Lect#11: Enzyme Mechanisms-III: Techniques for Drug Discovery

A. Drug Design
B. Techniques of Drug Discovery
   (1) Complexity of Drug Discovery
   (2) SARS and QSARS
   (3) Structure-based Drug Design
   (4) Combinatorial Chemistry and High-Throughput Screening
C. Introduction to Pharmacology
   (1) Pharmocokinetics
   (2) Toxicity and Adverse Reactions Eliminate Most Drug Candidates
      (a) Phase I
      (b) Phase II
      (c) Phase III
   (3) Drug Candidate Statistics
   (4) Cytochrome P450 Metabolizes Drugs
   (5) Many Drugs are Enzyme Inhibitors
      (a) Sulfadrugs
      (b) Viagara

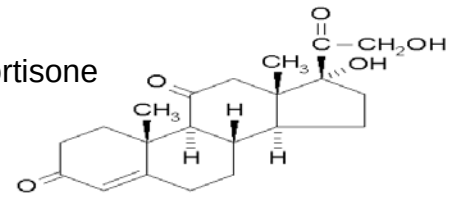
Lecture #11: Enzyme Mechanisms-III: Techniques for Drug Discovery

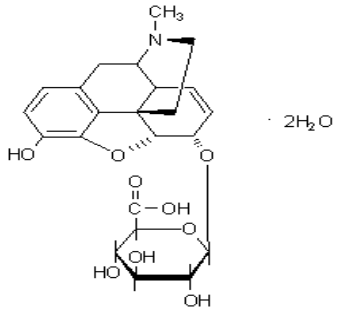
A. Drug Design:

- Improvements in medical care are largely attributed to **development** of wide variety of drugs

  - antibiotics
  - anti-inflammatory agents
  - analgesics and anesthetics,
  - antidepressants
  - antipsychotics,
  - immunosuppressants,
  - cancer drugs,
  - cardiovascular and stroke drugs, etc.

cortisone

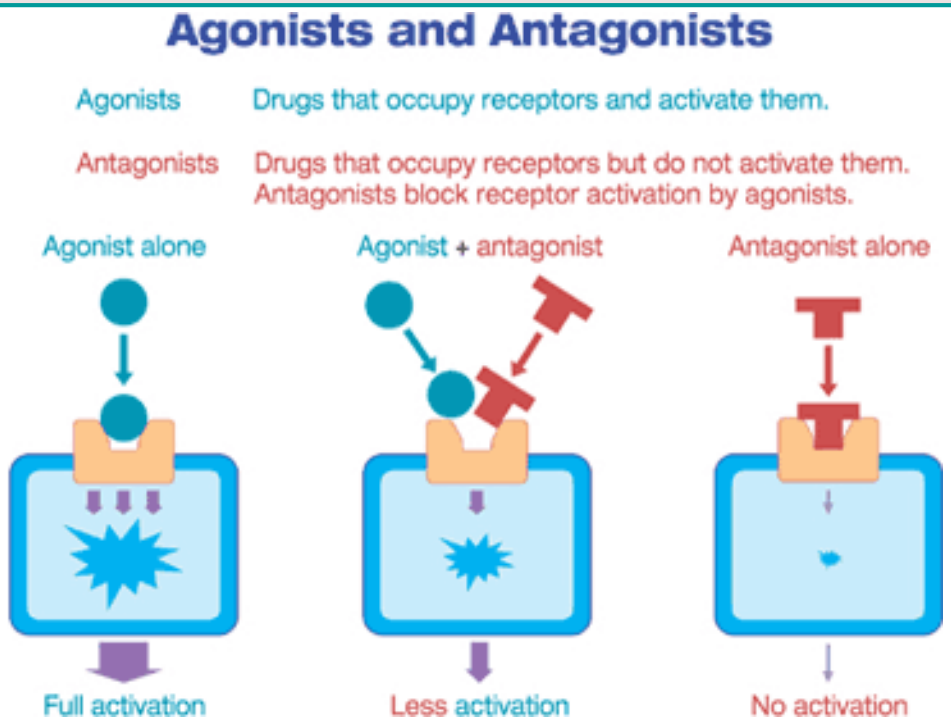




morphine

B. Techniques of Drug Discovery:

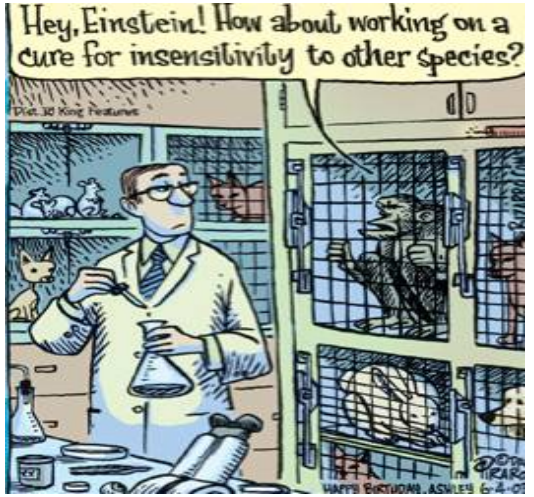
- drugs act by modifying the function of a particular **receptor** in the body or an **invading pathogen**
  - **receptor:** enzyme, channel or signalling protein
  - **Agonists:** compounds that modify the receptor function
  - **Antagonists:** compounds that bind without modifying function, but block agonist binding
- biochemical and physiological effects of a drug and its mechanism of action are called **pharmacodynamics**



1. Complexity of Drug Discovery

- drugs discovered by **screening large numbers** of synthetic compounds and natural products for the **desired effect**
- **natural products** are discovered by fractionation of the organism in which they occur and isolating the **"active ingredient"**
- *in vitro* screens are initially used such as the binding of a drug to a target enzyme implicated in the disease

- number of drug candidates is reduced to "effective" compounds
    - then **animal testing**
- **lead compound:** a drug candidate that exhibits a desired effect
    - binds to its target with a $K_d < 1\ \mu M$



- high affinity is necessary to minimize nonspecific binding to other macromolecules
    - Reduces potential side effects

Measures of drug effectiveness

IC_{50}: the [inhibitor] at which an enzyme exhibits 50% of its maximal activity ($v_i/v_o = 0.5$)

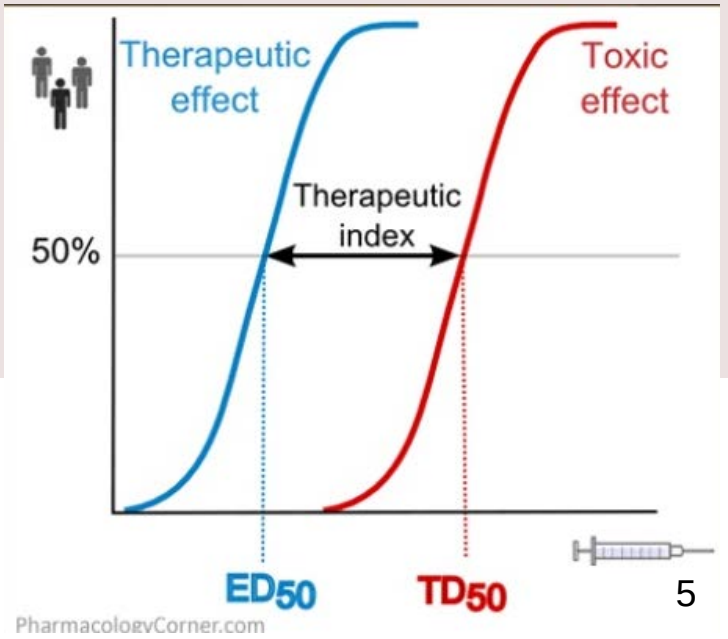
ED_{50}: the effective dose of a drug to produce a therapeutic effect in 50% of a test sample

