European Journal of Medicinal Chemistry 90 (2015) 897–915

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Additivity or cooperativity: Which model can predict the influence of simultaneous incorporation of two or more functionalities in a ligand molecule?

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ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form
10 October 2014
Accepted 27 November 2014
Available online 28 November 2014

Keywords:
Drug design
Lead optimization
Structure-activity relationship
Protein–ligand binding
Molecular recognition
Thermolysin

ABSTRACT

Predicting how binding affinity responds to ligand structural modifications in structure-activity relationship studies (SAR) is a major challenge in medicinal chemistry. This is particularly true when two or more of these modifications are carried out simultaneously. In this study, we present binding affinity data from several series of thermolysin inhibitors in which simultaneous structural modifications were investigated to determine whether they are cooperative or additive. Data revealed that, while additivity is at work in some cases, cooperativity is more commonly demonstrated. Cooperativity and additivity were then correlated with ligand descriptors, such as the spacing and the topological features of the modified groups, in a manner that may provide guidance as to when each model should be utilized. Cooperativity was particularly associated with contiguous groups and small unbranched hydrophobic side chain. Additivity, on the other hand, was associated with moderately distant hydrophobic group combinations and side chain branching. Such correlations can improve the predictability of SAR studies and can provide a starting point for additional investigations that may lead to further significant enhancements in the current scoring functions.

1. Introduction

Lead optimization involves cycles of structural modifications which aim at improving the lead's binding affinity or enhancing its pharmacokinetic properties. A typical structural modification might be the replacement of an H or a functional group with another. It is not uncommon for a medicinal chemist to perform more than one structural modification at a time in order to reduce the number of compounds to be synthesized. For example, the structural modifications A→X and B→Y could be carried out individually (two compounds are synthesized) and then combined in a third compound after evaluating whether these structural modifications move the process towards the desired goal. Alternatively, a medicinal chemist may opt to synthesize the third compound after evaluating only one or neither of these modifications (i.e. one or two compounds are synthesized). It should be noted that each of these choices could be misleading in one way or another. For instance, suppose the structural modifications A→X and B→Y are carried out, and one of them is found to be disadvantageous. The medicinal chemist might be discouraged from synthesizing the third compound that has both modifications, even though this third compound, if synthesized and evaluated, might display what has previously been termed “positive cooperativity” between the two modifications [1–3] and—as a consequence—might be good. On the other hand, skipping the evaluation of the individual modifications carries the risk of missing good modifications if the third compound is not good because the two modifications are negatively cooperative (i.e. the individual modifications are good, while the combination is bad). It is therefore crucial for medicinal chemists to be capable of accurately predicting not only the impact of the individual structural modifications on the binding affinity (or the pharmacokinetic property that is desired to be improved), but also the correct model that is to be employed when two, or even more, structural modifications are combined in a ligand.

Abbreviations: bn, benzyl; *i*-Bu, isobutyl; *n*-Bu, normal butyl; *sec*-Bu, secondary butyl; *tert*-Bu, tertiary butyl; Et, ethyl; Eq., equation; ITC, isothermal titration calorimetry; Me, methyl; *i*-Pr, isopropyl; *n*-Pr, normal propyl; TLN, thermolysin.

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http://dx.doi.org/10.1016/j.ejmech.2014.11.056
0223-5234/

