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Drug Design—A Rational Approach

Chapter 1

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1. INTRODUCTION

In the past few decades there has been a hiatus in the momentum of research and discovery of **'novel medicinal compounds'.** This particular trend in drug development perhaps is augmented due to **two** vital factors, namely : *first*, strict empirical and rational approach to drug design ; and *secondly*, high standards of safety and therapeutic efficacy together with tremendous increased costs of research and development and finally the clinical trials.

'**Drug design**' or **'tailor-made compound'** aims at developing a drug with high degree of chemotherapeutic index and specific action. It is a logical effort to design a drug on as much a rational basis as possible thus reducing to the minimum the trial and error approach. It essentially involves the study of biodynamics of a drug besides the interaction between drug molecules and molecules composing the biological objects.

Drug design seeks to explain :

(*a*) Effects of biological compounds on the basis of molecular interaction in terms of molecular structures or precisely the physico-chemical properties of the molecules involved.

(*b*) Various processes by which the drugs usually produce their pharmacological effects.

(*c*) How the drugs specifically react with the protoplasm to elicit a particular pharmacological response.

(*d*) How the drugs usually get modified or detoxicated, metabolized or eliminated by the organism.

(*e*) Probable relationship between biological activity with chemical structure.

In short, **drug design** may be considered as an integrated whole approach which essentially involves various steps, namely : chemical synthesis, evaluation for activity-spectrum, toxicological studies, metabolism of the drug, *i.e.*, **biotransformation** and the study of the various metabolites formed, assay procedures, and lastly galenical formulation and biopharmaceutics.

The **'drug design'** in a broader sense implies random evaluation of synthetic as well as natural products in bioassay systems, creation of newer drug molecules based on biologically-active-prototypes derived from either plant or animal kingdom, synthesis of congeners displaying interesting biological actions, the basic concept of isosterism and bioisosterism, and finally precise design of a drug to enable it to interact with a receptor site efficaciously.

In the recent past, another terminology **'prodrugs'** has been introduced to make a clear distinction from the widely used term **'analogues'**. **Prodrugs** are frequently used to improve pharmacological or biological properties. **Analogues** are primarily employed to increase potency and to achieve specificity of action.

2. ANALOGUES AND PRODRUGS

In the course of **drug design** the *two* major types of chemical modifications are achieved through the formation of **analogues** and **prodrugs.**

An **analogue** is normally accepted as being that modification which brings about a carbon-skeletal transformation or substituent synthesis. *Examples :* **oxytetracycline, demclocycline, chlortetracycline, trans-diethylstilbesterol** with regard to **oestradiol.**

The term **prodrug** is applied to either an appropriate derivative of a drug that undergoes *in vivo* hydrolysis to the parent drug, *e.g.*, **testosterone propionate, chloramphenicol palmitate** and the like ; or an analogue which is metabolically transformed to a **biologically active drug,** for instance : **phenylbutazone** undergoes *in vivo* hydroxylation to **oxyphenbutazone.**

3. CONCEPT OF 'LEAD'

Another school of thought views **'drug design'** as the vital process of envisioning and preparing specific new molecules that can lead more efficiently to useful drug discovery. This may be considered broadly in terms of two types of investigational activities. These include :

(*a*) **Exploration of Leads,** which involves the search for a new lead ; and

(*b*) **Exploitation of Leads,** that requires the assessment, improvement and extension of the lead.

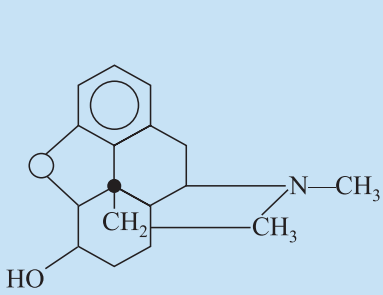
From the practical view-point it is the latter area wherein rational approaches to drug design have been mostly productive with fruitful results.

3.1 Examples

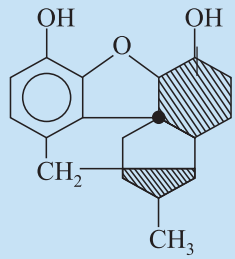
It is worthwhile to look into the right perspective of a few typical and classical examples of **drug design** as detailed below :

(*i*) **Narcotic Analgesics**

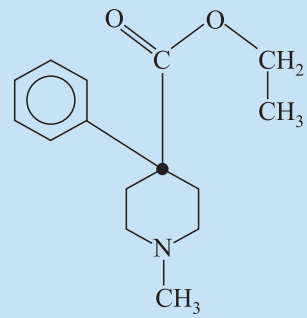
In the year 1939, Schaumann first identified and recognized the presence of a quaternary-carbon-atom in the morphine molecule, which eventually formed an altogether new basis and opened up a new horizon in the field of **drug design** of narcotic analgesics. Intensive research further led to the evolution of **pethidine** (**meperidine**) which incidentally combines both the properties of **morphine** and **atropine**. It possesses a quaternary carbon-atom and quite astonishingly a much simpler chemical structure to that of **morphine**.



Morphine

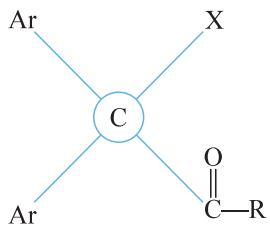


Morphine
(Schaumann's Model)



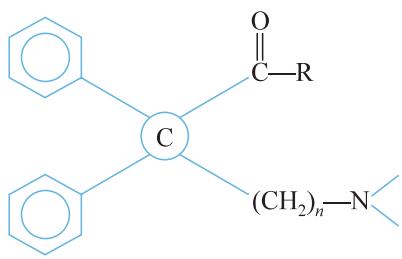
Pethidine
(Meperidine)

Ehrhardt suggested a **general formula** relevant to the analgesic activity in 1949 as stated below :



where, Ar is the aromatic ring, X the basic side chain and (—C—) carbonyl function in the form of an ester, ketone or an amide.

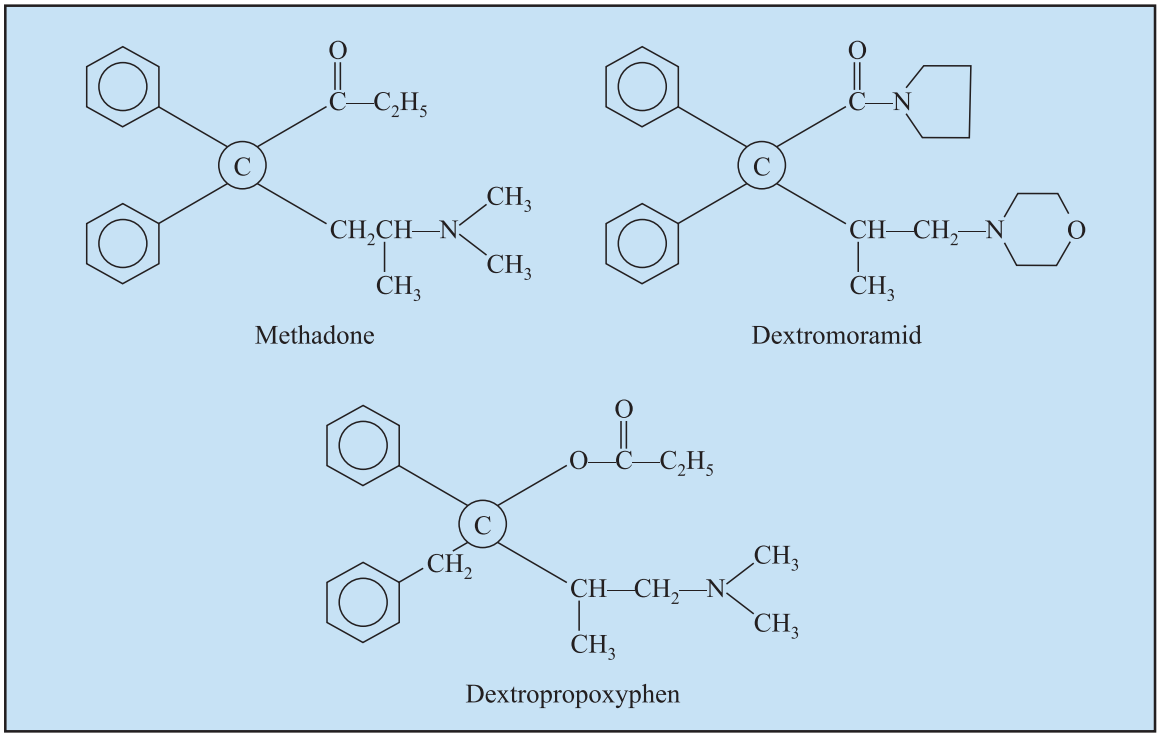
Later on, the above general formula was modified slightly as follows :



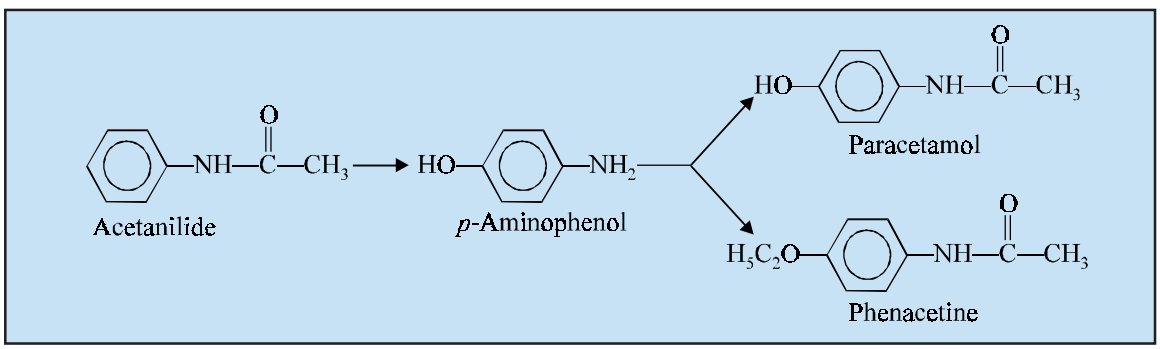
which successfully led to the development of the following *three* **narcotic analgesics,** namely : **methadone, dextromoramid** and **dextropropoxyphen.**

(*ii*) **Antipyretic Analgesics**

Another fruitful approach in **drug design** is the meticulous screening of the metabolite for probable pharmacological activity. The most interesting example is the bio-oxidation of acetanilide into *para*-aminophenol which subsequently on **chemical manipulation** has yielded better tolerated antipyretic-analgesics like **paracetamol** and **phenacetine.**

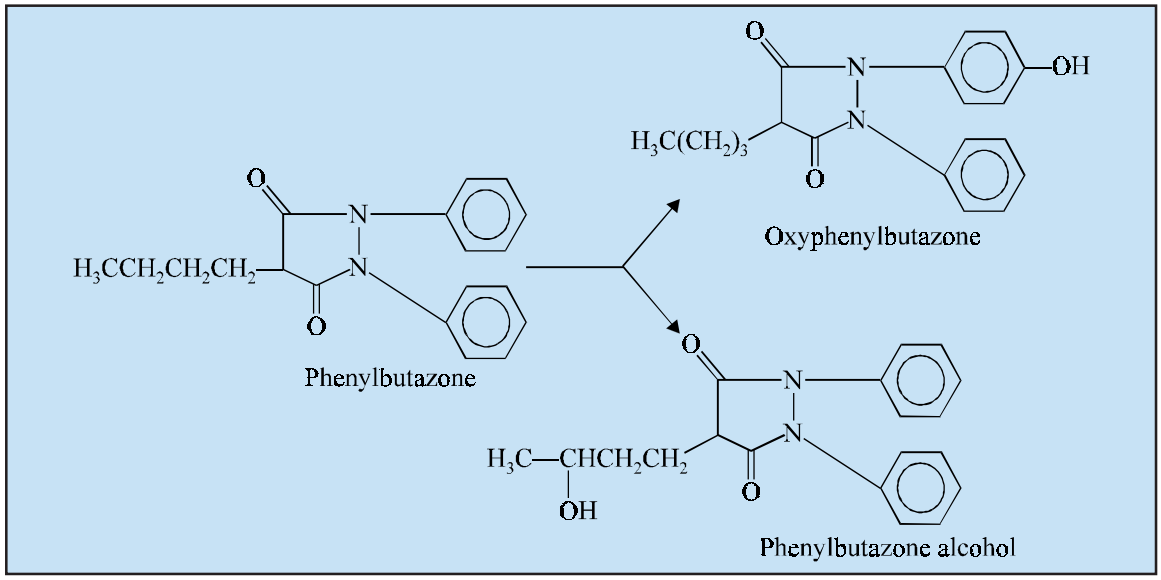


Quite recently **phenacetine** has been withdrawn completely because of its toxic after effects, though it dominated the therapeutic field for over 30 years as a potent antipyretic analgesics.



