1
The Binding Thermodynamics of Drug Candidates

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1.1
Affinity Optimization

The affinity optimization of drug candidates is a major goal in drug development. Most often, the starting points for optimization are compounds or fragments identified in screening campaigns. For full-size compounds, the top hits usually have binding affinities in the mid-micromolar range, while for fragments, hits with affinities as weak as millimolar are not uncommon. In both cases, the binding affinity needs to be improved by 5 orders of magnitude or more for a hit to become a reliable drug candidate. Five orders of magnitude improvement in affinity is equivalent to an additional binding energy of $-7.0\,\text{kcal mol}^{-1}$ ($\Delta G = -\text{RT}\ln(1/K_\text{d})$); that is, essentially doubling the binding energy of the starting compound. Performing this task in an efficient way and simultaneously improving or maintaining drug-like properties is not an easy task and, arguably, can be facilitated by an in-depth knowledge of the binding thermodynamics of a compound.

Affinity optimization is not a simple task because it needs to adhere to constraints that maintain or improve the drug-like character of the compound. A common framework is given by the Lipinski rules of five [1, 2], which limit the molecular weight and the number and type of functionalities that are present in the final compound. For screening hits that already have molecular weights around 500, improving the affinity to the required drug levels essentially means doubling the ligand efficiency (LE = ΔG/(number of heavy atoms)). For fragments ($M_\text{W} \sim 200$), it means that the chemical functionalities that are added to grow the compound must have a better LE than the starting fragment. Furthermore and in addition to binding affinity, other binding-related properties like selectivity or susceptibility to drug-resistant mutations need to be addressed.

Recently, researchers have become aware of the tendency for new drug candidates to be excessively hydrophobic, to exhibit low solubility and poor permeability, and correspondingly exhibit poor drug quality [3]. In order to identify high quality compounds at an early stage or to improve the quality of existing

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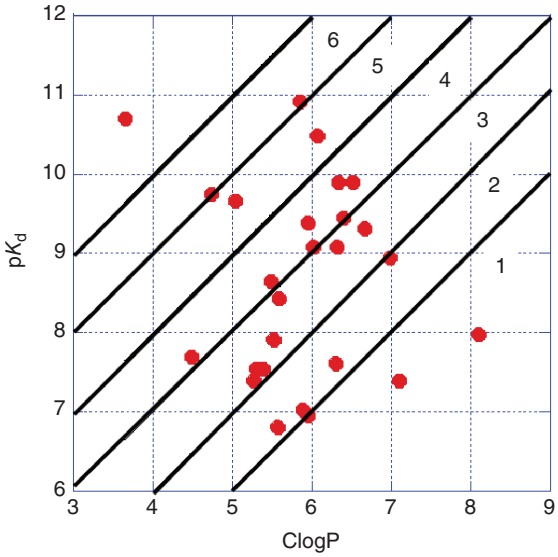

Figure 1.1 The logarithm of the binding affinity as a function of ClogP for a series of protease inhibitors analogs. The solid lines represent lines of constant LipE (indicated by the numbers). Compounds with the higher LipE arguably display the best drug-like properties.

leads, different metrics have been proposed. It has been realized that high quality compounds are those characterized by high potency and simultaneously low hydrophobicity [4]. In fact, if a plot is made of the logarithm of the potency of the compounds versus their ClogP (Figure 1.1) the high quality compounds cluster in the upper left corner. Those compounds are said to have a high lipophilic efficiency (LipE defined as $pK_d - ClogP$; for any given series pIC_{50} or pK_i can also be used in the analysis) [4]. From a fundamental standpoint, an important issue is to assess whether LipE and similar metrics have a solid thermodynamic foundation and how they can be implemented in a prospective way. This is the main topic of this chapter.

1.2 The Binding Affinity

The binding affinity is dictated by the Gibbs energy of binding ($\Delta G = -RTlnK_a$ or $\Delta G = -RTln(1/K_d)$), which in turn is the sum of the binding enthalpy (ΔH) and the binding entropy contribution ($-T\Delta S$), as shown in Figure 1.2. The bar graph in Figure 1.2, is called the thermodynamic signature [5, 6], and provides an instantaneous visual representation of the magnitude of the different interactions that contribute to binding. The thermodynamic signature can be measured by isothermal titration calorimetry (ITC) at any given temperature. Of all the techniques available to measure binding, ITC is the only one capable of measuring not only binding affinities but also the thermodynamic parameters that determine the