Now, how can we identify cooperativity or additivity between two structural modifications in a ligand? A typical analysis that has been commonly used to study cooperative phenomena is the double mutant cycle analysis [4–8]. This analysis has been used to determine whether ligand structural modifications are cooperative or additive with regard to their binding affinity/free energy [1,2,9]. To illustrate how this analysis works in general terms, consider Fig. 1. In this figure, the relationship between the structural modifications H→X and H′→Y is evaluated by comparing the binding free energy change (the differential binding energy)[10] occurring when both groups exist in the ligand ($\Delta\Delta G_{(H,H'\rightarrow X,Y)}$) with the sum of the binding free energy changes occurring when each group exists individually ($\Delta\Delta G_{(H,H'\rightarrow X,H')} + \Delta\Delta G_{(H,H'\rightarrow H,Y)}$). There are three possible outcomes: (1) $\Delta\Delta G_{(H,H'\rightarrow X,Y)} = \Delta\Delta G_{(H,H'\rightarrow X,H')} + \Delta\Delta G_{(H,H'\rightarrow H,Y)}$; (2) $\Delta\Delta G_{(H,H'\rightarrow X,Y)} < \Delta\Delta G_{(H,H'\rightarrow X,H')} + \Delta\Delta G_{(H,H'\rightarrow H,Y)}$; and (3) $\Delta\Delta G_{(H,H'\rightarrow X,Y)} > \Delta\Delta G_{(H,H'\rightarrow X,H')} + \Delta\Delta G_{(H,H'\rightarrow H,Y)}$. In the first case H→X and H′→Y demonstrate additivity, while in both the second and the third cases H→X and H′→Y are cooperative (The second is a case of positive cooperativity, and the third is a case of negative cooperativity). Alternatively, one can compare either the differential binding free energy associated with the replacement of the ligand H with group X in the presence ($\Delta\Delta G_{(H,Y\rightarrow X,Y)}$) and absence ($\Delta\Delta G_{(H,H'\rightarrow X,H')}$) of group Y, or the differential binding energy caused by the H'→Y replacement in the presence ($\Delta\Delta G_{(X,H'\rightarrow X,Y)}$) and absence ($\Delta\Delta G_{(H,H'\rightarrow H,Y)}$) of group X. If, for example, $\Delta\Delta G_{(H,Y\rightarrow X,Y)}$ and $\Delta\Delta G_{(H,H'\rightarrow X,H')}$ are equal, H→X and H′→Y are deemed additive. On the other hand, a more negative and a more positive $\Delta\Delta G_{(H,Y\rightarrow X,Y)}$ values indicate positive and negative cooperativities, respectively. Cooperativity may therefore be defined as a variation in $\Delta\Delta G_{(H\rightarrow X)}$ which occurs when a second group Y is incorporated in the ligand molecule.

1.1. Additivity/cooperativity and the partitioning of the differential binding energy

Fig. 2 illustrates a "three-dimensional" Born–Haber cycle which can be used to partition the binding of two ligands LH and LX to a biological target P [11,12]. These two ligands differ only in that ligand LX has the functional group X replacing an H in ligand LH. The differential binding energy caused by this functional group replacement is therefore represented by the free energy difference ($\Delta G_{LX} - \Delta G_{LH}$). Because each of these free energy terms can be partitioned into basic components as illustrated by Eq. (1) which represents the partitioning of ΔG_{LH}, the differential binding energy can be partitioned as well, and this partitioning is illustrated by Eq. (2). This equation describes the partitioning of the differential free energy $\Delta\Delta G_{(H\rightarrow X)}$ into three major components: the differential desolvation of the ligand ($\Delta G_{LX\text{-desolv}} - \Delta G_{LH\text{-desolv}}$), the differential ligand-protein association ($\Delta G_{LX\text{-assoc}} - \Delta G_{LH\text{-assoc}}$), and the differential ligand-protein complex resolvation ($\Delta G_{LX\text{-resolv}} - \Delta G_{LH\text{-resolv}}$). It should be noted that both Eq. (1) and Eq. (2) should include other terms if conformational or ionization changes occur in either the ligands or the target during the course of binding.

