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THE SYNTHESIS AND LACTAMASE INHIBITORY ACTIVITY OF 6-(CARBOXYMETHYLENE)PENICILLINS AND 7-(CARBOXYMETHYLENE)CEPHALOSPORINS

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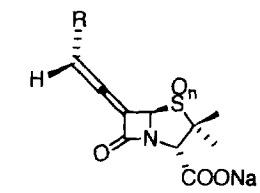
Abstract. A series of 6-(carboxymethylene)penicillinates and 7-(carboxymethylene)cephalosporanates were synthesized and evaluated as inhibitors of one type A and two type C β-lactamases. Disodium 6-(carboxymethylene)penicillinate sulfone (**15**) showed broad spectrum activity. A kinetic analysis demonstrated that **15** was a potent, partially irreversible inhibitor of the β-lactamase derived from *Enterobacter cloacae* P99.

We recently reported the synthesis and biological evaluation of several penicillin and cephalosporin derivatives having 6- and 7-exocyclic unsaturation, respectively. These compounds include the 6-vinylidenepenicillins (**1**),[1] the 7-vinylidenecephalosporins (**2**),[2] and the 7-alkylidenecephalosporins (**3**),[3] with representative examples from each class being evaluated as β-lactamase inhibitors of type A and type C β-lactamases. Different 6-alkylidenepenicillins with potent β-lactamase activity had previously been reported by other investigators. This includes 6-acetylmethylenepenicillanic acid (**4**),[4] 6-methoxymethylenepenicillanic acid (**5**),[5] the 6-(heterocyclyl)penicillin sulfones (**6**)[6] and the 6-(alkylidene)penems, such as BRL 42715 (**7**).[7]



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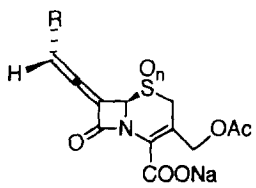

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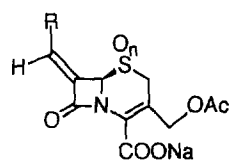

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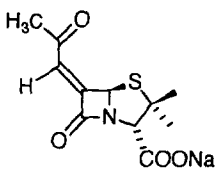

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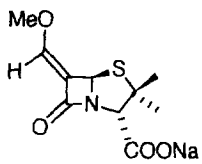

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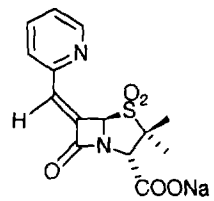

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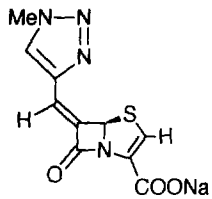

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During our analysis of the biological activity of 7-alkylidenecephalosporins, we noticed that 7-(*tert*-butoxycarbonyl)methylenecephalosporin sulfone (**19**) exhibits potent inhibitory activity of the β-lactamase derived from *Escherichia coli* WC3310. We thus decided to prepare and evaluate the corresponding penicillin analog. The synthesis (shown below) proceeded along the established route from 6-oxopenicillanic acid,[1] through the 6-alkylidenepenam sulfide. Oxidation cleanly afforded the corresponding sulfones. Deprotection, however, produced the doubly deprotected disodium salts (**14** and **15**) in addition to the desired *tert*-butyl esters (**12** and **13**). These disodium salts were homogenous by reverse phase chromatography, but extensively hydrated. The corresponding cephalosporins were prepared in an analogous fashion from 7-oxocephalosporanic acid.[2]

