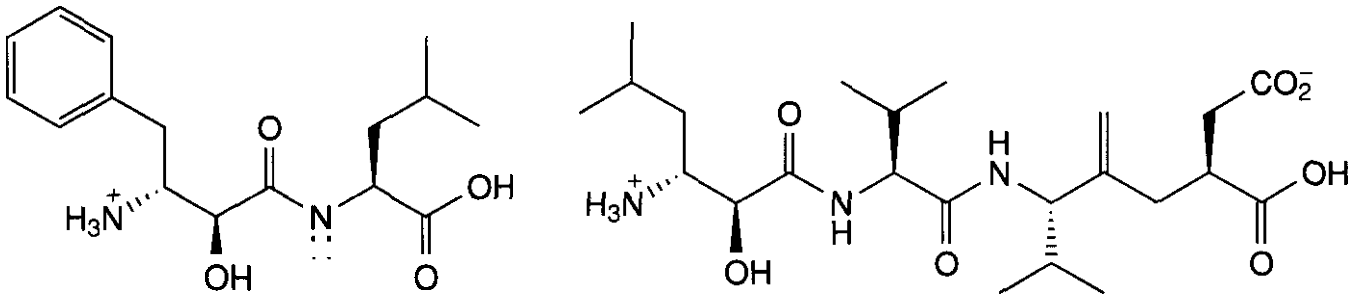


(17) (18)

Figure 17.15. Slow-tight-binding inhibitors of leucine aminopeptidase.

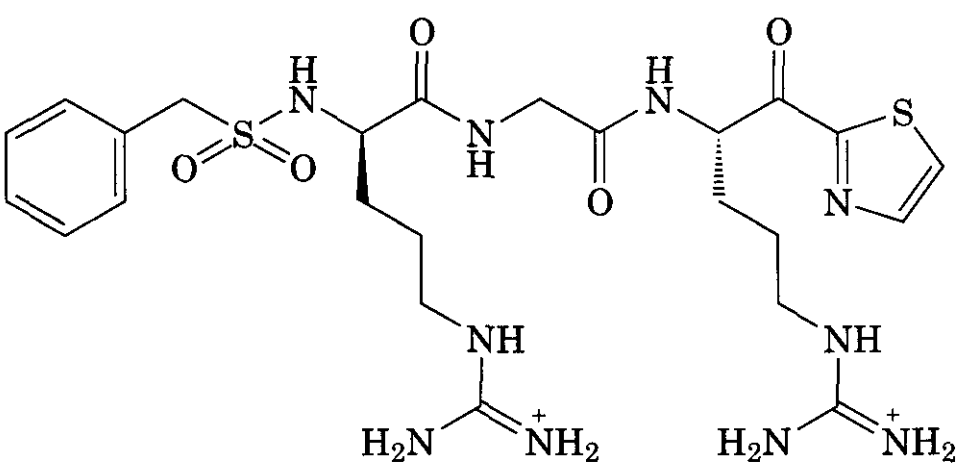
Bestatin (17) (Fig. 17.15) and amastatin (18) have been identified as slow-tight-binding inhibitors of LAP from porcine kidney, with K_i values in the low nanomolar range (84). Later, bestatin was shown to be a slow-binding inhibitor of LAP employing mechanism B, with a K_i value of 0.11 μM and a K_i^* value of 1.3 nM. Values of 1.5×10^{-2} s^{-1} and 2×10^{-4} s^{-1} were obtained for k, and k_{-4} (Equation 17.29), respectively (85). It was assumed that the inhibition of bovine lens leucine aminopeptidase (blLAP) by amastatin would also proceed by mechanism B. This prediction was supported by an X-ray crystallography study of the amastatin-blLAP complex (86), which suggested that (18) (and, by analogy, 17) initially binds to a Zn^{2+} atom in a groove in the active site. The slow step in binding was seen as a subsequent coordination to a second Zn^{2+} atom located deeper in the active site (86).

It is difficult to find clear-cut examples of slow-binding inhibition occurring by mechanism A. However, the inhibition of Factor Xa by a peptidyl-α-ketothiazole was found to be unusual because it appeared that the formation of E . I was partially rate limiting. Factor Xa is a trypsinlike protease found in the blood coagulation pathway, which cleaves prothrombin forming thrombin that, in turn, promotes blood clotting (Equation 17.33).

Inhibitors of Factor Xa activity offer potential as anticoagulants and several irreversible inhibitors of Factor Xa have been developed. One of the few tight-binding reversible inhibitors of Factor Xa is $BnSO_2$-D-Arg-Gly-Arg-ketothiazole (19).

The inhibitor could be displaced from Factor Xa by substrates and, based on steady-state assumptions, the dissociation constant for (19) was found to be 14 pM (87). However, the reaction progress curves indicated a slow-binding process, probably by mechanism B. Stopped-flow fluorescence studies, combined with kinetic analysis, showed that the isomerization step (E . I + E . I*) is unusually fast and that the formation of E · I is, at least, partially rate limiting.

In some instances the type of inhibition has been found to be isozyme specific. For example, inducibly expressed isozymes (iNOS) and constitutively expressed isozymes (cNOS) of nitric oxide synthase (NOS) all catalyze the conversion of L-arginine to L-citrulline and nitric oxide (Equation 17.34).



(19)