BPS2110 MIDTERM 1 NOTES

DRUGS: Chemical substances used in the treatment, prevention, or diagnosis of disease. Used to enhance/improve quality of life
Molecular entity: the active ingredient; the chemical substance itself used in pharmaceuticals

- World-wide, only about 30 new molecular entities are approved each year
- **Compendium of Pharmaceuticals and Specialties** has data on over 47 000 products, notices all bad interactions between different MOs.

Drug products: different forms/combinations of molecular entities.

- More common than molecular entities themselves: drugs can be sold in different doses, formulations, or combinations
- Thousands of products are licensed: FDA differentiates products from molecular entities
- Regulates the same drug at different doses as differently (sees them as different products

TYPES OF DRUGS
Biologic: manufactured using living things (plants, animals, humans, single cells)

- Vaccine, protein, antibodies, nucleic acids
- Normally large molecules with large molecular weights (>2000)
- Beginning to have more and more of a share of new drugs, industry tending towards making more of them

Small molecule: manufactured using chemical synthesis and from living things

- Low molecular weight (<2000)
- Normally synthesized from petrochemicals

THE PHARMACEUTICAL INDUSTRY: A multi-billion dollar industry with high risk, high profit margins (high costs make up for the loss of time/money in research)

- Highly regulated industry: more than any other industry (FDA, Health Canada) causes drugs to be extremely expensive to produce (~2 billion)

TYPES OF PHARMACEUTICAL COMPANIES
Ethical: companies investing heavily in research, discover/develop new molecular entities

- Large companies with hundreds of thousands of employees
- i.e. Johnson & Johnson, Novartis, Sanofi

Generic: limited research, focus on manufacturing products not protected by patent

- Large to medium sized companies; most people hired in quality control and lawyers
- Capable of charging less for a drug, since they did not need to pay for research
- i.e. Teva, Mylan, Allergan

Biotech: takes an idea from an academic lab, turn it into a single product

- Created to exploit academic discoveries, heavily research intensive

- Small company size (10's to 1000's), selling one to a few specialty products
- If successful, exists for short time and is bought out by a larger company

Contract research organization (CRO): provides a specialty service for the pharmaceutical industry

- These companies do not take on risk, they are paid passed on a service on contract (generally don't mind if what they're testing is "good" or not)
- Small to medium sized (100's to 10 000's)

DISCOVERING A NEW DRUG: 5 STEPS, 12 YEARS (a summary)

	LENGTH	SUMMARY	END
DISCOVERY	1 – 3 years	High throughput screening to find a **Drug Candidate** (a chemical structure on a piece of paper) - Beginning of the idea, identifying DC	Drug Candidate
DEVELOPMENT	1 – 2 years	Drug candidate becomes **an investigational New Drug (IND)**. DC is turned into a sellable product - Essentially asking permission to go into clinical trials (practically apply drug candidate)	IND
CLINICAL TRIALS	1 – 5 years	Drug is tested in humans for safety and efficacy - Test limits for dosing, efficacy, rare side effects, etc. - A **New Drug Application (NDA)** is filed	NDA
FDA APPROVAL	6m – 1.5y	Data review from clinical trials to ensure proper testing, lots of paperwork done - Verify data shows clear benefit outweighing risk	Market approval
MARKET	unlimited	Make money, continue safety testing to identify rare side effects - Find new indications	$$$$$

Discovery: **DRUG CANDIDATE**: a molecule identified as a potential drug

- Identified after 1-3 years research (synthesis/testing of up to 5000 new compounds), heavily driven by chemistry/biochemistry
- Structure is kept secret, no approval necessary for further testing and company can decide to continue (optimizing the candidate)
- Companies identify 3-4 backup compounds (primary fails, recovery time minimized)

Development: **INVESTIGATIONAL NEW DRUG (IND):** application made to FDA to enter clinical trial. ← compound survived development stages

- Animal pharmacology and toxicity data. This ensures safety in humans/used to validate clinical procedures
- Manufacturing information: proof that company can produce/supply consistent batches of the drug
    - Method of making compound
    - Purity profile ← proof purity is good/consistent
- Clinical protocols and investigator information (to prevent unnecessary risks)

FDA Approval: **NEW DRUG APPLICATION (NDA):** application made to FDA to enter market.

- Paperwork filed with FDA for market approval
- Includes all safety information, efficacy, appropriate labelling, manufacture being sound, controls in place to maintain quality, etc.

Generic drugs: **ABBREVIATED NEW DRUG APPLICATION (ANDA):** application made to FDA to enter market as a generic version of an ethical company's drug

- Drug not protected by patent
- Same identity, dose, formulation, route of administration, performance characteristics, intended use (essentially proof that you're making the same compound as the ethical company) ← animal/human data are not required

Discovery: **PROJECT INITIATION**

- Drug companies concerned with targeting markets (not diseases); a new drug costs more than $1 billion to develop, and companies must recover costs
- i.e. Nortel went bankrupt (though they had cutting edge devices): they were selling to a niche market

MARKET ANALYSIS

- Company makes an analysis of the market, to check if people will actually buy their product, type of potential customers (can they actually pay?)
- Nature of disease: if treating a life threatening condition, people will want to pay much more
- Acute vs. chronic: a chronic illness means long term treatment, which will make the company more money
    - A long term market with assured customers, and more profit
    - Top selling drugs on the market target long term, chronic conditions
    - Small molecule drugs: industry is shifting more resources in this direction, and these types of drugs are growing (aren't necessarily going to take over, however just growing recently)
- **Cancer:** 20-30% of new drugs entering market are for treating cancer; FDA cuts you some slack if you're developing a cancer drug
    - Cancer is a life threatening condition: people are willing to pay a lot to live

COMPETITVE ASSESSMENT

- If many other companies are testing/developing the same drug, it isn't worth the time/money to invest in the new drug (profit margins will not be as high)
- **Timing onto the market:** if there is already a cheaper version of drug already on market, it is not worth it to develop another one

    - 1st drug: makes the most money/profits
    - 2nd drug: less money overall, however spent less on development (high profit still)
    - 3rd drug: least amount of profit

Discovery: **FEASIBILITY in BCH**

- Companies look at the disease itself: if disease is well understood/a solvable issue, ease of diagnosis, treatable with drug
- Industry works on stuff with clear read outs, otherwise it stays in academia until something significant is found
- **Biochemical tools:** test assays for drugs, animal models
    - i.e. Humans are the only animals who get AIDS: there is no possible animal model, and as a result the disease is difficult to treat
- Do drugs already exist? Not only serves as a starting point for the company, but provides a proof of principle that a drug to treat disease is possible

BIOCHEMICAL STUDIES

- Proof of principle: is a drug possible to develop? Will it work? Must show experimental evidence showing the drug could work in the intended way
- Drug testing methods: a way to test candidate drugs, requires tools to test the new drug to make sure it works
- Development of animal models (of disease)

Discovery: **LEAD IDENTIFICATION:** medicinal chemistry requires a starting point

- If given a protein/enzyme we do not have the knowledge to simply design a compound fitting that protein right away (thus need to identify a lead)
- **Methods to identify leads:**
    - High throughput screening
    - Rational drug design
    - Natural products
    - Combinatorial chemistry
    - De-novo design
- A **LEAD COMPOUND**: has proven biological activity, and some specificity
    - Pattern of "drug-like" properties; can enter the body and can be made into a product
    - Must be a new drug/idea, so that a patent is possible
    - Must be possible to make the product (where synthetic organic chemists are necessary)

Identifying lead: **HIGH THROUGHPUT SCREENING (HTS):** 1000s compounds tested for activity

- Companies maintain large collections of compounds; test as many as they can
- Each time a new compound is made, it is separated & stored in DMSO for years (later testing), solid form (prevent degradation)
- Biological assay to test compounds is easily robot automated → modern robots screen >200 000 compounds/week (in an assembly line, gives 'yes' or 'no')
    - Most results are false positives (>99%), compounds show activity when tested more carefully

    - Counter screen used to sort false positives; using an assay related to biological function
    - Seeking compounds that are specific (active in one assay)
- HTS tests every single compound possible: drugs/biological molecules interact in unpredictable ways
- It is possible a certain compound may inhibit some enzyme in the future
- Not only test versions of the same compound, but a large variety of structures and chemical types

HTS TESTING: cont'd

- All compounds tested at same dose (typically 30uM) ← a starting point
    - Compound needs to be active at this dose in order to be applicable as a drug
    - If active dose too high, too much drug going into body (leading to possibility of high toxicity)
- Typical campaign tests >500 000 compounds, and generates approx. 500 hits (compound testing positive in HTS)

HIT TO LEAD ← REMOVES FALSE POSITIVES

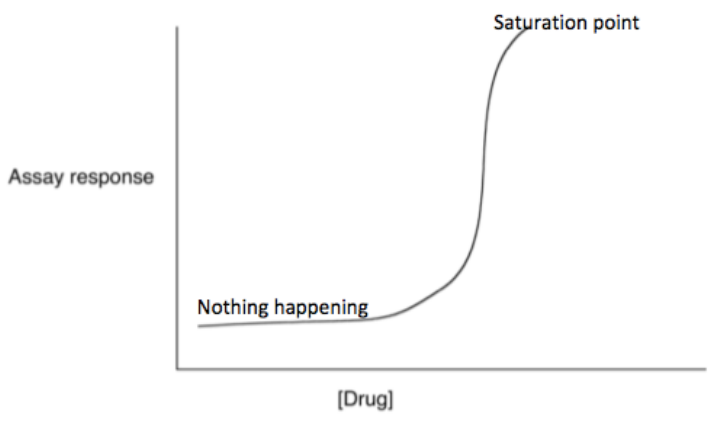
- **Possible false positives due to:**
    - Impurity ← hydrophobic impurity sticks to plastic of assay, giving false (+)
    - Decomposition ← decomposition in assay inhibited enzyme
    - Compound reactivity (detergent, redox rxn, strong electrophile/ nucleophile)

Impurity: **PAN-ASSAY INTERFERENCE COMPOUNDS (PAINS)**

- Promiscuous bioactive compounds, showing activity in virtually any biological test
- These compounds always give positive results:
    - Redox activity
    - Detergents
    - Strong acid/base
    - Strong nucleophile/electrophile
    - Photoreactive
    - Chelator
    - Highly lipophilic

Removing false (+): **STEP 1: RETESTING HITS**

- Re-test using purified samples, at multiple concentrations
- Compound should interact in a concentration-dependent manner



Dose response at multiple concentrations:
Small amount of drug = small activity
Increase amount = increase activity
Want a titration like curve, with noticeable difference (clear

cause/effect)

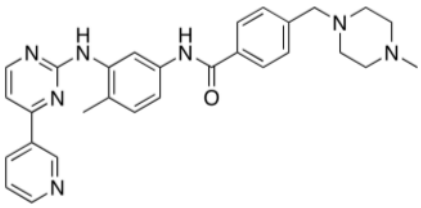
Additionally: test a sus positive in a different assay or check back in database for prev results

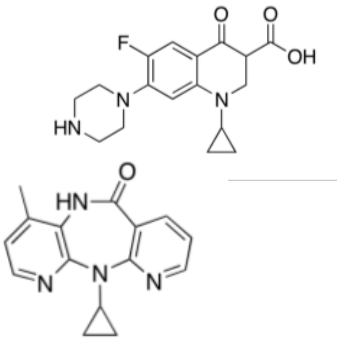
STEP 2: CONFIRMATION OF STRUCTURE

- Many compounds kept are very old: may have decomposed or improperly identified
- Re-synthesize and purify the compound, then test a series of compounds w related structures (to optimize)
    - Compounds in series should all be active (but show differences that can be related to structural patterns/differences)

STRUCTURES DEVELOPED BY HTS:

- Nevirapine
- Gleevec
- Quinolones





Lots of rings and heteroatoms (heterocycles, any atom in the ring of a cyclic compound, other than a carbon atom)

- Many aromatic molecules/rings with nitrogen → easy to assemble aromatic compounds with no chirality in a lab
- Nitrogen: has properties allowing it into body easily, and allow for aromatic chemistry
    - Nitrogen is able to pick up a positive charge to become polar, and can also be removed to become non-polar
- Chirality is expensive and may cause issues
- Organic, non-polar molecules with carbonyl groups
- No stereochemistry, totally flat and achiral ← <u>all characteristics make compound easy to assemble</u>

Identifying lead: **NATURAL PRODUCTS**

- Natural products: secondary metabolites isolated from living things (chemical not directly required for life; but produced for a secondary purpose i.e. poison, colour, fragrance)
- Difficult to perform hit-to-lead; chemical synthesis of related compounds difficult to synthesize
    - Thus limited to chemically modifying natural product

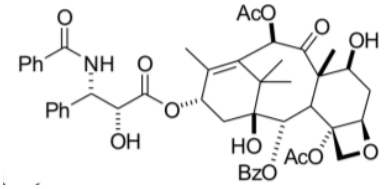
- Only done in academic labs (companies stopped in ~1980s, no return in money)

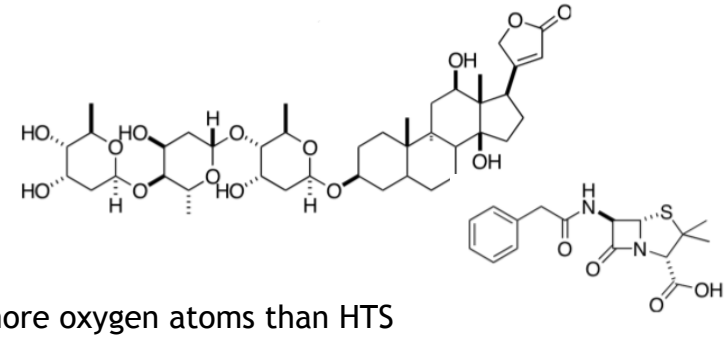
ISSUE IN NATURAL PRODUCTS: SUPPLY

- Organism of source must be farmable at an industrial scale
- Natural compounds are complex, difficult to manufacture (unless have access to the living organism producing it
- Living orgs don't make a sufficient quantity to provide for drug manufacture
- i.e. Spongistatin: low yields when harvesting, drove sponge to extinction

STRUCTURES IN NATURAL PRODUCT

- Penicillin (antibiotic)
- Taxol
- Digoxin





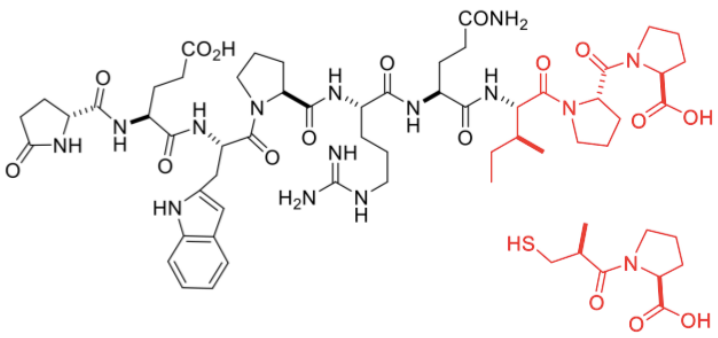
Natural product structures have a lot more oxygen atoms than HTS drugs

- Much larger, and much more stereochemistry/stereocenters
- HTS compounds avoid stereocenters, as it makes the process of synthesizing them much more difficult
    - Laboratories are unable to make things in a stereoselective manner very well (nature is able to do this)
- Not many aromatic rings, mostly aliphatic (carbon atoms forming open chains) ← aliphatic is a difficult to form in lab

Identifying lead: **RATIONAL DRUG DESIGN** ← from known natural material

- Design a lead using know/existing chemical structure:
    - Enzyme substrate
    - Natural inhibitor
    - Ligand for biological receptor
    - Existing drug
- Tweaking structure changes mechanism of action; design for improved properties and a patent advantage

CAPTOPRIL: resembles snake venom



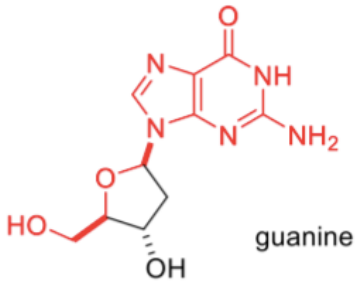
Only red part of the snake venom compound is used to reduce blood pressure (rest is "decoration")

Small red compound at bottom is captopril: all you need to lower blood pressure

Removes downsides of venom, and is practical to develop

- Snake venom is stripped down and re-purposed to a rationally designed drug

ACLYCLOVIR: resembles guanine



guanine

acyclovir

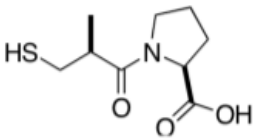
Acyclovir is similar to guanine in structure: rationally designed

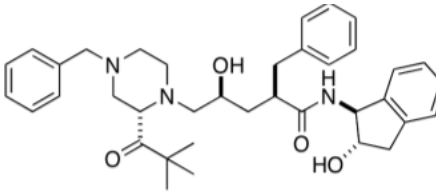
Herpes: uses guanine to replicate its genome

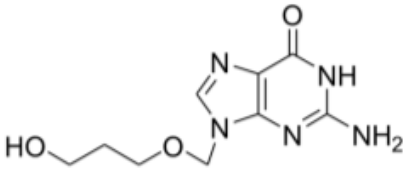
Aclyclovir selectively targets herpes

- It is a 'stripped down' version of guanine
- Great antiviral

STRUCTURES IN RATIONALLY DESIGNED DRUGS







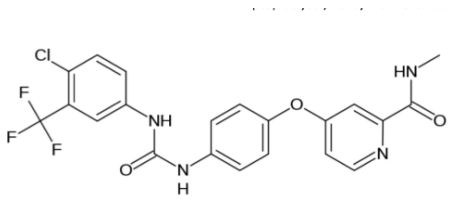
- Compounds typically contain nitrogen atoms, and a few oxygens (blend of the two)
- Aromaticity and aliphatics (variety of oxidation states)
- Few chiral centers (rare, difficult to form in lab)

Identifying lead: **COMBINATORIAL CHEMISTRY**

- Semi-random robotic synthesis of a large library of related compounds
- Test all compounds for biological activity, changes made based on Darwinian selection
    - Selection criteria kills off some molecules, where surviving ones are chosen for further testing
- Not successful as a lead drug discovery method in industry (used more in an academic sense)

COMBINATORIAL SEARCH

- Look for structures with common core structure, and add appendages to different parts of core (through chemical reactions)
- Creates multiple mixtures of functional groups, test them as a mixture or HTS methods
- Select molecule showing desired properties
- Sorafenib: the only drug discovered this way, treats kidney/liver cancer (rare disease)



Identifying lead: **DE-NOVO DESIGN** (computer designs structure) ← not very successful

- Computer designs a lead from scratch, requires a 3D structure of bio-target
- Starting with a protein structure, computer builds model of binding site
    - Then, computer designs a molecule that fits the pocket (all theoretical
- Hasn't been used to make a drug from start to finish; mainly used to fix issues w drugs and partial optimization

After discovering a lead: **OPTIMIZATION OF LEAD**

- Small changes made to structure of a drug, looking for changes in activity
    - Testing performed by biochemist, feedback given and drug design would be revised accordingly
- Emphasis on speed: companies usually try to optimize as many properties of a drug as possible at the same time
    - Every molecule made is tested against multiple different chemical properties
    - Patterns of results used to optimize the molecule

(SPR) STRUCTURE PROPERTY RELATIONSHIP

Properties of each compound synthesized:

- **Potency:** smallest dose possible for desired activity
- **Selectivity:** large enough dose to minimize off target activity
- **Solubility (water):** enter body & stay there
- **Lipophilicity:** soluble in non-polar solvents (fat)
    - Often, optimizing one of these property may diminish the other
- **Metabolism**: how liver affects drug (how it is broken down/metabolized)
- **Toxicity**
- **Ease of synthesis:** sometimes the "perfect molecule is too difficult to synthesize"

Getting drug into body: **DRUG-LIKE**

- Body recognizes drug as a poison; the hardest problem in med chem is getting the drug into body
- Certain properties correlate well with drug performance; try to optimize these at same time that potency is optimized
- End up with a drug candidate structure (from a lead product)

Development: **DRUG CANDIDATE TO A PRODUCT**

- Documentation
- Safety
- Large scale manufacture
- Formulation

Stage 1: **DOCUMENTATION AND PATENTS**

- Tight regulation at this stage; all research subjected to regulatory scrutiny
- When drug reaches market, mistakes made in documentation leads to lawsuits and patent breaking
- All data is sent to FDA (proper recordkeeping and lab book discipline important)

PATENT COVERAGE: makes sure the candidate and all future drugs in this class are protected.

