Organic Reactions of Drug Design, Development and Drug Action

(Student references)

Drug discovery

Medicinal chemistry deals with the discovery of new therapeutic chemicals and their development into useful medicine. Drugs are the molecules or chemicals used as medicine or as components in medicine to diagnose, cure, mitigate, treat or prevent disease. The discovery of a drug is a time-consuming (12-15 years) and expensive ($600-800 million) process before being introduced in the market. There are three phases of clinical trials prior to approval of drug in the market: (i) **Phase I** (usually one year) evaluates safety, tolerability, dosage level, side effects, pharmacokinetic properties and pharmacological effect in 20-100 volunteers; (ii) **Phase II** (1-3 years) determines the effectiveness and safety aspect of a drug and its doses in disease patients; (iii) **Phase III** (about 2-6 years) includes trials in several thousands of patients in clinics and hospitals to establish the efficacy of drug for the long time use. Finally, the new drug application (NDA) needs the approval of Food and Drug Administration (FDA) for commercial use which usually takes several years. However, the success rate for human clinical trials and hence availability of the 'drug' in the market is very low (<10 %).

Actually drugs are not discovered (it is a misnomer); what is being discovered is known as **lead** compound. It is defined as "a prototype compound that has a number of attractive characteristics, such as the desired biological or pharmacological activity, but may have other undesirable characteristics, for example, high toxicity, other biological activities, absorption difficulties, insolubility, or metabolism problems". The structure of the lead compound is modified synthetically to improve its clinical nature as drug candidate in terms of biological, --pharmacological and animal studies.

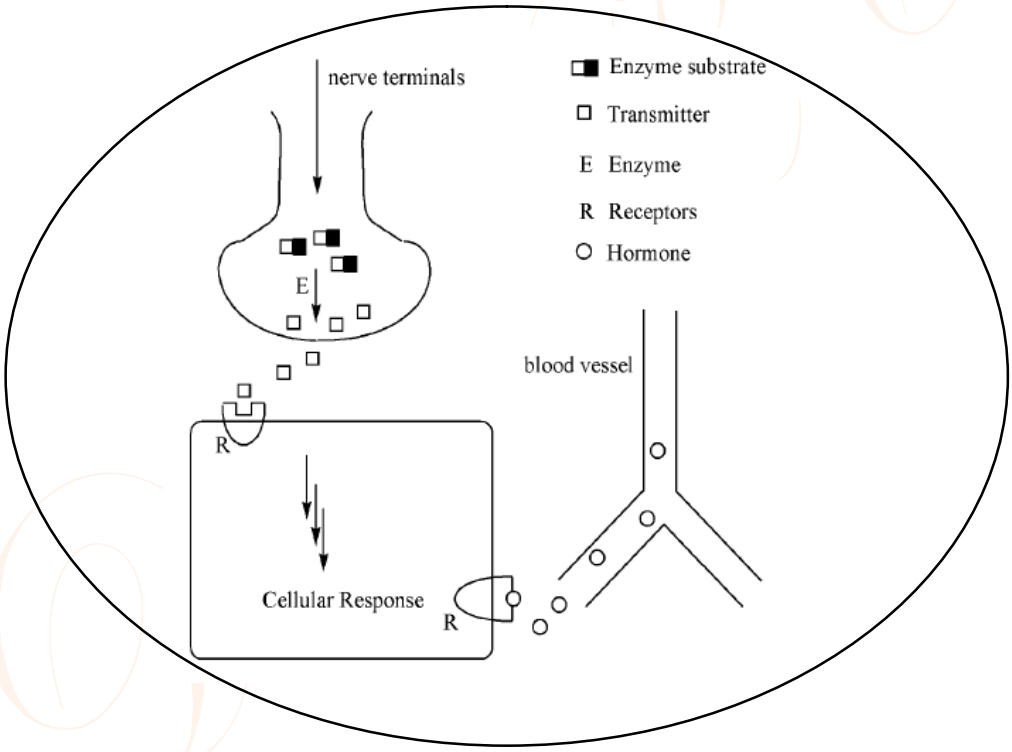
Despite our constant search for new drugs, nature is still an excellent source of new drug or the precursor of drug. Almost 40% of 520 new drugs approved in the market during 1980-2000 and more than 60% of anticancer and anti-infective agents in either clinical trials or in the market came from the natural sources (natural products or its derivatives). The reason is due to intrinsic nature of secondary metabolite to act in defense of their producing organism. For instance, fungal natural product is produced to defend microorganism, fungi, bacteria or cell replication of foreign organism. In addition, it is possible to modify those metabolites chemically to improve their properties.

Modern study of drug discovery focuses on genomics or proteomics research to access new drug targets by means of identifying important target genes and gene products (proteins expressed by genes) or by investigating new proteins in the organism's proteome.

Modern Drug Discovery

- Programme selection (choosing a disease to work on)
- Identification and validation a drug target
- Assay development
- Identification of a “lead compound”
- Lead optimization
- Identification of a drug candidate
- Clinical trials
- Release of the drug
- Follow-up monitoring

Principles of drug action



How specific have to be Drug-target interactions?

Let us consider, 1 mg drug having MW 200 $gmol^{-1}$. (1 mole = 6 x□10^{23})

So, 1 mg of drug provides (6.023 x□10^{23}) x□(10^{-3}/200) ≈ 3 x□10^{18} molecules.

A human has approximately 3□x 10^{13} cells which results average (3 x □10^{18}/3 x□10^{13}) = 1x□10^{5} number of drug molecules per cell.

An erythrocyte (a typical cell) contains approx 10^{10} molecules. This means 1 molecule of drug per (10^{10}/10^{5}) = 100,000 cellular molecules.

Suppose 500 mg of medicine with MW MW 200 $gmol^{-1}$ is taken for a symptom; meaning 1 molecule from medicine works per 1000 cellular molecules which eventually very high in number (because out of 1000 cellular molecules very few are active for the symptom).

Few important terminologies:

Lead Compounds:

A lead compound ("leading" compound or scaffold or template) in drug discovery is a chemical compound or a prototype compound that has a number of attractive characteristics, such as the desired biological or pharmacological activity, but may have other undesirable characteristics, for example, high toxicity, other biological activities, absorption difficulties, insolubility, or metabolism problems. Thus, a lead might have both pharmacological and biological activity likely to be therapeutically useful, and also its sub-optimal structure requires modification to fit better to the target. The structure of a lead is chemically modified to ‘drug-like or clinical-use’ molecules so that it improves potency, selectivity, or pharmacokinetic parameters.

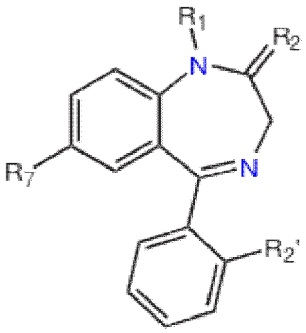
The lead compound provides a start for the drug design and development process. Natural products are the rich source of lead compounds. The agent responsible for biological activity of a natural extract is known as the **active principle**. Lead compounds have been isolated from plants, trees, microorganisms, animals, venoms, and toxin. Lead compounds have been generated from high throughput screening. Lead compounds can also provide a new way for therapeutic or diagnostic implication.

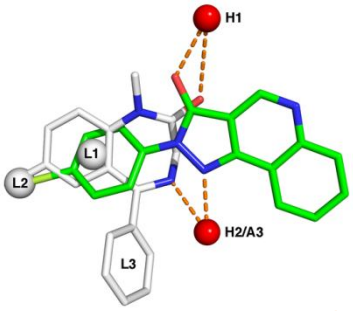
Pharmacophore:

Pharmacophore is a group of atom in molecule (to be the lead) or a vital part of a molecule or essential geometric arrangement of atoms or functional groups (stereochemical features) necessary to produce a given biological responses.

According to IUPAC definition 'A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response.'

Pharmacophore mapping is one of the major elements of drug design in the absence of structural data of the target receptor. The pharmacophore strategy was initially applied to discovery of lead molecules and now it has been extended to lead optimization.





Benzodiazepine binding site on the GABAA receptor

A pharmacophore model is a geometrical description of the chemical functionalities required of a ligand to interact with the receptor. A classic example of a pharmacophore model is the benzodiazepine binding site on the GABAA receptor. White sticks represent the carbon atoms of the benzodiazepine diazepam, while green represents carbon atoms of the nonbenzodiazepine CGS-9896. Red and blue sticks are oxygen and nitrogen atoms that are present in both structures. The red spheres labeled H1 and H2/A3 are, respectively, hydrogen bond donating and accepting sites in the GABAA receptor, while L1, L2, and L3 denote lipophilic binding sites. White structure representing benzodiazepine interacts with receptor more strongly than the green structure of nonbenzodiazepine.

The pharmacophore approach has proven to be successful, allowing (i) the perception and understanding of key interactions between a target and a ligand and (ii) the enrichment of hit rates obtained in experimental screening of subsets that have been obtained from *in silico* or virtual screening experiments.

Pharmacokinetics:

Pharmacokinetics (pharmakon = "drug" and kinetikos = "moving motion") determines the kinetics phenomena of a drug/lead/substance (pharmaceutical agents, hormones, nutrients, and toxins) from the moment it is administered externally to a living organism to excretion from the body. Pharmacokinetics evaluates the effect of a specific drug

action in the body. The pharmacokinetic phenomena includes the mechanisms of absorption and distribution, chemical changes of drugs in the body by metabolic enzymes (cytochrome P450 or glucuronosyltransferase enzymes), and the effects and routes of excretion of the metabolites of the drug. Pharmacokinetic is the result of following five processes.

Pharmacokinetics = Liberation + Absorption + Distribution + Metabolization + Excretion
L A D M E

The following most commonly pharmacokinetic matrix are measured.

1. Dose (amount of drug)
2. Time interval of dose administered
3. Effective concentration of drug after administration
4. Time to reach for a drug to act
5. Concentration of drug w.r.t a given volume
6. Excretion rate of drug
7. Half life of drug action
8. Bioavailability

Secondary metabolite:

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. It often plays an important role in plant defense against herbivory and other interspecies defenses. Humans also use secondary metabolites as medicines, flavorings, and recreational drugs. They are usually small molecules such as alkaloids, terpenoids, beta lactam, natural phenols, glycosides etc. they can be big small molecules like nonribosomal peptide, fatty acid, polyketides etc.

Bioassay: It is a means of determining the activity or potency or any screen in a biological system relative to a control or reference.

Activity and Potency: Activity is the particular pharmacological or biological effect such as antifungal activity, antibacterial activity, antiviral, anti tumor activity anticonvulsant activity, etc. and the potency is the strength of that effect.

Bioavailability: In pharmacology, bioavailability is the degree/fraction of drug or equivalent substances become available to the target (tissue, protein, enzyme, nucleic acid etc.) after administration. Bioavailability accounts the principal pharmacokinetic properties of drugs by means of its absorption and the part of an administered dose of unchanged drug being reached to target through systematic circulation. It is important for determining the doses of drugs. The bioavailability becomes 100% when a medication is administered intravenously.

Low bioavailability is the common phenomenon with oral dosage due to poor water soluble, slowly absorbtion. Orally administered drugs should pass through the intestinal wall followed circulation to the liver. Metabolism must occur before a drug enters to systemic circulation. Many drugs may be metabolized before adequate plasma concentrations are reached.

Drug discovery without a lead

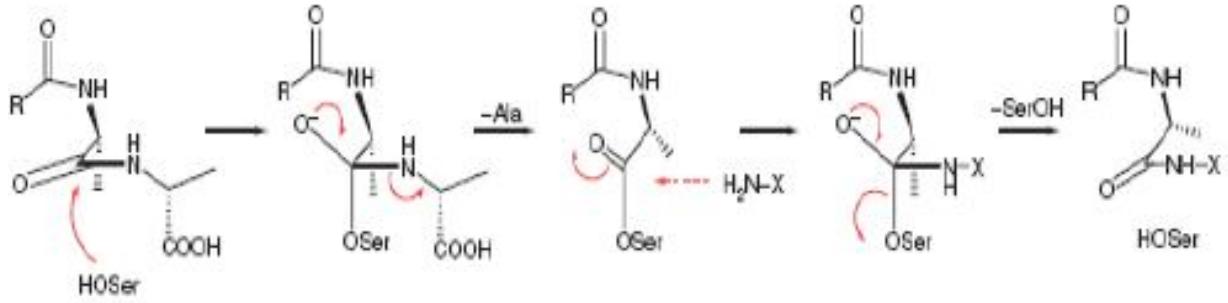
1. Penicillin (antibiotic)

Observation: In 1928, Alexander Fleming observed that the Staphylococcus bacteria was killed by a yellow-green mold that had accidentally contaminated the plate. He conferred that the mold must have released a substance that inhibited the Staphylococcus.

Isolation: In 1939, Howard Florey, Ernst Boris Chain purified penicillin which successfully treated bacterial infection in mice and later in patients. By the end of world war II, it had saved millions of lives from Pneumonia, syphilis, scarlet fever, wound, gonorrhea, diphtheria, etc.

Structure: Penicillin contains β-lactam moiety necessary for inhibition of bacterial action.

Mecahanism of action: Peptidoglycan layer confers mechanical strength on bacterial cell wall. Its integration is essential for bacterial survival. It is formed from cross-linked pentapeptides and sugars. Transpeptidase, a serine protease, catalyzes the formation of peptide bridges to cross-link neighboring strands.



Recognition/attack T.S 1 Acyl enzyme complex/attack T.S 2 Cross link

Figure: Mechanism of crosslinking by transpeptidase

Transpeptidase is also called penicillin binding protein (PBP). It is inhibited by following pathway using lactam ring. Each bacterium possesses a set of PBP which have different functions. Formation of second T.S is sterically prohibited and a water from vicinity of active site attacks.

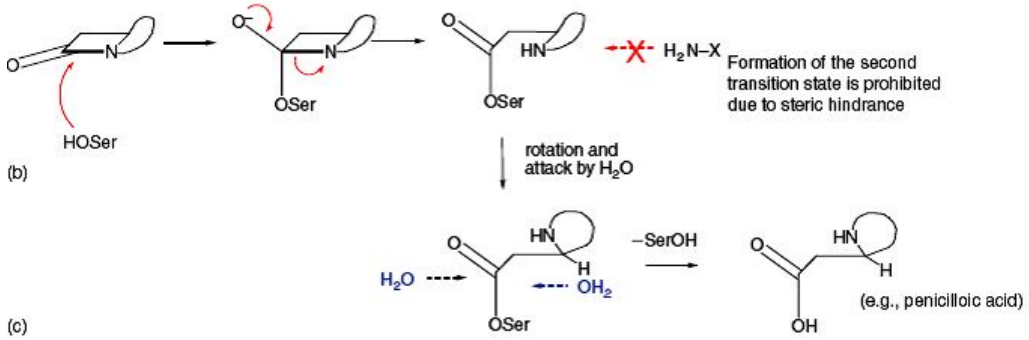


Figure: Inhibition of tranpeptidase by lactam

2. Librium (anxiolytic)

Librium is developed serendipitously from an inactive compound's which structure was mis-assigned and "shelved" and the project for new tranquilizer development was turned down. Later the compound (Chlordiazepoxide) was found active for the short-term (2–4 weeks) treatment of anxiety. Basically it is first benzodiazepines tranquilizer drug.

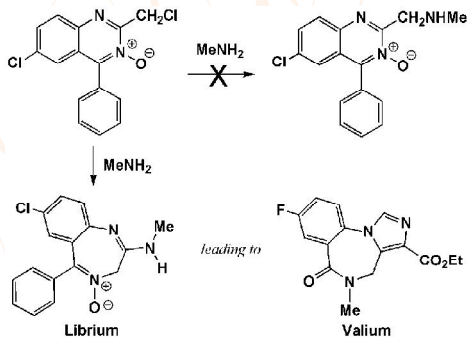


Figure: Formation of Librium

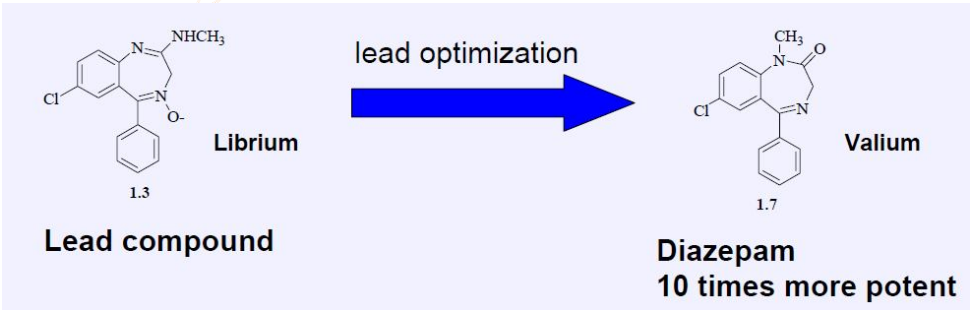


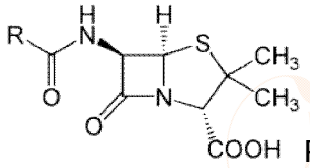
Figure: Lead optimization from Librium

How to find a lead? A process for lead identification:

Step-1: Choose a disease

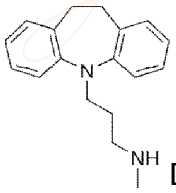
Step2: Select a suitable drug target for the disease

- where to target the drugs e.g specific organs, tissues, genes, proteins, receptor, enzyme, nucleic acid.
- specificity and selectivity of the target between species or within the body (to avoid undesirable side effect and toxicity)
  (*For instance, penicillin target β-Lactamase enzyme involved in bacterial cell wall biosynthesis. Mammalian cells do not have a cell wall. Thus, the enzyme is absent in human cells and penicillin has few side effects.*)



Penicillin

- a molecular target that is believed to influence a particular disease when affected by a drug.
- understanding the biomacromolecules involved in a particular disease state that allows the medicinal chemist whether agonist or antagonist to be designed for a particular receptor or whether inhibitors should be designed for a particular enzyme (keeping other enzyme unaffected).
  (***Cause:*** *Desipramine (tricyclic antidepressant) is known to inhibit the uptake of norepinephrine from nerve synapses. The drug also inhibits the uptake of serotonin. However, a search for selective serotonin uptake inhibitors has finally led to the discovery of Fluoxetine, the best selling antidepressant*.



Desipramine

Another point is that the various receptor subtypes are not uniformly distributed around the body, rather they are often concentrated in particular tissues. For example, adrenergic receptors in the heart are predominantly β1 subtype while those in the lungs are β2 subtype. If a drug acts on either, less side effects would be observed.)

- more than one target may need to be addressed for a particular ailment.

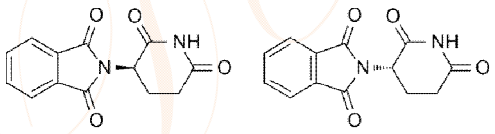
(*For instance, hypertension involves variety of receptors and enzymes such as β1-adrenoceptors, calcium ion channels, angiotessin-converting enzyme (ACE), and potassium ion channels that are targeted for treatment. Similarly, most of the current therapies for asthma involve a combination of bronchodilator (β2 agonist) and an anti-inflammatory agent such as a corticosteroid.*)

Step 3: Choice of bioassay and validation

- High-through screening,
- Affinity screening,
- Screening by NMR,
- Surface Plasmon resonance,
- Scintillation proximity assay,
- *in vitro* and *in vivo* tests

(*The bioassay should be simple, quick, relevant, cheaper, easier to carry out, less controversial as there are usually a large number of compounds to be analyzed. In vitro tests are suitable for that.*

In vivo analysis is comparatively slow and sometimes it causes animal suffering. Consequently, the pharmacokinetics has been misleaded. For example, penicillin methyl ester is hydrolyzed in mice into active penicillin, while it is not hydrolyzed in humans or rabbits. Also, thalidomide is teratogenic in rabbits and humans while it is not in mice.



Animal tests are not performed at the beginning. But it is equally important to alleviate the problem by eliminating the observable symptoms. For instance, the development of non-steroidal inflammatory drugs was carried out by inducing inflammation on test animals.

The animals used may be transgenic; meaning some mouse genes can be replaced by human genes so the mouse produces the human receptor or enzyme. The mouse's gene may be altered to be susceptible for some disease such as breast cancer.

Validation has to be done based on the observation in bioassay analysis. For instance, whether the antibacterial drug works or not can be tested by its efficiency to on kill the bacteria. Similarly, the validation of local anaesthetics is tested by their effect on blocking action potential in isolated nerve.)

Step4: Finding a lead

- screening of natural products e.g plant kingdom, microbial world, marine world, animal sources, venoms and toxins;
- natural ligands or modulators e.g. natural ligands for receptors, natural substrates for enzymes, enzyme products as lead compounds;
- high throughput screening (HTS) of synthetic compound " libraries" or combinatorial screening
- computer aided design (in silico or docking) or computerized searching of structural databases
- rational design
- designing lead compounds by NMR
- serendipity
- existing drugs,
- medical folklore.

(*Most biologically active natural products are secondary metabolites with quite complex structures. The only advantage is that they are extremely novel compounds.*

Plant kingdom offers lead compounds like morphine, cocaine, digitalis, quinine, tubocurarine, nicotine and muscarine, paclitaxel (Taxol, recent anticancer).

Microorganisms such as bacteria and fungi are rich for lead compounds (e.g. Antgimicrobial Drugs: pencillins, cephalosporines, tetracyclines, aminoglycosides, chloramphenicol, rifamycins)

Animal sources offers antibiotic peptides lead extracted from the skin of African clawed frog.

Teprotide obtained from venom of viper was the significant lead compound for the development of antihypertensive agents (Cilazapril & Captopril).

Medical folklore includes quinine (cinchona), reserpine (Rauwolfia), atropine (atropa beladona), morphine (opium poppy), digitalis (foxglove), emetine (ipeca), cocaine (coca) as important lead for drug action.

The target based natural ligands is used as the lead compound. For example, the natural neurotransmitters, adrenaline and noradrenaline, were used for development adrenergic β-agonists such as Salbutamol, dobutamine, xamoterol, H2 antagonists as cimetidine, and morphine.

Enzyme products act as lead compounds for an enzyme inhibition. For example, L-benzyl succinic acid inhibits enzyme catalyzed carboxy peptidase hydrolysis of peptides.

Natural modulators are natural or endogenous chemicals that exert allosteric control of receptor or enzymes and can also be as lead compounds. For example, Benzodiazepines modulate the receptor γ-aminobutyric acid (GABA) by binding to allosteric binding site. It leads to discovery of endogenous endozepines.

Workers in TNT factories always complained from headache due to dilatation of brain blood vessels. TNT was the basis to prepare nitro derivatives which were used in angina to dilate coronary blood vessels and alleviate pain. Mustard gas tanks used in second world war exploded in Italian harbor. They discovered that persons who survived and inhaled this gas lost their defense against microorganisms due to destruction of white blood cells. This led to the discovery of mustard like drugs which were used in leukemia to inhibit excessive proliferation of white blood cells.

HTS involves the miniaturization and automation of in vitro tests such that a large number of tests can be carried out in a short period of time. It involves testing of large number of compounds versus a large number of targets. The test should produce easily measurable effect. This effect may be cell growth, an enzyme catalyzed reaction which produces a color change (may be a dye) or displacement of radioactive labelled ligand from its receptors.

NMR screening mainly focuses on the detection of shorter relaxation times of ligands bound to a macromolecule than when they are unbound. Weak or strong binding can be measured and it is complimentary to HTS

Two visual methods in SPR (change in refractive index and reduction of emission of light) detect whether ligands bind to macromolecular targets).

Step 5: Isolation and purification of newly found lead

- Chromatography (column, HPLC etc.)

Step 6: Structural determination

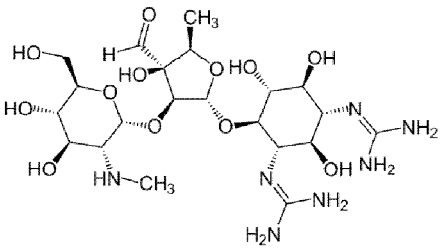
- Spectroscopy measurements (X-ray, NMR, Mass, UV, IR, Circular dichroism, Fluorescence, surface plamone resonance)

Step 7: Herbal medicine

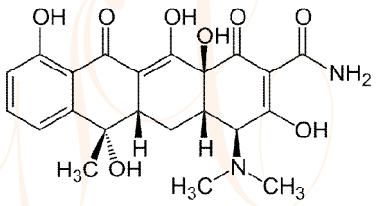
Drug Discovery with Lead

Random screening

In random screening, all compounds are tested without regard to the structure. It is an serendipitous discovery of drugs such as penicillin, benzodiazepine. Prior to discovery of sulfa drugs in 1935, random screening was the only option for drug development. Streptomycin (for tuberculosis) and tetracycline (for plague, fever, bacterial infection), the two antibiotics, are the outcome of random screening.



Streptomycin

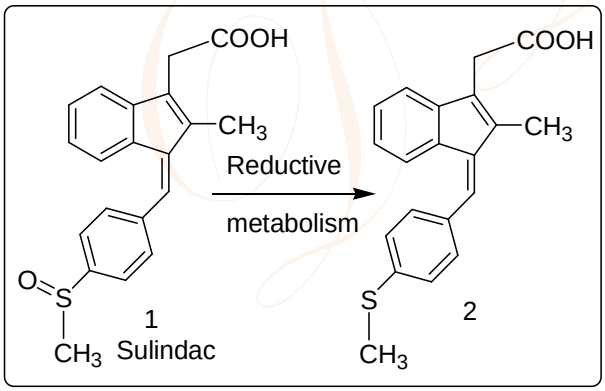


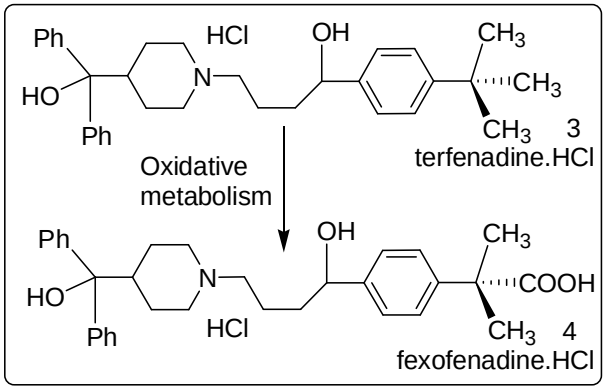
Tetracyclin

Nonrandom/Focused/Targeted Screening

A. Drug metabolism studies

Drug metabolism study infers that sometimes metabolites i.e drug degradation products generated *in vivo* are isolated as drug candidate. For instance, the following non-steroidal anti inflammatory drug (NSAID), sulindac (Clinoril, **1**), is not active rather its reduction product (**2**) is active. Similarly, antihistamine terfanadine (**3**), found in certain antifungal agents, causes abnormal heart rhythm. However, it's metabolic product, fexofenadine (**4**) is safer antihistamine drug.

