Organic Pharm. Chemistry IV

The Organic Chemistry of Drug Design and Drug Action

Lecture 8

24th December 2022

12:00- 2:00 PM

AlNoor University College

Pharmacy Department

1st Course/ 5TH Class

2022/2023

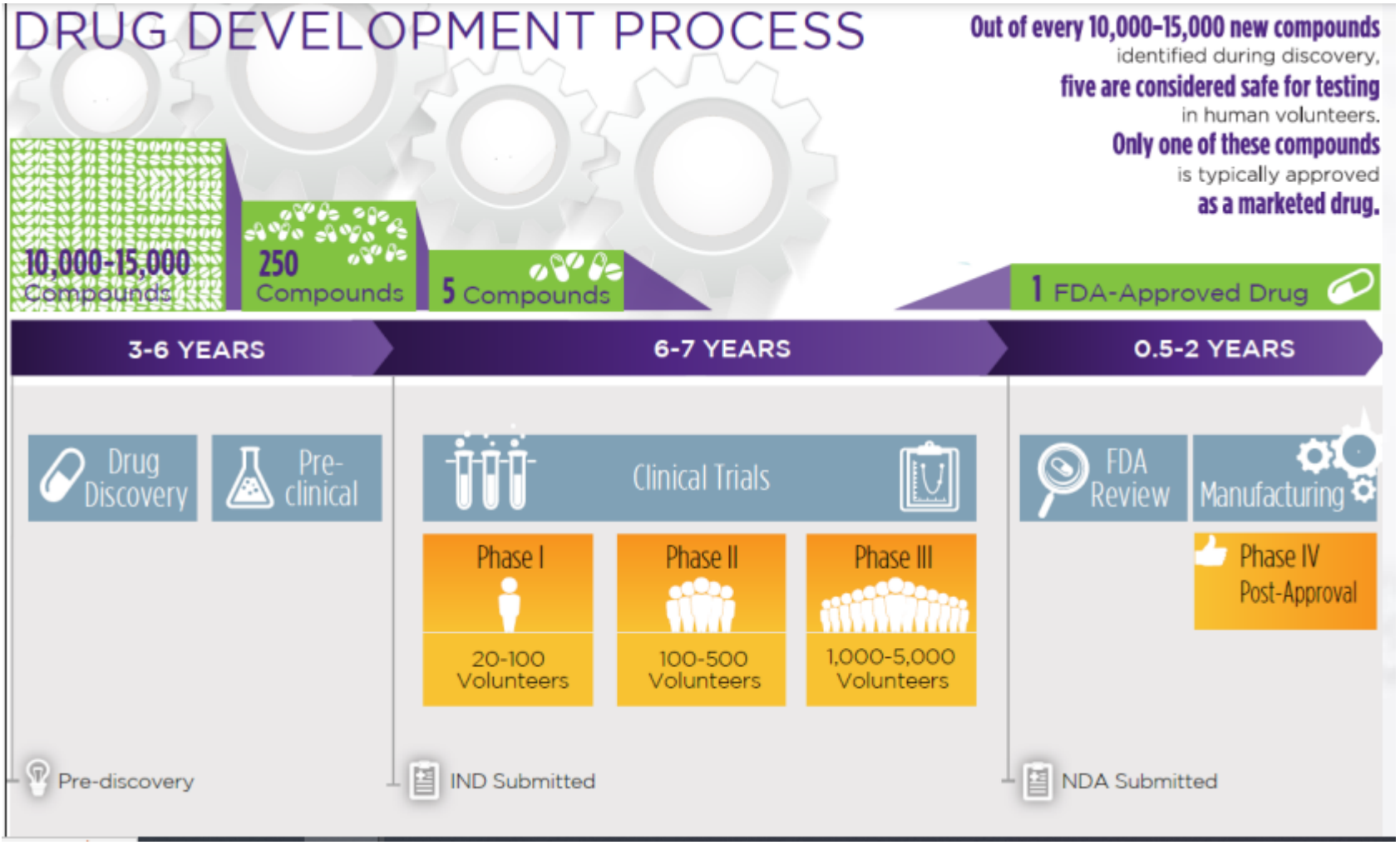
Quote of day :

Look who we are, we are the dreamers
We make it happen 'cause we believe it.

https://www.google.com/search?q=look+we+are+dreamer+lyrics&rlz=1C1GCEA_enIQ982IQ982&sxsrf=ALiCzsYmAkuXqlwR-7dO6trcoHueGqvZiQ:1671791916010&source=lnms&tbm=vid&sa=X&ved=2ahUKEwiG6Kqaxo_8AhW8W_EDHXYWCwcQ_AUoAXoECAEQAw&biw=1292&bih=735&dpr=0.8#fpstate=ive&vld=cid:0a5e6e01,vid:gfZChizkEuI

إذا كنت تستطيع أن تتخيل، يمكنك أن تحلق، إذا كنت تستطيع أن تحلم، تستطيع أن تكون.

Drug Development Process



Drug Designing…

Because of,

- the cost and the time
- the reasons for many diseases are not fully explained,

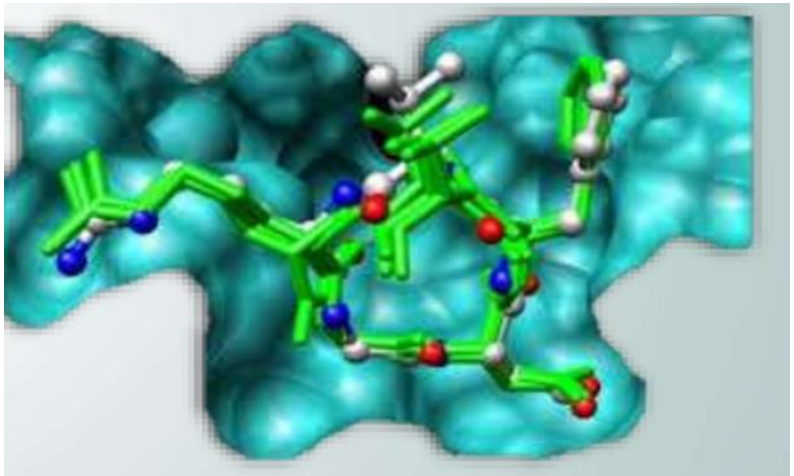
it has become necessary to design drugs in a rational way.

Drug design frequently but not necessarily relies on computer modeling techniques.

This type of modeling is sometimes referred to as computer -aided drug design.

Mechanism based drug design

When the disease process **is understood** at the molecular level and the target molecule(s) are defined, drugs can be designed specifically to interact with the target molecule in such a way as to disrupt the disease

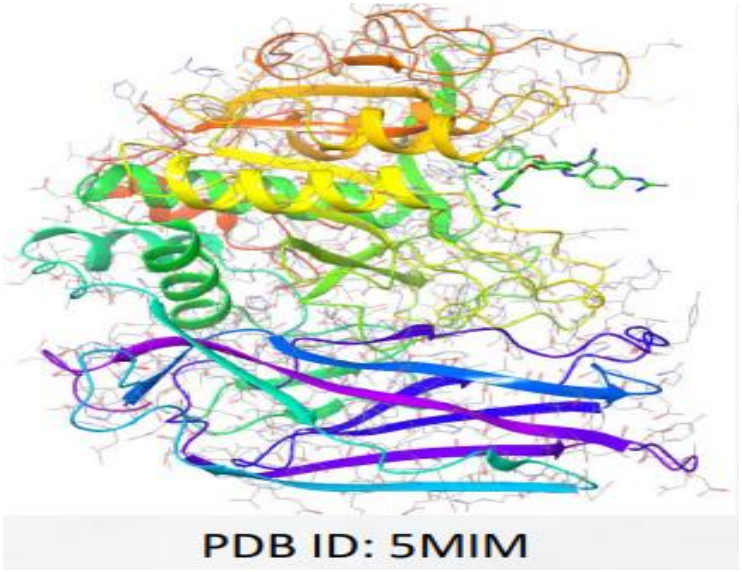


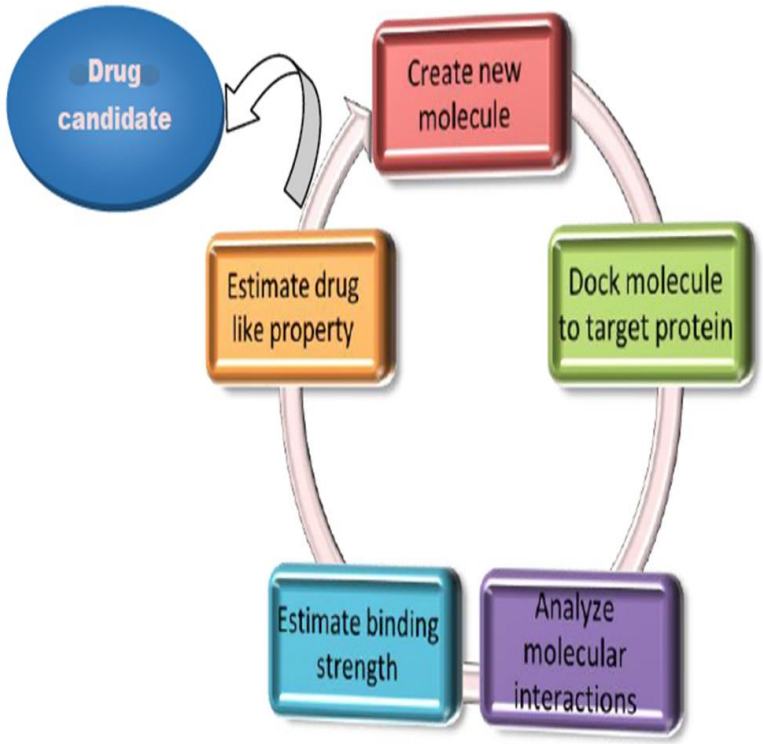
Computer-aided drug design(CADD)

CADD represents computational methods and resources that are used to facilitate the design and discovery of new therapeutic solutions.

Computer aided drug design techniques play an important role in;

1. Design of new chemical compounds which may be the drug active substances,
2. Reach more effective compounds
3. Define mechanism of action of the drugs

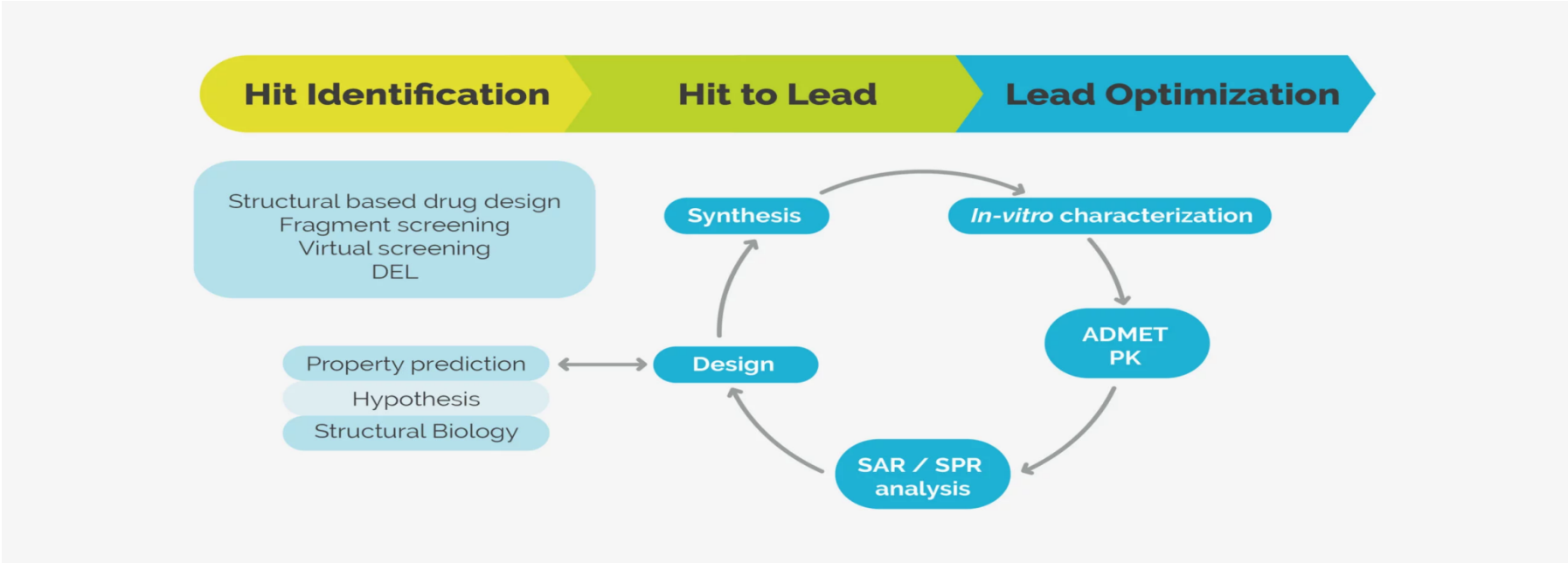




Introduction to CADD

Drug design with the help of computers may be used at any of the following stages of drug discovery:

- hit identification using **virtual screening** (structure- or ligand-based design)
- hit-to-lead (structure-based design, QSAR, etc.)
- lead optimization: optimization of other pharmaceutical properties while maintaining affinity.



Objective of CADD

To change from:

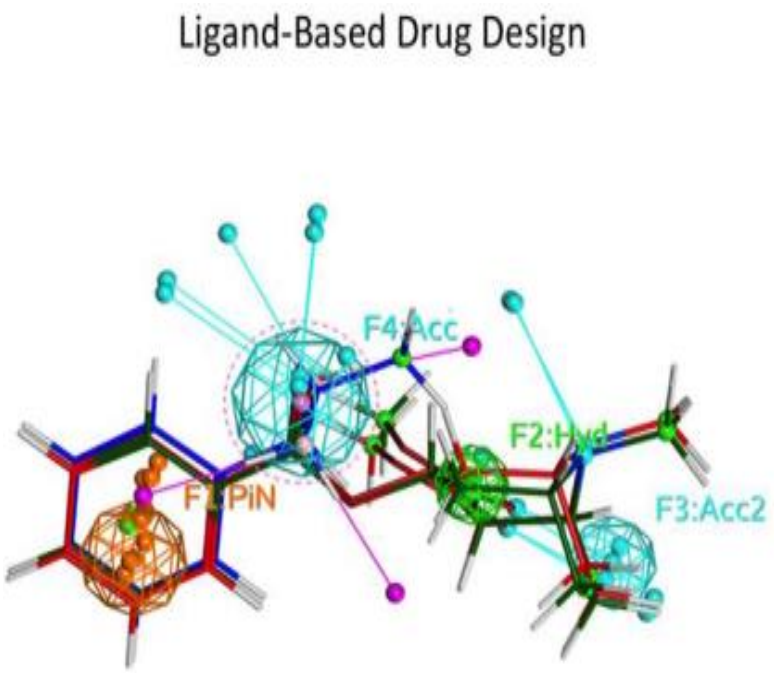
- ✓ Random screening against disease assays
- ✓ Natural products, synthetic chemicals

To:

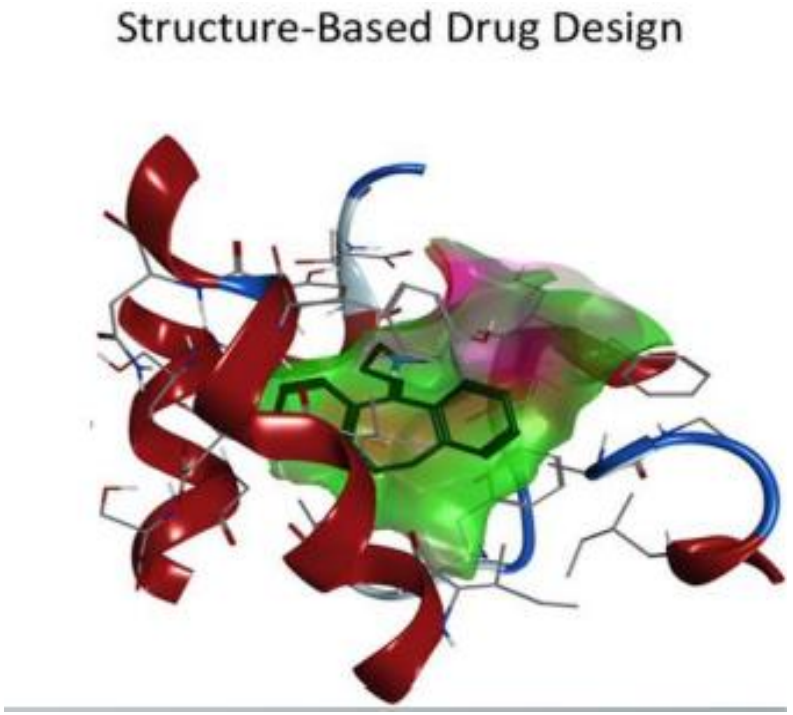
- ✓ Rational drug design and testing
- ✓ Speed-up screening process
- ✓ Efficient screening (focused, target directed)
- ✓ De novo design (target directed)
- ✓ Integration of testing into design process
- ✓ Fail drugs fast (remove hopeless ones as early as possible)

Types of drug design

1) Ligand based drug design



2)Structure based drug design



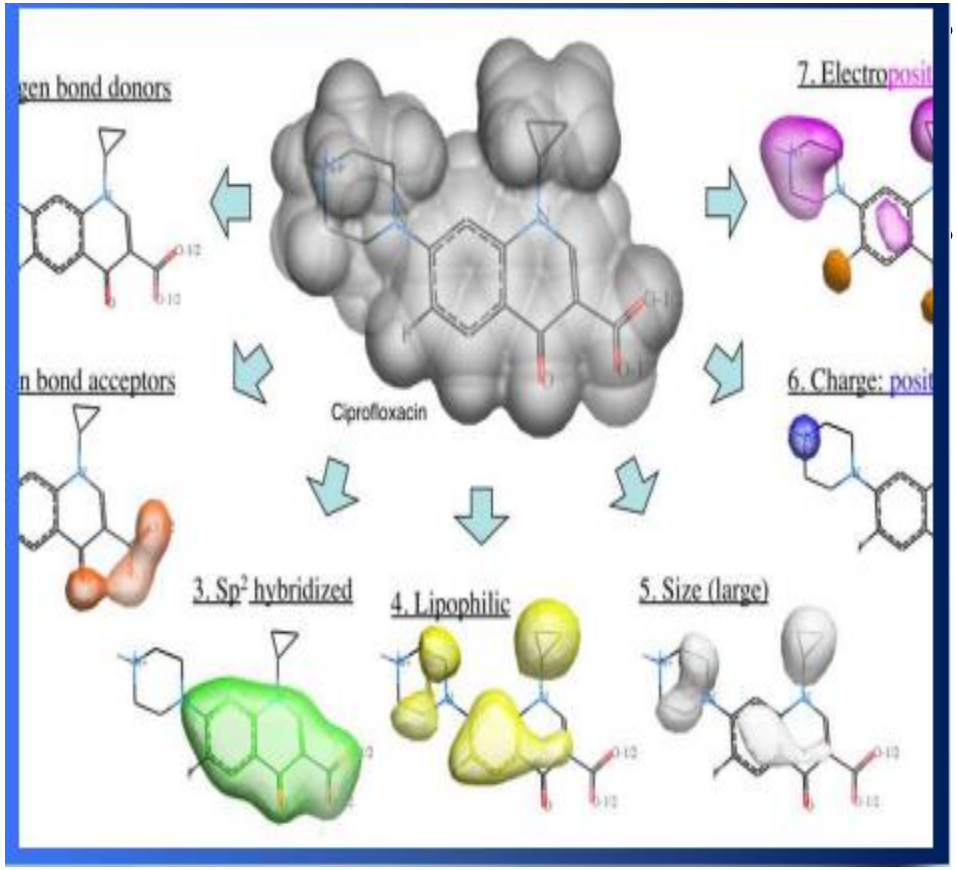
1- **Target = Receptors**, enzymes or nucleic acids

2- **Effector(ligand)** = There may be natural endogenous substances or drugs which occupy the active site of the target and affect the target positively or negatively.

STRUCTURE-BASED DESIGN

LIGAND BASED DESIGN

Ligand-based drug design



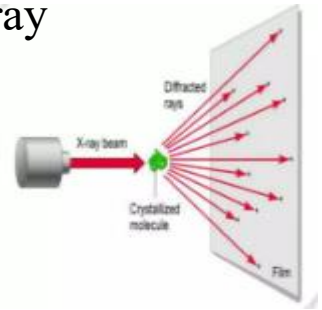
relies on knowledge of other molecules that bind to the biological target of interest.

used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.

1. Ligand-based drug design

- A model of the biological target may be built based on the knowledge of what binds to it, and this model in turn may be used to design new molecular entities that interact with the target.
- Alternatively, a quantitative structure-activity relationship (QSAR), in which a correlation between calculated properties of molecules and their experimentally determined biological activity, may be derived. These QSAR relationships in turn may be used to predict the activity of new analogs.

x-ray



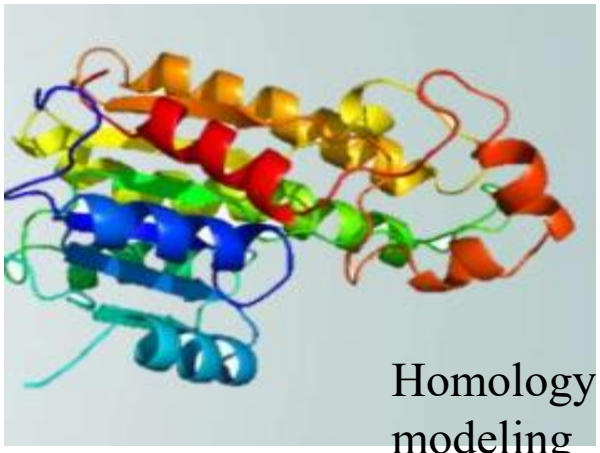
NMR

2. Structure-based drug design:

- If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.
- Homology modeling, also known as comparative modeling of protein, refers to constructing an atomic resolution model of the "target" and an experimental three-dimensional structure of a related homologous protein (the "template").

relies on knowledge of the three dimensional structure of the biological target obtained through :

1. x-ray crystallography
2. Nuclear Magnetic Resonance (NMR) spectroscopy.



Homology modeling

Structure-based drug design

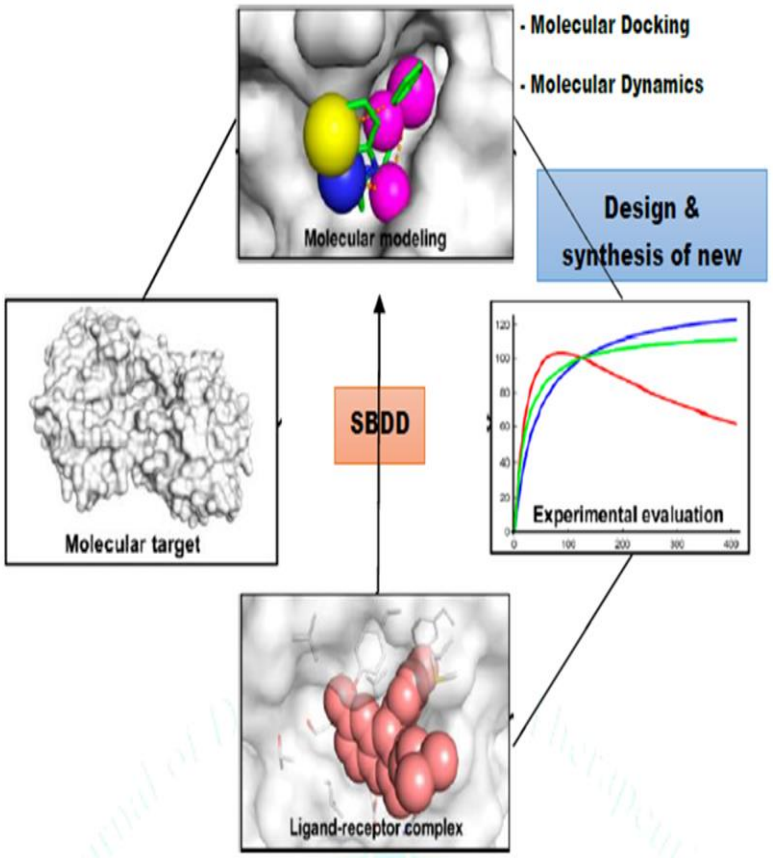
Using the structure of the biological target, candidate drugs that are predicted to bind with **high affinity** and **selectivity** to the target may be designed using:

- interactive graphics
- Intelligence of a medicinal chemist.
- various automated computational procedures may be used to suggest new drug candidates.

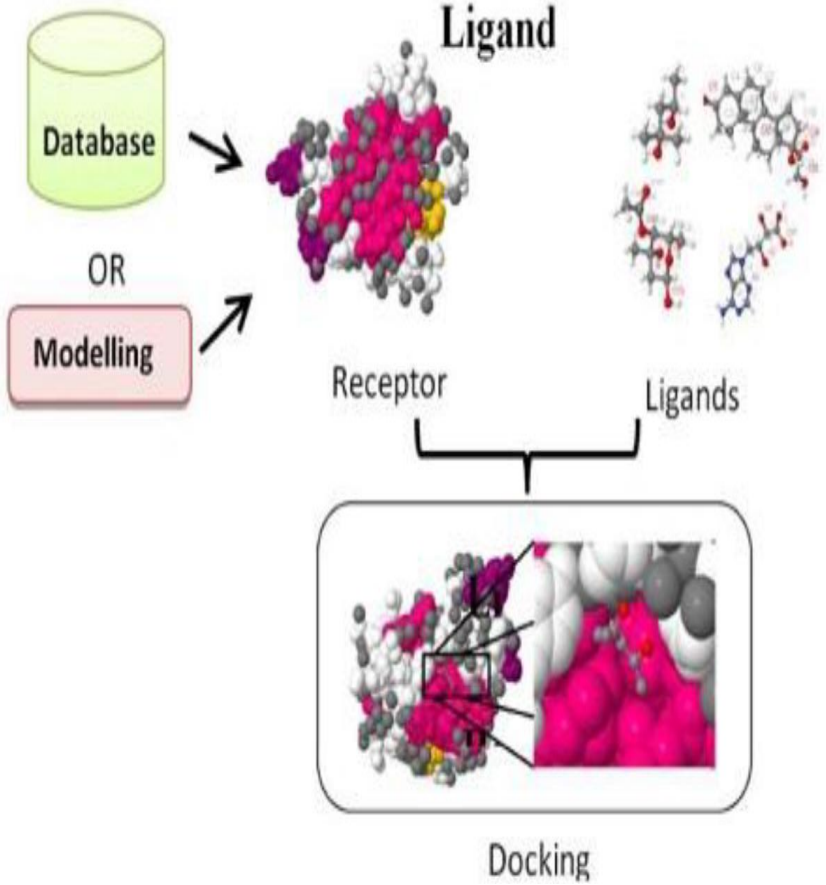
SBDD divided into :

1. Ligand Based DD (DataBase Searching)
2. Receptor Based DD

SBDD


- Molecular Docking
- Molecular Dynamics
Molecular modeling
Design & synthesis of new
Molecular target
SBDD
Experimental evaluation
Ligand-receptor complex

LBDD


Ligand
Database
OR
Modelling
Receptor
Ligands
Docking

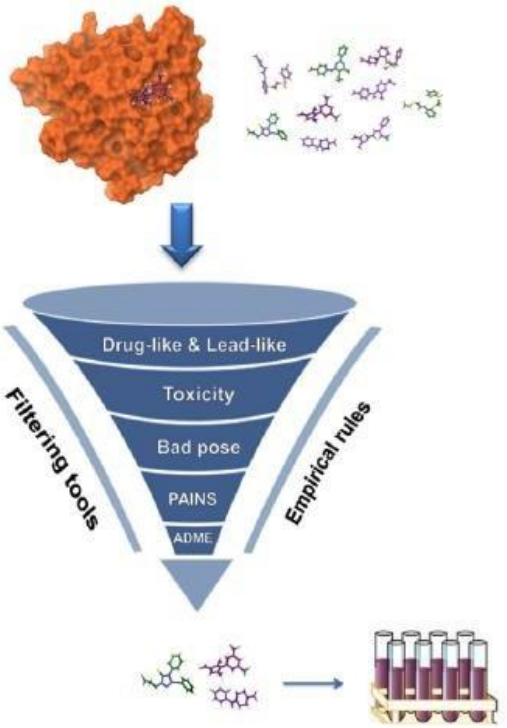
Different Approached based on Structural availability

Receptor	Ligand	Approach	Comments
known	known	DOCK receptor based	Programmes- AUTO-DOCK
known	unknown	De novo based	GROW, LEGEND
unknown	known	Ligand based	QSAR
unknown	unknown	Combinational based	

Methods

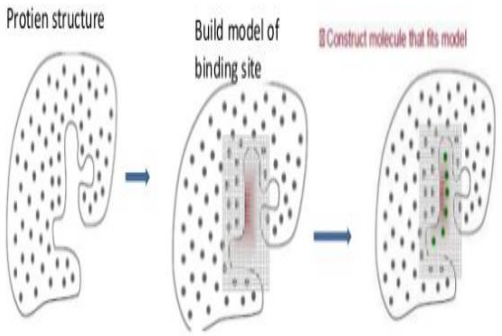
1) Virtual screening :

The first method is identification of new ligands for a given receptor by searching large databases of 3D structures of small molecules to find those fitting the binding pocket of the receptor using fast approximate docking programs.



2) de novo design of new ligands:

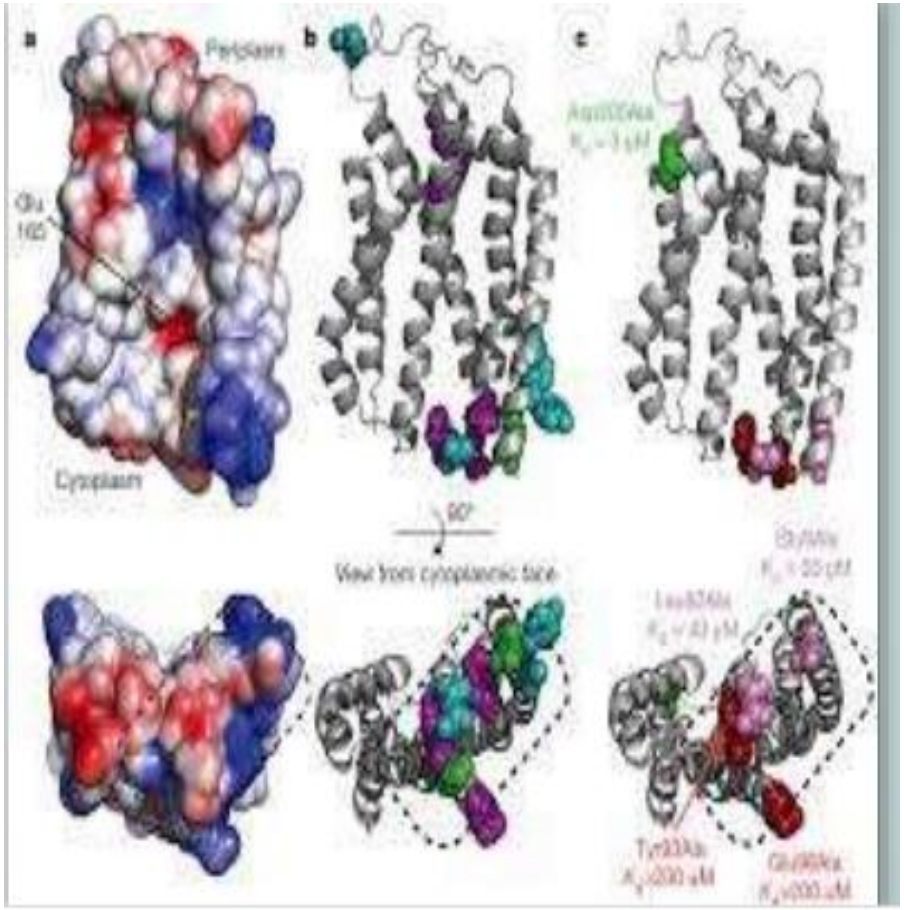
In this method, ligand molecules are built up within the constraints of the binding pocket by assembling small pieces in a stepwise manner. These pieces can be either individual atoms or molecular fragments. The key advantage of such a method is that novel structures can be suggested.



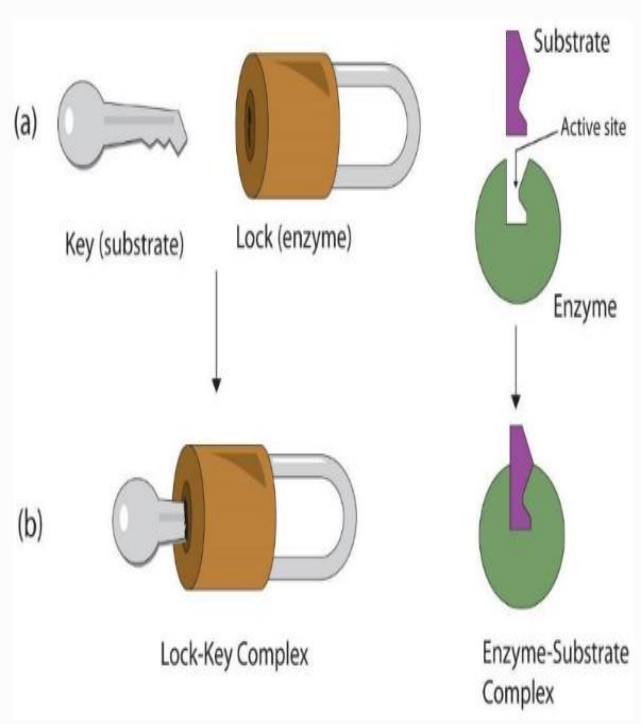
3) optimization of known ligands by evaluating proposed analogs within the binding cavity

Binding site identification

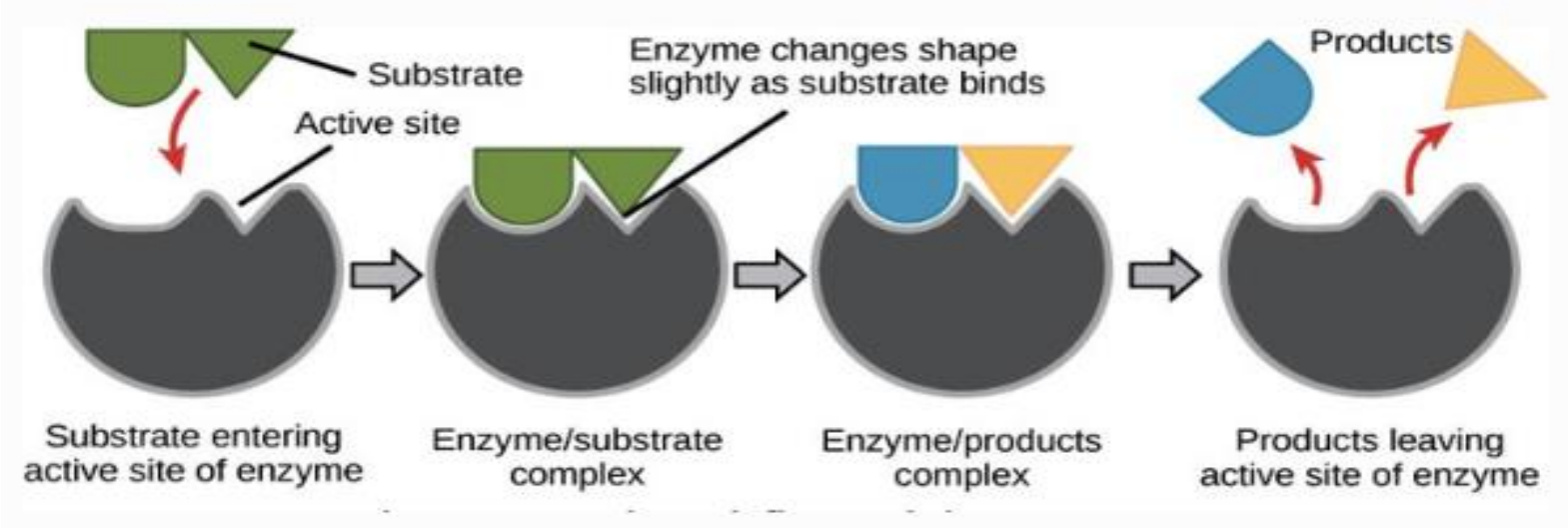
- It is the first step in structure based design.
- relies on identification of concave surfaces on the protein that can accommodate drug sized molecules that also possess appropriate **"hot spots"** (**hydrophobic surfaces, hydrogen bonding sites, etc.**) that drive ligand binding.



DOCKING

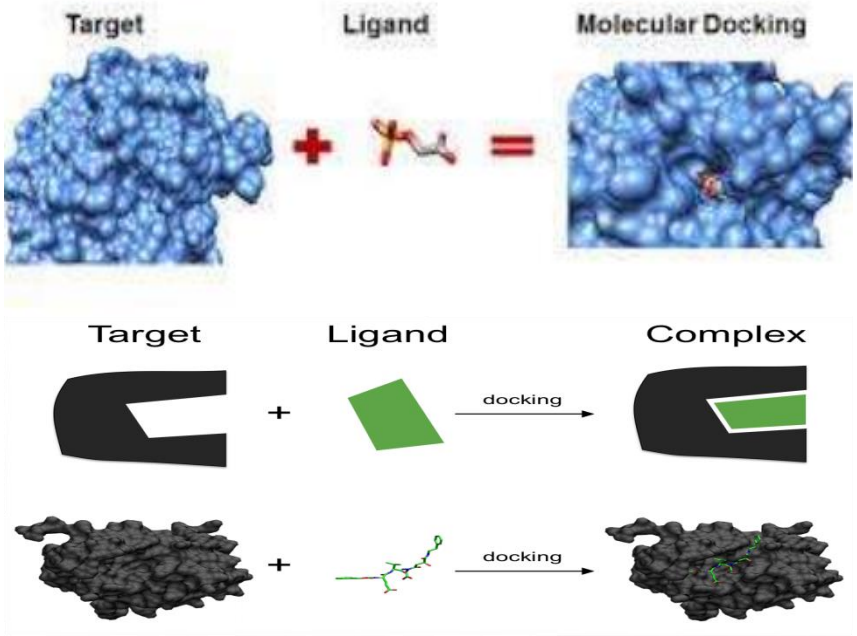


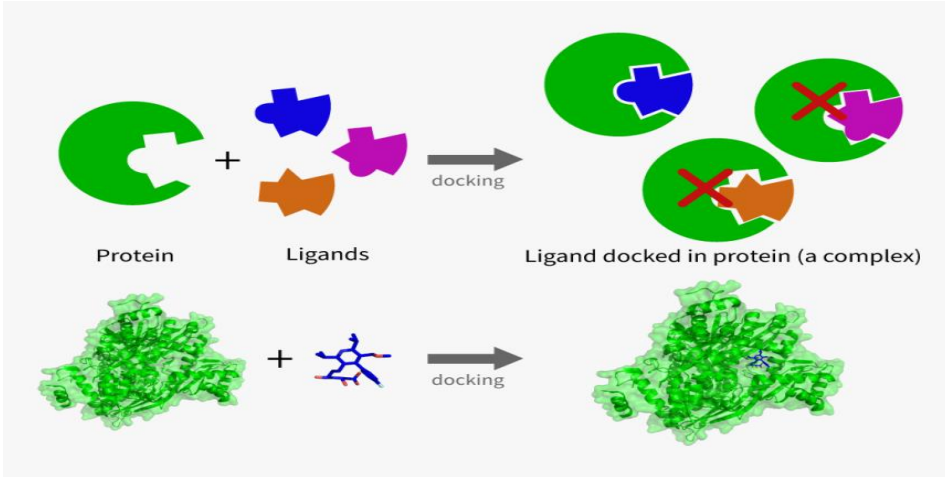
- **There are two distinct forms of docking.**
- Rigid docking.
- Flexible docking.



Docking & Scoring

- Docking attempts to find the "best" matching between two molecules
- It includes finding the Right Key for the Lock
- To place a ligand (small molecule) into the binding site of a receptor in the manners appropriate for optimal interactions with a receptor.
- To evaluate the ligand -receptor interactions in a way that may discriminate the experimentally observed mode from others and estimate the binding affinity.





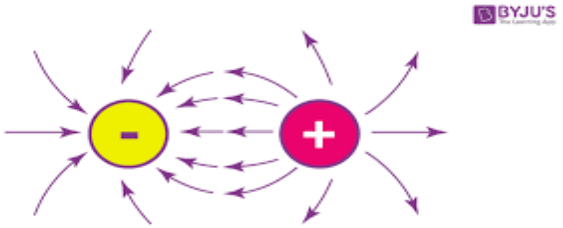
Drug-Receptor Binding: drugs bind to their respective receptor in a variety of ways depending on their characteristics

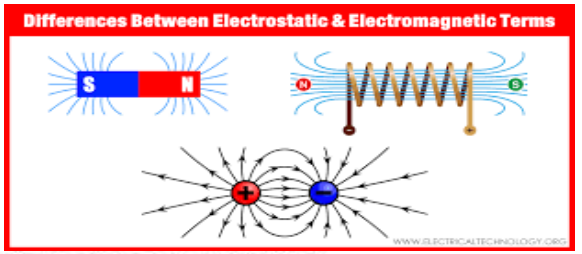
1. Electrostatic forces: forces with electrostatic origin are due to the changes residing in the matter

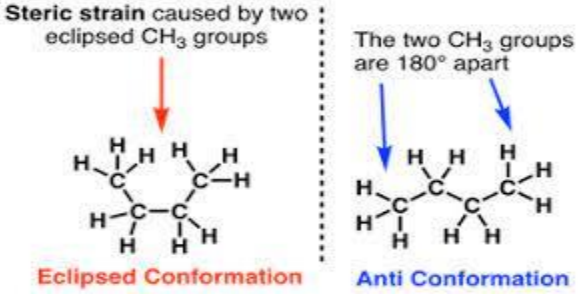
2. Electrodynemic Forces :The most widely known is probably the van der waals interaction

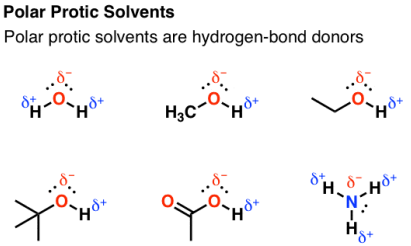
3. Steric Forces: These are caused by entropy . For example in cases where entropy is limited : there may be forces in minimized the free energy of the system

4. Solvents- related forces : These are due to structural changes of the solvent . These structural changes are generated , when ions, colloids , protein etc. are added into the structure of solvent. The most commonly are Hydrogen bond and Hydrophobic interaction .







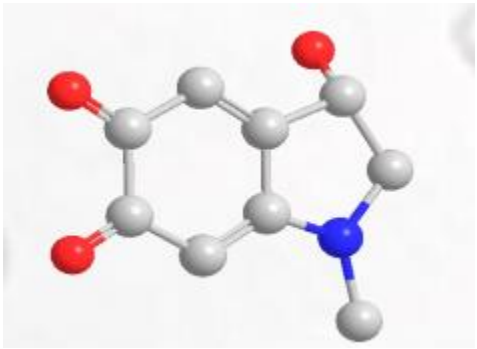


Categories of software

1. Databases & Draw Tools
2. Molecular Modeling &Homology Modeling
3. Binding site prediction & Docking
4. Ligand design Screening –QSAR
5. Binding free energy estimation
6. ADME Toxicity

COMPUTER-AIDED DRUG DESIGN

- 1. Molecular modeling studies



- 2. Quantitative structure activity relationship (QSAR)

Molecular modeling

The aim of molecular modeling is to understand

a. the basic relationship between chemical and physical properties,

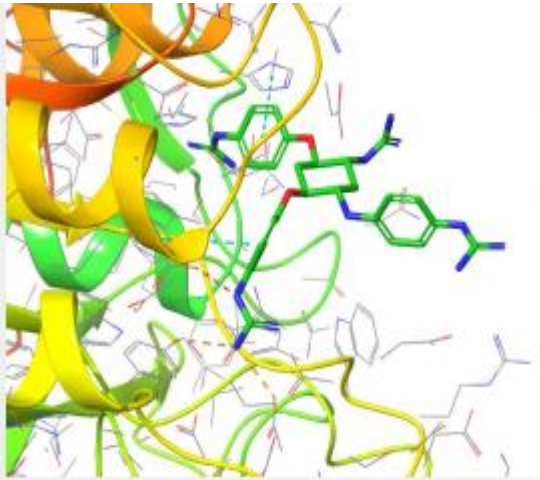
b. chemical structure and

c. 3D structure of a molecule.

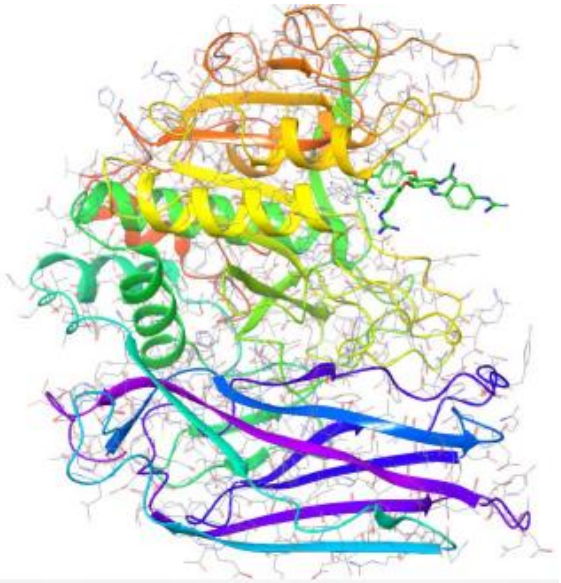
3-dimensional study is the definition of all properties of a compound in space.

Using molecular modeling techniques gathering information about;

1. 3D structure of the molecule
2. Physicochemical properties of the molecule
3. Comparison of a molecule with other molecules 4. Investigate the receptor-drug interactions



Close view



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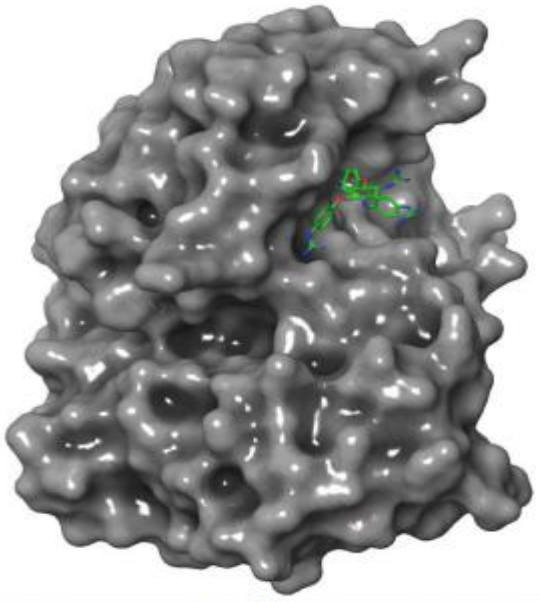
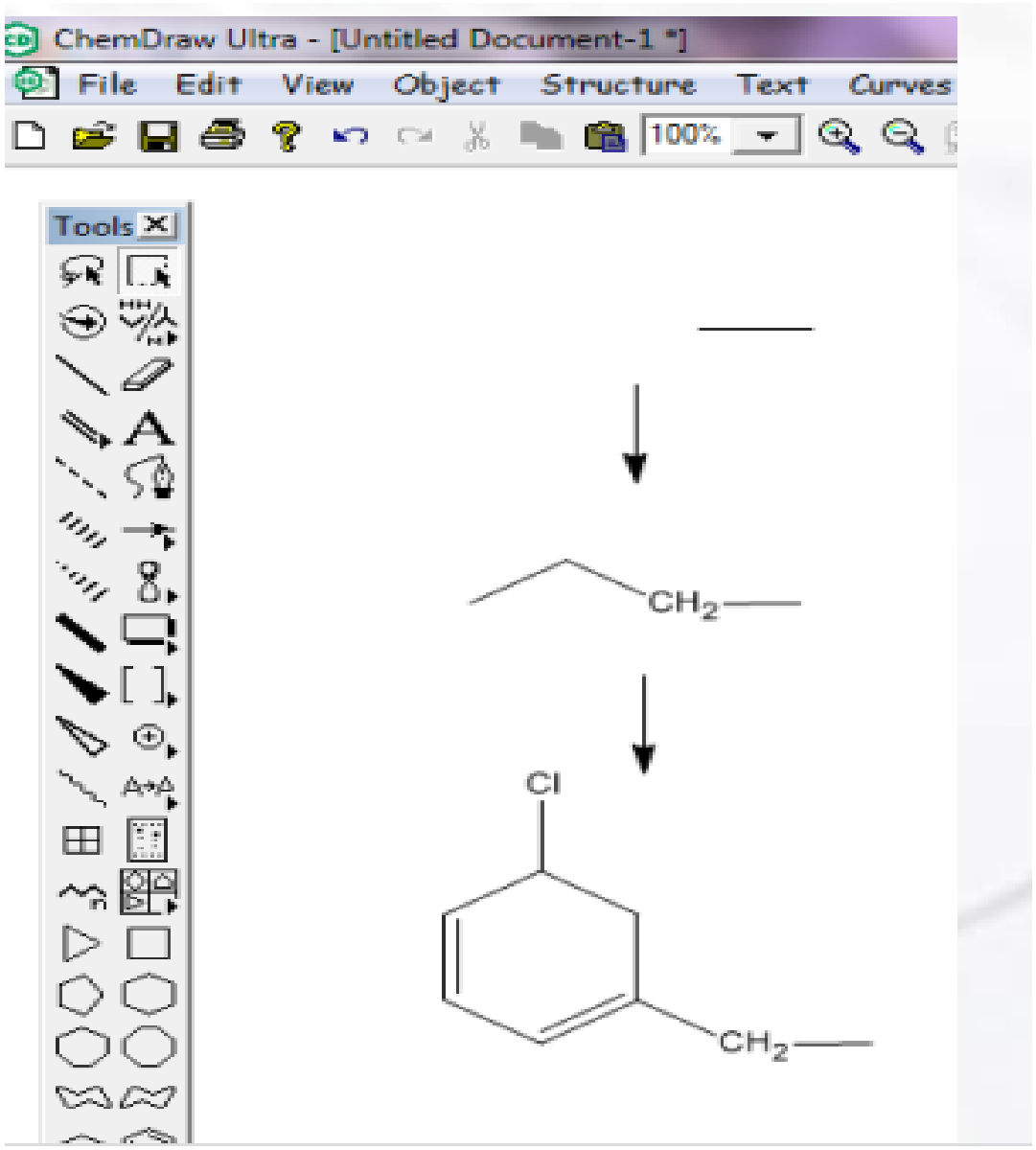
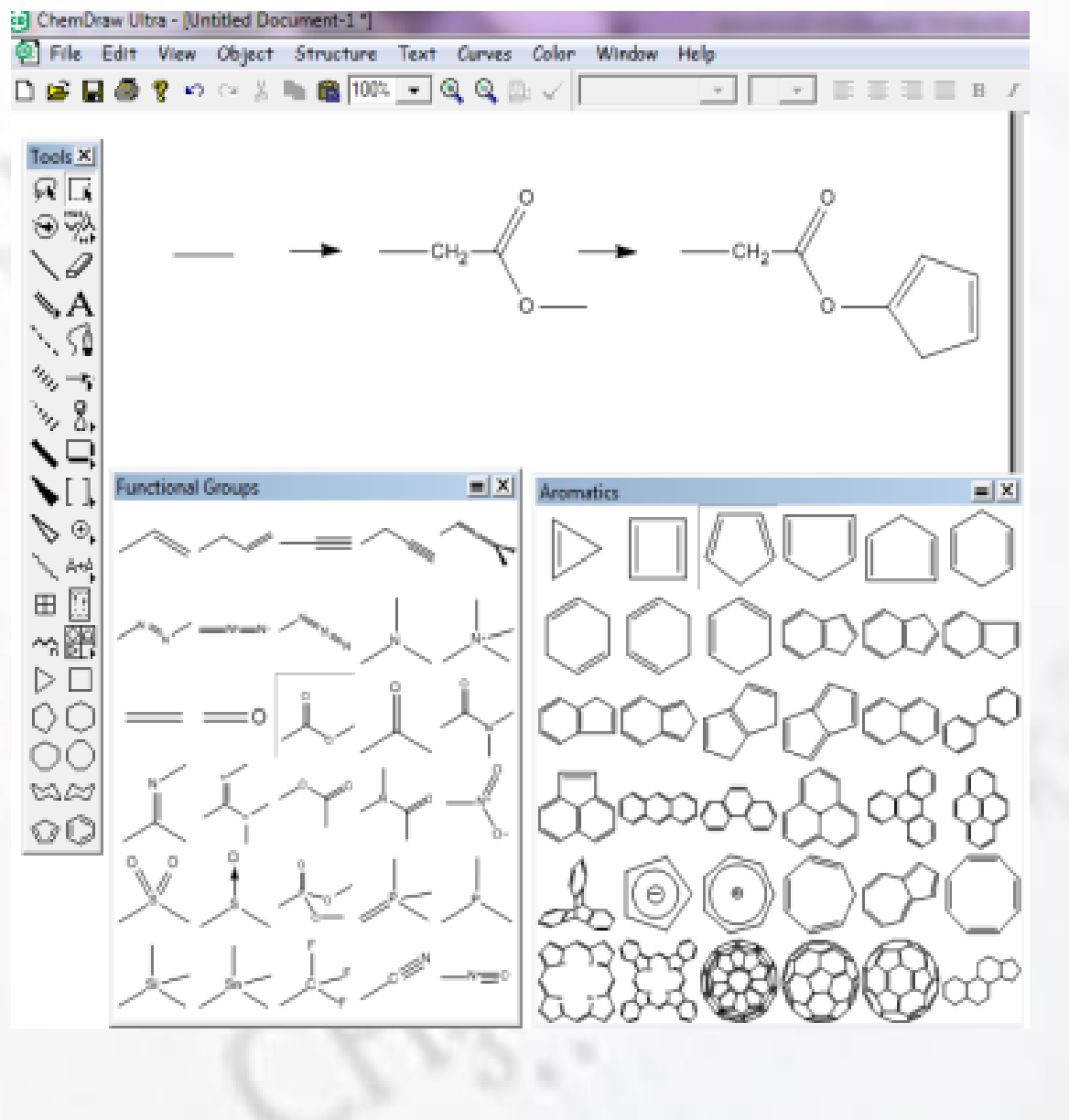


surface

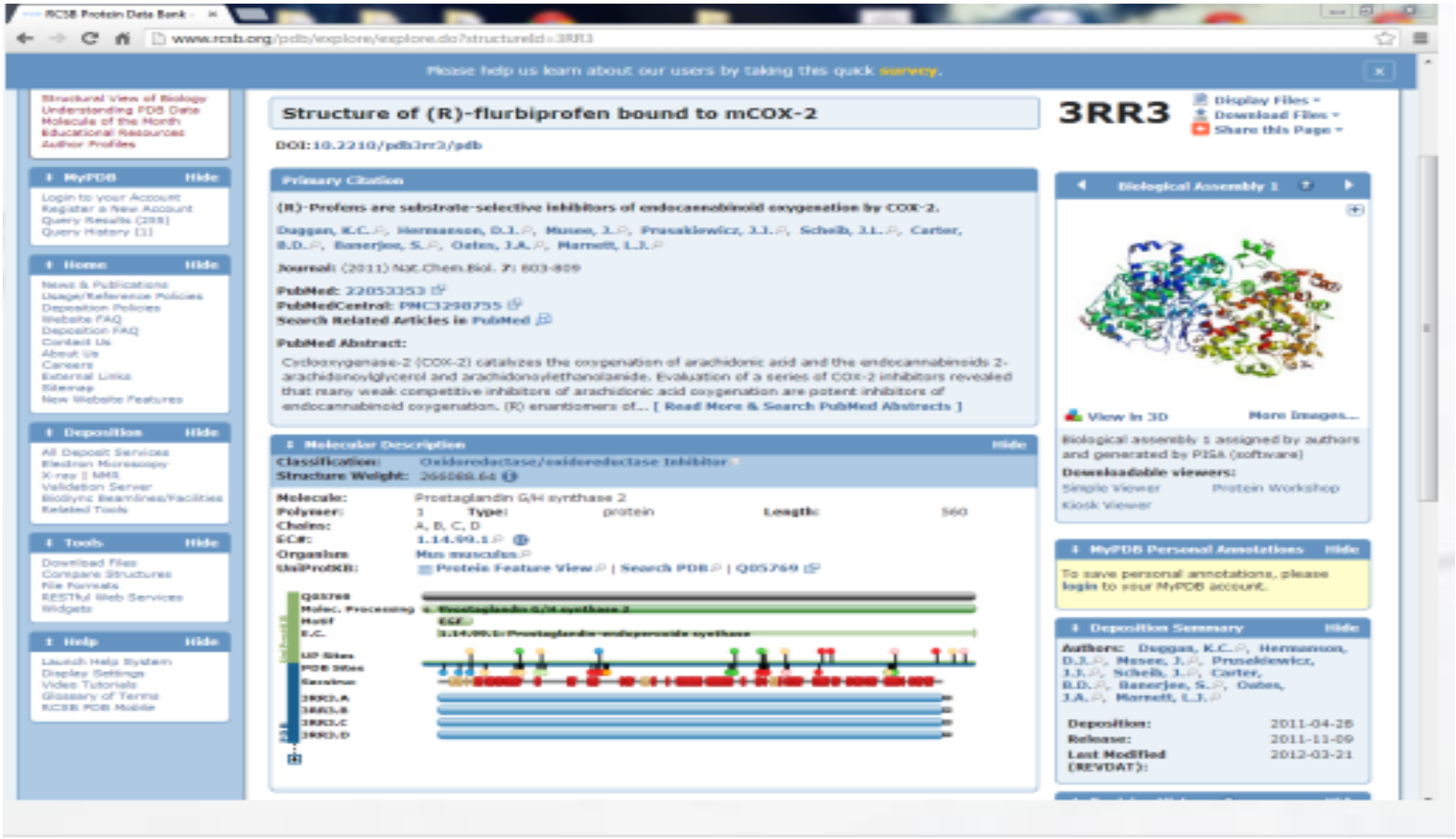
- 3D structure can be created by using the drawing features in the software

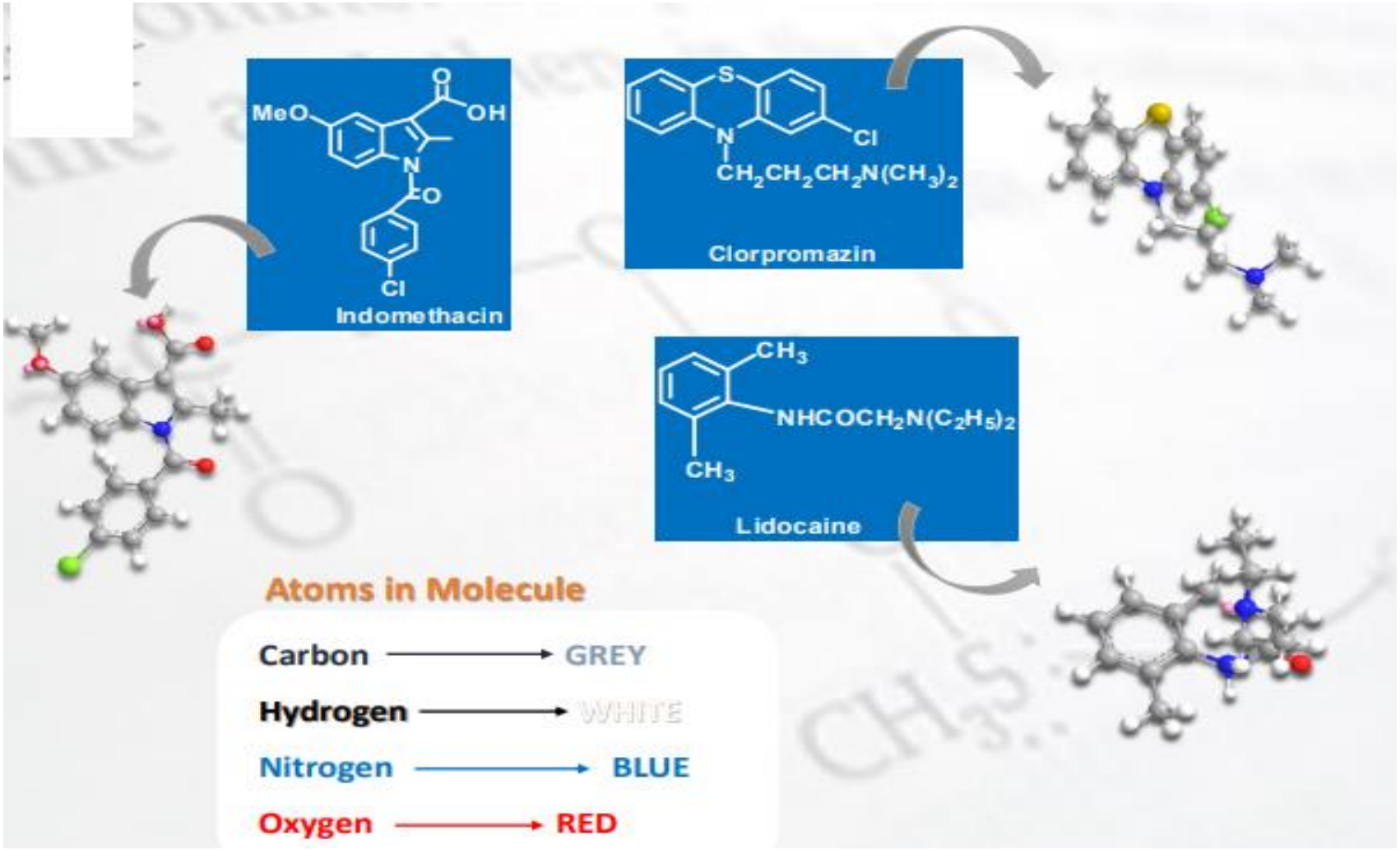


- 3D structures can be created by using the fragment data in a program (software).



The three-dimensional structure of the molecule can be taken directly from the data banks created by X-ray crystallography



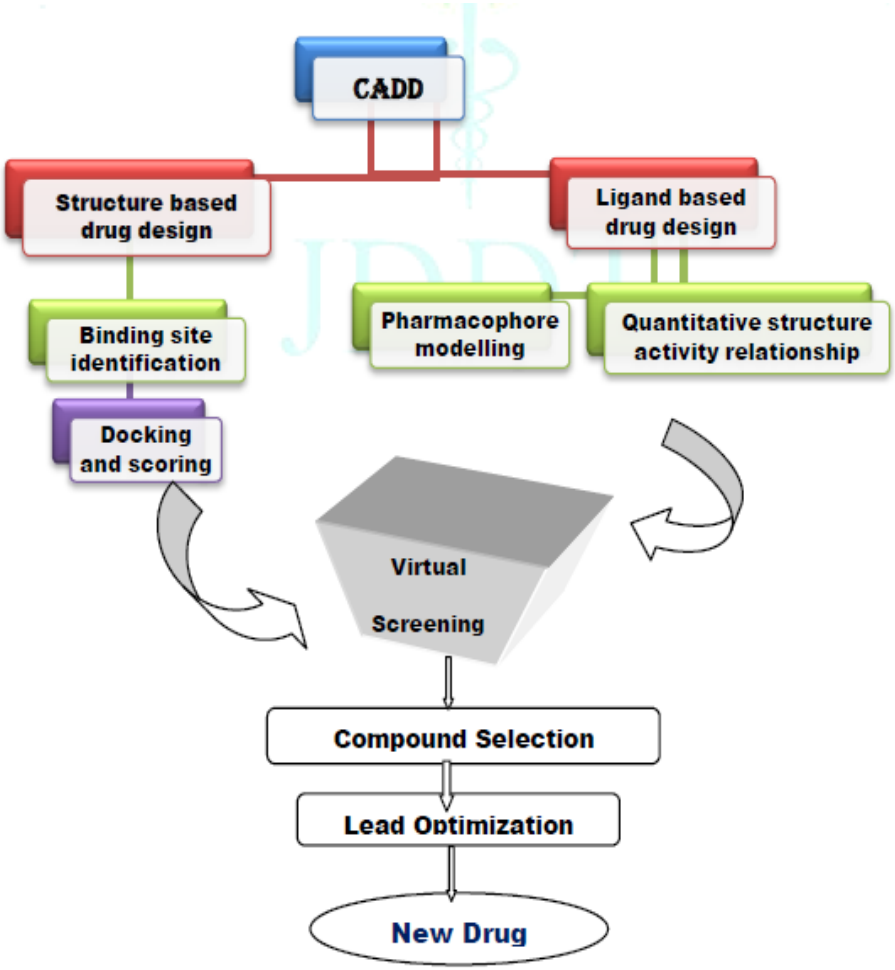

MeO
OH
N
O
Cl
Indomethacin
S
N
Cl
CH2CH2CH2N(CH3)2
Clorpromazin
CH3
NHCOCH2N(C2H5)2
CH3
Lidocaine
Atoms in Molecule
Carbon GREY
Hydrogen WHITE
Nitrogen BLUE
Oxygen RED

General Representation of workflow for CADD.

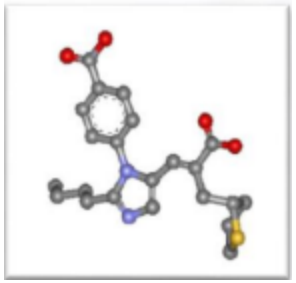
- Let's watch a video You can find the link below:

https://www.youtube.com/watch?v=IuJqbV4D8Cc&list=FLD5BNZVjYcwC62P KTJs47Gg

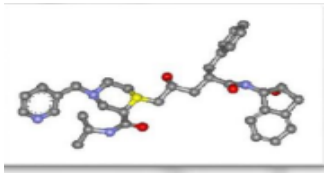
- This video is about SARS-CoV-2 Structure (COVID-19 Coronavirus)



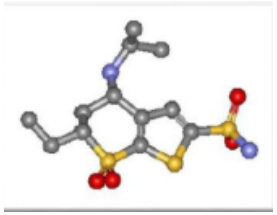
DRUGS DISCOVERED BY COMPUTER AIDED DRUG DESIGN METHODS



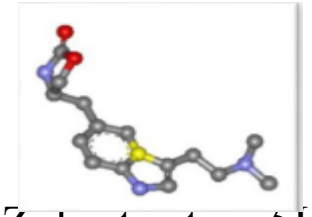
TEVETEN® for hypertension treatment-**Abbott** Eprosartan:
Angiotensin II receptor antagonist, Molecular Modeling



CRIXIVAN® for AIDS –**Merck** Indinavir:
HIV-1 Protease Inhibitor, X-ray crystallography, Moleculer Mechanics Calculations and Receptor Based Design.



TRUSOPT® for Glaucoma treatment-**Merck** Dorzolamide:
Carbonic anhydrase inhibitor ab inito Calculations and
Receptor Based Design



ZOMIG® for Migrain treatment-**Wellcome,** Zeneca
Zolmitriptan: 5HT1-agonist, Pharmacophore Analysis and Ligand Based Design


Thank you

The Organic Chemistry of Drug Design and Drug Action

BY:

Dr. Nohad A AlOmari (B.Pharm/MSc & PhD in Medicinal Chemistry)

Organic Pharm. Chemistry IV

The Organic Chemistry of Drug Design and Drug Action

Lecture 1

17th September 2023

10:30 (AM) - 12:30 PM

AlNoor University College

Pharmacy Department

1st Course/ 5TH Class

2022/2023

Lectures titles & Credit hours

Title of the course: *Organic Pharmaceutical Chemistry* **IV** Course number: **511**
Level: 5th Class, 1st Semester
Credit hours: **Theory 2 hours** **Laboratory** ------
Tutors:
Reference text: ***Wilson and Gisvold Textbook of Organic Medicinal and Pharmaceutical Chemistry; Delgado JN, Remers WA, (Eds.); Latest edition.***

Objectives: To give the students knowledge and experience in pro-drug and hormones as part of their medicinal and pharmaceutical field. I include classification, synthesis, biotransformation and/or formulation of certain drugs to improve their action as well as to avoid some side effect.

No	Lecture title	hours
1.	Basic concept of prodrugs; Covalent bonds (cleavable); Prodrugs of functional groups; Types of prodrugs.	6
2.	Chemical delivery systems; Polymeric prodrugs; Types and structure of polymers; Cross-linking reagents.	6
3.	Drug targeting.	4
4.	Project.	4
5.	Combinatorial chemistry; Peptides and other linear structures; Drug like molecules; Support and linker; Solution-phase combinatorial chemistry.	5
6.	Detection, purification and analgesics; Encoding combinatorial libraries; High-throughput screening; Virtual screening; Chemical diversity and library design.	5

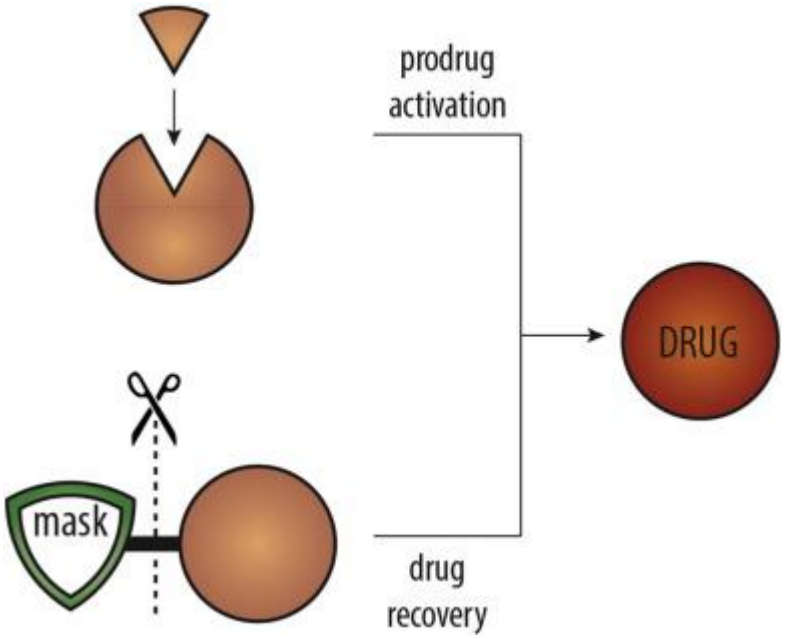
Learning Outcomes
At the end of this course ; students will be able to :

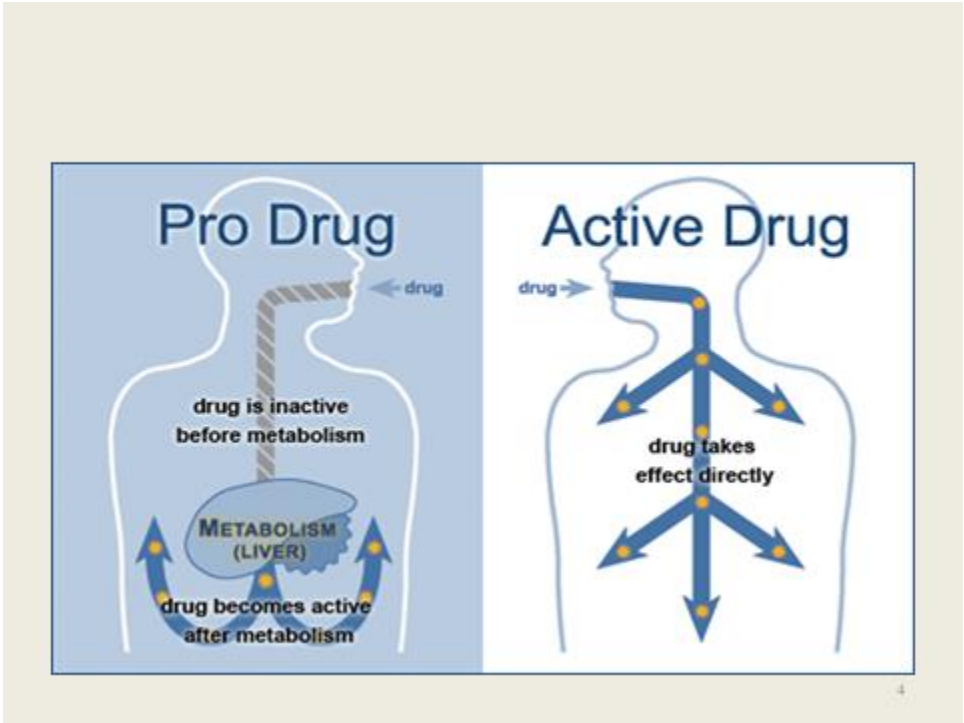
- Define and describe various applications of the prodrugs.
- Explain different types of prodrugs with different function groups
- Describe the carrier – linked prodrugs , Mechanisms of Prodrug Activation with examples.
- Define and explain macromolecules drug delivery
- Describe importance of mutual prodrugs.
- Explain bioprecursors with suitable examples
- Realize the meaning of macromolecular drug delivery , their function and roles.
- Site- directed Prodrug , what does it mean , their benefits and applications
- Definition of Combinatorial Chemistry, and its application to drug discovery .
- Virtual Screening and Computer- aided drug design

Prodrugs and Related Terms

Prodrug - a pharmacologically inactive compound that is converted to an active drug by a metabolic biotransformation

Ideally, conversion occurs as soon as the desired goal for designing the prodrug is achieved.