- Protects idea, manufactured product, path of synthesis, process of use of the compound, composition of matter
- Gives you right to prevent others from using your idea

REQUIREMENTS OF A PATENT:

- Novel: a new idea/product you discovered for the first time
- Utility: product should have an actual purpose, must be "real"
- Non-obvious: someone skilled in the art would not immediately recognize/think of it ← lawyers use this pillar to break patents

Stage 2: **SAFETY TESTING 1** ← in vitro

- Initial testing done with in-vitro (in glass) methods ← done on a biochemical assay to see if a drug will present bad side effects
    - In-vitro for initial testing; cheaper than animal
- Thousands of BCH assay tests done, looking for a **"clean profile"**
    - Want a minimum number of "positives" (some are acceptable)
    - "Deal breakers"; unacceptable side effects (carcinogenic, alters organ function, etc.)

Stage 2: **SAFETY TESTING 2** ← animal testing

- Animals: complex systems that cannot be artificially replicated (interactions with a biological system and unpredictable)
- FDA requires 2 species (at least 1 primate); in practice 3 are usually used (rat, dog, primate)
    - Rat: cheapest to maintain, small animal with consistent genetics
    - Dogs/others: more similar to human than a rat is, cheaper than a primate
    - Primate: most expensive, most similar to a human (as small as possible chosen)

Stage 3: **LARGE SCALE SYNTHESIS – PROCESS CHEMISTRY**

- Large-scale ≠ small scale, reactions do not work the same way on a large scale as on small scale (reactions working well in lab do not work industrially)
    - By nature of chemical reactions and heat transfer (i.e. spaghetti in a pot vs. spaghetti for 10 000 people)
- Methods of purification limited
    - Crystallization
    - Distillation

PROCESS CHEMISTRY: COMPANY GOALS

- Optimize synthesis for lowest cost:
    - Cost of goods
    - Energy costs
    - Labor costs
    - Cost of waste disposal
    - Safety costs
    - Transpo costs
- Reactions save money by limiting waste (expensive to get rid of), and also being more efficient

- Companies market their goals as environmentally friendly; in reality it is to lower costs of synthesis
- **Outsourcing processes:** ensuring lowest cost while not putting safety/energy on the line
    - Some reactions illegal in NA at large scale, however legal in China
    - Companies outsource processes to different countries to bypass own country's laws

REQUIREMENTS OF THE FDA: in development, hundreds of kg of drug are prepared

- Reliable: FDA wants the same product every time
- Consistent yield, purity ← must match every time the product is made
- Consistent purity profile (if impurities are present, ensure there are the same impurities in the same proportions each time)

FORMULATION OF THE FINAL PRODUCT → pill, liquid, etc.

- Active pharmaceutical ingredient is a small portion of product; also contains necessary excipients (non-medicinal ingredients)
    - STABILIZER: prevention of O_2 degradation ← usually an acid or a base, compounds normally contain unstable functional groups
    - PRESERVATIVE: drugs usually contain organic active ingredient, drawing bacteria/mold
    - FILLERS: added for consistent dosing, dilutes the active ingredient ← cellulose or something
    - DISINTEGRANTS: swells and "blows pill apart" in the stomach (aqueous environment) ← starch
    - BINDERS: holds drug together in mouth, holds ingredients together in pill
    - COLOUR: an important safety measure (identifying your own prescription); knowing distinct appearance of pill prevents incorrect prescriptions

TYPES OF FORMULATION

<u>MOST CONVENIENT FOR CONSUMER</u>: (must pass liver) ← liver may remove a lot of active ingredient during metabolism

- Pills/caplets
- Capsules
- Liquids

<u>LEAST CONVENIENT:</u> (bypasses liver) ← these do not get metabolized, and stay in body longer/ are most efficient in creating effect

- Topical cream
- Patch
- Injectable liquid
- Nasal spray
- Eye drops
- Suppository

A DRUG/PRODUCT SURVIVING ALL THESE STAGE BECOMES AN IND!

CLINICAL TRIALS ← A LARGE COST IN MANUFACTURE (60-770% of cost of new drug)

- Drug testing in humans to ensure safety, efficacy, dose range finding (optimal human does) ← thousands of kg required, made according to Good Manufacturing Practice (GMP)
- A large cost, making companies fund their own testing ensures that the best drug possible is put forward
- **DOUBLE BLIND STUDIES:** controls for placebo effect (in both patients and doctors)
    - Not used for serious illnesses: Cancer, AIDS
    - Is instead compared to existing treatments/products

PHASE I → SAFETY ONLY (<1 year)

- Small number of healthy volunteers (<100), focus on safety, range finding
    - Attempt to find maximum safe dose, starting near estimated minimum effective dose
    - 30% of IND fail here
- Not done for cancer drugs (themselves are carcinogenic, risk too high for healthy volunteers)

PHASE II → SAFETY AND EFFICACY (<1 year)

- Small number of patients (200-300), focus on establishing effective dose
- 70% of INDs fail this stage → new drug does not actually work (variety of reasons)
- Constant communication with FDA: when drug has been put through phase II you either notify proceeding into phase III, or withdraw application
    - Companies show drugs being in CT phase II for investments, if >1 year, the drug won't reach market

PHASE III → SAFETY, EFFICACY, RARE SIDE EFFECTS

- Large number of patients (1000s), focus on rare side effects
- High fail rate (70%) due to side effects, or drug not working in desired manner

IF DRUG PASSES ALL CLINICAL TRIALS, YOU MAY FILE AN NDA WITH THE FDA!!!

PHASE IV → MARKET (additional safety testing for rare side effects)

- Produce hundreds of tons each year, continue safety testing for very rare side effects
- Find new indications to increase potential markets
- Only 30 new drugs enter market per year, a small % will drop out

ORPHAN DRUGS → FOR VERY RARE CONDITIONS (fewer than 200 000 US patients)

- Smaller clinical trials (fewer patients available), lower standard of proof with FDA
- Tax credits up to 50% of research costs ← Orphan Drug Act (promotes therapy for rare conditions)
- Treating a rare condition with an urgent need, meaning safety is a lesser concern
- 7 year market exclusivity; drug is allowed on the market for much longer than usual since not many people buy it

NUREMBERG CODE FOR RESEARCH ON HUMANs

- Rules in place to cease experimentation if necessary:
    - Voluntary participation with informed consent
    - Prior animal studies
    - Benefit outweighs risk
    - Qualified scientists
    - No suffering
    - Experiment will stop when too dangerous
- Code of ethics created after WW2 → prior, complete trust was given to doctors performing experimentation, little restriction on what couldn't be done
- Nazis performed unnecessary and immoral experimentation → Nuremberg code stated one needed a good reason to perform an experiment

GOVERNMENT REGULATION & OVERSIGHT

- Drugs completely unregulated before 1907

Board of Food and Drug Inspection instated 1907

- First attempt to regulate drugs, required to label ingredients on a product only
- No regulation of therapeutic claims, no safety testing, no monitoring

MEDICATION IN 1800s

- "Patented" medication, leading consumer to believe the product was good ← a patent only protects an idea
- No control/testing on drugs, anyone could make/sell anything
- Most products contain alcohol/narcotics ("feel good" ingredients)
- Massengill disaster: sold sulfanilamide in a liquid form mixed with anti-freeze, caused a mass poisoning
    - Forced off the market due to incorrect labelling → labelled as an elixir
    - Prompted creation of the FDA

MODERN SAFETY STANDARDS IN ANIMALS

- At least 2 species of animal (1 primate)
- Must show drug is bio-available (drug actually gets into body) ←shown in blood test concentrations
- Must use relevant doses to what consumers will take later on
    - Companies attempt to "sneak under radar", giving lower doses to show drug is "safe"

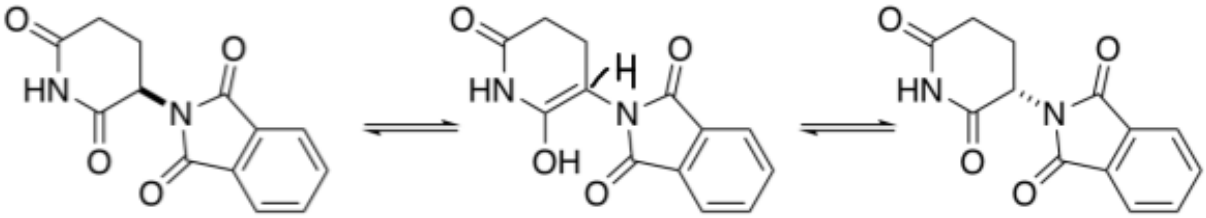
THALIDOMIDE ←a teratogen causing phocomelia

- A sedative with very few side effects, however tested insufficiently
- Drug was not marketed in the US: teratogenicity was discovered before FDA approval
- Thalidomide was tested in rats (did not give deformed pups)
    - A rat's uterus recognizes a malformed fetus, and is re-absorbed before birth can occur (in humans, defects, stillbirth and miscarriage occurs)

TERATOGENICITY OF THALIDOMIDE ←still unsure of actual correct answer

- Thalidomide manufactured as a racemic mixture of both left/right isomers
    - Both isomers need to be non-toxic; occasionally one isomer works while the other doesn't and vice versa

- R isomer was the active enantiomer, while S isomer was teratogenic vasoconstrictor (this theory was disputed, difficult to repeat experiment)
- Even if only R isomer was present in drug, the compound undergoes racemization in bodily pH:



1. Proton is removed; phenol (intermediate) is formed
2. Intermediate has no memory of what it was, has a 50% of protonating into either S or R isomer

Food and Drug Administration 1938 ← ensures safety of new drugs

- Ensures safety of drugs entering market (requires testing in animals, clinical trials for safety in humans)
- Directions for proper use required on label
- FDA does not do its own testing; responsibility of the company to fund its own testing, and send results to government
    - Requires companies to monitor their products: if an issue is reported with a prescription, the doctor reports issue to company (who then reports to FDA)
- Inspectors ensure data meets guidelines, statistics are verified, etc.
    - FDA employs inspectors for manufacturing, oversight agency sends inspectors to companies
    - Monitors for fraud, works hard to ensure honestly in industry
    - In the US less is spent on inspectors: this is an issue

INDUSTRY NEEDS REGULATION

- Ensures safe products, efficacy, and consumer protection
- A 3rd party requirement to ensure non-bias, and sell safe products to consumers
- Increases costs of drugs, and taxes (times spent researching and testing)

ADDITIONAL NOTES:

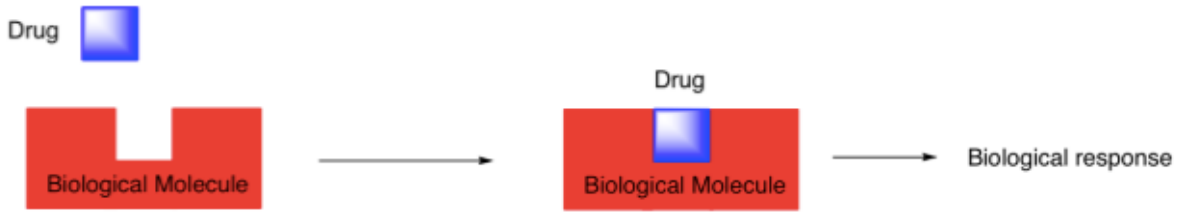
BIOLOGICAL TARGETS

BIOLOGICAL MOLECULES ARE MODULAR (easy to make)

- Smaller repeating subunits strung together create bigger molecules
  - Protein - amino acid
  - Nucleic acid - nucleotides
  - Polysaccharides - linear/branched sugars
  - Lipids - acetate or propionate
- Allows life to be possible; every chemical reaction in body requires a catalyst at physiological conditions
  - Stringing together biomolecules requires one enzyme (i.e. protein) that makes one kind of bond (i.e. amino acid)
- Modular structures are advantageous: biological systems easily build up/break down components for use and reuse elsewhere (also allows regulation in body)

Drugs produce effects by binding biomolecules:

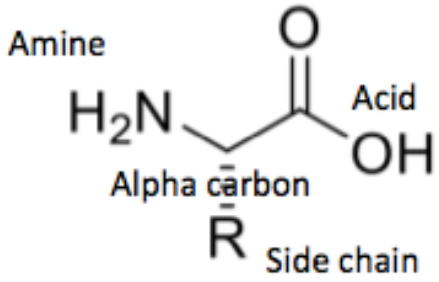
- Drug creates "drug-like" effects by interacting with biological molecules, making physical contact for effect to occur
- Enzymes (proteins) have well defined 3D shapes and create 3D chemical environments
  - Drug creates desired effect by binding bio-molecule



AMINO ACIDS <— 20 different types

- Amino acids mostly share same backbone/stereochemistry

- All amino acids in body are L, side chain going in to page
- Differ from each other due to side chain (chemical properties of side chains vary)



AMINO ACID SIDE CHAIN PROPERTIES (re-draw in ur notebook)

NON-POLAR SIDE CHAINS: differ in shape, though generally non-polar
ALKYL
Alanine Valine Leucine Isoleucine Methionine

AROMATIC
Phenylalanine Tyrosine Tryptophan

ACIDIC SIDE CHAINS: in physiological pH (7.4), side chains of acid de-protonate (become negatively charged) and behave as the weak conjugate base.
Aspartic acid Glutamic acid

BASIC SIDE CHAINS: (pH 7.4) bases protonate (gain an H+) and generally function as the weak conj. acid.
Lysine Arginine Histidine (can act as acid or base)

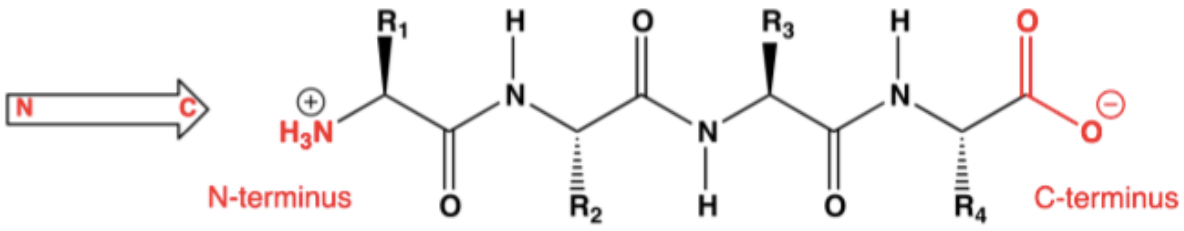
POLAR SIDE CHAINS: have lone pairs, hydrogen-dipole interactions
Asparagine Glutamine Serine Cysteine Threonine

Glycine (the only achiral amino acid)

Proline (the only one w a secondary amino) <- side chain loops around and forms ring with amino group

PRIMARY STRUCTURE OF PROTEINS <- sequence of amino acids in a protein

- Amino acids joined by peptide bonds (amide) from the N terminus to the C terminus
- ALWAYS in list sequence from N to C



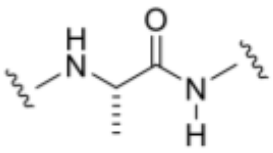
SECONDARY STRUCTURE: localizes areas of organizations in proteins

- Caused by conformational restrictions of proteins: all peptide bonds are single bonded, and rotation is possible in theory
    - Conformational restrictions b/w amide and a-carbon
    - Chemical environment restricts rotations, resulting in secondary structures
- Intermolecular forces acting in an intramolecular manner; side chain interactions within a region of the chain
- Form small- scale structures:
    - a - helix
    - B - sheet
    - loops

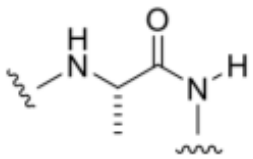
AMIDE BOND IS A SINGLE BOND WITH DOUBLE BOND CHARACTER

- the amide bond is a very flat structure; shaped as if it has a double bond
- Unlike double bond, C-N bond can rotate (slowly and favours 2 shapes)

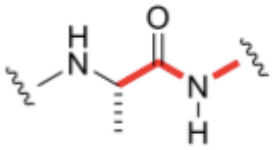
- C-N bond does not have unlimited rotation: it sits as either cis or trans
- Does not want to sit like a single bond: immediately it will rotate into a flat shape (either cis or trans)

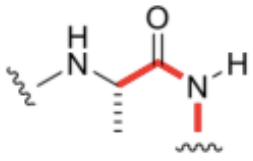


s-trans



s-cis





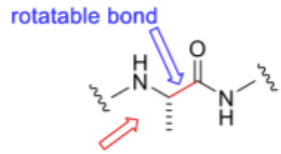
- Orbitals lock C-N bond into its flat shape; resonance between C=O and NH makes both sides sp2
- Bond is sp2 hybridized all the time; pretty much a double bond @ the amide bond (all structures flat an in same plane

