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Syntheses and biological evaluation of new cephalosporin-oxazolidinone conjugates[†]

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Abstract

Two cephalosporin-oxazolidinone conjugates were synthesized by incorporation of a carbamate linker at the 3'-position of the cephalosporin. These compounds show stability in aqueous media until specifically activated by a β -lactamase, and retain antibacterial activities profiles reflecting both the individual cephalosporin and oxazolidinone components.

Introduction

Since the discovery of penicillin, β -lactam antibiotics have been the most important family of antibacterial agents. Cephalosporins, a class of β -lactam antibiotics, are known to exert their biological activity by reacting with bacterial enzymes to open the β -lactam ring. This process is accompanied by liberation of the 3'-substituent, when the substituent can function as a leaving group (Scheme 1).¹ Examples are known where cytotoxic components, including mitomycin C,² doxorubicin,³ as well as antimicrobials, such as quinolones,⁴ have been incorporated at the 3' position. The cephalosporin-quinolone conjugates have been shown to exhibit a broad spectrum of antibacterial activity derived from both cephalosporin-like and quinolone-like components. These dual-action cepheids can also be used to overcome the destructive action of β -lactamases (β L) (Enz = β L, Scheme 1), a main cause of bacterial resistance to β -lactam antibiotics.⁵ While, as indicated, many cephalosporin prodrug conjugates have been described, linezolid (Fig. 1), a relatively new FDA approved drug for treatment of MRSA and VRE, or any of the related oxazolidinone antibiotics, have not been evaluated in an analogous fashion. Linezolid is the first oxazolidinone drug approved for the treatment of Gram-positive bacterial infections.⁶ Cephalosporin-oxazolidinone conjugates are anticipated to possess activity against both Gram-negative and Gram-positive bacteria. Herein we report the syntheses and biological evaluation of two cephalosporin-oxazolidinone congeners and demonstrate the controlled release of the oxazolidinone in the presence of a β -lactamase GES-II.

[†]Electronic Supplementary Information (ESI) available: Experimental procedures, characterization data and copies of ¹H NMR and ¹³C NMR spectra, protocols of antibacterial assays.

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Results and discussion

Based on the ample precedent mentioned earlier for the use of the β L-induced release process as depicted in Scheme 1, we considered using a carbamate linkage between the 3' position of a representative cephalosporin and piperazine-based linezolid analogs as depicted in Fig. 2. This strategy required the preparation and eventual conjugation of the appropriately functionalized cephalosporin and oxazolidinone components.

The syntheses of the component linezolid analogs **3a** and **3b** are shown in Scheme 2. The syntheses utilized considerable literature precedent⁷ and began with a S_NAr reaction of 3,4-difluoronitrobenzene and mono Boc-protected piperazine **4** to afford *para*-substituted nitrobenzene derivative **5** in 49% yield. Palladium catalyzed hydrogenation of compound **5** followed by reaction with CbzCl gave protected aniline **6**. Then, as reported,^{7a} compound **6** was sequentially treated with *n*BuLi and *R*-glycidyl butyrate at -78°C to form the key oxazolidinone **7** in 71% yield with defined stereochemistry at the C-5 position of ring A. Reaction of **7** with acetic anhydride in the presence of DMAP and pyridine afforded the corresponding C-5 acetate analog, **8**, in 95% yield. TFA-induced removal of the Boc group afforded the desired linezolid analog **3a** in 92% isolated yield. Alternatively, stepwise reaction of **7** with methanesulfonyl chloride, ammonia and acetyl chloride produced the acetamidomethyl-containing analog, **9**, in 61% overall yield for three steps. Analog **3b** was then obtained in good yield by treatment of **9** with TFA to remove the Boc group.

