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Anti-MRSA Cephems. Part 1: C-3 Substituted Thiopyridinium Derivatives

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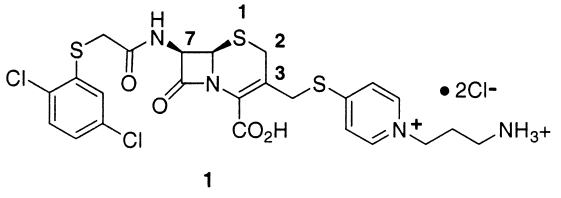
Abstract—Sixteen novel cephalosporin derivatives with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) are described. The compounds were synthesized using substituted thiopyridones, generated either by cyclization of functionalized precursors, or by direct alkylation of the enolate of 2-methyl substituted pyrones. The most active compound in vitro against a strain of MRSA (A27223) displayed an MIC of 0.5 μg/mL. The most efficacious compound in vivo had a PD_{50} of 2.1 mg/kg.

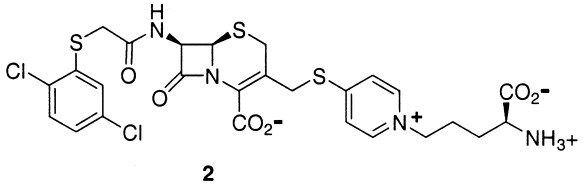
Nosocomial infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) have increased alarmingly in the past two decades.[1] Vancomycin remains the most effective treatment of MRSA in the clinic. Vancomycin resistance has developed in enterococci precipitating concern that transfer of this resistance from enterococci to MRSA would produce an extremely lethal and incurable pathogen.[2] Recent clinical isolates of MRSA with reduced susceptibility to vancomycin have given credence to this concern.[3] Consequently the search for new antibiotics with anti-MRSA activity remains of utmost importance to the future management of these infections. This search for new anti-MRSA compounds has recently been extended to include new derivatives with a cephem core structure.[4]

Early in our program to discover an injectable anti-MRSA cephalosporin we found that compounds with a lipophilic group at C-7, and a C-3 thio-linked pyridinium moiety, had excellent anti-MRSA activity both in vitro and in vivo.[5] One early compound of biological interest was the C-3 aminopropyl pyridinium derivative



1

MIC MRSA (A27223) = 0.125 μg/mL
MIC with 50% calf serum = 2 μg/mL
MIC_{90} MRSA = 2 μg/mL
PD_{50} MRSA (A27223) = 1.4 mg/kg



2

MIC MRSA (A27223) = 1 μg/mL
MIC with 50% calf serum = 4 μg/mL
MIC_{90} MRSA = 3.5 μg/mL
PD_{50} MRSA (A27223) = 4.1 mg/kg

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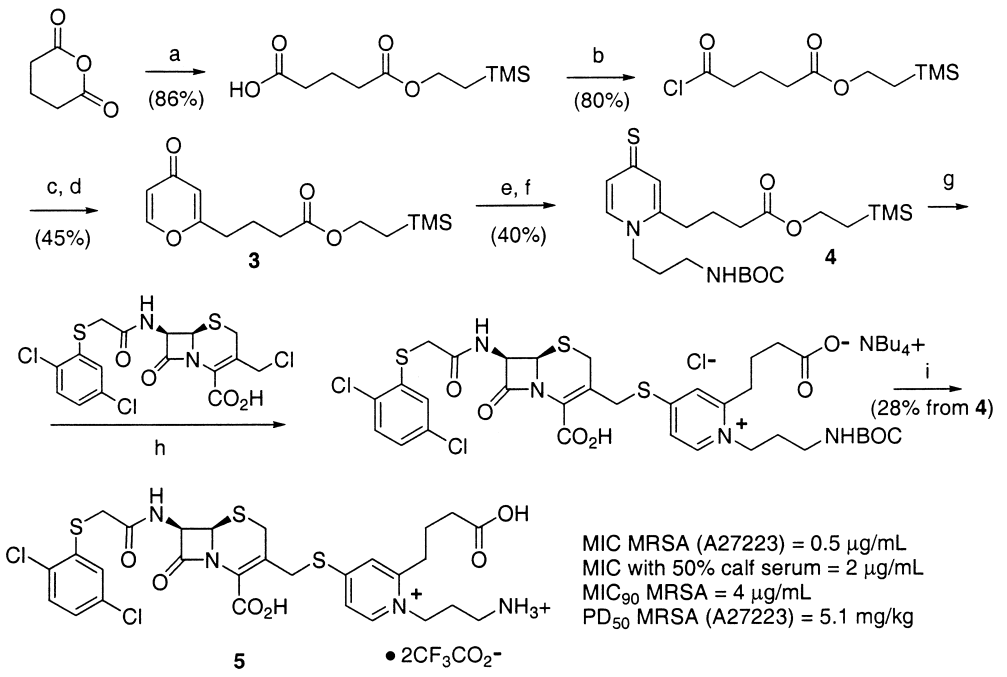


