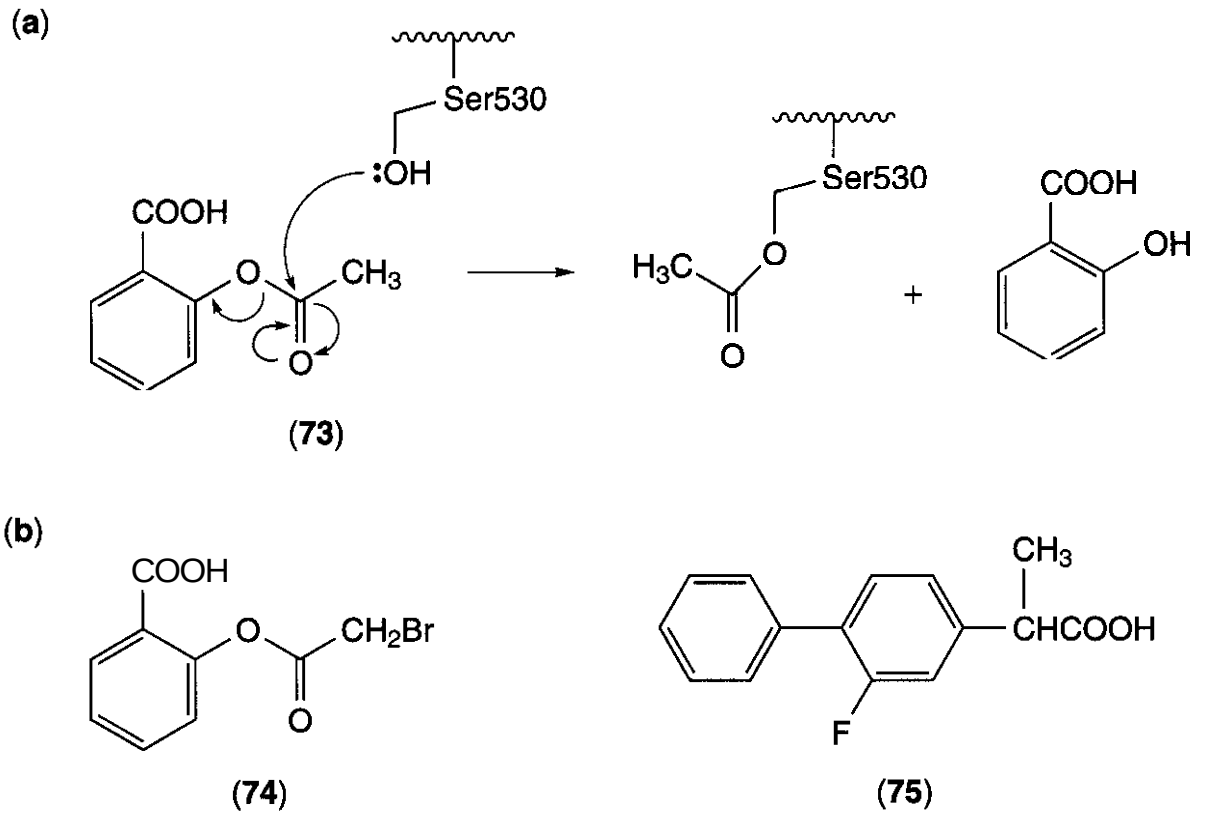

Figure 17.32. (a) Inactivation of prostaglandin H2 synthase by aspirin, and (b) inhibitors cocrystallized with prostaglandin synthase.

the molecule is arranged in an extended conformation, the reactive sulfonyl fluoride group would be found in a position analogous to that occupied by the y-phosphoryl group of ATP. This was initially used to explore the regulatory site of glutamate dehydrogenase (192) and the active site of pyruvate kinase (193). It has now been employed to label the NAD and ATP sites of more than 50 proteins (163). Modifications to 5'-FSBA have provided the fluorescent probe (78) as well as the bifunctional affinity label (79), which has a photoactivatable azido group as well as the electrophilic fluorosulfonyl moiety (163). The bromodioxybutyl compound (80)contains the adenine, ribose, and 5'-monophosphate of adenosine monophosphate (AMP). It is also water soluble and negatively charged at neutral pH. As described above, a bromomethyl ketone group will react with a number of nucleophiles, whereas the dioxo group can potentially react with arginine residues. This reagent has a structural similarity to adenylosuccinate (81) and was used to identify a critical arginine residue in the active site of adenylosuccinate lyase, an enzyme whose deficiency in humans leads to severe mental retardation and autism (163).

3.3 Mechanism-Based Inhibitors

Mechanism-based inactivators have great potential as drugs because they are designed to be specific toward their target enzyme. Furthermore, because these compounds are unreactive until activated within their target enzyme, they are expected to show little or no cellular toxicity. The design of mechanism-based inhibitors requires an understanding of the binding specificity requirements for the ligand-recognition site of the enzyme, to promote the formation of the initial noncovalent enzyme-inhibitor complex E . I (Equation 17.46). In addition, the choice of an appropriate latent functional group requires knowledge of the catalytic mechanism of the target enzyme with its normal substrate. Finally, covalent bond formation by the activated inhibitor (I′) will strongly depend on its inherent chemical reactivity, and its proximity to a susceptible amino acid residue or cofactor. A number of excellent reviews and monographs have appeared on the general design of mechanism-based inhibitors (166, 167, 194–201). The following examples have been chosen to emphasize both the potential for the use of