Structure and Function of Drugs

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Content

- Types, characteristics and classification of Drugs and APIs
  - Drug product forms and application methods
  - Molecular mechanism of APIs
- Structure-activity relationships
  - Path and fate of the drug in the body, toxicity
- Drug discovery
  - Strategies (HTS, design, fragment screen etc.)
  - Hit, Lead, DC
    - definitions, selection criterion
  - H2L, L2C optimization. Types, characteristics and classification of Drugs and APIs

Types, characteristics and classification of Drugs and APIs

Classification of drugs

Definitions

- **Finished Dosage Form (FDF) or Drug Product (DP)**

  A Finished Pharmaceutical Product (FPP), prepared for consumer applications, containing excipients and the Active Pharmaceutical Ingredient (API).

- **Active Pharmaceutical Ingredient (API)**

  A substance used in a Finished Pharmaceutical Product (FPP), intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to have direct effect in restoring, correcting or modifying physiological functions in human beings. (*WHO Technical Report Series, No. 961, Annex 10*)

- **Excipient**

  Any substances, other than the Active Pharmaceutical Ingredient (API), that have been appropriately evaluated for **safety** and are included in a **drug delivery system** to either aid the processing of the drug during its manufacture or protect, support or enhance **stability**, **bioavailability**, or **patient acceptability**, assist in product identification, or enhance any other attribute of the overall safety and effectiveness of the drug delivery system during storage and use.

Definitions cont.

- In Europe, the term is "medicinal product", and it is defined by **EU law** as:
  - "(a) Any substance or combination of substances presented as having properties for treating or preventing disease in human beings; or
  - (b) Any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.„
- In the US, a "drug" is:
  - A substance recognized by an official pharmacopoeia or formulary.
  - A substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease.
  - A substance (other than food) intended to affect the structure or any function of the body.
  - A substance intended for use as a component of a medicine but not a device or a component, part or accessory of a device.
  - Biological products are included within this definition and are generally covered by the same laws and regulations, but differences exist regarding their manufacturing processes (chemical process versus biological process.)

Classifications of Drugs

Types of classification

- Level of control
- Basis of their origin
- Mechanism of action
- Route of administration
- Biological system affected
- Anatomical Therapeutic Chemical Classification System (ATC system)
- Dosage form
- By patent status

Classifications of Drugs
Level of control

- ### Prescription drug

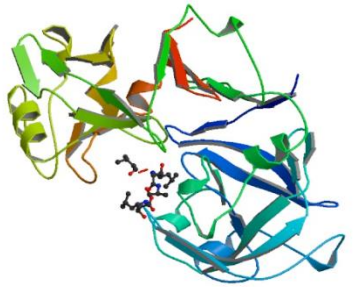
  Prescription drug is a pharmaceutical drug that legally requires a medical prescription to be dispensed.

  Prescription drugs are often dispensed together with a monograph (EP , USP, IP…) that gives detailed information about the drug.

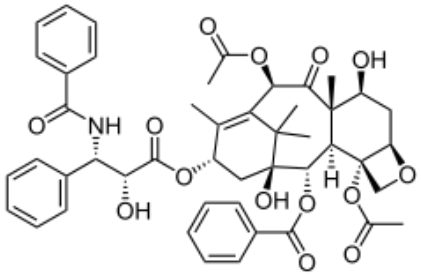
- ### Over-the-counter (OTC) drug

  OTC drugs are medicines sold directly to a consumer without a prescription, from a healthcare professional, as compared to prescription drugs, which may be sold only to consumers possessing a valid prescription. In many countries, OTC drugs are **selected by a regulatory agency** to ensure that they are ingredients that are **safe and effective** when used without a physician's care. OTC drugs are usually regulated by active pharmaceutical ingredients (APIs), not final products. By regulating APIs instead of specific drug formulations, governments allow manufacturers freedom to formulate ingredients, or combinations of ingredients, into proprietary mixtures.

Classifications of Drugs
Basis of their origin



- **Drug from natural origin:** Herbal or plant or mineral origin, some drug substances are of marine origin.
- **Drug from chemical as well as natural origin:** Derived from partial herbal and partial chemical synthesis Chemical, example steroidal drugs
- **Drug derived from chemical synthesis**
- **Drug derived from animal origin:** For example, hormones, and enzymes.
- **Drug derived from microbial origin:** Antibiotics
- **Drug derived by biotechnology genetic-engineering,** hybridoma technique for example
- **Drug derived from radioactive substances.**



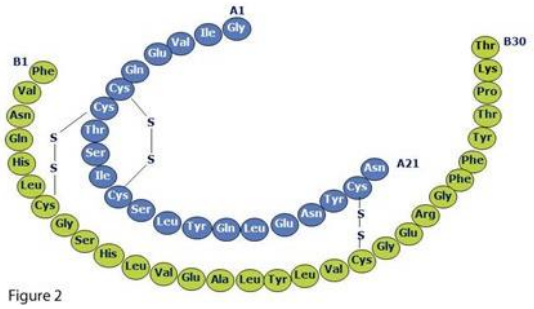
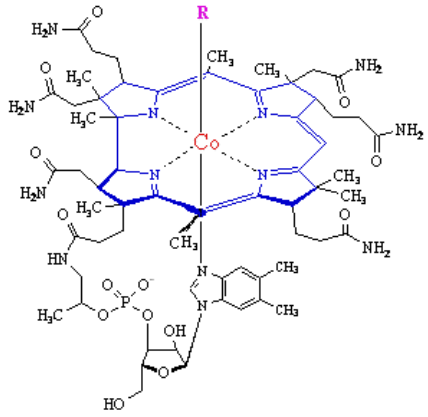


Figure 2



Classifications of Drugs
Mechanism of action

Mechanism of Action (MoA) refers to the specific biochemical interaction through which a drug substance produces its pharmacological effect.

- specific molecular targets to which the drug binds (enzyme or receptor)
- Receptor sites
- specific action

(A: ACE inhibitors with calcium channel blocking agents, ACE inhibitors with thiazides, adamantane antivirals, adrenal cortical steroids, adrenal corticosteroid inhibitors,adrenergic bronchodilators, agents for hypertensive emergencies,agents for pulmonary hypertension, aldosterone receptor antagonists, alkylating agents, allergenics, alpha-glucosidase inhibitors, alternative medicines, amebicides, aminoglycosides, aminopenicillins, aminosalicylates, AMPA receptor antagonists, amylin analogs, analgesic combinations, analgesics, androgens and anabolic steroids, angiotensin converting enzyme inhibitors, angiotensin II inhibitors with calcium channel blockers, angiotensin II inhibitors with thiazides, angiotensin receptor blockers, angiotensin receptor blockers and neprilysin inhibitors, anorectal preparations, anorexiants, antacids, anthelmintics, anti-angiogenic ophthalmic agents, anti-CTLA-4 monoclonal antibodies, anti-infectives, antiadrenergic agents (central) with thiazides, antiadrenergic agents (peripheral) with thiazides, antiadrenergic agents, centrally acting, antiadrenergic agents, peripherally acting, antiandrogens, antianginal agents,antiarrhythmic agents, antiasthmatic combinations, antibiotics/antineoplastics, anticholinergic antiemetics, anticholinergic antiparkinson agents, anticholinergic bronchodilators, anticholinergic chronotropic agents, anticholinergics/antispasmodics, anticoagulant reversal agents, anticoagulants, anticonvulsants, antidepressants, antidiabetic agents…)

Example:

Aspirin

The mechanism of action of aspirin involves irreversible inhibition of the enzyme cyclooxygenase; therefore suppressing the production of prostaglandins and thromboxanes, thus reducing pain and inflammation. This mechanism of action is specific to aspirin, and is not constant for all nonsteroidal anti-inflammatory drugs (NSAIDs). Rather, aspirin is the only NSAID that irreversibly inhibits COX-1

Classifications of Drugs
Route of administration

- **Oral**

  The most convenient and carries the lowest cost.

- **Topical**

  By delivering drugs almost directly to the site of action, the risk of systemic side effects is reduced. Skin irritation may result, the dosage is difficult to control.

- **Sublingual**

  This method refers to the pharmacological route of administration by which drugs diffuse into the blood through tissues under the tongue. For ex.: cardiovascular drugs, steroids, barbiturates, enzymes and increasingly, vitamins and minerals.

- **Inhalation**

  Inhaled medications can be absorbed quickly, and act both locally and systemically. Proper technique is necessary to achieve the correct dose. Inhalation is the most rapid way to deliver drugs to the brain, as the substance travels directly to the brain without being diluted in the systemic circulation.

- **Injection**

  The term injection encompasses **intravenous** (**IV**), **intramuscular** (**IM**), and **subcutaneous** (**SC**) administration.

  Injections act rapidly, with onset of action in 15–30 seconds for IV, 10–20 minutes for IM, and 15–30 minutes for SC, with 100% of bioavailability, and can be used for drugs that are poorly absorbed or ineffective when given orally

Classifications of Drugs
Biological system affected

- Gastrointestinal tract - digestive system
  - Upper digestive tract: reflux suppressants, proton pump inhibitors (PPIs), H2-receptor antagonists
  - Lower digestive tract: antispasmodics, antidiarrheals, bile acid sequestrants,
- Cardiovascular system
  - General: β-receptor blockers ("beta blockers"), calcium channel blockers, diuretics, vasodilators.
  - Affecting blood pressure/(antihypertensive drugs): ACE inhibitors, angiotensin receptor blockers, beta-blockers, calcium channel blockers,
  - Coagulation: anticoagulants, heparin, antiplatelet drugs
  - HMG-CoA reductase inhibitors: hypolipidaemic agents.
- Central nervous system
  - Hypnotics, anaesthetics, antipsychotics, antidepressants
- For pain and consciousness (analgesic drugs)
  - The main classes of painkillers are NSAIDs, opioids and Local anesthetics.
- Musculo-skeletal disorders
  - The main categories of drugs for musculoskeletal disorders are: NSAIDs (including COX-2 selective inhibitors), muscle relaxants, neuromuscular drugs

Classifications of Drugs
Biological system affected cont'

- For the eye
  - Antibacterial, antiviral, anti-fungal, anti-inflammatory, anti-allergy, anti-glaucoma drugs
- For the ear, nose
  - Antibiotics, antihistamines, NSAIDs, corticosteroids, antiseptics, local anesthetics
- Respiratory system
  - bronchodilators, antitussives, mucolytics, decongestants, inhaled and systemic corticosteroids,
- Endocrine system
  - androgens, antiandrogens, estrogens, corticosteroids, human growth hormone, insulin, antidiabetics, thyroid hormones, antithyroid drugs
- Reproductive and urinary system
  - antifungal, quinolones, antibiotics, cholinergics, anticholinergics, fertility medications
- For the skin
  - antifungals, disinfectants, systemic antibiotics, hormones, sunscreens, antiperspirants, corticosteroids, immune modulators
- For the immune system
  - Vaccines, immunoglobulins, immunosuppressants, interferons, monoclonal antibodies
  - For infections: antibiotics, antifungals, antituberculous drugs, antimalarials, antivirals, probiotics
  - For allergic disorders: anti-allergics, antihistamines, NSAIDs, Corticosteroids

Classifications of Drugs
By patent status

- Original drug
  - Discovered and Developped by a pharmaceutical company
  - Patented
    - Novelty
    - Inventive
    - Priority
  - Exlusivity on the market for 20 years (can be prolonged by 5 years
- Generic drug
  - Identical in dose, strength, route of administration, safety, efficacy, and intended use with original drug pruduct
  - Bioequivalent
    - Pharmacokinetic properties
    - Pharmacodinamic properties
  - Biosimilar
    - Generic biological drugs

Drug product forms and application methods

Target Product Profile (TPP)

- A Target Product Profile (TPP) is a planning tool for therapeutic candidates based on FDA *Guidance for Industry and Review Staff Target Product Profile — A Strategic Development Process Tool*
- Describes Ideal results and minimum acceptable results
  - Primary indication
  - Patient population
  - Treatment duration
    - Chronic for example
  - Delivery mode /Dosage form/Regimen
    - Oral/capsule/1-2X daily
  - Efficacy
  - Risk

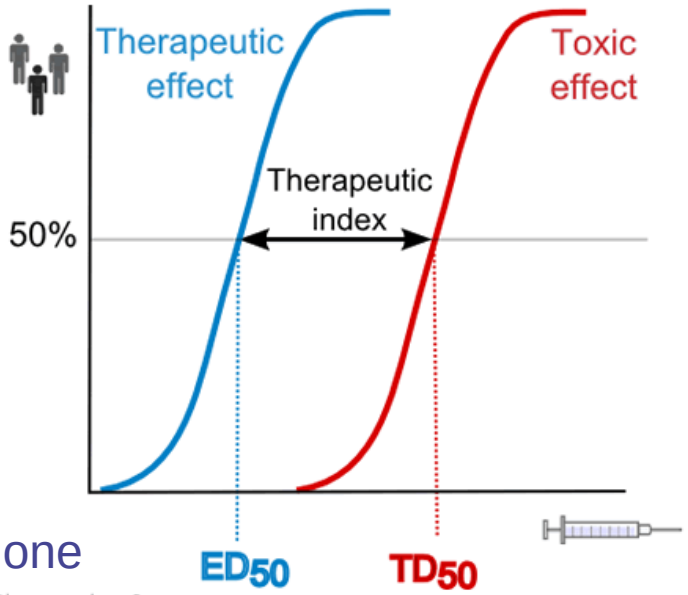
Therapeutic index

Therapeutic index (**TI**) is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxicity.

$$TI = TD_{50}/ED_{50}$$

TD_{50} - toxic dose in 50% of subjects
ED_{50} - efficacious dose in 50% of subjects

In contrast, in a drug development setting
TI is calculated based on plasma exposure levels.



A higher therapeutic index is preferable to a lower one

A drug with a narrow therapeutic range (i.e. having little difference between toxic and therapeutic doses) may have its dosage adjusted according to measurements of the actual blood levels achieved in the person taking it. This may be achieved through therapeutic drug monitoring (TDM) protocols. TDM is recommended for use in the treatment of psychiatric disorders with lithium due to its narrow therapeutic range.

Drug formulation

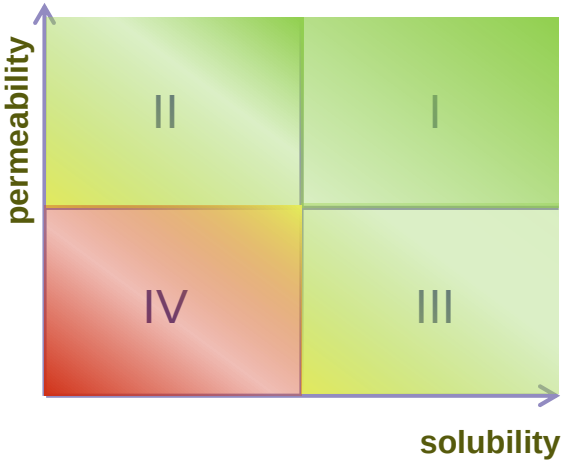
- API and exipients mixed in a controlled way to ensure proper bioavailability of the proper dosage and to enable the proper administration.
  - Shelf life depends on the stability of the API and the stability of the drug substance
  - Stability of the API within the given formula is depending on the compatibility of excipient and the API
  - Formulation often depends on phisicochemical properties of the API
    - Particle size distribution, pH, Polimorphism, solubility, bulk density
- Tablets, capsules
  - uniform appearance, with an acceptable taste, tablet hardness, or capsule disintegration
  - Wet granulation,
    - Water, ethanol, isopropanol
  - direct pressing, dry granulation
- Injectables
  - API is dissolved in a liquid
  - Often needs refrigaeration to keep stability

Tablets-composition and release

- Tablet composition
  - 5-10% of the drug: API
  - 80% of fillers, disintegrants, lubricants, glidants, and binders; and
  - 10% of compounds which ensure easy disintegration, disaggregation, and dissolution of the tablet in the stomach or the intestine.
- Sustained release
  - **Special coatings** can make the tablet resistant to the stomach acids resulting disintegration in the latter trackts of the gastointestinal system
  - Embeddign the active ingredient in an **insoluble porous matrix**, such that the dissolving drug must make its way out of the matrix before it can be absorbed.
  - Matrix swelling
    - To form a **gel** through which the drug exits.
  - Osmotic controlled-release oral delivery system where the active compound is encased in a water-permeable membrane with a laser drilled hole at one end. As water passes through the membrane the drug is pushed out through the hole and into the digestive tract where it can be absorbed.
- Coating
  - Sugar, varnish or wax to disguise the taste

BCS Classification
Solubility-Permeability

- Class I - High Permeability, High Solubility
- Class II - High Permeability, Low Solubility
- Class III - Low Permeability, High Solubility
- Class IV - Low Permeability, Low Solubility



- CLASS BOUNDARIES
  - A drug substance is considered HIGHLY SOLUBLE when the highest dose strength is soluble in < 250 ml water over a pH range of 1 to 7.5.
  - A drug substance is considered HIGHLY PERMEABLE when the extent of absorption in humans is determined to be > 90% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose.
  - A drug product is considered to be RAPIDLY DISSOLVING when > 85% of the labeled amount of drug substance dissolves within 30 minutes using USP apparatus I or II in a volume of < 900 ml buffer solutions.

