CHAPTER 18

Molecular Mechanism of Action (MMoA) in Drug Discovery

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Contents

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ABBREVIATIONS

ADME	absorption distribution metabolism excretion
BE	biochemical efficiency
CB	cannabinoid
EGF	epidermal growth factor
GABA	γ-aminobutyric acid
GR	glucocorticoid receptor
inhA	enoyl-ACP reductase of *Mycobacterium tuberculosis*
LBD	ligand binding domain
MK2	mitogen-activated protein kinase-activated protein kinase 2
MMoA	molecular mechanism of action
NA	neuroaminidase
NCoR	nuclear receptor co-repressor
NMDA	*N*-methyl-D-aspartate
NNRT	nonnucleotide reverse transcriptase
NSAIDs	nonsteroidal anti-inflammatory drugs
PDGFR	platelet-derived growth factor receptors
PPARγ	peroxisome proliferator-activated receptor
VEGFR	vascular endothelial growth factor receptor

1. INTRODUCTION-MOLECULAR MECHANISM OF ACTION

The molecular mechanism of action (MMoA) of a medicine is the connection of the molecular interactions between the therapeutic treatment and the biological target (*e.g.*, receptor, enzyme, etc.) that yields the physiological response. Pharmacological action begins with the interaction between these molecules as noted by Paul Ehlrich in 1913 that a substance will not work unless it is bound *corpora non agunt nisi fixata* [1]. However, the binding of the ''medicine'' to the target is not always sufficient for a substance to communicate the desired message to physiology. There are many facets of this interaction that ultimately result in the desired therapeutic outcome. For example, the site of interaction (allosteric *vs*. orthosteric), molecular descriptors of the binding interaction (affinity, binding kinetics), the functional impact (agonist, modulation, antagonism), and the specificity of the functional outcome (activation of specific signaling pathways) all contribute to the MMoA and impact the ultimate pharmacological response.

1.1. A significant factor in optimizing MMoA is understanding the potential for mechanism-based toxicity

Mechanism-based toxicity (on-target toxicity) is a potential concern with most human targets. MMoAs provide the means to minimize the mechanism-based toxicity while retaining the desired response [2–4]. Competition with endogenous effector (surmountable with fast on and off rates), uncompetitive inhibition, partial agonism, functional selectivity, and allosteric partial antagonism are mechanisms in which the physiological environment can help to shape the dose–response curves to minimize mechanism-based toxicity while retaining sufficient drug efficacy (see Section 4.1 for examples).

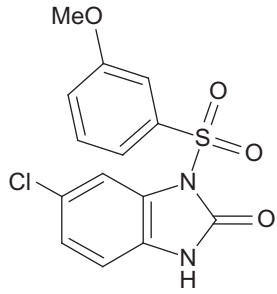
However, a drug such as an anti-infective agent with no potential for mechanism-based toxicity will maximize its therapeutic index *via* mechanisms in which drug binding is efficiently coupled to efficacy. Kinetic mechanisms that are irreversible, insurmountable, noncompetitive, full agonistic or slow dissociating will be safer because lower drug concentrations are required for efficacy. Lower drug concentrations minimize off-target toxicity and result in an increased therapeutic index. This is a principle driver for the desire for slow dissociation rates, long residence times, and irreversible inhibition.

1.2. Details of MMoA are not fully captured by IC_{50} and K_I

A challenge for drug discovery is that many of the molecular features important to an optimal MMoA are not captured by IC_{50} and K_I values in target-based screening assays. This is exemplified in a recent report on the molecular features that differentiate binding between functionally different ligands for the β_2-adrenergic receptor (β_2AR), a member of the G-protein-coupled receptor superfamily [5]. Wacker and colleagues found no discernable structural difference in binding between inverse agonist and antagonist. β_2AR bound to pharmacologically distinct ligands (antagonists and inverse agonists) has virtually identical backbone conformations in the crystal structures suggesting that the conformational changes capable of modifying signaling properties are very small, beyond the resolution of the obtained data. Alternatively, the major effect of inverse agonists and antagonists on β_2AR, which may be extrapolated to agonists, is not on modifying a specific conformation with large conformational changes, but on minor structural changes and a significantly larger contribution from receptor dynamics.

