyldimethylsilyl) protected amidine compound **7** following our previously described method [19, 20]. Compound **7** was then successfully coupled with 2-(1*H*-imidazol-1-yl)acetic acid using HATU in DMF/CH_2Cl_2 mixture as solvent in the presence of DIPEA. TBS protection in compound **8** was then removed by TBAF (tetrabutylammonium fluoride) leading to the formation of hydroxy derivative **9**, which was converted to the sodium salt of **4g** using the similar procedure described for **4a–4e**. All the final compounds **4a–4g** were purified by preparative HPLC, and the structures were confirmed by NMR spectra as well as HRMS data.

Substituted-amidine derivatives **4a–4g** were then analysed for their antibacterial activity in vitro alone, as well as in combination with meropenem, an existing antibiotic, using broth dilution method [29]. A number of bacterial species with different β-lactamases (Table 1), 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, were used as test bacterial species. Meropenem (MER) alone, and as a combination with avibactam, as well as with synthesized compounds **4a–4g**, were analysed for antimicrobial activity by determining their minimum inhibitory concentration (MIC, mg/dm^3) values. For comparison MIC values of avibactam and newly synthesized compounds **4a–4g** without meropenem were also determined.

It was observed that the compounds **4a–4g** are not antibacterial in action (MIC > 64 mg/dm^3) when used alone without β-lactam, but antibacterial activity of the β-lactam meropenem was changed upon combining the

compounds **4a–4g** individually (Table 1). This implies the formation of stable complex of the derivatives **4a–4g** with β-lactamases present in the bacterial strains, thus indicating the β-lactamase inhibitor potential of the synthesized compounds. In general, all compounds **4a–4g** enhanced potency of meropenem against all tested species with MIC values ranging from < 0.125 to 1 mg/dm^3 (compared to MER, MIC 2–4 mg/dm^3).

To our delight all compounds showed potency comparable to avibactam in combination with meropenem (MER + Avibactam), against *E. coli* clinical isolate, *E. coli* 8739, *K. pneumoniae* clinical isolate, *K. pneumoniae* 700603, *A. baumannii* clinical isolate, and *A. baumannii* 19606. All compounds except **4b** showed similar or better activity against *P. aeruginosa* clinical isolate (MIC 0.25–0.5 mg/dm^3) compared to avibactam (MIC 0.5 mg/dm^3). Among all bacterial species, *E. cloacae* strains proved the most resistant against all compounds with MIC values of 0.25 mg/dm^3 (for avibactam MIC is < 0.125 mg/dm^3), except for compound **4g** where MIC was comparable to avibactam against *E. cloacae* 700323 (MIC < 0.125 mg/dm^3).

It is worth mentioning that the stereochemistry of the final compounds does impact the antibacterial strength. This can be assessed by the activity comparison of the stereochemical isomers of compounds **4c** and **4d** previously reported by us. The compounds **4c** and **4d** are comparatively more active against all these bacterial strains in vitro [20]. From the data in Table 1, it is evident that compounds **4b** and **4c** showed better activity than avibactam against *A. baumannii* 19606, whereas compounds **4d**, **4e**, and **4f** were better than avibactam against *P. aeruginosa* clinical isolate. Moreover, it is clear that the compound **4g** is the most potent among all compounds and showed comparable potency to avibactam against eight out of ten strains and even better potency against one species.

Conclusion

We successfully synthesized seven substituted-amidine derivatives of diazabicyclooctane and the compounds were tested for antibacterial activity alone, and in combination with meropenem using bacterial species containing different β-lactamases. All compounds were not antibacterial in nature when tested alone, however all the compounds enhance the antibacterial strength of the antibiotic in vitro. This indicates the β-lactamase inhibition capability of the compounds allowing the drug to kill the bacteria, through a synergism between antibiotic and lactamase inhibitor. The compound **4g** proved to be the most potent among all and may be a lead compound for further development, and preclinical studies.

