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Optimization of novel monobactams with activity against carbapenem-resistant *Enterobacteriaceae* – Identification of LYS228

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ABSTRACT

Metallo-β-lactamases (MBLs), such as New Delhi metallo-β-lactamase (NDM-1) have spread world-wide and present a serious threat. Expression of MBLs confers resistance in Gram-negative bacteria to all classes of β-lactam antibiotics, with the exception of monobactams, which are intrinsically stable to MBLs. However, existing first generation monobactam drugs like aztreonam have limited clinical utility against MBL-expressing strains because they are impacted by serine β-lactamases (SBLs), which are often co-expressed in clinical isolates. Here, we optimized novel monobactams for stability against SBLs, which led to the identification of LYS228 (compound **31**). LYS228 is potent in the presence of all classes of β-lactamases and shows potent activity against carbapenem-resistant isolates of *Enterobacteriaceae* (CRE).

Infections caused by multi drug-resistant Gram-negative bacteria, such as carbapenem-resistant *Enterobacteriaceae* (CRE), are increasing in prevalence, are difficult to treat and present a serious public health threat.[1] While this threat may be addressed by the development of new antibiotic scaffolds against novel targets, that approach has been hampered by issues with compound permeability and efflux in Gram-negative organisms, a high bar for safety due to high projected efficacious doses and by resistance development against single-gene target therapies.[2] These risks can be mitigated by modification of a clinically validated scaffold against a validated target, if emerging resistance can be addressed.

β-Lactam antibiotics represent one of the most successful classes of drugs, with over 80 new chemical entities launched. They are generally safe and effective, but their utility against Gram-negative organisms has eroded due to development of resistance mediated by the expression of β-lactamases.[3] Mutations in the penicillin binding proteins (PBPs) have also been reported, but are not common.[4] In recent years, an increase in clinical strains able to degrade carbapenems through the expression of carbapenemases has been observed. This is alarming since carbapenems are frequently employed as the agents of last resort against multi

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drug-resistant (MDR) Gram-negative pathogens. Carbapenemases fall in two broad classes, the serine-type (e.g., *Klebsiella pneumonia* carbapenemase, KPC) and metallo-β-lactamases (MBLs) (e.g., New Delhi MBL, NDM-1). MBLs are especially worrisome, because they inactivate all classes of β-lactams, with the exception of the monocyclic β-lactams, such as monobactams.[5,6] Recently, increased research has focused on the discovery of inhibitors of MBLs, but, so far none have entered the clinical pipeline.

Aztreonam, the only monobactam drug registered in the United States and Europe, is stable to MBLs, but susceptible to many serine β-lactamases (SBLs). This limits its clinical use, as SBLs and MBLs are frequently co-expressed in clinical isolates of *Enterobacteriaceae*.[7] A combination of aztreonam with avibactam is currently undergoing Phase 2 clinical trials, and is expected to provide a valuable treatment option for organisms expressing SBLs and MBLs. We realized an opportunity to increase the stability of monobactams to SBLs, through structural modification of the monobactam (Fig. 1).

This approach allows for single agent coverage of SBL- and MBL-producers with the option of extending clinical usefulness in the face of evolving β-lactamase-mediated resistance by addition of a β-lactamase inhibitor in the future, if needed, as part of drug life cycle management.

Previous literature reports indicated that 4-*cis* substituted analogs of monobactams, such as carumonam (Takeda) and BO-1158 (Banyu Pharmaceutical Co.) (Fig. 2) are more stable to SBLs, relative to aztreonam.[8,9] An exception to this trend was noted for a β-lactamase from *Morganella morganii*, where the trans analogs seemed to be less impacted than *cis* analogs.[10] However, most of the current clinically relevant β-lactamases were not known at the time of these studies. To assess the stability of monobactams against a wider variety of current clinically relevant β-lactamases, we created a library of isogenic *E. coli* strains expressing individual β-lactamases from multi-copy plasmids. This approach allows data to be rapidly generated for a high number of compounds against many β-lactamases.[11]

We selected representative β-lactamases from over 1300 reported enzymes[3] based on prevalence in clinical isolates and functional relevance, in particular carbapenemases and extended spectrum β-lactamases (ESBLs), which were known to degrade aztreonam and other β-lactam drugs. First we profiled a set of known monocyclic β-lactams (Fig. 2) and imipenem in our isogenic strain panel. Representative data are provided in Table 1.