TD_{50}: the mean toxic dose of a drug to produce a particular toxic effect in one-half of the animals

LD_{50}: mean lethal dose of drug required to kill 50% of a test sample

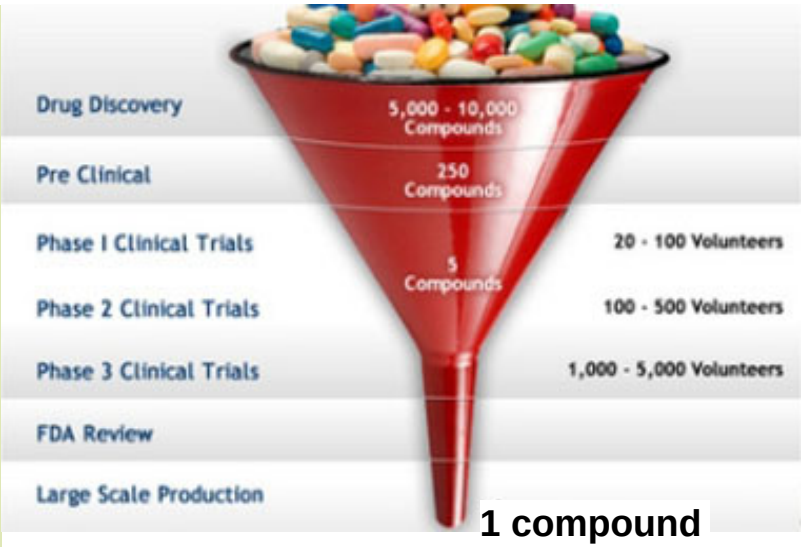
Therapeutic index: is the TD_{50}/ED_{50} ratio

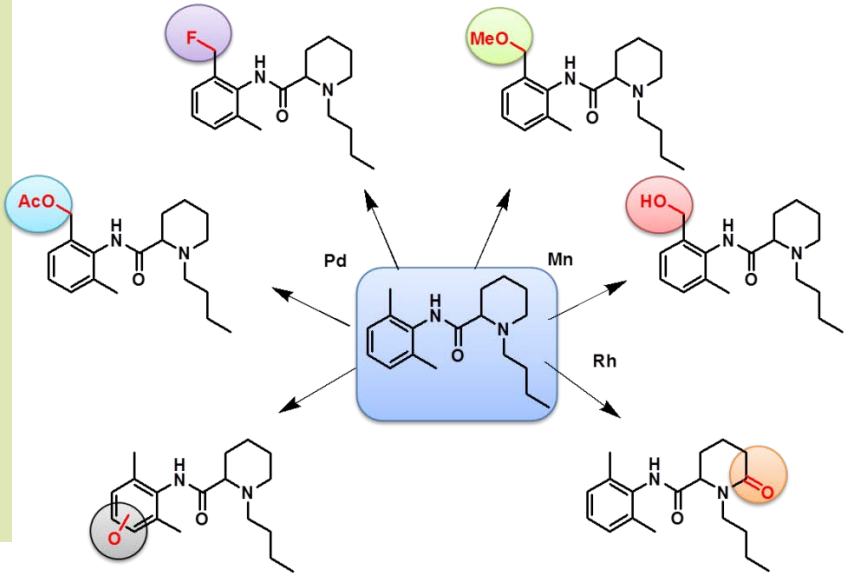
- Drugs that have **high** therapeutic indices are the **best**



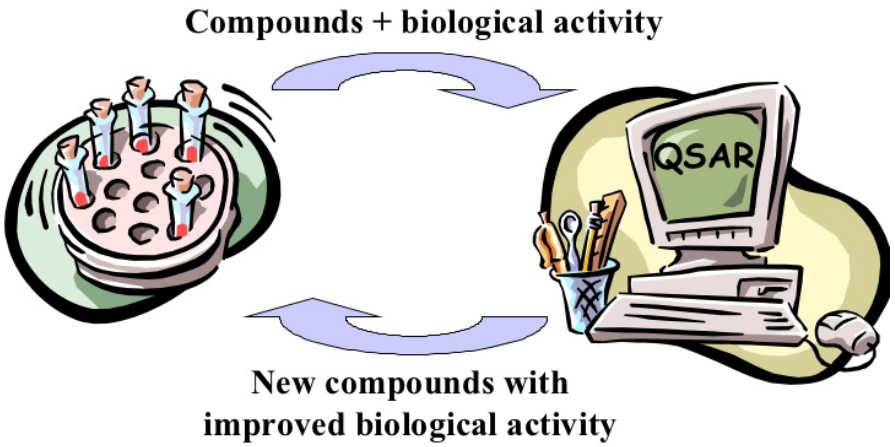
2. SARs and QSAR (Drug Design)

- **lead compound:** starting point to design more **efficacious** compounds
- **minor modifications** to a drug candidate can result in **major changes** in its pharmacological properties
- successful drugs are the result of 5,000 - 10,000 related compounds that were synthesized/tested
- systematic process
    - **structure-activity relationships (SARs)**
- **SARs:** synthesis and screening to determine which groups on a lead compound are important for drug function





QSAR and Drug Design



QSAR- quantitative structure-activity relationships

-there is a **simple mathematical relationship** between the biological activity of a drug and its physiochemical properties

- **hydrophobicity** of a drug is important for its biological activity
- changing the substituents on the drug to alter its nonpolar character **will affect its activity**

-hydrophobicity is measured by P (partition coefficient) between two immiscible solvents

$$P = [drug]_{oct}/[drug]_{H20}$$

$$\text{P = Partition Coefficient} = \frac{\text{Concentration dissolved in partition solvent}}{\text{Concentration dissolved in water}}$$

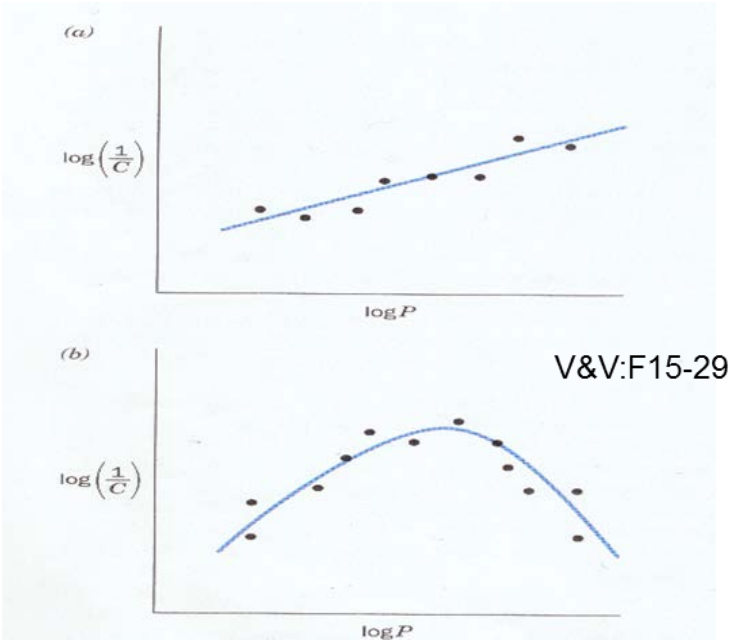
Conditions:
The solvents are "immiscible"
The system must be at equilibrium
All the solute must be dissolved
Temperature should be constant

