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A CLASSIFICATION OF ENZYME INHIBITORS[1]

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Abstract: Enzyme inhibitors that are primarily active-site directed are classified according to type within a tripartite scheme.

The design of active site directed enzyme inhibitors has become a rational activity inspired by the principles of organic chemical reactivity and knowledge of enzyme structure and mechanisms[2-4]. However, the categorization of such inhibitors has been somewhat arbitrary, despite a number of thoughtful proposals. A nomenclature that conveys mechanistically meaningful information succinctly, focussing on critical elements that epitomize the inhibitory process, is essential for clear thinking, so that trends and novelty can be easily discerned, and specific information can be coordinated into general principles for the development of new paradigms.

The classification in Table I is an attempt to provide a useful framework embracing the great majority of active site directed enzyme inhibitors.

SIMPLE SUBSTRATE ANALOGS (REVERSIBLE COMPETITIVE INHIBITORS)

The most straightforward approach to inhibitor design involves mimicking the structure of the substrate in the ground state according to the principles of isosterism in the hope of competing effectively with substrate for access to the active site of the target enzyme. Inhibitors assigned to this category exhibit competitive kinetics, are not processed by their target enzyme, mimic the structure of only a single substrate, and do not conform to criteria for inhibitors in Group I, vide infra. The inhibition of succinate dehydrogenase by malonate is a typical example of reversible competitive inhibition by a substrate analog[5].

GROUP I. TIGHT BINDING ENZYME COMPLEMENTS

Inhibitors in this group bind tightly to their enzyme target and their potency is critically dependent on the degree of complementarity between enzyme and inhibitor. Extremely tight binding to an enzyme catalyzing a single substrate reaction can be achieved by designing inhibitors that mimic the substrate in the transition state or high energy intermediates along the reaction pathways,[3,6] and criteria have been proposed to evaluate such inhibitors[7,8]. Recent examples of potent inhibitors that are regarded as transition state analogs include carbinol based peptides that mimic hydrate intermediates produced by the

action of renin[9] or HIV protease[10] on their substrates, phosphonyl based peptides that mimic hydrate intermediates produced by various metalloproteinases and their substrates[11], and Proscar™, which may be regarded as an amide mimic of a transient enolate produced during the course of catalysis by testosterone 5-alpha-reductase[12].

Other members in this group include multi-domain inhibitors which are targeted for more than one binding site or domain of the enzyme. These include multi-substrate analogs[13], ground state analogs in the sense proposed by Abeles[14] and essentially any small molecule[15] that has appreciable affinity for more than one binding domain of the enzyme.

GROUP II. MECHANISM-BASED INHIBITORS

Whereas thermodynamic priniciples provide the intellectual underpinnings for strategies classified in Group I, the rational design of inhibitors in Group II is rooted in the principles of organic chemical reactivity. Group II strategies involve the transformation of the inhibitor to an intermediate by its target enzyme with the establishment of a stable covalent bond between enzyme and inhibitor. Some authors have referred to inhibitors in Group II as mechanism-based or enzyme suicide inhibitors, more or less synonymously.

The cardinal features of enzyme suicide inhibition are latent reactivity, catalytic processing of inhibitor to a reactive intermediate, and the formation of an irreversible adduct as exemplified by the classic example of Bloch[16] in which a latent reactive ß,γ-decynoyl thioester is enzymatically converted to a reactive allene, which then traps an active-site histidine in a Michael reaction.

A mechanism-based inhibitor (or inactivator) is currently regarded "as a stable compound, which is treated as a substrate, and converted during the normal course of enzyme catalysis into a species, which without prior release from the active site, binds tightly to the enzyme"[17,18]. In this expanded definition the reagent has been generalized to embrace virtually any mode of inhibition stemming from a bond breaking step analogous to that which initiates the normal catalytic sequence. Since mechanism-based inhibitors are converted to inhibitory species during catalysis, Group II classifications need not be exclusive of those in Group I. As defined above, mechanism-based inhibitors are not restricted from giving rise to any of the inhibitor types in Group I. This term then covers an incredibly broad and diverse group of stable compounds that qualify as mechanism-based inhibitors merely by the target enzyme's ability to produce the actual inhibitory species during catalysis. Clearly, a classification which pinpoints the precise mode of action of the inhibitor

is desirable.

