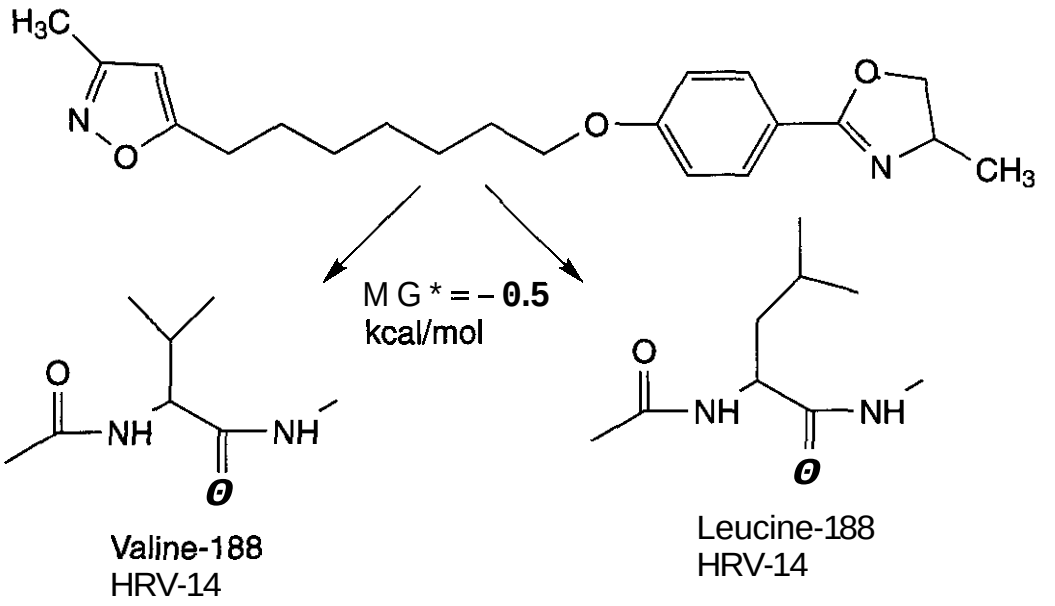

Figure 3.26. Calculated (330) relative affinity of a Sterling-Winthrop antiviral that binds to rhinovirus coat protein (HRV-14) and to the V188L mutant. Biological data indicate that V188L mutation drastically diminishes activity of the antiviral.

analyses of the multiple binding modes shown with thyroxine analogs (334) by transthyretin, a transport protein, and enkephalin analogs (335) by an FAB fragment have been made through crystallography. For this reason, the probability of correct answers with thermodynamic integration studies is directly related to the similarity in structure between the ligand of interest and the reference compound. All three-dimensional methods for predicting affinity require a fundamental assumption about the binding mode (in other words, an orientation rule for aligning compounds in the model). Examination of series of ligands binding to the same site usually includes examples of similar compounds that have different binding modes [e.g., the change in orientation (Fig. 3.25) of the C-terminal portion of the Roche HIV protease inhibitor compared with JG-365] (333). Molecular modeling is currently capable of distinguishing correctly in many cases between alternate binding modes of the same ligand. Many components (desolvation, entropy of binding, etc. of the ligand), which cloud the issue of direct calculation of affinities are constant when comparing binding modes of the same compound and, therefore, do not have to be evaluated. The computational costs of exploring possible binding modes within the active site is nontrivial, however, especially when the protein is capable of reorganizing to expose alternative sites, as was the case for a series of ligands for hemoglobin (181).

In a similar fashion, it is generally assumed from the competitive behavior for binding shown by many agonists and antagonists that they bind at the same site on the receptor (certainly, the simplest hypothesis). Recent studies on G-protein-coupled receptors indicates that agonists and antagonists often have different binding sites, given that mutations in the receptor can affect the binding of one and not the other. An example of such a study on the angiotensin II receptor has been published (336). This story is only beginning to unfold, but appears to be a general phenomenon in G-protein receptors (337, 338). Examples of this phenomenon have been reported with antagonists derived from screening where the structure of antagonist and agonist differ dramatically, but also where the antagonists were obtained by minor structural modification of the natural agonist.

3.5 Protein Structure Prediction

Prediction methods for generating the 3D structure of a protein based on its sequence alone fall into several categories. There are hierarchical methods that predict secondary structures and then attempt to fold those elements together. There are simulation methods that attempt to fold the protein through the use of models of reduced complexity and then refine the prediction by using them to constrain all-atom models. Additionally, there are hybrids of these approaches that rely heavily on heuristics. These methods have been successful in limited cases in the hands of their authors, but have generally been found lacking when tested by others in a more thorough and objective manner. Nevertheless, partial successes indicate that signal has begun to emerge from the smoke and mirrors.