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Drug-Target Residence Time: Critical Information for Lead Optimization

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Summary of recent advances

Failure due to poor *in vivo* efficacy is a primary contributor to attrition during the development of new chemotherapeutics. Lead optimization programs that in their quest for efficacy focus solely on improving the affinity of drug-target binding are flawed, since this approach ignores the fluctuations in drug concentration that occur *in vivo*. Instead the lifetime of the drug-target complex must also be considered, since drugs only act when they are bound to their targets. Consequently, to improve the correlation between the *in vitro* and *in vivo* activity of drugs, measurements of drug-target residence time must be incorporated into the drug discovery process.

Introduction

A primary source of attrition during drug discovery stems from poor *in vivo* efficacy [1]. An important factor that contributes to this problem is the major disconnect that exists between *in vitro* data and our ability to predict efficacy in humans. A detailed understanding of drug mechanism of action is important for improving the success of drug discovery, and we posit that a critical contributor to this understanding, and to modulating *in vivo* drug activity, is the lifetime of the drug-target complex. To provide this information the target must be known and assays must be available to assess both the thermodynamics *and kinetics* of drug-target interactions.

In order to appreciate how kinetic parameters can modulate drug activity, it is useful to consider the fundamental difference between drug behavior in a closed (*in vitro*) system and that in an open (*in vivo*) system [2••]. In a closed system drug, target and substrate are at equilibrium, and thus thermodynamic equilibrium constants such as K_d or K_i values, or more commonly IC_{50} values, accurately reflect the concentration of the drug-target complex and are appropriate metrics for differentiating potency. A similar argument can be made for whole cell assays, such as standard minimum inhibitory concentration (MIC) measurements of antibacterial activity, where activity is measured at fixed drug concentrations. However, *in vivo* systems are open systems in which drug concentration fluctuates with time, and in which the concentrations of both the endogenous substrate (ligand) for the target and the target itself can vary during normal function or in the presence of the drug. Clearly, if drug and target are not at equilibrium, measurements of *in vitro* potency based only on thermodynamic parameters are unlikely to reflect potency *in vivo*, and thus prioritizing

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compounds based on K_d, K_i or IC_{50} values is unlikely to be successful. Instead, in open systems it is more appropriate to consider the lifetime of the drug-target complex, since a drug will only exert its effect when it is bound to the target. In this case residence time (t_R), which is the reciprocal of the rate constant for dissociation of the drug-target complex (k_{off}), can be conveniently used to quantify the lifetime of the drug-target interaction [3••].

The importance of residence time in controlling the pharmacodynamics of drug action is illustrated in Figure 1 (see also [3••] [4••] [5]: for example, see Figure 2 in [3••]). Key considerations include (i) the concentration of drug at the target site (i.e. the pharmacokinetics), (ii) the thermodynamic dissociation constant of the drug-target complex and (iii) the rate constant for dissociation of the drug-target complex (k_{off}). In our analysis we have chosen a hypothetical situation in which the concentration of drug at the target site decreases exponentially with a half life of 1 h from a C_{max} value of 500 nM. For pimelic diphenylamide 106, which has a dissociation constant of 14 nM for histone deacetylase [6–7], the C_{max} is sufficient to inhibit 97 % of the target, assuming that the concentration of target is $\ll$ 500 nM and that no substrate is present. The percent inhibition of histone deacetylase has then been plotted as a function of time assuming that the drug does not rebind to the target and using the k_{off} value of 0.086 h^{-1} for this system [6–7], which corresponds to a half life for the drug-target complex of 8 h (t_R = 11.6 h). Thus after 12 h the enzyme target is still 37 % inhibited even though the free drug concentration has decreased by more than 2,000-fold and is now well below K_d. Also shown in Figure 1 is a similar analysis for two other hypothetical drugs that also have dissociation constants of 14 nM for their targets. For a drug that has a half-life of 72 h on its target (t_R = 104 h), the percent target occupancy is 87 % after 12 h. Conversely, for a rapid reversible drug the percent target occupancy is given simply by the dissociation constant of the drug-target complex and only 2 % of the target is occupied after 12 h. It can thus clearly be seen that residence time has a dramatic effect on percent target occupancy (i.e. on drug pharmacodynamics) in situations where the drug concentration fluctuates over the K_d for the target. In the case of a rapid reversible drug, the percent target occupancy is entirely dependent on the drug concentration at the target site. Drugs with elimination half-lives that are shorter than 1 h will cause the percent target occupancy to fall more rapidly than shown in Figure 1, while the percent target occupancy will fall more slowly for drugs with elimination half-lives that are longer than 1 h. As has been noted before, the analysis in Figure 1 also demonstrates how the difference in residence time of a drug on its target and on an off-target protein responsible for unwanted (toxic) side-effects will dictate how the therapeutic index of the drug varies with time [3••] [4••] [5]. For example, if the rapid reversible interaction depicted in Figure 1 represents the interaction of pimelic diphenylamide 106 with an off-target protein, then in our analysis this drug will have almost completely dissociated from the off-target protein after 12 h whereas the occupancy of the therapeutic target will still be 37 %.

