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The Synthesis and Evaluation of 2-Substituted-7-(alkylidene)cephalosporin Sulfones as β-Lactamase Inhibitors

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Abstract—A series of 2-substituted-7-(alkylidene)cephalosporin sulfones were prepared and evaluated as β-lactamase inhibitors. Compound **11c** showed excellent activity as an inhibitor of the class C β-lactamase derived from *Enterobacter cloacae*, strain P99.

The hydrolytic destruction of β-lactam antibiotics, mediated by the β-lactamase enzymes, represents the most common mechanism of bacterial penicillin-resistance.[1] The β-lactamases are divided into serine (A, C, and D) and metalloenzyme (B) classes.[2] Successful treatment of such resistant infections can often be effected by the co-administration of an antibiotic and a β-lactamase inhibitor.[2] Established inhibitors of the serine β-lactamases include clavulanate (**1**), which is co-administered with the antibiotic amoxicillin in the form of the product Augmentin® and tazobactam (**2**), co-administered with piperacillin in the product Zosyn® These inhibitors target the class A β-lactamases, which have, historically, been the most clinically important.

Recently, however, the class C enzymes (AmpC) have become an important clinical threat. Defined as cephalosporinases that are not inhibited by clavulanate,[4] the chromosomally encoded class C β-lactamase is present in 10 to 50% of patients infected with *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. Furthermore, plasmid-encoded class C β-lactamases[5] have also been described in isolates of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Salmonella senftenberg*.[6]

Our research group has recently described several new β-lactamase inhibitors, including cephalosporin-derived compounds (**3** and **4**),[7] as well as the 6-alkylidene-2′-substituted penams, **5**.[8] Especially in comparison to the penicillin series, relatively few cephalosporin-derived β-lactamase inhibitors are known.[9] We reported that **3** is a good inhibitor of the Class C β-lactamase, derived from *E. cloacae*, strain P99, while **4** inhibits the class A enzyme, TEM-1.[7] We recently undertook a project to design modifications of these inhibitors with the 3-fold goal of increasing their potency, broadening their spectrum, and furthering our understanding of the mechanism of inhibition. Our discovery of the enhanced activity and spectrum of the 2′-substituted-6-alkylidene-penams, **5**, has already been described.[8] In contrast to the penams (which possess few easily functionalized positions), the cephalosporin nucleus provides an excellent opportunity to create modifications at both the C-2 and C-3 positions on the six-membered ring. We now report the effect of selected C-2 modifications on the biological activity of **3** and **4**.

Synthesis

Three separate 7-aminocephalosporin derivatives (**6**), were converted to the corresponding 7-oxocephalosporinates (**7**), utilizing our earlier protocol.[10] Reaction of these with either (2′-pyridylmethylene)triphenylphosphorane or (*tert*-butoxycarbonyl)methylenetriphenylphosphorane generated five new compounds **8** (all exclusively of the *Z*-geometry, as shown). The reaction of compound **7c** with (2′-pyridylmethylene)triphenylphosphorane was unsuccessful.

These sulfides were then oxidized to the corresponding sulfones and treated with Eschenmoser's Salt[11] to produce the 2-exomethylidene cephems,[12] **10a**–**10e**. The

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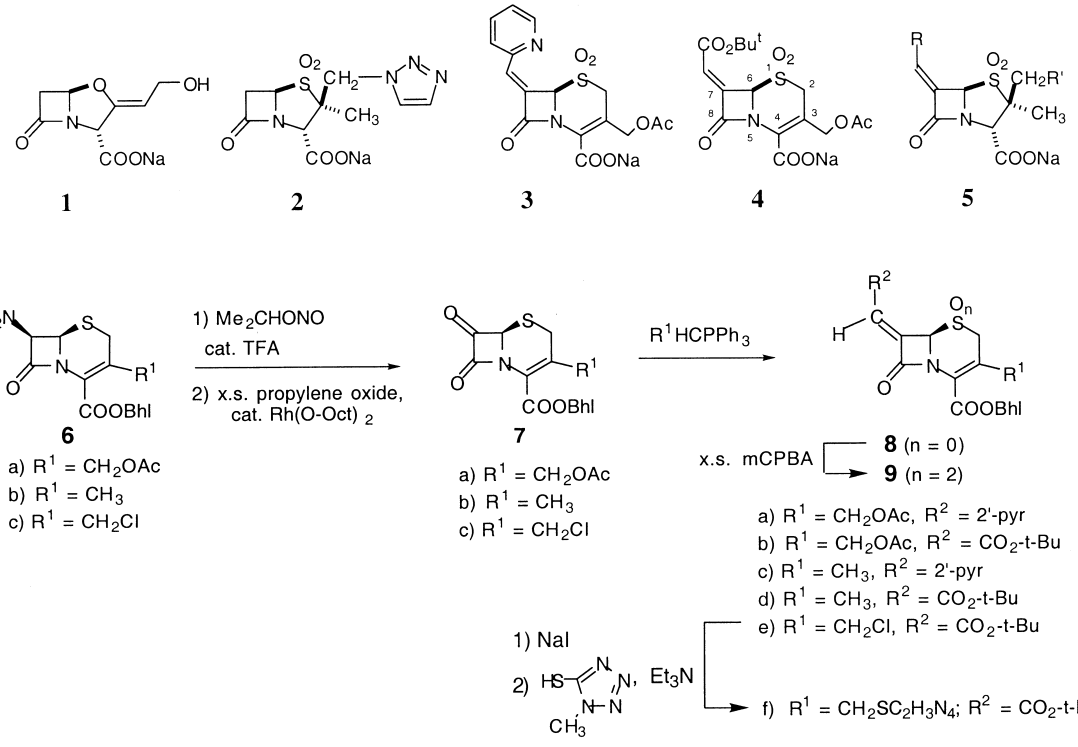
3′-chloride of **9e** was replaced (via the intermediate iodide) by *N*-methylthiotetrazole to produce **9f**, which was subsequently converted to **10f**. Lastly, the sulfone **9c** was treated with CS_2 in the presence of base and subsequently treated with methyl iodide to generate the dithiomethylidene analogue **10g**. All of these compounds were deprotected to generate the corresponding carboxylate salts. In the case of *tert*-butyl esters **10b** and **10d**, the deprotection also produced substantial quantities of the corresponding bis(carboxylate) salts **11h** and **11i**, respectively.

