

A Look at Combinatorial Chemistry and Physiochemical Concepts in Drug Design

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WRAIR

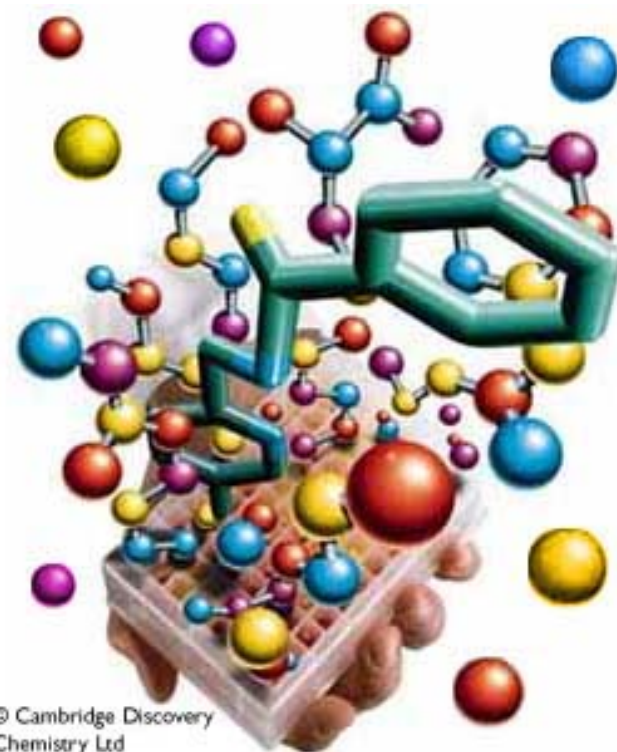
Monday, Oct 22, 2007

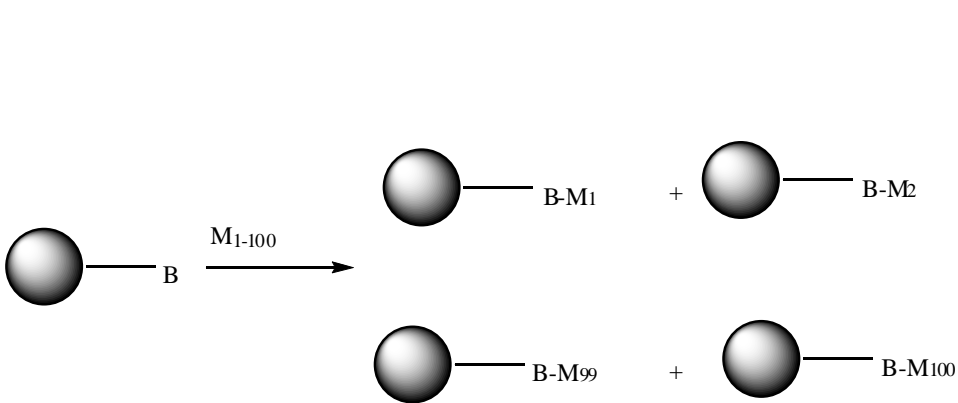
Outline

- Part 1: Combinatorial chemistry
 - What is it
 - Techniques
 - Synthetic combinatorial libraries
 - Diversity oriented synthesis and the future of combi. chem.
- Part 2: Physiochemical concepts in drug design
 - What are physiochemical properties
 - Which ones make a good drug
 - How can we change them
 - Mefloquine: a case study

What is combinatorial chemistry?

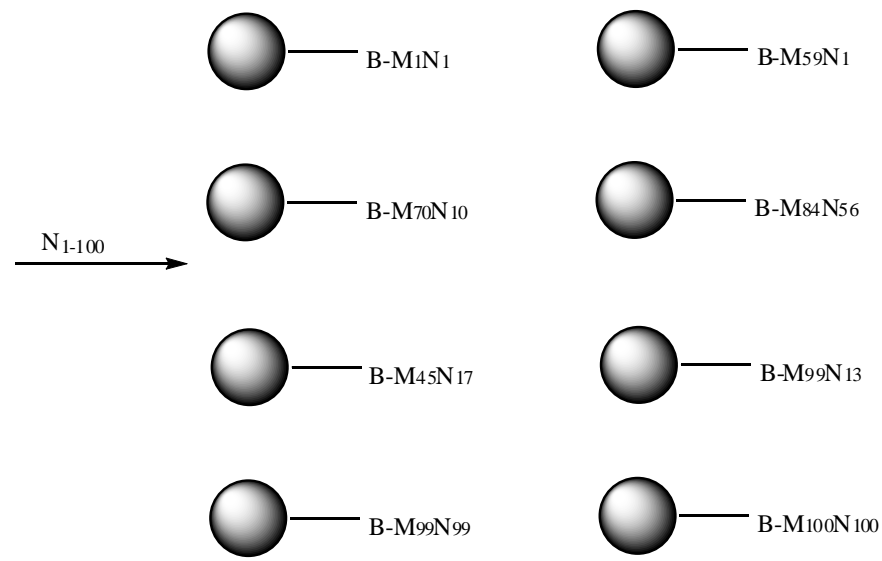
- Chemical technique of reacting large numbers of compounds to a central scaffold, repetitive process
- Caused by a push in the 80's & 90's to save money by screening more compounds, led to the generation of high through-put techniques including HTS
- Uses advanced robotics to automate chemistry techniques to remove the human element
- “the power of combinatorial chemistry lies in its ability to accelerate the drug discovery process through the rapid synthesis and subsequent screening of a larger number of compounds”





Equal probability of having any of the M molecules react with B

theoretical number of different molecules
100



Equal probability of having any of the 100 different B-M molecules reacting with any of the N molecules

theoretical number of different molecules
100x100= 10,000

Techniques

- Two approaches to Combinatorial Chemistry library generation
 - Phage library screening
 - Uses recombinant DNA to generate large numbers of peptides that are randomly expressed in phages or other vectors.
 - Restricted to natural amino acids
 - Have to be proficient in phage biochemistry
 - Synthetic Combinatorial Libraries (SCL)
 - Requires investment into robotics
 - Facilitates any building block of interest to be used
 - D amino acids, non natural amino acids, and carboxylic acids
 - More commonly used

Synthetic Combinatorial Libraries

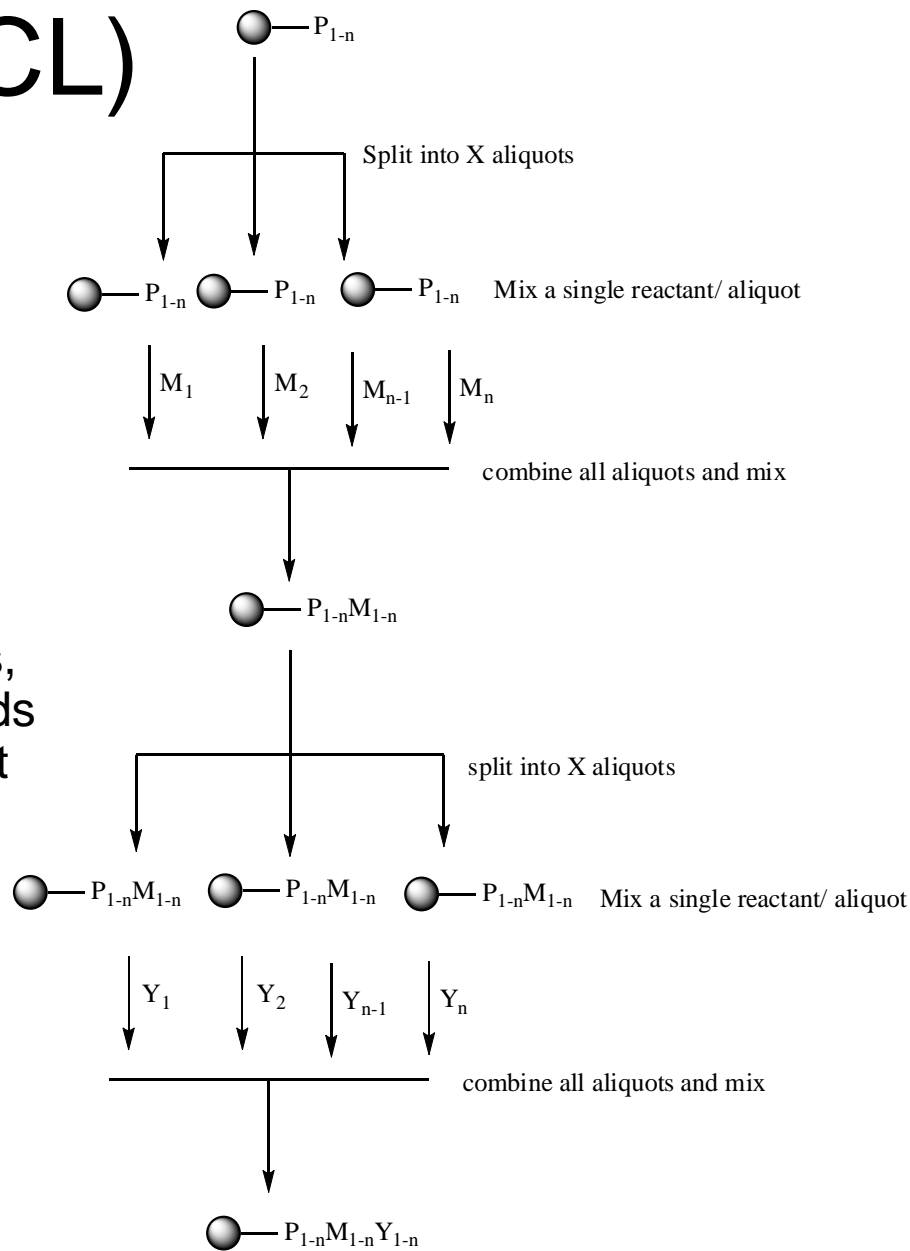
(SCL)

