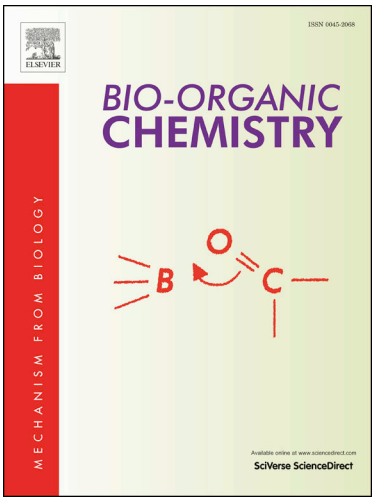
Journal Pre-proofs




# Synthesis and antibacterial evaluation against resistant Gram-negative bacteria of monobactams bearing various substituents on oxime residue

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# Synthesis and antibacterial evaluation against resistant Gram-negative bacteria of monobactams bearing various substituents on oxime residue

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**Abstract**

Based on the structural characteristics of aztreonam (AZN) and its target PBP3, a series of new monobactam derivatives bearing various substituents on oxime residue were prepared and evaluated for their antibacterial activities against susceptible and resistant Gram-negative bacteria. Among them, compounds **8p** and **8r** displayed moderate potency with MIC values of 0.125−32 μg/mL against most tested Gram-negative strains, comparable to AZN. Meanwhile, the combination of **8p** and **8r** with avibactam as a β-lactamases inhibitor, in a ratio of 1:16, showed a promising synergistic effect against both ESBLs- and NDM-1-producing *K. pneumoniae*, with significantly reduced MIC values up to 8-fold and >256-fold respectively. Furthermore, both of them demonstrated excellent safety profiles both *in vitro* and *in vivo*. The results provided powerful information for further structure optimization of monobactam antibiotics to fight β-lactamase-producing resistant Gram-negative bacteria.

**Keywords:**

monobactam; structure–activity relationship; antibacterial; Gram-negative bacteria; synergistic effect.

## 1. Introduction

Multidrug-resistant Gram-negative bacteria, especially *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumoniae*), *Escherichia coli* (*E. coli*) and *Acinetobacter baumannii* (*A. baumannii*), listed by the WHO as critical priority concerns [1], have become one of the most complex and gravest global threats [2–4]. One prevailing drug-resistant mechanism is the presence of β-lactamases including serine hydrolases (SBLs), extended spectrum β-lactamases (ESBLs) and New Delhi metallo-β-lactamase-1 (NDM-1), which could hydrolyze most clinically used β-lactam antibiotic and made more bacteria become resistant to even some of the strongest antibiotics. Despite of the limited therapeutic options for treating infections caused by Gram-negative bacteria, pharmaceutical research and development failed to meet the clinical needs, due to the "auto-obsolescence" of antibacterials [5–7]. Currently, discovering new antibiotics from medicinal chemistry efforts to improve the profiles of existing classes is still recognized as one of the most effective strategies, and the resulted derivatives can enhance potency and counter bacterial resistance mechanisms [8].

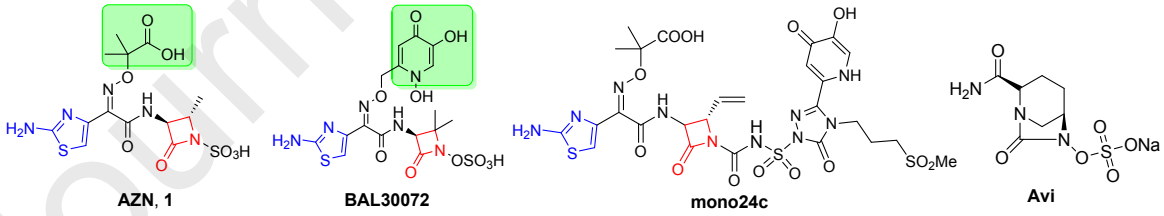



**Figure 1.** Structures of AZN, BAL30072, Mono24c and Avi

Aztreonam (AZN, **1**, Fig. 1), the only monobactam antibiotic in clinic [9,10], has been successfully used for the treatment of Gram-negative bacteria infections for several decades [11–15]. The combination of AZN and avibactam sodium (Avi, Fig.1) as a β-lactamase inhibitor has just been completed the phase II clinical trial for

treatment of infections caused by resistant Gram-negative bacteria [16]. Owing to the advantages of simple structure and strong efficacy against Gram-negative bacteria, AZN becomes an ideal parent for conducting structure optimization, and then several monobactam candidates, such as BAL30072 [17] and Mono 24c [18] (Fig. 1), are active in different stages of clinical or preclinical investigation.

As monobactams act upon bacteria by targeting penicillin-binding proteins (PBPs) located in periplasm [11,12], we first conducted the structural characteristics analyses between this kind of analogues and target protein PBP3. The results revealed there is a large space between oxime-linked group in monobactams and active site in PBP3[19], which is tolerable and can bear various substituents with different polarities or size [20a–c]. For AZN, as displayed in Fig. 2, salt bridge between carboxyl and amino acid residues Arg489 was formed and gem-dimethyl group interacted favorably with the hydrophobic wall (Tyr503-Tyr532-Phe533). On the other hand, 4-dihydroxypyridone moiety on the oxime side chain of BAL30072 located near the hydrophobic wall and a favorable interaction was well formed. The results suggested various substituents could be introduced on the oxime residue so as to improve the effect against Gram-negative bacteria.

Based on the structural characteristics mentioned above, in the present study, structural modification strategy was mainly focused on the nitrogen-based groups on oxime side-chain as depicted in Fig. 2. Various substituents, such as hydrophobic unsaturated aliphatic chains, phenyl groups and functional amino, carboxyl and siderophores catecholates fragments, were introduced respectively, by which several series of new monobactams were created. Herein, thirty-four new monobactam derivatives were synthesized and evaluated for their antibacterial activities using phenotypic screening assay. Additionally, the synergistic effects with β-lactamase

inhibitor, safety profiles of the representative compounds were also expounded.

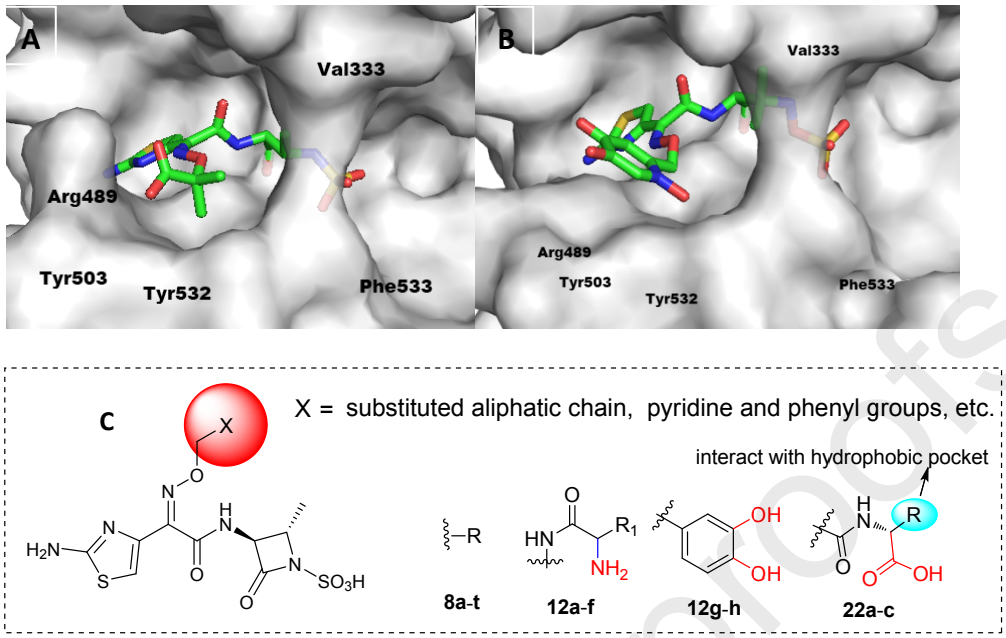
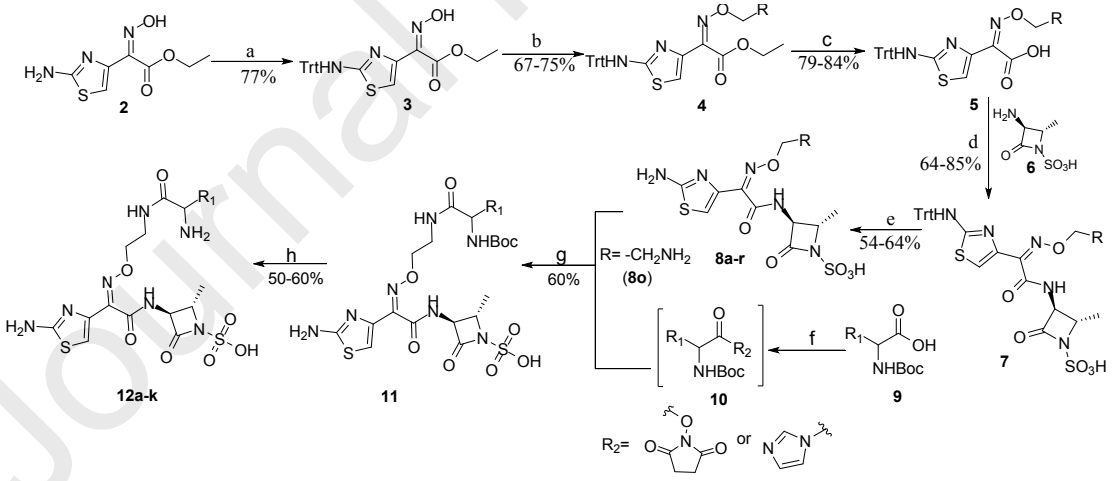



**Figure 2.** Crystallographic reported in literature of the complex of monobactam derivatives with PBP3 and structural modification strategy proposed. (A) Active site in **AZN**-acyl-PaePBP3 complex. **AZN** is shown in stick rendering with green carbons. (B) Active site of PaePBP3 bound to **BAL30072** colored in green. (C) Structural modification strategy proposed.