History

The term prodrug was introduced by *Albert* who used “prodrug” or “proagent”

Another term drug latentiation, which implies a time lag element or component, was coined by *Harper*.

Later, the concept of prodrug and latentiated drug for solving various problems was attempted and the definition of drug latentiation was extended to include non–enzymatic regeneration of parent compounds.

Drug meaning !!!

Prodrugs and soft drugs are opposite:

a prodrug is inactive - requires metabolism to **give active form**

a soft drug is active - uses metabolism to **promote excretion**

A pro-soft drug would require metabolism to convert it to a soft drug

Utility of Prodrugs

1. **Aqueous Solubility** - to increase water solubility so it can be injected in a small volume

2. **Absorption and Distribution** - to increase lipid solubility to penetrate membranes for better absorption

3. **Site Specificity** - to target a particular organ or tissue if a high concentration of certain enzymes is at a particular site or append something that directs the drug to a particular site---often tried to limit the toxicity of anticancer drugs.

Utility of Prodrugs (cont'd)

4. **Instability** - to prevent rapid metabolism; avoid first-pass effect

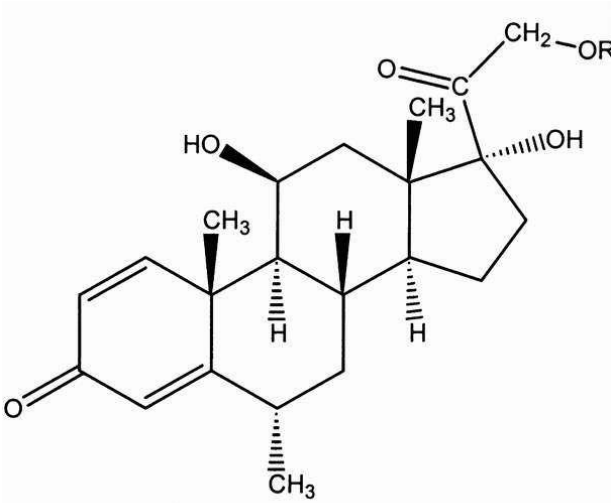
5. **Prolonged Release** - to attain a slow, steady release of the drug

6. **Toxicity** - to make less toxic until it reaches the site of action

7. **Poor Patient Acceptability** - to remove an unpleasant taste or odor; gastric irritation

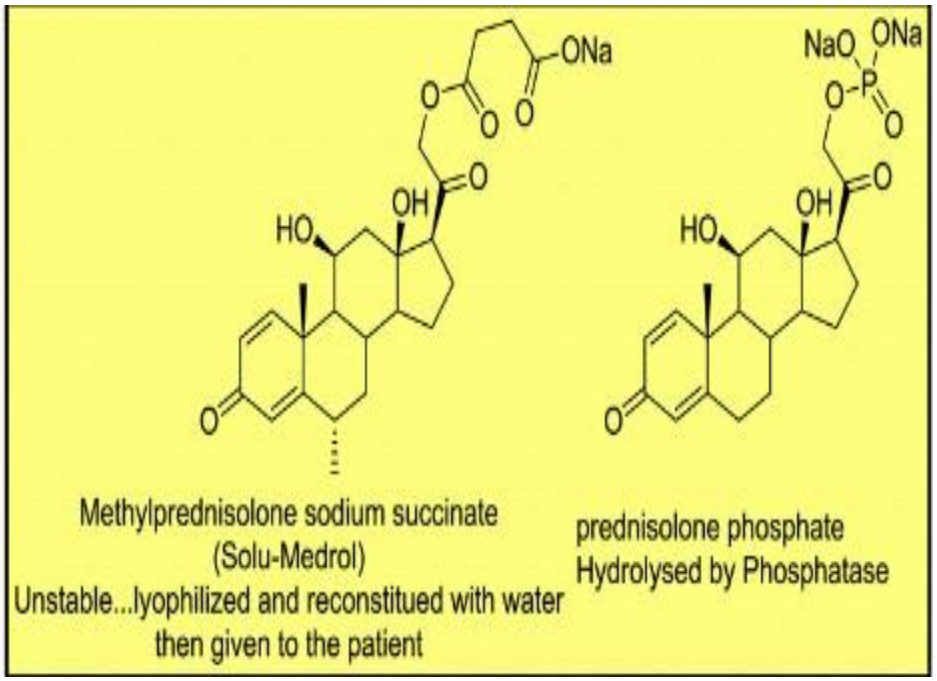
8. **Formulation Problems** - to convert a drug that is a gas or volatile liquid into a solid

1. **Aqueous Solubility** - to increase water solubility so it can be injected in a small volume



Methylprednisolone: R = H

Methylprednisolone Sodium Succinate: R = $C(=O)CH_2CH_2COO^-$ Na^+

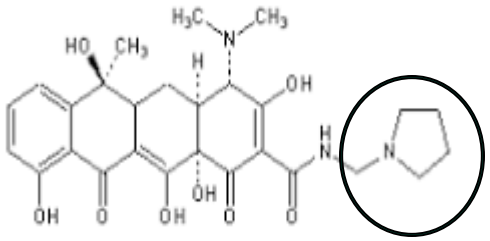


Methylprednisolone sodium succinate
(Solu-Medrol)
Unstable...lyophilized and reconstitued with water then given to the patient

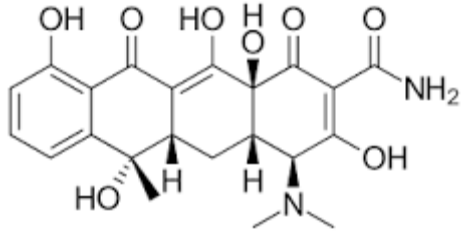
prednisolone phosphate
Hydrolysed by Phosphatase

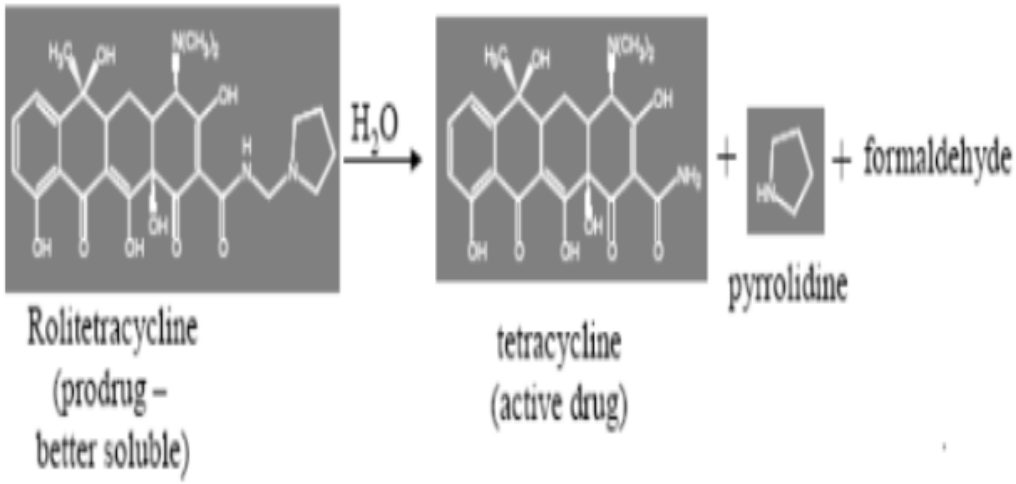
Phosphate ester of clindamycin (antibacterial) • Less painful on injection (HW)???

Lysine ester of oestrone • Lysine ester of oestrone is better absorbed orally than oestrone • Increased water solubility prevents formation of fat globules in gut • Better interaction with the gut wall • Hydrolysis in blood releases oestrone and a non toxic amino acid

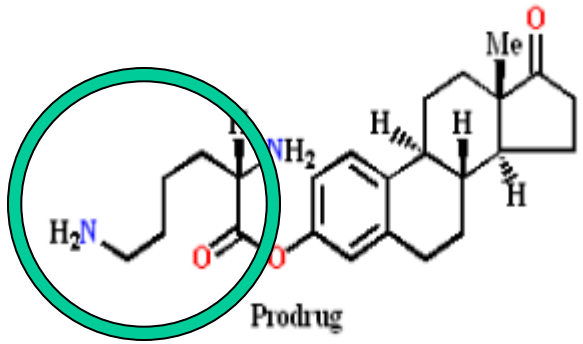


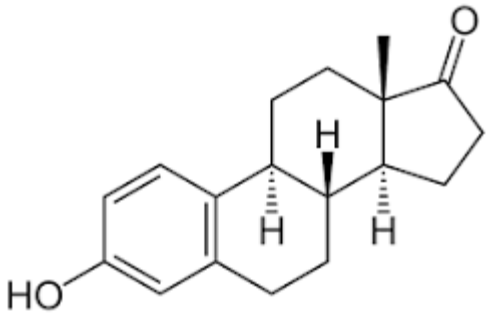
Rolitetracycline





Lysine ester of oestrone

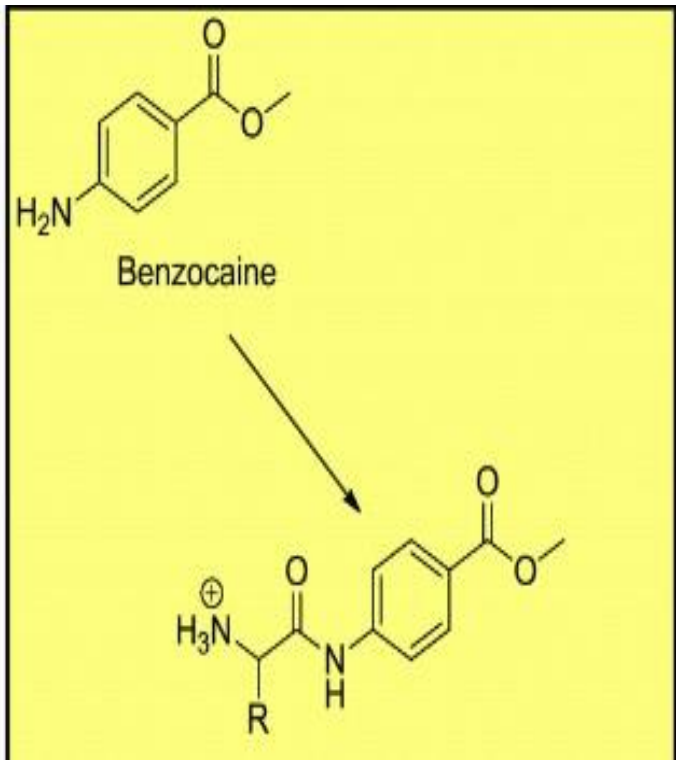




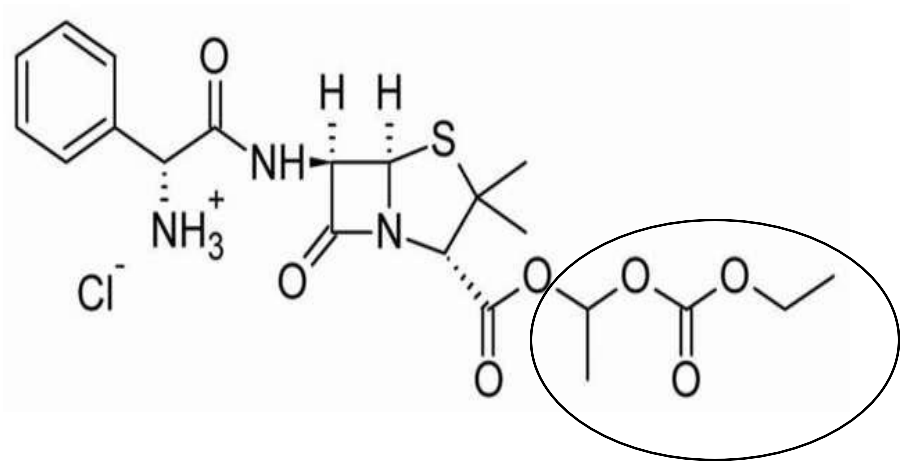
Prodrugs to increase water solubility

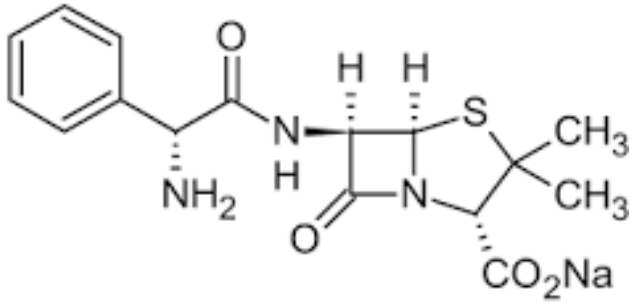
- Benzocaine coupled with amino acid ionized at physiological pH

Better water solubility



2. **Absorption and Distribution** - to increase lipid solubility to penetrate membranes for better absorption

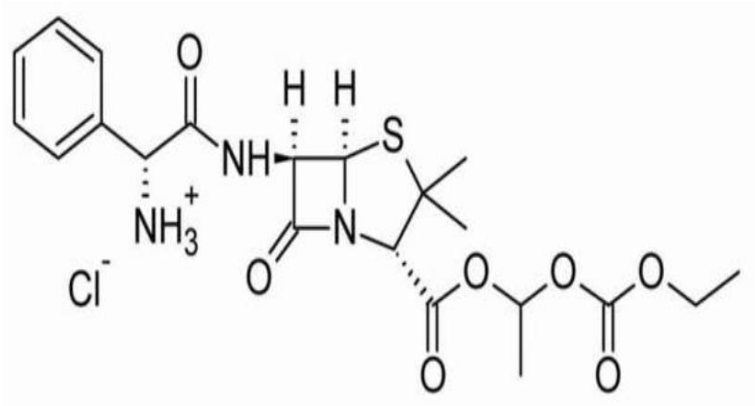




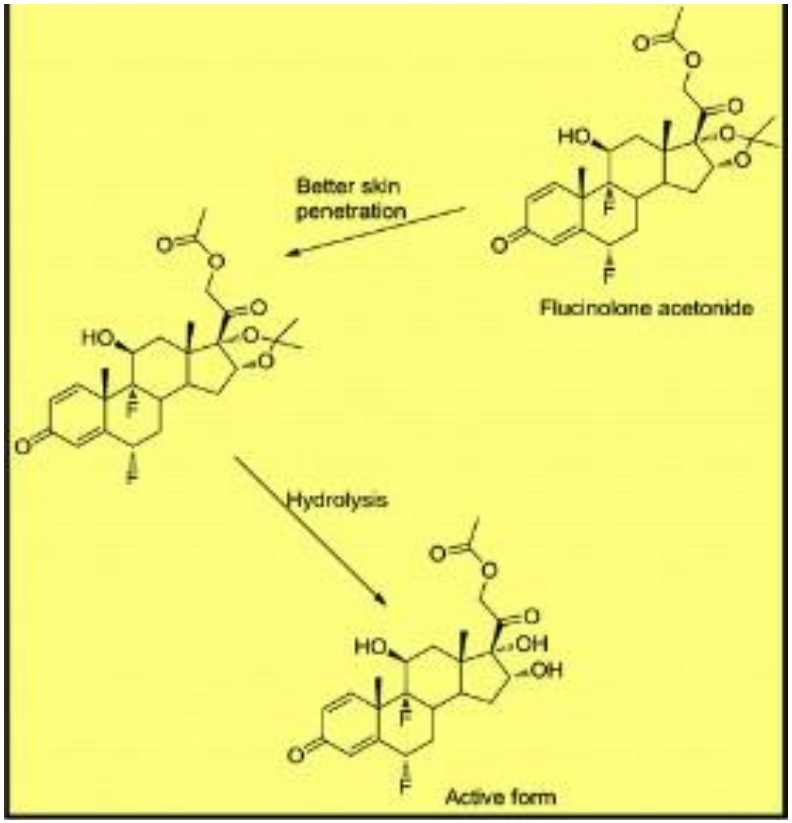
Ampicillin double ester

2. **Absorption and Distribution** - to increase lipid solubility to penetrate membranes for better absorption

a/ Ampicillin double ester



b/ Flucinolone acetonide

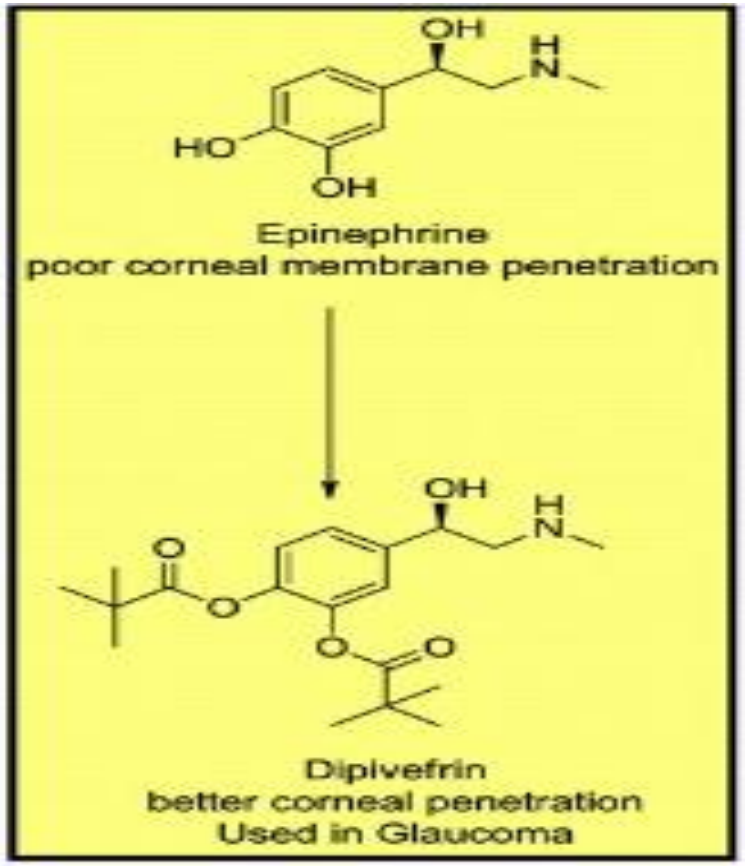


To increase absorption

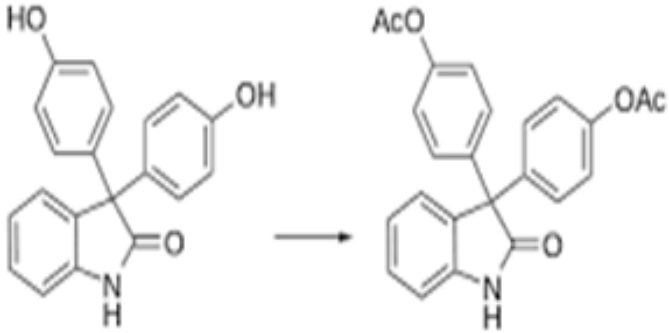
c/better corneal penetration

Dipivefrin better corneal penetration

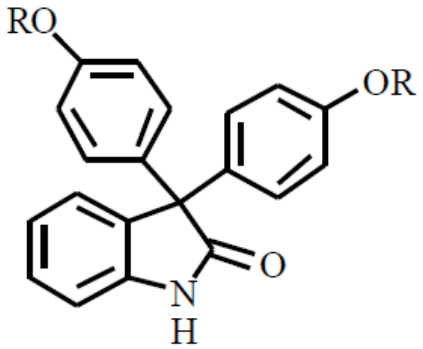
(used in Glaucoma)



3. **Site Specificity**



3. **Site Specificity** - to target a particular organ or tissue if a high concentration of certain enzymes is at a particular site or append something that directs the drug to a particular site---often tried to limit the toxicity of anticancer drugs.

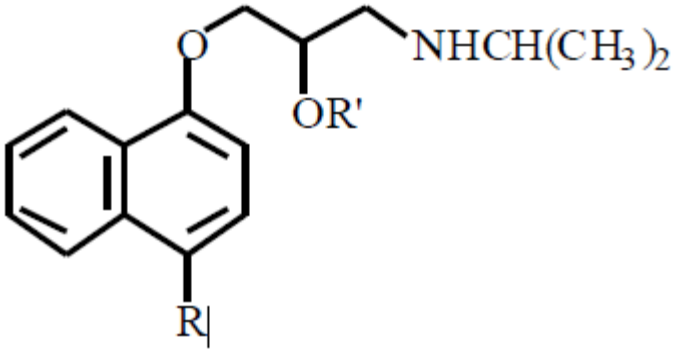


- **oxyphenisatin (R = H) (Rectaly)**

prodrug R = Acetyl(administer orally) hydrolyzed in intestines

4. **Instability**

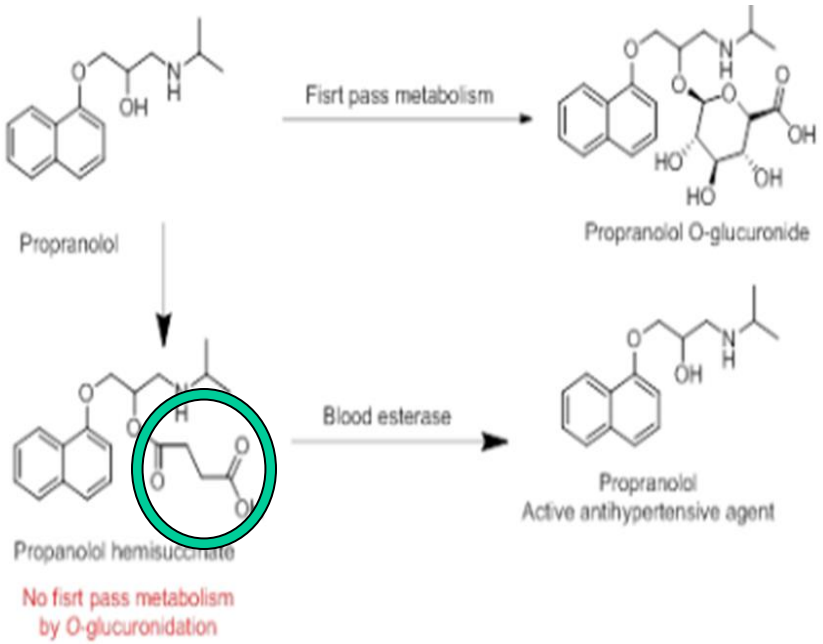
4. **Instability** - to prevent rapid metabolism; avoid first-pass effect



propanolol (R = R' = H)

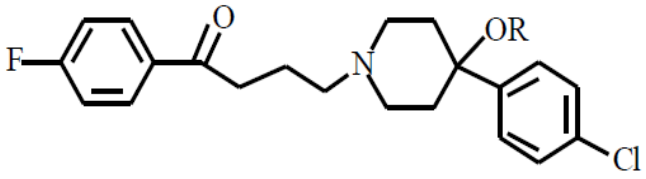
prodrug R' =

$OCCH_2CH_2COOH$



Propranolol undergo extensive first pass metabolism (glucuronidation)
So, adding ester group on alcohol will protect it from metabolism

5.Prolonged Release - to attain a slow, steady release of the drug

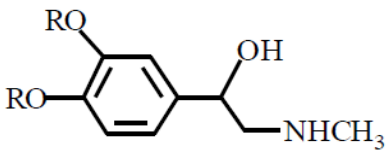


haloperidol (R = H)

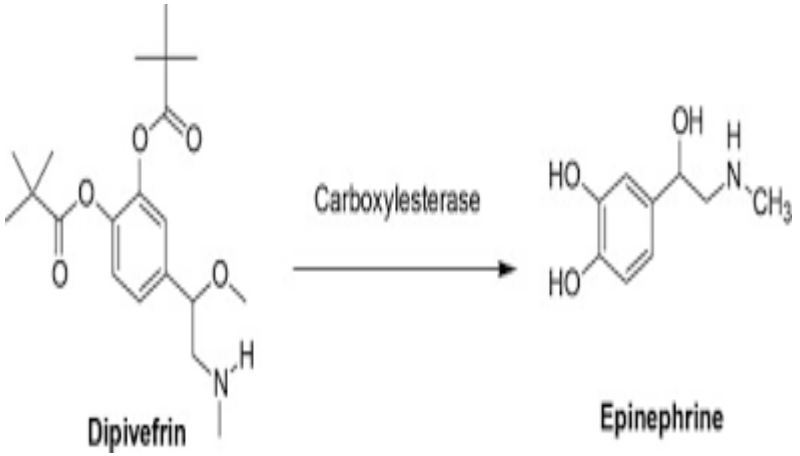
haloperidol decanoate (R = $CO(CH_2)_8CH_3$)

It is administered by injection into muscle at a dose of 100 to 200 mg once every 4 weeks or monthly.

6. **Toxicity** - to make less toxic until it reaches the site of action



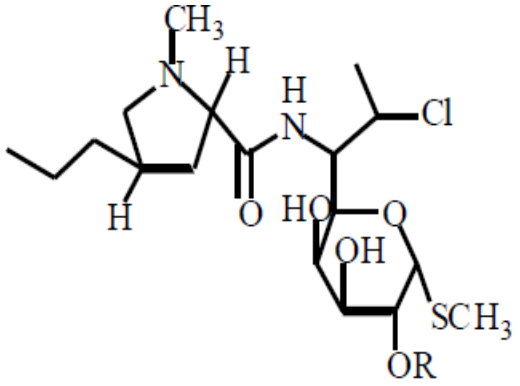
dipivefrin (R = Me_3CCO)
epinephrine (R = H)



Dipivefrin Epinephrine

7. **Poor Patient Acceptability** - to remove an unpleasant taste or odor; gastric irritation.

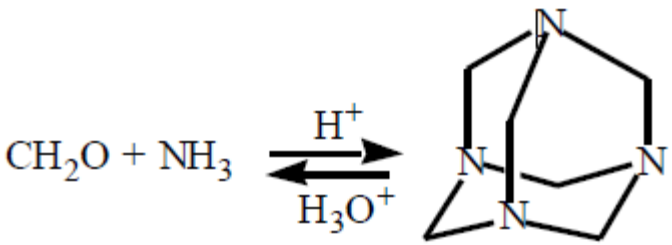
Clindamycin phosphate is inactive in vitro, rapid in vivo hydrolysis converts this compound to the antibacterially active clindamycin.



clindomycin (R = H)
clindomycin phosphate (R = PO_3H_2)
clindomycin palmitate (R = $O(CH_2)_{14}CH_3$)

8. **Formulation Problems** - to convert a drug that is a gas or volatile liquid into a solid (methenamine)

Methenamine hippurate is a prodrug that converts to formaldehyde in an acidic environment. Formaldehyde is the active substance that exerts bactericidal activity in the urine



Types of Prodrugs

Drug Latentiation - rational prodrug design

I. Carrier-linked prodrug

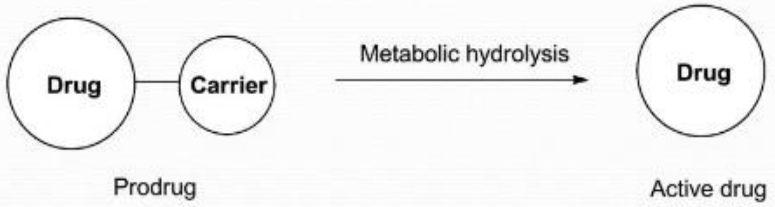
A compound that contains an active drug linked to a carrier group that is removed enzymatically

A. **bipartate** - comprised of one carrier attached to drug

B. **tripartate** - carrier connected to a linker that is connected to drug

C. **mutual** - two, usually synergistic, drugs attached to each other

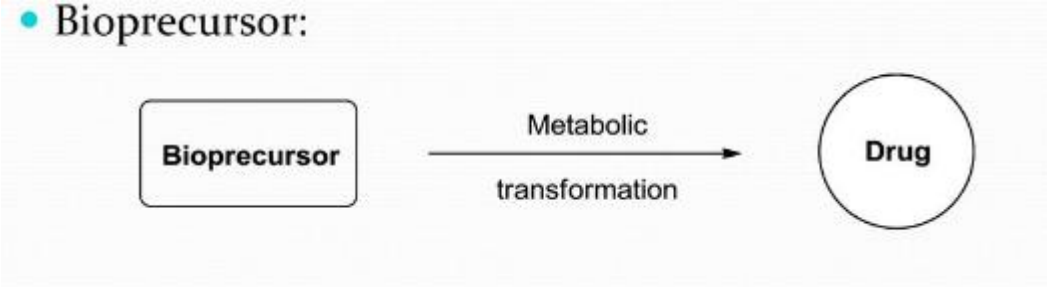
- Carrier-linked:



- The common carrier group is the ester (WHY?)
  - Availability of esterase enzyme
  - Possible of either increasing or decreasing the lipophilicity of the parent drug.
  - Can be either easily hydrolyzed or relatively stable ester

II. Bioprecursor prodrug

A compound metabolized by molecular modification into a new compound, which is a drug or is metabolized further to a drug - not just simple cleavage of a group from the prodrug—e.g., amine getting oxidized to CO2H, to afford the active.



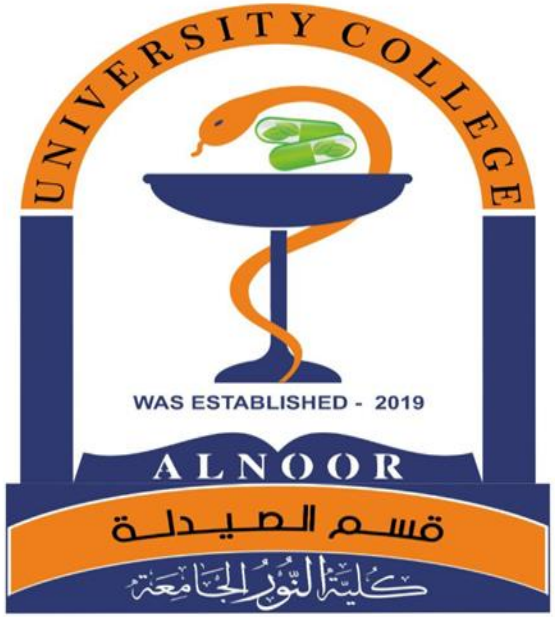
https://slideplayer.com/slide/15966920/

The Organic Chemistry of Drug Design and Drug Action

Lecture 2

1st October 2023

10:30 (AM) - 12:30 PM



AlNoor University College

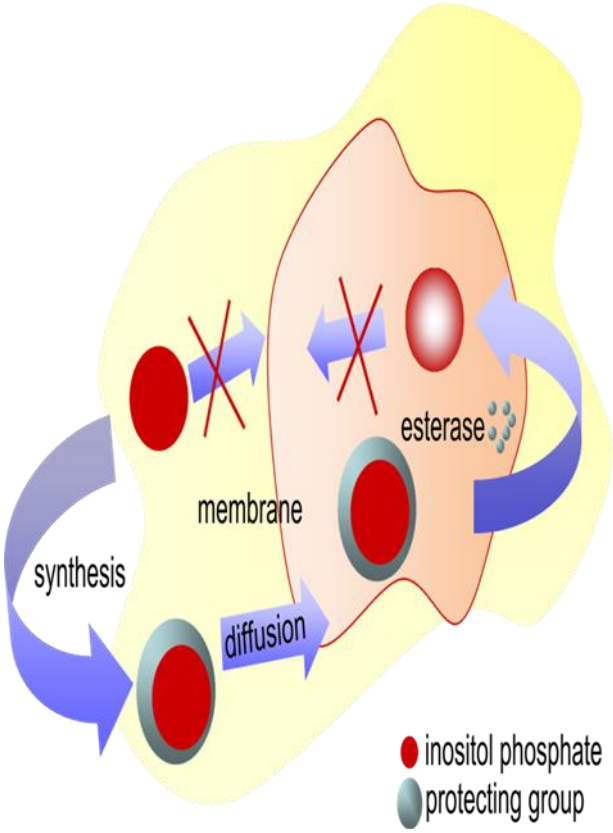
Pharmacy Department

1st Course/ 5TH Class

2023/2024

Recent prodrugs

- Approximately 10% of all marketed drugs worldwide can be considered prodrugs. Since 2008, at least 30 prodrugs have been approved by the FDA.[1] Seven prodrugs were approved in 2015 and six in 2017.
- Examples of recently approved prodrugs are such as: dabigatran etexilate (approved in 2010), gabapentin enacarbil (2011), sofosbuvir (2013), tedizolid phosphate (2014), isavuconazonium (2015), aripiprazole lauroxil (2015), selexipag (2015), latanoprostene bunod (2017), benzhydrocodone (2018), and tozinameran (2020).



- [1] Nature Reviews. Drug Discovery. 17 (8): 559–587

CLASSIFICATION:

Prodrugs can be classified into two major types, based on how the body converts the prodrug into the final active drug form.

Type I **prodrugs** are bioactivated **intracellularly**. Examples of these are anti-viral nucleoside analogs and lipid-lowering statins.

Type II prodrugs are bioactivated **extracellularly,** especially in digestive fluids or in the body's circulation system. Examples of these are antibody-, gene- or virus-directed enzyme prodrugs [ADEP/GDEP/VDEP] used in chemotherapy or immunotherapy.

Both major types can be further categorized into Subtypes, based on factors such as (Type I) whether the intracellular bioactivation location is also the site of therapeutic action, or (Type 2) whether or not bio activation occurs in the body's gastrointestinal fluids or its circulation system.

Steps in prodrug design:

- Identification of drug delivery problem.
- Identification of desired physicochemical properties-selection of transport moiety which will give prodrug desired transport properties and be readily cleaved in the desired biological compartment.

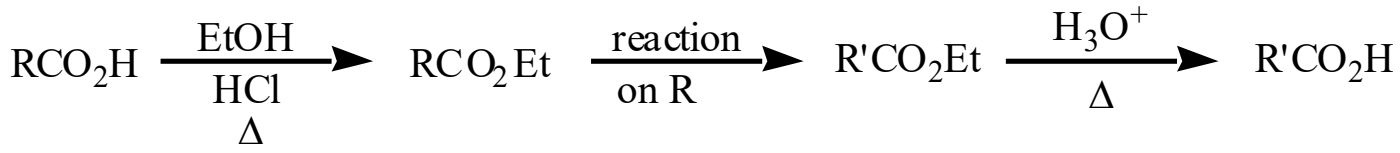
Design and Structure:

- Currently, many prodrugs employ primarily **hydroxyl, amine and carboxyl groups.** Esters are found most commonly in commercial prodrug, such as the drug oseltamivir (is an antiviral medication used to treat and prevent influenza A and influenza B)
- More atypical groups have been investigated for use in prodrugs, such as **thiols and imines**. The bioconversion of the prodrugs to their active parent drugs is by way of enzyme activity of **hydrolases**. Prodrugs can be designed to bioconvert based upon the specific characteristic of the enzymes that catalyze the reaction, specifically substrate recognition.

http://epublications.uef.fi/pub/urn_isbn_978-951-27-0634-1/urn_isbn_978-951-27-0634-1.pdf

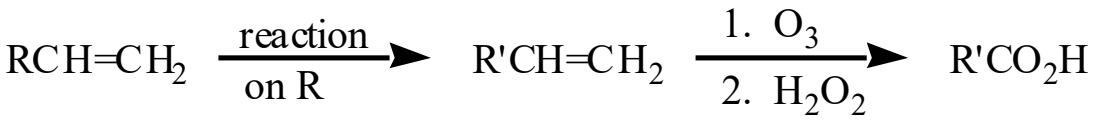
Imai, Teruko, and Masakiyo Hosokawa. "Prodrug Approach Using Carboxylesterases Activity: Catalytic Properties And Gene Regulation Of Carboxylesterase In Mammalian Tissue." Journal Of Pesticide Science 35.3 (2010): 229-239.

Protecting Group Analogy for the Concept of Prodrugs

A. 

analogous to carrier-linked

The ester group can be used To modify properties of The drug

B. 

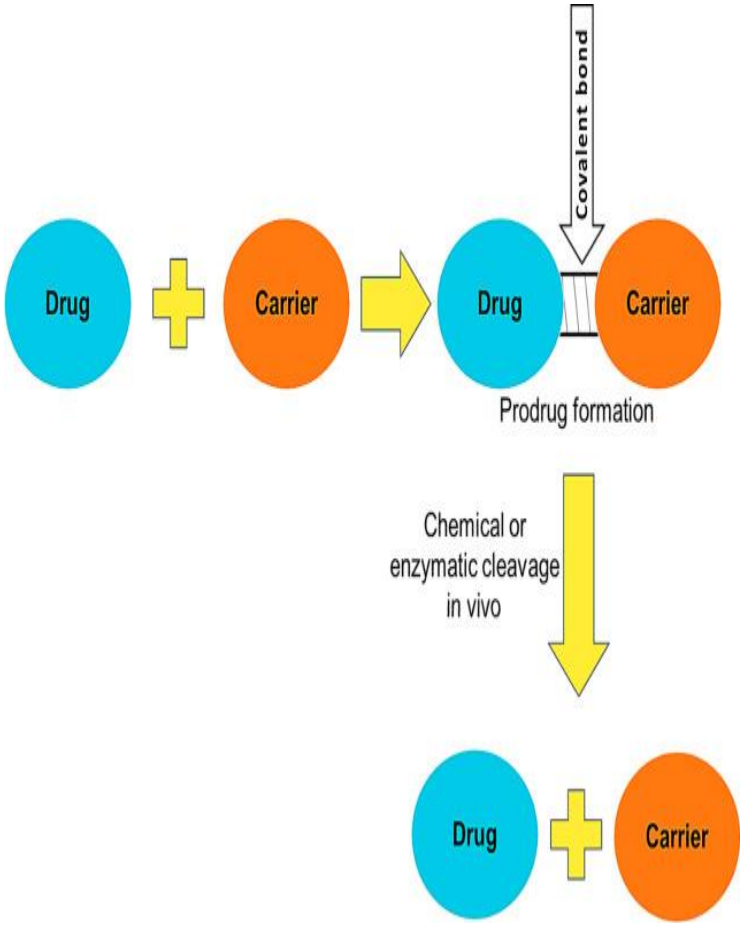
analogous to bioprecursor

Mechanisms of Prodrug Activation

Carrier-Linked Prodrugs

Most common activation reaction is hydrolysis.

Rate of hydrolysis can be modified by locating alkyl groups in area of the carbonyl group to Increase steric hindrance, and retard hydrolysis rate.



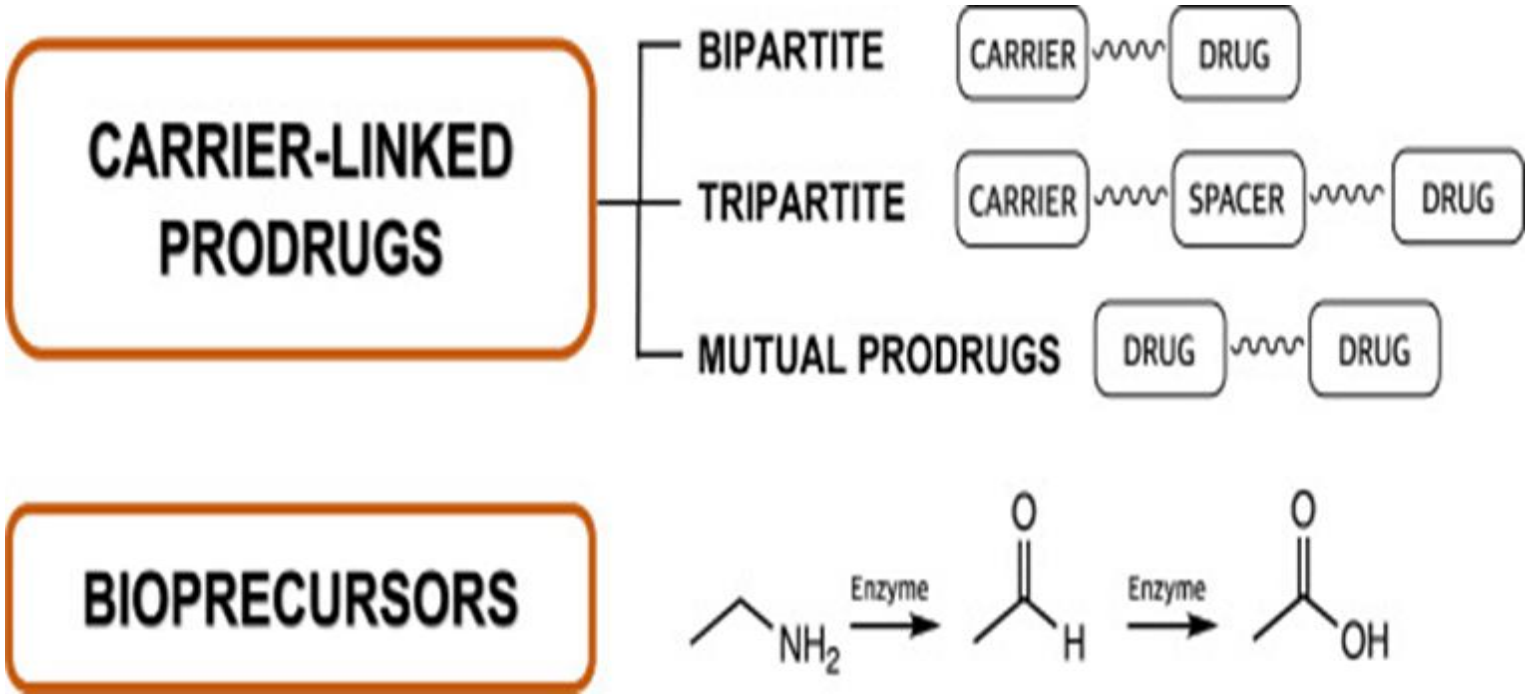
Steps in Prodrug Design

- - Identification of drug delivery problem and
- identification of desired physicochemical properties –
- Selection of transport moiety which will give prodrug desired transport properties and be readily cleaved in the desired biological compartment

Prodrugs are designed in two different ways. This includes:

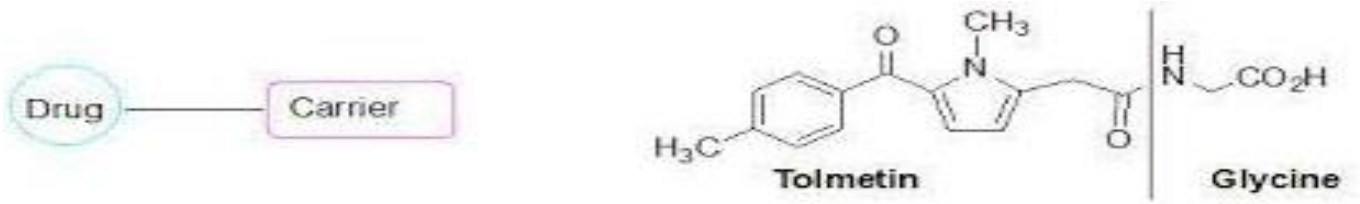
1) **carrier-Linked Prodrug:** This is a prodrug that's connected to an active medication. This connection breaks when it enters the body.

2) **Bioprecursor Prodrugs :** These are chemically-modified versions of a medication. Different enzymes transform them into active medications.

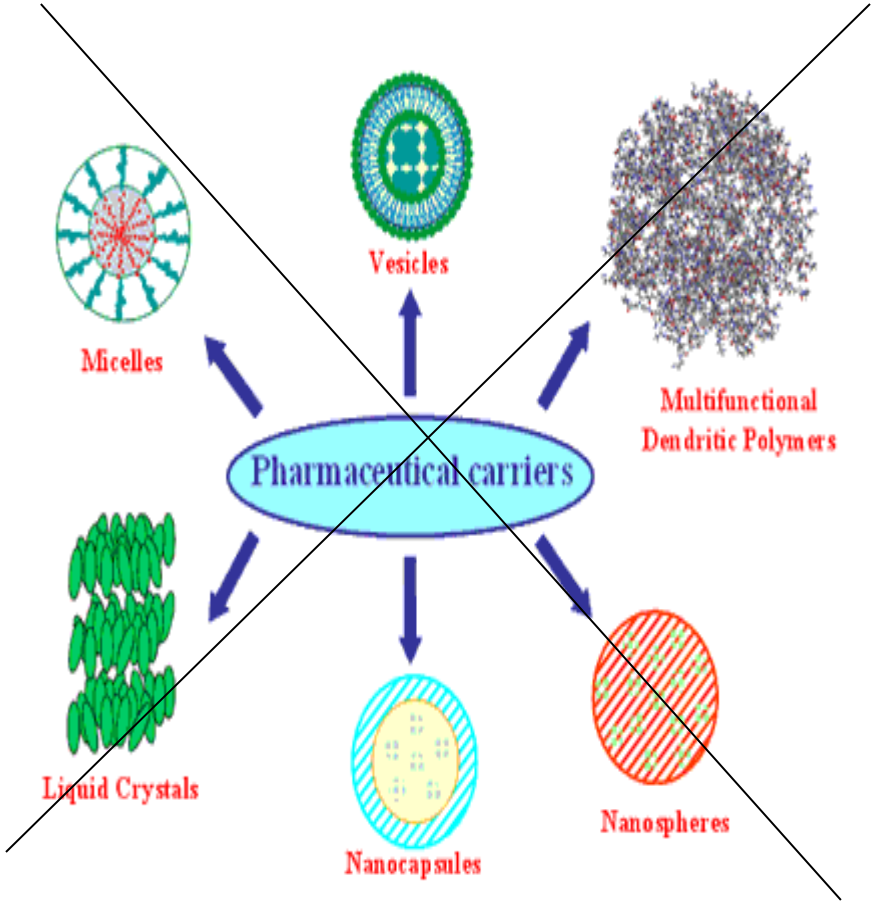


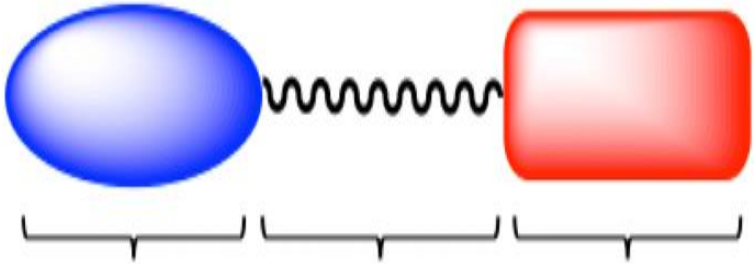
Ideal Drug Carriers

1. Protect the drug until it reaches the site of action
2. Localize the drug at the site of action
3. Allow for release of drug
4. Minimize host toxicity
5. Are biodegradable, inert, and nonimmunogenic
6. Are easily prepared and inexpensive
7. Are stable in the dosage form



Bipartite prodrug of tolmetin glycine.





Promoiety	Linker	Drug
- Chemical	- Ether	
- Peptide	- Carbamate	
- Aptamer	- Disulfide	
- Antibody	- Thioether	
...	...	

Carrier Linkages for Various Functional Groups

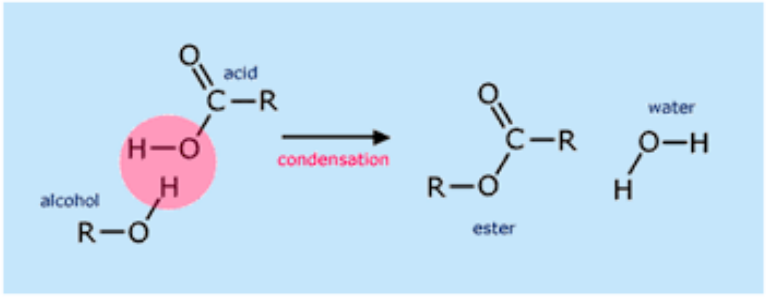
Alcohols, Carboxylic Acids, and Related Groups

Most common prodrug form is an ester

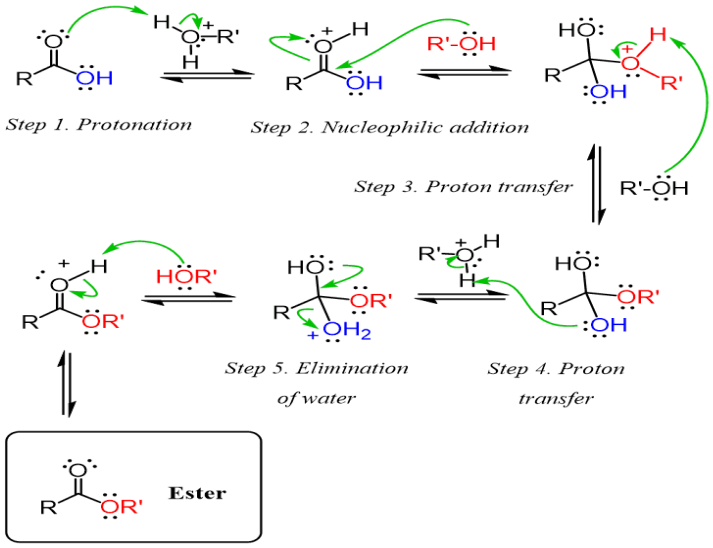
- esterases are ubiquitous
- can prepare esters with any degree of hydrophilicity or lipophilicity
- ester stability can be controlled by appropriate electronic and steric manipulations

Ester Formation & Hydrolysis

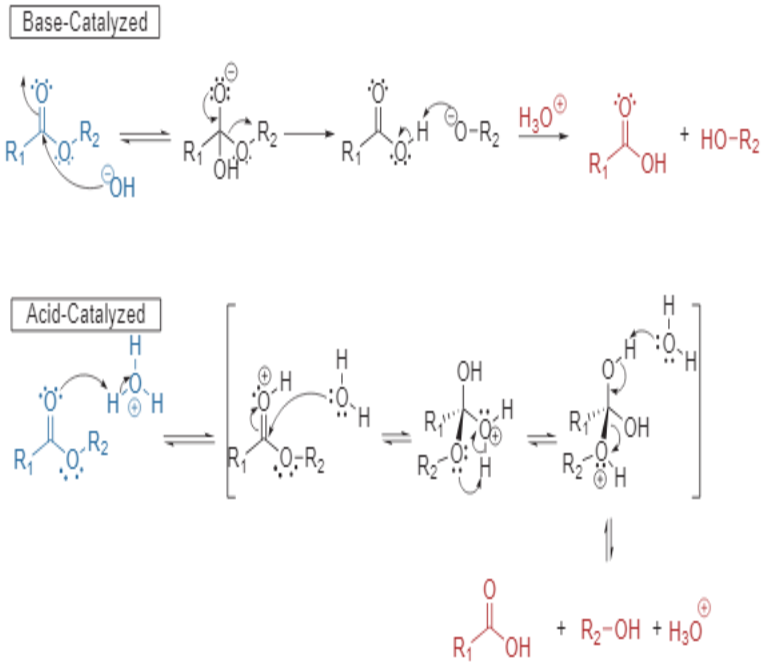
Ester formation and Mechanism of reaction



Fischer Esterification Mechanism



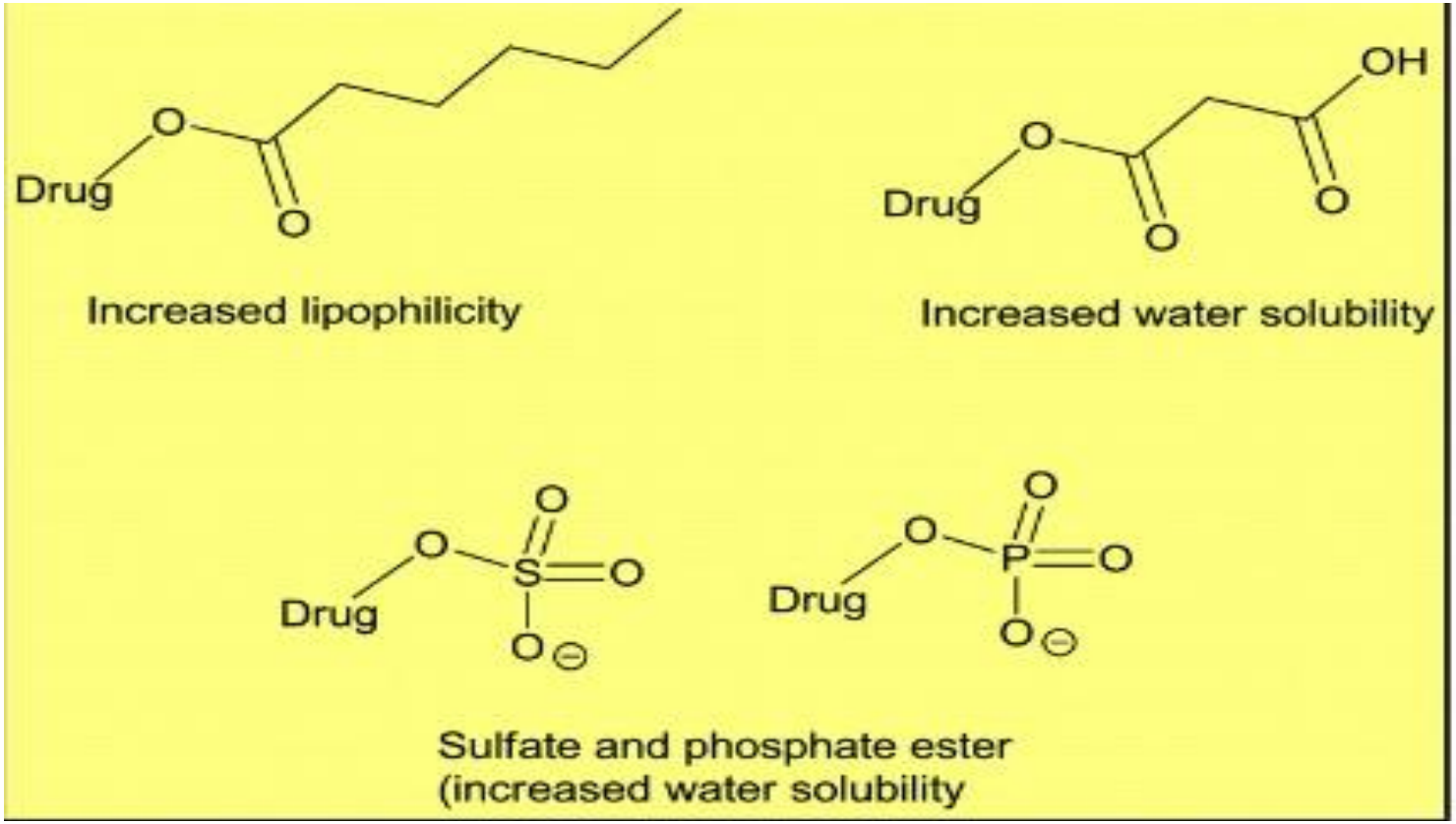
Ester Hydrolysis and Mechanism of reaction



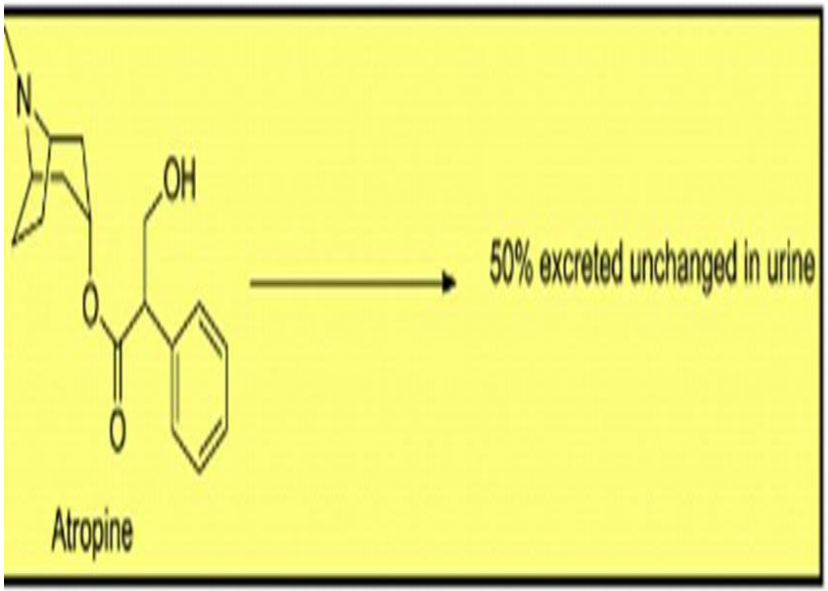
Prodrug functional groups- Esters

- ✓ The most common type of prodrug because of ease with which the ester can be hydrolyzed or formed.
- ✓ Esterase enzymes present in plasmaand other tissues that are capable of hydrolyzing a wide variety of ester linkage.
- ✓ Can add lipophilic or hydrophilicity to the drug.
- ✓ Increasing lipophilicity of the compound may yield a number of benefits , including increased absorption, decreased dissolution in the aqueous environment of the stomach , longer duration of action , and reducing bad taste .

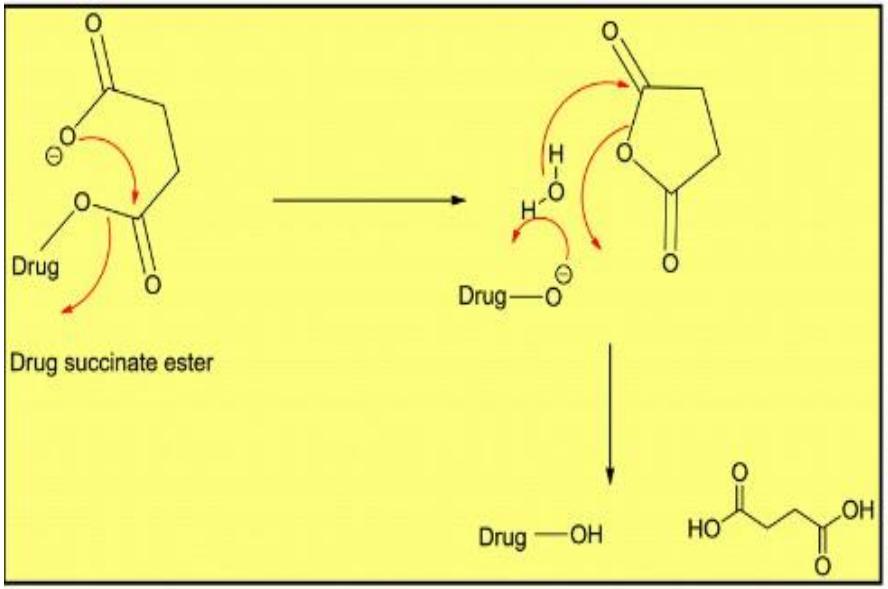
Drugs with Alcohol and carboxylic acid groups



Small sized ester may be easily hydrolyzed than sterically hindered esters.

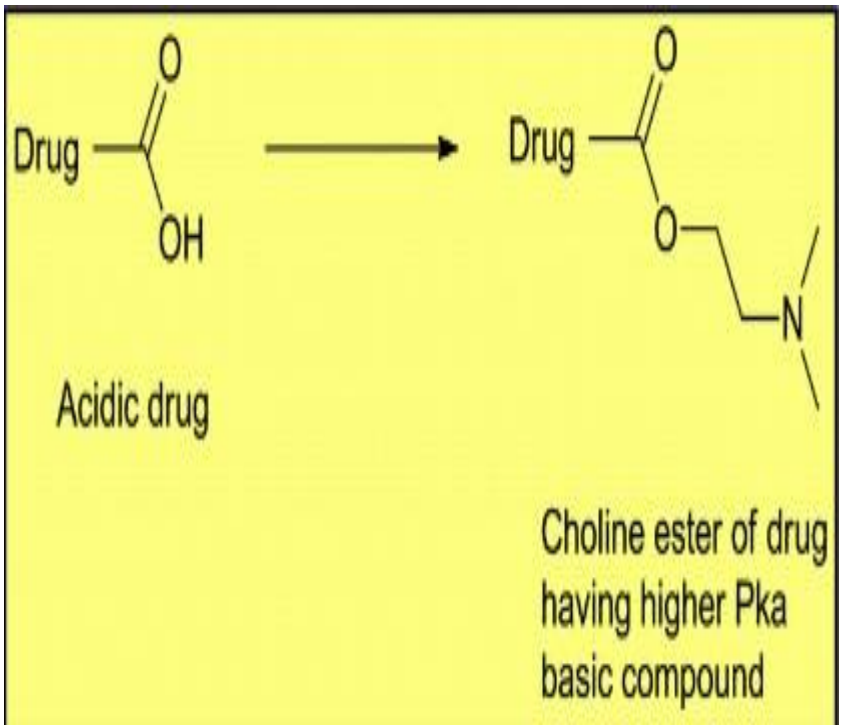


Electronic factors may play a role in the rate of hydrlysis



- Drugs having carboxylic group can be esterified to :

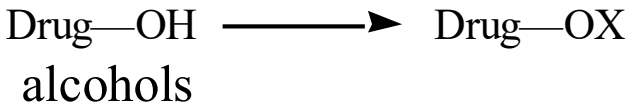
1. Increase lipophilicity
2. Increase Pka : this can be done by macking the choline ester of these drugs:

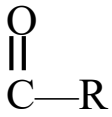
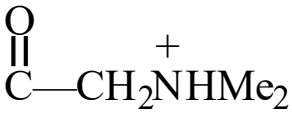
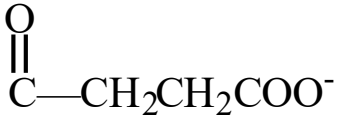
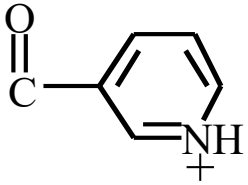
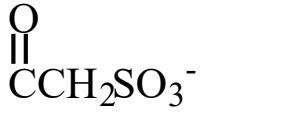


Prodrugs for Alcohol-Containing Drugs

Table 8.1

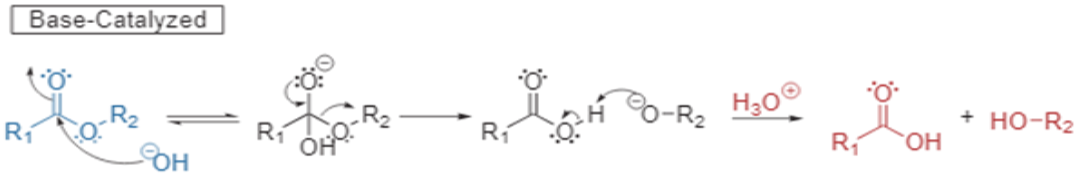
Ester analogs as prodrugs can affect lipophilicity or hydrophilicity

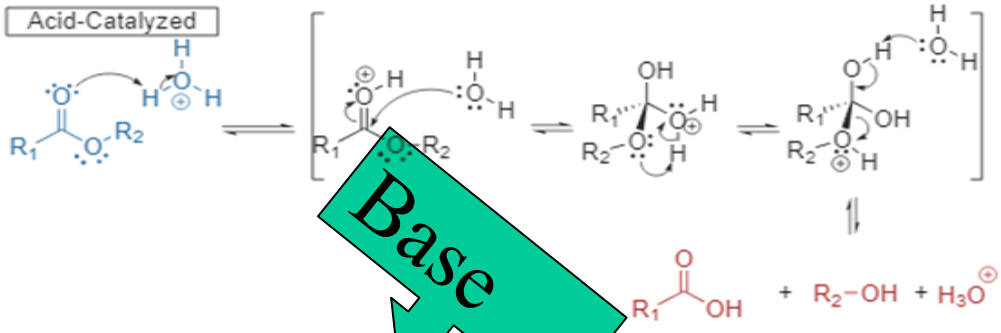


X	Effect on Water Solubility
	(R = aliphatic or aromatic) decreases (increases lipophilicity)
	increases (pK_a ~ 8)
	increases (pK_a ~ 5)
	increases (pK_a ~ 4)
$PO_3^{=}$ (phosphate ester)	increases (pK_a ~ 2 and ~ 6)
	increases (pK_a ~ 1)

To accelerate hydrolysis rate:

- attach an electron-withdrawing group if a **base hydrolysis mechanism is important**
- attach an electron-donating group if **an acid hydolysis mechanism is important**

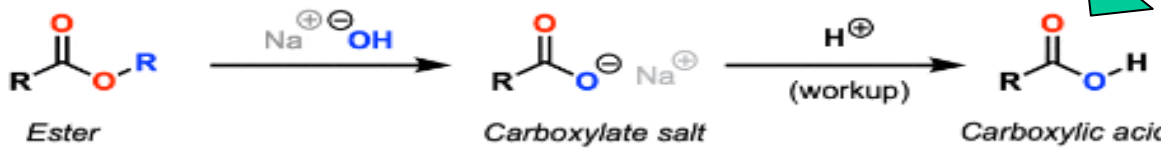




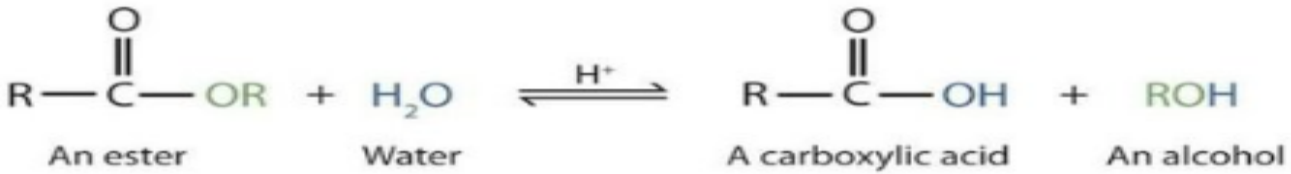
To slow down hydrolysis rate:

- make sterically-hindered esters
- make long-chain fatty acid esters

Base

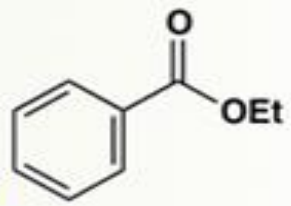


Acid

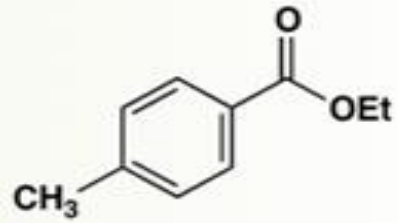


Which of the following esters is most reactive in alkaline hydrolysis

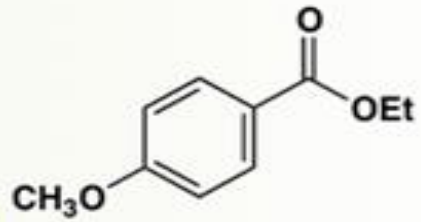
a)



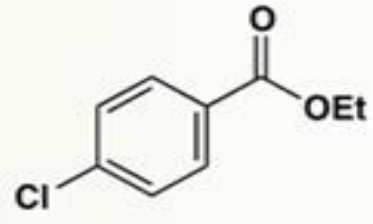
b)

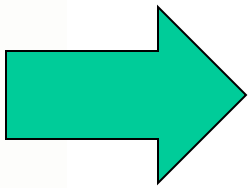


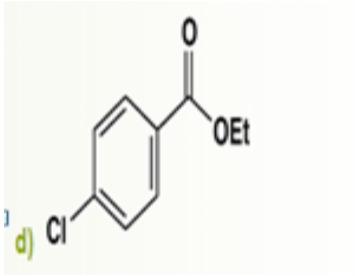
c)



d)



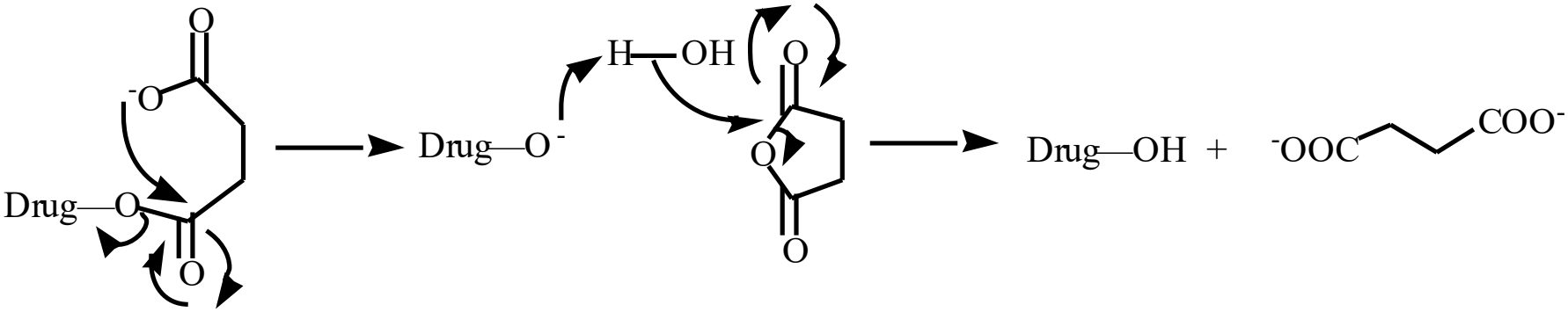




Another Approach to Accelerate Hydrolysis

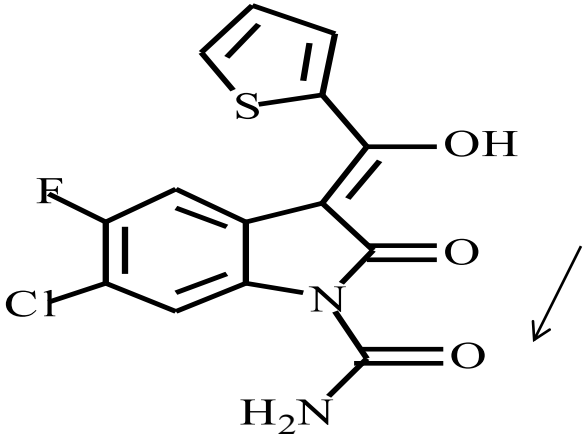
Intramolecular hydrolysis of succinate esters

Scheme 8.2



Also, acetals or ketals can be made for rapid hydrolysis in the acidic medium of the GI tract.

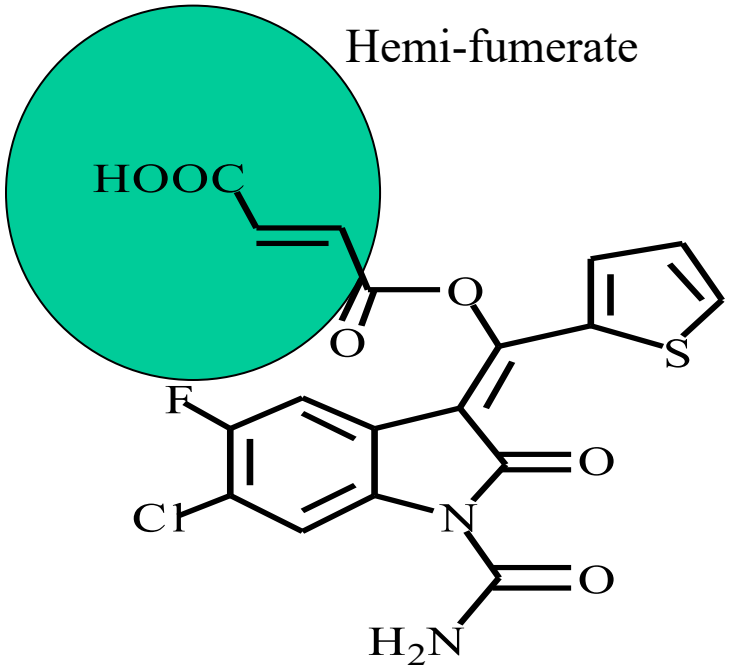
Enolic hydroxyl groups can be esterified as well.

