ter, producing acetate and regenerated enzyme. Agents such as parathion (101) and sarin (102) have found utility as insecticides and nerve gases, respectively, because they react with the enzyme to form the active-site serine-phosphate esters, (**103**) and (**104**). These esters are hydrolyzed extremely slowly by water, making the inhibition effectively irreversible (i.e., both k_{-}, and $k_{}$ are very small), although the inhibition can be overcome with high concentrations of strong nucleophiles such as hydroxylamine.

More recently, it has been established that inhibitors of acetylcholinesterase may play a role in the memory enhancement in patients with Alzheimer's disease (217). Unlike (101) and (**102**), carbamate inhibitors such as physostigmine (105) and rivastigmine (**106**) are classified as pseudoirreversible inhibitors because they react with AcChE to form a carbamylated serine (107). By comparison with the serine-phosphate ester, the carbamylated serine is rapidly hydrolyzed, thereby regenerating AcChE. For example, reactivation of the physostigmine-inactivated enzyme is rapid, with a $t_{1/2}$ of less than 40 min (218). Rivastigmine, a more useful therapeutic agent, is considerably longer acting, with a half-life of more than 10 h (217, 219). Overall, for pseudoirreversible inhibitors of this type, the effectiveness and duration of the "irreversible" inhibition will be controlled by the chemical nature of the groups transferred to the active-site nucleophile, making it readily amenable to manipulation.

In pseudoirreversible inhibitors of the second class, the enzyme is regenerated by the inhibitor simply dissociating from the enzyme; that is, the binding is covalent but reversible (k_{-2} * $k_{}$). This class can also be exemplified by an AcChE inhibitor. For example, the trifluoromethyl ketone (**108**) binds to AcChE as a slow-binding inhibitor (Section 2.4.1) with a K_i value of 0.06 nM, and a k_{off} value of 6.7×10^{-6} s^{-1} (220). A linear correlation was observed between K_i values of a series of fluoromethyl ketones and the V_{max}/K_i value for the corresponding substrate (220). This suggests (127) that the tetrahedral adduct (**109**), in effect, mimics the transition state (or a high-energy intermediate), thereby accounting for the high affinity (Section 2.5.3). The affinity of the inhibitor for AcChE could be decreased (with a concomitant increase in the value of $k_{}$), by sequentially reducing the number of fluorine atoms into the methyl group adjacent to the ketone (220). Finally, it should be noted that the two classes of pseudoirreversible inhibitor can be differentiated by examining the decomposition products of the inhibition reaction. When hydrolysis is required for enzyme regeneration, cleavage products, such as substituted carbamates, will be in evidence. Conversely, the trifluoromethyl ketones will not be broken down by AcChE and no decomposition products will be observed.

4 CONCLUSIONS

Enzyme inhibitors have long played an important role in medicine, pharmacology, and basic research. The advances in DNA technology have enabled cloning and overexpression of large numbers of enzymes, and the approaches described in this chapter have already led to the development of novel therapeutic agents. However, in the postgenomics era, large numbers of new targets have been identified. Although the drug discovery process moves toward structure-based drug design as its prime tool, even with high-throughput crystallography, not all target proteins will be readily accessible. The evolution of algorithms that can predict enzyme function and mechanism will ensure that the rational design of enzyme inhibitors not only complements structure-based approaches but continues to play a stand-alone role in the discovery of novel therapeutics.

REFERENCES

1. M. Sandler and H. J. Smith, Eds., ***Design of Enzyme Inhibitors as Drugs,*** Oxford University Press, Oxford, 1989.
2. M. Sandler and H. J. Smith, Eds., Design of Enzyme Inhibitors as Drugs, Vol. 2, Oxford University Press, Oxford, 1994.
3. P. Krogsgaard-Larsen, T. Liljefors, and U. Madsen, Eds., ***A Textbook of Drug Design and Deuelopment,*** 2nd ed., Harwood Academic, Amsterdam, 1996.