- Two types

- Resin mixtures

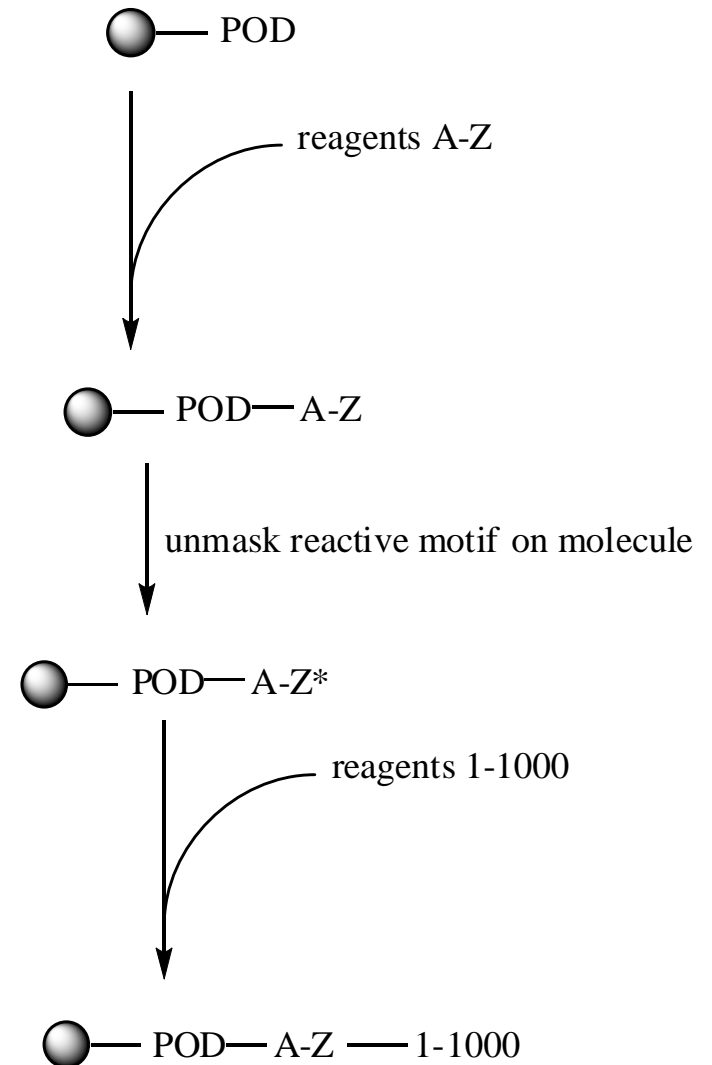
- Divide, couple, recombine (DCR) method

- AKA split resin method
- involves splitting beads, coupling reactants to beads, combining and mixing beads, followed by splitting the beads again and then coupling next reactant, and so on.
- Due to the nature of the process a bead will contain only one compound



SCL continued

- Reagent mixtures
 - Uses a predefined ratio of reagents in excess to accomplish equimolar incorporation of each reagent at a “position of diversity”
 - Needs large excess of reagent to accomplish pseudo first order kinetics
 - Reactivity rates of reagents need to be same
 - More commonly used because of easier automation



Deconvolution methods

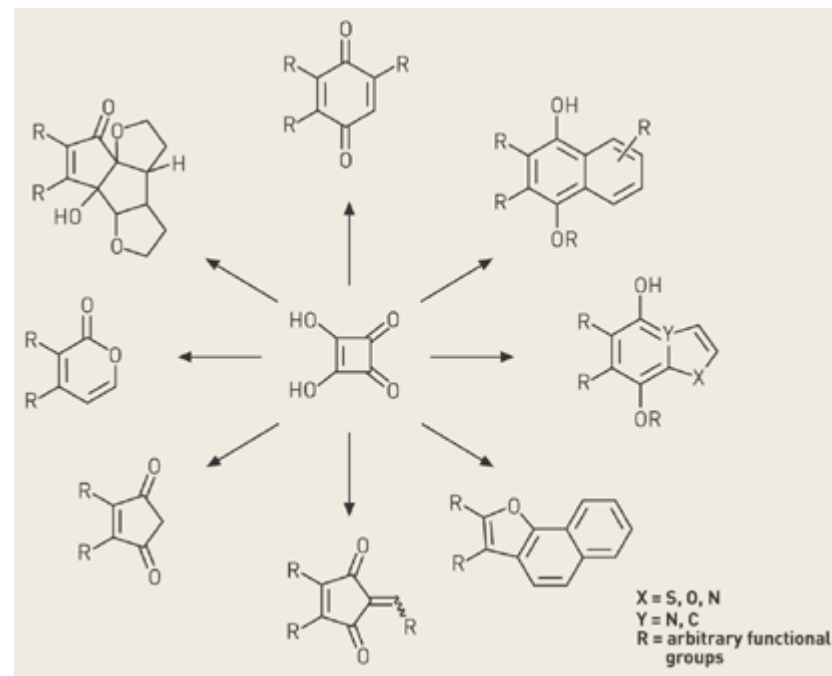
- Deconvolution is a method of identifying individual active compounds from the complex combinatorial mixture
- Three types
 - Iterative deconvolution
 - Positional scanning deconvolution
 - Tagging