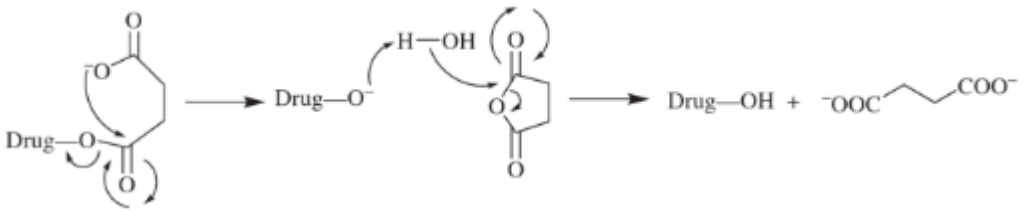


oxindole

antirheumatic agent

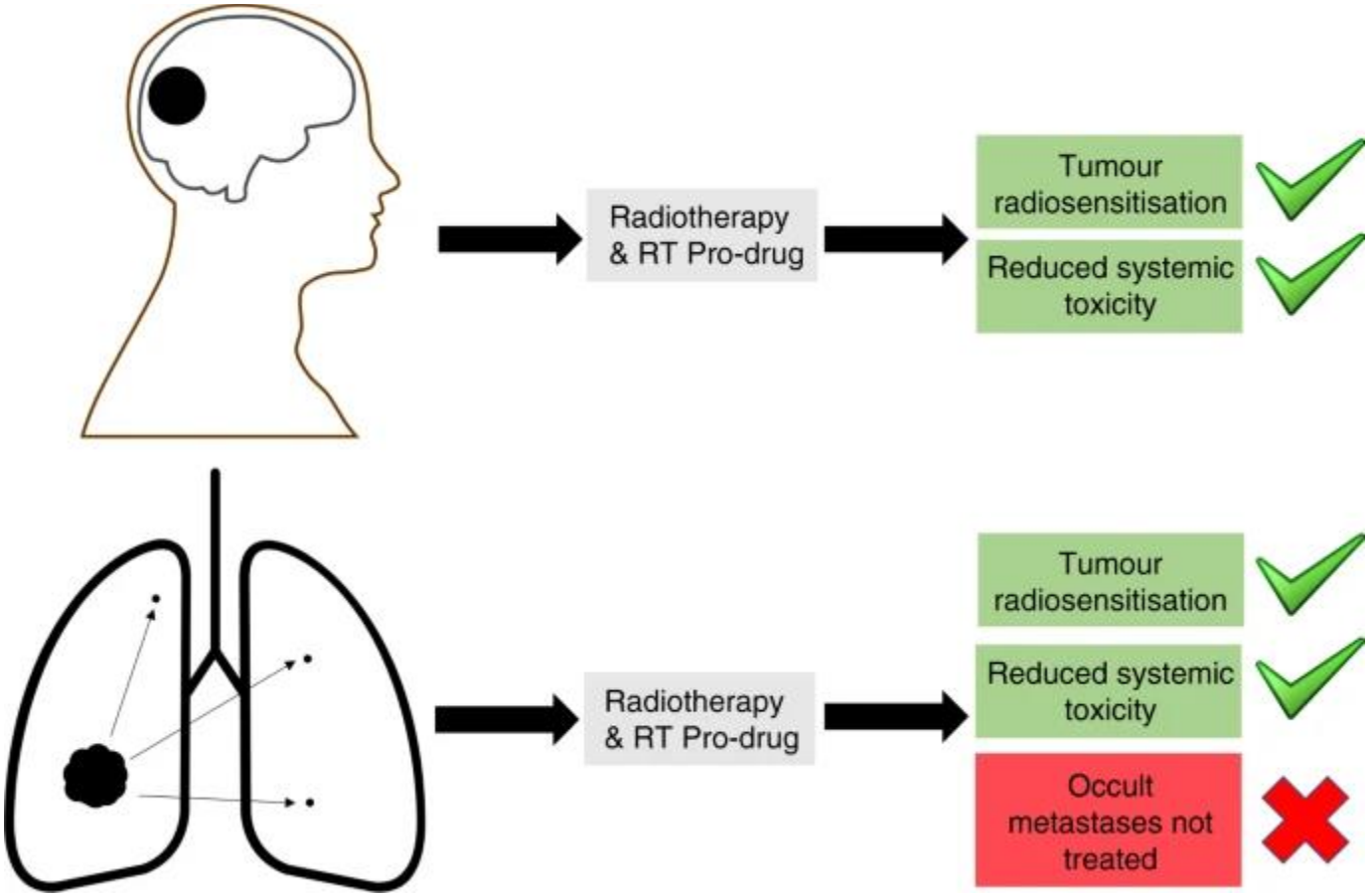
8.1





https://books.google.iq/books?id=yAbUAgAAQBAJ&pg=PA426&lpg=PA426&dq=oxindole+antirheumatic+chemistry+prodrug&source=bl&ots=2NCwZHtyVv&sig=ACfU3U2j_DRpm0zFu_ExQBelCjFe_GN4og&hl=en&sa=X&ved=2ahUKEwiYrIGhns2BAxWTQvEDHTo6Ckg4ChDoAXoECAMQAw#v=onepage&q=oxindole%20antirheumatic%20chemistry%20prodrug&f=true

Greatest benefit is perceived where chemotherapy is predominantly added to radiotherapy treatment (e.g. glioblastoma) to radiosensitise the localised tumour (top). Where chemotherapy plays an important role in treating occult metastatic disease (e.g. locally advanced small cell lung cancer, bottom), this will be less well-addressed.((**Not all prodrugs are activated by metabolic enzymes. Photodynamic therapy involve the use of an external light to activate prodrugs**.))



The potential benefit of radiotherapy-activated organic azide prodrugs by the clinical scenario. https://www.nature.com/articles/s41416-022-01746-1

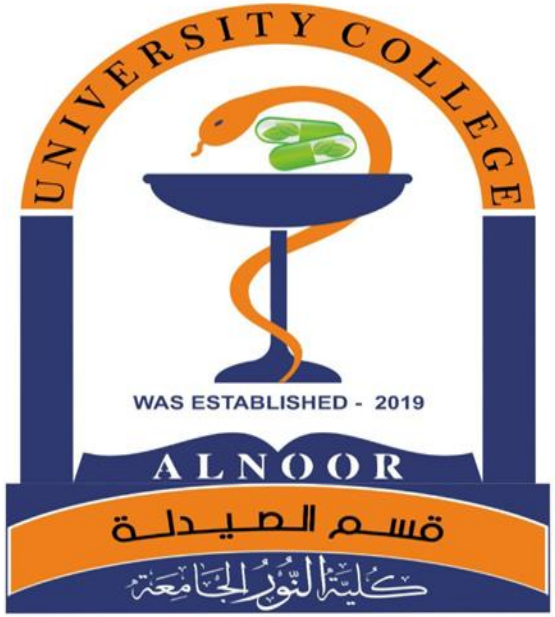
Organic Pharm. Chemistry IV

The Organic Chemistry of Drug Design and Drug Action

Lecture 3

29th October 2022

12:00- 2:00 PM



AlNoor University College

Pharmacy Department

1st Course/ 5TH Class

2022/2023

Be Positive

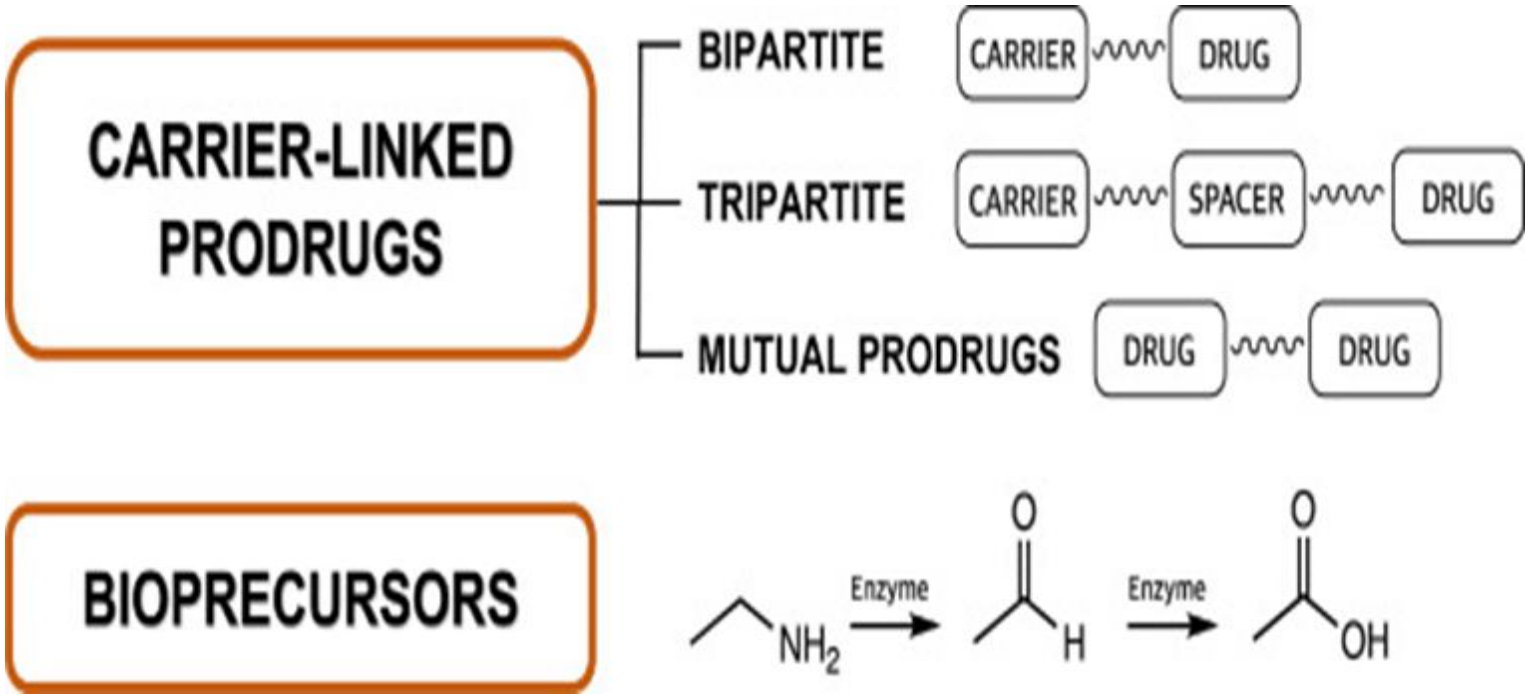
Shoot for the moon. Even if you miss, you'll land among the stars.“

- Les Brown

Prodrugs are designed in two different ways. This includes:

1) **carrier-Linked Prodrug:** This is a prodrug that's connected to an active medication. This connection breaks when it enters the body.

2) **Bioprecursor Prodrugs :** These are chemically-modified versions of a medication. Different enzymes transform them into active medications.



Types of Prodrugs

Drug Latentiation - rational prodrug design

I. Carrier-linked prodrug

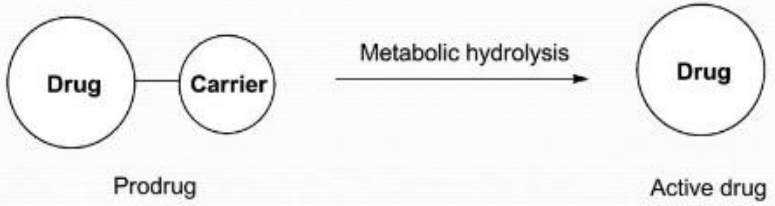
A compound that contains an active drug linked to a carrier group that is removed enzymatically

A. **bipartate** - comprised of one carrier attached to drug

B. **tripartate** - carrier connected to a linker that is connected to drug

C. **mutual** - two, usually synergistic, drugs attached to each other

- Carrier-linked:

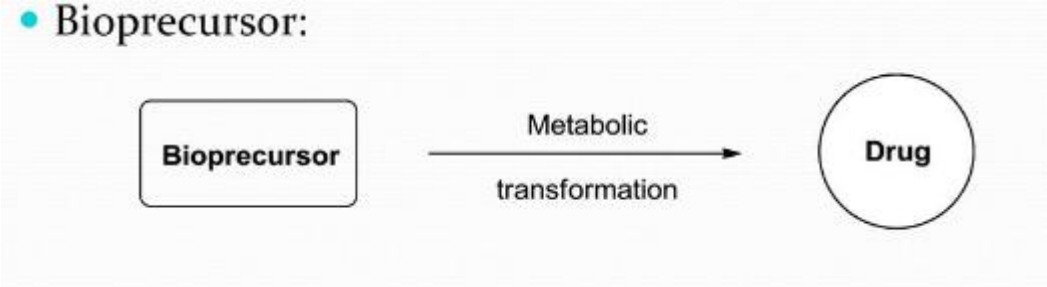


- The common carrier group is the ester (WHY?)
  - Availability of esterase enzyme
  - Possible of either increasing or decreasing the lipophilicity of the parent drug.
  - Can be either easily hydrolyzed or relatively stable ester

The ester prodrug is much more readily absorbed orally than the pharmacologically active carboxylic acid./ Why??

II. Bioprecursor prodrug

A compound metabolized by molecular modification into a new compound, which is a drug or is metabolized further to a drug - not just simple cleavage of a group from the prodrug—e.g., amine getting oxidized to CO2H, to afford the active.

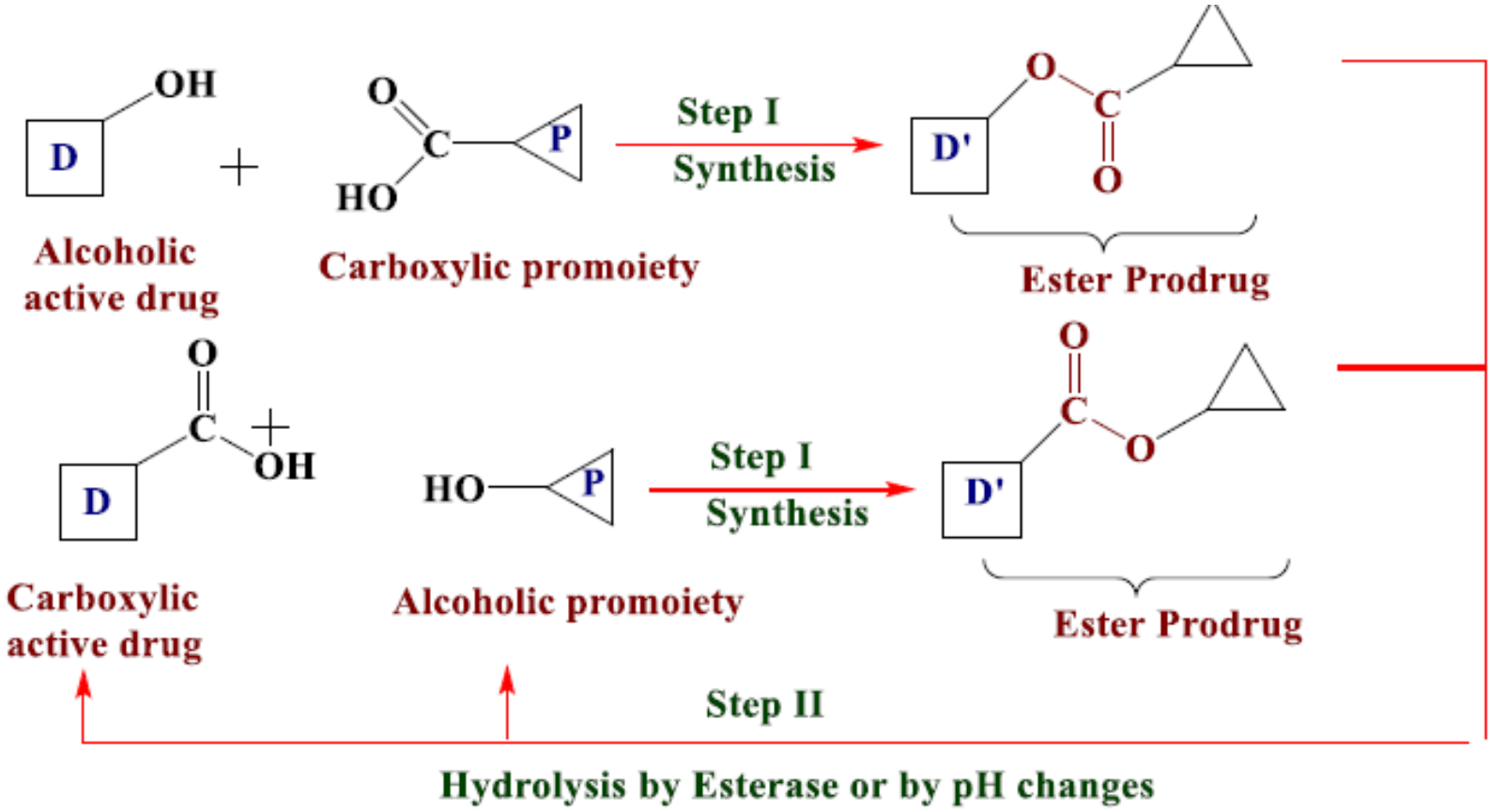


https://slideplayer.com/slide/15966920/

Carrier-Linked Prodrugs

- Carrier Linkages for Various Functional Groups
  Alcohols, Carboxylic Acids, and Related Groups

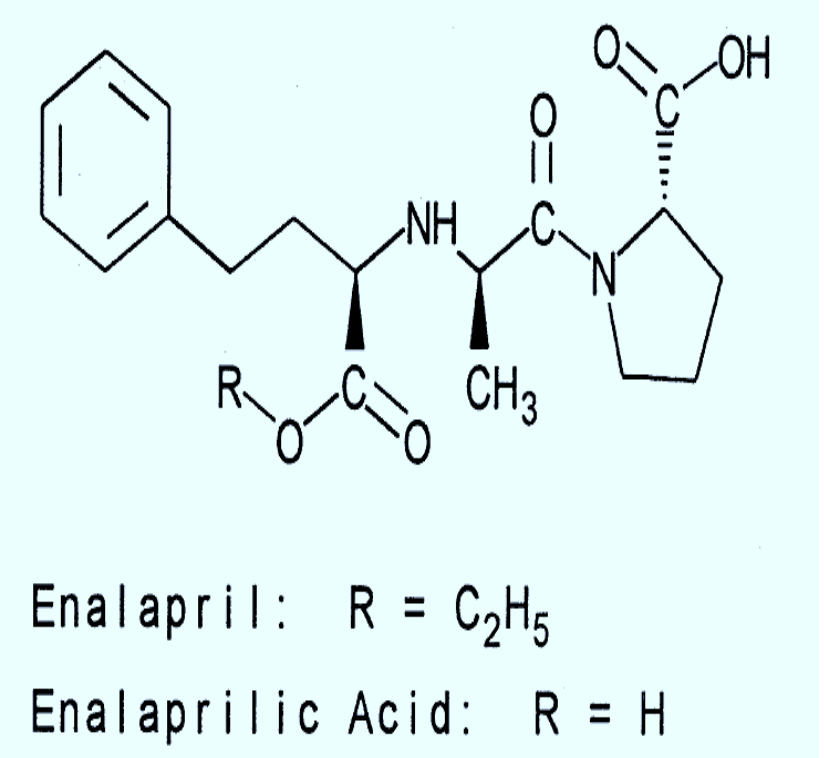
If the molecule contains either an alcohol or carboxylic acid functionality an ester prodrug may be easily synthesized



Carboxylic Acids

Enalapril is the ethyl ester of enalaprilic acid, an active inhibitor of angiotensin – converting enzyme (ACE).

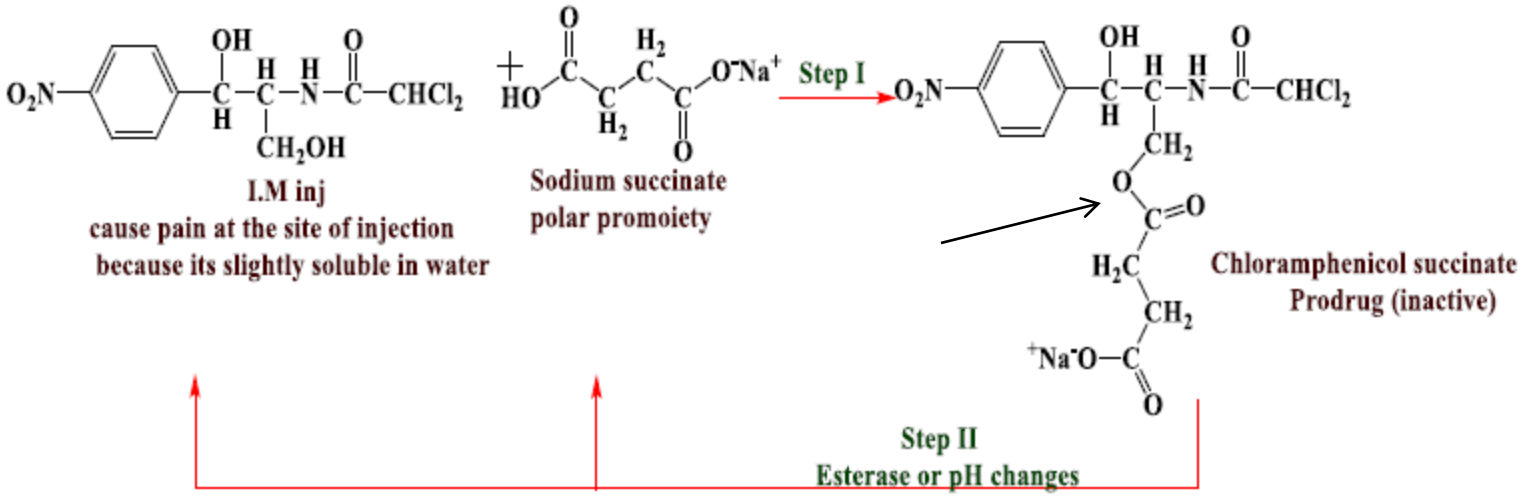
The ester prodrug is much more readily absorbed orally than the pharmacologically active carboxylic acid????



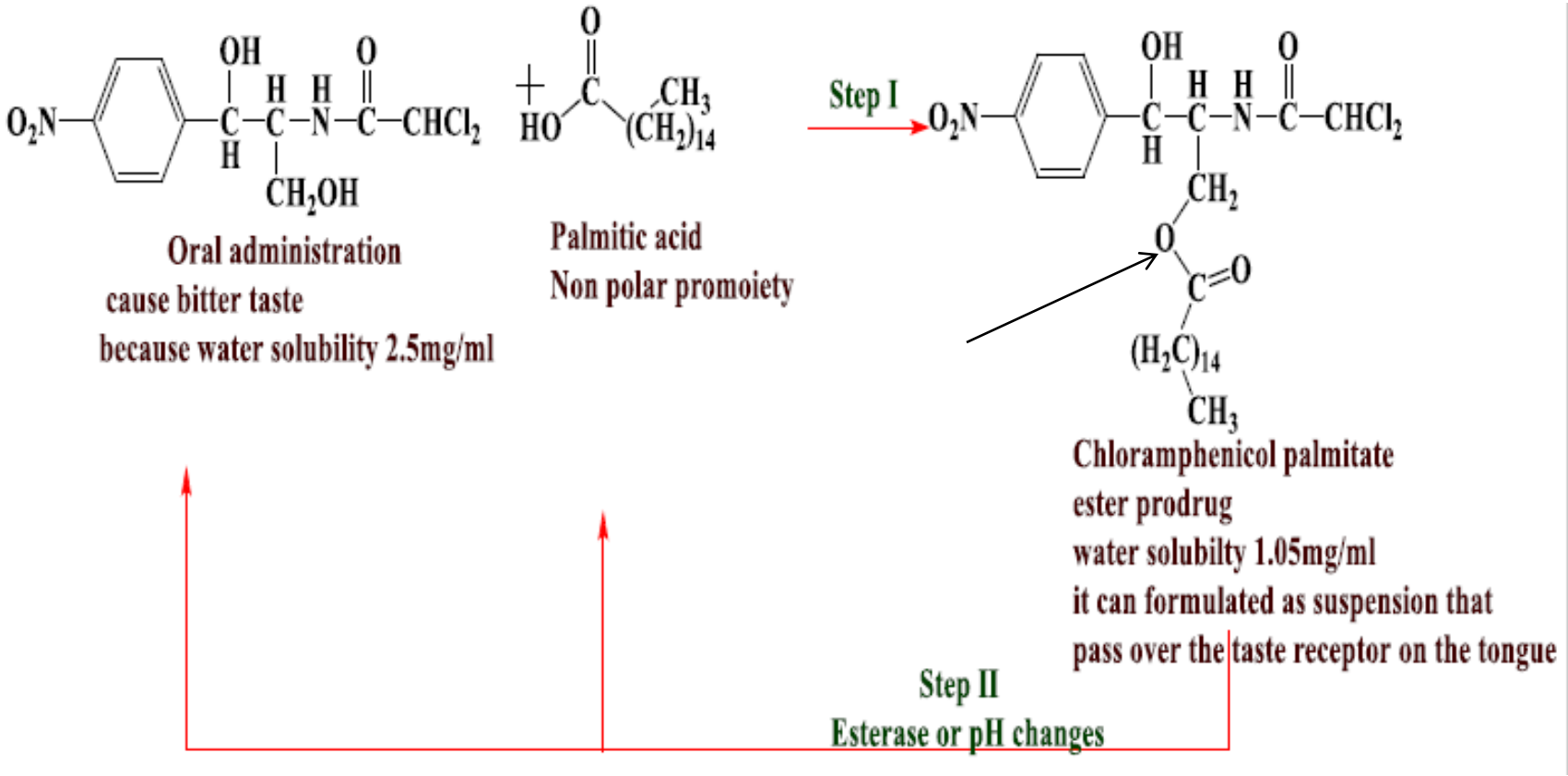
Chloramphenicol(antibiotic)

as Succinate

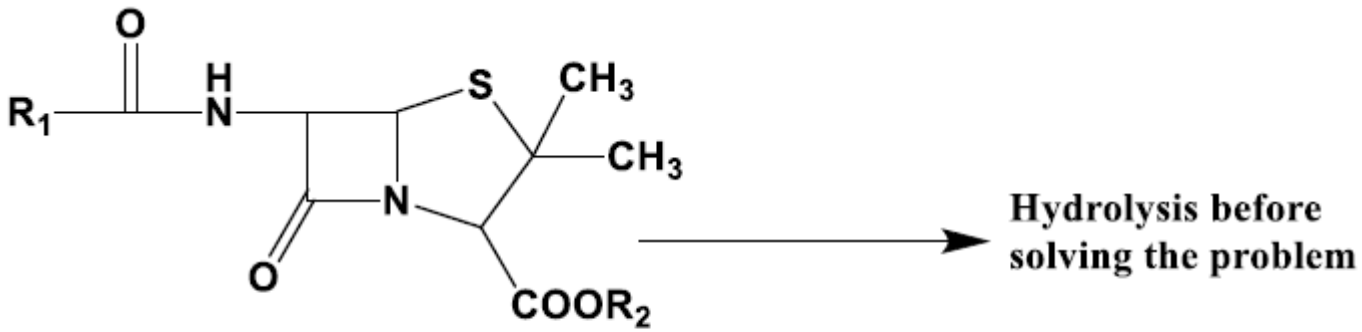
Chloramphenicol when given **parentally by IM inj. it is painful ,since it ppt. at site of injection because of its low water solubility,** so when **polar** functional group like **succinate** was added lead to increase water solubility and reduce pain.



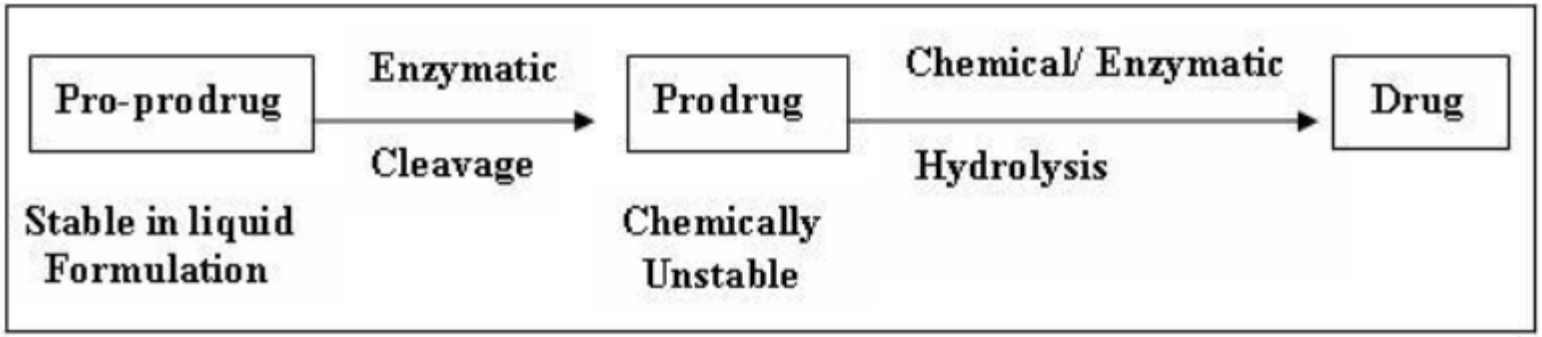
Chloramphenicol(antibiotic) as Palmitate



Double Ester-Prodrugs



R_2 = Ethyl, propyl, butyl, benzyl and phenyl

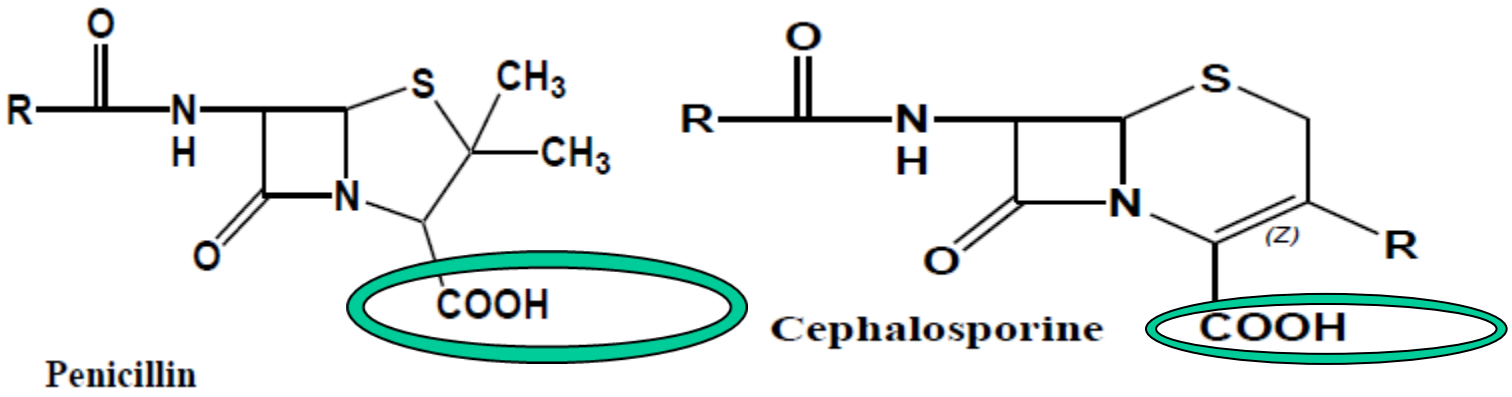


- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC105904/

Double ester prodrug

Prodrug approach is highly practiced to improve the drug delivery and drug targeting Target specific cleavage mechanism is followed in a prodrug design to encourage the site specific drug delivery However, it will not serve the purpose if it is not stable and possible to reach the target

Also, stability problems are observed in the prodrugs involving chemical release of active drug These problems can be improved through double prodrug approach in which enzymatic release mechanism is essential prior to the spontaneous release of the parent compound **Double prodrug also termed as Pro prodrug or Cascade Latentiated prodrug** is a prodrug further derivatized in such a fashion such that only enzymatic conversion to prodrug is possible before the latter can cleave spontaneously to release the active drug



The COOH group will be converted for example to ethyl, propyl, butyl, phenyl and benzyl esters, however, these esters are not stable at the GIT and they will release the parent drug and do not passes G.I.T, therefore, they are not good.

The carboxyl moiety is ionizable and quickly increase the water solubility of the compound in GIT, when the drug dissolute in stomach, it will be subjected to acid catalyzed decomposition?

Therefore, to decrease the ionization (and decomposition) of these antibiotics in the stomach, we have to convert the carboxylic functional group to more lipophilic moiety in order to decrease the solubility in the aqueous media and the drug will not dissolve in the acidic media of the stomach, thus there is decrement in the destruction of the molecule in the acidic media of the stomach

Pro-prodrug or Double Ester Strategy

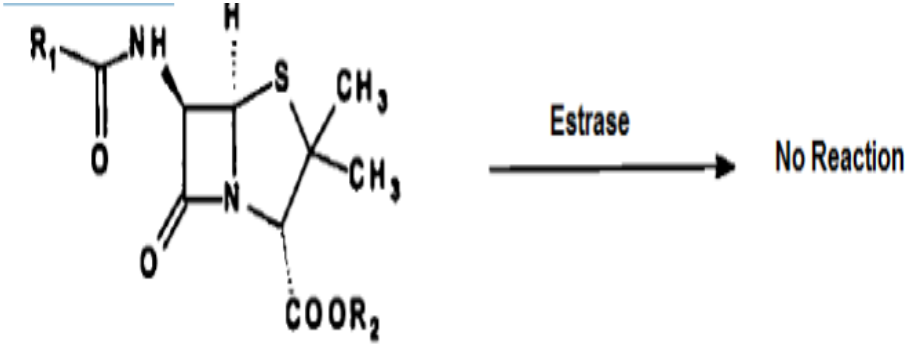
As a result a new method had been found to include a preparation of double ester of these compounds

Example on this method is a compound which is from the second generation of cephalosporines “ Cefpodoxime proxetil is a double ester prodrug

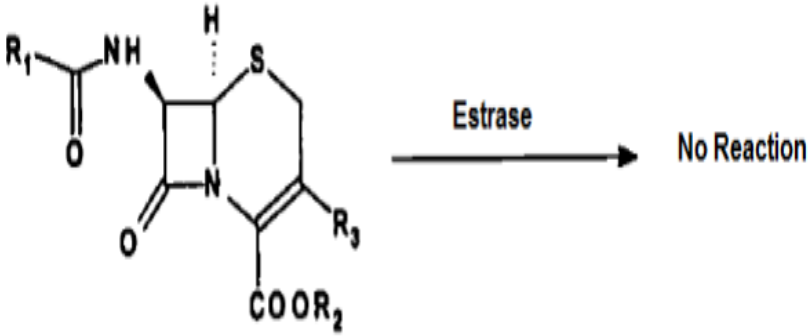
- The prodrug is highly labile to esterase enzyme

The problem of steric inhibition

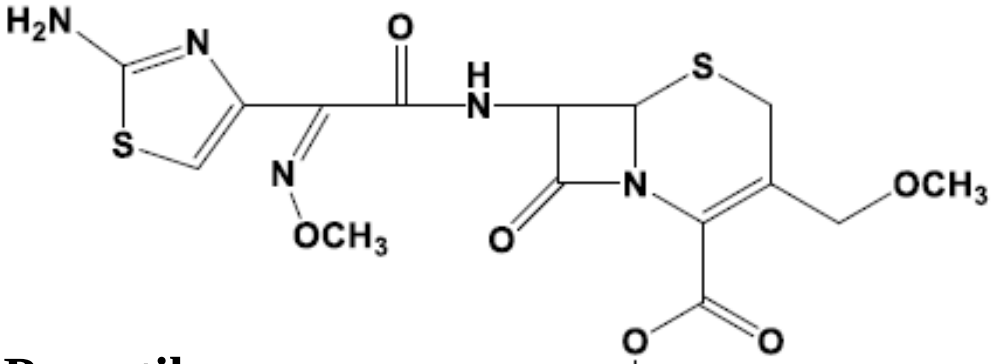
- Not all carboxylic esters are easily hydrolyzed in vivo. Steric inhibition around the ester in some cases prevents the prodrug from being hydrolyzed. This is seen in the β-lactams, in which it is often desirable to increase the hydrophobicity of the agent to improve absorption or prevent dissolution in the stomach where acid-catalyzed decomposition may occur. Simple esters of the carboxylic acid moiety, however, are not hydrolyzed in vivo to the active carboxylate.



Peniclin Esters: R_2 Ethyl, Propyl. Butyl, Phenyl



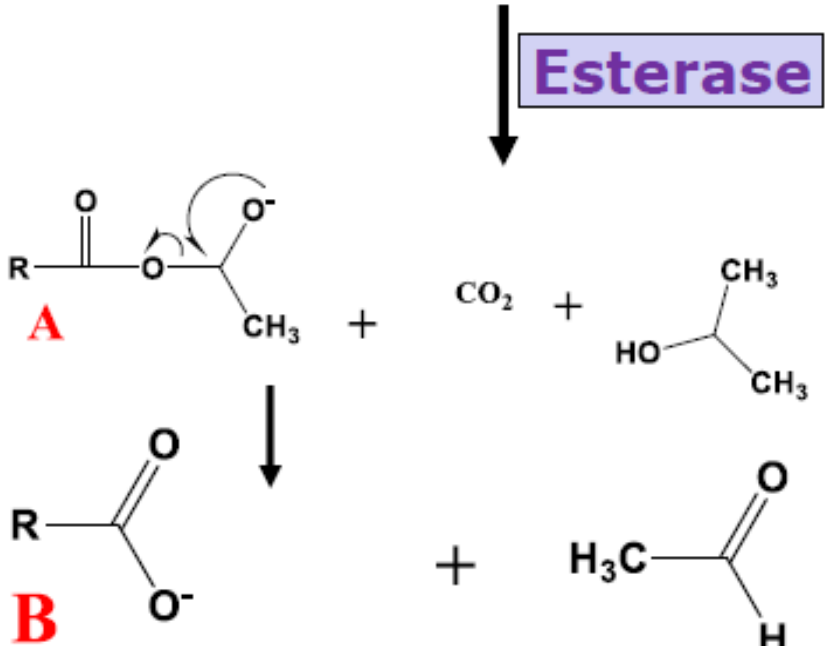
Ciphalosporin Esters: R_2 Ethyl, Propyl. Butyl, Phenyl



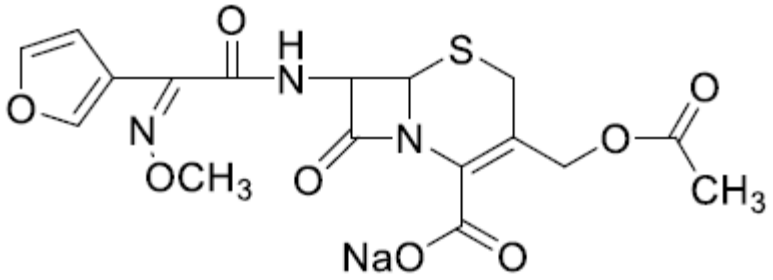
Cefpodoxime Proxetil

Compound (**A**) is highly susceptible to esterase enzyme and intramolecular hydrolysis giving the compound (**B**) with -COOH functional group which we need it for cephalosporin's biological activity, therefore, **this process is used to prepare prodrugs for penicillin and second and third generations of cephalosporin.**

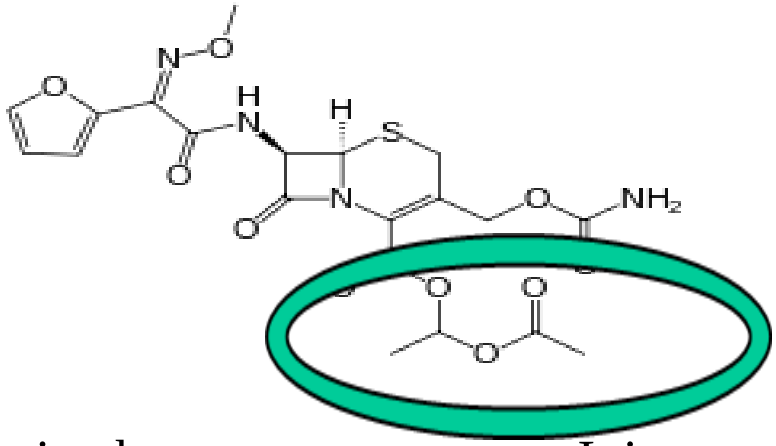
• **Advantage:** No destruction, decrease in the water solubility, pass and reach the blood and give the parent drug (**B**).



Cefuroxime Sodium (Salt)



Cefuroxime (2nd generation cephalosporin) is of parenteral administration (I.V) **thrice daily dosing**, it can cross BBB, therefore, it is indicated in meningitis, bronchitis, influenza and pneumonia in elderly patients.



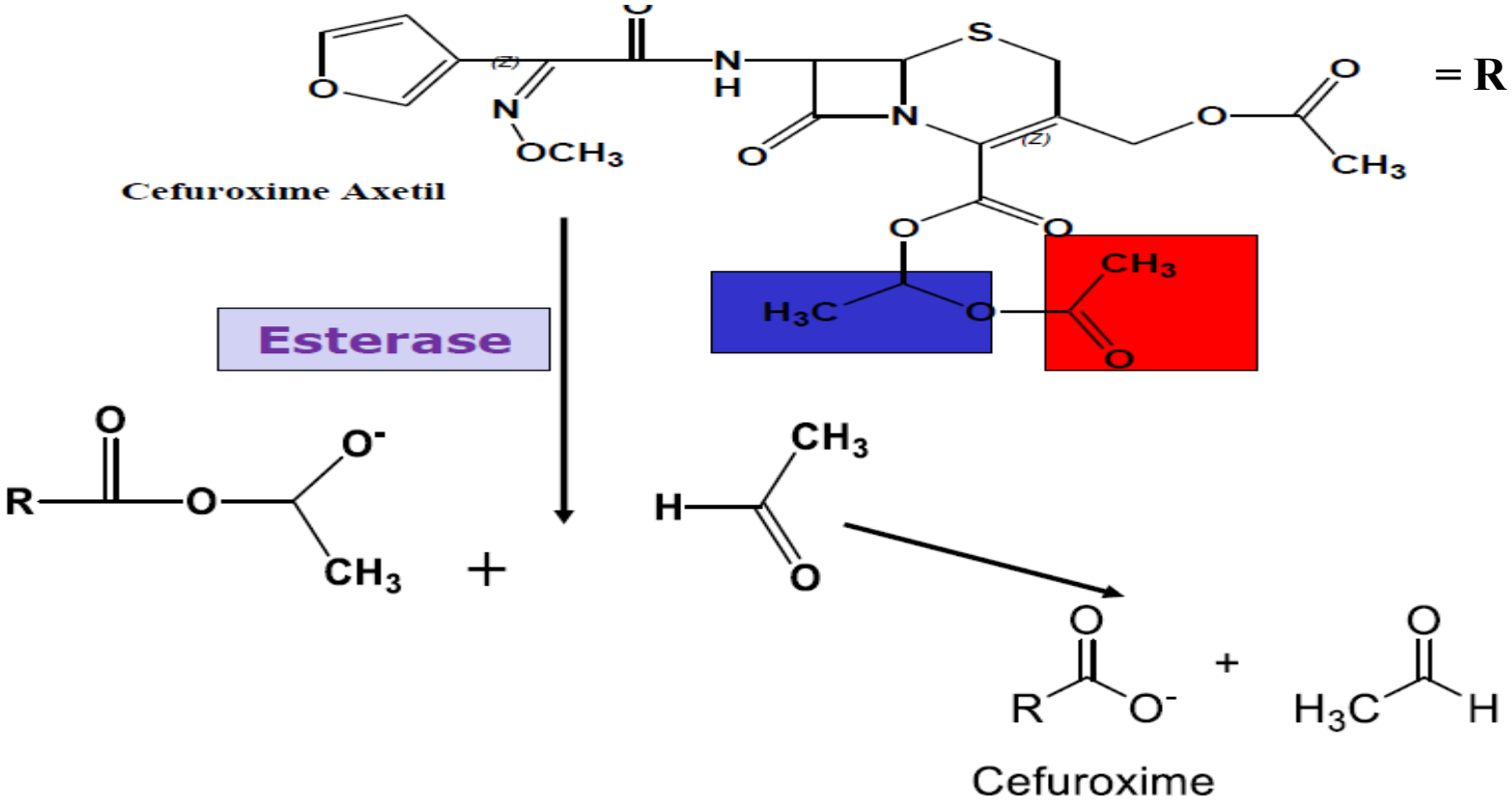
Cefuroxime axetil (Prodrug)

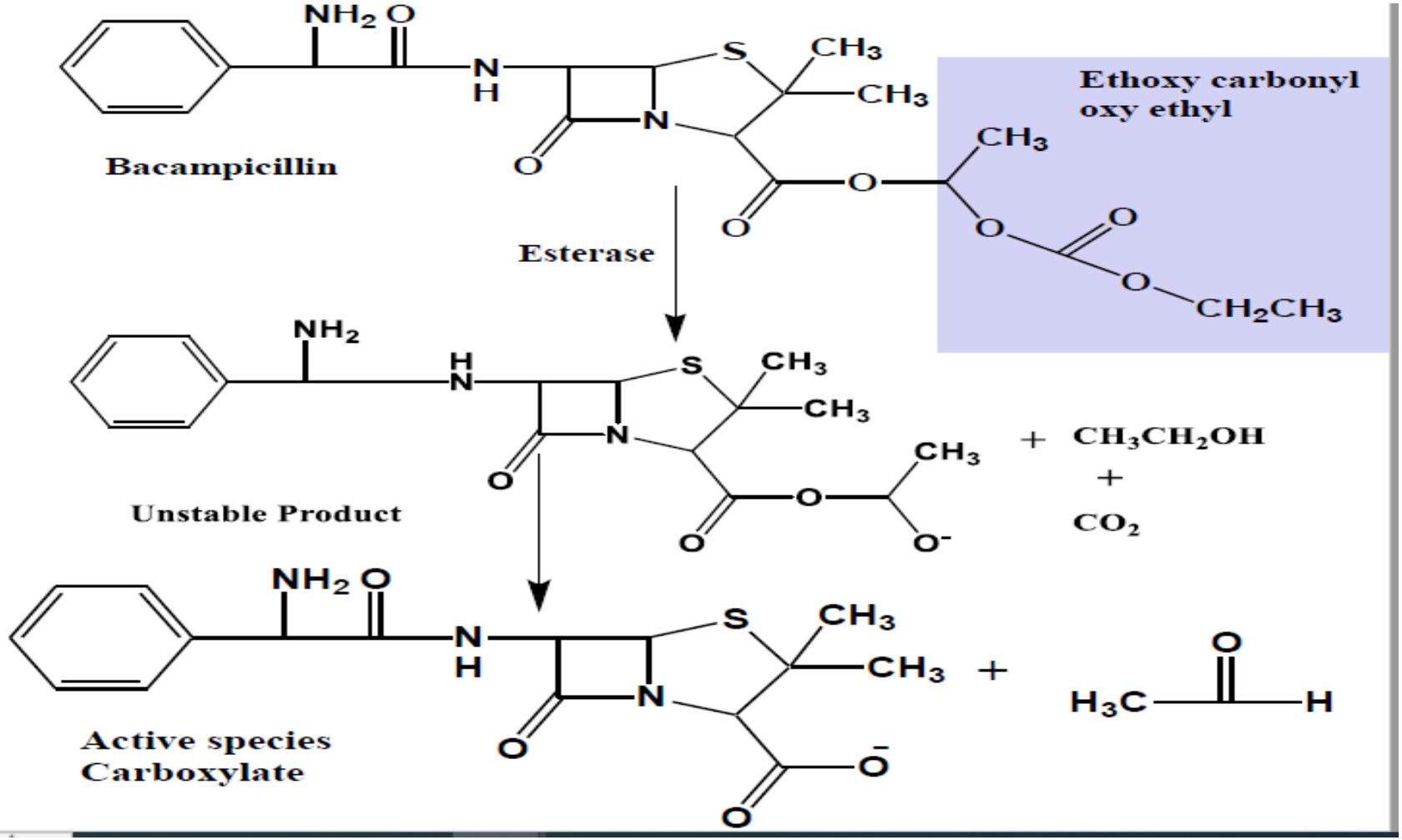
is a double prodrug of cefuroxime.

It is acid stable, lipophilic,

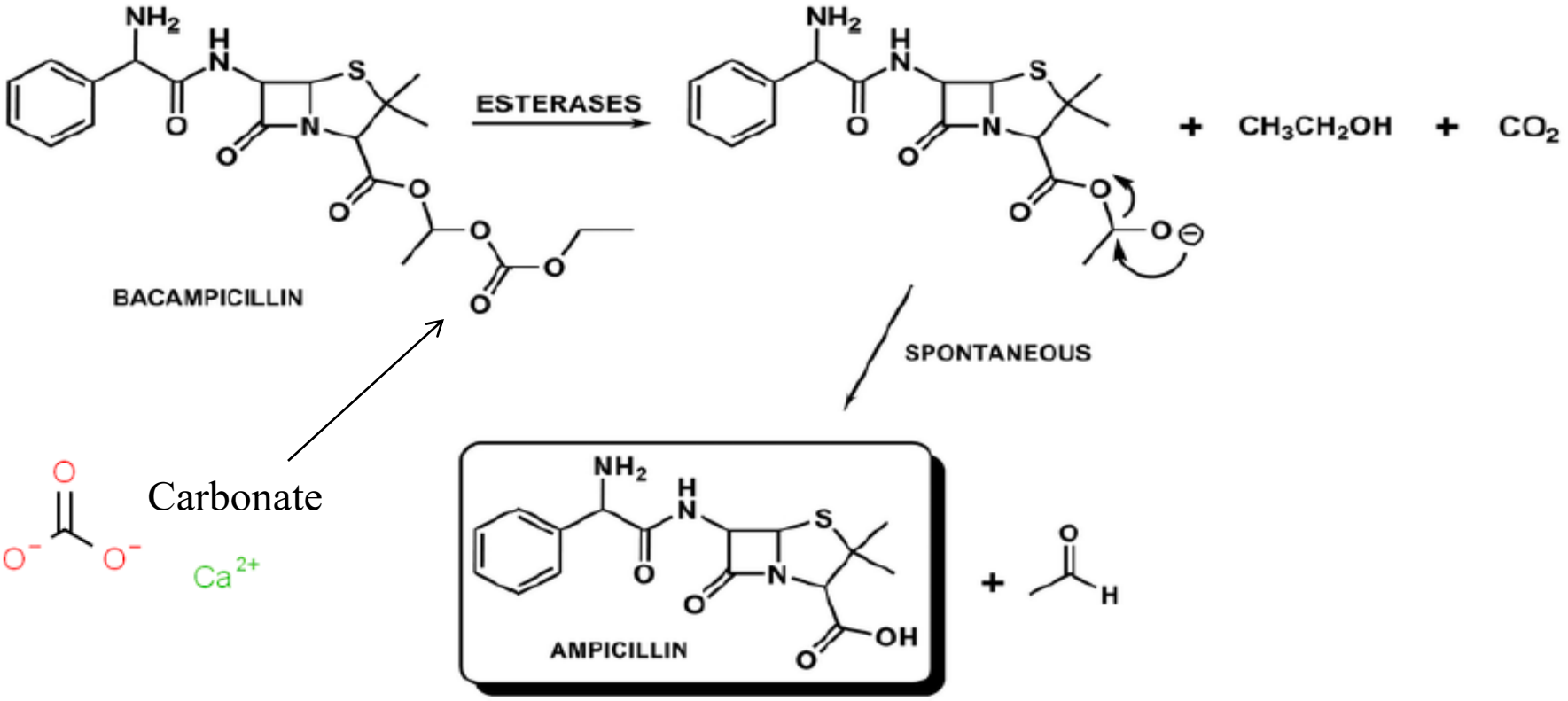
and hydrolyzed to cefuroxime after absorption by esterase enzymes. It is prepared for oral treatment of bacteria susceptible to it. **The double prodrug form permits twice a day dosing.**

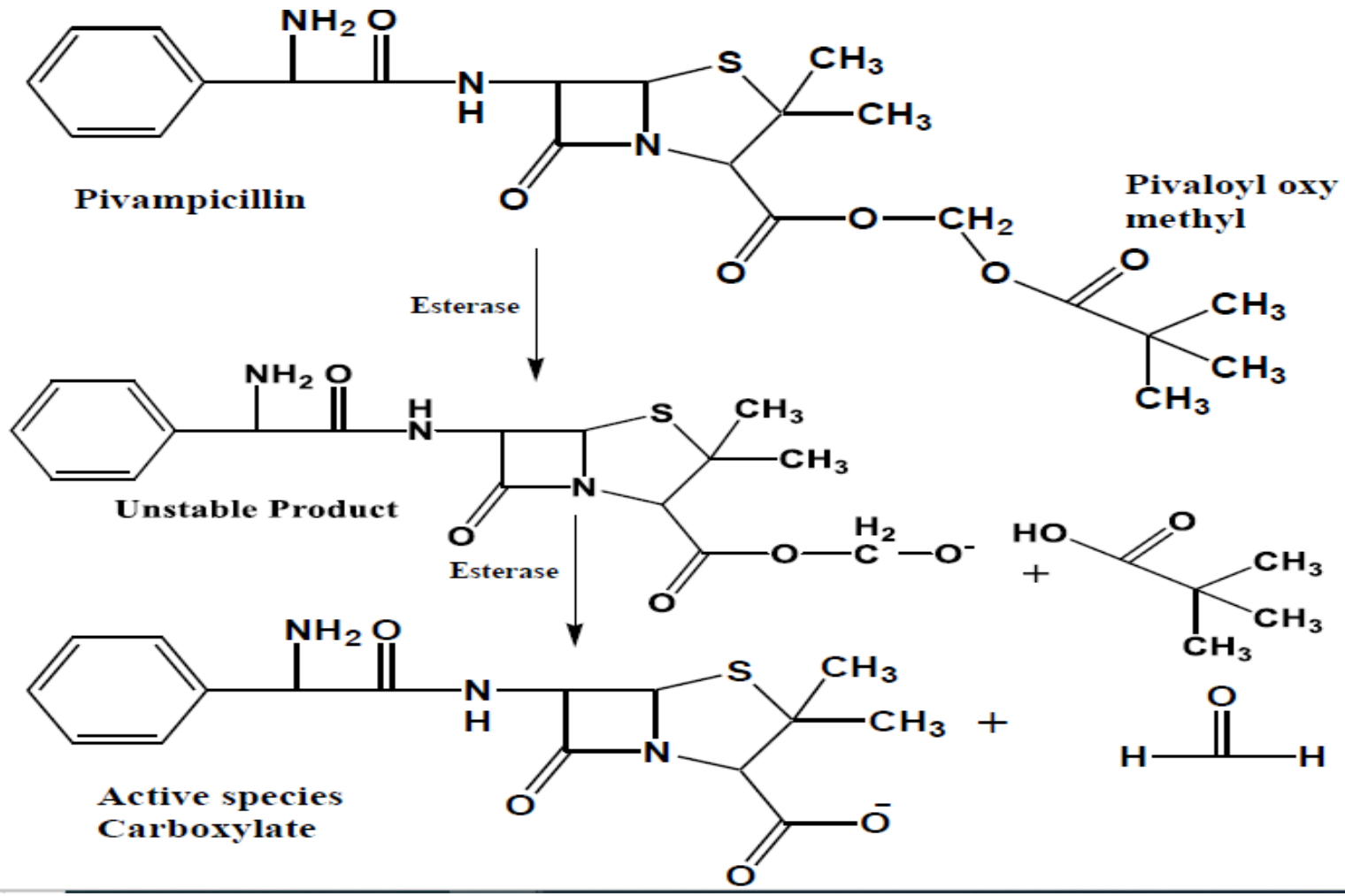
Cefuroxime axetil

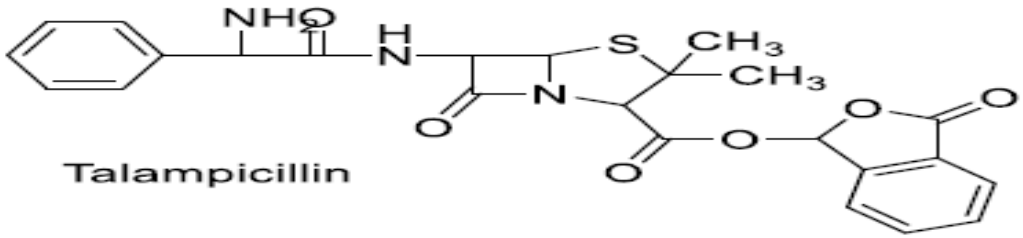



Bacampicillin
Ethoxy carbonyl oxy ethyl
Esterase
Unstable Product
+ CH3CH2OH
+
CO2
Active species
Carboxylate
+

As in cefpodoxime proxetil, where carbonate functionality was utilized, the carbonate is also susceptible to the action of esterase enzymes and the unstable product undergoes further reaction to give the active carboxylate species

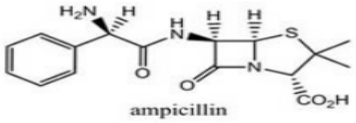


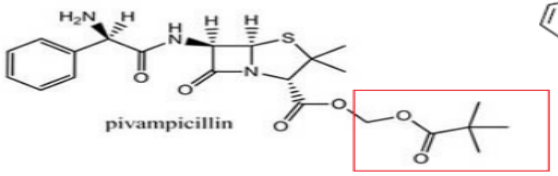

Pivampicillin
Pivaloyl oxy methyl
Esterase
Unstable Product
Esterase
Active species
Carboxylate

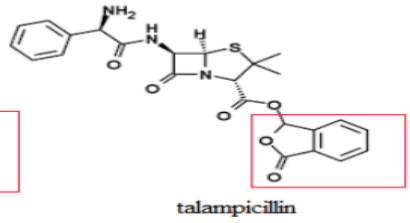


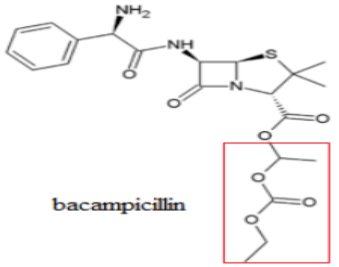
Bacampicillin, pivampicillin and talampicillin are prodrugs of ampicillin with no antibacterial activity, and after oral absorption they are hydrolyzed by plasma esterase, to form ampicillin.

• The oral absorption for all of the three double prodrugs is more rapid and more complete than that of ampicillin and less affected by food. Talampicillin has most rapid onset than the two others.

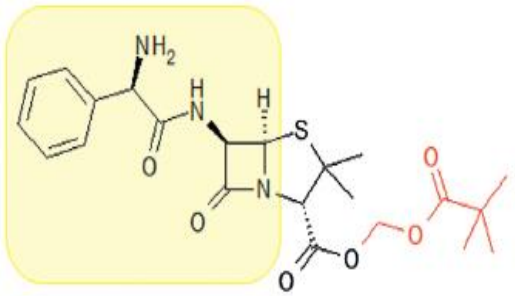




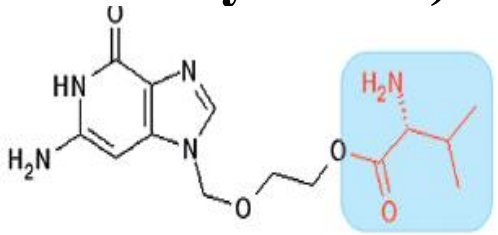




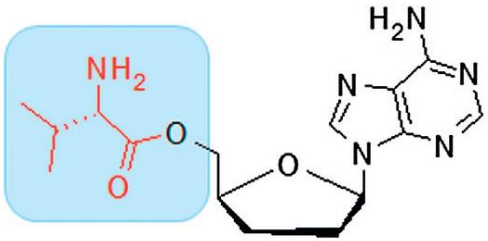
Recognition element inherent to pharmacological activity or conjugated as a promoiety (drug molecule in black; promoiety in red)



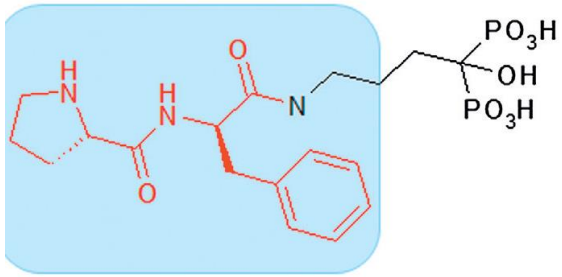
Pivampicillin: β-lactam antibiotic



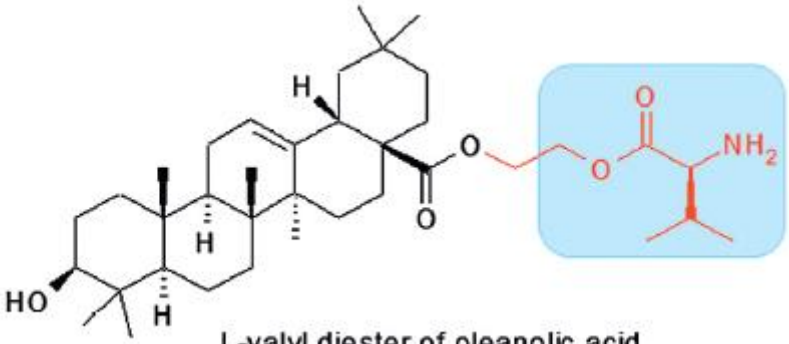
Valcyclovir- antiviral agent



L-valyl ester of didanosine



Pro-Phe-Alendronate



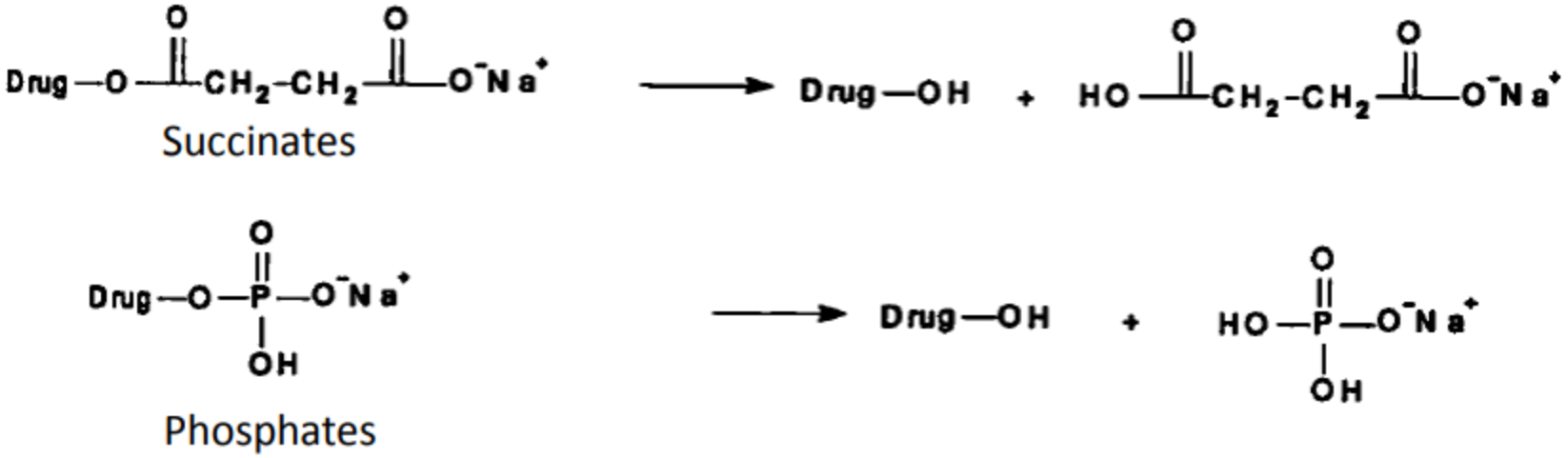
L-valyl diester of oleanolic acid

classical and recent examples of prodrugs where the PepT1 peptidic recognition element is part of the drug pharmacophore (highlighted in yellow) and examples where the recognition element is incorporated as a promoiety (highlighted in blue). The diversity of structures that are recognized by PepT1 is noteworthy, and the dipeptidyl prodrug of alendronate is a particularly interesting example.

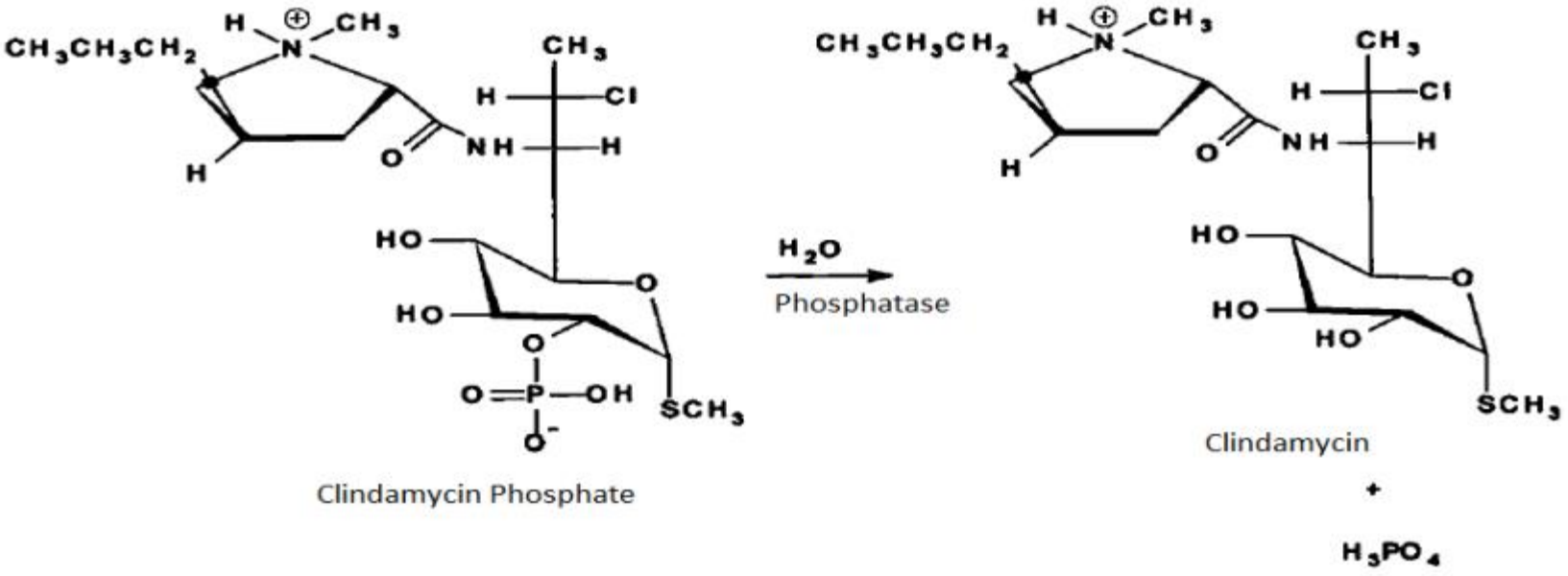
To increase the hydrophilicity of an agent

, several different types of ester prodrugs have been used, including succinates, phosphates, and sulfonates.

All are ionized at physiological pH and, therefore, increase the water solubility of the agents, making them more suitable for parenteral or oral administration when high water solubility is desirable



- Phosphate esters of alcohols offer another method of increasing the water solubility of an agent. The phosphates are completely ionized at physiological pH and generally hydrolyzed rapidly in vivo by phosphatase enzymes.
- Ionization of the phosphate function imparts high stability to these derivatives in solution, and solutions for administration can be stored for long periods of time without hydrolysis of the phosphate. Such an approach has been used to produce clindamycin phosphate, which produces less pain at the injection site than clindamycin itself.



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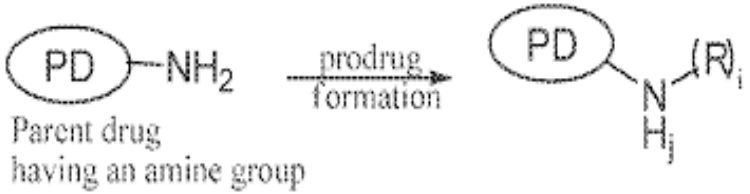
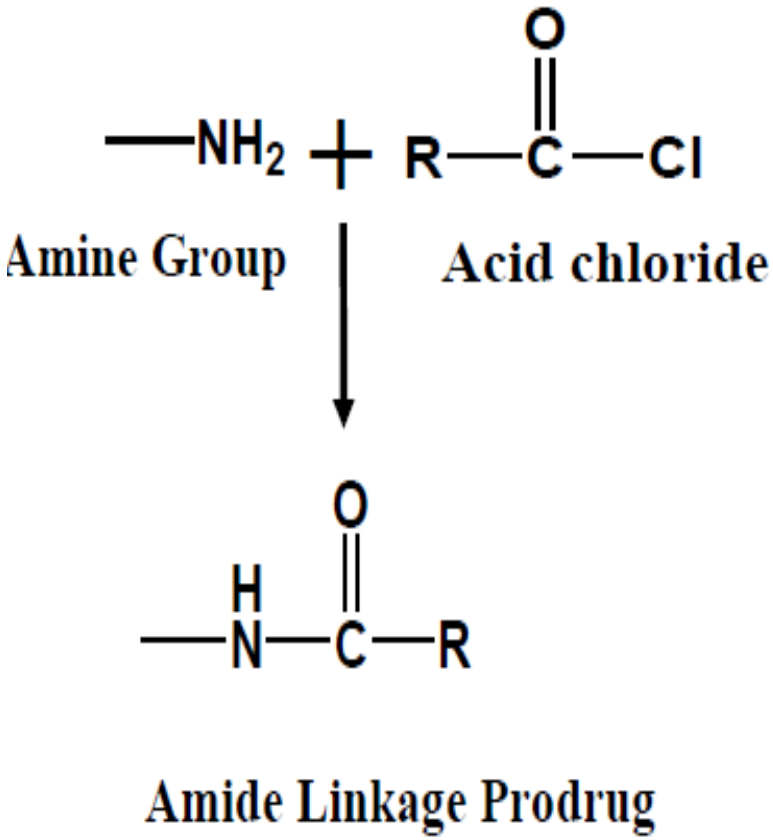
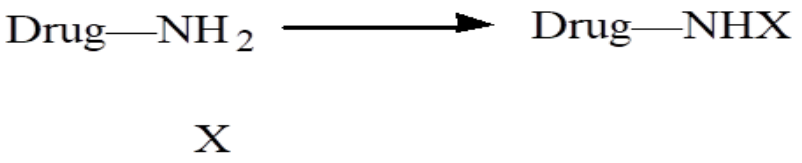


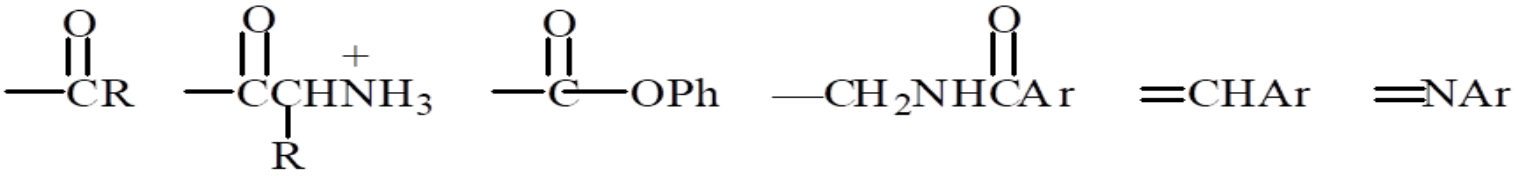
Parent drug having an amine group



Amide Linkage Prodrug

Amine Prodrugs





Amid Amide Active Carbamat Mannich base Imine Diazo

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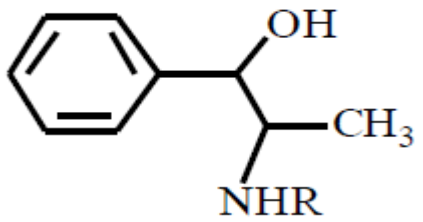
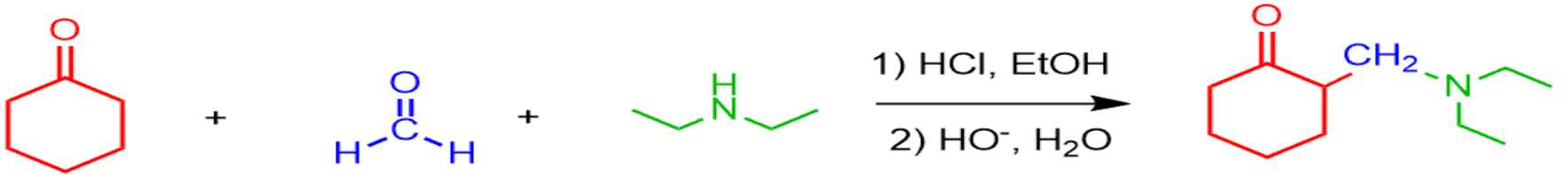
phenylpropanolamine hydrochloride (R = H HCl



The **Mannich reaction** is a condensation of an aldehyde or ketone in form of an enol with an iminium ion producing *β* - aminoalkyl carbonyl compounds.



ketone **aldehyde -** **2° amine** ***β* - aminoalkyl carbonyl**

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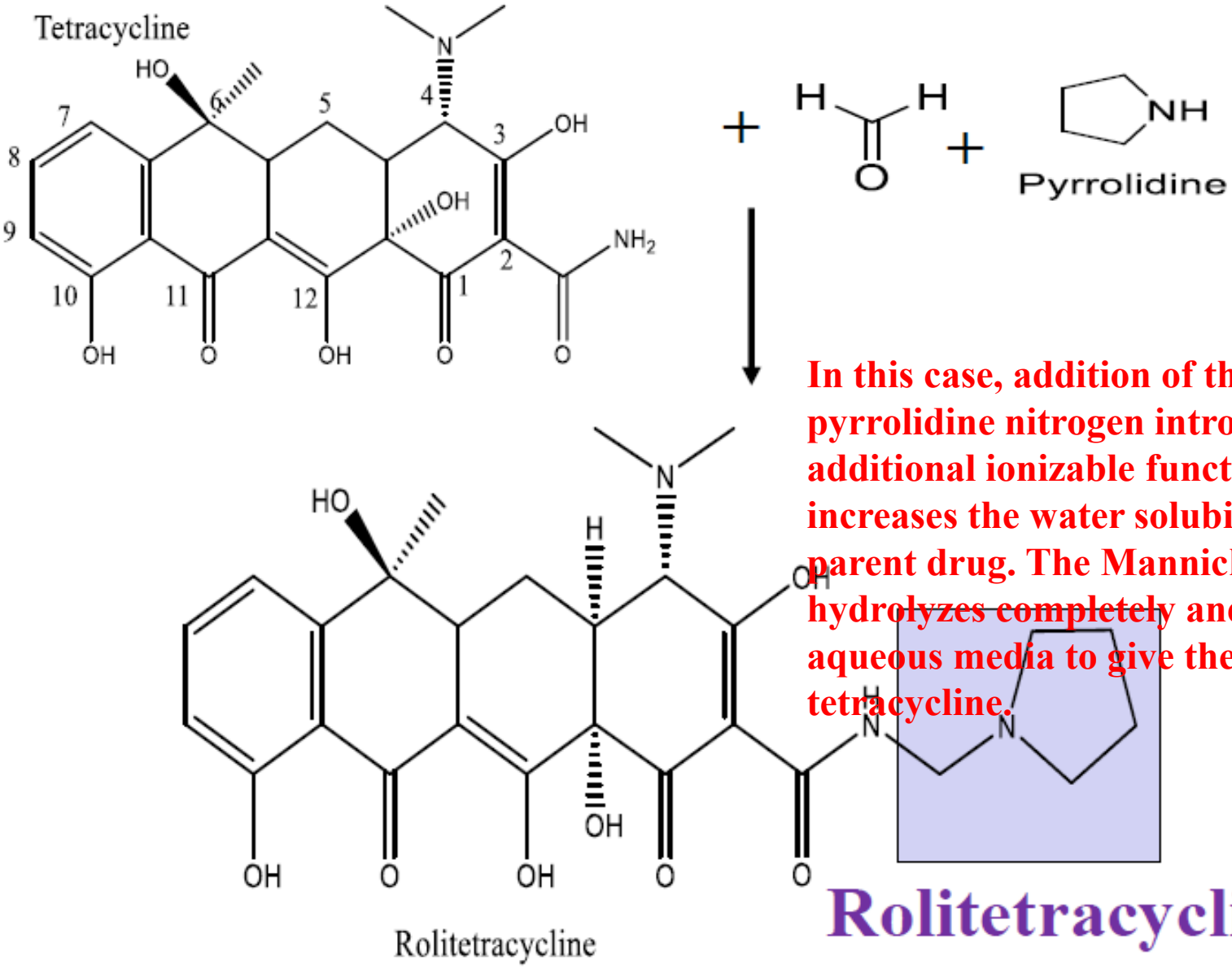

In this case, addition of the basic pyrrolidine nitrogen introduces an additional ionizable functionality and increases the water solubility of the parent drug. The Mannich base hydrolyzes completely and rapidly in aqueous media to give the active tetracycline.