(*iii*) **Antirheumatic Drugs**

The study of the metabolite conversion of the antirheumatic drug phenylbutazone resulted in the introduction of a better tolerated drug **oxyphenylbutazone** as an **antirheumatic drug** and **phenylbutazone** alcohol as an **uricosuric agent.**



4. FACTORS GOVERNING DRUG-DESIGN

A few cardinal factors governing the efficacy towards the evaluation of **drug design** include :

(*a*) The smaller the expenditure of human and material resources involved to evolve a new drug of a particular value, the more viable is the design of the programme.

(*b*) Experimental animal and clinical screening operations of the new drugs.

(*c*) Relationships between chemical features and biolgoical properties need to be established retrospectively.

(*d*) **Quantitative structure-activity relationships (QSARs)** vary to an appreciable extent in depth and sophistication based on the nature of evaluation of structure or activity. A purposeful relation of structural variables must include steric factors, electronic features of component functional groups and, in general, the molecule as a whole.

(*e*) The trend to synthesize a huge number of newer medicinal compounds indiscriminately for exploratory evaluation still prevails which exclusively reflects the creative genuineness and conceptual functions of a highly individualized expression of novelty by a medicinal chemist.

(*f*) Introduction of functional groups in a molecule that need not essentially resemble metabolites, but are capable of undergoing bonding interactions with important functional groups of biochemical components of living organisms affords an important basis for exploration.

(*g*) Disease etiologies and various biochemical processes involved prove useful.

5. RATIONAL APPROACH TO DRUG DESIGN

A **rational approach to drug design** may be viewed from different angles, namely :

5.1. Quantum Mechanical Approach

Quantum mechanics (or **wave mechanics**) is composed of certain vital principles derived from fundamental assumptions describing the natural phenomena effectively. The properties of protons, neutrons and electrons are adequately explained under **quantum mechanics**. The electronic features of the molecules responsible for chemical alterations form the basis of drug molecule phenomena.

5.2. Molecular Orbital Approach

Based on the assumption that electrons present in molecules seem to be directly linked with orbitals engulfing the entire molecule which set forth the molecular orbital theory. The **molecular orbital approach** shows a dependence on electronic charge as evidenced by the study of three volatile inhalation anaesthetics, and also on molecular conformation as studied with respect to acetylcholine by such parameters as bond lengths and angles including torsional angles.

Molecular orbital calculations are achievable by sophisticated computers, and after meticulous interpretations of results the molecular structure in respect of structure-activity analysis is established.

5.3. Molecular Connectivity Approach

This approach establishes the presence of structural features like cyclization, unsaturation, skeletal branching, and the position and presence of heteroatom in molecules with the aid of a series of numerical indices. **For example :** an index was determined to possess a correlative factor in the SAR study of amphetamine-type hallucinogenic drugs.

Molecular connectivity approach has some definite limitations, such as : **electronegativity variance between atoms**, **non-distinguishable entity of *cis-trans* isomerism.**

5.4. Linear Free-Energy Approaches

This method establishes the vital link between the proper selection of physicochemical parameters with a specific biological phenomenon. However, such a correlation may not guarantee and allow a direct interpretation with regard to molecular structure, but may positively offer a possible clue towards the **selection of candidate molecules for synthesis.**

6. DRUG-DESIGN : THE METHOD OF VARIATION

Under this method a new drug molecule is developed from a **biologically active prototype.** The various **advantages** are as follows :

(*a*) At least one new compound of known activity is found.

(*b*) The new structural analogues even if not superior may be more economical.

(*c*) Identical chemical procedure is adopted and hence, considerable economy of time, library and laboratory facilities.

(*d*) Screening of a series of congener (*i.e.*, member of the same gene) gives basic information with regard to pharmacological activity.

(*e*) Similar pharmacological technique for specific screening may be used effectively.

The **cardinal objectives** of the **method of variation** are :

- To improve potency

- To modify specificity of action
- To improve duration of action
- To reduce toxicity
- To effect ease of application or administration or handling
- To improve stability
- To reduce cost of production

In order to obtain a therapeutically potent and better-tolerated drug there exists invariably an apparent conflict of pure scientific objectives and practical objectives. **This may be expatiated by citing the instance of an exceedingly toxic congener (say an anti-neoplastic agent) that possesses a very high degree of specificity and the researcher may have in mind to prepare still more toxic compounds so as to develop the highest possible specificity of action.** On the contrary, absolutely from the practical aspect, the proposed clue may not be pursued solely depending on the policy of the organization and not the individual or group of researchers.

In fact, there are a few generalized approaches utilizing the method of variation. In this particular context, the familiarity with the molecular structure is of the prime importance. The various possible approaches in designing newer drugs by applying variation of a prototype are quite numerous. Once the molecular structure of the compound in question is drawn on the drawing board, one takes into consideration such information as the following :

(*a*) study of the core nucleus of the hydro-carbon skeleton ;

(*b*) variation of functional groups and their proximity to one another ;

(*c*) various probable rotational and spatial configurations ;

(*d*) possibility of steric hindrance between various portions of the molecule in different configurations in space ; and

(*e*) probability of electronic interactions between various portions of the molecule including such matters as inductive and mesomeric effects, hyper-conjugation, ionizability, polarity, possibility of chelation, asymmetric centres and zwitterion formation.

The application of the method of variation, depending on the considerations enumerated above, is exploited in two different manners to evolve a better drug. The two main approaches for this goal can be indicated as :

(*a*) drug design through disjunction ; and

(*b*) drug design through conjunction.

6.1. Drug Design through Disjunction

Disjunction comes in where there is the systematic formulation of analogues of a prototype agent, in general, toward structurally simpler products, which may be viewed as partial or quasi-replicas of the prototype agent.

The **method of disjunction** is usually employed in three *different* manners, namely :

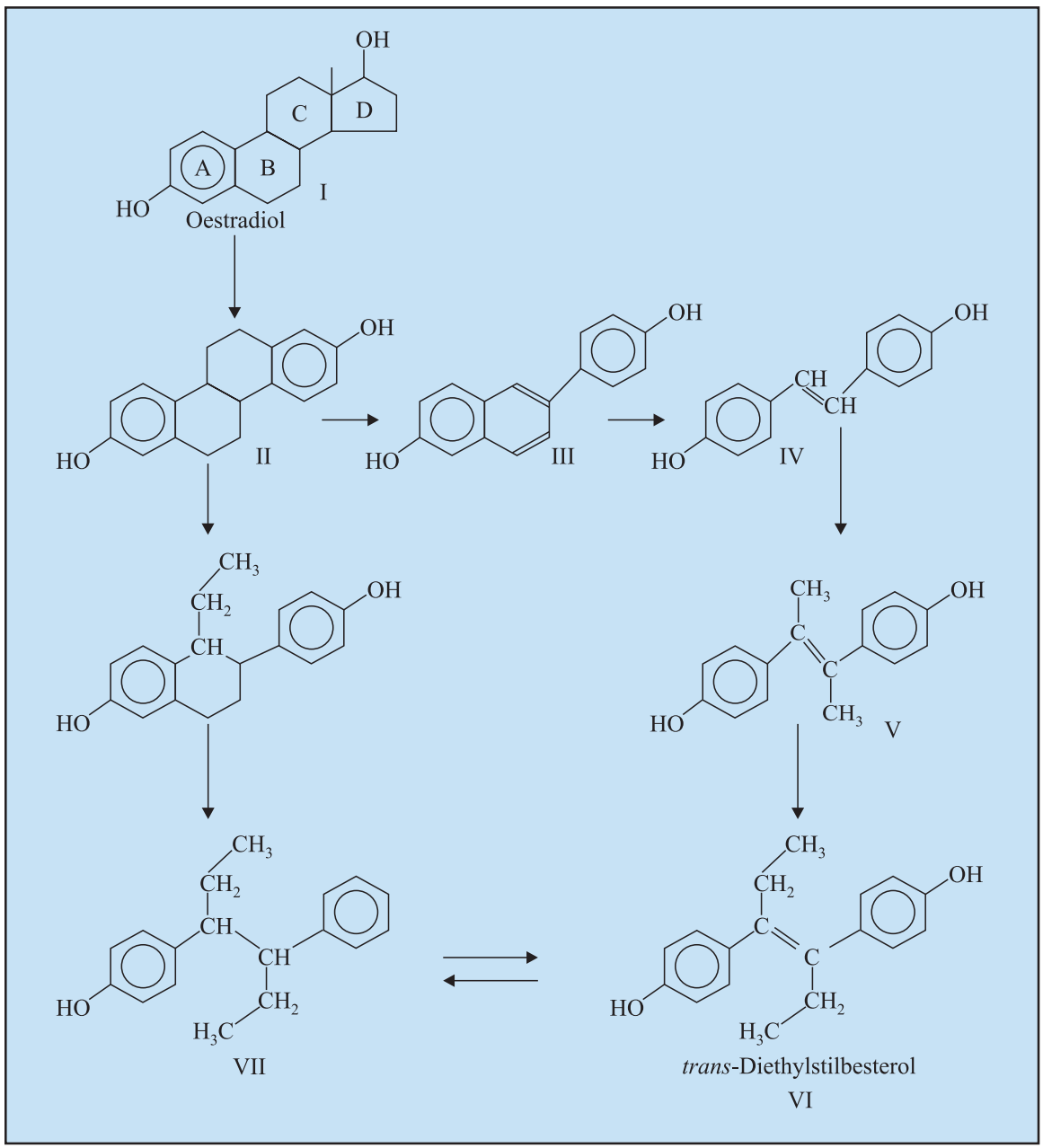
(*i*) unjoining of certain bonds ;

(*ii*) substitution of aromatic cyclic system for saturated bonds ; and

(*iii*) diminution of the size of the hydrocarbon portion of the parent molecule.

Example :

The extensive study on the estrogenic activity of oestradiol *via* drug design through disjunction ultimately rewarded in the crowning success of the synthesis and evaluation of *trans*-diethylstilbesterol. The **flow-sheet of estrogen design** is stated below :



Flow-sheet of Estrogen Design

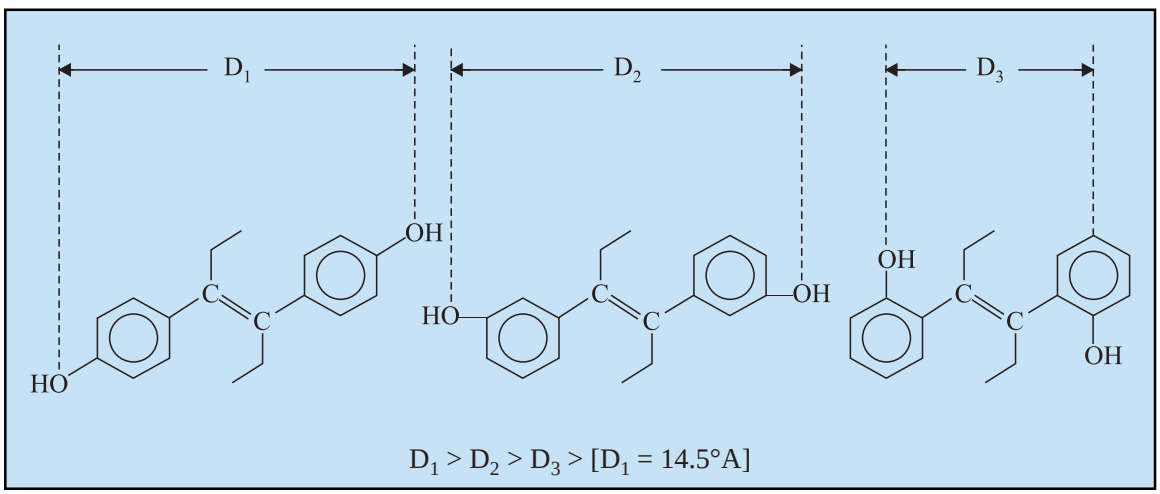
From the above the following *three* observations may be made. They include :

(*i*) Various steps in design of II to III to IV designate nothing but successive simplification through total elimination of the rings *B* and *C* in oestradiol (I).