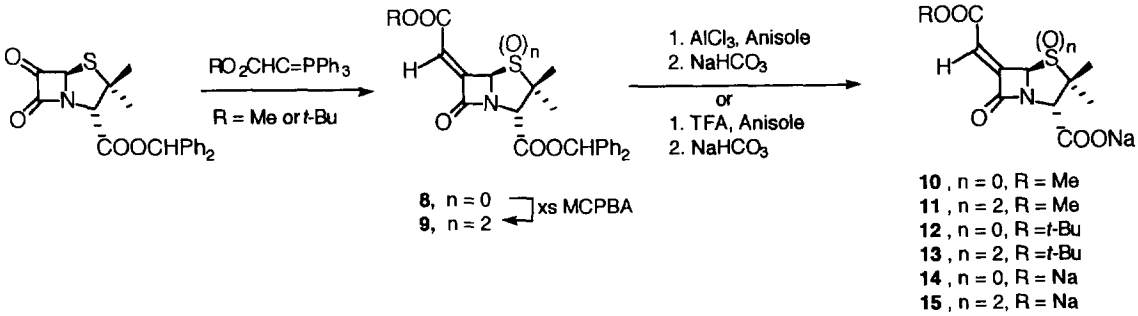
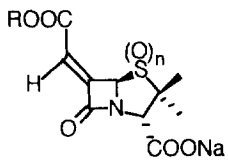


Table 1. β-Lactamase Inhibitory Activity, IC_{50} (nM)



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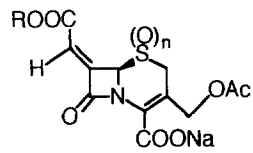

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Compound	n	R	*Ent. cloacae* P99	*E.coli* WC3310 TEM-2	*Ent. cloacae* SC 12368 E-2
tazobactam			943	25	4000
clavulanic acid			>20000	60	>20000
2a	2		130	>20000	260
3a	2		25	800	25
4	0		361	3	5415
10	0	CH_3	>20000	>20000	>20000
11	2	CH_3	154	308	615
12	0	$C(CH_3)_3$	8950	>20000	>20000
13	2	$C(CH_3)_3$	4090	136	13600
14	0	Na	13900	>20000	>20000
15	2	Na	45	120	91
16	0	CH_3	>20000	>20000	>20000
17	2	CH_3	>20000	37	>20000
18	0	$C(CH_3)_3$	2500	>20000	>20000
19	2	$C(CH_3)_3$	7800	5	5900
20	0	Na	>20000	>20000	>20000
21	2	Na	>20000	400	>20000

In Table 1,[8] we report the biological (β-lactamase inhibitory) activity of these compounds as inhibitors of β-lactamase enzymes derived from *Enterobacter cloacae* P99, *Escherichia coli* WC3310, and *Enterobacter cloacae*

P99. Included for comparison are known inhibitors, such as 6-acetylmethylenepenicillanic acid (**4**), clavulanic acid, and tazobactam, as well as our inhibitors **2a** (R = *t*-Bu, n = 2) and **3a** (R = 2'-pyridyl, n = 2).

Compound **15**[9] appeared to be a highly potent, relatively broad spectrum inhibitor, and we thus decided to explore its activity in more detail.[10] In the following study, *E. cloacae* P99 was chosen as the taget enzyme. Shown below are Kitz and Wilson[11] progress curves demonstrating the progressive inhibition characteristic of irreversible inhibitors and defining the second order rate constant of inactivation as 8.4 x 10^5 mol^{-1} min^{-1}.