Rolitetracycline

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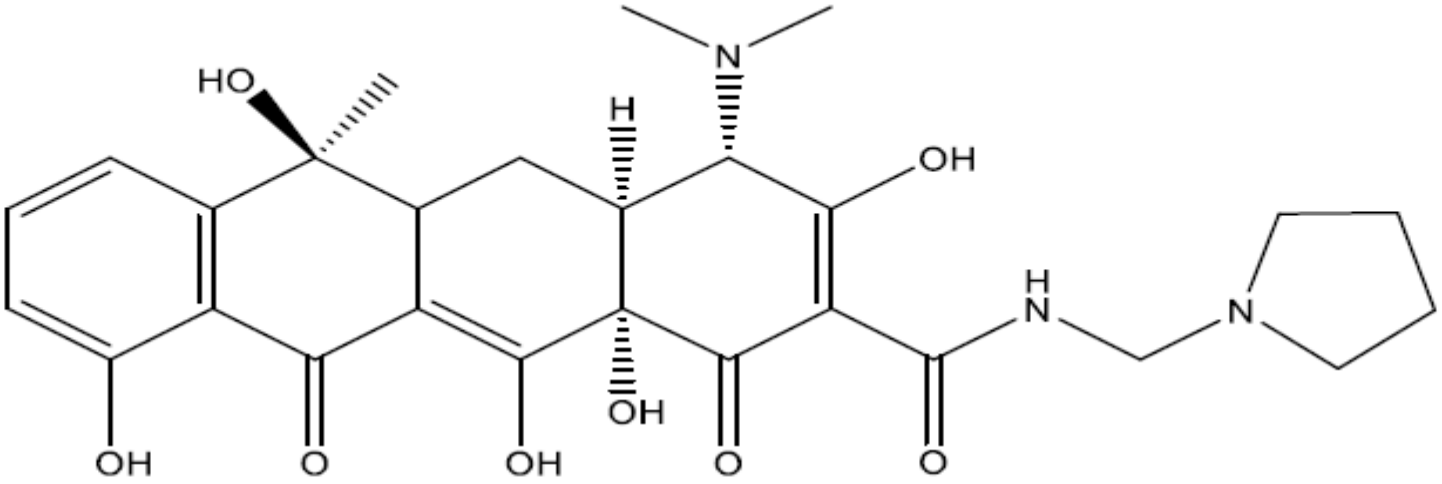


Rolitetracycline

Amidase

Tetracycline + Pyrrolidine + Formaldehyde

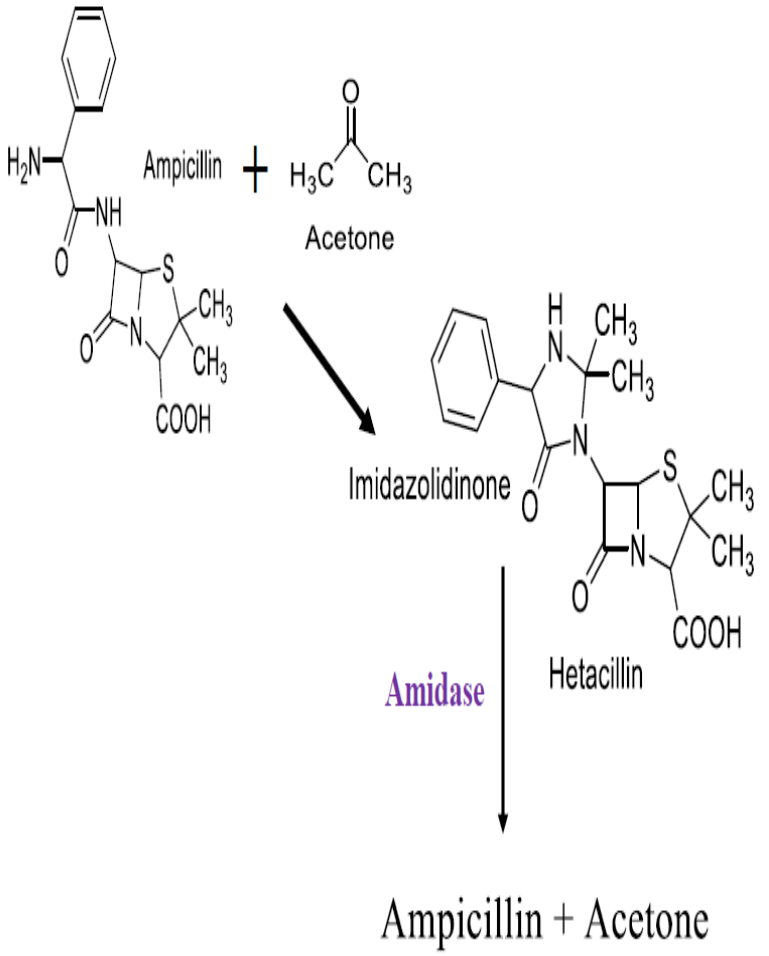
Hetacillin

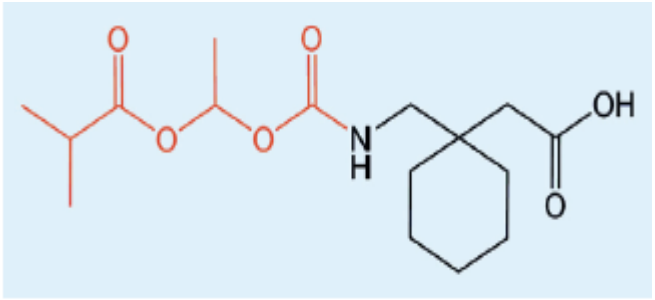
Mannich bases result from the reaction of two amines with an aIdehyde or ketone

The purpose of preparing hetacillin is to decrease the protonation of α amino group which has pKa of 7.3, i.e. readily protonated in acidic media then poorly absorbed.

Hetacillin is a prodrug form of ampicillin in which the amide nitrogen and α amino functionalities have been allowed to react with acetone to give an imidazolidinone ring system.

This has the effect of decreasing the basicity of α amino group and reducing protonation in small intestine so that the agent is more lipophilic





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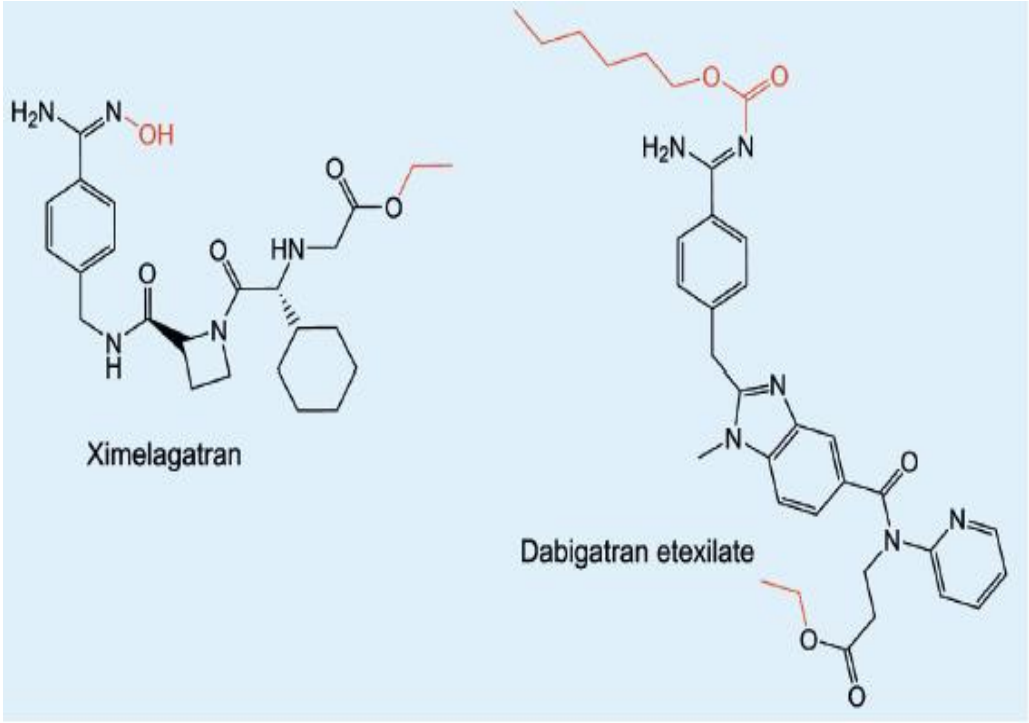
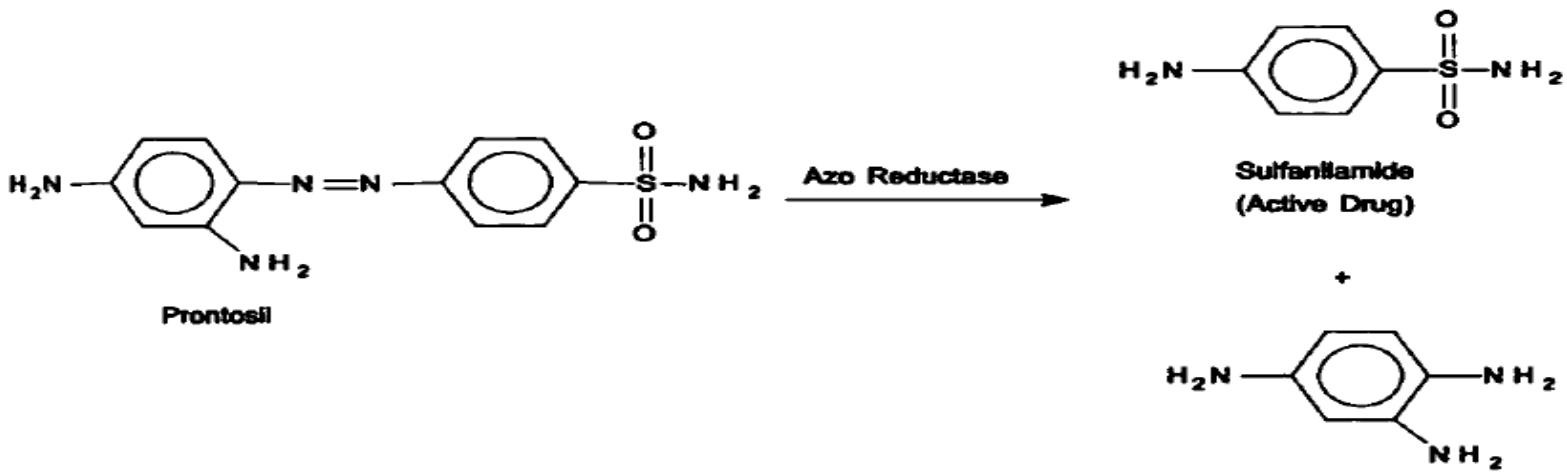


Recently approved Anticoagulants: A prodrug strategy to mask the polar basic group could improve absorption of the molecule from the gastrointestinal tract into the circulation, where the active drug molecule is then released by chemical or enzymatic cleavage. This approach has been successfully demonstrated in the area of thrombosis by the direct thrombin inhibitors.

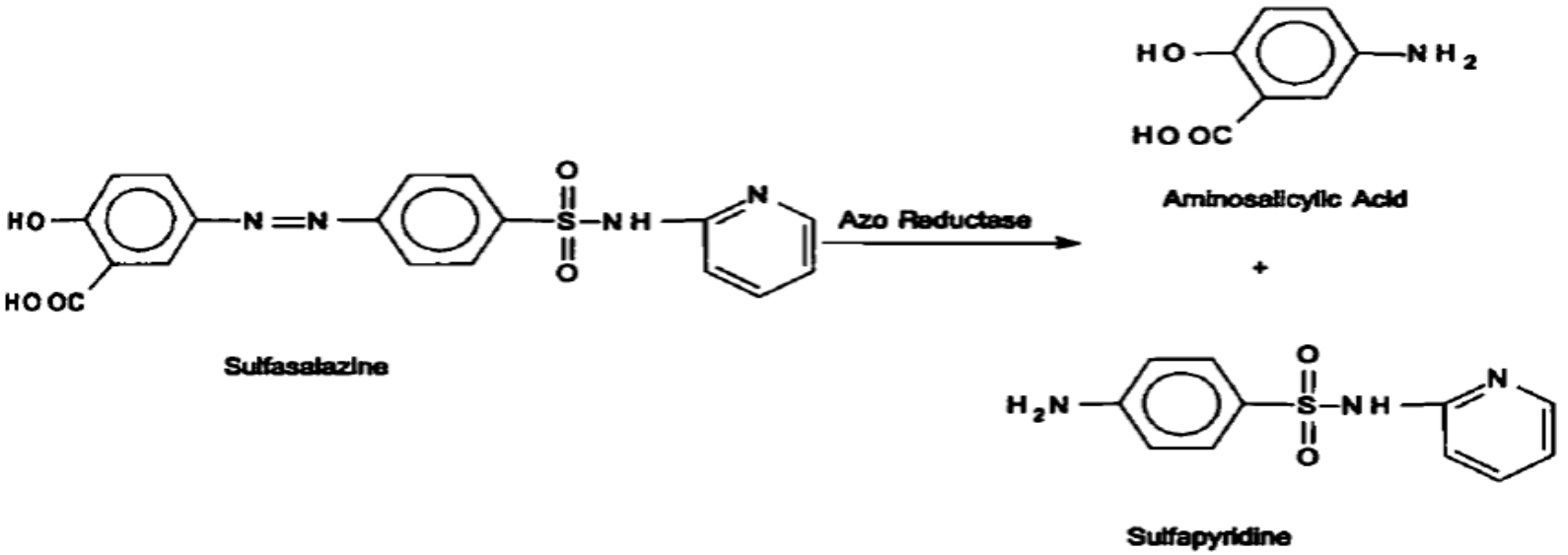
3- Azo Linkage

- Amines are derivatised to azolinkage prodrugs. Azo dye prontosil that led to the discovery of the sulfonamides as the first antibacterials to be used to treat systemic infections. Although prontosil itself was inactive in vitro, it was active in vivo and was converted by azo reductase enzymes in the gut to sutfanilamide the active species.



Although prontosil is no longer used as an antibacterial this type of linkage appears in sulfasalazine which is used in the treatment of ulcerative colitis.

The azo linkage is broken in the gut by the action of azo reductases produced by microflora. This releases the active agent, aminosalicylic acid



Sulfasalazine

Sulfapyridine

The **advantage** of this prodrug approach is that the combination of cleavage of the azo linkage and generation of aminosalicylic acid prior to absorption prevents the systemic absorption of the agent and helps concentrate the active agent at the site of action.

4- Carbonyl Compounds

- A number of different functionalities have been evaluated as prodrug derivatives of **carbonyls** (e.g., aldehydes and ketones), although this approach has not found wide clinical use. These have generally involved derivatives in which the sp2 hybridized carbonyl carbon is converted to an sp3 hybridised carbon attached to two heteroatoms such as oxygen, nitrogen, or sulfur. These prodrugs are reconverted to the carbonyl compounds by hydrolysis. Ex: **Methenamine** releases HCHO in the urine, which acts as an antibacterial agent

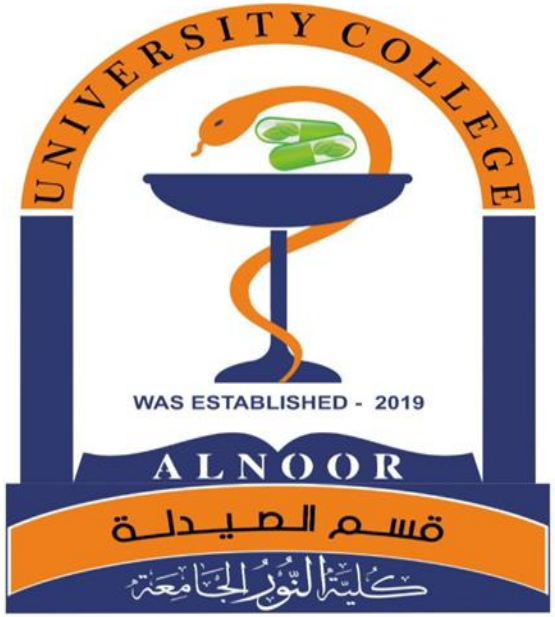
Organic Pharm. Chemistry IV

The Organic Chemistry of Drug Design and Drug Action

Lecture 4

5th November 2022

12:00- 2:00 PM



AlNoor University College

Pharmacy Department

1st Course/ 5TH Class

2022/2023

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يمكنك العودة وتغيير البداية

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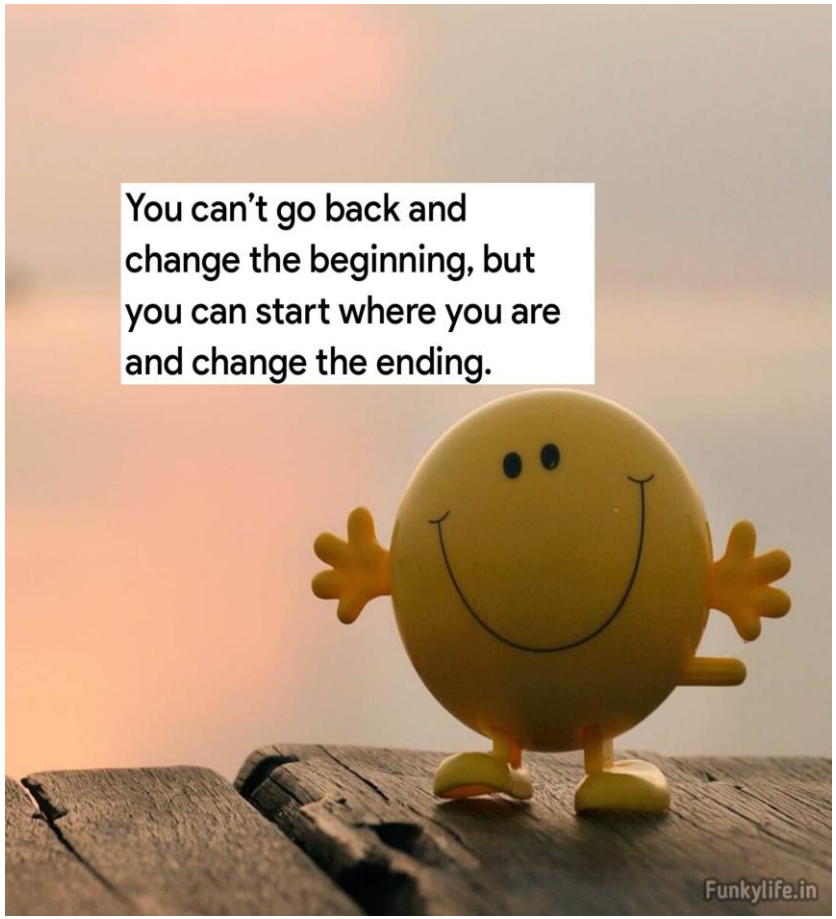

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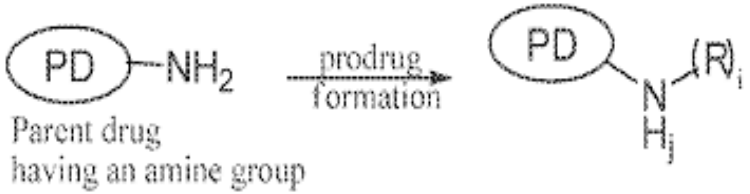
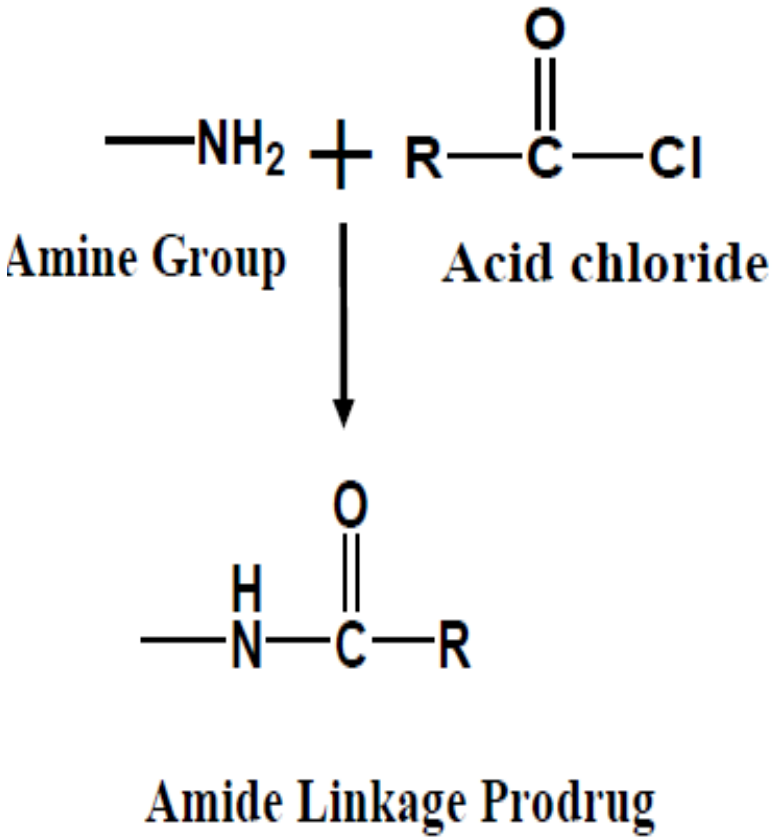


Parent drug having an amine group



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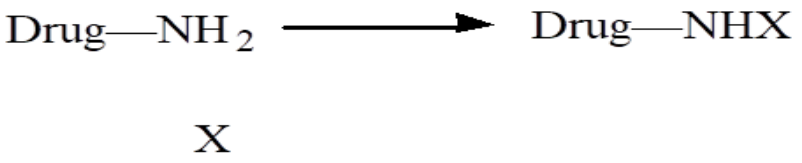

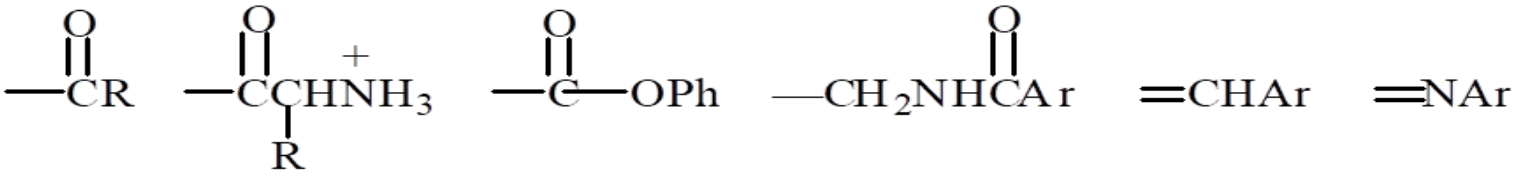

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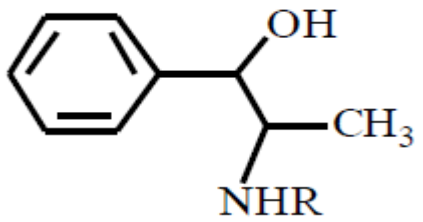

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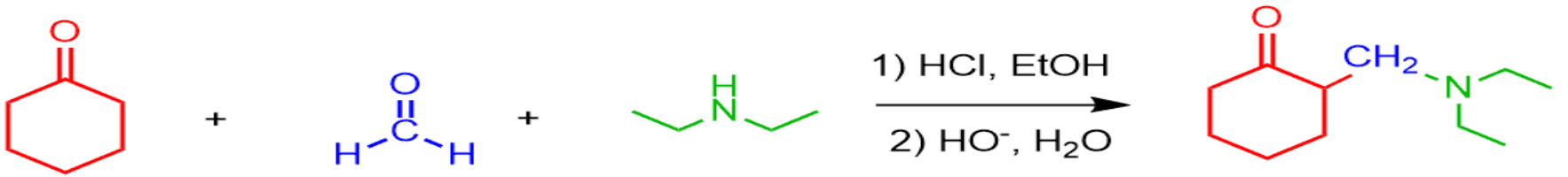

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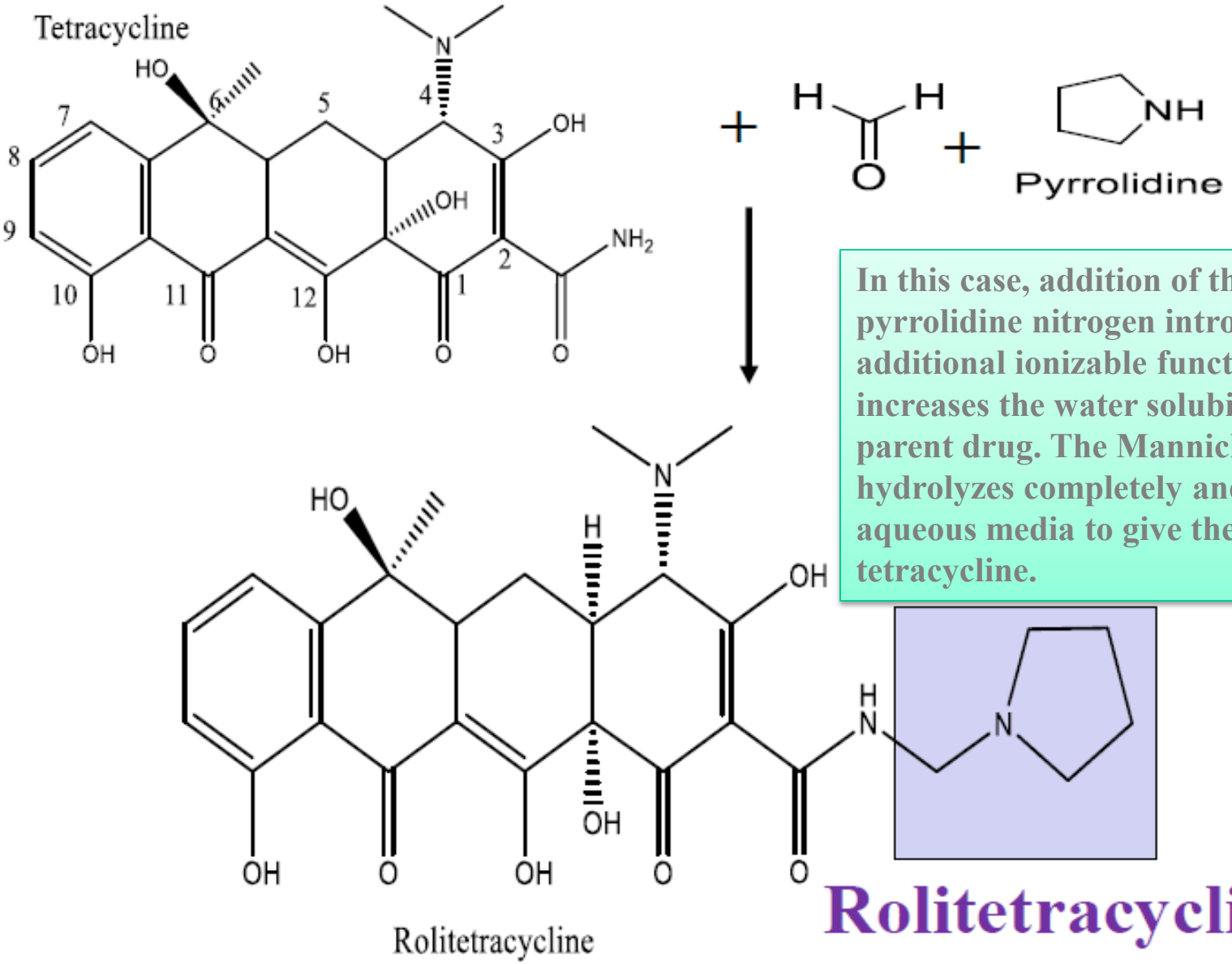

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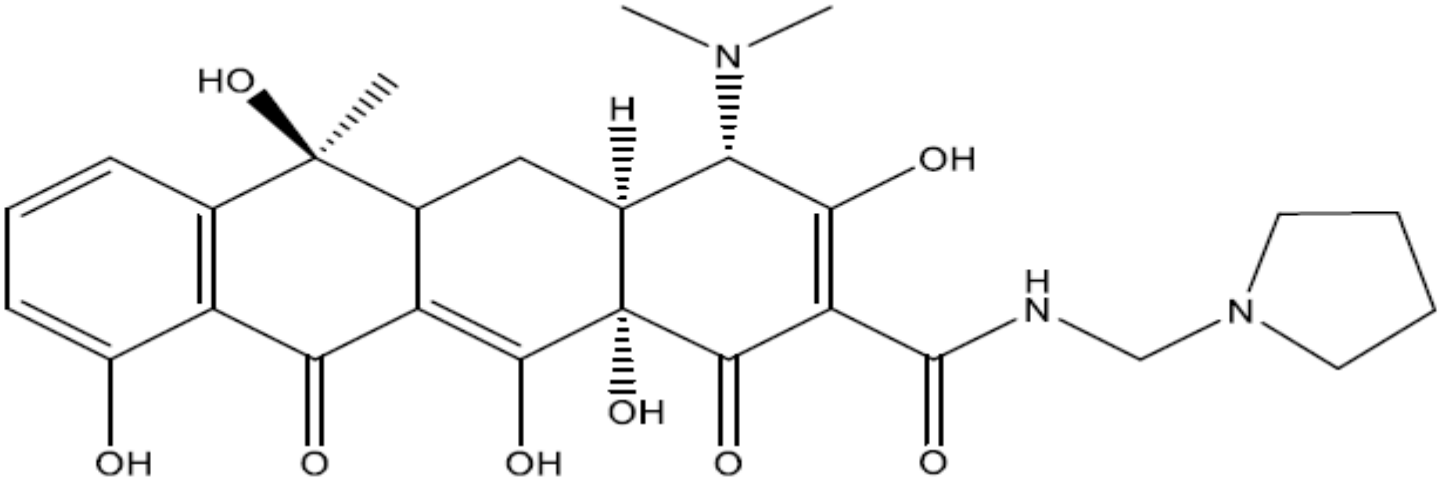
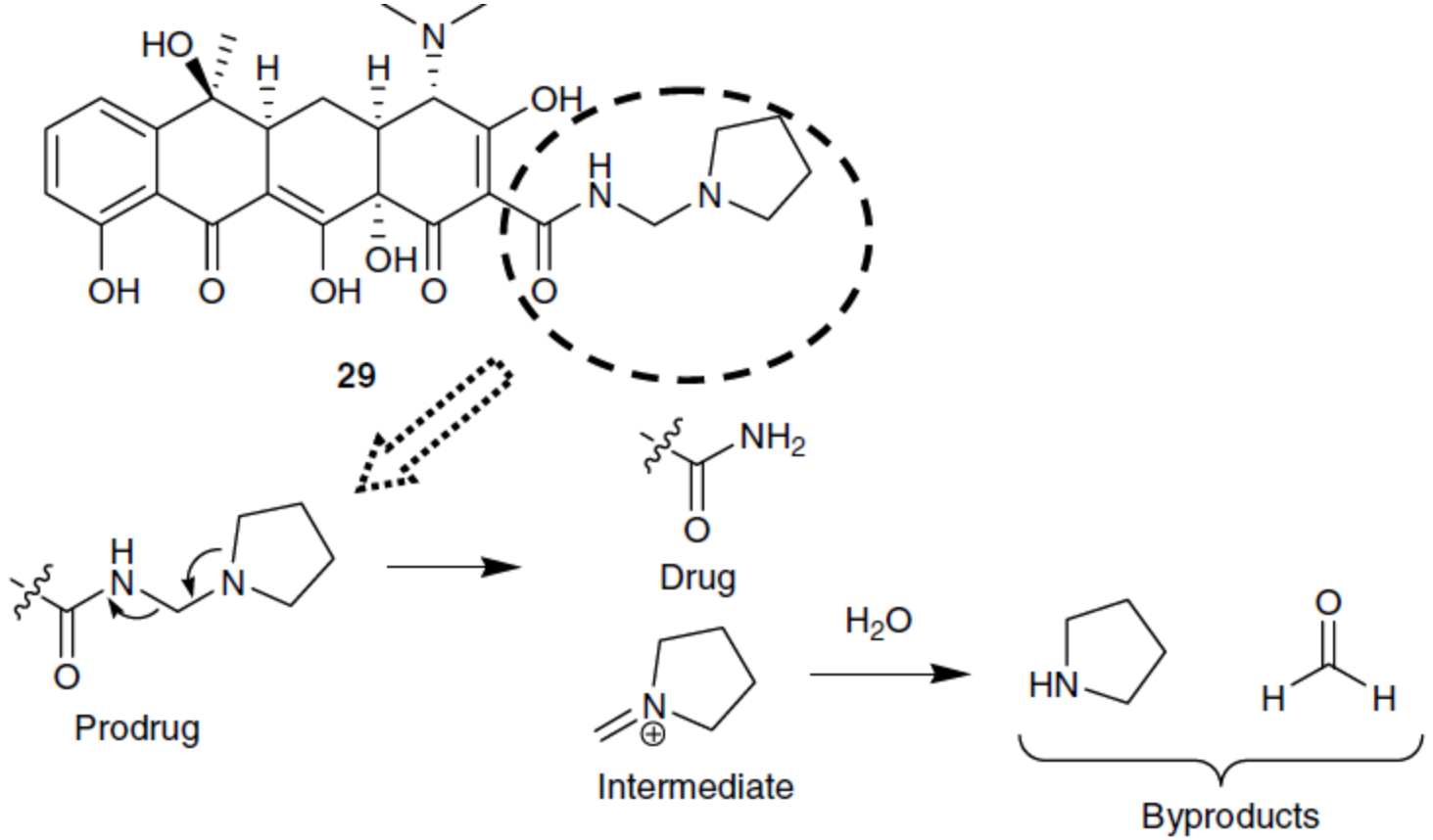


Rolitetracycline

Amidase

Tetracycline + Pyrrolidine + Formaldehyde

The general reconversion of an N-Mannich base prodrug using rolitetracycline as an example



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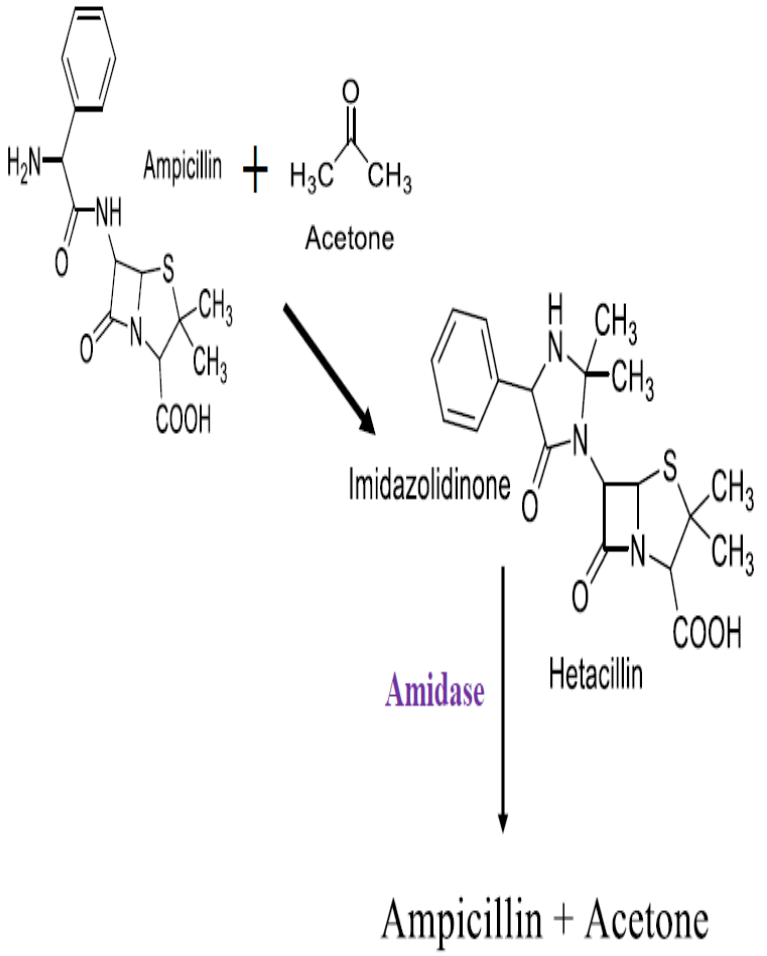

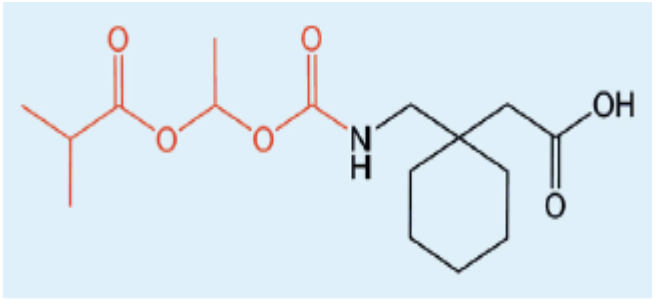

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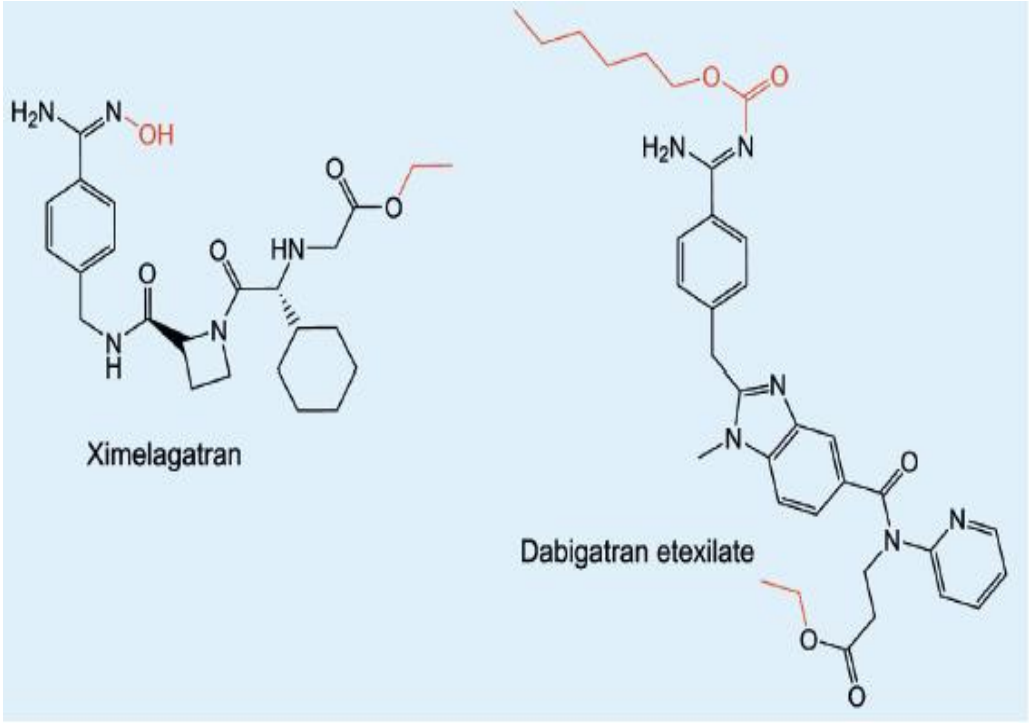

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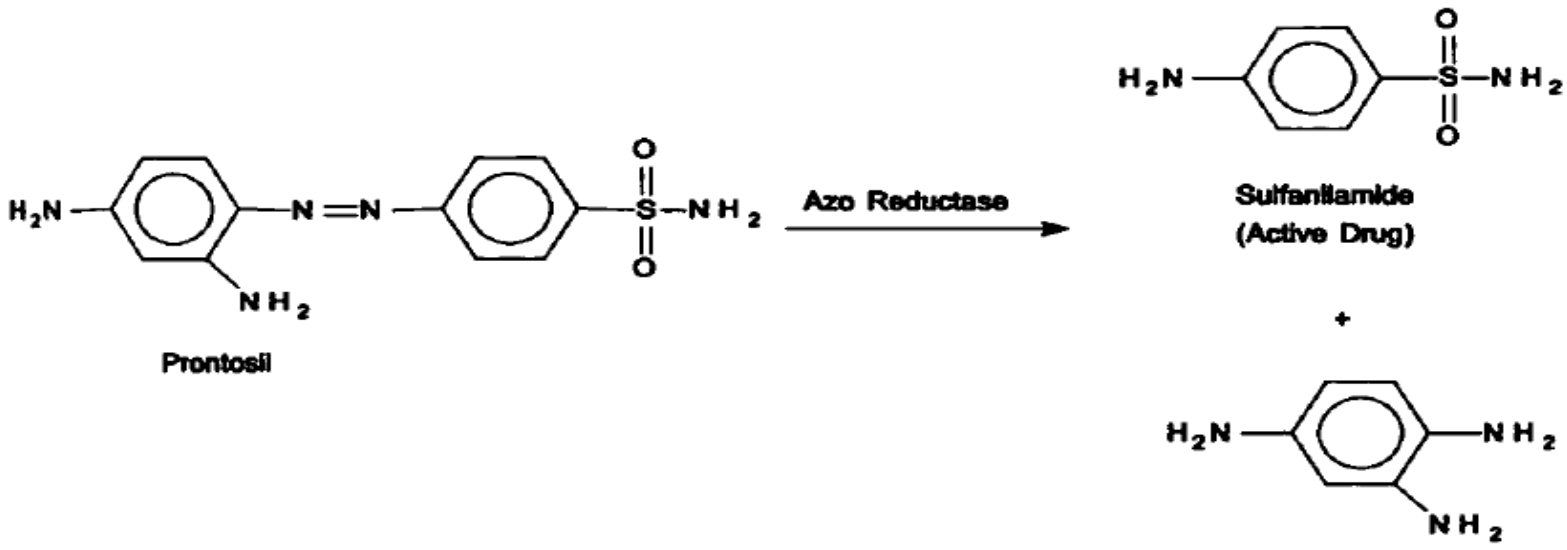
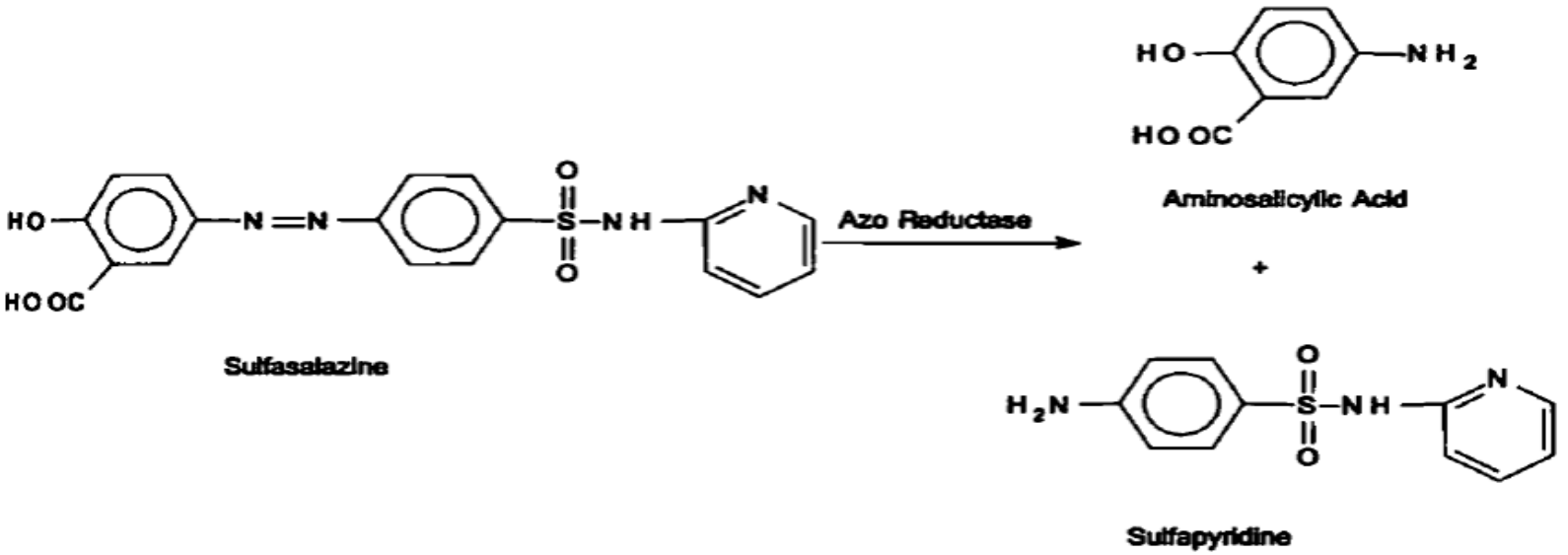


Although prontosil is no longer used as an antibacterial this type of linkage appears in sulfasalazine which is used in the treatment of ulcerative colitis.

The azo linkage is broken in the gut by the action of azo reductases produced by microflora. This releases the active agent, aminosalicylic acid



Sulfasalazine

Sulfapyridine

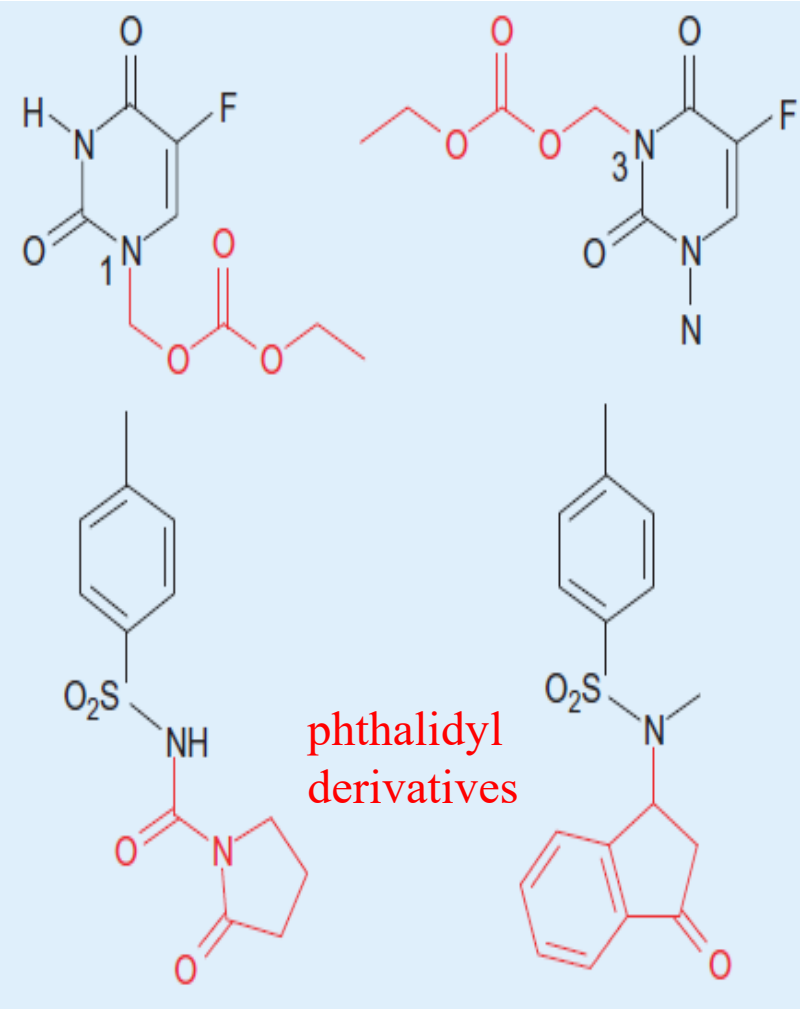
The **advantage** of this prodrug approach is that the combination of cleavage of the azo linkage and generation of aminosalicylic acid prior to absorption prevents the systemic absorption of the agent and helps concentrate the active agent at the site of action.

Prodrugs for Compounds with Acidic NH Functions.

Prodrugs obtained by N-alkoxycarbonyloxymethylation of 5-fluorouracil show improved delivery properties.

Both 1- and 3-alkoxycarbonyloxymethyl derivatives are hydrolyzed quantitatively to 5-fluorouracil, but the 3-substituted derivatives show a greater promise as prodrugs since they combine adequate stability in aqueous solution with a high susceptibility to hydrolysis in plasma .

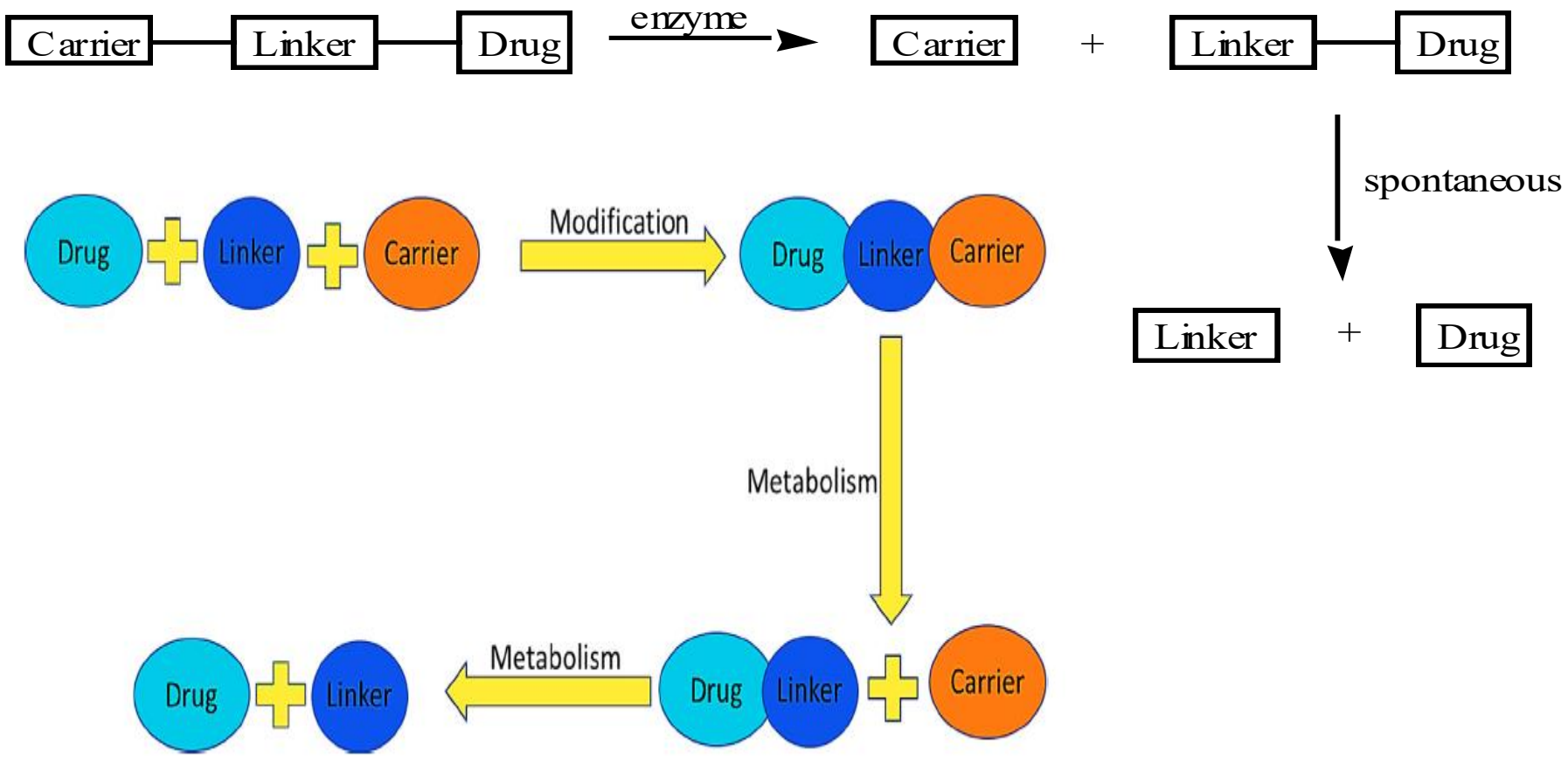
Sulfonamides, as well as carboxamides, carbamates, and other NH-acidic compounds (Figure), can be acylated with various groups or converted into phthalidyl derivatives



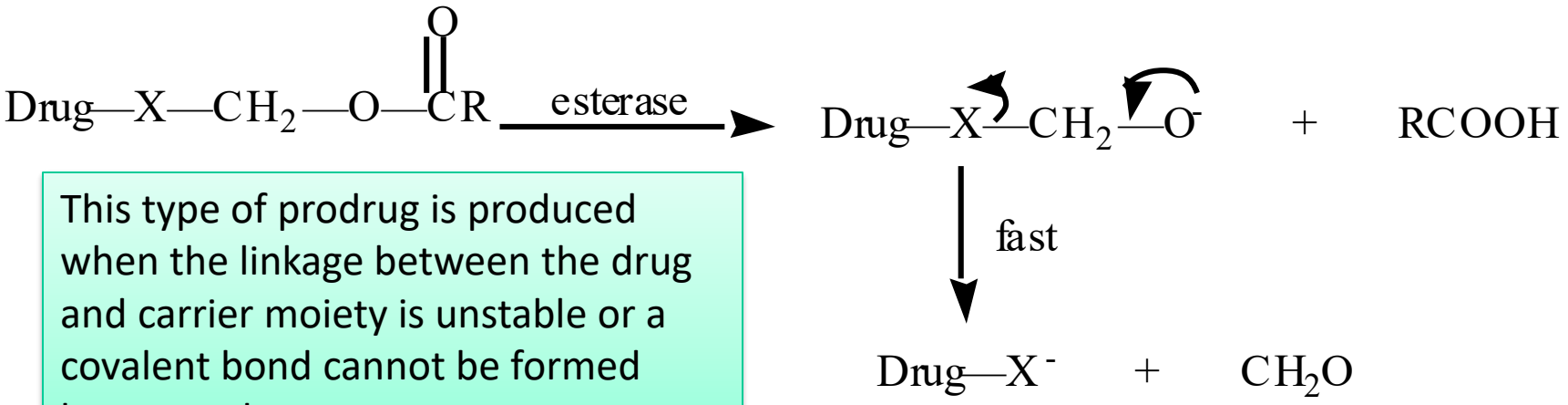
4- Carbonyl Compounds

- A number of different functionalities have been evaluated as prodrug derivatives of **carbonyls** (e.g., aldehydes and ketones), although this approach has not found wide clinical use. These have generally involved derivatives in which the sp2 hybridized carbonyl carbon is converted to an sp3 hybridised carbon attached to two heteroatoms such as oxygen, nitrogen, or sulfur. These prodrugs are reconverted to the carbonyl compounds by hydrolysis. Ex: **Methenamine** releases HCHO in the urine, which acts as an antibacterial agent

Tripartate Prodrugs



Typical Approach



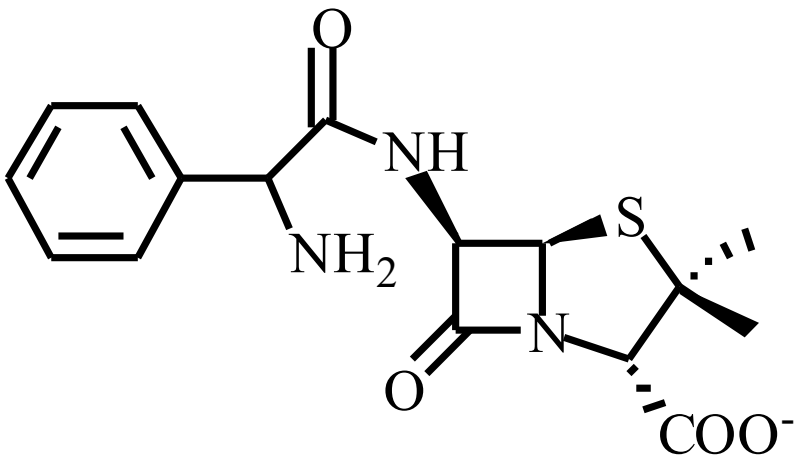
This type of prodrug is produced when the linkage between the drug and carrier moiety is unstable or a covalent bond cannot be formed between them.

Tripartate Prodrugs of Ampicillin

Poor oral absorption (40%)

Excess antibiotic may destroy important intestinal bacteria used in digestion and for biosynthesis of cofactors.

Also, more rapid onset of resistance.

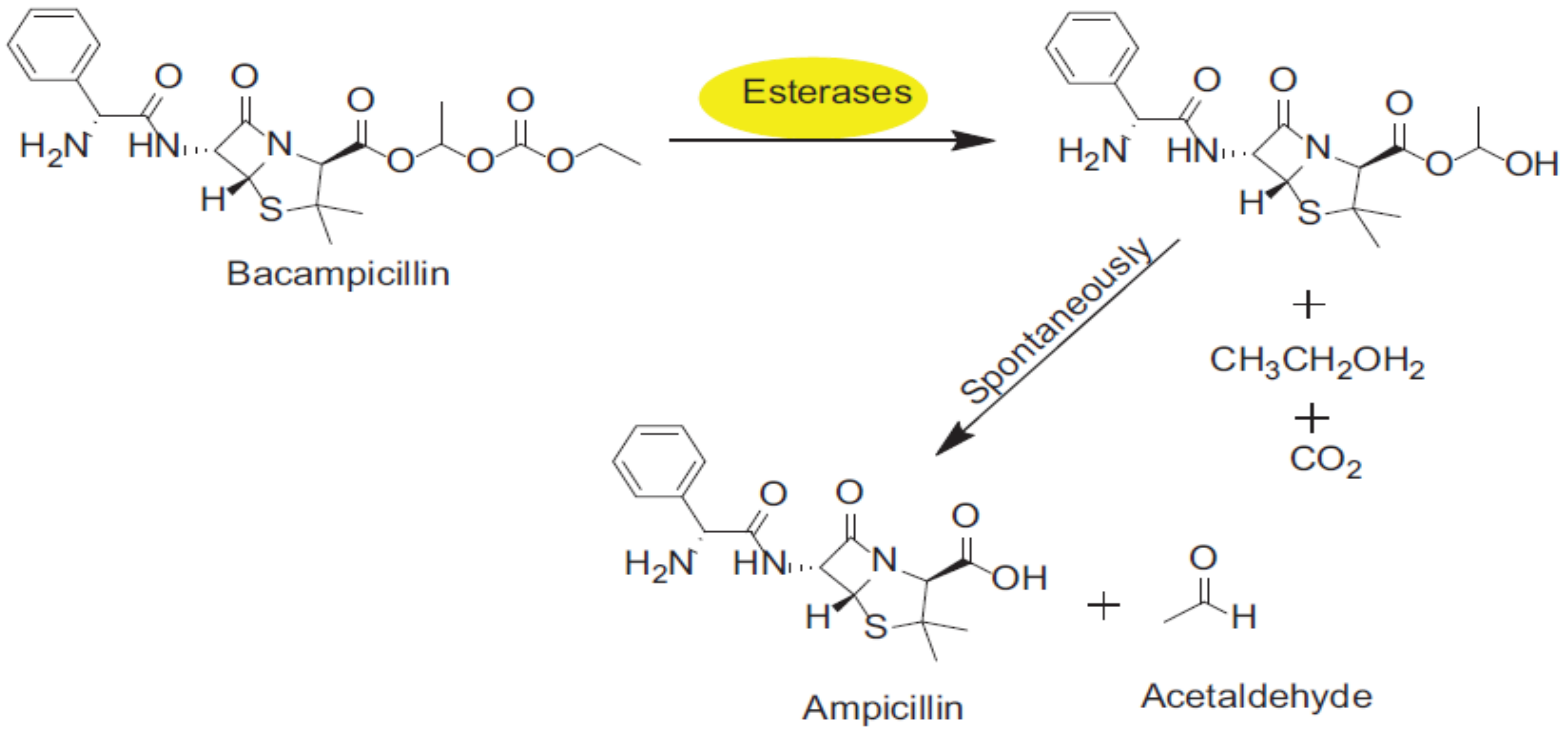


ampicillin
8.44
antibacterial

Various esters made were too stable in humans (although they were hydrolyzed in rodents) - thought the thiazolidine ring sterically hindered the esterase.

Tripartate Prodrugs of Ampicillin

The synthesis and metabolism of tripartite prodrugs
For example, Bacampicillin, a tripartite prodrug of ampicillin that gets hydrolyzed by esterase enzyme in vivo into ampicillin and ethanol. As shown in Fig. below, the linker moiety used is acetaldehyde which becomes spontaneously freed



98-99% absorbed

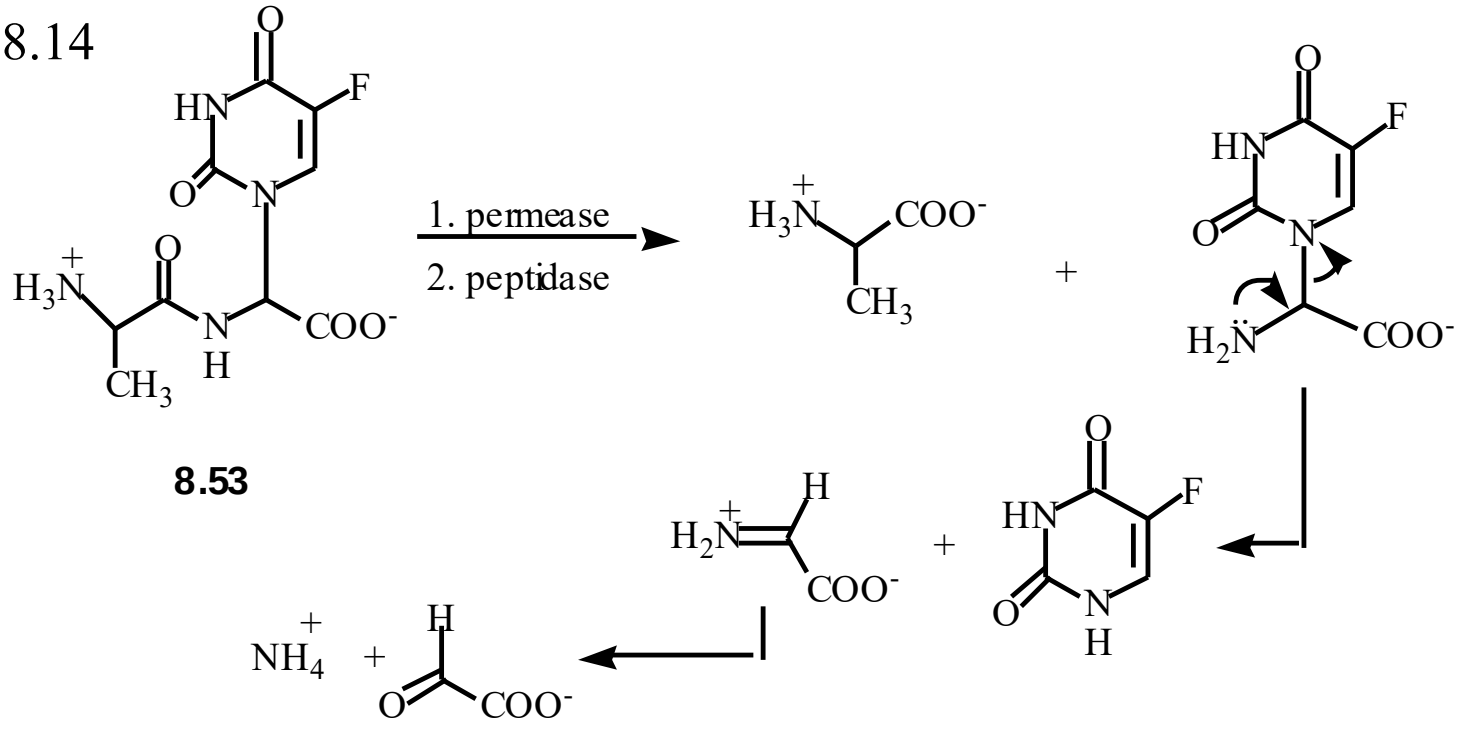
Ampicillin is released in < 15 minutes

Bioactivation of tripartite prodrug, that is, bacampicillin, by esterase enzyme into its active ampicillin form.

Tripartate Prodrug for Delivery of Antibacterials

Permeases are bacterial transport proteins for uptake of peptides.

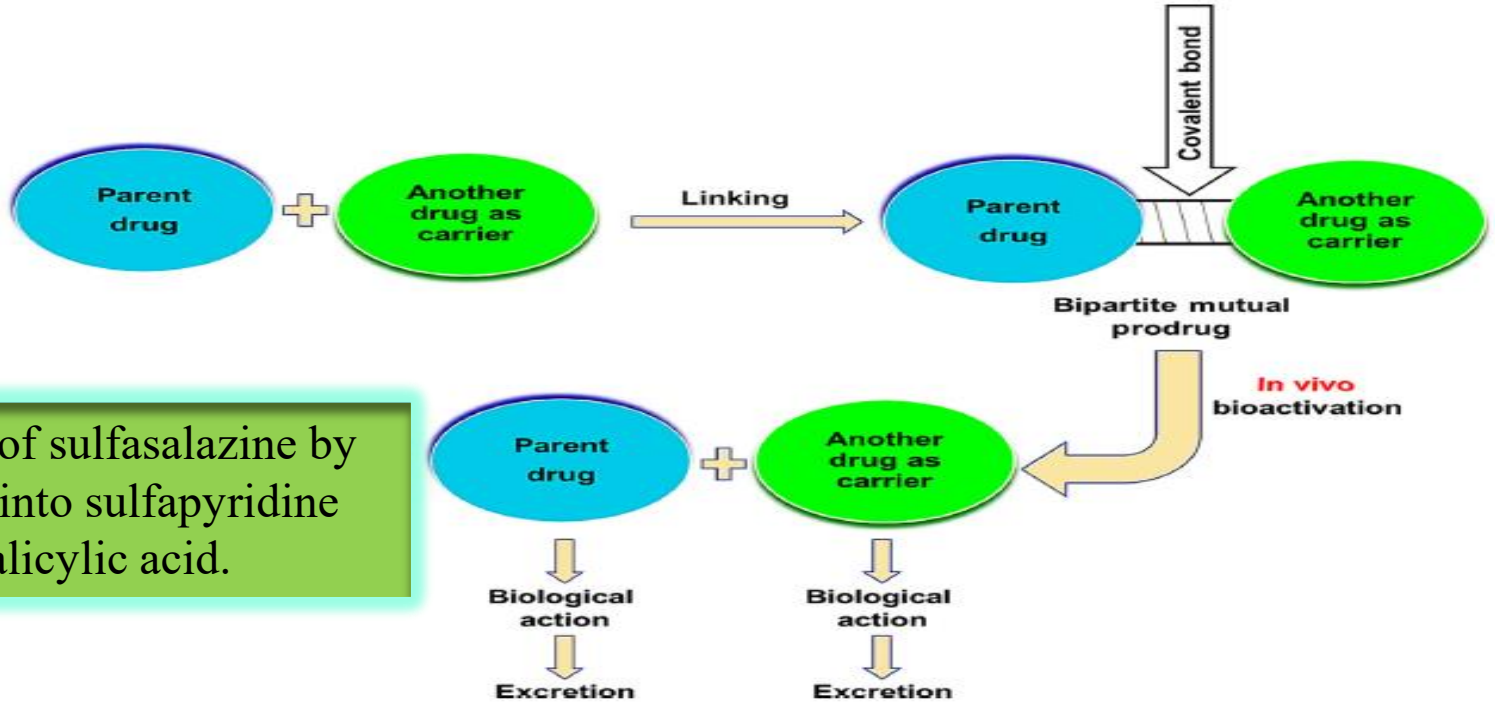
Scheme 8.14



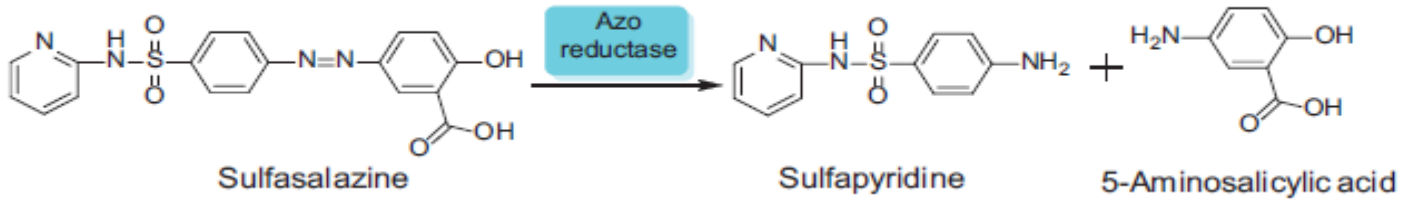
8.53

Only *L*,*L*-dipeptides are active

Schematic representation of the bipartite type of mutual prodrug formation and in vivo bioactivation of the same.



Bioactivation of sulfasalazine by azo reductase into sulfapyridine and 5-aminosalicylic acid.

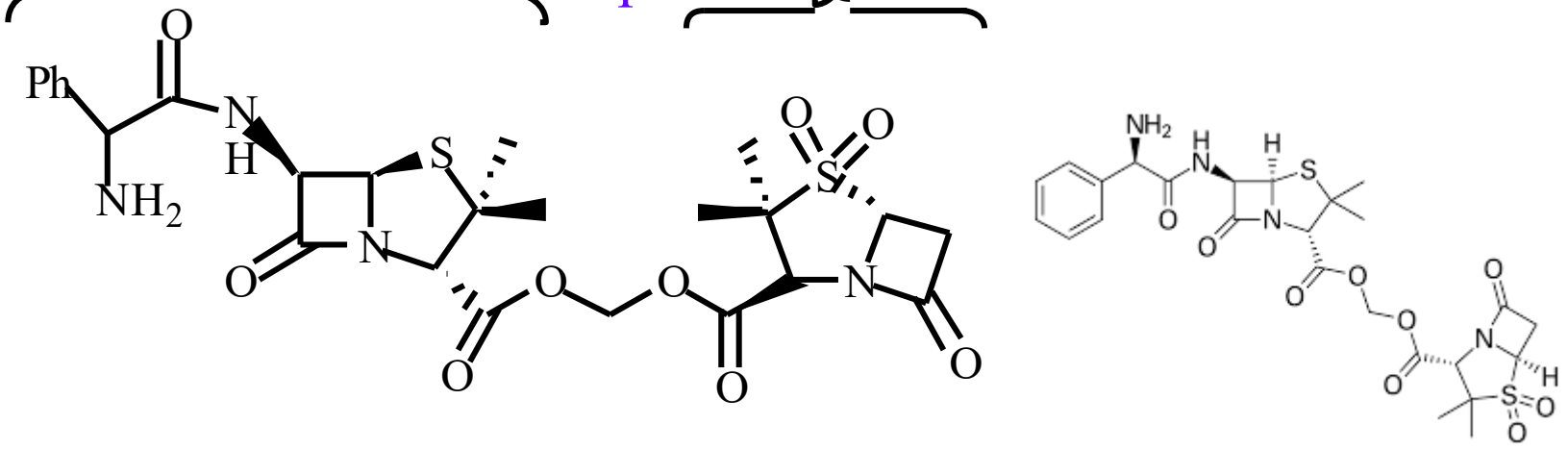


Mutual Prodrugs

A bipartate or tripartate prodrug in which the carrier is a synergistic drug with the drug to which it is linked.