(*ii*) The above manner of drug design finally led to successively less active products (*i.e.*, II, III, IV).

(*iii*) Upon plotting oestrogenic activity against various structures (I to VII) it was quite evident that the maximal activity in this series was attributed to ***trans*-diethylstilbesterol.**

It is, however, pertinent to mention here that in the following **three** different possible structures of **diethylstilbesterol analogues**, the oestrogenic potency decreases substantially as the distance '*D*' between the two hydroxyl groups decreases.



$D_1 > D_2 > D_3 > [D_1 = 14.5°A]$

6.2. Drug Design through Conjunction

This is known as the **systematic formulation of analogues of a prototype agent,** in general, toward structurally more complex products, which may be viewed as structures embodying, in a general or specific way, certain or all of the features of the prototype.

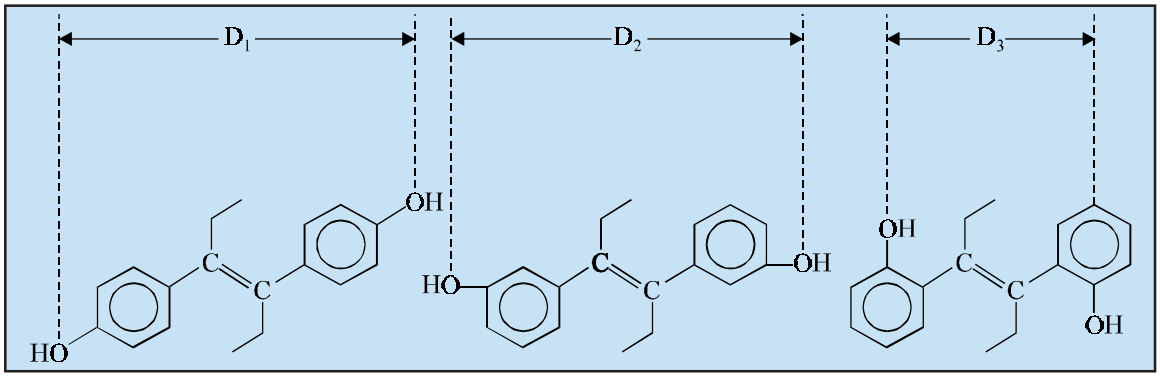
In this type of drug-design, the main principle involved is the **'principle of mixed moieties'.** A drug molecule is essentially made up with two or more pharmacophoric moieties embedded into a single molecule.

Example :

Ganglionic blocking agent—its development based on the principle of mixed moieties.

The principle of mixed moieties actually involve the conjunction of two or more different types of pharmacophoric moieties within a single molecule.

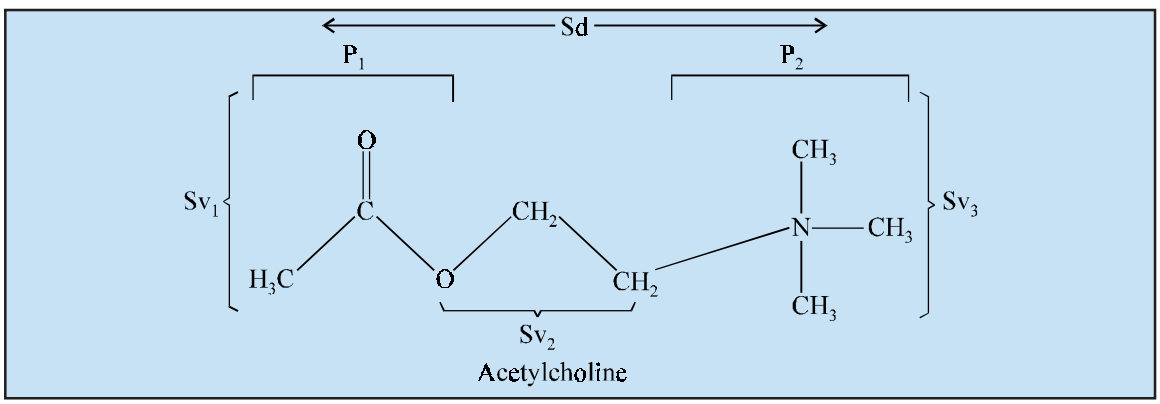
Acetylcholine is an effective postganglionic parasympathetic stimulant in doses that afford no appreciable changes in the ganglionic function ; whereas **hexamethonium** possesses only a slight action at postganglionic parasympathetic endings in doses that produce a high degree of ganglionic blockade.



(where Sv_1 = steric factor 1 ; Sv_2 = steric factor 2 ; Sv_3 = steric factor 3 ; Sd = steric distance factor ; P_1 = polarity factor 1 and P_2 = polarity factor 2).

The moiety requirements for postganglionic parasympathetic stimulant action (muscarinic moiety) have been duly summarized for convenience to the above structure of acetylcholine wherein the various operating factors have been highlighted.

The foregoing generalization of the muscarinic moiety on being studied in relation to the particular bisquaternary type of structure, *e.g.* hexamethonium, promptly suggests the following proposed design, thus embodying the ganglionic moiety and the muscarinic moiety into a single molecule.



It is, however, pertinent to mention here that the **'internitrogen distance'** essentially constitute an important factor in many series of bisquaternary salts that possess ganglionic blocking activity. It is worthwhile to note that this distance is almost similar to that present in hexamethonium in its most extended configuration.

However, the actual synthesis and pharmacological evaluation of the above **hexamethyl analogue** reveal the presence of both muscarinic stimulant and ganglionic blocking actions. Interestingly, the corresponding **hexaethyl analogue** possesses a ganglionic blocking effect and a weak muscarinic stimulant action.

7. DRUG DESIGN AND DEVELOPMENT : AN OVERVIEW

7.1. Preamble

The overwhelming qualified success in the evolution of **'ethical pharmaceutical industry'** in the twentieth century have not only registered an unquestionable growth in improving the fabric of society to combat dreadful diseases across the globe but also made a significant legitimate cognizance of an individual's quality of life and above all the life expectancy.

The twentyfirst century may obviously record and witness an apparent positive tilt in population demographics ultimately leading to a much healthier, stronger and happier elderly population.

However, in the 21st century, the **'ethical pharmaceutical industry'** has been fully geared towards the production of relatively safer, less toxic, more effective, higher therapeutic index, novel, innovative medicaments that will evidently help the mankind to afford a disease-free society ; besides, the elder ones with a glaring hope to live a still longer life span.

Following is the brief description in a chromological order for the development of **'ethical pharmaceutical industry'** in the world :

Year	Country	Historical Development
1600s	Japan	—Takeda in 1637*.
1800s	Europe and USA	—Fine chemical industries**.
1880s	Germany and UK	—Hoechst (Germany) and Wellcome (UK) for immunological drugs.
1889	UK	—Aspirin (as NSAID)
1990	France	—Rhone Poulenc
1914	Europe	—Engaged in US-operations
1929	USA	—Aureomycin (Lederle) ; Chloromycetin (Parke-Davis) ; Teramycin (Pfizer) ;
1950	France and Belgian	—Chlorpromazine [Rhone-Poulenc (France)] ; Haloperidol [Janssen (Belgium)]—both psychotropic drugs
1950s to 1970s	USA	—Pharmaceutical Industry showed a steady growth***
1970s	USA	—Greater advancement on molecular focus in the regimen of '**drug discovery**' picked up substantial momentum with the strategic induction of noted scientists in the US National Academy of Sciences, namely : Needleman P (Monsanto) ; Cuatrecasas P (Burroughs Wellcome) ; and Vagelos PR (Merck).

*Sneader WJ, **'Drug Discovery : The Evolution of Modern Medicines,'** John Wiley, Chichester, UK, 1997.

** Di Masi, d J *et al*. **Research and Development costs for new drugs for therapeutic category**, *Pharmaco.Econ.*, **7 :** *52, 169, 1995.*

***Drayer JI and Burns JP. From discovery to market : the development of pharmaceuticals. In : Wolff ME, ed, **Burgers Medicinal Chemistry and Drug Discovery**, 5th edn, Vol I, Wiley, New York, 1995, pp 251-300.

The various phases of transformations in **'ethical pharmaceutical industry'** between 1600 to 1970s brought about a sea-change with a significant shift from the core techniques of molecular pharmacology and biochemistry to those of molecular biology and genomics (biotechnology). Based upon these fundamental newer concepts amalgamated with various paradigm shifts resulted into the evolution of an exclusive progressive change in the scenario of both culture and the environment of the **'ethical pharmaceutical industry'** in developed as well as developing countries in the world.

7.2. Revolutions in Drug Discovery

A tremendous noticeable change in the **'process of drug discovery'** in the past three decades has been focused solely on the **'biotechnology revolution'.** In short, the techniques employed invariably in 'molecular biology' and 'biotechnology' opened up an altogether **'new trend in biomedical research'.**

In 1997, a staggering 1150 companies were established based on **'biotechnology'**, engaging three lacs research scientists working round-the-clock, and generated USD 12 billion. The six major biotech companies in USA, established in mid 1980s, now proudly enjoys the number one status not only in US but also in rest of the world, namely :

(*a*) **Genentech**—Presently subsidiaries of *Roche Biosciences ;*

(*b*) **Genetics Institute**—Presently subsidiaries of *American Home Products,*

(*c*) **Amgen ; Genzyme ; Chiron** and **Biogen—**Presently emerged as *major pharmaceutical companies.*

In the light of the huge accelerated costs for drug development, touching USD 359 million in 1991, to almost USD 627 million in 1995 and a projected USD 1.36 billion in 2000, have virtually pumped in lots of force geared towards superb efficacies and efficiencies in the pharmaceutical industry.* And this could only be accomplished through appreciable consolidation amalgamated with continued efforts of outsourcing of higher risk, early drug discovery to venture **capital-aided-biotech units ;** besides, clinical trials to the **clinical-research organizations** exclusively.

In order to significantly cut down the overhead expenses, and encash on sizable profitability various giants in the pharmaceutical industry have more or less adopted the following stringent measures to face the cut-throat competition in the global market and also survive gainfully, such as :

(*a*) To enhance the required productivity in the R and D activities of major pharmaceutical companies to sustain and maintain profitability,

(*b*) Increased productivity without enhancing R and D resources,

(*c*) Focusing on new research activities/strategies thereby creating a possible balance between internal research and external alliances,

(*d*) Merger and alliances in Pharmaceutical Industries dates back to 1970s with the formation of **Ciba-Geigy**** ; and till 2000 more than 20 such acquisitions/mergers have already been materialized across the globe.

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*Carr G : **The Alchemists : A survey of the Pharmaceutical Industry, Economist**, February 21, 1998, pp 3-18.

** de Stevens G., **Conflicts and Resolutions**., *Med. Res Rev.*, 1995, **15**, pp 261-275.

7.3. Research and Development Strategies

It has been proved beyond any reasonable doubt that the *'rate of success'* in **drug discovery** is exclusively dependent on the ability to identify, characterize novel, patentable newer **'target-drug-molecules'** usually termed as **New Chemical Entities** (**NCEs**), which essentially possess the inherent capability and potential in the management and control of a specific disease/ailment ; besides, being efficacious and safer in character. With the advent of latest technological advancements in the specialized areas related to **genomics and combinatorial chemistry** an appreciable advancement has been accomplished in the R & D strategies. It is, however, pertinent to mention here that a proprietary NCE status, position and recognition is an absolute must not only to ensure marketing exclusively but also to aptly justify the huge investment in the ensuing R & D process thereby making **medicinal chemitry** a more or less core element of the entire **'drug discovery process'.**

Interestingly, the **'drug discovery process'** may be categorized into **four** distinct heads, namely :

(*i*) Target identification and selection,

(*ii*) Target optimization,

(*iii*) Lead identification, and

(*iv*) Lead optimization.