Early combinatorial synthesis problems

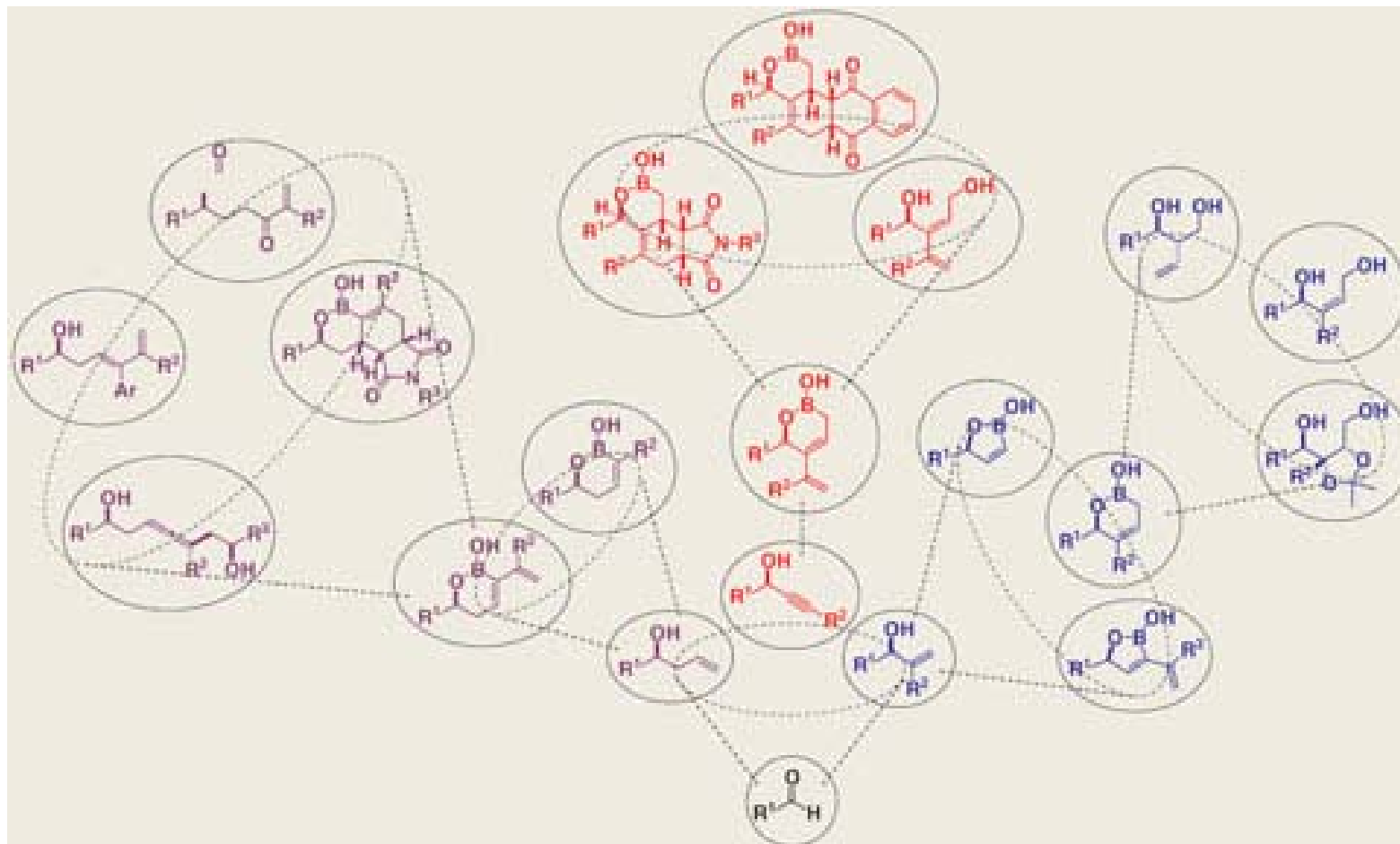
- Size limitation
 - Most combinatorial libraries are made up of smaller molecules- typically ranging in the trimer to pentamer range
- Undirected
 - No rationale for hit discovery
- Played a numbers game
 - Thought that if they made enough compounds that they would generate active leads
 - All peptide based molecules generated
 - Didn't realize that peptides generally do not make good drugs
 - Limited solubility, bioavailability, and quick degradation in vivo

Diversity Oriented Synthesis and the Future of Combi. Chem.

- Now combi. chem. is becoming more focused but still has its limitations
 - Not applicable to certain types of chemistry but more chemistry techniques are being discovered for DOS
 - More solution phased used
 - Incorporating more molecule types than just peptides
 - Using more rational in choosing targets for libraries
 - Diversity oriented synthesis by several academic labs is the future of CC.



DOS



"The small-molecule approach to biology: Chemical genetics and diversity-oriented organic synthesis make possible the systematic exploration of biology" Stuart L. Schreiber, *Chem. & Eng. News*, **2003**, *81*, 51-61

In Summary

- Combinatorial chemistry
 - Two types
 - Phage based
 - Synthetic based
 - Resin and reagent mixing types
 - Three types of deconvolution methods
 - Iterative, position scanning and tagging
 - Is a powerful technique when well thought out but can be less fruitful when misdirected
 - Led to the generation of high through put methods such as HTS
 - Newer methods (DOS) are becoming more widely accepted

Part 2: Physiochemical concepts

- Hypothetical question
 - You have a cold and you want to take medication for it, what properties are you looking for in a cold medication?
 - IV, IM, suppository, oral 1/hour, oral 1/day
 - Why?
 - What causes undesired properties/effects?

Part 2 Physiochemical Concepts in Drug Design

Physiochemical properties

- What are they?
 - They are physical or biological properties that a compound will display based on structural motifs or configurations of the compound
- Most common include
 - Solubility/Dissolution, oral absorption, uptake into the brain, plasma protein binding, distribution and metabolism
- Often linked to ADMET (absorption, distribution, metabolism, elimination and toxicity) profile

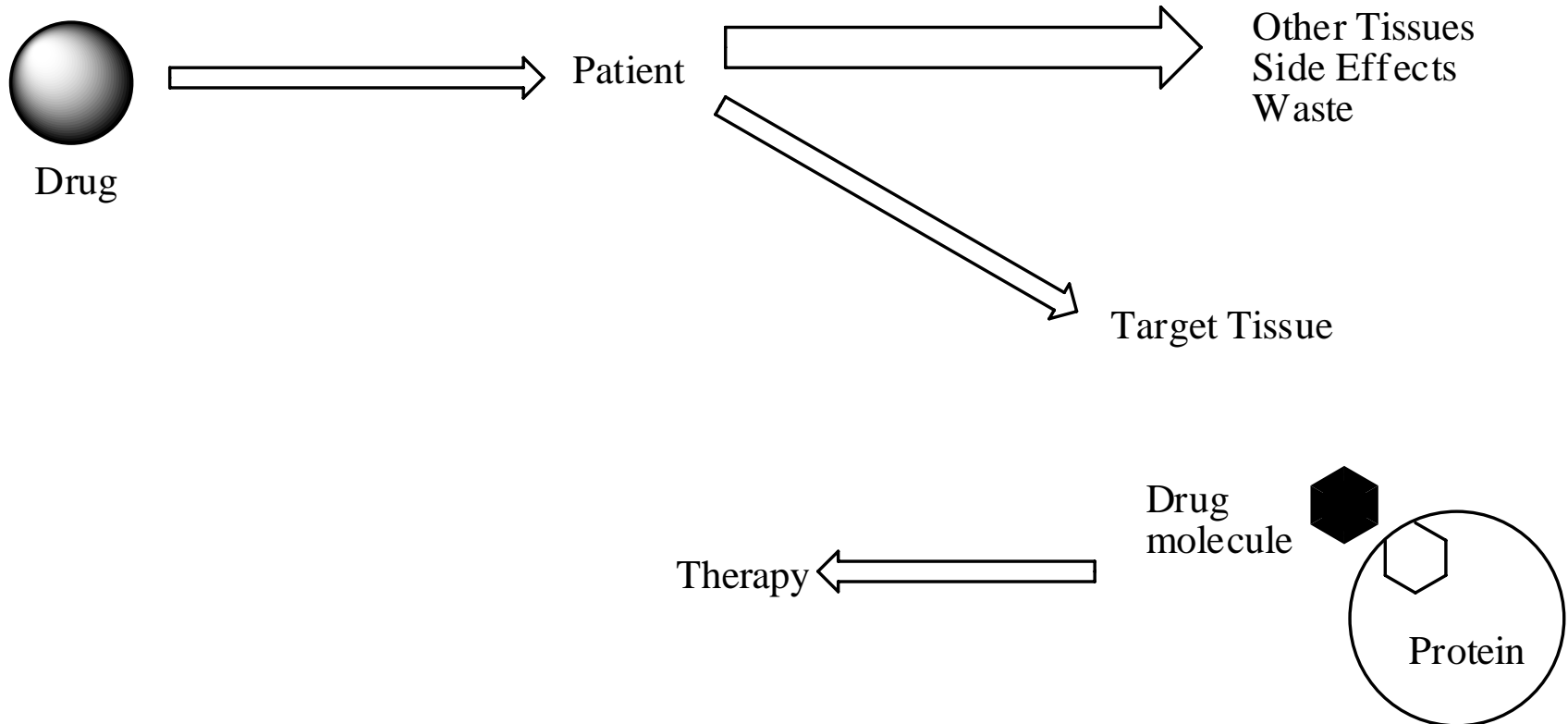
Physical Properties Vs Physiochemical Properties

- What is the difference between physical properties of a compounds and physiochemical properties?
- Physical property is any property that can be observed without changing the composition of the entity
- Physiochemical properties are any physical property of a molecule that when changed could potentially affect biological activity

Why are physiochemical properties important?