Table 1 Antibacterial activity of avibactam and compounds **4a–4g** alone as well as in combination with meropenem (MER)

Sample	MIC/mg dm^{-3}									
	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)
4a–4g and avibactam alone	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
MER alone	4	4	4	2	4	4	4	2	4	4
MER+avibactam	<0.125	<0.125	<0.125	<0.125	<0.125	<0.125	1	0.5	0.5	0.25
MER+**4a**	<0.125	<0.125	<0.125	<0.125	0.25	0.25	1	0.5	0.5	0.5
MER+**4b**	<0.125	<0.125	<0.125	<0.125	0.25	0.25	1	0.25	1	0.25
MER+**4c**	<0.125	<0.125	<0.125	<0.125	0.25	0.25	1	0.25	0.5	0.25
MER+**4d**	<0.125	<0.125	<0.125	<0.125	0.25	0.5	1	0.5	0.25	0.25
MER+**4e**	<0.125	<0.125	<0.125	<0.125	0.25	0.5	1	0.5	0.25	0.25
MER+**4f**	<0.125	<0.125	<0.125	<0.125	0.5	0.25	1	0.5	0.25	0.5
MER+**4g**	<0.125	<0.125	<0.125	<0.125	0.25	<0.125	1	0.5	0.25	0.5

[a]*E. coli* clinical isolate

[b]*E. coli* 8739

[c]*K. pneumoniae* clinical isolate

[d]*K. pneumoniae* 700603

[e]*E. cloacae* clinical isolate

[f]*E. cloacae* 700323

[g]*A. baumannii* clinical isolate

[h]*A. baumannii* 19606

[i]*P. aeruginosa* clinical isolate

[j]*P. aeruginosa* 9027

Experimental

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 (see supplementary figures). The NMR data were recorded either in D_2O or DMSO-d_6 containing 0.03% TMS as internal standard. Chemical shifts (δ) are given in parts per million (ppm) relative to TMS and coupling constant (J values) are given in Hertz (Hz). Signal multiplicities are reported as follows: s, singlet; brs, broad singlet; d, doublet; t, triplet and m, multiplet. IR spectra were measured on a SHIMADZU IRSpirit Fourier Transform Infrared Spectrophotometer. Melting points were measured on SGW X-4 (Laboratory device). Preparative HPLC was performed on an Agilent 1260 Infinity II System on Agilent 10 prep-C18 250×21.2 mm column, using an acetonitrile/aqueous 0.1% trifluoroacetic acid gradient, or an acetonitrile/aqueous 0.1% formic acid gradient, or an acetonitrile/water at 22 °C. Mass spectra were performed on an Agilent 1260II-6125 Separation Module using either ES$^-$ or ES$^+$ ionization modes. Column Chromatography was performed with using Qingdao Inc. Silica Gel: CC Grade (230-400 Mesh). Commercial solvents and reagents were generally used without further purification. All products were dried before characterization and used in subsequent synthetic steps.

General method for the synthesis of compounds 2a-2e; *N*-[[(2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl](imino)methyl]nicotinamide (2a, C$_{20}$H$_{21}$N$_5$O$_3$) To a solution of 500 mg **1** (1.82 mmol), 1.04g HATU (2.73 mmol), and 707 mg DIPEA (5.47 mmol) dissolved in 5 cm^3 DMF, 337 mg nicotinic acid (2.73 mmol) was added at room temperature under N_2. The reaction mixture was stirred for 16 h and was diluted with CH_2Cl_2 upon completion of the reaction. The organic phase was separated, and washed with brine and saturated NH_4Cl, dried over Na_2SO_4, and filtered. The organic layer was concentrated to give a residue, which was purified by silica gel column chromatography eluting with 50% EtOAc in hexane to give the title compound **2a** as a white solid, which was directly used for next step without further purification. Yield 75%; MS (LC/MS): $m/z = 380.4$ ([M + H]$^+$).

N-[[(2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1] octan-2-yl](imino)methyl]isonicotinamide (2b, $C_{20}H_{21}N_5O_3$) Compound **2b** was prepared from 337 mg isonicotinic acid (2.73 mmol) and 500 mg **1** (1.82 mmol) according to the general method. Yield 650 mg (94%) as a white solid; MS (LC/MS): $m/z = 380.4$ ([M + H]$^+$).