Another example of MMoA not captured by K_I demonstrates the contribution of association rate for the binding of benzimidazol-2-one derivatives (**1**) to HIV-1 reverse transcriptase [6]. The lysine 103 to asparagine mutant (K103N) has a minimal influence on the bound

conformation of NNRTIs, while it significantly affects the kinetics of the inhibitor-binding process. A detailed enzymatic analysis elucidating the molecular mechanism of interaction between benzimidazol-2-one derivatives and the K103N mutant of HIV RT showed that the loss of potency of these molecules toward the K103N RT was specifically due to a reduction of their association rate to the enzyme. Unexpectedly, these compounds showed a strongly reduced dissociation rate from the K103N mutant, as compared to the wild-type enzyme, suggesting that, once occupied by the drug, the mutated binding site could achieve a more stable interaction with these molecules. The K103N mutant has a minimal influence on the bound conformation of NNRTIs, while it significantly affects the kinetics of the inhibitor-binding process. Available structural and biochemical data strongly support the view that the mutated N103 residue slows the k_{on} rate for inhibitor binding through the formation of a hydrogen bond with the Y188 side chain.



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These important nuances of MMoA that are not captured by IC_{50} and K_I in binding assays highlight the gap between the molecular and physiological approaches to drug discovery. MMoA provides an opportunity to bridge the gap by mapping molecular changes to physiological outcomes. Molecular descriptors such as binding kinetics combined with metrics such as biochemical efficiency (BE) and discovery strategies based on increased understanding of the MMoA will help to bridge this gap, as described in the following sections.

2. MOLECULAR DESCRIPTORS

2.1. Equilibrium dissociation constant, K_I

The equilibrium dissociation constant measures the propensity for the bound drug/target complex to dissociate to free drug and target. Equilibrium dissociation constants are related to kinetic rate constants by the relationship $K_I = k_{off}/k_{on}$.

2.2. Reversible kinetic rates, k_{on}, k_{off}

The concentration at which the rate of dissociation equals the rate of association is equal to the equilibrium dissociation constant (K_I for inhibitors/antagonists, K_d for ligands). Association rate is concentration dependent in units of $M^{-1}\ s^{-1}$, and the dissociation rate is concentration independent with units in reciprocal time.

2.2.1. Association rates

There is a renewed interest in binding kinetics with an emphasis on residence time and dissociation rates [3,7,8]. Binding is not always diffusion controlled, and association rates can change within a series in a manner such that k_{off} does not correlate with K_I. Diffusion-controlled association rates for reactions in a cellular environment are estimated to be in the range of 10^5–$10^6\ M^{-1}\ s^{-1}$. The binding of all bimolecular reactions must initiate with a diffusion-controlled interaction. Transition to a stable ground state complex can involve local or global molecular movements that will slow the association rate of complex formation. When the timescale is very slow, this is termed slow binding. It can be observed by a lag in the approach to equilibrium as well as a shift in IC_{50} with preincubation time. It is straightforward to estimate if the association rate for the reaction is slower that diffusion controlled from the relationship $K_I = k_{off}/k_{on}$. A drug with a 1-nM K_I and diffusion-controlled reaction rate of $10^6\ M^{-1}\ s^{-1}$ will have a k_{off} rate of $0.001\ s^{-1}$ ($t_{1/2} = 693$ s from the relationship $t_{1/2} = 0.693/k$). Association rates of drugs with nanomolar K_I values and dissociation half-lives less than 10 min are most likely diffusion controlled, while the association rates of drugs with longer half-lives must involve a rate limiting conformational movement.