- Because poor properties lead to drug development failure
- Problems with Pk, bioavailability, and safety
 - Poor absorption, high first rate metabolism and toxicity
- Poor properties also lead to development issues
 - Expensive drug formulations
 - Increased development times
 - Patient problems because of higher dose or more frequent doses
- Because drug development is an expensive endeavor (0.6 to 1 B U.S. dollars spent on developing a new drug)

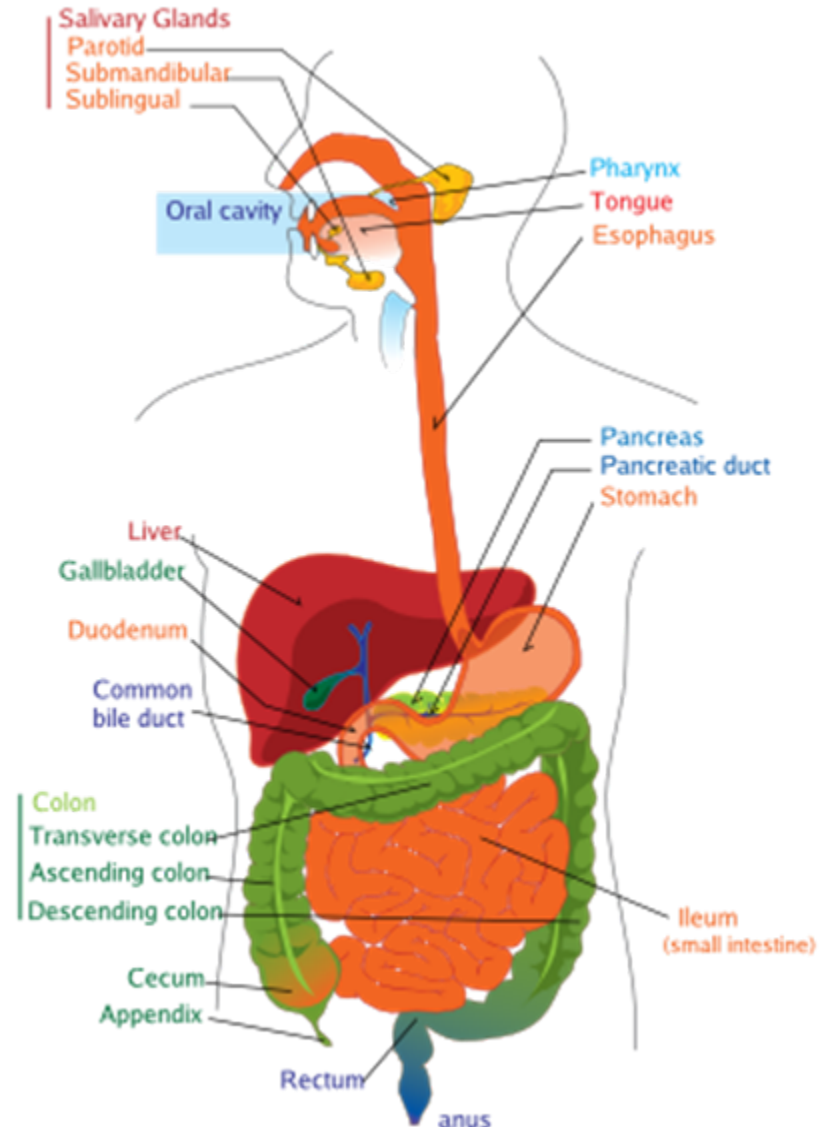
Drug delivery in vivo



properties determine how much drug reaches the target

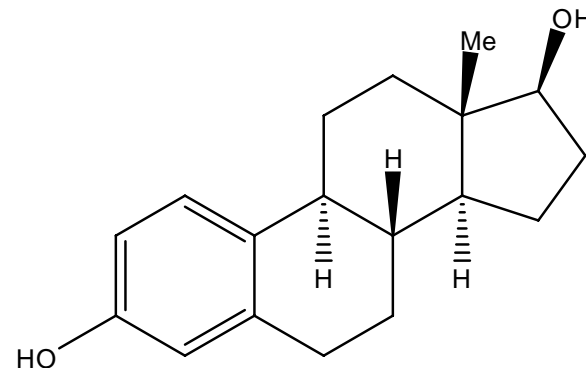
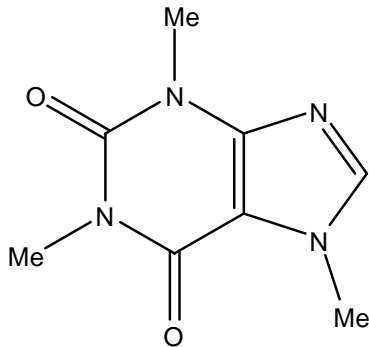
What physiochemical properties do we have to consider for an orally active drug?

- Solubility
- Stability to acidic conditions of the stomach
- Absorption from the gut
- Not a good substrate for PGP's
- Resistance to first pass metabolism
- Bioavailability



Oral drug considerations

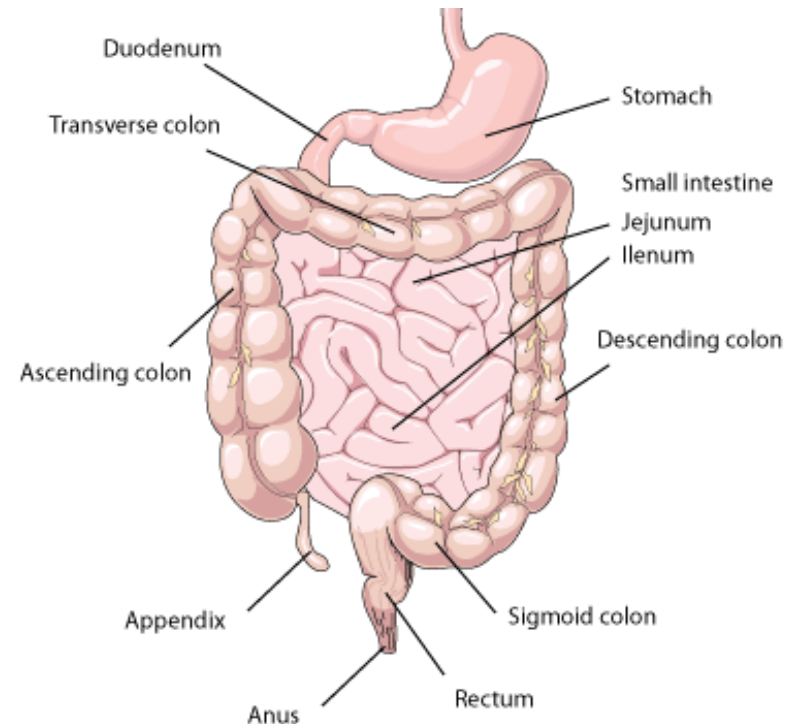
- Solubility- what do we mean by solubility?
 - Solubility in aqueous or organic solutions?
 - What structural motifs are likely to cause aq solubility? What about org solubility?
 - Is a polar molecule more likely to be aq or org soluble?



Acidity in the gut

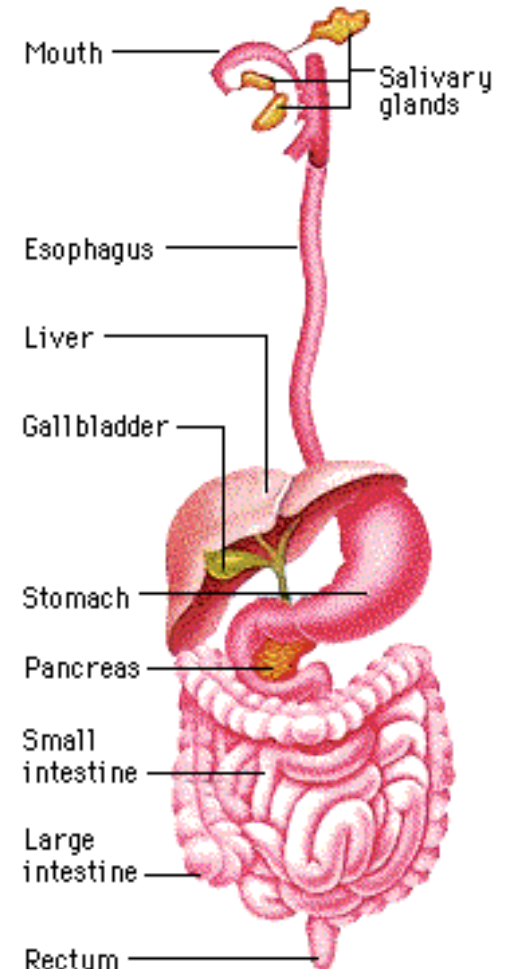
<u>GI Tract Location</u>	<u>Avg. pH Fasting</u>	<u>Avg pH Fed State</u>
Stomach	1.4-2.1	3-7
Duodenum	4.4-6.6	5.2-6.2
Jejunem	4.4-6.6	5.2-6.2
Lleum	6.8-8	6.8-8

Question- you make a drug that is charged at pH 4-9, is that going to be an orally available drug?