Shapes are restricted depending on side chain interactions:

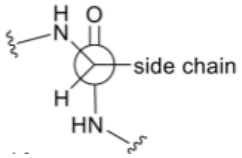
- Negative charges attract positive charges
- Hydrogen bonding between side chains and backbones of structures
- Non-polar side chains interact with other non-polar side chains

PREFERRED CONFORMATION: a-BOND

- Single C-N bond is free to rotate very slowly; amide bond is locked into s-trans conformation
- Restriction is given based on the preference of the rotamers
- The a-bond likes to adopt a conformation where the S chain is at 90° to the amide bond (side chain likes being perpendicular)

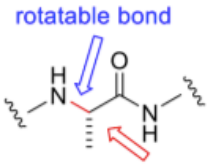


common conformation of α bond

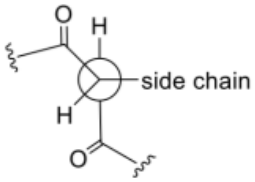


PREFERRED CONFORMATION: B-BOND

- Flatness of amide bond restricts rotation; this creates secondary structure in proteins



common conformation of β bond



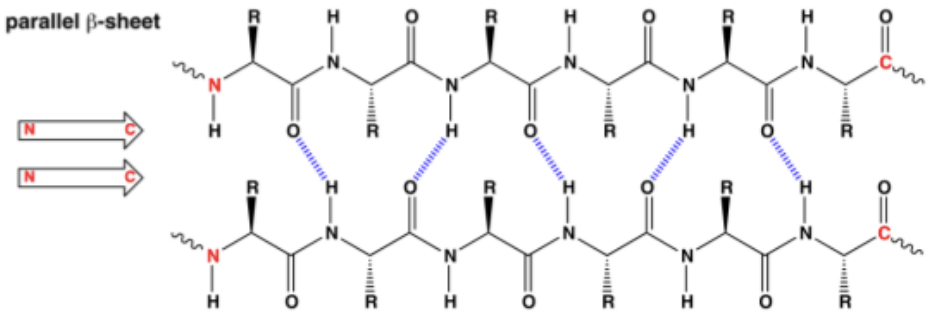
RESULT OF THESE CHEMICAL INTERACTIONS CREATES THE FOLDING OF THE AMINO ACID CHAIN; PRODUCING LARGE SCALE SECONDARY STRUCTURES

a-HELIXES FORM CYLINDER-CORKSCREW STRUCTURE

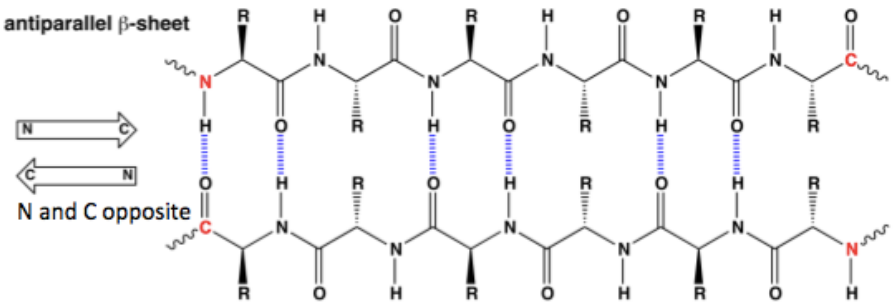
- each corkscrew represents 1 helix

B-STRUCTURES AND B-SHEETS

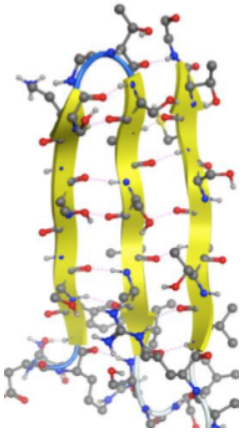
- Straight zig-zag of proteins; folds itself together with neighbouring beta sheet
- Can be single or double stranded



Anti-parallel more stable, and thus more favoured



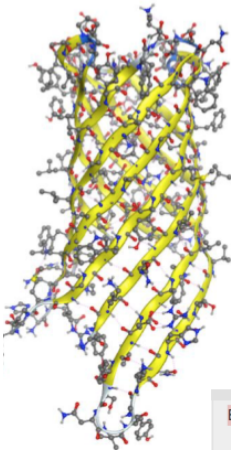
- Several strands can associate together (B-sheets), large sheets curl around themselves and form cylinders (B-barrels)
- Flatness of ribbon due to the flatness of amide bonds

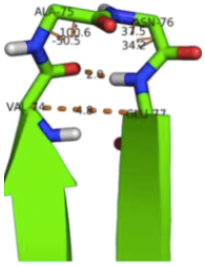


In anti-parallel sheets, one side of sheet can be polarized differently than the other side

Beta barrels are similar (same idea of inside vs. outside) however it is the inside of barrel has a different polarity than the surface

Beta barrels: have a helical component to them



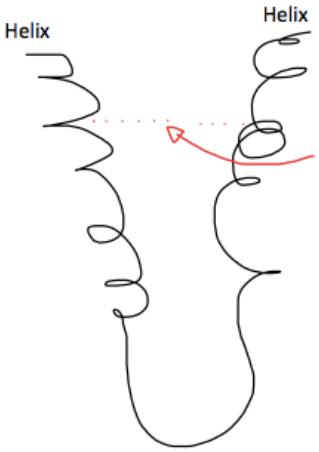


B-TURNS: portions in amino acid string with tight radii
-Flatness of the amide bond forces straight/flat structures
-Once in a while they will turn due to tight radii

PROLINE AND GLYCINE: tite turn due to flexibility of glycine + rigidity of proline
-Proline: has secondary amino, S-cis conformation preferred
-Glycine: has no side chain and is more bendy/flexible

LOOPS: proteins with no secondary structure (haven't adopted one)
- Looks like skinny noodles

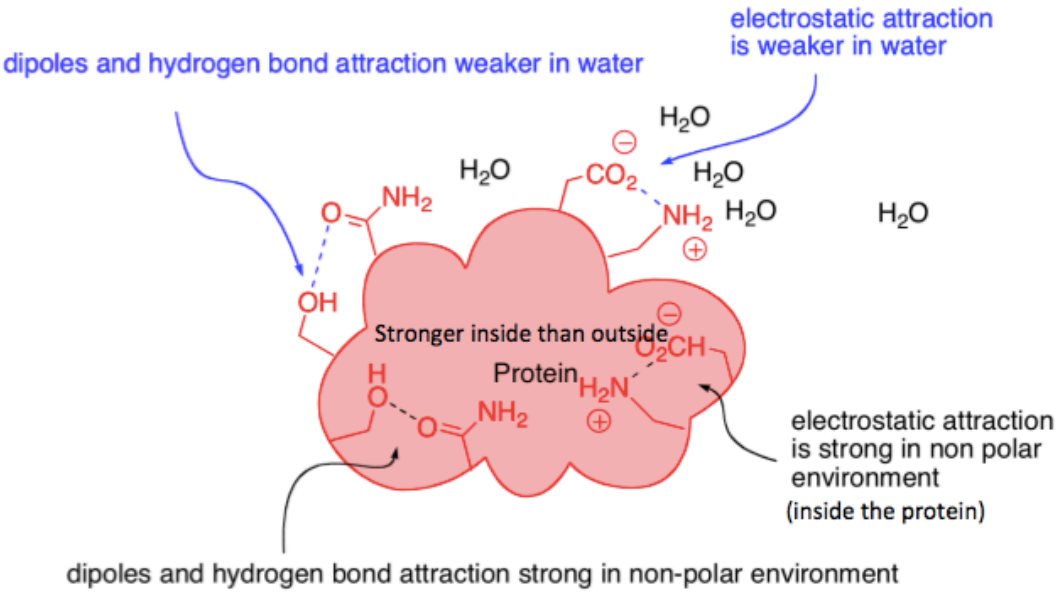
TERTIARY STRUCTURE: overall 3D shape of a protein (shit sticks together)
- Results from interactions b/w non-adjacent regions:
  - Amino acid side chain interactions
  - Two secondary structures (two helixes) interact
- Secondary structures adopted from interactions close by
- **Tertiary:** interactions between side chains far away from each other, due to intermolecular forces/interactions
- Contain regions of order (secondary structures) and less ordered regions (loops)



INTRAMOLECULAR INTERACTIONS
-Non-bonding (hydrogen, Van der Waals, etc.)
-Interactions between different parts on the same molecule

DISULPHIDE BONDS: something negative close by to something positive
H-BONDS: electronegative attraction between H+ and another molecule; between amino acid side chains
VAN DE WAALS: non-polar side chains attract to each other through random charge dispersion

- Van der Waals interactions are important for maintaining protein structure and drug interactions
- Protein fold on itself in a way to exclude water from inside:
    - Hydrophilic side chains fold towards outside (interacts with water)
    - Hydrophobic fold towards inside ("greasy" stuff inside)



- Polar groups on outside of molecule attract each other/water, groups on inside only interact with EACH OTHER
- Causes very strong intramolecular interactions (i.e. hydrogen bonding)
- Drug designers bury important molecule in grease, gets it away from water
- Van der waals important in keeping molecule safe/stabilized; VDW **strengthened by non-polar environment (weakened by polar)**

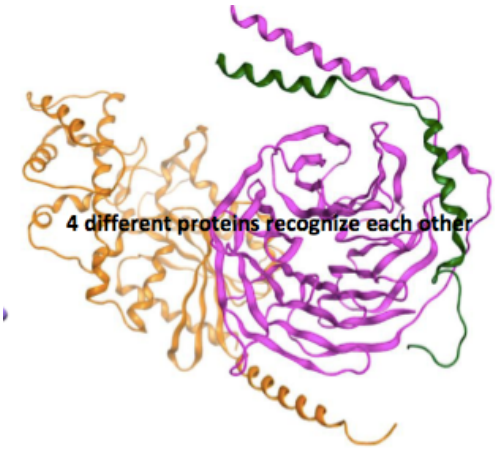
i.e. NaCl in H_2O: water is polar enough to form non-bonding interactions with Na+ and Cl- (we get saline solution

If solvent is switched for CH_4 (non-polar): unable to form non-bonding interactions

- Strengthens interactions between Na+ and Cl-, the hydrocarbon cannot insert between the two atoms
- Non-polar interactions ensure the strength of more powerful bonds to hold protein together

OCCASIONAL QUATERNARY STRUCTURES (two or more proteins bind together)

- Protein-Protein interaction strong: network of H, disulphide, VDW bonds holding proteins together
- Lots of surface contact area (hard to get between proteins), lots of chemical interactions (due to big surface area)



Large-spanned interactions

OVERALL PROTEIN STRUCTURE DETERMINES FUNCTION: most of molecule is scaffold

Much of the protein is there to keep the important amino acid (enzyme) in place

Protein holds the actual enzyme in its shape, so that it can still bind to specific substrate

TYPES OF PROTEIN TARGETS FOR DRUGS

ENZYMES (38%): most drug enzymes stop something from working (an inhibitor)

- Difficult to switch on a function in the body with a drug, can much easily stop a certain enzyme from working/stop formation of enzyme product

RECEPTORS (24%, 12%): activate (agonist) in-activate (antagonist)

- Some drugs can do both
- Drug exert effect by amplifying/suppressing receptor function

ION CHANNEL (8%): specialized channel receptor drug opens or closes

- Some perform both functions, same idea as receptors (very similar)

STRUCTURAL PROTEINS: uncommon

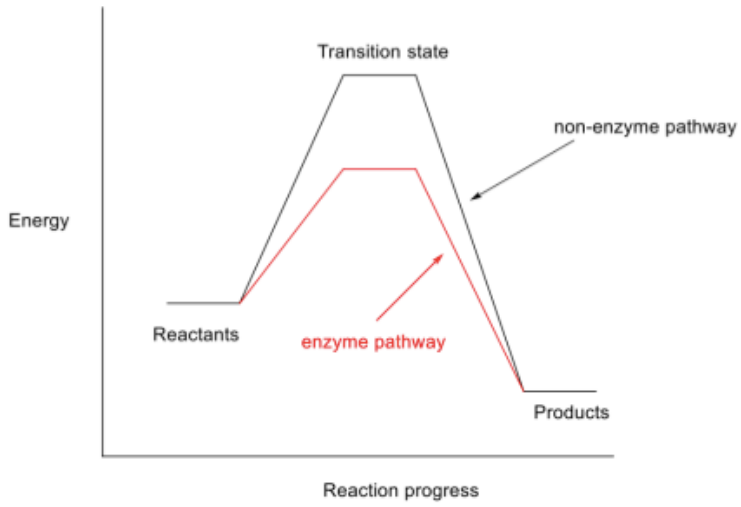
- Drug interferes with assembly/disassembly of certain protein (a few cancer drugs may do this

ENZYMES CATALYZE REACTIONS

- Enzyme provides a "designer solvent" for transition state of a reaction
  - Interested in the shape of enzyme at the transition state, when designing inhibitor (elaborate later)

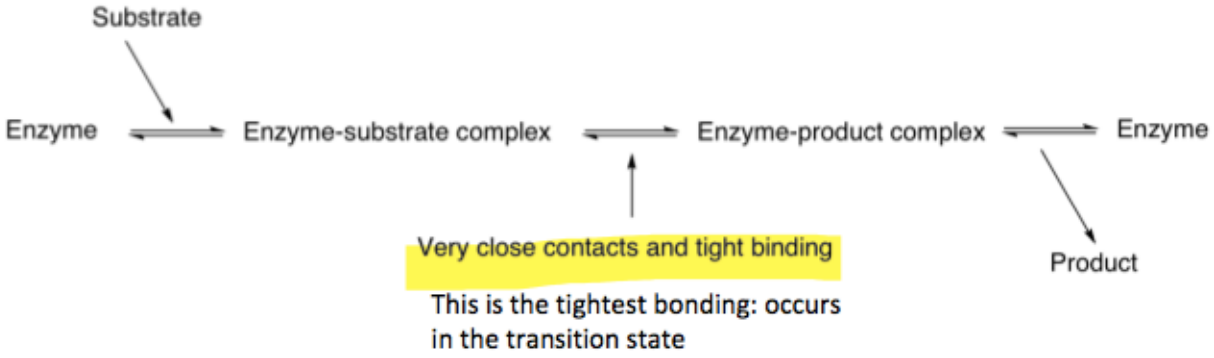
- Binds tightly to substrate and lowers the activation energy of the transition state

- Reaction is carried out at a faster rate and more stably



'DESIGNER SOLVENT' FOR TRANSITION STATE

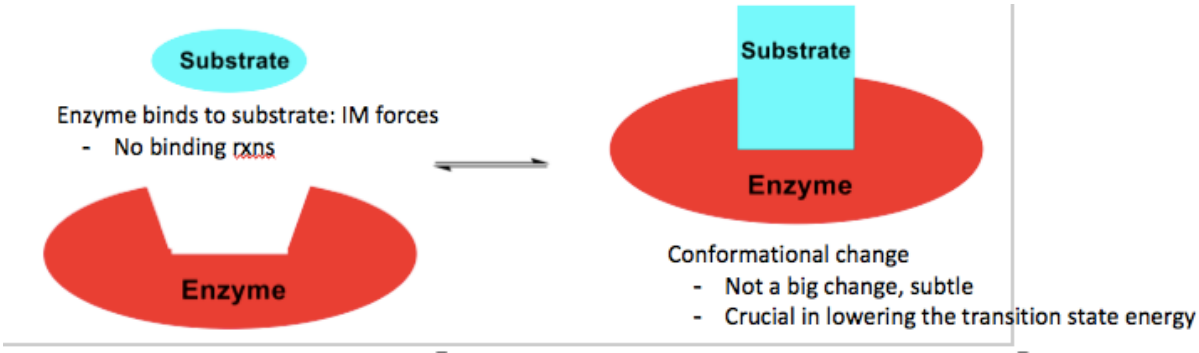
- How enzymes work is similar to how chemists plan/design a specific solvent for purposes in a rxn
- Enzymes create a specific chemical environment, favourable for the rxn it catalyzes:
  - H+ available at one proton where a neg. charge will form
  - Neg. charge on aspartic acid, for stabilizing a pos. charge on a substrate
- Without an enzyme: no specificity for molecules to interact
  - (notebook example) in order for rxn to occur, H_2O molecule must be positioned perfectly at a specific angle; O pointing to the pi* orbital of the C=O
  - random motion necessary for all components of molecule to align perfectly in 3D
  - THIS IS NEARLY IMPOSSIBLE AND TAKES FOREVER
- An enzyme has perfect alignment to the specific substrate
- Atoms are in right place automatically, and does not depend on random motion
- Much faster, more favourable reaction takes place
- Enzymes are similar to designer solvents: create a favourable reactions for substrate, stabilizes transition state



ENZYME ACTIVE SITE COMPLIMENTARY TO TRANSITION STATE

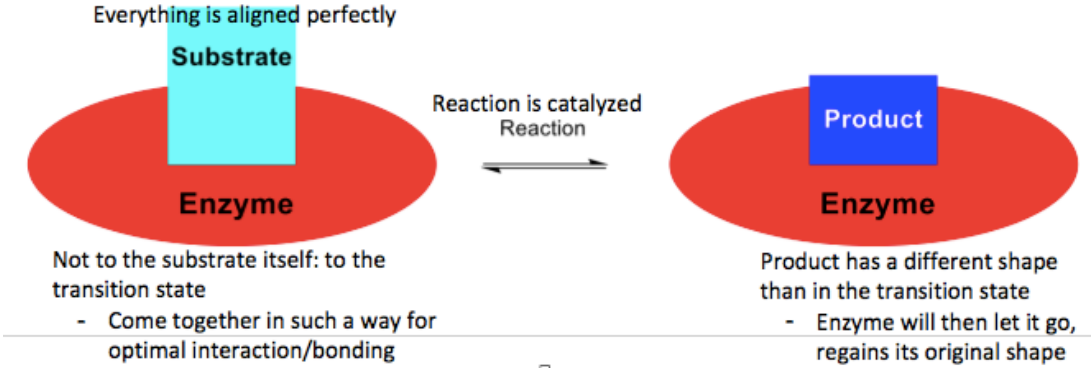
- Enzyme does not recognize substrate itself: recognizes transition state

ENZYME (E) BINDS SUBSTRATE (S): forms an ES complex
Enzyme/substrate both undergo slight conformation change to accommodate each other