The significance of drug-target residence time is highlighted by the large number of current drugs that have long residence times, stretching from minutes to days, on their targets [2••] [3••] [4••] [5] [8] [9•]. For example, investigation of 85 New Molecular Entities approved by the FDA between 2001 and 2004 showed that, for the 72 drugs for which the molecular target is known, 19 (26 %) are slow binding inhibitors [10•]. Furthermore, a survey of 50 drugs demonstrated that, in general, those compounds with longer residence time have better biological efficacy [11••]. In this review we add an additional 25 compounds (Table 1), most of which have been reported in the past 2 years, to the growing list of long residence time drugs, and present two specific examples in which there is a direct correlation between residence time and *in vivo* efficacy, and where thermodynamic assessments of potency are poor predictors of *in vivo* activity. We then comment on the significant hurdle to using mechanistic information for predicting and modulating residence time.

Mechanism of Drug-Target Complex Formation

Drug-target complex formation can occur through several different mechanisms [2••]. These include simple one step binding, induced fit, conformational selection, and irreversible inhibition. In each case the residence time (t_R) of the drug on the target is given by $1/k_{off}$, and for the one-step mechanism (Figure 2A), k_{off} is equal to the microscopic rate constant k_2. In the limiting situation where the k_{on} (k_1) values are diffusion controlled, then a drug that binds through the one-step mechanism with a K_d value of 1 nM will have a residence time of 10 s ($k_{off} = 0.1\ s^{-1}$) on the target (assuming $k_{on} = 10^8\ M^{-1}\ s^{-1}$). Thus, if an assumption is made concerning the value for k_{on} then k_{off} and hence residence time can be calculated from the thermodynamic equilibrium constant. However, k_{on} values for rapidly associating drugs are often smaller than the diffusion controlled limit [12], and pre-steady state kinetic methods using a rapid mixing system must be used to determine k_{off} values. For slow binding drugs both k_{on} and k_{off} are substantially smaller than the values given for the limiting case, and pre-steady state kinetic methods such as progress curve analysis that do not require rapid mixing can be used to determine drug-target residence times [13••].

Most slow on/off drugs operate through an induced fit mechanism (Figure 2B) in which the rapid formation of an initial drug-target complex (TD) is followed by a subsequent slow step leading to formation of the final drug-target complex (TD*). In general it is thought that this second step involves an isomerization of the target (and/or possibly also the drug) to a new conformation which is more complementary to the binding pair and in which drug-target interactions are optimized. The formation and breakdown of the initial complex is normally very fast, but the isomerization is slow and determines the overall drug-target interaction time. Because the final complex is thus much more stable than the initial complex, this type of binding is often associated with high affinity inhibition. Theoretically, residence time for this mechanism is a compilation of several rate constants (Figure 2B) [14••], however in most cases the reverse isomerization step, k_4, is rate determining and so $t_R \approx 1/k_4$.