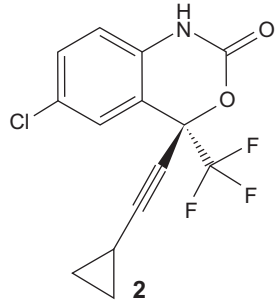
When binding is diffusion controlled within a structural series for a specific target, k_{off} will correlate with K_I. However, if the binding is more complex then K_I does not always correlate with k_{off}. Ligand binding to the M3 muscarinic receptor involves kinetically distinct conformational states. Sykes *et al*. [9] observed that the change in affinity for a series of antagonists better correlated with association rate as opposed to dissociation rate.

A recent report by Schrieber and coworkers describes some insights into the interactions involved in the association process [10]. They point out that there are two major classes of protein–protein interactions that describe most protein–protein association processes. One class is regulated primarily by electrostatic forces that provide both a long-range steering function and dominate the overall binding energy. The second class is more predominant and involves molecular contact interfaces having neutral or weakly charged surfaces in which the binding energy is governed primarily by hydrophobic forces. Studies based on weakly

charged interfaces by Schreiber and coworkers indicate the individual interface side chains have little or no effect on association rates [11,12]. For weakly charged interfaces, Horn and coworkers, using human growth hormone receptor as a model system, concluded that precise matching of surface terrains of the two molecules defines the competent transition state. Interestingly, they also concluded that final fine-tuning of the structure after the transition state contributes significantly to the thermodynamic character of the interaction [13].

2.2.2. Dissociation rates

Residence time and dissociation rates are important features of a medicine's action [3,7,8]. The rational chemical design of molecules with a desired residence time remains a great challenge. Efforts to improve activity almost invariably focus on maximizing binding through stabilizing the energy of the drug–target complex. However, achieving long residence time requires modulation of the energy between both ground states and transition states on the reaction coordinate. An excellent recent report by Lu and Tonge shows the relative contribution of both ground state and transition state to residence time [14]. Analysis of the relative contributions for many different molecules emphasizes the point that transition state energy can play a major role in modulating residence time. They also noted that compounds with long residence times such as efavirenz (4.1 h) (**2**) can have relatively low thermodynamic affinity for their targets (5 μM), stressing the potential disconnect between their thermodynamic stability of a drug–target complex and the lifetime of that complex.



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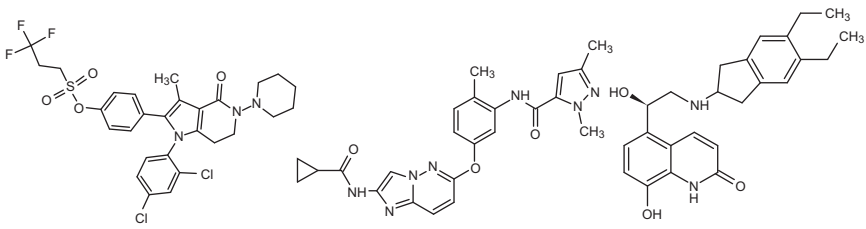
Recently, there were a number of reports of compounds with long residence times and long-lasting pharmacodynamic action attributed to slow dissociation rates including AZ12491187 (**3**) binding to the CB1 receptor [15], TAK-593 (**4**) a novel vascular endothelial growth factor receptor/platelet-derived growth factor receptors (VEGFR/PDGFR) inhibitor with a long residence time ($t_{1/2}$ from VEGFR2 with activity based assay was 5100 min) [16], and indacaterol (**5**) as an ultralong-acting inhaled β2-adrenoreceptor agonist [17].



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In another significant contribution, Lu and coworkers showed that the rate of breakdown of the enzyme–inhibitor complex of fatty acid synthase-II enoyl reductase from *Francisella rularensis* is a better predictor of *in vivo* activity than the overall thermodynamic stability of the complex [18].