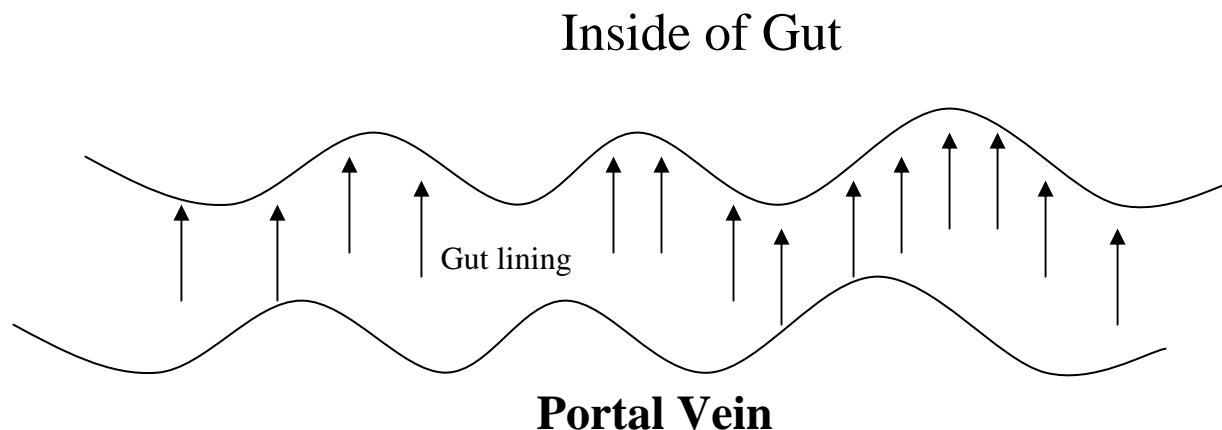
Absorption from the Gut

- Absorption mostly occurs in the small intestines, some in stomach and colon
- Drug gets absorbed directly into portal vein which takes it to the liver
- First pass metabolism occurs in liver
 - Making the compound more Polar cyp family of enzymes
- Elimination of metabolites and extracted drug gets mixed with bile and then is released back into intestines
- what makes it through is distributed via the blood to tissues



P-glycoproteins (PGP)

- Enzymes that belong to a family of active transporters and are located in many areas of the body
- Believed to have evolved as a protection mechanism to clear the body of harmful substances
- Play a large role in the elimination of drugs into the gut, also involved in transporting drugs out of the brain, and into urine and bile
- Very non-specific

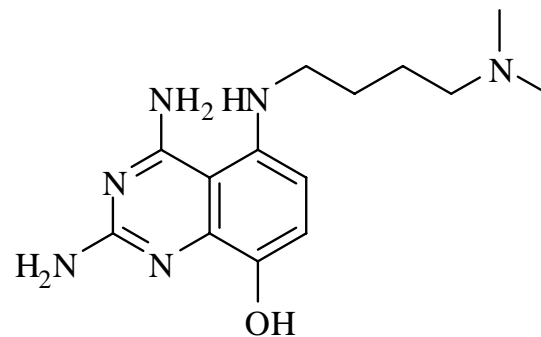


Oral drug considerations

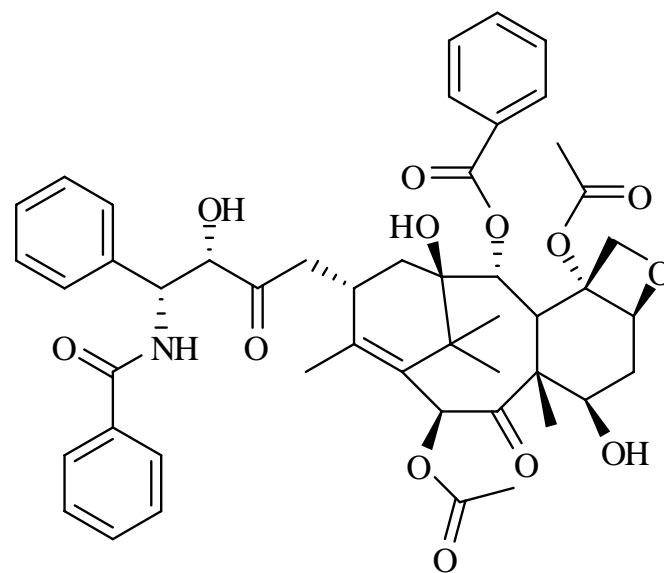
- Bioavailability- general term that describes the % of drug that gets into the systemic system without being changed

Lipinski's Rule of Five

- Good way of predicting likelihood that a compound will display oral activity
- Developed by Chris Lipinski in 1997 after observing that most medications are small and lipophilic molecules
 - Not more than 5 H-bond donors (OH or NH)
 - Not more than 10 H-bond acceptors (O or N)
 - MW less than 500 g/mol
 - A partition coefficient ($\log P$) less than 5
- Now there are many variants of the rule



Molecular Weight: 290.36

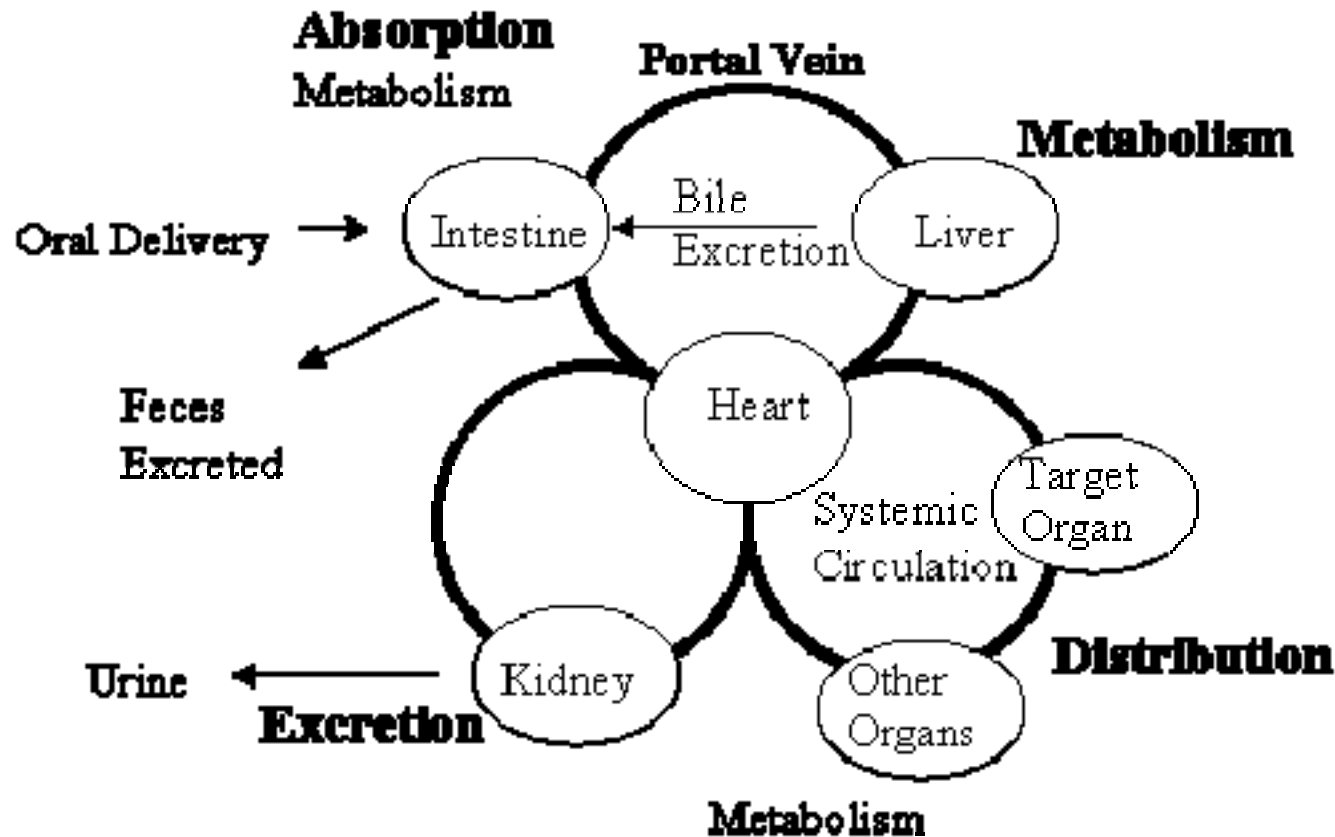


Molecular Weight: 851.93

What is the rationale for the Rule of Five?