(3S)-N-[[(2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]- octan-2-yl](imino)methyl]-1-methylpiperidine-3-carbox- amide (2c, $C_{21}H_{29}N_5O_3$) 215 mg (S)-1-methylpiperidine- 3-carboxylic acid (1.50 mmol) and 215 mg **1** (0.75 mmol) were reacted in 5 cm^3 CH$_2$Cl$_2$/DMF (3:2) for 30 h according to the general procedure. Yield 195 mg (65%); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.54$ (brs, 4H), 1.65–1.74 (m, 2H), 1.88–1.97 (m, 2H), 2.35 (brs, 3H), 2.78 (brs, 3H), 2.91 (brs, 1H), 3.04 (brs, 1H), 3.19 (brs, 1H), 3.35 (brs, 1H), 3.80–3.91 (m, 2H), 4.85 (brs, 2H), 5.66 (s, 1H), 6.76 (s, 2H), 7.36–7.39 (m, 3H), 7.49–7.51 (m, 2H) ppm; MS (LC/MS): $m/z = 400.2$ ([M + H]$^+$).

(3R)-N-[[(2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]- octan-2-yl](imino)methyl]-1-methylpyrrolidine-3-carboxa- mide (2d, $C_{20}H_{27}N_5O_3$) Compound **2d** was synthesized from 330 mg **1** (1.20 mmol) and 300 mg (R)-1-methylpyrrolidine- 3-carboxylic acid (2.33 mmol) in 6 cm^3 CH$_2$Cl$_2$/DMF (1:1) for 48 h, according to general method. Yield 204 mg (44%) brown solid; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.72$–1.79 (m, 1H), 1.90–2.03 (m, 5H), 2.22–2.27 (m, 1H), 2.60 (s, 3H), 2.95–2.97 (m, 1H), 3.16–3.27 (m, 3H), 3.48–3.51 (m, 1H), 3.82–3.87 (m, 1H), 3.90–3.97 (m, 1H), 4.85 (brs, 2H), 5.68 (s, 1H), 6.74 (s, 2H), 7.35–7.41 (m, 3H), 7.47–7.53 (m, 2H) ppm; MS (LC/MS): $m/z = 386.2$ ([M + H]$^+$).

N-[[(2S,5R)-6-(Benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]- octan-2-yl](imino)methyl]-2-(piperidin-1-yl)acetamide (2e, $C_{21}H_{29}N_5O_3$) Compound **2e** was prepared from 260 mg 2-(piperidin-1-yl)acetic acid (1.45 mmol) and 230 mg **1** (0.84 mmol) according to the general method. Yield 245 mg (73%) yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.33$–1.74 (m, 7H), 1.78–2.08 (m, 4H), 2.22–2.43 (m, 2H), 2.78 (t, $J = 12.1$ Hz, 1H), 3.22–3.32 (m, 3H), 3.75–3.85 (m, 1H), 3.94–4.09 (m, 1H), 4.34 (t, $J = 14.3$ Hz, 1H), 4.83–4.89 (m, 2H), 5.64 (s, 1H), 6.73 (s, 2H), 7.34–7.41 (m, 3H), 7.46–7.53 (m, 2H) ppm; MS (LC/MS): $m/z = 400.2$ ([M + H]$^+$).

General method for the synthesis of compounds 3a-3e; N-[[(2S,5R)-6-Hydroxy-7-oxo-1,6-diazabicy- clo[3.2.1]octan-2-yl](imino)methyl]nicotinamide (3a, $C_{13}H_{15}N_5O_3$) 100 mg of 10% wet Pd/C was added to a solu- tion of 200 mg compound **2a** (0.52 mmol) dissolved in 3 cm^3

THF containing few drops of triethylamine. The mixture was stirred under H$_2$ atmosphere at room temperature for 16 h. Later, the reaction mixture was filtered through a pad of celite, rinsed with EtOAc, and the filtrate was concentrated in vacuum. The crude product was purified by flash silica gel chromatography using 10% MeOH in CH$_2$Cl$_2$ to give the title compound **3a** (67 mg, 44% in two steps) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.75$–1.83 (m, 1H), 1.87–2.09 (m, 3H), 3.13–3.24 (m, 1H), 3.45–3.62 (m, 1H), 4.07–4.15 (m, 1H), 5.70 (s, 1H), 6.44 (s, 2H), 7.51 (dd, $J = 7.8, 4.8$ Hz, 1H), 7.93 (dt, $J = 7.8, 1.7$ Hz, 1H), 8.67– 8.71 (m, 2H), 9.24 (s, 1H) ppm; MS (LC/MS): $m/z = 290.2$ ([M + H]$^+$).

