Therapeutics I
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Drug Discovery

Objectives:

At the end of the next hour the student should:

1. be able to recall the similarities and differences between the following six methods of drug discovery:
   A. Serendipidous Random Screening
   B. Lead Discovery and Natural Products
   C. Lead Optimization
   D. Rational Drug Design
   E. Structure Based Drug Design
   F. Combinatorial Chemistry and High-throughput Screening

2. know an example of a drug that was discovered by each of the first five methods.

3. have been introduced to the concept of *in silico* drug discovery and ADME screening.

References:

History:

1. Principles of Medicinal Chemistry, W.O. Foye, T.L. Lemke, D.A. Williams 4th Edition, 1995, Chapters 1-4.

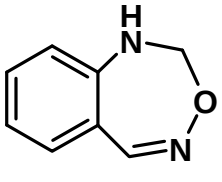
Neuroactive Steroids:

2. McNeil, R.G., Gee, K.W., Bolger, M.B., Lan, N.C., Wieland, S., Belelli, D., Purdy, R.H., and Paul, S.M., (1992) "Neuroactive Steroids that Act at $GABA_a$ receptors", Drug News & Perspectives 5(3):145-152.

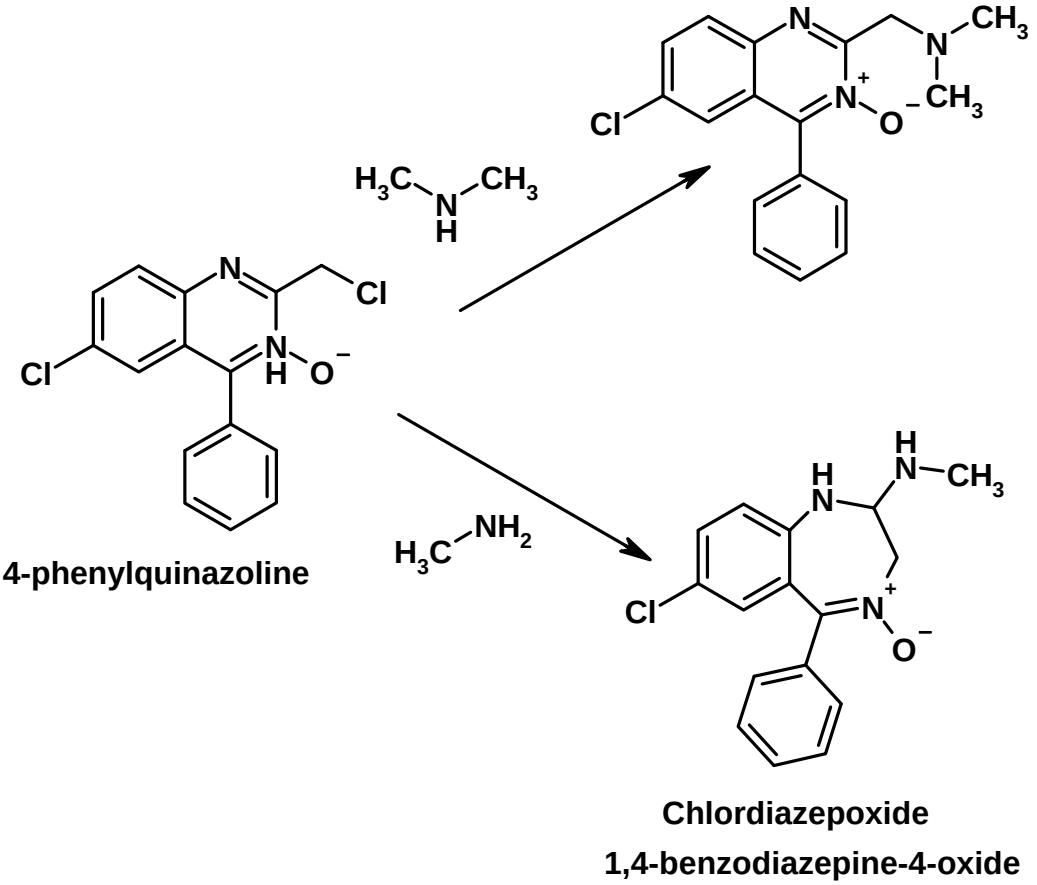
Serendipitous random screening

Discovery of Benzodiazepines (Valium™)

Hoffman-LaRoche in the 1930s, thought they had synthesized a series of 3,1,4-benzoxadiazepines.



