Small Molecule Development

From Inception to Market

MacMillan Group Meeting
1-11-12
by
Anthony Casarez

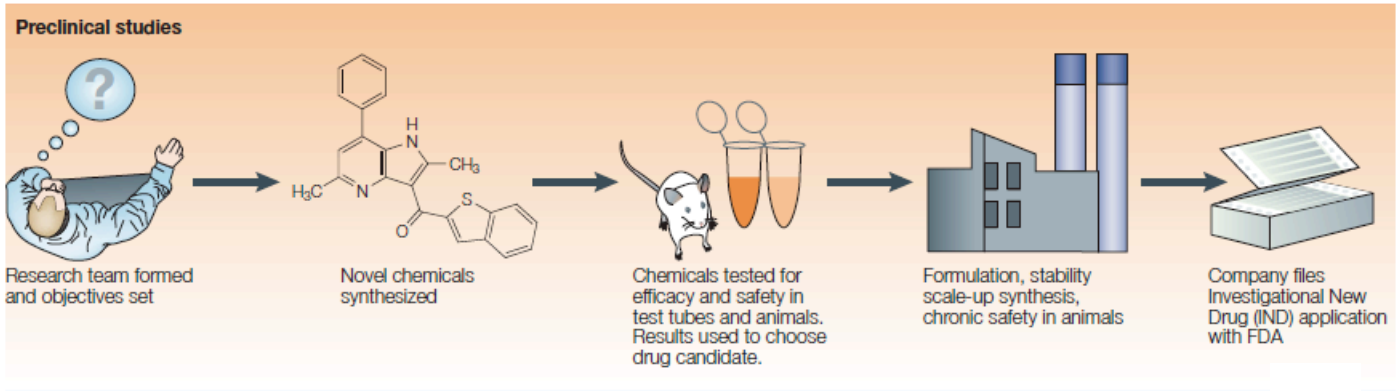
Major Objectives

- Familiarize you with the process of drug discovery and development.
- Provide detail about optimization parameters.
- Frame the medicinal chemist's role in the process.
- Relay opinions of leaders in the field regarding the future and direction of drug discovery.

Overview

The process can be divided into two major portions

- Preclinical



Lombardino, J.; Lowe, J. A. *Nature Rev. Drug Discov.* **2004**, *3*, 853.

Overview

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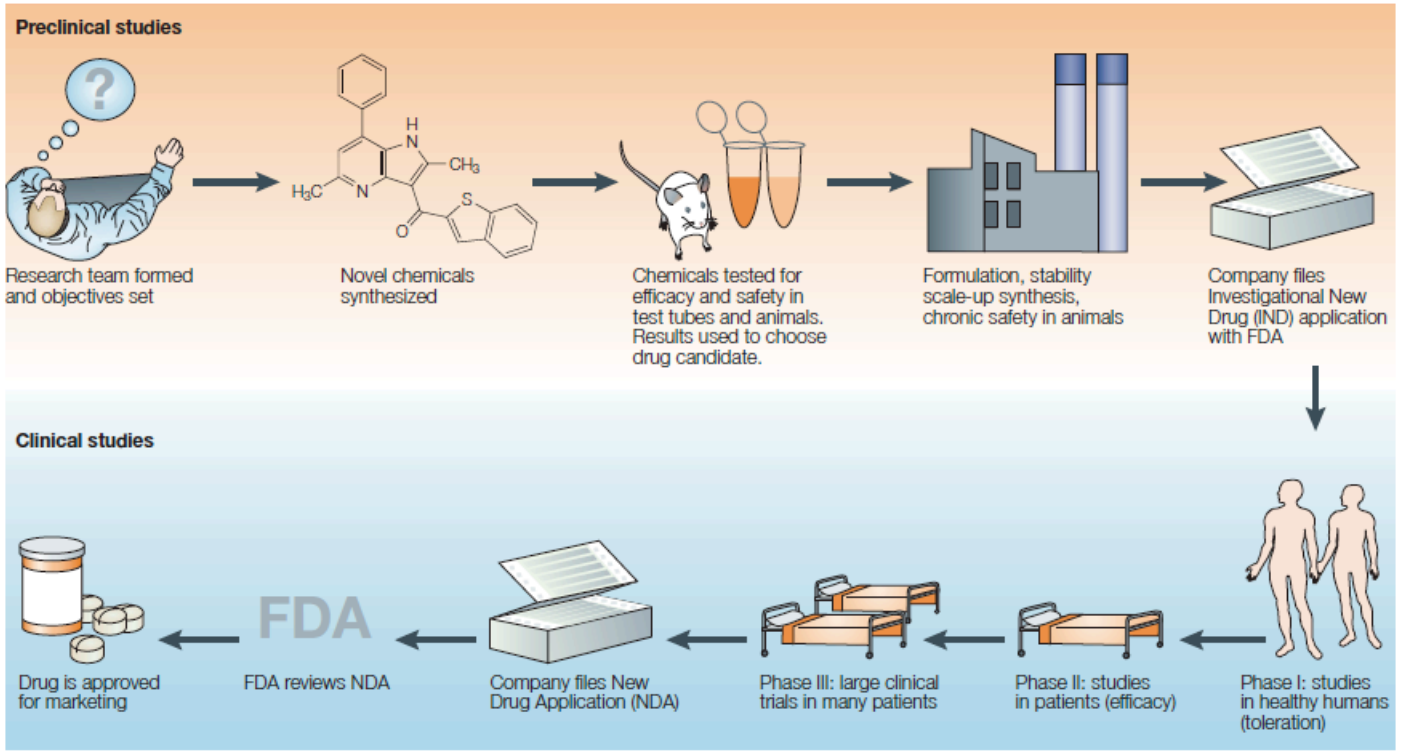
- Preclinical
- Clinical



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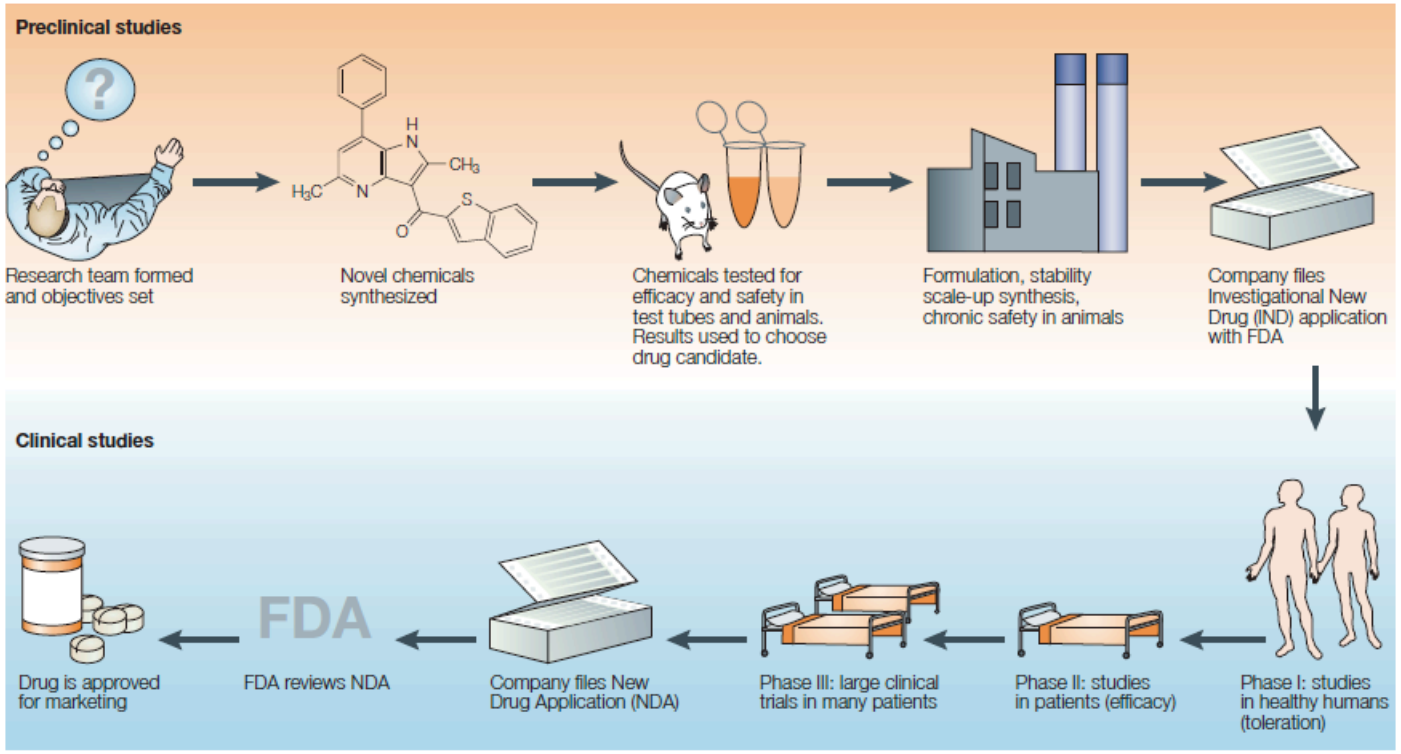
First Step

Target Discovery

- Preclinical



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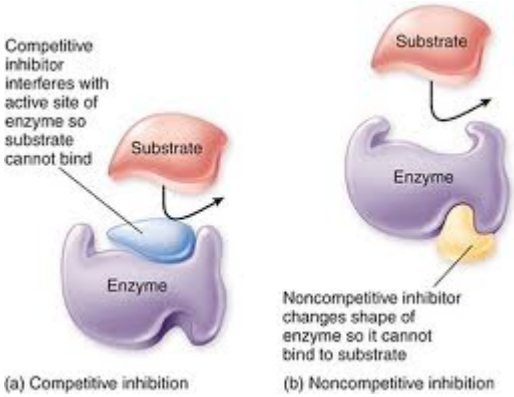
Target Identification

Target Discovery

- Identification of a suitable target is the first step of any small molecule agonist/antagonist program.

Is the target the active site?



Is the target the allosteric site?

- What is known about the protein in question regarding the disease mechanism?

Classes of Disease mechanisms

- Genetic disorders
- Infection
- Immune/autoimmune disease
- Trauma from injury or organ failure
- Multicausal disease

Eckstein, J. Alzheimer Research Forum. http://www.alzforum.org/drg/tut/ISOATutorial.pdf (accessed Oct 2011)

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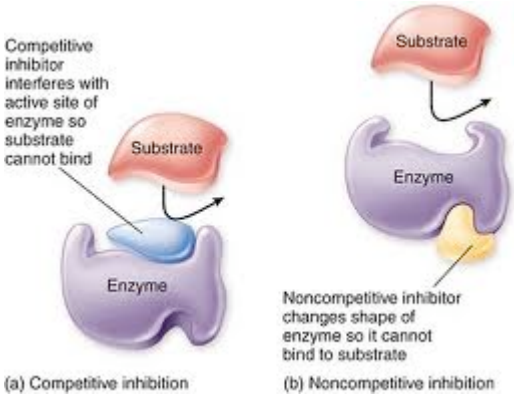

Is the target the allosteric site?

- What is known about the protein in question regarding the disease mechanism?
- Is the target "**druggable**?"

Druggability

- Access to cellular compartment
- Resistence suseptibility
- Transport mechanisms (cellular pumps)
- Side effects due to protein homology
- Toxicity from critical function inhibition

Eckstein, J. Alzheimer Research Forum. http://www.alzforum.org/drg/tut/ISOATutorial.pdf (accessed Oct 2011)

Nomenclature

Term	Definition	Therapeutic Use	Examples
Agonist	A ligand which increases the activity of a receptor, leading to increased receptor-mediated response.	To treat deficiency in endogenous agonist secretion or action (reduced receptor sensitivity) To modify the function of a tissue for symptomatic treatment.	*Insulin:* treatment of Type I diabetes. *Epinephrine:* b-adrenergic receptor agonist; smooth muscle relaxant for treatment of asthma and cardiac arrest
Inverse agonist	A ligand which decreases the constitutive activity of a receptor, leading to decreased receptor mediated response	To reduce excessive constitutive receptor activity.	*None:* Although many drugs are now known to have inverse agonist properties, there are no drugs marketed because of their inverse agonist properties.
Antagonist	A ligand which does not change the activity of a receptor but competes for residence time with the substrate in the active site	To block endogenous agonist action	*Atenolol:* b1-adrenergic antagonist used for treatment of hypertension, angina pectoris and acute myocardial infarction. *Loratadine:* H1 histamine receptor antagonist used for the treatment of allergic rhinitis
Allosteric modulator	A ligand that regulates receptor function by binding to a site distinct from that of the natural ligand.	To dampen or augment the activity of the endogenous agonist. Unlike the effect of antagonists, effects of allosteric modulators on endogenous agonist activity are saturable	*Cinacalcet:* positive allosteric modulator of the calcium-sensing receptor used for the treatment of secondary hyperparathyroidism
Functionally-selective agonist	A ligand that activates predominantly one of several responses coupled to a receptor	Improved therapeutic selectivity	*None:* Functionally selective ligands hold the promise of improved therapeutic efficacy with reduced adverse effects by targeting specific signaling pathways coupled to a single receptor subtype.