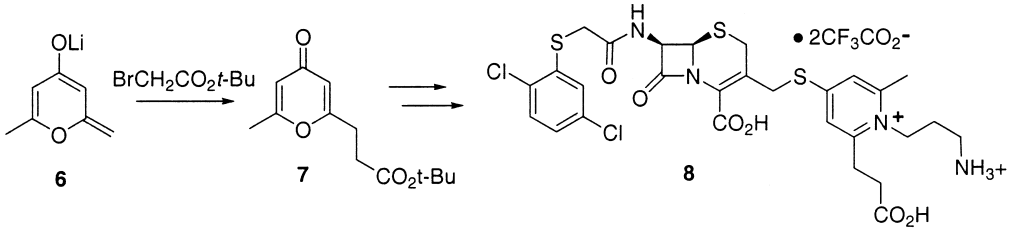
Scheme 1. (a) $TMSCH_2CH_2OH$, Et_3N, CH_2Cl_2; (b) $(COCl)_2$, PhH, reflux; (c) $MeOCH{=}CHC(O)CH_3$, LiHMDS, THF, −78 °C; (d) TFA, PhH, reflux; (e) Lawesson's reagent, toluene, 80 °C; (f) $H_2N(CH_2)_3NHBOC$, EtOH; (g) TBAF, THF; (h) MeOH, CH_2Cl_2; (i) TFA, CH_2Cl_2.

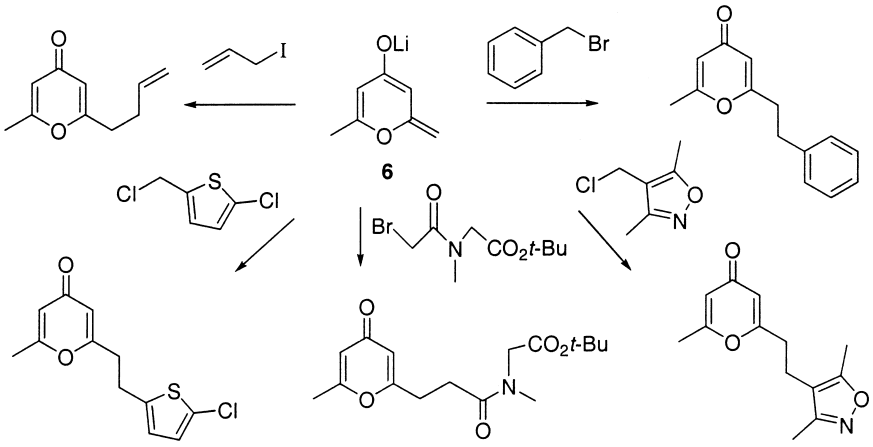
1. The MIC (minimum inhibitory concentration) of **1** against a homo-resistant MRSA strain (A 27223), was 0.125 μg/mL (2 μg/mL when assayed in the presence of 50% calf serum).[6] In a mouse systemic infection model, compound **1** showed a PD_{50} of 1.4 mg/kg.[7] Unfortunately, **1** was found to be acutely toxic to mice upon iv bolus administration at concentrations above the therapeutic dose.[8]

Subsequently, the ornithine-substituted C-3 pyridinium derivative **2** was synthesized and found to have good activity against MRSA. We were pleased to observe that this compound was much less acutely toxic in mice than **1**.[9] However, cephem **2** had poor aqueous solubility at neutral pH, likely a consequence of the compound's biszwitterionic nature.

