

# Drug Discovery



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## Drug Trivia

- USA: \$25 Billion/year for R&D of pharmaceuticals
  - 33% of this for clinical trials
- 10 to 15 years from concept to marketing
- Total cost to develop a drug (\$0.5B to \$0.9B)
- Example: Currently 19 Alzheimer's drugs in development
- 1 drug is FDA approved for every 5000 compounds tested
- 1 out of 100 drugs succeeds to market
- Estimate: 11,000,000 Americans with Alzheimer's by 2050 (currently about 4 million).
- Why are these facts important?
  - Drug development is time consuming and very expensive.
  - By using computers we hope to:
    - reduce costs
    - accelerate drug discovery.



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## Introduction



- A drug is a chemical entity with a known pharmacological effect.
  - For example, it may be used to:
    - counteract a disease state
    - maintain health
- It is regulated by the FDA in the USA (Food and Drug Administration).

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## Disease States



- Categories:
  - Genetic
    - over 3000 different genetic diseases due to a single mutational change
  - Viral
  - Bacterial
  - Toxic substances

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## Other Reasons for Taking Drugs



- Improvement of life expectancy
- Anti-depressants
- Weight loss
- Pain relief and anaesthetics
- Controlling hormonal function
- Control of cardiac function
- Controlling autoimmune diseases:
  - Rheumatoid arthritis
  - multiple sclerosis
  - Crohn's disease (a type of inflammatory bowel disease)
  - Systemic Lupus Erythematosus (lupus) (an autoimmune disease)
  - Type 1 diabetes mellitus
  - etc. (there are many others).
  - Idiopathic Thrombocytopenic Purpura (ITP)

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## Typical Focus Areas



- Cardiovascular System (CVS)
- Central Nervous System (CNS)
  - Includes anti-depressants
- Gastro-Intestinal
  - Eg. Cimetidine counteracts over production of acid
- Anti-Neoplastic agents
  - Anti-cancer drugs
- Respiratory agents
- Anti-Rheumatism agents
- Agents to amend metabolic disorders
- Anti-infective agents
- Anti-inflammatory agents
- Diagnostic agents

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## Drug Development and Computation



- The major emphasis is the development of computer algorithms to accelerate drug discovery.
  - We want an early elimination of drugs that have inadequate binding within the target receptor.
  - Ideally the drug is taken orally and survives any attempt at degradation so that it can get to the site where it is needed.
    - Computational procedures can help to predict this.
  - We want an early elimination of drugs that have harmful side effects.
    - This is more difficult computationally.

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## Drugs at the Molecular Level



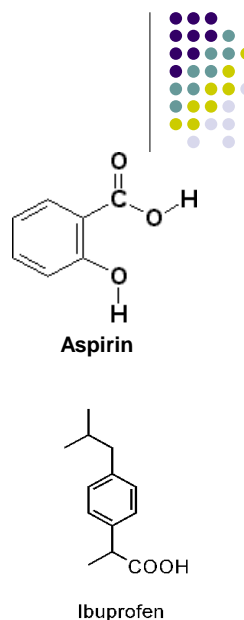
- Drugs are typically small molecules and may be derived from:
  - **Natural products**
    - plant extracts
    - animal fluids (Eg.: snake venoms)
    - fermentation broths
  - **Synthetic Chemicals**
    - derived from a pharmaceutical process

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## Drug Improvements

- Isolate natural compound
  - to get aspirin
  - ASA (acetyl salicylic acid)
- Manipulate structure to get a better drug with better efficacy and fewer side effects



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## Side Effects

Most drugs have side effects which are usually rare.

- Here are the side effects for tadalafil (cialis):
- “less common” - needing doctor’s attention:
  - Arm, back or jaw pain; blurred vision ; chest pain or discomfort; chest tightness or heaviness; chills; cold sweats; confusion; dizziness; fainting; faintness or lightheadedness when getting up from a lying or sitting position suddenly; fast or irregular heartbeat; headache; nausea; nervousness; pain or discomfort in arms, jaw, back or neck; pounding in the ears; shortness of breath; slow or fast heartbeat; sweating; unusual tiredness or weakness; vomiting.
- “less common” - no need for doctor’s attention unless they become “bothersome”:
  - Bloody nose; burning, crawling, itching, numbness, prickling, “pins and needles” , or tingling feelings; burning feeling in chest or stomach; burning, dry or itching eyes; body aches or pain; congestion; cough; diarrhea; difficulty in moving; difficulty swallowing; dry mouth; dryness or soreness of throat; eye pain; excessive eye discharge; fever; feeling of constant movement of self or surroundings ; feeling of warmth redness of the face, neck, arms and occasionally, upper chest; hoarseness; itching skin; joint pain; lack or loss of strength; loose stools; muscle aching or cramping; muscle pains or stiffness; nasal congestion; neck pain; pain in arms and/or legs; pain or burning in throat; rash; redness, pain, swelling of eye, eyelid, or inner lining of eyelid; reduced sensitivity to touch; runny nose; sensation of spinning; sleepiness or unusual drowsiness; sleeplessness; sores, ulcers, or white spots on lips or tongue or inside the mouth; spontaneous penile erection; stomach upset; swelling of eyelids; swelling or puffiness of eye or face; swollen joints; tearing; tender, swollen glands in neck; tenderness in stomach area; trouble sleeping; unable to sleep; upper abdominal pain; voice changes; watering of eyes.

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# Drug Discovery Overview



1. Target characterization:
2. Primary screening to get “hits”
3. Hits to leads
4. Structure modification for lead optimization
5. Clinical trials and approval

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## Drug Discovery Steps (1a)



- Target characterization:
  - Do research on the disease to the extent of generating a chemical, biological, and physiological set of hypotheses about the functionality of the genetic or biological target.
    - Targets are often **validated** by gene knock-out experiments.
    - Target functionality is studied wrt its reaction pathway.
  - Targets are often related to dysfunction due to disease:
    - Genetic disorders
      - Egs.: cystic fibrosis, phenylketonuria, Huntington disease, ...
      - A genetic disorder may give you a susceptibility to a disease (Eg.: BRCA and cancer).
    - Autoimmunity disorders
      - Egs.: rheumatoid arthritis, ITP, type 1 diabetes, systemic lupus erythematosus, ...
    - Infections (fungi, bacteria, viruses)
    - Injuries
      - Egs.: disease states due to high cholesterol, smoking, alcohol,...

