DRUG DISCOVERY AND DESIGN

Facultat de Farmàcia

1. Introduction.
2. Drug targets.
3. Basic concepts in the drug action.
4. Drug metabolism.
5. Design and development of new drugs.
6. Quantitative structure-biological activity relationships(QSAR).

VNIVERSITAT DE VALÈNCIA

DRUG DISCOVERY AND DESIGN

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UNIT 1. INTRODUCTION

1.1. Definitions: Pharmaceutical Chemistry, Drug, Medicine, Active Principle.

1.2. Relationships between Pharmaceutical Chemistry and other Sciences.

1.3. Classification of drugs.

1.4. Nomenclature of drugs.

1.5. The Pharmaceutical Industry.

1.1. DEFINITIONS

PHARMACEUTICAL (MEDICINAL) CHEMISTRY:

DEALS WITH THE STUDY OF DRUGS FROM A CHEMICAL POINT OF VIEW, INCLUDING THEIR DESIGN, SYNTHESIS AND STRUCTURAL ANALYSIS.

(As we shall see, this is a simple definition.)

DRUGS are chemicals that interact with a biological system to produce a biological effect.
This effect may be beneficial (pharmaceutical) or harmful (toxic), depending on the drug used and the dose administered.

In the above definition, drugs are chemicals used for medicinal purposes.

OBJECTIVES:

TO FIND, DEVELOP AND IMPROVE DRUGS THAT PREVENT, CURE OR ALLEVIATE DISEASES IN HUMANS AND ANIMALS.

1.1. DEFINITIONS

A DRUG SUBSTANCE, ACTIVE PRINCIPLE or ACTIVE PHARMACEUTICAL INGREDIENT (API):
Is a PURE compound that shows biological activity and can be used based on its therapeutic effects.
(In Spanish: *Fármaco*)

A MEDICINE results from the development of a drug. It may contain one or more **active principles** and excipients, solvents, stabilizers and/or preservatives may be also present.
Its commercialization must have been authorized after it has passed all analytical, pharmacological and toxicological controls.
(In Spanish: *Medicamento*)

In the European Union, the regulatory agency for drugs is the **European Medicines Agency (EMA)** *www.ema.europa.eu*

In Spain, the regulatory agency for drugs is the **AEMPS** (Agencia Española de Medicamentos y Productos Sanitarios) https://www.aemps.gob.es

1.1. DEFINITIONS

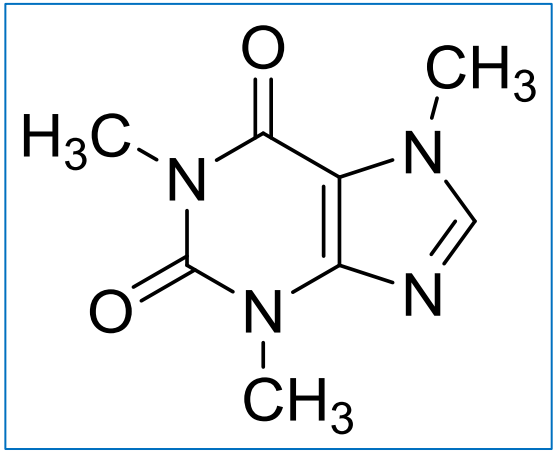
DRUG *(droga)*:

- **Coffee (1-2%)**
- **Tea (2-4%)**
- **Cola (0.6-3%)**
- **Guarana (2.5-5%)**
- **Mate (2%)**

PROPERTIES:

Diuretic
Anti-migraine
Stimulating

ACTIVE PRINCIPLE *(fármaco)*: CAFFEINE



Purine Antagonist
Phosphodiesterase Inhibitor

MEDICINE *(medicamento)*

- **Desenfriol ®**
- **Hemicraneal ®**
- **Calmante vitaminado ®**
- **Mejoral ®**
- **Analgilasa ®**
- **Frenadol Complex ®**
- **…**

1.1. DEFINITIONS

Definition of Medicinal Chemistry provided by a specialized commission of the IUPAC *(IUPAC= International Union of Pure and Applied Chemistry)*:

"Medicinal chemistry concerns the discovery, the development, the identification and the interpretation of the mode of action of biologically active compounds at a molecular level. Emphasis is put on drugs, but the interests of the medicinal chemist are not restricted to drugs, but include bioactive compounds in general. Medicinal chemistry is also concerned with the study, identification, and synthesis of the metabolic products of these drugs and related compounds".

DISCOVERY/RATIONAL DESIGN
STRUCTURE-ACTIVITY RELATIONSHIPS (SAR)
SYNTHESIS and DEVELOPMENT

1.1. DEFINITIONS

Case Studies in Modern Drug Discovery and Development, Edited by Xianhai Huang and Robert G. Aslanian, 2012 , John Wiley & Sons, Inc.

TARGET-BASED APPROACH: DISCOVERY PHASE

1) **Target Identification**: Biologist's identify a molecular target (e.g., enzyme or receptor) that influences the disease.
2) **Target Validation**: Confirm function and effects, and develop biological (in vitro/in vivo) assays
3) **Lead Identification**: A collection of molecules are screened for activity against the target.
4) **Lead Optimization**: Medicinal chemists modify the structure of the lead to optimize the biological and pharmacokinetic properties with ADMET considerations
5) **Development Candidate Preclinical Studies**: Select development candidate, carry out nonclinical safety assessment, pharmacokinetic and pharmacodynamic studies in animals and formulation and delivery system studies of the lead. Initial scale-up strategies investigated for delivery of toxic/clinical supplies.

(This concludes the discovery phase and initiates the development phase)

1.1. DEFINITIONS

DEVELOPMENT

Case Studies in Modern Drug Discovery and Development, Edited by Xianhai Huang and Robert G. Aslanian, 2012 , John Wiley & Sons, Inc.

1) Process and manufacturing chemists work on large scale synthesis, commercial route synthesis and long-term route synthesis to support toxicological studies. Phase I-IV clinical trials. Good Manufacturing Practice (GMP) is required in all of these processes. Patent filing.
2) Investigational New Drugs (IND) applications filed before beginning Phase I clinical trial. All clinical studies need to follow Good Clinical Practice (GCP) and work with regulatory authorities.
3) **Phase I Clinical Trial**: Assess human pharmacokinetic profile, safety, and tolerability in healthy human beings.
4) **Phase II Clinical Trial**: Further assess drug safety, dose range, and efficacy studies in a small number of patients.
5) **Phase III Clinical Trial**: Further assess drug safety, dose range, and efficacy studies in large number of patients with multiple trials.
6) **New Drug Application** (NDA) filed.
7) **Drug Approval.**
8) **Phase IV Clinical Studies** (if necessary): Post-marketing event, very large scale clinical studies to assess long term effect of the drug. Post-approval studies designed to assess the drug versus competitors, effectiveness, and qualify of life considerations.
9) **Drug Life Cycle Management.**

1.1. DEFINITIONS

G. L. Patrick. *An Introduction to Medicinal Chemistry*. Oxford Univ. Press., 5ª Ed. 2013

TARGET-BASED DRUG DESIGN AND DEVELOPMENT

a) Identifying the target disease
b) Identifying the drug target
c) Establishing testing procedures: finding a "hit" compound
d) Finding a lead compound
e) Establishing Structure-Activity Relationships (SAR)
f) Identifying a pharmacophore
g) Design: optimising target interactions and pharmaco-kinetic properties
i) Toxicological and safety tests
j) Chemical development and production of a candidate
k) Patenting and regulatory affairs
l) Clinical trials

(Medicinal chemists usually work in the stages highlighted in blue)

1.1. DEFINITIONS

There are three distinct phases in the path of a drug through the body:

- **The Pharmaceutical Phase**:
  from administration (oral or parenteral) to liberation of the active principle
  (medicinal chemists and pharmaceutical technologists)

- **The Pharmacokinetic Phase**:
  Absorption, Distribution, Metabolism, and Excretion (ADME)
  "*What the body does to the drug*"
  (medicinal chemists and biopharmacists)

- **The Pharmacodynamic Phase**:
  Interaction of the drug with the target that generates a biological response
  (medicinal chemists with biochemists and pharmacologists)

The chemical structure affects all these processes that influence biological activity!

1.1. DEFINITIONS

1. Hits are compounds that have confirmed *in vitro* activity on a target and are suitable for further optimization (also with regard to intellectual property (IP) rights).

(good pharmacodynamic properties)

2. Leads are compounds that have desired *in vitro* properties on ADME (absorption, distribution, metabolism, and excretion), are safe and patentable, and whose activity is confirmed first *in vivo* models.

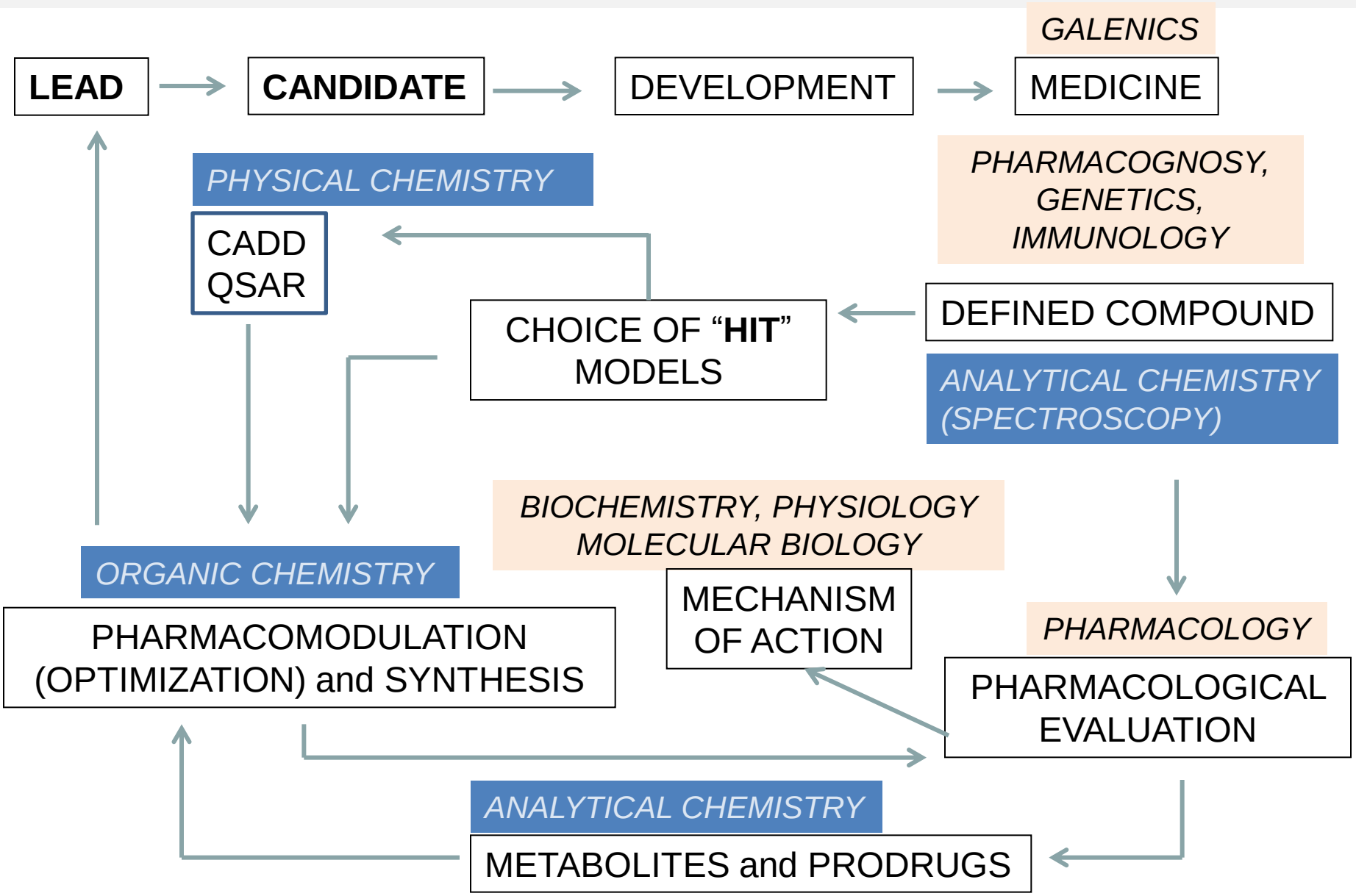
(good pharmacodynamic and pharmacokinetic properties)

3. Candidates are suitable for clinical development and are proven in disease-relevant *in vivo* models.

Green and Sustainable Medicinal Chemistry: Methods, Tools and Strategies for the 21st Century Pharmaceutical Industry, Edited by Louise Summerton, Helen F. Sneddon, Leonie C. Jones and James H. Clark
The Royal Society of Chemistry, 2016

(To be discussed in UNIT 5)

1.2. RELATIONSHIPS BETWEEN PHARMACEUTICAL CHEMISTRY AND OTHER SCIENCES



CADD (Computer-Aided Drug Design), QSAR (Quantitative Structure Activity Relationships)

1.3. CLASSIFICATION OF DRUGS

a. By chemical structure

Drugs with a common skeleton usually show the same biological action and mechanism of action, e.g. penicillins and cephalosporins, barbiturates and opioids.

These are called **structurally specific drugs**.

Sometimes compounds with similar chemical structures have very different effects in the body (e.g. sulfonamides and steroids). There may also be very different structures for the same effect (e.g. anaesthetics).

These are **structurally nonspecific drugs.**

b. By pharmacological effect

Pharmacodynamic agents (e.g. analgesics, antipsychotics, anti-hypertensives and anti-asthmatics) alter certain biological process in our cellules or systems. **Chemotherapy agents** (e.g. antibacterials and antivirals) are used against cancer or infectious diseases.

This is a useful criterion for determining the full scope of drugs available for a certain ailment but the drugs included are numerous and highly varied in structure.

(Patrick's)

1.3. CLASSIFICATION OF DRUGS

c. By target system

Drugs can be classified according to whether they affect a certain system in the body (e.g. the Central Nervous System).

A system usually has several targets with which drugs could interact (e.g. the brain, synapses, etc.).

Drugs in each group are likely to be varied in structure due to the different mechanisms of action involved (anti-cholinergic and anti-adrenergic drugs).

d. By target molecule

Some drugs are classified according to the molecular target with which they interact (anticholinesterases).

This is a more specific classification since it identifies the precise target upon which the drug acts.

We can expect a structural similarity between the agents involved as well as a common mechanism of action, though this is not an unbreakable assumption.

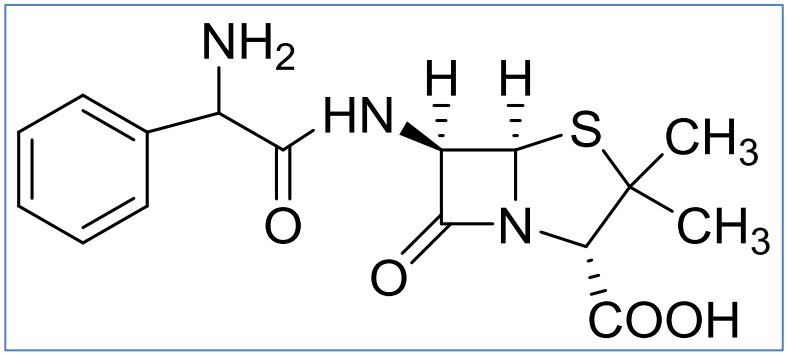
(Patrick's)

1.3. CLASSIFICATION OF DRUGS

c. antibacterial

d. enzyme inhibitor

a. specific



ampicillin

a. penicillin

b. chemotherapy

1.4. NOMENCLATURE OF DRUGS

DRUG NAMES

CHEMICAL NAME
IUPAC or IUBS
Very complex
Only used by specialists

WITH PROPRIETARY

WITHOUT PROPRIETARY

MEDICINES
Trade marks

CODES
PATENTS
Trade names

Generics
(Patent has expired)

ATC code

rINN (**WHO** since 1953)
Recommended International Non-proprietary Name
(to identify each drug with a specific name recognized in all countries)

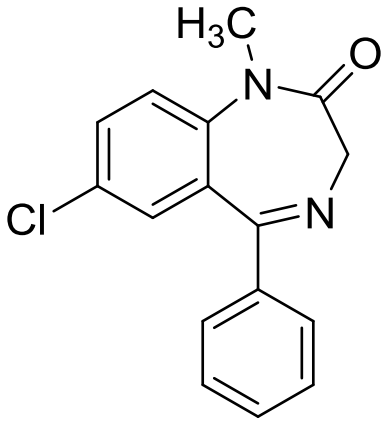
TABLES **PREFIXES**
AFFIXES
SUFFIXES

In Spain:
-D.C.I. (Denominación Común Internacional) defined by the WHO (equivalent to the rINN)
-D.O.E. (Denominación Oficial Española) Spanish version of the D.C.I. adopted by the Spanish Ministry of Health.

Diazepam (rINN): active principle name

Trade name (medicine): Valium®

ATC code:
N05BA01

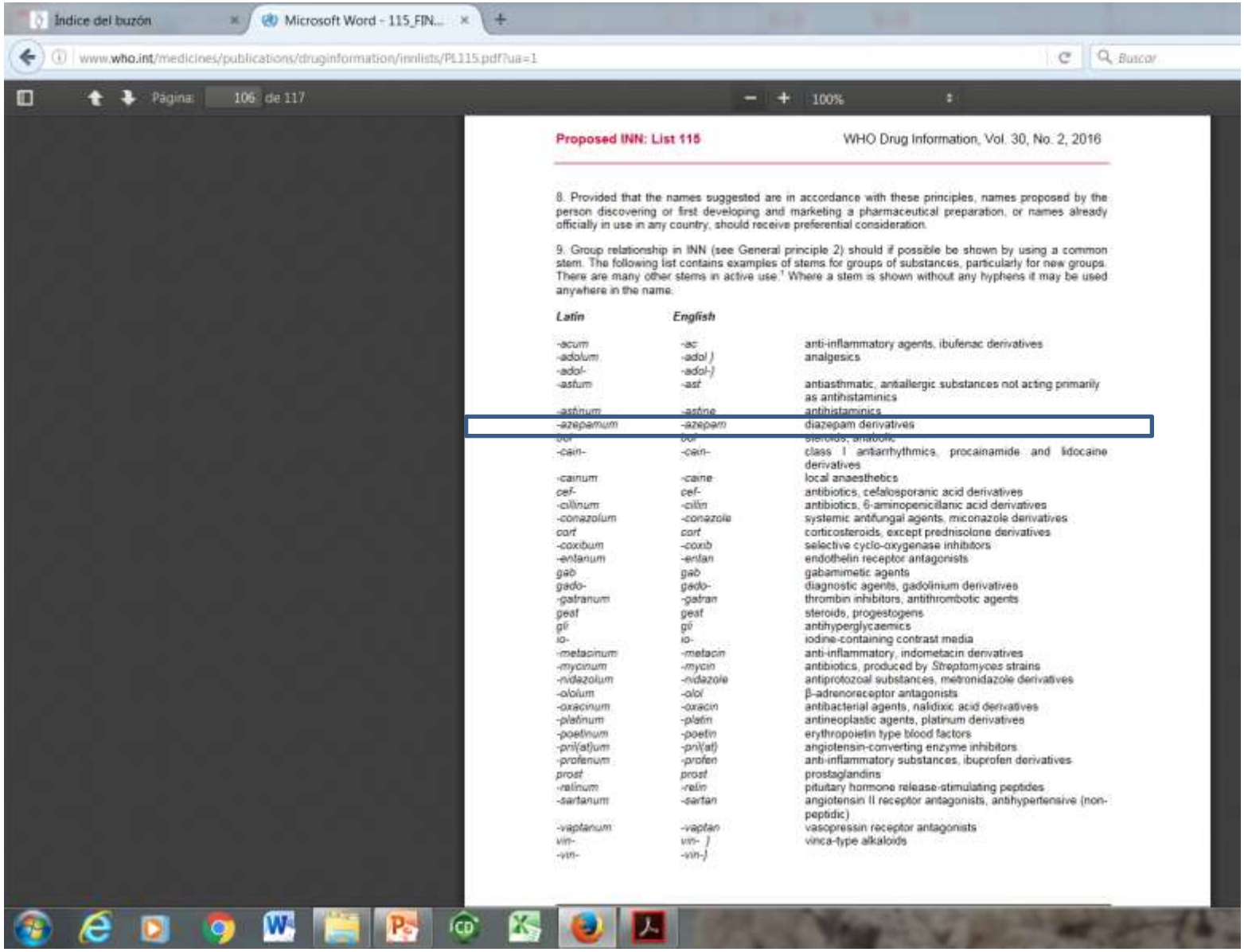


IUPAC name:

7-Chloro-1-methyl-5-phenyl-1,3-dihydro-benzo[e][1,4]diazepin-2-one

PAY ATTENTION TO THE CHEMICAL NAME!
It is important to see whether the name and the chemical structure match

1.4. NOMENCLATURE OF DRUGS



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Página: 106 de 117 — 100%

Proposed INN: List 115 — WHO Drug Information, Vol. 30, No. 2, 2016

8. Provided that the names suggested are in accordance with these principles, names proposed by the person discovering or first developing and marketing a pharmaceutical preparation, or names already officially in use in any country, should receive preferential consideration.

9. Group relationship in INN (see General principle 2) should if possible be shown by using a common stem. The following list contains examples of stems for groups of substances, particularly for new groups. There are many other stems in active use.[1] Where a stem is shown without any hyphens it may be used anywhere in the name.

Latin	*English*	
-acum	-ac	anti-inflammatory agents, ibufenac derivatives
-adolum	-adol)	analgesics
-adol-	-adol-)	
-astum	-ast	antiasthmatic, antiallergic substances not acting primarily as antihistaminics
-astinum	-astine	antihistaminics
-azepamum	-azepam	diazepam derivatives
-bol	-bol	steroids, anabolic
-cain-	-cain-	class I antiarrhythmics, procainamide and lidocaine derivatives
-cainum	-caine	local anaesthetics
cef-	cef-	antibiotics, cefalosporanic acid derivatives
-cillinum	-cillin	antibiotics, 6-aminopenicillanic acid derivatives
-conazolum	-conazole	systemic antifungal agents, miconazole derivatives
cort	cort	corticosteroids, except prednisolone derivatives
-coxibum	-coxib	selective cyclo-oxygenase inhibitors
-entanum	-entan	endothelin receptor antagonists
gab	gab	gabamimetic agents
gado-	gado-	diagnostic agents, gadolinium derivatives
-gatranum	-gatran	thrombin inhibitors, antithrombotic agents
gest	gest	steroids, progestogens
gli	gli	antihyperglycaemics
io-	io-	iodine-containing contrast media
-metacinum	-metacin	anti-inflammatory, indometacin derivatives
-mycinum	-mycin	antibiotics, produced by *Streptomyces* strains
-nidazolum	-nidazole	antiprotozoal substances, metronidazole derivatives
-ololum	-olol	β-adrenoreceptor antagonists
-oxacinum	-oxacin	antibacterial agents, nalidixic acid derivatives
-platinum	-platin	antineoplastic agents, platinum derivatives
-poetinum	-poetin	erythropoietin type blood factors
-pril(at)um	-pril(at)	angiotensin-converting enzyme inhibitors
-profenum	-profen	anti-inflammatory substances, ibuprofen derivatives
prost	prost	prostaglandins
-relinum	-relin	pituitary hormone release-stimulating peptides
-sartanum	-sartan	angiotensin II receptor antagonists, antihypertensive (non-peptidic)
-vaptanum	-vaptan	vasopressin receptor antagonists
vin-	vin-)	vinca-type alkaloids
-vin-	-vin-)	

http://www.who.int/medicines/publications/druginformation/innlists/en/

1.4. NOMENCLATURE OF DRUGS

ATC NOMENCLATURE
ANATOMICAL-THERAPEUTIC-CHEMICAL

This is used in the 'CATÁLOGO DE MEDICAMENTOS'
(Consejo General de Colegios Oficiales de Farmacéuticos)- SPAIN

One code for each drug

The **CAPITAL letter** denotes the principal active site
The **number and capital letter** denote the pharmacological action
The **alphanumeric code** designates the chemical structure and drug name

FIVE LEVELS

DIAZEPAM N05B A01
N Central nervous system - Main anatomic group
05 Psycholeplic - Pharmacological therapeutic group
B Tranquilizer - Pharmacological therapeutic subgroup
A Benzodiazepine derivative - Chemical subgroup
01 Diazepam - Final substance

http://www.whocc.no/atc/structure_and_principles/

http://www.who.int/medicines/publications/druginformation/WHO_DI_30-2_ATC-DDD.pdf

1.4. NOMENCLATURE OF DRUGS

The 1st Level (anatomical): indicates the anatomical main group. It consists of a capital letter denoting the organ or system on which the drug acts. There are 14 main groups.

A	DIGESTIVE AND METHABOLIC SYSTEM
B	BLOD AND HEMATOPOIETIC ORGANS
C	CARDIOVASCULAR SYSTEM
D	DERMATOLOGICAL MEDICINES
G	SEX HORMONES AND GENITOURINARY
H	SYSTEMIC HORMONAL PREPARATIONS, EXCL. SEX HORMONES
J	ANTIINFECTIOUS IN GENERAL FOR SYSTEMIC USE
L	ANTINEOPLASM AGENTS AND IMUNOMODULATORS
M	SKELETAL MUSCLE SYSTEM
N	NERVOUS SYSTEM
P	ANTIPARASITARY, INSECTICIDE AND REPELLENT PRODUCTS
R	RESPIRATORY SYSTEM
S	ORGANS OF THE SENSES
V	VARIOUS

DIAZEPAM N05B A01

https://en.wikipedia.org/wiki/Anatomical_Therapeutic_Chemical_Classification_System

1.4. NOMENCLATURE OF DRUGS

The 2nd Level indicates the therapeutic group and consists of two digits.

N05 Psycholeptics

The 3rd Level indicates the therapeutic or pharmacologic subgroup and consists of one capital letter.

N05B Anxiolytics

The 4th Level indicates the chemical subgroup and consists of one capital letter.

N05BA Benzodiazepine

The 5th Level indicates the chemical substance or pharmacological association and consists of two digits.

DIAZEPAM	N05B A01

N05BA01 Diazepam
N05BA02
N05BA03
N05BA04
N05BA05
etc…….

1.5. THE PHARMACEUTICAL INDUSTRY

REFERENCES:

The Pharmaceutical Industry in Figures (Key Data):
http://www.efpia.eu/uploads/Figures_2015_Final.pdf
https://www.efpia.eu/media/219735/efpia-pharmafigures2017_statisticbroch_v04-final.pdf
(EFPIA: European Federation of Pharmaceutical Industries and Associations)

Farmaindustria (Asociación Nacional Empresarial de la Industria Farmacéutica establecida en España)
http://www.farmaindustria.es/Farma_Public/Farmaindustria/Asociados/index.htm

http://www.pmfarma.es/colaboradores/mercado-mundial/1664-mercado-farmaceutico-mundial-a-agosto-2014.html

Electronic book (UV):
Green and Sustainable Medicinal Chemistry: Methods, Tools and Strategies for the 21st Century Pharmaceutical Industry
Edited by Louise Summerton, Helen F. Sneddon, Leonie C. Jones and James H. Clark. The Royal Society of Chemistry 2016
CHAPTER 9 Medicinal Chemistry: How ‘‘Green’’ is Our Synthetic Tool Box?
GOOD INTRODUCTION

1.5. THE PHARMACEUTICAL INDUSTRY

Some Features:

The pharmaceutical industry aims to turn fundamental research into innovative treatments that are widely available and accessible to patients.
It contributes to medical progress by researching, developing and bringing new medicines that improve health and quality of life for patients around the world.

It is one of Europe's top performing high-technology sectors:

- it directly employs more than 700,000 people (and generates three to four times more employment indirectly)
- it has the highest added-value per person employed (significantly higher than the average value for high-tech and manufacturing industries)
- it is the sector with the highest ratio of R&D investment to net sales.

The majority of drug discovery approaches in pharmaceutical companies still focus on target-based research activities.

1.5. THE PHARMACEUTICAL INDUSTRY

The number of new drugs approved (NDA) had decreased in recent decades:

- In the 1980s roughly 60 new drugs were approved annually.
- On average, FDA has approved 20-30 new drugs per year in the past two decades.
- Annual approvals in the past five years have been in the range of 40-50 new drugs (except in 2016). In 2018 a record number has been reached (59).

Expenditures are increasing, thus making the cost per approved drug much higher.

In recent decades the industry has shifted toward the biological as a drug class.

- Costly research and development (R&D) is conducted by pharmaceutical companies to introduce new medicines into the market.
- On average, **12-13 years** will elapse between the first synthesis of the new active substance and its commercialisation.
- In 2012 the cost of researching and developing a new chemical or biological entity was estimated at **€ 1,172 million** ($ 1,506 million in 2011 dollars).
- On average, only **one to two of every 10,000 substances** tested in laboratories will successfully pass all the stages of development required to become a marketable medicine.

Source: EFPIA

1.5. THE PHARMACEUTICAL INDUSTRY

Distribution of new approved drugs (NADs) between biologicals and small molecule drugs (USA)

2009: 7 of the 27 NDAs were biologicals (antibody and enzymes)

2010: 10 of the 21 NDAs were biologicals

2011: 11 of the 35 NDAs were biologicals

2018: 12 of the 59 NDAs were biologicals (C&EN 2019, 97, 3, 33-37)

Synthetic small molecules have been produced by medicinal chemists using methods of synthetic organic chemistry to assemble molecules in 6–12 or more steps of chemical synthesis.

More recently, small molecule drug discovery has incorporated computational chemistry, robotization and structural chemistry, etc.
Small molecules, with a molecular weight of 600 Da or less, are taken most often as oral tablets.

Protein drugs, which are injectable (intravenously or subcutaneously), are produced as recombinant proteins (without isolating or purifying them from organs and tissues, as was the case up to 1985). *Biotech companies.*

1.5. THE PHARMACEUTICAL INDUSTRY

An. Quím. **2006**, *102 (3)*, 13-22

GENERICS

When intellectual property protection rights have expired, a manufacturer who is not the inventor of the original product is allowed to produce and market similar medicines. These are called ‘generics’.

A **Generic Medicine** is developed to be the same as a medicine that has already been authorised (the ‘reference medicine’). It contains the same active substance as the originator medicine, and it is used at the same dose to treat the same disease as the reference medicine. However, the name of the medicine, its appearance (such as colour or shape) and its packaging can be different from those of the reference medicine.

http://www.medicinesforeurope.com/
European Generics Medicines Association (EGA)

1.5. THE PHARMACEUTICAL INDUSTRY

A **generic medicine "is any medicine that has the same qualitative and quantitative composition, active substance and pharmaceutical form as the originator product and whose bioequivalence with that product has been demonstrated with appropriate bioequivalence studies"**
Spanish Law of July 2006 on the Guarantees and Rational Use of Medicines and Sanitary Products (LGURMPS) **.**

A generic medicine contains the same active medicinal substance as an originator pharmaceutical product. Because it acts in the same way in the human body, it is interchangeable with the originator product. Generic medicines are launched when the originator product's patent has expired.

http://www.aeseg.es/en/ Spanish Generic Medicines Association

1.5. THE PHARMACEUTICAL INDUSTRY

Bioequivalence demonstrates **the interchangeability between a generic medicine and the originator medicine in terms of quality, security and efficacy**. Bioequivalence studies are done to prove that the generic medicines are equivalent and interchangeable with the originator product in terms of therapeutic efficacy.
These studies are much less expensive than clinical trials required for the originator product.

Generic medicines contribute to obtaining considerable savings, and effectively reduce the cost of medicines from 40 to 60%.

In Spain, the generic medicines market during the last year only represented **20% of the total pharmaceutical market in value and 40% in volume**. Generic medicines in Spain are still a long way from the European average, which is around 55% in volume.

In general, the market share of generics is significantly higher in newer EU Member States with historically low levels of intellectual property protection.

http://www.aeseg.es/en/ Spanish Generic Medicines Association
http://www.aeseg.es/documentos/

UNIT 2. DRUG TARGETS

2.1. Concept of drug target.

2.2. Drug-target interactions.

(Patrick's, 5th ed. chapter 1)

2.3. Chemical nature of the targets (proteins, lipids, nucleic acids and carbohydrates).

(Patrick's, 5th ed. chapters 2,3, 5 and 6)

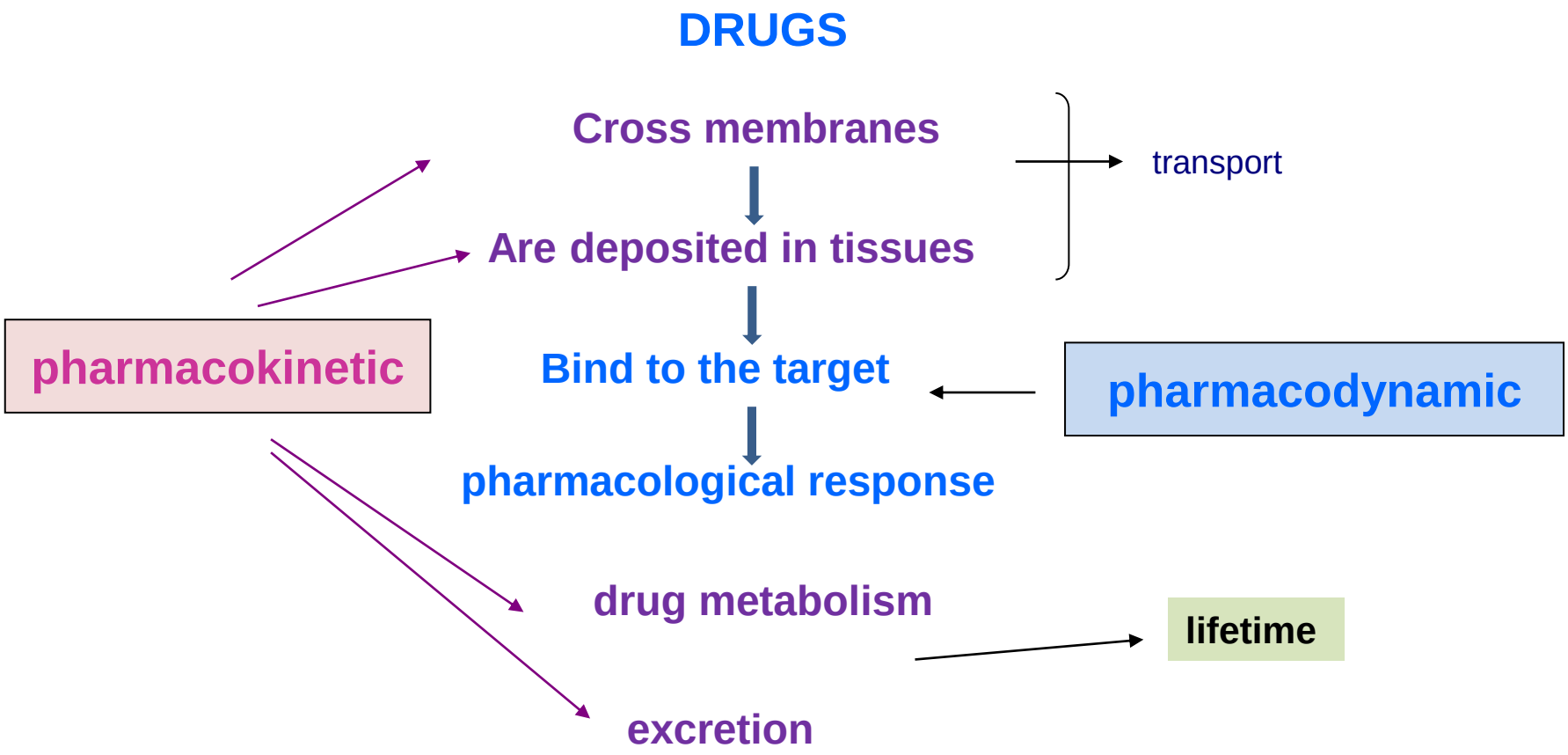
2.4. Examples of drugs that interact with the targets.

(Patrick's, 5th ed. chapters 4, 7-10)

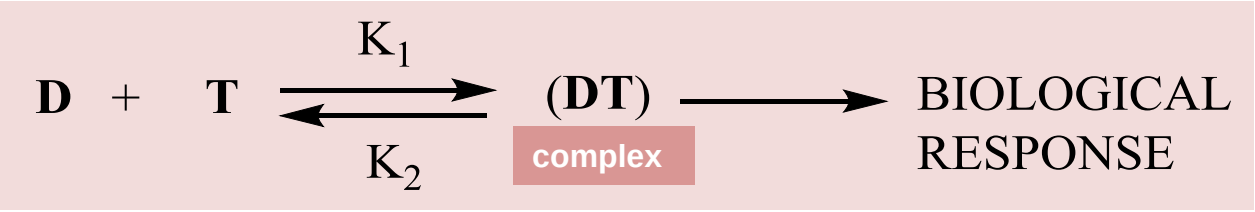
(see also Biochemistry I and II and Pharmacology I and II)

2.1. CONCEPT OF DRUG TARGET

Specificity is one of a drug's most important properties. We want the drug to perform a specific action (therapeutic, preventive or diagnostic). This is only possible if the active principle is able to recognise in the body those biomolecules that are related to the disease and trigger a response. These biomolecules are known as **drug targets**.



Arïens and Stephenson



Biological response = α[DT]

The **AFFINITY** of a drug (D) for a target (T) is a measure of how strongly the drug binds to the target. It is proportional to the equilibrium constant of complex formation.

EFFICACY (α) is a measure of the maximum biological effect that a drug can produce as a result of target binding.

2.1. CONCEPT OF DRUG TARGET

In the target-based approach, after a target disease has been identified, the first steps in (Rational) Drug Discovery are:

a) Target Identification
b) Target Validation (also establishing testing procedures)
d) Finding a lead compound

Target selectivity

Different species:

- Identify targets which are unique to the pathogen (bacteria, virus, fungi)
- Identify targets which are shared but which are different in structure

In Humans:

- different enzymes or different receptors etc.
- receptor types and subtypes
- isozymes
- also organ and tissue selectivity

- Tests are needed to find lead compounds and optimise the drug.
- The tests can be *in vivo* or *in vitro (*a combination of tests is often used)

2.1. CONCEPT OF DRUG TARGET

In vitro TESTS

- Are not carried out on animals/humans

  Target molecules

  Cells **Tissues** **Organs** **Micro-organisms**

- Involve fewer factors and are more suitable for routine testing (easier to rationalise).

- Measure the interaction of a drug with the target but not the ability of the drug to reach the target (do not demonstrate a physiological or clinical effect and do not allow the identification of possible side effects).

In vivo TESTS

- Are carried out on live animals or humans.
- Measure an observed physiological effect (also a drug's ability to interact with its target and its ability to reach that target).
- Possible side effects can be identified.
- Rationalisation could be difficult (many factors involved).

2.2. DRUG-TARGET INTERACTIONS

Drug targets are molecular structures that undergo a specific interaction with a drug (drug is a compound administered to treat, prevent or diagnose a disease). A change in the behaviour or properties of these targets can be detected as a consequence of this interaction.

- Drug targets are generally molecules that are much bigger than those of drugs.
- Drugs interact with their targets by binding to binding sites.
- Binding sites are located on the surface of macromolecules and consist typically of hydrophobic hollows that are recognised by the drug.
- Binding interactions involve **intermolecular bonds** between drug and target (or between targets and endogenous molecules or organic compounds in general).
- In the body, most drugs are in equilibrium (bound and unbound to their target).
- The functional groups on the drug that are involved in binding interactions are called binding groups.
- Specific regions within the binding site that are involved in binding interactions are called binding regions.

(Patrick's 5th ed., chapter 1)

$\Delta G = \Delta H - T\Delta S$ (Remember Organic Chemistry)

COMMON INTERMOLECULAR FORCES

Bond type	Bond energies **Energy** (KJ/mol)
Covalent	150-400
Ionic	20-25
Hydrogen bond	5-30
Ion-dipole	5-30
Dipole-dipole	5-30
Van der Waals	2-4
Hydrophobic	4
Charge transfer	5-30

Maximum energy available at room temperature 80 KJ/mol
Energy required to produce a conformational change 10 KJ/mol

2.2. DRUG-TARGET INTERACTIONS

Covalent bonds

- Are the strongest bonds (150-400 kJ mol^{-1}).
- Are considered "irreversible" interactions (the target is modified by the drug).
- Are not very common (they are found in some drugs used in chemotherapy).
- Are often related to toxicological effects.
- Usually involve a nucleophilic centre in proteins or nucleic acids that react with an electrophilic centre present in the drug.

Nucleophilic centres in proteins:

- Thiol group of cysteine
- S-atoms of methionine
- Primary amino-groups (e.g.: lysine, arginine)
- N-atom in the imidazol ring of histidine
- O-atom in serine

Nucleophilic centres in nucleic acids:

- Primary amino-groups of purine bases (e.g. adenine, guanine)
- N-atoms in rings of purine and pyrimidine bases (e.g. N7 of guanine)
- O-atoms of purine and pyrimidine bases (e.g. O6 of guanine)
- Phosphate O-atom (P=O)

Measurement and Estimation of Electrophilic Reactivity for Predictive Toxicology
J. A. H. Schwobel, Y. K. Koleva, S. J. Enoch, F. Bajot, M. Hewitt, J. C. Madden, D. W. Roberts, T. W. Schultz, and M. T. D. Cronin, *Chem. Rev*. **2011**, 111, 2562–2596

2.2. DRUG-TARGET INTERACTIONS

Common reaction mechanisms in the formation of covalent bonds

	Functional group present in drugs	Modified protein
• S_N1	(alkyl –X, X = leaving group)	Protein-Nu-R (alkyl)
• S_N2	(alkyl –X, X = leaving group)	Protein-Nu-R (alkyl)
• Acylation	(R-COX, X = leaving group)	Protein-Nu-COR (acyl)
• Imine (Schiff Base) Formation	(RCHO)	Protein-N=CH-R
• Michael (Conjugate) Addition	(C=C-X) (X = electron withdrawing group -CHO, -COR)	Protein-Nu-C –C- X
• S_NAr	(Ar –X, X = leaving group) (Ar: only if electron withdrawing groups are present NO_2, CN,…)	Protein-Nu - Ar

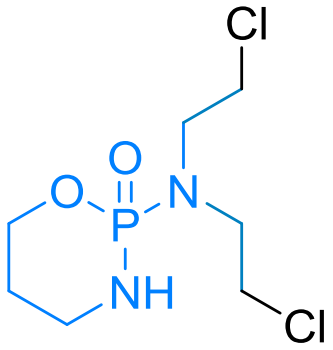
Measurement and Estimation of Electrophilic Reactivity for Predictive Toxicology
J. A. H. Schwobel, Y. K. Koleva, S. J. Enoch, F. Bajot, M. Hewitt, J. C. Madden, D. W. Roberts, T. W. Schultz, and M. T. D. Cronin*, *Chem. Rev*. **2011**, 111, 2562–2596

2.2. DRUG-TARGET INTERACTIONS

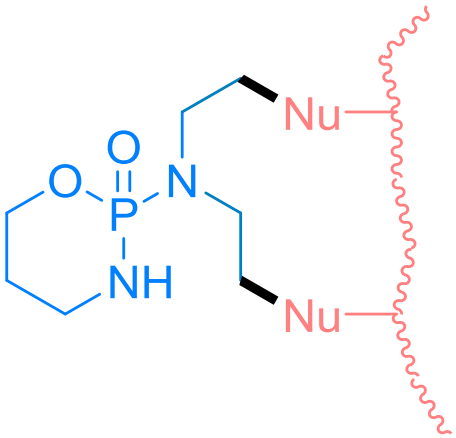
NITROGEN MUSTARDS

(ANTINEOPLASIC)

Cyclophosphamide



2-[Bis(2-chloroethylamino)]-tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide



TARGET

(Covalent bonds)

2.2. DRUG-TARGET INTERACTIONS

Electrostatic or ionic bonds

- These are the strongest of the intermolecular non-covalent bonds (20-40 kJ mol^{-1}).
- They take place between groups of opposite charge.
- Stronger interactions occur in hydrophobic environments.
- The strength of the ionic interaction is inversely proportional to the distance between the two charged groups but it drops less rapidly with distance than with other forms of intermolecular interaction.
- Ionic bonds are the most important initial interactions as the drug enters the binding site (e.g. proteins because several amino acids are charged at physiologic pH).

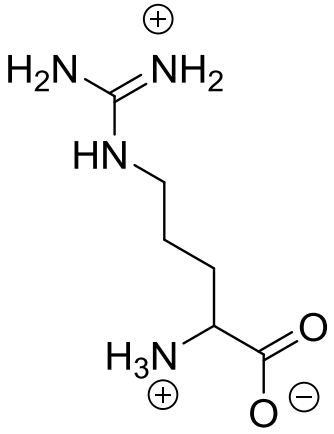
$$F = \frac{Q_1 \cdot Q_2}{K \cdot r^2}$$

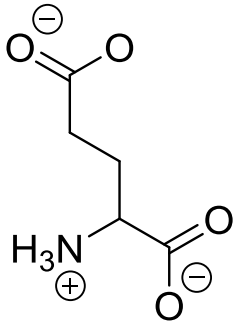
2.2. DRUG-TARGET INTERACTIONS

Electrostatic or ionic bonds

Charged Residues

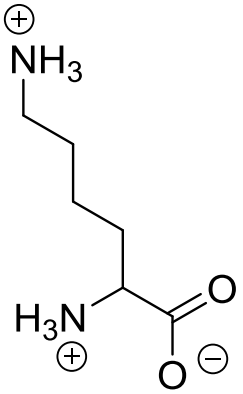
Arginine (Arg)

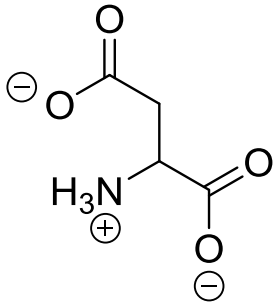




Glutamic Acid (Glu)

Lysine (Lys)





Aspartic Acid (Asp)

2.2. DRUG-TARGET INTERACTIONS

Hydrogen bonds

- These bonds vary in strength.
- They are weaker than electrostatic interactions but stronger than van der Waals interactions.
- A hydrogen bond takes place between an electron-deficient hydrogen (usually attached to a heteroatom (O or N) and an electron-rich heteroatom (N or O).
- The electron-deficient hydrogen is called a **hydrogen bond donor** (HBD).
- The electron-rich heteroatom is called a **hydrogen bond acceptor** (HBA).
- The interaction is directional and involves orbitals.
- Optimum orientation is where the X-H bond points directly to the lone pair on Y such that the angle between X, H and Y is 180^{o}.

2.2. DRUG-TARGET INTERACTIONS

Hydrogen bonds

- Examples of strong hydrogen bond acceptors:

  - COO^-, PO_4^-, $-NR_3$

- Examples of moderate hydrogen bond acceptors:

  - Oxygen atom in carbonyl groups:
    carboxylic acids, amides, ketones, esters
  - Oxygen atom in ethers, alcohols (phenols)

- Examples of poor hydrogen bond acceptors:

  - S, F, Cl, aromatic ring, nitrogen in amides, aromatic amines

- Example of good hydrogen bond donors:

  - ammonium ions when a H atom is present ($\underline{H}NR_3^+$)

See Appendix 8, Patrick's 5th Ed: Hydrogen Bonding Interactions

2.2. DRUG-TARGET INTERACTIONS

Ion-dipole interactions

They occur where the charge on one molecule interacts with the dipole moment of another.

- They are stronger than dipole-dipole interactions.
- The strength of these interactions falls less rapidly with distance than dipole-dipole interactions.

Dipole-dipole interactions

- These can occur if the drug and the binding site have dipole moments.
- Dipole alignment orients the molecule in the binding site.
- Orientation is beneficial if other binding groups are positioned correctly with respect to the corresponding binding regions and it is detrimental if the binding groups are not positioned correctly.
- The strength of the interaction decreases with distance more quickly than with electrostatic interactions but less quickly than with van der Waals interactions.

2.2. DRUG-TARGET INTERACTIONS

Ion-induced dipole interactions

They occur when the charge on one molecule induces a dipole on another (e.g. between a quaternary ammonium ion and an aromatic ring).

Van der Waals interactions

- These are very weak interactions (2-4 kJ mol^{-1}) but the overall contribution of van der Waals interactions can be crucial to binding.
- They occur between hydrophobic regions of the drug and the target because transient areas of high and low electron densities cause temporary dipoles.
- Interactions drop off rapidly with distance (the drug must be close to the binding region for interactions to occur).

2.2. DRUG-TARGET INTERACTIONS

De-solvation

- Polar regions of a drug and its target are solvated prior to interaction.
- De-solvation is necessary and requires energy.
- The stabilisation energy gained by drug-target interactions must be greater than the energy penalty required for de-solvation.

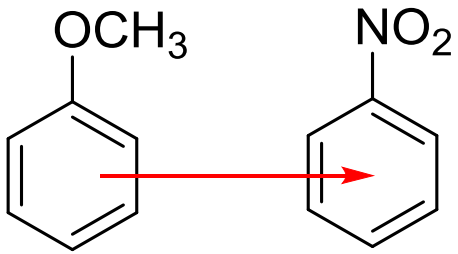
Hydrophobic interactions

- Are beneficial to binding energy.
- Hydrophobic regions of a drug and its target are not solvated.
- Water molecules interact with each other and form an ordered layer next to hydrophobic regions (negative entropy).
- Interactions between the hydrophobic regions of a drug and its target ‘free up’ the ordered water molecules and this process results in an increase in entropy.

2.2. DRUG-TARGET INTERACTIONS

Charge Transfer

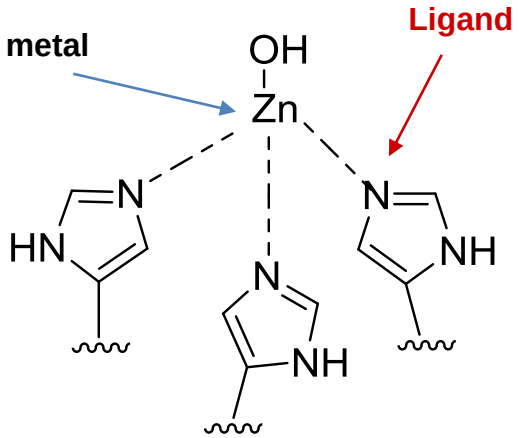
- Electrostatic interaction by π orbital overlapping between two molecules (donor and acceptor).
- Most common examples: electron-rich aromatic ring (methoxybenzene) and electron-poor aromatic ring (nitrobenzene). E.g. in nature: noradrenaline and ATP.



HOMO-LUMO interaction

Coordination

- Interaction between a metallic cation and a ligand atom or functional group acting as electron-donor (e.g. active site in carbonic anhydrase).



2.3. CHEMICAL NATURE OF DRUG TARGETS

A) Proteins

Structural proteins
Carrier (transport) proteins
Enzymes
Receptors

(Patrick's, 5th ed. chapters 2, 3, 5 and 6)

B) Nucleic acids: DNA and RNA

C) Lipids Membrane lipids

D) Carbohydrates in cell surface

Antigens and recognition molecules

These targets have different locations in cells. Many of them are located in membranes.

Cell Membrane

Remember:

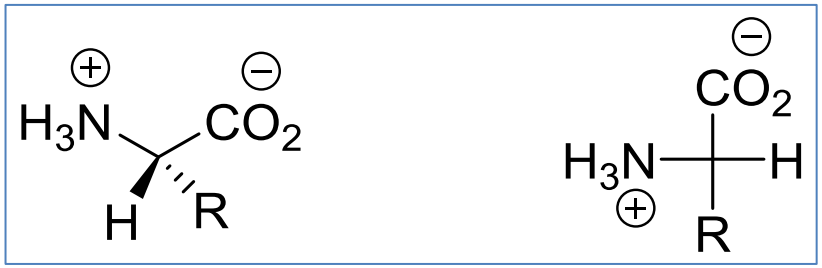
- The cell membrane is made up of a phospholipid bilayer.
- The polar head groups interact with water at the inner and outer surfaces of the membrane.
- The hydrophobic tails are hidden from the aqueous media (they interact with each other by van der Waals interactions).
- The cell membrane provides a hydrophobic barrier around the cell, preventing the passage of water and polar molecules.
- Proteins (ion channels, receptors, enzymes and transport proteins) are embedded in the cell membrane.

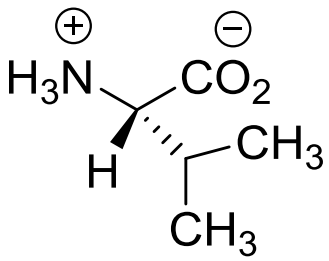
2.3. CHEMICAL NATURE OF DRUG TARGETS

Proteins

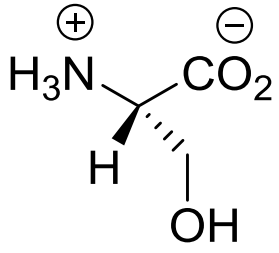
Remember:

- Amino acids are the building blocks for proteins.
- Each amino acid has an identical head group.
- Amino acids are chiral molecules (except Gly, R=H).
- Only *L*-amino acids are present in human biochemistry.
- The *L*-amino acids are *S*-enantiomers (except Cys; R = CH_2SH).

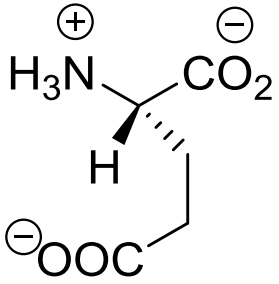




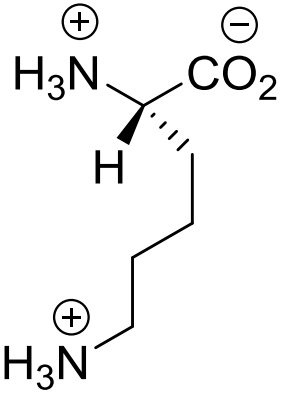
valine



serine



aspartate



lysine

2.3. CHEMICAL NATURE OF DRUG TARGETS

Proteins

The primary structure of proteins

Remember the codes for essential amino acids

Alanine	Ala	A	Tyrosine	Tyr	Y
Arginine	Arg	R	Histidine	His	H
Asparagine	Asn	N	Isoleucine	Ile	I
Aspartic acid	Asp	D	Leucine	Leu	L
Cysteine	Cys	C	Lysine	Lys	K
Glutamic acid	Glu	E	Methionine	Met	M
Glutamine	Gln	Q	Phenylalanine	Phe	F
Glycine	Gly	G	Threonine	Thr	T
Proline	Pro	P	Tryptophan	Trp	W
Serine	Ser	S	Valine	Val	V

- The primary structure is the order in which amino acids are linked together.
- Amino acids are linked through their head groups by peptide bonds to form a polypeptide chain or backbone.

2.3. CHEMICAL NATURE OF DRUG TARGETS

Proteins

The secondary structure of proteins

See Figure 2.5 The α-helix for proteins (Patrick's 5th ed.)

See Figure 2.6 The β-pleated sheet (Patrick's 5th ed.)

The tertiary structure of proteins

See Figure 2.10 Tertiary structure formation as a result of intramolecular interactions (Patrick's 5th ed.)

See Figures 2.11, 2 .12, 2.13 (Patrick's 5th ed.)

See Figures 2.14 and 2.16 (Patrick's 5th ed.)

Protein function:

Structural proteins

Transport proteins

Enzymes - life's catalysts

Receptors - life's communication system

2.3. CHEMICAL NATURE OF DRUG TARGETS

Protein

Enzymes

Remember:

They are globular proteins acting as the body's catalysts:

- Lower the activation energy of a reaction (but ΔG remains the same).
- Speed up time for reaction to reach equilibrium.

- Provide a reaction surface (the active site) and a suitable environment (hydrophobic).
- Bring reactants together and position them correctly for reaction.
- Stabilise the transition state with intermolecular bonds.

- Weaken bonds in the reactants and provide acid/base catalysis or nucleophilic groups

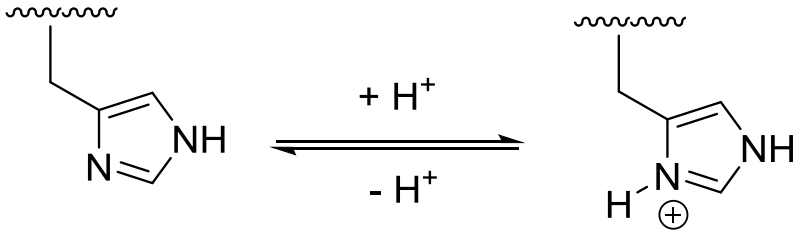
2.3. CHEMICAL NATURE OF DRUG TARGETS

Protein

Enzymes

- Histidine

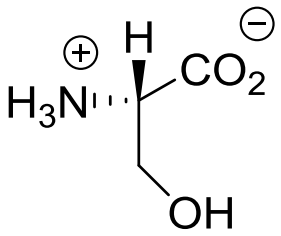
Acid/base catalysis



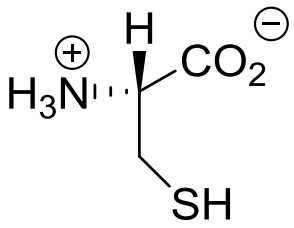
Non-ionised.
Acts as a basic catalyst

Ionised (stabilised by resonance).
Acts as an acid catalyst

Nucleophilic residues



L- Serine



L- Cysteine

2.3. CHEMICAL NATURE OF DRUG TARGETS

Protein

Enzymes

The Active Site

- Accepts reactants (substrates and cofactors).
- Is a hydrophobic hollow or cleft on the enzyme surface.
- Contains amino acids which bind reactants and participate in the enzyme-catalysed reaction.

Induced fit:
The active site changes shape to maximise intermolecular bonding

Overall Process of Enzyme Catalysis

Binding interactions must be strong enough to hold the substrate long enough for the reaction to occur, but they must be weak enough to allow the product to depart.

2.3. CHEMICAL NATURE OF DRUG TARGETS

Protein

Enzymes

Regulation of Enzymes

Enzymes with allosteric sites are often at the start of a biosynthetic pathway.

- The enzyme is controlled by the final product of the pathway.
- The final product binds to the allosteric site and switches off the enzyme.

- Designing molecules with stronger binding interactions can result in **enzyme inhibitors** which block the active site (**competitive** inhibitor).

- Some compounds are able to modify the activity of an enzyme by binding to an allosteric site. These are **allosteric** inhibitors (not competitive).

2.3. CHEMICAL NATURE OF DRUG TARGETS

Protein

Receptors

Structure and function

- Are globular proteins located mainly in the cell membrane.
- Receive messages from **chemical messengers** from other cells and transmit a message into the cell leading to a cellular effect.

There are specific receptors for different chemical messengers and each cell has a range of receptors in the cell membrane that make it responsive to different chemical messengers.

Chemical messengers are able to 'switch on' receptors without undergoing a reaction:

- Neurotransmitters are released from the end of a neuron to bind with a receptor on a target cell, such as a muscle cell or another neuron. They are usually short-lived and are responsible for messages between individual cells.
- Hormones are released from a cell or a gland which travels some distance throughout the body to bind with receptors on target cells.

2.3. CHEMICAL NATURE OF DRUG TARGETS

Protein

Receptors

Mechanism

- Receptors contain a binding site that is recognised by the chemical messenger.
- The binding of the messenger involves intermolecular bonds and it results in an induced fit of the receptor protein.
- This change in the receptor shape results in a ‘domino’ effect known as **Signal Transduction** and leads to a chemical signal being received inside the cell.
- The chemical messenger does not enter the cell and is not permanently bound.
- It **departs** the receptor **unchanged**.

Protein: Enzymes and Receptors

Receptors and enzymes. A Comparison

Similarities:

- A hydrophobic hollow or cleft on the surface of the receptor is equivalent to the active site of an enzyme.
- Both of them accept and bind a “ligand” compound (endogenous chemical messenger/ substrate or drug).
- Both of them are proteins (contain amino acids which bind the messenger/substrate or drug).
- The types of intermolecular interactions between substrate/enzyme and messenger/receptor are the same (and are the same with drugs).

Differences:

- A reaction or catalysis takes place on the active site of an enzyme, whereas no reaction takes place in the messenger/receptor complex (only a change in shape occurs that triggers the response in the cell).
- Receptors are usually located in cell membranes or within the nucleus, whereas enzymes can be found in every region of the body.

2.3. CHEMICAL NATURE OF DRUG TARGETS

Protein

Receptors

- Binding interactions in receptors must be strong enough to hold the messenger and long enough for signal transduction to take place.
- Interactions must be weak enough to allow the messenger to depart.
- Designing molecules with stronger binding interactions can result in drugs that block the binding site. These are called competitive **antagonists** (drugs which bind to a receptor without activating it) and are the opposite of **agonists** (drugs which produce the same response on a receptor as the natural messenger does).

These concepts will be developed later (see Unit 5)

Receptor Superfamilies

- ION CHANNEL RECEPTORS
- G-PROTEIN COUPLED RECEPTORS
- KINASE LINKED RECEPTOR
- INTRACELLULAR RECEPTORS

(to be developed in Pharmacology I)

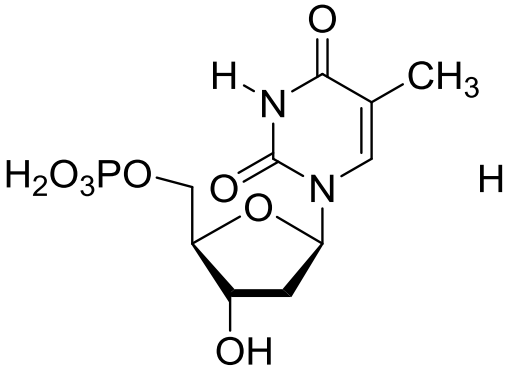
Nucleic Acids /DNA

Primary structure

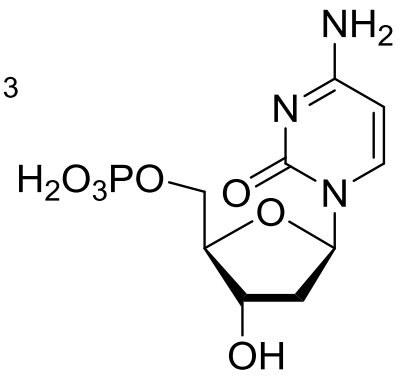
Building blocks (nucleotide):

Phosphate + sugar + nucleic acid (purine or pyrimine base)

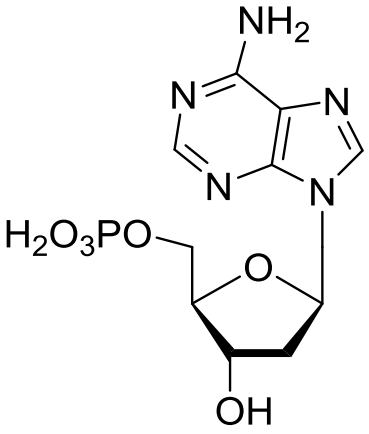
nucleoside



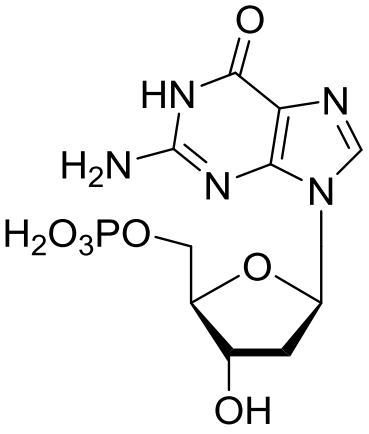
Deoxythymidine phosphate



Deoxycytidine phosphate



Deoxyadenosine phosphate



Deoxyguanosine phosphate

Nucleic Acids /DNA

Secondary structure

- The sugar phosphate backbone is ionised and faces outward because of favourable interactions with water.
- Nucleic acid bases point inward and pair up.
- Chains are complementary.
- Purine pairs with pyrimidine (constant diameter to helix) A-T or G-C.
- Base pairs are stacked (Van der Waals interactions between pairs).

See Fig 6.4. The secondary structure of DNA (Patrick's 5th ed.)

Nucleic Acids /DNA

Tertiary structure

- The double helix coils into a 3D shape.
- The double helix has to unravel during replication and unravelling leads to strain.
- The strain is relieved by enzyme-catalysed cutting and repair of the DNA chain (quinolone antibacterial agents inhibit this enzyme).

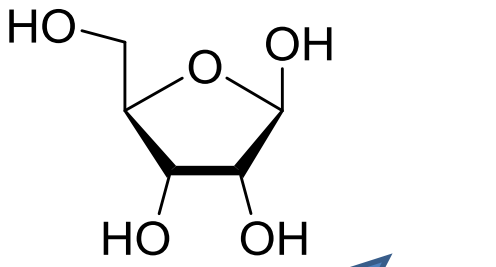
Topoisomerase II

- Topoisomerase II relieves the strain in the DNA helix by temporarily cleaving the DNA chain and crossing an intact strand through the broken strand.
- Tyrosine residues in the enzyme form covalent bonds to DNA.

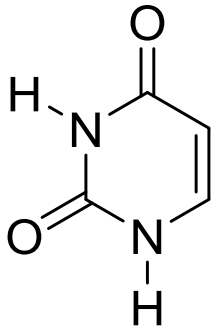
Nucleic Acids/RNA

2.1 Primary structure

Similar to DNA but ribose is used instead of deoxy-ribose and uracil is used instead of thymine.



Ribose



Uracil

2.2 Secondary structure

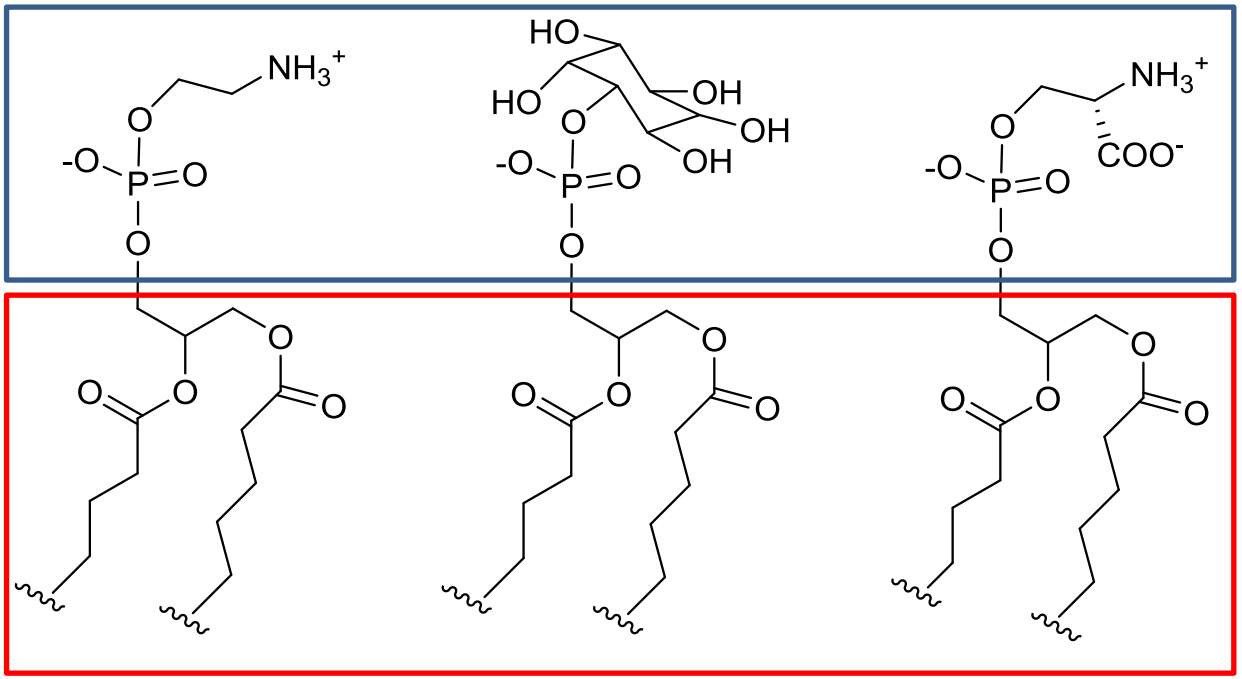
- RNA is single stranded but some regions of helical secondary structure exist due to base pairing within the same strand (t-RNA).
- Guanine pairs to cytosine, while adenine pairs to uracil.

Lipids in membranes

Phosphoglycerides

Polar heads

Fatty acids

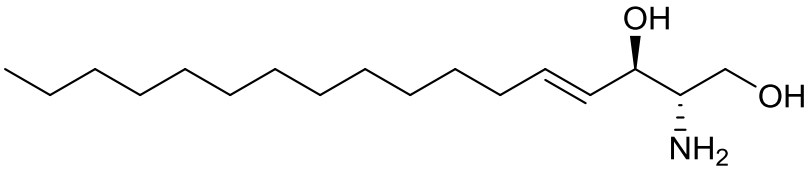


Polar head groups and fatty acids can be varied (ethanolamine, inositol, serine instead of choline, etc.).

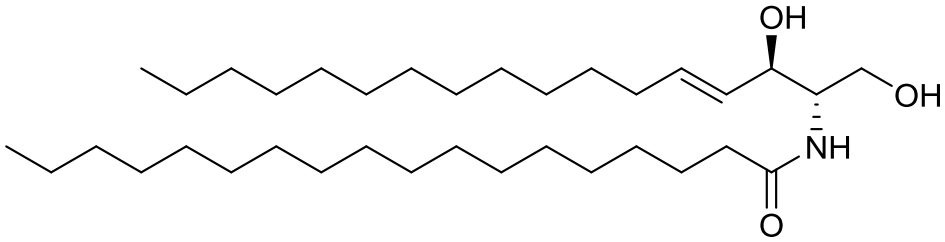
2.3. CHEMICAL NATURE OF THE TARGETS

Lipids in membranes

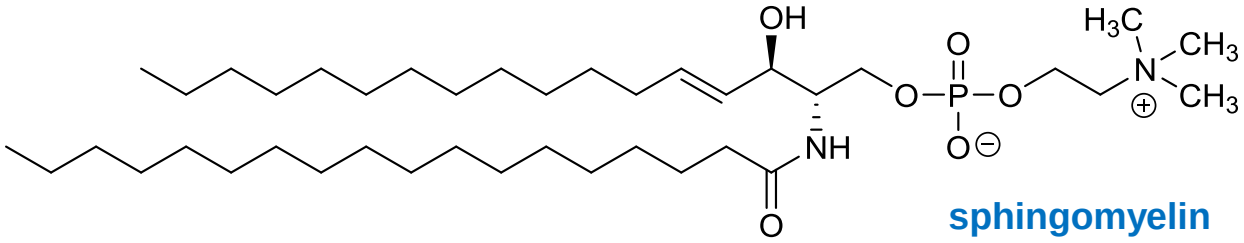
Sphingolipids



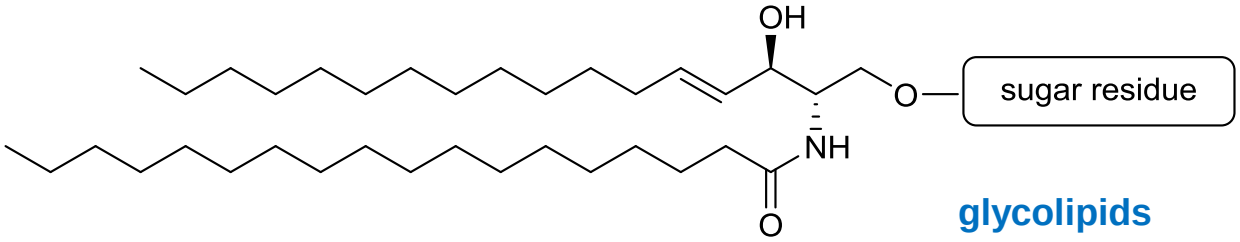
sphingosine



ceramide

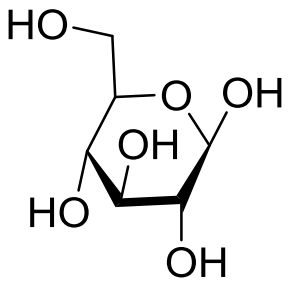


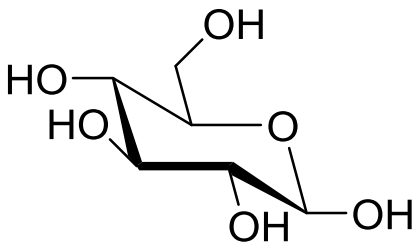
sphingomyelin



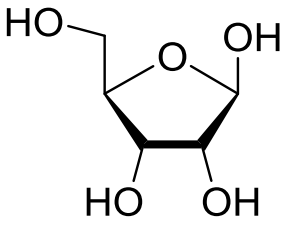
glycolipids

Carbohydrates

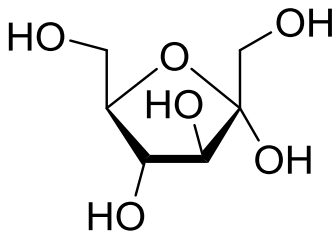




D- glucose



D-ribose



D-fructose

See also Figure 10.2.2. Cellulose, where glucosyl units are linked β-1,4. (Patrick's 5th ed).

- Carbohydrates play an important role in cell recognition, regulation and growth.
- They are potential targets for the treatment of bacterial and viral infections, cancer and autoimmune diseases (they act as antigens).

2.4. EXAMPLES OF DRUGS THAT INTERACT WITH THESE TARGETS

A) Proteins as drug targets

Structural proteins (tubulin) *(Patrick's 5th Ed. Chapter 10)*

Carrier (transport) proteins *(Patrick's 5th Ed. Chapter 10)*

Enzymes (*Patrick's 5th Ed. Chapter 7*)

Receptors *(Patrick's 5th Ed. Chapter 8)*

See UNIT 5

(More enzyme inhibitors are included in Pharmaceutical Chemistry II)
(Examples of agonists and antagonists are also included in Pharmaceutical Chemistry II)

B) Nucleic acids as drug targets *(Patrick's 5th Ed. Chapter 9)*

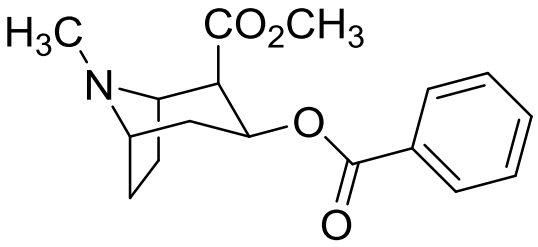
C) Lipids *(Patrick's 5th Ed. Chapter 10)*

D) Carbohydrates *(Patrick's 5th Ed. Chapter 10)*

PROTEINS:
Agents blocking transport proteins

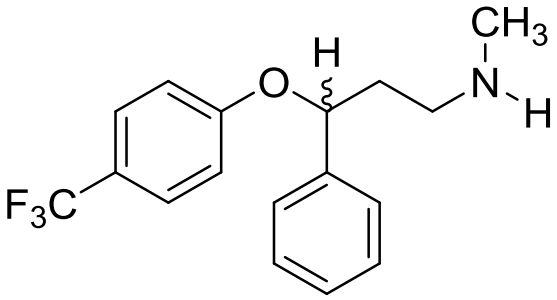
(Patrick's 5th Ed. Chapter 10)

- Prevent the re-uptake of neurotransmitters (e.g. dopamine, serotonin and noradrenaline).
- Result in increased levels of affected neurotransmitters.



cocaine

• Causes euphoric effects (reuptake inhibitor for dopamine in CNS) and suppresses hunger (reuptake inhibitor of noradrenaline in the peripheral system).

