Beijing Key Laboratory of Antimicrobial Agents[1], Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College; Department of Neurology[2], Beijing Friendship Hospital, Capital Medical University, Beijing, China

Synthesis and antibacterial evaluation of sulbactam derivatives against *Acinetobacter baumannii*

Yuanshuai Yang[1,†], Sheng Tang[1,†], Zhandong liu[2], Yanxiang Wang[1], Jing Pang[2], Xuefu You[1], Danqing Song[1], Yinghong Li[1,*], Xi Lu[1,*]

Received April 19, 2018, accepted May 18, 2018

**Corresponding authors: Yinghong Li, Xi Lu, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 10050, China.*
liyinghong@imb.pumc.edu.cn; luxi23@126.com
† These authors made equal contribution to this work.

Pharmazie 73: 433-437 (2018) *doi: 10.1691/ph.2018.8487*

Sulbactam, a known β-lactamase inhibitor, was found to own potent antimicrobial activity against *Acinetobacter baumannii* in clinical practice. Based on clinical evidence, a series of sulbactam derivatives were designed, synthesized and evaluated for antibacterial activity against *Acinetobacter baumannii*, taking sulbactam as the lead. Among them, compound **9** exhibited a good antibacterial activity against *Acinetobacter baumannii* with a MIC value of 4 μg/mL, slightly lower than that of sulbactam of 1 μg/mL. Especially, compound **9** displayed outstanding oral pharmacokinetic profiles with a maximum plasma concentration (C_{max}) of 19.6 μg/mL and area under the curve (AUC) of 10.8 μg·h/mL, much higher than those of sulbactam, and thus held the potential of being developed into an oral medication. These results provided the useful information for further structural modification and optimization of its kind, and compound **9** has been selected for the further investigation.

1. Introduction

Acinetobacter baumannii, an increasingly important hospital-derived Gram-negative cocobacillus, causes a variety of diseases including pneumonia and septicemia, and affects people with compromised immune systems, especially those in the intensive care unit (ICU) (Maragakis and Perl 2008). Mortality rates resulting from *A. baumannii* septicemia were reported to be 34.0–43.4% in the ICU and 16.3% outside the ICU (Howard et al. 2012). Therefore, it has been defined as the critical-priority in a global priority list of antibiotic-resistant bacteria published in 2017 by the World Health Organization (WHO) (Tacconelli et al. 2017). Clinically *A. baumannii* strains showed severe resistance to all commonly prescribed antibacterial drugs, such as β-lactams, fluoroquinolones, and glycopeptides, with different resistance mechanisms including a variety of efflux pumps and lactamases (Manchanda et al. 2010). Due to the increasing prevalence of infections and few effective antibacterial drugs for infectious diseases, there is an extremely urgent need for new options and medicines to control the infections caused by *A. baumannii* (Cai et al. 2016).

Sulbactam, as a β-lactamase inhibitor to protect antibiotics, has been widely used to fight bacterial infections in combination with β-lactam antibiotics (Adnan et al. 2013). Actually, sulbactam alone is not effective to most of the bacterial pathogens (Noguchi and Gill 1988). However, sulbactam had been found to exert potency in patients infected with *A. baumannii* in clinical practice and thus has been successfully used to treat diseases caused by *A. baumannii* (Garnacho-Montero et al. 2003; Corbella et al. 1998). Two possible mechanisms of sulbactam against *A. baumannii* were (i) binding to penicillin-binding protein 3 (PBP3) (Penwell et al. 2015); and (ii) traversing through the CarO β-barrel protein channel to kill bacteria (Wu et al. 2016). However, the poor oral absorption of sulbactam hampered its wide clinical use (English et al. 1990).

Based on the clinical evidence, structural modification and optimization were carried out taking sulbactam as the lead, in an effort to obtain new potent antibacterial candidates against *A. baumannii* with better oral pharmacokinetic (PK) profiles. As shown in Fig. 1, the structure–activity relationship (SAR) study was mainly focused on the substituents at the 2-, 4- and 6-position, and a series of sulbactam derivatives were designed according to the fragment growth principle in computer-aided design, targeting the active pocket of PBP3. The synthesis, SAR analysis and antimicrobial evaluation against *A. baumannii*, as well as PK properties of the representative compound were described in the present study.

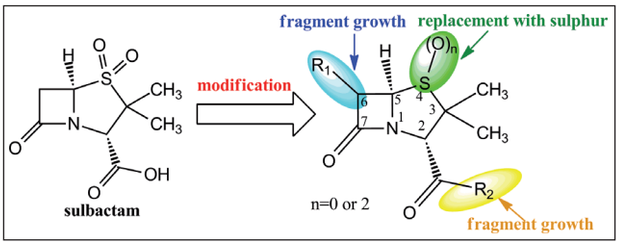
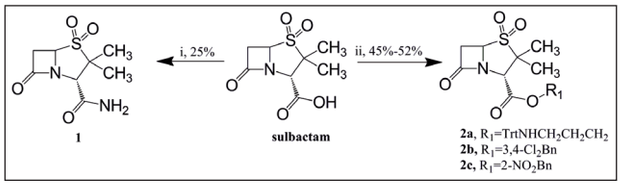