2.3. IC_{50}

The IC_{50} is the concentration of drug required for 50% inhibition. IC_{50} is an operational term dependent on the assay conditions. IC_{90} or IC_{99} is sometimes used when complete inhibition is required. Calculation of the fractional occupancy shows that IC_{90} concentration is approximately 10-fold greater than the IC_{50} concentration assuming one-site binding at equilibrium with a Hill coefficient of 1. The IC_{99} concentration is approximately 100-fold the IC_{50} concentration. The percent bound (also referred to as fractional occupancy) comes from the relationship percent bound $= [I]/[I] + K_I$, where K_I is the equilibrium dissociation constant for I and [I] is the concentration of inhibitor.

How do binding kinetics relate to the half maximal inhibitory concentration (IC_{50})? As noted above, IC_{50} is an operational term dependent on the assay conditions, whereas k_{on}, k_{off}, and K_I are intrinsic to a bimolecular interaction. For example, the IC_{50} for a competitive inhibitor will increase with increasing substrate concentrations even though the K_I, k_{on}, and k_{off} are unchanged. The change in IC_{50} due to competition is mathematically described by the Cheng–Prusoff relationship: $IC_{50}/K_I = 1 + [S]/K_m$, where [S] is the substrate concentration and K_m is the Michaelis constant. The IC_{50} is equal to K_I for noncompetitive inhibition.

2.4. Quantitation of irreversible inhibition

The effect of irreversible inhibition on a response is time dependent, and the rate of covalent modification is measured as the k_{inact}/K_I ($M^{-1}\ s^{-1}$), where k_{inact} is the kinetic rate of the covalent irreversible modification expressed in time and K_I is the equilibrium dissociation constant for initial binding of the inhibitor to the protein.

2.5. Conformational changes

There are no quantitative molecular descriptors for conformational changes. The effect of ligand-specific conformational changes is measured empirically in functional assays.

3. METRIC, BIOCHEMICAL EFFICIENCY

BE is defined as how effectively the binding of an inhibitor to the target provides the desired pharmacological response [3,4]. Quantitatively, BE is the ratio of the K_I obtained in a binding or enzyme assay to the IC_{50} in a physiologically relevant functional assay ($BE = K_I/IC_{50}$). A ratio of one indicates that binding is efficiently coupled to the physiological response. BE will directly influence the therapeutic index *via* the impact on the physiological drug concentrations required for pharmacodynamic response.

There are many factors that can influence the shift in dose–response curves between binding and functional assays, including

- Pharmacokinetics and ADME properties (solubility, cell penetration, efflux, metabolism, and protein binding)
- Assay relevance
    - Is the functional assay appropriate for the target?
    - Are the assays technically accurate?
- The target must be involved in the functional readout and biology.
- Molecular mechanisms of action

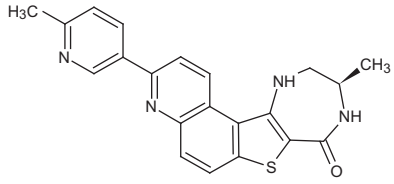
While all these can and do contribute to the relationship between binding affinity and the functional consequence, the role of MMoA is not always considered. The concept of biochemical efficiency was introduced to quantitative this possibility [3,4]. When using biochemical efficiency as a measure of an optimal MMoA, it is important that the other factors are also considered. For example, when evaluating for biochemical efficiency the assays must be run in the absence of serum (or plasma) to eliminate the shift in IC_{50} due to serum protein binding.

Confirmation of the importance of MMoA and the use of biochemical efficiency comes from the observation that most drugs are competitive with endogenous ligands (80%) [4] and also have good biochemical efficiency [3]. At first glance this is surprising, as endogenous competitive effectors will shift a dose–response curve to higher doses. Higher concentrations of drug will be required for equivalent occupancy and to achieve

the desired pharmacological response, resulting in reduced biochemical efficiency. Further analysis of the MMoAs revealed that the medicines have mechanistic features that minimize the negative effects of competition with endogenous effector.

