Unit
1

Drug Discovery and Development

INTRODUCTION

Humans used natural drugs before the emergence of written history. They used the constituents of plants as poisons for darts and arrows (hunting) and also used euphoriants in ritual functions for their psychoactive properties. The original documentation in natural drugs started from Sumerians and Akkadians in the 3rd century and Egyptian Ebers Papyrus in 1600 BC. The other documentations are Materia Medica and Pliny, the Elder in the first century AD, and by Galen in the second century. The pieces of evidence also exist with the same in Chinese traditional medicines and ayurvedic medicines as records. The documentation of medicinal plants in herbals 200 started 500 years ago. But the drugs from the laboratory started approximately time years ago, with the isolation of morphine from opium. Prompted by these inventions that time onwards organic chemistry was developed and considered as a separate scientific discipline. In the 19th century, alkaloidal drugs like cocaine, atropine, caffeine, quinine, nicotine and strychnine were isolated. In the mid of 20th century, many pharmacologically active alkaloids were purified from various sources and derivatized from the ergot fungus. The isolation of penicillin and its structurally related compounds developed the golden era of antibiotics to treat infectious diseases. Terrestrial microorganisms have been found and contributed to drug discovery and many compounds used now in a wide range of drugs for human ailments.

Boundless efforts were undertaken to discover and develop drug molecules from marine fauna and floura because oceans occupy 70% of the earth surface. Actions on these natural agents sparked off chemists to synthesize thousands of analogues to improve the natural products. Most of these procedures were carried out on a trial and error basis only.

In the present era, efficient drug design and discovery is achieved by adopting various advanced techniques like computational studies and combinatorial chemistry. The discovery of newer drug molecules also increased continuously and simultaneously by the different biotechnological processes.

The drug discovery process is very extensive and time-consuming. The average time required to introduce a new drug into the market ranges from 12–15 years at the average cost of 600–800 million. For obtaining one drug molecule in a market, approximately 10,000 compounds are tested in animal models. It may give less than 10 clinical candidates and are screened in human clinical trials.

Most of the drugs discovered are the prototype compounds constitute desired biological activities with many undesirable characteristics such as adverse effect, high toxicity, and ADME difficulties. Hence, the drug candidate is developed by altering its chemical structure by synthesis. The modified drug candidate contains extensive biological activity with less undesirable side effects. Once the NDA (new drug application) is submitted to the FDA, it requires tons of data to show the efficacy and safety of the drug to approve for commercial use.

Steps Involved in the Drug Discovery Process

The following are the various steps involved in the drug discovery and development process.

1. Selection of a disease
2. Selection of drug target
    a. Drug targets discovery
    b. Target specificity and selectivity between species
    c. Target specificity and selectivity within the body
    d. Targeting drugs to specific tissues and organs
3. Bioassay identification
    a. Bioassays
        i. *In vitro* tests
        ii. *In vivo* tests
    b. High throughput screening
        i. Protein-based biochemical assays
        ii. Cell-based assays
    c. Screening by nuclear magnetic resonance (NMR)
4. Identification of lead
    a. Identification of lead compounds from natural products or natural sources:
        i. Lead from plants
        ii. Lead from microorganisms
        iii. Lead from marine world
        iv. Lead from animals
        v. Lead from venoms and toxins
    b. Random screening
    c. Nonrandom screening or targeted or focused
    d. Drug metabolism studies
    e. Clinical observation
    f. Rational approaches to lead discovery
    g. Computer-aided drug design
    h. Serendipity and prepared mind
    i. Computerized searching of lead compounds of structural databases
    j. Lead design by NMR
5. Isolation and purification of lead compound
6. Determination of the chemical structure of the lead compound
7. Structure–activity relationship determination
    a. Binding role of hydroxyl groups
    b. Binding role of amino groups

   c. Binding role of double bonds
   d. Binding role of ketones
   e. Binding role of amines
   f. Binding role of amides
   g. Isosteres
   h. Testing procedure
8. Pharmacophore identification
9. Target-oriented drug design
10. Pharmacokinetic studies in drug design
11. Patenting of drug molecules
12. Drug metabolism studies
   a. Phase I or functionalization reactions
   b. Phase II or conjugation reactions
13. Designing the manufacturing process
14. Clinical trials implementation
   a. Phase I studies
   b. Phase II studies
   c. Phase III studies
   d. Phase IV studies
15. Marketing of drug

Now we are discussing all the procedures in detail.

Selection of a Disease

The first step of the drug design process is selecting a disease target. The pharmaceutical companies first decide the disease to which they can develop the drug. Hence, they preferably concentrate on the disease targets where there is a need for improved drugs or new drugs. In this case, they consider the economic as well as medical factors. A considerable amount of investments are required to develop a new drug. Hence the pharmaceutical industries must guarantee or secure that they acquire good financial return benefit for their investment. As a result, research projects are based on 'first world' diseases since this can be a risk (financial/time) to afford new drugs. The main area of research is focused on diseases like cancer, AIDS/HIV, tuberculosis, obesity and CVS ailments. A fewer research projects are focused on tropical diseases that threaten or reduce the life cycle of the thirties or forties. Selection of disease target is an essential step for developing drugs in pharmaceutical companies.

Selection of Drug Target

Once the particular disease target is selected, the next level is identifying a specific target of the drug. The target may be a receptor, enzyme or nucleic acid. The first step of the target selection is the basic knowledge about the nature of receptors that are involved in the particular diseases. The second step is the identification and designing of agonist or antagonist or inhibitor for the specific receptor. *For example*, serotonin receptor agonists are used to treat migraines, while dopamine antagonists are used as antidepressants. In some cases, it is necessary to check whether the selected target will be suitable or not for the selected disease. *For example*, tricyclic antidepressants inhibit nor-adrenaline uptake from nerve synapses. It also inhibits the uptake of another neurotransmitter, serotonin, which leads to the development of the best-selling drug fluoxetine.

a. Drug targets discovery

In earlier days, drug targets were discovered based on the action of drug or poison elicited by the chemical structure, which proves a molecular target. It indicates that the discovery of drug targets basically depends upon the drug discovery process.

In earlier days, drugs were primarily obtained from plants that were not synthesized, and it will interact with a receptor or enzyme present in the human body. Hence, the drug-receptor interactions occur by coincidence rather than design. Therefore drug discovery process in this manner was not pertinent.

In later years, the various chemical messengers present in the body were discovered as particular drug targets. The advancement in molecular genomics increases the discovery of potential receptors like DNA and various enzymes. The main challenge for a medicinal chemist is to find out a chemical compound, which will perfectly interact with these drug targets, identify their function, and whether they can act as suitable targets. This phenomenon is one of the main driving force for the development of combinatorial synthesis.

For example, the caspases, a group of enzymes, are the drug targets. The primary role of these enzymes is the hydrolysis of vital cellular proteins, and also it plays a crucial role in the inflammatory process and cell death.

The designing of new drugs (agonists) targeting these caspases are helpful for the treatment of cancer, autoimmune disorders and various viral diseases. The agents that inhibit the caspases activity decrease the prevalence of cell death, leading to the development of drugs for the treatment of trauma, neurodegenerative diseases, and cell death due to strokes.

b. Target specificity and selectivity between species

The target specificity and selectivity is the main factor for designing a drug. When the drug is more selective to its target, it has less chance to interact with various other targets, and the chances of producing undesirable side effects are also less. Examples:

i. The antimicrobial drugs, the selection of best targets are specific to microorganisms and are not present in humans.
ii. Penicillin inhibits the enzyme responsible for the cell wall synthesis of bacteria, but the same enzyme does not present in the human body. Hence penicillin produces fewer side effects.
iii. Sleeping sickness in Africans. The microorganism which induces sleeping sickness in Africans possesses a tail-like structure called flagellum. Hence, the drugs are designed to inhibit the proteins responsible for the production of the flagellum, which is not unique to human cells.

The other possible way to design a drug against the target is unique to both human and microbes, as long as the drug shows selectivity against the microbial target. This is due to the presence of a specific difference between the targets to allow such selectivity. A particular enzyme present in a bacterial cell significantly differs from the same enzyme present in human beings. Maybe the enzyme is derived from the common ancestor, but the evolution after several million years leads to significant structural differences.

For example, the antifungal agent fluconazole inhibits the enzyme fungal demethylase required for steroid synthesis in fungi. But the same enzyme is also present in the human being but not inhibited by fluconazole due to the significant structural differences between the two.

c. Target specificity and selectivity within the body

Selectivity against targets also plays an essential role in drugs acting against the receptors or enzymes. The drugs are designed so that they should specifically interact with a particular type of receptors. Nowdays, medicinal chemists are designing drugs with the highest range of target selectivity. Hence, the antagonists of various receptors are designed to act on the particular receptor and act against particular receptor subtypes.

For example, discovery of antipsychotic drugs with fewer side effects. The antipsychotic drugs are the dopamine receptor antagonists, which inhibit D_2 and D_3 receptors (totally 5 dopamine receptor subtypes). It is evident that inhibition of the D_2 receptor is responsible for the development of parkinsonian type side effect and hence current research is focused on finding out a selective antagonist.

d. Targeting drugs to specific tissues and organs

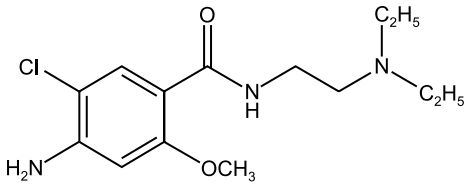
Sometimes the drugs are targeted against particular tissues or organs or particular areas of the brain. This is due to the dissimilar distribution of receptors in our body but is often or frequently concentrated in a specific tissue. *For example*, the type of receptor present in the heart is β_1 and in the lungs is β_2. This induces the design of new drug molecules that will act on the lungs with minimal or fewer side effects on the heart and *vice versa*.

For example, in Parkinson's disease, dopamine transmission is less or deficient in certain parts of the brain. But dopamine transmission usually functions elsewhere. A drug is given to mimic dopamine in the brain, which acts like a hormone and passed throughout the body to reach its target. Hence, the drug potentiates all the dopamine receptors and not only the one with dopamine deficiency. This kind of drug leads to a large number of side effects. Hence, it is prime to develop the drug or discover a drug with more selective to the particular subtype of dopamine receptors affected in the brain. This principle would target the drug more effectively towards the affected area and decrease the unwanted side effects in other parts of the body.

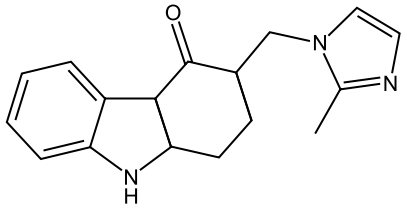
Risk or a word of caution: Human body is a highly complex system which contains lots of enzymes, messengers, receptors. Hence, there may be more than one enzyme, or receptor may be involved in the disease. Is it possible to say whether a specific receptor or enzyme plays a major role in a particular disease? The answer is no, because there is no simple cause for one ailment. *For example*, the development of hypertension. Hence the drug target for antihypertensive agents involves ACE receptors, calcium channel receptors, β_1-adrenoceptor, etc. It indicates that more than one target is required to address by a drug to treat a particular disease. *For example*, treatment of asthma involves a β_2-agonist (bronchodilator) and corticosteroids (anti-inflammatory agents).

However, sometimes a particular target is not important to treat a particular disease.

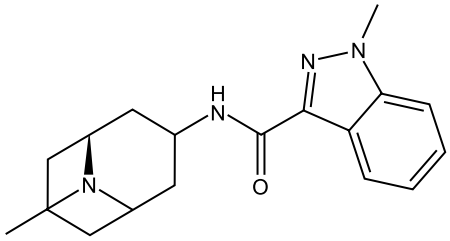
For example, previously, the D_2-dopamine receptor was thought to be responsible for inducing nausea. Hence, the D_2 receptor antagonist metoclopramide (more selective D_2 receptors) was discovered as an antiemetic agent. However, further developments indicates that more D_2 receptor-selective metoclopramide was less effective, and another receptor is significantly responsible for causing nausea. This leads to the discovery of 5-Hydroxy tryptamine (5-HT_3) antagonists such as granisetron and ondansetron.



Metoclopramide



Ondansetron



Granisetron

Bioassay Identification

a. Bioassays

These are quantitative assays used to measure the potency of agents by determining their effects on cell/tissue culture systems (*in vitro*) or living animals (*in vivo*).

The selection of particular bioassay method is a main key point to the success of drug design and research. A large number of compounds is tested normally, hence, the assay method should be easy, reliable, quick and straightforward. The bioassay tests are done in two ways.

i. *In vitro* tests (utilising isolated cells, tissues, enzymes or receptors)
ii. *In vivo* tests (utilizing animals)

In vitro tests are preferred over *in vivo* tests.

i. ***In vitro* tests:** *In vitro* tests use specific cells, tissues or enzymes. The efficiency of enzyme inhibitors can be studied on the pure enzyme in solution. Receptor agonists/antagonists are tested on isolated tissues or cells which contain the particular receptor on their surface.

For example, the bronchodilator activity can be tested by determining the inhibition ability on isolated tracheal smooth muscle contraction. Similarly, the binding affinity of a drug towards the receptor can be determined by radioligand studies.

[Many *in vitro* studies are also possible where the gene coding for a specific enzyme or receptor is identified, cloned and expressed in the fast dividing cells. *For example*, HIV *Protease* has been cloned and expressed in the bacteria *E. coli*].

ii. ***In vivo* tests:** Transgenic animals are typically used in *in vivo* assays. If possible, a transgenic animal in which genetic code is changed or altered means the animal genes are replaced by human genes. Hence, the animal produces similar enzymes produced by humans, which acts as a receptor, which permits the drug's test efficacy against this receptor. Alternatively, the animal's gene could be changed so that the animal became susceptible to cancer or particular ailments.

Validity of tests: The validity of tests is also one of the essential factors for designing a drug. Many times the validity of the testing procedures is clear-cut and straightforward. *For example*, the antibacterial activity can be determined by determining the MIC of drugs. Likewise, the local anaesthetic potency of the drug can be determined by measuring how well or to what extent it blocks the action potential in isolated nerve tissues. But in some cases, the testing methodology is a difficult one. *For example*, determination of the efficacy of new antipsychotic drugs. There is no exact animal model available for this test, and hence a simple *in vivo* method is not possible.

b. High throughput screening (HTS)

It is the process of testing a large number of diverse chemical structures against disease targets to identify HITS with the use of robotics, automation and miniaturization. It helps to screen 50000–100000 compounds in a short span of time against validated biological targets. It involves an automated operation system, a highly sensitive testing system, specific screening *in vitro* models, and abundant components library and a data acquisition and processing system. Characteristics of HTS: Simple, rapid and with good ligand-target interactions.

Drug companies use more than 500 targets. It includes:

- G-protein coupled receptors (45%)
- Enzymes (28%)
- Hormones (11%)
- Unknown (7%)
- Iron channels (5%)
- Nuclear receptors (2%)
- DNA (2%)

c. Screening by nuclear magnetic resonance (NMR)

NMR is used to determine the binding affinity of a compound towards a particular protein target. In NMR, the compound is radiated by a small amount of energy, and its nuclei shifted to an excited state, then the nuclei returned to the ground state with the emission of energy. This can produce a spectrum. The relaxation time of nuclei is calculated and it depends upon the size of the molecules *i.e.* small drugs have short relaxation time. Hence, it is possible to delay the measurement of energy emission such that only small molecules can be identified. It is the basic principle to detect the protein binding of drug molecules.

Identification of Lead

Once the selection of target and testing system is completed, the next step is the "identification of lead."

Lead is a compound or molecule with desired pharmacological or biological activity is called "lead." The lead compound or molecule provides a starting platform for the drug development process.

Various methods for the identification of lead molecules are given in detail below.

a. Identification of lead compounds from natural products or natural sources

A number of biologically active compounds or drugs are obtained from natural products only. They may be directly obtained from natural sources or developed from a lead molecule that is originally obtained from a natural origin. It is a problematic task to synthesize bioactive metabolities in the laboratory which have complex molecuar structure. Generally, nature provides novel chemical, biologically active compounds that no chemists would have dream to synthesize.

The lead compounds are obtained from various sources of the natural product as mentioned below.

i. **Lead from plants:** Plants act as the good source for providing various kinds of lead molecules with high molecular weight and diverse structures. The synthesis of these compounds in the laboratory is a difficult task. Numerous natural lead compounds are useful drugs. *For example*, morphine is used as narcotic analgesic and digitalis is used to treat CCF and quinine is used as an antimalarial agent.

The raw materials required for ayurvedic medicines are mainly obtained from natural products. They are collected in the form of crude drugs. Research reveals that nearly 70,000 species of higher plants exist in nature out of which only 10% of plants are used in traditional medicines, and 5% of plants are scientifically studied. Hence, studying or thorough knowledge about the chemical constituents present in the plants and screening of their biological activity provides the basic information for developing a lead compound. Natural products play a key role in drug discovery, and therefore it is termed as **cornerstones of drug discovery and development**. A large number of drugs are obtained from natural products. *For example*, Paclitaxel, an anticancer drug obtained from bark of the yew tree, *Taxes bravifolia*. Medicinal plants are used as:

1. Direct sources of bioactive compounds.
2. Bioactive fractions with complex structures are used to develop herbal-based complex semisynthetic compounds.
3. A source for lead discovery.
4. Bioactive markers for spectroscopic studies to discover novel molecules.

Some of the isolated active phytochemical ingredients are listed in Table 1.1.

Table 1.1: Isolated active pharmaceutical ingredients from plants

S. No	*Active ingredient isolated*	*Source*	*Year*	*Scientist*
1.	Morphine	Opium	1803	Friedrich Serturner
2.	Emetine	Ipecacuanha	1816	Pierre-Joseph Pelletier
3.	Digitoxin	Digitalis	1841	E Humolle and T Quevenne
4.	Cocaine	Cocca	1860	Albert Niemann
5.	Physostigmine	Calabar bean	1864	Albert Niemann

Various steps involved in the preliminary screening or lead identification in natural products are given in Fig. 1.1.

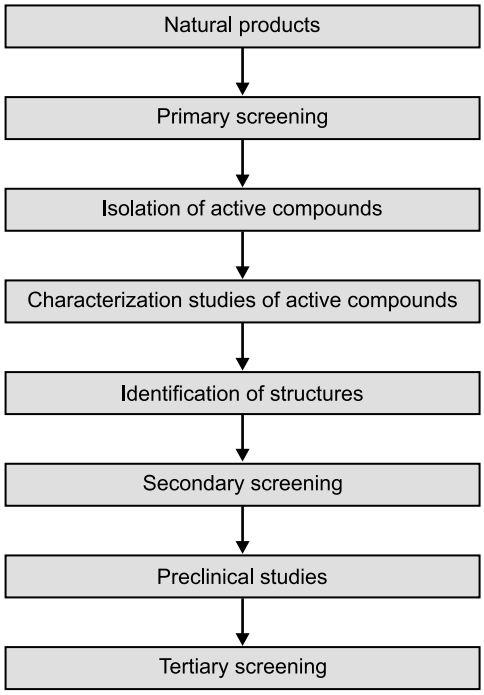
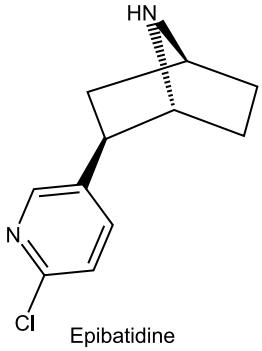


Fig. 1.1: Lead identification in natural product discovery

ii. **Lead from microorganisms:** Fungi are part of the human life cycle for more than 100 years. They are used as a food stuff (mushrooms) and used to prepare alcoholic beaverages (yeast) and medicines. The advanced or elaborate development in biotechnology and microbiology, extends the use of microbes to biological control, antibiotics and biologically active molecules.
One of the best examples of this is the famous discovery of the antibiotic penicillin from *Pencillium notatum*. The other examples are vancomycin from *Amycolaptosis orientaliis*, erythromycin from *Saccharopolyspora erythrae*, betulinic acid from *Betula pubescens,* an inhibitor of HIV replication.

iii. **Lead from marine world:** About 70% of the earth is covered by ocean, and it contains unique biodiversity and hence a possible source for the discovery and development of biologically active compounds.
Because of modern snorkeling use of manned submersibles, and the development of remotely operated vehicles, exploration of marine organisms such as sponges, algae, ascidians, tunicates and bryozoans became possible. These developments and exploration of the marine environment lead to the isolation of several structurally related bioactive lead compounds. *For example*, Ziconotide obtained from tropical cone snail is used as an analgesic. Plitidepsin, a depsipeptide obtained from *Aplidium albicans* is used to treat non-Hodgkin lymphoma and acute leukemia.

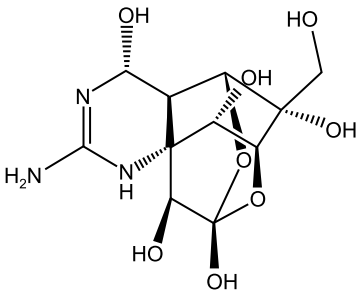
iv. **Lead from animals:** Sometimes animals are the excellent source for the identification and discovery of new lead compounds. *For example*, epibatidine, a potent analgesic that is obtained from the skin extracts of the Eucadorian poison frog and a group of peptides obtained from the extracted skin of the African clawed frog serve as antibiotics.