Berg, K. A.; Clarke, W. P. *Drug Discovery Today: Therapeutic Strategies.* **2009**, *3*, 4, 421.

Nomenclature

Term	Definition	Therapeutic Use	Examples
Agonist			
Inverse agonist			
Antagonist	A ligand which does not change the activity of a receptor but competes for residence time with the substrate in the active site	**To block endogenous agonist action**	*Atenolol:* b1-adrenergic antagonist used for treatment of hypertension, angina pectoris and acute myocardial infarction. *Loratadine:* H1 histamine receptor antagonist used for the treatment of allergic rhinitis
Allosteric modulator			
Functionally-selective agonist			

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Target Validation

Knockout Mice

- Transgenic mice can be a useful tool to study the function of a particular protein

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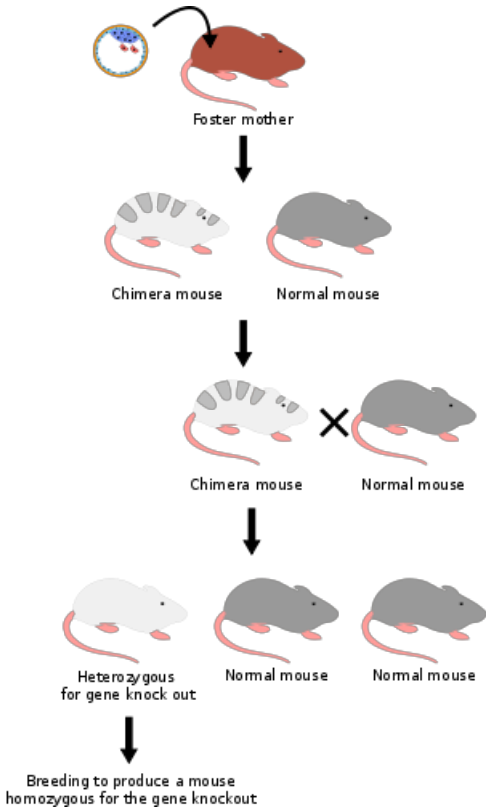
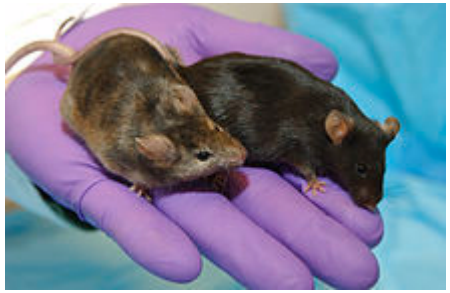


- Gene supression is indicated by a phenotypic response like hair color
- Heterozygous mice can be bred to produce homozygous mice with the gene fully knocked-out



Target Validation

Knockout Mice

- Transgenic mice can be a useful tool to study the function of a particular protein

Clinical Data

- Has the target been validated from previously developed therapeutics?
- Is there a safety or efficacy profile?

Chemical Biology

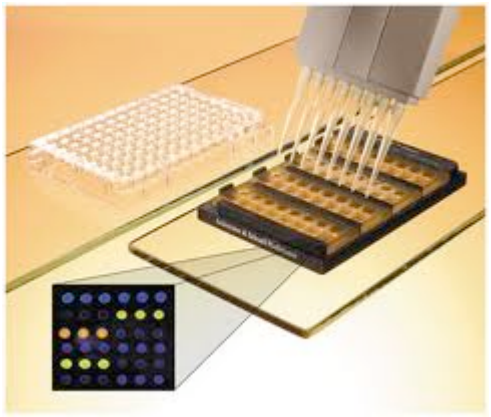
- What is known about the biological pathways of the disease?
- Has a small molecule chemical knockout produced a phenotypic response?

Eckstein, J. Alzheimer Research Forum. http://www.alzforum.org/drg/tut/ISOATutorial.pdf (accessed Oct 2011)

Assay Development

In Vitro

- Outside of the organism; isolated enzyme or cell based assays
- Monitors a surrogate readout (reporter)
- More cost effective than animal studies
- Can usually be performed in a High Throughput (HTS) Manor
- Does it correspond to the target directly?



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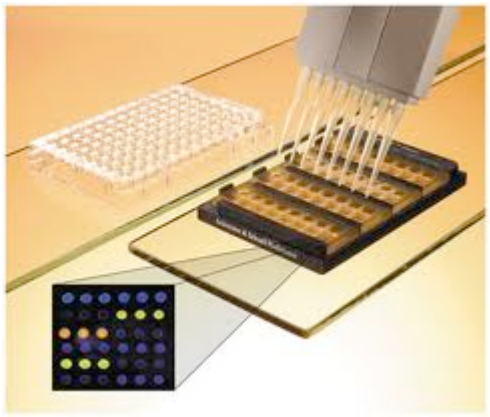

The key to successful assay is accurate target readout

Eckstein, J. Alzheimer Research Forum. http://www.alzforum.org/drg/tut/ISOATutorial.pdf (accessed Oct 2011)

Assay Development

In Vivo

- Inside the living organism
- Monitors biological efficacy and pharmacology in tandem
- The inadequacy of animal models is believed to be the major roadblock in pharmaceutical development

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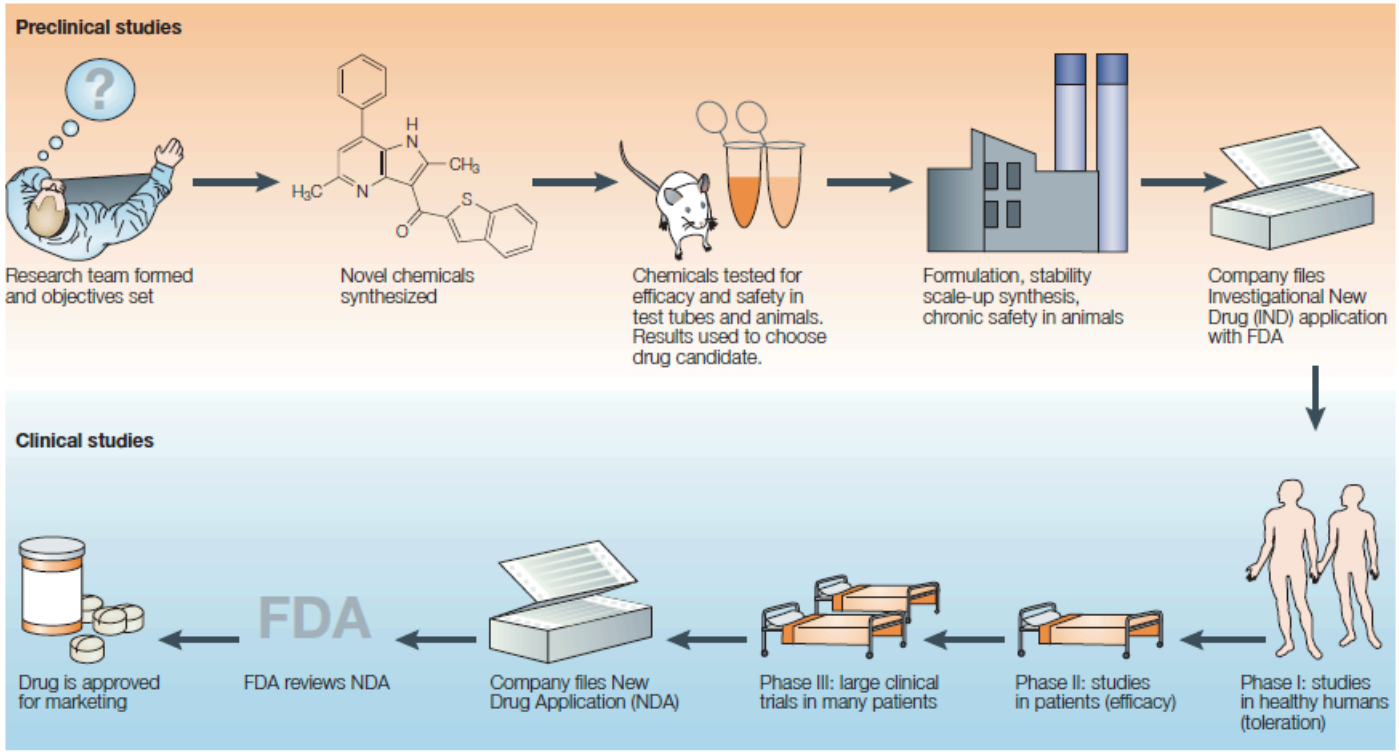
Hit to Lead

- Preclinical

Compound/Library Synthesis



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Hit Generation

High Throughput Screening

- Once a reliable in vitro assay is developed
- Aimed at rapidly screening a large compound collection(s)
- Can run 96, 384, or 1536 well plates

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Guidelines

- A large number of “hits” were coming from HTS screens that were not indicative of aqueous solubility (because they were dissolved in DMSO) which in turn possessed abysmal pharmacological properties
- The proceeding guidelines aimed to avoid producing further “hits” but more “leads”

Lipinski’s 5

- Not more than 5 hydrogen bond doners ($-NH_n$, or –OH)
- Not more than 10 hydrogen bond acceptors
- Molecular mass $\leq$ 500 daltons
- Partition coefficient (log *P*) $\leq$ 5

(substrates for biological transporters are exceptions)

- ***As these rules only address absorption, revisions have been made regarding truly successful development***

Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug. Deliv. Rev.* **1997**, *23*, 3.

Guidelines Revised

- Following **Lipinski's rules** for drug design has **significant limitations**
- **Rotatable bonds and polar surface** area are now commonly **included** in the guidelines
- Collection analysis showed that 65% of compounds with 7 or fewer rotatable bonds possessed ≥ 20% oral bioavailability and < 25% of compounds with > 10 rotatable bonds had ≥ 20% bioavailability. **Flexibility affects absorption**

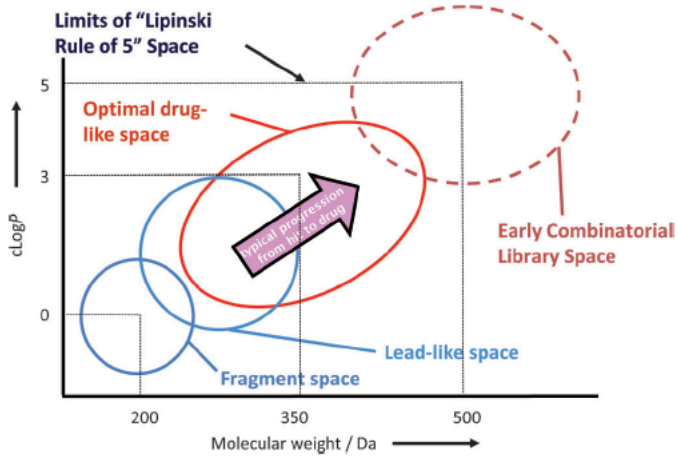

Figure 2. From recent analyses, optimal oral drug-like space can be defined in broad terms shown by the central, red oval. As optimization tends to progress by addition of complexity and lipophilicity (arrow), starting points should be in lead-like (or fragment-like) areas of property space.

Churcher et al.

- logP ≤ 3
- PSA > 75 Å,
- MW = 100-250 Da
- Aromatic rings ≤ 3
- Few sp^2 centers

Is prominent cross-coupling methodology biasing our strategy?

DeSimone et al. *Comb. Chem. High Throughput Screen.* **2004**, *7*, 473. Luthman et al. *J. Med. Chem.* **2003**, *46*, 558. Veber et al. *J. Med. Chem.* **2002**, *45*, 2615. Churcher et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 2

Hit to Lead

Prevailing Strategies

- **Target oriented synthesis (TOS)** – Accesses a precise region of chemical space usually based on a preexisting "privileged structure"
- **Combinatorial Chemistry** – Uses a common core structure with points of diversity e.g. R^1, R^2, and R^3 can generate N_{R1} x N_{R2} x N_{R3} possible structures
- **Diversity oriented synthesis (DOS)** – Aims to drastically explore chemical space utilizing complexity (**3-dimentional**) and diversity (**appendage, stereochemical, skeletal**) generating reactions.