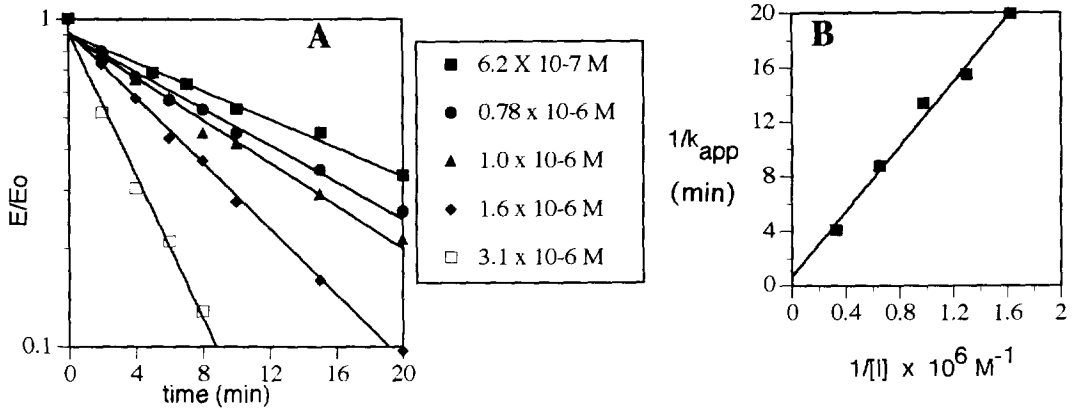


Figure 1. Panel A shows the progressive inhibition of enzyme at selected concentrations of inhibitor **15**. Panel B shows the double reciprocal plot of the progressive inhibition data which defines the value of $k_3' = 8.4 \times 10^5$ mol^{-1} min^{-1}

The two plots below demonstrate that, at a ratio of inhibitor:enzyme of 5:1, nearly complete inhibition of the lactamase was observed. Unlike most other inhibitors which usually exhibit some (usually slow) reactivation at these low [I]/[E] ratios, continued increasing inactivation was observed (over a 90 min interval) even at ratios as low as 1:1. The right plot below demonstrates that the turnover number of this inhibitor is approximately 5.

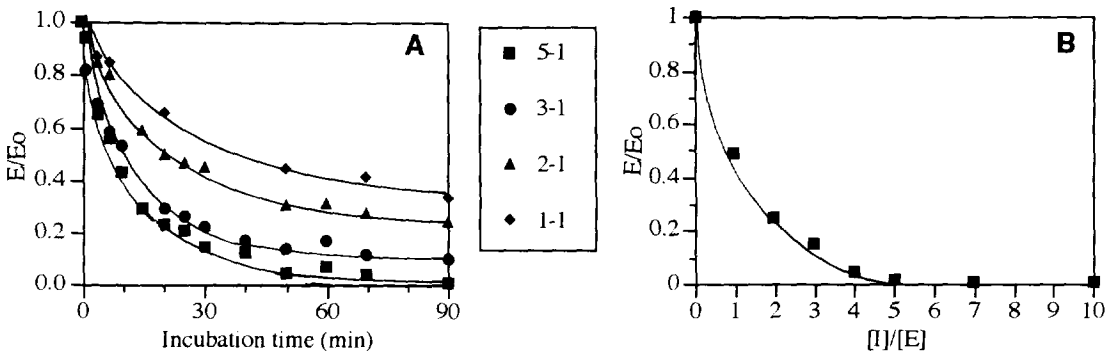


Figure 2. Panel A shows the remaining enzyme activity as a function of incubation time with inhibitor **15** at various [I]/[E]. Panel B shows the remaining enzyme activity following 90 min incubation at various [I]/[E].

Compound **15** is compared with **4** in the plot below (on the left). Even the extremely potent and broad spectrum inhibitor **4** exhibits a small amount of reactivation (turnover) during this 90 min interval. Under these conditions (in the presence of a very slight molar excess of inhibitor) such turnover is not seen for compound **15**. A more stringent test of the irreversibility of this inhibitor is the removal of all excess inhibitor by sephadex filtration (following incubation with excess of inhibitor). On the right, it is seen that, under these conditions, enzyme which has been inhibited with excess of **15** can, after sephadex filtration, recover approximately 40% of its activity slowly over a period of one to two days.