The concerted efforts encompassing various intangible and critical methodologies that ultimately relate to the activities, expertise, wisdom and integration of the individual scientist directly or indirectly involved in **'drug discovery process'** virtually leads to advance drug discovery profiles.*

In short, the qualified success in the **'drug discovery process'** predominantly revolves around the following cardinal factors, namely :

- Articulated project management processes
- Prioritization
- Well-defined aims and objectives
- Company organization(s) and culture
- Resourcing *modus operandi*
- Prompt decision making factors.

8. MOLECULAR HYBRIDISATION

The **molecular hybridisation** essentially embodies the synthesis of strategically designed of altogether newer breeds of **'bioactive agents'** either from two or even more compounds having different characteristic features by the aid of **covalent-bond synthesis.**

Necki (1886) first conceived the interesting **'salol principle',** whereby he exploited the beneficial properties of phenols and carboxylic acids possessing potent antibacterial characteristic features into the **'design'** of newer drug molecules with better and improved pharmacological activities by means of simple esterification.

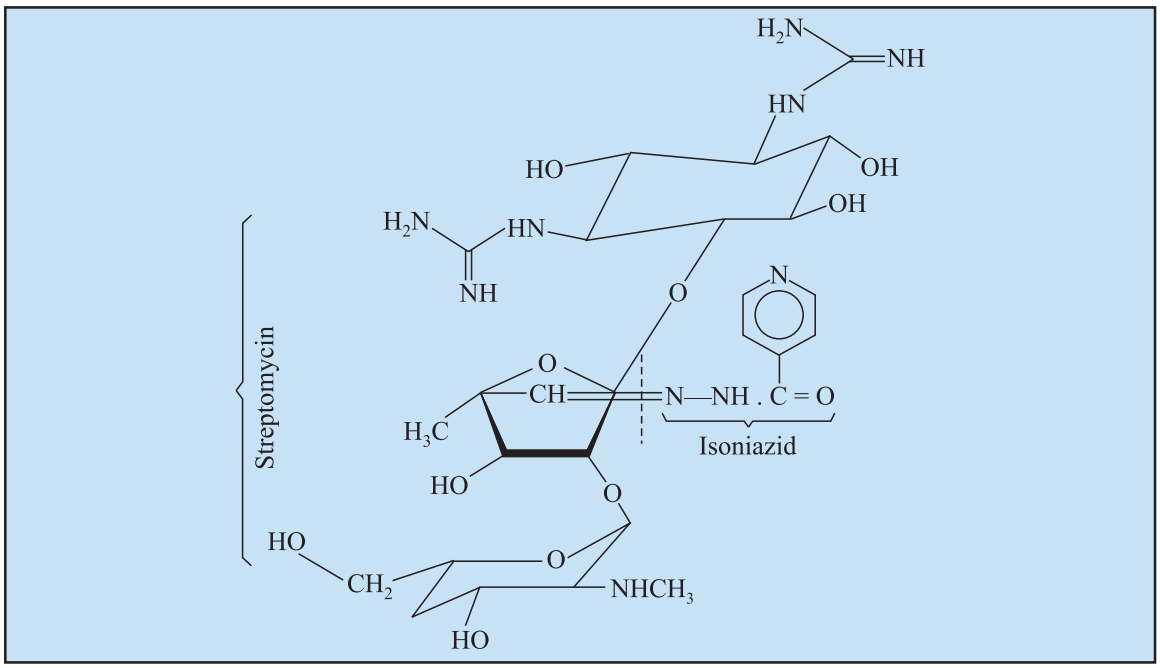
*Sapienza A.M., **Managing Scientists**, Wiley, New York, 1995.

A few typical examples wherein the hyberdisation was accomplished commencing from two **'bioactive entities'** *i.e.*, implementation of the **full-salol principle** occurred, as stated under :

Examples :

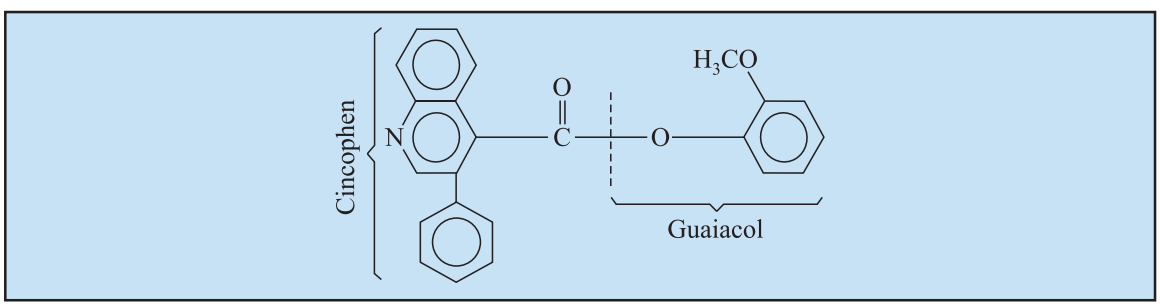
(*a*) **Antibacterial Agent :** *Streptoniazid ;*

A molecule of **streptomycin** and a molecule of **isoniazid** by means of a strong double bond between C and N with the elimination of a mole of water. **The 'hyberdised molecule'** exhibits a significant potentiated antibacterial and tuberculosstatic agent.



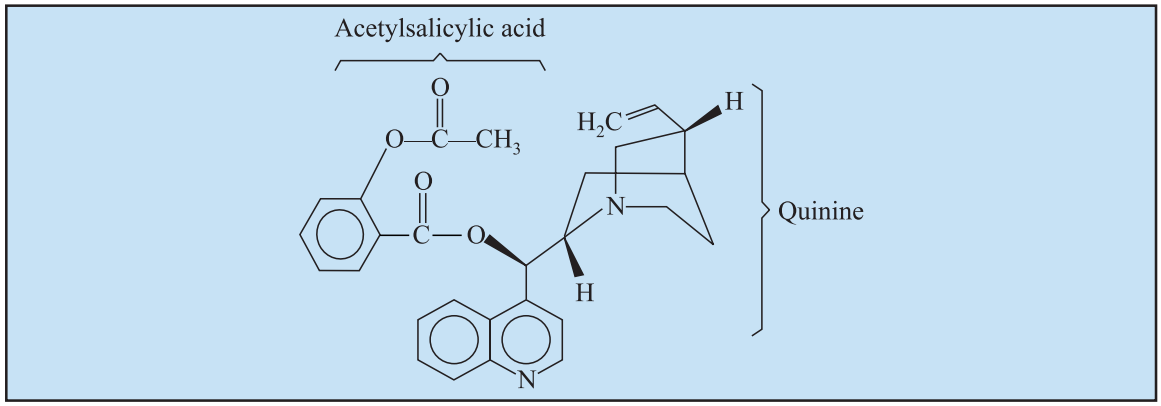
(*b*) **Antitussive Expectorant Drug :** *Guaicyl phenyl cinchoninate ;*

A mole each of cincophen and guaiacol gets hyberdised by forming an ester-linkage and losing a mole of water. The new product shows an improved antitussive and expectorant activity.



(*c*) **Antipyretic-Analgesic Agent :** *Quinine acetylsalicylate ;*

Hybridisation takes place between a mole of **acetylsalicylic acid** (*i.e.*, **aspirin**) and **quinine** (*i.e.*, a potent antimalarial agent) to lose a mole of water ; and the resulting hyberdised product potentiates the antimalarial activity along with substantial **antipyretic—analgesic activity**.



9. RIGIDITY AND FLEXIBILITY *Vs* DRUG DESIGN

It has been observed beyond any reasonable doubt whatsoever that the **structure-activity relationship** invariably affords certainly a molecular complementary prevailing evidently between the bioactive compound and the probable receptor site. At this point in time *two* different situations may usually crop up, namely :

(*a*) *increased rigidity* — that may ultimately lead to improved potencies ; and

(*b*) *increased flexibility*—that may give rise to better and improved activity.

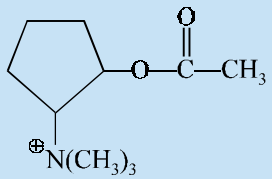
These two aforesaid situations shall now be discussed with typical examples so that one may have a better understanding of these aspects *vis-a-vis* drug design of **newer targetted** drug molecules.

9.1. Increased Rigidity

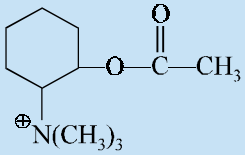
There are a plethora of **'drug molecules'** which are inherently flexible in nature *i.e.*, they can assume a wide-range of shapes **(spatial arrangements).** Of these structural variants quite a few are absolutely not so favourably acceptable for reaction at a specific **'receptor site'.** Therefore, the '**design**' or **'search'** for a relatively more rigid structural analogue essentially having the required, correct and desired **'dimensions'** must be looked into in order to obtain a more potent **drug substance.**

Besides, the actual distance existing between two vital functional moieties may be almost fixed arbitrarily in rigid molecular structural variants. These restructured and strategically positioned newer targetted-drug molecules may be subjected to vigorous and critical examinations by the aid of several sophisticated latest physicochemical analytical devices, such as : **X-Ray diffraction analysis** ; **Optical Rotary Dispersion (ORD) ; NMR-spectroscopy ; Mass Spectroscopy ; FTIR-Spectrophotometry and the like.**

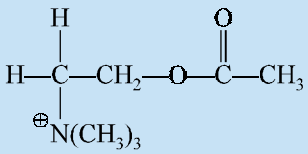
Examples : Structural analogues of **acetylcholine (ACh)** *i.e.*, a short-acting cholinergic drug, with **'increased rigidity'** having 5- or 6-membered saturated rings were synthesized ; and their activities were compared using **ACh as the reference drug** :



MODIFIED ACh WITH 5-MEMBERED CYCLIC RING (A)

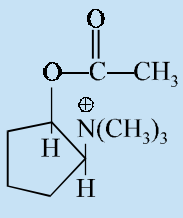


MODIFIED ACh WITH 6-MEMBERED CYCLIC RING (B)

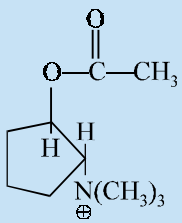


ACETYLCHOLINE ACh (AN OPEN-CHAIN COMPOUND)

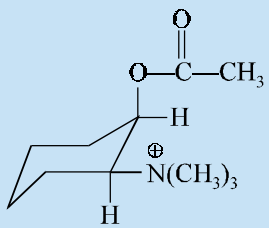
Interestingly, either of the *two* structural analogues (A) and (B) can be further resolved into their respective *trans*- and *cis*-isomers *i.e.*, spatially rearranged structures, as given below :



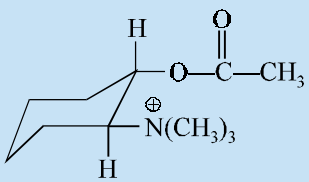
A-*cis*—



A-*trans*—



B-*cis*—



B-*trans*—

It has been observed that the **'intraatomic distance'** *between* 'O' and 'N' atoms for the *cis*-isomers (A & B) ranged between 2.5—2.9 Å ; whereas, between the corresponding *trans*-isomers (A & B) varied between 2.9—3.7 Å. Furthermore, the relative cholinergic activities of the *cis*-isomers were found to be greater than the corresponding *trans*-isomers using ACh as the reference drug.

The results of these findings have been summarized in the following table, wherefrom certain important clues may be derived with regard to some important functional group(s) located on the enzymes and the existing distances between such moieties.

S. No.	Drugs	Intra-Atomic Distances between 'O' and 'N' (Å)	Relative Cholinergic Activity
1	ACh	—	1.00
2	A-*cis*-	2.51	1.43
3	A-*trans*-	3.45	1.07
4	B-*cis*-	2.5—2.9	1.14
5	B-*trans*-	2.9—3.7	1.06

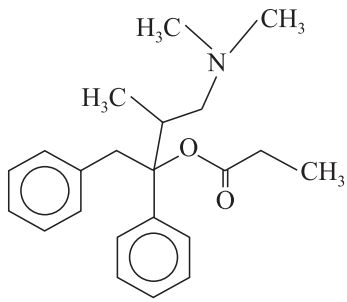
Thus, A-*cis*— is found to be almost 50% more active than ACh, and B-*cis*-only upto 15% than ACh. However, the corresponding *trans*-isomers of A and B did not show any improvement in their cholinergic activities.