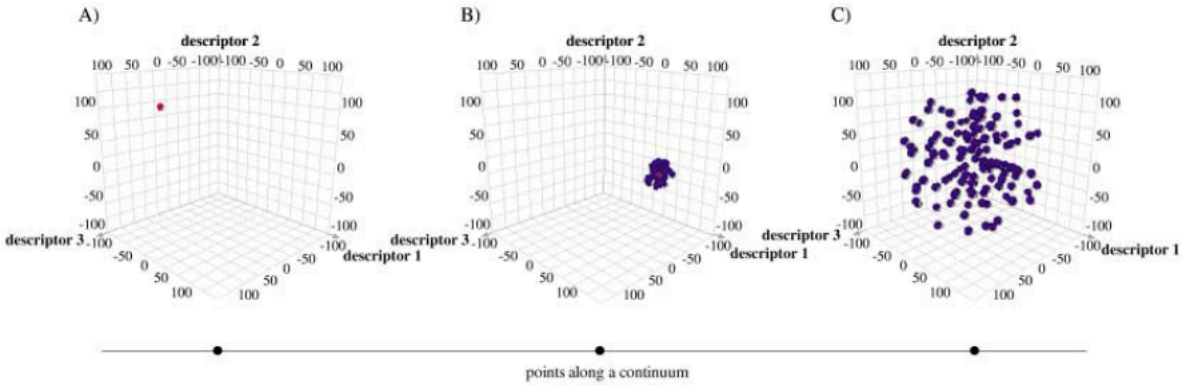

Figure 1. Comparison of TOS (A), medicinal and combinatorial chemistry (B), and DOS (C). Each three-dimensional plot is meant to represent the chemical product or collection of products derived from a single synthesis pathway. Each axis plots a calculable or measurable property of a small molecule (for example, molecular weight, solubility). A) The aim in TOS is to synthesize a single target structure having known or predicted properties (red sphere). B) The goal in medicinal and combinatorial chemistry is to synthesize a collection of analogues (blue spheres) of a target structure having known or predicted properties (red sphere). C) The aim in DOS is to populate chemistry space broadly with complex and diverse structures having unknown properties (blue spheres) as a first step in the small molecule discovery process. In some ways, these three approaches to synthesizing small-molecules represent points along a continuum.

Burke, M. D.; Schreiber, S. L. *Angew. Chem. Int. Ed.* **2004**, *43*, 46.

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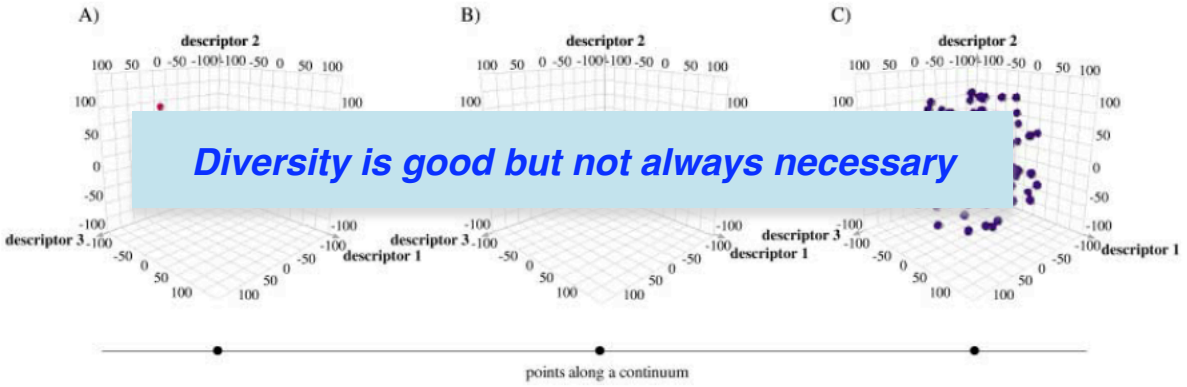

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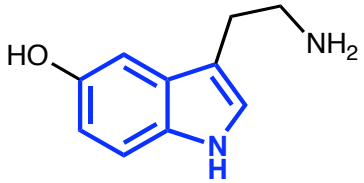
TOS

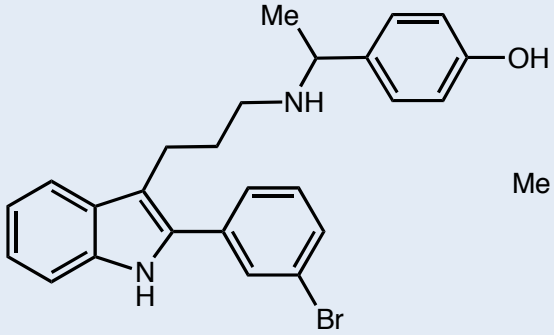
Target oriented synthesis (TOS) – Accesses a precise region of chemical space usually based on a preexisting "privileged structure"

- A "privileged structure" refers to a core, comprising of a significant portion of the molecule's size, that generally possess biological activity.
- Most privileged structures also possess 2 or more rings

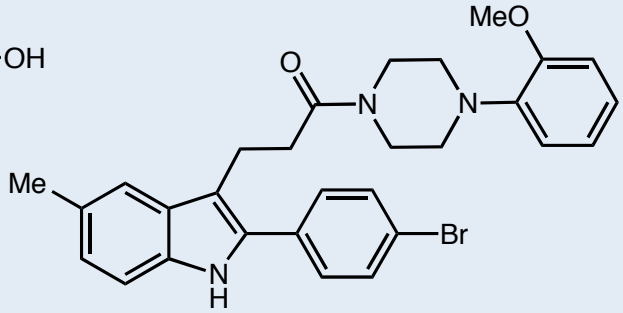


Serotonin

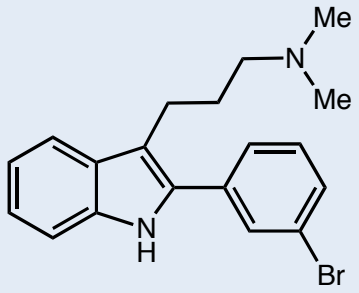
Indole Leads



NPY_3, 0.8 nM



NK1, 0.8 nM



$5Ht_6$, 0.7 nM

DeSimone R. W. et al. *Comb. Chem. High Throughput Screen.* **2004**, *7*, 473.

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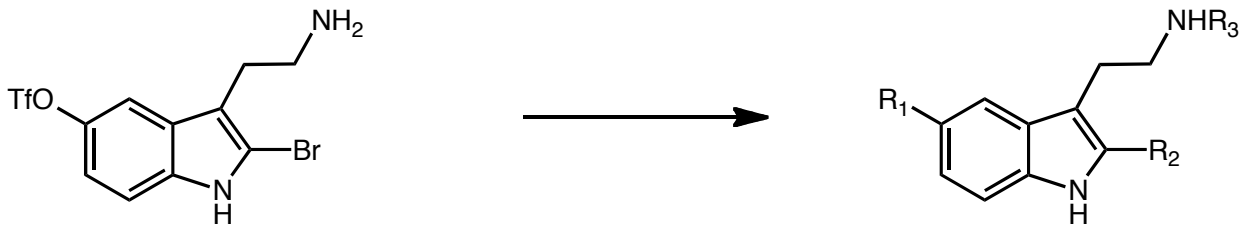

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Rational Design

These two approaches are not exclusive

Target oriented synthesis (TOS) – Accesses a precise region of chemical space usually based on a preexisting "privileged structure"

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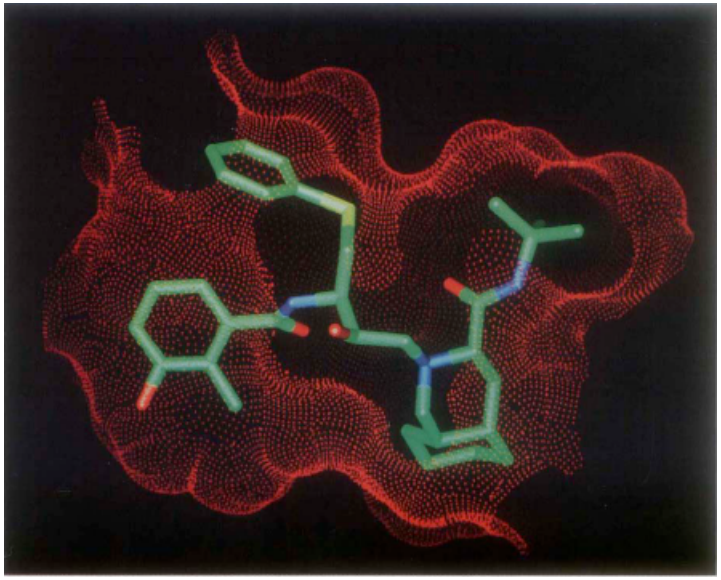
- Both can also implement rational design which is the design of a molecule or core structure based on key interactions predicted within a binding pocket
- Rational design requires X-ray or NMR structural data

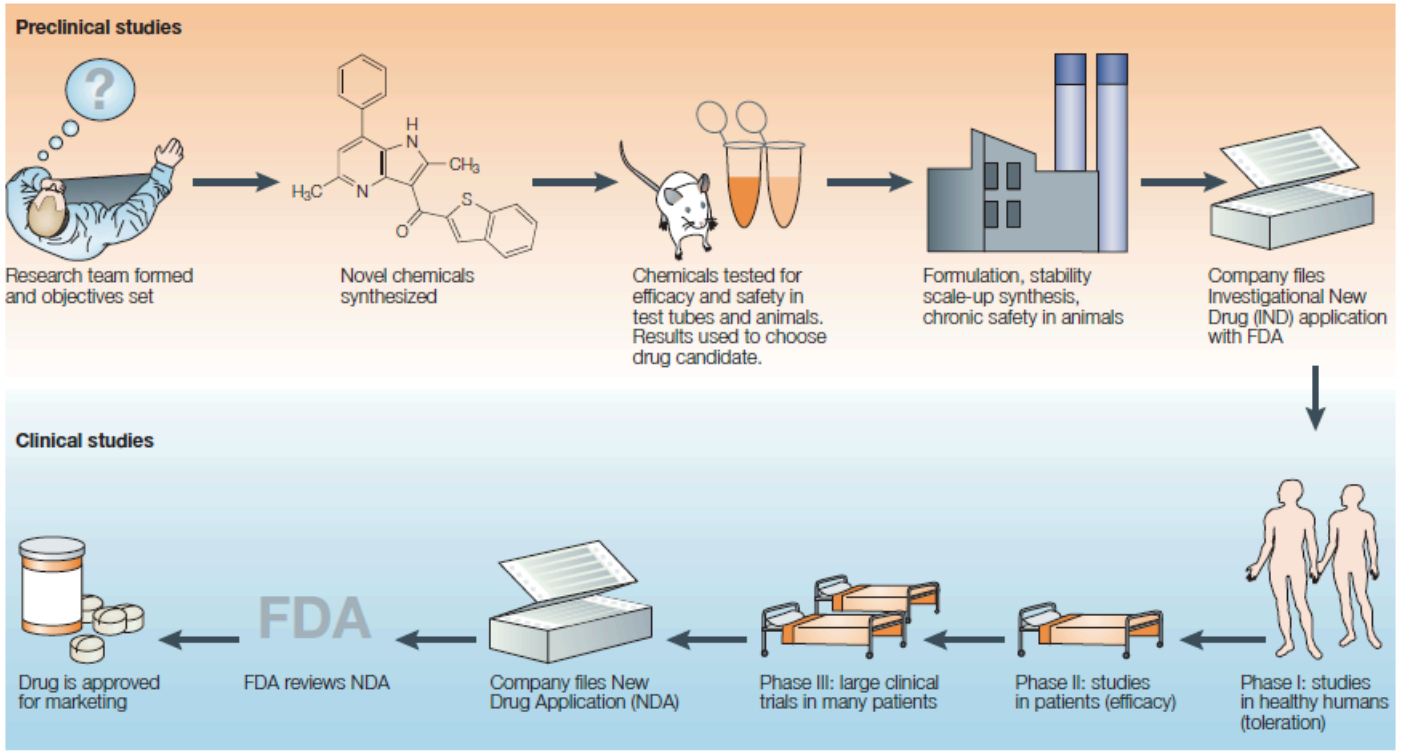


Agouron's AIDS drug nelfinavir (Viracept) was based on a rational design model

Hit to Lead

- Preclinical

Testing/Optimization



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Lead Optimization

ADMET

Absorption – The process by which a drug proceeds from the site of administration to the systemic circulation

Distribution – Movement of drug molecules from systemic circulation to the various tissue and organs of the body

Metabolism – Mechanism by which a drug is chemically converted to another substance, usually more polar and easily excreted

Exretion – Clearance of the unchanged drug (and possible metabolites) through the kidneys (urination), liver (fecal), or lungs (gas)

Toxicity – Drug or drug metabolites leading to organ/system failure and eventually permanent damage or death

Rowland, M.; Tozer, M. Section 1, Absorption and Dristribution Kinetics, In *Clinical Pharmacokinetics: Concepts and Applications.* Lippincott Williams & Wilkins 1995, pp. 11-50.
Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Absorption

Passive

- Involves dissolution and uptake through intestinal cell membranes through **passive diffussion**.
- Since most target administrations are oral (to maximize patient compliance) this is thought to be the **main mechanism** from which drugs enter the body

Carrier Mediated

- Involves active transport of a drug into the cell via membrane proteins. Authors of the below paper assert that **active transport** is actually predominant.