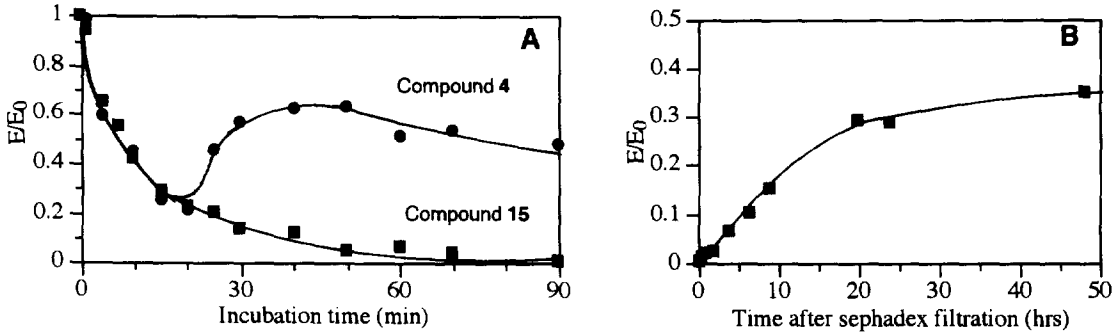
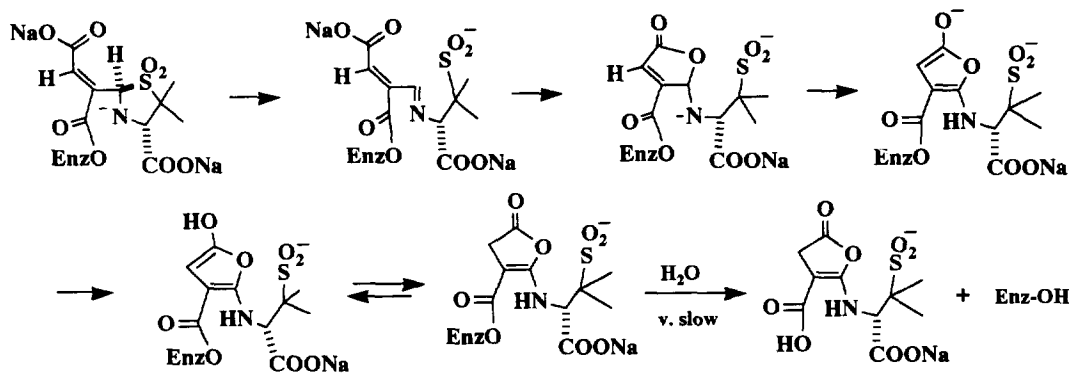


Figure 3. Panel A shows the relative inhibitory activity of both **15** (■) and **4** (●) as a function of incubation time (in each case [I]/[E] = 5). Panel B shows the slow recovery of enzyme activity after removal of excess inhibitor **15**.

A potential mechanistic proposal for this highly efficient inhibition is shown below. Following the precedent of Chen, we propose an intramolecular attack of the carboxylate on the intermediate imine. In a second step, the double bond is isomerized leading to the formation of a stabilized β-amino-β-acyloxyacrylate (vinylogous urethane) as an intermediate. Further mechanistic investigations are currently in progress.



In Table 2 we report the synergistic activity of our inhibitors with the known antibiotic piperacillin toward intact bacteria.[12] Synergy was observed for **15** with TEM-1 and OXA-1producing organisms. Compound **11** was synergistic with OXA-1 producing *E. coli* and for *S. aureus*. **17** was synergistic only with TEM-1 *E. coli*. The slightly lower *in vivo* activity (relative to their activity against the isolated enzymes) may indicate a somewhat reduced ability for these inhibitors to cross the cell membrane.

Table 2. Antibacterial Activity of Piperacillin: Inhibitors Synergy Study, MICs(μg/ml)

Inhibitor	***E. coli*** **TEM-1**	***E. coli*** **TEM-2**	***E. coli*** **OXA-1**	***E. cloacae*** **P99**	***E. cloacae*** **12368**	***S. aureus*** **ATCC29213**
Piperacillin	>128	>128	64	128	16	4
Tazobactam	>128	>128	>128	>128	>128	64
PIP: TZB	2	8	16	16	16	1
11	>128	>128	>128	>128	>128	>128
PIP: **11**	64	128	16	128	16	1
13	>128	>128	>128	>128	>128	128
PIP: **13**	128	>128	32	128	32	2
15	>128	>128	>128	>128	>128	>128
PIP: **15**	32	64	16	128	16	4
17	>128	>128	>128	>128	>128	>128
PIP: **17**	32	64	64	128	16	4
19	>128	>128	>128	>128	>128	4
PIP: **19**	64	128	64	128	16	4
21	>128	>128	>128	>128	>128	16
PIP: **21**	64	128	64	128	32	4

Acknowledgment. We are grateful to the National Institute of Health and the Robert A. Welch Foundation for support of this research. We are also indebted to Dr. Karen Bush (American Cyanamid) for providing penicillin-resistant bacterial strains (and valuable advice), to William Weiss (American Cyanamid) for performing the synergy studies, and to Dr. Christine Buchanan (Biology Dept., SMU) who directed our cultivation of the strains.