9.2. Increased Flexibility

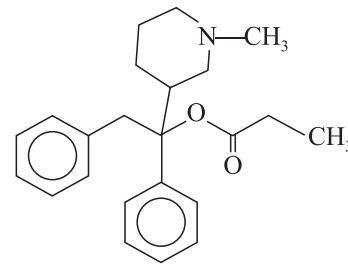
The problems encountered invariably with less flexible, rigid and compact molecules being that their manoeuvrability are comparatively much less. In other words, they either possess little or practically negligible capacity to have them rearranged to a more favoured conformation that may ultimately give rise to enhanced bioactivity.

Example :

Propoxyphene (I) is an open-chain structural analogue having narcotic analgesic activity ; whereas, its corresponding **cyclic analogue (II)** is almost found to be devoid of the pharmacological activity.



PROPOXYPHENE
(I)
(ACTIVE)



PROPOXYPHENE CYCLIC ANALOGUE
(II)
(INACTIVE)

10. 'TAILORING' OF DRUGS

With the advent of enormous in-depth knowledge of **'modern chemistry',** the **'tailoring'** of drugs has become a skilful art that may result fruitful results through specific modes of attack on a drug molecule.

Various **configurational and stereochemical changes** afford flexibility and overall dimension of a drug molecule. Such alterations may be conveniently achieved through different means and ways, namely : ring fission or fusion, formation of lower or higher homologues, introduction of optically active centres, formation of double bonds towards geometrical isomerism, and lastly introduction of bulky groups towards restricted rotation or the removal and replacement of such groups.

Alterations of various **physical and chemical characteristics** through the insertion of newer functional moieties or by the replacement of such groups already present by others that essentially differ in degree or in type. These types of changes may be effectively brought about by : isosteric replacement, changes of orientation or position of given moieties, introduction of polar character of given functional groups or replacement of other groups with different electrical features, and finally such changes which either promote or inhibit the presence of different electronic conditions achieved through inductive effects, mesomeric effects, tautomerism, chelation, hyperconjugation, etc.

11. GENERAL CONSIDERATIONS

Molecules, in general, may be viewed as dynamic electric entities. Hence, even the slightest alteration made in a relatively remote section of a molecule may cause either through spatial or through the overall matrix of the molecule, additional changes in some or all of its inherent characteristics.

An effective drug design from a biologically active prototype, whether approached through disjunction or conjunction or both, normally aims at modifying collectively all the moiety attributes that are absolutely essential capacities of a drug eliminated to a great extent which otherwise would have reduced its specificity of action, or interference with the primary type of action sought.

Probable Questions for B. Pharm. Examinations

1. Jutify the following statements :

(*a*) Drug design aims at developing a drug with high degree of chemotherapeutic index and specific action.

(*b*) From the practical view-point it is the '*Exploitation of Leads*' wherein rational approaches to drug-design have been mostly productive with fruitful results.

2. Discuss the various '*factors governing drug-design*'.

3. Eloborate the 'rational approach to drug design' with regard to Quantum Mechanics (or Wave Mechanics), Molecular Orbital Theory, Molecular Connectivity and Linear Free-Energy Concepts.

4. Enumerate the various cardinal objectives of '*The Methods of Variation*' giving appropriate examples.

5. The first synthetic oestrogen *trans*-diethylstilbesterol came into existence by applying the principle of 'drug-design through disjunction' from 'oestradiol'. Explain.

6. The development of 'ganglionic block agent' is exclusively based on the '*principle of mixed molecular*' as drug design through conjunction.

7. '*Tailoring of Drugs*' is the outcome of an unique blend of skill involving various configurational and stereochemical changes attributing its flexibility and overall dimension. Explain.

8. Discuss the various possible approaches in designing newer drugs by applying variation of a '*biologically active prototype*'.

9. Bio-oxidation and acetanilide and metabolic conversion of phenylbutazone gave rise to two better tolerated drug molecule used frequently and profusely in the therapeutic armamentarium. Explain.

10. Differentiate the basic concepts of '*analogues*' and '*prodrugs*' with the help of suitable examples of parent drug molecule(s).

RECOMMENDED READINGS

1. Blundell T., **'Structure-based Drug Design',** *Nature,* **384,** 23-26, 1996.

2. Kenny B.A. *et al*. **The Application of High Throughput Screening to Novel Lead Discovery,** *Prog. Drug Res.,* **41** : 246-269, 1998.

3. Williams M., **Strategies for Drug Discovery,** *NIDA Research Monograph,* **132** : 1-22, 1993.

4. Williams D.A. and Lemke T.L. (Eds.), **Foye's Principles of Medicinal Chemistry,** Williams DA and Lemke T.L. (Eds.), Lippincot Willams & Wilkins, New York, 5th edn., 2002.

5. Wolff M.E. (Ed.) **Medicinal Chemistry and Drug Discovery,** John Wiley & Sons, New York, 5th edn., 1995.

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2

Physical-Chemical Factors and Biological Activities

Chapter

2 Physical-Chemical Factors and Biological Activities

1. INTRODUCTION

The quest for knowledge to establish how the drugs act in a living system has been a thought-provoking topic to scientists belonging to various disciplines such as medicinal chemistry, molecular pharmacology and biochemistry. Since the turn of the twentieth century these researches have more or less established the basis of drug action on a more scientific and logically acceptable hypothesis. With the advent of such newer fields of study, for instance : **tracer techniques, genetic engineering, bio-technology, electron microscopy** and **computer-aided physico-chemical methods,** a new direction has been achieved towards more vivid explanation of the intricacies of drug interaction sequel to drug design.

In the recent past, receptors and drug-receptor interactions theories have highlighted the importance of physical and chemical characteristics with regard to drug action. Such salient features may include : partition coefficients, solubility, degree of ionization, isosterism and bio-isosterism, surface activity, thermodynamic activity, intramolecular and intermolecular forces, redox potentials, stereochemistry and interatomic distances between various functional groups.

Medicinal chemistry undoubtedly rests its main focus on the broad based variations embracing the influence of numerous possible manipulations with regard to the chemical structure on the biological activity. In the light of the above statement of facts supported by copious volumes of scientific evidences reported in literatures, it is almost important and necessary for the **'medicinal chemist'** to decepher and logically understand not only the **'mechanism of drug action'** *in vivo* by which a **drug substance** exerts its effect, but also the overall physicochemical properties of the molecule. In a rather most recent conceptualized theoretical basis the specific terminology **'physicochemical characteristics'** invariably refers to the cognizable influence of the plethora of organic functional moieties strategically positioned within a **drug substance,** namely : acid/base characteristics, partition coefficient, water solubility, lipoidal solubility, crystal structure, stereochemistry, chirality to name a few. It is, however, pertinent to mention here that most of the aforesaid properties covertly and overtly exert a significant influence upon the various biological phenomenon *in vivo*, such as : **absorption, distribution, metabolism** and **excretion** (ADME) of the newer **'target-drug molecule'.**

Therefore, a creative **'medicinal chemist'** should ponder over the intricacies, complexities and legitimate presence of each functional moiety to the overall physical chemical properties of the **'target-drug molecule'** with a view to arrive at or design safer, better and efficacious medicinal agents. Nevertheless, such critical studies have to be carried out in a rather methodical and systematic manner

vis-a-vis their affect upon biological activities. Generally, such elaborated studies are commonly referred to as **'structure-activity relationship' (SAR)** ; and more recently as **'quantitative-structure-activity relationship' (QSAR).**

It would be worthwhile to look into the physical and chemical aspects of the drug separately and an attempt made to establish the relation of such properties to biological activities.

2. PHYSICAL PROPERTIES

A plethora of physical properties play an important role in modifying the biological activities of a good number of medicinal compounds. A few such properties are : **features governing drug action at active site, factors governing ability of drugs to reach active site, dissociation constants, isosterism and bio-isosterism.**

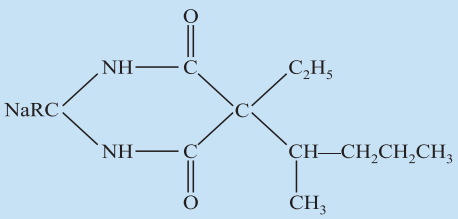
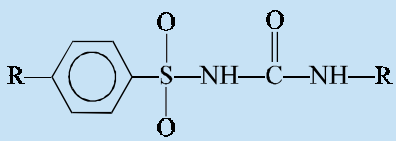
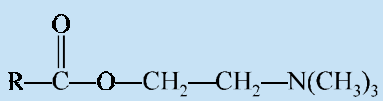
2.1. Features Governing Drug Action at Active Site

The various factors that govern the action of drugs at the active site may be due to **structurally specific and non-specific drugs**.

2.2. Structurally Specific Drugs

A number of compounds that possess remarkable pharmacological actions are essentially the **structurally specific drugs**. Though the physical characteristics of the drug play an important role in the biological activity, yet the chemical properties do exert their justified influence on the activity.

Effects of Minor Structural Modifications on Biological Activity

Structure	Biological Activity	Pharmacological Classification
(*a*) 		
R = O, (pentobarbitone sodium)	Short-acting	Hypnotic
R = S, (thiopental sodium)	Ultra-shortacting	
(*b*) 		
R = CH_3, R′ = C_4H_9 (tolbutamide)	Short-acting	Hypoglycemic
R = Cl, R′ = C_3H_7 (chloropropamide)	Long-acting	
(*c*) 		
R = CH_3 (acetylcholine)	Short-acting	Cholinergic
R = NH_2 (carbamylcholine)	Long-acting	

The apparent effect of structure on biological activity may be observed in the following examples where such a change is mainly due to minor group alterations. For instance, alterations in groups in parent structures bring about appreciable difference in the hynotic, hypoglycemic and cholinergic activities. It is pertinent to mention here that such changes only affect the duration of action without any influence on the biological response.

2.3. Structurally Non-specific Drugs

The **structurally non-specific drugs** include general anaesthetics, hypnotics together with a few bactericidal compounds and insecticides. However, it is important to note here that the biological characteristic of such drugs is solely linked with the physical properties of the molecules rather than the chemical feature.

It has been reported that the toxic depressant concentration of such a drug bears a close resemblance with the physical features, namely : partition coefficient, solubility, vapour pressure and surface activity. As most of these characteristics are entirely based on an equilibrium phenomenon, the ultimate effects of the drug on the biological system are directly linked to an equilibrium model.

2.4. Thermodynamic Activity

Structurally non-specific action is usually due to the accumulation of a drug in an important part of a cell which possesses dominant lipid characteristics. Substances like alkanes, alkenes, alkynes, ketones, amides, chlorinated hydro-carbons, ethers and alcohols display narcotic activity which is directly proportional to the partition coefficient of each individual substances.

2.5. Meyer-Overton and Meyer-Hemmi Theory

Meyer and Overton in 1899* observed that the **narcotic efficacy of drugs** was directly related to their **partition coefficients between oil and water**. In other words, the degree of narcosis produced by a chemically indifferent substance solely depends on its ability to attain a certain molar concentration specifically in cell lipids. Such a concentration of a drug in cell lipids is usually governed by two major factors, namely : *first*, the partition coefficient of the drug ; and *secondly*, the least molar concentration of the drug need to be present in the extracellular fluids to cause narcosis in a test animal. Thus the lipid concentration may now be attained by the multiplication of the molar concentration of the narcotic present in the extracellular fluids by its partition coefficient.

For instance, **phenobarbital**, has narcotic concentration for tadpoles (moles/litre of water) 0.008 and partition coefficient (oil/water) 5.9 gives rise to the value of lipid concentration for narcosis (moles/litre) ($0.008 \times 5.9 = 0.048$).

The results achieved by means of the above experimental approach fully coincides with the theory of Meyer and Hemmi.

