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Finding a better path to drug selectivity

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Abstract

Extremely high affinity and selectivity are two of the most sought-after properties of drug molecules. Selectivity has been difficult to achieve, especially for targets that belong to large families of structurally and functionally related proteins. There are essentially two ways [AU1] by which selectivity can be improved during lead optimization: a chemical modification of the lead compound that improves the affinity towards the target to a higher extent than to off-target molecules; and a chemical modification that lowers the affinity of the lead compound towards off-target molecules. Maximal selectivity is achieved when both mechanisms can be combined synergistically. As we discuss here, analysis of several protease inhibitors that vary in a single functionality indicates that nonpolar functionalities preferentially follow the first mechanism, whereas polar functionalities follow the second, and that those features are imprinted in their thermodynamic signatures.

Introduction

The binding affinity of a drug for its target, K_a , is dictated by the Gibbs energy of binding (ΔG), $K_a = e^{-\frac{\Delta G}{RT}}$. [AU2]Maximal binding affinity occurs when ΔG is large and negative. For example, picomolar binding affinity corresponds to -16.4 kcal/mol, whereas nanomolar affinity corresponds to -12.3 kcal/mol in Gibbs energy. A binding energy between -12 and -16 kcal/mol is the usual goal in lead optimization. For a starting compound with micromolar affinity, this goal implies the addition of an extra -8.2 kcal/mol to the binding energy of the compound. Given that the Gibbs energy of binding is composed of two contributions, the enthalpy (ΔH) and entropy (ΔS) changes ($\Delta G = \Delta H - T\Delta S$), the required energy is obtained by optimizing the enthalpy and entropy contributions to binding [1–4].

In general, extremely high-binding affinity requires favorable enthalpic and entropic contributions. Given that binding implies transferring a compound from an aqueous environment into the binding pocket of a protein, the energy of desolvation as well as the energy of interaction of the drug molecule and its target need to be taken into consideration. The energies of all bonds that are made and broken during the process contribute to the enthalpy change, whereas all ordering and/or disordering processes in the protein, compound and solvent contribute to the entropy change. In the binding process, different chemical functionalities (polar or nonpolar) also contribute differently to the enthalpy and entropy changes. The specific enthalpic and entropic contributions associated with the binding of a compound to a target define its thermodynamic signature. Fortunately, the thermodynamic

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signature can be measured experimentally by isothermal titration calorimetry (ITC). Having access to the thermodynamic signature provides a useful roadmap for the optimization of a compound, because it immediately identifies the forces that require optimization [3,5].

The binding affinity of a drug molecule for its target results from the ultimate balance of desolvation and attractive forces. As a rule, polar and nonpolar chemical functionalities contribute differently to affinity and selectivity owing to differences in their thermodynamic signatures. Whereas the desolvation of both polar and nonpolar groups is coupled to favorable entropy changes, the desolvation of polar groups carries unfavorable enthalpy changes that are one order of magnitude larger than those of nonpolar groups [6]. In addition to desolvation changes, binding involves enthalpic and entropic changes resulting from interactions of the compound with the target. Nonpolar groups can establish van der Waals contacts with the target that result in small enthalpic gains, whose magnitude depends on the degree of shape complementarity between compound and target. Polar groups are able to establish hydrogen bonds, which might contribute more significantly to the binding enthalpy. In general, only strong hydrogen bonds are able to overcome the unfavorable desolvation enthalpy of polar groups and contribute favorably to the binding enthalpy. ITC measurements of different compounds that vary by a single functionality and for which crystallographic structures of their bound complexes are available (e.g. [7,8]), indicate that strong hydrogen bonds contribute anywhere between -4 and -5 kcal/mol to the enthalpy change, whereas van der Waals interactions associated with a methyl group can contribute as much as -1 kcal/mol.

The interactions between the drug molecule and its target also result in a loss of conformational entropy owing to the structuring of protein residues and the drug molecule itself. The loss of conformational entropy of the compound can be minimized by introducing conformational constraints. All else being equal, a conformationally constrained compound will have a better binding affinity and also better selectivity than its flexible counterpart. Conformational constraints will restrict the ability of the compound to accommodate to binding site variations found in homologous off-target molecules. In this respect, Knight and Shokat [9] noted that selective kinase inhibitors are usually entropically constrained with four or fewer rotatable bonds connecting any two ring systems.