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## Drug Discovery Steps (1b)



- Target characterization:
  - Identify the protein and the active site on that protein.
    - What is the normal and/or dysfunctional activity of this site?
    - What will be the role of the drug? Usually an inhibitor.
    - There are two cases:
      - Structure-based design:
        - We have structural information about the binding site.
        - We can use docking strategies for a drug design project.
        - Using “de novo” design we can construct a drug to match the binding site.
      - Ligand-based design:
        - Without knowledge of the binding site we study ligands that have a high binding affinity for the binding site and try to find their patterns of molecular similarity (QSAR, pharmacophores).

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## Drug Discovery Steps (1c)

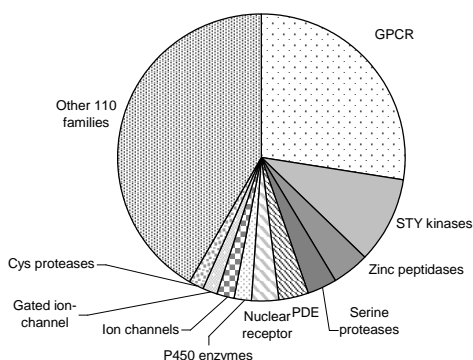


- Target characterization:
  - Drugs usually inhibit or block the actions of regulatory proteins. Here are four possibilities:
    - **Enzymes**
      - Eg.: Celecoxib inhibits cyclooxygenase-2.
    - **Carriers**
      - Eg.: Fluoxetine (Prozac) is converted to norfluoxetine which inhibits serotonin transporters.
    - **Receptor proteins**
      - Eg.: Researchers are investigating drugs that will inhibit PPAR  $\delta$  a protein that is involved in the meta-static spread of breast cancer.
    - **Ion channels**
      - Eg.: Calcium channel-blocking drugs are useful in the elimination of heart arrhythmias.

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## Drug Discovery Steps (1d)



Hopkins, A.L., Groom, C.R.,  
The druggable genome,  
*Nature Reviews, Drug Discovery*,  
(1) 2002, pp. 727-730.

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### ● Drug Targets:

- Over 400 proteins have been used as drug targets.
- Most targets are in a few major families:
  - GPCR's
    - G-Protein-Coupled Receptors
  - kinases
  - proteases
  - peptidases

## Drug Discovery Steps (2)



### ● Primary screening to get “hits”:

- Initial active compounds may come from:
  - traditional remedies
  - drugs that work for similar diseases
- HTS (High Throughput Screening)
  - HTS tries to rapidly assess the activity of a large number of compounds using assays that are run in parallel.
  - Identify “hits” (compounds that achieve binding in the nanomolar to micromolar range).
- Design an assay to measure function of the target.
  - An assay may be *in vitro* or *in vivo* (animal model) based.
- Use the assay to look for modulators of target's function.

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## Drug Discovery Steps (3a)



- Hits to leads
  - Primary screening identifies hits.
    - To increase the chances that we eventually get a compound that meets the many constraints that will be imposed, this list of hits has to be enlarged with more compounds that are similar to these hits.
    - This is often done using:
      - Screening of synthesized compound libraries
      - In-silico screening of virtual libraries

Chemists may synthesize as many as 10,000 different compounds before getting a drug that will make it to market.

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## Drug Discovery Steps (3b)



- Screening of synthesized compound libraries
  - Various compound libraries are in use:
    - Random collections of synthesized compounds.
    - Collections of synthesized compounds that meet some established criteria (Eg.: compounds that are likely to get through the blood brain barrier.)
    - Combinatorial libraries.
      - These are compound collections that have been synthesized in a combinatorial fashion to produce a family of *analogs*.
      - For example, we start with a “scaffold” that has  $m$  places (points of diversity)  $R_1, R_1, \dots, R_m$ , where side groups can be varied.
      - If point  $R_i$  has  $N_i$  different substituents then we can rapidly generate  $N_1 \times N_2 \times \dots \times N_m$  compounds.
      - Generated by industrial processes using robotic technologies.

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## Drug Discovery Steps (3c)



- In-silico screening of virtual libraries
  - Virtual screening can sift through millions of virtual compounds that meet the specified constraints of the researcher.
    - Constraints may place a range on: molecular weight, charge, hydrophobicity, hydrogen bond acceptors and donors, etc.
    - Machine learning strategies can be used to predict whether or not these compounds meet various ADMET constraints (to be discussed later).

## Drug Discovery Steps (3d)



- A library will contain of thousands of variations with respect to a fixed template:
  - Typically organized as a “scaffold” with a small number of functional “R-groups” attached to the scaffold.
  - You too can download millions of compounds from: <http://pubchem.ncbi.nlm.nih.gov/>, also small datasets are freely available from: <http://michem.disat.unimib.it/chm/>.
- Good libraries span large areas of chemical and conformational space.
  - i.e. high molecular diversity
  - diversity across: steric, electrostatic, hydrophobic interactions...
- Libraries may also be designed for specific objectives.
- Computer aided combinatorial library design is still in its infancy.

## Drug Discovery Steps (3e)



- Counter-screens
  - Additional screening can involve assays that involve related proteins from the same family or proteins with very similar binding sites.
  - This check is to verify that the compound is sufficiently selective that it has an affinity only for the target protein.
    - We do not want “promiscuous inhibitors”.
    - The idea is to eliminate unwanted side effects if possible.
    - Example: the Cox-2 and Cox-1 proteins have very similar binding sites.
      - (Isoleucine in Cox-1 and valine in Cox-2)
      - Non-steroidal anti-inflammatory drugs should bind to Cox-2.
      - NSAIDs are an annual \$13 Billion market...

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## Drug Discovery Steps (3f)



- Iterations
  - If desired virtual screening can be used to inform the choices that are made in the construction of synthesized compound libraries.
  - It is also possible that screening assays of a compound library can be used to generate the families of virtual compounds that are subjected to virtual screening.
  - Consequently, it is to be expected that assays of compound libraries and virtual screening are done in an alternating fashion, the idea being to constantly refine the set of candidate drug leads.