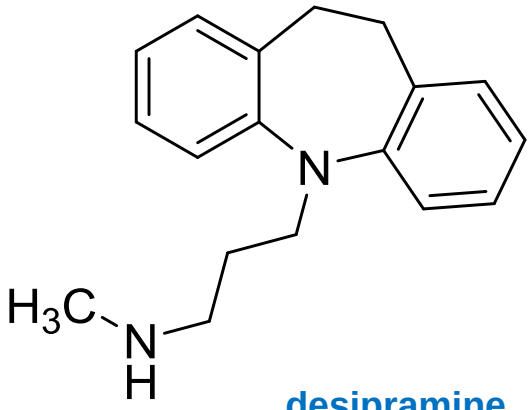


fluoxetine (Prozac®)

Selective serotonin reuptake inhibitor (SSRI) used as an antidepressant.

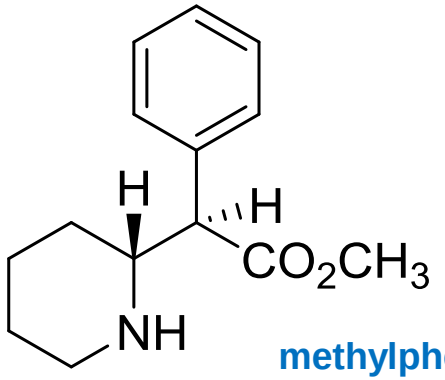
PROTEINS:
Agents blocking transport proteins

(Patrick's 5th Ed. Chapter 10)



desipramine

Tricyclic antidepressant (it was the principle treatment for depression from 1960 to 1980) and it is a non-selective reuptake inhibitor for noradrenaline.



methylphenidate (Ritalin®)

Is a reuptake inhibitor for noradrenaline and dopamine and is used to treat attention deficit hyperactivity disorder.

Drugs acting on nucleic acids

(Patrick's 5th Ed. Chapter 9)

Classification based on the mechanism of action:

1. **Intercalating agents**
2. **Topoisomerase poisons (non-intercalating)**
3. **Alkylating agents**
4. **Metallating agents**
5. **Chain cutters and chain terminators**

Drugs acting on DNA

(Patrick's 5th Ed. Chapter 9)

Intercalating agents

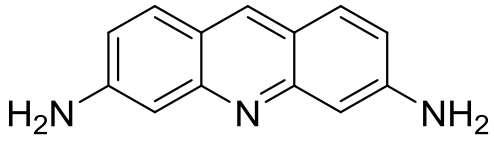
Mechanism of action

- Intercalating agents contain planar aromatic or heteroaromatic ring systems that slip between the layers of nucleic acid pairs and disrupt the shape of the helix.
- Intercalation prevents replication and transcription and can inhibit topoisomerases.
- Preference is often shown for the minor or major groove.

Drugs acting on DNA

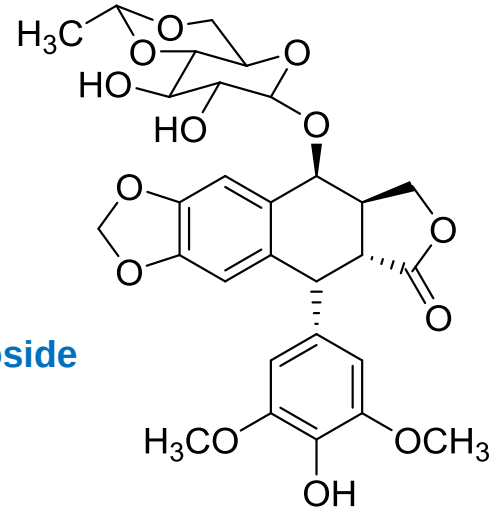
Intercalating agents

Proflavine (aminoacridine)
Antibacterial



Proflavine:
- has a planar tricyclic system.
- the amino substituents are protonated and charged.
- was used as a topical antibacterial agent in the second world war.
- targets bacterial DNA.
- is too toxic for systemic use.

Etoposide and **teniposide**
- Stabilise the complex between DNA and topoisomerase enzymes.
- Are used as anticancer agents.

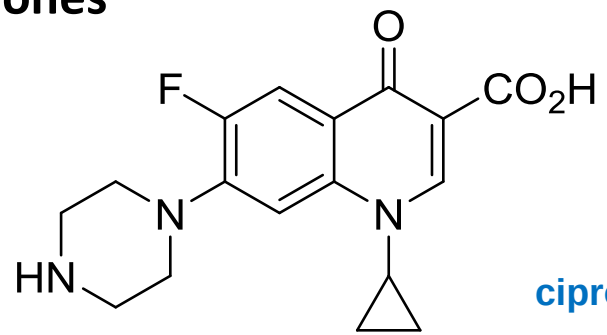


etoposide

Drugs acting on DNA

Topoisomerase poisons (non-intercalating)

Quinolones and fluoroquinolones



ciprofloxacin

- Four drug molecules are stacked in the bound complex.
- They are bound to DNA and enzyme by hydrogen and ionic bonds.

See also Fig 9.6. Complex formed between DNA, the topoisomerase enzyme and fluoroquinolones. (Patrick's 5th ed.)

Drugs acting on DNA

(Patrick's 5th Ed. Chapter 9)

Alkylating and metallating agents

- Contain highly electrophilic groups and form **covalent bonds** to nucleophilic groups in DNA (7-N of guanine).
- Prevent replication and transcription and are useful anticancer agents.
- Have toxic side effects (e.g. alkylation of proteins).
- Can cause inter-strand and intra-strand cross-linking (in double helix) if two electrophilic groups are present
- The alkylation of nucleic acid bases can result in miscoding.

Examples:

Nitrogen mustards
Nitrosoureas
Methanesulfonates
Cisplatin

Drugs acting on DNA

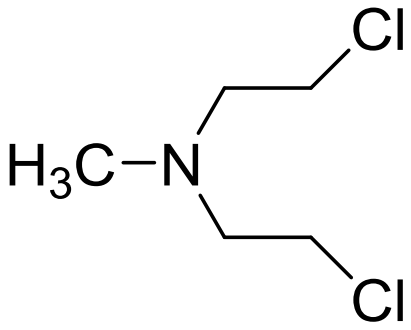
Alkylating and metallating agents

See Patrick's 5th ed. Fig 9.7. Nucleophilic groups on adenine, guanine and cytosine.

See Patrick's 5th ed. Fig 9.8. Normal and abnormal base-pairing of guanine.

Nitrogen mustards:

chlormethine
(mechlorethanamine)



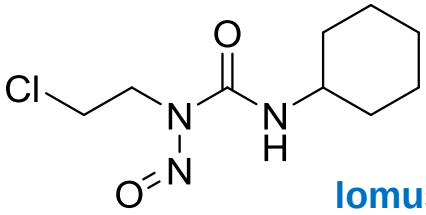
- Were used medicinally in 1942.
- Cause intra-strand and inter-strand cross-linking.
- Prevent replication.
- Mono-alkylation of guanine is also possible.
- Analogues with better properties have been prepared.

See Patrick's 5th ed. Fig 9.9. Alkylation of DNA by chlormethine.

Drugs acting on DNA

Alkylating and metallating agents

Nitrosoureas

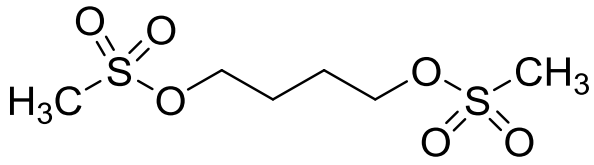


lomustine

Nitrosoureas decompose in the body to form an alkylating agent and a carbamoylating agent.

See Patrick's 5th ed. Fig 9.11. Mechanism of action for nitrosoureas.

Methanesulfonates:



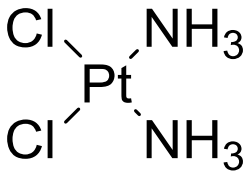
busulfan

- Is a synthetic agent used as an anticancer agent.
- Causes inter-strand cross-linking.

See Patrick's 5th ed. Fig 9.13. Cross-linking mechanism involving busulfan.

Drugs acting on DNA

Alkylating and metallating agents



Cisplatin

- Is a neutral inactive molecule that acts as a “prodrug”.
- Platinum is covalently linked to chloro substituents and ammonia molecules act as ligands.
- Is activated in cells with low chloride ion concentration where chloro substituents are replaced with neutral water ligands.
- Produces positively charged species.

See Patrick’s 5th ed. Fig 9.14. Activation of cisplatin and intrastrand cross-linking of DNA.

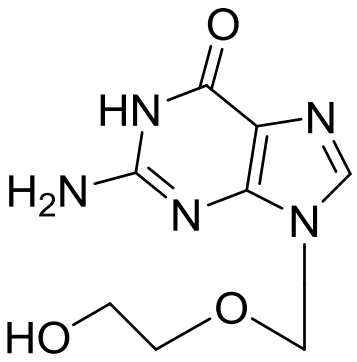
- Binds to DNA in regions rich in guanine units.
- Links intrastrand rather than interstrand.
- Localised unwinding of DNA double helix.
- Inhibits transcription.

Drugs acting on DNA

Antiviral

Chain terminators

- Act as “false substrates”.
- Are incorporated into the growing DNA chain during replication.
- Once they have been incorporated, the chain can no longer be extended.



acyclovir

See Patrick’s 5th ed.

Fig 9.20a . The normal replication mechanism.

Fig 9.20b. A drug acting as a chain terminator.

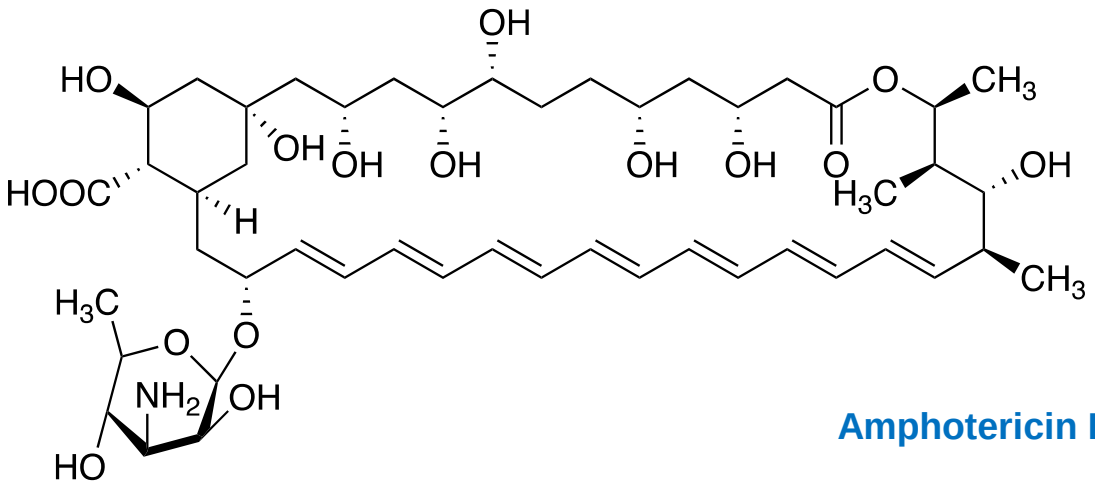
Fig 9.21. Structure of aciclovir, aciclovirtriphosphate and deoxyguanosine. P = Phosphate.

Drugs acting on lipids

(Patrick's 5th Ed. Chapter 10)

- The number of drugs that interact with lipids is relatively small (anaesthetics and antibiotics).
- In general, all act in the same way by disrupting the lipid structure of cell membranes.

"Tunnelling molecule": **Amphotericin B** (antifungal agent) builds tunnels through the membrane and drains the cell.



Amphotericin B

See Patrick's 5th ed. Fig 10.12 (a) Ion channel pore through the cell membrane formed by amphotericine; (b) Interaction between amphotericin and ergosterol in the ion pore channel.

Drugs acting on lipids

Tunnelling molecules

Gramicidin (Antibiotic)

Val-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NH-CH_2-CH_2-OH

Gramicidin is a peptide antibiotic that is thought to form a helix in the cell membrane.

- Two helices aligned end to end form an 'escape tunnel'.
- The hydrophobic exterior interacts with cell membrane lipids.
- The hydrophilic interior allows the uncontrolled passage of ions.

See Patrick's 5th ed. Fig 10.14 . Gramicidin helices aligned end-to-end to traverse the cell membrane.

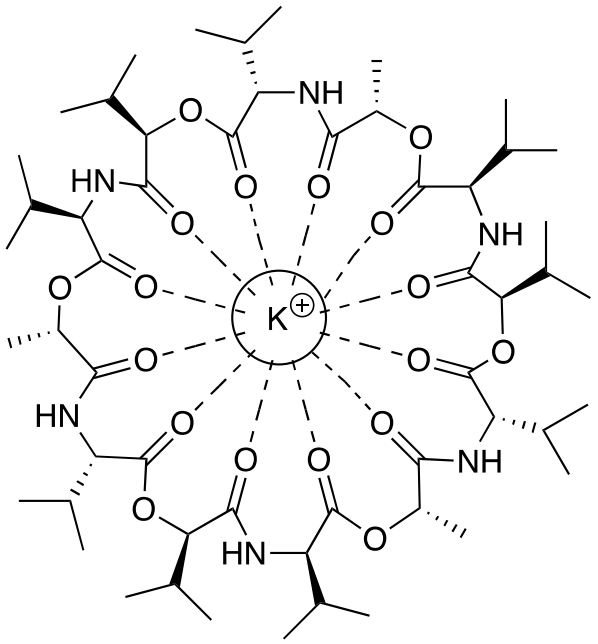
Drugs acting on lipids

Ion carriers

Valinomycin acts as an «inverted detergent»

Valinomycin has a cyclical structure with alternating ester and amide links and it allows uncontrolled escape of potassium ions from cell.

•Hydrophobic residues are placed on the exterior.
• Polar carbonyl groups are placed in the interior.



Potassium ion in the hydrophilic centre of valinomycin: carbonyl groups interact with potassium ion.

UNIT 3. BASIC CONCEPTS IN DRUG ACTION

3.1. Membranes. Physicochemical models that explain the transport across membranes.

3.2. Physicochemical properties and pharmacological activity: water solubility, degree of ionization, lipid solubility and partition coefficient.

3.3. Molecular topology and biological activity. Concept of structure, constitution, configuration and conformation: implications for pharmacological activity.

3.4. Stereoselectivity in drug activity and pharmaco-kinetics.

Patrick's 5th ed. chapters 5, 11, 13 , 14 and 18
Foye's 7th ed. chapter 2

Delgado's 2nd ed. chapter 3
Silverman's, chapters 2 and 3

3.1. BASIC CONCEPTS IN DRUG ACTION

- The most potent drug at its target site may be useless clinically.
- Active drugs *in vitro* may be inactive *in vivo* if a drug cannot reach its target site.
- Drug design should consider both binding interactions and pharmacokinetics.

Factors to be considered (ADME)

- Drug Absorption
- Drug Distribution
- Drug Metabolism
- Drug Excretion

} PHARMACOKINETICS

(see Biopharmacy and Pharmacokinetics)

Bioavailability refers to how quickly and how much of a particular drug reaches the blood supply once all the problems associated with absorption, distribution, metabolism and excretion have been taken into account.

Oral bioavailability is the fraction of the ingested dose that survives to reach the blood supply. This property should be considered alongside the pharmacodynamics of the drugs.

3.1. BASIC CONCEPTS IN DRUG ACTION

DRUG ABSORPTION

Drug absorption refers to the route or method by which a drug reaches the blood supply.

- To reach the blood supply, orally taken drugs must cross the gut wall.
- Most orally active drugs pass through the cells lining the gut wall.
- Drugs are therefore required to cross two fatty cell membranes.
- A balance between hydrophilic and hydrophobic character is required (two different environments: blood supply / cell membranes).

DRUG DISTRIBUTION

- After crossing the gut wall, the drug enters the blood vessels.
- The cells lining the blood vessels are loose fitting and there is no need for the drug to cross the cell membranes.
- The drug can cross blood vessel walls quickly through pores between the cells.

DRUG DISTRIBUTION

- Drugs absorbed orally are first taken to the liver.
- Chemical modification of the drug is possible by enzymes in the liver (**drug metabolism**).
- Drug metabolism in the liver deactivates a certain percentage of the absorbed drug before distribution occurs around the body (**first pass effect**).

- Distribution around the body is uneven due to uneven blood supply.
- Distribution from blood vessels to tissues and organs is rapid.
- If the target is within the cell the drug has to enter a cell.
- Blood concentration drops rapidly after absorption due to distribution, macromolecular binding and storage in fat tissue (e.g. barbiturates) or bone.
- The blood brain barrier hinders polar drugs from entering the brain:

The polarity of peripherally acting drugs can be increased to reduce CNS side effects.

3.1. BASIC CONCEPTS IN DRUG ACTION

For the drug to reach the site of action it must be able to interact with two environments:

- a lipophilic environment (e.g. membranes).
- an aqueous environment (e.g. cytoplasm inside the cell, extracellular fluid outside the cell).

- Drugs must be sufficiently polar to be soluble in aqueous conditions.
- Drugs must be sufficiently polar/fatty to interact with binding sites.
- Drugs must be sufficiently lipophilic to cross cell membranes.
- Drugs must be sufficiently lipophilic to avoid rapid excretion.
- Drugs must have both hydrophilic and lipophilic characteristics.

- Very polar drugs can be administered by injection.
- Very polar drugs can be useful in gut infections.

3.1. TRANSPORT ACROSS MEMBRANES

https://www.youtube.com/watch?v=RPAZvs4hvGA

(Remember: *Physiology I*)

Transport	Molecules moved	Uses energy?	Example transporter
Simple diffusion	Small, nonpolar	No	-
Facilitated diffusion	Polar molecules, larger ions	No	GLUT4
Primary active transport	Molecules moving against their gradient coupled to the hydrolysis of ATP	Yes	Sodium-potassium pump Proton pump
Secondary active transport	Molecules going with + molecules going against gradient	Yes	Sodium-calcium exchanger

https://www.khanacademy.org/test-prep/mcat/cells/transport-across-a-cell-membrane

Passive Diffussion

FICK'S LAW

Relates the diffusive flux to the concentration under the assumption of steady state.

$$-\frac{dC}{dt} = \frac{K \cdot A\,(C_1 - C_2)}{d}$$

- $-dC/dt$ = diffusion velocity through membrane
- K = diffusion constant
- A = diffusion area
- C_1 and C_2 = concentrations on either side of the membrane
- d = membrane thickness

3.1. TRANSPORT ACROSS MEMBRANES

Structural requirements for drugs taken orally: "Drug-like properties"

Lipinski's Rule of Five

Orally active drugs generally show a balance between hydrophilic and hydrophobic properties and obey at least three of the following rules:

- **MW < 500**
- **No more than 5 HBD groups**
- **No more than 10 HBA groups**
- **log P < +5**

This rule is not foolproof – there are several exceptions (active transport, pinocytosis,…).

See: J. Med. Chem. 2011, 54, 6405–6416
dx.doi.org/10.1021/jm200504p

Veber's parameters

• Molecular flexibility and the polar surface of the molecule are important to drug absorption.

• Too many rotatable bonds is bad for absorption.

(Molecular weight is not a factor)

Total number of HBDs and HBAs ≤ 12
Number of rotatable bonds ≤ 10

or

Polar surface area < 140 Angstroms
Number of rotatable bonds ≤ 10

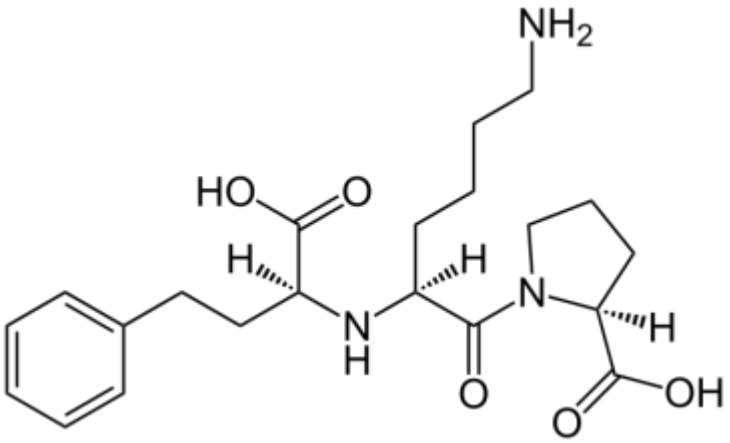
3.1. TRANSPORT ACROSS MEMBRANES

Some highly polar drugs are absorbed from the digestive system.

- **Pinocytosis** - a process allowing the passage of **large polar drugs** into a cell without actually crossing the cell membrane.

- **Small polar molecules** (MW <200) cross the gut wall through small pores between cells (do not cross membranes).

- **Polar molecules** bearing a *structural resemblance* to some building blocks such as amino acids or nucleic acid bases are carried across the membrane by transport proteins (e.g. lisinopril).



lisinopril

3.2. PHYSICOCHEMICAL PROPERTIES

- **Water Solubility**
- **Degree of Ionization**
- **Lipid Solubility (Lipophilicity) and Partition Coefficient**

- The degree of ionization has an important influence on water solubility and lipophilicity.
- The ionization state of a drug depends on the pK_a values of the ionizable groups and the pH of the medium with which it has to interact.

Foye's, Silverman's and Delgado's (Spanish)

3.2. PHYSICOCHEMICAL PROPERTIES

Water Solubility

Depends mainly on two factors:

- Hydrogen bonds with water molecules.
- Ionization (ion-dipole interactions with water molecules).

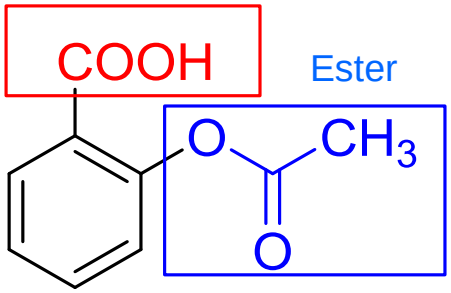
Predicting the drug water solubility by an empiric approach (Lemke):

- Is based on the *carbon-solubilizing potential* of organic functional groups.
- If the potential of the functional groups exceeds the total number of C atoms, the compound is considered water-soluble.

Functional group	Monofunctional group	Polyfunctional group
Alcohol	5-6 carbon atoms	3-4 carbon atoms
Phenol	6-7 carbon atoms	3-4 carbon atoms
Ether	4-5 carbon atoms	2 carbon atoms
Aldehyde	4-5 carbon atoms	2 carbon atoms
Ketone	5-6 carbon atoms	2 carbon atoms
Amine	6-7 carbon atoms	3 carbon atoms
Carboxylic acid	5-6 carbon atoms	3 carbon atoms
Ester	6 carbon atoms	3 carbon atoms
Amide	6 carbon atoms	2-3 carbon atoms
Urea, Carbonate, Carbamate		2 carbon atoms
Charge (cationic and anionic)	20-30 carbon atoms	

3.2. PHYSICOCHEMICAL PROPERTIES

Carboxylic acid



- *Polyfunctional molecule*
- *Two functional groups (2 x 3 carbons = 6 carbons)*
- *Molecular formula: $C_9H_8O_4$*
- *6 < 9 (solubilizing potential is less than carbon content)*

INSOLUBLE

Water Solubility

The ionized forms of carboxylic acids (carboxylates) and amines (ammonium) are much more soluble in water than the neutral forms.

Molecules containing intramolecular ionic or hydrogen bonds may be less soluble than expected (e.g. tyrosine).
A compound is considered soluble in water when the water solubility is above 1g/10mL (10%) (In USP 3.3-10%).

3.2. PHYSICOCHEMICAL PROPERTIES

Water Solubility

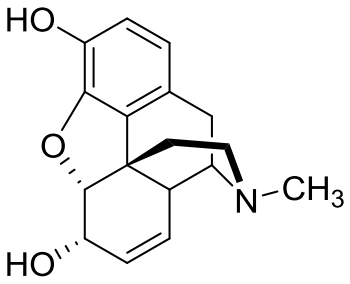
Definition of approximate drug solubility by US pharmacopoeia

Term	Parts of the solvent required for one part of solute
Very soluble	Less than 1 part (1 : 1)
Freely soluble	1 to 10 part (1 : 1-10)
Soluble	10 to 30 part (1 : 10-30)
Sparingly soluble	30 to 100 part (1: 30-100)
Slightly soluble	100 to 1000 part (1 : 100-1000)
Very slightly soluble	1000 to 10000 part (1 : 1000-10000)
Practically insoluble	More than 10000 part (1:>10000)

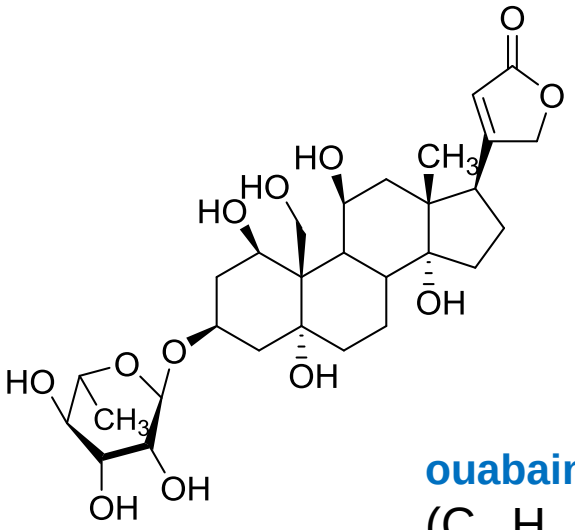
3.2. PHYSICOCHEMICAL PROPERTIES

Water Solubility

Are *morphine* and *ouabain* water-soluble drugs?
Is the protonated form of *morphine* a water-soluble drug?



morphine
$(C_{17}H_{19}NO_3)$

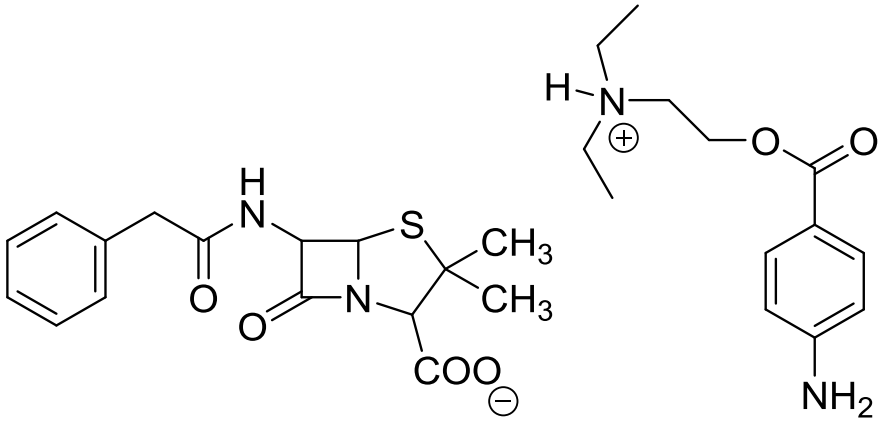


ouabain
$(C_{29}H_{44}O_{12})$
poisonous glycoside

(see Delgado's book)

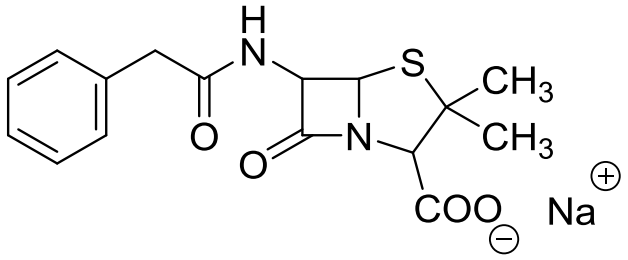
3.2. PHYSICOCHEMICAL PROPERTIES

Water Solubility



Penicillin G procaine

1g / 250 mL



Penicillin G sodium

1g / 40 mL

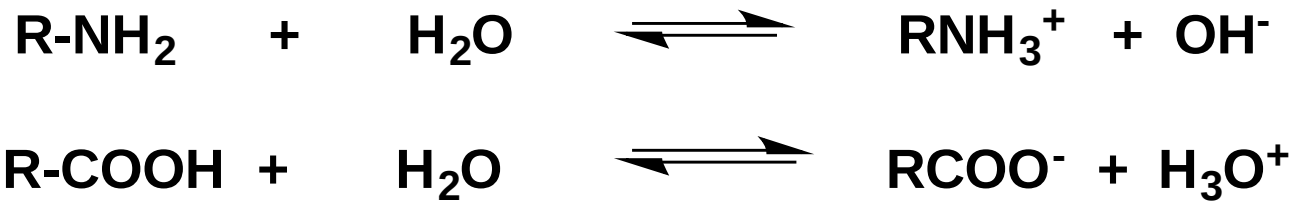
Water solubility in salts depends on the chemical structure of both components (cation and anion).

(from Foye's)

3.2. PHYSICOCHEMICAL PROPERTIES

Degree of ionization

- Ionization can have a profound effect not only on a drug's interaction with a target but also on its lipophilicity.
- The ionization of the drug can favour binding to the receptor but can also make crossing membranes difficult prior to reaching the target.
- An equilibrium can be established between the neutral form and the ionized form.
- E.g. many drugs contain an amine functional group. Amines are weak bases and form ammonium cations in water (pK_a = 9-10 for aliphatic amines).



For carboxylic acids (pK_a = 4-5), an equilibrium with the corresponding carboxylate anion can be established at physiological pH.

3.2. PHYSICOCHEMICAL PROPERTIES

Degree of ionization

$$AH \rightleftharpoons A^- + H^+$$

$$K_a = [A^-]\,[H^+] / [AH]$$

$$BH^+ \rightleftharpoons B + H^+$$

$$K_a = [B]\,[H^+] / [BH^+]$$

$$pK_a = - \log K_a$$

REMEMBER: when we refer to the pK_a of a basic substance we are referring to the pK_a of its conjugate acid (RNH_3^+ for a primary amine).

See Figure 11.1. Equilibrium between the ionized and non-ionized form of an amine (Patrick's 5th Ed.).

Only non-ionized forms are able to cross membranes by diffusion.

Once in the blood, the two forms are also in equilibrium.

3.2. PHYSICOCHEMICAL PROPERTIES

Degree of ionization

- For an ionizable substance with a given pK_a, the extent of ionization depends on the pH.
- At a particular pH the degree of ionization can be determined by the Henderson-Hasselbach equation, which is derivable from the acid dissociation constant equation:

Henderson-Hasselbach equation

Acidic drug

$$pKa = pH + \log [AH] / [A^-]$$

or

$$pH = pKa + \log [A^-] / [AH]$$

Basic drug

$$pKa = pH + \log [BH^+] / [B]$$

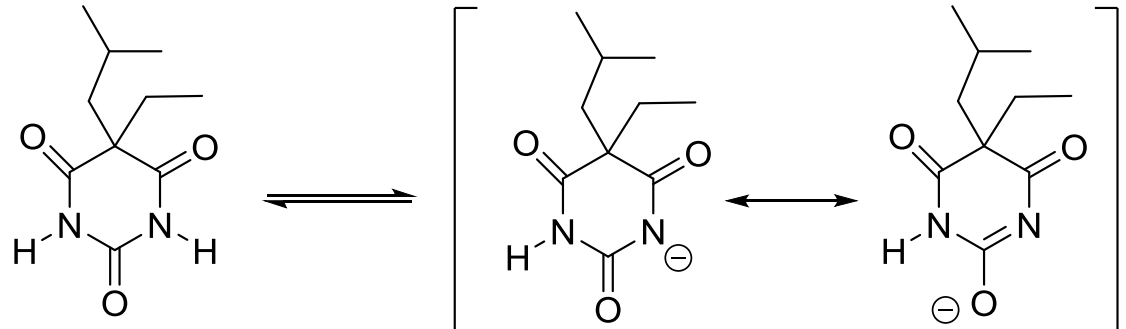
or

$$pH = pKa + \log [B] / [BH^+]$$

3.2. PHYSICOCHEMICAL PROPERTIES

Degree of ionization

Calculating the ratio of ionization of *amobarbital* at pH 7.4:



amobarbital
pKa = 8.0

conjugate base

$$pKa = pH + \log [Acid] / [Base]$$

Weak acid:

The ionized form is the base

$$[AH] / [A^-] = 10^{pKa - pH} \qquad [AH] = 10^{pKa - pH} [A^-]$$

$$[AH] + [A^-] = 100$$

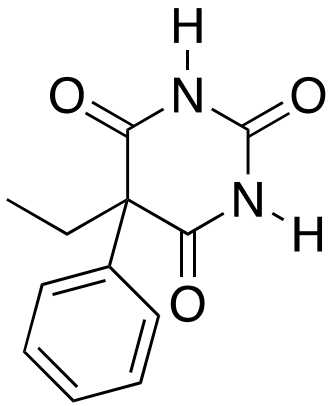
$$[AH] = 3.98 [A^-]$$

$[A^-] = 20$
$[AH] = 80$

20% in ionized form

3.2. PHYSICOCHEMICAL PROPERTIES

Degree of ionization



pKa = 7.3

Phenobarbital. Degree of ionization at different pHs.

pH	% [H-D]	% [D-]
2.0	100	0
6.0	96.17	3.83
7.0	72.53	28.47
8.0	20.07	79.93
10.0	0.25	99.75

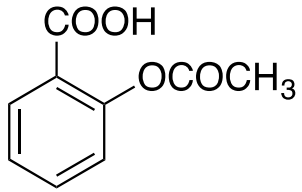
	pH
Stomach	1-3
Small intestine	6-8
Large intestine	5-7

Fallingborg J. Intraluminal pH of the human gastrointestinal tract. Dan Med Bull. 1999;46(3):183-96.

3.2. PHYSICOCHEMICAL PROPERTIES

Degree of ionization

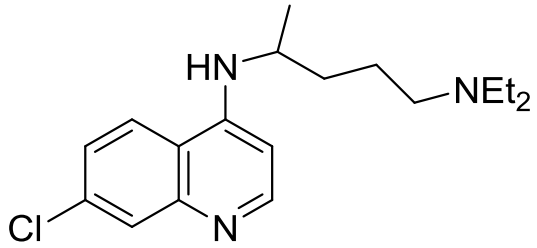
aspirin pKa = 3.5 (weak acid)
100 mg oral



$AH \rightleftharpoons A^- + H^+$

[NI] **[I]**

chloroquine pKa = 10.1 (weak base)
100 mg oral



$B + HCl \rightleftharpoons BH^+ + Cl^-$

[NI] **[I]**

<10 = [I]

Stomach
pH = 2

> 90 = [NI]

Blood
pH = 7.4

[NI]

Aspirin is well absorbed in the stomach.

>>99.99 = [I]

Stomach
pH = 2

<<0.01 = [NI]

Blood
pH = 7.4

[NI]

Chloroquine is not absorbed in the stomach.

3.2. PHYSICOCHEMICAL PROPERTIES

Degree of ionization

(Rule of Thumb)

Weak ACIDS

pH = pKa ~ 50% ionized

pH = pKa + 1	~ 90% ionized	pH = pKa - 1	~ 90% non-ionized
pH = pKa + 2	~ 99% ionized	pH = pKa - 2	~ 99% non-ionized
pH = pKa + 3	~ 99.9% ionized	pH = pKa - 3	~ 99.9% non-ionized
pH = pKa + 4	~ 99.99% ionized	pH = pKa - 4	~ 99.99% non-ionized

Example: pKa of ASPIRIN is 3.5

Physiological pH = 7.4	pH = pKa + 4	% ionization = 99.99% ionized
Stomach pH = 2	pH = pKa – 1.5	% ionization = 1-10% ionized

Weak BASES

pH = pKa ~ 50% ionized

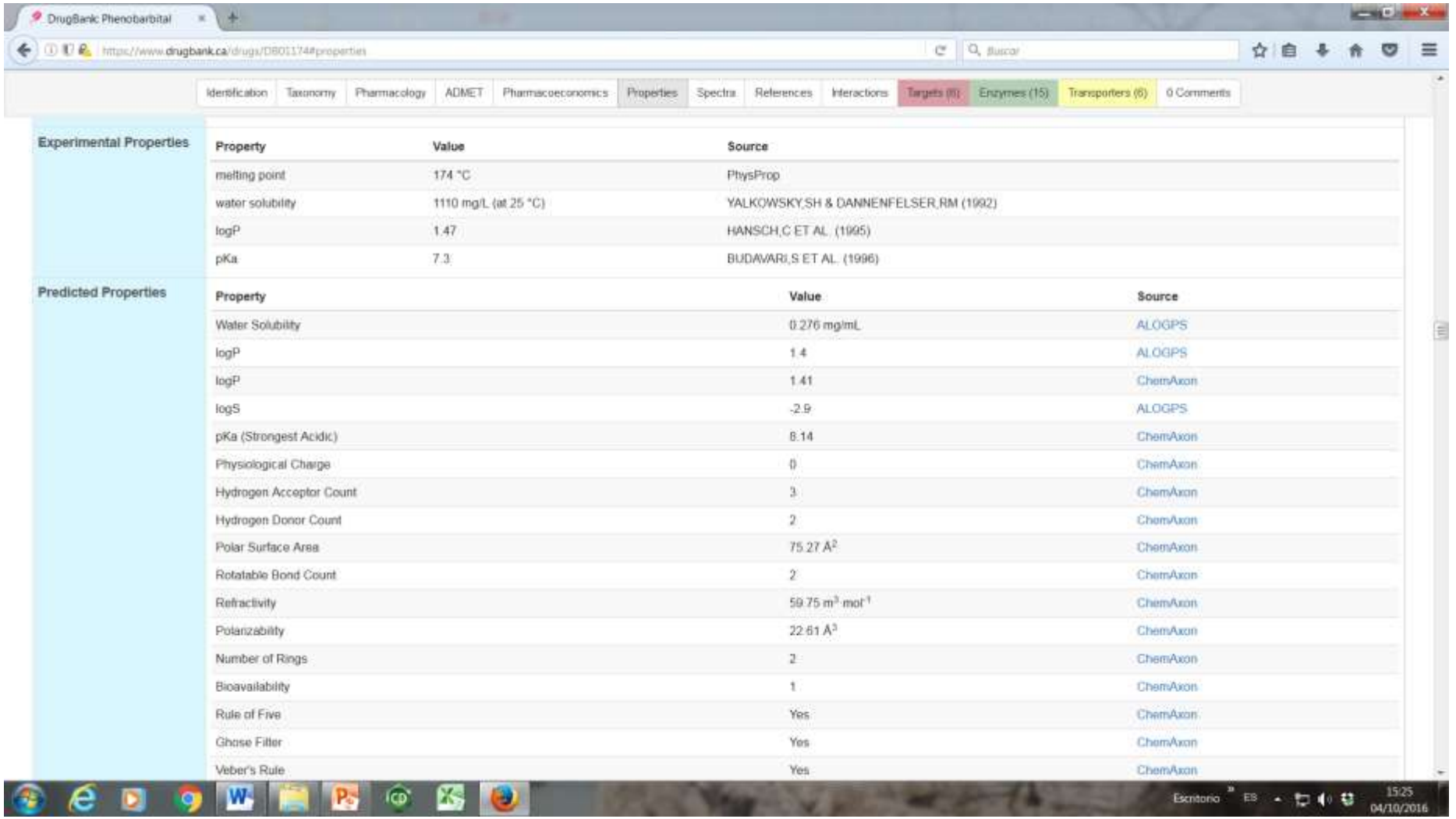
pH = pKa - 1	~ 90% ionized	pH = pKa + 1	~ 90% non-ionized
pH = pKa - 2	~ 99% ionized	pH = pKa + 2	~ 99% non-ionized
pH = pKa - 3	~ 99.9% ionized	pH = pKa + 3	~ 99.9% non-ionized
pH = pKa - 4	~ 99.99% ionized	pH = pKa + 4	~ 99.99% non-ionized

Example: pKa of CHLOROQUINE is 10.1

Physiological pH = 7.4	pH = pKa - 3	% ionization = ~ 99.9% ionized
Stomach pH = 2	pH = pKa – 8	% ionization = >> 99.99% ionized

3.2. PHYSICOCHEMICAL PROPERTIES

Degree of ionization



Experimental Properties

Property	Value	Source
melting point	174 °C	PhysProp
water solubility	1110 mg/L (at 25 °C)	YALKOWSKY,SH & DANNENFELSER,RM (1992)
logP	1.47	HANSCH,C ET AL. (1995)
pKa	7.3	BUDAVARI,S ET AL. (1996)

Predicted Properties

Property	Value	Source
Water Solubility	0.276 mg/mL	ALOGPS
logP	1.4	ALOGPS
logP	1.41	ChemAxon
logS	-2.9	ALOGPS
pKa (Strongest Acidic)	8.14	ChemAxon
Physiological Charge	0	ChemAxon
Hydrogen Acceptor Count	3	ChemAxon
Hydrogen Donor Count	2	ChemAxon
Polar Surface Area	75.27 $Å^2$	ChemAxon
Rotatable Bond Count	2	ChemAxon
Refractivity	59.75 $m^3 \cdot mol^{-1}$	ChemAxon
Polarizability	22.61 $Å^3$	ChemAxon
Number of Rings	2	ChemAxon
Bioavailability	1	ChemAxon
Rule of Five	Yes	ChemAxon
Ghose Filter	Yes	ChemAxon
Veber's Rule	Yes	ChemAxon

https://www.drugbank.ca/drugs

3.2. PHYSICOCHEMICAL PROPERTIES

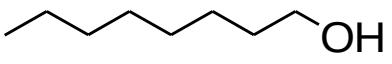
Lipophilicity & Partition Coefficient

- Several authors (e.g. Richet, Overton and Meyer) have found a good correlation between lipid solubility and biological activity in certain drug series.

Measuring lipophilicity:

- Hansch proposed the **partition coefficient (P)** between 1-octanol and water:

$$\mathbf{P} = [\text{compound}]_{oct} \; / \; [\text{compound}]_{aq}$$



- 1-Octanol has properties that simulate those of natural membranes (long saturated alkyl chain and a hydroxyl group for hydrogen bonding) and dissolves water to saturation (1.7 M).
- The value of P is determined experimentally (e.g. using a shaking device like a separatory funnel or a reverse-phase HPLC method) and varies slightly with temperature and the concentration of the solute.
- With neutral molecules in dilute solutions and small temperature changes, variations in P are minor.

3.2. PHYSICOCHEMICAL PROPERTIES

Lipophilicity & Partition Coefficient

$$\mathbf{P} = [\text{compound}]_{oct} \; / \; [\text{compound}]_{aq}$$

P > 1 (log P > 0) lipophilic
P < 1 (log P < 0) hydrophilic

- The more positive the log P, the more lipophilic the compound.
- The larger the value of P, the more the drug will interact with the lipid phase (e.g. membranes).
- With very high values of P, the drug will be unable to cross the aqueous phase and will localize in the first lipophilic phase with which it comes into contact.
- As P approaches zero, the drug will be so water-soluble that it will not be capable of crossing the lipid phase and will localize in the aqueous phase (remember there are exceptions for the absorption of some very polar drugs).
- A parabolic relationship between potency (log 1/C) and log P is often found for a series of structurally related drugs:

$$\log 1/C = - k (\log P)^2 + k' (\log P) + k''$$

- In these cases there is a value of log P which corresponds to the maximum: $\log P_o$ (E.g. in a series of nonspecific hypnotics, Hansch found that all active compounds had a similar log P, approximately 2).

3.2. PHYSICOCHEMICAL PROPERTIES

Lipophilicity & Partition Coefficient

When the compound has ionizable groups, the equation changes in order to take into account the ionization degree (α) in water, which is calculated from ionization constants:

$$\mathbf{P} = \frac{[\text{compound}]_{oct}}{[\text{compound}]_{aq}\ (1 - \alpha)}$$

- Ionization makes the compound more soluble in water than the structure appears.
- The degree of ionization depends not only on the pKa of the substance but also on the pH of the aqueous phase.
- The pH inside the gastrointestinal tract varies widely from the stomach (pH 1-2) through to the end of the small intestine (pH ~ 8).
- The term log D (D = distribution coefficient, between 1-octanol and aqueous buffer) describes the log P of an ionizable compound at a particular pH.
- E.g. $\log D_{4.5}$ is the log P of an ionizable compound at pH 4.5

3.2. PHYSICOCHEMICAL PROPERTIES

Lipophilicity & Partition Coefficient

Predicting the drug lipophilicity from the structure:

- This is possible if we know the lipophilicities of substituents and atoms.
- Hansch and coworkers derived substituent constants for the contribution of individual atoms and groups to the partition coefficient.
- The lipophilicity constant for an atom or group X, π_X, is defined by:

$$\pi_X = \log P_X - \log P_H = \log (P_X / P_H)$$

  - P_X is the partition coefficient for the compound with substituent X.
  - P_H is the partition coefficient for the parent molecule.

- Log P calculated from the lipophilic constants for substituents is called Clog P:

$$\text{Clog P} = \sum \pi$$

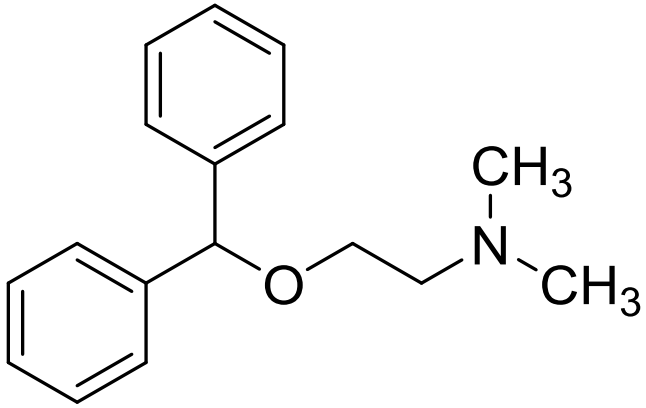
3.2. PHYSICOCHEMICAL PROPERTIES

Lipophilicity & Partition Coefficient

- π_X depends on the characteristics of the group/atom: inductive, resonance and steric effects are important (a positive value means that the substituent increases lipophilicity and a negative value means the opposite).
- The values of π for the most common substituents are tabulated .

(see Chapter 6)

diphenhydramine
(antihistamine)



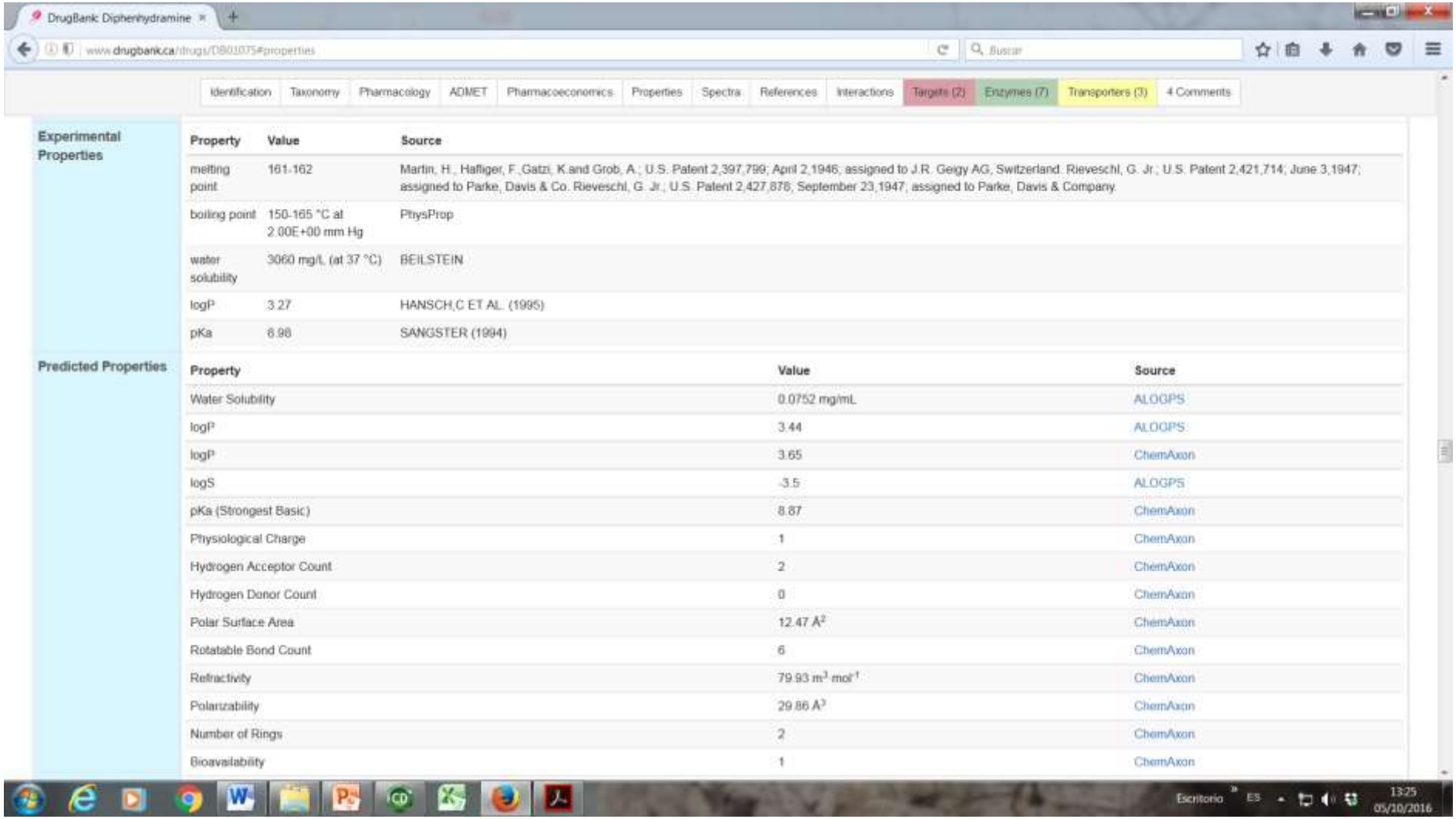
Calculated log P $= 2\pi_{Ph} + \pi_{CH} + \pi_{OCH2} + \pi_{CH2} + \pi_{NMe2} - 0.2 =$
$= 2\ (2.13) + 0.50 - 0.73 + 0.50 - 0.95 - 0.2 = 3.38$

Numerous software packages are now commercially available (ChemDraw, etc.) but the results can differ widely and also differ from the experimental value.

3.2. PHYSICOCHEMICAL PROPERTIES

Lipophilicity & Partition Coefficient

Diphenhydramine (properties from Drugbank)



DrugBank: Diphenhydramine

www.drugbank.ca/drugs/DB01075#properties

Identification | Taxonomy | Pharmacology | ADMET | Pharmacoeconomics | Properties | Spectra | References | Interactions | Targets (2) | Enzymes (7) | Transporters (3) | 4 Comments

Experimental Properties

Property	Value	Source
melting point	161-162	Martin, H., Hafliger, F.,Gatzi, K.and Grob, A.; U.S. Patent 2,397,799; April 2,1946; assigned to J.R. Geigy AG, Switzerland. Rieveschl, G. Jr.; U.S. Patent 2,421,714; June 3,1947; assigned to Parke, Davis & Co. Rieveschl, G. Jr.; U.S. Patent 2,427,878; September 23,1947; assigned to Parke, Davis & Company
boiling point	150-165 °C at 2.00E+00 mm Hg	PhysProp
water solubility	3060 mg/L (at 37 °C)	BEILSTEIN
logP	3.27	HANSCH,C ET AL. (1995)
pKa	8.98	SANGSTER (1994)

Predicted Properties

Property	Value	Source
Water Solubility	0.0752 mg/mL	ALOGPS
logP	3.44	ALOGPS
logP	3.65	ChemAxon
logS	-3.5	ALOGPS
pKa (Strongest Basic)	8.87	ChemAxon
Physiological Charge	1	ChemAxon
Hydrogen Acceptor Count	2	ChemAxon
Hydrogen Donor Count	0	ChemAxon
Polar Surface Area	12.47 Å^2	ChemAxon
Rotatable Bond Count	6	ChemAxon
Refractivity	79.93 m^3 mol^{-1}	ChemAxon
Polarizability	29.86 Å^3	ChemAxon
Number of Rings	2	ChemAxon
Bioavailability	1	ChemAxon

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http://www.drugbank.ca/drugs/DB01075#properties

3.3. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

- *Targets are chiral biomolecules (e.g. carbohydrates, amino acids, cholesterol, etc.).*
- *Carrier proteins in membranes are chiral (absorption).*
- *Proteins in blood supply are chiral (distribution).*
- *Enzymes are chiral (metabolism).*

- Most drugs currently on the market are some type of isomeric mixture. For many of these drugs the biological activity resides in one isomer.
- If critical functional groups in the drug molecule do not occupy the proper spatial region, the desired pharmacological activity cannot be achieved because productive interactions with the biological target will not be possible.

Stereochemistry involves the study of the relative spatial arrangement of atoms that form the structure of molecules.

3.3. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

The three-dimensional (3D) nature of the **functional groups that contribute to the pharmacological activity of the drug** is important in predicting drug potency and potential side effects.

These important binding groups that are required for activity and their relative positions in space with respect to each other are called **PHARMACOPHORE**.

Molecular topology is a part of mathematical chemistry dealing with the algebraic description of chemical compounds so allowing a unique and easy characterization of them. The totality of information about the mutual connectedness of all pairs of atoms (chemically bonded and not chemically bonded) in a molecule determines the topology of the respective molecule.

(Journal of Chemical Education , 1995,72, 12, 1059)

3.3. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

There are different levels to define the structure of organic molecules:

- **Constitution** (atoms present)
- **Connectivity** (order in which the atoms are connected) ⟶ isomers
- **Configuration** (spatial distribution of atoms and groups) ⟶ stereoisomers

changes in configuration require bond cleavage and bond formation

(different compounds)

- **Conformation** (flexibility/rotatable bonds) ⟶ conformers (stereoisomers)

changes in conformation do not require bond cleavage (same compound)

Different constitution and connectivity = different properties

Same constitution and connectivity but different configuration:

Double bonds or rings: **diastereomers**

Chiral compounds: **enantiomers** and diastereomers

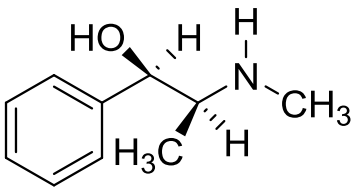
Same constitution, connectivity and configuration:

Flexible compounds (single bonds): different **conformations**

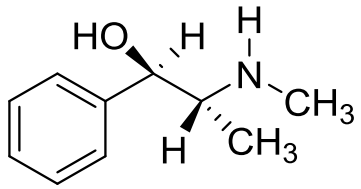
3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Diastereomers:

- Molecules are non-superimposable, non-mirror images.
- Are possible in molecules with two or more asymmetric centres and/or double bonds and/or rings.
- The physicochemical properties are different (water solubility, partition coefficient, pKa, etc.)
- The distances and angles between non-connected atoms are different.
- Differences in biological activity are usually observed.

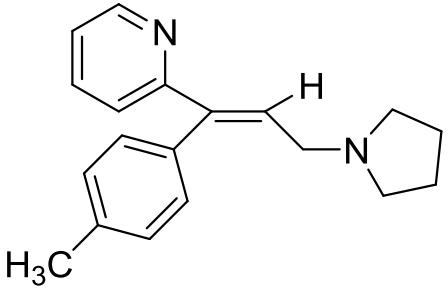


(1R, 2S) - (-) - **ephedrine**



(1R, 2R) - (-) - **pseudoephedrine**

α- and β-adrenergic agonists
Used to treat various diseases



***E*-tripolidine**
antihistamine
Z-tripolidine is inactive

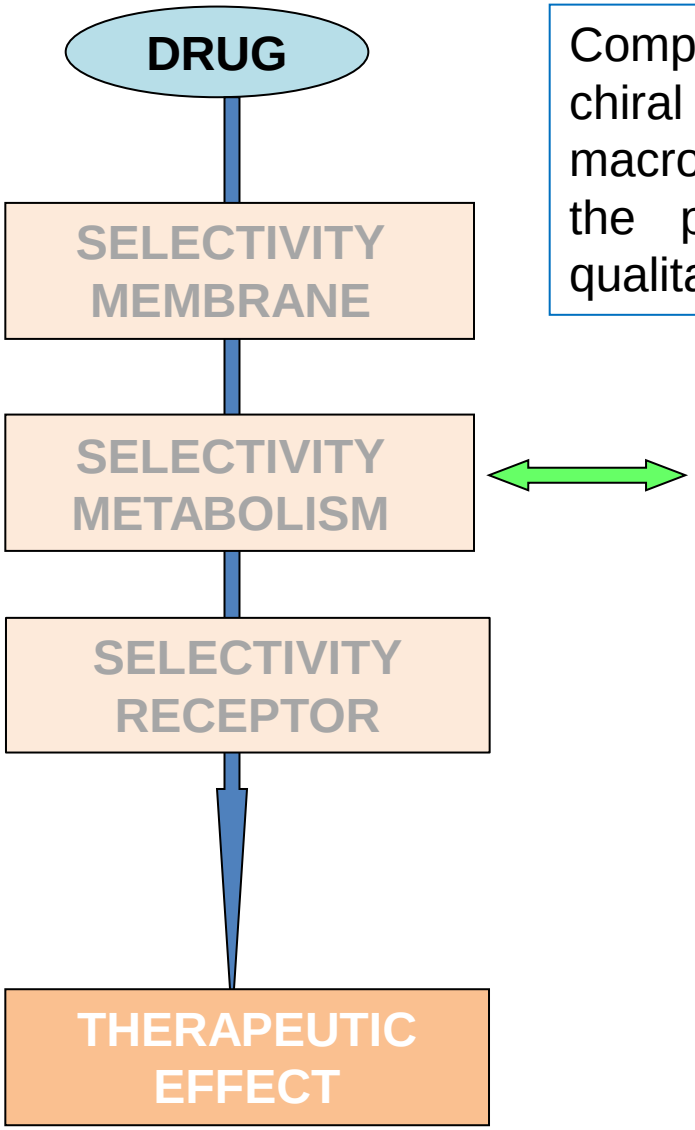
3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Enantiomers:

- Molecules that are non-superimposable, mirror images.
- They are possible when the molecule is chiral (asymmetrical centres, etc.).
- The physical properties are identical (water solubility, partition coefficient, pKa, etc.) except for interaction with the polarized light.
- There are no differences in distances or angles between non-connected atoms.
- Different biological activity (enantioselectivity) may be observed because of the different interaction with the target (asymmetrical nature).
- Each enantiomer may also experience selective absorption, metabolism, distribution and/or excretion determining a different biological activity or potency.
- Such processes may also contribute to the adverse effects of a particular enantiomer (e.g. if a different protein is involved in side effects or toxicity).

3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Enantiomers:



Complexes of both enantiomers of a chiral drug with the target or other chiral macromolecules are diastereomeric, so the pharmacological activity may be qualitatively and quantitatively different.

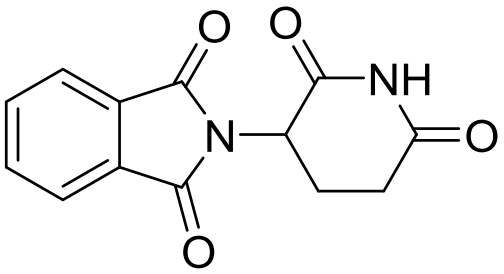
SELECTIVITY NONSPECIFIC RECEPTORS/ DISTRIBUTION

Enantioselectivity

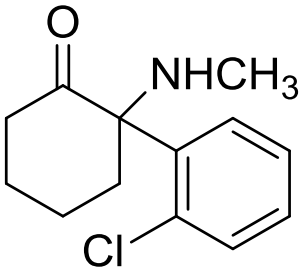
The more active enantiomer is called the **eutomer.**
The less active enantiomer is called the **distomer.**

3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

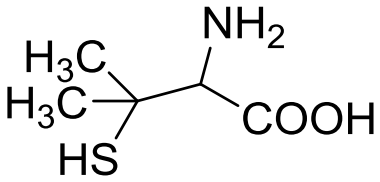
Enantiomers: *Distomer showing toxicity or side effects.*



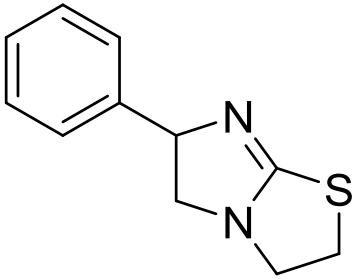
thalidomide
(R) – (+) sedative
(S) – (-) teratogen



ketamine
(S) – (+) anaesthetic
(R) – (-) hallucinogenic



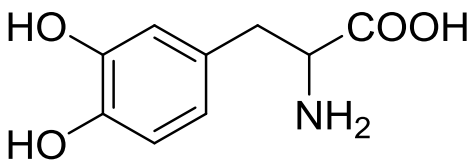
penicillamine
(S) – (-) chelating agent (in metal poisoning)
(R) – (+) toxic



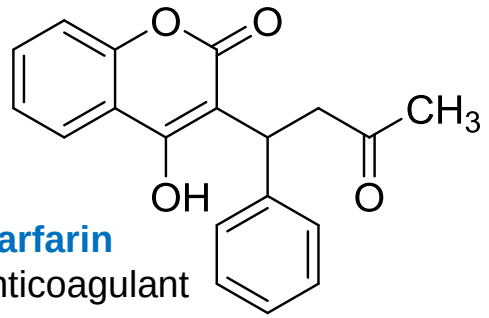
tetramisol
(S) – (-) anthelmintic
R) – (+) side effects

3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Enantiomers:



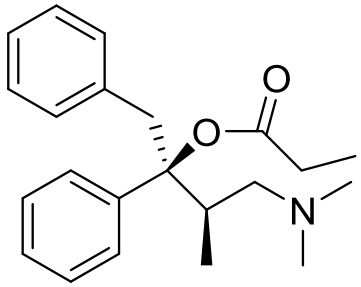
DOPA
Precursor of dopamine
Used to treat Parkinson's disease



Warfarin
Anticoagulant

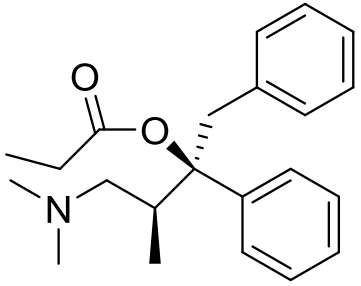
Only the (S) enantiomer is absorbed.

The (S) enantiomer binds to the human serum albumin and is distributed.



(1S,2R) - (+)

Dextropropoxyphene
DARVON
(analgesic)



(1R,2S) - (-)

Levopropoxyphene
NOVRAD
(antitussive)

3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Enantiomers:

EUTOMER (MORE ACTIVE)

DISTOMER (enantiomer)

THE EASSON-STEDMAN HYPOTHESIS

In order to show enantioselectivity, the more potent enantiomer must be involved in at least three intermolecular interactions with the binding site on the target (three binding regions) and the less potent enantiomer must only interact with one or two regions.

Eudismic Ratio

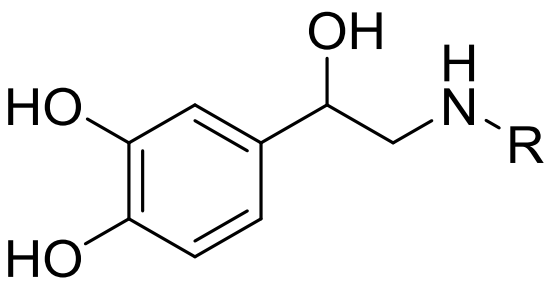
$$E.R. = \frac{\text{activity of eutomer}}{\text{activity of distomer}}$$

Affinity rather than activity is very often used in this equation.

3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Enantiomers:

Differences in bronchodilator activity between some catecholamines:

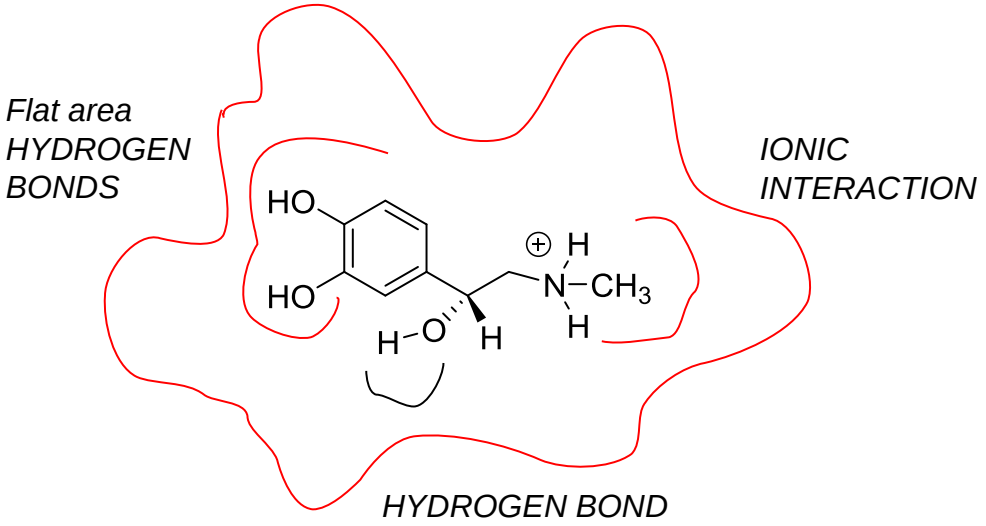


R = H **noradrenaline**
R = Me **adrenaline**
R = ^{i}Pr **isoprenaline**

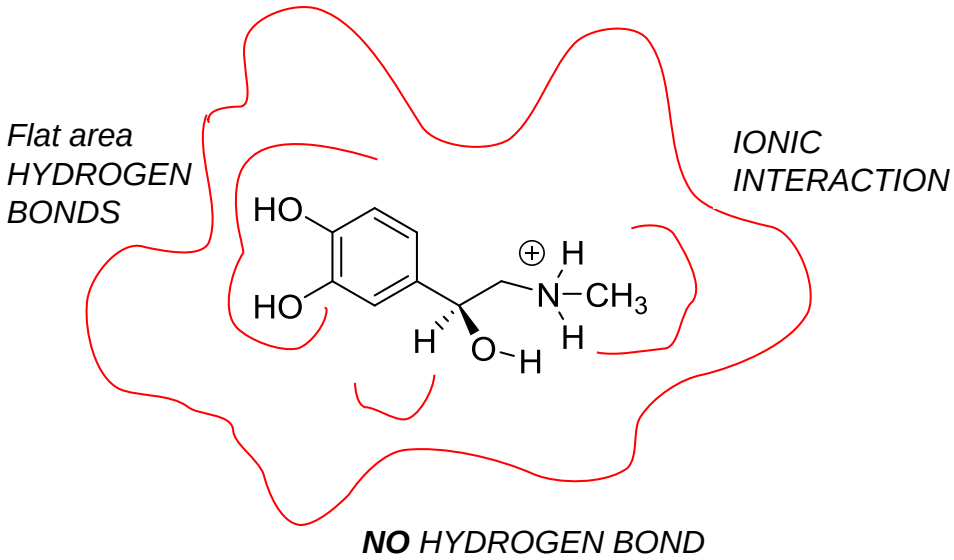
	Bronchodilator activity relative to (R)-(-) noradrenaline	Eudismic ratio
(R)-(-)-noradrenaline	100	R/S = 71
(S)-(+)-noradrenaline	1.4	
(R)-(-)-adrenaline	5800	R/S = 45
(S)-(+)-adrenaline	130	
(R)-(-)-isoprenaline	27000	R/S = 818
(S)-(+)-isoprenaline	33	

3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Enantiomers:



Schematic representation of the interactions of R and S adrenaline with the receptor.



3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

- Most chiral drugs contain two or more asymmetric centres.
- The racemate of a chiral drug may be less potent than the eutomer and may have more side effects.

Isomers in a mixture that do not contribute to the desired action are called **ISOMERIC BALLAST**.

Benefits of using optically pure drugs:

- Required dose is smaller.
- Fewer side effects are observed.
- Pharmacological activity is improved.

3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Chiral synthetic compounds as drug candidates:

First tests are carried out with racemic mixtures

If activity is observed, a decision has to be made to develop the racemic mixture or one enantiomer.

One single enantiomer must be developed when:

The pharmacological activity depends on one enantiomer (**eutomer**).

The less active enantiomer (**distomer**) is toxic.

The therapeutic index (TI) is small.

The racemic mixture can be marketed when:

Both enantiomers show activity (synergic or additive).

The distomer has no toxicity.

The therapeutic index is high.

It is important to know the margin of safety that exists between the dose needed for the desired effect and the dose that produces unwanted and possibly dangerous side effects. This relationship, known as the **therapeutic index**, is defined as the ratio LD_{50}/ED_{50}. In general, the narrower this margin, the more likely it is that the drug will produce unwanted effects.

3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

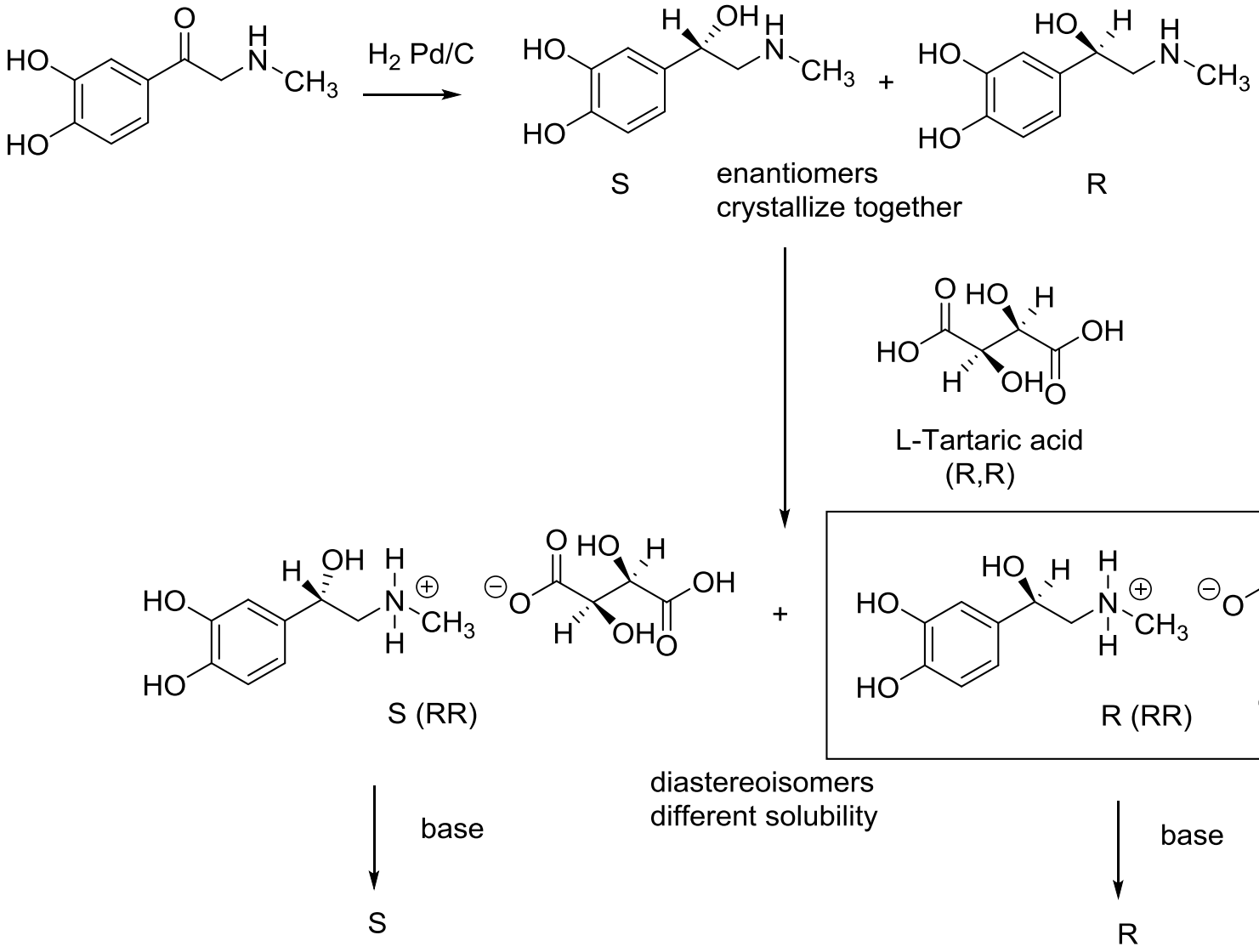
- Producing an enantiomer of a chiral drug is more expensive than producing a racemate.
- Pure enantiomers can be obtained by the **resolution** of a racemic mixture or by an **asymmetric synthesis.**

- A racemic mixture can be resolved into its two enantiomers in different ways:
  - Preferential crystallization (by adding a small amount of pure enantiomer to the racemic mixture).
  - Chromatography (a chiral compound linked to the silica support or cellulose as the stationary phase).
  - The formation of diastereomeric derivatives followed by the separation and recovery of one enantiomer (salts: a chiral drug containing a carboxylic acid + single enantiomer of an optically active amine or the opposite).
  - Kinetic resolution: with enzymes (lipases hydrolyze one enantiomeric ester rather than the other).