Meyer and Overton further expanded their theory and suggested that the correlation may be established and observed between lipid solubility and the central nervous system (CNS) depressant activity profile. The CNS-depressant activity is found to be directly proportional to the partition coefficient of the **'drug substance'.**

Example : *Three* widely different drug substances having altogether divergent chemical structures, such as : **thymol, valeramide-OM and 2-nitroaniline** exhibited partition coefficient ranging between 0.030 to 950.0 ; and the calculated depressant concentration in cellular lipids varying between 0.021 to 0.045 as summarized below :

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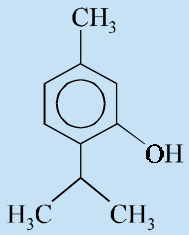
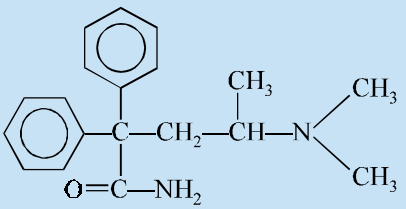
*Meyer, H. *Arch Exptl. Pathol. Pharmakol*, **42,** 109, 1899.

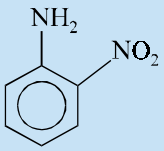
Overton, E. *Viertljahrsschr. Naturforsch. Ges. Ziirich*, **44,** 88, 1899.



Thymol



Valeramide-OM



2-Nitroaniline

S.No.	Compounds	Partition coefficient (n-Octanol/Water)	Required conc. to immobilise Tadpole	Calcd. Depressant conc. in cellular lipids
1	**Thymol**	950.0	4.7×10^{-5} moles	0.045 [*i.e.*, $950 \times 4.7 \times 10^{-5}$]
2	**Valeramide-OM**	0.030	0.07 moles	0.021 [*i.e.*, 0.30×0.07]
3	**2-Nitroaniline**	14.0	0.0025 moles	0.035 [*i.e.*, 14×0.0025]

From the above *three* structural variants one may infer that the value of 0.03 mole in the cellular lipids strongly indicates the required concentration for **'immobilization of tadpoles'.** In short, it adequately confers the **bioactivity of a drug substance to its inherent hydrophobicity and hydrophilicity**.

2.6. Ferguson's Theory

Ferguson observed that a number of **physical characteristics**, namely : **partition coefficients**, **vapour pressure**, **solubility in water**, **effect on the surface tension of water**, **and capillary activity**, are usually altered in accordance with the geometric progression in stepping up a homologous series. A plot between the logs of geometric progression values and the number of carbon atoms will form a straight line. Thus, each of the physical characteristics stated earlier, designates a heterogenous phase distribution at equilibrium. According to Ferguson—**"the molar toxic concentrations in a homologous series change on ascending the series not by equal steps but that instead their logarithms decrease by equal steps, it is to be concluded that they are largely determined by a distribution equilibrium between heterogenous phase — the external circumambient phase where the concentration is measured and a biophase (i.e., the phase at the site of action) which is the primary seat of toxic action".**

The thermodynamic activity of a non-volatile drug may be calculated from the expression *S/So*, where *S* is the molar concentration of the drug and *So* its solubility. Likewise, the thermodynamic activity values of volatile substances may be calculated by using the expression *P/Po*, where *P* is the partial pressure of the substances in solution and *Po* is the saturated vapour pressure of the substance. These findings coined the Ferguson's principle which states that — **"substances which are present at the same proportional saturation in a given medium have the same degree of biological action."**

2.7. Van der Waal's Constants

Van der Waal's equation may be expressed as :

$$(P + a/V^2)\,(V - b) = RT,$$

where P = Pressure,

V = Volume,

T = Temperature,

R = Gas constant,

a = Constant for attractive forces between molecules and

b = Constant for volume occupied by molecules of gas.

It has been proved that in the case of narcotic agents the activity enhanced proportionately with the increased volume and size of the molecules. This may further explain their activity in terms of their ability to fit into chemical structures inside the cell membranes thereby checking the passage of essential elements.

2.8. The Cut-off Point

It has been observed at several instances that the biological activity of a homologous series of synthesized analogous may not increase endlessly. Normally one comes across a point at which the biological activity starts falling very rapidly as one ascends a homologous series. This particular situation is termed as the **'cut-off point'.** Such a peculiar behaviour may be caused due to either the solubility of the drug in water or the minimum concentration required in water to exhibit the biological response.

Examples : The various examples expatiating the importance and utility of **'cut-off point'** may be observed in the following typical instances :

(1) In a series of **alkylated resorcinols** the length of the *alkyl chain* is directly proportional to the **antibacterial activity,**

(2) Likewise, in a series of **para-aminobenzoic acid esters** the length of the alkyl chain is directly proportional to the **local anaesthetic activity,** and

(3) Similarly, in a homologous series of **n-alkanols** correlation between the length of chain and **antibacterial** activity could be observed.

However, it has been reported that a positive enhancement in the **physical characteristics** usually take place with the ascending homologous series, such as : **viscosity**, **surface activity**, **boiling point** and above all the **partition coefficient** ; but the **water-solubility** decreases appreciably.

Salient Features : The various salient features of **'cut-off point'** are as stated under :

(*a*) In ascending homologous series of **'alkylated resorcinols'** the maximum antibacterial activity, in terms of the **phenol coefficient,** is found to be with *6-carbon atoms* located in the side-chain. Hence, the **cut-off point** in this specific instance stands at when $n = 5$.

(*b*) A logical explanation may be given by virtue of the fact that increase in number of C-atoms in the side-chain increases the antibacterial activity, which is due to the enhanced partition coefficient ; and like ultimately affords an enhancement in **penetration of the cell wall.**

(*c*) A situation, when $n = 6$, the antibacterial activity falls sharply due to the drastic poor water solubility of the resultant compounds.

(*d*) Ferguson plotted 'log of aqueous solubility' *Vs* 'number of carbon-atoms in the side-chain' and observed that as the number of C-atoms in the alkyl group increases, a stage is reached when an **'interaction'** between the **"saturation line S"** and **"log water solubility"** takes place, as shown in Fig. 2.1.

(*e*) Fig. 2.2 represents a plot between **"Log conc. (Mole $\times 10^{-6}$ L^{-1}) for bactericidal action"** *Vs* **"Number of C-atoms".** At 'X', when there are 6-carbon atoms in the side chain, the water solubility decreases than the previous compound having 5-C-atom. Similarly, at 'Y', when there are 9-carbon atoms, the water-solubility dips down considerably. Therefore, the **'cut-off-point'** for the microorganisms are essentially located between (X + Y) when there are 6 and 9 C-atoms present in the alkyl group respectively.

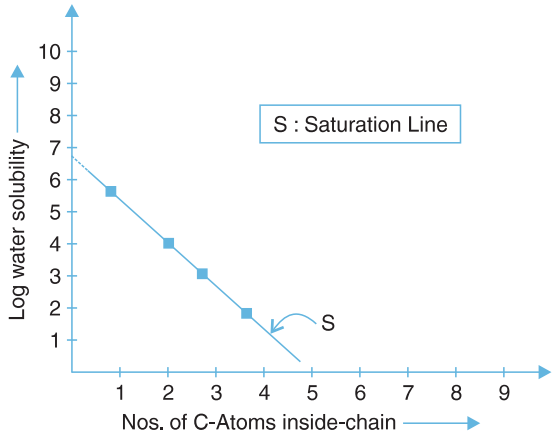


Fig. 2.1. Interaction between 'Saturation-Line S' and 'Log Water Solubility'.

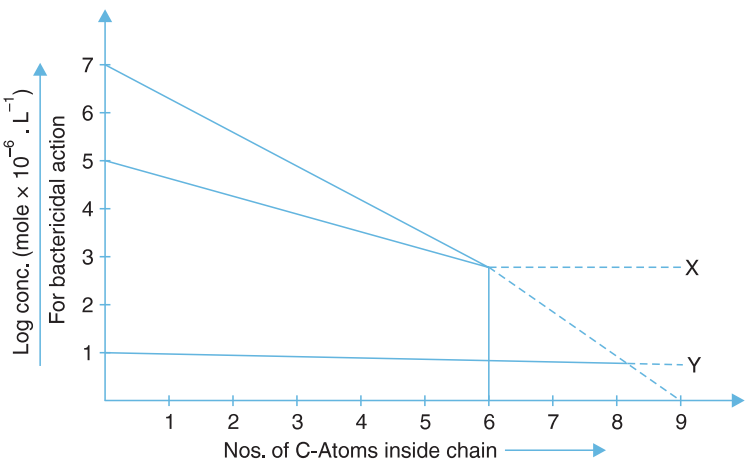


Fig. 2.2. Plot between Log Conc. and Nos. of C-Atoms Inside-chain.

2.9. Steric Factors

Interestingly, it is absolutely necessary for a **'drug molecule'** to engage into a viable and plausible interaction either with a **drug receptor** or with an **enzyme**, it has got to *first* approach ; and *secondly*, attach to a binding site. Obviously, this essentially demands certain specific criteria that a '**drug molecule**' must fulfil, for instance : bulk, size and shape of the **'drug'.** Precisely, the '**bulky substituent**' more or less serve as a shield that eventually hinders the possible and feasible interaction taking place between a **'drug'** and a **'receptor'.**

Meticulous and intensive in-depth studies in this particular aspect has practically failed to *justify* and *quantify* steric characteristics in comparison to quantifying either electronic or hydrophobic

characteristics. In fact, a plethora of methodologies have been tried and tested to ascertain the steric factor(s). In the present context only *three* such methods shall be discussed briefly, namely :

(*a*) Taft's Steric Factor (Es),

(*b*) Molar Refractivity (MR), and

(*c*) Verloop Steric Parameter.

2.9.1. Taft's Steric Factor (Es)

An attempt has been made to quantify the steric features of various substituents (*i.e.,* functional moieties) by the help of **Taft's steric factor (Es).**

In fact, there are *three* predominant constants, namely :

(*i*) Hammett substitution constant (σ),

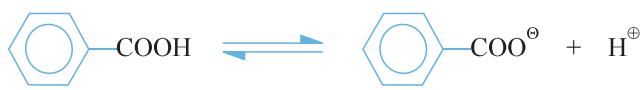
(*ii*) Resonance effect (R), and

(*iii*) Inductive effect (F).

can only be employed for aromatic substituents ; and are hence suitable exclusively for such **'drugs'** that contain *aromatic rings.*

A. Hammett Substitution Constant (σ). It is a measure of either the **electron-withdrawing** or **electron-donating** capability of a substituent (*i.e.,* the functional moiety). **Hammet substitution constant** may be determined conveniently by actual measurement of the dissociation of a series of benzoic acid substituted derivatives *vis-a-vis* the dissociation of pure benzoic acid itself.

However, benzoic acid being a **'weak-acid'** gets partially ionized in an aqueous medium (H_2O) as depicted under :



Explanation. An equilibrium is established between the two distinct species *i.e.,* the *ionized* and *non-ionized* forms. Thus, the relative proportion of the said two species is usually termed as the **'dissociation'** or **'equilibrium'** constant ; and invariable designated by K_H (wherein the *'subscript H'* represents/signifies that there is no substituents normally attached to the aromatic nucleus *i.e.,* the phenyl ring).

$$\therefore \qquad K_H = \frac{[PhCOO^{\ominus}]}{[PhCOOH]}$$

As soon as a substituent is strategically positioned on the aromatic (phenyl) ring, this **'equilibrium'** gets imbalanced. At this juncture *two* situations may crop up distinctly by virtue of the fact that :

(*i*) An electron-withdrawing moiety, and

(*ii*) An electron-releasing (donating) moiety

could be present in the aromatic ring thereby giving rise to **altogether different electronic status** to the **'Aryl Nucleus'.**

(*a*) **Electron-Withdrawing Moiety.** A host of **electron-withdrawing groups**, such as : NO_2, CN, COOH, COOR, $CONH_2$, CONHR, $CONR_2$, CHO, COR, SO_2R, SO_2OR, NO ; cause and result in the aromatic ring (with a π electron cloud both on its top and bottom) having a marked and stronger electron withdrawing and stabilizing influence on the carboxylate anion as illustrated below. Hence,

the overall equilibrium shall influence and shift more to the ionized form thereby rendering the '**substituted benzoic acid**' into a **much stronger acid** (benzoic acid as such is a weak acid). The resulting substituted benzoic acid exhibits a larger K_X value (where, X designates the substituent on the aromatic nucleus) (see Fig. 2.3).