Residence Time as a Predictor of *In Vivo* Efficacy

The observation that many drugs have long residence times on their targets suggests that drug-target residence time is important for determining drug efficacy *in vivo* [3••] [4••] [5]. However, in many cases data for only a single drug-target pair are available, and in order to directly demonstrate the importance of residence in modulating *in vivo* efficacy it is most appropriate to consider examples from within a compound series where correlations between residence time and *in vivo* efficacy are revealed. In our own work we are developing inhibitors of the bacterial enoyl-ACP reductase (FabI) enzyme in the bacterial fatty acid biosynthesis from organisms such as *Mycobacterium tuberculosis* and *Francisella tularensis.* Studies with the FabI enzyme from *M. tuberculosis* (mtFabI, InhA) are an interesting case in point for the current review because it is known that the front-line tuberculosis drug isoniazid is a slow binding inhibitor of this enzyme with a residence time of 62 min [15]. In the FabI enzymes it is thought that slow binding inhibition is coupled to ordering of a substrate recognition loop close to the active site [16] and this information has been used to develop slow binding inhibitors (Figure 3A) of both the mtFabI enzyme [17] and also of the *F. tularensis* FabI (ftuFabI) [18••]. For *F. tularensis*, the MIC values of our inhibitors range from 0.00018 μg/ml to 2.5 μg/ml and a linear correlation was observed between logMIC and $\log K_i$, supporting the hypothesis that ftuFabI is the primary cellular target of these compounds. However no correlation was observed between either K_i or MIC values and the *in vivo* efficacy of the compounds in an animal model of tularemia infection. Instead, a linear correction was found between t_R and *in vivo* efficacy (Figure 3B).

A second example is given by antagonists of the Neurokinin 1 receptor (NK_1R) [19] which, together with its tachykinin substrate P (SP), have been reported to be involved in many human diseases [20]. Aprepitant, CP-99994 and ZD6021 are competitive antagonists of NK_1R with almost identical thermodynamic affinities for the receptor [19]. However, efficacy studies using a gerbil foot tap (GFT) assay, revealed that the *in vivo* activity of the three compounds decreased in the order aprepitant > ZD6021 > CP-99994 which correlated with the residence time of the compounds on the receptor (Table 1). Thus, in both this study as well as the FabI inhibitor discovery program, drug-target residence time, rather than thermodynamic affinity, is a better predictor of *in vivo* drug activity.

Optimizing Residence Time

The above discussion makes it clear, in our opinion, that measurements of drug-target residence time should be incorporated into the standard drug discovery paradigm during the lead optimization phase. It should be possible to screen for long residence time compounds in a high-throughput format, for example by determining whether IC_{50} values shift following preincubation of drug and target or by introducing a washing step to remove transiently bound drug from immobilized target following preincubation of drug and target, and it is straightforward to measure residence time for smaller subsets of more advanced compounds in order, for example, to prioritize compounds for characterization in animal models of disease. However, the rational modulation of residence time remains a much greater challenge. Almost invariably, in their efforts to improve activity lead optimization programs have focused solely on maximizing the affinity of drug-target binding - that is on stabilizing the energy of the drug-target complex. However, as we will see below, achieving a long residence time often requires modulation of the energy of both ground states and transition states on the reaction coordinate. Thus, different approaches to assessing the activity of ligand analogs are needed if optimizing residence time is included as a goal.

The free-energy profiles in Figure 2 illustrate the fundamental point that the rate constants that control the lifetime of a drug-target complex depend on the relative free energies of the ground and transition states on the reaction coordinate. Thus, an increase in residence time will occur by a combination of factors that lead to a decrease in k_{off}, *and indeed these changes might even include a decrease in the stability of the drug-target complex*. For example, in our study of the ftuFabI inhibitors, compound **5** has a much higher free energy for the final TD* complex than compound **3** (triclosan) (K_i* 2.7 and 0.051 nM, respectively). The longer residence time of **5** on the enzyme (143 min compared to 40 min) is achieved by a large increase in the free energy of the transition state. In other words, changes in the structure of **5** compared to **1** actually destabilized the TD* complex, but also destabilized the transition state leading to TD* to an even greater extent. In the case of ftuFabI we know that conversion of TD to TD* involves the ordering of a loop of amino acids close to the active site [16–17,21], and we are currently probing the structure of the transition state for this process in order to rationally improve residence time in this series. In order to visualize the changes in the ground state and transition state energies and how these contribute to alterations in residence time, we have calculated the free energy changes of the ground state and transition state for each ftuFabI inhibitor relative to compound **1**, which has the shortest residence time. In Figure 3C it can be seen that for compounds **3** and **4** the increase in residence time is primarily a result of ground state stabilization relative to **1**, whereas for **2** and **5** transition state destabilization is the primary contributor. Thus, even within this single compound series changes in the free energies of both the ground state and transition state are important for modulating residence time.