3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

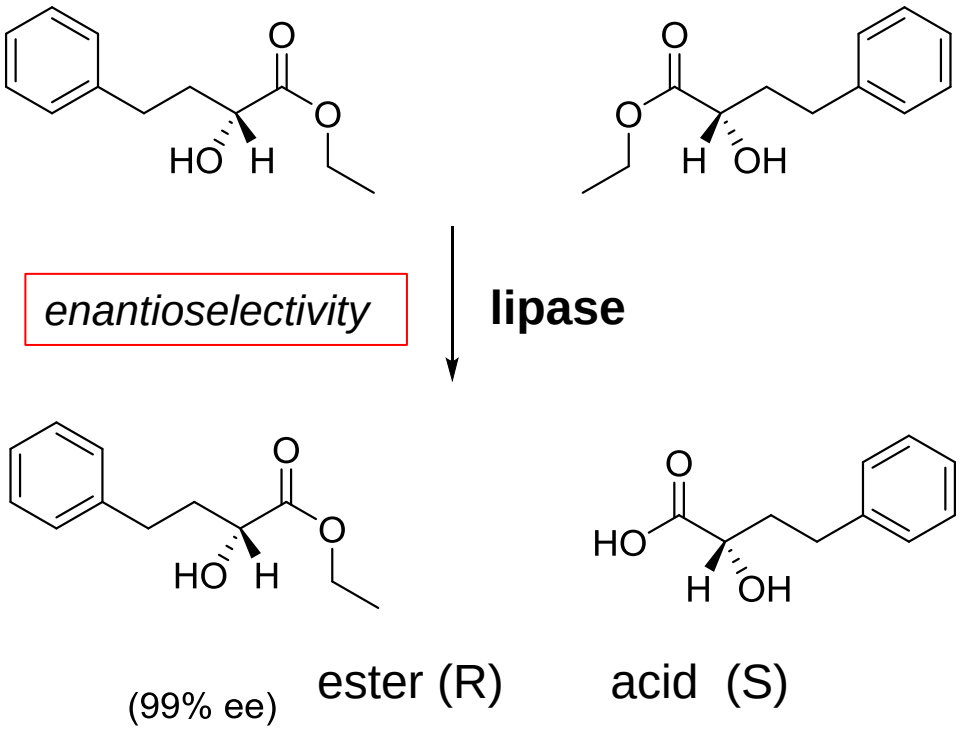
Enantiomers: Resolution

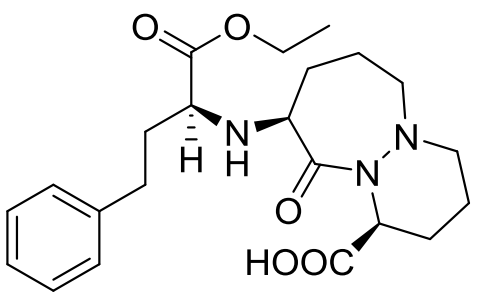
Synthesis of (R)-adrenaline



Enantiomers: enzymatic resolution

Enantiomers





cilazapril

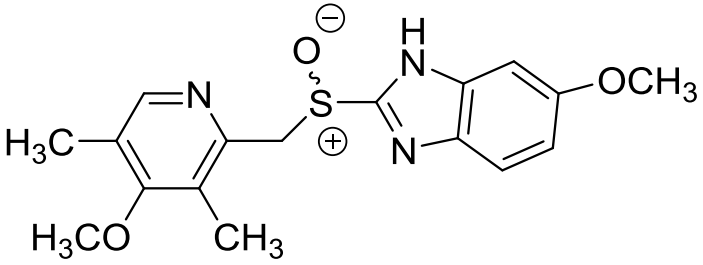
angiotensin-converting enzyme inhibitor (ACE inhibitor)
used to treat hypertension and congestive heart failure

Starting material in the synthesis of cilazapril.

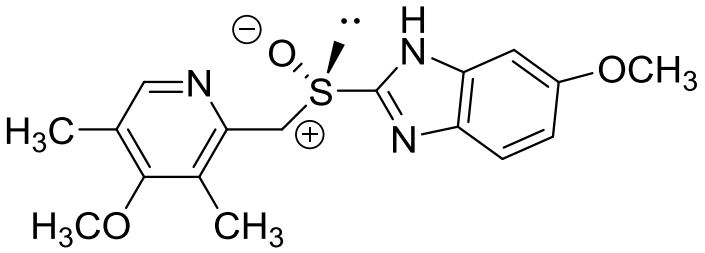
3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Enantiomers

- An asymmetric synthesis can be achieved by starting with an optically pure starting material that already contains the required asymmetric centres or by including an asymmetric reaction.
- An **asymmetric reaction** produces a new asymmetric centre with selectivity for one stereoisomer over the other.



omeprazole (racemic)
It was the first proton pump inhibitor to reach the market (1988). In Europe the patents expired in 1999.



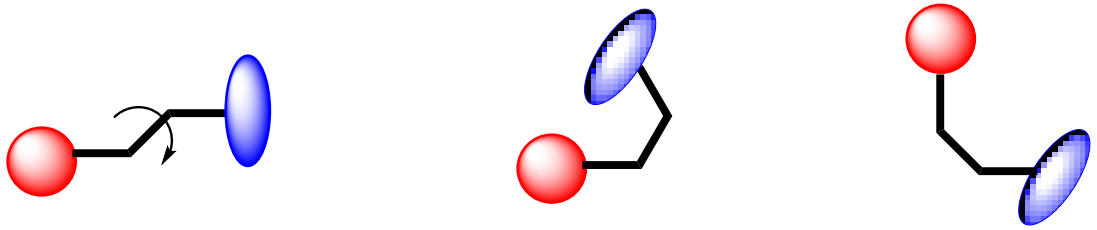
S-enantiomer (**esomeprazole**)
It was launched in 2000 on the basis that it has therapeutic advantages over omeprazole (superior in terms of pharmacokinetic profile).

It is an example of **chiral switching**, whereby a racemic drug is replaced on the market with a single enantiomer.

Conformation and biological activity

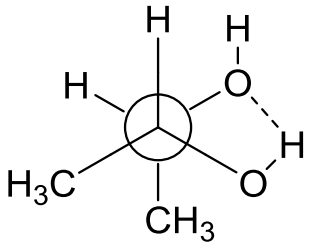
- Many endogenous compounds are very simple and flexible (e.g. adrenaline, acetylcholine).
- They interact with different targets because they are able to adopt different conformations (e.g. different types and subtypes of adrenergic and cholinergic receptors).

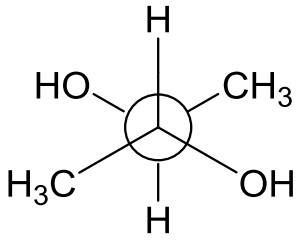
flexible chain: different conformations



Remember:
Conformations in butane: staggered, partially eclipsed, gauche, fully eclipsed

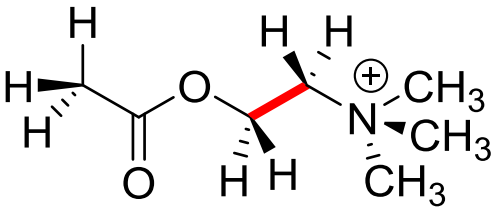
These conformations have different energy depending on the steric and electrostatic interactions (see 2,3-butanediol)

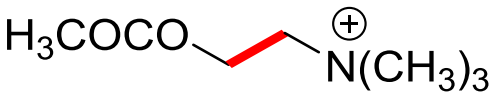


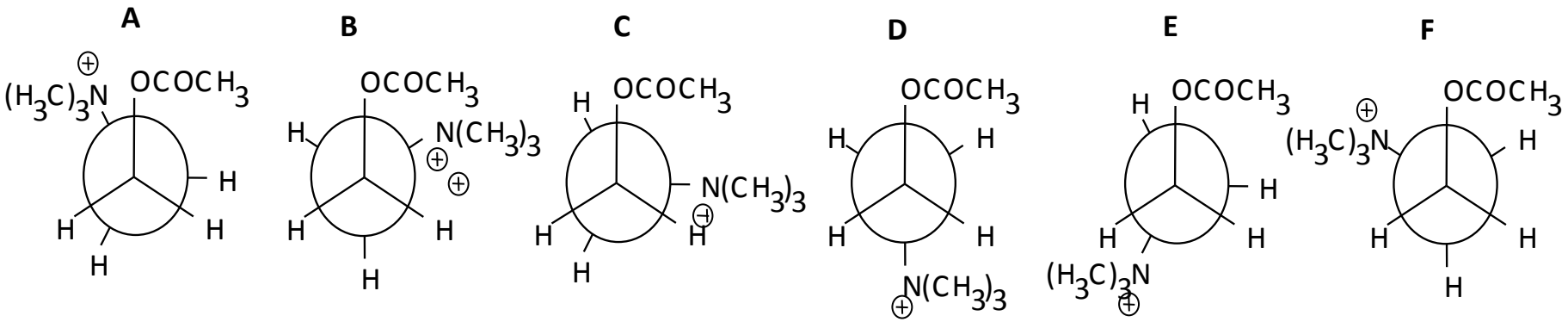


3.4. STEREOCHEMISTRY AND BIOLOGICAL ACTIVITY

Acetylcholine:







- It is difficult to know what conformation a flexible molecule must have in the complex with the target in order to trigger the biological response (X-ray, NMR studies, etc.).
- This conformation is not necessarily the most stable conformation in water.
- This conformation is called **active or pharmacophore conformation**.

Pharmacophore

- Defines the important groups involved in binding and tthe relative positions of the binding groups.
- Needs to know the active conformation.
- Is important for drug design and for drug discovery

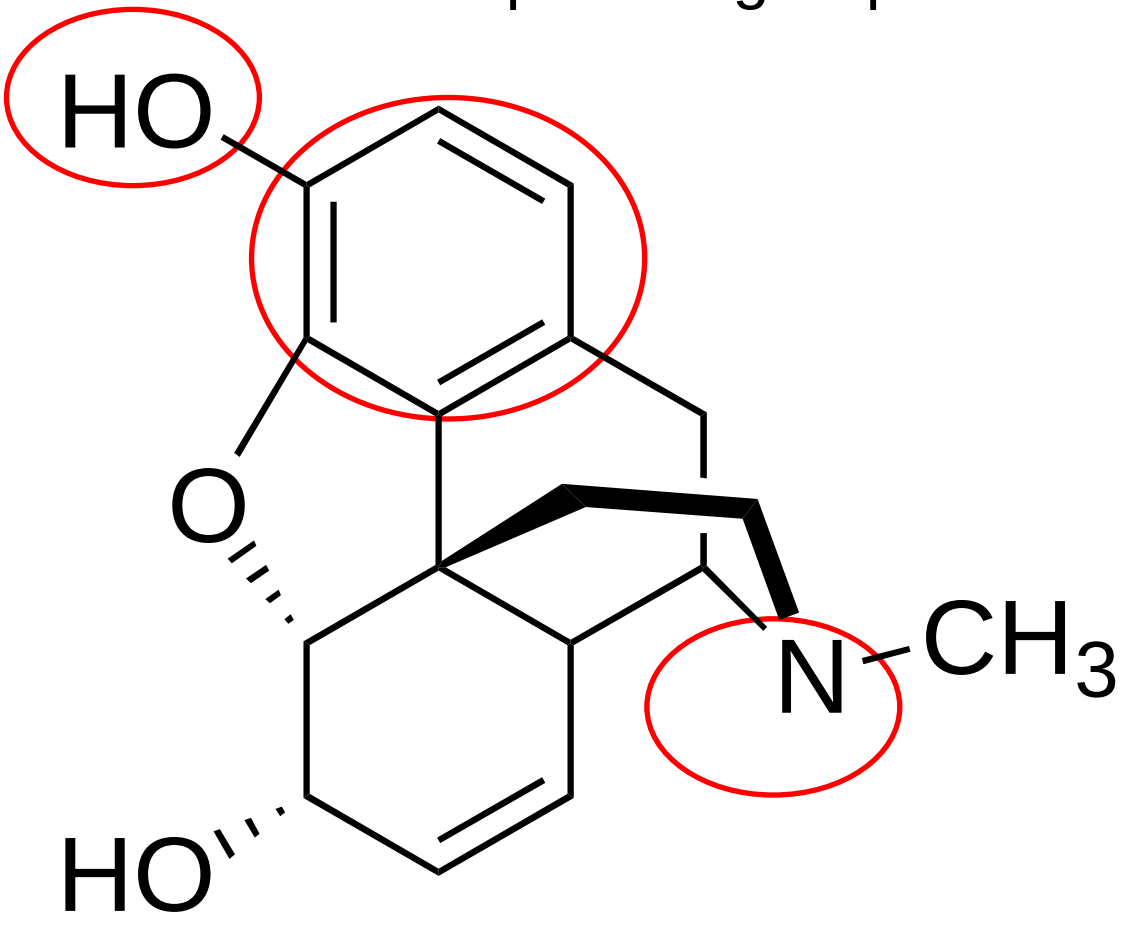
2D Pharmacophore
Defines the minimum skeleton that connects important binding groups
(from structure-activity relationships (SAR) studies in analogous compounds).

3D Pharmacophore:
Defines relative positions in space of important binding groups
(computational techniques and SAR studies).

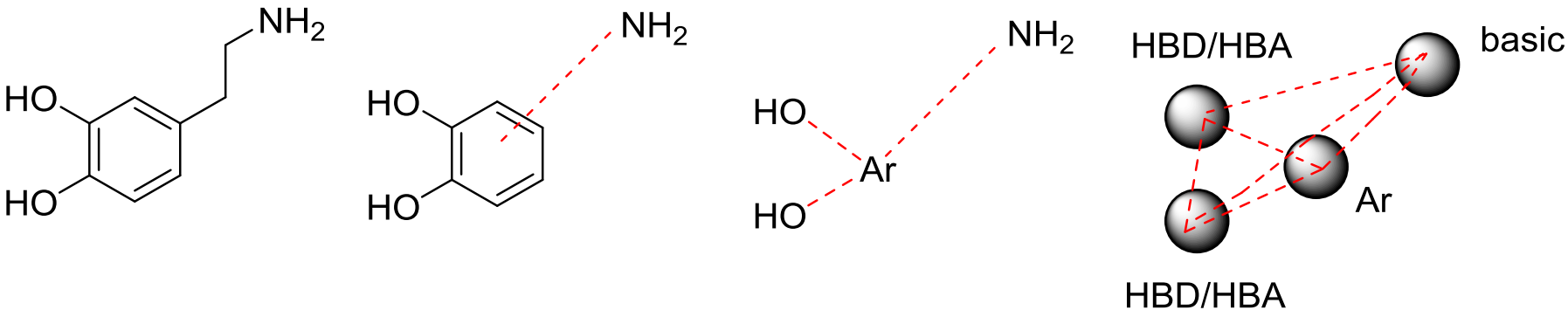
2D pharmacophore

morphine:

Important groups for activity



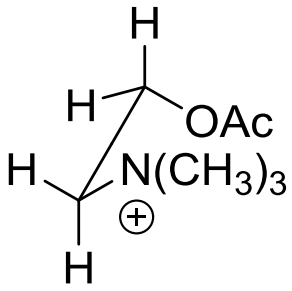
3D Pharmacophore - triangles for dopamine



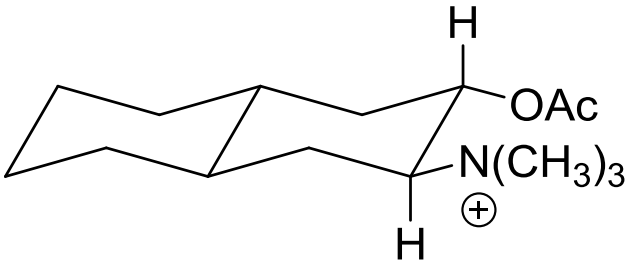
- The active conformation must be identified in order to identify the 3D pharmacophore.
- Conformational analysis identifies possible conformations and their stabilities.
- Conformational analysis is difficult for flexible molecules with large numbers of conformations.
- It is easier to compare activities of **rigid analogues**.

Rigidification: introducing rings

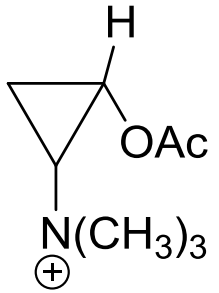
Rigid analogues of acetylcholine



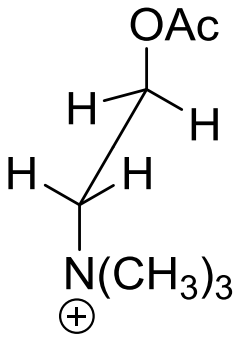
The “cisoid” conformation of acetylcholine



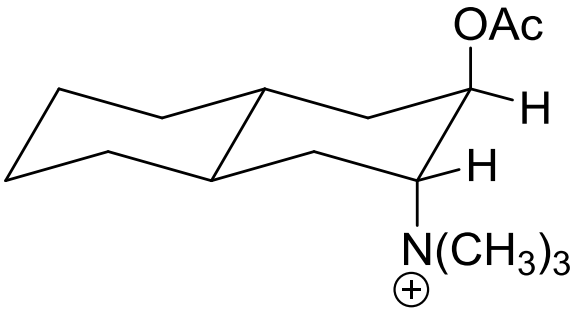
The rigid trans-decalin analogue



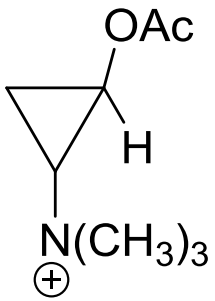
The rigid cyclopropyl analogue



The “transoid” conformation of acetylcholine



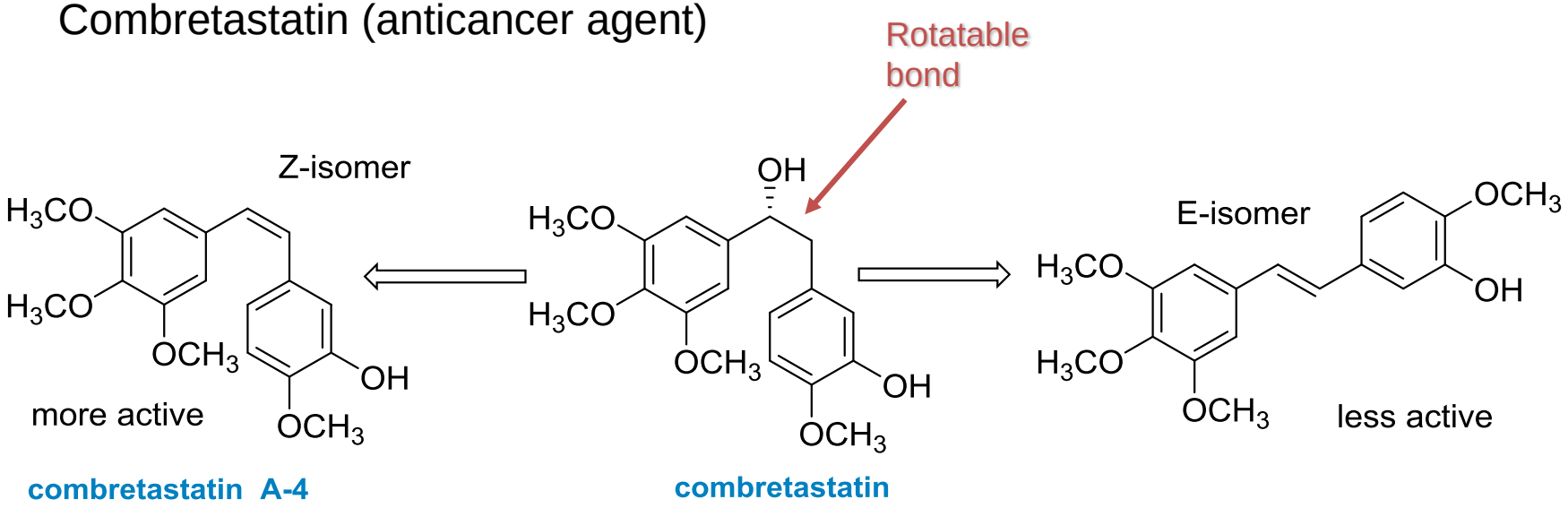
The rigid trans-decalin analogue



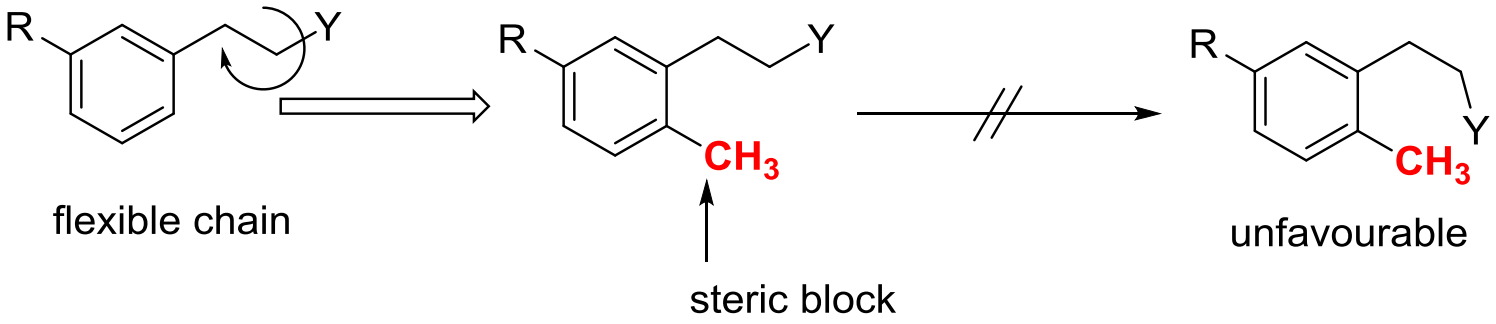
The rigid cyclopropyl analogue

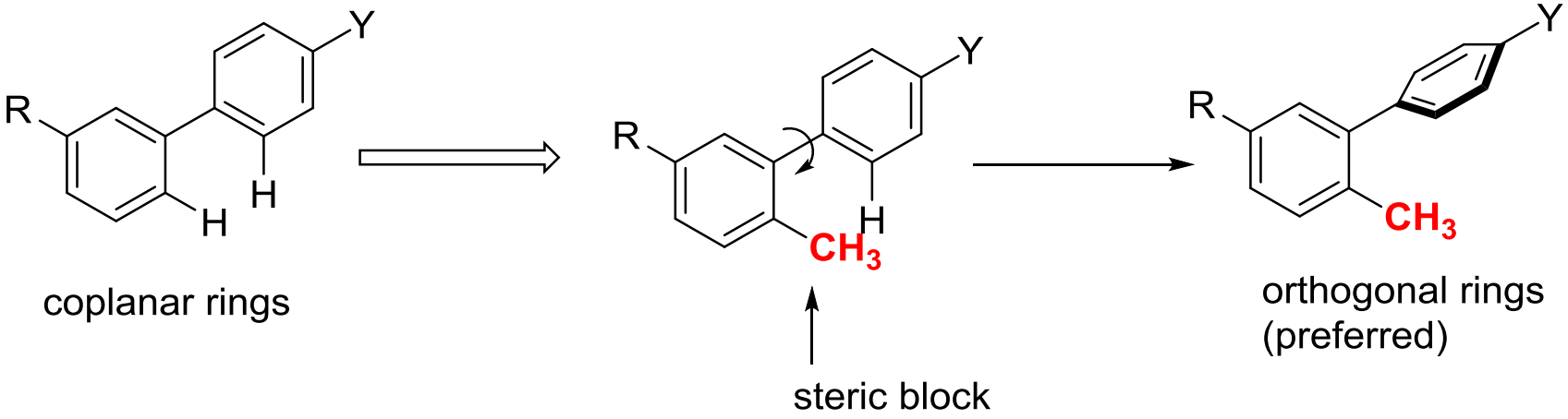
Rigidification: introducing double bonds

Combretastatin (anticancer agent)



Rigidification: introducing steric blocks





UNIT 4. DRUG METABOLISM

4.1. Phase I: Transformation reactions.

4.2. Phase II: Conjugation reactions.

Patrick's 5th ed. chapter 13 and Delgado's chapter 5

4.3. Bioreversible derivatives: prodrugs and bioprecursors.

- Patrick's, 5th ed. chapter 14 (14.6)
- Silverman's, 3rd ed. chapters 8 and 9
- Avendaño's (chapter 8 - Spanish)

4.4. "Soft" drugs and "hard" drugs.

Prodrugs – from Serendipity to Rational Design
K.M. Huttunen, H. Raunio, and J. Rautio, *Pharmacol. Rev.* 63:750 –771, 2011

4. DRUG METABOLISM - INTRODUCTION

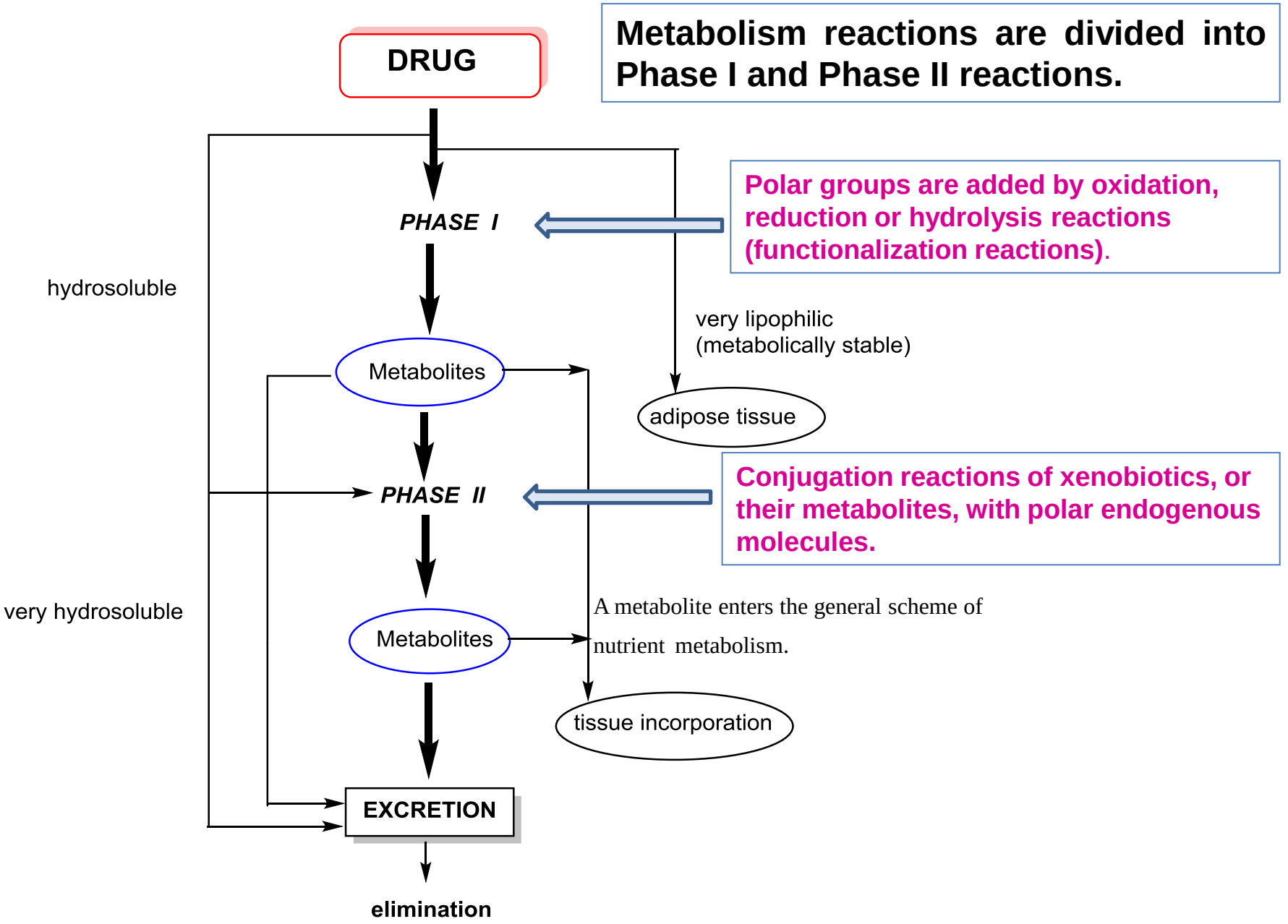
- Drug metabolism is considered a **detoxification** pathway by which molecules are chemically modified to make them more polar, thereby facilitating elimination from the body.
- Drug metabolism or **biotransformations** are the chemical reactions responsible for converting drugs into other products (called metabolites) within the body before and/or after they have reached their sites of action.

- Almost all these reactions are enzyme-catalysed and will therefore exhibit the general characteristics of enzyme-controlled processes (they will tend to be stereospecific and will be affected by substrate concentration, pH, and temperature).
- There are differences between mammals, differences between the same species, differences in age, sex, pregnancy, etc.
- It is important to understand the metabolism of newly developed drugs:

  -metabolism plays a central role in bioavailability and the half-life of a drug (influencing how much (dose) and how often (frequency) a drug should be administered).

  -in order to establish the best administration route.

4. DRUG METABOLISM - INTRODUCTION



4. DRUG METABOLISM - INTRODUCTION

Phase I Enzymes:

Cytochrome P450 enzymes	(microsomes)
Flavin monooxygenases	(microsomes)
Xanthine oxidase and aldehyde oxidase	(cytosol)
Monoamine oxidases (MAO-A and MAO-B)	(mitochondria)
Dihydrodiol dehydrogenase	(cytosol)
Alcohol and aldehyde dehydrogenases	(cytosol)
Esterases/amidases	(microsomes, cytosol, plasma)
Epoxide hydrolase	(microsomes and cytosol)
Sulfur reductase	(cytosol)
Quinone reductase	(microsomes and cytosol)
Azo/nitro reductase	(microsomes, cytosol and gut)

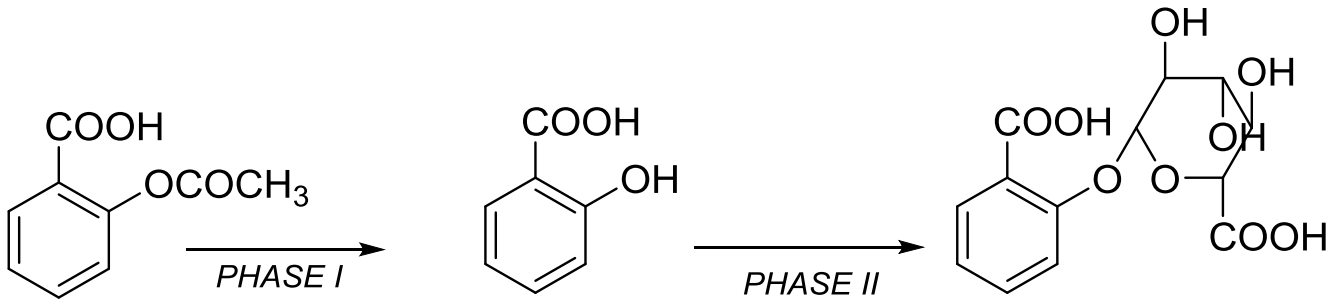
Phase II Enzymes:

Glucuronosyl transferases	(microsomes)
Sulfo-transferases	(cytosol)
Methyl transferases	(microsomes and cytosol)
Acetyl transferases	(cytosol)
Glutathione transferases	(microsomes and cytosol)
Amino acid transferases	(microsomes)

Drug Discovery: Practices, Processes and Perspectives
John Wiley and Sons, p 126

4. DRUG METABOLISM - INTRODUCTION

Example:



aspirin salicylic acid glucuronide

- Most reactions add a polar 'handle' to the molecule.
- Increasing the polarity of a compound increases the rate of drug excretion.

- Most enzyme-catalysed reactions occur in the liver (detoxification).
- Metabolic reactions also occur in blood, the gut wall and other organs.
- Drug metabolites are usually less active or inactive (with the exception of prodrugs).
- Modification of a structure may interfere or prevent binding interactions with a target (pharmacodynamics).

4.1. PHASE I TRANSFORMATIONS

4.1.1. Microsomal **oxidations**

Carbon sp^3 oxidations
Allylic and benzylic oxidations
Oxidative dealkylations. Amines, ethers and thioethers.
Oxidative dehydrohalogenation
Oxidative N-dealkylation and deamination
Alkene and alkyne oxidations
Aromatic hydroxylation
Amine and sulphur derivative oxidations

4.1.2. Non-microsomal **oxidations**

Alcohol and aldehyde oxidations catalyzed by dehydrogenases
Oxidative deamination catalyzed by monoamine oxidase
Purine oxidation

4.1.3. **Reductions**

Azo reductions
Ketone reductions
Nitro-derivative reductions
Alkene reductions
Sulfoxide reductions

4.1.4. **Hydrolytic reactions**

Hydrolysis of nitriles, esters, and amides
Hydration of epoxides

4.1. PHASE I TRANSFORMATIONS

Microsomal oxidations: monooxygenases

These are catalyzed by mixed-function oxidases in the liver: cytochrome P-450 and flavin-containing monooxygenase (FMO).

Cytochrome enzymes use an oxygenated heme (or haem) prosthetic group, while the FMO family uses FAD to oxidize its substrates.

Cytochrome P-450
The heme residue is protoporphyrin IX containing a coordinated Fe(III).

- It is located in the liver.
- There are at least 12 families in human biochemistry.
- Individuals differ in the types of Cyt P-450 enzymes present.
- Patient variability in drug metabolism complicates dose levels and leads to different susceptibilities to drugs.

4.1. PHASE I TRANSFORMATIONS

Microsomal oxidations: monooxygenases

Cytochrome P-450

Drug-drug interactions

Some drugs affect the activity of cytochrome P450 enzymes:
- phenobarbitone enhances activity.
- cimetidine inhibits activity.

These drugs may affect metabolism of other drugs (e.g. warfarin) and the result can be overdose or under-dose of the affected drug.

Drug-food interactions

Certain foods affect the activity of cytochrome P450 enzymes:
- brussel sprouts & cigarette smoke enhance activity.
- grapefruit juice inhibits activity.

Microsomal oxidations: monooxygenases

Cytochrome P-450 (CYP450)

The most common reaction catalysed by cytochromes P450 is the insertion of one atom of oxygen (**monooxygenation**) while the other oxygen atom from molecular oxygen is reduced to water.

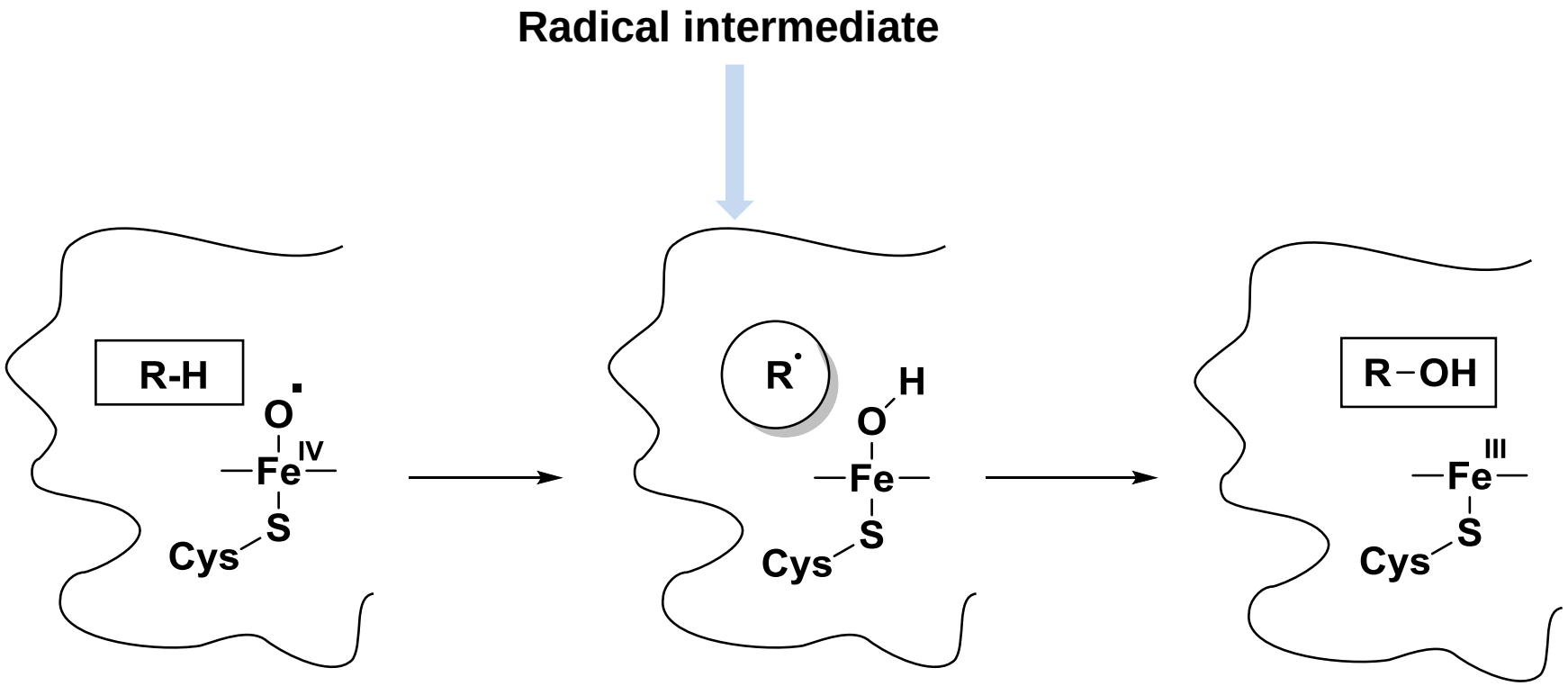
This insertion can take place into the **aliphatic position of an organic substrate (RH)**.

$$\mathbf{R\text{-}H + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+}$$

In the oxidative process, a reduction of Fe^{3+} to Fe^{2+} occurs before the molecular oxygen is bound. The electron is provided by NADPH, which is oxidized to $NADP^+$ by means of a flavoprotein.

4.1. PHASE I TRANSFORMATIONS

CYP450 oxidations of Csp^3-H bonds



Remember:

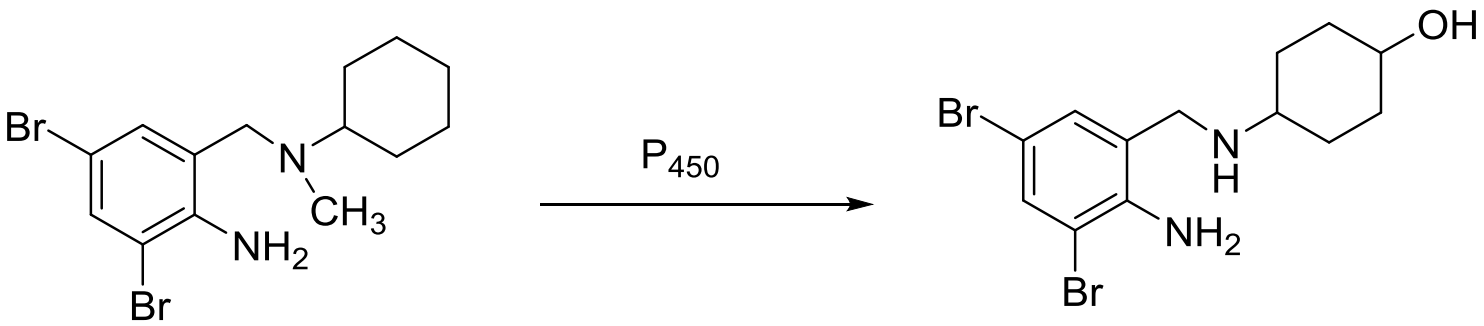
Relative radical stability
Steric effects are important in enzymatic catalysis

4.1. PHASE I TRANSFORMATIONS

CYP450 oxidations of Csp^3-H bonds

METABOLITES

- **primary alcohols**
- **secondary alcohols**
- **allylic alcohols**
- **benzylic alcohols**
- **propargylic alcohols**

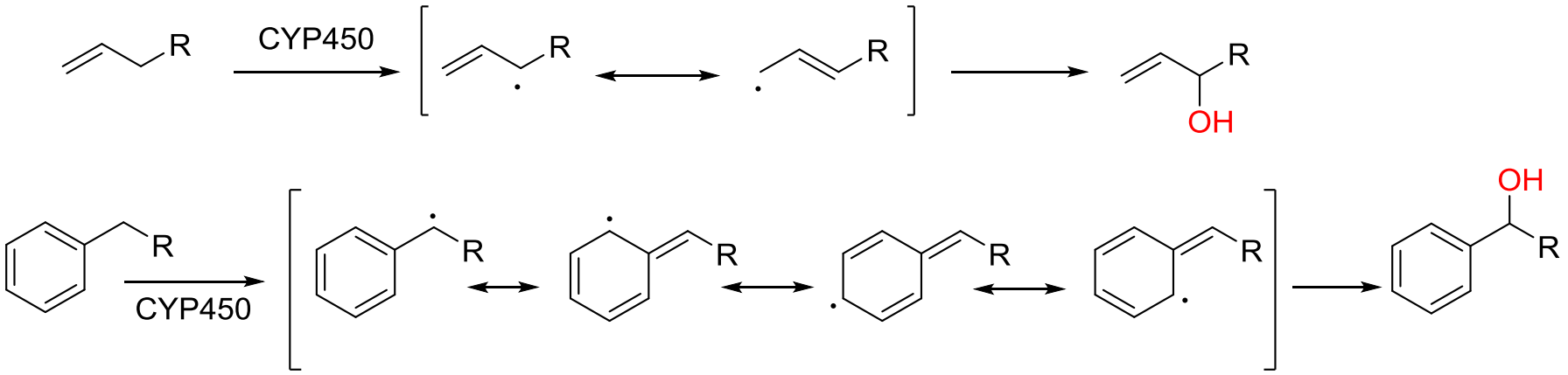


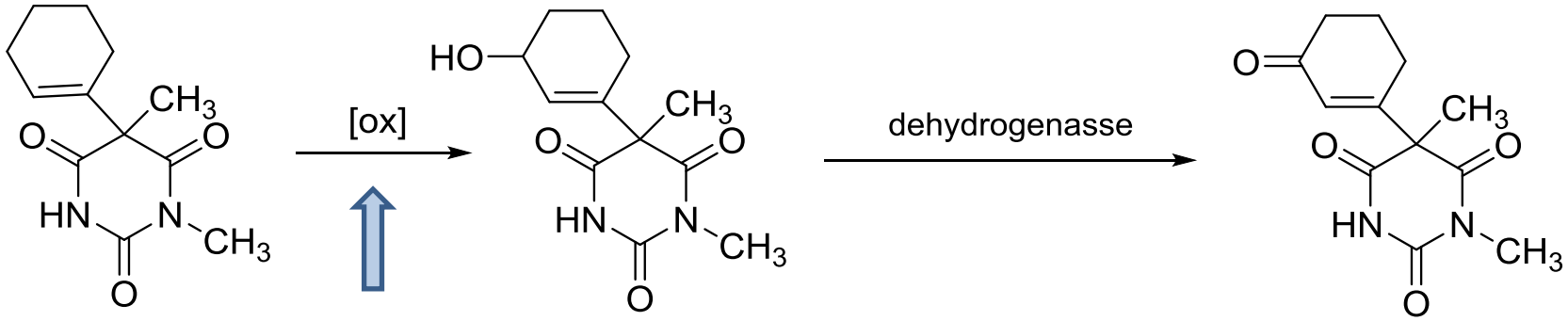
bromhexine
(mucolytic)

ambroxol
(also active)

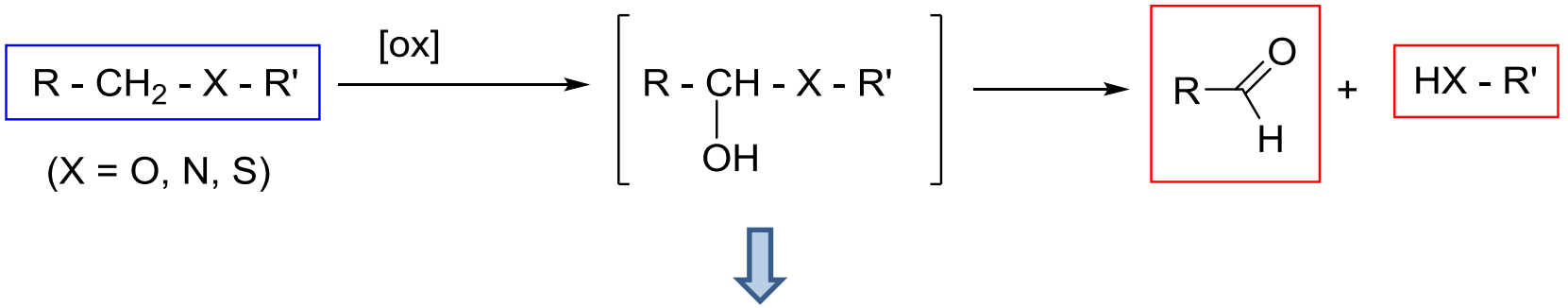
4.1. PHASE I TRANSFORMATIONS

CYP450 oxidations of Csp^3-H bonds: allylic and benzylic positions





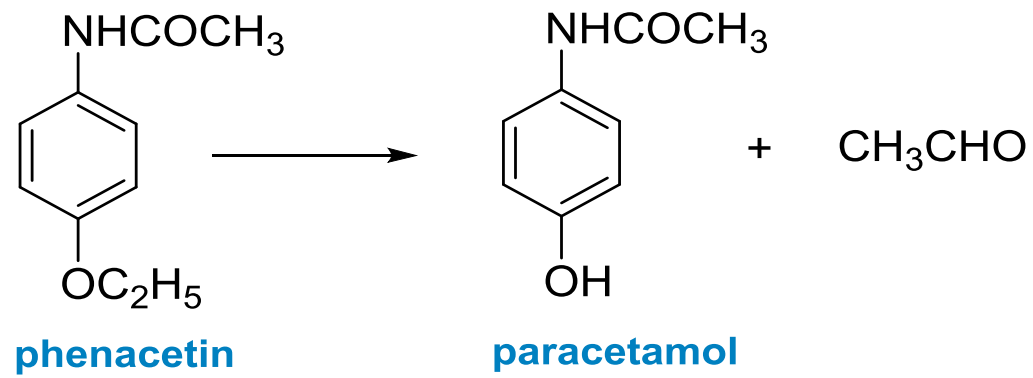
CYP450 oxidations at Csp^3-H bonds vicinal to a heteroatom: N-, O- and S-dealkylation



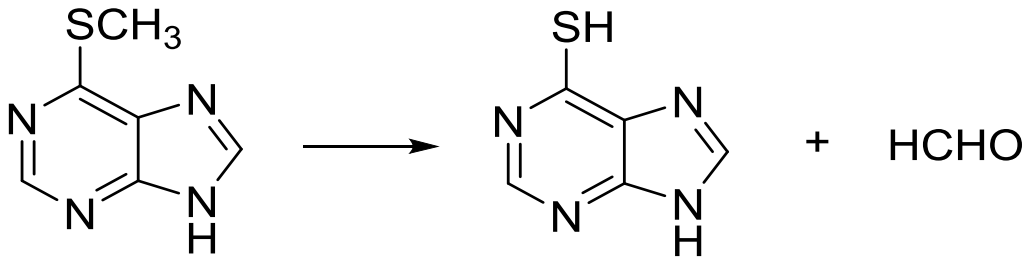
Unstable metabolites:
Give aldehydes or ketones and the corresponding amine, alcohol or thiol

The mechanism of the second reaction depends on the heteroatom (X).

CYP450 oxidations at Csp^3-H bonds vicinal to a heteroatom: N-, O- and S-dealkylation

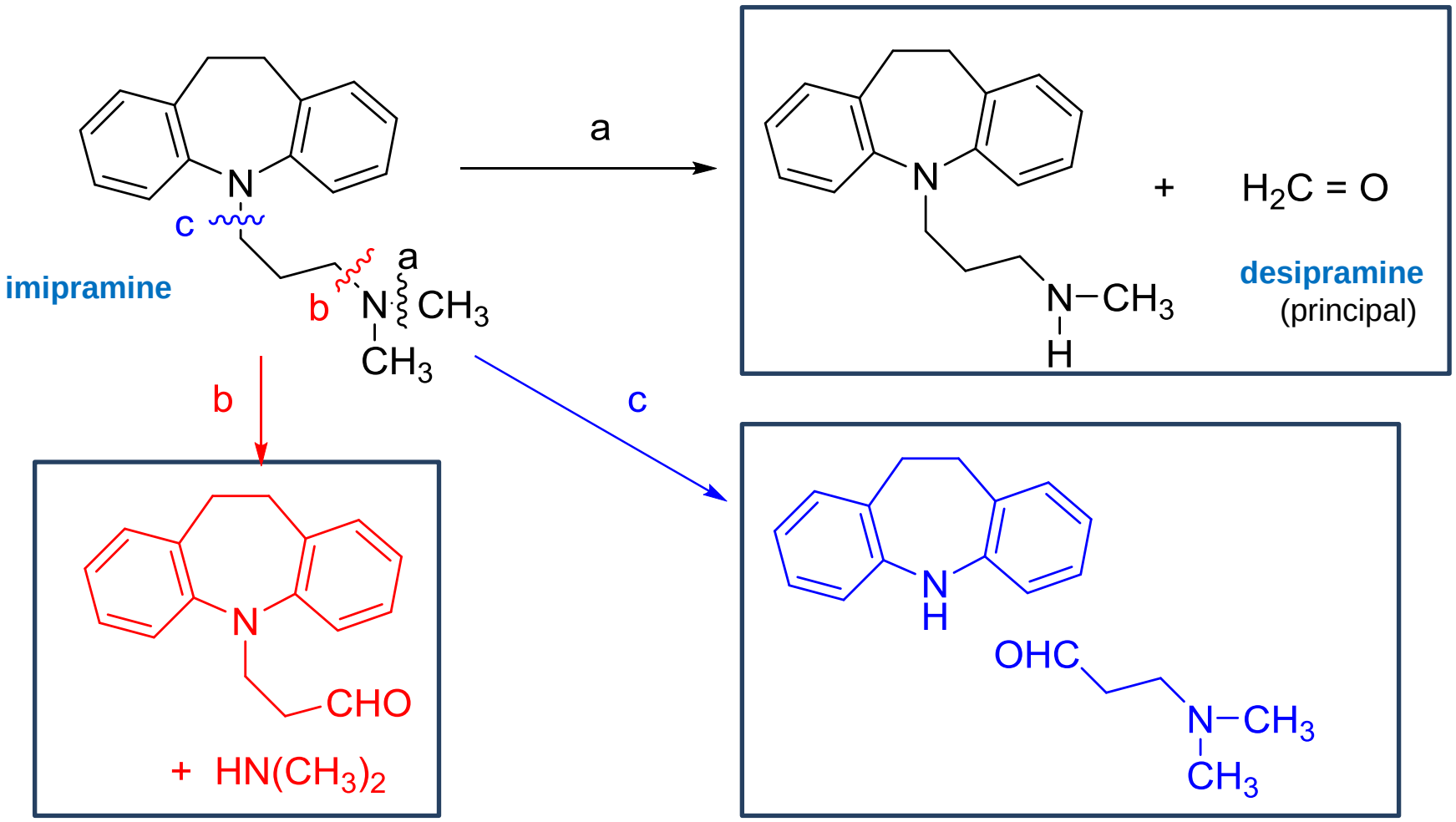


phenacetin paracetamol



6-methylthiopurine 6-mercaptopurine

CYP450 oxidations at Csp^3-H bonds vicinal to a heteroatom: N-, O- and S-dealkylation



(minor products)

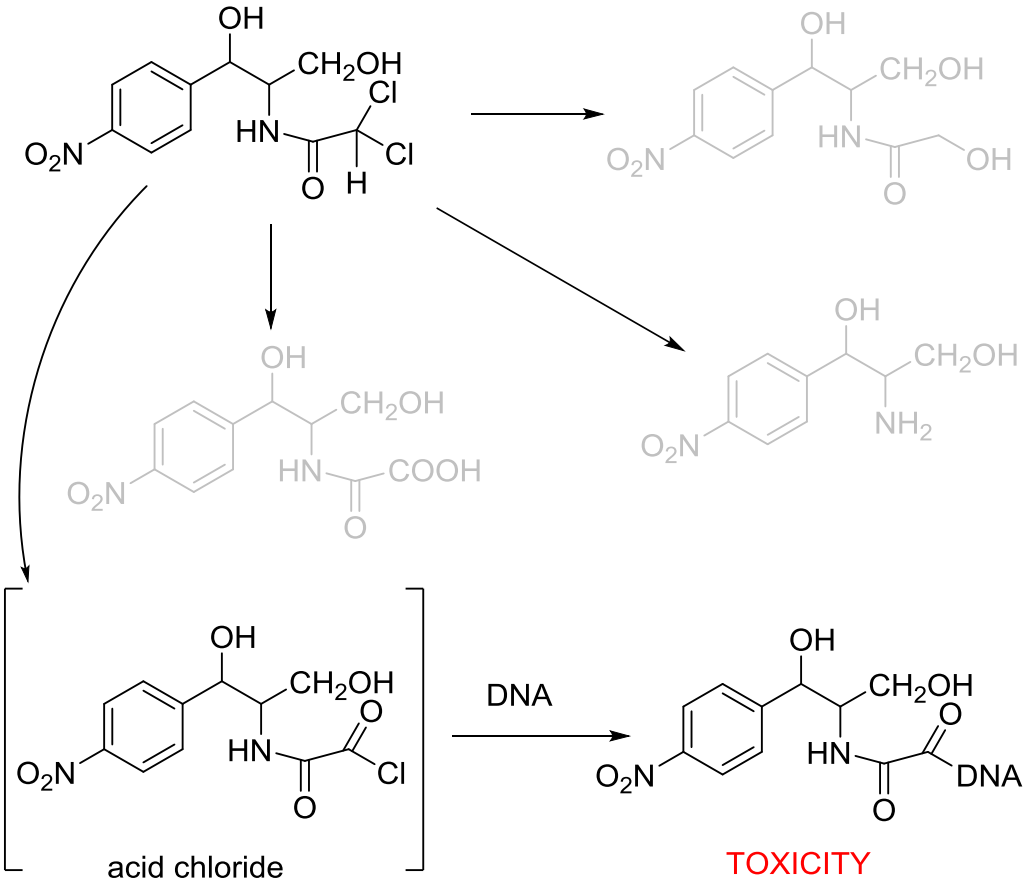
4.1. PHASE I TRANSFORMATIONS

CYP450 oxidations at Csp^3-H bonds vicinal to a halogen atom

Dehalogenation of alkyl chlorides

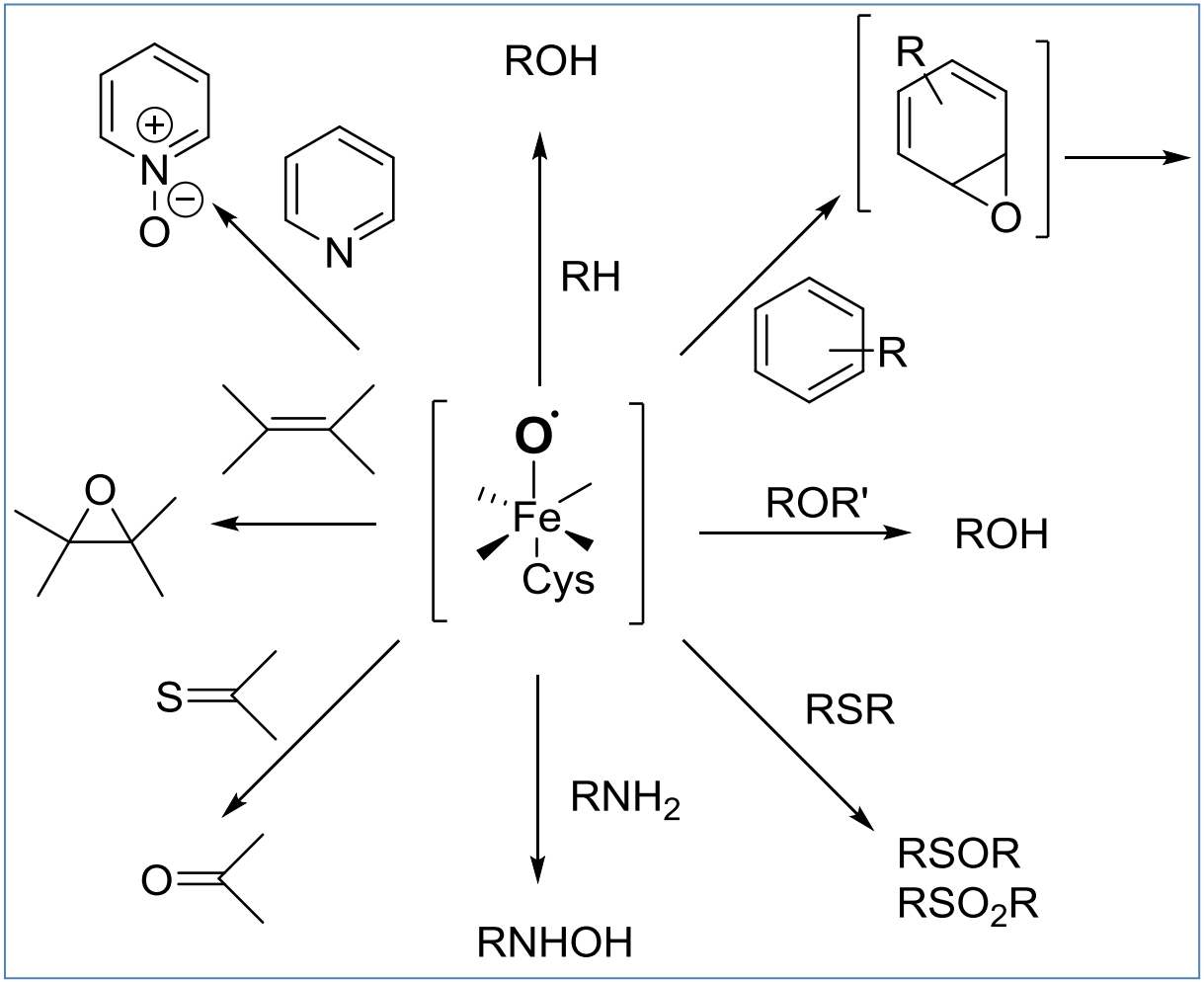
RR'CHX ⟶ RR'C(OH)X ⟶ RCOR' + HX

chloramphenicol
(antibiotic)



4.1. PHASE I TRANSFORMATIONS

Double bonds, aromatic rings and heteroatoms can also be oxidized by CYP450.

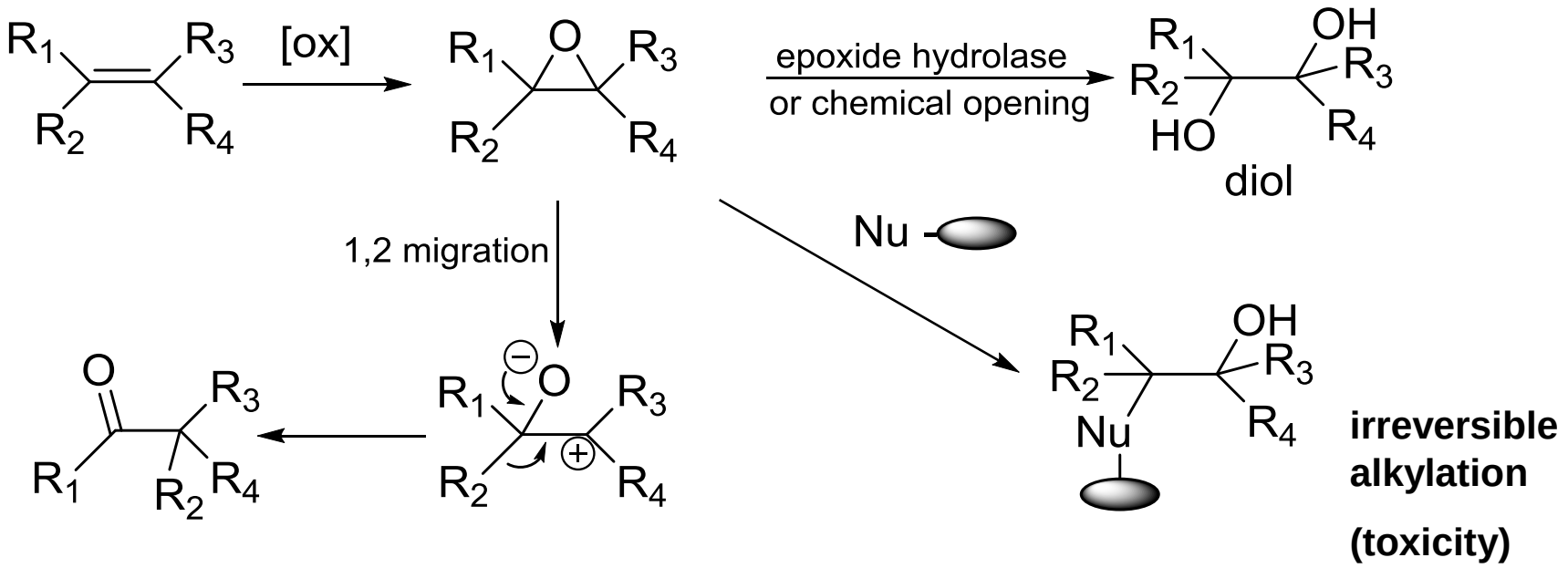


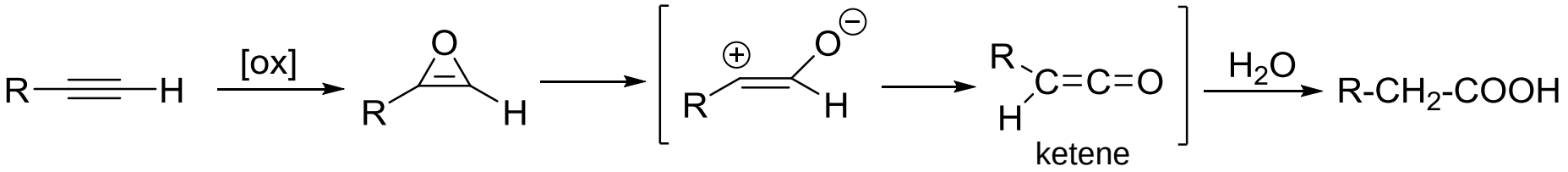
Epoxides are electrophilic: Nucleophilic addition from amino, thiol or hydroxyl groups from proteins or nucleic acids can take place (**toxicity**).

Drug + O_2 + NADPH + H^+ → Oxidized drug + H_2O + $NADP^+$

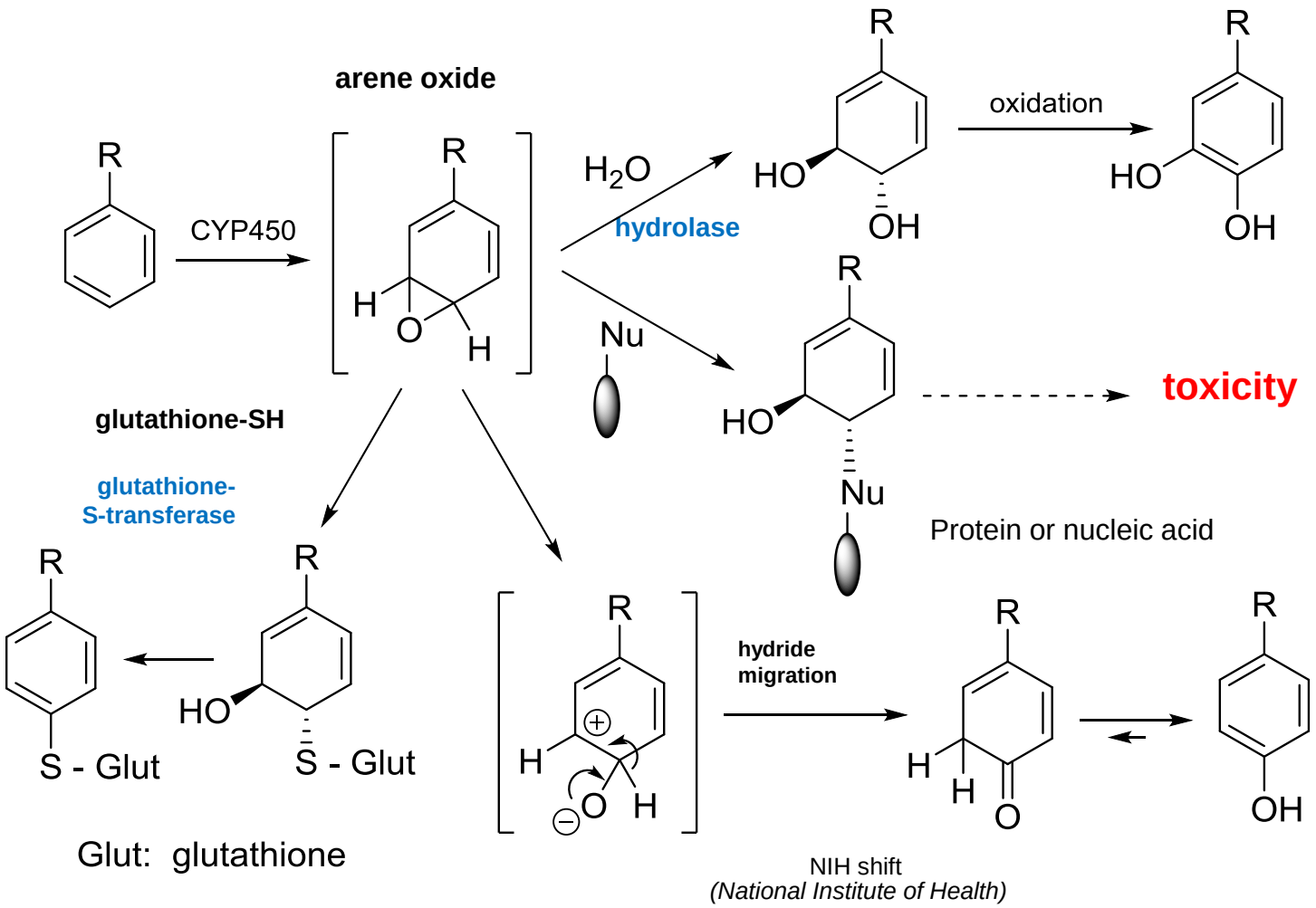
4.1. PHASE I TRANSFORMATIONS

CYP450 oxidations of double and triple bonds





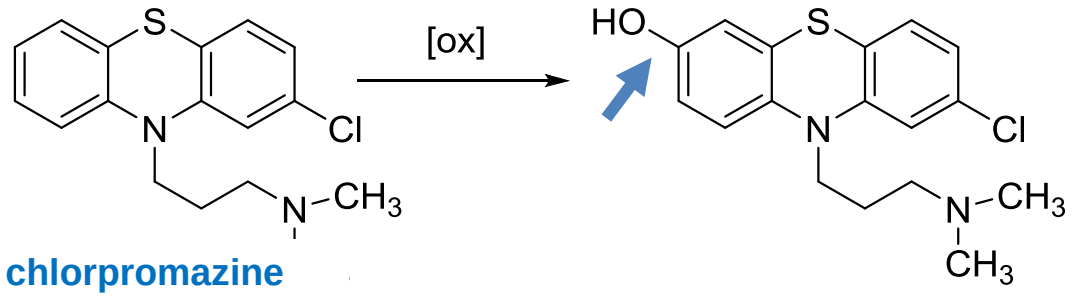
CYP450 oxidations of aromatic rings: epoxides, phenols and dihydroxybenzenes



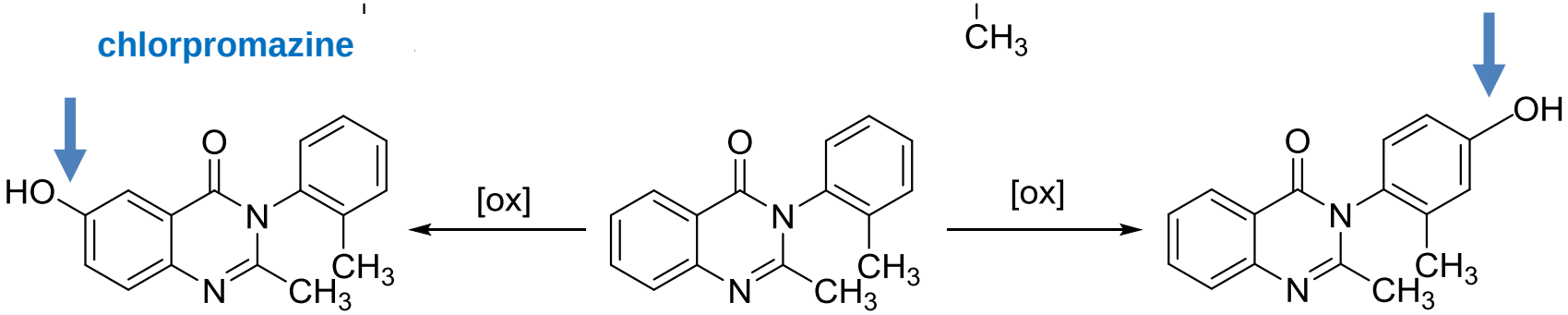
Aromatic hydroxylation mechanism

4.1. PHASE I TRANSFORMATIONS

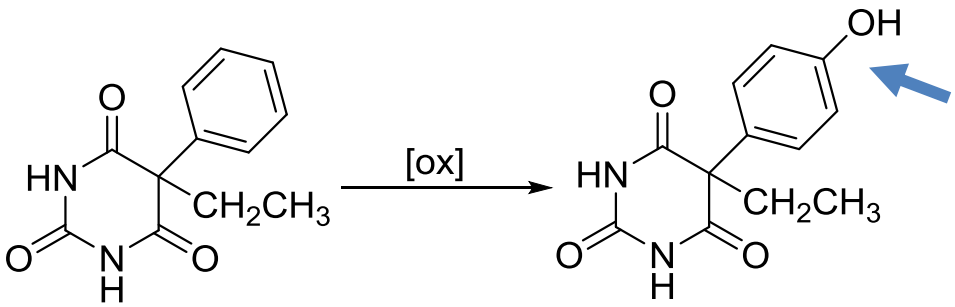
CYP450 oxidations of aromatic rings:phenols



chlorpromazine



methaqualone



phenobarbital

NOTE: Aromatic rings that are highly deactivated for SEAr or hindered are stable and are not oxidized (see TCDD: tetrachlorodibenzodioxin).

CYP450 oxidations at nucleophilic nitrogen atoms

Tertiary amines → N-Oxides

Secondary amines → Hydroxylamines

Aliphatic primary amines → Hydroxylamines → Imines → Nitro

Aromatic primary amines → Hydroxylamines → Nitroso → Nitro

Pyridines → N-Oxides

Primary amides → N-Hydroxylamides

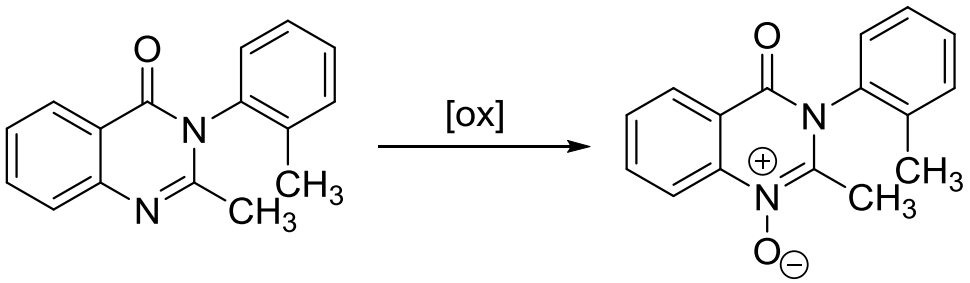
Secondary amides → N-Hydroxylamides

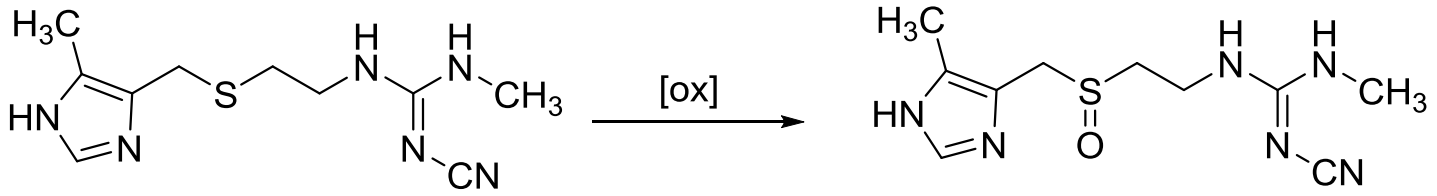
4.1. PHASE I TRANSFORMATIONS

CYP450 oxidations at nucleophilic sulphur atoms

Thiol (x 2)	→	Disulphide
Thiol	→	Sulfoxide → Sulphone
Thioketone	→	Ketone (desulphurization)

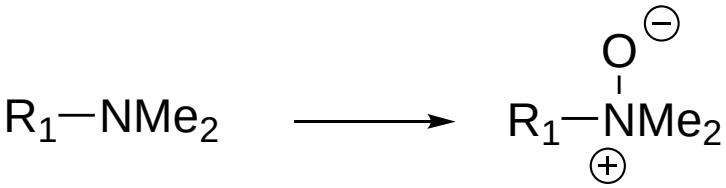
metaqualone (hypnotic)

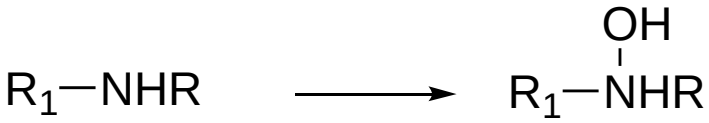


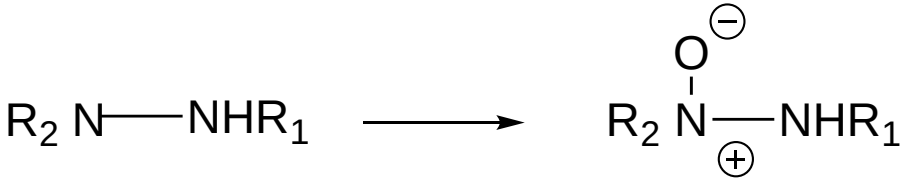


cimetidine (treatment of peptic ulcers)

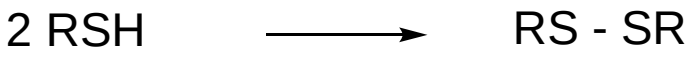
Phase I reactions catalysed by flavin-containing monooxigenases







Many of these reactions are also catalysed by cytochrome P450 enzymes.