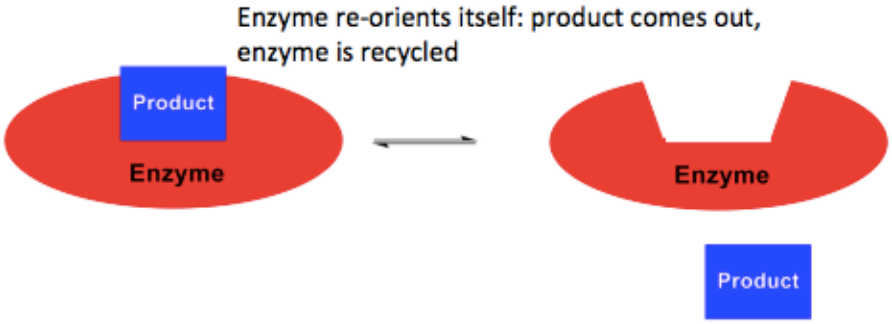


REACTION OCCURS

- Components in ES complex in correct alignment to facilitate a rxn
- Substrate converted into product @ active site, enzyme may change shape during this process



PRODUCT IS RELEASED



- Conformational change causes enzyme to spit out product
- Enzyme is recycled and can catalyze another rxn

THEORIES OF ENZYMATIC CONFORMATIONAL CHANGE

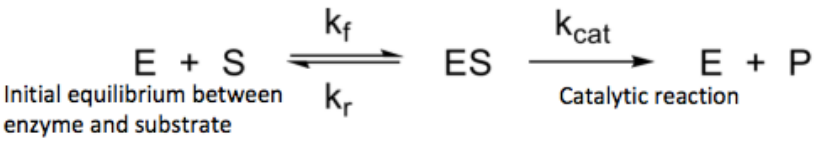
LOCK AND KEY: enzyme binding pocket is the exact shape for substrate

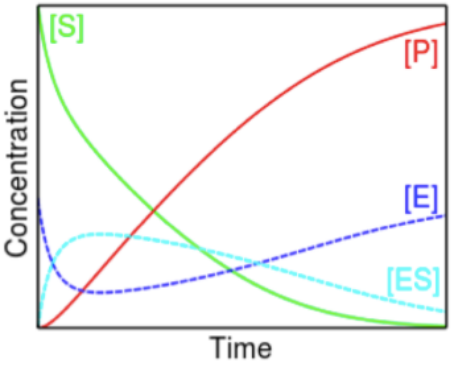
- Incorrect: enzymes and substrates are not in correct shape for each other before contact

INDUCED FIT: binding of substate to exhume changes conformation of enzyme

- Change “activates”/“deactivates” enzyme
- Important to remember that both enzyme and substrate are flexible & change conformation during binding
- Enzyme/substrates are flexible and can accommodate to each other, but that means they can also fit many others
    - Constantly “flexing” and wiggling, shape changes are not instantaneous or symmetrical (recall wiggly video)

KINETICS OF SIMPLE ENZYME FUNCTION

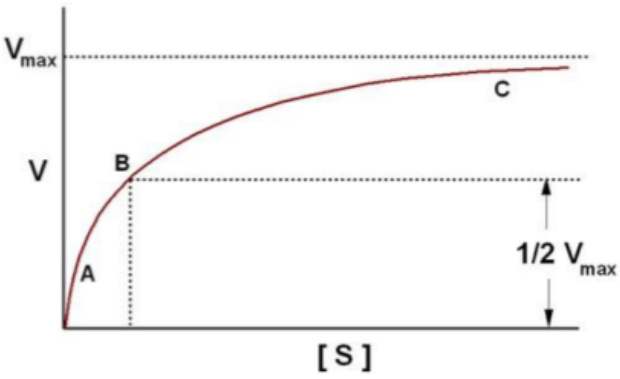




- Track shows how things change from start to end of rxn
- 0-100 product, 100-0 substrate
- tracks ES complex

MICHAELIS-MENTON KINETICS

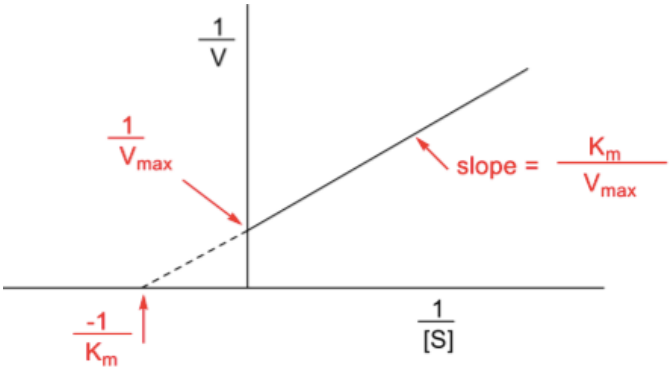
$$V = \frac{dP}{dt} = \frac{V_{max}[S]}{K_m + [S]}$$



Measures velocity of reaction (rate of formation of products)

- Speed of rxn depends on amount of substrate available (adding substrate increases velocity
- K_m: concentration of substrate, when you are at half max. velocity
- K_{cat}: turnover rate; measures how efficiently ES complex produces product (bigger K_{cat} = easier reaction)
- K_{cat}/K_m measures index enzyme efficiency
- K_d: strength of binding, disassociation constant
    - How much enzyme sticks to substrate; if substrate tightly binds to enzyme, difficult to design a drug to compete/kick out natural substrate

RELATIONSHIP CAN BE RE-ARRANGED INTO LINEAR

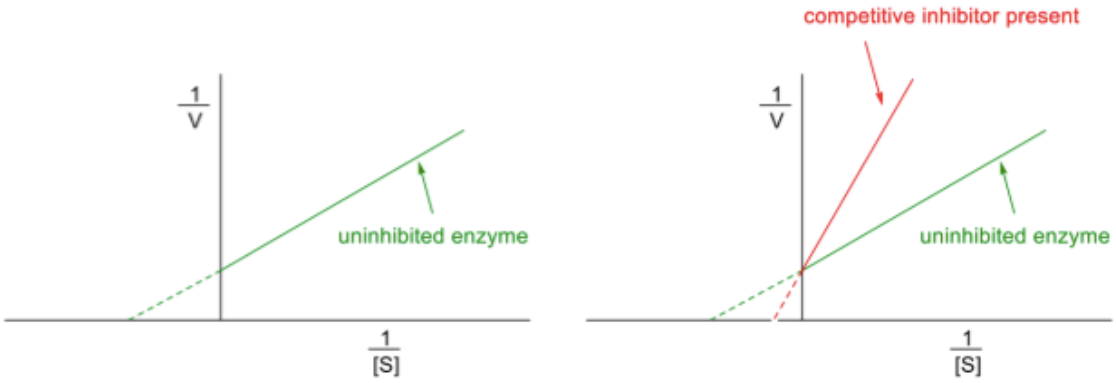


$$\frac{1}{V} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

ENZYME INHIBITION

COMPETITIVE INHIBITION: drug (inhibitor) competes with substrate molecule for active site

- Binds at the active site, substrate cannot bind (inhibitor occupies space)
- Inhibitor is approximately same shape as substrate, can bind same pocket
- Competitive does not change V_{max}, intercept of graph unchanged
- Changes K_m, amount of substrate needed to reach Vmax

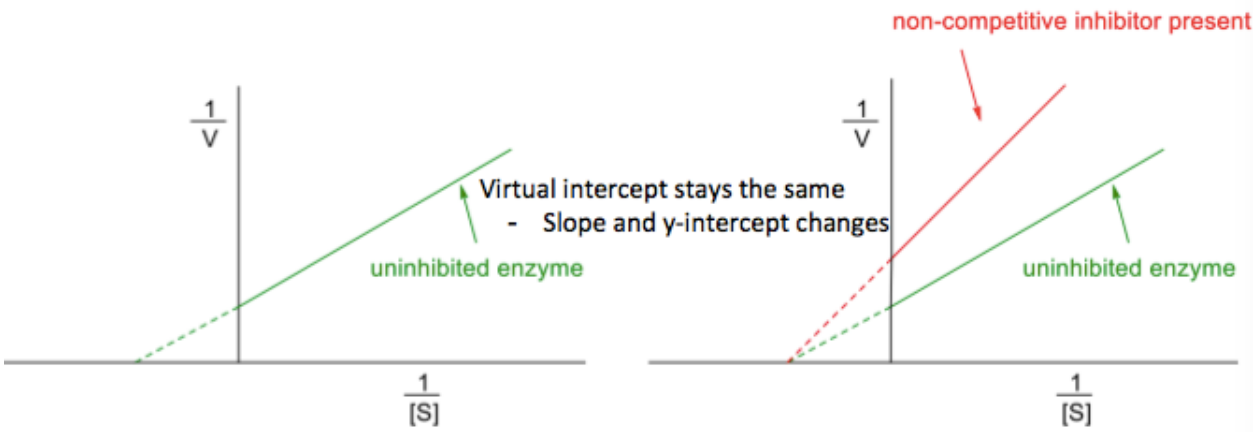


Competitive: DISULFIRAM <— FOR ALCOHOLICS

- Blocks aldehyde dehydrogenase, causes build up of acid aldehyde (super hang over)

NON-COMPETITIVE: Inhibitor binds to different site than the active

- Substrate still binds to active site, inhibitor prevents ES complex from forming (ALLOSTERIC INHIBITION)
- Prevents/alters conformation change (when enzyme+substrate bind); cannot bind as tightly/properly
- Changes slope and intercept, doesn't alter K_m
    - o same amount of substrate binds)
    - o V_{max} is lowered

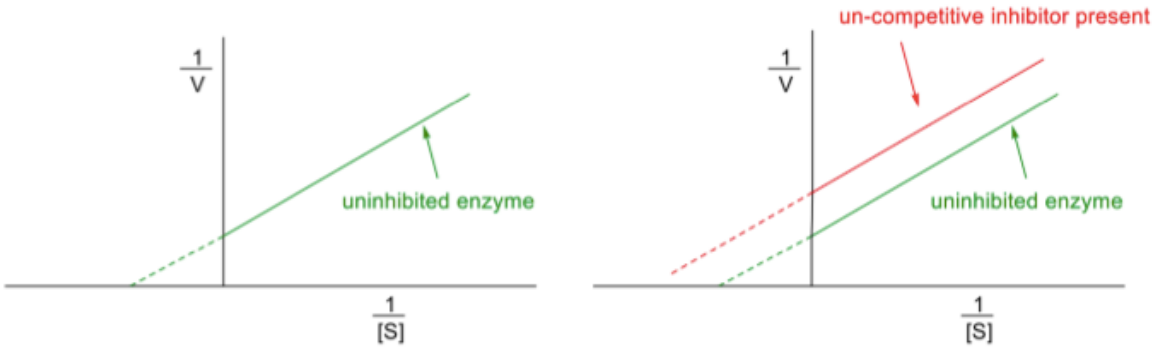


Non-competitive: FLUCONAZOLE (antifungal)

- Changes shape of protein enough such that when binding substrate, not nearly as efficient
- SIDE EFFECT: interferes w other drugs taken (acetly-co p450? a liver enzyme)
- Inhibits interaction of liver enzyme; causes more absorption of a different drug than anticipated
- Drug-drug interaction creates possibility of high toxicity/overdose

UN-COMPETITIVE (very rare): inhibitor binds to actual ES complex

- Inhibitor cannot actually bind to native enzyme; instead to an imaginary non-existent pocket
- Destroys catalytic ability of ES complex
- Slope remains the same: shifts it up and changes intercept
- Max efficiency is lowered as well as amount of substrate needed to reach 1/2 velocity (K_{cat})



Uncompetitive: LITHIUM <— inhibits enzymes in body uncompetitively

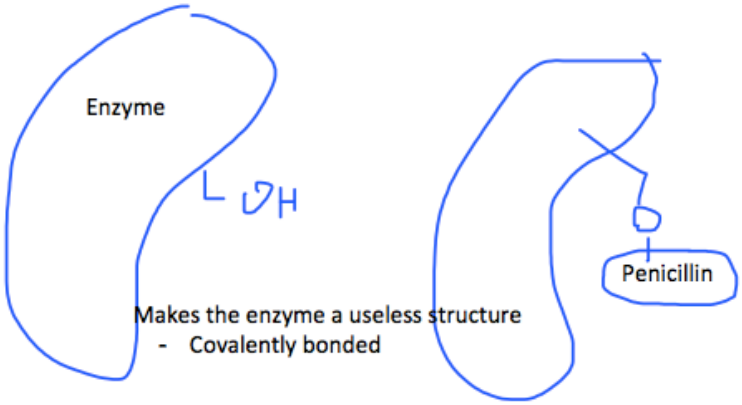
- Blocks inositol monophosphatase, treats manic depression
- Exact mechanism unknown

IRREVERSIBLE INHIBITORS: covalent bond permanently changing conformation

- Inhibitor usually bonds to active site
- An irreversible conformation change, shape is permanently altered

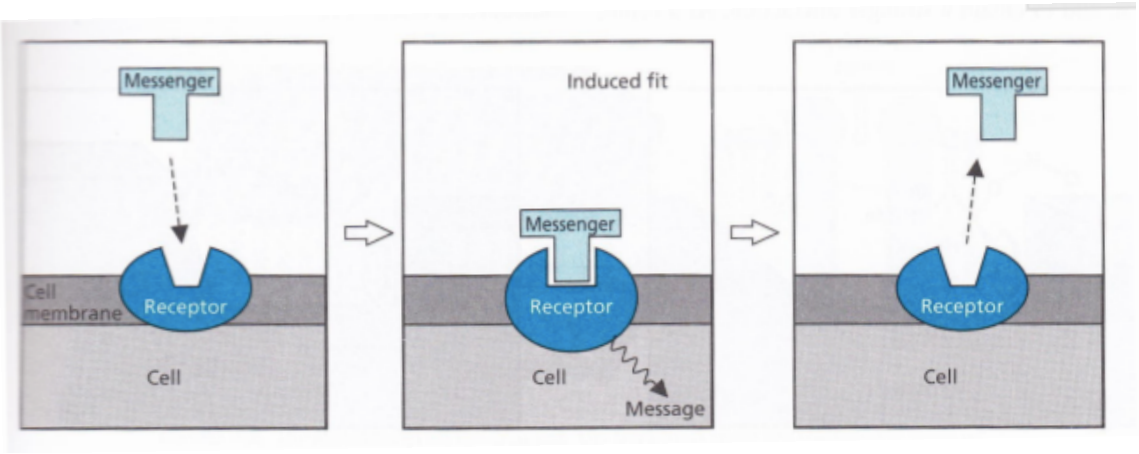
PENICILLIN REACTS COVALENTLY WITH TRANSPEPTIDASE

- Breaks two bonds, forms a bond between NH_2 and D-Ala; irreversible bonding to transpeptidase
- Bacteria secretes viscous liquid around itself, needs this shit to cement it into a shell
- Irreversible bond to transpeptidase causes bacteria to die
- Humans only have L-amino acids, and thus are unaffected

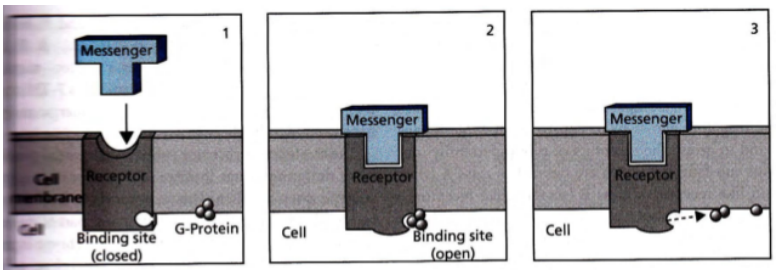


The 2nd key target of drugs in body is receptors (found on cell membranes)
How cells communicate with environment: **MESSENGER INDUCES SHAPE CHANGES**

- Messenger binds receptor + shape change + transfer of information
- Normally a protein carrying information(messenger) to another protein wedged in cell membrane
- When messenger binds to receptor, receptor changes conformation at "top" and "bottom" of molecule
- Chemical signal is sent from receptor to inside of cell (cascading events)



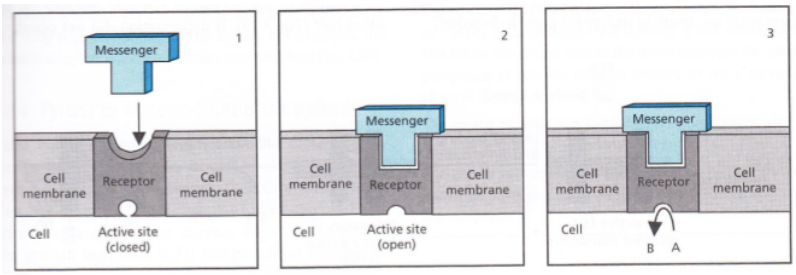
VIA BINDING: something bound to enzyme, shape change on other side of enzyme allows binding of a secondary messenger



Then unbinds and relays a message

Shape change allows receptor to connect to molecules on other end

VIA CATALYSIS: receptor is an enzyme with an active site inside cell



Enzyme otherwise not in correct shape to act

Binding of messenger prot. causes conformation change

Receptor now in correct shape to undergo rxn

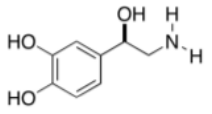
Activating/deactivating a catalyst

AGONISTS: simulate normal messenger

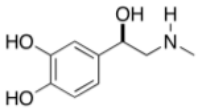
- Binding at same location of messenger (active site), creating came conformational change (switches inner function on)
- Some enzymes **bind allosterically**; to site other than the active (ALLOSTERIC MODULATOR)
- Sends information in absence of messenger; simulates pathway without original substrate

ASTHMA DRUGS: noradrenaline mimics adrenaline

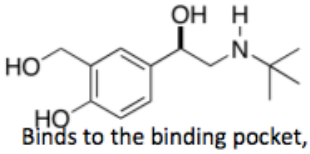
- Shaped similarly to adrenaline and binds active site of noradrenaline receptor (adrenaline agonist)



Adrenaline



Noradrenaline



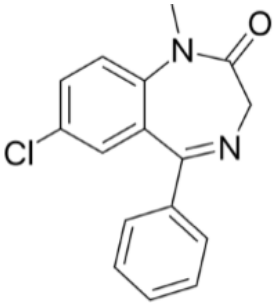
Levalbuterol (Salbutamol)

Binds to the binding pocket, causes receptor to wrap around it similar to the normal molecule

- Information still gets sent

BENZODIAZEPINE: allosteric modulator

- Drug binds allosterically to $GABA_A$ ion channel
- GABA: inhibitory neurotransmitter; in presence of GABA and benzodiazepine, channel will open more readily/stay open longer



Shape of ion channel changed enough so that binding threshold of messenger is reduced

