A KINETIC BASIS FOR THE HANSCH EQUATION

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ABSTRACT
The Hansch equation is a key mathematical relationship and a conceptual guiding principle (QSAR) in the practice of modern medicinal chemistry. Recent years have apparently witnessed a debate on the utility of QSAR, hence it seems opportune to explore its fundamental origins. The Hansch equation leads to a parabolic relationship between drug activity and hydrophobicity. Currently, this is explained on the basis of more efficient drug-receptor interaction at low to moderate hydrophobicity, and decreasing aqueous solubility of the drug at moderate to high hydrophobicity. Herein is presented an alternative kinetic model, essentially based on the rate of the drug-receptor interaction; thus, binding is rate determining up to moderate levels of hydrophobicity, beyond which drug release is rate determining. The overall model is based on the idea that the release of the drug occurs concurrently with a physiological response, although alternative variants are also discussed. Overall, it is argued that QSAR essentially indicates the primacy of electronic over steric effects. This has fundamental implications for the classical theory of drug-receptor binding, which may need to be appropriately reassessed. Thus, the observed structure-activity relationships possibly apply to the kinetics of drug-receptor binding, likely involving substrate-induced conformational changes within the receptor, prior to the binding event. Recent developments in receptor-based drug design methodology apparently support these views.

Key words
Drug; Hammett equation; Hansch equation; hydrophobic; receptor
INTRODUCTION

Quantitative structure activity relationships (QSAR) are now considered to epitomize the principles of drug action in particular, and biomolecular interactions in general.\textsuperscript{1-7} Thus, QSAR is at once a practical tool with the potential to alleviate human suffering and a great generalizing principle with deep theoretical roots spread across the chemical-biological divide. Since its first insightful enunciation by Hansch – now enshrined in an eponymous equation – it has evolved into one of the defining advances in chemical biology, both profoundly conceptual and eminently utilitarian.

QSAR – in its essence – traces biological activity to hydrophobicity. The stark simplicity of this causal relationship should in itself be a cause for wonderment, given the sheer complexity of biological phenomena and the imperfections of molecular science! Yet, QSAR has recently come under intense critical scrutiny, amidst widespread disappointment at its perceived under-performance in churning out one magic bullet after another! Perhaps one sees in this an excess of Occamistic zeal, but – on the other hand – the need for reliable drug discovery protocols cannot, of course, be overstated!

The Hansch equation defines the fundamental basis of QSAR, and mathematically relates drug activity to hydrophobicity. The resulting parabolic curve is currently believed to reflect the efficiency of drug-receptor binding: apparently, this is directly proportional to the hydrophobicity parameter, but beyond a certain point decreasing bio-availability overwhelms binding. Although this model is ostensibly compelling, the explanation at high levels of hydrophobicity is apparently unsubstantiated. Hence, it is worth exploring alternative mechanistic rationales for the observed effects. An interesting kinetic model of drug action is thus presented below.

QSAR and the Hansch equation derive from the principles of linear free energy relationships (LFER). These, in particular the Hammett equation, are well known in physical organic chemistry and are widely employed to make sense of structure-property and structure-reactivity relationships. The extension of LFER into the biological domain, apparently, represents a significant reductionist step towards explaining life in physico-chemical terms.

DISCUSSION

\textit{LFER in biology: the Hansch equation}. It is noteworthy that the first hints of a possible association between molecular structure and biological activity were being obtained as early as the late 19\textsuperscript{th} century. And – with a prescience that now appears stunning – these early studies apparently implicated hydrophobicity too! Thus, it was known that cytotoxicity was inversely
related to water solubility and that narcotic action was related to the oil/water partition coefficient; the relation between acid dissociation and bacteriostatic activity soon followed. (Clearly, these elegant studies were perforce limited by the physical techniques available then, but they do display a remarkable intuition!)