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## Drug Discovery Steps (4)



- Structure modification for lead optimization
  - The drug structure is manipulated to increase potency.
  - This is often an iterative process:
    1. Synthesize and co-crystallize with the protein.
    2. Follow up with computer-aided analysis and design of more drug candidates.
      - a) What happens when functional groups are modified or removed?
      - b) Should the scaffold be more flexible or more rigid?
      - c) Extra groups may be added to stop interactions with other proteins (these interactions causing side effects).
    3. Optimization is multiple objectives and changes may be necessary to meet ADMET constraints.
  - Repetition of these steps leads to a small set of final drug candidates.

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## Drug Discovery Steps (5)



- Clinical trials and approval
  - The previous discovery steps can take over 2 years (typically ongoing with testing of other drugs)
    - Or watch the competition: "Me Too!" drugs.
  - Preclinical testing
    - Lab and animal testing
  - Phase 1
    - Limited number of healthy volunteers are given the drug
    - Safety and dosage determination
  - Phase 2
    - 100 to 300 patient volunteers
    - Testing for efficacy and side effects
  - Phase 3
    - 1000 to 5000 patient volunteers
    - Testing for long term reactions
  - FDA review and approval
  - Post-marketing testing

Total Cost of all stages:  
600 to 800 (Millions, U.S. \$)  
(A disputed estimate. See:  
"The Truth About the Drug  
Companies" by M. Angell, M.D.)

Time: 7 to 15 years!

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## Drug Development and Computation



- The major emphasis is the development of algorithms to accelerate drug discovery.
  - We want an early elimination of drugs that have inadequate binding within the target receptor.
  - Ideally the drug is taken orally and survives any attempt at degradation so that it can get to the site where it is needed.
    - Computational procedures can help to predict this.
  - We want an early elimination of drugs that have harmful side effects.
    - This is more difficult computationally.

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## Typical Drug Lead Screening



- Start with 10's of thousands of compounds.
- Narrow this down to 2 or 3 hundred lead candidates for preclinical testing.
- Less than 10 candidates in clinical testing.
  - 80% pass phase 1
  - 30% of these pass phase 2
  - 80% of these pass phase 1
- Usually end up with one drug approved by the FDA.

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## Computational Issues in Drug Discovery



- **Target Identification**
  - What protein do we need to target?
  - Where on this protein is the receptor site?
- **Lead Discovery and Optimization**
  - Can the drug get to the protein receptor?
  - What is the molecule that will bind to this site?
- **Toxicology**
  - Does it do harm to the patient?
  - What are the side effects?

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## Drug Assays



- An early and expensive approach:
  - Put an infectious agent such as a bacteria into thousands of tiny vials.
  - Add a drug lead compound to each well.
    - This may involve several thousand leads...
  - Test to see which drugs kill the infectious agent.
- New less expensive approach:
  - Do computational screening of drugs.
  - This will involve calculations that predict docking.

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## Early Drug Discovery (1)



- We concentrate on modern techniques but past strategies are worth noting:
  - Traditional and “Folk medicine”
    - Only recently have researchers begun to fully appreciate the contribution of traditional medicines.
      - Aspirin
      - digoxin from digitalis from foxglove
      - quinine from cinchona bark for treating malaria
      - morphine from opium poppy (first isolated in 1803)
        - but dates back to 4000 BC (ancient Sumerians)
      - Many, many other examples (herbs, etc.)

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## Early Drug Discovery (2)



- Serendipity
  - Making a discovery while you were looking for something else.
    - See: Kubinyi, H. Chance Favors the Prepared Mind – From Serendipity to Rational Drug Design, *Journal of Receptor and Signal Transduction Research* 19(1-4), 1999, pp. 15-39.
    - Kubinyi lists 53 different drugs discovered serendipitously.
  - Fleming accidentally contaminated a Petri dish containing a bacteria with a mould that produced a chemical that came to be known as penicillin.
  - Amphetamine (originally a nasal decongestant)
  - LSD (originally for cardiovascular treatment)
  - Warfarin (low toxicity of rat poison in attempted suicide)
  - Viagra (sildenafil citrate) Pfizer was originally looking for an antihypertensive. This drug was almost eliminated from clinical trials when some men noted an unexpected side effect related to vasodilation.

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## Lead Optimization – more details



- Leads are optimized for:
  - Activity
  - Specificity
  - ADMET properties
- Optimization of activity and specificity is done by modifying functional groups to enhance binding affinity.

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## ADMET



- A successful drug needs more qualifications than the ability to bind at some receptor site:
  - **A**bsorption
  - **D**istribution
  - **M**etabolism
  - **E**limination
  - **T**oxicity

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## ADMET: Absorption



- Absorption deals with the passage of the drug into the blood stream after it is ingested.
  - It must pass through various membranes
    - In general neutral molecules are more easily passed through membranes than are charged molecules.

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## ADMET: Distribution (1)



- Where will the drug go?
  - Spread of the drug is generally via the bloodstream although the lymphatic system can also distribute a drug.
    - Drugs may travel in the bloodstream either in “free form” or attached to a plasma protein (in which case they can do nothing until they become unattached).
  - Distribution is also affected by solubility and stability.
    - Lipophilicity of a drug implies that it will go to fatty tissues.

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## ADMET: Distribution (2)



- Prodrugs and stability:
  - Prodrugs are compounds that are stable but pharmacologically inert.
  - They are converted by an enzyme to become the needed drug.
  - Examples:
    - A P450 liver enzyme may do the conversion.
      - fluoxetine (Prozac) is metabolized by the liver to produce norfluoxetine which is a serotonin reuptake inhibitor.
      - levodopa is the prodrug for dopamine a neurotransmitter used in the treatment of Parkinson's syndrome.