The above analysis has been extended to the compounds in Table 1, where we have plotted the changes in ground state and transition state energies for each compound relative to a

standard state with an overall dissociation constant of 1 μM and a t_R of 0.01 s (Figure 4). Compounds where ground state stabilization is the primary contributor are shown on the left while those where transition state destabilization is the primary factor are on the right. It can be seen that for DADM3-ImmH, PT70 and DFPP-DG, stabilization of TD* is the primary driver for increasing residence time whereas for Bis-AMT the increase in residence time is primarily due to destabilization of the transition state. Although comparisons within a compound series would be more rigorous, and the choice of standard state is arbitrary, this figure again makes the point that changes in transition state energy can play a major role in modulating residence time. In addition, it is also important to note that compounds with long residence times on their targets, such as efavirenz (t_R = 4.1 h) and Bis-AMT (t_R = 12.6 h), can have relatively low thermodynamic affinity for their targets (5 μM and 3.7 μM, respectively), stressing the potential disconnect between the thermodynamic stability of a drug-target complex and the lifetime of that complex. The data in Table 1 thus reinforce the importance of quantitating both the thermodynamics and kinetics of drug-target interactions.

Conclusion

The goal of lead optimization is to improve the *in vivo* properties of compounds identified early in the drug discovery process so that they can be used to treat disease in humans. A significant component of current effort is focused on improving the affinity of the lead for the drug-target, and also decreasing the affinity of the lead for off-target proteins if these are known. However, current approaches that rely exclusively on thermodynamic equilibrium constants are flawed since fundamental differences between closed and open systems are ignored, and in order to improve predictions of *in vivo* drug efficacy, measurements of drug-target residence time must also be incorporated into the drug discovery process. Critically, the residence time of a drug on its target results from the *difference* in free energy between the relevant ground and transition states on the reaction coordinate. Thus, efforts to improve the thermodynamic affinity of a drug for its target in systems where long residence times are desired, may actually reduce *in vivo* drug efficacy if stabilization of the drug-target ground state is offset by correspondingly larger stabilization of the rate limiting transition state, leading to a reduction rather than an increase in residence time. In this review we have operated from the standpoint that longer residence times are preferred, and this is certainly true in situations where a complete and sustained abrogation of activity is required, such as inhibition of an anti-infective target. However we recognize that there are therapeutic targets where short drug-target interactions are optimal, for example where the activity of a human enzyme must be transiently modulated. Importantly, in both cases knowledge of residence time will be critical for understanding and predicting *in vivo* drug activity.

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• of special interest

•• of outstanding interest

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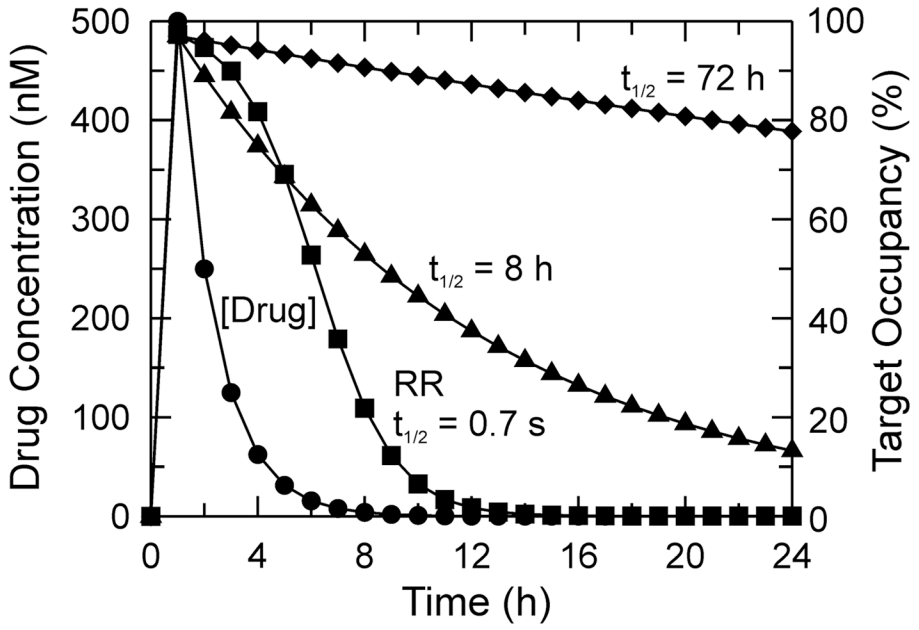

