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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP OF C-3 BENZOYLOXYMETHYL CEPHALOSPORINS EXHIBITING ANTI-MRSA ACTIVITIES

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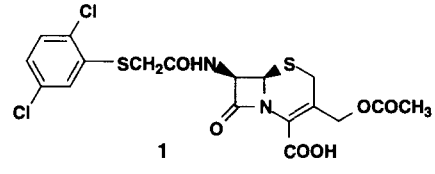
Abstract: A series of cephalosporins bearing C-3 benzoyloxymethyl groups were prepared and evaluated for their anti-MRSA activity and plasma stability. They exhibit excellent in vitro activity (MIC = 0.06 ~2 μg/mL) against MRSA and excellent stability in human plasma. © 1997 Elsevier Science Ltd.

Methicillin resistant *Staphylococcus aureus* (MRSA) is a gram positive organism responsible for common nosocomial infections. Since the first report on MRSA as an important clinical problem in the 1960's, the proportion of *S. aureus* resistant to methicillin has increased significantly over the past three decades.[2] Currently, vancomycin, a glycopeptide, is the drug of choice for the treatment of infections caused by MRSA. Although vancomycin is highly effective against MRSA, there is an urgent need for new antibiotics as alternative therapy due to the alarming potential for the emergence of vancomycin resistant *S. aureus* strains.[3]

For the above reasons, we have been investigating a new class of anti-MRSA cephalosporins. In our early studies, we found that C-3 acetoxymethyl cephalosporin **1**[4] (Figure 1) exhibits good in vitro (MIC = 2 μg/mL) and in vivo (PD_{50} = 2 mg/kg) activity against MRSA (strain A 27223).[5] However, a C-3 acetoxymethyl group has been a concern for metabolism due to its susceptibility to enzymatic hydrolysis.[6]

Figure 1

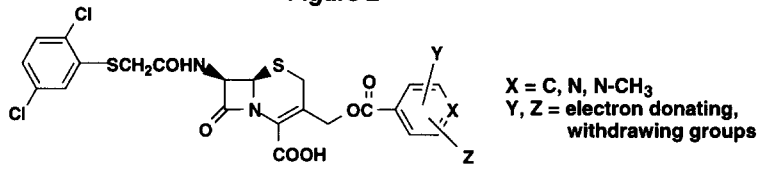


1

As an initial strategy to overcome this problem, we investigated a series of C-3 benzoyloxymethyl cephalosporin derivatives. Takaya et al. reported that C-3 benzoyloxymethyl cephalosporins bearing a C-7

aminothiazolyl side chain are more stable to enzymatic cleavage than the corresponding C-3 acetoxymethyl cephalosporins.[7] Therefore, we were hopeful that C-3 benzoyloxymethyl cephalosporins bearing 2,5-dichlorophenylthioacetamido C-7 side chain would improve the metabolic stability of **1**. Our challenge was to identify a C-3 benzoyloxymethyl cephalosporin possessing good anti-MRSA activity as well as acceptable plasma stability. Herein we report the synthesis and the in vitro anti-MRSA activity of the C-3 benzoyloxymethyl cephalosporins as well as the stability in human plasma for selected derivatives (Figure 2).

Figure 2



Chemistry

The cephalosporin derivatives in Table 1 were prepared by an efficient five step synthetic route as described in Scheme 1. The C-7 amino cephalosporin **2**[8] and the 2,5-dichlorophenylthioacetic acid **3**[9] were coupled using DCC to produce the cephalosporin **4**. Compound **4** was then oxidized to the sulfoxide **5** in order to avoid the isomerization of the cephem double bond.[10, 11] We improved the yield of the conversion of **5** to **6** significantly by utilizing dibenzo-18-crown-6, which presumably accelerated the reaction by generating a naked carboxylate anion.[12] Reduction of the sulfoxide **6** to the corresponding sulfide **7**,[10] followed by deprotection of DPM (diphenylmethyl) ester afforded the C-3 benzoyloxymethyl cephalosporins **8** in good yields.

Results and Discussion

The in vitro activity of the C-3 benzoyloxymethyl cephalosporins was evaluated by determination of minimum inhibitory concentration (MIC) values by utilizing the standard broth dilution method. The in vivo activity was evaluated by using a MRSA systemic infection model in mice and was expressed by protective dose (PD_{50}). The in vitro plasma stability was evaluated by incubating a cephalosporin in human plasma at 37 °C and was expressed as a half-life.