For example, a classification with subtypes such as mechanism-based inhibitor/transition state analog to describe a compound that is catalytically processed to a transition state analog, not only indicates that the original reagent has been modified during the course of catalysis, but actually stipulates the type of activity directly responsible for the inhibition.

THE NEED FOR MULTIPLE DESIGNATIONS

There are at least four categories of mechanism-based inhibitors that can be envisaged. Catalytic processing by enzymes may serve to convert inhibitors in Group II,

1) to tight binding active-site complements (mechanism-based inhibitor/Group I inhibitor; not formulated in Table I). This category embraces inhibitors such as methionine sulfoximine[19] and phosphinothricin[20], which are converted by glutamine synthase to transition state analogs (mechanism-based inhibitor/transition state analog), as well as peptidyl aldehyde inhibitors of serine[21] and cysteine proteinases[22] (but vide infra 3)).

2) to reactive intermediates that combine with the enzyme irreversibly in a step that lies outside normal catalysis (mechanism-based inhibitor/reactive intermediate, or suicide inhibitor), as in the classic example of Bloch[16], as well as a large number of documented examples involving vitamin B_6 and flavin-dependent enzymes[23].

3) to compounds that are converted to stable analogs that are unable to proceed to product because they lack the requisite functionality for further processing (dead-end inhibitors). This designation should be applied to inhibitors that are processed by their target enzymes in the normal course of catalysis, but lack the required structural elements to proceed to product, and are trapped in potential energy wells. A large number of inhibitors have been described in the literature as dead-end inhibitors that lack critical structural elements and do not undergo catalytic processing at all[24-25]. It may be more useful to classify these non-substrates kinetically, as competitive, non-competitive, uncompetitive or slow binding inhibitors, and according to whether they are substrate, transition-state, or product analogs, while reserving the term dead-end inhibitors for compounds that have followed a path that affords no ready outlet. Herein a dead end inhibitor is exemplified by 5-fluorouridylate

acting on thymidylate synthase[26] to give a stable ternary complex consisting of enzyme, coenzyme, and inhibitor, from which free enzyme is only very slowly regenerated; since the fluorine substituting for the essential hydrogen cannot be eliminated in the manner of a proton, the cycle cannot be completed. To be maximally informative, a further designation is required to specify the reasons why dead-end inhibition is effective for each inhibitor. For example, peptidyl aldehydes which have been regarded as transition state analog inhibitors of serine and cysteine proteinases should be described as dead-end inhibitors/transition-state analogs.

4) to stable analogs that have the potential for conversion to product, but are trapped in potential energy wells (alternate substrate inhibitors). As opposed to dead-end inhibitors, which simply lack structural features for substrate turnover, alternate substrate inhibitors give rise to stable complexes during the normal course of catalysis because the rate of one or more steps in the normal cycle has become extremely slow. Prime examples of alternate substrate inhibitors include (1) 5-substituted 2-alkylamino-4H-(3,1)-benzoxazin-4-ones[27] which inhibit human leukocyte elastase by forming sterically encumbered acyl enzymes in which the ester function is shielded from nucleophilic attack by flanking ortho groups, (2) p-guanidinobenzoic acid esters[28] which inhibit tryptic enzymes by virtue of powerful electronic attractions in the acyl enzyme between the positively charged guanidinium function and an active-site aspartate, and (3) anticholinesterases such as physostigmine[29], which undergo transcarbamoylation to form a stable linkage between enzyme and substrate, that is only slowly hydrolyzed.

GROUP III. AFFINITY LABELS

In common usage, a classical affinity label is an active-site directed reagent that contains a chemically reactive group which gives rise with its target enzyme to an adduct that is irreversible to gel filtration and to exhaustive dialysis. The term "chemically reactive" is ambiguous. To medicinal chemists reactive connotes the likelihood of non-specific irreversible reactions under physiological conditions over a twelve hour period (on the order of the lifetime of a drug in vivo). Thus, molecules containing chloromethyl ketone functionality that react with glutathione under such conditions are considered to be chemically reactive, and would be expected to react with divers thiol(ate) functions in a biochemical millieu, and are to be regarded as classical (chemically reactive) affinity labels.

