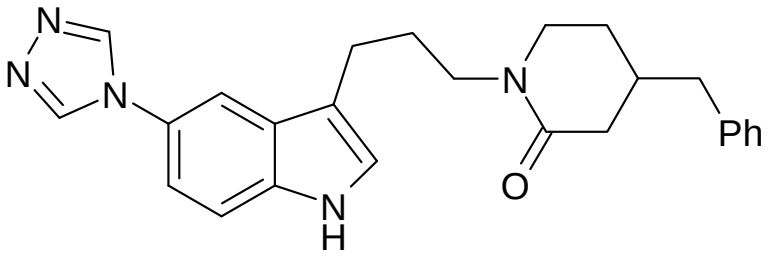
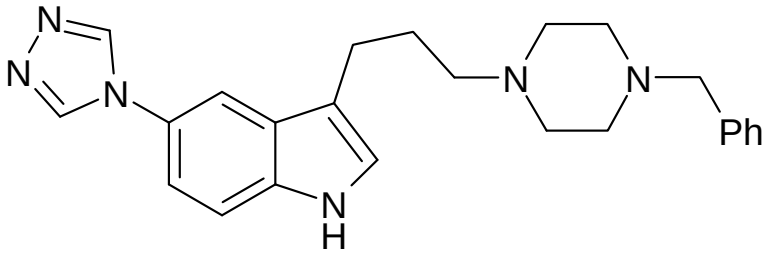
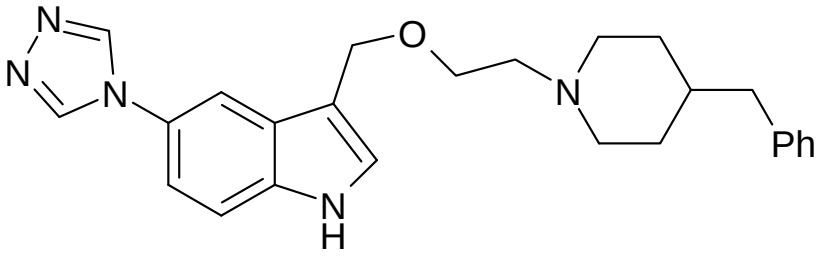
Which one would you make……?



A



B

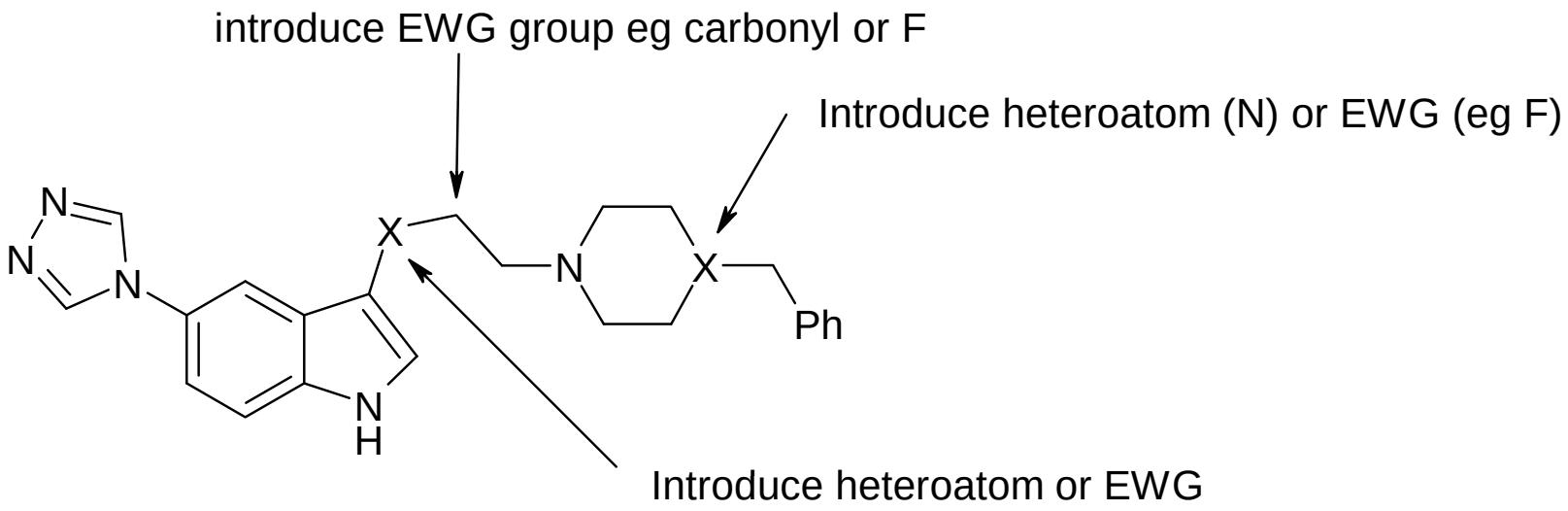


C

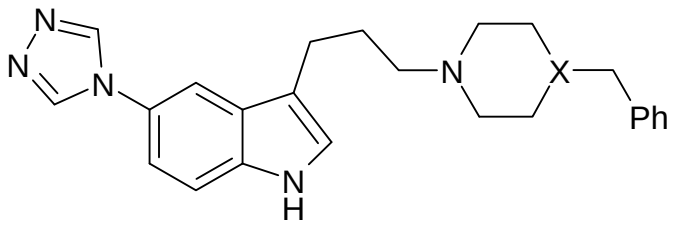
Hypothesis: Lower pKa of basic N to influence absorption

Effect of lowering pKa is to increase logD and decrease % of ionized compound at gastric pH - both will favour membrane permeability

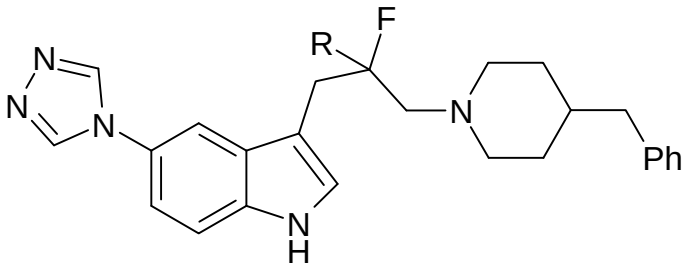
Strategy: Introduce heteroatoms, EWG's β or γ to nitrogen



What was tried…….



1 : X = CH
2 : X = CF

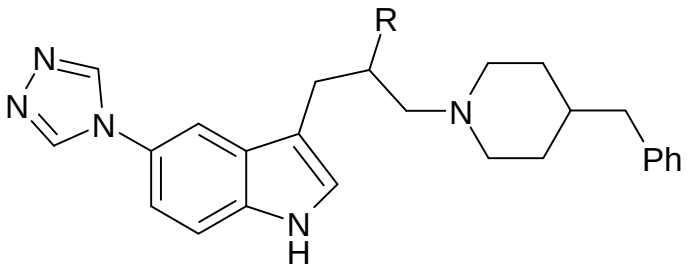


3 : R = H
4: R = F

Compound	5-HT1D Ki	pKa	cLogD	Concentration in rat plasma HPV sampling 0.5h after 3 mg/kg p.o.
1	0.3 nM	9.7	2.5	25 ng/ ml
2	0.9 nM	8.8	3.5	570 ng /ml
3	0.9 nM	8.7	3.5	781 ng/ ml
4	78 nM	6.7	4.7	ND

- **Lowering pKa improves permeability and oral absorption**
- **Fluorine atoms have minimal steric influence on structure**
- **NB: fluoropiperidines are possibly toxic but any heteroatom β or γ to a nitrogen will lower pKa**

But.....



1 : R = H
3 : R = F

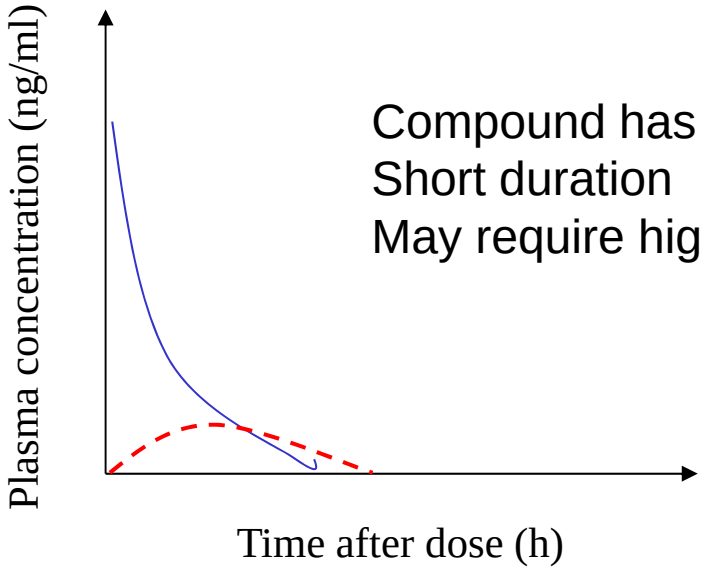
				Concentration in rat plasma 0.5h after 3 mg/kg p.o.	
Compound	**5-HT1D Ki**	**pKa**	**cLogD**	**HPV sampling**	**systemic (cardiac) sampling**
1	**0.3 nM**	**9.7**	**2.5**	**25 ng/ ml**	**< 2 ng/ ml**
3	**0.9 nM**	**8.7**	**3.5**	**781 ng/ ml**	**196 ng/ ml**

Increase in lipophilicity leads to extensive first pass metabolism
- lower than expected systemic exposure……

Metabolism

Metabolism and Clearance

How do you know you have a problem?