- if C is [drug] to achieve a specified level of biological function then activity may be expressed as 1/C
  - plot of log(1/C) vs log P for a **small range of log P values** gives a **linear relationship**

$$\log(1/C) = k_1 \log P + k_2$$

- where k_1 and k_2 are constants whose **optimum values** in QSAR can be determined by **computerized curve fitting**
- compounds with a **larger range** of log P values
  - plot of log1/C vs log P will have maximum value and will be described by a **quadratic equation**

$$\log(1/C) = k_1(\log P)^2 + k_2 \log P + k_3$$



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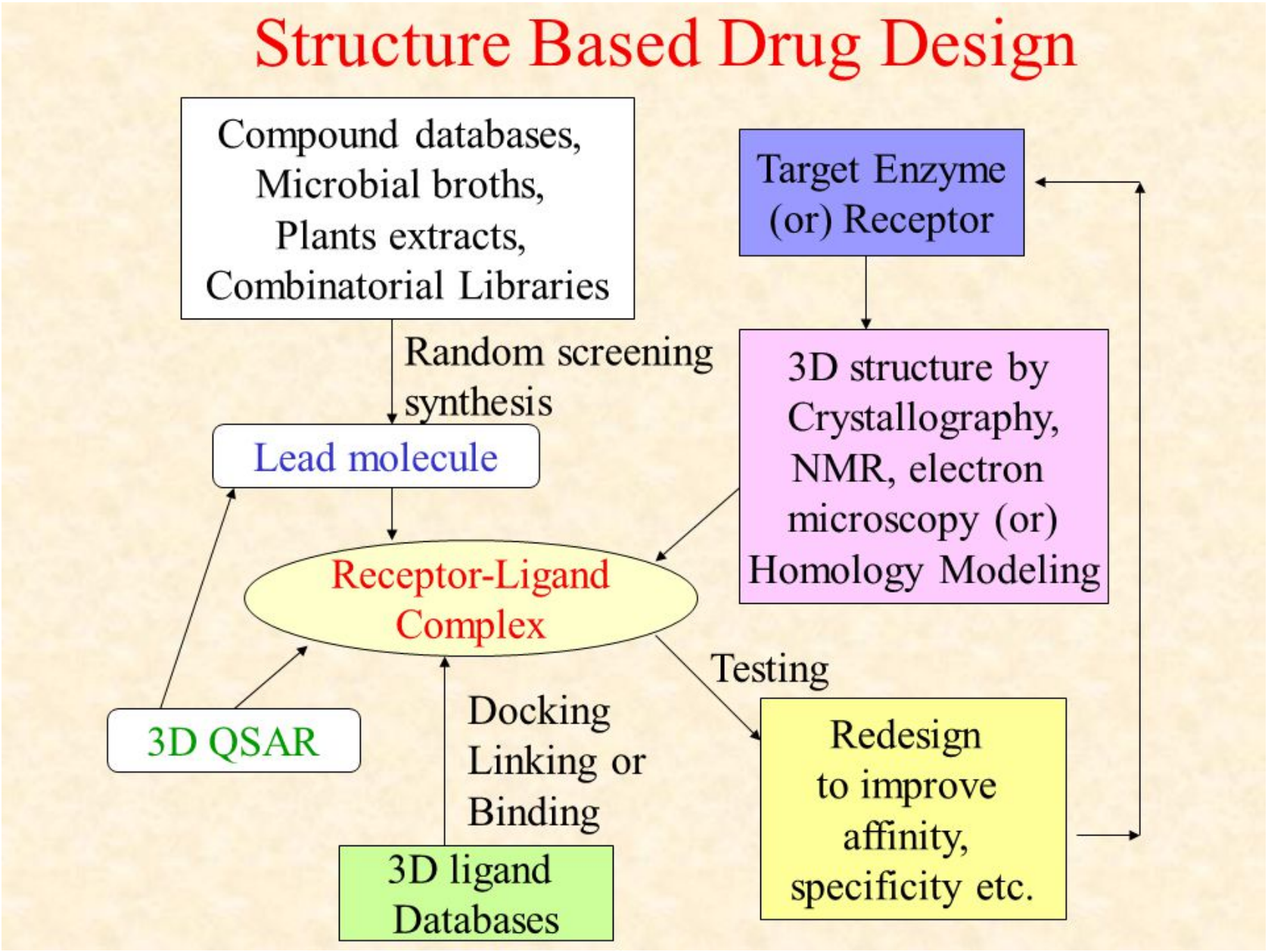
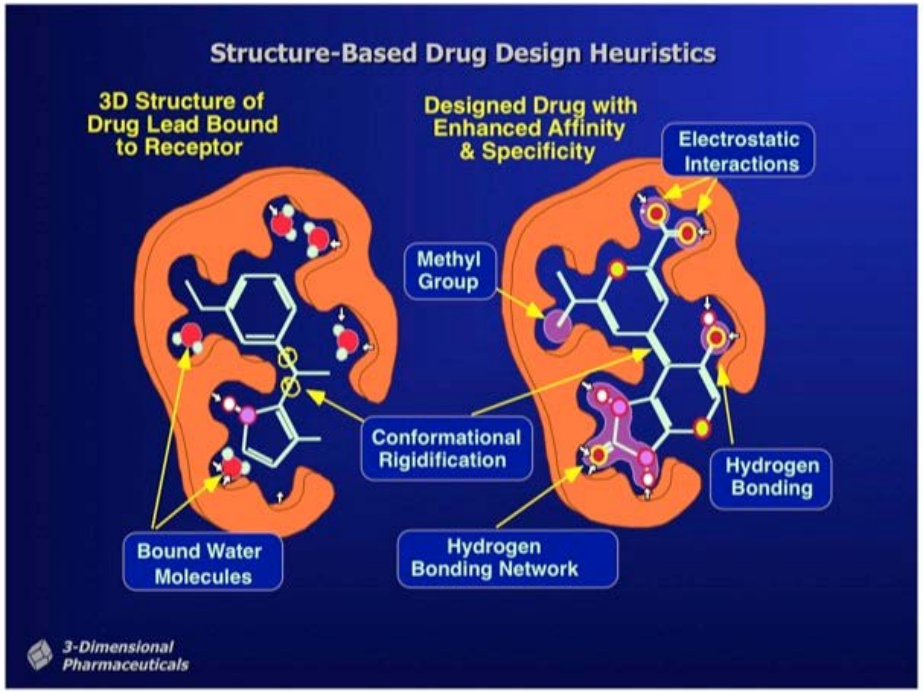
-typically QSAR depends on the chemical properties of a variety of substituents

