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Do enthalpy and entropy distinguish first in class from best in class?

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A drug molecule should bind to its target with high affinity and selectivity. Because the binding affinity is a combined function of the binding enthalpy and the binding entropy, extremely high affinity requires that both terms contribute favorably to binding. The binding enthalpy, however, is notoriously more difficult to optimize than the binding entropy, a fact that has resulted in thermodynamically unbalanced molecules that do not achieve optimal potency. In fact, with current technologies, the enthalpic optimization of drug candidates may take years and only appear in second-generation products. Within that context, it is not surprising that structure/activity relationships (SARs) that explicitly incorporate the interplay between enthalpy and entropy and accelerate the optimization process are being developed and gaining popularity.

Introduction

Binding affinity, K_a, is dictated by the Gibbs energy of binding (ΔG), $K_a = e(-(\Delta G/RT))$. However, ΔG is the sum of two different terms ($\Delta G = \Delta H - T\,\Delta S$) and, consequently, extremely high affinity is only achieved when both enthalpy (ΔH) and entropy (ΔS) contribute favorably to binding [1–5]. Although the simultaneous optimization of enthalpy and entropy is the clear goal, the experience of many pharmaceutical laboratories has shown that this goal is difficult to achieve. Several complicating factors are present. First, the forces that contribute to the binding enthalpy are difficult to optimize and second, if an enthalpic improvement is actually made, it is often not reflected in better affinity, because the enthalpy gain is compensated by an entropy loss. The binding entropy on the contrary, being dependent primarily on the hydrophobic effect, is easier to optimize and is less affected by compensating enthalpy changes. As a result, the recent trend has been toward increasingly hydrophobic, poorly soluble, entropically optimized drug candidates [6–9]. Nevertheless, examination of the evolution of FDA-approved HIV-1 protease inhibitors as well as statins, the two classes of drugs for which complete thermodynamic information has been published, suggests that best in class compounds that come into the market after several years are enthalpically better optimized than the original first in class compounds. Although the primary motivation to develop best in class compounds is certainly not a better binding enthalpy, but rather, much better potency, higher selectivity, better pharmacokinetics or a superior drug resistance profile, it is noteworthy that at the end, the resulting compounds have more favorable binding enthalpies. A better enthalpic character also indicates a transformation in the type of interactions that determine binding. It appears that the molecular interactions reflected in a better binding enthalpy are critical for the development of improved drugs. If this is the case, why are drug candidates not enthalpically optimized from the start? Why not make the first in class also the best in class? New thermodynamic-based platforms are beginning to address those issues.

The difficulties in enthalpic optimization

Two different classes of forces determine the binding of a drug molecule to its target: attractive forces like van der Waals and hydrogen bonding interactions between drug and protein and repulsive forces, like the hydrophobic effect that tends to force the drug out of the aqueous solvent into a hydrophobic cavity. Because these forces contribute differently to the enthalpy and entropy changes, the thermodynamic signature, that is the proportion by which the enthalpy and entropy contribute to binding

