

M.Phil Pharmaceutical Chemistry

Session 2020-2022

DRUG DESIGN & DRUG DEVELOPMENT

Course Code-PHC-701

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Course Outline

1. History of drug and drug designing
2. Systematic drug development and rational research methods
3. General and theoretical aspects of drug design
4. Development of new drugs
 - a. Sources of drugs
 - b. Random screening
 - c. Extraction from natural sources
 - d. Molecular modifications
 - e. Evaluation of intermediate products

Recommended text books

- I. Drug discovery and development (Technology in transition)- H P RANG
- II. Medicinal chemistry a molecular and biochemical approach 3rd edition, Thomas Nogrady, Donald F. Weaver.
- III. An introduction to medicinal chemistry –Graham L Patrick.

History of drug and drug designing

DRUG:

"Drug is a small molecule design to bind, interact and modulate the activity of specific biological receptors."

As per WHO:

"It is a natural or synthetic substance which (when taken into the living body) affects its functioning."

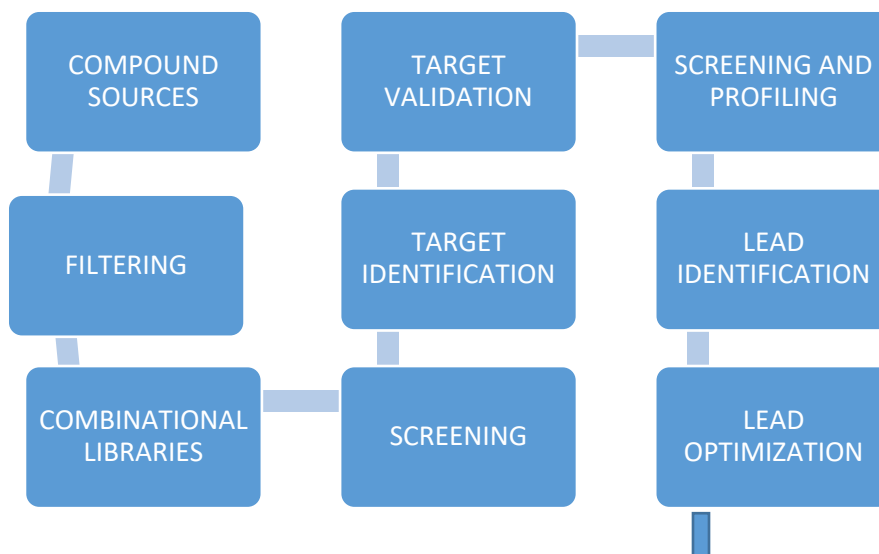
RECEPTORS:

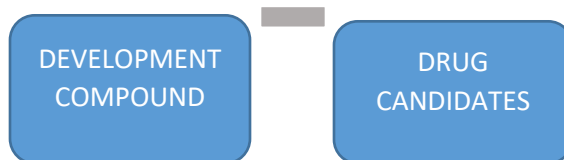
"Receptors are proteins that bind and interact with other molecules to perform the different functions required for the maintenance of life." The role of drug is to correct the functioning of these receptors to remedy the resulting medical condition.

PHARMACOPHORE:

"The three dimensional arrangement of atoms within a drug molecule that permits a specific binding interaction with a desired receptor is called pharmacophore." The pharmacophore is that portion of molecule that establishes intermolecular interactions with the receptor site.

STAGES OF DRUG DISCOVERY:





DRUG DESIGN:

Drug design or rational drug design or simply rational design, is the "*inventive process of finding new medications based on the knowledge of a biological target.*"

Drug design involves the design of small molecules that are complementary in shape and charge to the bio-molecular target with which they interact and therefore will bind to it.

Ways of Drug Designing:

- Development of ligands with desired properties for the targets having known structure and function.
- Development of ligands with pre-defined properties for the targets whose structural information may be or may not be known.

PRINCIPLES OF DRUG DESIGN:

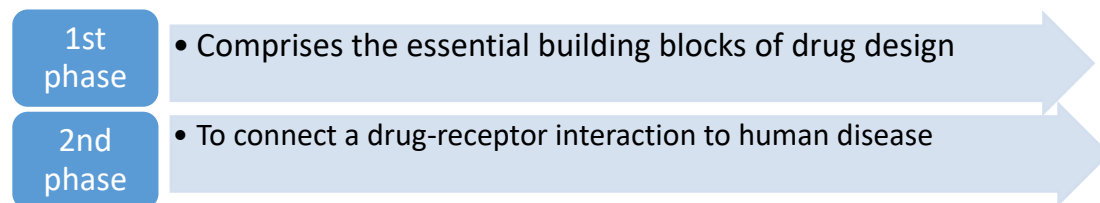
The basic principle of the medicinal chemistry is to design the new chemical entities (NCEs) and their optimization and development as useful drug molecule for the treatment of the disease.

This can be done by determining the:

- ✓ Design of new molecules.
- ✓ How they interact with biological macromolecules such as proteins and nucleic acids.
- ✓ The relationship between their chemical structure & biological activities.
- ✓ Conduction of structure activity analysis.
- ✓ Their ADME throughout the body.

DRUG DESIGN APPROACHES:

Drug design may be divided into two phases. Elementary concepts about drugs, receptors and drug-receptor interactions are applied to human diseases.



1ST PHASE: This phase is further divided into three steps.

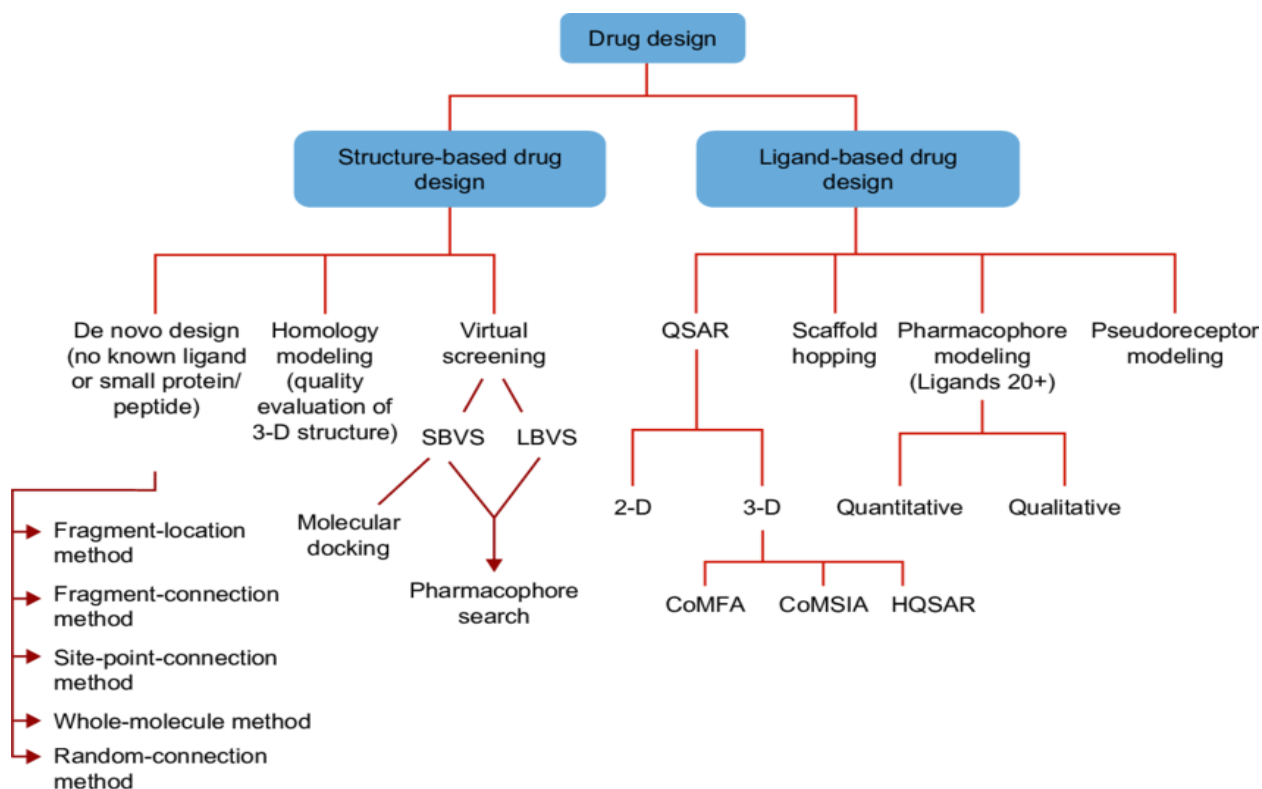
Step 1: Involves knowing what property turns a molecule into a drug. All drugs may be molecules but all molecules are not certainly drugs. Drug molecules are “small” molecules (molecular weight usually below 800g per mol. Often below 500). e.g. Penicillin, acetylsalicylic acid and morphine are all small organic molecules.

STEP II: Involves knowing what properties turn a macromolecule into a receptor. All receptors may be macromolecules, but all macromolecules are certainly not receptors. Receptor macromolecules are frequently proteins or glycoproteins.

STEP III: Involves knowing how to design and synthesize a drug to fit into a receptor. This prototype compound is referred to as a lead compound.

2ND PHASE: Once the basics of drug design are obtained, the drug designer next focuses upon the task of connecting a drug-receptor interaction to human disease. This is the goal of the second phase. This phase requires an understanding of biochemistry and of the molecular pathology of the disease being treated.

TYPES OF DRUG DESIGN



1) Ligand Based Drug Design:

Ligand based drug design relies on knowledge of other molecules that bind to the biological target of interest which may be used to derive a pharmacophore model. In other words, a model of the biological target may be built based on the knowledge of what binds to it and this model in turn may be used to design new molecules.

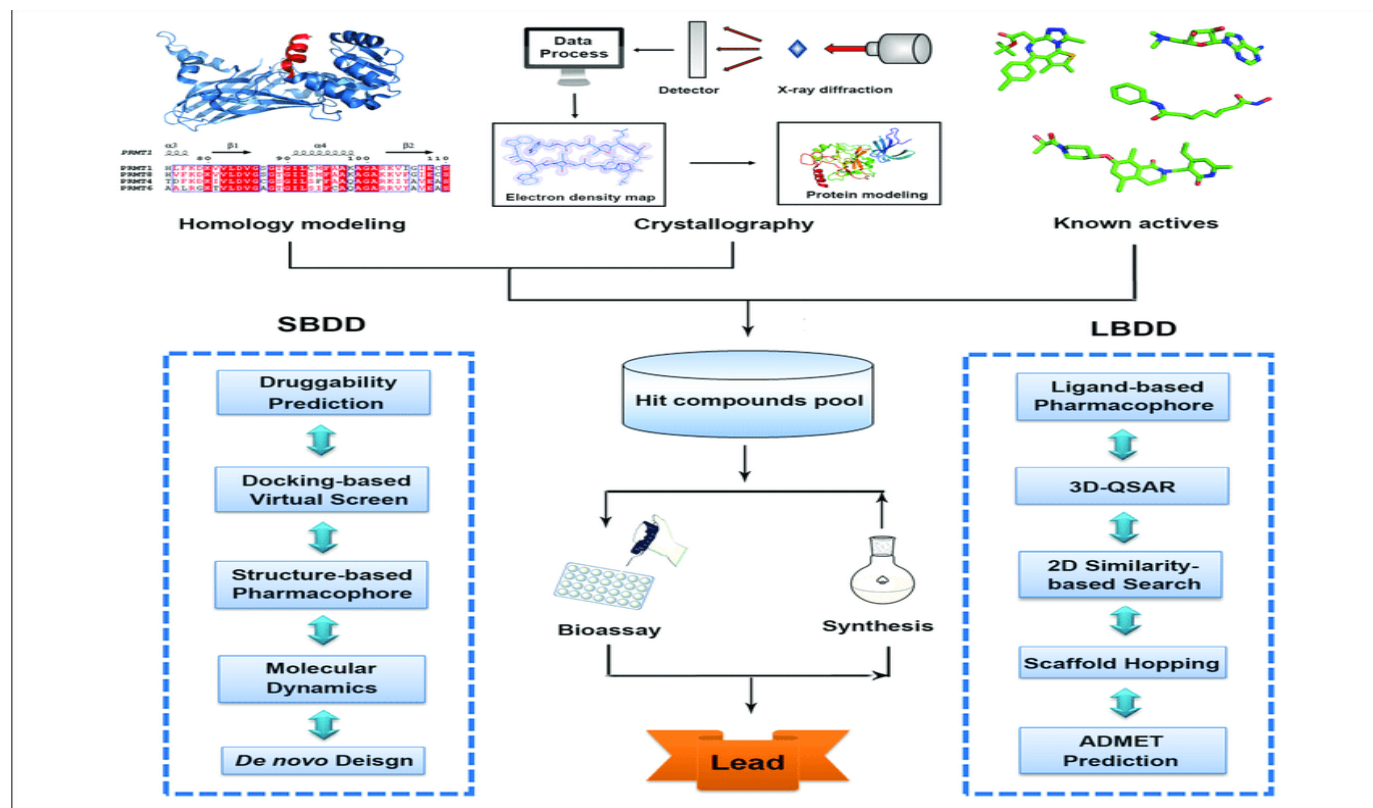
2) Structure based drug design:

Structure based drug design relies on knowledge of three dimensional structure of the biological target obtained through methods such as *X-ray crystallography* and *NMR spectroscopy*. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.

e.g., Indinavir is an example of very potent peptidomimetic compound discovered using the elements of 3D structure and structure activity relationship.

A typical SBD approach commonly contains the following steps:

1. The x-ray crystal structure of the target in its biologically active form is first determined.
2. A ligand is co-crystallized with the target protein and the complex is analyzed to reveal the binding domain.
3. Essential binding interactions between ligand and protein and other binding pockets which are not optimally exploited, are identified.
4. The biological relevance of the identified interactions is often confirmed by the use of site-directed mutagenesis of the target protein.



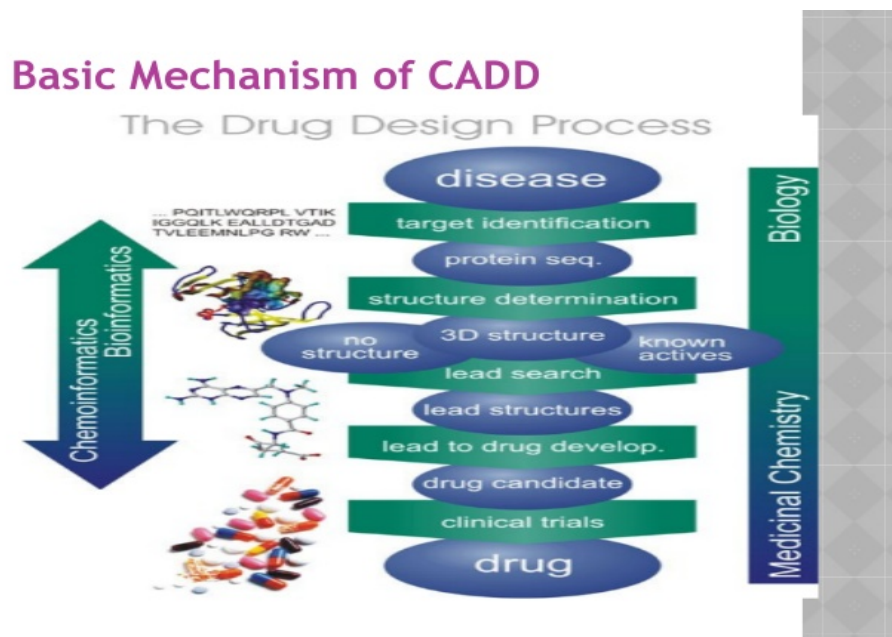
3) **Rational Drug Design:** Rational drug design begins with a hypothesis that modulation of a specific biological target may have therapeutic value compared to traditional design that relies in Trial-and Error. In order to select a biomolecule as a drug target, two essential pieces of information are required.

- **THE FIRST** is modulation of the target will have therapeutic value.
- **THE SECOND** is that the target is “drug able”. This means that it is capable of binding to a small molecule and that its activity can be modulated by the small molecule.

Rational drug design develops fewer compounds compared to High-throughput Screening. However, these compounds are very specific to the target and use computer based modelling to achieve this specificity.

4) Computer Aided Drug Design:

It represents computational methods and resources that are used to facilitate the design and discovery of new therapeutics. CADD is an exciting and diverse discipline in which various aspects of applied and basic research merge. Molecular mechanics and dynamics are used to predict the confirmation of small molecules.



Applications of CADD:

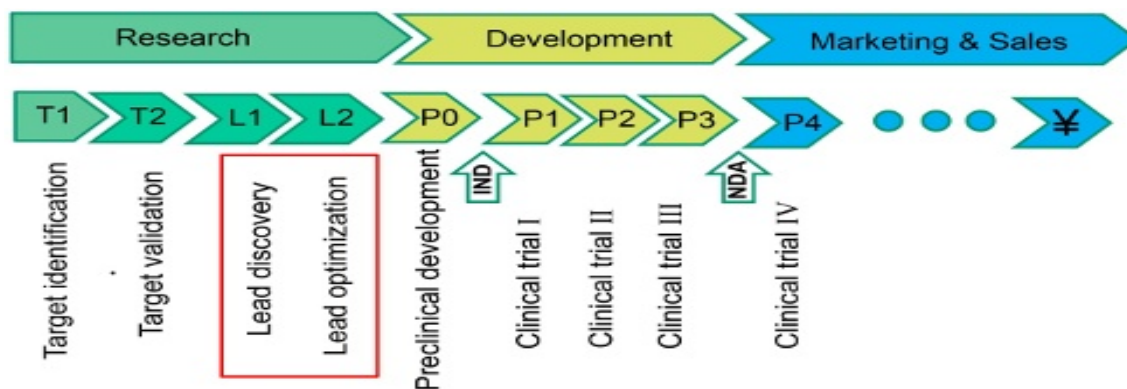
- Elimination of compounds with undesirable effects.
- Identify and optimize new drugs.

TARGET IDENTIFICATION:

Drug targets are usually proteins but are in some cases small regions of DNA or RNA.

The choice of a drug target is primarily made on a biological and biochemical basis.

- ❖ The target should be unique.
- ❖ The ideal target macromolecule is one that is closely linked to human disease and binds small molecule in order to carry out a function.
- ❖ Homology modeling or comparative modeling is the most reliable method for target structure prediction that builds 3D structure for unknown proteins based on the known homologous protein structure (i.e. >40% similarity).



EVALUATION OF IDENTIFIED TARGET:

Once a target has been identified, it is necessary to obtain accurate structural information. There are three primary methods for structure determination that are useful for drug design: x-ray crystallography, NMR and homology modeling. Crystal structure is the most common source of structural information for drug design and the method is useful for proteins that range in size from a few amino acid.

TARGET VALIDATION: Target validation is a process by which the predicted molecular target (i.e., protein or nucleic acid) of a small molecule is verified. The binding site is a small region, where ligand molecule can best fit or bind to activate the receptor and produce the desirable effects.

- Recognizing the binding site or the active site residue in the target structure is of high importance in SBDD. Because the proteins are capable of undergoing conformational changes to recognize the accurate binding site residue is difficult.
- It is an accepted statement that proper selection of chemical compounds, with minimal potency and specificity, during the early phases of drug discovery plays a vital part in the success SBDD.

LEAD DRUG IDENTIFICATION: A lead compound is an organic molecule that act as a prototype drug around which further optimization is centered and focused. Once a small molecule has been identified as binding to the target molecule, it must be evaluated before

proceeding to further stages. The task to identify leads is the first essential step for medicinal chemist in drug discovery. It requires two essential components: compounds to be test and test system in the form of screening assays.

In principle, two strategies can be used for identifying leads, namely screening and design.

- **PHYSICAL SCREENING** of larger & smaller lead compound libraries. Leads are first evaluated visually with computer graphics and can often be optimized at this step for increased affinity.
- **DESIGNED MOLECULES**. The design may be based on structures (generally endogenous molecules or competitor drugs) known to be active on the target in question (ligand-based design) or on the known structure of the target.
- **COMPUTATIONAL METHODS** are also important. They are used in many ways, for example:
 - To compute the estimates of physicochemical properties, based on chemical structure.
 - To create and rank 'virtual libraries' of compounds that are unavailable or not yet synthesized.
 - To perform 'virtual screening' on structurally defined targets.
 - To generate pharmacophore models, based on established SAR's.

LEAD OPTIMIZATION:

Lead optimization is that part of drug discovery process in which a defined lead compound is optimized to generate a drug candidate for pre-clinical development.

Lead optimization is actually the hardest and least Road-Mapped aspect because multiple requirements have to be satisfied simultaneously.