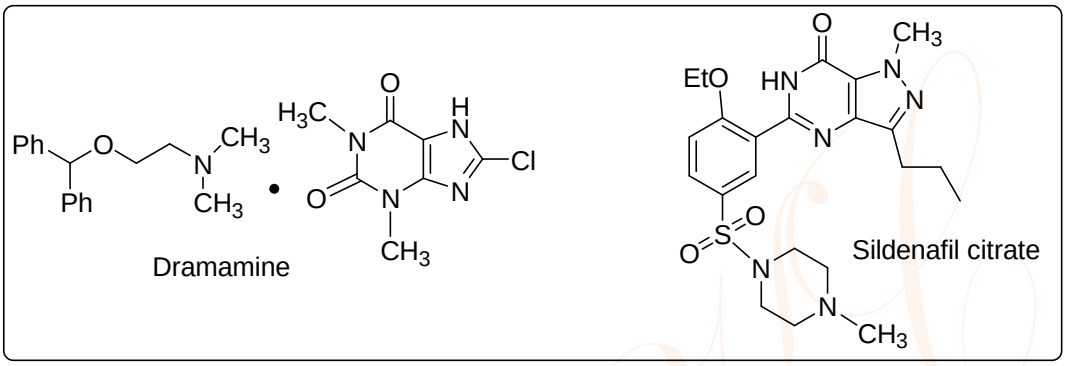




B. Clinical observations

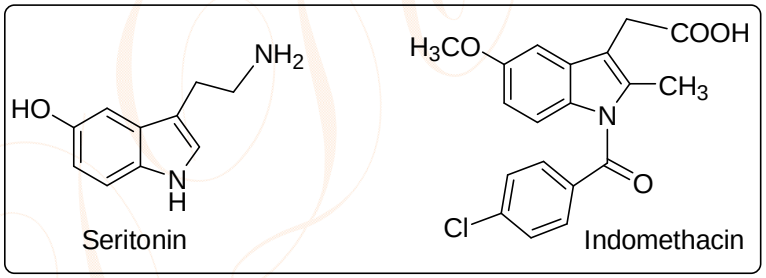
Some drugs exhibit more than one pharmacological activity during clinical trials. It produces a side effect. That side reaction can be used as drug lead. For instance, an

antihistamine Dramamine was tested for allergy treatment. However, clinical observation was found to be effective in the treatment of any kind of motion sickness (seasickness, carsickness, airsickness). Another instance is the clinical discovery of impotence drug sildenafil citrate (Viagra) which was wrongly designed and presumed to be active for the treatment of angina and hypertension.



C. Rational approach for drug discovery

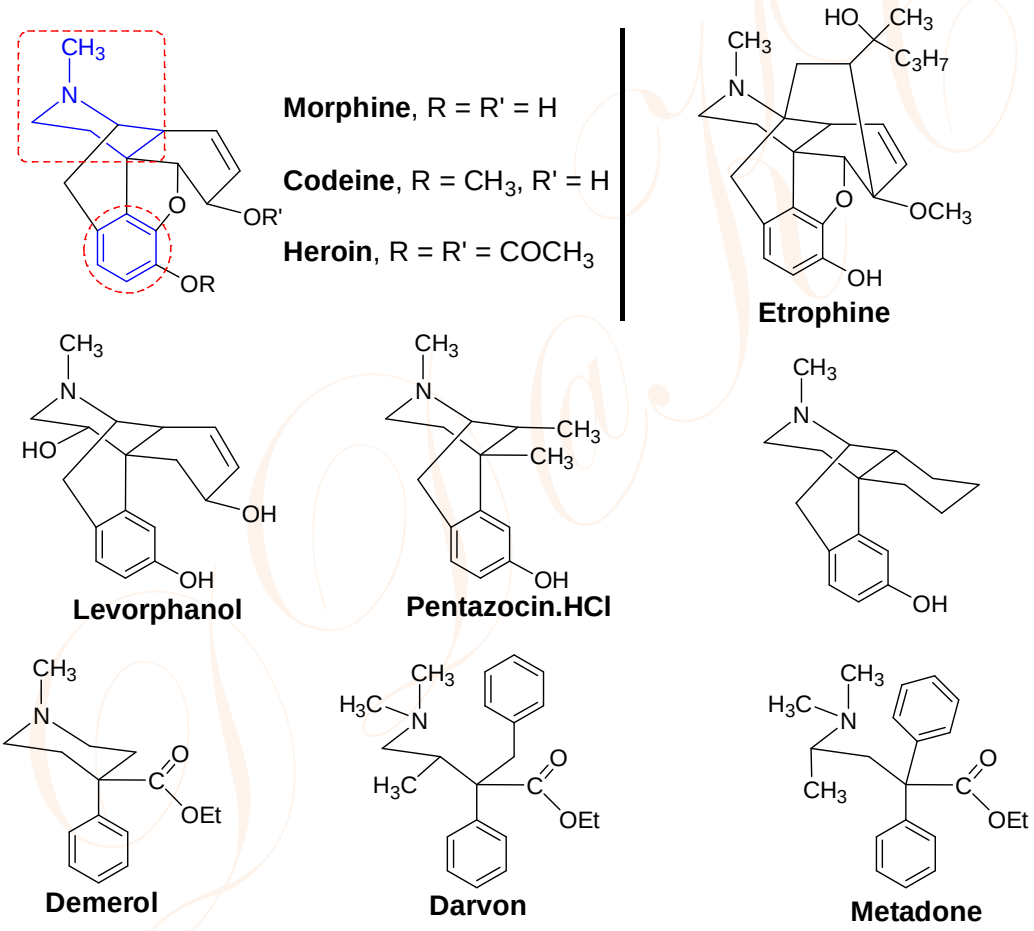
Rational drug design is intended to decrease the number of compounds that need to synthesize and to find high affinity, low toxicity compounds faster and more inexpensively. It is now become the major route to lead discovery. For instance, the NSAID indomethacin (Indocin) was developed based on rational approach in which serotonin was believed to be the mediator of inflammation and a lead for NSAID.



Lead Modification

A. *Identification of active part of lead: Pharmacophore*

Lead modification deals with the pharmacodynamic activity (LADME of pharmacokinetics). By removal or addition of groups in the lead gives an understanding about the pharmacophoric group and auxophoric group. Let us take an example of analgesic morphine which is an important compound used to treat both acute and chronic severe pain, acute pulmonary edema etc. and try to find out the part necessary for pharmacophoric and auxophoric activity in morphine.



The following points for pharmacophoric analysis:

i. The morphine family of analgesic binds to the μ-opioid receptor (similar to somatostatin receptor and a group of inhibitory G protein-coupled receptors with opioids as ligands and found mostly in brain, spinal cord and digestive tract). *The decrease in potency on removal/addition of the groups indicated in morphine structure suggests that it is pharmacophoric whereas an increase in potency is known as auxophoric with the*

interference in proper binding. Essentially no alteration in potency indicates that it is auxophoric having no interference with binding.

ii. *Auxophoric effect:* The dihydrofuran oxygen atom that connects between benzene and cyclohexene rings is not in the pharmacophore. Removal of oxygen atom results in change in conformation of cyclohexane as well as enhances degree of freedom of the whole molecule. The derived compound, levorphanol, was found 4 times more potent than morphine as analgesic and retains its addictive properties. It is assumed that the conformational mobility allows the molecule to have bioactive conformation necessary to bind μ-opioid receptor.

iii. *Pharmacophoric effect:* Removal of half of the chair form (pentazocin.HCl) or addition of fused cyclohexane drastically drops the analgesic potency from morphine, but has some addictive property.

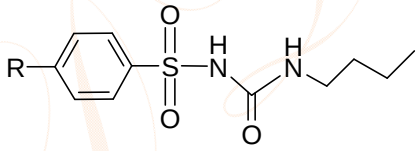
iv. Removal of all fused rings (Demerol) sustains its analgesic which is 10-12% of the overall potency of morphine.

v. *Auxophoric effect:* An acyclic analogue, Darvon, is two third as potential as codeine and binds effectively with μ-opioid receptor.

vi. *Auxophoric effect:* Addition of groups to lead structure of morphine e.g etropine increases the analgesic potency by 3200 times more than morphine. It means imposing additional rigidity in morphine geometry increases the ability to bind to the μ-opioid receptor.

B. Functional group modification

Carbutamide (R = NH_2) is an effective antibacterial agent. It also has the antidiabetic activity. If it is being used as antidiabetic drug, this could be lead to bacterial resistance. However, the simple change in functional group from amine to methyl (tolbutamide) leads to absolutely an antibacterial agent.



Carbutamide, R = NH_2
Tolbutamide, R = CH_3

C. Structure Activity Relationship (SAR)

Drugs are classified as:

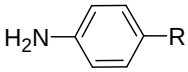
i) Structurally specific (acts on specific sites e.g receptor or enzyme and their activity and potency are susceptible to small change in structure)

ii) Structurally non-specific (no specific binding sites and usually have lower potency e.g drugs for anesthetic, sedatives, antiseptic, disinfectants).

Both specific and non specific drug design includes in SAR studies.

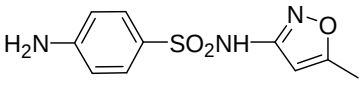
Case-I

The development of antibacterial sulfonamide drugs (Sulfa drugs) are the excellent examples for SAR studies. Variation in structures (changing R group or substitution in amine) with different functional groups produces different activity and potency in cell.

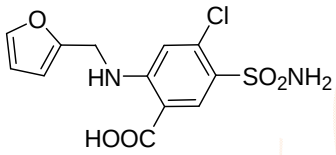


Sulfa drug

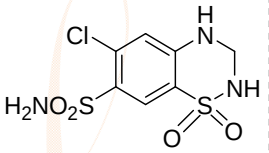
R = SO_2NH_2, antibacterial
R = $SO_2NHCONHR'$, antidiabetic
R = thiazides, diurete
R = SO_2NHR, antimicrobial
R= SO_3H, antimicrobial



sulfamethoxazole



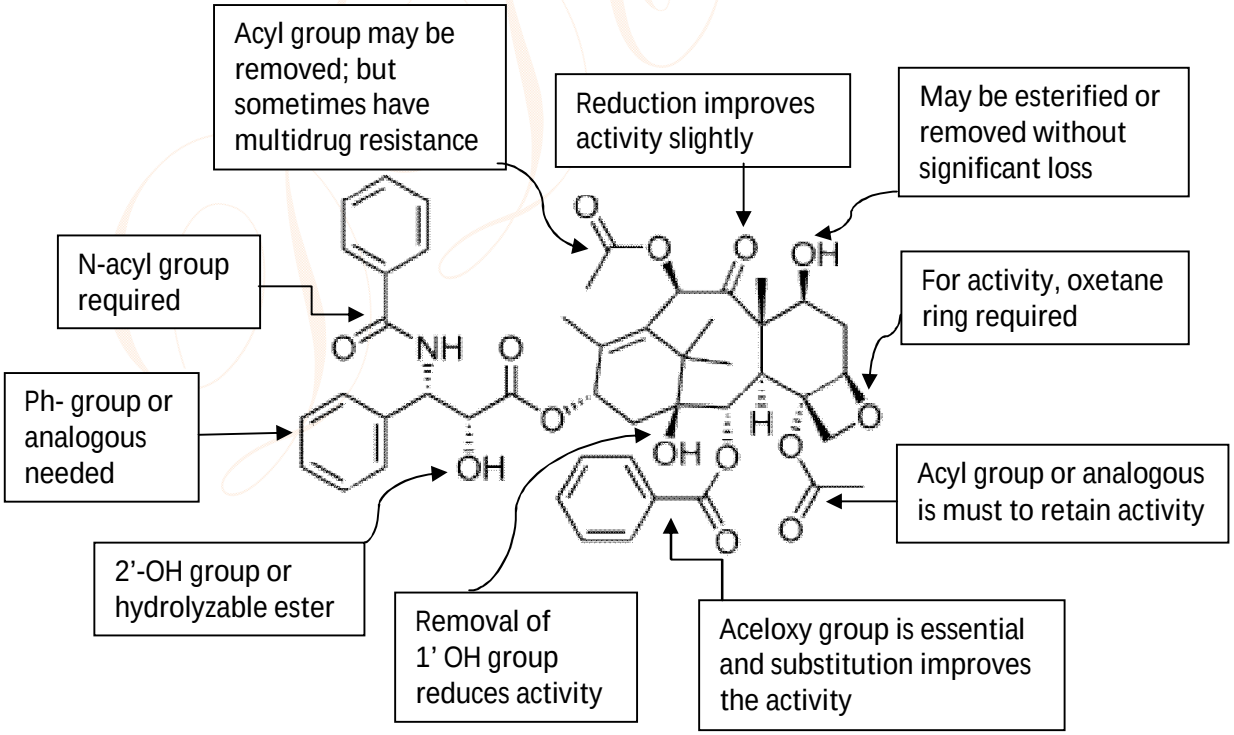
Furosemide



Hydrochlorthiazide

Case-II

Another example of SAR studies is the remarkable development of anticancer drug, paclitaxel (Taxol) that blocks the mitosis by promoting assembly of tubulin into microtubules. Many structural modifications (structure-potency relationship) were made to improve its potency.



D. *Privileged structure and drug-like molecules*

See the drug discovery without lead "Librium" for details.

E. Structure modification using different parameters:

1) Homologation

The increase in homologation of a lead increases its potency, but to a certain extent. For instance, the % of spasmolytic activity of a mandalate ester increases with increase in methtylene group upto nine i.e n-nonyl ester. Further increase in methylene group decreases its activity quite drastically. This can be explained by means of increase in lypophilicity of the molecule upon increase in homologation that permits penetration in cell membranes until it lowers water solubility. But it is not necessarily be true. Sometimes, high lipophilicity decreases the drug transport across the intestinal membrane.

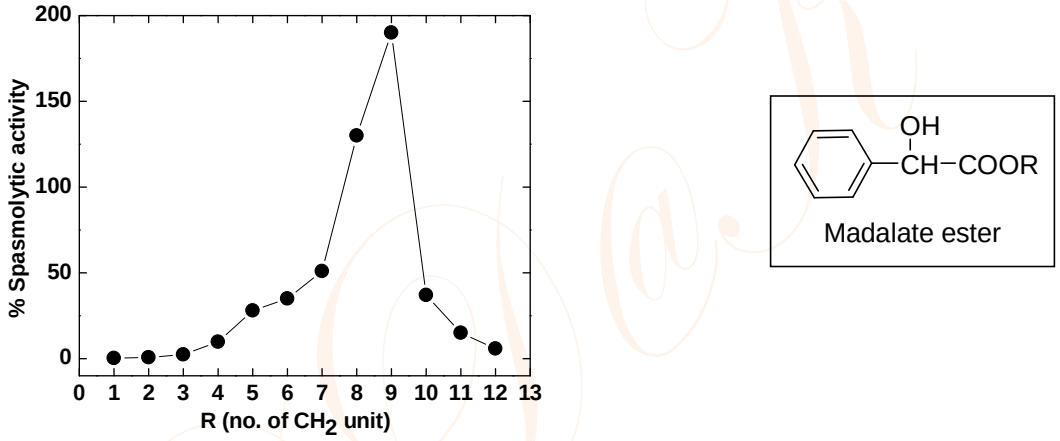


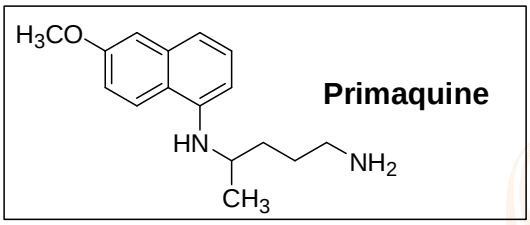
Figure: Spasmolytic activity vs. homologation in 'R' group of madalate ester

N.B: *Lipophilicity* is the ability of a chemical compound to dissolve in lipophilic solvents such as fats, oils, lipids, non-polar solvents (hexane or toluene). They interact to each other or other lipophilic substances through the London dispersion force (usually a part of Van-der Waals forces or a weak intermolecular force arising from quantum-induced instantaneous polarization multipoles in molecules). The lipophilic substances do not have or have very little capacity to form hydrogen bonds. They have invariably large partition coefficients in o/w (octanol/water).

2) Chain branching

The branched alkyl chain has larger molar volume and size which makes them less lipophilic as compared to their corresponding straight alkyl chain analogues. This usually affects the potency of the drug molecule. Another explanation is related to the pharmacodynamic phenomenon in which a branch chain may interfere with the receptor

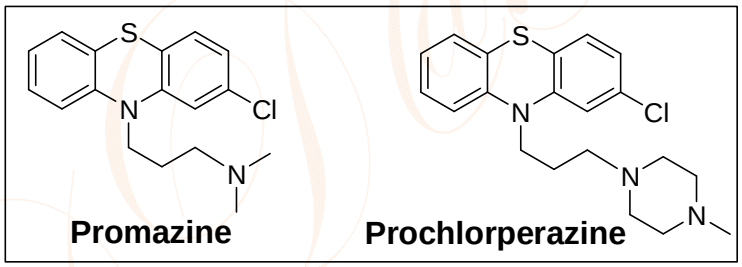
during binding. For instance, $PhCH_2CH_2NH_2$ is an excellent substrate for monoamine oxidase; but $PhCH(CH_3)CH_2NH_2$ (amphetamine) is not. Similar reason can be attributed for the potency of amine analogues i.e primary > secondary > tertiary. The example includes an antimalarial drug primaquine (8-aminoquinoline group of drugs) phosphate which is more potent in its primary amine stage over secondary and tertiary homologues.



Primaquine

3) Ring chain transformation

Ring chain transformation could lead a better pharmacokinetic effect in terms of increasing lipophilicity and decreasing metabolism. It could make the drug more effective *in vivo*. Again, by connecting substituents in the ring, pharmacodynamic properties could be enhanced by constraining the group into favorable conformation (however, it is not true in all cases). For example, prochlorperazine is better tranquilizer than promazine as the formar has extra methylpiperazine ring.



Promazine **Prochlorperazine**

4) Bioisosterism

Bioisosteres are substituents or groups that have physical or chemical similarities and which produce broadly similar biological properties. Bioisosterism is an important lead modification approach and has significant role in alteration of pharmacokinetics of lead by attenuating toxicity or modifying the activity of a lead.

Evolvement of Bioisosterism

- In 1919, Langmuir stated that the chemical behavior and reactivity of determined substances possessing atoms or groups with the same number of valence electrons (isoelectronic) i.e. are called isosterism. Isosteres are isoelectronic species.

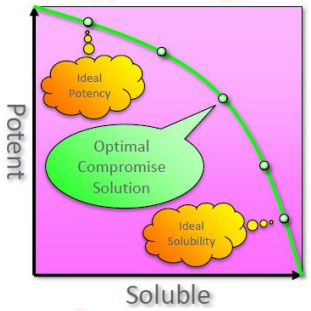
O^{-2} x F^{-} x Ne x Na^{+} x Mg^{+2}

ClO_4^{-} x SO_4^{-2} x PO_4^{-3}

N=N x C=O

CO_2 x NO_2

N=N=N x N=C=O^{-}



- In 1925, Grimm formulated the Hydride Displacement Law, an empiric rule which states that the addition of a hydrogen atom with a pair of electrons (i.e. hydride) to an atom, produces a pseudoatom presenting the same physical properties as those present in the column immediately behind on the Periodic Table of the Elements for the initial atom, showing that any atom belonging to groups 4A, 5A, 6A, 7A on the Periodic Table change their properties by adding a hydride, becoming isoelectronic pseudoatoms.

	Group 4A	Group 5A	Group 6A	Group 7A	Group 8A	
N° of e^{-}	6	7	8	9	10	11
	C	N	O	F	Ne	Na^{+}
H-	CH	NH	OH	FH		
H-	CH_2	NH_2	OH_2	FH_2^{+}		
H-	CH_3	NH_3	OH_3^{+}			
H-	CH_4	NH_4^{+}				

- In 1932, Erlenmeyer proposed a broadening of the term isosterism in which he defined isosteres as elements, molecules or ions that represent the same number of electrons at the valence level. His considered that elements of the same column on the Periodic Table are isosteres among themselves (e.g. C x Si x Ge) and the creation of a concept of rings electronically equivalent, later broadened to the term ring bioisosterism.

- In 1951, Friedman , defined bioisosters as compounds that fit the definitions of isosteres and which exercise their biological activity of bioreceptor, whether through agonist or antagonist actions. He introduced the term bioisosterism to describe the phenomenon observed between substances structurally related which presented similar or antagonistic biological properties.

- In 1979, Thornber proposed a broadening of the term bioisosteres, defining them as subunits or groups or molecules which possess physicochemical properties of similar biological effects.

- According to Burger in 1991, compounds or groups that possess near-equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties

Bioisosters are mainly two types: Classical and non classical isosteres.

Classical isosteres

1. Monovalent atoms or groups
2. Divalent atoms or groups
3. Trivalent atoms or groups
4. Tetrasubstituted atoms
5. Ring equivalents

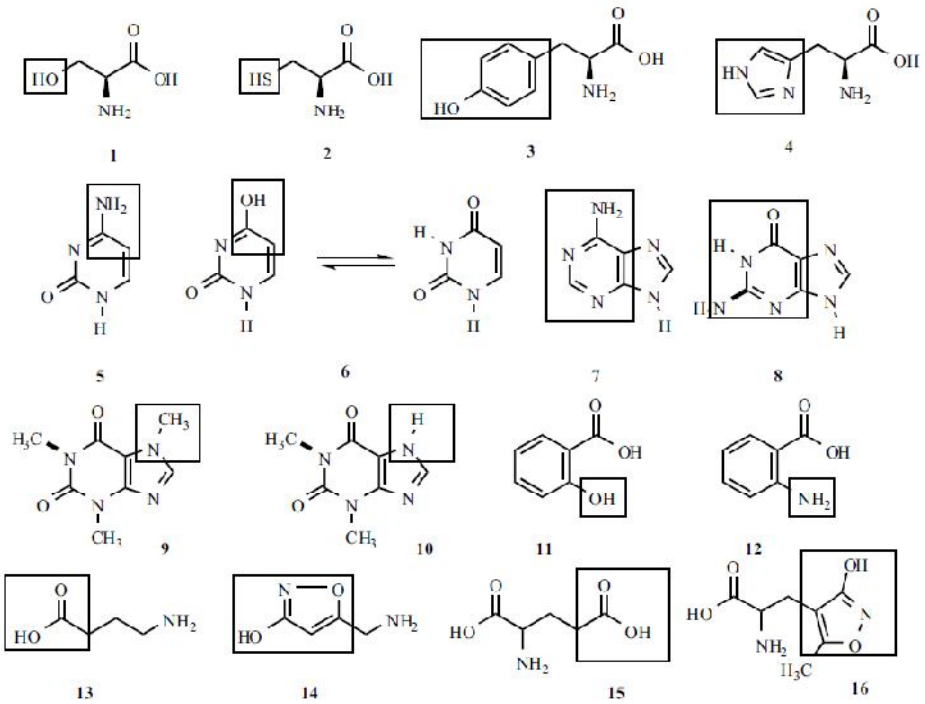
Monovalent	*Divalent*	*Trivalent*	*Tetravalent*
, -OH, $-NH_2$, $-CH_3$, -OR	$-CH_2-$	=CH-	=C=
-F -Cl, -Br, - I, -SH, $-PH_2$,	-O-	=N-	=Si=
$-Si_3$, -SR	-S-	=P-	$=N^+=$
	-Se-	=As-	$=P^+=$
	-Te-	=Sb-	$=As^+=$
			$=Sb^+=$

Non-Classic Bioisosteres

1. Cyclic vs Noncyclic
2. Functional groups
3. Retroisosterism

-CO-	-COOH	$-SO_2NH_2$	-H	-CONH-	-COOR	$-CONH_2$
$-CO_2-$	$-SO_3H$	$-PO(OH)NH_2$	-F	-NHCO-	-ROCO-	$-CSNH_2$
$-SO_2-$ $-SO_2NR-$	-tetrazole $-SO_2NHR$ $-SO_2NH_2$		-OH $-CH_2OH$		-catechol	
-CON-	-3-hydroxyisoxazole				-benzimidazole	
-CH(CN)-	-2-hydroxychromones		$-NHCONH_2$			C_4H_4S
R-S-R			$-NH-CS-NH_2$			$-C_5H_4N$
(R-O-R')	=N-					$-C_6H_5$
R-N(CN)-	C(CN)=R'		$-NH-C(=CHNO_2)-NH_2$ $-NH-C(=CHCN)-NH_2$			$-C_4H_4NH$
-halides	 $-CF_3$ -CN $-N(CN)_2$					
	$-C(CN)_3$					

In nature, many examples of classic bioisosterism in the form of broadening chemodiversity have been identified. This includes essential amino acids serine (**1**) and cysteine (**2**), tyrosine (**3**) and histidine (**4**) among the pyrimidine and purine bases cytosine (**5**) and uracile (**6**), adenine (**7**) and guanine (**8**); among the xanthines caffeine (**9**) and theophyline (**10**). Salicylic (**11**) and anthranilic (**12**) acids are the origination of two classes of non-steroid anti-inflammatory drugs, e.g. acetylsalicylic acid and mefenamic acids, respectively. Furthermore, examples of the application of non-classic bioisosterism are also found in nature - such as the bioisosteric relationship existing between γ-aminobutyric acid (GABA) (**13**) and muscimol (**14**), between the neurotransmittors glutamate (**15**) and AMPA (**16**).



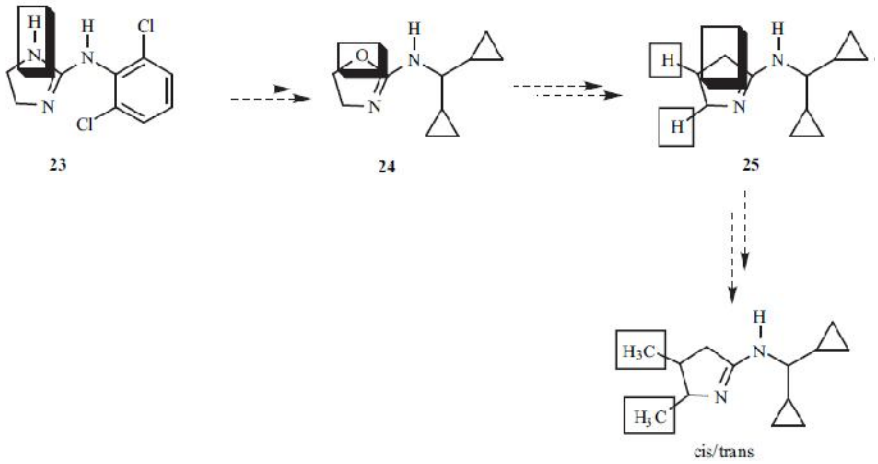
Bioisosterism as a strategy of molecular modification

The classic bioisosterism is less numerous and is used for the structural design of new drugs while non-classic bioisosterism is significantly predominant in designing new pharmacotherapeutically attractive substances such as selective receptor antagonist or agonist drugs, enzymatic inhibitors or anti-metabolites. The correct use of bioisosterism demands physicochemical properties such as physical, chemical, electronic and conformational parameters:

i. ***Structural:*** size, volume and electronic distribution of the atoms or the considerations on the degree of hybridization, polarizability, bonding angles and inductive and mesomeric effects
ii. ***Receptor interactions:*** degree of lipidic and aqueous solubility (such as logP and pKa);
iii. ***Pharmacokinetics:*** chemical reactivity of the functional groups or bioisosteric structural subunits, mainly to predict significant alterations in the processes of pharmacodynamic activity (ADME) including for the eventual alteration of the toxicity profile relative to the main metabolites. Then lipophilicity, hydrophobicity, pK_a, hydrogen bonding becomes important.
iv. ***Metabolism:*** conformational factors influencing blocking or aiding metabolism. The differential capacity of inter- or intramolecular hydrogen bonds formation should be considered.

