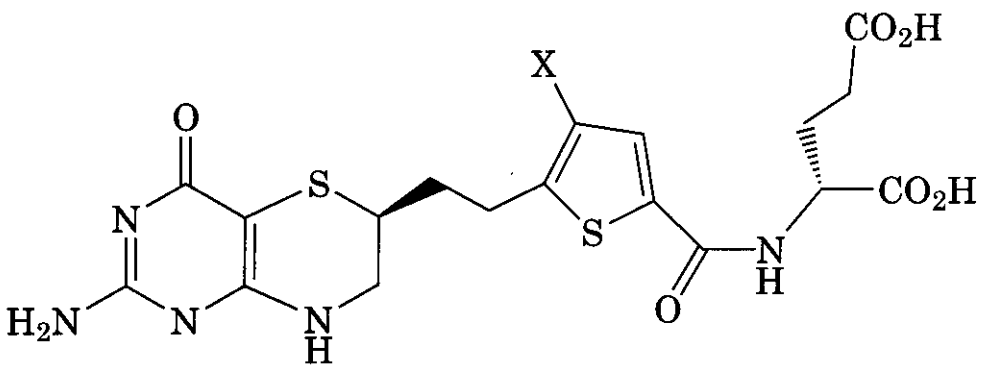

(26) X = H
(27) X = methyl

2.4 Proteases

2.4.1 Angiotensin-Converting Enzyme and the Discovery of Captopril. The design of captopril was a landmark in the application of structural models for developing enzyme inhibitors (81,82). This discovery rapidly led to the development of a family of therapeutically useful inhibitors of angiotensin-converting enzyme for the treatment of hypertension (83). The story has been reviewed thoroughly (for a historical perspective, see either Ref. 84 or Ref. 85), and is briefly summarized here. Angiotensin II, a circulating peptide with potent vasoconstriction activity, is generated by the C-terminal hydrolytic cleavage of a dipeptide from angiotensin I, catalyzed by angiotensin-converting enzyme. Therefore, inhibitors of angiotensin-converting enzyme are vasodilators. ***[An important aside:*** Angiotensin I is generated from a precursor by the action of renin, another exopeptidase that is an aspartyl protease. An orally available renin inhibitor remains an elusive goal, although there are still efforts under way that use SBDD methods (86). Renin inhibitors were early tools in the study of the essential aspartyl protease of human immunodeficiency virus (HIV), which is discussed later.]

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu → Asp-Arg-Val-Tyr-Ile-His-Pro-Phe + His-Leu
Angiotensin I — Angiotensin II

A key tool in the discovery of captopril at Squibb was the use of a model for the active site of angiotensin-converting enzyme (Fig. 10.8). This model was based on the already known X-ray structure of bovine pancreatic carboxypeptidase A. Both enzymes are C-terminal exopeptidases that require zinc ion for activity, but differ in that carboxypeptidase A releases an amino acid, rather than a dipeptide. Hence, the binding site for the angiotensin-converting enzyme was postulated to be longer, and to contain groups to interact with the central peptide linkage. The suggestion had been made (87) that the inhibition of carboxypeptidase A by benzylsuccinate could be explained by viewing benzylsuccinate as a "by-product analog" (Fig. 10.8, top). The hypothesis was that one of the carboxylates bound into a cationic site, whereas the other interacted with the active site zinc. If this were true, then a similar model for angiotensin-converting enzyme predicted that slightly longer diacids, designed with some regard for the sequence preferences of the converting enzyme, should inhibit that enzyme. This hypothesis was quickly confirmed by the inhibitory activity of succinyl-proline (**28a**).

Peptide sequences related to those of snake venom peptides had already been used to define the structural requirements for peptide inhibitors of angiotensin-converting enzyme. Peptides are unstable *in vivo* and poorly absorbed intestinally, and thus are not good drug candidates. However, the best peptide inhibitor was 500-fold more potent than (**28a**). The