Molecular mechanism of API

Types of APIs

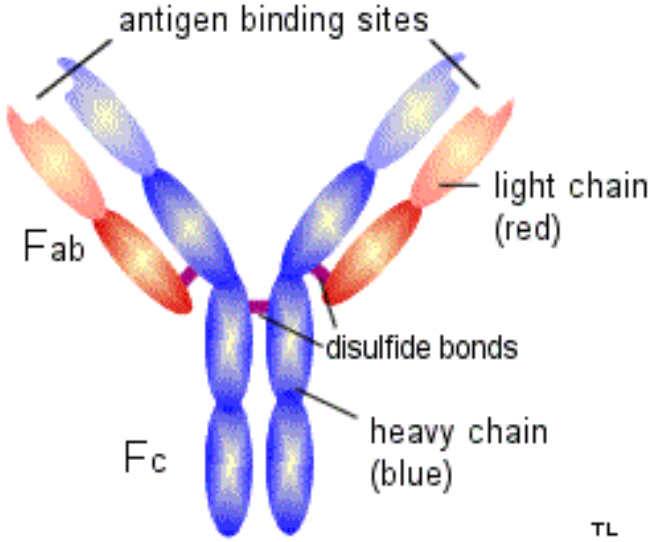
- Biologicals
  - Enzymes
  - Monoclonal antibodies
  - siRNAs
  - Large peptides
- Vaccines
- Small molecules
  - Enzyme inhibitors
  - Receptor antagonists
  - Receptor agonists
  - Hormones
  - Kinase blockers
  - Effectors on RNA transcription

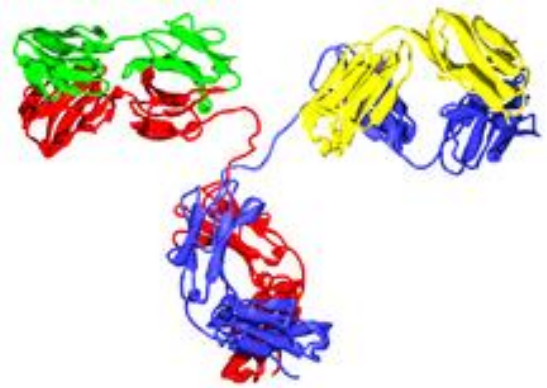
Enzyme therapy

- Pompe disease
  - Pompe disease is a rare inherited neuromuscular disorder that causes progressive muscle weakness in people of all ages
  - Pompe disease is caused by a defective gene that results in a deficiency of an enzyme, acid alpha-glucosidase (**GAA**). The absence of this enzyme results in excessive buildup of glycogen, stored in a specialized compartment of muscle cells.
- Hurler syndrome
  - Genetic disorder that results in the buildup of glycosaminoglycans due to a deficiency of alpha-L iduronidase.
- Hunter syndrome
  - Very similar to Hurler syndrome. Deficiency of iduronate-2-sulfatase (I2S). The accumulated substrates in Hunter syndrome are heparan sulfate and dermatan sulfate
- Gaucher disease
  - The disorder is characterized by bruising, fatigue, anemia, low blood platelet count and enlargement of the liver and spleen, and is caused by a hereditary deficiency of the enzyme glucocerebrosidase

Antibodies (immunoglobulins IGs)

- Types of antibodies
  - Chimeric
  - Recombinant
  - Fully human
  - Humanized
- Production of antibodies
  - Fermentation





Purification of antibodies

Antibody purification involves selective enrichment or specific isolation of antibodies from serum (polyclonal antibodies), ascites fluid or cell culture supernatant of a hybridoma cell line (monoclonal antibodies).

Purification methods range from very crude to highly specific and can be classified as follows:

- Physicochemical fractionation – differential precipitation, size-exclusion or solid-phase binding of immunoglobulins based on size, charge or other shared chemical characteristics of antibodies in typical samples. This isolates a subset of sample proteins that includes the immunoglobulins.
- Class-specific affinity – solid-phase binding of particular antibody classes (e.g., IgG) by immobilized biological ligands (proteins, lectins, etc.) that have specific affinity to immunoglobulins. This purifies all antibodies of the target class without regard to antigen specificity.
- Antigen-specific affinity – affinity purification of only those antibodies in a sample that bind to a particular antigen molecule through their specific antigen-binding domains. This purifies all antibodies that bind the antigen without regard to antibody class or isotype.

Monoclonal antibodies

- Effective in protein protein interactions
  - Large surfaces with many weak interactions separated in space but resulting in very specific and strong interaction
  - Difficult and in most cases impossible to influence by small molecules
- Main indications
  - Cancer treatment
  - Autoimmune diseases
  - Anti-inflammatory
  - Diagnostics
- Potential side effects of antibodies
  - Antibodies are much more selective than small molecules, hence less adverse effect are associated with them
  - Risk of a side effect associated with an antibody is related to the long half-life in the body
  - Potential side effect might be higher rate of infections compared to control group taking placebo

Examples
Anti-inlammatory Mabs

infliximab	•rheumatoid arthritis •Crohn's disease •Ulcerative Colitis •ankylosing spondylitis	inhibits TNF-α	**chimeric**
adalimumab	•rheumatoid arthritis •Crohn's disease •Ulcerative Colitis •ankylosing spondylitis	inhibits TNF-α	**human**
basiliximab	•Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	**chimeric**
daclizumab	•Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	**humanized**
ignasimab	•Bistue's Syndrome	inhibits Manent receptor on activated T cells	**humanized**
omalizumab	•moderate-to-severe allergic asthma	inhibits human immunoglobulin E (IgE)	**humanized**

Examples
Anticancer drugs

gemtuzumab	•relapsed acute myeloid leukemia	targets myeloid cell surface antigen CD33 on leukemia cells	**humanized**
alemtuzumab	•B cell leukemia	targets an antigen CD52 on T- and B-lymphocytes	**humanized**
rituximab	•non-Hodgkin's lymphoma •rheumatoid arthritis	targets phosphoprotein CD20 on B lymphocytes	**chimeric**
trastuzumab	•breast cancer with HER2/neu overexpression	targets the HER2/neu (erbB2) receptor	**humanized**
nimotuzumab	•Approved in squamous cell carcinomas, Glioma •Clinical trials for other indications underway	EGFR inhibitor	**humanized**
cetuximab	•Approved in squamous cell carcinomas, colorectal carcinoma	EGFR inhibitor	**chimeric**
bevacizumab	•Anti-angiogenic cancer therapy	inhibits VEGF	**humanized**

siRNA

- Many diseases develop from the undesirable production of specific
- Proteins. Protein production in the cell begins with transcription. This process generates a messenger RNA (mRNA), which is then translated intoprotein in the cytoplasm.
- Typical mRNA produces approximately 5,000 copies of a protein. Consequently, targeting mRNA rather than the protein itself is potentially a much more efficient approach to block protein function.
  - RNAs of 21-23 nucleotides in length, called small interfering RNAs (siRNAs), are snipped from longer dsRNA chains by an enzyme called Dicer. The antisense strand of the siRNA is used by an RNA-induced silencing complex (RISC) to guide messenger RNA (mRNA) cleavage, so promoting mRNA degradation.
  - siRNA associates with RISC and directs it to the target mRNA. The siRNA-associated RISC binds to the target mRNA through a base-pairing interaction and degrades it. RISC complex is catalytic and can cleave multiple target mRNAs.
- Administration
  - Synthetic RNA (siRNA) can be injected into the cell
  - A viral vector encoding a short hairpin RNA (shRNA) can be used to deliver siRNA into the cell
  - siRNA -coding DNA constructs can be incorporated into the genome

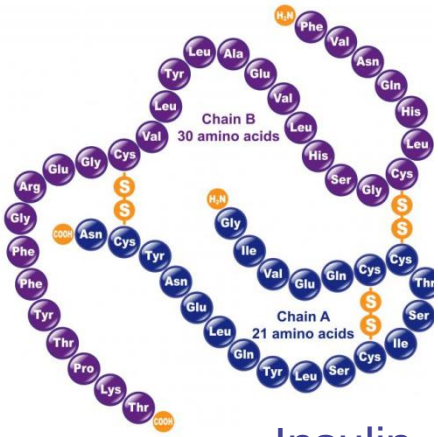
Peptides

More than 7000 naturally occurring peptides have been identified, and often have crucial roles in human physiology, including actions as hormones, neurotransmitters, growth factors, ion channel ligands, or anti-infectives. In general, **peptides are selective and efficacious signaling molecules** that bind to specific cell surface receptors, such as G protein-coupled receptors (GPCRs) or ion channels, where they trigger intracellular effects. Given their attractive pharmacological profile, peptides represent an excellent starting point for the design of novel therapeutics and their specificity has been seen to translate into **excellent safety, tolerability, and efficacy profiles in humans**.

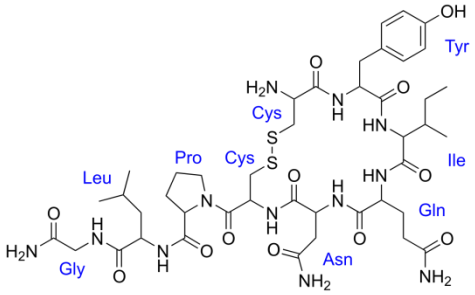
Naturally occurring peptides are often not directly suitable for use as convenient therapeutics because they have intrinsic weaknesses, including poor chemical and physical stability, and a short circulating plasma half-life. Some of these weaknesses have been successfully resolved through what we term the 'traditional design' of therapeutic peptides (see table: **SWOT analysis**). Besides traditional design, a range of peptide technologies has been emerging that represent the opportunities and future directions within the peptide field. These include multifunctional and cell penetrating peptides, as well as peptide drug conjugates.

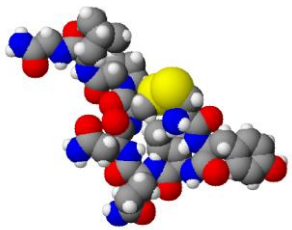
S	**Strengths** • Good efficacy, safety, and tolerability • High selectivity and potency • Predictable metabolism • Shorter time to market • Lower attrition rates • Standard synthetic protocols	**W**	**Weaknesses** • Chemically and physically instable • Prone to hydrolysis and oxidation • Tendency for aggregation • Short half-life and fast elimination • Usually not orally available • Low membrane permeability
O	**Opportunities** • Discovery of new peptides, including protein fragmentation • Focused libaries and optimized designed sequences • Formulation development • Alternative delivery routes besides parental • Multifunctional peptides and conjugates	**T**	**Threats** • Immunogenicity • New advancements in genomics, proteonimics, and personalized medicine • Significant number of patent expiries • Price and reimbursement environment • Increasing safety and efficacy requirements for novel drugs

Examples for peptide therapeutics

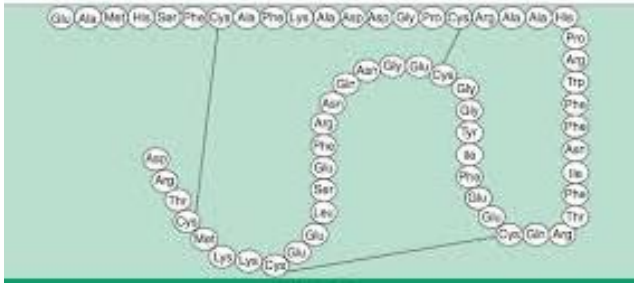


Insulin





Oxytocin



Ecallantide

is a drug used for the treatment of hereditary angioedema (HAE) and in the prevention of blood loss in cardiothoracic surgery.It is an inhibitor of the protein kallikrein and a 60-amino acid polypeptide.

Vaccines

A vaccine is a biological preparation that provides active acquired immunity to a particular disease.

Types

- Inactivated

  These vaccines contain inactivated, but previously virulent, micro-organisms that have been destroyed with chemicals, heat, radiation, or antibiotics. E.g. influenza, cholera, bubonic plague, polio, hepatitis A, and rabies.

- Attenuated

  Some vaccines contain live, attenuated microorganisms. Many of these are active viruses that have been cultivated under conditions that disable their virulent properties, or that use closely related but less dangerous organisms to produce a broad immune response. E.g. yellow fever, measles, rubella, and mumps, and the bacterial disease typhoid.

  Attenuated vaccines have some advantages and disadvantages. They typically provoke more durable immunological responses and are the preferred type for healthy adults. But they may not be safe for use in immunocompromised individuals, and may rarely mutate to a virulent form and cause disease.

- Toxoid

  Toxoid vaccines are made from inactivated toxic compounds that cause illness rather than the micro-organism. E.g. tetanus and diphtheria. Toxoid vaccines are known for their efficacy.

- Subunit

  Protein subunit – rather than introducing an inactivated or attenuated micro-organism to an immune system (which would constitute a "whole-agent" vaccine), a fragment of it can create an immune response. Examples include the subunit vaccine against Hepatitis B virus that is composed of only the surface proteins of the virus (previously extracted from the blood serum of chronically infected patients, but now produced by recombination of the viral genes into yeast), the virus-like particle (VLP) vaccine against human papillomavirus (HPV) that is composed of the viral major capsid protein, and the hemagglutinin and neuraminidase subunits of the influenza virus. Subunit vaccine is being used for plague immunization.

Vaccines
cont'

- Conjugate

  Conjugate – certain bacteria have polysaccharide outer coats that are poorly immunogenic. By linking these outer coats to proteins (e.g., toxins), the immune system can be led to recognize the polysaccharide as if it were a protein antigen. This approach is used in the Haemophilus influenzae type B vaccine.

- Experimental (vaccines under development)
  - Dendritic cell vaccines combine dendritic cells with antigens in order to present the antigens to the body's white blood cells, thus stimulating an immune reaction. (brain tumors and malignant melanoma)
  - DNA vaccination – an alternative, experimental approach, created from an infectious agent's DNA. The proposed mechanism is the insertion of viral or bacterial DNA into human cells. Some cells of the immune system that recognize the proteins expressed will mount an attack against these proteins and cells expressing them. Because these cells live for a very long time, if the pathogen that normally expresses these proteins is encountered at a later time, they will be attacked instantly by the immune system.
  - T-cell receptor peptide vaccines are under development for several diseases such as atopic dermatitis. These peptides have been shown to modulate cytokine production and improve cell mediated immunity.

- Valence

  Vaccines may be monovalent or multivalent. A monovalent vaccine is designed to immunize against a single antigen or single microorganism. A multivalent or polyvalent vaccine is designed to immunize against two or more strains of the same microorganism, or against two or more microorganisms.

- Heterotypic

  These are vaccines that are pathogens of other animals that either do not cause disease or cause mild disease in the organism being treated. The classic example is Jenner's use of cowpox to protect against smallpox. A current example is the use of BCG vaccine made from Mycobacterium bovis to protect against human tuberculosis.

Small molecules enzyme inhibitors

- An **enzyme inhibitor** is a molecule that binds to an enzyme and decreases its activity
  - Competitive inhibition
    - Affinity towards the active site of the enzyme
  - Uncompetitive inhibition
    - inhibitor binds only to the substrate-enzyme complex
  - Non-competitive inhibition
    - binding of the inhibitor to the enzyme reduces its activity but does not affect the binding of substrate
  - Mixed inhibition
    - Allosteric binding
- Examples
  - Ritonavir
    - Peptidomimetic HIV-1 protease inhibitor
  - Tipranavir
    - Non-peptide based HIV-1 protease inhibitor
  - Captopril
    - ACE (Angiotensin Converting Enzyme) inhibitor
  - Axitinib
    - Protein kinase inhibitor

Small molecules
Receptor agonists, antagonists

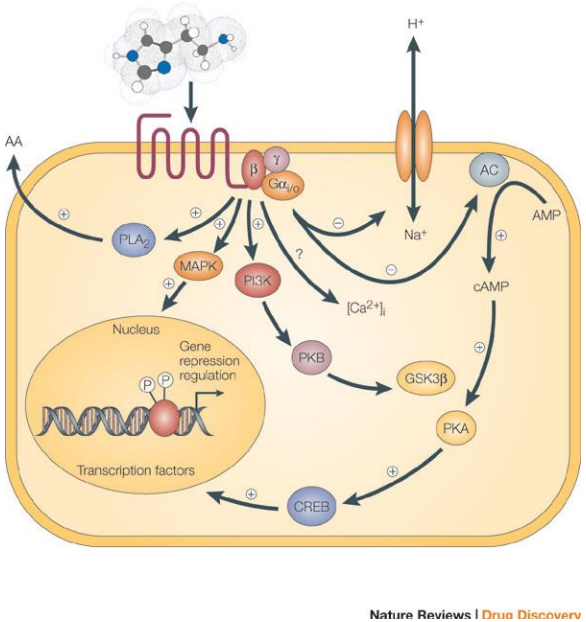
- Receptors are protein molecules with the ability of receiving chemical signal (ligand binding) and upon the chemical signal they induce a response or a cascade of responses (signal transduction

- Types of receptors
  - Based on location
    - External - Cell surface receptors
    - Plasma receptors
    - Nuclear receptors
  - Based on molecular actions
    - GPCR (G-protein coupled receptor)
    - Ionotropic receptors
    - Tyrosine kinase linked receptors, Enzyme linked receptors
    - Nulear receptors