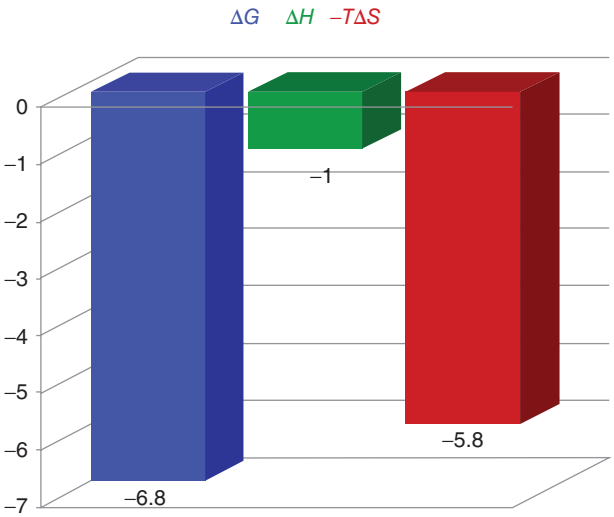

Figure 1.2 The thermodynamic signature is a representation of the binding thermodynamic parameters that permit a rapid assessment of the forces that determine the binding of a compound.

binding energy. Since the enthalpy and entropy changes originate from different types of interactions, having access to those quantities provide an indication of the forces that drive the binding of a compound, and simultaneously delineate paths for optimization. In the past, ITC has been primarily used retrospectively rather than prospectively as a guiding tool for lead optimization. This situation is changing due to two factors: improved understanding of the relationships between thermodynamic forces (enthalpy, entropy, and heat capacity) and chemical structure, and a new generation of instruments with reduced sample requirements, better sensitivity, and much faster throughputs [7, 8].

A nanomolar affinity corresponds to a Gibbs energy close to -12.5 kcal mol^{-1}, while a picomolar affinity corresponds to a Gibbs energy close to -16.5 kcal mol^{-1}. In a typical scenario, a common design goal is engineering a compound with a binding affinity on the order of 0.1 nM, which is equivalent to a Gibbs energy of -14 kcal mol^{-1}. If the starting compound identified in a screen has a 10 μM affinity (equivalent to -6.8 kcal mol^{-1} as in the example in Figure 1.2) its affinity optimization will require an additional -7 kcal mol^{-1}. This additional -7 kcal mol^{-1} of binding affinity can be achieved by any possible enthalpy and entropy combinations that add up to the required amount. While in a test tube, the precise enthalpy/entropy balance may be irrelevant, in real life it is very important as the enthalpy and entropy changes originate from different types of interactions and, consequently, compounds with different thermodynamic signatures will have otherwise different properties. From the point of view of drug development, the particular way by which the additional -7 kcal mol^{-1} are achieved will determine to a large extent the drug quality of the compound.

1.3 The Enthalpy Change

Drug molecules are composed of polar and nonpolar (carbon) atoms and they contribute very differently to the enthalpy change. In binding, two processes occur simultaneously: desolvation and the formation of drug/protein interactions. The desolvation of polar groups is highly unfavorable whereas the desolvation of nonpolar groups is favorable. There is a penalty of 6.2 kcal mol^{-1} for desolvating a hydroxyl group, for example, and a gain of −0.76 kcal mol^{-1} for desolvating a methyl group. The penalty of 6.2 kcal mol^{-1} is equivalent to more than 4 orders of magnitude in binding affinity, indicating that unless polar groups establish very strong interactions with the protein they are going to contribute unfavorably or very little to binding affinity. Furthermore, the desolvation penalty of polar groups is of an enthalpic origin. In fact, the desolvation enthalpies of polar groups are on the order of 5–9 kcal mol^{-1}, which is about 1 order of magnitude higher than that of nonpolar groups. Unfavorable binding enthalpies are usually associated with polar groups that become desolvated and do not establish strong interactions with the protein. Table 1.1 summarizes enthalpies for the desolvation (transfer from aqueous solution to gas phase) [9] of common chemical functionalities used in lead optimization.

