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DRUG DISCOVERY TODAY TECHNOLOGIES

Drug Discovery Today: Technologies

Vol. 17, No. null 2015

The role of binding kinetics in drug discovery

On-rate based optimization of structure–kinetic relationship – surfing the kinetic map

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In the lead discovery process residence time has become an important parameter for the identification and characterization of the most efficacious compounds *in vivo*. To enable the success of compound optimization by medicinal chemistry toward a desired residence time the understanding of structure–kinetic relationship (SKR) is essential. This article reviews various approaches to monitor SKR and suggests using the on-rate as the key monitoring parameter. The literature is reviewed and examples of compound series with low variability as well as with significant changes in on-rates are highlighted. Furthermore, findings of kinetic on-rate changes are presented and potential underlying rationales are discussed.

Section editor:
Xavier Barril – Facultat de Farmacia, Universitat de Barcelona, Spain.

Introduction

Over the past decade multiple groups have been emphasizing the value of residence time in drug discovery. Among them Swinney and Copeland have been the first in trying to establish a connection between binding kinetics and efficacy of compounds successfully applied in the clinic [1–3]. Whereas more recently others have brought forward that there is no correlation between binding kinetics and *in vivo* efficacy, but instead pointed out that the latter depends on pharmacokinetic stability [4]. The underlying rationale of the concept of long residence times has been convincingly argued from a theoretical point of view, that is, compounds binding to a target will have an extended effect on its function. However, there is evidence that certain requirements must be fulfilled to benefit from slow binding kinetics and that other factors will limit such an application [5,6]. While the discussion of optimizing compounds with desired residence time during hit identification and lead optimization is ongoing, there is no general agreement on an appropriate parameter for structure–kinetic relationships (SKR).

Binding kinetics and structure–kinetic relationship

For the characterization of a compound *in vitro*, where the system is in equilibrium, the affinity is determined by K_d or IC_{50}. These parameters have been guiding medicinal chemistry efforts in the process of optimizing compound activity. By contrast, in an open *in vivo* system the compound concentration is not constant but becomes dependent on other parameters. Thereby potency measures such as K_d will no longer be the only relevant parameter guiding efficacy.

The concept of optimizing compounds for longer residence time suggests that in the nonequilibrium situation the dissociation rate constant k_{off} of the target–ligand complex is the relevant parameter. While the concentration of free ligand in

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 http://dx.doi.org/10.1016/j.ddtec.2015.08.003

the vicinity of the target might have decreased below a concentration capable to influence efficacy, the fraction of target inhibited by the ligand can still be high enough to repress the overall target activity to a significant extent. This can last until the target–ligand complex concentration decays to a point where sufficient amount of free target becomes available by ligand unbinding or target resynthesis and thereby restores its function. This decay of the target–ligand complex is defined by the off-rate and is also expressed by its reciprocal parameter, the residence time.

As a consequence, a long residence time on a target has a beneficial effect on efficacy, while a short residence time on an off-target results in an improved safety profile.

Structure–kinetic relationship and how to determine it

For the task of lead optimization, structure–activity relationship (SAR) is one of the major tools for medicinal chemistry. During the course of a project the affinity is usually characterized by K_d or IC_{50} and is commonly improved from low to high affinity by optimizing and maximizing protein–ligand functional group complementarity. In an ideal situation, SAR can be applied as a system for medicinal chemistry which allows incremental affinity changes to be attributed to various functional groups being studied within a compound series. In the case of SKR, which is used in a similar fashion to SAR, a variety of different parameters have been proposed. One of the common underlying assumptions is that such parameters capture the relationship between incremental changes to a compound and its kinetic behavior as in the case of compound changes and the affinity for SAR. More importantly, the parameter that is chosen to describe SKR should be independent from affinity to be able to distinguish SKR from SAR.

General introductions to kinetics

The most simplistic model describing the binding mechanism is the single step binding model which assumes that ligand (L) and receptor (R) form the ligand–receptor complex without any stable intermediate. In this case the complex formation and complex decay can be described with the two rate constants k_{on} and k_{off} and the thermodynamic constant K_d as the ratio of k_{off}/k_{on}. Two more complex models with an additional step are the 'induced fit' [7] and the 'conformational selection' model [8]. In the former case it is assumed that after formation of the first protein–ligand encounter complex (RL) a conformational rearrangement of the receptor to a more stable R*L complex occurs. The latter involves two interconverting receptor conformations of which only one is capable to bind the ligand.