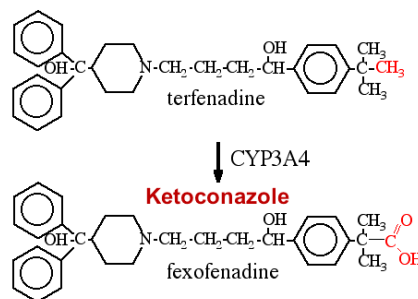
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## Toxicity of a Prodrug



- Seldane a very popular antihistamine had worldwide sales of over a billion dollars
  - 100 million patients; but withdrawn in 1998 after 17 deaths.
  - It is a prodrug that is rapidly metabolized by CYP3A4 to give fexofenadine.
  - The prodrug is a potent blocker of potassium channels and can cause ventricular arrhythmias.

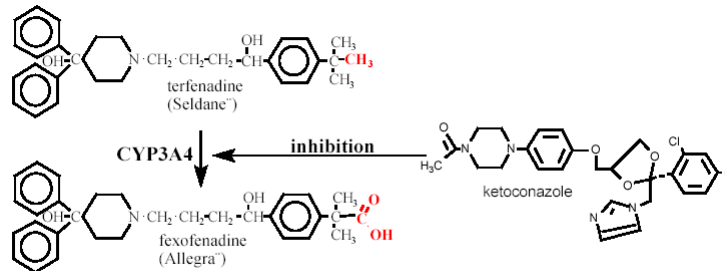


CYP3A4: cytochrome P450, family 3, subfamily A, polypeptide 4

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## Toxicity of a Prodrug (cont.)



- The conversion to fexofenadine can be compromised if the liver is not fully functional or if the enzymes are inhibited by ketoconazole (an antifungal agent) or erythromycin (a common antibiotic).
- Result: syncope, ventricular arrhythmia, cardiac arrest  
dizziness irregular heartbeat death

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## ADMET: Metabolism



- Metabolism is the biotransformation of the drug into other compounds (metabolites).
  - Usually more water soluble and consequently may be excreted from the body via the urinary system.
  - Occurs mainly in the liver but can also occur in the blood.
  - If a drug is rapidly metabolized there will not be enough left to have a therapeutic effect.
    - Also, we must be careful that a metabolite is not toxic.

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## ADMET: Elimination



- Elimination is the removal of the drug from the system by degradative metabolism and excretion.
  - mostly kidney and bowel
    - although exhalation, sweating, and even breast milk are also possibilities.
  - Elimination is an important factor in lessening the possibility of bad side effects due to over dosing.

Worst side effect in history (teratogenesis)  
thalidomide in the 1960's produced over 10,000 severely malformed children.

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## ADMET: Toxicity



- A chemical may be directly toxic or produce toxic effects after metabolism.
  - A toxic metabolite may be temporary and will do no harm **if** it is rapidly metabolized to harmless compounds OR
  - it may be a toxic metabolite that is the product of a final reaction and hence may stay around long enough to do damage.

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## ADMET References



- Good starting points:
  - van de Waterbeemd, H., Gifford, E., ADMET In Silico Modelling Towards Prediction Paradise?, *Drug Discovery, Nature Reviews*, Vol. 2, 2003, 192-204.
  - Hodgson, J. ADMET – Turning Chemicals into Drugs, *Nature Biotechnology*, Vol. 19, 2001, 722-726.

## Drug Delivery



- Drugs can enter the body through various routes:
  - Oral ingestion (pill)
  - Inhalation (powder)
  - Parenteral (needle)
  - Rectal (suppository)
  - Transdermal (patch)

## Bioavailability (1)



- Bioavailability refers to the percentage of the drug dose that manages to get into the systemic circulation after oral ingestion.
- Controlling factors include:
  - Absorption
    - solubility
    - H-bonding properties
    - polar surface area
    - MW
    - partition / distribution coefficient
    - pK<sub>a</sub>
  - Metabolism
    - liver
    - extrahepatic metabolism due to bacteria in the intestinal mucosa

Some research has been done to characterize bioavailability using a computation model such as a neural network or a decision tree.

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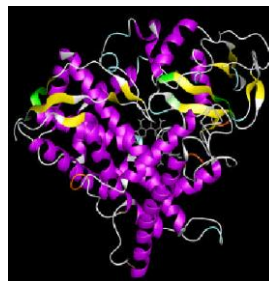
## Bioavailability (2)



- Hepatic clearance:
  - Drugs taken orally go down into the gastrointestinal tract and are subsequently absorbed by the intestinal mucosa and are carried to the liver via the hepatic portal vein.
    - Fresh blood goes to the liver via the hepatic artery.
  - Metabolism in the liver involves the cytochrome P-450 system.
    - A family of enzymes that try to break down chemicals recognized as foreign to the body.
    - Examples: CYP1A2, CYP2C9, CYP2C8, CYP3A4, many more.
      - CYP1A2 breaks down caffeine,
      - CYP2D6 breaks down fluoxetine,
      - CYP2E1 breaks down ethanol
      - CYP3A4 breaks down cyclosporin, terfenadine, erythromycin, grapefruit juice, ...

...

CYP2D6:



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## Bioavailability (3)



- Besides the detoxification done by hepatic clearance the liver has various other responsibilities:
  - storage of glycogen
  - metabolism of cholesterol and fat
  - cleansing bacteria from blood
    - The hepatic portal vein is loaded with bacteria from the gut!
  - elimination of worn-out red blood cells
  - many other duties...

## Movement of a Drug Through the Body

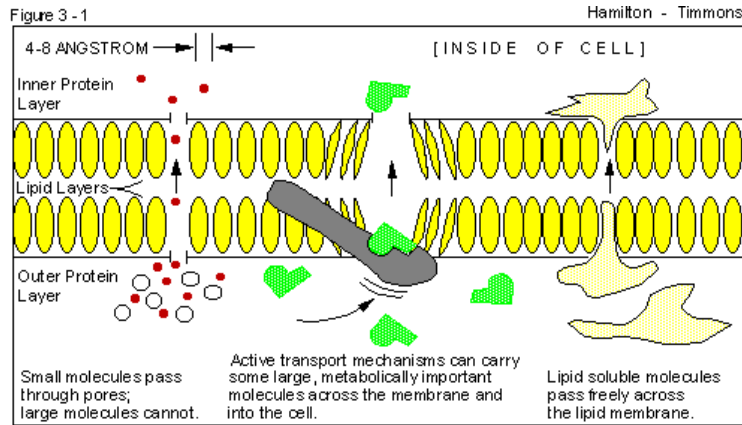


- As noted last day, we need more than a high binding affinity.
  - The drug must get to its tissue destination.
  - This may depend on:
    - size of the molecule
    - the charge of the molecule
    - the lipo-solubility
    - the nature of the cells lining the blood vessels.