1930s



1955

Chlordiazepoxide was submitted for testing in 1957 and was found to be hypnoic, and anticonvulsant. It was given the trade name Librium and marketed in 1960. Valium (5 – 10 more potent) was marketed in 1963.

Lead Discovery and Natural Products

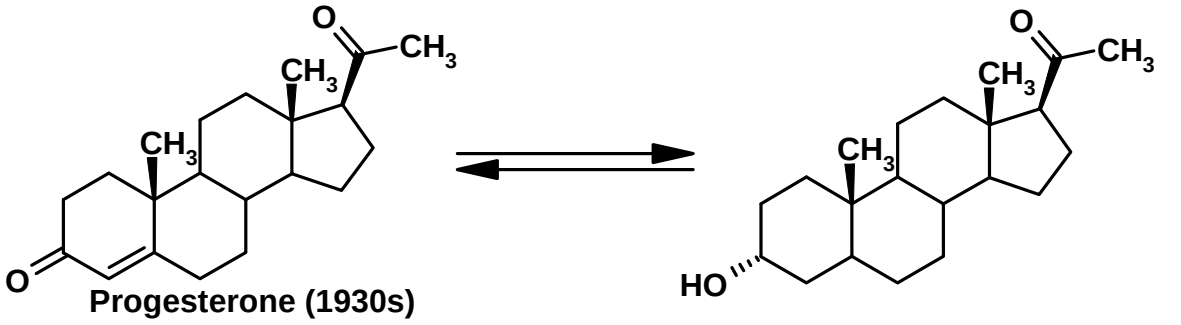
Discovery of the Epalons.

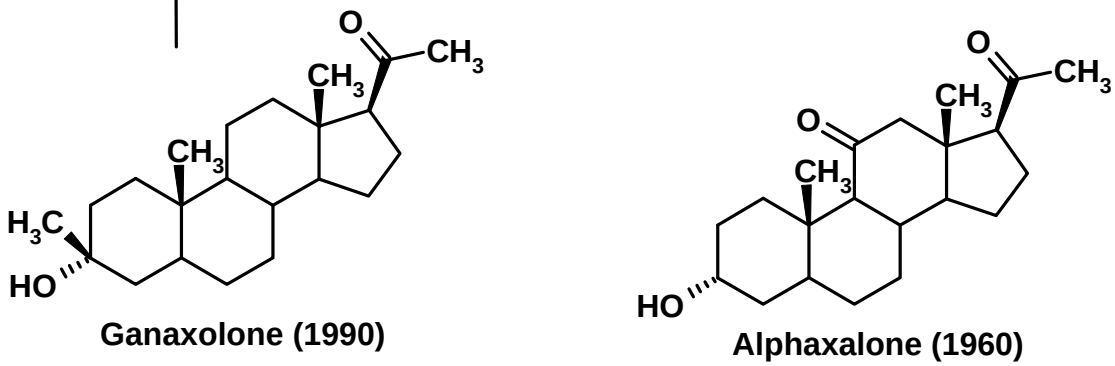
Russell E. Marker in 1930's working with Dioscorea (Mexican Yam)

Led to the first commercial synthetic procedure for progesterone

In 1937 Marker isolated epiallopregnaolone (Epalon) from 10,000 gallons of human pregnancy urine (US Patent 2,231,017).

Late 1960's. produced Alphaxalone as a non-volatile general anesthetic





Lead Optimization

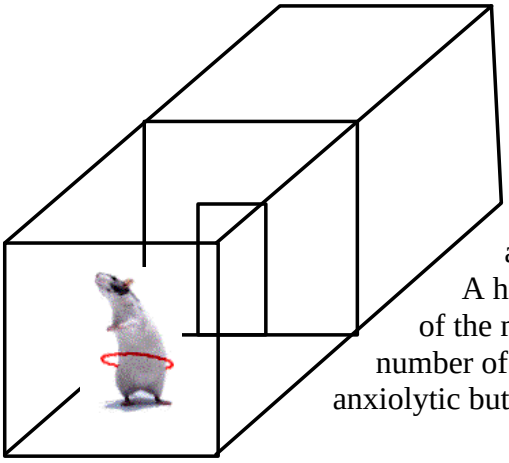
Late 1980s, CoCensys discovered the pharmacology, chemistry, and molecular site of action (at the $GABA_A$ receptor in the CNS) of the Epalons.