Acute toxicity data from many more of our cephem derivatives led us to formulate an empirical 'rule of thumb' for predicting toxicity in our series of analogues. In general, compounds with a net positive charge at neutral pH (such as **1**) produced rapid death in mice upon iv dosage. Neutral compounds (such as **2**) were usually much safer, while derivatives with a net negative charge were generally nontoxic when injected into mice.[10] Unfortunately, cephems carrying a net negative charge usually had weak activity against MRSA. As a consequence, we were often obliged to stay within the manifold of cephems that were overall neutral in charge, and attempt to maximize intrinsic antibacterial activity while improving solubility and reducing toxicity.

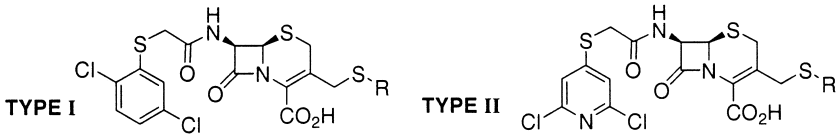
One of our approaches to improve the solubility of compounds such as **2** was to prepare derivatives that had the ammonium and carboxylate moieties of the ornithine side chain at C-3 separated by some distance. It was hoped that the separation of these charged species might have a beneficial effect on solubility by attenuating their intramolecular association, and favoring their interaction with aqueous media. Scheme 1 illustrates the synthesis of our first target based on this thesis, cephem **5**. Key thiopyridone **4** is generated by a cyclization strategy in a few steps, and allowed to react with a C-3 chloromethyl cephalosporanic acid intermediate to provide the C-3 pyridinium derivative.[11] Deprotection of the silylethyl ester prior to this step, and the BOC group afterwards, ultimately yielded target **5**. We were gratified to observe that cephem **5** appeared to be more soluble than compound **2** at varied pH, and in the presence of added saline. For example, at pH 7 in water the solubility of **5** was found to be 6 mg/mL, while the solubility of **2** was 1.5 mg/mL.[12] At pH 9, the solubility of **5** rose to 16 mg/mL, while only 2.4 mg/mL of **2** was in solution. When **5** was dissolved in 0.9% saline at pH 9, the solubility was found to be 15 mg/mL, indicating a negligible salt effect on solubility.

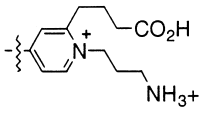
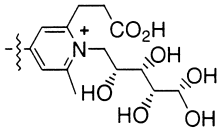
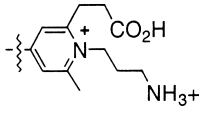
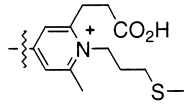
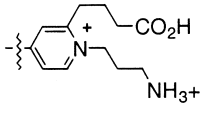
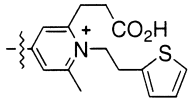
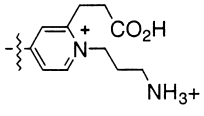
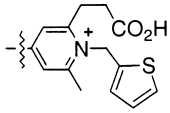
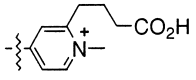
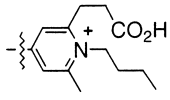
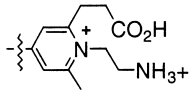
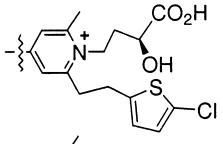
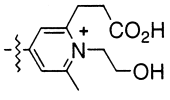
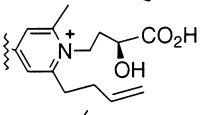
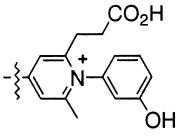
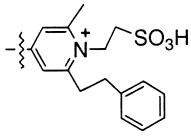




Scheme 2.