N-[[(2S,5R)-6-Hydroxy-7-oxo-1,6-diazabicyclo[3.2.1] octan-2-yl](imino)methyl]isonicotinamide (3b, $C_{13}H_{15}N_5O_3$) Compound **3b** was synthesized from 650 mg **2b** (1.71 mmol) using general method. Yield 36% white solid; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.75$–1.82 (m, 1H), 1.87–2.11 (m, 3H), 3.16–3.25 (m, 1H), 3.33–3.43 (m, 1H), 4.04–4.13 (m, 1H), 5.76 (s, 1H), 6.43 (s, 2H), 7.47 (d, $J = 4.9$ Hz, 2H), 8.70 (d, $J = 4.9$ Hz, 2H), 9.20 (s, 1H) ppm; MS (LC/MS): $m/z = 290.2$ ([M + H]$^+$).

(3S)-N-[[(2S,5R)-6-Hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]- octan-2-yl](imino)methyl]-1-methylpiperidine-3-carbox- amide (3c, $C_{14}H_{23}N_5O_3$) Compound **3c** was synthesized according to general procedure from 195 mg compound **2c** (0.49 mmol). Yield 155 mg (quantitative) white solid, which was directly used for next step without further purifi- cation. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.46$–1.79 (m, 4H), 1.83–2.04 (m, 4H), 2.21 (s, 3H), 2.70–2.97 (m, 5H), 3.16–3.26 (m 1H), 3.73–3.82 (m, 1H), 3.87–3.98 (m, 1H), 5.65 (s, 1H), 6.46 (s, 2H), 9.28 (s, 1H) ppm; MS (LC/MS): $m/z = 310.2$ ([M + H]$^+$).

(3R)-N-[[(2S,5R)-6-Hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]- octan-2-yl](imino)methyl]-1-methylpyrrolidine-3-carbox- amide (3d, $C_{13}H_{21}N_5O_3$) Compound **3d** was prepared form 200 mg **2d** (0.52 mmol) according to the general procedure. Yield 160 mg (quantitative) white solid. Compound **3d** was directly used for next step without further purification. MS (LC/MS): $m/z = 296.2$ ([M + H]$^+$).

N-[[(2S,5R)-6-Hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]- octan-2-yl](imino)methyl]-2-(piperidin-1-yl)acetamide (3e, $C_{14}H_{23}N_5O_3$) 240 mg compound **2e** (0.60 mmol) was hydro- genated according to the general procedure to get the 190 mg compound **3e**. Yield quantitative, white solid. Compound **3e** was directly used for next step without further purification. MS (LC/MS): $m/z = 310.2$ ([M + H]$^+$).

General method for the synthesis of compounds 4a-4e; Sodium (2S,5R)-2-(N-nicotinoylcarbamimidoyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (4a, C$_{13}$H$_{14}$N$_5$NaO$_6$S)