The foundations of the modern LFER approach and methodology, however, were laid in the 1960’s by the extensive work of Hansch and coworkers. In particular, the activity of plant growth regulators was seen to be a function of the Hammett $\sigma$ along with a newly defined hydrophobicity parameter ($\pi$, Eqn. 1), based on the oil/water partition coefficients $P_x$ and $P_H$ (for a reference compound). It was thus seen that $\pi$ and $\sigma$ were weighted components in a quantitative relation (Eqn. 2) involving the activity of the drug, defined as $\log(1/C)$ in terms of the concentration ($C$) required for eliciting a certain response. Further extensive work, however, uncovered a more complicated parabolic relationship that was valid across a much larger variety of substrates (Eqn. 3 and Fig. 1, based in $P$ rather than $\pi$).

$$\pi_e = \log P_x - \log P_H \quad (1)$$
$$\log(1/C) = a\sigma + b\pi + ck \quad (2)$$
$$\log(1/C) = a\log P - b(\log P)^2 + c\sigma + k \quad (3)$$

This monumental contribution to chemical biology is, appropriately, well served by several masterly reviews. Such precedent allows us to pick up the action at an advanced stage in the evolution of QSAR. In particular, the parabolic curve (Fig. 1) that is so successfully addressed by the extended Hansch treatment can now be subjected to mechanistic analysis, hopefully leading to the roots of QSAR per se. Hence, this is an opportune juncture to enquire into the possible mechanistic basis of Eqn. 3, in terms of the fundamental interaction between a drug and its target receptor.

*Mechanistic variants possibly leading to the Hansch equation.* The extended Hansch equation (Eqn. 3) is currently believed to be a comprehensive representation of both drug transport and binding in quantitative terms. Also noteworthy is the presence of the Hammett $\sigma$ on the right hand side of Eqn. 3, implying that it is essentially a LFER in itself as discussed further in the next section.
**Fig. 1.** The parabolic relationship between drug activity and hydrophobicity [represented by $-\log(C)$ and $\log(P)$ respectively], based on the well-known Hansch equation (Eqn. 3)

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**Scheme 1.** The reversible binding of a drug (D) to a receptor (R), with the formation of a drug-receptor complex ($C_{DR}$) and its active form ($C_{DR}^*$); this breaks down with the release of a physiological response ($P_R$), along with D and R (the $k$'s are rate constants for the indicated step)

An important feature of LFER theory is that a break in the correlation implies a change in the reaction mechanism. Graphically, this manifests as a change in the slope of the LFER plot, thus indicating a change in the $\rho$ value at the point of inflexion. The analogy with the parabolic curve implied by Eqn. 3 now becomes obvious. In fact, these features can be addressed on the basis of the following generalized mechanism of drug action (Scheme 1).

Scheme 1 depicts the binding of the drug (D) to a receptor (R) to form the drug-receptor complex ($C_{DR}$), which undergoes a transformation to an active form ($C_{DR}^*$). This activated complex then breaks down irreversibly, concomitantly issuing a physiological response ($P_R$) and releasing the drug (D). Interestingly, the overall mechanism would vary depending on the hydrophobicity of the drug, as discussed below.

The kinetics of the mechanism shown in Scheme 1 can be addressed with the help of the steady-state approximation to arrive at Eqn. 4 (a full derivation is to be found in the Appendix section). Importantly, it is assumed that the activity of the drug, expressed in terms of $\log(1/C)$, is
proportional to the rate of breakdown of the activated drug-receptor complex, \( i.e. k_3 [C_{DR^*}] \). This is based on the assumption that the physiological response \( (P_R) \) is issued in this step.

\[
\log(1/C) \propto k_3 [C_{DR^*}] = (k_1 k_2 k_3 [D][R])/(k_2 k_3 + k_1(k_2 + k_3))
\]  

(4)

Note that the ‘\( P_R \)’ is to be understood in a broad sense, e.g. to include enzyme inhibition. Two simplifying cases may now be considered.