We initially investigated the effect of varying the nature of the para-substituent of the benzoate group on the anti-MRSA activity (Table 1). We were also interested in pyridyl substituted esters such as isonicotinic (**8j**) and nicotinic (**8k**) esters. In addition to the unique electronic properties of a pyridyl group, this pyridyl derivatives provide a pathway into another class of cephalosporins, namely quaternized cephalosporins (**8l** and **8m**).[13]

All C-3 benzoyloxymethyl cephalosporins (**8a** ~ **8i**) in Table 1 exhibit excellent in vitro activity against MSSA and MRSA with MICs ranging from 0.007 to 2 μg/mL. The in vitro activity of these cephalosporins is much improved compared to that of C-3 acetoxymethyl cephalosporin **1**. It appears that the increased lipophilicity at C-3 side chain contributes to the improved activity of the C-3 benzoyloxymethyl cephalosporins. The electronic effect of the para substituent on the benzoate group has a slight to moderate influence on the anti-MRSA activity. Cephalosporins with electron donating groups (**8f**, **8g**, **8h** and **8i**) are two to eight fold more active in vitro against MRSA than the unsubstituted analog (**8a**) and those with electron withdrawing groups (**8b**, **8c** and **8d**). However, **8e**, possessing an electron withdrawing acetyl group on the phenyl ring, exhibits exceptional in vitro activity which is comparable to that of cephalosporins with electron donating groups. It is possible that the acetyl group is readily hydrolized to the corresponding phenol (**8i**).

Scheme 1

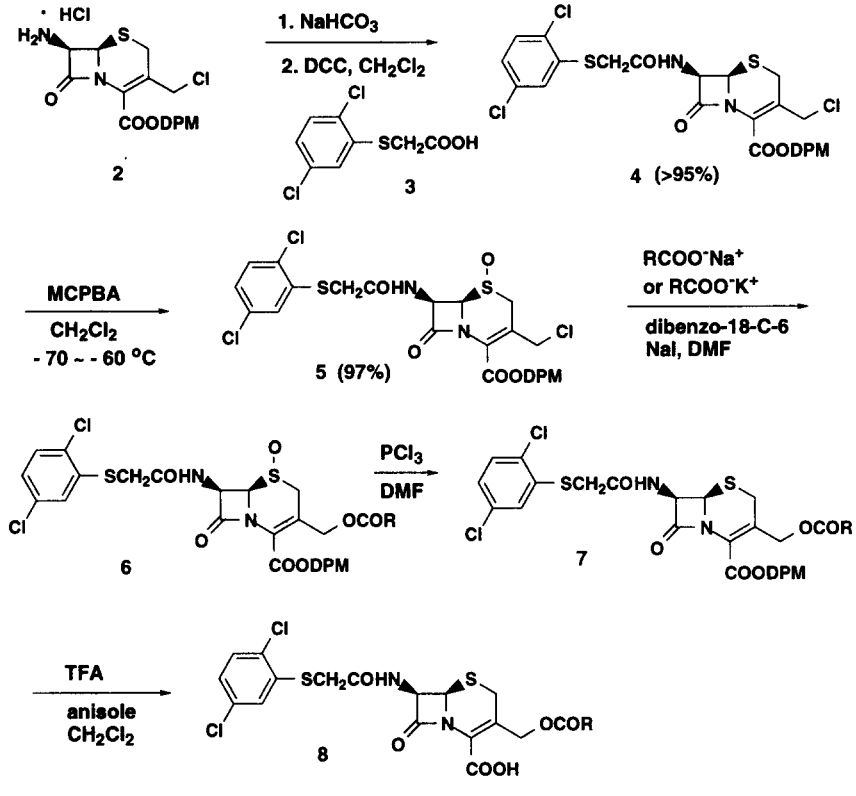
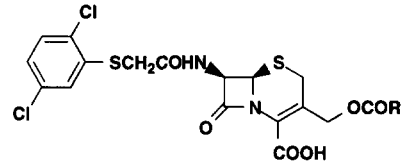
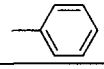
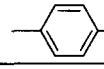
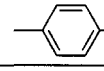
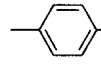
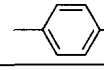
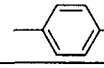
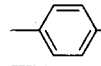
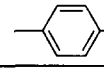
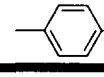
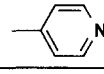
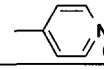


Table 1



	R	MIC (μg/mL)				
		MSSA (A15090)	MRSA (A27217)[14]	MRSA (A27218)[15]	MRSA (A27223)[5]	MRSA (A27621)[16]
8a		0.06	1	0.5	1	1
8b	 NHCOCH$_3$	0.06	0.5	0.5	1	1
8c	 CN	0.03	0.125	0.125	1	0.5
8d	 NO$_2$	0.06	0.25	0.25	2	2
8e	 OCOCH$_3$	0.007	0.06	0.06	0.125	0.125
8f	 CH$_3$	0.125	0.5	0.06	0.5	0.25
8g	 OCH$_3$	0.06	0.06	0.125	0.5	0.5
8h	 N(CH$_3$)$_2$	0.06	0.125	0.125	0.25	0.25
8i	 OH	0.007	0.06	0.06	0.25	0.5
8j	 N	0.06	1	0.5	8	4
8k	 N	0.125	2	2	4	4
8l	 N- CH$_3$ ⊕	0.125	8	2	32	32
8m	 N ⊕ CH$_3$	0.5	64	32	64	64
ref.	vancomycin	0.25	0.25	0.25	0.25	0.25