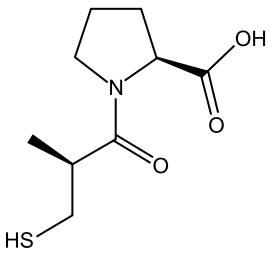
Epibatidine

v. **Lead from venoms and toxins:** Venoms and toxins obtained from plants, insects, microbes and animals also often serve as lead compounds. These are highly potent because they specifically interact with their receptors with strong affinity in the body. Hence, they are used as an important tool for studying enzymes, receptors, etc. Most of these toxins are polypeptides such as α-bungarotoxin from cobras. But the nonpeptide toxins are more potent. *For example*, tetrodotoxin from pufferfish. Venoms and toxins are used as lead compounds for the development of various kinds of drug molecules as Teprotide, a peptide derivative obtained from the Brazilian viper venom served as a lead compound for the development of the antihypertensive drugs Captopril and Cilazapril.

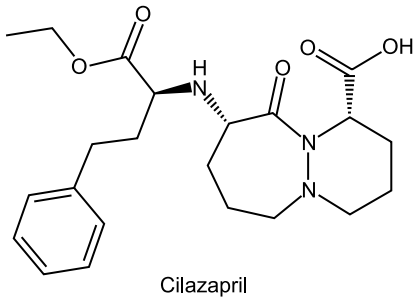
Clostridium botulinum, the bacterium secrets botulinium, a neurotoxin responsible for serious and severe food poisoning, shows specific uses. It can be injected into a particular muscle (those containing the eyelids) to prevent spasms. They inhibit cholinergic transmission, hence serves as the lead for the development of cholinergic antagonists.



Tetrodotoxin



Captopril



Cilazapril

b. Random screening

This is a valuable method primarily used when the known drugs are not available, but other compounds possess desired biological activities. All compounds are tested in the assay irrespective of their chemical structure. Before discovering sulpha drugs (before 1935), random screening is the only approach to find out the lead compounds. But till today, it is a crucial method to identify the lead compound because now-a-days millions of compounds are rapidly screened by high throughput screening. This is the method of choice when the drug-receptor details are not known. Two important kinds of compounds screened by random screening are natural products and synthetic compounds.

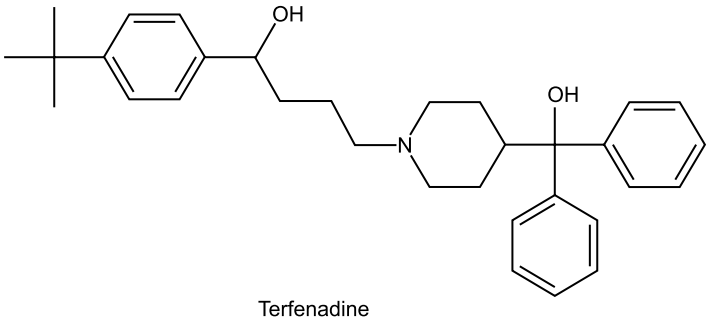
c. Nonrandom screening (targeted or focused)

It is a modified method of random screening. In this method, the compounds uncovered in random screening possesses weak activity, or compound with different functional groups than leads are selectively tested. *For example*, screening of anticancer compounds as cancer is not a single disease.

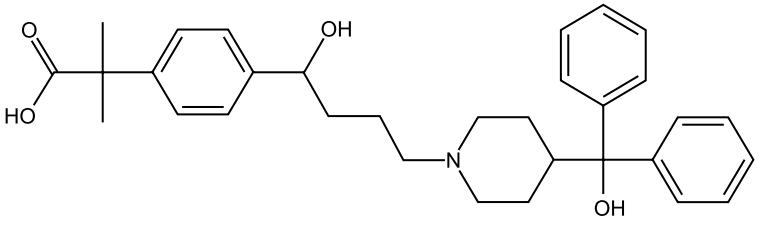
d. Drug metabolism studies

Metabolism or detoxification is a process from which the drugs are eliminated from our body after eliciting their pharmacological activity. This process is occurred by the action of enzymes. Metabolic biotransformation occurs on the drugs by increasing their polarity. Sometimes metabolites act as lead molecules for the structural modification and identification of the active compounds. During these studies, the metabolites are isolated and screened if the drug molecule or metabolites exhibit the activity.

Example 1: Fexofenadine hydrochloride (Allegra), a non-sedating antihistamine prodrug and a metabolite of terfenadine hydrochloride. The former leads to abnormal heart rhythms if taken alongwith an antifungal agent (inhibit the terfenadine metabolizing enzymes). But the latter one, fexofenadine metabolism, is not affected by the antifungal agent. Hence, it is a safer drug, and metabolites can be screened for other kinds of activities.

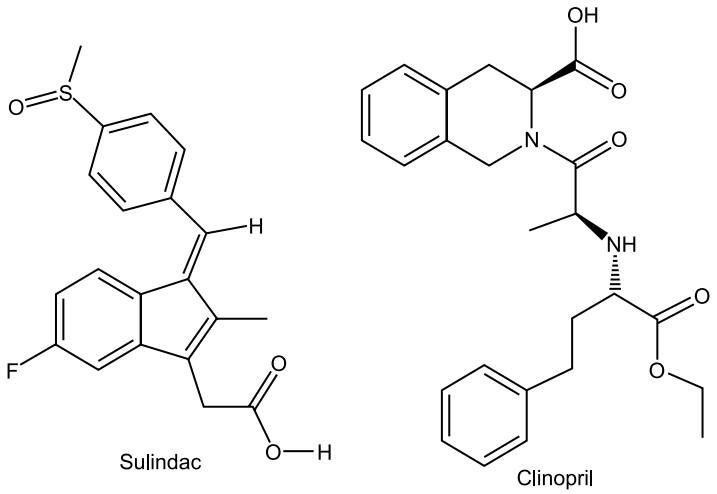


Terfenadine



Fexofenadine

Example 2: Sulindac, an anti-inflammatory drug is not responsible for its activity, but clinopril, its metabolic product, produces the required activity.

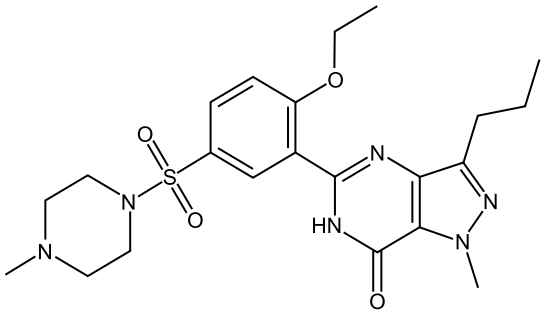


Sulindac Clinopril

e. Clinical observation

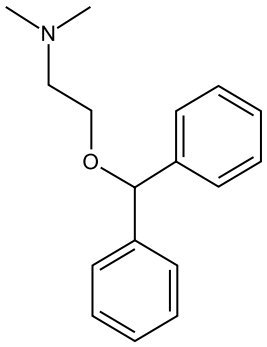
Sometimes, during clinical trial phases, some drugs can elicit more than one pharmacological activity and considered as a side effect. This kind of compound later serves as a lead compound for their secondary pharmacological activities, which was considered as a side effect.

Example 1: Sildenafil is a drug of choice for impotence. It was designed first for the treatment of angina and hypertension. But in phase II clinical trial, it has shown increased erectile function and possessed weak antianginal activity. Hence, it was designed as a drug for the treatment of impotence.



Sildenafil citrate

Example 2: The drug diphenhydramine an antihistaminic agent first used to treat patients with car sickness problems in 1947. After that, it was found that it can also be used to treat air sickness and sea sickness. Hence, it has become widely used for the treatment of all kinds of motion sickness problems.

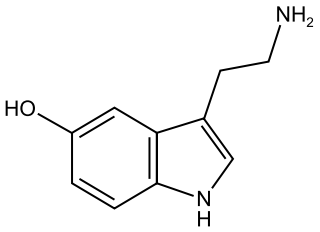


Diphenhydramine

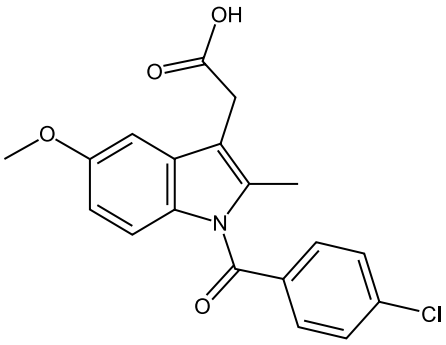
f. Rational approaches to lead discovery

All the above methods discussed do not entirely discover the major rational component. After the lead compound is obtained from the simple screen techniques (random/ nonrandom screening) or drug metabolism studies or clinical investigations, then the question arises can we design a lead compound with specific pharmacological activity? Many kinds of diseases are produced due to the imbalance of particular chemicals in the body, or entering of foreign organisms or excess cell growth. Once a relevant biochemical system is identified, initial lead compounds become the natural receptor ligands or enzyme substrates.

Example: Serotonin used as a lead for the development an anti-inflammatory agent indomethacin, because serotonin itself possesses weak anti-inflammatory property.



Serotonin



Indomethacin

It is not easy to discover a drug with much accuracy without side effects. Hence, rational approaches are much directed to lead discovery. Once the identification of the

lead compound is completed, its structure can be altered or modified until a drug is obtained with promising activity and minimum side effects.

g. Computer-aided drug design

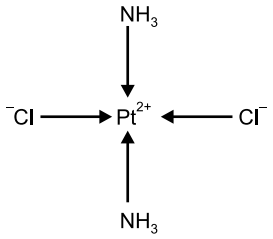
Complete knowledge about the drug binding target site significantly leads to the design and development of novel lead compounds. In CADD, molecular modeling software programmes are used to study the nature of the binding site and design molecules that will fit and bind. In some cases, the targets, i.e. receptors or enzymes, are crystallized; it is possible to determine the protein structure and its binding site by X-ray crystallography. Suppose the chemical structure and its analogue binding to protein have been determined to be used for the model generation of a particular protein.

h. Serendipity and prepared mind

More often, lead compounds are found by chance or serendipity. Serendipity has played a large part in the discovery of drugs. *For example*, the development of penicillin by Florey and Chain was possible only because of Alexander Fleming, who noted the inhibition of Staphylococcus by *Penicillium notatum.*

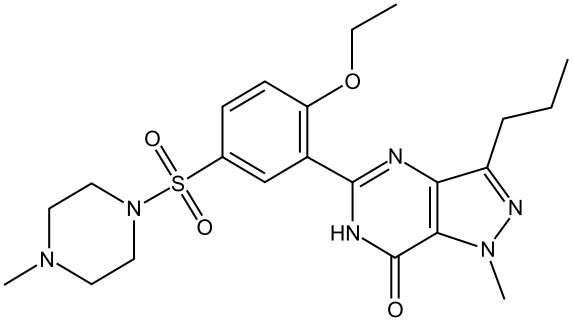
Examples:

i. **Discovery of cisplatin:** The discovery of cisplatin is an interesting one that arises from the research carried out to determine the effect of electrical current on bacterial growth. During this research, the bacterial cell wall synthesis was inhibited due to the release of some kind of agent for the hydrolysis of platinum electrodes. This compound was later identified as *cis*-diamminedichloroplatinum (II), called cisplatin.



Cisplatin

ii. **Discovery of sildenafil (viagra):** It was by discovered by chance during the research aimed to develop a novel drug to treat heart disease. Later it was found to increase blood supply by dilating blood vessels and hence used to treat erectile dysfunction and high blood pressure.



Sildenafil citrate

Vincristine and Vinblastine: These drugs were developed by chance when the research was carried out to develop drugs to reduce blood sugar levels. Now Vincristine and Vinblastine are used for the treatment of Hodgkin's disease.

i. Computerized searching of lead compounds of structural databases

Novel lead analogues are found out by databank searching. To carry out this type of search, one should know about the pharmacophore of the particular drug candidate. This kind of database searching is called *database mining*.

j. Lead design by NMR

In the previously described **serendipity** method, the lead compounds can be discovered from either natural or synthetic sources. However, it has the main disadvantage that there is no guarantee that such a lead compound would be taken to the final clinical candidate. In recent days, NMR spectroscopy is used to design a novel lead instead of discovering one. This method uses a small molecule known as epitopes that will bind to a particular but different region of the target protein. These epitopes do not elicit any pharmacological response themselves but only bind to the receptor. If a large molecule is designed and attached to these epitopes together, which acts as a lead compound and it binds to the whole binding site of the protein which may show activity.

This approach can be used to develop proteins of known structure that are labelled with ^{15}N such that each amide bond in the protein has an identifiable peak. The 2D NMR spectrum will be helpful to identify each peak matching ^{15}N with ^{1}H (Fig. 1.2).

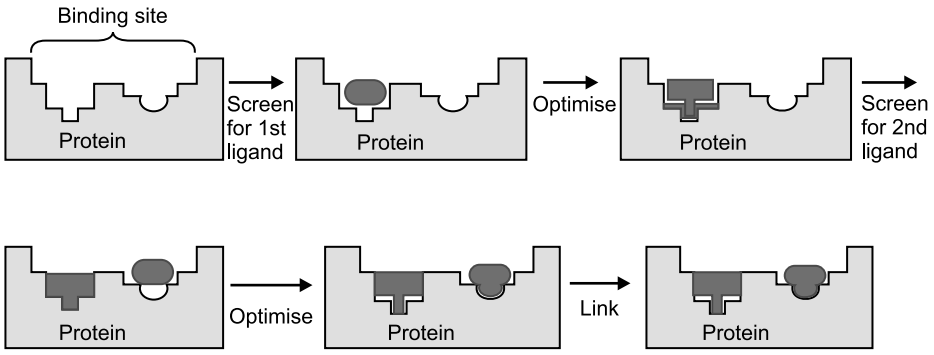


Fig. 1.2: Epitope mapping

This method utilizes a building block approach, but this is differing from combinatorial synthesis. The building blocks are optimized in the former one, and a small number of epitopes are then prepared. However, in combinatorial synthesis, many linkers are made from different building blocks and further incorporated into synthesis.

Lead generation by NMR is limited to small protein targets of molecular weight less than 40,000 and obtained in quantities more than 200 mg and labelled with ^{15}N.

Isolation and Purification of Lead Compound

Isolation and purification of the lead molecule are necessary when the lead is obtained/present in the mixture of other compounds. The mixture is obtained from a natural source

or combinatorial synthesis. The principle of isolation and purification depends on the structure of the lead, stability, solubility, and the lead compound present in that mixture. *For example*, isolation and purification of penicillin was a problematic process. Still, penicillin is an excellent antibiotic with fewer side effects in human beings. It was stable in solution form; when the solvent was removed, penicillin was destroyed (instability of penicillin). The successful isolation of penicillin was achieved after the discovery of freeze-drying process.

A number of other methods are also available for the isolation and purification of active principles. Different chromatographic techniques are used now-a-day to isolate and purify the active compounds from the natural products. *For example*, paper chromatography, TLC, HPLC, HPTLC, LC-MS, GC-MS, etc.

Determination of the Chemical Structure of Lead Compound

In previous decades, the determination of the structure of a chemical molecule was challenging and a big hurdle to overcome. A novel structure that may now take a week's works to determine would have taken 2–3 decades of work in the past. *For example*, a microanalysis of cholesterol was carried out in 1888 to get its molecular structure. The chemical structure was not fully determined until an X-ray crystallographic study was carried out in 1932.

Although, now-a-days structural determination is straight forward, and it is applicable when the natural product is obtained in minute quantities. The most valuable techniques are X-ray crystallography, NMR spectroscopy, and MASS spectroscopy.

Structure-Activity Relationship Determination

SAR studies of the synthesized compound aim to discover the parts that are important to biological activity. It is easily achieved by chemical transformations to add or remove or alter the particular group, which alters the binding or pharmacological activity.

a. **The binding role of –OH groups:** Hydroxyl groups are responsible for hydrogen bond. Conversion or modification of this group into ether or ester affect the hydrogen bond affinity or weakens the bond. Primarily, hydrogen atom present in –OH group is responsible for hydrogen bonding to the receptor, and if it is removed, there is no formation of a hydrogen bond (Fig. 1.3).

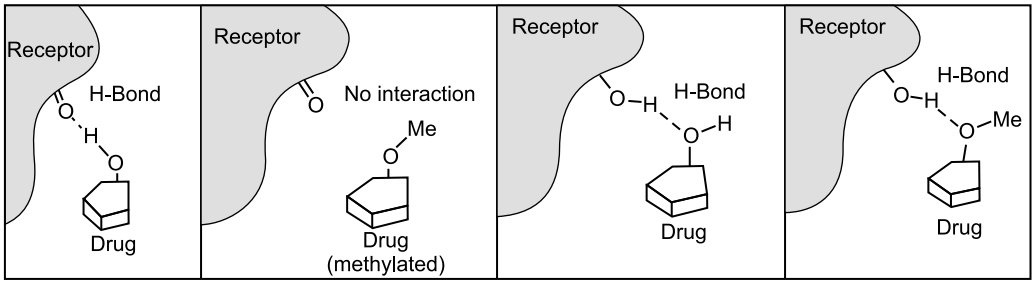


Fig. 1.3: Possible hydrogen bonding

b. **Binding role of amino group:** Aromatic rings are commonly responsible for the van der Walls interactions with hydrophobic regions of the receptor site. The benzene ring can be converted into cyclohexane by flat hydrogenation reaction. The cyclohexane

structure is no longer flat; further, it affects the receptor affinity to their target (Fig. 1.4).

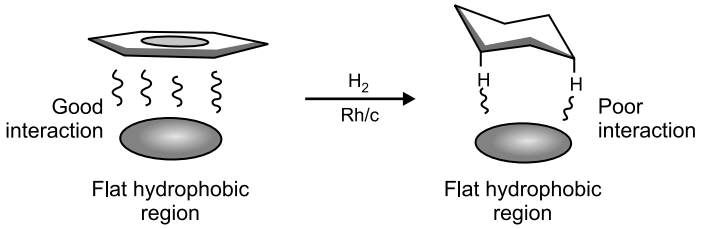


Fig. 1.4: Hydrogenation of benzene

Replacement of aromatic ring with bulky alkyl group also leads to reduced steric interactions.

c. **Binding role of double bonds:** Reduction of double bonds occur quickly, and a significant effect on the shape of the molecule weakens van der Walls interaction with the receptors (Fig. 1.5).

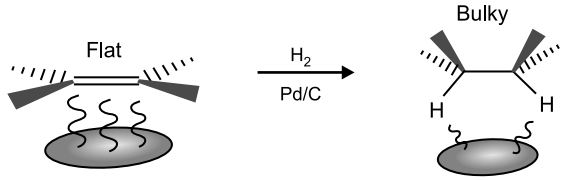


Fig. 1.5: Hydrogenation of alkene

d. **Binding role of ketones:** A ketone group is generally found in many drug molecules. It interacts with the receptor through dipole-dipole interactions or hydrogen bonding (Fig. 1.6). It is easy to reduce the ketone group to alcohol, which alters the geometry from planar to tetrahedral alcohol. This change in geometry reduces the strength of earlier dipole-dipole interactions and also weakens the hydrogen bonding.

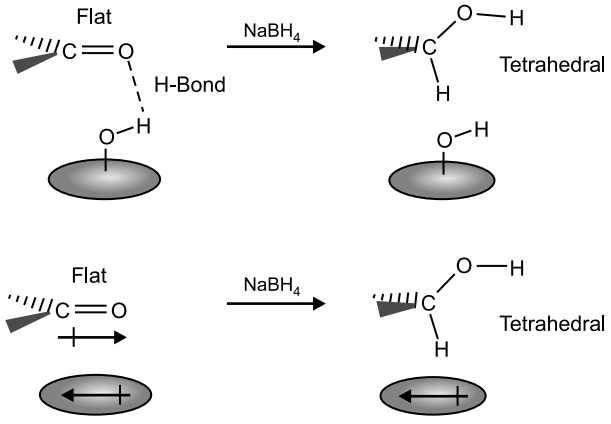


Fig. 1.6: Reduction of ketones

e. **Binding role of amines:** Amine groups present in the drugs are involving in ionic bonding or hydrogen bonding. Conversion of the amino group into amide group prevent the participation of lone pair of electrons of the nitrogen atom in hydrogen bonding and weakens the bond or prevents to accept a proton to form ion. Tertiary amines are dealkylated before conversion to amides which is carried out by chloroformate or vinyloxy carbonyl chloride or cyanogen bromide (Fig. 1.7).

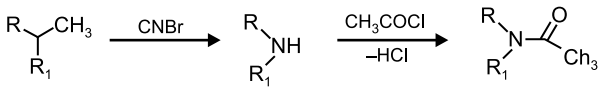


Fig. 1.7: Binding role of amines

f. **Binding role of amides:** Amides have interacted with protein targets via hydrogen bonding. The modification of this group is achieved by hydrolysis, which divides the molecule into two compounds, i.e. amine and carboxylic acid. Any loss of activity is due to the loss of other critical binding groups, or the reduction of amide leads to amine, which weakens the hydrogen bond interactions associated with carbonyl oxygen (Fig. 1.8).