Fig. 1: Chemical structure of sulbactam, and our structure modification strategy

2. Investigations and results

2.1. Chemistry

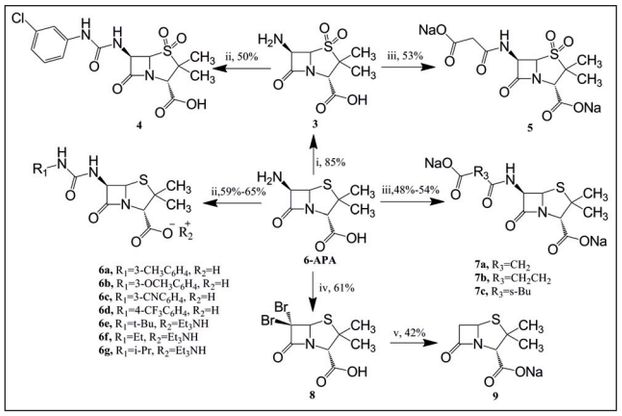
The synthetic routes to prepare all the target compounds from sulbactam or 6-aminopenicillanic acid (6-APA) were depicted in Schemes 1 and 2, respectively. As depicted in Scheme 1, the target product **1** was obtained by the amination of sulbactam with NH_4HCO_3 in the presence of Boc_2O and pyridine (Pozdnev 1995) in a 25% yield. The reaction of sulbactam with 3-bromo-*N*-trityl-propanamine or benzyl bromide in the presence of triethylamine in anhydrous DMF provided the products **2a–c** (Oh and Jung 2007) in 45–52% yields.



Scheme 1: Reagents and conditions: (i) Boc_2O, NH_4HCO_3, pyridine, acetonitrile, rt, 3 h; (ii) 3-bromo-*N*-tritylpropanamine or benzyl bromide, Et_3N, DMF, rt, 16 h.

As shown in Scheme 2, 6-APA was oxidized effectively to compound **3** by potassium permanganate ($KMnO_4$) in a sulfuric acid medium (Sandanayaka et al. 2003). Compound **3** was treated with an isocyanate in the presence of triethylamine in dichloromethane to gain the desired compound **4** (Demirci et al. 2014) in a 50% yield. Compound **5** was obtained by the treatment of compound **3** with anhydride (Yin et al. 2016) in 53% yield. Similarly, the reaction of 6-APA and a variety of isocyanates gained the 6-urea penicillanic acid derivatives **6a–g** with yields of 59–65%, and the reaction of 6-APA and Meldrum's acid gained the penicillanic acid derivatives **7a–c** (Recherche et Industrie Therapeutiques 1978) with yields of 48–54%. 6,6-Dibromopenicillanic acid (**8**) was prepared from 6-APA using the procedure of Padayatti et al. (2006) in 61% yield. The target compound **9** was obtained by the reduction of compound **8** with magnesium powder at 0 °C (Orchid Chemicals and Pharmaceuticals Ltd 2004) in 42% yield.

The structures of all the newly synthesized compounds were established by ^1H NMR, ^{13}C NMR spectra and ESI high-resolution mass spectra.



Scheme 2: Reagents and conditions: (i) $KMnO_4$, H_2SO_4, H_2O, acetonitrile, 0 °C, 1 h; (ii) isocyanate, Et_3N, DCM, rt, 3 h; (iii) anhydride or Meldrum's acid, BSA, ethyl acetate, rt, 12 h; (iv) Br_2, $NaNO_2$, H_2SO_4, DCM, 0 °C, 1 h; (v) Mg, HCl, ethyl acetate, sodium 2-ethylhexanoate, 0 °C, 4 h.

2.2. Molecule design

Fragment-based drug discovery (FBDD) is gathering momentum in the pharmaceutical industry because it is more likely to produce better optimized drug like compounds with lower molecular weight. 1087 molecules were generated through the fragment growth at the 2- and 6- position performed on the Discovery Studio 4.5 software. Based on the calculated results, three types of sulbactam derivatives with the higher ludi scores than that of sulbactam (ludi score = 212), such as 6-aminoalkanoic acid, 6-urea and O-benzyl as shown in Fig. 2A, were selected as the aimed skeleton structures for next investigation. The calculated binding modes of the three representative structures at the active site of the PBP3 (PDB code: 3UE3) domain, as shown in Fig. 2B, all these structures matched the domain very well. The hydrogen bond contributed mainly to the strong interaction between the aimed molecules and the domain.

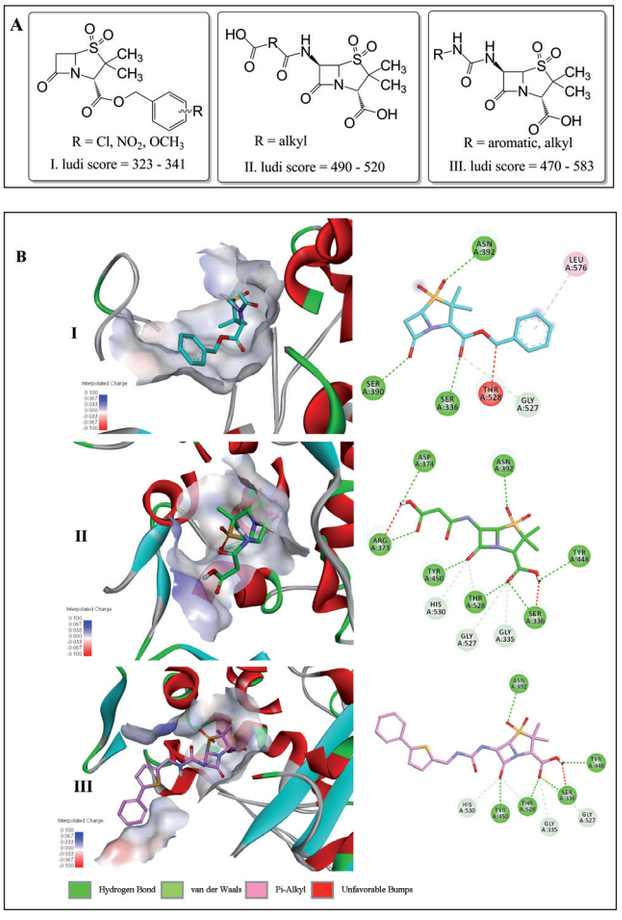
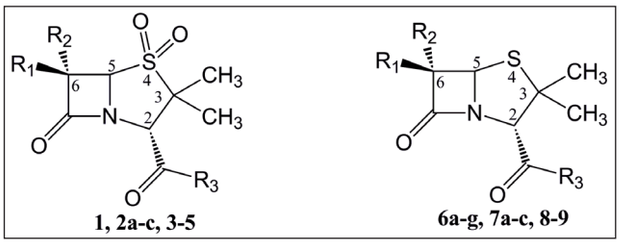


Fig. 2: (A) The skeleton structures of these small molecules; (B) Binding modes within the receptor PBP 3 active pocket. These Figures were produced using the Discovery Studio 4.5 software. The receptor structure was shown in surface form. The small molecules were presented as stick structure. Hydrogen bonds were indicated by green dashed lines between the atoms involved, while hydrophobic contacts were represented by an green circle. The unfavorable bumps were shown with red dashed lines.