4.1. PHASE I TRANSFORMATIONS

4.1.1. Microsomal **oxidations**

Carbon sp^3 oxidations
Allylic and benzylic oxidations
Oxidative dealkylations. Amines, ethers and thioethers.
Oxidative dehydrohalogenation
Alkene and alkyne oxidations
Aromatic hydroxylation
Amine and sulphur derivative oxidations

4.1.2. Non-microsomal **oxidations**

Alcohol and aldehyde oxidations catalyzed by dehydrogenases
Oxidative deamination catalyzed by monoamine oxidase
Purine oxidation

4.1.3. **Reductions**

Azo reductions
Ketone reductions
Nitro-derivative reductions
Alkene reductions
Sulfoxide reductions

4.1.4. **Hydrolytic reactions**

Hydrolysis of nitriles, esters, and amides
Hydration of epoxides

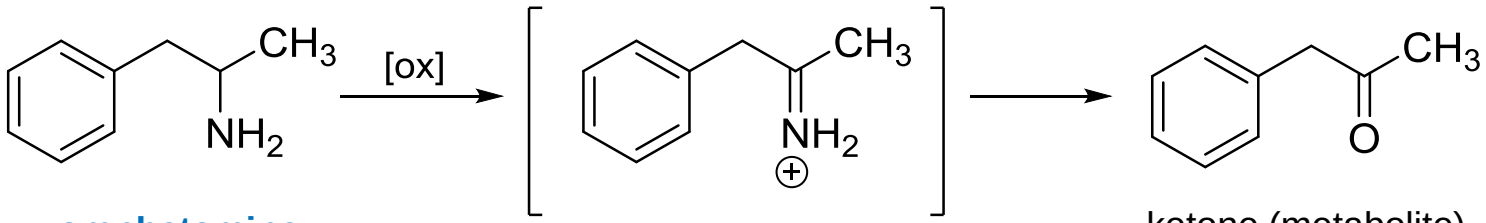
4.1.2. Non-microsomal oxidations: alcohol and aldehyde oxidation

Primary alcohols $\xrightarrow[NAD^+]{\text{Alcohol dehydrogenase}}$ Aldehydes $\xrightarrow[NAD^+]{\text{Alcohol dehydrogenase}}$ Carboxylic acids

Secondary alcohols $\xrightarrow[NAD^+]{\text{Alcohol dehydrogenase}}$ Ketones

Primary amines $\xrightarrow{\text{Monoamine oxidases}}$ Aldehydes

4.1.2 Non-microsomal oxidations: oxidative deamination of primary amines

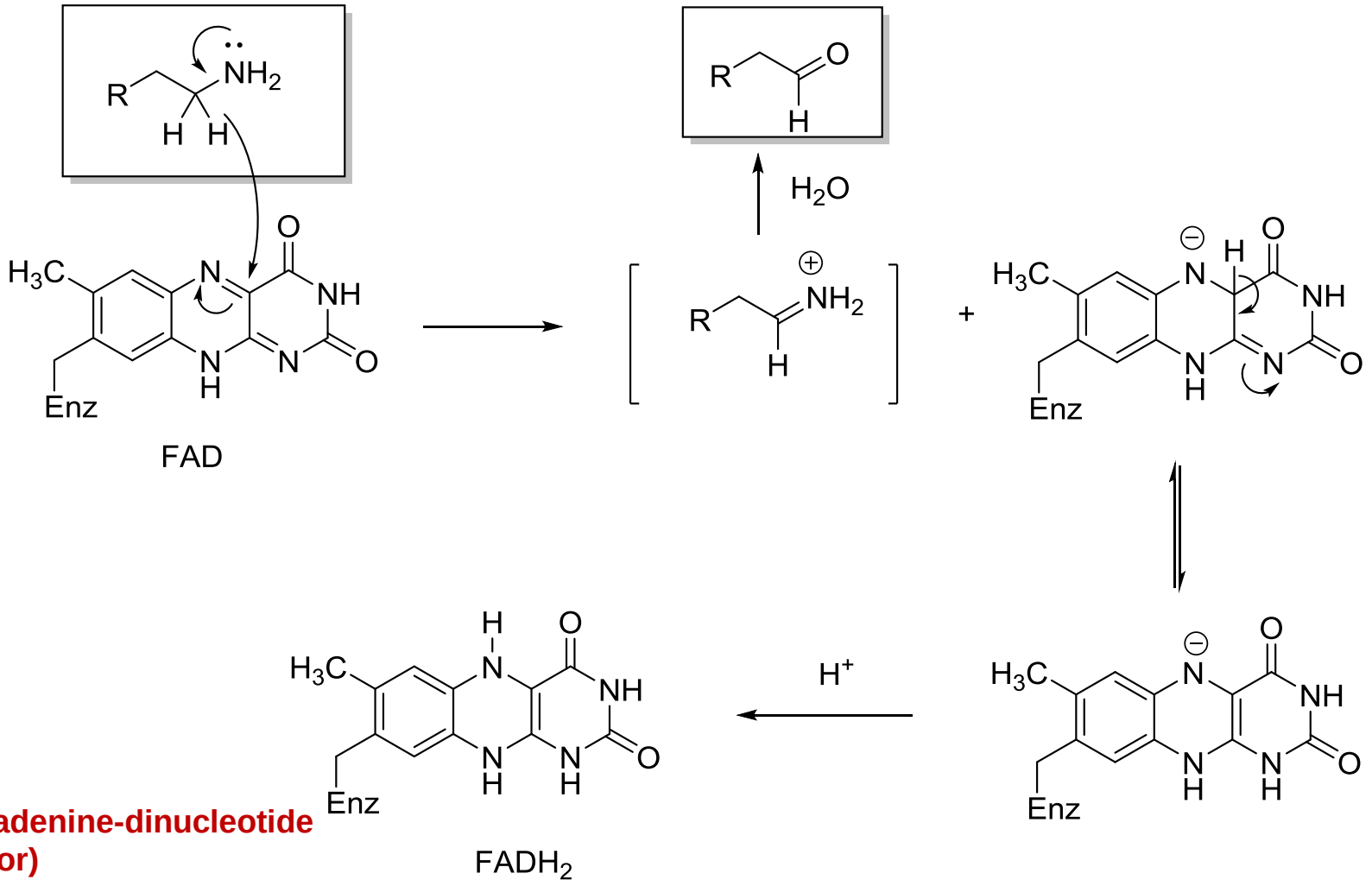


amphetamine

ketone (metabolite)

4.1. PHASE I TRANSFORMATIONS

Non-microsomal oxidations: oxidative deamination of primary amines (MAO)



Flavin adenine-dinucleotide (cofactor)

Compare with oxidative N-dealkylation

4.1.3. Reductions: cytochrome P-450 enzymes and other reductases

Aldehydes —(Alcohol dehydrogenase)→ Primary alcohols

Ketones —(Reductases / NADPH)→ Secondary alcohols

α,β-unsaturated ketones → Saturated ketones

Alkyl halides → Alkanes

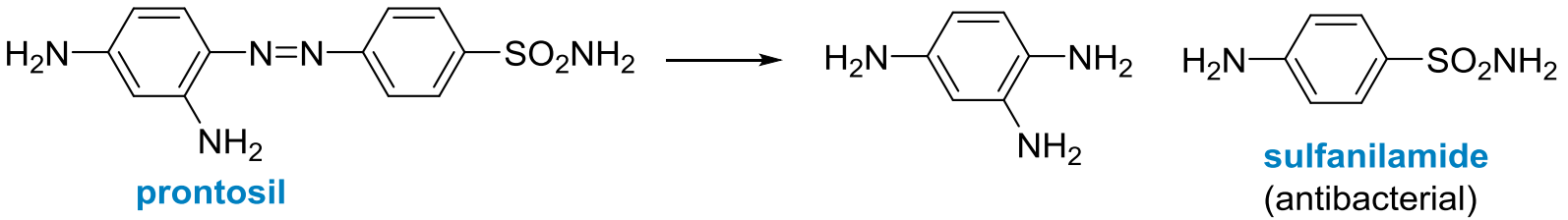
Aromatic nitro → Aromatic primary amines

Ar-N=N-Ar (azo) → Ar-NH-NH-Ar → $Ar\text{-}NH_2$ + $H_2N\text{-}Ar$

Disulphides → Thiols

Sulphoxides → Thioethers

4.1.3. Reductions: cytochrome P-450 enzymes and other reductases

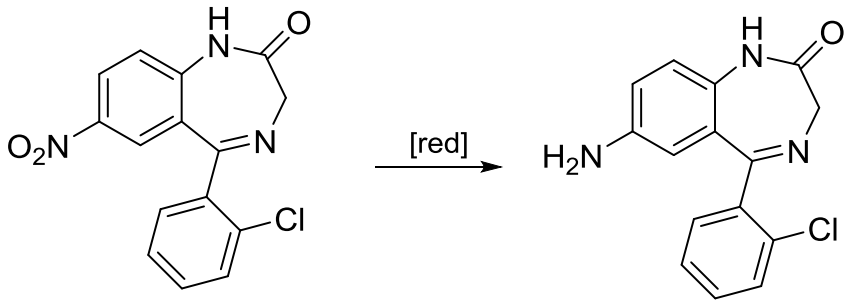


prontosil

sulfanilamide
(antibacterial)

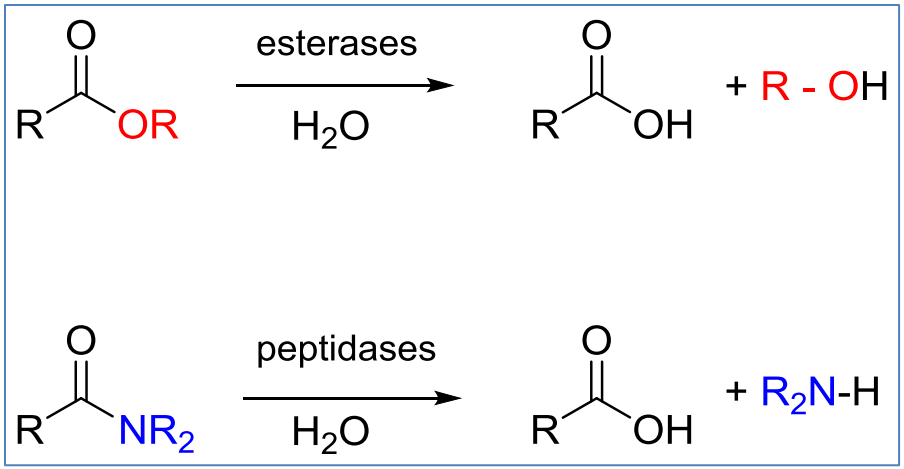
(R)-warfarin
(anticoagulant)

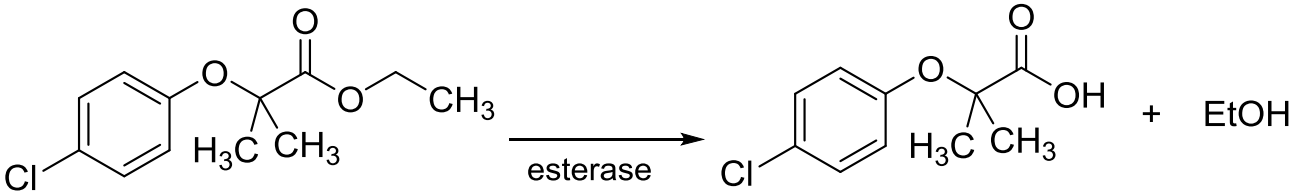
(R,S)-warfarinol



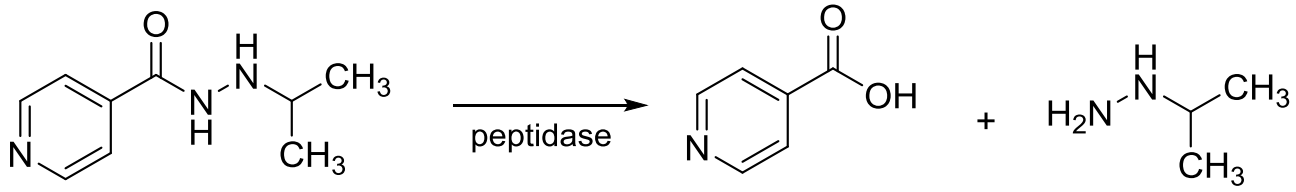
clonazepam

4.1.4. Hydrolysis



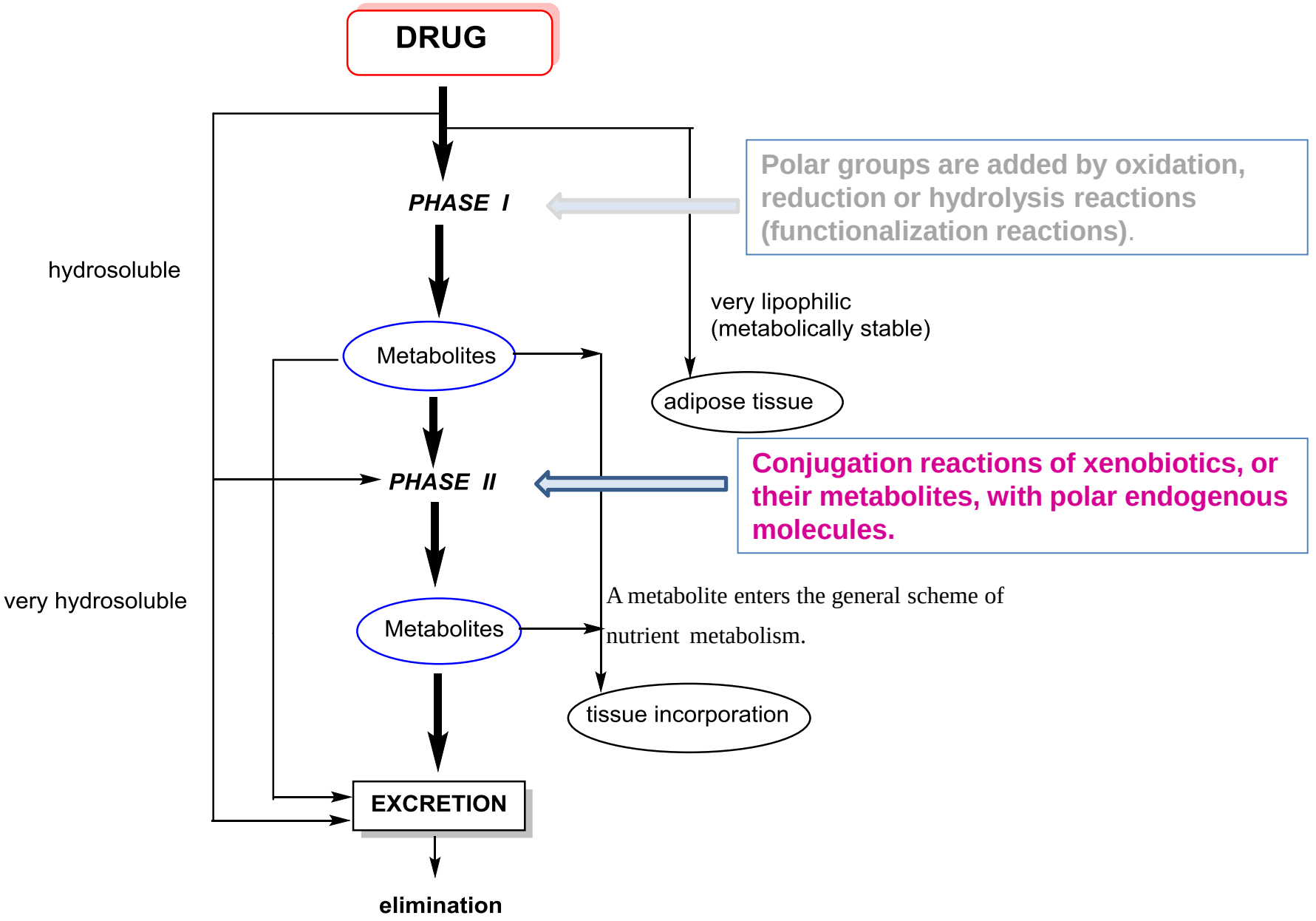


Clofibrate (hypolipidemic)



Iproniazid
(antidepressant)

4.2. PHASE II TRANSFORMATIONS



4.2. PHASE II TRANSFORMATIONS

4.2.1. Conjugation with glucuronic acid

4.2.2. Conjugation with sulphate

4.2.3. Conjugation with glutathione

4.2.4. Conjugation with amino acids

4.2.5. Acylation reactions

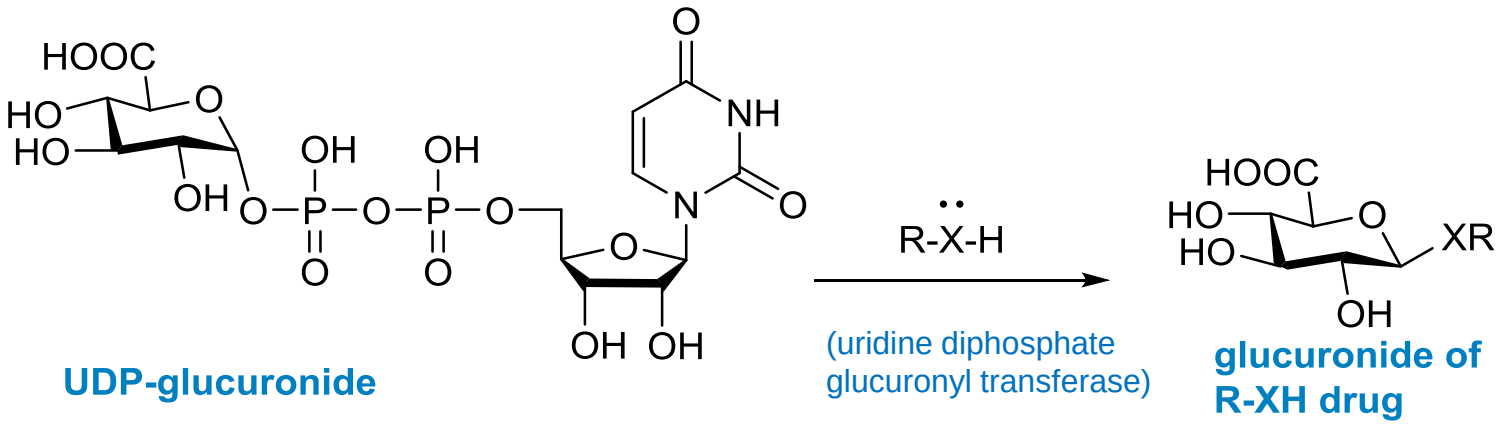
4.2.6. Methylation reactions

Enzymes in phase II reactions:

1. *Uridine diphosphate glucuronyl transferase (UDP-glucuronyl transferase)*
2. *3'-Phosphoadenosine-5'- phosphosulphate transferase*
3. *Glutathione transferase*
4. *Amino acid N-acyl transferase*
5. *N-Acyl transferase*
6. *Methyl transferase*

4.2. PHASE II TRANSFORMATIONS

4.2.1. Glucuronides (nucleophilic atoms in drugs)



UDP-glucuronide

(uridine diphosphate glucuronyl transferase)

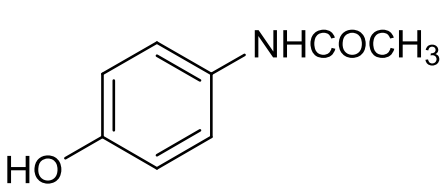
glucuronide of R-XH drug

R-X-H:

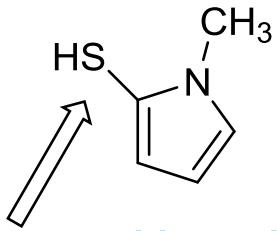
- Alcohols and amines
- Hydroxylamines **R_2N-O**-H
- Thiols **R-S**-H
- Phenols **Ar-O**-H
- Carboxylic acids **RCO-O**-H
- Sulphonamides **R-SO_2-NR'-**H

4.2. PHASE II TRANSFORMATIONS

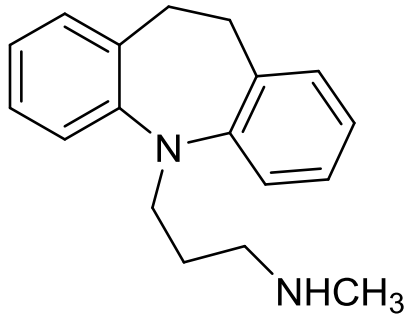
4.2.1. Glucuronides (nucleophilic atoms in drugs)



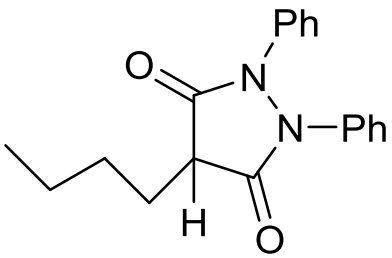
paracetamol



methimazole

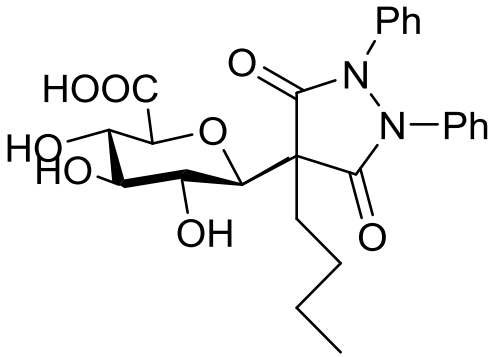


demethylimipramine

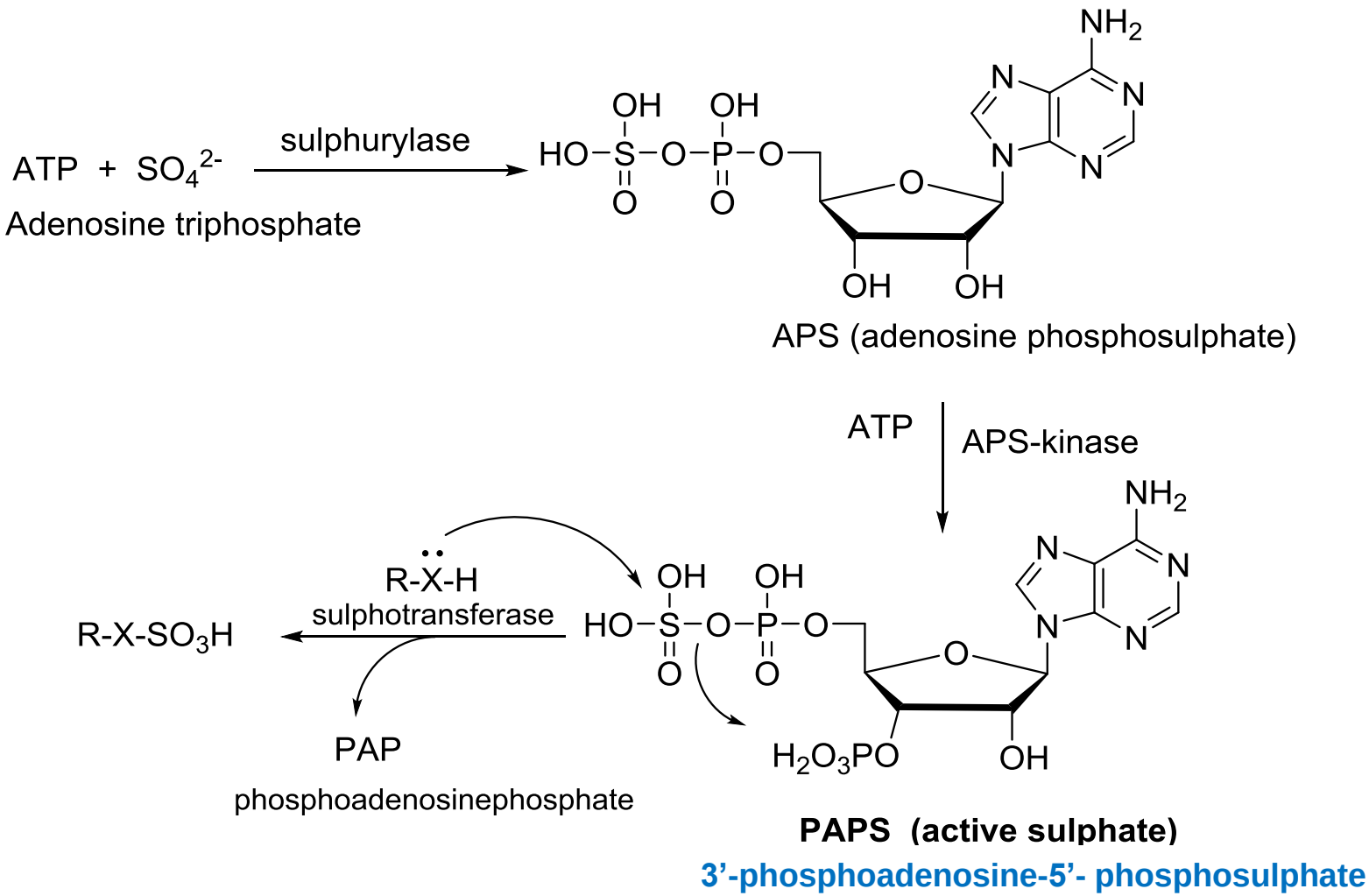


acid C-H

UDP-glucuronide



4.2.2. Sulphates (nucleophilic atoms in drugs)



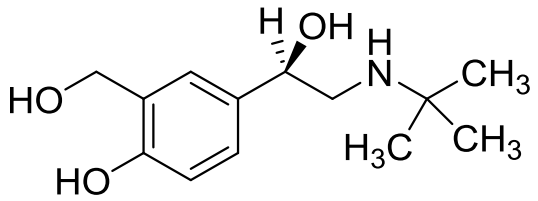
4.2. PHASE II TRANSFORMATIONS

4.2.2. Sulphates (nucleophilic atoms in drugs)

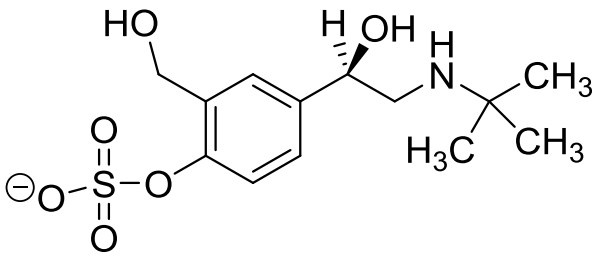
Ar – OH
Phenols
—Sulphotransferase, PAPS→
Ar – O – SO_3^-
sulphate conjugate

R – OH
Alcohols
—Sulphotransferase, PAPS→
R – O – SO_3^-
sulphate conjugate

R_2N – H
Amines
—Sulphotransferase, PAPS→
R_2N – SO_3^-
sulphate

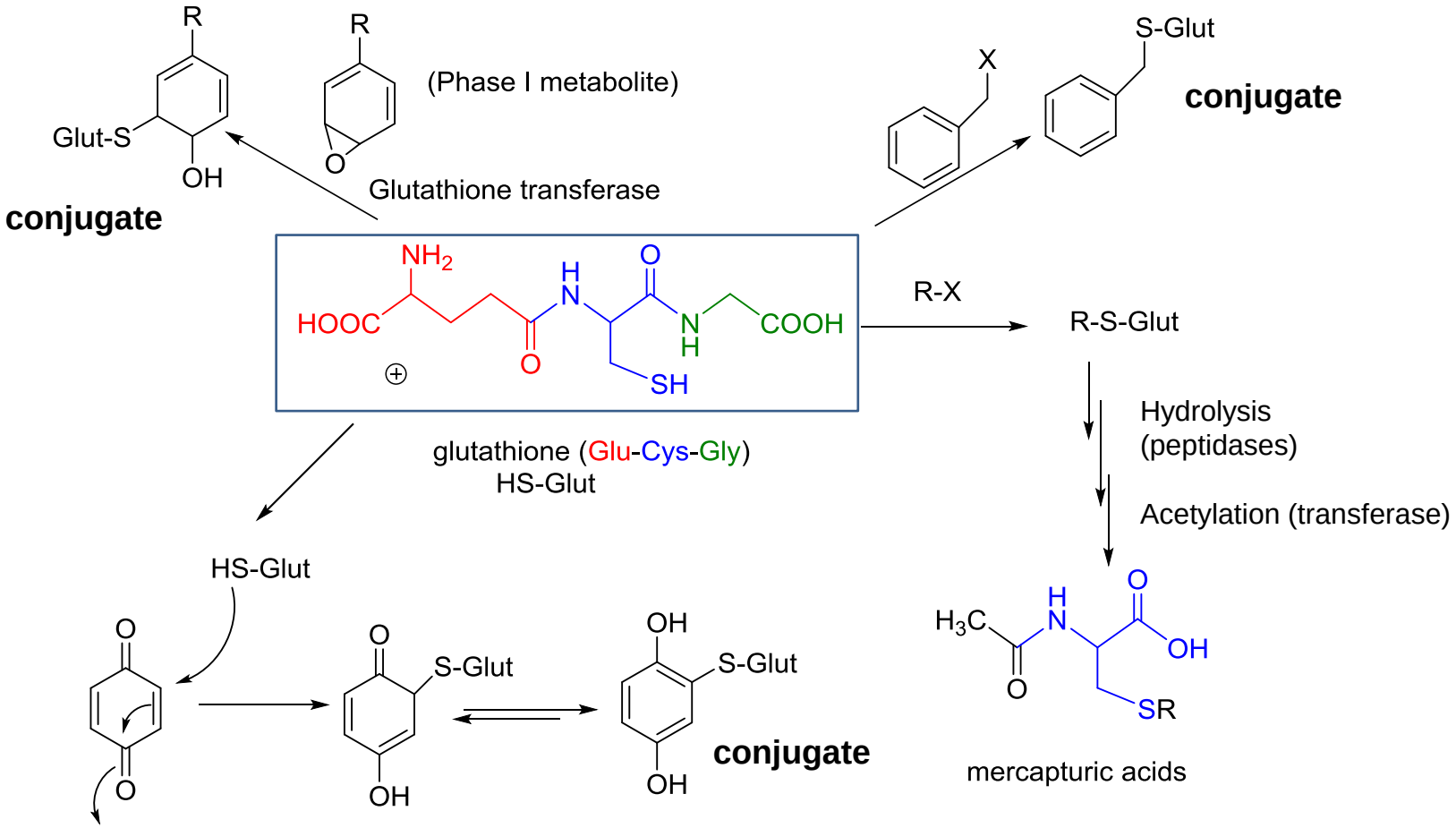


salbutamol



Ionized at physiological pH

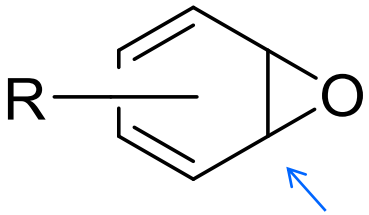
4.2.3. Conjugation with glutathione (electrophilic centres in drugs)



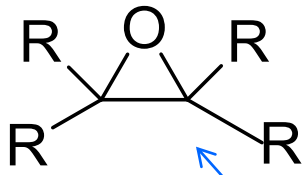
Electrophilic functional groups:
alkyl and benzyl halides, epoxides, quinones, α,β-unsaturated carbonyl compounds.

4.2.3. Conjugation with glutathione (electrophilic centres in drugs)

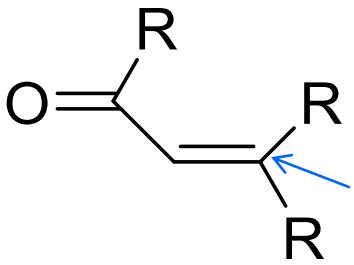
Propose the structure of the products that result from the reaction of Glut-SH with the following substrates:



HS-Glut

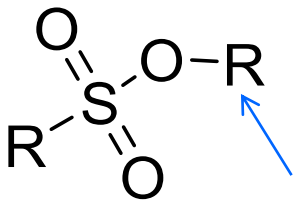


HS-Glut



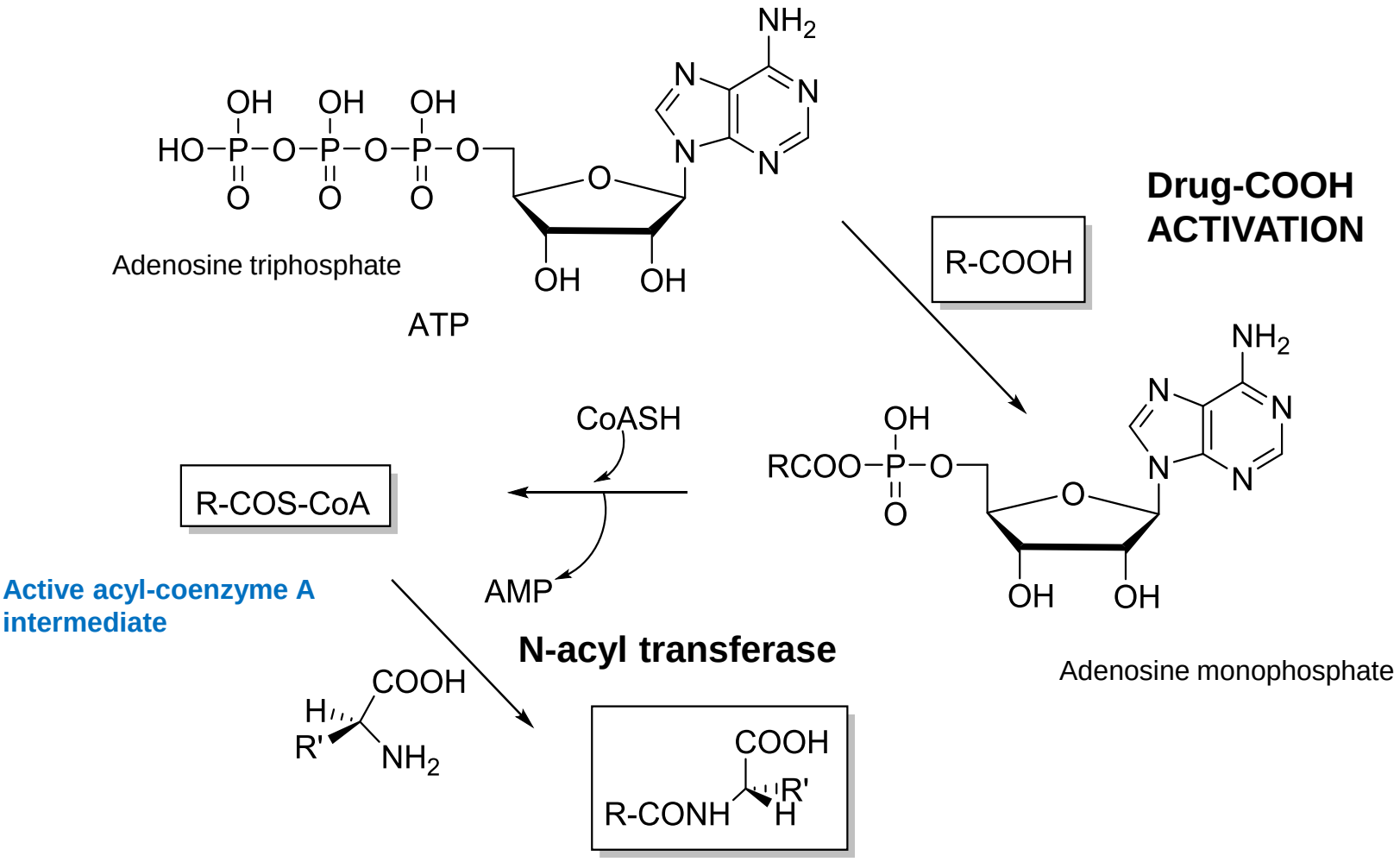
HS-Glut

(conjugate addition)



HS-Glut

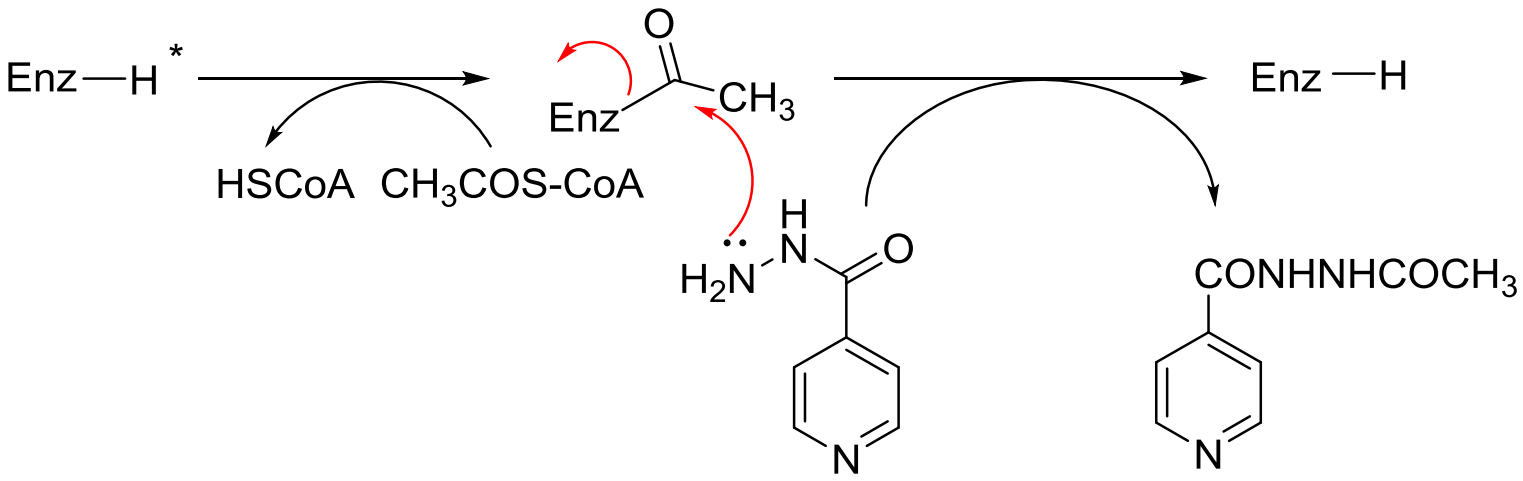
4.2.4. Conjugation with amino acids (aromatic and arylacetic acids)



4.2.5. Acylation (amines, sulphonamides, hydrazines and hydrazides)

Primary amines (RNH_2 or $ArNH_2$) —(N-Acyltransferases; **Acetyl**-SCoA)→ Acetamides (RNH**Ac** or ArNH**Ac**)

Hydrazines ($RNHNH_2$) —(N-Acyltransferases; **Acetyl**-SCoA)→ Hydrazides (RNHNH**Ac**)



* N-acetyl transferase

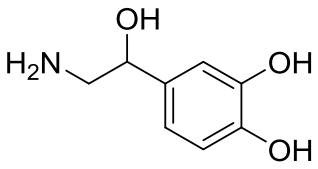
isoniazid

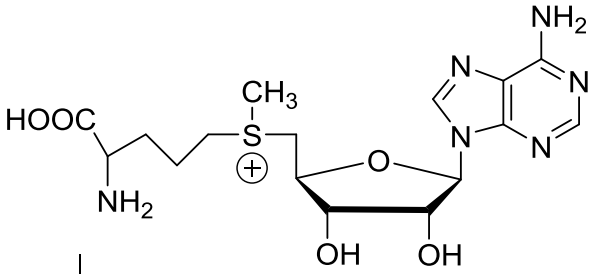
4.2.5. Methylation (amines, thiols, pyridines and phenol groups)

Ar – OH (Phenols) —Methyltransferase / S-Adenosylmethionine→ **Ar – O - Me**

R – SH (Thiols) —Methyltransferase / S-Adenosylmethionine→ **R – S - Me**

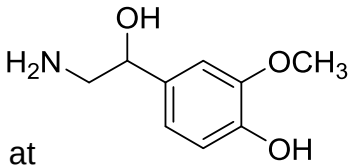
R_2N – H (Amines) —Methyltransferase / S-Adenosylmethionine→ **R_2N – Me**



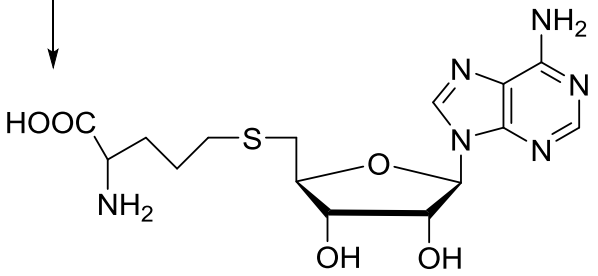


S-5'-adenosylmethionine (SAM) (coenzyme)

COMT
(catecholmethyltransferases)



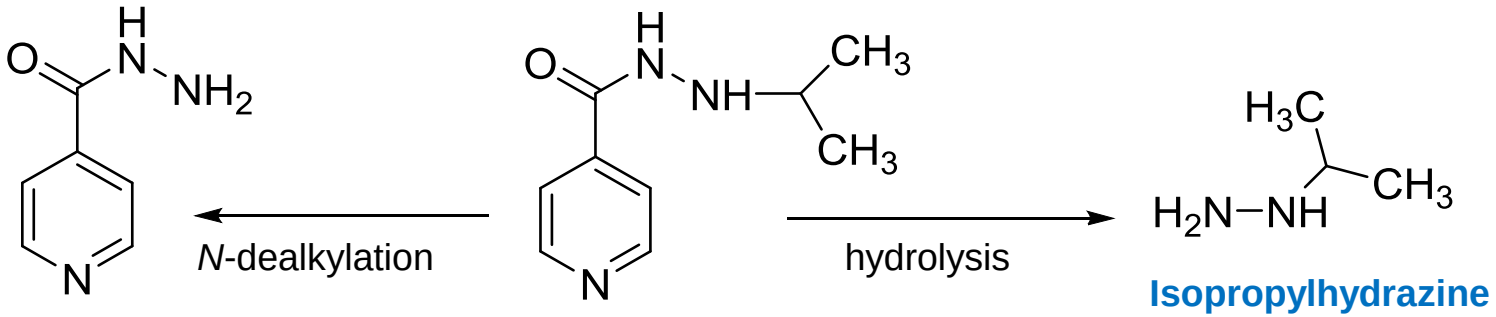
Methylated the hydroxyl group at *meta* position to the alkyl substituent



S-5'-adenosylhomocysteine

Consequences of drug metabolism

- Deactivation
  - Phenobarbital (aromatic hydroxylation)
  - Procaine (ester hydrolysis)
- Bioactivation (prodrugs)
  - Phenacetin (paracetamol)
  - Prontosil (sulfanilmide)
  - Imipramine (desipramine)
- Activity change (iproniazid)



isoniazid
(tuberculostatic)

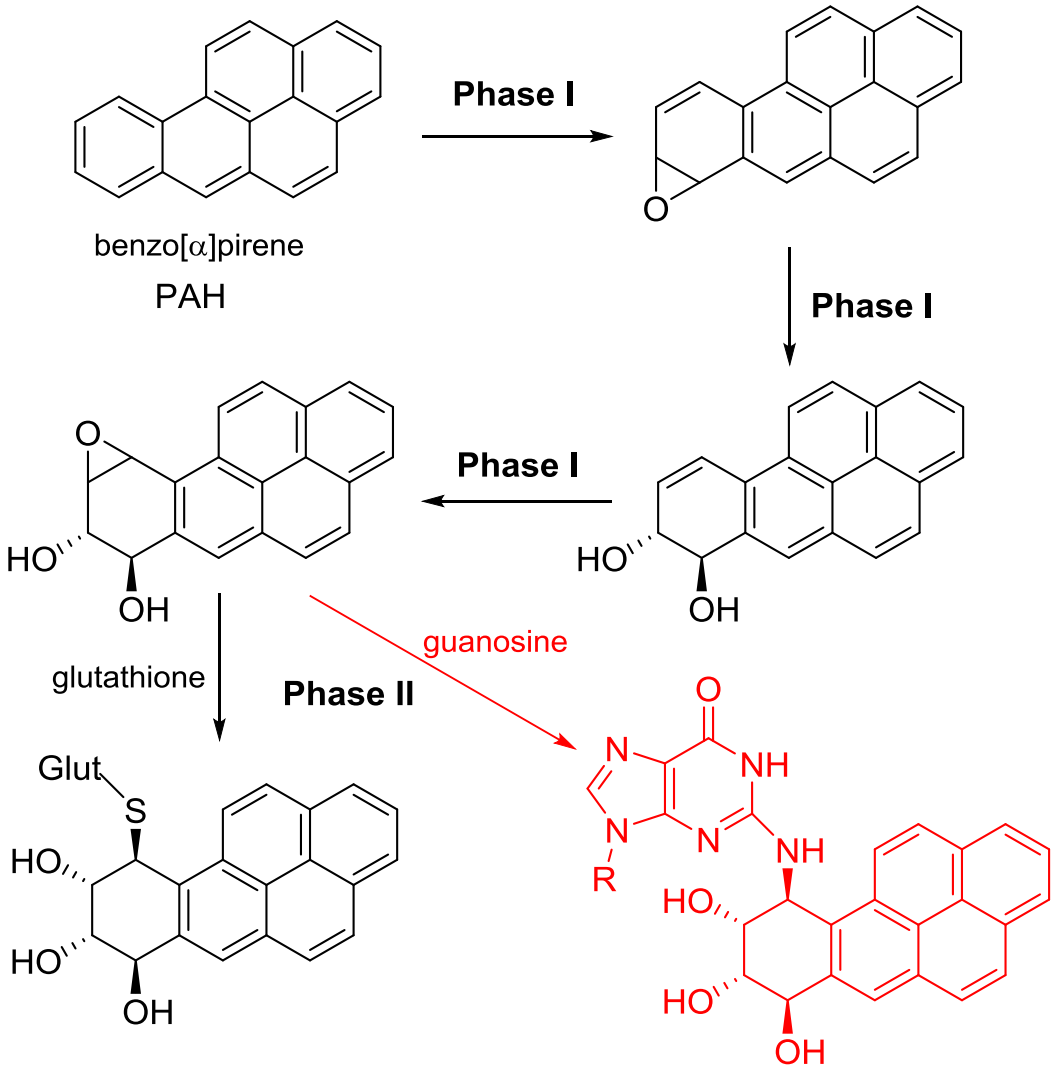
Iproniazid
(antidepressant)

Isopropylhydrazine

Consequences of drug metabolism

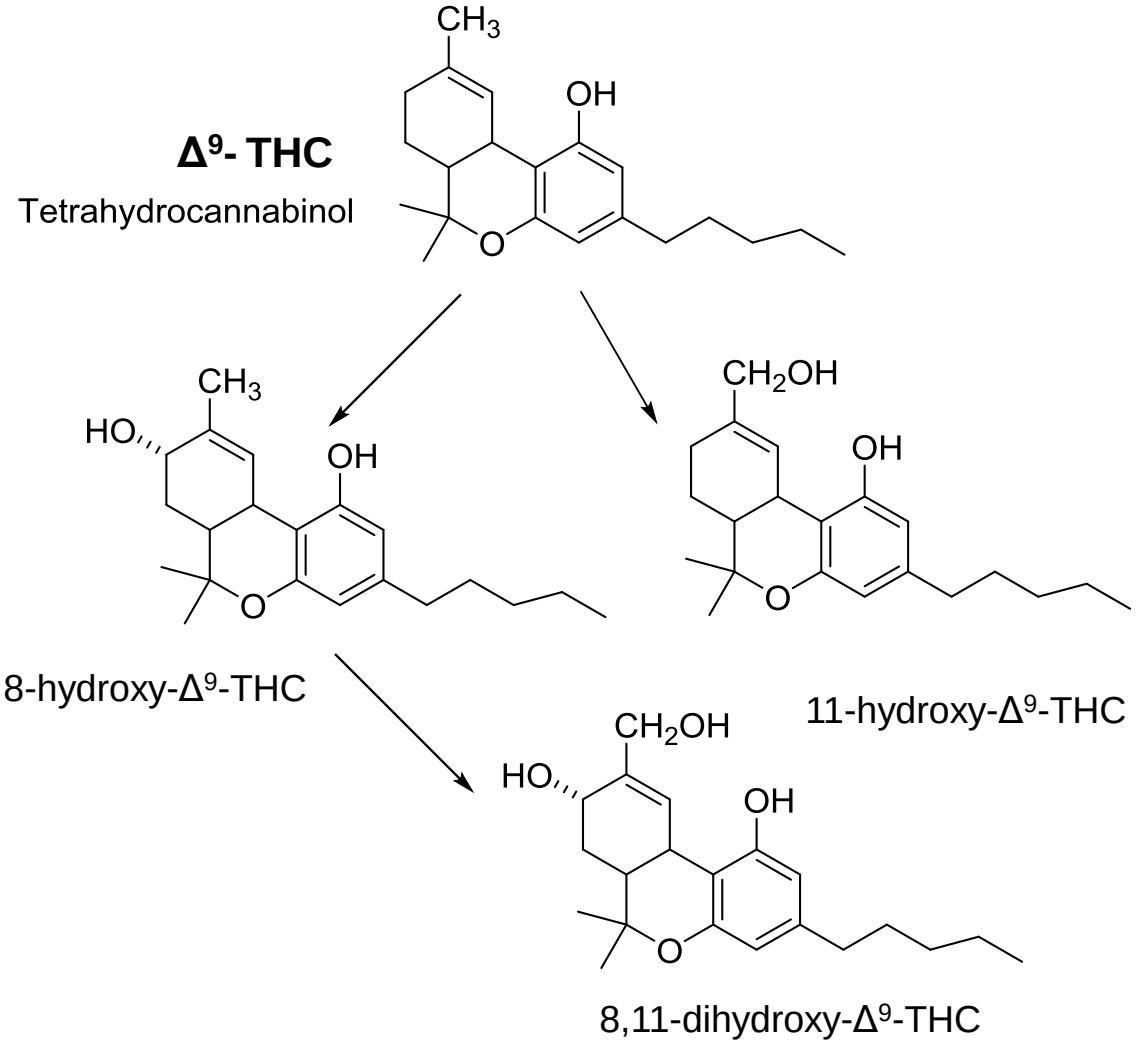
Formation of toxic metabolites

Polycyclic aromatic hydrocarbons (PHA): carcinogenic



Consequences of drug metabolism

Formation of toxic metabolites



More active in CNS

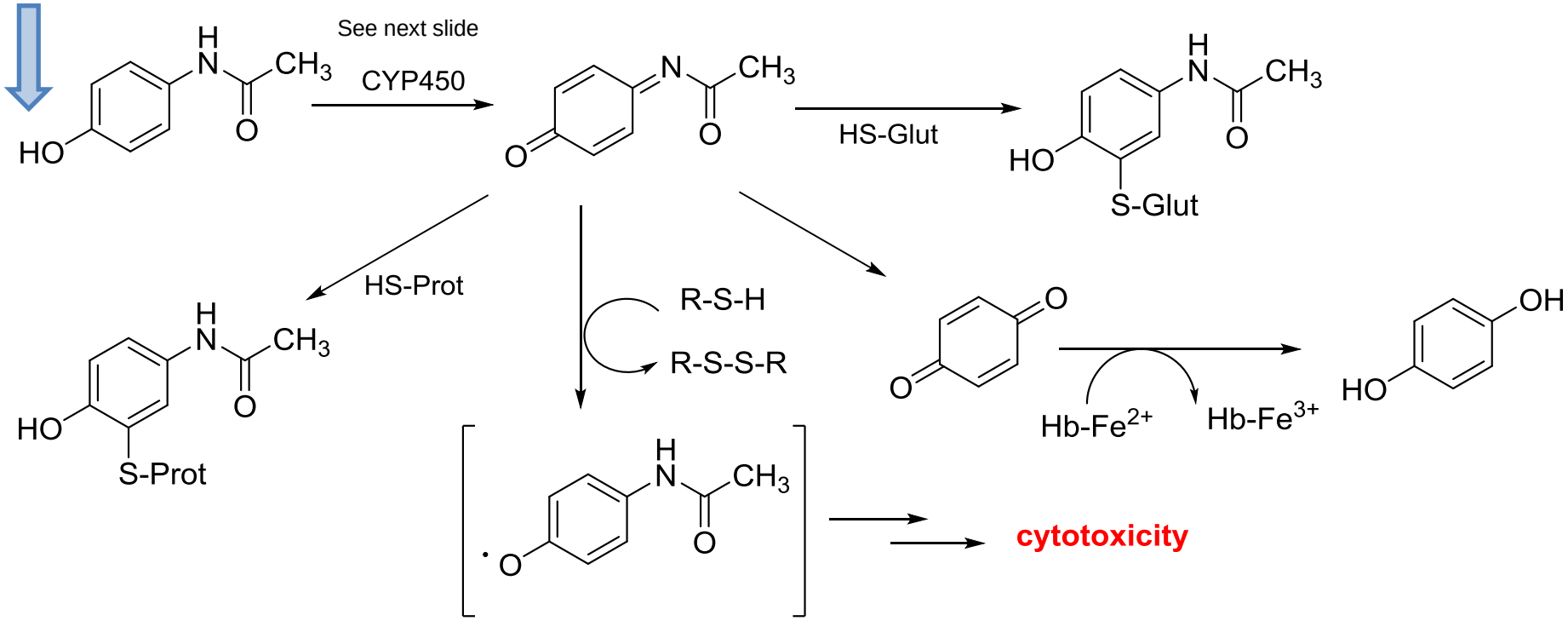
Consequences of drug metabolism

Formation of toxic metabolites

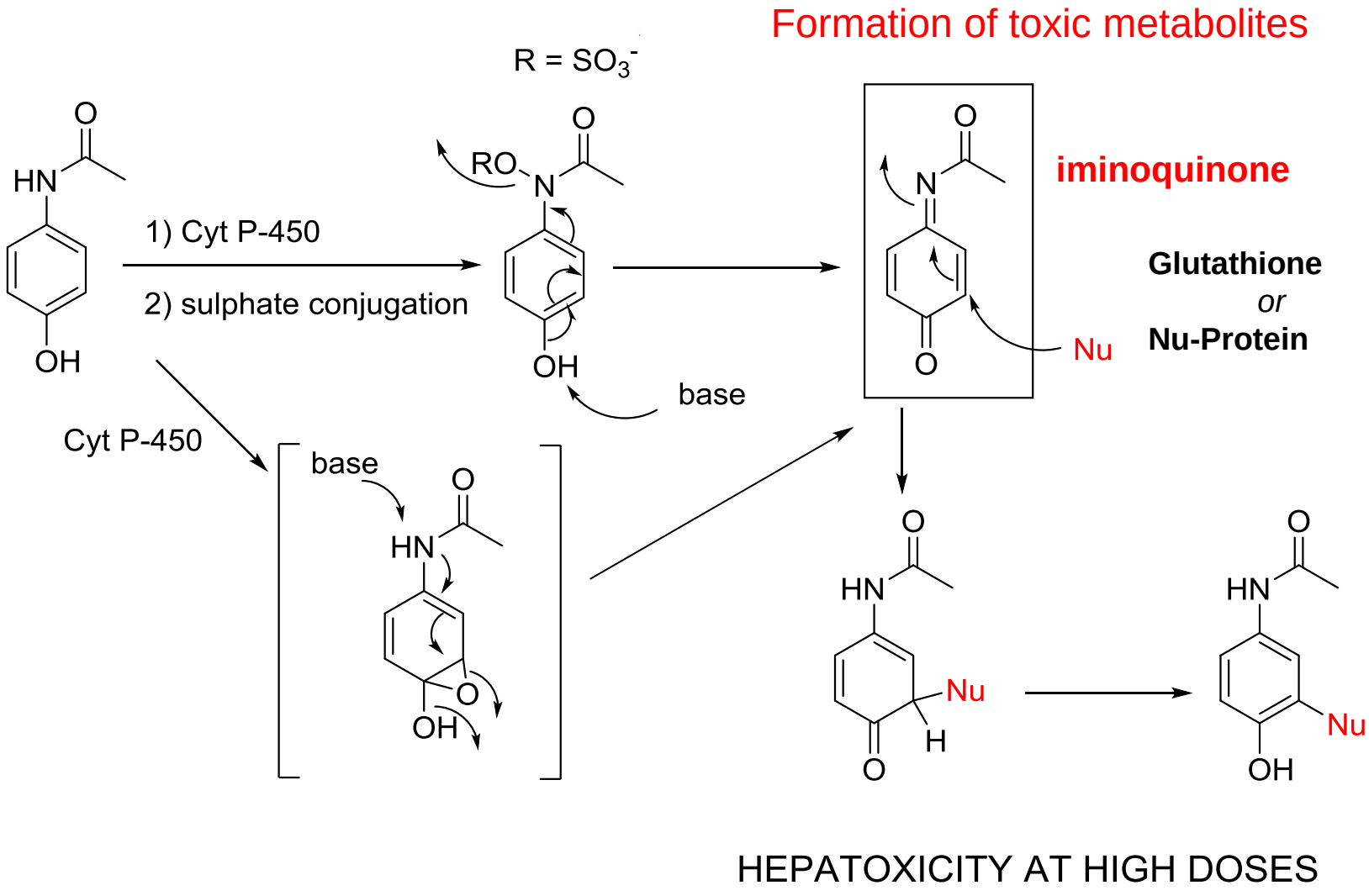
Paracetamol:

conjugates (sulphate and glucuronide, excretion, detoxification)

oxidation in liver: toxicity

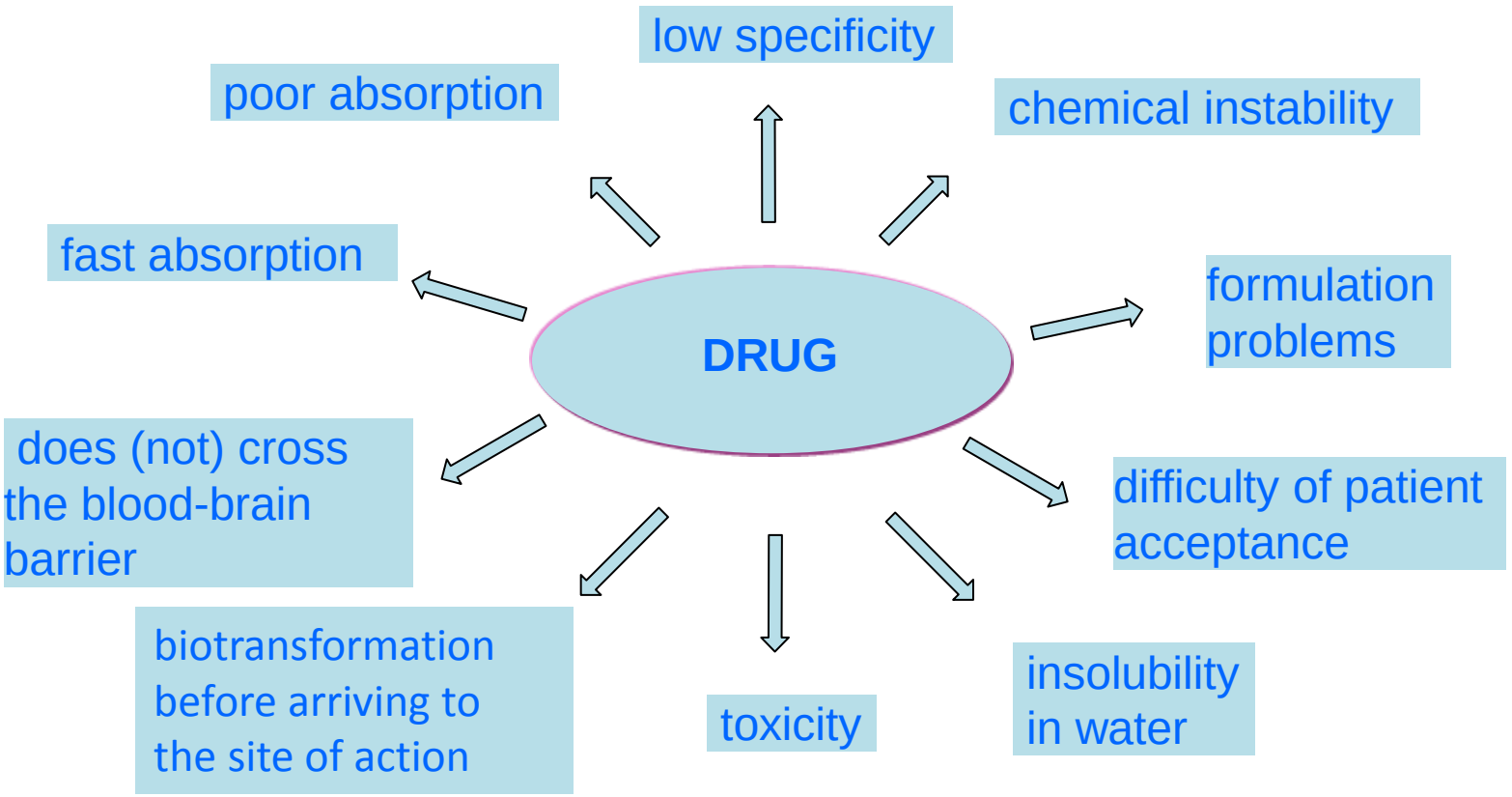


Consequences of drug metabolism



4.3. BIOREVERSIBLE DERIVATIVES

SEVERAL PROBLEMS CAN LIMIT THE EFFECTIVENESS OF A POTENTIAL DRUG
pharmacodynamics / pharmacokinetics / chemistry / galenics



4.3. BIOREVERSIBLE DERIVATIVES

SOLUTIONS TWO APPROACHES :

PHARMACOMODULATION: DESIGNING IMPROVED ACTIVE ANALOGUES: Structural changes are incorporated into active compounds in order to maintain activity and eliminate or decrease undesirable properties.

(*TO BE DEVELOPED IN UNIT 5*)

BIOREVERSIBLE DERIVATIVES:
Structural changes are intended to obtain INACTIVE derivatives which will be activated in our body (bioactivation) by means of a metabolic process.

Bioreversible derivative is synonymous with prodrug or latent drug.

Prodrug

"An inactive compound which is converted into an active compound in the body" (Albert 1951)

Prodrugs are masked forms of active drugs that are designed to become activated after an enzymatic or chemical reaction after they have been administered in the body. There are two types of prodrugs:

- **carrier-linked prodrugs**
- **bioprecursor prodrugs**

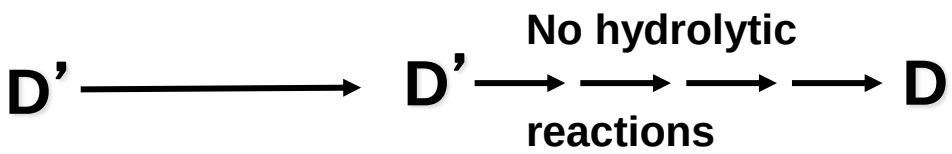
4.3. BIOREVERSIBLE DERIVATIVES

A **carrier-linked prodrug** is a prodrug that contains a temporary linkage of a given active substance with a transient carrier group that can be easily removed *in vivo*, usually by a hydrolytic reaction.

A **bioprecursor prodrug** is a prodrug that results from a molecular modification of the active principle generating a new compound that is able to be transformed metabolically or chemically. The resulting compound is the active principle. Modification does not involve linkage to a carrier group.

CARRIER-LINKED PRODRUGS

BIOPRECURSOR PRODRUGS



D = active drug
D - C = carrier-linked prodrug, inactive
D' = inactive derivative

4.3. BIOREVERSIBLE DERIVATIVES

Carrier-linked prodrugs

DRUG AND CARRIER

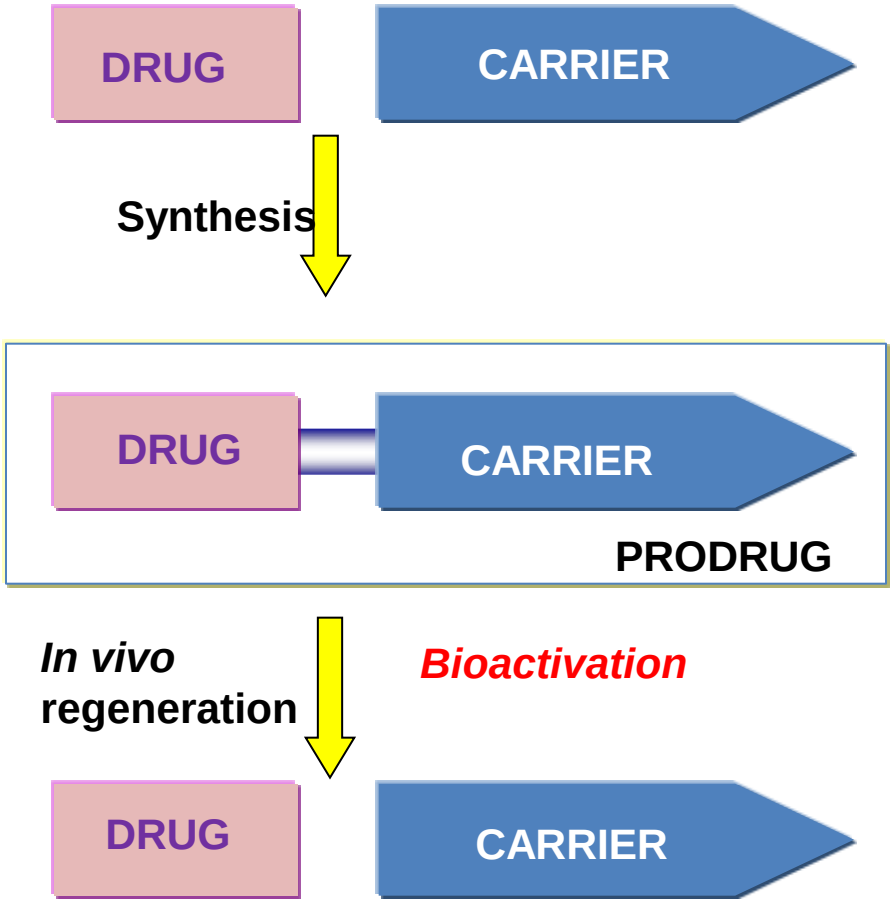
1) Union by covalent bond (non-ionic).

2) The prodrug must be inactive or much less active than the drug.

3) The bond must be broken *in vivo.*

4) The prodrug and the carrier must be non-toxic.

5) The regeneration of the drug in the target must be fast so as to avoid alternative metabolism.



When, because of steric hindrance or functional properties, the desired prodrug carrier cannot be attached directly to the parent active molecule, conventional carrier-linked prodrugs can have a synthetic handle (a spacer or linker) between the active drug and the carrier.

CARRIER-LINKED PRODRUGS

- A labile additional fragment is temporarily attached to the drug.
- Prodrugs facilitate drug localization/distribution.
- Alteration of lipophilicity in relation to the active drug.
- Metabolic activation is done by (chemical or enzymatic) hydrolysis.

BIOPRECURSOR PRODRUGS

- There is no additional labile fragment.
- There are no major differences in lipophilicity between the bioprecursor prodrug and the active drug.
- Activation occurs primarily by redox processes (phase I metabolic reactions).

4.3. BIOREVERSIBLE DERIVATIVES

Are used

- to improve membrane permeability
- to prolong activity
- to mask toxicity and side effects
- to vary water solubility
- to target drugs
- to improve chemical stability

(see examples in Patrick's 5th ed. Chapter 14)

Prodrugs used to improve membrane permeability

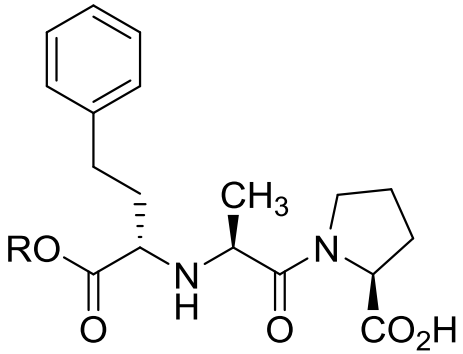
Esters

- Are used to mask polar carboxylic acids, alcohols or phenols.
- Are hydrolysed by esterases (different esterases in different organs or tissues).
- Are used when a carboxylic acid, alcohol or phenol is required for target binding.
- The leaving group (alcohol or carboxylic acid) should ideally be non-toxic.

Example

enalapril (R = Et)
enalaprilate (R = H)

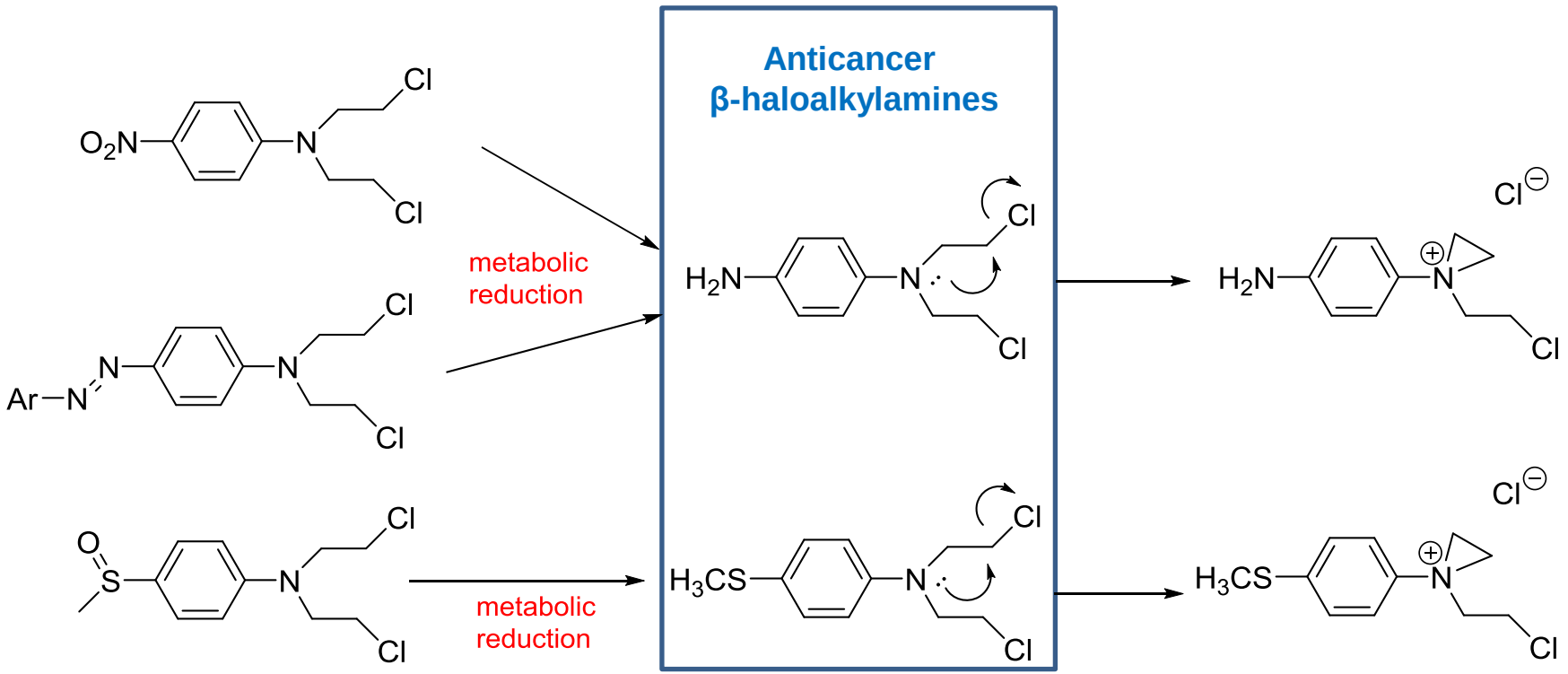
(antihypertensive)



4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs used to target drugs

These *para* groups withdraw electron density from the nitrogen atom.

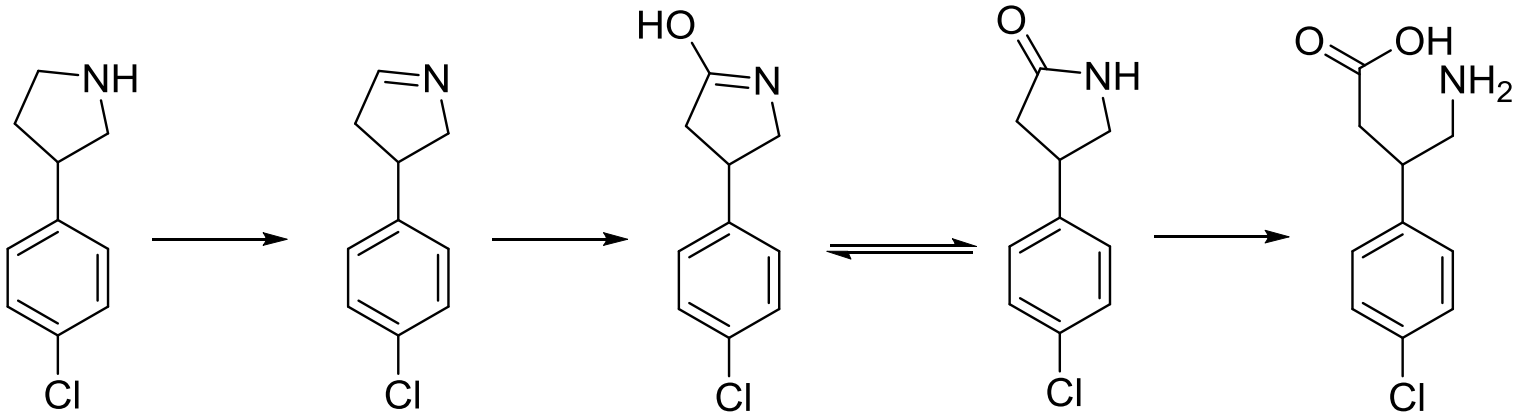


INACTIVE

the active compound is the aziridinium salt

4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs used to target drugs



Precursor that
can cross the brain
membrane

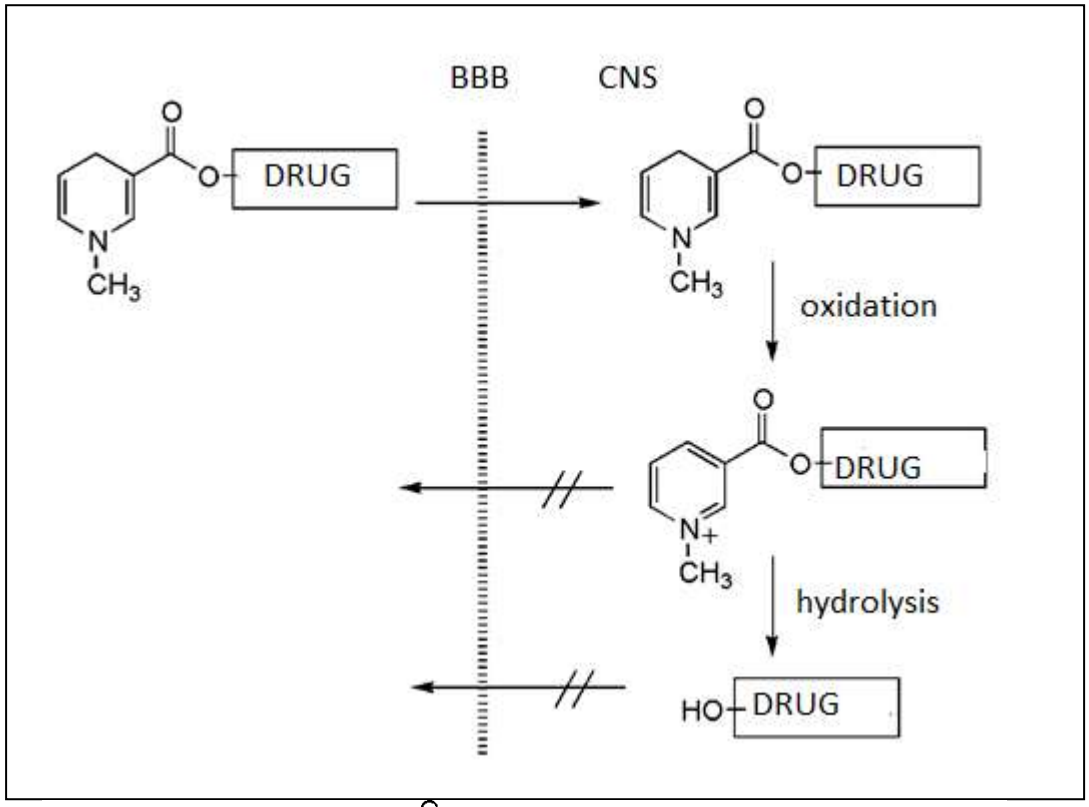
baclofen

Active on the CNS
(central nervous
system)

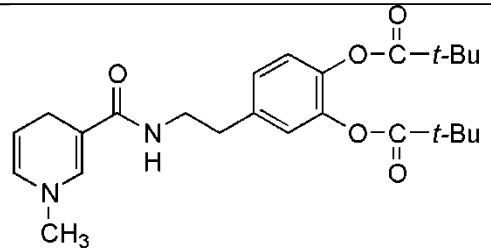
4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs used to target drugs

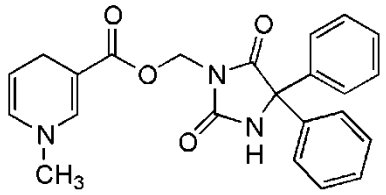
«mixed» prodrugs for selective distribution in the CNS



BBB = blood-brain barrier
CNS = central nervous system



dopamine



phenytoin

"mixed" prodrugs

4.3. BIOREVERSIBLE DERIVATIVES

Types of carrier-linked prodrugs by functional group linked to the carrier

- **From carboxylic acid**
  - Esters
  - Acyloxymethyl esters
  - Acyloxymethyl carbonates
  - Cyclic carbonates
- **From amino and amido**
  - Amide
  - Phenol carbamates
  - N- Acyloxymethyl carbamates
  - Mannich Base derivatives
  - Imine/Enamine
- **From ketones**
  - Acetals
  - Oxazolidines/Thiazolidines
  - O-Carboxymethyl oximes
- **From hydroxyl group (alcohol and phenol)**
  - Esters
  - Ethers
  - Carbamates
  - Acyloxymethyl ethers
- **From thiol (thiol and thiophenols)**
  - Thioesters
  - Acyloxymethyl thioesters
  - Disulfides

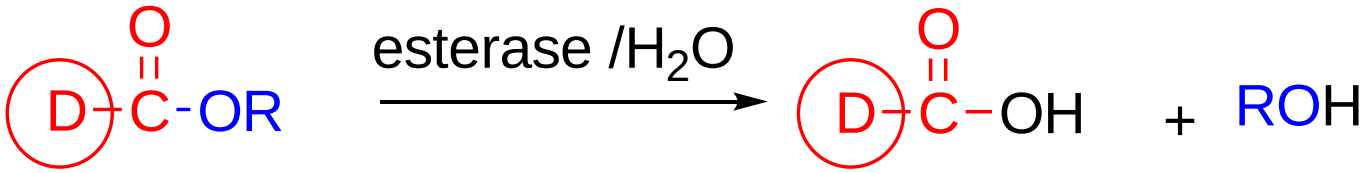
(Avendaño)

4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs from carboxylic acids

ESTERS

- Bioactivation is by esterases: there is a wide distribution in the body.
- They can be also hydrolysed by enzymes of bacterial microflora.
- Esters may be prepared with different degrees of lipophilicity/hydrophilicity.
- The hydrolysis rate can be controlled by choosing carriers with suitable stereoelectronic properties.