Figure 1. Residence time, pharmacokinetics and pharmacodynamics

An analysis that demonstrates how residence time affects the amount of drug-target complex (pharmacodynamics) as a function of time. The drug is assumed to reach a maximum concentration (C_{max}) of 500 nM at the target site 1 h after dosing, and to have an elimination half-life of 1 h (pharmacokinetics) so that the drug concentration at time t is given by $D(t) = C_{max} * 2^{(-t/1)}$ (●). The fractional occupancy of the target by the drug, given as a percentage, is shown for three drugs all of which have equilibrium dissociation constants of 14 nM for the final drug-target complex (DT for a rapid reversible inhibitor, DT* for a slow-onset inhibitor) so that at C_{max} (500 nM), the target in each case is 97 % occupied by drug (500/(500+14)). This assumes that the concentration of target is ≪ 500 nM and that no substrate is present to compete for binding to the target. For the histone deacetylase inhibitor pimelic diphenylamide 106 [6–7], the percent target occupancy has been plotted as a function of time using a drug-target complex half-life ($t_{1/2}$) of 8 h (the k_{off} value for this inhibitor is 0.086 h^{-1} and t_R = 11.6 h) (▲). The percent target occupancy at time t is then given by $\%occupancy = 97 * 2^{(-t/8)}$ assuming that the drug does not rebind to the target (i.e independent of the free drug concentration). A similar analysis has been performed for a hypothetical drug with $t_{1/2}$ = 72 h (t_R = 104 h) (◆). Also shown is the percent target occupancy for a rapid reversible (RR) drug calculated directly from the K_d value of 14 nM where $\%occupancy = 97 * (D(t)/(D(t)+K_d))$ and D(t) is the drug concentration at time t as calculated previously (■).