2.3. Antimicrobial activity of target compounds and SAR analysis

The structures and activities of all the target compounds against multidrug-resistant *A. baumannii* ATCC 19606 strain that was purchased from ATCC are listed in Table 1. Minimum inhibitory concentrations (MICs) assay of the target compounds were determined by the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS) guidelines (CLSI 2009) with sulbactam as the reference.

Table 1: MIC values of target compounds against *A. baumannii* ATCC 19606 strain



Code	R_1	R_2	R_3	MIC[a]
1	H	H	NH_2	> 128
2a	H	H	$TrtNH(CH_2)_3O$	> 128
2b	H	H	$3,4\text{-}Cl_2BnO$	> 128

Code	R_1	R_2	R_3	MIC[a]
2c	H	H	2-NO_2BnO	32
3	NH_2	H	OH	8
4	*3*-$ClC_6H_4NHCONH$-	H	OH	> 128
5	NaO_2CCH_2CONH-	H	ONa	> 128
6a	*3*-$CH_3C_6H_4NHCONH$-	H	OH	> 128
6b	*3*-$OCH_3C_6H_4NHCONH$-	H	OH	> 128
6c	*3*-$CNC_6H_4NHCONH$-	H	OH	> 128
6d	*4*-$CF_3C_6H_4NHCONH$-	H	OH	> 128
6e	*t*-BuNHCONH-	H	O$^-Et_3NH^+$	> 128
6f	$CH_2CH_2NHCONH$-	H	O$^-Et_3NH^+$	> 128
6g	*i*-PrNHCONH-	H	O$^-Et_3NH^+$	> 128
7a	NaO_2CCH_2CONH-	H	ONa	> 128
7b	$NaO_2CCH_2CH_2CONH$-	H	ONa	> 128
7c	NaO_2C-*s*-Bu-CONH-	H	ONa	> 128
8	Br	Br	OH	> 128
9	H	H	ONa	4
Sul[b]	—	—	—	1

Abbreviations: [a] MIC, minimum inhibitory concentration (μg/mL); [b] Sul, Sulbactam.

SAR study was first focused on the substituent on position 2 in sulbactam. The 2-carboxyl group was replaced by ester or amide, by which compounds **1** and **2a–c** were generated and measured. As displayed in Table 1, all of them showed a complete loss of antimicrobial activity regardless the size of the substituent, suggesting that carboxyl group at the 2-position might be essential for the activity.
Then, 2-carboxyl was retained, SAR analysis was moved on the substituents at the 6-position. Different substituents were introduced on position 6, such as amino, aminoalkanoic acid and urea, and a series of 6-substitued sulbactam and penicillanic acid derivatives were generated. As shown in Table 1, compound **3** possessing the 6-amino group displayed a moderate antimicrobial activity with a MIC value of 8 μg/mL, weaker than that of sulbactam with 1 μg/mL. Compounds **4**, **5**, **6a–g**, **7a–c** and **8** with a large 6-substituent, such as aminoalkanoic acid or urea, caused a complete loss of antimicrobial activity with the MIC values of > 128 μg/mL, which hinted that the introduction of a bulk substituent at the 6-position was not beneficial for activity, and correspondingly, a small substituent at the 6-position might be more favorable. This result was basically consistent with the reported mechanism that small molecules could more easily traverse through CarO β-barrel protein channel to kill bacteria.
Based on these SAR results, penicillanic acid **9** with no substituent at the 6-position was generated, and its antibacterial activity against *A. baumannii* was evaluated in this study for the very first time. As expected, compound **9** afforded an inspiring activity against *A. baumannii* with a MIC value of 4 μg/mL, only slightly weaker than that of sulbactam. Therefore, compound **9** was selected as the representative compound for the following investigations. Compared with sulbactam, compound **9** might reduce its polarity with sulphur atom instead of sulphone at the 4-position, and result in an improvement in oral absorption.

2.4. In vivo pharmacokinetic profile of compound 9 and sulbactam

PK studies of the compound **9** and sulbactam were then performed on a Sprague-Dawley (SD) rat model at the dosage of 25 mg/kg *via* oral route. The preliminary PK curve was shown in Fig. 3, and PK parameters were displayed in Table 2. Compared with sulbactam, compound **9** had a higher area under the curve (AUC) value of 10.8 μg·h/mL and a longer half-life ($T_{1/2}$) of 13.10 h. Especially, the maximum plasma concentration (C_{max}) of compound **9** (19.6 μg/mL) was approximately 50 times higher than that of sulbactam (0.37 μg/mL), and well above its MIC value of 4 μg/mL, while the C_{max} of sulbactam was below its MIC value of 1 μg/mL. These data collectively provided solid evidence on the higher oral absorption of compound **9**, therefore, it holds the potential of being developed into an oral medication.

