Walter Ward
Consultancy & Training

Helping You With
Enzymology & Drug Discovery
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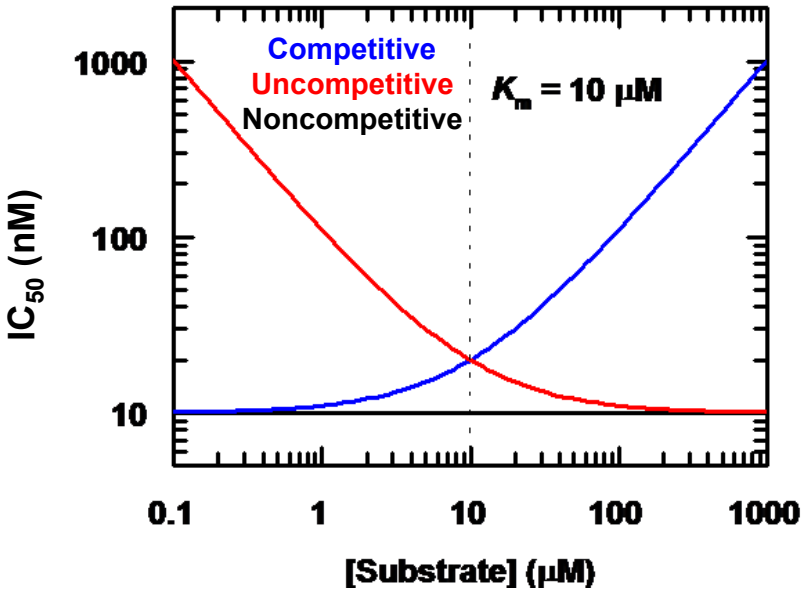
Kinetics, Binding & Biophysics in Drug Discovery

Workshop at The Trinity Centre, Cambridge Science Park
12 -13 September 2016

- Identification & evaluation of active compounds
- Evaluation of reagents & assays
- Obtaining diverse hits with good physicochemical properties
- Avoiding & eliminating pan-assay interference compounds
- Dose-response & mechanism
- Understanding SAR
- Diverse hits for differentiated options
- Kinetics & thermodynamics of binding
- Thermal shift & biosensors
- Covalent inhibitors & residence times



- Tutor: Walter Ward, wal.ward@hotmail.co.uk
- Examples from 29 years experience
- 88% participants consider workshops to be excellent or good value
- Clients in USA, UK, Switzerland, Sweden, France, India & China
- Formerly Senior Principal Scientist at AstraZeneca
- Supported launch of 4 new medicines

Fees up to 7 August: £150 not-for-profit, £300 industrial
including a copy of the presentations
£50 supplement after 7 August
Advance registration required,
Please contact: Gwyn Richards gwynprichards@gmail.com

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Programme

Monday 12 September

0930 Welcome & registration with tea and coffee

Fundamental principles

1000 Introduction
1020 Binding thermodynamics & isothermal titration calorimetry
1100 Discussion & tea / coffee break
1130 Biophysical assays & affinity screens
1230 Discussion & break (no lunch provided)
1400 Kinetics of binding, enzymes & receptors
1500 Discussion & tea / coffee break
1530 Experimental design & dose response analysis
1615 Discussion
1630 Finish

Tuesday 13 September

0930 Welcome with tea and coffee

Identification of active compounds

1000 Assay development
1040 Identification & evaluation of fragment hits
1100 Discussion & tea / coffee break
1130 Nonspecific inhibition & pan-assay interference compounds

Evaluation of active compounds

1200 Transition state analogues, slow binding & covalent inhibition
1220 Discussion & break (no lunch provided)
1400 Evaluation of slow binding & irreversible inhibitors
1445 Significance of drug-target residence times
1500 Discussion & tea / coffee break
1530 Tight binding inhibition
1550 Mechanism of inhibition, SAR & selectivity
1630 Discussion
1700 Finish

Overview

This workshop helps you to apply rigorous science in drug discovery. It focuses on the identification and evaluation of enzyme inhibitors, also highlighting the relevance when targeting receptors or protein-protein interactions. By combining kinetics, activity assays and biophysical measurements, the workshop addresses problems which cause many drug discovery projects to struggle, such as insufficient quality in lead series, difficulties in understanding structure-activity relationships (SAR), insufficient efficacy *in vivo* and misleading compounds that function through spurious mechanisms. The workshop is based on fundamental principles, which can be applied widely across different target proteins. These principles are described and their application is illustrated using examples from personal experience and the literature.