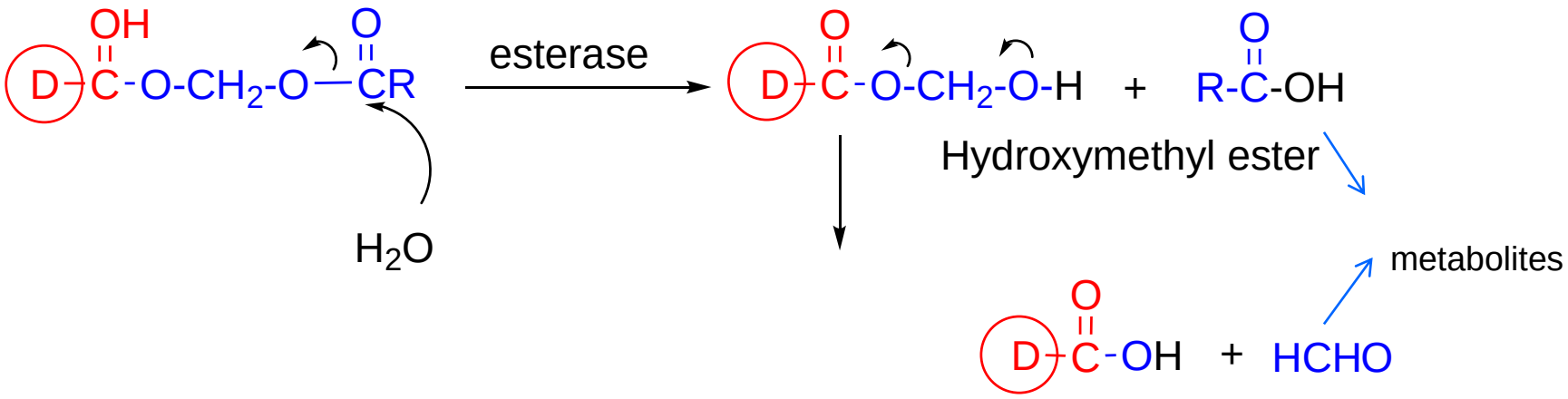
Some esters are too stable *in vivo*.
For steric reasons, the carbonyl group may find it difficult to access the active centre in some esterases: spacers are often used between the drug and the carrier.

Remember the methods you know for synthesising esters.

Prodrugs from carboxylic acids

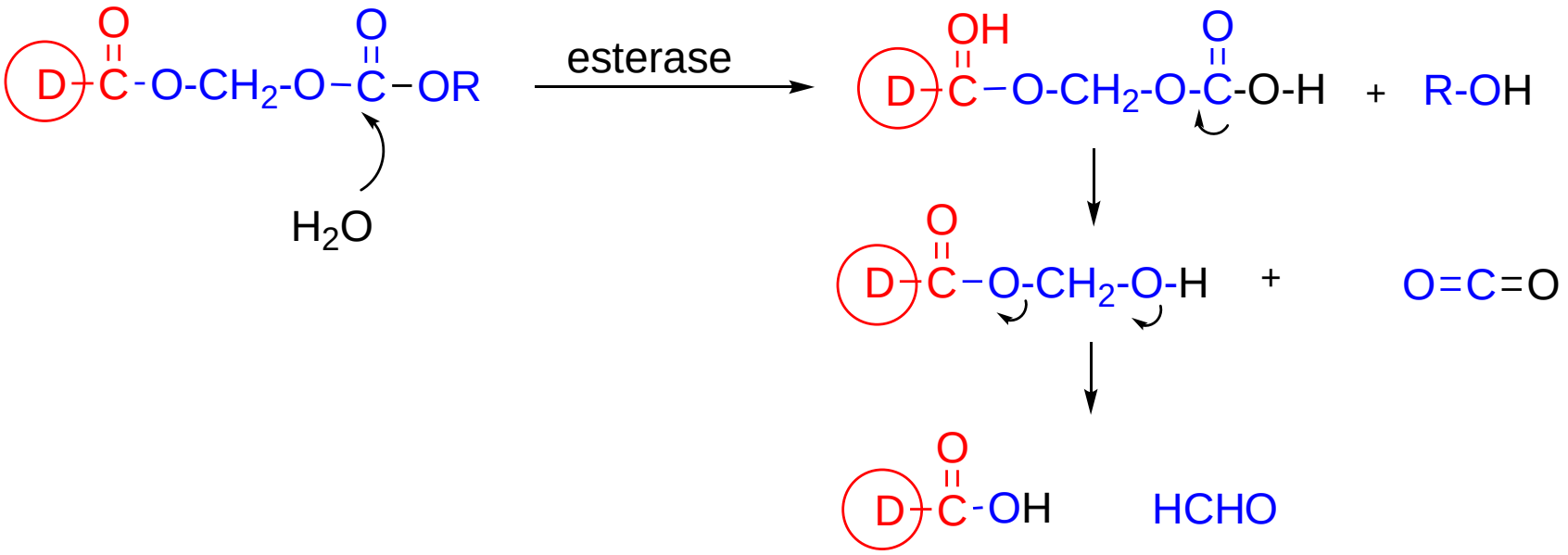
ACYLOXYMETHYL ESTERS

Bioactivation: the external ester group gains easier access to the esterase active site:



Prodrugs from carboxylic acids

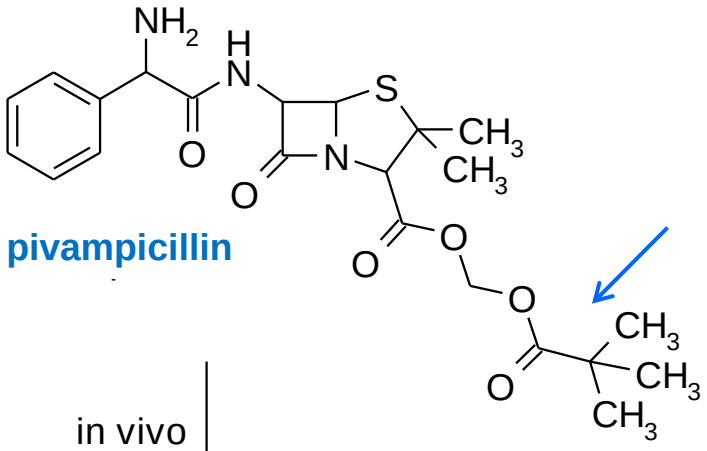
ACYLOXYMETHYL CARBONATES



4.3. BIOREVERSIBLE DERIVATIVES

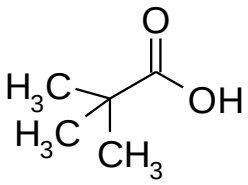
Prodrugs from carboxylic acids

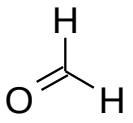
Example: ampicillin prodrug derivatives

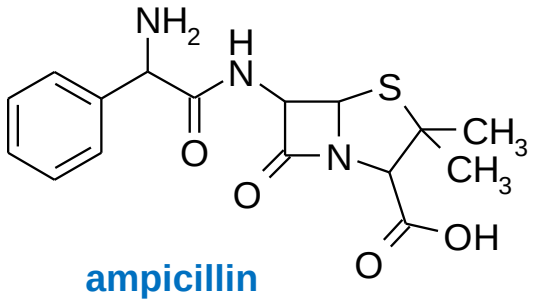


pivampicillin

in vivo

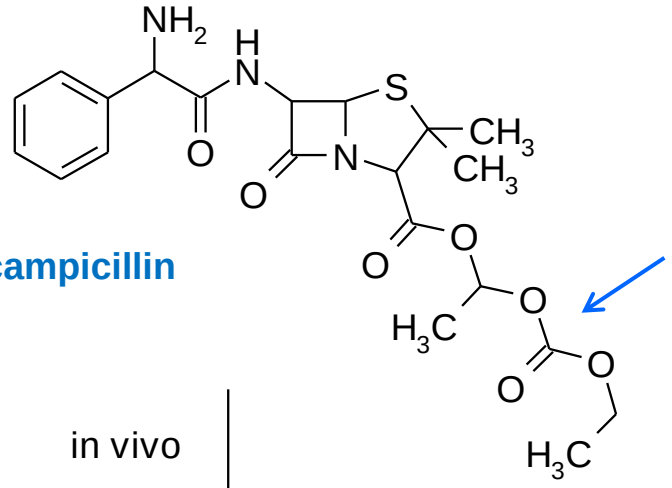






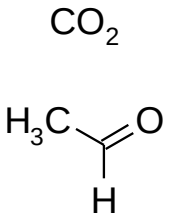
ampicillin

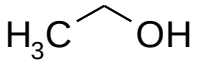
Broad spectrum antibiotic



bacampicillin

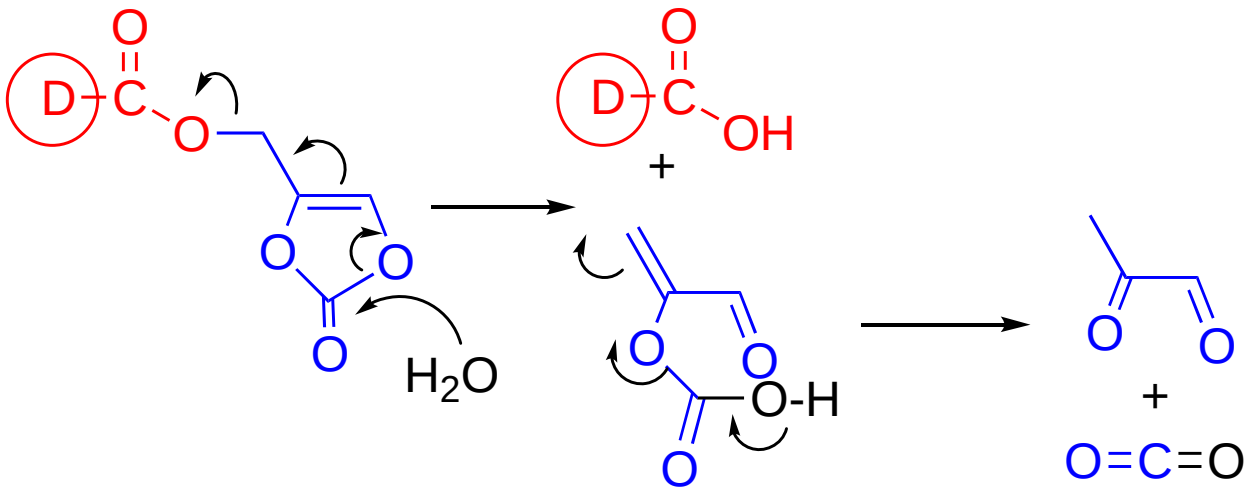
in vivo





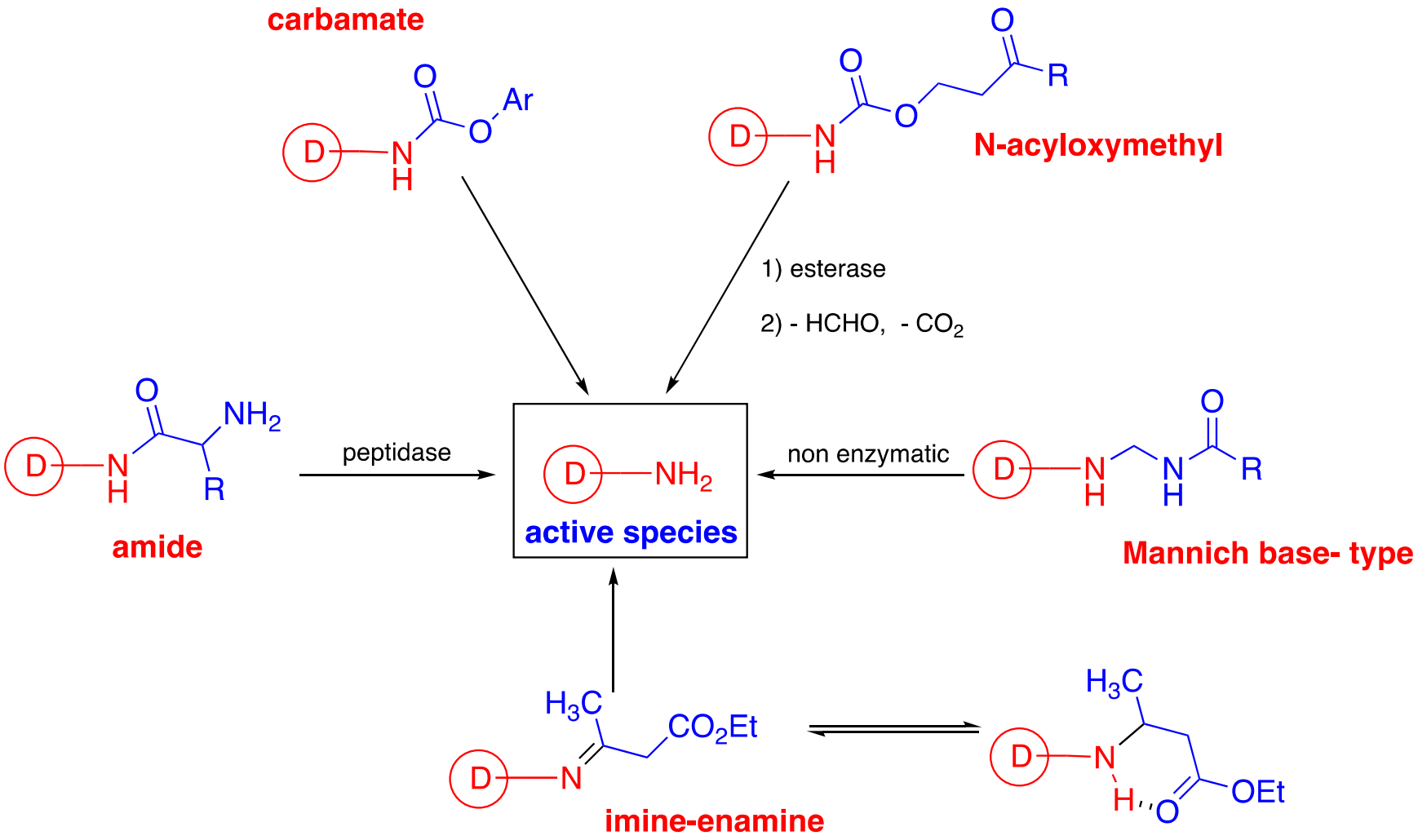
Prodrugs from carboxylic acids

CYCLIC CARBONATES



4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs from amine groups

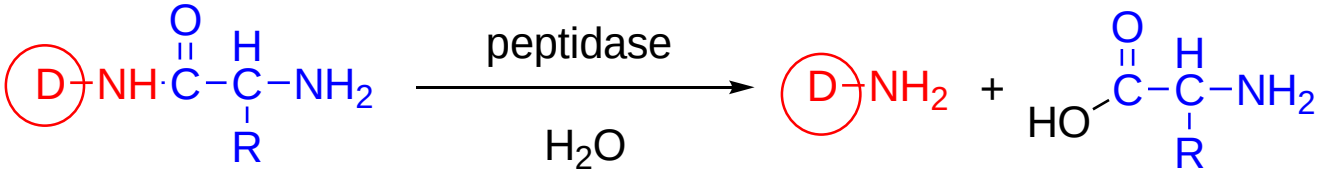


4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs from amine groups

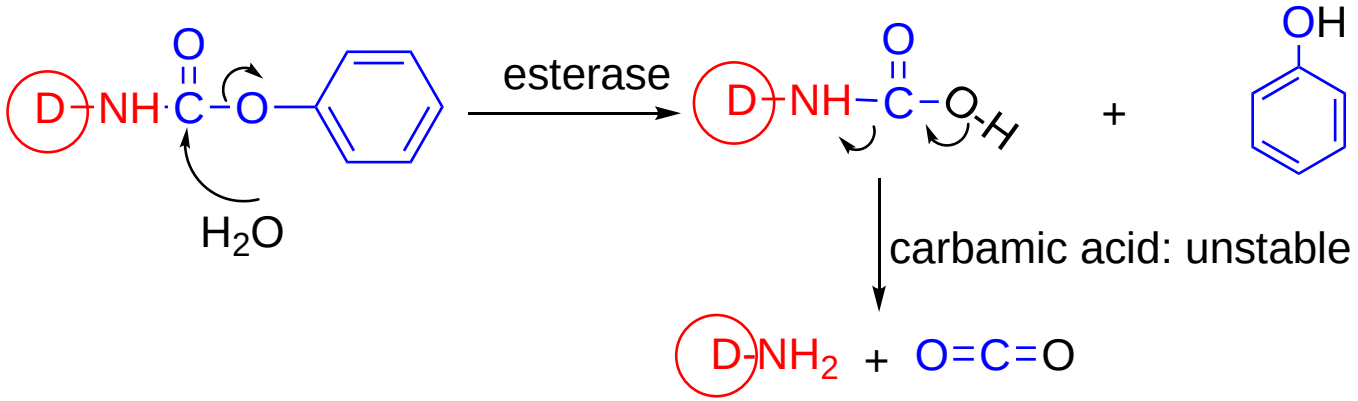
AMIDES

Amides are difficult to hydrolyse except if the carboxylic part is one amino acid (in such case, the amides can be degraded by peptidases):



PHENOL CARBAMATES

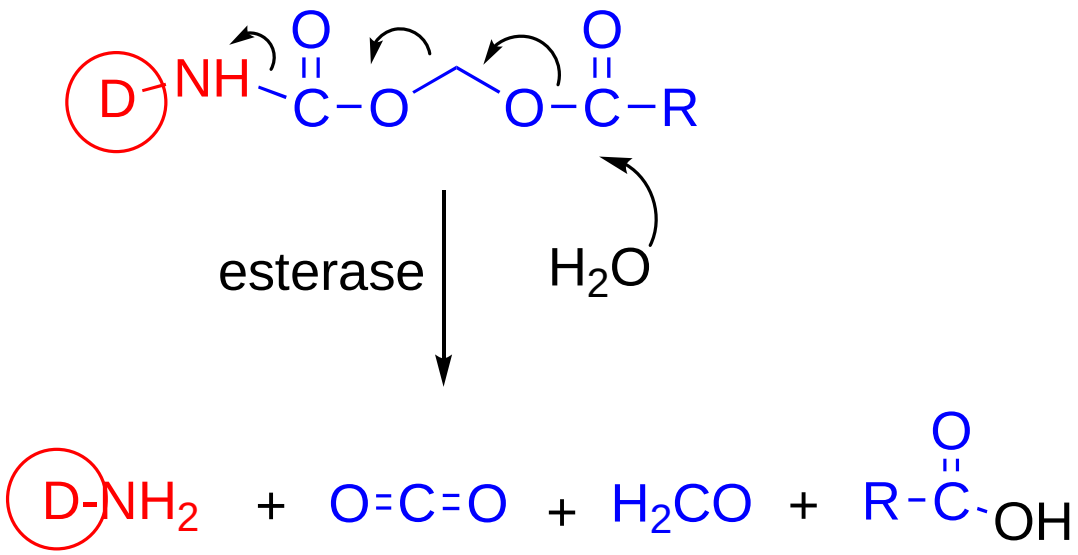
Carbamates are generally quite stable but phenol carbamates ($RNHCO_2Ph$) are easily hydrolysed by plasmatic esterases:



4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs from amine groups

N-ACYLOXYMETHYL CARBAMATES



4.3. BIOREVERSIBLE DERIVATIVES

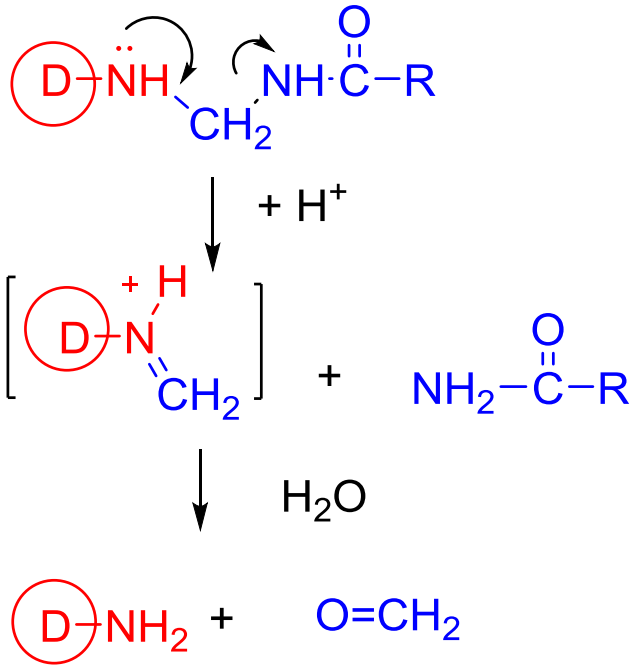
Prodrugs from amine groups

MANNICH BASES

These are obtained by the reaction of a drug (amine), formaldehyde and an amide.

The nitrogen basicity decreases in 2-3 units and the N atom is not protonated at physiological pH. Consequently, lipophilicity increases.

Bioactivation:

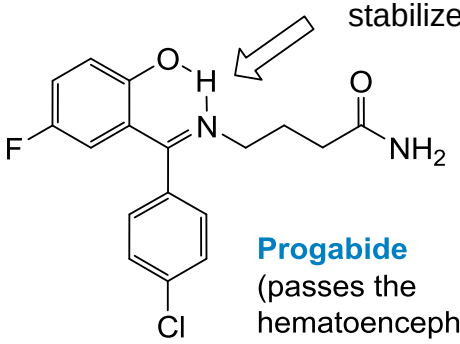


4.3. BIOREVERSIBLE DERIVATIVES

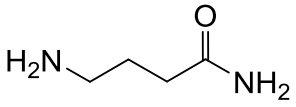
Prodrugs from amine groups

stabilized imines

Hydrogen bond that stabilizes the imine

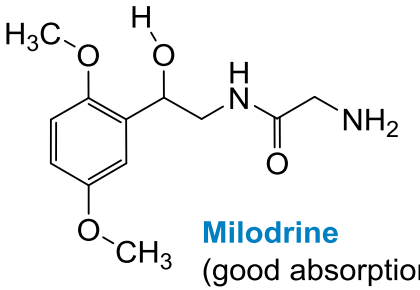


Progabide
(passes the hematoencephalic barrier)

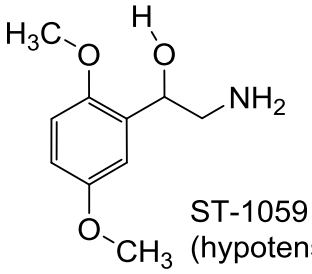


Gabamide
(inhibits convulsions)

amides



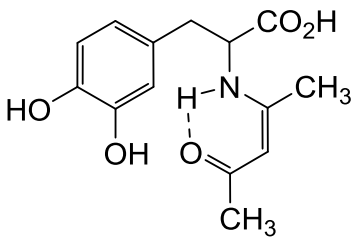
Milodrine
(good absorption)



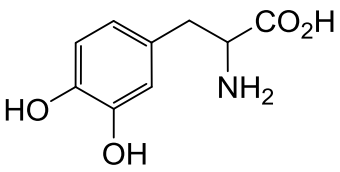
ST-1059
(hypotensive)

Glycine amide derivative
Easy to hydrolyse by peptidases

enamines



Dopa enamine



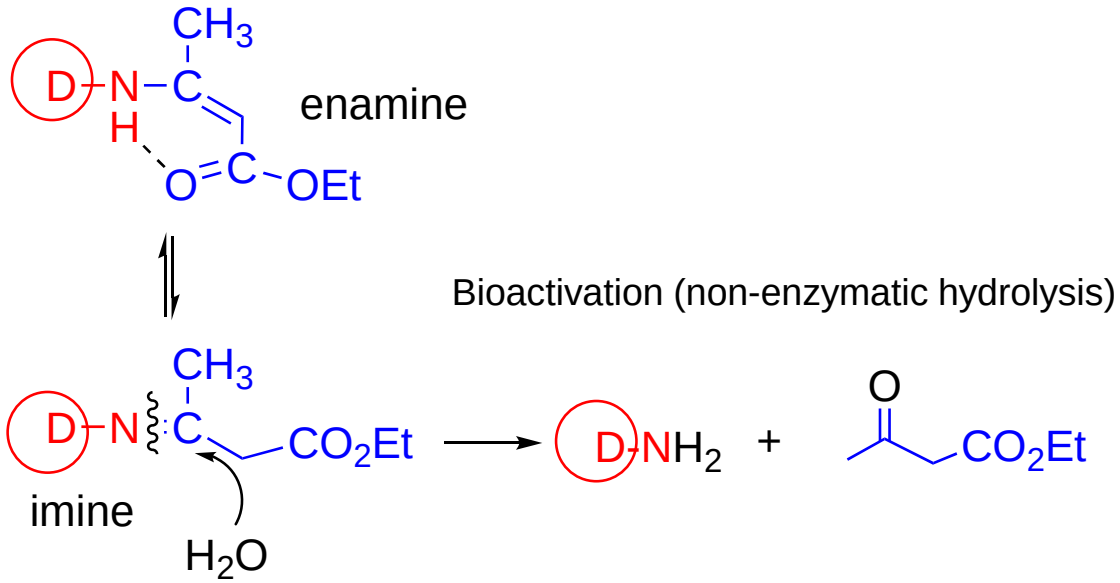
DOPA
(anti parkinsonian)

4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs from amine groups

IMINES : ENAMINES

Those derived from β-dicarbonyl compounds are stabilized by intra-molecular hydrogen bond.

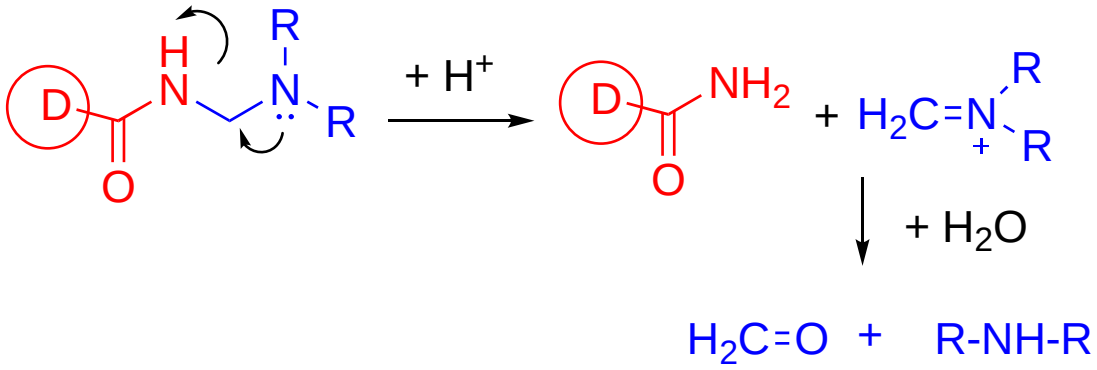


4.3. BIOREVERSIBLE DERIVATIVES

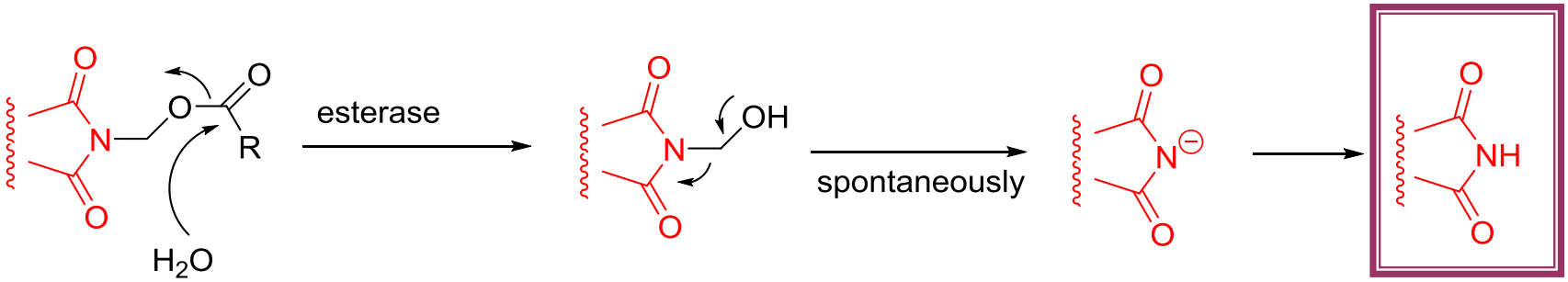
Prodrugs from amide and imide groups

AMIDES: *MANNICH-BASE PRODRUGS*

FORMATION: drug + formaldehyde + secondary amine.
BIOACTIVATION: does not need enzymatic hydrolysis (very unstable).

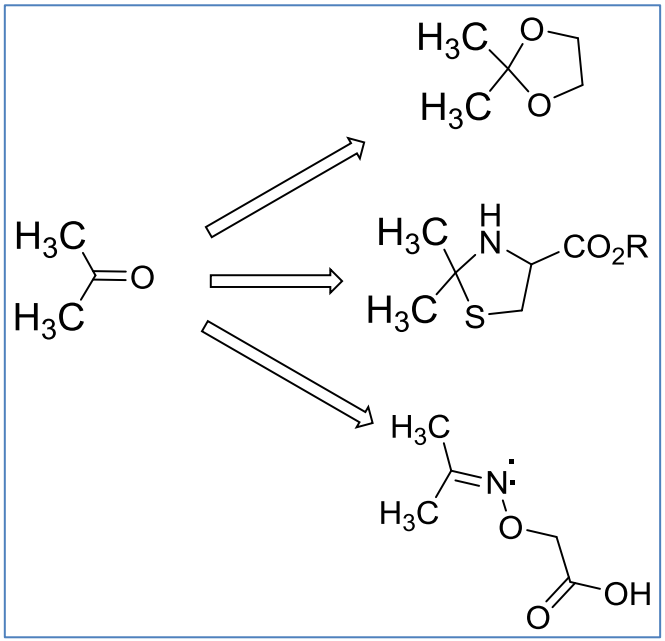


IMIDES: *ACYLOXYMETHYL DERIVATIVES*

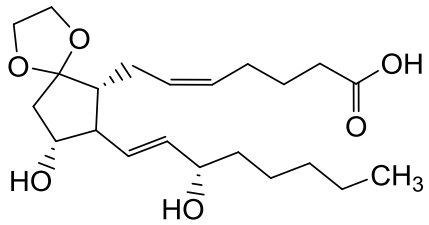


4.3. BIOREVERSIBLE DERIVATIVES

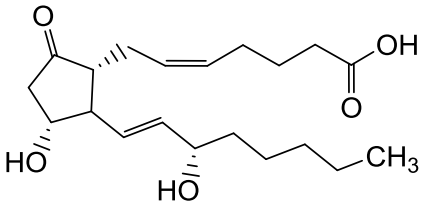
Prodrugs from carbonyl groups



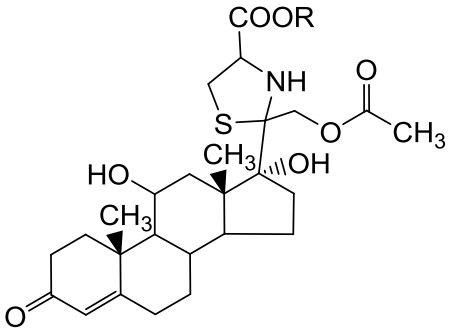
Acetals
Thiazolidines/ oxazolidines
O-carboxymethyl oximes



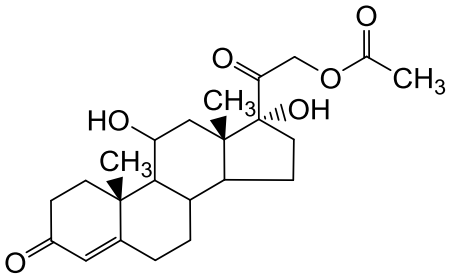
Stable derivative in solid state



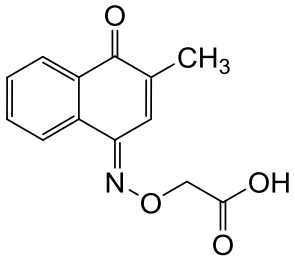
PGE2



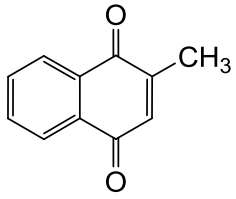
Prodrug for topical use
(accumulates in the skin)



hydrocortisone acetate



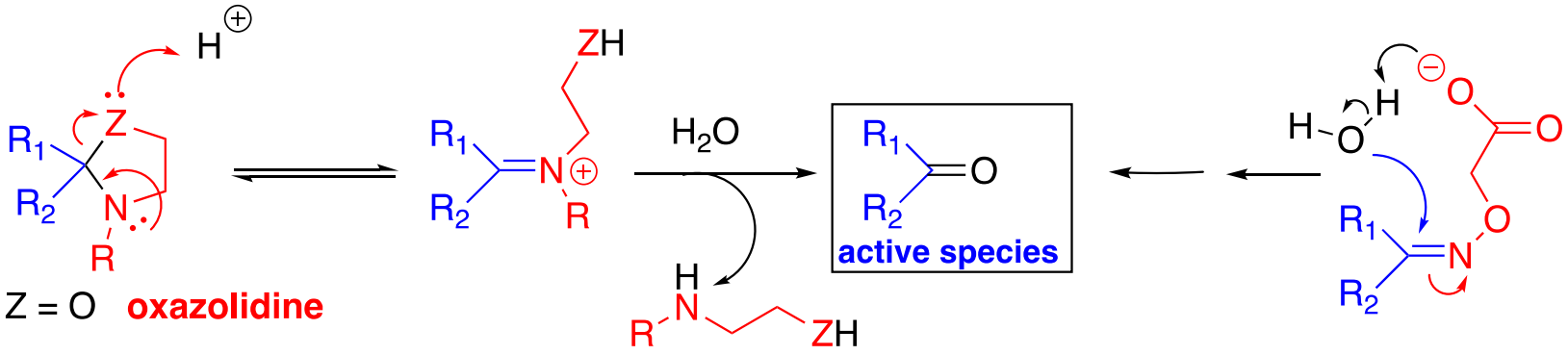
menadoxime
water soluble



menadione
(antihaemorrhagic)

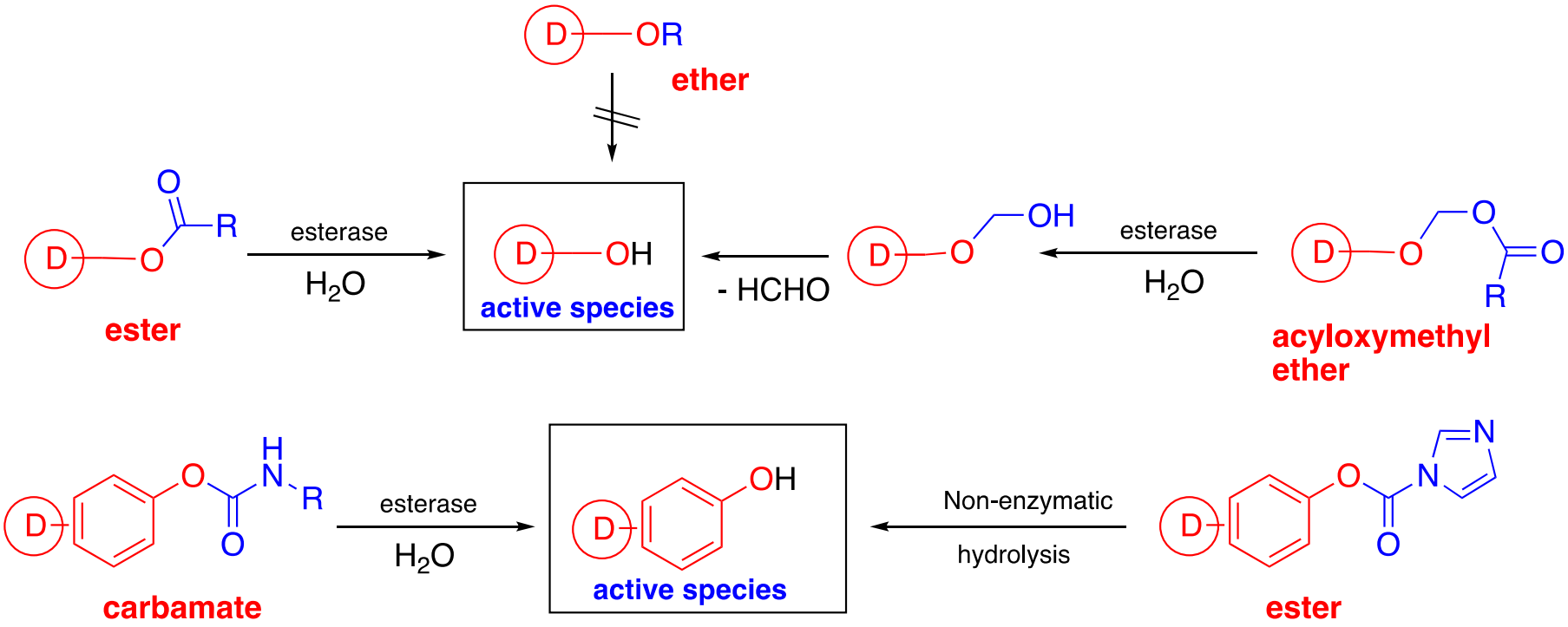
Prodrugs from carbonyl groups

Bioactivation



4.3. BIOREVERSIBLE DERIVATIVES

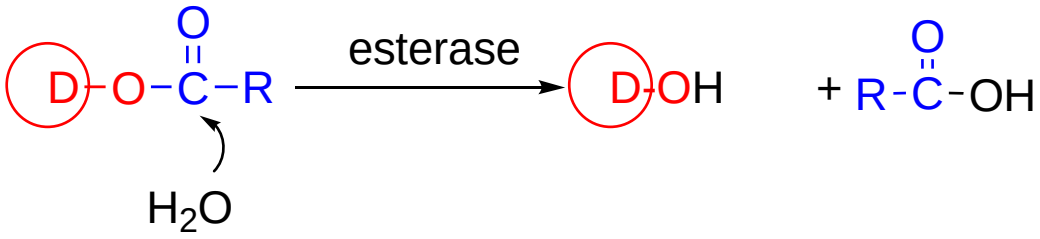
Prodrugs from alcohols and phenols



4.3. BIOREVERSIBLE DERIVATIVES

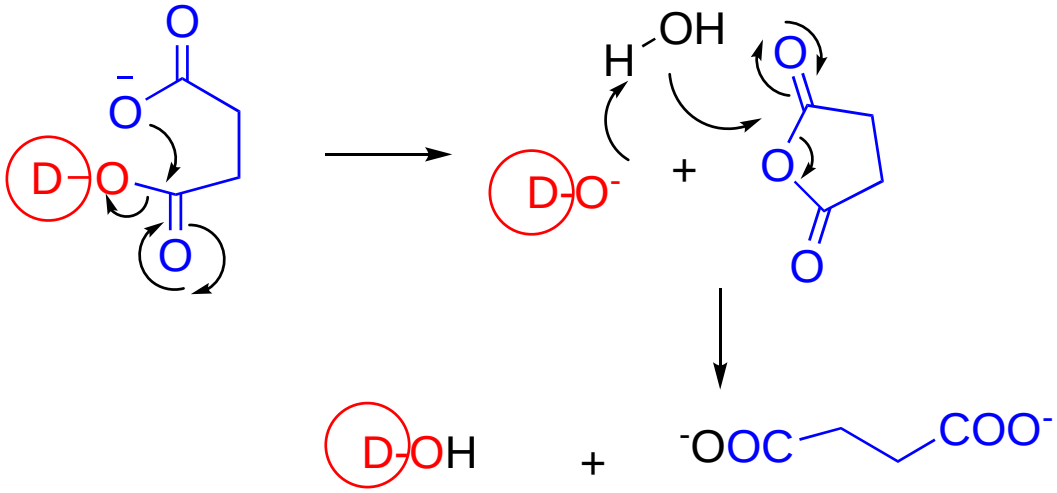
Prodrugs from alcohols and phenols

ESTERS



EXCEPTION: SUCCINATE HEMI-ESTERS

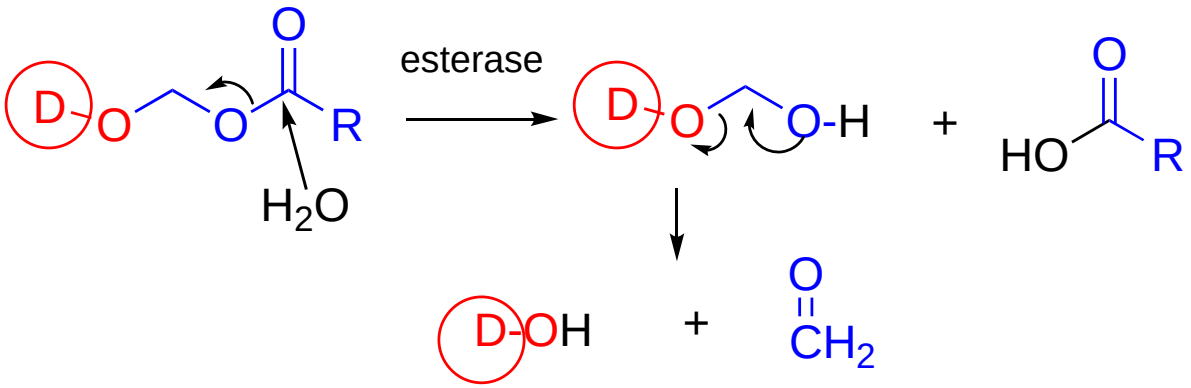
Hydrolysis does not require esterases because it is facilitated by intra-molecular attack from the free carboxylate group:



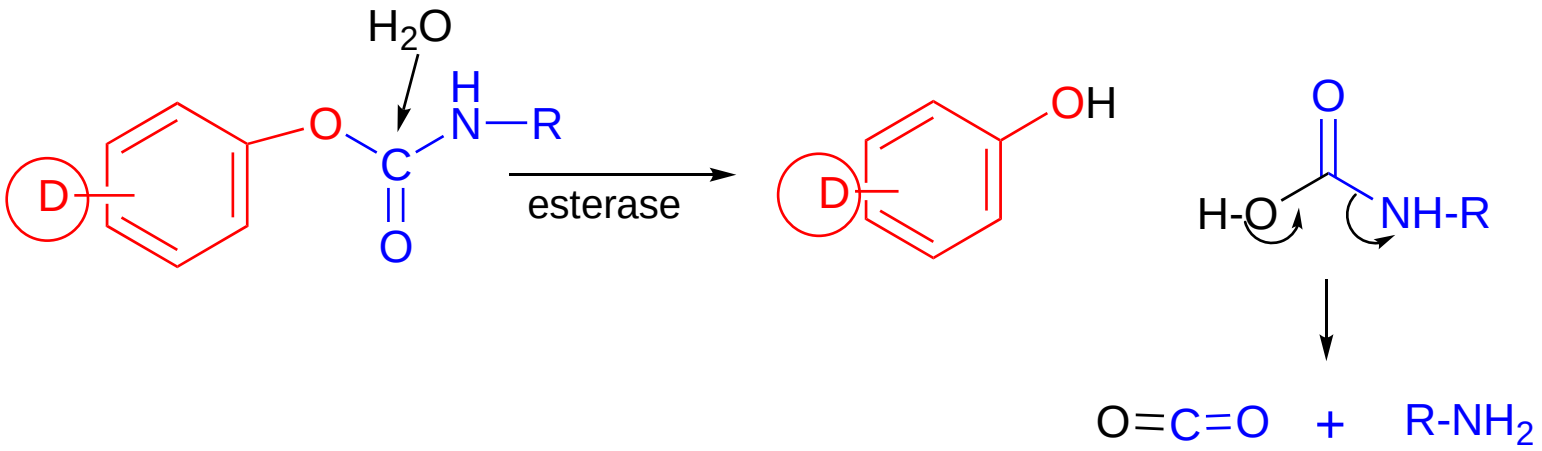
4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs from alcohols and phenols

ACYLOXYMETHYL ETHERS



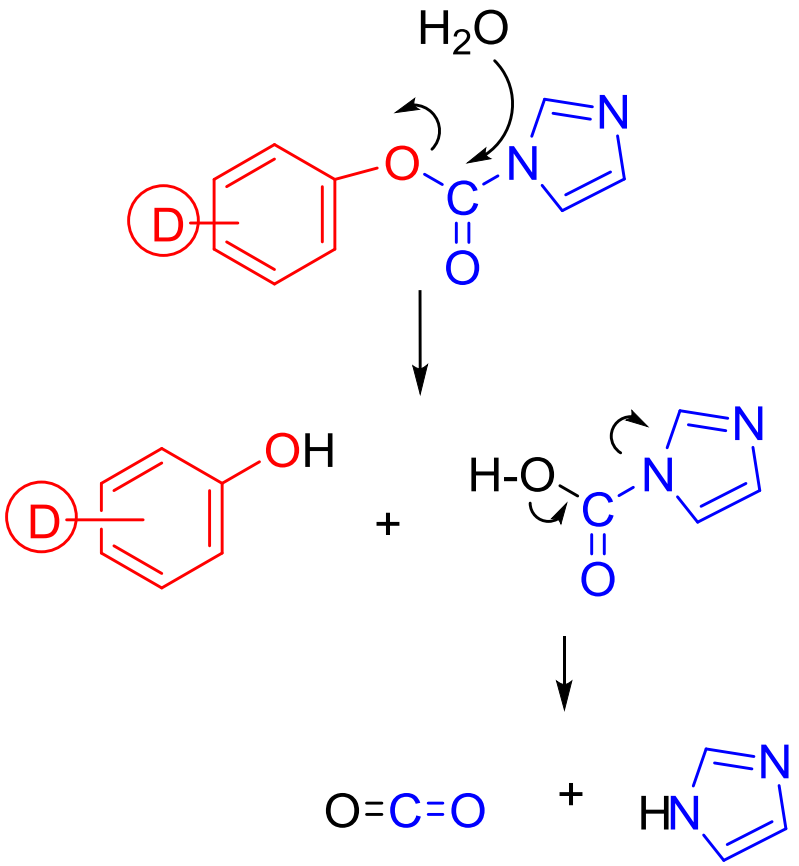
PHENOLS:
CARBAMATES *(also esters and acyloxymethyl ethers such as alcohols)*



4.3. BIOREVERSIBLE DERIVATIVES

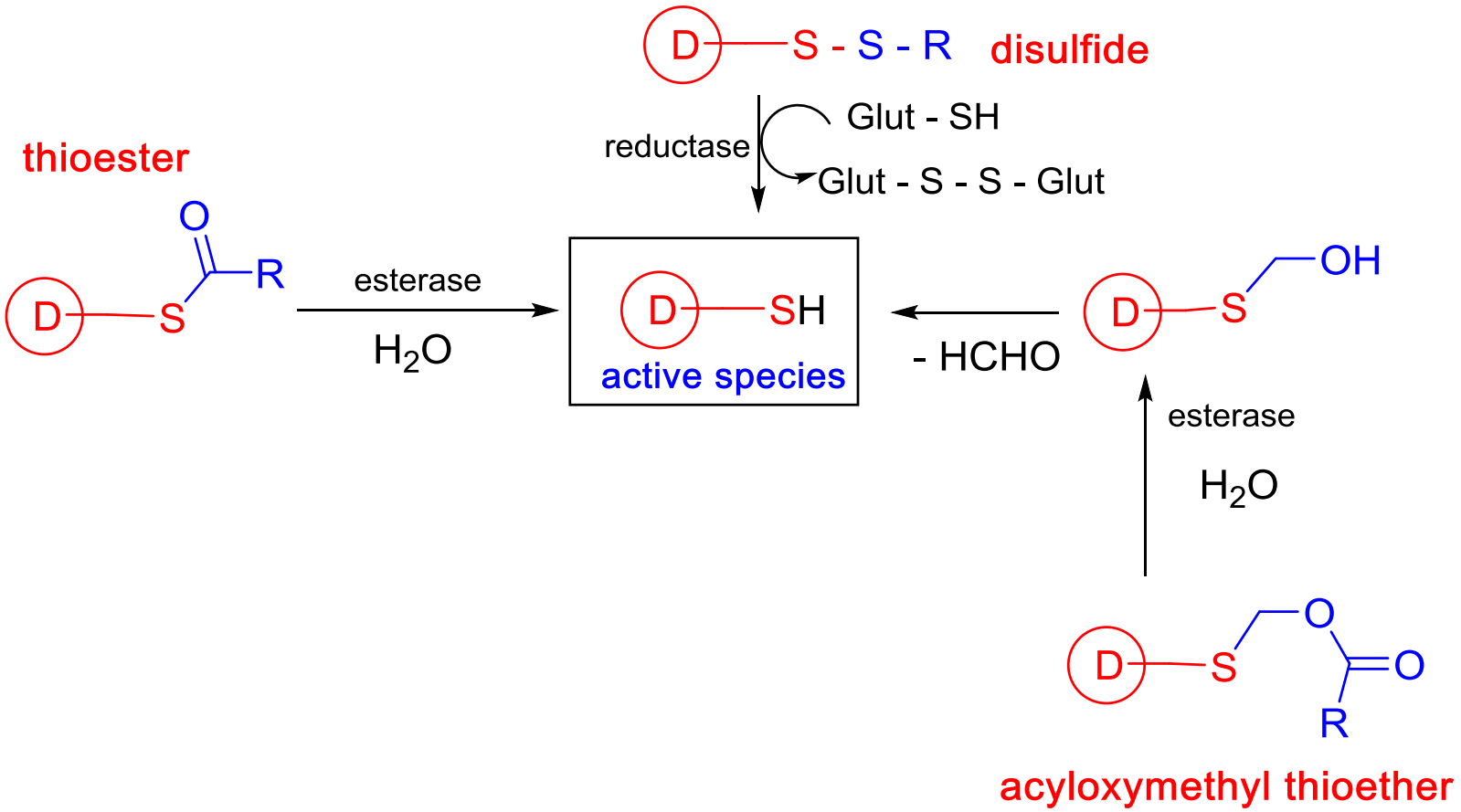
Prodrugs from phenols

Imidazol-derived phenol carbamates:
the phenolic drug is liberated by non-enzymatic hydrolysis.



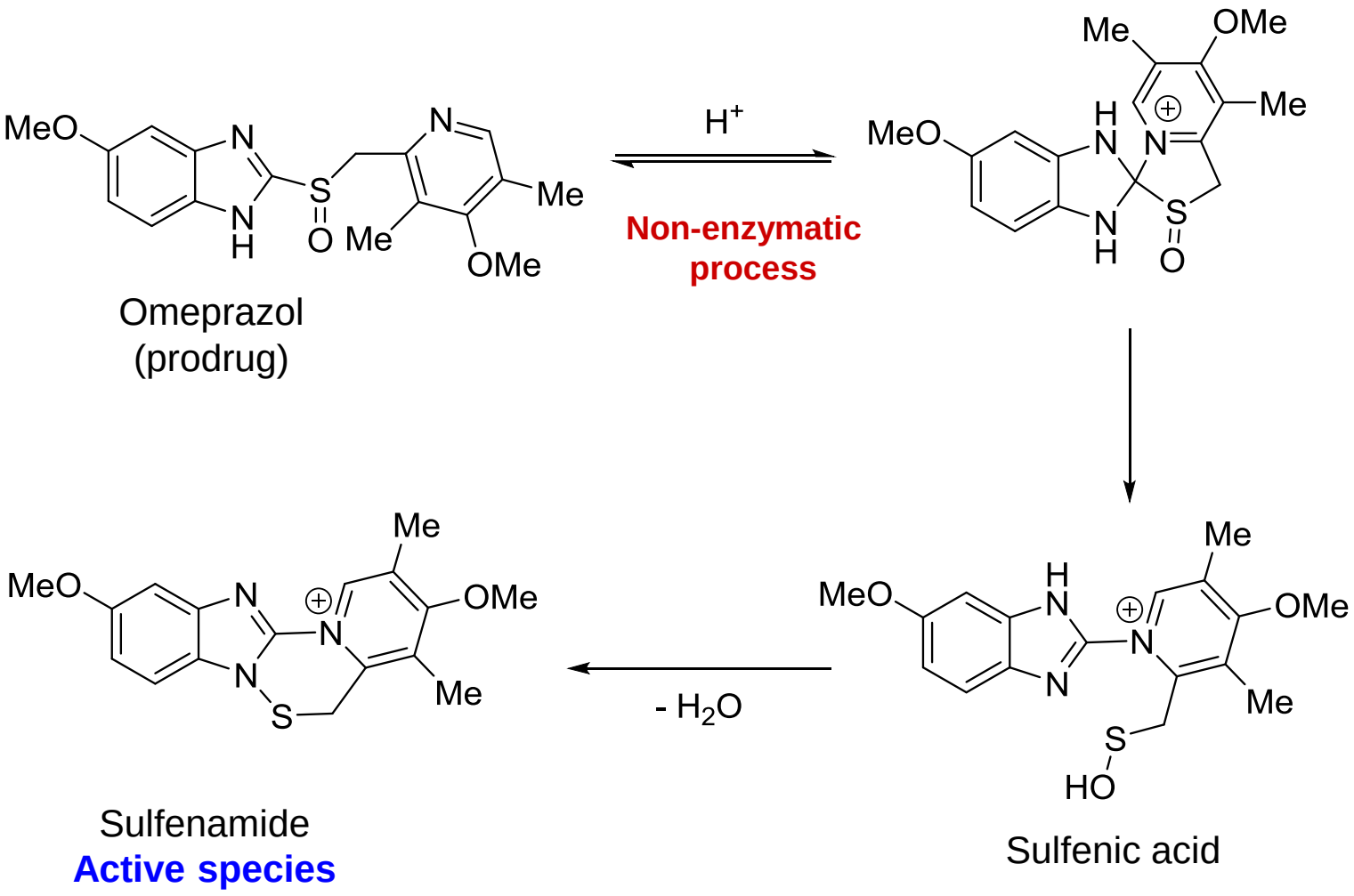
4.3. BIOREVERSIBLE DERIVATIVES

Prodrugs from thiols and thiophenols



4.3. BIOREVERSIBLE DERIVATIVES

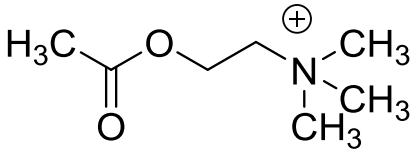
Singular example: activation of omeprazol (proton pump inhibitor in the stomach)



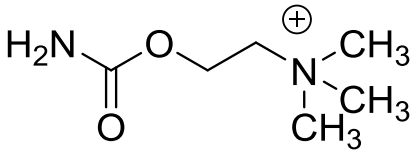
4.4. HARD DRUGS AND SOFT DRUGS

Drugs may be defined as hard or soft depending on their metabolic susceptibility.

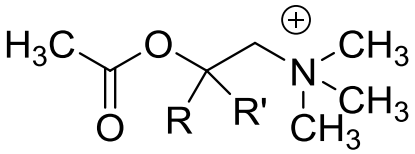
Hard drugs are biologically active drugs that are resistant to metabolism and remain unchanged in the body.
They are poor substrates for the metabolizing enzymes.
The potentially metabolically-sensitive parts of these drugs are either sterically hindered or the hydrogen atoms are substituted for halogens to block oxidation.



acetylcholine



carbachol



R	R’	hydrolysis rate
H	H	500
CH_3	H	15
CH_3	CH_3	0

Electronic or steric changes in the structure of acetylcholine prolong its action.

4.4. HARD DRUGS AND SOFT DRUGS

Soft drugs (also called **antedrugs**) are biologically active drugs that are designed to have a predictable, controlled metabolism to non-toxic products after they have achieved their desired pharmacological effect.

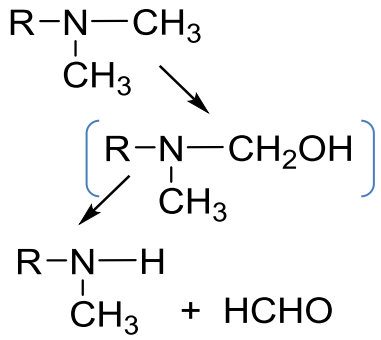
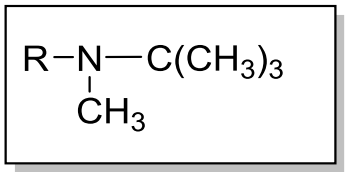
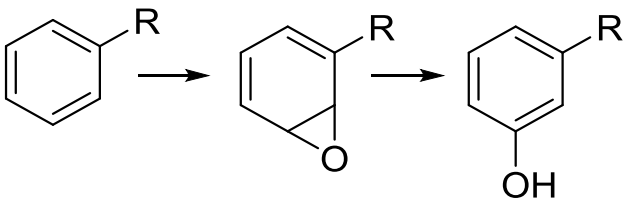
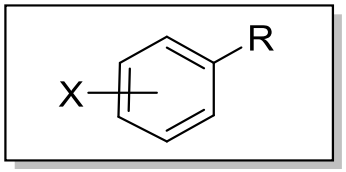
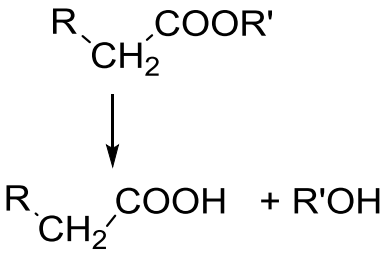
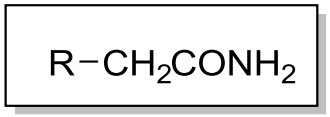
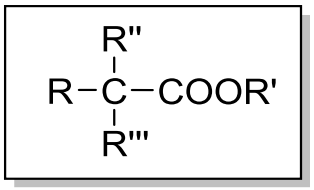
General characteristics of soft drugs:

- They have a close structural similarity to the lead compound.
- A metabolically sensitive moiety is built into the lead structure.
- The changes do not affect the overall physicochemical or steric properties of the lead compound.
- The built-in metabolism is the major (or preferably the only) metabolic route for drug deactivation.
- The rate of the predictable metabolism can be controlled.
- The products resulting from the metabolism are non-toxic and have no other biological activities.
- The predicted metabolism does not lead to highly reactive intermediates.

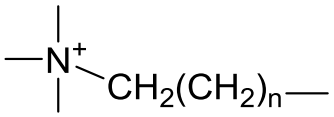
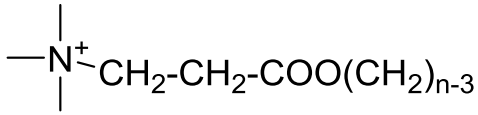
Advantages of soft drugs:

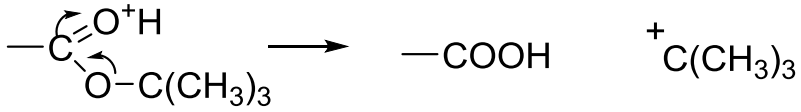
a) As there are no toxic metabolites, the therapeutic index of the drug is higher.
b) Pharmacologically active metabolites are avoided.
c) Drug interactions resulting from metabolite inhibition of enzymes are eliminated.
d) Pharmacokinetic problems caused by multiple active species are simplified.

4.4. HARD DRUGS AND SOFT DRUGS

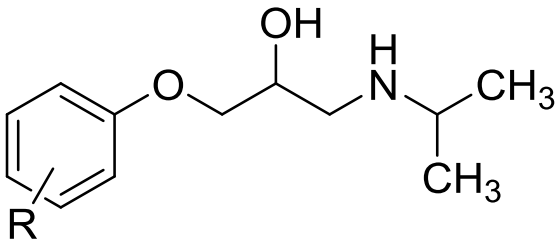
METABOLIC REACTION	HARD DRUG: STRUCTURAL MODIFICATION	OBJECTIVE
N-dealkylation 		steric hindrance in hydroxylation step
aromatic hydroxylation 	 X= CF_3, SO_2NH_2, SO_3H and halogen atoms	deactivation of aromatic ring by electron withdrawing substituents and steric hindrance
hydrolysis of esters 		more stable
		steric hindrance

4.4. HARD DRUGS AND SOFT DRUGS

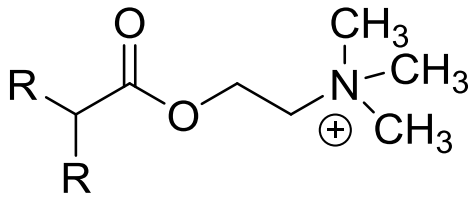
FUNCTIONAL GROUP IN LEAD COUMPOUND	FUNCTIONAL GROUP IN SOFT DRUG	OBJECTIVE
—$(CH_2)_n$—	—COO—$(CH_2)_{n-2}$ **Esters**	Hydrolytic metabolism
—$COO(CH_2)_nN^+R_3$ $n>_1$	—$COOCH_2N^+R_3$ **Acyloxymethyl ammonium derivative**	Chemical instability
	 **Introduction of a carbonyl at β position to a quaternary ammonium**	Favoured degradation (carbonyl group as electron withdrawing group)
R-COOR'	R—$COOC(CH_3)_3$ **Tert-butyl esters**	Easier hydrolysis due to tert-butyl cation stability



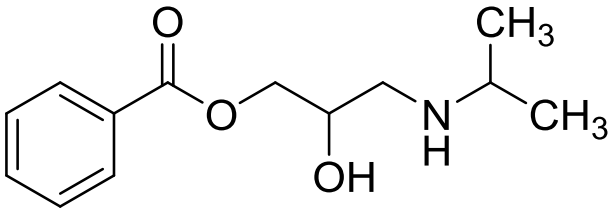
4.4. HARD DRUGS AND SOFT DRUGS



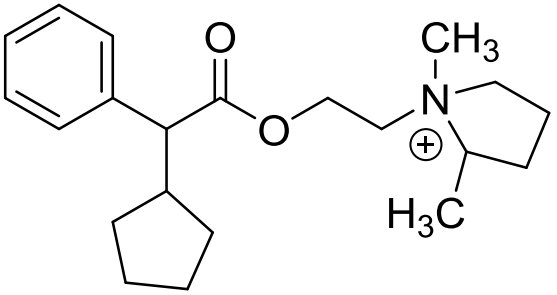
aryloxypropanolamine



acetylcholine esters

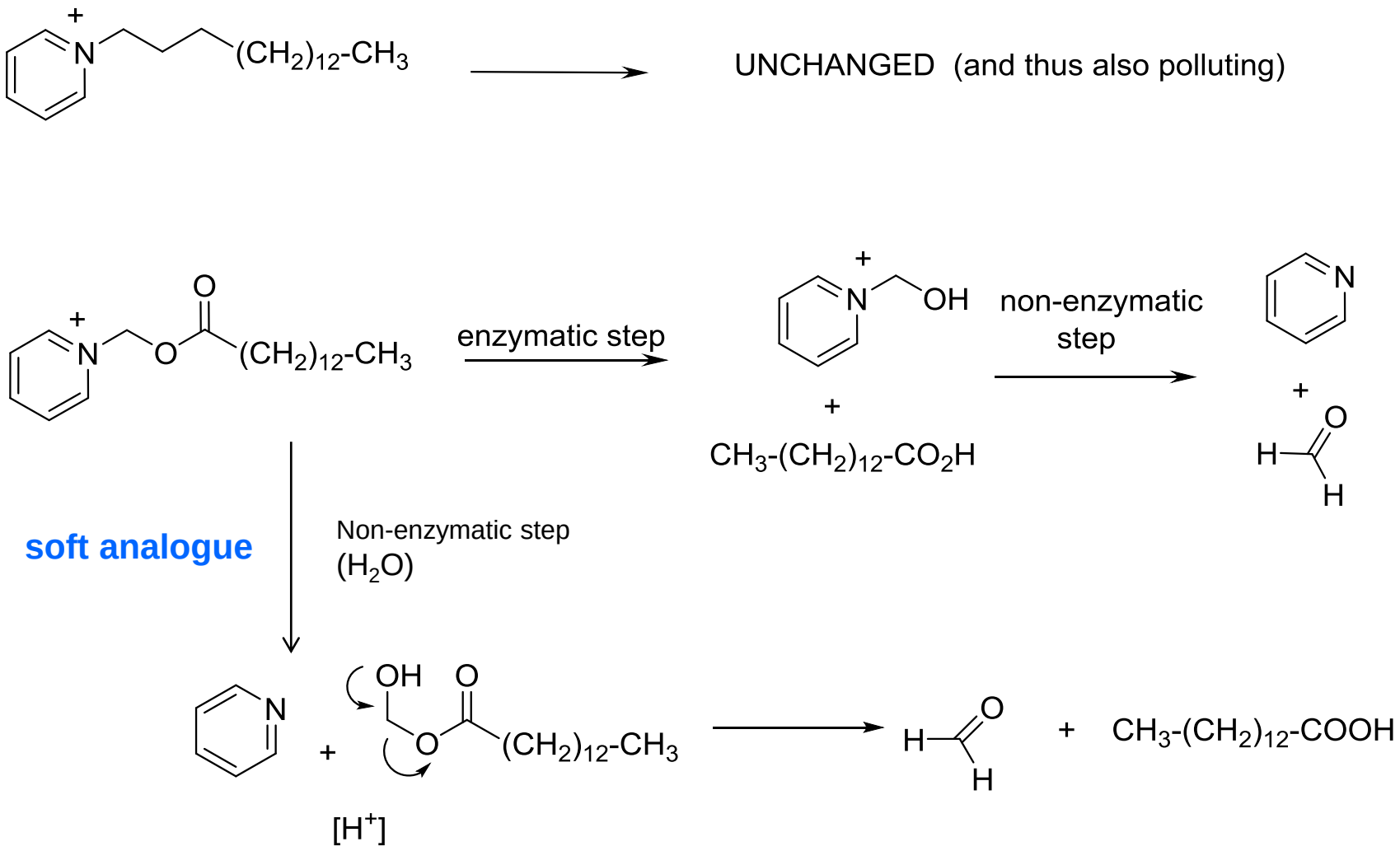


Soft analogue of an aryloxypropanolamine



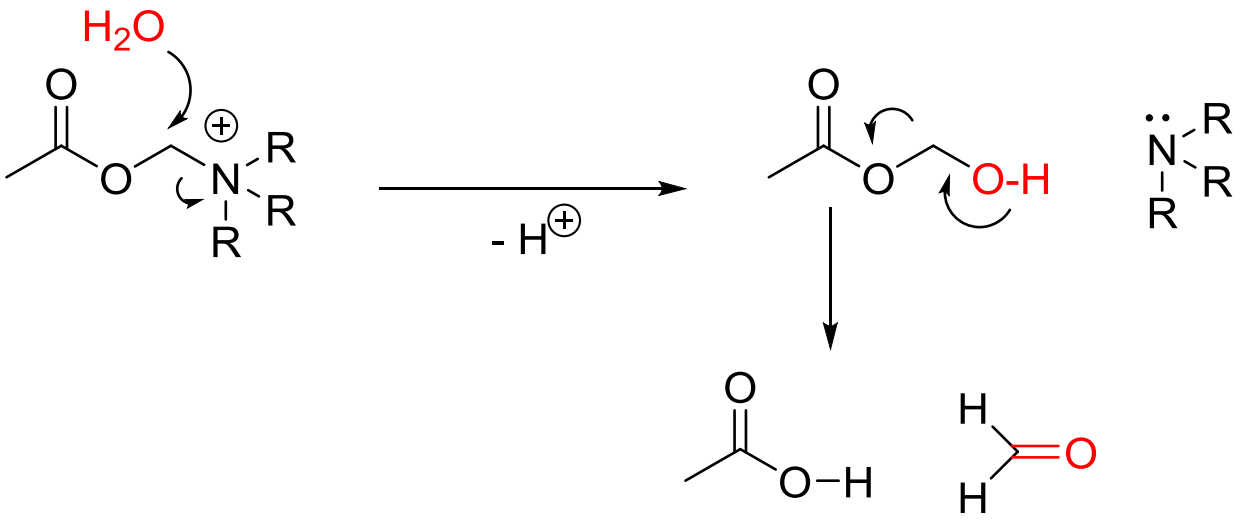
Soft analogue of an anticholinergic

Cetylpyridinium salts to local-use antiseptics

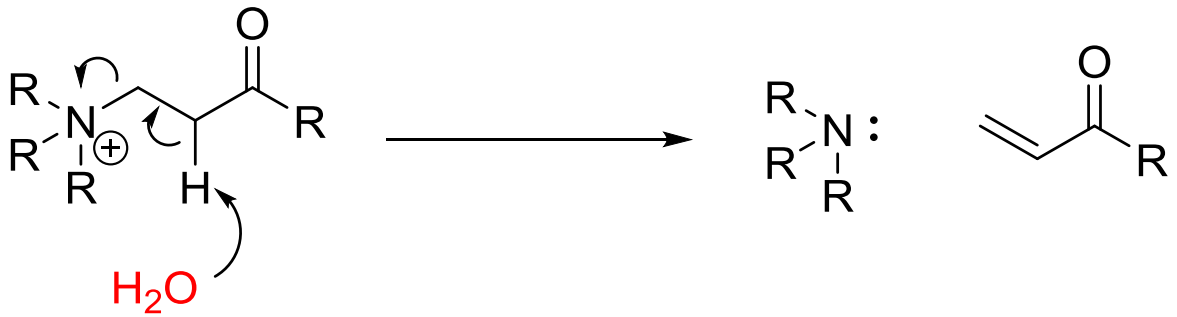


4.4. HARD DRUGS AND SOFT DRUGS

Acyloxymethyl amonium derivatives



Introduction of a β–carbonyl to to quaternary ammonium



UNIT 5. DESIGN AND DEVELOPMENT OF NEW DRUGS

5.1. Evolution of research and drug discovery methods. Current methods for discovering lead compounds.

(Patrick's 5th ed. Ch. 12 and 16; Silverman's 3rd ed. Ch. 1 and 2, Wermuth's 4th ed. Ch. 4-8)

5.2. Qualitative relationships chemical structure-biological activity (SAR). Concept of pharmacophore and auxophoric group. Drug design based on molecular modelling.

5.3. Pharmacomodulation.

(Patrick's 5th ed. Ch. 13, 14; Silverman's 3rd ed. Ch. 2, Wermuth 4th ed Ch. 4 and 12)

5.4. Examples of rational strategies in drug design: receptor activation and/or deactivation and enzyme inhibition.

(Patrick's 5th ed. Chapters 7 and 8)

5.1. EVOLUTION OF RESEARCH AND DRUG DISCOVERY

Medicinal chemistry involves Lead discovery, Lead optimization, Process chemistry and development, and determination of the mechanisms of drug action.

It is a discipline at the intersection of Synthetic Organic Chemistry and Pharmacology that focuses on small organic molecules (not on biologics and inorganic compounds).

The Past

DRUGS or Lead Compounds ⟶ **Targets (mechanism)**

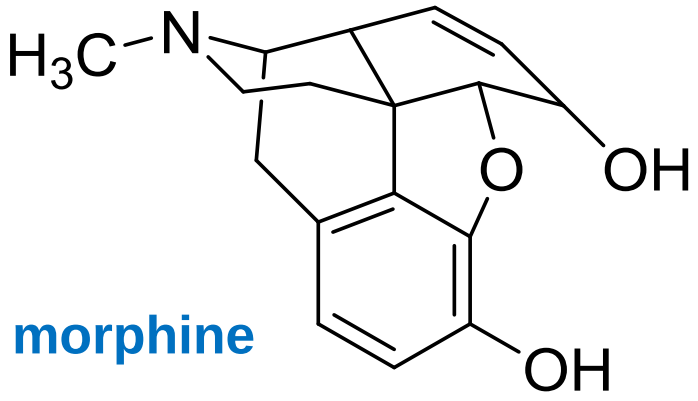
Historically, compounds produced by medicinal chemists have been screened by pharmacologists *in vivo* in whole-animal models of disease.
Many important drugs, such as aspirin and penicillin, were marketed without knowledge of their mechanism of action.

Drugs discovered without rational design

- Medicinal chemistry folklore
- Serendipitous drug discovery
- Discovery through metabolism studies
- Discovery through clinical observations

- **Without rational design: Medicinal chemistry folklore**

Papaver somniferun → **OPIUM** → **morphine**



PAIN TREATMENT

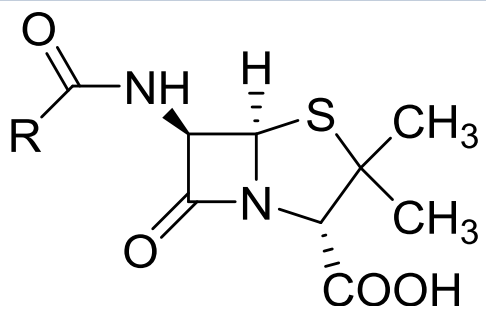
Also:
reserpine, ephedrine, scopolamine, quinine, digoxin, atropine tubocurarine, etc.