On the other hand, acyloxymethylketones (i.e., mesitoyloxyketones)[30] which contain a sluggish leaving group, and are demonstrably stable in the pH range 5-8 to standard bionucleophiles such as glutathione, are considered to be chemically unreactive to nucleophilic attack. In conjunction with an appropriate affinity group, such acyloxymethylketones can specifically alkylate active site thiols of papain type cysteine proteinases at physiological temperatures and pH. Compounds which exhibit such behaviour are to be regarded as quiescent affinity labels, if they exploit intrinsic binding to lower the overall free energy of activation of an "aberrant" chemical path and lead to facile enzyme inactivation, with each step being distinct from those which the enzyme has evolved to catalyze. Additional examples of quiescent affinity labels may include the inhibition of isopentenyl diphosphate isomerase by 3-(fluoromethyl)-3-butenyldiphosphate[31] and the cysteine proteinase inhibitor, E-64[32].

MAJOR POINTS

1. Enzyme inhibitors can be categorized according to whether they are chemically reactive, catalytically processed, or simply bound by their target enzyme.
2. Especially tight binding inhibitors are distinguished from simple substrate analogs in that they mimic the substrate in the transition state or interact at more than one binding domain of the target enzyme.
3. The term mechanism-based inhibitor merely indicates that the initial reagent has been modified as a consequence of enzyme catalysis. The type of activity directly responsible for the inhibition should be stipulated by subtypes related to whether or not a reactive intermediate is formed during catalysis or to what degree the inhibitor is capable of being processed as a substrate.
4. In principle, affinity labels may be of two types, classical or quiescent, which differ in their intrinsic chemical reactivity.

Table I: Classification of Enzyme Inhibitors

type	binding[a] determinants	chemically reactive[b] group	minimal mechanism	rationale
Substrate analog	S	no	$E + S' \rightleftarrows E{\cdot}S'$	structural mimicry
Group I. Tight Binding Enzyme Complements				
Transition State Analog	t.s.	no	$E + I_{ts} \rightleftarrows E{\cdot}I_{ts}$	E binds T.S. tightly
Multi-substrate Analog	$S_1 + S_2$	no	$E + S_1\text{-}S_2 \rightleftarrows E{\cdot}S_1\text{-}S_2$	multiple binding effects
Ground State Analog	S + R	no	$E + S'\text{-}R \rightleftarrows E{\cdot}S'\text{-}R$	"
Group II. Mechanism-based Inhibitors				
Suicide Inhibitor	S	latent	$E + S' \rightleftarrows E{\cdot}S' \rightleftarrows E{\cdot}I_r \rightarrow E\text{-}I_r$	unmasking of latent reactive group
Dead-end Inhibitor	S	no	$E + S' \rightleftarrows E{\cdot}S' \rightarrow E'\text{-}S'$	S' lacks structural elements for turnover
Alternate Substrate Inhibitor	S	no	$E + S'' \rightleftarrows E{\cdot}S'' \rightleftarrows E'\text{-}S'' \rightleftarrows E'\text{-}P \rightleftarrows E{\cdot}P \rightleftarrows E + P$	stabilize E'-S", E'-P or E·P
Group III. Affinity Labels				
Classical Affinity Label	S	yes	$E + S_{rx} \rightleftarrows E{\cdot}S_{rx} \rightarrow E\text{-}S' + X^-$	high effective concentration of reactive group at active site
Quiescent Affinity Label	S	no	$E + S_x \rightleftarrows E{\cdot}S_x \rightarrow E\text{-}S' + X^-$	differential reactivity: chemical vs. enzymatic

Table legend

[a] *refers to initial reagent*; S = *substrate*; t.s. = *transition state*; $S_1 + S_2$ = cosubstrates; R = entity with affinity for non-catalytic site.

[b] S' = pseudosubstrate; I_{ts} = transition state analog; I_r = reactive intermediate; $E\text{-}I_r$, E-S', E'-S', E'-S", and E'-P = enzyme-inhibitor adduct; S" = alternate substrate inhibitor; P = product; S_{rx} = reactive affinity label; S_x = quiescent affinity label; $S_1\text{-}S_2$ = multisubstrate analog; S'-R = ground state analog.

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