The criteria for the promotion of a compound to a drug candidate include properties related to pharmacokinetics and safety as well as potency and selectivity.

Various approaches are employed in order to improve the desired pharmacological properties of the lead nucleus.

○ Identification of pharmacophore:

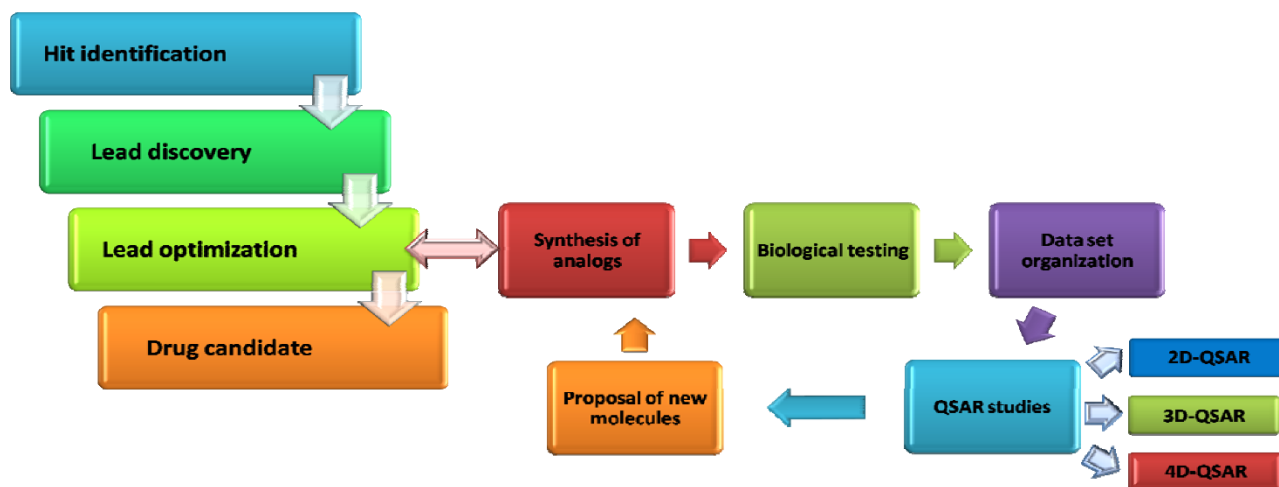
Any drug molecules consist of both essential and nonessential parts. Essential part is important in governing pharmacodynamics (drug-receptor interaction) property. Nonessential part influences on pharmacokinetic features. Once such pharmacophore is identified, structural modification can be done to improve pk properties of the drug.

○ Functional group optimization:

The activity of a drug can be correlated to its structure in terms of the contribution of its functional groups to the lipophilicity and electronic features of the drug.

○ Structure activity relationship studies:

SAR studies usually involve the interpretation of activity in terms of the structural features of the drug molecule.



Drug Development

Stages of drug developments are;

Stage 1: Drug Discovery

The 1st stage of drug development is drug discovery. In past some drugs like penicillin have been discovered accidentally. Today, more symptomatic approaches are used, such as:

High-Throughput screening: which allows scientists to test thousands of potential targets with thousands of diverse chemical compounds to identify a new drug-target combination.

Rational drug design: which involves designing and synthesizing compounds based on the known structure of a specific target molecule.

Stage 2: Pre-Clinical development

Pre-clinical testing aims to establish how drugs are absorbed and distributed in the body, and how they are broken down and removed from the body. The results of pre-clinical testing are used to determine how to best formulate the drug for its intended clinical use.

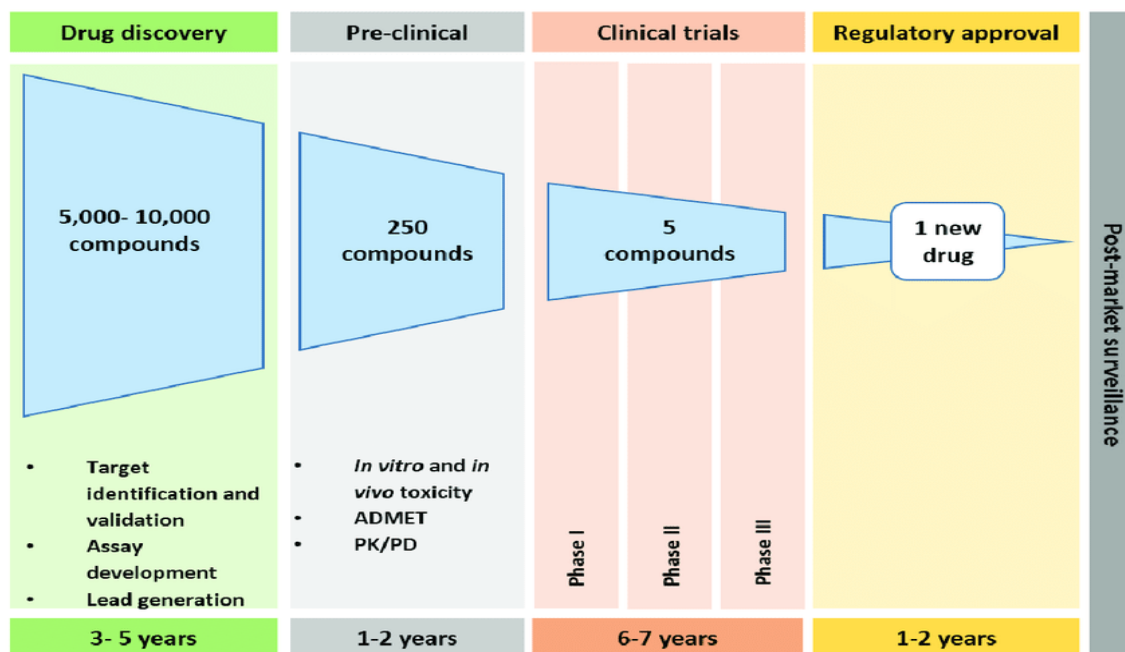
Stage 3: Clinical trials

This phase is divided into Phase 0, I, II, III and IV. This phase involves the testing of the drug on human volunteers to provide more information about its safety and effectiveness. By the end of clinical trial

phase, most of the investigational new drugs will have been eliminated on the grounds of safety and effectiveness.

Stage 4: Regulatory approval

After a drug has been approved, pharmaceutical companies have short period where only they have the rights to market the drug and before other companies can market the same drug. The period is used to regain the massive investment required to develop and launch the new drug.



TECHNIQUES OF DRUG DESIGN

There are various techniques of drug design like:

- QSAR
- Docking
- X-ray crystallography
- NMR
- Homology modeling

QSAR:

QSAR is a mathematical relationship between a biological activity and physico-chemical properties of drug. A general formula for the QSAR can be given by the following:

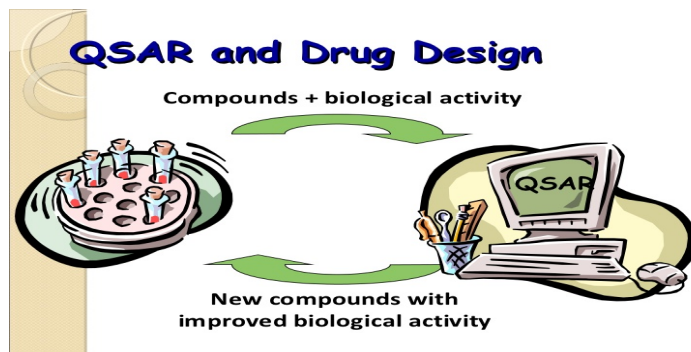
$$\text{Biological activity} = f(\text{Physico-chemical properties})$$

So, it can be used to evaluate the activity of new compounds.

Mathematical models are built based on structural parameters to describe.

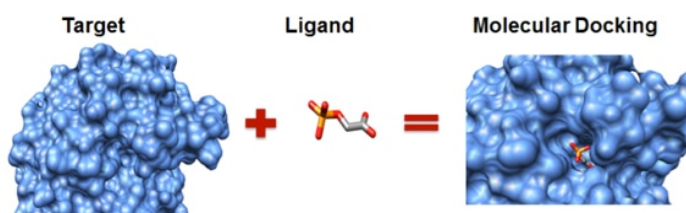
Earlier 2D-QSAR, but 3D-QSAR have been adopted.

3D-QSAR methodologies are CoMFA, CoMSIA and HQSAR.

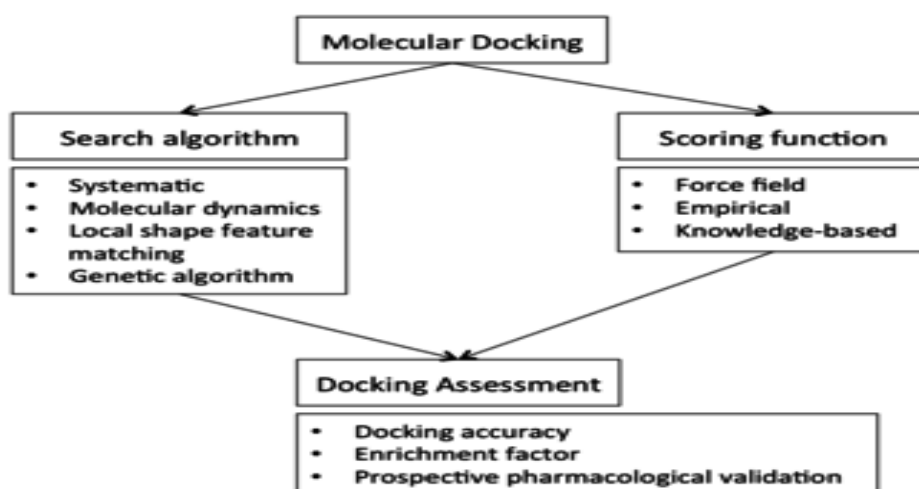


DOCKING:

“Docking is the method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex.”



Molecular docking is one of the most frequently used method in structure based drug designing due to its ability to predict the binding confirmation of small molecule ligands to the appropriate target binding site. Docking can be achieved through two interrelated steps: 1st by sampling confirmation of the ligand in the active site of the protein; 2nd then ranking these confirmations via a scoring function.



Applications of docking:

- Hit identification
- Lead optimization
- Bioremediation

Uses of Docking:

- Drug target
- Protein ligand interaction
- Protein therapies
- Engineered protein enzymes
- Better understand the machinery of life
 - ✓ Enzyme-inhibitor class
 - ✓ Antibody-antigen class
- For predicting both the strength and type of produced signal.

X-ray Crystallography:

X-ray crystallography is a technique used for identifying the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of incident X-rays to diffract into many specific directions.

By measuring the angles and intensities of these diffracted beams, a crystallographer can produce a three dimensional picture of the density of electrons within a crystal. From this electron density, we can determined:

- a) Position of atoms within a crystal
- b) Chemical bonds
- c) Disorder
- d) and various other information's

NMR:

A variety of nuclear magnetic resonance applications have been have been developed for structure-based drug discovery. NMR provides many advantages over other methods, such as the ability to directly observe chemical compounds and target biomolecules and to be used for ligand-based and protein-based approaches.

Homology modeling:

A common challenge in CADD research is determining the 3D structure of proteins because the 3D structure for only a small fraction of the protein is known.

Bioinformatics software tools are then used to predict the 3D structure of the target based on the known 3D structure of the templates.

REFERENCES:

- IV. Drug discovery and development (Technology in transition)- H P RANG
- V. Medicinal chemistry a molecular and biochemical approach 3rd edition, Thomas Nogrady, Donald F. Weaver.
- VI. An introduction to medicinal chemistry –Graham L Patrick.

Systematic Drug Development and Rational Drug Methods

Systematic Drug Development:

To establish a genuine research work is a modern day task. Till 1970, new active chemicals were identified on the basis of erratic variables .e.g.

- Incidental examination
- Unexpected results
- Arduous shortlisting

of lot of plants and animals. But all these approaches are not acceptable because identification of new substance is not confirmed. Methods of drug development can be classified into four categories.

1. **Changes and Enhancement in already present chemicals (Analogue Development)**
2. **Scientific and structured shortlisting (Systematic screening)**
3. **Use of biological data**
4. **Organized research and logical attitude(Planned research and rational approaches)**

Approach 1. Analogue Development:

The most common approach to develop a new drug is analogue development. That approach deals with modification of already present compounds by many chemical processes. The already verified compounds are selected for modifications like

- Enhancing action
- Better specificity of action
- Improves therapeutic window

- Product line extension
- Ease in administration of dosage forms
- Improves patient compliance

Some modal examples are as follows:

Derivatives of Losartan

Derivatives of Conazol

Pharmaceutical industries prefer this approach because of economic and competitive factors of market. To compete the sale of patented products, pharmaceutical industries search for analogues of already existing drugs.

Types of Analogues:

The definition of analogue leads to establishment of three types of analogues.

1. Direct analogues (having chemical and pharmacological similarities)
2. Structural analogues(having structural similarities only)
3. Functional analogues(Chemically different compounds showing almost same pharmacological activities)

Features of Analogue Drug design:

Synthetic derivatives have required pharmacological and therapeutic effects.

During analogue drug design some new properties of compounds are also identified as a result some new compound may be discovered.

Approach 2. Scientific Shortlisting or Systematic screening:

This approach involves testing of hypothetical actions of compounds whether synthetic or natural on modal of organism or testing biologically. In vivo testing includes

- Binding assays
- Enzyme inhibition testing
- Reaction with isolated organ or cell cultures

Types of screening:

1. **Broad screening (extensive Screening):**

This type of screening involves studying pharmacological actions on various systems extensively. It involves new chemical substance from original research. Pharmacological activity on CNS, CVS, GIT, antiviral, antibacterial or chemotherapeutic activity is checked and huge investment is required for this purpose. E.g. Sternbach identified Benzodiazepines this way

2. Random screening:

In this type of screening pharmacological activity is fixed to be tested by a compound but several hundreds and thousands chemicals are tested against a single biological activity. E.g. during world war II chickens infected with *Plasmodium gallinaceum* were used for identification of antimalarial activity of thousands of compounds. The main objective was to overcome the shortage of natural compound used i.e. quinine.

In the same manner this approach was implemented in Europe and USA for identification of anticancer activity.

Ethambutol(anti tubercular agent) and Lovastatin (hypocholestrolemic agent) were identified this way.

3. High throughput screening:

During 1980's, using software and advanced in-vitro techniques above approaches are combined and testing is done. E.g. screening of various compounds on large number of biological targets (ELISA) , also applicable in case of insulin.

Features:

- Unavoidable diversity
- High cost
- Chances of less output
- Possible active drugs can be identified with combinatorial chemistry in combination with this approach from larger libraries. E.g. Neviraprine, Efavirins

4. Screening of Synthetic Intermediates:

Products formed during the chain reactions to formulate a compound are chemically related to final product as they belong to same class of compounds. Hence most expectedly these compounds have same pharmacological activity.e.g Isoniazid is used in the synthesis of sulphathiazoles and later it is revealed that it has itself antituberculosic activity.

5. SOSA Approach:

SOSA stands for selective optimization of side activity. This is the improved version of high throughput screening. This approach is based on taking an idea from old drugs.

SOSA approach includes screening of selective number of compounds on specific pharmacological receptors. Positive results are then analyzed to prepare synthetic derivatives and in this way side effect is converted into main effect.

Approach 3. Use of Biological Information:

This approach is mainly involved in identification of new lead compounds by following ways:

1. Use of actions in humans

The action of external compound on the humans can be examined by many methods:

Ethnopharmacology:

This terminology explains the use of natural products for the treatment of diseases. These natural products constitute most used active constituents now.

Ethnopharmacology explains sources of natural products used as drugs or as medicinal substance such as

- Digitalis is the source of cardiotonic glycosides.
- Opium is the source of opiates.
- Cinchona is the source of cinchona alkaloids.
- Rauwolfiaserpentina is used for its tranquilizing activity for many years so reserpine was characterized from it. In the same manner compounds were isolated and purified from plants such as Atropine from

Atropine from *Atropa belladonna*, pilocarpine from *Pilocarpus jaborandi*, ephedrine from *Ephedra* and in the same manner theophylline, nicotine and cocaine were extracted.

Over a period, use of these medicines poses side effects and unexpected side effects lead to the formation of new active compounds for some new activity such as

- Anti-histamine (Promethazine) has sedative effects as side effect which is utilized in research leading to establishment of new class of neuroleptics (Chlorpromazine).
- In the same manner sildenafil (anti-hypertensive and cardiotonic) has side effects led to the establishment of its use for erectile dysfunction.

Other examples include

- Hypoglycemic effects of some sulphonamides.
- Anti-depressant effect of isoniazid.
- Use of Minoxidil (Anti-hypertensive) for baldness.

New utilization of existing drug:

Sometimes a new clinical activity of drug was observed and as a result that compound used in new medication. E.g. Amiodarone was introduced as a coronary dilator. Due to thyroid dysfunction and skin discoloration it is withdrawn from the market but later its anti-arrhythmic activity was identified.

Unexpected Identification of Actions of Industrial Chemicals:

During the manufacture of sulpha drug sulphathiazole, one of the starting material i.e. 2-amino thiazole was identified having anti-thyroidal activity so that led to the discovery of aminothiazoles for the treatment of thyroidal gland hyperactivity.

2. Use of Actions in Animals:

Preparation from the plant Vincarosea was used for anti-diabetic activity. Experimentation on rats of this preparation led to death of rats due to leucopenia. So, later on vincristine and vinblastine were isolated to treat human leukemia. Another example is the discovery of acetyl cholinesterase inhibitors during the research studies of organophosphorus insecticidal compounds at Bayer Laboratory.

3. Use of Actions in Plants and Microbiology:

Indole acetic acid (IAA) is metabolite of tryptophan and act as a growth hormone in plants. 5-hydroxylated analogue of IAA is principle urinary metabolite of serotonin. On the the basis of following observations Shen from Merck Laboratories designed anti-inflammatory compounds from IAA. The observations are as follows:

- Possible role of serotonin in inflammatory processes.
- Increase in urinary metabolite of tryptophan in rheumatic patients.

Among these derivatives Shen discovered powerful NSAID i.e. Indomethacin in 1963 In the same manner that led to discovery of many antibiotics from microbes like Penicillin, Chloramphenicol, streptomycin

Approach 4.Planned research and rational approaches:

A more scientific method is based on understanding of incriminated molecular targets, e.g.

Enzymes

Receptors

Ion channels

Signaling proteins

Transport protein or DNA

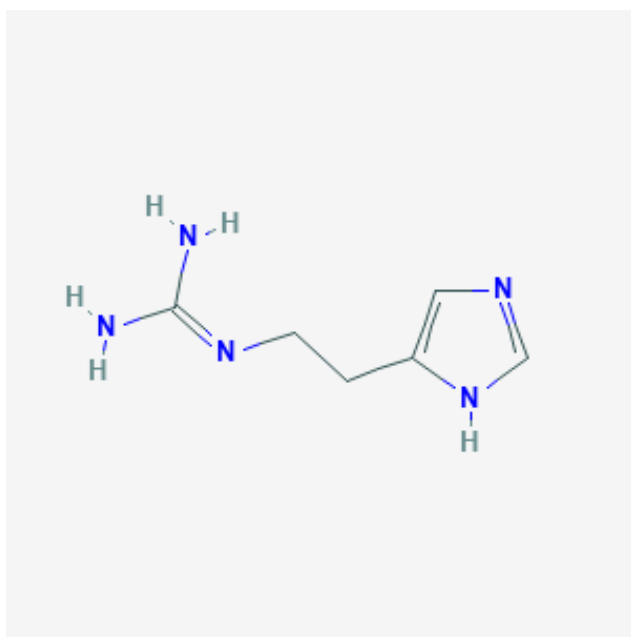
L-DOPA & Parkinsonism:

In Parkinson's disease dopamine level decreases in the brain or basal ganglion so the therapy is administering patient with L-DOPA. This amino acid is able to cross blood brain barrier and then to decarboxylase itself into dopamine by DOPA-decarboxylase. Later on it was discovered that 95%OF DOPA administered by oral route is decarboxylated by peripheral decarboxylase enzyme. To prevent this

unwanted degradation a peripheral inhibitor of DOPA decarboxylase was added in therapy.

H2 Receptor Antagonists:

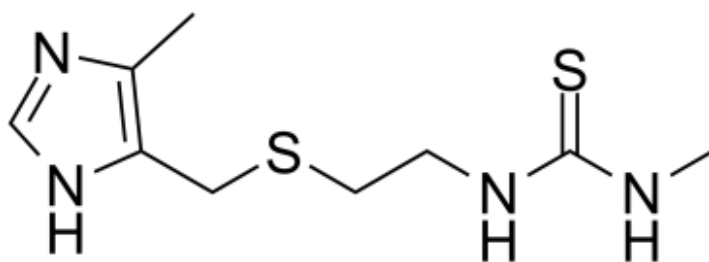
H1 receptor antagonists were not capable of to antagonize the effects of gastric secretions caused by histamine. Scientists identified the existence of unknown class of histamine receptors and initiated a program of systematic research of specific research of antagonists for these receptors. The first compound that showed weak anti-histamine property for gastric secretion was N-guanyyl histamine.