A mixture of 406 mg compound **3a** (1.45 mmol) and 569 mg SO$_3$-pyridine (7.27 mmol) dissolved in 20 cm^3 pyridine was stirred at room temperature for 16 h. The reaction mixture was concentrated to give a residue, which was purified by preparative HPLC on an Agilent 10 prep-C18 250×21.2 mm column and lyophilized, followed by ion exchange using column filled with Dowex-50wx Na$^+$ resin, to give **4a**. Yield 12 mg (2%) white solid; m.p.: 182.3 °C; ^{1}H NMR (400 MHz, D$_2$O): δ = 1.85–2.09 (m, 2H), 2.11–2.23 (m, 2H), 3.50–3.61 (m, 1H), 3.77–2.86 (m, 1H), 4.04–4.13 (m, 1H), 5.75 (s, 1H), 7.53 (dd, J = 5.0, 7.8 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 8.59 (d, J = 1.5 Hz, 1H), 8.62 (d, J = 5.0 Hz, 1H) ppm; ^{13}C NMR (100 MHz, D$_2$O): δ = 26.5, 27.8, 41.6, 57.2, 61.1, 116.9, 124.1, 129.4, 136.2, 146.8, 150.7, 162.6, 170.1 ppm; FT-IR (KBr): $\bar{v}$ = 3487, 3355, 2963, 2250, 1592, 1058, 856, 741, 597 cm^{-1}; MS (LC/MS): m/z = 368.1 ([M-Na]$^-$); MS (HR-EI-MS): m/z = 368.0660.

Sodium (2S,5R)-2-(N-isonicotinoylcarbamimidoyl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (4b, C$_{13}$H$_{14}$N$_5$NaO$_6$S)

200 mg compound **3b** (0.72 mmol) was reacted with 569 mg SO$_3$-pyridine (3.58 mmol) as described in general method to afford compound **4b**. Yield 5 mg (2%) white solid; m.p.: 190.1 °C; ^{1}H NMR (400 MHz, D$_2$O): = δ 1.92–2.07 (m, 2H), 2.10–2.23 (m, 2H), 3.49–3.58 (m, 1H), 3.67–3.75 (m, 1H), 4.01–4.10 (m, 1H), 5.76 (s, 1H), 7.46 (d, J = 5.0 Hz, 2H), 8.61 (d, J = 4.7 Hz, 2H) ppm; ^{13}C NMR (100 MHz, D$_2$O): = δ 26.5, 27.6, 41.4, 58.8, 57.1, 116.8, 121.0, 121.6, 141.8, 149.2, 149.5, 162.5, 169.7 ppm; FT-IR (KBr): $\bar{v}$ = 3475, 3392, 2965, 1691, 1648, 1058, 845, 741, 600 cm^{-1}; MS (LC/MS): m/z = 368.1 ([M-Na]$^-$); MS (HR-EI-MS): m/z = 368.0659.

Sodium (2S,5R)-2-[N-[(S)-1-methylpiperidine-3-carbonyl]-carbamimidoyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (4c, C$_{14}$H$_{22}$N$_5$NaO$_6$S)

Compound **4c** was synthesized from 155 mg **3c** (0.5 mmol) as described in general procedure. Yield 134 mg (70%) white solid; m.p.: 184 °C; ^{1}H NMR (400 MHz, D$_2$O): δ = 1.75–2.03 (m, 6H), 2.08–2.18 (m, 2H), 2.82 (s, 3H), 2.86–2.95 (m, 1H), 3.02–3.11 (m, 1H), 3.37–3.67 (m, 4H), 3.95–4.08 (m, 2H), 5.70 (s, 1H) ppm; ^{13}C NMR (100 MHz, D$_2$O): δ = 23.8, 26.3, 34.0, 37.1, 40.7, 41.0, 43.4, 53.9, 54.0, 54.3, 54.5, 117.0, 162.7, 173.7 ppm; FT-IR (KBr): $\bar{v}$ = 3467, 3378, 2963, 1695, 1648, 1053, 972, 799, 560 cm^{-1}; MS (LC/MS): m/z = 388.2 ([M-Na]$^-$); MS (HR-EI-MS): m/z = 388.1296.

Sodium (2S,5R)-2-[N-[(R)-1-methylpyrrolidine-3-carbonyl]-carbamimidoyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (4d, C$_{13}$H$_{20}$N$_5$NaO$_6$S)