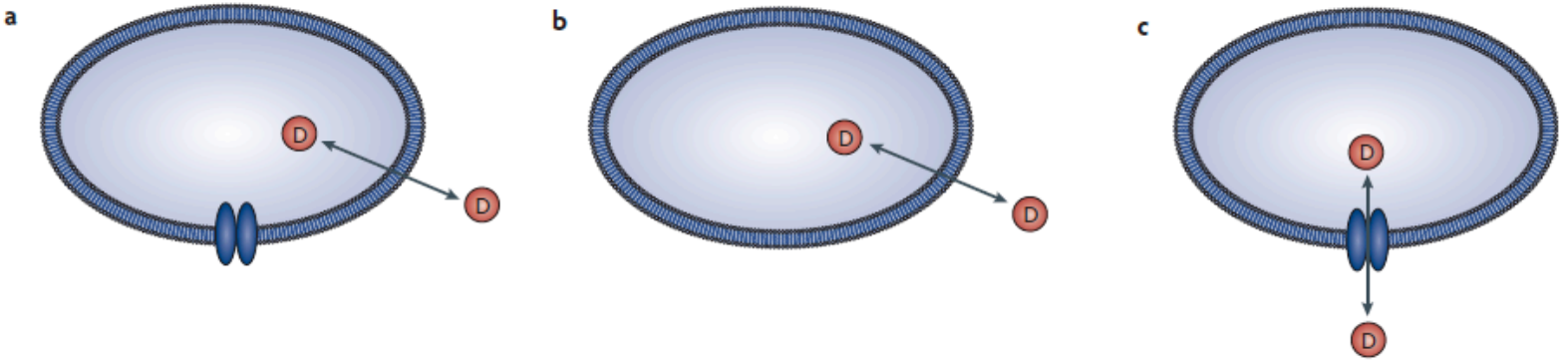


Figure 1 | **Transmembrane transport of drugs. a** | The membrane-bounded compartment is taken to consist of a lipid bilayer in which proteinaceous carriers are embedded. The drug (D) partitions into the lipid bilayer portion of the membrane roughly according to logP (the octanol–water partition coefficient) and redissolves in the intracellular fluid. **b** | In this view, drug transport occurs via transfer across the bilayer membrane exactly as it might do in a phospholipid membrane lacking any proteins (although we note that these may more readily admit passage via aqueous pore defects that do not occur so readily in a protein-containing natural biomembrane). **c** | In an alternative view, which is the focus of this article, most or all of the drug transport occurs via proteinaceous carriers that exist in the membrane and that normally transport natural cellular and extracellular metabolites (that is, those biosynthetically produced by the organsim) but which also show activity in transporting xenobiotics. Models (**b**) and (**c**) are not mutually exclusive and could in principle occur together in the same membrane. Overall, the steady-state, free intracellular concentration of a drug will reflect an interplay between passive uptake and the activities of influx and efflux transporters.

Dobson, P. D.; Kell, D. B. *Nature Rev. Drug Discov.* **2008**, *7*, 205.

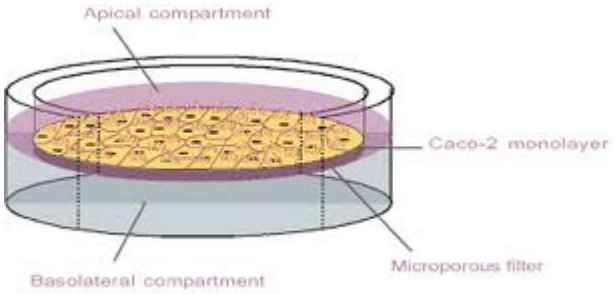
Absorption
In Vitro Models

Caco-2 cell line

- Derived from human colorectal carcinoma; widely used due to expression of **tight junctions**, **mirovilli**, and a number of enzymes (**peptidases, esterases, p-glycoprotein, uptake transporters**), and **bile acids** that are characteristic intestinal absorptive cells
- **Expression** of P-glycoprotein (PgP) or MDR1 (**multidrug resistance protein 1**) provides information about active drug efflux
- In vivo correlation with passive drug uptake has been well established though requires **21 day incubation** period

MDCK cell line

- Similar to Caco-2 but derived from dog kidney; only requires **3 day incubation**
- Can be transfected with **human MDR1**, providing useful data



Lack of CYP3A4, a metabolizing enzyme, limits predictive power

Best when used in combination with metabolic stability (hepatocyte) assays

Autursson, P.; Karlsson, J. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 880.
Autursson, P.; Palm, K.; Luthman, K. *Adv. Drug. Deliv. Rev.* **2001**, *46*, 27.
Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Absorption
In Vivo

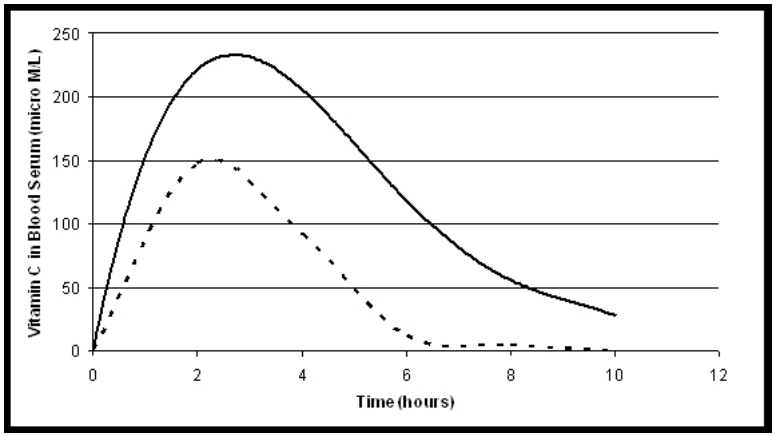
Bioavailability (F)

- A measure of the amount of **drug** that is actually **absorbed** from a given dose.
- Limited by **dissolution**, **permeability**, **gut motility**, **ionization**, and **first pass** (loss of drug as it passes through sites of elimination before entering circulation) effects.
- When **metabolism** is mainly **hepatic** (liver) then F can be represented as:
  $F = F_a(1-E_h)$ where F_a = intestinal fraction absorption, E_h = metabolic hepatic extraction ratio

Area Under the Curve (AUC)

- Directly derived from **concentration of drug** in systemic **circulation** from either oral or intravenous doses
- Comparison of AUC_{oral} to AUC_{iv} allows for the calculation of F where
  $F = (AUC_{oral}/Dose_{oral})/(AUC_{iv}/Dose_{iv})$

Formulation can matter



---- Vitamin C absorption from powdered form
—— Vitamin C absorption from liposomal encapsulation

(data from Azantis Inc.)

Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.
Shargel, L; Yu. A.B.C. Ch 9-10 Pharmacokinetics of Drug Absorption, Bioavailabitlity and Bioequivalence. In *Biopharmaceuticals and Pharmakokinetics*. Appleton & Lange **1993**, pp. 169-223.

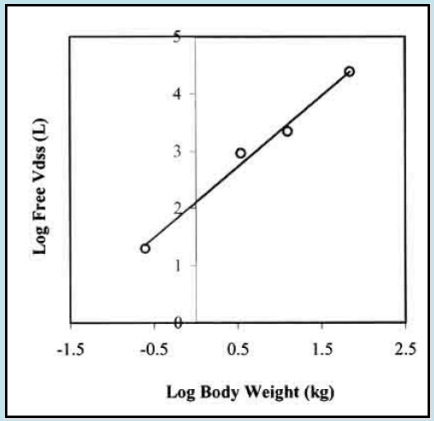
Distribution

Volume of Distribution

- Apparent volume of distribution (Vdss), inferred from system exposure or by actual tissue measurement, is a **theoretical volume** to estimate **drug distribution**
- Defined by the **steady state of drug entry** and **exit** from the central compartment to the tissue compartment
- In units of L/kg of body weight and allows for the calculation of drug half-life ($T_{1/2}$) from $T_{1/2}$ = 0.693*Vdss/Cl where Cl is clearance

Predictive Methods

Allometry scaling: Human Vdss is extrapolated as the responding body weight compared to anatomical, physiological, and biochemical similarities in mammals



Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.
Shargel, L; Yu. A.B.C. Ch 9-10 Muticomponent Models In *Biopharmaceuticals and Pharmakokinetics*. Appleton & Lange **1993**, pp. 169-223.

Distribution

Volume of Distribution

- Apparent volume of distribution (Vdss), inferred from system exposure or by actual tissue measurement, is a **theoretical volume** to estimate **drug distribution**
- Defined by the **steady state of drug entry** and **exit** from the central compartment to the tissue compartment
- In units of L/kg of body weight and allows for the calculation of drug half-life ($T_{1/2}$) from $T_{1/2}$ = 0.693*Vdss/Cl where Cl is clearance

Predictive Methods

Allometry scaling: Human Vdss is extrapolated as the responding body weight compared to anatomical, physiological, and biochemical similarities in mammals

Proportionality method: Compares free fraction (f_P) of drug in plasma of dog to that of human to estimate Vdss

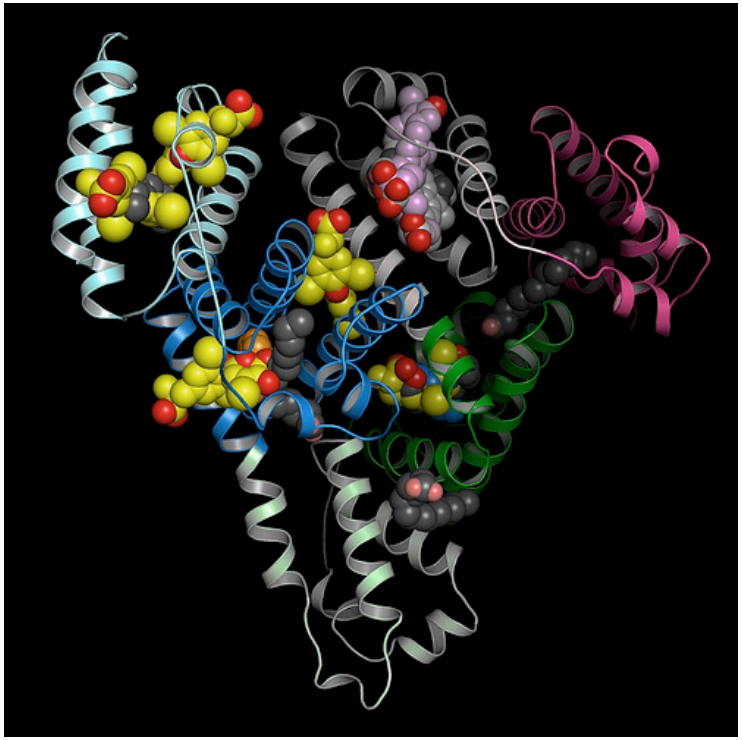
Average Fraction Unbound in Tissue Method: After (f_{ND}) of tissue is calculated from the Oie-Tozer equation for each preclinical species, the average value is assumed for humans which can then be used to calculate Vdss

Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.
Shargel, L; Yu. A.B.C. Ch 9-10 Muticomponent Models In *Biopharmaceuticals and Pharmakokinetics*. Appleton & Lange **1993**, pp. 169-223.

Distribution

Drug-Protein Binding

- Called **free fraction (*f*)** which is the extent that a drug binds to tissue (f_{ND}) or serum (f_P) proteins
- In serum the two major proteins of concern are **α1-acid glycoprotein** (44,000 D) and **serum albumin** (65,000 D); macromolecular complex formation restricts distribution to tissue
- **Equilibrium dialysis** and **ultracentrifugation** are **methods** used to asses (*f*)



Rendered by Prof. Stephen Curry Imperial College, London

- Serum albumin with simltaneous (unrealistic) molecules of known transport

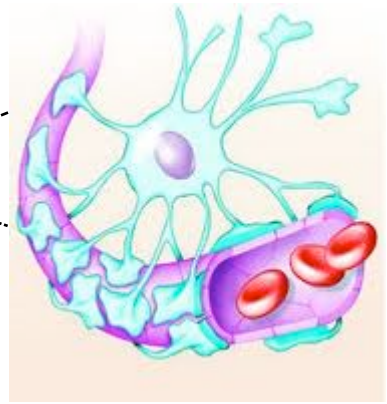
- Rendering shows actual location of binding for various biomolecules (some fatty acids) but in reality would only bind one at a time

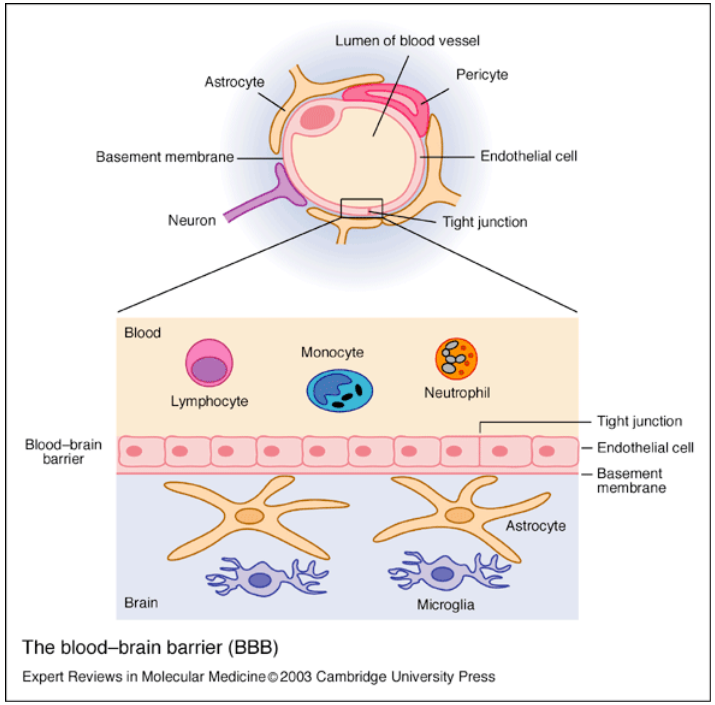
Innis et al. *J. Cereb Blood Flow and Metab.* **2007**, *27*, 1533.
Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Distribution

Brain Penetration

- The brain is separated from the circulatory system by the **blood-brain barrier (BBB)**, preventing the uptake of many drugs
- Physiochemical properties such as **charge**, **molecular weight**, and **lipophilicity** are of utmost importance when designing CNS drugs
- Along with Caco-2 and MDCK cell lines, **models** to study uptake include include **brain-blood partioning, brain perfusion, the indicator dilution technique, brain uptake index, the capillary depletion technique, and intracerebral microdialysis**.