The thermodynamic signature provides a rapid evaluation of the enthalpic contribution to binding. If the enthalpy is unfavorable, the first task is to identify the origin of this behavior and, if necessary, localize the problematic polar groups and eliminate them (see, for example, [10, 11]). If high-resolution structural

Table 1.1 Desolvation free energies and enthalpies for chemical functionalities commonly used in lead optimization.[a)]

Group	ΔG desolvation (kcal mol^{-1})	ΔH desolvation (kcal mol^{-1})
NH_2	5.8	7.9
NH	6.0	9.4
N	5.9	9.3
$N_{aromatic}$	4.1	4.9
NO_2	4.8	4.7
O	3.8	5.2
OH	6.2	8.7
CO	5.5	5.5
COO	4.9	5.4
COOH	7.7	8.4
CH_3	−0.76	0.57
CH_2	−0.18	0.77

Values from Cabani *et al.* [9].

a) Enthalpies associated with the transfer of different chemical functionalities from aqueous solution to the gas phase.

information is not available, conventional structure activity relationships based upon the thermodynamic signature of compounds can provide the required information to identify unwanted polar groups.

Polar groups that establish strong hydrogen bonds with the protein, usually contribute favorably on the order of −4 to −5 kcal mol^{-1} to the binding enthalpy [12]. Figure 1.3 shows the thermodynamic signature for two pairs of compounds that vary by a single functionality. In the top panel, the change of a thioether to a sulfonyl results in a strong hydrogen bond and an enthalpic gain of 3.9 kcal mol^{-1}. In the bottom panel, the change of a methyl group to a hydroxyl group results in an enthalpic gain of 4.4 kcal mol^{-1}. It must be noted, however, that in both cases the enthalpic gains are accompanied by compensating entropic losses. In the first case, the entropic loss is larger than the enthalpic gain resulting in a small drop in binding affinity. In the second case, the entropic loss is smaller than the enthalpic gain resulting in a twofold gain in binding affinity. These results provide the rationale for the common observation in lead optimization that the introduction of polar groups that establish strong hydrogen bonds often results in no binding-affinity gains. These results also indicate that in order to obtain binding-affinity gains with polar groups, it is necessary to overcome the ubiquitous phenomenon of enthalpy/entropy compensation [12].

Contrary to polar groups, the introduction of nonpolar groups usually results in small enthalpy and entropy gains that bring about moderate improvements in binding affinity. The cumulative improvements may result in high affinity but also highly hydrophobic compounds. Figure 1.4 shows a typical situation in which a methyl group is added to a compound. In this case, favorable contributions of 0.8 and 0.5 kcal from the enthalpy and entropy changes result in a binding-affinity gain of 8.7-fold. In our work, we have observed situations like this many times and provide a rationale to the practice of using (or abusing) hydrophobic groups to improve binding affinity. The introduction of nonpolar functionalities is devoid of the large compensatory enthalpy/entropy changes that greatly difficult lead optimization with polar groups.

1.4 The Entropy Change

Two terms are of major concern from an engineering point of view: the solvation/desolvation entropy and the conformational entropy. The desolvation of both polar and nonpolar groups is favorable to binding [9]. On the other hand, the ordering or structuring of side chains or backbone in the protein or the drug molecule contributes unfavorably to the binding entropy. In most situations, the desolvation entropy dominates and the observed binding entropy is favorable. The exceptions are those cases in which the binding reaction is coupled to large structuring processes like the refolding of disordered domains in proteins [14]. This situation is illustrated in Figure 1.5 for the binding of the inhibitor NBD-556