Compound has a short $T_{1/2}$ (half life)
Short duration
May require high or multiple dosing to see a biological effect

Compound A iv bolus injection 1 mg/kg

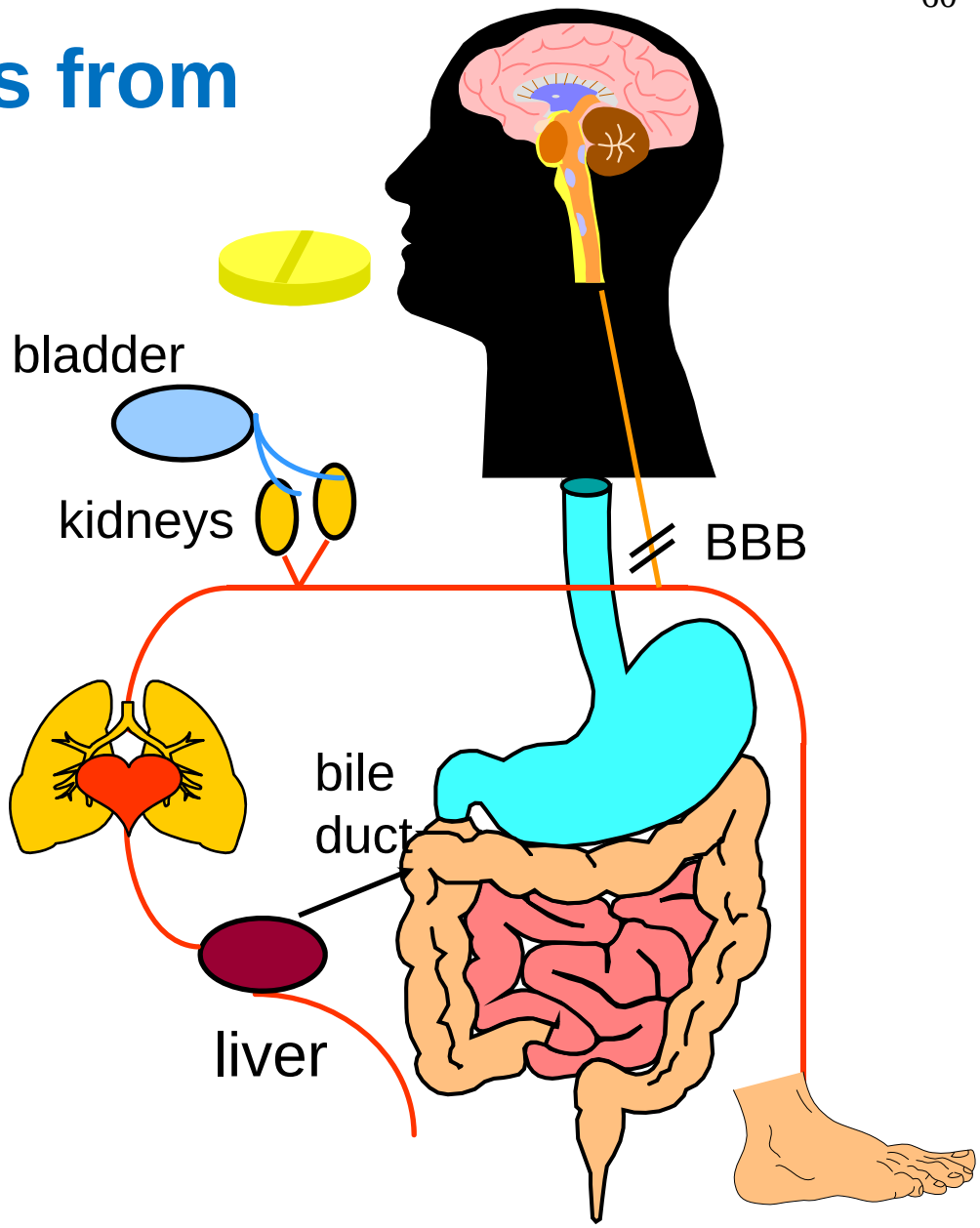
- **Low plasma exposure (area under curve AUC)**
- **Drug disappears rapidly from plasma**

Compound A oral dose (po) 10 mg/kg – jugular vein detection

- **Low plasma exposure (AUC)**
- **Extensive first pass metabolism**

“Clearance” of drugs from plasma

- Successfully entered blood
- survive blood contents (hydrolysis etc)
- Survive extraction from blood by liver
- survive metabolism in liver (oxid. and conj.)
- avoid active transport to bile
- avoid excretion by kidneys

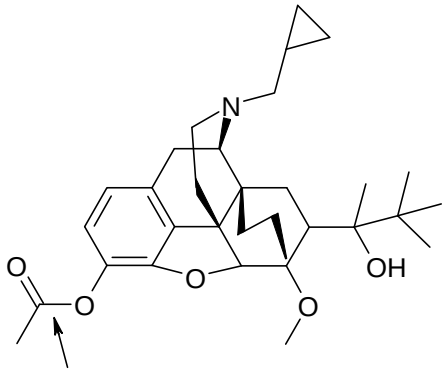


Common sources of the problem

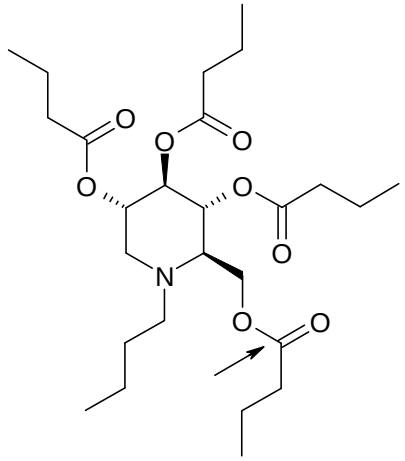
- Plasma instability
- Biliary elimination of compound unchanged
- Metabolism by the liver
- Renal elimination of compound unchanged

Blood Instability

Enzymatically mediated, usually hydrolases and peptidases
Therefore compounds containing esters and some electrophilic amides can be a concern
Rates of hydrolysis usually (but not always) faster in rodents than man



Isocarbacyclin



glycovir

Human blood T1/2	17min	45min

Rates are hard to predict but are sensitive to electrophilicity, sterics and lipophilicity
J Med Chem, 1999, 42, 5161

GI Tract & Liver

Plumbing and liver physiology

Clearance - an important concept

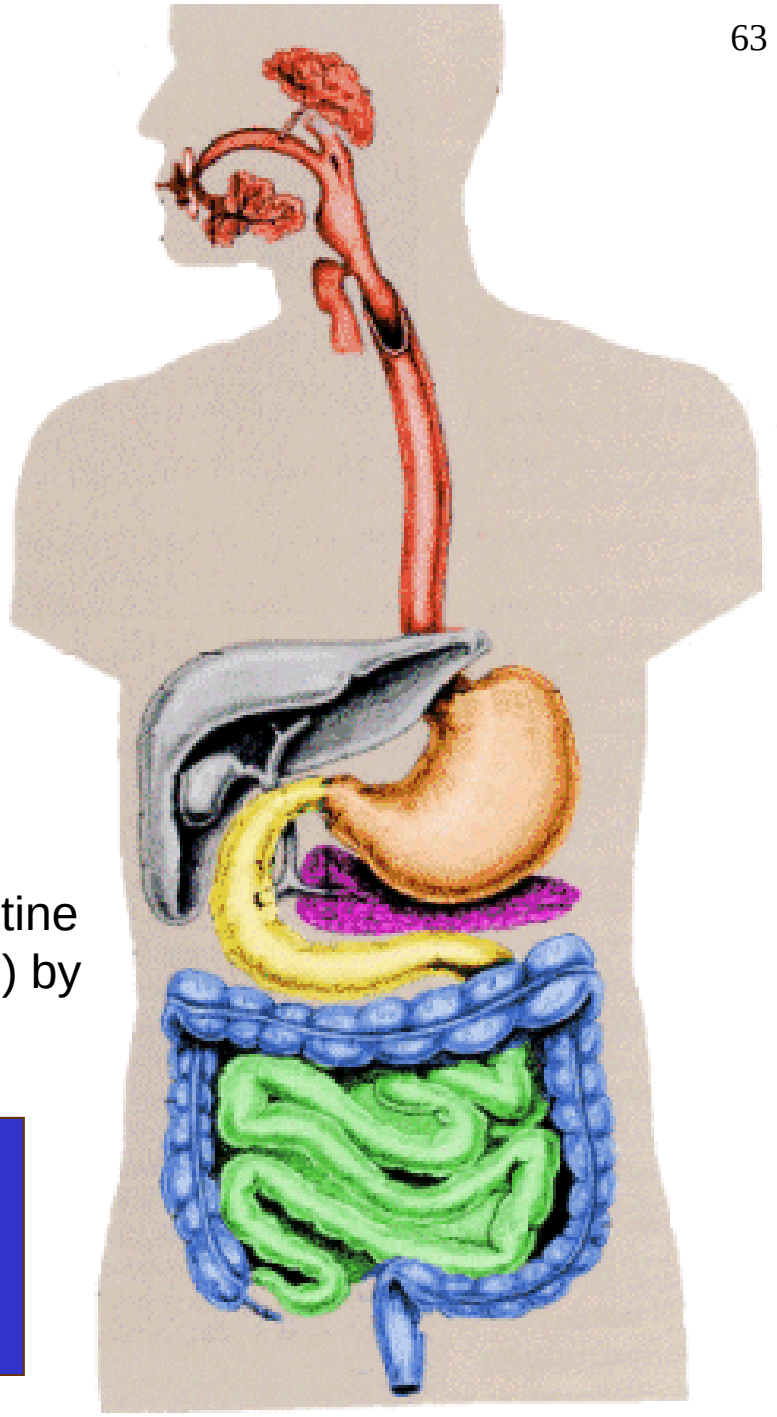
Double whammy – first pass and every pass