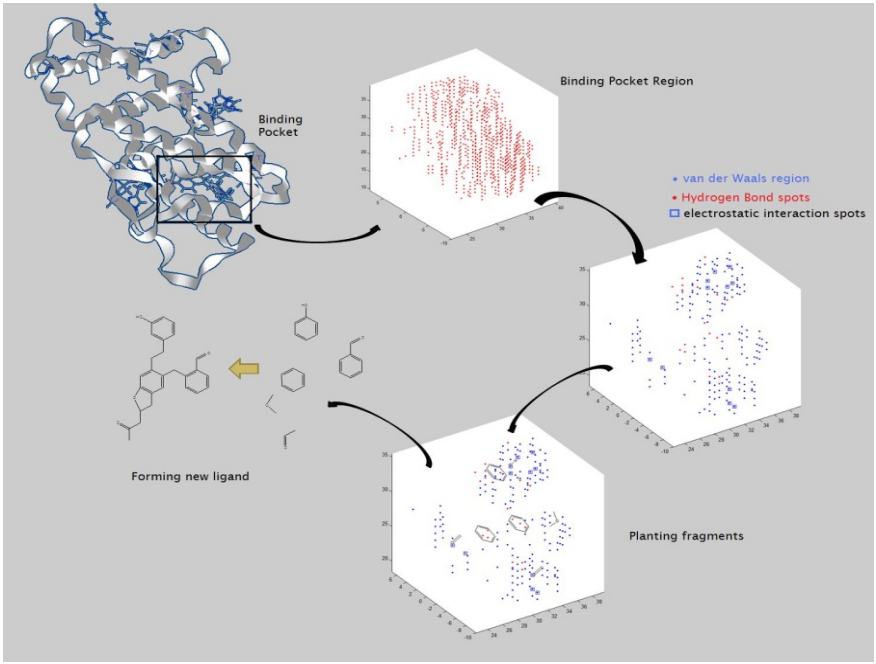
-pK values, van der Waals radii, H-bonding energy, and conformation

3. Structure-based drug design





- also called **rational drug design**
  - uses the <u>structure</u> of a receptor in complex with a drug candidate to **guide compound development**
- based on **X-ray crystallography and NMR structure determination**

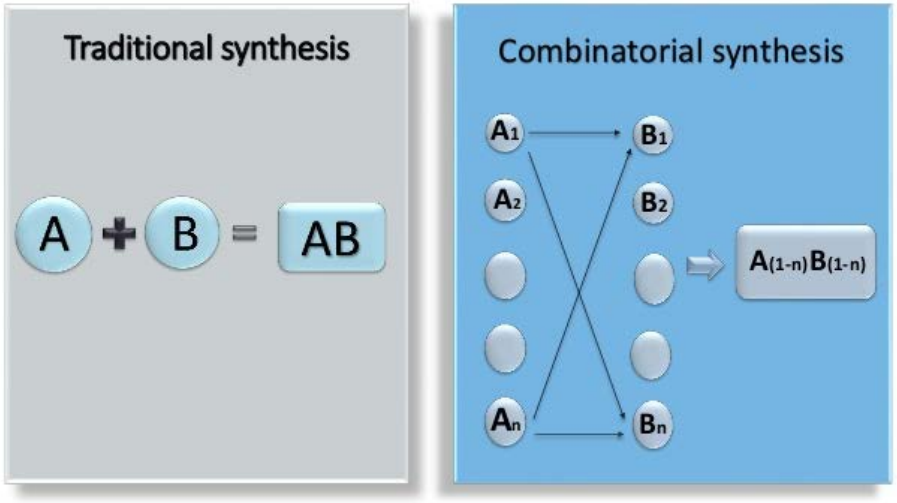


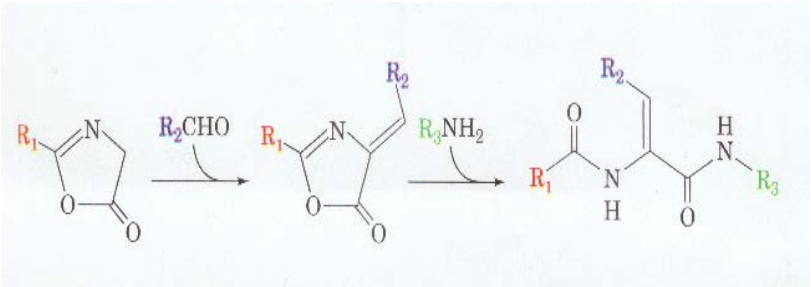
-may be supplemented with molecular modelling tools

- energy minimization
- quantum mechanical calculations
- docking simulations
- iterative process: structure of receptor with improved compounds

4. Combinatorial Chemistry and High-Throughput Screening

- **combinatorial chemistry:** rapidly and inexpensively synthesize large numbers of compounds in a "**make-many-compounds-and-see-what-they-do approach"**





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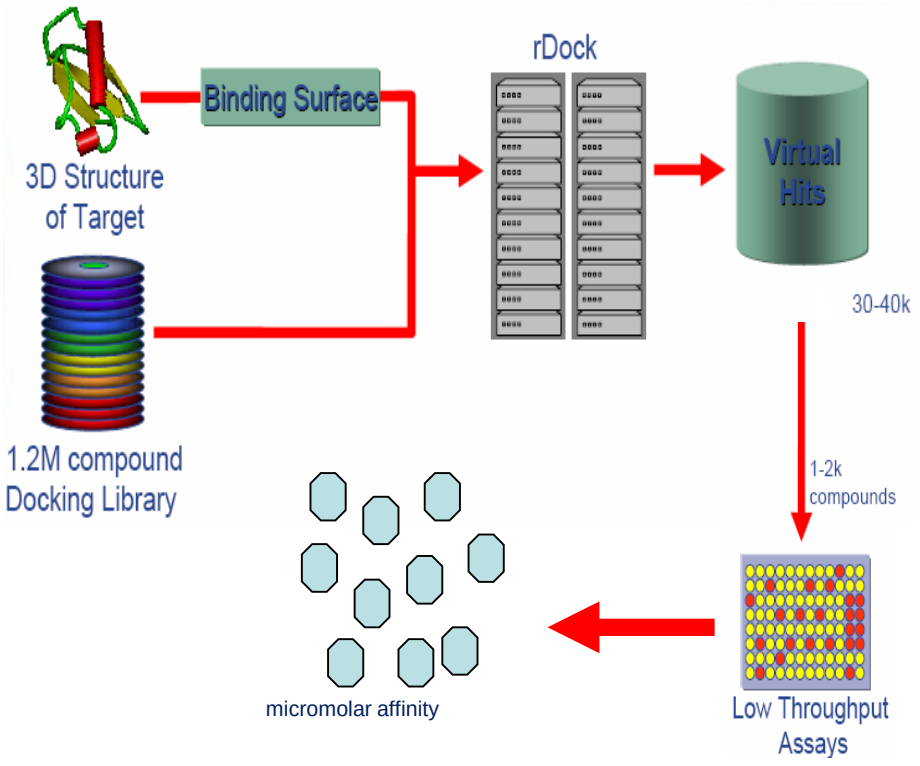
Arylidene diamides

- combinatorial synthesis approach: 100 different groups may be added to a single position on a drug scaffold

Virtual screening

Structure-based virtual screening



Methods **71**, 135-145 (2015)

Structure-based

- more effective at detecting novel chemical scaffolds
- need high-resolution structure of target

Ligand-based

- use SARs data from previous active molecules
- can increase structural diversity of compounds with similar activity
  - QSAR and pharmacophore modelling
- Disadvantage: discover molecules that are more limited in scaffold diversity

C. Introduction to Pharmacology

- *in vitro* development of an effective drug candidate is **the first step** in the development process
- **useful drug** must be delivered in **sufficient concn to the target receptor** in the human body without **unacceptable side effects**