Figure 1 summarizes typical contributions to the thermodynamic signature of a drug molecule. The figure is by no means exhaustive. For example, if binding is associated with extensive protein structuring or folding, as is the case with intrinsically disordered proteins, the unfavorable conformational entropy might predominate, giving rise to an overall unfavorable entropy [10,11]. In addition, protonation and/or deprotonation effects that are buffer dependent need to be deconvoluted from the observed binding energy [12,13]. Because different forces have different thermodynamic profiles, it is possible for two compounds with similar affinities to have different selectivity profiles. From a design point of view, the goal is to maximize favorable interactions with the target while simultaneously minimizing or even making unfavorable the interactions of the compound with off-target molecules. Understanding the molecular origin of the selectivity differences of compounds with similar binding affinities becomes crucial to the design of more selective drug molecules. It is evident that all the forces that contribute to binding affinity do not contribute equally to selectivity. A tight fit between the ligand and its binding target not only maximizes van der Waals interactions, but also reduces the probability that the ligand will be accommodated equally well in off-target molecules. Affinity gains with off-target proteins will not be as large as with the target.

Hydrogen bonds are also major contributors to selectivity owing to their stringent distance and angle constraints. A different arrangement of donors and acceptors within the binding

pocket not only weakens or eliminates the favorable energy of hydrogen bonds, but also leaves intact the severe desolvation penalty that originates from the burial of unsatisfied polar groups, thus lowering the affinity towards off-target molecules. In this respect, a few strong hydrogen bonds can be expected to provide better selectivity than a larger number of weak, partially satisfied hydrogen bonds that can be reshuffled and be energetically similar in an off-target protein. Given that hydrophobicity, or desolvation entropy in general, represents an exclusion from the solvent rather than an attractive force to the target, it does not contribute to selectivity even though it might contribute strongly to affinity. As shown in Figure 1, in most situations, two terms are responsible for most of the unfavorable contributions to the binding energy, the positive enthalpy associated with the desolvation of polar groups and the unfavorable entropy associated with the conformational structuring of the protein and its target. By contrast, the terms that contribute most favorable are the negative enthalpy associated with hydrogen bonds and van der Waals interactions and the favorable desolvation entropy of both polar and nonpolar functionalities.

In drug development, a lead compound or fragment is optimized by modifying or adding different chemical functionalities. These functionalities are usually derived from structure–activity relationships that rely on the binding affinity or a related parameter (K_i , IC_{50} , EC_{50} , etc.). Affinity and selectivity are generally correlated; however, the spread in selectivity for any given affinity can be larger than two orders of magnitude even at the highest affinity levels [14]. In general, selectivity has been elusive even for high-affinity inhibitors of targets that belong to families of highly homologous proteins, such as serine proteases (thrombin) and kinases [15–20]. This situation prompts the general question: what is the best strategy to improve the selectivity of a compound that has already achieved extremely high affinity against its target?

Previously, we considered the role of binding enthalpy during the evolution of drug molecules from first in class to best in class [21,22]. We concluded that more enthalpic compounds preferentially occupied best-in-class positions, and that they exhibited characteristics beyond favorable binding affinity, such as improved selectivity or better drug-resistant profiles, in the case of anti-retrovirals [1], which propelled them to prominence. Here, we analyze selectivity gains obtained by the introduction of different functionalities with different thermodynamic signatures. In particular, we show that strong enthalpic interactions can improve selectivity even if they do not contribute to an improvement in binding affinity. This observation directly addresses the issue of improving the selectivity of a compound that already exhibits extremely high binding affinity. Because standard structure–activity relationships do not reveal enthalpic or other energetic differences between compounds of equal affinity, ITC becomes a crucial methodology to profile the selectivity of potential drug candidates.

Selectivity owing to nonpolar functionalities

One of the most ubiquitous ways of improving binding affinity is by the introduction of nonpolar functionalities that fill a binding cavity. This approach usually produces small gains in binding enthalpy owing to van der Waals interactions that are coupled to entropic gains associated with desolvation. A typical situation is shown in Figure 2. In this case, the introduction of two methyl groups results in a gain of two orders of magnitude in binding affinity owing to combined enthalpy and entropy gains [8]. An important question is: how is this affinity improvement reflected in the selectivity of a compound towards related off-target molecules? In the case of targeting the HIV-1 protease, pepsin and cathepsin D are two human aspartic proteases to which off-target binding needs to be minimized and, consequently, were chosen for selectivity measurements. Figure 3 shows the changes in selectivity associated with the introduction of the methyl groups. Selectivity is defined as the

ratio of the affinity of the compound towards the off-target protein relative to the target protein (K_d ratio = $K_{d, \text{off target}}/K_{d, \text{target}}$). The larger the K_d ratio, the better the selectivity.