## 2. Results and discussion

### *2.1 Chemistry*

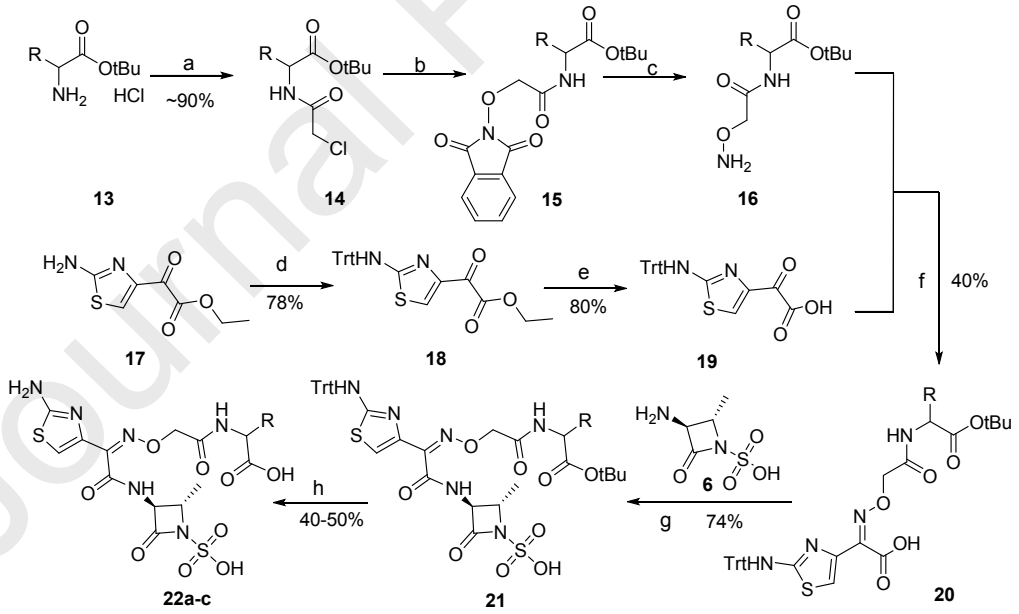



**Scheme 1** (a) $Ph_3CCl$, TEA, DMF, r.t.; (b) $ArCH_2Br$, $K_2CO_3$, NaI, DMF, r.t.; (c) 2 N NaOH (aq.); then 1N HCl, pH = 1−2; (d) DIC, HOBt, DMAP, DMF, r.t.; (e) TFA, $SiEt_3H$, DCM, −10 ℃; (f) EDCI, NHS, anhydrous DMF, r.t. or CDI, anhydrous DMF, r.t.; (g) Anhydrous DMF, r.t.

The synthesis route of target analogues **8a**−**t** was shown in Scheme 1, taking commercially available (*Z*)-ethyl 2-(2-aminothiazol-4-yl)-2-(hydroxyimino)acetate **2** as the starting material. Firstly, the bare amino group in **2** was protected by

tri-phenylmethyl (Trt) to achieve intermediate **3** with the yield of 77%. Then, the key intermediate **5** was generated by a two-step sequence including an etherification with corresponding halides and an ester hydrolysis in ideal yields. Then condensation of **5** and (2*S*,3*S*)-3-amino-2-methyl-4-oxoazetidine-1-sulfonic acid **6** generated intermediate **7**, which underwent an amino deprotection to achieve the desired products **8a**−**t** in fair yields.

Next, to increase the substituent diversity on the oxime side chain, another series of target compounds **12a**−**k** were obtained taking **8o** with a terminal amino group as the starting material in overall yields of 30−36%, as shown in Scheme 1. First, the carboxylic acid group in amino acids **9a**–**k** was activated by EDCI/NHS or CDI to form intermediate **10a**−**k**., which coupled with **8o** in anhydrous DMF directly without further purification to produce intermediate **11a-k**. Removal of the BOC protective group led to the final products **12a**−**k**.




**Scheme 2** (a) Chloroacetyl chloride, TEA, DCM, 0°C; (b) PhtNOH, $K_2CO_3$, DMF, 40 °C; (c) Hydrazine hydrate, in MeOH, r.t.; (d) $Ph_3CCl$, TEA, DMF, r.t.; (e) 2 N NaOH; then 1 N HCl, pH = 1-2; (f) MeOH, r.t; (g) DIC, HOBt, TEA, DMF, r.t.; (h) TFA, $SiEt_3H$, DCM, −10 °C.

Scheme 2 illustrated the synthesis of **22a**−**c** taking the commercially available amino acids (**13a**–**c**) as the starting material. The treatment of **13a**–**c** with chloroacetyl

chloride gave **14a**–**c**, which were converted into **15a**–**c** through nucleophilic substitution with *N*-hydroxyphthalimide. After removal of the phthaloyl group, the key intermediate alkoxyamine **16a-c** were obtained, which were then condensed with 2-oxo-2-(2-(tritylamino)thiazol-4-yl) acetic acid **19** generated by a two-step procedure from ethyl 2-oxo-2-(2-(amino)thiazol-4-yl) acetate **17**, to give Z-configuration **20a**–**c** as stable isomers. On one hand, the strong steric hindrance of triphenylmethyl aminothiazoly is not conductive to the formation of *E*-isomer. On the other hand, the intra-molecular hydrogen bond between carboxyl and the oxygen of oxime makes Z-isomer more stable [21a–c]. Then, the condensation of **6** and **20a**–**c** rendered intermediates **21a**–**c**, which underwent a Boc removal to afford the target compounds **22a**−**c** in moderate yields of 40–50%.

*2.2. SAR analysis for the antibacterial activity*

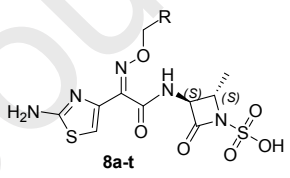
The structures and activities against Gram-negative bacteria of the total 32 target compounds were listed in Table 1. Minimum inhibitory concentration (MIC) was determined by the agar dilution assay described by the Clinical and Laboratory Standards Institute with AZN as the reference drug. Bacterial strains including drug-resistant *E. coli, K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and drug-susceptible *Enterobacter cloacae* (*E. cloacae*), *Enterobacter aerogenes* (*E. aerogenes*) were from the ATCC collection and clinical isolates from Chinese hospitals.

Firstly, a series of hydrophobic groups including substituted pyridine, phenyl, alkene and alkyne were respectively incorporated on the oxime side chain of AZN, and a set of monobactam analogues (**8a**−**k, 8p**−**t**) were prepared and measured for their activities against drug-susceptible and resistant Gram-negative bacteria. Compounds **8a**−**h** with substituted pyridine moieties displayed decreased antibacterial

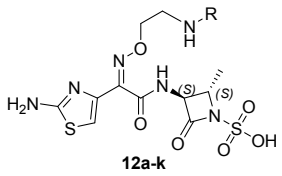
activities against the tested Gram-negative strains with MICs values ranging from 8 to >32 μg/mL, compared with the lead AZN. Only compound **8k** gave moderate activity against tested *E. coli* and *E. cloacae* strains with MICs values in the range of 1 to 2 μg/mL.

At the meantime, compounds **8l**−**n** bearing saturated heterocyclic substituents instead also exhibited reduced antibacterial potencies against all tested strains, compared with AZN. To our excitement, compounds **8p** and **8r** bearing a unsaturated allyl and propargyl group respectively showed comparable potencies to AZN with MIC values of 0.125−0.5 μg/mL against tested *E. coli* strain. More importantly, both of them exhibited a higher potency against *A. baumannii* strain than AZN with the MIC value of 8 μg/mL. Then the unsaturated allyl was retained, and methyl and bromo atom were respectively introduced on the double bond, by which new compounds **8q**, **8s** and **8t** were then created. However, a significant decrease in antibacterial potency was witnessed, compared with **8p**, which might originate from the steric hinderance of the extra substitutions on the alkene bond. Therefore, it was suggested that the presence of free allyl and propargyl moieties might be beneficial for antibacterial potency.

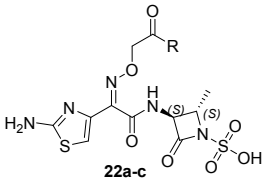
**Table 1 Antibacterial activities of the target compounds against Gram-negative bacteria**



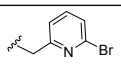
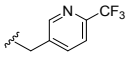

8a-t

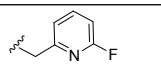
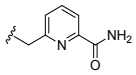
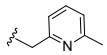
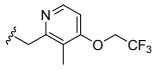
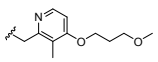
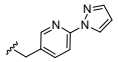
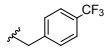
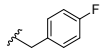
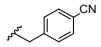
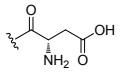
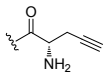
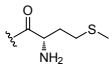
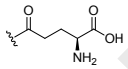
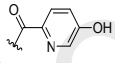
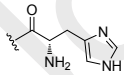
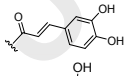
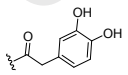
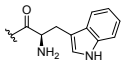
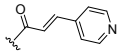



12a-k




22a-c

| Comp. | R | Minimum Inhibitory Concentration (MIC*, μg/mL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *E. coli*[a] | | | *K. pneum*[b] | | | *E.cloa*[c] | *E. aerog*[d] | *A. baum*[e] | *P. aeru*[f] | |
| | | ATCC 25922 ESBLs[a](-) | CDC 1001728 NDM-1(+) | 13-66 | ATCC 700603 ESBLs(+) | 2146 NDM-1(+) | 16-14 | ATCC 43560 | ATCC 13048 | ATCC 19606 | ATC C 27853 BL(+) | 16-11 CRE |
| **8a** |  | 32 | 64 | >64 | >32 | >32 | >64 | 32 | >32 | >32 | >32 | >64 |
| **8b** |  | >32 | 64 | >64 | >32 | >32 | >64 | >32 | >32 | >32 | >32 | >64 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **8c** |  | 16 | 16 | >64 | >32 | >32 | >64 | 8 | 32 | >32 | >32 | >64 |
| **8d** |  | 8 | >32 | >32 | >32 | >32 | >32 | 8 | 16 | >32 | >32 | >32 |
| **8e** |  | 16 | 16 | >64 | >32 | >32 | >64 | 16 | 32 | >32 | >32 | >64 |
| **8f** |  | >32 | 64 | >64 | >32 | >32 | >64 | >32 | >32 | >32 | >32 | >64 |
| **8g** |  | >32 | 64 | >64 | >32 | >32 | >64 | >32 | >32 | >32 | >32 | >64 |
| **8h** |  | 16 | 16 | 32 | >32 | >32 | >64 | 16 | 32 | >32 | >32 | >64 |
| **8i** |  | 64 | 32 | >64 | >128 | >128 | >64 | 64 | 128 | 128 | >128 | >64 |
| **8j** |  | 8 | 8 | 64 | >128 | >128 | >64 | 4 | 16 | 64 | >128 | >64 |
| **8k** |  | 2 | 1 | >64 | >64 | >64 | >64 | 1 | 32 | 64 | >64 | >64 |
| **8l** |  | 16 | 64 | >64 | >128 | >128 | >64 | 4 | 64 | >128 | >128 | >64 |
| **8m** |  | 4 | 4 | >64 | >128 | >128 | >64 | 2 | 4 | >128 | >128 | >64 |
| **8n** |  | 16 | 32 | >64 | >64 | >64 | >64 | 4 | 32 | >64 | >64 | >64 |
| **8o** |  | 1 | 2 | 4 | 8 | 128 | 4 | 1 | 2 | >128 | >128 | >64v |
| **8p** |  | 0.25 | 16 | 16 | 4 | >128 | 8 | 0.125 | 0.25 | 8 | 32 | 64 |
| **8q** |  | 1 | 32 | 32 | 128 | >128 | 32 | 0.25 | 2 | >128 | 64 | >64 |
| **8r** |  | 0.5 | 16 | 16 | >128 | >128 | 16 | 0.125 | 0.5 | 8 | 128 | >64 |
| **8s** |  | 2 | 8 | 32 | >64 | >64 | 64 | 1 | 2 | 64 | 64 | >64 |
| **8t** |  | 4 | 8 | >64 | >64 | >64 | >64 | 4 | 8 | >64 | >64 | >64 |
| **12a** |  | >32 | 32 | >64 | >32 | >32 | >64 | >32 | >32 | >32 | >32 | >64 |
| **12b** |  | >32 | 16 | >64 | >32 | >32 | 64 | 4 | 4 | 32 | >32 | >64 |
| **12c** |  | >32 | 16 | >64 | >32 | >32 | 64 | 2 | 16 | >32 | >32 | >64 |
| **12d** |  | >32 | 32 | >64 | >32 | >32 | >64 | 2 | 4 | >32 | >32 | >64 |
| **12e** |  | >32 | >64 | >64 | >32 | >32 | 64 | 16 | 32 | >32 | >32 | >64 |
| **12f** |  | >64 | >64 | >64 | >64 | >64 | >64 | >64 | >64 | >64 | >64 | >64 |
| **12g** |  | >32 | >64 | >64 | >32 | >32 | >64 | 8 | 2 | >32 | >32 | >64 |
| **12h** |  | >32 | >64 | >64 | >32 | >32 | >64 | >32 | 32 | >32 | >32 | >64 |
| **12i** |  | >32 | 64 | >64 | >32 | >32 | >64 | >32 | 32 | >32 | >32 | >64 |
| **12j** |  | >32 | 64 | >64 | >32 | >32 | >64 | 8 | 32 | >32 | >32 | >64 |
| **12k** |  | >32 | 64 | >64 | >32 | >32 | >64 | 16 | 32 | >32 | >32 | >64 |
| **22a** |  | >128 | 32 | >64 | >128 | >128 | 32 | >128 | >128 | >128 | >128 | >64 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **22b** |  | >128 | 32 | 32 | >128 | >128 | 8 | >128 | >128 | >128 | 128 | >64 |
| **22c** |  | >128 | >64 | 64 | >128 | >128 | >64 | >128 | >128 | >128 | >128 | >64 |
| **AZN** | - | ≤0.25 | 4 | 16 | 4 | 64 | 2 | 0.5 | ≤0.25 | >32 | 8 | 64 |