(*b*) **Electron-Donating Moiety :** A plethora of **electron-donating groups**, for instance : R, Ar, F, Cl, I, Br, SH, SR, O^-, S^-, NR_2, NHR, NH_2, NHCOR, OR, OH, OCOR, influence and render the ensuing aromatic ring into a distinctly much less stable to stabilize the *carboxylate ion.* Thus, the equilibrium gets shifted to the left overwhelmingly ; thereby ultimately forming a relatively **much weaker acid** having a smaller K_X value (see Fig. 2.3).

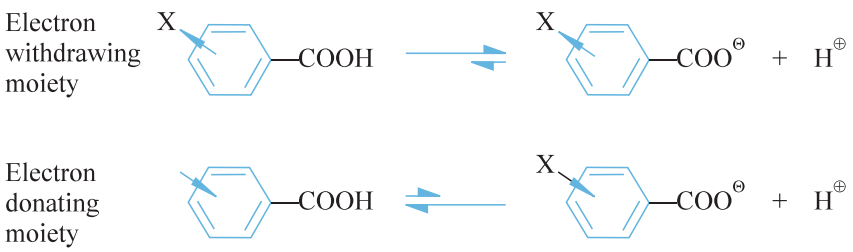


Fig. 2.3 : Influence of Substituent Moiety X on the Status of Equilibrium in Reaction.

Now, the **Hammett substitution constant [σ_X]** with reference to a specific substituent X is usually defined by the following expression :

$$\sigma_X = \log \frac{K_X}{K_H} = \log K_X - \log K_H$$

Therefore, for all benzoic acids essentially possessing electron-withdrawing substituents shall have larger K_X values than the parent benzoic acid itself (K_H) ; thereby the value of **Hammett substitution constant σ_X** for an electron-withdrawing substituent shall be always **positive**.

Similarly, for most benzoic acid variants essentially having electron-donating substituents shall have comparatively smaller K_X values than benzoic acid itself ; and, therefore, the value of **Hammett substitution constant σ_X** for an electron-donating substituent will always be **negative**.

Furthermore, the **Hammett substitution constant** essentially and importantly takes cognizance of *two* vital and critical supportive effects, such as : **resonance effect**, and **inductive effect**. Consequently, the value of σ with respect to a specific substituent may exclusively depend upon whether the attached '*substituent*' is located either at *meta*-or at *para*-position. Conventionally, such particular substituent is invariably indicated by the subscript *m* or *p* first after the symbol σ.

Example : The nitro ($-NO_2$) substituent on the benzene nucleus has two distinct σ values, namely : $\sigma_m = 0.71$ and $\sigma_p = 0.78$.

Explanation. From the σ values, one may evidently observe that the electron-withdrawing strength at the *para*-position is solely contributed by both **'inductive'** and **'resonance'** effects combinedly which justifies the greater value of σ_p, as shown in Fig. 2.4(*a*). Likewise, the *meta*-position, only affords the electron-withdrawing power by virtue of the **'inductive'** influence of the substituent ($-NO_2$ group), as shown in Fig. 2.4(*b*).

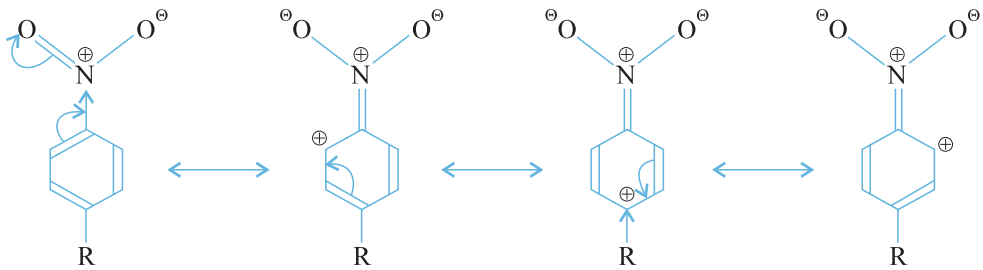


Fig. 2.4(*a*) Electronic Influence on R Caused due to Resonance and Inductive Effects of *p*-nitro Function.

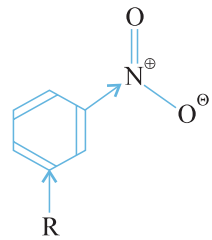


Fig. 2.4(*b*) Electronic Influence on R Caused due to Inductive Effect alone of *m*-nitro Function.

B. Resonance Effect (R) : It has been observed that **'resonance'** mostly gives rise to an altogether **different distribution of electron density** than would be the situation if there existed absolutely no resonance.

Examples : The resonance effects, as observed in *two* electron donating functional moieties, such as : $-NH_2$ (amino) ; and –OH (hydroxyl), attached to an aromatic nucleus, are depicted in Fig. 2.5(*a*) and (*b*) as under :

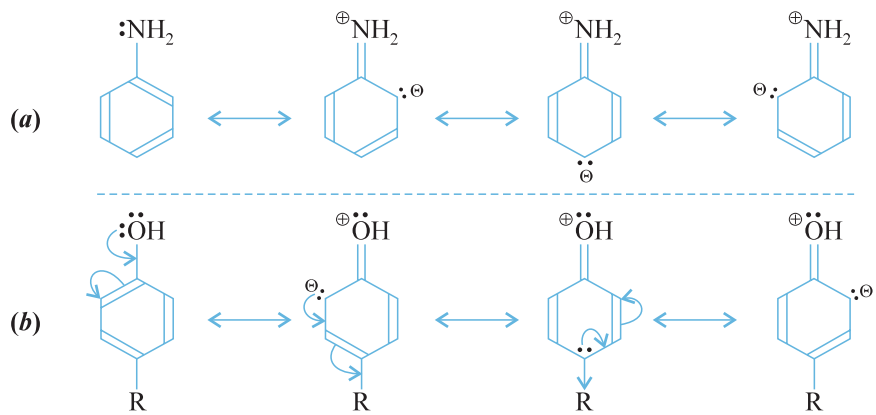


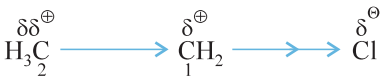
Fig. 2.5(*a*) :Resonance Structures of Aniline

Fig. 2.5(*b*) Electronic Influence on R Exclusively Dominated by Resonance Effects.

Explanations : For Resonance Structures of Aniline [Fig. 2.5(*a*)] : In case, the **first structure** happened to be the **'actual structure of aniline',** the two unshared electrons of the N-atom would certainly reside exclusively on that particular atom. However, in true sense and real perspective the **first structure** is **not** the ideal and only structure for aniline but a **hybrid one** which essentially includes contributions from several canonical forms as shown, wherein the density of electrons of the unshared pair does not reside necessarily on the N-atom but gets spread out around the phenyl ring. In nut shell, this observed density of electron at one particular position (with a corresponding enhancement elsewhere) is invariably known as the **'resonance'** or **'mesomeric effect'.**

For Resonance Structures of Phenol [Fig. 2.5(*b*)] : Here, the influence of R at the *para* position, and the electron-donating effect caused due to resonance is more marked, pronounced and significant as compared to the electron-withdrawing influence due to induction.

C. Inductive Effect (F) : The C—C single bond present in **'ethane'** has practically no polarity as it simply connects two equivalent atoms. On the contrary, the C—C single bond in '**chloroethane**' gets solemnly polarized by the critical presence of the electronegative *chlorine-atom.* In fact, the prevailing polarization is actually the sum of *two* **separate effects.** *First,* being the C-1 atom that has been duly deprived of a part of its electron density evidently by the greater electronegativity of Cl. It is, however, compensated partially by drawing the C—C electrons located closer to itself, thereby causing polarization of this bond and consequently rendering a slightly positive charge on the C-2 atom as shown below :



Chloroethane

Secondly, the effect is caused not through bonds, but directly either through *space* or *solvent molecules,* and is usually termed as the **field effect.***

2.9.2. Molar Refractivity (MR)

Another vital and equally important criterion to measure the **'steric factor'** is adequately provided by a parameter called as **molar refractivity (MR)**. It is usually designated as a simple measure of the volume occupied either by an individual atom or a cluster (group) of atoms. However, the **MR** may be obtained by the help of the following expression :

$$MR = \frac{(n^2 - 1)}{(n^2 + 2)} \times \frac{MW}{d}$$

where, n = Index of refraction,

MW = Molecular Weight,

d = Density,

MW/d = Volume and

$\frac{n^2 - 1}{n^2 + 2}$ = Correction factor (*i.e.*, how easily the substituent can undergo polarization)

Molar refractivity is specifically significant in a situation when the substituent possesses either π *electron* or *lone pairs of electrons.*

*Roberts ; Moreland., *J. Am. Chem. Soc.,* **75,** 2167, 1953.

2.9.3. Verloop Steric Parameter

The unique revelation and wisdom of a latest computer researched programme termed as **sterimol** has indeed helped a long way in measuring the **steric factor** to a reasonably correct extent. It essentially aids in the calculation of desired steric substituent values (otherwise known as **Verloop steric parameters**) based on various standard physical parameters, such as : Van der Waals radii, bond lengths, bond angles, and ultimately the proposed most likely conformations for the substituent under examination. It is, however, pertinent to mention here that unlike the **Taft's steric factor (E_S)** (see Section 2.9.1) the Verloop steric parameters may be measured conveniently and accurately for any substituent.

Example : **Carboxylic acid (say, Benzoic Acid) :** The ensuing **Verloop steric parameters** for a carboxylic acid moiety are duly measured as shown in Fig. 2.6 below, where L represents the length of the substituent, and $B_1 - B_4$ designate the radii (*i.e.*, **longitudinal** and **horizontal**) of the *two* functional groups *viz.*, *carboxyl and hydroxyl* (—O—H).

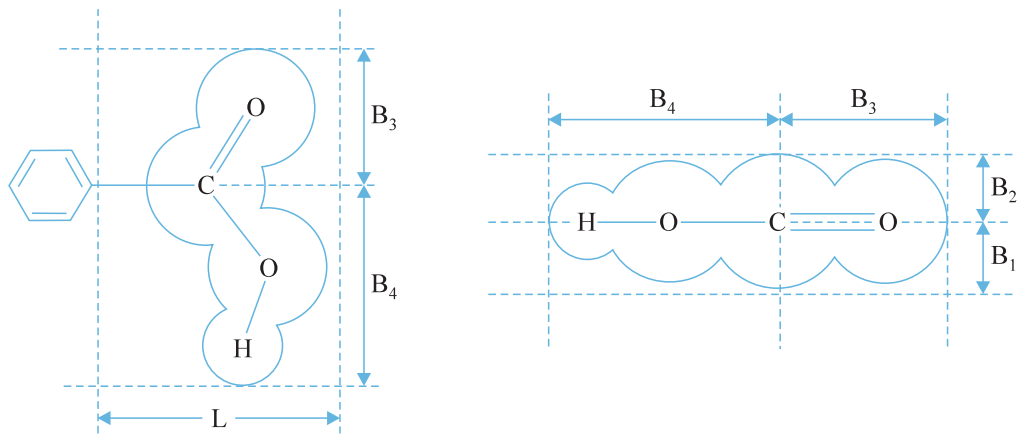


Fig. 2.6 Verloop Steric Parameters for a Carboxylic Acid (—COOH) Moiety.

Interestingly, most **quantitative structural activity relationship (QSAR)** studies usually commence by considering σ (**Hammett substitution constant**) and, in case there exists more than one substituent, the σ values are represented in a summed up manner as Σσ. Keeping in view the enormous quantum of **synthetic newer target drug molecules,** it has now become almost necessary and possible either to modify/refine or fine tune-up the QSAR equation. In fact, a substituent's **resonance effect (R)** and **inductive effect (F)** may be quantified as far as possible with the help of available **'tables of constants'.** In certain instances one may evidently observe that :

- a substituent's effect on biological activity is solely on account of F rather than R, and *vice versa.*
- a substituent exerts a more prominent and appreciable activity when strategically located at a specific position on the aromatic nucleus ; and moreover it may also be embeded in the '**equation**' appropriately.