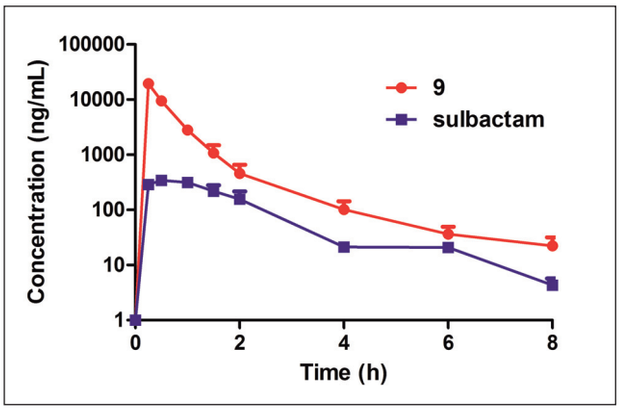


Fig. 3: Time-concentration curves of compound **9** and sulbactam at 25 mg/kg p.o.

Table 2: Main PK parameters of 9 and sulbactam at po 25 mg/kg, SD rat

	9	Sulbactam
C_{max} (μg/mL)	19.6	1.60
T_{max} (h)	0.25	0.67
$T_{1/2}$ (h)	13.1	3.24
AUC_{0-last} (μg·h/mL)	10.8	0.73
AUC_{0-inf} (μg·h/mL)	11.2	0.74
MRT_{0-inf} (h)	2.59	2.74

3. Discussion

Sulbactam, as a β-lactamase inhibitor, was found to own potent antimicrobial activity against *Acinetobacter baumannii* in clinical practice. Based on clinical evidence, taking sulbactam as the lead, a series of sulbactam derivatives defined to 2- and 6-substituents were designed, synthesized and evaluated for activity against *A. baumannii*. The SAR analysis revealed that (i) small volume groups at the 6-postion might be helpful for the potency, and (ii) 2-carboxyl is an important element to keep the compound potent. Among them, compound **9** showed moderate activity against *A. baumannii* with MIC of 4 μg/mL, a little bit weaker than that of sulbactam. Importantly, compound **9** displayed an excellent *in vivo* PK properties with C_{max} of 19.6 μg/mL and AUC of 10.8 μg·h/mL, much higher than those of sulbactam, suggesting a potential for an oral medication. The results provided the useful information for structural optimization, and compound **9** has been chosen for further investigation. This study provides a good example of "Bed-side to Bench" process, and the use of sulbatam derivatives as a novel family of antibaterial agents against *A. baumannii* might be greatly expanded.

4. Experimental

4.1. Chemistry

4.1.1. Material and methods

All reagents and anhydrous solvents were obtained from commercial sources and used without further purification. Melting points (mp) were obtained with CXM-300 melting point apparatus. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance (600 MHz, 500 MHz and 400 MHz for ^1H NMR; 151 MHz, 126 MHz and 101 MHz for ^{13}C NMR). *J* values are given in Hz. The ^1H chemical shifts were referenced to the solvent peak: DMSO-d_6 (2.49 ppm), and the ^{13}C chemical shifts were referenced to the solvent peak: DMSO-d_6 (40.5 ppm). ESI high-resolution mass spectra (HRMS) were recorded on an AutospecUltima-TOF spectrometer. Flash column chromatography was performed on Combiflash Rf 200, particle size 0.038 mm.
Compounds **3** (Sandanayaka et al. 2003) and **8** (Padayatti et al. 2006) were prepared according to the procedures reported.

4.1.2. Synthesis of compound **1**

To a stirred solution of sulbactam (2.33 g, 10 mmol), pyridine (0.5 ml) and Boc_2O (4.36 g, 20 mmol) in acetonitrile (10 mL), NH_4HCO_3 (2.37 g, 30 mmol) was added and the mixture was stirred for 3 h. After the reaction was complete, excess NH_4HCO_3 was removed by filtration and the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel with 20 : 1 dichloromethane/methanol as eluent to give compound **1**.

3,3-Dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamide 4,4-dioxide (**1**). White solid (25% yield), m.p. 210.5–211.1 °C. ^{1}H NMR (600 MHz): δ 7.72 (s, 1H, $CONH_2$), 7.64 (s, 1H, $CONH_2$), 5.00 (s, 1H, 5-H), 4.00 (s, 1H, 2-H), 3.62–3.52 (m, 1H, 6-H), 3.24 (d, *J* =2.0 Hz, 1H, 6-H), 1.45 (s, 3H, 3-CH_3), 1.34 (s, 3H, 3-CH_3); ^{13}C NMR (151 MHz): δ 172.7, 168.8, 63.4, 62.9, 60.9, 37.8, 19.9, 18.8. ESI-HRMS m/z: calcd for $C_8H_{12}N_2O_4S$ [M–H]$^-$: 231.0445, found: 231.0448.

4.1.3. Synthesis of compounds **2a–c**

Sulbactam (10 mmol) was added to a solution of triethylamine (2.6 mL) in anhydrous DMF (10 mL), and the resulting mixture was stirred for 1 h at room temperature. After the solid was dissolved, 3-bromo-*N*-tritylpropanamine or benzyl bromide (12 mmol) in anhydrous DMF (10 mL) was added dropwise slowly and then the mixture was stirred for 16 h at room temperature. After the reaction was completed, the mixture was extracted with ethyl acetate (30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by flash column chromatography on silica gel with 3 : 1 petroleum ether/ethyl acetate as eluent to give the target compounds **2a–c**.