Antibacterial
ampicillin

β-lactamase inactivator
penicillanic acid sulfone

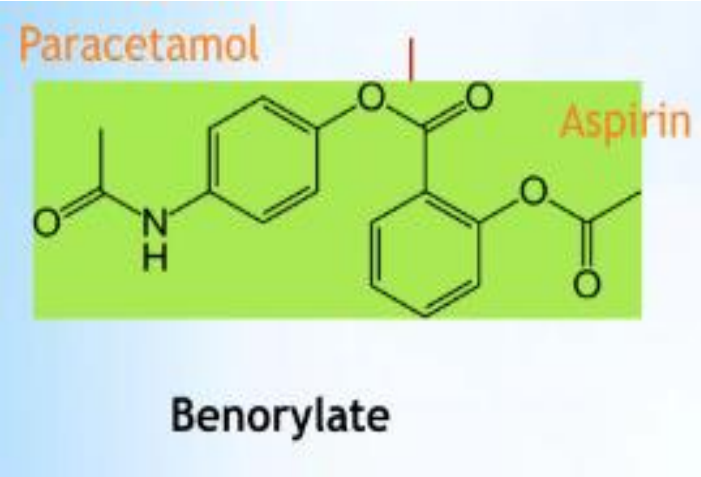


sultamicillin
8.59

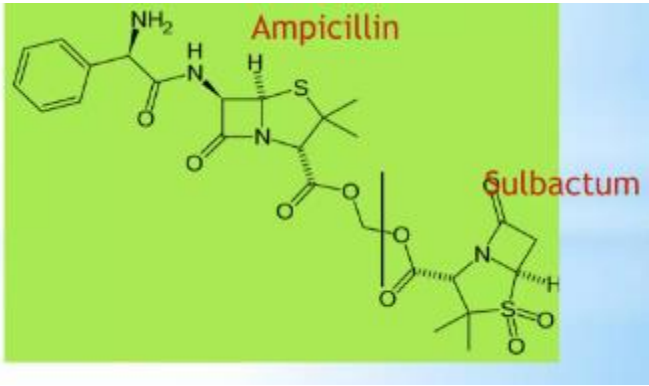
Hydrolysis gives 1:1:1 ampicillin : penicillanic acid sulfone : formaldehyde

Mutual Prodrugs

Benorylate



Sultamicillin



Ideal Mutual Prodrugs

- Well absorbed
- Both components are released together and quantitatively after absorption
- Maximal effect of the combination of the two drugs occurs at 1:1 ratio
- Distribution/elimination of components are similar

UNIQUE APPROACHES TO CARRIER PRODRUG DESIGN

A. Site-specific Delivery

Tissue-selective drug delivery has enormous potential to improve the safety and efficacy profile of drug molecules. Prodrug strategies have been investigated to target cell/tissue delivery. In principle, two targeting

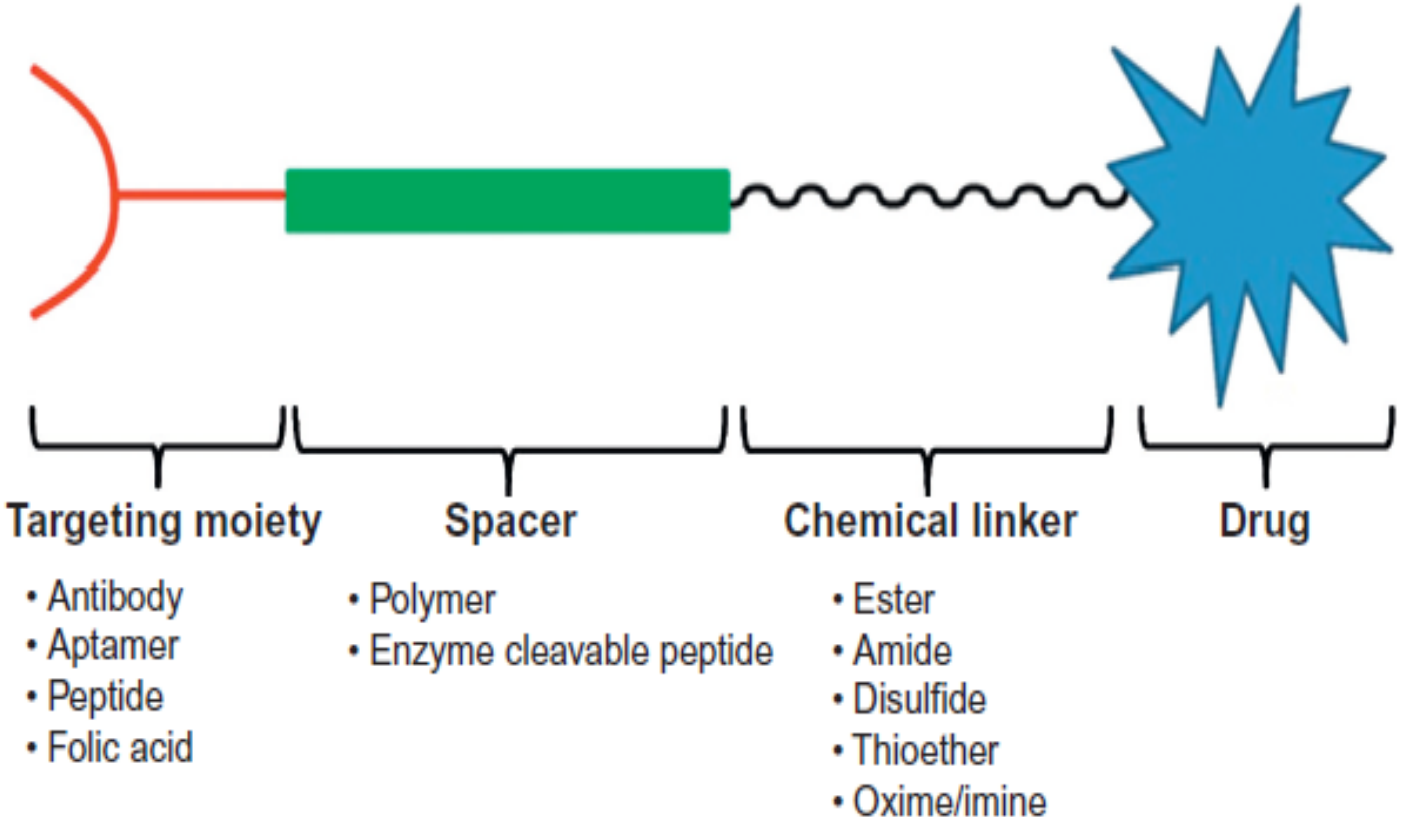
possibilities can be considered:

(1) site-directed drug delivery ,whereby prodrugs/conjugates are transported to target cells/tissues by tissue-specific transporters or cell surface receptors; and

(2) site-specific drug release, in which the prodrug is distributed everywhere but is activated by enzymes preferentially localized in target cells/tissues. In either case, there are several components to consider when designing a prodrug, such as the targeting moiety, spacer and/or cleavable linker, and the drug molecule, as illustrated in Figure


Forest= Body
Knight= Drug
Weapons=
physicochemical
properties
Dragon= receptor

General design aspects of a targeted drug conjugate.



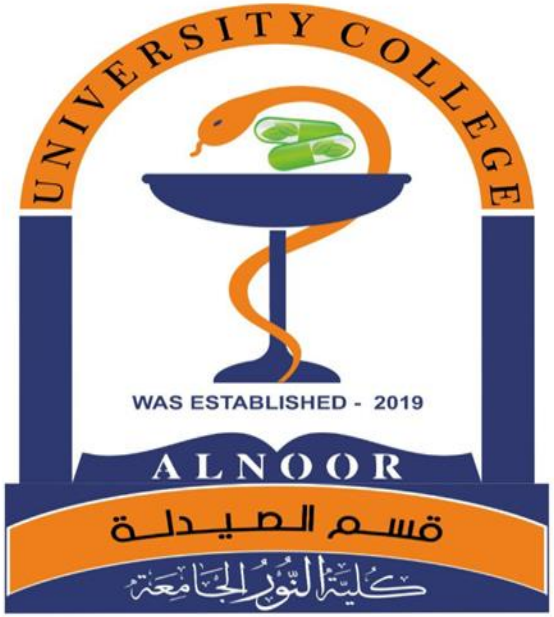
Organic Pharm. Chemistry IV

The Organic Chemistry of Drug Design and Drug Action

Lecture 5

12th November 2022

12:00- 2:00 PM



AlNoor University College

Pharmacy Department

1st Course/ 5TH Class

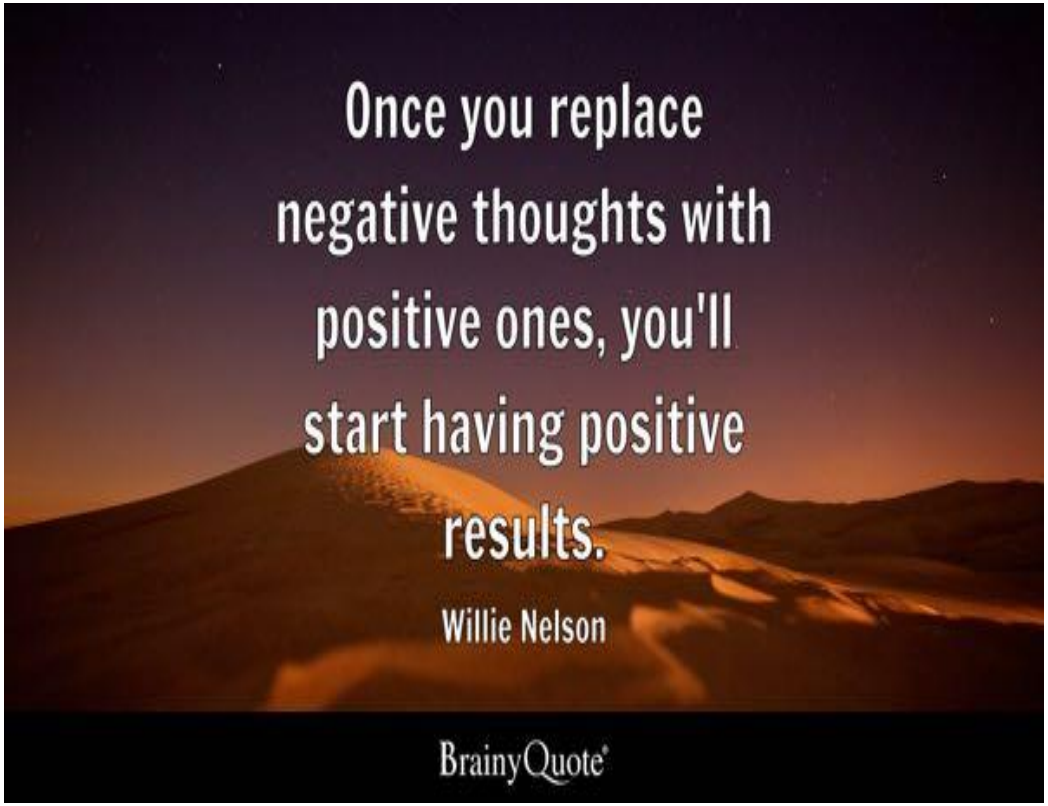
2022/2023


STAY
positive

Be (+)


NEGATIVE
POSITIVE


THINK
POSITIVE
TALK
POSITIVE
FEEL
POSITIVE


Once you replace
negative thoughts with
positive ones, you'll
start having positive
results.
Willie Nelson
BrainyQuote

UNIQUE APPROACHES TO CARRIER PRODRUG DESIGN

A. Site-specific Delivery

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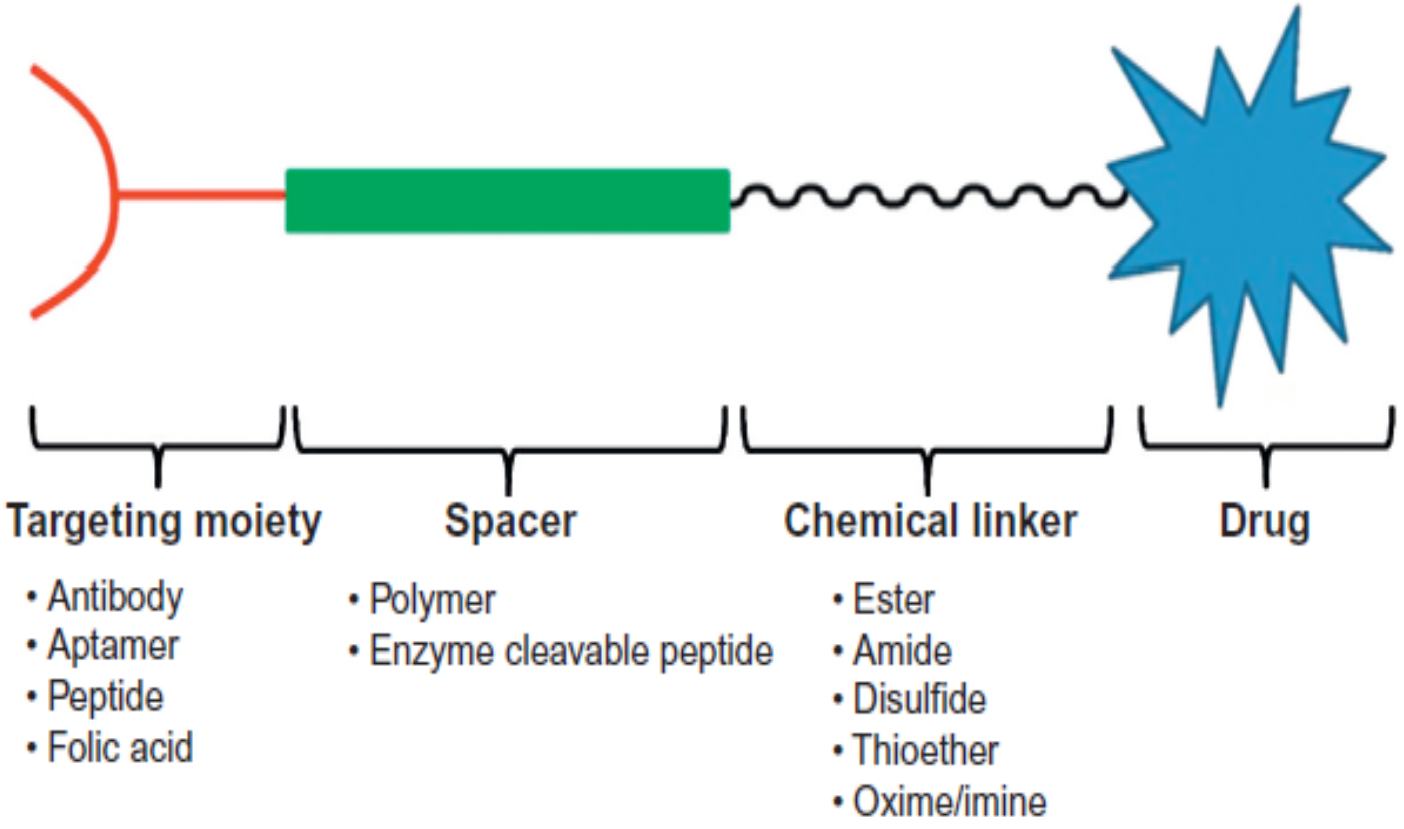
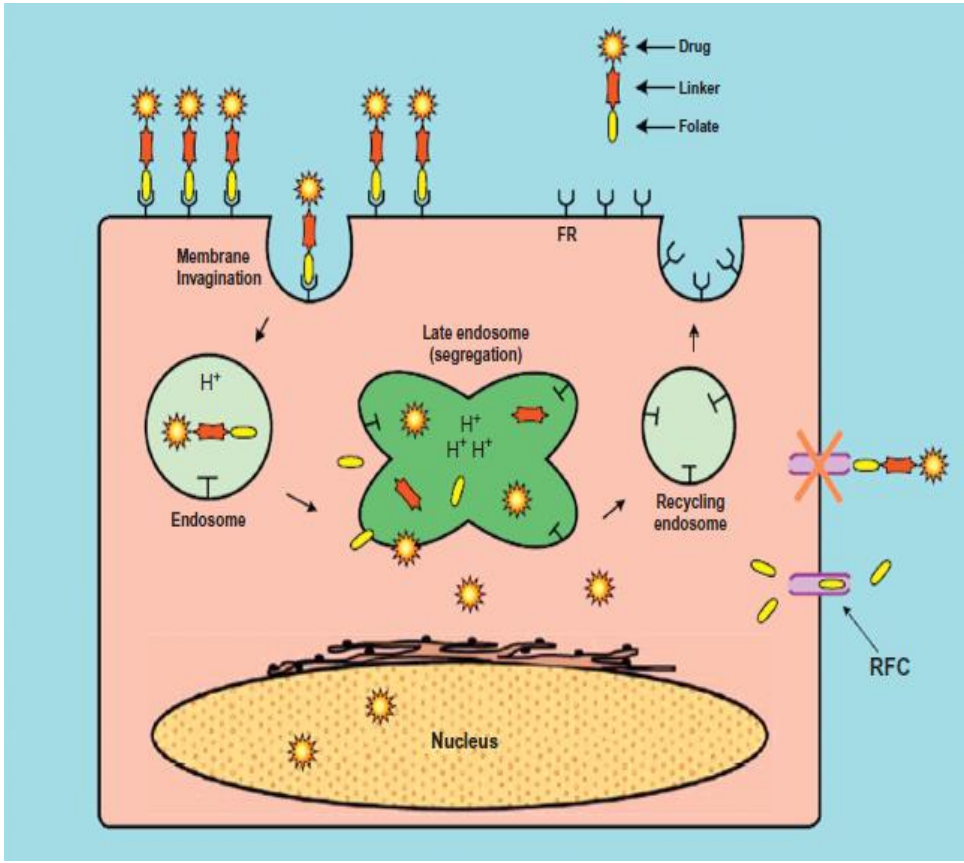
General design aspects of a targeted drug conjugate.



Representation of targeted delivery and folic acid-drug conjugate uptake via endocytosis.

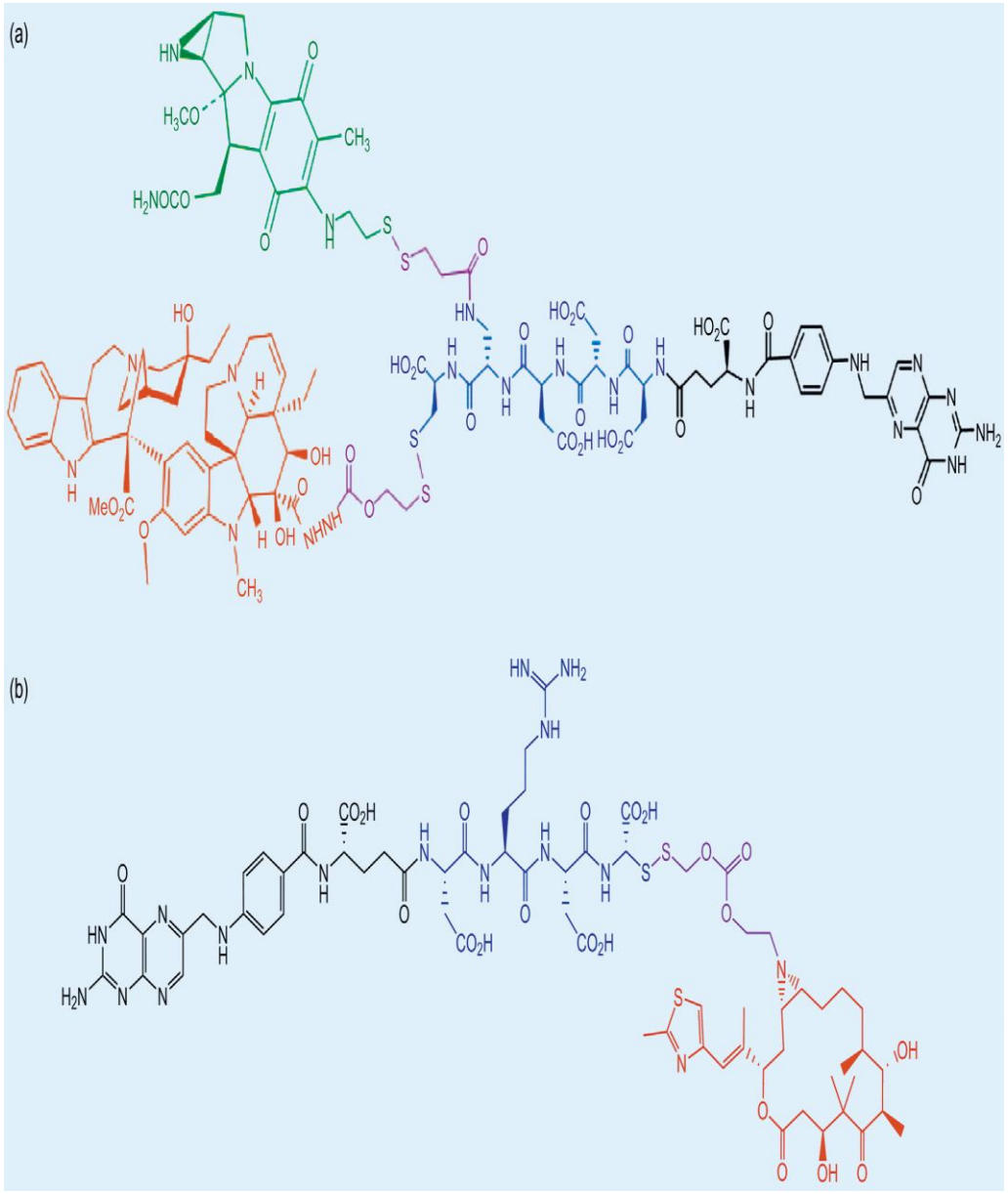
- The folic acidddrug conjugate binds specifically to the FR with high affinity. The plasma membrane invaginates to form an intracellular vesicle that migrates toward the perinuclear region and fuses with other endosomal elements. The mature endosome becomes acidic, and the receptor changes conformation and releases the conjugate, which cleaves to yield the drug molecule [85]. Reprinted with permission from Elsevier. Copyright 2004.



A promising strategy identified by researchers at Endocyte targets folate receptors (FR), which are limited to tissues that are responsible for retention of folates such as **kidney, placenta, and liver, and more importantly to specific pathological tissues such as tumors**

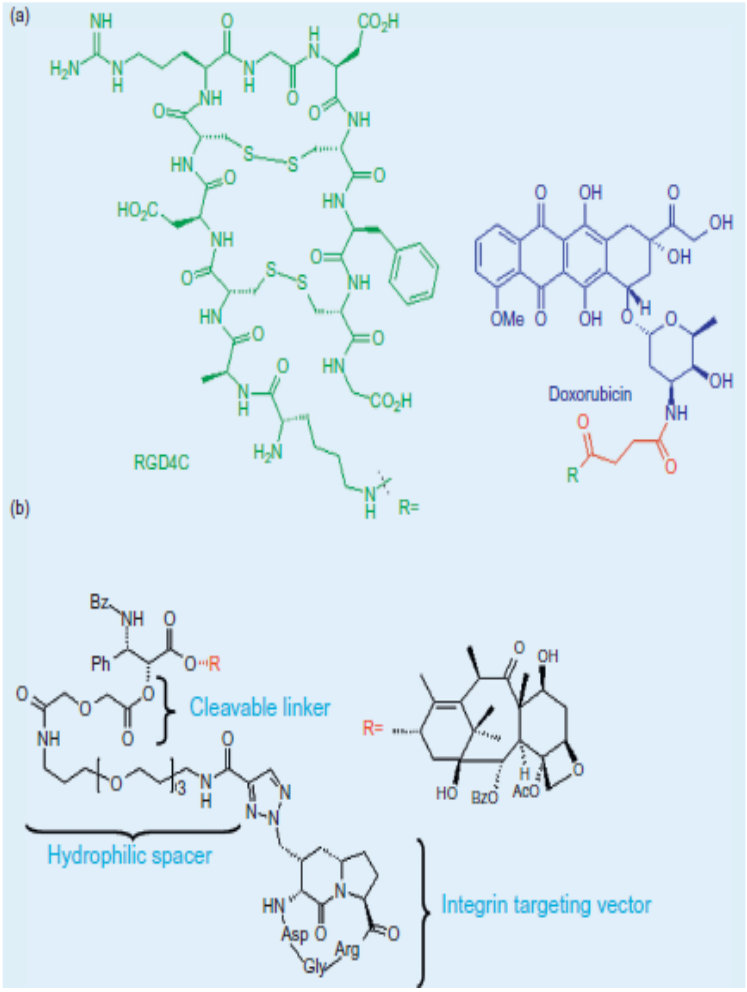
(ovarian, endometrial, renal, lung, and breast carcinomas) and chronic inflammatory sites.

The potential for increased therapeutic index and reduced toxicity.



(a) Chemical structure of dual drug conjugate, EC0255. Folic acid is shown in black, the hydrophilic peptide spacer is shown in blue, the biologically cleavable linker is shown in purple, desacetyl vinblastine is shown in red and mitomycin is shown in green [8185,87]. Reprinted with permission from reference. Copyright 2007 American Chemical Society. (b) Epothilonefolate conjugate (epothilone in red).

Integrin-targeting prodrugs utilizing the RGD peptide motif

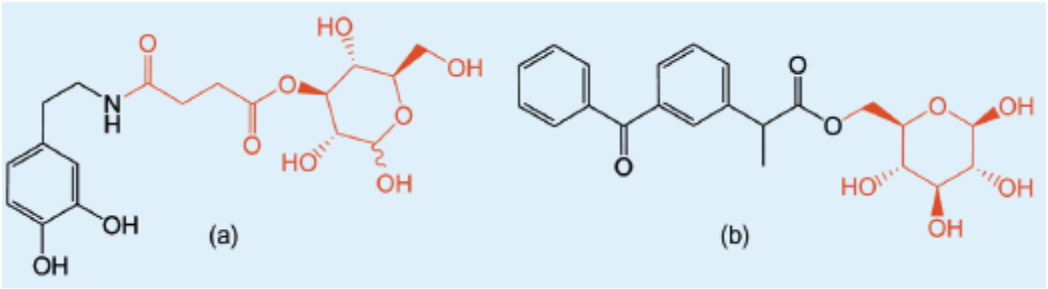


Another tumor-targeting strategy **utilizes the arginine-glycine-aspartic acid (RGD) peptide motif**, which displays a strong binding affinity and selectivity to integrin αvβ3. Integrins are a family of heterodimeric transmembrane glycoproteins involved in cell-to-extracelluar matrix and cell-to-cell interactions. The ability of integrins to be internalized by cells on activation with anchoring ligands suggests they can be used to facilitate the delivery of therapeutics.

Induction of multidrug resistance by doxorubicin in combination with its nonspecific toxicities has restricted DOX-based chemotherapy, and thus a targeting approach may expand its use. Among the different RGD peptide analogs, doxorubicinRGD4C conjugate (Figure a)

Besides doxorubicin, paclitaxel was covalently attached via a cleavable ester linkage to an integrin-recognizing cyclic RGD vector (Figure b). The ability of the conjugate to inhibit growth of tumor cells was assessed using a growth inhibition assay in which the effect of free paclitaxel was compared. The conjugate demonstrated low nanomolar IC50 values that are comparable or even superior to paclitaxel

B. PRODRUGS AIMED AT BRAIN DELIVERY



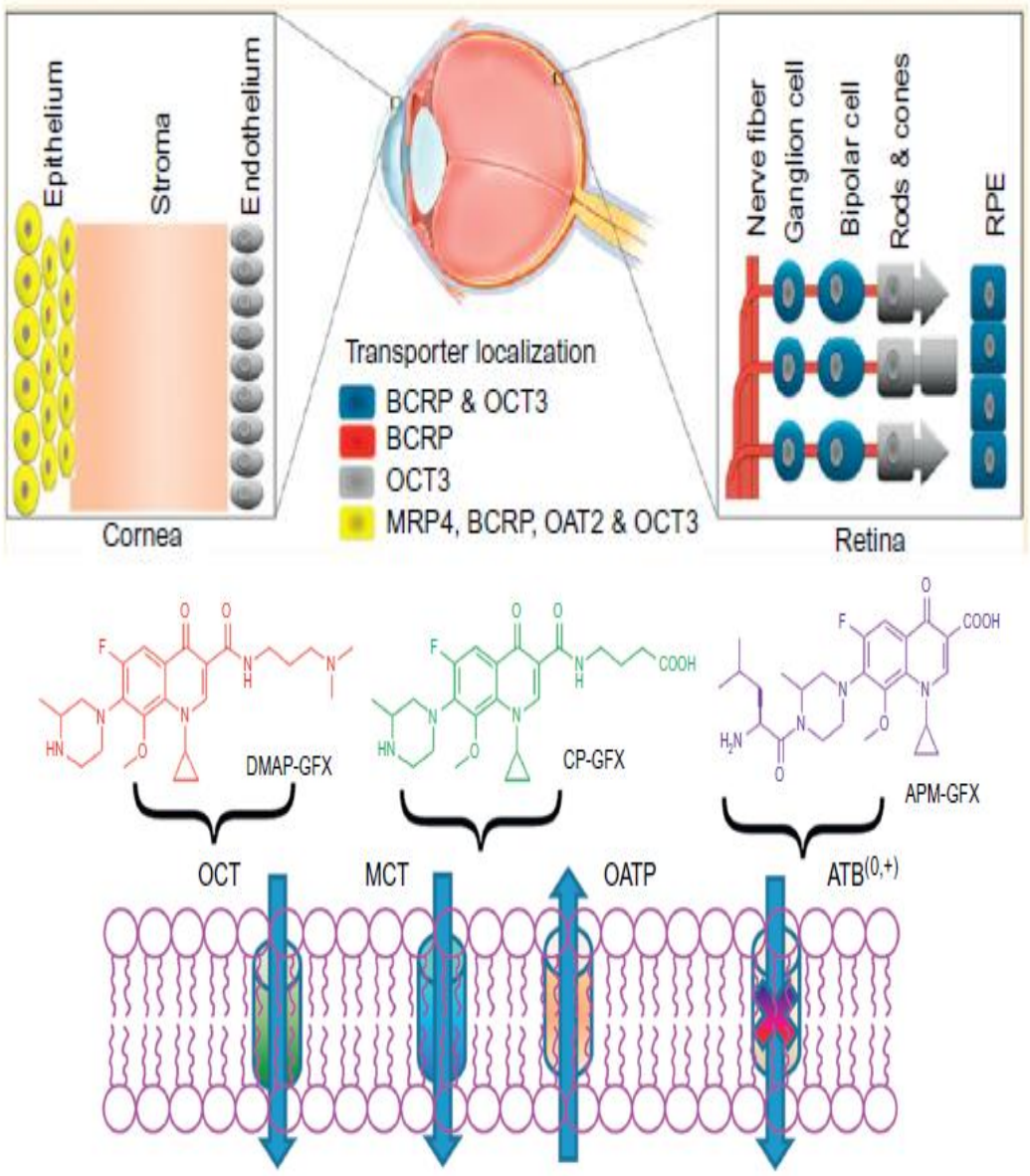
Two interesting prodrug strategies utilize transporter-mediated and receptor-mediated delivery. GLUT-1 is a transporter that mediates the uptake of glucose from the bloodstream to the CNS and is located in the membrane of brain capillary endothelial cells. L-Dopa, a dopamine precursor that (unlike dopamine) crosses the BBB, is the standard treatment for Parkinson's disease. Unfortunately, the premature conversion of L-dopa to dopamine in the peripheral tissues is the cause of unwanted side effects. Thus a dopamine prodrug using a succinyl linker to D-glucose was prepared (Figure a).

uptake of the prodrug was greatly reduced in the presence of glucose, whereas dopamine had no effect. Transporter-mediated uptake may explain why glu-dopamine is able to be absorbed into the CNS from the bloodstream while dopamine is not. Gynther et al demonstrated that the ketoprofen-D-glucose conjugate (Figure b) possesses

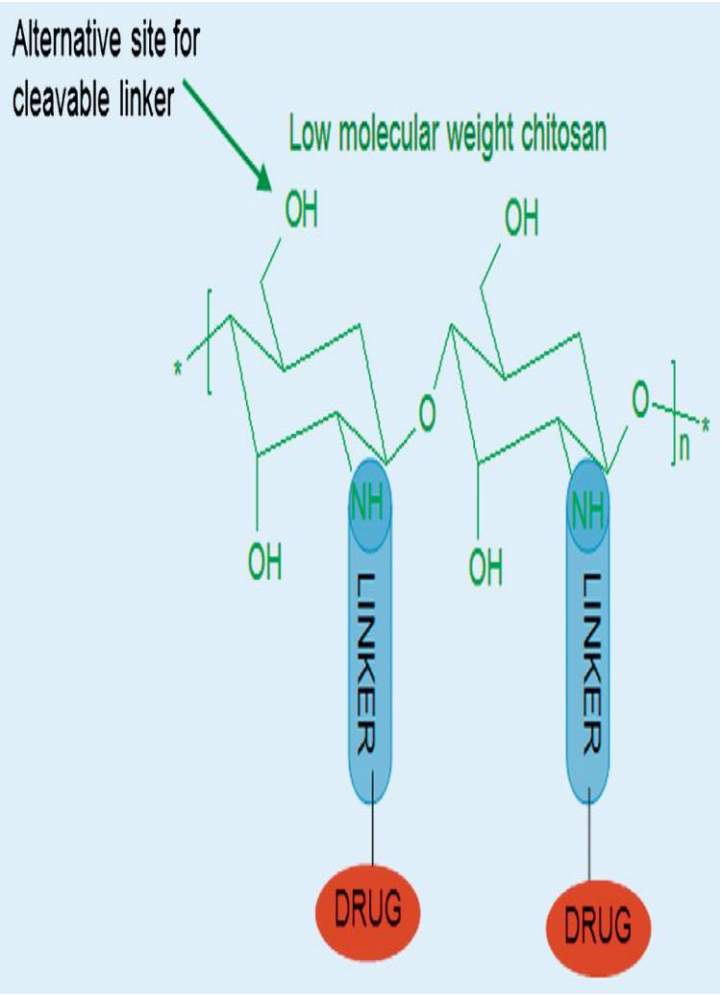
good chemical stability in aqueous solution at pH 7.4, its uptake by the brain is mediated by GLUT-1, and the ester prodrug undergoes bioconversion to ketoprofen and glucose in the brain tissue.

C. OCULAR DIRECTED PRODRUGS

Access to the posterior segments of the eye requires site-specific drug delivery systems .Topical delivery does not provide enough drug to this region, and even systemically administered drugs have limited access to the retina and vitreous due to the blood-retinal barrier (BRB). The BRB is the major barrier to drug delivery to the posterior chamber of the eye. The BRB is composed of the RPE (outer BRB) and endothelial cells of the retinal blood vessels (inner BRB). Transporters expressed in the corneal epithelium and RPE play vital roles in mediating the transport of drugs, xenobiotics, and nutrients into and out of the anterior and posterior regions of the eye (Figure)



D. KIDNEY DIRECTED PRODRUGS



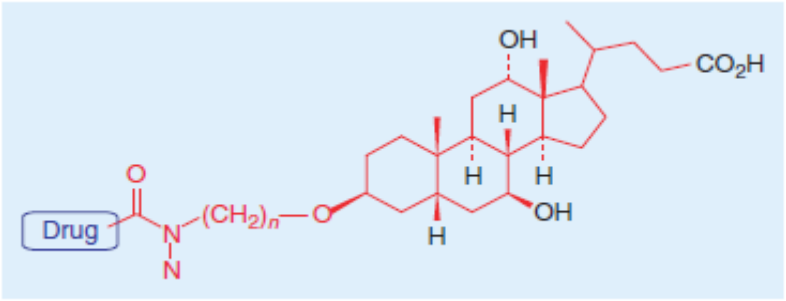
Kidney-targeted delivery is important when trying to reduce extra-renal toxicity of the drug and to improve its therapeutic efficiency for diseases of the kidney.

the use of low-molecular-weight protein (LMWP) appears most promising. The LMWP approach is based on drug attachment to a protein (,30kD) that is freely filtered through the glomerulus and accumulates specifically in the kidney, in particular in the proximal tubular cells, through a luminal reabsorption mechanism and is stable in circulation but is digested in the lysosomes of the proximal tubular cell to release the drug .Among the different types of LMWPs, lysozyme, cytochrome-c, aprotinin, and chitosan have been shown to be potential renal-specific drug carriers. Chitosan has been extensively used in the pharmaceutical field because it is biocompatible, biodegradable, and nontoxic. LMW chitosan is especially useful because it can quickly and reversibly open the tight junctions between intestinal epithelial cells , a useful feature for a drug given by oral route of administration

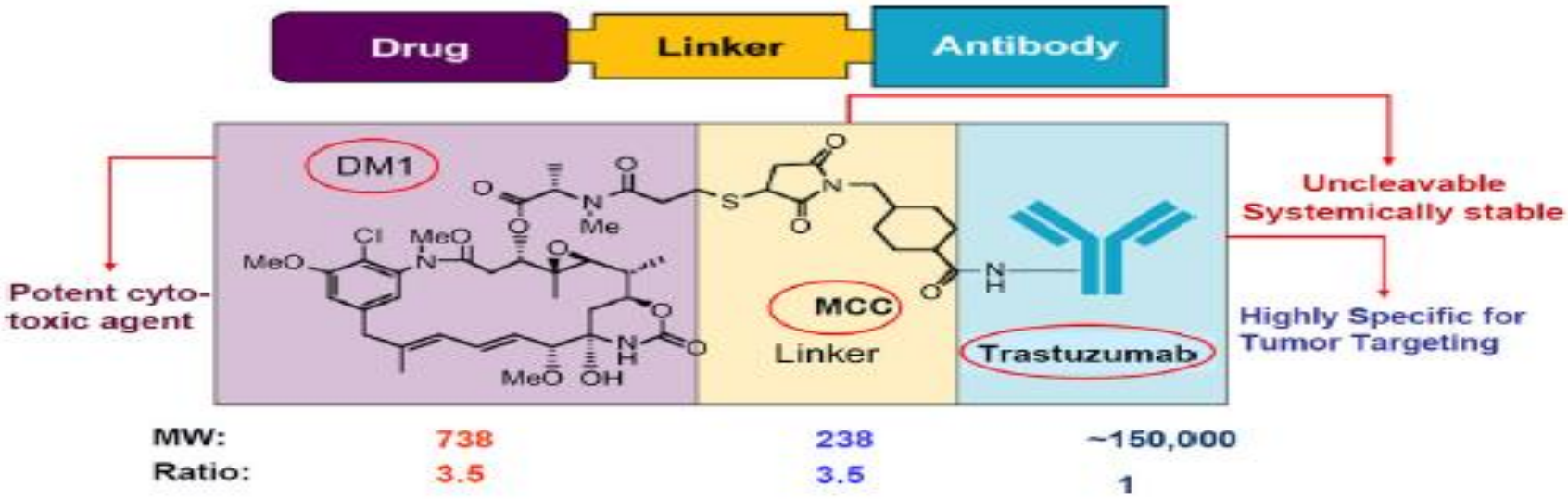
E. LIVER DIRECTED PRODRUGS

Bile acids for liver-specific targeting

- Coupling of drugs to modified bile acids was proposed for liver specific targeting .The rationale is based on the recognition of bile acid-linked drugs by the endogenous bile acid transport system. Chlorambucil (an alkylating cytostatic agent), HR-780 (an inhibitor of hydroxymethylglutaryl-CoA reductase), and an oxaproline peptide (an inhibitor of prolyl-4-hydroxylase) were chosen for conjugation to bile acids (Figure)



Trastuzumab-DM1 (T-DM1) combines the approved anti-HER2 mAB (Herceptin) with the microtubule destabilizing agent emtansine (DM1) via a succinimidyl 4-[N maleimidomethyl]cyclohexane-1-carboxylate (SMCC) linker attached to the ε-amino group of the lysine residue of the antibody (Figure). The impact of the linker on the efficacy and pharmacokinetics of an ADC is based on the empirical assessment of different linkers with the individual drug molecule and antibody in the context of the disease . In this case, a thioether linkage provided the widest margin between the minimum efficacious dose and the best safety profile in an in vivo animal model.



T-DM1 antibody-directed conjugate .Copyright 2010 Genentech/Roche. DM15emtansine5Derivative of Maytansine, a microtubule destabilizing agent. MCC5[maleimidomethyl]cyclohexane-1-carboxylate, a nonreducible thioether linkage.Trastuzumab5Herceptin.

2. Site-Specific Drug Release

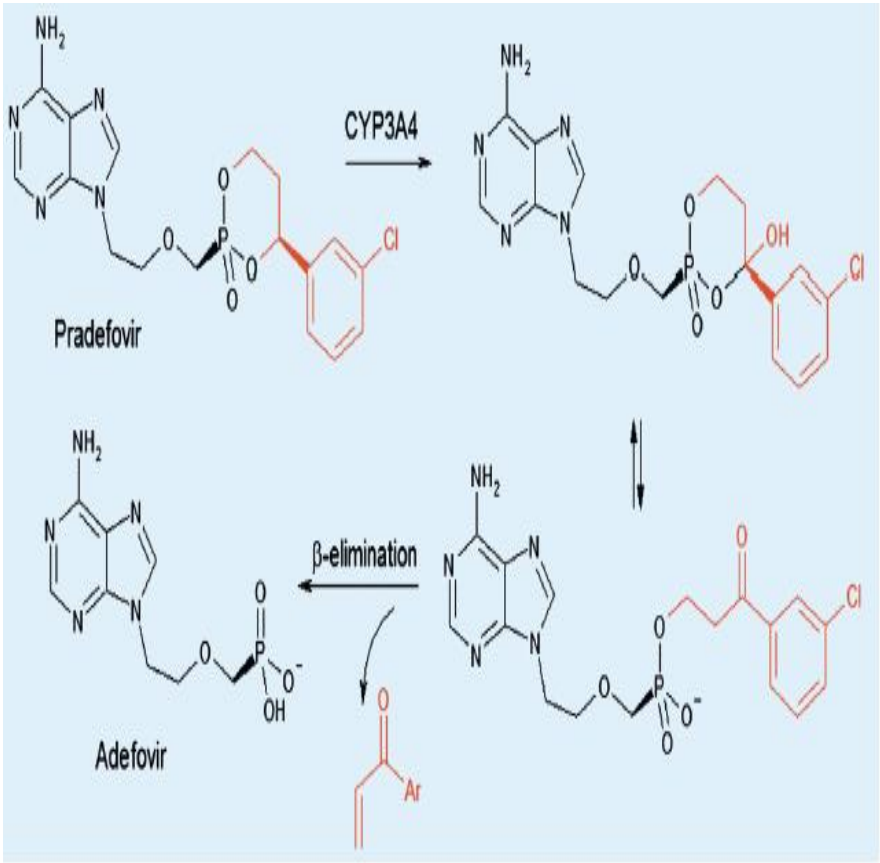
Targeting drugs to specific organs, tissues, or cells is an attractive strategy for improving drug efficacy and reducing side effects. A complementary approach is to use prodrugs, which distribute widely but cleave intracellularly to the active drug by a tissue-specific enzyme. The whole strategy of site-specific release of a given drug lies in the identification of an enzyme present in high concentrations in the target tissue and nearly absent elsewhere. In addition, the enzyme is required to efficiently cleave a broad range of structurally diverse substrates in order for this approach to be generally applicable. An appropriate prodrug can then be designed using the selective cleavage possibility offered by the enzyme. The examples below demonstrate how this approach has been used to target the liver, kidney, and tumor cells.

A related concept that should be mentioned is antibody-directed enzyme-prodrug therapy (ADEPT) .This approach utilizes an antibody or antibody fragment directed at a tumor-associated antigen to carry an enzyme that has no human homologue to cancer sites. After clearance of the enzyme in the blood, a nontoxic prodrug that is a substrate for the enzyme is given, and a potent cytotoxic agent is released within the tumor site by the catalytic action of the enzyme.

A. RELEASE IN THE LIVER

Attempts to target the liver using liver-specific receptors and transporters have not been as successful as utilizing liver-specific enzymes such as the cytochrome P450 (CYP) enzymes. The liver is the major site for CYP mediated reactions, although CYP enzymes are also expressed to a lesser extent in adrenal gland, small intestine, brain, and kidney .Attention must also be given for potential interpatient variability in exposure of the drug due to differences in expression levels of the enzyme among patients and also for drugdrug interactions that may occur through inhibition or induction of CYPs, especially CYP3A4. These aspects that could affect prodrug conversion must be taken into account when developing prodrugs activated by CYPs

What is the type of strategy used ??



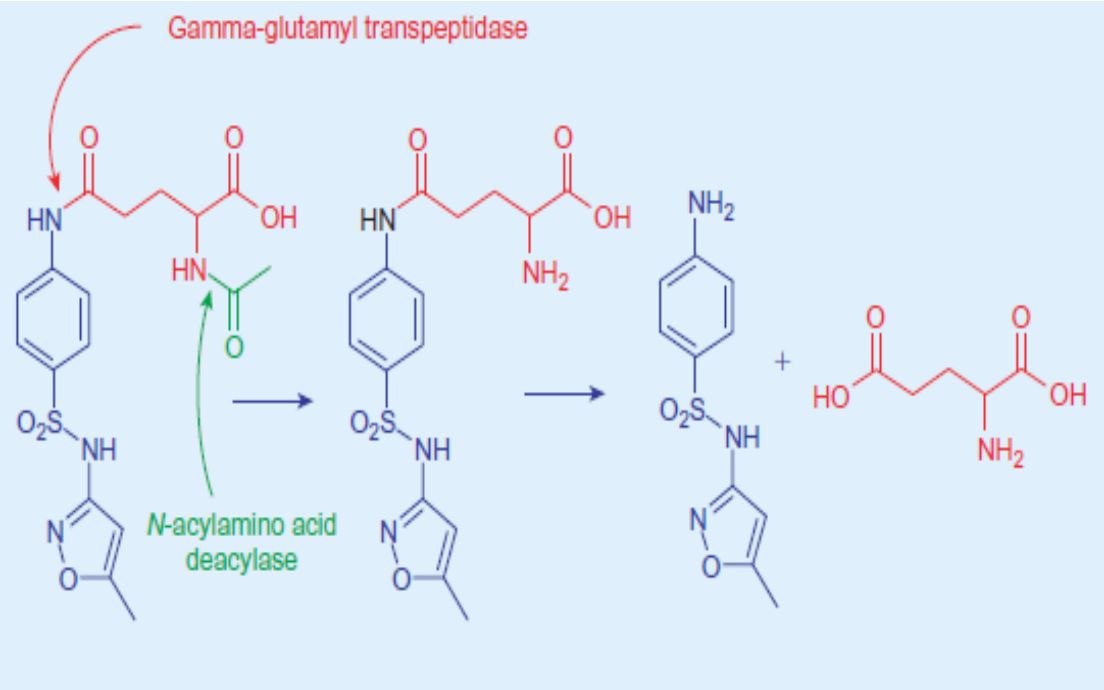
HepDirect prodrug Pradefovir

Pradefovir is the most advanced prodrug utilizing this approach to deliver adefovir to the liver for chronic hepatitis B

B. RELEASE IN THE KIDNEY

It is possible to obtain a kidney-selective accumulation of sulfamethoxazole by administering the drug in the form of N-acetyl-γ-glutamate .The regeneration of the free sulfamide requires the initial deacylation of the glutamic moiety by an N-acylamino acid deacylase, which is also present in the kidneys in high concentrations (Figure).The γ-glutamyl strategy for confining drug action to the kidney and the urinary tract requires that the prodrug under consideration function as a substrate for γ-glutamyl transpeptidase and, eventually, for N-acylamino acid deacylase.

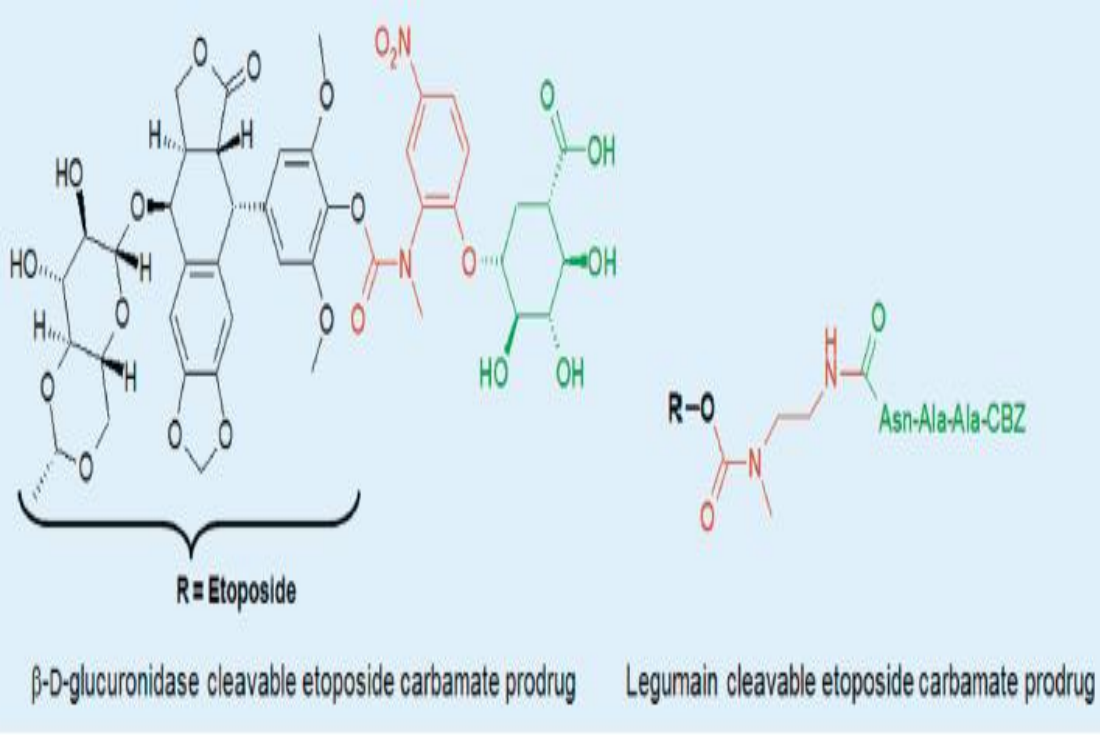
Kidney-selective release of sulfamethoxazole



C. RELEASE IN TUMOR SITES

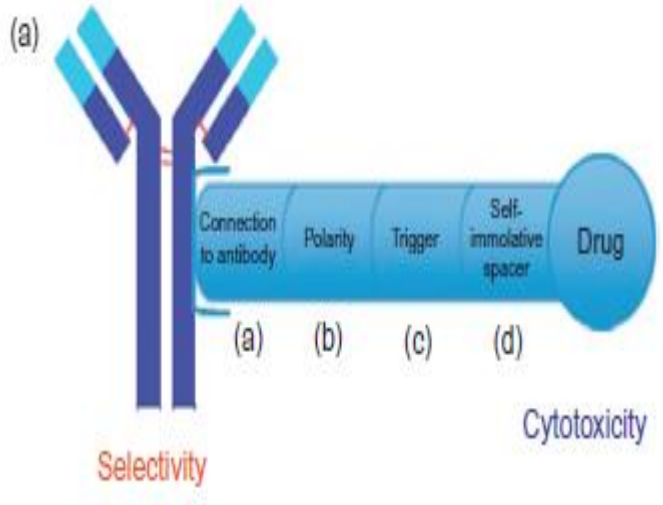
Enzyme-prodrug approaches to cancer therapy have the potential to achieve tumor-selective drug delivery resulting in less toxic side effects, more effective antitumor activity, and perhaps the delay of the development of drug resistance. Three examples that highlight this concept are the use of uridine phosphorylase, legumain, and β-D-glucuronidase cleavable prodrugs.

In various tumor tissues, the activity of the enzyme uridine phosphorylase is markedly higher than in the surrounding normal tissues. This observation prompted the synthesis of 5-fluorouracil prodrugs. Among them, 50-deoxy-5-fluorouracil shows high antitumor activity and less host toxicity compared to fluorouracil. This favorable therapeutic index is attributed to a preferential bioactivation by uridine phosphorylase in tumor cells.



3. Antibody-Drug Conjugates as Macromolecular Prodrugs

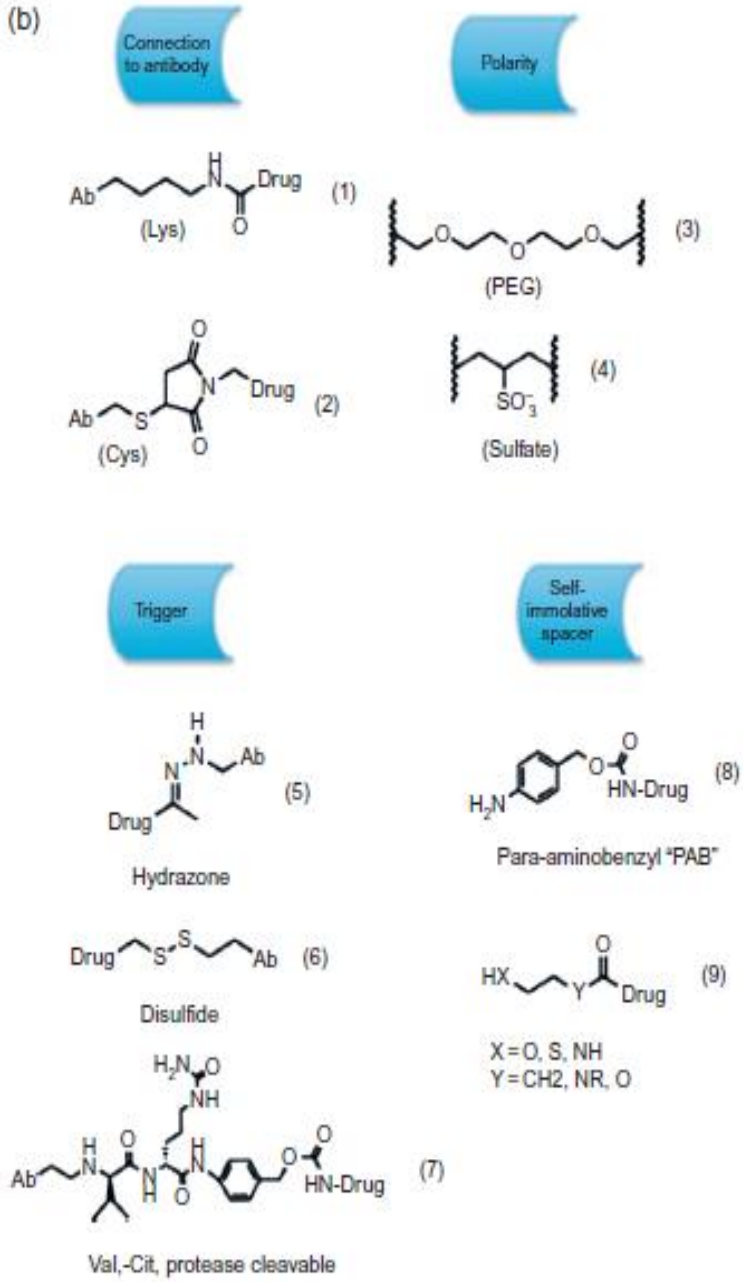
Antibody-drug conjugates can be considered both a site-directed and site-specifically released macromolecular prodrug. An antibodydrug conjugate (ADC) is essentially a three component system comprising a potent drug substance linked via a biodegradable linker to an antibody mAb (Figure a).



(a) Architecture of an ADC (b) The components of the linker portion of the ADC:

(a) the connection of the linker to the antibody; (b) the polarity of the linker; (c) the trigger that initiates the cleavage of the drug; and (d) the self-immolative portion that liberates

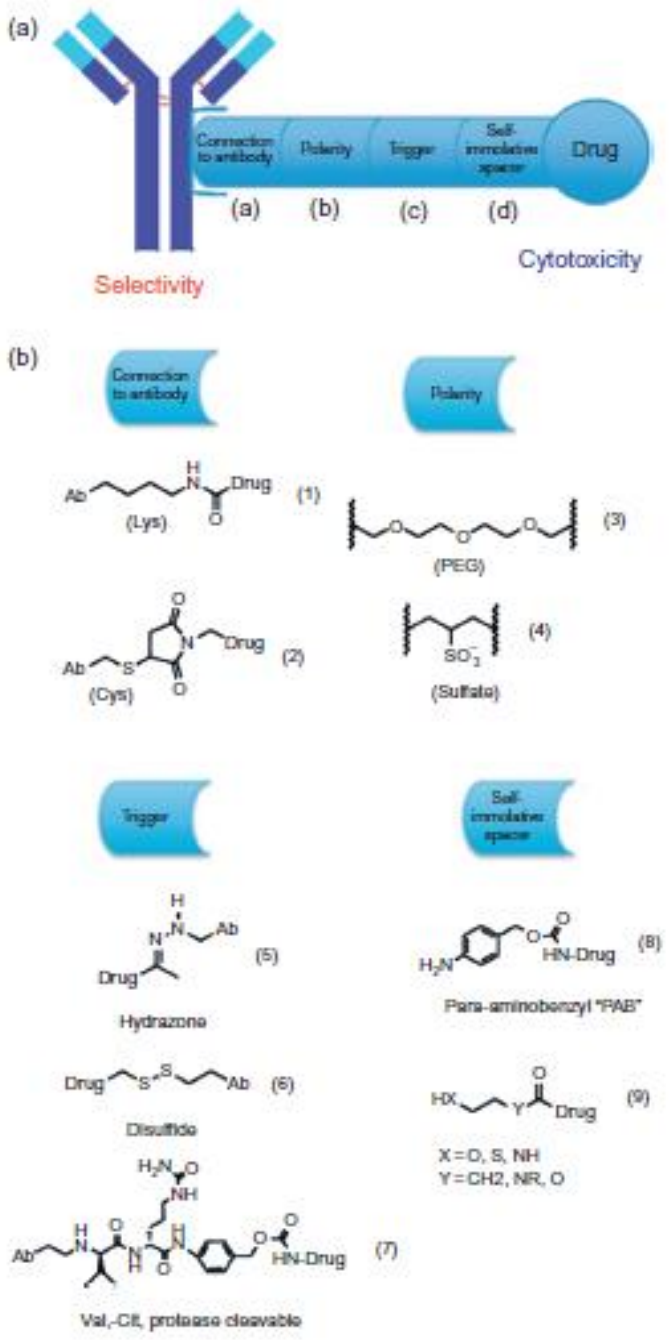
the drug



(1) The connection of the drug to the antibody is usually via a lysine or cysteine amino acid residue on the antibody. Antibodies have been engineered with two site-specific (light or heavy chain) cysteine residues to allow two drugs per antibody when a payload connected to a maleimide is added to the thio-engineered antibody. The drug conjugate retains the interchain disulfide bonds, while only the engineered cysteine residue forms a bond to the drug molecule. This process provides a uniform and well-characterized ADC.

This is only one example of how this has been done to control drug loading. In most cases, the sites of attachment and the number of attachments vary to give an average drug-to-antibody ratio (DAR). (2) The polar component imparts hydrophilicity to the inherently hydrophobic ADC, thus preventing aggregation and/or precipitation.

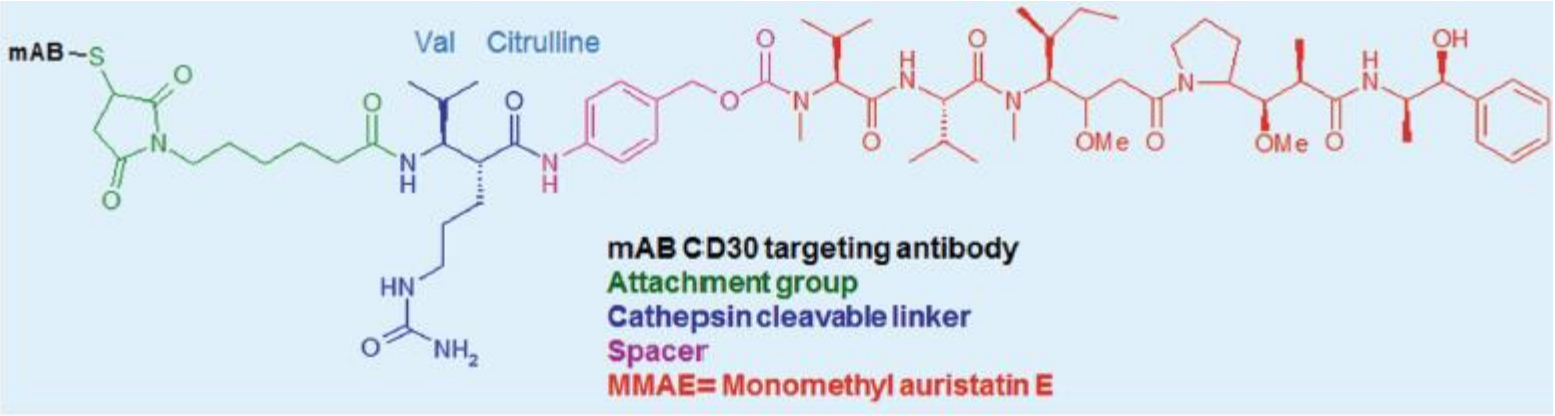
(3) The trigger is the part of the linker that initiates cleavage and release of the drug. The three trigger mechanisms that have been exploited in the ADCs that went into the clinic are hydrazone hydrolysis in the acidic lysosomal



More than twenty ADCs currently in clinical trials and recent approvals highlight the significant advances made in the field.

Seattle Genetic's SGN-35 is an antibody-directed prodrug therapeutic designed to target the CD30 receptor, a defining marker for Hodgkins lymphoma. The potent antimitotic agent monomethylauristatin E (MMAE) is attached via a self-immolative spacer to a novel cathepsin-cleavable linker (valine-citrulline), which in turn is attached to the sulfhydryl group of a cysteine residue on the antibody.

Maleimide is the attachment group used to add selectively to one of the eight available sulfhydryl groups on the antibody



Structure of brentuximab vedotin (Adcetris).

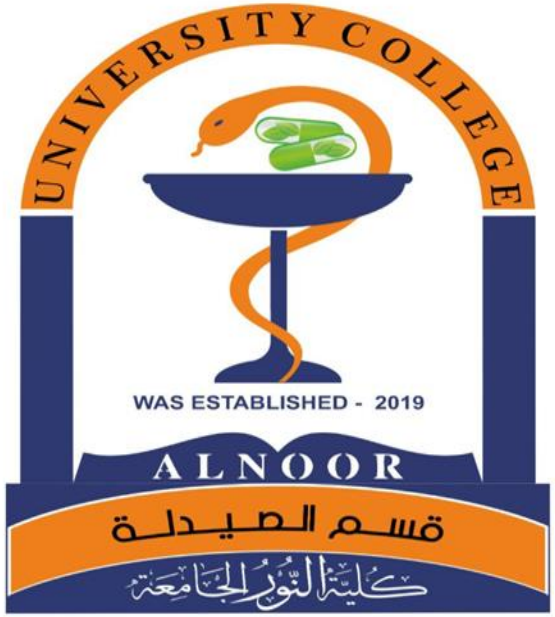
Organic Pharm. Chemistry IV

The Organic Chemistry of Drug Design and Drug Action

Lecture 6

19th November 2022

12:00- 2:00 PM



AlNoor University College

Pharmacy Department

1st Course/ 5TH Class

2022/2023

Be (+)


لَا تَحْزَنْ إِنَّ اللَّهَ مَعَنَا
"Do not grieve; indeed
Allah is with us."

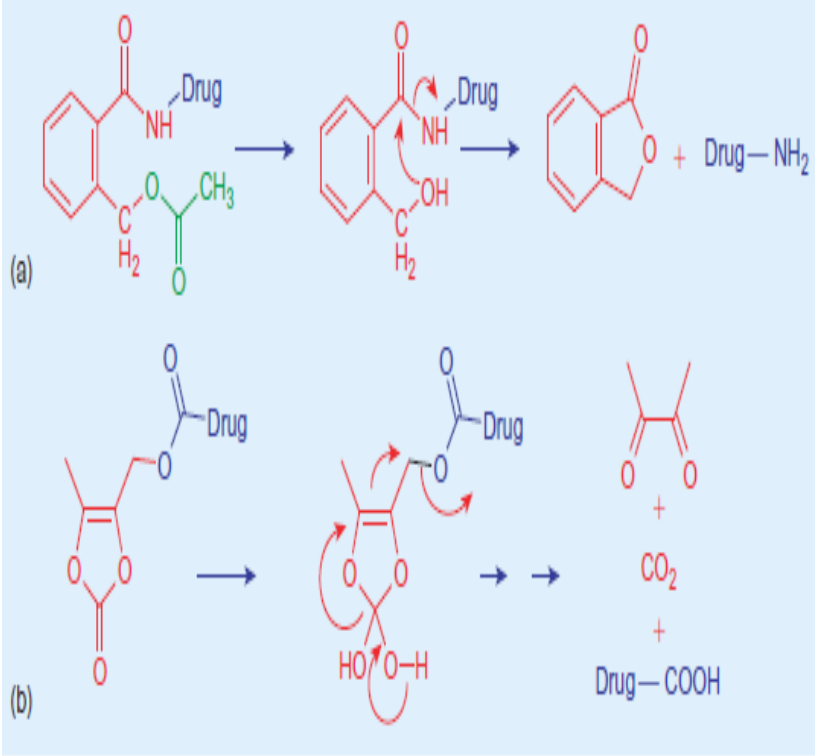

NEW
OLD HABITS

B. Use of Cascade Prodrugs

Most of the prodrugs described to this point are classical carrier-linked prodrugs. Classical carrier-linked prodrugs may sometimes be ineffective because the prodrug linkage is too stable (amides, nonactivated esters). In such cases, a β-assistance provided by an easily in vivo generated nucleophile can represent an interesting solution.

The release of the active molecule from the prodrug proceeds through a two-step trigger mechanism, for which the name "cascade latentiation" was coined by Cain in 1975 .

The concept, also called distal hydrolysis or the double prodrug concept , is illustrated by the use of 2-acyloxymethylbenzoic acids as amine-protective functions, providing amides with the lability of esters (Figure a), and by the use of substituted vinyl esters [5(2-oxo-1,3-dioxol-yl)methyl esters] as lipophilic cascade carriers for carboxylic acid-containing drugs such as ampicillin ,α-methyldopa , or various cephalosporins (Figure b) .



Cascade latentiation:

(a) 2-Acyloxymethylbenzoic acids provide amides the lability of esters;

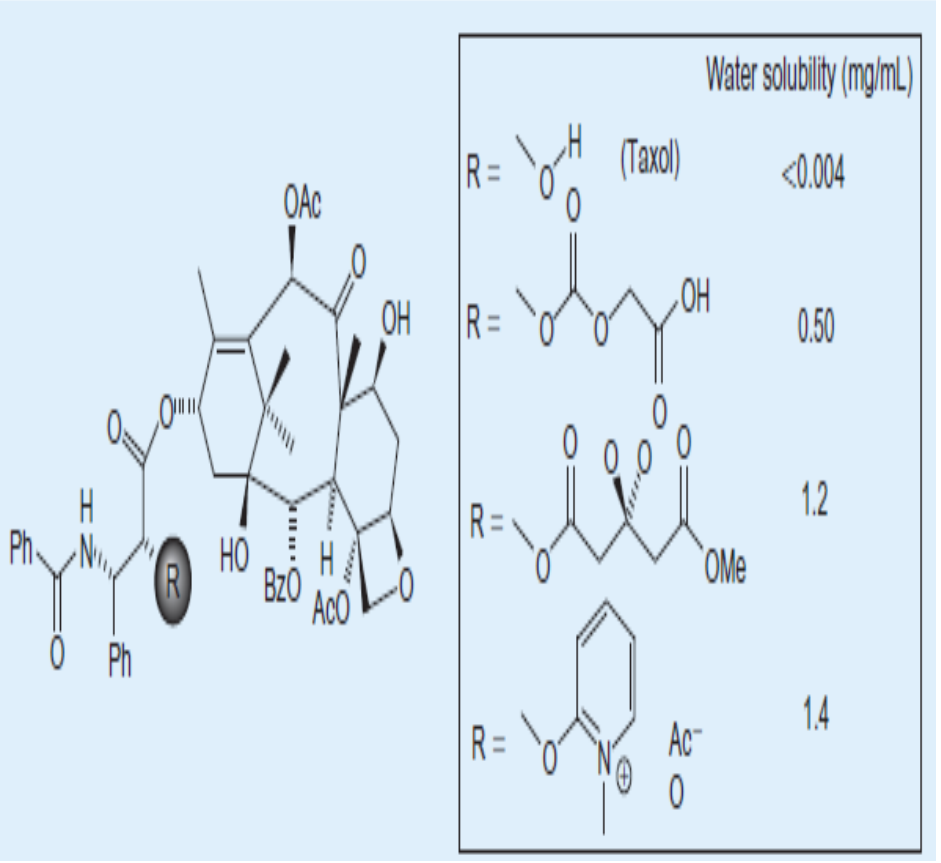
.(b) Substituted vinyl esters as lipophilic cascade carrier for carboxylic acid-containing drugs .

1. Water-Soluble Paclitaxel Prodrugs

Paclitaxel (sold under the name Taxol) is a potent microtubule-stabilizing agent that has been approved for cancer treatment. Despite paclitaxel's therapeutic promise, its aqueous insolubility (,0.004 mg/mL) hampers its

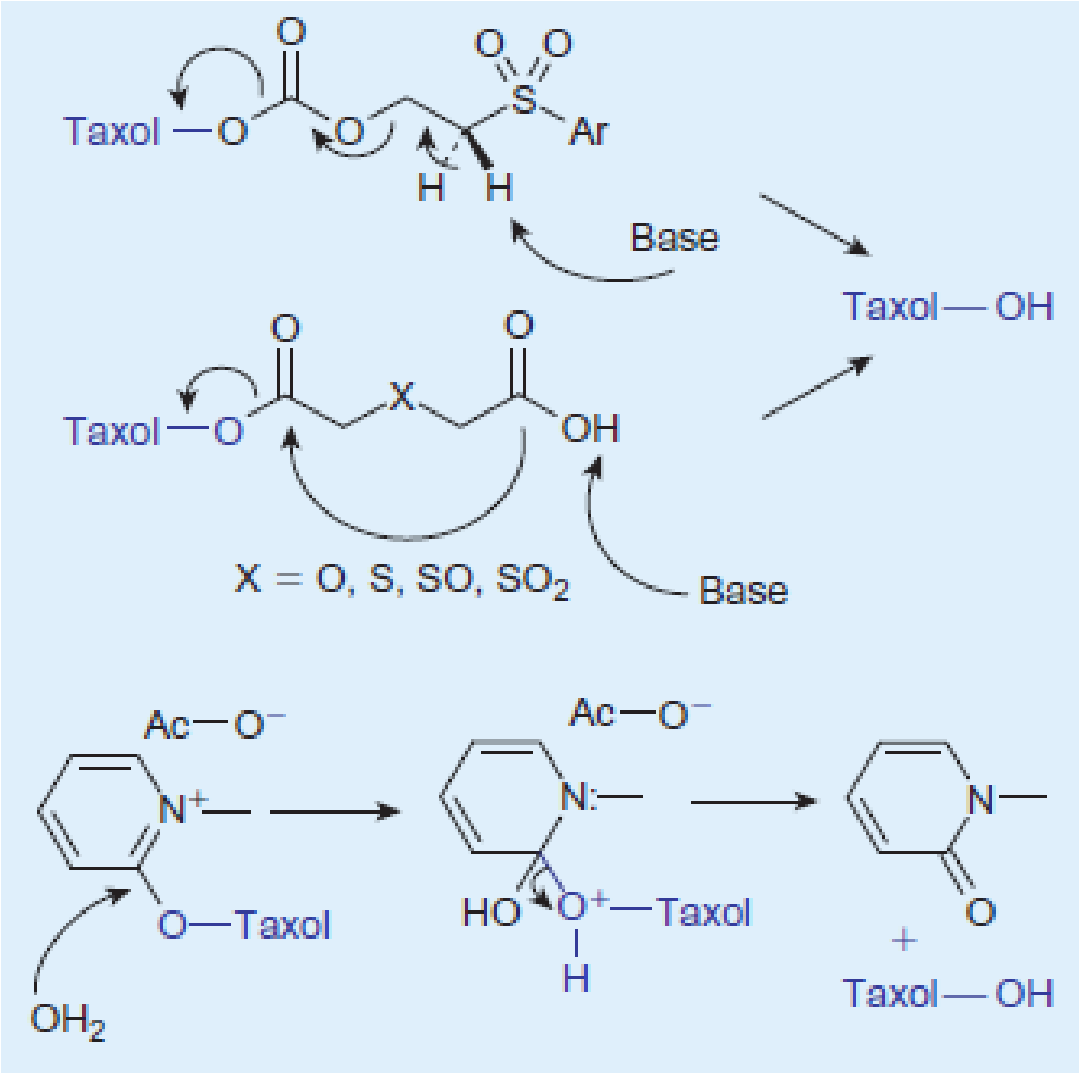
clinical application. Nicolaou et al] report the design, synthesis, and biological activity of prodrugs

designed to improve water solubility that can also be considered as cascade prodrugs (Figure).