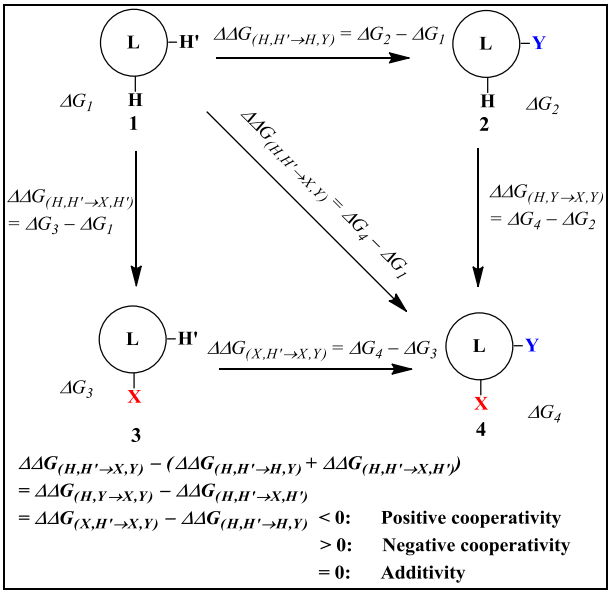

Fig. 1: A general double mutant cycle showing how cooperativity vs. additivity could be identified by comparing the differential binding energies of the H→X structural modification in presence and absence of group Y (Y vs. H′). Also cooperativity could be identified by comparing the differential binding energies of the H'→Y structural modification in presence and absence of group X (X vs. H).

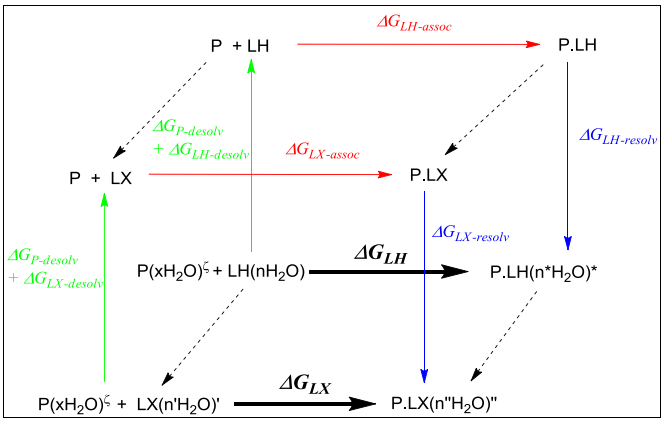

Fig. 2: A "three-dimensional" Born–Haber cycle representing the binding of two ligands LH and LX to a biological target P. These two ligands differ only in that the H of LH is replaced by a functional group X. The pre-association events are simplified to involve only the desolvation of the ligand and the receptor (no conformational or ionization changes). Additional terms would need to be included in Eqs. (1) and (2) if conformational or ionization changes occurred.

$$\Delta G_{LH} = \Delta G_{LH-\text{desolv}} + \Delta G_{P-\text{desolv}} + \Delta G_{LH-\text{assoc}} + \Delta G_{LH-\text{resolv}} \quad \text{(Eq. 1)}$$

$$\begin{aligned}\Delta\Delta G_{(H\rightarrow X)} &= \Delta G_{LX} - \Delta G_{LH} \\ &= \left(\Delta G_{LX-\text{desolv}} - \Delta G_{LH-\text{desolv}}\right) + \left(\Delta G_{LX-\text{assoc}} \right. \\ &\quad \left. - \Delta G_{LH-\text{assoc}}\right) + \left(\Delta G_{LX-\text{resolv}} - \Delta G_{LH-\text{resolv}}\right)\end{aligned} \quad \text{(Eq. 2)}$$

Given that additivity and cooperativity were previously defined in terms of variation in the differential free energy, these phenomena can be explained through the differential free energy partitioning. Additivity, for instance, exists when the differential free energy of a structural modification (e.g. H→X) is the same, no matter whether the initial or the final group of the second modification exists in the ligand (e.g. H′ or Y; Fig. 1): $\Delta\Delta G_{(H,H'\rightarrow X,H')} = \Delta\Delta G_{(H,Y\rightarrow X,Y)}$. This case could be obtained if (1) none of the differential free energy components illustrated in Eq. (2) changes when the structural modification H→X is carried out in presence of the H′ or the Y of the modification H'→Y; or (2) in the presence of Y vs. H′, more than one of these free energy components change in opposite directions so that no net change in the differential free energy is produced (e.g. ($\Delta G_{LX\text{-desolv}} - \Delta G_{LH\text{-desolv}}$) and ($\Delta G_{LX\text{-assoc}} - \Delta G_{LH\text{-assoc}}$) change in opposite directions but with