(PHARMACOGNOSY)

These natural products are used as drugs but have also inspired the design of synthetic analogues. *(MEDICINAL CHEMISTRY)*

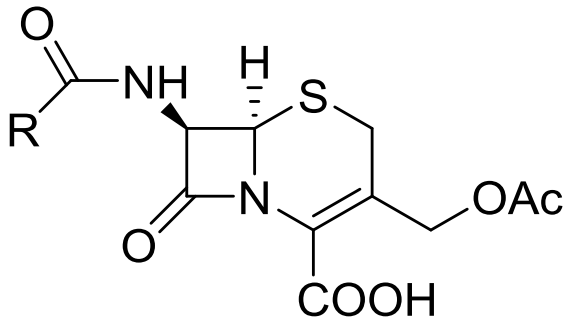
5.1. EVOLUTION OF RESEARCH AND DRUG DISCOVERY

- **Without rational design: serendipity (observations in microorganisms)**



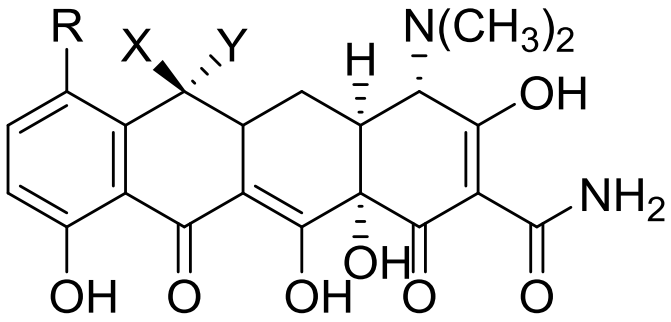
penicillins

(*Penicillium notatum*)

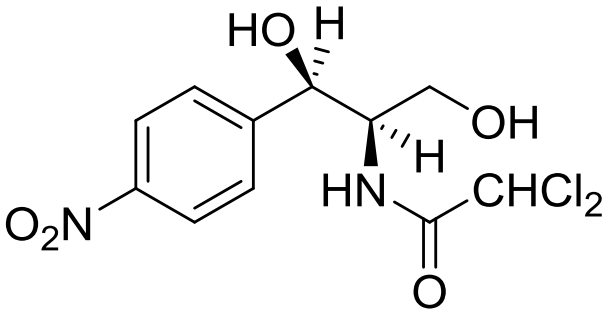


cephalosporins

From penicillin to new antibiotics: semi-synthetic analogues were designed and systematic screening from natural sources was developed.



tetracyclines

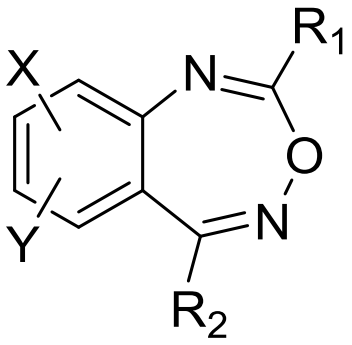


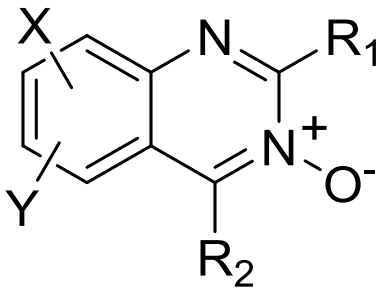
chloramphenicol

- **Without rational design: serendipity (synthetic compounds)**

Serendipitous discovery of benzodiazepines

- Roche set out to prepare a series of benzheptoxdiazines as potential new tranquilizer drugs (1955).
- The actual structure was found to be that of a quinazoline 3-oxide.
- No active compounds were found, so the project was abandoned.

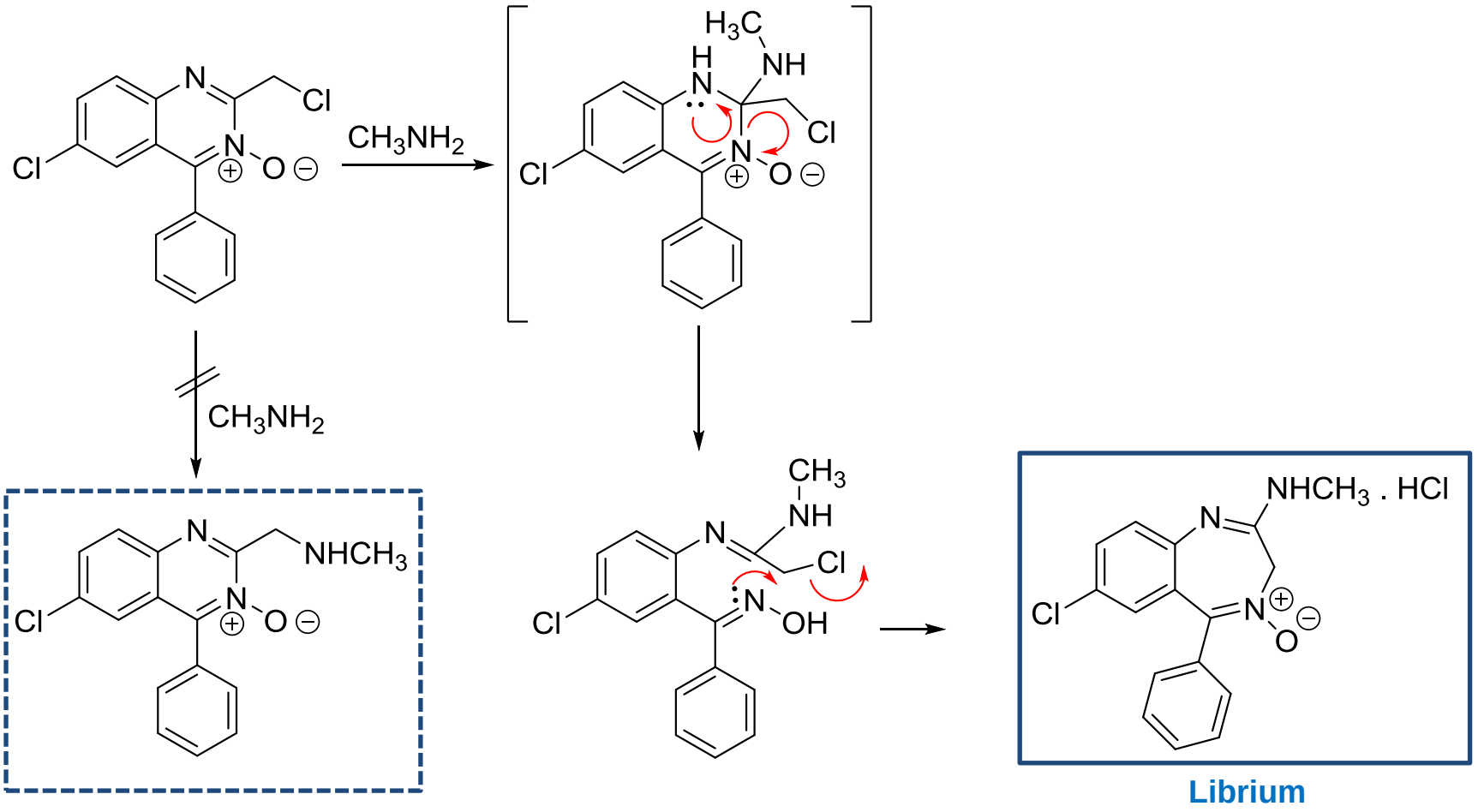




In 1957, during a lab cleanup, a vial containing what was thought to be the latter compound (X = 7-Cl, $R^1 = CH_2NHCH_3$, $R^2 = C_6H_5$) was sent for testing. It was highly active.

- **Without rational design: serendipity (synthetic compounds)**

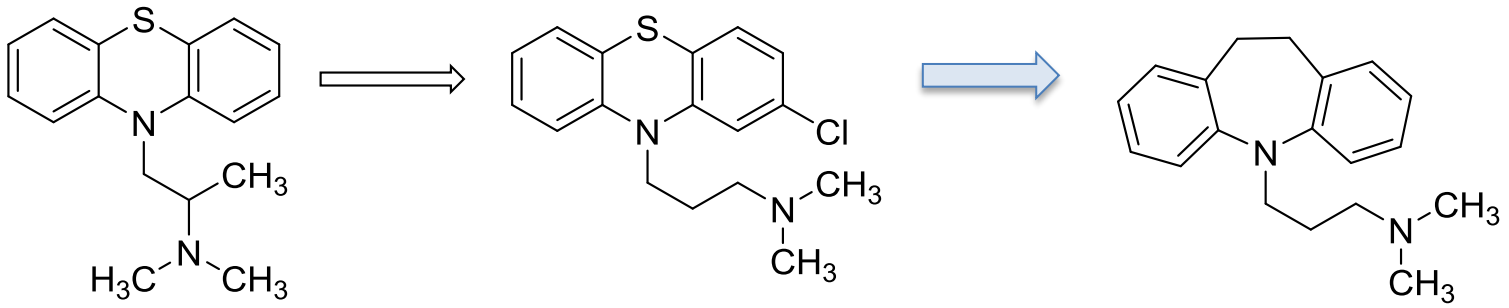
The actual structure of the compound was benzodiazepine 4-oxide, **Librium®**, (obtained from an unexpected reaction of the corresponding chloromethyl quinazoline 3-oxide with methylamine).



Librium

5.1. EVOLUTION OF RESEARCH AND DRUG DISCOVERY

- **Without rational design: clinical observations (observations in humans)**



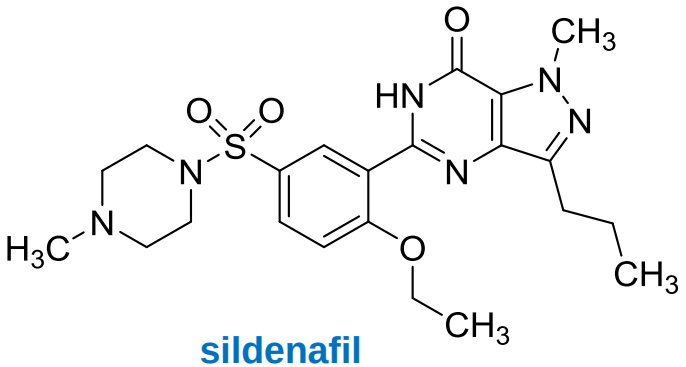
promethazine
(antihistamine
with sedative effects)

chlorpromazine
(neuroleptic
prototype)

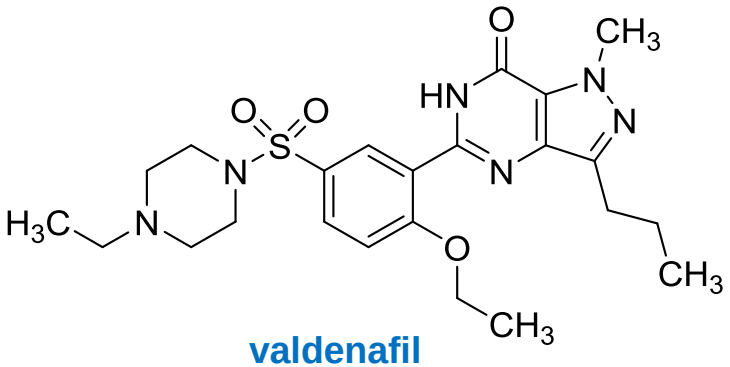
imipramine
(antidepressant)

Also: chlorothiazide (antidiabetic sulphonamide) from antibacterial sulphanilamide

More recently: sildenafil (Viagra), ritonavir, aztreonam, etc.



sildenafil



valdenafil

(The SOSA approach : Selective Optimization of Side Activities)

5.1. EVOLUTION OF RESEARCH AND DRUG DISCOVERY

- **Without rational design: serendipity and side effects (synthetic compounds)**

• The use of **nitrous oxide** and **ether** as narcotic gases in surgery resulted from the observation that people who inhaled these chemicals [at parties] did not experience any pain after injury.

• The vasodilator activity of **amyl nitrite** and **nitroglycerin** was discovered by chemists who developed strong headaches after inhaling or ingesting small amounts.

• A wrong working hypothesis on chloral hydrate, which was supposed to degrade metabolically to narcotic **chloroform**, led to its application as a strong sedative (in reality, the metabolite trichloroethanol is the active form).

• **Warfarin** was used as rat poison.

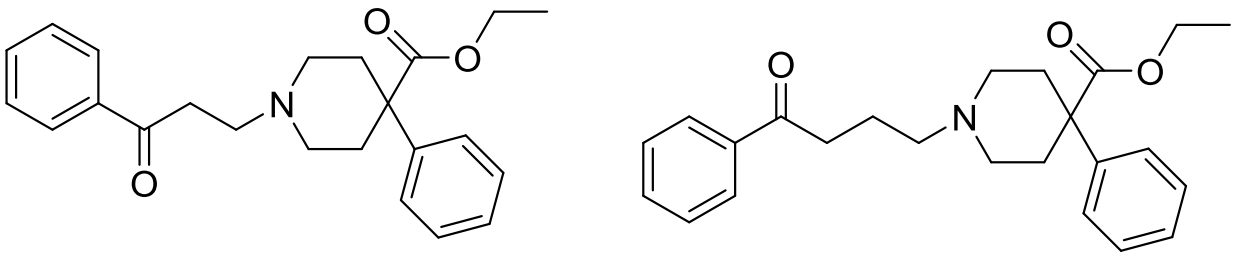
(Find more examples on page 207 of Patrick's 5th ed.)

Without rational design: observations in animals

- Organophosphorous acetylcholine inhibitors (Bayer)

Discovery of **haloperidol**

- Replacing the N-methyl group of pethidine with butyrophenones led to the production of certain potent analgesics that also calmed mice.
- Haloperidol emerged in 1958 as the most potent tranquilizer (Janssen).



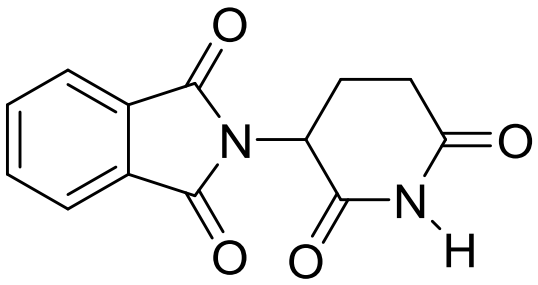
haloperidol

(Wermuth's 4th ed. Ch. 4)

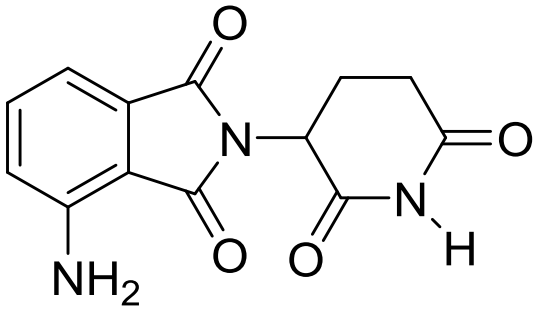
- **Without rational design: clinical observations (observations in humans)**

New Uses for Old Drugs

(Wermuth's 4th ed. Ch. 4)



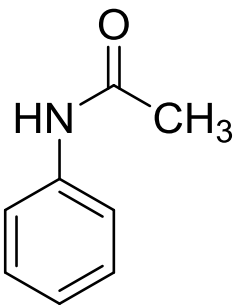
thalidomide



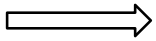
panalidomide

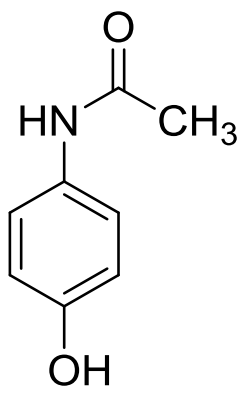
Thalidomide was launched as sedative/hypnotic drug but was withdrawn because of its teratogenicity.
It is now used to treat a complication in the chemotherapy of leprosy. Some analogues have also been obtained.

- **Without rational design: metabolism studies**

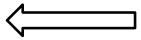


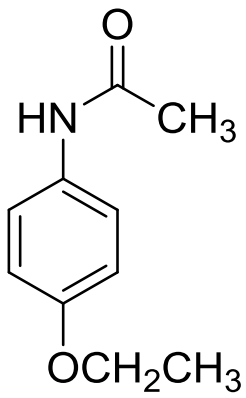
acetanilide





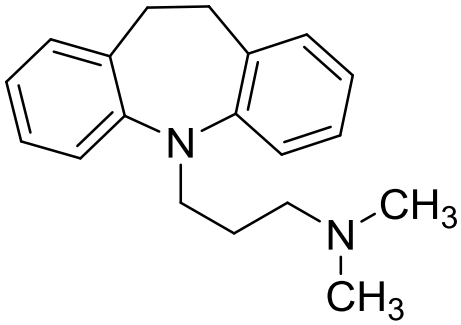
paracetamol



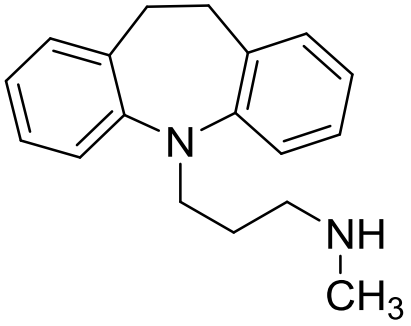


acetophenetidin

ANALGESICS



imipramine



desipramine

ANTIDEPRESSANTS

5.1. EVOLUTION OF RESEARCH AND DRUG DISCOVERY

(Wermuth's 4th ed. Ch. 4)

- *Exploitation of Biological Information*
  - Observations Made in Humans
  - Observations Made in Animals
  - Observations Made in the Plant Kingdom and Microbiology

- Biological information constitutes a rich source of new lead molecules for research.
- It offers creative approaches that do not depend on routine pharmacological models.
- Once the lead **molecule is identified**, it is immediately the object of thorough research to determine its **molecular mechanism of action**.
- Simultaneous synthesis of structural analogues and establishment of structure-activity relationships (SAR) proceed in order to optimize all indispensable parameters for their development (potency, selectivity, metabolism, bioavailability, toxicity and cost price).
- New lead compounds have often arisen form research projects conducted in a different field (keeping an open mind is crucial when testing biological activities).
- Even if the initial discovery was purely fortuitous, a great effort of rationalization is necessary in subsequent research.

5.1. EVOLUTION OF RESEARCH AND DRUG DISCOVERY

- In the past most drugs were “discovered“ either by identifying the active ingredient from traditional remedies or by serendipitous discovery rather than “rational” drug discovery.
- Diseases are controlled at the molecular and physiological levels.
- New technologies are helping to identify new targets.
- The shape of molecules at the atomic level is well understood.
- Very few drugs are now “discovered” without an optimization process from the discovery of an active compound. Finding a **lead** is an important step in drug discovery.

Since the last few decades of the 20th century:

The most common approach to drug discovery has been based on **mechanisms of action** (screening or designing compounds for their effect on a particular biological target).

Disease → Target ⟶ **Lead compounds to DRUGS**

5.1. EVOLUTION OF RESEARCH AND DRUG DISCOVERY

- 113 first-in-class drugs were approved by the FDA from 1999 to 2013.
- 78 were discovered through a hypothesis-driven molecular/pathway **target-based approach** (45 small molecules and 33 biologicals).
- 8 were discovered by **phenotypic screening** (using an assay that monitors a phenotypic change – non-isolated and identified target).
- The remaining drugs were discovered:
  - by identifying an active substance from a natural source.
  - by derivatizing a pharmacologically active natural substance (hormone).

Remember:
Between the first positive assays of an active substance and the substance reaching the market, an average of **12-13 years** elapse.

(Wermuth's 4th ed)

5.1. EVOLUTION OF RESEARCH AND DRUG DISCOVERY

(Wermuth's 4th ed)

Active compounds are called "hits" when only activity in in vitro assays has been tested. Once other important features have been evaluated, the term "lead" should be used.

Lead optimization provides "drug candidates" that can finally be considered drugs when they are approved and reach the market.
(see unit 1).

In this section we will use the term "lead" even when "hit" may be more appropriate in some cases (a "hit –to-lead" optimization process would be necessary in these cases).

The main paradigm for discovering lead compounds has been based on the "one target-one disease" philosophy (see Unit 1).

It uses *in vitro* high-throughput technologies (HTS) and computer-aided drug design.

More recent strategies are based on advances in NMR or X-Ray crystallography.

Some diseases are still inadequately treated by the one target-one disease approach.

Alternatives to the target-based drug-discovery approach

Phenotypic screening

Two approaches for identifying molecules with particular biological effects in cell-based assays or animal models:

- Based on **function** : screening compounds for their ability to induce or normalize functions, such as growth processes, hormone secretion, or cell death (apoptosis).
- Based on **physiology** (screening compounds in isolated organ systems or animal models of disease in order to reduce symptoms of the disease).
  - this was the first drug-discovery approach.
  - it is now used as a last resort because of its low screening capacity, its cost, and the difficulty in identifying the mechanism of action.

E.g. using cellular assays to measure the levels of various proteins or the effects on characteristics such as cell proliferation.

There is also a recent trend for designing ligands that act selectively on multiple targets ("**designed multiple ligands**").

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Strategies

(different books have different classification criteria)

- From existing drugs: "me too" and "me better" and the SOSA approach
- **Screening**
  - **Screening** of natural products
    - Medicinal folklore and more
  - **Screening** the "libraries" of synthetic compounds
    - Combinatorial and parallel synthesis
  - Computerized searching of structural databases
- **Rational design**
  - **Design** from natural ligands or modulators
  - Computer-aided **design**
  - Fragment-based lead discovery (based on NMR spectroscopy, X-ray crystallography, etc.)
- Serendipity and prepared mind

(Patrick's 5th ed. Chapter 12; Silverman's 3rd ed. Chapters 1 and 2)

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Strategies

(Wermuth's 4th ed. Ch. 4)

- Analogue Design : "me-too" compounds (chemical and pharmacological similarity)

- Systematic Screening:
  - Extensive Screening
  - Random Screening
  - High-Throughput Screening
  - Screening of Synthesis Intermediates
  - New Leads from Old Drugs: The SOSA Approach

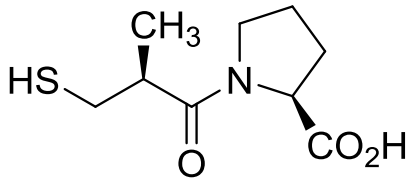
- Exploitation of Biological Information
  - Observations Made in Humans
  - Observations Made in Animals
  - Observations Made in the Plant Kingdom and Microbiology

- Planned Research and Rational Approaches

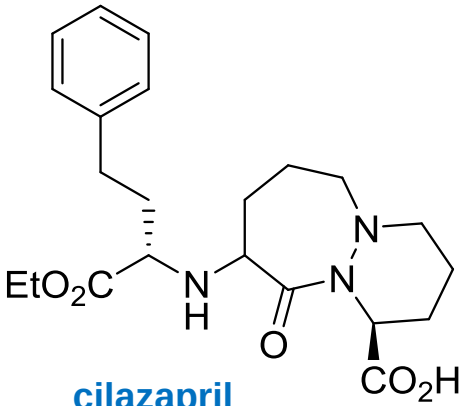
- Applying Biophysical Technologies and Computational Methods
  - Biophysical Technologies
  - Computational Methods

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

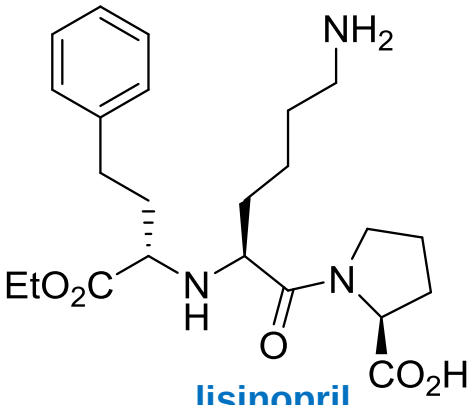
Analogue design



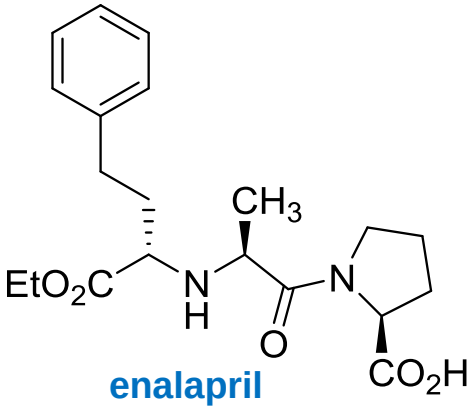
captopril



cilazapril
Hoffmann-LaRoche



lisinopril
Merck



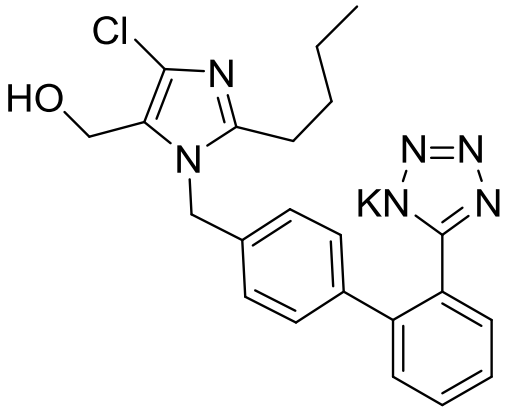
enalapril
Merck

- Established drugs are used as lead compounds by competitor companies in order to design a drug with the same profile (same market area).
- The aim is to modify the structure enough to avoid patent restrictions while retaining its activity (*me too)* and, if possible, improving its therapeutic properties (*me better)*.

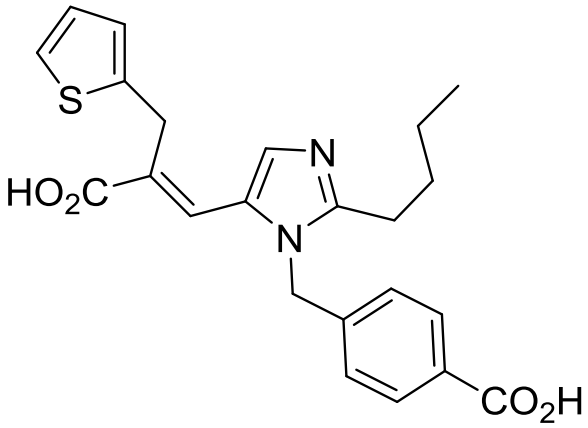
5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Analogue design

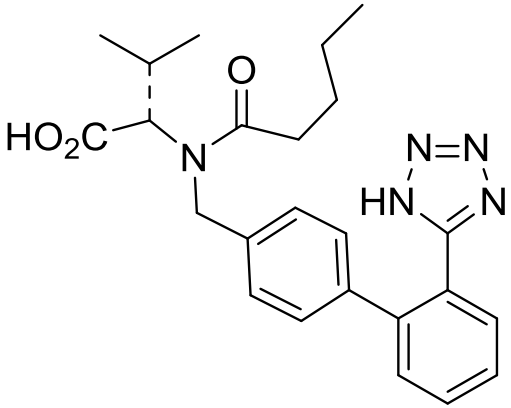
- Analogues from **losartan** (DuPont), an angiotensin AT1 antagonist launched as a vasodilator in 1994 (patented in 1986).



losartan
DuPont



eprosartan
SmithKline Beecham



valsartan
Novartis

(Wermuth's 4th ed. Ch. 4)

Systematic screening

- Involves screening new molecules on an animal model or in a biological test without having any hypothesis regarding its therapeutic potential.
- Uses *in vitro* rather than *in vivo* tests (binding assays, enzyme inhibition measurements, activity on isolated organs or cell cultures).

- Can be achieved in two ways:
  - **Extensive screening**: exhaustive pharmacological research on a small number of chemically sophisticated and original molecules.

    (central nervous, cardiovascular, pulmonary and digestive systems, antiviral, antibacterial, or chemotherapeutic properties, etc.).

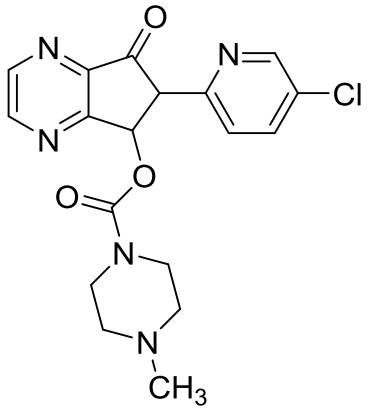
    A successful systematic screening campaign: **paclitaxel** (Taxol).
  - **Random screening**: from a vast number of molecules (hundreds or thousands), the aim is to find one that may be active in a given indication. The therapeutic objective is set in advance.

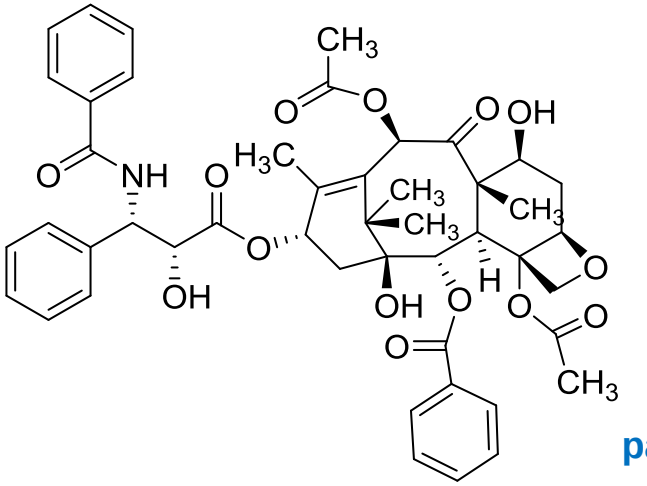
    This was very useful for discovering new antibiotics (samples of earth collected in countries from all over the world).

    More recent successes include the discovery of **lovastatin**.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Systematic screening

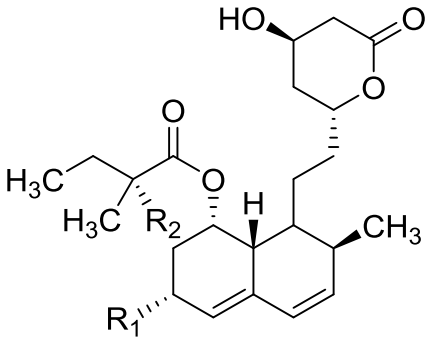


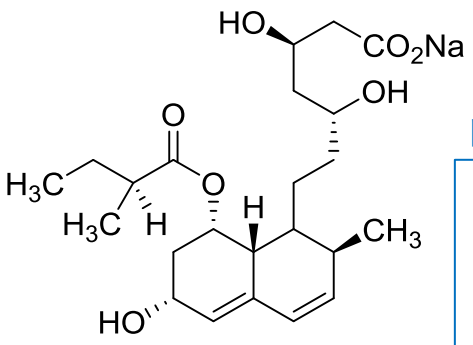


Drugs discovered by random screening.

paclitaxel

zopiclone (hypnotic - used in insomnia)





pravastatin (semi-synthetic)

$R_1 = R_2 = H$ **mevastatin** (natural product)
$R_1 = Me$, $R_2 = H$ **lovastatin** (natural product)
$R = Me$ **simvastatin** (semi-synthetic analogue)

- Statins are used for lowering cholesterol
- Pravastatin is less lipophilic and presents fewer central side-effects.
- The ring-opened form is the actual active *in vivo*.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

High-throughput screening (HTS)

- This started in the 1980s with the arrival of robotics and the miniaturization of *in vitro* testing methods.
- It is a combination of the two earlier approaches.
- It involves the automated testing of large numbers of compounds (libraries) versus a large number of targets (typically, several thousand to a million compounds can be tested at once in 30-50 biochemical tests).
- The test must produce an easily measurable effect which can be detected and measured automatically (radioligand-based and fluorescence-based assays).
- Positive hits are compounds with an activity in the μM to nM range.
- There are many false-positive hits :
  - promiscuous inhibitors – poor selectivity
  - chemically reactive agents – chemical reaction with the target protein

  (alkylating and acylating agents should not be included if only reversible interactions are desired)

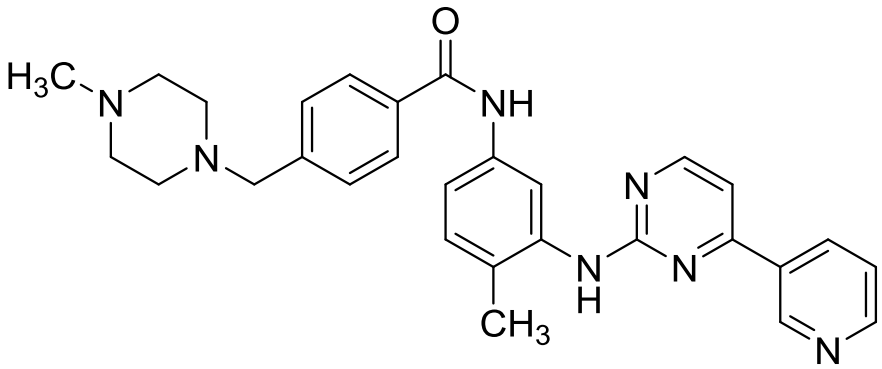
Limitations of HTS:

- Coverage of the molecular diversity, even when the size of libraries is increased.
- The quality and management of the collections.
- Management of the results.
- Financial implications.

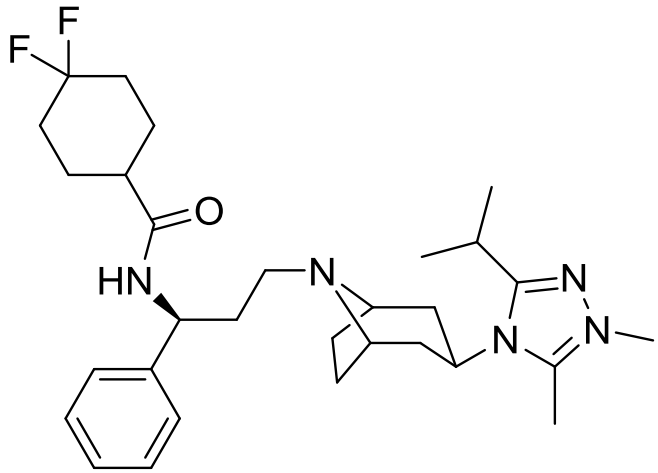
5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

High-throughput screening (HTS)

This strategy has delivered numerous drugs:



imitanib
anticancer (tyrosine kinase inhibitor)



maraviroc
antiretroviral
treatment of HIV infection

Failure rate is high between the number of hits and those compounds which are identified by HTS as authentic lead compounds (in same cases screening highlights no hits because the selected target is not clinically relevant or the hits do not show appropriate physicochemical properties).

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

High-throughput screening (HTS)

- For a random HTS, a major consideration is the source of the large number of compounds that are usually required. Preparing the compound collection for screening is crucial.
- **Targeted screening** implies applying prior knowledge in order to select compounds that are considered most likely to interact with the target.

More rational design (HTS)

- Several cheminformatics and modelling tools can be used (*in silico* screening) to generate better collections:
  - Diversity:
    - Several representatives of each compound scaffold.
  - Removing unwanted compounds:
    - Filters (Lipinsky's rule of five for oral bioavailability and many others).
  - Designing target-focused compound collections:
    - When protein crystal structures are available:
      - Library based on structural information using computers.

        Known active compounds on these targets can be used as starting points for a **ligand-based design** search and the corresponding generation of specialized collections.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of compounds for screening

A) Natural

- Plants
- Microorganisms
- Animals
- Biochemicals
- Marine life

B) Synthetic

- Chemical synthesis (traditional)
- Combinatorial synthesis

C) Virtual

- Computer-aided drug design

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Identifying lead compounds

Natural compounds and those obtained by “traditional” organic synthesis were isolated and their structure determined before testing.

VERY EXPENSIVE AND TIME CONSUMING!!!!

A) Isolation and purification

- solvent-solvent extraction
- chromatography
- crystallisation
- distillation

B) Structure determination

- elemental analysis
- molecular weight
- mass spectrometry
- infrared spectroscopy
- ultraviolet spectroscopy
- NMR (^{1}H, ^{13}C, ^{2}D) spectroscopy
- X-ray crystallography

Thousands or millions of compounds are required in very small amounts for HTS: chemical libraries and combinatorial and parallel synthesis have been developed.

(Patrick’s 5th ed. chapter 16)

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

The Synthetic World

Chemical libraries:

- Collection of stored chemicals and associated databases.
- Pharmaceutical companies ask academic researchers for their products (e.g. Lilly: https://openinnovation.lilly.com).
- Based on organic chemistry.

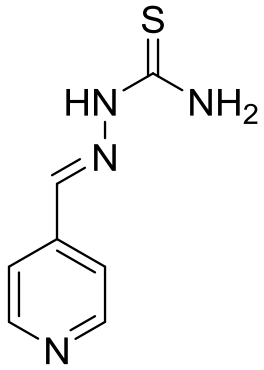
5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

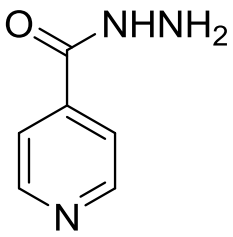
Screening of synthesis intermediates

The Synthetic World

- Synthesis intermediates are chemically connected to final products.
- It is prudent to submit these compounds for pharmacological evaluation.
- Tuberculostatic semicarbazones were initially used in the synthesis of antibacterial sulfathiazoles. Testing of isonicotinic acid hydrazide revealed the powerful tuberculostatic activity of the precursor **isoniazid**.
- **Azidothimidine** (AZT**,** 3'-azido-3'-deoxythymidine) was an intermediate in the synthesis of the corresponding primary amine.

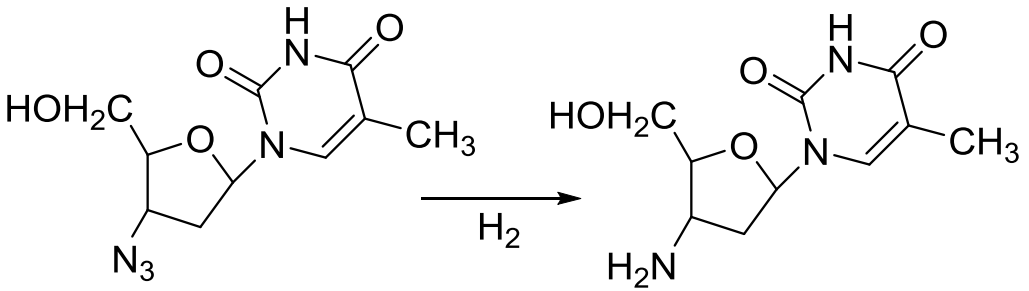


isonicotinaldehyde thiosemicarbazone



isoniazid

reverse transcriptase inhibitor
AIDS treatment



azidothimidine (AZT) 3'-amino-3'-deoxythymidine

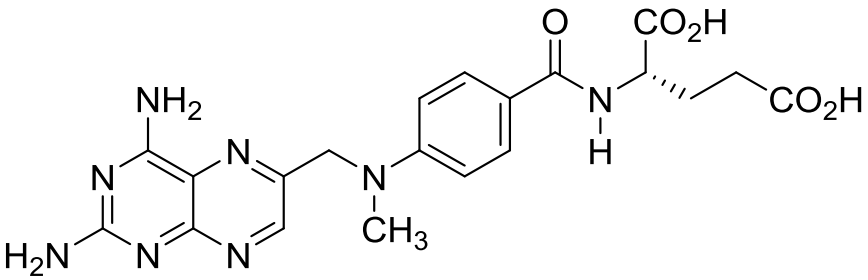
5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

The Synthetic World

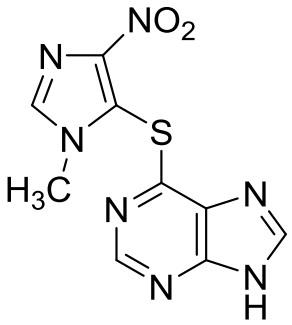
Screening of synthesis intermediates

- **Mercaptopurine** is an intermediate in the synthesis of methotrexate and is also active as an inhibitor of dihydrofolate-reductase. However, it is toxic. Subsequent optimization led to azathioprine (a prodrug that releases mercaptopurine *in vivo*).
- **Azathioprine** is a potent immunosuppressive agent that was used in all organ transplantation until the advent of cyclosporine.
- **Allopurinol** also inhibits xanthine-oxidase and is therefore used to treat gout.

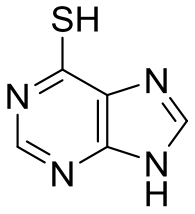


methotrexate

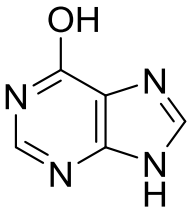
(Wermuth's)



azathioprine



mercaptopurine



allopurinol

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

The Synthetic World

Combinatorial and parallel synthesis:

- Enables the use of a defined reaction route to produce a large number of structurally related compounds in a short space of time.
- The full set of compounds produced in this way is called a compound library.
- Reactions are conducted on a small scale and the process can be automated or semi-automated.
- Reactions are conducted in several reaction vessels at the same time under identical conditions using different reagents for each vessel.
- Is based on solid-phase techniques.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

Combinatorial and parallel synthesis. Solid-Phase Techniques

The Synthetic World

Requirements for Solid-Phase Techniques:

- A resin bead or a functionalised surface to act as a solid support.
- An anchor or linker.
- A bond linking the compound to the linker. The bond must be stable to the reaction conditions used in the synthesis.
- A way to cleave the product from the linker at the end.
- Protection of functional groups not involved in the synthesis.

See Fig 16.4. The principles of an anchor/linker. X, Y and Z are functional groups (Patrick's 5th ed).

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

The Synthetic World

Parallel synthesis

- Reactions are carried out in several reaction vessels at the same time under identical conditions using different reagents for each vessel.
- Reactions are conducted on a small scale and the process can be automated or semi-automated.
- A single product is formed in each reaction vessel: identity is known.
- Is useful for producing analogues in drug optimisation and finding lead compounds.

Automated parallel synthesis

- Automated synthesisers are available with 42, 96 or 144 reaction vessels or wells.
- Reactions and work-ups are carried out automatically.
- The same synthetic route is used with different reagents for each vessel.
- A different product is obtained in each vessel.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

The Synthetic World

Combinatorial synthesis

- This is the automated synthesis of a large number of compounds in a short space of time using a defined reaction route and a large variety of reactants.
- It is normally conducted on a small scale using solid phase synthesis and automated synthetic machines.
- It is useful for finding lead compounds.
- A standard synthetic route is used to produce a large variety of analogues.

- Each reaction vessel contains a mixture of products.
- The products are physically distinct (attached to different beads).
- The identities of the structures in each vessel are not known with certainty.
- Enables the rapid synthesis of large numbers of compounds.
- Each mixture is tested for activity (as the mixture).
- Inactive mixtures are stored in compound libraries.
- Active mixtures are studied further in order to identify the active component.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

The Synthetic World

Combinatorial synthesis

The Mix and Split Method

Example - Synthesis of all possible dipeptides using 5 amino acids
Standard methods would involve 25 separate syntheses.
The mix and split procedure involves five separate syntheses.

Standard method

Glycine (Gly)
Alanine (Ala)
Phenylalanine (Phe)
Valine (Val)
Serine (Ser)

25 separate experiments →

Gly-Gly	Ala-Gly	Phe-Gly	Val-Gly	Ser-Gly
Gly-Ala	Ala-Ala	Phe-Ala	Val-Ala	Ser-Ala
Gly-Phe	Ala-Phe	Phe-Phe	Val-Phe	Ser-Phe
Gly-Val	Ala-Val	Phe-Val	Val-Val	Ser-Val
Gly-Ser	Ala-Ser	Phe-Ser	Val-Ser	Ser-Ser

Note:
Each experiment involves the protection of amino acids, coupling and deprotection.

Mix and split strategy

See Fig 16.29. Synthesis of five different dipeptides using the mix and split strategy (Patrick's 5th ed.).

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

The Synthetic World

Combinatorial synthesis

Identifying structures from combinatorial synthesis

Recursive deconvolution

- Is a method for identifying the active component in a mixture.
- Is quicker than separately synthesising all possible components.
- Needs to retain samples before each mix-and-split stage.

Planning a Combinatorial Synthesis

Aims

- To generate a large number of compounds.
- To generate a diverse range of compounds.
- To increase the chances of finding a lead compound that will bind to a binding site.

The synthesis is based on producing a molecular core or "scaffold" with functionality attached.

Target molecules should obey certain criteria.
E.g. Lipinski's 'Rule of Five' for oral activity

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

The Synthetic World

Combinatorial synthesis

Scaffolds

'**Spider' scaffolds** are preferable for exploring conformational space.

Enable variation of functional groups around a whole molecule in order to increase the chances of finding suitable binding interactions.

'Tadpole' scaffolds

- variation is restricted to a specific region around the molecule.
- there is less chance of favourable interactions with a binding site.

Privileged scaffolds

Scaffolds which are common in medicinal chemistry and are associated with a diverse range of activities:

(benzodiazepines, hydantoins, benzenesulphonamides, etc.)

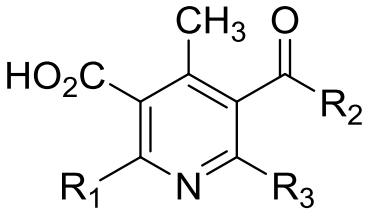
5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

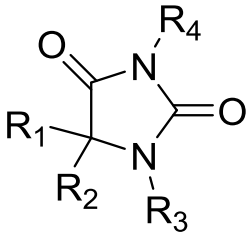
The Synthetic World

Combinatorial synthesis

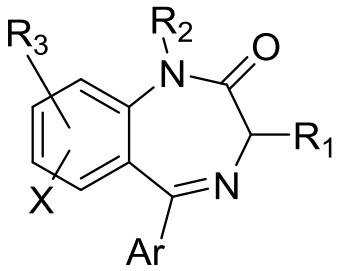
Scaffolds



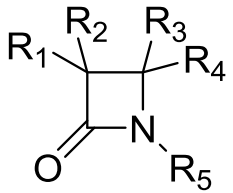
pyridines



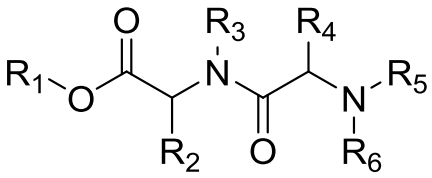
hydantoins



benzodiazepines



β-lactams



dipeptides

(captopril, enalapril)
orally active

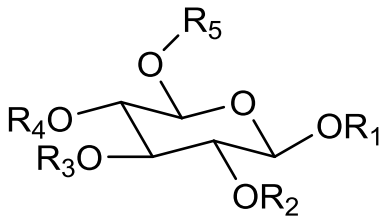
- Good scaffolds
- Spider-like
- Low molecular weight
- Variety of synthetic routes available

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

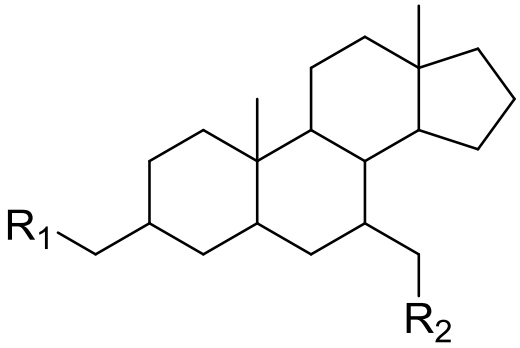
The Synthetic World

Combinatorial synthesis Scaffolds



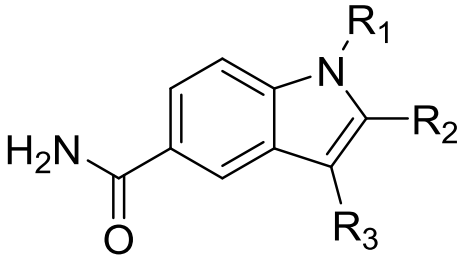
glucose

- Spider-like and small molecular weight
- Multiple OH groups
- Difficult to vary R_1-R_5 independently



steroid

- MW relatively high
- Restricted number of functional groups allowed for MW
- Relatively few positions where substituents can be added



indole

- Tadpole-like scaffold
- Restricted region of variability

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

Diversity-oriented synthesis

The Synthetic World

Comparison of Target-Oriented, Combinatorial, and Diversity-Oriented Syntheses

Target-oriented synthesis and combinatorial chemistry use retrosynthetic analysis to interrogate a single point in chemical space or its immediate surroundings, respectively.

Diversity-oriented synthesis tries to maximize skeletal diversity and thus coverage of chemical space.

From http://www.brandon-russell.com/pdf/dos.pdf

THE BASICS OF DIVERSITY-ORIENTED SYNTHESIS
Kieron M. G. O'Connell, Warren R. J. D. Galloway, and David R. Spring
Diversity-Oriented Synthesis: Basics and Applications in Organic Synthesis, Drug Discovery, and Chemical Biology
First Edition. Edited by Andrea Trabocchi

http://www-spring.ch.cam.ac.uk/publications/pdf/2013_DOS_1.pdf

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Sources of lead compounds for screening

The Virtual World

In silico screening or virtual screening:

- Uses a set of computational methods to analyse large databases or collections of known compounds in order to identify potential hit candidates by predicting compound binding to a drug target.
- Is less expensive than HTS.
- Can complement HTS or be performed prior to experimental screening.

Two main approaches:

- *Ligand-based virtual screening*: 2D or 3D chemical structures or molecular descriptors of known active and inactive molecules are used to retrieve other compounds of interest from a database by seeking a common substructure, pharmacophore, or shape parameters.
- *Target-based virtual screening*: the library of database compounds are docked into a binding site and then ranked using one or several scoring functions (knowledge of the 3D structure of the target and binding site is required).

(Wermuth's 4th Chapter 6)

Sources of lead compounds for screening

The Virtual World

***In silico* screening or virtual screening**:

It is important to filter out undesirable molecules in a pre-screening process because of the magnitude of the drug-like chemical space (10^6 compounds).

By applying filters the overall quality of the query database is improved:
-synthetic accessibility is problematic.
-reactive groups are present.
-predicted oral bioavailability is poor (non-compliance with Lipinski's rule of five).

When both target and ligand structures are known, combinations of this information can be used for computed-assisted compound screening.

Only for promising compounds is biological activity assessed using *in vitro* and/or *in vivo* tests.

This is one of the **computer-assisted drug-discovery techniques**, which are based on molecular informatics.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

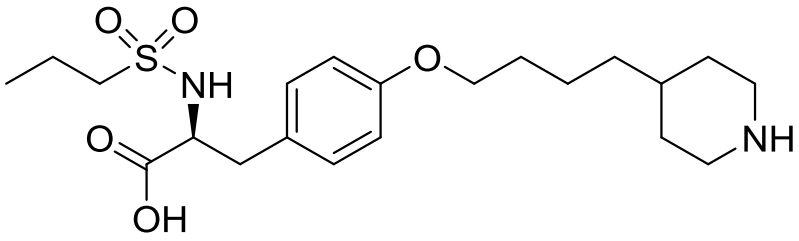
- **Strategies in the search for new lead compounds**

The Virtual World

***In silico* screening or virtual screening**:

(Wermuth's 4th ed. Ch. 4)

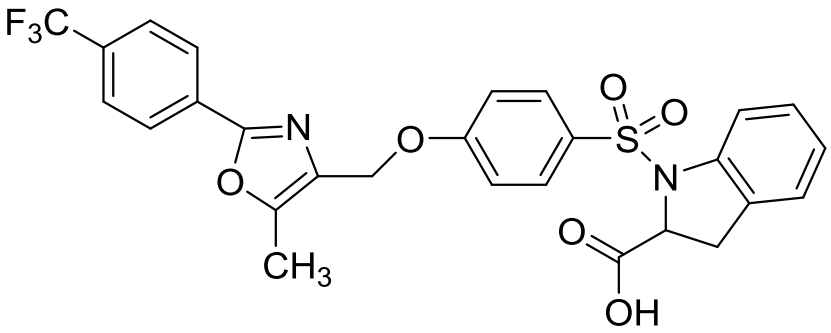
Many molecules have been developed with the help of *in silico* approaches.



tirofiban

reversible antagonist of fibrinogen
Antiplatelet

One of the first FDA-approved drugs developed from a pharmacophore-based virtual screening design.



cevoglitazar

peroxisome-proliferator activated receptor (PPAR- α/γ) dual agonist
Antidiabetic

Developed by combining the virtual screening of 3D databases and structure-based drug design.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Current methods for discovering lead compounds

(different books have different classification criteria)

- From existing drugs: "me too" and "me better" and the SOSA approach
- **Screening**
  - **Screening** of natural products
    - Medicinal folklore and more.
  - **Screening the** "libraries" of synthetic compounds
    - Combinatorial and parallel synthesis.
  - Computerized searching of structural databases.

- **Rational design**
  - **Design** from natural ligands or modulators.
  - Computer-aided **design**.
  - Fragment-based lead discovery

    (based on NMR spectroscopy, X-ray crystallography, etc.).

- Serendipity and prepared mind.

(Patrick's 5th ed. Chapter no. 12; Silverman's 3rd ed. Chapters 1 and 2)

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Rational Design: Design form Natural Ligands

(Wermuth's 4th ed. Ch. 4)

This is based on the knowledge of the incriminated (previously identified and characterized) molecular target (enzyme, receptor, ion channel, transport protein, DNA, etc.). (Advances in molecular and structural biology).

Natural ligands, substrates or modulators can be used as leads.

- Adrenaline and noradrenaline were the starting points for the development of adrenergic β-agonists (salbutamol, dobutamine, etc.) and antagonists (pronethalol).
- Histamine was used as the lead compound in the design of H_2 antagonists (cimetidine).
- 5-Hydroxytriptamine was the starting point for the development of sumatriptan.

When the natural ligand for a receptor is not known, searching for it can be a major project. If the search is successful, a brand-new area of drug design opens up (e.g. identification of opioid receptors led to the discovery of endorphines and enkephalins as lead compounds.) Enzyme inhibitors have also been designed based on the structure of the corresponding substrate.

In section 5.4 we will develop the strategies and discuss some examples

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Computer-Assisted Drug Design (CADD)
" De novo" Drug Design

(Wermuth's 4th ed. Ch. 6)
(Patrick's 5th ed. Ch. 17)

De novo design generates virtual compounds for computational analysis as an extension to the *in silico* screening of physically available compounds stored in databases. The main aim is to find innovative scaffolds for further development.

De novo design can also be divided into **structure-based** approaches (when the 3D structure of the target is known) and **ligand-based** approaches.

Manual

- An operator directs the study.
- It enables the input of the designer's ideas.
- It is useful for identifying a single lead compound.
- It is slow and limited to the designer's originality.

Automated

- The program is automated and therefore much faster.
- No bias is introduced by the operator.
- It is useful for generating a large number of possible lead compounds.
- It produces novel structures.
- It may generate impractical structures for synthesis.
- Scoring structures for binding strengths are unreliable.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Computer-Assisted Drug Design (CADD)
" De novo" Drug Design

(Wermuth's 4th ed. Ch. 6)
(Patrick's 5th ed. Ch. 17)

Structure-based approaches

Procedure:

- Crystallise protein + ligand.
- Determine crystal structure by X-ray crystallography.
- Download for molecular modelling studies.
- Identify the binding site.
- Identify binding interactions.
- Identify other potential binding regions in the binding site.
- Remove ligand *in silico.*
- **Design** ligands to fit and bind to the binding site *in silico.*
- Calculate the strength of binding.
- Synthesize and test promising structures.
- Optimise by structure-based drug design.

This approach can also be used in the lead-modification step.

(Wermuth's 4th ed. Ch. 6)
(Patrick's 5th ed. Ch. 17)

Computer-Assisted Drug Design: " De novo" Drug Design

Points to consider

- Designs of structures that fill the binding site should be avoided:
  - experimental error in crystal structure.
  - different binding modes from those predicted.
  - Space is needed for drug optimisation.
- Flexible molecules are better than rigid ones:
  - they allow for alternative binding modes.
- Synthetic feasibility.
- Stable conformation for binding.
- Energy of desolvation of the ligand should be taken into account.
- There are structural differences in targets from different species.

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Computer-Assisted Drug Design (CADD)
" De novo" Drug Design

(Wermuth's 4th ed. Ch. 6)
(Patrick's 5th ed. Ch. 17)

Several computer software programs are available:

LUDI: works by fitting molecular fragments to different regions of the binding site and then linking the fragments together.

SPROUT: the interaction sites used consist of atom-sized spheres.

LEGEND: does not use fragments to generate skeletons. The skeletons are grown one atom at a time using random choices at each stage of the process.

GROW: is limited to peptides.

SYNOPSIS:

- Is an automated program.
- Is a fragment-based process.
- Generates synthetically feasibly structures.
- Synthetic rules are incorporated into the structure-building process.
- Fragments must be commercially available.
- Fragments are only linked if synthetically feasible.
- The program provides a possible synthetic route.

See Case study no 5. (Patrick's 5th ed).

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Fragment-Based Drug Design (FBDD)

(Wermuth's 4th ed. Ch. 7)
(Patrick's 5th ed. Ch. 12)
(Silverman's 3rd ed. Ch. 2)

Uses several techniques, including NMR spectroscopy, X-ray crystallography or mass spectrometry, to discover and optimise the lead.

To design the lead, this method searches for small molecules (fragments or **epitopes** with a molecular mass in the 100-300 range) which will bind to specific but different regions of a protein's binding site.

The molecules will have no activity in themselves as they only bind to one part of the binding site. However, if a larger molecule is designed which links these epitopes together, then a lead compound may be created.

Origin

- (1981) Theory**:** binding efficiencies can be thought of as a combination of two or more moieties of the molecule.
- (1985) Experimental evidence: the potency of the first statin (mevastatin) can be rationalized as a combination of two "fragments" that bind into separate but adjacent binding pockets.
- (1996) Researchers from Abbott Laboratories reported a new technique called "SAR by NMR".

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Fragment-Based Drug Design (FBDD)

(Wermuth's 4th ed. Ch. 7)
(Patrick's 5th ed. Ch. 12)
(Silverman's 3rd ed. Ch. 2)

NMR

Is applied to proteins of known structure which are labelled with ^{15}N (or ^{13}C) (the amide bond in the protein will have an identifiable peak) even if they are not studied by X-Ray crystallography.

- Overexpression of these proteins in microorganisms is possible when $^{15}NH_4Cl$ is used as their sole nitrogen source.
- The complete 3D and 4D of the purified protein is determined so that the position of every amino acid residue in the protein is known.

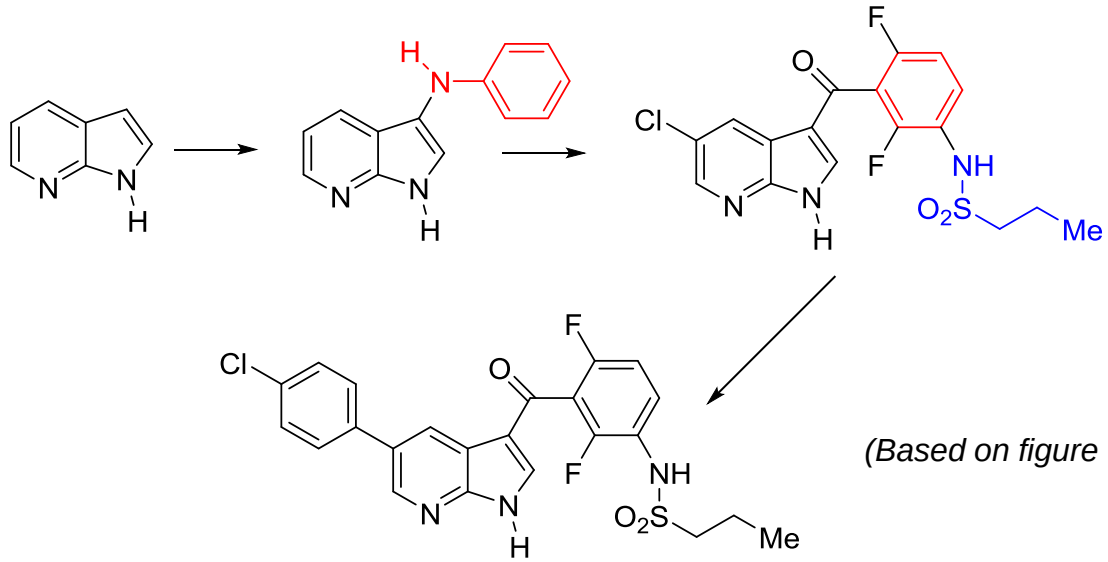
- The first step involves screening a library of small compounds (10 at a time) by observing the ^{15}N chemical shift (δ) for a specific amide nitrogen of the protein.
- Once a fragment is identified that causes a notable change in δ, a library of similar analogues is screened to identify compounds with optimal binding at this site.
- A second library of compounds is then screened in the presence of an excess of the first optimized ligand to find a compound that binds a nearby site.
- The location and orientation of each ligand is determined, and compounds are synthesized in which the two ligands are covalently attached.

Fragment-Based Design (FBDD)

NMR

Figure 12.16. Epitope mapping. (Patrick's 5th ed.)

- **Vemurafenib** (Zelboraf®) is a selective kinase B-Raf enzyme inhibitor that is used for the treatment of metastatic melanoma.
- It is the first drug approved that was derived from a hit found using FBDD (2012 in Europe).
- It is a good example for fragment elaboration where selectivity was achieved during the lead optimization process from the suitable selection of fragments during FBDD.



(Based on figure 7.16 Wermuth's 4th ed. Ch. 7)

5.1. METHODS FOR DISCOVERING LEAD COMPOUNDS

Fragment-Based Drug Design (FBDD)

(Wermuth's 4th ed. Ch. 7)
(Patrick's 5th ed. Ch. 12)
(Silverman's 3rd ed. Ch. 2)

Attractive features (compared to HTS):

- A higher proportion of the atoms of a fragment can directly interact with the target.
- The number of fragments that need to be screened is lower.
- The subsequent structural optimization of a hit fragment has many more options and can result in a higher success rate for generating novel chemical structures.
- Starting with a low molecular mass and a low lipophilic fragment is likely to produce leads with small, simple structures (lipophilicity can be increased during the lead optimization process). Small drugs can also be used as fragments and see if they have other activities (SOSA approach) (good pharmacokinetics and non-toxic).

Limitations:

- Fragments do not always orient in the same way individually as when combined together in an optimized structure: induced conformational changes can occur.
- In-house and commercial sources for ligands can lack extensive structural diversity.
- Very good understanding of the target binding sites through NMR or X-ray crystallography is needed.
- Characterization of some targets (membrane proteins) is difficult.

5.2. STRUCTURE-ACTIVITY RELATIONSHIPS (SAR)

1868 - Crum-Brown and Fraser

- Examined neuromuscular blocking effects of a variety of simple quaternary ammonium salts to determine whether the quaternary amine in curare was the cause for its muscle paralytic properties.
- Conclusion: the physiological action is a function of chemical constitution.

Structurally specific drugs (most drugs):

- Act at specific sites: targets (this idea was proposed by Langley in 1878 and developed by Ehrlich and Fischer - receptors).
- Activity/potency susceptible to small changes in structure.

Structurally nonspecific drugs:

- Have no specific site of action.
- Present similar activities with varied structures (various gaseous anesthetics, sedatives, antiseptics).

5.2. STRUCTURE-ACTIVITY RELATIONSHIPS (SAR)

- For **structurally specific drugs** acting at specific sites:
  - interactions of drugs with targets are highly specific: highly specific 3D binding elements exist in targets that serve as complementary binding sites for the active molecules.
  - compounds exerting similar activities on the same target possess closely related binding properties.

- These molecules present structural elements of identical chemical features in sterically consistent locations and their activity and potency are highly susceptible to small changes.
- Depending on how the lead was discovered, only a small part of the lead compound may be involved in the appropriate interactions.
- Small variations in these parts of the molecule can dramatically influence the activity, whereas variations in other parts cause only minor changes in biological activity.
- A multitude of molecular modifications could be made on the structure of a lead.
- Important binding interactions between the lead compound and its target can be identified after crystallization of the complex. When this is not possible:

Structure-Activity Relationships (**SAR**) studies

5.2. STRUCTURE-ACTIVITY RELATIONSHIPS (SAR)

SAR

QUALITATIVE

Determining the relationships between the structure of a drug and its activity

The aim is to identify which parts of the molecule are important for binding and biological activity and which parts are not.

PHARMACOPHORE

QUANTITATIVE QSAR (see Unit 6)

For a series of similar compounds: the relationships between several parameters related to the structure and biological activity are expressed by an equation for predicting whether a new molecule will be active.

Studies of qualitative **Structure-Activity Relationships** (SAR) involve synthesizing analogues of the lead compound and testing them to determine how the structural changes affect biological activity.

5.2. STRUCTURE-ACTIVITY RELATIONSHIPS (SAR)

Traditional methodology for determining qualitative SAR:

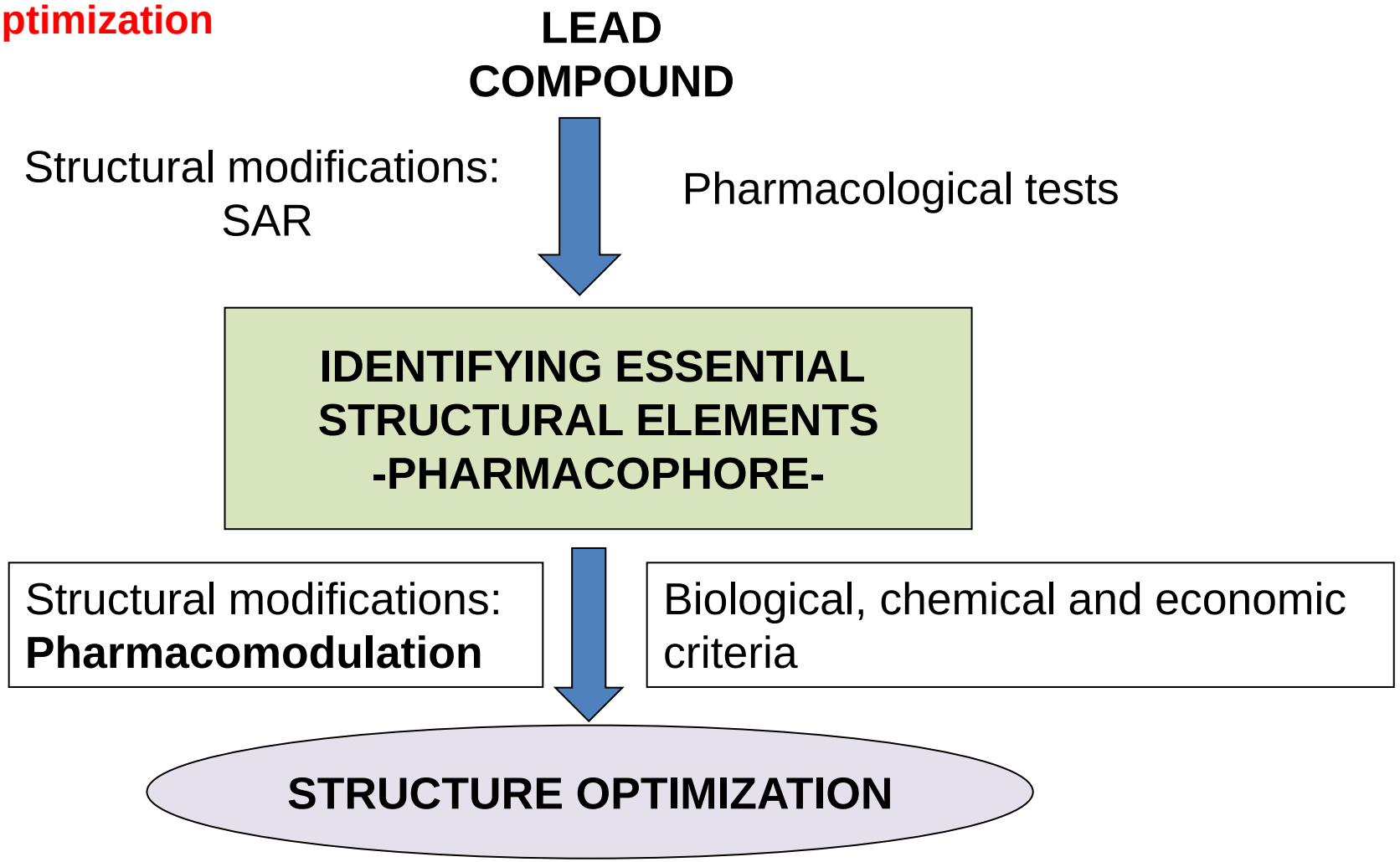
- Obtain structural analogues (alter, remove or mask a functional group).
- Test the analogues for activity.
- Conclusions will depend on the method of testing:
  - *in vitro* – tests for binding interactions with target.
  - *in vivo* – tests for target binding interactions and/or pharmacokinetics.
- If *in vitro* activity drops, the group is important for binding.
- If *in vivo* activity is unaffected, the group is not important for binding.

- Modifications may disrupt binding due to electronic/steric effects.
- The easiest analogues to make are those made from the lead compound.
- Possible modifications may depend on other groups present.
- Some analogues may have to be made via a full synthesis:
  (e.g. replacing an aromatic ring with a cyclohexane ring).
- Enables the pharmacophore to be identified. *(review Unit 3)*

Patrick's 5th ed. Chapter 13.1: Binding interactions that are possible for different functional groups and analogues that may be synthesized to establish whether they are involved in binding.

5.2. STRUCTURE-ACTIVITY RELATIONSHIPS (SAR)

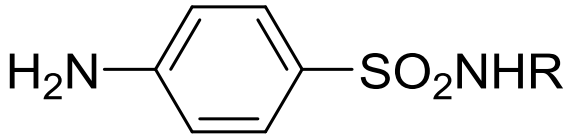
Lead optimization



Designing analogues to obtain compounds with better pharmacological profiles, simpler structures and simpler synthetic processes.

5.2. STRUCTURE-ACTIVITY RELATIONSHIPS (SAR)

SAR: Example



SULFA DRUGS

Thousands of analogues were synthesized.

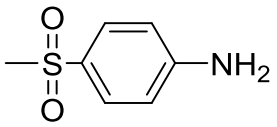
Clinical trials have shown that various analogues possess three activities:

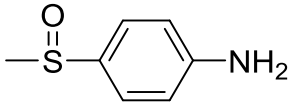
Antimicrobial – Diuretic - Antidiabetic

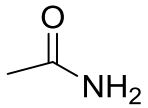
Lead: sulfanilamide (R = H)

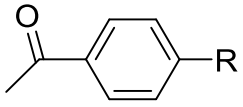
For **antibacterial activity**:

- Groups must be *para.*
- The amino group must be NH_2 (or converted to NH_2 *in vivo* (prodrugs)).
- Replacing the benzene ring or adding substituents decreases or abolishes activity.
- R can be









(but potency is reduced).

- R = $SO_2NR'_2$ produces inactive compounds.

5.2. PHARMACOPHORE AND AUXOPHORE

The highest common denominator of a group of ligands that exhibit a similar biological effect recognized by the same binding site is called a "**pharmacophore**".

Official definition for **pharmacophore** recommended by the IUPAC:

"*A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interaction with a specific biological target structure and to trigger (or block) its biological response*". *(Wermuth's chapter 21)*

A **pharmacophoric element** or feature is generally defined as an atom or groups of atoms common to active compounds with respect to a target and essential for the activity (e.g. an amino group, an aromatic group, an oxygen atom, etc.)

The other atoms of the molecule are commonly referred to as *auxophore* and may be modified to improve pharmacodynamics or pharmacokinetics in the lead optimization process.

5.2. PHARMACOPHORE AND AUXOPHORE

A **pharmacophore model** may be regarded as the representation of a collection of pharmacophore features.

The active conformation must be identified in order to identify the 3D pharmacophore. For flexible molecules with large numbers of conformations it is easier to compare activities of **rigid analogues**.

3D pharmacophore models can be used for database screening in order to retrieve new compounds based on the similarity of pharmacophoric features. (Pharmacophore-Based Virtual Database Screening).

Parallel screening against several pharmacophores in order to predict multitarget effects is also possible and very useful.

5.2. PHARMACOPHORE AND AUXOPHORE

Pharmacophore modelling in computer-aided drug design

(Wermuth's chapter 21)

- Computers and modelling programs enable 3D structures to be displayed and manipulated.
- Automated pharmacophore discovery programs have been developed: (reliable 3D structural and biological data for all of compounds under study must be available: e.g. atomic valences, bond orders, protonation state, stereochemistry, consideration of possible tautomers, etc.).

- The steps for automated pharmacophore identification are:
  a) Select a set of active ligands known to bind the same binding site.
  b) Perform conformational analysis of all the ligands.
  c) Assign pharmacophoric features.
  d) Perform molecular superimposition of the ligand conformations in order to develop a common 3D pharmacophore.

5.2. PHARMACOPHORE AND AUXOPHORE

Pharmacophore modelling in computer-aided drug design

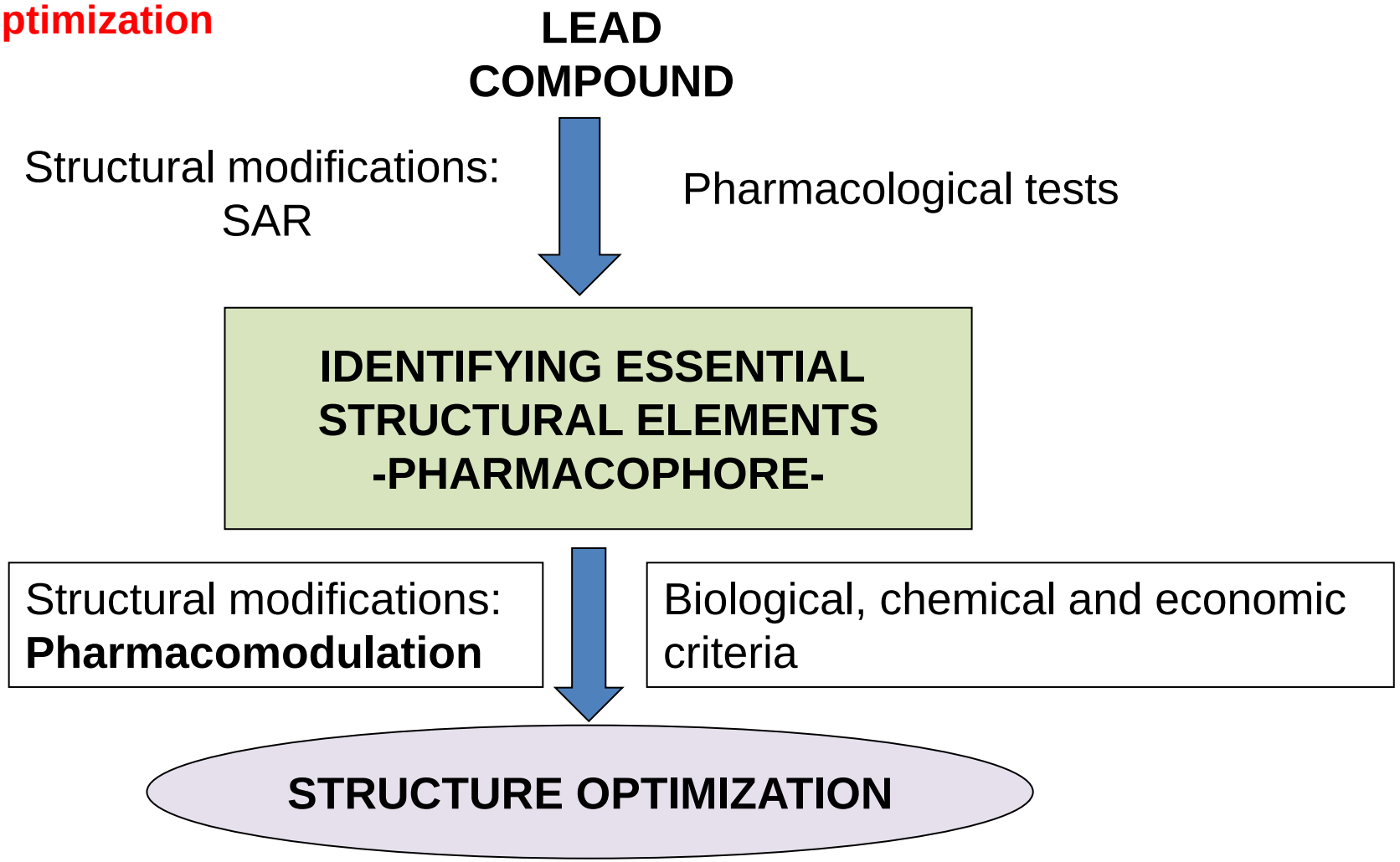
(Wermuth's chapter 21)

- Starting from a preliminary pharmacophore model, a hypothetical receptor consisting of individual amino acid residues can be constructed surrounding a set of superimposed ligands.
- A complex system can be generated guided by permanent correlation of biological data and model-derived calculated free energies of binding.
- This complex can be useful as a model for the interaction pattern of a real binding site.
- The resulting hypothetical receptor model is called a "mini-receptor" or "**pseudo-receptor**".
- The pseudo-receptor can be used to obtain a model that can predict the biological activities of new ligands and enable the design of novel molecules that fit the model (3D Quantitative Structure-Activity Relationships).
- The number of examples that successfully predict biological activity using pharmacophore methods is constantly growing.