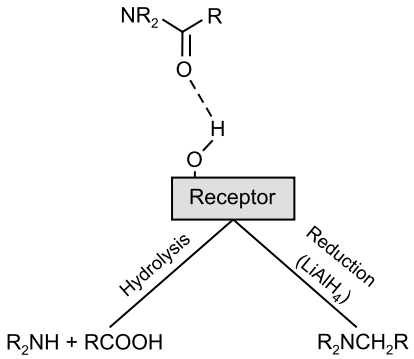


Fig. 1.8: Binding role of amides

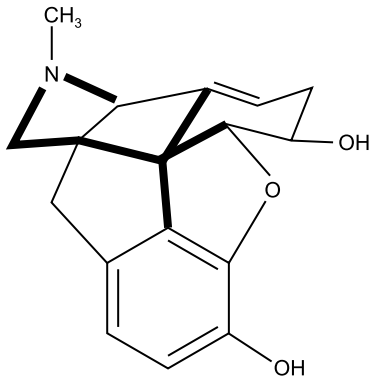
g. **Isosteres:** Friedman first coined the term bioisosterism, and he defines "bioisosters are functional groups/molecules which possess similar physical and chemical properties producing broadly similar biological activities". Burger expanded this definition as "Bioisostere are compounds, have near equal shapes and volumes, the approximately same distribution of electrons and exhibit similar biological properties". Bioisosterism is an essential phenomenon for modifying lead compounds that is used to alter the pharmacological activity or attenuate the toxicity.

h. **Testing procedure:** The biological testing should contain *in vitro* and *in vivo* studies. It has shown that which groups are essential in drug-target interactions. The main disadvantage of *in vitro* testing is that it identifies groups that are important in protecting or assisting the drug in its passage through the body.

Pharmacophore Identification

Pharmacophore is defined as the part of the chemical structure responsible for the pharmacological activity and their relative positions in space concerning each other. *For example*,

morphine, the pharmacophore of morphine is highlighted in the structure given below.



Morphine

Medicinal chemist often removes any group or atoms and tests for their pharmacological activity to identify the pharmacophore. If the removal of atoms and functional groups decreases the activity, they are likely to be a part of the pharmacophore. If the removal increases the activity, then it indicates that the groups were not a part of the pharmacophore, they are considered auxophore that inhibits the pharmacophore's binding. However, during this process, potency is only not affected, it also sometimes alters the activity.

Target-Oriented Drug Design

Once the pharmacophore is identified, the next step is whether it is possible to synthesize lead analogues because very few naturally available drugs are effective and ideal, and many of the drugs produce serious side effects.

Increasing the drug-target interactions increases the pharmacological activity, while increase in target selectivity will decrease the side effects. The main principle of target-oriented drug design is to modify the lead so that it acts more strongly and selectively with its molecular targets in the body.

Pharmacokinetic Studies in Drug Design

The compounds with better binding interactions may not act as a good drug, as some of the drugs are active in *in vitro* tests but not active in *in vivo* test. This is due to the passage of drugs through various barriers in the *in vivo* trials. Pharmacokinetic drug design is used for designing drugs to overcome these barriers.

Patenting of Drug Molecules

Research and development utilize a tremendous amount of money and time. Therefore a pharmaceutical company quite rightly wants to recoup its costs and reap the benefit. Consequently, it requires having the authentication or rights to manufacture and sell the products for a reasonable period of time at a price that generates enough profits for further research and development. Without these rights, any competitive company could prepare the same product and sell the same at a much lower price.

Patents permit pharmaceutical companies to get the rights. A patent will grant the pharmaceutical company to get an exclusive right to their novel products for a limited period of time to get the profit.

Patent is a complete and preciously written document that describes the invention so that a person skilled in the same field can reproduce the same invention after the patent expires. Suppose a pharmaceutical company or a person wants to get an approval or copyright then, it has to apply with the specified agencies, and the patent is issued only after its approval. Moreover, each country has a unique patent procedure, so the pharmaceutical company has first decided in which country it is going to market its novel drug and file the patents. Patent laws also differ from one country to another. Hence the patent application was also submitted to authorities with the help of patent specialists, i.e. patent lawyers and patent attorneys.

Once the patent has been filed and submitted, the patent authorities then decide whether the claims within the patent are novel or not and they satisfy the requirements. However, the product (lead) discovered must not have revealed at any platform. Hence, the pharmaceutical companies only show their work at conferences or in scientific journals after the structures have been patented.

The patent should be filed after the invention as soon as possible; hence the competition by other companies is prevented. Generally, the patent is filed before the research team had the chance to start all the extensive tests which need to be done on novel drugs. It may not even have synthesized all the possible structures which it intends to make. Hence the team is not able to identify which specific compound in a series is likely to be the best drug candidate. Due to this, most of the patents include all analogues belonging to a particular category. This inhibits the synthesis of a very close analogue of the best compound by another company and selling it in competition (patent can cover certain products and uses of the products and synthesis of the products or all the aspects).

Types of patents

1. *Utility patents:* It includes various discoveries such as machines, articles of manufacturing process, etc.
2. *Plant patents:* Products obtained from plants.
3. *Design patent:* Particular design prepared.

The Patent Cooperation Treaty (PCT, Fig. 1.9) helps applicants who are seeking patent protection internationally. The schematics of various procedures followed in different countries for filing patents is given in Figs 1.10–1.12.

Drug Metabolism Studies

Testing for Drug Metabolites

The metabolites of drugs can be identified by testing them into animals and humans. It is essential to determine the nature of the metabolite as toxic or non-toxic. Ideally, the formed metabolites should be inactive and can easily excreted from the body. Based on the metabolites, structural modification can be performed to obtain a new drug with a more potent and less toxic profile. These drug metabolism studies are helpful in the designing of new drugs.

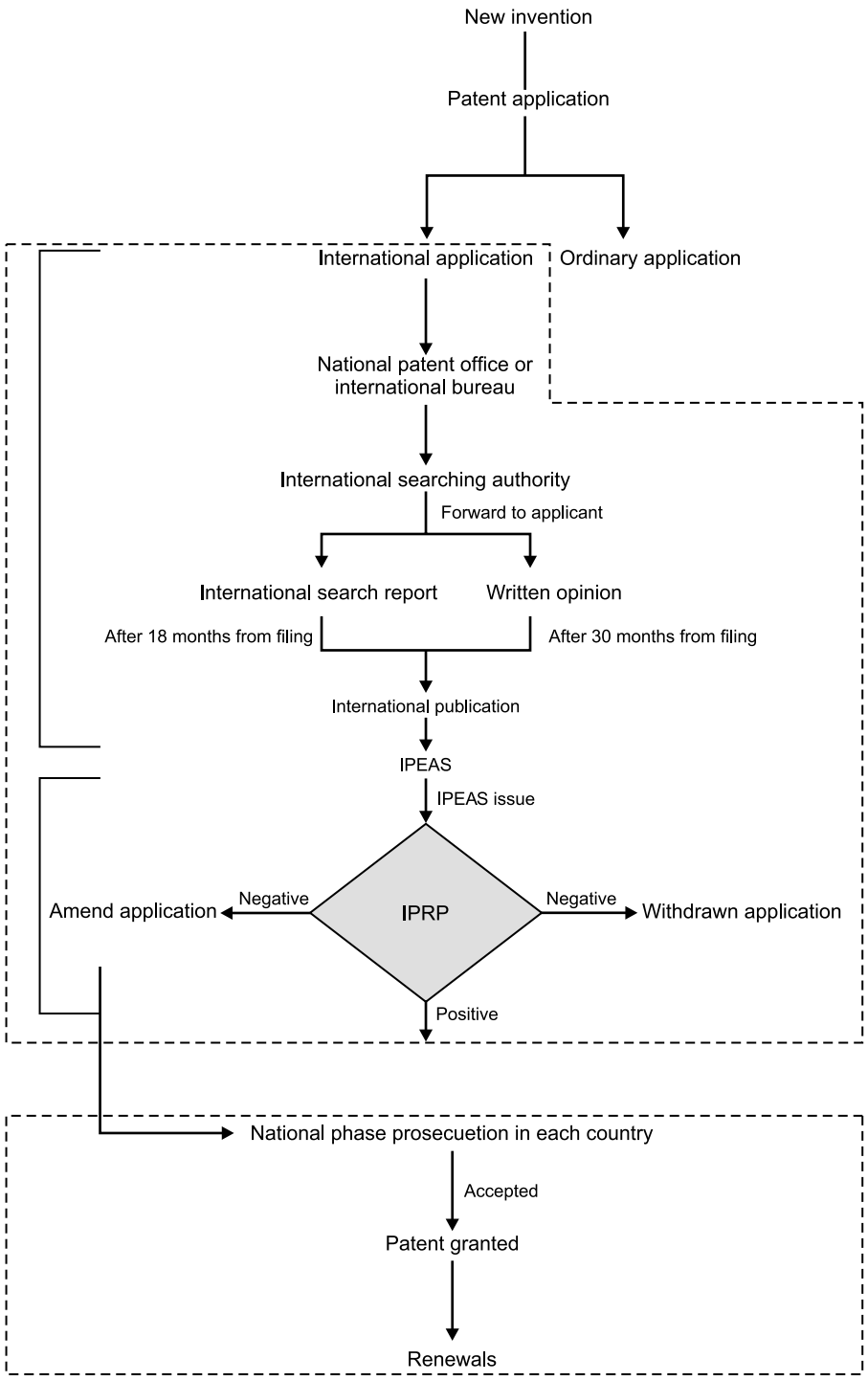


Fig. 1.9: PCT filing procedure

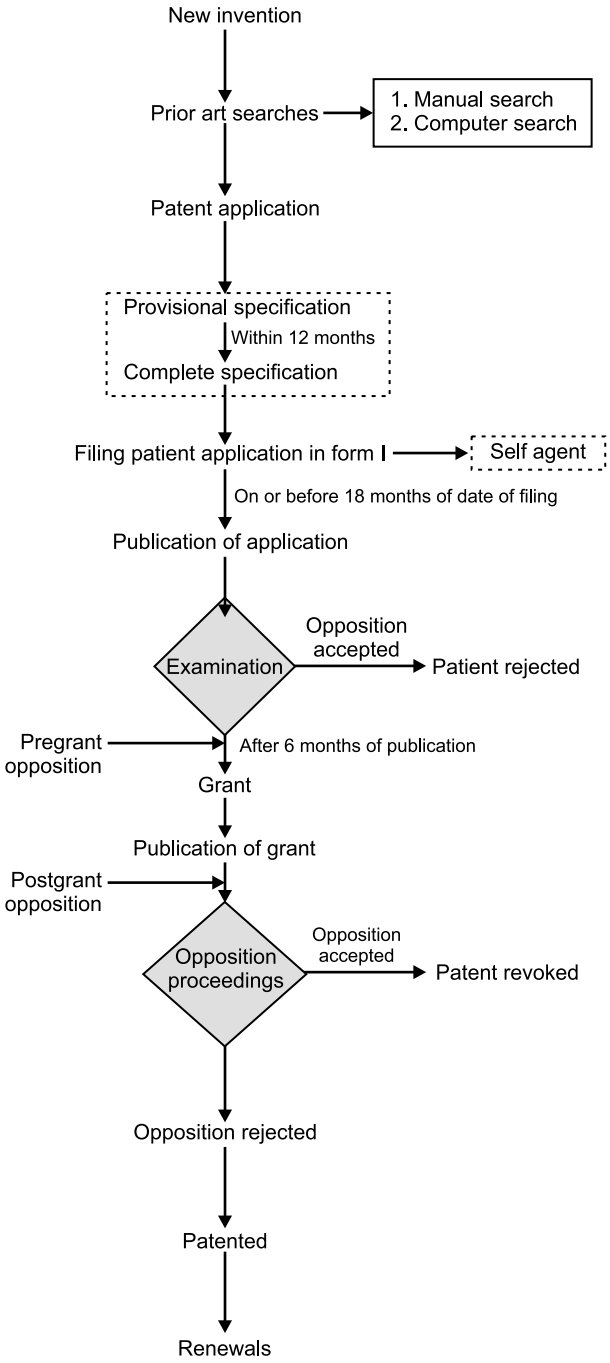


Fig. 1.10: Patent filing procedure in India

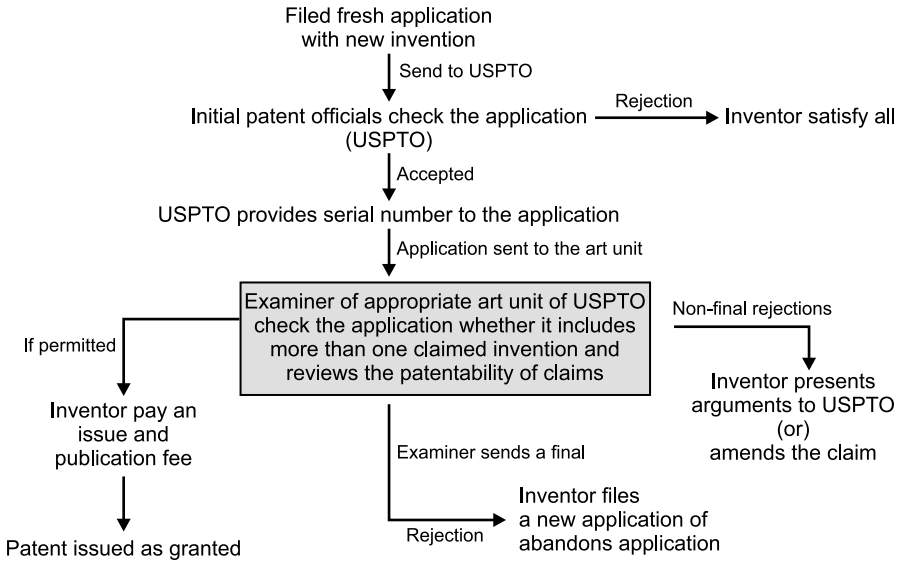


Fig. 1.11: Patent filing procedure in the USA

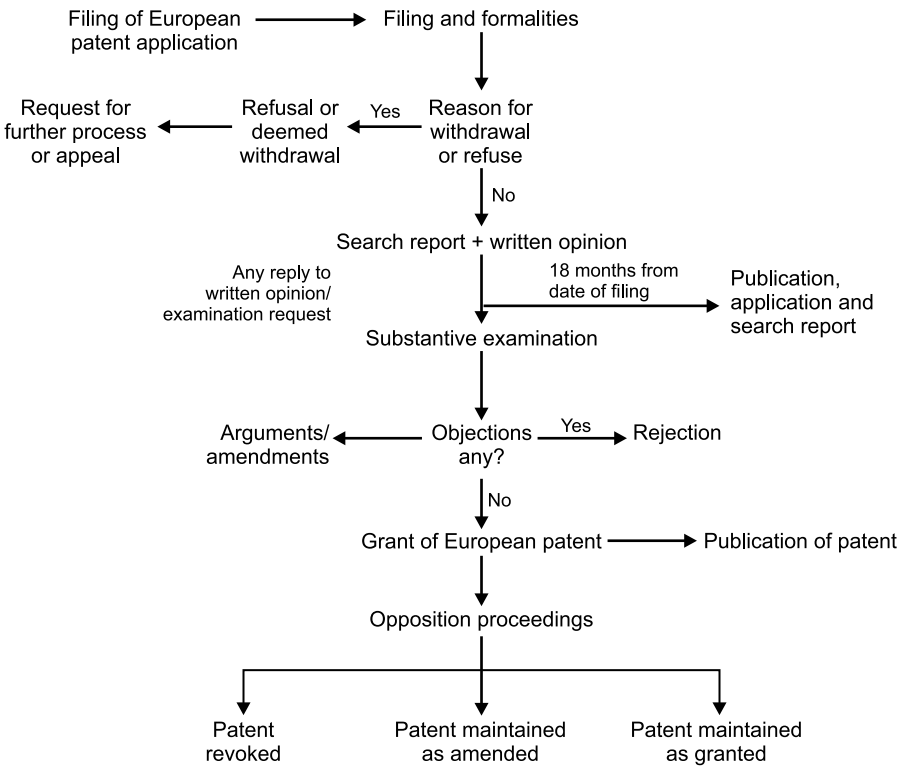


Fig. 1.12: Patent filing procedure in Europe

The **process of drug metabolism or biotransformation** is described briefly (Fig. 1.13).

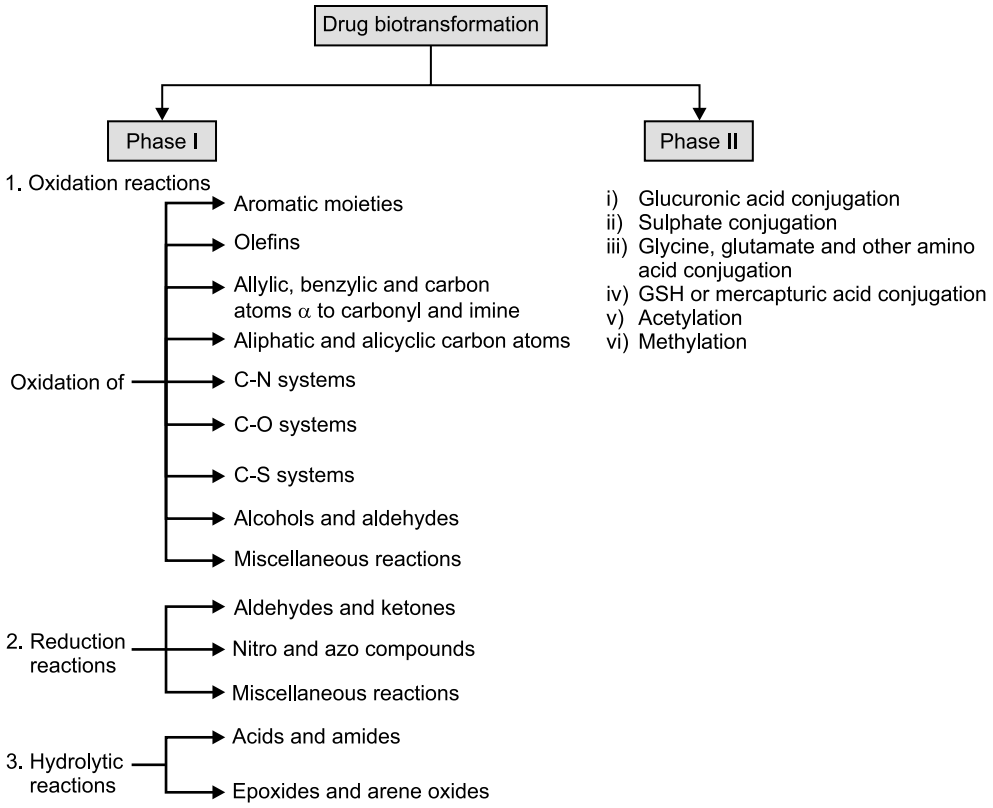


Fig. 1.13: Process of drug metabolism/biotransformation

The biochemical process of converting drugs or xenobiotics into non-toxic agents in the body is called biotransformation or metabolism. Hence, drug metabolism is also known as the process of **detoxification.** During this process, the drugs are converted into water-soluble conjugates and eliminated through the kidney.

Importance of metabolism

Most of the organic drugs are highly lipophilic when administered via the oral route, they pass the lipoprotein members of GIT and reach the bloodstream. Once the drugs enter into various target organs elicit their pharmacological response. Due to reabsorption in the renal tubule, the lipophilic drugs are not excreted from the body via urine. Hence, enzymes perform various metabolic changes and render them more water-soluble and then eliminated. During the metabolism, not all drugs are converted into an inactive form.

General pathways of drug metabolism

Drug metabolism reactions are broadly classified into two types.

a. Phase I reactions or functionalisation reactions
b. Phase II reactions or conjugation reactions

a. **Phase I or functionalization reactions:** The main principle of this reaction is to introduce or attach polar functional groups such as –OH, –COOH, $–NH_2$ into the xenobiotic and convert them into a highly water-soluble compounds. The phase I reactions involve hydroxylation, oxidation, reduction and hydrolytic transformation reactions.
b. **Phase II reactions or conjugation reactions:** These reactions help to attach small, polar and easily ionizable endogenous substances such as glucuronic acid, sulphates, glycine, and other amino acids into the functional group of phase I metabolites or parent compounds that already possess suitable functional moieties to form highly water-soluble conjugate products. These conjugates generally do not have any pharmacological activity and are non-toxic to humans, and are readily excreted by the urine. The phase II reactions also includes acetylation and methylation, which do not increase water solubility but attenuate or terminate the pharmacological activity. In contrast, glutathione conjugation protects the body from the effect of chemical reactive conjugates or metabolites.

   Both phases I and II reactions are competent to one another; thus, detoxifying and facilitating the elimination of drugs and xenobiotics from the body occurs. Let us discuss phase I and phase II reactions in detail.

Phase I reactions or functionalization reactions

Characteristic properties of phase I reaction is that it improves the hydrophilicity, it facilitates conjugation and reduce stability.

Types of phase I reactions: These are classified into three types.

- Oxidative reactions
- Reductive reactions
- Hydrolytic reactions.

Phase II reactions (or) true drug detoxification process (or) conjugation reactions

Phase I reactions do not yield fully hydrophilic or pharmacologically inactive metabolites. However, phase II reactions convert the metabolites from phase I reactions into highly polar and water-soluble products. The phase II conjugation reactions required many numbers of conjugative enzymes. The main role of these enzymes is attaching small, polar and ionizable endogenous molecules such as, glucuronic acid, glycine, sulphate conjungation and glutamine to the phase I metabolites or products or parent xenobiotics. The resulting conjugates formed are relatively water-soluble and excreted rapidly from the body. The conjugates formed are non-toxic and pharmacologically inactive.

Types of phase II reactions.

- Glucuronidation
- Sulphate conjugation
- Conjugation with glycine, glutamine and other amino acids
- Conjugation with GSH or mercapturic acid conjugates
- Acetylation
- Methylation

Designing the Manufacturing Process

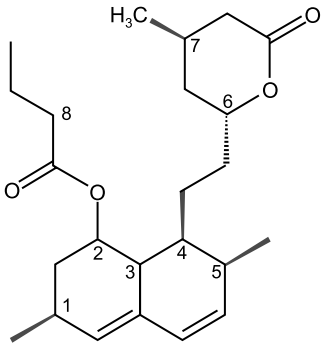
Sometimes, the most active drug is not taken to clinical practice because of the cost of synthesizing it. If a choice must be made between two drugs where one is slightly less

active than the other, but it is easier to synthesize, then the less active structure may be well selected for clinical trials.