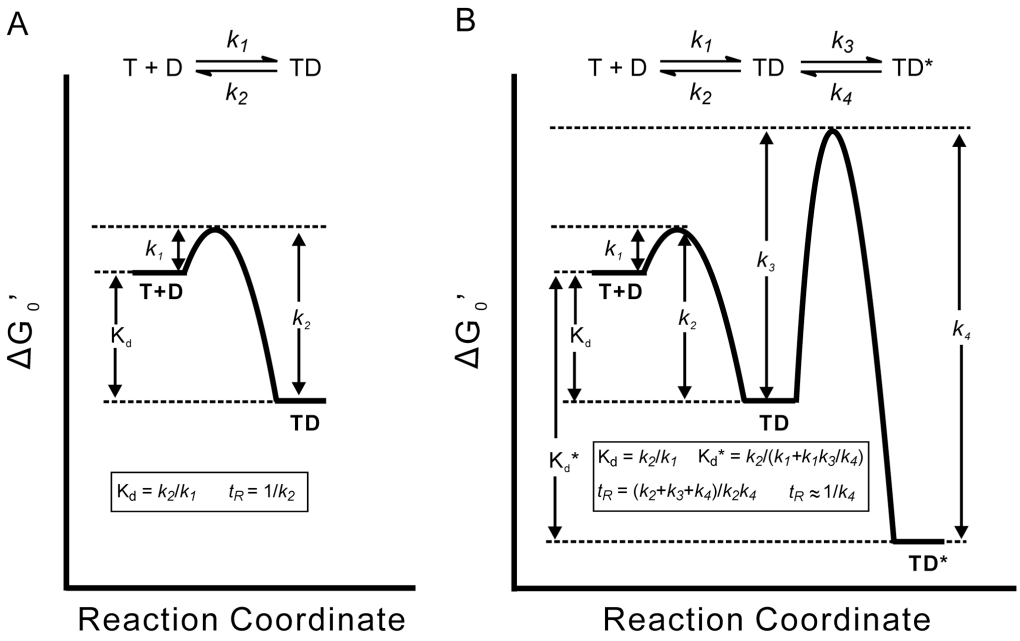

Figure 2. Free energy profiles for drug-target interactions

T and D refer to target and drug respectively. **A**) One-step binding mechanism, in which k_1 and k_2 are the forward and reverse rate constants, respectively, and K_d is the dissociation constant. **B**) Two-step induced fit binding mechanism. k_1 and k_2 are the rate constants for formation of the initial TD complex, while k_3 and k_4 are the rate constants for the isomerization step leading to the final TD* complex. In the example shown k_3 and k_4 are small so that formation and breakdown of TD* is slow. K_d is the dissociation constant of TD, while K_d* is the dissociation constant of TD* and determines the true affinity of the drug for its target.