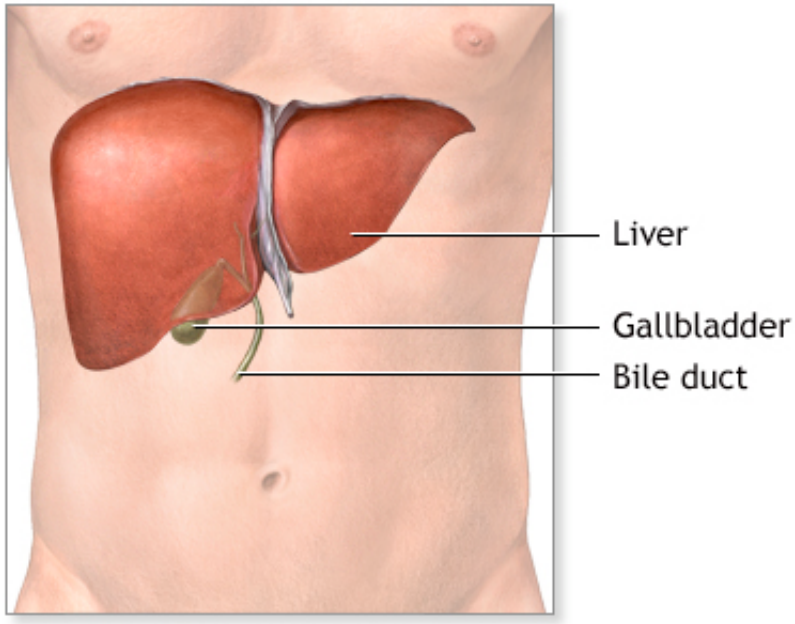


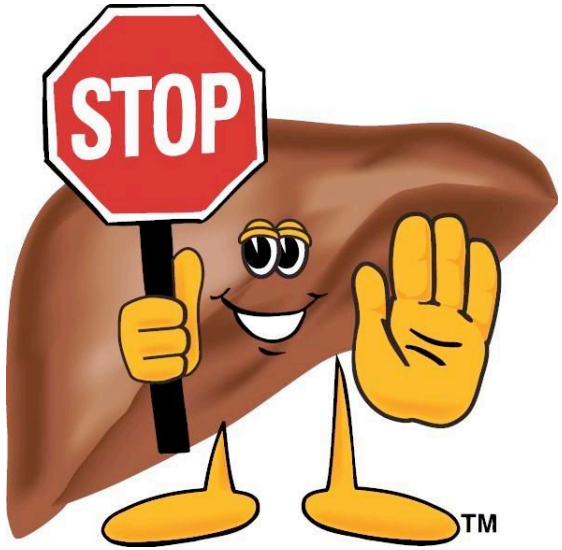
Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Metabolism

Phase I

- Often in the form of direct **hydrolysis, reduction,** or **oxidation** performed in the **liver** (though metabolism does happens in other tissues)
- Commonly performed **hydrolases, reductases,** the **cytochrome P450 enzyme family,** monoamine oxidases **(MAO's),** and flavin-containing monooxygenases **(FMO's)**





Excessive alcohol consuption = compromised liver function

Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Metabolism

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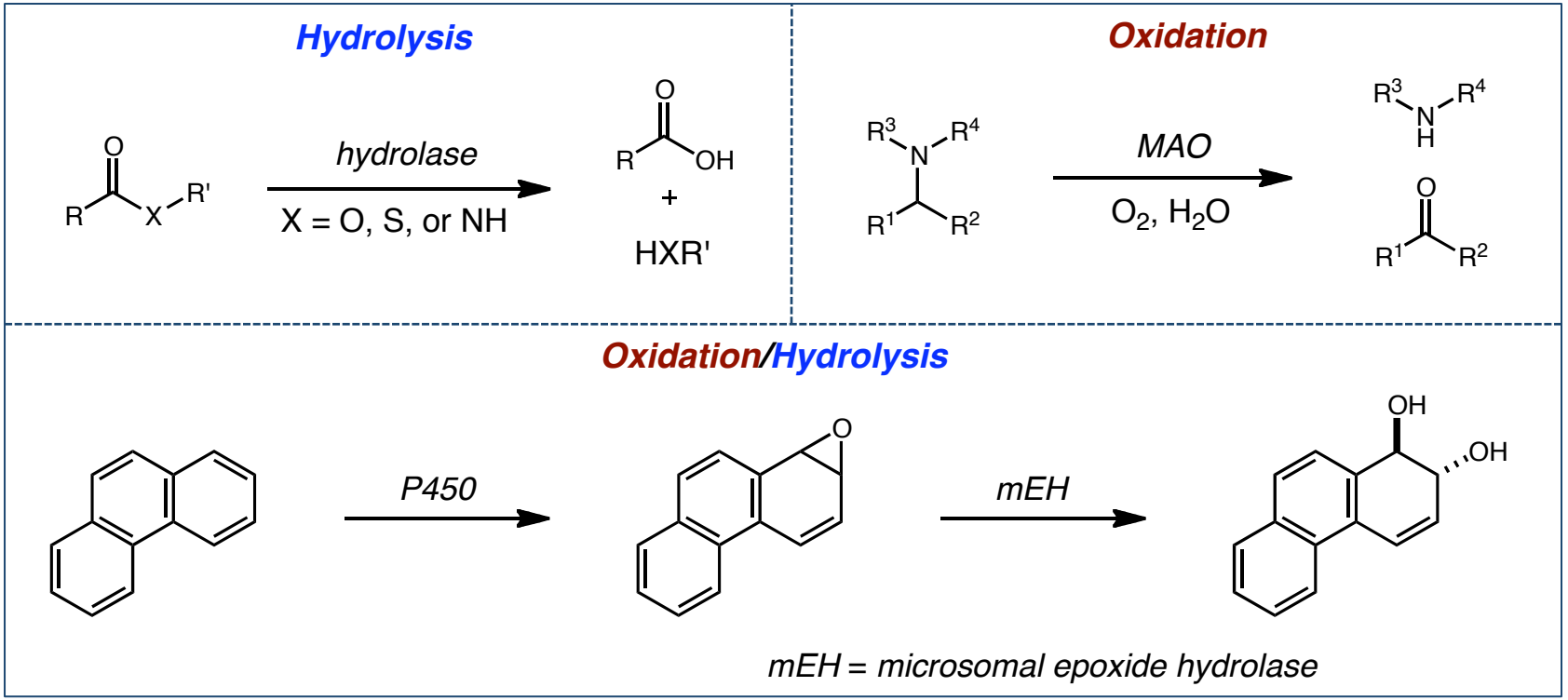


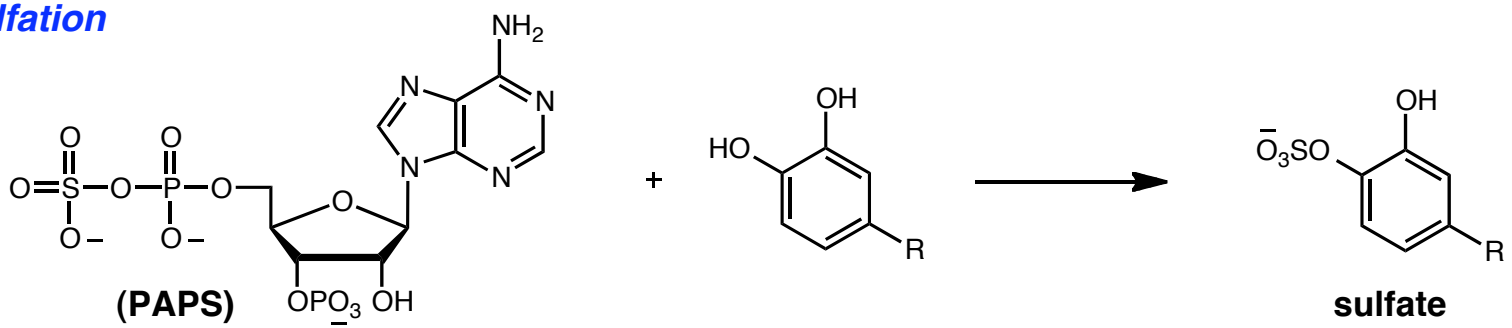
Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Metabolism

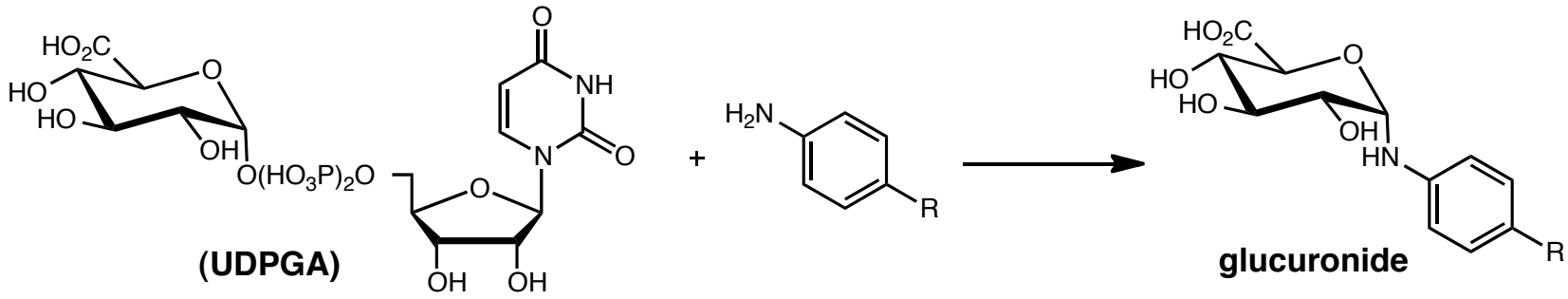
Phase II

- Usually **conjugating reactions**, appending a polar moiety to either the parent substrate or that after Phase I metabolism
- The two most prominent forms of conjugation are **sulfation** and **glucuronidation**

Sulfation



Glucuronidation



Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125. Weinshiboum, R.; Raftogianis, R.B. Sulfotransferases and Methyltransferases. In Metabolic Drug Interactions. Levy, R.H.; Thummel, K.E.; Trager, W.F.; Hansten, P.D.; Eichelbaum, M. Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, **2000,** pp.191-204

Metabolism

Phase I

- Often in the form of direct **hydrolysis, reduction, or oxidation performed in the liver** (though metabolism does happens in other tissues)
- Commonly performed **hydrolases, reductases, the cytochrome P450 enzyme family, monoamine oxidases (MAO's),** and **flavin-containing monooxygenases (FMO's)**

Phase II

- Usually conjugating reactions, appending a polar moiety to either the parent substrate or that after Phase I metabolism
- The two most prominent forms of conjugation are **sulfation** and **glucuronidation**

Predictive Methods

- **LCMS, LCMS/MS, and NMR** can all be used to identify metabolites
- Hepatocytes (**liver cells**), microsomes (**endoplasmic reticulum fragments**), and the **cytosol** can all be used in vitro to determine metabolites
- Intrinsic metabolic clearance (CL_{int}) can be calculated from microsome and hepatocyte scaling factors

Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Excretion

Renal (Kidney)

- Filters xenobiotics **directly** from the **blood stream** via passive diffusion or active transport by three processes: glomular filtration, tubular secretion, and tubular reabsorption
- The kidney directs filtrates into the **bladder** which allows for **urinary excretion**
- **Renal excretion** is the sum of the rate of filtration plus secretion minus rate of reabsorption and can be calculated by: Cl_R = excreted amount/time interval/[mean plasma]