# Access of Drugs to Body Tissues



- Size of drug and passage through a membrane:



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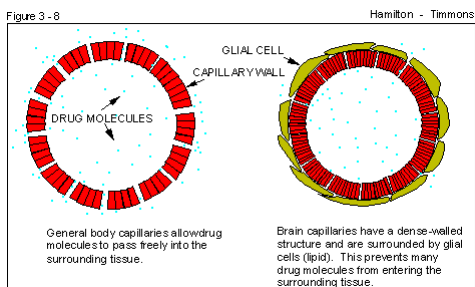
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# Blood Brain Barrier



- BBB:

- See: <http://www.rci.rutgers.edu/~lwh/drugs/ch03-08.htm>



- The gaps between endothelial cells in capillaries of the brain are extremely small.
  - Gaps for liver and kidney are larger.
- This forms a structure called the blood brain barrier.
- Polar molecules do not enter the brain unless they are actively transported.
  - This is a design issue for drugs that must go to the brain.
  - Enzymes in the endothelial cells also give protection.

Side notes:

1. The brain is 2% of the body by weight but it uses 25% of the energy and 20% of the blood flow.
2. A fetus has more brain blood flow, a weaker BBB and weaker enzyme system.

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## Lipinski's "Rule-of-Five"



- Which properties make drugs different from other chemicals?
  - Lipinski did a study, analyzing the World Drug Index (WDI) and came up with his rules (actually more like guidelines):
    1. MW < 500 Daltons
    2. Calculated octanol/water partition coefficient (CLOGP) < 5
    3. Number of hydrogen bond donors < 5
    4. Number of hydrogen bond acceptors < 10.

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## Little Things Mean A Lot



- Recall that a virtual library often consists of a scaffold with various sites that are modified to produce different drugs.
- What looks like a small difference can significantly change what a molecule does:
  - As an example consider Xanthine and its methylxanthine derivatives acting as stimulants and diuretics:

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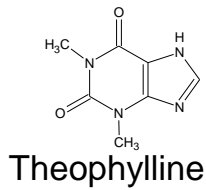
# Xanthines



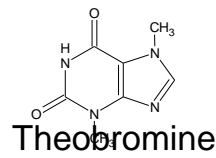
The parent compound



Coffee:  
CNS stimulation



Drug for asthma  
(Bronchodilation)



Chocolate:  
CNS stimulation

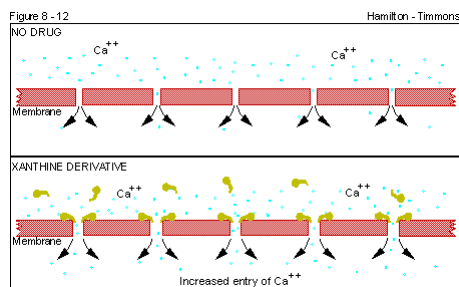
All xanthines have a diuretic effect.

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# Xanthine Derivatives

- Xanthine derivatives increase  $Ca^{++}$  permeability.
  - $Ca^{++}$  entry increases levels of excitation for a cell.
    - Other suspected modes of action deal with inhibition of an enzyme that breaks down cAMP which mediates a neurotransmitter called catecholamine.



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## TIBO Derivatives



- Another example of a scaffold + R groups:
  - TIBO analogs are HIV reverse transcriptase inhibitors.
  - There are various computational papers that discuss techniques for predicting binding affinity:
    - Huuskonen, J. QSAR Modeling with the Electrotopological State: TIBO Derivatives, *J. Chem. Inf. Comput. Sci.* 41, 2001, 425-429.
    - Hannongbua, S., Pungpo, P., Limtrakul, J., Wolschann, P. Quantitative Structure-Activity Relationships and Comparative Molecular Field Analysis of TIBO Derivatized HIV-1 Reverse Transcriptase Inhibitors, *J. Computer-Aided Molecular Design*, 13, 1999, 563-577.
    - Both papers include affinity data.

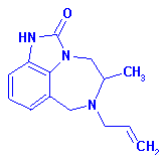
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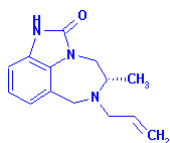
## Some Examples of TIBO Analogs



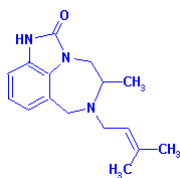
TIBO is an acronym for: tetrahydroimidazo[4,5,1-jk][1,4]benzodiazepinone



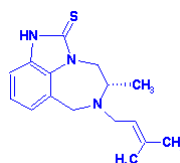
- R 14458
- Chemical name:  
Imidazo[4,5,1-jk][1,4]benzodiazepin-2(1H)-one,  
4,5,6,7-tetrahydro-5-methyl-6-(2-propenyl)-, (1)-



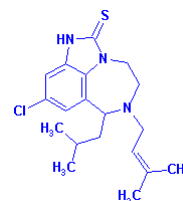
R78304



R79882



R82150



9CI-7-iBu-6-(DMA)  
Thione deriv.

Drug Discovery

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## Drug Design History (1)



- ? – 1960
  - Serendipity
    - chlordiazepoxide (librium), aspartame (sweetener), ether, acetylsalicylic acid, dicoumarol, etc.
  - Clinical observations
    - warfarin, LSD (hallucinogenic instead of cardiovascular activity), iproniazid (antidepressant instead of tuberculostatic activity), etc.
  - Natural products
    - quinine, taxol, morphine, digitaline, etc.

