

Unit 4: Rational Design of Enzyme Inhibitors

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4.1 Enzyme kinetics in brief

Enzymes are the second great class of drug targets, and inhibitor design starts from their kinetics. For the simple scheme in which enzyme E binds substrate S, forms the ES complex and releases product P, the **Michaelis-Menten equation** gives the rate:

$$v = V_{\max} \times [S] / (K_m + [S])$$

K_m : substrate concentration at half maximal rate; low K_m means tight substrate binding

$V_{\max}$: rate when the enzyme is saturated with substrate

k_{cat} : turnover number, products per enzyme per second at saturation ($V_{\max} = k_{cat} \times \text{total enzyme}$)

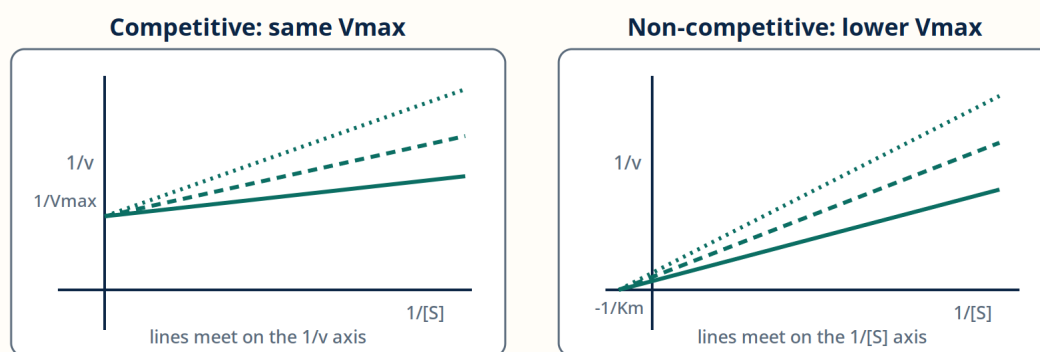
Reciprocal plotting (Lineweaver-Burk: $1/v$ against $1/[S]$) turns the curve into a straight line whose intercepts give K_m and $V_{\max}$, and, more importantly for this unit, turns each inhibition pattern into a recognisable picture (section 4.2). An inhibitor is quantified by its IC_{50} in an assay and, more fundamentally, by its inhibition constant K_i .

4.2 Principles of enzyme inhibition

Type	Binds	Effect on K_m	Effect on $V_{\max}$	Diagnostic picture (Lineweaver-Burk)
Competitive	Active site, free enzyme only	Increased (apparent)	Unchanged	Lines meet on the $1/v$ axis
Non-competitive	Separate site, enzyme or ES complex	Unchanged	Decreased	Lines meet on the $1/[S]$ axis
Uncompetitive	ES complex only	Decreased	Decreased	Lines are parallel
Mixed	Either, with different affinities	Changed either way	Decreased	Lines meet off both axes
Irreversible	Covalent bond at or near the active site	Time dependent loss of active enzyme	Falls with time	Activity does not return on dilution or dialysis

Two distinctions cut across the table. **Reversible versus irreversible** is about the bond: non-covalent complexes dissociate, covalent ones do not. **Suicide substrates (mechanism based inactivators)** are a special irreversible class: the enzyme's own catalytic machinery converts the harmless inhibitor into the reactive species that destroys it, as clavulanic acid does to beta-lactamases (section 4.6).

Reading Inhibition on Lineweaver-Burk Plots



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Figure: Inhibition patterns are read off reciprocal plots. Where the lines cross tells you where the inhibitor binds.

4.3 Enzyme inhibitors in medicine

Inhibitor drug	Enzyme inhibited	Mode	Clinical use
Enalaprilat, lisinopril	Angiotensin converting enzyme	Competitive, zinc binding	Hypertension, heart failure
Atorvastatin and other statins	HMG-CoA reductase	Competitive with HMG-CoA	Cholesterol lowering
Benzylpenicillin (penicillin G)	Bacterial DD-transpeptidase (penicillin binding protein)	Irreversible acylation of the active serine	Bacterial infection
Aspirin	Cyclo-oxygenase	Irreversible acetylation	Pain, inflammation, antiplatelet
Allopurinol	Xanthine oxidase	Converted to oxypurinol, a tight binding product	Gout
Methotrexate	Dihydrofolate reductase	Tight binding competitive	Cancer, rheumatoid arthritis
Clavulanic acid	Beta-lactamases	Suicide substrate	Protects amoxicillin
Saquinavir and later protease inhibitors	HIV protease	Transition state mimic	HIV infection

The statin story shows lead discovery inside this unit: compactin (mevastatin), isolated from a fungus, already contained the HMG-like portion that blocks the reductase; synthetic statins kept that warhead geometry and improved everything around it.