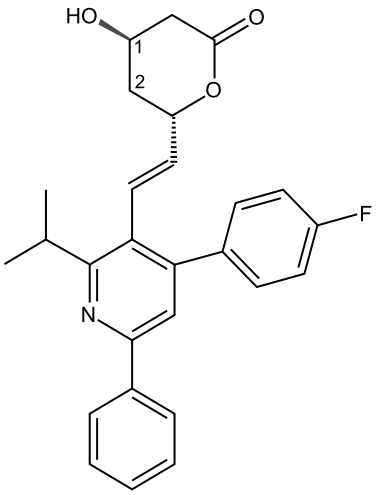
The industrial synthesis of drugs is efficient and economical. But these priorities are less critical during drug design. The main focus of drug design is to synthesize the drug as soon as possible; hence less consideration is given during drug design. But once it enters into large-scale production, economic factors, cost and safety of reagents, experimental procedures become more important and change the original synthetic method used to get a drug.

Example 1: Synthesis of chiral drugs. The easiest and cheapest method of synthesis of chiral drugs is to make racemates. If a chiral drug is marketed as a racemate, then there is a necessity to test both enantiomers, which increases the number of tests to be done and test cost. The enantiomers may have different activities. Hence, the usage of enantiomers are minimized, and the manufacturing process focused on separating the enantiomers or to carry out the asymmetric synthesis. Thus the drug goes back to the drug design phase to see whether it can be altered to reduce asymmetric centers.

Example 2: Cholesterol-lowering agent mevinolin has eight asymmetric centres, but later development leads to the formation of the drug candidate HRY80 with fewer asymmetric centres.



Mevinolin



Newer derivative of mevinolin

We may not be able to synthesize all drugs; many of the drugs obtained from natural sources are complex structures that are too difficult and more expensive to synthesize on an industrial scale.

Example, Penicillin, Taxol, etc. These kind of compounds obtained from natural sources is time-consuming, tedious, and expensive and further leads to the wastage of natural products. *For example*, a minimum of four mature yew trees are cutted for obtaining and the amount of taxol used to treat one person. Sometimes the semisynthetic process is used to counterpart the above process. The biosynthetic intermediate is obtained from the natural source, and the final compound is prepared by the conventional synthetic method. The intermediate can be easily obtained from the natural source in high yield by this method than the final compound and hence we can synthesize various analogues of the final compound. *For example*, synthesis of semi-synthetic Penicillins and Taxols.

Toxicity testing: Before entering the drug into the clinical trials phase, it should be tested for its toxicity. It should be done by *in vivo* and *in vitro* testing on transgenic mice or genetically modified cell cultures to examine the effects on cell-reproduction and carcinogenicity.

The toxicity of the drug can be determined by LD_{50} value; other than this, various toxicity tests are available.

Clinical Trials Implementation

It involves testing the drugs on volunteers and patients. There are four phases of clinical trials.

a. **Phase I studies:** Healthy volunteers are used to take the drug to check the potency, pharmacokinetics, and side effects.
b. **Phase II studies**: Drugs are tested on a small number of patients to check any effect and used to calculate the dose.
c. **Phase III studies:** In this study, the drug is tested on many patients and compared with placebo treatment or other alternative treatments. The comparative studies are carried out without bias and random selection of patients. The trials are carried out by double-blind technique in which neither the patient nor the investigators knows what treatment is applied.
   Phase III studies are used to determine whether the drug is really effective and also to study the beneficial effects or psychological effects. It is also used to identify and calculate the optimum dose.
d. **Phase IV studies:** In this phase, the drug is placed on the market to moniter the drug's effectiveness and any unexpected side effects.

Ethical issues: In phases I–III clinical trials, the patient's permission is essential and mandatory. It is obtained from the patient by a consent form.

Marketing of Drug

The successful products are prepared in a suitable formulation and distributed.

Historical Approaches in Drug Discovery

Today's system of treatments and medicines is based on traditional medicines exhibited in every continent of the world and every cultural part. The most popular ones are ayurvedic medicines in India, galenic medicines in Europe and traditional medicines in East-Asia (China).

Each one of these is inter-related, and they possess their principles and basic philosophy. Chinese-medicines practice is based on five important symbolic elements in the world. They are wood, fire, earth, metal and water. The generation of positive cycle proceeds as follows. Wood burns into the fire, and fire forms ashes that generate earth. Metal can be mined from the earth. If metal is heated, it melts like water that initiates plants' growth, and again plants give wood. The negative susceptible to change cycle is complementary to the positive one. The Chinese medicines and treatments are based upon this Ying-Yang or five elements theory. They are considered the vital internal organs of the human body and are related to these five elements.

In India, the medical treatment is based upon the ayurvedic, Siddha and Unani systems of medicines. The word 'Ayurveda' described that Ayu means 'life' and Veda

means "knowledge." Compared to modern allopathy medicines, ayurveda composed of some fundamental principles called darshanas, representing the seven dhatus. They are considered as human body constituents. The three critical doshas are vata, pitta and kapha. A balance of these three doshas is essential for maintaining normal health and any imbalance in these doshas leads to disease. The ayurvedic treatment is based not only on these three doshas but also on the individual constitution and various environmental factors.

The traditional European medicine system is based on Egyptian and Babylonian Assyrian culture.

Around 500 BC, the Greek philosophers Aristotle, Pythagoras and Empedocles were developed and influenced the European medicine. The basic principle behind these traditional European medicines involves three elements such as water, air, fire and its relation to man. Any disturbances of these four lead to disease. In 400 BC, the famous physicians Hippocrates and Galenus influences and dominated Western medicines; hence the basic principle is known as Galenism. During this period, the great physician Hippocrates developed four humors that are parallel to four fundamentals. The four humors were:

1. Blood (coming from the heart)
2. Phlegm (supposed to come from the brain)
3. Yellow bile (coming from the liver) and
4. Black bile (coming from the stomach and spleen).

Each one of these are connected with others.

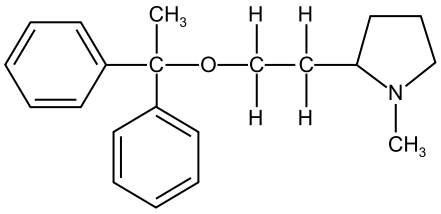
The European medicine system has been greatly influenced by the Arabian system of medicine as particularly of the physician Ali Ibn Sina. In many countries, the canon of medicine affect European medicines. In the 12th century, saint Hildegard of Bingen was a famous pharmacist and physician who influenced European medicine. She wrote many books about humans, their illness, and treatment with herbal drugs. In the 15th century, Paracelsus (Theophrastus Bombastus) reformed the European system of medicines. The Latin term "Ubimalum ibiremedium" indicates that the herb's shape or colour describes against which disease the plant drug can be used.

When compared with Ayurveda and Chinese medicines, the European medicine system no longer exists, and it became the history of medicine. Now-a-days the modern European medicine is based on allopathic system such as modern western medicine.

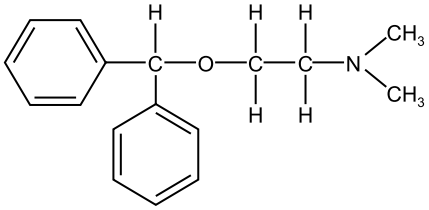
Bioisosterism

The correlation between biological properties and physicochemical properties of individual atoms, functional moiety molecules is difficult because the simultaneous involvement of many properties and the quantification is also difficult. Friedman first coined the term *bioisosterism*, and defined as "**functional groups/molecules which possess similar physical and chemical properties producing broadly similar biological activities**." Burger expanded this definition as "**bioisosters are compounds which have near equal shapes and volumes, the approximately same distribution of electrons and exhibit similar biological activities**." These compounds interact with the same biological systems or targets as agonists or antagonists hence produces similar biological activities. Bioisosterism is an important phenomenon for modifying the lead compound used to modify the pharmacological activity or attenuate the toxicity.

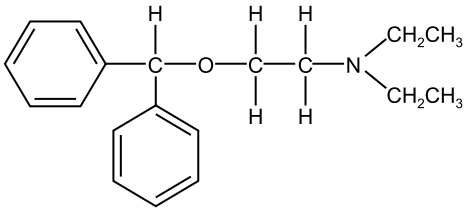
For instance, it is always preferable to have small compact substituents on the terminal nitrogen among antihistamines.



A Clemastine pyrrolidine analogue



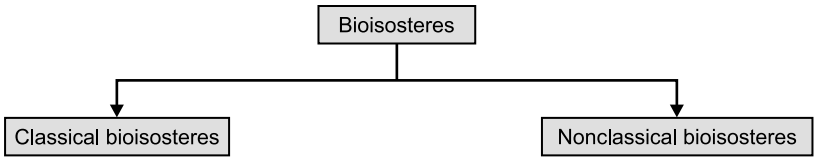
B Diphenhydramine dimethyl analogue



C Diethyl analogue of diphenhydramine

In the above three structural analogues, it has been observed that '**A**' possesses twice the activity of '**B**'. In contrast, it shows an activity many times greater than that of the open-chain diethylamino analogues '**C**'.

Classification



A. Bioisosteric monovalent atoms and groups
1. Hydrogen atom vs fluorine atom replacement
2. Bioisosteric replacement of -OH groups with amino group
3. Interchange of hydroxyl and third groups
4. Replacement of chlorine with methyl

B. Divalent atoms and groups
C. Trivalent atoms and groups
D. Tetravalent atoms and groups
E. Ring equivalents
1. Divalent ring equivalents
2. Trivalent ring equivalents

A. Cyclic vs Noncyclic
B. Nonclassical bioisosteric replacement of functional groups
1. Bioisosteric replacement of carboxylic acid group
   (a) Replacement involves -OH group
   (b) Replacement involving -COOH group
2. Bioisosteric replacement of carbonyl group
3. Bioisosteric replacement of catechol ring system
4. Bioisosteric replacement of ester
5. Bioisosteric replacement of hydroxyl group
6. Bioisosteric replacement of halogen atoms
7. Bioisosteric replacement of thioether
8. Bioisosteric replacement of thiourea
9. Bioisosteric replacement of azomethine
10. Bioisosteric replacement of spacer group

Classical bioisosteres

Functional moieties that satisfy the original conditions of Longmuir and Grimm rule are known as classical bioisosteres. In 1919, Irving Langmuir, described "**compounds or groups of atoms with the same number of atoms and electrons possess similar physico-chemical properties**." *For example*, N_2 and CO, N_2O and CO_2, etc. These are known as isosteres. In 1920, H G Grimm further developed this and found out, minimizing or inhibiting the capacity of some chemical groups over the other groups. Accordingly, 'the addition of a hydride ion to an atom gives the resulting pseudo atom', the properties of the atom with the next highest atomic number. The Grimms hydride displacement law is indicated in Table 1.2.

Table 1.2: Grimms hydride displacement law

C	N	O	F	Ne	Na^+
	CH	NH	OH	FH	–
		CH_2	NH_2	OH_2	FH_2^+
			CH_3	NH_3	OH_3^+
				CH_4	NH_4^+

In Table 1.2, each vertical column indicates an isostere.

Hans Erlenmeyer, in 1932 extended the definition given by Grimm to "**isosteres as atoms, groups, ions and molecules in which the valence electrons are identical**." It is described in Table 1.3.

Table 1.3: Number of valance electrons and the concerned atoms

Number of valance electrons and the concerned atoms				
4	*5*	*6*	*7*	*8*
N^+	P	S	Cl	ClH
P^+	As	Se	Br	BrH
S^+	Sb	Te	I	IH
As^+		PH	SH	SH_3

Bioisosteric monovalent atoms and groups

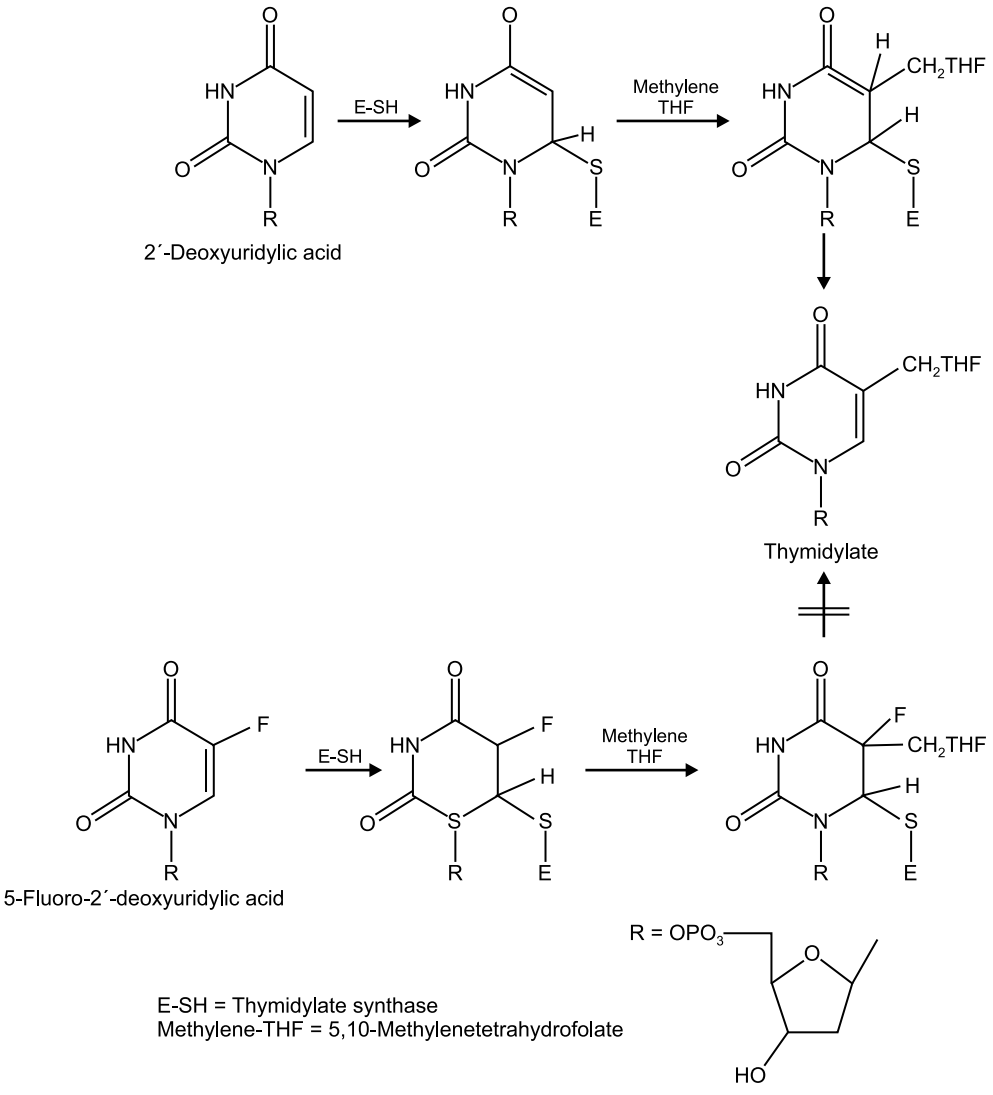
- F, H
- OH, NH
- F, OH, NH or CH_3 for H
- SH, OH
- Cl, Br, CF_3

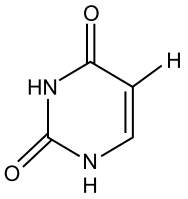
Let us discuss these monovalent atoms and groups in detail.

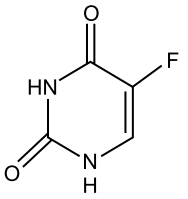
1. **Hydrogen atom vs fluorine atom replacement:** Both H and F atoms are with steric parameters but differ in their electronic configuration. Fluorine atom is a highly electronegative element than hydrogen in the periodic table. This difference leads to a significant difference in their properties. Due to high electronegativity of fluorine,

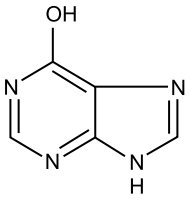
it exhibits inductive effect (electron attraction) as well as mesomeric effect (electron donation/lone pair donation). The pharmacological differences are due to the effect of electron-withdrawing property that fluorine substitution leads to the interaction with a biologically active target and influences the drug metabolism.

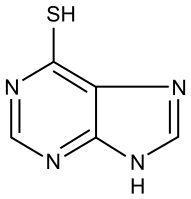
For example, 5-Fluoro uracil, i.e. 5-Fluoro-2-deoxy uridylic acid is responsible for the inhibition of thymidylate synthase enzymes involved in converting uridylic acid to thymidilic acid and is critical for DNA synthesis. The increased activity of 5-Fluoro-2-deoxy uridylic acid relative to 5-FU is due to the inductive effect of "F" atom and forms a strong covalent bond with the enzyme than hydrogen thymidylate synthetase.



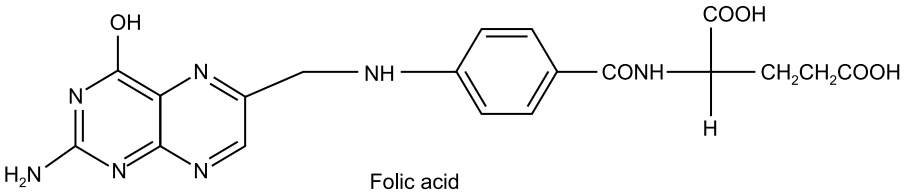

Uracil

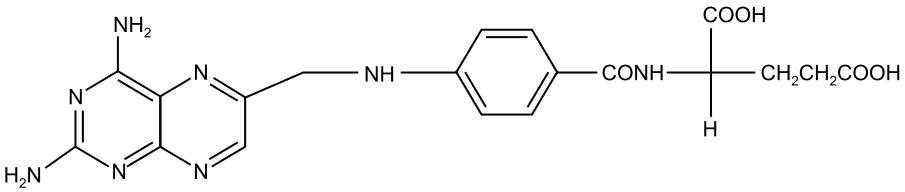

5-Fluorouracil


Hypoxanthine


6-Mercaptopurine

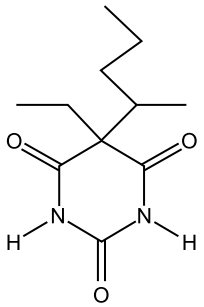
2. **Bioisosteric replacement of –OH group with the amino group:** The biosteric replacement of –OH group by amino group increases the pharmacological activity. The presence of electron-donating groups such as –N in heterocycles leads to tautomerization (C–OH to C=O). The ability of amino group to form hydrogen bond with the enzyme leads to increased metabolic activity.

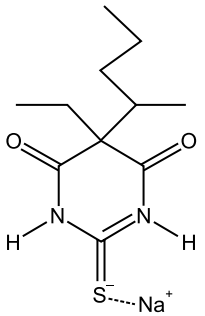

Folic acid


Aminopterin

Aminopterin –NH_2 group facilitates hydrogen bond with enzyme DHR.

3. **Interchange of hydroxyl and thiol group:** The replacement of –OH group with thiol group enhances the activity due to the size of the substituent, van der Walls radius and hydrogen bonding.
   For example, barbiturates like pentobarbital and thiopental sodium.

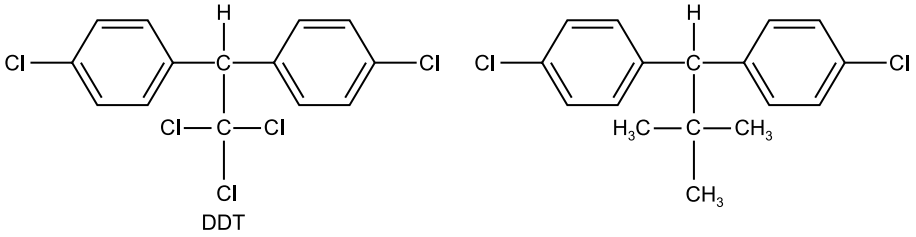

Pentobarbital


Thiopental sodium

Thiopental sodium is more active than pentobarbital with shorter duration of action.

4. **Replacement of chlorine with methyl:** The chlorine atom is isosteric and lipophilic with –CH_3 group because its ability to alter the metabolism. This replacement

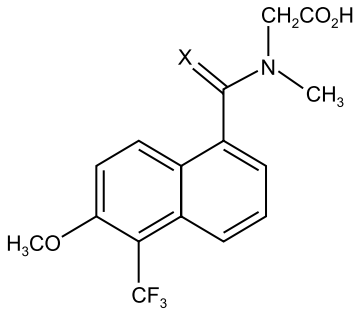
increases the xenobiotic metabolic rate. Lipid soluble chemicals are distributed into the adipose tissue unless they are metabolized. They tend to accumulate for more extended periods. *For example*, in DDT, the replacement of 'Cl' by *tert*-butyl group, increases metabolic degradation and reduces the persistence of pesticide effect.