Clearance also affects bioavailability (F) because of first pass extraction

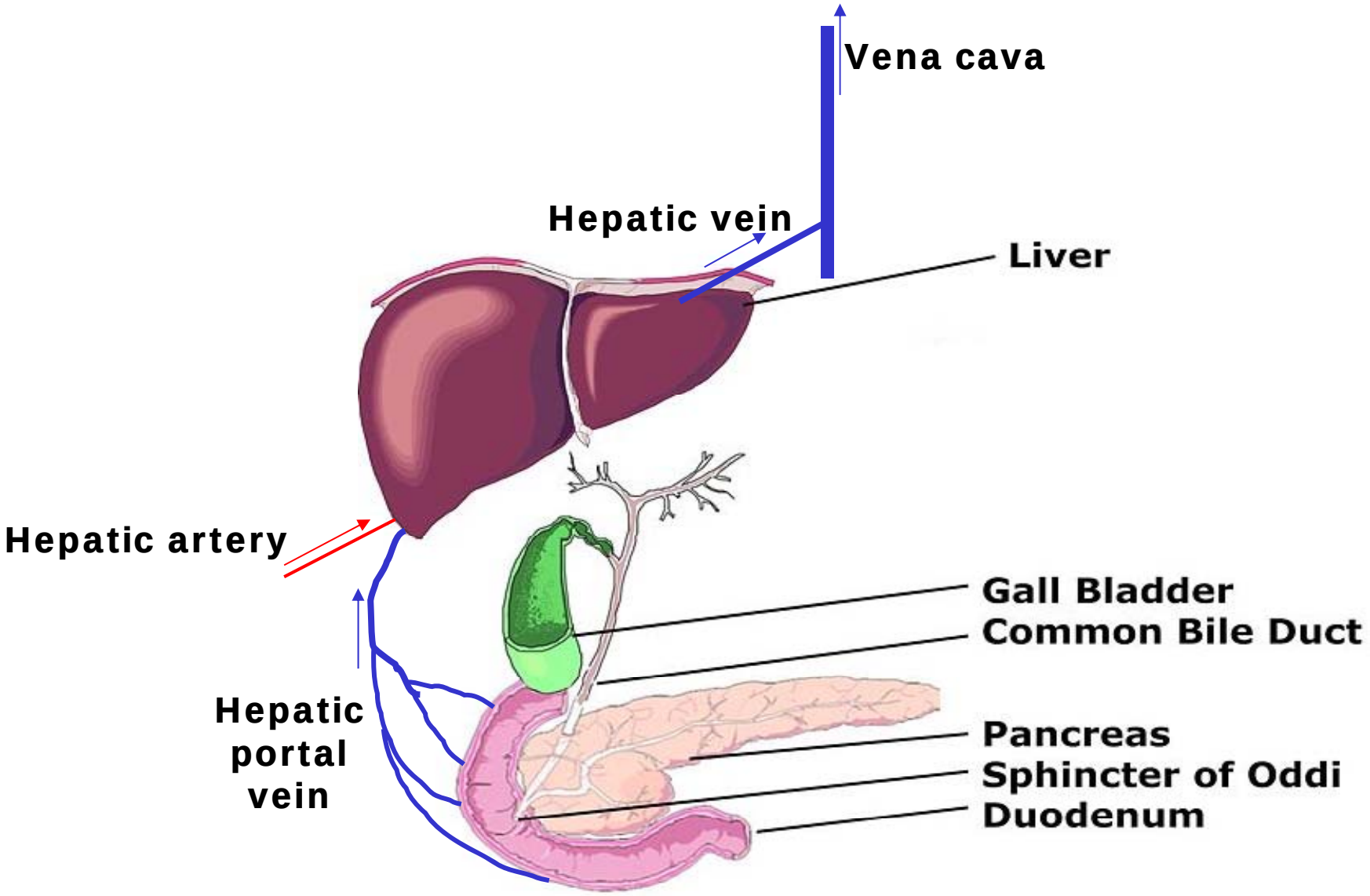
$F = F_{abs} * F_{gut} * F_{hep}$ where

F_{abs} = fraction absorbed
F_{gut} = fraction which survives metabolism in the intestine
F_{hep} = fraction which survives extraction (metabolism) by the liver

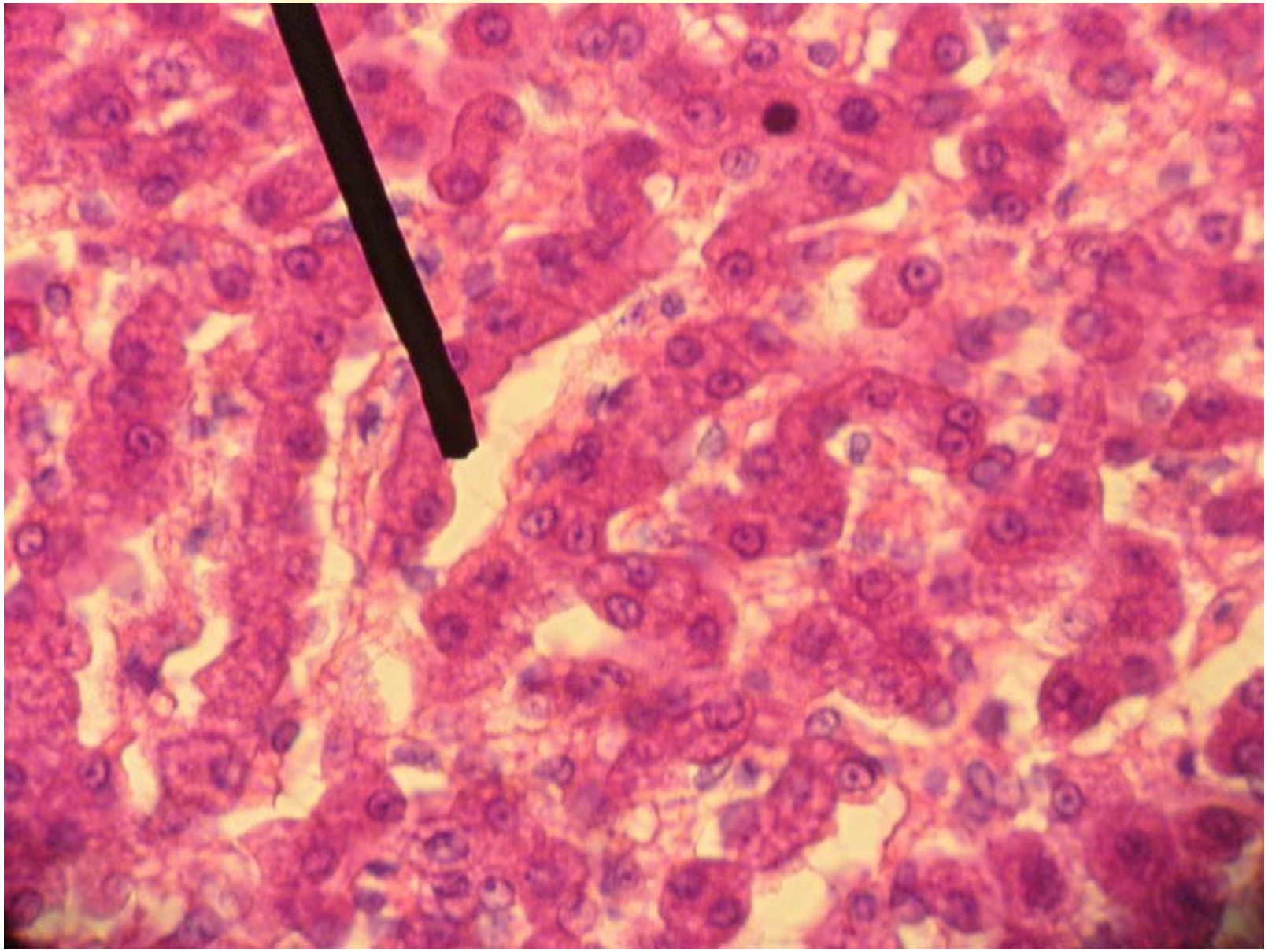
- Video explanation of anatomy of liver
- http://www.nottingham.ac.uk/nursing/sonet/rlos/bioproc/liveranatomy/index.html
- Dr Viv Rolfe, Uni Nottingham



Liver & its Connections



Liver Histology



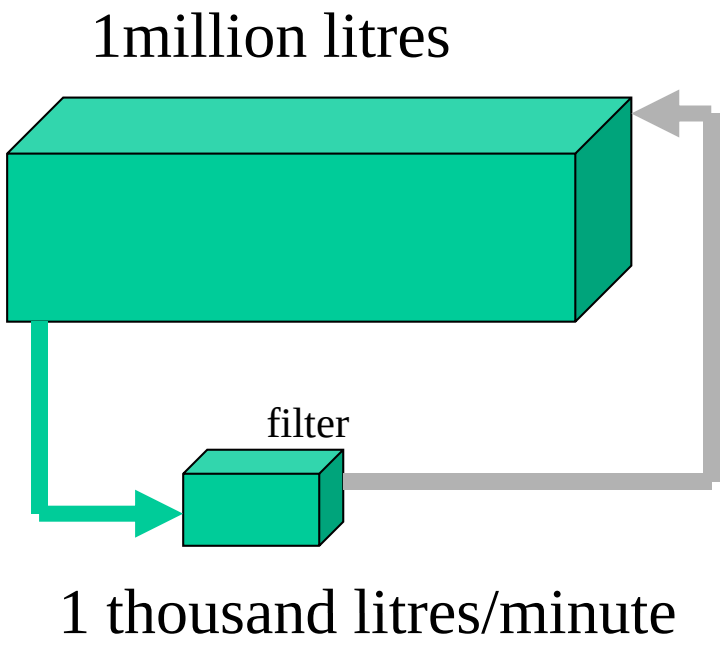
Plasma clearance – an analogy

Imagine a swimming pool.

Drop green ink into it and mix it.

A pump sends water through a filter.

The filter destroys the ink and returns clean water to the pool.



The flow rate is the CLEARANCE.

The half-life = $\log_e(2)$ x volume / clearance =
0.693x1000000/1000 = **693 minutes**!!!

Plasma clearance – an analogy

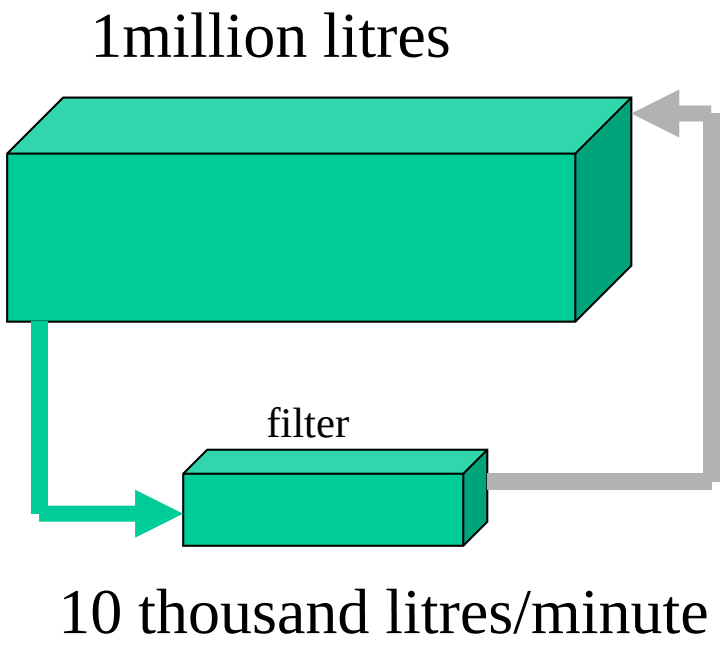
So fit a bigger pump and filter!