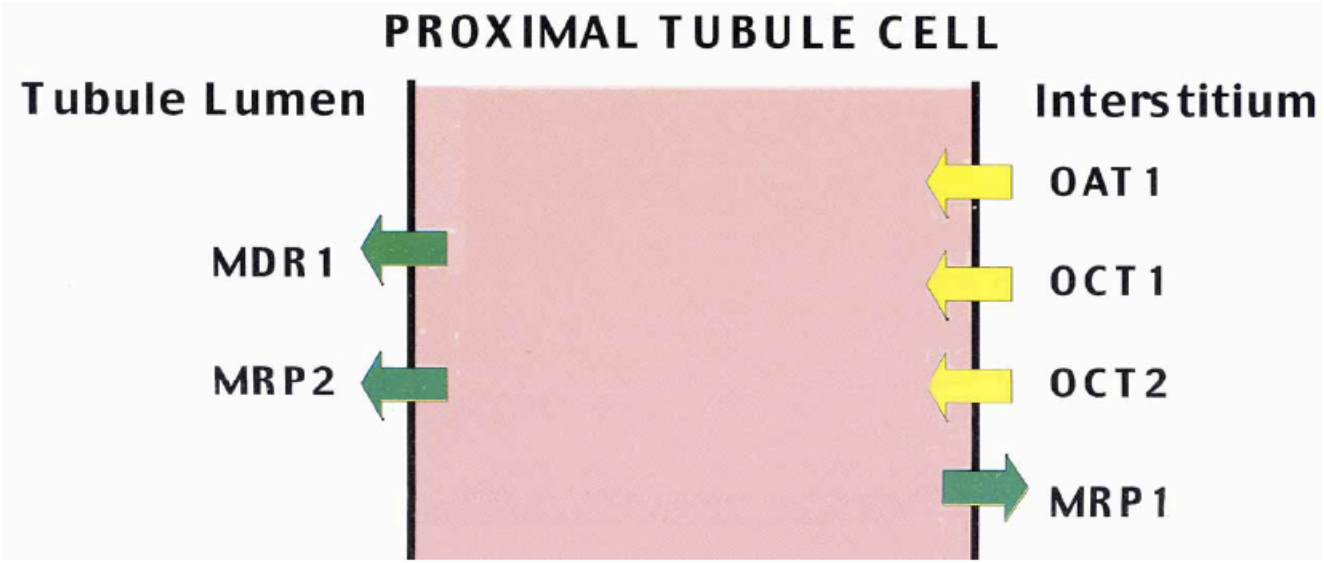


Fig. (13). Drug transporters located in the renal tubule cells showing both uptake transporters (yellow arrows) and efflux transporters (green arrows). Only transporters that have been shown to transport drugs are shown; there are additional transporters that transport endogenous compounds, but these are outside of the scope of this review. OAT, organic anion transporter; OCT, organic cation transporter; MRP, multidrug resistance related protein; MDR, multidrug resistance protein.

Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Excretion

Hepatic (Liver)

- Excretion can happen prior to or after metabolism of a xenobiotic (foreign substance) via passive diffusion or active transport into the **bile duct**
- The bile duct leads to the duodenum (small intestine) which allows for **fecal excretion**
- **Biliary clearance** can be calculated by measuring bile flow and drug concentration in plasma and bile: CL_B = [bile]*[bile flow]/[plasma]

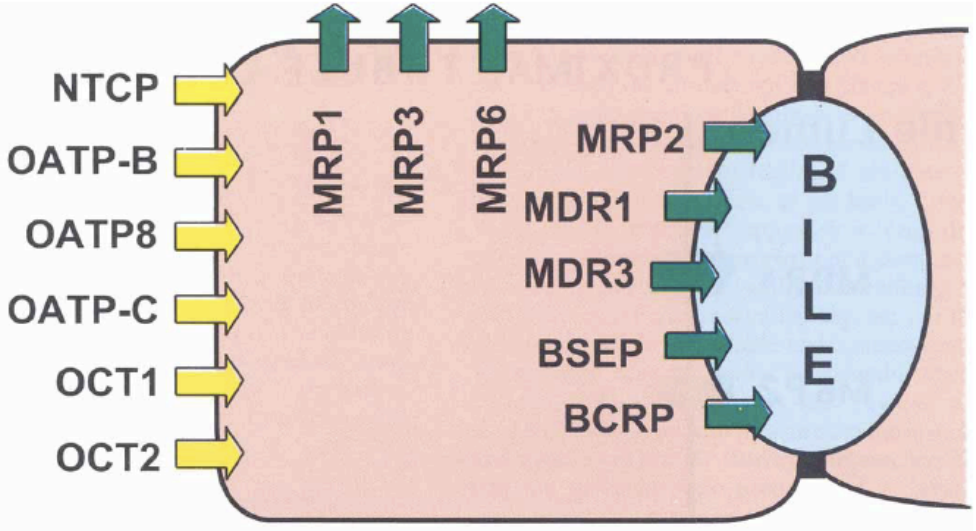
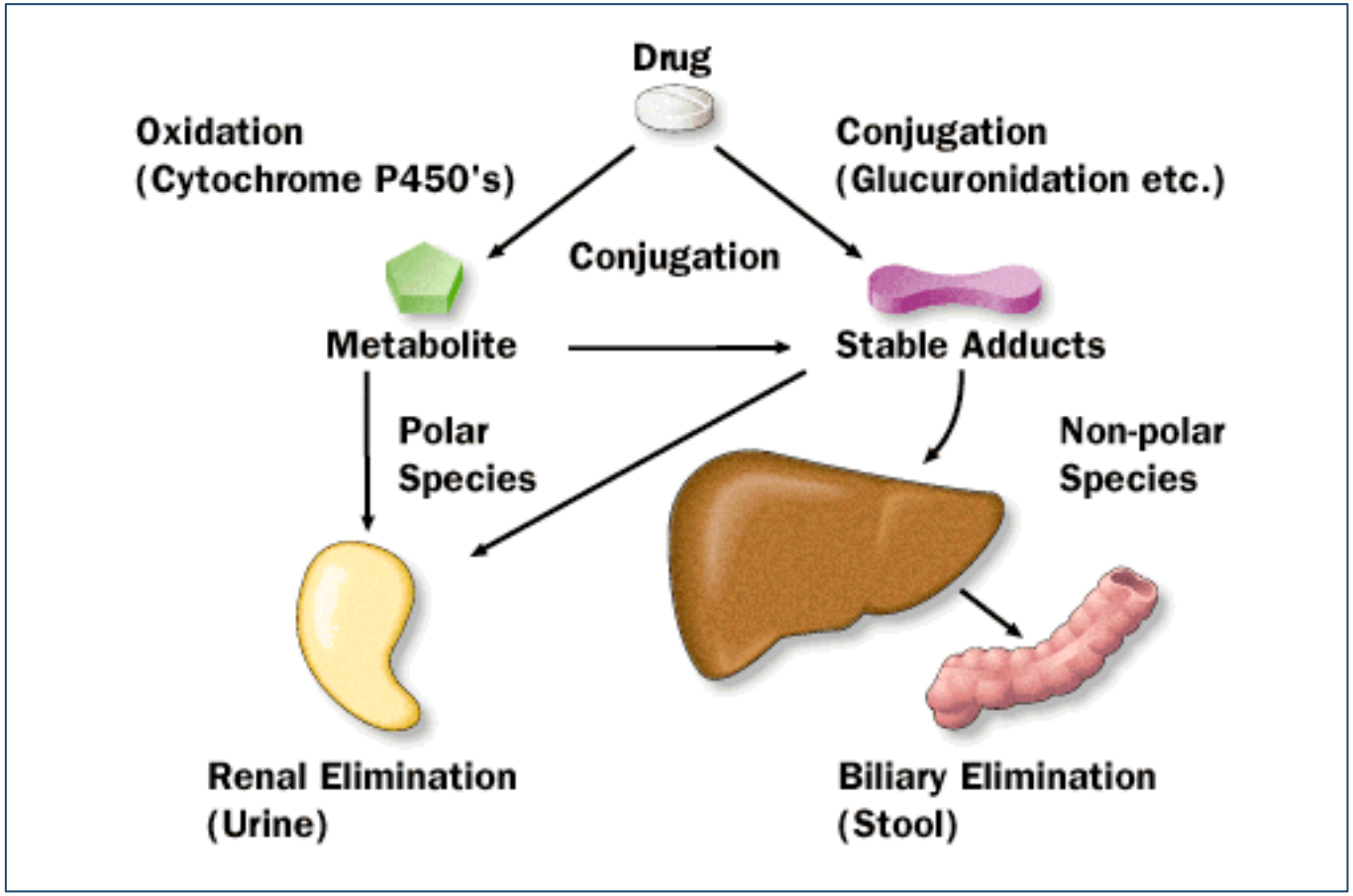


Fig. (12). Drug transporters located in the hepatocytes showing both uptake transporters (yellow arrows) and efflux transporters (green arrows). Only transporters that have been shown to transport drugs are shown; there are additional transporters that transport endogenous compounds, but these are outside of the scope of this review. NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; MRP, multidrug resistance related protein; MDR, multidrug resistance protein; BSEP, bile salt export pump; BCRP, breast cancer related protein.

Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Excretion

Summary of Excretion Pathways



Flexner, C. D.; Piscitelli, S. C. Medscape Education. http://www.medscape.org/viewarticle/421137_1 (accessed Jan 2012)

Toxicology

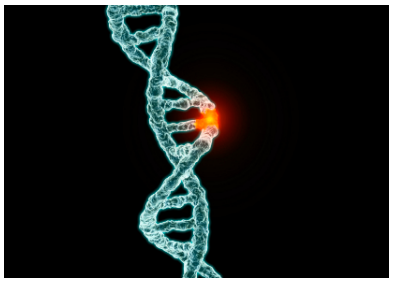
Organ Toxicity

- Function of three main determinants: **Intrinsic toxic** property of a chemical, **local concentration** within an **organ,** capability of **host defense** to **detoxify** and **cope** with **chemical injury**
- Medicinal chemists should be aware of **traditional toxic functionalities** but those liabilities are not always severe depending on ADME for that particular compound
- All measures should be taken to **assess toxicity** in vitro before administration to a mammal

Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.
For an excellent review on problematic moieties see: Kalgutkar et al. *Chem. Res. Toxicol.* **2011**, *24*, 1345

Toxicity

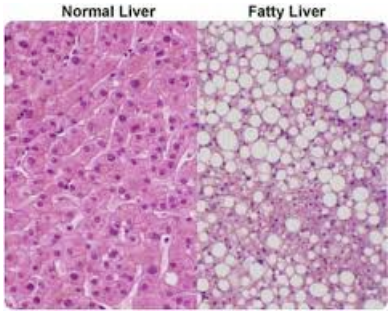
Genetic Toxicity



- **Carcinogens** can be mutagenic or non-mutagenic.
- Mutagenic carcinogens induce **DNA sequence mutations**
- The **Ames test and VITOTOX® assay** both test for mutagenic toxicity base on a bacterial genotoxic response.

Hepatic Toxicity

- A **major cause** of post-market **withdrawal** of **medications**. Many conditions contribute to this withdrawal though four major ones are steatosis, cholestasis, phospholipidosis, and reactive intermediates
- **Steatosis is the accumulation of fatty acids** and is caused by the inhibition of β-oxidation of long-chain fatty acids which increases triglyceride concetration within the body
- **Cholestasis is the impairment of bile flow** and can lead to jaundice which hospitalizes 2-5% of cases and ~20% of the ederly
- **Reactive metabolites (electrophiles, free radicals)** can form covalent bonds with biomolecules leading to reduced function and genetic mutation
- **Phospholipidosis** involves the **accumulation of excess phospholipids** in cells accompanied by coincidental toxitities



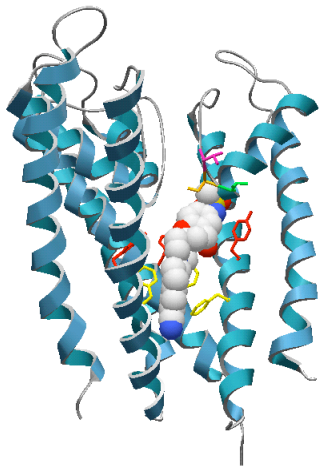
Steatosis

Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.

