Communication

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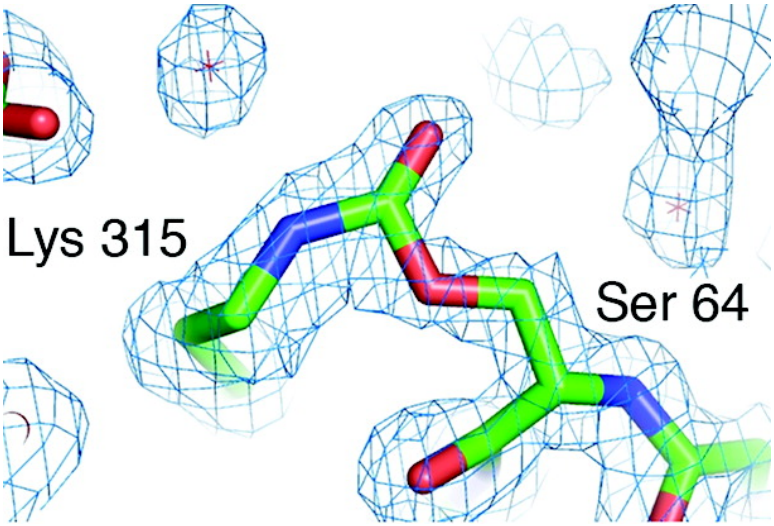

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O-Aryloxycarbonyl Hydroxamates: New β-Lactamase Inhibitors That Cross-Link the Active Site

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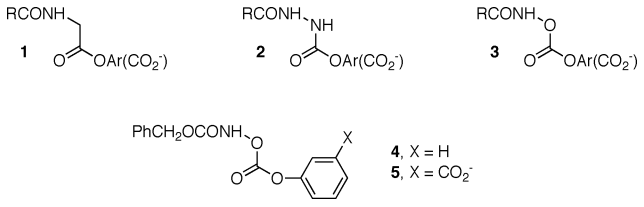
The effective lifetime of β-lactams as antibiotics can be extended by concurrent treatment of patients with β-lactamase inhibitors.[1,2] The β-lactamase inhibitors in commercial production at present, however, are of limited spectrum and are largely specific to the class A enzymes.[1] New classes of inhibitor with broader specificity covering classes B, C, and D β-lactamases would be welcome since the prevalence of these enzymes, and thus β-lactam resistance among bacteria, continues to grow. We describe in this communication a new type of β-lactamase inhibitor with an unusual mechanism of action involving the covalent cross-linking of active side residues.

The depsipeptides of general structure **1** are β-lactamase substrates.[3,4] Although the aza analogues **2** display little or no substrate activity,[5] we were encouraged to try the oxa analogues **3** because of the inhibitory properties of vanadate/ hydroxamic acid complexes.[6] Compounds **4** and **5** were therefore obtained from careful reaction of the appropriate hydroxamic acid and chloroformate (the latter carboxyl-protected in the case of **5**) in the presence of imidazole (Supporting Information). An alkoxy side chain was chosen since the alkyl or aryl analogues were unstable to the Lössen rearrangement.[7] An NMR spectrum of ^{15}N-**4** in DMSO-d_6 showed a ^{15}N resonance at 161.1 ppm coupled (J = 93 Hz) to a proton at 11.75 ppm. This clearly identifies the product as the *O*-acyl rather than *N*-acyl hydroxamic acid. Compounds **4** and **5** hydrolyzed in aqueous buffer (20 mM MOPS, pH 7.5), yielding benzyl *N*-hydroxycarbamate, the phenol, and, presumably, bicarbonate; pseudo-first-order rate constants (k_0) of 2.5 × 10^{-4} and 2.78 × 10^{-4} s^{-1}, respectively, were obtained.



Compound **4** inhibited, essentially irreversibly, the class C β-lactamase of *Enterobacter cloacae* P99 in a time-dependent fashion, as evident from Figure 1. At low inhibitor/enzyme concentration ratios, the final activity of the enzyme was not zero, which suggested that some turnover accompanied the inhibition reaction (background hydrolysis of **4** was not sufficient to explain the final activity). A greater excess of inhibitor did completely inactivate the enzyme (Figure 1). A plot of residual activity versus concentration of **4** (Figure 2) suggested that about two turnovers accompanied inhibition. These data were fitted to Scheme 1, where EI is likely to be a hydrolyzable acyl enzyme[4] which can also partition to a dead end complex EI′. These fits, shown as solid lines in the figures, yielded k_1 and k_2/k_3 values of 6.1 ± 0.2 × 10^3 s^{-1} M^{-1} and 2.0 ± 0.1, respectively.