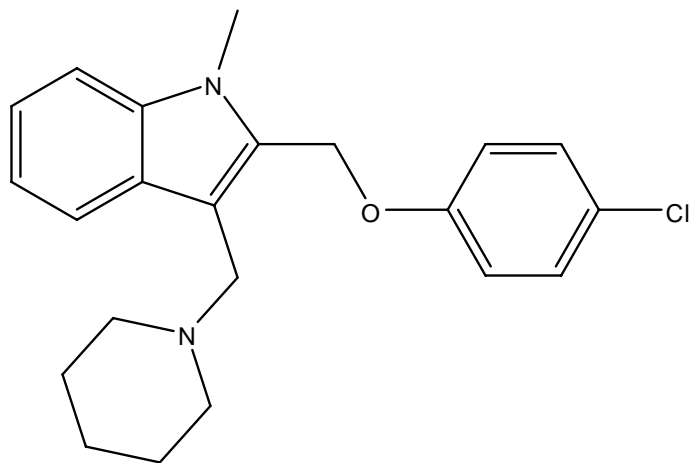
- Hydrogen bonds
 - increase water solubility
 - break H bonds to move into lipid bilayer
- Molecular Weight
 - greater molecular weight increases size
 - must create larger cavity in water or lipid membrane
 - lower aqueous solubility
 - harder to move through tightly packed lipid aliphatic side chains
- Log P
 - greater log P reduces water solubility
 - compound must be dissolved to reach intestinal membrane surface to be absorbed
- Transporter substrates
- Enhance uptake or efflux

Quick recap on ADME

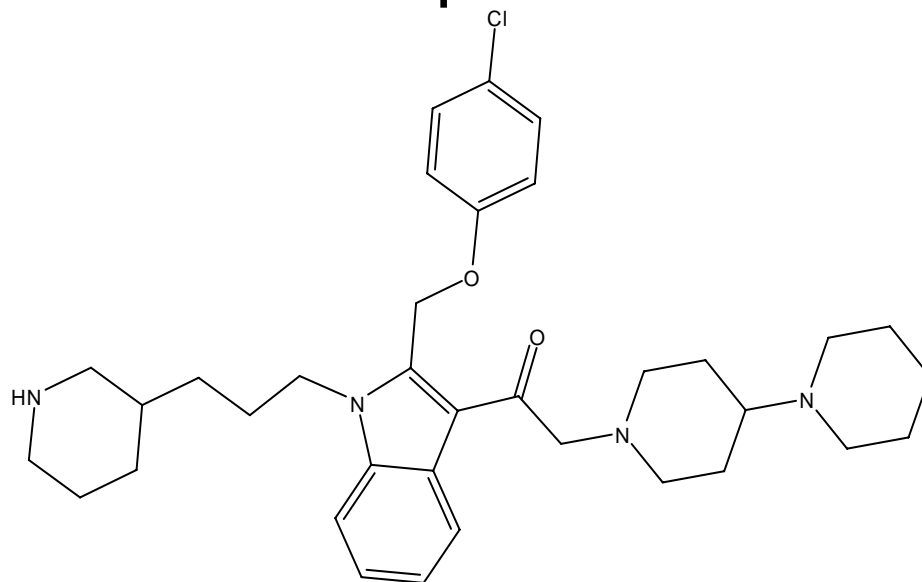


How can we combat poor physiochemical properties?

- Depends on which property is problematic but generally the only way to do this is to modify structure or formulation of the compound



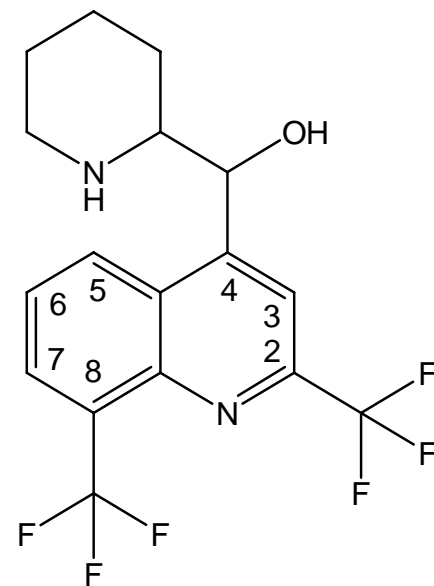
In vitro potency 2 μ M
MW 369



In vitro potency 1 nM
MW 591
Poor oral properties

A case study in physiochemical properties

- Mefloquine is an antimalarial that was developed by the WRAIR in the 70-80's but has shown to be neurotoxic
- Can we change the structural motifs of the molecule enough to change the biological action?
- What causes a molecule to cross the BBB?



Mefloquine

Evaluation of alkylaminoquinoliny-methanols as new antimalarials

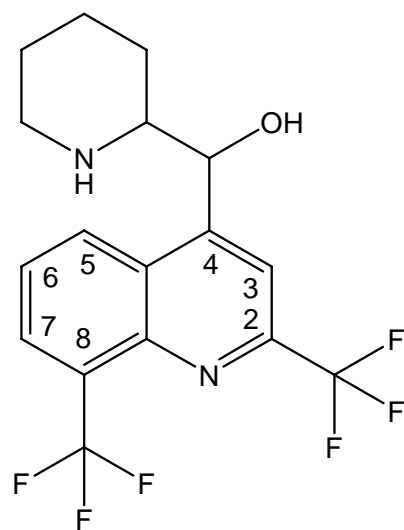
- **GOALS:**
 - Identification of a quinoline methanol with fewer CNS effects than Mefloquine.
 - Compound would serve as a functional replacement for Mefloquine.
 - Dual role as a once weekly prophylactic drug and as a treatment agent for malaria in combination with other antimalarials.

- **WHY REPLACE MEFLOQUINE?**
 - CNS effects.
 - Resistance.
 - Cost.

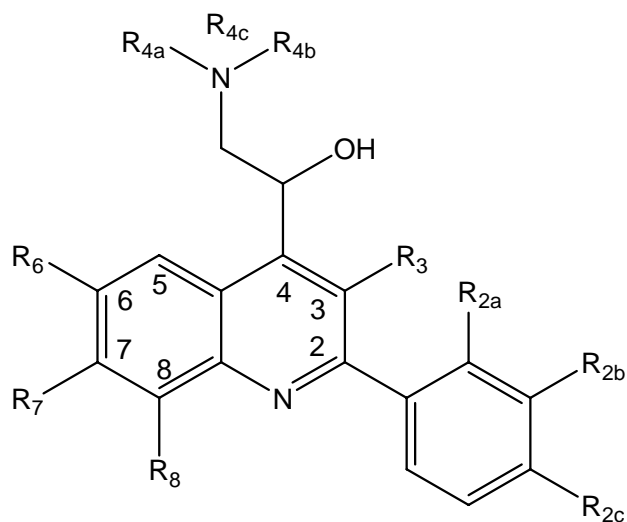
Desired Properties of Mefloquine Replacement

- Long biological half-life.
- **More favorable CNS profile than Mefloquine.**
- Greater activity than Mefloquine against MQ-resistant strains of *P. falciparum*.

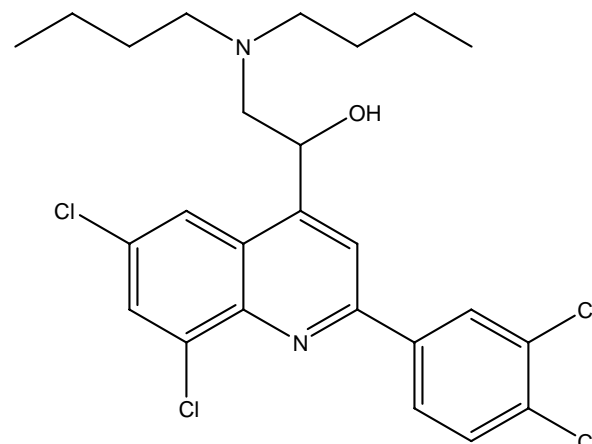
Alkylaminoquinolinyl methanols as replacement drugs for Mefloquine?