*MIC (μg/mL) was determined by agar dilution; [a]*E. coli*; [b]*K. pneumoniae*; [c]*E. cloacae* 435 60; [d]*E. aerogenes* 13048; [e]*A. baumannii*; [f]*P. aeruginosa*.

In addition, it is worth to mention that compound **8o** with propylamine was effective against both NDM-1-producing *E. coli* strain and ESBLs-producing *K. pneumonia* with MIC values of 2−8 μg/mL. Thus, the substituents with siderophores catecholate fragments [22] which might easily penetrate the cell membranes, free amine groups and carboxyl groups were introduced through flexible and long linkers in compound **8o** respectively. Compounds **12a**−**k** were synthesized and screened accordingly as described in Table 1. However, most of them exhibited obviously decreased activities against all Gram-negative strains. Only compounds **12b** and **12d** showed moderate potencies against some drug-susceptible strains, such as *E. cloacae* and *E. aerogenes* with MICs of 2−4 μg/mL. It seemed the flexible substituents would be detrimental to maintain antibacterial potency.

Finally, to simulate the salt bridge and Pi-Alkyl interactions between PBP3 and AZN, three analogues **22a**−**c** bearing different amino acid fragments on oxime side chain were prepared. However, compounds **22a**−**c** showed minimal activity against the tested strains as depicted in Table 1. Based on these results, compounds **8p** and **8r** were selected as the representative compounds for further investigations.

*2.3. Safety evaluations of compounds **8p** and **8r** in vitro and in vivo*

To evaluate the safety profiles of the representative compounds, the cytotoxic effects of the compounds **8p** and **8r** on H460 cells were carried out using MTT assay. As displayed in Fig. 3, both of them showed none cytotoxicity up to the concentration of 30 μg/mL to give the $CC_{50}$ values of over 30 μg/mL, suggesting high safety

profiles *in vitro*.

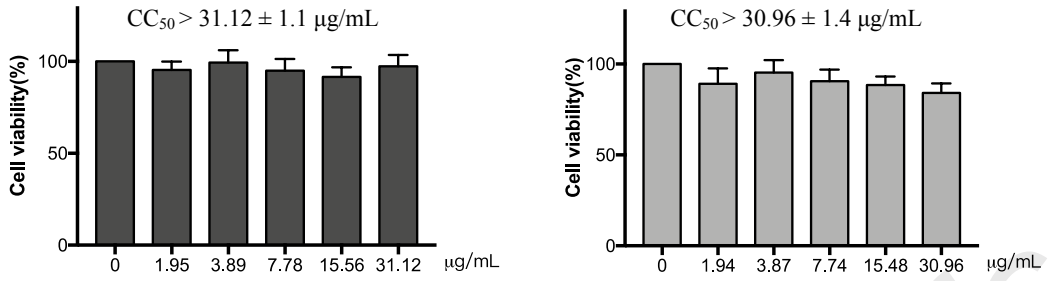



**Figure 3.** Cytotoxic effects of compounds **8p** and **8r** on H460 cells. Following pre-treatment with compounds **8p** and **8r** at the indicated concentrations for 24 h, the viability of cells was determined by MTT assay. Control cells were treated with 0.4 % (v/v) DMSO.

Acute toxicity of compounds **8p** and **8r** in mice was evaluated as well. Compounds **8p** and **8r** were given intravenously in a single-dosing experiment at 100, 200, 300 or 400 mg/kg, respectively. The mice were closely monitored for 7 days. No mouse died in the 7-day observation duration, indicating that the $LD_{50}$ of **8p** and **8r** via intravenous route was more than 400 mg/kg, suggesting that both of them had a wide therapeutic window and deserved further investigations.

**Table 2.** Acute toxicity of compounds **8p** and **8r** in mice

| Compd | $LD_{50}$ (mg/kg) |
|---|---|
| **8p** | > 400 |
| **8r** | > 400 |

*2.4. Molecular docking*

In order to further understand their modes of interactions with *P. aeruginosa* PBP3, molecular docking between *P. aeruginosa* PBP3 and compounds **8p** and **8r** were performed with Discovery Studio 4.5 docking program. As depicted in Fig. 4, Pi-alkyl interactions between allyl of compound **8r** and hydrophobic wall composed of Tyr-Tyr-Phe were well formed. These interactions might be helpful for inducing-fit conformational changes (Figure 4 IIA/IIB) and contribute to the formation of different hydrogen bonds between **8r** and other residues (SER294, LYS484, SER485, etc.) in

the active site of PBP3. And compound **8p** interacted in the hydrophobic pocket in a similar way to **8r** (Fig. 4, IA/IB). In addition to hydrophobic interaction of Pi-alkyl with PBP3, several other factors could contribute to the antibacterial effect, such as permeability, binding to PBP3, efflux pump. These data may be helpful to include PBP3 binding data for those specific compounds **8p** and **8r**.

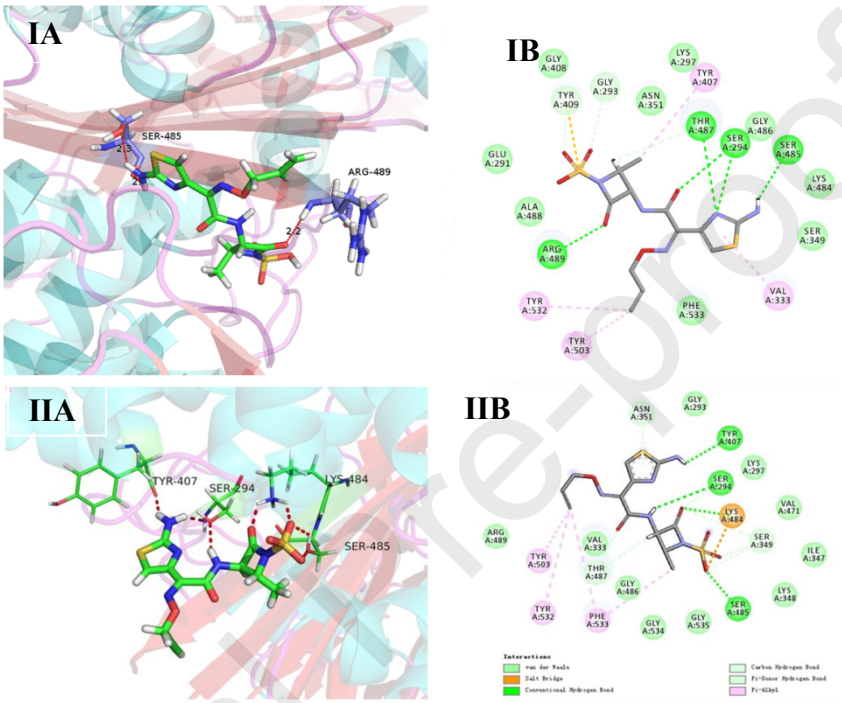



**Figure 4.** Interactions of compounds **8p**, **8r** in the active site of the *P. aeruginosa* PBP3 (Docking with Discovery Studio). (I A/B) compound **8p**; (II A/B) compound **8r**; Compounds are shown as stick models with green carbon atoms, blue nitrogen atoms, red oxygen atoms, and yellow sulfur atoms. Hydrogen bonds are depicted as red dashes in three-dimensional view and green dashes in two-dimensional view; Salt bridge interactions are depicted as brown dashes; Pi-Sulfur interactions are depicted as yellow dashes; Pi-Alkyl interactions are depicted as purple dashes.

*2. 5. Synergic Studies*

The combination of antibiotics and β-lactamase inhibitor has been widely used in clinic studies owing to their exciting synergy effect [23]. To find the strategy against resistant bacteria, synergistic studies of compounds **8p** and **8r** with β-lactamase inhibitor Avi were conducted on ESBLs- or NDM-1-producing *K. pneumoniae* strains, using microdilution checkerboard technique [24−26]. As shown in Table 3, taking AZN as the positive reference, significant synergies were observed in

combinations of **8p** or **8r** and Avi respectively, based on the fractional inhibitory concentration index (FICI) was ≤ 0.5. The combination of **8p**/**8r** with avi in a ratio of 1:16, showed a promising synergistic effect against two drug-resistant *K. pneumoniae* strains, with significantly reduced MIC values up to 8-fold and >256-fold. It also confirmed that β-lactam ring hydrolysis by β-lactamase might be the main resistant mechanism of its kind [27], other than the outer membrane permeability barrier and efflux pump mechanism.