**Fig. 2.** Gibbs free energy (G) profile diagram for case (i) below, involving rate-determining formation of \( C_{DR} \) (cf. Scheme 1 and Eqn. 5)

**Case (i): rate-determining formation of \( C_{DR} \).** In this case, \( k_3 \gg k_2 \) and \( k_2 \gg k_1 \), represented by the free energy profile in Fig. 2. Thus, the further breakdown of \( C_{DR} \) and \( C_{DR^*} \) is much faster than their reversion to free drug and receptor, and Eqn. 4 reduces to Eqn. 5.

\[
\log(1/C) \propto k_3 [C_{DR^*}] = k_1 [D][R]
\]  

(5)

The activity of the drug would then essentially depend on the rate of formation of the drug-receptor complex \( (C_{DR}) \), events ‘downstream’ from this being relatively rapid. Furthermore, the view that the interior of the receptor is essentially hydrophobic leads to the mechanistic implications discussed below.

To the extent that an increase in the hydrophobicity of \( D \) leads to an increase in the stability of \( C_{DR} \), a corresponding increase in the value of \( k_1 \) may also be expected. This derives more formally from Hammond’s postulate, noting the proximity of \( C_{DR} \) to the preceding rate-
determining transition state TS$_1$. Therefore, this case corresponds to the left-hand side of the parabolic curve in Fig. 1.

**Case (ii): rate-determining breakdown of C$_{DR}^*$.** In this case, $k_{-1} \gg k_2$ and $k_2 \gg k_3$, represented by the free energy profile in Fig. 3. Thus, the further breakdown of C$_{DR}^*$ determines the overall kinetics, so both C$_{DR}$ and C$_{DR}^*$ are formed in relatively rapid pre-equilibria. Eqn. 4 then reduces to Eqn. 6.

\[
\log(1/C) \propto k_3[C_{DR}^*] = (k_1/k_{-1})(k_2/k_{-2})k_3[D][R]
\]  \hspace{1cm} (6)

Now, the equilibrium constants for the formation of C$_{DR}$ and C$_{DR}^*$, represented by $(k_1/k_{-1})$ and $(k_2/k_{-2})$ respectively, would expectedly be enhanced by an increase in the hydrophobicity of D. However, this would also lead to a decrease in the rate of breakdown of C$_{DR}^*$ ($k_3$). Essentially, this is because part of the hydrophobic binding energy enjoyed by C$_{DR}^*$ is lost in the transition state corresponding to its cleavage from the receptor.

Clearly, as $k_3$ represents the rate-determining step, an increase in the hydrophobicity of D would lead to a decrease in its activity. This case, therefore, corresponds to the right-hand side of the parabolic curve in Fig. 1.

It is noteworthy that current explanations invoke a decrease in water solubility, and hence bio-availability, for this part of the curve. However, although water solubility and hydrophobicity would indeed be inversely related, a causal relationship between these and bio-availability
cannot be assumed. This would be particularly true at low drug levels, \textit{i.e.}, below the solubility limit (corresponding to the upper part of the profile in Fig. 1).

In fact, the solubility based explanations ignore a possible ‘compensation effect’, by which more hydrophobic substrates would be more active at lower concentrations! In such a case, a profile resembling a plateau would be expected. However, the highly symmetrical profile in Fig. 1 indicates that drug activity is unlikely to be controlled by a multiplicity of effects. Also, the solubility ranges over which the study is performed would be different for different sets of drugs, and the solubility limits need not necessarily be breached in all cases.

Clearly, the role played by solubility in the activity of a drug would be complex and idiosyncratic. Therefore, alternative explanations are worth having in hand, until firm evidence either way emerges from the extensive studies that would be required.

**Case (iii): the transition region.** An interesting variation involves the case of \( k_3 \sim k_2 \sim k_1 \), \textit{i.e.} the three transition states (TS\(_1\), TS\(_2\) and TS\(_3\)) are equal in energy (Fig. 4). This case would correspond to a transition between cases (i) and (ii) above and thus represent the relatively flat part of the parabolic curve in Fig. 1. In this regime, an infinitesimal increase in the hydrophobicity of D would lead to a lowering of TS\(_1\) but raise TS\(_3\), thus cancelling out the effect overall.

