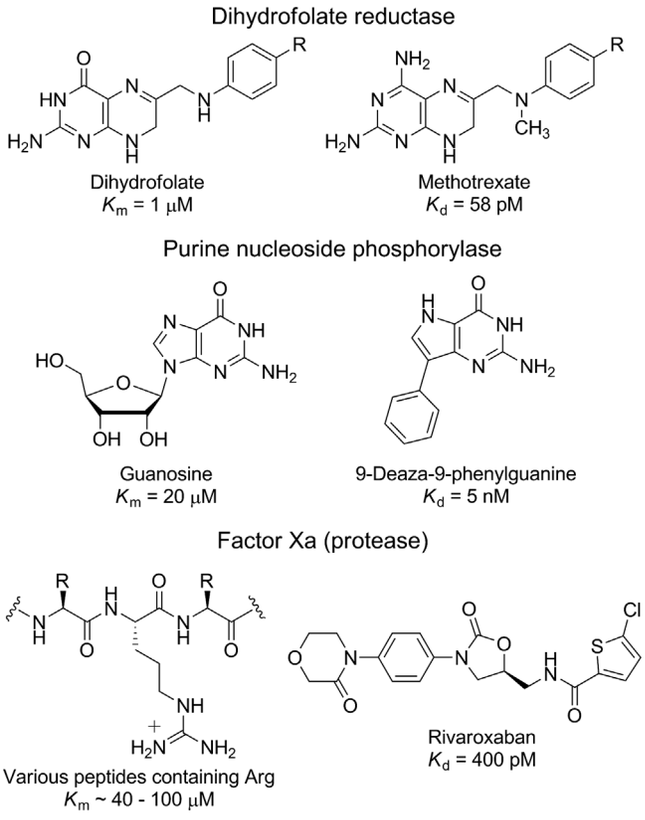

Figure 15.6. Tight-binding inhibitors of dihydrofolate reductase and purine nucleoside phosphorylase that are not transition-state analogs. The substrates and K_m values are given, along with the dissociation constants (K_d) for methotrexate,[138] 9-deaza-9-phenylguanine,[139] and rivaroxaban.[140]

the position of the transition state in the reaction coordinate of chemical reactions.[22,23] Substrate specificity usually limits the utility of this method in enzyme-catalyzed reactions, however. Tight-binding inhibitors, as mentioned above, have also been used to survey transition-state structure, whereby particularly potent inhibition (low nM or pM) is proposed to reflect features of the transition state. However, without direct information on transition-state structure, one cannot be confident that inhibition is due to transition-state mimicry. Tight-binding inhibitors that are not transition-state analogs are well known (Figure 15.6).

Kinetic isotope effects

Features of the transition state, however, can be captured in the vibrational frequencies of atomic stretching and bending modes within the substrate at the transition state relative to those in the ground state. It has long been recognized that isotopic substitution at specific positions in the substrate often result in different reaction rates for the light and heavy species; these phenomena are known as kinetic isotope effects (KIEs; Figure 15.7). When the bonding environment surrounding the atom of interest becomes less restrained at the transition state, the gap in zero point energies (ZPEs) of the bonds to the light and heavy isotopes becomes smaller; thus, the rate constant for the light

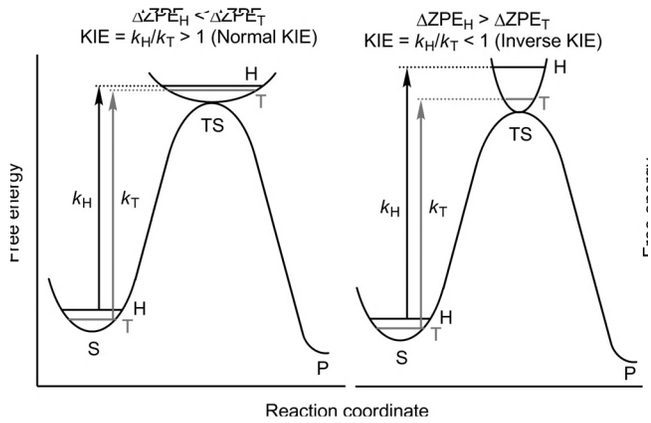

Figure 15.7. Origin of normal and inverse kinetic isotope effects (KIEs). Differences in the zero-point energies (ZPEs) of bonds to light (H) and heavy (T) isotopes at the ground state (S) and the transition state (TS) result in differences in the corresponding rate constants, k_H and k_T. For a looser bond at the transition state (left diagram), the ZPEs of the light and heavy isotopes are closer, giving rise to a normal KIE ($k_H/k_T > 1$). In contrast, for a stiffer bond at the transition state (right diagram), the ZPEs are more greatly separated, yielding an inverse KIE ($k_H/k_T < 1$).

species is greater than that for the heavy species, and a normal KIE (i.e., $k_{light}/k_{heavy} > 1$) is obtained. In contrast, when the bonding environment becomes more restrained at the transition state, the difference in ZPEs becomes larger, yielding an inverse KIE (i.e., $k_{light}/k_{heavy} < 1$). The magnitude of the KIE indicates the degree to which the bonding environment has changed between the ground state and the transition state. This information reports on the extent of bond formation/cleavage, as well as geometrical changes at and remote from the reactive center. If KIEs are determined for multiple positions in the substrate, one can deduce a structure for the transition state with the aid of computational modeling.[21,24]

Methods for the measurement of enzymatic KIEs and their interpretation in relation to transition-state structure have been well described in the literature.[25–29] Two major approaches for the measurement of KIEs are the competitive and noncompetitive (direct) methods. The direct approach involves measurement of the reaction kinetics for the light and heavy isotopologs in separate experiments, and the ratio of the rate constants gives the experimental KIE. The competitive method, in contrast, involves a mixture of the heavy (e.g., ^{3}H) and light (e.g., ^{1}H with remote ^{14}C) isotopologs, with the remote label acting as an internal standard. A normal KIE will cause an enrichment in the light, faster-reacting isotope (i.e., larger ^{14}C/^{3}H ratio) in the product, whereas the isotopic ratio of unreacted substrate will decrease. Experimental KIEs are calculated from comparison of the ratios before reaction and after partial enzymatic conversion. Because the reaction conditions are always identical for both species in the competitive method, it is an order of magnitude more precise than the direct method and is a superior technique usually employed in transition-state analysis.[26,30]