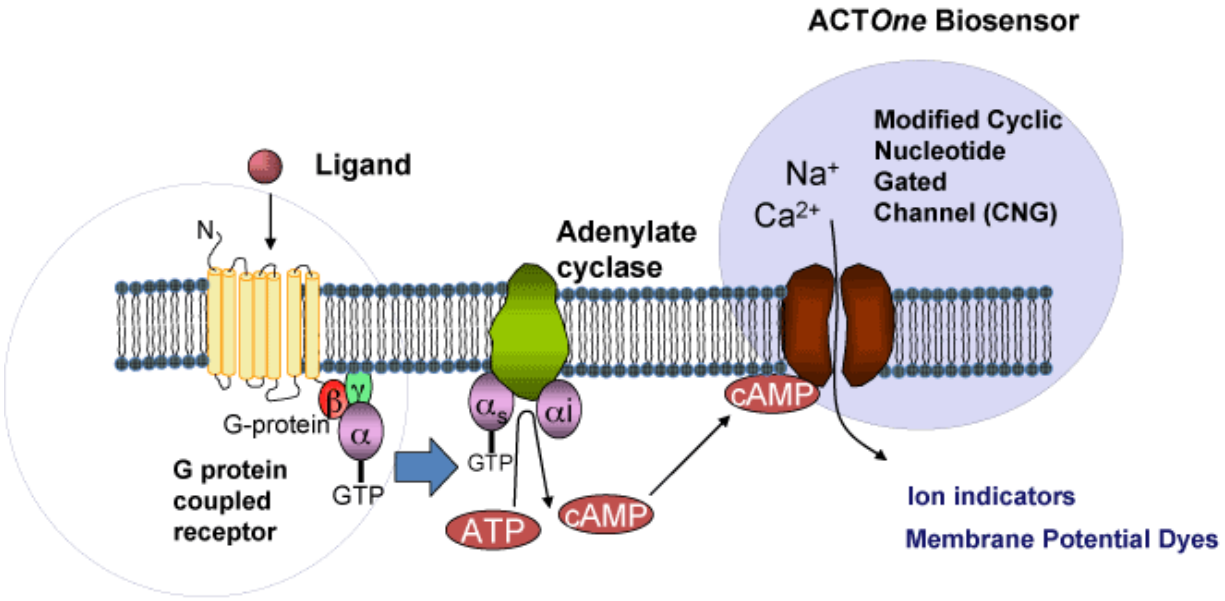
Small molecules
Receptor ligands

- Agonist
  - Activates the receptor resulting in a maximal biological response
- Partial agonist
  - Activates the receptor resulting in a partial or none maximal response
- Inverse-agonist
  - Blocks the constitutive activity of the receptor
    - A receptor which is capable of producing a biological-response in the absence of a bound-ligand is said to display "constitutive-activity".
- Antagonist
  - Binds to the receptor but do not activates it. Inhibits the binding of agonist or inverse-agonist
    - Reversible (competitive)
    - Irreversible (covalent binding)
- Allosteric modulators
  - They do not bind to the agonist-binding site of the receptor but instead on specific allosteric-binding sites, through which they modify the effect of the agonist, e.g. benzodiazepines (BZDs) bind to the BZD-site on the GABA-A receptor and potentiate the effect of endogenous-GABA

GPCR
Signal transduction



Nature Reviews | Drug Discovery



- A GPCR is a protein with 7 transmembrane domains
- Upon the ligand binding G-protein is released and dissociates
- Alfa domain of G-protein induces adenylate cyclase activity (ATP is transformed to c-AMP)
- C-AMP induces ion-flux by activating ion channels Na+ or Ca+
- G-protein can also induce other mechanism resulting in kinase activity and arrestin activity

Examples of ionotropic receptors

- Extracellular
  - Nicotinic acetylcholine receptor
    - Acetylcholine, Nicotine
      - Na+, K+, Ca2+
  - Glycine receptor (GlyR)
    - Glycine, Strychnine
      - Cl− HCO3-
  - GABA receptors:
    - GABA-A, GABA-C GABA
      - Cl− HCO3−
  - Glutamate receptors: NMDA receptor, AMPA receptor, and Kainate receptor
    - Glutamate
      - Na+, K+, Ca2+
  - 5-HT3 receptor
    - Serotonin
      - Na+, K+
  - P2X receptors
    - ATP
      - Ca2+, Na+, Mg2+

Examples of ionotropic receptors cont.

- Intracellular
  - cyclic nucleotide-gated ion channels
    - cGMP, cAMP and cGTP
      - Na+, K+
  - IP3 receptor
    - IP3
      - Ca2+
  - Intracellular ATP receptors
    - ATP (closes channel)
      - K+
  - Ryanodine receptor
    - Ca2+
      - Ca2+

Small molecules
Ion channel activators / blockers

- **Ion channels** are pore-forming membrane proteins whose functions include establishing a resting membrane potential, shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane
- Ligand gated
  - Ionotropic receptors
- Voltage gated
  - Sodium
  - Calcium
  - Potassium
  - Proton
- Examples
  - Calcium channel blockers for indications such as cardiovascular diseases
    - Dihydropyridines, phenylalkylamines, benzothiazepines
  - Sodium channel blockers
    - Antiepileptics
      - Carbamazepine

Small molecules
Effectors on RNA transcription

- Drugs that modulates gene transcription
  - Hence translation to and expression of proteins
  - Selectivity is major issue
- Immunsupressants
  - Immunosuppressants inhibit T-cell activation and proliferation, which play a central role in both immune responses and autoimmune diseases.
- Estrogen agonists-antagonists
- Antiinflammatory drugs
  - Aspirin and salicilates

Structure Property Relationship

R&D flow

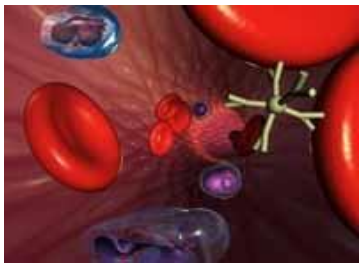
Research

Development

Biological target identification → Biological target validation → "LEAD" identification → "LEAD" optimization → Preclinical development → Clinical development → Registration

2-3 years | 3-4 years | 2 years | 6-8 years | 1-2 years

~300 M$ | ~200 M$ | ~100 M$ | ~400 M$



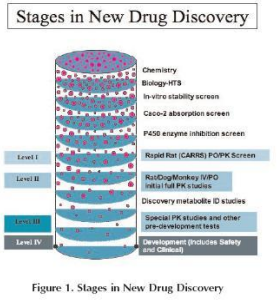
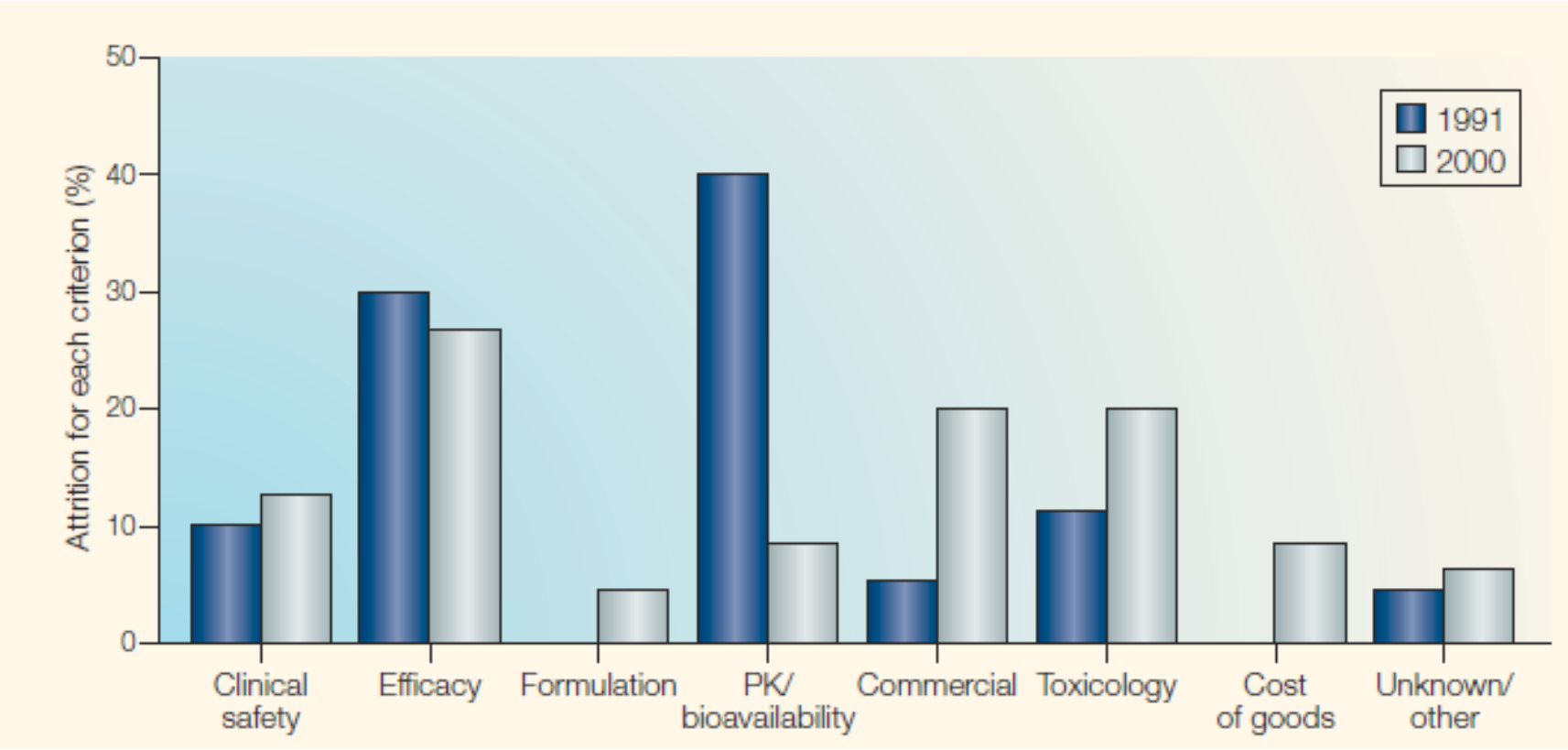


Figure 1. Stages in New Drug Discovery

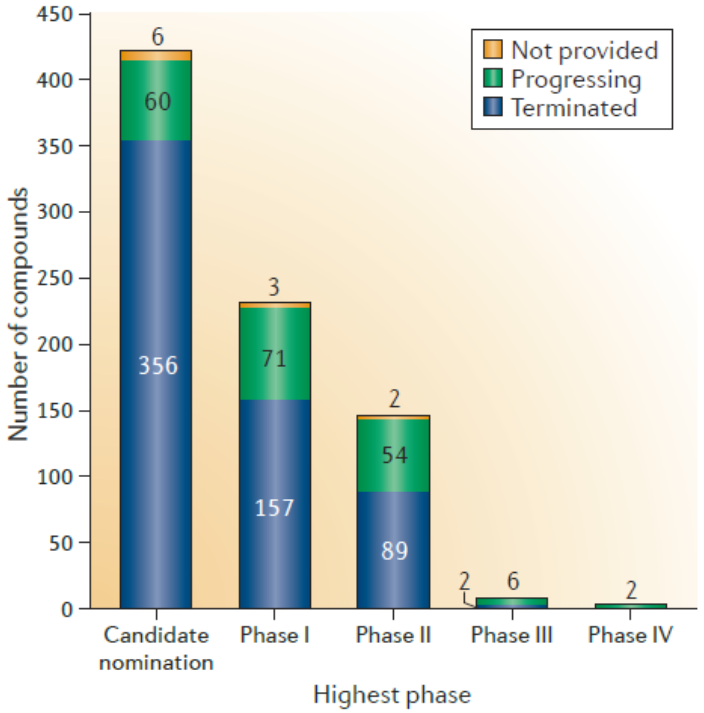
~ 15-20 years and ~ 1000 M$

Comparison of reasons for attrition

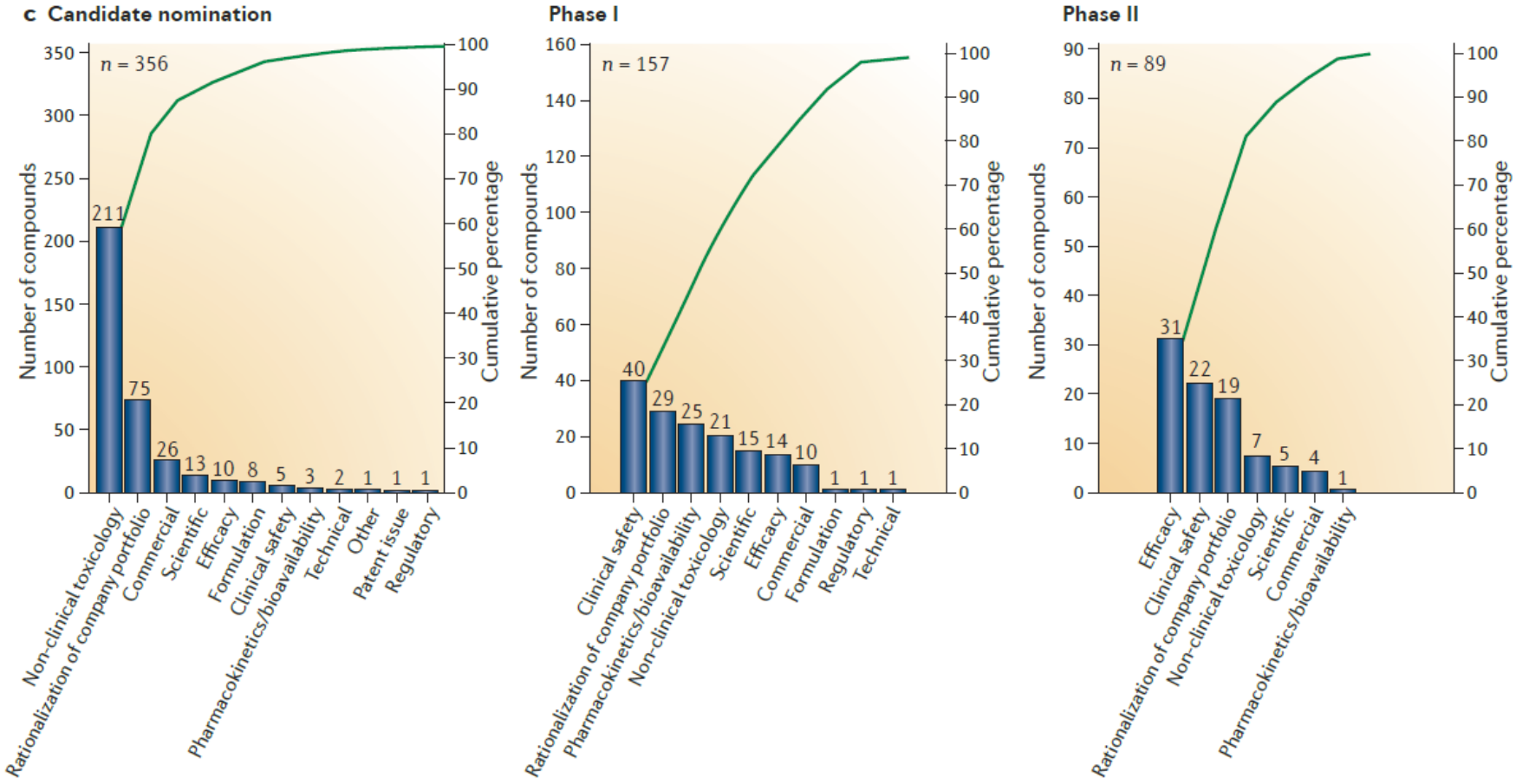


Attrition rates

- Attrition of drug candidates in clinical pipeline is extremely high
- Fail fast policy
  - Preclinical phase
- Despite the growing efforts of analysing data of reason for attrition and growing effort of prediciton adverse effects the attrition rate is high
  - Steady state
  - Which can be interpreted as a success because of the stricter and stricter acceptance criterion



Reasons for attrition in development stages



Drug like properties

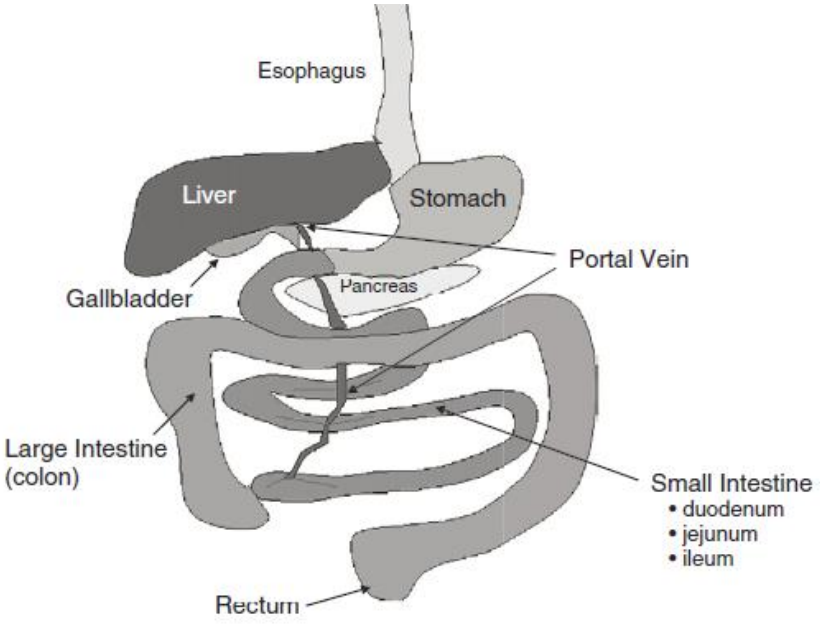
- Lipinski's rule of five
  - Based on empirical and statistical examination of oral drugs on the market
  - Not taking into account the biologocal activity
  - In general, an orally active drug has no more than one violation of the following criteria:
- No more than **5 hydrogen bond donors** (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- No more than **10 hydrogen bond acceptors** (all nitrogen or oxygen atoms)
- A molecular mass less than **500 daltons**
- An octanol-water partition coefficient (**log *P)* not greater than 5**

ADME/tox

- **A**bsorption
  - Permeability through the epithelial cell membrane
- **D**istribution
  - Distribution in the body, Blood, Different organs and tissues, Blood Brain Barrier, etc
  - Determined by using radioactive labeled compound
- **M**etabolism
  - Modification by enzymes
- **E**xcretion
  - Ellimination from the body
- **T**oxicology
  - Adverse effects

Absorption

- Mouth
  - Limited time
- Stomach
  - Relatively short time
  - Relatively small surface
- Small intestine
  - Large surface
  - Longer residence time
  - pH gradient (from acidic to basic)
- pH dependent solubility and stability is a key factor throghout the whole gastrointestinal tract on absorption
  - Neutral molecules show greater permeability than ionic compounds



Absorption cont.