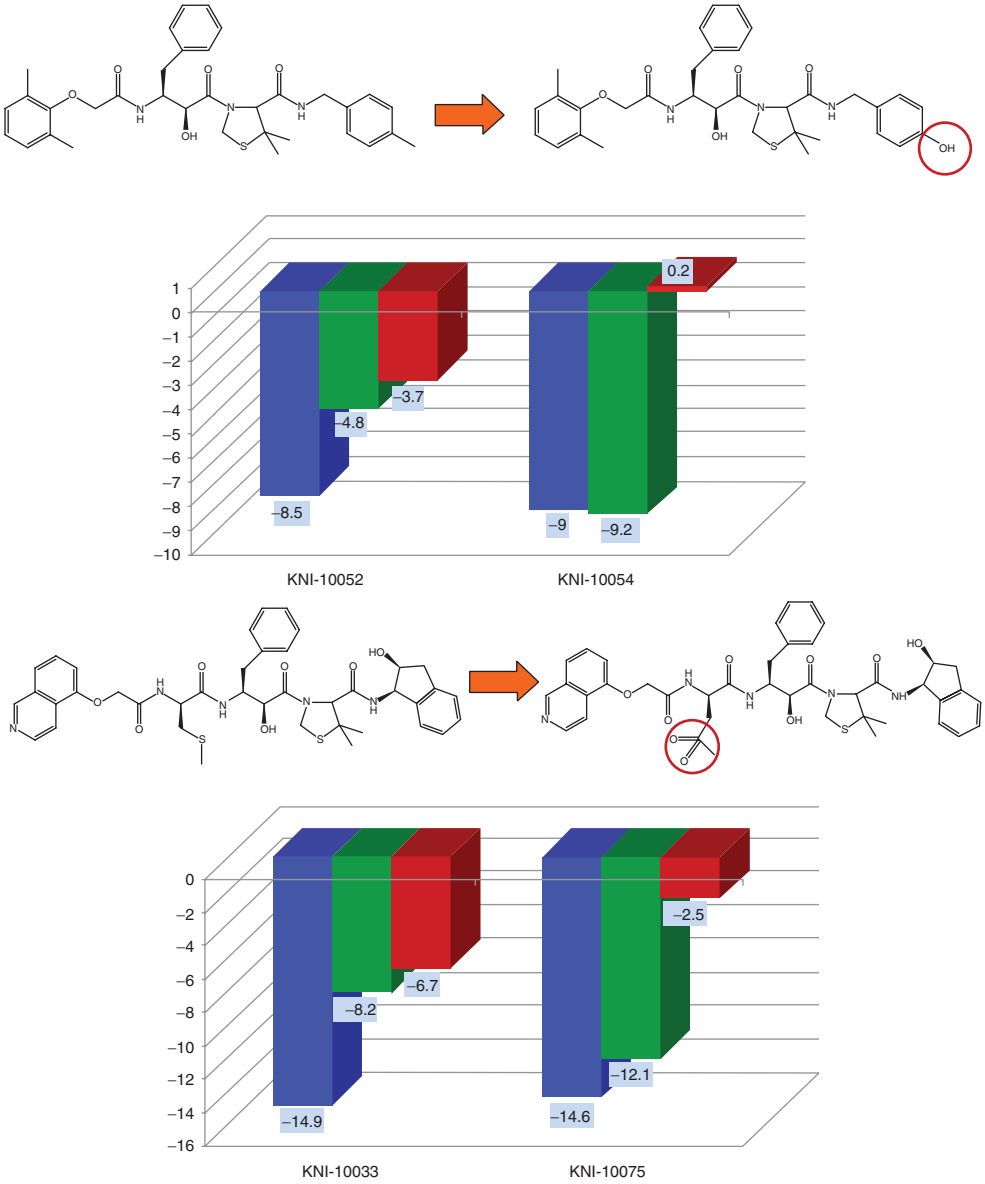

Figure 1.3 The thermodynamic signatures for two pairs of protease inhibitors that vary by the addition of a single polar group that establish a strong hydrogen bond with the target. The formation of the hydrogen bond results in an enthalpic gain of −4 to −5 kcal mol^{-1}. The enthalpic gain is compensated to different extents by opposite entropy loses [12].