![Gibbs free energy profile diagram](Fig. 4)  
**Fig. 4.** Gibbs free energy (G) profile diagram for case (iii) above, representing the flat part of the parabolic curve in Fig. 1 (\textit{cf.} Scheme 1 and Eqn. 4)
Generally, it is assumed that the interconversion between C\textsubscript{DR} and C\textsubscript{DR}\textsuperscript{*} is relatively rapid and less affected by the hydrophobicity of D \( (i.e. k_2 \sim k_{-2} \gg k_3 \text{ and } k_{-1}) \). In fact, it is necessary to posit the incursion of C\textsubscript{DR}\textsuperscript{*} to distinguish the simple breakdown of the drug-receptor complex from its breakdown with concomitant release of a physiological response.

**Two-stage release: an alternative model.** The above model is based on the simplifying assumption that the physiological response is issued concomitant to the release of the drug (Scheme 1). However, in the general case, one would have to consider the possibility that the two events are not necessarily linked, but occur sequentially.

Thus, the breakdown of the active form of the drug-receptor complex (C\textsubscript{DR}\textsuperscript{*}) could occur in two stages, with the physiological response being issued first along with the formation of a deactivated drug-receptor complex (C\textsubscript{DR}\textsuperscript{'}); this then breaks down to the free drug (D) and receptor (R), as shown in Scheme 2 below.

Furthermore, either the issue of the physiological response or the breakdown of C\textsubscript{DR}\textsuperscript{'} can be rate determining. In the former case \( (k_3 < k_4) \), the effectiveness of the drug would be proportional to the rate of issue of the physiological response, and thus the concentration of C\textsubscript{DR}\textsuperscript{*}. The consequences would then be similar to cases (i) – (iii) above. Thus, an increase in hydrophobicity could lead to either an increase or a decrease in activity, depending on the relative magnitudes of \( k_1, k_{-1}, k_2, k_{-2} \) and \( k_3 \), as discussed in detail above.

On the other hand, the possibility that the breakdown of C\textsubscript{DR}\textsuperscript{'} is rate determining \( (k_3 > k_4) \) would be analogous to case (ii) above, involving rate-determining breakdown of C\textsubscript{DR}\textsuperscript{*}. Interestingly, however, this would correspond to the right hand side of the parabola in Fig. 1 only if the concentration of the free receptor (R) is far lower than that of the drug (D). For only then would the breakdown of C\textsubscript{DR}\textsuperscript{'} determine the availability of the free receptor (noting there is no excess of R and the physiological response has been issued in the previous step).

\[
\begin{align*}
D + R & \xrightarrow{k_1} C_{\text{DR}} \xrightarrow{k_2} C_{\text{DR}}^* \xrightarrow{k_3} C_{\text{DR}}' \xrightarrow{k_4} (D + R) \\
& \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \ Quad
In other words, the parabolic relationship (Fig. 1) would then be observed at relatively high drug levels. That the relationship in Fig. 1 is often observed possibly indicates that studies are generally carried out with \([D] \gg [R]\). In the case of \([D] \ll [R]\), the effectiveness of the drug would depend on the concentration of \(C_{DR}^*\), as this would determine the strength of the physiological response. Hence, the effectiveness of the drug would generally increase with its hydrophobicity. Intermediate cases, \(i.e. [D] \sim [R]\), would represent a transition between these cases.

Interestingly, this concentration dependence would not apply to case (ii) above, as the issue of the physiological response itself is rate determining (as noted above). As this occurs concomitantly with the release of the drug, the overall effectiveness of the drug is inversely related to its hydrophobicity. In any case, these arguments are predicated on the validity of the assumptions on which the above model is based, so each case study would have to be considered on its own merits.

**Kinetic model of drug action.** Eqns. 4-6 essentially represent a kinetic model of drug action, with the stability of the drug-receptor complex being only obliquely relevant to the activity (cf. Hammond’s postulate mentioned above). In other words, the activity of a drug depends on its rate of binding to and subsequent release from the receptor.

