Optimization of a β -Lactam Scaffold for Antibacterial Activity via the Inhibition of Bacterial Type I Signal Peptidase

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S Supporting Information

ABSTRACT: β -Lactam antibiotics, one of the most important class of human therapeutics, act via the inhibition of penicillin-binding proteins (PBPs). The unparalleled success in their development has inspired efforts to develop them as inhibitors of other targets. Bacterial type I signal peptidase is evolutionarily related to the PBPs, but the stereochemistry of its substrates and its catalytic mechanism suggest that β -lactams with the 5S stereochemistry, as opposed to the 5R stereochemistry of the traditional β -lactams, would be required for inhibition. We report the synthesis and evaluation of a variety of 5S penem derivatives and identify several with promising activity against both a Gram-positive and a Gram-negative bacterial pathogen. To our knowledge these are the first 5S β -lactams to possess significant antibacterial activity and the first β -lactams imparted with antibacterial activity via optimization of the inhibition of a target other than a PBP. Along with the privileged status of their scaffold and the promise of bacterial signal peptidase I (SPase) as a target, this activity makes these compounds promising leads for development as novel therapeutics.

KEYWORDS: SPase, secretion, penem, penetration, antimicrobial

Y. pestis MIC = 16 μ g/mL
S. epidermidis MIC = 4 μ g/mL
IC₅₀ = 1.8 μ g/mL
Human cell cytotoxicity > 128 μ g/mL

The discovery of the β -lactam penicillin in 1928 and the subsequent development of other antibiotics over the next decades revolutionized health care. Today, however, the therapeutic development pipeline has all but gone dry,¹ and with the relentless evolution of drug resistant bacteria and the very real potential for untreatable bacterial infections,² the discovery of new antibiotics, especially those with novel targets and activity against both Gram-positive and Gram-negative pathogens, has re-emerged as an urgent need.

The β -lactams were not only among the first antibiotics discovered, their scaffold has been the most successfully diversified, both in nature and the pharmaceutical industry, and these constitute the most widely used family of drugs to treat infections. The privileged status of the β -lactams, and the similarity between their targets, bacterial penicillin-binding proteins (PBPs), and other serine proteases,³ such as elastase,^{4,5} thrombin,⁶ chymase,^{7–10} human cytomegalovirus protease,^{11–14} prostate specific antigen,^{15,16} the cysteine protease 3C from rhinovirus,¹⁷ and bacterial ClpP¹⁸ has inspired efforts to develop them as inhibitors of these targets, as well. However, with few exceptions,^{12,19} success has only been demonstrated with *in vitro* biochemical inhibition assays, and no β -lactam with antibacterial activity has been discovered via the optimization of binding to a target other than a PBP.

The need to export proteins across the cytoplasmic membrane is shared by all bacteria and for most proteins this is accomplished by the general secretory pathway, the final step of which is the release of the mature proteins via the action of type I signal peptidase (SPase). SPase is essential,²⁰ and thus it

is highly conserved across bacteria, and correspondingly, inhibitors of SPase have the potential for broad-spectrum activity. Indeed, SPase has been appreciated as a promising target for antibiotic therapy for decades.^{21–30}

Interestingly, SPase is evolutionarily related to PBPs and both are Ser-Lys dyad proteases.³¹ However, PBPs catalyze *si*-face attack on D-amino acid substrates^{32–38} whereas SPase catalyzes *si*-face attack on L-amino acid substrates. This suggests that β -lactams with the 5S stereochemistry, as opposed to the 5R stereochemistry possessed by the canonical β -lactams, might inhibit SPase.

In the 1990s, SmithKline Beecham (SKB) Pharmaceuticals synthesized racemic mixtures of penems in an effort to discover SPase inhibitors. Upon the resynthesis of variants with *in vitro* activity, they found that the activity resided entirely with the 5S enantiomers.²⁷ A large number of variants with substituents at the C2, C3, and C6 positions were synthesized and examined, and although a number were found to inhibit the activity of purified SPase, none were found to have any useful *in vivo* activity against intact bacteria, and the efforts appear to have been terminated sometime before 1998. In 2003, Hu and co-workers reported their efforts to optimize the same 5S scaffold at Procter and Gamble Pharmaceuticals, in this case via cyclization of the C2 and C3 substituents.³⁹ Again, while potent *in vitro* inhibitors were discovered, none possessed significant

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activity against intact bacteria, and these efforts also appear to have been abandoned

The structure of *E. coli* SPase bound to the allyl-protected 5S penem **1**, one of the most potent 5S β -lactam variants discovered at SKB, has been reported (Figure 1).⁴⁰ The

Figure 1. (A) Structure of compound **1**. (B) Structure of SPase complex with 5S penem **1** (PDB ID 1B12) with C2 indicated.

structure confirmed the *si*-face attack of the catalytic Ser90 side chain. While the structure revealed multiple interactions between the penem dihydrothiazole ring and C6 acetoxyethyl substituent, it also revealed that a C2 substituent, not present in **1**, would be oriented into solvent. The C2 substituents of the conventional 5R β -lactams are similarly oriented into solvent when bound to a PBP. Nonetheless, substituents at this position have been identified that optimize activity of the 5R scaffold,^{41,42} suggesting that the scaffolds were at least in part limited by penetration through the cell wall and/or the Gram-negative outer membrane and that the substituents optimized penetration. Because their physicochemical properties are expected to be similar, and because the previous pharmaceutical efforts identified compounds with potent *in vitro* activity but no activity against intact bacteria, we suspected that the activity of the 5S penem scaffold might be similarly limited and thus might be optimized by similar substituents. While various C2 substituents were examined previously, few of these were analogous to those found to optimize the 5R scaffold. Thus, we were interested in exploring whether more similar substituents might optimize the 5S scaffold for antibacterial activity.