Drop green ink into it and mix it again.

The filter destroys the ink and returns clean water to the pool.

The flow rate is the CLEARANCE.

The half-life = $\log_e(2)$ x volume / clearance =
0.693x1000000/10000 = **69.3 minutes**

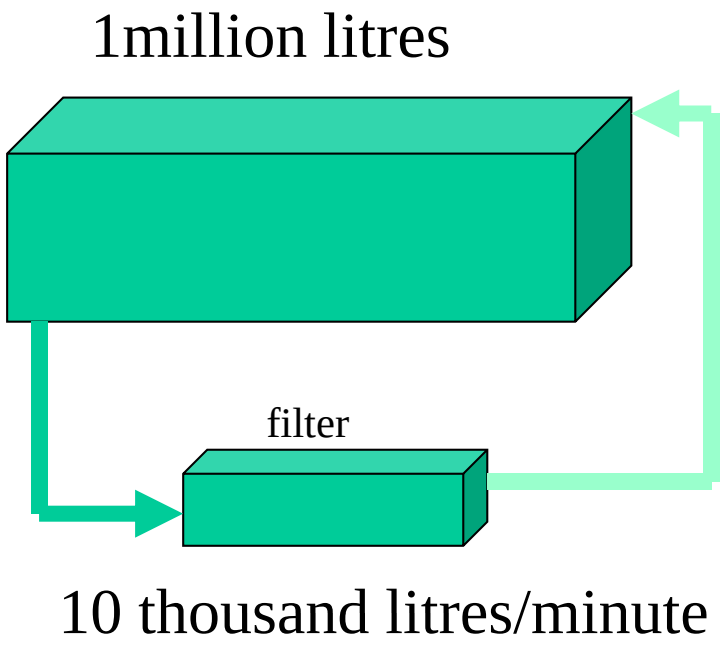


Plasma clearance – an analogy

Suppose the filter is only 50% efficient (extraction ratio E_h = 0.5).

Now the CLEARANCE is 5000 litres/minute

The half-life is doubled to:
0.693x1000000/5000 = **138.6 minutes**



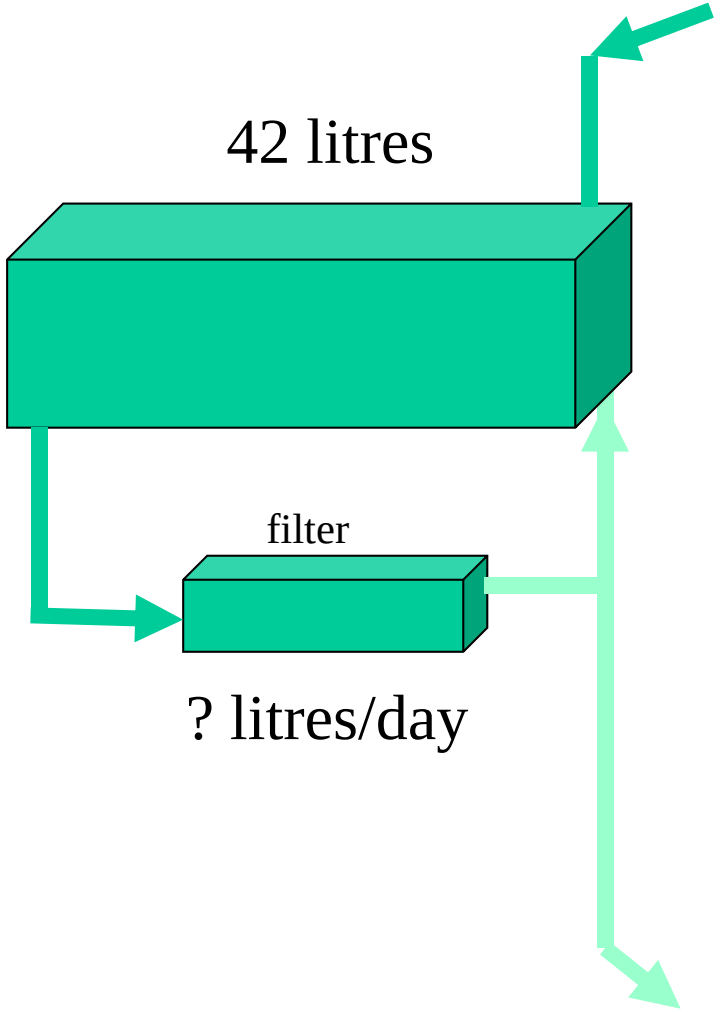
Clearance – worked example

Total body water is 42L (70kg person)

The half-life of water is 8 days

What is the clearance of water?

CL = $\log_e(2)$ x volume / half-life



Clearance – worked example

Total body water is 42L (70kg person)

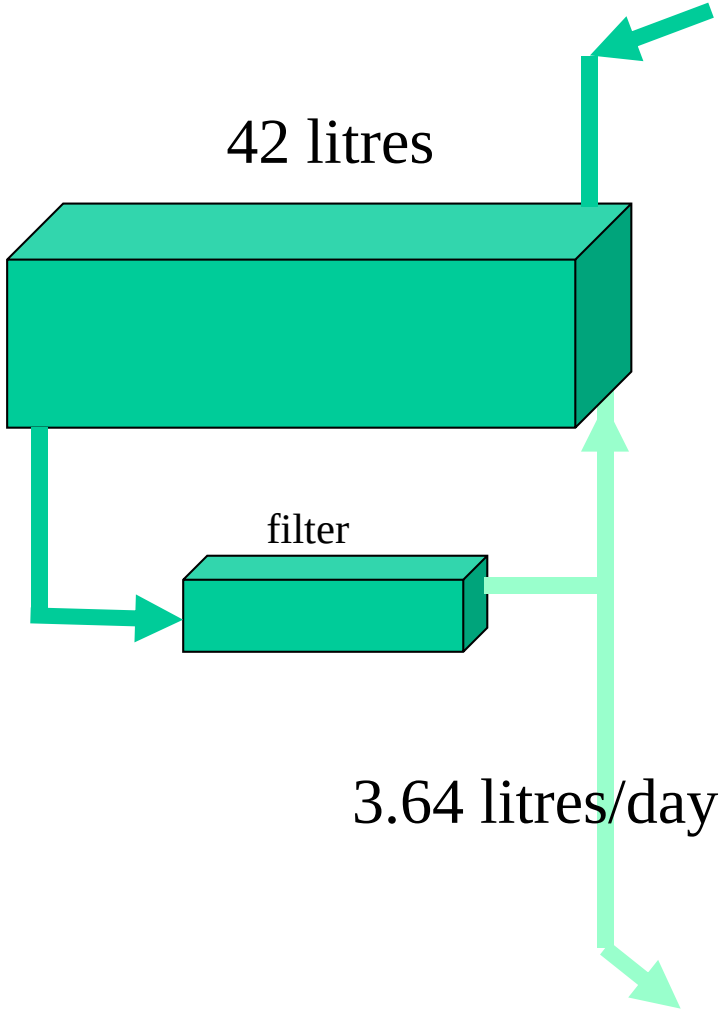
The half-life of water is 8 days

What is the clearance of water?

CL = $\log_e$ (2) x volume / half-life

= 0.693 x 42 / 8 = **3.64L/day**

- *Is this reasonable?*
- *Where does it come from?*
- *Where does it go?*



Clearance – worked example

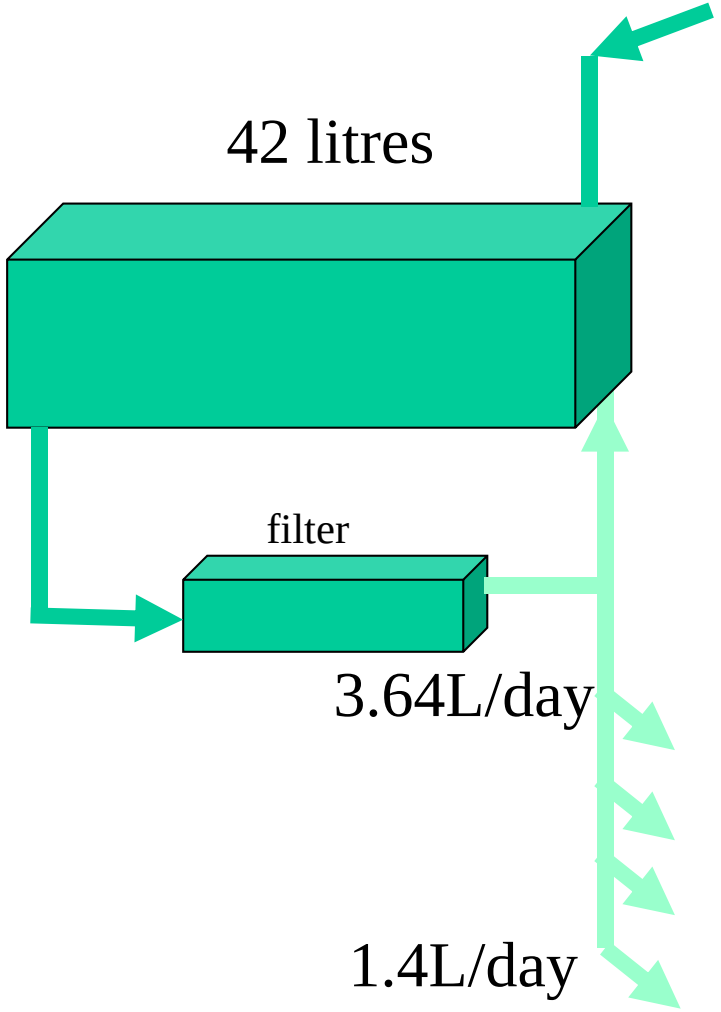
Total body water is 42L (70kg person)

The half-life of water is 8 days

What is the clearance of water?