Watersoluble prodrugs of paclitaxel

The mechanistic rationale for the design of the first two paclitaxel prodrugs lies in the spontaneous decomposition of the carbonate ester after the abstraction of one of the activated protons or of an acidic proton (Figure). The release of paclitaxel from the pyridinium prodrug (paclitaxel -2₀-methyl- pyridinium acetate; paclitaxel -2₀-MPA) is presumed to be the result of a nucleophilic attack by water or another nucleophile at the 2₀-position of the pyridinium moiety



- Paclitaxel release mechanisms from paclitaxel prodrugs

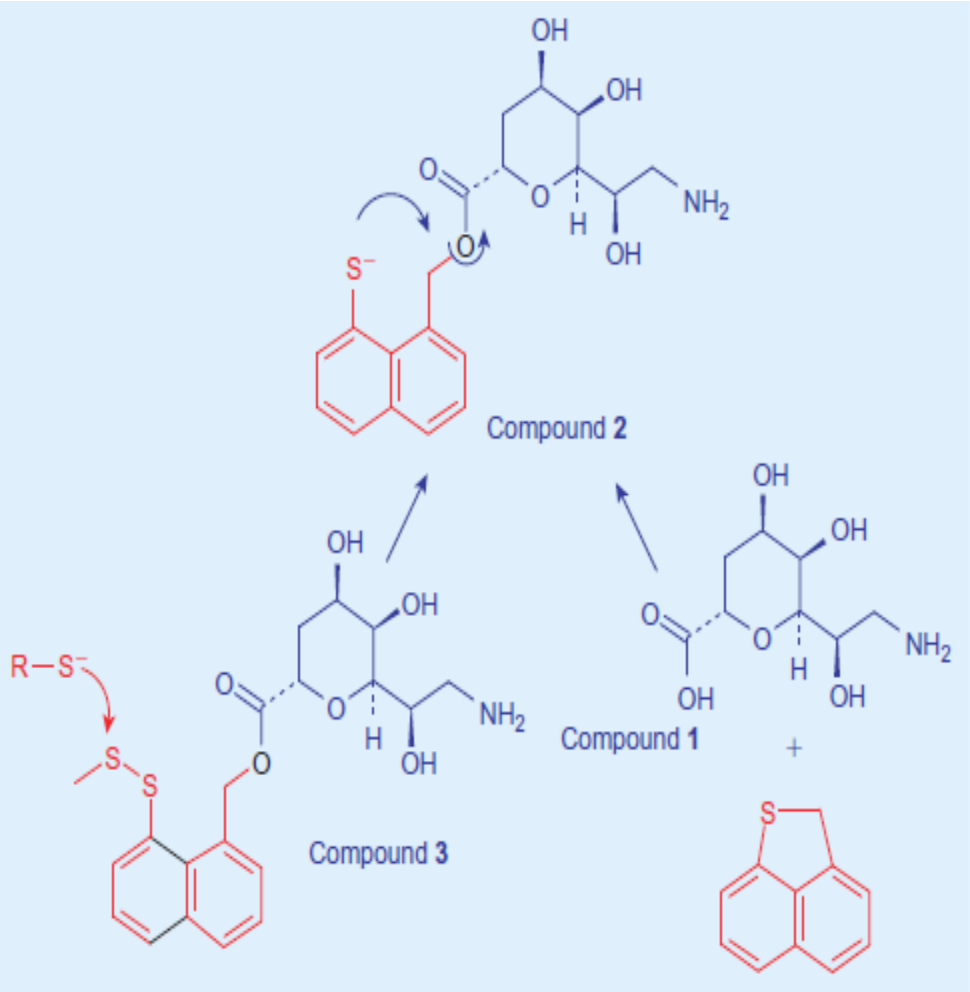
2. Bioactivation of an Antibacterial Prodrug

Although amino acid 1 is a potent inhibitor of CMPKDO synthase (Figure), a key enzyme in the

biosynthesis of the lipopolysaccharide of Gram-negative bacteria, it is unable to reach its cytoplasmic target and

is therefore inactive as an antibacterial agent. Simple lipophilic esters are not useful to enhance the delivery of amino acid 1 since they are not cleaved by the bacteria. On the other hand, double prodrug 3 has been found to solve the problem .

Upon entry into bacterial cells, the disulfide bond in compound 3 is reduced by sulfhydryl compounds present in the intracellular milieu, resulting in the formation of thiol 2. This is highly unstable, and the active amino acid 1 is formed by a rapid, intramolecular displacement.

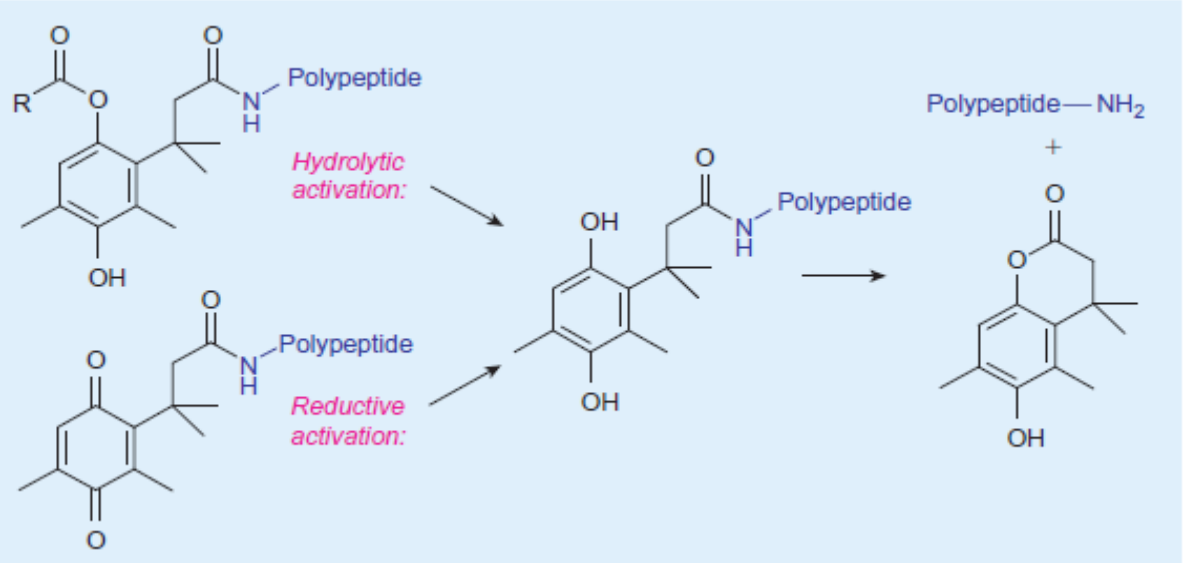


Bioactivation of the antibacterial prodrug of an impermeant inhibitor of 3- deoxy-D-manno-2-octulosonate cytidylyl- transferase

3. Double Prodrugs for Peptides

Amsberry and Borchardt , have applied Cain's cascade concept to prepare lipophilic polypeptide prodrugs. The amine functionality of the polypeptide is coupled to 20-acylated derivatives of 3-(20,50-dihydroxy-40, 60-dimethylphenyl)-3,3-dimethylpropionic acid (Figure). Under simulated physiological conditions, the parent amine is regenerated in a two-step process: enzymatic hydrolysis of the phenolic ester, followed by a nonenzymatic intramolecular cyclization, leading to the release of the free amine (polypeptide) and a lactone. The lactonization step is highly favored because of the steric pressure created by the three methyl groups (the "trimethyl lock" concept). An alternative to the hydrolytic first step involves a bioreductive generation of the intermediate phenolic amide

Proposed conversion of esterase-sensitive and redox-sensitive double prodrugs of peptides

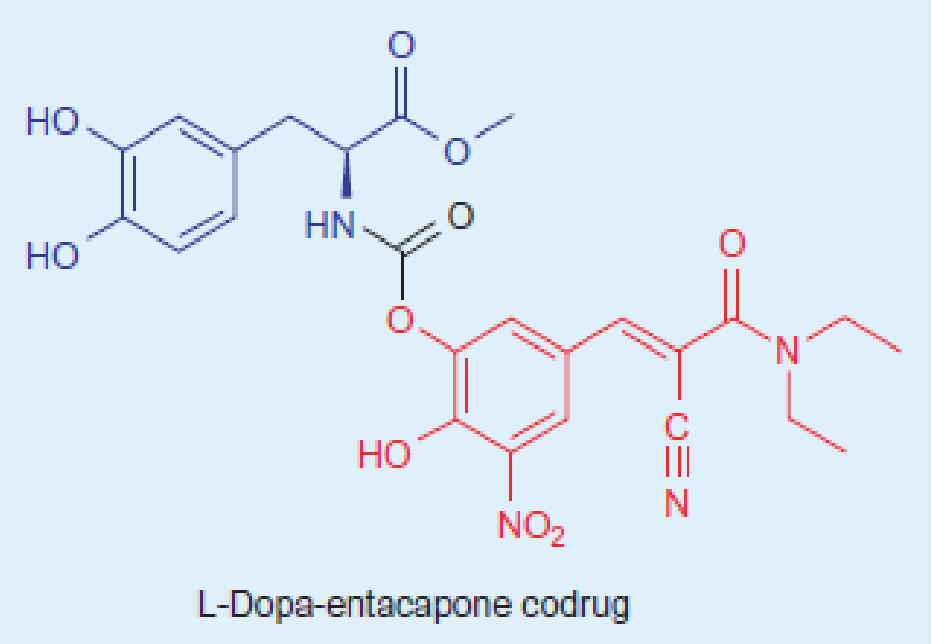


C. Codrugs or Mutual Prodrug

Codrugs are also named “mutual prodrugs.” Their design consists of the linking, in a unique molecule, of at least two different synergistic drugs that are released in vivo at the desired site of action.

An example is found in

the association of L-dopa to the catechol O-methyltransferase (COMT) inhibitor entacapone (Figure)

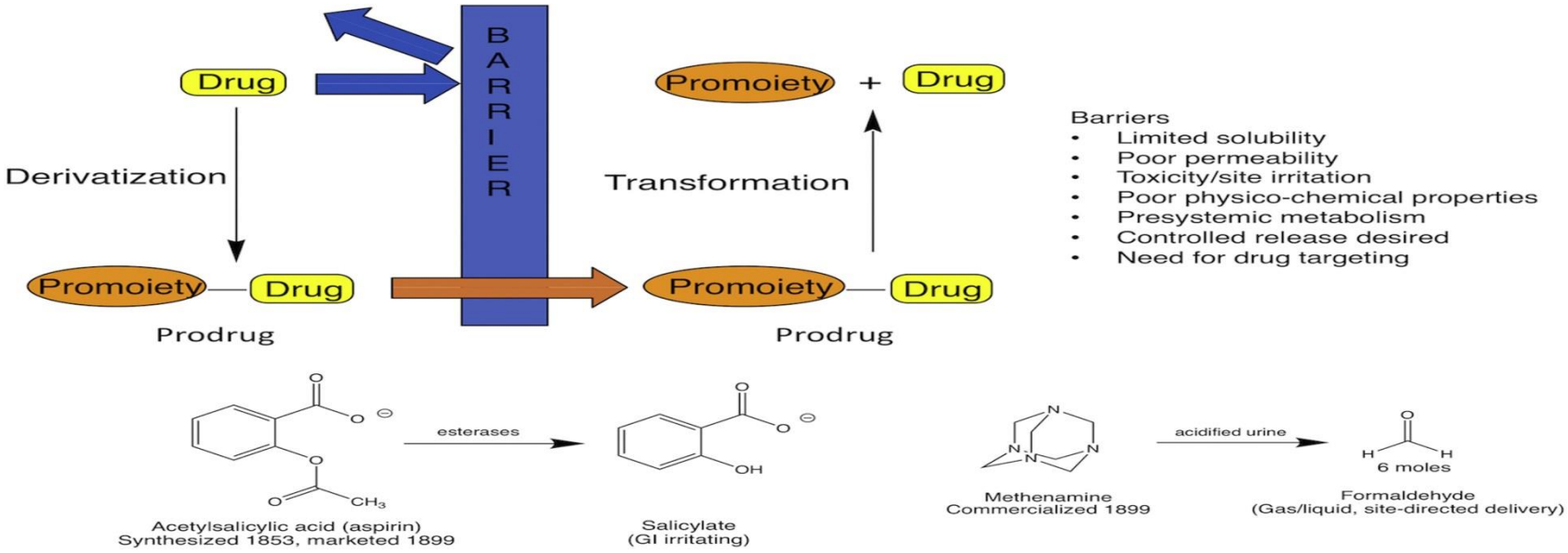


L-Dopa-entacapone codrug

The amino functional group of L-dopa is linked to the phenolic function of entacapone by means of a carbonyl group; thus a carbamate function links the two active agents.

V. BIOPRECURSOR PRODRUG EXAMPLES

The following examples illustrate the bioprecursor prodrug approach. The intentional use of bioprecursor design is relatively recent, and—in some cases—there are doubts about the prospective or retrospective character of the design. The first examples relate to oxidative bioactivations. These are followed by examples of reductive bioactivations and mixed-type bioactivations. In general, the active species results from a cascade of metabolic reactions involving oxidative as well as reductive processes, complicated by hydrolytic reactions or hydrationdehydration sequences.

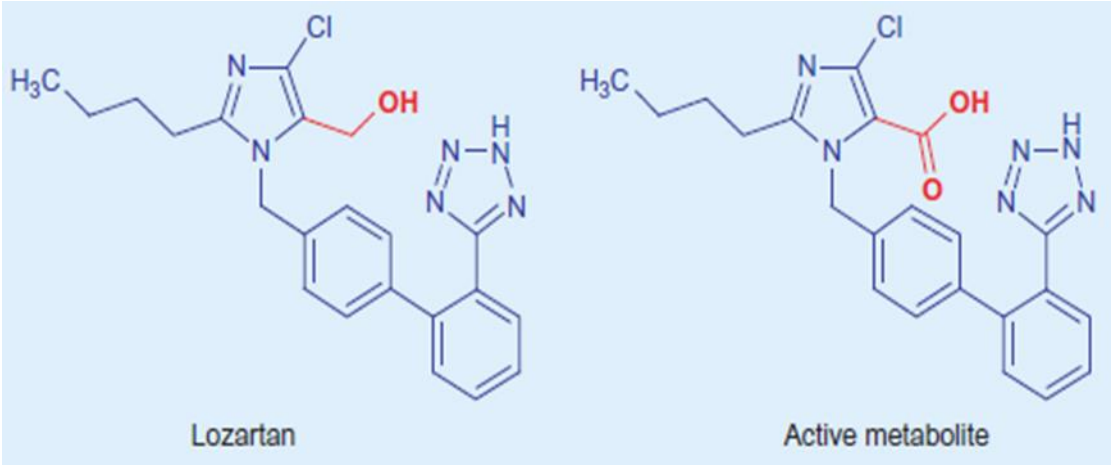


A. Oxidative Bioactivations

1. Oxidative Bioactivation of Losartan

A classic example of a bioprecursor prodrug is found in losartan, a nonpeptide angiotensin II receptor antagonist used as an antihypertensive medication .It can also be considered a bioprecursor prodrug insofar as, in vivo, the primary alcohol is oxidized to a carboxylic acid (Figure), which represents the actual active principle

Oxidative bioactivation of losartan

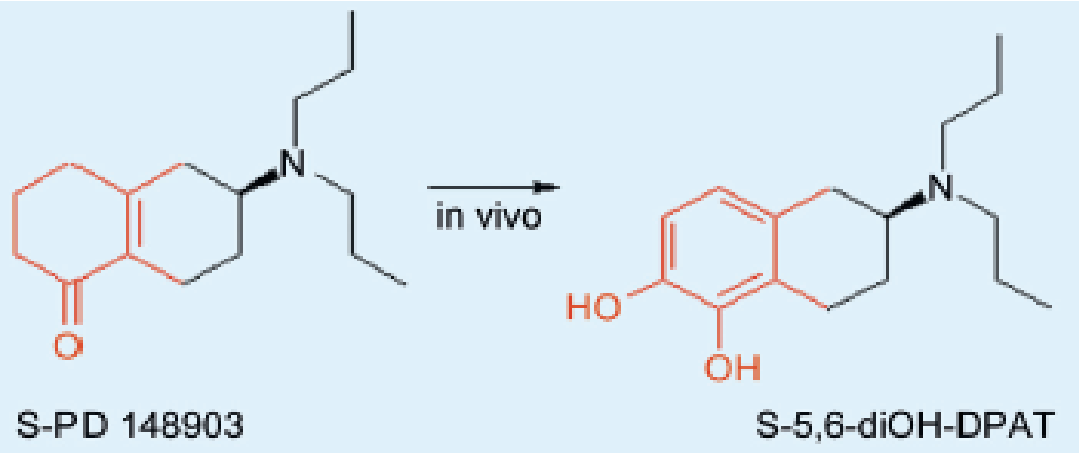


2. Conjugated Cyclohexeneones as Bioprecursors of Catecholamines

Venhuis et al observed a particularly original oxidative bioactivation mechanism by which an α, β-unsaturated cyclic ketone is converted to the corresponding catechol and delivered enantioselectively into the CNS (Figure).

This concept can be generalized and has the potential to lead to new anti-Parkinson's treatments [

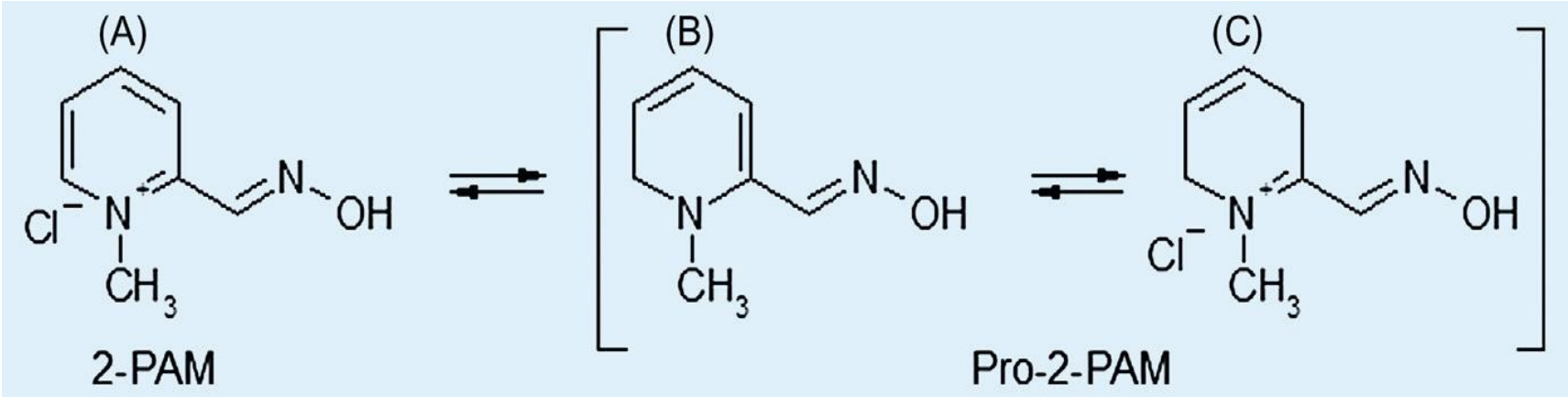
Oxidative bioactivations leading to catechols



3. Site-Specific Delivery of Acetylcholine-Esterase Reactivator 2-PAM to the Brain

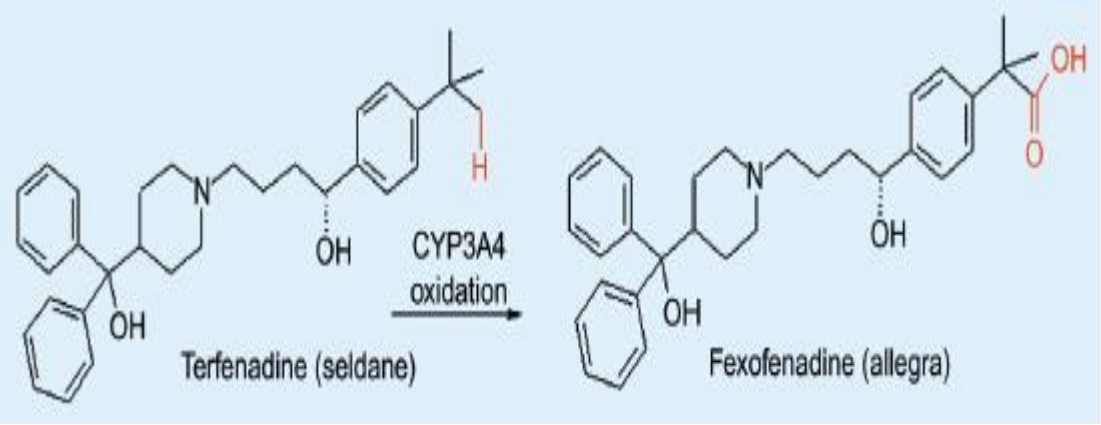
N-methylpyridinium-2-carbaldoxime (2-PAM5a; Figure) constitutes the most potent reactivator of acetylcholinesterase poisoned by organophosphorus acylation. However, due to its quaternary nitrogen, 2-PAM penetrates the biological membranes poorly and does not appreciably cross the BBB. For this compound, it was designed a novel dihydropyridinepyridinium salt type of redox delivery system. The active drug is administered as its 5,6-dihydropyridine derivative (Pro-2- PAM5b), which exists as a stable immonium salt .

The lipoidal b (pKa56.32) easily penetrates the BBB, where it is oxidized to the active a. A dramatic increase in the brain delivery of 2-PAM by the use of Pro-2-PAM is thus achieved, resulting in a re-activation of phosphorylated brain acetyl-cholinesterase in vivo



4. Oxidation of Terfenadine by CYP3A4

Terfenadine, a bioprecursor prodrug, is an antihistamine used for the treatment of allergic conditions. It is completely metabolized in the liver by the enzyme cytochrome P450 3A4 isoform to give the active metabolite fexofenadine (Figure). Terfenadine was taken off the market due to the risk of cardiac arrhythmia caused by QT prolongation. The active metabolite is not cardiotoxic, however, and is now sold under the brand name Allegra. This is an example where the active component is better administered than a bioprecursor prodrug due to its pharmacologically undesirable activity.

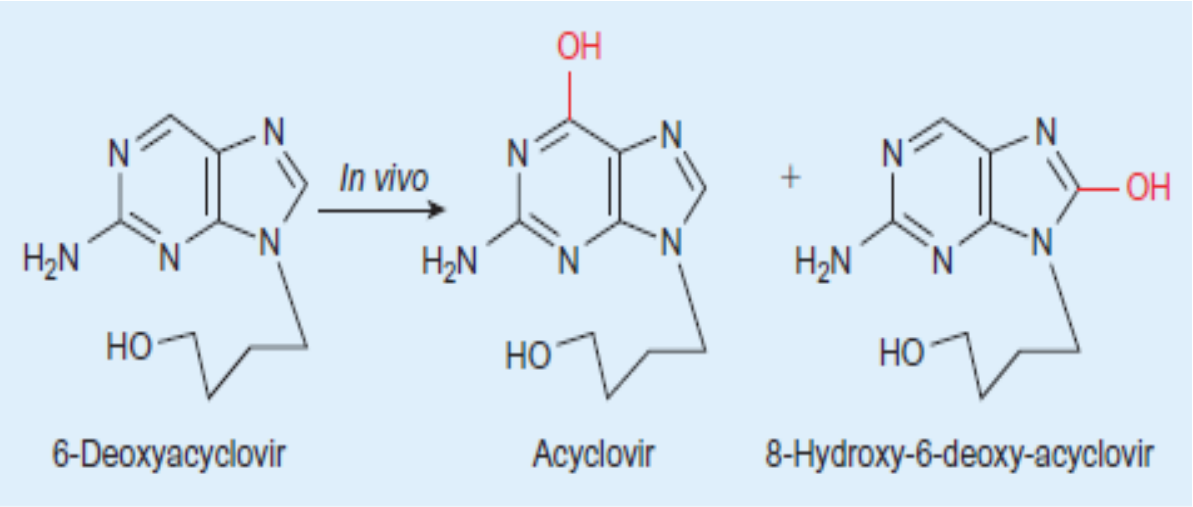


CYP3A4 oxidation of terfenadine

5. 6-Deoxyacyclovir as a Bioprecursor of Acyclovir

The antiherpetic agent acyclovir suffers from poor oral bioavailability; only 1020 percent of an oral dose is absorbed in humans. This is ascribed to low water solubility due to strong interaction forces in the crystal lattice.

The corresponding deoxo derivative (6-deoxyacyclovir) was shown by Krenitsky to be eighteen times more water soluble and to be rapidly oxidized in vivo by xanthine oxidase to the parent drug (Figure). Studies in rats and in human volunteers showed that orally administered 6-deoxyacyclovir has a 56 times greater bioavailability than acyclovir

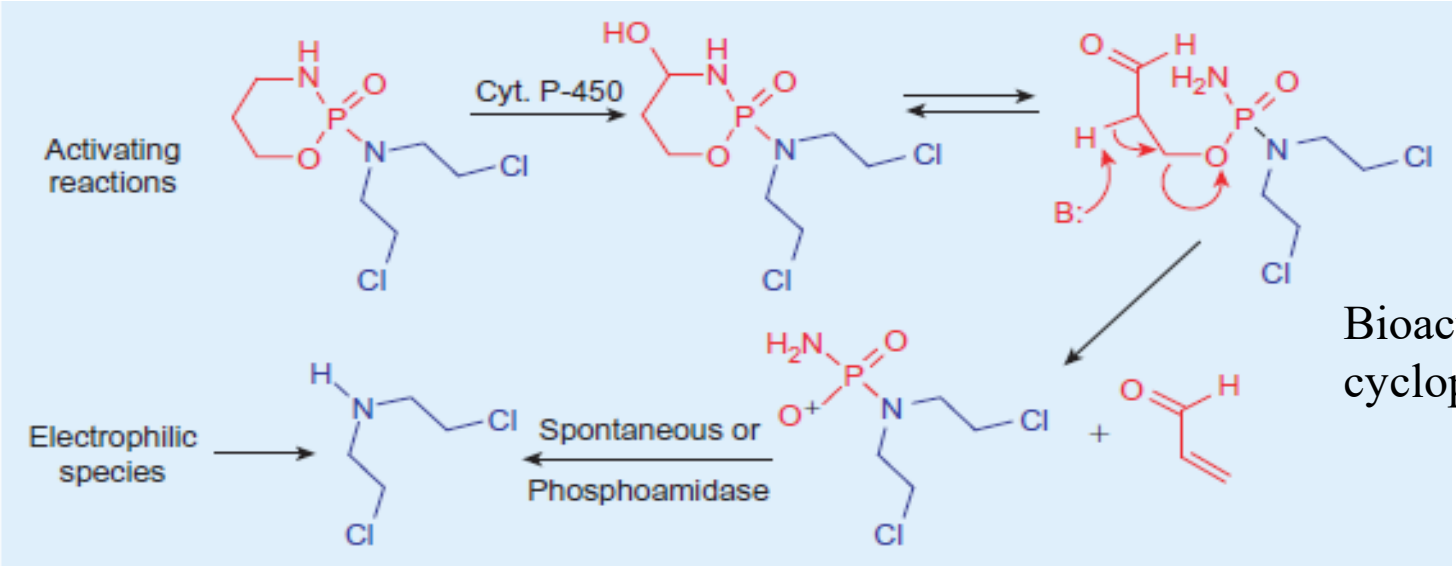


6-Deoxyacyclovir as a bioprecursor of acyclovir

6. Bioactivation
Cyclophosphamide

Cyclophosphamide is a cytotoxic (cytostatic), nonspecific cell cycle, antiproliferative agent, which is used in such diverse medical problems as neoplasia, tissue transplantation, and inflammatory diseases .Chemically, it is an inert bioprecursor for a potent nitrogen mustard alkylation agent (Figure).

Cyclophosphamide was synthesized by Arnold et al in the hope that it would be inert until activated by an enzyme present in the body, especially in a tumor. The activation mechanism is believed to require an initial oxidative dealkylation, followed by a spontaneous or phosphoramidase-catalyzed hydrolysis to the parent nitrogen mustard



Bioactivation of cyclophosphamide

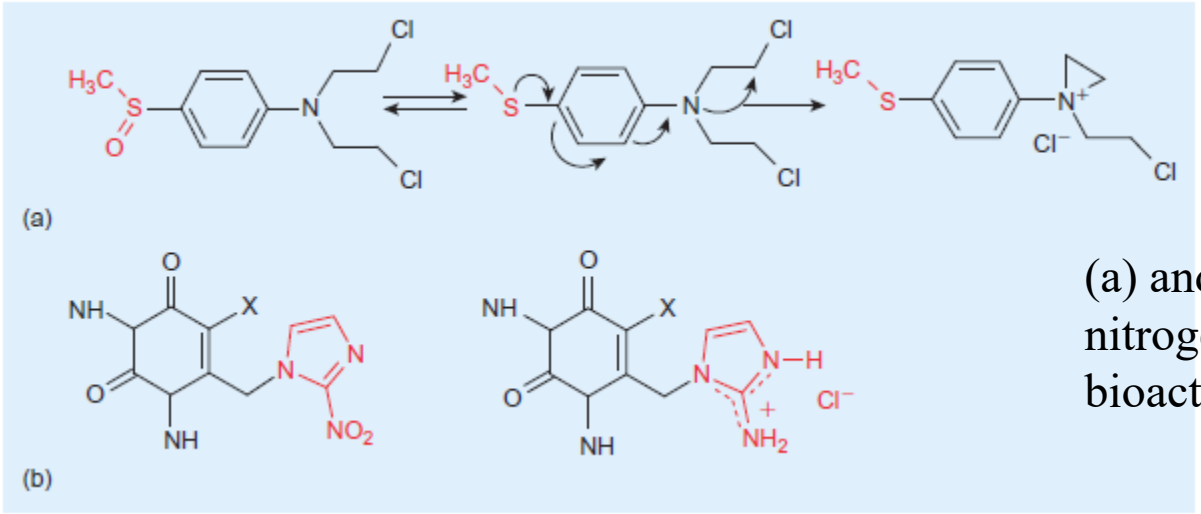
- B. Reductive Bioactivations

1. Reductive Bioactivation of Nitrogen Mustards

Many conventional anticancer drugs display relatively poor selectivity for neoplastic cells, and solid tumors are particularly resistant both to radiation and to chemotherapy. However, solid tumors possess a few unique and important microenvironmental properties such as localized hypoxia, nutrient deprivation, and low pH .

On the other hand, as shown above for sulindac, sulfoxides can undergo two major biotransformations: reversible reduction to the sulfide and irreversible oxidation to the sulfone. The oxidation to the sulfone is the dominant process under normal physiological conditions, but the reduction to the sulfide becomes significant under anaerobiotic conditions .Taking advantage of these findings, Kwon et al devised a hypoxia-selective

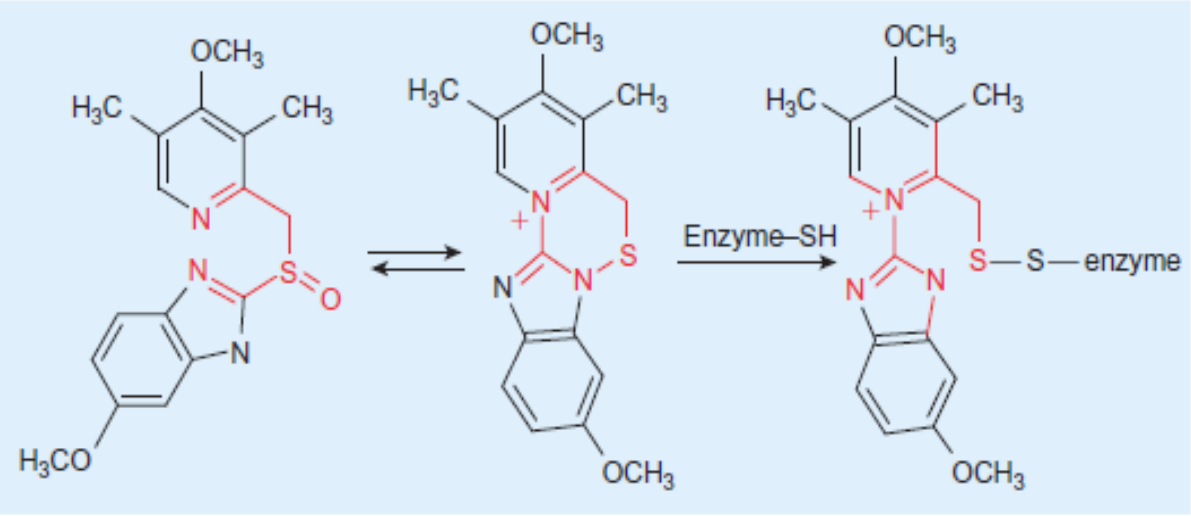
alkylating bioprecursor prodrug (Figure)



(a) and (b) Hypoxia-selective nitrogen mustard and bioactivated TP inhibitors

2. Reductive Bioactivation of Nitroimidazolylmethyluracils

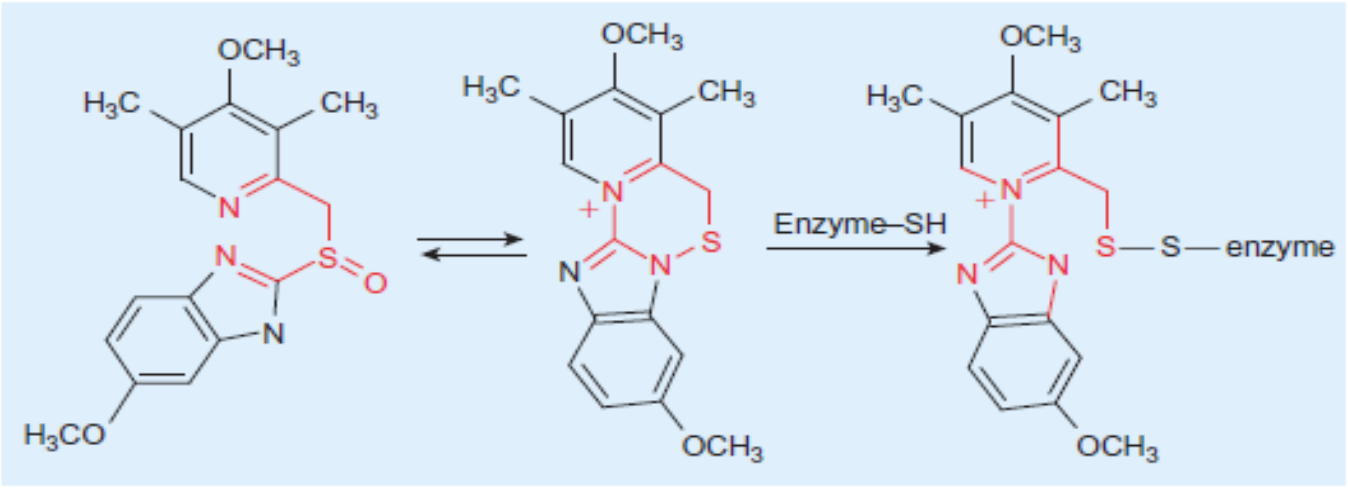
Thymidine phosphorylase (TP) is an angiogenic growth factor and a target for anticancer drug design (Figure 28.46b). Docking studies of the modeled TP predicted that the binding of aminoimidazolyl methyluracils was energically more favored than that of the corresponding nitro counterparts [159]. Effectively, the passage from the nitro to the amino analog was accompanied by a 1,000-fold increase in TP inhibition.



- Reductive bioactivation of omeprazole

3. Reductive Bioactivation of Omeprazole

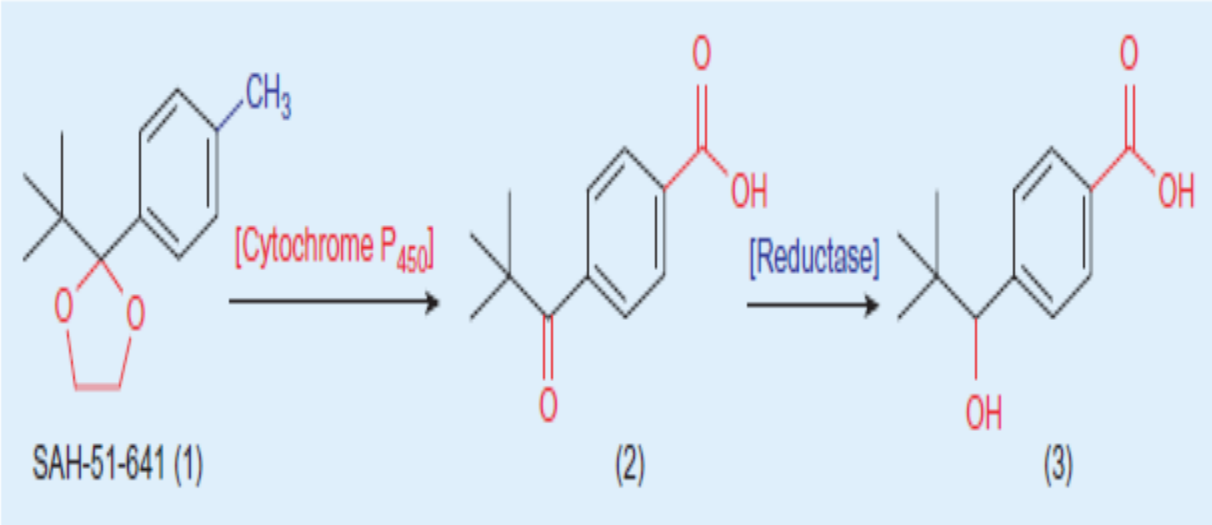
Omeprazole effectively inhibits gastric secretion by inhibiting the gastric H_1, K_1-ATPase . This enzyme is responsible for gastric acid production and is located in the secretory membranes of parietal cells. Omeprazole is an anti-ulcerative drug, used especially in the treatment of ZollingerEllison syndrome . In vivo, omeprazole is transformed into the active inhibitor, a cyclic sulfenamide (Figure)which forms disulfide bridges with the thiol groups of the enzyme and thus inactivates it . The high specificity in the action of omeprazole (pKa54.0) is due to its preferential concentration in the rather acidic parietal cells where it is activated. In neutral regions of the body, omeprazole is rather stable and is only partially converted to the active species.



Reductive bioactivation of omeprazole

C. Mixed Bioactivation Mechanisms

Certain bioactivation mechanisms involve several chemical sequences, some of them being oxidative and others being reductive. The prodrug SAH 51-641 (1) (Figure) illustrates an example of a mixed oxidativereductive mechanism. SAH 51-641 is a potent hypoglycemic agent, which acts by inhibiting hepatic gluconeogenesis via inhibition of fatty acid oxidation .This compound is metabolized by a sequential oxidation/reduction to the corresponding keto-acid (2) and the hydroxyl acid (3). Compound (3) is a substrate for medium-chain fatty acyl CoA ligase and represents the actual active agent



- Mixed oxidative/reductive bioactivation of dioxolanes.

Bioprecursors Versus Carrier Prodrugs

A comparative balancesheet established for the two prodrug approaches led us to the following conclusions

(Table below):

- • The bioavailability of carrier prodrugs is modulated by using a transient transport moiety. Such a linkage is not implied for bioprecursors, which result from a molecular modification of the active principle itself.
- • The lipophilicity is generally the subject of a profound alteration of the parent molecule in the case of carrier prodrugs, whereas it remains practically unchanged for bioprecursors.
- • The bioactivation process is exclusively hydrolytic for carrier prodrugs. It involves mostly redox systems for bioprecursors.
- • The catalysis leading to the active principle is hydrolytic (either through general catalysis or through extrahepatic enzymes) for carrier prodrugs. For bioprecursors, it seems largely restricted to Phase I metabolizing enzymes

Table: Bioprecursors Versus Carrier Prodrugs

	Prodrugs	
	Carrier prodrugs	Bioprecursors
Constitution	Active principle + carrier group	No carrier group
Lipophilicity	Strongly modified	Usually slightly modified
Bioactivation	Hydrolytic	Mostly oxidative or reductive
Catalysis	Chemical or enzymic	Only enzymic

DIFFICULTIES AND LIMITATIONS

The introduction of prodrugs in human therapy gave successful results in overcoming undesirable properties such as poor absorption, rapid biodegradation, or formulation problems. It can be expected that an increasing number of medicinal chemists will be tempted by this approach. However, they must keep in mind that prodrug design can also give rise to a large number of new difficulties, especially in the assessment of pharmacological, pharmacokinetic, toxicological, and clinical properties.

At the pharmacological level, for example, because bioactivation is necessary to create the active species, these compounds cannot be submitted to preliminary in vitro screening tests, namely, binding studies, neurotransmitter re-uptake, measurements of enzymatic inhibition, and activity on isolated organs.

- The measurements of pharmacokinetic parameters can lead to numerous misinterpretations. Thus, pivampicillin has a half-life of 103 min in a buffered aqueous solution at 37C, but it falls to less than one min after addition of only 1 percent of mouse or rat serum. In the presence of human serum (10 percent), however, the half-life is fifty min, whereas in whole human blood it is only five min. These results exemplify the care required to avoid incorrect conclusions. In addition, when a prodrug and the parent molecule are compared, one must take into account the differences in their respective time courses of action. The maximum activity can appear later for the
- prodrug than for the parent compound, and often the comparison of the AUC could constitute a better criterion.

DIFFICULTIES AND LIMITATIONS (Cont.)

At the toxicological level, even when prodrugs derive from well-known active principles, they have to be regarded as new entities. Undesirable side effects can appear that are directly related to the prodrug (e.g., allergy to bucloxic acid) or derived from the bioactivation process (e.g., formation of unwanted or unexpected metabolites), or which can be attributed to the temporary transport moiety (e.g., digestive intolerance to pivampicillin, antivitamin- PP activity of nicafenine). This latter case is particularly illustrative. An apparently innocent carrier group such as

N-hydroxyethylnicotinamide appeared as a promising candidate for improving the absorption of acidic antiinflammatory drugs or clofibric acid .However, during the clinical studies, side effects similar to vitamin PP deficiency appeared, suggesting that N-hydroxyethylnicotinamide could function as a nicotinamide antimetabolite.

The compounds then had to be withdrawn (H. Cousse, Pierre Fabre & Co, personal communication).

In a review of potential hazards of the prodrug approach, Gorrod [167] cites four toxicity mechanisms:

1. Formation of a toxic metabolite of the total prodrug that is not produced by the parent drug.

2. Consumption of a vital constituent (e.g., glutathione) during the prodrug activation process. As L-cysteine is

needed for the biosynthesis of glutathione, a supply with L-cysteine prodrugs can eventually confer some

protection of the hepatic cells [168].

3. Generation of a toxic derivative from a transport moiety supposed to be "inert."

4. Release of a pharmacokinetic modifier (causing enzymatic induction, displacing protein-bound molecules,

altering drug excretion, etc.).

Eventually, at the clinical stage, the predictive value of animal experiments is also questionable. Thus, for two prodrugs derived from α-methyldopa, the active doses in rats were identical, but they turned out to be very different during clinical investigations. One compound was just as potent as α-methyldopa, whereas the other one was 34 times more potent .

Finally, a patent application for a new prodrug should take into account all these aspects, and because the biological profile of each individual prodrug will differ depending on the linker/spacer and promoiety, it should in no way be regarded just as a complement to the main file. The question of patentability, however, is becoming more difficult to define as more prodrug examples appear in the literature, thus requiring inventors to meet the non-obvious criteria by demonstrating an unexpected result from the prodrug.

Recent prodrugs designed for targeted delivery will certainly fulfill the requirements for patentability: novelty, utility, and non-obviousness.

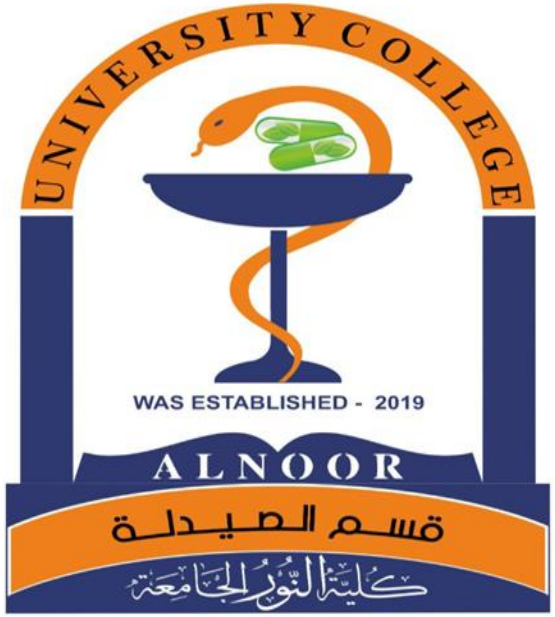
Organic Pharm. Chemistry IV

The Organic Chemistry of Drug Design and Drug Action

Lecture 6

3rd December 2022

12:00- 2:00 PM



AlNoor University College

Pharmacy Department

1st Course/ 5TH Class

2022/2023

Be (+)


لَا تَحْزَنْ إِنَّ اللَّهَ مَعَنَا
"Do not grieve; indeed
Allah is with us."


NEW
OLD HABITS

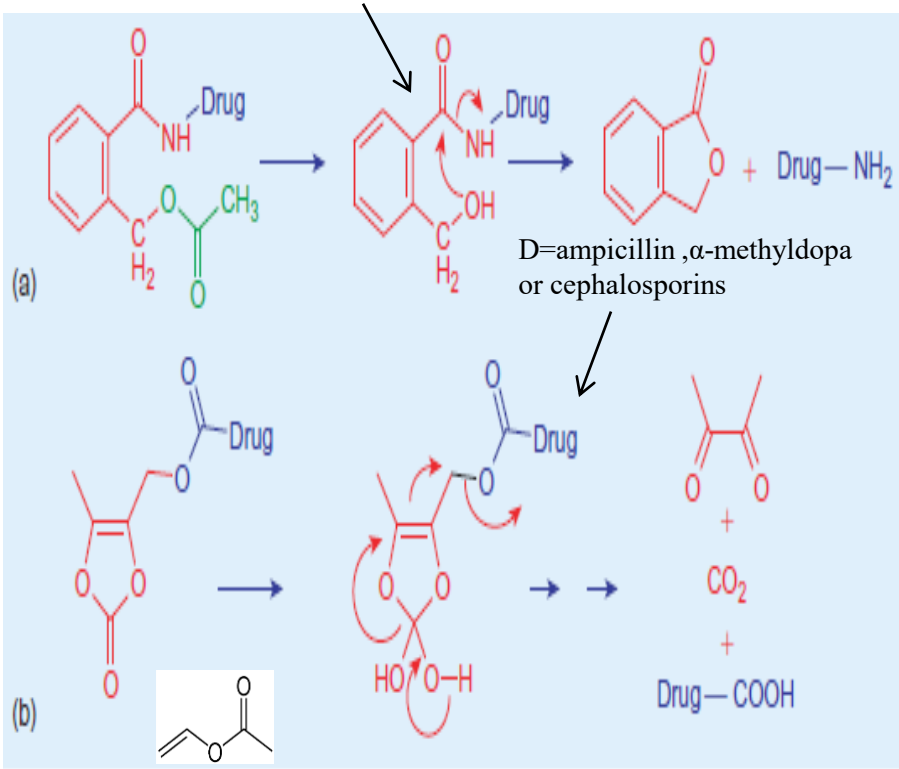
B. Use of Cascade Prodrugs

Most of the prodrugs described to this point are classical carrier-linked prodrugs. Classical carrier-linked prodrugs may sometimes be ineffective because the prodrug linkage is too stable (amides, nonactivated esters). In such cases, a β-assistance provided by an easily in vivo generated nucleophile can represent an interesting solution.

The release of the active molecule from the prodrug proceeds through a two-step trigger mechanism, for which the name "cascade latentiation" was coined by Cain in 1975 .

The concept, also **called distal hydrolysis or the double prodrug concept** , is illustrated by the use of 2-acyloxymethylbenzoic acids as amine-protective functions**, providing amides with the lability of esters (Figure a),** and by the use of substituted vinyl esters [5(2-oxo-1,3-dioxol-yl)methyl esters] as lipophilic cascade carriers for carboxylic acid-containing drugs such as ampicillin ,α-methyldopa , or various cephalosporins (Figure b) .

2-acyloxymethylbenzoic acids

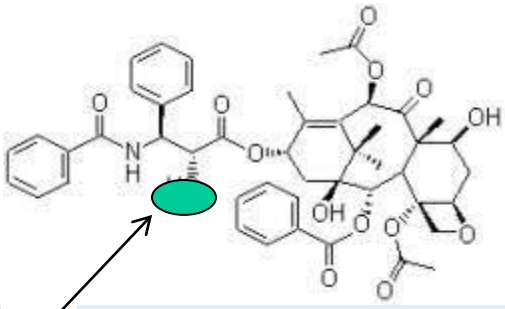


Cascade latentiation:

(a) 2-Acyloxymethylbenzoic acids provide amides the lability of esters;

.(b) Substituted vinyl esters as lipophilic cascade carrier for carboxylic acid-containing drugs .

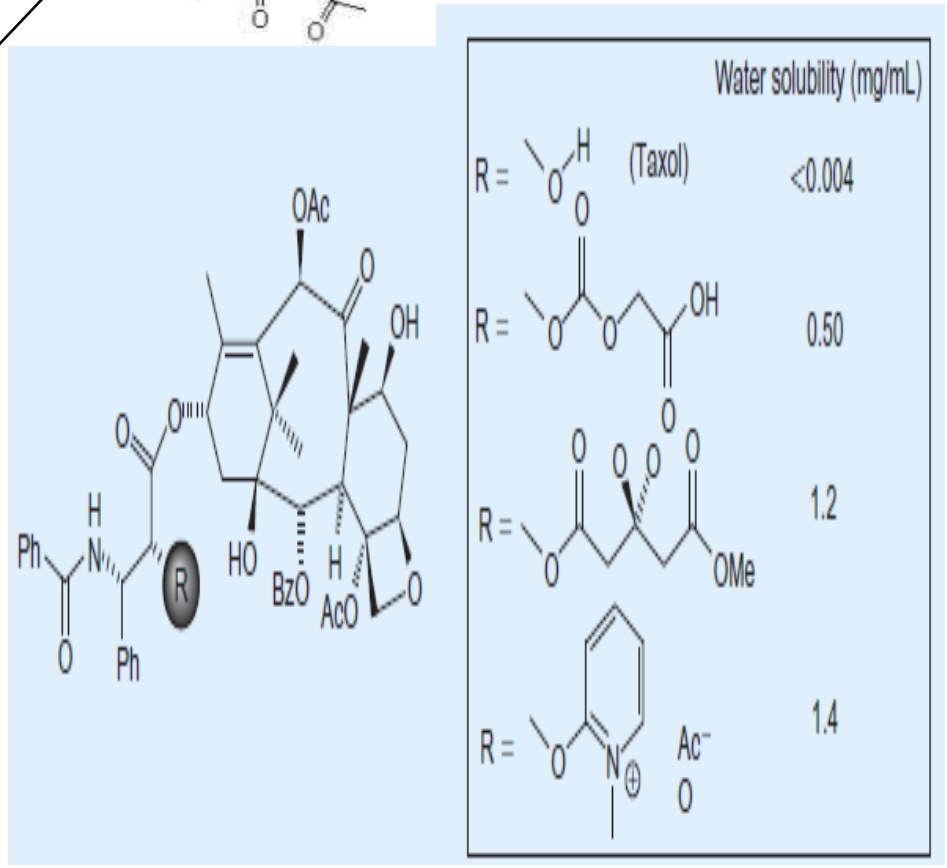
1. Water-Soluble Paclitaxel Prodrugs



Paclitaxel (sold under the name Taxol) is a potent microtubule-stabilizing agent that has been approved for cancer treatment. Despite paclitaxel's therapeutic promise, its aqueous insolubility (,0.004 mg/mL) hampers its

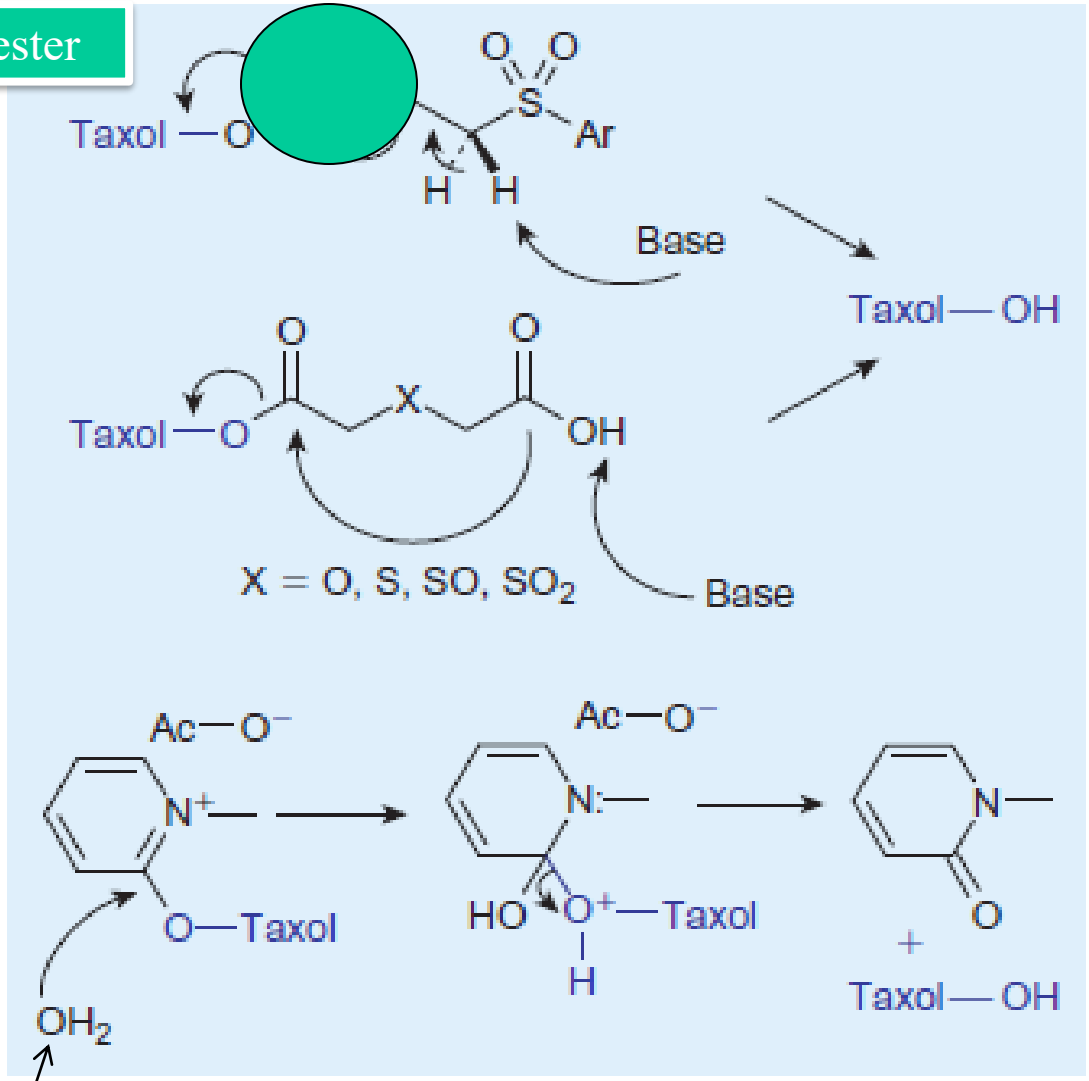
clinical application.

Nicolaou et al] report the design, synthesis, and biological activity of prodrugs designed to improve water solubility that can also be considered as cascade prodrugs (Figure).



Water soluble prodrugs of paclitaxel

Carbonate ester



The mechanistic rationale for the design of the first two paclitaxel prodrugs lies in the **spontaneous decomposition of the carbonate ester** after the abstraction of one of the activated protons or of an acidic proton (Figure).

The release of paclitaxel from the **pyridinium prodrug** (paclitaxel -2₀-methyl- pyridinium acetate; paclitaxel -2₀-MPA) is presumed to be the result of a **nucleophilic attack by water or another nucleophile at the 2₀-position of the pyridinium moiety**

- Paclitaxel release mechanisms from paclitaxel prodrugs

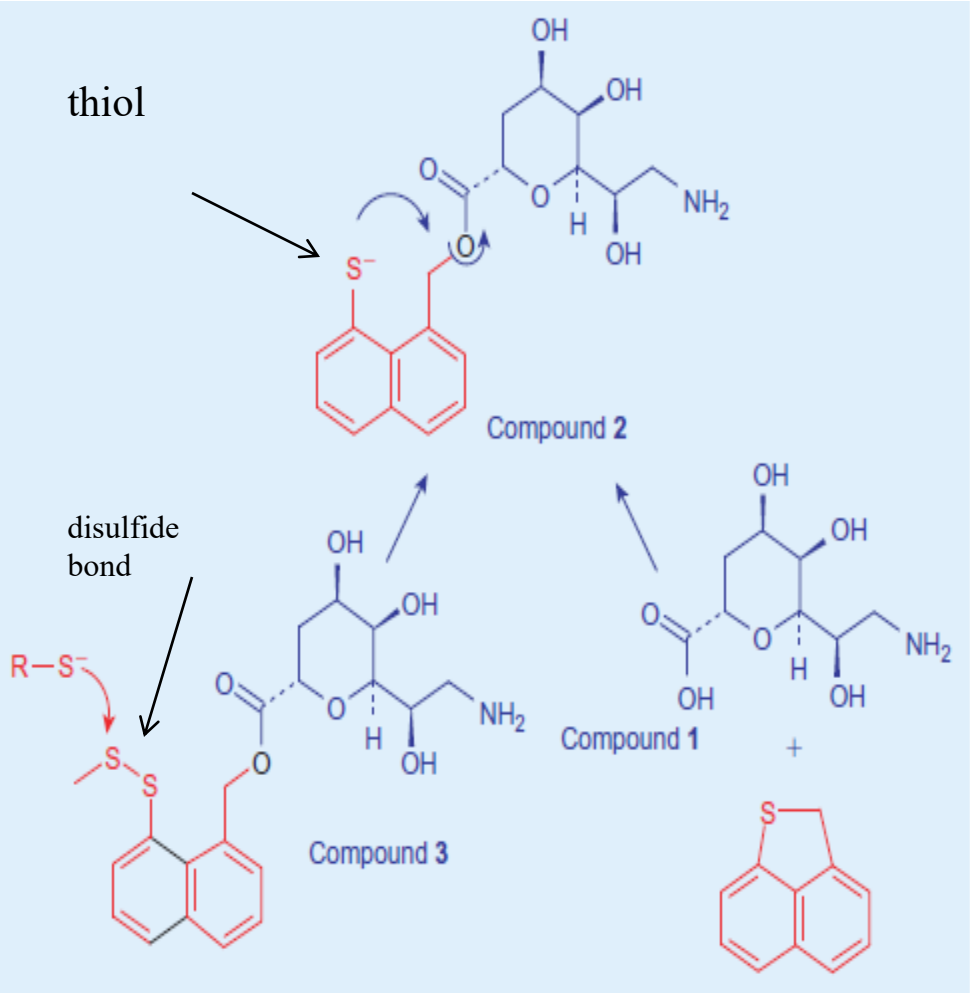
Water molecule

2. Bioactivation of an Antibacterial Prodrug

Although amino acid 1 is a potent inhibitor of CMP/KDO synthase (Figure), a key enzyme in the biosynthesis of the lipopolysaccharide of Gram-negative bacteria, it is unable to reach its cytoplasmic target and is therefore inactive as an antibacterial agent.

Simple lipophilic esters are not useful to enhance the delivery of amino acid 1 since they are not cleaved by the bacteria. On the other hand, double prodrug 3 **has been found to solve the problem .**

Upon entry into bacterial cells, the disulfide bond in compound 3 is reduced by sulfhydryl compounds present in the intracellular milieu, resulting in the formation of thiol 2. This is highly unstable, and the active amino acid 1 is formed by a rapid, intramolecular displacement.



Bioactivation of the antibacterial prodrug of an impermeant inhibitor of 3- deoxy-D-manno-2-octulosonate cytidylyl- transferase

3. Double Prodrugs for Peptides

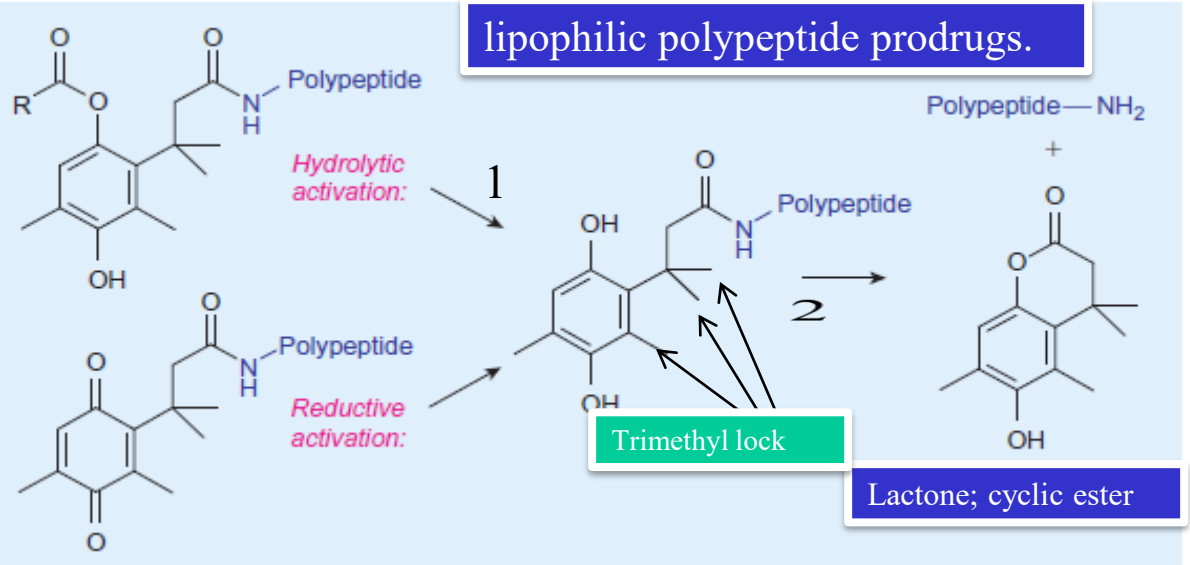
Amsberry and Borchardt , have applied **Cain's cascade concept** to prepare lipophilic polypeptide prodrugs. The amine functionality of the polypeptide is coupled to 20-acylated derivatives of 3-(20,50-dihydroxy-40, 60-dimethylphenyl)-3,3-dimethylpropionic acid (Figure).

Under simulated physiological conditions, the parent amine is regenerated in **a two-step process: (1)enzymatic hydrolysis of the phenolic ester, followed by a (2) nonenzymatic intramolecular cyclization, leading to the release of the free amine (polypeptide) and a lactone**.

The lactonization step is highly favored because of the steric pressure created by the three methyl groups (**the "trimethyl lock" concept)**. An alternative to the hydrolytic first step involves a bioreductive generation of the intermediate phenolic amide

Phenolic ester

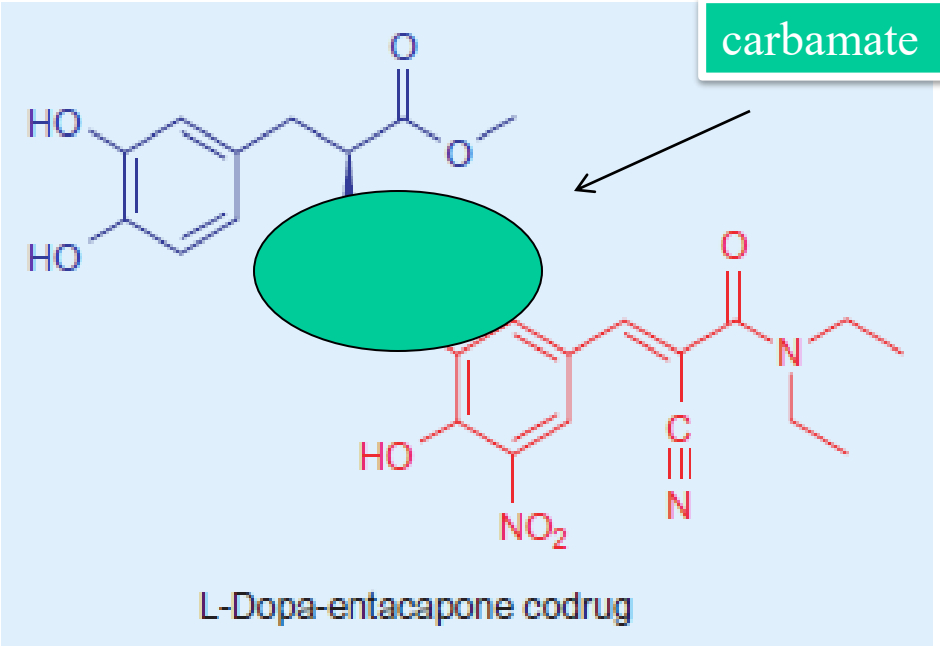
Proposed conversion of esterase-sensitive and redox-sensitive double prodrugs of peptides



C. Codrugs or Mutual Prodrug

Codrugs are also named “mutual prodrugs.” Their design consists of the linking, in a **unique molecule, of at least two different synergistic drugs that are released in vivo at the desired site of action.**

An example is found in the association of L-dopa to the catechol O-methyltransferase (COMT) inhibitor entacapone (Figure)



L-Dopa-entacapone codrug

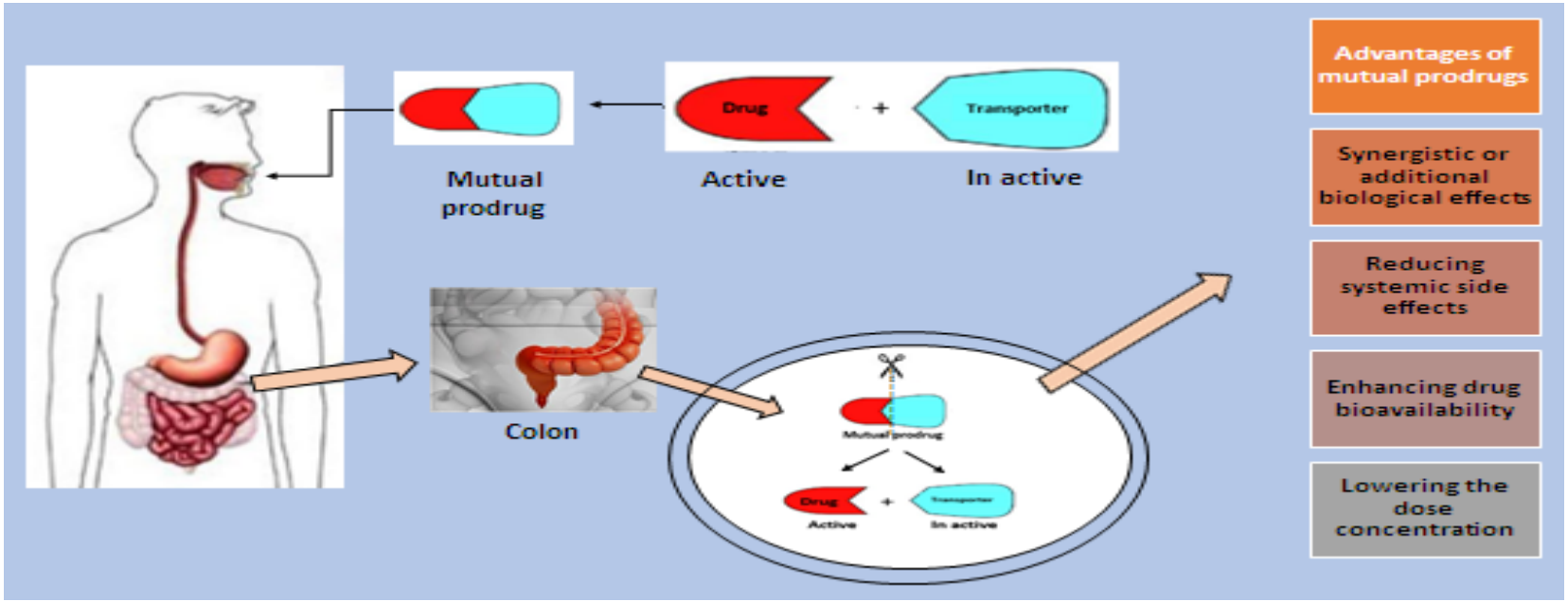
The amino functional group of L-dopa is linked to the phenolic function of entacapone by means of a carbonyl group; thus a **carbamate function** links the two active agents.

V. BIOPRECURSOR PRODRUG EXAMPLES

The following examples illustrate the bioprecursor prodrug approach. The intentional use of bioprecursor design is relatively recent, and—in some cases—there are doubts about the prospective or retrospective character of the design.

The first examples relate to oxidative bioactivations.

These are followed by examples of reductive bioactivations and mixed-type bioactivations. In general, the active species results from a cascade of metabolic reactions involving oxidative as well as reductive processes, complicated by hydrolytic reactions or Hydration -dehydration sequences.

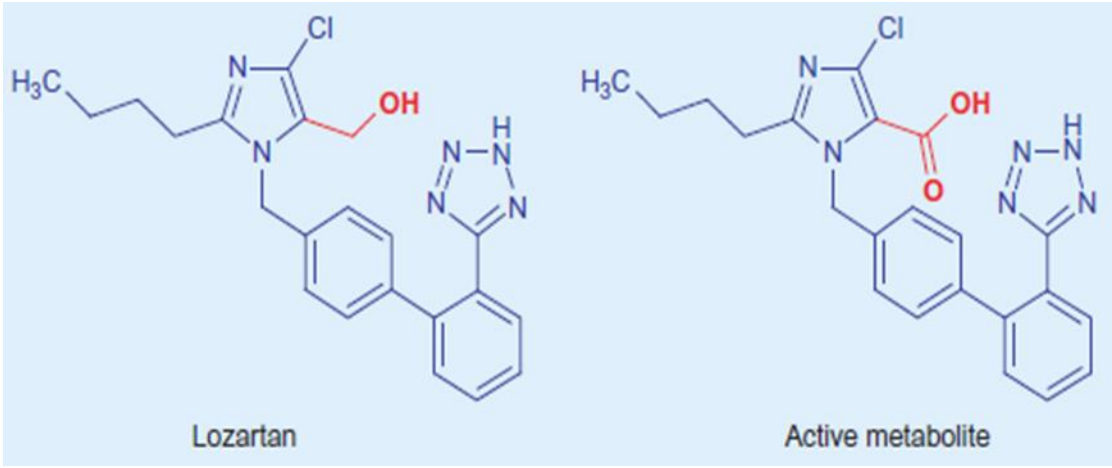


A. Oxidative Bioactivations

1. Oxidative Bioactivation of Losartan

A classic example of a bioprecursor prodrug is found in **losartan, a nonpeptide angiotensin II receptor antagonist used as an antihypertensive medication** .It can also be considered a bioprecursor prodrug insofar as, in vivo, the primary alcohol is oxidized to a carboxylic acid (Figure), which represents the actual active principle

Oxidative bioactivation of losartan

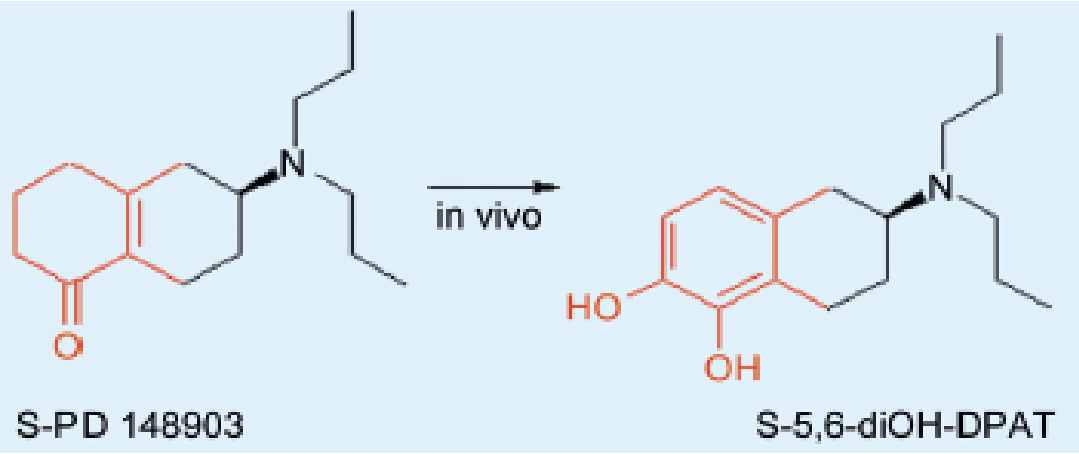


2. Conjugated Cyclohexeneones as Bioprecursors of Catecholamines

Venhuis et al observed a particularly original oxidative bioactivation mechanism by which an α, β-unsaturated cyclic ketone is converted to the corresponding catechol and delivered enantioselectively into the CNS (Figure).