- Solubility
  - pH dependent
- Stability
  - Hydrolitic processing
    - Acidic pH
  - Enzymatic processing
    - Esterase, peptidase, lipase, aldolase, dehydrogenase, phosphatase
- Permeability
  - Passive transport
- Active transport
- First pass metabolism
  - Metabolism in the gut wall
  - Liver (portal vain delivers the compound to the liver before it reaches the rest of the body)
- Efflux

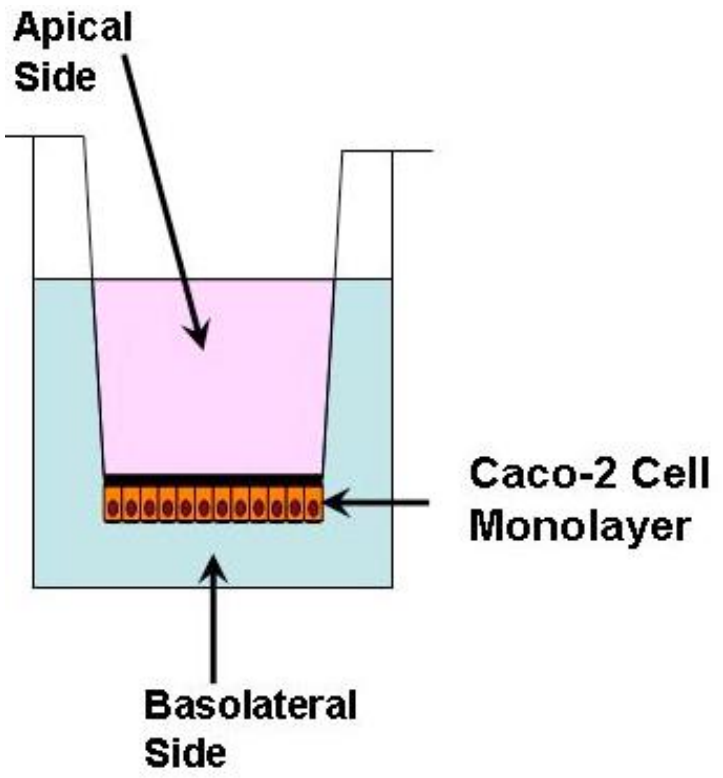
Predictive models

- Caco-2
  - The **Caco-2** cell line is a continuous cell of heterogeneous human epithelial colorectal adenocarcinoma cells
  - Although derived from a **colon carcinoma**, when cultured under specific conditions the cells become differentiated and polarized such that their phenotype, morphologically and functionally, resembles the **enterocytes lining in the small intestine**.
  - Caco-2 cells **express** *tight junctions*, a number of *enzymes* and *transporters* that are characteristic of such enterocytes
    - **peptidases, esterases, P-glycoprotein (efflux pump), uptake transporters for amino acids, bile acids, carboxylic acids**
  - Caco-2 cells are most commonly used not as individual cells, but as a monolayer on a cell culture insert filter

Caco-2 measurement

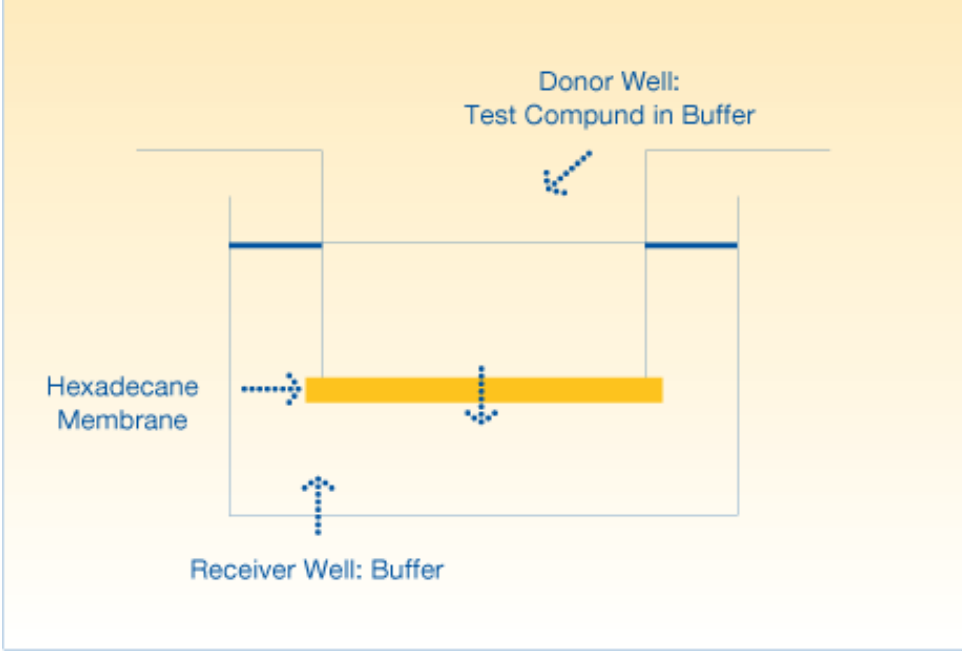
- Effective permeability (Peff) is measured in the apical -> basolateral (A/B) direction
  - Samples from the receiver side of the chamber are taken at 30, 50, 70, and 90 minutes post experiment initiation. Analysis is performed using LC-MS, HLPC-UV or LSC (liquid scintillation counter). Peff is calculated using the following formula:

- Peff (cm/sec) = $(dX/dt)/(A*C_0*60)$
  - where X = mass transported, A = surface area and C_0 = initial donor drug concentration



Predictive models

- PAMPA (Parallel Artificial Membrane Permeability Assay)
  - *In vitro* **non-cellular model** for passive transcellular permeation
  - Avoids the complexity of active transport
  - Advantage
    - Screening
    - Lower cost



Bioavailability

In pharmacology, **bioavailability (BA)** is a subcategory of absorption and is the fraction of an administered dose of unchanged drug that reaches the systemic circulation, one of the principal pharmacokinetic properties of drugs.

By definition, when a medication is administered intravenously, its bioavailability is 100%.

However, when a medication is administered via other routes (such as orally), its bioavailability generally decreases or may vary from patient to patient.

Bioavailability is one of the essential tools in pharmacokinetics, as bioavailability must be considered when calculating dosages for non-intravenous routes of administration.

Absolute bioavailability

Absolute bioavailability compares the bioavailability of the active drug in systemic circulation following non-intravenous administration (i.e. oral, rectal), with the bioavailability of the same drug following intravenous administration.

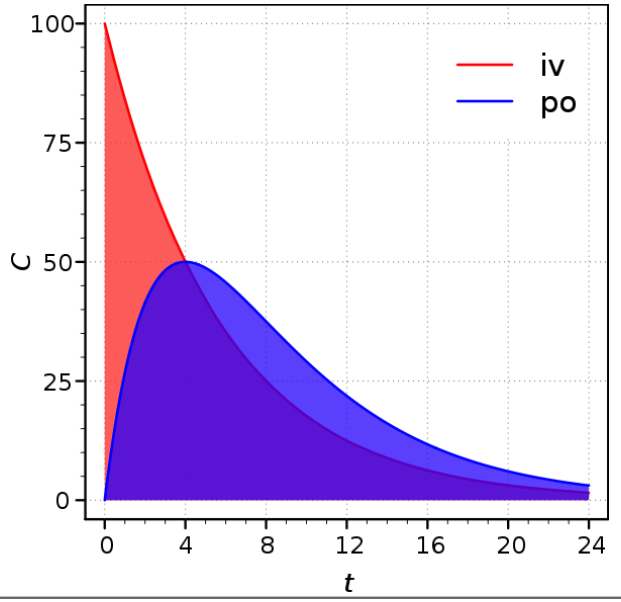
Comparison must be dose normalized.

In order to determine absolute bioavailability of a drug, a pharmacokinetic study must be done to obtain a plasma drug concentration vs time plot for the drug after both intravenous (**iv**) and extravascular (i.e. oral - **po**) administration.

Absolute Bioavailability (F_{abs}) is the dose-corrected area under curve (**AUC**) non-intravenous divided by AUC intravenous.

$$F_{abs} = 100 \times (AUC_{po} \times D_{iv}) / (AUC_{iv} \times D_{po})$$

Therefore, a drug given by the intravenous route will have an absolute bioavailability of 100% (f=1), whereas drugs given by other routes usually have an absolute bioavailability of less than one. If we compare the two different dosage forms having same active ingredients and compare the two drug bioavailability is called ***comparative bioavailability***.



Absolute bioavailability cont'

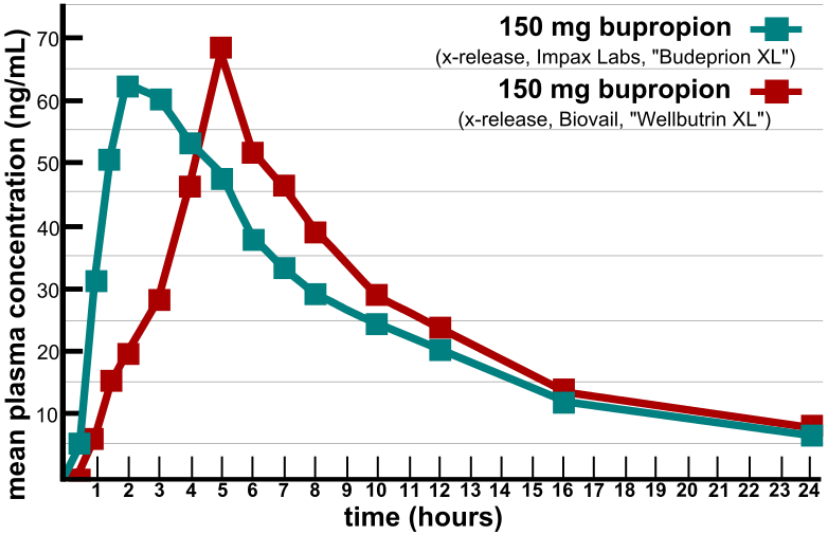
- Bioavalability determination is costly
  - Usually not performed as routine measurement or screening
  - High sensitivity detection
    - LC-MS/MS
- Usually performed on specific set of molecules
  - Affinity towards the target
  - Acceptable Caco-2 or PAMPA data
  - Acceptable metabolc liability
  - Acceptable solubility
    - Preformulation in case of low solubility
    - Suspension or solubilization
- Low bioavailability can occur sometimes despite acceptable data set on predicitve models (predictive models are quite liable in practice)

Relative bioavailability and bioequivalence

Relative bioavailability measures the bioavailability (estimated as the AUC) of a formulation (A) of a certain drug when compared with another formulation (B) of the same drug, usually an established standard, or through administration via a different route. When the standard consists of intravenously administered drug, this is known as absolute bioavailability.

$$F_{rel} = 100 \times (AUC_A \times D_B) / (AUC_B \times D_A)$$

Relative bioavailability is one of the measures used to assess **bioequivalence (BE)** between two drug products. For FDA approval, a generic manufacturer must demonstrate that the 90% confidence interval for the ratio of the mean responses (usually of AUC and the maximum concentration, C_{max}) of its product to that of the original is within the limits of 80% to 125%. While AUC refers to the extent of bioavailability, C_{max} refers to the rate of bioavailability. When T_{max} is given, it refers to the time it takes for a drug to reach C_{max}.

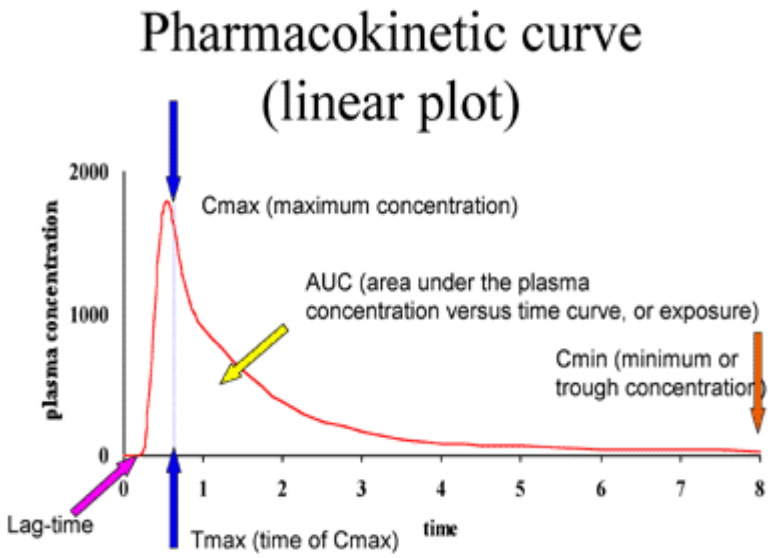


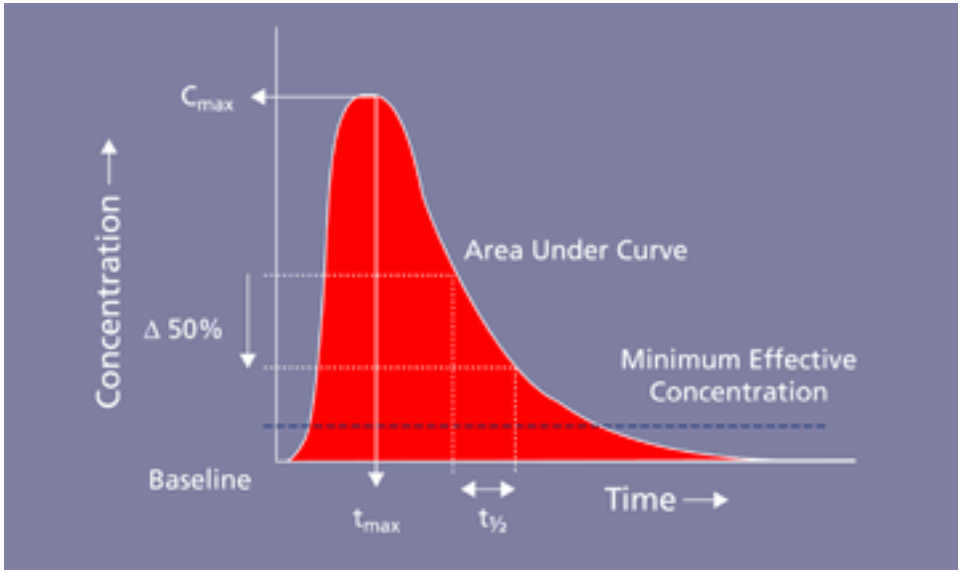
A **bioequivalency (BE) profile** comparison of 150 mg extended-release bupropion as produced by Impax Laboratories for Teva and Biovail for GlaxoSmithKline.

Pharmacokinetic metrics

Characteristic	Description	Example value	Symbol
Dose	Amount of drug administered	500mg	D
Dosing interval	Time between drug dose administrations	24h	T
C_{max}	The peak plasma concentration of a drug after administration	50 mg/L	C_{max}
t_{max}	Time to reach C_{max}	10-12h	t_{max}
C_{min}	The lowest concentration that a drug reaches before the next dose is administered	20 mg/L	C_{min}
Volume of distribution	The apparent volume in which a drug is distributed	5,0 L	VD
Concentration	Amount of drug in a given volume of plasma	100 mg/L	C_0
Elimination half-life	The time required for the concentration of the drug to reach half of its original value	18 h	$t_{1/2}$
Area under the curve	The integral of the concentration-time curve	1,50 mg/L · h	AUC
Clearance	The volume of plasma cleared of the drug per unit time	0,50 L/h	CL
Bioavailability	The systemically available fraction of a drug	0,9	f

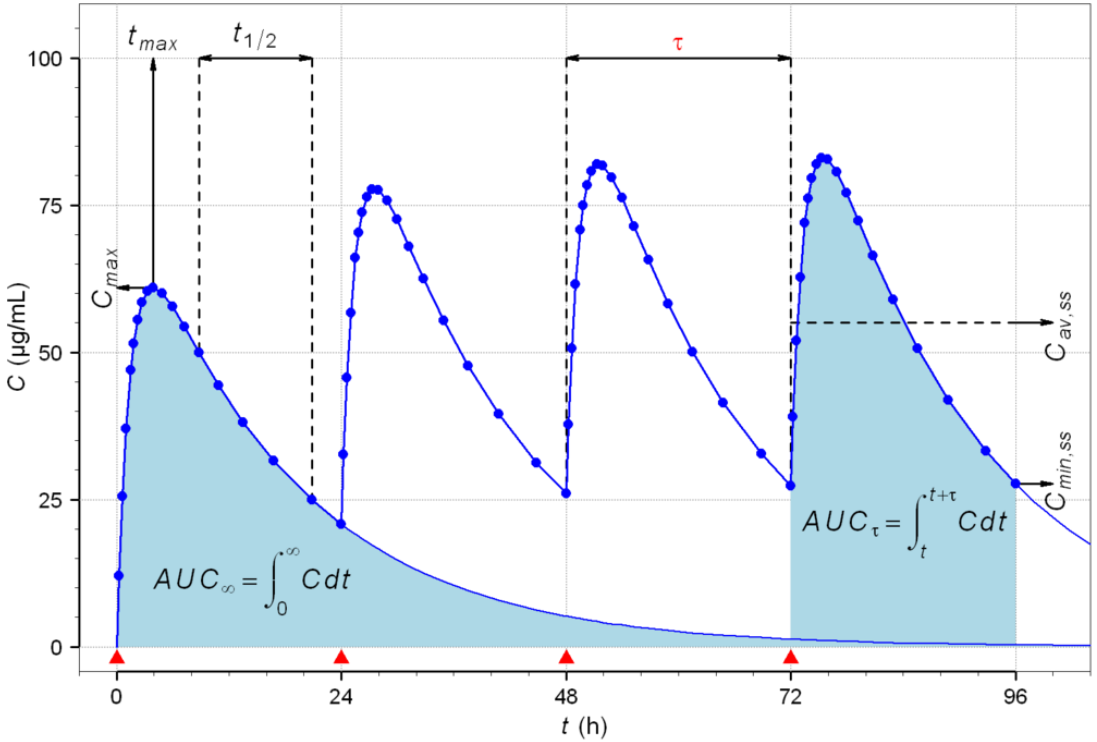
Pharmacokinetic curve and Half-life





Steady state

In pharmacokinetics, *steady state* refers to the situation where the overall intake of a drug is fairly in dynamic equilibrium with its elimination. In practice, it is generally considered that steady state is reached when a time of 4 to 5 times the half-life for a drug after regular dosing is started.



