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SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIP OF C-3 QUATERNARY AMMONIUM CEPHALOSPORINS EXHIBITING ANTI-MRSA ACTIVITIES

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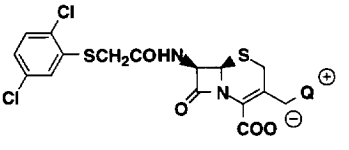
Abstract: A series of cephalosporins bearing C-3 quaternary ammonium groups were prepared and evaluated for their anti-MRSA activity. They exhibit good to excellent in vitro activity (MICs = 1–8 μg/mL) against MRSA. © 1997 Elsevier Science Ltd.

Nosocomial infections caused by gram-positive bacteria are increasing and becoming a serious threat to antimicrobial chemotherapy.[2] Methicillin-resistant *Staphylococcus aureus* (MRSA) is at the center of particular concern, since it is resistant to all current antibiotics except vancomycin. Although vancomycin is highly effective against MRSA, there is a need for new antibiotics due to the alarming potential for the emergence of vancomycin resistant strains of MRSA.[3]

We have been investigating a new class of anti-MRSA cephalosporins and have found that C-3 benzoyloxymethyl cephalosporins bearing a lipophilic 2,5-dichlophenylthioacetamido group at C-7 exhibit excellent in vitro anti-MRSA activity. However, these cephalosporins suffer from poor in vivo activity.[4] Fourth generation cephalosporin antibiotics possessing C-3 quaternary ammonium groups are known to exhibit increased in vitro and in vivo activity against gram positive organisms.[5] Therefore, we were interested in evaluating the anti-MRSA potential of new class of C-3 quaternary ammonium cephalosporins bearing a lipophilic 2,5-dichlorophenylthioacetamido C-7 side chain (Figure 1).

Figure 1



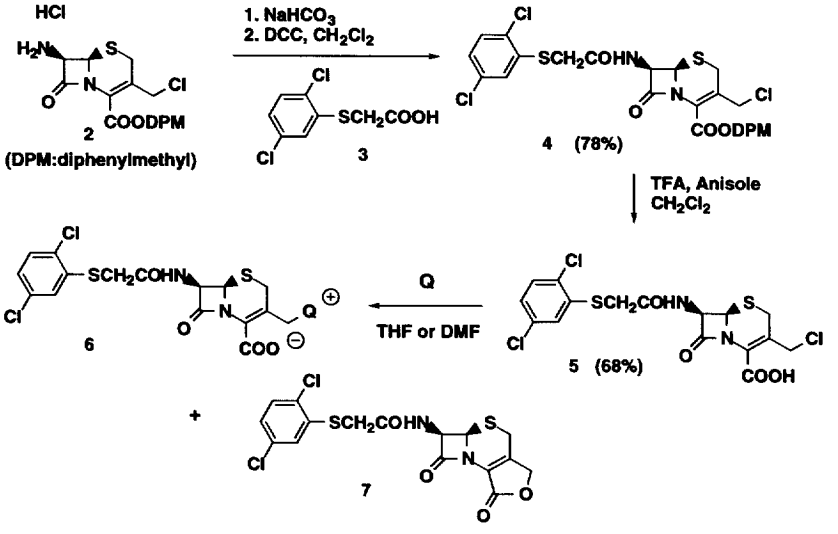
Q = aliphatic bicyclic or heteroaromatic bicyclic quaternary ammonium group

Chemistry

The C-3 quaternary ammonium cephalosporins listed in Table 1 and Table 2 were prepared by the synthetic route described in Scheme 1. The C-7 amino cephalosporin **2** was coupled with 2,5-dichlorophenylthioacetic acid **3** by using DCC to give the C-7 2,5-dichlorothiophenylacetamido cephalosporin **4**. The DPM ester group in **4** was removed by treatment with trifluoroacetic acid to yield the corresponding acid **5**. The C-3 quaternary ammonium cephalosporins **6a–6s** were readily obtained from the quaternization of compound **5** with a variety of amines. For the preparation of compounds **6a–**

6i, sodium iodide was added to generate the C-3 iodomethyl cephalosporin intermediate in situ, since these heteroaromatic amines are weak nucleophiles.[6] On the other hand, compounds **6j–6s** were obtained without the assistance of sodium iodide by reaction with aliphatic tertiary amines.[7] The quaternization step produced variable amounts of the lactone cephalosporin **7** as a side-product.[6,7] The lactone cephalosporin **7** was readily separated from C-3 quaternized ammonium cephalosporins **6** by trituration with acetone.

Scheme 1



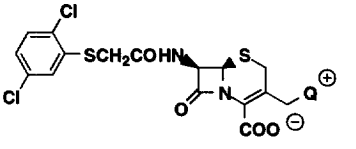
Results and Discussion

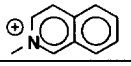
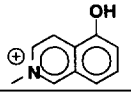
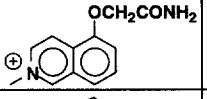
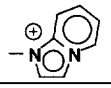
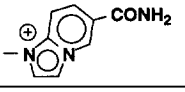
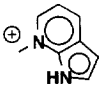
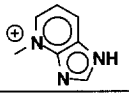
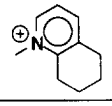
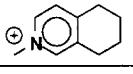
The in vitro activity of quaternary ammonium cephalosporins was evaluated by determination of minimum inhibitory concentration (MIC) values by the standard broth dilution method. The in vivo efficacy was evaluated by utilizing a MRSA systemic infection model in mice and was expressed as protective dose (PD_{50}).