**Table 3.** Antibacterial activities of compounds **8p** and **8r** alone and in combination with Avi against *K. pneumoniae* strains (MIC[a])

| | **alone** | | | | **combination** | | |
|---|---|---|---|---|---|---|---|
| **Strain** | **8p** | **8r** | Avi | AZN | **8p (+Avi)[b]** | **8r (+Avi)[b]** | **AZN (+Avi)** |
| 700603 ESBLs (+) | 4 | >128 | 32 | 4 | 0.5 | 0.5 | 0.5[c] |
| 2146 NDM-1(+) | >128 | >128 | 32 | 64 | 0.5 | 0.5 | 0.5[d] |

a, MIC (μg/mL) was determined by agar dilution; FICI<0.5; b, combination ratio in 1:16 (samples/Avi, m/m); c, combination ratio in 1:8; d, combination ratio in 1:2

## 3. Conclusion

In summary, thirty-four new monobactam derivatives were prepared and evaluated for their antibacterial activities against Gram-negative bacteria, taking AZN as the lead. Among them, compounds **8p** and **8r** showed satisfactory potencies with MIC values of 0.125−32 μg/mL against most tested Gram-negative strains. Both of them displayed high safety profiles both *in vitro* and *in vivo*. Synergistic effect of **8p**/**8r** combined with Avi significantly enhanced their antibacterial activity against ESBLs and NDM-1-producing *K. pneumoniae*, with significantly reduced MIC value up to 8-fold and >256-fold in a ratio of 1:16. It further confirmed that main resistant mechanism of its kind might originate from β-lactam ring hydrolysis by β-lactamase. Further structure modifications and optimizations of this kind for overcoming β-lactamase-producing resistant Gram-negative bacteria are actively underway in our laboratory.

## 4. Experimental section

### 4.1 Chemistry

Melting points (mp) were measured with an MPA 100 OptiMelt automated melting point system (Stanford Research Systems, California, USA). $^{1}$H NMR and $^{13}$C NMR spectra were performed on a Varian Inova spectrometer (San Francisco, CA, USA) or a Bruker Avance III spectrometer (Bruker Co., Zurich, Switzerland) in DMSO-$d_6$ with $Me_4Si$ as the internal standard. ESI high-resolution mass spectrometry (HRMS) was performed on an AutospecUltima-TOF mass spectrometer (Micromass UK Ltd, Manchester, UK). Flash chromatography was performed on a CombiFlash Rf 200 system (Teledyne, Nebraska, USA), with a particle size of 38 μm. Lyophilization was performed on CHRIST ALPHA 2-4 LD plus freeze drier (Marin Christ, Osterode, Germany).

### 4.2 General procedures for synthesis of compounds 8a–r

*4.2.1 2S,3S-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[(6-bromopyridin-2-yl)methoxy]imino] acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8a**)*. $Et_3SiH$ (0.06 g, 0.53 mmol) was added to a suspension of **7a** (0.2 g, 0.26 mmol) in DCM (5 mL) under Nitrogen at –15 °C. The reaction mixture was allowed to stir at –15 °C for 20 min, then TFA (1.8 g, 15.75 mmol) was added. After stirring at –15 °C for 5 h, the solvent was evaporated in vacuo at r.t.. EA and petroleum ether (1:1, 10 mL) was added to the residue and stirred at r.t. for 1 h. The mixture was filtered to give the title product **8a** (65.0 mg, 48%) as off-white solid, mp: 180.8–182.1 °C. $^{1}$H NMR (500 MHz) δ 9.65 (s, 1H), 7.81 (t, $J$ = 7.8 Hz, 1H), 7.60 (d, $J$ = 7.9 Hz, 1H), 7.48 (d, $J$ = 7.6 Hz, 1H), 6.97 (d, $J$ = 3.4 Hz, 1H), 5.28 (s, 2H), 4.51 (dd, $J$ = 7.7, 2.3 Hz, 1H), 3.80–3.62 (m, 1H), 1.54–1.31 (d, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 170.3, 162.3, 161.5, 159.7, 141.2, 141.0,

128.4, 128.2, 127.7, 127.3, 121.4, 112.1, 76.5, 61.0, 57.6, 18.7. HRMS: calcd for $C_{15}H_{16}BrN_6O_6S_2$ $[M+H]^+$ 518.9751, found 518.9760.

*4.2.2 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[[6-(trifluoromethyl)pyridin-3-yl]methoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8b**)*. Compound **8b** was prepared from **7b** (0.30 g, 0.40 mmol) by the procedure as described for **8a** as off-white solid (99.0 mg, 49%), mp: 158.5–160.0 °C. $^1$H NMR (500 MHz) δ 9.61 (d, *J* = 6.7 Hz, 1H), 8.80 (s, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 6.96 (s, 1H), 5.39 (s, 2H), 4.50 (d, *J* = 7.1 Hz, 1H), 3.71 (d, *J* = 4.1 Hz, 1H), 1.42 (d, *J* = 5.8 Hz, 3H). $^{13}$C NMR (151 MHz) δ 170.8, 170.1, 162.1, 161.3, 149.6, 146.3(t), 137.7, 137.6, 123.1, 121.2, 121.0, 111.9, 73.3, 60.8, 57.4, 40.4, 40.3, 40.1, 40.0, 39.9, 39.7, 39.6, 18.4. HRMS: calcd for $C_{16}H_{16}F_3N_6O_6S_2$ $[M+H]^+$ 509.0519, found 509.0528.

*4.2.3 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[(6-fluoropyridin-2-yl)methoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8c**)*. Compound **8c** was prepared from **7c** (0.20 g, 0.29 mmol) by the procedure as described for **8a** as off-white solid (70.6 mg, 54%), mp: 169.5–171.4 °C. $^1$H NMR (500 MHz) δ 9.65 (d, *J* = 7.7 Hz, 1H), 8.04 (dd, *J* = 15.9, 8.0 Hz, 1H), 7.39 (d, *J* = 7.3 Hz, 1H), 7.12 (d, *J* = 6.8 Hz, 1H), 6.97 (s, 1H), 5.24 (s, 2H), 4.51 (dd, *J* = 7.7, 2.3 Hz, 1H), 3.74–3.71 (m, 1H), 1.43 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (126 MHz) δ 170.3, 163.9, 162.3, 162.1, 161.4, 157.0, 143.6, 119.7, 112.1, 109.2, 108.9, 76.4, 61.0, 57.6, 18.7. HRMS: calcd for $C_{15}H_{16}N_6O_6FS_2$ $[M+H]^+$ 459.0551, found 459.0557.

*4.2.4 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[(6-carbamoylpyridin-2-yl)methoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8d**)*. Compound **8d** was prepared from **7d** (0.30 g, 5.0 mmol) by the same procedure as described for **8a** as off-white solid (0.22 g, 54%). mp:169.3–170.1 °C. $^1$H NMR (500 MHz) δ 9.65 (d, *J* = 7.5 Hz, 1H), 8.07 (s, 1H), 8.02 (t, *J* = 7.7 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.67 (s,

1H), 7.62 (d, *J* = 7.7 Hz, 1H), 6.95 (s, 1H), 5.37 (s, 2H), 4.52 (dd, *J* = 7.7, 2.4 Hz, 1H), 3.77–3.71 (m, 1H), 1.42 (d, *J* = 8.8 Hz, 3H). $^{13}$C NMR (151 MHz) δ 170.6, 169.9, 166.3, 162.2, 159.0, 157.1, 150.1, 138.8, 129.5, 124.4, 121.2, 111.5, 76.7, 60.9, 57.4, 18.5. HRMS: calcd for $C_{16}H_{18}N_7O_7S_2$ $[M+H]^+$ 484.0704, found 484.07092

*4.2.5 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[(6-methylpyridin-2-yl)methoxy] imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8e**)*. Compound **8e** was prepared from **7e** (0.25 g, 0.35 mmol) by the procedure as described for **8a** as off-white solid (0.11 g, 70%). mp: 152.0–154.0 °C. $^1$H NMR (500 MHz) δ 9.57 (d, *J* = 7.7 Hz, 1H), 8.34 (t, *J* = 7.8 Hz, 1H), 7.74 (d, *J* = 7.8 Hz, 3H), 6.92 (s, 1H), 5.42 (s, 2H), 4.49 (dd, *J* = 7.6, 2.2 Hz, 1H), 3.72 (dd, *J* = 6.1, 2.3 Hz, 1H), 2.69 (s, 3H), 1.43 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (126 MHz) δ 169.8, 162.3, 162.0, 158.9, 155.4, 153.3, 150.8, 144.8, 126.3, 122.6, 112.3, 73.1, 61.0, 57.6, 21.1, 18.7. HRMS: calcd for $C_{16}H_{19}N_6O_6S_2$ $[M+H]^+$ 455.0802, found 455.0808.

*4.2.6 (2S,3S)-2-Methyl-3-{(Z)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl] methoxy]imino]-2-[2-(tritylamino)thiazol-4-yl]acetamido}-4-oxoazetidine-1-sulfonic acid (**8f**)*. Compound **8f** was prepared from **7f** (0.30 g, 0.37 mmol) by the procedure as described for **8a** as off-white solid (0.11 g, 54%), mp: 156.7–158.9 °C. $^1$H NMR (500 MHz) δ 9.41 (d, *J* = 7.8 Hz, 1H), 8.82 (d, *J* = 6.7 Hz, 1H), 7.69 (d, *J* = 6.8 Hz, 1H), 6.84 (s, 1H), 5.43 (s, 2H), 5.22 (d, *J* = 8.5 Hz, 2H), 4.44 (dd, *J* = 7.8, 2.5 Hz, 1H), 3.66 (dd, *J* = 6.2, 2.6 Hz, 1H), 2.29 (s, 3H), 1.40 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.3, 167.1, 162.2, 162.0, 158.7, 150.8, 142.6, 125.7, 124.7, 116.9, 112.0, 110.1, 71.0, 66.4, 60.8, 57.4, 18.4, 10.5. HRMS: calcd for $C_{18}H_{20}F_3N_6O_7S_2$ $[M+H]^+$ 553.0782, found 553.0789

*4.2.7 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[[4-(3-methoxypropoxy)-3-methyl pyridin-2-yl]methoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8g**)*.

Compound **8g** was prepared from **7g** (0.30 g, 0.38 mmol) according to the procedure as described for **8a** as off-white solid (0.11 g, 52%), mp: 142.8–144.4 °C. $^1$H NMR (500 MHz) δ 9.38 (d, *J* = 7.8 Hz, 1H), 8.73 (d, *J* = 6.8 Hz, 1H), 7.62 (d, *J* = 6.9 Hz, 1H), 6.83 (s, 1H), 5.41 (s, 2H), 4.51–4.35 (m, 3H), 3.67 (dd, *J* = 6.1, 2.5 Hz, 1H), 3.52 (t, *J* = 6.1 Hz, 2H), 3.26 (s, 3H), 2.27 (s, 3H), 2.12–2.02 (m, 2H), 1.41 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (126 MHz) δ 169.3, 162.1, 158.8, 151.8, 149.0, 141.8, 141.3, 125.8, 117.3, 114.9, 111.9, 109.5, 70.4, 68.5, 60.7, 58.5, 57.4, 28.8, 18.5, 10.6. HRMS: calcd for $C_{20}H_{27}N_6O_8S_2$ $[M+H]^+$ 543.1326, found 543.1334

*4.2.8 (2S,3S)-3-{(Z)-2-[[[6-(1H-Pyrazol-1-yl)pyridin-2-yl]methoxy]imino]-2-(2-aminothiazol-4-yl)acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8h**)*. Compound **8h** was prepared from **7h** (0.30 g, 0.40 mmol) by the procedure as described for **8a** as off-white solid (0.10 g, 50%), mp: 156.7–158.9 °C. $^1$H NMR (500 MHz) δ 9.55 (d, *J* = 7.8 Hz, 1H), 8.63 (d, *J* = 2.4 Hz, 1H), 8.51 (d, *J* = 1.4 Hz, 1H), 8.02 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.94 (s, 1H), 7.84 (s, 1H), 6.93 (s, 1H), 6.59–6.58 (m, 1H), 5.29 (s, 2H), 4.48 (dd, *J* = 7.8, 2.5 Hz, 1H), 3.76–3.59 (m, 1H), 1.40 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 170.7, 170.0, 162.8, 162.2, 151.0, 148.2, 142.8, 139.7, 131.4, 127.6, 119.8, 112.1, 111.4, 108.8, 73.6, 60.8, 57.4, 18.4. HRMS: calcd for $C_{18}H_{19}N_8O_6S_2$ $[M+H]^+$ 507.0864, found 507.0875