In drug discovery, measurements of IC_{50}, K_i, K_d and pA_2 are used routinely to build SAR. However, the magnitudes of these values may depend upon the identity or concentration of the reagents, and may vary according to the timing or sequence of steps in the assays. The workshop explains how these factors influence results and how they can be exploited in assay design. Also, guidance is given on the interpretation of measured values. Increasing awareness of the importance of kinetics has led to an EU initiative "Kinetics for Drug Discovery", www.k4dd.eu/.

Further information

Introduction. Similarities between enzymes, receptors & protein-protein interactions; using IC_{50} values in understanding structure-activity relationships (SAR); assays at different stages of drug discovery; improving success rates; increasing efficiency.

Fundamental principles

Binding thermodynamics & isothermal titration calorimetry. Affinity, free energy, enthalpy & entropy; isothermal titration calorimetry to measure binding thermodynamics; enthalpy-entropy compensation; interpretation of binding thermodynamics; binding to probe mechanism; evaluation of proteins, assays & active compounds.

Biophysical assays & affinity screens. Using fluorescence to follow binding; binding increases protein stability; screening for shifts in melting temperature (Tm); optimization & analysis of Tm data; unfolding with denaturants; Biacore & other biosensors; design & validation of biosensor measurements; survey of affinity screens.

Kinetics of binding, enzymes & receptors. Enzyme forms: free enzyme, complexes with substrates, intermediates & products; different enzyme forms are diverse targets; pre-steady state kinetics: binding & dissociation; measurement of rate constants & half-lives; kinetic probe competition assays; significance of k_{off} & k_{on}; steady state kinetics: definition & limitations; co-operativity & allosteric binding; derivation of steady state equations: dose-response analysis & mechanism of inhibition; comparison of IC_{50}, K_i & K_d.; similarities between enzymes, receptors & protein-protein interactions; receptor states & ligand types.

Experimental design & dose-response analysis. Pitfalls in linear transformations (eg Lineweaver-Burk); non-linear fitting to estimate IC_{50}; confidence intervals; pIC50 (= -log IC_{50}) is a useful metric; investigating mechanism by fitting alternative rate

equations; *P* values can be misleading; alternative equations to estimate IC_{50}; comparison of IC_{50}s to measure SAR & selectivity; maximum information from minimum data: efficient experimental designs.

Hit identification

Assay development. Reliability, relevance & efficiency; importance of target protein stability; choice of substrates; formatting assays to identify diverse hits; troubleshooting variation in IC_{50}; aspects of assays for receptors and protein-protein interactions.

Identification & evaluation of fragment hits. Physical properties & Rule of 3; small compounds as efficient probes of chemical space; selection of fragments to test; ligand efficiency; test cascade to identify & evaluate fragment hits; comparison with HTS.

Hit evaluation

Non-specific inhibition & pan assay interference compounds. IC_{50}s give misleading SAR if inhibition is nonspecific, slow binding, irreversible or tight binding ($IC_{50} \approx 50\%$ enzyme concentration); definition of specificity & selectivity; nonspecific inhibitors commonly occur as "false actives"; avoiding, recognising & removing of nonspecific hits.

Mechanisms of slow binding & irreversible inhibition. Mechanisms of drugs acting by enzyme inhibition; transition state analogues; covalent inhibitors: affinity labels & enzyme activated suicide inhibitors; covalent inhibition of P450s, adverse drug reactions & drug-drug interactions.

Evaluation of slow binding & irreversible inhibitors. Reversibility tests: recognition of perturbed IC_{50}s; IC_{50} has limited relevance for covalent inhibitors; evaluation of SAR for reactive or slow binding inhibitors.

Significance of drug-target residence times. Influence of binding kinetics & pharmacokinetics on pharmacodynamics, dose interval, efficacy & safety.

Tight binding inhibition. $IC_{50} \approx 50\%$ enzyme concentration; recognition of perturbed IC_{50}s; measuring high affinities; slow & tight binding.

Mechanism of inhibition (MoI): SAR, potency & selectivity. Information on mechanism helps understanding of SAR, & insight into efficacy & toxicity; determination & interpretation of MoI; efficient mechanistic profiling; relationships between IC_{50}, K_i & K_d; IC_{50} depends on mechanism, K_m & substrate concentration: implications for potency & selectivity; complementarity between crystal structures & kinetic mechanisms.