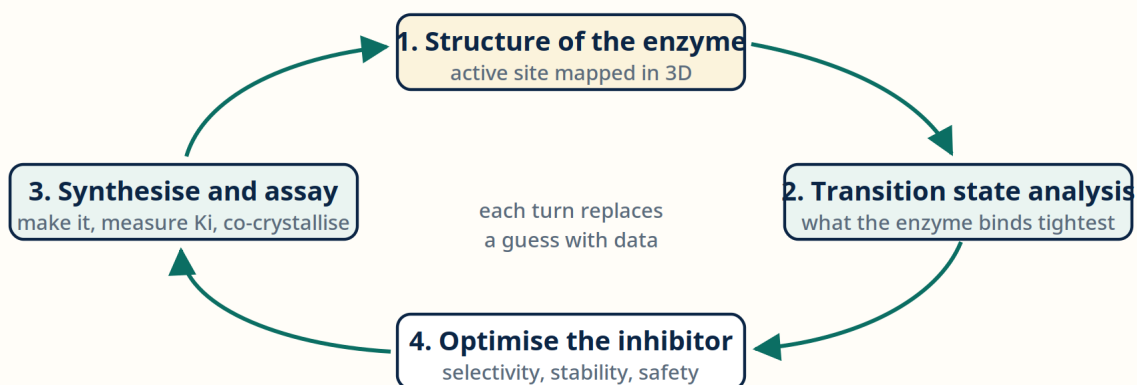
4.4 Enzyme inhibitors in basic research

Inhibitors are also laboratory instruments. A selective inhibitor lets a researcher switch one enzyme off in a living system and read its function from what stops, which is target validation done chemically (section 1.3). Affinity labels and activity based probes, irreversible inhibitors carrying a reporter group, tag the active enzymes in a mixture and are used to map enzyme families and to fish out the unknown target of a drug found by screening. Kinetic patterns (section 4.2) run in reverse are how an unknown inhibitor's binding site is located before any structure is available. Many drug targets were first taken seriously because a research inhibitor showed a clean, interpretable effect in cells.

4.5 Rational design of non-covalent inhibitors

The design cycle, once the target enzyme is chosen and validated:

Rational Design of Enzyme Inhibitors: The Cycle



Saquinavir and oseltamivir both came out of this cycle as transition state mimics.

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Figure: The design cycle. Every turn replaces a guess with a structure and an assay result.

The strongest non-covalent strategy is the **transition state analog**: enzymes bind the transition state of their reaction far more tightly than the substrate, so a stable molecule shaped and charged like the transition state is bound with exceptional affinity. **Saquinavir** carries a hydroxyethylene unit that mimics the tetrahedral intermediate of peptide bond cleavage by the HIV protease; **oseltamivir** (section 3.9) mimics the flattened sialic acid transition state of neuraminidase. Other non-covalent strategies design into **allosteric pockets** beside the active site (the non-nucleoside pocket of HIV reverse transcriptase) or exploit a single differing residue between two related enzymes to buy selectivity, as the COX-2 side pocket was exploited in section 3.6.

4.6 Rational design of covalent inhibitors

Covalent inhibition was avoided for decades from fear of indiscriminate reactivity, then returned deliberately once the design rules were understood:

- **Two step logic**: the inhibitor first binds reversibly and selectively in the pocket (a good non-covalent inhibitor), and only then does a mildly reactive **warhead** group react with a nearby amino acid side chain. Selectivity comes from the binding step; the covalent step adds duration.
- **Warheads and targets**: the acrylamide group of afatinib and osimertinib reacts with a cysteine at the rim of the EGFR ATP site; ibrutinib's acrylamide reacts with Cys481 of Bruton's tyrosine kinase. Aspirin's acetyl group and penicillin's beta-lactam ring are the classical covalent warheads, and the nitrile of some modern inhibitors (for example in cathepsin and DPP-4 chemistry) forms reversible covalent bonds, a halfway house.
- **Advantages**: potency at low dose, pharmacological action that outlives the drug's plasma presence (effect ends when the body resynthesises the enzyme, as with aspirin and platelets), and activity against some resistant mutants because the bond does not depend on binding affinity alone.
- **Risks, stated honestly**: a warhead that is too reactive modifies off target proteins and can trigger immune toxicity; a cysteine that mutates removes the anchor (the C481S mutation is a known ibrutinib resistance route). Modern covalent design therefore keeps warheads weakly reactive and lets binding do the work.

- **Suicide substrates as the limiting case:** clavulanic acid is processed by beta-lactamase into a species that permanently disables it, so the enzyme destroys itself with its own mechanism; gamma-aminobutyric acid transaminase is inactivated the same way by vigabatrin.

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