A mixture of 160 mg compound **3d** (0.54 mmol), 147 mg SO$_3$-NMe$_3$ (1.04 mmol), and 0.22 cm^3 TEA (1.55 mmol) in 6 cm^3 THF/water (1:1) was stirred at room temperature for 21 h. The reaction mixture was concentrated under reduced pressure to provide a residue. The residue was further purified by Dowex-50wx Na$^+$ resin, using water as an elution solvent to give **4d**. Yield 75 mg (38%) white solid; m.p.: 170.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.75–1.85 (m, 1H), 1.96–2.06 (m, 2H), 2.07–2.23 (m, 3H), 2.58–2.68 (m, 1H), 2.90 (s, 3H), 3.54–3.62 (m, 1H), 3.64–3.71 (m, 2H), 3.75–3.83 (m, 1H), 3.87–4.08 (m, 3H), 5.66 (s, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): = δ 23.7, 27.2, 28.0, 41.2, 45.4, 47.6, 55.2, 55.7, 55.8, 56.9, 118.0, 161.5, 171.2 ppm; FT-IR (KBr): $\bar{v}$ = 3466, 3370, 2967, 1695, 1654, 1052, 972, 799, 621 cm^{-1}; MS (LC/MS): m/z = 374.1 ([M-Na]$^-$); MS (HR-EI-MS): m/z = 374.1138.

Sodium (2S,5R)-7-oxo-2-[N-[2-(piperidin-1-yl)acetyl]-carbamimidoyl]-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (4e, C$_{14}$H$_{22}$N$_5$NaO$_6$S)

Compound **4e** was obtained from 190 mg **3e** (0.6 mmol) using the procedure described for compound **4d**. Yield 65 mg (65%) white solid; m.p.: 172.1 °C; ^{1}H NMR (400 MHz, D$_2$O): δ = 1.67–1.87 (m, 6H), 1.90–2.01 (m, 1H), 2.04–2.23 (m, 3H), 3.25–3.38 (m, 2H), 3.48–3.60 (m, 2H), 3.71–3.78 (m, 1H), 3.97–4.05 (m, 1H), 4.13–4.25 (m, 2H), 4.35 (t, J = 7.1 Hz, 1H), 5.63 (s, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 21.9, 22.8, 23.2, 27.2, 28.5, 41.1, 45.2, 54.0, 55.1, 117.9, 161.5, 163.4 ppm; FT-IR (KBr): $\bar{v}$ = 3447, 2958, 1671, 1293, 1055, 972, 799, 621, 598 cm^{-1}; MS (LC/MS): m/z = 388.1 ([M-Na]$^-$); MS (HR-EI-MS): m/z = 388.1298.

tert-Butyl [3-[(2S,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboximidamido]-3-oxopropyl]-carbamate (5, C$_{22}$H$_{31}$N$_5$O$_5$)

To a solution of 301 mg **1** (1.10 mmol), 70 mg DMAP (0.57 mmol), and 358 mg DCC (1.73 mmol) dissolved in 40 cm^3 THF, 335 mg of 3-[(tert-butoxycarbonyl)amino]propanoic acid (1.77 mmol) was added at room temperature. The reaction mixture was then stirred for 16 h under nitrogen. After completion, the reaction mixture was concentrated to dryness, diluted with EtOAc, washed with saturated NH$_4$Cl, brine, dried over Na$_2$SO$_4$, and filtered. The organic layer was concentrated to give a residue, which was purified by silica gel column chromatography eluting with 50–75% EtOAc in petroleum ether to give the title compound **5**. Yield 440 mg (90%) white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.37 (s, 9H), 1.68–1.75 (m, 1H), 1.80–2.00 (m, 3H), 2.43–2.51 (m, 2H), 3.09–3.17 (m, 2H), 3.20–3.25 (m, 1H), 3.72–3.81 (m, 1H), 3.89–3.98 (m, 1H), 4.84 (s, 2H), 5.65 (s, 1H), 6.72 (s, 3H), 7.35–7.41 (m, 3H), 7.47–7.52 (m, 2H) ppm; MS (LC/MS): m/z = 446.2 ([M+H]$^+$).