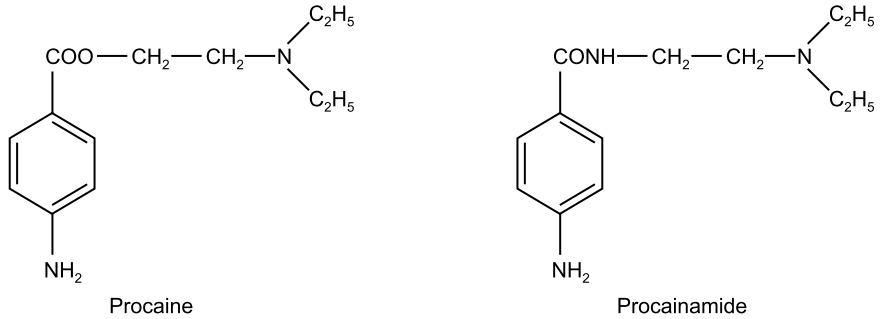
Divalent atoms and groups:

–C=S, –C=O, –C=NH, –C=C–

The replacement of the C=S group with C=O group lead to an increase in pharmacological activity. *For example*, Tolrestat and Oxotolrestat. Drugs inhibit the aldose reductase activity due to the replacement C=S by C=O in oxotolrestat resulted in lesser inhibition of activity in both *in vitro* and *in vivo*.



Replacement of C=O by C=NH

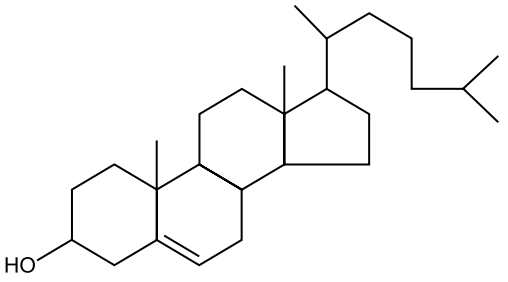


Trivalent atoms and groups:

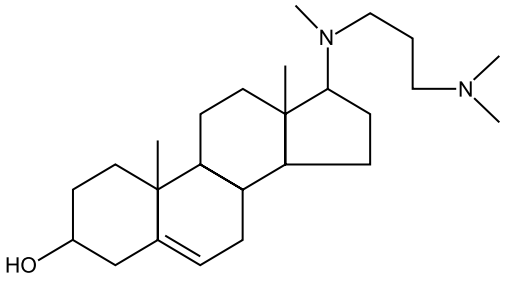
–CH=, –N=

–P=, –As=

Replacement of –CH= with –N: The replacement of CH= with –N in cholesterol leads to 20, 25-Diazacholesterol, a potent inhibitor of cholesterol synthesis due to the greater electronegativity of nitrogen atom.

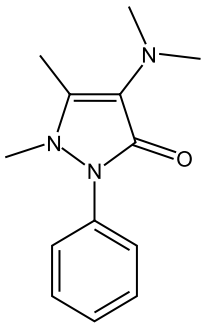


Chloesterol

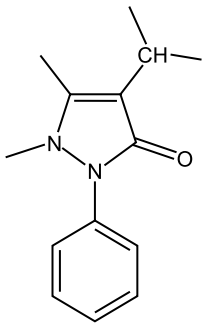


20,25-Diazacholesterol

4-Dimethylamino antipyrine and its carbaisostere possesses equal antipyretic activity.



4-Dimethylaminoantipyrine



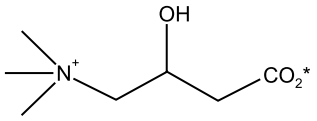
4-Isopropylantipyrine

Tetravalent atoms and groups

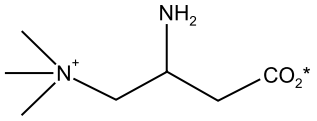
$=N^{+}=$, $=C=$, $=P^{+}=$, $=As^{+}=$

Replacement of tetravalent trimethyl ammonium group by *tert*-butyl group.

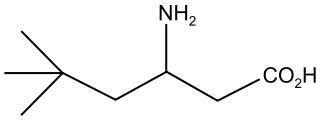
Acyl carnitine analogues: These are carnitine acyl transferase inhibitors. The bioisosteric replacement of –OH group of carnitine by amino group (b) and replacement of tetravalent trimethyl ammonium group with a *tert*-butyl group (c) alters the inhibitory activity.



(**a**)



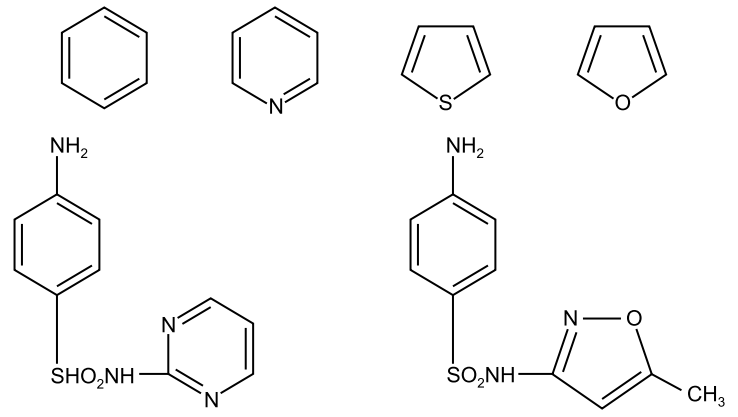
(**b**)

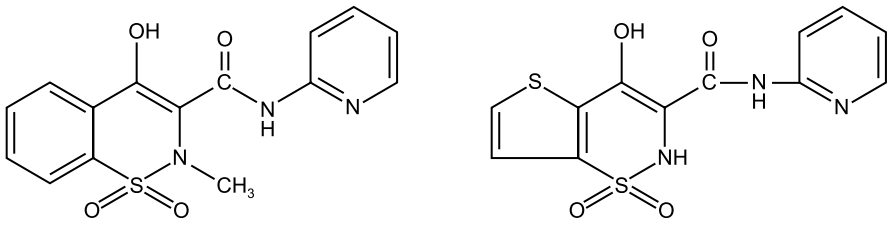


(**c**)

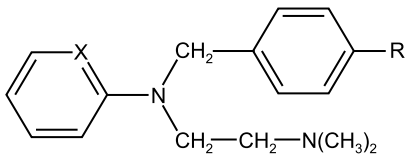
Terta-substituted atoms

Ring equivalents: The classical isosteric replacement principle iso is applied to the ring system leads to the development of effective biosteres. The bioisosteric replacement of benzene, pyridine, thiophene gives analogues with different series of pharmacological activities. *For example*, sulfamethoxazole is more potent than sulfadiazine.



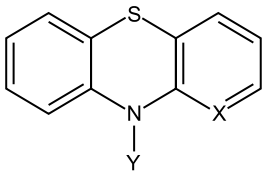


Tenoxicam is more potent than Piroxicam.



Antergan X = CH, R = H
Mepyramine X = N, R = OCH_3

Mepyramine is more potent than antergan.



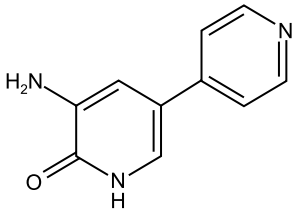
Promethazine X = Ch, Y = $CH_2CH(CH_3)N(CH_3)_2$
Isothipendyl X = N, Y = $CH_2CH(CH_3)N(CH_3)_2$
Promazine X = CH, Y = $CH_2CH(CH_3)N(CH_3)_2$
Prothipendyl X = N, Y = $CH_2CH(CH_3)N(CH_3)_2$

The replacement of benzene by pyridine increases the activity in tricyclic antihistamines. *For example,* promethazine and isothipendyl.

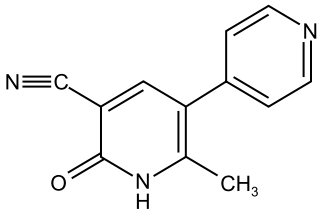
In neuroleptics, the replacement of benzene by pyridine reduces both sedative and extrapyramidal activity, example promazine and prothipendyl.

Ring equivalent of bioisosteres are classified into two types.

1. **Divalent ring equivalents**: The bioisosteric replacement of divalent ring equivalents with different ring systems alters the biological activity. *For example*, amrinone and milrinone-cardiotonic agents. The positive vasodilatory action of amrinone and milrinone is due to adenosine-3′, 5′-cyclic phosphate phosphodiesterase-III enzyme inhibition. SAR studies of amrinone shows that the free amino group is not required for the *in vitro* cAMP PDE-III activity.

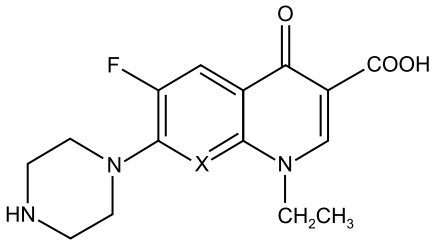


Amrinone



Milrinone

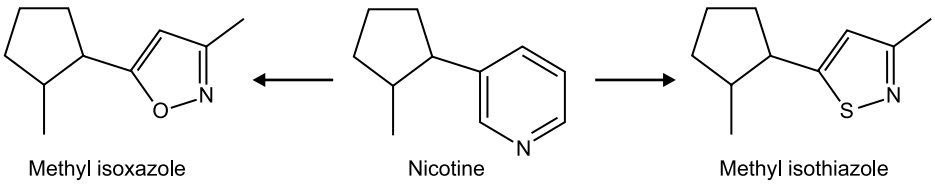
2. **Trivalent ring equivalents**: Example: Norfloxacin and Enoxacin within antibacterial activity.



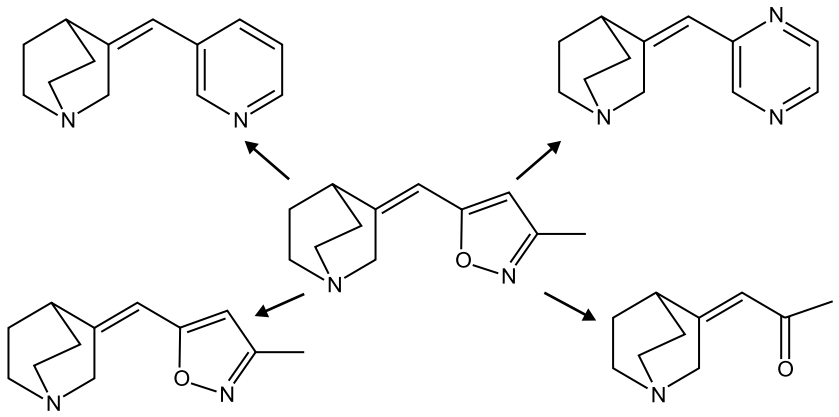
Norfloxacin X = –CH–
Enoxacin X = –N–

Other examples for ring equivalent bioisosteres

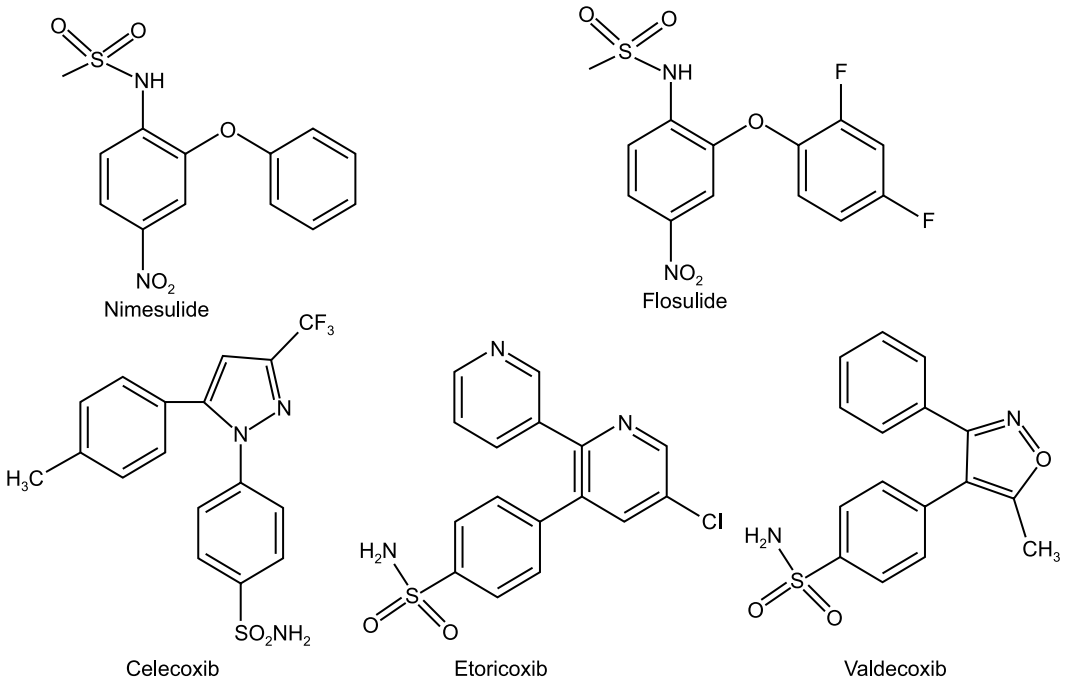
Bioisosteres of pyridine: The pyridine ring present in nicotine is replaced by other rings such as methyl-iso-oxazole or methyl isothiazole. The bioisosteric replacement of isoxazole ring in the 3-methyl-5-isoxazolyl by pyridine, pyrazine, oxadiazole or acyl group increases the affinity to some nicotinic receptors.



Methyl isoxazole Nicotine Methyl isothiazole



Bioisosteres of other heterocycles: Bioisosteres of some other heterocycles are mentioned below.

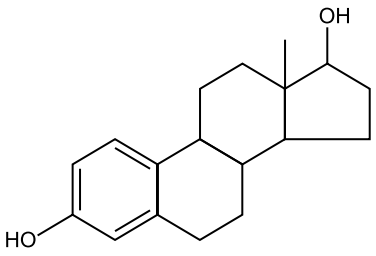


Nonclassical bioisosteres

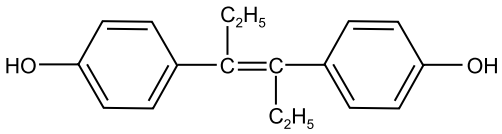
This class of isosteres includes all the bioisosteric replacements which are not defined. These are having the ability to maintain similar biological activity.

Cyclic vs noncyclic: The replacement of rings B and C of the oestradiol leads to hexesterolin, which depends on the configurational aspects. The diastereomer E is superior to diastereomer Z with less estrogen activity. A similar activity was also observed in the dehydrogenated diethyl stilbesterol. *For example*, diethyl stilbesterol (DES), the central bond is important for the exact binding of ethyl groups to the estrogen receptors. But the other

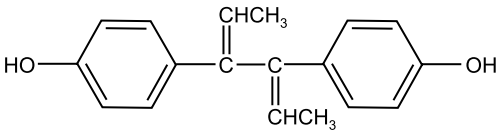
analogues (C-2 to C-4) possess equipotent activity whereas estradiol nonrigid analogues (C-5 to C-7) have no estrogenic activity.



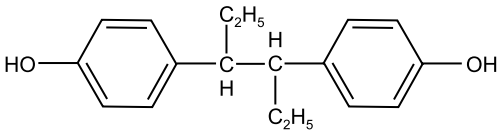
Oestradiol
(1)



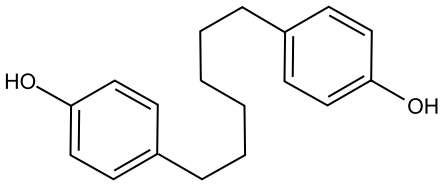
Diethylstilbestrol
(2)



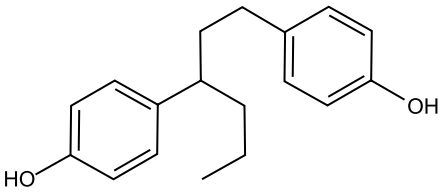
Dienestrol
(3)



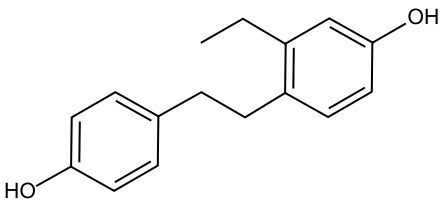
Hexesterol
(4)



1,6-bis-(*p*-hydroxyphenyl)hexane
(5)



1,6-bis-(*p*-hydroxyphenyl)hexane
(6)

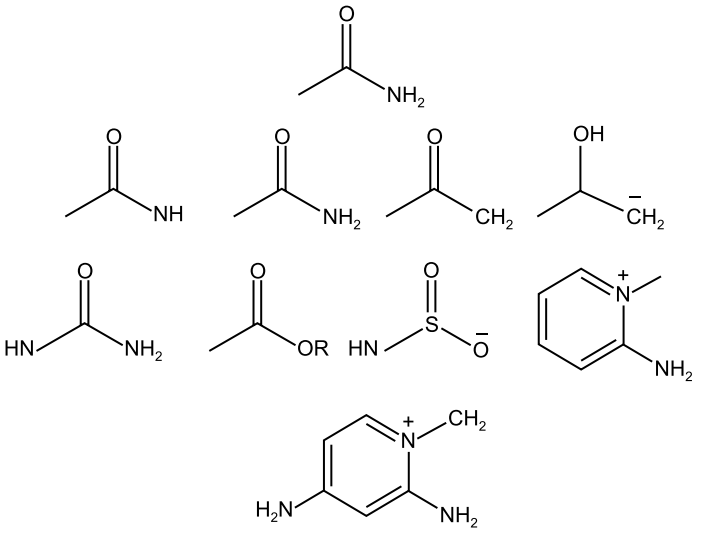


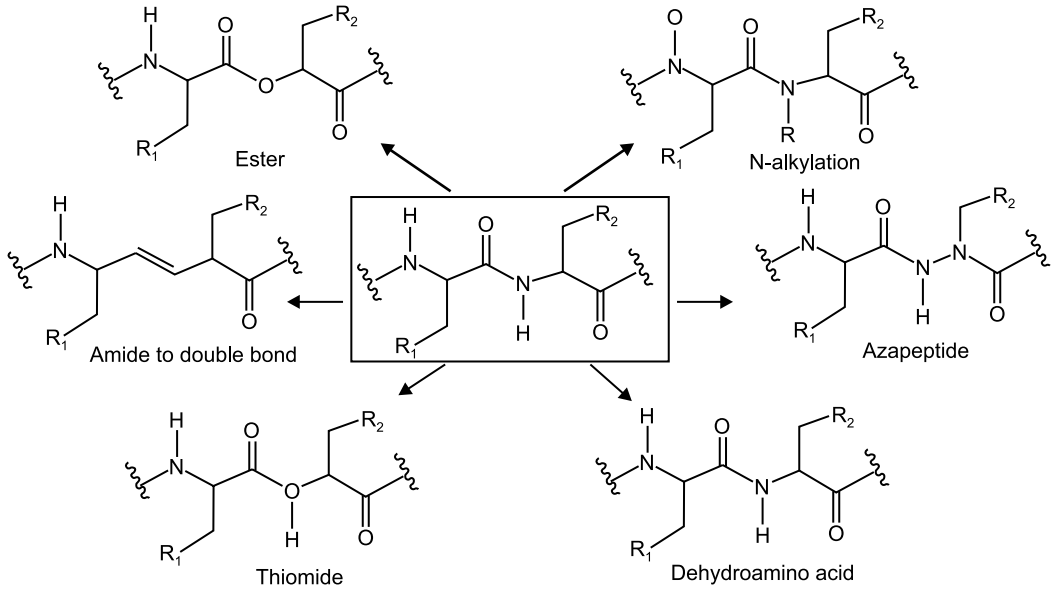
1,6-bis-(2-ethyl-4-hydroxyphenyl)ethane
(7)

Biosteric replacement for the amide is done because of its implications in peptide chemistry. Peptide bonds and peptide fragments are replaced with a variety of structural moieties to covalent peptides into chemically stable and orally available molecules.

Nonclassical bioisosteric replacement of functional groups

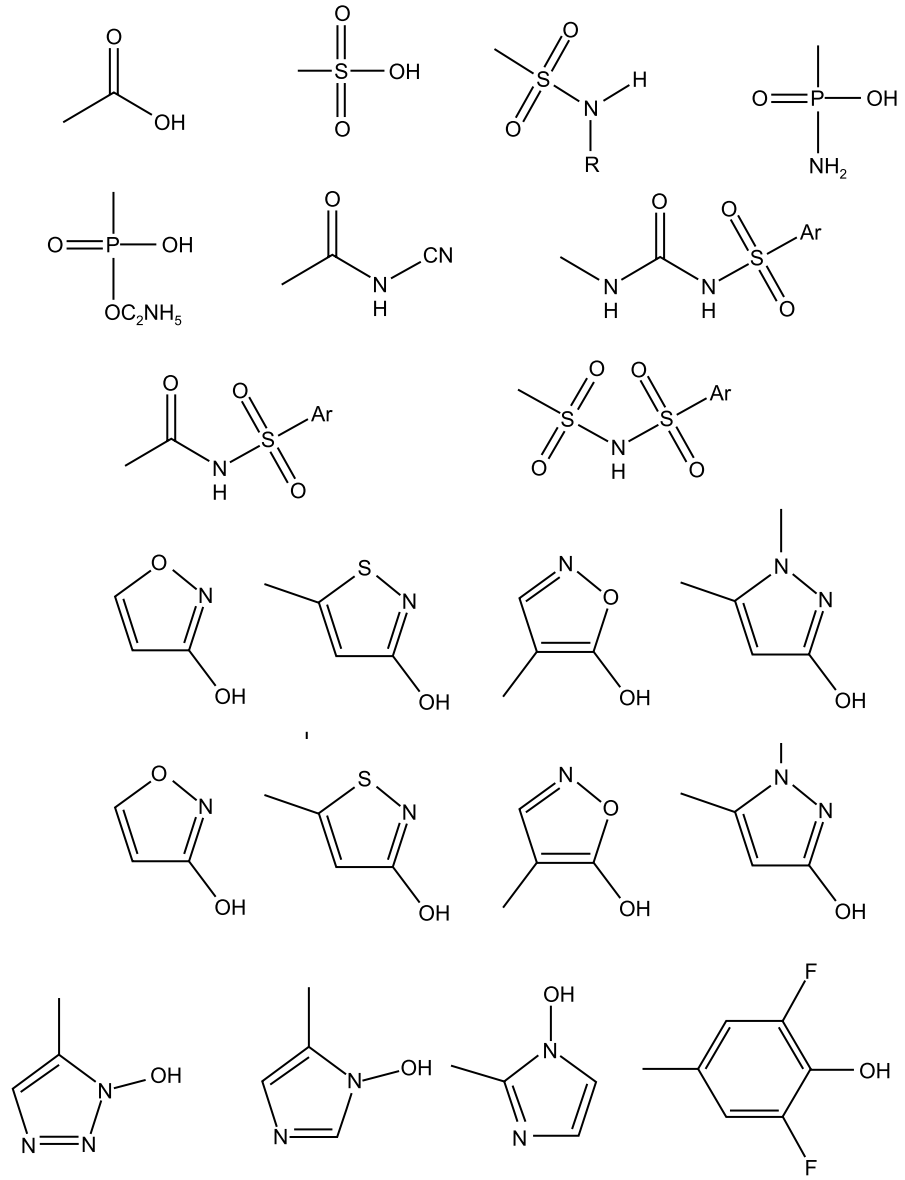
1. *Bioisosteric replacement of amide group:* Bioisosteric groups for amides are described below.