References and Notes

1. Buynak, J. D.; Borate, H. B.; Lamb, G. W.; Khasnis, D. D.; Husting, C.; Isom, H.; Siriwardane, U. *J. Org. Chem.* **1993**, *58*, 1325.
2. Buynak, J. D.; Khasnis, D.; Bachmann, B.; Wu, K.; Lamb, G. *J. Am. Chem. Soc.* **1994**, *116*, 10955.
3. Buynak, J. D.; Wu, K.; Bachmann, B.; Khasnis, D.; Hua, L.; Nguyen, H. K.; Carver, C. L. *J. Med. Chem.* **1995**, *38*, 1022.
4. a) Arisawa, M.; Then, R. L. *J. Antibiot.* **1982**, *35*, 1578. b) Arisawa, M.; Then, R. *Biochem. J.* **1983**, *209*, 609. c) Arisawa, M.; Adam, S. *Biochem. J.* **1983**, *211*, 447.
5. Brenner, D. G.; Knowles, J. R. *Biochemistry* **1984**, *23*, 5839.
6. a) Chen, Y. L.; Chang, C. W.; Hedberg, K. *Tetrahedron Lett.* **1986**, *27*, 3449. b) Chen, Y. L.; Chang, C. W.; Hedberg, K.; Guarino, K.; Welch, W. M.; Kiessling, L.; Retsema, J. A.; Haskell, S. L.; Anderson, M.; Manousos, M.; Barrett, J. F. *J. Antibiot.* **1987**, *40*, 803.
7. a) Bennet, I. S.; Brooks, G.; Broom, N. J. P.; Calvert, S. N.; Coleman, K.; Grancois, I. *J. Antibiot.* **1991**, *44*, 969. b) Farmer, T. H.; Page, J. W. J.; Payne, D. J.; Knowles, D. J. C. *Biochem. J.* **1994**, *303*, 825.
8. The data was obtained by incubating the enzyme with inhibitor at high concentration for 10 min prior to the addition of a small portion of this mixture to a dilute solution of nitrocefin. The rate of change in the absorbance at 482 nm was followed for one min and compared with the corresponding rate of change in the absence of inhibitor. The IC_{50} value is the concentration of inhibitor needed to achieve a 50% reduction in the catalytic activity of the enzyme. For comparison, tazobactam and clavulanic acid were also evaluated.
9. Data for **15:** IR (KBr) 1712, 1694, 1627, 1379, 1315, 1124, 632, 490 cm^{-1}. ^1H NMR (D_2O) δ 6.53 (1 H, s), 5.59 (1 H, s), 4.11 (1 H, s), 1.42 (3 H, s), 1.31 (3 H, s). High-resolution mass spectrum for $[C_{10}H_9NO_7SNa]^+$ i.e. (M-Na) m/z calcd. 309.9997, found. 310.0008.
10. a) While this work was in progress, it came to our attention that compound **14** (the sulfide of **15**) had been prepared by other investigators and found (in agreement with us) that this compound was lacking significant β-lactamase inhibitory activity: Häbich, D.; Metzger, K. *Heterocycles* **1986**, *24*, 289. b) Another related article involving the preparation of 6-alkylpenams is: Adam, S.; Then, R.; Angehrn, P. *J. Antibiot.* **1993**, *46*, 641.
11. Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245.
12. The inhibitors were tested alone for antibacterial activity and then tested as a 1:1 combination with piperacillin (PIP). This combination was then compared with a similar combination of tazobactam (TZB) and piperacillin.

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