MIC (minimum inhibitory concentration); MSSA (methicillin-susceptible *S. aureus*); MRSA (methicillin-resistant *S. aureus*)

All C-3 benzoyloxymethyl cephalosporins (**8a~8i**) exhibited moderate to poor in vivo anti-MRSA activity (PD_{50} = 6~25 mg/kg) in mice, regardless of the electronic nature of the substituent on the phenyl ring. As an effort to improve the in vivo activity, we examined the effect of the lipophilicity at C-3 side chain on in vivo activity by introducing a pyridyl group as in **8j** and **8k**. We further decreased lipophilicity at C-3 side chain by quaternizing the pyridyl nitrogen atom in **8j** and **8k** to generate the C-3 quaternary cephalosporins **8l** and **8m**, respectively. However, none of these compounds exhibits acceptable anti-MRSA activity in vitro and in vivo.

Two cephalosporins (**8a** and **8h**) were evaluated for their stability in human plasma. The half-life of **8a** and **8h** is > 600 min. in human plasma (in vitro), whereas that of C-3 acetoxymethyl cephalosporin **1** is ~250 min. It appears that the C-3 benzoyloxymethyl cephalosporins bearing 2,5-dichlorophenylthioacetamido C-7 side chain are more stable to enzymatic hydrolysis than the corresponding C-3 acetoxymethyl cephalosporin.

In summary, C-3 benzoyloxymethyl cephalosporins are more potent in vitro than C-3 acetoxymethyl cephalosporin against MRSA, presumably due to the increased lipophilicity at C-3 side chain. They also exhibit improved in vitro stability in human plasma.

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References and Notes

1. Current Address: Mitotix, Inc., Cambridge, MA 02139
2. Gayner, R. P.; Culver, D. H.; Horan, T. C.; Henderson, T. S.; Martone, W. J. *Infectious Disease in Clinical Practice* **1993**, 2, 452.
3. Turik, M. A.; Zeckel, M. L. *Exp. Opin. Invest. Drugs* **1995**, 4, 889.
4. (a) US 3907784, **1975**, Huffman, G. W. (b) Pfeil-Doyle, J.; Draheim, S. E.; Kukolja, S.; Ott, J. L.; Counter, F. T. *J. Med. Chem.* **1988**, 31, 1993.
5. A27223: Methicillin resistant strain of *S. aureus* (homogeneous)
6. (a) For metabolism of cephalothin : Sullivan, H. R.; McMahon, R. E.; Heiney, R. E. *J. Antibiot.* **1971**, 24, 375. (b) For metabolism of cephacetrile : Nakai, Y.; Kanai, Y.; Fugono, T.; Tanayama, S. *ibid.* **1976**, 29, 81. (c) For metabolism of cephapirin : Cabana, B. E.; Van Harken, D. R.; Hottendorf, G. H. *Antimicrob. Agents & Chemother.* 1976, 10, 307. (d) For metabolism of cefotaxime : Schrinner, E.; Limbert, M; Penasse, L; Lutz, A. *J. Antimicrob. Chemother.* **1980**, 6, suppl. A : 25.

7. Takaya, T.; Takasugi, H.; Murakawa, T.; Nakano, H. *J. Antibiot.* **1981**, 34, 1300.
8. The compound **2** was purchased from Otsuka Chemical Co., Ltd.
9. The compound **3** was prepared from the coupling of 2,5-dichlorothiophenol with bromoacetic acid.
10. Kaiser, G. V.; Cooper, R. D. G.; Koehler, R. E.; Murphy, C. F.; Webber, J. A.; Wright, I. G.; Van Heyningen, E. M. *J. Org. Chem.* **1970**, 35, 2430.
11. Wei, C. C.; Bartkovitz, D.; West, K. F. *ibid.* **1992**, 57, 4027.
12. For compound **8a**, the yield of the displacement step was improved from 33% to 70% by utilizing dibenzo 18-crown-6.
13. Compounds **8l** and **8m** were prepared by quaternizing the corresponding C-3 pyridyl derivitives **7l** and **7m**, respectively, with CH_3I in acetone and subsequent deprotection of DPM ester.
14. A27217: Methicillin resistant strain of *S. aureus* (heterogeneous)
15. A27218: Methicillin resistant strain of *S. aureus* (heterogeneous)
16. A27621: Methicillin resistant strain of *S. aureus* (homogeneous)

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