1. Pharmacokinetics

- how a drug interacts with various cell barriers
- most convenient form of drug delivery is oral
- drugs must pass a series of barriers
- chemically stable in the acidic pH of stomach and not be degraded by digestive enzymes
- absorbed into the bloodstream and pass several membranes
- can't bind too tightly to other body substances
- must survive modification by liver enzymes
- avoid rapid excretion by the kidneys
- must pass from capillaries to the target tissue
- must pass blood-brain barrier (if brain is target)

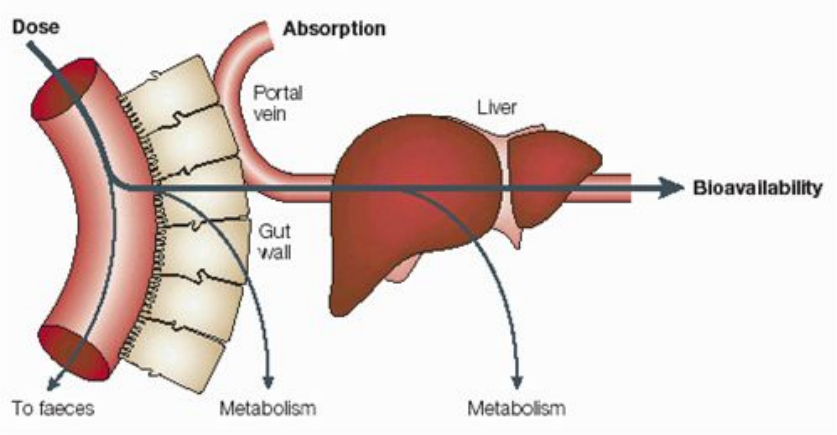
  -must pass through the plasma membrane (intracellular target)

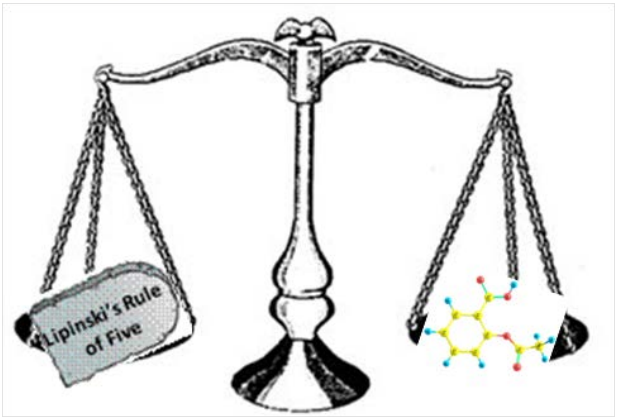
- **Bioavailability:** does the drug reach site of action?
  - depends on the dose given and its pharmacokinetics

Lipinski´s Rule of 5

Combination of descriptors to estimate intestinal absorption.
Insufficient uptake of compounds, if

Molecular weight > 500	slow diffusion
logP > 5.0	too lipophilic
> 5 H-bond donors (OH and NH)	too many H-bonds with the
>10 H-bond acceptors (N and O atoms)	head groups of the membrane

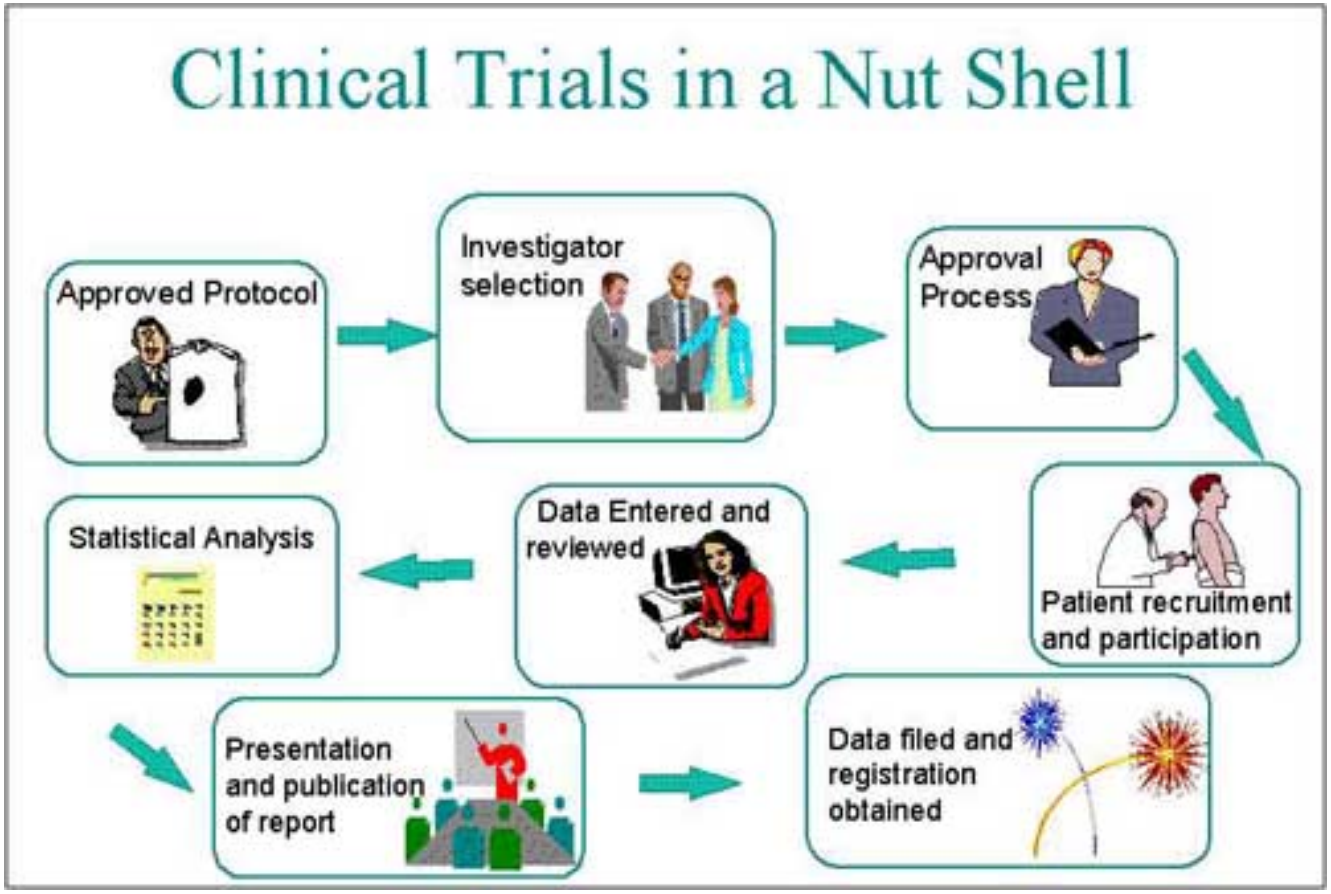




Lipinski, C.A. Adv. Drug Deliv. Rev. (2016) **101:** 34-41

2. Toxicity and Adverse Reactions Eliminate Most Drug Candidates

- drug candidate must be **safe and efficacious** in humans
- initial test in animals but ultimately the drug must be tested in **clinical trials**
- in US, clinical trials are monitored by the FDA and have 4 phases