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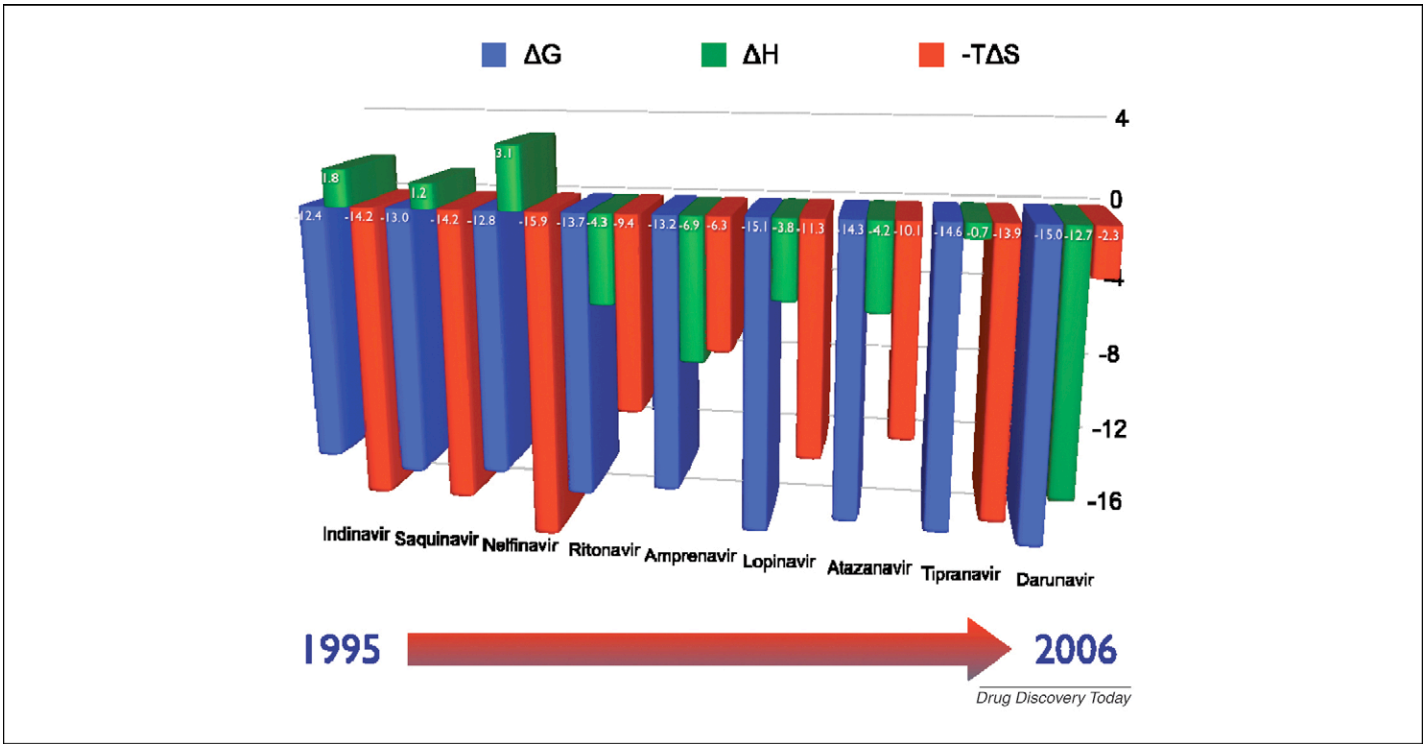

FIGURE 1

The thermodynamic signature of all HIV-1 protease inhibitors approved by the FDA. It is apparent that better enthalpies have accompanied the search for inhibitors with better binding affinity, selectivity and drug resistance profile. All protease inhibitors with low picomolar affinity have favorable binding enthalpies. Data from [1,14].

[9,10] provides a unique experimental way of characterizing the binding mode of a drug molecule.

The enthalpy change associated with the interaction between drug and protein is difficult to optimize because it is composed of two major conflicting contributions[1]: the favorable enthalpy associated with the formation of hydrogen bonds and van der Waals contacts and the unfavorable enthalpy associated with the desolvation of polar groups. van der Waals interactions are maximized by a perfect geometric fit between drug and target, while the strength of hydrogen bonds is maximal when the distance and angle between acceptors and donors are optimal. If the distance and angle are suboptimal, the enthalpic contribution of a hydrogen bond does not simply become smaller and eventually approach zero, it actually becomes unfavorable. The reason behind this observation is that hydrogen bond donor and acceptor groups in the compound are hydrogen-bonded to water before binding. In binding energetics the real question is, how strong is the hydrogen bond that any given group forms with the protein, relative to the hydrogen bond that the same group forms with water before binding? The strength of the bonds with water are reflected in the enthalpy of desolvating those groups. The enthalpy penalty associated with the desolvation of polar groups commonly used in drug design is in the order of 8 kcal/mol at 25°C (1 cal = 4.18 J), which is about one order of magnitude higher than that of nonpolar groups (see review and compilation of experimental values in [12]). Therefore, a favorable interaction enthalpy is an indication that the drug establishes good interactions with the target and that those interactions are strong enough to compensate the unfavorable enthalpy associated with desolvation. Conversely, an unfavorable binding enthalpy usually indicates that polar groups are not forming strong bonds with the target and that the desolvation penalty dominates. Structure-based drug design is not yet capable of engineering hydrogen bonds down to the tenths of 1 Å that are required to achieve a favorable enthalpy contribution. On the contrary, structure/activity relationships (SARs) extended to three dimensions by the incorporation of enthalpy and entropy data in addition to binding affinity are capable of identifying optimal locations for hydrogen bond donors and acceptors.