2-(Tritylamino)ethyl 3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate 4,4-dioxide (**2a**). Light yellow solid (52% yield), m.p. 56.8–57.3 °C. ^{1}H NMR (600 MHz): δ 7.96 (s, 1H, NH), 7.47–7.35 (m, 6H, arom), 7.28 (t, *J* = 7.6 Hz, 6H, arom), 7.18 (t, *J* = 7.3 Hz, 3H, arom), 5.10 (dd, *J* = 4.6, 1.7 Hz, 1H, 5-H), 4.29–4.20 (m, 2H, $COOCH_2$), 3.64 (dd, *J* = 16.5, 4.6 Hz, 1H, 2-H), 3.24 (dd, *J* = 16.5, 1.7 Hz, 1H, 6-H), 2.83 (m, 1H, 6-H), 2.16–2.00 (m, 2H, CH_2CH_2NH), 1.85 (m, 2H, CH_2CH_2NH), 1.31 (s, 3H, 3-CH_3), 1.23 (s, 3H, 3-CH_3); ^{13}C NMR (151 MHz): δ 172.5, 167.5, 146.3 (3), 128.9 (6), 128.2 (6), 126.6 (3), 70.7, 66.3, 62.7, 61.0, 42.7, 37.9, 20.1, 18.3 (3). ESI-HRMS m/z: calcd for $C_{30}H_{32}N_2O_3S$ [M + H]$^+$: 533.2104, found: 533.2107.

3,4-Dichlorobenzyl 3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate 4,4- dioxide (**2b**). Light yellow solid (45% yield), m.p. 64.2–64.7 °C. ^{1}H NMR (500 MHz): δ 7.58 (d, *J* = 8.0 Hz, 2H, arom), 7.52-7.45 (m, 1H, arom), 5.45 (d, *J* = 2.7 Hz, 2H, $COOCH_2$), 5.20 (dd, *J* = 4.7, 1.7 Hz, 1H, 5-H), 4.51 (s, 1H, 2-H), 3.69 (dd, *J* = 16.5, 4.6 Hz, 1H, 6-H), 3.26 (dd, *J* = 16.5, 1.7 Hz, 1H, 6-H), 1.42 (s, 3H, 3-CH_3), 1.35 (s, 3H, 3-CH_3); ^{13}C NMR (151 MHz): δ 172.5, 167.0, 136.5, 132.6, 130.4, 129.3 (3), 62.9, 62.7, 62.6, 61.0, 37.8, 20.0, 18.0. ESI-HRMS m/z: calcd for $C_{15}H_{15}Cl_2NO_3S$ [M + H]$^+$: 389.9980, found: 389.9949.

2-Nitrobenzyl 3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanes-2-carboxylate 4,4-dioxide (**2c**). Light yellow solid (46% yield), m.p. 72.1–72.9 °C. ^{1}H NMR (500 MHz): δ 8.16–8.14 (m, 1H, arom), 7.90–7.76 (m, 2H, arom), 7.67 (m, 1H, arom), 5.57 (s, 2H, $COOCH_2$), 5.21 (dd, *J* = 4.6, 1.7 Hz, 1H, 5-H), 4.62 (s, 1H, 2-H), 3.70 (dd, *J* = 16.5, 4.6 Hz, 1H, 6-H), 3.28 (dd, *J* = 16.5, 1.7 Hz, 1H, 6-H), 1.47 (s, 3H, 3-CH_3), 1.36 (s, 3H, 3-CH_3); ^{13}C NMR (151 MHz): δ 172.5, 167.0, 162.7, 148.1, 134.7, 130.8, 130.3, 125.4, 64.7, 62.8, 62.7, 61.0, 37.8, 20.0, 18.0. ESI-HRMS m/z: calcd for $C_{15}H_{16}N_2O_7S$ [M + H]$^+$: 367.0599, found: 367.0594.

4.1.4. Synthesis of compound **4**

Compound **3** (Sandanayaka et al. 2003) (10 mmol) was added to a solution of triethylamine (2.6 mL) in dichloromethane (20 mL), and the resulting mixture was stirred for 1 h at room temperature. After the solid was dissolved, isocyanate (12 mmol) was added dropwise slowly and then the mixture was stirred for 3 h at room temperature. After the reaction was complete, the mixture was adjusted with 2 N HCl to reach the pH 1.5–2.0 and washed with brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by flash column chromatography on silica gel with 20 : 1 dichloromethane/methanol as eluent to give the target compound **4**.

6-(3-(3-Chlorophenyl)ureido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid 4,4- dioxide (**4**). White solid (50% yield), m.p. 205.1–205.9 °C. ^{1}H NMR (600 MHz): δ 13.82 (s, 1H, COOH), 9.50 (s, 1H, NH), 7.67–7.66 (m, 1H, NH), 7.31–7.28 (m, 1H, arom), 7.20–7.18 (m, 1H, arom), 7.08–6.97 (m, 2H, arom), 5.99 (dd, *J* = 10.3, 4.6 Hz, 1H, 6-H), 5.46 (d, *J* = 4.6 Hz, 1H, 5-H), 4.42 (s, 1H, 2-H), 1.52 (s, 3H, 3-CH_3), 1.40 (s, 3H, 3-CH_3); ^{13}C NMR (151 MHz): δ 176.3, 168.5, 153.8, 141.4, 133.7, 130.9, 122.2, 117.8, 116.8, 66.3, 64.6, 63.7, 57.2, 19.9, 17.6. ESI-HRMS m/z: calcd for $C_{15}H_{16}ClN_3O_6S$ [M–H]$^-$: 400.0375, found: 400.0370.

4.1.5. Synthesis of compound **5**

Compound **3** (10 mmol) was added to a solution of *N*, *O*-bis(trimethylsilyl)acetamide (20 mmol) in ethyl acetate (20 mL), and the resulting mixture was stirred overnight at room temperature. After the solid was dissolved, the anhydride or Meldrum's acid (11 mmol) was added and the mixture was stirred for an additional 3 h. After the reaction was completed, the mixture was adjusted with $NaHCO_3$ to reach the pH 7.0–8.0 and extracted with water (20 mL). The combined aqueous layers were adjusted with 2 N HCl to reach the pH 1.5–2.0 and extracted with ethyl acetate (20 mL). The organic layer was washed with brine (30 mL), and added sodium 2-ethylhexanoate (22 mmol) in ethyl acetate (10 mL). The precipitate formed was collected by filtration. The product was purified by recrystallization in ethanol to give the target compound **5**.