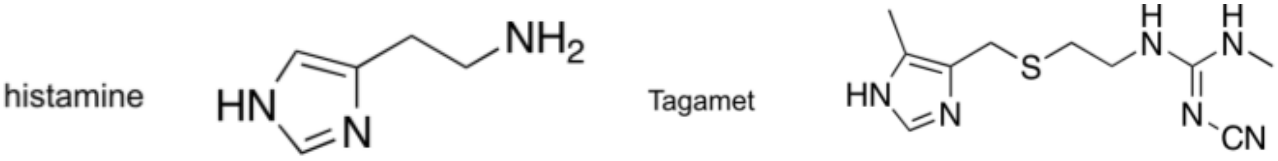
Now, less concentration of messenger protein required to send information

ANTAGONIST: blocks normal receptor function

- Antagonist binding induces abnormal shape change no longer able to transmit properly (no single is sent)
- May bind at the active site & block messenger from entering
- May bind allosterically & ruin molecule (i.e. take away receptor's flexibility)

TAGAMET: binds to histamine binding site

- Drug used to reduce amount of stomach acid produced
- Distance b/w main binding domains larger than histamine; active site is stretched and antagonism produced



In histamine, enzyme/receptor grabs amine ring and NH_2 to activate, needs to bend to reach

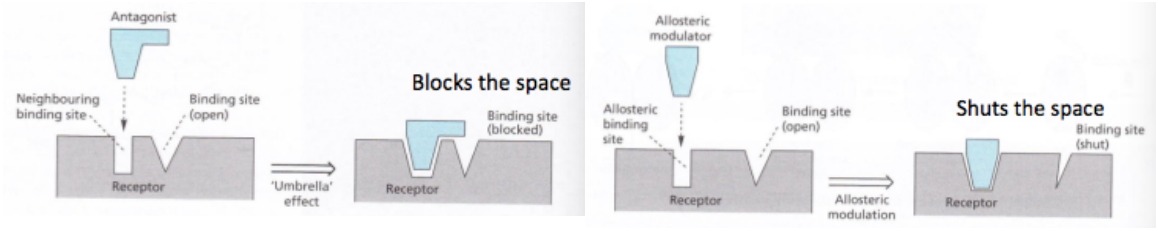
- Produces HCl

In Tagamet, spacing b/w two ends much longer. When enzyme grabs both ends, shape of active site is changed/unfit for activity (not a big change)

- A few extra carbon atoms enough to space molecule out & ruin receptor (everything sensitive to overall shape)

ALLOSTERIC ANTAGONIST: binds near or partly inside active site

- Shape of receptor is changed, normal molecule can no longer come in



- Allosterics are uncommon: usually things bind to active site

PARTIAL AGONIST: agonist binds receptor, produces non-ideal conformation change

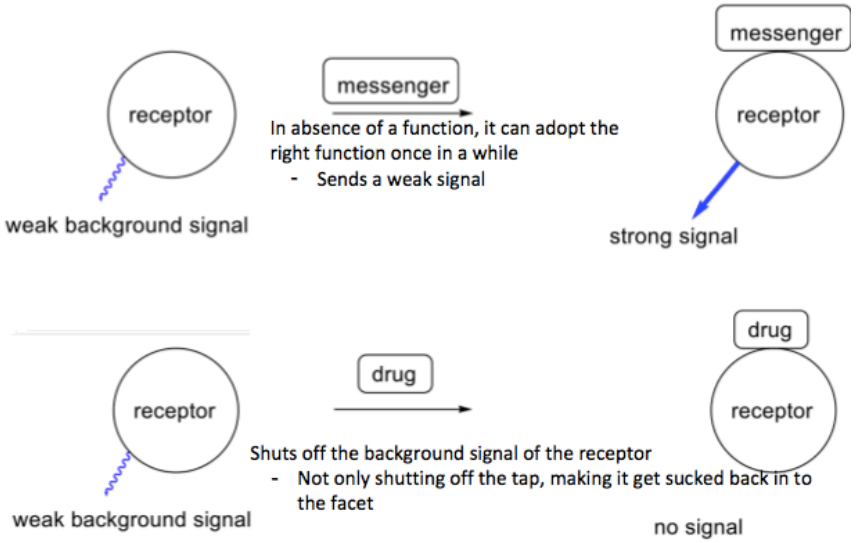
- Change allows subsequent messenger, however much weaker (change dissassociation constant, fewer molecules sent out)
- Capable of binding receptor in more than one way, to give agonism or antagonism

NALOXONE: partial agonist of opioid receptor

- Blocks effects of opioid poisoning; produces opioid level effects at a much smaller rate
- Thus blocks effects of poisoning; when people overdose they won't die

INVERSE AGONIST: actually an antagonist

- Not only antagonizes action of enzyme/receptor, causes it to act in reverse
- Receptors have background activity: show a weak function even w absence of messenger
- Inverse agonist will change receptor's normal function, shutting background function off
- Results in apparent **reversal in response;** occurs due to weak inherent signal of the receptor



CLOZAPINE: shows inverse agonist behaviour

- Originally thought to be weak D_2 antagonist, actually inverse antagonize

MEASURING DRUG BEHAVIOUR: uses biological assays

- Qualitative (is it working yes or no)
- Quantitative (a number, how well its working
- In vitro: in glass, no animal (this is preferred bc cheap)
  - Can also isolate chemicals as pure enzyme, giving cleaner information
- IN vivo: testing in living animals

GENERAL ASSAY TYPES

- HTS (used qualitatively) <— emphasis on speed
  - Fully automated, usually fluorescence detection, 384 or 1536 plate

- Routine SAR work (used quantitatively)<— emphasis on accuracy
    - Semi-auto or manual (collection of compounds small enough), usually fluorescence detection, some may use radioactivity
- Kinetics/special studies
- Cell-based (used quantitatively, requires skilled technicians)
    - Used for antibiotics, antivirals, metabolism studies
    - Usually slow & difficult to interpret results
    - Assays purchased, used for multiple tests after drug has been developed
- Tissue based (tests permeability)

ADDITIONAL NOTES:

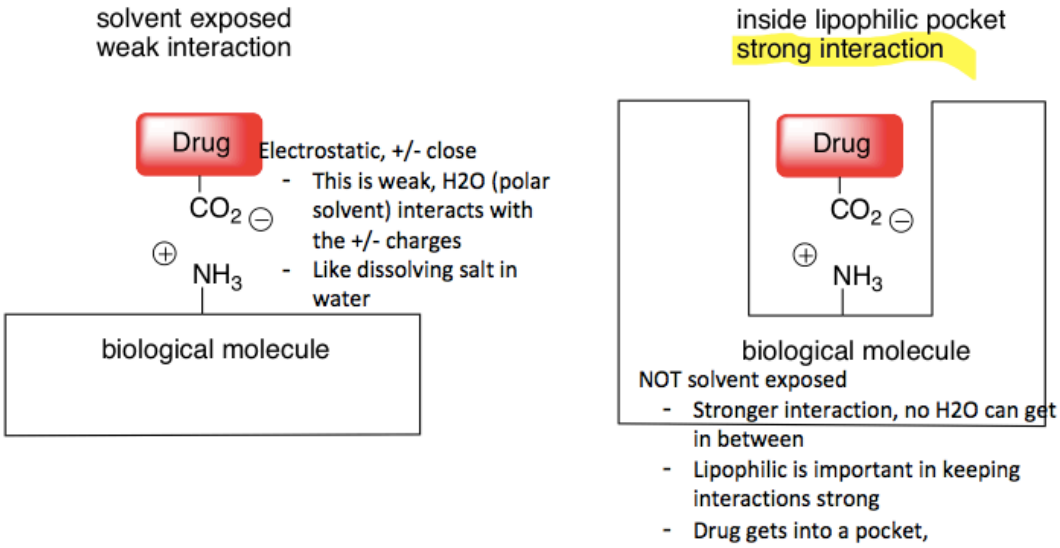
STRUCTURE-ACTIVITY RELATIONSHIP

Drugs produce effects by physically binding to bio-molecules
- Physical contact: drug floats around in body, encounters bio-molecule, binds, response occurs

Binds using IM forces:
- Fits into enzyme pocket in a specific way, altering shape of bio molecule
- Electrostatics (+ and -) <— strongest type
- Hydrogen bond
- Dipole-dipole
- VDW (the same thing holding proteins together, keeps drugs stuck to protein)

ELECTROSTATIC INTERACTION: strong bond b/w point charges
- Strength dependent on surroundings; bond stronger in hydrophobic environment
- Non-directional interaction, only factor in strength of bond is distance
- How charges are pointed has no effect

LOCATION IMPORTANT:



HYDROGEN BOND: also strong
- Dependent on surrounding environments, bond is also DIRECTIONAL
- Strongest when X-H bond points directly in a straight axis towards lone pair acceptor
- If H atom comes in at an angle, axis goes in different direction
- Interaction no longer co-linear and results in weaker interaction (see notes for clarity)

HYDROGEN BOND DONORS: provide the hydrogen

- X-H group, the thing w a hydrogen on it
- Vas majority of donors OH, NH. Variety of functional groups: OH, NH_2, NHR, CO_2, CONHR, etc.
- C-F bond acts as a donor (rarely) <— C-F bond is sort of an H-bond interaction
    - Electronegative fluorine attracts lone pairs, even though it tends to be the negative part of dipoles

HYDROGEN BOND ACCEPTORS: have a lone pair that attracts hydrogen

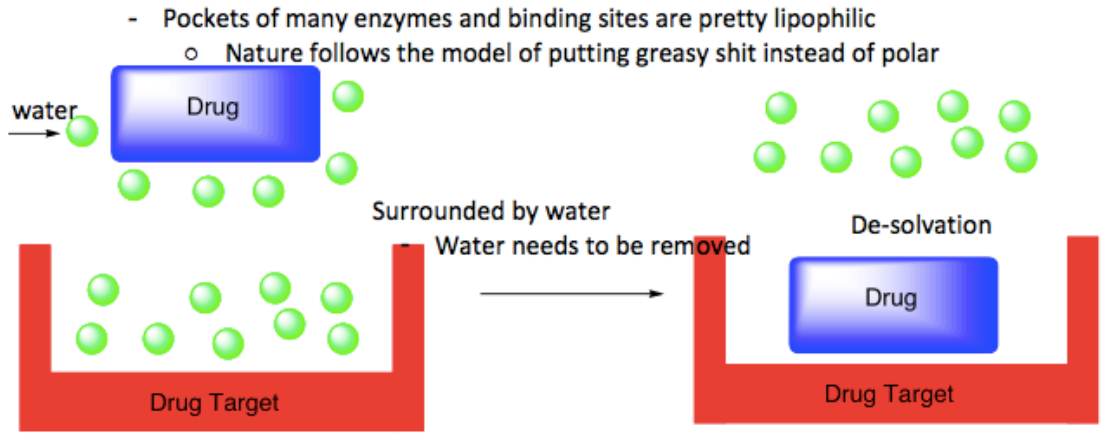
- Good acceptors (have lone pair): Oxygen, Nitrogen
- Weak acceptor: Sulfur

DIPOLE-DIPOLE INTERACTION: moderate strength interaction

- Depends on surrounding environment; strengthened by hydrophobic
- Usually carbonyl groups; strong electron withdrawing groups

VAN DER WAALS (DISPERSION): a weak interaction

- Dependent on higher surface area
- Important in de-solvation of binding pockets; lipophilicity significantly improves potency of drugs
    - Drying the pocket easier with a non-polar drug (than polar one)
    - Water molecules get stripped away by VdW as drug enters binding site
    - Binding pockets often lipophilic, facilitating water removal & ligand binding
- Water gets excluded from 2 areas attracted by VdW; water gets squeezed out from between non-polar molecules



Potency is linked to lipophilicity; most drugs are highly lipophilic (lots of C-H bonds)

POTENCY: concentration of drug needed in order for effects to be apparent

- Low potency: more effective, "takes a low amount of drug"
- Drug aims for low potency: more convenient for consumer to ingest

SPR/SAR - STRUCTURE PROPERTY RELATIONSHIP

- Structural changes made to molecule, test & measure **various** properties of molecule
- Relate effects/function to change in structure, use information for further compound design
- SPR: 10-15 different measurements taken for optimization every time structure is made (rather than only optimizing one parameter)

GOAL: DRUG-LIKE (user friendly)

- Potent: easy to take, small dose needed for desired effect (easy to optimize)
- Bioavailable: getting drug actually into body
  - Drug % entering the blood stream after oral dosing
- Chemical behaviour: pattern of chemical activity
  - Solid, liquid
  - Stability
  - Ease of synthesis

COMMON PROPERTY MEASUREMENTS

- Solubility: how it dissolves in water
- pKa: Acid/base nature of drug is useful in getting it into body
- LogP or LogD: ability to cross membranes, its lipophilicity
- Molecular weight: solubility and ability to cross membranes
- Permeability: ability to cross membranes
- Melt point: want a goldilocks (not too solid not too liquid)
  - Solids easier to manufacture/purify
- Metabolism: amount entering body, lifetime
- Protein binding:
  - Amount circulating body, lifetime in body, availability for activity

PROPERTY OPTIMIZATION: drug needs to pass a number of bodily barriers

- Drug cannot bind its target unless it actively enters body & gets there
- Chemical properties of drug controls how it passes barriers
- Want to maximize amount of drug reaching target thus must optimize other properties along with its potency

<u>GI - TRACT</u>

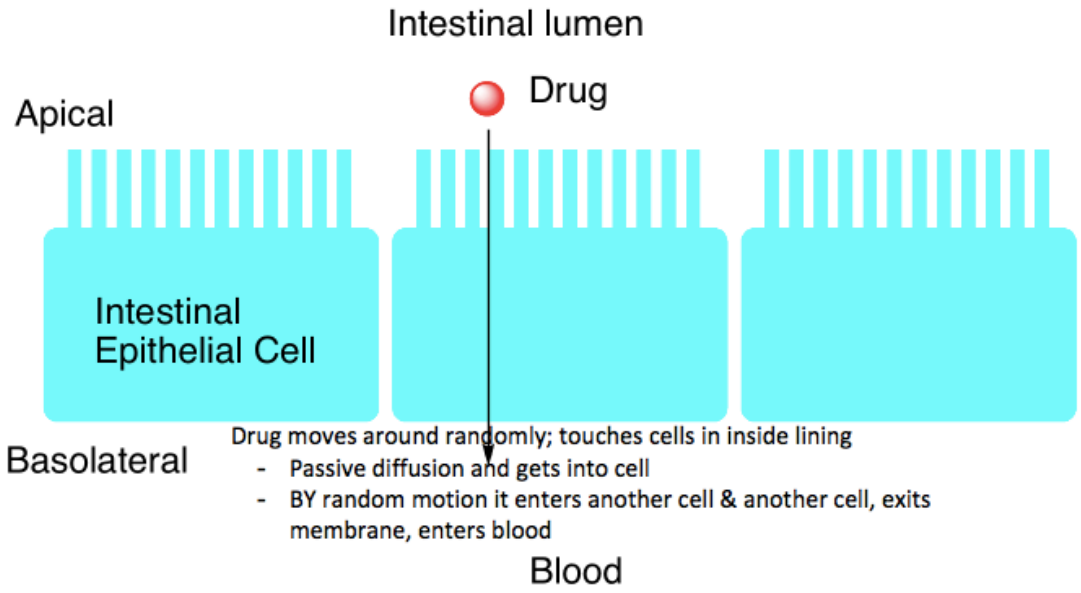
STOMACH HAS pH 1.4 - 2.1: drugs are not absorbed in the stomach

- Only enters bloodstream when reach small intestine
- Drug must be water soluble and capable of resisting strong acid

INTESTINAL ENVIRONMENT: mildly acidic, 4.4 - 6.8 (close to neutral pH)

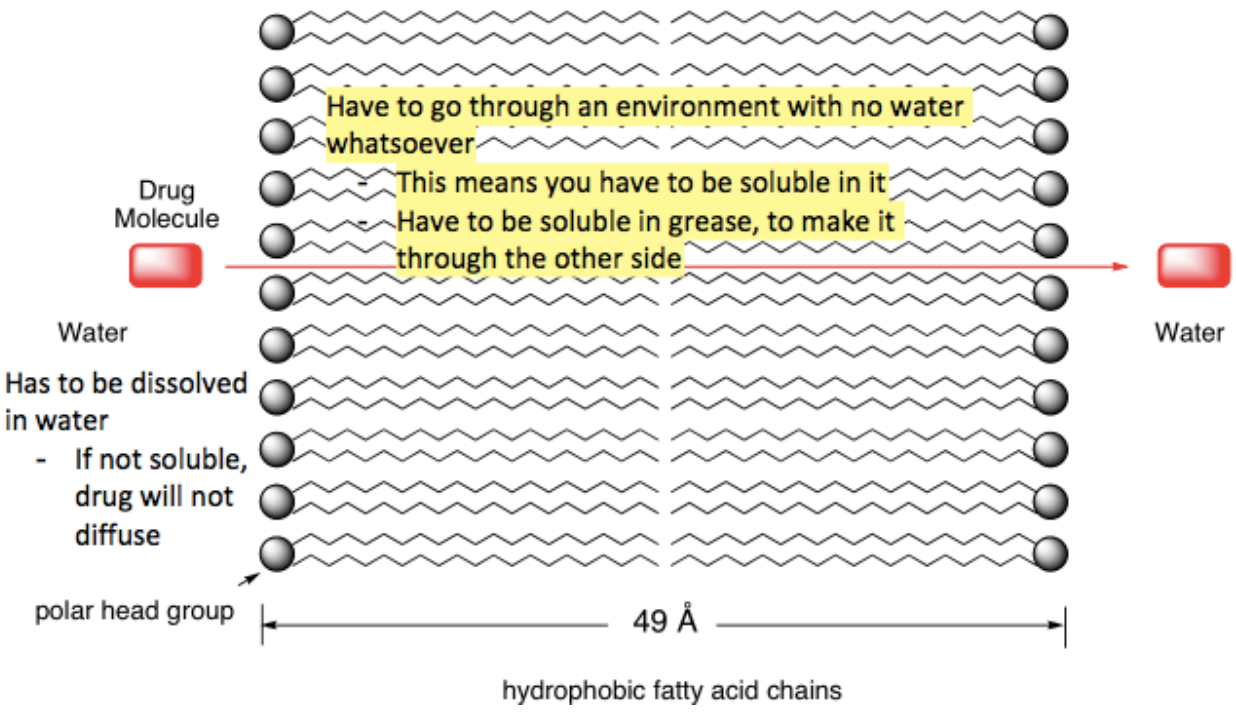
- Bile salts stick to drug, aid in digestion (solubilize lipophilic drug)