Structure.1 N-Guanyyl Histamine

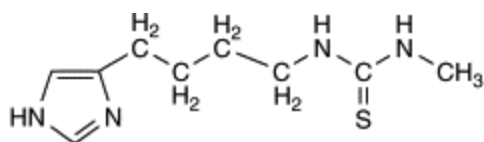
Later on lengthening of side chain of this compound resulted in increase in antagonistic activity of H2 receptors but still there is not complete blockade of receptors. Further Burimamide was obtained by replacing basic guanidine group with neutral thiourea. But Burimamide was rejected due to its low bioavailability.

Further addition of methyl group in position 4 of imidazole ring followed by attachment of an electron withdrawing group i.e. sulphur atom in the side chain led to formation of compound with more acceptable properties i.e. active, less ionized and with improved absorption. The derivative was named as Metiamide which is 10 times more potent than Burimamide. But due to this thio urea group side effects like agranulocytosis and nephrotoxicity were observed that limited its clinical uses.

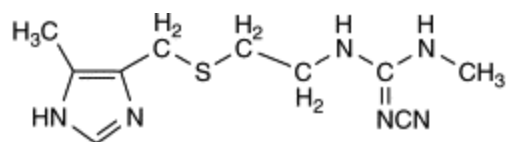


Structure.2 Metiamide

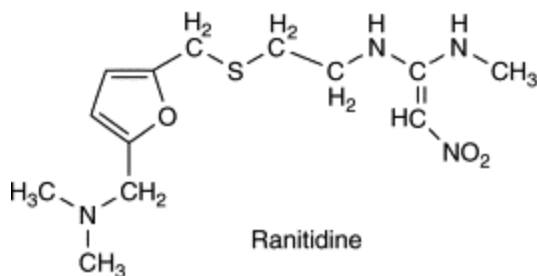
Later on it was discovered that imidazole ring was not indispensable to H₂ antagonistic activity. Thus Ranitidine which possess a furan ring appeared to be even more potent than cimetidine.



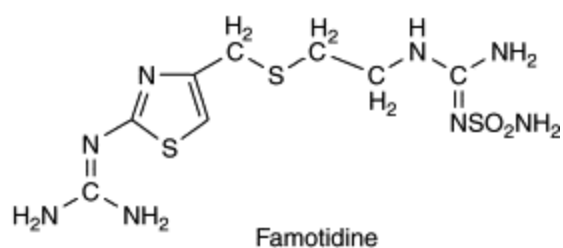
Burimamide



Cimetidine



Ranitidine



Famotidine

Rational Research Methods:

Generally the rational development of a new drug follows a three step process:

- Initially a target has to be identified relating to a particular diseases state
- Then it is fully characterized
- Finally a molecule is designed that binds to it

High-Throughput screening(HTS):

- Is the process of testing a large number of diverse chemical structures against disease targets to identify “Hits” (compounds with desired effects).
- HTS is characterized by its simplicity, rapidness, low cost and high efficiency.
- HTS allows a researcher to quickly conduct millions of pharmacological tests.
- Through this process one can rapidly identify active compounds.
- The results of HTS provides starting point of drug design.

- **Fluorescence based techniques** are likely to be among the most important detection approaches used for HTS due to their high sensitivity as they speed up assays.
- The application of **NMR technology** to HTS is another recent trend in drug research.
- One advantage afforded by NMR technology is that it can provide direct information on the affinity of the screening compounds and binding location of protein.
- The structure activity relationship acquired from NMR analysis can sharpen the library design which will be important in furnishing HTS with well-defined drug candidates.

Visualizing Molecules and their properties:

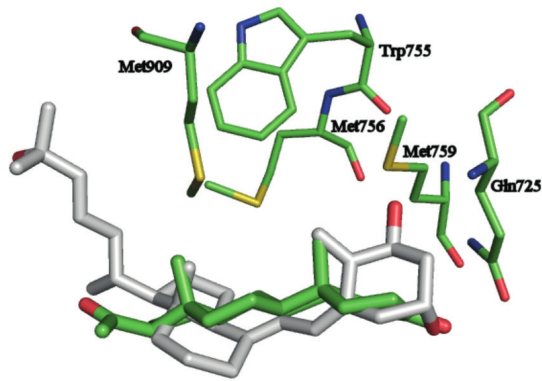
- Molecular modeling software was utilized from 1970s but the view was black and white.
- Modern computers with specialized software allow scientists to step inside a room and view a virtual display of a biological system of interest.
- Interactive devices allow scientists to virtually push a ligand into its target active site to develop a better understanding of its interaction.

Mechanics of Visualization:

- The graphic display of molecules and their properties is accomplished by dividing a complex display into small parts.
- The smallest part of graphics is called "Primitive" and consists of points, vectors and polygons.
- Molecular modeling software combines three primitives into objects such as atoms, bonds or surfaces
- The final display observed may be built up of hundreds or thousands of objects.

Stick Display:

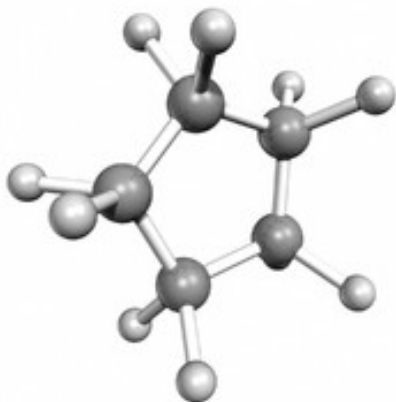
It shows structure in a simple, nearly transparent form.



Structure.3 Stick display of different amino acids

Ball and Stick Display:

Structure.4Cyclopentane



Computational Chemistry Overview:

Computational chemistry method is the use of theory and computer technology to calculate molecular properties.

The greatest potential power of computational chemistry is the domain of making predictions prior to experimental work.

It solves chemical problems and calculates the structure and properties of compounds.

In general computational chemistry refers to energy based methods

Approaches of Computational Chemistry:

Computational chemistry approaches can be divided into two broad categories:

- 1. Quantum Mechanics Based**
- 2. Classical Mechanics Based**

Approach that gives result in shortest possible time is used.

- 1. Quantum Mechanics Based**

It covers the area of Ab initio (high volume data processing applications)

Density functional theory

- 2. Classical Mechanics Based**

It refers to force field (molecular mechanics) calculations .

Molecular dynamics simulations

Computer Aided Drug Design (CADD):

Computer aided drug design is an encompassing term. It includes

Energy based calculations

QSAR

Data base searching

Pharmacophore perception methods

Structure Based Methods

Structure based methods are principle analogues to HTS in that both target and ligand structure information is imperative. Structure based approaches include:

Ligand docking

Pharmacophore

Ligand design Methods

Ligand Based Methods

Ligand based methods use only ligand information for predicting activity depending on its similarity / dissimilarity to previously known active ligands. The method has advantage of requiring minimal compound design or prior knowledge and technologies.

Force Field Methods

In past force field methods were known as Westheimer Method. Calculations of force field method depend on fundamental concept that a ball and spring model may be used to approximate a molecule.

References:

1. MEDICINAL CHEMISTRY An Introduction by GARETH THOMAS (SECOND EDITION)
2. Drug Discovery Research (New Frontiers in the Post-Genomic Era) by Ziwei Huang

Theoretical aspects of drug design

- This is the non experimental study of drug design.
- Structure activity relationship (SAR) and quantitative structure activity relationship (QSAR) collectively known as Q(SAR) are the theoretical models that can be used to predict the physiochemical, biological and environmental fate properties of molecules.
- The quantitative aspects of the biological activity and the mathematical relationships existing between the biological activity (BA), chemical structure (C) and physicochemical properties (P) must be understood for the drug design.
- Aim of these models is to develop correlations between biological or pharmacological activity and physicochemical properties of a set of molecules.

Quantitative structure activity relationship

This non experimental part of drug design comprising study of both

- structure-activity relationship studies
- activity-property relationship studies
- Biological activity of a drug depends on the types and magnitude of interactions between the receptor and the drug molecule'. Various structural attributes of the drug molecule like electronic distribution, steric feature, etc., are the determining factors regulating the interactions. All Quantitative Structure-Activity Relationship (QSAR) studies are based on the notion that BA is function of C and/or P.
$$BA = f(C, P)$$

Goals of QSAR

The goals of QSAR studies include

- better understanding of the mode of action
- Prediction of new analogs with better activity
- Optimization of the lead compound to reduce toxicity and increase selectivity

▪ Activity-property relationship studies

The first quantitative correlation of biological activity was made with physical property rather than the structure. Probably the reason was that the concept of structure was ill defined till 1929 when the symmetric structure of benzene was confirmed by X ray studies.

- In 1901, Meyer and Overton drew attention to the significance of lipid solubility as a determinant of biological activity. They showed that narcotic effect of a wide variety of compounds could be correlated with their partition coefficient.
- In 1939, Ferguson showed that parameters like relative solubility obtained by applying simple thermo-dynamic principles could also be used for correlation with narcotic or depressant effects. This is known as **Ferguson principle**.

In 1940, Hammett showed that chemical reactivity of meta- and para-substituted benzenes derivatives could be correlated by the following equation.

$$\text{Log (Kx/Kh)} = \sigma\rho$$

σ = Hammett constant (electronic distribution of electrons)

ρ = parameter representing the specific reaction type
 K_x = benzoic acid substituted by the group x
 K_h = unsubstituted benzoic acid (parent compound)

According to which if an electron withdrawing group is attached to the aromatic ring of benzoic acid, it would increase the acid strength.

- **Partition coefficient** is another aspect of drug design which is explained by byHansch substituent constant

By analogy with Hammett equation, in 1963, **Hansch** proposed

$$\text{Log} (P_x/P_h) = \rho \pi$$

P_x = partition coefficient for substituted compound
 P_h = partition coefficient for parent compound
 π =lipophilicity contribution of the substituent
 ρ = has a value 1 for n-octanol-water system

Partition coefficient is the ratio of concentration of compound in two immiscible phases in equilibrium condition.
partition coefficient shown by = n-octanol / water

- It tell us about either the drug is lipophilic or hydrophilic. Higher value of partition coefficient shows its lipophilic nature and it will move towards lipid membranes of body
- ...on the other hand low value shows that drug is hydrophilic in nature and will move towards the hydrophilic part like blood stream. So it will tell us about the distribution behavior of drug in body.

Hansch model is one of the most successfully applied methods in the field of QSAR and ROD. It was developed, based on the following postulates:

- Drug reaches near the receptor site by "**random walk**", i.e. crossing various lipid barriers by passive diffusion process.
- Drug binds with the receptor (critical reaction site) forming a complex.

- The drug-receptor complex may undergo chemical reaction or conformational changes for the desired activity.
- The drugs in a congeneric series act through same mechanism of action .

Hansch model is a linear free energy related (LFER) model. It considers that each substituent has a specific influence on the equilibrium and rate constants of a reaction via changes in electron density and steric effects at the reaction centre and this can be described in terms of linear free energy relationships that do not follow immediately from the law of thermodynamics (extrathermodynamic relations).

- Hansch model also used electronic and steric parameters and this has considerable predictive value and diagnostic potential and it gives an insight into mode of action and location of the receptor.

Electronic parameters

- Apart from various electronic substituent constants like Hammett constants, hansch constant etc., various experimental quantities expressing intermolecular binding are
 - dipole moment (μ) (electric dipole moment)
 - ionization potential (I) (ionization energy)
 - polarizability (P_E)
- and also various quantum chemical parameters (like energies of highest occupied and lowest unoccupied molecular orbitals, electron density, electrophilic and nucleophilic localization energies.

Steric parameters

➤ The various steric parameters which are commonly used in Hansch analysis are

- molecular connectivity
- Taft steric parameters (E_s)
- van der Waals volume (V)

Taft substituent constant

is used to measure the polar effects of substituents in aliphatic compound when the group does not form a part of conjugated system. It is defined by the following equation :

$$\sigma^* = (1/2.48) [\log(K/K_o)B - \log(K/K_o)A]$$

σ^* = Taft substituent constant

K = rate constant for hydrolysis of substituted compound

K_o = rate constant for the hydrolysis of the methyl group

A = acid hydrolysis

B = base hydrolysis

Van der Waals dimensions

- Van der Waals volume (v) or van der Waals radius(r) explain the actual dimension of the group.
- Van der Waals radius is defined as “the distance of the group protrudes from the bulk of the parent molecule”. It can be correlated with biological results in the same way as Taft's steric constant.

▪ Structure- activity relationship studies

Free- Wilson model

- Also known as additively model or de novo approach.
- It is the true structure-activity relationship model.
- This method is based on the assumption that “ the introduction of a particular substituent at a particular molecular position always contributes in the same way to the biological potency of the whole molecule”, as expressed by the equation:

log BA = contribution of unsubstituted (parent) compound + contribution of corresponding substituents

$$\log BA = \mu + \sum A_p A_s$$

A_p= no of positions at which groups attached

A_s=number of substituents at that position

μ = overall average

- This equation is solved by MLR using the presence (1) and absence (0) of the different substituents as independent parameters, while the measured activity serves as dependent variable.

❖ **Fujita- Ban modification** of free-Wilson model is now used commonly instead of original method. An arbitrary reference compound is chosen and activity contributions of various structural features are found out in relation to that present in the reference compound

Topological scheme

- These are based on graph theoretic approach and mostly deal with hydrogen suppressed graphs. Topo-logical consideration includes number and types of atoms and bonds, interatomic connections (adjacency count), paths, branching, molecular size, shape, functionality, etc.

- Among the various topological schemes, molecular connectivity indices (MCI) of Kier and Hall are most successful. These indices encode various structural features of molecules that are obtainable from two dimensional representations of molecular structures and have been successfully correlated with various physicochemical and biological parameters.

Kier and Hall, have formulated, more recently, two other schemes applying the basic concept of topological schemes. One of these is Kappa shape index, an index for molecular shape, and the other is electro topological state atom index, which has been claimed to have power

- ❖ to identify important atoms or fragments necessary for a particular biological activity

TAU scheme (pal et al)

- Offers some advantages over MCI from the point of view of diagnostic features of these indices and presence of scope of its application for molecules with higher complexity.

Molecular negentropy

- A global index calculated based on the information theory of Shannon and Weaver applied on total molecular graph, has also been used in structure-activity correlations

Other Substructural Approach

Other methods involving structural parameters include

- Cramer's sub structural analysis.
- Statistical-Heuristic method for automated search of drugs for screening.
- The logico-structural approach.
- Heuristic approach to topological pharmacophores.

Steps involved in QSAR studies

The QSAR methodology enables the development of mathematical models.

- It can be used to predict the biological activity of newly designed compounds.
- There are three steps involved in this procedure:
 1. Creation of a database in which calculation of various physiochemical and structural parameters of congeneric take place.
 2. Regression analysis leading to model development between biological activities versus derived physiochemical descriptors.
 3. Validation of the models and prediction of the biological activity of the designed compounds.

Statistical methods used in QSAR analysis:

- Statistical methods are an essential component of QSAR work.
- They help to build models.
- Estimate models predictive abilities.
- Validate an already existing model.
- Find the relationships and co relationship among the variables and the activities.
- Data analysis methods are used to recombine data into forms and groups and observations into hierarchies.

1. Regression Methods

- It is mathematical procedure, which correlates dependent variable with the independent variables.

- There can be different forms of regression analysis:

I. Simple linear regression analysis :

- An independent variable is correlated with a dependent variable and produces a linear one term equation. It is useful for discovering some of the most important descriptor

II. MLR analysis :

- More than one independent variable is correlated with a dependent variable and a single multiterm equation is formed.

Stepwise linear regression analysis:

This is useful when the number of independent variables is very high and is thus correlated in a stepwise number with the dependent variable producing a multiterm linear equation.

2. Partial Least Square :

- Hundreds or even thousands of independent variables can be correlated with one or several dependent variables. Often perfect correlations are obtained in PLS analysis because of the usually large number of X variables.

3. Genetic Function Approximation :

- It provides multiple models that are created by evolving random initial models using a genetic algorithm. Models are improved by performing a crossover operation to recombine better sorting models .This method is used when dealing with large number of descriptors.
- The GFA algorithm uses a genetic algorithm to perform a search over the space of possible QSAR/QSPR models to estimate the fitness of each model. Such evolution of a population of randomly constructed models leads to the discovery of highly predictive QSARs/QSPRs.

4. Genetic Partial Least Squares :

The method combines the best of GFA and PLS. The G/PLS algorithm uses GFA to select appropriate basis functions to be used in the model of the data.

Application of G/PLS allows the construction of larger QSAR equations while avoiding overfitting and eliminating most variables.

Statistical Measures commonly used in Regression analysis

- **Correlation coefficient (r):**

It indicates how well data fit a statistical model. A high value of correlation coefficient indicates the statistical significance of regression equation.

- **Standard error of the estimate (S) :**

Its value considers the number of objects n and the number of variables k . The smaller the value of S the better is the QSAR.

- **Predicted sum of squares (PRESS) :**

The sum of all compounds of the square difference between the actual and predicted values of dependent variables.

- **Cross validation :**

It is an approach for assessing the predictive value of a model. The cross validation is generated during a validation procedure.

Model Development Procedures:

2D QSAR Analysis:

- The 2D descriptors are usually developed by using the atoms and connective information of the molecules.
- In 2D QSAR physicochemical parameters such as hydrophobic, steric, hydrogen acceptor, hydrogen donor, electronic field effect or resonance are used.
- In addition to these parameters de novo constants or indicator variables with 0 to 1 value denoting the absence or presence of certain features are also used.

3D QSAR analysis:

- 3D QSAR are quantitative models that relate the biological activity of small molecules with their properties calculated in 3D space.
 - Hence 3D properties of a molecule are considered rather than that of individual substituents.
 - The 3D structures are usually generated from 2D OR 2D with configurational information or 3D structure database.
 - Among all the 3D QSAR techniques, CoMFA is the most widely used and has shown unprecedented accuracy in prediction.
 - Some of these approaches to QSAR are based on the statistical analysis of the 3D interaction fields.
 - These are generated by measuring over a regular 3D grid the interaction energy between a small probe atom or a group and the ligands.
-
- Quantitative drug design; a critical introduction. 2nd edition
 - Organic chemistry of drug design and drug action. 3rd edition
 - Computer-Aided Drug Design: An Innovative Tool for Modeling by
Pranita P. Kore, Madhavi M. Mutha, Rishikesh V. Antre*, Rajesh J. Oswal,
Sandip S. Kshirsagar.

SOURCES OF DRUGS

DRUG:

Drug is defined as any natural or chemical substances used in the investigation, diagnosis, treatment, cure or management of different diseases in human and animals is called drug.

CRUDE DRUG:

A crude drug is any naturally occurring unrefined substance derived from the organic and inorganic sources such as plants animal bacteria,organ or organism intended for use in diagnosis cure treatment or prevention of disease in humans and other animals is called crude drug.