Toxicity

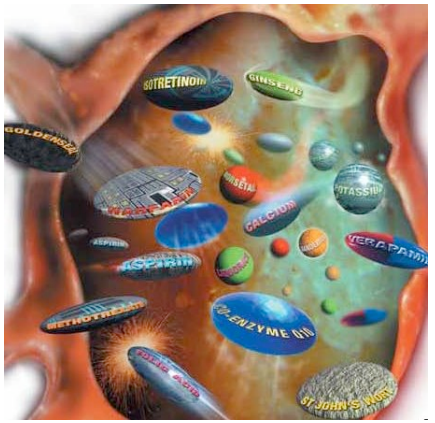
Cardio Toxicity

- **Interruption** of the **repolarization phase** of the ventricular cells due to interference of the drug with cardiac **potassium channels** (action potential repolarization)
- **Binding** of a number of drugs to the **hERG** (the human *Ether-à-go-go*-Related Gene) K+ channel has been shown to exhibit significant **cardio toxicity** and is now tested for in vitro via a competitive inhibition assay



Drug-Drug Interactions

- **Inhibition of CYP3A4** (a cytochrome P450 enzyme) confers potential inability to metabolize other ingested drugs
- **Inhibition of PgP** (multidrug resistance protein) inhibits cell efflux of potential toxins

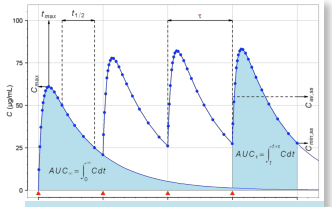


Lin et al. *Curr. Top. Med. Chem.* **2003**, *3*, 1125.
Sanguinetti, M.; Ng, K. H. The Ion Channel lab, University of Utah http://www.cvrti.utah.edu/ms-lab/blockers.htm (accessed Jan 2012)

Medicinal Chemistry Feedback Loop

Synthesis of New Compounds

3-6 Years



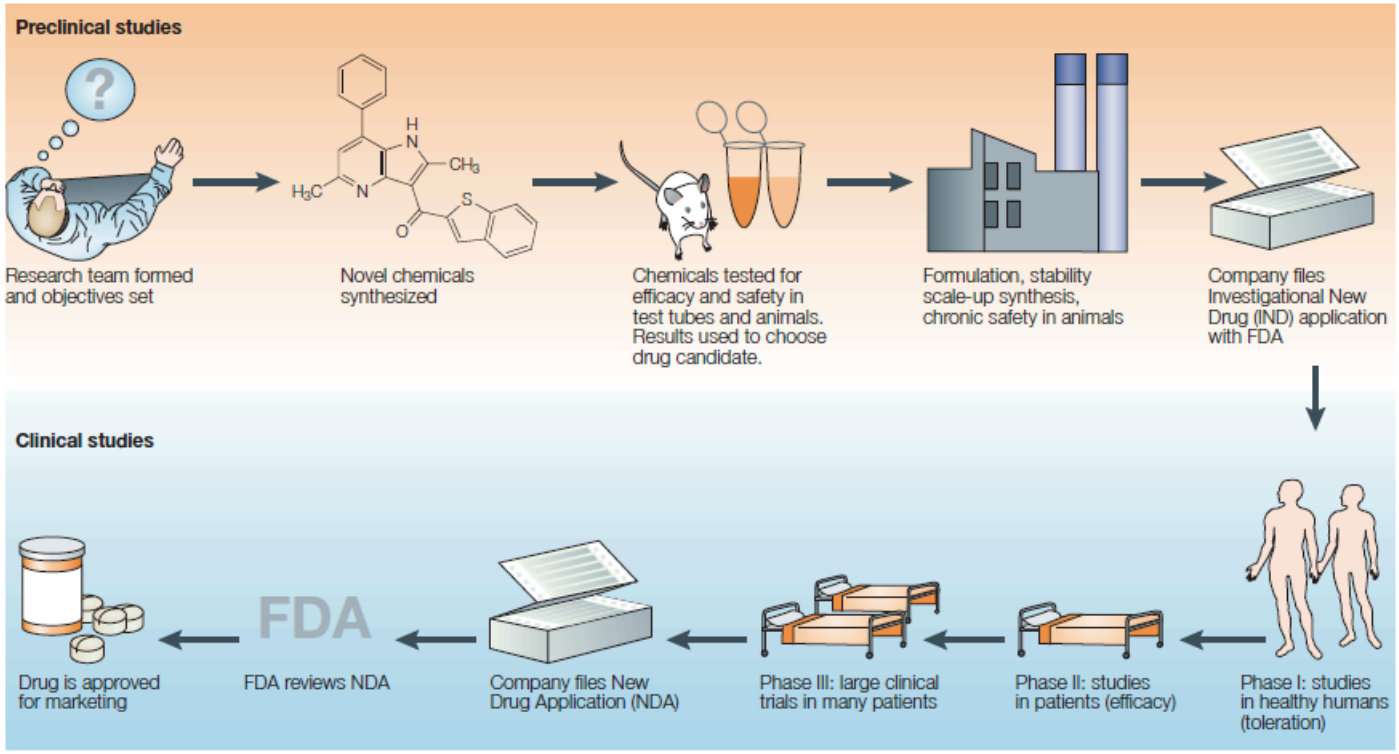
ADMET Data

Rational Modifications

Development

Process Optimization

- Preclinical



Lombardino, J.; Lowe, J. A. *Nature Rev. Drug Discov.* **2004**, *3*, 853.

Development

Pre-Clinical FDA Requirements

- Pharmacological profile of drug
- Perform acute toxicity in at least two animal species
- Short term toxicity studies based on duration of clinical trials

Process Chemistry

- Availability of cost-effective starting materials
- Minimization of synthetic and purification steps
- Scale-up validation
- Reduced cost of goods (COGS)

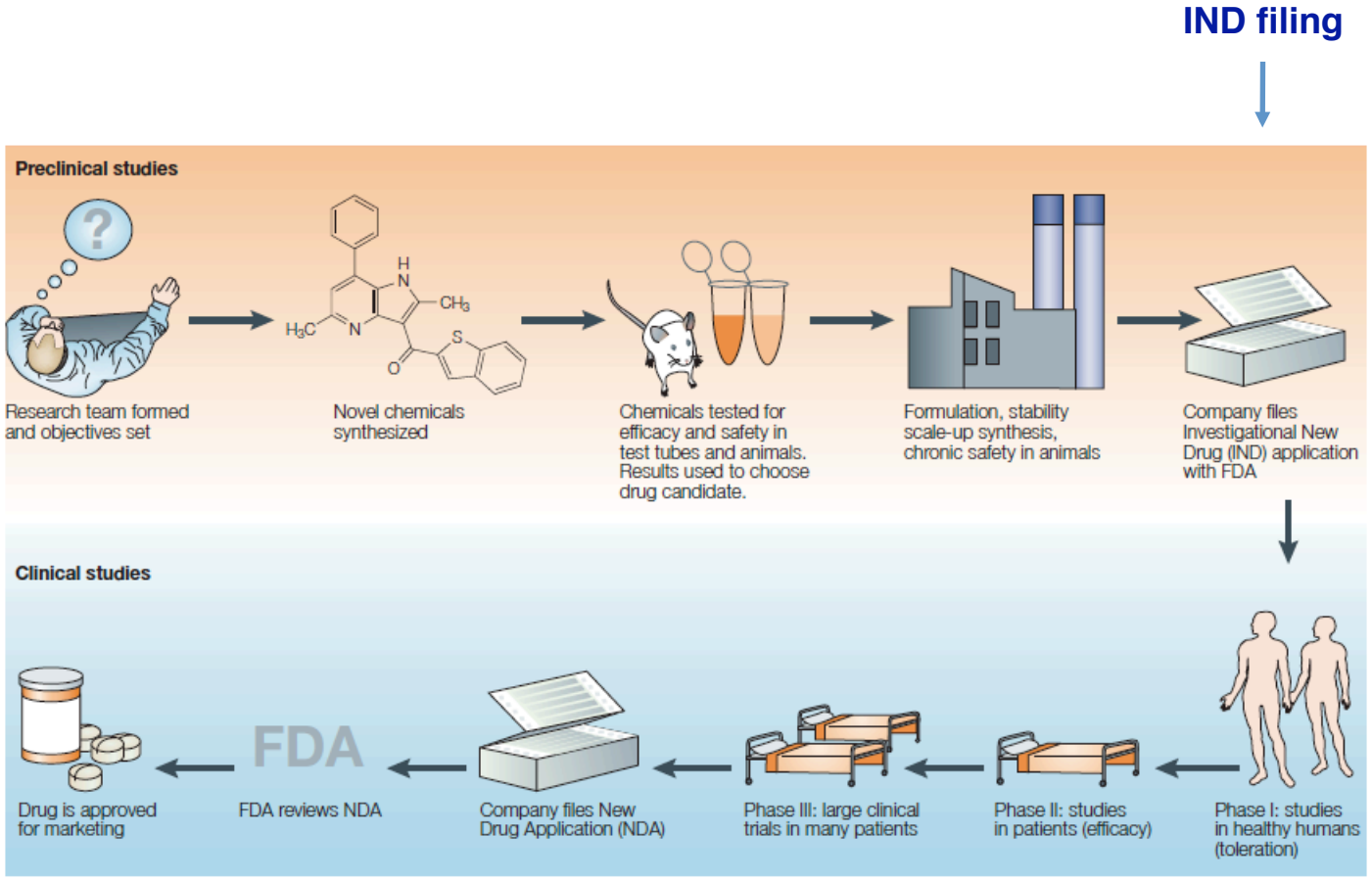
Chemistry, Manufacturing and Controls (CMC)

- Active Pharmaceutical Ingredients (API)
- Characterization
- Manufacturer and method of manufacture
- Process controls
- Specifications and purity profiles
- Container system for drug substance and storage
- Stability

Eckstein, J. Alzheimer Research Forum. http://www.alzforum.org/drg/tut/ISOATutorial.pdf (accessed Oct 2011)

New Drug Investigation Filing

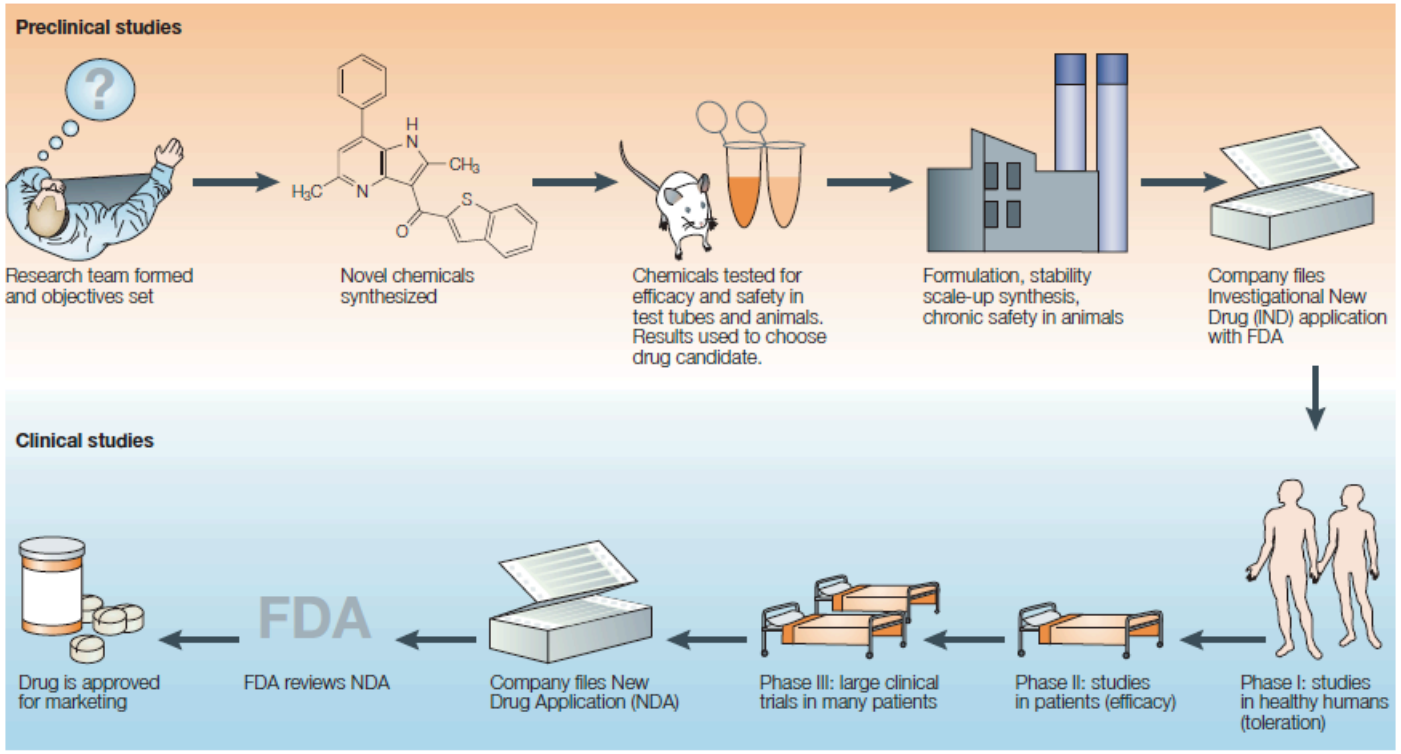
- Preclinical



Lombardino, J.; Lowe, J. A. *Nature Rev. Drug Discov.* **2004**, *3*, 853.

Clinical Trials

- Clinical



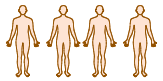
Human Testing

Lombardino, J.; Lowe, J. A. *Nature Rev. Drug Discov.* **2004**, *3*, 853.