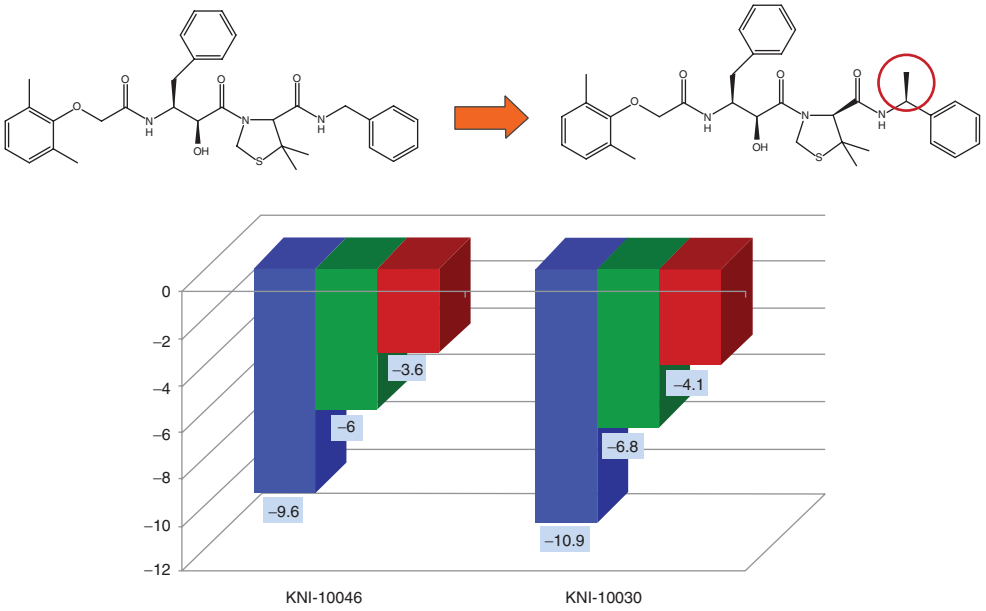

Figure 1.4 The thermodynamic signature for a pair of protease inhibitors that vary by the addition of a single methyl group. A well-packed methyl group is usually associated with small enthalpy and entropy gains resulting in a binding affinity gain [13].

to gp120, the envelope glycoprotein of HIV-1 [14]. In this case, the large negative enthalpy and large unfavorable entropy are associated with the structuring of regions of gp120 that are intrinsically disordered in the unliganded state.

Because nonpolar groups exhibit a favorable entropy of desolvation and a very small enthalpic penalty that can be easily overcome by van der Waals interactions with the target, they have been a favorite tool for optimization [6, 15, 16], and consequently drug candidates have become more hydrophobic in recent years. The binding of these compounds is dominated by large favorable entropies and enthalpies that are often unfavorable or only slightly favorable. In fact, it was noticed earlier for HIV-1 protease inhibitors as well as statins [6, 15, 16] that enthalpically optimized compounds appeared only after the first-in-class drugs had already been in the market for a while. This observation is a testimony to the difficulties in enthalpic optimization.

1.5 Engineering Binding Contributions

It is obvious that the main complication during optimization arises from the introduction (location and type) of polar groups. There are different situations in which polar groups can be found. (i) Polar groups can be introduced as solubilizers of

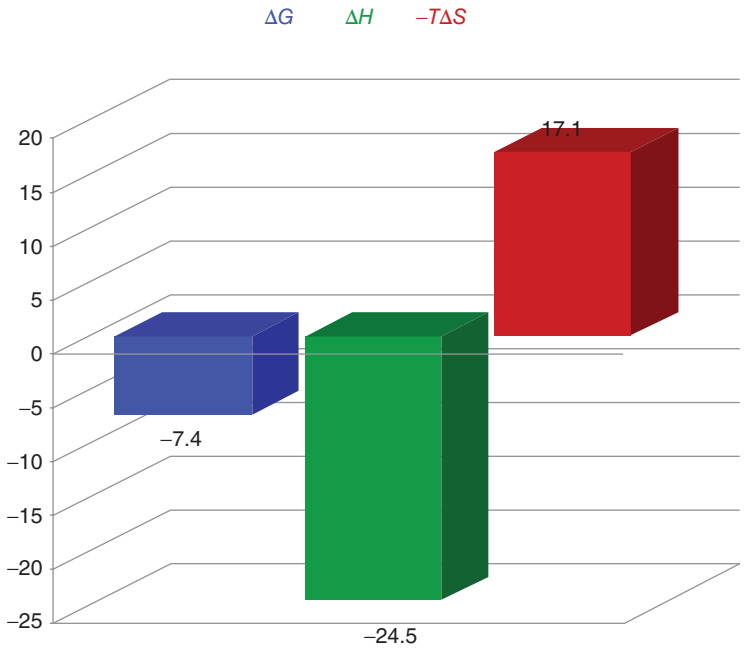

Figure 1.5 The thermodynamic signature of NBD-556a HIV-1 gp120/CD4 inhibitor. The binding of this inhibitor is coupled to a large refolding (structuring) of gp120 and consequently the thermodynamic signature is dominated by a large unfavorable binding entropy and an equally large favorable enthalpy attributed to the refolding of the protein [14].

hydrophobic compounds. To be effective solubilizers, they should remain exposed to the solvent and not pay the desolvation penalty. (ii) Polar groups that become desolvated but do not establish strong interactions with the protein. These groups can be identified by their unfavorable contribution to the binding enthalpy; they should be eliminated as they only pay the desolvation penalty without contributing favorably to binding. (iii) Polar groups that establish strong hydrogen bonds but do not contribute to binding affinity. These groups are characterized by strong favorable contributions to the binding enthalpy (−4 to −5 kcal mol^{-1}, Figure 1.3) but equally large entropic compensation that negates any gain in affinity. Even though these groups do not contribute to affinity, they do contribute to selectivity and should be preserved [13]. (iv) Polar groups that establish strong hydrogen bonds and contribute favorably to the binding affinity. These groups are the most important ones as they contribute both to binding affinity and selectivity.