SOURCES OF DRUG

Drugs are obtained from six major sources

1. Plant sources
2. Animal sources
3. Mineral/earth sources
4. Semisynthetic/synthetic sources
5. Microbial sources
6. Recombinant DNA technology

1. Plant Source

- Plant source is the oldest source of drugs.
- Most of the drugs in ancient times were obtained from plants
- Almost all parts of the plants are used e.g leaves , stem ,bark , fruits and root

PLANT SOURCE



Source	Plant	Drug	Use
Leaf	Digitalis	Digoxin	CHF
Bark	Cinchona	Quinine	Malaria
Fruit	Opium	Morphine	Analgesic
Seed	Eserin	Anticholinestrerase	M.G

Contd.,

Pharmacological active principle in plants are;

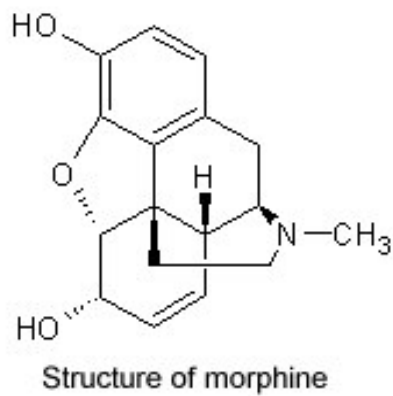
1. Alkaloids
2. Glycosides
3. Oils
4. Resins
5. Gums
6. Tannins

i. ALKALOIDS:

These are basic nitrogenous compounds of plant origin having definite pharmacological activity, e.g

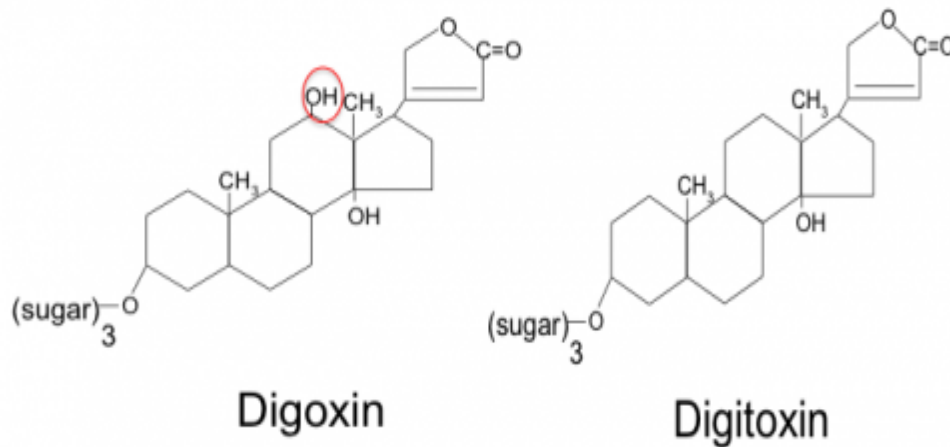
Atropine Atropa belladonna

Morphine Papaver somniferum



ii. GLYCOSIDES:

Non reducing sugar which on hydrolysis yield sugar part (glycone) and non-sugar part (aglycone) are called glycosides. Therapeutic activity of glycosides is due to aglycone part e.g digoxin, digitoxin are obtained digitalis plant.



iii. OILS:

Generally three types of oils are used for medicinal purpose

1. Essential oils(volatile oils)
2. Fixed oils
3. Mineral oils

1. **Essential oils**: These are the oils which are obtained from various parts of the plants by distillation process

- They are highly aromatic and are slightly soluble in water
- No food value e.g Ginger oils , peppermint oil

USES: Carminative, antiseptic, Flavoring agent

2. **FIXED OILS**: These are the esters of long chain fatty acids and alcohols

- Obtained by solvent extraction of crushed seeds
- Non volatiles
- Saturated from animals
- Unsaturated from plants
- E.g Cotton seed oil, Oliveoil, Caster oil.

USES: as purgative, as pharmaceutical vehicles, as lubricant

3. **MINERAL OIL**: Obtained by dry distillation of wood. E.g liquid paraffin

USES: lubricant laxatives.

3. **RESIN**: These are complex chemical substances formed in the shizogenous ducts of plants

- Insoluble in water
- Soluble in alcohol and organic solvents
- E.g: benzoin, rosin ,cannabis
- **USES**: Carminative , Anti spasmodic, Analgesic

4. **GUMS**: These are the secretory products of plants . soluble in water and form gummy mass.

- example: gum tragacanth ,gum acacia

USES:

- Emulsifying and suspending agent

5. **TANNINS**: These are complex non nitrogenous phenolic derivative obtained from plants

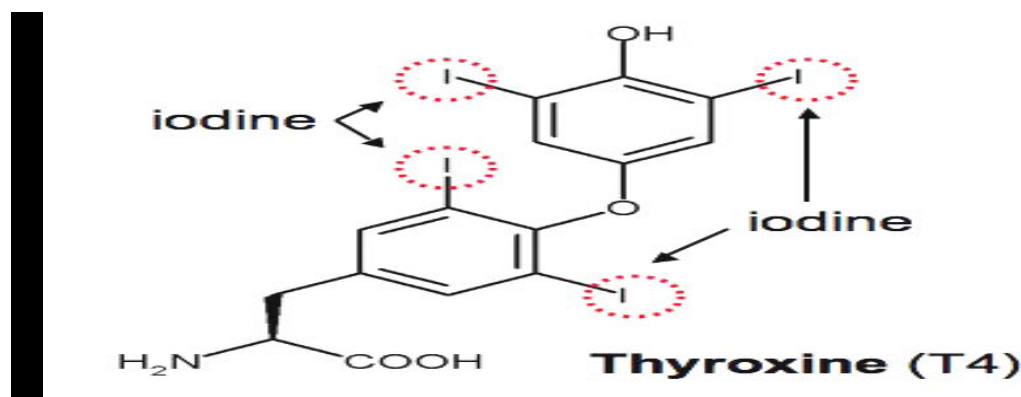
- Non crystallisable compounds
- Soluble in water and sparingly soluble in organic solvents

E.g: black catechu, palecatechu

USES: as astringent, Antiseptic, Anti carcinogenic

2. ANIMAL SOURCES

Various organs and tissues of animals were used in the past without understanding their mechanism of action. Active principles of animal drugs are protein, fats, oils, enzymes and hormones. e.g. Heparin is obtained from leech, Insulin from pork pancreas, Thyroxine from Sheep.



Similarly urokinase enzyme which is used as thrombolytic agent is released from human kidney cells in human urine .

3. MINERAL SOURCES

A mineral is a naturally occurring inorganic solid with a definite chemical composition and ordered atomic arrangement.

- They are not made by humane.
- Solid not liquid.
- Ex: Fe ,Zn ,Mg,Mn
- USES: as antacid, antioxidant.

4. MICROORGANISM SOURCE:

Bacteria fungi molds are important source of many lifesaving drugs

- These are obtained by microorganism and used to kill microorganism

- EX: Penicillin is obtained from *penicillium notatum* fungus. Streptomycin is obtained from *Streptomyces griseus* bacteria.



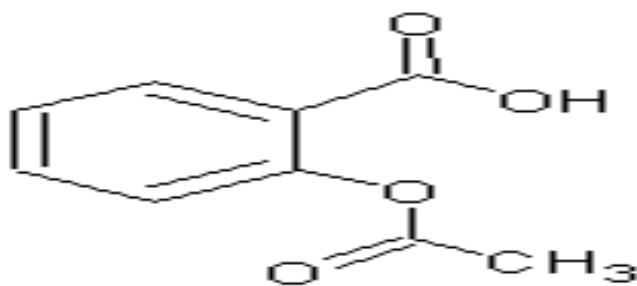
5. SYNTHETIC SOURCE

Synthetic drugs are the chemical compounds which are produced in the laboratory e.g aspirin.

Presently majority of drugs are produced by this method

Advantages:

- quality can be controlled
- Process is easier and cheaper
- Large scale production



Aspirin

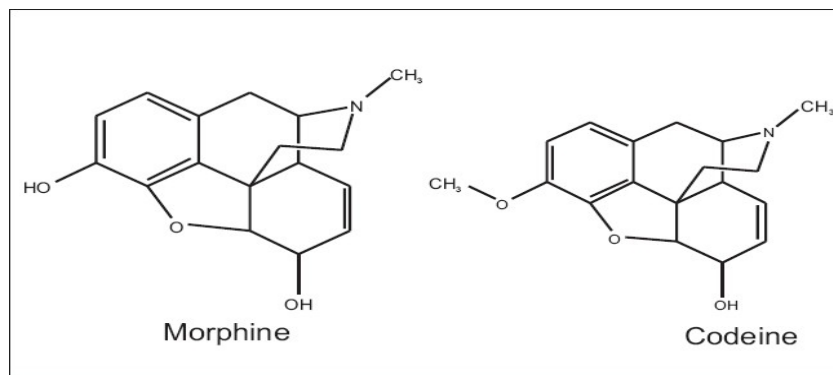
Acetylsalicylic Acid

$C_9H_8O_4$

6. SEMISYNTHETIC DRUGS

These are obtained by changing the chemical structure of naturally occurring drugs.

EX: Codeine is obtained by the methylation of Morphine



7. GENETIC ENGINEERING

Now a days lot drugs are being produce by genetic engineering which involve rDNA technology DNAalteration, gene slicing etc.

Example: Hepatitis –B vaccine, Insulin by recombinant DNA technology.

References:

1. <https://link.springer.com/chapter/>
2. Slideshare.net

Development of new drugs (Random Screening)

Definition:

“ **Random screening** involves no intellectualization; all compounds are tested in the bioassay without regard to their structures.”

Drug discovery in earlier days was made by **random screening** of higher plants. Thousands of new organic compounds are synthesized and subjected to pharmacological **screening**. This process of **random screening**, though inefficient, has led to the identification of new lead compounds.

Crude plant drugs like opium, senna, belladonna, reserpine, ephedrine, etc., were in use for centuries.

With the serendipitous discovery of penicillin came the screening of microorganisms, resulting in a large number of antibiotics from bacterial and fungal sources.

Prototypes of these antibiotics enabled medicinal chemists to modify them and yield better antibacterials with improved therapeutic profiles.

Thousands of new organic compounds are synthesized and subjected to pharmacological screening. This process of random screening, though inefficient, has led to the identification of new lead compounds. Optimization of the lead compounds has resulted in good clinical drug candidates.

For Example:

Sulfanilamide testifies this, as many sulfonamides have resulted in drugs ranging from antibacterial through anti-malarial, anti-diabetic, diuretic, and sulfas with activity for typhoid fevers.

Automated high-throughput screening systems have increased the efficiency of random screening. Combinatorial chemistry has accelerated synthetic methods and facilitated synthesis of a huge library of compounds which is subjected to high throughput screening for deciphering the biological activity of the compounds. Although synthesis was fast, this technique has not produced compounds with the status as drugs. [1]

Combinatorial chemistry:

Combinatorial chemistry involves the generation of a large array of structurally diverse compounds, called a chemical library, through systematic, repetitive and covalent linkage of various “building blocks”. Once prepared, the compounds in the chemical library can be screened, concurrently, for individual interactions with biological targets of interest. Positive compounds can then be identified, either directly (in position-addressable libraries) or via decoding (using genetic or chemical means). [2]

Combinatorial chemistry has been used for both drug lead discovery and optimization. [3][4][5]

Screening of Combinatorial Libraries:

The screening of a combinatorial library can be divided into two categories:

- 1) Virtual screening.
- 2) Experimental real screening.

1) Virtual screening:

It uses computational methods to predict or simulate how a particular compound interacts with a given target protein. The three virtual screening methods used in modern drug discovery include

- a) Molecular docking.
- b) Pharmacopoeia mapping.
- c) Quantitative structure-activity relationships.

Disadvantage:

The disadvantages of virtual screening are that it cannot replace real screening, and generated hits may be very difficult to chemically synthesize.

2) Real screening approaches:

Such as high-throughput screening (HTS), can test the activity of hundreds of thousands of compounds experimentally, providing real results; **Disadvantage:**

These methods are far more expensive and slower than virtual screening methods.

High-throughput screening (HTS):

High-throughput screening (HTS) is a key process used in drug discovery to identify hits from compound libraries that may become leads for medicinal chemistry optimization. [6]

Reference:

- 1) Giridhar R. Drug discovery: Past and present. J Adv Pharm Technol Res. 2012 Jan;3(1):2. doi: 10.4103/2231-4040.93554. PMID: 22470886; PMCID: PMC3312722.
- 2) Liu R, Li X, Lam KS. Combinatorial chemistry in drug discovery. Curr Opin Chem Biol. 2017 Jun;38:117-126. doi: 10.1016/j.cbpa.2017.03.017. Epub 2017 May 8. PMID: 28494316; PMCID: PMC5645069.
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- 4) Kennedy JP, Williams L, Bridges TM, Daniels RN, Weaver D, Lindsley CW. Application of combinatorial chemistry science on modern drug discovery. J Comb Chem. 2008;10:345–354. [PubMed] [Google Scholar]
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Extraction from Natural Sources

Background:

In the past extract from natural sources were used to treat and eradicate the diseases. These extracts obtained from plants, animals & micro-organisms (bacteria, fungi, algae). Natural products have positive impact on human civilization.

Extraction of natural products was used since with discovery of fire. Every Production department including perfume industries, cosmetic, pharmaceutical, bio fuel, food, and fine chemical industries use extraction processes.

Almost 50% of drugs registered by FDA are derived from natural sources.

Importance of Extraction:

Quantitative Determination:

Potency of drug can be controlled in extract than in crude form

More Stable form:

Deterioration by enzyme action is diminished due to separation from bulk

Enhanced Organoleptic Characteristics:

More palatable and more elegant

Easy Formulation:

Tableting of crude material may not be possible.

Different Route of Drug Administration:

Injection of crude material may be undesirable and dangerous

Natural Products:

Natural products are anything that is produced by life.

Classification Based on Function:

1. Primary Metabolites:

metabolites that have intrinsic functions. These have functions like energy production, nutrient assimilation, growth and development. E.g carbohydrates, lipids, amino acids and nucleic acids.

2. Secondary Metabolites:

Are not essential for survival and dispensable. Alkaloids, phenylpropanoids, polyketides and terpenoids.

Classification Based on Sources:

1. Prokaryotic:

Bacteria: Produce anti-infective natural product. For example Clostridium botulinum produce botulinum toxin used as anti-infective.

Archaea: exist in extreme environments like polar regions, hot springs and deep oceans. For example cellulose, amylase, xylanases etc.

2. Eukaryotic:

Fungi: examples

Penicillium chrysogenum produce cephalosporins used as antibacterial drug

Animals: Animals are also the source of natural products especially the venomous animals.

Plants: the main source of natural product are plants.

For example Galanthus produce galantamine which is used as antimalarial drug.

Extraction:

Extraction is the method by which active constituents are separated from solids or liquids with help of solvents.

Extraction is the first step of separation of active constituents or potential substances from crude form.

Extract:

Preparation of crude drugs which contain all constituents which are soluble in solvent.

Marc:

Solid residue obtained after extraction.

Menstruum:

Mixture of solvent used for extraction.

General Procedure of Extraction:

- Solvent penetrate into solid matrix
- Solute dissolves in the solvent
- Solute diffuse out of the solid matrix
- The extracted solutes are collected

Factors facilitating extraction process:

- Properties of extraction solvent: polar solvents to separate polar solutes and non polar to separate non polar solutes

- Particle size of raw material: if particle size is finer then better will be the extraction too fine particle size also cause adsorption to the solid and difficult to filter.
- Solvent to solid ratio is high the higher the extraction yield but require long time to concentrate the extract
- Extraction temperature: high temperature cause increase in solubility of solute in solvent but to high temperature also cause loss of solvent and degradation of thermolabile components
- Extraction duration increase extraction efficiency up till equilibrium

Selection of solvent:

Selection of solvents is based on law of similarity and intermiscibility or like dissolves like.

Solvents with polarity value near to polarity value of solute are used for extraction.

Alcohols eg. Ethyl alcohols and metyl alcohols are universal solvents used for extraction in phytochemistry.

Types of extraction:

Conventional extraction method:

- **Maceration**
- **Percolation**
- **Reflux extraction**

Modern extraction method:

- **Soxhlet extraction**
- **Pressurized liquid extraction**
- **Supercritical fluid extraction**
- **Ultrasound assisted extraction**
- **Microwave assisted extraction**
- **Pulsed electric field extraction**
- **Enzyme assisted extraction**

A brief summary of various extraction methods

Method	Solvent	Temperature	Pressure	Time	Volume of organic solvent consumed	Polarity of natural products extracted
Maceration	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Long	Large	Dependent on extract- ing solvent
Percolation	Water, aqueous and non-aqueous solvents	Room temperature, occasionally under heat	Atmospheric	Long	Large	Dependent on extract- ing solvent
Decoction	Water	Under heat	Atmospheric	Moderate	None	Polar compounds
Reflux extraction	Water, aqueous and non-aqueous solvents	Under heat	Atmospheric	Moderate	Moderate	Dependent on extract- ing solvent
Soxhlet extraction	Organic Solvent	Under heat	Atmospheric	Long	Moderate	Dependent on extract- ing solvent
Pressurized liquid extraction	Water, aqueous and non-aqueous solvents	Under heat	High	Short	Small	Dependent on extract- ing solvent
Supercritical fluid extraction	Supercritical fluid (usually S-CO ₂), sometimes with modifier	Near room tempera- ture	High	Short	None or small	Nonpolar to moderate polar compounds
Ultrasound assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature, or under heat	Atmospheric	Short	Moderate	Dependent on extract- ing solvent
Microwave assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Short	None or Moderate	Dependent on extract- ing solvent
Pulsed electric field extraction	Water, aqueous and non-aqueous solvents	Room temperature, or under heat	Atmospheric	short	Moderate	Dependent on extract- ing solvent

Enzyme assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature, or heated after enzyme treatment	Atmospheric	Moderate	Moderate	Dependent on extracting solvent
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Maceration:

It is very simple extraction method with the disadvantages of long extraction time. Maceration is an isocratic extraction method and cold extraction methods. It is suitable for extraction of thermolabile compounds. This method involves extraction of constituent materials from plants in solvent by immersing the plant sample in a particular solvent. It is done at room temperature at steady state.

Procedure of Maceration:

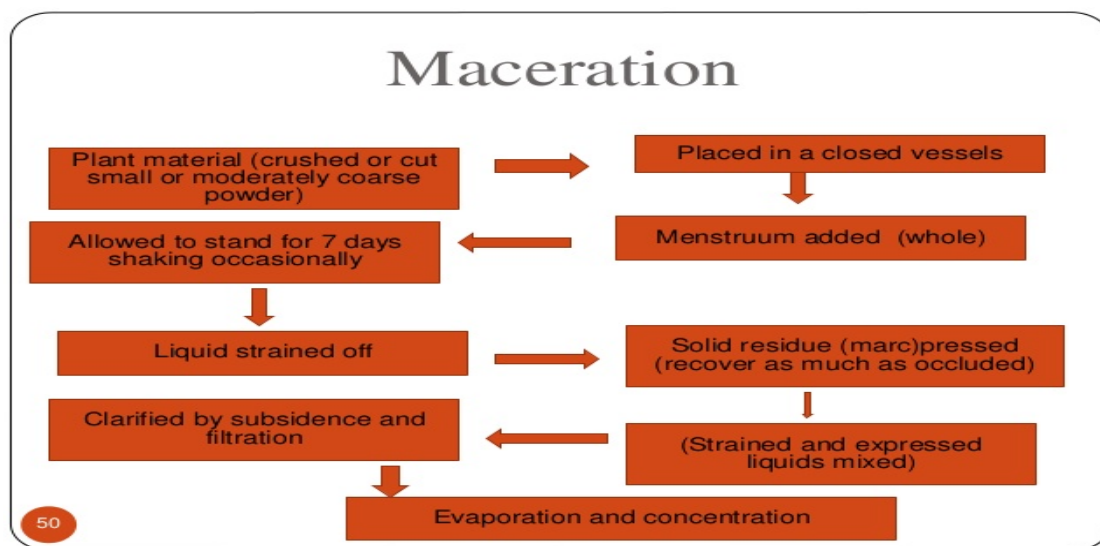
STEP 1: Cleaned and air dried crushed plant material or coarsely powdered is dipped in an appropriate solvent called menstruum in a closed container and allowed to stand still for 4-7 days under complete steady state at room temperature with occasional agitation and opening the lid times to times to release the developed pressure and shake until the soluble matter has dissolved.

STEP 2: The damp solid material is filtered off using a funnel with a cotton plug and then the marc is further pressed to recover as much as occluded solution as possible. Sufficient time is provided for coagulation and settling and the settled matter is then filtered using filter paper.

STEP 3: The resultant extract is then concentrated under reduced pressure to obtain the crude extract of the plant.

STEP 4: The extracted crude is then used for further analysis. As the system is stationary in the maceration process, the extraction process works on principle of molecular diffusion which is a time consuming process and ensues dispersal of the concentrated solution accumulation around the surface of the particles and bringing fresh solvent to the surface of particles for further extraction. Also a closed process is used to avoid the evaporation of solvent from the process.

The solvent used for maceration methanol, methanol water or any other organic solvent
Following is the Figure showing the flow for process of Maceration:



Types of Maceration:

- ☐ **Simple Maceration:** Simple Maceration is basically used for organized and unorganized crude drugs.
 - ☐ E.g. tincture of orange, lemon, and squill.
- ☐ **Double Maceration :** It is used for concentrated infusion of orange.
- ☐ **Triple Maceration:** The maceration process may be carried out with the help of heating or stirring
 - ☐ E.g. concentrated infusion of Quassia and Senna.

Merits and Demerits: Maceration requires small sample size. It has strong swelling properties or high mucilage and it is an energy saving process. But on the other hand unable to extract the drug exhaustively, It is very slow process and the amount of solvent required is more.

Percolation:

It is continuous downward displacement of the solvent through the bed of crude drug material to get extract. It is most frequently used to extract active ingredients in the preparation of tinctures and fluid extracts. It is a method of short successive maceration or process of the displacements. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used.