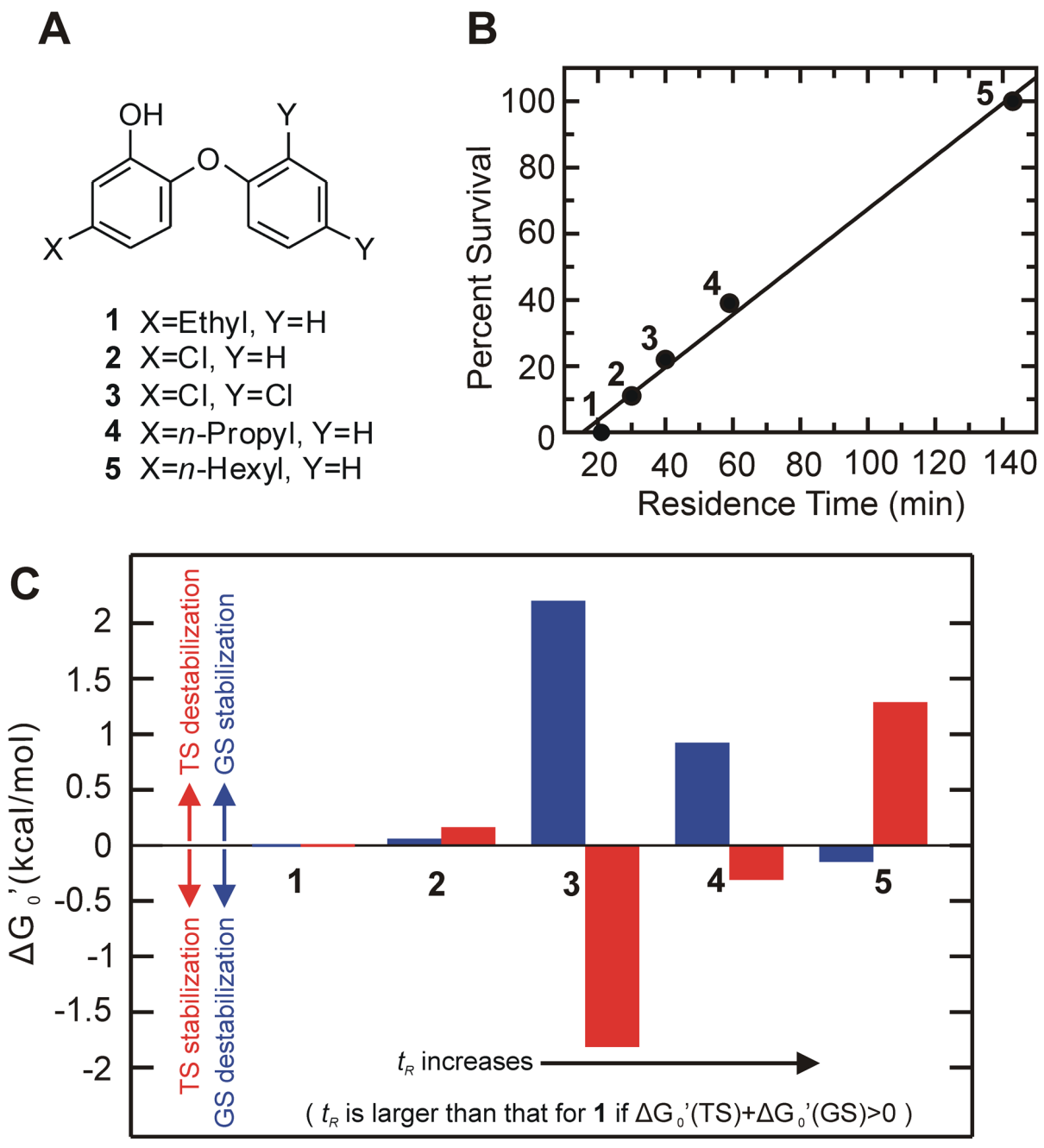

Figure 3. *In vitro* and *in vivo* data for the *F. tularensis* FabI inhibitors

A) Structures of the diphenyl ether inhibitors. **B)** Linear correlation between t_R and *in vivo* efficacy [18]. **C)** Ground state (GS) and transition state (TS) contributions to the residence time of each compound. Changes in the free energy of the ground state have been calculated at 298K relative to compound **1** which has the shortest residence time. The change in ground state free energy of compound x relative to the compound 1 is given by $\Delta G_{GS} = -RT\ln(K_{i(x)}/K_{i(1)})$, where $K_{i(1)}$ and $K_{i(x)}$ are the equilibrium dissociation constants for the two compounds from ftuFabI, and R=1.986 $calK^{-1}mol^{-1}$. In order to determine how the change in transition state free energy contributes to the change in residence time, the change in transition state free energy between the two compounds is given by ΔG_{TS} =

$-RT(\ln(K_{i(1)}/K_{i(x)})-\ln(k_{off(1)}/k_{off(x)}))$, where $k_{off(1)}$ and $k_{off(x)}$ are the dissociation rate constants of the two compounds from ftuFabI. Ground state (GS) contributions are shown in blue, where a positive value indicates the ground state has been stabilized relative to the standard state while a negative value reflects a relative destabilization of the ground state. Transition state (TS) contributions are shown in red, where positive values indicate transition state destabilization and negative values indicate transition state stabilization relative to the standard state.

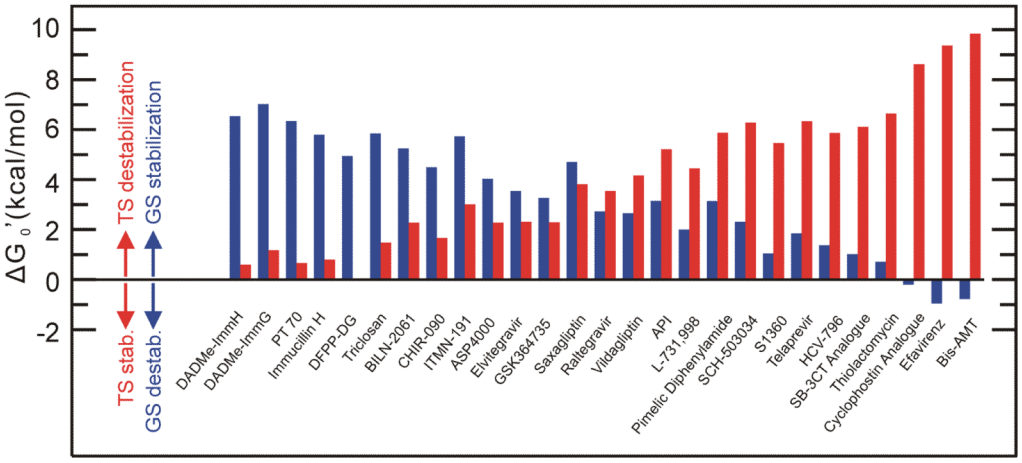

Figure 4. Ground state and transition state contributions to changes in residence time for the compounds in Table 1

Changes in the free energy of the ground state (GS) and transition state (TS) for each compounds in Table 1 has been calculated relative to a transient-binding drug with a thermodynamic affinity of 1 μM for its target and a drug-target residence time (t_R) of 0.01s (k_{off} = 100 s^{-1}). ΔG_{GS} and ΔG_{TS} have been calculated as described in the legend to Figure 2: $\Delta G_{GS} = -RT\ln(K_{i(x)}/1\times10^{-6})$, and $\Delta G_{TS} = -RT(\ln(1\times10^{-6}/K_{i(x)})-\ln(100/k_{off(x)}))$. Ground state (GS) and transition state (TS) contributions are shown in blue and red, respectively, as defined for Figure 2.