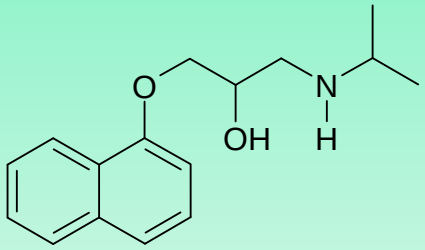
CL = 3.64L/day = 2.5mL/min

Urine flow = 1.4L/day = 1mL/min

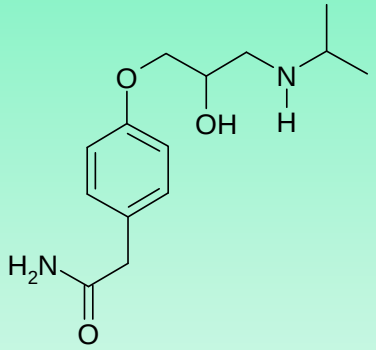


Renal Clearance

- Typically only relevant for low lipophilicity compounds e.g. log D ≤0
- Therefore not very common!



Propanolol (logD = 1.5)
99% of clearance by metabolism



Atenolol (clogP = -1.9)
> 90% excreted unchanged in urine

Physicochemical Determinants of Human Renal Clearance. Journal of Medicinal Chemistry (2009), 52(15), 4844-4852.

Biliary Elimination of Compound

Once believed to be solely a function of molecular weight
(MW >500 for human)

However, now more widely regarded as an “active transport” problem

Can affect acids, bases and polar neutrals; bile is alkaline and this can “attract” acid drugs

Concentration gradient from bile to plasma can be 10000 to 1 for low permeability drugs

Difficulty - need to surgically cannulate rats and look for drug in bile fluid
- bile is not the easiest matrix to analyse

Biliary Clearance

We think we are starting to understand what controls it……

Most drugs are sufficiently lipophilic for membrane permeability and oral absorption
Compounds which are less lipophilic tend to experience active transport.
Probably active transport is the norm, but permeable compounds can leak out again

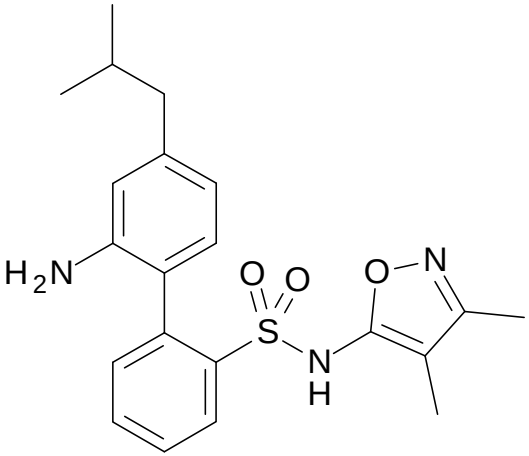
So increase in PSA →
decrease in permeability →
increased likelihood of biliary clearance

Details of specific transporters are hard to get and harder to interpret.
We have seen compounds which are
>99% plasma bound
E_h ~ 1 (mostly biliary)
bile / plasma ratio ~ 1000:1 (bile unbound plasma ~ 100,000:1!)

Biliary clearance often leads to a high concentration inside hepatocytes, blocking transport of bile acids or other toxins → hepatotoxicity

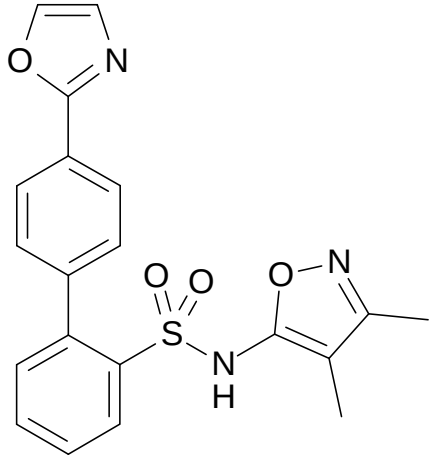
Efflux in Caco-2 assay or increasing PSA increase the risk of hepatic uptake

Example of biliary clearance: BMS ET_A antagonists



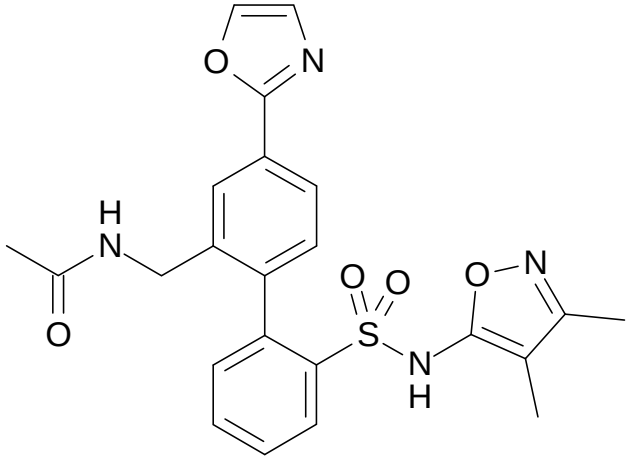
BMS-187308

moderately fast
in vitro & in vivo



BMS-193884

slow in vitro
slow in vivo

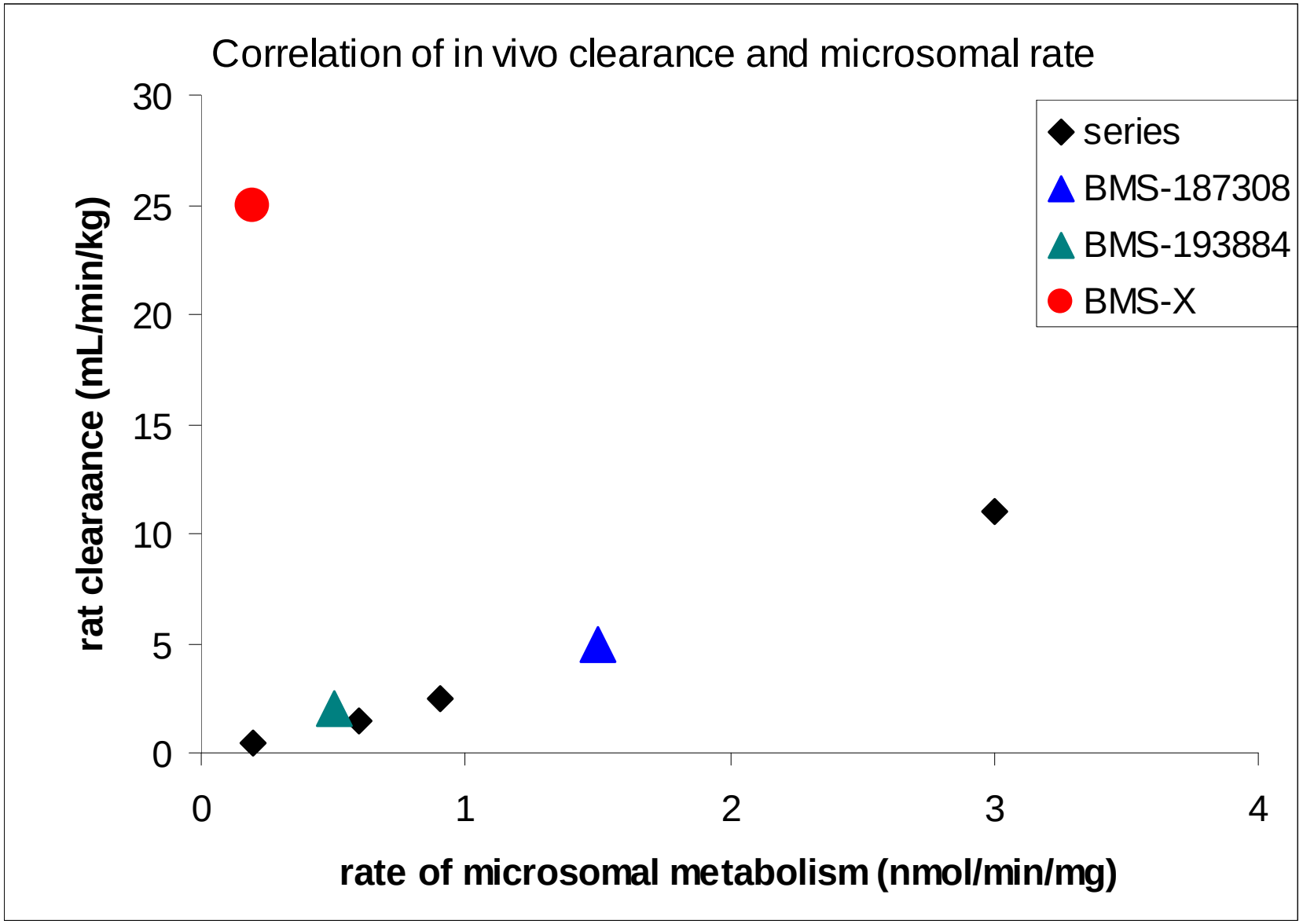


BMS-X

very slow in vitro
very fast in vivo -
biliary clearance!

WG Humphreys *et al*, Xenobiotica, 33 (11), 1109-23, 2003

Example of biliary clearance



Metabolism and Clearance

Most drugs are sufficiently lipophilic for membrane permeability and oral absorption
Metabolism in the liver is therefore the major route of clearance.

Where metabolism in the liver is the principal method of elimination then

$$\text{Clearance } (CL_H) = Q_H E_h \text{ ml/min/kg}$$

Q_H is the blood flow through the liver
E_h is the liver extraction ratio = $(C_A - C_V)/ C_A$

C_A = Concentration of drug entering liver. C_V = Concentration of drug leaving liver

Metabolism and Clearance

What are high and low clearance values?