Contrary to the enthalpy, the binding entropy is much easier to optimize. Two major terms contribute to the entropy of binding, the desolvation entropy change and the conformational entropy change. The desolvation entropy is favorable and originates from the release of water molecules as the drug molecule and the binding cavity undergo complete or partial desolvation upon binding. Favorable desolvation entropy is the predominant force

[1] In this article only enthalpy contributions that can be engineered in the design process are considered. Other contributions like those due to protein conformational changes or linked protonation/deprotonation of protein groups are usually beyond the reach of the drug designer and define a constant background for a given target. In some cases that background is enthalpically unfavorable, in which case the important parameter is the binding enthalpy relative to that of the initial compound in the optimization process. Also, all enthalpy and entropy values must be reported at the standard reference temperature of 25°C, at which solvation and other energetic parameters are tabulated in the literature. Owing to heat capacity changes, values measured at different temperatures vary and cannot be compared directly with those at 25°C [11]. In general, binding enthalpies measured at higher temperatures are more negative.

associated with the binding energy of hydrophobic groups. In fact, it has been estimated that burying a carbon atom from the solvent contributes in the order of 25 cal/mol Å^2 to the binding affinity [13]. The conformational entropy change, on the contrary, is almost always unfavorable, as the binding process involves the loss of conformational degrees of freedom for both the drug molecule and the protein molecule. Drug designers, however, have learned to minimize the conformational entropy loss, by engineering conformational constraints that make the free conformation of the drug molecule similar to its bound conformation.

In addition to the intrinsic difficulties associated with the engineering of atomic interactions, the optimization of a lead candidate needs to be performed by following very rigorous constraints related to oral bioavailability, toxicology and so on. Functional groups cannot be added at will, because the molecular weight of successful drug candidates should generally not be substantially higher than 500 Da. Also, hydrophobicity needs to be kept under certain limits ($C\log P < 5$) and all the hydrogen bonds need to be made with fewer than five donors and ten acceptors [6–8]. Finally, all enthalpy gains need to be translated into affinity gains, a task that is not straightforward.

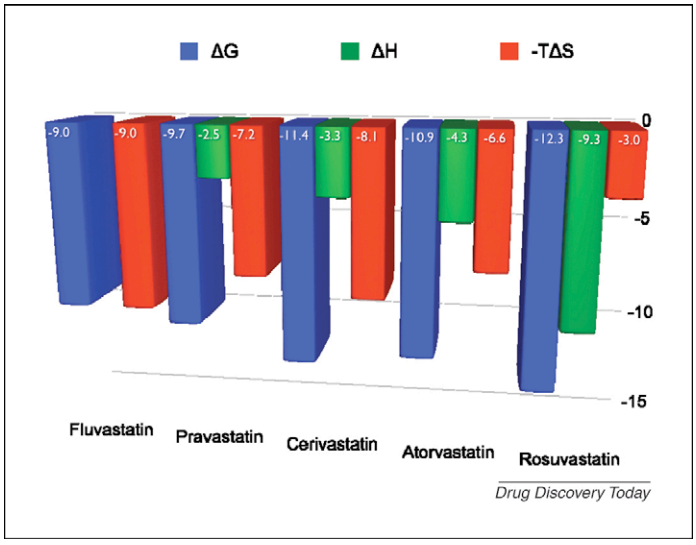

FIGURE 2

The thermodynamic signature of the statins, the cholesterol lowering drugs. Newer, more potent statins are enthalpically better optimized than older ones. Data from [2].