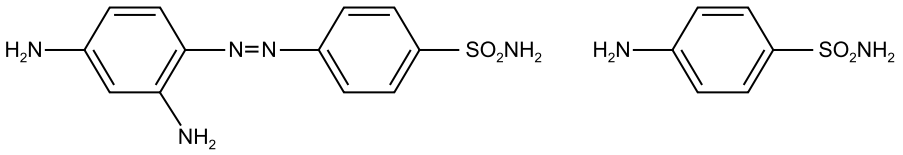


Amide group bioisoteres

2. *Bioisosteric replacement of carboxylic acid group:* Non-classical bioisosteric replacement of carboxylic group involves the following.

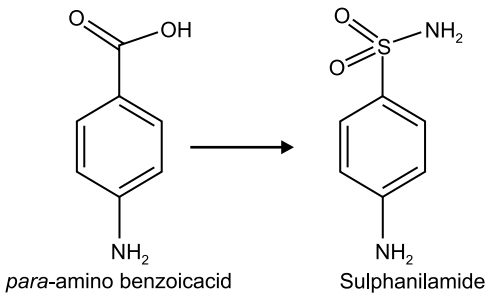


a. **Replacement involves only—OH portion:** Not altering the pharmacological activity.
b. **Replacement involving—COOH group:** Prontosil and sulphanilamide



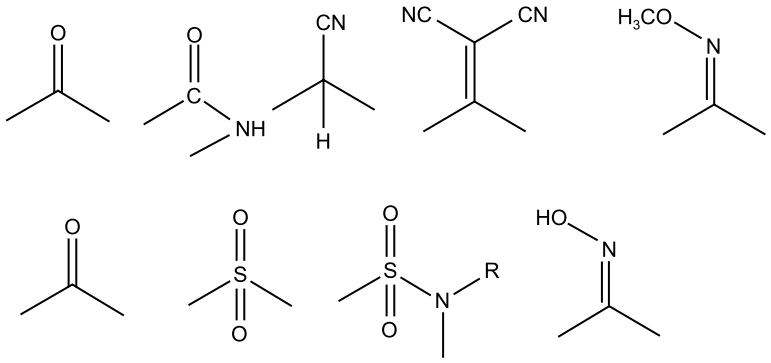
Prontosil

Sulphanilamide



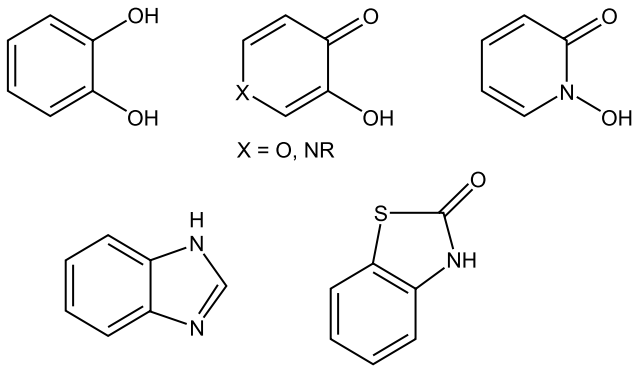
Carboxylic group bioisosteres

3. *Bioisosteric replacement of carbonyl group:* The bioisosteric replacement of carbonyl group used primarily for the aldehyde and ketone moiety. Some of the groups are depicted below.

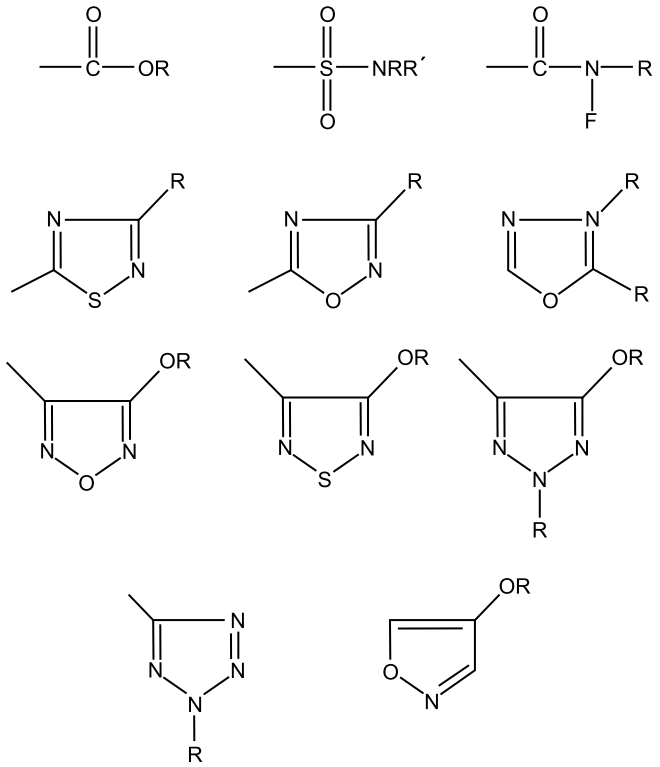


For example, non-insulin-dependent diabetes mellitus treated by sulphonyl ureas, which possess different activity.

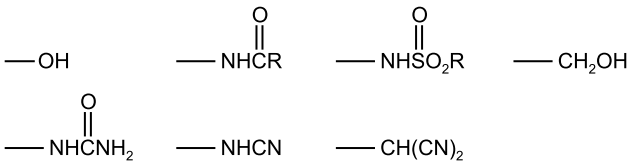
4. *Bioisosteric replacement of catechol ring system:* Catechol ring can be replaced by the following groups.



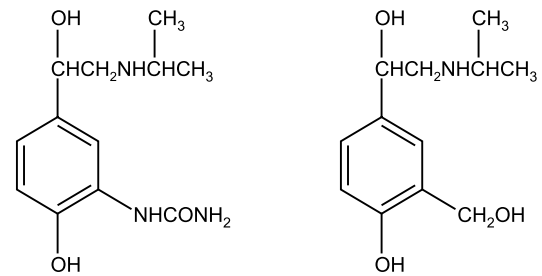
5. *Bioisosteric replacement of esters:* Esters are replaced by the following groups.



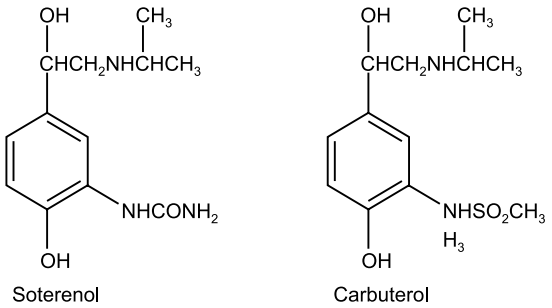
6. *Bioisosteric replacement of hydroxyl group:* Hydroxyl group can be replaced by the following groups.



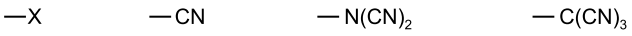
Drugs example: Adrenoceptor agonist, isoproterenol is a bronchodilator, in which a 3-hydroxyl group is replaced with a biosteric group, leading to albuterol, isoproterenol and carbuterol, leads to the derivatives with potent and selective activities.

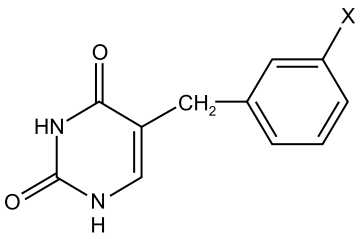


Isoproterenol Albuterol

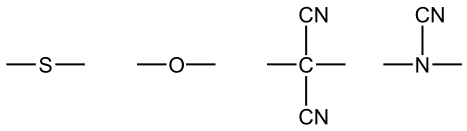


7. *Bioisosteric replacement of halogen atoms:* Halogen atoms are replaced by the electron-withdrawing groups such as cyano or trifluoromethyl group. *For example*, 1-[(2-hydroxy ethoxy)methyl-5-benzyl uracil tested for inhibition of uridine phosphorylase is an enzyme responsible for reversible phosphorolysis pyrimidine nucleosides. It catalyzes the degradation of 5-fluoro-2′-deoxyuridylic acid. In 5-benzyl uracils, the C-3 position is substituted with electron-withdrawing group which decreases the activity. The replacement of the 'Cl' atom by the strong electron-withdrawing groups such as CN, CF_3 group leads to less potent analogues.

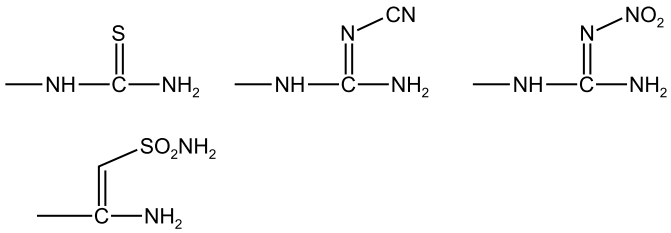




8. *Bioisosteric replacement of thioether:* Thioether group can be replaced by the following groups as mentioned below.

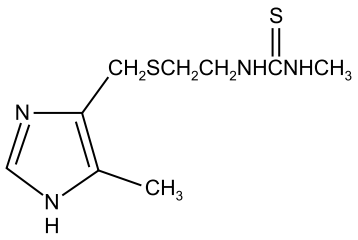


9. *Bioisosteric replacement of thiourea:* It is successfully applied to the development of H_2-receptor antagonist involved in peptic ulcer disease.

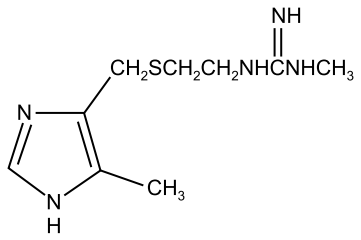


Drugs example: Metiamide (1) contains thiourea moiety that is responsible for the agranulocytosis, replacement of this group with guanidine group (2) leads to

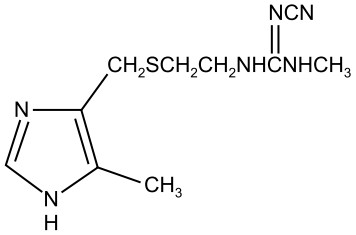
absorption problems because of its high degree of ionization at physiological pH. Further, bioisosteric replacement of guanidine group with cyano guanidino group leads to cimetidine (3) which is two times more active than metiamide in gastric acid inhibition. The other examples are famotidine, ranitidine and nizatidine.



(1)

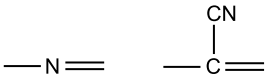


(2)

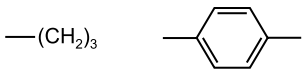


(3)

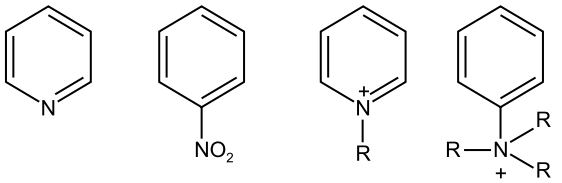
10. *Bioisosteric replacement of azomethine:* Azomethine group can be replaced by the following groups as mentioned below.



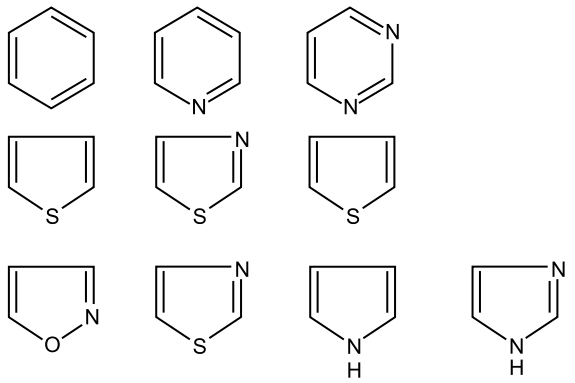
11. *Bioisosteric replacement of spacer group:* Spacers can be replaced by the following groups as mentioned below.



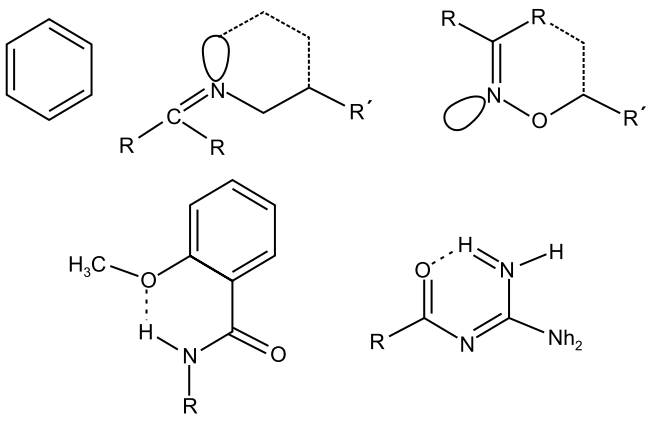
Pyridine



Benzene



Ring Equivalents



Effect of bioisosteric modifications

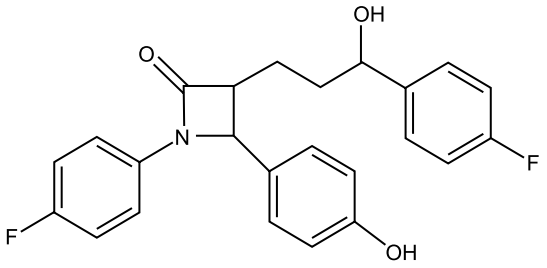
Bioisosteric modifications convert a molecule that may have one or more of the following effects.

1. *Structural:* If a functional group is replaced by a bioisostere that has a structural role containing other moieties in a particular geometry, then shape, size, and hydrogen bonding will be important.
2. *Receptor interaction:* When the moiety that is replaced is involved in a specific interaction with receptors or a particular enzyme, except lipid and water solubility, the other factors will be necessary.
3. *Pharmacokinetics:* If the moiety replaced is needed for pharmacokinetics' properties such as absorption, transportation and excretion, the compounds should have hydrophilicity, lipophilicity, pKa, and hydrogen bonding will be important.
4. *Metabolism:* If the functional moiety or group replaced is involved in preventing or aiding metabolism, then the chemical reactivity will be important.

Some other examples for bioisosteric replacement:

1. *Replacement of hydrogens/OCH_3 by fluorine:* The replacement of hydrogen by fluorine is used to increase the drug's metabolism by altering the pKa value of the nitrogen atom

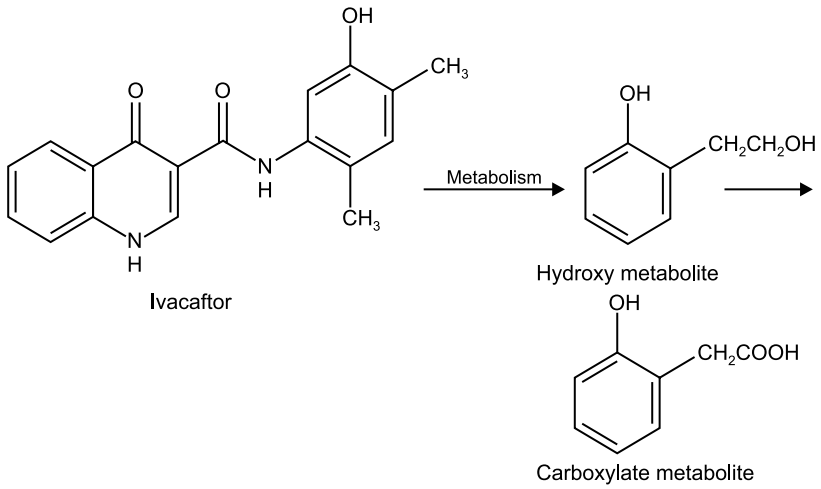
present. This replacement is used to increases solubility. *For example*, fluorine atom present in ezetimibe (anticancer agent) with its metabolite decreases the oxidative metabolism potential.



Ezetimibe

ED_{50} in dog = 0.007 mg/Kg

2. *Replacement of hydrogen by deuterium:* This type of bioisosteric replacement is commonly used to decrease the metabolic rate of the drug. *For example*, Ivacaftor used to treat mutated cystic fibrosis. It is metabolized and yields hydroxy and carboxylate metabolites, which are less active, but the ivacaftor's deuterated analogue possesses similar *invitro* activity and reduced metabolic rate. It does not exceed the level of the parent compound also.

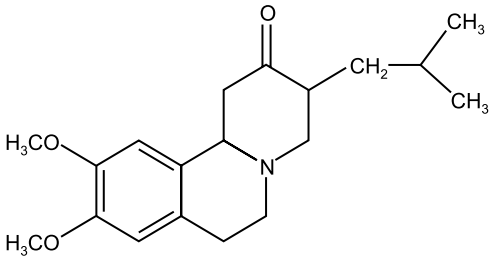


Ivacaftor

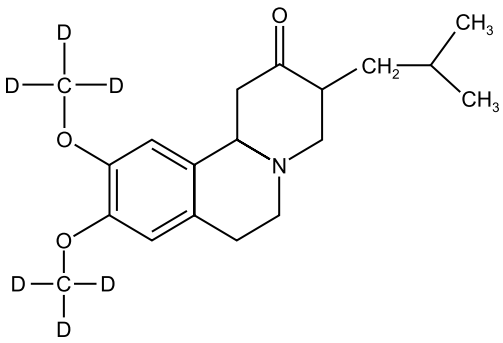
Hydroxy metabolite

Carboxylate metabolite

Example: **Tetrabenazine:** It is a drug used for the treatment of Huntington's disease. The drug is absorbed well, and it has less bioavailability. But $t_{1/2}$ of deutetrabenazine is double than that of the parent drug; hence it can be administered three times a day at lower doses. By this, the toxicity is reduced, and the efficacy of the drug can be increased.

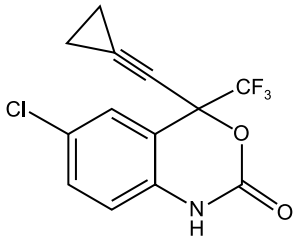


Tetrabenazine



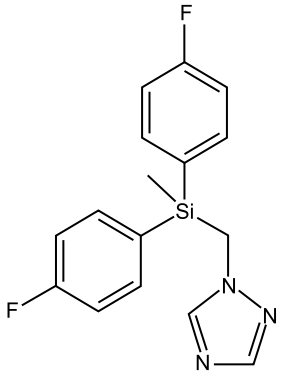
Deutetrabenazine

Example: **Efavirenz:** An anti-HIV drug undergoes metabolism and gives glutathione conjugate, which produces nephrotoxicity. But the deutrated analogue (deutration at cyclopropyl group) decreases the formation of toxic metabolite.

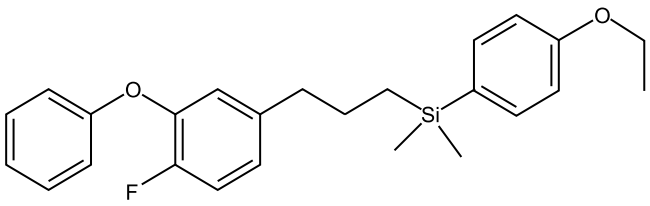


Efavirenz

3. *Replacement of carbon by silicon:* Flusilazole is used as an antifungal agent, but its bioisostere silafluofen is used as an insecticide.



Flusilazole



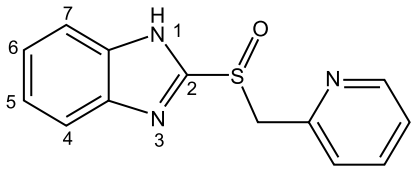
Silafluofen

Case Studies

Drug discovery and development of pantoprazole as proton pump inhibitors

Proton pump inhibitors (PPIs) are the drugs of choice for the treatment of peptic ulcer and gastro-oesophageal disease. They act by binding with H^+/K^+ ATPase and inhibit the acid secretion in the stomach. They also bind with the proton pumps present in the cancerous cells and reduce the drug resistance in chemotherapy.