(To learn *more about QSAR, see Unit 6)*

5.3. PHARMACOMODULATION

Lead optimization

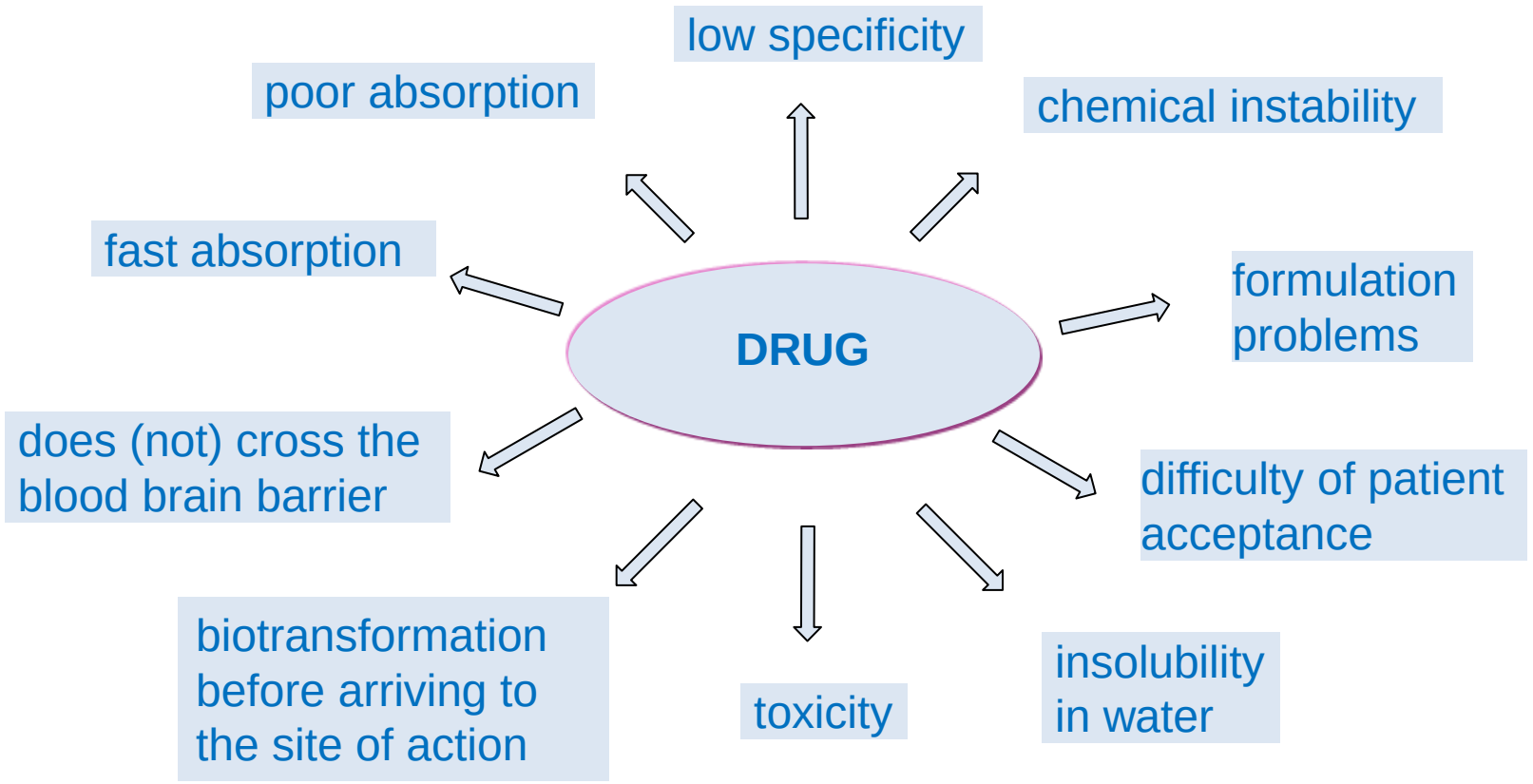


Designing analogues to obtain compounds with better pharmacological profiles, simpler structures and simpler synthetic processes.

5.3. PHARMACOMODULATION

Lead optimization

PROBLEMS LIMITING THE EFFECTIVENESS OF A POTENTIAL DRUG
pharmacodynamics / pharmacokinetics / chemistry / galenics



Lead modification: strategies

The strategy for designing the analogues we will synthesize and test depends on the size and degree of complexity of the lead's structure and our main objective.

Approaches with regard to changes in the lead structure size or complexity:

- **simplification** (**disjunctive approaches**):
  - Involves deleting functions, structural elements or cycles.
  - Is also used in SAR studies (to identify the pharmacophoric elements).
  - Is useful for complex substances (many natural compounds).

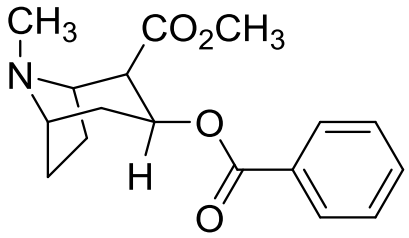
- **conservation** of the same level of **complexity** (**modulative approaches**):
  - Involves interconversion of atoms and functional groups, etc.
  - Is also used in SAR studies.

- enlargement by **adding elements (conjunctive approaches**):
  - Attaching additional structural elements (**extension**).
  - Association of two separate drugs (**association**).

5.3. PHARMACOMODULATION

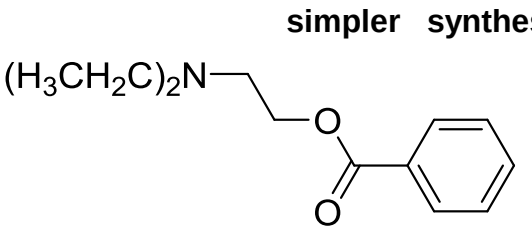
Lead modification: examples

simplification



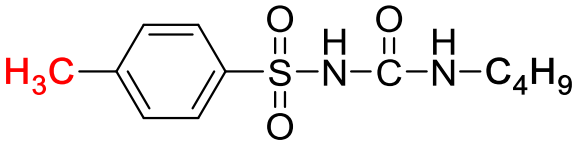
cocaine

simpler synthesis

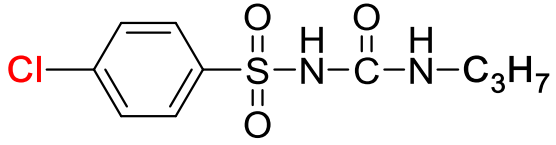


procaine

modulation

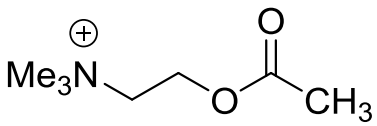


tolbutamide

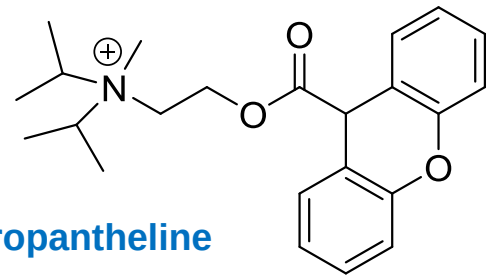


chlorpropamide

extension



acetylcholine



propantheline

muscarinic antagonist

5.3. PHARMACOMODULATION

Lead modification: strategies

Changes aimed at optimizing:

- the interaction of a drug with its target (potency and selectivity).
- the **pharmacokinetic properties** (ADME): access to the target.
- **chemical** stability and synthesis.

Optimizing binding interactions with the target

Reasons

- To increase activity and reduce dose levels.
- To increase selectivity and reduce side effects.

Strategies

Vary alkyl substituents	Vary aryl substituents
Extension	Chain extensions/contractions
Ring expansions/contractions	Ring variation
Vary atoms or groups (isosteres)	Simplification

Rigidification *(see Unit 3 on active conformation)*

(see Patrick's 5th ed. chapter 13)

5.3. PHARMACOMODULATION

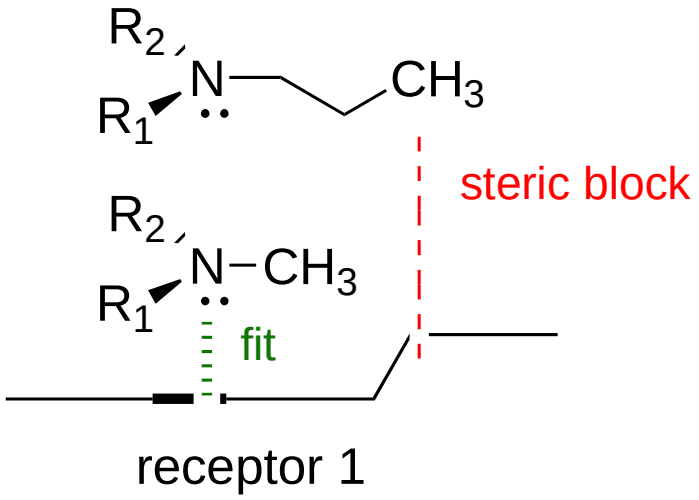
Lead modification: strategies To optimize binding interactions with the target

Vary alkyl substituents

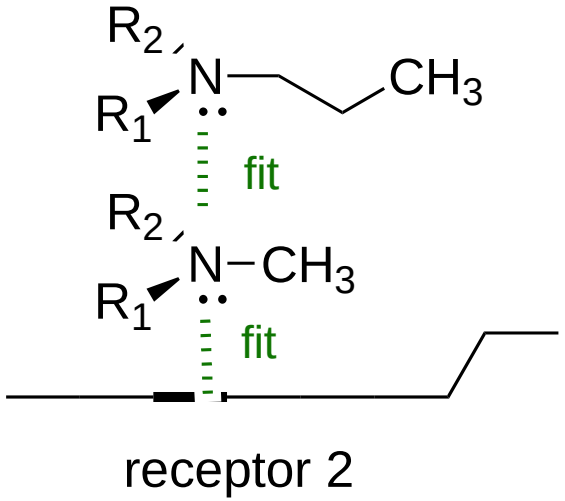
- The alkyl group in the lead compound may interact with the hydrophobic region in the binding site.
- Varying the length and bulk of the group could optimise interaction or introduce selectivity.

Example:

Selectivity of adrenergic agents for β-adrenoceptors rather than for α-adrenoceptors.



receptor 1



receptor 2

5.3. PHARMACOMODULATION

Lead modification: strategies

Varying alkyl substituents

Homology

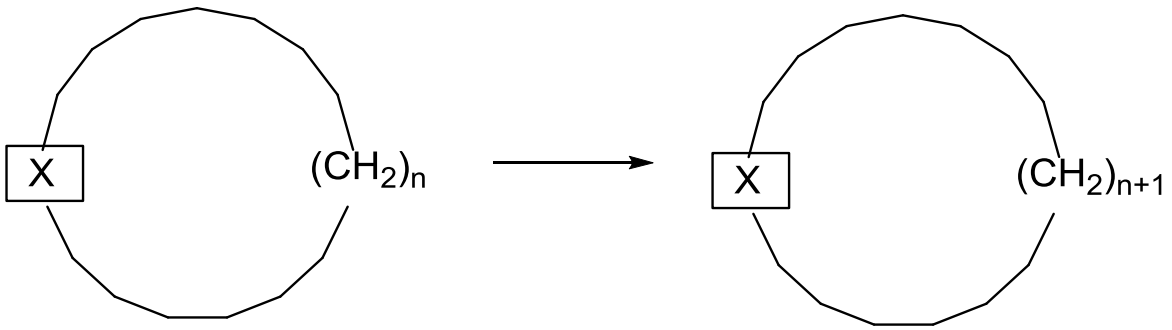
- The similarity of the organic compounds of a series in which each member differs from its adjacent compounds by a fixed increment, e.g. by CH_2.
- Compounds in the series are named **homologues**.

Monoalkylated derivatives

$$R - X \longrightarrow R - CH_2 - X \longrightarrow R - CH_2 - CH_2 - X \longrightarrow \text{etc.}$$

Vary length

Cyclomethylene derivatives



Ring's extension or contraction

Difunctional derivatives

$$X - (CH_2)_n - Y \longrightarrow X - (CH_2)_{n+1} - Y$$

Vary length
(distance between groups)

5.3. PHARMACOMODULATION

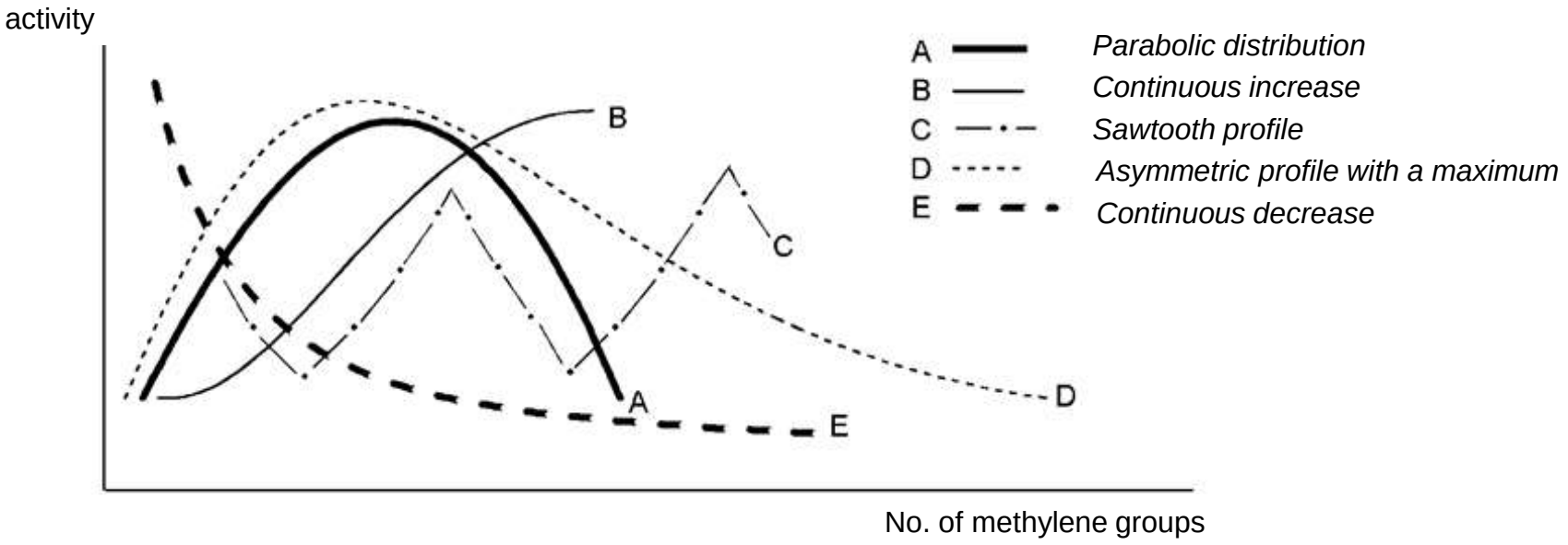
Lead modification: strategies

Varying alkyl substituents

Homology

The effect that increasing the methylene groups has on the activity of a drug depends on the importance of the modified alkyl chain: whether the chain takes part in binding or not.

Changes in lipophilicity are also important.

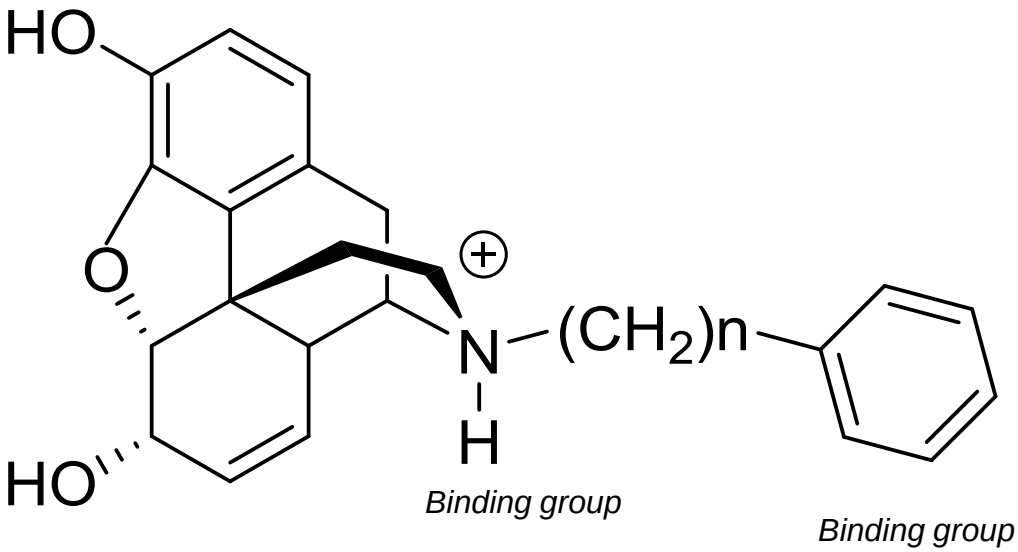


Lead modification: strategies

Optimizing binding interactions with the target

Chain Extension/Contraction - Homologation

This strategy is employed to find the ideal length for the best interaction by shortening or lengthening the chain length when two important binding groups are linked together by a chain



Optimum chain length n = 2

Lead modification: strategies Optimizing binding interactions with the target

Varying alkyl substituents

Synthetic feasibility of analogues

- It is feasible to replace alkyl substituents on heteroatoms with other alkyl substituents.
- It is difficult to modify alkyl substituents on the carbon skeleton of a lead compound.
- Total synthesis is usually required to vary alkyl substituents that are on the carbon skeleton.

See Fig 13.32. Methods of modifying an alkyl group (Patrick's 5th ed).

Ring expansion/contraction - Homologation

Expanding or contracting a ring puts other rings in different positions relative to each other.
Better interactions are possible.

See Fig 13.40. Ring expansion (Patrick's 5th ed).

Lead modification: strategies

Optimizing binding interactions with the target

Ring variations

Replacing aromatic/heterocyclic rings with other ring systems sometimes results in improved properties.
It is often done for patent reasons.

Varying aryl substituents

- Varying substituents or the substitution pattern

Nitroanilines:

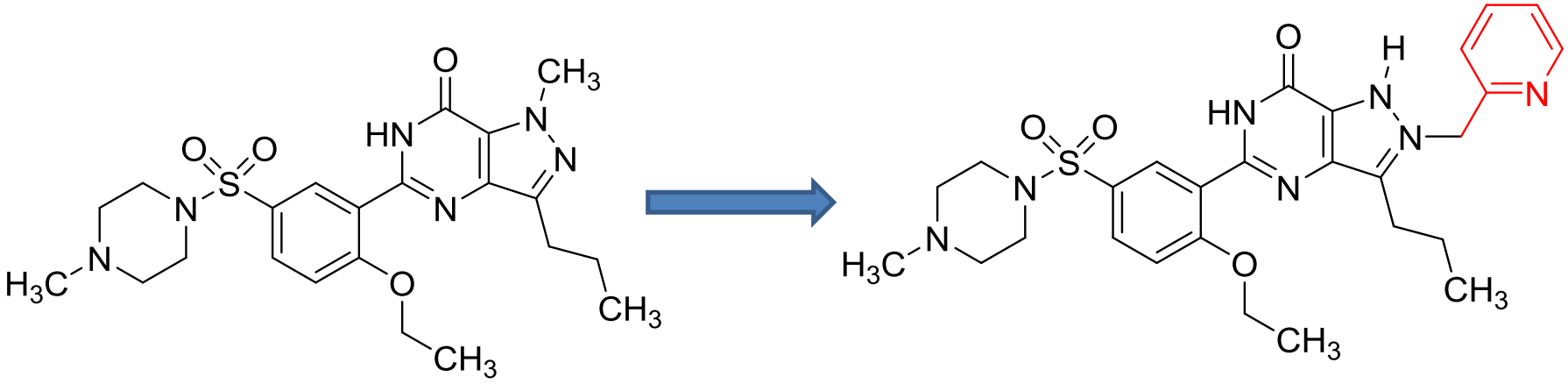
- The binding strength of NH_2 as HBD is affected by the relative position of NO_2.
- This effect is stronger when NO_2 is at the *para* position.
- Amine N is a weaker HBA due to these effects.

Lead modification: strategies — Optimizing binding interactions with the target

Extension

Exploring the target binding site for further binding regions to achieve additional binding interactions.

Example: Second-generation anti-impotence drugs



Sildenafil (Viagra®)

- Extension – addition of pyridine ring
- Extra van der Waals interactions and HBA
- Increased target selectivity

5.3. PHARMACOMODULATION

Lead modification: strategies — Optimizing binding interactions with the target

Isosteric Replacement

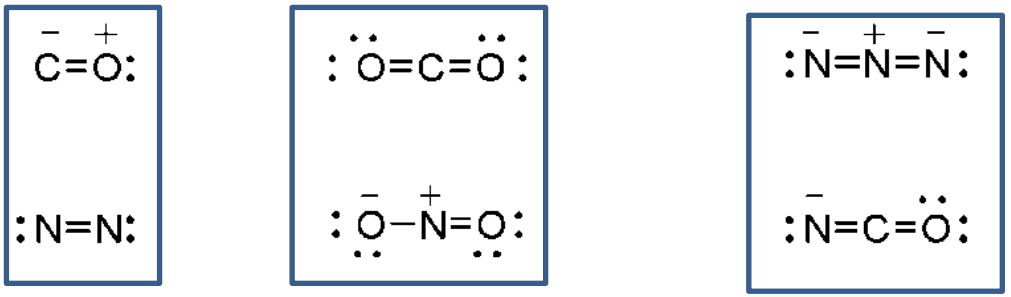
Replacing a functional group with a group of the same valence (isostere)

e.g. OH replaced by SH, NH_2, CH_3 or O replaced by S, NH, CH_2

- Leads to more controlled changes in steric/electronic properties.
- May improve binding but may also modify pharmacokinetics, toxicity, stability and synthetic processes.

Classic Isosterism (Langmuir, 1919)

Isosteres are atoms, ions or molecules that contain the same number and arrangement of electrons and the same number of atoms.



5.3. PHARMACOMODULATION

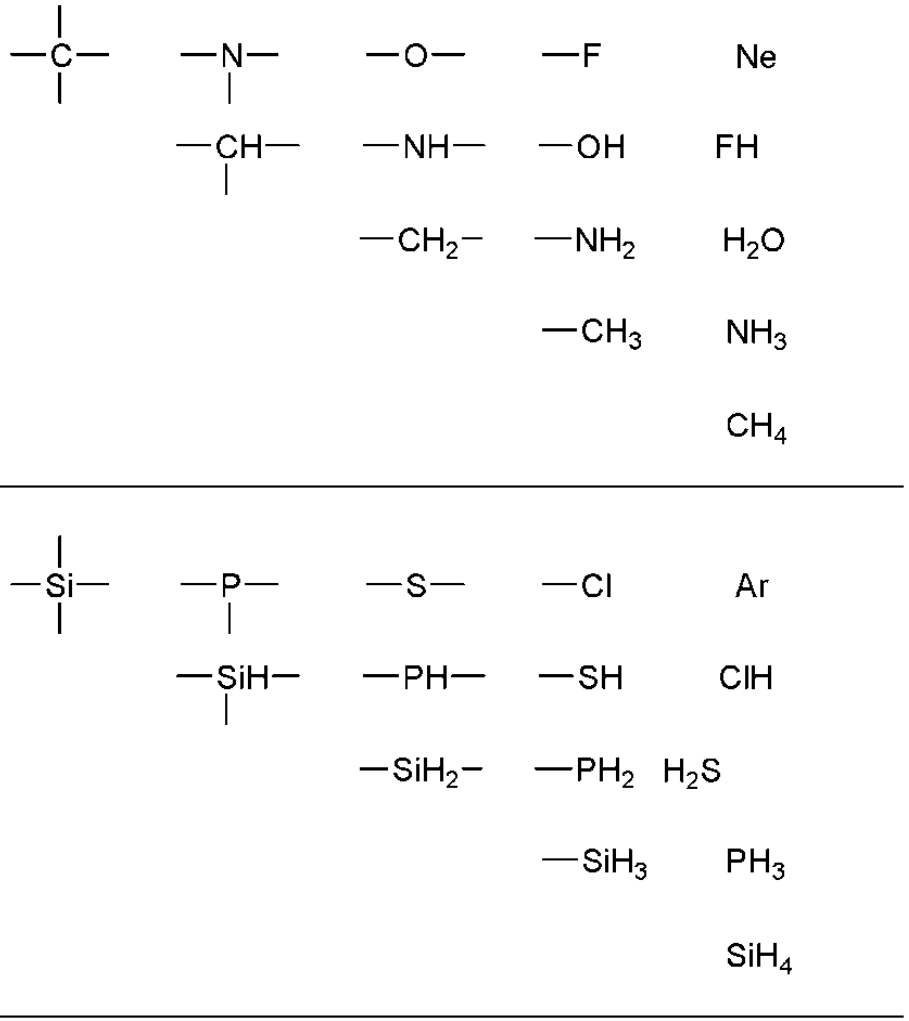
Lead modification

Classic isosterism

Grimm's Hydride Displacement Law (1925)

Addition of hydride to an atom gives to the resulting *pseudoatom* the properties of the atom with the next highest atomic number.

Isosteric and Bioisosteric Replacement



5.3. PHARMACOMODULATION

Lead modification Isosteric Replacement

Classic isosterism

Erlenmeyer's Definition (1932):
"Isosteres are elements, molecules or ions in which the peripheral layers of electrons may be considered identical".

Expansions:

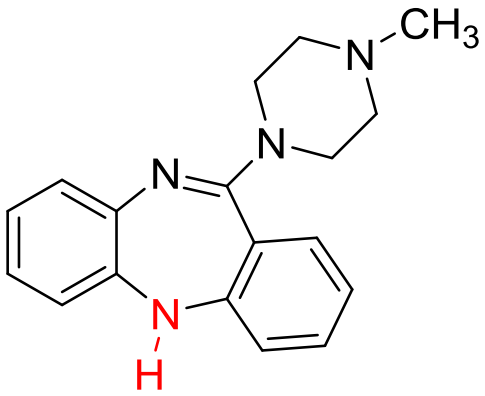
- The whole group of elements present in a given column of the periodic table
  E.g.: Si and C , S and O
- Pseudoatoms (from Grimm's law and including groups which seem totally different but which possess similar properties)
  E.g.: -Cl and -CN, -CN and -SCN
- The ring equivalents: equivalence between –CH=CH- and -S-
  E.g.: benzene and thiophene

*Isosterism criteria***:**

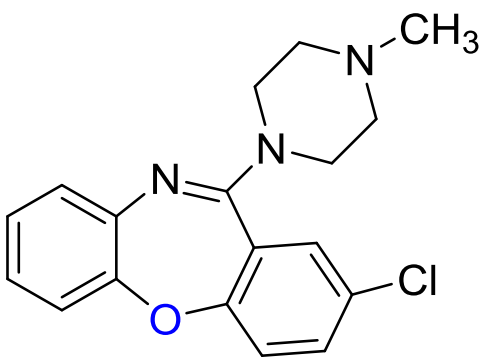
Isosteric molecules must present similar (if not identical) volume, shape and physical properties.
Some properties may be different: e.g. dipolar moment, polarity, size, etc.

Lead modification

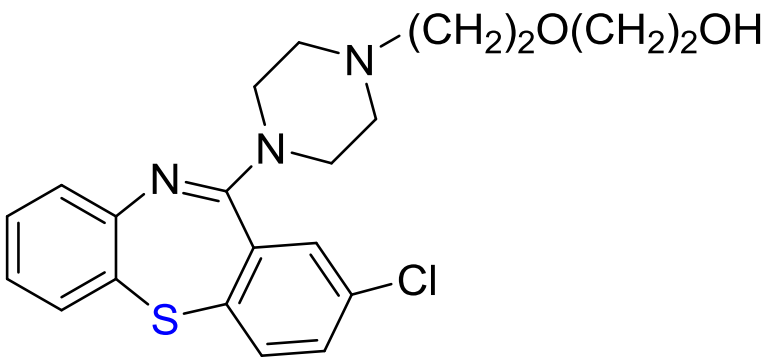
Isosteric replacement: classical (bio)isosteres



clozapine



lozapine



quetiapine

5.3. PHARMACOMODULATION

Lead modification Isosteric and Bioisosteric Replacement

Bioisosterism

Friedman (1951):

Bioisosteres are compounds that fit the broadest definition for isosteres and have the same type of biological activity (**classical bioisosteres**).

Thornber (1979):

Bioisosteres are groups or molecules which have chemical and physical similarities producing broadly similar biological effects.

(antagonism is included)

A bioisostere is a group that can be used to replace another group while retaining the desired biological activity.

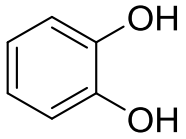
Non-classical bioisosteres:

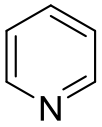
Do not have the same number of atoms or electrons and do not fit the rules of classical isosteres but show similar biological activity.

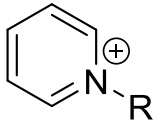
5.3. PHARMACOMODULATION

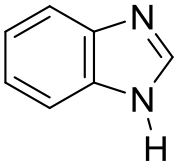
Lead modification: strategies Bioisosteric Replacement

BIOISOSTERES BY ELECTRONIC EFFECTS OR HYDROGEN BOND ABILITY

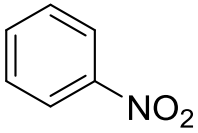




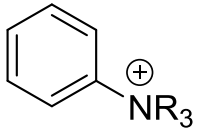




catechol/benzimidazole (electron pairs, hydrogen bonds)

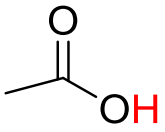


pyridine/nitrobenzene (electron density)

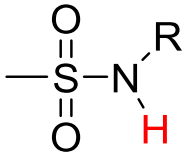


pyridinium/anilinium (charge density)

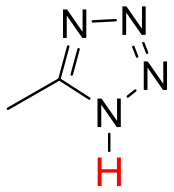
BIOISOSTERES BY ACIDIC CHARACTER



carboxyl



sulphonamide



5-tetrazolyl

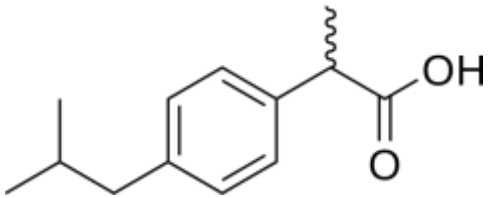
- They are ionized at physiological pH.
- They have a plane structure.
- Tetrazole is more lipophilic (better absorption).

E.g. losartan

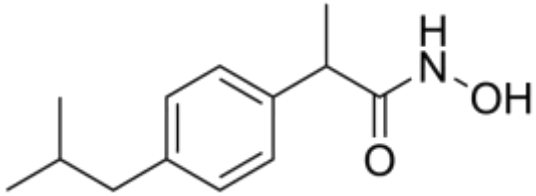
5.3. PHARMACOMODULATION

Lead modification: strategies — Bioisosteric Replacement

Carboxylic acids analogues

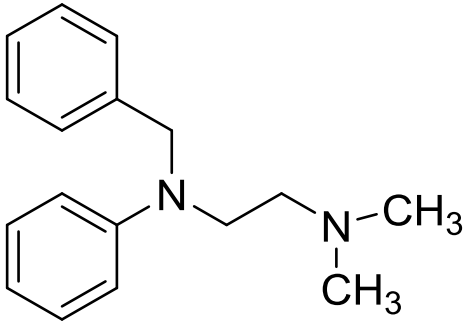


ibuprofen

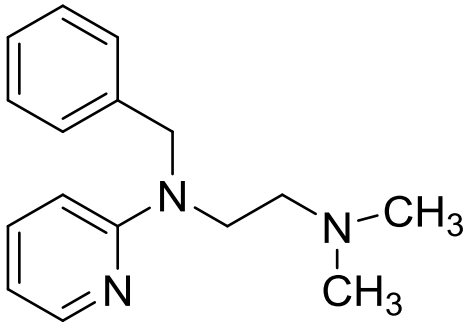


ibrupoxam
(hydroxamic acid)

Ring variation



phenbenzamine



tripenelamine

Lead modification: strategies — Isosteric and Bioisosteric Replacement

Two bioisosteric groups are similar in some properties but may differ in others.

Properties that may be affected by bioisosteric replacements:

a) Electronic distribution
b) Hybridation angles
c) Size and volume of the groups
d) Solubility
e) Acidity
f) Ability to form hydrogen bonds

5.3. PHARMACOMODULATION

Lead modification: strategies Optimizing binding interactions with the target

Simplification

- Simpler structures may fit the binding site more easily and increase activity.
- Simpler structures may be more selective and less toxic if excess functional groups are removed.
- Lead compounds are often complex and difficult to synthesize.
- Simplifying the molecule makes the synthesis of analogues easier, quicker and cheaper.

- Oversimplification may result in decreased activity and selectivity.
- Simpler molecules have more conformations.
- Simpler molecules are more likely to interact with more than one target binding site.
- Oversimplification may result in more side effects.

Methods:
•Retain the pharmacophore.
•Remove any unnecessary rings, functional groups or asymmetric carbons in small steps.

See Fig 13.50. Glipine analogues (Patrick's 5th ed).

Lead modification: strategies

Changes aimed at optimizing:

- the interaction of a drug with its target (potency and selectivity)
- the **pharmacokinetic properties** (ADME): access to the target
- **chemical** stability and synthesis

To optimize binding interactions with the target

Reasons

- To increase activity and reduce dose levels.
- To increase selectivity and reduce side effects.

Strategies

Varying alkyl substituents
Extension
Ring expansions/contractions
Varying atoms or groups (Isosteres)

Varying aryl substituents
Chain extensions/contractions
Ring variation
Simplification

Rigidification *(see Unit 3 on active conformation)*

(see Patrick's 5th ed. chapter 13)

5.3. PHARMACOMODULATION

Lead modification: strategies **Optimizing pharmacokinetic properties**

To improve membrane permeability

Varying alkyl substituents

larger alkyl groups increase hydrophobicity (homologous).
Caution: may interfere with target binding for steric reasons.

Masking or removing polar groups

increases the hydrophobic character.
Caution: do not modify the necessary polar groups for target binding (see prodrugs).
Methods:
ethers from alcohols, amides from amines, esters from acids, etc.

Bioisosteric replacement (e.g. –COOH and tetrazole ring).

(see Patrick's 5th ed. chapter 14)

5.3. PHARMACOMODULATION

Lead modification: strategies **Optimizing pharmacokinetic properties**

To improve solubility or distribution

Adding polar groups

- decreases the hydrophobic character.
- is useful for targeting drugs versus gut infections (sulphamides).
- is useful for reducing CNS side effects.

  Caution: may introduce unwanted side effects.

Varying pKa

- in order to obtain the required ratio of ionized to unionized drug.

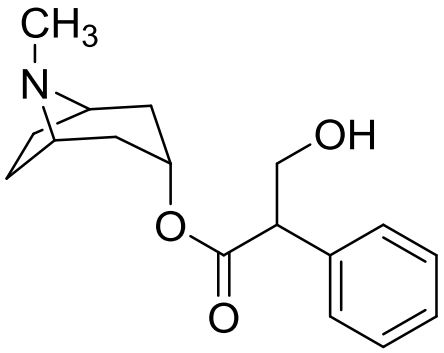
  Caution: may affect binding interactions.

Methods:

- varying alkyl substituents on amine nitrogen.
- varying aryl substituents in aromatic amines or aromatic carboxylic acids
- bioisosteres.

Lead modification: strategies Optimizing pharmacokinetic properties

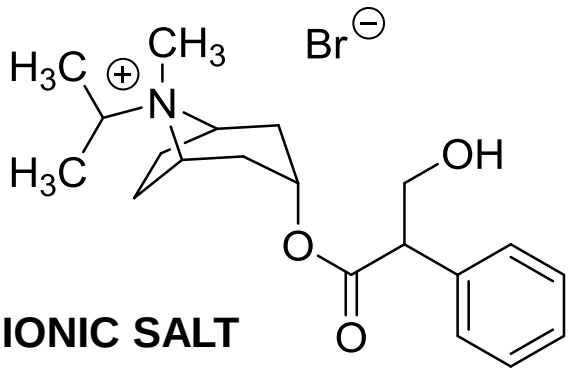
To improve solubility or distribution



atropine

central and peripheral anticholinergic

Crosses the blood brain barrier and has important side effects.



ipratropium bromide

exclusively peripheral anticholinergic

5.3. PHARMACOMODULATION

Lead modification: strategies — Optimizing pharmacokinetic properties

To increase drug stability

Steric shields

Introduce bulky group as a shield.
Protect a susceptible functional group from hydrolysis.
Hinder attack by nucleophiles or enzymes.

Electronic stabilization of labile functional groups

e.g. Bioisosteres. Replacing esters with carbamates or amides.

Steric and electronic effects in combination

(See the example in questions from procaine to lidocaine.)

Metabolic blockers

Introduce groups at a susceptible site to block the reaction.
Bioisosteres (e.g. replacing H with F).

Removing or replacing a susceptible group with a metabolically more stable one

Bioisosteres (replacing methyl groups with Cl).
e.g. modification of tolbutamide to chlorpropamide.

Shifting susceptible metabolic groups that are important for binding

e.g. salbutamol.

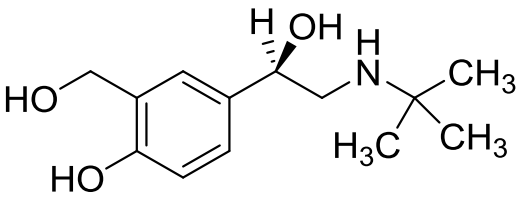
(see Unit 4 – soft and hard drugs)

Lead modification: strategies

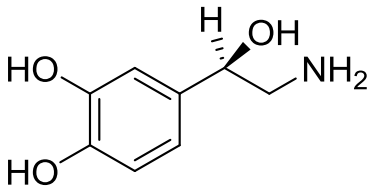
Optimizing pharmacokinetic properties

To increase drug stability

Shifting a susceptible OH phenolic group



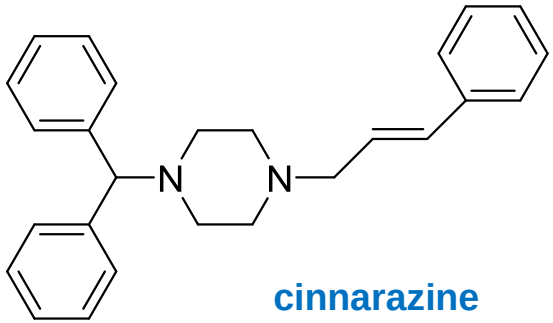
salbutamol



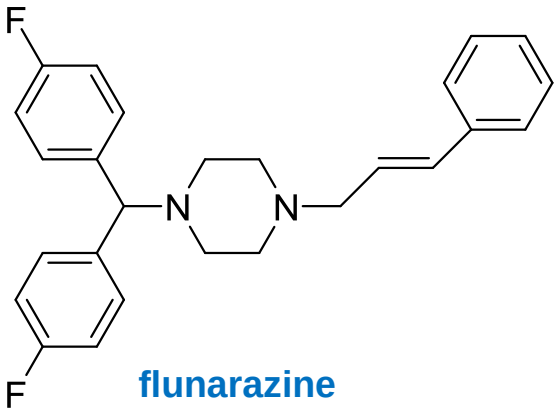
noradrenaline

Benzylic alcohol is not a good substrate for COMT.
The presence of a HBD is important for activity.

Bioisosterism between H and F



cinnarazine



flunarazine

Cerebral vasodilator

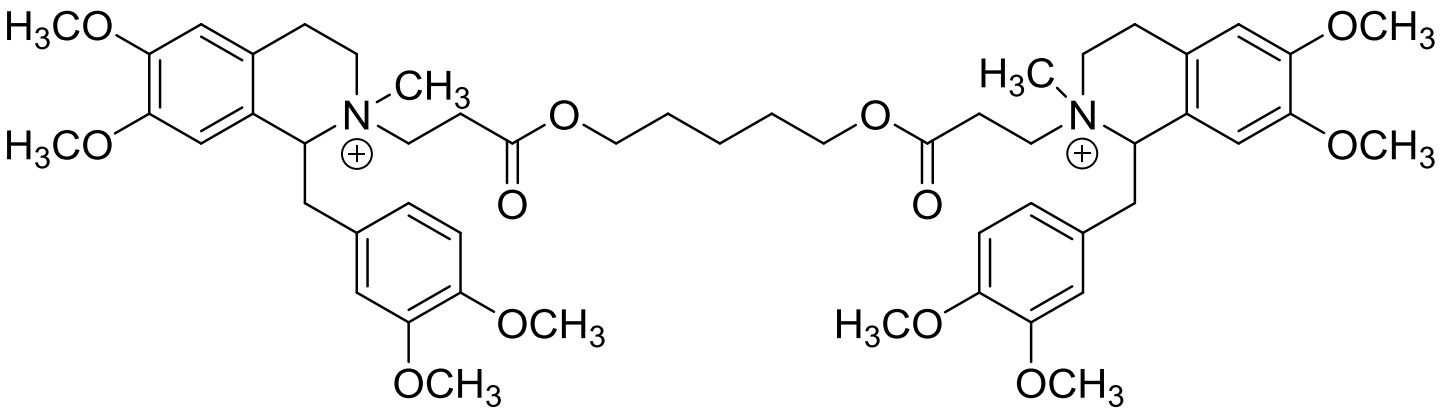
Fluorine atoms prevent para-hydroxylation.

Lead modification: strategies Optimizing pharmacokinetic properties

To decrease drug stability

(see Unit 4 on soft and hard drugs)

Introducing metabolic groups susceptible to Phase I or Phase II metabolic reactions or non-enzymatic reactions:
e.g. atracurium (self-destruct drug) a neuromuscular blocking agent.



- Is stable at acid pH and unstable at blood pH (slightly alkaline).
- Self-destructs by Hoffmann elimination and has a short lifetime.
- Enables the anaesthetist to control dose levels accurately.
- Post-surgical recovery times are short.

5.3. PHARMACOMODULATION

Lead modification: strategies Optimizing pharmacokinetic properties

To reduce drug toxicity *(TOXICOLOGY)*

Removing or replacing functional groups known to be toxic or varying the positions of substituents e.g.:

aromatic nitro groups	aromatic amines
bromoarenes	hydrazines
polyhalogenated groups	hydroxylamines

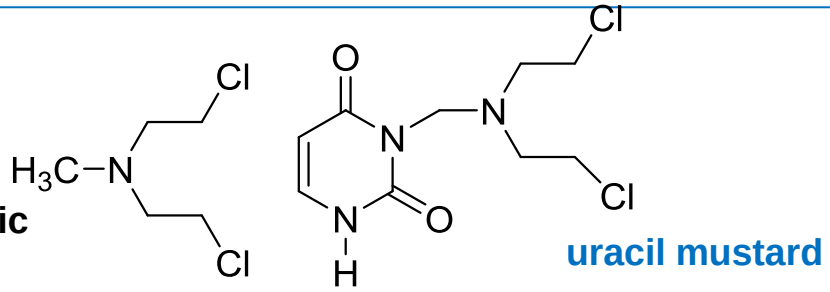
Targeting drugs

Linking a drug to a biosynthetic building block (amino acid, nucleic bases, antibodies)

Carrier proteins introduce the modified drug into the cells.

(The concept is the same as with prodrugs. The difference is that the analogue must be active.) E.g. anticancer drugs.

Non-selective/toxic



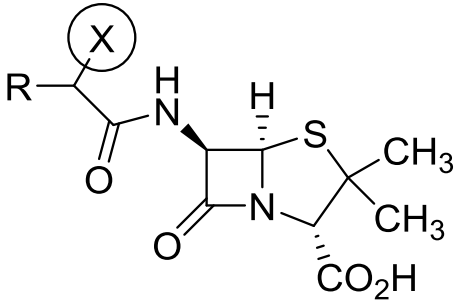
uracil mustard

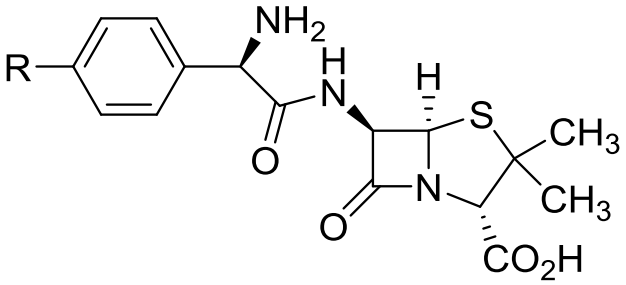
Increasing the ionization degree: gut infections, peripheral over central activity, etc.

5.3. PHARMACOMODULATION

Lead modification: strategies Increasing chemical stability

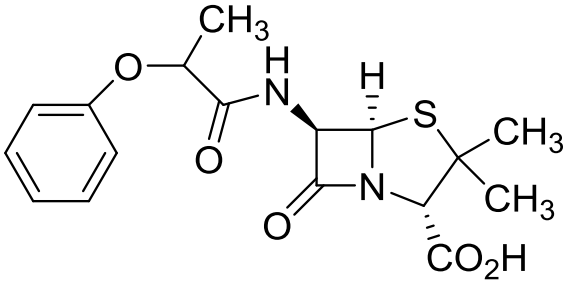
Electron withdrawing group
by inductive effect





R = H **ampicillin**

R = OH **amoxicillin**

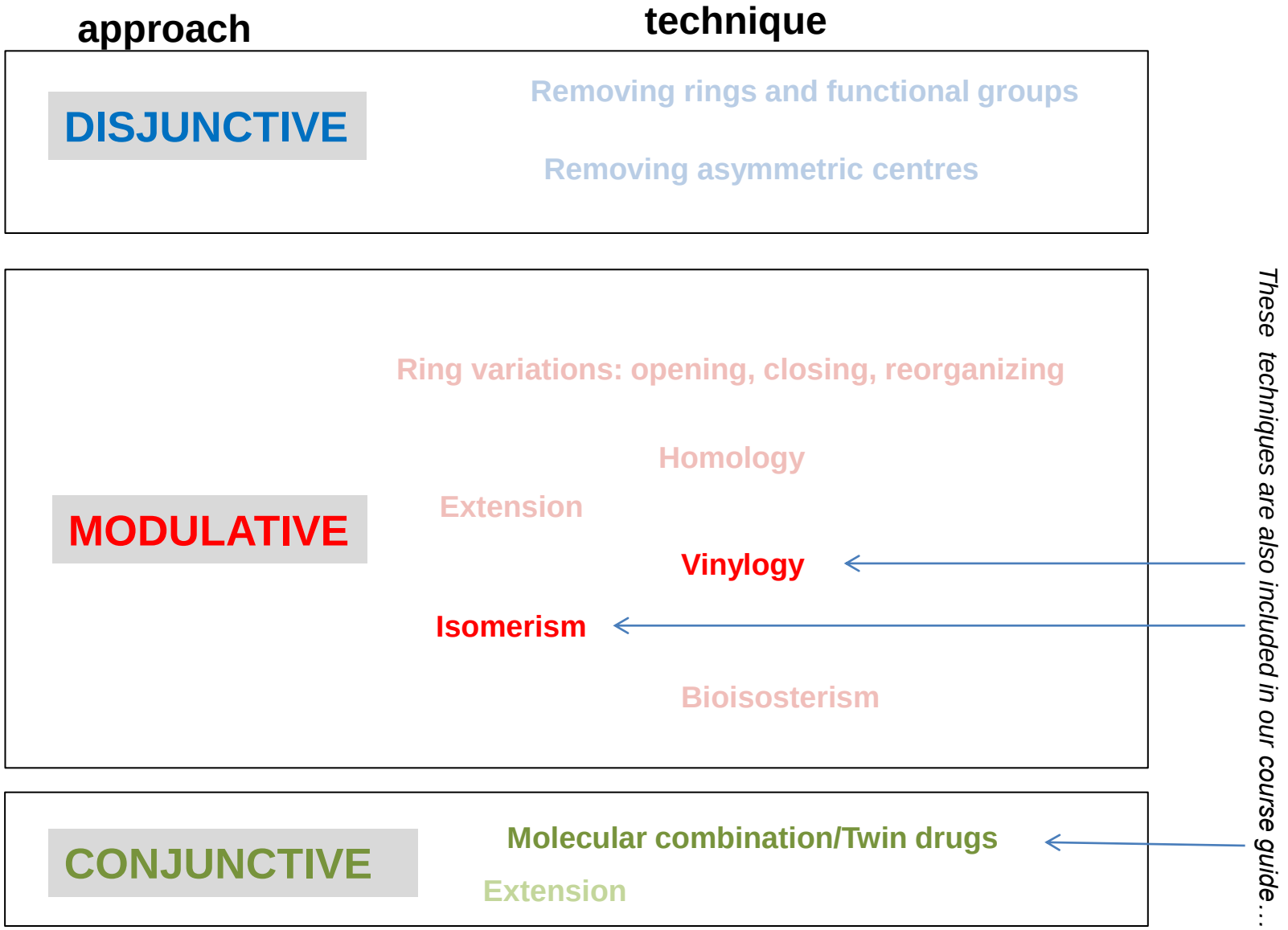


phenethicillin

PENICILLINS Increase resistance to acid hydrolysis.

5.3. PHARMACOMODULATION

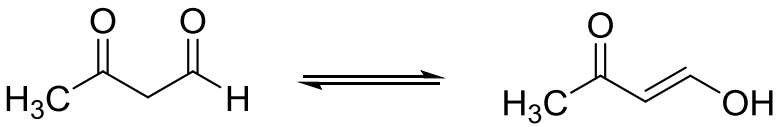
Lead modification: techniques



Lead modification: techniques

Vinylogy

Modulative approaches

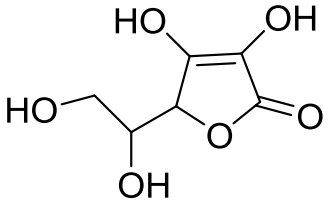


Formylacetone
(3-oxobutanal)

Enol form

Acetic acid (pKa = 4,7)

Formylacetone (enolic form) and ascorbic acid are comparable in acidity to carboxylic acids.



Ascorbic acid (pKa = 4,7)

The vinyl group in these molecules plays the role of an electron-conducting channel between the carbonyl and the hydroxyl group
(resonance or mesomeric effect).

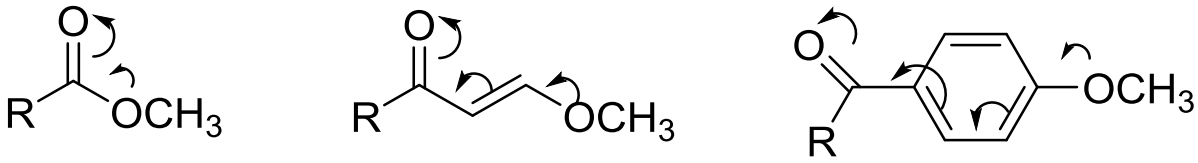
Vinylogy is the transmission of electronic effects of the atoms or groups through an unsaturated organic bonding system.
(Double and triple bonds and aromatic rings, including the presence of heteroatoms).

Lead modification: techniques

Vinylogy

Modulative approaches

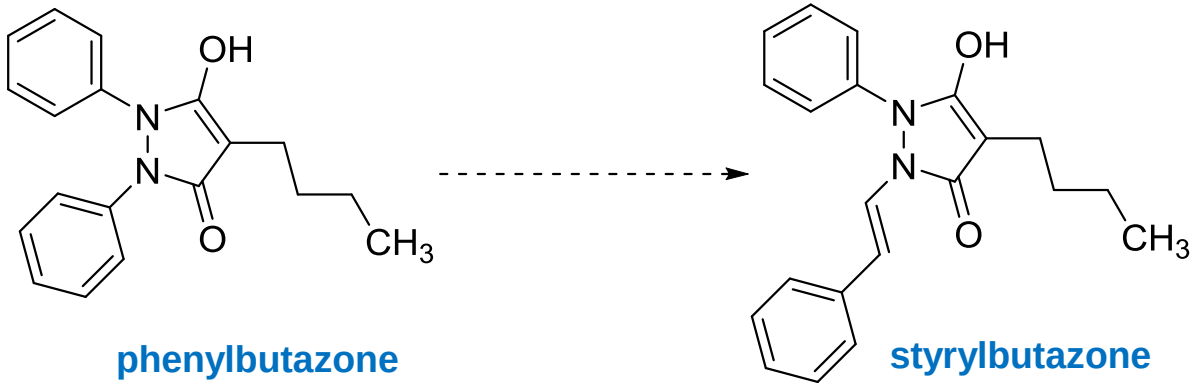
X - Y When X and Y are groups where electrons can be delocalized.



Vinylogous **Arenologous**

The electronic properties of vinylogues are similar but the lipophilicity, shape and flexibility of the molecule are modified.

Example:



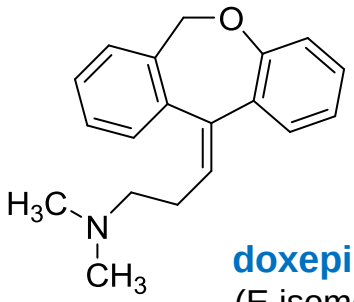
phenylbutazone **styrylbutazone**

Lead modification: techniques

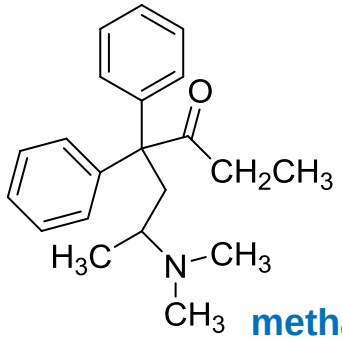
Modulative approaches

Isomerism

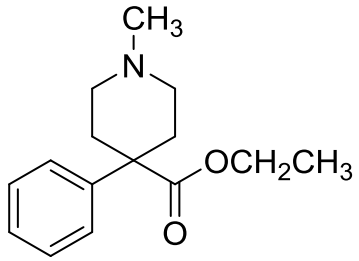
Important changes in the shape and position of certain groups



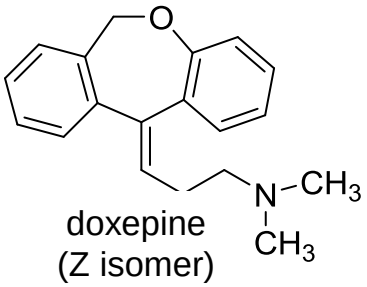
doxepine
(E isomer)



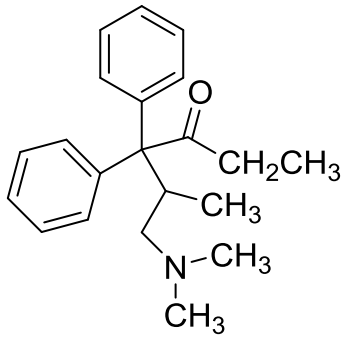
methadone



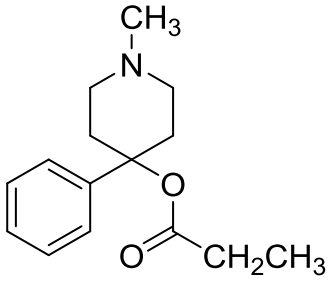
pethidine



doxepine
(Z isomer)



isomethadone



alphaprodine

double bond

position

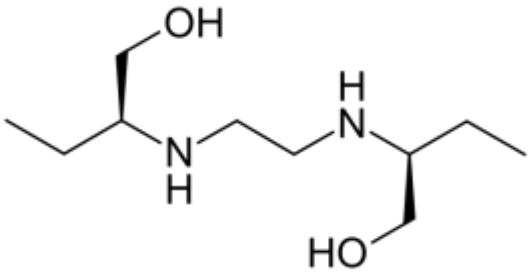
inverse ester

5.3. PHARMACOMODULATION

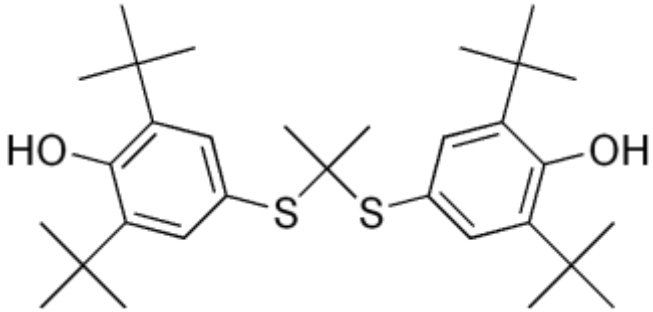
Lead modification: techniques

Drug duplication: symmetrical twin drugs

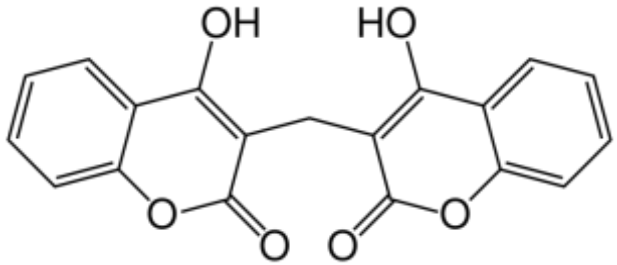
Conjunctive approaches



ethambutol
(tuberculostatic)



probucol
(anti-hyperlipidemic)

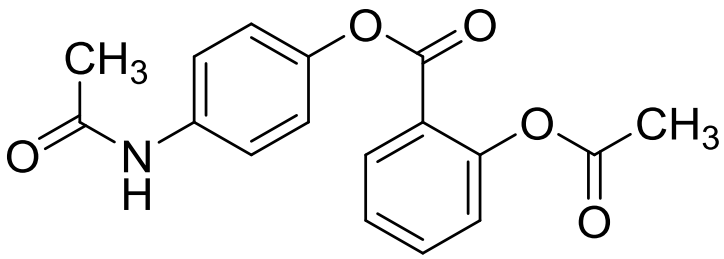


dicumarol
(anticoagulant)

Metabolically non-reversible – different phamacokinetics

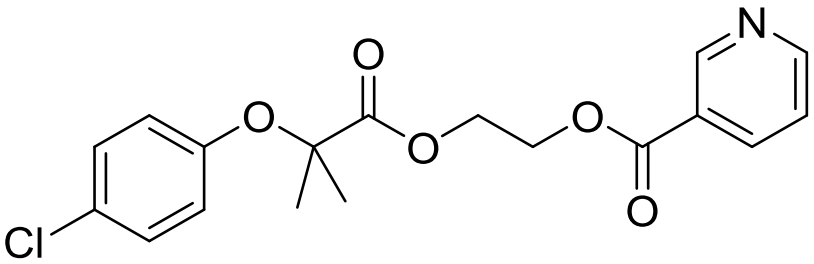
Lead modification: techniques

Drug association: non-symmetrical twin drugs **Conjunctive approaches**



benorilate
aspirine + paracetamol
(analgesic)

Co-drugs or hybrids



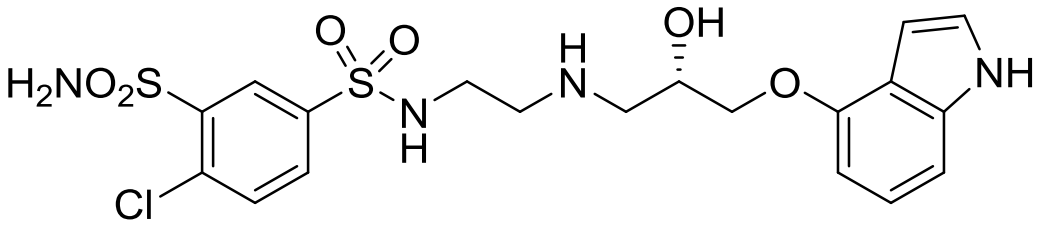
etofibrate
clofibric acid + nicotinic acid
(cholesterol and triglyceride reducer)

Metabolically reversible:
Prodrugs whose bioactivation leads to two different drugs with synergic affects.

5.3. PHARMACOMODULATION

Lead modification: techniques

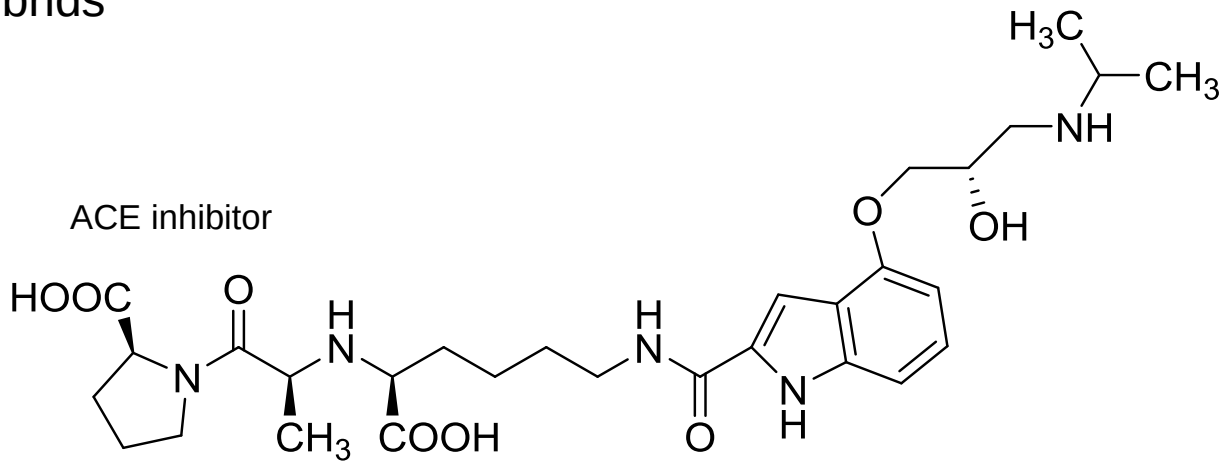
Drug association: non-symmetrical twin drugs **Conjunctive approaches**



chlorothiazide derivative
(diuretic)

pindolol
(adrenergic β_1 blocker)

Co-drugs or hybrids



ACE inhibitor

antihypertensive

Metabolically non-reversible: multi-target drugs *(take a single tablet)*

5.3. PHARMACOMODULATION

The lead optimization process: guidelines

- The possibilities for molecular variations around the lead structure are immense.
- Deciding which compounds to prepare and which ones to reject is not easy.

- If information about the ligand-target interactions is available from experimental or computational data:

  Is the lead relevant to a computer-aided design?
- When no information is available:

  design analogues to identify which molecular features are favourable to the activity and which are detrimental (not only activity against the target but also other important properties of the future drug molecules with regard to pharmacokinetics, chemical stability and toxicity).

1. The minor modification rule

When designing analogues, first use simple organic reactions (hydrogenations, methylations, acetylations, changes to substituents, and isosteric replacements, etc.).

Wermuth's 4th ed, chapter no 12

5.3. PHARMACOMODULATION

The lead optimization process: guidelines

2. The biological logic rule

Make the earliest possible use of biological data.

3. The structural logic rule

As soon as certain structural data are available (distances, conformations, substituent orientations, etc.), they should be fed back into the drug design.
E.g. use pharmacophore identification or receptor mapping.

4. The right substituents

It is important to choose the substituents based on their lipophilic, electronic or steric properties.

5. The easy organic synthesis rule

Synthesising new compounds is a costly and lengthy process so first prepare those whose synthesis is easiest and for which intermediates are commercially available). The synthesis of heterocycles has many advantages.

Wermuth's 4th ed, chapter 12

The lead optimization process: guidelines

6. Eliminate chiral centres

Racemates and both enantiomers are usually three different pharmacological entities (pharmacological, toxicological and clinical research is required before it can be decided whether it is advantageous to use racemates or enantiomers in clinical practice).

7. The Pharmacological Logic Rule

When designing analogues, compare the results for your compounds with those of reference compounds published by competitors (resynthesize if necessary).

When SAR studies enable the molecular features associated with high activity to be identified, also prepare at least one compound that, according to your results, should be inactive.

Wermuth's 4th ed, chapter 12

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

Chemical messengers as leads

MODULATION of the activity of chemical messengers can take place on different levels:

ACTIVATION AND/OR RECEPTOR BLOCKING RECEPTOR

AGONISTS AND ANTAGONISTS

AUTORECEPTOR
modulating neurotransmitter release and synthesis

STORAGE CARRIER PROTEINS REUPTAKE PROTEINS

ENZYMES (BIOSYNTHESIS AND DEGRADATION)

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

RECEPTORS: Chemical messengers as leads

Design of Agonists

- Important binding interactions in natural messengers must be identified.
- Agonists are designed to have functional groups capable of the same interactions.
- An agonist usually requires the same number of interactions.
- Modulative pharmacomodulation techniques are usually used.

Requirements

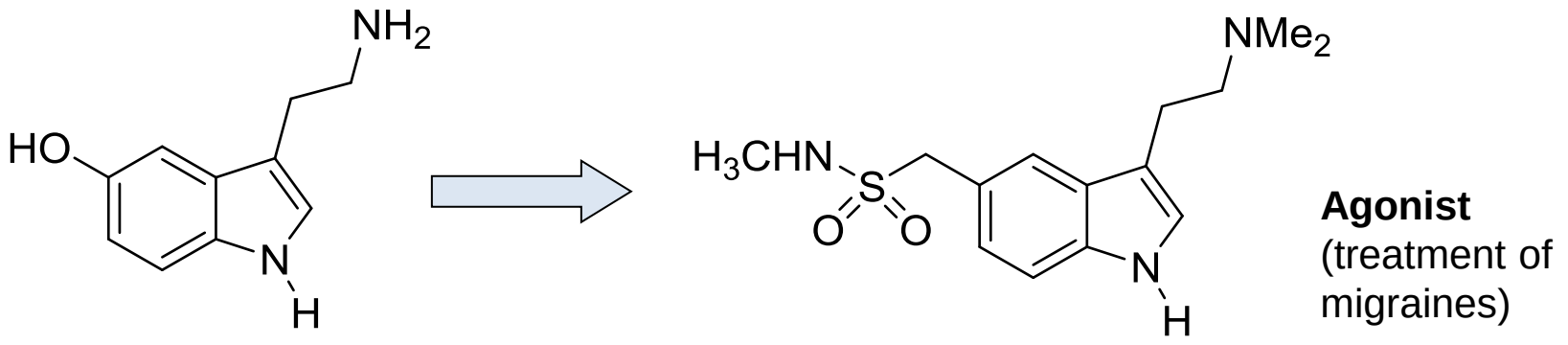
- The agonist must have the **correct binding groups**.
- The binding groups must be correctly **positioned** in order to interact with complementary binding regions.
- The drug must have the correct **shape** to fit the binding site.

- One enantiomer of a chiral drug normally binds more effectively than the other (eutomer and distomer).
- Different enantiomers are likely to have different biological properties.

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

RECEPTORS: Chemical messengers as leads

Design of Agonists



5-hydroxytryptamine

sumatriptan

Agonist
(treatment of migraines)

Many examples of drugs that act as agonists have been developed from the corresponding chemical messengers.

Allosteric modulators

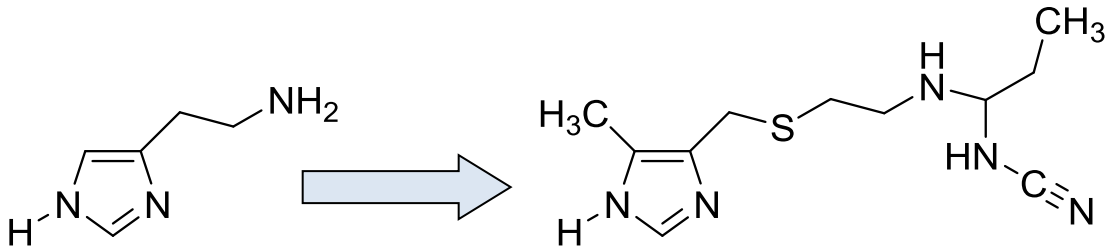
These are agents which enhance receptor activity by binding to an allosteric binding site rather than to the messenger binding site.
The structure of allosteric modulators does not have to be similar to that of the agonist since they do not share the same binding site (the steric and electronic requirements are different).

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

RECEPTORS: Chemical messengers as leads

Reversible Antagonists

- Antagonists bind to the binding site but fail to produce the correct induced fit (the receptor is not activated).
- The normal messenger is blocked from binding.
- Antagonists can form binding interactions with binding regions in the binding site that is not used by the natural messenger (new elements added: extension).



histamine **cimetidine** (anti-ulcer)

See Patrick's 5th ed. Chapter 25

(Wikipedia):

Cimetidine was the prototypical histamine H2 receptor antagonist from which the later members of the class were developed. Cimetidine was the culmination of a project at Smith, Kline and French (SK&F; now GlaxoSmithKline) by James W. Black, C. Robin Ganellin and others to develop a histamine receptor antagonist to suppress stomach acid secretion. **This was one of the first drugs discovered using a rational drug design approach.** Sir James W. Black shared the 1988 Nobel Prize in Physiology or Medicine for the discovery of propranolol (selective adrenergic antagonist) and also is credited for the discovery of cimetidine

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

RECEPTORS: Chemical messengers as leads

Irreversible Antagonists

- The antagonist binds irreversibly to the binding site.
- A different induced fit means that the receptor is not activated.
- A covalent bond is formed between the drug and the receptor.
- The messenger is blocked from the binding site.
- Increasing the messenger concentration does not reverse antagonism.
- **Irreversible antagonists are often used to label receptors** (not in therapeutics).
- **They have a similar structure** to the chemical messenger.
- **They have reactive functional groups:** depending on the groups that act in the catalysis.

Allosteric Antagonists

- The antagonist binds reversibly to an allosteric binding site.
- Intermolecular bonds are formed between the antagonist and the binding site.
- The induced fit alters the shape of the receptor.
- The binding site is distorted and is not recognised by the messenger.
- Increasing the messenger concentration does not reverse antagonism.
- **The structure of allosteric antagonists does not have to be similar to that of the chemical messenger** since they have different binding sites (the steric and electronic requirements are different).

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

Enzymes	Enzyme targets for useful medications
1 **Antibacterial agents (unit 8)**	Dihydropteroate synthetase, transpeptidase
2 **Antiviral agents**	HIV reverse transcriptase, HIV protease, viral DNA polymerase
3 **Anti-inflammatory agents**	Cyclooxygenase
4 **Cholesterol lowering agents**	HMG-CoA reductase
5 **Antidepressants** (unit 11)	Monoamine oxidase
6 **Anticancer agents**	Tyrosine kinase, dihydrofolate reductase, thymidylate synthase, aromatase, etc.
7 **Antihypertensive agents**	Renin, angiotensin converting enzyme
8 **Treatment of male erectile dysfunction**	Phosphodiesterase
9 **Anti-gout agents**	Xanthine oxidase
10 **Anti-ulcer agents**	Proton pump
11 **Alzheimer disease**	Cholinesterases
12 **Diuretics**	Carbonic anhydrase

Enzyme Inhibitors

reversible inhibition

By structural analogy with substrate: antimetabolites
By structural analogy with transition state

irreversible inhibition

(or ENZYMATIC INACTIVATION)

Addressed to the active centre
Suicide inhibitors

Reversible inhibitors

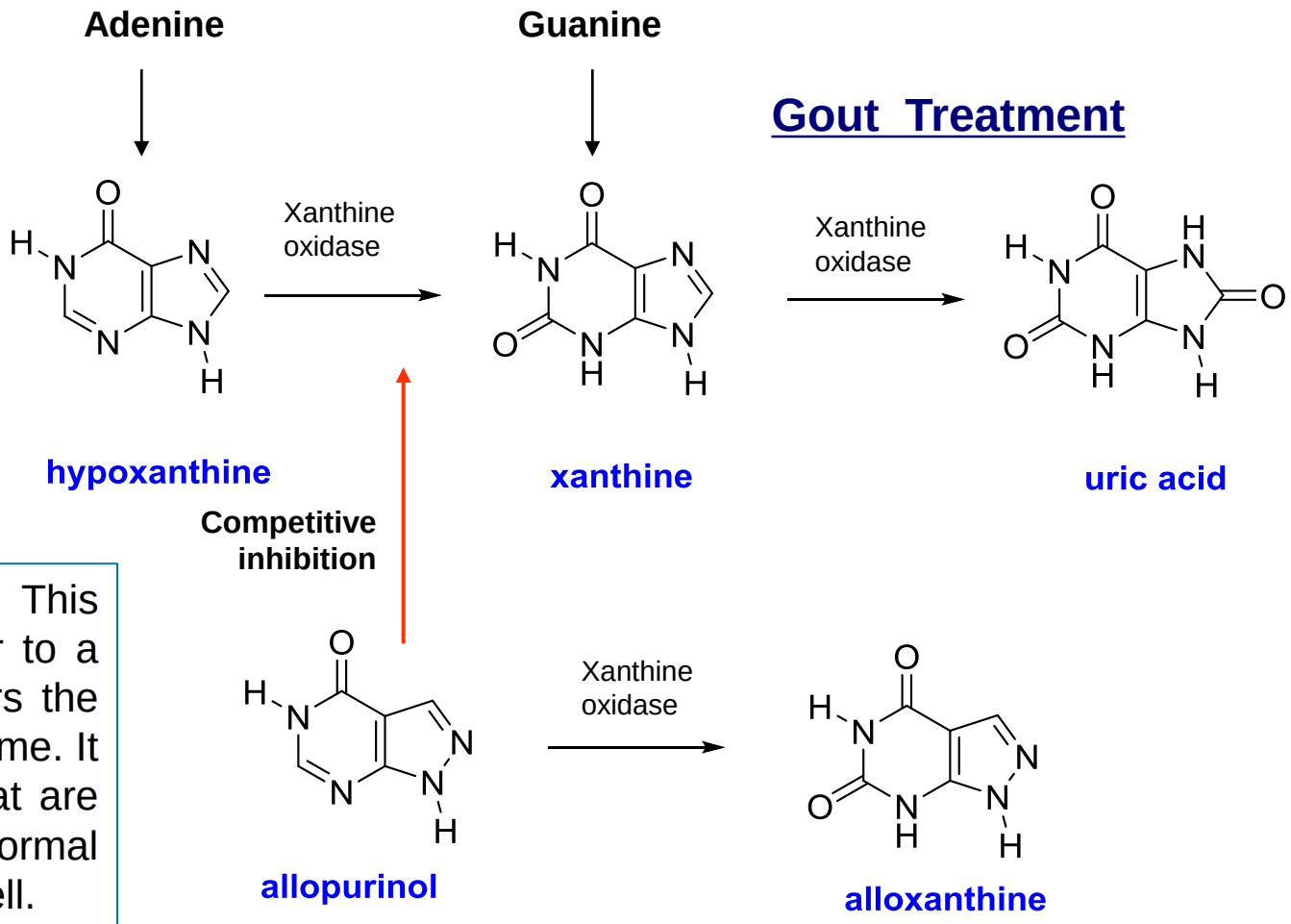
- The inhibitor binds reversibly to the active site.
- Intermolecular bonds are involved in binding.
- The inhibitor undergoes no reaction.
- Inhibition depends on the strength of inhibitor binding and inhibitor concentration.
- The substrate is blocked from the active site.
- Increasing the substrate concentration reverses inhibition.
- The inhibitor is likely to be similar in structure to the substrate, product or cofactor.