With oxazolidinones **3a** and **3b** in hand, the syntheses of carbamate-linked cephalosporin-oxazolidinone compounds **1** and **2** were carried out as shown in Scheme 3. The syntheses began with a controlled hydrolysis to remove the acetyl group from the 3'-hydroxymethyl substituent of commercially available 7-amino cephalosporanic acid (7-ACA). Reaction with phenylacetyl chloride followed by treatment with freshly prepared diphenyldiazomethane⁸ provided cephem **10** in 29% overall yield for three steps.⁹ Subsequent reaction with tetrachloroethyl chloroformate gave intermediate carbonate **11** in 81% yield. Reaction of the activated carbonate **11** with **3a** in the presence of pyridine and DMAP was attempted. Desired coupling product **12** was generated; however, as often during reactions of cephalosporins, partial isomerization of the cephem nucleus occurred,¹⁰ to give a nonseparable mixture of the ³ and ² double bond isomers (**12** and **12b**, Fig. 3). Cephalosporin ² isomers **12b** are not effective antibiotics^{10c,11} and also are not substrates for the planned β -lactamase induced prodrug process depicted earlier in Scheme 1. Further studies of the coupling reaction revealed that without any basic additives, reaction between **11** and **3a** still took place and provided the desired protected conjugate **12** in 40% yield. No ²–³ isomerization was observed. Subsequent removal of the benzhydryl protecting group from **12** gave the desired conjugate **1** in 28% yield after crystallization. With this approach validated, cephalosporin-oxazolidinone conjugate **2** was also synthesized in a similar manner, involving the formation of conjugate **13** with a C-5 acetamidomethyl substitute and subsequent removal of benzhydryl group of **13**.

To determine whether cephalosporin-oxazolidinone conjugates **1** and **2** could be specifically activated in the presence of β -lactamase as planned, a LC/MS assay was performed (Fig. 4).¹² Conjugates **1** and **2** were separately incubated with catalytic amounts of β -lactamase

GES-II (0.4 mol%) in phosphate buffered saline (50 mM, pH 7.0) at room temperature. We were pleased to find that in the presence of β -lactamase, conjugates **1** and **2** were completely cleaved to the hydrolyzed cephalosporin and the oxazolidinone components **3a** and **3b**, respectively, after 4 h. Control experiments were also conducted by treatment of **1** and **2** under the same conditions in the absence of β -lactamase. As expected, conjugates **1** and **2** were stable even after 24 h. These data demonstrate that compounds **1** and **2** are good substrates for β L GES-II and are stable in a neutral aqueous environment until specifically activated by the β -lactamase.

Cephalosporin-oxazolidinone conjugates **1** and **2**, as well as linezolid analogs **3a** and **3b**, and ciprofloxacin as a control, were tested for their antibacterial activities against various strains of Gram-positive and Gram-negative bacteria as well as *Mycobacterium vaccae*, using an agar diffusion assay (Table 1). Cephalosporin derivative **14** was synthesized from 7-ACA (Scheme 4) and included in the assay as an additional control. In general, conjugates **1** and **2** were found to retain similar biological profiles compared to cephem **14** as well as individual oxazolidinones **3a** and **3b**. More interestingly, for most organisms tested, conjugates **1** and **2** displayed extended activity relative to linezolid itself. For example, they exhibited good activity against the Gram-negative strain *Pseudomonas aeruginosa* K799/61, while linezolid was relatively inactive. Conjugates **1** and **2**, as well as components **3a** and **3b**, were also evaluated against MRSA and VRE, and good activity was observed. Again, conjugate and components matched in activity. All compounds, including conjugates **1** and **2**, as well as oxazolidinone **3a** and **3b**, also exhibited antimycobacterial activity and could potentially be useful for treatment of *M. tuberculosis* as they induced large inhibition zones against *M. vaccae*, a common model for *M. tuberculosis*.¹³ Interestingly, linezolid components **3a** and **3b** were roughly equipotent *in vitro* with linezolid against several Gram-positive organisms, including *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Micrococcus luteus*. These biological data suggested that the cephalosporin-oxazolidinone conjugates might possess additive biological activity corresponding to that of both the cephem and oxazolidinone components.