The quaternary ammonium cephalosporins reported in this paper can be classified as (1) C-3 heteroaromatic bicyclic quaternaries (Table 1: **6a–6i**) and (2) C-3 aliphatic bicyclic quaternaries (Table 2: **6j–6s**). All of the heteroaromatic quaternary ammonium cephalosporins in Table 1 exhibit good in vitro activity with MICs ranging from 0.125 to 1 μg/mL against MSSA and 1 to 4 μg/mL against MRSA. In compounds **6a–6e**, introduction of a hydroxyl group (**6b**) or a carbamoyl group (**6c**, **6e**) had little effect on the in vitro activity relative to **6a** and **6d**. In compounds **6d–6g**, introduction of additional nitrogen atoms to the C-3 aromatic bicyclic ring did not influence the in vitro activity significantly. Heteroaromatic quaternary derivatives with a fused aliphatic ring (**6h** and **6i**) also had good in vitro activity comparable to **6a**. Compounds **6a–6i** in Table 1 exhibit a broad range of in vivo activity (PD_{50} = 1–25 mg/kg) against

MRSA. Among those, compounds **6a**, **6b**, **6c**, and **6g** stand out with good in vivo activity (PD_{50} = 1–4 mg/kg). Compounds **6h** and **6i** where one of the bicyclic ring is replaced by an aliphatic ring have the least potent in vivo activity in this series (PD_{50}s = 25 mg/kg).

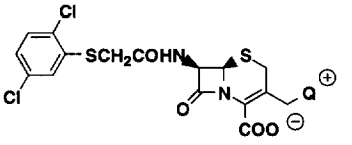
Table 1

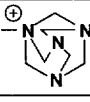
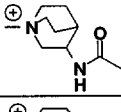
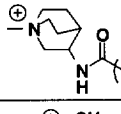
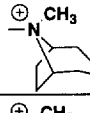
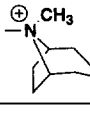
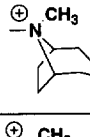
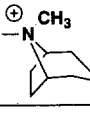


	Q	MIC (μg/mL)					
		MRSA (A27217)	MRSA (A27223)	MRSA (A27621)	MRSA (A27226)	MSSA (A15090)	MSSA (A20241)
6a		2	2	2	2	0.5	0.5
6b		1	1	1	1	1	1
6c		2	2	2	2	1	1
6d		2	4	2	1	0.25	0.5
6e		2	2	2	2	0.5	1
6f		1	2	2	2	1	1
6g		1	2	2	1	0.125	0.125
6h		1	2	2	1	0.5	0.5
6i		2	4	4	2	0.5	0.5

A27217 (heterogeneous strain); A27223 (homogeneous strain); A27621 (homogeneous strain); A27226 (homogeneous strain)

Table 2



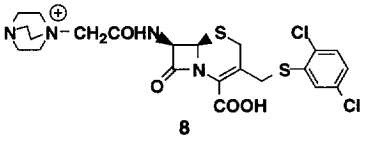
	Q	MIC (μg/mL)					
		MRSA (A27217)	MRSA (A27223)	MRSA (A27621)	MRSA (A27226)	MSSA (A15090)	MSSA (A20241)
6j		2	2	4	4	1	1
6k		1	1	1	1	0.25	0.5
6l		2	4	4	4	0.25	0.5
6m		4	8	8	8	0.125	0.125
6n		4	4	4	2	0.5	0.5
6o		2	4	4	4	1	1
6p		2	2	2	2	0.25	0.25
6q		2	4	4	4	0.5	0.5
6r		1	2	2	2	0.25	0.25
6s		1	4	2	4	0.125	0.125

The aliphatic bicyclic aliphatic quaternary ammonium cephalosporins in Table 2 (**6j–6s**)[8] also exhibit good in vitro anti-MRSA activity with MICs ranging from 0.125 to 1 μg/mL against MSSA and 1

to 8 μg/mL against MRSA. Overall, the C-3 heteroaromatic quaternary ammonium cephalosporins (**6a–6i**) are slightly more potent in vitro against MRSA than the C-3 aliphatic bicyclic quaternary ammonium cephalosporins (**6j–6s**). In compounds **6j–6o**, introduction of a hydroxyl group (**6k**) improved the in vitro activity of **6j**. On the other hand, introduction of additional amino groups in the ring (**6l** and **6m**) or in the side chain (**6n** and **6o**), slightly decreased in vitro activity against MRSA compared to **6j** and **6k**. For the tropane series (**6p–6s**), a carbonyl group (**6q**) or a hydroxyl group (**6r** and **6s**) in the side chain has a minimal effect on the in vitro activity relative to **6p**. The C-3 aliphatic bicyclic quaternary ammonium cephalosporins (**6j–6s**) exhibit excellent to poor in vivo activity against MRSA with PD_{50}s ranging from 0.8 to 25 mg/kg. Among these cephalosporins, compounds **6l** and **6r** exhibit excellent in vivo activity (PD_{50}s = 0.8–1.0 mg/kg). It is interesting to note that the compound **6s**, which possesses an β-OH tropane C-3 side chain, exhibits moderate in vivo efficacy (PD_{50} = 9.5 mg/kg) relative to the α-isomer **6r**.