MEFLOQUINE



AAQMS

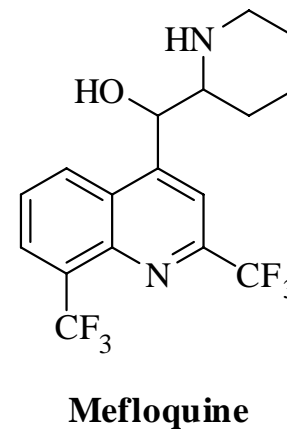
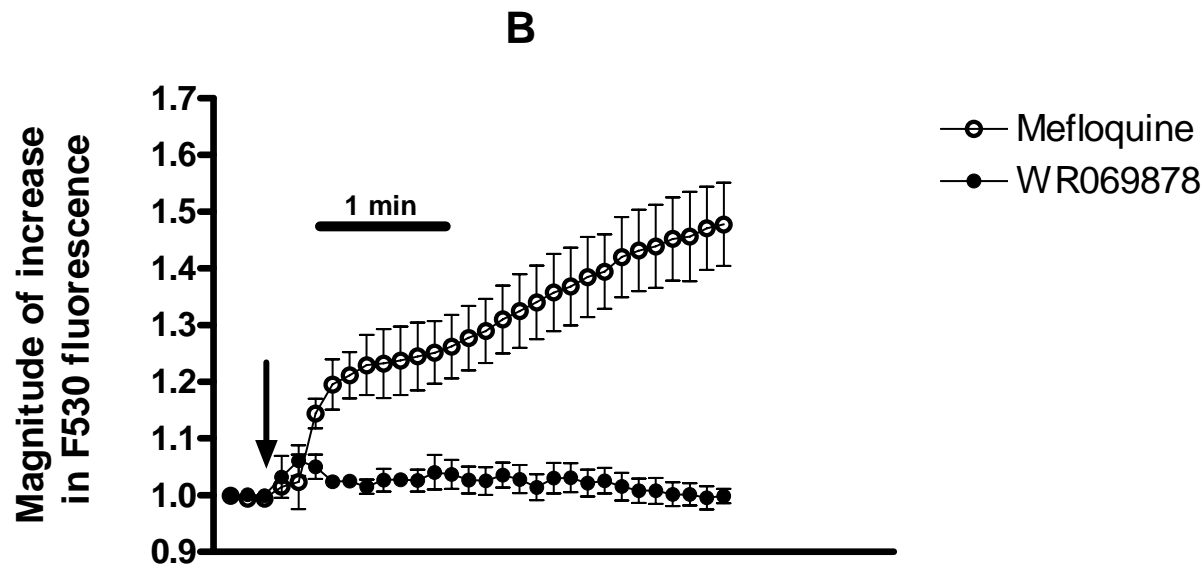
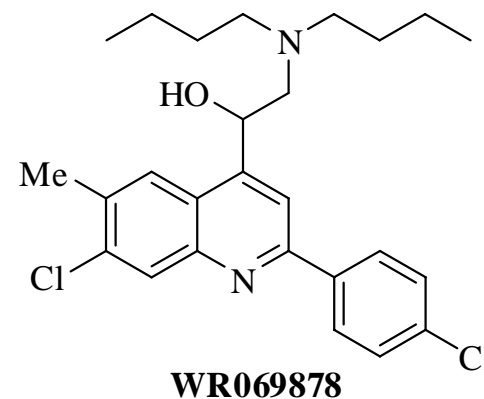
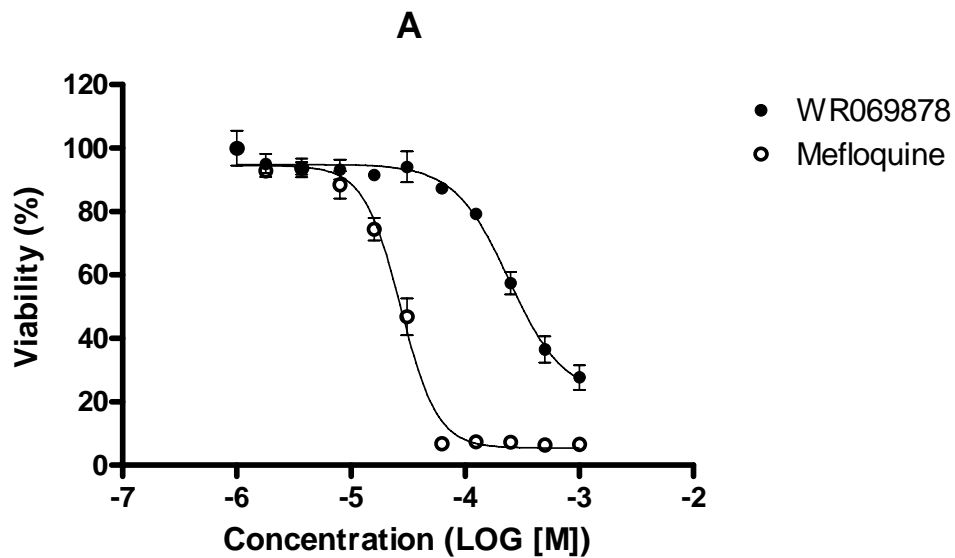


WR030090

Key Pharmacological Properties of AAQMs

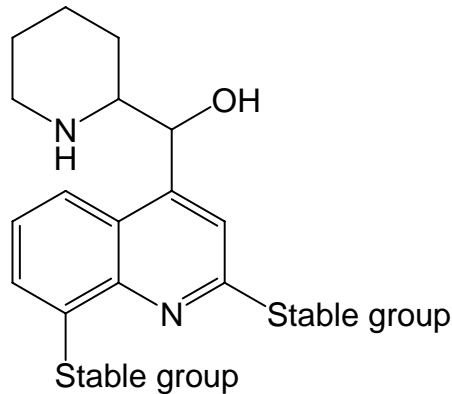
COMPOUND	SOLUBILITY	LOGP	P. f. IC50	Neurons TD50	Met. stab H-life	IC50 2D6	TOPKAT	P. b. MIN CUR DOSE - SC	P. b. MIN CUR DOSE - PO
Mefloquine	8.29	2.87	15	20	> 120	18.6	10	20	5 ⁱ
WR030090	0.004	8.18	11	2000	> 120	NT	10	20	5, 5 ⁱ
WR069878	0.019	8.04	1.5	200	38	18.2	15	20	2.5, 5 ⁱ
WR176399	1.11	5.47	0.48	600	> 120	3.5	15	40	10
WR007524	0.28	5.46	0.63	60	> 120	2.2	14	80	< 1.25,
WR041294	1.59	5.04	1.1	60	> 120	0.6	18	>640	2.5 ⁱ
WR081049	0.088	7.01	1.6	200	24	0.24	17	160	10
WR035058	0.037	7.18	2.7	600	38	0.3	16	40	2.5
WR098656	19.0	4.98	3.3	60	> 120	0.34	30	10	1.25
WR074086	0.043	7.10	3.4	2000	46	2.8	16	20	< 1.25
WR211925	0.090	7.29	5.2	600	54	0.65	15	160	2.5 ⁱ
WR029252	0.004	7.74	7.6	600	> 120		14	5	5.0
WR211679	0.024	8.63	7.7	2000	64		9	ND	2.5 ⁱ
WR177973	0.43	6.78	7.9	200	15		14	320	
WR106752	0.029	8.17	8.0	2000	NT		15	40	> 40
WR053188	0.003	8.23	18	2000	89		11	80	
WR030091	0.006	9.29	19	3000	>120		9	160	
WR030464	0.007	9.20	19	3000	>120		9	160	
WR106751	0.2	6.39	22	60	8		16	>640	
WR149105	0.003	8.48	36	600	NT		12	10	
WR029656	0.002	9.86	117	3000	> 120		7	160	
WR131739	0.19	6.42	181	200	34		16	>640	
WR091884	47	1.48	277	60	> 120		11	>640	
WR140089	0.003	8.43	500	3000	NT		11	40	

Neurotoxicity of WR069878 v Mefloquine



Why Are AAQMS Less Neurotoxic Than Mefloquine?