The time course of drug plasma concentrations over 96 hours following oral administrations every 24 hours. Note that the AUC in steady state equals AUC∞ after the first dose

Volume of distribution

Volume of distribution (VD) is defined as the distribution of a medication between plasma and the rest of the body after oral or parenteral dosing.

The VD of a drug represents the degree to which a drug is distributed in body tissue rather than the plasma. VD is directly correlated with the amount of drug distributed into tissue; a higher VD indicates a greater amount of tissue distribution. *A VD greater than the total volume of body water (approximately 42 liters in humans) is possible, and would indicate that the drug is highly distributed into tissue.*

Drugs with a high lipid solubility, low rates of ionization, or low plasma binding capabilities have higher volumes of distribution than drugs which are more polar, more highly ionized or exhibit high plasma binding in the body's environment.

$$VD = \frac{\textbf{total amount of drug in the body}}{\textbf{drug blood plasma concentration}}$$

Drug dosing

- Short half life
  - More frequent dosing
- Low bioavailability
  - Higher dose

Drug metabolism

Drug metabolism (xenobiotic metabolism) is the biochemical modification of pharmaceutical substances by living organisms, through specialized enzymatic systems. Drug metabolism often converts lipophilic chemical compounds into more readily excreted hydrophilic products.

These reactions often act to detoxify poisonous compounds; *however, in some cases, the intermediates in xenobiotic metabolism can themselves be the cause of toxic effects.*

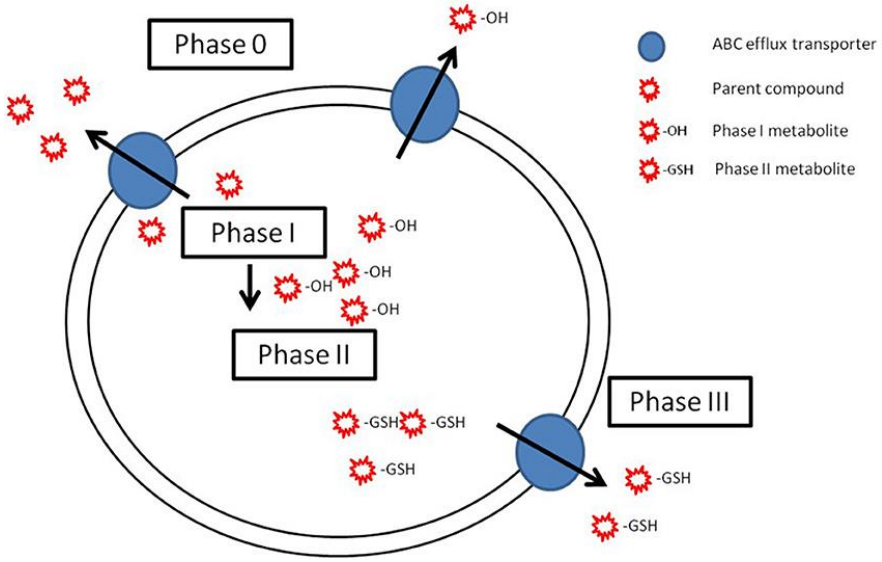
The reactions in these pathways are of particular interest in medicine as part of drug metabolism and as a factor contributing to multidrug resistance in infectious diseases and cancer chemotherapy. The actions of some drugs as substrates or inhibitors of enzymes involved in xenobiotic metabolism are a common reason for hazardous drug interactions.

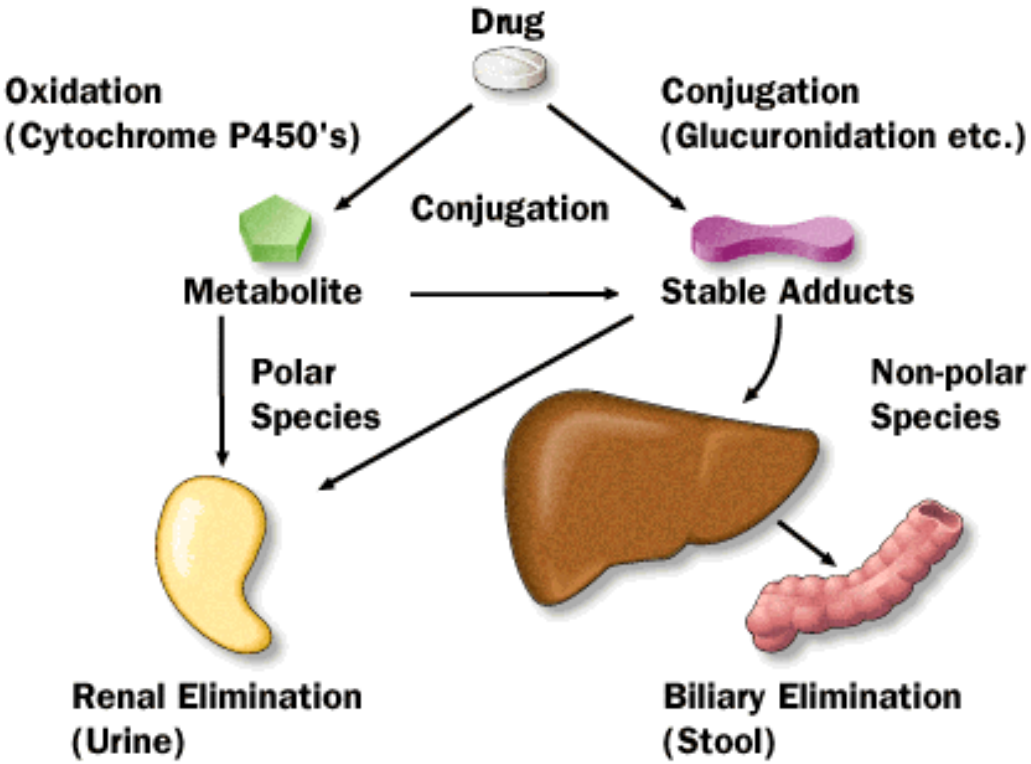
Drug metabolism is divided into three phases:

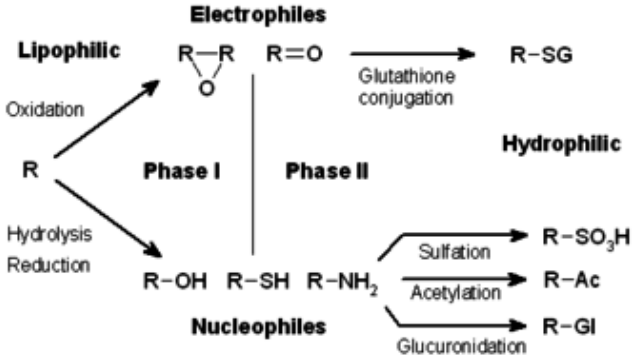
In ***phase I***, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into xenobiotics.

In ***phase II***, these modified compounds are then conjugated to polar compounds. These reactions are catalysed by transferase enzymes such as glutathione S-transferases.

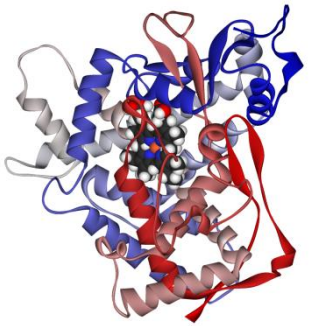
Finally, in ***phase III***, the conjugated xenobiotics may be further processed, before being recognised by efflux transporters and pumped out of cells.



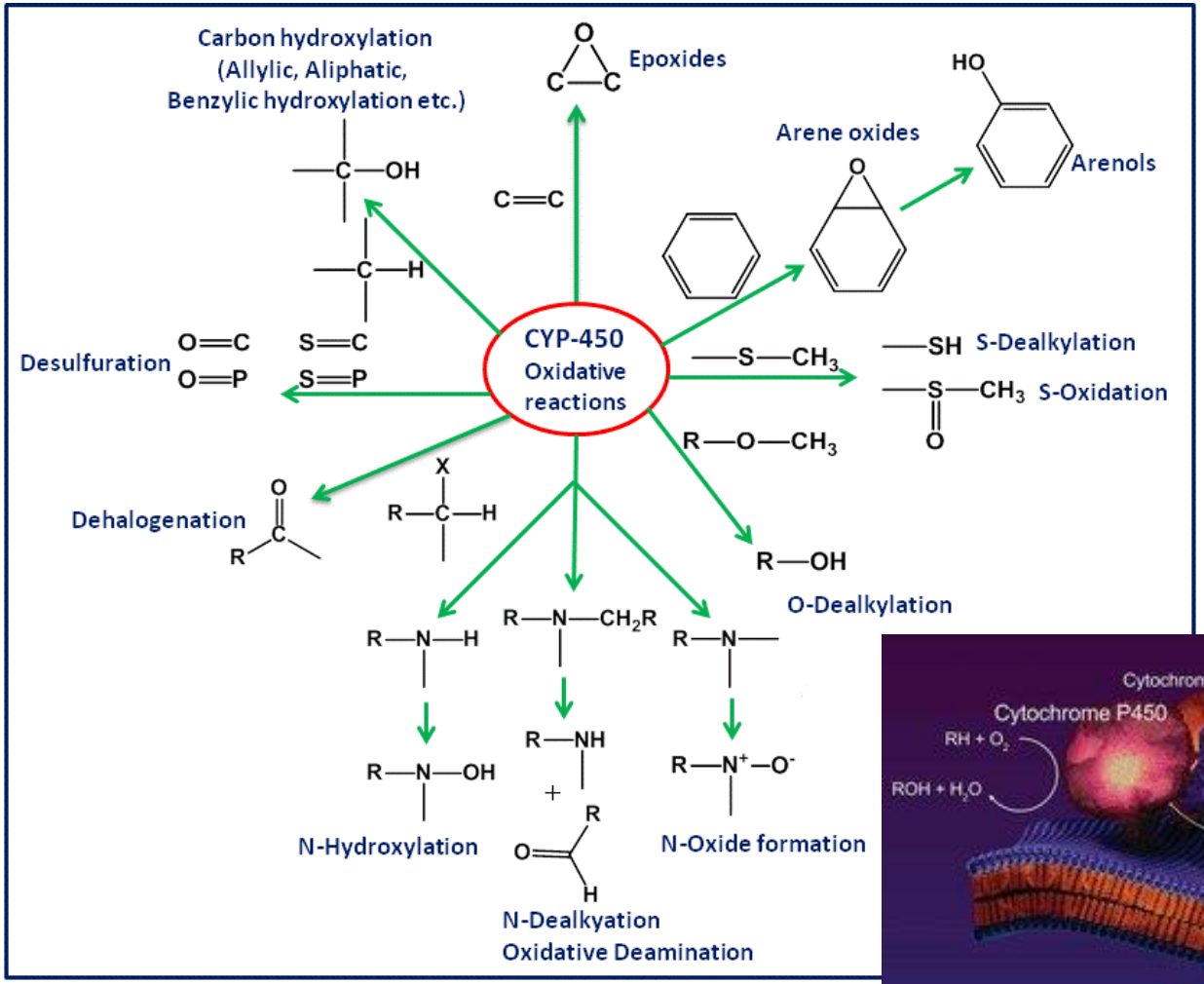

Drug
Oxidation
(Cytochrome P450's)
Conjugation
(Glucuronidation etc.)
Conjugation
Metabolite
Stable Adducts
Polar
Species
Non-polar
Species
Renal Elimination
(Urine)
Biliary Elimination
(Stool)

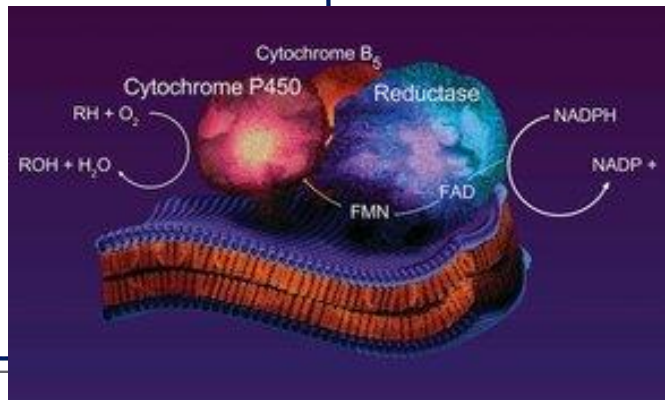

Electrophiles
Lipophilic
R—R
O
R=O
Glutathione
conjugation
R–SG
Oxidation
Hydrophilic
R
Phase I
Phase II
Hydrolysis
Reduction
R–OH
R–SH
R–NH2
Sulfation
R–SO3H
Acetylation
R–Ac
Nucleophiles
Glucuronidation
R–Gl