Expression of the carbapenemases NDM-1, KPC-2 and OXA-48 caused a shift in the minimal inhibitory concentration (MIC) for

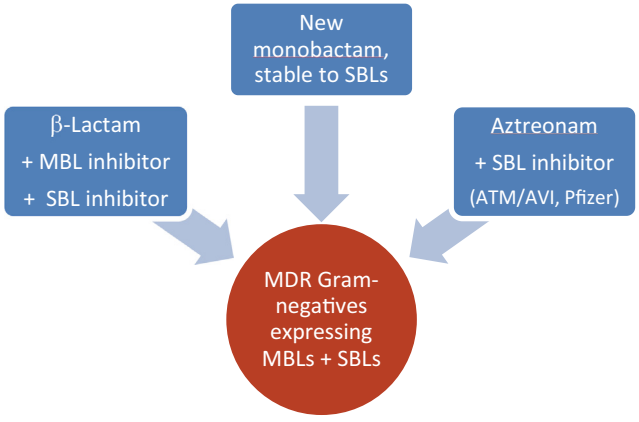

Fig. 1. Approaches considered for addressing resistance in MBL-expressing Gram-negative organisms. MBL, metallo-β-lactamases; SBL, serine β-lactamases; ATM, aztreonam; AVI, avibactam; MDR, multi drug-resistant.

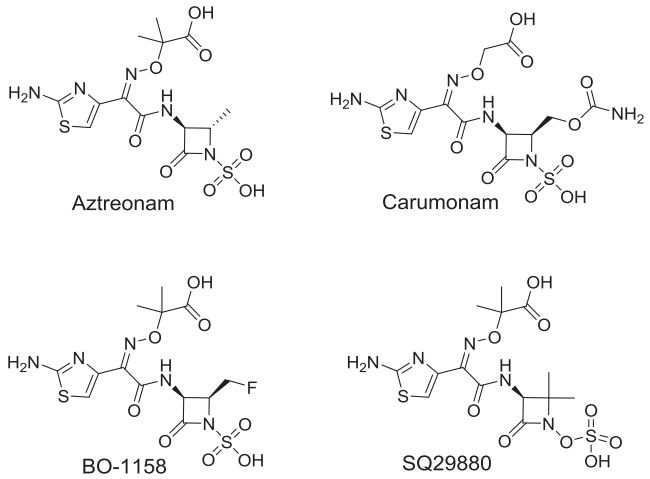

Fig. 2. Monocyclic β-lactam drugs and reference compounds.

imipenem relative to the activity against the parent strain, confirming expression of functional carbapenemases in these strains. All monocyclic β-lactams were found to be stable to MBLs, including NDM-1. Aztreonam was strongly affected by expression of ESBLs, like SHV-12 and CTX-M-15, the serine carbapenemase KPC-2, and to a lesser extent by class C enzymes like AmpC. The 4-*cis* substituted analogs carumonam and BO-1158 were less affected by KPC-2 and CTX-M-15 than aztreonam, but remained highly susceptible to SHV-12, with similar susceptibility to AmpC as aztreonam. The 4-dimethyl sulfactam SQ29880 appeared stable to KPC-2 and AmpC and was minimally affected by CTX-M-15, but was highly susceptible to SHV-12. We chose not to explore sulfactams like SQ29880 further, because of the significantly lower hydrolytic chemical stability relative to monobactams, which we considered a risk for modifications of the sulfactam core. None of the monocyclic β-lactams were affected by the class D carbapenemase OXA-48. Data for additional β-lactamases are provided in Table S2, which shows that other class A enzymes of the TEM and VEB type, as well as other class C and some class D enzymes (e.g., OXA-146[12]) degraded aztreonam and carumonam as well.

These data indicated that class A and class C serine β-lactamases seemed to be the main liability of existing monobactam drugs. We therefore set out to determine if stability towards these enzymes could be improved in 4-*cis* substituted monobactams. We focused on KPCs, CTX-Ms, SHVs, TEMs, AmpC and CMY-2, which are frequently present in drug-resistant clinical strains of *Enterobacteriaceae*.[13,14] We chose not to utilize siderophore containing moieties in our design, because of the reported concerns for resistance development.[15–17] However, others are exploring this strategy, and recently Shionogi advanced a siderophore-containing cephalosporin (cefiderocol) into phase 3 studies, contesting the concerns for adaptation-mediated resistance.[18]

Initially, as part of an amide library, we prepared 4-substituted amide analogs **6** and **7**, and noticed that the *N*-methyl-substituted analog **7** was less affected than the parent **6** by SHV-12 and KPC-2 (Table 2).

However, this came with the cost of significantly reduced antibacterial activity for **7**. We hypothesized that steric effects may be important and that cyclizing the tertiary amide in **7** to a lactam may allow for fine tuning of activity and β-lactamase stability. The 4-, 5- and 6-membered lactams (compounds **8**–**10**) were prepared and the 5-membered lactam **9** was found to be optimal, with increased activity relative to **7**, while maintaining good β-lactamase stability, except against AmpC. This finding prompted us to explore broadly 5-membered heterocyclic substitution in the 4-position. The synthesis of the analogs is described in detail in the