Drug Discovery

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## Drug Design History (2)



- 1960 – 1980
  - Structure-activity relationships and screening
    - Most modern drugs
- 1980 – 1995
  - Rational design: ligand-based
    - enalapril (high blood pressure and heart failure), cimetidine
  - Rational design: protein-based
    - HIV-1 protease inhibitors, TS (thymidylate synthetase) inhibitors (cancer therapeutics).
- 1995 – present
  - Automated High-Throughput Screening (HTS), combinatorial chemistry, genomics & proteomics
    - in clinical development

Drug Discovery

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## Rational Design



- Issue: Is the 3D-structure of the target protein known?
  - No => Ligand-Based design
  - Yes => Protein-Based design  
also called Structure-Based design

Drug Discovery

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## Ligand-Based Screening



- Steps:
  1. We generate a pharmacophore.
  2. Use this to build a “fingerprint” based on 2D or 3D information.
    - Hashed fingerprints are a common strategy.
  3. Do a similarity search.
    - partial least squares, GAs, NNs, ...
    - 3D Databases: ACD, CSD, NCI, MDDR, ...
    - Major issue: What is similarity?
      - biological similarity vs. chemical similarity
  4. Collect the hits.
- Software:
  - DISCO, CATALYST, MACCS-3D

Drug Discovery

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# Peptidomimetism

A Type of Protein-Based Design



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## Introduction



- Peptidomimetism
  - Peptidomimetism is the design of a drug that will mimic the functionality of a short peptide (protein) sequence that interacts with a receptor site in a protein-protein interaction.
    - Usually a small sequence
      - (say 3 to 15 peptides in length)
    - It should induce the same pharmacological effect as the longer protein.

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## Examples (1)

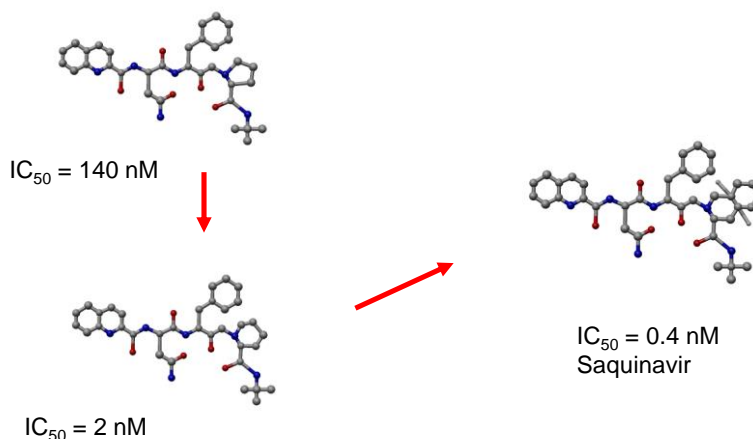


- Protease inhibitors
  - HIV protease is a protein that normally binds to another protein (the poly-protein that must be cleaved when the virus matures).
    - We want to produce a drug that resembles the protein that HIV protease uses as a substrate.
    - The drug binds with the protease and disables it.
    - In addition to AIDS inhibitors, drugs have been designed to treat hypertension, malaria, and Alzheimer's disease.
    - See: Bursavich, M.G., and Rich, D.H., Designing Non-Peptide Peptidomimetics in the 21<sup>st</sup> Century: Inhibitors Targeting Conformational Ensembles, *Journal of Medicinal Chemistry*, Vol. 45, No. 3, Jan. 2002, 541- 558. (Includes some discussion of movement of the "Flap" region in HIV protease).
- Another potential target would be the SARS corona virus site that binds to the CD13 protein.

Drug Discovery

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## Lead Optimization to Get Saquinavir



Drug Discovery

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## Examples (2)



- Examples of other drug targets
  - any protein-protein interaction that is used by a disease causing process:
    - cancer
    - viral invasion
    - parasites
    - bacterial infections
    - inflammation
    - cardiovascular disease
  - Of course the drug must target these processes and not interfere with normal function.

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## Examples (3)



- Physiological roles of peptides:
  - Angiotensin II      Blood pressure regulation
  - Endothelin          Blood pressure regulation
  - Fibrinogen          Blood platelet aggregation
  - Leu-enkephalin      Ligands of the morphine  
    Met-enkephalin      receptor (analgesics)
  - Neuropeptide Y      Blood pressure regulation
  - Substance P          Broncho-constriction

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## Drug Requirements



- If the drug is to act like a small peptide sequence, why not just use a small peptide sequence?
  - Proteins are subject to proteolysis in the gut.
    - If the results of the proteolysis are not used by the body they are rapidly excreted.
  - Even if small fragments escape proteolysis they typically have poor bioavailability characteristics.
  - In Summary:
    - Biologically unstable
    - Poorly absorbed
    - Rapidly metabolized

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## Strategy



1. Start with high-resolution crystal structures so that a model of the binding site can be developed.
2. Use combinatorial chemistry to develop drug leads.
3. Carry out depeptidization or *de novo* design using computer programs.

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## Goals of Peptidomimetism



- Primary goal:
  - Find a non-peptide that has the same functional capabilities.
- Secondary goals deal with optimization:
  - specificity
  - oral bioavailability
  - ADMET properties
  - These are achievable with a non-peptide but not achievable with a peptide.
- \$\$\$\$ 😊
  - Very significantly: the final result will be a proprietary molecule with a strong patent position.

Drug Discovery

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## Two Strategies for Peptidomimicry



- Goals:
  - We want to take a peptide-base molecule and design a molecule that is non-peptidic but functionally similar and with good ADMET properties.
- Two strategies:
  - Depeptidization
    - We can perform successive modifications of the peptide making it less and less peptide like.
      - The approach is conceptually straightforward but requires continual chemical syntheses to make sure the effort is on track.
  - *De-Novo* design:
    - We analyze the 3D pharmacophore and use this information to design the drug “from new”.
      - This strategy is more complicated because we start with nothing but the pharmacophoric specification and we must then have an algorithm that best utilizes this information to design the drug.