This concept can be generalized and has the potential to lead to new anti-Parkinson's treatments .

Oxidative bioactivations leading to catechols

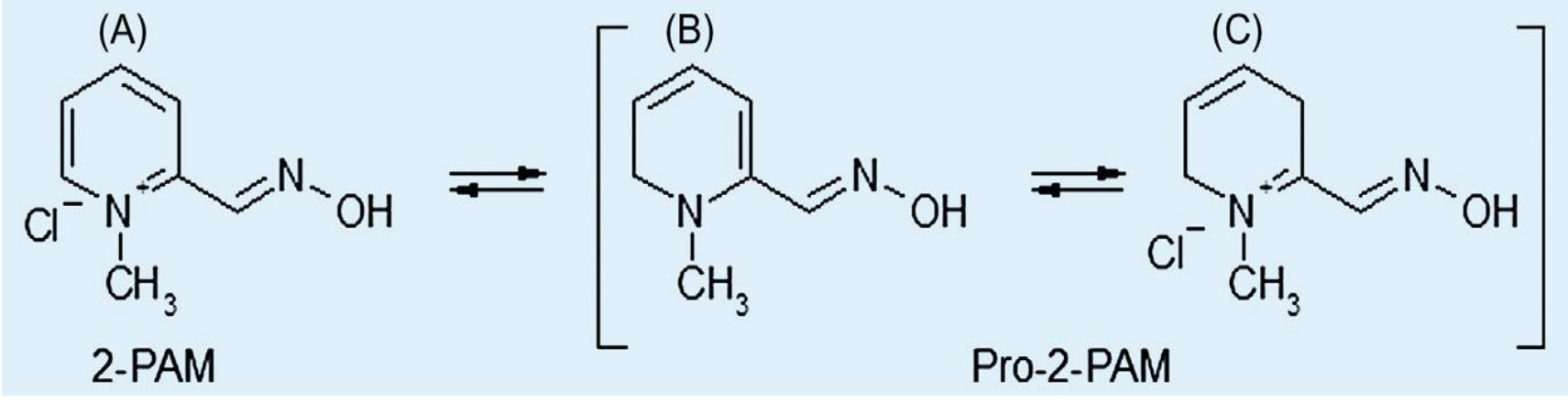


3. Site-Specific Delivery of Acetylcholine-Esterase Reactivator 2-PAM to the Brain

N-methylpyridinium-2-carbaldoxime (2-PAM; Figure(A)) constitutes the most potent reactivator of acetylcholinesterase poisoned by organophosphorus acylation. However, due to its quaternary nitrogen, **2-PAM penetrates the biological membranes poorly and does not appreciably cross the BBB**.

For this compound, it was designed a novel dihydropyridinepyridinium salt **type of redox delivery system.** The active drug is administered as **its 5,6-dihydropyridine derivative (Pro-2- PAM(B), which exists as a stable immonium salt .**

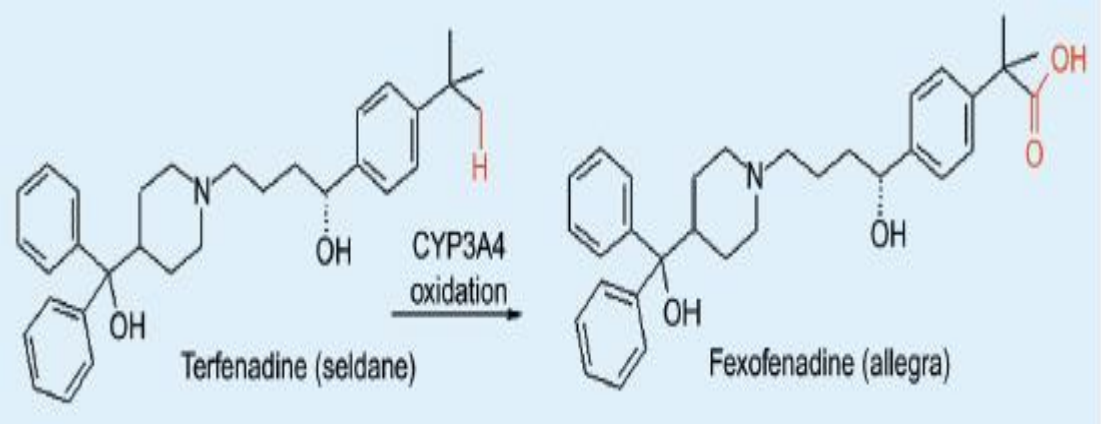
The lipoidal b (pKa56.32) easily penetrates the BBB, where it is oxidized to the active a. A dramatic increase in the brain delivery of 2-PAM by the use of Pro-2-PAM is thus achieved, resulting in a re-activation of phosphorylated brain acetyl-cholinesterase in vivo



4. Oxidation of Terfenadine byCYP3A4

Terfenadine, a bioprecursor prodrug, is an antihistamine used for the treatment of allergic conditions. It is completely metabolized in the liver by the enzyme cytochrome P450 3A4 isoform to give the active metabolite fexofenadine (Figure).

Terfenadine was taken off the market due to the risk of cardiac arrhythmia caused by QT prolongation. The active metabolite is not cardiotoxic, however, and is now sold under the brand name Allegra. This is an example where the **active component is better administered than a bioprecursor prodrug due to its pharmacologically undesirable activity**.



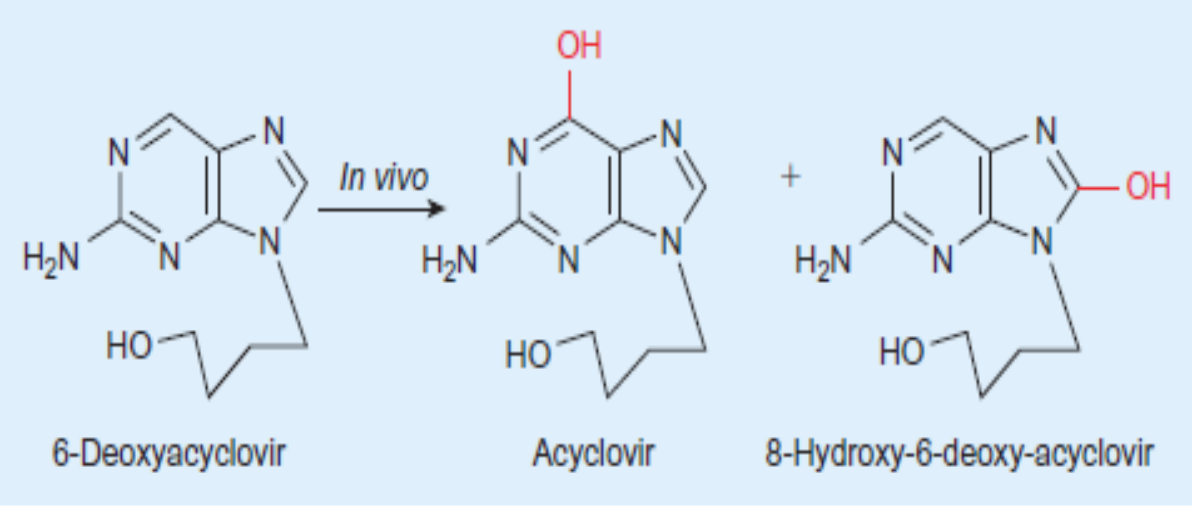
CYP3A4 oxidation of terfenadine

5. 6-Deoxyacyclovir as a Bioprecursor of Acyclovir

The antiherpetic agent acyclovir suffers from poor oral bioavailability; only 10-20 % of an oral dose is absorbed in humans. This is ascribed to low water solubility due to strong interaction forces in the crystal lattice.

The corresponding deoxo derivative (6-deoxyacyclovir) was shown by Krenitsky to be eighteen times more water soluble and to be rapidly oxidized in vivo by xanthine oxidase to the parent drug (Figure).

Studies in rats and in human volunteers showed that orally administered 6-deoxyacyclovir has a 56 times greater bioavailability than acyclovir

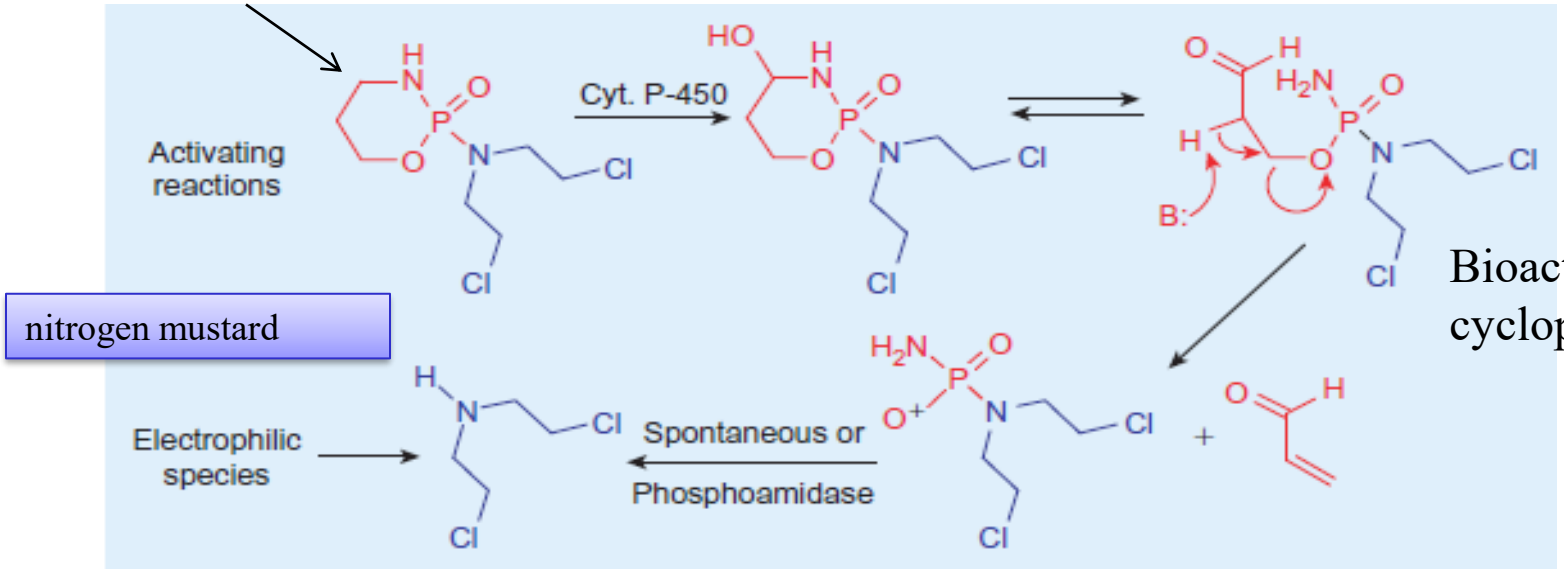


6-Deoxyacyclovir as a bioprecursor of acyclovir

6. Bioactivation
Cyclophosphamide

Cyclophosphamide is a cytotoxic (cytostatic), nonspecific cell cycle, antiproliferative agent, which is used in such diverse medical problems as neoplasia, tissue transplantation, and inflammatory diseases .Chemically, it is an inert bioprecursor for a potent nitrogen mustard alkylation agent (Figure).

Cyclophosphamide was synthesized by Arnold et al in the hope that it would be inert until activated by an enzyme present in the body, especially in a tumor. The activation mechanism is believed to require **an initial oxidative dealkylation, followed by a spontaneous or phosphoramidase-catalyzed hydrolysis to the parent nitrogen mustard**



Bioactivation of cyclophosphamide

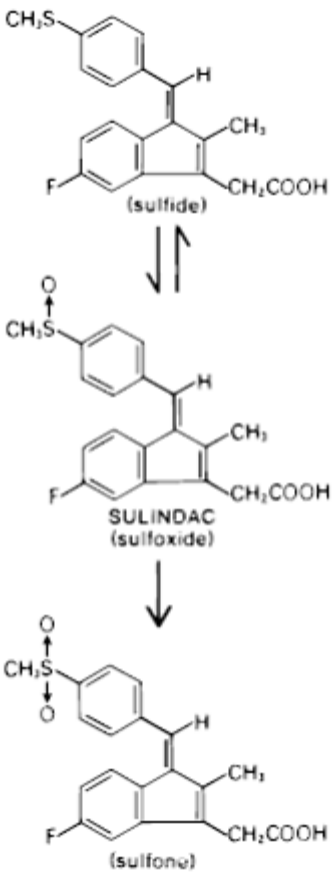
B. Reductive Bioactivations

1. Reductive Bioactivation of Nitrogen Mustards

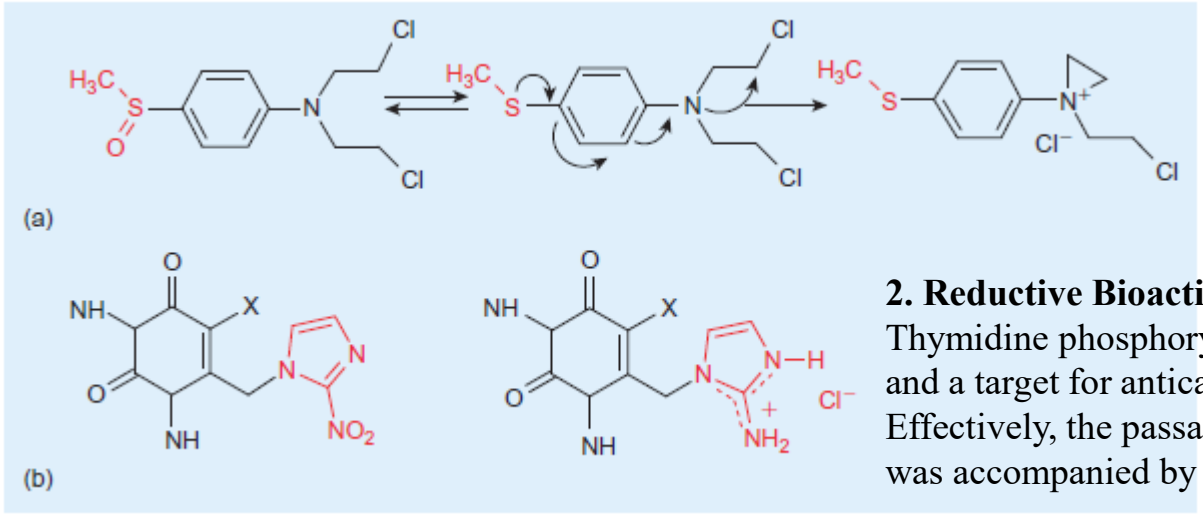
Many conventional anticancer drugs display relatively poor selectivity for neoplastic cells, and solid tumors are particularly resistant both to radiation and to chemotherapy. However, **solid tumors possess a few unique and important microenvironmental properties such as localized hypoxia, nutrient deprivation, and low pH .**

On the other hand, as shown previously for sulindac, sulfoxides can undergo two major biotransformations: reversible reduction to the sulfide and irreversible oxidation to the sulfone.

The oxidation to the sulfone is the dominant process under normal physiological conditions, but the reduction to the sulfide becomes significant under anaerobiotic conditions .Taking advantage of these findings, Kwon et al devised a hypoxia-selective alkylating bioprecursor prodrug (Figure)



(a) and (b) Hypoxia-selective nitrogen mustard and bioactivated TP inhibitors



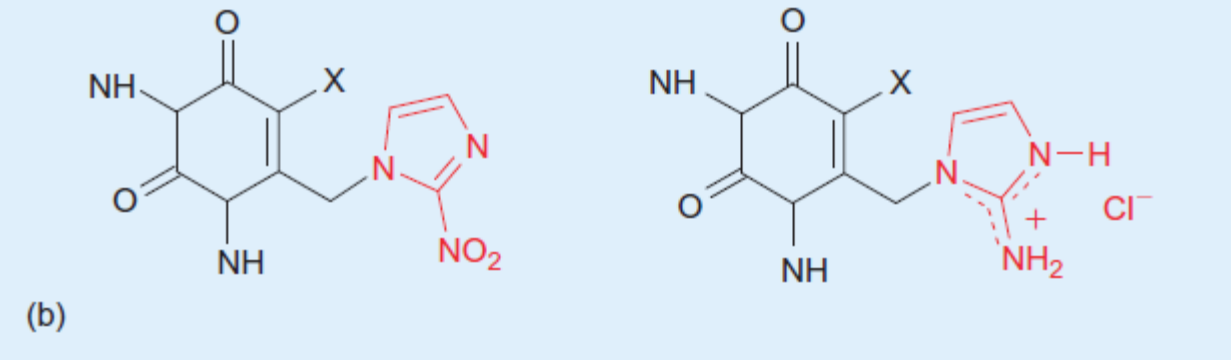
2. Reductive Bioactivation of Nitroimidazolylmethyluracils

Thymidine phosphorylase (TP) is an angiogenic growth factor and a target for anticancer drug design

Effectively, the passage from the nitro to the amino analog was accompanied by a 1,000-fold increase in TP inhibition.

2. Reductive Bioactivation of Nitroimidazolylmethyluracils

Thymidine phosphorylase (TP) is an angiogenic growth factor and a target for anticancer drug design (Figure(b). Docking studies of the modeled TP predicted that the binding of aminoimidazolyl methyluracils was energically more favored than that of the corresponding nitro counterparts .Effectively, the passage from the nitro to the amino analog was accompanied by a 1,000-fold increase in TP inhibition.



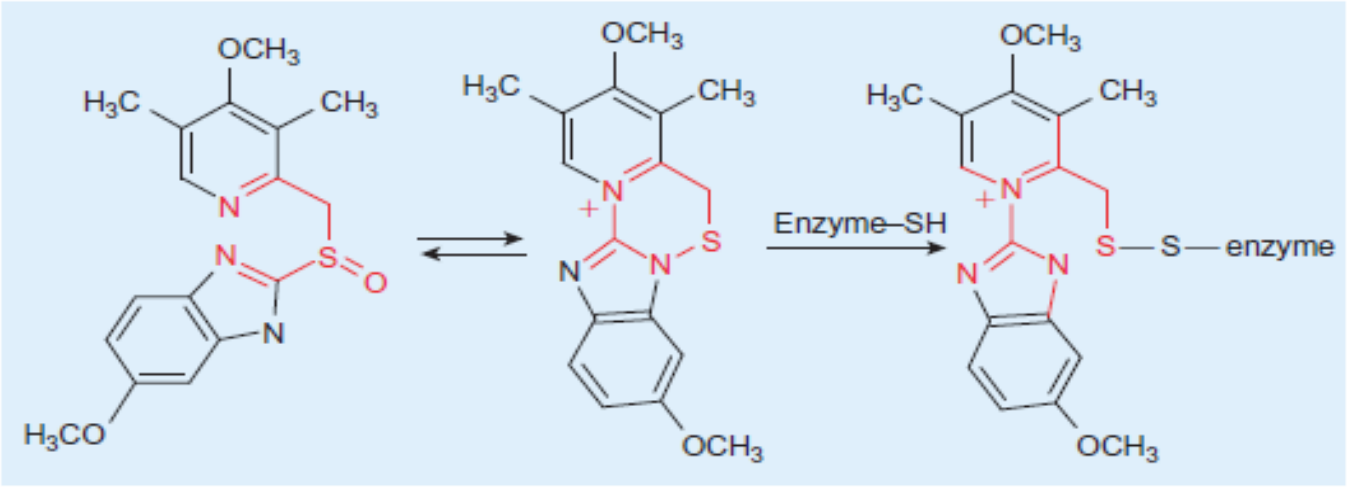
(b)

3. Reductive Bioactivation of Omeprazole

Omeprazole effectively inhibits gastric secretion by inhibiting the gastric H_1, K_1-ATPase . This enzyme is responsible for gastric acid production and is located in the secretory membranes of parietal cells. Omeprazole is an anti-ulcerative drug, used especially in the treatment of ZollingerEllison syndrome .

In vivo, omeprazole is transformed into the active inhibitor, a cyclic sulfenamide (Figure)which **forms disulfide bridges with the thiol groups of the enzyme and thus inactivates it .**

The high specificity in the action of omeprazole (pKa54.0) is due to its preferential concentration in the rather acidic parietal cells where it is activated. In neutral regions of the body, omeprazole is rather stable and is only partially converted to the active species.

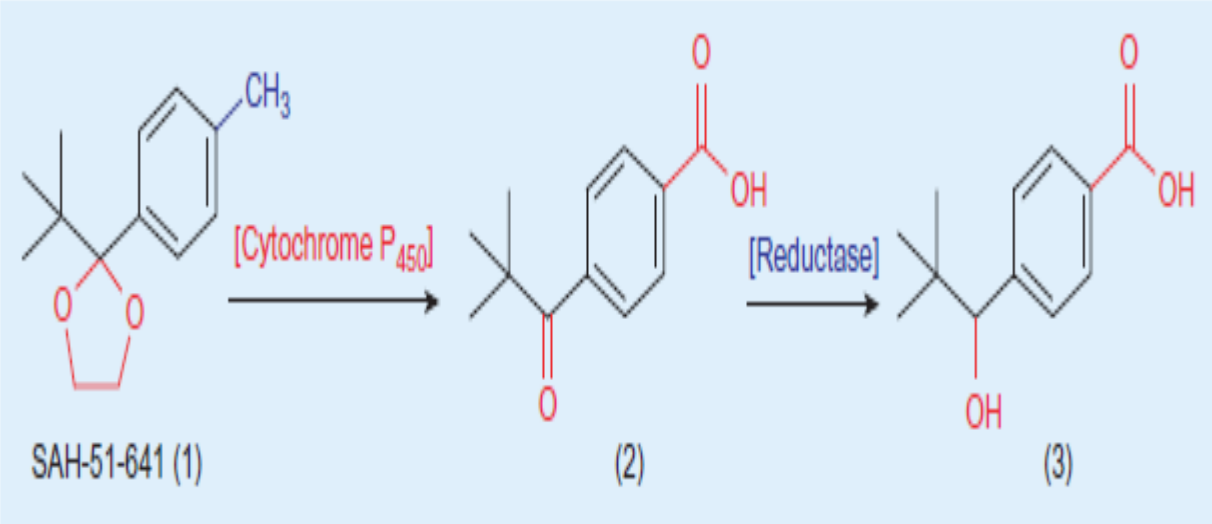


Reductive bioactivation of omeprazole

C. Mixed Bioactivation Mechanisms

Certain bioactivation mechanisms involve several chemical sequences, some of them being oxidative and others being reductive. The prodrug SAH 51-641 (1) (Figure) illustrates an example of a mixed oxidative reductive mechanism.

SAH 51-641 is a potent hypoglycemic agent, which acts by inhibiting hepatic gluconeogenesis via inhibition of fatty acid oxidation .This compound is metabolized by a sequential oxidation/reduction to the corresponding keto-acid (2) and the hydroxyl acid (3). Compound (3) is a substrate for medium-chain fatty acyl CoA ligase and represents the actual active agent



- Mixed oxidative/reductive bioactivation of dioxolanes.

Bioprecursors Versus Carrier Prodrugs

A comparative balance sheet established for the two prodrug approaches led us to the following conclusions

(Table below):

- • The bioavailability of carrier prodrugs is modulated by using a transient transport moiety. Such a linkage is not implied for bioprecursors, which result from a molecular modification of the active principle itself.
- • The lipophilicity is generally the subject of a profound alteration of the parent molecule in the case of carrier prodrugs, whereas it remains practically unchanged for bioprecursors.
- • The bioactivation process is exclusively hydrolytic for carrier prodrugs. It involves mostly redox systems for bioprecursors.
- • The catalysis leading to the active principle is hydrolytic (either through general catalysis or through extrahepatic enzymes) for carrier prodrugs. For bioprecursors, it seems largely restricted to Phase I metabolizing enzymes

Table: Bioprecursors Versus Carrier Prodrugs

	Prodrugs	
	Carrier prodrugs	Bioprecursors
Constitution	Active principle + carrier group	No carrier group
Lipophilicity	Strongly modified	Usually slightly modified
Bioactivation	Hydrolytic	Mostly oxidative or reductive
Catalysis	Chemical or enzymic	Only enzymic

DIFFICULTIES AND LIMITATIONS

The introduction of prodrugs in human therapy gave successful results in overcoming **undesirable properties such as poor absorption, rapid biodegradation, or formulation problems.** It can be expected that an increasing number of medicinal chemists will be tempted by this approach. However, they must keep in mind that prodrug design can also give rise to a large number of new difficulties, especially in **the assessment of pharmacological, pharmacokinetic, toxicological, and clinical properties**.

At the pharmacological level, for example, because **bioactivation is necessary to create the active species, these compounds cannot be submitted to preliminary in vitro screening tests, namely, binding studies, neurotransmitter re-uptake, measurements of enzymatic inhibition, and activity on isolated organs.**

The measurements of **pharmacokinetic parameters** can lead to numerous misinterpretations. Thus, **pivampicillin has a half-life of 103 min in a buffered aqueous solution at 37C, but it falls to less than one min after addition of only 1 percent of mouse or rat serum.** In the presence of human serum (10 percent), however, the half-life is fifty min, whereas in whole human blood it is only five min. These results exemplify the care required to **avoid incorrect conclusions.** In addition, when a prodrug and the parent molecule are compared, one must take into account the differences in their respective time courses of action. The maximum activity can appear later for the prodrug than for the parent compound, and often the comparison of the AUC could constitute a better criterion.

DIFFICULTIES AND LIMITATIONS (Cont.)

At the **toxicological level,** even when prodrugs derive from well-known active principles, they have to be regarded as new entities. Undesirable side effects can appear that are **directly related to the prodrug (e.g., allergy to bucloxic acid) or derived from the bioactivation process (e.g., formation of unwanted or unexpected metabolites), or which can be attributed to the temporary transport moiety (e.g., digestive intolerance to pivampicillin, antivitamin- PP activity of nicafenine).** This latter case is particularly illustrative. An apparently innocent carrier group such as

N-hydroxyethylnicotinamide appeared as a promising candidate for improving the absorption of acidic antiinflammatory drugs or clofibric acid .However, during the clinical studies, side effects similar to vitamin PP deficiency appeared, suggesting that N-hydroxyethylnicotinamide could function as a nicotinamide antimetabolite.

The compounds then had to be withdrawn (H. Cousse, Pierre Fabre & Co, personal communication).

In a review of potential hazards of the prodrug approach, Gorrod cites four toxicity mechanisms:

1. Formation of a toxic metabolite of the **total prodrug** that is not produced by the parent drug.

2. Consumption of a **vital constituent** (e.g., glutathione) during the prodrug activation process. As L-cysteine is needed for the biosynthesis of glutathione, a supply with L-cysteine prodrugs can eventually confer some protection of the hepatic cells .

3. Generation of a toxic derivative from a **transport moiety** supposed to be “inert.”

4. Release of a pharmacokinetic **modifier** (causing enzymatic induction, displacing protein-bound molecules,altering drug excretion, etc.).

Eventually, at the clinical stage, the predictive value of animal experiments is also questionable. Thus, for two prodrugs derived from **α-methyldopa,** the active doses in rats were identical, but they turned out to be very different **during clinical investigations**. One compound was just as potent as α-methyldopa, whereas the other one was 34 times more potent .

Finally, a patent application for a new prodrug should take into account all these aspects, and **because the biological profile of each individual prodrug will differ depending on the linker/spacer and promoiety, it should in no way be regarded just as a complement to the main file**. The question of patentability, however, is becoming more difficult to define as more prodrug examples appear in the literature, thus requiring inventors to **meet the non-obvious criteria by demonstrating an unexpected result from the prodrug.**

Recent prodrugs designed for targeted delivery will certainly fulfill the requirements for patentability: **novelty, utility, and non-obviousness.**


THANK
YOU

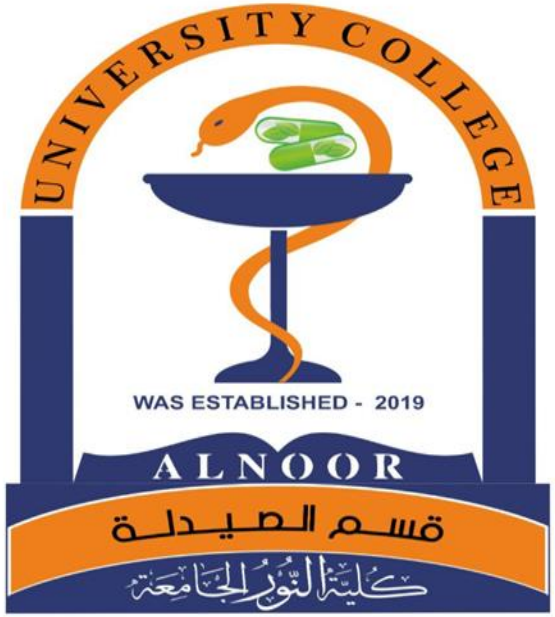
Organic Pharm. Chemistry IV

The Organic Chemistry of Drug Design and Drug Action

Lecture 6

3rd December 2022

12:00- 2:00 PM



AlNoor University College

Pharmacy Department

1st Course/ 5TH Class

2022/2023

Just Be Positive

(وَمَنْ يَتَّقِ اللَّهَ يَجْعَلْ لَهُ مِنْ أَمْرِهِ يُسْرًا)

الطلاق 4

Have a Look to the following YouTube:

- https://www.google.com/search?q=combinatorial+chemistry&rlz=1C1GCEA_enIQ982IQ982&sxsrf=ALiCzsZk7-LUImeTJAFv4Yj623Jmt0HaA:1670525954050&source=lnms&tbm=vid&sa=X&ved=2ahUKEwjZ3tCQ2ur7AhUoSvEDHUD1AXgQ_AUoAnoECAIQBA&biw=1292&bih=735&dpr=0.8#fpstate=ive&vld=cid:b2c6b60f,vid:MVgsX7PM4F4

Outlines

De novo Drug Discovery and Development

Drug design approach:

1. Classical approach
2. **Combinatorial chemistry**
3. Computer-aided drug design

Combinatorial Library : Scaffold-based vs. Backbone-based libraries

Methods of combinatorial Chemistry Synthesis

Synthetic methods & techniques:

De novo Drug Discovery and Development

The estimated time and main steps in de novo drug discovery and development and drug repurposing for cancer therapy. De novo drug discovery and development for cancer therapy takes 10–17 years and comprises basic discovery, drug design, in vitro and in vivo experimentation (including identifying safety and efficacy), clinical trials and finally drug registration into the market. In contrast, drug repurposing for cancer therapy takes only 3–9 years as it can bypass several processes that have been completed for the original indication if the anticancer potential of the candidates is confirmed

Drug design approach:

1. Classical approach :

make a change on an existing compound or synthesize a new structure and see what Happens. Most drug compounds were synthesized in milligram quantities in a serial one at a time fashion After synthesis, the compound was sent to a biologist, who tested it in several in vitro assays and returned the results to the chemist Based on the assay results, the chemist would apply some structure activity relationship (or use chemical intuition to decide what changes to make in future versions of the molecule to improve activity

Using this iterative process, a chemist would be able to synthesize only a handful of structures per week. Since the yield of marketable drugs from compounds synthesized and tested is only about 1 in 10,000, the **road to success has been a long and expensive one, taking 6 to 12 years and costing $500 to $800 million per drug**.

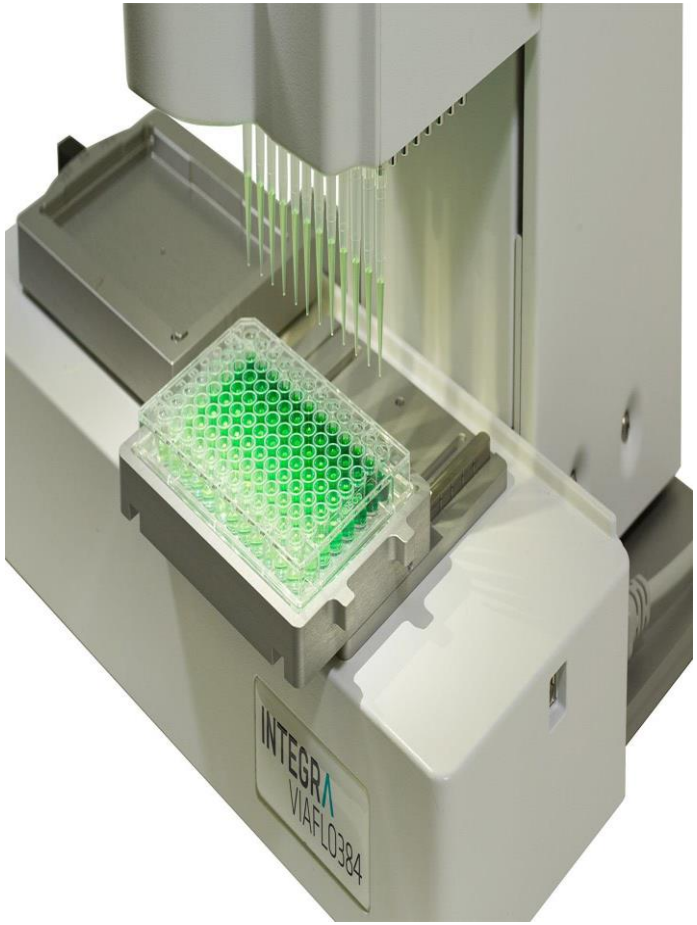
2. Combinatorial chemistry approach

In the mid-1980s, classical approach to drug synthesis changed dramatically with the introduction of **combinatorial chemistry.**

The drug discovery process became a highly **parallel one**, in which hundreds or even thousands of structures could be synthesized at one time.

Interestingly, biologists had for some time been **using high-throughput screening (HTS)** to perform their in vitro assays, running assays in 96-well microtiterplates and even using laboratory robotics for pipetting and analysis.

HTS :a key process used in drug discovery to identify hits from compound libraries that may become leads for medicinal chemistry optimization.



The term combinatorial chemistry was coined to refer to the parallel generation of all possible combinations of substituents or components in a synthetic experiment. Whereas the yield from a serial synthesis is a single compound, **the yield from a combinatorial synthesis is a chemical library.**

Figure 1 shows two common types of chemical libraries:

1.Generic library, based on a single parent or scaffold structure and multiple substituents or residues.

2.Mixture library, containing a variety of structure types.

The total number of structures in a library is either the product of the various numbers of substituents (for a generic library) or the total number of structures in a mixture.

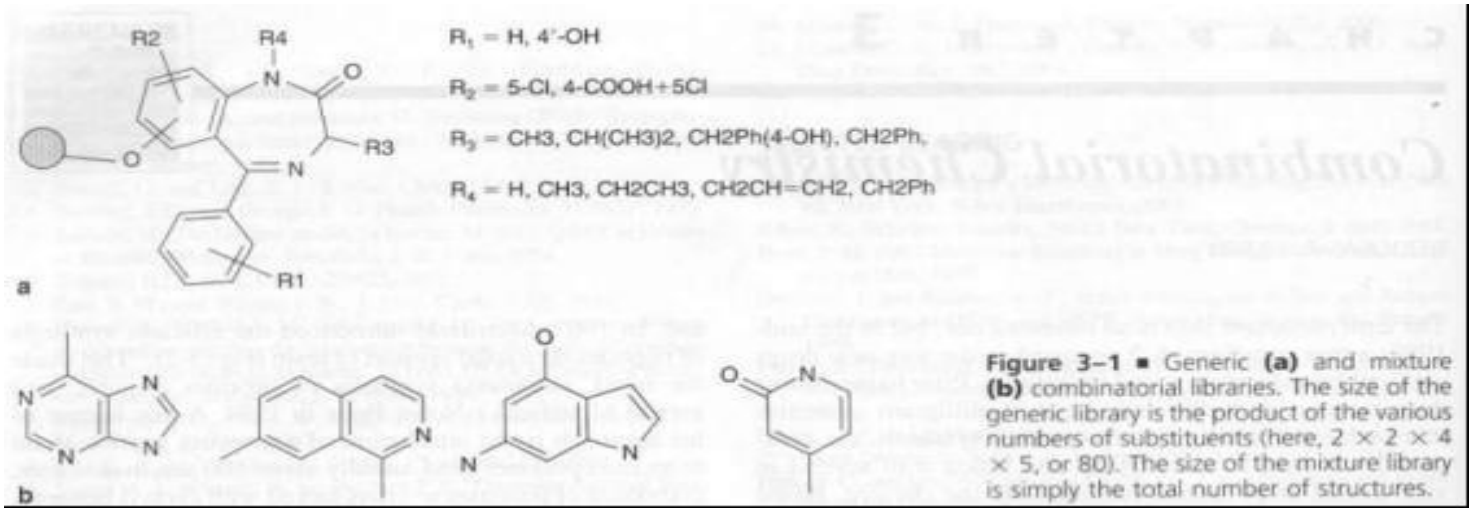


Figure 3–1 ▪ Generic **(a)** and mixture **(b)** combinatorial libraries. The size of the generic library is the product of the various numbers of substituents (here, 2 × 2 × 4 × 5, or 80). The size of the mixture library is simply the total number of structures.

Rational" approach:

The medicinal chemist can select subsets of substituents that vary in **lipophilicity , steric bulk, induction, and resonance effects**

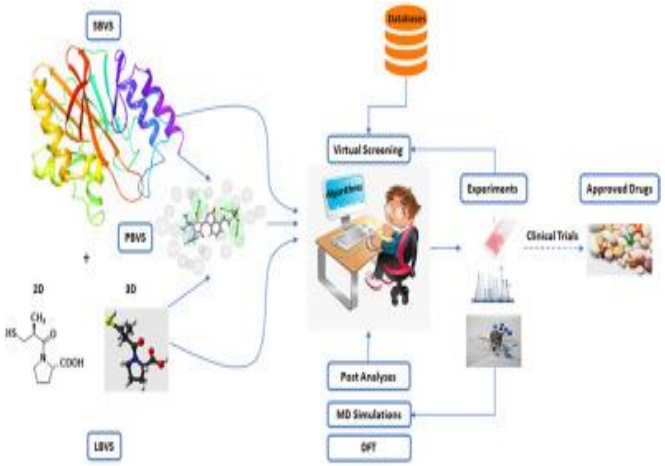
This **rational" approach** to drug design assumes that there is some understanding of the target receptor and that there is a lead molecule, commonly called the prototype molecule.

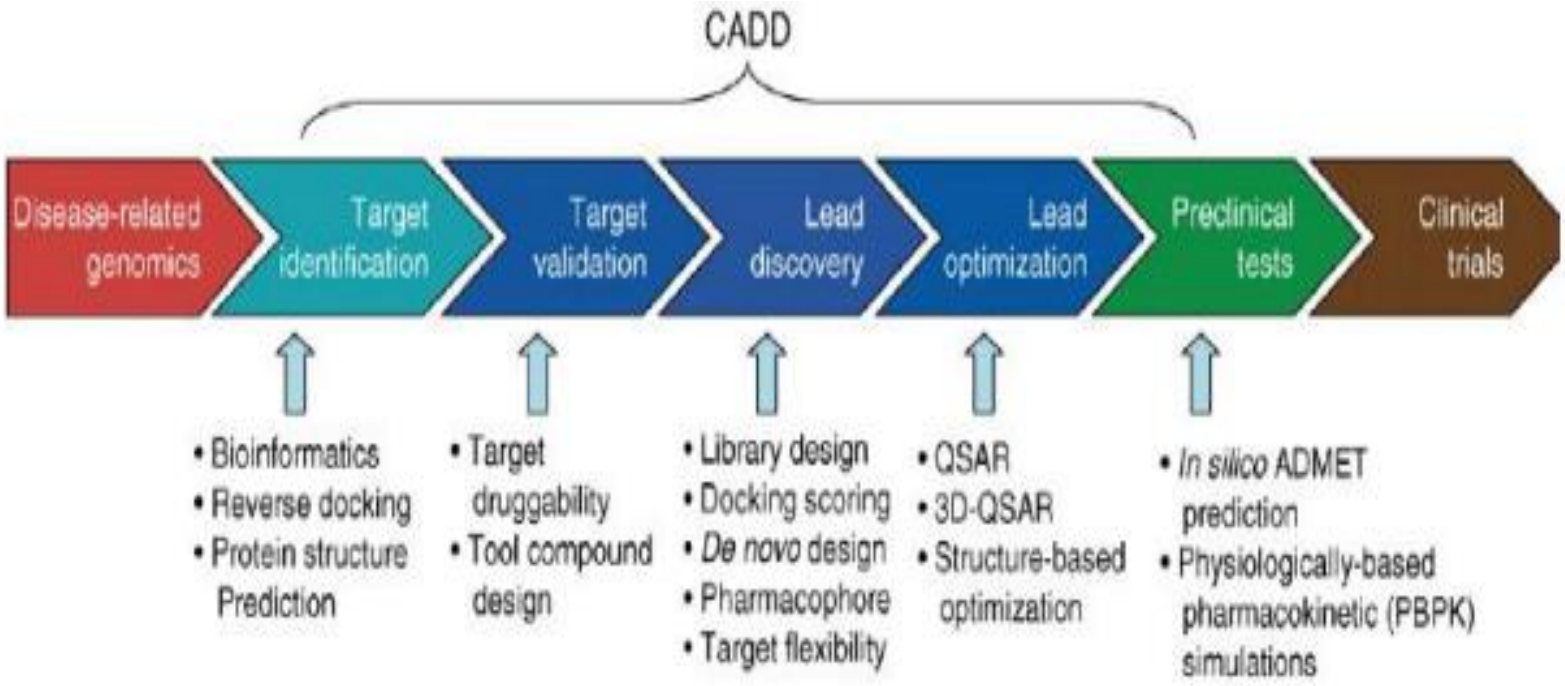
A classic example is **the dihydrofolate reductase** inhibitor methotrexate, which has been one of the prototypes that laboratories have used to synthesize and test new inhibitors.

The goal of combinatorial chemistry is to be able to synthesize, purify, chemically analyze, and biologically test all the structures in the library, using as few synthetic experiments as possible

3 . Computer aided drug design:

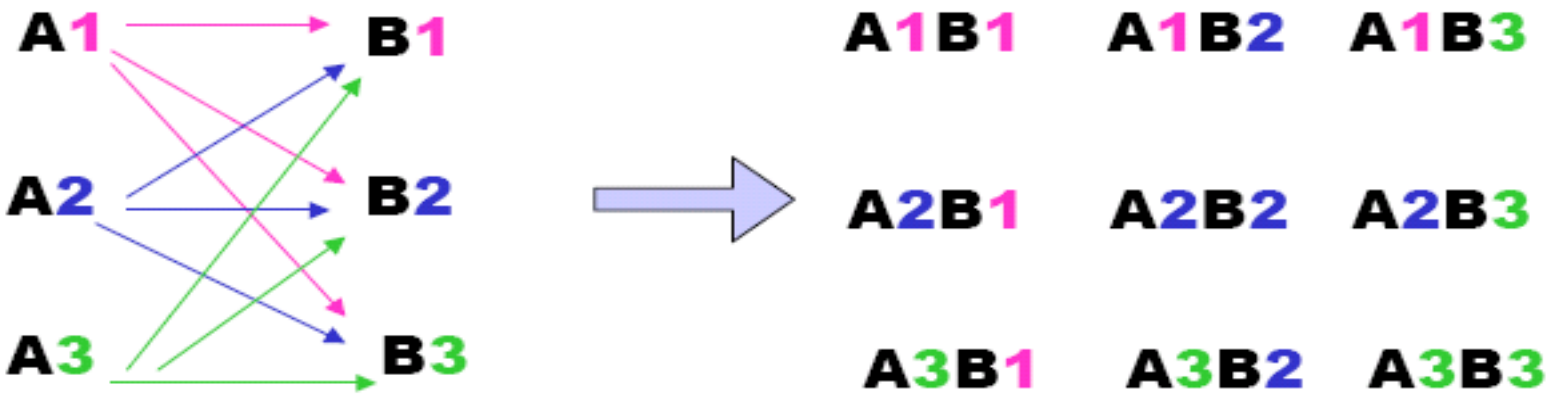
drug design increasingly is based on modern computational chemical techniques; it also uses sophisticated knowledge of disease mechanisms and receptor properties.





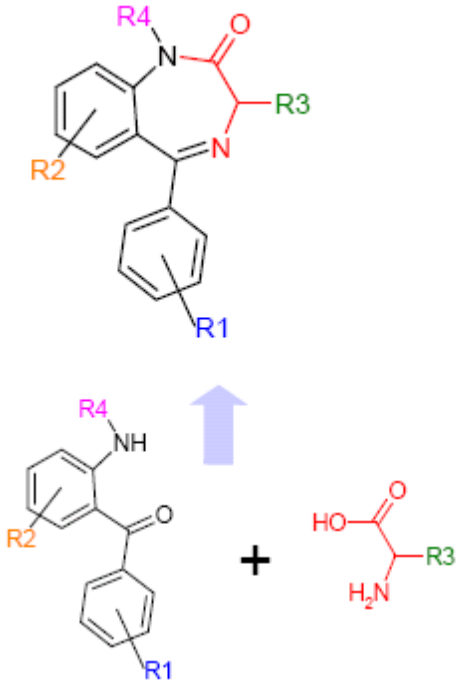
Combinatorial Libraries:

- Def.: Collection of finally synthesized compounds
- Size: depends on the number of **building blocks** used per reaction and the number of reaction steps, in which a new building block is introduced
- Typical: 10^2 up to 10^5 compounds



9 different compounds

Distinction: Scaffold-based vs. Backbone-based libraries



1. Scaffold-based libraries:

Definition: Core-structure, which all compounds of the library have in common

- Scaffold can consist of several single building blocks (here: Amino acid and Aminobenzophenone)

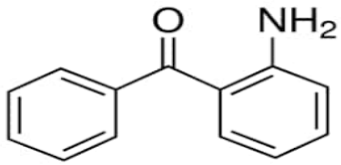
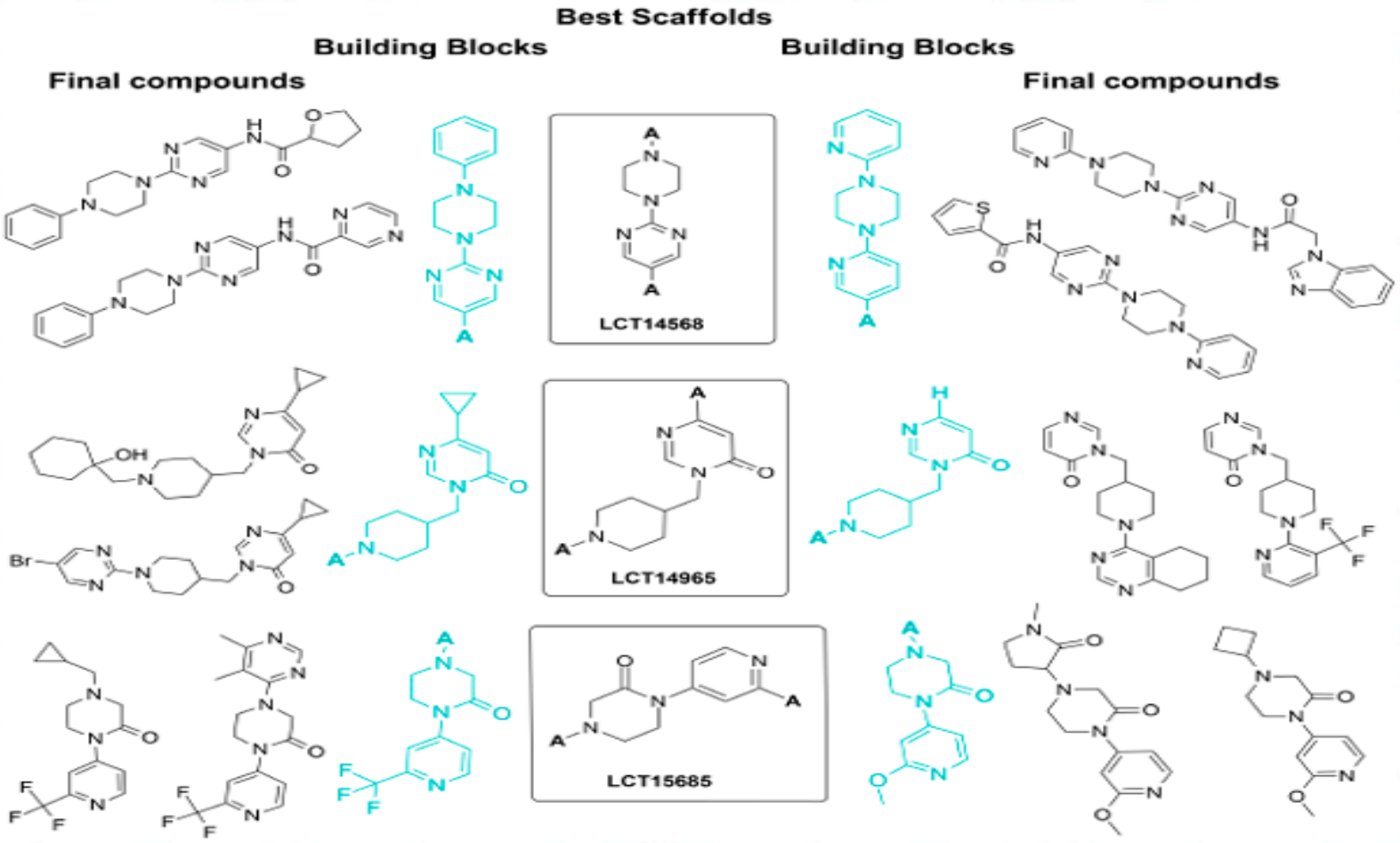
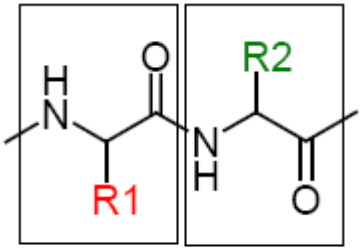


Figure . Example of final compounds designed with the use of molecular scaffolds through the preparation of intermediate building blocks.



2. Backbone-based libraries:

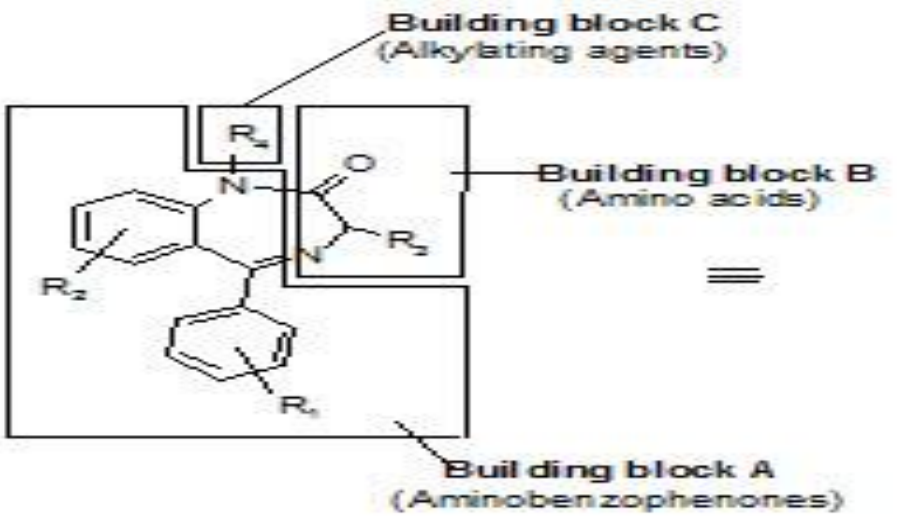


Building block A Building Block B

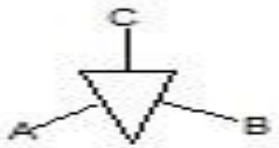
- Further Examples:

Nucleic Acids ,Carbohydrates

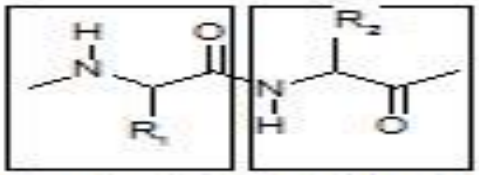
Scaffold-based vs. Backbone-based libraries



≡



(A)



≡ A - B (B)

Methods of combinatorial Chemistry Synthesis

1. Solid Phase

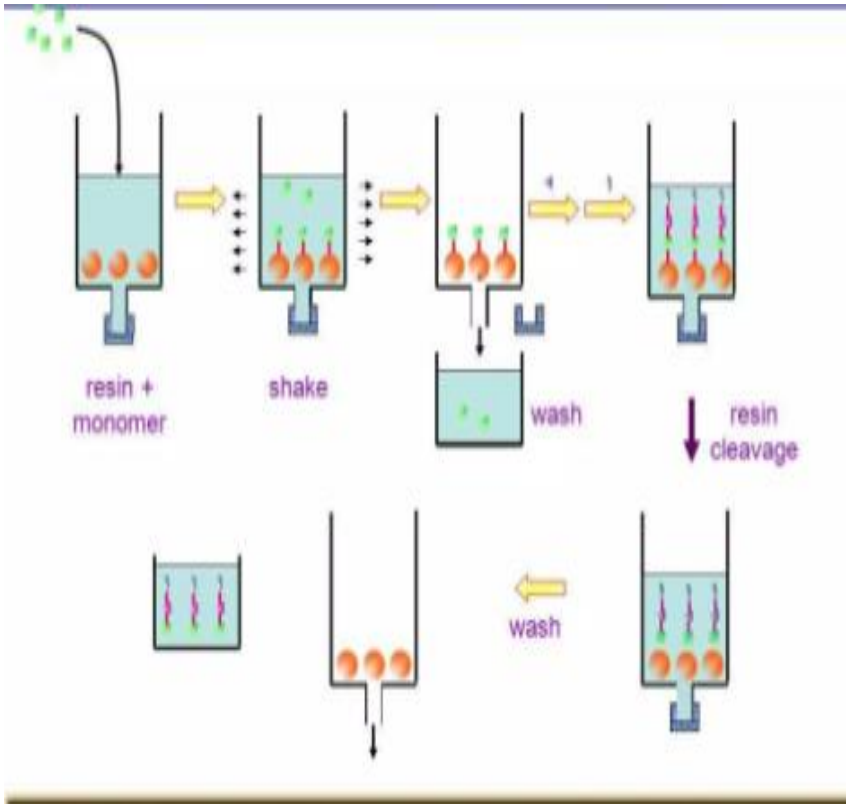
(resin bead)

2. Solution Phase

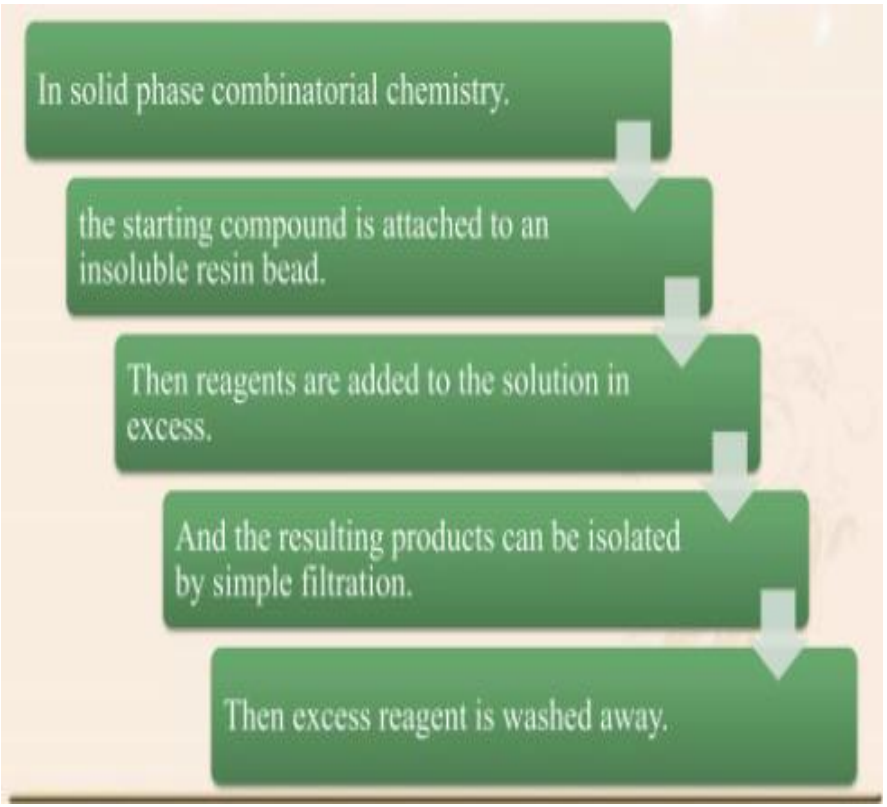
(solvent in reaction flask)

Solid Phase

Solid-phase synthesis

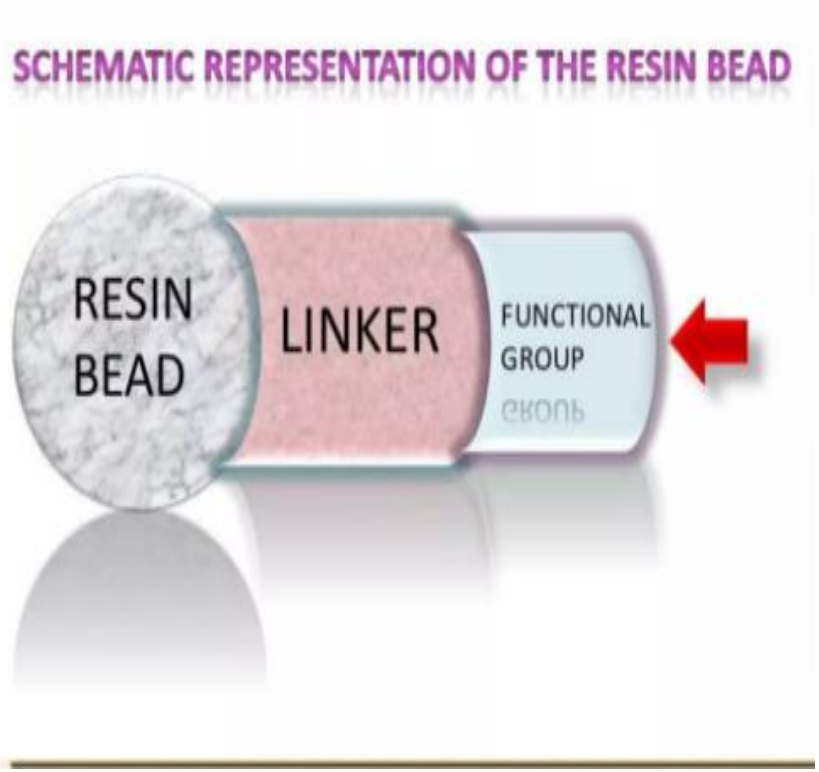


Solid phase combinatorial chemistry



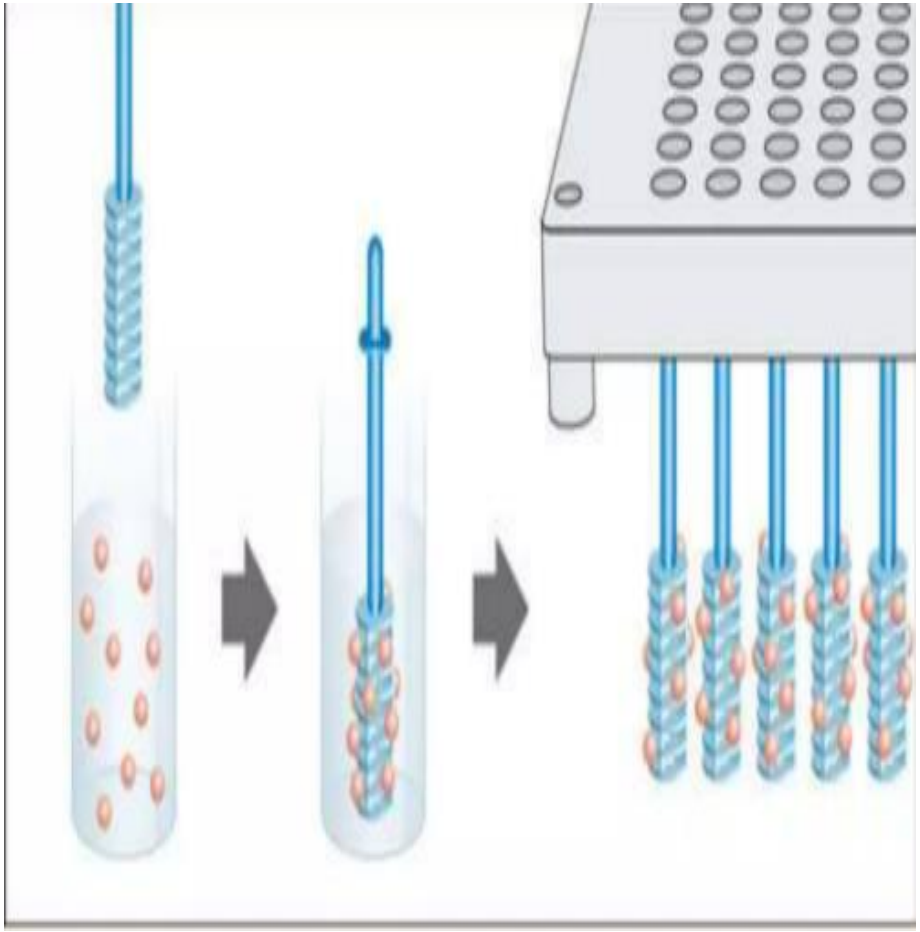
The use of solid support for organic synthesis relies on three interconnected requirements

- Polymeric solid support
- A linker
- Protecting group



Solution phase combinatorial chemistry

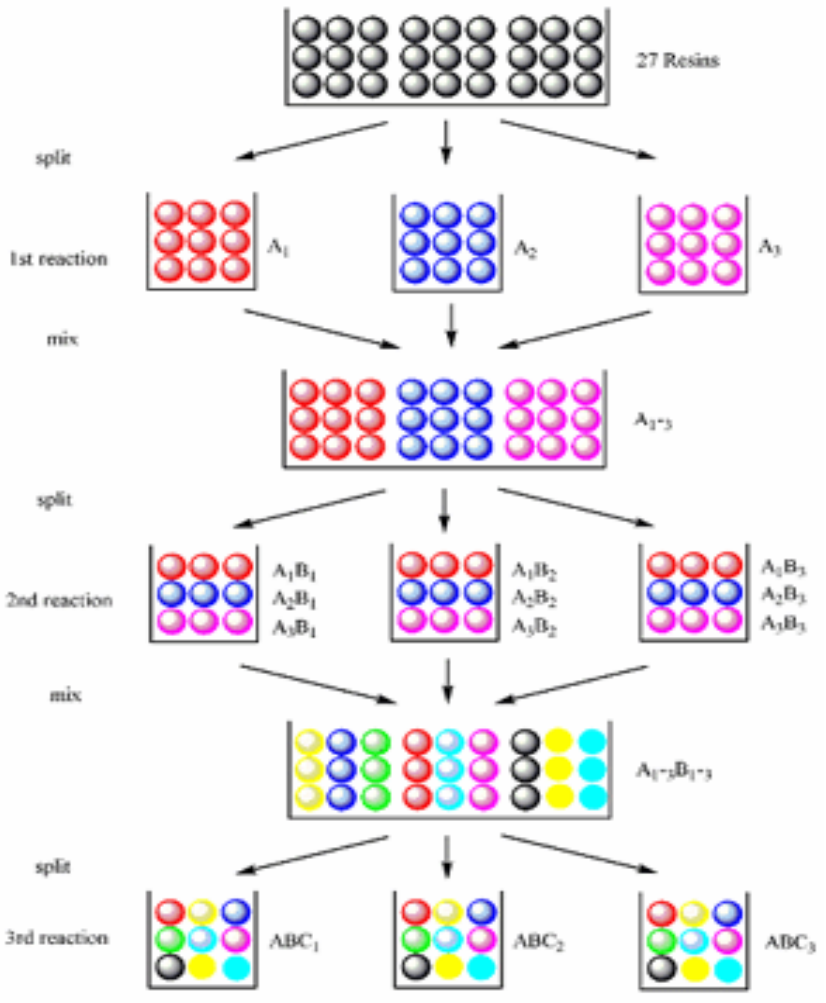
- Reaction proceeds in Solution.
- Can be used to produce libraries that consist of single compounds or mixtures.
- Single compound libraries are prepared using parallel synthesis.
- Easy characterization of intermediates as well as end product.
- Standard analytical protocols can be used to characterize products between each reaction step.