Table 1.



No. type	R	MIC[a]	PD_{50}[b]	No. type	R	MIC[a]	PD_{50}[b]
5,I		0.5 (4)	5.1	**15,I**		8 (16)	>20
8,I		1 (4)	2.5	**16,I**		2 (4)	>25
9,II		2 (4)	4.3	**17,I**		4 (8)	>23
10,I		2 (2)	2.1	**18,I**		4 (8)	>23
11,I		4 (8)	5.2	**19,I**		4 (8)	>23
12,I		2 (2)	3.9	**20,I**		8 (8)	>23
13,I		2 (4)	8.4	**21,I**		4 (8)	19.2
14,I		4 (8)	>25	**22,I**		2 (4)	>25

[a]MIC (in μ/mL) versus MRSA A27223, value in parentheses is MIC in presence of 50% calf serum. For details see ref 6.
[b]PD_{50} (in mg/kg) for activity against MRSA A27223 in a mouse model of systemic infection. For details see ref 7.

While the chemistry of Scheme 1 delivered ample quantities of **5** for initial in vitro and in vivo assay, the overall sequence to key 4-thiopyridone **4** suffered from low yields.[13] We therefore desired a more simple and general route to substituted thiopyridones to facilitate analogue synthesis in this branched (relative to **1** and **2**) pyridinium series of compounds. We found 2,6-dimethylpyran-4-one to be an inexpensive starting material, and decided to attempt the direct γ-alkylation of one of the methyl groups via the extended enolate ion.[14] We were pleased to observe that dienolate **6** reacted smoothly with *tert*-butyl bromoacetate to afford substituted pyrone **7** in 84% yield. The conversion of **7** to cephem **8** was accomplished using chemistry similar to that depicted in Scheme 1.

We found that alkylation of **6** was efficient with the activated halides shown in Scheme 2.[15] Using this approach, and the chemistry of Scheme 1, we synthesized the cephems shown in Table 1.[16]

Some SARs in this series of cephems can be gleaned from the data presented in Table 1. In general, we have found that cephems of Type II, containing a dichlorothiopyridyl substituent at C-7, are usually 2–4 times less active in vitro than the corresponding dichlorothiophenyl substituted cephems of Type I . This effect is observed by the loss of activity for **9** relative to **5** against our marker strain of MRSA A27223. Interestingly, **9** is just as active as **5** in vivo. This has often been observed in our program for cephems of Type II relative to Type I, and may result from an improved pharmacokinetic or metabolic profile for compounds containing the dichlorothiopyridyl substituent at C-7.

Table 2. MIC data in μg/mL

	Organism	A No.	MIC of compounds		
			5	**8**	**10**
1	*S. pneumoniae*/Pen. I	A27881	0.007	0.06	0.003
2	*S. pneumoniae*/Pen. Resist.	A28272	0.06	0.25	0.06
3	S. pyogenes/*Todd Hewitt*	A9604	0.0005	0.0005	0.003
4	*E. faecalis*	A20688	0.25	0.5	0.5
5	*E. faecalis*/+50% calf serum	A20688	1	4	1
6	*E. faecium*	A24885	1	2	2
7	*E. faecium*/Amp. R, Vanco. S	A28156	32	64	64
8	*S. aureus*/Pen.−	A9497	0.001	0.007	0.007
9	*S. aureus*/Pen.+	A9606	0.06	0.125	0.125
10	*S. aureus*/+50% calf serum	A9606	0.25	0.25	0.125
11	*S. aureus*/MS/Pen.+	A20241	0.06	0.25	0.125
12	*S. aureus*/Hetero MR	A27218	0.5	0.5	0.5
13	*S. aureus*/+50% calf serum	A27218	0.5	1	1
14	*S. aureus*/Hetero MR	A27217	0.25	0.5	0.5
15	*S. aureus*/Homo MR	A27223	0.5	1	2
16	*S. aureus*/+50% calf serum	A27223	2	4	2
17	*S. aureus*/Homo MR	A27621	0.5	0.5	1
18	*S. aureus*/Homo MR	A27295	1	2	4
19	*S. aureus*/Homo MR	A27226	0.25	0.5	1
20	*S. aureus*/MR, P−	A27225	0.5	0.5	1
21	*S. epidermidis*	A24548	0.015	0.03	0.03
22	*S. epidermidis*/MR	A25783	0.03	0.25	0.25
23	*S. epidermidis*/Imipenem R	A27368	0.125	0.5	0.25
24	*S. haemolyticus*	A21638	0.03	0.125	0.06
25	*S. haemolyticus*/Homo MR	A27229	1	2	2