Drugs are absorbed through passive diffusion (occurs through random motion)



DIFFUSION ACROSS LIPID BILAYER:

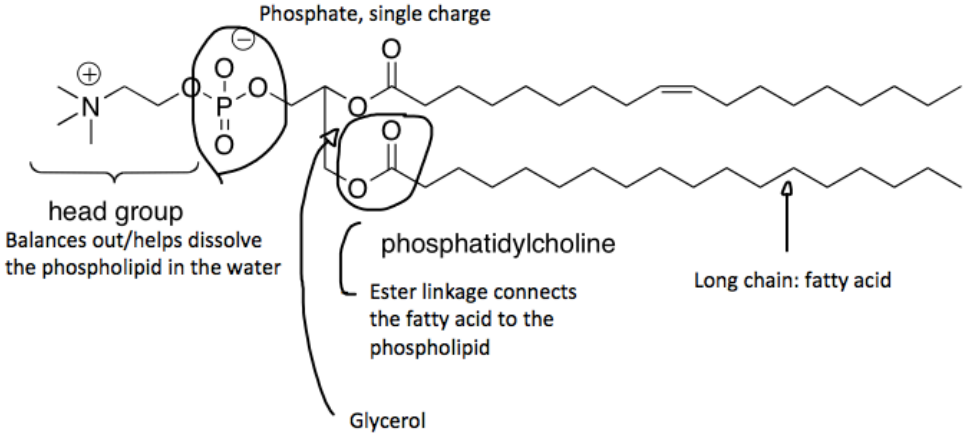
- Interior very non-polar, IM interactions (primarily VdW)



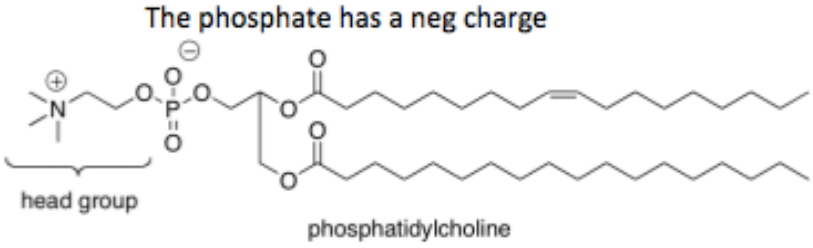
Drugs require simultaneous opposite properties: dissolves in water (polar), also dissolves in lipid environment (non-polar)

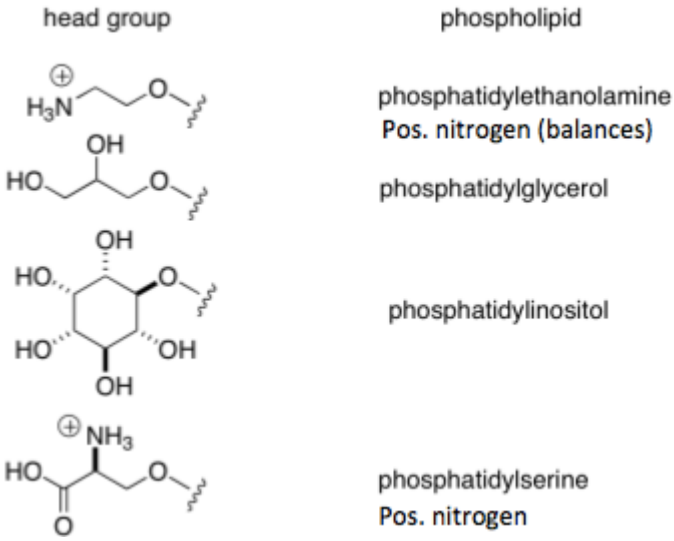
MEMBRANE PHOSPHOLIPIDS: drug must withstand in opposite chemical environments

- Water: very polar medium, lots of hydrogen bonding/dipole interactions
- Hydrophilic & lipophobic
- Hydrocarbons: non-polar medium, only VdW interactions
- Lipophilic & hydrophobic

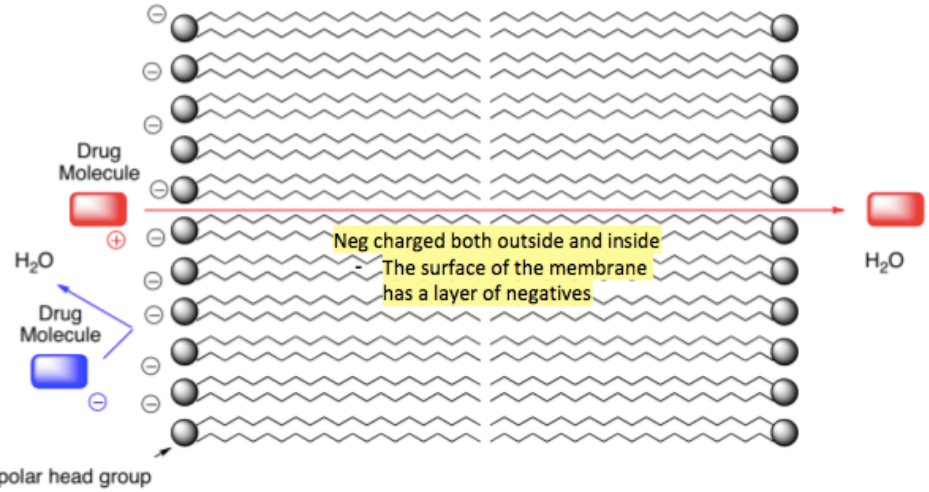


HEAD GROUPS: balances negative charge of phosphate





Negatively charged drugs have extra difficulties



MOST DRUGS IONIZABLE:
either acids or bases
Basic 75%: will act positive at physiological pH
Acidic 20%: will act negatively at physiological pH
Neutral 5%
Acid or base equilibria allows easy conversion to neutral form, to pass membranes

METABOLISM

DRUGS MUST PASS LIVER: designed to "detoxify" foreign molecules

- Liver recognizes molecular entity as "not human"; metabolizes it to remove it from blood
- Metabolism:
  - Phase I: oxidation
  - Phase II: conjugation
- Hydrophobic molecules highly metabolized; difficult to get things past liver without being chewed up
  - Most drugs hydrophobic materials
- Liver will add things on to hydrophobic material + make them hydrophilic, so that kidney can secrete

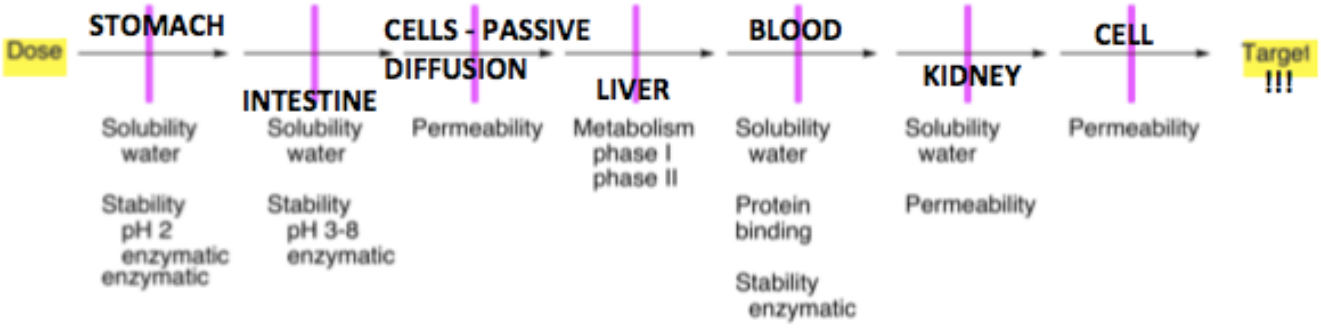
DRUG TRANSPORTED TO BODY BY BLOOD

- Blood is mostly water, here want hydrophilic material
- Lipophilic molecule binds to carrier proteins in blood (detox purpose)
- Plasma protein binding (carrier molecules) can hinder drug and make it unusable in body
  - Can also protect drug; if bound to carrier then liver will not metabolize
- Blood contains hydrolytic enzymes: protects against poisons
  - Esterases
  - Proteases

KIDNEY CLEARS SUBSTANCES FROM BLOOD

- Hydrophilic compounds are easily cleared from blood
  - If drug is too hydrophilic, lifetime in body won't be long
  - Eliminated once in kidney
- Hydrophobic tend not to be cleared

DRUG MUST ENTER TARGET ORGAN: sufficient amount of drug must passively diffuse from blood to organ (to produce response)



PROPERTIES MEASURED ASSOCIATED WITH ADME

- Absorption, distribution, metabolism, excretion (things often work opposite to each other) <— molecule must switch back & forth
- Hard to manufacture drug to pass all these targets

LIPINSKI'S RULE OF FIVE: estimation of if something can get in to body (if bio-available)

- Predicts poor absorption/permeation by looking at drug's chemical structure
- Fast, no cost, well documented and widely used
- CONDITIONS VIOLATING 2 OR MORE OF CRITERIA will likely not be bio-available
    - >5 hydrogen bond donors (OH's and NH's) <— count number of H's bonded to O or N
    - >10 hydrogen bond acceptors (N's and O's) <— only considers O and N (O and N can be bonded to something already)
    - >500 Molecular weight
    - >5 LogP

RATIONALE

- H-bonds contribute to solubility of drug; too many H-bonds will not allow drug to pass through bio-membrane
- Large molecules are less soluble, create larger cavity in solvent, do not pass easily through packed membrane lipids

- **LogP** higher, molecule is more lipophilic
    - Log of partition coefficient between octane and water for **drug in neutral form**

$$\text{LogP} = \text{Log}\left(\frac{[\text{Drug}_{\text{octanol}}]}{[\text{Drug}_{\text{water}}]}\right)$$

Solubility of compound in octanol

Solubility in water

    - High value = lipophilic; drug too lipophilic, it will not get to the membrane in the first place (wouldn't have been able to dissolve in water)
    - LogP measured at a pH where drug in neutral form
- Drugs must balance lipophilicity & hydrophilicity (Goldilocks)
- **LogD** uses same eq'n as LogP, however done at a specific pH (normally physiological, 7.4)
- In rule of 5, can replace LogP criteria w LogD:
    - $1 < \text{LogD}_{7.4} < 3$ (upper and lower limits on LogD)

MEASUREMENT METHODS OF LOGP AND LOGD

Solutions: make standard solution in water, add equal volume octanol, shake

- Separate layers, measure amount of drug in one layer

HPLC (high performance liquid chromatography): run sample through HPLC column

- Retention time used to calculate LogP

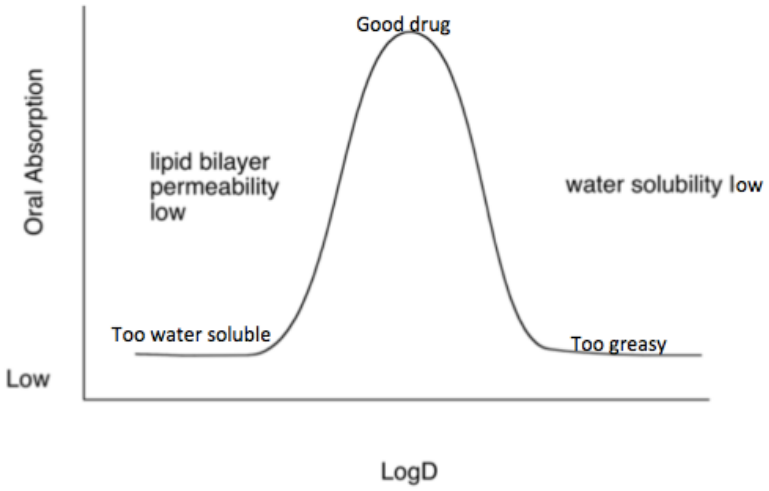
ADVANTAGE/DISADVANTAGE LOGP

- Advantage: methods well established, correlates well to Rule of Five
- Disadvantage: measurements not applicable to physiological pH
    - Each molecule requires a different buffer (must measure when molecule in neutral form)

LOGD

- Advantage: well established, easier to measure fixed pH, correlates to Rule of Five, relevant to physiological conditions
- Disadvantage: less widely used than LogP

LOOKING FOR BALANCE IN $LogD_{7.4}$

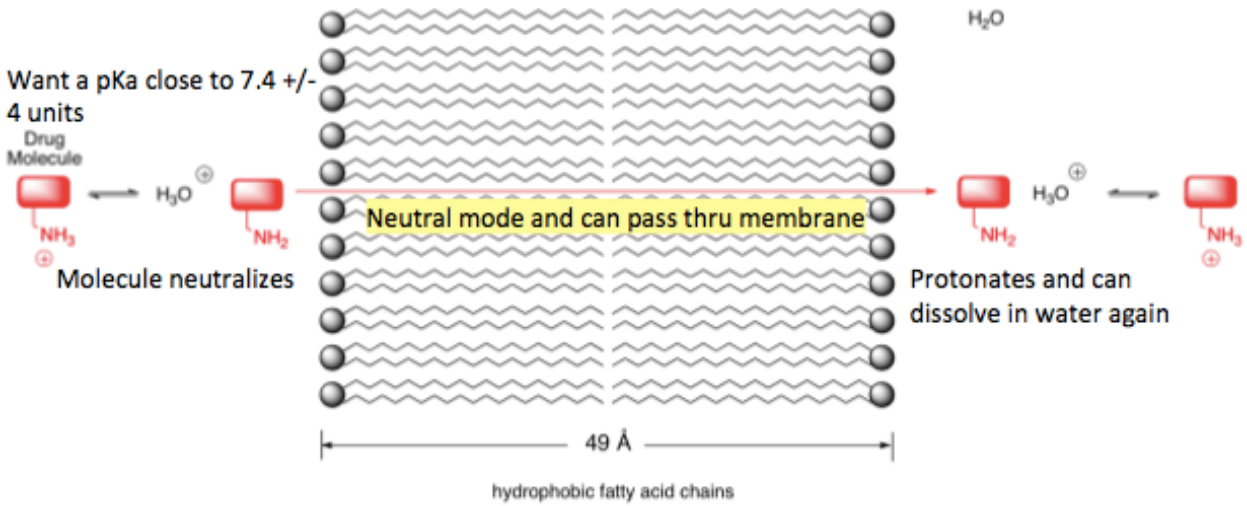


pKa CONTROLS WATER SOLUBILITY

- Recall most drugs contain ionizable groups (acids/bases) <— ionization increases water solubility
- 75% basic, 20% acidic, 5% non-ionizable

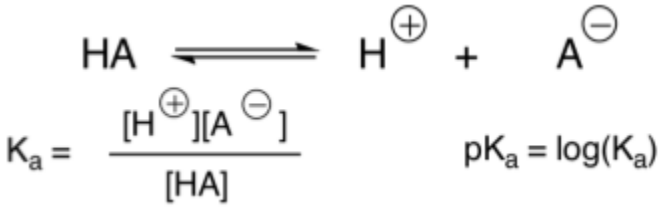
IONIZABLE DRUGS HAVE HIGHER CHANGE OF GETTING IN TO BODY

- Want drug to be charged to be soluble & dissolve in body
- Also want it to convert to neutral form to pass membranes

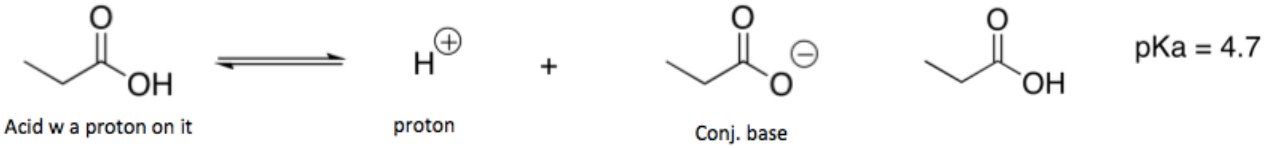


pKa DESCRIBES BOTH ACIDITY AND BASICITY

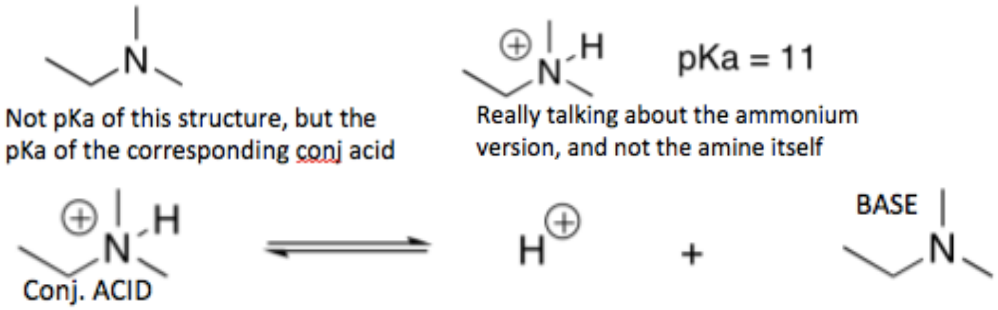
- Equilibrium constant related to removal of H+; pKa = strength of acid



pKa OF ACID: refers to acid form (left side of eq'n)



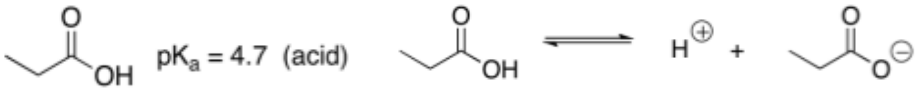
pKa OF BASE: value refers to corresponding conjugate acid



Put base structure on the right hand side of eq'n, the conj. acid on the left hand side is the pKa of interest

MOLECULE GETS PROTONATED WHEN pH < pKa
ACID:

- Neutral when pH < pKa, charged when pH > pKa

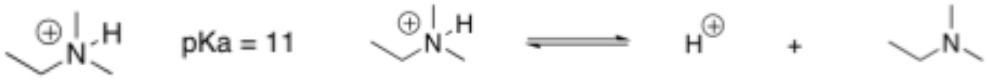


Acid: its neutral form is the "protonated" form, you remove an H+ atom when the pH > pKa (becomes negatively charged)

- Acids behave as weak bases at physiological pH of (7.4)
- Example above: phys. pH > 4.7, so the acid would lose a proton

BASES:

- Positively charged when pH < pKa, neutral when pH > pKa

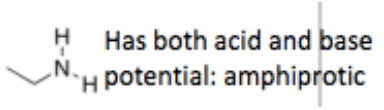


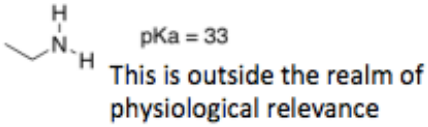
Bases (in physiological pH) behave as their conjugate acid: pH < pKa, and base gains an H+