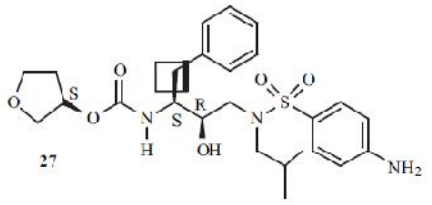
Examples of Classical Bioisosterism

- A pathway of classic bioisosterism in search for new antihypertensive drug

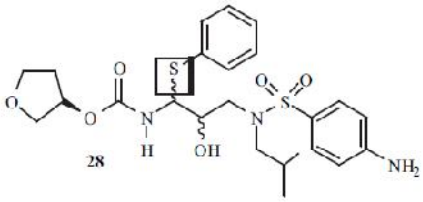


The extended modification (from 23 to 25) through classic bioisosterism leads to greater binding with I_1 imidazoline receptor (I_1R) and reduced action on α_2-adrenoceptors.

- A pathway of classic bioisosterism in search for new anti-HIV drug



27

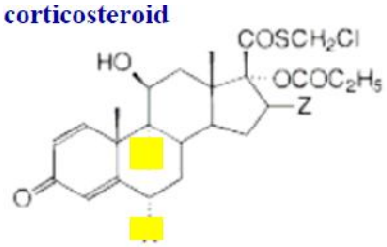


28

28a *S, S, R* = IC_{50} = 1,4 µM
28b *S, S, S* = IC_{50} = 11,6 µM
28c *S, R, S* = IC_{50} = 12,5 µM
28d *S, R, R* = IC_{50} = 16,7 µM
27 *S, S, R* = IC_{50} = 0,001 µM

Rocheblave and coworkers developed new HIV-protease inhibitors using bioisosteric exchange between bivalent groups. The exchange of the methylene group (CH_2), present in the structure of the lead compound amprenavir (**27**), by sulfur atom (S) in **28** was found little effective as observed in IC_{50} value. However, Knowing noticed that the isosteric replacement induces several modifications in terms of size, shape, electronic distribution, chemical reactivity, lipophilicity and hydrogen bonding capacity. Four diastereoisomers (**28 a-d**) were tested as weak inhibitors of recombinant HIV protease due to the high sensitivity to hydrolysis of thioisoster (half life 10 minutes). Rather, amprenavir (**27**) was proteolytically stable even after 1440 min.

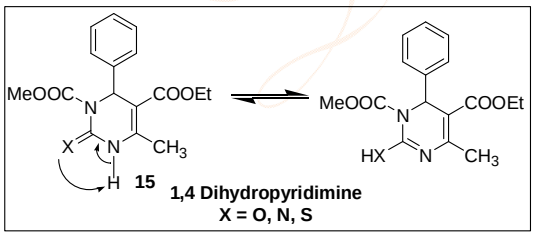
- Topical anti-inflammatory activity by corticosteroid using bioisosterism



compound	X	Y	Z	topical anti-inflammatory activity[a]
3a	H	F	$=CH_2$	42
3b	F	F	$=CH_2$	108
3c	H	H	β-CH_3	27
3d	H	F	β-CH_3	41

In case of compound **3b**, the highest anti-inflammatory activity was observed. The presence of alkene (Z group) maintains the planarity to avoid steric interactions and minimize the eclipsing effect of five membered ring. The electronegative fluorine atoms as both X and Y groups at β to the middle ring enhance the recognition with receptors.

- Calcium blocking activity: An effect of Bioisosterism through tautomerization

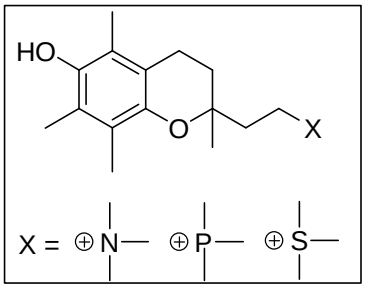


compound	X	van der Waal's radius[24] (Å)	IC_{50} (nM)[a]
15a	=O	1.40	140
15b	=NH	1.50	160
15c	=S	1.85	17

[a] Concentration that produced 50% inhibition and determined for the vasorelaxant activity with potassium-depolarized rabbit thoracic aorta.

1,4 dihydropyrimidine undergoes tautomerization to its another isosteric structure. The role of heteroatom is significant in establishing calcium blocking activity. Due to high v-d-w radius of 'S' atom, it resulted pronounced calcium blocking activity (IC_{50} = 17 µM).

- *In vito* and *in vivo* inhibition of lipid autooxidation in mice heart homogenate

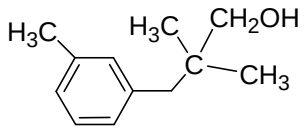


compound	X	*in vitro*[a] IC_{50} (μM)	*ex vivo*[b] ID_{50} (μM/kg)	IC_{50}/ID_{50}
45a	$-N^+(CH_3)_3$	19	11	1.7
45b	$-P^+(CH_3)_3$	10	8	1.3
45c	$-S^+(CH_3)_2$	7	6	1.3

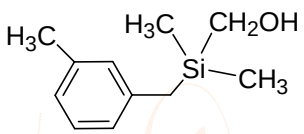
[a] Concentration that inhibits thiobarbituric acid reactive substances (TBARS) formation by 50%. [b] Dose that inhibits TBARS formation by 50% 1 h after sc administration.

The *in vito* and *in vivo* inhibition of lipid autooxidation was effective when 'N' is replaced by 'P' or 'S'. It may be due to the more polarizability of 'P' and 'S' that inhibits the substrate (TBARS) formation. [N.B. Thiobarbituric acid reactive substances (TBARS) are formed as a byproduct of lipid peroxidation (i.e. as degradation products of fats). It is detected by the TBARS assay using thiobarbituric acid as a reagent.]

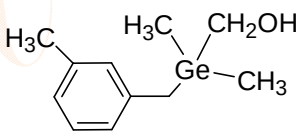
- A study on C/Si/Ge bioisosterism



Majantol,
strong, fresh aldehydic,
lily-of-the-valleyflower odor

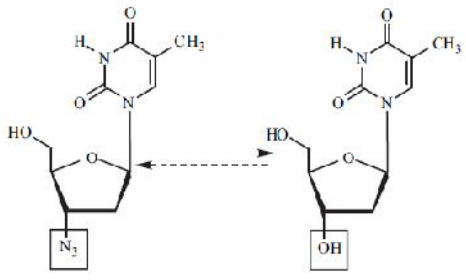


Sila-Majantol,
lily-of-the-valleyflower odor
but strong terpenoil odor



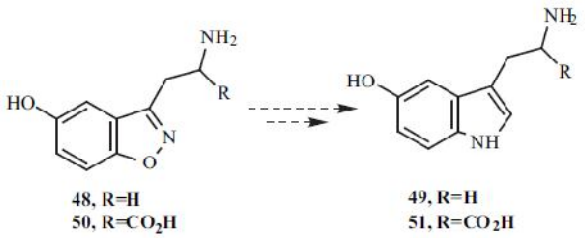
Germa-Majantol,
Weak and no
characteristics odor

- AZT: an example for bioisosterism



Zidovudine (AZT, **43**), isolated from seaweed, is an important chemotherapeutic resource available for the treatment of acquired immune deficiency syndrome. AZT is an inhibitor of reverse transcriptase (RT) enzyme. There is a classic bioisosteric relationship of monovalent groups (azide in **43** and OH in **44**, although azide and OH have dramatic electronic difference**)** between nucleoside thiamine (**44**, endogeneous substrate for DNA or RNA synthesis) and AZT (**43**).

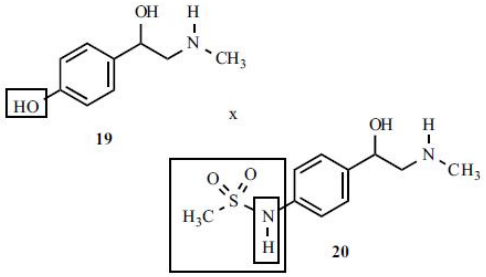
- Ring Bioisosterism



Benzisoxazole ring in **48** and **50** has no type of serotoninomimetic activity and not even anti-serotonine. The indole ring in **49** and **51** showed activity when tested with the substrate for serotonine decarboxilase enzyme. This is an example of bioisosteric relationship between two ring nucleuses based on the receptor site involved.

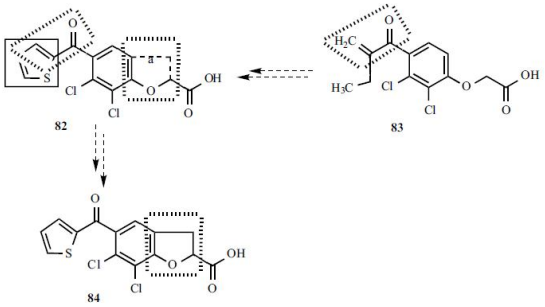
Examples of Non classical Bioisosterism

- An adrenergic derivative structurally similar to catecholamine



These two compounds have comparable acidity (similar pKa; 9.1 for compound **19** and 9.6 for compound **20**). It means they should have equivalent interactions with receptor through ionic and hydrogen bonding. The acidic groups OH in **19** and NH in **20** are monovalent. However, actual study says that the two adrenergic derivatives are different in their way of molecular recognition to the receptor.

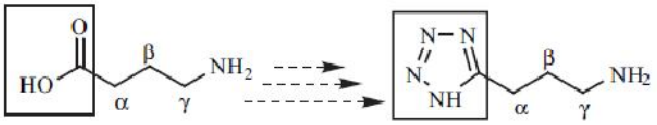
- Cyclic vs. non cyclic replacement



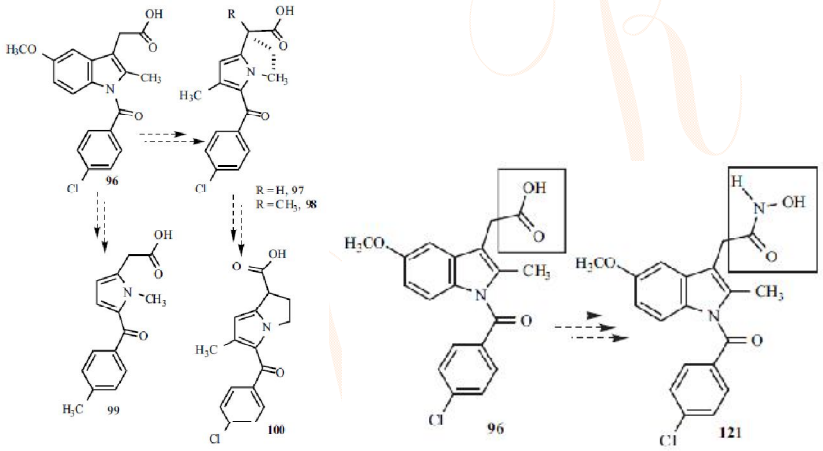
A non-classic biisosterism representing ring closing or anelation (furan and thiophene moiety) choose the definition for new lead compound for diuretic drugs (**84**). Inclusion of

thiophene eliminates the Michael acceptors whereas furan strengthens the conformation. Ring cyclization of indometacin, a NSAID, leads **96** (only pyrrole) to **99/100** (5-fused-5 pyrrole moiety) to better anti-inflammatory property.

- Non-classic Bioisosterism with functional group changes

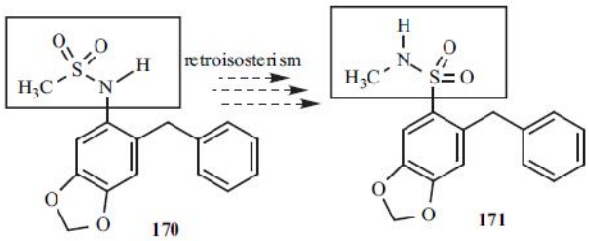


Tetrazole group (**113**) mimic carboxylic acid (**13**) in terms of physicochemical property related to acidity. However, the former is more stable and lipophilic. This modification leads greater possibility to overcome blood brain barrier with the type of tropism favorable to desired activity.



On the other hand, carboxylic acid in indometacin (**96**) modified with hydroxyl amine (**121**) has proved to be metabolically stable.

- Retroisomerism



Due to retroisomerism between methylsulfonylamine (**170**) and methylsulfonamide (**171**) which differs their pKa values (**170** is more acidic to **171**) makes 170 a better COX-2 inhibitor.

5. Combinatorial Chemistry

Combinatorial chemistry is a robust laboratory technique to synthesize and tests millions of structurally different molecule (*Chemical libraries*) at a time for the purpose of biological screening such as lead discovery or lead modification. It is a powerful alternative method to optimize initial lead and to generate new lead against a specific target. Thus, combinatorial chemistry is`1 defined as systematic and repetitive covalent assembly of a set of different structural *building blocks* to produce diverse array of molecule with a common scaffold.

To find a lead quickly and efficiently, combinatorial synthesis provides a means of producing large quantity of compounds.

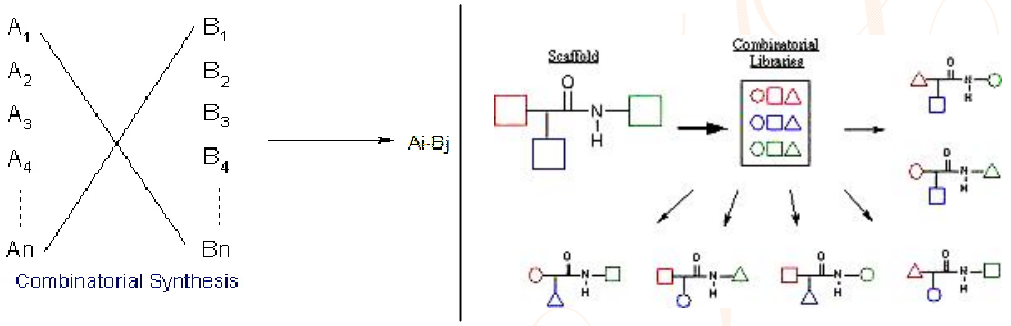


Figure: Schematic representation of combinatorial synthesis

Combinatorial libraries contain:

a) Size: number of building blocks used per reaction and number of reaction steps
b) Typical: 10^2 to 10^5 compounds

Conventional Reaction: **A + B → A-B**

Combinatorial Chemistry: **A(1-n) + B(1-n) → A(1-n) – B(1-n)**

The number of possible different compounds in a library (N) is determined by the number of building blocks used in each step (b) and the number of synthetic steps (x).

$N = b^x$; if equal number of building block used in each step.

N = bcd; if the number of building blocks in each step is varied (b, c, d for three step synthesis).

The number of compounds (N) in combinatorial library of all the pentapeptides (synthetic steps x =5) comprising 20 essential amino acids (building blocks b = 20) would be: **$N = 20^5$** or 3.2 million different pentapetides.

<u>There are two approaches by which combinatorial library can be generated:</u>

I. <u>Biological library approach</u>
   a) Plasmid approach
   b) Filamentous phage approach
   c) Polysome apprpoach

II. <u>Spatially addressable parallel solid phase library approach</u>
   a) Multi-pin methodology (synthesis of peptides on a polypropylene pin functionalized with acrylic acid arranged in 96 microtiter well)
   b) Tea-bag methodology (synthesis of peptides by using resin in sealed plypropylen bag which is coupled to each activated amino acid solution one by one and deprotection between every coupling step)
   c) SPOTS membrane methodology (cellulose paper is used as solid support to synthesize peptides)
   d) Light directed (photochemically) peptide synthesis of solid support (use of amide linker)

<u>Advantages:</u>

a) Faster synthesis of large population of structurally different molecules called chemical libraries in short time
b) One time screening against variety of targets using high throughput screening (HTS)
c) Reduced the time and cost in producing effective and new competitive drugs.
d) Use of solid support to carry out the reaction in presence of excess reagents (so that purification is done simply by filtration and those are not attached/reacted to the solid support, is also removed due to insolubility of solid support)
e) Diversity of the molecule can be attended
f) Applicable in organic, inorganic, organometallic, polymer chemistry

<u>Disadvantages:</u>

a) Difficulty of the scaling up the reaction and sluggishness of the reaction

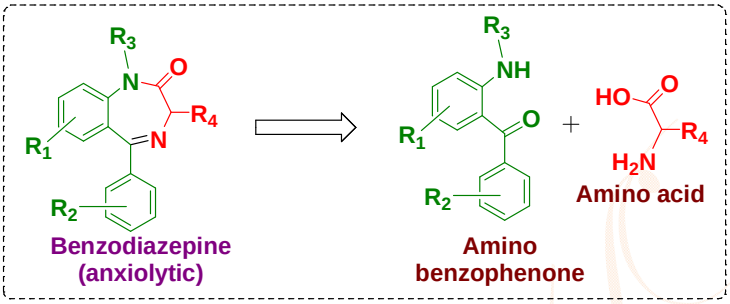
<u>Any alternative:</u>

a) *<u>Covalent scavenger technology</u>* could be used where the reactions are performed in solution with excess reagents and it is then scavenged with polymer supported scavenger after the completion of the reaction.
b) Use of polymer-supported reagents with solution reaction

Distinction: Scaffold-based vs. Backbone-based libraries

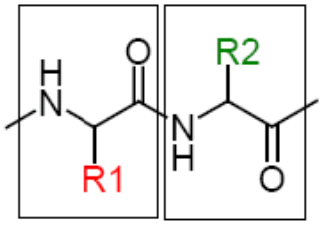
Scaffold-based

For anxiolytic drug development, the scaffold for efficient lead compound consists of two single building blocks: Aminobenzophenone and Aminoacid and.



Backbone-based

For peptide library, the backbone of peptides i.e amino acids is used for library design.



Building block A Building Block B

6. 'Split and mix' or 'Split and pool' or 'divide-couple-recombine' method to produces 'one bead-one compound' distribution

'Split and mix' or 'Split and pool' or 'divide-couple-recombine' method is also a type of combinatorial synthesis. It involves the following steps in synthesizing peptide library:

a) dividing the resin beads (solid support) into few portions,
b) generating building blocks in each resin portion by coupling separately with different amino acids,
c) recombine those resin portions.

The result of split synthesis is a collection of polymer beads where each bead contains one library member. Such concept of 'Split and pool' is known as "one bead−one peptide" distribution. One serious limitation is: each bead carries only 100-500 pmol of product which makes structure determination difficult. So, mass spectrometric method is usually employed.

Table: Split and mix synthesis of peptide libraries

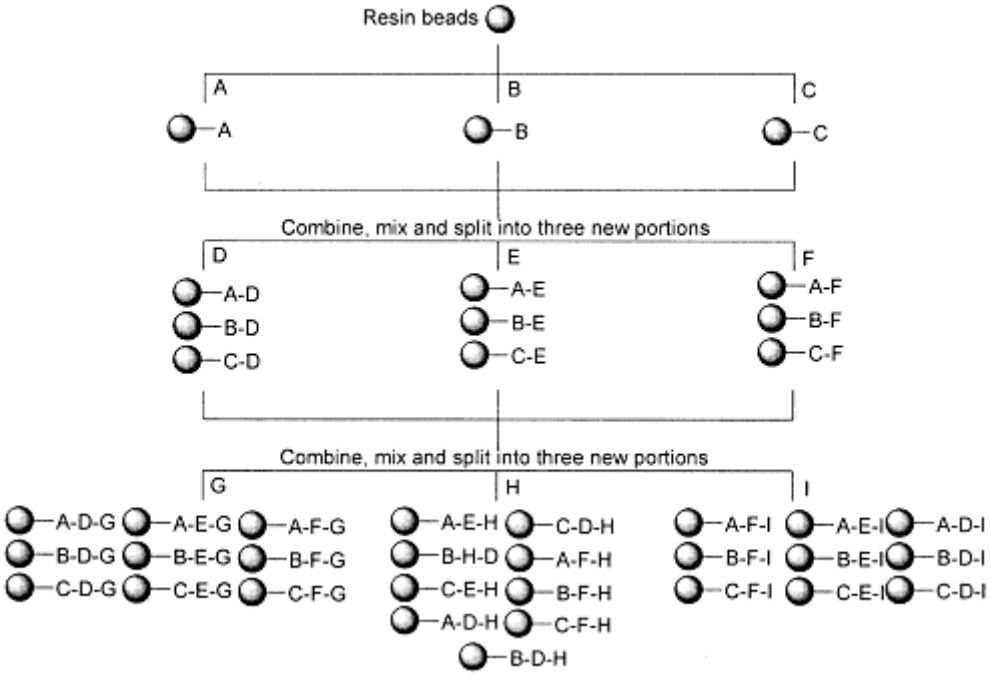
Step number	Number of amino acids	Number of reaction vessels	Number of cycles in one step	Total number of cycles	Number of peptides
1	3	3	3	3	$3^1=3$
2	3	3	3	6	$3^2=9$
3	3	3	3	9	$3^3=27$
4	3	3	3	12	$3^4=81$

Advantages:

- Only few reaction vessels required
- Method of choice for large libraries (up to 10^5compounds)

Disadvantages:

- Three-fold amount of resin beads necessary
- Only little amounts of the synthesized compounds available



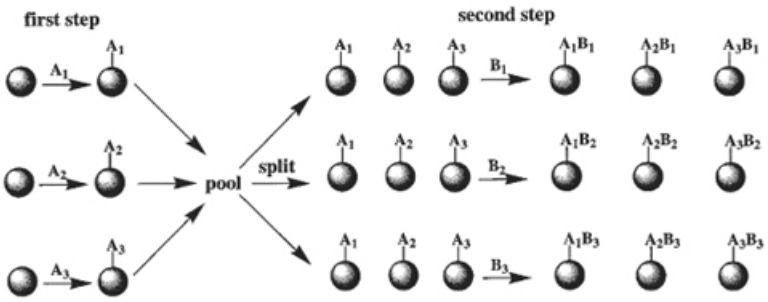


Figure: A schematic representation of 'Split and mix' or 'Split and pool' or 'divide-couple-recombine' method

7. Encoded combinatorial synthesis

In encoded combinatorial chemical libraries, each chemical sequence is labeled by an appended "genetic" tag, itself constructed by chemical synthesis. A "retrogenetic" way is implemented to specify each chemical structure. In case of encoded 'Split and mix' or 'Split and pool' or 'divide-couple-recombine' method for the synthesis of peptide library, resins are first coupled with unequal amount of two amino acids (say 60:40 or 70:30) and then divided to follow conventional 'split and pool' technique. Finally, the encoded peptide is measured by mass spectrometry.

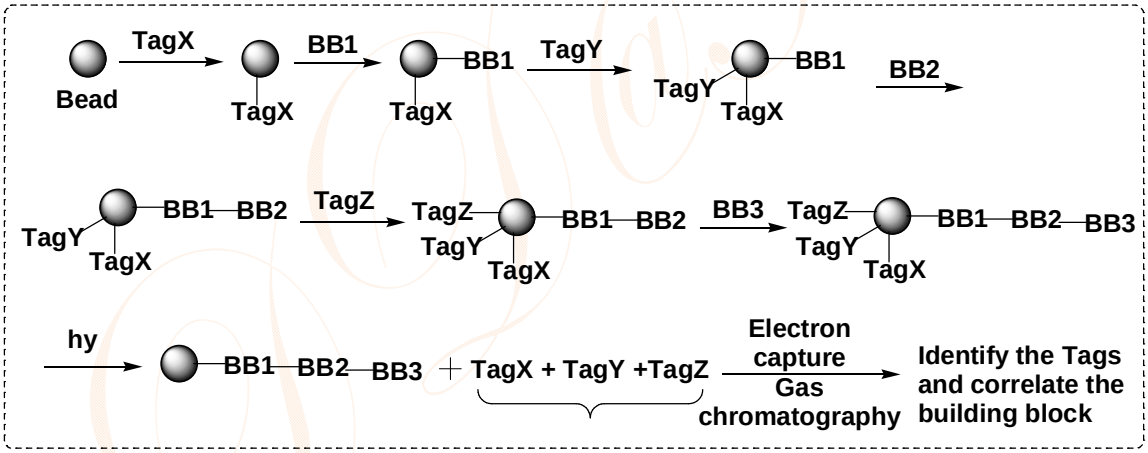


Figure: Still methodology for encoding combinatorial peptide libraries on a polymer bead

The process is summarized as follows:

Step 1: begin with some appropriate linker, LINK
GGGCCCTITTCTT&G-LI NK
Step 2: This product is divided into two aliquots for parallel synthesis.
CACATGGGGCCCTATTCTT&G-LINK-Gly

ACGGTAGGGCCCTITTCTTAG-LINK-Met

Step 3: The elongated products are pooled and again split into two parts for parallel synthesis.
CACATGCACATGGGGCCCTATTCTTAG-LINK-Gly-Gly
CACATGACGGTAGGGCCCTATTCTTIG-LINK-Met-Gly
ACGGTACACATGGGGCCCT&TTCTTLG-LINK-Gly-Met
ACGGTAACGGTAGGGCCCT&TTCTT&G-LINK-Met-Met

Step 4: Once more the products are pooled and divided into two aliquots for parallel synthesis.

8. Parallel synthesis

Execution of the chemical reactions takes time and during that time not only one but a series of reactions can be realized. Each synthetic reaction is started in a different reaction vessel and all the necessary operations are executed in parallel. Compounds are synthesized in parallel using spatially separated compartments. It is like 'one vessel -one compound' philosophy. The synthesis is feasible in solid supported as well as solution chemistry is possible

Advantages:

- Reaction time for synthesis of multiple compounds is similar to one synthesis (solvent, reagents are serially transported into each reaction vessel)
- Each compound is substantially 'pure' in its location
- Defined location provides the structure of a certain compound
- Easier biological evaluation
- No deconvulation is required
- No risk of synergistic effect leading to false positive result during screening

Disadvantage:

- Applicable only for medium libraries (several thousand compounds)

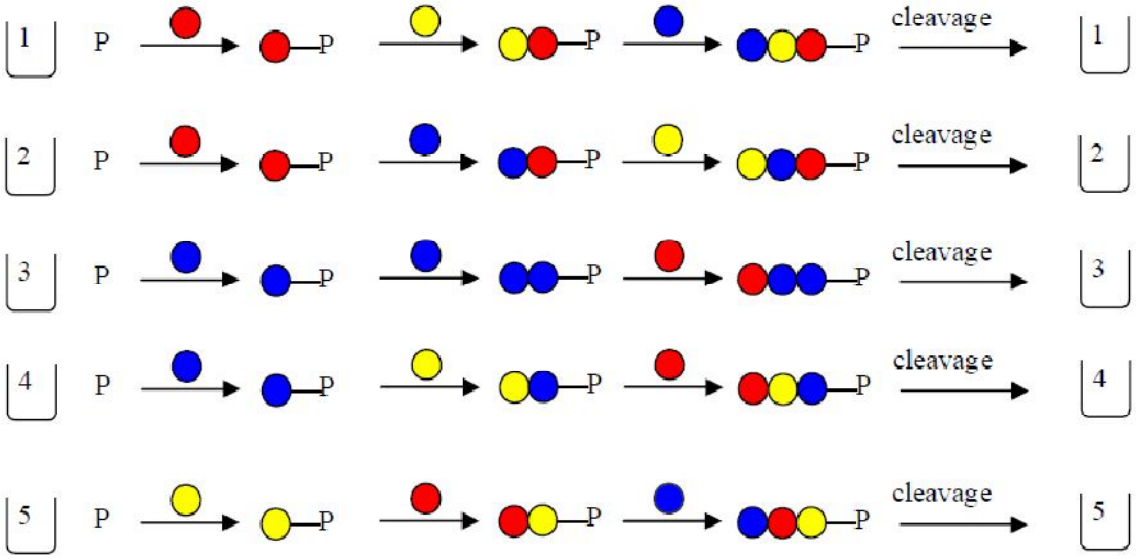
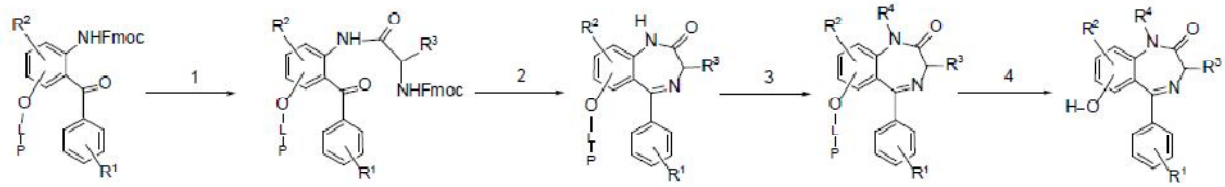


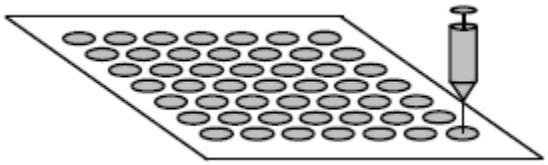
Figure: Parallel synthesis of five trimers in five reaction vessels. Each circle represents amino acid for the synthesis of peptide library.