Phase I – CYP-450 oxidative reactions

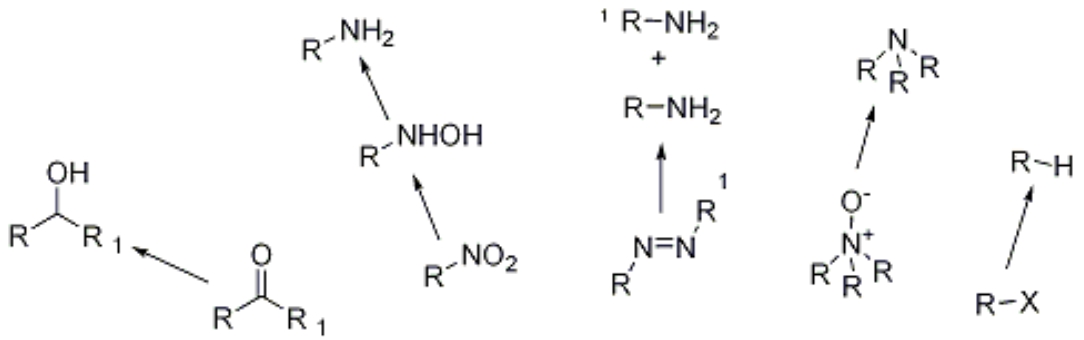


Cytochrome P450 oxidase

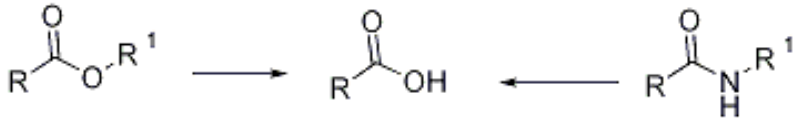




Phase I – Reduction and hydrolysis



Drug Metabolic Pathway Reduction

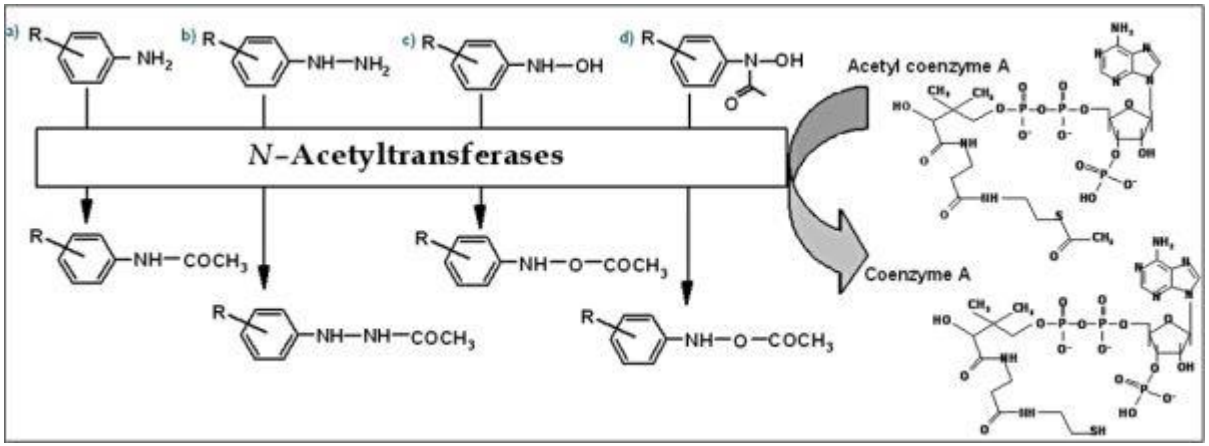


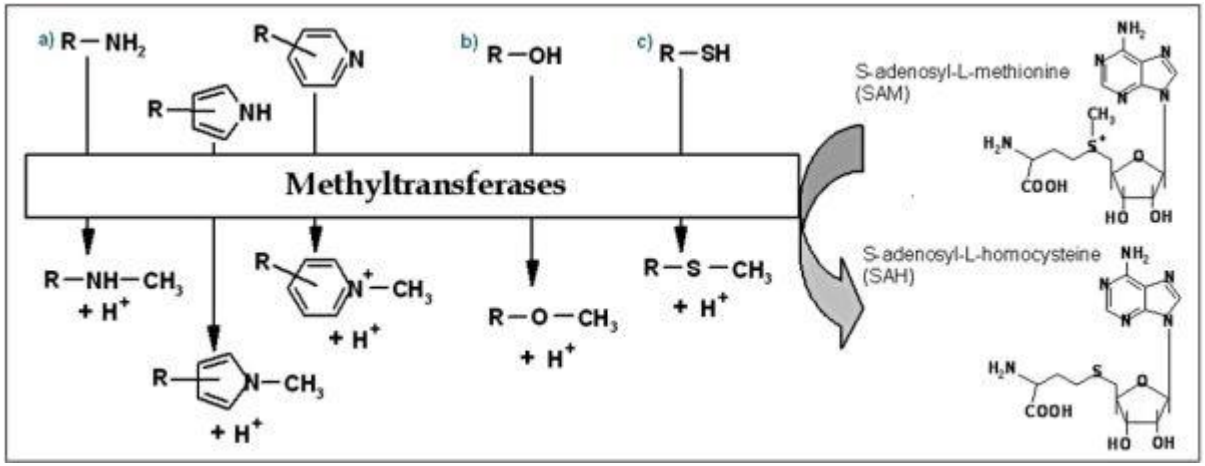
Drug Metabolic Pathway Hydrolysis

Phase II reactions - conjugation

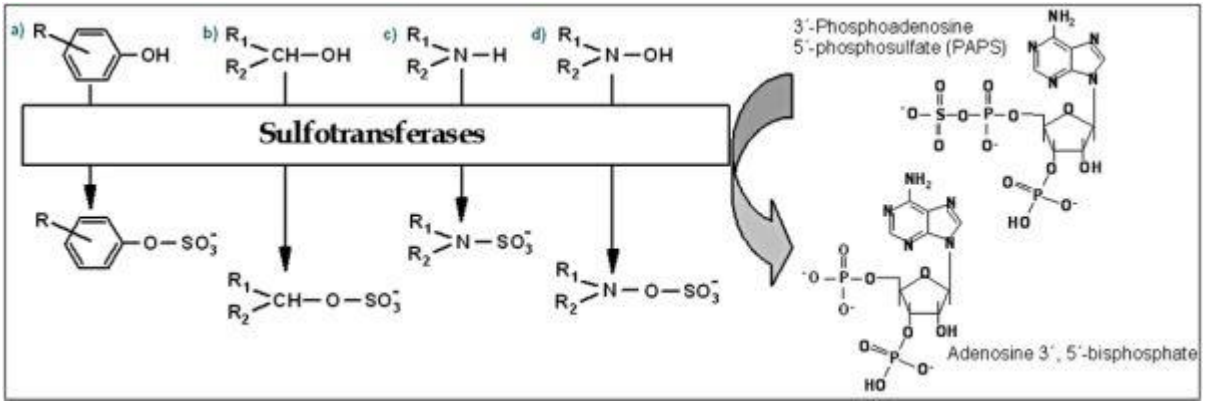
Enzymes	Cosubstrates	Functional groups
UDP-glucuronosyltransferases	UDP-glucuronide	–OH, –NH_2
sulfotransferases	PAPS (phosphoadenosine phosphosulfate)	–OH, –NH_2
glutathione-S-transferases	glutathione	epoxy groups, double bonds
acetyltransferases	acetyl-CoA	–OH, –NH_2
methyltransferases	SAM (S-adenosyl methionine)	–OH, –NH_2, –SH
epoxide hydrolase	H_2O	epoxide groups
aminoacyltransferases	amino acids	–COOH

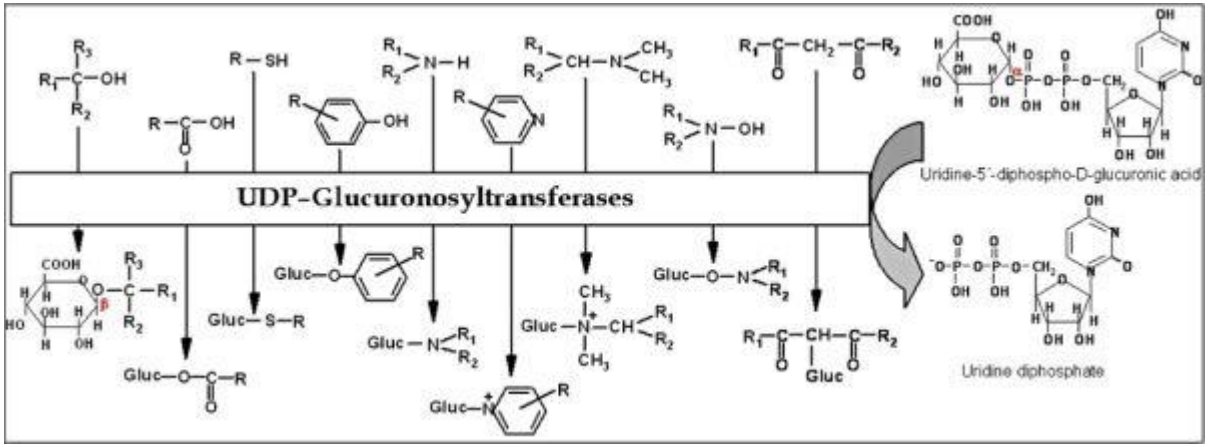
Phase II reactions – acetyltransferases and methyltransferases



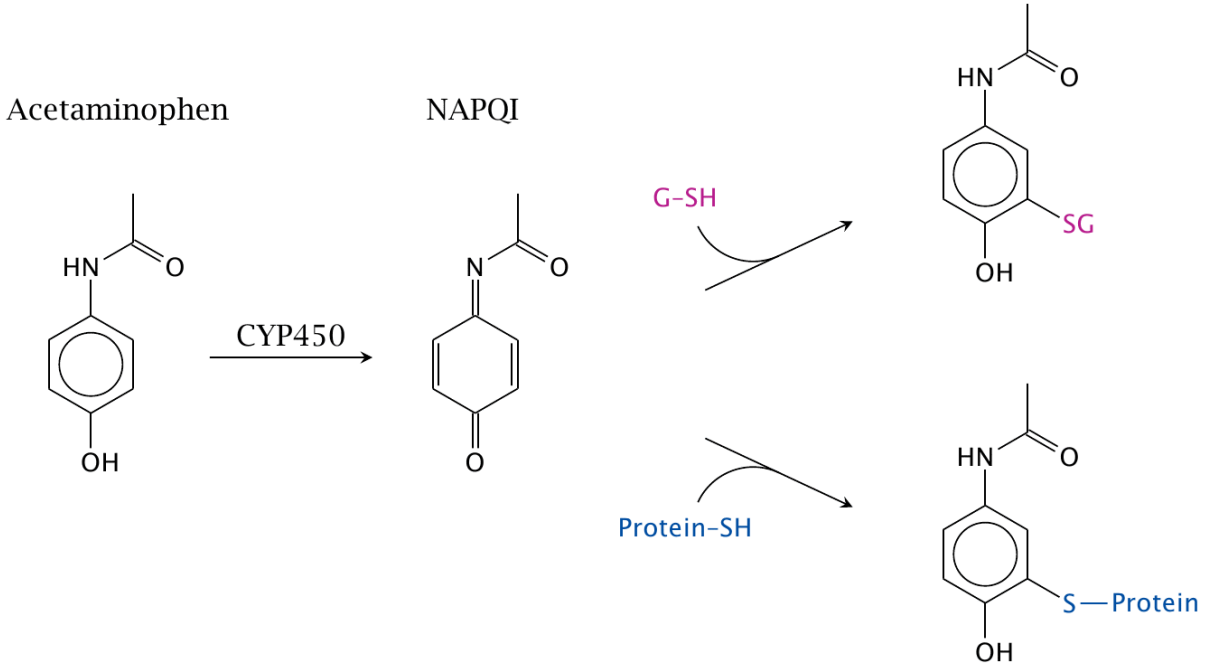


Phase II reactions - UDP-glucuronosyltransferases and sulfotransferases





Example – Metabolism of acetaminophen



Acetaminophen also undergoes successive phase I and phase II reactions. The initial CYP-catalyzed reaction yields N-acetyl-p-benzoquinone imine (NAPQI). This molecule is also quite reactive towards nucleophiles, particularly sulfhydryl groups. ***Glutathione*** is the most abundant intracellular thiol, and while supplies last will neutralize most NAPQI. However, once glutathione has been depleted, NAPQI will start reacting with cellular macromolecules and cause cytotoxicity. This mostly affects the liver, since it has the highest activity of cytochrome P450 enzymes and therefore will produce the most NAPQI.

Acetaminophen is well tolerated when applied at dosages that will not deplete glutathione. However, it turns toxic rapidly once the safe dosage limit is exceeded.

Clearance

Clearance is a pharmacokinetic measurement of the volume of plasma that is completely cleared off of a substance per unit time (ml/min).

Total body clearance = renal clearance + hepatic clearance + lung clearance

For many drugs the clearance is simply considered as the renal excretion ability (*the rate at which waste substances are cleared from the blood by the kidney*). In these cases clearance is almost synonymous with renal clearance.

Each substance has a specific clearance that depends on its filtration characteristics.

Clearance is a function of glomerular filtration, secretion from theperitubular capillaries to the nephron, and re-absorption from the nephron back to the peritubular capillaries.

It can refer to the amount of drug removed from the whole body per unit time, or in some cases the inter-compartmental clearances can be discussed referring to redistribution between body compartments such as plasma, muscle, fat.

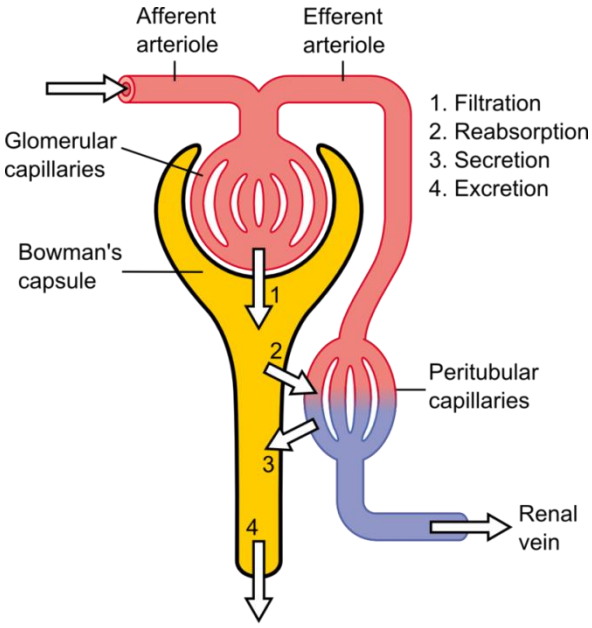


Diagram showing the basic physiologic mechanisms of the kidney

Toxicity

- Toxicity is a degree of adverse effects caused by a compound to
  - Living organism
    - Like animals (pesticides), plants or bacteria (antibacterial agents)
    - Often species dependent
  - Organs
    - Like hepatotoxicity
  - Cells
    - Cytotoxicity
- Toxicity is dose dependent
- Examples for categories
  - **Respiratory sensitizers** cause breathing hypersensitivity when the substance is inhaled.
  - **Skin sensitizers** cause allergic response from a dermal application.
  - **Carcinogens** induce cancer, or increase the likelihood of cancer occurring.
  - **Reproductively toxic** substances cause adverse effects in either sexual function or fertility
  - **Specific-target organ** toxins damage only specific organs

Toxicity

- Target related
  - Difficult to predict
    - Knockout animals
    - Tool compounds (or antibodys)
- Non-target related
  - Specificity issues
  - Predictive assays
    - hERG (potassium ion channel mediating repolarization in the heart)
    - CYP inhibition
    - CEREP panels
- Other predictive models
  - MNT (micronucleus test) screening for **genotoxic** compounds
  - AMES (potential **mutagenic** compounds)
  - FETAX (adverse or toxic effect on fertility)

Toxycity

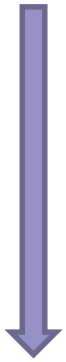
- Animal models
  - Rat
  - Mouse
  - Rabbit
  - Dog
  - Pig
  - Monkey
- Important to know some properties of the compound
  - affinity towards the same biological target in the given animal
  - Metabolism in the given animal
  - PK/PD (acute or chronic dosing)
- Predicition of the therapeutic window
- Association of toxicity with target or non target related toxicity
  - Different series of molecules (with different core structure) can be checked
    - Costly (time and money)

Optimization

- Structure Activity Relationship (SAR)
  - Based on binding affinity for the target
- Structure-Property Relationships (SPR)
  - More complex
  - Based on all properties influencing the PK

General issue of drug candidate finding

- HTS (High troughput screening)
  - Hit identification
    - Series of molecules synthesized around hits
  - Lead selection
    - Series of molecules synthesized around teh Lead
  - DC selection



Higher Mw

- Synthesized molecules are added to the compound library
  - Growing number of compounds
  - But also growing Mw of compound
- Larger and larger Hits and Leads are identified
- More and more difficult to find Drug candidates with drug like properties

Optimization for biological activity

- Ligand-Receptor affinity is high when the Gibbs energy is high for the given complex

$$G(p,T) = H-TS$$

- H: Enthalpy factor
  - Interactions between the ligand and the receptor
    - Strength of interactions (H-bonds, Van der Waals,)
  - Interactions between the ligand and media
    - Removal of non-wanted interactions
- S: Enthropy factor
  - Minimizing of the flexibility of the ligand molecule

Properties
difficult to influence

- Active transport
- Efflux pumps
- Plasma Protein binding
- Enzymatic degradation in the GI tract
- Plasma enzyme hydrolizys
  - Pro-drugs

Optimization of properties

Low absorption, owing to low solubility or permeability

High clearance, owing to metabolism

Clearance by hydrolysis in the GI tract or blood

Efflux that opposes uptake in many membranes and enhances extraction in the liver and kidney

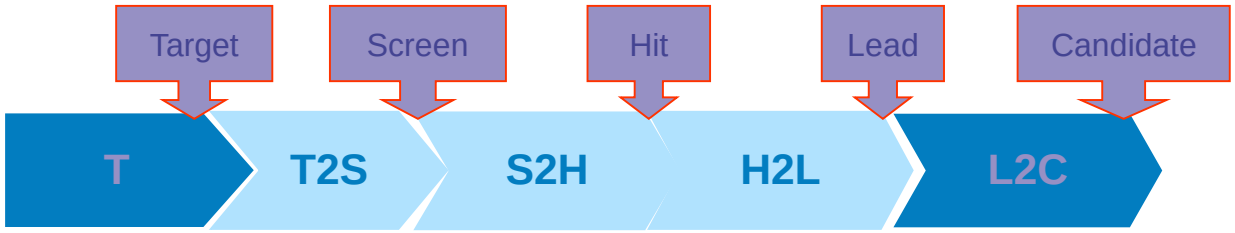
High protein binding that limits free drug at the target

Poor penetration of a blood–organ barrier at the target organ

High volume of distribution due to lipophilicity

Drug Discovery

Drug discovery stages



- Go-NoGo decisions between stages
  - Criterion setting
    - Criterion for target selection
      - Level of clinical validation
      - Innovativeness
      - Competition
    - Criterion for hits
    - Criterion for Leads
    - Criterion for Drug Candidate (DC)