Alternatively, we sought to incorporate single-bonded substituents at C-2. Introduction of methyl, for example, might make the cephem more penam-like and thus broaden its spectrum as an inhibitor of the class A penicillinases. We also desired to explore the activity of compounds with potential leaving groups at this position.

Our initial attempts to incorporate a methyl (or other alkyl) group at C-2 by generation of the allylic anion, followed by alkylation, were unsuccessful. In cases where some alkylation product could be isolated, it appeared that the alkylation had occurred at C-4, with allylic transposition of the double bond. An alternative, and successful, strategy is shown below. Reaction of the 2-methylidene compound **10c** with 9-BBN, followed by treatment with acetic acid, produced a 2:1 mixture of the β:α isomers of **12**, respectively.[12] These compounds were then deprotected to the corresponding carboxylate salts, **13**.

We also explored the incorporation of heteroatom substituents at C-2. Reaction of compound **9c** with NBS in the presence of Et_3N produced bromide **14**[13] which was converted to the thiotetrazole **15**[14] by reaction with 5-mercapto-1-methyltetrazole. This was successfully deprotected to produce carboxylate **16**.



1 **2** **3** **4** **5**

6

a) $R^1 = CH_2OAc$
b) $R^1 = CH_3$
c) $R^1 = CH_2Cl$

7

a) $R^1 = CH_2OAc$
b) $R^1 = CH_3$
c) $R^1 = CH_2Cl$

a) $R^1 = CH_2OAc$, R^2 = 2′-pyr
b) $R^1 = CH_2OAc$, $R^2 = CO_2$-t-Bu
c) $R^1 = CH_3$, R^2 = 2′-pyr
d) $R^1 = CH_3$, $R^2 = CO_2$-t-Bu
e) $R^1 = CH_2Cl$, $R^2 = CO_2$-t-Bu
f) $R^1 = CH_2SC_2H_3N_4$; $R^2 = CO_2$-t-Bu

10

a) $R^1 = CH_2OAc$; R^2 = 2′-pyr; R^3, R^4 = H
b) $R^1 = CH_2OAc$; $R^2 = CO_2$-t-Bu; R^3, R^4 = H
c) $R^1 = CH_3$; R^2 = 2′-pyr; R^3, R^4 = H
d) $R^1 = CH_3$, $R^2 = CO_2$-t-Bu; R^3, R^4 = H
e) $R^1 = CH_2Cl$; $R^2 = CO_2$-t-Bu; R^3, R^4 = H
f) $R^1 = CH_2SC_2H_3N_4$; $R^2 = CO_2$-t-Bu; R^3, R^4 = H
g) $R^1 = CH_3$; R^2 =2′-pyr; R^3, $R^4 = SCH_3$

11

a) $R^1 = CH_2OAc$; R^2 = 2′-pyr; R^3, R^4 = H
b) $R^1 = CH_2OAc$; $R^2 = CO_2$-t-Bu; R^3, R^4 = H
c) $R^1 = CH_3$; R^2 = 2′-pyr; R^3, R^4 = H
d) $R^1 = CH_3$; $R^2 = CO_2$-t-Bu; R^3, R^4 = H
f) $R^1 = CH_2SC_2H_3N_4$, $R^2 = CO_2$-t-Bu, R^3, R^4 = H
g) $R^1 = CH_3$; R^2 = 2′-pyr, R^3, $R^4 = SCH_3$
h) $R^1 = CH_2OAc$; $R^2 = CO_2Na$; R^3, R^4 = H
i) $R^1 = CH_3$; $R^2 = CO_2Na$; R^3, R^4 = H