A comparison of the data for compound **5** relative to **10** indicates a slight preference in vitro for compounds with a three-carbon link between the acid group and the pyridinium ring, rather than a two-carbon link. However, the effect of this structural change on the in vivo efficacy of these two compounds is the opposite; a preference for the two-carbon link is observed. The data for compounds **8** and **10** indicate that an extra methyl substituent on the pyridinium ring is well tolerated. Compounds **14**–**22** were found to be generally inactive in vivo even though **16** and **22** had good in vitro activity.[17] It is clear from the data presented in Table 1 that the most active compounds in vivo contain an alkylammonium group on the pyridinium nitrogen, as compared to a neutral or anionic group. Further representative MIC data for the most interesting compounds in this series is given in Table 2. As Table 1 indicates, these compounds are quite active against a variety of streptococci, staphylococci, and enterococci. (The cephems in this class are primarily active against Gram-positive bacteria, with little activity against the representative Gram-negative organisms (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis, P. aeruginosa*) included in our screening panel.)

In summary, we have synthesized a set of substituted thiopyridinium cephems via thiopyridones derived from either acyclic precursors (Scheme 1), or direct alkylation of 2,6-dimethyl-pyran-4-one (Scheme 2). Many of these compounds have excellent activity against MRSA in vitro and in vivo, and remain interesting leads in the quest for agents to combat these important pathogens. Additionally, the methods described here should prove useful to those engaged in the synthesis of compounds containing substituted thiopyridinium moieties.

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6. Antibacterial MICs were determined in broth according to the standard conditions recommended by the National Committee for Clinical Laboratory Standards (NCCLS). MIC assays against MRSA utilized Mueller-Hinton broth + 2% NaCl, a bacterial inoculum size $\sim 5\times10^5$ CFU/mL, and were incubated at 35 °C for 24 h. MIC was defined as the lowest drug concentration inhibiting all visible growth. MIC_{90}s were determined against a panel of 58 strains of MRSA. Compounds bearing the dichlorothiophenyl amide at C-7 often have good activity against penicillin-binding protein 2a (PBP2a). IC_{50}s for inhibition of PBP2a were determined for three of the compounds discussed here: **1** = 1.2 μM; **2** = 2.8 μM; **5** = 3.4 μM.

7. The PD_{50} values reported represent the concentration of the compound that protects 50% of the infected animals from death in a mouse model of systemic infection. Mice were given (ip) a lethal dose of MRSA A27233 (homogeneous strain, 2.4×10^8 CFU) on day 1, and the test compound was administered (im) twice on day 1 at 0.2 h and 2 h post infection. Animals were monitored until the end of day five for survival.

8. Compounds were assayed for acute toxicity in mice as follows: The test compound was dissolved in 5% aqueous dextrose at a concentration between 5 and 25 mg/mL. The solution was filtered through a 0.2 μ filter, and between 0.1 and 1 mL of the solution was injected (in usually less than a min) into the tail vein of three mice. The usual toxic reaction observed after injection of these types of compounds was red coloration of the feet, ears, tail and muzzle of the mice, followed by respiratory distress and death. Death, when observed, usually occurred within min of injection. Mice surviving 1 h post injection usually recovered with no obvious adverse effects. Compound **1**, given at a dose of ~200 mg/kg, resulted in death to all three mice tested.