A recent publication by Yun and coworkers on the epidermal growth factor (EGF) receptor exemplifies the physiological consequences of biochemical efficiency [19]. The diminished ATP affinity of the oncogenic EGF receptor mutants opens a ''therapeutic window (index),'' which renders them more easily inhibited relative to the wild-type EGF receptor and other kinases. The authors describe how resistance mutations in the ATP binding site restore the affinity of the kinase for ATP to wild-type levels. The mutations enable ATP to compete more effectively with the inhibitor. The work also provides an explanation for why EGF receptor covalent irreversible inhibitors are insensitive to the mutations. Due to a lack of competition with ATP, the dose–response curves for the irreversible inhibitors are not shifted to higher concentrations.

The challenge to overcome poor BE can make a specific target intractable by a specific mechanistic approach. Mourey *et al*. [20] described the pharmacologic properties of a benzothiophene MK2 inhibitor, PF-3644022, with good activity but poor BE (**6**). PF-3644022 is a potent freely reversible ATP-competitive compound that inhibits MK2 activity ($K_I = 3$ nM) with good selectivity when profiled against 200 human kinases. They noted that of the MK2 inhibitor chemotypes reported, few have submicromolar potency at inhibiting TNFα production in cells, perhaps because of poor physiochemical properties, poor cell permeability, poor BE, or inadequate enzyme potency. Of several MK2 chemotypes investigated, only the benzothiophenes have cellular IC_{50} values less than 500 nM. PF-3644022 is a highly permeable and potent MK2 inhibitor ($K_I =$ 3 nM), yet it exhibits poor BE with at least 30-fold weaker activity at inhibiting TNFα production in cells. Given that PF-3644022 is an ATP-competitive inhibitor, the shift in cellular potency may be caused by competition with high cellular concentrations of ATP (∼5 mM). The binding constant of MgATP for nonactive MK2 is 30 μM. The authors believe that the best K_I values achievable with MK2 are low nanomolar because they were unable to achieve further potency even after gaining additional interactions in the ATP pocket. They also developed several irreversible MK2 inhibitors as tool compounds that did in fact exhibit BEs near one but had insufficient selectivity to explore as drug leads. Although the MK2 knockout mouse validated MK2 as a very attractive target for TNFα inhibition, the very low BE suggests a low probability of success developing MK2 inhibitors as drugs.



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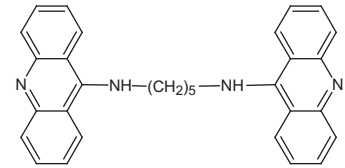

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4. STRATEGIES FOR AN OPTIMAL MMOA

The strategy for an optimal MMoA is dependent upon the potential for mechanism-based toxicity.

4.1. Strategies for minimizing mechanism-based toxicity

Fast kinetics can minimize mechanism-based toxicity by enabling equilibrium competition that may be advantageous if sufficient efficacy can be maintained. This is exemplified by a number of medicines including atypical antipsychotics working through the D_2 receptor [21], NSAIDs such as ibuprofen [22], and *N*-methyl-D-aspartate (NMDA) antagonists [23]. Effective and safe NMDA receptor antagonists for the treatment of neurodegenerative disorders are required to leave the NMDA channel quickly when the membrane is depolarized under physiological conditions. To meet this criterion, the antagonist is expected to inhibit the channel at negative membrane potentials, then leave the channel quickly and have no effect at depolarized membrane potentials. Luo and coworkers [24] recently reported that bis(propyl)-cognitin (**7**), a novel dimeric acetylcholinesterase inhibitor and γ-aminobutyric acid subtype A receptor antagonist, is an uncompetitive NMDA receptor antagonist with a fast off-rate (UFO). In cultured rat hippocampal neurons, they demonstrated that **7** voltage-dependently, selectively, and moderately inhibited NMDA-activated currents. The inhibitory effects of **7** increased with the rise in NMDA and glycine concentrations. Kinetics analysis showed that the inhibition was of fast onset and offset with an off-rate time constant of 1.9 s.