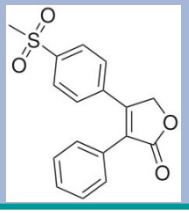
Clinical Trial Phases

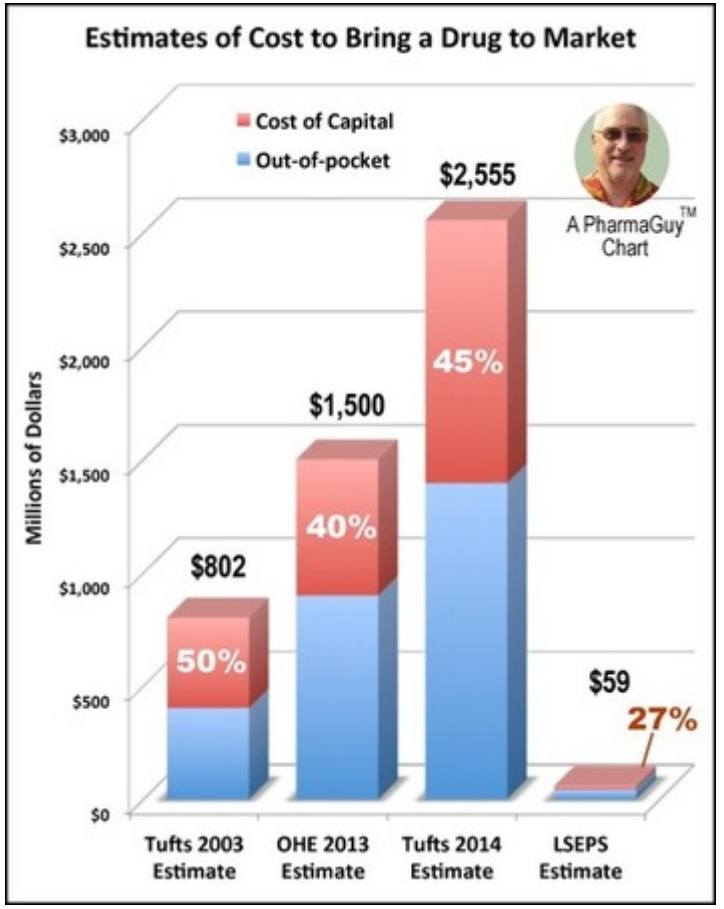
	Phase I	Phase II	Phase III	Phase IV
Primary Goal	• Establish the overall safety	• Establish the activity of a drug for a specific group of patients with a specific disease	• Confirm the safety and effectiveness of a drug for a specific group of patients with a specific disease	• Monitor ongoing safety in large populations and uncontrolled use of drug
Secondary Goals	• Establish the maximum tolerated dose • Determine serious side-effects • Determine the metabolism and pharmacologic actions of drugs	• Determine the common short-term side effects and risks.	• Evaluate the overall risk-benefit ratio	• Identify additional, unusual side-effects • Identify additional potential uses of the drug

https://www.cbc.ca/natureofthings/episodes/brain-magic-the-power-of-the-placebo

3. Drug Candidate Statistics

- **5** drug candidates in **5000** that enter preclinical trials reach clinical trials
- **1 of the 5** is approved for clinical use
- **40%** of candidates pass **Phase I** trials
- **50%** of those passing Phase I trials pass **Phase II** trials
- most drugs that enter Phase III **pass this trial**
- preclinical portion of drug discovery process **averages 3 years**
- successful clinical trials usually **require 7 – 10 years**
    - average **cost is $2.6 billion USD**
- drug may be **withdrawn some months or years later** if causes unanticipated life-threatening side effects in as few as **1 in 10,000 individuals**
    - (eg., Vioxx withdrawn by Merck, 2004)





4. Cytochrome P_{450} Metabolizes Drugs

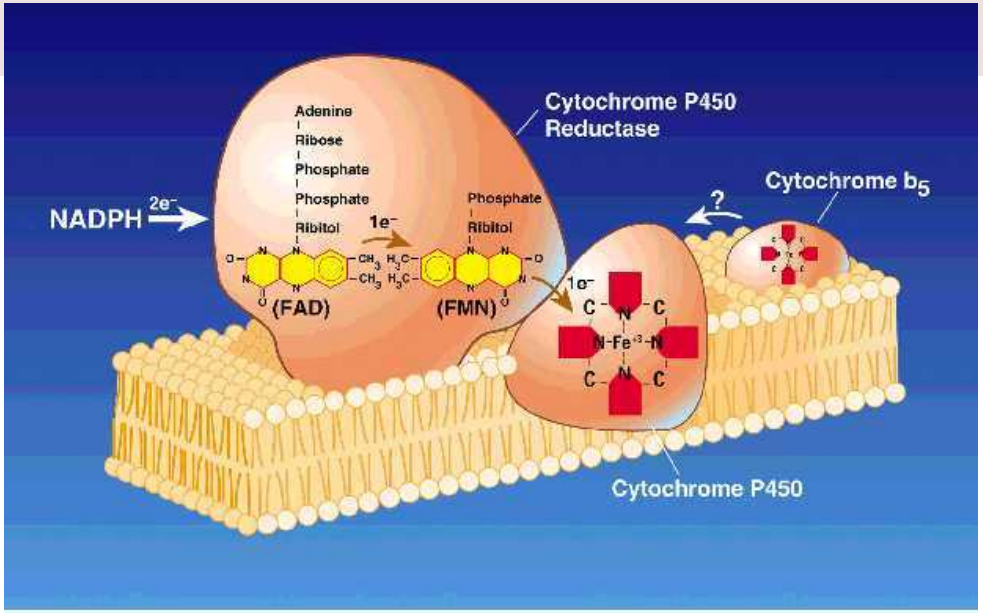
-why is it that a drug that is **well tolerated** by most patients can pose a **danger** to others?

-**differences in reactions to drugs** arise from genetic differences, different disease states, other drugs that they are taking, age, sex, and environmental factors

-cytochrome P450 role is to detoxify xenobiotics and assist in metabolic clearance of drugs

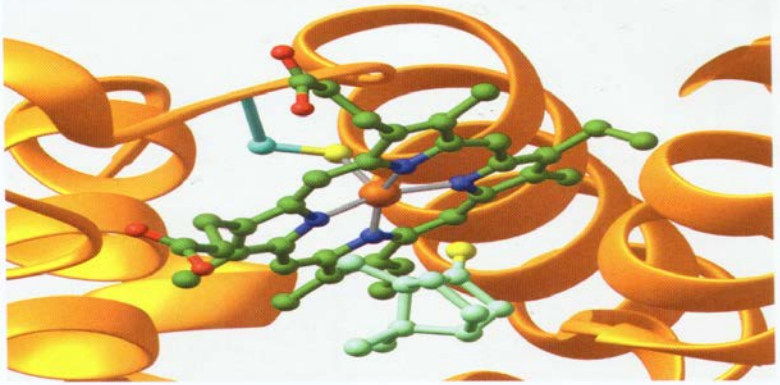
-humans express ca. 100 isoenzymes that catalyze the general reaction:

$$RH + O_2 + 2H^+ + 2e^- \leftrightarrow ROH + H_2O$$



-many cytochrome P_{450} in humans are **polymorphic** with several common alleles