9. Compound **2**, given at a dose of ~300 mg/kg, was safe to all three mice tested.

10. We did not observe a direct correlation between a compound's solubility profile and its acute toxicity. Most of our compounds appeared to form microparticulate aggregates that were observed by polarized light microscopy and/or laser light scattering spectrometry. These aggregates often passed through the 0.2 μ filter employed prior to the dosing of the compound in the toxicity assay. Many safe compounds carrying a net zero or net negative charge were found to be insoluble and aggregated at neutral pH; therefore we cannot definitively conclude that insolubility and toxicity are directly linked for our compounds. The most direct correlate for toxicity was that of overall charge of the molecule, as stated in the text. (We do not have an explanation for this correlation, thus the use of the term 'empirical'. We cannot rule out that toxic cholinergic responses may be involved; however, we have not performed any experiments to determine the cholinergic properties of our positively charged derivatives. We also cannot rule out that a strong interaction with blood constituents (such as cells, serum proteins and platelets) is operative for our positively charged compounds, followed by formation of emboli in the capillaries of the lung. For an example of these latter effects regarding the toxicity of positively charged macromolecular species, see: Moreau, E.; Ferrari, I.; Drochon, A.; Chapon, P.; Vert, M.; Domurado, D. *J. Contr. Rel*. **2000**, *64*, 115.)

11. For a synthesis of the C-3 chloromethyl cephalosporanic acid intermediate see ref 4i.

12. For solubility determinations, a known quantity of test compound is dissolved in water or 0.9% saline and the pH adjusted to the desired point by the addition of minute amounts of 1 N NaOH. The solutions are stirred for 2 h, and then filtered through a 0.2 μ filter. The filtrate is then assayed by HPLC and the concentration of dissolved compound determined by comparison to a standard curve.

13. (a) Our synthesis of pyrone 3 was based on the chemistry reported by Koreeda and Ganem. The presence of the pendant ester group led to reduced yields through this sequence. For syntheses of pyrones such as **3**, see: Koreeda, M.; Akagi, H. *Tetrahedron Lett*. **1980**, *21*, 1197. (b) Morgan, T. A.; Ganem, B. *Tetrahedron Lett*. **1980**, *21*, 2773.

14. (a) For previous reports of alkylation of the dienolate of 2,6-dimethyl-pyran-4-one, see: Yamamoto, M.; Sugiyama, N. *Bull. Chem. Soc. Jpn*. **1975**, *48*, 508. (b) Smith, A. B., III; Scarborough, R. M., Jr. *Tetrahedron Lett*. **1978**, *19*, 4193. (c) West, F. G.; Fisher, P. V.; Arif, A. M. *J. Am. Chem. Soc*. **1993**, *115*, 1595. (d) West, F. G.; Amann, C. M.; Fisher, P. V. *Tetrahedron Lett*. **1994**, *35*, 9653.

15. Isolated yields after chromatography were in the range of 20–80% for the products illustrated in Scheme 2 and are unoptimized. The use of chloromethyl methyl sulfide and 1-iodopropane as alkylating agents for this reaction did not provide any detectable product. Attempted Michael addition of the dienolate to *t*-butyl acrylate was similarly unsuccessful.

16. The thiopyridone required for the synthesis of cephem **10** was made via alkylation of the dienolate anion of the known 2-methyl-pyran-4-one. For a synthesis of this pyrone, see: Dorman, L. C. *J. Org. Chem*. **1967**, *32*, 4105.

17. In general, compounds that were found to be inactive in vivo were not evaluated in mouse pharmacokinetic or pharmacodynamic studies. Thus, there remain many possible (but unevaluated) explanations for the inactivity of compounds **14**–**22**. Each individual compound may have different metabolism and distribution properties due to the nature of the pendant groups on the C-3 thiopyridinium ring. A particularly simple explanation could be that inactive compounds remain localized at the site of intramuscular injection limiting the exposure of the animal to the test drug. This could either be a consequence of low solubility, or poor absorption into the vascular system from the muscle tissue.