1987 patent on Ganaxolone and many other related 3-beta-substituted Epalons epiallopregnanolone owned by USC.

Currently, Ganaxolone is in Phase II clinical trials for treatment of infantile spasm, complex partial seizures (petite mal epilepsy), and migrane.

Rotorod apparatus: Used to study the sedative-hypnotic properties of drug molecules. Mice are injected with a control substance or the test drug and placed on the rotating dowel. Mice who are dosed with control or low doses of an anesthetic steroid will "ride" the dowel like a lumber-jack rolling logs. Mice who feel sleepy will fall off the rod indicating an active drug.

Light – Dark Transition: Used to test the anxiolytic (anti-anxiety) properties of a test drug. Two chambers are connected by a small door. One chamber is dark and the other is open to the ambient light. Mice generally spend more time in the dark, but are curious about the lighted compartment. The number of transitions between the dark and light compartments is proportional to the degree of anxiolytic activity of the test drug.



A high **therapeutic ratio** (TD_{50} = Dose required for half of the mice to fall of the rotorod / Dose for half maximal number of transitions) indicates a drug molecule that will be anxiolytic but not produce day-time sedation.

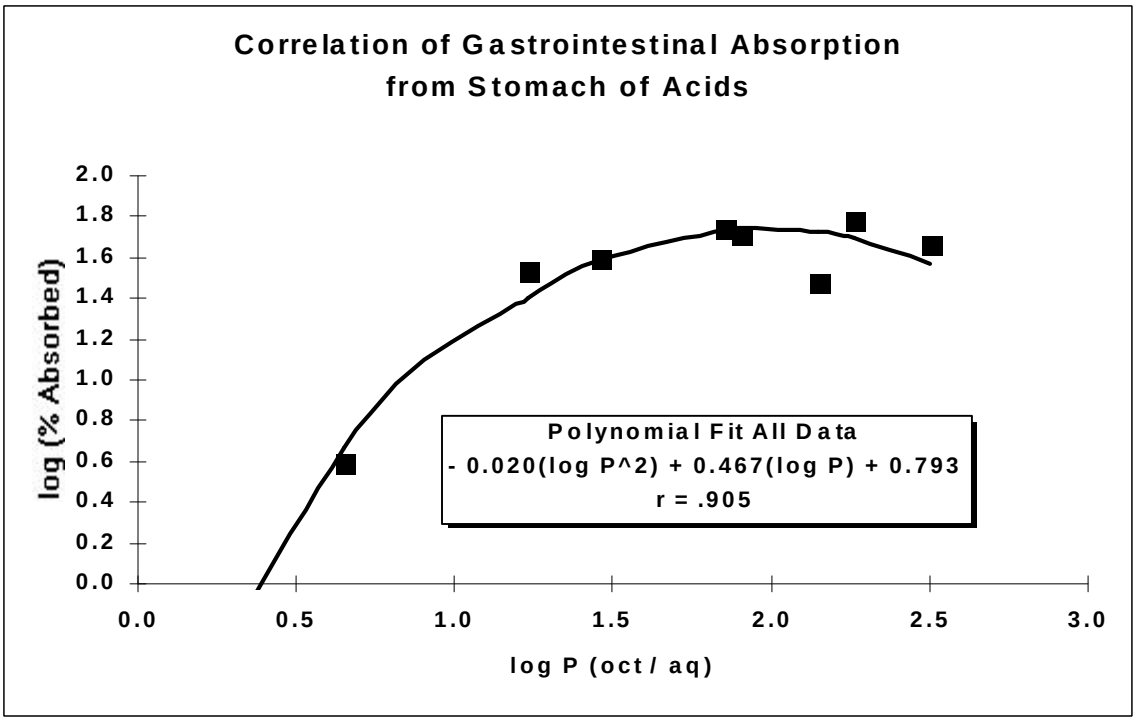
Rational drug design and (QSAR)

Pioneer of quantitative-structure-activity-relationships (QSAR) was Corwin Hansch in the 1960s.