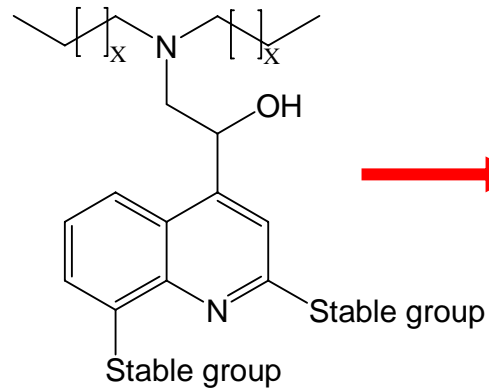
4-Quinoline Carbinolamines



Biological Data

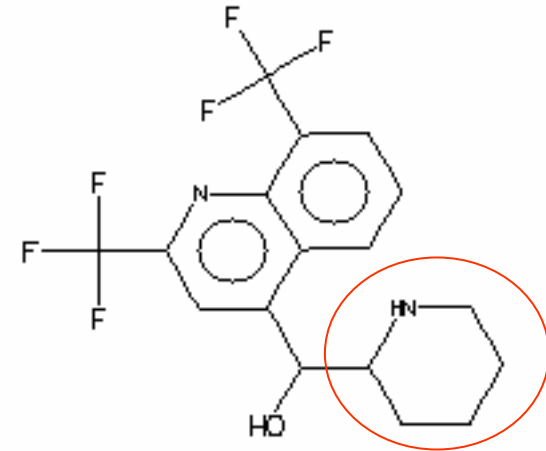
Neurotoxicity **32**
TM91C235 **38**
Relative therapeutic
index **1**

AAQMS



Biological Data

Neurotoxicity **600**
TM91C235 **16**
Relative therapeutic
index **32**

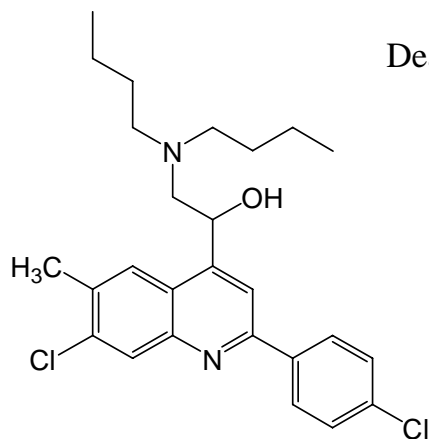


Mefloquine

In Vitro Data – *P. falciparum*

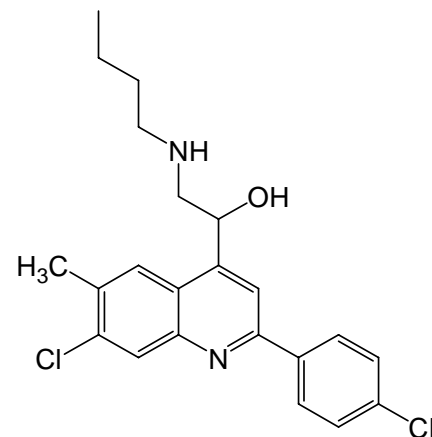
	W2	D6	TM91C235	TM90C2A
	IC90 (ng/ml)			
Mefloquine	3.9	20	89	101
WR069878	0.49	5.3	11.7	16

WR069878 - Metabolism



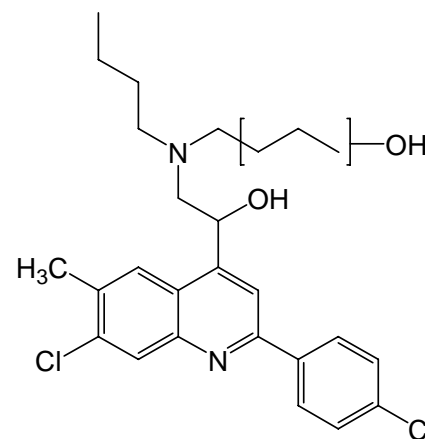
WR069878
 $C_{26}H_{32}Cl_2N_2O$
Exact Mass: 458.19
 m/z 459.2 [M+H]⁺

Dealkylation (> 92%)



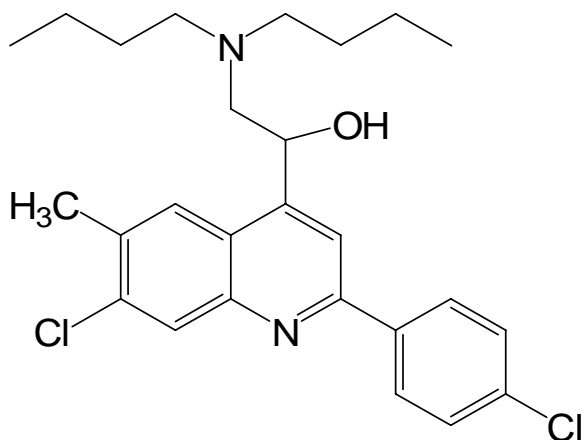
$C_{22}H_{24}Cl_2N_2O$
Exact Mass: 402.13
 m/z 403.1

Hydroxylation



$C_{26}H_{32}Cl_2N_2O_2$
Exact Mass: 474.18
 m/z 475.1

LEAD - WR069878



Biological Data

Neurotoxicity (IC₅₀) **200 uM**
TM91C235 (IC₉₀) **12 ng/ml**
ED₅₀ in mice **5-10 mg/kg x 3 PO**
Aotus cure dose **40 mg/kg x 3 PO**

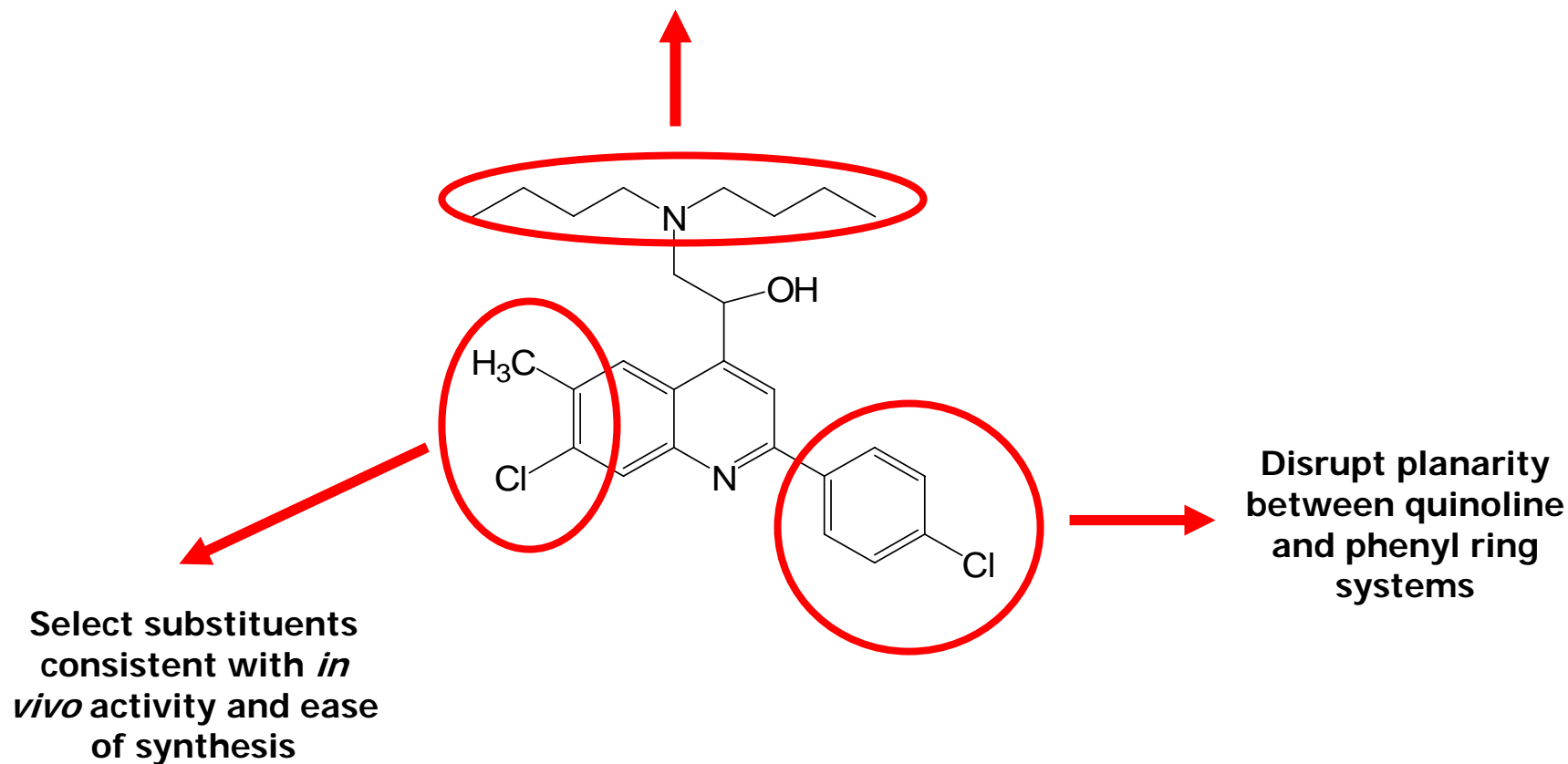
Potential for phototoxicity

Shorter in vitro half-life than
Mefloquine

Low plasma conc. – Poor
bioavailability

How Do We Identify an NGQM?

Open piperidine ring to improve antimalarial activity and mitigate neurotoxicity, select substituent resistant to N-dealkylation



Questions?