Steps in Percolation:

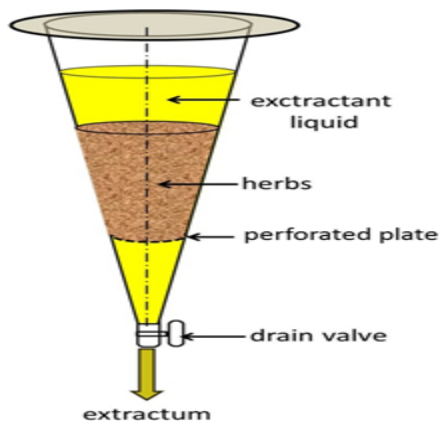
STEP 1: Size reduction: The drug to be extracted is subjected to suitable degree of size reduction, usually from coarse powder to fine powder.

STEP 2: Imbibition: During imbibition the powdered drug is moistened with a suitable amount of menstruum and allowed to stand for four hours in a well closed container.

STEP 3: Packing: After imbibition the moistened drug is evenly packed into a percolator

STEP 4: Maceration: After packing sufficient menstruum is added to saturate the material. The percolator is allowed to stand for 24-25 hours to macerate the drug.

STEP 5: Percolation: The lower tap is opened and liquid collected therein is allowed to drip slowly at a controlled rate until 3/4th volume of the finished product is obtained.



Types of Percolation:

☐ Simple Percolation:

E.g. Tincture of Belladonna and compound tincture of cardamom.

☐ **Modified Percolation:** Repeated maceration is more effective than simple one. Multiple maceration- solvent is divided into equal multiple time considering the solvent retained by plant tissue. It is basically used to prepare concentrated preparation.

☐ **Reserved Percolation:** In this case the extraction is done through the general percolation procedure. At the last, evaporation is done under reduced pressure in equipment like a climbing evaporator to the consistency of a soft extract (semi solid) such that all the water is removed. This is then dissolved in the reserved portion which is strongly alcoholic and easily dissolves the evaporated portion with any risk of precipitation.

Merits and Demerits: It requires less time than maceration. Extraction of thermolabile constituents can be possible. But it requires more time than any other type of extraction. Requires more solvent and skilled persons.

Decoction:

Decoction is a method of extraction by boiling herbal or plant material to dissolve the chemicals of the material, which may include stems, roots, bark and rhizomes.

- In this process the crude drug is boiled in a specified volume of water 1:4 for a defined time
- Volume is reduced to 1/4th the original
- It is then cooled and filtered
- It is used for water soluble and heat stable constituents
- Eg tea and coffee

Reflux Extraction:

Reflux extraction is a solid-liquid extraction process at a constant temperature with repeatable solvent evaporation and condensation for a particular period of time without the loss of solvent. The system is widely used in herbal industries as it is efficient, easy to operate and cost effective.

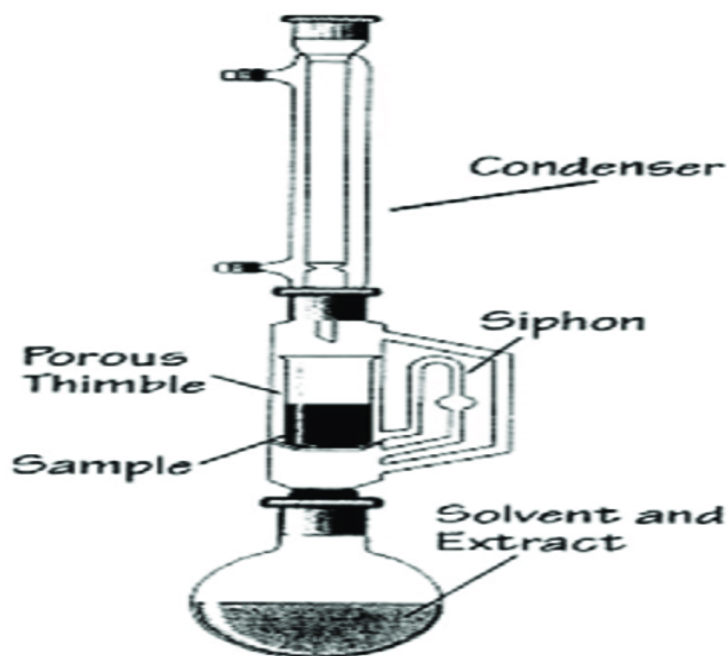


- Place extraction material in the flask along with boiling stones
- Attach condenser and connect hoses so water travel against gravity
- There should be no leakage in the connection of condenser and flask
- Circulate water through the condenser and begin heating the flask
- Reflux ring is seen
- At the end of reflux turn down the heat
- Keep circulating the water in the condenser until flask is warm enough to touch
- Put the flask under tap water and filter the extract

Soxhlet extraction:

This extraction is used where compound has limited solubility in a solvent and impurity is insoluble in solvent.

- Solid material containing some desired components is placed inside a thimble made from thick filter paper. Which is loaded in the main chamber of soxhlet chamber. The extractor is then placed in a flask containing extraction solvent. Soxhlet is then equipped with condenser
- The solvent is heated to reflux. Solvent vapours travel up the distillation arm condensed by condenser and travels back into the chamber holding the thimble of solid.
- Chamber filled with warm solvent and dissolve some of desired components. The chamber is automatically emptied down the distillation chamber by siphon side arm.
- This cycle repeated many times for hours or days
- Use only single batch of solvent
- The solvent is removed by means of rotary evaporation
- The non soluble part remains in the thimble and discarded



Pressurized liquid extraction (PLE):

Pressurized liquid extraction is a sample preparation technique that combines elevated temperature and *pressure* with *liquid* solvents to achieve fast and efficient *extraction* of the components from the solid matrix.

- High pressure solvent extraction

- PLE applies high pressure in extraction. High pressure keeps solvents in a liquid state above their boiling point resulting in a high solubility and high diffusion rate of lipid solutes in the solvent, and a high penetration of the solvent in the matrix
- PLE dramatically decreased the consumption of extraction time and solvent and had better repeatability compared to other methods.



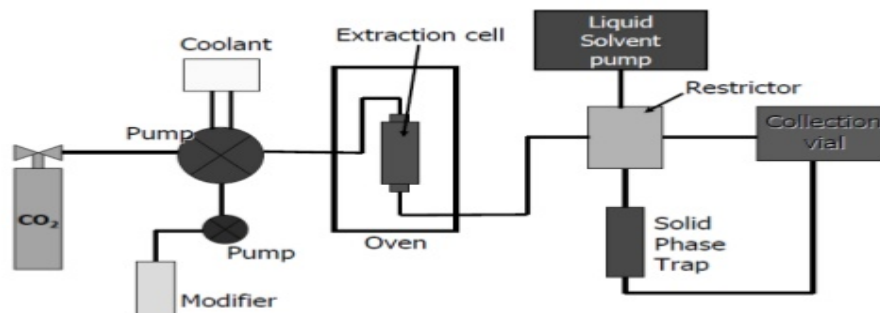
Supercritical fluid extraction (SFE):

Supercritical fluid extraction represents an alternative technique to conventional solid liquid extraction with lower solvent consumption and lower working temperature. It is form of liquid extraction where the usual liquid solvent phase has been replaced by a supercritical fluid- a substance that is above its critical point. Amongst a wide variety of supercritical fluids, carbon-dioxide is essentially the only convenient supercritical extraction solvent used because of its comparatively low critical temperature.

An organics solvent (also called modifier) may be added to the supercritical fluid to enhance its solvating properties. In case of CO₂ as the supercritical fluid, extraction can be performed under mild condition, thus reducing both the risks of thermal degradation and poor collection efficiencies of volatile analytes.CO₂ is most effective for dissolving organic compounds particularly molecules displaying some degree of lipophilicity, such as esters and lactones. The modifier component may introduce into the fluid either using a separate pump and suitable mixing devices or may be added to the sample matrix in the extraction cell prior to pressuring with CO₂. Frequently, an off-line valve is incorporated between the pump and the extraction vessel and between the vessel and the restrictor. In this set-up static or dynamic extraction or a combination of the two may be performed. The restrictor maintains the pressure within the extractor vessel by the flow control. The use of SFE both at the analytical and processing scales is quite widespread in the food industry for extraction of fats and oils from seeds, foodstuffs, and other materials, the technique has also been applied to the extraction of the active compounds from medicinal plants, such as steroids, terpenes, alkaloids, various oxygen containing heterocyclic compounds, as well as aromatics and phenolic compounds.

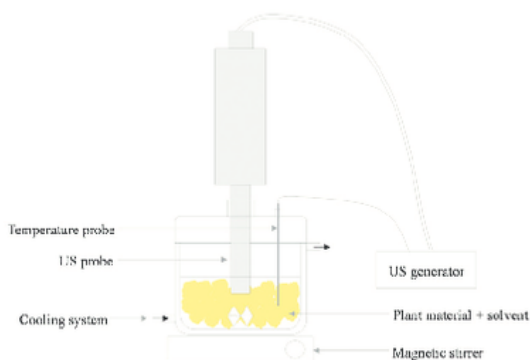
SUPERCritical FLUID EXTRACTION (SFE) CONTD..

Supercritical fluid extraction is the process of separating one component from another (the matrix) using supercritical fluids as the extracting solvent



Ultrasound assisted extraction (UAE):

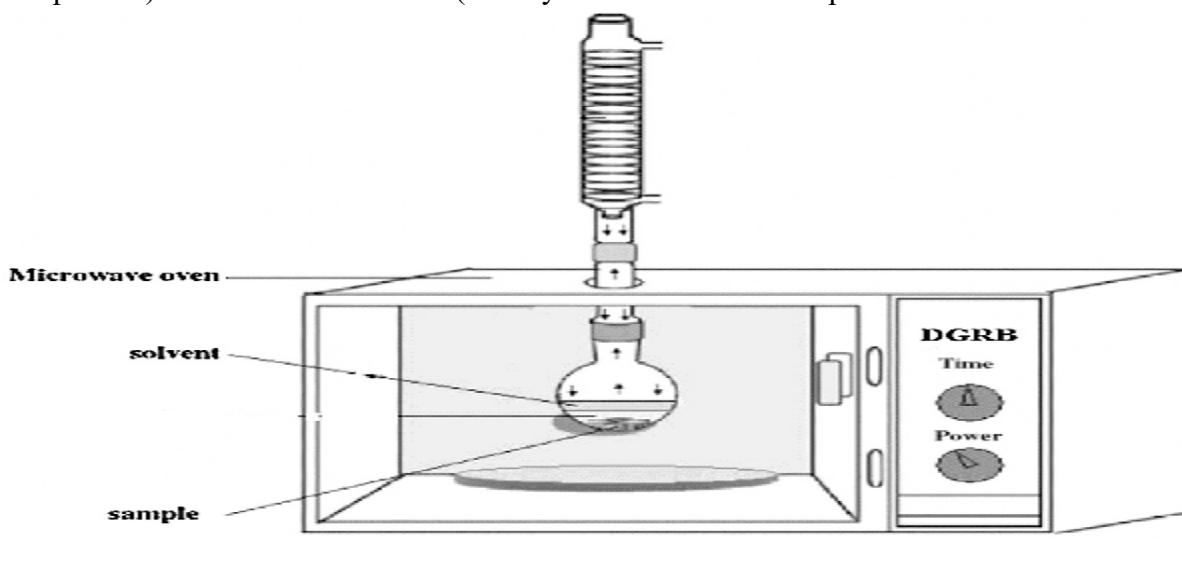
- Ultrasonic extraction or sonication, uses ultrasonic wave energy in the extraction.
- Ultrasound in the solvent producing cavitation accelerates the dissolution and diffusion of the solute as well as the heat transfer, which improves the extraction efficiency.
- The other advantage of UAE includes low solvent and energy consumption, and the reduction of extraction temperature and time.
- UAE is applicable for the extraction of thermolabile and unstable compounds. UAE is commonly employed in the extraction of many types of natural products



Microwave assisted extraction (MAE):

is a process of using **microwave** energy to heat solvents in contact with a sample in order to partition analytes from the sample matrix into the solvent.

- Microwaves generate heat by interacting with polar compounds such as water and some organic components in the plant matrix following the ionic conduction and dipole rotation mechanisms.
- The transfers of heat and mass are in the same direction in MAE, which generates a synergistic effect to accelerate extraction and improve extraction yield.
- Application of MAE provides many advantages, such as increasing the extract yield, decreasing the thermal degradation and selective heating of vegetal material. It reduces the usage of organic solvent.
- There are two types of MAE methods: solvent-free extraction (usually for volatile compounds) and solvent extraction (usually for non-volatile compounds).



Pulsed electric field (PEF) extraction:

PEF is the application of short time pulsed with high voltage into the food product placed between two electrodes, thus promoting the modification of membrane permeability and the increase of the extraction yield. Recently, PEF treatment has been applied in order to recover pigments from beetroot.

- Pulsed electric field extraction significantly increases the extraction yield and decreased the extraction time because it can increase mass transfer during extraction by destroying membrane structures.
- The effectiveness of PEF treatment depends on several parameters including field strength, specific energy input, pulse number and treatment temperature.
- PEF extraction is a non-thermal method and minimizes the degradation of the thermolabile compounds.

Enzyme assisted extraction (EAE):

The structure of the cell membrane and cell wall, micelles formed by macromolecules such polysaccharides and protein, and the coagulation and denaturation of proteins at high temperatures during extraction are the main barriers to the extraction of natural products. The extraction efficiency will be enhanced by EAE due to the hydrolytic action of the enzymes on the components of the cell wall and membrane and the macromolecules inside the cell which facilitate the release of the natural product. Cellulose, α -amylase and pectinase are generally employed in EAE.

References:

1. Zhang, Qing-Wen & Lin, Li-Gen & Ye, Wen-Cai. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Medicine. 13. 10.1186/s13020-018-0177-x.
2. Pradnya Ingle, et.al , (2019). “Techniques Adopted for Extraction of Natural Products ,Extraction Methods: Maceration, Percolation, Soxhlet Extraction, Turbo distillation, Supercritical Fluid Extraction,”. International Journal of Advanced Research in Chemical Science (IJARCS), 6(4), pp.1-12. DOI: [http://dx. doi.org / 10.20431/ 2349-0403.0604001](http://dx.doi.org/10.20431/2349-0403.0604001).
3. <https://en.wikipedia.org/wiki/Decoction>
4. <https://www.slideshare.net/AbarnaAbi1/soxhlet-apparatus>

MOLECULAR MODIFICATION IN DRUG DEVELOPMENT

Definition:

Molecular modification is chemical alteration of a known and previously characterized lead compound for the purpose of enhancing its usefulness as a **drug**.

This method of obtaining Drugs is also called as

- Molecular manipulation
- Method of variation
- Selective Process or Approach

In molecular modification, a well-established chemical substance of known biological activity is known as lead compound or prototype.

Importance:

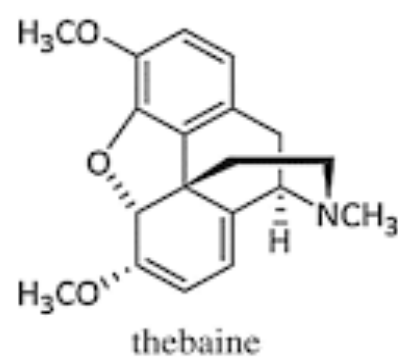
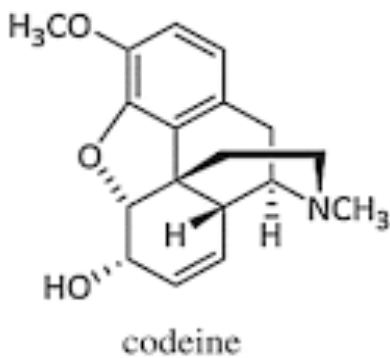
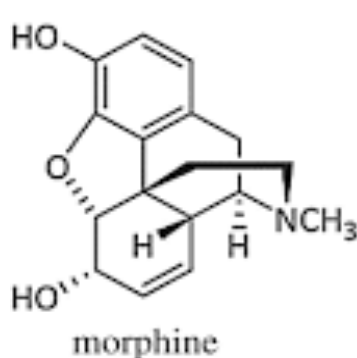
- enhancing its specificity for a particular body target site
- increasing its [potency](#)
- improving its rate and extent of [absorption](#)
- modifying to advantage its time course in the body
- reducing its [toxicity](#)
- changing its [physical](#) or [chemical properties](#) (like [solubility](#)) to provide desired features.

Example:

- molecular modification in nature include morphine, codeine, and the baine among the opium alkaloids;
- atropine, scopolamine, and cocaine among the tropine alkaloids
- testosterone, progesterone, and estradiol among the sex hormones.
- Through structure modification the medicinal chemist has developed local anesthetics from cocaine, useful agents from atropine and scopolamine

Opium Alkaloids

Opium, the sun-dried latex of the unripe fruit of *Papaver somniferum*, cultivated from early times for this drug, contains at least 23 alkaloids. Of the major alkaloids three—morphine, codeine, and thebaine—contain the morphinan ring system.



Morphine for over a century has been the most important agent for the relief of pain. Codeine, with its phenolic hydroxyl group protected by methyl, has about one tenth the analgesic activity of morphine, but as such has found its place in the relief of mild pain and as an antitussive agent.

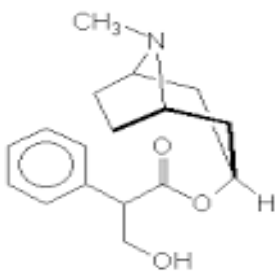
Thebaine, which differs from codeine by the addition of methylene groups and the removal of two hydrogen atoms, is neither an analgesic nor an antitussive. Instead, it resembles strychnine and brucine in its spinal convulsant properties. It is not used in medicine; it has utility, however, in the synthesis of codeinone derivatives, some of which are useful as analgesics. The chemist has unraveled the morphine-analgesic pattern and has gone

far beyond nature in his quest for an analgesic with the power of morphine

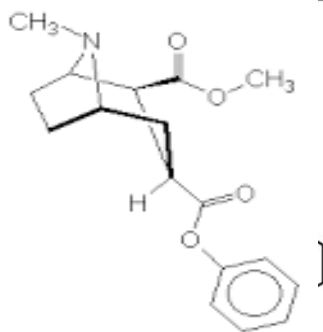
but without its liabilities.

Tropine Alkaloids

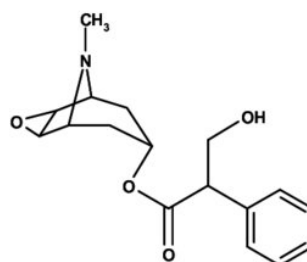
The tropine alkaloids are another illustration. Atropine, scopolamine, and cocaine are structurally related, each having the tropine nucleus. While atropine and scopolamine overlap in pharmacodynamic activities, cocaine has uniqueness in this series, being a topical anesthetic and a potent addicting agent.



Atropine



Cocaine



Scopolamine

In this case, nature's molecular modification cannot be attributed to a special metabolism within a species. Cocaine and atropine are not related botanically. They originate from different plants. Extension of Tropane Alkaloids. Here again, medicinal chemists have gone

beyond nature by molecular modification. While cocaine possesses topical anesthetic activity but no local infiltration value as an anesthetic, a number of very useful, local anesthetic agents such as procaine and lidocaine have been derived from our knowledge of the structure of cocaine. Chemists have broadened the scope of usefulness of the cocaine structure.

ATROPINE and SCOPOLAMINE

- ANTISPASMODICS
- ANTIULCER
- ANTIDIARRHEAL
- ANTIPARKINSONISM
- MYDRIATICS
- CNS DRUGS

COCAINE

- LOCAL ANESTHETICS

Atropine and its related oxide, scopolamine, have given rise to a number of important agents useful in the treatment of a number of disease conditions. The drugs applicable to each class of pharmacodynamic activity are all anticholinergics and block the parasympathetic nervous system

with varying degrees of specificity. By molecular modification, it has been possible to produce a series of compounds having qualitative effects resembling cutting of the parasympathetic nerve to a particular organ.