*4.2.9 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[[4-(trifluoromethyl)benzyl]oxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8i**)*. Compound **8i** was prepared from **7i** (0.24 g, 0.32 mmol) according to the procedure as described for **8a** as white solid (0.12 g, 68%), mp: 190.0–191.3 °C. $^1$H NMR (500 MHz) δ 9.43 (d, *J* = 7.9 Hz, 1H), 7.73 (d, *J* = 8.1 Hz, 2H), 7.58 (d, *J* = 8.1 Hz, 2H), 7.25 (s, 2H), 6.76 (s, 1H), 5.24 (s, 2H), 4.47 (dd, *J* = 7.9, 2.5 Hz, 1H), 3.72 (dd, *J* = 6.1, 2.6 Hz, 1H), 1.39 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.0, 162.9, 162.6, 150.8, 143.3, 142.9,

128.7, 128.3(2), 125.8, 125.8, 125.8, 125.8, 125.6(2), 110.6, 74.9, 60.8, 57.3, 18.44. HRMS: calcd for $C_{17}H_{17}F_3N_5O_6S_2$ $[M+H]^+$ 508.0567, found: 508.0577.

*4.2.10 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[(4-fluorobenzyl)oxy]imino] acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8j**).* Compound **8j** was prepared from **7j** (0.25 g, 0.36 mmol) by the procedure as described for **8a** as off-white solid (0.1 g, 65%). mp: 198.5–200.3 °C. $^1$H NMR (500 MHz) δ 9.38 (d, *J* = 7.9 Hz, 1H), 7.46–7.37 (m, 2H), 7.31 (s, 2H), 7.24–7.15 (m, 2H), 6.75 (s, 1H), 5.12 (s, 2H), 4.44 (dd, *J* = 7.9, 2.6 Hz, 1H), 3.67 (qd, *J* = 6.1, 2.6 Hz, 1H), 1.36 (d, *J* = 6.2 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.1, 163.0, 162.9, 162.5, 161.3, 150.2, 134.3, 130.2, 130.1, 115.6, 115.4, 110.3, 75.2, 60.8, 57.3, 18.4. HRMS: calcd for $C_{16}H_{17}FN_5O_6S_2$ $[M+H]^+$ 458.0599, found: 458.0615.

*4.2.11 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[(4-cyanobenzyl)oxy]imino]acetami do}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8k**).* Compound **8k** was prepared from **7k** (0.40 g, 0.56 mmol) by the procedure as described for **8a** as off-white solid (0.15 g, 56%), mp: 176.5–178.3 °C. $^1$H NMR (500 MHz) δ 9.54 (d, *J* = 7.8 Hz, 1H), 7.85 (d, *J* = 8.2 Hz, 2H), 7.56 (d, *J* = 8.2 Hz, 2H), 6.86 (s, 1H), 5.29 (s, 2H), 4.48 (dd, *J* = 7.8, 2.5 Hz, 1H), 3.72–3.68 (m, 1H), 1.40 (d, *J* = 6.0 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.8, 169.7, 162.2, 161.9, 144.5, 143.9, 132.8, 128.3, 119.3, 111.3, 110.8, 75.2, 60.8, 57.4, 18.5. HRMS: calcd for $C_{17}H_{17}N_6O_6S_2$ $[M+H]^+$ 465.0646, found 465.0655.

*4.2.12 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[(2-morpholinoethoxy)imino]acetam-ido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8l**).* Compound **8l** was prepared from **7l** (0.30 g, 0.43 mmol) by the procedure as described for **8a** as white solid (0.10 g, 52%), mp: 147.0–148.1 °C. $^1$H NMR (600 MHz) δ 9.16 (d, *J* = 8.0 Hz, 1H), 7.60 (s, 1H), 4.56 (dd, *J* = 16.3, 4.5 Hz, 2H), 4.42 (dd, *J* = 8.0, 2.6 Hz, 1H), 3.82 (s, 4H), 3.75–3.72 (m, 2H), 3.62–3.52 (m, 2H), 3.33 (s, 4H), 1.45–1.36 (d, *J* = 1.41Hz, 3H). $^{13}$C NMR

(151 MHz) δ 167.4, 163.2, 162.4, 129.5, 128.8, 117.1, 69.6, 63.8(2), 61.1, 57.3, 55.4, 52.2(2), 18.5. HRMS: calcd for $C_{15}H_{23}N_6O_7S_2$ $[M+H]^+$ 463.1064, found 463.1073.

*4.2.14 (2S,3S)-3-{(Z)-2-(2-aminothiazol-4-yl)-2-[[2-(piperazin-1-yl)ethoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid, trifluoroacetic acid salt (**8m**)*. Compound **8m** was prepared from **7m** (0.30 g, 0.37 mmol) by the procedure as described for **8a** as white solid (0.14 g, 65%), mp: 192.7–194.0 °C. $^1$H NMR (600 MHz) δ 9.37 (d, *J* = 7.4 Hz, 1H), 8.93 (s, 2H), 6.89 (s, 1H), 4.48–4.37 (m, 3H), 3.80 (dd, *J* = 6.1, 2.4 Hz, 1H), 3.40 (s, 10H), 1.43 (t, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.3, 162.8, 159.1, 158.9, 115.4, 111.3, 70.0, 61.0, 57.3(2), 55.5, 49.4(2), 41.1, 18.5. HRMS: calcd for $C_{15}H_{24}N_7O_6S_2$ $[M–CF_3COOH+H]^+$ 462.1224, found 462.1236。

*4.2.15 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[2-(piperidin-1-yl)ethoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8n**)*. Compound **8n** was prepared from **7n** (0.30 g, 0.43 mmol) by the procedure as described for **8a** as off-white solid (0.11 g, 56%), mp: 150.3–152.1 °C . $^1$H NMR (600 MHz) δ 9.16 (d, *J* = 8.0 Hz, 1H), 7.57 (s, 1H), 4.53 (t, *J* = 4.6 Hz, 2H), 4.42 (dd, *J* = 7.9, 2.4 Hz, 1H), 3.72 (dd, *J* = 6.1, 2.4 Hz, 1H), 3.49 (s, 4H), 2.97 (s, 2H), 1.67 (s, 3H), 1.41 (d, *J* = 6.2 Hz, 3H). $^{13}$C NMR (151 MHz) δ 167.3, 163.3, 162.4, 129.5, 117.0, 115. 6, 69.6, 61.1, 57.3, 55.1, 53.2, 23.0, 21.5, 18.5. HRMS: calcd for $C_{16}H_{25}N_6O_6S_2$ $[M+H]^+$ 461.1272, found: 461.1278.

*4.2.16 (2S,3S)-3-{(Z)-2-(2-aminothiazol-4-yl)-2-[(2-ammonioethoxy)imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonate (**8o**)*. $Et_3SiH$ (0.10 g, 0.82 mmol) was added to a suspension of **7o** (0.30 g, 0.41 mmol) in DCM (10 mL) under nitrogen at –15 °C. The reaction mixture was allowed to stir at -15 °C for 20 min, then TFA (5.0 mL) was added. After stirring at –15 °C for 5 h, the mixture was evaporated in vacuo at r.t.. The

mixture of EA and petroleum ether (1:1, 10 mL) was added to the residue, stirred for 1 h. The mixture was filtered to give crude product. The crude solid was dissolved the in water, and purified by HP20 resin column. The column was rinsed with water and finally 20% MeOH in water to elute the target product. MeOH was evaporated under vacuum at 36 °C. The residual water solution was lyophilized to give the title product **8o** (0.1 g, 64%) as white solid, mp: 230.1 °C (dec.) $^1$H NMR (600 MHz) δ 9.21 (s, 1H), 7.75 (s, 3H), 7.23 (s, 2H), 6.84 (s, 1H), 4.48 (s, 1H), 4.23 (d, *J* = 4.5 Hz, 2H), 3.76–3.63 (m, 1H), 3.16–3.01 (m, 2H), 1.43 (d, *J* = 5.7 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.1, 162.7, 162.7, 151.2, 142.7, 110.8, 70.8, 60.8, 57.7, 38.8, 18.5. HRMS: calcd for $C_{11}H_{17}N_6O_6S_2$ $[M+H]^+$ 393.0646, found 393.0651.

*4.2.17 (2S,3S)-3-{(Z)-2-[(Allyloxy)imino]-2-(2-aminothiazol-4-yl)acetamido}-2-methy -l-4-oxoazetidine-1-sulfonic acid (**8p**)*. Compound **8p** was prepared from **7p** (0.30 g, 0.47 mmol) by the procedure as described for **8o** as white solid (0.12 g, 65%), mp: 189.2–190.1 °C. $^1$H NMR (600 MHz) δ 9.47 (d, *J* = 7.6 Hz, 1H), 6.88 (s, 1H), 6.02–5.95 (m, 1H), 5.36–5.23 (m, 2H), 4.65 (d, *J* = 5.0 Hz, 2H), 4.43 (d, *J* = 7.7 Hz, 1H), 3.70–3.60 (m, 1H), 1.38 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ170.2, 162.0, 161.2, 146.6, 134.1, 118.2, 111.1 75.7, 60.8, 57.4, 18.5. HRMS: calcd for $C_{12}H_{16}N_5O_6S_2$ $[M+H]^+$ 390.0537, found: 390.0541.

*4.2.18 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[(2-methylallyl)oxy]imino]aceta mido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8q**)*. Compound **8q** was prepared from **7q** (0.30 g, 5 mmol) by the procedure as described for **8a** as off-white solid (0.10 g, 56%), mp: 181.4–183.2 °C. $^1$H NMR (600 MHz) δ 9.45 (d, *J* = 7.7 Hz, 1H), 6.86 (s, 1H), 4.94 (d, *J* = 40.8 Hz, 2H), 4.56 (s, 2H), 4.46 (dd, *J* = 7.8, 2.4 Hz, 1H), 3.76–3.59 (m, 1H), 1.71 (s, 3H), 1.41 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ

170.0, 162.2, 161.6, 141.7, 112.9, 110.8, 100.0, 78.3, 60.8, 57.4, 19.7, 18.5. HRMS: calcd for $C_{13}H_{18}N_5O_6S_2$ $[M+H]^+$ 404.0693, found 404.0698.