Few example of parallel synthesis

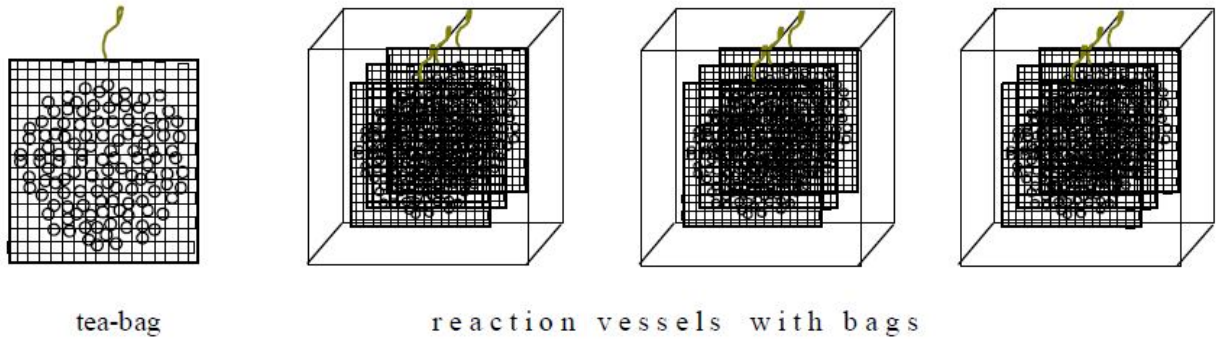
Multipin technique using parallel synthesis



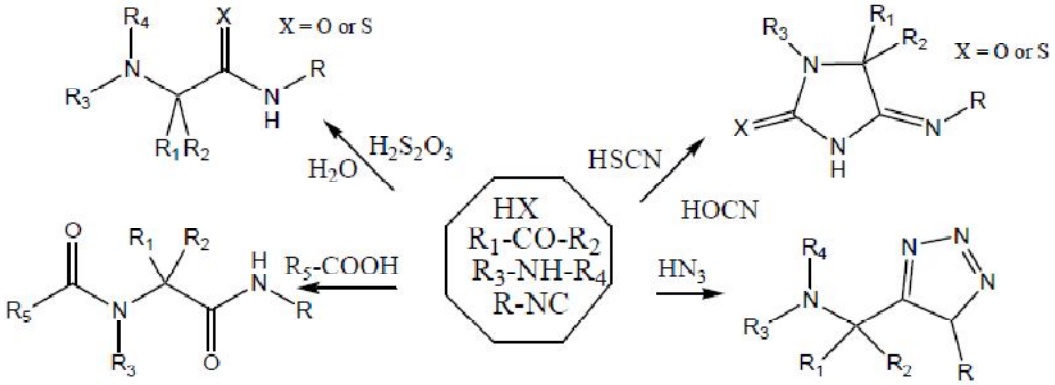
SPOT technique using parallel synthesis



Tea-bag technique using parallel synthesis



Ugi muticomponent (U-nCR) reactions as parallel synthesis



Determining the most bioactive substance of a mixture

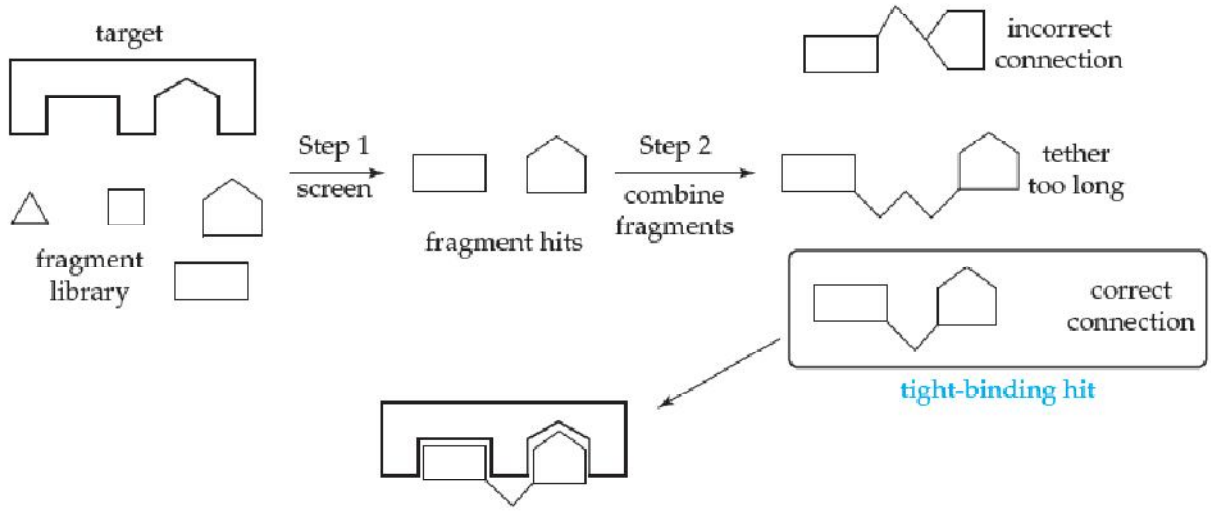
Methods 1: On-bead screening

a) Compounds are still covalently attached to the solid support
b) The solid-bound library is treated with a labelled biological target (receptor)
c) Selection of the labelled beads (highly automated methods!) followed by structural characterisation
d) Requirement: Solid support/ Linkers have to be water-soluble

Method 2: Deconvulation

a) Preparation of sets of sublibraries (Each of them contains compounds, where the identity of one single building block is known; the building blocks at the remaining positions contain all possible variations)
b) Screening of sublibrariesprovides the mixture with high bioactivity
c) Iterative deconvulation or deconvulation by position scanning

F. *Fragment based drug discovery*



Scheme: Hit development through fragment based screening

The key to discover the successful hits through fragment-based screening requires following considerations:

1) Targets with two or more (multiple) active site pockets, in which each can accommodate the respective fragment (based on recognition, size, affinity etc.).
2) Identifying the binding fragments.
3) Proper connections or tethering between fragments (correct length and position to fit with target) to enable a single strongly binding hit (vide scheme).
4) Model for fragments and target interactions.

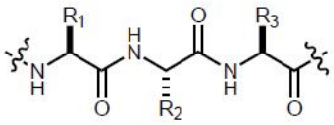
<u>Inhibition of Stromelysin</u>

According to Prof. George Whitesides at Harvard University, a linker should be longer than the necessary simply fold upon themselves to bring the fragments closer for binding a target. The poor choice for a linker is one that is too short to span the distance between fragment binding sites. For instances, the development of inhibitors of Stromelysin was carried out using fragment based strategy. (*Stromelysin is a zinc-dependent protease that is responsible for breaking down and re-forming connective tissues which dysfunction including related enzymes causes arthritis and tumor activity*).

Peptidomimetics

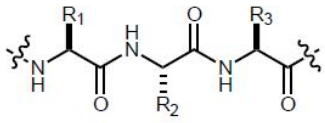
Peptidomimetics is the way to mimic an endogeneous peptide backbone by non-peptidic fragments while retaining the appropriate topography, conformation and electronic properties (pharmacokinetic properties) of the lead peptide for binding to a particular target biomolecule. The design of peptidomimetics can be a lead optimization approach for drug development.

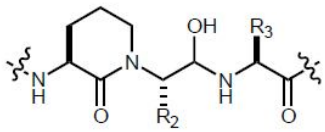
Replacement of a peptide (both backbone and side chain of peptide) brings the conformational restriction and lipophilicity into the peptide. The lipophilicity of the peptide enhances the bioavailability of the molecules. As a result of peptidomimetics, the proteolytic enzyme e.g peptidase is unable to recognize the molecule and hence is stable under enzymatic condition.



1. What is the conformation of the biologically active core peptide?
2. How can the peptide be modified to rigidify that conformation?
3. Do these modifications increase the peptide's bioavailability, receptor selectivity, and resistance to degradation?

Q. Why peptidomimetics is needed for drug potency?





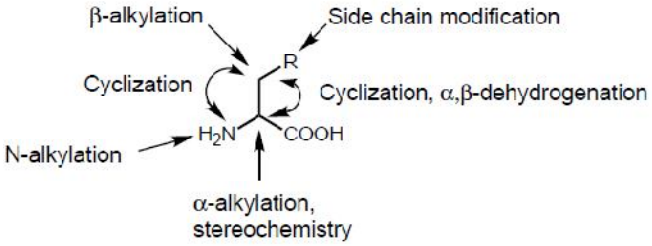
Disadvantages of Peptides as Drugs

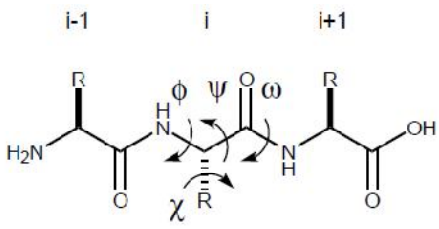
1. Limited stability towards proteolysis by peptidases in the gastroeintesinal tract and in serum ($t_{1/2}$ on the order of minutes)
2. Poor transport properties from the intestines to the blood and across the blood-brain barrier due to high MW and lack of specific transport systems
3. Rapid excretion through the liver and/or kidneys
4. Inherent flexibility enables interaction with multiple receptors besides the target, and could result in undesired side-effects

Advantages of Peptidomimetics as Drugs

1. Conformationally restrained structures can minimize binding to non-target receptors and enhance the activity at the desired receptor.
2. Addition of hydrophobic residues and/or replacement of amide bonds results in better transport properties through cellular membranes.
3. Isosteres, retro-inverso peptides, cyclic peptides and non-peptidomimetics all reduce the rate of degradation by peptidases and other enzymes.

The way of peptidomimetics

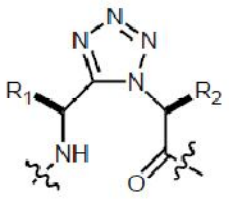




Modification	Conformational effect
1. Backbone *N*-alkylation	ϕ, ψ, χ are constrained, facilitates cis-trans amide bond isomerism
2. Backbone C_α-alkylation	ϕ, ψ are constrained to a helical or extended linear structure
3. D-Amino acid/proline substitution	Favors formation of β-turn structures
4. Peptide bond isosteres	ω can be fixed at 0 or 180° (olefins), or allowed greater freedom of rotation (i.e. -CH_2 S-)
5. Cyclic amino acids	ω can be biased to 0 or 180°, ϕ, ψ are biased towards formation of β-turns or γ-turns, χ can also be affected
6. Dehydroamino acids	Fix χ at 0 or 180°
7. β-alkylation	Constrain χ, may also affect backbone conformation

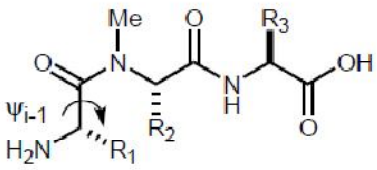
Examples of peptidomimetics

Tetrazoles



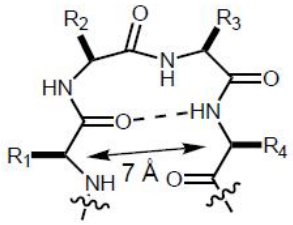
- The tetrazole locks the amide in a cis configuration, and is more easily synthesized than a cis-olefin isostere.
- Similar or enhanced activity of a tetrazole peptidomimetic indicates a cis-amide bond is favorable for receptor binding.
- Absence of activity can be due to the increased steric bulk of the tetrazole, not necessarily that a cis-amide bond is not the bioactive conformation.

N-Methyl amides



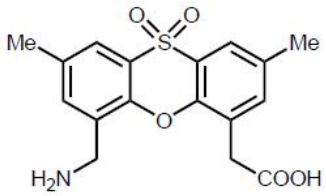
- The cis-amide is only 0.6 kcal/mol higher in energy than the trans isomer.
- The increase in flexibility is tempered by the steric restrictions imposed by the Me group. ψ_{i-1} is restricted to $60<\psi<180$ in both the cis- and trans-amide.

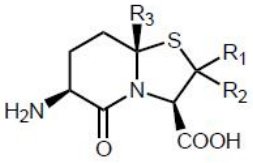
Representative β-turn mimetics



β-turn

Representative β-turn mimetics

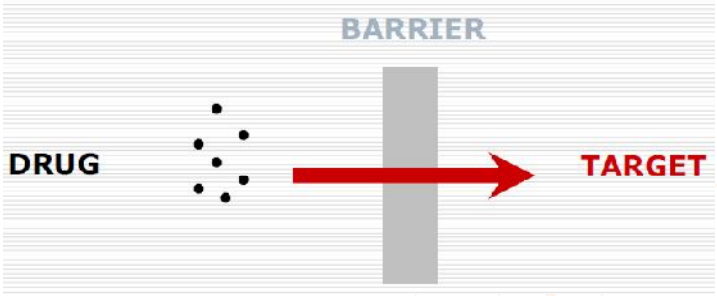




Concept of lipophilicity for drug action

Physiological barriers

When a drug molecule encounters a barrier, the amount of drug reaching the other side is diminished. The penetration of drugs to the therapeutic target is slowed and attenuated by the barrier. How molecules behave at each barrier determines the rate at which molecules progress to the target site.



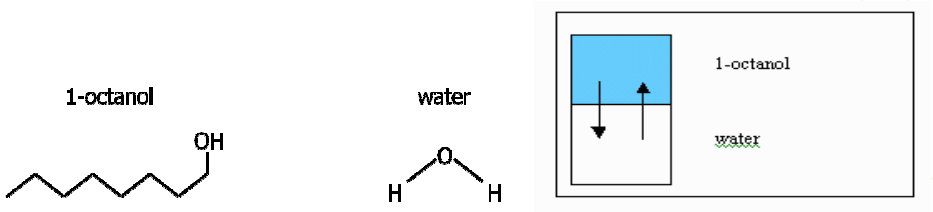
Importance of Lipophilicity

Hansched proposed that the first step in overall drug process is the diffusion where drug molecules make its way from a dilute solution outside of the cell to a particular site in a cell. This is a slow process and it depends on the molecular structure of the drug. To reach the site of action, a drug molecule has to interact with two environments: (i) lipophilic (membranes) and (ii) aqueous (exobiophase such as cytoplasm). The functions of membranes (non aqueous lipophilic) are to protect the cell (cytoplasm) from water-soluble substances and to form surface where enzyme or proteins are attached that separates solution of different electrochemical potential. For example, *blood-brain-barrier*, a membrane surrounded by capillaries of the circulating system in a brain that protect passive diffusion of polar chemicals from the blood stream and blocks the delivery of central nervous system (CNS)drug to their site of action.

According to Fluid mosaic model for the structure prediction of the membrane, there are polar end group (water soluble) connected by hydrocarbon chain (hydrophobic, water insoluble or organic soluble) of the lipid and the integral proteins are embedded within lipid bilayer. The main constituent of the lipid membrane are cholesterol and ionic phospholoipid. Thus, all lipid membranes are amphiphatic in nature. The hydroxyl group of cholesterol is polar hydrophilic ends and steroid/hydrocarbon part is lipophilic ends. The membrane is stabilized by ion-dipole interactions with water and from association from non-polar groups. Increasing lipophilicity may increase target protein binding at the expense of aqueous solubility & metabolic stability.

Measurement of lipophilicities

Hansch measured the lipophilicity of the compounds according to their solubility in membrane or vesicle. He set a scale of lipophilicity of organic molecules based on the solubility in membrane. The model for first step of drug action is such that the solubility of the compound in 1-octanol which stimulates a lipid membrane, relative to that in water. [1-octanol dissolves water to the extent of 1.7 M saturation].



Participation coefficient, a measure of the solubility in octanol in water

$$P = [compound]_{oct} / [compound]_{aq} (1-\alpha) \quad \text{.....(1)}$$

The relative potencity of a drug in terms of lipophilicities is expressed by following equation:

$$\log 1/C = -k(\log P)^2 + K^{I} (\log P) + k^{II} \quad \text{.....(2)}$$

where C is concentration of drug and P = partition coefficient

From eq **1**, if P < 1 (more soluble in water), log P is negative. Hence the drug interaction with lipid phase is less.

From eq **1**, if P > 1 (more soluble in water), log P is positive. The more positive P more would be the lipophilic. Hence the drug interaction with lipid phase is more.

Hansch proposed that the partition coefficient is contributed by individual atom and group. The lipophilicity of the substituent is determined by the following equation:

Lipophilicity of substituent constant, $\pi = \log P_X - \log P_H = \log P_X / \log P_H$

P_X is partition coefficient for the compound with substituents; P_H is partition coefficient of the parent molecule.

Lipinski's 'the rule of five' (RO5)

The pharmacokinetics of improving oral bioavailability during lead modification is based on Lipinski's 'the rule of five'. According to the rule, a good drug candidate (druglikeness) should have following characteristics:

- The molecular weight < 500 daltons
- An octanol-water partition coefficient, log P < 5
- No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)

Note that all numbers are multiples of five, which is the origin of the rule's name (rules of thumb). Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria.

Rational of RO5:

- Molecular Weight is related to the size of the molecule. As molecular size increases, a larger cavity must be formed in water to solubilise the compound thus decreasing solubility. Increasing size also impedes passive diffusion through the tightly packed aliphatic side chains of the lipid bilayer membrane.
- Increasing logP decreases aqueous solubility which reduces absorption. Membrane transporters can either enhance or reduce compound absorption by either active uptake transport or efflux respectively. This means that transporters can have a strong impact on increasing or decreasing absorption.
- Hydrogen bonds increase solubility in water and must be broken in order for a compound to permeate the lipid bilayer membrane. Thus increasing the number of hydrogen bonds reduces partitioning from the aqueous phase into the lipid bilayer membrane for permeation by passive diffusion.

The rule has many extensions:

- Partition coefficient log P = from −0.4 to +5.6 range
- Molar refractivity = from 40 to 130
- Molecular weight = from 180 to 500
- Number of atoms = from 20 to 70 (includes H-bond donors [e.g.; OH's and NH's] and H-bond acceptors [e.g.; N's and O's])
- Polar surface area < 140 ´^2
- Number of rotatable bond < 10

A drug's pharmacokinetics in the human body is described by this rule which includes their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not have the scope to predict whether a compound is pharmacologically active.

Antibiotics, antifungals, vitamins and cardiac glycosides are the exception of the rule because they often have active transporters to carry them across the membrane, so lipophilicity is not relevant.

The Structural Properties of the Molecule

- ✓ Hydrogen Bond Forming Moieties
- ✓ Lipophilicity
- ✓ Molecular Weight
- ✓ Polar Surface Area
- ✓ Shape
- ✓ Reactivity
- ✓ pKa

The Physico-Chemical properties

- ✓ Properties
- ✓ Solubility
- ✓ Permeability
- ✓ Chemical Stability

The Biochemical properties

- ✓ Metabolism (Phases 1 & 2)
- ✓ Protein and Tissue Binding
- ✓ Transport Modality

Pharmacokinetics & Toxicity

- ✓ Clearance
- ✓ Half-Life
- ✓ Bioavailability
- ✓ Drug-Drug Interactions

Realization that drug design studies based on affinity & potency alone

- ✓ Candidates may be too polar to
- ✓ penetrate BBB and reach CNS targets
- ✓ Candidates may be unstable & rapidly
- ✓ cleared through first pass metabolism
- ✓ Candidates may be too soluble to be absorbed from the intestine

A diverse ensemble of physicochemical and biochemical processes is encountered by drug molecules

- ✓ Cell Membranes
- ✓ Metabolic Enzymes
- ✓ Solution
- ✓ pH

- ✓ Efflux Transporters
- ✓ Binding Proteins

Poor drug properties may include

- ✓ Low absorption- low solubility or permeability
- ✓ High clearance- owing to metabolism
- ✓ High clearance by hydrolysis- in GIT or blood
- ✓ Efflux- opposes uptake in many membranes which enhances extraction in liver & kidney
- ✓ High protein binding- limits free drug at target
- ✓ Poor penetration of a blood-organ barrier at the target organ
- ✓ High volume of distribution due to lipophilicity

LogP

- ✓ Molecular Volume (related to molecular weight & affects the size of the cavity that must be formed in the solvent to solubilize the molecule)
- ✓ Dipolarity (polar alignment of the molecule with the solvent)
- ✓ Hydrogen Bond Acidity
- ✓ Hydrogen Bond Basicity

Lipophilicity (changes with the condition of the phases)

- ✓ Partitioning solvents
- ✓ pH
- ✓ Ionic strength
- ✓ Buffer
- ✓ Co-solutes/Co-solvents

Pka effects

- ✓ Ionised molecules are more soluble in aqueous media than neutral molecules because they are more polar
- ✓ Solubility is determined both by the intrinsic solubility of the neutral molecule and that of the ionized species which is much greater.
- ✓ Ionised molecules are less permeable than neutral molecules
- ✓ The neutral molecules are more lipophilic than their ionized counterparts and are considered to be the dominant form that permeates by passive diffusion

Drugs and their pK_a

Acids	pK_a
Penicillin V	2.7
Salicylic Acid	3.0;13.8
Acetylsalicylic Acid	3.5
Diclofenac	4.1
Sulfathiazole	7.1
Phenobarbital	7.4;11.8
Phenytoin	8.3
Acetaminophen	9.9
Caffeine	14

Bases	pK_a
Caffeine	0.6
Quinidine	4.1;8.0
Tolbutamide	5.3
Cocaine	8.4
Ephedrine	9.4
Imipramine	9.5
Atropine	9.7

Therapeutic index

IC_{50}: The concentration at which 50% inhibition of an enzyme or protein or antagonism of a receptor take place.

LD_{50}: The lethal dose for 50% of the test animal population

ED_{50}: The dose required for 50% of maximal effect (or the effective dose that produces maximum therapeutic effect in 50% of the test animals)

Thus, the therapeutic index = LD_{50} / ED_{50}

The larger the therapeutic index, the greater the margin of the safety of the compound.

There is no specific minimum value for therapeutic index before a drug is approved. Because, it depends on the disease and also whether other therapies are already available or the side effect is minimal. A low therapeutic index is tolerable for lethal disease like cancer or AIDS (where the therapeutic index value in between 1-5) especially if no other treatment is available or it produces minimum side effect. For less threatening disease, the therapeutic indices are in the order of 10-100.
For example, the therapeutic index of antitumor agent chlorambucil (Leukeran) is found 23.
According to Hansch equation, the lipophilicity and electronic factors are related to the concentration by following equation.

$$\log 1/C = -k(\log P)^2 + K^{I} (\log P) + \rho\sigma + k^{II} \quad \ldots\ldots\ldots\ldots\ldots(2)$$

$$\log 1/C = -k\,\pi^2 + K^{I}\,\pi + \rho\sigma + k^{II} \quad \ldots\ldots\ldots\ldots\ldots(2)$$

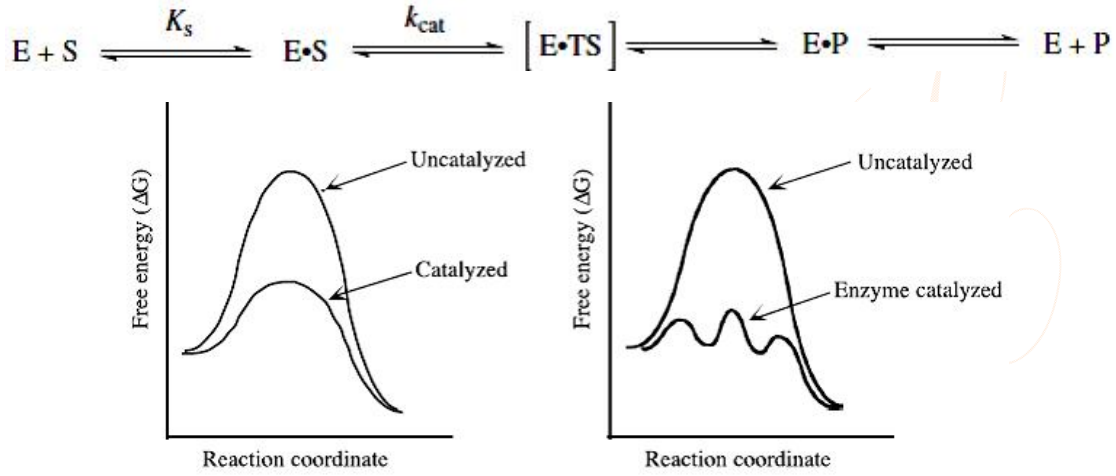
The lipophilicity (**logP**) and electronic properties (**σ, π**) are related to the molar concentration (**C**) that elicit the standard biological response (**IC_{50} , LD_{50} , ED_{50}**).

Q. The LD_{50} for a potential antiobesity compound was found to be 10 mg/kg and the ED_{50} was 2mg/kg. Is it an important drug candidate? Why?

Ans: Therapeutic index = LD_{50} / ED_{50} = 10 $mgkg^{-1}$/ 2 $mgkg^{-1}$ = 5
Since the obesity is considered as less threatening disease, the therapeutic index should be in between 10-100. Hence, the antiobesity compound can not be considered as potent drug candidate.