***tert*-Butyl [3-[(2*S*,5*R*)-6-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboximidamido]-3-oxopropyl]carbamate (6, $C_{15}H_{25}N_5O_5$)** Hydrogenolysis of 440 mg compound **5** (0.99 mmol) was performed by the method described for **3a** in 20 cm^3 EtOAc with a few drops of TEA. The mixture was hydrogenated under 45 psi pressure at room temperature for 1 h, filtered through a pad of celite, rinsed with EtOAc. The filtrate was concentrated to give a residue, which was purified by silica gel column chromatography eluting with 5% MeOH in CH_2Cl_2 to give the title compound **6**. Yield 245 mg (69%) yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.37$ (s, 9H), 1.67–1.77 (m, 2H), 1.83–1.91 (m, 1H), 1.94–2.01 (m, 1H), 2.44–2.51 (m, 2H), 3.09–3.21 (m, 3H), 3.66–3.72 (m, 1H), 3.93–4.03 (m, 1H), 5.65 (s, 1H), 6.45 (s, 2H), 6.72 (t, $J = 5.1$ Hz, 1H), 9.27 (s, 1H) ppm; MS (LC/MS): $m/z = 378.2$ ([M + Na]$^+$).

Sodium (2*S*,5*R*)-2-[*N*-(3-aminopropanoyl)-carbamimidoyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (4f, $C_{10}H_{16}N_5NaO_6S$) Compound **4f** was prepared according to modified procedure described for **4a**. 245 mg compound **6** (0.69 mmol) was reacted with 159 mg SO_3-pyridine (1 mmol) in 10 cm^3 pyridine, to form the intermediate sulfonic acid (332 mg, 94%) as a white foam. The intermediate was then treated with 1.4 cm^3 TFA in 6 cm^3 anhydrous CH_2Cl_2. The mixture was stirred for 3.5 h at 0 ºC, and then concentrated under reduced pressure to give a residue. The residue was dissolved in 15 cm^3 CH_2Cl_2 and extracted with water (2 × 10 cm^3). The aqueous layer was freeze-dried and purified by Dowex-50wx Na$^+$ resin, using water as an elution solvent. Yield 22 mg (10%) white powder; m.p.: 203.5 °C; ^{1}H NMR (400 MHz, D$_2$O): $\delta = 1.71$–1.80 (m, 1H), 1.89–1.97 (m, 1H), 2.02–2.11 (m, 2H), 2.77–3.87 (m, 2H), 3.17 (t, $J = 6.0$ Hz, 2H), 3.46–3.54 (m, 1H), 3.91–4.00 (m, 2H), 5.64 (d, $J = 4.6$ Hz, 1H) ppm; ^{13}C NMR (100 MHz, D$_2$O): $\delta = 22.6$, 26.5, 29.9, 35.2, 40.9, 45.0, 57.4, 117.5, 162.8, 171.1 ppm; FT-IR (KBr): $\bar{v} = 3462$, 3174, 2974, 1468, 1447, 1244, 1053, 972, 742, 598 cm^{-1}; MS (LC/MS): $m/z = 334.1$ ([M-Na]$^-$); MS (HR-EI-MS): $m/z = 334.0829$.

***N*-[[(2*S*,5*R*)-6-(*tert*-Butyldimethylsilyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl](imino)methyl]-2-(1*H*-imidazol-1-yl)-acetamide (8, $C_{18}H_{30}N_6O_3Si$)** 704 mg HATU (1.85 mmol) and 0.82 cm^3 DIPEA (4.08 mmol) were added to a solution of 490 mg 2-(1*H*-imidazol-1-yl)acetic acid (1.87 mmol) and 279 mg amidine **7** [19] (0.94 mmol) in 7 cm^3 DMF/CH_2Cl_2 (1:1). The reaction mixture was then stirred at room temperature for 24 h. The reaction was quenched with saturated aq. NaHCO$_3$ and extracted with EtOAc. The organic layer was washed with water, then brine, dried over Na$_2$SO$_4$ and filtered. The filtrate was concentrated to give a residue, which was purified by silica gel column chromatography eluting with 5% MeOH in CH_2Cl_2 containing 0.2% TEA to give the title compound **8.** Yield 220 mg (57%) yellow solid. MS (LC/MS): $m/z = 407.2$ ([M + H]$^+$).