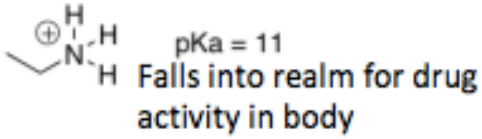
- Example above: base would not be in its neutral form: it protonates to conj. acid on the left side

SOME GROUPS AMPHIPROTIC: (can act as acid or base)

- In calculation, check both pKa values and choose the one falling in realm of physiological conditions

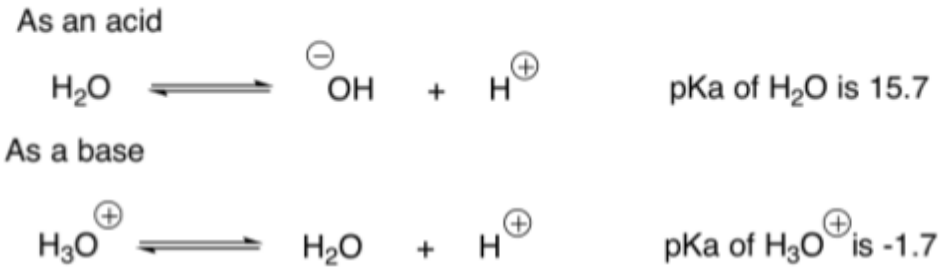






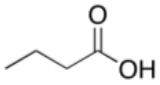
ACCEPTABLE RANGE FOR pKa IS AQUEOUS RANGE FOR WATER

- pKa of -1.7 - 15.7
- Amphiprotic water acts as base or acid
- Do not worry about pKa values outside this range; they are considered "non-contributing"/"insignificant"

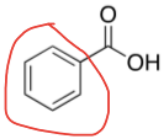


USING pKa TABLE: must consider conjugation and induction

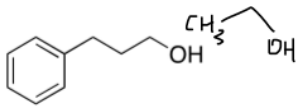
- Double bond beside group gives possibility of resonance, can affect pKa majorly



- Aromaticity has big impact on pKa —> look on table for closest thing under carboxylic acid category

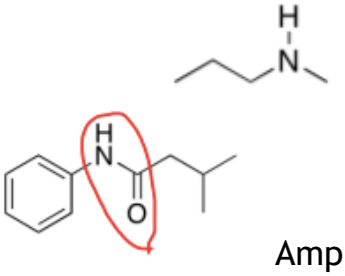


- If aromatic is too far away, it won't impact pKa of group

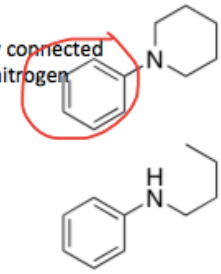


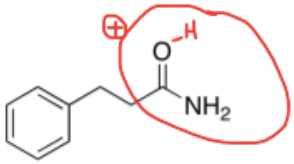
OTHER EXAMPLES

Alkyl group connected to nitrogen
- Look up closest thing in table



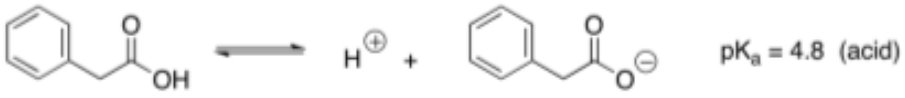
Aryl (phenol)
- Directly connected to the nitrogen

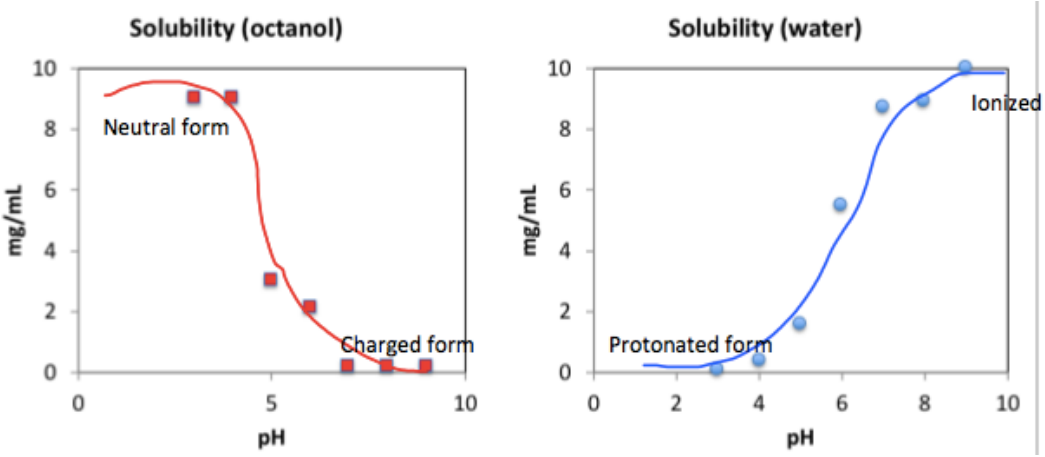




Amphiprotic functional group; nitrogen and oxygen connected together, pick oxygen (the thing connected to double bond)

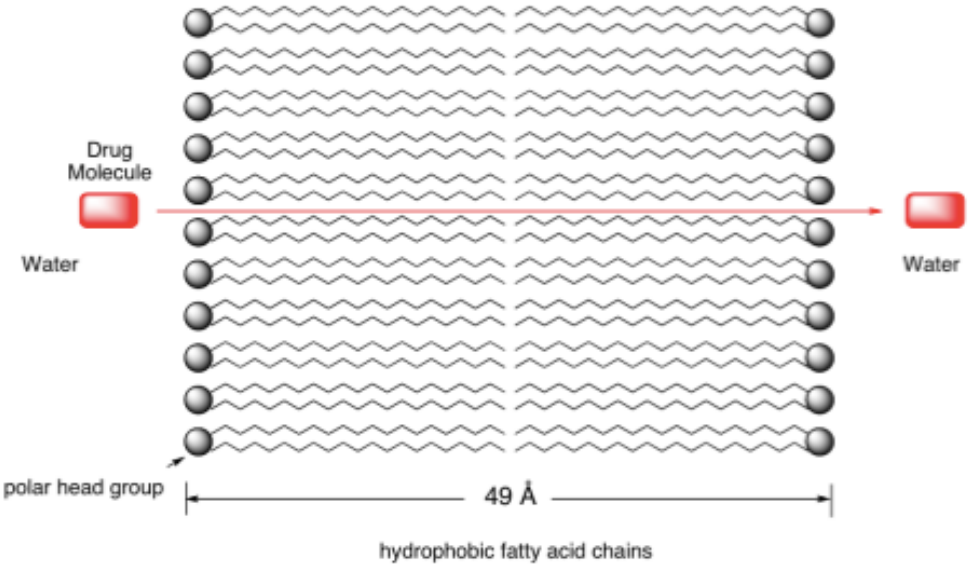
pH HAS OPPOSITE EFFECTS ON WATER & LIPID SOLUBILITY





DRUG PERMEABILITY: rate drug passes through lipid membranes

- Includes solubility, pKa LogP, molecular weight
- Measures amount of time it takes to get from one side to other:



PERMEABILITY METHODS

Caco-2: uses human colon carcinoma cells (similar to intestinal membrane)

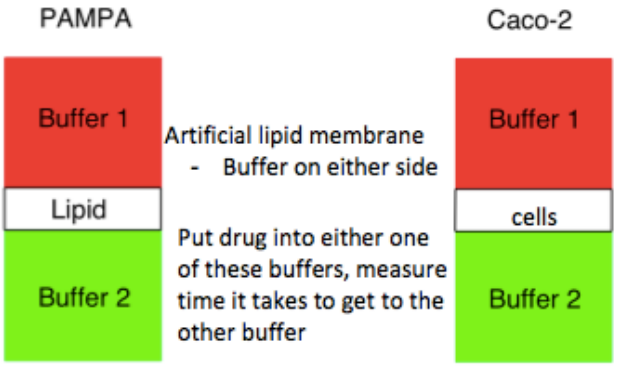
- Results more relevant to clinic, however assay difficult to do

PAMPA: artificial membrane (less similar to real human)

- Assay easier to do

Method:

- Place sample in buffer 1, measure rate at which it transports to buffer 2



BENCHMARK VALUES OF COMMERCIAL DRUGS
(Biopharmaceuticals Classification System)

	High Solubility	Low Solubility
High Permeability	Class 1	Class 2
Low Permeability	Class 3	Class 4

Class 1 compound: ideal for oral dosing
- Soluble and permeable
- FDA gives you a waiver for Bio-equivalence/bioavailability studies

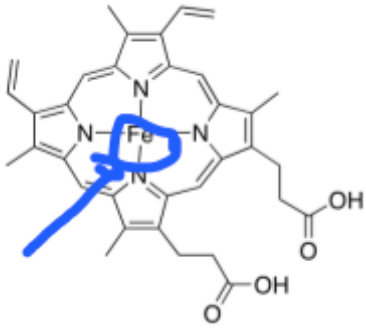
Class 2 compound: permeable, not very soluble
- Tend to be lipophilic, extra ingredients (formulations) need to be added for water solubility

Class 3 compound: soluble, not very permeable
- Tends to be hydrophilic
- Prodrug (inactive form that body converts to active) used to improve permeability

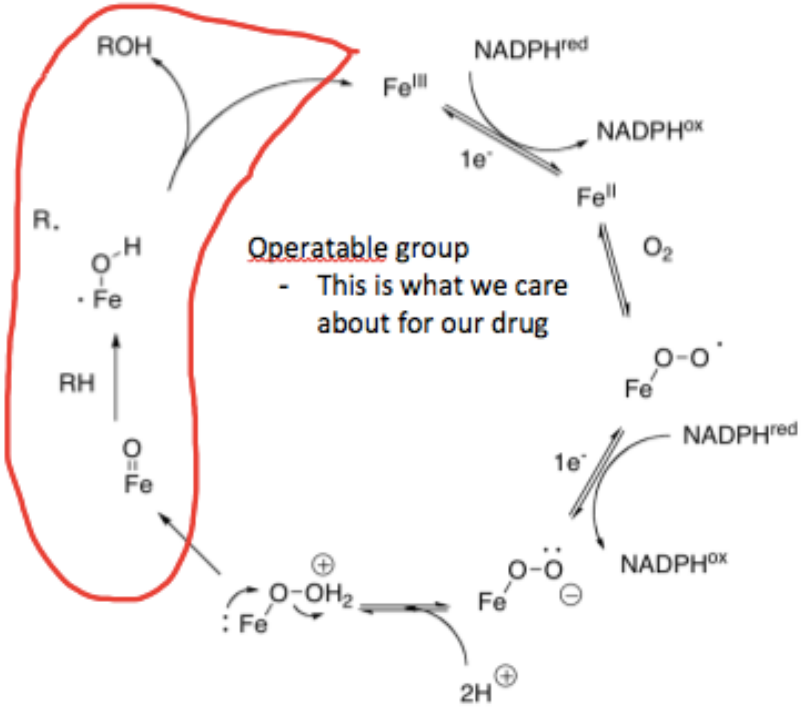
Class 4: low solubility and permeability
- Expensive and risky development (high fail rate)

METABOLIC STABILITY: Liver turning lipophilic material into hydrophilic

Phase I: adds polar functional groups onto molecule to reduce lipophilicity
- Oxidation of aliphatic/aromatic groups from electron rich sites
- Carried out by CYP_{450} enzyme in liver (heme iron)
- Controlled rust reaction occurring in enzyme active site
- Takes "grease" of drug, sticks OH groups on it



CATALYTIC CYCLE OF THE ENZYME

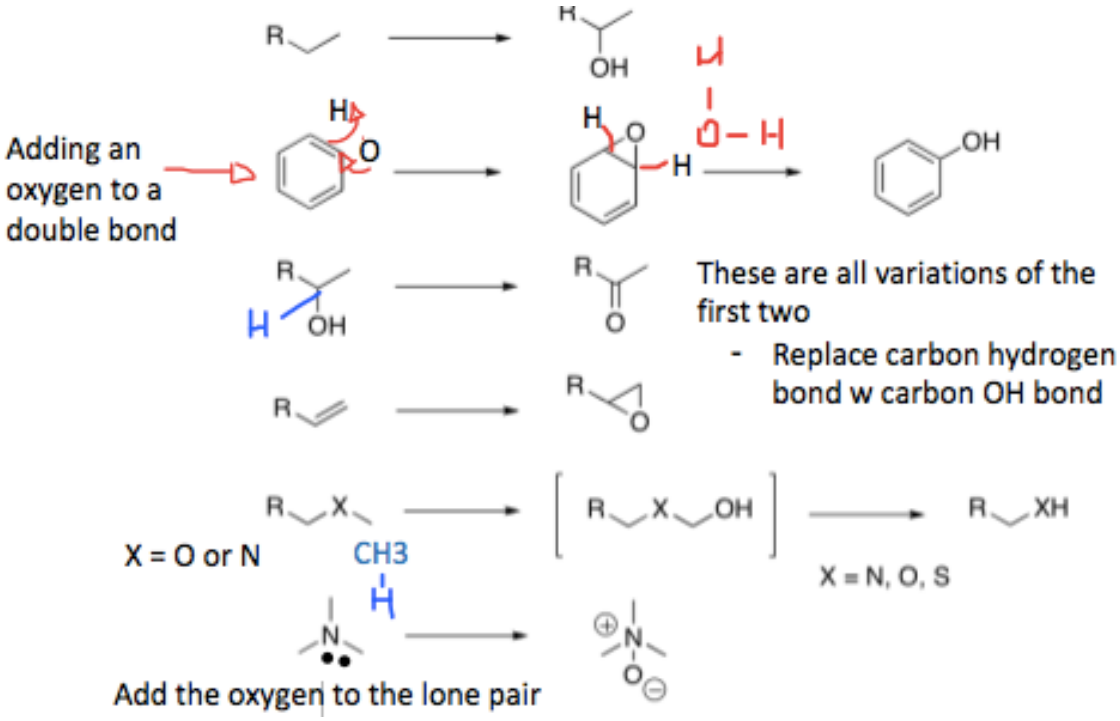


Iron is converted to its various oxidation states

Transfer of oxygen while removing electrons (classic redox)

OXIDATION FROM SUBSTRATE POINT OF VIEW: loss of electrons at electron rich sites (part of molecule with most electrons)

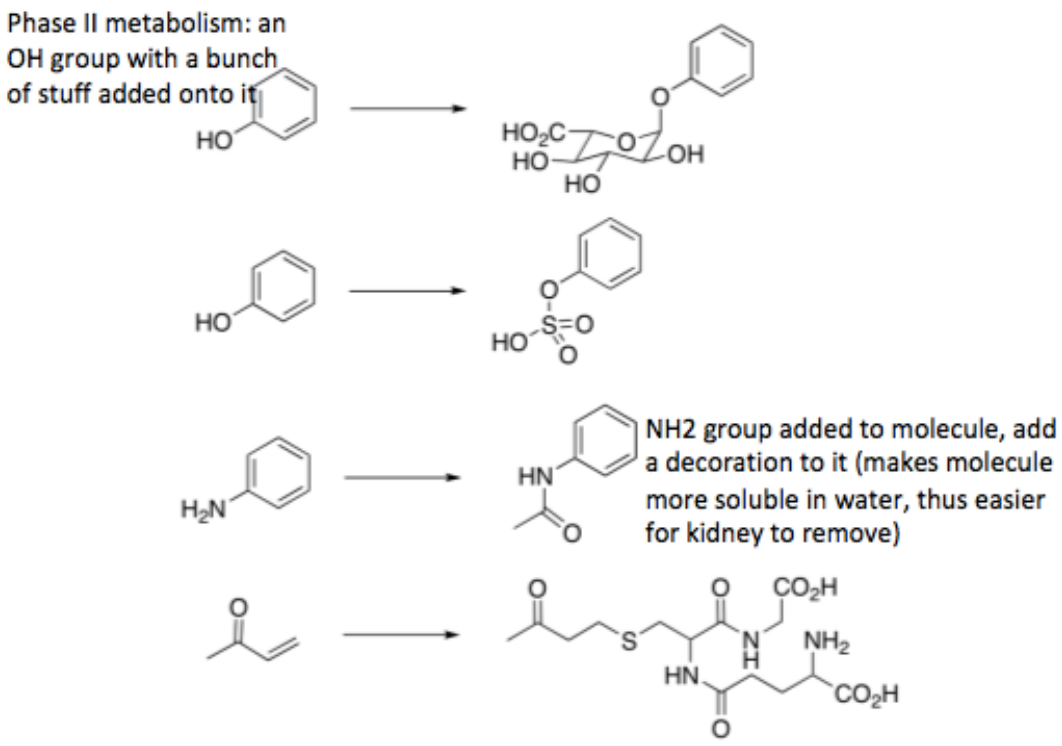
- When designing a drug to limit metabolism, really focus on limiting oxidation
- Looks for location of molecule with lots of electrons (lone pairs, alkyl groups, etc.)
- Chart: a series of oxidation reactions (replacing H+ with OH)



Phase II: conjugation to improve water solubility (adds polar groups)

- Things added to chemical structure to make easier for kidney to dispose
  - Glucuronic acid
  - Sulfonation
  - Acetylation
  - Glutathione
- These groups solubilize molecules

COMMON PHASE II REACTIONS



COMMON MEASUREMENTS METABOLISM (amount of time for liver to chew molecule up)

- Liver microsomes: half life measured ($T_{1/2}$)
- Plasma stability: also ($T_{1/2}$)
    - Metabolic enzymes in blood plasma do same thing as liver

PLASMA PROTEIN BINDING: blood contains proteins binding to drugs

- Drugs bound to plasma protein allow drug to be soluble in water
- When drug is stuck to plasma protein, it is unable to diffuse into cells
    - Reduces effective concentration of drug in blood
    - Reduces metabolism/clearance from blood
- Lipophilic drugs most likely to bind

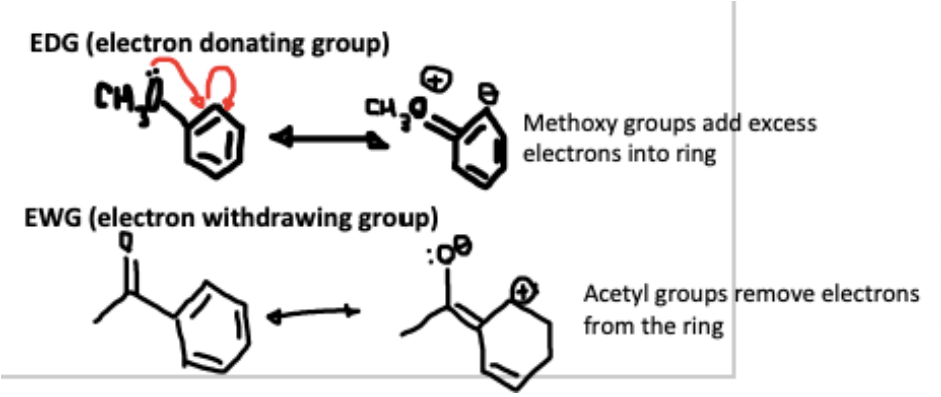
MEASURE OF PROTEIN BINDING <— % of drug bound