Enzymes

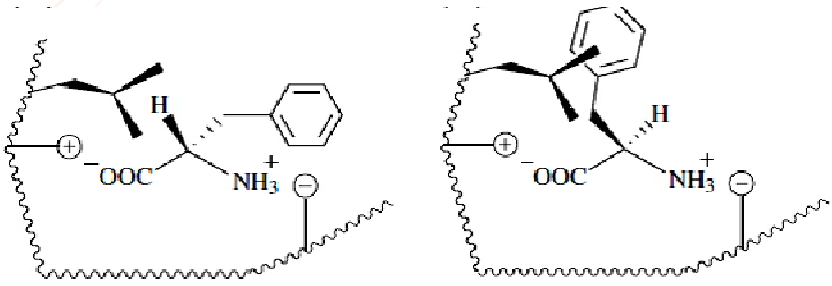
Enzymes function by lowering transition state energies and energetic intermediates and by raising the ground state energy (ground state destabilization). Among 21 hypotheses, an enzyme-catalyzed reaction is always initiated by the formation of an enzyme–substrate (or E·S) complex from which catalysis begins.



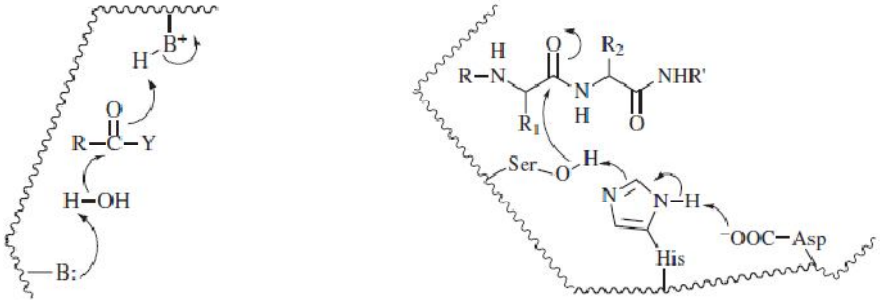
Enzyme catalysis is characterized by two features: specificity and rate acceleration. The active site contains moieties that are responsible for both of these properties of an enzyme, namely, amino acid residues and, in the case of some enzymes, cofactors. A cofactor, also called a coenzyme, is an organic molecule or a metal ion that binds to the active site, in some cases covalently and in others noncovalently, and is essential for the catalytic action of those enzymes that require cofactors.

Approximation is the rate enhancement by proximity. The enzyme serves as a template to bind a substrate in close proximity. As a result, there is a loss of rotational and translational entropies of the substrate upon binding to the enzyme. However, this entropic loss is offset by a favorable binding energy of the substrate, which provides the driving force for catalysis.

Differential binding interactions by Enantiomers: binding pocket for the (S)-isomer (left), stertic hindrance with the (R)-isomer (right).



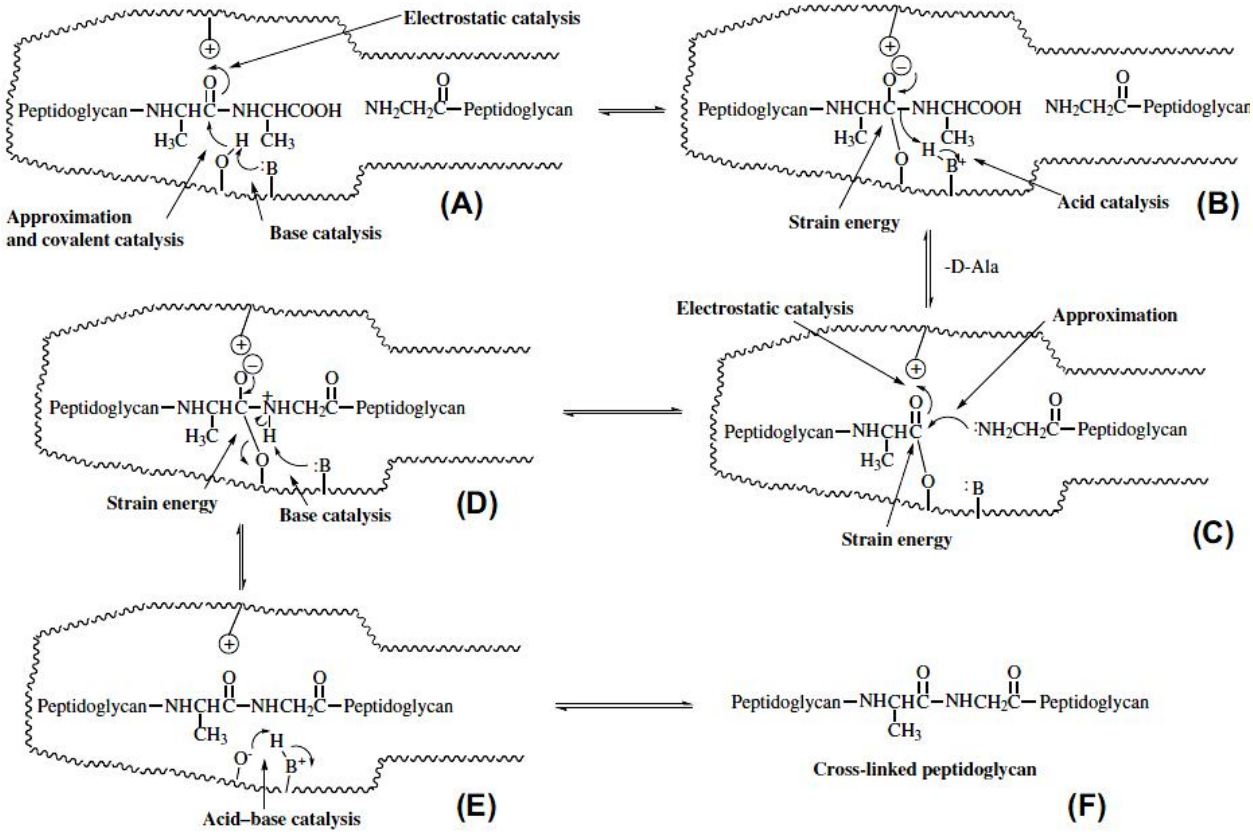
Simultaneous acid and base enzyme catalysis (left) and Charge relay system for activation of an active-site serine residue (right)



Mechanism of enzyme catalysis

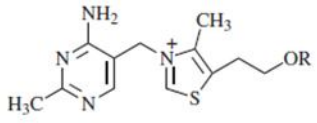
Peptidoglycan transpeptidase, the bacterial enzyme catalyzes the cross-linking of peptidoglycan strands to make the bacterial cell wall. Most bacterial peptidoglycan transpeptidases are embedded in the cell membrane, making them difficult to isolate in their active form.

Hypothetical mechanism for peptidoglycan transpeptidase

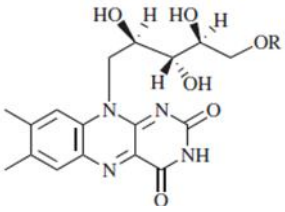


Coenzyme catalysis

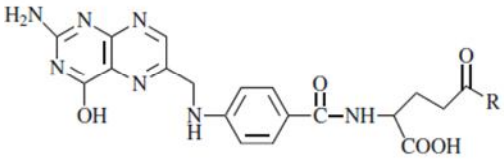
A coenzyme, or cofactor, is any organic molecule or metal ion that is essential for the catalytic action of the enzyme. The usual organic coenzymes are generally derived as products of the metabolism of vitamins that we consume. There are coenzymes which are not derived from vitamins rather assists enzyme functions.



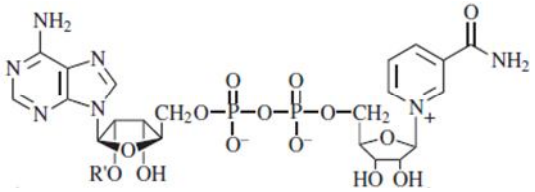
Thiamin diphosphate
(of Vitamin B_1/ thiamin)



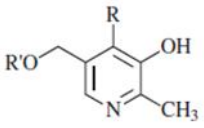
Flavin mononucleotide
(of Vitamin B_2/Riboflavin)



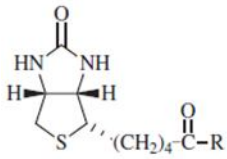
Tetrahydrofolate
(of Vitamin B_9/Folic acid)



NAD+
(of Vitamin B_3/Nacinamide)



Pyridoxal 5′-phosphate
(of Vitamin B6/ pyridoxine)



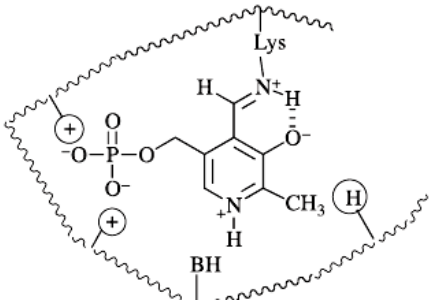
Biotin

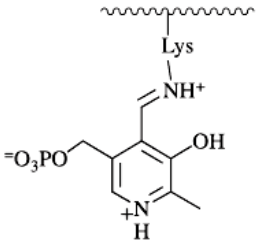
Vitamins are essential nutrients. Human metabolism is incapable of producing them. Deficiency in a vitamin implicate in the shutting down of the catalytic activity of various enzymes that require the coenzyme made from the vitamin. This leads to certain disease states.

Pyridoxal 5′-Phosphate (PLP)

Pyridoxal 5′-phosphate (PLP) is the versatile coenzyme which catalyzes several reactions of amino acids such as recimization, decarboxylation, α—cleavage, β—elimination, γ—elimination, transamination etc.

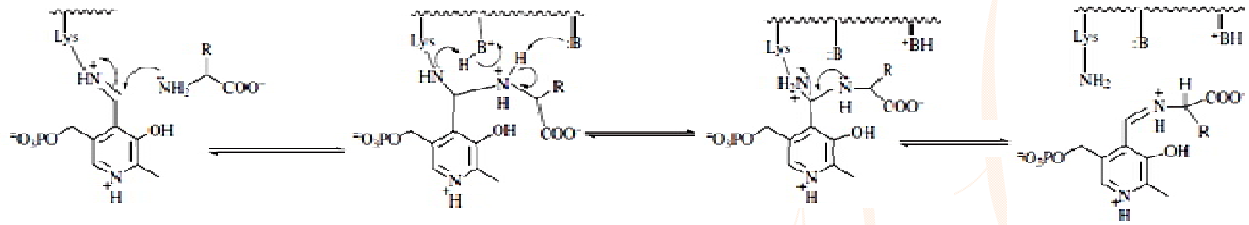
The major interaction of PLP is to bind with the active site is covalent bond which is shown below.



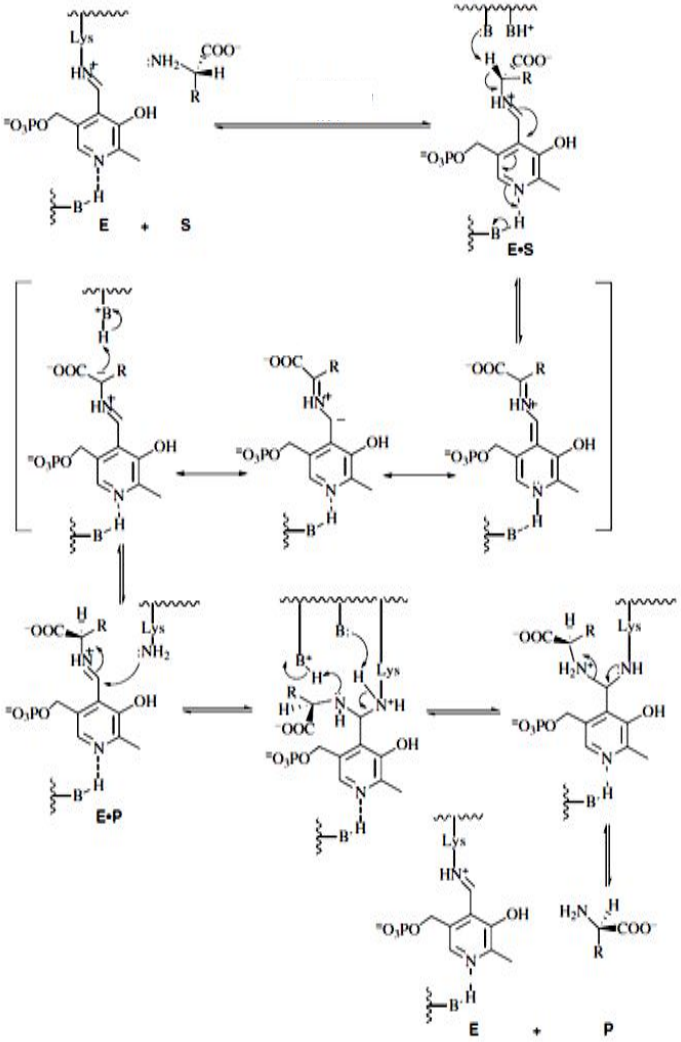


The most important features of PLP is that the aldehyde group of the PLP is held tightly at the active site by a Schiff base (iminium) linkage to a lysine residue. Thus, the first step in all PLP-dependent enzyme transformations of amino acids is a transimination reaction, i.e., the conversion of the lysine–PLP imine to the substrate–PLP imine. The pyridinium group acts as an electron sink to stabilize electrons for which it allows nonenzymatic reaction.

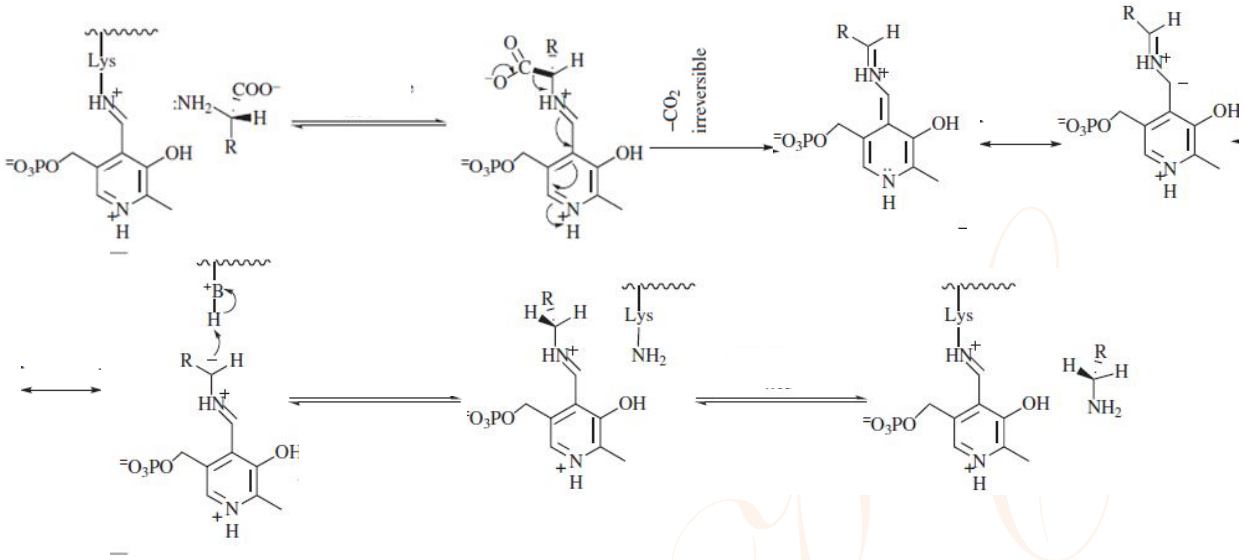
PLP dependent enzymatic reaction



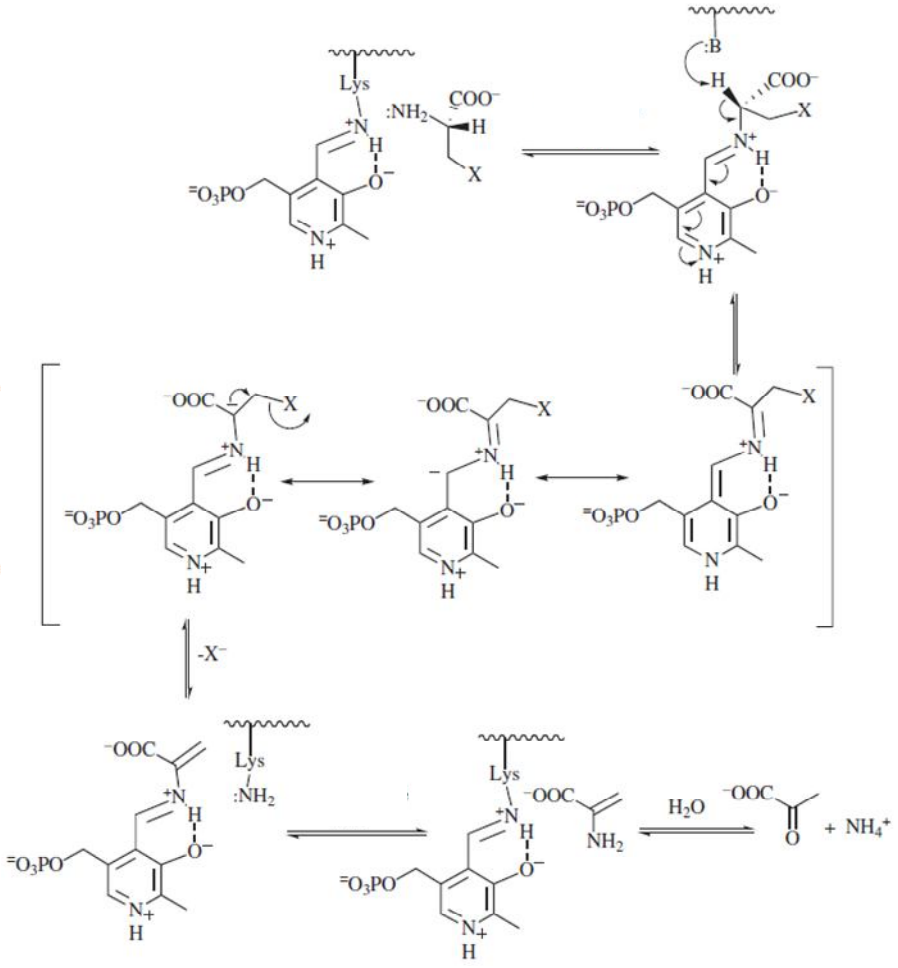
PLP dependent racemization



PLP dependent decarboxylation

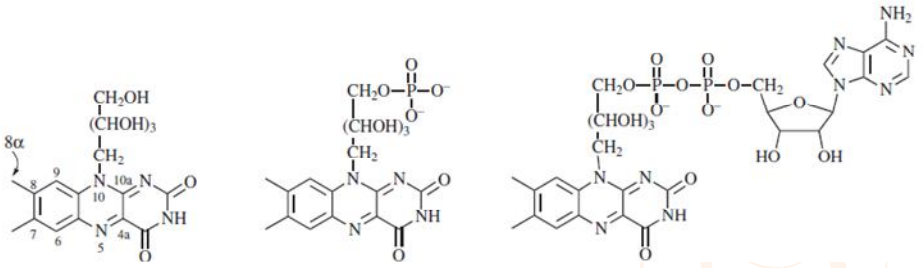


PLP dependent β—elimination



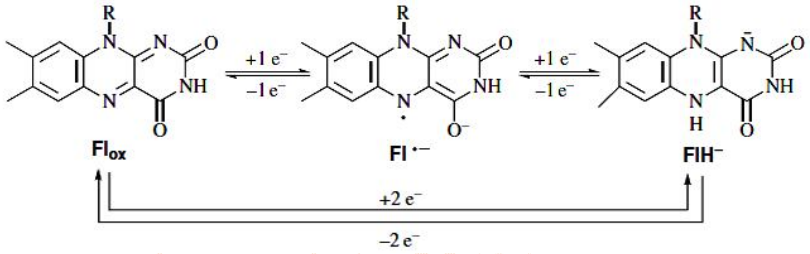
Flavin

Different forms of flavin coenzymes are derived from riboflavin (vitamin B_2) which is enzymatically converted to two other forms, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Most enzymes use both forms. flavin coenzymes catalyze a wide variety of redox and monooxygenation reactions. Some flavoenzymes are called oxidases and others dehydrogenases.

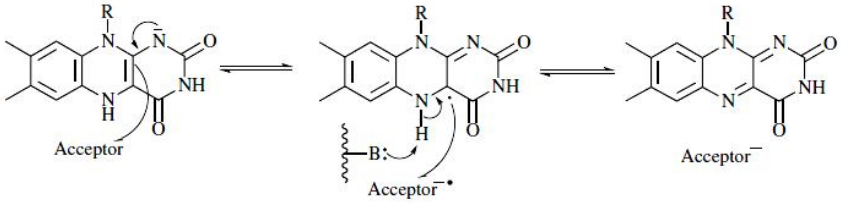


Riboflavin (Vitamin B_2) FMN FAD

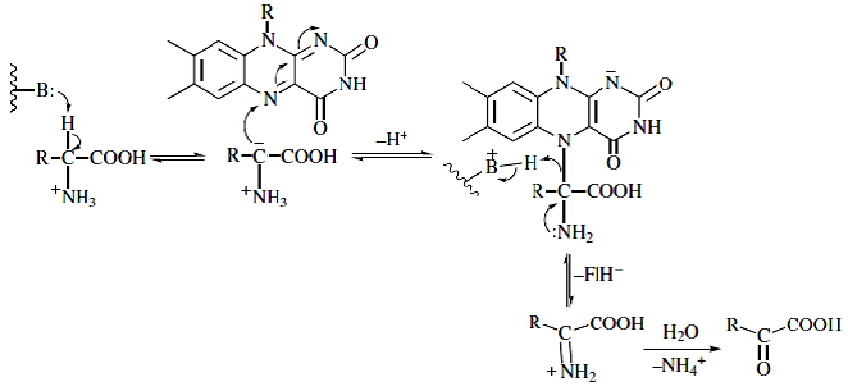
One- and two-electron reductions of flavins



Mechanism for dehydrogenase-catalyzed flavin oxidation



Two-electron mechanism for flavin-dependent D-amino acid oxidase-catalyzed oxidation of d-amino acids

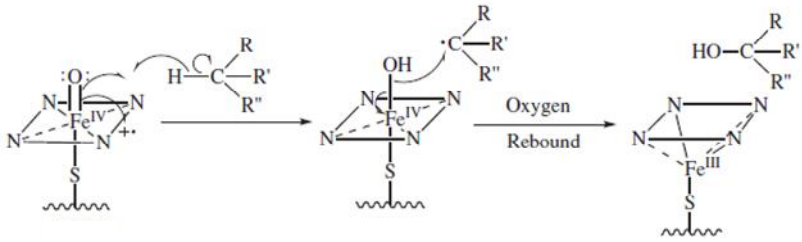


Heme

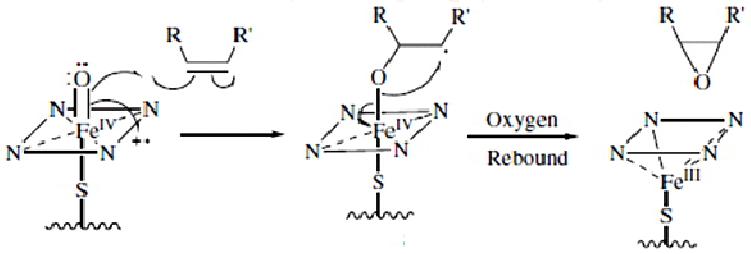
Heme, or protoporphyrin IX, is an iron(III)-containing porphyrin cofactor for a large number of liver microsomal mixed function oxygenases principally in the cytochrome P450 family of enzymes. Like flavin monooxygenases, these enzymes are important in the metabolism of xenobiotics, including drugs.

Molecular oxygen binds to the heme cofactor (after reduction of Fe^{3+} to Fe^{2+}) and is converted into a reactive form, which is used in a variety of oxygenation reactions, especially hydroxylation and epoxidation reactions.

Mechanism for heme-dependent hydroxylation reactions of alkanes



Mechanism for heme-dependent epoxidation reactions of alkenes



Enzyme inhibition

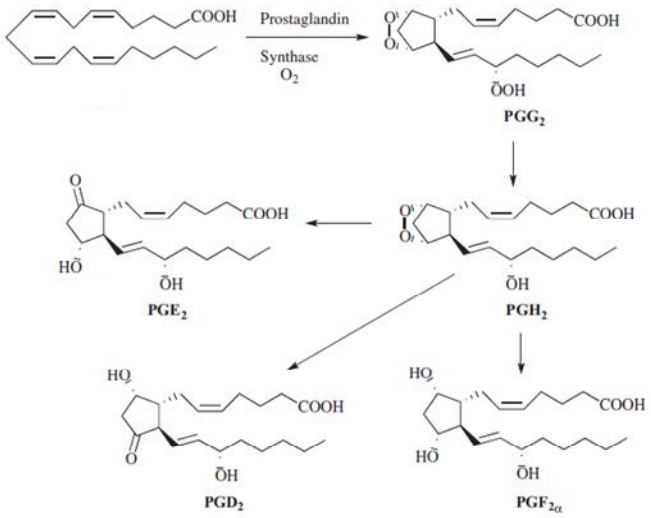
Many diseases, or at least the symptoms of diseases, arise from a deficiency or excess of a specific metabolite in the body, from an infestation of a foreign organism, or from aberrant cell growth. Many disease states can be addressed by specific enzyme inhibition. The compound that blocks or slows down enzyme catalysis is an ***enzyme inhibitor***. If the interaction with the target enzyme is irreversible (covalent), then the compound of that enzyme inhibitor referred to as ***an irreversible enzyme inhibitor*** or ***enzyme inactivator***. So, if enzyme activity is blocked, the substrates for that enzyme cannot be metabolized, and the metabolic products are not generated.

Situation 1**:** If a cell has a deficiency of the substrate for the target enzyme, a disease state arises as a result of that deficiency. Then inhibition of that enzyme would prevent the degradation of the substrate, thereby increasing its concentration. For instance, inhibition of the enzyme that degrades γ-aminobutyric acid (GABA), namely, GABA aminotransferase (GABA-AT), leads to an anticonvulsant effect.

Situation 2: If there is an excess of a particular metabolite that produces a disease state, then inhibition of an enzyme on the pathway for biosynthesis of that metabolite should diminish its concentration. Excess uric acid can lead to gout.

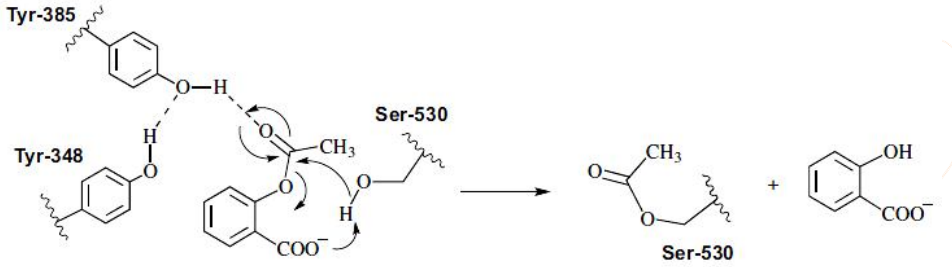
Enzyme inhibition is a promising approach for the rational discovery of new leads or drugs. Prostaglandins (PGs) are important hormones that are involved in the pathogenesis of inflammation and fever. Elevation of body temperature during infection is also mediated by the release of PGs. Microsomal enzymes catalyze the biosynthesis of PGs, and that PGs are always released when cells are damaged. Inhibition of PG synthase [cyclooxygenase (COX)] results in antiinflammatory, antipyretic, and analgesic effects.