Sodium 6-(2-carboxylatoacetamido)-3,3-dimethyl-7-oxo-4-thia-1- azabicyclo[3.2.0] heptane-2-carboxylate 4,4-dioxide (**5**). White solid (54% yield), m.p. 213.1–213.6 °C. ^{1}H NMR (500 MHz): δ 10.17 (s, 1H, NH), 5.41 (s, 1H, 6-H), 5.36 (d, *J* = 4.1 Hz, 1H, 5-H), 3.86 (s, 1H, 2-H), 3.46–3.42 (m, 1H, $OCCH_2CO$), 2.81–2.74 (m, 1H, $OCCH_2CO$), 1.55 (s, 3H, 3-CH_3), 1.46 (s, 3H, 3- CH_3); ^{13}C NMR (151 MHz): δ 174.7, 173.4, 173.2, 170.8, 74.2, 67.2, 64.5, 57.7, 47.8, 31.9, 27.8. ESI-HRMS m/z: calcd for $C_{11}H_{12}N_2Na_2O_8S$ [M–2Na]$^-$: 333.0387, found: 333.0383.

4.1.6. Synthesis of compounds **6a–d**

Compounds **6a–d** were synthesized from 6-APA as described for the preparation of compound **4**.

3,3-Dimethyl-7-oxo-6-(3-(m-tolyl)ureido)-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (**6a**). White solid (59% yield), m.p. 213.7–214.3 °C. ^{1}H NMR (500 MHz): δ 13.39 (s, 1H, COOH), 8.76 (s, 1H, NH), 7.21 (s, 1H, NH), 7.17–7.08 (m, 2H, arom), 6.84–6.76 (m, 2H, arom), 5.59–5.54 (m, 1H, 6-H), 4.29 (s, 1H, 5-H), 3.66 (s, 1H, 2-H), 2.25 (s, 3H, Ar-CH_3), 1.62 (s, 3H, 3-CH_3), 1.51 (s, 3H, 3-CH_3). ^{13}C NMR (151 MHz): δ 172.0, 170.9, 156.5, 138.7, 132.5, 129.1, 128.9, 127.5, 124.1, 73.4, 66.7, 63.2, 59.9, 27.3, 26.7, 21.3. ESI-HRMS m/z: calcd for $C_{16}H_{19}N_3O_4S$ [M–H]$^-$: 348.1023, found: 348.1021.

6-(3-(3-Methoxyphenyl)ureido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (**6b**). White solid (62% yield), m.p. 206.2–206.8 °C. ^{1}H NMR (600 MHz): δ 11.62 (s, 1H, COOH), 9.11 (s, 1H, NH), 7.15 (s, 1H, NH), 7.13 (d, *J* = 8.2 Hz, 1H, arom), 6.94 (d, *J* = 8.9 Hz, 1H, arom), 6.87 (dd, *J* = 8.0, 2.0 Hz, 1H, arom), 6.51 (dd, *J* = 8.2, 2.5 Hz, 1H, arom), 5.48–5.45 (m, 2H, 5-H and 6-H), 4.02 (s, 1H, 2-H), 3.71 (s, 3H, Ar-OCH_3), 1.59 (s, 3H, 3-CH_3), 1.51 (s, 3H, 3-CH_3); ^{13}C NMR (151 MHz): δ 175.4, 170.7, 160.1, 154.1, 141.5, 130.0, 110.5, 107.5, 104.0, 73.2, 68.2, 64.7, 59.0, 55.3, 32.5, 27.6. ESI-HRMS m/z: calcd for $C_{16}H_{19}N_3O_5S$ [M–H]$^-$: 364.0972, found: 364.0976.

6-(3-(3-Cyanophenyl)ureido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (**6c**). White solid (61% yield), m.p. 213.5–213.8 °C. ^{1}H NMR (600 MHz): δ 13.03 (s, 1H, COOH), 8.80 (s, 1H, NH), 7.98–7.91 (m, 1H, NH), 7.83–7.61 (m, 4H, arom), 5.10–4.96 (m, 1H, 6-H), 4.34 (s, 1H, 5-H), 3.77 (s, 1H, 2-H), 1.58 (s, 3H, 3-CH_3), 1.22 (s, 3H, 3-CH_3); ^{13}C NMR (151 MHz): δ 171.6, 170.8, 155.7, 133.4, 131.8, 131.5, 130.8, 129.9, 118.4, 112.2, 73.3, 66.5, 63.4, 59.9, 27.3, 26.7. ESI-HRMS m/z: calcd for $C_{16}H_{16}N_4O_4S$ [M–H]$^-$: 359.0819, found: 359.0825.

3,3-Dimethyl-7-oxo-6-(3-(4-(trifluoromethyl)phenyl)ureido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**6d**). White solid (61% yield), m.p. 213.1–213.6 °C. ^{1}H NMR (600 MHz): δ 13.00 (s, 1H, COOH), 8.79 (s, 1H, NH), 7.87 (d, *J* = 8.3 Hz, 2H, arom), 7.61 (d, *J* = 8.3 Hz, 2H, arom), 5.10–5.02 (m, 1H, 6-H), 4.39–4.32 (m, 1H, NH), 4.23 (s, 1H, 5-H), 3.79 (s, 1H, 2-H), 1.59 (s, 3H, 3-CH_3), 1.21 (s, 3H, 3-CH_3); ^{13}C NMR (151 MHz): δ 171.6, 170.8, 155.8, 136.2, 128.3, 127.1 (2), 126.4, 125.3, 123.5, 73.4, 66.6, 63.3, 60.0, 27.3, 26.6. ESI-HRMS m/z: calcd for $C_{16}H_{16}F_3N_3O_4S$ [M–H]$^-$: 402.0740, found: 402.0739.