The methyl group substitutions improve the affinity against the target by approximately two orders of magnitude. This affinity increase is achieved by more favorable enthalpy and entropy changes originating from better shape complementarity (i.e. improved van der Waals interactions) and larger desolvation surface for the methyl groups [8]. Against both pepsin and cathepsin D, the affinity also increases but to a lesser extent than to the target protease, resulting in a selectivity gain in addition to the improvement in target-binding affinity. The selectivity increases by a factor of 34 against pepsin and 13 against cathepsin D. Burying hydrophobic groups from the solvent will increase the binding affinity even if the shape complementarity with the binding pocket is not optimal. In general, maximal affinity gains with hydrophobic groups are achieved when the shape complementarity is optimal and the desolvation surface is maximized. Of course, if the off-target molecule has a smaller cavity and the hydrophobic functionality does not fit, a decrease in off-target binding affinity leading to better selectivity could be observed. This example represents a typical optimization situation in which selectivity gains are observed after improving the binding affinity of the compound from 12 nM to 0.14 nM using nonpolar functionalities.

Selectivity owing to polar groups

Strong hydrogen bonds contribute favorable binding enthalpies to the binding energetics; however, they do not necessarily improve the binding affinity because the enthalpy gains can be completely overcome by opposing entropy changes [7]. Compensating entropy changes arise most often from conformational entropy losses associated with the molecular structuring induced by the hydrogen bonds themselves and/or by a reduced desolvation [7]. Figure 4 illustrates the situation created by the replacement of a thioether by a sulfonyl group in an otherwise identical HIV-1 protease inhibitor. The sulfonyl group establishes a strong hydrogen bond with Asp 30 of the protease, as evidenced in the crystal structure and by a gain of 4 kcal/mol in the binding enthalpy [7]. In this case, the enthalpic gain is completely compensated by an entropic loss of comparable magnitude, resulting in inhibitors with similar binding affinities (13 pM for the thioether compound and a slightly weaker affinity, 21 pM, for the sulfonyl compound). These two compounds already exhibit extremely high affinity in the mid-picomolar level and the optimization issues no longer involve binding affinity. The selectivity of the two compounds was checked against pepsin and cathepsin D, as shown in Figure 5. In this case, addition of the hydrogen-bonding functionality increased the selectivity by a factor of 7 against pepsin and 9 against cathepsin D. It is noteworthy that the most enthalpic compound has better selectivity despite having slightly lower binding affinity for the target. As shown in Figure 5, the sulfonyl substitution improves selectivity by lowering the binding affinity of the compound towards the off-target proteins. This is a distinct mechanism to the one observed with the nonpolar substitutions in Figure 3.

Hydrogen bonds have very rigorous distance and angular constraints. If those constraints are not satisfied, the enthalpic penalty associated with desolvation of a polar group (5–9 kcal/mol) will predominate, yielding a net unfavorable enthalpy [6]. A strong hydrogen bond between the compound and target protein, similar to the one established by the sulfonyl compound above, contributes a favorable enthalpy on the order of –4 kcal/mol. If the placement of the hydrogen bond donor and/or acceptor group in the off-target protein is absent or displaced with respect to the one in the target protein, hydrogen bonding will not occur or will be weak. Under those conditions, the enthalpic penalty of desolvation will predominate and result in a significant drop in the affinity of the compound against the off-target protein. Additional measurements of the binding enthalpy of the two compounds to

pepsin confirm that the binding enthalpy of the sulfonyl compound (Figure 4) decreases by 1.5 kcal/mol in binding enthalpy when compared to the thioether compound. These experiments suggest that the enthalpic desolvation penalty of polar groups should be considered explicitly when optimizing a compound for selectivity. More importantly, the drug design approach can be successful even if the hydrogen bond made by the polar functionality does not improve the binding affinity of the compound to the target.

Maximal selectivity will be achieved by establishing a few very strong hydrogen bonds towards unique sites in the intended target protein. If one of those bonds is not satisfied with an off-target protein, the energetic penalty will be significant, causing a large decrease in binding affinity to the off-target protein. A large number of weak hydrogen bonds, by contrast, will combine favorable and unfavorable interactions that can be satisfied in different ways (a kind of a 'Velcro effect') that contributes little to selectivity. Even though no precise calorimetric data are available, the above conclusions are consistent with structural features that have been associated with selectivity for other target proteins. For example, Thal *et al.* [23] noticed that compounds with fewer hydrogen bond donors and acceptors have better selectivity against G protein-coupled receptor kinase 2.