Biosynthesis of prostaglandins (PGs) from arachidonic acid



Therefore, inhibition of platelet COX is particularly effective in blocking PG biosynthesis because unlike most other cells, platelets cannot regenerate the enzyme. When PG synthesis is blocked, there is less stimulation of the pain-sensitive nerve endings, resulting in less aching of muscles and joints, as well as less relaxation of blood vessels in the head, so fewer headaches.

PG synthase or COX (different isoform 1-3 of COX) was found to be irreversibly inactivated by NSAID (non-steroidal antiinflamatory drug) including aspirin which transfers an acetyl group to each enzyme molecule. A hypothetical mechanism for acetylation of prostaglandin synthase by aspirin is shown below.

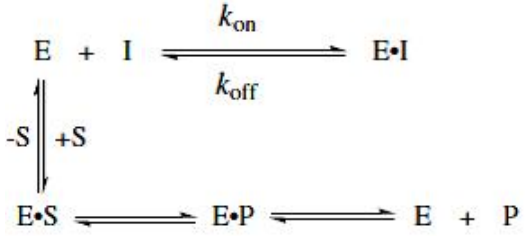


Enzyme inhibitors can be grouped into two general categories:

a) Reversible inhibition
b) Irreversible inhibitors

Reversible Enzyme Inhibition

The most common enzyme inhibitor drugs are the reversible type, particularly ones that compete with the substrate for active site binding. These are known as ***competitive reversible inhibitors***, typically compounds that have structures similar to those of the substrates or products of the target enzymes and which bind at the substrate binding sites, thereby blocking substrate binding. Typically, these inhibitors establish their binding equilibria with the enzyme rapidly, so that inhibition is observed as soon as the enzyme is assayed for activity.



$$IC_{50} = \left(1 + \frac{[S]}{K_m}\right) K_i$$

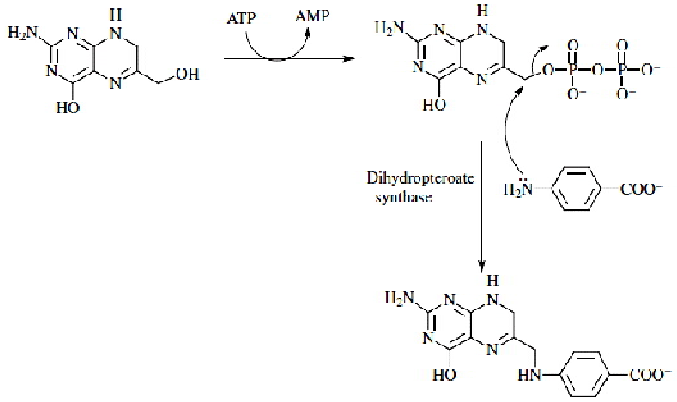
Interaction of the inhibitor with the enzyme can occur at a site other than the substrate-binding site (i.e., at an allosteric binding site).

An inhibitor-induced conformational change in the enzyme to give a form of the enzyme that does not bind the substrate properly, then the inhibitor is a noncompetitive reversible inhibitor.

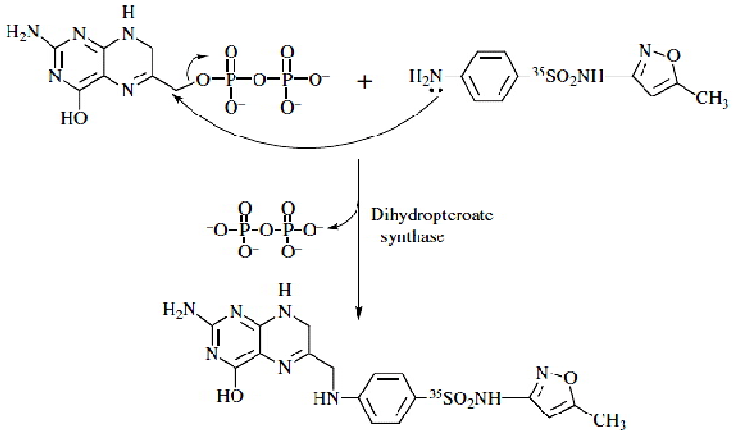
Sulfa drug: A Competitive reversible inhibitor

Mechanism of action

Biosynthesis of bacterial dihydrofolic acid.



Dihydropteroate synthase uses sulfamethoxazole in place of para-aminobenzoic acid.

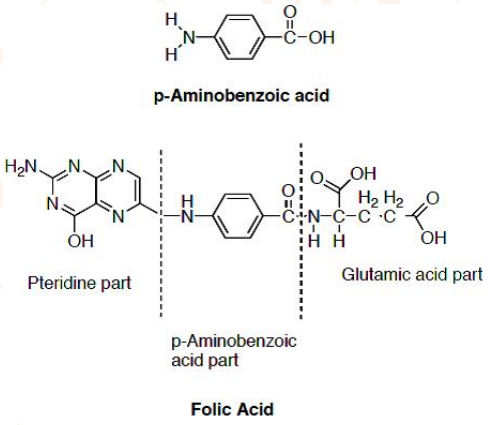


This is an example of competitive reversible inhibition in which the inhibitor also is a substrate. Because of the structural similarity of sulfanilamide to p-aminobenzoic acid, it is a potent competitive inhibitor of the enzyme. The product cannot produce dihydrofolate, and, therefore, the organism cannot get the tetrahydrofolate needed as a coenzyme to make purines which are needed for DNA biosynthesis. This is why the sulfonamides are bacteriostatic, not bacteriocidal. Inhibition of tetrahydrofolate biosynthesis only inhibits replication; it does not kill the existing bacteria.

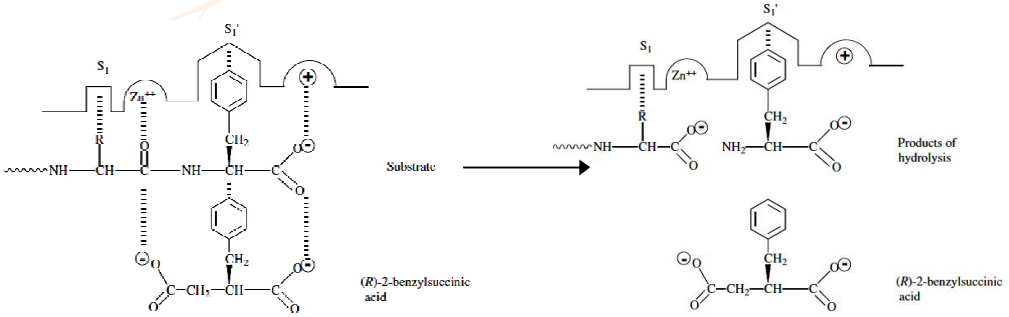
Inhibitors of dihydropteroate synthase, however, have no effect on humans, because we do not biosynthesize folic acid and, therefore, do not have that enzyme. Folic acid is a vitamin and must be eaten by humans. Furthermore, because bacteria biosynthesize their folic acid, they do not have a transport system for it. Consequently, we can eat all the folic acid we want, and the bacteria cannot utilize it. This is another example of selective toxicity. Sulfonamides are not effective with pus-forming infections because pus contains many compounds that are the end products of tetrahydrofolate-dependent reactions, such as purines, methionine, and thymidine. Therefore, inhibition of folate biosynthesis is unimportant, and pus can contribute to bacterial sustenance.

Illustration of competitive inhibition by sulfa drug

The mode of action of the sulfa drugs is well established. Higher animals, like humans, do not synthesize folic acid and hence must acquire it in their food. Bacteria must synthesize folic acid for growth. Bacteria synthesize folic acid using several enzymes in our body. One of the most crucial is dihydropteroate synthetase (DHPS), which catalyzes the attachment of p-aminobenzoic acid to a pteridine ring system. When sulfanilamide is present, it competes with the p-aminobenzoic acid for the active site on the enzyme owing to structural similarity. This phenomenon is competitive inhibition. Once the active site of DHPS is occupied, folic acid synthesis is prevented and the bacterial growth or multiplication stops.



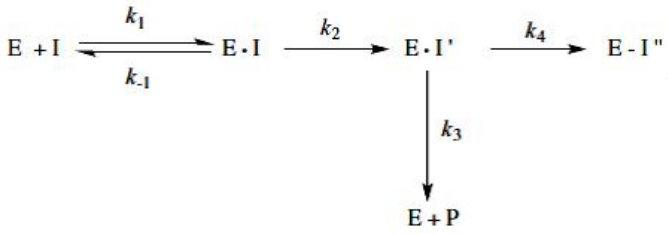
Inhibition of carboxypeptidase by (R-2-benzylsuccinic acid)



Mechanism-Based Enzyme Inactivators

An unreactive compound, bearing structural similarity to the substrate for a specific enzyme, binds to active sites and is converted to product by normal catalytic mechanism. The compound is called ***mechanism-based enzyme inactivator***. It inactivates the enzyme prior to escape from the active site.

Inactivators act initially as substrates for the target enzyme and the product often forms a covalent bond to the target enzyme (may be a tight-binding inhibitor). k_2 is the inactivation rate constant. The partition ratio k_3/k_4 indicates or a released product per inactivation event.

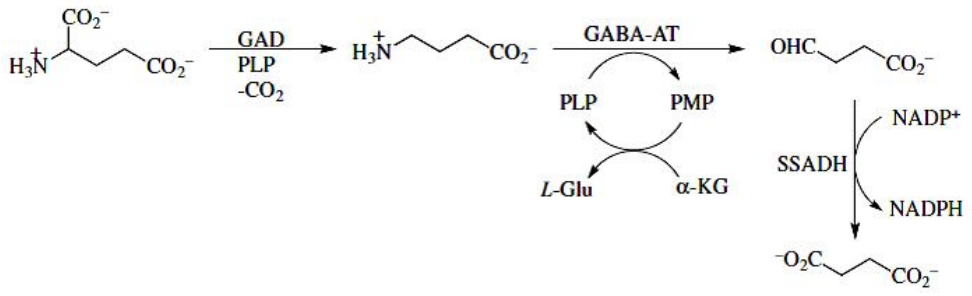


Example of Mechanism-Based Enzyme Inactivators: Vigabatrin, an Anticonvulsant Drug

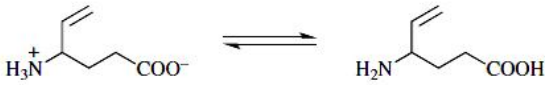
Epilepsy is a family of CNS diseases which arises due to imbalance of two principle neurotransmitters in the brain: L-glutamic acid, an excitatory neurotransmitter, and GABA, an inhibitory neurotransmitter.

The concentrations of L-glutamic acid and GABA are regulated by two PLP-dependent enzymes, L-glutamic acid decarboxylase (GAD), which converts glutamate to GABA, and GABA-AT, which degrades GABA to succinic semialdehyde with the regeneration of glutamate. The dysfunction in GABA system has been implicated in the symptoms like epilepsy, Huntington's disease and Parkinson's disease. If the concentration of GABA is abated below a threshold limit in the brain, convulsions begin.

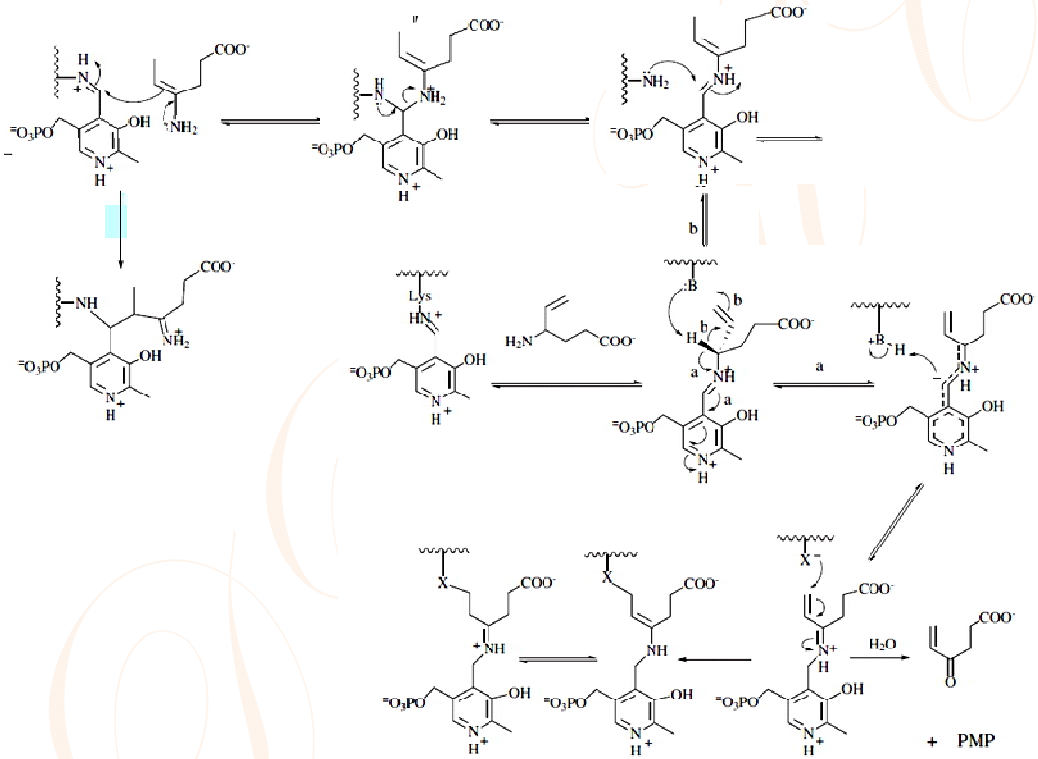
Approach 1: So, GABA might have been considered as anticonvulsant agent. But no such effect was evident as it does not cross the blood-brain-barrier, a membrane that surrounds the capillaries of the circulatory system in the brain and protects it from passive diffusion of undesirable (generally hydrophilic) chemicals from the bloodstream.



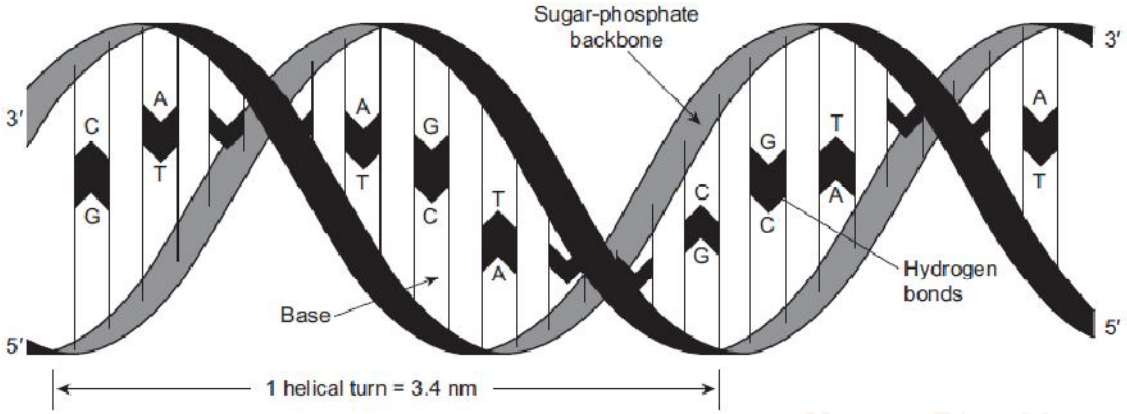
***Approach 2*:** To increase brain GABA level, that compound may be suitable for anticonvulsant property if it crosses the blood–brain barrier as well as inhibits GABA-AT.

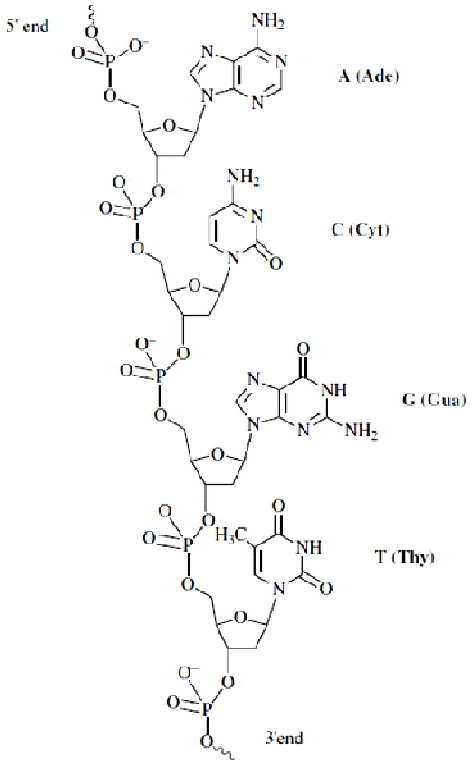


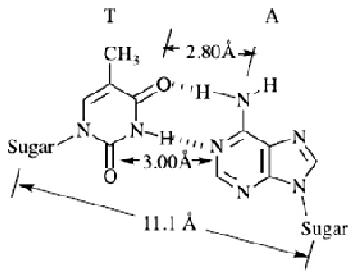
4-amino-5-hexenoic acid (vigabatrin) is the first rationally designed mechanism-based inactivator drug. Vigabatrin, which is also a small charged molecule, can diffuse through that lipophilic membrane because the electron-withdrawing vinyl substituent lowers pK_a of the amino group and increases the lipophilicity and hence increases the concentration of the nonzwitterionic form which is more lipophilic. Hypothetical mechanism for the inactivation of GABA aminotransferase by vigabatrin is shown.

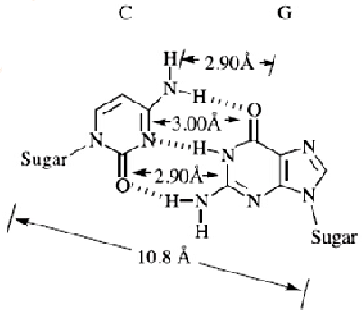


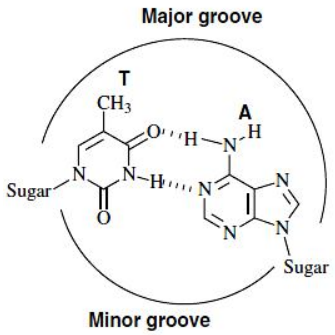
DNA structure and DNA interactive agents

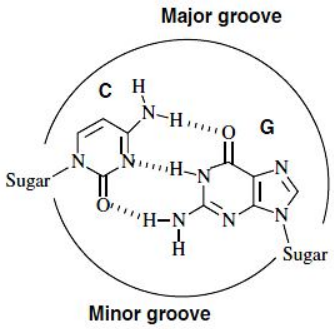




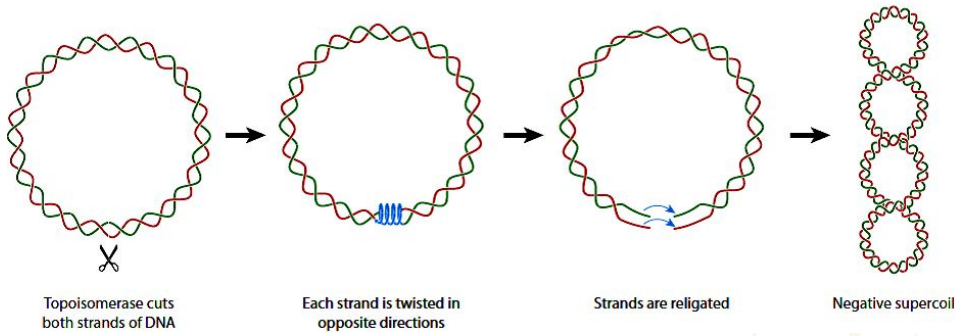




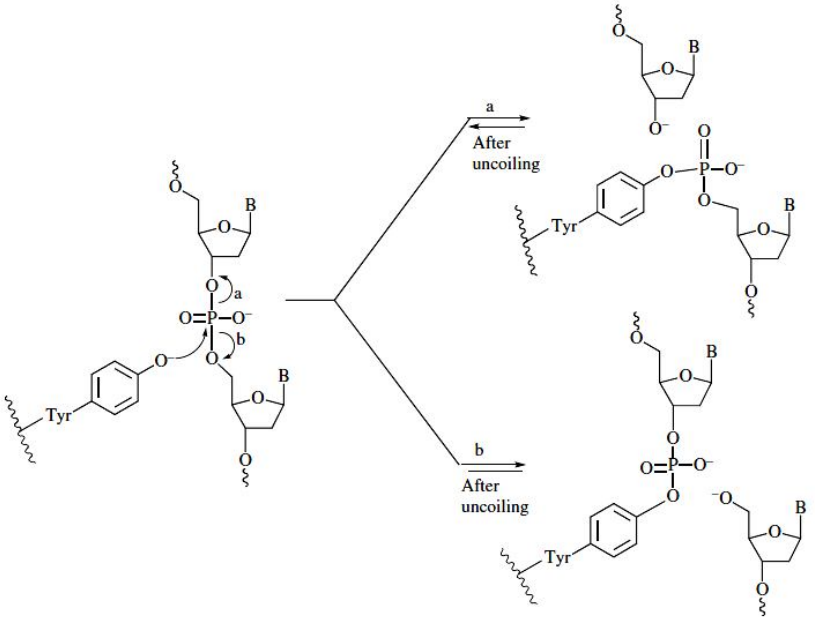




Duplex DNA ⟶ **Supercoiled DNA**



DNA topoisomerase-catalyzed strand cleavage



DNA-Interactive Drugs

The overall shape and chemical structure of DNA found in normal and abnormal cells is nearly indistinguishable. However, cancer cells undergo a rapid, abnormal, and uncontrolled cell division than normal cells. Genes coding for differentiation in cancer cells are inadequately expressed, while genes coding for cell proliferation are expressed as usual. Because these cells are continually undergoing mitosis, there is a constant need for rapid production of DNA. Drugs that interact with cancer cell (DNA-interactive drugs) will react to normal cells resulting in toxicity. The p53 can sense DNA damage in normal cells. Thus, the activation of p53 responses to cellular functions including upregulation of DNA repair systems, cell cycle arrest (to allow time for DNA repair to occur), or programmed cell death (apoptosis). Such cell cycle arrest or apoptosis in response to DNA are not possible in tumor cells. Therefore, cancer cells are more sensitive to DNA-damaging agents.

The DNA is constantly being damaged, which leads to 80–90% of human cancers. Thus, these DNA lesions must be excised. To repair the modified bases, variety of mechanisms involving cross-links, DNA adducts, double-strand breaks, such as base excision, direct reversal, nucleotide excision and recombination.

In general, anticancer drugs that target DNA are most effective against malignant tumors with a large proportion of rapidly dividing cells, such as leukemias and lymphomas. Unfortunately, the most common tumors are solid tumors, which have a small proportion of rapidly dividing cells.

Toxicity of DNA-interactive agents

The clinical effectiveness of a cancer drug requires that it generally be administered at doses in the toxic range so that it kills tumor cells but allows enough normal cells in the critical tissues, such as the bone marrow and GI tract, to survive, thereby allowing recovery to be possible. There is some evidence that the nausea and vomiting that often occurs from these toxic agents are triggered by the central nervous system rather than as a result of destruction of cells in the GI tract. Besides, chemotherapy-induced hair loss is also prevalent toxicity.Even though cancer drugs are very cytotoxic, they must be administered repeatedly over a relatively long period of time to be assured that all of the malignant cells have been eradicated.

Drug Interactions

The most significant problem associated with the use of combination chemotherapy is drug interactions; overlapping toxicities are of primary concern. An example (unrelated to DNA-interactive drugs) is the synergistic effects that are obtained when methotrexate (an inhibitor of dihydrofolate reductase) precedes 5-fluorouracil (an inhibitor of thymidylate synthase.

Classes of DNA-interactive drugs

There are three major classes of clinically important DNA-interactive drugs.

a) ***Reversible binders:*** interact with DNA through the reversible formation of noncovalent interactions.
b) ***Alkylators:*** react covalently or alkylates with DNA bases
c) ***DNA strand breakers:*** generate reactive radicals that produce cleavage of the polynucleotide strands.

The ideal DNA-interactive drug may turn out to be a nonpeptide molecule that is targeted for a specific sequence and site size. To become an effective anticancer drug, traditional cancer chemotherapy demands significant amounts of DNA damage as required eliciting the cell killing.

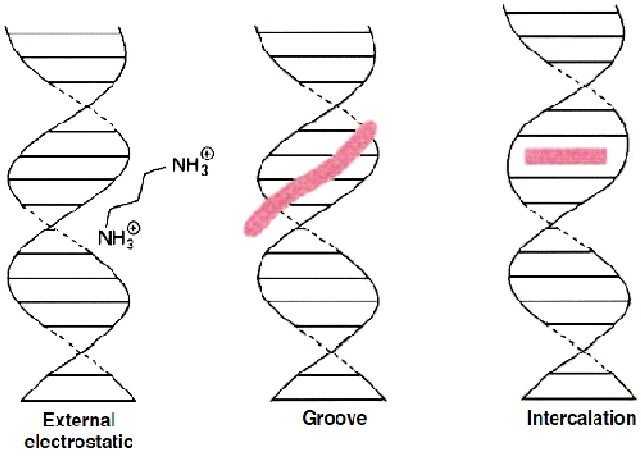
Proteins are examples of molecules that exhibit unambiguous DNA sequence recognition as a result of complementary hydrogen bonding between amino acid residues on the protein and nucleic acid bases in the major and minor grooves of DNA. Proteins generally use major groove interactions with B-DNA because there are more donor and acceptor sites for hydrogen bonding than in the minor groove.

The question arises `how drug molecules interact with DNA. DNA in the cell is packed as chromatin. The outer surface of the DNA is directly accessible to small molecules. Larger molecules also can interact or bind with DNA after uncoiling since nucleosomes are in dynamic equilibrium with uncoiled DNA. Drug binding to uncoil DNA interferes with the binding of the DNA to the histone.

Reversible DNA Binders

Nucleic acids inside the cell interact with a variety of small molecules, including water, metal cations, small organic molecules, and proteins, all of which are essential for stabilization of the nucleic acid structure. Interference with these interactions can disrupt the DNA structure. There are three important ways that small molecules can reversibly bind to duplex DNA and lead to interference of DNA function:

(1) by electrostatic binding along the exterior of the helix;
(2) by interaction with the edges of the base pairs in either the major or minor groove;
(3) by intercalation between the base pairs



(1) External Electrostatic Binding

Duplex DNA contains negatively charged polyanionic sugar-phosphate backbone which affects DNA structure and functions. Release of bound cationic counter ions of the negative phosphate groups upon binding of specific cation ligands can provide both favorable (increased entropy) and unfavorable (decreased enthalpy because of loss of specific ionic interactions) contributions to the overall free energy, leading to disruption of the DNA structure.

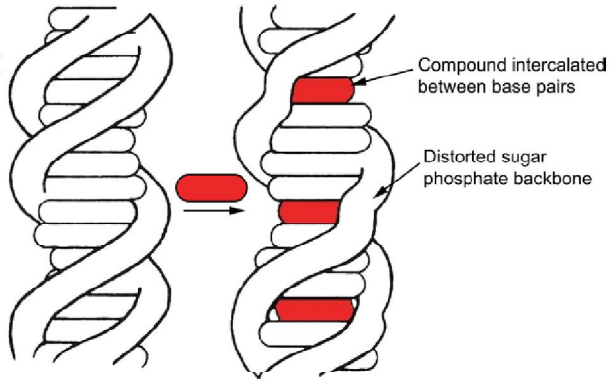
(2) Groove Binding

The major and minor grooves differ significantly in their hydrogen bonding, electrostatic potentials, steric effects, and degree of hydration. Proteins specifically bind to major groove while small molecules prefer minor groove binding. Minor groove binding molecules generally have aromatic rings connected by single bonds that allow for torsional rotation in order to fit into the helical curvature of the groove with displacement of water molecules. The minor groove is generally not as wide in A–T-rich regions relative to G–C-rich regions; therefore, A–T regions might be more amenable to flat aromatic molecule binding than are G–C regions. Groove binders do not necessarily unwind DNA base pairs. Cationic groups undergo electrostatic interactions with the negative electrostatic potential in the minor groove.