We first focused on confirming the SKB results and synthesized compound **2** (Scheme 1). Briefly, synthesis commenced with the three-step conversion of the commercially available azetidinone to the desired penem scaffold and then proceeded via photoisomerization, TBAF deprotection, and *m*-CPBA oxidation to produce the corresponding penem sulfoxide, which was then deprotected via zinc reduction (Supporting Information). With compound **2** in hand, half maximal inhibitory concentrations (IC_{50} 's) were determined with *Escherichia coli* SPase and the fluorogenic peptide substrate, (Dabcyl)-AGHDAHA↓SET-(EDANS) (where EDANS is the fluorescence donor, 5-((2-aminoethyl)amino)-naphthalene-1-sulfonic acid, and Dabcyl is the fluorescence acceptor, 4-(4-dimethylaminophenylazo)benzoic acid; the cleavage site is indicated with an arrow) (Table 1 and Supporting Information). Consistent with the SKB results, we observed that penem **2** inhibits purified *E. coli* SPase (IC_{50} = 3.0 μ g/mL). Using the standard CLSI broth microdilution assay,⁴³

Scheme 1. Synthesis of Compound **2**^a

^aReagents and conditions: (a) $h\nu$, toluene, 33% (RSM: 39%). (b) TBAF, AcOH, THF, 57%. (c) *m*-CPBA, CH_2Cl_2 , 78%. (d) Zn, AcOH, THF, 46% (RSM: 26%). (e) $Pd(PPh_3)_4$, dimedone, THF, 48%.

Table 1. Structures, Biochemical Data, and Antibacterial Data

Compound		IC_{50} (μ g/mL)	MIC (μ g/mL)	
			<i>S. epidermidis</i>	<i>Y. pestis</i>
2		2.99 $\pm$ 0.30	128	128
3		0.63 $\pm$ 0.22	16	16
4		1.04 $\pm$ 0.20	8	16
5		6.37 $\pm$ 2.76	>128	>128
6		3.95 $\pm$ 0.94	>128	>128
7		1.31 $\pm$ 0.30	16	32
8		1.16 $\pm$ 0.16	16	32
9		1.49 $\pm$ 0.09	8	32
10		0.96 $\pm$ 0.08	4	16
11		1.22 $\pm$ 0.19	32	64
12		0.80 $\pm$ 0.10	8	8
13		1.75 $\pm$ 0.47	4	16

we also confirmed that **2** has no activity against intact *E. coli* (strain MG1655).

To further explore the potential of the 5S penems, we extended our analysis to include the Gram-negative pathogen

Yersinia pestis (strain KIM6+) and the Gram-positive pathogen *Staphylococcus epidermidis* (strain RP26A), which had not been examined previously. Interestingly, we found that **2** had weak but detectable activity against both pathogens, inhibiting their growth with MICs of 128 $\mu\text{g/mL}$.

To begin to explore the potential optimization of this activity, we first synthesized the 5S penem derivative **3** (Scheme 2), which bears the C2 ethylamine substituent of thienamycin

Scheme 2. Synthesis of Compound **3**^a

^aReagents and conditions: (a) *m*-CPBA, CH_2Cl_2 , 68%. (b) NaOMe, NEt_3 , 82%. (c) TBAF, AcOH, THF, 81%. (d) $\text{Pd}(\text{PPh}_3)_4$, dimedone, THF, 50%.

and which adds a positive charge that could increase penetration through the cell wall or the Gram-negative outer membrane. The IC_{50} of **3** with *E. coli* SPase was found to be 0.63 $\mu\text{g/mL}$, 5-fold reduced relative to compound **2** (Table 1). More importantly, **3** showed 8-fold increased activity against both *Y. pestis* and *S. epidermidis* (MICs of 16 $\mu\text{g/mL}$; Table 1).