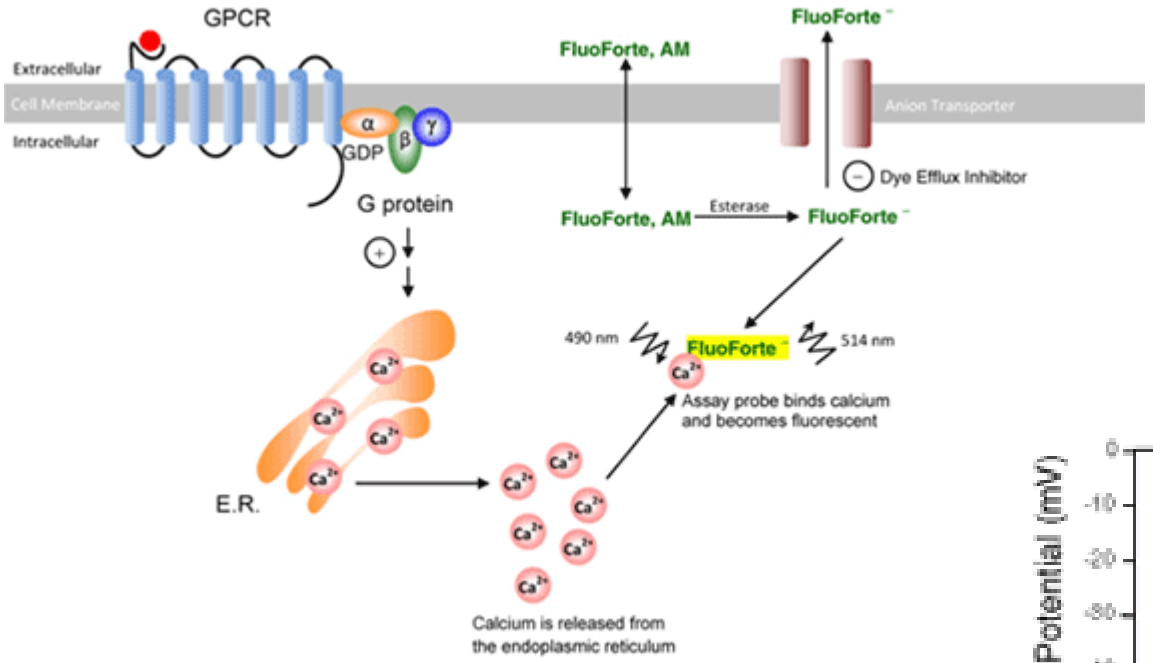
Hit finding strategies

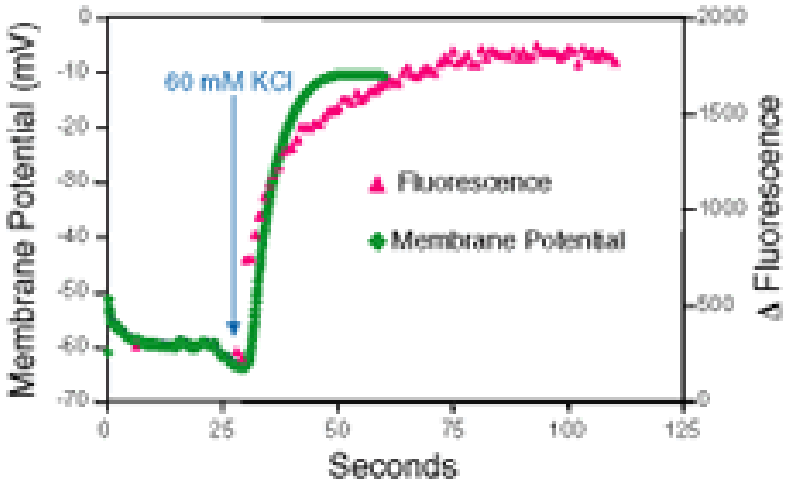
- High throughput screening (HTS)
  - Internal library
  - External libraries
    - Non-exclusivity
- Natural products
  - Analogues by design
  - Screening of smaller sets of molecule collection synthesized by parallel synthesis
    - So-called: Arrays of molecules
- Academic groups

High throughput screening

- An automated method for testing biological activity of millions of compounds in cost and time efficent way
- Robotic screening in a single dose
- Automated detection
  - Binding assay (radioactivity),
  - FLIPR (fluorimetric imaging plate reader)
  - Microscope (image analysis)
- Automated data analysis
- Timeframe 3 to 6 weeks
- Continuous evolution
  - Ultrahigh-troupghput screening 100 millions of reaction in 10 hours enabled by microfluidics

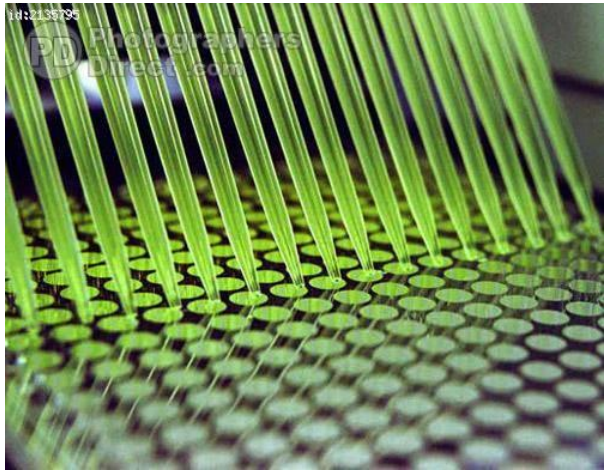
FLIPR assay


GPCR
Extracellular
Cell Membrane
Intracellular
α
β
γ
GDP
G protein
FluoForte, AM
FluoForte⁻
Anion Transporter
Dye Efflux Inhibitor
FluoForte, AM
Esterase
FluoForte⁻
490 nm
FluoForte⁻
514 nm
Ca2+
Assay probe binds calcium
and becomes fluorescent
E.R.
Calcium is released from
the endoplasmic reticulum

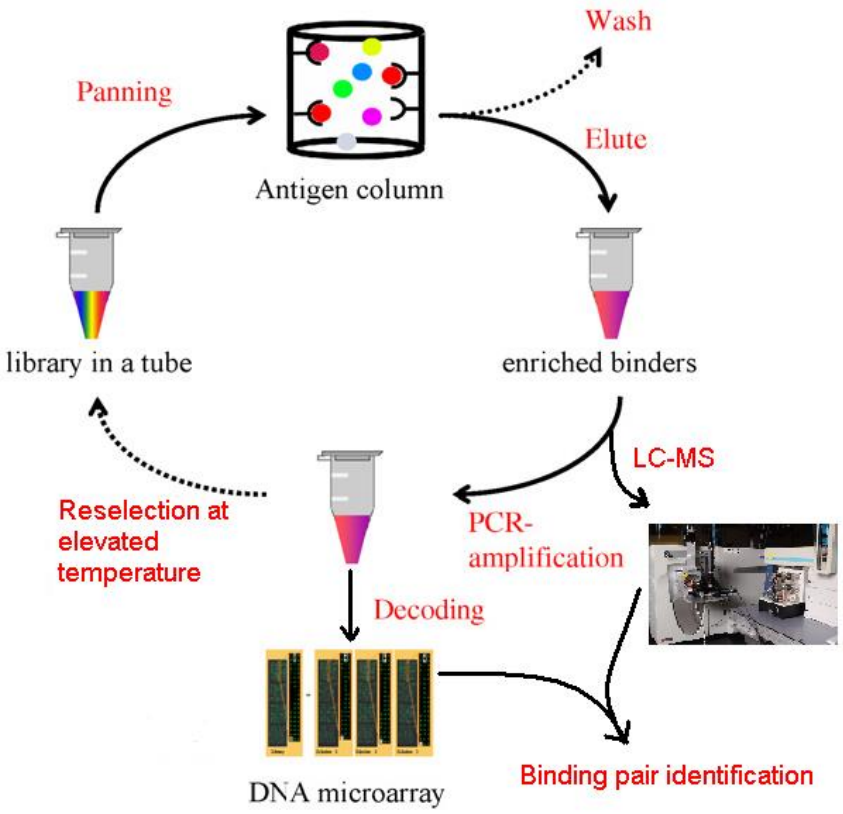

Membrane Potential (mV)
0
-10
-20
-30
-40
-50
-60
-70
60 mM KCl
Fluorescence
Membrane Potential
2000
1500
1000
500
0
Δ Fluorescence
0
25
50
75
100
125
Seconds

High throughput screening cont.

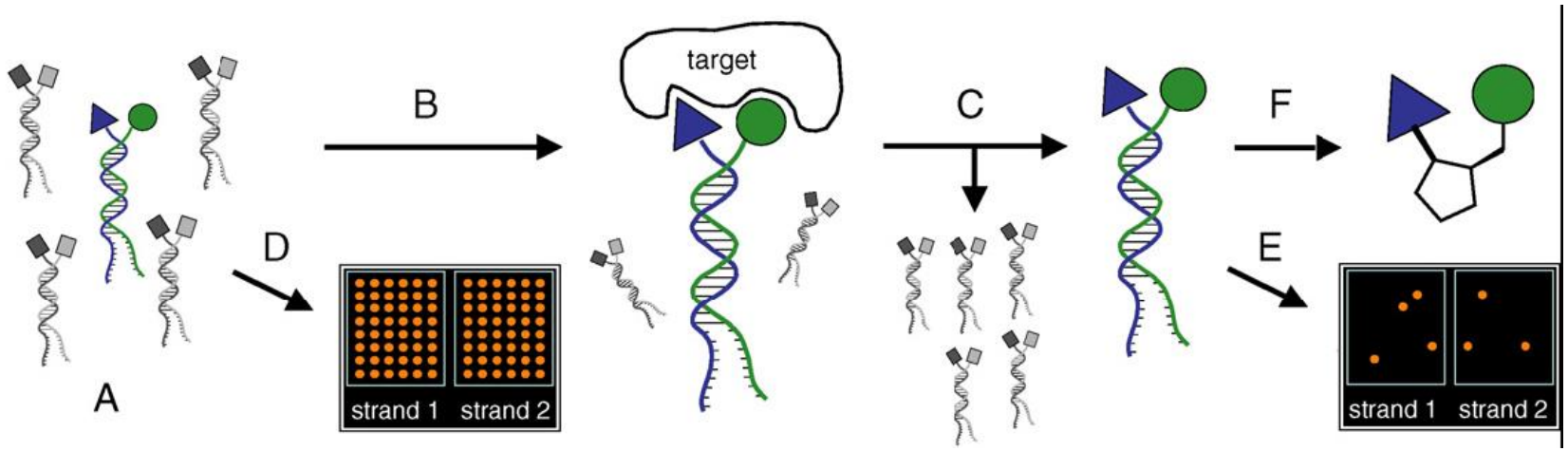
- Key elements
  - Assay suitable for automation
  - Robotic sample treatment
    - Caroussel system to store assay plates for high storage capacity and high speed access
    - Plates: multiplets of traditional 96 well-plates (384, 1536, 3456 well-plates)
  - Counter-screen
    - To filter out false positives
  - Quality control
    - High speed, automated system
  - Backscreen
  - IC50 determination
    - Multiple concentration points
    - Few hundreds to few tousands of compounds



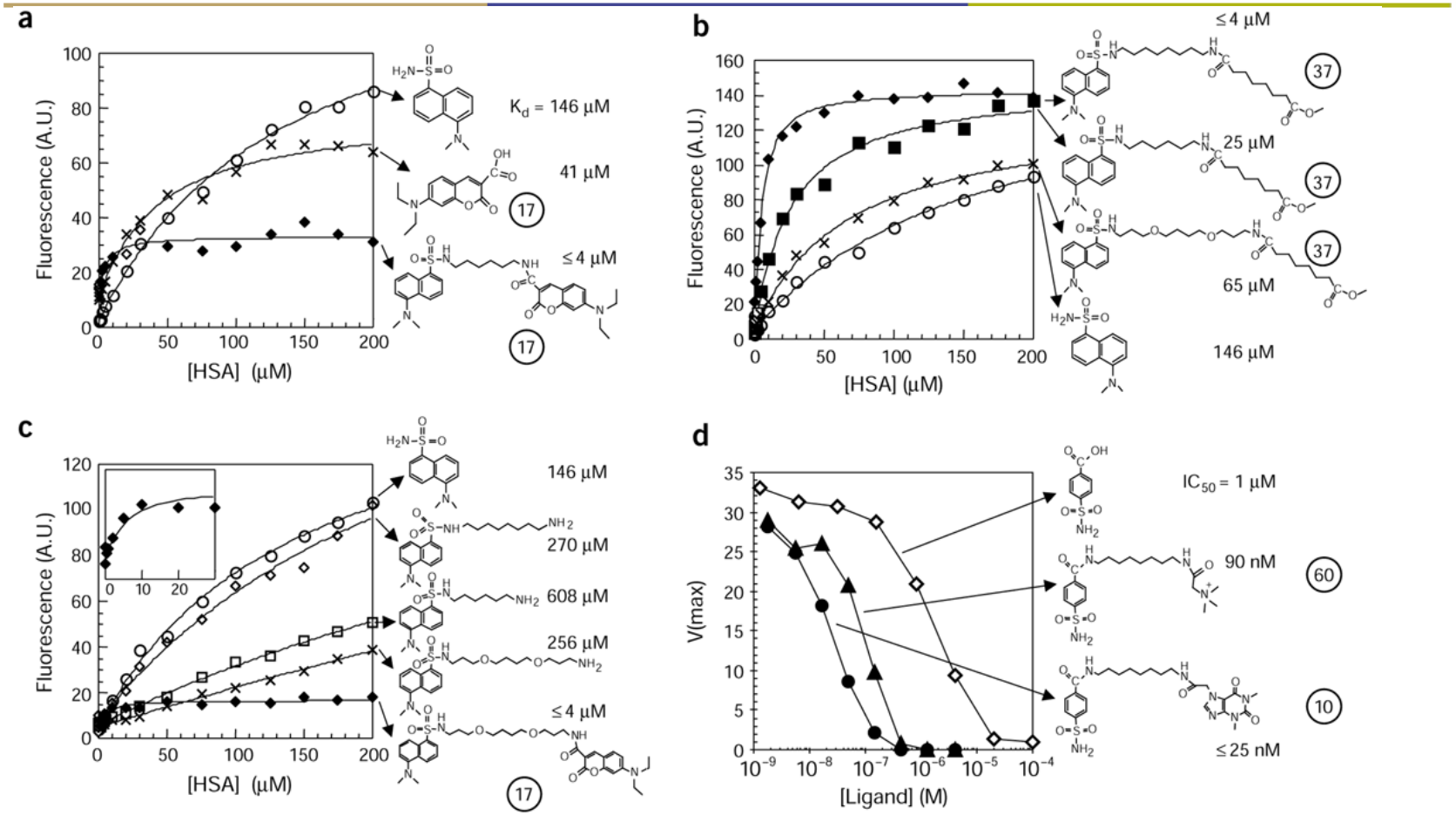
DNA-encoded libraries



DNA-encoded libraries



Examples for hits by DNA-encoded library screen



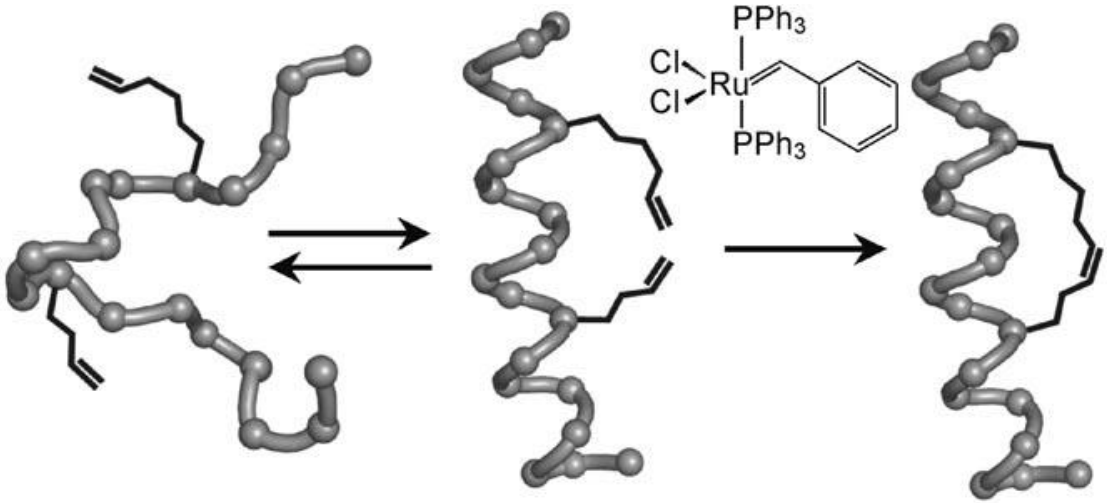
Covalent conjugation of selected binders improves the affinity towards the target

Natural products

- Taxol
  - Yew tree
- Aspirin
  - Salicilin from willow tree
  - Salicilic acid
  - Acetyl salicilin
- Penicillin
  - Isolated from Penicillium fungi
- Morfin
  - From opium which is extracted from poppy plant
- Captopril
  - Derived from Bradykinin potentiating factor

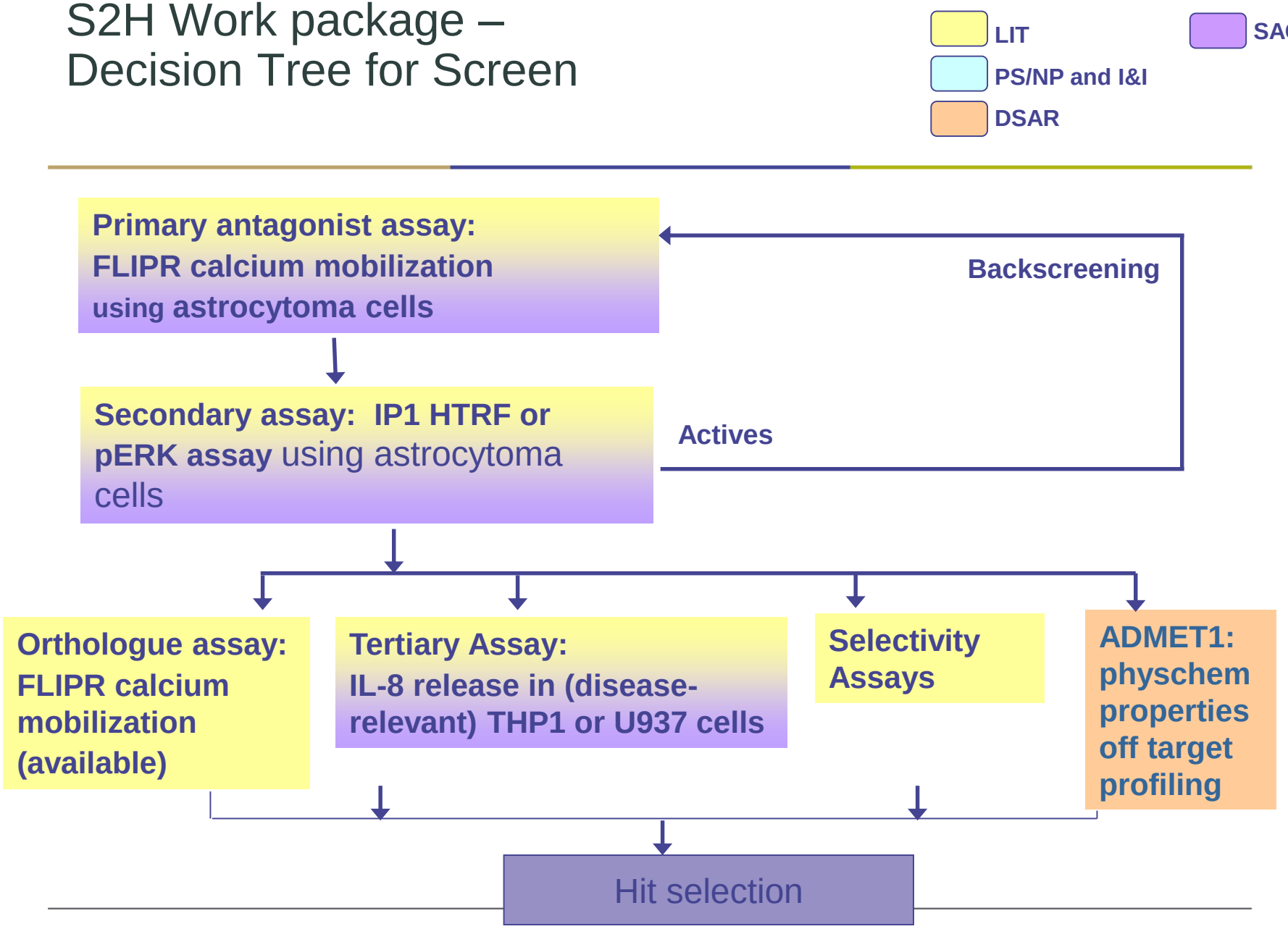
Academic groups-Example

- Protein-Protein interaction often occurs via the interaction of alfa-helical elements of the proteins
  - The truncated peptide in itself does not intend to form a stable helix and is more prone to enzymatic digestion
- A hydrocarbon cross-linking system was reported to stabilize the helical structure of the peptide together with enhancing metabolic stability