First in class and best in class

The role of binding enthalpy during the evolution of drug molecules can be appreciated by considering two literature examples for which thermodynamic data for complete series of drugs are available. The progression of HIV-1 protease inhibitors, major components in the chemotherapy of AIDS and the progression of the statins, cholesterol-lowering drugs that perform their function by inhibiting the enzyme HMG-CoA reductase and are among the most widely prescribed drugs in the world. Figure 1 shows the thermodynamic signature of all FDA-approved HIV-1 protease inhibitors [1,14]. There are several conclusions that can be derived from the data. First, the binding affinity of the inhibitors has increased from K_i's close to nanomolar for the first inhibitors approved in 1995–1996 to K_i's in the low picomolar range for those approved in 2005–2006. It is evident that, after ten years of optimization, the potency increase is due to improved binding enthalpies and that all protease inhibitors with picomolar binding affinity have favorable binding enthalpies. Figure 2 shows the thermodynamic signatures of the statins [2]. In this case, it is also apparent that the binding affinity increase observed in the newer statins correlates with a more favorable binding enthalpy. It is apparent that, in both cases, the compounds that were first in class were not enthalpically optimized, whereas subsequent ones exhibit more favorable binding enthalpies. Also, the enthalpic evolution for both classes of drugs has taken more than ten years, a process that could be substantially reduced if accurate thermodynamic guidelines were available. Obviously, the goal to be achieved in the development of best in class compounds is not a better enthalpy *per se*; however, those compounds with extremely high affinity, good selectivity and superior drug resistance profiles are those with favorable binding enthalpies. These observations identify the molecular interactions reflected in the binding enthalpy as critical variables in lead optimization. Thermodynamic signatures like those shown in Figs 1,2, provide a convenient way to visualize the thermodynamic balance of a compound during optimization and are being increasingly used in the literature [15–17].

How much enthalpy is necessary?

Although HIV-1 protease inhibitors like darunavir have strong binding enthalpies (−12.7 kcal/mol) and derive most of their binding affinity from enthalpy, it appears that more modest enthalpic goals can also bring significant benefits for compounds characterized by large favorable binding entropies. Goals as modest as avoiding an unfavorable binding enthalpy can have significant consequences. Consider, for example, the case of tipranavir and indinavir. Both inhibitors have similar entropic contributions to binding (close to −14 kcal/mol); however, indinavir has an unfavorable binding enthalpy of 1.8 kcal/mol, whereas tipranavir has a slightly favorable binding enthalpy of −0.7 kcal/mol. The net enthalpic difference of 2.5 kcal/mol is enough to increase the affinity of tipranavir by a factor of 70 to 19 pM. This example demonstrates that, even for compounds with an entropically dominated binding, the elimination of an unfavorable binding enthalpy results in a significant binding affinity improvement. An unfavorable binding enthalpy decreases the binding affinity by a factor of 10 for every 1.4 kcal/mol. Conversely, every gain of 1.4 kcal/mol increases the affinity by one order of magnitude. In addition, enthalpic and affinity gains due to hydrogen bonds are also likely to improve selectivity owing to their strict distance and geometric constraints. As a rule of thumb, we can estimate that −14 kcal/mol is the maximum that the binding entropy can contribute to affinity before a compound becomes completely insoluble. This entropic contribution of −14 kcal/mol is equivalent to a binding affinity of 55 pM if the binding enthalpy were zero, a goal extremely difficult to achieve for an entropically optimized compound. It is apparent that the binding enthalpy needs to be controlled during lead optimization even for entropically driven compounds. Because the most important source of unfavorable enthalpy is the desolvation penalty of polar groups, it is important to measure binding enthalpy changes every time that