Conclusions

We have described straightforward syntheses of cephalosporin-oxazolidinone conjugates. The conjugates retained antibacterial activity comparable to individual cephalosporin and oxazolidinone components, and showed stability in aqueous media until specifically activated by a β -lactamase. Future work will focus on *in vitro* enzyme assays to examine the release of oxazolidinone in the presence of β -lactamase producing bacteria. Additional structural analogs are also under consideration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- The LC/MS analyses were carried out on a Waters ZQ instrument consisting of chromatography module Alliance HT, photodiode array detector 2996, and mass spectrometer Micromass ZQ, using a 3 × 50 mm Pro C18 YMC reverse phase column (Waters, Milford, MA, www.waters.com). Mobile phases: 10 mM ammonium acetate in HPLC grade water (A) and HPLC grade acetonitrile (B). A gradient was formed from 5% to 80% of B in 10 min at 0.7 mL/min. The MS electrospray source operated at capillary voltage 3.5 kV and a desolvation temperature 300 °C.
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Fig. 1.
Linezolid oxazolidinone antibiotic.

Fig. 2.
Cephalosporin-oxazolidinone conjugates.

Fig. 3.
General structures for Δ^3 – Δ^2 double bond cephem isomers.

Fig. 4. HPLC trace of compounds **1**, **2** and products **3a**, **3b** released by β -lactamase activation. Left to right: (*) **3a** (with β L, 4 h), (♦) **1** (no β L, 24 h), (●) **3b** (with β L, 4 h), (■) **2** (no β L, 24 h).

Scheme 1.

Drug-release process from cephalosporin nucleus

Scheme 2.
Syntheses of linezolid analogs **3a** and **3b**

Scheme 3. Syntheses of cephalosporin-oxazolidinone conjugates **1** and **2**

Scheme 4.
Synthesis of cephem **14**

Table 1

Antibacterial activity of conjugates **1** and **2** in the agar diffusion assay

Growth inhibition zones in mm (9 mm well diameter)											
Gram-positive bacteria						Gram-negative bacteria					
compds ^a	<i>B. subtilis</i> ATCC 6633 ^b	<i>S. aureus</i>			<i>E. faecalis</i> 1528 ^c VRE	<i>M. luteus</i> ATCC 10240 ^b	<i>P. aeruginosa</i>			<i>M. vaccae</i> IMET	
		SG 511 ^b	Efs1 ^c	Efs4 ^c			K799/WT ^c	K799/61 ^c	<i>E. coli</i> SG 458 ^b		
linezolid	33/37p ^d	35	34/39p	35/39p	42	34	0	10P	23/28p	52	
14	46/52p	39	43	35	13	23	11h ^f	23	35	0	
3a	24/26p	21	31	19/23P	29	25	15h	16/26h	13P	42	
3b	26	18	31/35p	14/18P	25	25	11P/18h	20/30h	14P	35	
1	43/47p	36	40	31	27	26	17h	22/31p	33	44	
2	42/45p	34	40	30	17	23	15P/20h	24/33h	33	30	
cipro	31	18	0	0	0	15	29	35	34	23	

^a Exactly 50 µL of a 2.0 or 1.0 mM solution in DMSO : MeOH (1 : 9) of each compound was filled in 9 mm wells in agar media (Standard I Nutrient Agar, Serva or Mueller Hinton II Agar, Becton, Dickinson and Company). Inhibition zones read after incubation at 37 °C for 24 h. Cipro (ciprofloxacin) was dissolved in H₂O to give a 5 µg/mL solution.

^b 1.0 mM solution of linezolid, **1–2**, **3a–3b**, and **14** was used.

^c 2.0 mM solution of linezolid, **1–2**, **3a–3b**, and **14** was used.

^d p, partially clear inhibition zone/colonies in the inhibition zone.

^e P, unclear inhibition zone/many colonies in the inhibition zone.

^f h, faint indication of inhibition zone.