4.1.7. Synthesis of compounds **6e–g**

6-APA (10 mmol) was added to a solution of triethylamine (2.6 mL) in dichloromethane (20 mL), and the resulting mixture was stirred for 1 h at room temperature. After the solid was dissolved, isocyanate (12 mmol) was added dropwise slowly and then the mixture was stirred for 3 h at room temperature. After the reaction was completed, the precipitate was collected by filtration and washed with dichloromethane (50 mL) to give the target compounds **6e–g**.

Triethylammonium 6-(3-(tert-butyl)ureido)-3,3-dimethyl-7-oxo-4- thia-1-azabicyclo [3.2.0]heptane-2-carboxylate (**6e**). White solid (65% yield), m.p. 205.4–205.9 °C. ^{1}H NMR (600 MHz): δ 6.31 (s, 1H, NH), 6.25 (s, 1H, NH), 5.40–5.37 (m, 2H, 5-H and 6-H), 3.96 (s, 1H, 2-H), 2.90 (q, *J* = 7.3 Hz, 6H, $(CH_2CH_3)_3N$), 1.55 (s, 3H, 3-CH_3), 1.48 (s, 3H, 3-CH_3), 1.22 (s, 9H, $(CH_3)_3C$), 1.12 (t, *J* = 7.3 Hz, 9H, $(CH_2CH_3)_3N$); ^{13}C NMR (151 MHz): δ 176.6, 170.8, 155.8, 73.2, 68.5, 64.6, 58.9, 49.7, 45.4 (3), 32.2, 29.5 (3), 27.7, 9.4 (3). ESI-HRMS m/z: calcd for $C_{19}H_{36}N_4O_4S$ [M–Et_3NH]$^-$: 314.1180, found: 314.1179.

Triethylammonium 6-(3-ethylureido)-3,3-dimethyl-7-oxo-4-thia-1- azabicyclo[3.2.0] heptane-2-carboxylate (**6f**). White solid (64% yield), m.p. 200.6–201.3 °C. ^{1}H NMR (500 MHz): δ 6.51–6.42 (m, 1H, NH), 6.35–6.33 (m, 1H, NH), 5.43–5.31 (m, 2H, 5-H and 6-H), 3.95 (s, 1H, 2-H), 3.06–2.97 (m, 2H, CH_2CH_3), 2.91 (q, *J* = 7.3 Hz, 6H, $(CH_2CH_3)_3N$), 1.56 (s, 3H, 3-CH_3), 1.48 (s, 3H, 3-CH_3), 1.12 (t, *J* = 7.3 Hz, 9H, $(CH_2CH_3)_3N$), 0.99 (t, *J* = 7.2 Hz, 3H, CH_2CH_3); ^{13}C NMR (151 MHz): δ 176.2, 171.1, 156.7, 73.4, 68.4, 64.5, 59.1, 45.3 (3), 34.5, 32.1, 27.7, 15.9, 9.2 (3). ESI-HRMS m/z: calcd for $C_{17}H_{32}N_4O_4S$ [M–Et_3NH]$^-$: 286.0867, found: 286.0868.

Triethylammonium 6-(3-Isopropylureido)-3,3-dimethyl-7-oxo-4-thia- 1-azabicyclo [3.2.0]heptane-2-carboxy late (**6g**). White solid (65% yield), m.p. 208.2–208.9 °C. ^{1}H NMR (600 MHz) δ 6.34 (d, *J* = 9.9 Hz, 1H, NH), 6.27 (d, *J* = 7.4 Hz, 1H, NH), 5.39–5.38 (m, 2H, 5-H and 6-H), 3.96 (s, 1H, 2-H), 3.66–3.63 (m, 1H, CH), 2.91 (q, *J* = 7.3 Hz, 6H, $(CH_2CH_3)_3N$), 1.55 (s, 3H, 3-CH_3), 1.48 (s, 3H, 3-CH_3), 1.12 (t, *J* = 7.3 Hz, 9H, $(CH_2CH_3)_3N$), 1.03 (m, 6H, $(CH_3)_2CH$); ^{13}C NMR (151 MHz): δ 176.3, 170.8, 156.0, 73.3, 68.5, 64.6, 59.1, 45.3 (3), 41.5, 32.3, 27.7, 23.5 (2), 9.4 (3). ESI-HRMS m/z: calcd for $C_{18}H_{34}N_4O_4S$ [M–Et_3NH]$^-$: 300.1023, found: 300.1024.

4.1.8. Synthesis of compounds **7a–c**

Compounds **7a–c** were synthesized from 6- APA as described for the preparation of compound **5**.

Sodium 6-(2-carboxylatoacetamido)-3,3-dimethyl-7-oxo-4-thia-1- azabicyclo[3.2.0] heptane-2-carboxylate (**7a**). White solid (56% yield), m.p. 136.3–137.1 °C. ^{1}H NMR (500 MHz): δ 10.33 (s, 1H, NH), 5.47 (dd, *J* = 8.8, 4.1 Hz, 1H, 6-H), 5.38 (d, *J* = 4.1 Hz, 1H, 5-H), 3.91 (s, 1H, 2-H), 2.87 (s, 2H, $OCCH_2CO$), 1.55 (s, 3H, 3-CH_3), 1.46 (s, 3H, 3-CH_3); ^{13}C NMR (126 MHz): δ 175.1, 171.5, 170.8, 169.5, 74.1, 67.4, 64.8, 57.9, 44.0, 32.0, 27.9. ESI-HRMS m/z: calcd for $C_{11}H_{12}N_2Na_2O_6S$ [M–2Na]$^-$: 301.0499, found: 301.0501.