- Fu = fraction of unbound drug
- Fu = [unbound drug]/[total drug]
- A percentage of drug bound, telling you the free drug concentration

SAR/SPR - Structure property relationship

- Every time new molecule is designed, different properties are tested & used as a guide for design/optimization

- Changes in structure correlated to change in property; next molecule is designed using this guide (done multiple times, looking at how structure improves properties)
- SPR: effect of structure on molecular properties:
    - Potency
    - Solubility
    - Permeability
    - pKa
    - Melt/boil point
    - Bioavailability
    - Metabolic/chemical stability



PROCESS OF DRUG OPTIMIZATION:

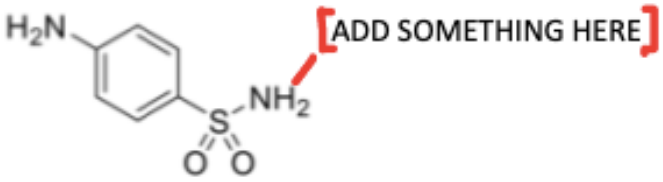
1. Lead structure (drug candidate)
2. Make related compounds, small changes to the structure
    - Change only 1 factor at a time, to be able to see what the change does
3. Measure potency/properties of compound
    - All properties of compound are measured, many parameters are optimized at same time
4. Patterns in data used to identify site on molecule:
    1. to change/not change
    2. modifications that are good/bad

TECHNIQUES OF MOLECULAR MODIFICATION

- Additions/deletions
- Substitutions
- Chain extension/contraction
- Ring expansion/contraction
- Ring variations
- Ring fusions
- Simplification
- Rigidification: having a rigid structure prevents shape changes in drug

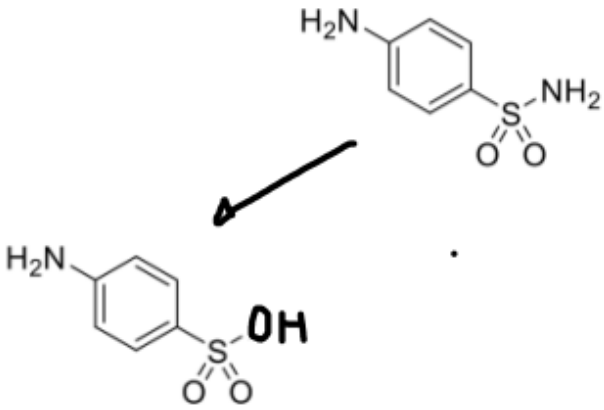
ADDITIONS/DELETIONS: adding/removing a group

- Gives steric information: if you are able to add something, it tells you if the pocket is open, or if there is something there



- i.e. Adding an R group onto a region, tells you how large pocket is, what the max range of the pocket is
- This information can be useful for future optimization

SUBSTITUTIONS: replacing one group w another (isostere)



- Replace the NH2 group with OH group; these molecules have different properties

-If H-bonding occured with the NH2 group but not with the OH group, it gives you functional information

Addition of isostere provides information on/gets rid of activity

Also tells you if area the molecule is on is lipophilic/hydrophilic

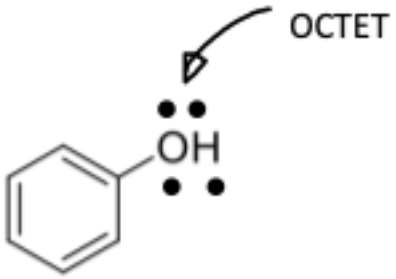
- Tells you about nature of interactions (i.e. by replacing a donor w an acceptor

ISOSTERES: functional group replacements

- Groups of atoms with similar sterics/electronics
  - Gives similar biological activity, produces similar shapes/sizes
- Classical: isostere has same valency
- Bio-isostere: has same/similar chemical properties

CLASSICAL ISOSTERE: same valency & similar size

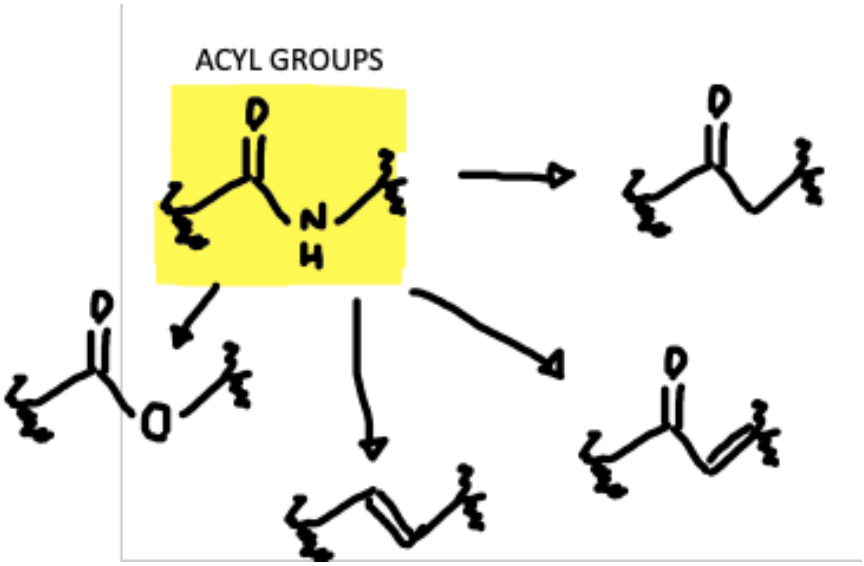
- Helps determine if group is important to binding or not (alters properties in controlled way)



-i.e. if replacing a group causes activity/no more activity, it gives information on the group's purpose

- ISOSTERES OF OH: different heteroatom like F, Cl, SH, NH2 (or CH3, because similar size)
- Choosing atoms in the same row (same # electrons) or column (same chemical properties & behaviour)

ACYL GROUPS



EXAMPLE OF N-C ISOSTERE

-Amide bonds act like double bonds

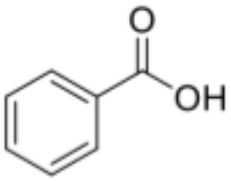
-Amide groups can be replaced w a trans double bond

-If a double bond works as a replacement, it tells you only the pi nature of the bond matters for function

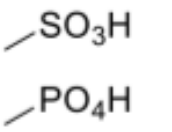
NON-CLASSICAL ISOSTERE (BIO-ISOSTERE)

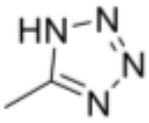
- Atoms/groups showing similar chemical properties (differ in overall electronics and sterics)
- i.e. you can replace COOH (carboxylic acid) with acids of different 3D shape
    - Acids have same properties (negatively charged at biological pH)
    - Could observe different abilities of getting into body

isosteres of CO_2H



Series of replacements w relatively low pKA
- Can replace carboxylic acids

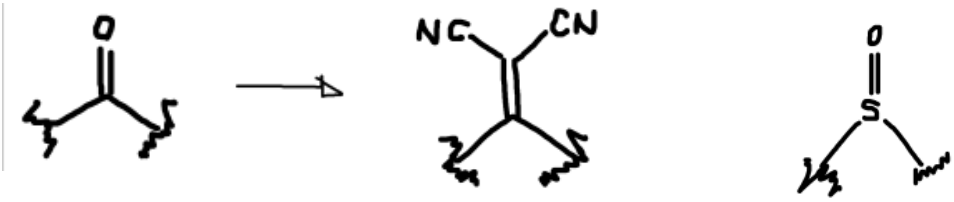




Acids

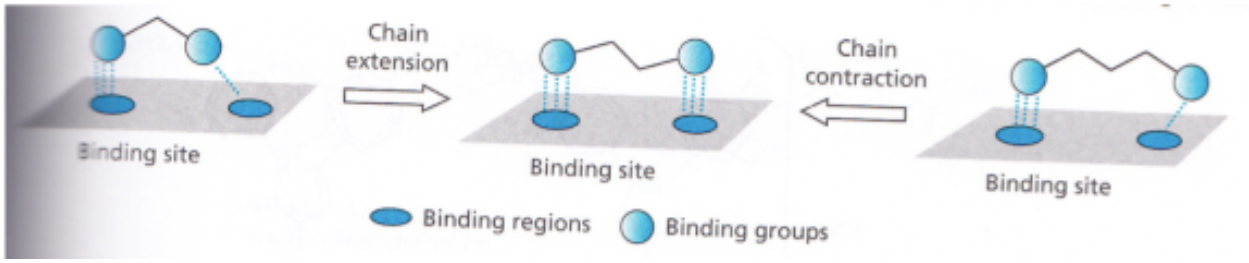
<-- this is a tetrazole, heterocycle with 4 Nitrogens, works the same from acid base point of view as carboxylic acid, has acidity comparable to acetic acid
- This heterocycle (due to the ring), is good as acting as a base
- pKa is similar to carboxylic acid, and can replace it

EXAMPLES OF NON-CLASSICAL ISOSTERES



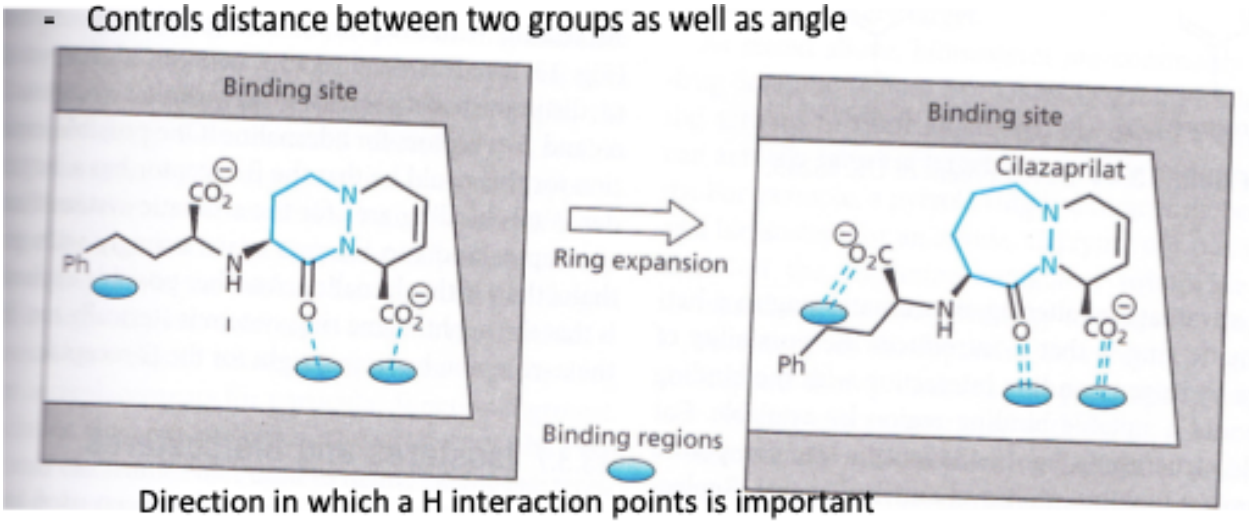
To an enzyme, the =O portion of molecule and the cyanide look like the same compound (though on paper they look very different)
- Non-classical isosteres do not fit classical definition
- Still work as a functional replacement to a group on a molecule

CHAIN EXTENSION/CONTRACTION
- Addition or removal of an atom from a chain may make a big difference
- i.e. Reaching the binding site of a group; better alignment (stronger, optimal bond)
- Change is made, looking for effects on activity



RING EXPANSION/CONTRACTION: systematic ring enlargement/contraction
- Making a ring bigger may angle groups better to regions, for optimal binding
- Heteroatoms are used for size and synthetic simplicity

- Controls distance between two groups as well as angle



Direction in which a H interaction points is important

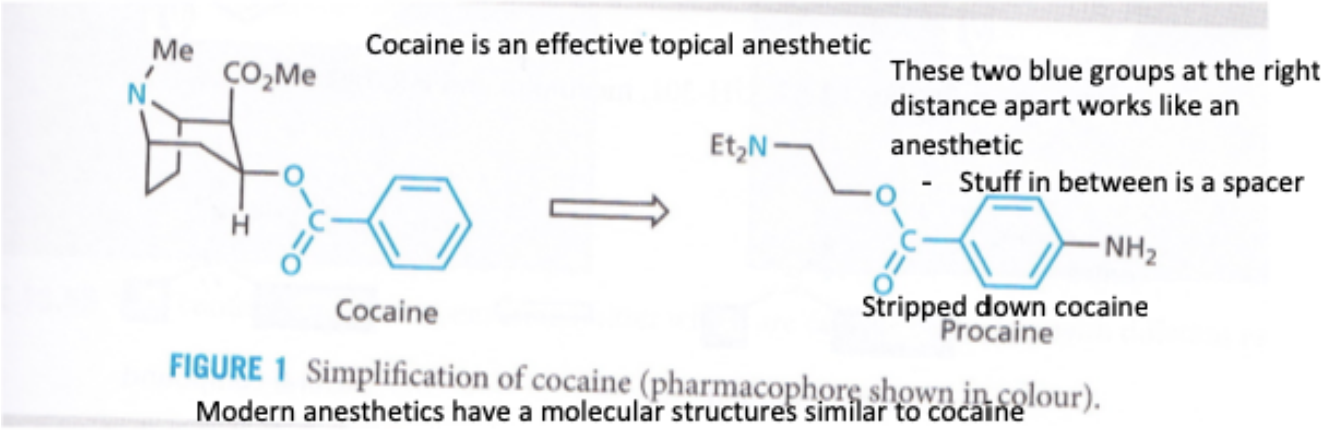


FIGURE 1 Simplification of cocaine (pharmacophore shown in colour).

Modern anesthetics have a molecular structures similar to cocaine

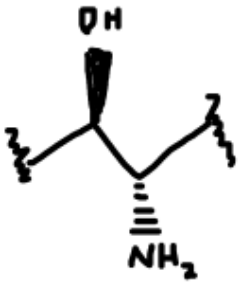
^**STRUCTURE SIMPLIFICATION**: remove parts that do not affect potency

- Procaine is an anaesthetic engineered from cocaine
  - Parts shown in blue are important for deadening nerve pain
  - The two groups at a specific distance apart are the key to making it work
  - Stripping away the other parts of the molecule (removing the narcotic effects), while still getting numbing

REMOVING STEREOCENTRES

- Biological molecules are chiral: S and E enantiomers seen as different molecules/drugs
- Absolute configuration has huge effects
- Even when reacting the same way, body sees different enantiomers as completely separate (1 may be seen as foreign)
- Encourages production of single enantiomer when possible
- Stereocentres increase manufacturing complexity:
  - Difficult to make single enantiomer in pure form
  - When a molecule w stereocentres are made, both configurations must be tested separately
  - Less work involved with fewer stereocentres

- Racimates are easier to make/test: considers enantiomers as separate compounds
- Racimate: mixture with equal amounts of either isomer
    - both configurations can be easily separated



This molecule (w a stereo centre) has 4 isomers
-the body sees each isomer as a completely different molecule
-Each isomer must be tested for toxicity

PHARMACOPHORE: groups important for drug activity

- Pharmacophores are groups on your drug that actually touch the receptor (molecule you're interacting with)
    - 3D summary of the things you want to touch in the body
    - If your hand represented the drug, and you touch piano keys, the tips of your fingers are the pharmacophore
    - They are all the different important parts on your drug, that touch the enzyme
    - The way pharmacophores are connected doesn't matter, however you just need to know how they are spatially arranged

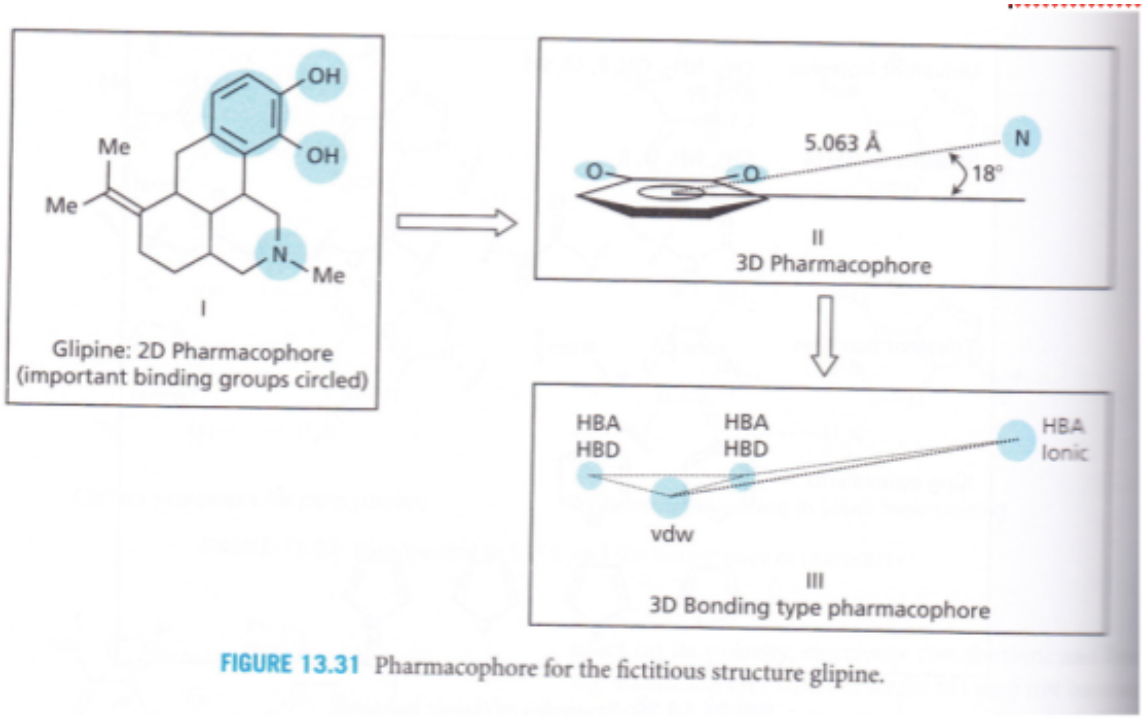


FIGURE 13.31 Pharmacophore for the fictitious structure glipine.