Interestingly, the rate of uptake of a drug by the receptor, rather than the binding constant, may indeed be a better indicator of the efficacy of the drug. This is because drug uptake would be a dynamic process, as it would have to compete with excretion and possible metabolic deactivation of the drug. In fact, the total physiological response would be independent of the binding constant, so a purely equilibrium model may well be dubious. (In any case, rates often reflect corresponding equilibrium constants, so the rate of uptake of a drug also indicates its binding constant.)

Also noteworthy is the fact that the activity of the drug is represented by \(\log(1/C)\), which is itself proportional to \(\log[P]\) (Eqn. 3). Hence, it may be expected that the key rate constants in the above treatment (\(i.e. k_1 \text{ and } k_3\)) would then be similarly related to the hydrophobicity of \(D\) via \(\log[P]\). In fact, these rate constants are almost certainly composites of other rate constants, as the binding of the drug and its subsequent ejection would be complex multi-step processes. Thus, Scheme 1 and Eqn. 4 constitute a simplified representation comprising the essential features of the proposed mechanism.

Thus, described above is a certain model of drug action that is compatible with the time-tested Hansch equation (Eqn. 3 and Fig. 1). To the extent that the assumptions involved in the
derivation of the key equation (Eqn. 4) are valid, the arguments possibly constitute a mechanistic proof of Eqn. 3. However, it is perhaps best to view the empirically-derived Eqn. 3 and the mechanistic model represented by Eqn. 4 as being related in a complementary or ‘symbiotic’ sense. They are thus mutually reinforcing, with Eqn. 4 representing a possible conceptual basis for Eqn. 3, although only further work would indicate the accuracy of the proposed model.

The Hansch equation as LFER. The concluding discussion in the previous section also leads to certain interesting questions. Thus, Eqn. 3 relates \(-\log C\) to a variety of quantities on the right hand side, which raises the question of compatibility in terms of units. The partition coefficients \((P)\) and the Hammett \(\sigma\) are dimensionless quantities, so the constants \(a, b, c\) and \(k\) would then take on the units corresponding to \(-\log C\). Also, the partition coefficients \((P)\), being equilibrium constants, can be related to corresponding Gibbs free energy changes. Along with the presence of the Hammett \(\sigma\), this implies that the right hand side of Eqn. 3 is composed of Gibbs free energy terms (with the exception of \(k\)).

\[
\mu_C = \mu_o + RT \ln C \quad (7)
\]
\[
-\log C = (\mu_o - \mu_C)/2.303RT \quad (8)
\]

Furthermore, \(\log C\) can be expressed in terms of chemical potentials via Eqns. 7 and 8, wherein \(\mu_C\) is the chemical potential of the drug at concentration \(C\) and \(\mu_o\) its standard potential. This implies that Eqn. 3 is overall a linear free energy relationship in itself (as previously noted).

Possible spatial consequences of the kinetic model. The kinetic model of drug action, proposed above as a possible basis for the Hansch equation, apparently accords with an intriguing phenomenon that was observed in the early years of QSAR. Thus, it has been known for long that, in certain cases, biological activity may correlate with hydrophobicity with a remarkable disregard for molecular size and shape. In fact, the successful application of the Hansch equation is based on an independent correlation between hydrophobicity and molecular structure. Thus, structural units with estimated contributions to the overall hydrophobicity lead to possible molecular structures for synthesis and testing. This exercise implies a tenuous relationship of the overall structure with hydrophobicity (a variety of structures can lead to the same \(\log P\) value), and hence with activity.

The kinetic model proposed above indicates that drug action is related to the rate of binding of the drug to the receptor. Interestingly, this could possibly imply that the effectiveness of a drug is related to the complex process involving the unfolding of the macromolecular receptor, followed
by the entry of the drug molecule into the receptor interior and the refolding of the receptor around the drug molecule.

This overall process leading to the formation of the drug-receptor complex is presumably initiated by the drug molecule itself, which thus acts as a catalyst for the initial unfolding of the receptor. It now appears that this is possibly the key to the overall effectiveness of the drug. It is noteworthy that the catalysis of the unfolding of the receptor likely requires the binding of the drug molecule initially on the receptor surface. This is likely less geometrically demanding in terms of molecular structure than the formation of the drug-receptor complex itself, and is perhaps related to the hydrophobicity of the drug molecule more than its overall shape.