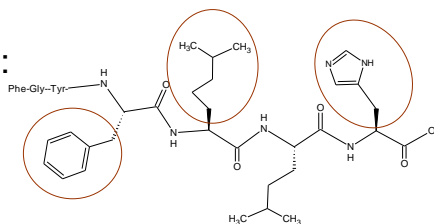
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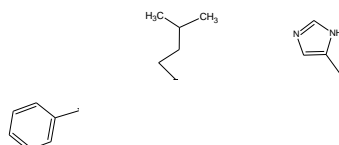
## De-Novo Design Overview

- A possible procedure:

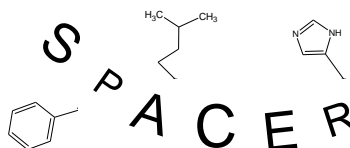
1. Identify the most important groups:



2. Remove all atoms not contributing to functionality:



3. Build a non-peptide spacer that will leave the groups in the same positions.



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## Examples of *de novo* Design Algorithms

- There are many programs that are currently used to aid the drug design process:

- BUILDER, CAVEAT, CONCERTS, DLD, GENSTAR, GROUPEBUILD, GROW, GROWMOL, HOOK, LEGEND, LUDI, MCDNLG, MCSS, MOLMAKER, NEWLEAD, PRO-LIGAND, PRO-SELECT, SKELGEN, SME, SMOG, SPLICE, SPROUT, TOPAS.

- See: Gisbert Schneider and Hans-Joachim Böhm, Virtual screening and fast automated docking methods, *Drug Design Today*, Vol. 7, No. 1 Jan. 2002, 64-70.

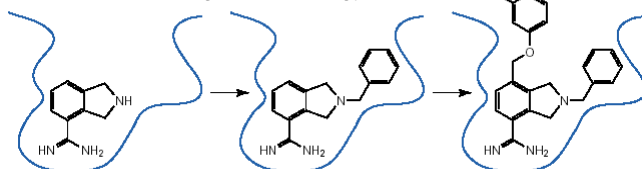
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## Two Approaches for Structure-Based Molecule Assembly from Fragments

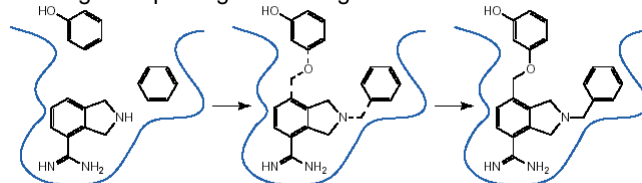


(a) The sequential growth strategy:



The solid line represents the Ligand-binding site

(b) Fragment-placing and linking:



*Drug Discovery Today*

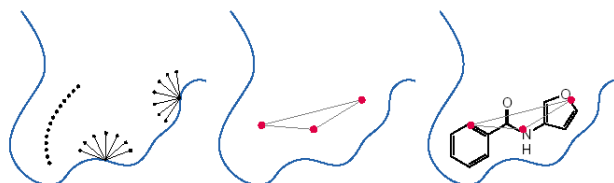
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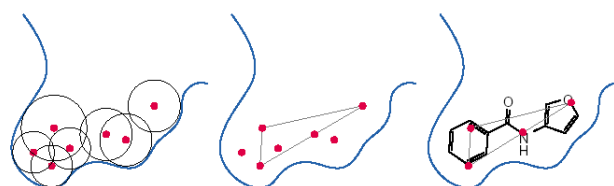
## Other Strategies for Filling the Pocket



(a)



(b)



*Drug Discovery Today*

**Figure 2.** Schematic illustration of (a) the FlexX algorithm and (b) the DOCK algorithm. FlexX matches triangles of interaction sites onto complementary ligand atoms. The program DOCK fills the binding site with spheres, and sphere centers are then matched to the ligand atoms to determine plausible ligand-receptor complexes. The surface of the receptor pocket is drawn as a solid line. The dotted line represents potential lipophilic interaction points, and the fan-like structures indicate potential hydrogen-bonding sites.

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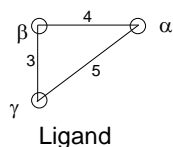
72

## Fragment Placement and Clique Search

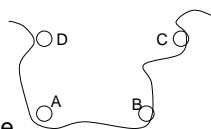


- The placement algorithm builds a **distance compatibility graph**:

- Assume the ligand has features  $\alpha$ ,  $\beta$ , and  $\gamma$  with separation distances defined in this diagram:



Assume further that the receptor site has complementary protein features labelled as A, B, C, and D with the distances between these features presented in the table to the right:



AB	4
AC	6
AD	3
BC	3
BD	5
CD	7

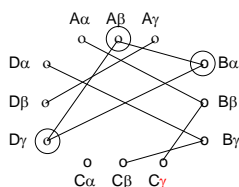
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## Fragment Placement & Clique Search (cont.)



- We wish to do a docking of the ligand into this site so that the ligand is placed with  $\alpha$ ,  $\beta$ , and  $\gamma$  matching with a subset of A, B, C, and D in such a way that distances are properly matched.
  - We will not consider any other issues, for example: chemical features.
  - This can be done using a distance compatibility graph:



Connect  $Xa$  to  $Yb$  if distance from  $X$  to  $Y$  is same as distance from  $a$  to  $b$ .

The required fragment placement is revealed by finding a clique in this graph.

A clique is fully connected subgraph.

In this example, a clique is highlighted using large circles.

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## FLEXX



- Reference:
  - Rarey, Kramer & Lengauer (1995) Time-Efficient Docking of Flexible Ligands into Active Sites of Proteins, ISMB-95.
- Problem description:
  - They assume the receptor site is rigid, but the ligand is flexible.
  - The goal is to predict the geometry of the binding between ligand and receptor site and to predict the binding affinity.
  - They want a docking strategy that is fast and reliable.

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## FLEXX



- FLEXX uses an incremental construction strategy with three phases:
  - Base selection:
    - The user must select a connected part of the ligand.
  - Base placement:
    - The base fragment is the first fragment to be docked into the receptor site.
  - Construction:
    - The entire ligand is constructed in the receptor site.
      - Remaining fragments are attached to the base fragment and placed into the site so that lowest energy is obtained.

Drug Discovery

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## FLEX X



- Base selection trade-off issue:

- The base fragment should have a reasonably large set of interacting groups so that its placement in the receptor site is likely to be the same, or at least very similar, to that achieved when the full ligand docks in the actual receptor.