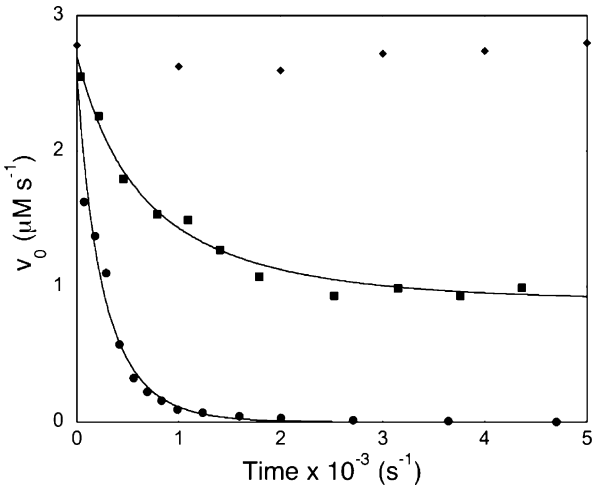


Figure 1. Activity of the P99 β-lactamase (0.25 μM) as a function of time in the presence of **4** (0 μM, ◆; 0.5 μM, ■; 2.5 μM, ●).

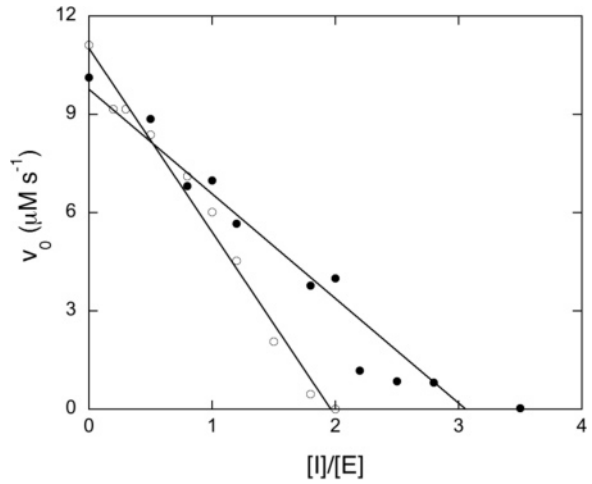
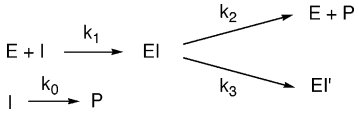


Figure 2. Activity of the P99 β-lactamase (0.25 μM) after complete reaction with **4** (●) and **5** (○) at various concentrations (0−1.0 μM).

Scheme 1



Compound **5** was also an inhibitor of the P99 enzyme (Figure 2). Experiments analogous to those described above yielded values of k_1 and k_2/k_3 of 5.4 ± 0.3 × 10^3 s^{-1} M^{-1} and 1.00 ± 0.05, respectively. It is interesting that **5**, bearing the *m*-carboxy sub-

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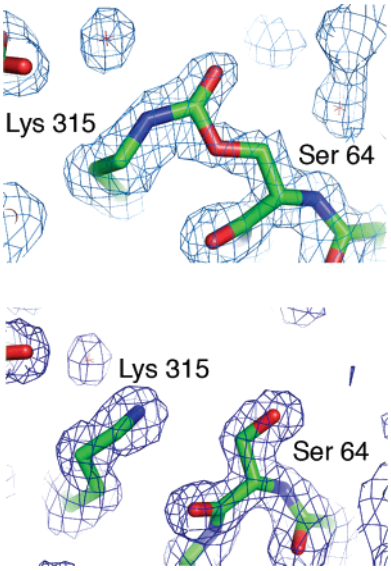


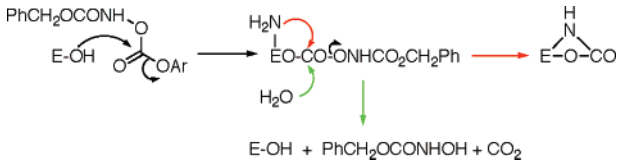
Figure 3. Top: crystal structure of the active site of the AmpC β-lactamase after inhibition by **4**, showing the carbamate cross-link between Ser64 and Lys315. The electron density is contoured at the 3σ level. Bottom: the same view of the wild-type enzyme,[10] showing a clear gap between Ser64 and Lys315.