While these three different models of ligand target association are usually discussed to fit experimental data [9], the true underlying mechanism is potentially a combination of 'conformational selection' and 'induced fit' and thereby even more complicated [10,11].

Single step binding mechanism

In a significant number of cases the data is fitted to the less complex single step mechanism. Depending on the true underlying mechanism such an approximation can be wrong and therefore reported data such as k_{on} and k_{off} should be thought of as being macroscopic rate constants indicative of a more complex process.

In this simplified situation when considering the energy profile of the reaction coordinate (Fig. 1) the activation energy of the bimolecular binding process is associated with k_{on}. The energy difference between the transition state and the stable RL-complex is related to k_{off} and thereby to residence time, while the energy difference of the unbound state (R + L) and the RL-complex state is related to the affinity K_d. Figure 1 illustrates that the residence time or off-rate has two contributing factors, namely the activation energy associated with the on-rate and the affinity associated with K_d. While there exists a good understanding of how to describe structural variations of the ligand resulting in changes of affinity (SAR) the description of structural modifications with respect to kinetics (SKR) is difficult if the residence time or the corresponding off-rate is used.

Limitations of on-rates

On-rates can vary significantly for different protein–ligand complexes but are limited at the upper end by the rate of diffusion. This rate is in the range of 10^8–10^9 M^{-1} s^{-1} and defines an insurmountable limitation. On the lower end there are practical limitations that have to be considered. While the occupation of a target depends on the on-rate and the ligand concentration, incubation time and free ligand concentration determine the time required for reaching equilibrium target occupation. A simulation of target occupation

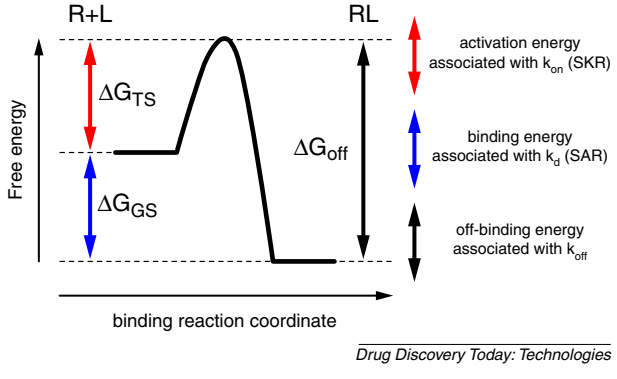

Figure 1. Free energy profile of the two state binding model. The energy barriers which separate the unbound from the bound state are highlighted as red and black arrows for the forward and reverse process. The free energy of binding is indicated by the blue arrow.