Results and Discussion

As shown in Table 1, these new compounds were evaluated in cell-free systems as inhibitors of the two class A β-lactamases TEM-1 and PC1, derived from *Staphylococcus aureus*, as well as the class C enzyme, P99, derived from *E. cloacae*. Both **11a** and, especially, its corresponding 3′-desacetoxy analogue **11c**, display improved inhibition of the class C enzyme, relative to the C-2 unsubstituted compound **3**. The 3′-acetate is known to activate the β-lactam carbonyl toward nucleophiles[15] (such as the active site serine) and to behave as a leaving group subsequent to enzyme acylation. In fact, Pratt has shown[16] that, upon enzymolysis of typical cephalosporin antibiotics, loss of the 3′-leaving group results in the formation of a stabilized acyl-enzyme (leading, for example, to transient inhibition of the PC1 lactamase). In the present case, however, incorporation of a 3′-acetate, as exemplified by comparison of **11a** and **11c**, decreased the inhibitory potency.[17] The data indicate that addition of the 2-methylidene group, while resulting in improved inhibitory activity of the 7-(2″-pyridylmethylidene)cephems toward the class C P99 β-lactamase, degraded their

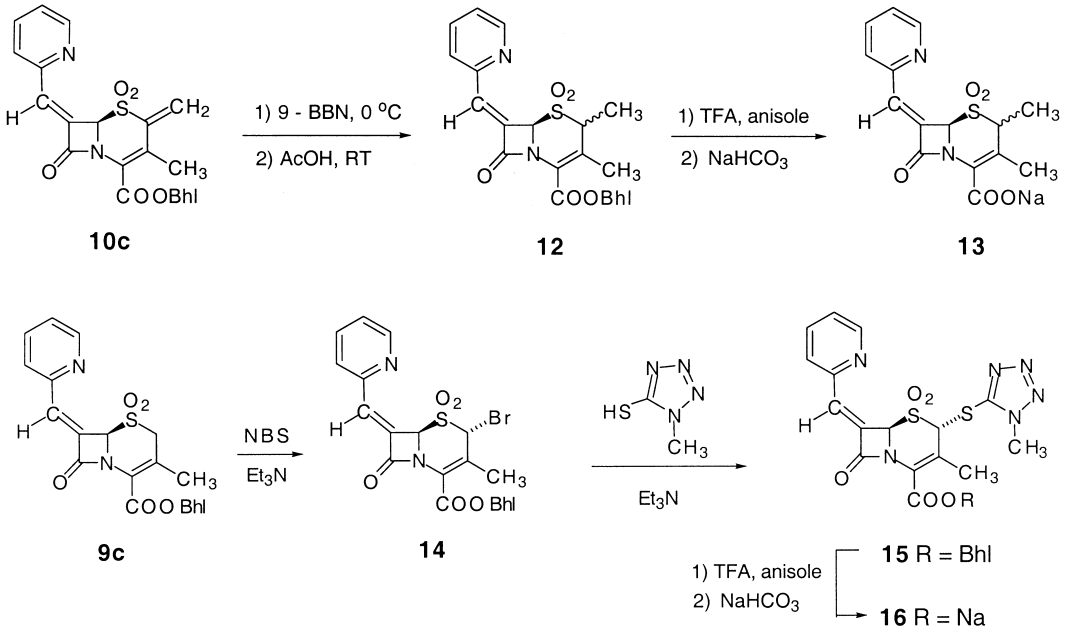
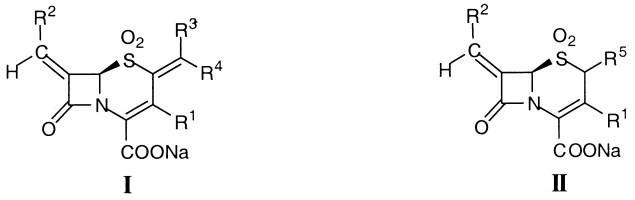
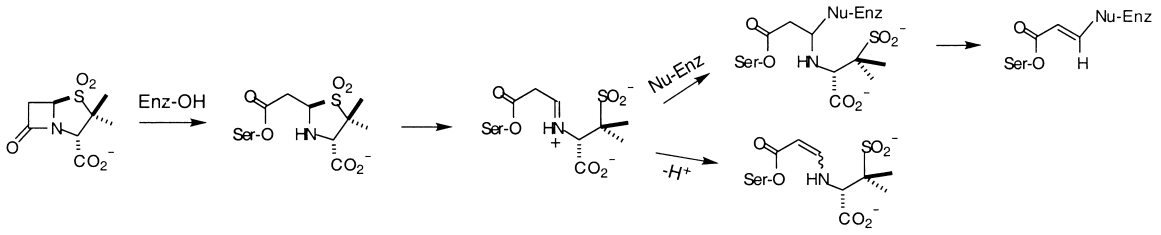