Clearance (CL_H) = Q_H * E
For drugs where hepatic elimination is high then E $\rightarrow$ 1 and CL ~ Q_H
Clearance is high and approaches hepatic drug flow

	Rat	Dog	Man
Hepatic blood flow (ml/min/kg)	90	40	21
High clearance; E > 0.7 (ml/min/kg)	>63	>28	>15
Low clearance; E < 0.3 (ml/min/kg)	<30	<12	<7

Clearance is measured after an iv dose of compound (all the dose is “absorbed”)

Clearance also affects bioavailability (F) because of first pass extraction

$F = F_{abs} * F_{gut} * F_{hep}$ where

F_{abs} = fraction absorbed
F_{gut} = fraction which survives metabolism in the intestine
F_{hep} = fraction which survives extraction (metabolism) by the liver

(21mL/min/kg = 2100L/day!!!)

Metabolism

Why are drugs metabolised?

- **High molecular weight or high lipophilicity**
  - metabolism makes compounds more polar and more water soluble
  - trend for metabolism to increase with lipophilicity

- **Reactive/ labile groups eg:**
  - benzylic or allylic positions,
  - electron rich aromatic rings
  - N-methyl or O-methyl groups, Sulphur atoms
  - acidic OH or NH groups

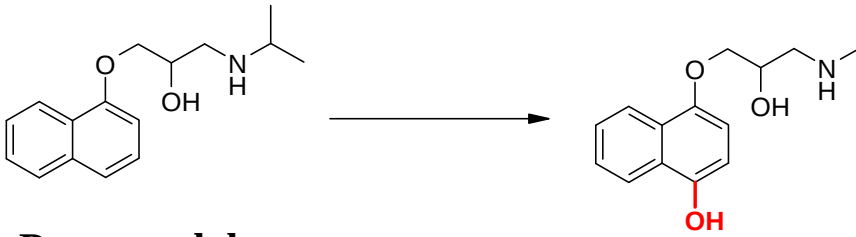
- **High affinity for metabolising enzyme**
  - Good fit into active site, specific interactions

Phase I Metabolism

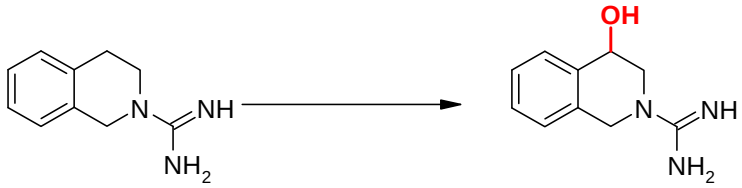
- Principally by:-
- (i) Oxidation
  - Aliphatic or aromatic hydroxylation
  - N-, or S-oxidation - cycling
  - N-, O-, S-dealkylation
- (ii) Reduction
  - Nitro reduction to hyroxylamine/ amine
  - Carbonyl reduction to alcohol - cycling
- (iii) Hydrolysis
  - Ester or amide to acid and alcohol or amine
  - Hydrazides to acid and substituted hydrazine

Examples of Phase I Metabolism

- Oxidation

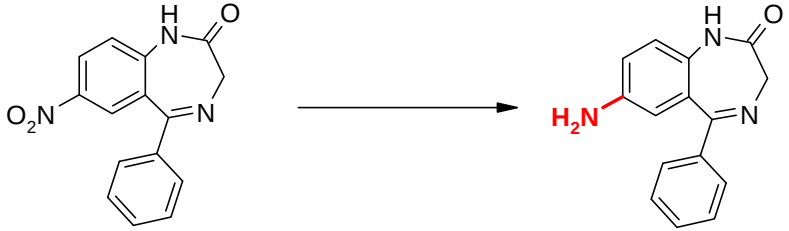


Propranolol (β-blocker)



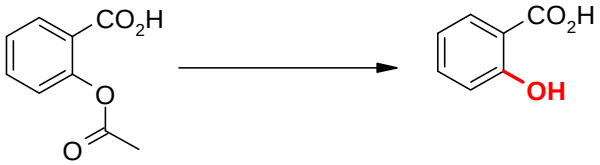
Debrisoquine (anti-hypertensive)

- Reduction



Nitrazepam (hypnotic)

- Hydrolysis



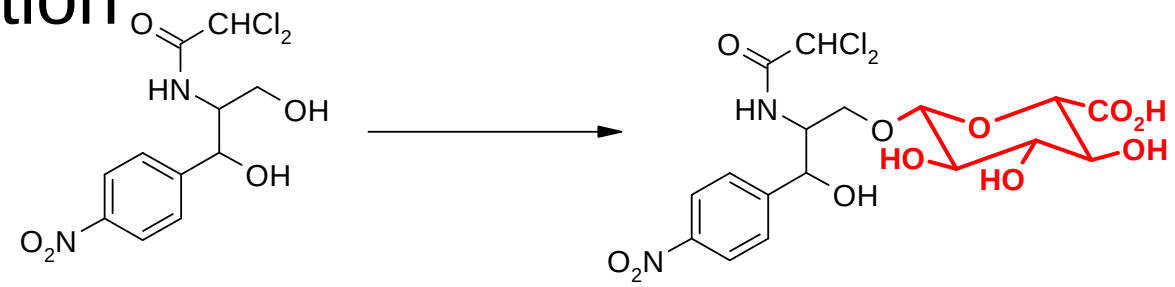
Aspirin (Analgesic)

Phase II Metabolism

- Principally by:-
- (i) Glucuronidation
  - Carboxylic acid, alcohol, phenol, amine
- (ii) Sulphation
  - Alcohol, phenol, amine
- (iii) Acetylation
  - Amines
- (iv) Amino acids
  - Carboxylic acids
- (v) Glutathione conjugation (gly-cys-glu)
  - Halo-cpds, epoxides, arene oxides, quinone-imine

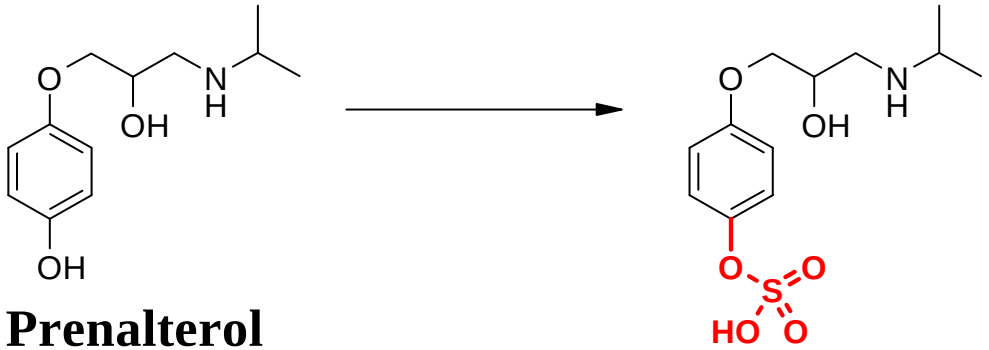
Examples of Phase II Metabolism

- Glucuronidation



Chloramphenicol (antibiotic)

- Sulphation



Prenalterol (β-blocker)

In vitro measurement of metabolism

- **Microsomes (species)**
  - **A subcellular fraction obtained by centrifugation of liver cells. Mainly composed of the endoplasmic reticulum**
  - ➢ **Perform Phase I reactions only**
- **Hepatocytes (species)**
  - **Isolated whole liver cells. (must be used fresh)**
  - **Harder to get hold of human hepatocytes**
  - ➢ **Capable of performing both Phase I and II reactions**

➢ **Purified metabolising enzymes can be prepared**

➢ **Rates of metabolism are generated**

➢ **Metabolite identification may be possible**

➢ **Extrapolation from *in vitro* to *in vivo* is possible (with caution!)**

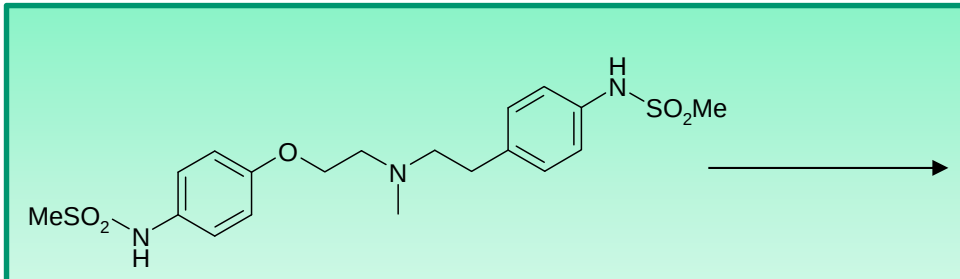
Metabolism

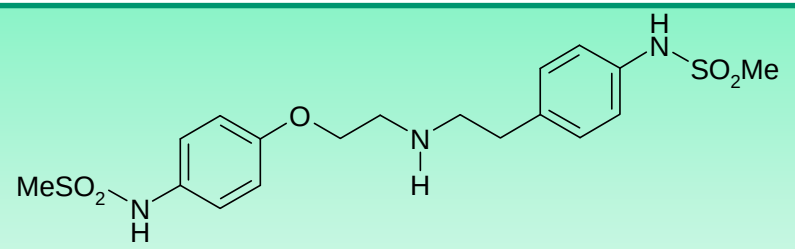
Identification of metabolites

Knowing the exact structure of major metabolite(s) is a powerful aid to the medicinal chemist

- metabolism can be blocked/ suppressed
- potential toxicity can be predicted
- predict if the same metabolites formed in human as rat/ dog

- Advances in LCMS, MS/MS and NMR have allowed minute quantities of metabolites to be identified
- *In vitro* liver preparations (microsomes, hepatocytes)
- *Ex vivo* analysis of plasma or tissue samples
- Analysis of urine/ faeces
- ***In vitro* and *in vivo* metabolite profiles may be different eg: dofetolide**





Only metabolite *in vitro*
One third of metabolites formed *in vivo*

What can *you* do?