COMBINATORIAL CHEMISTRY

ON SOLID PHASE	IN SOLUTION
– large excess of reagents allowed	– all organic reactions can be used
– multistep synthesis allowed	– no chemistry assessment
– easy workup-isolation	– no linker/cleavage chemistry
– mix and split possible	– unlimited product quantities

Synthetic methods & techniques:
1. Split-Pool-Synthesis
2. Parallel Synthesis



1. Split-Pool-Synthesis

- Splitting of the resin, coupling with building block A1-A3
- Pooling, washing, deprotection
- Splitting, coupling with B1-B3
- Pooling, washing, deprotection
- Splitting, coupling with C1-C3

- After a Split-Pool-synthesis: just one single compound is bound to each resin bead

  ⇨ „one- bead- one- compound“ library

- Split-Pool-Procedure requires a solid support

Advantages:

•Only few reaction vessels required

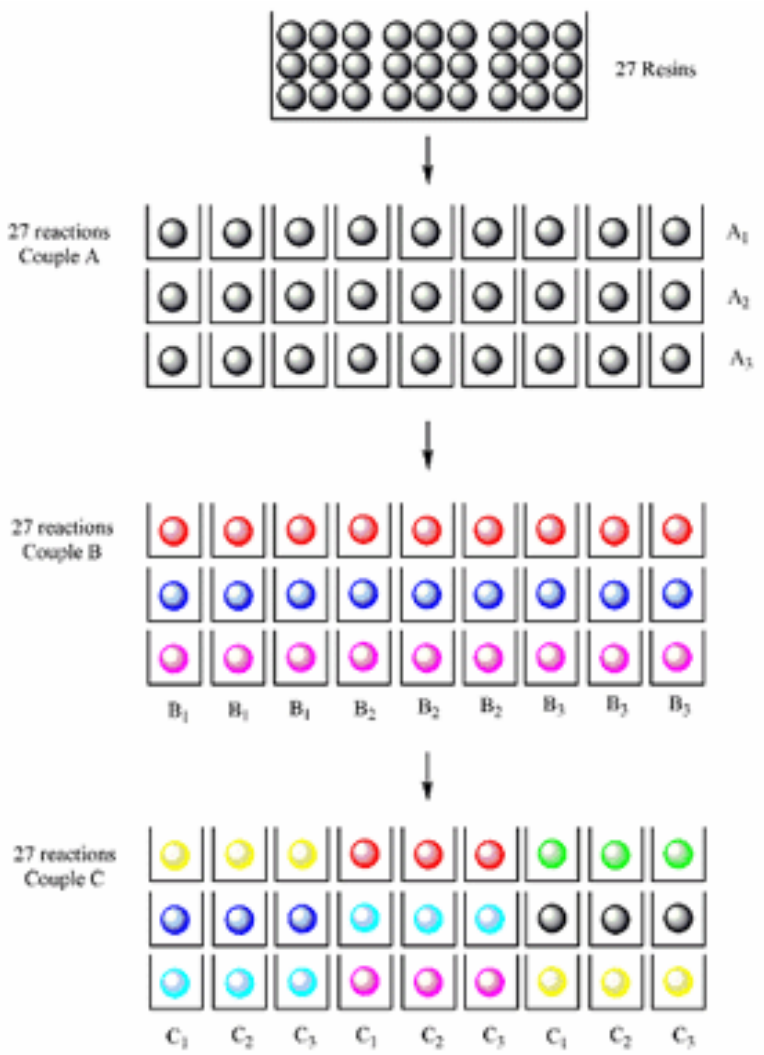
•Method of choice for large libraries (up to 105compounds)

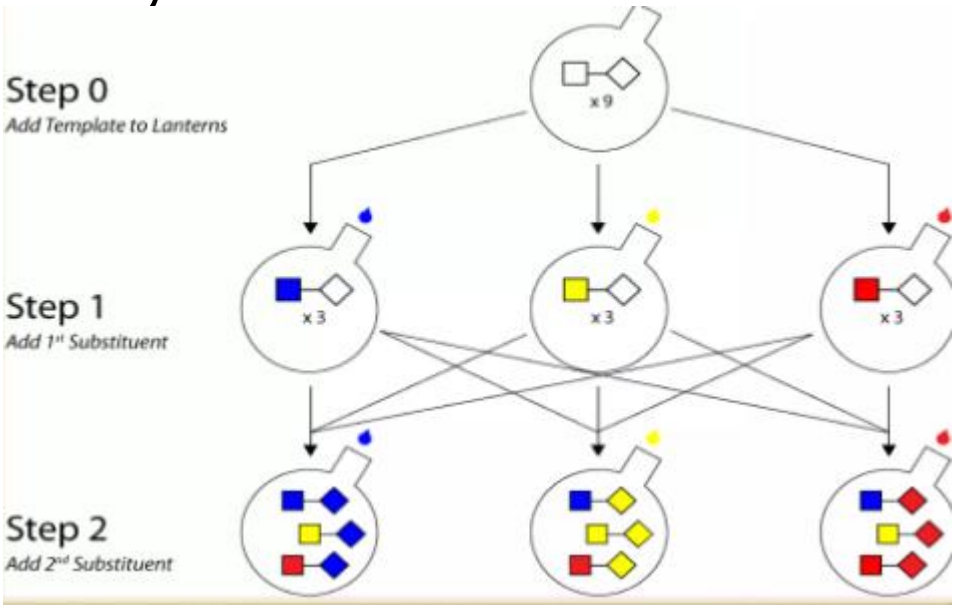
Disadvantages:

•Threefold amount of resin beads necessary

•Only little amounts of the synthesized compounds available

2. Parallel Synthesis





- Coupling with building block A1-A3(1/3 of the resin beads for each building block), then washing, deprotection
- Coupling with building block B1-B3(1/3 of the resin beads for each building block), then washing, deprotection
- Coupling with building block C1-C3(1/3 of the resin beads for each building block), then washing, deprotection

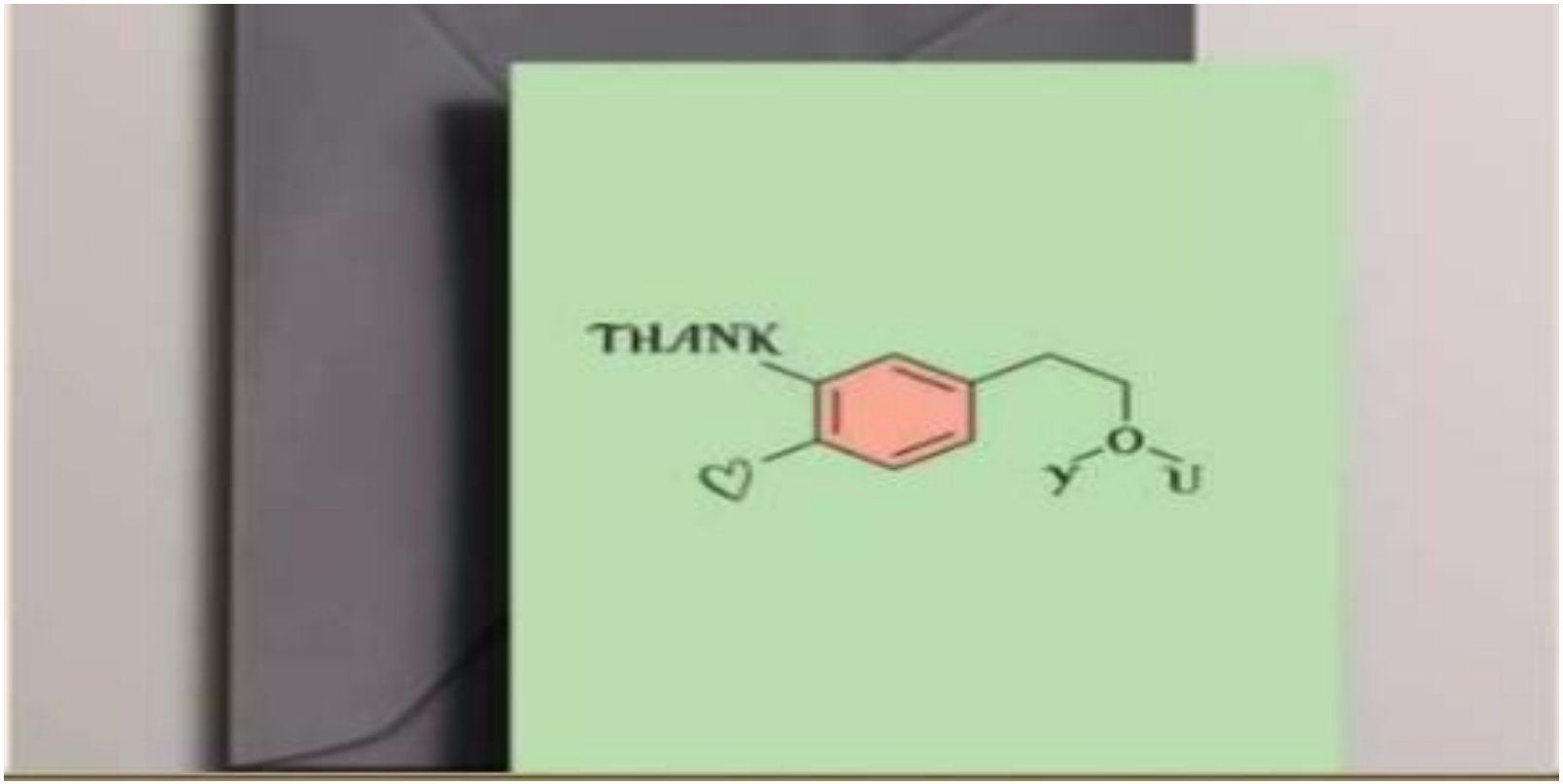
Sources:

•G. Jung: Combinatorial Chemistry, Synthesis, Analysis, Screening, p. 1-34, Wiley VCH, Weinheim 1999 (Chemistry Library: 86/VK5500J95)

•Pictures: http://www.nvu.edu/classes/ytchang/book/c007.html

https://www.google.com/search?q=split-pool-synthesis+combinatorial+chemistry&rlz=1C1GCEA_enIQ982IQ982&sxsrf=ALiCzsYYlyRIL3mooplTuLcHeeAUOcp3w:1671214978004&source=lnms&tbm=vid&sa=X&ved=2ahUKEwjNv-344P77AhVhS_EDHWUBBvIQ_AUoAnoECAEQBA&biw=1707&bih=781&dpr=0.8#fpstate=ive&vld=cid:c8a848f7,vid:BVQHw3BG7r0

https://www.google.com/search?q=combinatorial+chemistry&rlz=1C1GCEA_enIQ982IQ982&sxsrf=ALiCzsZk7-LUI-meTJAFv4Yj623Jmt0HaA:1670525954050&source=lnms&tbm=vid&sa=X&ved=2ahUKEwjZ3tCQ2ur7AhUoSvEDHUD1AXgQ_AUoAnoECAIQBA&biw=1292&bih=735&dpr=0.8#fpstate=ive&vld=cid:67ef75cd,vid:hU7UmrHo0O4



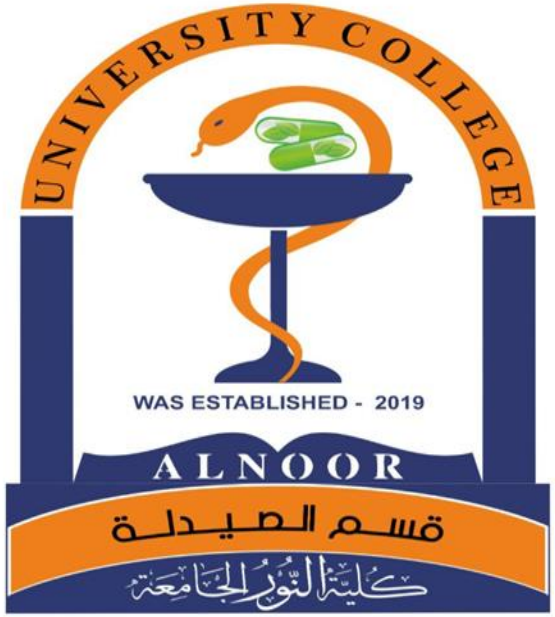
Organic Pharm. Chemistry IV

The Organic Chemistry of Drug Design and Drug Action

Lecture 10

31st December 2022

12:00- 2:00 PM



AlNoor University College

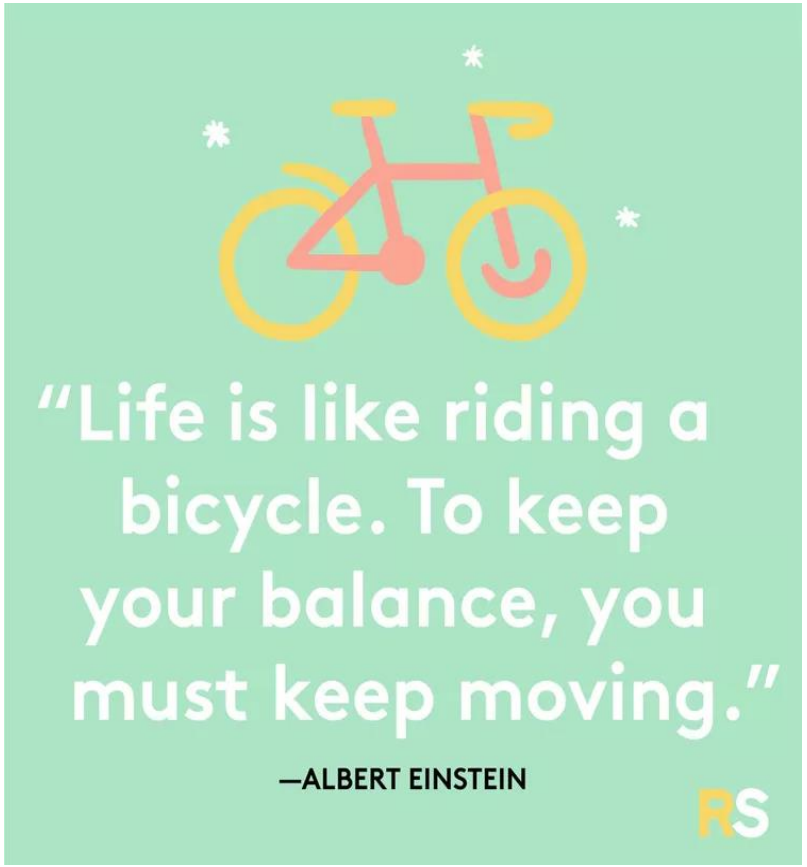
Pharmacy Department

1st Course/ 5th Class

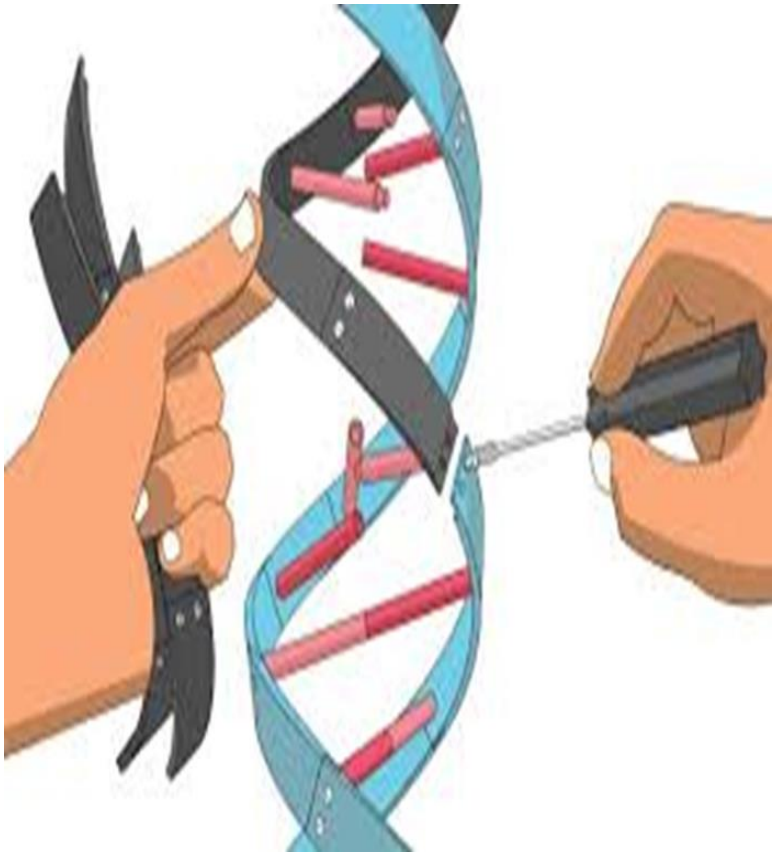
2022/2023

Be a Positive (B+)

- "Life is like riding a bicycle. To keep your balance, you must keep moving."



Recombinant DNA Technology

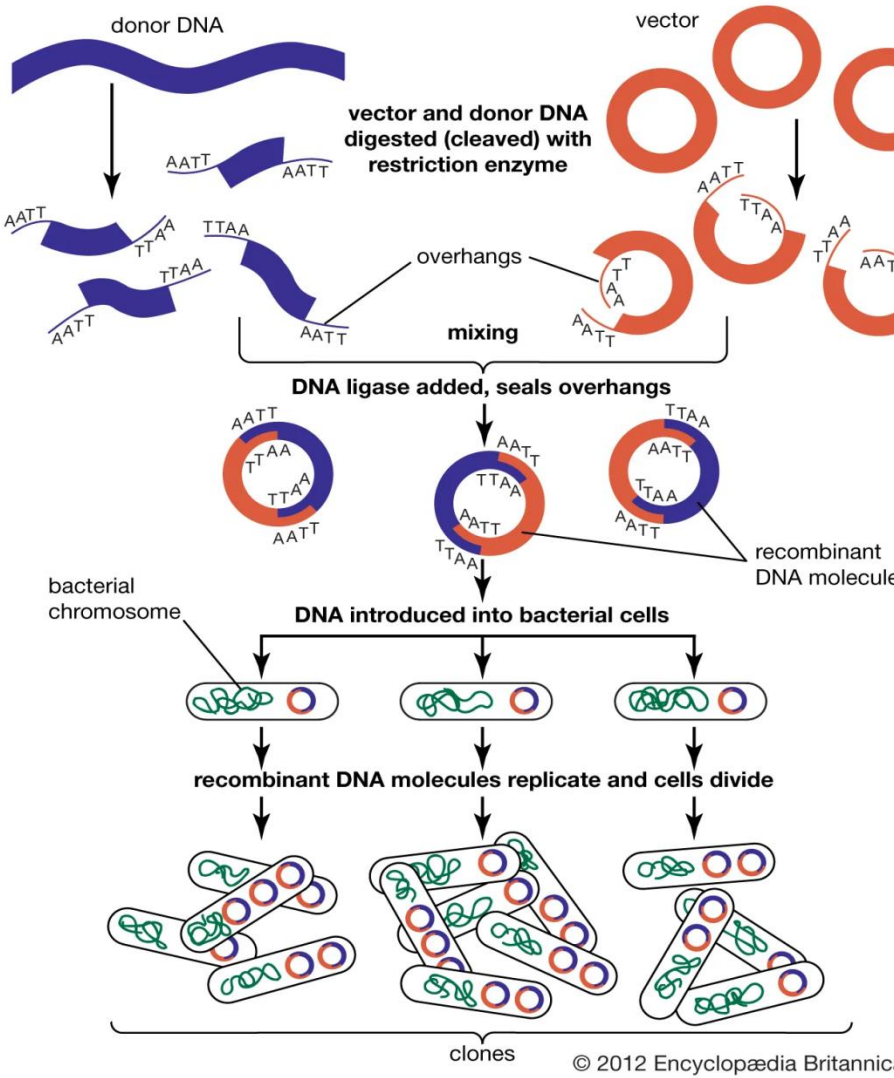


Please have a look to this YouTube

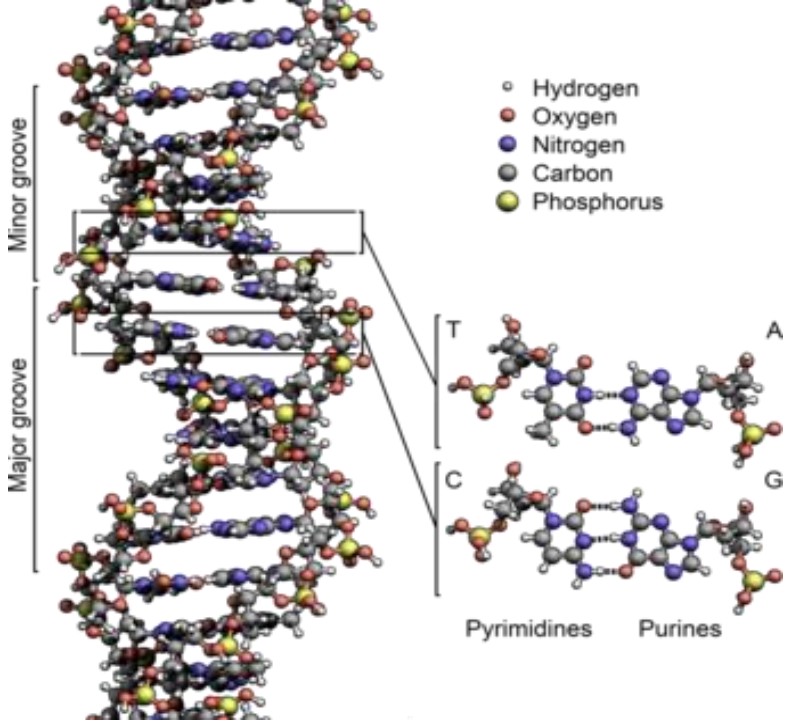
https://www.youtube.com/watch?v=C6DS20GktCI&t=276s&ab_channel=BoatofKnowledgeOhioUniversity

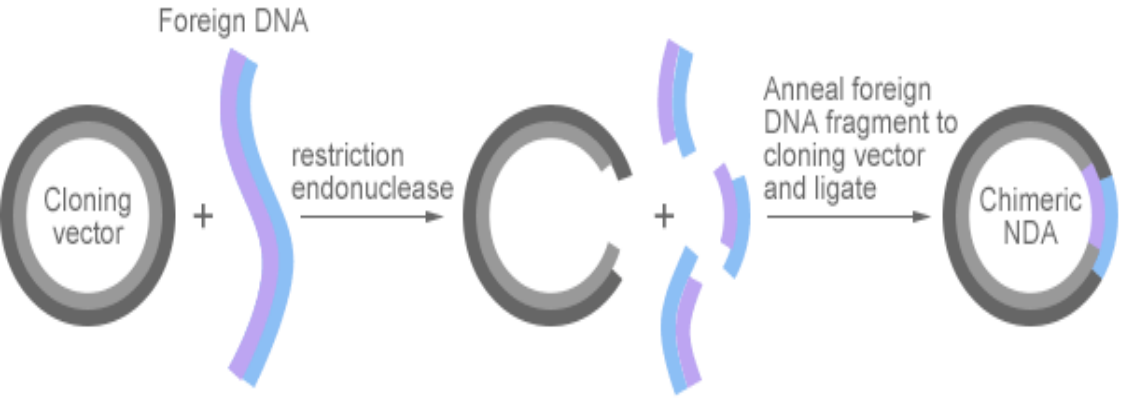
Recombinant DNA technology

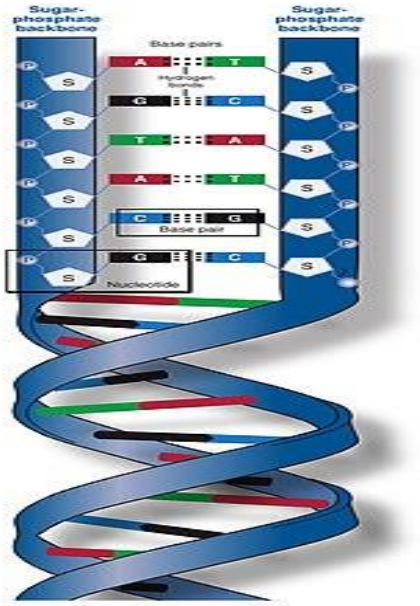
- Recombinant DNA technology involves **using enzymes and various laboratory techniques to manipulate and isolate DNA segments of interest**. This method can be used to combine (or splice) DNA from different species or to create genes with new functions. The resulting copies are often referred to as recombinant DNA



- Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, differing only in the nucleotide sequence. Recombinant DNA molecules are sometimes called **chimeric DNA** because they can be made of material from two different species like the mythical chimera..



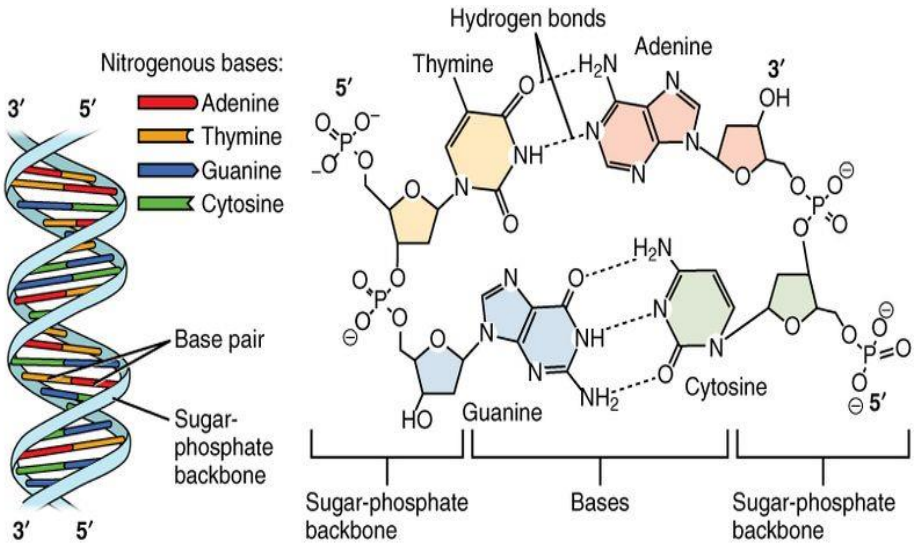


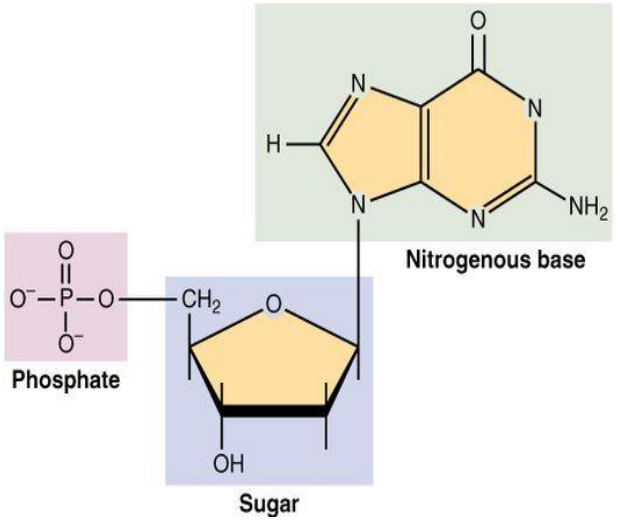


the arrangement of nucleotides within the structure of nucleic acids:

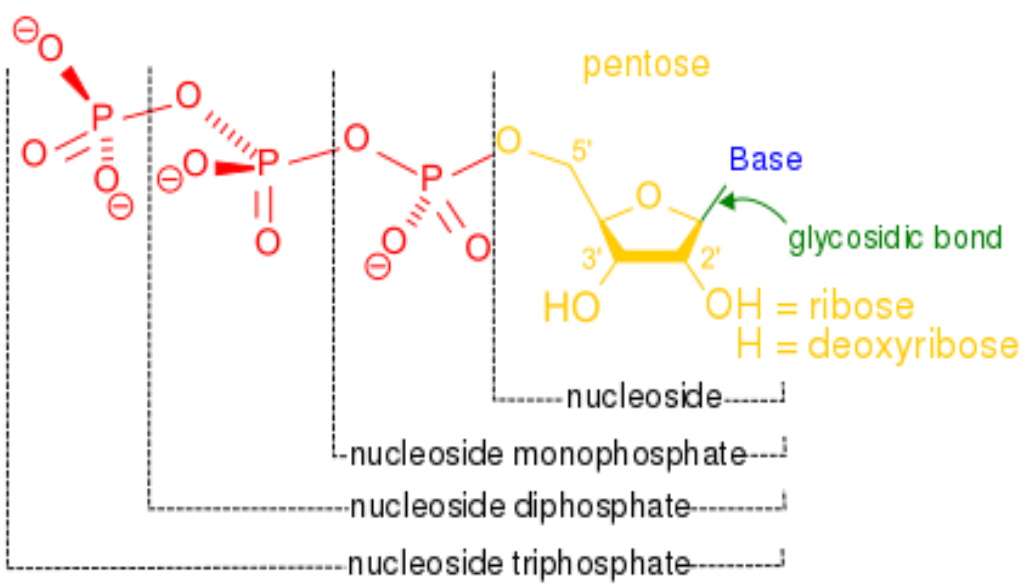
- A nucleotide is composed of three distinctive chemical sub-units: a five-carbon sugar molecule, a nucleobase (the two of which together are called a nucleoside), and one phosphate group.

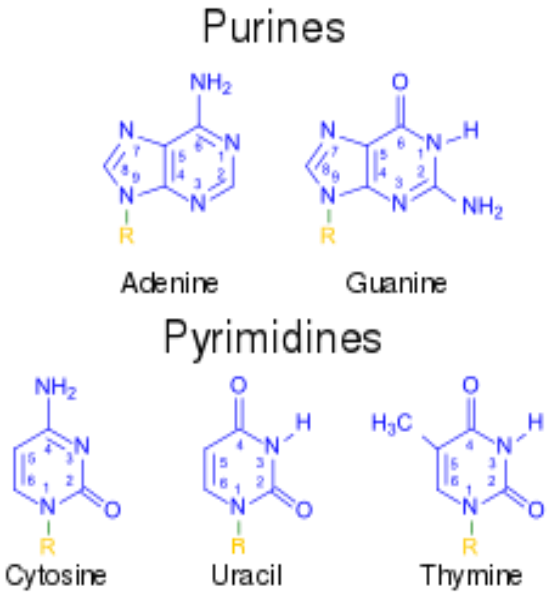
- With all three joined, a nucleotide is also termed“ nucleoside *mono*phosphate", "nucleoside *di*phosphate" or "nucleoside *tri*phosphate", depending on how many phosphates make up the phosphate group.

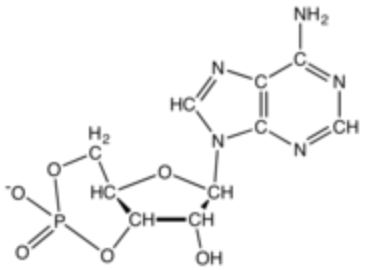


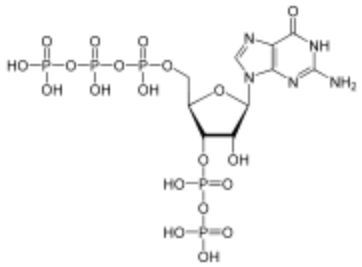


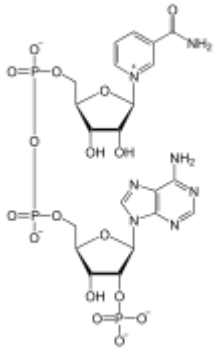
Structural elements of three nucleotides—where one-, two- or three-phosphates are attached to the nucleoside (in yellow, blue, green) at center:

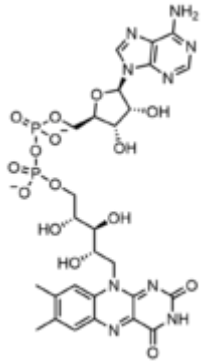












Examples of non-nucleic acid nucleotides

History

The idea of recombinant DNA was first proposed by **Peter Lobban**, a graduate student of Prof. **Dale Kaiser** in the Biochemistry Department at **Stanford University** Medical School. The first publications describing the successful production and intracellular replication of recombinant DNA appeared **in 1972** and 1973, from Stanford and UCSF.

In 1980 Paul Berg, a professor in the Biochemistry Department at Stanford and an author on one of the first papers was awarded the Nobel Prize in Chemistry for his work on nucleic acids "with particular regard to recombinant DNA". Werner Arber, Hamilton Smith, and Daniel Nathans shared the 1978 Nobel Prize in Physiology or Medicine for the discovery of restriction endonucleases which enhanced the techniques of rDNA technology.

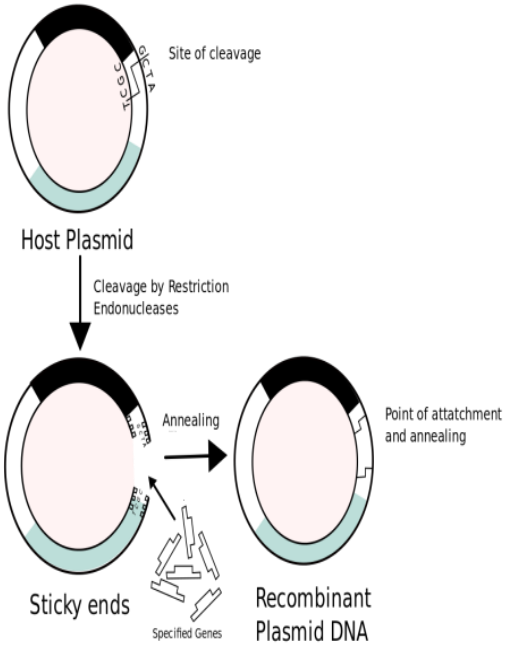
Stanford University applied for a US patent on recombinant DNA in 1974, listing the inventors as Herbert W. Boyer (professor at the University of California, San Francisco) and Stanley N. Cohen (professor at Stanford University); this patent was awarded in 1980.The first licensed drug generated using recombinant DNA technology was human insulin, developed by Genentech and licensed by Eli Lilly and Company

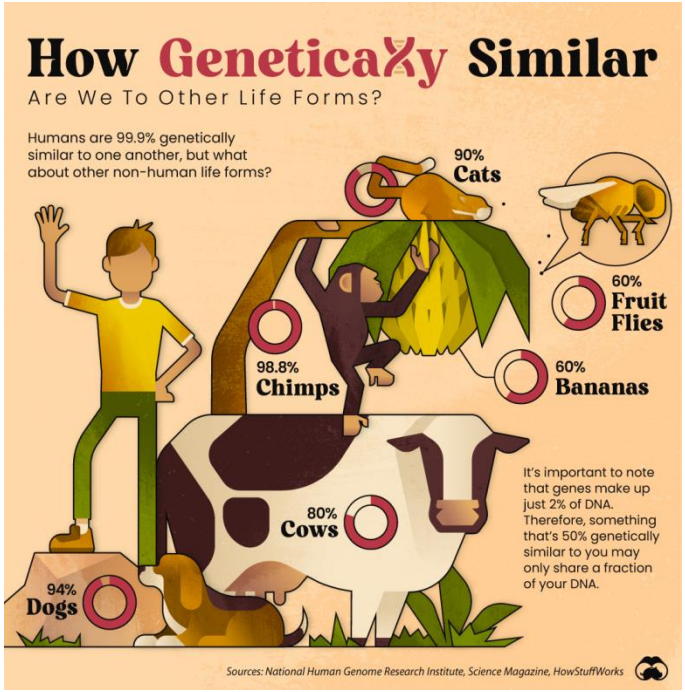
Introduction

- The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA can be joined to bacterial DNA, or human DNA can be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature can be created by the chemical synthesis of DNA and incorporated into recombinant DNA molecules. Using recombinant DNA technology and synthetic DNA, any DNA sequence can be created and introduced into living organisms.

- Proteins that can result from the expression of recombinant DNA within living cells are termed *recombinant proteins*. When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced. Expression of foreign proteins requires **the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences**

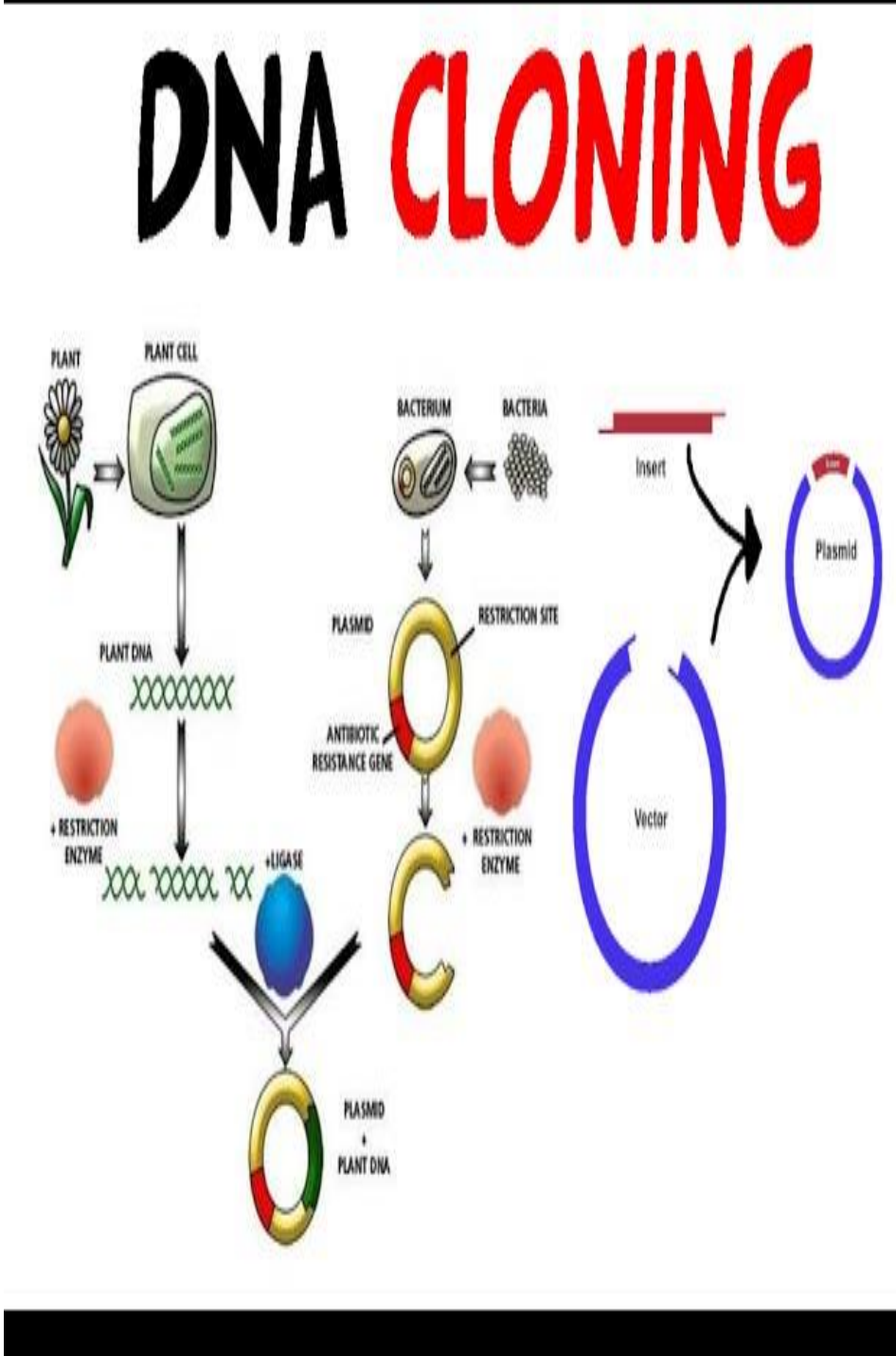
- **Recombinant DNA differs from genetic recombination in that the former results from artificial methods while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.**





Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as **molecular cloning**) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.

• Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure. They differ only in the nucleotide sequence within that identical overall structure.

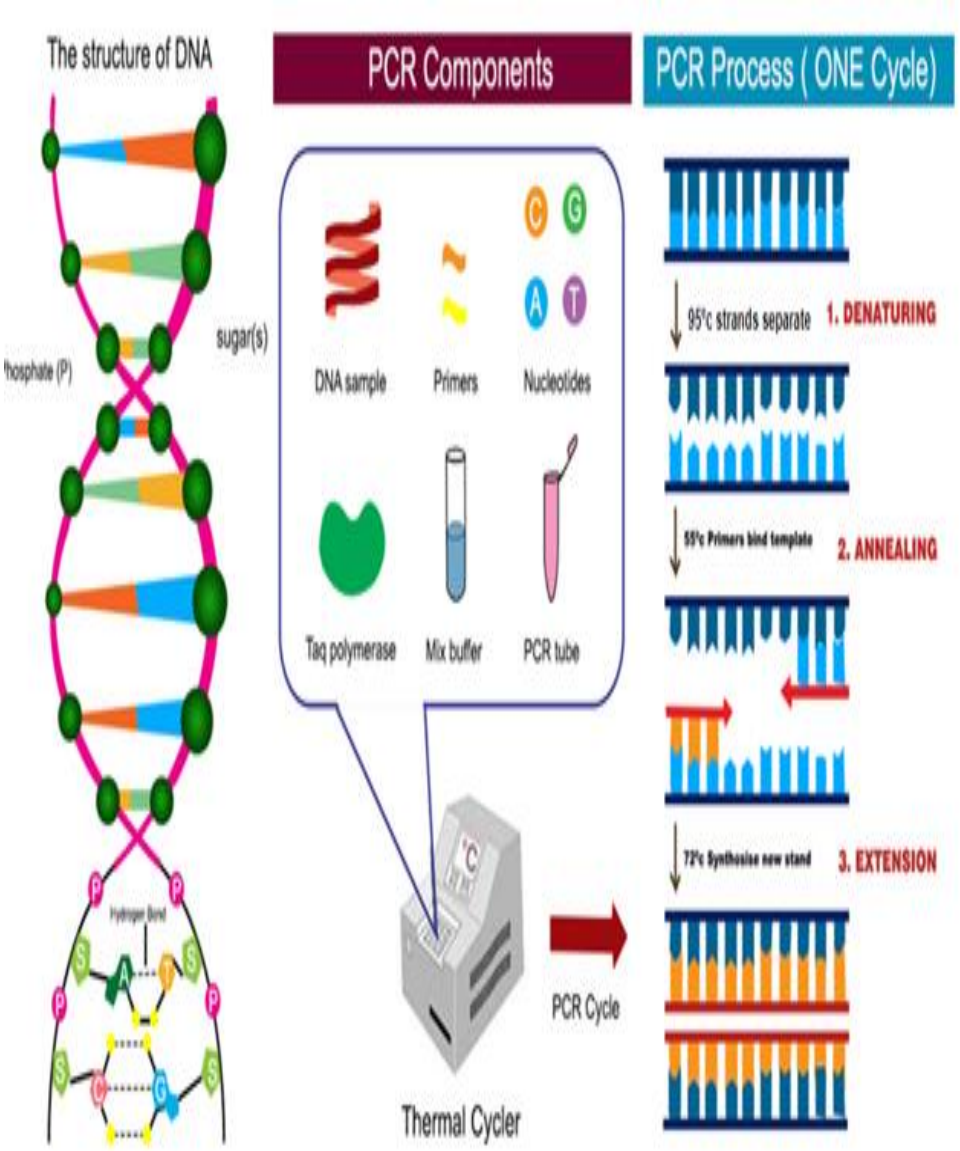


DNA creation

Molecular cloning is the laboratory process used to create recombinant DNA. It is one of two most widely used methods, along with polymerase chain reaction (PCR), used to direct the replication of any specific DNA sequence chosen by the experimentalist.

There are two fundamental differences between the methods. **One** is that molecular cloning involves **replication of the DNA within a living cell**, while PCR **replicates DNA in the test tube, free of living cells.**

The other difference is that cloning involves **cutting and pasting DNA sequences,** while **PCR amplifies by copying an existing sequence.**



In standard cloning protocols, the cloning of any DNA fragment essentially involves **seven steps:**

(1) Choice of host organism and cloning vector,

(2) Preparation of vector DNA,

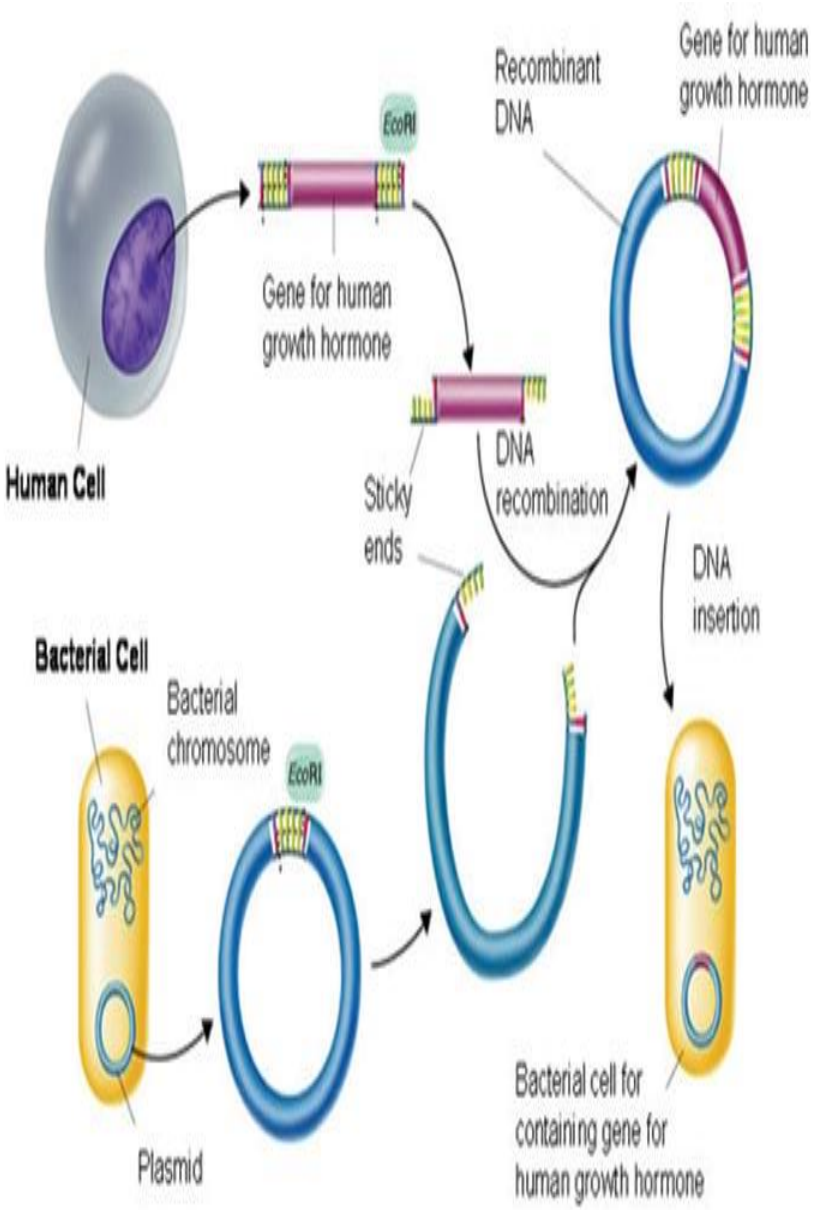
(3) Preparation of DNA to be cloned,

(4) Creation of recombinant DNA,

(5) Introduction of recombinant DNA into the host organism,

(6) Selection of organisms containing recombinant DNA, and

(7) Screening for clones with desired DNA inserts and biological properties.

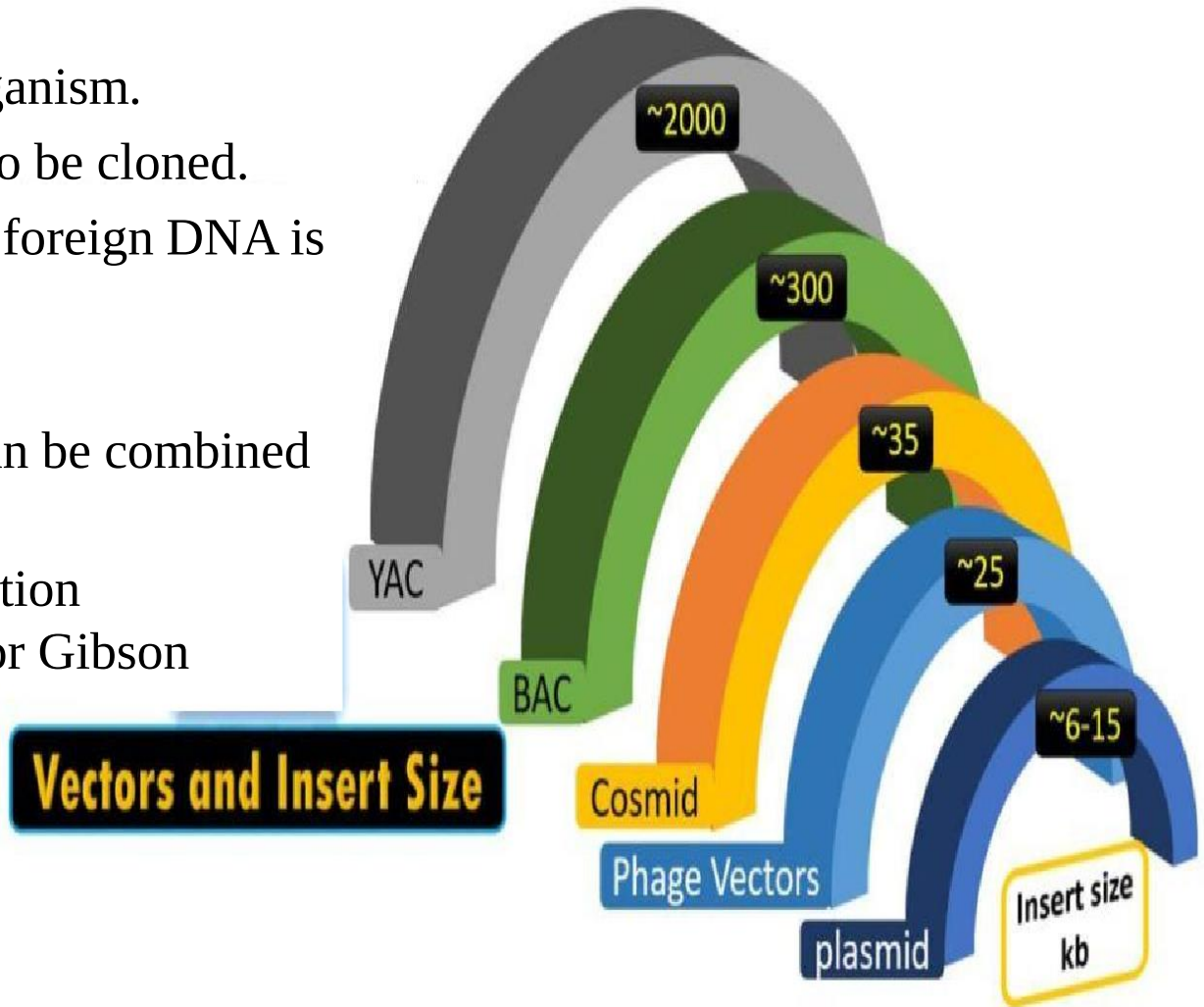


Choice of Vector

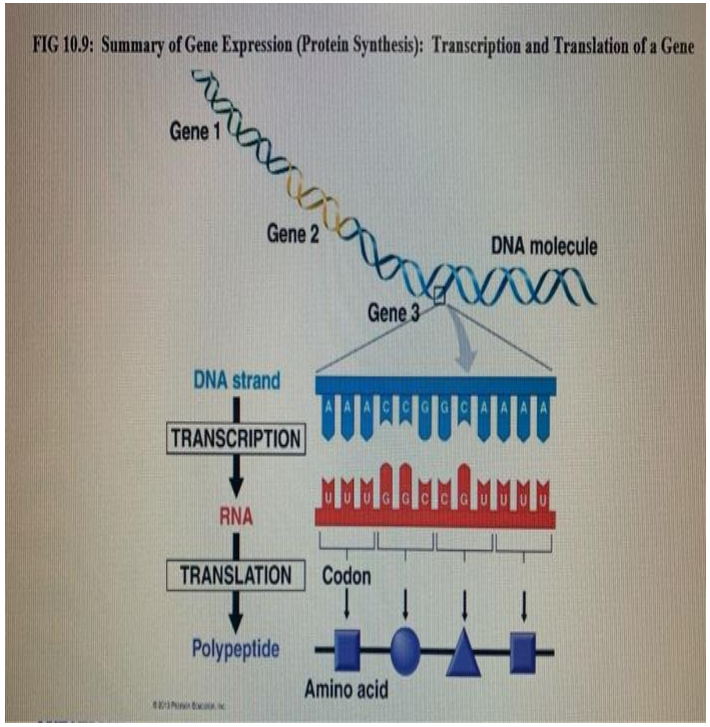
The choice of vector for molecular cloning depends on:

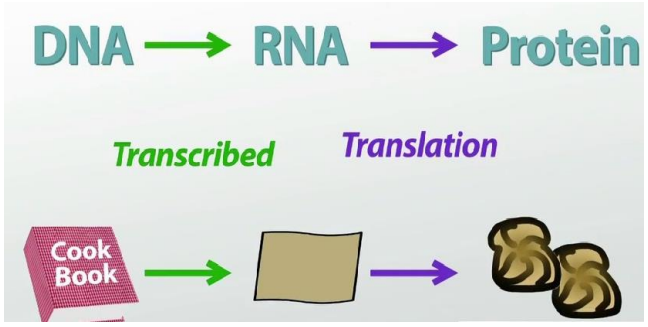
1. the choice of host organism.
2. the size of the DNA to be cloned.
3. whether and how the foreign DNA is to be expressed.

• The DNA segments can be combined by using a variety of methods, such as restriction enzyme/ligase cloning or Gibson assembly.



DNA expression





Following transplantation into the host organism, the foreign DNA contained within the recombinant DNA construct may or may not be expressed.

That is, **the DNA may simply be replicated without expression**, or it may be transcribed and translated and a recombinant protein is produced. Generally speaking, expression of a foreign gene requires restructuring the gene to include sequences that are required for producing an mRNA molecule that can be used by the host's translational apparatus (e.g. promoter, translational initiation signal, and transcriptional terminator).

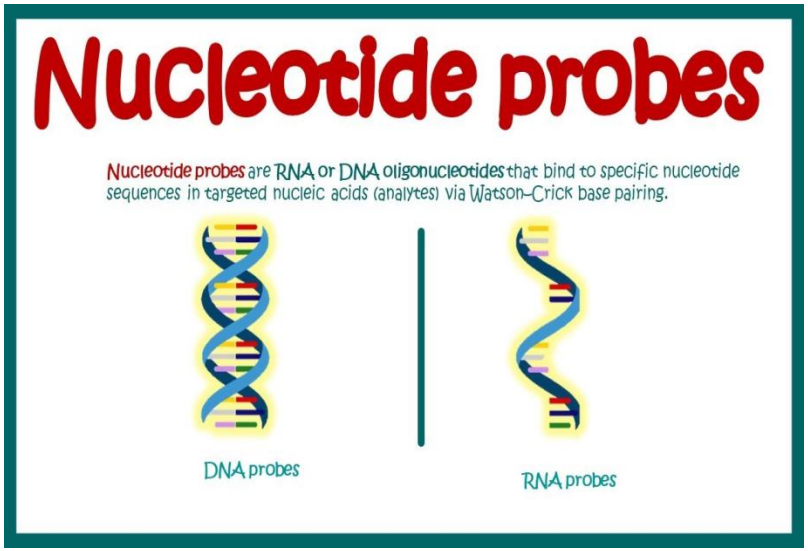
Specific changes to the host organism may be made to improve expression of the ectopic gene. In addition, changes may be needed to the coding sequences as well, to **optimize translation, make the protein soluble, direct the recombinant protein to the proper cellular or extracellular location, and stabilize the protein from degradation.**

Applications of recombinant DNA

- Recombinant DNA is widely used in biotechnology, medicine and research.
- Today, recombinant proteins and other products that result from the use of DNA technology are found in essentially every **western pharmacy, physician or veterinarian office, medical testing laboratory, and biological research laboratory.**
- In addition, organisms that have been manipulated using recombinant DNA technology, as well as products derived from those organisms, have found their way into many farms, supermarkets, home medicine cabinets, and even pet shops, such as those that sell GloFish and other genetically modified animals.

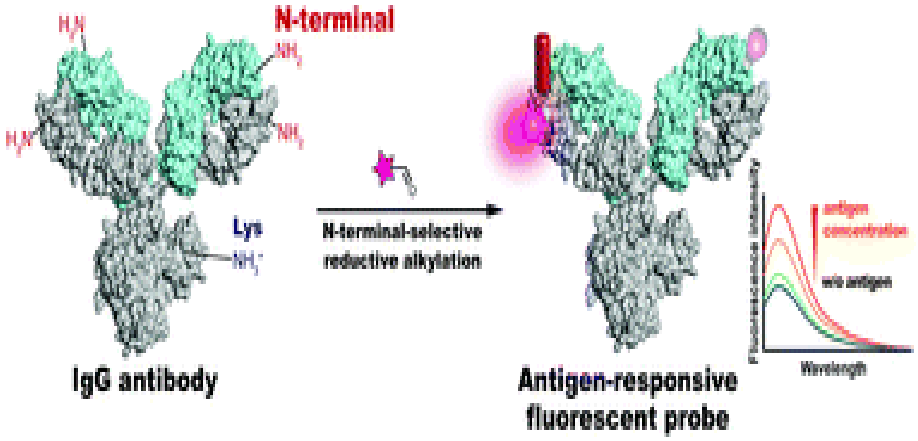
Applications of recombinant DNA

The most common application of recombinant DNA is in basic research, in which the technology is important to most current work in the biological and biomedical sciences. Recombinant DNA is used to identify, map and sequence genes, and to determine their function. **rDNA probes** are employed in analyzing gene expression within individual cells, and throughout the tissues of whole organisms.



Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organisms.

Many additional practical applications of recombinant DNA are found in industry, food production, human and veterinary medicine, agriculture, and bioengineering.

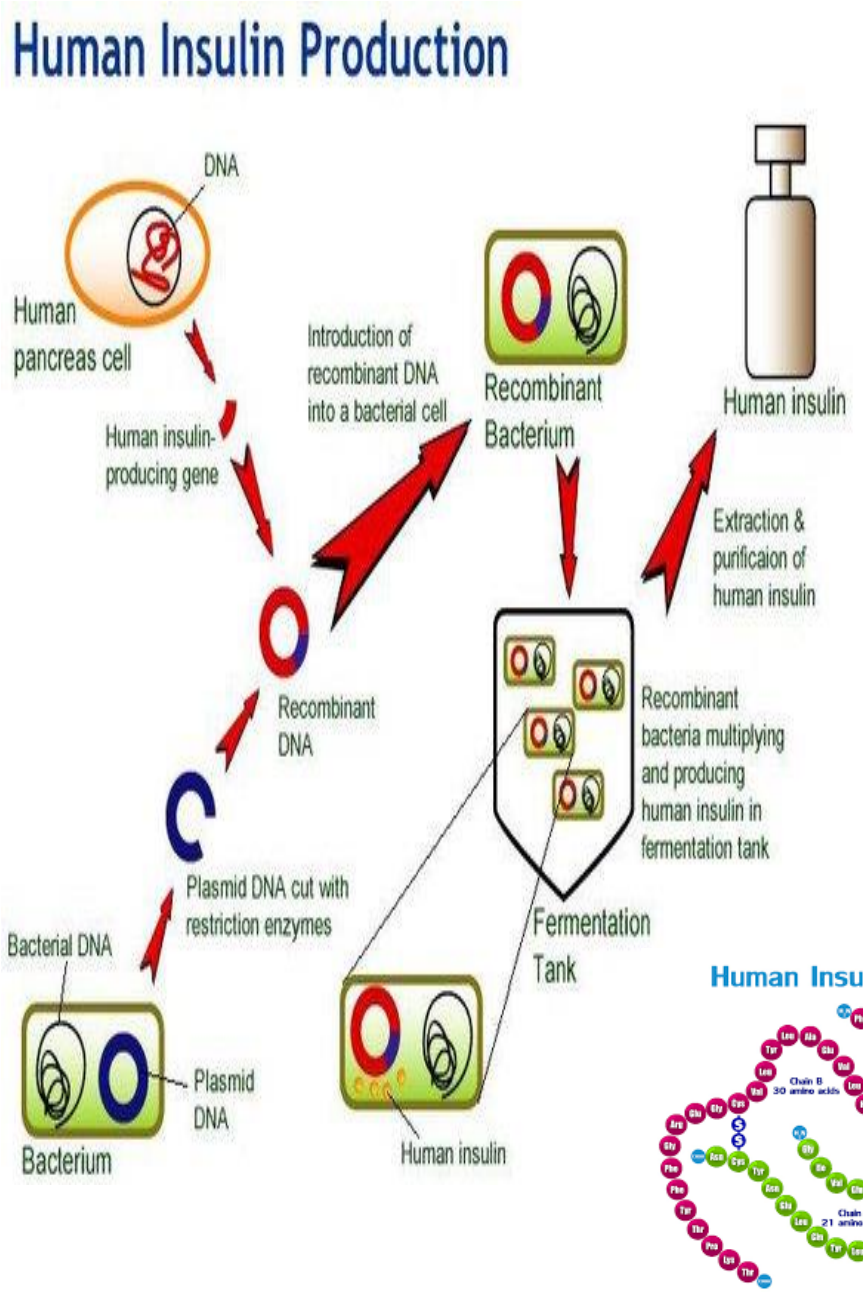


Recombinant human insulin.

Almost completely replaced insulin obtained from animal sources (e.g. pigs and cattle) for the treatment of insulin-dependent diabetes.

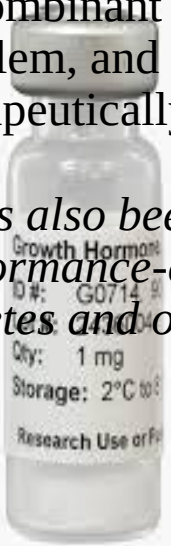
A variety of different recombinant insulin preparations are in widespread use.

Recombinant insulin is synthesized by inserting the human insulin gene into *E. coli*, or yeast (Saccharomyces cerevisiae) which then produces insulin for human use.

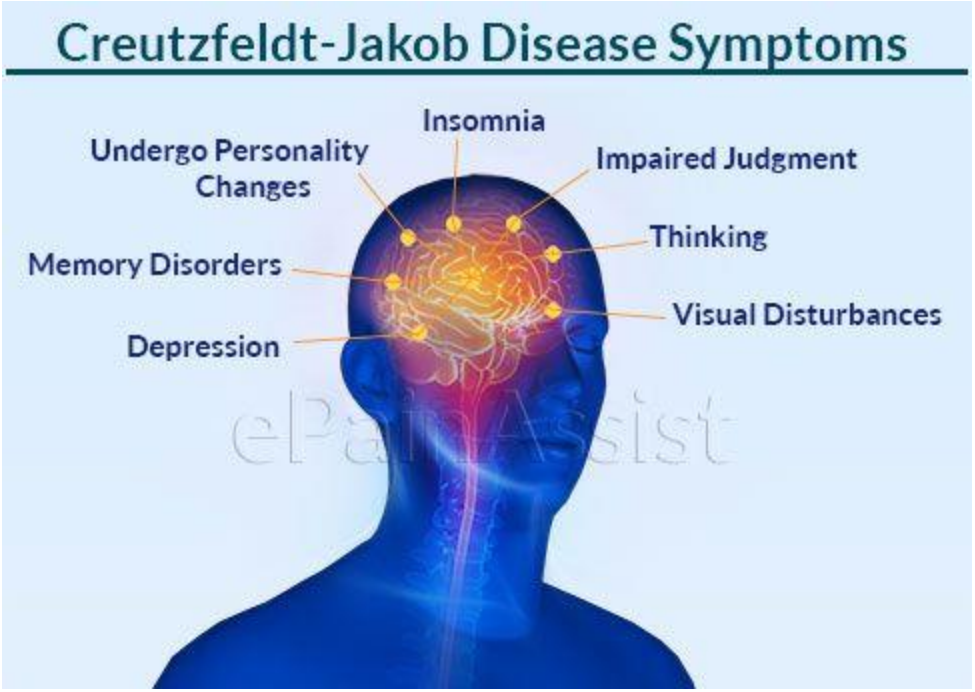


Recombinant human growth hormone (HGH,

(Somatotropin)Administered to patients whose pituitary glands generate insufficient quantities to support normal growth and development. Before recombinant HGH became available, HGH for therapeutic use was obtained from pituitary glands of cadavers جثث. This unsafe practice led to some patients developing Creutzfeldt–Jakob disease. Recombinant HGH eliminated this problem, and is now used therapeutically.



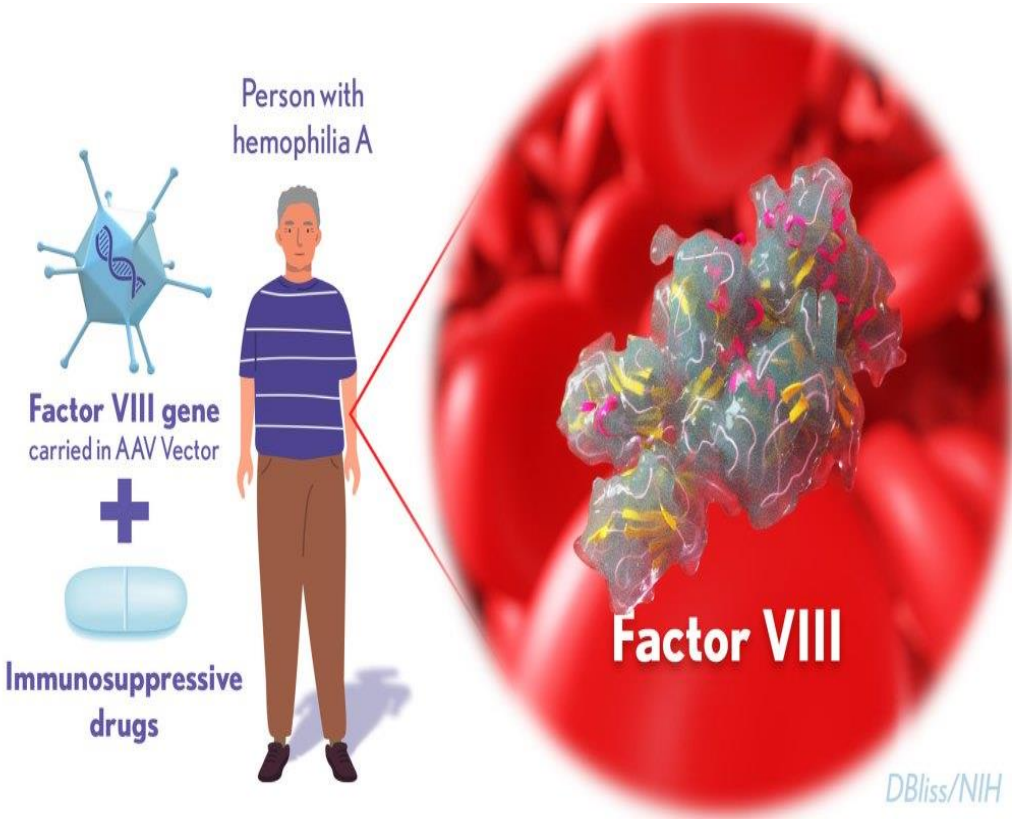
It has also been misused as a performance-enhancing drug by athletes and others.



Recombinant blood clotting factor VIIIA

blood-clotting protein that is administered to patients with forms of the bleeding disorder **hemophilia**, who are unable to produce **factor VIII** in quantities sufficient to support normal blood coagulation.

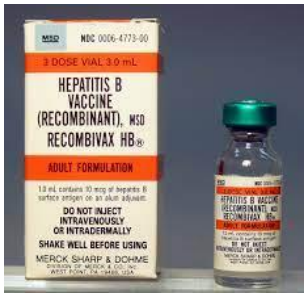
Before the development of recombinant factor VIII, the protein was obtained by processing large quantities of human blood from multiple donors, which carried a very high risk of transmission of blood borne infectious diseases, for example HIV and hepatitis B.

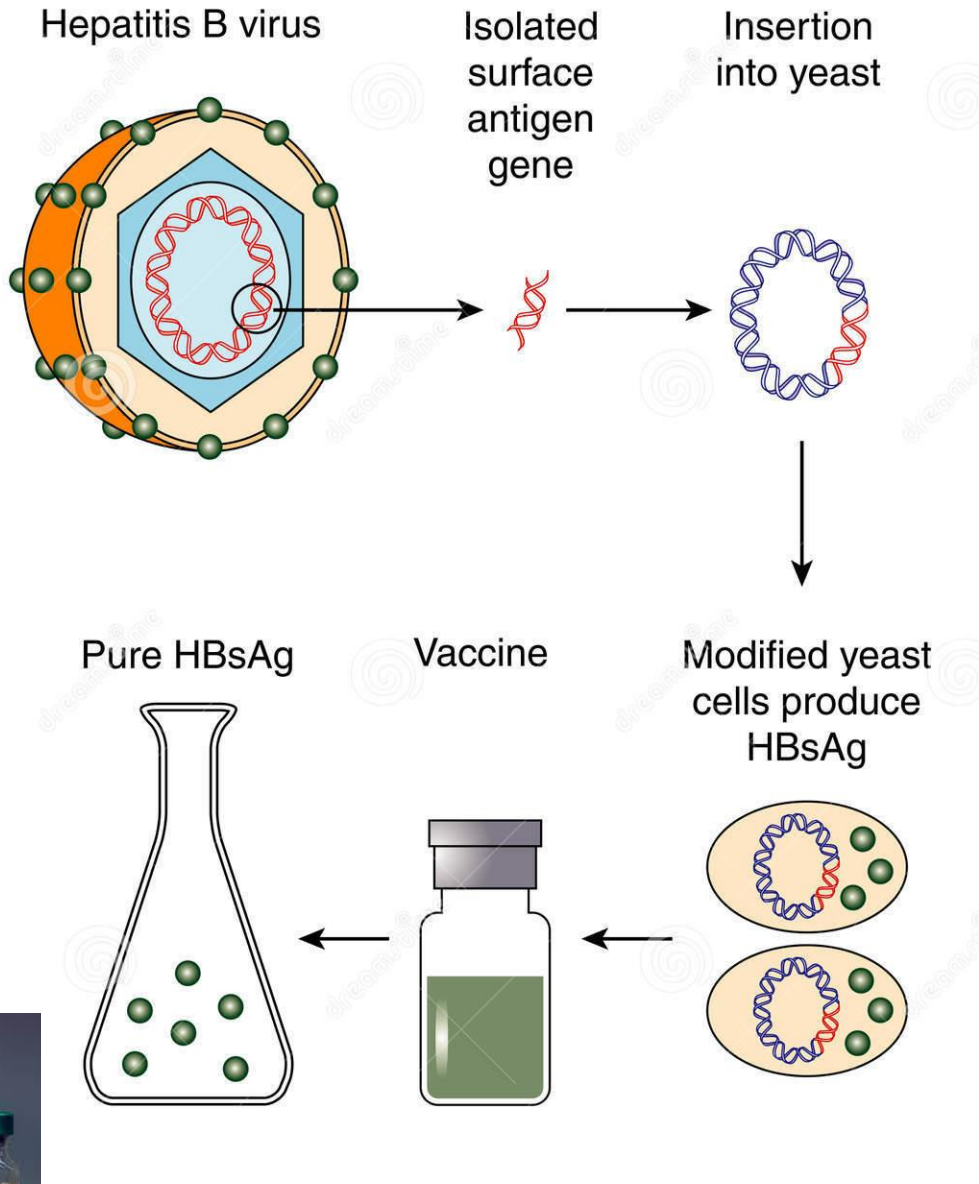


Recombinant hepatitis B vaccine Hepatitis B

infection is controlled through the use of a recombinant hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells.

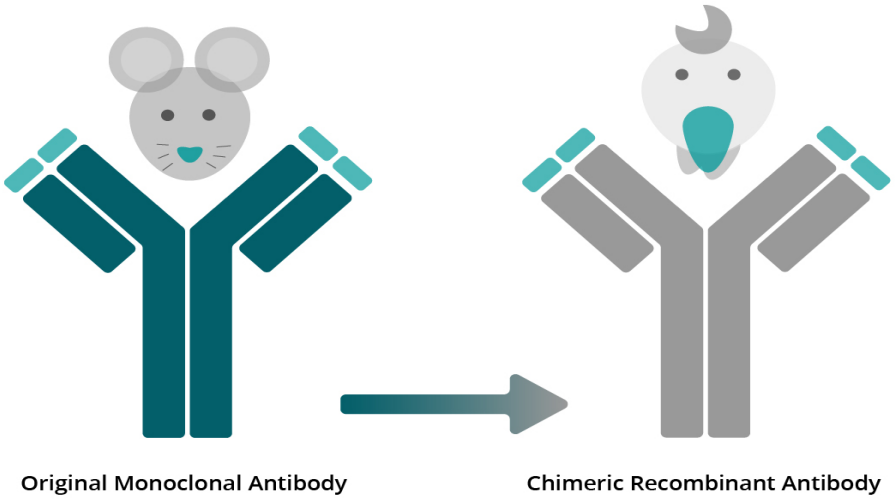
The development of the recombinant subunit vaccine was an important and necessary development because hepatitis B virus, unlike other common viruses such as polio virus, cannot be grown in vitro





Recombinant antibodies

Recombinant antibodies (rAbs) are produced in vitro by the means of expression systems based on mammalian cells.



Their mono specific binding to a specific epitope makes rAbs eligible not only for research purposes, but also as therapy options against certain cancer types, infections and autoimmune diseases.

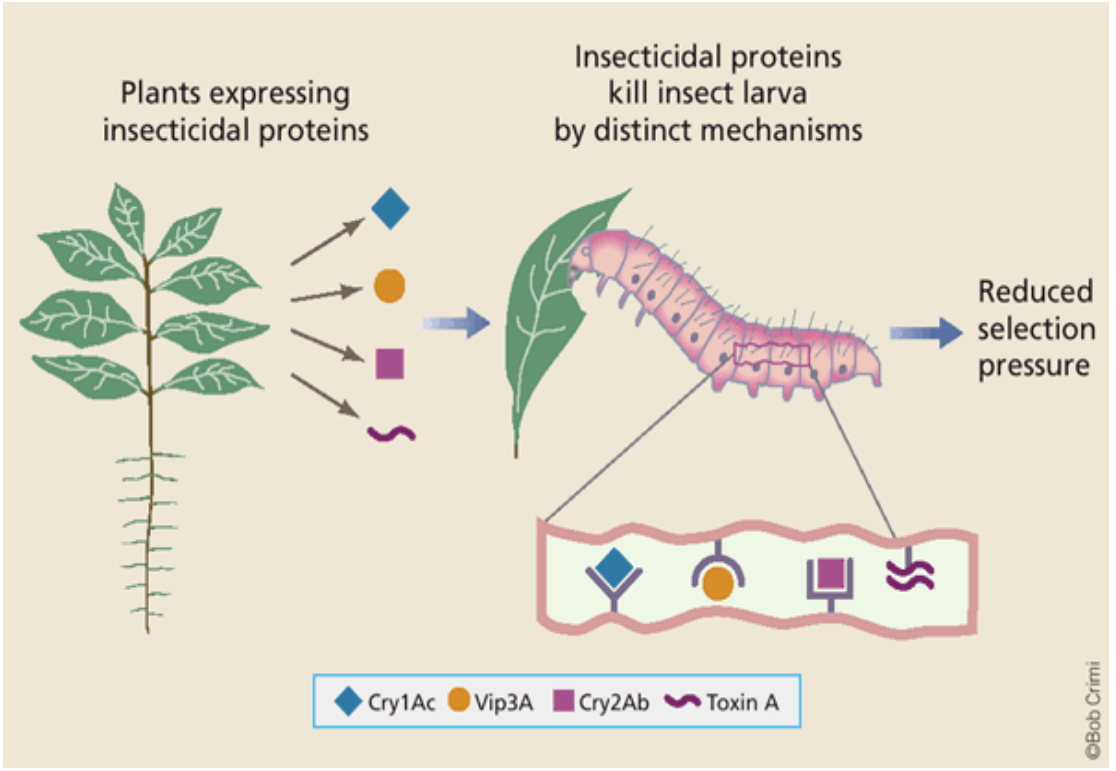
Golden rice

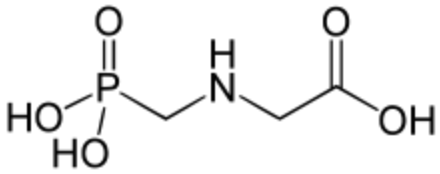
A recombinant variety of rice that has been engineered to express the enzymes responsible for β-carotene biosynthesis.This variety of rice holds substantial promise for reducing the incidence of vitamin A deficiency in the world's population.

Golden rice is not currently in use, pending the resolution of regulatory and intellectual property issues.

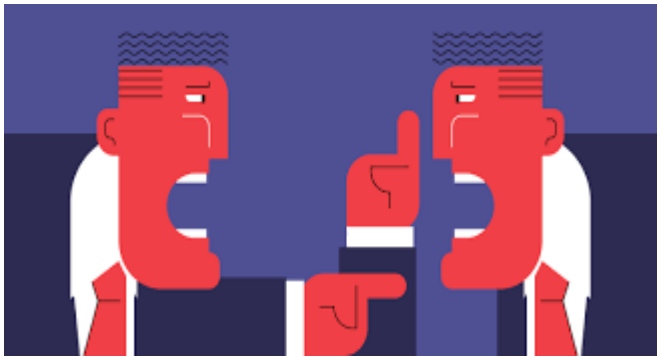
Herbicide-resistant crops

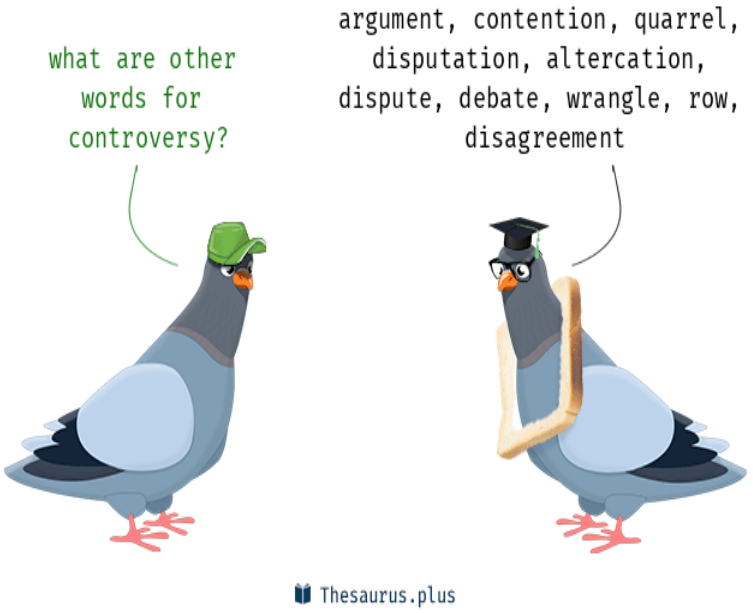
Commercial varieties of important agricultural crops (including soy, maize/corn, sorghum, canola, alfalfa and cotton) have been developed that incorporate a recombinant gene that results in resistance to the herbicide glyphosate (trade name *Roundup*), and simplifies weed control by glyphosate application. These crops are in common commercial use in several countries.





Controversy





- Scientists associated with the initial development of recombinant DNA methods recognized that the potential existed for organisms containing recombinant DNA to have undesirable or dangerous properties.
- At the 1975 Asilomar Conference on Recombinant DNA, these concerns were discussed and a voluntary moratorium on recombinant DNA research was initiated for experiments that were considered particularly risky. This moratorium was widely observed until the National Institutes of Health (USA) developed and issued formal guidelines for rDNA work.
- **Today, recombinant DNA molecules and recombinant proteins are usually not regarded as dangerous.**

However, concerns remain about some organisms that express recombinant DNA, particularly when they leave the laboratory and are introduced into the environment or food chain.

Furthermore, there are concerns about the **by-products** in biopharmaceutical production, where recombinant DNA result in specific protein products.

The major by-product, termed host cell protein, comes from the host expression system and poses a threat to the patient's health and the overall environment

Any Question??