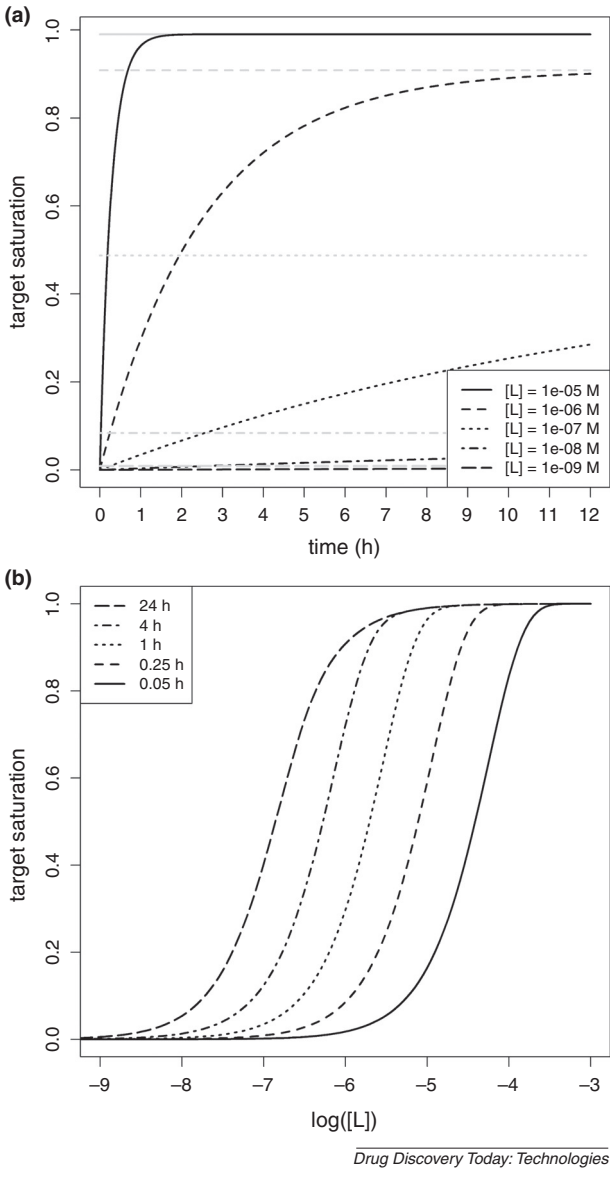

Figure 2. **(a)** Plot of the analytical solution of the binding process for the two state model using R for a hypothetical compound with $k_{on} = 10^2$ M^{-1} s^{-1}, $K_d = 10^{-7}$ M and $k_{off} = k_{on}*K_d = 10^{-5}$ s^{-1}. The receptor concentration was set to 10^{-8} M and the time courses of the target saturation for five different ligand concentrations were calculated independently. Initial conditions were set to [RL] = 0 M, [R] = [R_{tot}] and [L] = [L_{tot}], that is, no complex is present at $t = 0$. **(b)** Using the same compound and initial conditions as in (a) 160 ligand concentrations in 0.05 log increments from −3 to −11 (from 10^{-11} to 10^{-3} M) were calculated independently for five different time points. The target saturations at these five time points were connected to yield the inhibition curves shown in the plot.

(Fig. 2a) with various ligand concentrations indicates that the target occupation at a ligand concentration of 1 μM becomes crucial with on-rates below 10^3 M^{-1} s^{-1}. It shows that with an association rate of 10^2 M^{-1} s^{-1} at 1 μM ligand concentration the target reaches less than 60% occupation after two hours of incubation. This limitation can be circumvented for compounds with even lower on-rates by compensation with higher ligand concentrations *in vitro*. However, *in vivo* ligand binding might be limited in terms of reaching sufficient free ligand concentration. As another consequence of such incomplete target occupation the measured IC_{50} will shift toward higher values when pre-incubation times are chosen too short (Fig. 2b).

Making sense of kinetic data

Residence time and other metrics

Investigations into the effect of compound properties using binding kinetics of large data collections have demonstrated that long residence times correlate with lipophilicity (clogP), molecular weight (MW), number of rotatable bonds (NRB), ionization states and slow on-rates [12,13]. Dependency on clogP, MW and NRB is expected as these parameters are typically identified as descriptors of ligand affinity which is associated with SAR. In fact, residence time is a function of binding affinity.

It is important to understand that residence time is the crucial parameter for an extended effect of target inhibition. But in terms of guiding medicinal chemistry along the optimization process, residence time is not necessarily the best descriptor for SKR, in particular when comparing compounds with different affinities for a target.

Several metrics were suggested to be used for SKR-based optimization one of which is kinetic efficiency [14]. In analogy to the definition of ligand efficiency [15], kinetic efficiency was defined as the residence time divided by the number of heavy atoms. It is questionable if the comparison of the atom-normalized residence time gives sufficient insight into kinetics or whether it still resembles a parameter that is influenced at least in part by binding affinity. As an example we calculated kinetic and ligand efficiencies for three ligands from a DOT1L program (SI Table 1) [16]. In this example the increase in kinetic efficiency is paralleled with an increase in ligand efficiency and an improvement of K_d values. The compound evolution shows a trend that is essentially affinity-dependent for both metrics normalized by the number of heavy atoms. We conclude that ligand efficiency and kinetic efficiency are not independent of one another and that in this case the kinetic efficiency gain just mirrors the affinity gain. Another approach has been applied in the analysis of kinetic data on CDK8 inhibitors by comparing residence time with the number of hydrophobic contacts in the structural context of the protein–ligand structures [17]. This approach again has an affinity bias on the kinetic parameter since it monitors the ratio between residence time and the number of contact atoms observed in the ligand–protein crystal structure, which usually is a descriptor of thermodynamic stability.