Rational drug design is intended to decrease the number of compounds that need to synthesized and to find high affinity, low toxicity compounds faster and more inexpensively.

Table 1. Physicochemical properties and % Absorbed for Acidic Molecules
Ref: E.J. Lien, (1985) Env. Tox. and Chem. 4:259-271.

Drugs	Log P	Log (% Abs)		
ACIDS	(Oct/Aq)	Obsd	Calcd	PKa
Phenol Red	-1.16	0.30	-4.36	7.9
Barbital	0.65	0.60	0.66	7.8
Acetylsalicylic Acid	1.23	1.54	1.41	3.5
Phenol	1.46	1.60	1.59	9.9
Benzoic Acid	1.85	1.74	1.74	4.2
p-hydroxypropiophenone	1.85	1.74	1.74	7.8
5-Nitrosalicylic Acid	1.90	1.72	1.74	2.3
Secobarbital	2.15	1.48	1.73	7.9
Salicylic Acid	2.26	1.79	1.69	3.0
Thiopental	2.50	1.66	1.57	7.6



The data shown above illustrates that the gastrointestinal absorption of organic acids increases with increasing lipophilicity with a peak absorption for compounds with a logP of ~2.0. According to the polynomial fit, the absorption of organic acids will decrease as the logP value becomes greater than 2. In addition, all of these organic acids have pKa values that result in > 90% un-ionized species at pH~1.0.

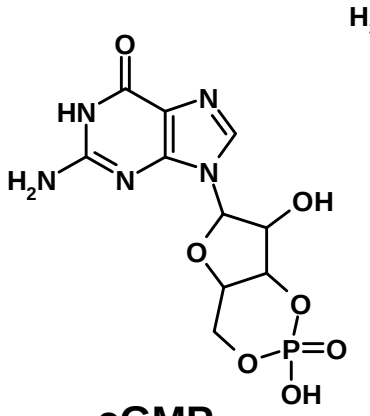
Viagra

Sildenafil (Viagra™) developed by using rational drug design.

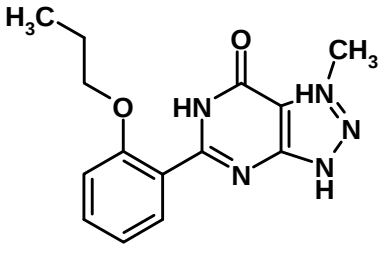
Pfizer team started in 1986 with the structure of cGMP and a very weak non-selective inhibitor of cGMP phosphodiesterase (PDE) called Zaprinast

Zaprinast was modified to increase its activity as an inhibitor of PDE 5 and to improve its solubility and decrease lipophilicity.

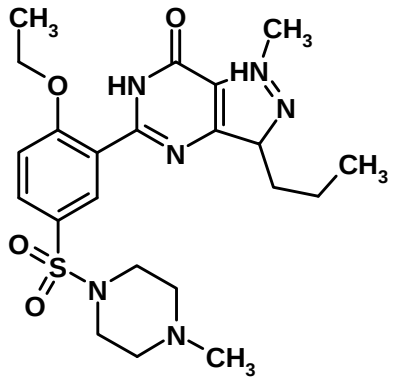
Sildenafil (UK-92,480) in 1989 was a potent selective inhibitor of cGMP PDE 5



cGMP



Zaprinast



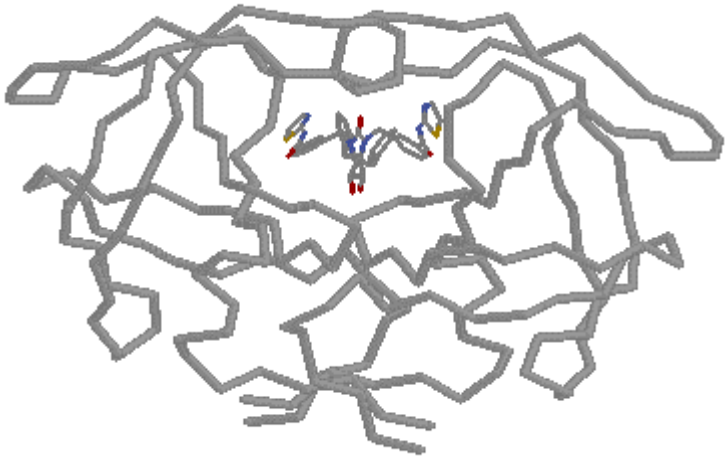
UK-92,480 (Viagra)