Clinical Trials

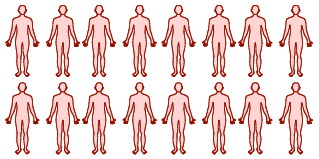
Phase I

- **Healthy humans (20-80)**
- Determine human **ADMET** values and **side effects** from increasing dose.
- Phase **1a** is a **shorter**, safety assessment period
- Phase **1b** is a **longer** evaluation which may include patients
- 40% failure rate



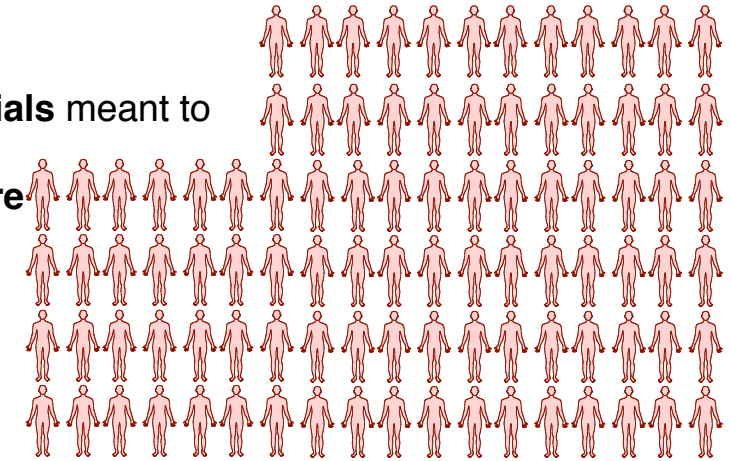
Phase II

- Tested in affected patients by randomized controlled trials (RCT)
- Determines **efficacy** along with **short and long term side effects**
- Phase **2a** involves **100-300 patients**, often hospitalized to determine dose response, patient type, frequency of dosing
- Phase **2b** is more controlled and **rigorously demonstrates efficacy**
- 62% failure rate of successful phase I passes



Phase III

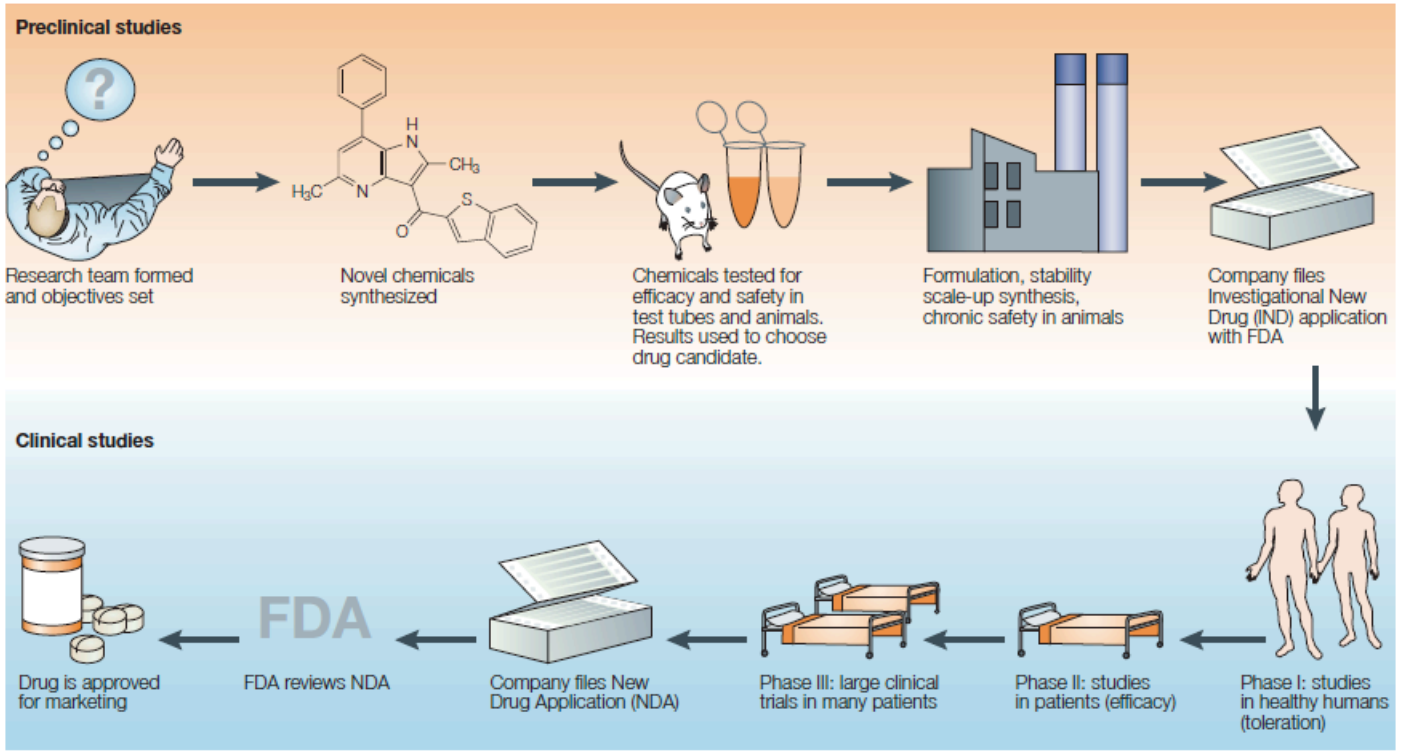
- Expanded with both **controlled** and **uncontrolled trials** meant to allow **extrapolation** to the general **population**
- Must be controlled and compared to **standard of care**
- Use **double-blind** study **when** practical and **ethical**
- Be randomized and of **adequate size**
- **40% failure** of successful **phase II** passes
- **23% failure** of successful **phase III** passes at registration



Eckstein, J. Alzheimer Research Forum. http://www.alzforum.org/drg/tut/ISOATutorial.pdf (accessed Oct 2011)

New Drug Application Filing

- Clinical



NDA Filing

Lombardino, J.; Lowe, J. A. *Nature Rev. Drug Discov.* **2004**, *3*, 853.

New Drug Application

Major Requirements

- Chemistry, manufacturing and control, samples
- ADMET data both clinical and non-clinical
- Clinical data (efficacy, safety, dosing, etc.)
- Safety update 120 days after NDA application
- Statistical analysis, case report tabulations and forms
- Patent information including certification

Review

- Assesses sponser's claims about drug safety and effectiveness.
- Reviewers can request reanalysis of drug performance in a patient subset or in the original population pool
- After a rigorous vetting process an action letter is sent of approval, approvable, or non-approvable with sufficient justification

Phase IV (overview)

- Monitors safety and long-term side effect after is it approved for prescription
- Evaluates effectiveness in larger population pools (general public)
- Retraction of an approved drug is common

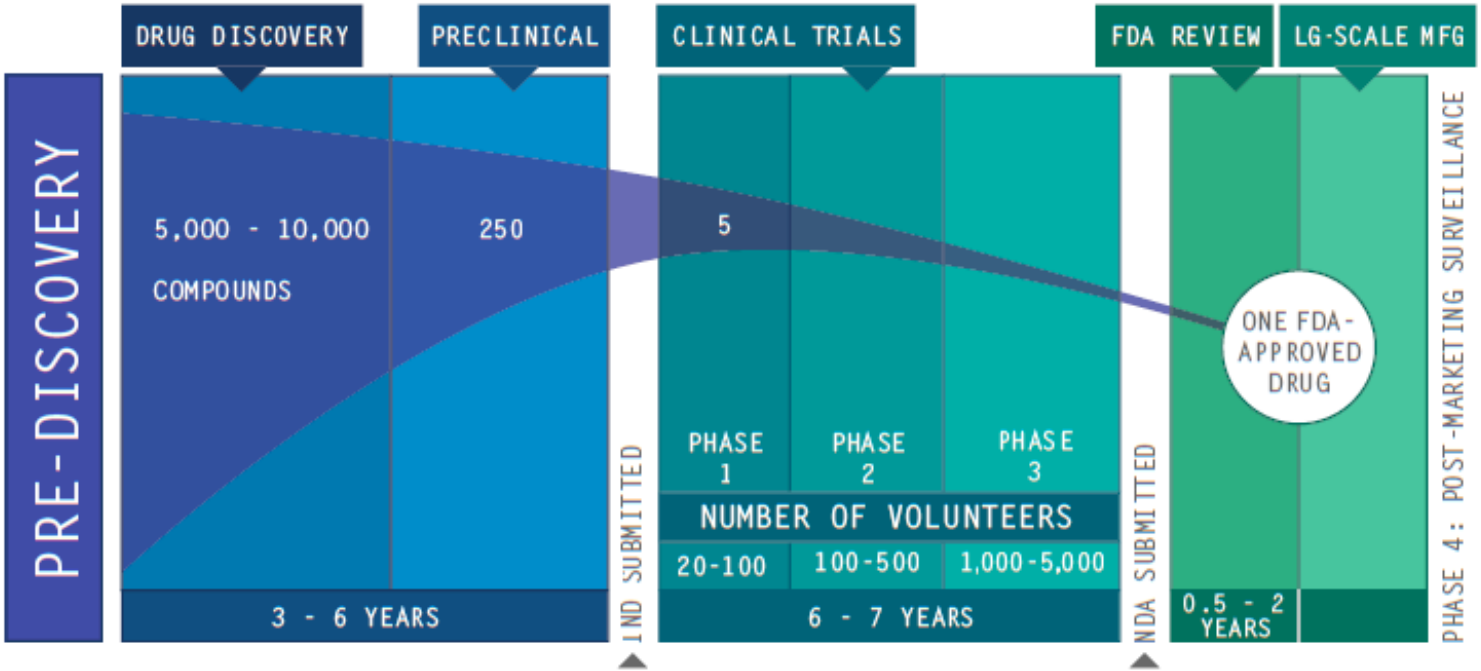
Eckstein, J. Alzheimer Research Forum. http://www.alzforum.org/drg/tut/ISOATutorial.pdf (accessed Oct 2011)

Phase IV Withdrawals

Drug (Indication)	Approved	Withdrawn	Years Delay	Reason Drug Is Pulled	Company
Fenfluramine (weight loss)	1973	1997	24	Pulmonary hypertension, heart valve disease	Wyeth-Ayerst
Posicor (hypertension, angina)	1985	1998	13	Reduced liver enzymes	Roche
Seldane (allergies)	1985	1997	12	Heart problem when taken with other drugs	Hoescht Marion Roussel
Hismanal (allergies)	1988	1999	11	Heart arrhythmia	Janssen Pharmaceutica
Propulsid (nocturnal heartbeat)	1993	2000	7	Cardiac arrhythmia	Janssen Pharmaceutica
Vioxx (pain)	1999	2004	5	Heart attack, stroke	Merck
Baycol (anti-cholesterol)	1997	2001	4	Muscle deterioration	Bayer
Rezulin (anti-diabetes)	1997	2000	3	Liver toxicity	Pfizer
Razar (antibiotic)	1997	1999	2	Severe cardiovascular problems	Glaxo
Raplon (airway muscle relaxant)	1999	2001	2	Bronchospasm	Organon
Duract (pain)	1997	1998	1	Hepatitis, liver failure	Wyeth-Ayerst
Lotronex (IBD)	2000	2000	9 months	Ischemic colitis, constipation	Glaxo

Eckstein, J. Alzheimer Research Forum. http://www.alzforum.org/drg/tut/ISOATutorial.pdf (accessed Oct 2011)

The Numbers



- It requires between **800 million and 1.4 billion dollars** of investment for **one** approved **drug**
- Of all of the **millions upon millions** of compounds screened, only **one** FDA approved drug (**sunitinib**, Pfizer, renal carcinoma) came from a **combinatorial library**
- **Natural product** derived structures (parent compounds, derivatives, analogues, and mimics) still comprise of **57.7%** of all FDA **approved drugs**

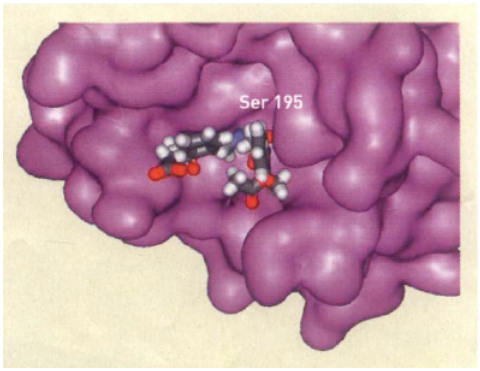
"People who say natural product research has had its day are being arbitrary and are limiting their options for no good reason" –Barry Trost

http://www.innovation.org/index.cfm/InsideDrugDiscovery (accessed Oct 2011). Ojima, I. *J. Med. Chem.* **2008**, *51*, 2587. Borman, S. Charting Better Routes to Drugs. *CE & N*, June 28, 2004, 37.

The Future

Drug Targets

- At present day **~3,000 human proteins are "druggable"** but only a fraction of these will be safe and efficacious drug targets with **~200 targeted by current therapies**
- Many **new protein targets** should be identified with **high throughput crystallography** and **functional genomics**
- Proteomics will always be a source of target production



New Drugs

- A **modification** of **Lipinski's rules** will allow chemists to produce more **lead-like molecules**
- **New methodologies** that tolerate **polar functional groups** will help to reduce the synthesis of more lipophilic (poor solubility) libraries
- **Structure based drug design** will increase as more **crystallography data** is produced
- **Pharmaceutical** companies **and synthetic chemists** will need to better **collaborate** in order to define and **address** the biggest **challenges** plaguing **drug discovery**
- **The Grand Challenge** (proposed by Schreiber)**:** Building a **comprehensive database** of **bioactive compounds** and **screening data,** finding a **small molecule modulator** of each function of **every human protein**
- Pharmaceutical companies will gladly take, but will they share?

Borman, S. Charting Better Routes to Drugs. *CE & N*, June 28, 2004, 37. Churcher et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 2. Kramer, R.; Cohen, D. *Nature Rev. Drug Discov.* **2004**, *3*, 965.