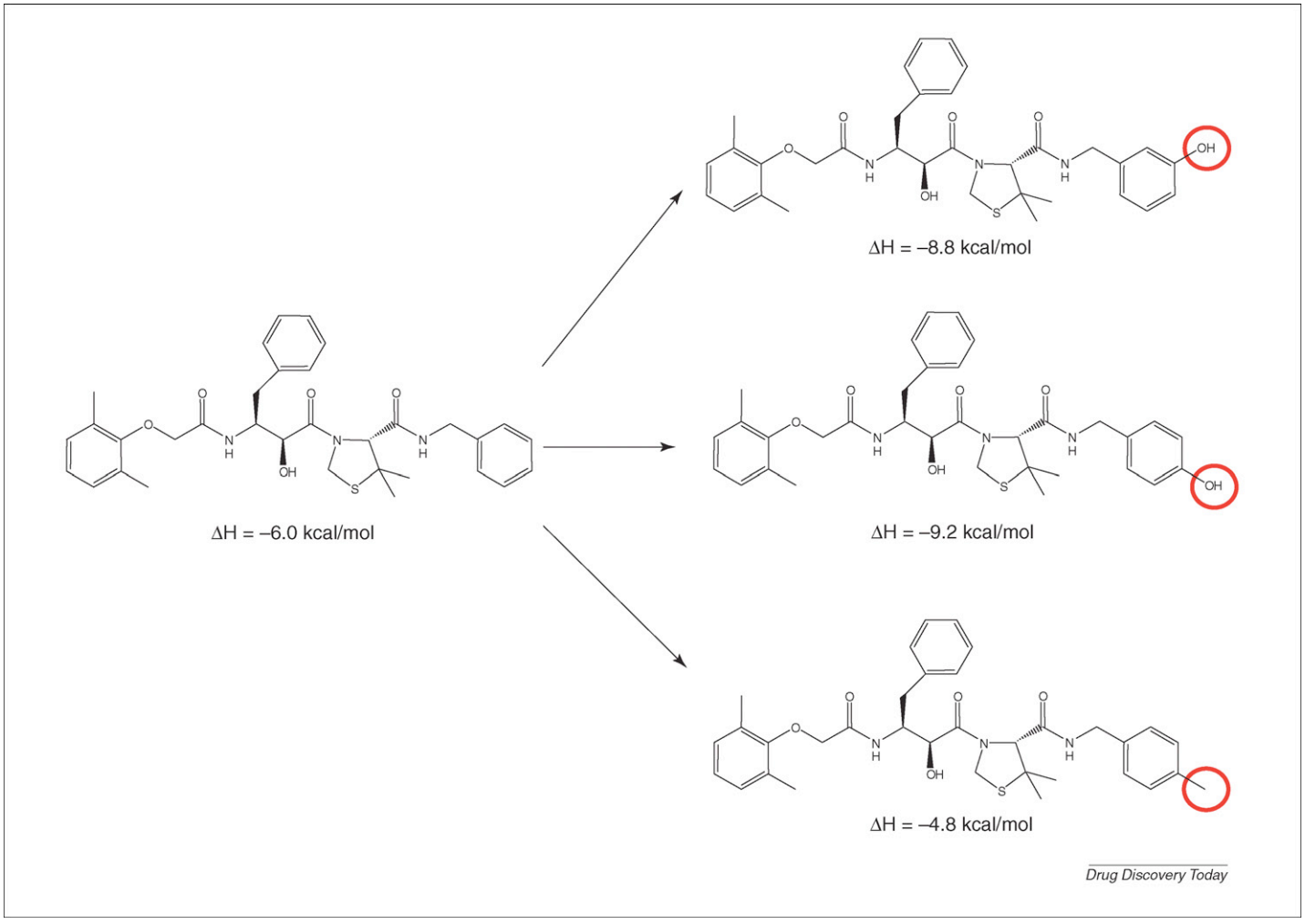

FIGURE 3

Isothermal titration calorimetry (ITC) is the best tool to identify the most appropriate location for hydrogen bond donors or acceptors as shown for a series of plasmepsin II inhibitors. The best location for the hydrogen donor (highlighted hydroxyl group) is identified by a more favorable binding enthalpy. A negative control experiment performed by placing a methyl group at the same position results in a drop in binding enthalpy of 4.4 kcal/mol. Unpublished data from this laboratory (Ruben, A. and Freire, E.).

a hydrogen bond donor or acceptor or other polar functionality is added or repositioned within the compound.

Identifying strong hydrogen bonding interactions

Enthalpic contributions to binding are notoriously more difficult to engineer than entropic contributions. A weak hydrogen bond will not add a little to the binding enthalpy; it will actually oppose it because the unfavorable enthalpy change associated with the desolvation of polar groups is very large (~8–9 kcal/mol [12]). Experimentally, the best way to identify strong hydrogen bonds is by directly measuring the binding enthalpy by isothermal titration calorimetry (ITC) [18–21]. Figure 3 illustrates a situation encountered during the optimization of plasmepsin II inhibitors, a novel antimalarial target [3]. In this example, a hydrogen bond donor is placed at different positions in a phenyl ring and the enthalpic response followed by ITC. As shown in the figure, the best location for the hydroxyl group (*para* position) is immediately detected by a more favorable enthalpy. A negative control experiment performed by placing a methyl group at the same position results in a drop in binding enthalpy of 4.4 kcal/mol, confirming that the best functionality at that location is a hydrogen bond donor. This experimental approach is much faster and more accurate than any available structure-based computational algorithm.

Transforming enthalpy gains into affinity gains

A strong hydrogen bond, however, does not guarantee an improvement in binding affinity, as the enthalpic gain can be totally compensated by an entropy loss. Figure 4 illustrates this situation for the pair of HIV-1 protease inhibitors KNI-10033 and KNI-10075 [22]. KNI-10033 is a potent experimental HIV-1 protease inhibitor with picomolar affinity against the wild-type enzyme (K_d = 13 pM). The binding affinity of the inhibitor is the result of favorable enthalpic ($\Delta H = -8.2$ kcal/mol) and entropic ($-T\,\Delta S = -6.7$ kcal/mol) interactions. The replacement of the thioether group in KNI-10033 by a sulfonyl group (KNI-10075) results in a strong hydrogen bond with the amide of Asp 30B of the HIV-1 protease. No significant changes in hydrogen bonding or conformation of inhibitor and protein are observed in the crystallographic structures [22]. This additional hydrogen bond improves

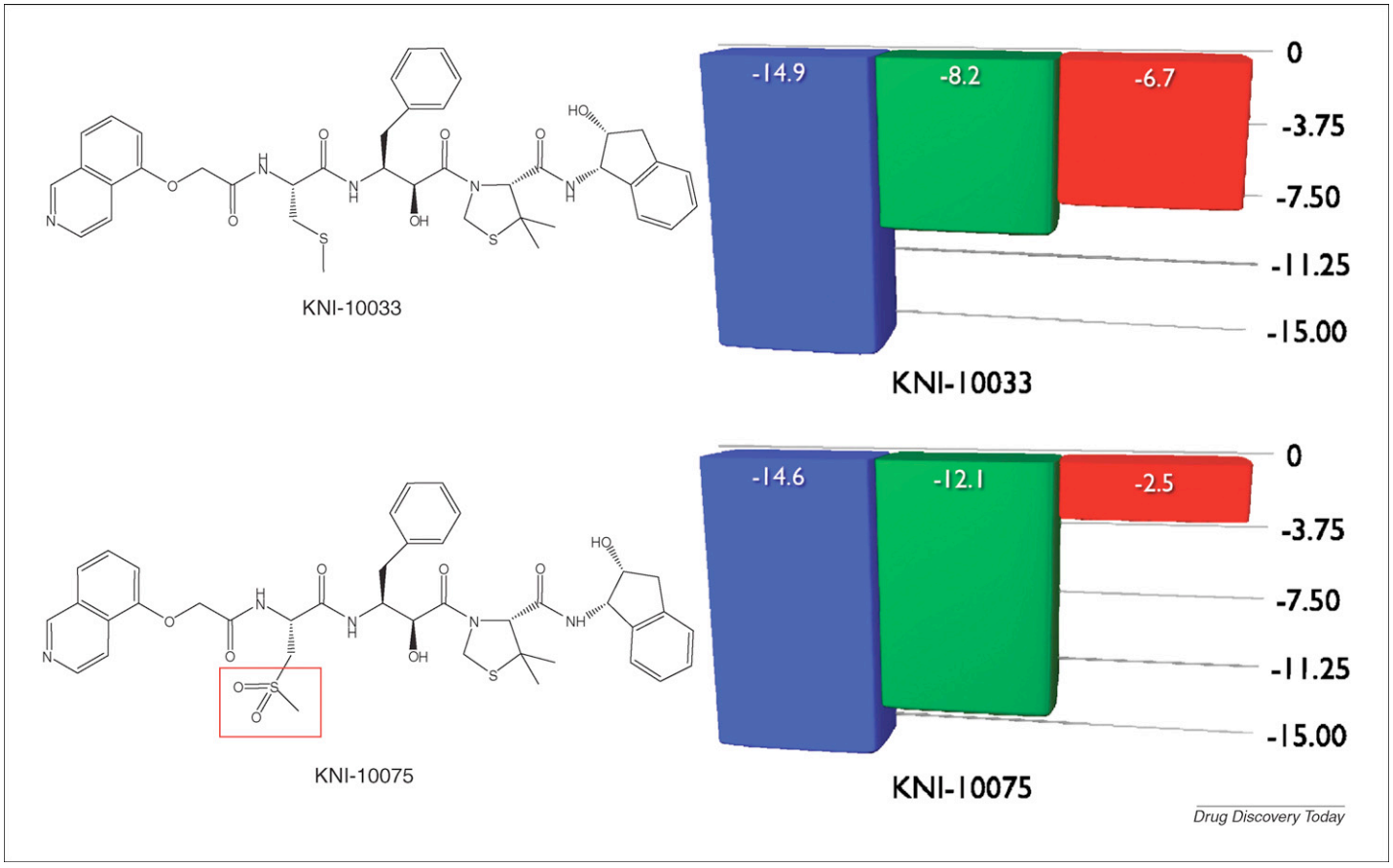

FIGURE 4

A good hydrogen bond does not necessarily bring about an improvement in binding affinity because the enthalpy gain can be compensated by an entropy loss. For the example in the figure, crystallographic and thermodynamic analyses indicate that the structuring induced by the hydrogen bond is ~65% responsible for the entropy loss, the remaining being due to a diminished desolvation [22].

the binding enthalpy by −3.9 kcal/mol; however, the enthalpy gain is completely compensated by an entropy loss, resulting in no affinity change. Crystallographic and thermodynamic analysis of the inhibitor/protease complexes indicate that the compensating entropy originates from a conformational entropy loss resulting to the structuring induced by the hydrogen bond and by a smaller desolvation entropy [22]. This observation emphasizes the fact that an enthalpy gain is necessary but not sufficient for an affinity gain. The key question is, where do we place hydrogen bonds to minimize any compensating entropic effects.

Overcoming enthalpy/entropy compensation

The phenomenon of enthalpy/entropy compensation has been discussed in the literature for many years [23–27]. It essentially says that a change in enthalpy is compensated by a change in entropy and vice versa. However, it cannot be considered as an absolute law. If enthalpy/entropy compensation were an inevitable phenomenon, it would be impossible to improve the binding affinity of a compound. At the thermodynamic level, the role of the drug designer is to overcome enthalpy/entropy compensation, because improving the binding affinity necessarily means defeating enthalpy/entropy compensation. For the designer, for example, it is necessary to know where to place hydrogen bonds, so they make a maximal contribution to affinity. The rules are relatively simple. First, a hydrogen bond must contribute significantly to enthalpy. In our experience, a well-placed hydrogen bond can make a favorable enthalpic contribution on the order of −4 to −5 kcal/mol, which is equivalent to a 1000–5000-fold increase in affinity if the entropy change was zero. Second, the enthalpic contribution should not be neutralized by a compensatory entropy change. As the main cause of compensatory entropy originates from structuring regions of the protein adjacent to the bond, hydrogen bonds should be aimed at already structured regions of the protein. Structured regions within binding sites can be recognized from either crystallographic B factors, hydrogen–deuterium exchange [28] or computational approaches [29] and can be used to identify the general location of hydrogen bond acceptors or donors. Alternatively, a similar effect can be achieved by directing several hydrogen bonds against the same location in the protein such that the first one pays the entropy penalty and subsequent ones bind to an already structured region [30]. Once the general location is identified, it can be more precisely defined by experiments like those shown in Fig. 3. Another source of compensatory entropy is the forced solvent exposure of hydrophobic groups that may occur upon formation of a hydrogen bond. For example, a hydrogen bond donor/acceptor functionality attached to a large aromatic ring can force part of the ring to be exposed to the solvent. This additional exposure, relative to the

one existing in the absence of the hydrogen bonding functionality, brings about a drop in the favorable desolvation entropy that partially or completely neutralizes any enthalpic gains due to hydrogen bond.

Conclusions

Binding is a process controlled by thermodynamics. For many years, the underlying thermodynamic variables (ΔH, ΔS) have not been utilized as guiding tools in drug development, partly because of a lack of adequate microcalorimetric instrumentation and appropriate formalisms for data interpretation and analysis. This situation is rapidly changing, as better instruments with the required sensitivity, throughput and data processing capabilities have become available. It is evident that the enthalpic optimization of a compound is critical for achieving extremely high affinity. In addition, because the enthalpy and entropy changes reflect different types of interactions, other drug properties, like selectivity, are also affected by the enthalpy/entropy balance (thermodynamic signature) of a compound. Enthalpic optimization is difficult but can be facilitated by monitoring the enthalpic and entropic consequences of introducing or modifying different chemical functionalities. Traditionally, optimization has been driven by affinity; however, a multidimensional approach that also tracks the enthalpic and entropic contributions to affinity can yield faster and better results.

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