stituent, is not a better inhibitor than **4**. This result is contrary to what would be expected from comparable substitution in the depsipeptides **1**. A *m*-carboxy group in **1** is thought to interact specifically with the P99 active site.[8] The results suggest that **1** and **5** may not bind to the active site in the same way. It should be noted, however, that inactivation of the enzyme by **5** was competitively inhibited by *p*-nitrobenzene boronic acid, which is itself a competitive inhibitor of the P99 enzyme.[9]

An electrospray mass spectrum of the inhibited enzyme was obtained. Enzyme (10 μM) and **4** (5 mM) were incubated together in MOPS buffer (above) for 5 min, after which time the enzyme was inactive. The protein was then precipitated with trichloroacetic acid, washed, and dried, and an ES+ mass spectrum obtained. The spectrum showed an increase in protein mass of 29, in good agreement with the mechanism of inactivation described below.

A 1.8 Å resolution crystal structure of the inhibited AmpC class C β-lactamase was also obtained, as described in the Supporting Information. The only observable difference from the structure of the native enzyme[10] was at the active site. In monomer A of the structure, the O_γ oxygen of Ser64 is flipped some 180° ($Ser64C_\alpha C_\beta O_\gamma C$) and forms part of an unprecedented carbamate bridge to N_ξ of Lys315 (Figure 3). Tyr150 has moved aside slightly to accommodate insertion of a carbonyl, but O_ξ remains within hydrogen-bonding distance of the inserted carbonyl oxygen (Figure S1, Supporting Information). Lys73 remains hydrogen bonded to Tyr150 O_ξ. Coordinates of the structure have been deposited in the RCSB protein data bank as entry 2P9V.

Scheme 2



The mechanism of inhibition of the P99 β-lactamase by **4** and **5** can thus, from the data available at present, be represented by the sequence shown in Scheme 2. This represents a novel cross-linking of the active site and a previously unobserved specific modification of one of the two conserved lysine residues of the β-lactamase active site. Inhibition of class A β-lactamases by clavulanic acid and penicillin sulfones has been shown to involve cross-linking of the active site serine to the conserved Ser130.[11,12]

We have also observed that **4** and **5**, and other derivatives of these compounds, inhibit the class A TEM β-lactamase. We plan further experiments to determine the scope of these compounds against β-lactam-recognizing enzymes.

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Supporting Information Available: Synthetic procedures for compounds **4** and **5** and the kinetics methods. Details of the crystallographic procedures and statistics are also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Georgopapadakou, N. *Exp. Opin. Invest. Drugs* **2004**, *13*, 1307.
(2) Buynak, J. D. *Biochem. Pharmacol.* **2006**, *31*, 930.
(3) Pratt, R. F.; Govardhan, C. P. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1302.
(4) Govardhan, C. P.; Pratt, R. F. *Biochemistry* **1987**, *26*, 3385.
(5) Cabaret, D.; Garcia Gonzalez, M.; Wakselman, M.; Adediran, S. A.; Pratt, R. F. *Eur. J Org. Chem.* **2001**, 141.
(6) Bell, J. H.; Pratt, R. F. *Biochemistry* **2002**, *41*, 4329.
(7) Renfrow, W. B., Jr.; Hauser, C. R. *J. Am. Chem. Soc.* **1937**, *59*, 2308.
(8) Ahn, Y.-M.; Pratt, R. F. *Bioorg. Med. Chem.* **2004**, *12*, 1539.
(9) Nagarajan, R.; Pratt, R. F. *Biochemistry* **2004**, *43*, 9664.
(10) Usher, K. C.; Blaszczak, L. C.; Weston, G. S.; Shoichet, B. K.; Remington, S. J. *Biochemistry* **1998**, *37*, 16082.
(11) Brown, R. P. A.; Aplin, R. T.; Schofield, C. J. *Biochemistry* **1996**, *35*, 12421.
(12) Kuzin, A. P.; Nukaga, M.; Nukaga, Y.; Hujer, A.; Bonomo, R. A.; Knox, J. R. *Biochemistry* **2001**, *40*, 1861.

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