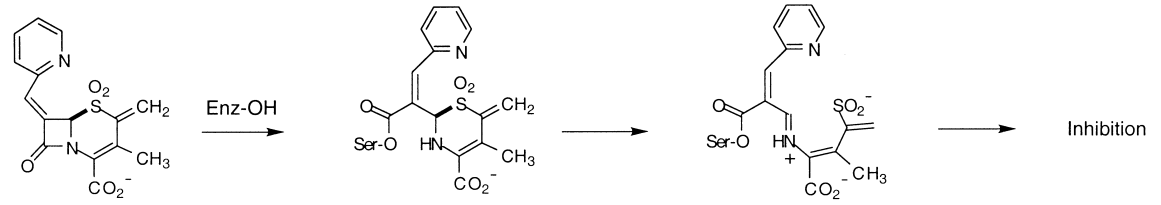
Table 1. Inhibition of three representative serine β-lactamases[19]



Compd	Type	R^1	R^2	R^3, R^4	R^5	IC_{50} (μM)		
						P99	TEM-1	PC1
2	Tazo	—	—	—	—	51.9	0.29	2.57
3	II	CH_2OAc	2′-pyr	—	H	0.498	34.8	402
11a	I	CH_2OAc	2′-pyr	H, H	—	0.165	99.2	733
11c	I	CH_3	2′-pyr	H, H	—	0.039	91.1	1860
11g	I	CH_3	2′-pyr	SCH_3, SCH_3	—	0.51	173	NT
13	II	CH_3	2′-pyr	—	CH_3	3.9	74.1	102
16	II	CH_3	2′-pyr	—	$SC_2H_3N_4$	29.0	31.6	844
4	II	CH_2OAc	CO_2-*t*-Bu	—	H	6.56	0.03	977
11b	I	CH_2OAc	CO_2-*t*-Bu	H, H	—	1.55	0.932	NT
11d	I	CH_3	CO_2-*t*-Bu	H, H	—	48.6	56.5	NT
11f	I	$CH_2SC_2H_3N_4$	CO_2-*t*-Bu	H, H	—	2.38	1.13	NT
11h	I	CH_2OAc	CO_2Na	H, H	—	2.49	498	230
11i	I	CH_3	CO_2Na	H, H	—	36.8	277	NT



Scheme 1.



Scheme 2.

ability to inhibit both of the class A enzymes. This trend is also evident in comparing inhibition of the 7-[(*tert*-butoxycarbonyl)methylidene]cephems **4**, **11b**, **11d** and **11f**.[18] In the latter series, however, the extremely poor overall activity of the desacetoxy cephem **11d** indicates a mechanistic requirement for a leaving group at the 3′-position, in contrast to the 7-(pyridylmethylidene)-cephem series.

In the case of the penicillin sulfones (**5**), good inhibitors have been prepared with R = 2′-pyridyl,[20] and also with R = COONa[8b] (with selected 2′-position modifications improving activity in both series).[8a] While the inhibitory activity of the corresponding 2′-pyridylmethylidene cephalosporin (**3**), is also high, that of the 7-position carboxylate is not.[7] Similarly, in the present 2′-methylidene series, the 7′-carboxymethylidenes **11h** and **11i** lack significant inhibitory activity.

The present data imply that the 7-(pyridylmethylidene)- and the 7-[(*tert*-butoxycarbonyl)-methylidene]cephalosporins, which are established inhibitors of the class C and class A β-lactamases, respectively, operate by different inhibitory mechanisms. The penicillin sulfones, including sulbactam and tazobactam, represent the closest available mechanistic analogy of the present cephalosporin sulfones. In the former case, it is believed that inhibition results from the series of chemical transformations shown in Scheme 1.[21]

An analogous mechanism for the cephalosporin sulfones is shown in Scheme 2. We anticipated that a ring opening of the six-membered ring might be promoted by the presence of a C-2 methylidene group. We are currently engaged in crystallographic and kinetic studies to clarify the nature of the chemical transformations leading to inhibition.

Acknowledgements

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19. Assay method involves 4 min incubation of a solution of inhibitor and enzyme, followed by transfer of an aliquot into a dilute solution of the substrate nitrocefin. Hydrolysis is monitored spectrophotometrically at 480 nm for 1 min. The rate is constant throughout this period. Error is $\pm 10\%$, based on multiple experiments. The purity of all compounds and intermediates was verified by NMR.
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