-gives rise to tremendous variation in drug metabolism among individuals



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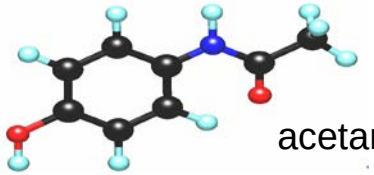
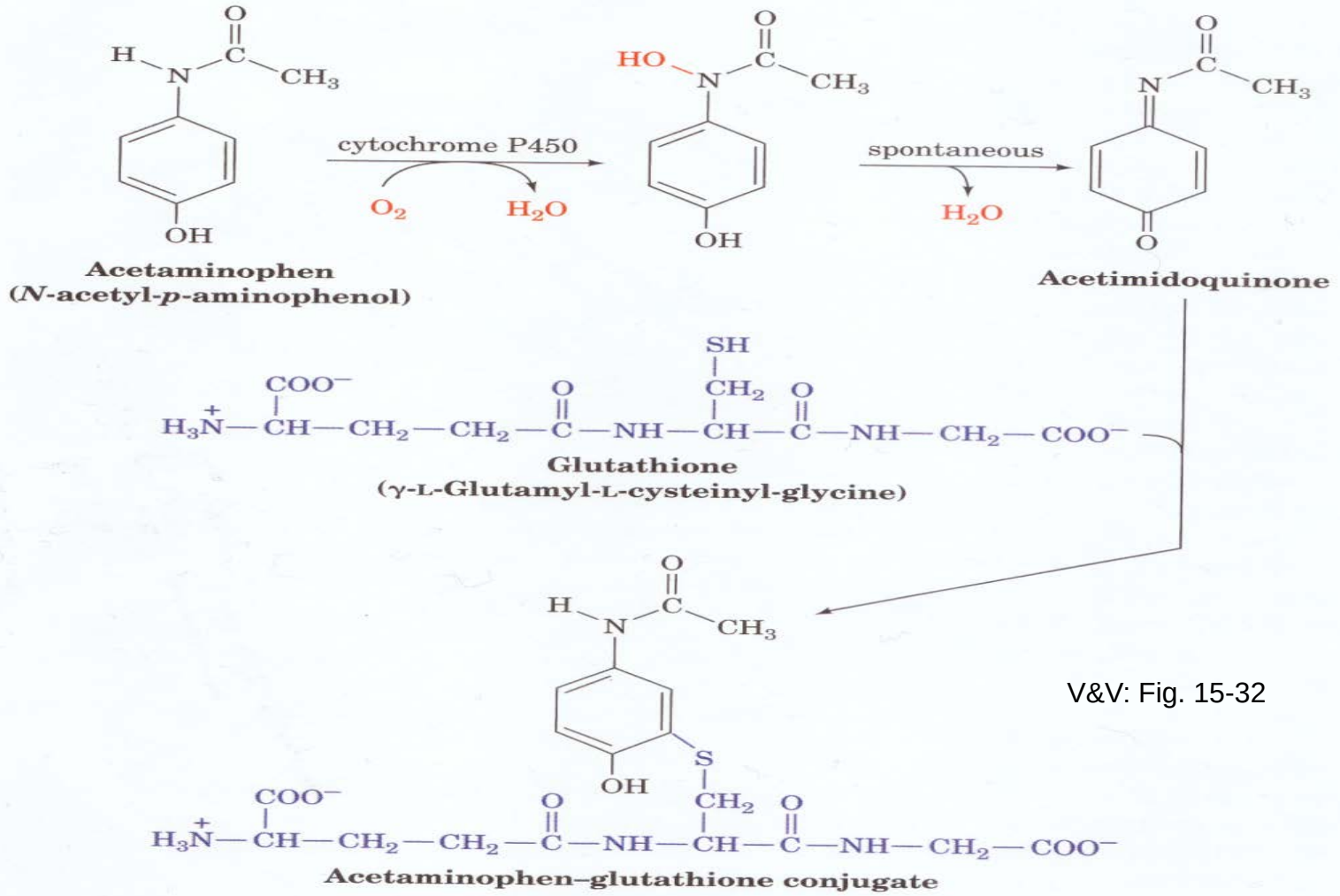


acetaminophen

- **acetaminophen** is a widely used analgesic and antipyretic
- safe in therapeutic doses (1.2 g/day; but at 10 g/day) = **toxic**
- acetaminophen (95%) is enzymatically glucuronidated or sulfated to form conjugates which are readily excreted
- 5% is converted by cytochrome P450 (CYP2E1) to **acetimidoquinone** which is conjugated with glutathione
- **larger doses** of acetaminophen saturates excretion pathways and the cytochrome P450-mediated pathway becomes significant
- if hepatic glutathione is rapidly **depleted then** acetimidoquinone conjugates with the protein sulfhydryl groups **= fatal hepatotoxicity**


cytochrome P450
O_2
H_2O
spontaneous
H_2O
Acetaminophen
(N-acetyl-p-aminophenol)
Acetimidoquinone
Glutathione
(γ-L-Glutamyl-L-cysteinyl-glycine)
Acetaminophen–glutathione conjugate

V&V: Fig. 15-32


Advil
TYLENOL
$5.99

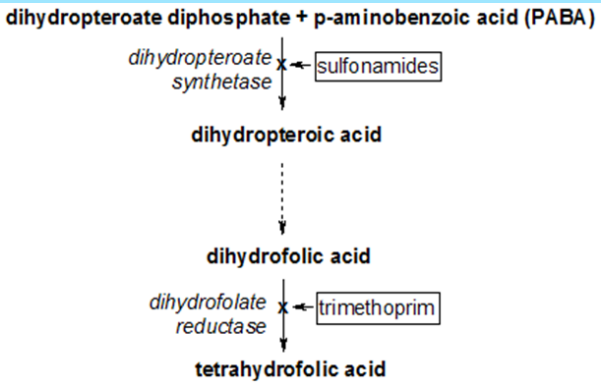
5. Many Drugs are Enzyme Inhibitors

-modern drug therapy is based on concepts of enzyme inhibition

-toxicity is **unavoidable** because, with the exception of cell wall synthesis in bacteria, most drugs that kill tumors, viruses, and bacteria also **kill normal eukaryotic cells** within the host

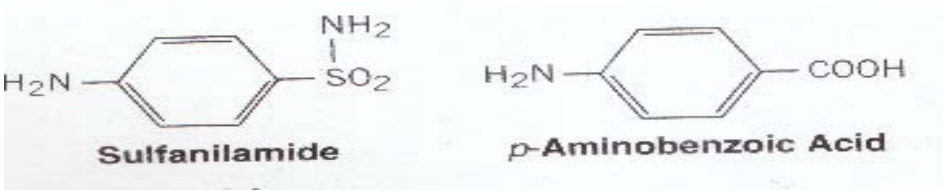
-relatively **short generation time** of the microbial organisms and tumor cells **can be exploited**

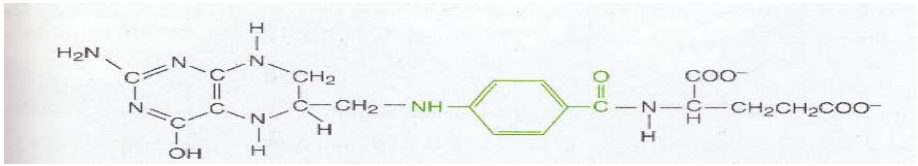
-these cells will be **more sensitive** to metabolic inhibitors than the host eukaryotic cells



a. Sulfa Drugs

-sulfanilamide is an antibacterial agent because it competes (competitive inhibitor) with p-aminobenzoic acid (PABA), essential for bacterial growth