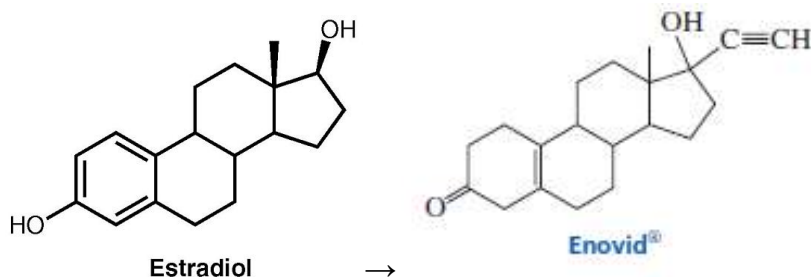
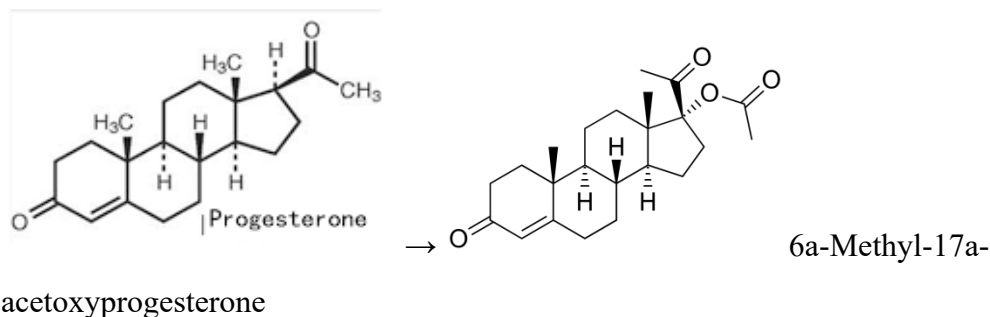
Sex Hormones

Nature is at its best in the sex hormone field. Here are three hormones containing steroid nuclei, each of which has a very specific pharmacological activity and a very specific role in sex physiology. Testosterone, the testicular hormone, is identified as a male hormone.

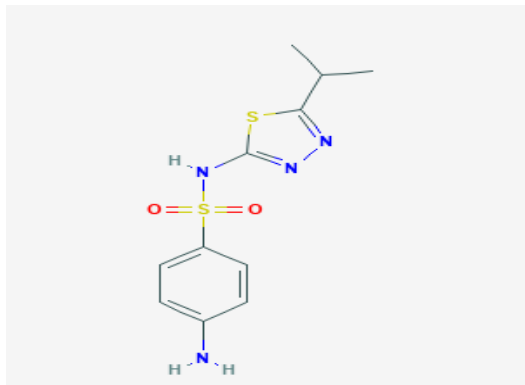
Estradiol (17β), which differs from testosterone only by loss of a methyl group and aromatization of ring A, is the ovarian hormone. Progesterone is the pro gestational hormone and is essential for fertilization and maintenance of pregnancy. Once again medicinal chemists, by further alteration of structures, have made more potent oral and more useful progestins. 6a-Methyl-17a-acetoxypregesterone is at least 100 times as potent as progesterone by the oral route.

Norethynodrel (Enovid), also derived through molecular modification of the estradiol type structure, is the first significant orally active an ovulatory agent and oral contraceptive. Up to now, I have placed emphasis on molecular architecture in nature and how medicinal chemists elaborated on nature. Let us now turn to some

structures discovered in chemical laboratories, structures conceived and synthesized by man.



Development of Anti-diabetic Drug



5 isopropyl 2 sulphanilamide 1,3,4 thiadiazole

In 1942, Patients with typhoid fever under treatment with an isopropyl-thiadiazole derivative of sulphanilamide, 5 isopropyl 2 sulphanilamide 1,3,4 thiadiazole and problems associated with this drug were

- Weakness and dizziness
- Stimulated Insulin Release
- Was ineffective in the absence of functional islets

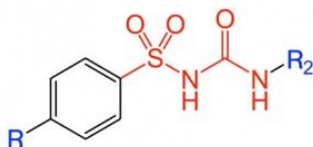
1st Generation anti-diabetic

Study show that hypoglycemic action dependent primarily upon the urea like structures formed by a N & C atoms of the thiadiazide ring and the N-atom of the sulphonamide grouping.

Basic Structure for Activity:

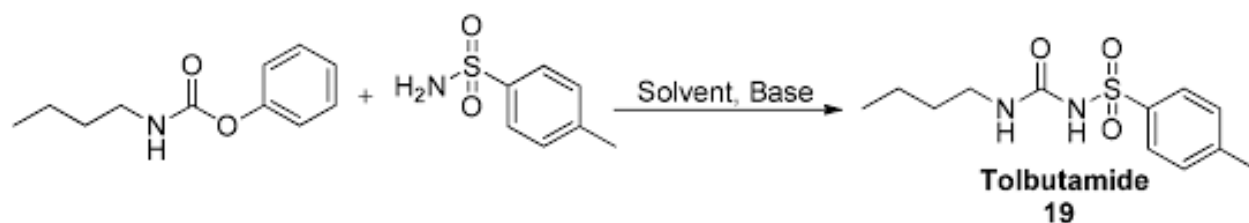
Ar-SO₂-NHCONH-R (Ar-Aryl group) (R-Alkyl Group)

Sulphonyl Ureas



Drug	Structure
Carbutamide	R1: NH ₂ , R2:C ₄ H ₉

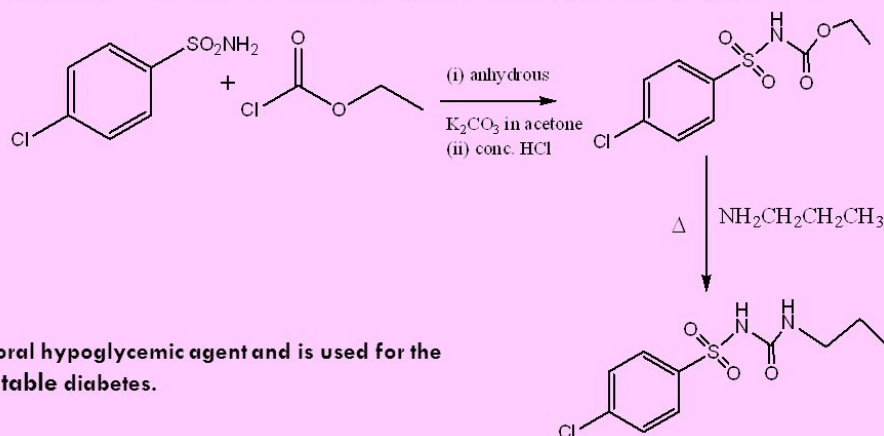
Tolbutamide	R1:CH ₃ , R2: C ₄ H ₉
Chlorpropamide	R1:-Cl , R2:C ₃ H ₇



Synthesis of Tolbutamide

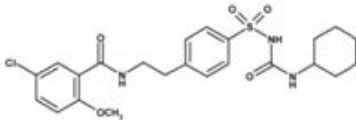
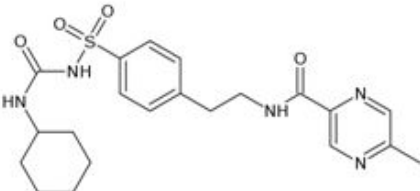
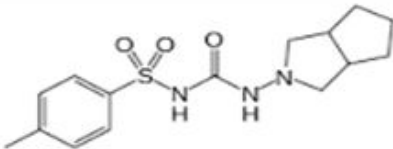
CHLORPROPAMIDE

(ii) **Preparation** : Chlorpropamide is prepared by refluxing p-chlorophenyl sulphonamide (I) with ethyl chloroformate (II) in the presence of anhydrous K₂CO₃ in acetone. Ethyl-(p-chlorophenyl sulphonyl)-carbamate (III) thus formed is heated with n-propyl amine to get chlorpropamide.

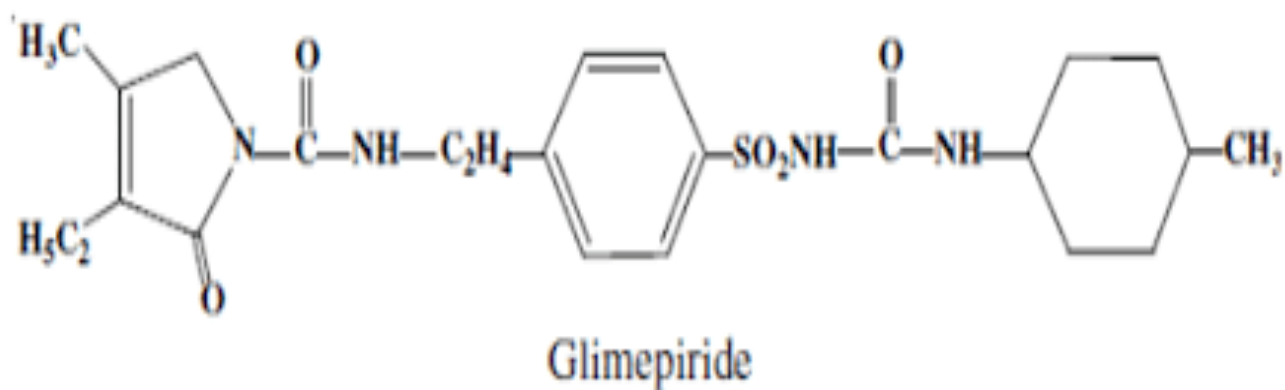


Synthesis of Chlorpropamide

2nd Generation Anti-Diabetic

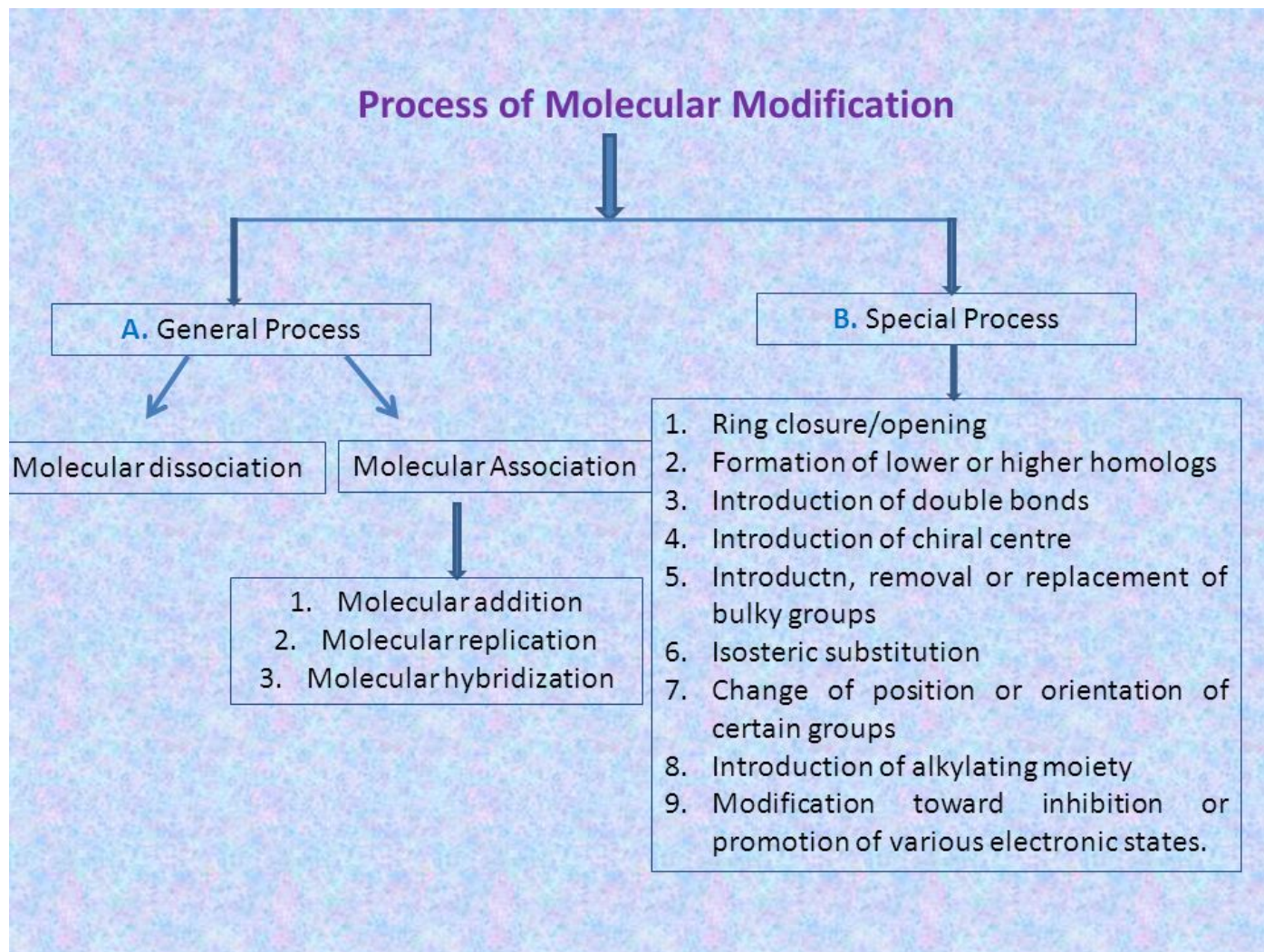
Drug	Structure
Glyburide	 Glyburide
Glipizide	
Gliclazide	

3rd Generation Anti-Diabetic



Glimepiride is structural analogue of 2nd generation of sulphonylureas in which amide moiety of the 4-aryl substituent has been replaced with the heterocyclic

Uredio group. This structural modification is reported to result binding to different region of beta cell receptor and in enhanced potential and increase duration. Most Potent- Lowest dose of sulfonylureas.

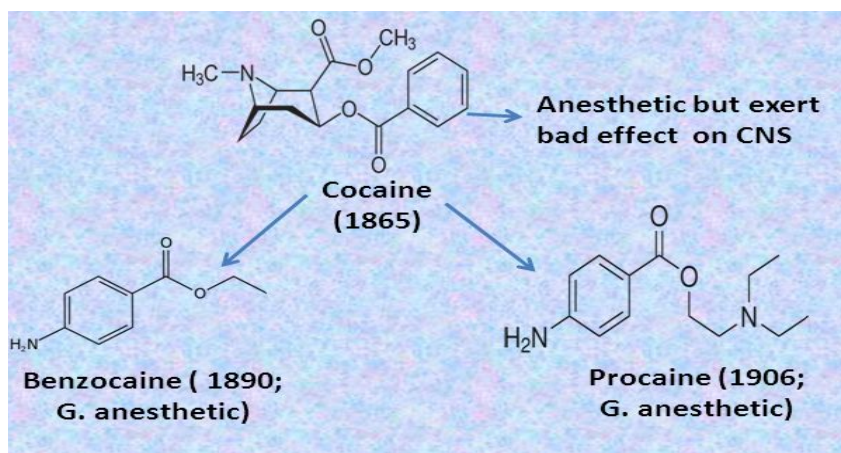


Process of Molecular Modification:

General Process

1. Molecular Dissociation

It is systemic synthesis and evaluation of simpler analog of the lead compound. These analogs are partial or virtual replicas of the prototype drugs; which is actually natural product of very intricate chemical structure.



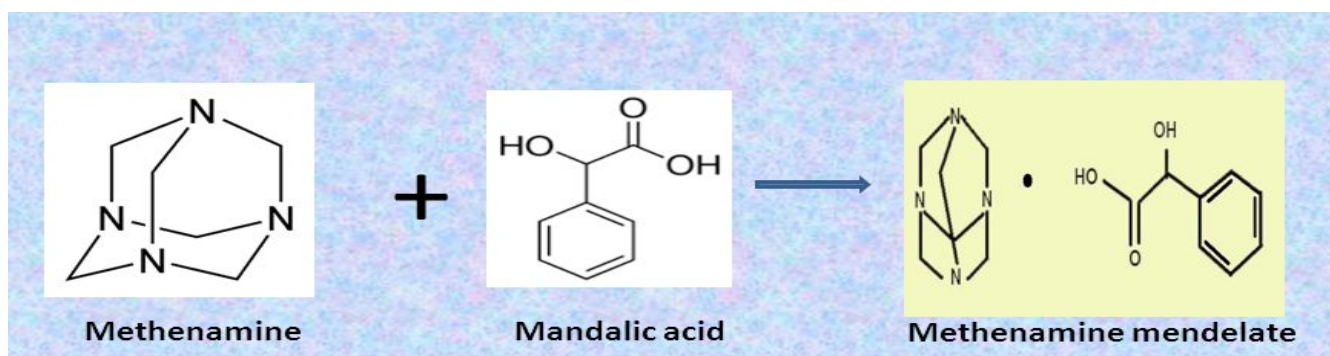
2. Molecular association:

It is synthesis and evaluation of one or more & more complex analog of the prototype. Three main type of association has been distinguish.

- I. Molecular addition
- II. Molecular replication
- III. Molecular hybridization

➤ Molecular addition:

Addition of different moieties through weak forces, such as electrostatic attraction and H-bonding.



➤ Molecular Replication:

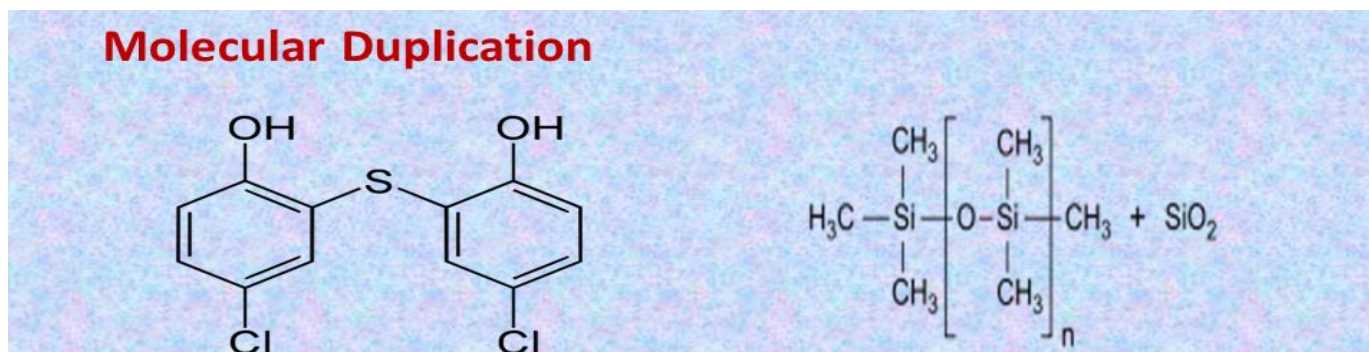
Addition of identical moieties through covalent bonding.

Duplication: association of two moieties

Triplication: association of three moieties

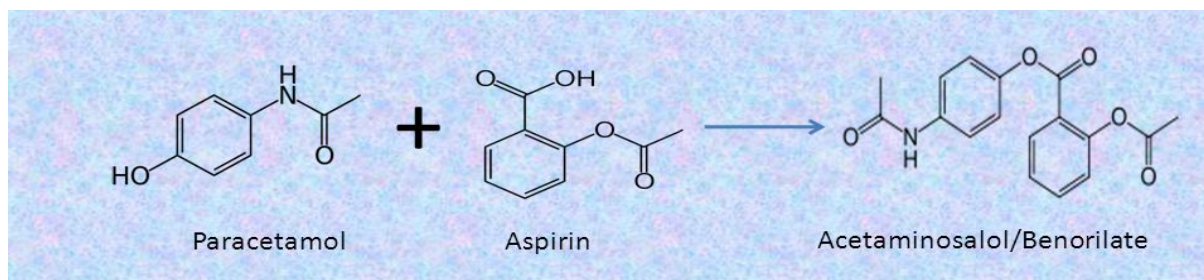
Tetraplication: association of four moieties

N-plication: association of n moieties



➤ **Molecular Hybridization:**

Association of different or mixed moieties through covalent bonding.



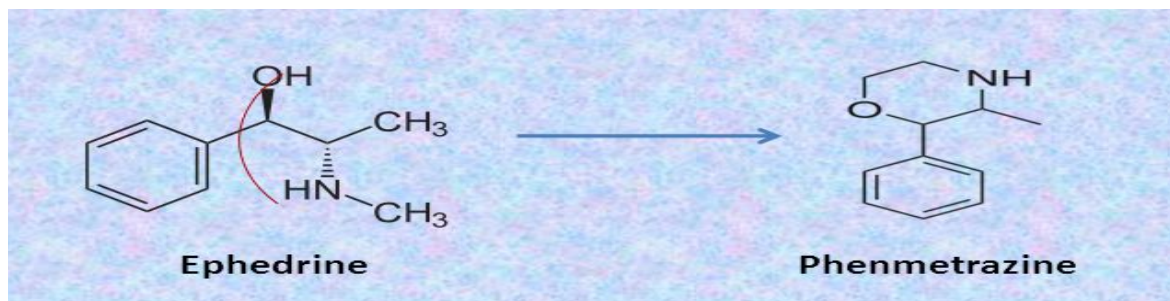
Special Process

Two types

1. Alternation which increase or decrease the dimension and flexibility of a molecule
2. Alternation of physical and chemical properties through the introduction of new groups or the replacement of different moieties by different ones.