*4.2.19 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[(prop-2-yn-1-yloxy)imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**8r**)*. Compound **8r** was prepared from **7r** (0.30 g, 0.47 mmol) by the procedure as described for **8o** as light-yellow solid (0.10 g, 56%), mp: 180 °C (dec.). $^1$H NMR (500 MHz) δ 9.32 (d, *J* = 7.7 Hz, 1H), 7.22 (s, 2H), 6.72 (s, 1H), 4.66 (s, 2H), 4.39 (d, *J* = 7.8 Hz, 1H), 3.70–3.64 (m, 1H), 3.47 (s, 1H), 1.38 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.1, 162.4, 162.3, 150.1, 150.1, 110.9, 80.4, 78.3, 62.3, 60.9, 57.3, 18.5. HRMS: calcd for $C_{12}H_{14}N_5O_6S_2$ $[M+H]^+$ 388.0380, found 388.0384.

*4.2.20 (2S,3S)-3-((Z)-2-(2-aminothiazol-4-yl)-2-(((2-bromoallyl)oxy)imino) acetamido)-2-methyl-4-oxoazetidine-1-sulfonic acid (**8s**)*. Compound **8s** was prepared from **7s** (0.30 g, 5.0 mmol) by the same procedure as described for **8a** as off-white solid (0.12 g, 60%). mp:166.3–167.1 °C. $^1$H NMR (600 MHz) δ 9.47 (d, J = 7.4 Hz, 1H), 6.86 (s, 1H), 6.04 (s, 1H), 5.69 (s, 1H), 4.74 (s, 2H), 4.45 (d, J = 5.4 Hz, 1H), 3.71 (d, J = 3.6 Hz, 1H), 1.41 (d, J = 5.8 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.58, 162.18, 161.88, 128.26, 118.95, 111.39, 77.58, 60.81, 57.37, 18.49. HRMS: calcd for $C_{12}H_{13}N_5O_6S_2Br$ $[M-H]^-$ 465.9493, found 465.9489

*4.2.21 (Z)-3-(2-(2-aminothiazol-4-yl)-2-(((3-methylbut-2-en-1-yl)oxy)imino) acetamido)-2-methyl-4-oxoazetidine-1-sulfonic acid (**8t**)*. Compound **8t** was prepared from **7t** (0.30 g, 5.0 mmol) by the same procedure as described for **8a** as off-white solid (0.10 g, 53%). mp:153.3–54.1 °C. $^1$H NMR (600 MHz) δ 9.41 (d, J = 7.7 Hz, 1H), 6.87 (s, 1H), 5.41 – 5.39 (m, 1H), 4.64 (d, J = 6.8 Hz, 2H), 4.44 (dd, J = 7.8, 2.6 Hz, 1H), 3.69 – 3.66 (m, 1H), 1.74 (s, 3H), 1.68 (s, 3H), 1.41 (d, J = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 170.07, 162.09, 138.17, 128.24, 127.99, 127.10, 120.09, 110.56,

71.90, 60.81, 57.39, 25.95, 18.58, 18.44. HRMS: calcd for $C_{14}H_{18}N_5O_6S_2$ $[M-H]^-$ 416.0702, found 416.0712

**4.3 General procedures for synthesis of compound 12a–k**

*4.3.1 (S)-3-Amino-4-{[2-[[(Z)-[1-(2-aminothiazol-4-yl)-2-[[(2S,3S)-2-methyl-4-oxo-1-sulfoazetidin-3-yl]amino]-2-oxoethylidene]amino]oxy]ethyl]amino}-4-oxobutanoic acid (**12a**)*. EDCI (86.0 mg, 0.45 mmol) and NHS (48.0 mg, 0.42 mmol) was added to a solution of Boc-*L*-aspartic acid 4-tert-butyl ester (0.10 g, 0.35 mmol) in anhydrous DMF (1.0 mL). The reaction mixture was allowed to stir at r.t. until the reaction fininsed. **8o** (0.15 g, 0.30 mmol) in dry acetonitrile (3.0 mL) and absolute DIEA (76.6 mg, 0.59 mmol) was added to above reaction mixture dropwise. After stirring at r.t. overnight, the mixture was diluted with water (5.0 mL) and extracted with EA (10.0 mL×3). The organic layers were combined and washed with brine (20 mL×3), concentrated under vacuum to give crude product **11a**.

$Et_3SiH$ (0.07 g, 0.60 mmol) was added to the suspension of **11a** (0.20 g, 0.30 mmol) in DCM (10 mL) under nitrogen at –15 °C. The reaction mixture was allowed to stir at -15 °C for 20 min, then TFA (5.0 mL) was added thereto. After stirring at –15 °C for 5 h, the mixture was evaporated in vacuo at r.t.. EA and petroleum ether (1:1, 10 mL) was added to the residue and stirred in for 1 h, then filtered to give the title product **12a** (0.10 g, 65%) as off-white solid. mp: 172.5–173.2 °C. $^1$H NMR (500 MHz) δ 9.34 (d, *J* = 7.7 Hz, 1H), 8.52 (t, *J* = 5.4 Hz, 1H), 8.19 (s, 4H), 6.89 (s, 1H), 4.46 (dd, *J* = 7.7, 2.4 Hz, 1H), 4.14 (s, 2H), 4.10–4.01 (m, 1H), 3.78–3.69 (m, 1H), 3.48–3.39 (m, 2H), 2.86–2.74 (m, 2H), 1.43 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 171.3, 169.8, 168.3, 162.5, 158.9, 117.0, 115.0, 110.8, 72.9, 60.9, 57.5, 49.5, 39.0, 35.8, 18.5. HRMS: calcd for $C_{15}H_{22}N_7O_9S_2$ $[M+H]^+$ 508.0915, found 508.0923.

*4.3.2 (2S,3S)-3-{(Z)-2-[[2-[(S)-2-Aminopent-4-ynamido]ethoxy]imino]-2-(2-amino thiazol-4-yl)acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**12b**)*. Compound **12b** was prepared from **8o** (0.15 g, 0.30 mmol) and boc-*L*-methionine (0.10 g, 0.47 mmol) by the procedure as described for **12a** as off-white solid (0.10 g, 70%), mp: 161.5–163.3 °C. $^1$H NMR (500 MHz) δ 9.38 (d, *J* = 7.7 Hz, 1H), 8.54 (t, *J* = 5.6 Hz, 1H), 8.28 (d, *J* = 4.0 Hz, 4H), 6.93 (d, *J* = 9.0 Hz, 1H), 4.47 (dd, *J* = 7.7, 2.5 Hz, 1H), 4.24–4.09 (m, 2H), 3.98–3.90 (m, 1H), 3.76–3.71 (m, 1H), 3.56–3.37 (m, 2H), 3.11 (t, *J* = 2.6 Hz, 1H), 2.80–2.67 (m, 2H), 1.43 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.9, 167.7, 162.5, 159.0, 116.8, 114.9, 111.0, 78.1, 75.9, 73.1, 60.9, 57.5, 51.3, 38.9, 21.6, 18.5. HRMS: calcd for $C_{16}H_{22}N_7O_7S_2$ $[M+H]^+$ 488.1017, found 488.1026.

*4.3.3 (2S,3S)-3-[(S,Z)-5-Amino-12-(2-aminothiazol-4-yl)-6-oxo-10-oxa-2-thia-7,11-diazatridec-11-enamido]-2-methyl-4-oxoazetidine-1-sulfonic acid (**12c**)*. Compound **12c** was prepared from **8o** (0.15 g, 0.30 mmol) and boc-*L*-methionine (87.3 mg, 0.35 mmol) by the procedure as described for **12a** as off-white solid (0.10 g, 65%), mp: 164.5–165.9 °C. $^1$H NMR (500 MHz) δ 9.46 (d, *J* = 7.5 Hz, 1H), 8.60 (s, 1H), 8.21 (d, *J* = 3.8 Hz, 4H), 6.96 (s, 1H), 4.47 (dd, *J* = 7.7, 2.4 Hz, 1H), 4.20 (t, *J* = 5.2 Hz, 2H), 3.95–3.86 (m, 1H), 3.76 (dd, *J* = 6.1, 2.5 Hz, 1H), 3.46 (dd, *J* = 9.8, 4.8 Hz, 2H), 2.04 (s, 3H), 1.98 (dd, *J* = 16.0, 6.3 Hz, 2H), 1.43 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 173.2, 170.2, 168.9, 162.4, 161.0, 144.6, 111.2, 73.5, 60.9, 57.5, 52.2, 39.0, 31.4, 28.7, 18.5, 14.9. HRMS: calcd for $C_{16}H_{26}N_7O_7S_3$ $[M+H]^+$ 524.1050, found 524.1060.

*4.3.4 (S)-2-Amino-5-{[2-[[(Z)-[1-(2-aminothiazol-4-yl)-2-[[(2S,3S)-2-methyl-4-oxo-1-sulfoazetidin-3-yl]amino]-2-oxoethylidene]amino]oxy]ethyl]amino}-5-oxopentanoic acid (**12d**)*. Compound **12d** was prepared from **8o** (0.15 g, 0.30 mmol) and boc-*L*-glutamic acid 1-tert-butyl ester (0.10 g, 0.33 mmol) by the procedure as described for **12a** as off-white solid (89.2 mg, 57%), mp: 166.5–167.9 °C. $^1$H NMR

(500 MHz) δ 9.20 (d, *J* = 8.2 Hz, 1H), 8.20 (d, *J* = 3.6 Hz, 4H), 7.90 (t, *J* = 5.7 Hz, 1H), 6.83 (s, 1H), 4.52 (dd, *J* = 8.2, 2.5 Hz, 1H), 4.07 (t, *J* = 5.5 Hz, 2H), 3.94 (d, *J* = 5.4 Hz, 1H), 3.79–3.67 (m, 1H), 3.40–3.23 (m, 2H), 2.42–2.22 (m, 2H), 2.13–2.03 (m, 1H), 1.96 (dd, *J* = 10.8, 5.6 Hz, 1H), 1.43 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 171.7, 171.3, 169.3, 162.9, 162.5, 158.9, 158.6, 110.3, 73.2, 60.7, 57.8 52.2, 38.7, 26.3, 18.4. HRMS: calcd for $C_{16}H_{24}N_7O_9S_2$ $[M+H]^+$ 522.1071, found 522.1078.

*4.3.5 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[2-(5-hydroxypicolinamido)ethoxy] imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**12e**)*. To the solution of 5-hydroxypicolinic acid (0.10 g, 0.72 mmol) in DMF (1.0 mL) was added CDI (0.12 g, 0.75 mmol) and stirred at r.t. for 1 h. The above solution (0.75 mL) was added dropwise into the solution of **8o** (0.10 g, 0.20 mmol) in acetonitrile (3.0 mL) and stirred at r.t. overnight. Removal of solvent was carried out to give a brown residue, which was subjected to flash column chromatography (eluent: from 0–15% of methanol in DCM) to afford **12e** (75.0 mg, 73%), mp: 193.1–194.0 °C. $^1$H NMR (500 MHz) δ 10.60 (s, 1H), 9.28 (d, *J* = 8.0 Hz, 1H), 8.61 (t, *J* = 6.0 Hz, 1H), 8.16 (d, *J* = 2.7 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 1H), 7.31–7.24 (m, 3H), 6.74 (s, 1H), 4.44 (dd, *J* = 8.0, 2.6 Hz, 1H), 4.15 (t, *J* = 6.0 Hz, 2H), 3.79–3.74 (m, 1H), 3.62–3.48 (m, 2H), 1.36 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.0, 164.6, 163.0, 162.6, 156.7, 150.3, 143.2, 141.6, 137.2, 123.8, 123.1, 109.9, 73.0, 60.8, 57.2, 39.0, 18.4. HRMS: calcd for $C_{17}H_{20}N_7O_8S_2$ $[M+H]^+$ 514.0809, found 514.0815.