Timoprazole: The drug discovery of pantoprazole is a good example of step-by-step discovery and development of PPIs. In 1975 the first drug timoprazole was discovered for its inhibitory action against gastric acid secretion. But it induces thyroid gland enlargement due to iodine intake inhibition and thymus gland atrophy. Timoprazole is a pyridyl methyl sulphinyl benzimidazole structure. Optimization of this benzimidazole moiety such as mercapto benzimidazole had no effect on iodine uptake; hence the substitution -SH group into timoprazole reduces the toxicity without affecting the antisecretory efficacy.



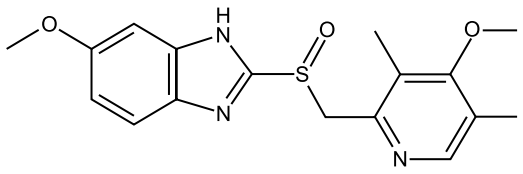
Timoprazole

Hence, the optimization of benzimidazole moiety of timoprazole was focused on developing other PPIs. The introduction of a trifluoromethyl group to benzimidazole leads to active compounds with different solubility. Further, the stability and half-life also increased by the replacement of CF_3 by fluoroalkoxy group. Further studies have shown that the active moiety responsible for the action of PPIs was cyclic sulphonamides, which are formed in acidic conditions, indicate minor modifications on the backbone of timoprazole lead to developing a promising class of PPIs. But the intramolecular rearrangement of benzimidazole into sulphonamides includes several geometric constraints; hence the optical molecules were stable at neutral pH but easily activated at low pH. Since 1985, it was aimed to develop a compound with good stability at neutral pH, which can sustain the higher level of stability at lower level pH 5 but being rapidly activatable at lower pHs and combined level of H^+/K^+ ATPase inhibition. Due to the synthesis and evaluation of numerous compounds, the compound with highly fulfilled criteria, the most important candidate, pantoprazole and its salts were developed.

In 1986, the sesquihydrate salt form of pantoprazole was developed and synthesized; from 1987 onwards, the sodium salt was synthesized, which is highly stable and more compatible with other excipients used in the formulation. Finally, after 14 years of discovery and development (identification: 7 years and clinical trials: 7 years) pantoprazole was first introduced in 1994 to the German market. The good solubility and high solution stability of pantoprazole allowed it to become the first marketed PPI for IV infusion in critical care patients.

Development of various PPIs are described below.

Omeprazole



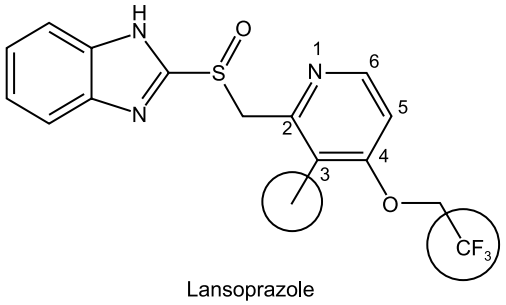
Omeprazole

In 1988, omeprazole was the first marketed PPI drug. This was developed by introducing two methoxy groups at the C-6 and C-4 position of the benzimidazole and pyridine, respectively as well as two methyl groups at C-3 and C-5 of the pyridine system of the omeprazole. It is available in the form of 1:1 racemate form.

Lansoprazole

It was the second PPI drug introduced in the European market in 1991 and the USA market in 1995.

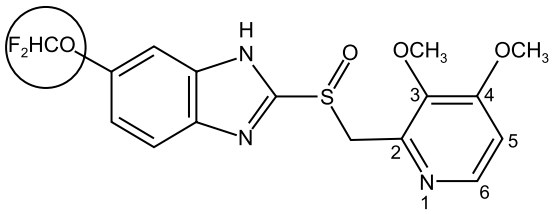
The introduction of the methyl group and the trifluoromethoxy group at C-3 and C-4 position of pyridine ring gives Lansoprazole. There is no substituent at benzimidazole ring. It is also available as a 1:1 ratio of racemic mixture (enantiomer) such as Levolansoprazole and Dexlansoprazole.



Lansoprazole

Pantoprazole

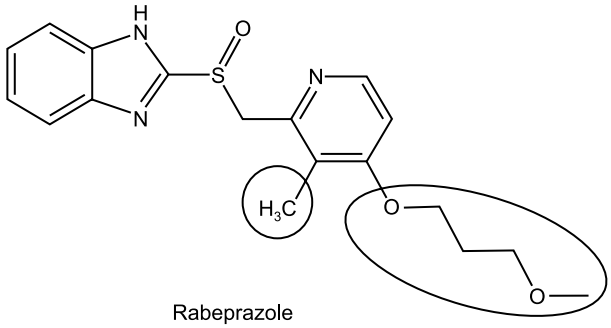
It was the third PPI and first introduced into the German market in 1994. The introduction of difluoromethoxy (-$OCHF_2$) group at benzimidazole moiety and two methoxy (-OCH_3) groups at C-3 and C-4 position of pyridine ring leads to the development of pantoprazole.



Pantoprazole

Rabeprazole

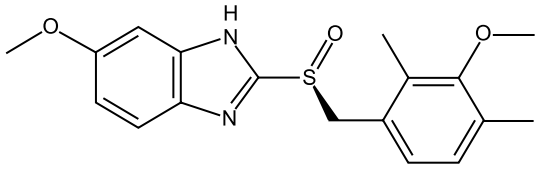
It was introduced into the USA market in 1999. Introduction of methyl group at C-3 and methoxypropoxy substitution at C-4 positions of pyridine ring gives rabeprazole. It is marketed as sodium salt.



Rabeprazole

Esomeprazole

It was introduced to the USA market in 2001. It is an *S*-enantiomer of omeprazole with enhanced efficacy and higher solubility.

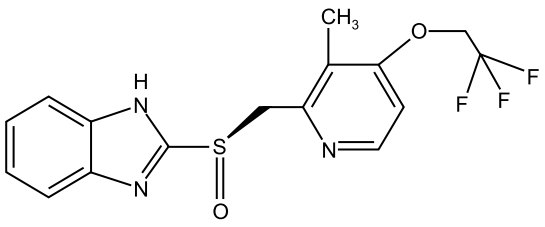


Esomeprazole

(*S*)-(–)-isomer is more active in humans, while *R*-(+)-isomer is more active in rats and both enantiomers show equipotent activity in dogs.

Dexlansoprazole

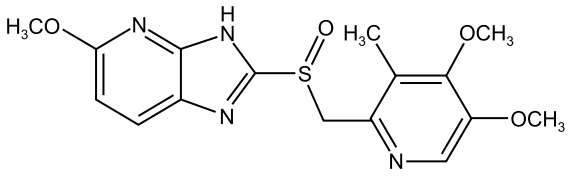
It was introduced to the market in 2009. It is a (*R*)–isomer of lansoprazole. The main advantage of this drug is it reaches 80% circulating drug availability after oral administration and it has also dual release technology. The first quick release of the drug leads to plasma peak concentration within one hour of application and the 2nd retarded release leads to another peak about 4 hours later.



Dexlansoprazole

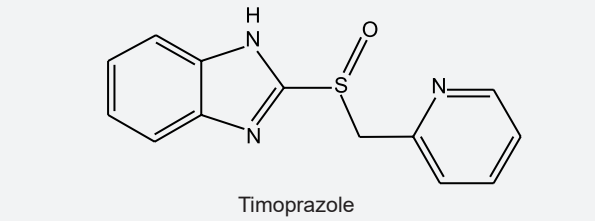
Tentoprazole (imidapyridines)

It was developed by the bioisosteric development of the benzimidazole ring of PPIs by imidazo [4,5*b*]pyridine moiety. It is a novel class of PPI drug with 7 hours of plasma half-life. The imidazo [4,5*b*] pyridine ring decreases the metabolic rate, increases the plasma residue time and decreases the pKa.



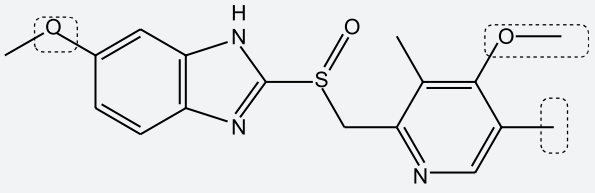
Tentoprazole

Schematic representation of the development of proton pump inhibitors



Timoprazole

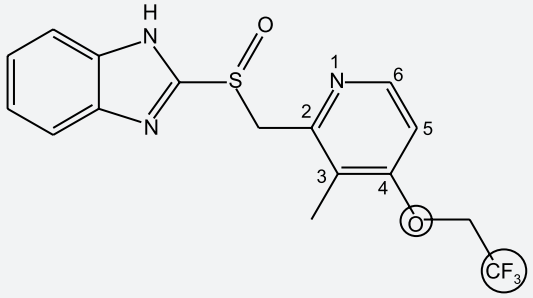
Served as the lead compound



Omeprazole

Omeprazole developed by the introduction of two methoxy groups at the C-6 and C-4 position of the benzimidazole and pyridine respectively. Two methyl groups at C-3 and C-5 of the pyridine backbone of the timoprazole. Available in the form of 1:1 racemate.

Side effects: Abdominal pain, diarrhoea, headache, dizziness.

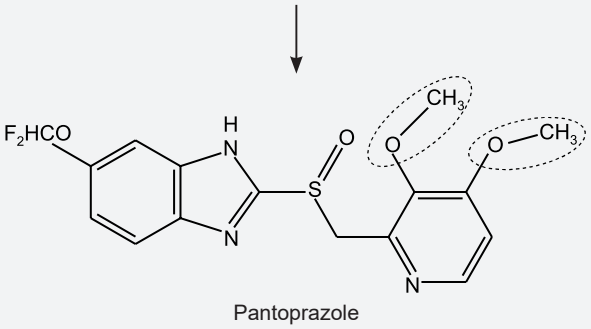


Lansoprazole

Introduction of methyl group and the trifluoroethoxy group at C-3 and C-4 position of pyridine ring gives lansoprazole. There is no substituent at benzimidazole ring. It is also available as 1:1 ratio racemate form.

Side effects: Headache, feeling sick, diarrhoea, stomach pain

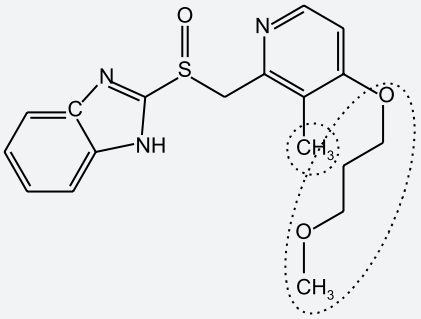
Contd.



Pantoprazole

Introduction of difluoromethoxy (-$OCHF_2$) group at benzimidazole moiety and two methoxy (-OCH_3) group at C-3 and C-4 position of pyridine ring yielded pantoprazole.

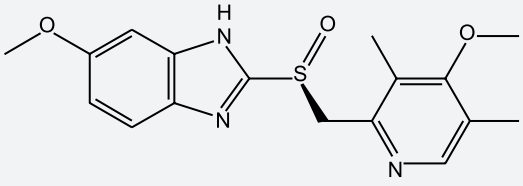
Side effects: Headache, diarrhoea, stomach pain.



Rabeprazole

Introduction of methyl group at C-3 and methoxy propoxy substitution at C-4 positions of pyridine ring yielded rabeprazole

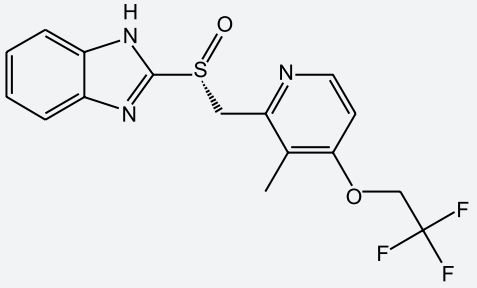
Side effects: Bloating or swelling of the face, arms, hands, lower legs, or feet.



Esomeprazole

Esomeprazole is an (*S*)-(-) isomer of omeprazole is more active in humans, while *R*-(+)-isomer is more active in rats and both enantiomers show equipotent activity in dogs.

Side effects: Blistering, peeling, or loosening of the skin, bloating, darkened urine and drowsiness.



Dexlansoprazole

It is a (*R*)-isomer of lansoprazole. The main advantage of this drug is it reaches 80% circulating drug availability after oral administration and it has dual release technology.

Side effects: Burning, crawling, itching, or tingling feelings, chest pain, discomfort, tightness, stomach pain.

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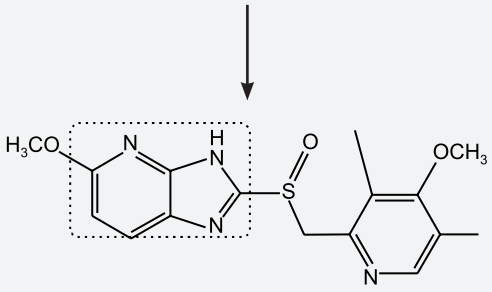


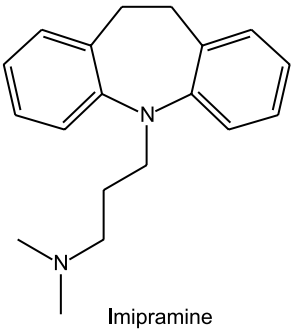
Tentoprazole

The biosteric development of the benzimidazole ring of PPIs by imidazo [4,5-b] pyridine moiety leads to tentoprazole. Imidazo [4,5-b] pyridine ring decreases the metabolic rate, increases the plasma residue time and decreases the pKa. ($t_{1/2} = 7$ hours).

Side effects: Cause weak bones and a deficiency of minerals such as magnesium, stomach pain, constipation.

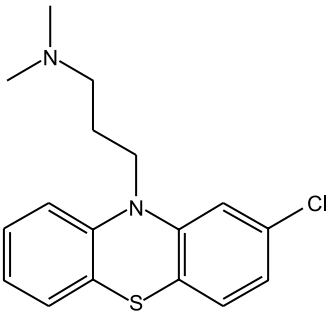
Design and Development of Psychoactive Agents

Bioisosteric replacement is an essential tool that plays a significant role in the design of psychoactive agents. Let us discuss psychoactive agents development by using the antidepressant drug Imipramine (dibenzaepine) derivative served as the lead compound.



Imipramine

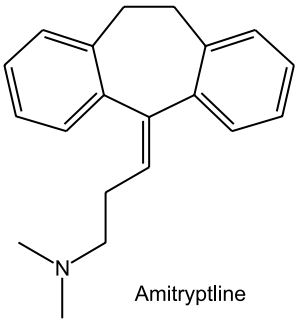
The bioisosteric replacement of the central azepine ring by thiazine ring afforded the antipsychotic drug Chlorpromazine.



Chlorpromazine

Both of the above drugs possess a different mode of action, pharmacological activities, and therapeutic utility.

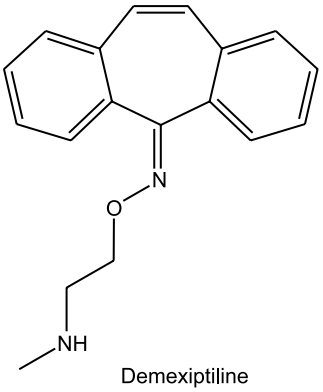
The bioisoteric replacement of the nitrogen atom of imipramine by exocyclic double bond afforded the drug amitryptline.



Amitryptline

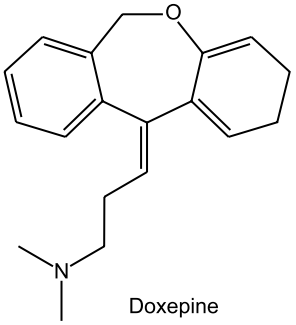
The minor structural modification in the imipramine leads to the development of various antidepressant agents as described here, with changes in their duration of action.

Introduction of unsaturation in the tricyclic ring and bioisosteric replacement of 'N' of the azepine nucleus with 'C' and addition of exocyclic double bond, bioisosteric replacement of 'C' with 'O' in side-chain configured demexiptiline.



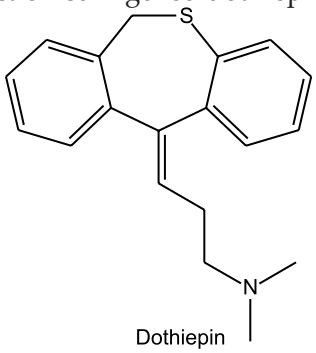
Demexiptiline

Bioisosteric replacement of azepine nucleus of dibenzazepine with loxapine alongwith exocyclic double bond introduction configured doxepine.



Doxepine

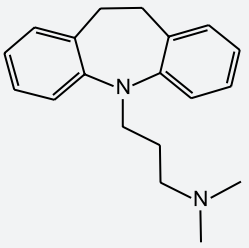
Bioisosteric replacement of azepine nucleus of dibenzazepine with thiapine alongwith exocyclic double bond introduction configured dothiepin.



Dothiepin

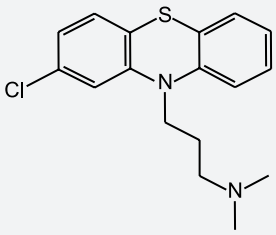
In the above compounds, Z-isomer of oxepine acts as a more potent anti-depressant than *E*-isomer, but the drug is marketed as a racemic mixture (demexiptiline).

Schematic representation of the development of psychoactive agents



Imipramine

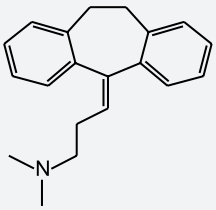
Served as the lead compound.



Chlorpromazine

The bioisosteric replacement of the central azepine ring by thiazine ring yielded antipsychotic drug chlorpromazine.

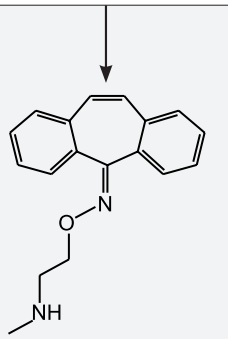
Side effects: Tachycardia, hypertension, dysrythmias.
Advantage: Changes in duration of action



Amitryptilin

The bioisoteric replacement of the nitrogen atom of imipramine by exocyclic double bond afforded the drug amitryptiline.
Side effects: Tachycardia, drowsiness, and dizziness

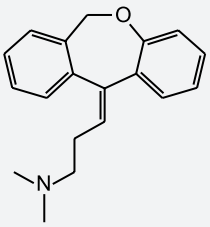
Contd.



Demexiptiline

Introduction of unsaturation in the tricyclic ring and bioisosteric replacement of azepine nucleus with 'C' and addition the 'N' at exocyclic double bond, and bioisosteric replacement of 'C' with 'O' in side chain afforded the compound demexiptiline.

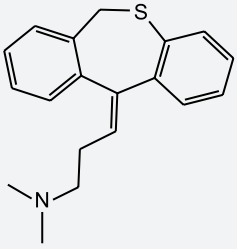
Side effects: Tachycardia, drowsiness and dizziness



Doxepine

Bioisosteric replacement of azepine nucleus of dibenzazepine of imipramine with loxapine alongwith exocyclic double bond introduction afforded the compound doxepine.

Side effects: Antiadrenergic effects of abnormal heart rhythms, low blood pressure, and anticholinergic effects of dry mouth, constipation



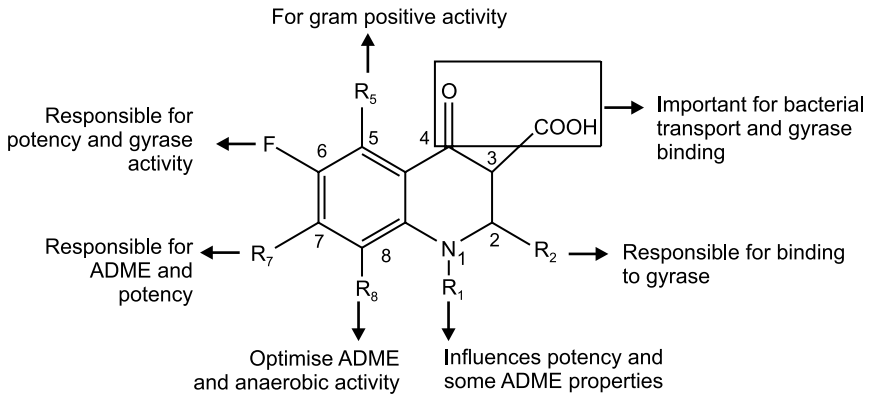
Dothiepine

Bioisosteric replacement oxapine with thiapine afforded the compound dothiepine.

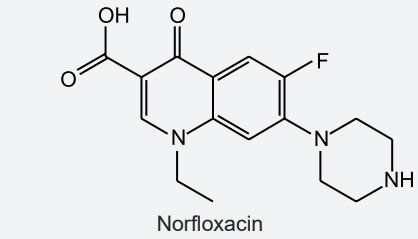
Side effects: Headache, dizziness and constipation

Development of Quinolone Antibiotics

Quinolone antibiotics were discovered in 1960. They are mainly used for the treatment of UTI. These are ideal antibiotics with good bioavailability and high potency. The large volume of distribution of these drugs reduces the toxic potential. The first drug that belongs to this category was nalidixic acid. In 1980, the other derivatives such as ciprofloxacin and ofloxacin were marked. But recently, nearly 10000 molecules are patented. Quinolines are derived from the quinine nucleus. The basic structure belongs to this class of molecules is given hereafter.

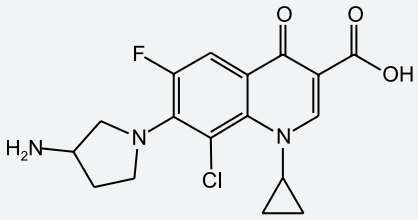


Schematic representation of the development of quinolone antibiotics



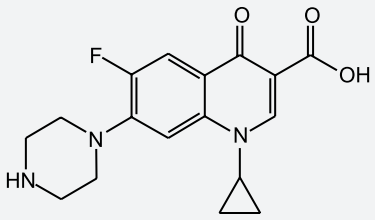
Norfloxacin

This compound was developed by the introduction of piperazine ring at C-7 position of the basic ring. Norfloxacin shows good gram negative antibacterial activity which is due to the main role of piperazine ring which inhibits efflux mechanism.