Example for a Lead generation plan

S2H Work package – Decision Tree for Screen



S2H Work package

- Compound Identification
  - Full HTS screen
  - Full HTS screen of Combichem
  - Virtual screening to be included

- Series identification, expansion and prioritisation
  - QC of all actives (AnSci)
  - Clustering and similarity search
  - Chemical backscreening of 3000+ cmpds
  - Combichem hit exploration and backscreen
  - Setup/application of selectivity assays for hit selection
    - Closest members in the receptor family
  - Phys-chem properties (solubility, chemical stability) (AnSci)
  - Assessment of major ADMET1 liabilities (DSAR)

Hit criteria

- **Active in human primary assay: IC50 < 10µM***
- **Active in secondary assay: IC50 < 10µM***
- **>= 10fold selectivity over closest members in the given receptor family**
- **Some preliminary SAR**
- **Synthetic feasibility**
- **Chemically stable cpds**
- **Assessment of chemical structure novelty**
- **Assessment of physicochemical parameters**
- **Assessment of off-target profile**
- **Assessment of ADMET liabilities**

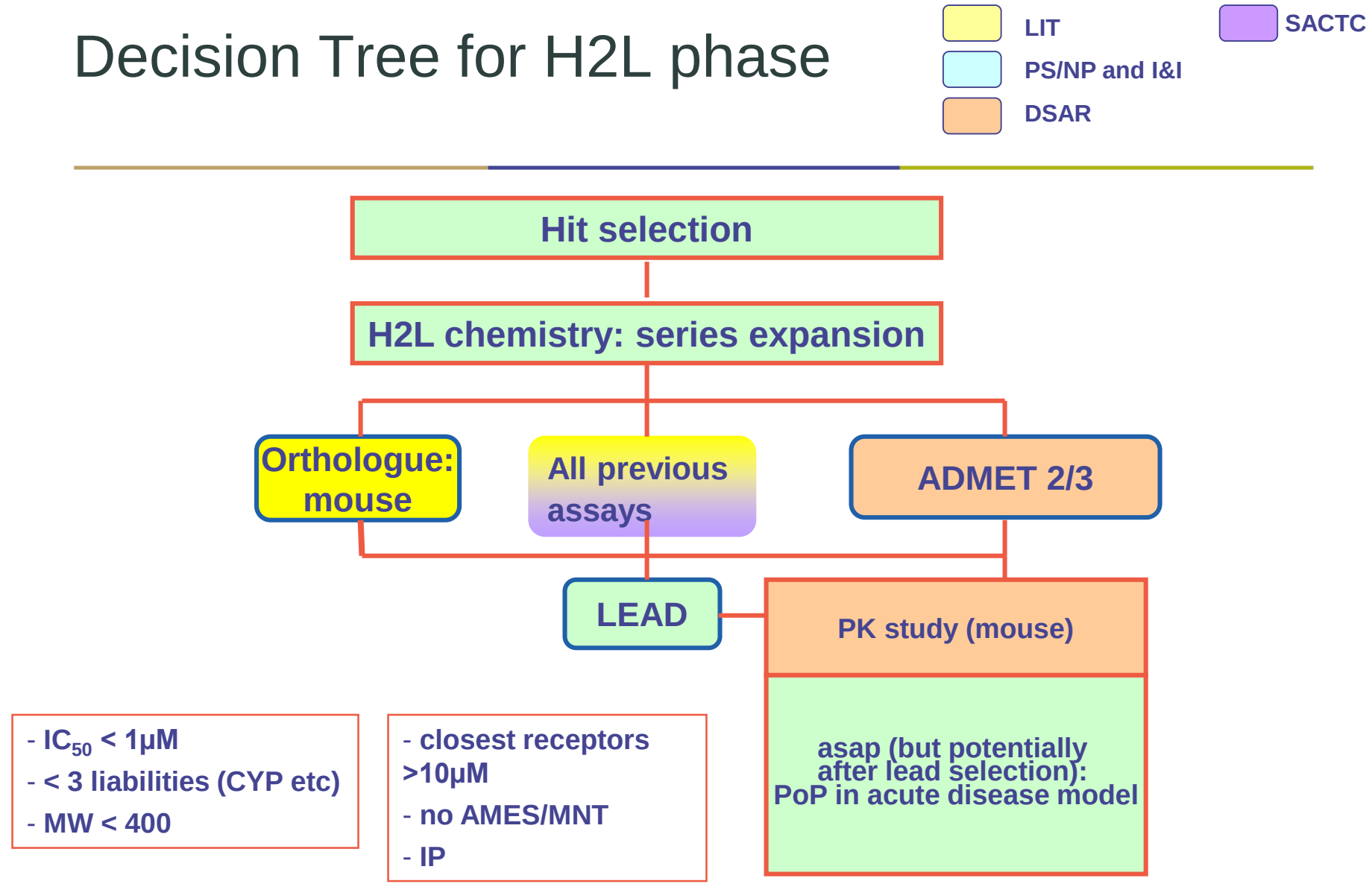
***to be reduced if assay sensitivity higher**

Work Package – Hit to Lead – H2L

- Activities to be performed
  - **Chemical synthesis**
  - **Combichem hit optimization**
  - **Biological testing**
  - **Profiling/DSAR**
  - **AMES/MNT/DSAR**

- Deliverables, Timelines and Critical Issues to Candidate Identification
  - **Lead series identification**
  - **Issues identified to fix during optimization**
    - **Proposal for early Lead optimization**
  - **Lead Review**

Decision Tree for H2L phase



Work Package – Hit to Lead – H2L

- Series expansion
  - Hit to Lead chemistry: series selection, scaffold design; HT parallel synthesis of 30-100 compounds per month at steady state
  - Biological profiling including multiple assays as in screening tree:
    - Primary assay run every week depending on # of cpds delivered
    - Selectivity assays run as needed
    - Secondary assay (IP1 assay) run as needed
    - Tertiary assay (IL8 assay) run as needed
  - Management of compound logistics
  - Phys.-chem. property measurements
  - ADMET2 by DSAR to understand relative series optimisability
  - Off-Targets-2: Specificity data on Hit analogues & Lead nomination package
- Assessment of potential to modulate critical optimisation parameters
- Patentability assessment
- Synthesis scale up of 80 mg
- ADMET3 profile & lead package by DSAR

Lead Criteria

- Active in human primary assay: IC50 <1μM*, SAR
- Active in secondary assay: IC50 <10μM*
- Specificity for target by selectivity assays (>over 10 fold difference)
- Patentability assignment by 1st Tier searches/MARPAT
- MW <= 400
- Negative MNT/AMES (“go/no go”)
- Selectivity against available CEREP/kinase panels
- < 3 Optimisation parameters for the following:
  - Solubility (pH 7.4) >10 μM from solid
  - CaCO2 permeability: high
  - Microsomal lability h/r: <40%
  - Standard CYP inhibition (IC50):>10μM; no MBI for 3A4
  - CYP induction: <40%
  - hERG: IC50 >10 μM or <50% at 1 & 10 μM
  - Adequate plasma exposure in the mouse based on compound potency

Lead optimization

Affinity improvement

- Identify the pharmacophore surface and elements
- Identify relevant strong bonds
  - Most improrant acid-base interaction H-bonds aromatic(pi-pi) interactions, apolar interactions
- Try to establish the knowledge about the sizes of binding pockets
- Identify parts of the molecules which does not take place in interaction or point towards outer surface
  - Minimize these parts
- Try to minimize flexibility of the molecule

- Key tools
  - Parallel synthesis
  - Co-crystals (crystal structure of receptor-ligand)
  - Computational methods
    - Conformations
    - Docking methods

Permeability and metabolic liability improvement

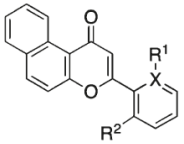
- Good correlation between calculated logD and Caco-2
  - Higher the logD higher the permeability
- Good correlation between calculated logD and metabolism
  - Lower the logD lower (better) the metabolism
    - Metabolism should be between 20% and 40%

- The issue is usually that the better the Caco-2 data the worst the metabolism

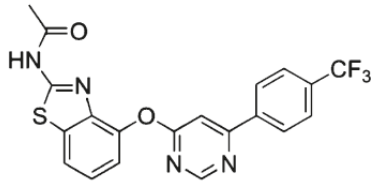
- The window where one can find good compound with high Caco-2 value and acceptable metabolism depends on chemical series or the core-structure
  - Within the given series one should establish the correlation between calc logD Caco-2 and metabolism
  - Optimization thereafter should be assisted by calculation of logD

Solubility improvement

- pH dependent
- Polarity
- H-bond donors and acceptors
  - May have negative effect on biological activity
  - May have negative effect on permeability
- Planarity of the molecule
  - Breaking planarity will improve solubility
  - Lower tendency towards stacking

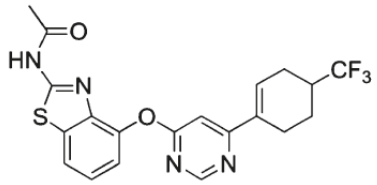


R^1	R^2	X	EROD EC_{50} (μM)	solubility[a] (μg/mL)	melting point (°C)	calcd dihedral angle[b] (deg)	CLogP[c]
H	H	C	1.4	84.6	165−167	17.8	4.7
H	Me	C	>10	262	135−137	37.9	4.9
Me	Me	C	>10	1270	92	70.0	5.1
F	H	C	0.33	153	157	9.1	4.8
F	F	C	0.20	248	150	40.5	4.9
OMe	H	C	0.27	45.8	192−193	18.5	4.1
	H	N	0.45	299	187−188	0.0	3.4



1

rat TRPV1 (CAP) IC_{50}: 0.9 nM
rat TRPV1 (acid) IC_{50}: 0.5 nM
solubility (0.01 M HCl): <1 μg/mL
CLogP: 4.6
melting point: 219-221 °C



2

rat TRPV1 (CAP) IC_{50}: 27 nM
rat TRPV1 (acid) IC_{50}: 2.4 nM
solubility (0.01 M HCl): 13 μg/mL
CLogP: 3.7
melting point: 130-131 °C

J. Med. Chem. 2011, 54, 1539–1554

Optimization of hERG, CYP inhibition or CYP induction issues

- Similar to Caco-2 and metabolism issues
- Improve polarity
- Typically leads to a compromise between Caco-2 and CYP induction or CYP inhibition properties
- Routine measurement of CYP inh and CYP ind is typically more expensive and cycle time is loger than measuring metabolic liability

- The best solution in most cases to move on to a back-up series

DC criteria

- **Patent protection**
  - Search results (closest prior art) and follow-up plans
  - Position paper from patent department
- **Critical evaluation of the synthetic procedure including: -**
  - Yields
  - Identification of potentially limiting issues e.g. COG,
  - FTO of intermediates
- **Initiate salt screening as early as possible**
  - Perform screening on multiple candidates prior to identification of the final DC
  - DC Nomination is not contingent on identification of final salt form but preferred

DC criteria

- **Structural characterization**
  - **NMR,**
  - **LCMS**
  - **HRMS & MS/MS**
  - **IR**
  - **Single crystal XRD**
- **Elemental Analysis**
  - **CHN**
  - **KF – water content**
  - **counterions**
- **Chemical purity**
  - **RP-HPLC, 220 nm**
- **Enantiomeric purity (if applicable)**
  - **Chiral HPLC**
- **Specific rotation**

DC criteria

- **Measured Physico-chemical properties (preferred values)**
  - **Molecular Weight (<500)**
  - **logP (<5)**
  - **LogD (in the range 1-3)**
  - **pKa – Measured but no specific value preferred**
- **Equilibrated solubility (in H_2O, pH 1.1, 4.5, 6.5 & 7.4; other GRAS vehicles used in biological experiments based on the DSAR agreed list; other media and/or pH's could be tested according to the program needs). Preferred solubility >1mg/ml in any one aqueous media between pH 1 – 7.4**
  - **Water**
  - **HCl 0.1N**
  - **acetate pH 4.5**
  - **50 mM phosphate pH 6.5**
  - **50 mM phosphate pH 7.4**
  - **Vehicles used in biology**

DC criteria

- **Chemical stability (same media as for solubility; with co-solvent for insoluble drugs)**
  - **Protected from daylight at t0, 12h, 24h & 48h @ RT, (<2% decomposition preferred; >5% unacceptable)**
  - **In daylight (photo stability) at t0, 12h, 24h & 48h @ RT, (<2% decomposition preferred; >5% unacceptable)**
  - **If compound degrades in the 2 - 5% range repeat at 37°C (>5% decomposition unacceptable).**
- **Chiral stability, if applicable (same media as for stability; with co-solvent for insoluble drugs)**
  - **After 48hrs, RT, protected from light (<2% decomposition preferred; >10% unacceptable).**
- **Solid state characterization**
  - **XRPD**
  - **optical microscopy**
  - **DSC & TGA**
  - **DVS**

DC criteria

- **Solid state stability**
  - **Physical stability of the solid by TGA/DSC/XRPD (<2% decomposition preferred; >5% unacceptable)**
  - **Chemical stability of the solid by HPLC**
  - **Chiral stability of the solid by HPLC/SFC if applicable**
  - **Photo stability by HPLC**
- **Intestinal permeability *in vitro* (Caco-2/TC-7)**
  - **Preferred $P_{app} \geq 20x10^{-7}$cm/s**
- **Pharmacokinetics in species used for pharmacological evaluation (doses, form, vehicle and route of administration to mirror those used in pharmacology)**
- **Inter-species comparison of liver microsomal metabolism (pharmacological and toxicological species versus human). Preferred range for human is 20-40% metabolized.**
- **Plasma stability (>20% Degraded after 4h @ 37°C is unacceptable)**
- **Determination of metabolic clearance in human hepatocytes (Preferred range 0.12-0.04 ml*hr-1*10-6 hep)**

DC criteria

- **Contribution of CYP2D6 and -3A4 to overall metabolic clearance in human hepatocytes and supersomes (under standard s-a conditions >75% contribution of CYP3A4 or >50% contribution of CYP2D6 is unacceptable).**
- **Competitive inhibition of CYP2C9, CYP2D6 and CYP3A4 on pooled human microsomes**
- **CYP2B6 and 3A4 induction using human hepatocytes (For 3A4 Imax >40% is unacceptable)**

DC criteria

- ***In vitro* Pharmacology (Receptor, human and ortholog)**
  - **Primary assay: binding EC_{50} in human and ortholog**
    - **At least a rough idea of k_{off} from radioligand binding assay (~zero, fast, slow..,)**
  - **Secondary assay: 1-2 of applicable signaling assays:**
    - **cAMP**
    - **calcium release**
    - **MAP kinase**
    - **GTP-gammaS**
    - **Beta-arrestin**
    - **Mechanism from signaling assay – competitive-noncompetitive/agonist- antagonist/partial agonist/inverse agonist**
  - **Tertiary assay: Phenotypic assay in native cell with native receptor or signaling assay**

DC Criteria

- ***In vivo* Pharmacology**
  - **Demonstration that the compound impacts an acute, *in vivo* response linked to the target.**
  - **Supporting PK is required to aid with dosing regimen and data interpretation (i.e., PK/PD).**
  - **Pharmacological profile in model(s) predictive of the targeted therapeutic indication(s) (Prophylactic and/or therapeutic regimen) by the proposed clinical route and using pharmaceutically acceptable vehicle.**
    - **When tools are available (e.g. knock-out mice), studies demonstrating that the compound exerts its *in vivo* (acute mechanistic or disease models) effects through the proposed target should be performed.**
  - **Supporting PK is required to aid with dosing regimen and data interpretation (i.e., PK/PD).**
- **SAFETY STUDIES**
  - **In Silico Evaluation**
  - **Genetic Toxicology**
    - **AMES II**
    - **In vitro MNT**

Thank you