*4.3.6 (2S,3S)-3-{(Z)-2-[[2-[(S)-2-Amino-3-(1H-imidazol-4-yl)propanamido]ethoxy] imino]-2-(2-aminothiazol-4-yl)acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid* (***12f***). Compound **12f** was prepared from **8o** (0.10 g, 0.20 mmol) and boc-*L*-histidine (0.24 mmol) by the procedure as described for **12e** as off-white solid (74.1 mg, 70%), mp: 149.9–152.8 °C. $^1$H NMR (500 MHz) δ 9.28 (d, *J* = 7.9 Hz, 1H), 9.01 (s, 1H),

8.29 (s, 3H), 8.19 (t, *J* = 5.6 Hz, 1H), 7.49 (s, 1H), 6.86 (s, 1H), 4.53 (dd, *J* = 7.9, 2.5 Hz, 1H), 4.21 (t, *J* = 6.6 Hz, 1H), 4.12–4.04 (m, 2H), 3.86–3.81 (m, 1H), 3.41 (dd, *J* = 10.3, 5.4 Hz, 2H), 3.33–3.05 (m, 3H), 1.45 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.2, 167.8, 163.9, 162.7, 135.1, 127.0, 119.8, 118.8, 115.8, 110.7, 72.7, 60.8, 57.9, 51.8, 39.0, 26.8, 18.3. HRMS: calcd for $C_{17}H_{24}N_9O_7S_2$ $[M+H]^+$ 530.1235, found 530.1245.

*4.3.7 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[2-[(E)-3-(3,4-dihydroxyphenyl) acrylamido]ethoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**12g**).* Compound **12g** was prepared from **8o** (0.15 g, 0.30 mmol) and caffeic acid (57.0 mg, 0.32 mmol) by the procedure as described for **12e** as off-white solid (0.10 g, 60%), mp: 223.7 °C (dec.). $^1$H NMR (500 MHz) δ 9.37–9.16 (m, 2H), 7.68 (s, 1H), 7.46–7.30 (m, 3H), 7.26 (s, 2H), 7.02 (d, J = 13.7 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.76 (d, *J* = 1.9 Hz, 1H), 6.31 (d, *J* = 15.9 Hz, 1H), 4.47 (dd, *J* = 9.0, 3.5 Hz, 1H), 4.10 (t, *J* = 6.1 Hz, 2H), 3.76–3.70 (m, 1H), 3.38–3.31 (m, 2H), 1.42 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.0, 162.9, 162.5, 162.1, 156.2, 152.2, 150.3, 143.1, 139.6, 126.3, 123.8, 121.5, 117.2, 116.2, 110.1, 72.8, 60.8, 57.4, 46.1, 18.5. HRMS: calcd for $C_{20}H_{21}N_6O_9S_2$ $[M-H]^-$ 553.0805, found 553.0819.

*4.3.8 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[2-[2-(3,4-dihydroxyphenyl)acetamido]ethoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**12h**).* Compound **12h** was prepared from **8o** (0.10 g, 0.20 mmol) and 3,4-dihydroxy phenylacetic acid (37.0 mg, 0.22 mmol) by the procedure as described for **12e** as off-white solid (73.8 mg, 68%), mp: 194.5–195.4 °C. $^1$H NMR (500 MHz) δ 9.26 (d, *J* = 7.6 Hz, 1H), 7.96 (s, 1H), 7.41 (s, 2H), 6.77 (s, 1H), 6.67 (d, *J* = 1.6 Hz, 1H), 6.63 (d, *J* = 7.9 Hz, 1H), 6.49 (d, *J* = 7.8 Hz, 1H), 4.46 (dd, *J* = 7.7, 2.4 Hz, 1H), 4.03 (s, 2H), 3.74–3.71 (m, 1H), 3.30 (d, *J* = 5.5 Hz, 2H), 3.23 (s, 2H), 1.42 (d, *J* = 6.0 Hz,

3H). $^{13}$C NMR (151 MHz) δ 173.2, 171.6, 169.1, 162.9, 162.6, 145.3, 144.2, 127.5, 120.3, 116.9, 115.8, 110.2, 73.0, 60.8, 57.4, 49.1, 42.5, 38.7, 18.5. HRMS: calcd for $C_{19}H_{23}N_6O_9S_2$ $[M+H]^+$ 543.0962, found 543.0963.

*4.3.9 (2S,3S)-3-{(Z)-2-[[2-[(R)-2-Amino-3-(1H-indol-3-yl)propanamido]ethoxy] imino]-2-(2-aminothiazol-4-yl)acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (12i)*. Compound **12i** was prepared from **8o** (0.15 g, 0.30 mmol) and *N*-(tert-butoxy carbonyl)-D-tryptophan (0.10 g, 0.33 mmol) by the procedure as described for **12a** as off-white solid (92 mg, 53%), mp: 156.9–158.5 °C. $^1$H NMR (500 MHz) δ 10.98 (d, *J* = 1.8 Hz, 1H), 9.31 (d, *J* = 7.8 Hz, 1H), 8.49 (t, *J* = 5.6 Hz, 1H), 8.08 (d, *J* = 4.3 Hz, 4H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.23 (d, *J* = 2.3 Hz, 1H), 7.09 (t, *J* = 7.2 Hz, 1H), 7.01 (t, *J* = 7.2 Hz, 1H), 6.89 (s, 1H), 4.47 (dd, *J* = 7.8, 2.5 Hz, 1H), 4.13–3.92 (m, 3H), 3.78–3.74 (m, 1H), 3.38 (dd, *J* = 11.2, 5.6 Hz, 2H), 3.23–3.12 (m, 2H), 1.42 (t, *J* = 8.8 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.7, 169.2, 162.6, 159.0, 158.8, 136.7, 127.6, 125.4, 121.6, 119.0, 118.9, 117.0, 115.0, 111.9, 110.9, 107.4, 72.8, 60.8, 57.5, 53.4, 38.8, 27.7, 18.5. HRMS: calcd for $C_{22}H_{27}N_8O_7S_2$ $[M+H]^+$ 579.1439, found 579.1439.

*4.3.10 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[2-[(E)-3-(pyridin-4-yl)acrylamido] ethoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (12j)*. Compound **12j** was prepared from **8o** (0.15 g, 0.30 mmol) and 4-pyridineacrylic acid (49.2 mg, 0.33 mmol) according to the procedure as described for **12e** as off-white solid (72.2 mg, 46%), mp: 175.4–176.5 °C. $^1$H NMR (600 MHz) δ 9.24 (d, *J* = 8.1 Hz, 1H), 8.61 (d, *J* = 4.5 Hz, 2H), 8.16 (s, 1H), 7.70 (d, *J* = 4.2 Hz, 2H), 7.43 (d, *J* = 15.8 Hz, 1H), 7.25 (s, 2H), 6.96 (d, *J* = 15.8 Hz, 1H), 6.77 (s, 1H), 4.51 (d, *J* = 6.0 Hz, 1H), 4.11 (s, 2H), 3.75 (m, 1H), 3.55–3.40 (m, 3H), 1.40 (d, *J* = 5.9 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.0, 164.9, 163.0, 162.8, 150.5, 149.7(2), 142.9, 136.7, 127.7, 122.8(2), 110.2,

73.0, 60.7, 57.5, 38.8, 18.4. HRMS: calcd for $C_{19}H_{22}N_7O_7S_2$ $[M+H]^+$ 524.1017, found 524.1025.

*4.3.11 (2S,3S)-3-{(Z)-2-(2-Aminothiazol-4-yl)-2-[[2-(pyrimidine-4-carboxamido) ethoxy]imino]acetamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**12k**)*. Compound **12k** was prepared from **8o** (0.15 g, 0.30 mmol) and 4-pyrimidinecarboxylic acid (39.7 mg, 0.32 mmol) according to the procedure as described for **12e** as off-white solid (80.8 mg, 54%), mp: 185 °C (dec.). $^1$H NMR (500 MHz) δ 9.37 (s, 1H), 9.27 (d, *J* = 8.1 Hz, 1H), 9.10 (t, *J* = 5.9 Hz, 1H), 9.06 (d, *J* = 5.0 Hz, 1H), 8.03 (d, *J* = 5.0 Hz, 1H), 7.26 (s, 2H), 6.73 (s, 1H), 4.47 (dd, *J* = 8.1, 2.5 Hz, 1H), 4.23–4.15 (m, 2H), 3.82–3.77 (m, 1H), 3.68–3.49 (m, 2H), 1.33 (d, *J* = 6.1 Hz, 3H). $^{13}$C NMR (151 MHz) δ 169.0, 163.3, 162.9, 162.7, 160.1, 158.5, 156.7, 150.4, 143.1, 118.9, 110.0, 72.7, 60.7, 57.3, 39.3, 18.4. HRMS: calcd for $C_{16}H_{19}N_8O_7S_2$ $[M+H]^+$ 499.0813, found 499.0818.

**4.4 General procedures for synthesis of compounds 22a–c**

*4.4.1 (2S,3S)-3-{(S,Z)-5-(tert-Butoxycarbonyl)-7-oxo-11-[2-(tritylamino)thiazol-4-yl]-9-oxa-2-thia-6,10-diazadodec-10-enamido}-2-methyl-4-oxoazetidine-1-sulfonic acid (**21a**)*. Chloroacetyl chloride (0.29 g, 2.53 mmol) was added to a solution of (S)-1-(*tert*-butoxy)-4-(methylthio)-1-oxobutan-2-aminium chloride **13** (0.47 g, 1.94 mmol) and DIEA (0.63 g, 4.86 mmol) in DCM (10 mL) dropwise at 0 °C. After stirring for 5 min, the reaction mixture was allowed to warm to r.t.. Extracted with EA (10 mL×3) and combined organic layers was dried over anhydrous sodium sulfate, filtrated and concentrated under vacuum to give a brown oil residue **14** (0.51 g, 93%).

$K_2CO_3$ (0.32 g, 2.33 mmol) was added to a solution of PhtNOH (0.35 g, 2.14 mmol) in DMF (5.0 mL) . The reaction mixture was allowed to stir at r.t. for 30 min, then there was plenty of solid precipitated out of solution. Compound **14** in DMF (1.0

mL) was added to above reaction mixture. After stirring at 40 °C overnight, the reaction mixture was allowed to cool to r.t.. Water (10.0 mL) was added dropwise into the mixture, white solid precipitated out, filtered and collected the cake to give the product **15** (0.83 g, 95%).

Hydrazine hydrate (1 mL) was added to the solution of compound **15** (0.83 g) in DCM (5.0 mL). The mixture was stirred at r.t. for 6 h, the filtered. The filtration was washed with water (10 mL×3) and the organics were combined and evaporated to give the oil **16** (0.57 g).