It is also noteworthy that rigid lock-and-key ideas have gradually given way to the induced-fit theory, involving a relatively flexible macromolecular receptor that wraps itself around the drug molecule. This implies that molecular shape and size may not play a dominant role in the formation of the drug-receptor complex, as generally assumed. Indeed, the importance of receptor flexibility is being increasingly recognized in modern drug design strategies.\textsuperscript{11-13}

These arguments, apparently, are limited to those cases where hydrophobicity plays a major role in the action of a drug. However, even in cases wherein hydrophobicity plays a minor role, it is unclear to what extent molecular shape per se determines effectiveness. As a whole host of properties can be related to molecular structure, it is a daunting challenge to disentangle pure geometrical effects possibly related to the formation of the drug-receptor complex. Further work would possibly illumine this essential problem, but in the meanwhile the above kinetic model – which would apply at least to select cases – is worthy of consideration.

CONCLUSIONS

An exploration into the origins of the Hansch equation leads to a possible kinetic basis of drug action. Although the observed validity of the Hansch equation has been established beyond doubt, its phenomenal origins remain to be clarified. The kinetic model proposed herein is apparently a viable alternative to current explanations. In this model, the rate of incorporation of the drug into the receptor is a function of the hydrophobicity of the drug up to moderate values of the hydrophobicity parameter. However, at high values of the hydrophobicity parameter the release of the drug from the receptor is slowed down, along with the physiological response, leading to a decrease in effectiveness.

Also, the fact that the Hansch equation is a linear free energy relationship in itself, with a dominant contribution from the hydrophobicity parameter, indicates that drug activity largely correlates with electronic effects. This implies that steric and geometric criteria generally play a
secondary role in such regimes. This may be interpreted in terms of the binding of the drug to the receptor surface, which initiates the entry of the drug to the interior of the receptor, as determining drug activity. This apparently accords with current views of drug-receptor interactions as involving relatively flexible receptor macromolecules.

**APPENDIX**

*Derivation of Eqn. 4 (cf. Scheme 1).* By the steady state approximation the rate of formation of C_{DR} is equal to the rate of its collapse, leading to Eqs. A1 and A2:

\[
k_1[D][R] + k_2[C_{DR}^*] = k_3[C_{DR}] + k_3[C_{DR}^*] \quad (A1)
\]

\[
[C_{DR}] = \frac{(k_1[D][R] + k_2[C_{DR}^*])}{(k_1 + k_2)} \quad (A2)
\]

Analogous treatment of C_{DR}^* leads to Eqn. A3:

\[
k_2[C_{DR}] = k_2[C_{DR}^*] + k_3[C_{DR}^*] = (k_2 + k_3)[C_{DR}^*] \quad (A3)
\]

Combining Eqs. A2 and A3 leads to Eqn. A4, thence to Eqns. A5-A7:

\[
k_3(k_1[D][R] + k_2[C_{DR}^*])/(k_1 + k_2) = (k_2 + k_3)[C_{DR}^*] \quad (A4)
\]

\[
k_2k_1[D][R] + k_2k_2[C_{DR}^*] = (k_2 + k_3)(k_1 + k_2)[C_{DR}^*] \quad (A5)
\]

\[
k_1k_2[D][R] = (k_2k_3 + k_1(k_3 + k_2))[C_{DR}^*] \quad (A6)
\]

\[
[C_{DR}^*] = k_1k_2[D][R]/((k_2k_3 + k_1(k_3 + k_2)) \quad (A7)
\]

As per the key assumption, the activity of the drug in terms of log(1/C) is proportional to k_3[C_{DR}^*]. Hence, by Eqn. A7:

\[
\log(1/C) \propto k_3[C_{DR}^*] = \frac{(k_1k_2k_3[D][R])}{(k_2k_3 + k_1(k_2 + k_3))} \quad (4)
\]

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