The medicinal chemist can reduce metabolic clearance by altering chemical structure

- knowing what structural features or properties favour metabolism
- knowing/ predicting the structure of metabolites

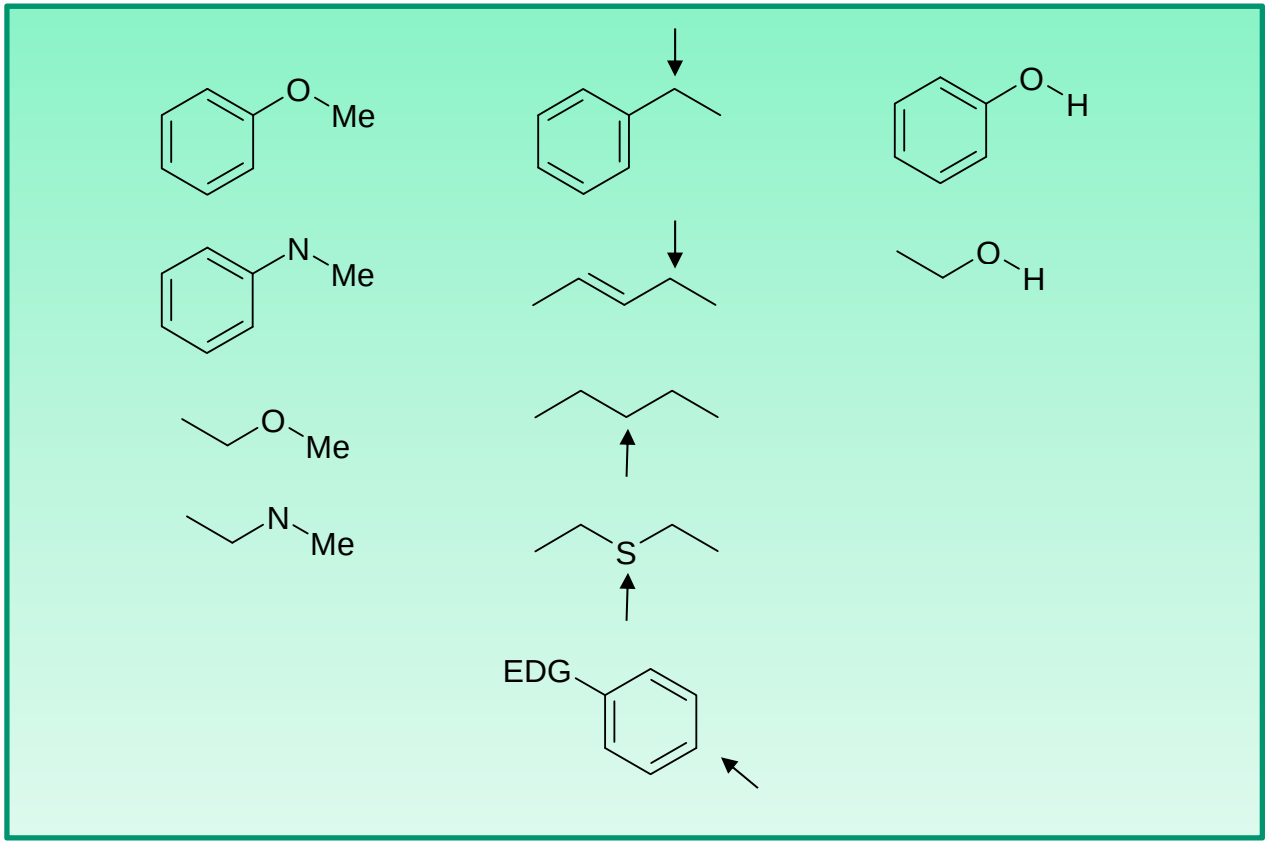
- **Lower the overall lipophilicity of a compound**
  - introduce polar atoms/ groups, basic or acidic groups
  - remove/ modify highly lipophilic regions (polyalkyl chains, unsubstituted aryl rings)

- **Block / sterically hinder sites of metabolism**

- **Remove reactive/ labile sites or replace with bioisoteres**

- **Make aryl rings more electron deficient**

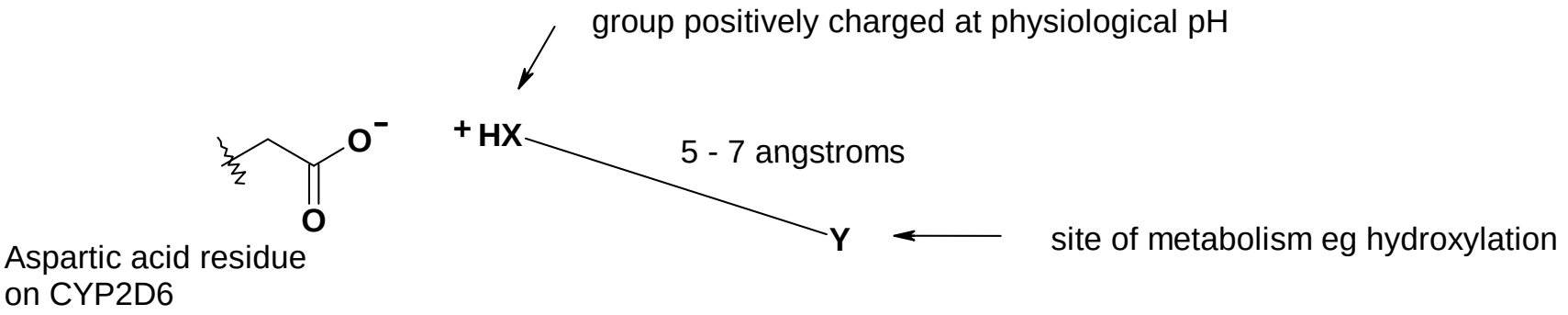
Summary of common metabolic soft spots

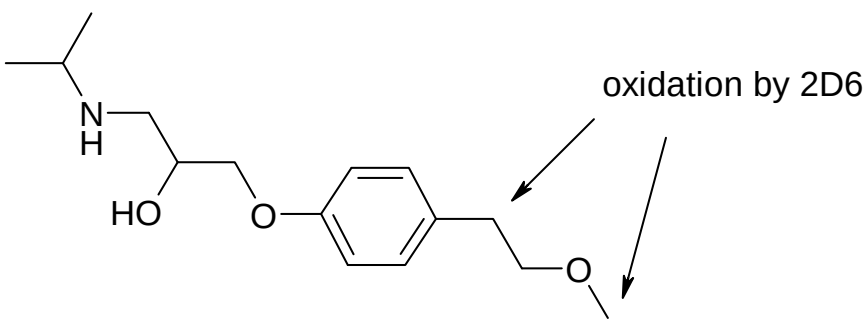


Block sterically (adjacent substituent or bigger than methyl)
or electronically (reduce/remove electron density) with halogens,
heteroatoms, EWGs

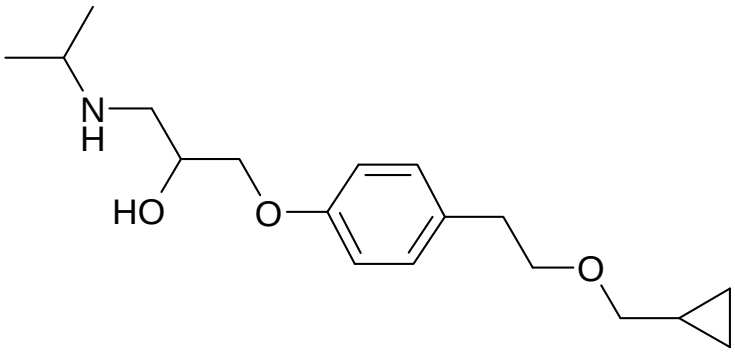
Metabolism by CYP2D6

Model of CYP2D6 and substrate





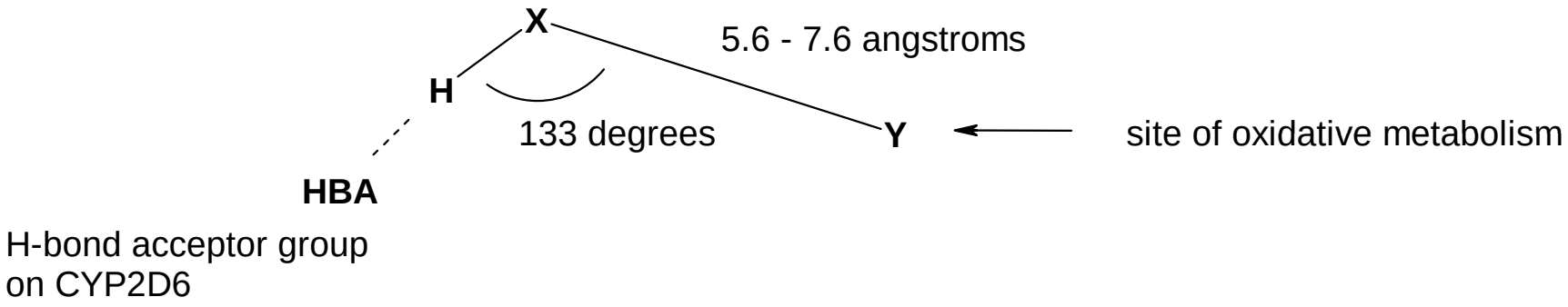
Metoprolol
38% oral bioavailability
Clearance 15 ml/min/kg