***N*-[[(2*S*,5*R*)-6-Hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]-octan-2-yl](imino)methyl]-2-(1*H*-imidazol-1-yl)acetamide (9, $C_{12}H_{16}N_6O_3$)** 0.65 cm^3 TBAF (0.65 mmol) was added to a solution of 220 mg compound **8** (0.54 mmol) dissolved in 5 cm^3 THF at 0 °C, and stirred at 0 °C for 1.5 h. The mixture was concentrated to dryness, added 5 cm^3 of water, extracted with EtOAc and DCM. The organic layer was dried over Na$_2$SO$_4$, and filtrated. The filtrate was concentrated to give the title compound **9**. Yield 188 mg (98%) brown solid, which was directly used for next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.68$–1.78 (m, 2H), 1.90–2.05 (m, 2H), 3.19–3.25 (m, 1H), 3.68–3.77 (m, 1H), 4.05–4.13 (m, 1H), 5.08 (d, $J = 17.1$ Hz, 1H)), 5.14 (d, $J = 17.1$ Hz, 1H), 5.60 (s, 1H), 6.32 (s, 2H), 6.85 (s, 1H), 7.06 (s, 1H), 7.53 (s, 1H) ppm; MS (LC/MS): $m/z = 293.1$ ([M + H]$^+$).

Sodium (2*S*,5*R*)-2-[*N*-[2-(1*H*-imidazol-1-yl)acetyl]-carbamimidoyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (4g, $C_{12}H_{15}N_6NaO_6S$) Compound **4g** was prepared from 188 mg compound **9** (0.64 mmol), 177 mg SO_3-NMe$_3$ (1.27 mmol), and 0.25 cm^3 TEA (1.79 mmol) in 6 cm^3 THF/water (1:1) as described for compound **4d**. Yield 95 mg (44%) white solid; m.p.: 180.2 °C; ^{1}H NMR (400 MHz, D$_2$O): $\delta = 1.77$–1.89 (m, 1H), 1.95–2.03 (m, 1H), 2.07–2.17 (m, 2H), 3.60–3.67 (m, 1H), 3.92–3.98 (m, 1H), 4.05–4.14 (m, 1H), 5.22 (d, $J = 17.4$ Hz, 1H), 5.29 (d, $J = 17.4$ Hz, 1H), 5.63 (d, $J = 5.3$ Hz, 1H), 7.20–7.23 (m 2H), 8.17 (s, 1H) ppm; ^{13}C NMR (100 MHz, D$_2$O): $\delta = 22.3$, 26.3, 41.3, 44.4, 48.8, 57.0, 116.7, 122.0, 123.6, 137.5, 162.7, 167.5 ppm; FT-IR (KBr): $\bar{v} = 3467$, 3376, 3151, 2970, 1679, 1480, 1463, 1053, 972, 799, 613, 560 cm^{-1}; MS (LC/MS): $m/z = 371.1$ ([M-Na]$^-$); MS (HR-EI-MS): $m/z = 371.0774$.

Determination of antibacterial activity

Five wild type bacterial strains, namely *E. coli* clinical isolate (TEM-1), *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 (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) expressing variable β-lactamases were indigenously isolated and cultured from patients at Ningxia Medical University hospital, P.R. China.

A standard procedure was employed for these studies using the broth microdilution method according to the guidelines of the Clinical Laboratories and Standards Institute [29]. In a typical experiment, meropenem as a test antibiotic compound was dissolved in DMSO and diluted in microbial growth medium (Mueller–Hinton Broth II, cation adjusted) to a final concentration range of 0.125–64 mg/dm^3 in serial two-fold dilution. In all cases the final DMSO concentration was less than 0.5%. Bacteria were added to 96-well microtiter plates containing the serial two-fold dilutions of the compounds; the final cell density was approximately 5×10^5 colony forming units/cm^3 (CFU/cm^3). Plates were incubated at 37 ℃ for 18–24 h and read visually. The MIC of the test compound that inhibited visible growth of the bacteria was recorded. The same assay conditions were used when the compounds **4a–4g** and avibactam alone and as a combination with test meropenem as an antibiotic compound (as two controls) were tested for MIC (mg/dm^3). While meropenem was serially diluted as described above, a constant concentration (4 mg/dm^3) of inhibitor compounds **4a–4g** was used. The test results listed in Table 1.

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