Sodium 6-(3-carboxylatopropanamido)-3,3-dimethyl-7-oxo-4-thia- 1-azabicyclo [3.2.0]heptane-2-carboxylate (**7b**). White solid (53% yield), m.p. 213.5–213.8 °C. ^{1}H NMR (500 MHz): δ 9.29 (s, 1H, NH), 5.32–5.28 (m, 2H, 5-H and 6-H), 3.83 (s, 1H,

2-H), 2.30–2.27 (m, 2H, OCC$\underline{H}_2$CH$_2$CONH), 2.12–2.10 (m, 2H, OCCH$_2$C$\underline{H}_2$CONH), 1.55 (s, 3H, 3-CH$_3$), 1.44 (s, 3H, 3-CH$_3$); ^{13}C NMR (126 MHz) δ 176.6, 174.3, 174.0, 170.5, 74.6, 67.6, 64.7, 58.3, 34.1, 33.2, 32.1, 28.1. ESI-HRMS m/z: calcd for $C_{12}H_{14}N_2Na_2O_6S$ [M–2Na]$^-$: 315.0656, found: 315.0657.

Sodium 6-(3-carboxylato-2-methylbutanamido)-3,3-dimethyl-7-oxo- 4-thia-1-azabicyclo[3.2.0]heptane-2-car boxylate (**7c**). White solid (50% yield), m.p. 211.1–211.6 °C. ^{1}H NMR (400 MHz): δ 8.97 (s, 1H, NH), 5.39–5.29 (m, 2H, 5-H and 6-H), 3.88 (s, 1H, 2-H), 2.44–2.35 (m, 1H, OC(CH$_3$C$\underline{H}$CHCH$_3$)CONH), 2.26–2.16 (m, 1H, OC(CH$_3$CHC$\underline{H}$CH$_3$)CONH), 1.56 (s, 3H, 3-CH$_3$), 1.46 (s, 3H, 3-CH$_3$), 1.08–1.03 (m, 6H, CH$_3$CHCHCH$_3$); ^{13}C NMR (151 MHz): δ 175.5, 171.6, 170.8, 169.4, 73.4, 70.7, 68.3, 64.5, 63.3, 59.4, 31.7, 27.3, 271, 26.6. ESI-HRMS m/z: calcd for $C_{14}H_{18}N_2Na_2O_6S$ [M–2Na]$^-$: 343.0969, found: 343.0968.

*4.1.9. Synthesis of compound **9***

Magnesium powder (40 mmol) was added to a solution of compound **8** (Padayatti et al. 2006) (10 mmol) in ethyl acetate (20 mL) and water (10 mL), and the resulting mixture was stirred for 4 h at 0 °C while maintaining the pH at 4.0–4.5 with 2 N HCl. After the reaction was completed, the mixture was filtered. The pH of filtrate was adjusted to 1.5–2.0 with 2 N HCl and extracted with ethyl acetate (20 mL) for three times. The combined ethyl acetate layers were dried over Na_2SO_4 and evaporated. The oily residue was purified by flash column chromatography on silica gel with 5:1 dichloromethane/methanol as the eluent and then salified by sodium 2-ethylhexanoate (22 mmol) in ethyl acetate (10 mL). The desired compound **9** was collected by filtration.

3,3-Dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**9**). White solid (42% yield), m.p. 195.8–196.2 °C. ^{1}H NMR (400 MHz): δ 5.13 (s, 1H, 5-H), 3.87 (s, 1H, 2-H), 3.45–3.40 (m, 1H, 6-H), 2.81 (d, J = 15.4 Hz, 1H, 6-H), 1.54 (s, 3H, 3-CH$_3$), 1.44 (s, 3H, 3-CH$_3$); ^{13}C NMR (101 MHz): δ 172.3, 170.2, 74.4, 66.2, 59.9, 45.3, 32.7, 27.8. ESI-HRMS m/z: calcd for $C_8H_{10}NNaO_3S$ [M–Na]$^-$: 200.0375, found: 200.0381.

4.2. Molecule design

The crystal structure of PBP3 protein was recovered from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) (PDB code: 3UE3). Fragment growth was performed to design PBP 3 inhibitors using the Discovery Studio 4.5 software. We used sulbactam as ligand scaffold as well as 2- and 6-positions as link points. The fragments were taken from interior fragments library installed on the Discovery Studio 4.5 software. Ludi score function was used to evaluate the affinity for PBP3 (Bohm HJ 1992).

4.3. Antibacterial assays

The studied compounds were evaluated for their antibacterial activities against *A. baumannii* ATCC 19606 strain by using the agar dilution assay at various concentrations of 128.0, 64.0, 32.0, 16.0, 8.0, 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, 0.06 and 0.03 mg/mL described by the Clinical Laboratory Standards Institute (CLSI 2009), with sulbactam as the positive control. The test medium was Mueller-Hinton agar, and the inoculum was 104 colony forming units (cfu)/spot. Culture plates were incubated at 35 °C for 18 h, and MICs were defined as the lowest concentrations that prevented visible growth of the bacteria.

4.4. PK assay in SD rats

Single dose PK studies on compound **9** and sulbactam were performed using male SD rats in full compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council (US) 2011). After oral administration (25 mg/kg), blood samples were collected at specified intervals (0.25, 0.5, 1, 1.5, 2, 4, 6 and 8 h) with heparin sodium as anticoagulate. Centrifugation of blood samples gave the plasma fraction. PK parameters were calculated with noncompartment model using WinNonlin 5.2 based on the LC–MS/MS quantitation data.

Acknowledgments: This work was supported by the National Natural Science Foundation of China (81361138020 and 81621064) and the CAMS initiative for innovative medicine (2017-12M-1-012) and the CAMS Graduate Innovation Fund (2017-1007-19).

Conflicts of interest: The authors declare no conflict of interest.

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