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 site 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 chlorofor-mate (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 Lo¨ssen rearrangement.7 An NMR spectrum of 15 N-4 in DMSO-d6 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 (k0) 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 enzyme4 which can also partition to a dead end complex EI′. These fits, shown as solid lines in the figures, yielded k1 and k2/k3 values of 6.1 ± 0.2 × 103 s−1 M−1 and 2.0 ± 0.1, respectively.

Compound 5 was also an inhibitor of the P99 enzyme (Figure 2). Experiments analogous to those described above yielded values of k1 and k2/k3 of 5.4 ± 0.3 × 103 s−1 M−1 and 1.00 ± 0.05, respectively. It is interesting that 5, bearing the m-carboxy sub-
An electrospray mass spectrum of the inhibited enzyme was obtained. Enzyme (10 μM) and 5 (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 was at the active site. In monomer A of the structure, the Oκ oxygen of Ser64 is flipped some 180° (Ser64Cα-Cα/Oκ,C) and forms part of an unprecedented carbamate bridge to Nε of Lys315 (Figure 3). Tyr150 has moved aside slightly to accommodate insertion of a carbonyl, but Oκ remains within hydrogen-bonding distance of the inserted carbonyl oxygen (Figure S1, Supporting Information). Lys73 remains hydrogen bonded to Tyr150 Oε. Coordinates of the structure have been deposited in the RCSB protein data bank as entry 2P9V.

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. 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.

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

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