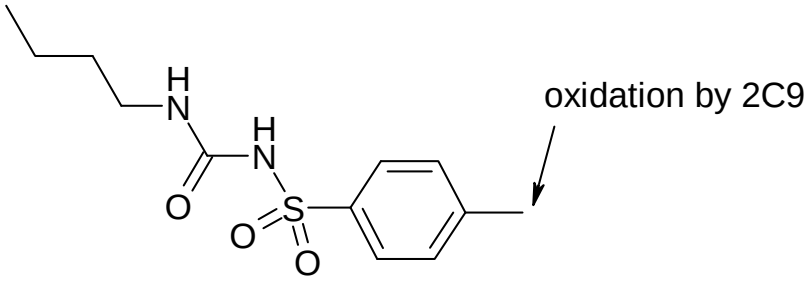


Betaxolol
89% oral bioavailability
Clearance 4.7 ml/min/kg

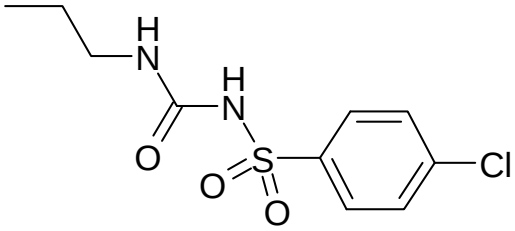
Metabolism by CYP2C9

Model of CYP2C9 and substrate





Tolbutamide
Half life = 5 hours

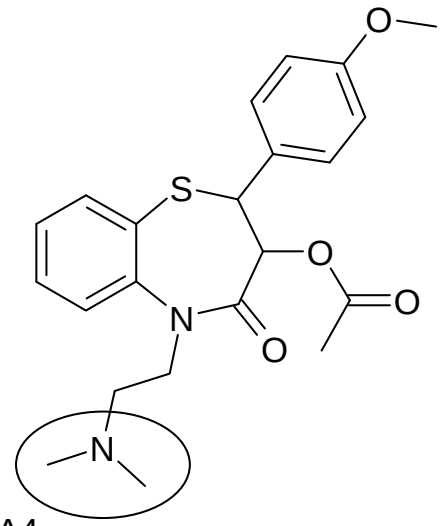


Chlorpropamide
Half life = 35 hours

Metabolism by CYP3A4

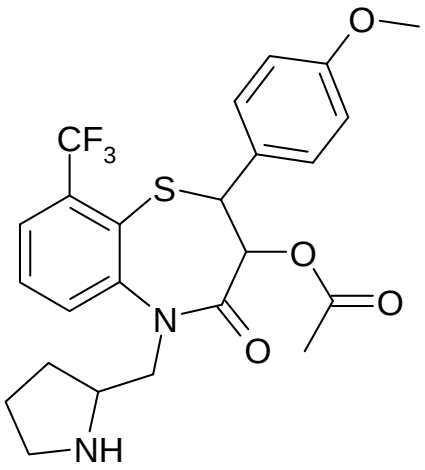
Substrates of CYP3A4 – lipophilic neutral or basic compounds

Sites of metabolism – allylic positions, nitrogen atoms (eg N-dealkylations)



N-dealkylation by CYP3A4

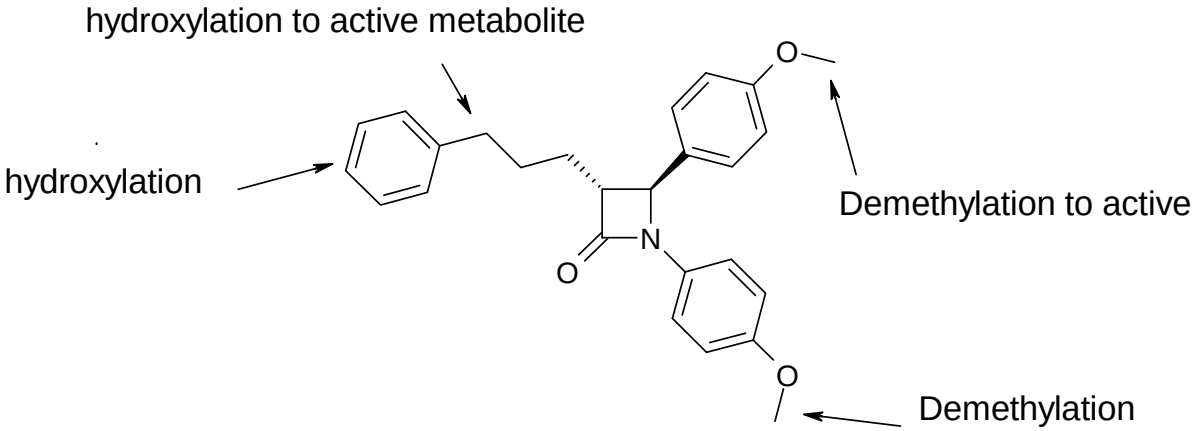
Diltiazem



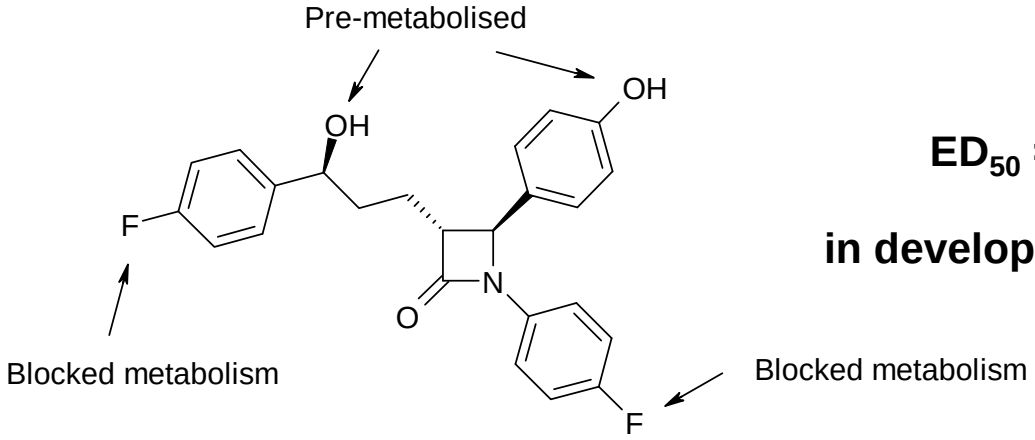
Reduced metabolism by 3A4

Use of metabolite identification to drive medicinal chemistry

Cholesterol absorption inhibitors (J. Med. Chem. 2004, 47, 1-9)



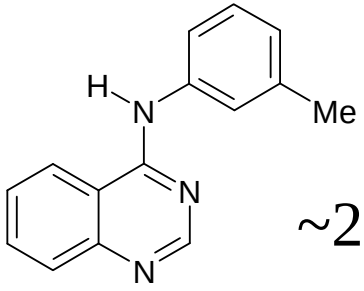
ED_{50} = 0.2 mg/Kg p.o. (Monkey)



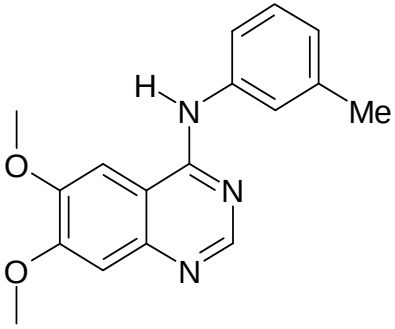
ED_{50} = 0.0005 mg/Kg p.o. (Monkey)
Ezetimibe
in development as cholesterol lowering agent

- Metabolites identified and synthesised
- Tested to identify active and inactive metabolites
- Sites of deactiviating metabolism blocked, sites of productive metabolism incorporated

Discovery of Iressa…

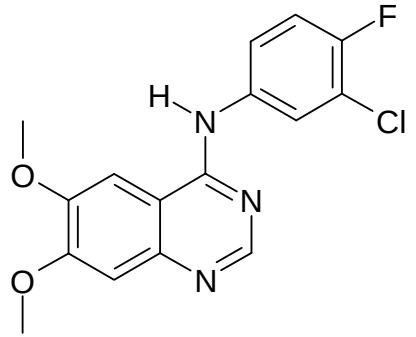


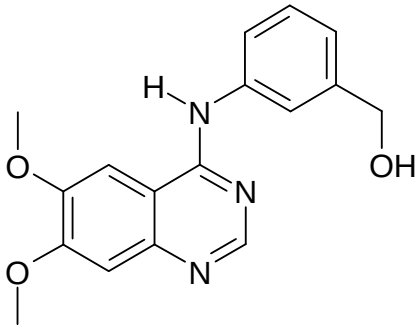
~200nM lead

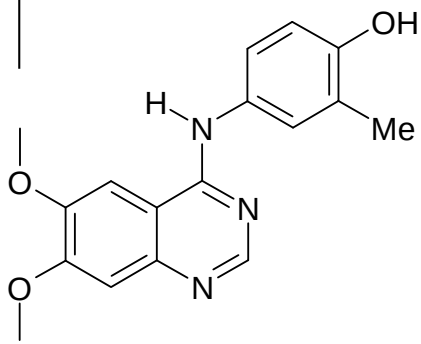


~5nM

Metabolised in vivo to

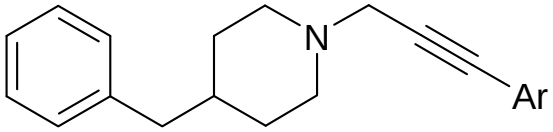


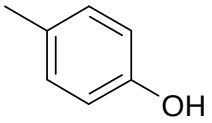
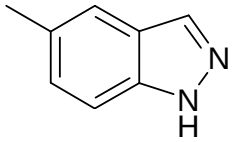
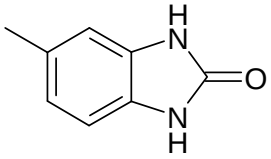




Blocking Phase II conjugation processes

- Exploration of phenol bioisosteres in a series of NMDA (NR1A/2B) receptor antagonists
- Phenol has low oral exposure and no oral activity due to extensive glucuronide formation
- Correctly placed phenol bioisostere is resistant to glucuronidation

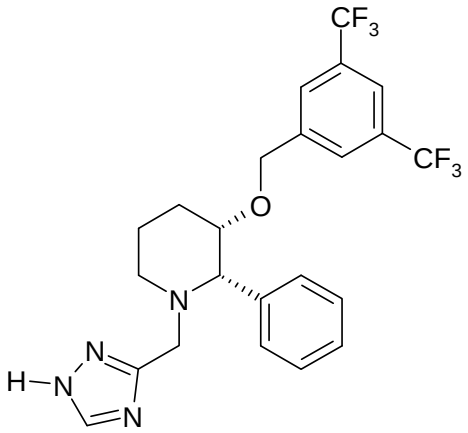


Ar	NR1A/2B IC_{50} nM	In vivo activity
	100	Inactive po
	38	NT
	5.0	active @ 10 mg/kg po

Brainteaser – NK-1 receptor antagonists

(J. Med. Chem. 1996, 39, 2907-2914 and J. Med. Chem. 1998, 41, 4607-4614)

How would you attempt to increase the duration of action of this lead compound?



CLUE: cLogD = 5.2

NK-1 IC_{50} = 0.18 nM
Biological effect at 8 hours (guinea pig): 55% inhibition @ 1mg/kg po
24 hours: 0%

CLUE: A major metabolite was identified as: