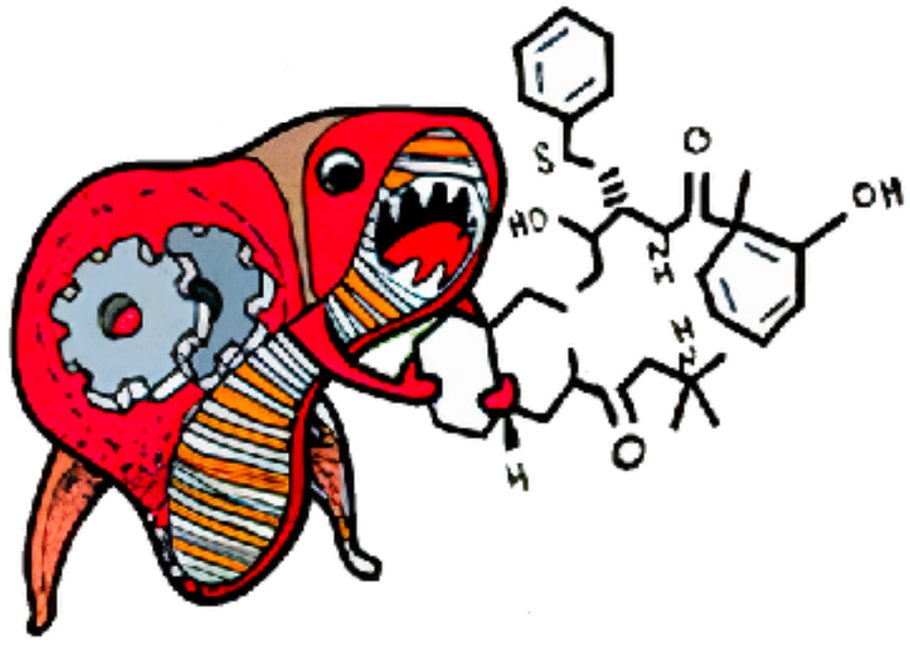
Drug Metabolism and Toxicity



Edna Mao

Group Meeting Literature Talk

October 13th, 2021

Drug discovery and development

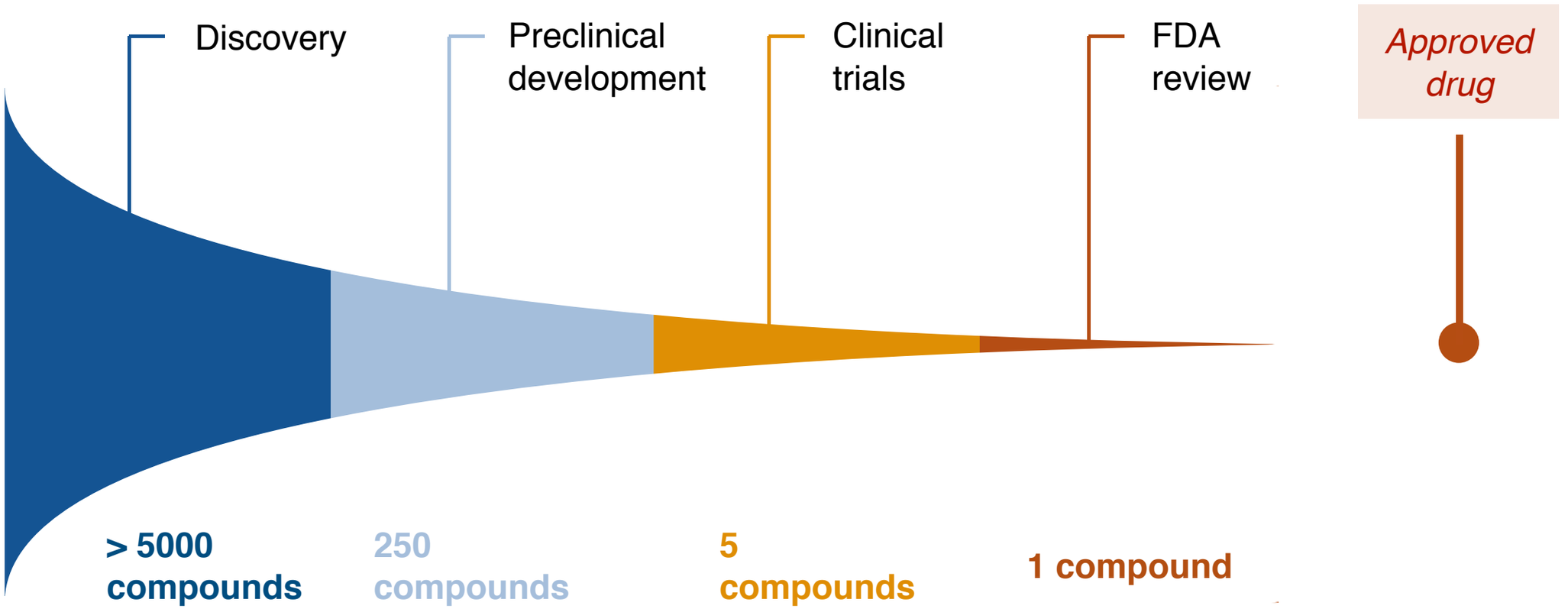
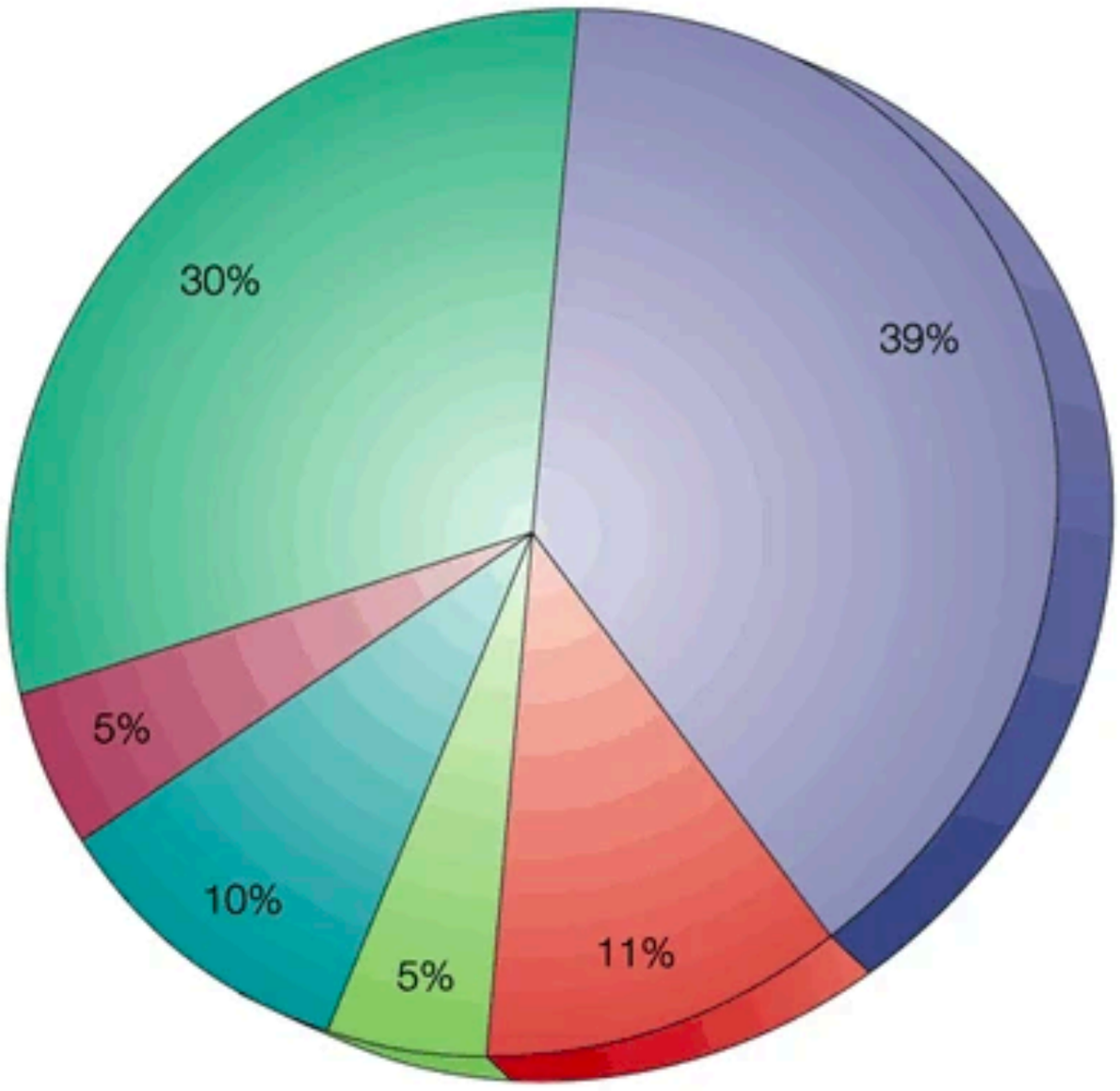


Image credit: Ian B. Perry

Reasons drugs fail in the pipeline



- Most common reason for failures in drug discovery attributed to pharmacokinetic properties
- Harmful toxicity another major concern in drug development

Van de Waterbeemd, H.; Gifford, E. *Nat. Rev. Drug. Discov.* **2003**, *2*, 192–204.

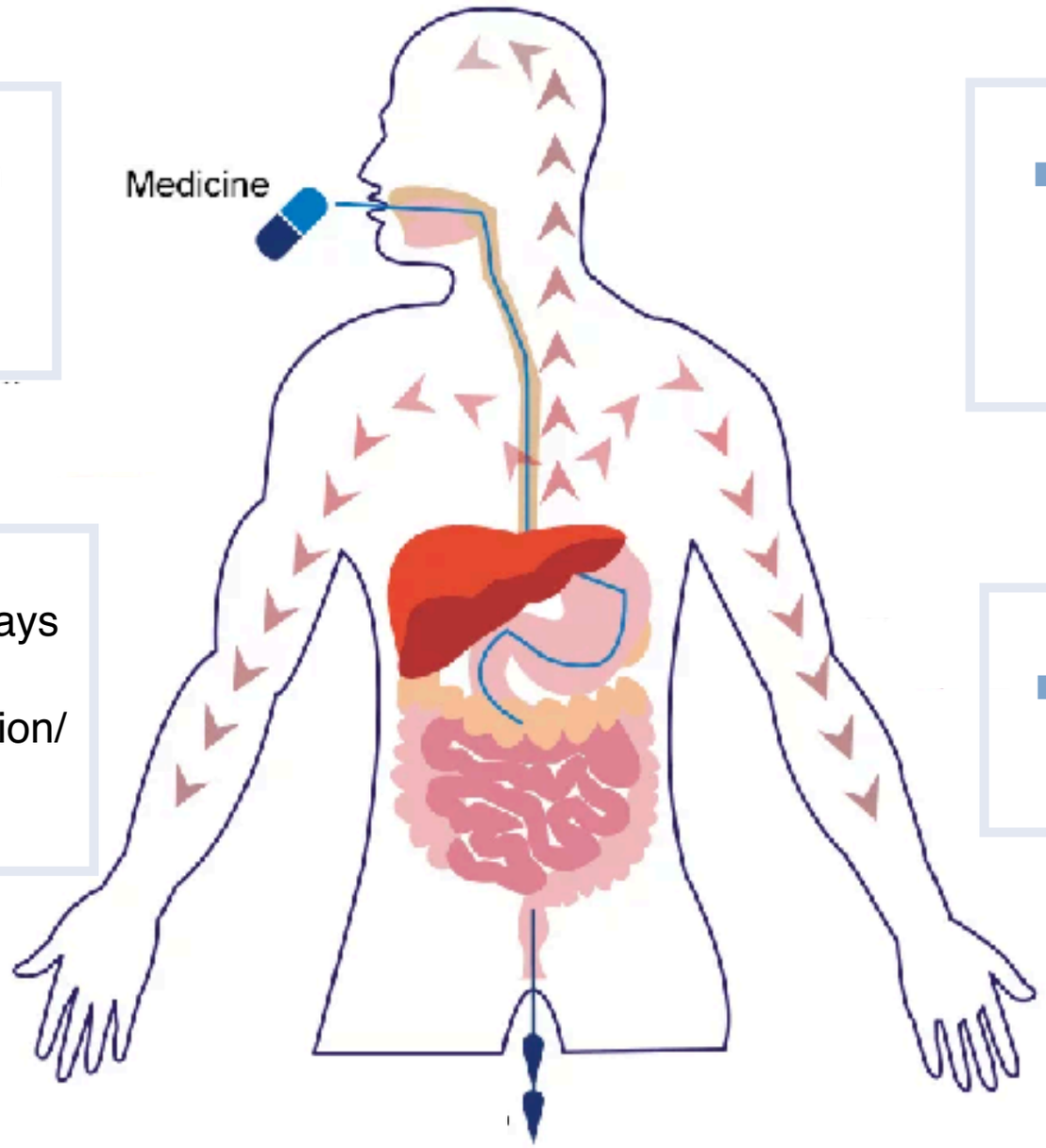
Fundamentals of pharmacokinetics

Pharmacokinetics

The study of the bodily processes that affect the movement of drugs across the body

Absorption

- Route of administration
- Bioavailability



Distribution

- Transfer of drug through body by bloodstream
- Sites of accumulation

Metabolism

- Biotransformation pathways
- Mechanism of detoxification/ active species formation

Excretion

- Method and rate of drug clearance from body

Outline

Introduction to drug metabolism

- Common biotransformations

Metabolism-induced toxicity

- Mechanisms of adverse reactions
  - Reactive intermediates and structural alerts

Applications to drug design

- Designing around metabolism

Applications to drug design

- Designing around drug-induced toxicity
- Challenges in predicting adverse effects

Outline

Introduction to drug metabolism

- Common biotransformations

Metabolism-induced toxicity

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Applications to drug design

- Designing around metabolism

Applications to drug design

- Designing around drug-induced toxicity
- Challenges in predicting adverse effects

Introduction to drug metabolism

Xenobiotic metabolism

The chemical alteration of non-endogenous compounds within the body

The early studies of metabolism

1841-1842

- Ure and Keller independently ingest benzoic acid
- Observed compound in urine in “copious amounts”
- Compound similar to benzoic acid, but contained nitrogen

1845

- Compound identified by Dessaignes as hippuric acid (glycine–benzoic acid conjugate)
- Glycine conjugation first metabolic reaction to be described!

1848-1893

- Methylation, aromatic oxidation, and conjugation reactions characterized
- Mechanisms considered “absolutely puzzling”, a “biochemical curiosity”

Early 1900’s

- Metabolism found to occur primarily in liver
- Liver microsome isolated in 1943 by Claude

1945

- Lipmann elucidates role of coenzyme A in acetylation reactions
- Discovery wins Nobel Prize in Medicine in 1953

1947

- Richard Williams publishes “Detoxification mechanisms”, solidifying drug metabolism as a defined field of study

1960’s

- Discovery and characterization of cytochrome P450’s and their role in drug metabolism

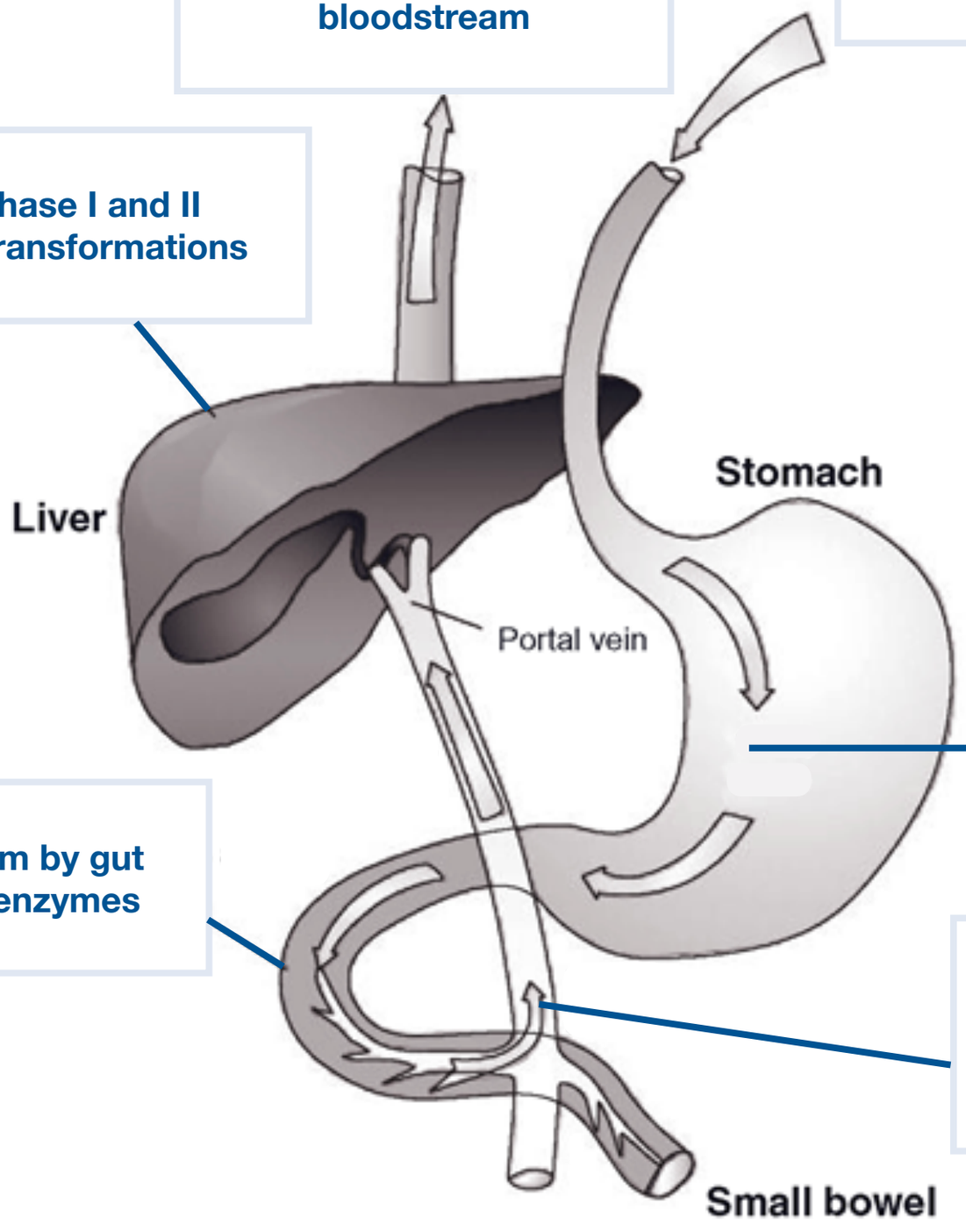
King, R. S. *Drug Metabolism Handbook: Concepts and Applications.;* John Wiley & Sons, 2009; pp 3–9.

First pass metabolism

Fraction of dose enters bloodstream

Orally administered drug

Phase I and II biotransformations



The concentration of orally administered drugs is greatly reduced by “first pass metabolism” before reaching systemic circulation

Metabolism by gastrointestinal enzymes

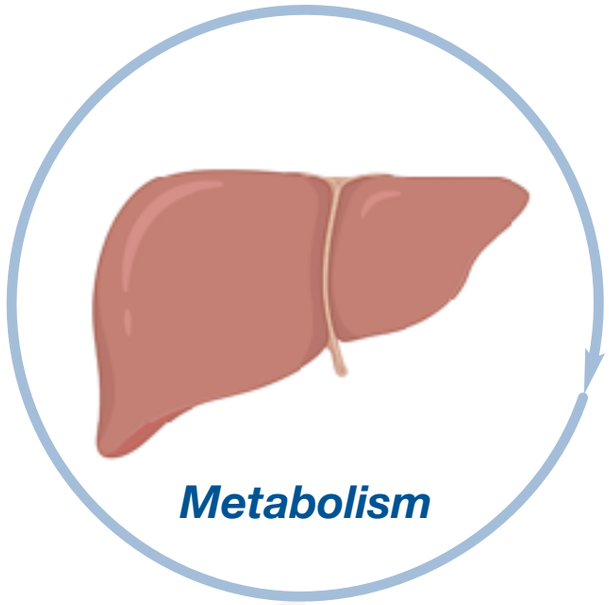
Metabolism by gut bacterial enzymes

All orally administered drugs pass through portal vein into liver before distribution to the rest of body

Ionescu, C.; Caira, M. R.; *Drug Metabolism: Current Concepts.;* Springer, 2005.

Phases of drug metabolism

Drug molecule
Lipophilic, not excretable



Modified compound
Polar, hydrophilic

Phase 1: Modification

Introducing functional handles to drug

Phase 2: Conjugation

Attaching highly hydrophilic groups

Excretion
Bile, urine

Phases of metabolism

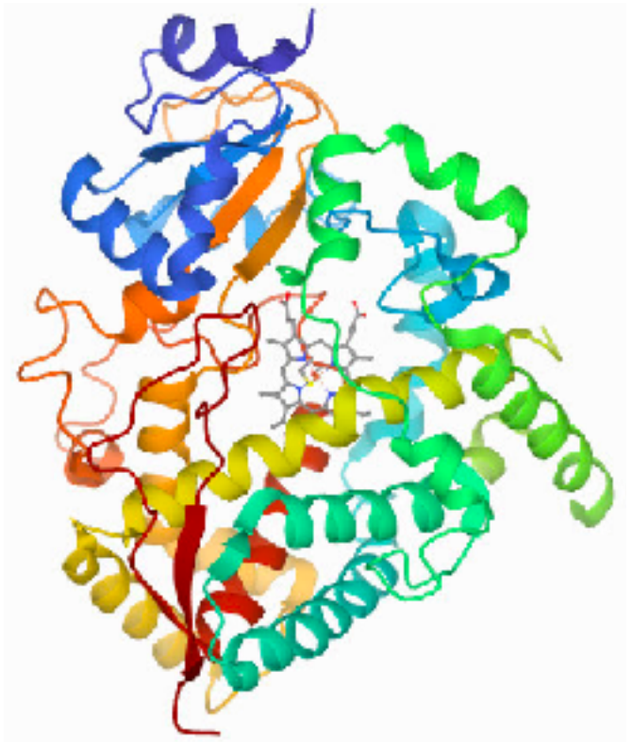
Phase I metabolism

- Initial modifications to drug molecules
- Adding or uncovering reactive functional groups
- Mostly facilitated by CYP enzymes in liver

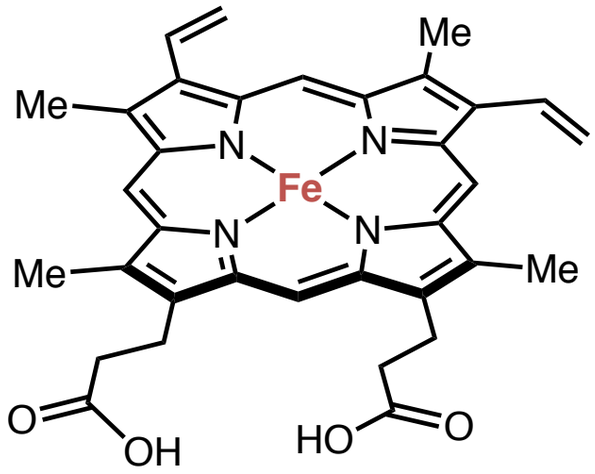
Phase II metabolism

- Conjugation of polar functional groups, usually resulting in deactivation
- Facilitated by wide variety of transferase enzymes
- Conjugated molecules are recognized by transporters and excreted

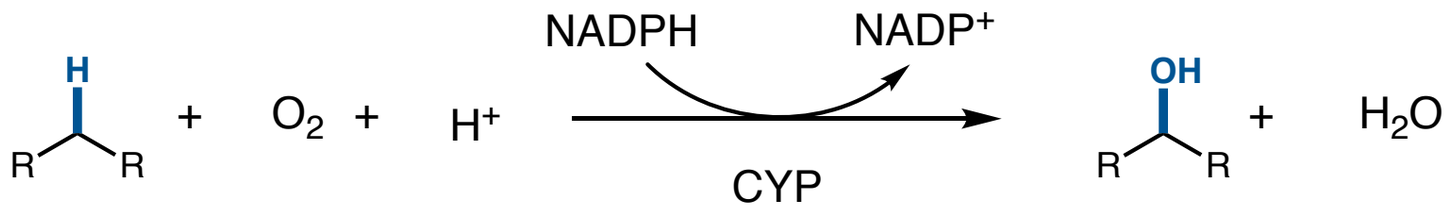
Cytochrome P450s



Superfamily of heme-containing monooxygenase enzymes

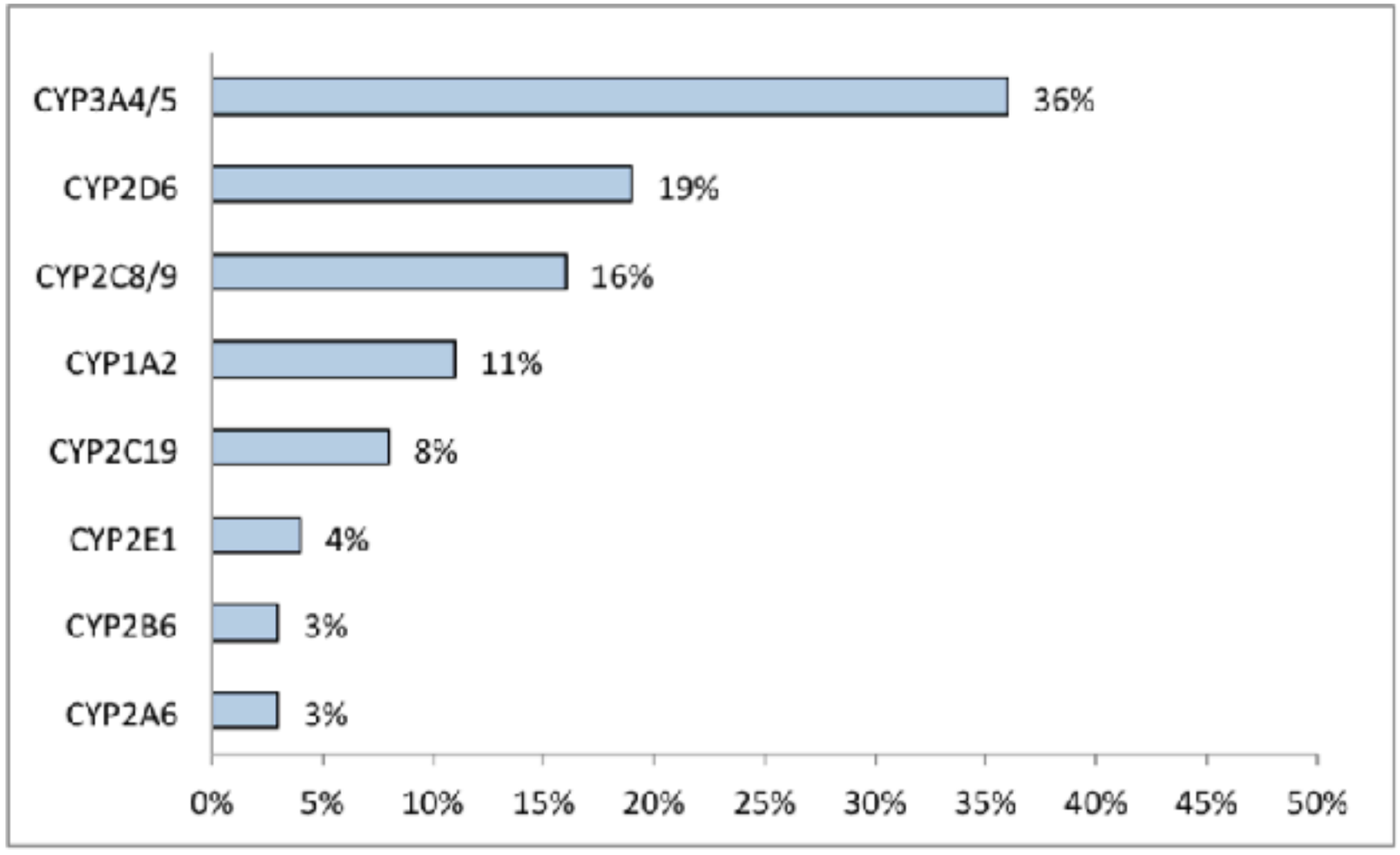


Heme B



Ionescu, C.; Caira, M. R.; *Drug Metabolism: Current Concepts.;* Springer, 2005.

The Cytochrome P450-dependent mixed-function oxidase system



- In human microsomes, P450s found on in mitochondrial and endoplasmic reticulum membranes
- CYP enzymes responsible for 75% of metabolism

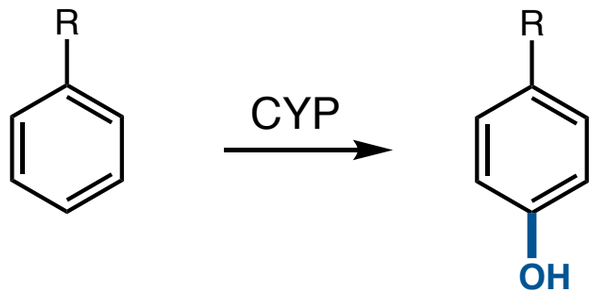
Other enzymes that facilitate metabolism

- Flavin-containing monooxygenases (FMO)
- Alcohol dehydrogenase (ALD)
- Aldehyde oxidases (AO)
- Monoamine oxidases (MAO)

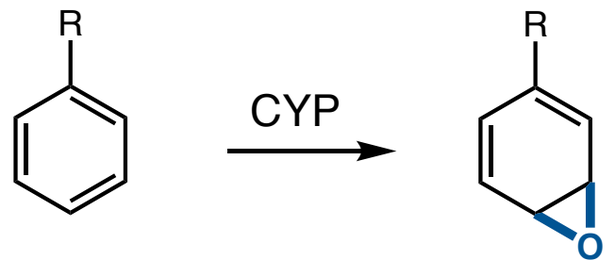
Ionescu, C.; Caira, M. R.; *Drug Metabolism: Current Concepts.;* Springer, 2005.

Phase I biotransformations - Oxidations at carbon centers

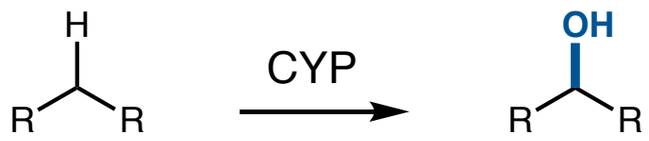
Aromatic hydroxylation



Epoxidation

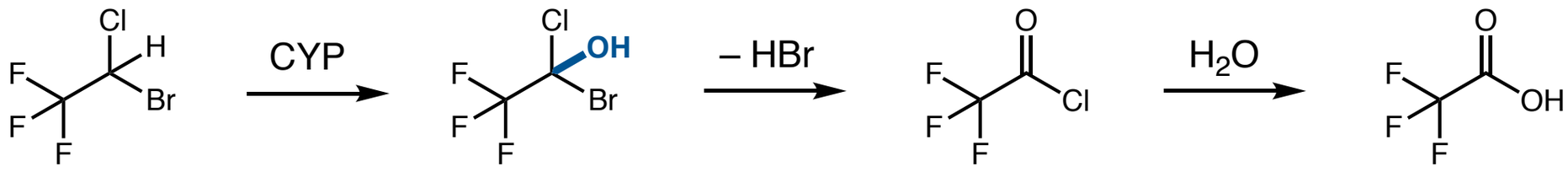


Aliphatic hydroxylation



Alcohol oxidation

Oxidative dehalogenation

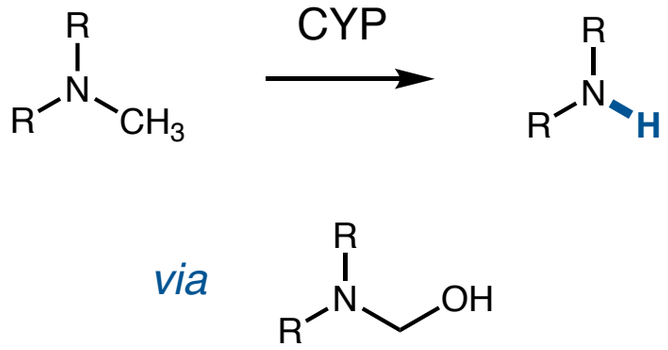


Halothane

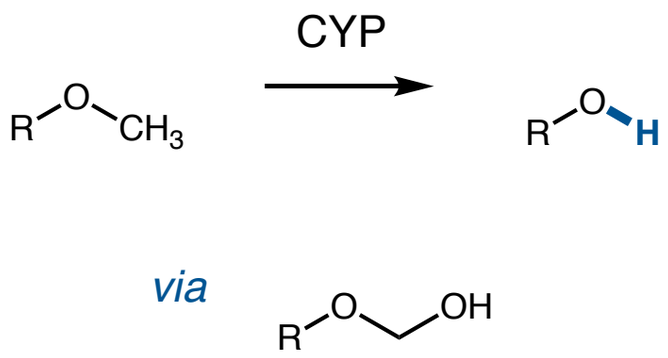
Ionescu, C.; Caira, M. R.; *Drug Metabolism: Current Concepts.;* Springer, 2005.

Phase I biotransformations - Oxidations at heteroatoms

N-dealkylation

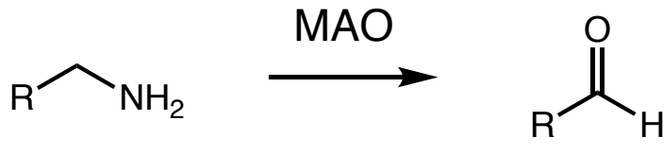


O-dealkylation



N-oxidation

Deamination



- Responsible for the inactivation of endogenous neurotransmitters
- Targeted by class of antidepressant and anti-anxiety medications known as “MAO inhibitors”

Ionescu, C.; Caira, M. R.; *Drug Metabolism: Current Concepts.;* Springer, 2005.

Phases of metabolism

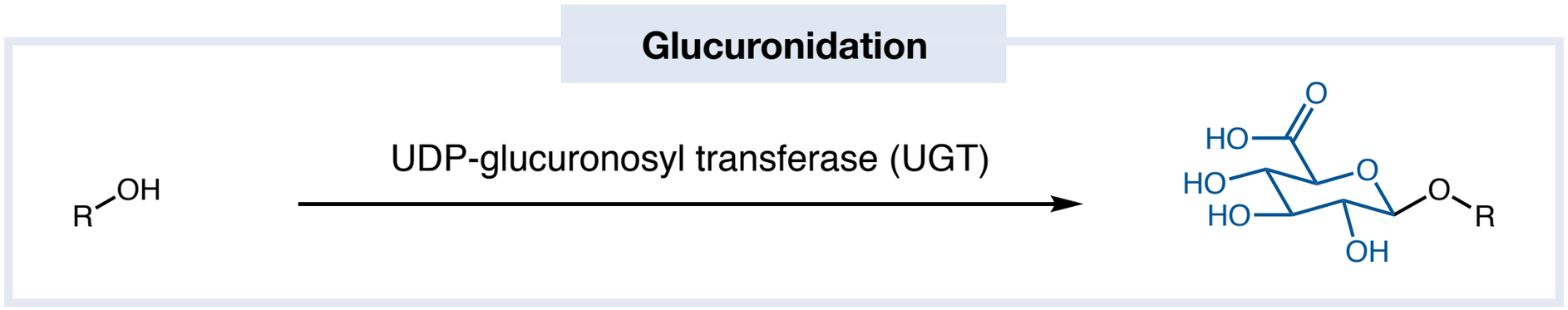
Phase I metabolism

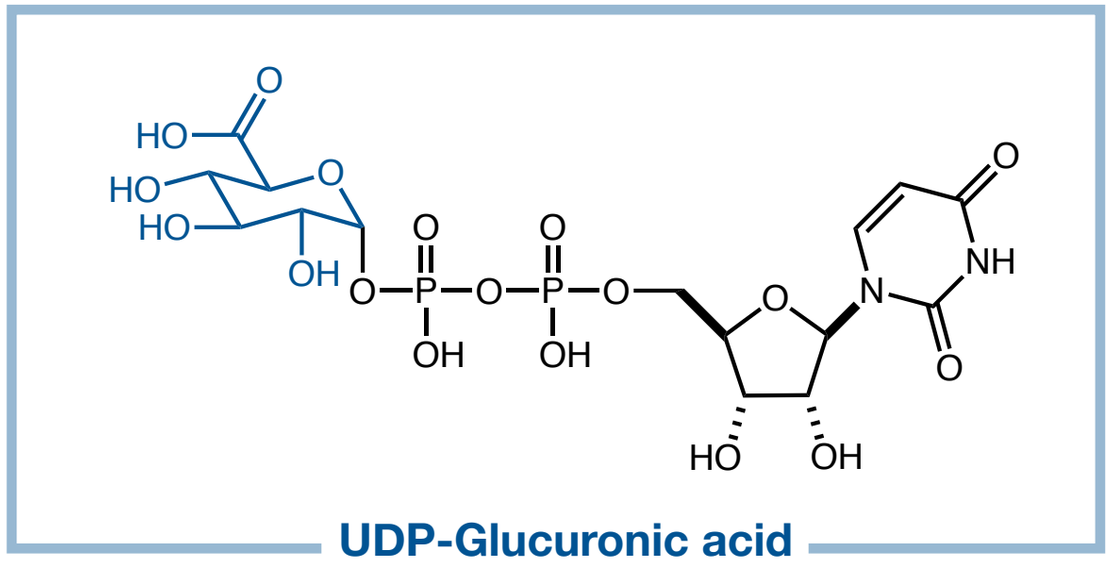
- Initial modifications to drug molecules
- Adding or uncovering reactive functional groups
- Mostly facilitated by CYP enzymes in liver

Phase II metabolism

- Conjugation of polar functional groups, usually resulting in deactivation
- Facilitated by wide variety of transferase enzymes
- Conjugated molecules are recognized by transporters and excreted

Phase II conjugations: glucuronidation

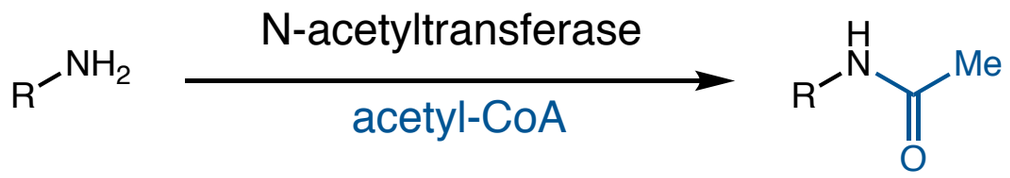




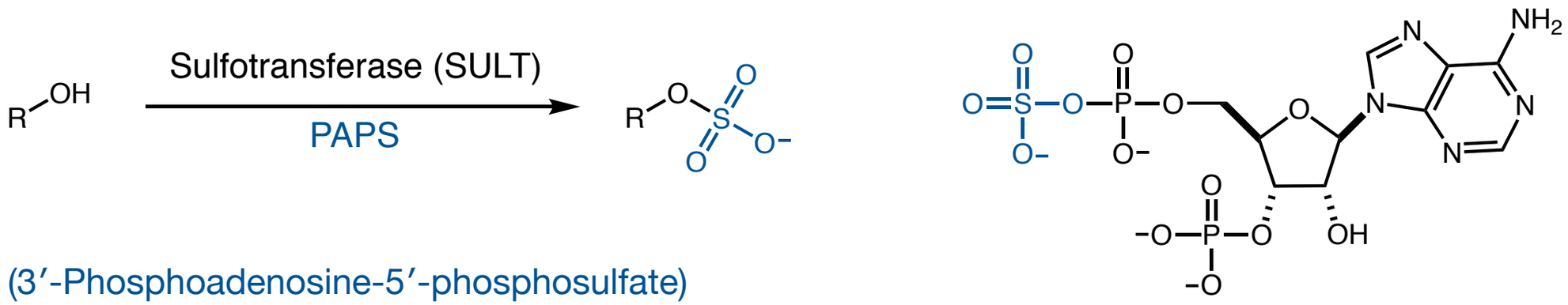
Ionescu, C.; Caira, M. R.; *Drug Metabolism: Current Concepts.;* Springer, 2005.

Phase II conjugations

Acetylation

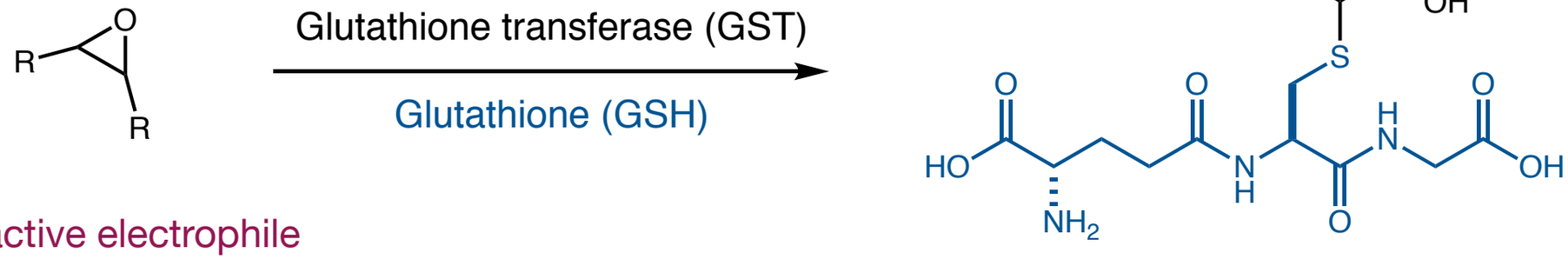


Sulfation



(3′-Phosphoadenosine-5′-phosphosulfate)

Glutathione conjugation

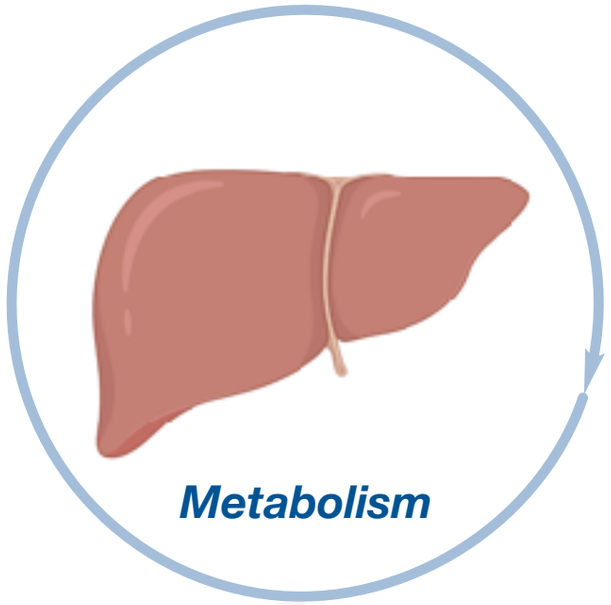


Reactive electrophile

Ionescu, C.; Caira, M. R.; *Drug Metabolism: Current Concepts.;* Springer, 2005.

Phases of drug metabolism

Drug molecule
Lipophilic, not excretable



Modified compound
Polar, hydrophilic

Phase 1: Modification

Introducing functional handles to drug

Phase 2: Conjugation

Attaching highly hydrophilic groups

Excretion
Bile, urine

Implications of metabolism on drug design

Drugs need to reach their targeted site and remain in the system for a meaningful amount of time in order to elicit the desired pharmacological effects.

Pharmacokinetic properties which are impacted by metabolism:

- Clearance
- Half life
- Bioavailability

Prodrugs

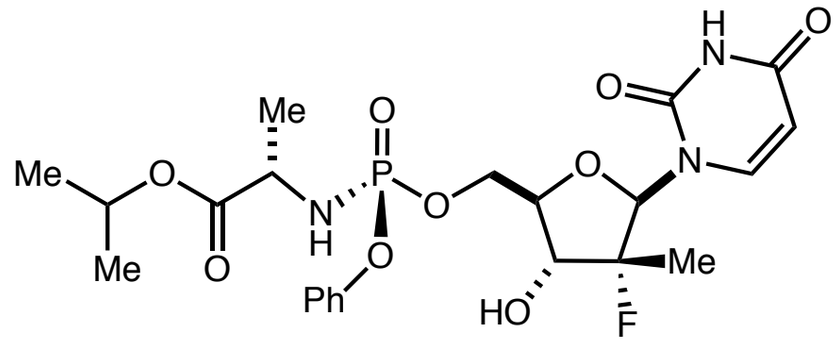
Prodrugs: a class of drugs administered in a pharmacologically inactive form, which upon administration is enzymatically converted into its active form *in vivo*

Prodrug strategy can overcome challenges in :

- Absorption
- Route of administration
- Low bioavailability

Case study:
Sofosbuvir (Pharmasset/Gilead)

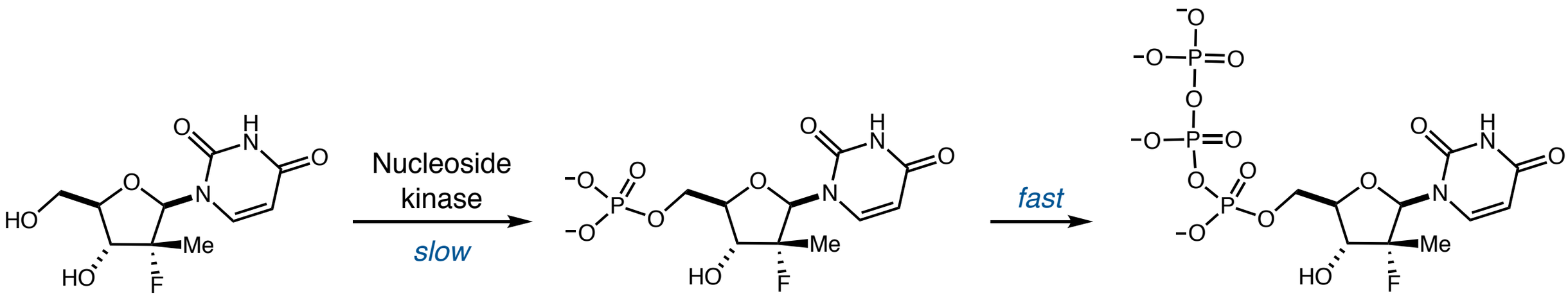
- Approved in 2013 for hepatitis C
- WHO List of Essential Medications
- Nucleotide prodrug (ProTide)



Sofosbuvir

The ProTide approach: Sofosbuvir

Nucleoside analogue drugs tend to suffer from low potency due to slow first phosphorylation



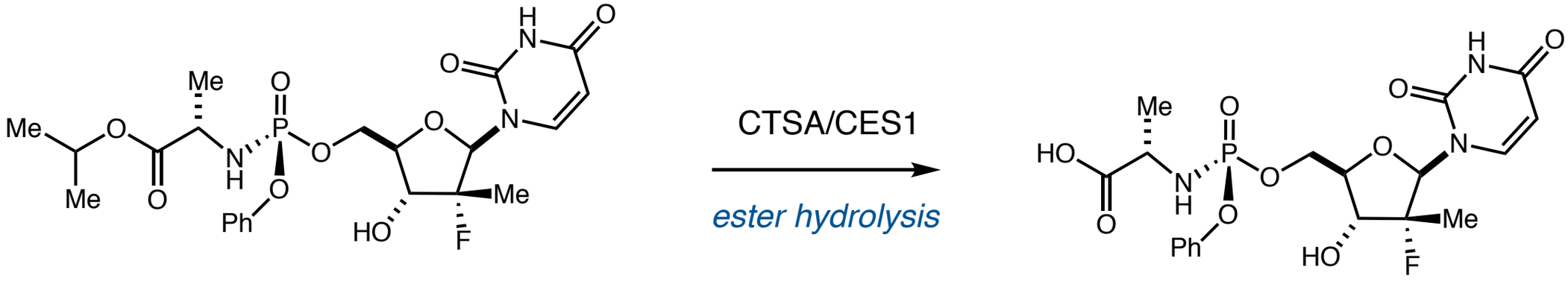
Nucleoside **Nucleoside monophosphate** **Nucleoside triphosphate**

Active form of drug

Nucleosides delivered as the monophosphate suffer from poor absorption and bioavailability due to charged phosphate

Rautio, J.; Meanwell, N. A.; Di, L.; Hageman, M. J. *Nat. Rev. Drug. Discov.* **2018**, *17*, 559–587.

The ProTide approach: Sofosbuvir

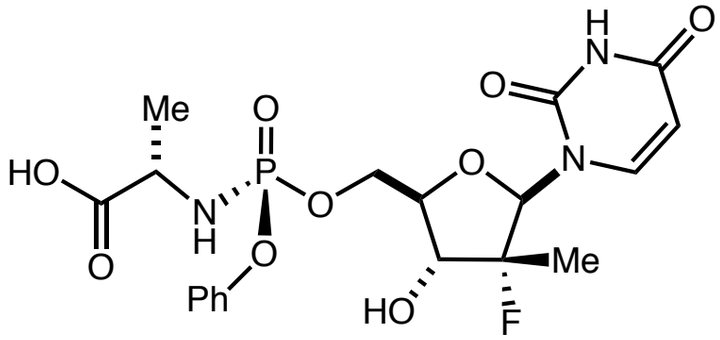


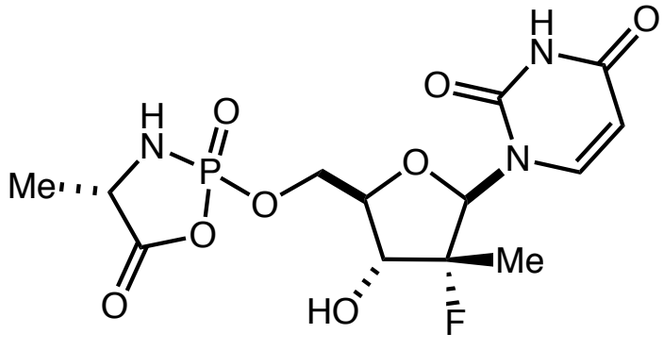
Sofosbuvir

- Phosphate group masked
- Effectively distributed to hepatocyte cytosol (cells expressing high levels of CTSA/CES1)
- ProTide hydrolyzed intracellularly (no metabolite detected in plasma)

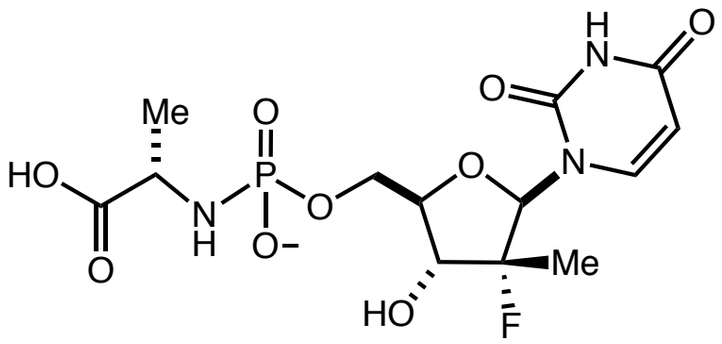
Rautio, J.; Meanwell, N. A.; Di, L.; Hageman, M. J. *Nat. Rev. Drug. Discov.* **2018**, *17*, 559–587.

The ProTide approach: Sofosbuvir

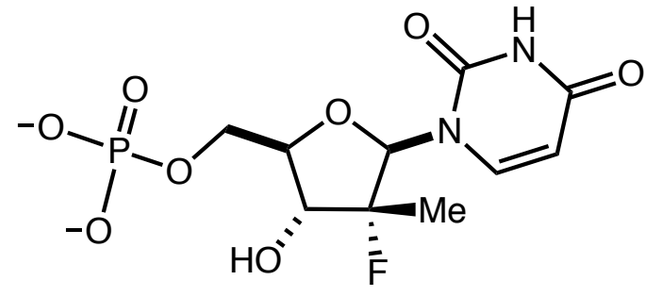




–PhOH

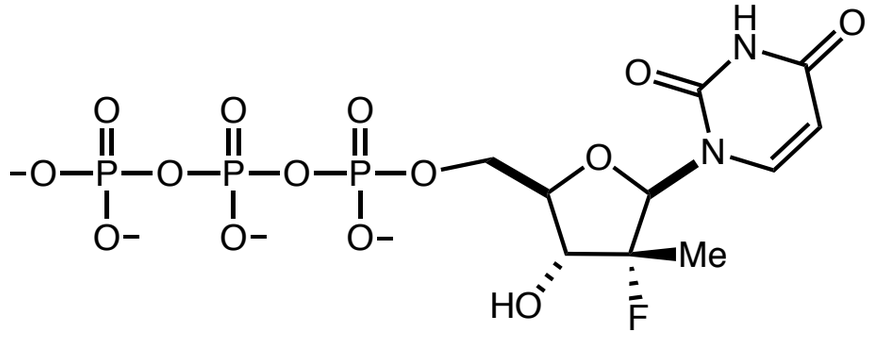


H_2O



HINT1

UMP-CMP kinase,
NDPK



Sofosbuvir triphosphate

- Recognized by HCV NS5B polymerase
- Incorporated into RNA strand, leading to chain growth termination

Rautio, J.; Meanwell, N. A.; Di, L.; Hageman, M. J. *Nat. Rev. Drug. Discov.* **2018**, *17*, 559–587.

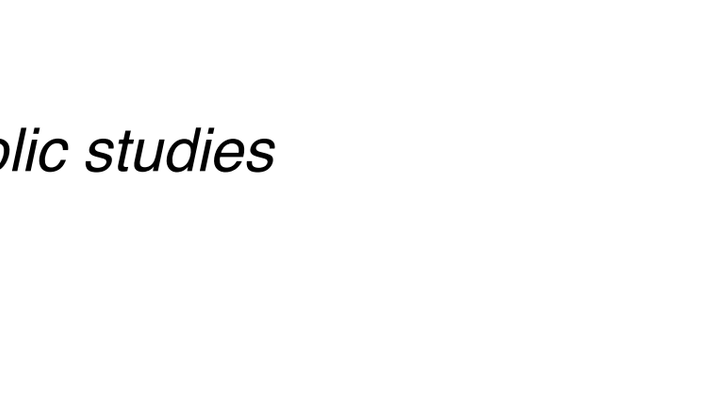
Lessons learned from metabolic studies

Information from metabolic studies can be used for rational drug design

Case study:

Ezetimibe (Schering-Plough/Merck)

- Approved in 2002 for hypercholesterolemia
- 125th most prescribed medication in 2018

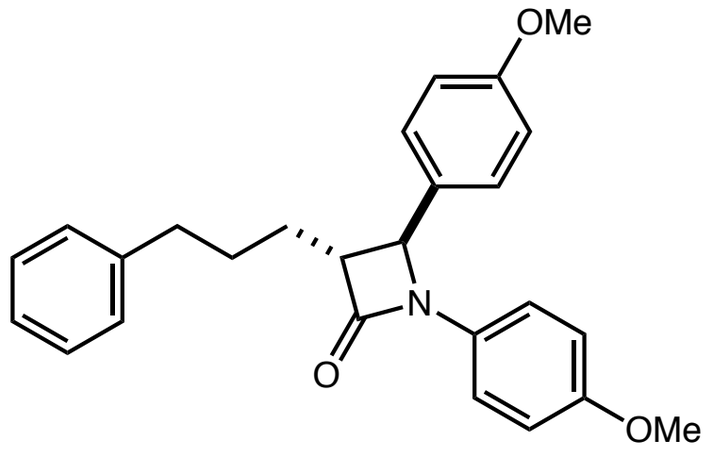


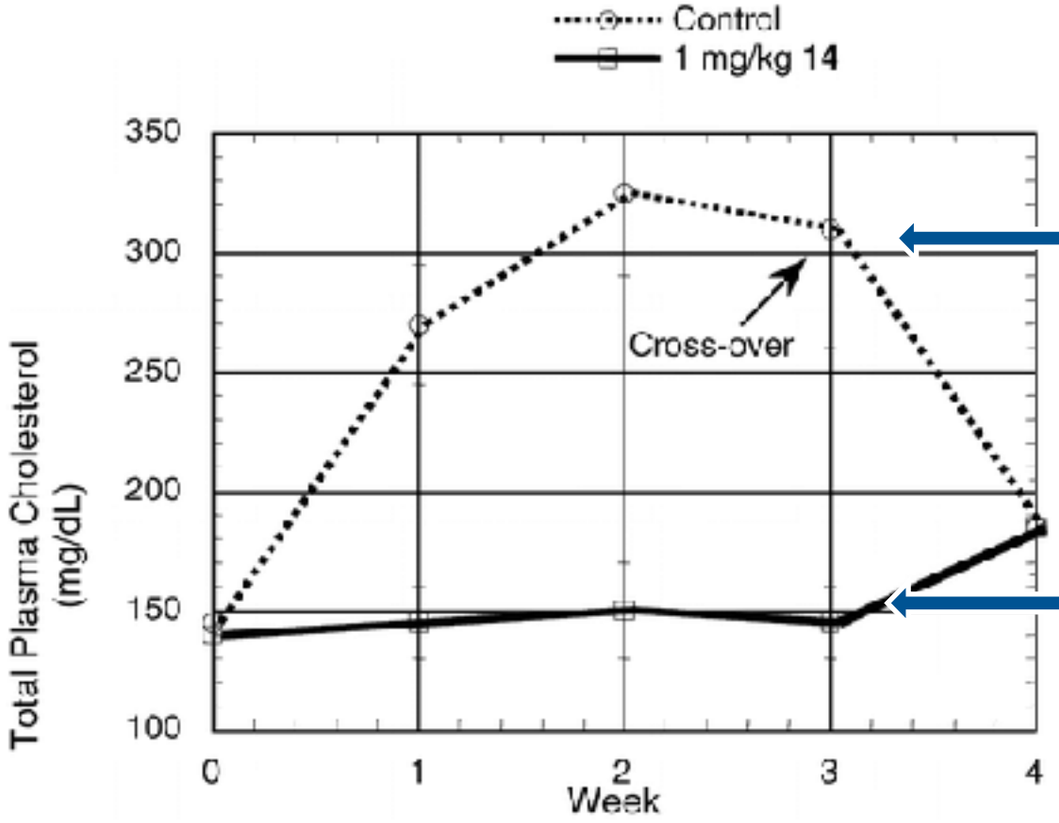
Lead compound: SCH-48461

Clader, J. W. *J. Med. Chem.* **2004**, *47* (1), 1–9.

Case study: the development of Ezetimbe

Lead compound: SCH-48461



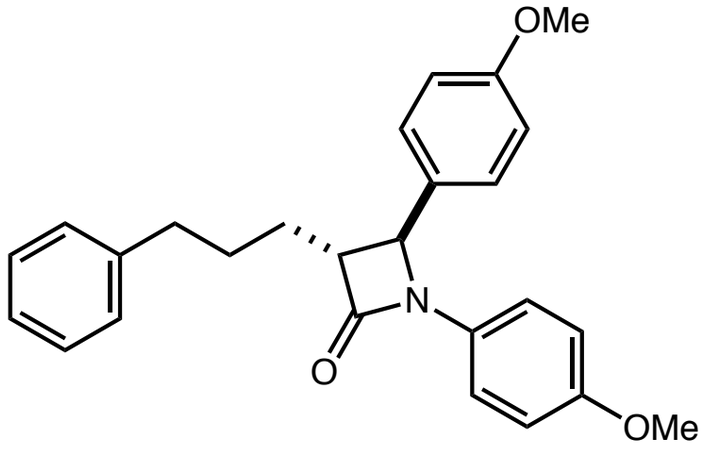


Initial results with cholesterol-fed hamsters showed promising efficacy

- Control group showed no decrease in plasma cholesterol until dosed with compound in week 3
- Cholesterol-fed hamsters dosed with 1 mg/kg compound displayed a lower total plasma cholesterol level
- Dosed group showed increase in plasma cholesterol when dosing stopped at week 3

Clader, J. W. *J. Med. Chem.* **2004**, *47* (1), 1–9.

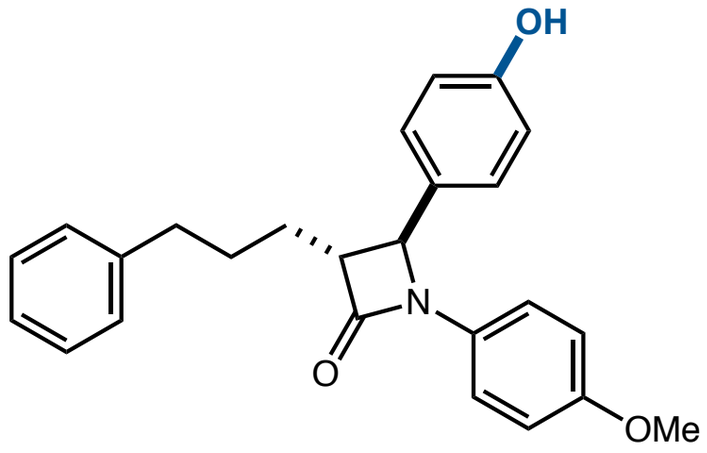
Case study: the development of Ezetimbe



Lead compound
ED_{50} = 2.2 mg/kg/day

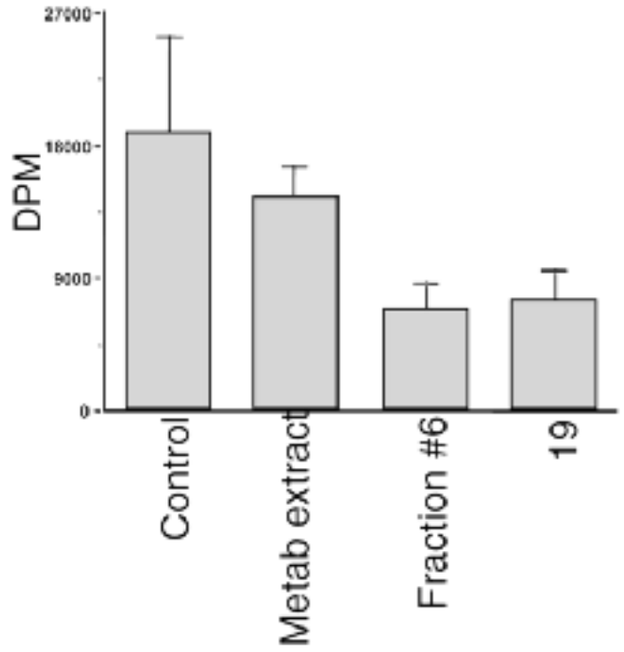
Compound is rapidly metabolized *in vivo,* resulting in poor half life

Relatively high dose needed



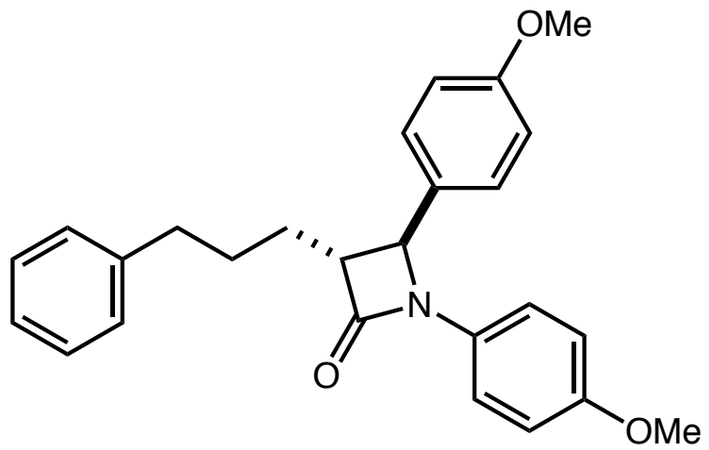
Metabolite isolated from bile (19)

- Hamsters fed ^{14}C cholesterol
- ^{14}C cholesterol levels in plasma measured
- Demethylated metabolite accounted for majority of activity



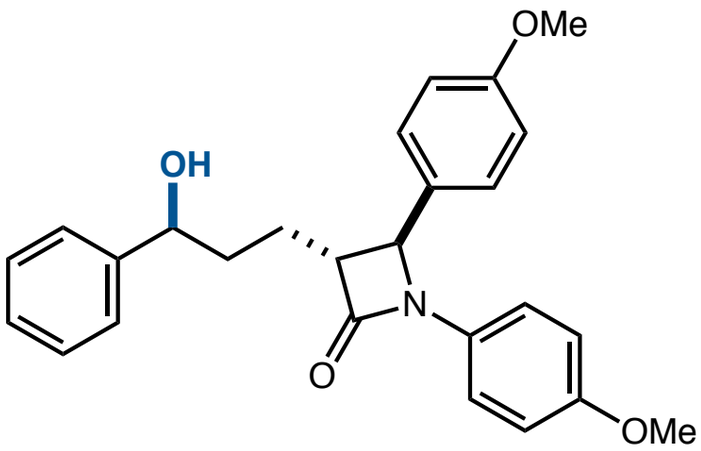
Rosenblum, S. B., *et. al. J. Med. Chem.* **1998,** *41* (6), 973–980.
Clader, J. W. *J. Med. Chem.* **2004**, *47* (1), 1–9.

Case study: the development of Ezetimbe

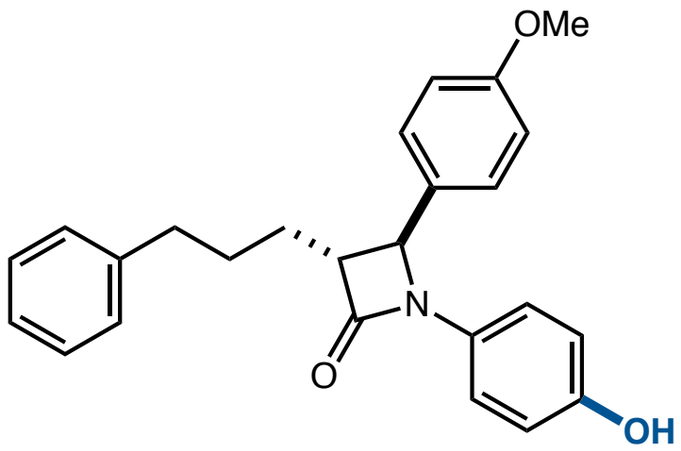


Lead compound
ED_{50} = 2.2 mg/kg/day

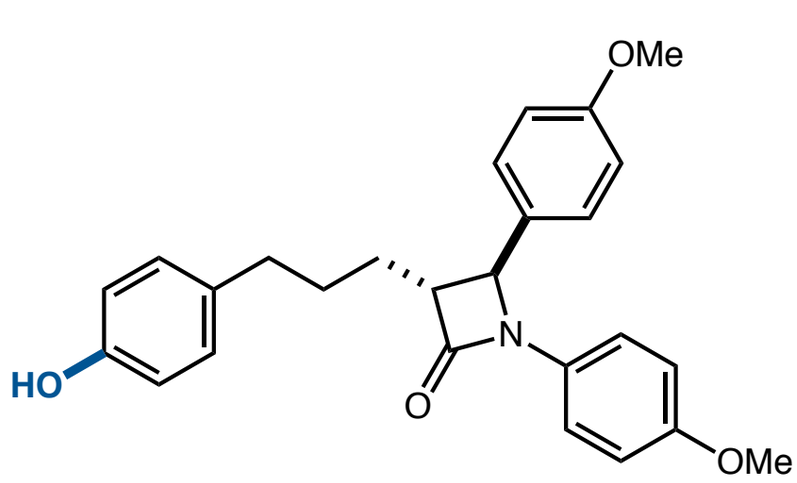
SAR studies



_(S)_-benzylic hydroxylation
ED_{50} = 0.9 mg/kg/day
2x more potent than lead
5x more potent than (R)-hydroxylation



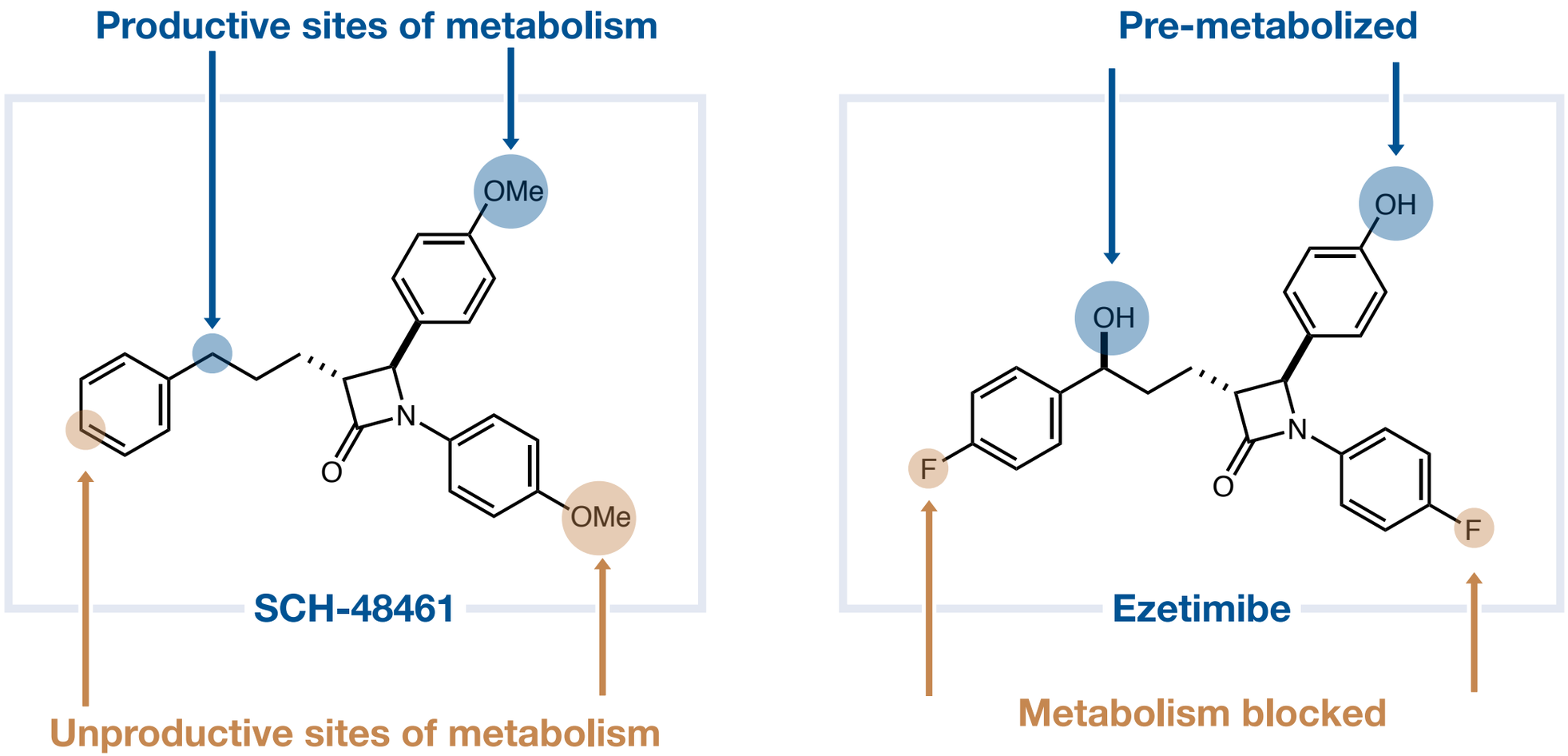
N-Aryl hydroxylation
-78% LCE @ 50 mg/kg/day
Slight decrease in activity



C3 Aryl hydroxylation
-16% LCE @ 50 mg/kg/day
Large decrease in activity

Rosenblum, S. B., *et. al. J. Med. Chem.* **1998,** *41* (6), 973–980.
Clader, J. W. *J. Med. Chem.* **2004**, *47* (1), 1–9.

Case study: the development of Ezetimbe



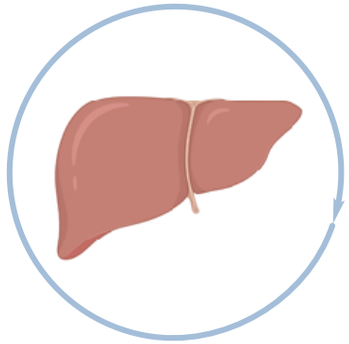
ED_{50} (mg/kg)		
2.2	**Hamster**	0.04
0.1	**Dog**	0.007
0.2	**Monkey**	0.0005

Rosenblum, S. B., *et. al. J. Med. Chem.* **1998,** *41* (6), 973–980.

Outline

Introduction to drug metabolism

- Common biotransformations



Metabolism-induced toxicity

- Mechanisms of adverse reactions
  - Reactive intermediates and structural alerts

Applications to drug design

- Designing around metabolism

Applications to drug design

- Designing around drug-induced toxicity
- Challenges in predicting adverse effects

Adverse drug reactions (ADRs)

Adverse drug reaction:

a response to a drug that is **noxious**, **unintended** and **occurs at doses normally used in man**

Type A

- Associated with primary pharmacology of drug
- Usually detected in animal models/clinical trials
- Can be mitigated through dose adjustments

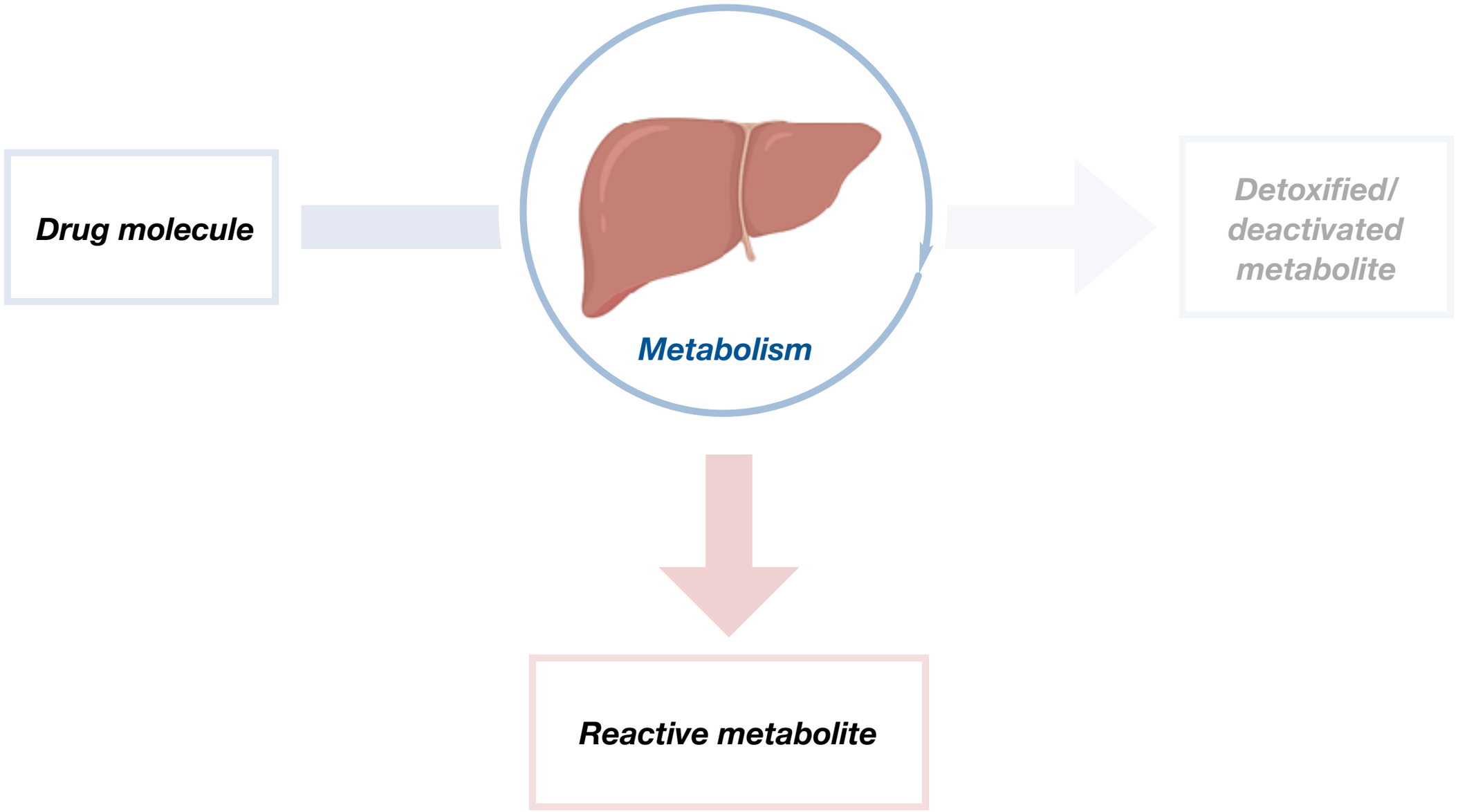
Type B (idiosyncratic ADRs)

- Not associated with primary pharmacology, mechanism usually not understood
- Does not occur at any dose for most people
- Poor correlation with animal models
- Often not detected until drug has been exposed to a large population of patients

Attia, S. M. *Oxid. Med. Chem. Longev.* **2010,** *3* (4), 238–258.

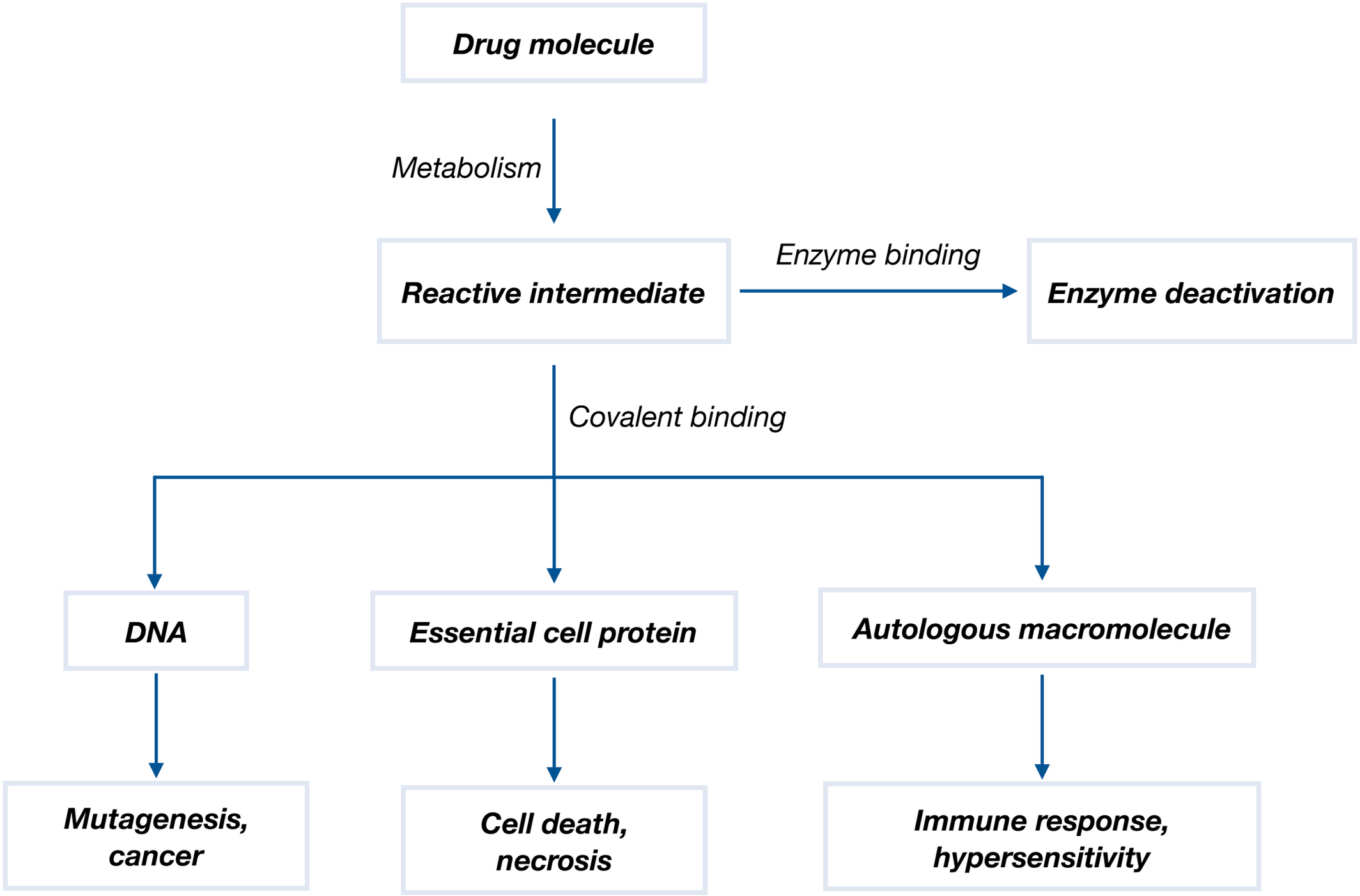
Bioactivation to reactive metabolites

Bioactivation to reactive metabolites

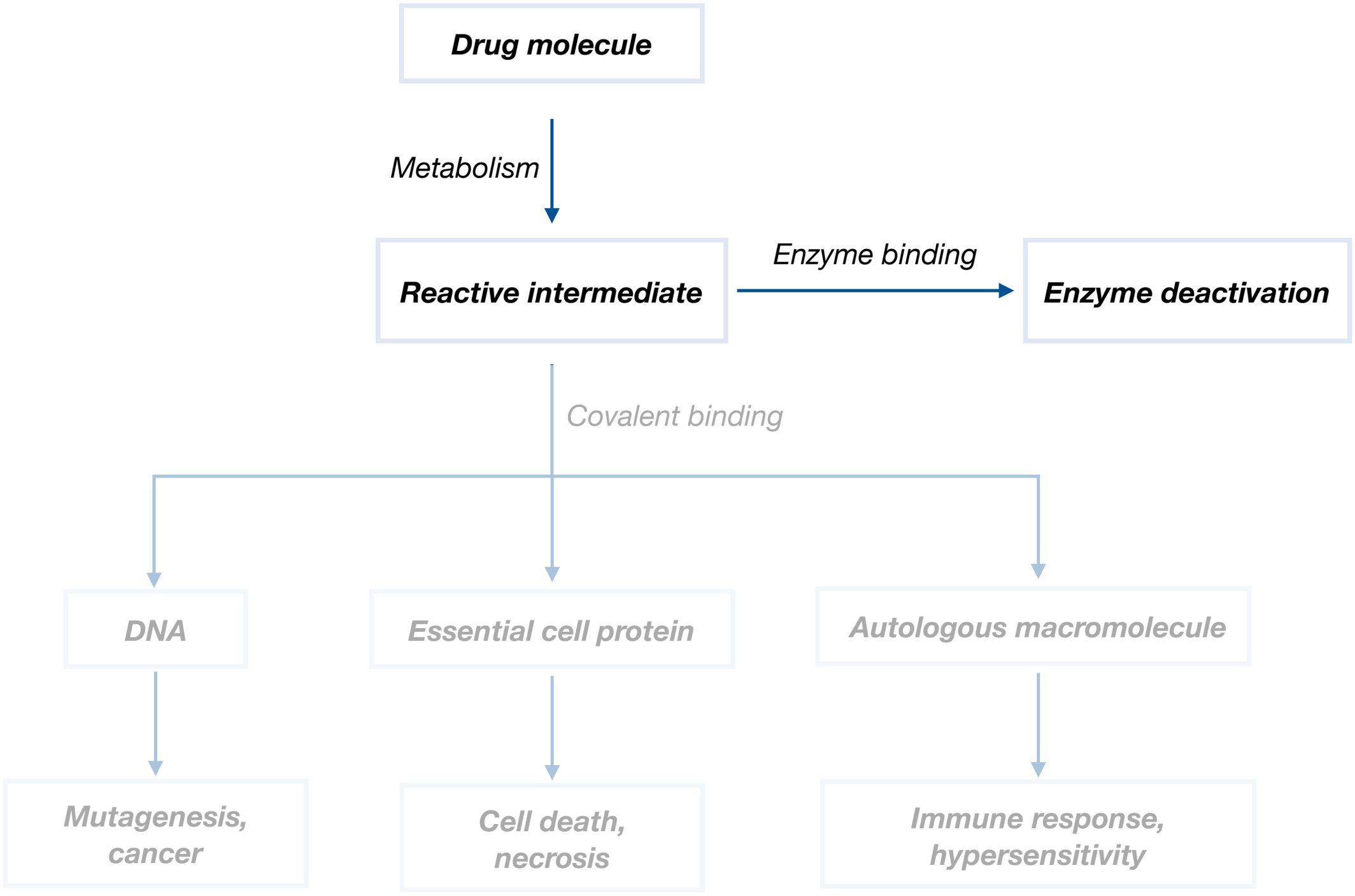


Bioactivation is usually the initial event in most drug-induced toxicities

Mechanisms of adverse reactions

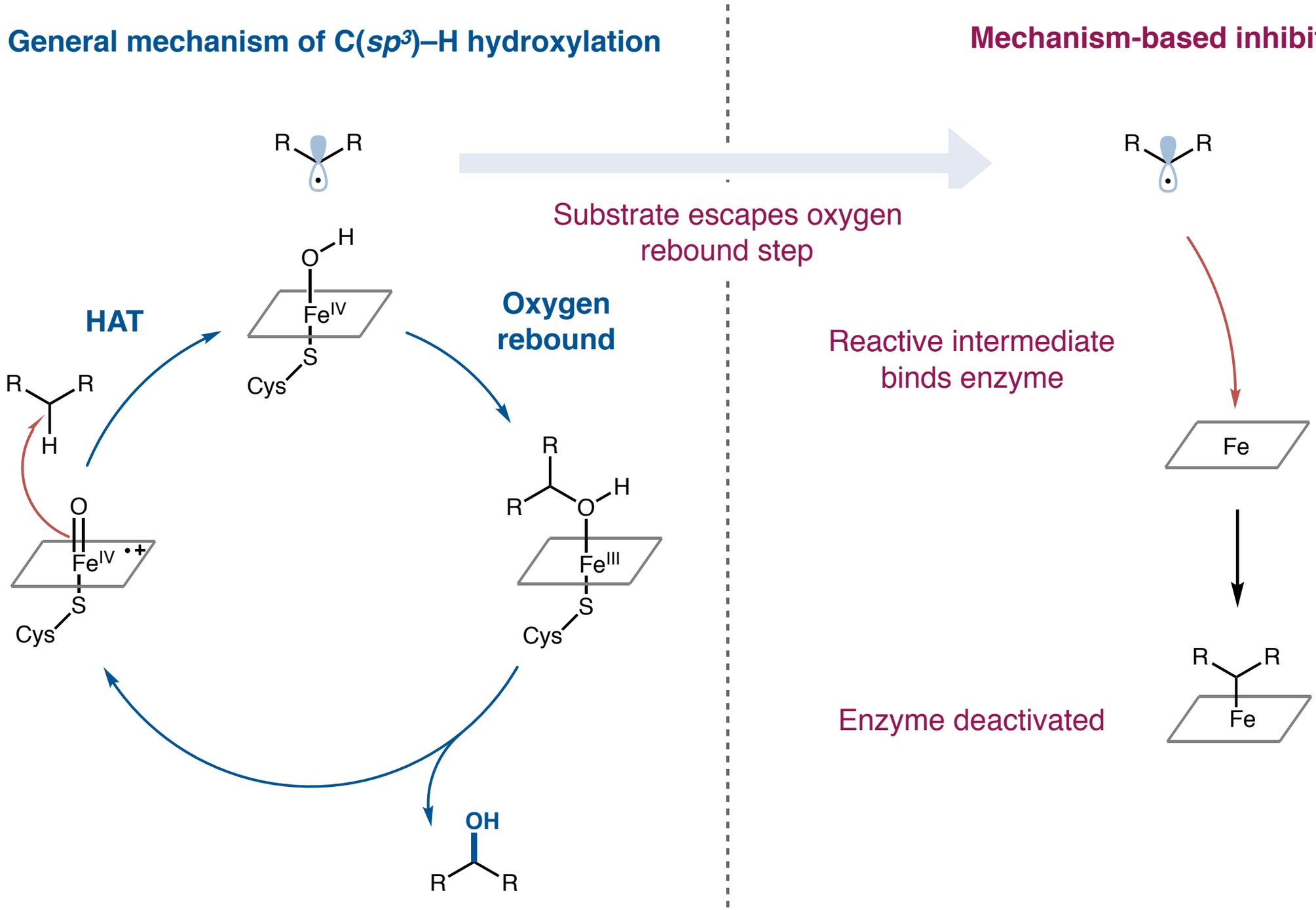


Mechanisms of adverse reactions



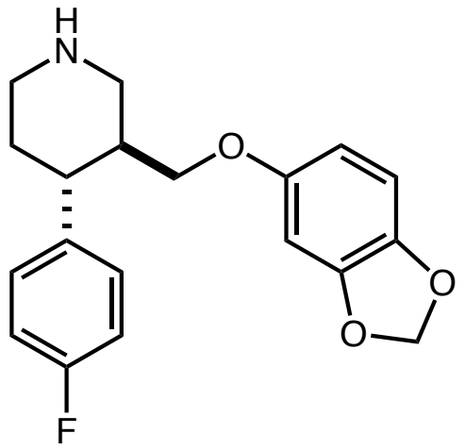
Mechanism-based inhibition

In some instances, a substrate can escape the oxygen-rebound step, binding CYP and leading to inactivation

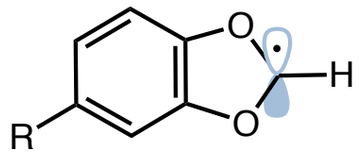


De Montenallo, O.; *Cytochrome P450: Structure, Mechanism, and Biochemistry. 3rd ed.;* Springer, 2005.

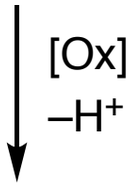
Mechanism-based inhibition by paroxetine

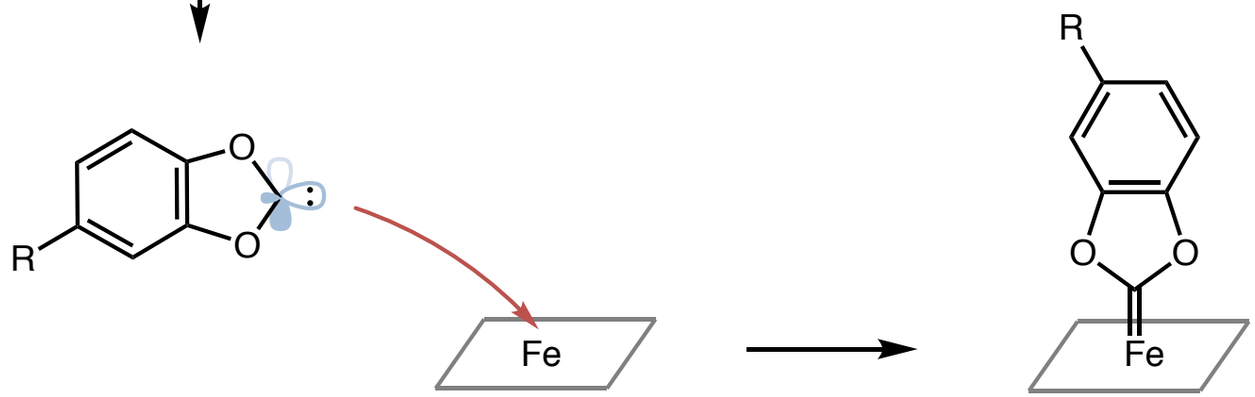


Paroxetine



Formed during metabolism





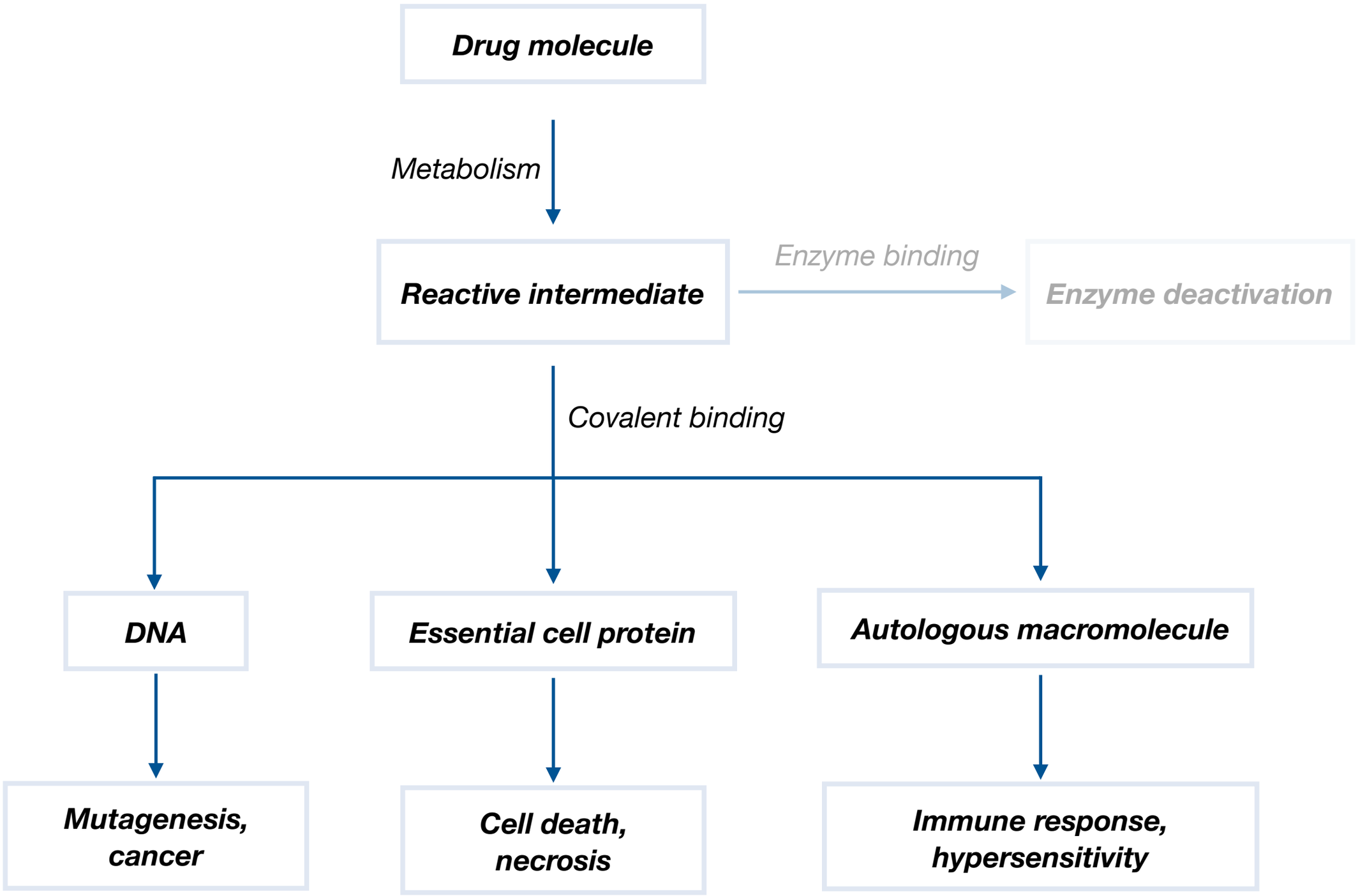
Strong inhibition of CYP2D6 and CYP2B6

- Approved in 1992 for use as antidepressant
  - 74th most prescribed drug in 2018
  - Known to cause drug-drug interactions

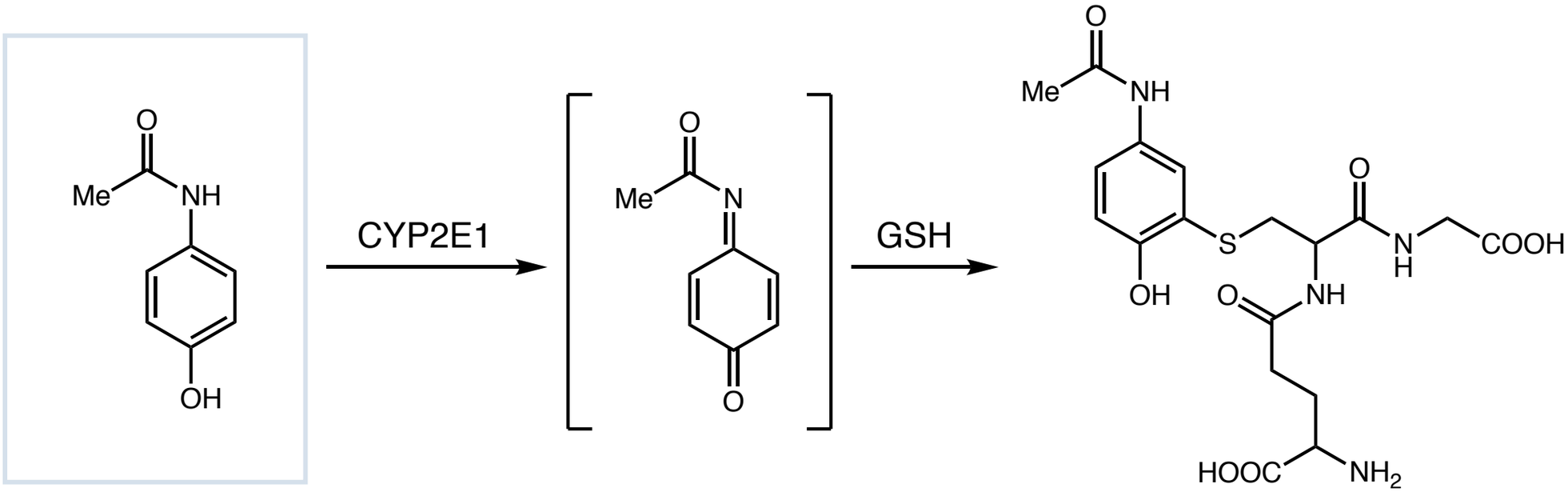
Prescribing guidelines warn that paroxetine is not to be taken with certain other drugs

De Montenallo, O.; *Cytochrome P450: Structure, Mechanism, and Biochemistry. 3rd ed.;* Springer, 2005.

Mechanisms of adverse reactions



Classic example: drug-induced liver damage from acetaminophen



Acetaminophen

Reactive quinone intermediate

Glutathione conjugate

- Most commonly used medication for pain and fever in the US and Europe
- On WHO List of Essential Medicines
- Leading cause of acute liver failure and drug overdoses in Western countries

Endogenous GSH levels depleted

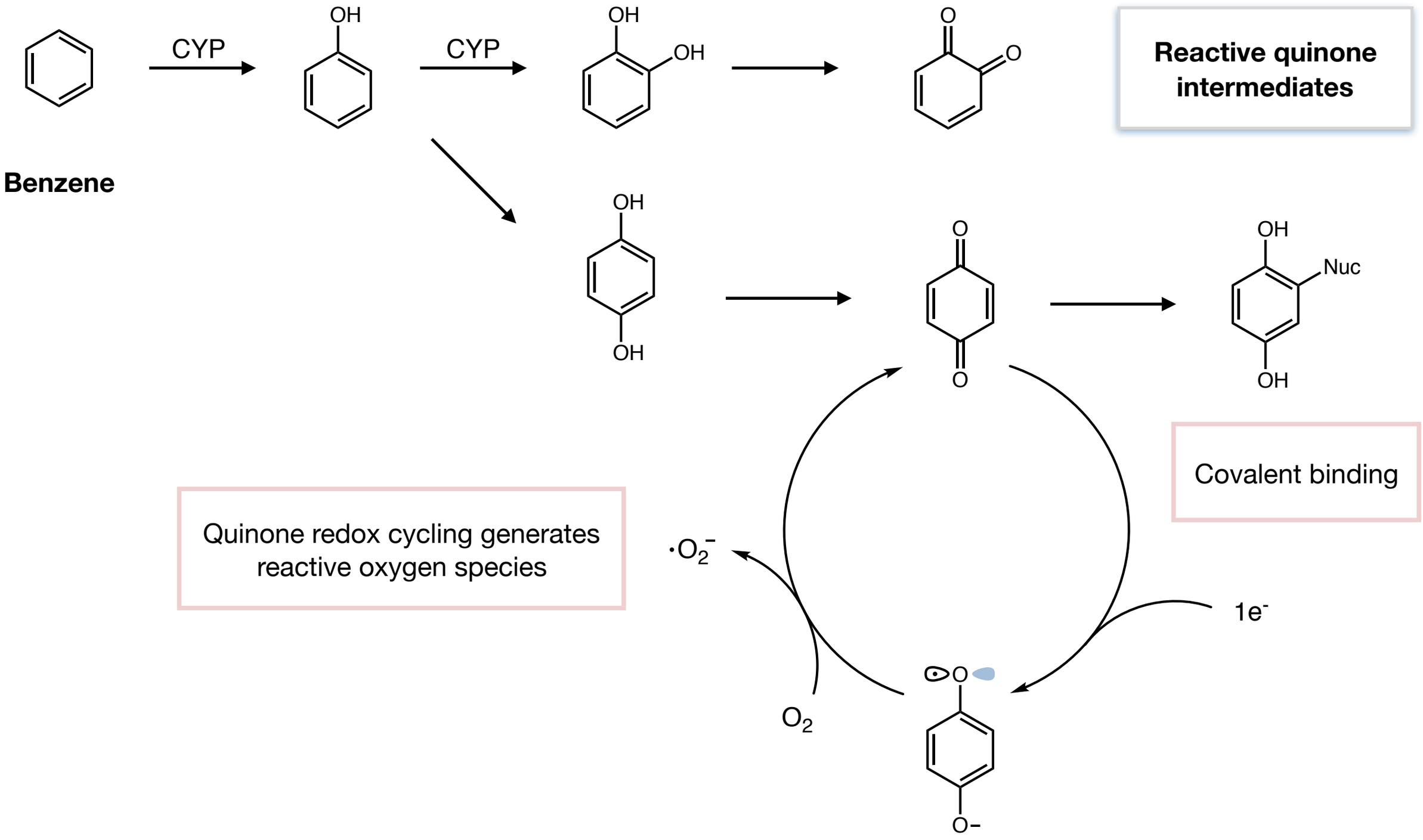
Treatment of overdose may involve administrating of N-acetylcysteine (glutathione precursor)

Covalent binding to hepatic proteins

Cell death, liver damage

Kalgutkar, A. S. *J. Med. Chem.* **2020,** *63* (12), 6276–6302.

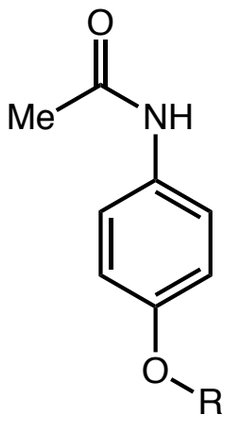
Classic example: Toxicity of benzene



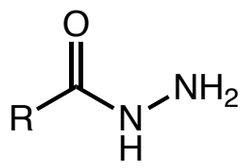
Bolton, J. L.; Dunlap, T. *Chem. Res. Toxicol.* **2017,** *30* (1), 13–37.

The structural alert concept

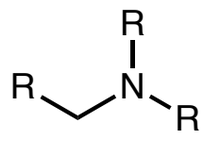
***Structural alerts (toxicophores)**: functional groups frequently found in drugs associated with adverse reactions*



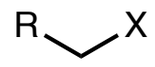
Acetanilides



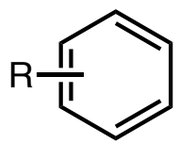
Hydrazides



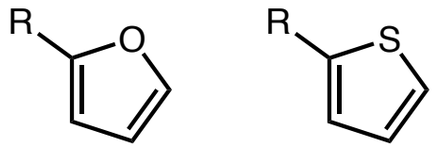
Amines



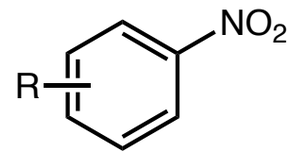
Halogenated compounds



Phenyl rings



Heteroaromatics

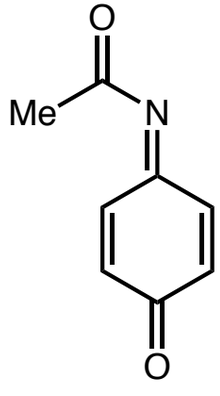


Nitroaromatics

Nelson, S. D. *J. Med. Chem.* **1982,** *25* (7), 753–765.

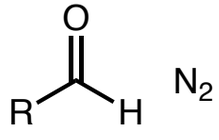
The structural alert concept

***Structural alerts (toxicophores)**: functional groups frequently found in drugs associated with adverse reactions*



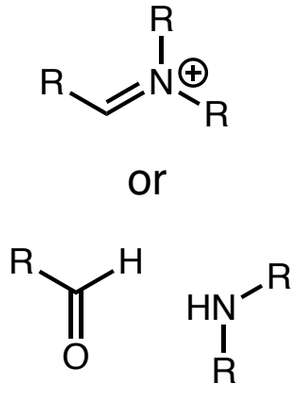
Acetanilides

Michael acceptors

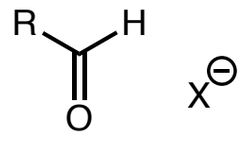


Hydrazides

Alkylating/acylating agents

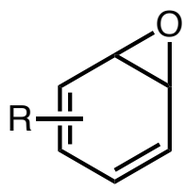


Amines

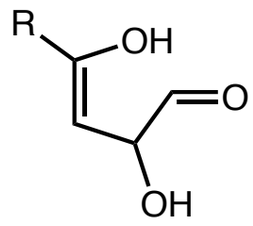


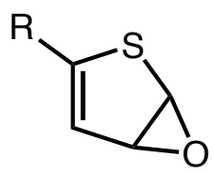
Halogenated compounds

Acylating agents, possible fluoride toxicity



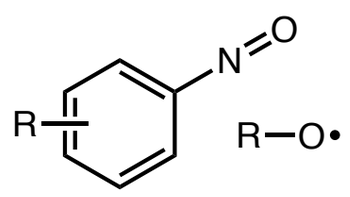
Phenyl rings





Heteroaromatics

Epoxidation leading to reactive intermediates

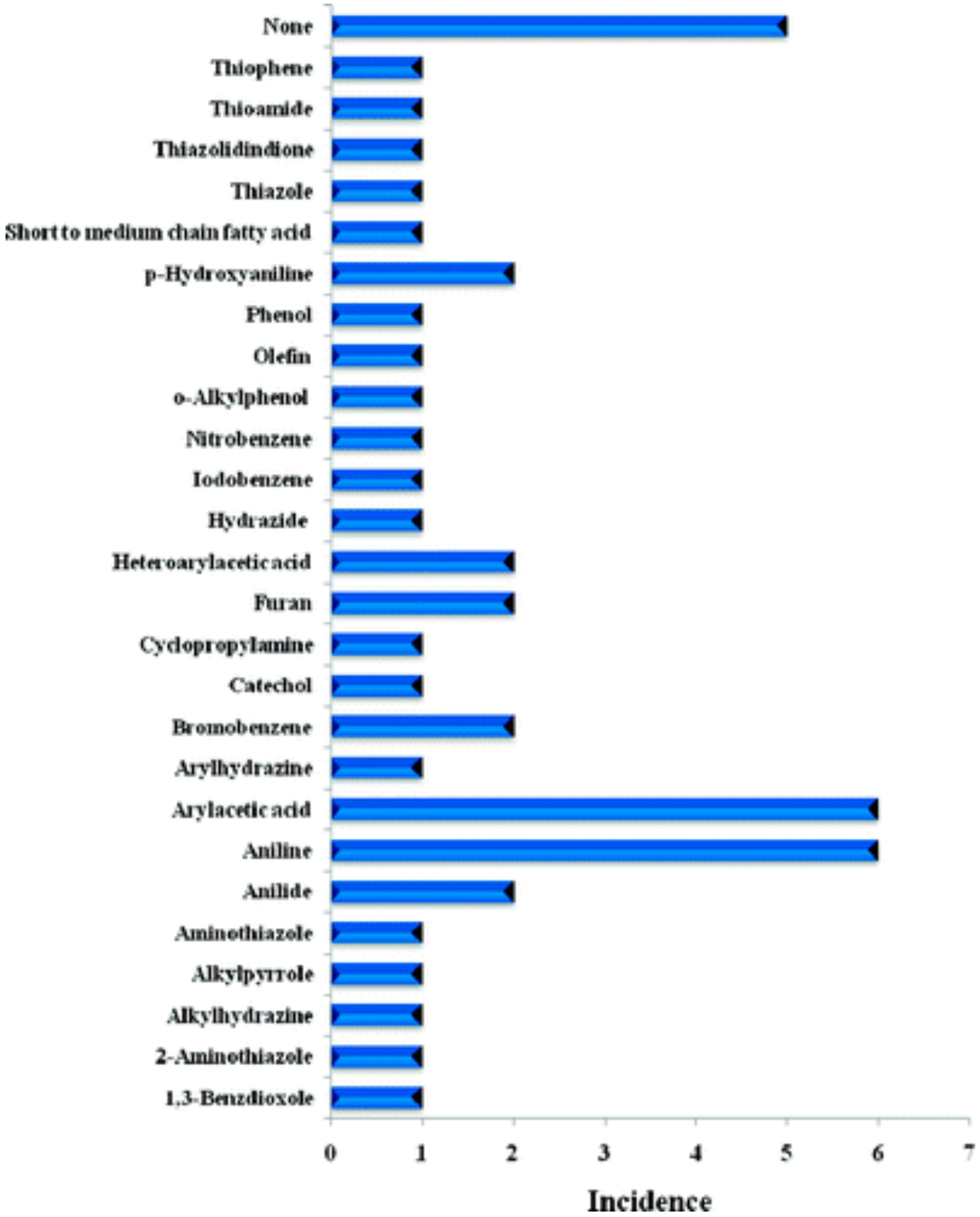


Nitroaromatics

Reactive oxygen species

Nelson, S. D. *J. Med. Chem.* **1982,** *25* (7), 753–765.

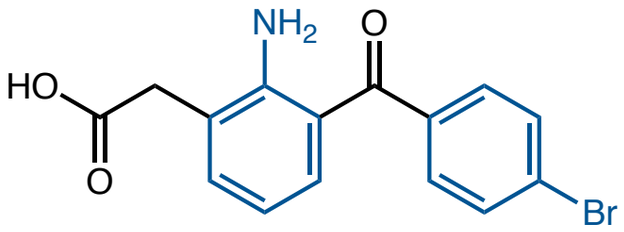
Structural alerts as a method for identifying potential toxicity



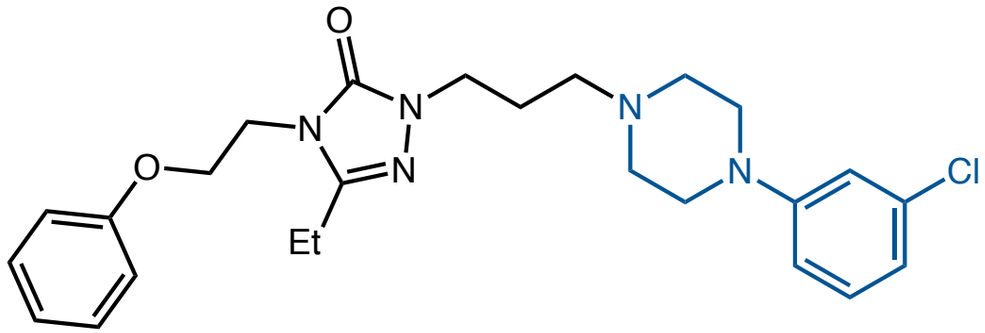
- Survey of withdrawn drugs found structural alerts in 55 out of 68 drugs (80.8%)
- The propensity to form reactive metabolites was observed in 36 out of the 55 drugs (65%)

Stepan, A. F.; Walker, D. P.; Bauman, J.; Price, D. A.; Baillie, T. A.; Kalgutkar, A. S.; Aleo, M. D. *Chem. Res. Toxicol.* **2011**, *24*, 1345– 1410.

Drugs recalled due to idiosyncratic drug-induced liver injury

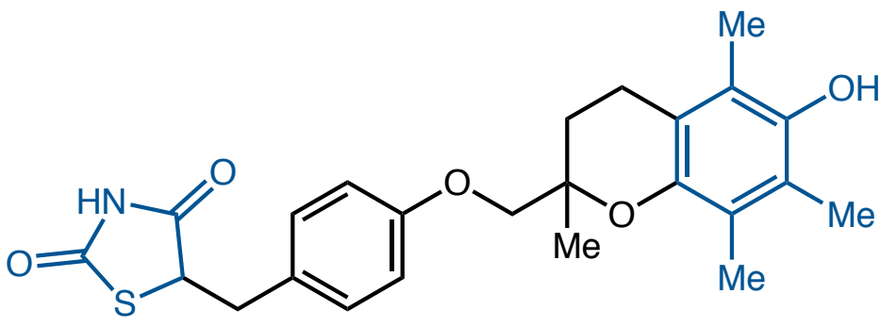


Bromfenac
Structural alerts: Aniline, bromobenzene
Withdrawn in: 1998

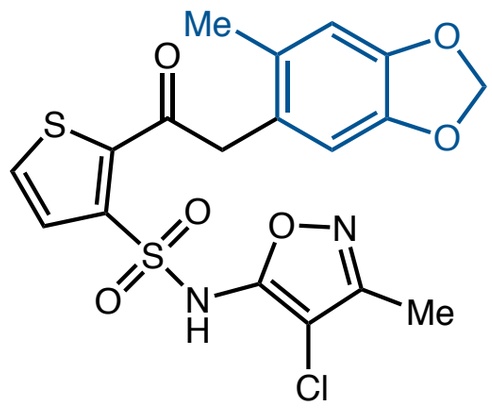


Nefazodone
Structural alerts: *N*-aryl piperazine
Withdrawn in: 2004

Hepatotoxicity is leading cause of idiosyncratic adverse drug reactions



Troglitazone
Structural alerts: Thiazolidinedione, phenol
Withdrawn in: 2000
Linked to 63 cases of liver-failure deaths

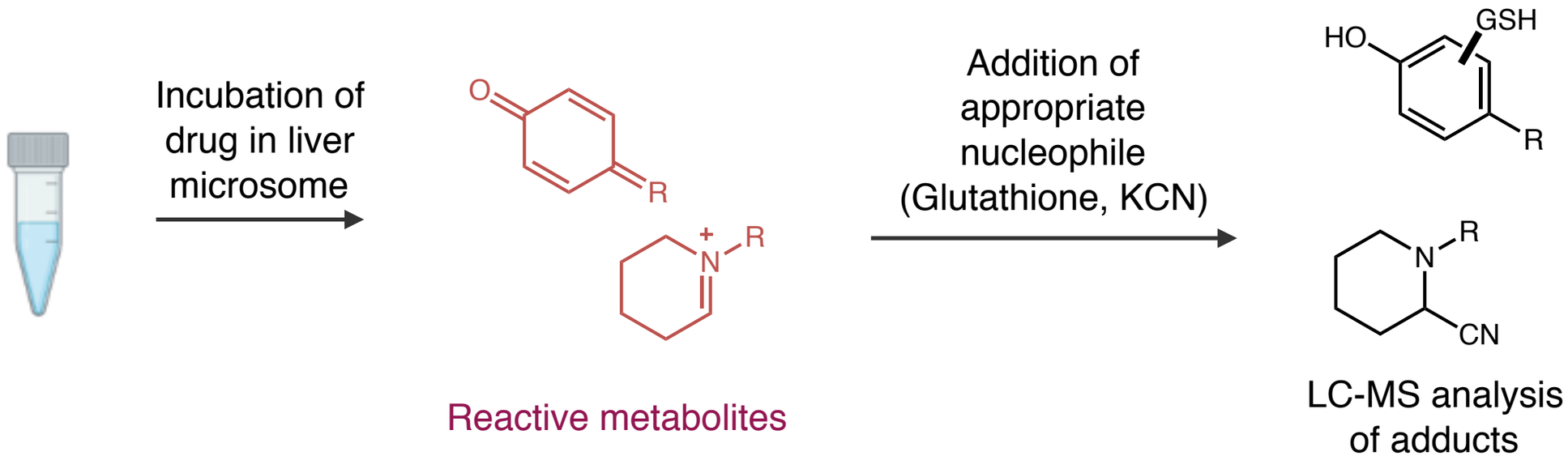


Sitaxentan
Structural alerts: 1,3-benzodioxole
Withdrawn in: 2010

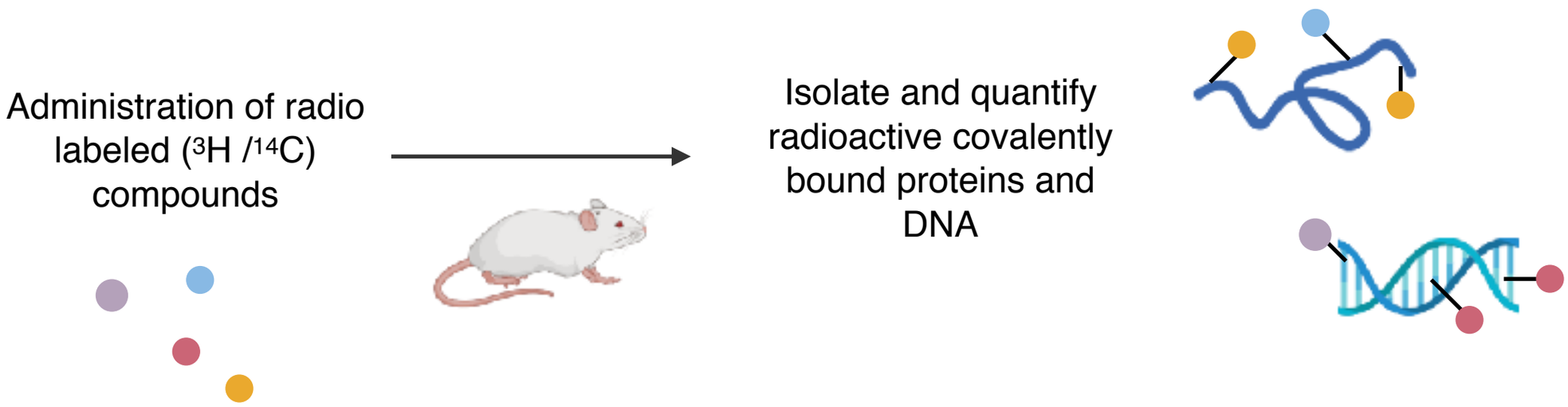
Kalgutkar, A. S. *J. Med. Chem.* **2020,** *63* (12), 6276–6302.

Assaying formation of reactive metabolites

Reactive metabolite trapping assays

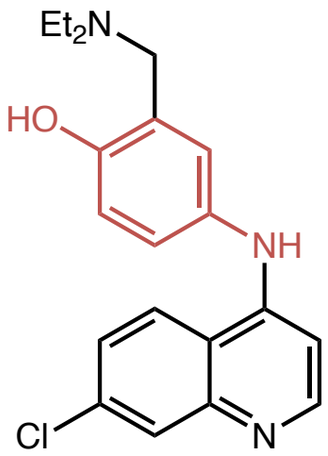


Radiolabeling assays



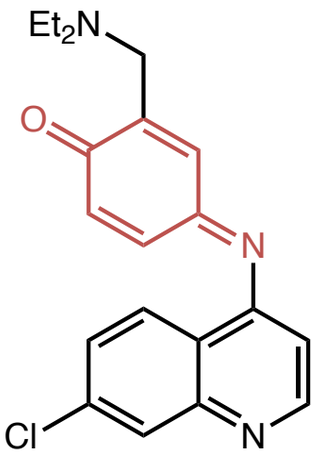
Evans, D. C., *et. al. Chem. Res. Toxicol.* **2004,** *17* (1), 3–16.

Strategies for mitigating effects of structural alerts



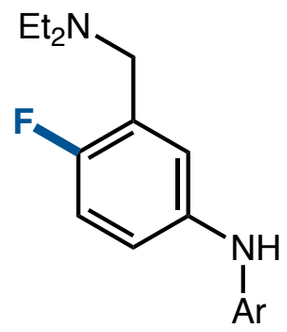
Amodiaquine

CYP

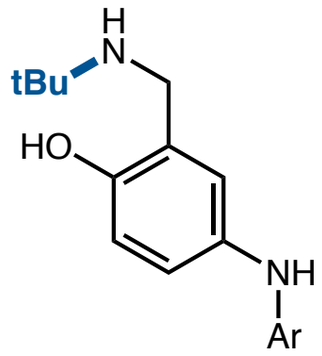


Reports of hepatotoxicity, agranulocytosis

Analogues with improved safety profile



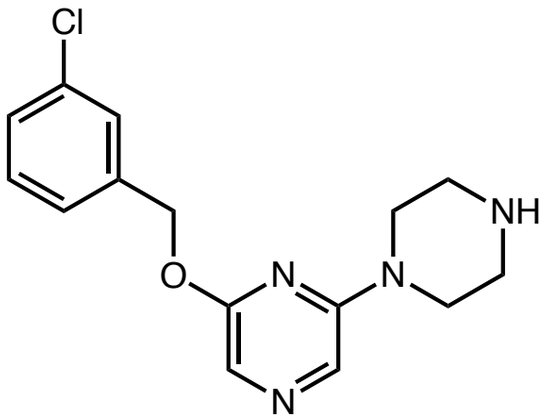
- Reduce electron density of aromatic rings



- Steric blocking of metabolically active sites

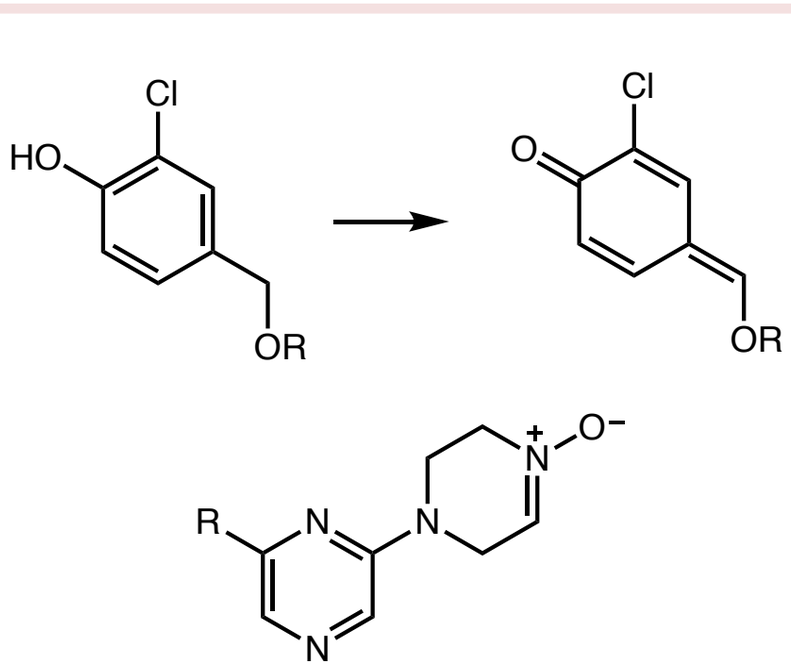
O'Neill, P. M., *et. al. J. Med. Chem.* **2009,** *52* (5), 1408–1415.

Strategies for mitigating effects of structural alerts



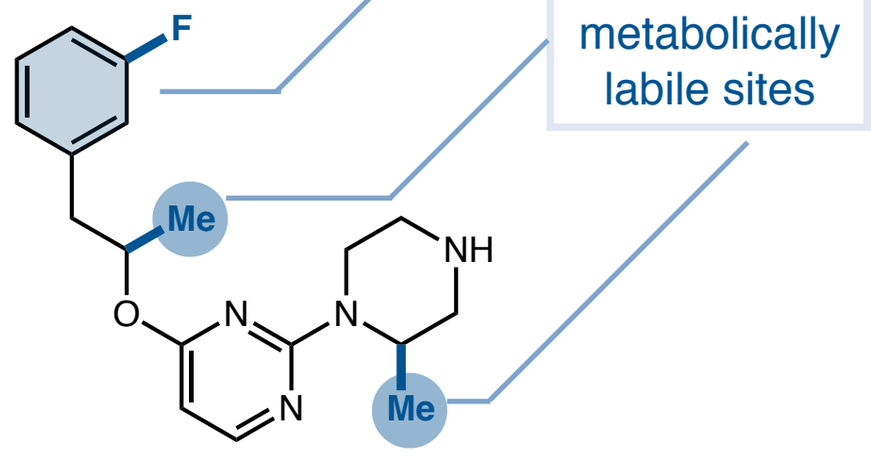
5-HT$_{2C}$ (serotonin) receptor agonist
(Pfizer)

Ames positive (mutagenic)



Reactive intermediates observed

- Remove structural alert
- Block metabolically labile sites

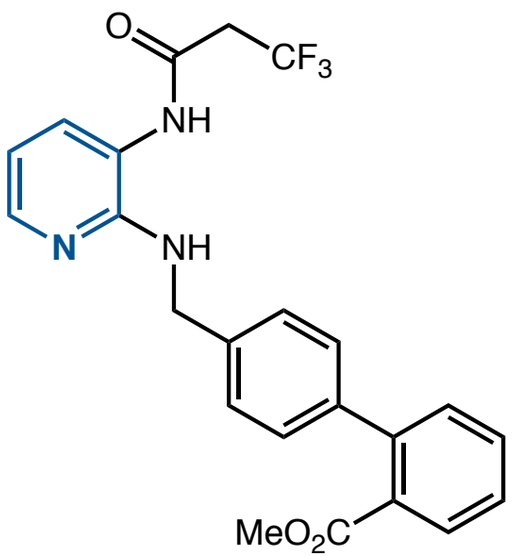


Redesigned compound

Ames negative

Kalgutkar, A. S., *et. al. Bioorg. Med. Chem. Lett.* **2009,** *19* (6), 1559–1563.

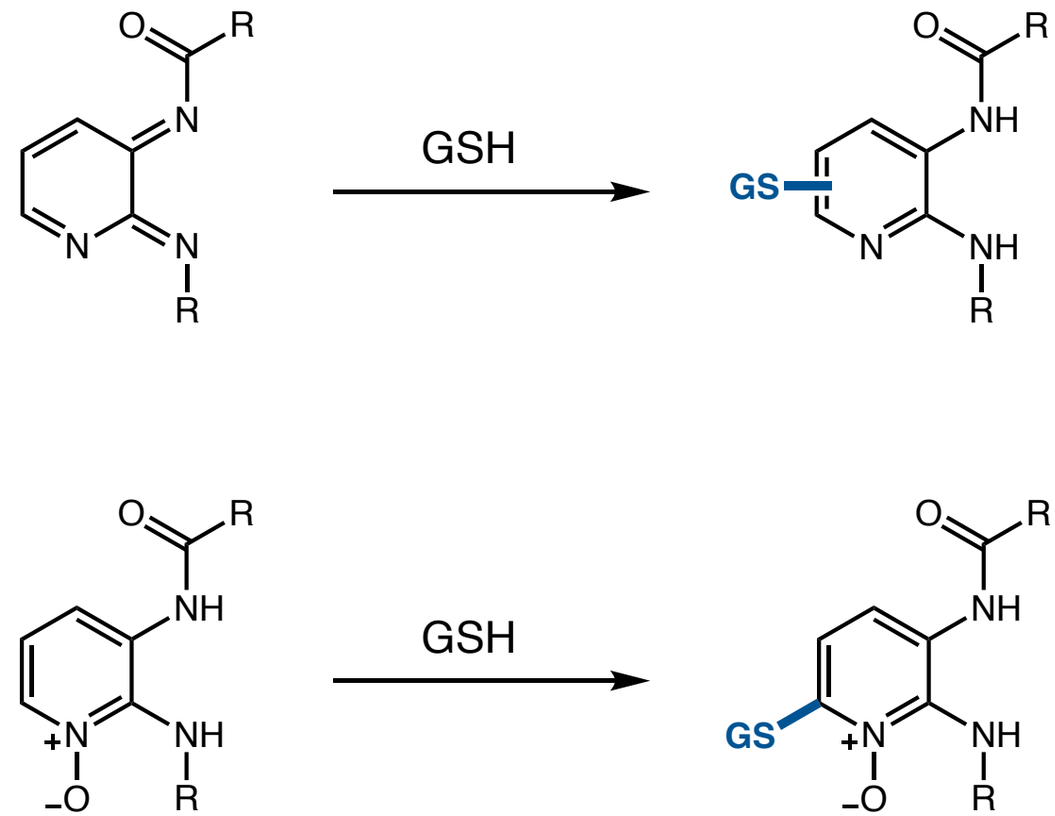
Strategies for mitigating effects of structural alerts

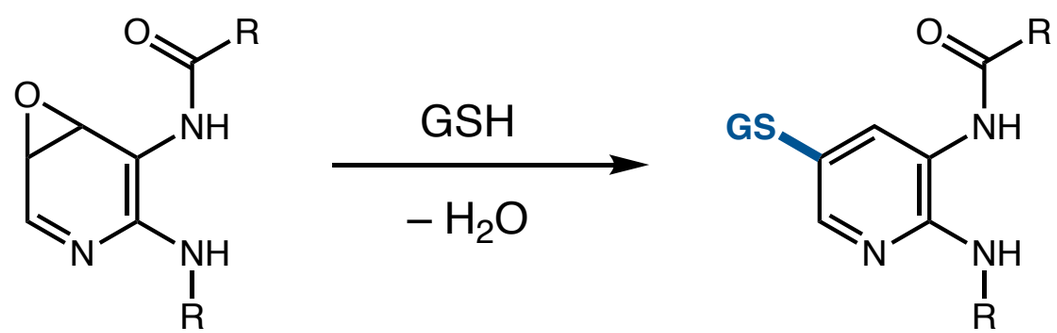


Investigated as bradykinin B_1 antagonist
(Merck)

2,3-diaminopyridine moiety determined to be safety liability

Incubation with human and rat liver microsome

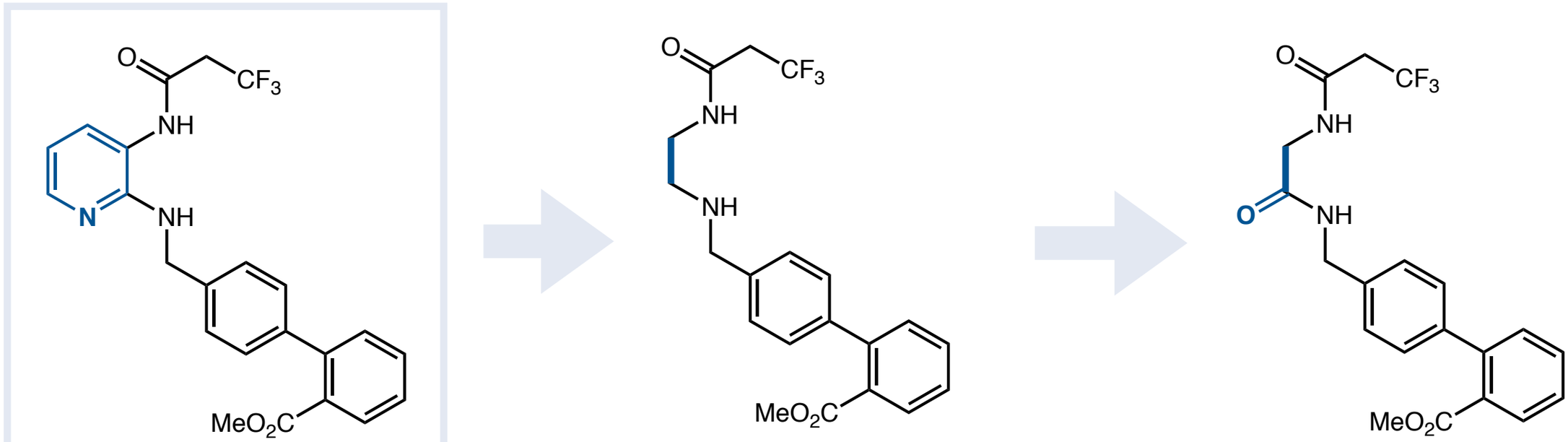




Irreversible binding to liver microsomal proteins

Tang, C., *et. al. Chem. Res. Toxicol.* **2005**, *18* (6), 934–945.

Bioisosteric replacement of structural alerts



hBK B_1 K_i = 0.012 µM

(Human bradykinin B_1 inhibitor constant)

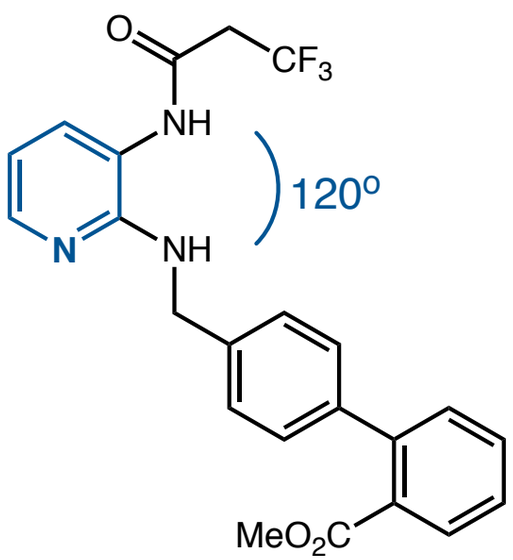
Structural modification

hBK B_1 K_i = 52 µM

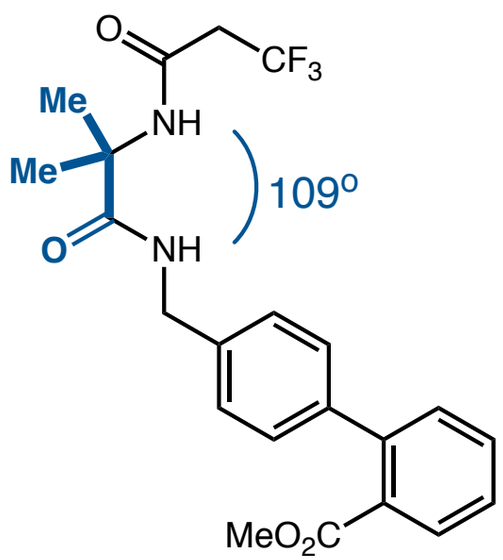
Inactive

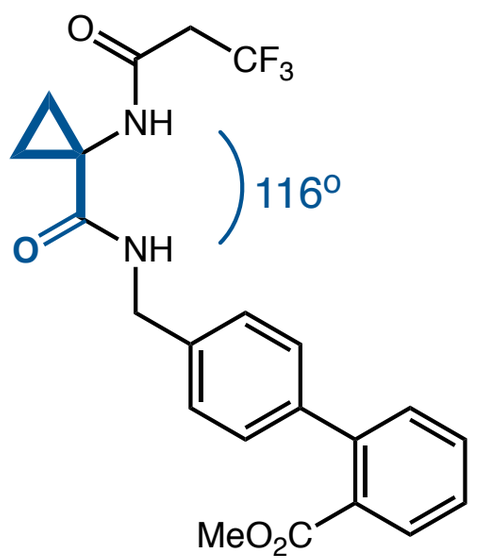
Tang, C., *et. al. Chem. Res. Toxicol.* **2005,** *18* (6), 934–945.

Bioisosteric replacement of structural alerts



Lead compound





Redesigned

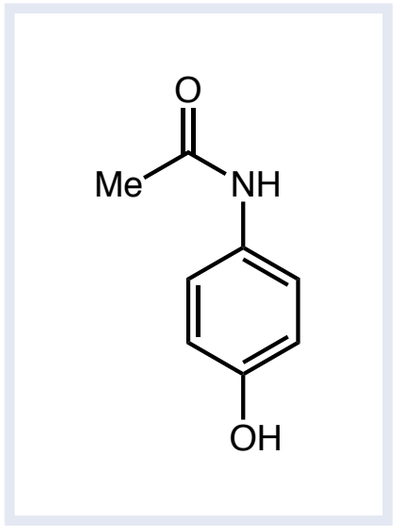
- Thorpe-Ingolde effect allows for bioisosteric mimicry of 2,3-diaminopyridine bond angle
- Large improvement in rat pharmacokinetic profile due to heightened metabolic stability

Wood, M. R., *et. al. J. Med. Chem.* **2006,** *49* (4), 1231–1234.

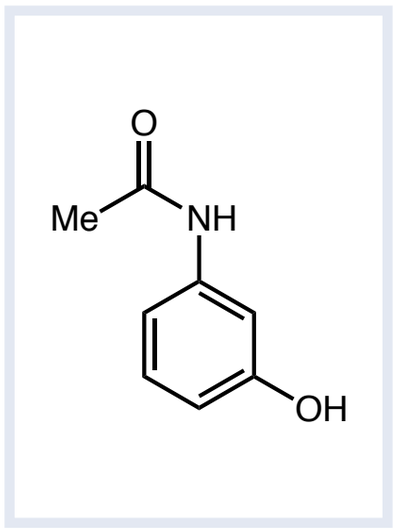
Challenges in predicting adverse effects

The ability to predict *a priori whether* drug bioactivation will lead to toxicity in humans is ***very limited***

Challenges in predicting adverse effects



Acetaminophen (APAP)



AMAP

Dose normalized to give comparable levels of *in vivo* covalent binding in mice liver

Adduction of "critical proteins" is required to cause organ toxicity

Not all protein binding is deleterious

Hepatotoxic

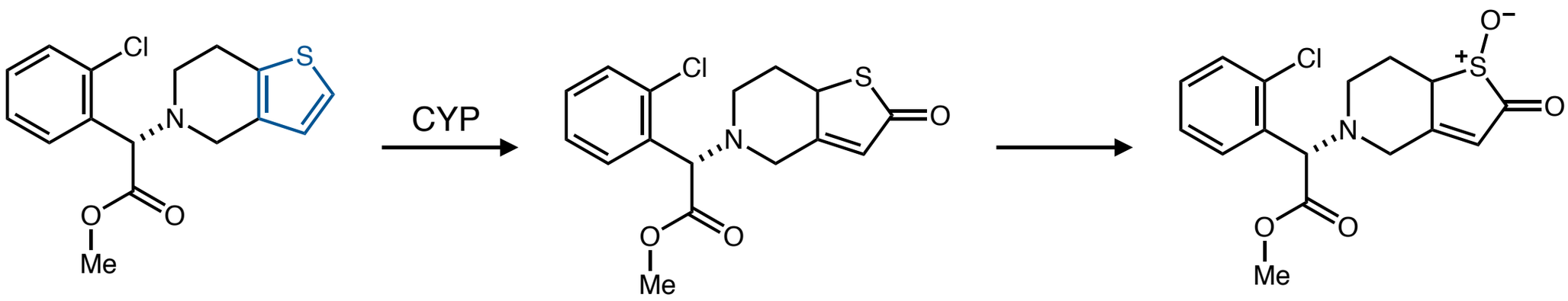
Nonhepatotoxic

Tirmenstein, M. A., Nelson, S. D. *J. Biol. Chem.* **1989,** *264*, 9814–9819.

Shortcomings of the structural alert concept

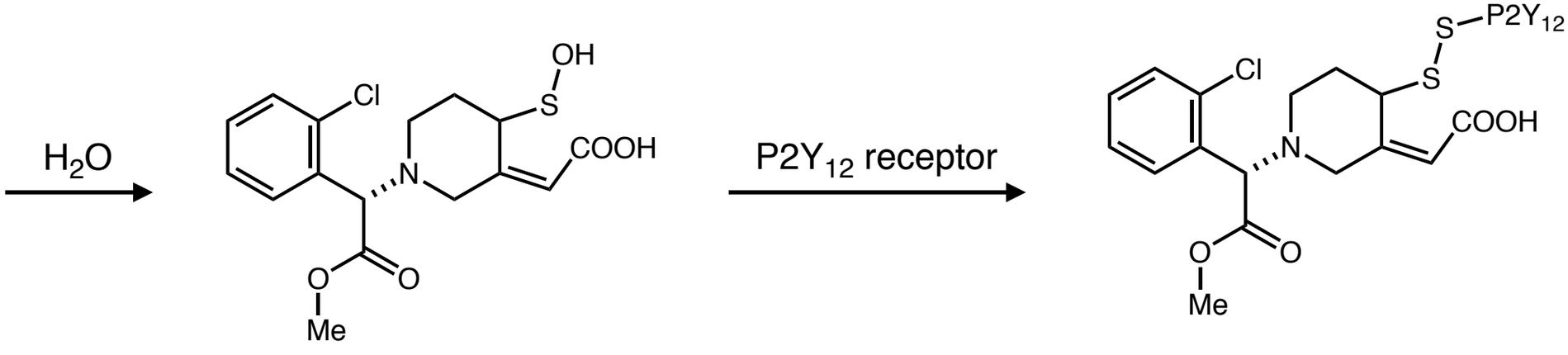
Not every structural alert results in toxicity

- 53% of 108 of the most prescribed drugs in 2009 possessed structural alerts
  - Evidence of reactive metabolite formation in 41% of those cases



Clopidogrel

Thiophene structural alert



Driscoll, J. P.; Sadlowski, C. M.; Shah, N. R.; Feula, A. *J. Med. Chem.* **2020,** *63 (12)*, 6303–6314.

Shortcomings of the structural alert concept

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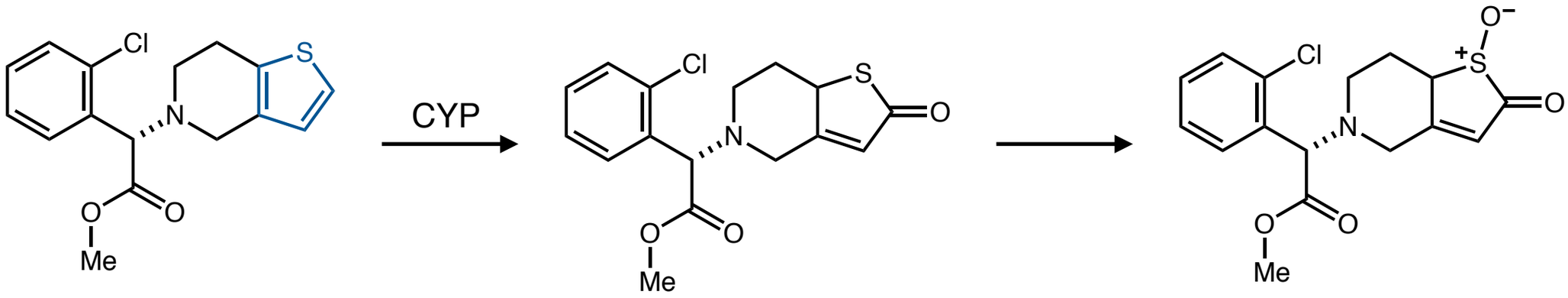
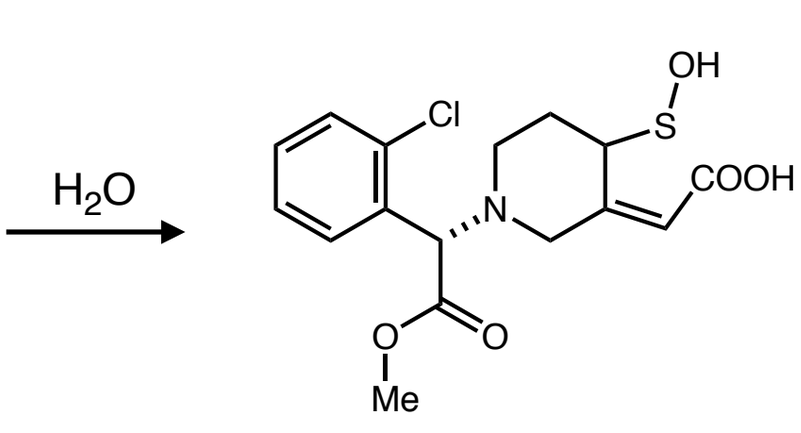
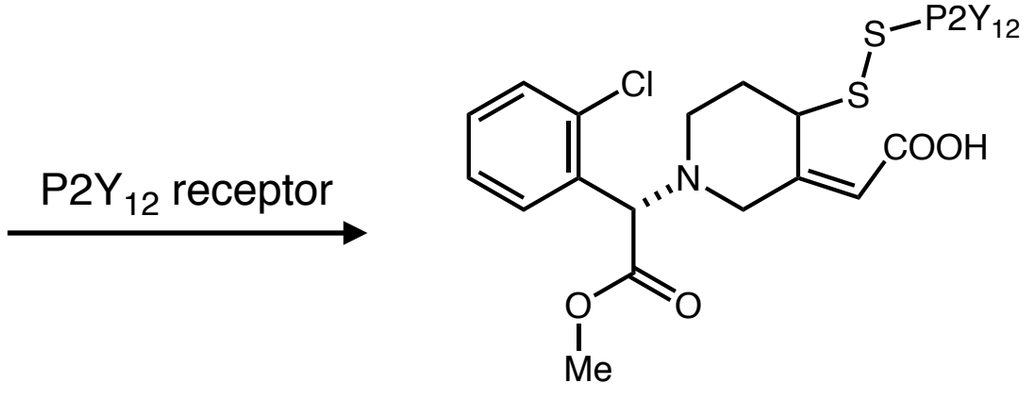


Clopidogrel

Thiophene structural alert



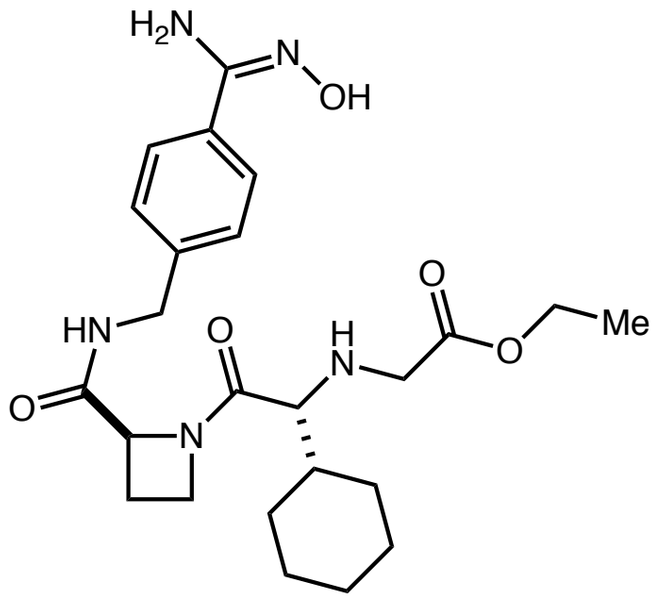


Irreversible inhibition of platelet aggregation

Driscoll, J. P.; Sadlowski, C. M.; Shah, N. R.; Feula, A. *J. Med. Chem.* **2020,** *63 (12)*, 6303–6314.

Shortcomings of the structural alert concept

Not every IADR associated with a structural alert



Ximelagatran

Does not contain any known structural alerts

Rejected for FDA approval in 2009 after clinical trials due to idiosyncratic drug-induced liver injury

Cannot completely eliminate structural alerts

“If medicinal chemists abstained from the synthesis of phenyl ring-containing compounds because the phenyl ring is considered as a structural alert, humanity would be deprived of countless life-saving drugs”

Kalgutkar, A. S. *J. Med. Chem.* **2020,** *63 (12)*, 6276–6302.

Challenges in predicting adverse effects

- *Lack of thorough characterization distinguishing critical and noncritical proteins*
- *Structural alert analysis often inaccurate*
- *Inter- and intra-species variation in drug metabolism*

The ability to predict *a priori whether* drug bioactivation will lead to organ or immune toxicity is ***very limited***

Drug discovery and development

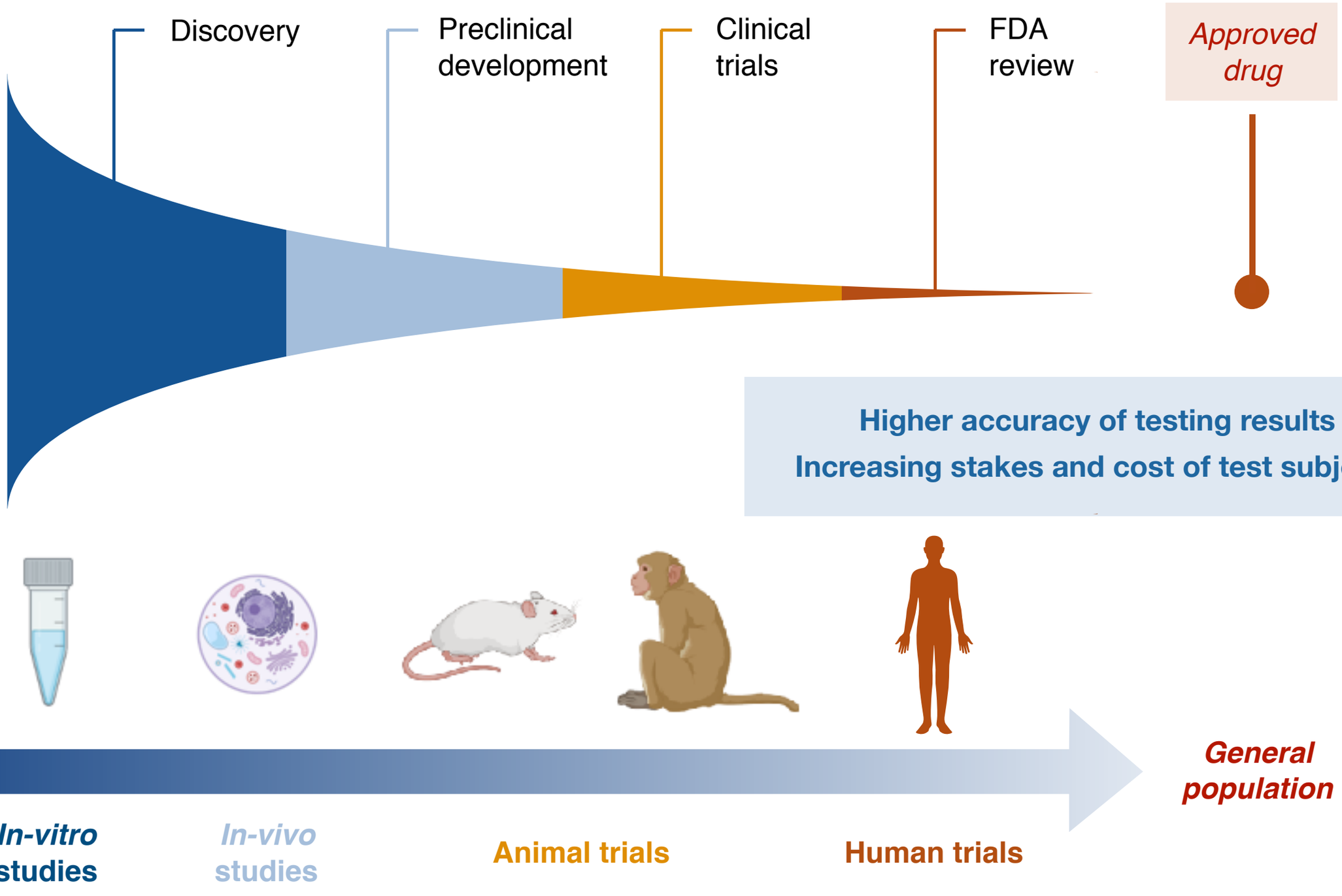
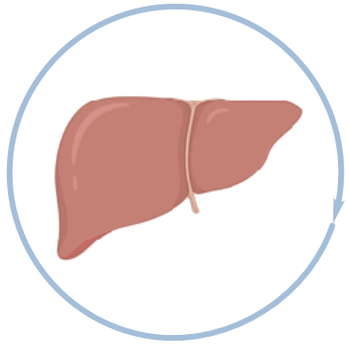


Image credit: Ian B. Perry

Outline

Introduction to drug metabolism

- Common biotransformations



Metabolism-induced toxicity

- Mechanisms of adverse reactions
  - Reactive intermediates and structural alerts

Applications to drug design

- Designing around metabolism

Applications to drug design

- Designing around drug-induced toxicity
- Challenges in predicting adverse effects