Table 1

Long residence time in drugs and inhibitors reported in the past two years.

Inhibitor	Target	Dissociation Constant (nM)	Residence Time	Indication	Ref.
PT 70	Enoyl-ACP Reductase, *M. tuberculosis*	0.022	23 min	Bacterial Infection	[17]
Triclosan	Enoyl-ACP Reductase, *F. tularensis*	0.051	40 min	Bacterial Infection	[18]
Thiolactomycin	β-Ketoacyl-ACP Synthase, *M. tuberculosis*	300	42 min	Bacterial Infection	[22]
API	Chitinase A *S. marcescens*	4.9	3.8 h	Bacterial Infection	[23]
Bis-AMT	Peptide Deformylase *E. Coli*	3700	12.6 h	Bacterial Infection	[24]
CHIR-090	UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc deacetylase *E. Coli*	0.5	5.6 min	Bacterial Infection	[25–26]
Pimelic Diphenylamide 106	Human Histone Deacetylase 3	14	11.6h	Breast, Prostate, Lung and Stomach Cancer	[6–7]
SB-3CT Analogue	Human Gelatinase (MMP-2)	180	28 min	Breast and Prostate Cancers (Tumor Metastasis)	[27–29]
Cyclophostin Analogue	Human Acetylcholinesterase	1386	4.2 h	Alzheimer's Disease	[30]
Efavirenz	HIV-1 Reverse Transcriptase	5000	4.1 h	HIV	[31]
Elvitegravir	HIV Integrase	4	2 min	HIV	[32]
Raltegravir	HIV Integrase	10	6.7 min	HIV	[32]
GSK 364735	HIV Integrase	2.5	3.3 min	HIV	[32]
L-731,988	HIV Integrase	34	9.1 min	HIV	[32]
S1360	HIV Integrase	170	>10 min	HIV	[32]
Telaprevir	Hepatitis C Virus Nonstructural Protease (NS3)	43.4	2.9 h	Hepatitis C	[33]
SCH-503034	Hepatitis C Virus Nonstructural Protease (NS3)	20	5.7 h	Hepatitis C	[33]
BILN-2061	Hepatitis C Virus Nonstructural Protease (NS3)	0.14	56 min	Hepatitis C	[33]
ITMN-191	Hepatitis C Virus Nonstructural Protease (NS3)	0.062	7.3 h	Hepatitis C	[34]
HCV-796	Hepatitis C Virus RNA polymerase	98	34 min	Hepatitis C	[35]
ASP4000	Human Dipeptidyl-peptidase IV	1.1	7.1 min	Type II Diabetes, and Obesity	[36]
Vildagliptin	Human Dipeptidyl-peptidase IV	11.3	17 min	Type II Diabetes, and Obesity	[36]
Saxagliptin	Human Dipeptidyl-peptidase IV	0.35	5.1 h	Type II Diabetes, and Obesity	[37]
CP-99994	Human Nonpeptide Human Neurokinin I Receptor (GPCR)	ND	< 30 min	Antiemetic and Parkinson's Disease	[19]
ZD6021	Human Neurokinin I Receptor (GPCR)	ND	1 h	Antiemetic and Parkinson's Disease	[19]
Aprepitant	Human Neurokinin I Receptor (GPCR)	ND	> 1 h	Antiemetic and Parkinson's Disease	[19]
DFPP-DG	Human Purine Nucleoside Phosphorylase	0.237	0.69 min	T-cell Cancer and Autoimmune Diseases	[38]

Inhibitor	Target	Dissociation Constant (nM)	Residence Time	Indication	Ref.
Immucillin	Human Purine Nucleoside Phosphorylase	0.056	12 min	T-cell Cancer and Autoimmune Diseases	[39]
DADMe-ImmH	Human Purine Nucleoside Phosphorylase	0.016	29 min	T-cell Cancer and Autoimmune Diseases	[39]
DADMe-ImmG	Human Purine Nucleoside Phosphorylase	0.007	2.9 h	T-cell Cancer and Autoimmune Diseases	[39]