Drugs that act as allosteric modulators can offer significant advantages over classical agonists and antagonists. For example, the benzodiazepines exhibit large therapeutic indexes, probably because they enhance the action of endogenous γ-aminobutyric acid (GABA) without activating

the receptor directly. The low toxicity of the benzodiazepines has contributed to their usefulness in a wide variety of clinical contexts, ranging from anxiety to epilepsy. [25].

Receptor ligands can induce or stabilize ligand-specific conformations that couple to specific functional responses and minimize mechanism-based toxicity. Selective estrogen receptor modulators (SERMs) are the prototype for this MMoA [26]. It is now clear that many receptor modulators bind to a target and stabilize specific conformations that drive ligand-specific and selective functional responses. This new understanding has changed the pharmacological dogma from a linear one ligand/one target/one response paradigm into one in which multiple ligands can interact with one target to produce ligand-specific responses [27,28].

The molecular details that drive this diversity are not as yet fully understood. For example, mifepristone is known to induce mixed passive antagonism, active antagonism, and agonism effects *via* the glucocorticoid receptor (GR) pathway. Schoch and coworkers [29] report the crystal structure of a ternary complex of the GR ligand binding domain (GR-LBD) with mifepristone and a receptor-interacting motif of NCoR. The structures of three different conformations of the GR-LBD mifepristone complex show how the 11β substituent in mifepristone triggers the helix 12 molecular switch to reshape the coactivator site into the co-repressor site. Two observed conformations exemplify the active antagonist state of GR with NCoR bound. In another conformation, helix 12 completely blocks the coregulator binding site and explains the passive antagonistic effect of mifepristone on GR.

4.2. Strategies to maximize efficacy

When there is minimal risk due to mechanism-based toxicity, a MMoA that maximizes efficacy is desirable. A nonequilibrium reversible inhibitor with long residence times will reduce a competition dependent shift in dose–response curves (see Figure 1, left curves). This phenomena has been called insurmountable and pseudo-irreversible. The consequence of this increased BE is a maximized therapeutic index due to the requirement for lower drug concentrations to achieve the efficacious response. This is exemplified in a report by Behm and coworkers [30] with GSK1562590 (**8**), a slowly dissociating urotensin-II receptor antagonist. While optimizing HTS hits, they identified two compounds, GSK1440115 (**9**) and GSK1562590 which exhibited differential characteristics consistent with rapidly and slowly reversible modes of action, respectively. Both compounds were high-affinity ligands; however, GSK1440115 was a

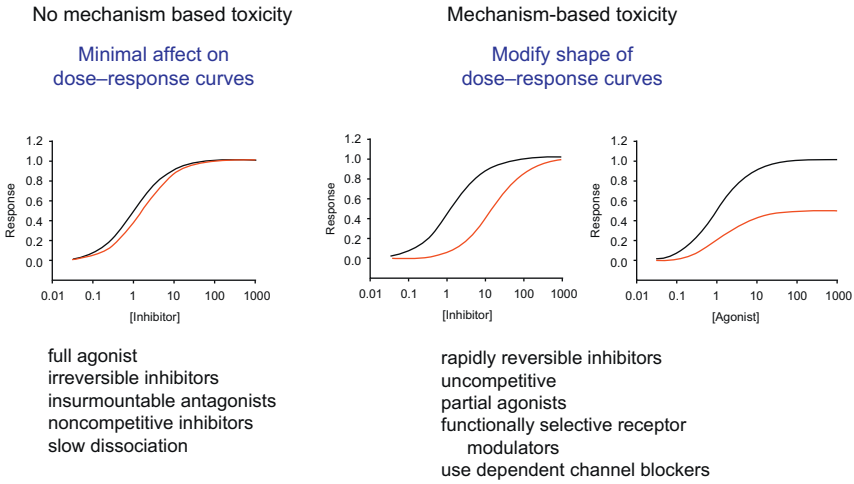
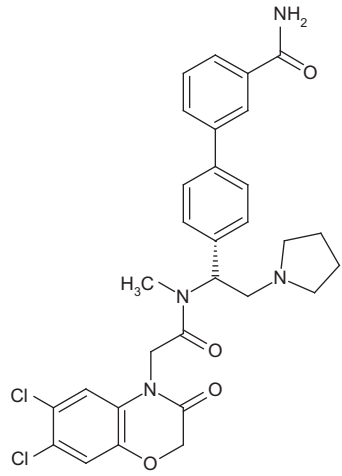