Concluding remarks

Figure 6 summarizes the different ways in which nonpolar and polar functionalities can affect selectivity. A nonpolar functionality that fills a cavity with good shape complementarity can be expected to improve affinity to the target by combined enthalpy and entropy gains. Against off-target molecules, those gains are not expected to be as large, therefore contributing to improved selectivity. In some situations, for example, if the functionality does not physically fit in the off-target cavity, a decrease in affinity to the off-target molecule can be observed. A polar functionality that makes a strong hydrogen bond with the target molecule contributes favorably to the binding enthalpy by as much as -4 to -5 kcal/mol. This enthalpy gain does not necessarily translate into an affinity gain because it can be totally or partially compensated by an entropy loss. Even if a strong hydrogen bond does not contribute to affinity, it might contribute significantly to selectivity. If the hydrogen bonding partner is not present or if its location is not similar in an off-target protein, the enthalpic desolvation penalty will predominate, causing a decrease in binding affinity to the off-target molecule. In the absence of affinity differences between compounds, the main criterion for selectivity screening becomes the thermodynamic signature, binding enthalpy and binding entropy, and emphasizes the role of calorimetric techniques, such as ITC, in profiling drug candidates.

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Figure 1.

Typical contributions to the thermodynamic signature in drug design. Favorable enthalpic interactions (ΔH ; green bars) originate primarily from hydrogen bonding and van der Waals interactions. Unfavorable enthalpic interactions, by contrast, originate from the desolvation of polar groups. Favorable entropic contributions ($T\Delta S$, red bars) are due to desolvation of nonpolar and polar functionalities, whereas unfavorable entropic contributions originate from the structuring of residues in the protein or the compound itself. Abbreviation: ΔG , Gibbs energy of binding (blue bars).

Figure 2.

Two inhibitors that fill a cavity in the HIV-1 protease to a different extent. The inhibitors are identical except at the R positions, which are either hydrogen (H) or methyl (CH₃) groups. The larger CH₃ groups fill the cavity better, increasing the binding affinity by two orders of magnitude owing to combined enthalpy (ΔH ; green bars) and entropy ($T\Delta S$; red bars) gains [8]. Abbreviation: ΔG , Gibbs energy of binding (blue bars).

Figure 3.

The effects of the methyl substitutions for the inhibitors shown in Figure 2 on the K_d for the target (HIV-1 protease) and the off-target proteases, pepsin and cathepsin D. The selectivity changes (K_d ratio = $K_{d, \text{off target}}/K_{d, \text{target}}$) are shown on top of the bars. The improvement in selectivity is due to the larger increase in affinity to the target than to the off-target molecules (arrows). Key: hydrogen (H), pink bars; methyl (CH₃), blue bars.

Figure 4.

Two inhibitors that vary at a single position by the presence of a hydrogen-bonding functionality. The inhibitors are identical except at the R position, which is either SCH_3 or SO_2CH_3 . The sulfonyl group forms a strong hydrogen bond with Asp 30, which is reflected in an enthalpic gain of -4 kcal/mol (ΔH ; green bars). The enthalpic gain does not induce an affinity gain, as it is totally compensated by an entropy loss ($T\Delta S$, red bars) [7]. Abbreviation: ΔG , Gibbs energy of binding (blue bars).

Figure 5.

The effects of the sulfonyl substitution for the inhibitors shown in Figure 4 on the K_d for the target (HIV-1 protease) and the off-target proteases, pepsin and cathepsin D. The selectivity changes ($K_d \text{ ratio} = K_{d, \text{off target}}/K_{d, \text{target}}$) are shown on top of the bars. The improvement in selectivity is due to a decrease in the affinity of the compound to the off-target molecules (arrows). Introduction of the hydrogen bonding to the target can improve the selectivity of the compound even if it does not improve the binding affinity to the target. Key: SCH₃, pink bars; SO₂CH₃, blue bars.

Figure 6.

Expected thermodynamic outcomes associated with the introduction of nonpolar (a) and polar (b) functionalities in a lead compound. A nonpolar group that fills a cavity with good shape complementarity will increase the binding affinity to the target protein (blue) by combined enthalpic and entropic gains (a; top). If the functionality does not fill the cavity tightly for an off-target protein (a, center) a smaller affinity gain will be observed, thus contributing to selectivity. If the cavity in the off-target protein is smaller and the functionality does not physically fit, an affinity decrease can be observed with a larger selectivity gain. (b, top) shows the effects of a polar functionality that establishes two strong hydrogen bonds with the target protein (blue) that is partially compensated by an opposite entropic penalty (this situation is common, but other outcomes are also possible). If one of the hydrogen-bonding partners is not present or if its location is not similar in an off-target protein (b, bottom), the enthalpic desolvation penalty will predominate, causing a decrease in binding affinity to the off-target molecule. Key: target molecules, blue; off-target molecules, green; $\Delta\Delta G$, Gibbs energy of binding (blue bars); $\Delta\Delta H$, enthalpy (green bars); $T\Delta\Delta S$, entropy (red bars).