PERSPECTIVE

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Quantifying Biological Activity in Chemical Terms: A Pharmacology Primer To Describe Drug Effect

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ABSTRACT Drugs can initiate, inhibit, modulate, or potentiate basal activity in cells to produce physiological effects. The interplay between the fundamental affinity and efficacy of drugs with the functional texture imposed on the receptor by the cell (*e.g.*, variation in basal set points or cytosolic signal proteins) generates behaviors for drugs in different tissues that can cause apparently capricious variation between tissues under various physiological conditions. This poses a problem for pharmacologists studying drugs in test systems to predict effects in therapeutic ones. De-emphasis of tissue-specific drug behaviors by reducing drug effects to chemical terms can, to a large extent, reduce the effects of variances in biological systems (changing basal set points, genetic and biochemical variability, *etc.*). This Perspective discusses the application of four major pharmacodynamic parameters (affinity, efficacy, orthosteric *vs* allosteric binding, and rate of dissociation of drug from the biological target) to the quantification of biological activity to furnish chemical structure–activity relationships (SARs). These four parameters can be used to quantify effects in test systems and predict subsequent activity in a therapeutic setting. Because at least three different SARs are involved in the drug discovery process (primary therapeutic activity, pharmacokinetics, and safety), with more possible if target selectivity is required, some simple statistical approaches to multivariate structure–activity studies (*i.e.*, primary activity plus selectivity data) also are considered. In total, these data can provide system-independent data to characterize biological activity of molecules in chemical terms that can greatly reduce biologically induced variability.

Quantitative description of drug effect is essential in pharmacology because it forms the basis of drug structure–activity relationships (SARs). At the heart of this approach are stable scales that can quantify change that are not subject to biological variation. As an example, chemical scales such as melting points and NMR coupling constants fit this description because they do not vary with experimental conditions. However, in the process of describing chemical effects on living biological systems, pharmacologists must contend with cellular expressions of drug activity that change because of a variety of system-dependent factors (cellular metabolism, species, genetic variability, *etc.*), and this leads to reliance on less absolute scales of activity. On a superficial level, drug effects can be described by overt observation of effects on specific tissues (full agonism, partial agonism, *etc.*), but such a system soon falls into chaos because the same drug produces different behaviors in different tissues. With techniques based on null methods (whereby equal cellular effects are compared, thereby canceling differences in cellular density of receptors and efficiencies in receptor coupling) and measurement of system-independent scales of activity, it is possible for pharmacologists to reduce apparently variable effect to consistent parameters rooted in chemistry. Therefore, to avoid reliance on variable drug behavior, drug receptor theory has produced a theoretical framework to describe biological effect with a minimum of key parameters. Theoretically, these parameters can be employed to predict drug behavior in all tissues. This is imperative in the drug discovery and development process because nearly all drugs are tested and optimized in test systems far removed from the therapeutic one. Under

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these circumstances, activity scales must be able to predict the activity of molecules in *all* biological systems irrespective of what system generates the original SAR.

A therapeutic need usually defines the primary activity required in a molecule for drug therapy. However, in addition, a successful drug must be absorbed and able to reach the primary biological target, remain there for a time adequate to produce therapeutic effects (have adequate pharmacokinetics), and be tolerable to the host (do no harm). The rules governing the effects of chemical structure on these various effects (SAR) need not be, and often are not, the same for each of these criteria. This poses complications for chemical optimization of overall drug activity because, in addition to primary activity (pharmacodynamics), attention must be paid to favorable pharmacokinetics (where the drug goes in the body and how long it stays in the body) and lack of toxicity; in total this spectrum of activities is referred to as a druglike profile. This Perspective will concentrate on defining the pharmacodynamic properties of a prospective drug and the definition of its primary activity in relation to other activities (selectivity).

KEYWORDS

pIC_{50}: Logarithm of the molar concentration of antagonist producing 50% inhibition of a defined pharmacological process. This can be used to quantify antagonist potency, although it has system-dependence that is not operable for pK_B estimates. There are pharmacological procedures to convert pIC_{50} values into pK_B values, thus canceling the experimental influences on the measurement.

pK_B: Logarithm of the equilibrium dissociation constant of an ant antagonist–receptor complex. This parameter quantifies the potency of the antagonist.

Affinity: A measure of the forces that cause a molecule to bind and stay bound to a receptor, inversely proportional to the equilibrium dissociation constant of the ligand–receptor complex (defined as k_2/k_1; k_2 = rate of dissociation of the molecule from the receptor surface in s^{-1}, k_1 = rate of association of molecule to receptor in s^{-1} mol^{-1}).

Agonist: A molecule possessing efficacy such that the behavior of the receptor toward its host cell is altered upon binding.

Allosteric: Binding of molecules to separate sites on the receptor to induce an interaction between them caused by a change in the protein conformation of the receptor.

Antagonist: A molecule that binds to the receptor to prevent the activation of that receptor by an agonist.

Efficacy: The ability of a molecule to cause the receptor to change its behavior toward its host cell.

Full agonist: An agonist that produces the maximal response that matches the maximal capability of the assay to return response.

Chemistry and Pharmacodynamics. Drugs have intrinsic properties that relate to their chemical structure and cause them to be active in biological systems. When this occurs, the systems take on various behaviors in response to these drugs. Much of the dissimulation in the drug discovery process is the use of behavioral scales for drugs as intrinsic scales of classification, that is, a given drug can exhibit a range of different behaviors in various cell types. For example, the β-adrenoceptor ligand prenalterol can block the effects of β-adrenoceptor stimulation in some tissues (be an antagonist in canine coronary artery), produce partial β-adrenoceptor stimulation in others (be a partial agonist in guinea pig left atria), and produce full β-adrenoceptor stimulation in yet others (be a full agonist in guinea pig right atria) (*1, 2*). Therefore, labeling this molecule with observed behaviors (antagonist, partial or full agonist) is confusing because the tissue type would then have to be designated, and specific information about the systems would need to be known to fully describe the activity. This type of apparently conflicting nomenclature for drug activity can lead to confusion in the lead optimization process unless the root molecular properties causing those behaviors are assessed and quantified. Thus, biologists need to characterize what their molecules *are*, not just what they *do*, in particular systems. In the case of prenalterol, this is done by simply referring to the molecule as a high-affinity, low-efficacy ligand for β-adrenoceptors.

The following scales can quantify biological activity in a system-independent manner:

Affinity. This is a chemical term that quantifies the ability of a molecule to bind to a biological target. It is unique for the molecule and target and transcends differences in cell type.

Efficacy. This is a uniquely pharmacological term describing the change in behavior of the biological target toward its host (cell) upon binding of the molecule. Like affinity, it can be quantified as a target-specific but system-independent ratio.

Orthosteric vs Allosteric Interaction. When two molecules interact at a biological target (*i.e.*, antagonism), either they can compete for a common binding site on the target (orthosteric interaction) or they each bind to the target with their own specific binding site, such that the interaction between the two molecules occurs through a change in the conformation of the protein (allosteric interaction). It is important to differentiate these mechanisms as orthosteric and allosteric ligands have different ranges of behavior in biological systems.

Kinetics of Offset. Whereas potency determines the relationship between drug concentration and target occupancy, target coverage (sustained association of the drug with the target *in vivo*) is what is important therapeutically. This involves how quickly the ligand washes off of the target in an open system (whole-body pharmacokinetics). Thus, k_2 (the rate of dissociation of a mol-

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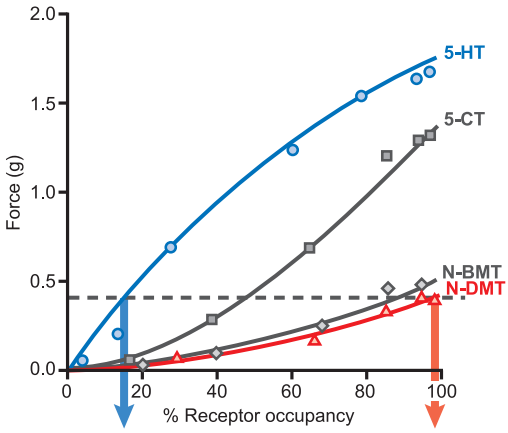


Figure 1. Agonist efficacy. Occupancy response curves (rat ileum contraction to serotonin agonists 5-HT (●), 5-cyanotryptamine (■), *N*,*N*-dimethyltryptamine (▲), and *N*-benzyl-5-methoxytryptamine (◆), expressed as the percent of the total number of receptors the agonists occupy. Abscissa: percent receptor occupancy by the agonist as calculated by mass action and the equilibrium dissociation constant of the agonist–receptor complex. Ordinate: force of contraction in grams. Data drawn from ref 4.

ecule from its target) should be quantified to fully characterize its therapeutic potential.

It is worth exploring the various tools in the biologist's armamentarium available to provide these data to chemists in the lead optimization process.

Affinity. Decades of technological advancement basically have not affected the model used to quantify biological affinity, namely, the Langmuir adsorption isotherm. Devised by the chemist Irving Langmuir to quantify adsorption of molecules onto metal surfaces, this model defines the concentration of ligand that binds to 50% of the available binding sites (see Box). Pharmacological assays can be used to measure affinity of antagonists (ligands that do not produce excitation of cells but rather occupy receptors to prevent activation by agonists) to yield pK_B estimates (−log equilibrium dissociation constants of antagonist–receptor complexes) that are unique for antagonist−receptor pairs in all tissue types. This enables quantification of antagonist potency in test tissues that will be predictive of antagonist affinity in therapeutic systems. Although there are procedures that quantify the observed affinity of agonists, the fact that these molecules abstract the receptor to an active form (*vide infra*) can affect this estimate of affinity, making it tissue-dependent. For this reason, agonist affinity is better quantified through the operational model to be discussed later.

Efficacy. A second fundamental property of all drugs is efficacy. It is a term introduced into pharmacology by Stephenson (*3*) as a scale of the direct activity of drugs that produces a physiological effect (such drugs are called agonists). Although no absolute value with units can be measured that has meaning between different organ systems, the *relative* efficacy of two agonists can be defined in any one given system. This relative value defines the "power" a given agonist has to induce response. For example, Figure 1 shows the contraction of rat ileum in response to various serotonin receptor agonists expressed as a percentage of the receptor occupancy induced (calculated with the adsorption isotherm utilizing the concentration of the agonist and the equilibrium-dissociation constant of the agonist–receptor complex). It can be seen that whereas the agonist *N*-benzyl-5-methoxytryptamine (N-BMT) requires nearly 90% of the receptors to induce a response of 0.4 g tension, the same response can be produced by 5-hydroxytryptamine (5-HT) through occupation of only 17% of the receptor population. Thus, there must be something intrinsically different about the chemical structure of 5-HT compared with that of N-BMT to make it a more powerful agonist; efficacy was introduced into pharmacology to account for such differences.

Pharmacological tools enable the quantification of cellular activation data; unlike enzymology or other protein-

KEYWORDS

Null effects: These yield system-independent measures of agonist activity by comparison of drug potencies at concentrations that produce equal effect. It is assumed that if two agonists are tested in a given tissue, then the tissue factors controlling the process of cellular response production from receptor stimulation will be the same for both agonists. This, in turn, allows cancellation of these cellular effects (namely, receptor density and efficiency of receptor coupling) to cause the relative potency of the agonists to be due only to the molecule-dependent activity of the agonists. Therefore, the relative potency becomes a function only of the drug-dependent properties of affinity and efficacy.

Operational model: A theoretical framework to describe agonism in pharmacological systems based on receptor occupancy and a Michaelis–Menten coupling of the receptor to cellular response producing machinery.

Orthosteric: Binding of molecules to a common site such that they compete for occupancy on the receptor.

Partial agonist: A molecule that produces a maximal response that is below what the assay can return as a system maximal response.

Pharmacodynamics: The study of drug interaction with biological targets, that is, receptors.

Pharmacokinetics: The study of drug movement in the body (absorption, distribution, metabolism, and excretion of drugs *in vivo*).

Rate of dissociation: Rate of diffusion of the molecule away from the receptor once bound (in s^{-1}). This is a measure of the persistence of the ligand occupancy on the receptor and propensity to wash off the receptor once the concentration in the compartment is zero.

Stimulus–response coupling: The various biochemical pathways that link the cell surface receptor to cell metabolism to provide an observable response to receptor activation.

based biochemical techniques, a 50% maximal cellular response can occur at concentrations of agonist much lower than those required to bind to 50% of receptors in a cell membrane. This is because of the agonist-specific property of efficacy (power to induce response) and the fact that cells may contain a large number of receptors and those may be extremely efficiently coupled to cellular biochemical mechanisms that amplify activation (referred to as stimulus-response coupling reactions) (Figure 2). This leads to a phenomenon referred to as receptor reserve, which produces a discontinuity between drug occupancy and tissue response.

The operational model of drug action formulated by Black and Leff (*5*) is the major tool for the quantitative comparison of cellular drug effect. It defines agonist [A] response as

$$\text{response} = \frac{[A]\cdot\tau\cdot E_{max}}{[A](\tau + 1) + K_A} \qquad (5)$$

where K_A is the previously defined equilibrium dissociation constant of the agonist–receptor complex (inverse of affinity) and τ is a transduction function describing both the sensitivity of the organ to stimulation and the efficacy of the agonist causing the stimulation. If the τ values for two agonists are determined in a given tissue, then the tissue-specific aspects of τ cancel and the ratio of those τ values can be considered a ratio of the efficacy of the two agonists. This can confer a type of prescience to the test systems used to quantify drug effects, because the ratios of τ can be used to predict the relative response to the two agonists in *any* tissue system, *including the therapeutic one*, if the response to one of the agonists is known in the therapeutic system (*e.g.*, a standard therapy targeted for improvement). Under these circumstances, the effect of new agonists in that same therapeutic system can be predicted with the operational model (*6*).

Table 1 illustrates the power of this model. Equation 5 was used to fit concentration–response curves to isoproterenol and prenalterol in thyroxine-treated guinea pig right atria with K_A isoproterenol = 220 nM, K_A prenalterol 50 nM, and τ for isoproterenol = 1000 (data from ref *7*). These parameters gave good agreement between the experimental and fit curves for isoproterenol; a τ value of 4 (ratio of τ for prenalterol to τ for isoproterenol = 0.004) allowed the model to fit the experimental curve for prenalterol. This data set then set the ratio of efficacies (τ values) for isoproterenol and prenalterol for this receptor; these should remain constant for *every* tissue in which both of these agonists is tested. To fit the different experimental concentration–response curves for isoproterenol in the various tissues, different τ values for isoproterenol were assigned (this reflects differences in the tissue-dependent aspects of τ, namely, the receptor density and efficiency of receptor coupling). Thus, the data were fit with τ values for isoproterenol for the different tissues of 450, 350, 140, 50, and 14 (see Table 1). The importance of the operational model stems from the fact that the model then

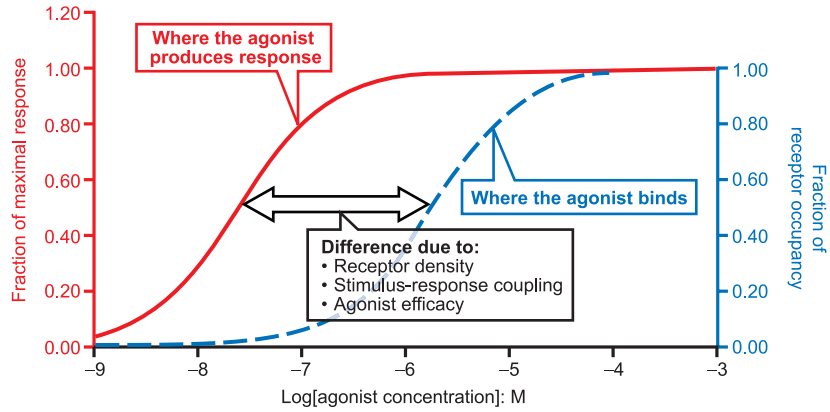


Figure 2. Impact of efficacy on potency of agonists. Blue broken line represents the binding of the agonist to the receptors, and the red line represents the actual potency of the agonist as it produces pharmacological response. The difference is the ability of the molecule to induce physiological response as it binds to receptors referred to as efficacy.

TABLE 1. Prediction of prenalterol responses in different tissues using operational theory[a]

	Isoproterenol		Prenalterol					
	Exptl pEC_{50}[b]	Predicted pEC_{50}[b]	Exptl max response[c]	Predicted max response[d]	Exptl pEC_{50}[b]	Predicted pEC_{50}[b]	τ_{iso}	τ_{pren}
GP R atria[e]	9.66	9.6	0.85 ± 0.05	0.8	7.9	8.0	1000	4
Cat L atria	9.34	9.3	0.62 ± 0.09	0.64	7.83	7.75	450	1.8
Rat L atria	9.2	9.2	0.58 ± 0.08	0.58	7.75	7.7	350	1.4
Feline papillary	8.8	8.8	0.34 ± 0.13	0.36	7.69	7.62	140	0.56
GP L atria	8.4	8.37	0.16 ± 0.06	0.17	7.55	7.4	50	0.2
Canine coronary artery	7.9	7.8	0	0.05	na[f]	7.5	14	0.056

[a]Columns on the extreme right indicate τ values for isoproterenol and prenalterol in each preparation. Of note is the fact that the ratio of these τ values is constant (*i.e.*, $\tau_{pren}/\tau_{iso} = 0.004$). This fact allows prediction of the dose–response curve to prenalterol in any tissue where the dose–response curve to isoproterenol is defined. Data from ref *7*. [b]Negative logarithm of EC_{50} (concentration producing 50% compound maximal response). [c]Experimentally determined response to isoproterenol considered maximal. [d]Experimentally determined maximal response as fraction of isoproterenol maximal response. [e]Thyroxine-treated. [f]No measurable response.

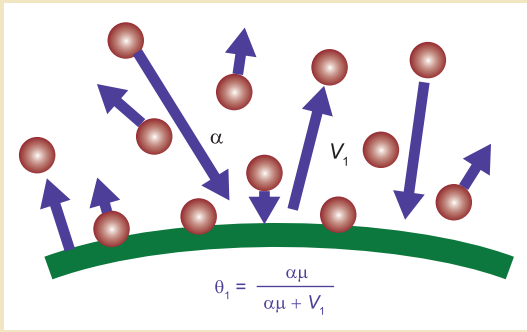
predicts that the concentration–response curves for prenalterol in those same tissues should be accurately fit with those same τ values multiplied by the ratio 0.004. As can be seen in Table 1, the ratio of 0.004 provided an excellent fit for the experimental curves for prenalterol in all tissues. Thus, in an unknown system, if the curve to isoproterenol were to be determined, then fitting the data with $K_A = 220$ nM (this is a molecular constant) and a τ value from a computer fit for isoproterenol would automatically allow prediction of the curve to prenalterol using a value for τ of 0.004 times the one for isoproterenol and a K_A value of 50 nM. The measurement of agonist relative efficacy can be very important because it can predict when agonists will not produce therapeutic agonism in tissues of low sensitivity. An example of this type of prediction is shown in Box 2 (*8*).

An added advantage of quantifying agonist efficacy and affinity separately is that the SARs for these two molecular properties can be quite different. Thus, instead of simply conveying changes in potency to chemists as biological data for structure–activity characterization, biologists can employ specific tools to separately assess affinity and efficacy as two system-independent scales. This approach has yielded high therapeutic gain as in the selective diminution of efficacy and increase in affinity of ligands for histamine receptors through chemical analogs to produce the clinically valuable H_2 histamine receptor antagonists for treatment of ulcers (*9*).

As more sophisticated pharmacologic assays become available to detect the effects of molecules on receptors, it is evident that there are numerous "efficacies" that can be described. For example, catecholamines have efficacy for β-adrenoceptors that leads to activation of $G_{\alpha s}$ proteins to increase intracellular cyclic AMP; the β-blocker propranolol binds to the same receptor but does not produce the same activation (*i.e.*, it has no "efficacy" to elevate cyclic AMP) (*10*). However, a different type of pharmacological assay shows that propranolol activates extracellular signal regulated kinases through β-adrenoceptor binding, so from this standpoint propranolol has efficacy for this physiological action (*11*). These type of data illustrate how "efficacy" is defined by the assay utilized to detect it (*12*) and that ligands can have a number of different efficacies while activating a given receptor (referred to as pleuridimensional efficacy (*13*)). Despite the assay-dependent nature of efficacy, once a therapeutically defined efficacy is identified, the operational model has the capability of quantifying the capability of a given molecule to produce the defined physiological response in a system-independent manner. This, in turn, allows the prediction of therapeutic efficacy from experiments done in test systems.

Orthosteric *vs* Allosteric Molecular Interactions. A third important property of drugs that is extremely valuable as a molecular descriptor is whether a molecule interacts orthosterically or allosterically with a biological

Box 1. The Langmuir adsorption isotherm.



Irving Langmuir, a chemist working for General Electric, devised the adsorption isotherm to quantify the adsorption of chemicals into metal surfaces for the production of light bulb filaments. The derivation, published in 1918, defines an "intrinsic rate of condensation" toward the surface for a molecule (denoted α) and a rate of "evaporation" of the molecule away from the surface (denoted V_1). The rate of offset of diffusion away from the surface is given by

$$dV_- = V_1\theta_1 \quad (1)$$

where θ_1 is the fractional surface area already bound by the molecule. The rate of diffusion toward the surface is given by

$$dV_+ = \alpha\mu(1 - \theta_1) \quad (2)$$

where the concentration of the molecule is μ and the fraction of the remaining surface area remaining to be bound is $1 - \theta_1$. At equilibrium, these rates are equated as eq 1 = eq 2 to yield the following relation (denoted in Langmuir's original nomenclature):

$$\theta_1 = \frac{\alpha\mu}{\alpha\mu + V_1} \quad (3)$$

The fraction of protein bound by a ligand A is rewritten in biological terms with the equilibrium dissociation constant of the protein–ligand complex (denoted K_A and defined as $k_2/k_1 = V_1/\alpha$):

$$\rho_A = \frac{[A]}{[A] + K_A} \quad (4)$$

Langmuir received the Nobel Prize for his work in surface chemistry in 1932; his isotherm still forms the basis of all models of biological affinity today.

target. Molecules can either compete for the binding site of endogenous hormones or neurotransmitters (thereby precluding their action through steric hindrance; referred to as orthosteric interaction) or bind to their own site on the target protein to modify the effects of hormones and neurotransmitters (through a change in the conformation of the protein; referred to as allosteric interaction) (Figure 3). These two types of molecular interactions lead to completely different pharmacological behaviors (*14*). For instance, in orthosteric systems there never is a protein species with both agonist and antagonist binding simultaneously. Thus, such systems are pre-emptive in that high concentrations of antagonist will completely negate agonist effects and make the receptor inoperative. Another property of such systems is that the antagonism will be identical for all agonists interacting with the receptor.

In contrast, allosteric effects are permissive in that the pharmacologically unique protein species is the receptor bound to both the agonist and the allosteric modulator. Three characteristic effects of allosteric modulators result in pharmacological behavior different than that of orthosteric systems. First, allosteric effects are saturable. This occurs because allosteric modulators bind to their own site on the receptor to induce their effect, and an asymptotic maximum of effect occurs when the allosteric site is fully bound. If the allosteric effect is a reduction in agonist affinity, then the effect need not be complete. Thus, while high concentrations of orthosteric antagonist necessarily eliminate agonist response, a saturating concentration of allosteric modulator may only partially reduce sensitivity of the receptor to the agonist, allowing partial function. For example, the allosteric modulator UCB35625 produces only a maximal 20% reduction in the binding of the radioactive chemokine ^{125}I-CCL4 to the CCR1 chemokine receptor (*15*). Second, allosteric modulators can demonstrate probe-dependence. For example, the allosteric modulator alcuronium reduces the affinity of muscarinic radiolabeled antagonist [^{3}H]-methyl-QNB but increases the affinity to the radioactive antagonist [^{3}H]-atropine (*16*). This can offer distinct therapeutic advantages over orthosteric ligands. For example, a study of 1064 HIV-1 infected individuals indicates that an active chemokine system operating through CCL3L1 activation of the CCR5 receptor is protective and delays progression to AIDS (*17*). Allosteric inhibition of CCR5-mediated HIV-1 infection shows nearly a 500-fold variation in the relative activity in blocking HIV-1 entry and beneficial CCL3L1-mediated effects (*18*); such differential activity would not be possible with orthosteric antagonism of HIV-1 entry. Such probe-selectivity opens

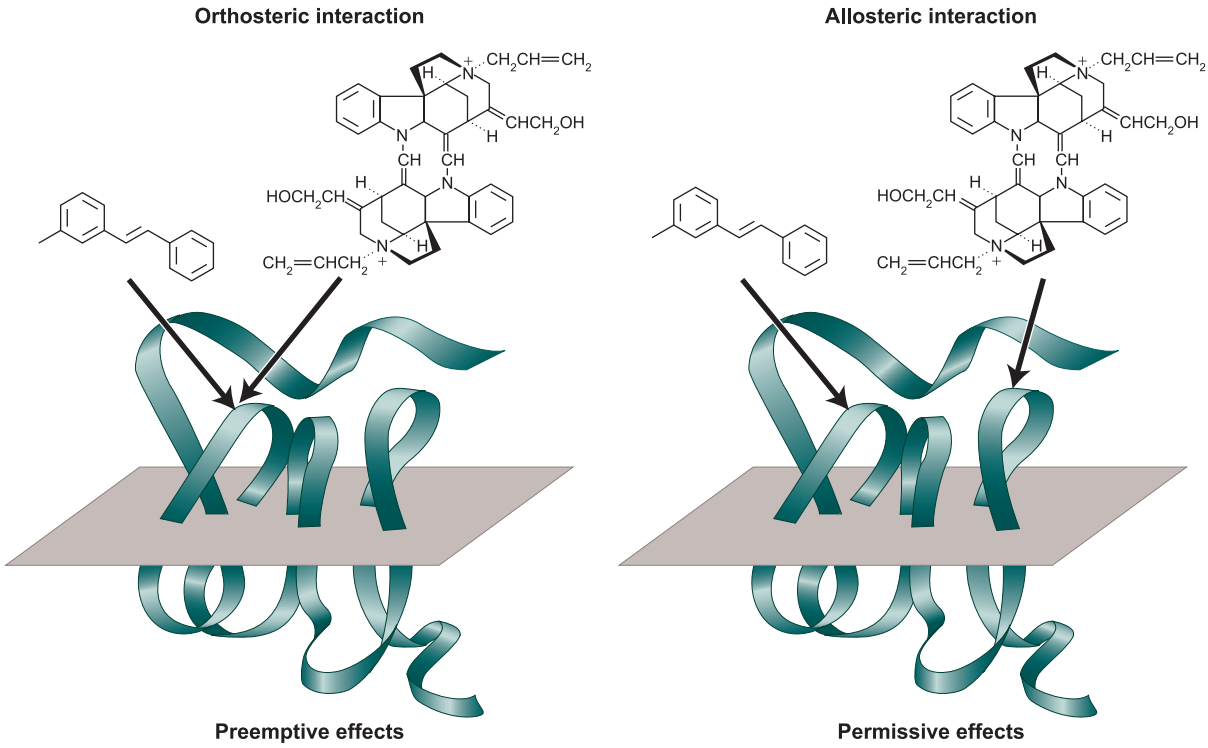


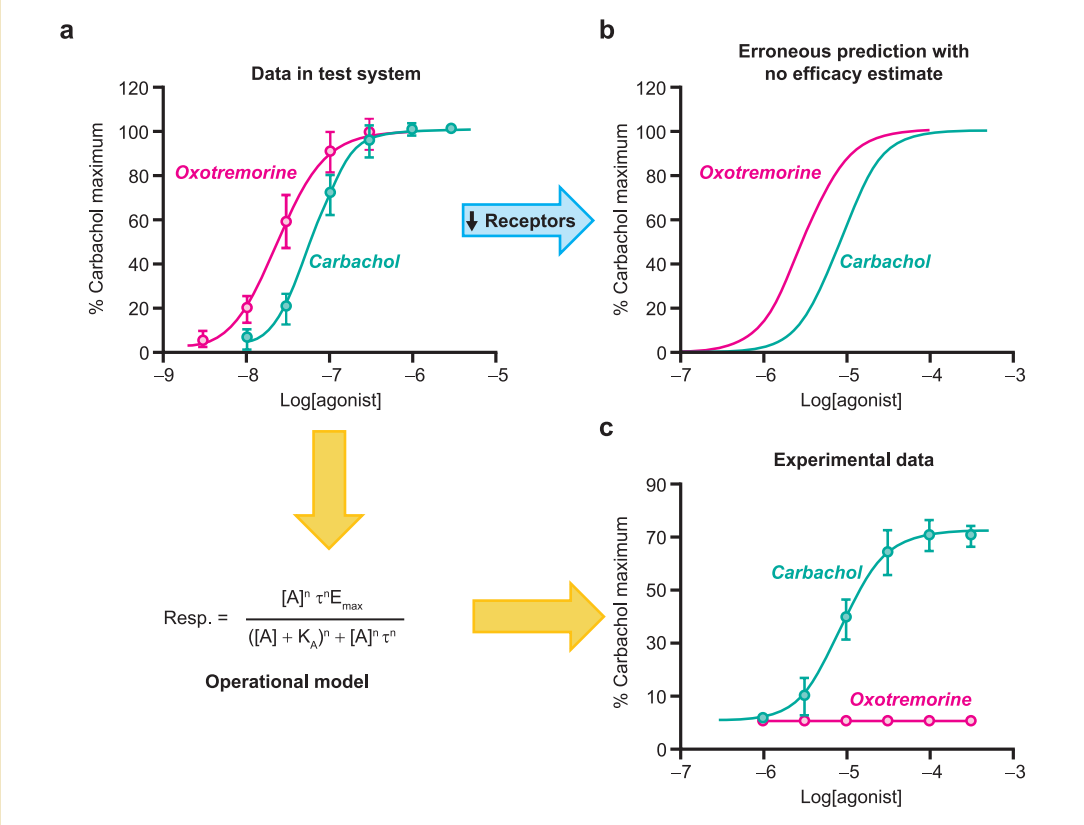
Figure 3. Two fundamentally different ways in which molecules can interact with receptors. In orthosteric systems, there is never a protein species with both molecules residing simultaneously, thus the only effects can be from one or the other drug. In an allosteric permissive system, there are proteins with both drugs binding simultaneously, and thus one drug can modify the effects of the other. Allosteric systems are considerably more versatile in controlling physiological response.

the possibility of producing "function-sparing" receptor blockers that block the pathogenic but not normal physiologic function of receptors (*18*). Finally, allosteric modulators can have separable effects of agonist affinity and efficacy. For example, the allosteric CCR5 HIV-1 entry inhibitor aplaviroc minimally affects the binding of radioactive ^{125}I-CCL5 to the receptor but completely negates CCL5 efficacy in producing calcium response (*19*).

Allosterically mediated changes in receptor conformation can lead to texture in receptor antagonism, and this can have favorable therapeutic consequences. In cases of tolerance to HIV-1 entry inhibition (where the virus may mutate to utilize the allosterically altered receptor for viral entry), therapy with a different allosteric modulator may overcome the viral resistance by inducing a new conformation (*19*). In cases where partial sensitivity of a biological receptor target is required, allosteric modulators have the capability of reducing but not eliminating agonist response. The separate effect on agonist affinity and efficacy allows for the possibility of autoadjustment of antagonist potency in response to the level of physiological tone. For example, the potency of the NMDA receptor allosteric antagonist ifenprodil actually increases with increased NMDA agonism because ifenprodil increases the affinity but decreases the efficacy of NMDA on the receptor (*20*). Finally, because allosteric modulators bind to auxiliary sites on the receptor protein removed from the agonist binding site, the potential for receptor subtype selectivity is greater than for orthosteric antagonists, which bind to the conserved natural agonist binding site (*21*).

The Pharmacokinetic-Pharmacodynamic Interface: Impact of Kinetics. A major dissimulation in drug discovery is caused by the fact that drug activity is quantified in test systems *in vitro* under equilibrium conditions and then used therapeutically under transient kinetic

Box 2. Predicting agonism: the power of prescience through operational analysis.



Panel a shows the relative responses to two muscarinic receptor agonists, carbachol and oxotremorine; it can be seen that oxotremorine has twice the potency of carbachol in this test system, namely, guinea pig ileum. A naïve prediction of the relative effects of these agonists in a less sensitive system is shown to the right of this panel. According to this prediction, in another tissue that is 300 times less sensitive and where the potency of carbachol (as quantified by the concentration producing 50% maximal response, EC_{50}) is 10 μM, it might be supposed that oxotremorine would follow suit with a 300-fold decrease in potency and have a potency of 3 μM. The true pattern of responses is shown in panel c, where it can be seen that the dose–response curve to carbachol is indeed shifted 300-fold to the right but the curve to oxotremorine disappears completely.

The apparently unexpected pattern is in fact predicted completely through quantification of the relative efficacies and affinities of these agonists with the operational model. Specifically, it was found that oxotremorine has a 100-fold greater affinity but 1/40 of the efficacy of carbachol. Under these circumstances, the loss of response to an agonist that gains its primary potency through high affinity (*vs* high efficacy) is expected, and the absence of agonism can be predicted in all low sensitivity systems. The quantification of the relative affinities and efficacies of oxotremorine and carbachol in the test system with the operational model enables prediction of the relative effects of both agonists in all systems.

nonequilibrium conditions (*22*). Thus while *in vitro* drug assays are conducted in assay wells where the concentration at the drug target is known, *in vivo* the therapeutic target responds to a moving stream of drug, that is, as the drug is absorbed it immediately begins to leave the body either through hepatic clearance or direct renal excretion. An example of the kinetic profiles of drug concentration *in vitro* and *in vivo* is shown in Figure 4. Therefore, it is important to gauge some measure of the way drugs will perform under these transient condi-

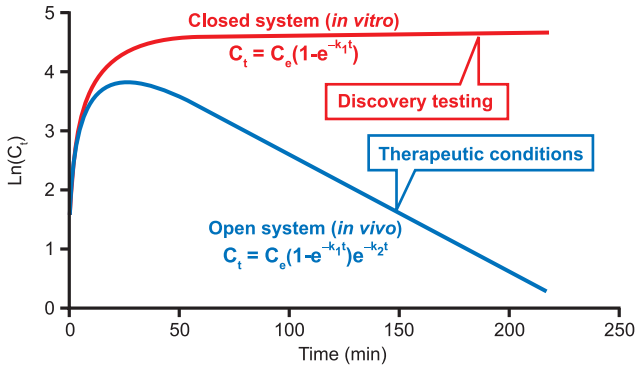


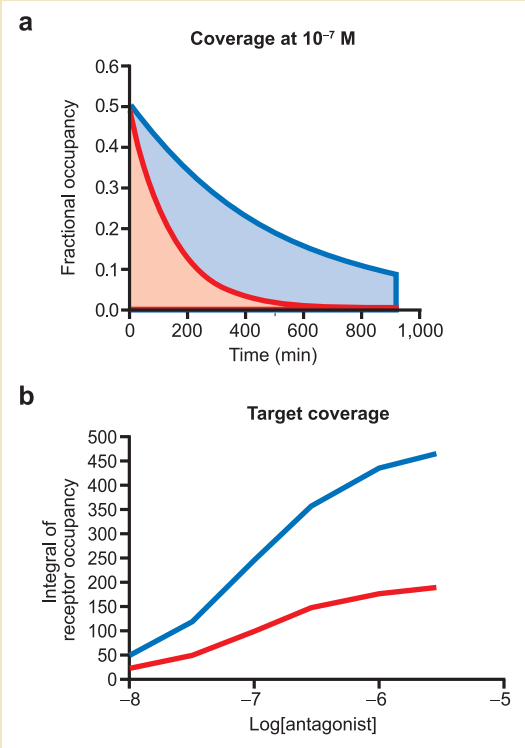
Figure 4. Time course for drug concentration in a closed system (*i.e.*, filling a well in an assay plate for an *in vitro* assay) and an open system (*in vivo*) likened to filling a cup with fluid that has a hole in it. *In vivo*, the drug is eliminated by hepatic or renal clearance immediately upon entry into the bloodstream (central compartment).

tions; this is done through quantifying the rate of dissociation of the drug from the receptor. The rate at which a drug washes off the target is the determinant of therapeutic utility and can compensate for problems in pharmacokinetics. For example, if a receptor antagonist has a very slow rate of receptor dissociation, then a rapid clearance may not necessarily preclude therapeutic use because a short exposure to the drug may load the receptors, and occupancy will last beyond pharmacokinetic presence in the central compartment. The target coverage produced by a fast and slow offset drug is contrasted in Box 3.

The characterization of antagonist value through measurement of potency in closed systems falls short of adequately describing antagonist behavior because it will not predict what will happen in an open *in vivo* system. For example, the equilibrium dissociation constant (denoted K_B) of an antagonist is the ratio of the rate of dissociation from the receptor (k_2) divided by the rate of association to the receptor, k_1: $K_B = k_2/k_1$. Therefore, one antagonist (designated antagonist A) may have a $K_B = 50$ nM from $k_2/k_1 = 0.005\ s^{-1}\ M/10^5\ s^{-1}$ while another antagonist (antagonist B) may be equipotent with $K_B = 50$ nM from $k_2/k_1 = 0.03\ s^{-1}\ M/6 \times 10^5\ s^{-1}$. Both are equiactive in the *in vitro* equilibrium assay, but the rate of dissociation of antagonist B is 6 times faster than that of antagonist A. Therefore, in an open system, antagonist A will provide a much better target coverage because it will wash off of the receptor much more slowly than antagonist B. Since offset kinetics cannot be estimated in standard equilibrium potency measurements; an additional but simple assay is required. However, the potential gains from such an exercise are considerable because adequate target exposure to a slow offset rate drug can, to a certain extent, cancel rapid clearance and poor pharmacokinetics and yield a superior therapeutic profile. Specifically, a slowly dissociating antagonist may bind the target during a short pharmacokinetic exposure to provide target coverage long after washout of drug out of the target compartment. An added bonus is the fact that the rate of onset of effect also can be estimated because it is the quotient of the rate of dissociation divided by the potency (equilibrium dissociation constant). Therefore, an experiment to quantify the rate of dissociation can yield estimates of the rate of onset and duration of effect.

Single and Multivariate SARs. The most simple SAR relates chemical structure to a single number that quantifies biologic activity, that is, pIC_{50} for enzyme or receptor inhibition where pIC_{50} is the negative logarithm of the molar concentration producing 50% effect. A useful procedure to accomplish this is to obtain multiple independent replicates of test compound potency, calculate a standard deviation, and then use that estimate of accuracy to calculate statistical confidence limits. For example, a mean pIC_{50} value of 8.2 with standard deviation of 0.22 for $n = 5$ replicates yields 95% confidence limits of 7.6–8.8; this means that the true value will likely be between those values 95% of the time. Confidence limits are much better estimates of error and accuracy because they incorporate the number of estimates used in the calculation. Under these circumstances, if a discovery program de-

Box 3. Kinetic target coverage.



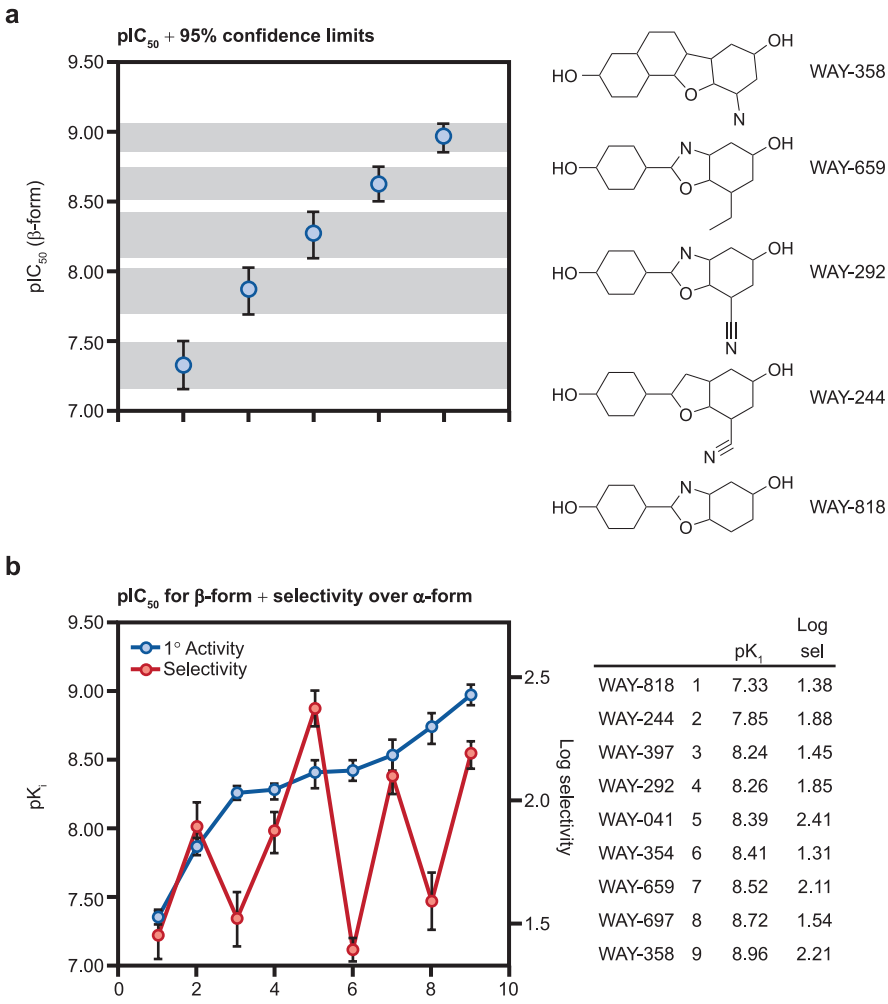
The affinity of a molecule for a protein is the ratio of the rate of offset (*k*) after binding divided by the rate of onset leading to binding. Two antagonists can be equiactive in terms of equilibrium potency but still have very different abilities to stay bound to the target in open systems where the compartment is continually emptied due to washing with drug-free medium (as encountered *in vivo*).

Panel a shows the rate of offset of protein occupancy (ρ_t) for equiactive antagonists (fast offset in red and slow offset in blue) at concentrations that occupy 50% of the receptors (ρ_e) given by the relationship $\rho_t = \rho_e e^{-kt}$. The integral of this relationship over time (shaded areas in panel a) is a measure of how long the target is occupied by the molecule. This integral is shown in panel b, where it can be seen that, though the molecules are equally potent, the blue antagonist does a much better job of occupying receptors in an open *in vivo* system.

cides that it can accept a 5% chance of being incorrect about a given estimate, then meaningful improvements in compound structure can be gauged by those compounds that exceed the previous compound's 95% confidence limit. Therefore, in the previous example, we could be 95% certain that a compound with a mean sample pIC_{50} of 8.85 would be a significantly more potent compound than the previous one and that the structural changes made were correct for optimizing activity. Figure 5 shows SARs for inhibitors of the β-form of the estrogen receptor (*23*); it can be seen that the changes in chemical structure shown produce increases in pIC_{50} values that exceed the 95% confidence limits of the previous compound.

A single variate (one pIC_{50} value to follow) SAR is relatively simple but rarely adequate. As discussed at the beginning of this Perspective, at least two other important SARs are involved in the making of a successful drug, namely, the SAR for optimized pharmacokinetics (absorption, distribution, metabolism, and excretion) and optimized safety. When other SARs are involved (referred to as a multivariate SAR), then complications may arise in that changes in chemical structure that optimize one activity may diminish another. Figure 5, panel b, shows a collateral and equally sought after activity for the inhibitors of the β-form of the estrogen receptor, namely, selectivity over the α-form of the receptor. Progress in producing selectivity can be assessed with a slightly modified statistical procedure for exceeding 95% confidence for selectivity ratios (*23*). It can be seen from Figure 5, panel b, that changes in structure that optimize primary (β-form potency) activity do not necessarily optimize selectivity (ratio of β- to α-activity).

Conclusions. The main premise of this Perspective is that a minimal amount of data obtained from *in vitro* assays early on in drug discovery programs can be used to characterize biological activity in molecular terms to the extent that effects can be predicted in all systems. Thus, for an antagonist the measurement of a pK_B characterizes its potency, determination of orthosteric *vs* allosteric mode of action determines what types of interaction with the endogenous agonist it will have, and the measurement of rate of dissociation (k_2) will estimate persistence in target coverage. In addition, testing in a range of assays that detect direct biological response will predict whether a direct effect will be seen *in vivo*. With respect to agonists, comparison of agonist dose–response curves with standard agonists can quantify relative affinity and efficacy of the test agonist (with the operational model) to the point where the magnitude of response may be predicted in the therapeutic system (if the effects of a comparison standard agonist are known in the therapeutic system). In addition, determination of allosteric *vs* orthosteric binding can predict the



		pK_i	Log sel
WAY-818	1	7.33	1.38
WAY-244	2	7.85	1.88
WAY-397	3	8.24	1.45
WAY-292	4	8.26	1.85
WAY-041	5	8.39	2.41
WAY-354	6	8.41	1.31
WAY-659	7	8.52	2.11
WAY-697	8	8.72	1.54
WAY-358	9	8.96	2.21

Figure 5. Single and multivariate structure–activity relationships. Antagonists of β-estradiol receptors. a) Single variate SAR. pIC_{50} values (negative logarithm of the molar concentration producing 50% inhibition of binding) of five compounds. Each compound has a potency that exceeds the 95% confidence limits of the potency of the previous compound in the series, thereby indicating statistically significant improvement in potency. Shaded bars show 95% confidence limits. b) Multivariate SAR. β-Estradiol antagonist potency (blue line; as for panel a) in ascending order, as well as the concomitant selectivity of the compounds for the β- over the α-form (red line). The symbols in red represent the ratio of IC_{50} values for potency on β-estrogen receptor *vs* α-estrogen receptor. Thus, a value of log selectivity of 2.11 (compound 7) means that the molecule is $10^{2.11} = 128$ times more potent as an inhibitor of the β-form of the estrogen receptor than the α-form. Although the SAR for primary activity is uniform (for the order chosen), the selectivity does not necessarily follow suit. Data from ref 23.

interaction the agonist will have with the endogenous physiological tone in the *in vivo* system. The aim of such studies is to take the characterization of biological activity out of the realm of using system-dependent behaviors in test assays of varying sensitivity and into the realm of chemical descriptors of drug activity.

Additional Comments. This topic is fully discussed in the American Chemical Society short course "Pharmacokinetics and Pharmacodynamics: Principles and Applications in Pre-Clinical Drug Development" and the ACS web course "A Pharmacology Primer for Chemists".

REFERENCES

1. Kenakin, T. P., and Beek, D. (1984) The measurement of the relative efficacy of agonists by selective potentiation of tissue responses: studies with isoprenaline and prenalterol in cardiac tissue, *J. Auton. Pharmacol. 4*, 153–159.
2. Kenakin, T. P. (1985) Prenalterol as a selective cardiostimulant: differences between organ and receptor selectivity, *J. Cardiovasc. Pharmacol. 7*, 208–210.
3. Stephenson, R. P. (1956) A modification of receptor theory, *Br. J. Pharmacol. 11*, 379–393.
4. Leff, P., Martin, G. R., and Morse, J. M. (1986) *Br. J. Pharmacol. 89*, 493–499.
5. Black, J. W., and Leff, P. (1983) Operational models of pharmacological agonist, *Proc. R. Soc. London, Ser. B 220*, 141–162.
6. Kenakin, T. P. (2003) Predicting therapeutic value in the lead optimization phase of drug discovery, *Nat. Rev. Drug Discovery 2*, 429–437.
7. Kenakin, T. P. (1985) Prenalterol as a selective cardiostimulant: differences between organ and receptor selectivity, *J. Cardiovasc. Pharmacol. 7*, 208–210.
8. Kenakin, T. P. (1997) *The Pharmacologic Analysis of Drug Receptor Interaction* 3rd ed., p 1491, Lippincott-Raven, NY.
9. Black, J. W. (1996) A personal view of pharmacology, *Annu. Rev. Pharmacol. Toxicol. 36*, 1–33.
10. Black, J. W., Duncan, W. A., and Shanks, R. G. (1965) Comparison of some properties of pronethalol and propranolol, *Br. J. Pharmacol. Chemo. 25*, 577.
11. Azzi, M., Charest, P. G., Angers, S., Rousseau, G., Kohout, T., Bouvier, M., and Piñeyro, G. (2003) β-Arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G-protein-coupled receptors, *Proc. Natl. Acad. Sci. U.S.A. 100*, 11406–11411.
12. Kenakin, T. P. (2008) Pharmacological onomastics: what's in a name? *Br. J. Pharmacol. 153*, 432–438.
13. Galandrin, S, and Bouvier, M. (2006) Distinct signaling profiles of β_1 and β_2 adrenergic receptor ligands toward adenylyl cyclase and mitogen-activated protein kinase reveals the pluridimensionality of efficacy, *Mol. Pharmacol. 70*, 1575–1584.
14. Christopolous, A., and Kenakin, T. P. (2002) G-Protein coupled receptor allosterism and complexing, *Pharmacol. Rev. 54*, 323–374.
15. Sabroe, I., Peck, M. J., Van Keulen, B. J., Jorritsma, A., Simmons, G., Clapham, P. R., Williams, T. J., and Pease, J. E. (2000) A small molecule antagonist of chemokine receptors CCR1 and CCR3, *J. Biol. Chem. 275*, 25985–25992.
16. Hejnova, L., Tucek, S., and El-Fakahany, E. E. (1995) Positive and negative allosteric interactions on muscarinic receptors, *Eur. J. Pharmacol. 291*, 427–430.
17. Gonzalez, E., Kulkarni, H., Bolivar, H., Mangano, A., Sanchez, R., Catano, G., Nibbs, R. J., Freedman, B. I., Quinones, M. P., Bamshad, M. J., Murthy, K. K., Rovin, B. H., Bradley, W., Clark, R. A., Anderson, S. A., O'connell, R. J., Agan, B. K., Ahuja, S. S., Bologna, R., Sen, L., Dolan, M. J., and Ahuja, S. K. (2005) The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility, *Science 307*, 1434–1440.
18. Muniz-Medina, V. M., Jones, S., Maglich, J. M., Galardi, C., Hollingsworth, R. E., Kazmierski, W. M., Ferris, R. G., Edelstein, M. P., Chiswell, K. E., and Kenakin, T. P. (2009) The relative activity of 'function sparing' HIV-1 entry inhibitors on viral entry and CCR5 internalization: is allosteric functional selectivity a valuable therapeutic property, *Mol. Pharmacol.* In press.
19. Watson, C., Jenkinson, S., Kazmierski, W., and Kenakin, T. P. (2005) The CCR5 receptor-based mechanism of action of 873140, a potent allosteric non-competitive HIV entry-inhibitor, *Mol. Pharmacol. 67*, 1268–1282.
20. Kew, J. N. C., Trube, G., and Kemp, J. A. (1996) A novel mechanism of activity-dependent NMDA receptor antagonism describes the effects of ifenprodil in rat cultured cortical neurons, *J. Physiol. 497*, 761–772.
21. Tucek, S., and Proska, J. (1995) Allosteric modulation of muscarinic acdetylcholine receptors, *Trends Pharmacol. Sci. 16*, 205–212.
22. Copeland, R. A., Pompliano, D. L., and Meek, T. D. (2006) Drug target residence time and its implications for lead optimization, *Nat. Rev. Drug Discovery 5*, 730–739.
23. Manas, E. S., Unwalla, R. J., Xu, Z. B., Malamas, M. S., Miller, C. P., Harris, H. A., Hsiao, C., Akopian, T., Hum, W-T., Malakian, K., Wolfrom, S., Bapat, A., Bhat, R. A., Stahl, M. L., Somers, M. S., and Alvarez, J. C. (2004) Structure-based design of estrogen receptor-β-selective ligands, *J. Am. Chem. Soc. 126*, 15106–15119.