Compound	PDE-1	PDE-3	PDE-5
Zaprinast	9,400 nM	58,000 nM	2,000 nM
Viagra	260 nM	65,000 nM	**3.6 nM**

Structure based drug design

1970s computer software and hardware was developed to allow the display and manipulation of three dimensional models of drug molecules.

Today we have 9232 three-dimensional X-ray crystallographic structures of drugs and receptors.



Inhibitors of human immunodeficiency virus (HIV-1) protease.

Indinavir sulfate was designed with the help of an X-ray crystallographic structure and molecular mechanics calculations.

http://199.105.131.24/phys/html/interact/mech/intro.asp
http://199.105.131.24/phys/html/interact/history/intro.asp

Pharmaceutical Targets and Human Genome Project

X-ray crystallographers can not keep pace

Computational techniques have been developed to predict the structures of pharmaceutical target molecules starting from just sequence information.

Single Nucleotide Polymorphisms (SNPs)

Sickle cell anemia is caused by the variation of a single base pair in the β-globin gene..

Fiber optic based SNP detection, 400,000 hybridized DNA on a fiber optic bundle (1.3 cm^2).http://www.illumina.com/.

Combinatorial Chemistry

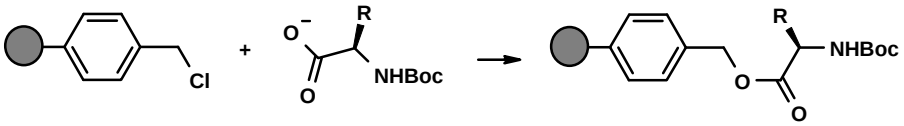
Combinatorial chemistry is based on the hope that if you synthesize and test enough compounds you are surely going to find a winner somewhere in the haystack.

Robert Bruce Merrifield

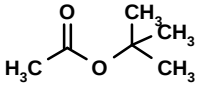
Automated peptide-synthesis technology was invented in the 1960s by biochemist Robert Bruce Merrifield was awarded the Nobel Prize in chemistry "for his development of methodology for chemical synthesis on a solid matrix."

Merrifield's Invention

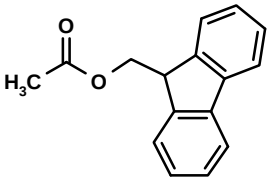
"Our idea was to put the first amino acid of the peptide chain on a solid support, a polystyrene resin bead," says Merrifield. "You put it on by a rather stable bond. Then you carry out the addition of the second amino acid, so that you now have a dipeptide. You filter these polystyrene beads and wash them thoroughly to effect a partial purification. Then you're all ready to add the next amino acid, and you go through the same procedure." In this way, the peptide is built one amino acid at a time to the desired length. At the end of the synthesis, the peptide is removed from the solid support to be purified and characterized (Merrifield 1963).