Alternation which increase or decrease the dimension and flexibility of a molecule

● **Ring Closure or opening**



- **Formation of lower or higher homologs**

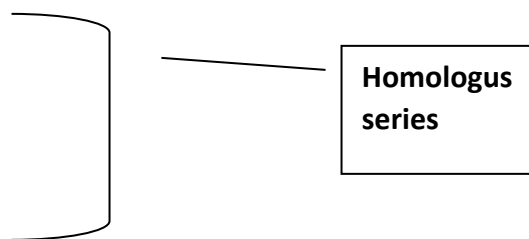
Example

CH₄

CH₃-CH₃

CH₃-CH₂-CH₃

CH₃-CH₂-CH₂-CH₃



In alkanes & polymethylene series, the following general types of changes were observed

- ✧ Activities increase regularly until a max is reached, higher member being almost or entire inactive

Example:

Structurally non specific drug

- ✓ General anesthetic
- ✓ Volatile insecticide
- ✓ Disinfectants

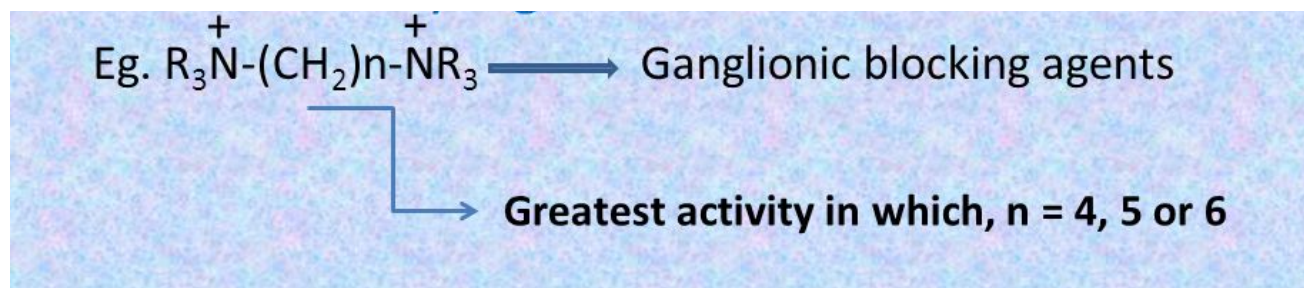
Structurally Specific drug

Local anaesthetic

- ✧ Activities increase irregularly, reaches a maximal value and then decrease again irregularly

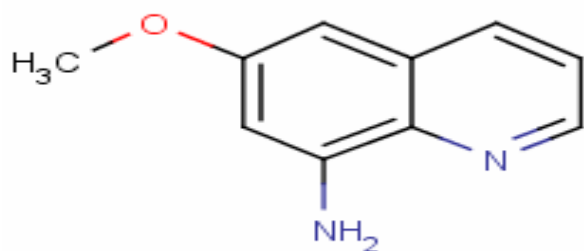
Example: Benzylic esters & Atropinic properties

- ✧ Activities increase or decrease reaches a relatively high or low value and then remain constant for a few or many higher member.

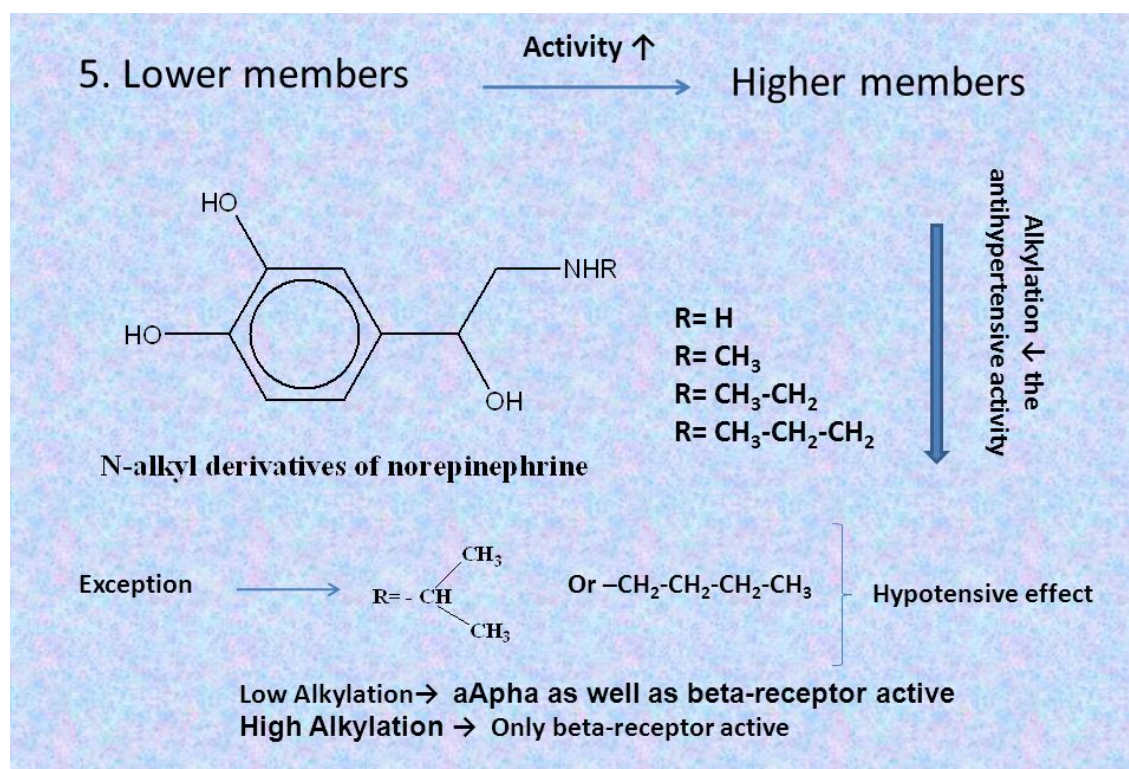


- ✧ Activity alternates, compounds having odd number of carbon item have more active than neighbouring member with an even number of carbon item and vice versa

Example: antimalarial from 6-methoxy-8-aminoquinoline



6-methoxy-8-aminoquinoline



Application:

- ✓ Greater probability of homologs, analogs having pharmacological properties similar to those of the prototype than the compound selected or synthesized random.
- ✓ Possibility of obtaining pharmacologically superior products.
- ✓ Likelihood of the production of new drug of being more economical.
- ✓ Data gather may help to elucidate SAR
- ✓ Use of same method of biological assay use for the prototype
- ✓ To obtain drugs having more desirable properties than the prototype in terms of potency, specificity, duration of action, ease of application, administration, stability and cost of production

References:

- https://en.wikipedia.org/wiki/Molecular_modification
- <https://pubs.acs.org/doi/pdf/10.1021/ba-1964-0045.ch001>
- <https://pubs.acs.org/doi/abs/10.1021/ba-1964-0045.ch001>
- <https://slideplayer.com/slide/10752607/>

Evaluation of Intermediate Products

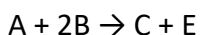
Intermediate Products

An intermediate is a substance formed during a middle step of a [chemical](#) reaction between [reactants](#) and the desired [product](#).

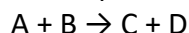
An intermediate or reaction intermediate is a substance formed during a middle step of a chemical reaction between [reactants](#) and the desired [product](#). Intermediates tend to be extremely reactive and short-lived, so they represent a low concentration in a chemical reaction compared with the amount of reactants or products. Many intermediates are unstable [ions](#) or free radicals.

Examples:

- In the chemical equation



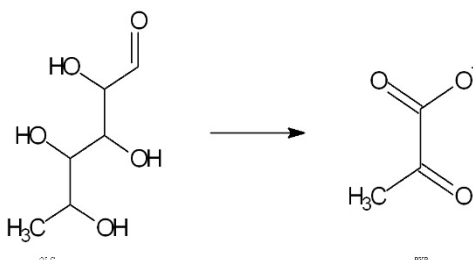
The steps could be

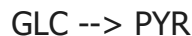


The D chemical would be an intermediate chemical

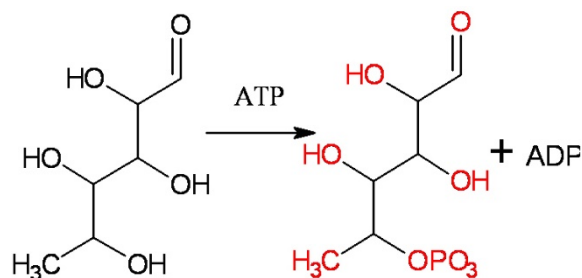
Most chemical reactions are [stepwise](#), that is they take more than one [elementary step](#) to complete. An intermediate is the reaction product of each of these steps, except for the last one, which forms the final [product](#).

The overall balanced equation of glycolysis is





Glucose (GLC) is the beginning reactant and is converted through a series of steps to form the final product, pyruvate (PYR). However, the intermediate “appears in the mechanism of the reaction but is not in the overall balanced equation.” One example of an intermediate in a glycolysis step reaction is the conversion of:



Glucose --> Glucose-6-phosphate

RED = intermediate

The first step of glycolysis yields glucose-6-phosphate from a glucose molecule. However, in the overall balanced reaction of glycolysis, there is no presence of glucose-6-phosphate written because it exists for a short time before it is consumed in the next reaction.

Properties

Many intermediates are

- ❖ short-lived
- ❖ high energy
- ❖ highly reactive

Reaction intermediates are often free radicals or unstable ions.

Oxidizing radicals (COOH and OH) found in combustion reactions are so reactive that a high temperature is required to constantly produce them, in order to compensate their disappearance, or the combustion reaction will cease.

Types of Reaction Intermediates

1. Carbanions

In organic chemistry, a carbanion (referred to as a carbonium ion in some texts) is a reaction intermediate in which there is a negative one charge located on a carbon atom. Carbanions are formed by treating an organic compound with a VERY strong base. Consider as an example the reaction of butane with a base. When the base pulls off a hydrogen atom from butane, a carbanion is formed.

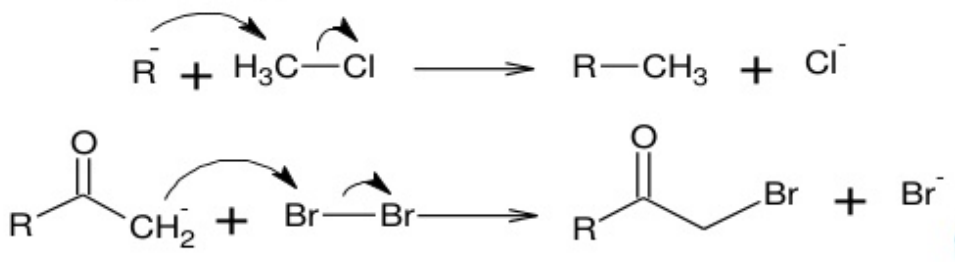


Reaction of butane with a base to form a carbanion intermediate

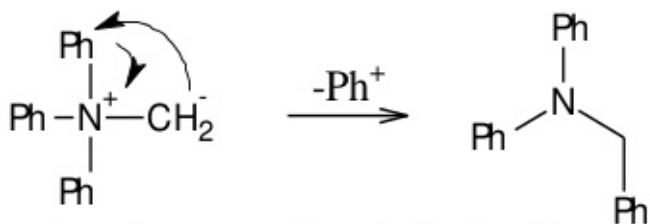
Carbanions are extremely reactive, and once they are formed in a chemical reaction they typically don't last very long. Usually they go on further to react with a positive species in the reaction to form the final product of the reaction. This should make sense to us because we are forming a negatively charged intermediate, meaning it will be attracted to something else that has some sort of positive character to it.

Reaction of Carbanion:

- Carbanions often act as nucleophiles to react with electrophilic species.



- In rare cases, the carbanions may undergo cationotropic 1,2-shift to give rearranged products, for eg;



- The carbanions may be oxidized to free radicals. For eg;



Stability:

Stability is directly related to the strength of the conjugate acid.

The weaker is the acid the stronger the base strength and lower the stability of carbanion
 Carbanion is stable by the field effect if any heteroatom is connected with the carbanion atom, provided that the heteroatom bears a positive charge in at least one important conical form

Factors determining the stability and reactivity of carbanion :

The inductive effect: Electronegative atom adjacent to the charge will stabilize the charge

Hybridization: of the charge bearing atom. the greater the sp^3 character of the charge bearing atom the more stable the anion.

The extent of conjugation of anion: resonance effect can stabilize the anion.

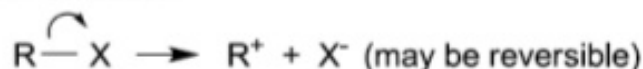
2. Carbocation:

a carbocation is positively charged carbon atom. It may be carbenium ion (trivalent positive species) CH_3^+ , $C_2H_5^+$ or carbonium ion (Pentavalent positive species)

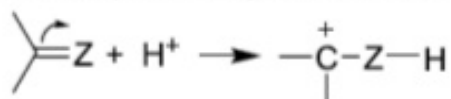
II. THE GENERATION AND FATE OF CARBOCATIONS

Two general ways to form carbocations:

i. A direct ionization:



ii. Addition of a positive species to an unsaturated system:

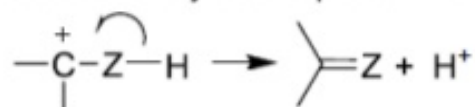


The reaction of carbocations:

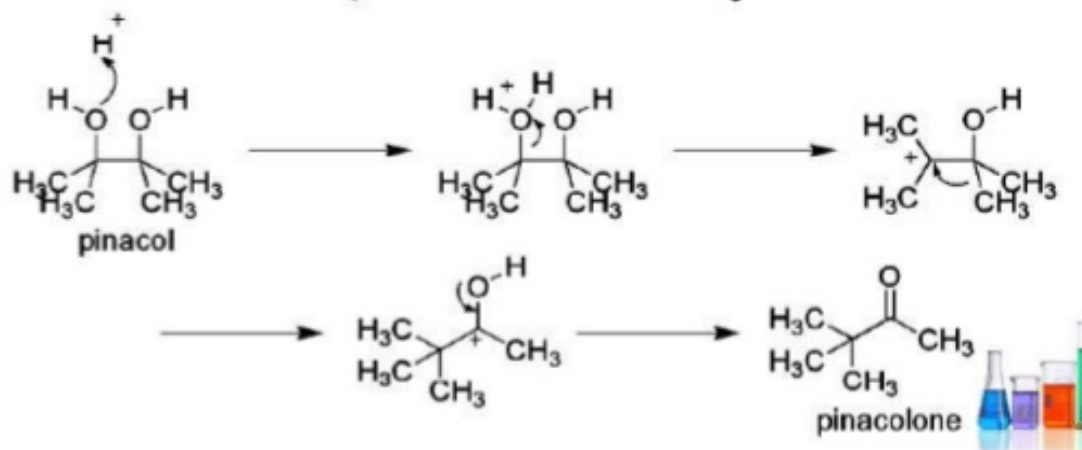
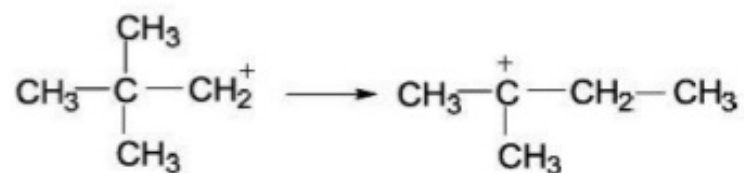
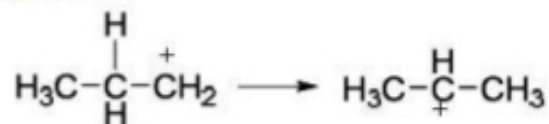
i. Combination with a species possessing an electron pair.



ii. The carbocation may lose a proton from the adjacent atom.

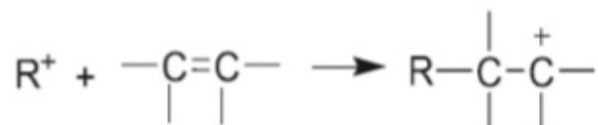


iii. Rearrangement

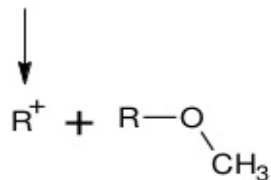
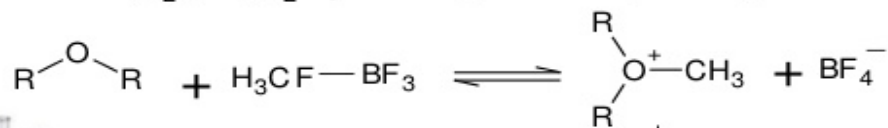
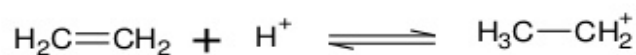
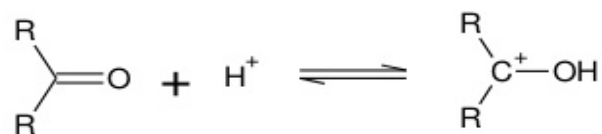


iv.

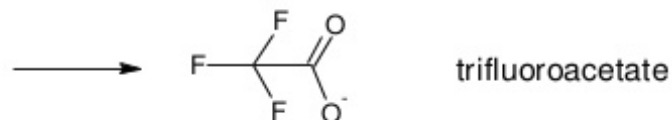
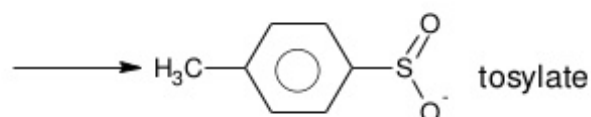
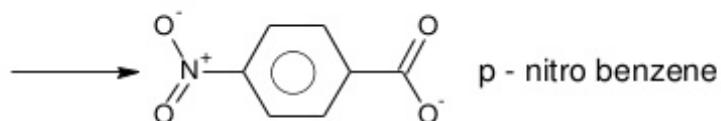
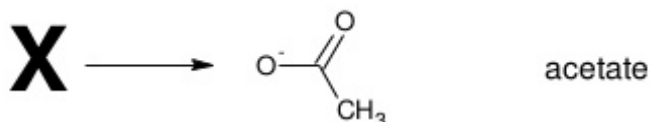
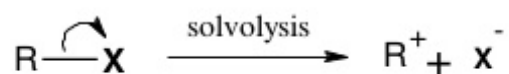
Addition



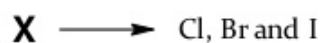
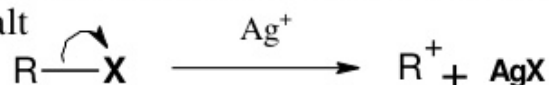
By the addition of a cation to a neutral molecule.



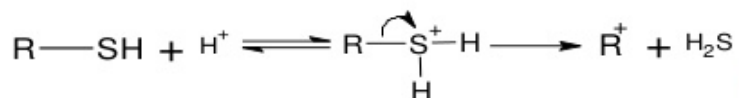
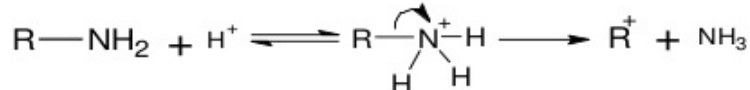
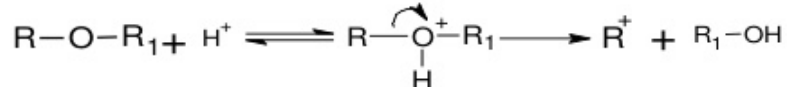
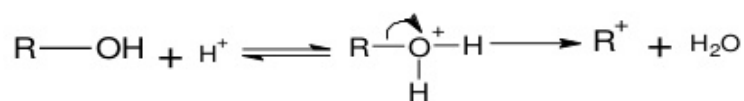
V. By the heterolytic fission of a C-heteroatom bond.



Vi. By the heterolysis fission of a C - heteroatom bond to form onium salt



Onium salt Carbocation

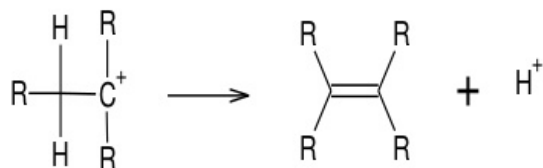


Reaction of Carbocation:

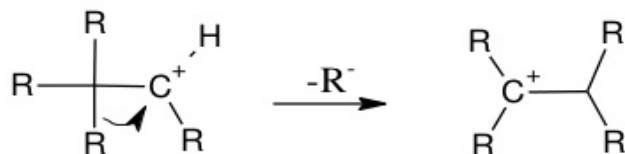
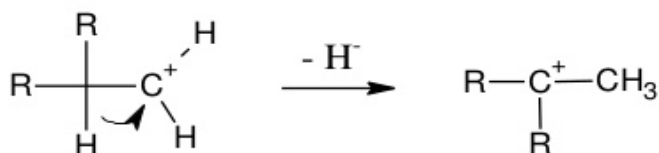
- R^+ cation act as an electrophile to react with nucleophiles. For Eg;



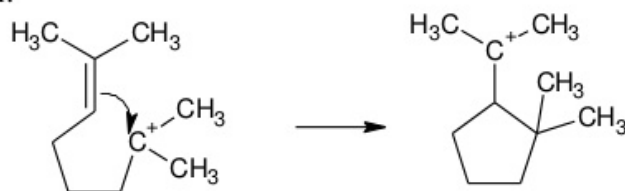
- Some carbocations act as Bronsted acid to lose a proton. For Eg;



- 1° or 2°-carbocation often undergo Wagner –Meerwein rearrangement by an anionotropic 1,2- shift of a hydride or an alkyl anion. For Eg;



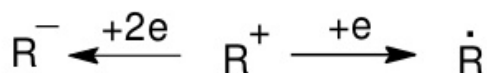
- Internal alkylation of a C=C bond sometimes may take place with a carbocation.