(3) Intercalation

Intercalation is a noncovalent interaction in which the drug is held rigidly perpendicular to the helix axis. This causes the base pairs to separate vertically, thereby distorting the sugar-phosphate backbone and decreasing the pitch of the helix. Example includes the intercalation of ethidium bromide into B-DNA. Flat, generally aromatic or heteroaromatic molecules bind to DNA by inserting (i.e., intercalating) and stacking between the base pairs of the double helix. The principal driving forces for intercalation are stacking and chargetransfer interactions, but hydrogen bonding and electrostatic forces also play a role in stabilization. The van der Waals forces that hold the intercalated molecules to the base pairs are stronger than those found between the stacked base pairs.

Intercalators do not bind between every base pair. The neighbor exclusion principle states that intercalators can, at most, bind at alternate possible base-pair sites on DNA. Binding in one site causes a conformational change in the adjacent site which prevents binding of the intercalator in that adjacent site (***negative cooperativity***). Therefore, intercalation does not disrupt the Watson–Crick hydrogen bonding, but it does destroy the regular helical structure, unwinds the DNA at the site of binding in general. Intercalation of ethidium bromide into B-DNA is shown below.



DNA Alkylators

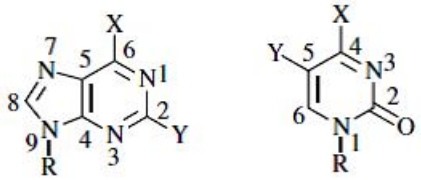
According to Ross, a biological alkylating agent is a compound that can replace a hydrogen atom with an alkyl group under physiological conditions (pH 7.4, 37 °C, aqueous solution). These alkylation reactions are basically substitution reactions by N, O, and S heteroatomic nucleophiles with the electrophilic alkylating agent. In general, the relative rates of nucleophilic substitution at physiological pH are in the order:

thiolate > amino > phosphate > carboxylate

The DNA alkylators (irreversible inhibitors) react with the DNA (enzyme) to form covalent bonds.

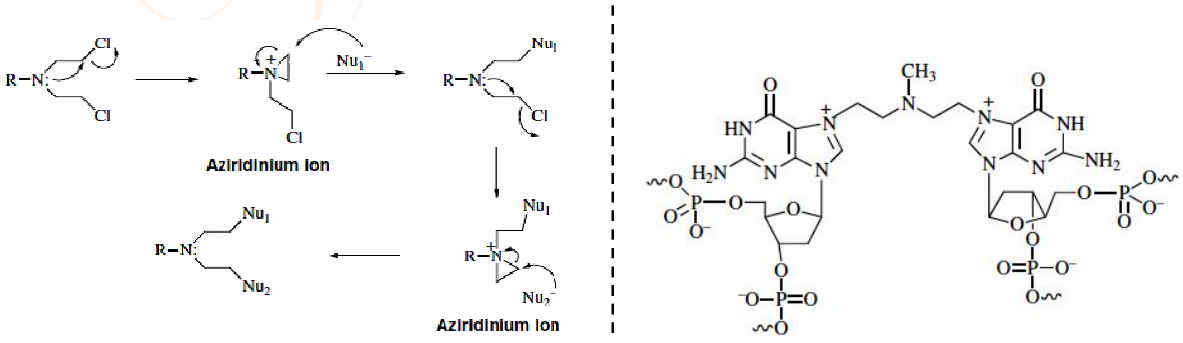
The most reactive nucleophilic sites for DNA are:

N-7 of guanine > N-3 of adenine > N-7 of adenine > N-3 of guanine > N-1 of adenine > N-1 of cytosine (as indicated in purine and pyrimidine structure).

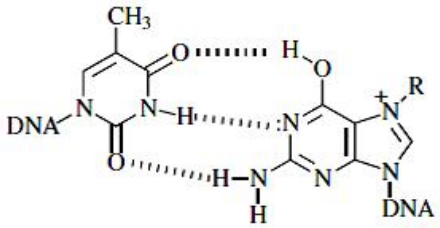


The reactivity of various nucleophilic sites on DNA is strongly controlled by steric, electronic, and hydrogen bonding effects.

Nitrogen mustards are the effective alkylating agent for DNA. They have a N-nucleophile capable of anchimeric assistance (neighboring group participation). Normally, DNA undergoes both intrastrand and interstrand cross-linking quite effectively with a bifunctional alkylating agents (two electrophilic sites) than monofunctional alkylating agents. ***Mechlorethamine***, being a bifunctional alkylating agent, reacts strongly with the N-7 of two different guanines in DNA producing an interstrand cross-link.

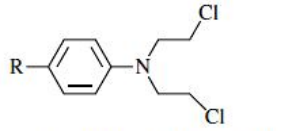


The above alkylation of guanidine at N-7 position shifts the equilibrium to enol tautomeric form which, in turn, allows the base pairing with thymine residues instead of cytosine. As a result, miscoding during replication occurs.



Most anticancer drugs including alkylating agents does common type of damage by modifying DNA at N-7 guanine residues through hydrolysis that produces an abasic site on DNA in which the heterocyclic nucleobase has been displaced. This results in the destruction of the purine nucleus and in DNA strand scission.

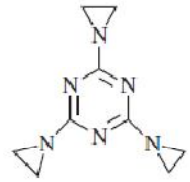
To get selectivity in alkylation via aziridine ion formation, the methyl substitution of mechlorethamine by electron withdrawing aryl substitution in Chlorambucicl could be the way to slow down anchimeric assistance and hence the rate of alkylation. As a result of this stabilization, some of these compounds could be administered orally, and they would be able to undergo absorption and distribution (LADME) before extensive alkylation occurred. Furthermore, simple aryl substituted nitrogen mustards are not water adequate soluble for intravenous administration.



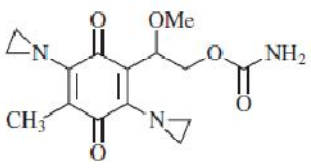
Chlorambucil (R = $(CH_2)_3CO_2H$)
Melphalan (R = $CH_2CH(NH_2)CO_2H$)

In addition to interstrand cross-links, it also is possible for the second chloroethyl group of the nitrogen mustard to react with a thiol or amino group of a protein, resulting in a DNA–protein cross-link.

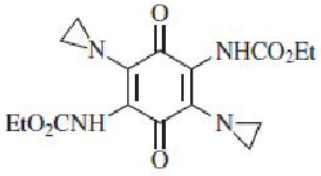
Examples of antitumor ethylenimines include triethylenemelamine, carboquone and diaziquone which are less reactive aziridines than nitrogen mustards under physiological pH.



Triethylenemelamine

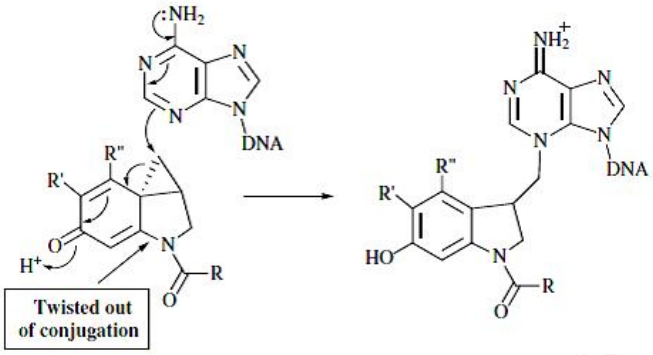


Carboquone

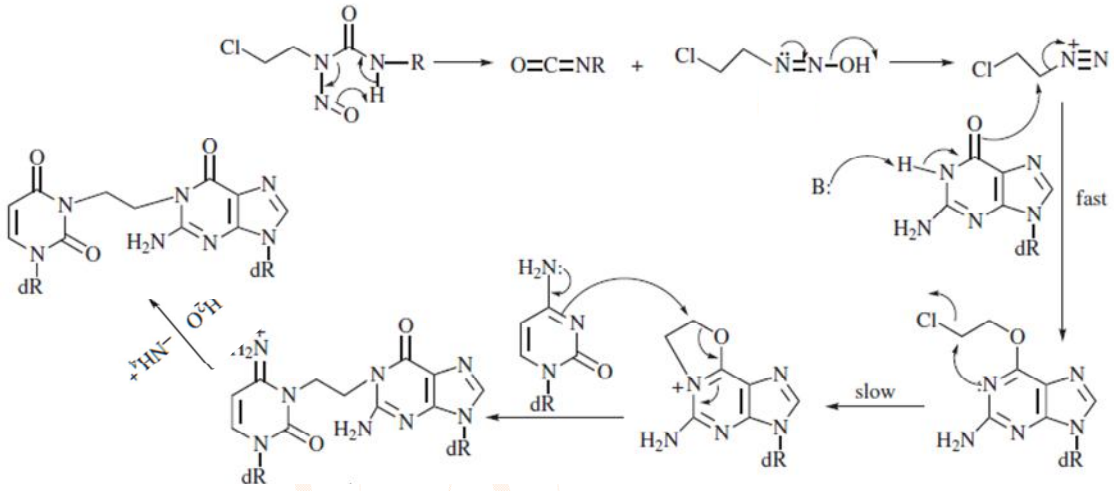


Diaziquone

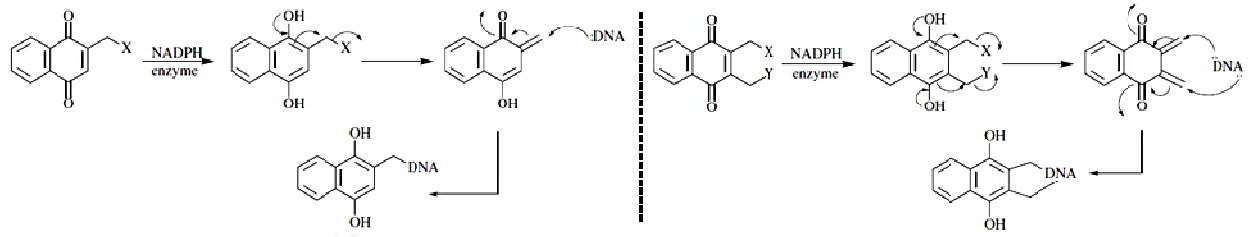
N-3 adenine alkylation



Mechanism proposed for cross-linking of DNA by (2-chloroethyl)nitrosoureas

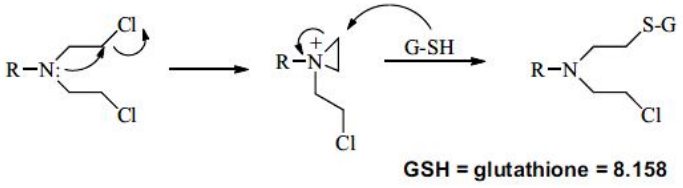


Bioreductive mono and bis-alkylating agents

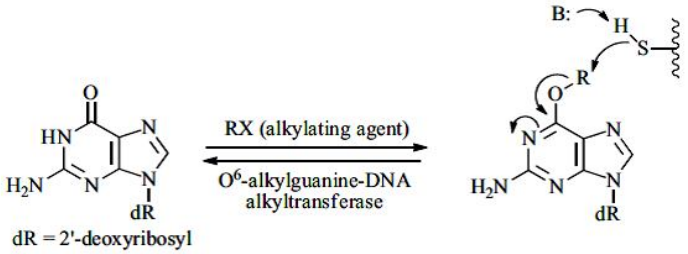


Drug resistance of DNA alkylator

It is important to note that Glutathione (GSH), a small tripeptide containing cysteine (γ-glutamylcysteinylglycine) inactivates nitrogen mustard to produce a glutathione-drug adduct (right above). Increased secretion of glutathione or increased level of the enzyme glutathione S-transferase catalyzed reaction can be a mechanism for resistance to DNA alkylating agents such as nitrogen mustards.



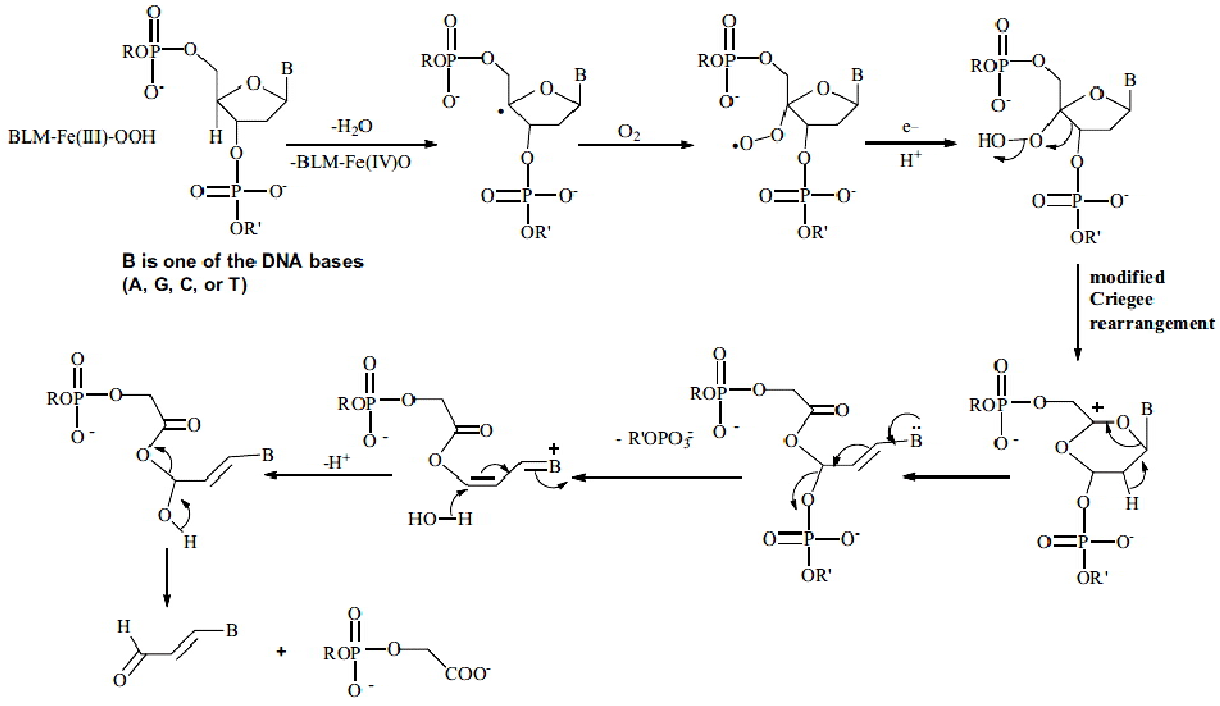
Dealkylation of these adducts is promoted by O^6-alkylguanine-DNA alkyltransferase, and high levels of the transferase are correlated with resistance to drugs that act by this mechanism.



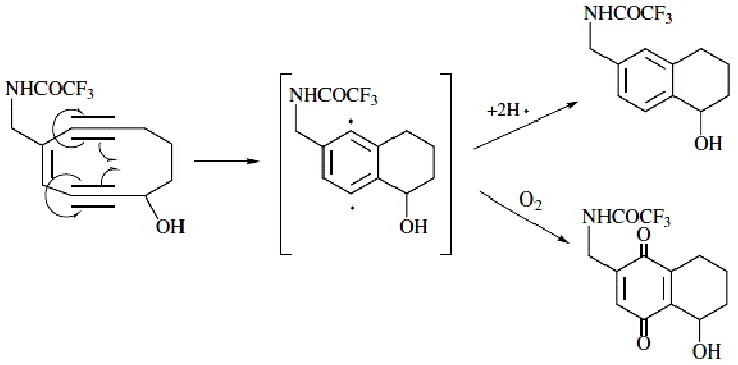
DNA Strand Breakers

DNA-interactive drugs initially intercalate into DNA. But under certain conditions, they react in such a way as to generate radicals. These radicals typically abstract hydrogen atoms from the DNA sugar-phosphate backbone or from the DNA bases, leading to DNA strand scission. Therefore, these DNA-interactive compounds such as anthracycline, bleomycin, enedynes etc. are metabolically activated radical generators.

Bleomycin mediated DNA strand scission : Modified Criegee mechanism



Enediyne can play the same DNA scission by using radicals

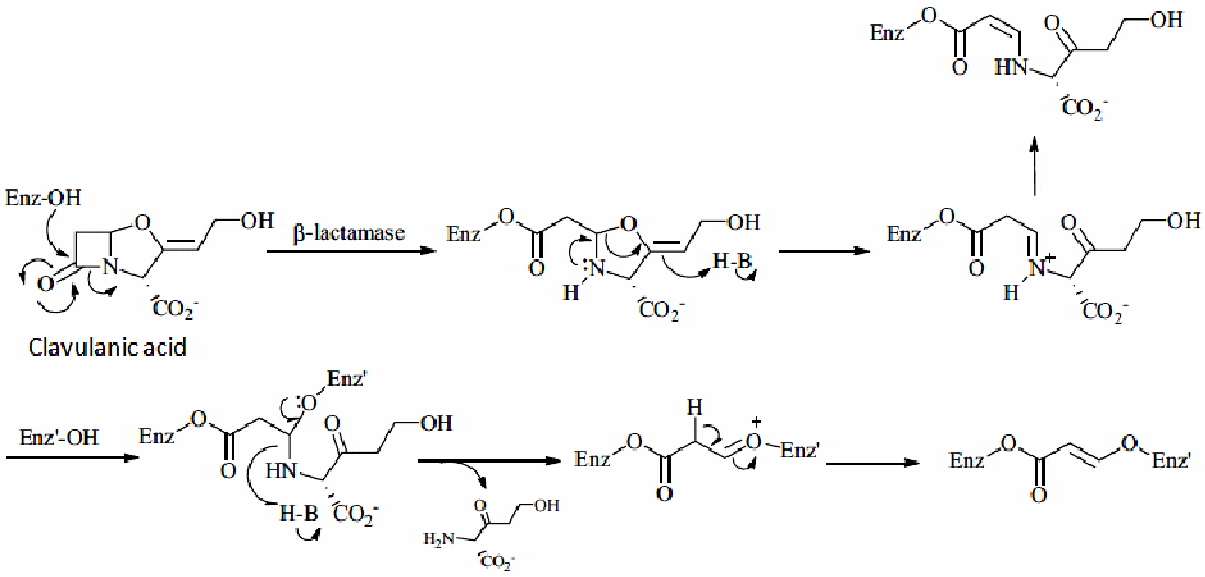


Drug Synergism

Drug synergism arises when the therapeutic effect of two or more drugs used in combination is greater than the sum of the effects of the drugs administered individually. Synergism applies if there is resistance to a drug that is administered alone and a synergistic response arises when coadministration of the primary drug with a second compound inhibits the resistance mechanism.

Cause of penicillin resistance: The major cause for resistance to penicillins is the excretion of β-lactamases which hydrolyzes penicillins. Naturally, drug synergism would be the combination of penicillin and a β-lactamase inhibitor such as Clavulanic acid, amoxicillin. These β-lactamase inhibitors do not have naturally occurring β-lactam structure; but act as potent mechanism-based inactivators of β-lactamases inhibitor. The β-lactamase inhibitors have no antibiotic activity, but they protect the penicillin from destruction so that it can interfere with cell wall biosynthesis. For instance, the combination of amoxicillin with clavulanate is sold as Augmentin and ampicillin plus sulbactam are in Unasyn.

The proposed mechanism of action of clavulanate as a β-lactamase inhibitor is shown below. The product is finally a mechanism-based inactivator.



The drug-destroying enzymes expressed by bacteria come from plasmids. If these plasmids were eliminated from the bacterium, it would become susceptible to antibiotics. Plasmid incompatibility occurs when two plasmids containing the same origin of replication are in the same bacterium. They are forced to compete for proteins and RNA needed for replication, so the most efficient of the two can drive out the other.

Drug metabolism

Q. When a foreign organism enters the body, the immune system produces antibodies to interact with and destroy it. Small molecules, however, do not stimulate antibody production. So how has the human body evolved to protect itself against low-molecular-weight environmental pollutants?

The principal mechanism is the use of nonspecific enzymes that transform the foreign compounds (xenobiotics, which are often highly nonpolar molecules) into polar molecules that are excreted by the normal bodily processes. The enzymatic biotransformation of drugs is known as ***drug metabolism***. This process is always highly desirable in response to all foreign molecules including for drug molecules as foreign agent which needs to enter and be retained in the body sufficiently long to be effective. Owing to structural similarity with endogenous compounds, many drugs may get metabolized by specific and nonspecific enzymes.

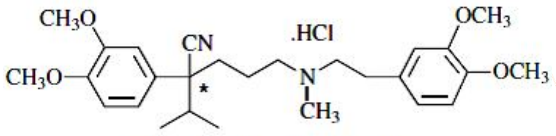
Methods of drug administration and metabolism

- ☑ The principal site of drug metabolism is the liver, the kidneys, the lungs, and the gastrointestinal (GI) tract. After taking a drug orally, it is usually absorbed through the mucous membrane of the small intestine or from the stomach. Once out of the GI tract, it is carried by the bloodstream (via the portal vein) to the liver where it is usually first metabolized. Metabolism by liver enzymes prior to the drug reaching the systemic circulation is called the ***presystemic or first-pass effect***, which may result in substantial or even complete deactivation of the drug. If a large fraction of the drug is metabolized, then larger or multiple doses of the drug will be required to get the desired effect. Another undesirable effect of drug metabolism is that occasionally the metabolites of a drug may be toxic, even though the drug is not.
- ☑ Intravenous (IV) injection introduces the drug directly into the systemic circulation to be used when a rapid therapeutic response is desired. It takes 15-20 sec for total blood circulation.
- ☑ A subcutaneous injection delivers the drug through the loose connective tissue of the subcutaneous layer of the skin.
- ☑ Intramuscular injection is used when large volumes of drugs need to be administered, if slow absorption is desirable, or if the drug is unstable in the gastric acid of the stomach.
- ☑ A sublingual route (the drug is placed under the tongue) bypasses the liver. After absorption through the buccal cavity, the drug enters the systemic circulation. For instance, nitroglycerin (Nitrostat), a drug is used for the treatment of angina.
- ☑ The rectal route, in the form of a solid suppository or in solution as an enema, leads to absorption through the colon mucosa. For instance, Ergotamine (Ergomar), a drug for migraine headaches is administered in this way.

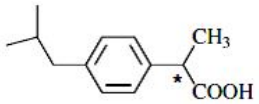
- ☑ Another method of administration, particularly for gaseous or highly volatile drugs such as general anesthetics, is by pulmonary absorption through the respiratory tract. The asthma drug, isoproterenol (Isuprel), is metabolized in the intestines and liver, but administration by aerosol inhalation is effective in getting the drug directly to the bronchi.
- ☑ Topical application of the drug to the skin or a mucous membrane is used for local effects; few drugs readily penetrate the intact skin.

Factor influencing drug metabolism: The pathway and rate of drug metabolism are influenced by species, sex, age, strain, hormones, pregnancy, and liver diseases such as cirrhosis, hepatitis, porphyria, and hepatoma.

***Stereoselectivity of drug metabolism*:** The drug metabolism pathways are also highly stereoselective. Stereoselectivity may arise through the conversion of an achiral drug into a chiral metabolite. Alternatively, a particular enantiomer may undergo metabolized to a greater extent by one pathway while the other enantiomer follows predominantly by another pathway. For instance, the racemic antiarrhythmic drug, Verapamil is 16 times more potent when administered intravenously than when taken orally and its (−)-isomer is 10 times more potent than the (+)-isomer. The therapeutically inactive R-isomer of the analgesic ibuprofen (Advil) is converted enzymatically in the body to the active S-isomer and then racemization occurs initially.



Verapamil hydrochloride



Ibuprofen

Therefore, the function of drug metabolism is to convert a molecule that can cross biological membranes into one that is cleared, generally in the urine; each progressive metabolic step usually reduces the lipophilicity of the compound.

Drug metabolism studies are essential to evaluate the potential safety and efficacy of drugs. Consequently, prior to approval of a drug for human use, an understanding of the metabolic pathways and disposition of the drug in humans and in preclinical animal species is required.

Metabolism studies can also be a useful lead modification approach. For example, after many years on the drug market, terfenadine (Seldane) was removed because it was found to cause life-threatening cardiac arrhythmias.

With a general understanding of important metabolic pathways, it is often possible to design a compound that is inactive when administered, but which utilizes the metabolic

enzymes to convert it into the active form. Drug metabolism can convert a pharmacologically inactive ***prodrug*** into an active drug.

Types of drug metabolism reactions: Two types: a) ***Phase I*** and b) ***Phase II*** reactions.

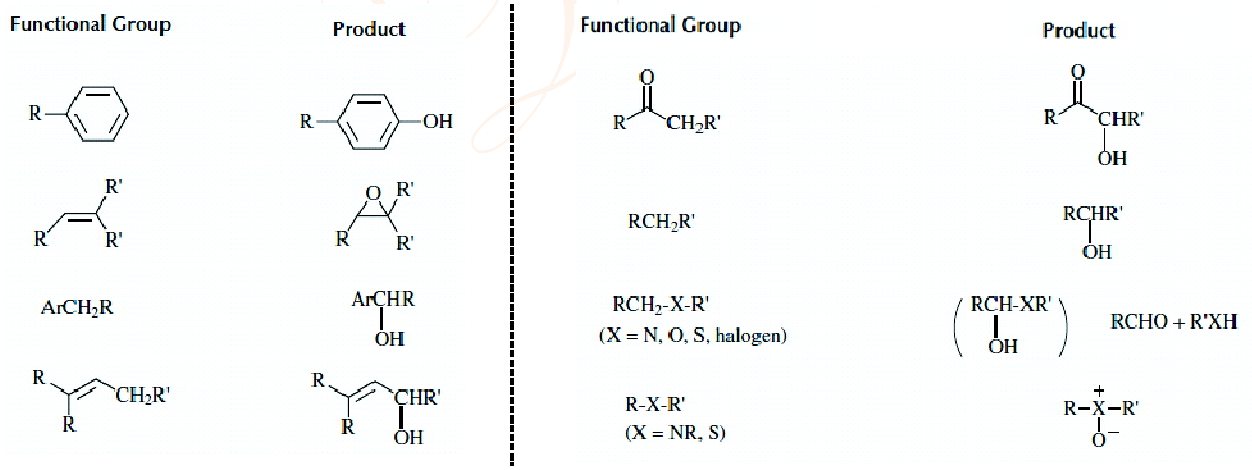
a) ***Phase I transformations*** involve reactions that introduce or unmask a functional group, such as oxygenation or hydrolysis.
b) ***Phase II transformations*** mostly generate highly polar derivatives (known as conjugates), such as glucuronides and sulfate esters, for excretion in the urine.