In this new series of cephalosporins (Table 1 & 2), we have generated compounds **6a**, **6b**, **6c**, **6g**, **6l**, and **6r** that exhibit excellent in vitro and in vivo anti-MRSA activity. We achieved this anti-MRSA activity by introducing the lipophilic 2,5-dichlorothiophenylacetamido group as a C-7 side chain and the hydrophilic quaternary ammonium groups as a C-3 side chain. We have questioned if these two side chains are interconvertible. Therefore, we prepared the compound **8**[9] and compared its anti-MRSA activity (Table 3) with that of the compound **6l** (Table 2). Compound **8** is devoid of any activity against MRSA (MIC = 128 μg/mL, PD_{50} = 25 mg/kg). It appears that the lipophilic 2,5-dichlorophenyl-thioacetamido group should be linked to the C-7 side chain, whereas the hydrophilic quaternary ammonium group should be positioned at the C-3 side chain in order to generate good anti-MRSA activity.

Table 3

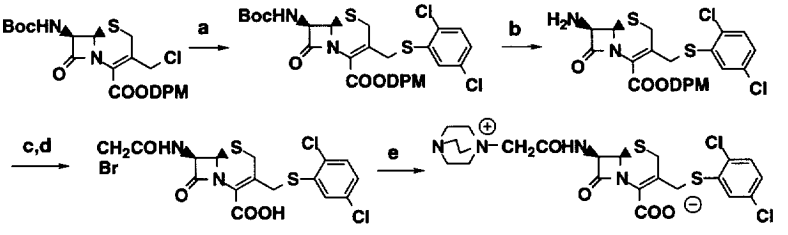
	MIC (μg/mL)					
	MRSA (A27217)	MRSA (A27223)	MRSA (A27621)	MRSA (A27226)	MSSA (A15090)	MSSA (A20241)
 **8**	128	128	128	128	4	4

In summary, the C-3 quaternary ammonium cephalosporins in Table 1 and Table 2 exhibit good to excellent in vitro activity against MRSA. While certain cephalosporin derivatives are highly potent in vivo, many derivatives in this series exhibit moderate to poor in vivo efficacy. It is possible that differences in pharmacokinetic properties and in vivo stability may contribute to this high variability in in vivo efficacy. In the series of C-3 heteroaromatic quaternary ammonium cephalosporins (Table 1), compounds **6a**, **6b**, **6c**, and **6g** exhibit the most promising anti-MRSA activity. In the series of C-3 aliphatic bicyclic quaternary ammonium cephalosporins (Table 2), compounds **6l** and **6r** stand out with their excellent anti-MRSA activity.

Acknowledgments: We are grateful to Bristol-Myers Squibb analytical chemistry group at Wallingford for the spectroscopic analysis of compounds listed in Table 1, 2 and 3. We also thank the microbiology group at Wallingford for providing in vitro (Dr. J. C. Fung-Tomc's group) and in vivo (Dr. Y. H. Tsai's group) data.

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(6)*, 452.
3. (a) Turik, M. A.; Zeckel, M. L. *Exp. Opin. Invest. Drugs* **1995**, *4(9)*, 889. (b) *Morbidity & Mortality Weekly Report*, **1997**, *46(27)*, 624.
4. Kim, O. K.; Ueda, Y.; Mansuri, M. M.; Russell, J. W.; Bidwell, V. W. *Bioorg. Med. Chem Lett.* **1997**, *7(14)*, 1945.
5. (a) Bryskier, A. *J. Clin. Microbiol. & Infect.* **1996**, *1(1)*, 1. (b) Periti, P. *J. Chemother.* **1996**, *8*, 112.
6. Yields of the quaternization step for compounds **6a–6i** range from 12 to 49%. The lactone cephalosporin **7** was obtained as a major product for the preparation of the compounds **6a–6i**.
7. Yields of the quaternization step for compounds **6j–6s** range from 27 to 86%. The lactone cephalosporin **7** was also observed as a minor product.
8. Compounds **6k** and **6n–6s** were obtained as a mixture of two diastereomers (~1:1).
9. The compound **8** was prepared as shown below.



a. 2,5-dichlorobenzenethiol, 2,6-lutidine, DMF, rt, 5 h, 86% b. p-TsOH, CH_3CN, rt, 5 h, 51% c. $BrCH_2COBr$, THF, N-methyl-morpholine, 0 °C, 2 h, 75% d. TFA, anisole, CH_2Cl_2, 0 °C to rt, 1.5 h, quant. e. DABCO, THF, 0 °C to rt, 2 h, 47%

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