To a solution of key intermediate **19** (0.56 g, 1.37 mmol) in MeOH (5.0 mL) was added the above hydroxylamine **16** (0.6 g, 2.16 mmol), then stirred at r.t. for 1 h. Concentrated the reaction mixture to afford the light-yellow residue. The residue was purified by flash column chromatography (eluent: from 0–12% of MeOH in DCM) to afford the intermediate **20** as white solid (0.47 g, 51%).

To a solution of **20** (0.30 g, 0.44 mmol) in anhydrous DMF (6 mL) was added DIC (95.0 mg, 0.76 mmol) and HOBT (0.1 g, 0.76 mmol) successively. After stirred at r.t. for 1 h, a solution of **6** (0.11 g, 0.58 mmol) in dry DMF (2.0 mL) with absolute triethylamine (0.14 g) was added thereto, then stirred at r.t. overnight. The mixture was diluted with water (20 mL) and extracted with EA (50 mL×3). The organic layers were combined and washed with brine (50 mL×3), concentrated under vacuum to give a brown oily residue. The residue was purified via flash column chromatography (eluent: from 0–10% of methanol in DCM) to afford **21a** (0.23 g, 78%) as foamy solid. $^1$H NMR (400 MHz) δ 9.53 (d, $J$ = 7.7 Hz, 1H), 8.88 (s, 1H), 7.77 (d, $J$ = 8.0 Hz, 1H), 7.36–7.22 (m, 15H), 6.83 (s, 1H), 4.55 (q, $J$ = 15.9 Hz, 2H), 4.41 (dd, $J$ = 7.7, 2.4 Hz, 1H), 4.36–4.31 (m, 1H), 3.67–3.62 (m, 1H), 2.47–2.34 (m, 2H), 1.96 (s, 3H), 1.94–1.84 (m, 2H), 1.50–1.30 (m, 12H).

*4.4.2 (S)-2-{2-[[(Z)-[1-(2-Aminothiazol-4-yl)-2-[[(2S,3S)-2-methyl-4-oxo-1-sulfo azetidin-3-yl]amino]-2-oxoethylidene]amino]oxy]acetamido}-4-(methylthio)butanoic acid (**22a**)*. Compound **22a** was prepared from **21a** (0.20 g, 0.23 mmol) according to the procedure as described for **12f** as off-white solid (87.5 mg, 68%), mp: 156.9–158.1 °C. $^1$H NMR (400 MHz) δ 9.61 (d, *J* = 7.3 Hz, 1H), 7.80 (d, *J* = 7.7 Hz, 1H), 6.89 (s, 1H), 4.77–4.32 (m, 4H), 3.79–3.67 (m, 1H), 2.48–2.34 (m, 2H), 2.00–1.92 (m, 5H), 1.44 (d, *J* = 4.7 Hz, 3H). $^{13}$C NMR (151 MHz) δ 173.3, 169.6, 169.1, 162.0, 146.4, 128.8, 111.8, 73.4, 60.8, 57.5, 51.1, 31.1, 30.1, 18.5, 15.0. HRMS: calcd for $C_{16}H_{23}N_6O_9S_3$ $[M+H]^+$ 539.0683, found 539.0689.

*4.4.3 (S)-2-{2-[[(Z)-[1-(2-Aminothiazol-4-yl)-2-[[(2S,3S)-2-methyl-4-oxo-1-sulfo azetidin-3-yl]amino]-2-oxoethylidene]amino]oxy]acetamido}-2-phenylacetic acid (**22b**)*. Compound **22b** was prepared according to the procedure as described for **22a** as off-white solid (0.10 g, 32% overall yield), mp: 178.8–180.7 °C. $^1$H NMR (400 MHz) δ 9.58 (d, *J* = 7.5 Hz, 1H), 8.36 (d, *J* = 7.6 Hz, 1H), 7.46–7.28 (m, 7H), 6.90 (s, 1H), 5.45 (d, J = 7.4 Hz, 1H), 4.66 (q, *J* = 15.6 Hz, 2H), 4.48 (d, J = 5.8 Hz, 1H), 3.73 (d, *J* = 5.7 Hz, 1H), 1.42 (d, *J* = 5.7 Hz, 3H). $^{13}$C NMR (151 MHz) δ 171.9, 171.9, 170.8, 169.5, 168.5, 162.1, 137.5, 137.4, 129.0, 129.0, 128.4, 128.0, 111.6, 73.2, 60.9, 57.5, 56.1, 18.5. HRMS: calcd for $C_{19}H_{21}N_6O_9S_2$ $[M+H]^+$ 541.0805, found 541.0812.

*4.4.4 (R)-2-{2-[[(Z)-[1-(2-Aminothiazol-4-yl)-2-[[(2S,3S)-2-methyl-4-oxo-1-sulfo azetidin-3-yl]amino]-2-oxoethylidene]amino]oxy]acetamido}-3-phenylpropanoic acid (**22c**)*. Compound **22c** was prepared according to the procedure as described for **22a** as off-white solid (90.5 mg, 34% overall yield), mp: 163.1–164.4 °C. $^1$H NMR (400 MHz) δ 9.71 (d, *J* = 7.8 Hz, 1H), 7.86 (d, *J* = 8.3 Hz, 1H), 7.37–7.08 (m, 7H), 6.97 (s, 1H), 4.67–4.39 (m, 4H), 3.77–3.72 (m, 1H), 3.14–3.07 (m, 1H), 2.91 (dd, *J* = 13.7, 9.9 Hz, 1H), 1.44 (d, *J* = 6.1 Hz, 2H). $^{13}$C NMR (151 MHz) δ 173.0, 170.8, 169.7,

168.9, 162.1, 137.9, 129.6, 128.7, 128.6, 126.9, 126.8, 111.87, 73.4, 60.8, 60.2, 57.3, 37.1, 18.5. HRMS: calcd for $C_{20}H_{23}N_6O_9S_2$ $[M+H]^+$ 555.0962, found 555.0967.

**4.5 Antimicrobial Assay**

MICs of the purified target compounds were determined 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 μg/mL as described by the Clinical Laboratory Standards Institute. Organisms used in this study included strains from the ATCC collection and clinical isolates from Chinese hospitals. AZN was used as positive control drug. The test medium used was Mueller-Hinton agar, and the inoculum was $10^4$ 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.

**4.6 Safety Evaluation Assay**

Cell suspensions (100 μL) of H460 cells at concentration of 50% confluence were seeded into the 96-well plates, and treated with various concentrations of compounds **8p** and **8r**. After incubation for 24 h, the MTT solution (20 μL, 1 mg/mL) was added into each well and incubated for 4 h at 37 °C with 5% $CO_2$. Subsequently, the culture supernatant was replaced with 150 μL DMSO. The optical density (OD) were measured at 550 and 630 nm using a microplate reader. The net absorbance ($OD_{630}$–$OD_{550}$) indicates the enzymatic activity of mitochondria and provides information on cell viability. Control cells were treated with 0.4 % (v/v) DMSO.

Female Kunming mice with weight of 20.0 g (±1.0 g) were fed with regular rodent chow and housed in an air-conditioned room. The mice were randomly divided into different groups with 8 mice each. Compounds **8p** and **8r** were given intravenously in a single-dosing experiment at 100, 200, 300 or 400 mg/kg,

respectively, and the control mice were given physiological saline with the same volumes. All mice were closely monitored for 7 days. Body weight as well as survival were monitored.

**4.7 Molecule Docking Assay**

The ligand–receptor docking was performed with Discovery Studio 4.5 software using the default settings. The PBP3 crystal structure (PDB code: 3PBS, resolution: 2.0 Å) was recovered from the RCSB Protein Data Bank (http://www.rcsb.org/structure/3PBS). 3D structures of compounds **8p**/**8r** were generated and energy minimization was performed. Before docking, protein structure and ligand were prepared. In the course of protein preparation, ligands and water molecules were removed from the complex. CDOCKER program was employed. The docking conformation with the highest docking score was finally chosen to evaluate the affinity of the receptor–ligand interaction.

**4.8 Studies for Synergy**

CAMH (cation-adjusted Mueller-Hinton) broth was used for checkerboard assay. Colony counts were determined using tryptic soy agar (TSA; BD, Cockeysville, MD) plates. The combinations including AZN-Avi, **8p**-Avi, and **8r**-Avi were evaluated on two drug-resistant *K. pneumoniae* strains. One was ESBLs-producing strain ATCC 700603, and the other was NDM-1 producing-strain ATCC BAA-2146, using microdilution method. Briefly, final concentrations of $5\times10^5$ CFU/mL of bacterial inocula were added to wells of 96-well microtiter plates containing two-fold diluted Avi and the other antimicrobial AZN, **8p** or **8r** in CAMH broth. The concentration ranges of compounds tested were 0.125−128 μg/mL for **8p** and **8r**, and 0.5−32 μg/mL for Avi. After incubating at 35 °C for 18 h, the combined effects of Avi with each antibacterial were analyzed by calculating FICI value as follows: FICI = (MIC of drug

A in the combination/MIC of drug A alone) + (MIC of drug B in the combination/MIC of drug B alone). The combination of two compounds was demonstrated to be synergistic when the value of FICI was ≤ 0.5; indifferent when 0.5 < FICI < 4; antagonistic when FICI ≥4 [24]. The experiments were performed in duplicate on different days.

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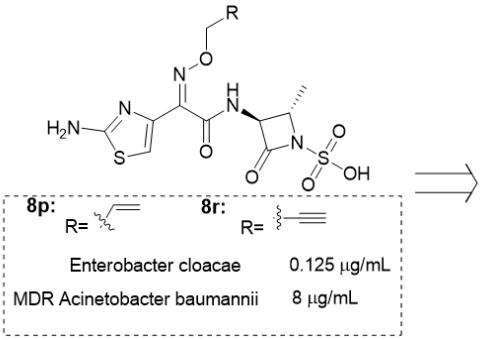
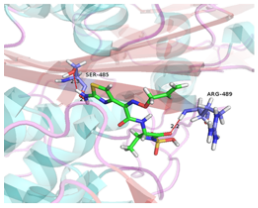
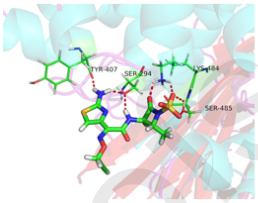
## Graphical abstract





| *K. pneumoniae* Strain | alone | | | | combination | | |
|---|---|---|---|---|---|---|---|
| | 8p | 8r | Avi | AZN | 8p (+Avi) | 8r (+Avi) | AZN (+Avi) |
| 700603 ESBLs (+) | 4 | >128 | 32 | 4 | 0.5 | 0.5 | 0.5 |
| 2146 NDM-1(+) | >128 | >128 | 32 | 64 | 0.5 | 0.5 | 0.5 |

**Highlights**

- 34 new monobactam derivatives are synthesized and evaluated for anti- Gram-negative bacteria.
- Compounds **8p**, **8r** showed comparable activities with AZN, with MIC values of 0.125−32 μg/mL.
- They exhibited good synergistic effect on enzyme-producing $G^-$ bacteria combined with Avibactam.
- Compounds **8p, 8r** displayed excellent safety profiles both *in vitro* and *in vivo*.

# Synthesis and antibacterial evaluation against resistant Gram-negative bacteria of monobactams bearing various substituents on oxime residue

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**Conflict of interest**

The authors declare no conflict of interest.