As mentioned earlier, the enthalpy gain associated with strong hydrogen bonds can be opposed by a large entropy loss resulting in no gain or even a loss in binding affinity. Often, the origin of the entropy loss is due to: (i) the structuring effect triggered by the newly formed hydrogen bond (loss in conformational entropy);

and/or (ii) losses in desolvation entropy if the new hydrogen bond forces some groups to be more exposed to water.

Improving binding affinity with hydrogen bonds (i.e., polar groups) is difficult because it requires overcoming large unfavorable entropic effects. Hydrogen bonds should be directed to structured regions of the protein in order to minimize structuring effects and their associated compensating entropy changes. If the crystallographic structure of the target protein is known, structured regions can be identified either computationally or by examining the B-factors in the pdb structure file [12]. On the other hand, unfavorable entropy changes due to diminished desolvation can be overcome by modifying the size/geometry of the group or its stereochemistry [17].

The effects on binding affinity of polar groups that establish strong hydrogen bonds are always the difference between a large favorable enthalpy and a large unfavorable entropy changes. For example, a strong hydrogen bond that improves binding affinity by 1 order of magnitude (-1.4 kcal mol^{-1}) will often contribute a favorable enthalpy of -4.5 kcal mol^{-1} and be opposed by an unfavorable entropy contribution of 3.1 kcal mol^{-1}. On the other hand, hydrophobic functionalities are usually characterized by small favorable enthalpy and entropy changes. Thermodynamically, it is not surprising that hydrophobic groups represent the fastest way of optimizing affinity. This example illustrates the qualitative difference of improving affinity by nonpolar and polar functionalities. Enthalpy, being more difficult to optimize, has appeared only as the dominant driving force in second generation or "best in class" drugs [6]. Obviously, problems associated with highly hydrophobic compounds like solubility, bioavailability, and selectivity, to name a few, have been recognized and provide the rationale and the incentive for the development of enthalpically optimized compounds.

1.6 Lipophilic Efficiency and Binding Enthalpy

The LipE is essentially a measurement of the binding affinity minus the hydrophobicity of a compound. From a thermodynamic standpoint, it will be proportional to the amount of binding affinity that originates from forces other than the hydrophobic effect; that is, to the proportion of the binding affinity that is contributed by polar groups. Since polar groups that contribute to affinity also contribute strongly to the binding enthalpy, we hypothesized that the LipE and the binding enthalpy must be correlated. Also, a highly polar compound will have a small ClogP but high affinity only if the polar groups establish strong hydrogen bonds and contribute favorably to the binding enthalpy. Figure 1.6 illustrates the correlation between LipE and binding enthalpy for three different cases. Measuring the binding enthalpy provides an immediate account of the enthalpic efficacy of the polar functionalities (i.e., their specific contribution to binding affinity as very often entropy compensation negates or diminishes the

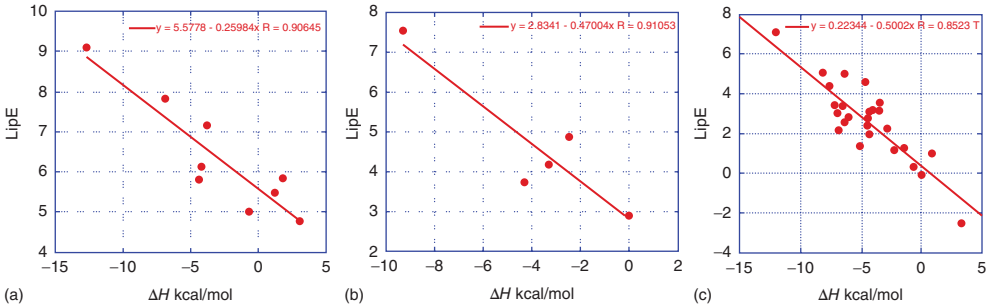

Figure 1.6 Correlation of binding enthalpy with LipE for FDA approved HIV-1 protease inhibitors (a), statins (b), and a series of HIV-1 protease analogs belonging to the same chemical scaffold (c).

contributions to affinity of groups that contribute significantly to the binding enthalpy [12, 13]) in a compound and delineates optimization strategies. The LipE/enthalpy correlation provides a solid foundation for these metrics and a tool for faster optimization.

Acknowledgments

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