5,6,7,8-tetrahydrofolate

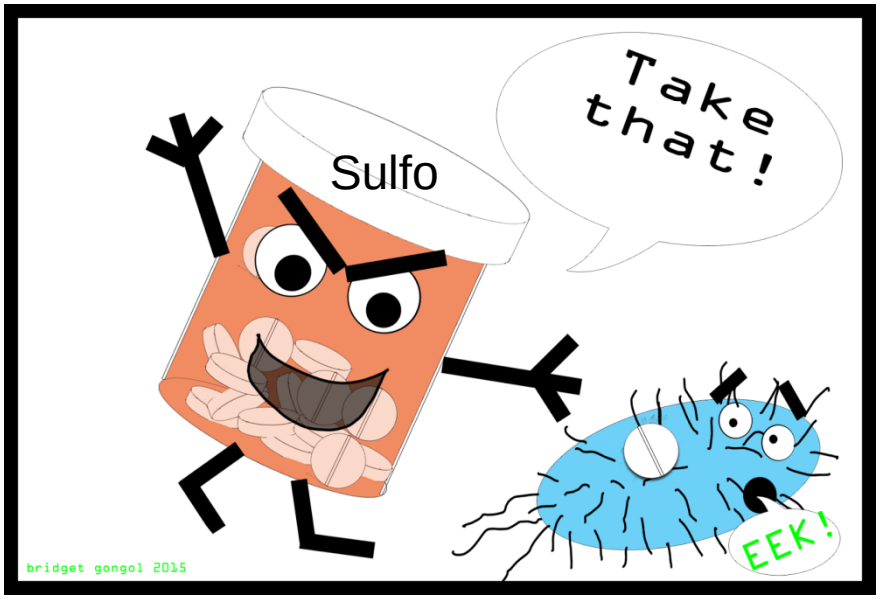
Source of folate

-bacteria can’t absorb folic acid but must synthesize it

-bacterial dihydropteroate synthase is tricked into making an intermediate containing sulfanilimide that can’t be converted to folate

-bacteria is starved of the required folate and can’t grow or divide

-since humans obtain folate from dietary sources, the sulfanilimide is not harmful at doses that kill the bacteria



b. Viagra®-an unexpected outcome in a drug-design program (Pfizer)

-sometimes the outcome of a drug design program is unanticipated

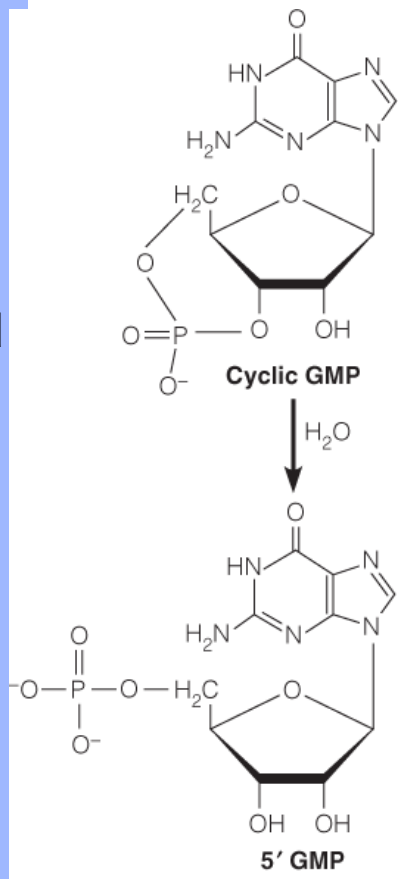
-e.g., penicillin discovery by Fleming

-relaxation of smooth muscle cells of blood vessels is controlled by intracellular ↓ [Ca^{2+}] as triggered by ↑ [cGMP]

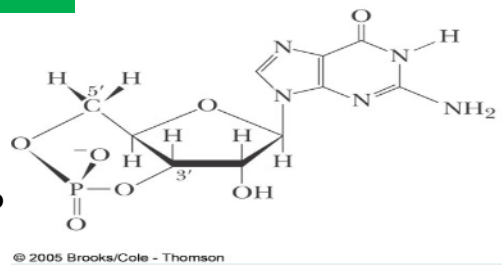
-cGMP is hydrolyzed by phosphodiesterases (PDE) to form 5'-GMP and let muscles contract again

-Pfizer designed **PDE inhibitor** against PDE5 isotype, a dominant form in human vascular tissue

-no significant benefits for **angina or hypertension** but some men in clinical trials reported **penile erection**

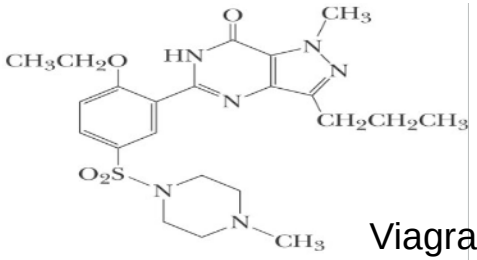


PDE substrate



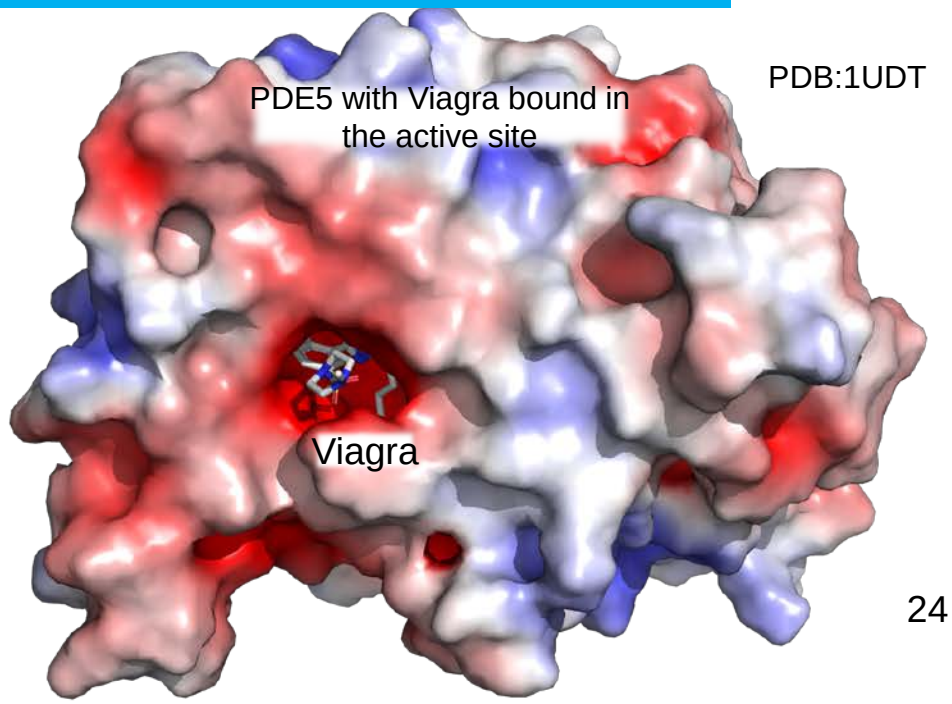
cGMP

PDE inhibitor



Viagra®

-apparently, Viagra® causes an increase in [cGMP] in penile vascular tissue allowing vascular muscle relaxation, improved blood flow and erection. A drug was born!



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