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

ENZYMES: substrates as leads

Reversible inhibitors

By structural analogy with substrate



Antimetabolite: This compound is similar to a substrate that suffers the reaction by the enzyme. It inhibits enzymes that are crucial to the normal metabolism of the cell.
It is used in antibacterial and anticancer therapy.

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

ENZYMES: substrates as leads

Reversible inhibitors

Transition state inhibitors

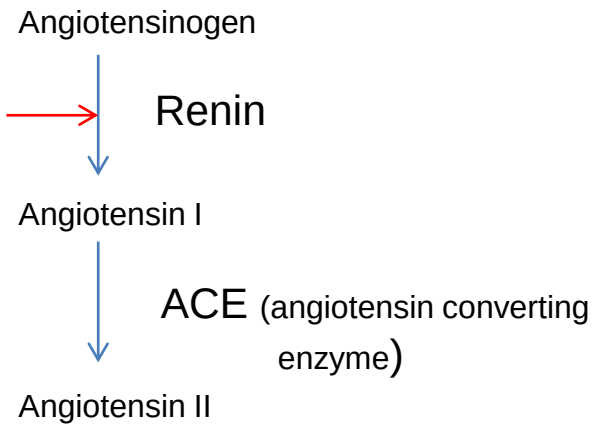
Drugs designed to mimic the transition state of an enzyme-catalysed reaction:

- Are likely to bind more strongly than drugs that mimic the substrate or product.
- Are high-energy, transient species that cannot be isolated or synthesised.

Drug design may be based on reaction intermediates which are closer in character to transition states than to substrates or products.

The drug mimics the stereochemistry and binding properties of the reaction intermediate but is stable.

Renin inhibitors



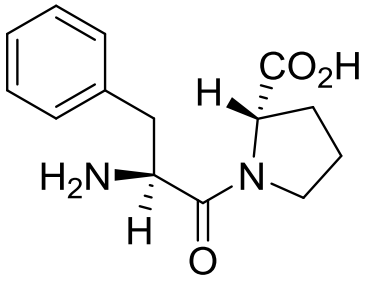
•Renin inhibitors block the synthesis of angiotensin I and II.
• Angiotensin II constricts blood vessels and raises blood pressure.
• Renin inhibitors act as antihypertensives (drugs to lower blood pressure).

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

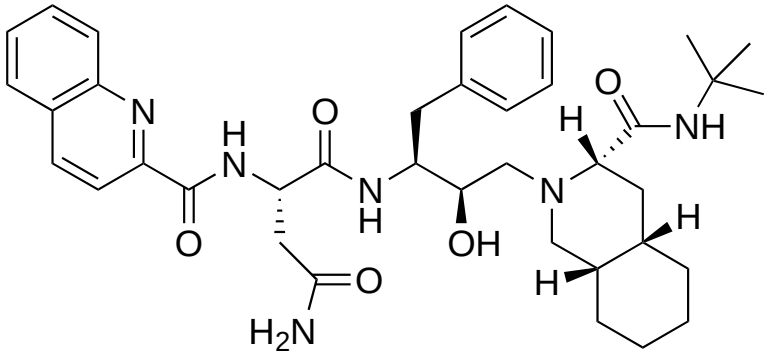
ENZYMES: substrates as leads

Reversible inhibitors

Transition state inhibitors



L-Phe-L-Pro



saquinavir

It is important to know the structure of the active site and the mechanism of the catalytic reaction.

Examples of reversible inhibitors

ACE Inhibitors
Protease inhibitors
Kinase inhibitors

Diuretics
Sulphonamides

Statins
Antidepressants

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

ENZYMES: substrates as leads

Irreversible inhibitors

The inhibitor binds irreversibly to the active site:
- A covalent bond is formed between the drug and the enzyme.
- The substrate is blocked from the active site.
- Increasing substrate concentration does not reverse inhibition.
- The inhibitor is likely to be **similar** in **structure** to the substrate.
- **Electrophilic functional groups are** present in irreversible inhibitors:

Alkyl halides	**Epoxides**	**α,β-Unsaturated ketones**
Fluorophosphonates	**β-lactams**	**Lactones**

Hydroxyl, amino or thiol groups from amino acids in the active site of the enzyme react with these groups.

Examples

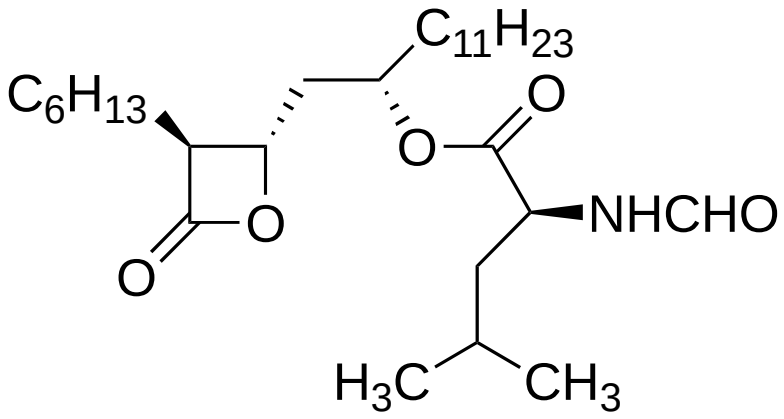
Nerve gases	Penicillins	Disulfuram	Cephalosporins
Proton pump inhibitors		Orlistat	Aspirin

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

ENZYMES: substrates as leads

Irreversible inhibitors

Orlistat is an anti-obesity drug that inhibits pancreatic lipase.



- The enzyme is blocked from digesting fats in the intestine.
- Fatty acids and glycerol are therefore less absorbed.
- Biosynthesis of fat in the body is reduced.

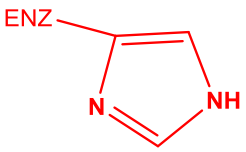
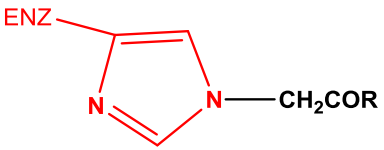
See Box 7.2 Patrick's 5th ed. Chapter 7 p.90

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

ENZYMES: substrates as leads

Irreversible inhibitors

More examples

NUCLEOPHILE	ELECTROPHILIC GROUP OF THE INHIBITOR	PRODUCTS
ENZ- NH_2	RCOOCOR (anhydrides)	RCONH-ENZ
	ROCR'=NH (imidates)	HN=CR'NH-ENZ
	$ArSO_2X$ (arenosulphonyl hydrides)	$ArSO_2$NH-ENZ
ENZ-OH	$(RO)_2PO(X)$ (phosphates)	$(RO)_2PO$-O-ENZ
	XCH_2COR (2-haloketones)	
ENZ-SH	RCH=CH-C=O (conjugated carbonyls)	ENZ-S-$CHRCH_2C$=O

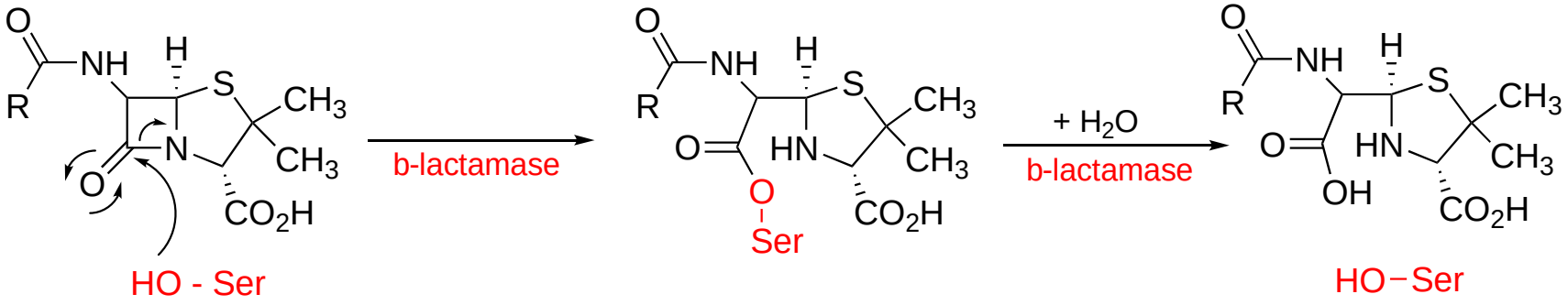
5.4. RATIONAL STRATEGIES IN DRUG DESIGN

ENZYMES: substrates as leads

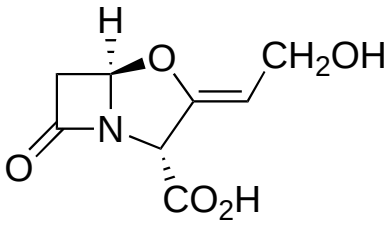
Irreversible inhibitors

Suicide substrates

Penicillin is an irreversible inhibitor of bacterial transpeptidase. Penicillin is a substrate of β-lactamase.



Clavulanic acid is a suicide inhibitor of β-lactamase with a similar structure and reactive latent functionality.



See Fig 7.10 (Patrick's 5th ed). Clavulanic acid acting as a suicide substrate

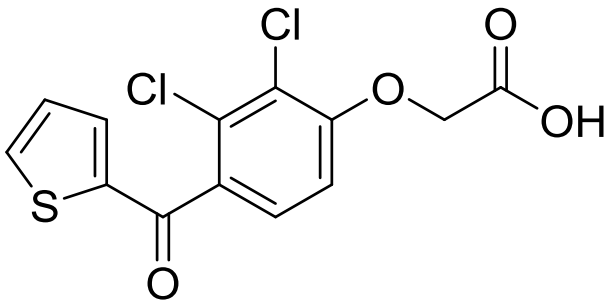
5.4. RATIONAL STRATEGIES IN DRUG DESIGN

ENZYMES: substrates as leads

Irreversible inhibitors

Suicide substrates

Inhibition of cyt P450 : toxicity



tienilic acid

- Tienilic acid was marketed as a diuretic.
- It was withdrawn due to interaction with cytochrome P450 enzymes.
- The agent acts as a suicide substrate on cytochrome P450 enzymes.

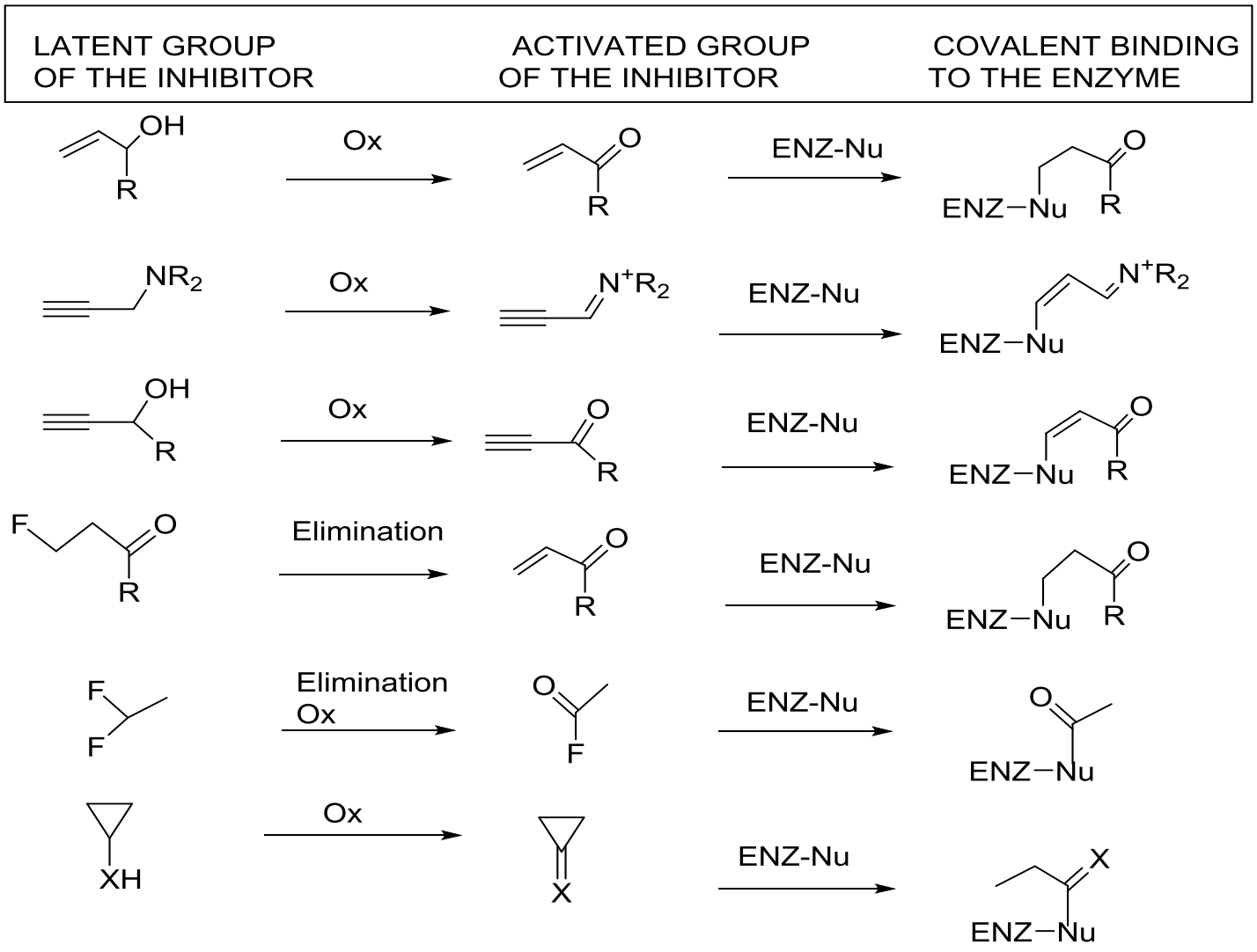
See Box 7.3 Patrick's 5th ed.

5.4. RATIONAL STRATEGIES IN DRUG DESIGN

ENZYMES

Irreversible inhibitors

Suicide substrates



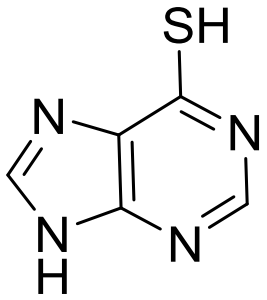
5.4. RATIONAL STRATEGIES IN DRUG DESIGN

ENZYMES

Inhibitors acting at allosteric binding sites

- The inhibitor binds reversibly o irreversibly to the allosteric site.
- Induced fit alters the shape of the enzyme and the active site is distorted and not recognised by the substrate.
- Increasing the substrate concentration does not reverse inhibition.
- **The inhibitor is not similar in structure to the substrate**.
- **The inhibitor can be similar in structure to the final product of a multistep metabolic route.**

6-Mercaptopurine



- Inhibits the first enzyme in the biosynthesis of purines.
- Blocks the biosynthesis of purines and DNA.
- Is used to treat leukaemia.

UNIT 6. QUANTITATIVE STRUCTURE - BIOLOGICAL ACTIVITY RELATIONSHIPS (QSAR)

6.1. Introduction.

6.2. Physicochemical parameters: Hammett equation (electronic effects), Taft equation (steric factors) and Hansch equation (hydrophobic factors).

6.3. QSAR examples. Methods used to correlate physical and chemical parameters with biological activity. Examples.

Patrick's 5th ed. Ch. 17 and 18

Silverman's 3rd ed. Ch. 2

Delgado's Ch. 8

6.1. INTRODUCTION TO QSAR

ALREADY SEEN IN PREVIOUS UNITS:

- Changes in shape or changes in functional groups or substituents can improve pharmacodynamics and/or pharmacokinetics.
- Synthesising analogues of a lead compound is one strategy used in the design of drugs.
- The number of possible analogues is almost infinite.

THIS UNIT:

- Quantitative structure-activity relationships (QSAR) are rational approaches for deciding which substituents to use.
- Classical QSAR consider only 2D structures and approaches attempt to identify and quantify the physicochemical properties or structural elements of a drug and determine whether any of them affect the drug's biological activity.
- 3D-QSAR analysis (Correlative Molecular Fields Analysis (CoMFA)) starts from 3D structures and correlates biological activities with 3D-property fields.

6.1. INTRODUCTION TO QSAR

Historical Overview

1868: Crum-Brown and Fraser predicted that a mathematical relationship between structure and activity would be expressed.

1962: Hansch and Fujita attempted to quantify the effects of certain substituent modifications and developed QSARs.

1988: Cramer developed 3D QSAR.

Hansch paradigm

1. Biological activity depends on the drug structure.
2. The drug structure involves certain global properties such as hydrophobicity, charge and volume in a certain position of the molecule.
3. These global and local properties can be quantified by physicochemical parameters.
4. There is always a function which relates changes in biological activity with changes in global and local properties, though it may be neither simple nor obvious.

6.1. INTRODUCTION TO QSAR

QSAR is an attempt to remove the element of luck from drug design by establishing a mathematical relationship, in the form of an equation, between biological activity and measurable physicochemical parameters that represent properties that have a major influence on the drug's activity.

Biological Activity (BA) = **f (drug properties)**

BA can be expressed in $1/LD_{50}$, $1/ED_{50}$, 1/C, log1/C

Biological activity can be expressed as the reciprocal of the concentration of drug required to achieve a defined level of biological activity (1/C).

BA = F (parameters) =
***f* (physicochemical) + *g* (structural) + *h* (theoretical) + etc.**

- The equation usually includes log *P* and electronic and steric factors.
- The typical equation for a wide range of log *P* is parabolic.

$$\text{Log } (1/C) = - k_1 (\log P)^2 + k_2 \log P + k_3\sigma + k_4 E_s + k_5$$

Hansch Equation

6.1. INTRODUCTION TO QSAR

Basic Requirements in QSAR Studies:

- The compounds studied must be structurally related.
- They act at the same target and bind in a comparable manner.
- They exert the same mechanism of action.
- Binding affinity is correlated to interaction energies.
- Biological activities are correlated to binding affinity.
- Correct testing procedures must be used.

(E.g. for enzyme inhibitors, *in vivo* tests are not valid since both pharmacodynamic and pharmacokinetics factors come into play. It is better to use isolated enzymes).

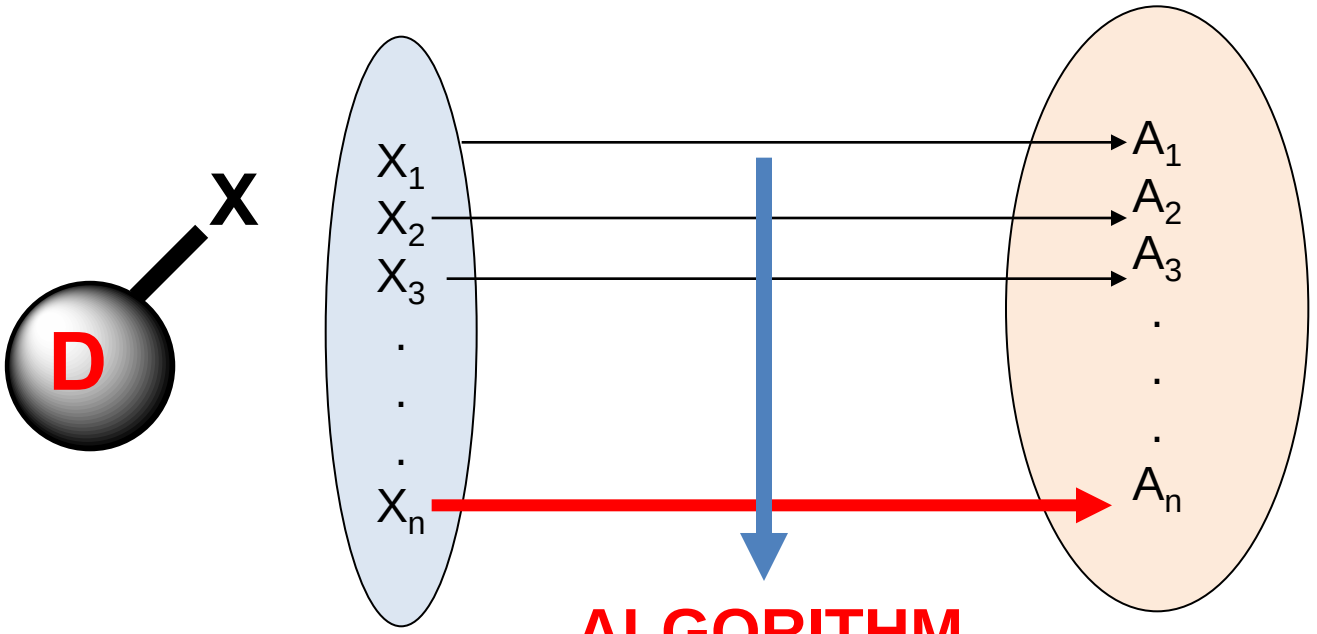
Applications

- TO **PREDICT** the pharmacological **activity** of compounds that have not yet been synthesised and are structurally related with other already prepared and tested compounds.

- TO **DESIGN drugs** with optimal activity.

- TO **STUDY** a drug's **mechanism of action**.

6.1.- INTRODUCTION TO QSAR



In classical QSAR, quantitative measurements for biological and physicochemical properties are required.

Molecular Properties and Their Parameters

Molecular Property	Corresponding Interaction	Parameters
Electron density	Ionic bonds, dipole-dipole interactions, hydrogen bonds, charge transfer interactions	**σ, R, F**, κ
Lipophilicity	Hydrophobic interactions	**Log P, π**, f, R_M
Polarizability	Van der Waals interactions	**MR, MV**
Topology	Steric hindrance, geometric fit	**E_S, r_V, L, B**, distances, volumes, etc.

Physicochemical parameters: electronic, lipophilic, steric, etc.
Structural parameters: size, VDW radius, etc.
Theoretical parameters: topological indices

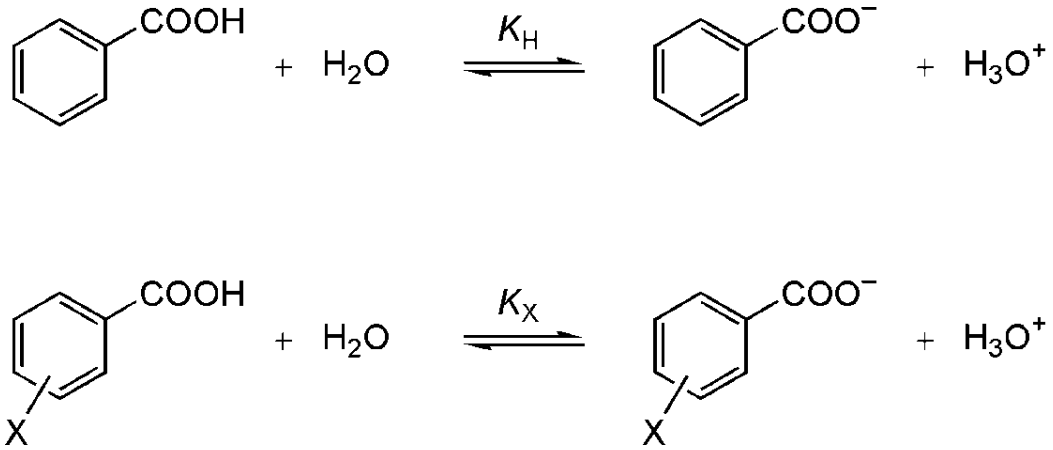
6.2. HAMMETT EQUATION. ELECTRONIC FACTORS

Electronic effects: Hammett Substituent Constant (σ). Aromatic substituents

- The constant (σ) is a measure of the e-withdrawing or e-donating influence of substituents.
- It can be measured experimentally and tabulated.

E.g. For aromatic substituents, σ is measured by comparing the dissociation constants of substituted benzoic acids with benzoic acid.

1933: Hammett Structure/Reactivity Relationship



$$\sigma_X = \log \frac{K_X}{K_H} = pK_{a(H)} - pK_{a(X)}$$

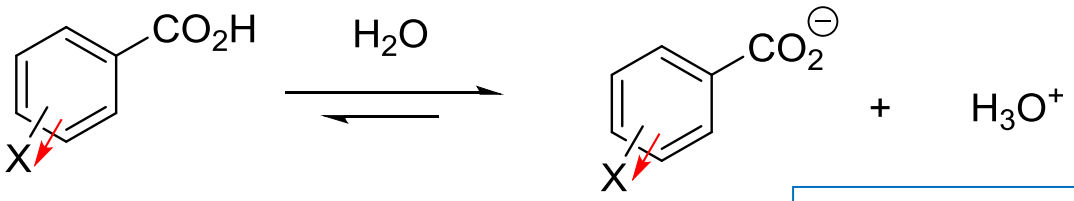
$\sigma_X > 0$: electron withdrawing

$\sigma_X < 0$: electron donor

6.2. HAMMETT EQUATION. ELECTRONIC FACTORS

Electronic effects: Hammett Substituent Constant (σ). Aromatic substituents

X= electron-withdrawing group (e.g. NO_2, CN)



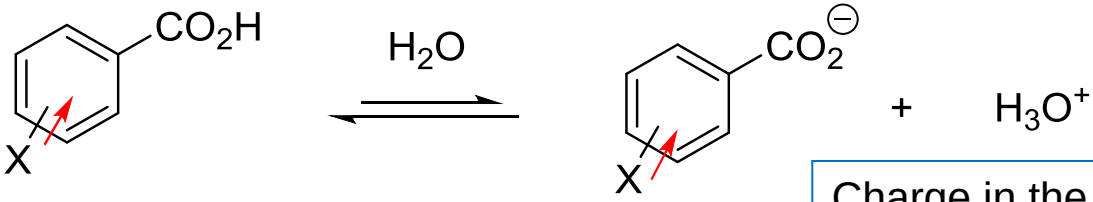
X = electron-withdrawing group

Charge in the anion is stabilised by X.
Equilibrium shifts more to the right.
$K_X > K_H$

$$\sigma_x = \log K_X / K_H = \log K_X - \log K_H$$

σ Positive value

X= electron-donating group (e.g. alkyl chain)



X = electron-donating group

Charge in the anion is destabilised.
Equilibrium shifts more to the left.
$K_X < K_H$

σ Negative value

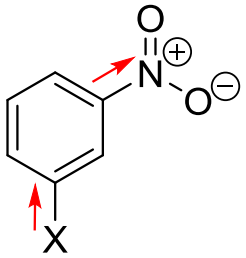
6.2. HAMMETT EQUATION. ELECTRONIC FACTORS

Electronic effects: Hammett Substituent Constant (σ). Aromatic substituents

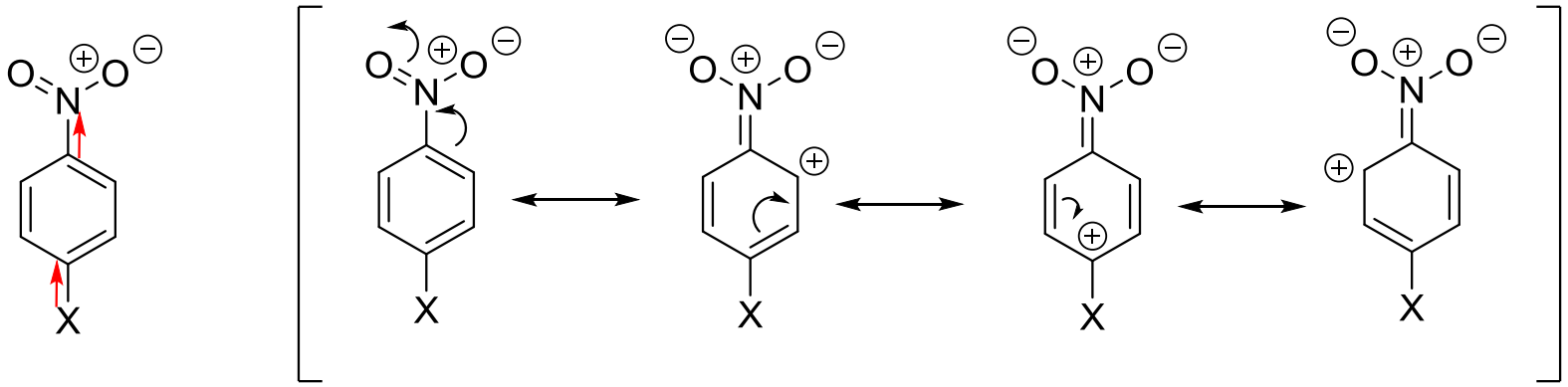
σ value depends on inductive and resonance effects.
σ value depends on whether the substituent is *meta* or *para.*
ortho values are invalid due to steric factors.

Examples: $\sigma_m\,(NO_2) = 0.71$ $\sigma_p\,(NO_2) = 0.78$

meta NO_2: electronic influence on X is inductive (electron-withdrawing: positive value)



para NO_2: electronic influence on Y is due to inductive and resonance effects

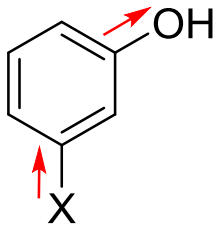


6.2. HAMMETT EQUATION. ELECTRONIC FACTORS

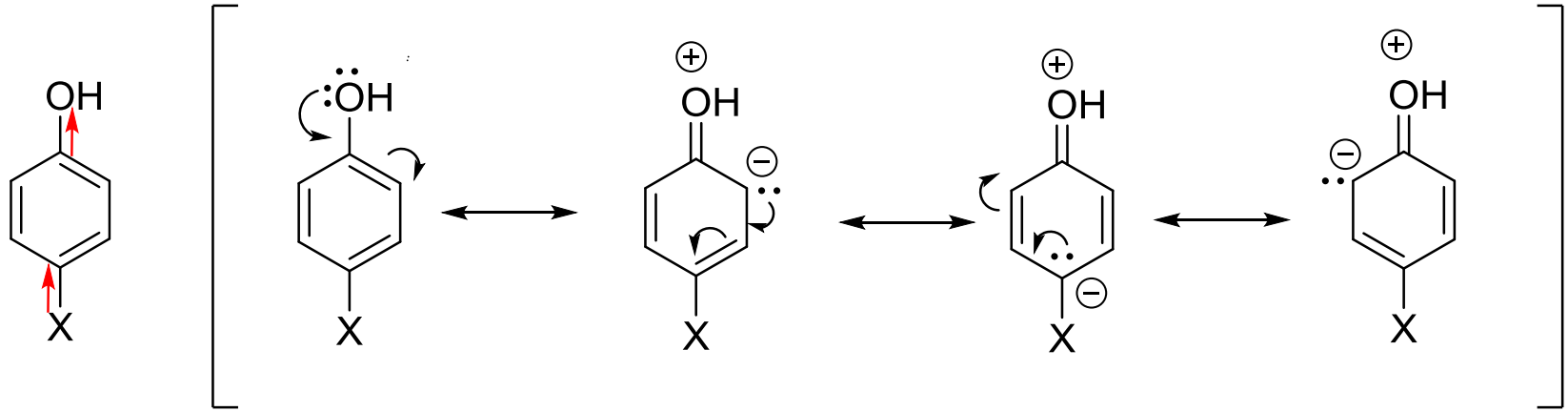
Electronic effects: Hammett Substituent Constant (σ). Aromatic substituents

EXAMPLES: $\sigma_m\,(OH) = 0.12$ $\sigma_p\,(OH) = -0.37$

meta OH : electronic influence on X is inductive (electron-withdrawing: positive value)



para OH: electronic influence on X is dominated by resonance effects (electron-donating)



6.2. HAMMETT EQUATION. ELECTRONIC FACTORS

Electronic effects: Hammett Substituent Constant (σ). Aromatic substituents

substituent	σ_m	σ_p	substituent	σ_m	σ_p
H	0,00	0,00	CN	0,56	0,66
Br	0,39	0,23	CO_2^-	- 0,10	0,00
Cl	0,37	0,23	CHO	0,35	0,42
F	0,34	0,06	CO_2H	0,37	0,45
I	0,35	0,18	CF_3	0,43	0,54
NO_2	0,71	0,78	$C{=}O(NH_2)$	0,28	0,36
NH_2	- 0,16	- 0,66	CH_3	- 0,07	- 0,17
NH_3^+	0,86	0,60	CH_2OH	0,00	0,00
O^-	- 0,47	- 0,81	$COCH_3$	0,38	0,50
OH	0,12	- 0,37	C=O(OEt)	0,37	0,45
OCH_3	0,12	- 0,27	$C(CH_3)_3$	- 0,10	- 0,20
$OC{=}O(CH_3)$	0,39	0,31	C_6H_5	0,06	- 0,01

(Delgado's book, chapter 8)

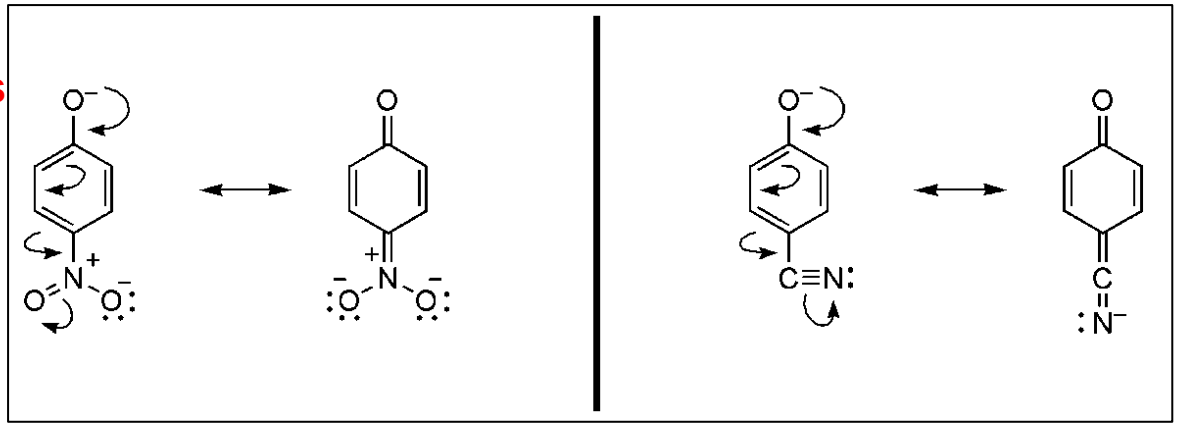
6.2. HAMMETT EQUATION. ELECTRONIC FACTORS

Electronic effects: Hammett Substituent Constant (σ). Aromatic substituents

Some deviations are observed for substituents that are directly conjugated to the reaction site: constants σ^+ and σ^- are defined to correct them.

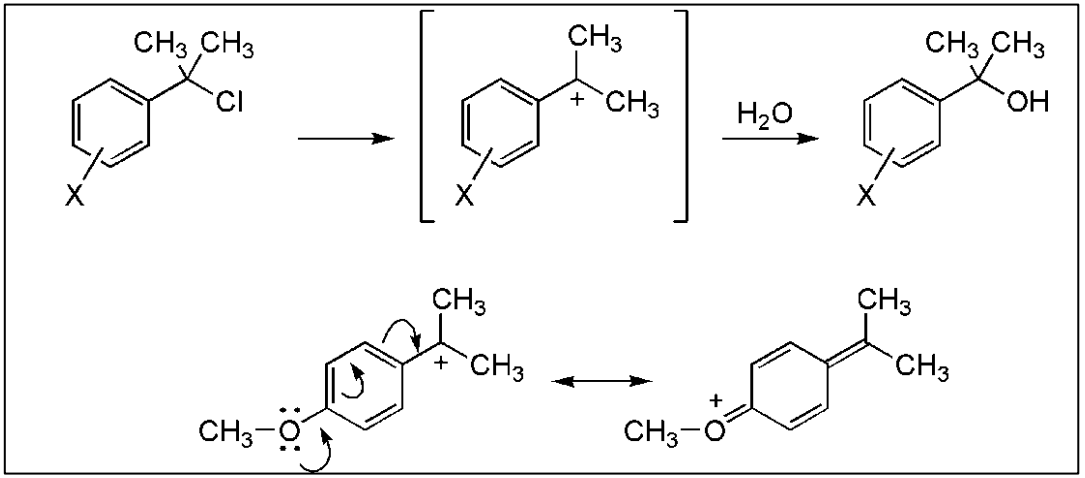
σ^- for withdrawing substituents

Phenol ionization



σ^+ for electron donor substituents

Benzyl chlorides solvolysis



(Delgado's book, chapter 8)

6.2. HAMMETT EQUATION. ELECTRONIC FACTORS

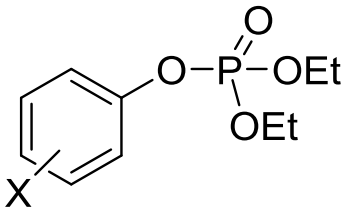
Electronic effects: Hammett Substituent Constant (σ). Aromatic substituents

substituent X	σ_p^-	σ_p
NO_2	1,27	0,78
CHO	1,03	0,43
CN	0,88	0,66
$COCH_3$	0,84	0,50
COOEt	0,68	0,45

substituent X	σ_p^+	σ_p
NMe_2	- 1,70	- 0,83
NH_2	- 1,30	- 0,66
MeO	- 0,78	- 0,27
CH_3	- 0,31	- 0,17
C_6H_5	- 0,18	- 0,01

(Delgado's, chapter 8)

Example: QSAR Equation for insecticidal activity of diethyl phenyl phosphates:



(Patrick's)

$$\log (1/C) = 2.282\sigma - 0.348$$

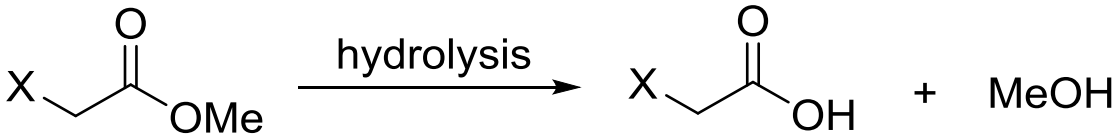
Conclusion?

r is close to 1 (95% of the data is accounted for by the σ parameter).

This is one of the few examples where activity is related to electronic factors alone (which is a good indication that the drugs do not have to pass into or through a cell membrane to have activity). Acetyl cholinesterase is the target and is situated on the outside of cell membranes.

Electronic Effects: Hammett Substituent Constant (σ)
Aliphatic substituents

- Are defined by σ_I
- Produce purely inductive effects.
- Are obtained experimentally by measuring the **rates of hydrolysis** of aliphatic esters (methyl ethanoate is the parent ester).
- Hydrolysis **rates** are measured under basic and acidic conditions.



X= electron donating **Rate↓** **$\sigma_I < 0$**

X= electron withdrawing **Rate↑** **$\sigma_I > 0$**

Basic conditions: Rate is affected by steric and electronic factors. Gives σ_I after correction for steric effect.
Acidic conditions: Rate is affected by steric factors only (see E_s).

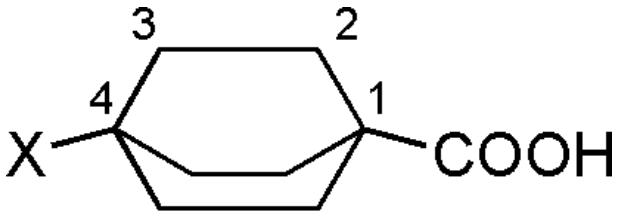
6.2. HAMMETT EQUATION. ELECTRONIC FACTORS

Electronic Factors *R* & *F*

In the Hammett constant (σ), both resonance and inductive effects are involved. These effects can influence pharmacological activity in several ways. Swain-Lupton constants represent the contributions made by the inductive (F) and mesomeric or resonance (R) components of the Hammett constant.

- $\mathscr{R}$ **- Quantifies substituent's resonance effects**
- $\mathscr{F}$ **- Quantifies substituent's inductive effects**

$$\sigma = r\,\mathscr{R} + f\,\mathscr{F}$$



F is calculated by taking into account the effect of one X substituent over the ionization of [2.2.2]octanecarboxylic acids. There is no resonance effect.

6.2. HAMMETT EQUATION. ELECTRONIC FACTORS

Electronic Factors *R* & *F*

SWAIN and LUPTON CONSTANTS

substituent	*F*	*R*	*substituent*	*F*	*R*
H	0,00	0,00	$OC{=}O(CH_3)$	0,41	- 0,07
Br	0,44	- 0,17	CN	0,51	0,19
Cl	0,41	- 0,15	CO_2^-	- 0,15	0,13
F	0,43	- 0,34	CF_3	0,38	0,19
I	0,40	0,19	$C{=}O(NH_2)$	0,24	0,14
NO_2	0,67	0,16	CH_3	- 0,04	- 0,13
NH_2	0,02	- 0,68	CH_2OH	0,00	0,00
NH_3^+	0,94	- 0,27	$COCH_3$	0,32	0,20
O^-	- 0,35	- 0,49	C=O(Et)	0,33	0,15
OH	0,29	- 0,64	$C(CH_3)_3$	- 0.07	- 0,13
OCH_3	0,26	- 0,51	C_6H_5	0,08	- 0,08

(Delgado's, chapter 8)

F and R have negative values for electron donating substituents and positive values for electron withdrawing substituents

6.2. TAFT'S EQUATION. STERIC FACTORS

Steric Factors

- The interaction of a drug with a target involves the mutual approach of two molecules.
- Another important parameter for QSAR is the steric effect.
- Steric properties are difficult to quantify.
- Several parameters have been used.

Taft's Steric Factor (E_s)

•Is obtained by comparing **the rates of hydrolysis** of substituted aliphatic esters against a standard ester under acidic conditions.

$E_s = \log k_x - \log k_o$

k_x represents the rate of hydrolysis of the substituted ester.
k_o represents the rate of hydrolysis of the parent ester.

•Is limited to substituents which interact sterically with the tetrahedral transition state for the reaction (not by resonance or internal HB).

•May undervalue the steric effect of groups in an intermolecular process (e.g. a drug binding to a receptor).

•The reference ester is X=Me. Smaller substituents have positive values.

Substituent	H	F	Me	Et	n-Pr	n-Bu	i-Pr	iBu	cyclopentyl
E_s	1.24	0.78	0	-0.07	-0,36	-0,39	-0.47	-0.93	-0,51

6.2. TAFT'S EQUATION. STERIC FACTORS

Steric Factors

Molar Refractivity (*MR*) - a measure of a substituent's volume

MR is the molar volume corrected by the refractive index. It represents the size and polarizability of a fragment or molecule.

$$MR = \underbrace{\frac{(n^2 - 1)}{(n^2 - 2)}}_{\text{Correction factor for polarisation}} \times \underbrace{\frac{MW}{d}}_{\text{Defines volume}}$$

- MR is defined by the Lorentz-Lorentz equation, where "*n*" is the index of refraction at the sodium D line, *MW* is the molecular weight, and *d* is the density of the compound.
- The greater the positive MR value of a substituent, the larger the steric or bulk effect.
- MR also measures the electronic effect and may therefore reflect dipole-dipole interactions at the receptor site.

6.2. LIPOPHILICITY FACTORS

From Unit 3:

- The hydrophobic character of a drug is crucial for crossing cell membranes and may be important in receptor interactions.
- The hydrophobic character of a drug can be measured experimentally by testing the drug's relative distribution in an *n*-octanol/water mixture. The relative distribution is known as the **PARTITION COEFFICIENT (P)**.
- Another parameter commonly used to relate distribution with biological activity is the **LIPOPHILICITY SUBSTITUENT CONSTANT (π)**.
- The **P** parameter refers to the whole molecule whereas π is related to substituent groups.

Hydrophobicity of the molecule

$$\text{PARTITION COEFFICIENT} \quad P = \frac{[\text{Drug}]_{\text{octanol}}}{[\text{Drug}]_{\text{water}}}$$

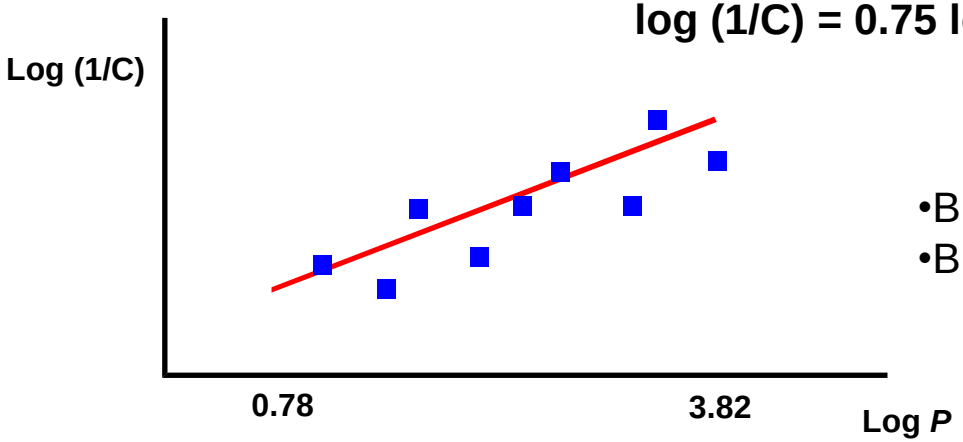
High P ⟶ High lipophilicity

6.2. LIPOPHILITICY FACTORS

Hydrophobicity of the molecule

•The activity of drugs is often related to *P.*
e.g. the binding of drugs to serum albumin (straight line – limited range of log *P*).

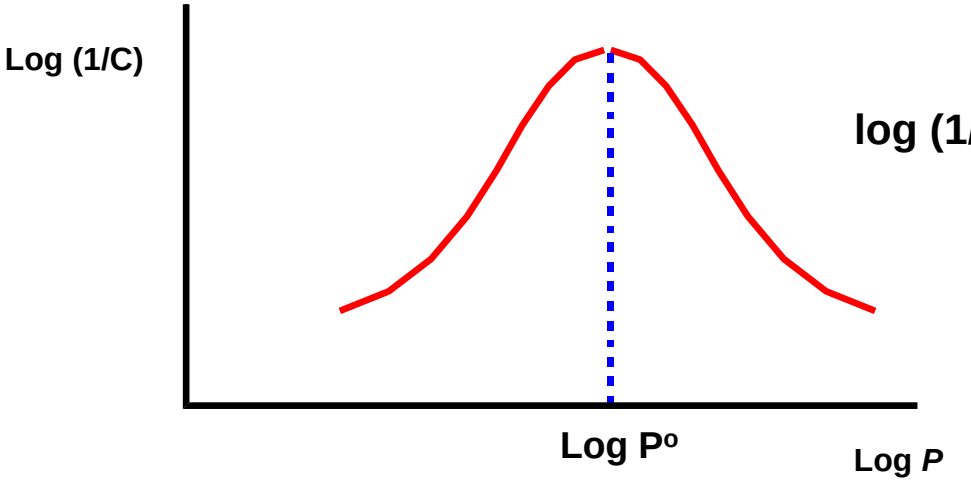
log (1/C) = 0.75 log P + 2.30



(n = 42, *r* = 0.960)

•Binding increases as log *P* increases.
•Binding is greater for hydrophobic drugs.

General anaesthetic activity of ethers (parabolic curve – wider range of log *P* values).



log (1/C) = -0.22(log P)^2 + 1.04 log P + 2.16

Optimum value of log *P* for anaesthetic activity = log *P*°

6.2. LIPOPHILITICY FACTORS

From unit 3

Substituent lipophilicity constants - Hansch and Fujita parameter

- Substituent lipophilic constants (π_X) are also known as hydrophobic constants.
- They represent the contribution made by a group or atom to the partition coefficient and were defined by Hansch and co-workers by the equation:

$$\pi_X = \log P_X - \log P_H = \log (P_X / P_H)$$

P_X is the partition coefficient for the compound with substituent X.

P_H is the partition coefficient for the parent molecule.

- Log P calculated from the lipophilic constants for substituents is called c log P.

$$c \log P = \sum \pi \quad \text{(p values depend on the pattern structure)}$$

6.2. LIPOPHILITICY FACTORS

Substituent hydrophobicity constant (π)

From unit 3

- *π_X depends on the characteristics of the group/atom: inductive, resonance and steric effects are important (a positive value means that the substituent increases the lipophilicity and a negative value means the opposite).*
- *The values of π for the most common substituents are tabulated*

Substituents more hydrophilic than hydrogen have negative π values.

Substituents more lipophilic than hydrogen have positive values.

- Numerous software packages are now commercially available (ChemDraw, etc.) but the results can differ widely and also differ from the experimental value.

6.2. LIPOPHILITICY FACTORS

Substituent hydrophobicity constant (π)

$$\pi_X = \log \frac{P_X}{P_H}$$

susbstituent	$\pi_{arom.}$	$\pi_{alif.}$	substituent	$\pi_{arom.}$	$\pi_{alif.}$
H	0,00		OC=O(CH_3)	- 0,64	- 0,27
Br	0,86	0,60	CN	- 0,57	- 0,84
Cl	0,71	0,39	CO_2^-	- 4,36	- 4,67
F	0,14	- 0,17	C=O(NH_2)	- 1,49	- 1,71
I	1,12	1,00	CH_3	0,56	0,50
NH_2	- 1,23	- 1,19	$COCH_3$	- 0,55	
NH_3^+		- 4,19	C=O(Et)	0,51	
O^-	- 3,87		$C(CH_3)_3$	1,98	
OH	- 0,67	- 1,12	C_6H_5	1,96	
OCH_3	- 0,02				

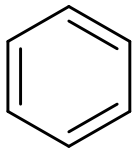
(Delgado, chapter 8)

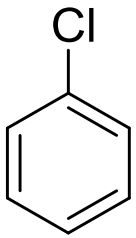
Example:

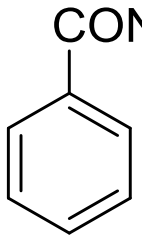
Benzene Log P = 2.13

Chlorobenzene Log P = 2.84

Benzamide Log P = 0.64







π_{Cl} = **0.71** (arom)

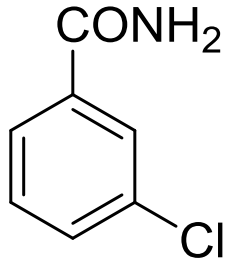
π_{CONH_2} = **-1.49** (arom)

6.2. LIPOPHILITICY FACTORS

Substituent hydrophobicity constant (π)

- Log *P* can be calculated using π values.
- The value of π is valid only for parent structures.

Example:



***meta*Chlorobenzamide**

$$\text{Log } P_{(\text{calculated})} = \log P_{(\text{benzene})} + \pi_{Cl} + \pi_{CONH_2}$$
$$= 2.13 + 0.71 - 1.49$$
$$= 1.35$$
$$\text{Log } P_{(\text{observed})} = 1.51$$

- A QSAR equation may include both *P* and π.
- *P* measures the importance of a molecule's overall hydrophobicity (relevant to absorption, binding etc.).
- π identifies specific regions of the molecule that may interact with hydrophobic regions in the binding site.

6.2. PHYSICOCHEMICAL PARAMETERS

Summary:

Electronic effects

Hammett constant σ

$\sigma > 0$ more electron withdrawing than H

$\sigma < 0$ more electron donating than H

Steric factors

Taft parameter E_s

$E_s < 0$ group bigger than CH_3

$E_s > 0$ group smaller than CH_3

Lipophilicity

Hansch and Fujita parameter π

$\pi < 0$ substituents more hydrophilic than H

$\pi > 0$ substituents more lipophilic than H

$P = [drug]_{org}/[drug]_{aq}$

$P > 1$ (log $P > 0$) more lipophilic than H

$P < 1$ (log $P < 0$) more hydrophilic than H

6.3. METHODS USED IN QSAR

HANSCH and FUJITA EQUATION (physicochemical parameters)

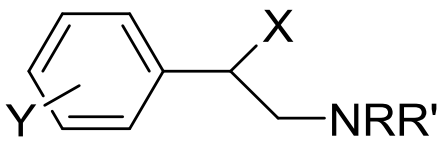
Parabolic equation: when the log P values are spread over a wide range

$$\log (1/C) = - a\pi^2 + b\pi + \rho\sigma + \delta E_S + c$$

Linear equation: when the range of lipophilicity values (or hydrophobicity) is limited.

$$\log (1/C) = b\pi + \rho\sigma + \delta E_S + c$$

Example: Adrenergic blocking activity of β-halo-β-arylamines.



$$\text{Log } (1/C) = 1.22\ \pi - 1.59\sigma + 7.89$$

Conclusions

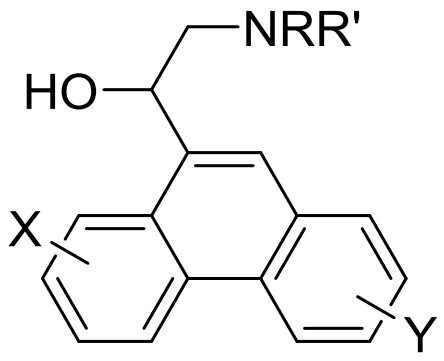
Activity increases if π is positive (i.e hydrofobic substituents).

Activity increases if σ is negative (i.e. electron-donor substituents).

6.3. METHODS USED IN QSAR

HANSCH and FUJITA EQUATION (physicochemical parameters)

Example: Antimalarial activity of phenanthrene aminocarbinols



$$\text{Log } (1/C) = = -0.015\,(\log P)^2 + 0.14 \log P + 0.27 \sum \pi_X + 0.40 \sum \pi_Y + 0.65 \sum \sigma_X + 0.88 \sum \sigma_Y + 2.34$$

Conclusions:

- Activity increases slightly as log *P* increases (the constant is small).
- The parabolic equation implies an optimum log P^o value for activity.
- Activity increases for hydrophobic substituents (especially in ring Y).
- Activity increases for e-withdrawing substituents (especially in ring Y).

6.3. METHODS USED IN QSAR

HANSCH and FUJITA EQUATION (physicochemical parameters)

Choosing suitable substituents

Substituents must be chosen to satisfy the following criteria:

- There must be a range of values for each physicochemical property studied.
- Values must not be correlated for different properties (i.e. they must be orthogonal in value).
- At least 5 structures are required for each parameter studied.

Substituent	H	Me	Et	n-Pr	n-Bu
π	0.00	0.56	1.02	1.50	2.13
MR	0.10	0.56	1.03	1.55	1.96

Correlated values.
Are any differences in activity due to π or MR?

Substituent	H	Me	OMe	$NHCONH_2$	I	CN
π	0.00	0.56	-0.02	-1.30	1.12	-0.57
MR	0.10	0.56	0.79	1.37	1.39	0.63

No correlation in values
Valid for analysing the effects of π and MR.

HANSCH and FUJITA EQUATION (physicochemical parameters)

PREDICTIVE VALUE OF THE HANSCH EQUATION

Practical development of the Hansch equation is governed by the following criteria:

a) The selection of independent variables.

b) Justification of the choice of variables.

c) Principle of parsimony: When statistical significance is equal, we choose the simplest model.

d) The number of studied compounds must be related to the number of variables.

HANSCH and FUJITA EQUATION (physicochemical parameters)

SCOPE AND LIMITATIONS OF HANSCH'S METHOD

Advantages:

a) Simple parameters are used.
b) Quantitative predictions are made.
c) The method is economical and easy to use.
d) The conclusions can be extended to unused substituents.

Limitations:

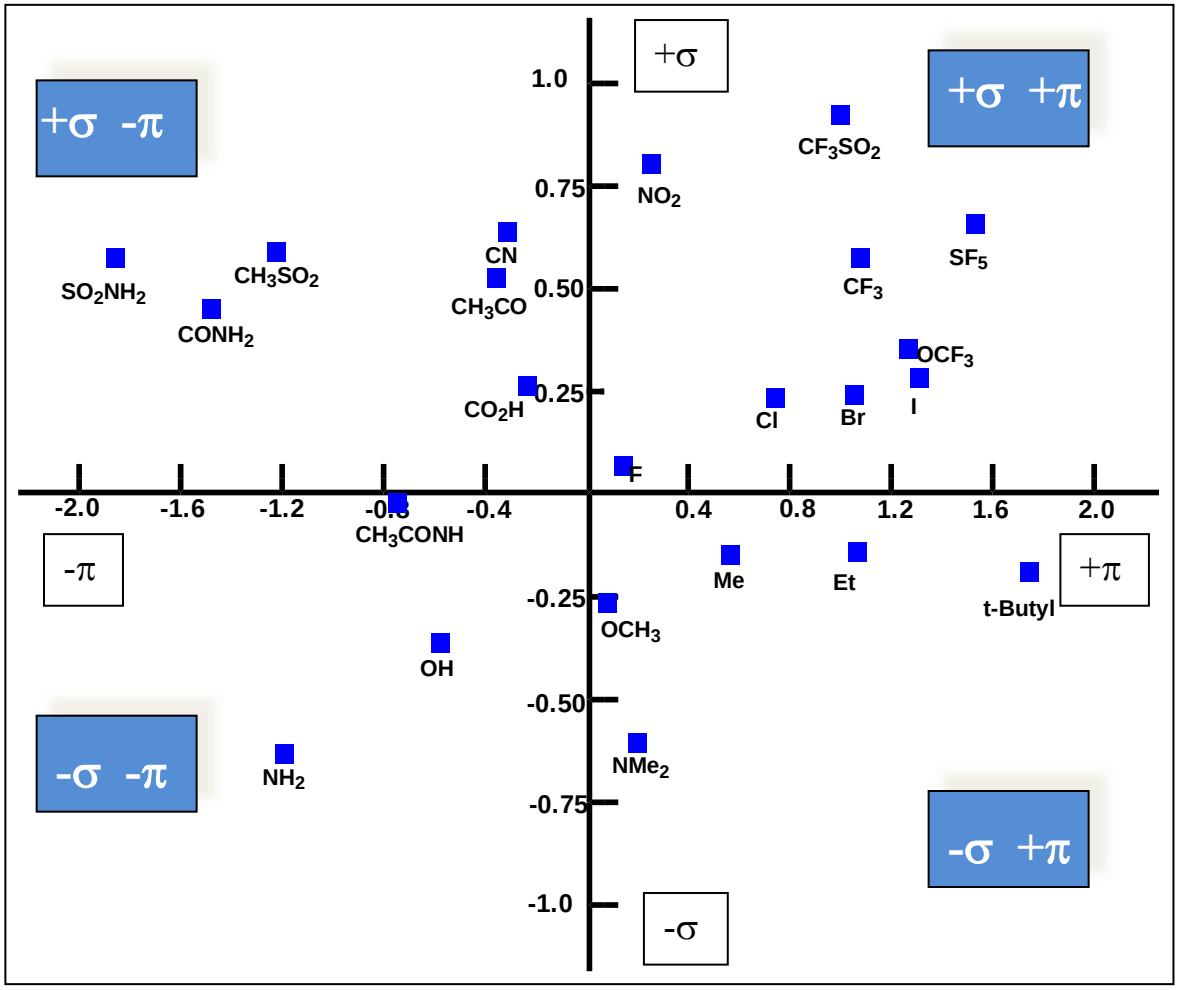
The values of the parameters must be known.
a) A large number of compounds is required for statistical significance.
c) Results obtained with small molecules may be imperfect models of biological systems.

6.3. METHODS USED IN QSAR

Craig Plot

The Craig plot shows values for two different physicochemical properties for various substituents.

- Allows easy identification of suitable substituents for a QSAR analysis which includes both relevant properties.
- A substituent is chosen from each quadrant in order to ensure orthogonality.
- Substituents are chosen with a range of values for each property.

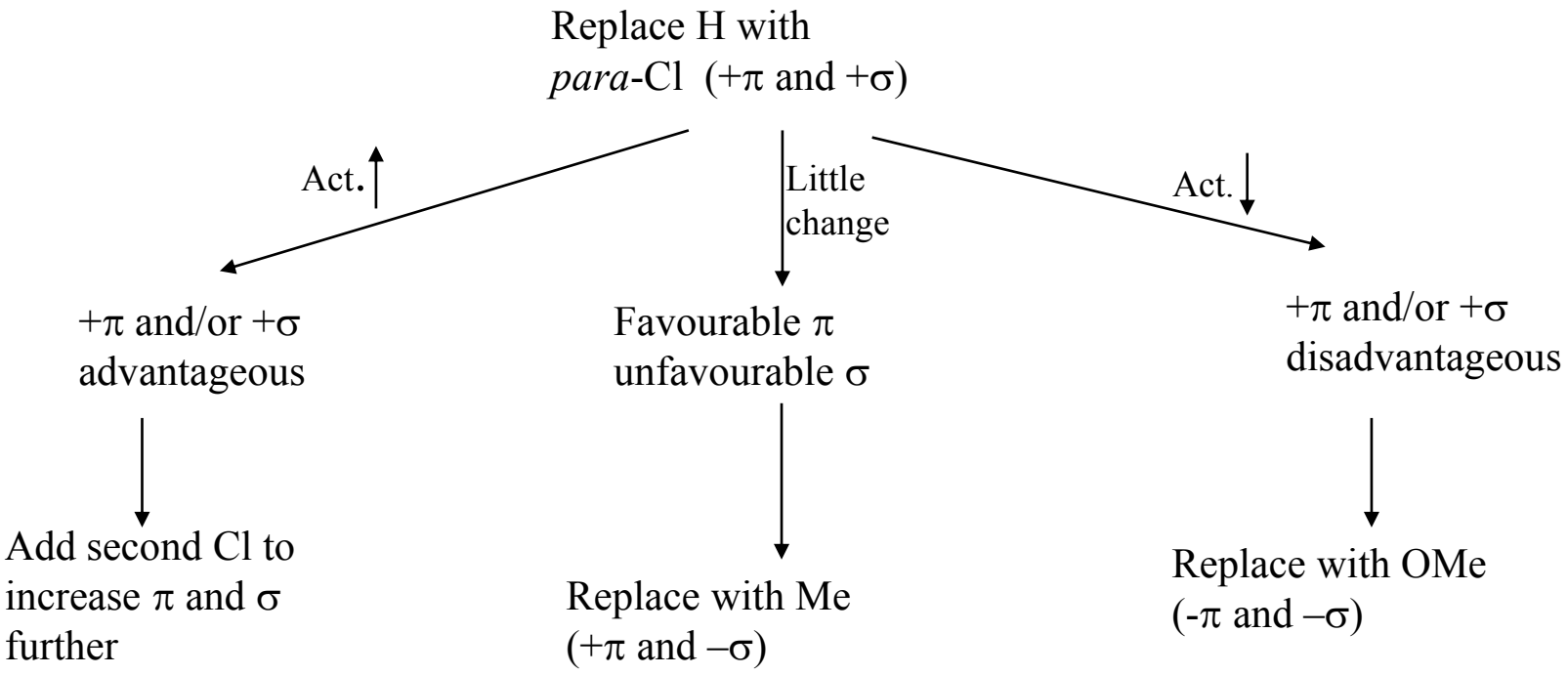


6.3. METHODS USED IN QSAR

Topliss Scheme

This is used to decide which substituents to use if compounds are optimised one by one (where synthesis is complex and slow).

Example: Aromatic substituents



Further changes suggested based on arguments of π, σ and steric strain.

Bioisosteres

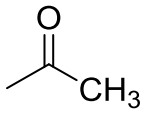
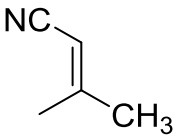
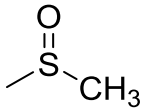
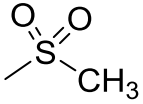
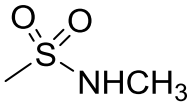
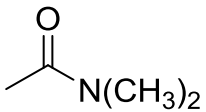
substituent						
π	-0.55	0.40	-1.58	-1.63	-1.82	-1.51
σ_p	0.50	0.84	0.49	0.72	0.57	0.36
σ_m	0.38	0.66	0.52	0.60	0.46	0.35
MR	11.2	21.5	13.7	13.5	16.9	19.2

Figure 18.17 Physicochemical parameters for six substituents (Patrick's 5th ed.)

- Substituents with similar physicochemical properties (e.g. CN, NO_2 and COMe may be bioisosteres).

- Choose bioisosteres based on the most important physicochemical property

(e.g. COMe & SOMe are similar in σ_p; SOMe and SO_2Me are similar in π)

Free-Wilson Approach

Method

- The biological activity of the parent structure is measured and compared with the activity of analogues bearing different substituents.
- An equation is derived that relates biological activity to the presence or absence of particular substituents.

$$\textbf{Activity} = k_1X_1 + k_2X_2 + \ldots k_nX_n + Z$$

- X_n is an indicator variable which is given the value 0 or 1 depending on whether the substituent (n) is present or not.
- The contribution of each substituent (n) to activity is determined by the value of k_n.
- Z is a constant representing the overall activity of the structures studied.

Free-Wilson Approach

Advantages

- Physicochemical constants or tables are not required.
- It is useful for structures with unusual substituents.
- It is useful for quantifying the biological effects of molecular features that cannot be quantified or tabulated by the Hansch method.

Disadvantages

- A large number of analogues need to be synthesised in order to represent each different substituent and each different position of a substituent.
- It is difficult to rationalise why specific substituents are good or bad for activity.
- The effects of different substituents may not be additive (e.g. intramolecular interactions).

Free-Wilson/Hansch Approach

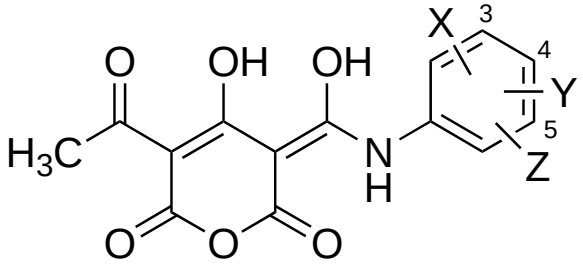
Advantages

- Indicator variables may be used as part of a Hansch equation.

6.3. METHODS USED IN QSAR

Free-Wilson / Hansch Approach

Case Study (Patrick's chapter 18)



QSAR analysis of pyranenamines (SK & F)
(anti-allergy compounds)

Stage 1 19 structures were synthesised to study π and σ

$$\text{Log}\left(1/C\right) = -0.14\,\Sigma\pi - 1.35(\Sigma\sigma)^2 - 0.72$$

$\Sigma\pi$ and $\Sigma\sigma$ = total values for π and σ for all substituents

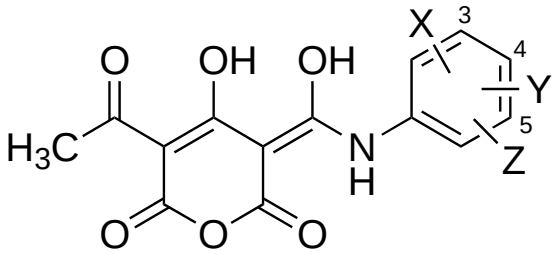
Conclusions:

- Activity drops as π increases.
- Hydrophobic substituents are bad for activity (this is unusual).
- Any value of σ results in a drop in activity.
- Substituents should not be e-donating or e-withdrawing (activity falls if σ is + value or - value).

6.3. METHODS USED IN QSAR

Free-Wilson/Hansch Approach

Case Study



Stage 5

$$\text{Log } (1/C) = -0.034(\Sigma\pi)^2 - 0.33\Sigma\pi + 4.3\,(F\text{-}5) - 1.3(R\text{-}5) - 1.7(\Sigma\sigma)^2 + 0.73(3,4,5\text{-HBD})$$

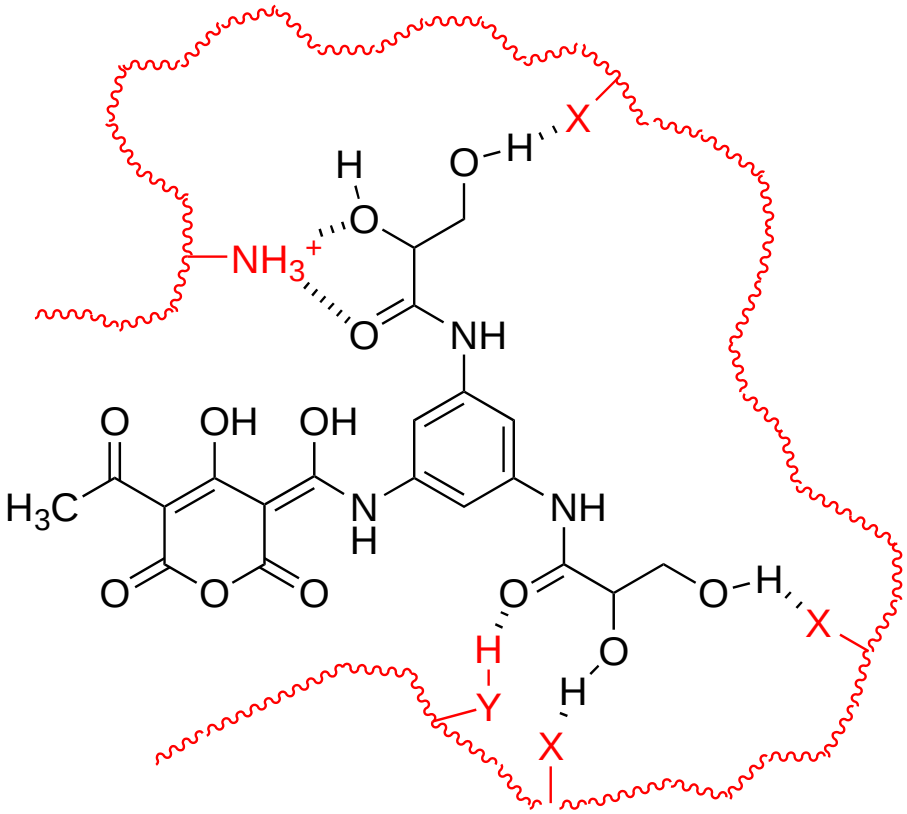
$$- 0.86\,(\text{HB-INTRA}) - 0.69(NHSO_2) + 0.72(4\text{-OCO}) - 0.59$$

Conclusions:

- Increasing the hydrophilicity of substituents allows the identification of an optimum value for π ($\Sigma\pi$ = -5). The equation is now parabolic (-0.034 $(\Sigma\pi)^2$).
- The optimum value of $\Sigma\pi$ is very low and implies a hydrophilic binding site.
- *R*-5 implies that resonance effects are important at position 5.
- HB-INTRA equals 1 for H-bonding groups *ortho* to each other (act. drops -0.86).
- HB-INTRA equals 0 if H-bonding groups are not *ortho* to each other.
- The steric parameter is no longer significant and is not present.

Free-Wilson / Hansch Approach

Stage 6 Optimum Structure and binding theory



Based on Figure 18.19 Hypothetical binding interactions between a pyranenamine and the target binding site (Patrick's 5th ed.)

6.4. 3D-QSAR

Molecular modelling has previously been described in lead discovery and lead optimization. E.g.:

-virtual screening from a database of chemical structures.

-identification of the 3D-pharmacophore in SAR studies.

Classical QSAR methods use computational methods in lead modification but consider only 2D structures.

In **three-dimensional quantitative structure-activity relationships** (3D-QSARs), the 3D properties of a molecule are considered as a whole rather than considering individual substituents or moieties.

General approach:

- Select a group of molecules, each of which has been assayed for a particular activity.
- Align the 3D conformations of the molecule according to some predetermined orientation rules.
- Calculate a set of spatially dependent parameters for each molecule determined in the receptor space surrounding the aligned series.
- Derive a function that relates each molecule's spatial parameters to its respective biological property.
- Establish self-consistency and predictability of the derived function.

6.4. 3D-QSAR (CoMFA)

Advantages over QSAR

- It does not rely on experimental values.
- It can be applied to molecules with unusual substituents.
- It is not restricted to molecules of the same structural class.

Comparative Molecular Field Analysis (CoMFA)

This is the most used method (developed by Cramer).

Assumption: the most important features about a molecule are its overall size and shape and its electronic properties. If these features can be defined, it is possible to study how they affect biological properties.

- Physical properties are calculated using computer software.
- No experimental constants or measurements are involved.
- Properties are known as ‘fields’.
- The steric field defines the size and shape of the molecule.
- The electrostatic field defines electron-rich/poor regions of molecule.
- Hydrophobic properties are relatively unimportant.

The molecule-receptor interaction is then represented by the steric and electrostatic fields exerted by each molecule.

Comparative Molecular Field Analysis (CoMFA)

•The results are represented as a 3D contour map in which contours of various colours represent locations on the structure where lower or higher steric or electrostatic interactions would increase or decrease binding.

Limitations of Comparative Molecular Field Analysis:

- The molecules are assumed to bind with similar orientations in the target, which may not be the case.
- Correct alignments are almost impossible for compounds with a large number of rotatable bonds.

See Chapter 18 (Patrick's 5th ed.)

Alternative Ligand-Based Approaches are dependent on alignment rules:

Comparative Molecular Similarity Indices Analysis (CoMSIA):

- Uses a different potential function.
- Not only steric and electrostatic but also hydrophobic fields are calculated.

Comparative Binding Energy Analysis (COMBINE):

- Is based on structural data of ligand-receptor complexes.
- Hypothesis: the free energy of binding can be correlated with a subset of energy components calculated from the structures of ligand and receptors in bound and unbound forms.

Another popular 3D-QSAR method is **topomer similarity searching**.

A *topomer* is a molecular descriptor that focuses on the shape of a molecule as represented by a combination of the shapes of different fragments (used to search for 3D molecular structures in conventional structural databases).

CoMFA and topomer similarity have been merged into a new 3D-QSAR method called **Topomer CoMFA**:

- Structures in a series are each broken into two or more fragments that are common to the series.
- Topomer 3D models are constructed for each fragment.
- A set of steric and electrostatic fields is generated for each topomer set.

Advantages:

- This method minimizes the preparation needed for 3D-QSAR analysis.
- It automates the creation of models for predicting biological activity.
- It is more user-friendly than traditional CoMFA analysis.