Figure 1 A primary driver of the impact of binding mechanism on the therapeutic index is the potential for mechanism-based toxicity. The curves show the relationship of concentration to binding (black) versus function (red). When there is no mechanism-based toxicity the binding should be efficiently coupled to function and the concentration–response curves will optimally be overlapping (left). When there is potential for mechanism-based toxicity, the functional curves may be shifted to the higher concentrations to limit mechanism-based toxicity (center); this is what would be expected with rapidly reversible competitive inhibitors at equilibrium. A decrease in maximal response as seen with partial agonists is another mechanism to minimize mechanism-based toxicity (right). Source: Swinney [2] reproduced with permission from Wolters Kluwer© 2008.

surmountable antagonist of urotensin in arteries in all species tested, whereas GSK1562590 was an insurmountable antagonist in all species with the exception of monkey. GSK1562590 was more than two orders of magnitude more potent than GSK1440155 in suppression of the maximal contractile response for human urotensin-II in rat isolated aorta. In addition, a minimal difference in potency between binding and functional assays (BE) was observed for GSK1562590 as compared to ~10-fold for GSK1440155. The authors hypothesize that the slow receptor off-rate might be important for urotensin antagonists to counteract the pseudo-irreversible binding characteristics of urotensin-II.



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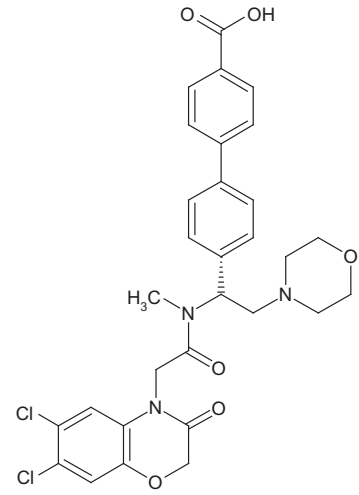

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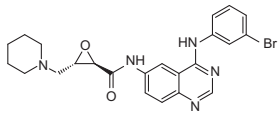
Irreversible inhibition is another strategy to limit competition and increase BE. Novel irreversible inhibitors have been reported for EGFR [31], peroxisome proliferator-activated receptor γ (PPARγ) [32], and VEGFR [33] tyrosine kinases. Carmi and coworkers [31] reported irreversible EGFR kinase inhibitors can circumvent acquired resistance to first-generation reversible, ATP-competitive inhibitors in the treatment of non-small-cell lung cancer. They contained both a driver group, which assures target recognition, and a warhead, generally an acrylamide or propargylamide fragment that binds covalently to Cys797 within the kinase domain of EGFR. They performed a systematic exploration of the role for the warhead group introducing different cysteine-trapping fragments at the 6-position of a traditional 4-anilinoquinazoline scaffold. They identified different groups that were able to irreversibly bind to EGFR through nucleophilic addition (epoxides), nucleophilic substitution (phenoxyacetamides), carbamoylation (carbamate), Pinner reaction (nitrile), and disulfide bond formation (isothiazolinone, benzisothiazolinone, thiadiazole). They also highlighted another interesting warhead represented by phenoxyacetamides which in principle can release a leaving group on nucleophilic substitution. The lead molecule from this work (**10**) showed promising biological result with efficacy at lower doses than gefitinib.



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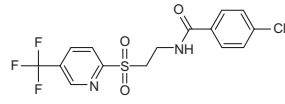

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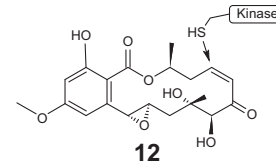

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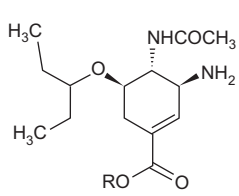
In other reports of irreversible inhibitors, Shearer and coworkers [32] identified a series of trifluoro-methyl-2-pyridylsulfones (**11**) as covalent binders of PPARγ and Barluenga *et al.* [33] reported modifications of the natural product hypothemycin (**12**) and related resorcylic acid lactones bearing a *cis*-enone moiety as irreversible VEGFR kinase inhibitors and with *in vivo* efficacy.

Positive allosteric modulators provide another MMoA to maximize efficacy. Allosteric modulators are ligands that bind to allosteric sites to alter the biological properties of the endogenous orthosteric ligand *via* changing its affinity (generally through the dissociation rate), its efficacy, or both [34,35]. A recent finding by Valant and coworkers identifies a novel and largely unappreciated mechanism of ''directed efficacy,'' whereby functional selectivity may be engendered in a GPCR (muscarinic receptor) by utilizing an allosteric ligand to direct the signaling of an orthosteric ligand encoded within the same molecule [36].

5. CHEMISTRY OF BINDING KINETICS

A challenge for medicinal chemists is that many of the molecular interactions that contribute to binding kinetics and MMoA are dynamic involving structural movements and conformational rearrangements. As described below, some insights are provided by studies with influenza B neuraminidase, which reveals rotations in residues important for binding [37] and InhA, the enoyl-ACP reductase of *Mycobacterium tuberculosis*, which shows a loop ordering involved in the slow kinetics [38].

A mechanism of slow-binding inhibition for oseltamivir, **13**, is revealed by a mutation in neuroaminidase (NA) from influenza B virus. Loss of slow binding is generally associated with mutations in the NA active site, leading to NA inhibitor resistance. Upon binding, residue E275 of B/PerthNA fails to rotate to allow binding of the sec-pentyl moiety to the aliphatic portion of this residue as observed in the equivalent residue (E276) in N1 and N9 NAs. The authors conclude that the rotation of residue E275 needed for high-affinity binding of oseltamivir also does not occur in the current strains of influenza B wild-type NAs, which may possibly lead to decreased clinical efficacy of oseltamivir in children [37].



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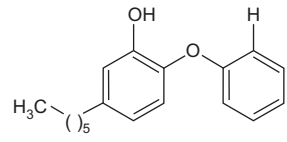

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Luckner and coworkers [38] have used structure-based design to develop a slow onset inhibitor that directly targets InhA. Previous work resulted in the development of a series of alkyl-diphenyl ethers that are nanomolar inhibitors of InhA. The best inhibitor of this series, 8PP (**14**), is active against drug-sensitive and drug-resistant strains of MTB. Although these inhibitors have high affinity for InhA, they are still rapid reversible inhibitors. Luckner and coworkers rationally modified the alkyl-diphenyl ethers to promote interactions between the inhibitor and the loop that becomes ordered during slow onset inhibition. The ordered substrate-binding loop covers the entrance to the binding pocket and thereby locks the inhibitor into the cavity and increases its residence time. It is conceivable that the conformational change of the loop is responsible for the slow step observed in the binding studies.

6. CONCLUSIONS

MMoA is a feature of drug action that bridges the gap between specific molecular interactions and pharmacological activity. Progress is being made to better understand and use binding kinetics, and ligand-specific conformational changes to help design and discover molecules with an increased chance to become therapeutically useful medicines.

ACKNOWLEDGMENT

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