Clinafloxacin

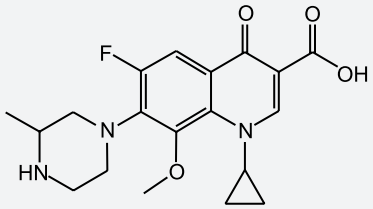
The introduction of pyrrolidinyl group yielded clinafloxacin which shows good activity against gram positive organisms.



Ciprofloxacin

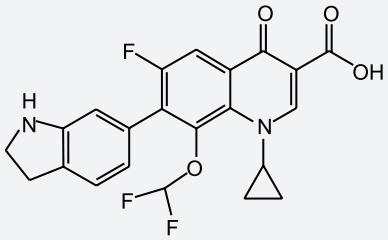
The addition of piperazine ring at C-7 and introduction of cyclopropyl group at N-1 position leads to the compound ciprofloxacin. Both substituents increased the potency of the compounds.

Contd.



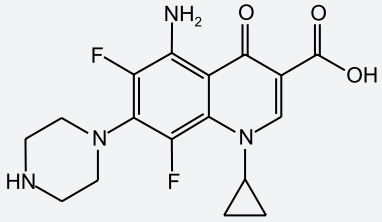
Gatifloxacin

Introduction of $-OCH_3$ group at C-8 yielded gatifloxacin



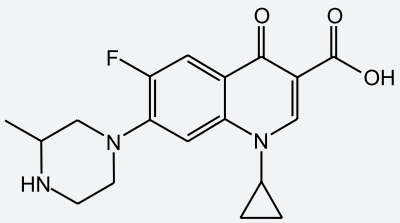
Garenofloxacin

Replacement of piperazine with benzimidazole and fluoro substitution at C-8 resulted in the formation of garenofloxacin



Sparfloxacin

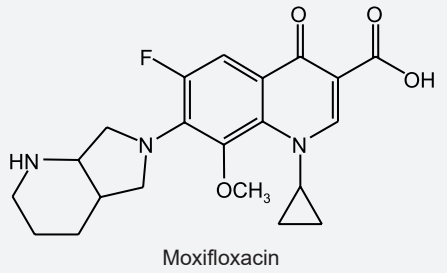
The introduction of NH_2 at C-5 position lead to the development of sparfloxacin with good gram positive activity



Grepafloxacin

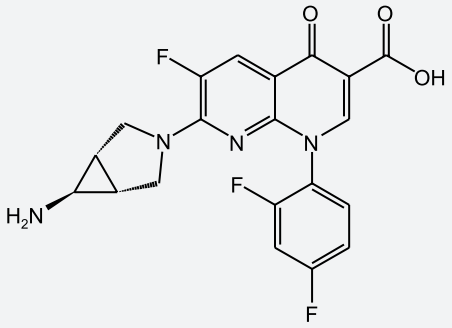
Introduction of CH_3 at C-5 position gave this drug which possesses improved gram positive activity than ciprofloxacin

Contd.



Moxifloxacin

These are developed by the introduction of azabicyclo group at C-7 position which increases the lipophilicity



Trovofloxacin

Broad anerobic coverage
Side effects: Nausea, headache

Development of Morphine Analogues

Opium class of drugs were used in china about 2000 years ago and was known in Mesopotamia. Various strategies are used for the development of morphine analogues, as mentioned below.

1. Variation of substituents
2. Drug extension
3. Simplification
4. Rigidification

Let us discuss in detail.

1. **Variation of substituents:** The alkyl substituents on the phenolic group lead to inactive or least active compounds. It indicates that the free phenolic group is essential for analgesic activity.
2. **Drug extension:** It is one of the strategies where the addition of extra binding groups extends the drug molecule. The aim here is to probe for other binding regions which might be available in the interaction between the drug and its receptor (Fig. 1.14).

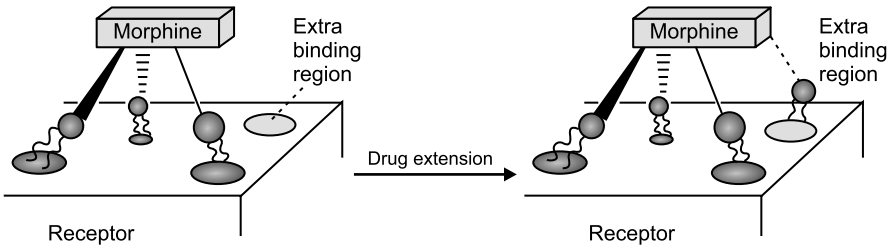
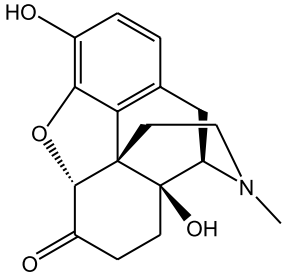


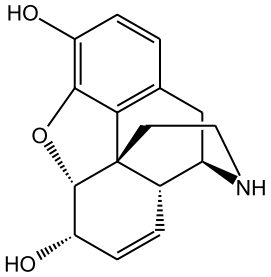
Fig. 1.14: Drug extension of morphine

The newly added extra functional groups lead to the development of many analogues. The important analogues among that is the oxymorphone obtained by the introduction of the hydroxyl group at C-14.



Oxymorphone

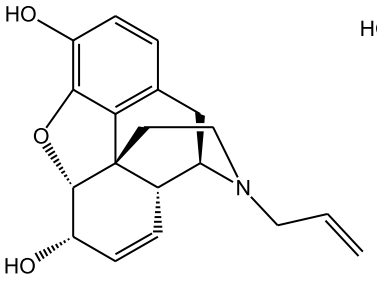
It possesses 2.5 times more active metabolism than morphine due to the possible effective interaction between the suitable amino acids on the receptor site. The addition of alkyl substituents at the nitrogen produces pronounced effects in the activity. Removal of the alkyl group from the morphine leads to the formation of normorphine.



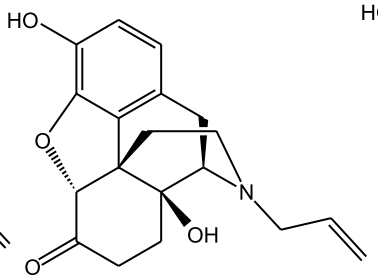
Normorphine

Alkylation of normorphine with various alkyl halides gives various alkylated morphine with a different pharmacological action potential. If the substituents increased from carbon chain length C-1- to C-4, i.e. methyl to butyl, there is no activity. But if pentyl or hexyl group is present, the activity is slightly increased. If morphine is substituted with phenyl ethyl group, the activity increases upto 14 fold. This is due to the strong interaction of the group on the atom influencing the activity spectrum of morphine.

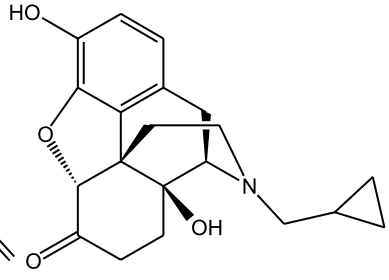
If the alkyl substituents attached to the N atom are allyl or cyclopropyl group leads to the development of another series of morphine analogues, which possess opposite activity to that of morphine. *For example,* opioid antagonists naloxone and naltrexone and the partial agonist nalorphine. It is due to the blocking of morphine receptors.



Nalorphine (partial agonist)



Naloxone (antagonist)

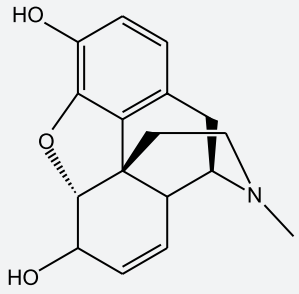


Natrexone (more active than naloxone)

The development of nalorphine have the following advantages.

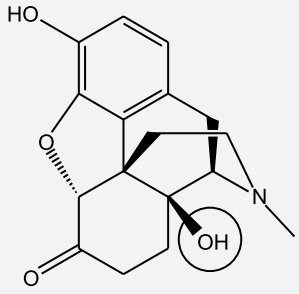
Narlorphine is an antagonist but produces weak analgesic activity with no side effects like morphine. It gave the idea for the development of addictive safe analgesics might be possible. How does this type of principle is possible? The answer is, there are 3 types of analgesic receptors present. Nalorphine binds with two receptors strongly and loosly binds with the 3rd receptor responsible for the weak analgesic activity. But the main side effect of nalorphine is the development of hallucinogenic effect.

Schematic representation of the development of morphine analogues



Morphine

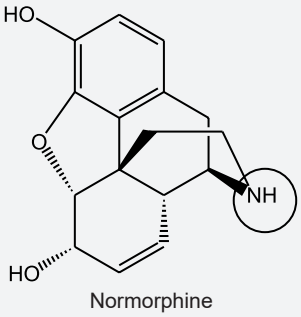
- Lead compound of the series (morphine)



Oxymorphone

- It is obtained by the introduction of hydroxyl group at C-14.
- It is 2.5 times more active than morphine.

Side effects: Cardiac arrest, anticholinergic effect (dryness of mouth, fast heart rate, insomnia)



Normorphine

- By the removal of methyl group from morphine leads to the formation of normorphine
- If morphine is substituted with phenethyl group instead of methyl, the activity increases upto 14 fold.

Side effects: Dysphoria, myoclonus

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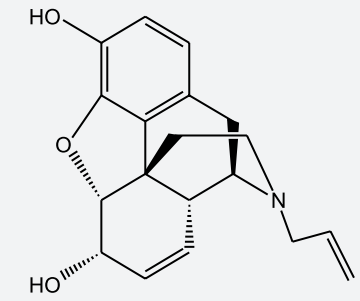
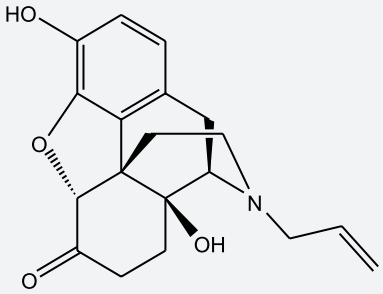


Nalorphine

- When the alkyl substitutents attached to the N atom is allyl group, which possess opposite activity to that of morphine.
- It is acting as partial agonist too
- It produces weak analgesic activity with no side effects like morphine

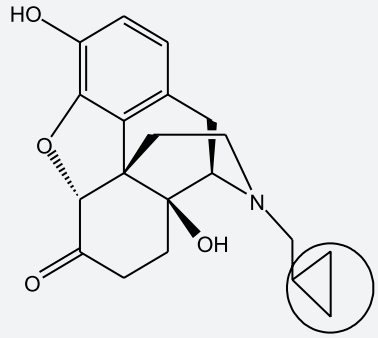
Side effects: The development of hallucinogenic effect, dysphoria, anxiety, and confusion



Naloxone

- When –OH group is replaced by oxygen yielded Naloxone
- It is acts as antagonist
- It is 7 times more active than nalorphine in antagonizing the effect of morphine.

Side effects: Nervousness, seizures (in babies), stomach cramps



Natrexone

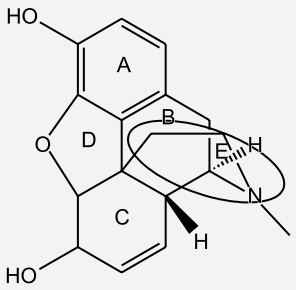
- When allyl group of naloxone is replaced by the cyclopropyl gives natrexone
- It is more active than naloxone due to cyclopropyl ring
- Its opoid antagonist activity is reported to be 2 to 9 times that of naloxone and 17 times of nalorphine.

Side effects: Anxiety, trouble sleeping, tiredness

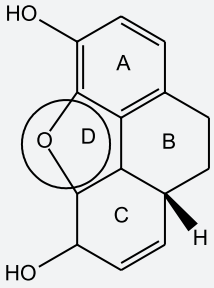
Simplification of Drug Dissection

Morphine molecule contains five rings. Many kind of morphine analogues are developed by the dissection of any one of the rings.

Schematic representation of the development of morphine analogues by simplification



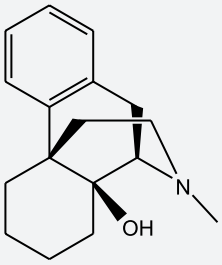
Morphine



Morphine analogues

Removal of E ring

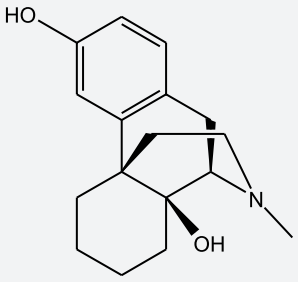
- Removal of E Ring
- Removal of E ring of morphine leads to morphine analogues.
- These analogues with complete loss of activity



N-Methyl morphinan

Removal of D ring

- The dissection of ring D from morphine leads to the development of new class of analogues known as morphinan
- It was the first compound tested in this series and possessed 20% activity as morphine

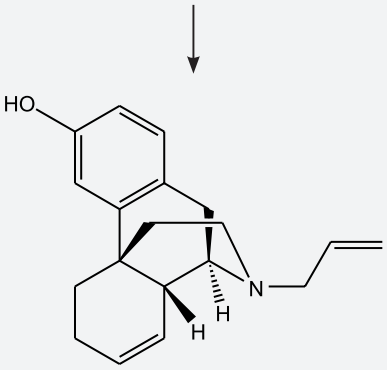


Levalorphanol

- It was developed by the addition of CH_3 group in N atom of morphinan
- It is 5 times more analgesic than morphine
- It can be taken orally and the duration of action is more compared to morphine

Side effects: Heart burn, vision problem, difficulty in urinating

Contd.



Levallorphan

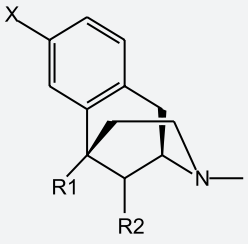
- Introduction of allyl group on nitrogen atom yields levallorphan.
- It is 5 times more potent anatagonist than nalorphine. Substitution of phenyl group on nitrogen atom gives the analogues which are 15 times more potent than morphine.
- It resembles nalorphine in its pharmacological action and is about 5 times more effective as a narcotic antagonist

Side effects: Psychomimetic effect, dysphoria

Removal of Rings C and D

Schematic representation of the development of morphine analogues by dissection of rings C and D

Dissection of rings C and D developed new analogues called as benzomorphine

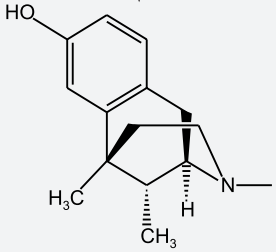


Benzomorphin

Removal of rings C and D

- It is a chemical compound that is the base for a series of drugs which variably act on the opioid kappa and sigma receptors

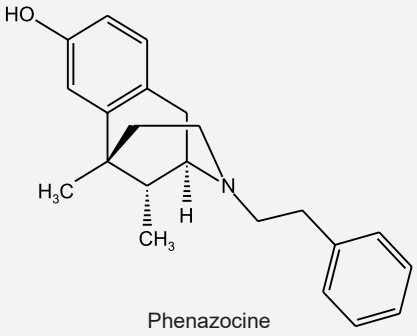
Side effects: Psychomimetic effect, respiratory depression



Metazocine

- Addition of methyl group developed the drug metazocine. It possess equipotent activity with morphine

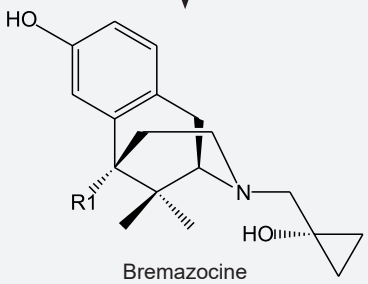
Contd.



Phenazocine

- Replacement of N-methyl group of metazocine with phenyl ethyl yield phenazocine.
- It is 4 times more potent than morphine without addiction properties

Side effects: Dysphoria, heart burn



Bremazocine

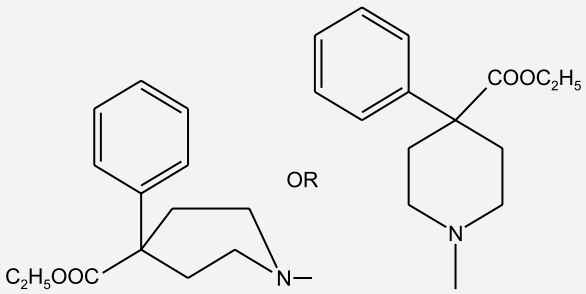
- Introduction of cyclopropanol on N-methyl group of the metazocine, ethyl at C-5, methyl group at C-9 position yielded bremazocine
- It is 200 times more active than morphine with the absence of addiction and breathing depression.

Side effects: Sedation, diuresis, respiratory depression

Removal of Rings B, C and D

Schematic representation of the development of morphine analogues by dissection of rings B, C and D.

Dissection of rings B, C and D led to the development of compounds called as 4-Phenyl piperidines

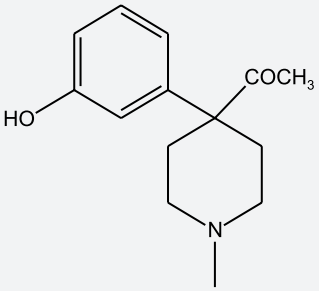


Pethidine Mepiridine

Pethidine is 20 times more active than morphine

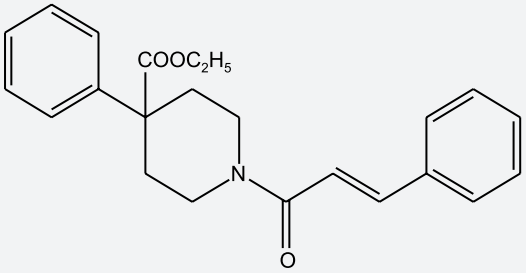
Side effects: Light headedness, mood changes, extreme calm

Contd.

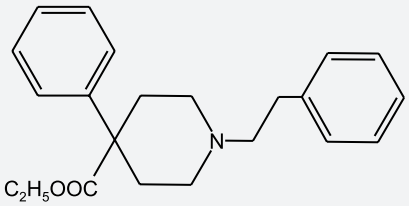


Ketobemidone

- The introduction of OH group into the phenyl group and replacement of ester to ketone moiety yielded ketobemidone.
- It is found to have 6 times more activity.



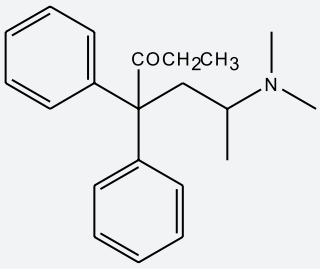
- Introduction of cinnamic acid moiety in place of methyl group of pethidine molecule, the activity was increased upto 30 fold.



Fentanyl citrate

- It was developed by replacement of N-methyl group by phenethyl group with the absence of phenolic –OH group.
- It is 100 times more potent than morphine.

Side effects: Dyskinesia, convulsion



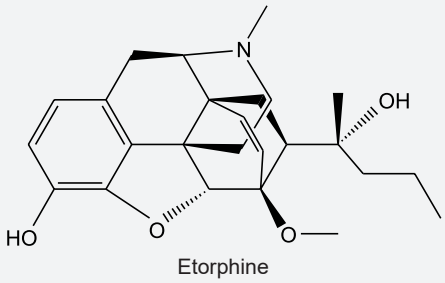
Methadone

- **Dissection of the rings B, C, D and E** rings developed the series of analogues called as methadone
- Methadone was proved as a useful analgesic but it produces morphine like side effects
- The withdrawal effect of methadone is less severe

Side effects: Constipation, sleepiness

Rigidification

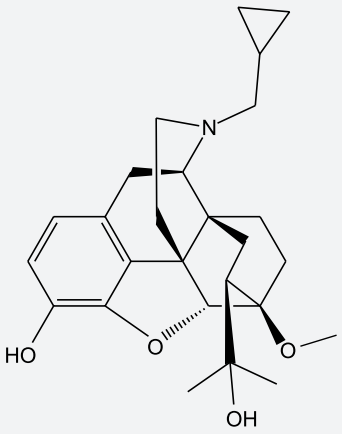
Schematic representation of the development of morphine analogues by rigidification



Etorphine

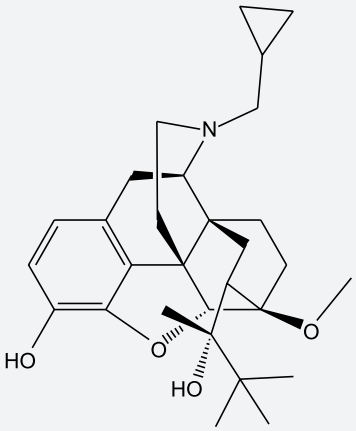
Etorphine is 10000 times more potent than morphine and 300 times easily crosses the BBB

Side effects: Cardiopulmonary depression



Diprenorphine

- Introduction of cyclopropyl group yielded a powerful antagonist known as diprenorphine.
- It is 100 times more potent than nalorphine



Buprenorphine

- Replacing the methyl group by treating with Grignard reagent and introduction of tertiary butyl group yielded the drug buprenorphine.
- It possesses analgesic activity
- It is 100 times more active than morphine and 4 times more active than nalorphine.

Side effects: Abdominal pain, tooth decay, drowsiness