Phase I Transformations

Oxidative Reactions

Cytochrome P450 is an essential enzyme for metabolism. Cytochrome P450 is a superfamily of enzymes (also referred to as isozymes) containing a heme cofactor with structurally variable active sites that catalyze the same reaction on different substrates, e.g. the oxidation of steroids, fatty acids, and xenobiotics. The primary localization of these enzymes is the liver. However, they also remain in the lung, kidney, gut, adrenal cortex, skin, brain, aorta, and other epithelial tissues. Cytochrome P450 is associated with another enzyme, NADPH-cytochrome P450 reductase, a flavoenzyme that contains one molecule each of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN).

Classes of Substrates for Cytochrome P450



***Physiochemical property of drug for Cytochrome P450 metabolism*:** The presence of an ionizable group and lipophilicity of drug molecules play key role in binding to the active site of Cytochrome P450 following regioselectivity of the metabolic reaction and

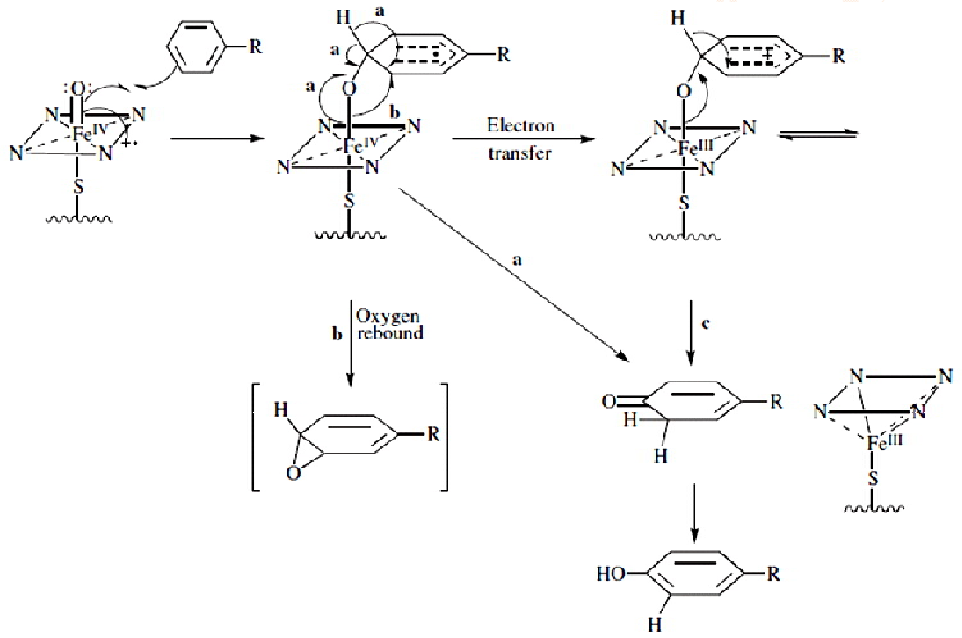
increased metabolic clearance respectively. The reaction and site of reaction catalyzed by cytochrome P450s are determined by

(1) the topography of the active site of the particular isozyme,

(2) the degree of steric hindrance of the heme iron-oxo species to the site of reaction, and

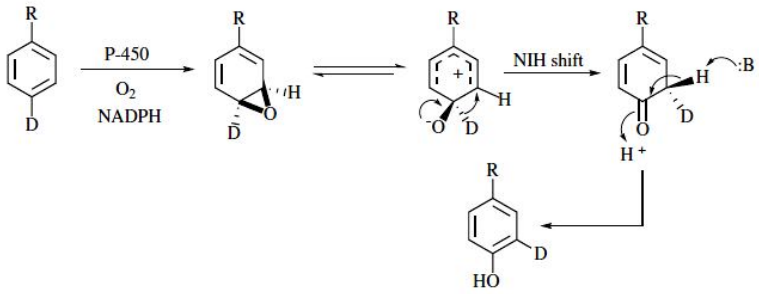
(3) the ease of hydrogen atom abstraction or electron transfer from the compound that is metabolized by the isozyme.

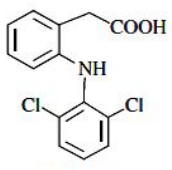
Addition–rearrangement mechanism for arene oxide formation



Rearrangement of arene oxides to arenols (NIH shift)

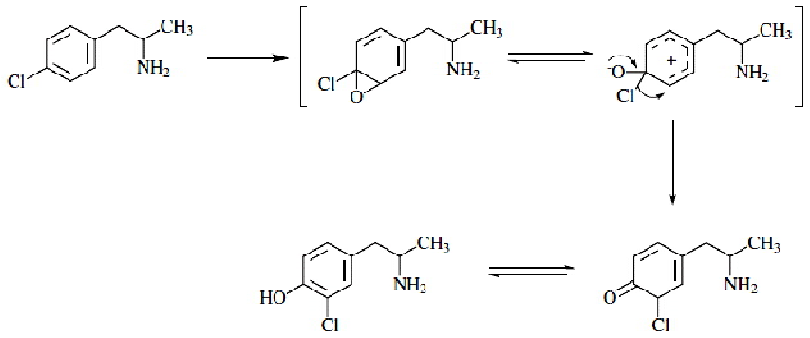
The rearrangement of an arene oxide to an arenol is known as the NIH shift because a research group at the NIH proposed this mechanism based on studies with specifically deuterated substrates. For instance, antiinflammatory drug diclofenac (Voltaren) is metabolized to 4-hydroxydiclofenac with a half-life of 1 h.



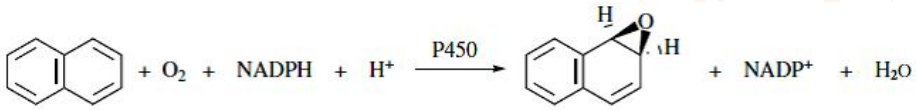


Diclofenac

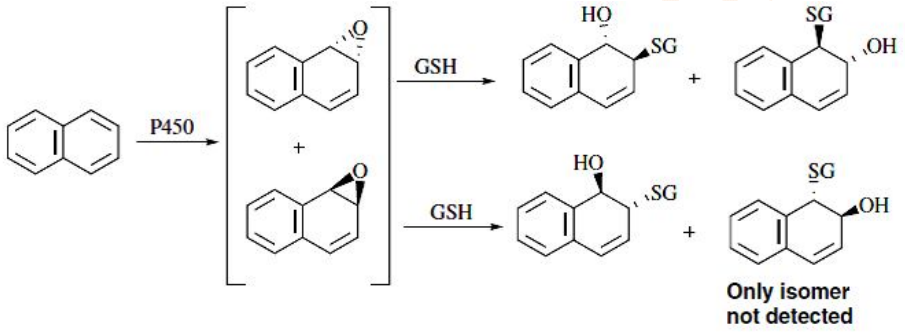
NIH shift of chloride ion



Formation of glutathione adducts from naphthalene oxides

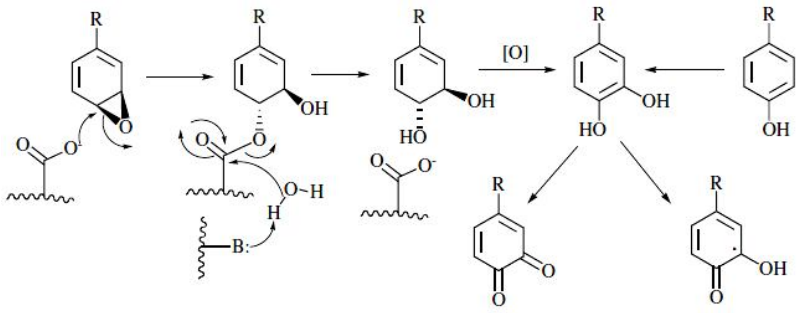


Glutathione S-transferase is an important enzyme that protects the cell from the electrophilic arene oxide metabolites.



Metabolic formation and oxidation of catechols

Aromatic hydroxylation, as is the case for all metabolic reactions, is species specific. The *para*-hydroxylation is a major route of metabolism for many phenyl-containing drugs. Because of the reactivity of arene oxides, they can undergo rapid reactions with nucleophiles.

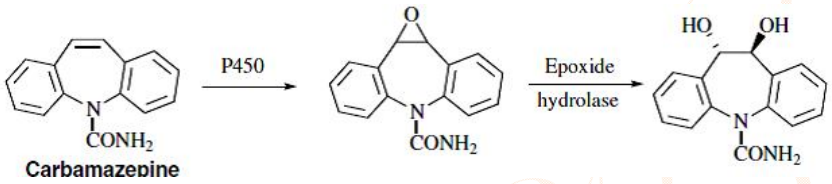


Alkene epoxidation

Alkenes are more reactive than aromatic π-bonds and the former are metabolically epoxidized.

Metabolism of carbamazepine

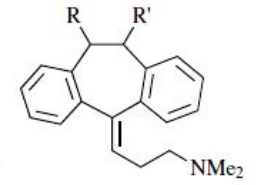
The metabolite derived from the epoxidation of Carbamazepine by Cytochrome P450 is responsible for the anticonvulsant activity of carbamazepine. The epoxide is converted stereoselectively into the corresponding (10S,11S)-diol by epoxide hydrolase.



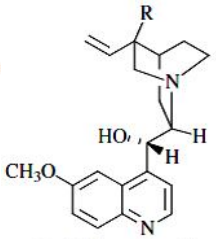
Carbamazepine

Allylic/benzylic oxidation

A typical cytochrome P450 of benzylic oxidation is the metabolism of the antidepressant drug amitriptyline (R = R′ = H, Elavil), which is oxidized to a metabolite (R = H, R′ = OH and R = OH, R′ = H). P450-catalyzed metabolism of the antiarrhythmic drug quinidine (R = H, Quinidex) leads to allylic oxidation (R = OH).



Amitriptyline (R = R' = H)

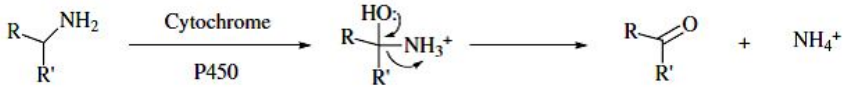


Quinidine (R = H)

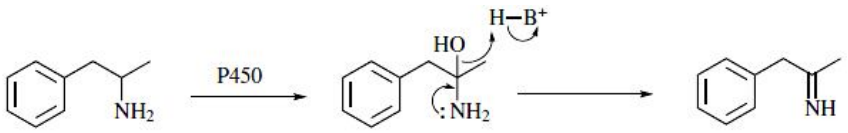
Oxidation of Carbon Nitrogen system

Oxidative deamination of primary amines

The oxidative cleavage of ammonia from the primary amine is known as oxidative deamination.

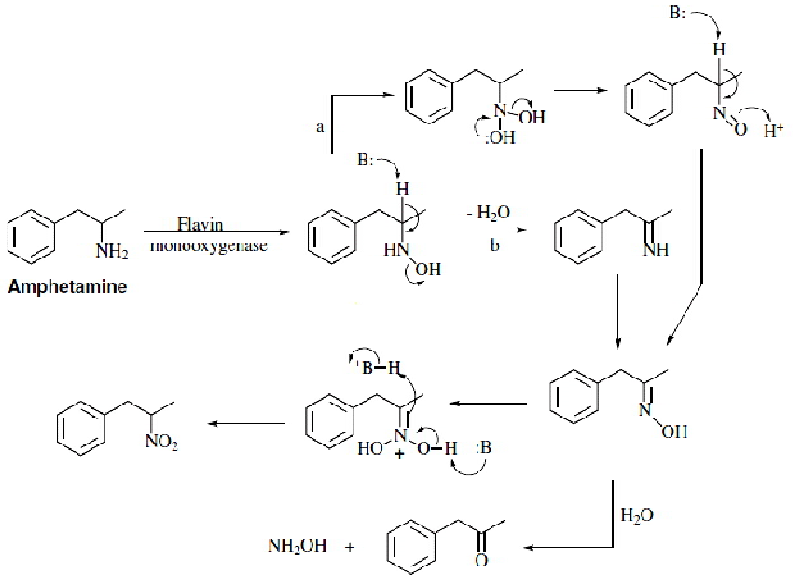


Amphetamine imine formation via the carbinolamine



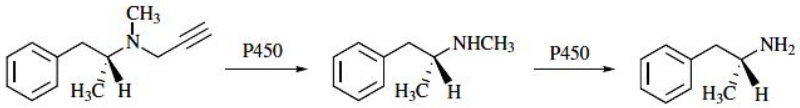
N-Oxidation pathways of amphetamine

It has been suggested that basic amines (pKa 8–11) are oxidized by the flavoenzymes; nonbasic nitrogen-containing compounds, such as amides, are oxidized by cytochrome P450 enzymes; and compounds with intermediate basicity, such as aromatic amines, are oxidized by both enzymes.

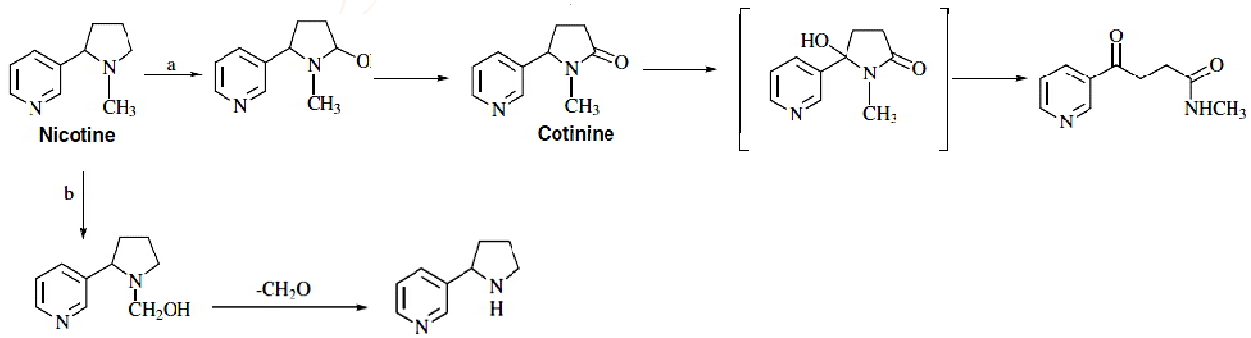


Metabolism of selegiline (deprenyl)

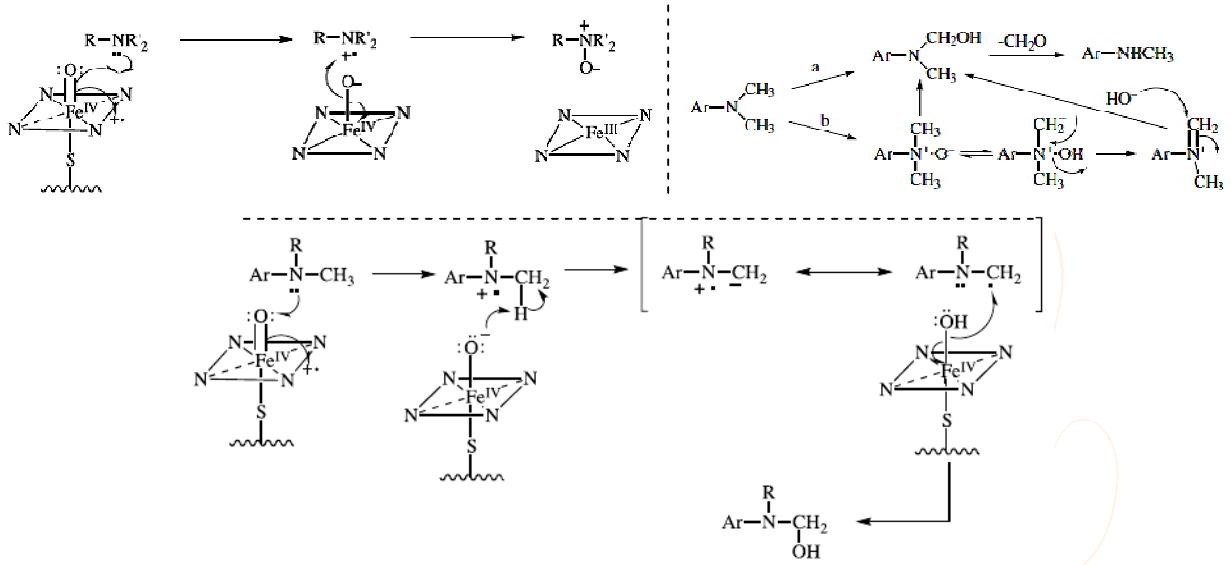
The antiparkinsonian drug selegiline (R-(−)-deprenyl, Eldepryl) is a potent inactivator of monoamine oxidase B (MAO B). It is metabolized by various isozymes of cytochrome P450 to R-(−)-amphetamine enantiomer (no change in chiral centre) which is specifically used in the treatment of Parkinson's disease.



Oxidative metabolism of nicotine

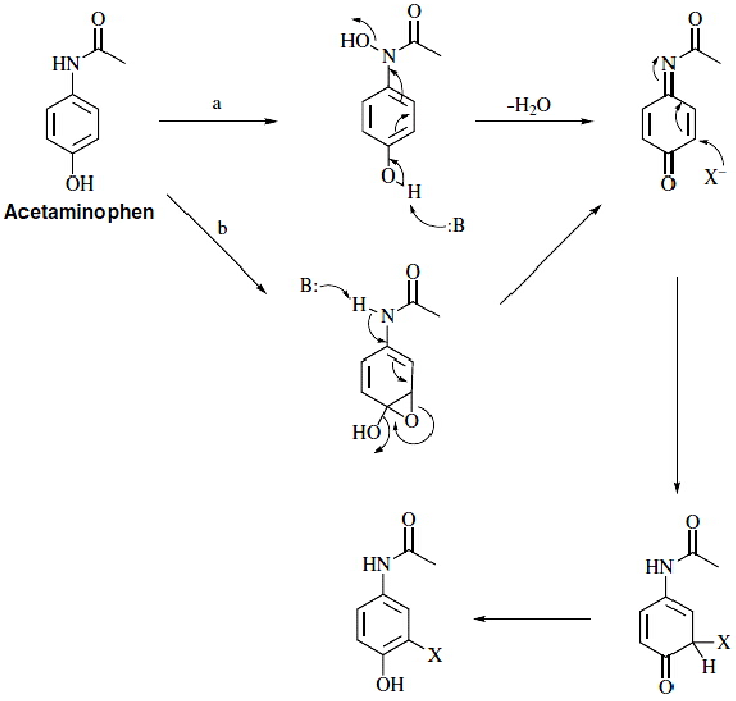


Mechanism of cytochrome P450-catalyzed N-oxidation of tertiary aromatic amines



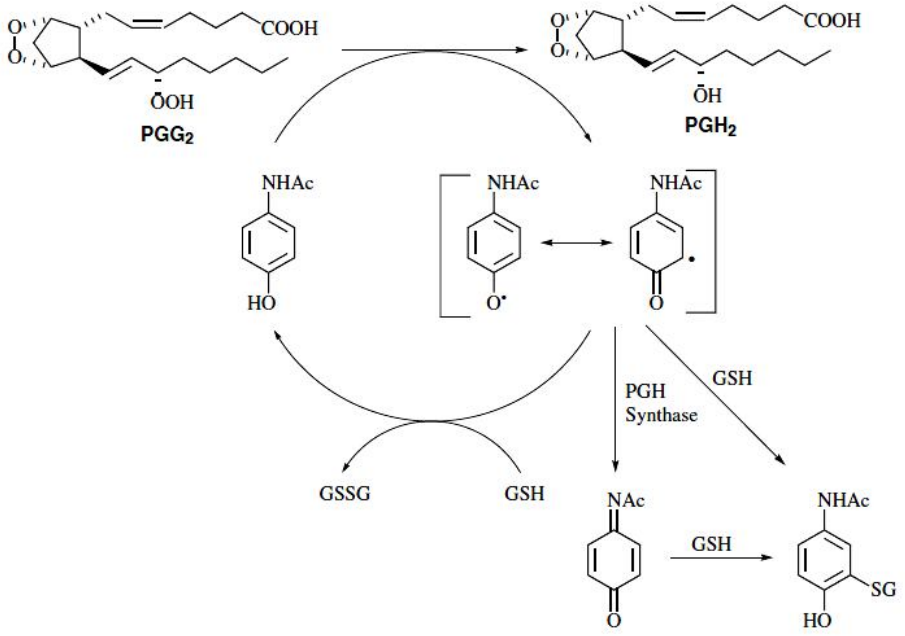
Initial proposals for bioactivation of acetaminophen

The analgesic agent acetaminophen (Tylenol) is relatively nontoxic at therapeutic doses, but in large doses, it cause hepatoxicity. Isozymes of cytochrome P450 are responsible for the conversion of acetaminophen to electrophilic metabolites which reacts with glutathione causing depletion of liver glutathione level.



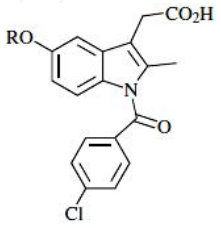
Proposed bioactivation of acetaminophen by prostaglandin H synthase

The enzyme Prostaglandin synthase (also called prostaglandin H synthase, prostaglandin-endoperoxide synthase, or cyclooxygenase) catalyzes the cyclooxygenation of arachidonic acid to prostaglandin G_2 (PGG_2) followed by the reduction of PGG_2 to PGH_2. It is present in high concentrations in the kidneys. During the reduction of PGG2 to PGH2, prostaglandin H synthase can simultaneously co-oxidize acetaminophen to metabolites that reacts with glutathione to form the glutathione conjugate. When the concentration of cytochrome P450 is low, Prostaglandin H synthase may act as important drug-metabolizing enzyme in tissues.

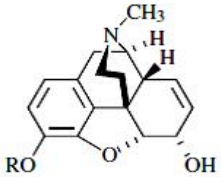


Oxidations of Carbon–Oxygen Systems

Oxidative O-dealkylation is a common biotransformation which is catalyzed by microsomal mixed function oxidases. For instance, a major metabolite of the antiinflammatory drug indomethacin (R = CH_3, Indocin) is the O-demethylated compound (R = H). Similarly, the narcotic analgesic codeine (R = CH_3) is O-demethylated to morphine.



Indomethacin (R = CH_3)



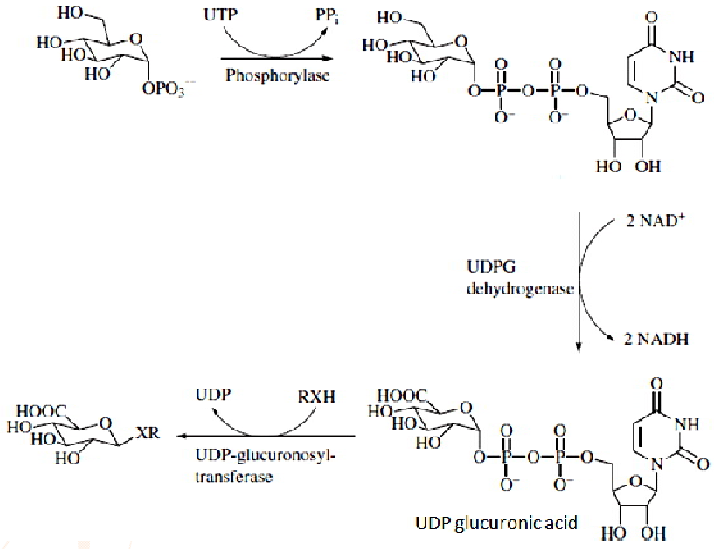
Codeine (R = CH_3)
Morphine (R = H)

Phase II Transformations: Conjugation

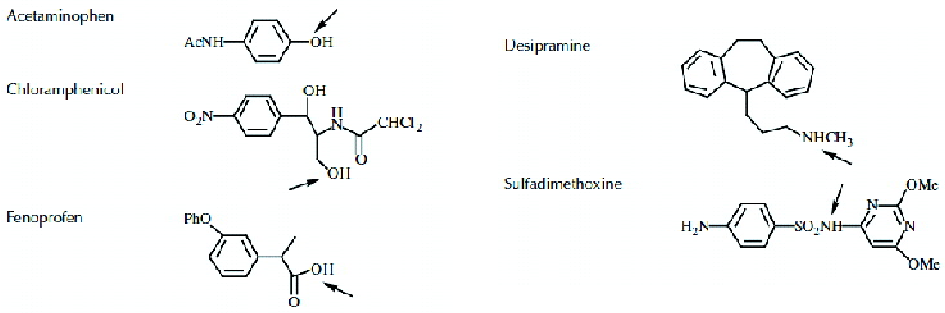
Phase II or conjugating enzymes catalyze the attachment of small polar endogenous molecules such as glucuronic acid, sulfate, or amino acids to drugs or, more often, to metabolites arising from Phase I metabolic processes. This Phase II modification further deactivates the drug, changes its physicochemical properties, and produces water-soluble metabolites that are readily excreted in the urine or bile.

Glucuronidation is the most common mammalian conjugation pathway. The coenzyme form of glucuronic acid, namely, uridine 5′-diphospho-α-D-glucuronic acid (UDP glucuronic acid) is biosynthesized from α-D-glucose-1-phosphate. Drug molecules conjugate with UDP glucuronic acid by UDP-glucuronosyltransferase to form glucuronide which are readily excreted due to hydrophilic nature in presence of carboxylate and hydroxyl groups.

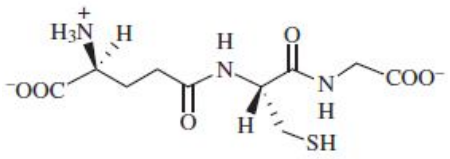
It is suggested that UDP-glucuronosyltransferase is closely associated with cytochrome P450 so that as drugs become oxidized by Phase I cytochrome P450 reactions, the metabolites are efficiently conjugated.



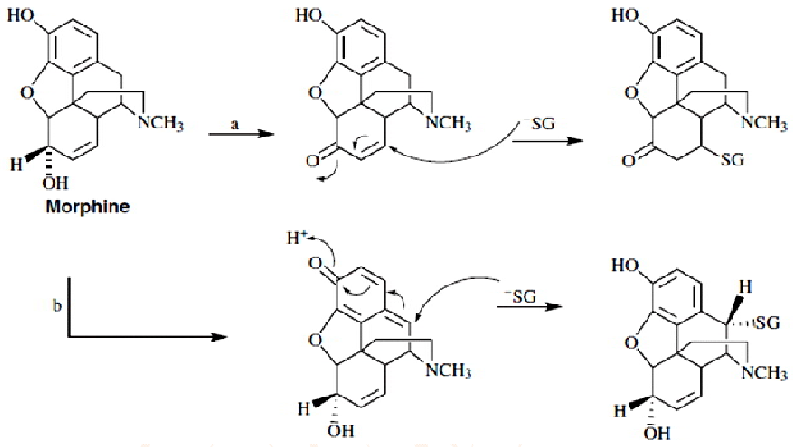
Location of Glucuronides formation by drug molecules



Glutathione Conjugation



By virtue of its thiol group, the tripeptide glutathione can easily conjugate electrophilic nature of metabolites produced in phase I. However, it is rarely excreted by urine due large molecular weight and amphiphilic character; rather it is eliminated through bile. Conjugation with glutathione occurs in the cytoplasm of most cells, especially in the liver and kidney where the glutathione concentration is 5–10 mM.



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4. **Walters, W. P.; Stahl, M. T.; Murcko, M. A. Drug Discovery Today 1998, 3, 160.**
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