- Fragmentation of carbon chain of carbocation is also known.



- Carbocations can be reduced to a free radical or carboanions by cathodic reduction.



Stability:

In solution, the carbocation may be free or it may exist as may be as an ion pair.

Simple alkyl cation:

Tertiary > secondary > Primary

The most stable of all alkyl cation is tertiary butyl cation.

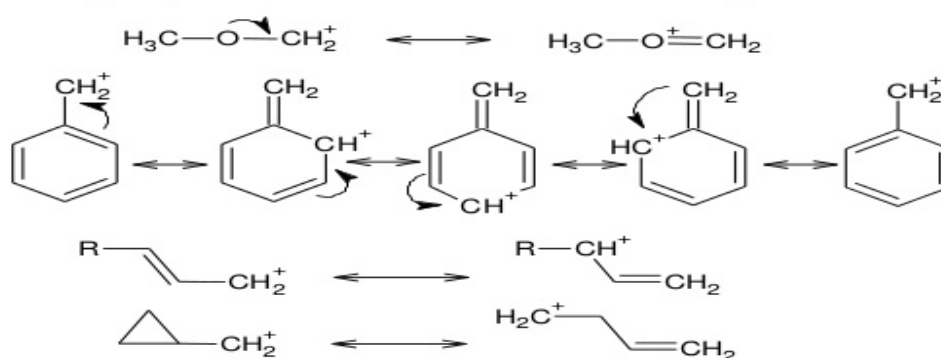
Methane, ethane and propane treated with superacid also yield tertiary butyl cation as a main product.

The electron donating effect of alkyl groups increase the electron density at the charge bearing carbon, reducing the net charge on the Carbon, and in effect spreading the charge over the α carbon.

Hyperconjugation:

Tertiary > secondary > Primary (stability)

- +R groups stabilize the carbocations. For eg;



- Some carbocations are stabilized due to aromatization. For eg;



Cyclopropenyl cation is stable due to aromatization



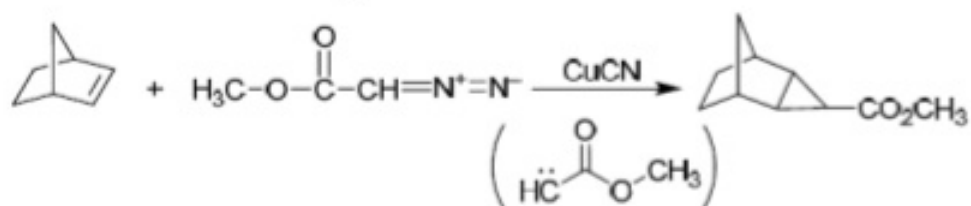
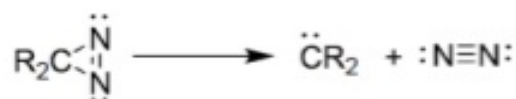
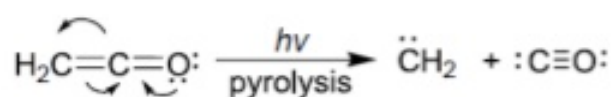
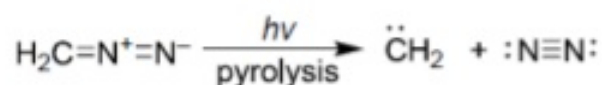
3. Carbene:

a carbene is a highly reactive species containing a carbon atom with six valence electrons and having the general formula $\text{RR}'\text{C}$.

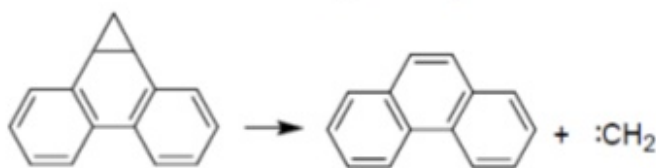
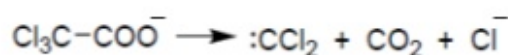
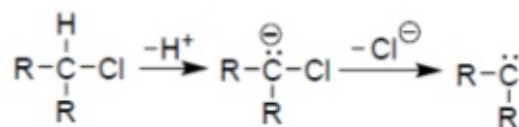
Triplet carbene is stable in the gaseous state while singlet carbene occurs mostly often in aqueous medium.

Generation and fate of Reaction of Carbene:

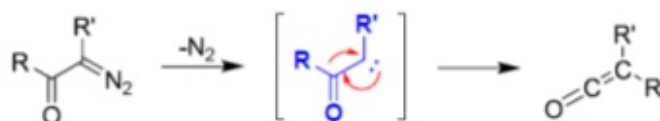
- Disintegration of diazoalkanes and their analogs, via photolytic, thermal, or transition metal (Rh, Cu)-catalyzed routes.



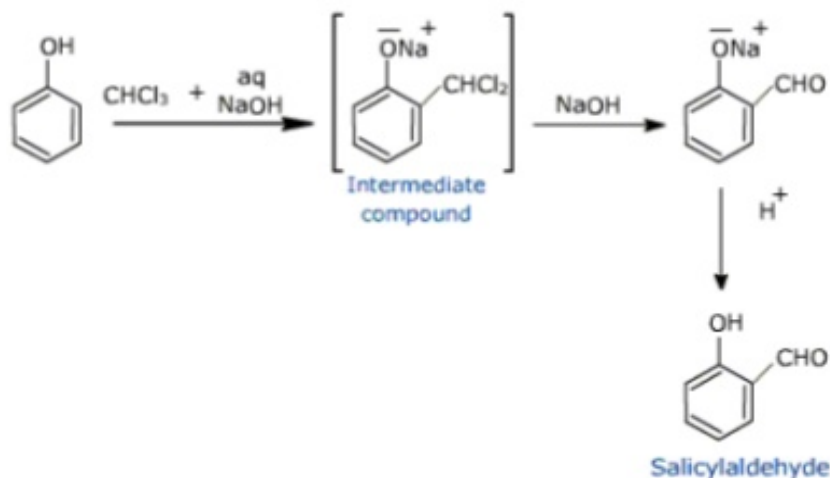
- Base-induced elimination



- Carbenes are intermediates in the **Wolff rearrangement**.

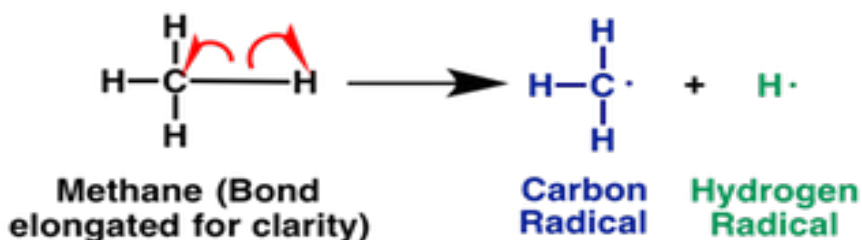


- Carbenes being the electrons-deficient species may take part in electrophilic aromatic substitution reactions. For eg; in Riemer –Tiemann reaction.



4. Free Radicals

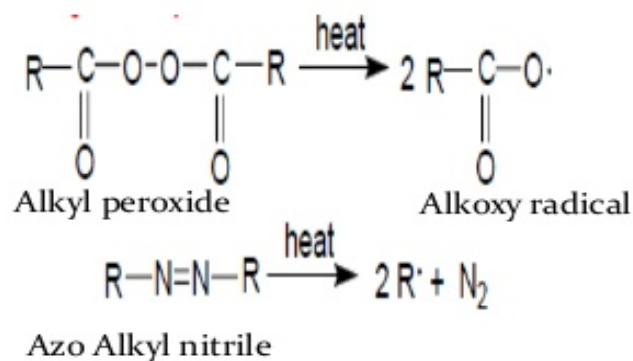
Another common class of reaction intermediates are free radicals. Free radicals contain a single, unpaired electron. They result when a covalent bond (a bond composed of two electrons) is broken and each atom takes one electron from the bond. For example, when a carbon-hydrogen bond in methane is broken, one of the electrons from the bond goes to carbon and the other electron goes to hydrogen. Notice that when we are representing free radicals, we use single dots on the atom in which the radical is located.



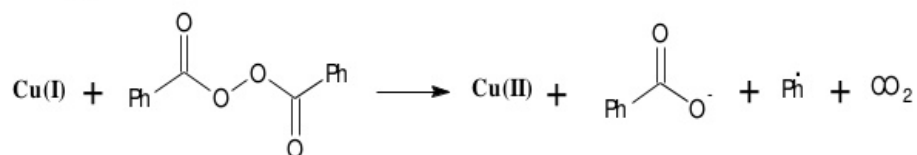
Formation of a carbon radical and a hydrogen radical from methane

Generation and fate of Reaction of Free Radical:

- Thermolysis or photolysis of organic peroxides and azo compounds generates free radicals.

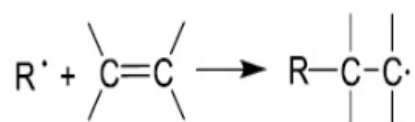


- Bimolecular redox reactions also generate free radicals. For eg;

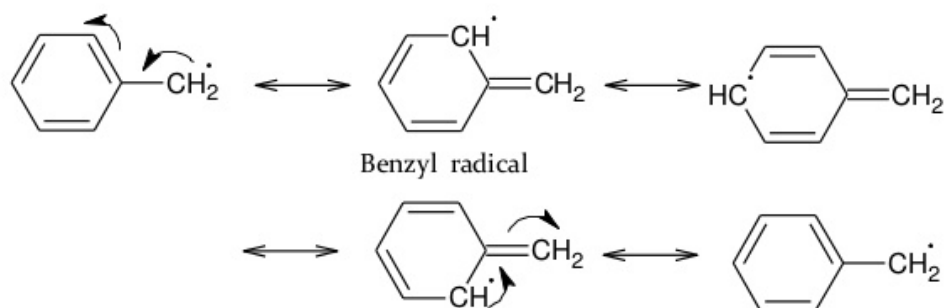


Stabilization of free radicals:

Resonance effect due to conjugation stabilizes the free radicals.

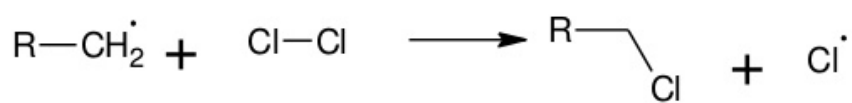
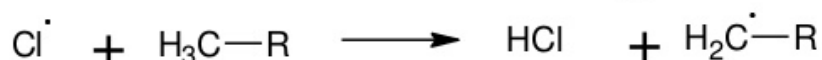
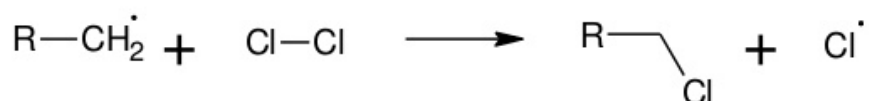
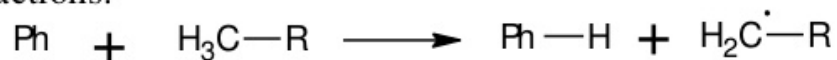


- For aromatic

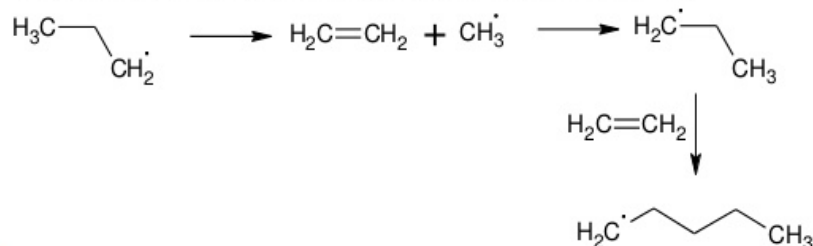


Reaction of Free Radical:

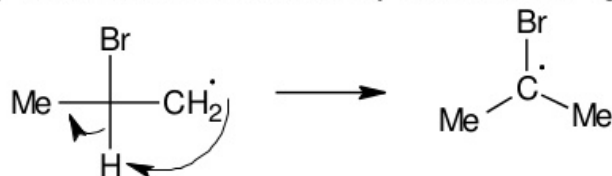
- Free radical often take part in radical-propagating reactions.



- In some cases, the free radical itself may be fragmented and trigger the propagation of a chain reaction. For eg;



- Suitably substituted free radical may isomerize. for eg;

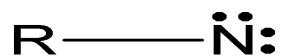


- Disproportionation and radical coupling are the common reactions of the termination of free radicals. For eg;



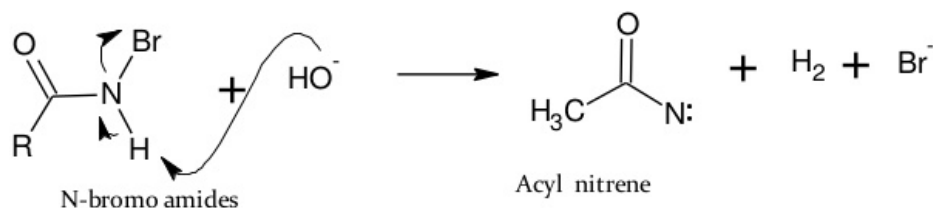
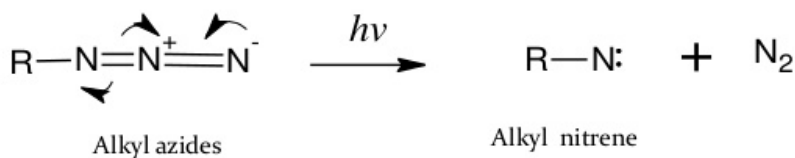
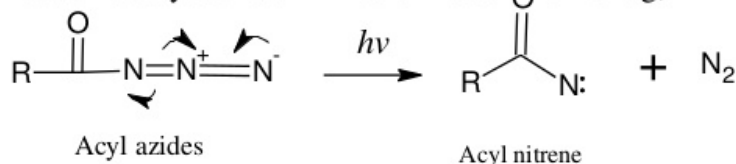
5. Nitrenes

These are the neutral reaction intermediate where the central nitrogen atom is electron deficient and has sextet of electron.

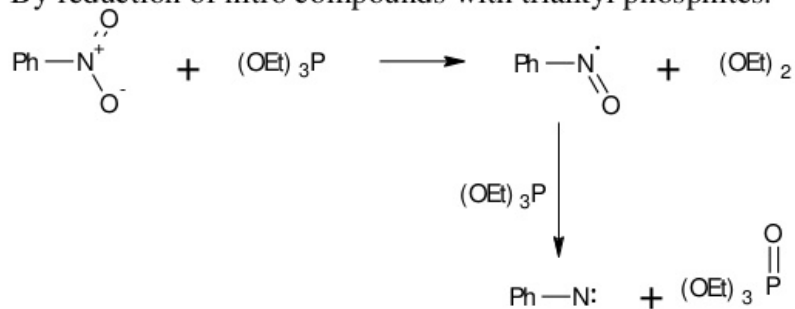


GENERATIONS OF NITRENES

- Like carbenes, these are also generated by protolytic, thermal, or base-catalysed α -elimination reactions. For eg;

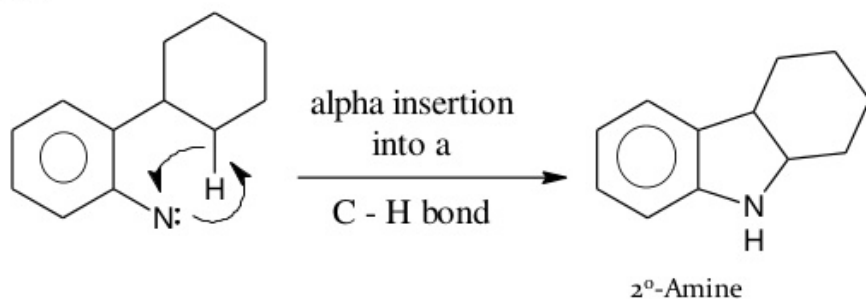


- By reduction of nitro compounds with trialkyl phosphites.

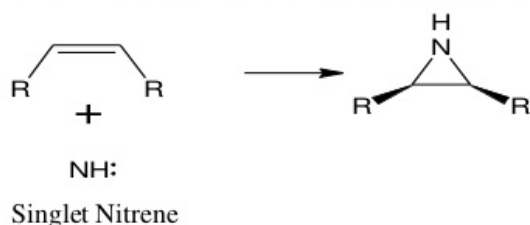


Reaction of Nitrene:

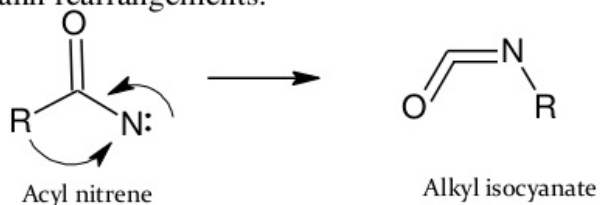
- Singlet nitrene undergoes a σ -insertion to give 2° - amines. For eg;



- π - Insertion of nitrenes into a C=C bond gives aziridines.



- Acyl nitrenes undergo skeletal rearrangement to give alkyl isocyanates. This rearrangement is involved in Curtius and Hoffmann rearrangements.



Screening of synthesis intermediates

As synthesis intermediates are chemically connected to final products, and as they often present some common groupings with them, it is not inconceivable that they share some pharmacological properties.

Examples

- Inhibitors of the enzyme dihydrofolate-reductase such as methotrexate are used in the treatment of leukaemia. During the search for methotrexate analogues a very simple intermediate, mercaptopurine, was also submitted to testing. It proved to be active but relatively toxic.

- ▶ Subsequent optimization led to azathioprine, a prodrug releasing mercaptopurine in vivo. Azathioprine was found to be more potent as an immunosuppressive agent than
- ▶ previously used corticoids and was systematically used in all organ transplantations until the advent of cyclosporine.
- ▶ Another intermediate in this series, allopurinol, inhibits xanthine-oxidase and is therefore used in the treatment of gout

One of the important example is the tuberculostatic semicarbazones: they were initially used in the synthesis of antibacterial sulfathiazoles. Subsequent testing of isonicotinic acid hydrazide, destined for the synthesis of a particular thiosemicarbazone, revealed the powerful tuberculostatic activity of the precursor which has since become a major antitubercular drug (isoniazide).

Subsequent testing of isonicotinic acid hydrazide, destined for the synthesis of a particular thiosemicarbazone, revealed the powerful tuberculostatic activity of the precursor which has since become a major antitubercular drug (isoniazide).

Laboratory Synthesis of Pharmaceutical Intermediate Products

- ▶ synthesis of pharmaceutical intermediates in the group of antibiotics and anticancerogenic drugs
- ▶ The technology has been developed to obtain (2R,3S)-3-phenylisoserine hydrochloride - an amino acid, witch is a main semi product in the synthesis of anticancer drug - Paclitaxel.
- ▶ A process has been developed to extract natural dyes like green dyes (chlorophyll, chlorophyllin) from spinach and red dyes (β -carotene) from carrot roots.

Reference:

- https://en.wikipedia.org/wiki/Reaction_intermediate
- [https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_\(Physical_and_Theoretical_Chemistry\)/Kinetics/Rate_Laws/Reaction_Mechanisms/Reaction_Intermediates](https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Kinetics/Rate_Laws/Reaction_Mechanisms/Reaction_Intermediates)
- <https://www.thoughtco.com/definition-of-intermediate-605251>
- <https://study.com/academy/lesson/what-is-a-reaction-intermediate-definition-examples.html>
- <https://www.slideshare.net/saiswathivarma/reactions-intermediate>
- Advance Organic Chemistry BS Bahal
- Textbook of Organic Chemistry By O.P Agarwal
- Organic Chemistry by R.T Morrison and R.N Boyed