However, the number of low-energy conformations of the base fragment should be few in number because each such conformation will be treated separately in the algorithm.

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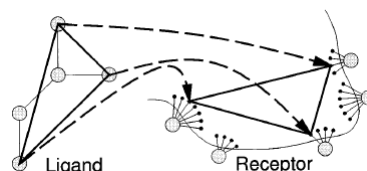
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## FLEX X



- Placement issues:

- There can be many possibilities for placement of the ligand in the receptor site.
- The algorithm must find three simultaneously occurring “interactions” between the base fragment and the receptor.



- There may be many possibilities to be investigated.
- An interaction may be electrostatic, H-bonding, etc.
  - Steric collisions have to be avoided and surface complementarity supported.
  - Within a small margin, the distances of the triangle formed by the three interaction sites in the ligand must match the corresponding distances in the receptor.

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## Depeptidization



- Recall that in this approach we wish to modify the peptide so that we maintain the needed functional groups in the correct position and try to get good ADMET properties.

## A Range of Possibilities

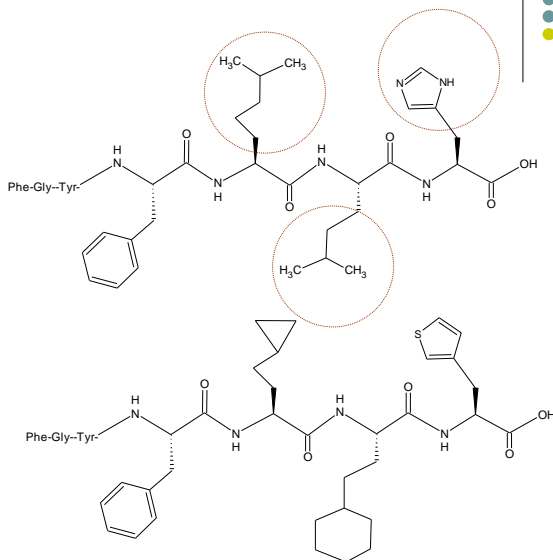


- Researchers doing peptidomimeticism categorize ligands into three groups (see Ripka & Rich):
  - Type I:
    - These are peptide molecules that still have a peptide backbone. We typically have non-natural amino acids.
  - Type II:
    - These are small non-peptide molecules that bind to the receptor and produce the biological effect, but they do not necessarily mimic the structure of the native ligand.
  - Type III:
    - These mimic the peptide ligand by employing a non-peptidic scaffold to properly position key binding elements for interaction with the receptor.



## Mimics that are Peptide Based

The top structure is a peptide that has been studied by the biochemist who has determined that the circled side groups will be replaced by unnatural amino acid replacements

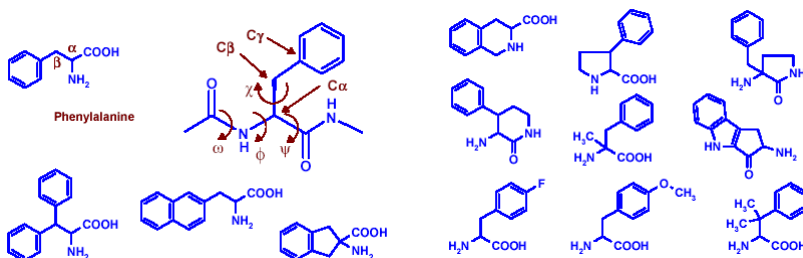


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## Amino Acid Analogs

- The idea is to replace a natural amino acid with a synthetic amino acid that has similar functionality but better ADMET properties.
  - Such an amino acid is called an *analog*.
  - Example: Analogs for phenylalanine.

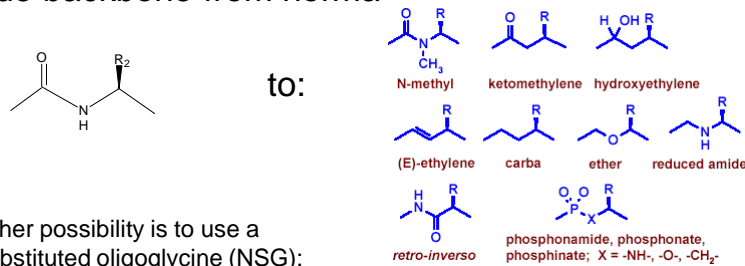


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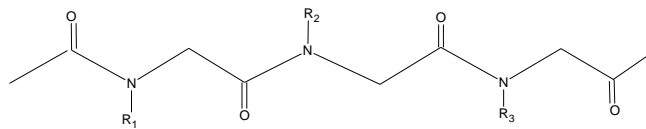
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## Replacement of Peptide Bonds

- The medicinal chemist may also change the peptide backbone from normal:



- Another possibility is to use a N-substituted oligoglycine (NSG):



- Notes: 1) No amide protons => decreased polarity => increased bioavailability  
 2) This backbone is more flexible than the normal peptide backbone.

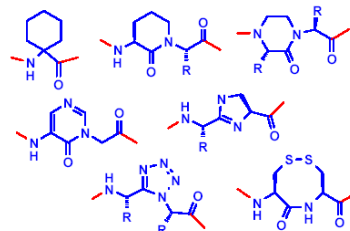
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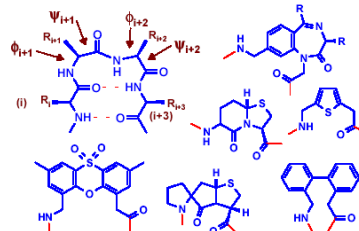
## Adding Cycles Stabilizes the Molecule

- Drugs are more specific to a binding site if they are more rigid and designed for that site.

- Ring structures can be inserted into a backbone to enhance its conformational stabilization.



- A beta turn can also be subject to mimetics:



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## Other Directions



- See:
  - Preissner, R., et al., Matching Organic Libraries with Protein-Substructures, *Journal of Computer-Aided Molecular Design*, 15, 2001, 811-817.
  - They use a strategy that allows automatic identification of sub-structures in proteins that resemble given 3D templates.
    - There is an emphasis on beta-turn mimetics.
    - The analysis ties in with the usual secondary structure categorizations (helix and coil).