Boc (t-butyloxycarbonyl) =



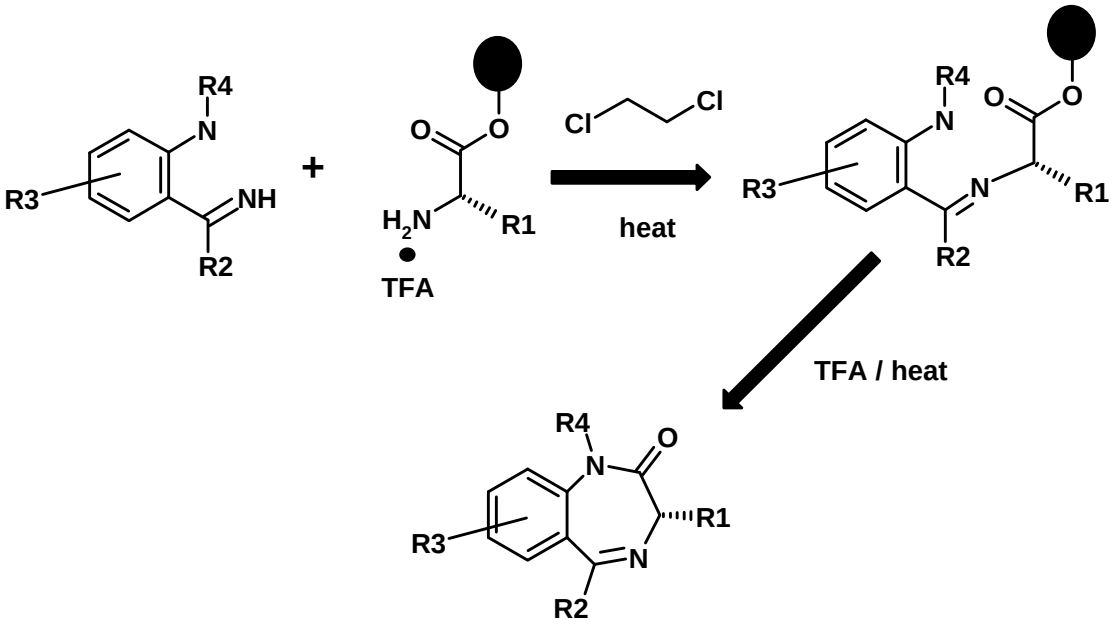
Fmoc (9-fluorenylmethyloxycarbonyl) =



Jonathan Ellman

Found a method to make benzodiazepines derivatives using combichem in 1992

Example of parallel solid-phase synthesis of benzodiazepines:



Benzodiazepines are well-know β-turn peptide mimetics, and structural variation leads to a wide range of biological activities..

Mix and Split Synthesis (combinatorial libraries)

Brute force method relies on the "numbers game"

If you make enough compounds, you will surely get lucky

Richard Houghten and colleagues found an all D-amino acid opioid peptide by synthesis of 52,128,400 hexapeptides.

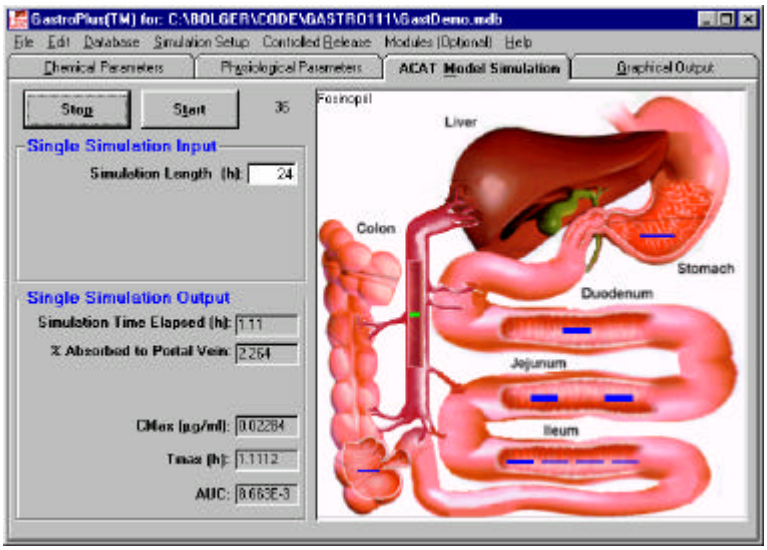
High throughput screening

Current robotic methods have been developed to allow *in vitro* screening of 100,000 compounds per day.

See: http://www.sphinxpharm.com/history.html

Early ADME screening & *in silico* prediction

Holy grail of drug design is to be able to design and screen new drug molecules *in silico* without the need to synthesize or perform *in vitro* screening.



Several companies have been established to develop sophisticated computer models of organ systems. Physiome Inc. has developed a sophisticated cardiovascular model that promises to allow drug companies to test cardiovascular drugs *in silico.*

Simulations Plus, Inc. has developed a complex simulation of drug absorption in the gastrointestinal tract called "GastroPlus™". In combination with QMPRPlus (Quantiative Molecular Permeability Relationships), GastroPlus will be able to predict the absorption, distribution, metablism and elimination of combinatorial libraries prior to synthesis or *in vitro* testing.