On-rate as metric

To be able to detect and actively navigate kinetic effects by medicinal chemistry independent from affinity, it would be

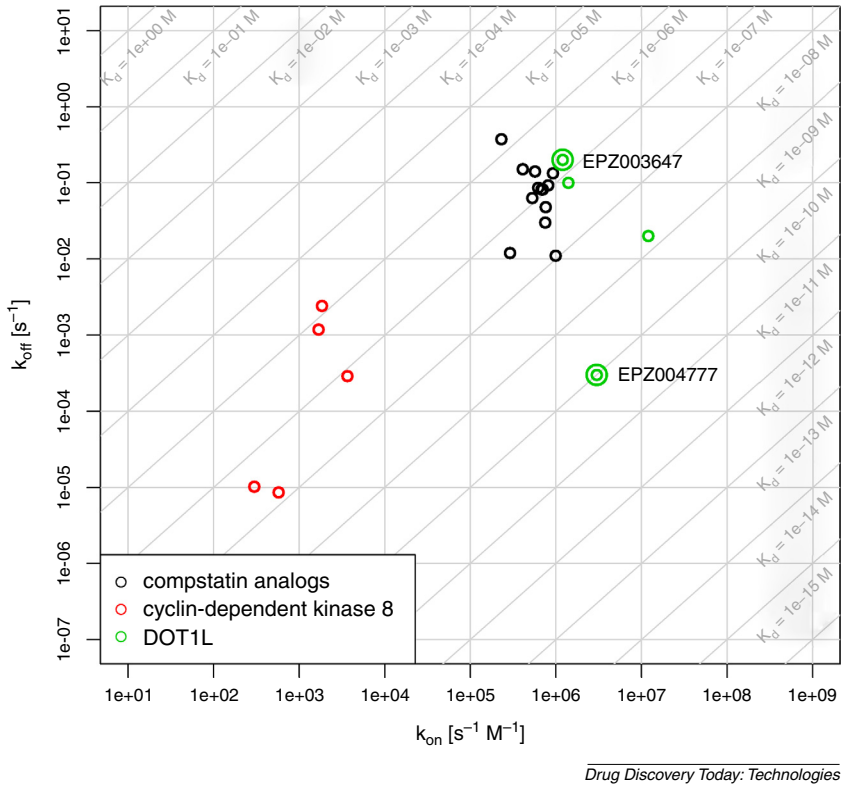

Figure 3. Kinetic map of three compound series with limited variability in their on-rate.

suitable to utilize a parameter independent from K_d. Lu and Tonge emphasized the importance of quantifying both the thermodynamic and kinetic properties of ligand binding [18]. For thermodynamics they defined ΔG_{GS}, a ground state change compared to a reference of 1 μM affinity and for kinetics ΔG_{TS}, a transition state change compared to a reference residence time of 0.01 s^{-1}. In this analysis the transition state energy is separated from the ground state binding energy by the underlying relationship that both contribute to the unbinding energy ΔG_{off} ($\Delta G_{off} = \Delta G_{TS} + \Delta G_{GS}$). Applying this concept we suggest to transform this equation ($\Delta G_{TS} = -RT \ln(k_{off}) + RT \ln(K_d)$) and to use the ratio of k_{off}/K_d as the guiding parameter for medicinal chemistry. For the simple situation of a single step binding mechanism this parameter is identical to k_{on} because $k_{off}/K_d = k_{on}$.

Using the free energy profile of the reaction coordinate (Fig. 1), k_{off}/K_d is related to the energy contribution of reaching the transition state which is independent of the energy level of the ligand–target complex. Thus studying kinetics should resolve around how transition state energies can be changed, whether and how they can be elevated or decreased. Combining this understanding together with the planned compound design and the associated potency of the final targeted compound one should be able to forecast the residence time using these two parameters. This means that part of the final residence time is given by the compound affinity (K_d) and part is given by the energy level that is associated with the transition state and thereby with the on-rate.

Visualization of kinetic data utilizing the kinetic map

Following the simplistic model of the one step binding mechanism k_{on}, k_{off} and K_d are interdependently connected and can be plotted in a 2D-kinetic map [19], where K_d can be derived from the two kinetic rates. The advantage of this kinetic map is the visualization of multiple compounds and their kinetic properties k_{on}, k_{off} and implicitly K_d.

Plotting the compounds from the DOT1L program [16] (Fig. 3) one can see that off-rates and residence times were improved in a stepwise manner by a factor of 660. Furthermore the map shows that the improvement of residence time between EPZ003647 and EPZ004777 is achieved via an affinity gain while the on-rates remain almost constant.

Examples, observations and rationales on kinetic behaviors

The evaluation of multiple data sets has shown that the off-rate usually correlates better with the affinity than with the on-rate [20]. Low variability of on-rates has also been observed in other compound series [21,22]. This observation might have led to the misinterpretation that k_{on} is of less relevance compared to k_{off}.

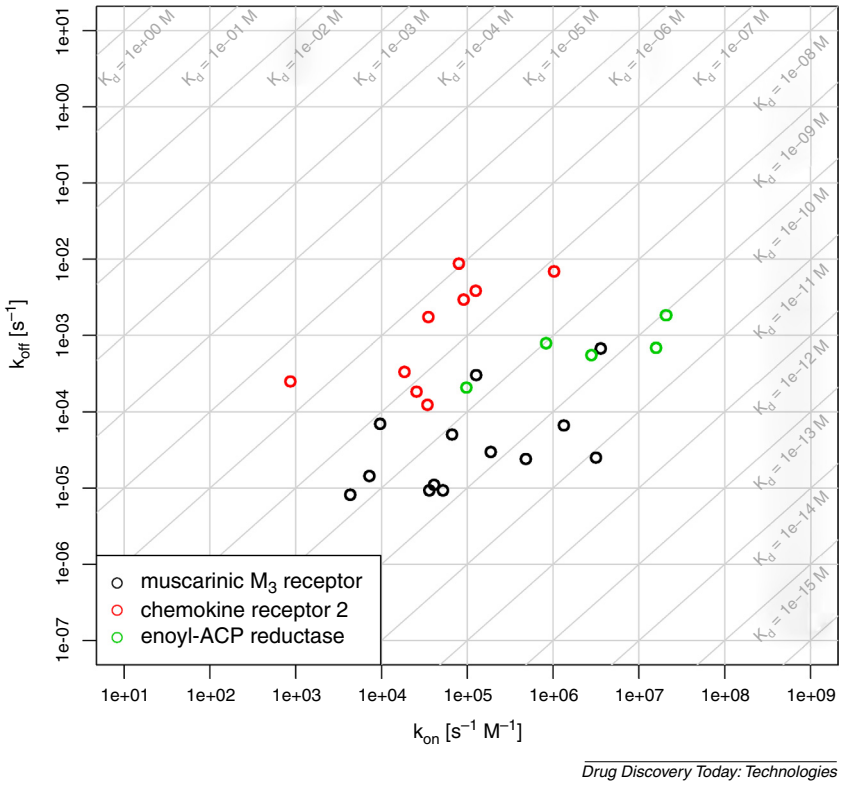

Figure 4. Three compound series of three distinct targets which display significant differences in their on-rates.

Considering that the range of potential on-rates varies between the diffusion limit (10^9 M^{-1} s^{-1}) and a lower practical limit of approximately 10^3 M^{-1} s^{-1}, this range seems to offer some room for optimization in medicinal chemistry. For the generation of long residence time compounds, with respect to an ideal 'sweet spot' of on-rate kinetics, values between 10^3 and 10^5 M^{-1} s^{-1} seem most attractive since they are interlinked with residence times of multiple hours without the need of affinity optimization into the subnanomolar range. Tiotropium, a muscarinic M_3 receptor antagonist, shows a residence time of multiple hours under nonphysiological conditions as a consequence of very high affinity (K_d = 7.9 pM) with a rather fast on-rate [23]. By contrast the p-38 inhibitor BIRB-796 exhibits its long residence time of 33 hours through a combination of high affinity (K_d = 0.1 nM) and a comparably slow on-rate (8.5×10^4 M^{-1} s^{-1}) [24]. Similar residence times are found for HDAC inhibitors with much lower on-rates (3.5×10^2 M^{-1} s^{-1}) while the potency contribution is less pronounced (compound 1: K_d = 38 nM) [25]. These examples demonstrate that there is variability in on-rates across different targets. However, medicinal chemists have been confronted with the question if it is possible to design on-rates for a given target into a given compound series and whether general optimization strategies are available. Figure 3 shows three targets with one set of compounds each, where on-rates seem to be less variable within the individual series [16,17,21]. One could conclude that the limited variability is associated with the target or the target binding site itself. By contrast, the collection of kinetic data on HIV-protease inhibitors has demonstrated a broader range of on-rate variability across a variety of compound classes [19]. Further examples have been reported [26–28]. Three compound series compiled in a kinetic map (Fig. 4) show an on-rate variability on the targets enoyl-ACP reductase [29], muscarinic M_3 receptor [23,30] and chemokine receptor 2 [31].

The knowledge of on-rate variation across different targets opens up an important research field in the search of the understanding which target–ligand interactions will result in either fast or slow association rates. Observations such as protein conformational rearrangements, protein desolvation, steric or other effects between ligands and proteins need to be considered as major drivers for these variances in association rates.

General observations and approaches for kinetic optimization

In the search for a molecular understanding it is often highlighted that structure information is essential to rationalize kinetic behaviors. Based on the fact that protein–ligand X-ray crystal structures resemble a thermodynamic minimum, it can be rather difficult to predict kinetic properties based on the activation energy of a transition state not observable by X-ray crystallography.

Nonetheless, as an example for a kinetic change based on protein conformations an induced fit mechanism of tau-tubulin kinase 1 (TTBK1) specifically observed by a flip of the XDFG-amide of the protein backbone was associated with a change in the kinetic signature that resulted in a 200-fold drop in on-rate [32]. With a more dramatic conformational change of an αC-helix movement, the ERK2 kinase inhibitor SCH772984 showed slow on-rate binding [33]. In general, strategies that address different target binding sites or sub-pockets can be promising in manipulating on-rates. This is the case for the kinase target family which contains a conserved DFG motif. Some kinases can undergo a conformational transition from DFG-in to DFG-out opening a deeply buried pocket. Binding to the latter is associated with slower on-rates, as in the case for the aforementioned compound BIRB-796.

Absence of a visible protein conformational change in an X-ray structure is however not indicative of a particular kinetic behavior. This is the case for the aforementioned HDAC where hydroxamate SAHA (PDB: 4LXZ) and benzamide compound 1 (PDB: 4LY1) show no major difference in the protein structure. Still, the more sterically demanding compound 1 exhibits an on-rate that is slower by 2 orders of magnitudes. One can assume that binding into the buried binding site of compound 1 is only possible if protein rearrangements occur within the binding event. In addition, binding into deep binding pockets requires the desolvation of water molecules and in the case of HDAC waters bound to the zinc ion are also replaced by the ligand, which results in a deceleration of the binding process.

Desolvation processes have been described in other cases and have been argued to be responsible for decelerated association rates [34,35]. The reasoning being that if ligands bind via hydrogen bonding in a binding site that is shielded from bulk water and thus electrostatically deshielded, the enthalpic hydrogen bonding strength becomes kinetically stabilized. Thus, water which occupies this binding site prior to ligand binding exchanges at slower on-rates. In consequence, water shielded hydrogen bonds display a kinetic barrier. This principle has been underlined in an example of thermodynamic and kinetic data from a set of HSP90 ligands [36]. Furthermore others have shown that calculated desolvation energies correlate with kinetic data of the association step [37,38]. In these cases protein–ligand structure information, especially when resolved with tightly bound waters, might be key to the interpretation of kinetic changes.

Conclusion

Designing binding kinetics into a compound remains a challenging task for medicinal chemistry within lead optimization. Ultimately, optimizing for long residence times requires either to improve the affinity by lowering the ground state of the receptor–ligand complex or to elevate the energy level of the transition state. As a guiding tool we suggest to monitor a parameter that can be derived from the ratio k_{off}/K_d or by k_{on} *per se* if the situation can be described with a one-step model. Multiple examples, where the on-rate has been monitored show that the variability in SKR can be limited. Thus the strategy for medicinal chemistry in optimizing for long residence times can be the focus on optimizing affinity or to explore additional binding epitopes that are associated with larger kinetic barriers.

Conflict of interest

Proteros is a service provider offering the determination of binding kinetics and X-ray structures. The authors confirm that this article content has no other conflict of interest.

Acknowledgement

The authors thank Andreas Bergmann for helpful discussions and proof reading of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ddtec.2015.08.003.

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