Encouraged by these results we synthesized and characterized derivatives **4**–**13** (Table 1), which possess a range of C2 substituents culled from previously developed 5R β -lactams or their derivatives. Each was prepared from the sulfoxide precursor and the corresponding thiol in a fashion analogous to that used to prepare **3** (Supporting Information). Conversion of **3** to **4**, which bears the imipenem-like amidine modification, resulted in a slightly reduced IC_{50} , and while it had no effect on activity against *Y. pestis*, it increased activity 2-fold against *S. epidermidis*. Simple addition of methyl groups to the added amine of **3** yielding the mono- and dimethylated derivatives **5** and **6**, resulted in a 10- and 6-fold reduced binding *in vitro* and a complete loss of antibacterial activity. The pyridine moiety of **7** resulted in a 2-fold decrease in IC_{50} , retention of activity against *S. epidermidis*, but a 2-fold loss in activity relative to **3** against *Y. pestis*. The sulfone side chain of **8**, drawn from the sulopenem structure, modestly reduced biochemical activity relative to **3** and showed indistinguishable antibacterial activity against both *S. epidermidis* and *Y. pestis*. Derivative **9**, with the C2 amine replaced with a hydroxy group, had 2-fold decreased biochemical activity, and relative to **3**, 2-fold increased and decreased antibacterial activity against *S. epidermidis* and *Y. pestis*. When the hydroxyl group was replaced with a carbamate group, the resulting derivative **10** had a similar biochemical and antibacterial activity against *Y. pestis*, as did **3**, but a 4-fold further increased antibacterial activity against *S. epidermidis* (MIC = 4 $\mu\text{g/mL}$).

Addition of the more elaborate meropenem C2 side chain, resulting in analog **11**, had a modest effect on biochemical activity and it resulted in a 4- and 8-fold decrease in antibacterial activity, relative to **3**. Truncation of the

meropenem side chain to its pyrrole unit (**12**) or its enantiomer (**13**) again had only modest effects on biochemical activity. However, relative to **3**, compound **12** showed 2-fold further increased activity against both *S. epidermidis* and *Y. pestis* and MICs of 8 $\mu\text{g/mL}$. While compound **13** showed the same activity against *Y. pestis*, it was 4-fold more active than **3** against *S. epidermidis*, inhibiting its growth with an MIC of 4 $\mu\text{g/mL}$.

In general, the C2 modifications had relatively modest effects on the inhibition of SPase biochemical activity *in vitro*, consistent with the structural data suggesting that they are disposed into solvent. However, we found that the parental scaffold had antibacterial activity against *Y. pestis* and *S. epidermidis* and that the modifications optimized this activity up to 32-fold. To confirm that the antibacterial activity of the 5S penems results from the inhibition of SPase, we examined the activity of the derivatives against *Y. pestis* strain KIM6+ harboring an arabinose inducible plasmid-borne copy of the gene encoding SPase.⁴⁴ As a positive control, we also examined the activity of arylomycin A-C₁₆, a semisynthetic member of the arylomycin class of natural product inhibitors of SPase^{25,45,46} currently under development,^{47–49} and as a negative control, the conventional 5R β -lactam PBP inhibitor imipenem. Without induction, the arylomycin and imipenem inhibited the growth of *Y. pestis* with an MIC of 4 and 1 $\mu\text{g/mL}$, respectively. With the addition of 2% arabinose, the MIC of imipenem was unchanged, consistent with activity that is independent of SPase, unlike the MIC of the arylomycin, which was shifted to 128 $\mu\text{g/mL}$.

Under identical conditions, the induction of SPase resulted in MICs of 64 $\mu\text{g/mL}$ for compounds **7** and **10**, and 32 $\mu\text{g/mL}$ for **8** and **12**, suggesting that as with arylomycin, their activity results from the inhibition of SPase, but since the reduction in activity is somewhat less than that observed with the arylomycin, it is possible that these compounds also inhibit another target. However, for derivatives **3**, **4**, **9**, and **13**, the induction of SPase resulted in an MIC of 128 $\mu\text{g/mL}$, identical to that observed with the arylomycin A-C₁₆ control, confirming that the activity of these derivatives results from the inhibition of SPase. Thus, **3**, **4**, **9**, and **13** are the first β -lactams imparted with significant antibacterial activity via optimization of the inhibition of a target other than a PBP.

To confirm that the activities of the 5S penems are specific for bacteria and to explore their potential toxicity, we examined their effects on human HEK293 cells. Consistent with their apparently SPase-specific effects, at concentrations up to 128 $\mu\text{g/mL}$, **3**, **4**, **9**, and **13** showed no effect on growth (Supporting Information). Thus, the reasonable antibacterial activity of these compounds appears to come with little toxicity.

SPase is a novel and promising target for antibacterial therapy, and there is no scaffold that has yielded more successful therapeutics than the β -lactam scaffold. Thus, the identification of β -lactams with reasonable activities against the Gram-positive and Gram-negative pathogens, *S. epidermidis* and *Y. pestis*, represents promising leads for further development. The most promising of these analogs seems to be **13**, which while having no apparent human cell toxicity, inhibits the growth of the Gram-positive and the Gram-negative pathogens with MICs of 4 and 16 $\mu\text{g/mL}$, respectively. As expected based on the crystallographic data, the C2 substituents explored had only modest effects on SPase binding and likely mediate their effects via altered penetration through the outer-membrane and/or cell wall. In contrast, C6-substituents are expected to access the substrate binding pocket, and thus, future effects will

focus on introducing such modifications to the C2-optimized derivatives discovered here.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00064.

Methods, supporting references, NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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