2.10. Hansch Equation

Integrating various factors, namely : **Taft's steric factor, resonance, inductive, Verloop steric parameters** with the partition behaviour of **'drug molecules'** Hansch* and Fujita** exploited these principles in determining the establishing **quantitative structure-activity relationship (QSAR)** of **drugs,** which has undergone a sea change both in expansion and improvement with the help of **computer researched soft-wares**.

The **hydrophobic characteristic,** designated by π_x, may be correlated to a **drug's distribution pattern**, within which a given substituent '*x*' affects molecular behaviour and conduct with regard to its :

- ➤ distribution and transport, and
- ➤ drug-receptor activities.

The hydrophobic characteristic π_x of a drug substance may be expressed as :

$$\pi_x = \log P_x - \log P_{yH}$$

where, log P = logarithm of 1-octanol-water partition coefficient

y = A parent compound (*i.e.*, an unsubstituted reference compound/drug).

Salient Features : The various salient features of **Hansch equation** are as enumerated under :

(1) Value of π is indicative, to a certain extent, the behavioural pattern of a *'substituent'* contributing to the solubility behaviour of a molecule under investigation. It also reflects upon the manner it gets partitioned between lipoidal and aqueous interfaces in the reputed compartments it happens to cross as a **'drug'** so as to reach the **'site of action'** ultimately.

(2) It is, however, not very clear and definite whether the solid surface of a *'drug'* undergoes adsorption on colloidally suspended plasma proteins while establishing the **hydrophobic characteristics π.**

(3) Interestingly, the concurrent considerations of π and σ **(Hammett's constant)** has evolved gainful vital correlations existing between the biological activities of quite a few drug substances with their corresponding physical properties and chemical structures.

Therefore, **Hansch's correlations** piece together valuable information(s) of a newly designed **'drug molecule'** in a more plausible, predictive and quantifiable manner than before — and apply it to a biological system more logistically and judiciously. This particular concept and idea was further substantiated and expanded by assuming that all the *three* substituents *viz.*, π, σ and Es, exert a significant effect on the efficacy and hence the potency of a **'drug substance'** ; and are found to be additive in nature independently. Therefore, it has given rise to the underlying **linear Hansch equation** :

$$\log\left(\frac{1}{C}\right) = a \log P + b\, E_S + \rho(\sigma) + d$$

where , C = Concentration of drug producing the biological response being measured,

log P = Substituent constant for solubility (*i.e.*, π),

E_S = Taft constant (for steric effects),

ρ = (rho) Proportionality constant designating the sensitivity of the reaction to electron density.

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* Hansch *et al. J. Am. Chem. Soc.*, **85,** 2817, 1963, *ibid*, **86**, 1616, 1964 ;

** Fujita *et al. J. Am. Chem. Soc.*, **86,** 5175, 1964.

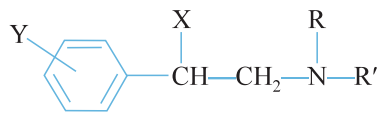
σ = Hammett substitution constant

a, *b*, *d* = Constants of the system (which are determined by computer to obtain the **'best fitting line').**

It is pertinent to state at this juncture that *not* all the parameters shall necessarily be significant.

Example : **β-Halo-arylamines :** The **adrenergic blocking profile** of **β-halo-arylamines** was observed to be solely related to the two constants, π and σ ; and specifically excluded the steric factor altogether.

i.e., $$\log\left(\frac{1}{C}\right) = 1.22\,\pi - 1.59\,\sigma + 7.89$$

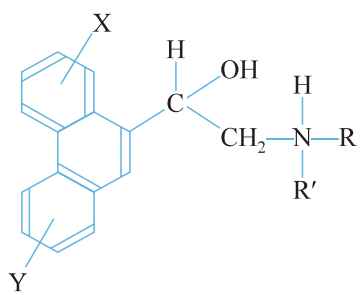


The aforesaid equation offers a dictum that the **'biological response'** gets enhanced if the substituents possess a positive π value and a negative σ value ; or more explicitely the substituents must preferentially be both hydrophobic in nature and electron donating in character.

It has been established beyond any reasonable doubt that there exists no correlation between the **π factor** and the **P value** ; therefore, it is quite feasible to have **Hansch equations** essentially comprising of these two stated components :

Example : **Phenanthrene aminocarbinols :** An analogous series of more than one hundred **phenanthrene aminocarbinols** were successfully synthesized and subsequently screened for their **antimalarial profile**. Interestingly, the analogous series fitted appropriately into the following version of **Hansch equation** :

$$\log\left(\frac{1}{C}\right) = -0.015\,(\log P)^2 + 0.14\log P + 0.27\,\Sigma\pi_x + 0.40\,\Sigma\pi_y + 0.65\,\Sigma\sigma_x + 0.88\,\Sigma\sigma_y + 2.34$$



PHENANTHRENE AMINOCARBINOL

Salient Features : The various characteristic **salient features** that may be derived from the above equation are, namely :

(1) As the **hydrophobicity** of the molecule (P) enhances there exists a very nominal increase in the **antimalarial activity**.

(2) The corresponding constant is low (0.14) which reflects that the increase in **antimalarial activity** is also low.

(3) The value of $(\log P)^2$ evidently reveals that there prevails a **maximum P value for activity**.

(4) Further the above equation suggests that the **antimalarial activity** gets enhanced appreciably when the hydrophobic moieties are strategically located either on ring 'X' or more specifically on ring 'Y'. It further ascertains that the **hydrophobic interaction(s)** are virtually taking place at these sites.

(5) The **electron-withdrawing substituents on rings 'X' and 'Y'** contribute enormously to the **antimalarial activity** ; however, the effect is more on ring 'Y' than in ring 'X'.

2.11. The Craig Plot

The **Craig plot** is nothing but an actual plot between the **'π factor'** taken along the **X-axis** and the **'σ factor'** taken along the **Y-axis**, thereby having a clear and vivid idea with regard to the relative properties of different functional moieties (substituents).

Fig. 2.7 illustrates the **Craig plot** of various *para* aromatic substituents for the σ and π factors respectively.

Salient Features : The various advantageous **salient features** of a **Craig plot** are enumerated as under :

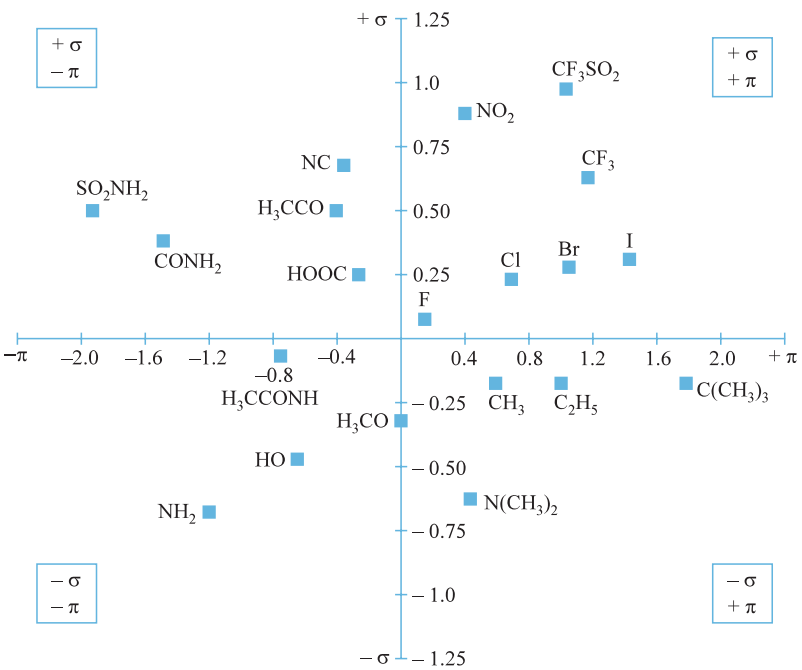


Fig. 2.7 The Craig Plot for the σ and π Factors of *para*-Aromatic Substituents.

(1) The **Craig plot** in Fig. 2.6 evidently depicts that there is absolutely no clearly defined overall relationship between the two **'key factors'** σ and π. However, the various functional moieties (*i.e.*, substituents) are strategically positioned around all the four quadrants of the plot based on their inherent physicochemical status and integrity.

(2) From the above **Craig plot** one may obviously identify the substituents that are particularly responsible for +ve π and σ parameters, –ve π and σ parameters, and lastly one +ve and one –ve parameter.

(3) Further it is quite convenient and easy to observe which substituents have nearly identical π values, such as : *dimethyl-amino*, *fluoro* and *nitro* on one hand ; whereas, *ethyl*, *bromo*, *trifluoromethyl*, and *trifluoromethyl sulphonyl* moieties on the other are found to be almost located on the same **'vertical line'** on the **Craig plot.** Thus, theoretically all these functional groups are legitamately interchangeable on a newly designed drug molecule wherein the major critical and principal factor that significantly affects the **'biological characteristics'** is essentially the π factor.

Interestingly, in the same vein, the various functional moieties that are located on the **'horizontal line'**, for instance : methyl, ethyl *tert-butyl* on one hand ; whereas, carboxy, chloro, bromo, and iodo moieties on the other can be regarded and identified as being *iso-electric* in nature or possessing identical σ values.

(4) **QSAR studies** are exclusively and predominantly governed and guided by the **Craig plot** with regard to the various substituents in a new drug molecule. Therefore, in order to arrive at the most preferred **'accurate equation'** essentially consisting of π and σ, —the various structural analogues must be synthesized having appropriate substituents pertaining to each of the four quadrants.

Examples :

(*i*) **Alkyl Moieties :** These substituents contribute exclusively +ve p values and –ve s values.

(*ii*) **Acetyl Moieties :** These are responsible for attributing –ve π values and +ve σ values.

(*iii*) **Halide Groups :** These functional moieties essentially enhance both electron-withdrawing characteristics and hydrophobicity in the **'drug molecule'** by virtue of their +ve σ and +ve π effects.

(*iv*) **Hydroxy Groups :** These functional moieties exert progressively more hydrophilic and electron-donating characteristics on account of the –ve π and –ve σ effects.

(5) Importantly, the very establishment and derivation of **Hansch equation** will certainly give a better reliable and meaningful clue with regard to attaining a reasonably good biological property based on the fact whether π and σ must be –ve or +ve in character. However, further improvements in the **'drug molecule'** could be accomplished by exploring various other possible substituents picked up judiciously from the relevant quadrant (see Fig. 2.6).

Example : In case, the **Hansch equation** rightfully demands that +ve σ and –π values are an absolute necessity, additional relevant substituents must be picked up from the top-left quadrant.

(6) The **Craig plot** may also be exploited to compare the **MR and hydrophobicity**.

2.12. The Topliss Scheme

Keeping in view the enormous cost incurred with regard to the synthesis of a large range of structural analogues necessarily required for a **Hansch equation,** it has become almost necessary to restrict the synthesis of a relatively lesser number of drug molecules that may be produced in a limited span of time having viable biological activity. Based on the actual outcome of the biological activity *vis-a-vis* the actual structure of the '**drug**' ultimately helps to determine the next analogue to be synthesized.

The **Topliss scheme** is nothing but an organized **'flow diagram'** which categorically permits such a procedure to be adopted with a commendable success rate.

In actual practice, however, there are *two* distinct **Topliss Schemes**, namely : (*a*) For **aromatic substituents ;** and (*b*) For **aliphatic side-chain substituents.** It is pertinent to mention here that the said *two schemes* were so meticulously designed by taking into consideration both **electronic** and **hydrophobicity** features (*i.e.*, substituents) with a common objective to arrive at the **'optimum biological active substituents'.**

It may be made abundantly clear and explicit that the **Topliss Schemes** are not a replacement for the Hansch analysis. Hence, the former may be made useful and effective only when a good number of tailor-made structures have been designed and synthesized.

[A] Fig. 2.8 represents the **Topliss Scheme for Aromatic substituents ;** and has been based on the assumption that the **'lead compound'** essentially possesses a single monosubstituted aromatic ring and that it has already been screened for its desired biological activity.

Salient Features : The various **salient features** with respect to the **Topliss scheme** for aromatic substituents are as described below :

(1) 4-chloro derivative happens to be the '*first structural analogue*' in this particular scheme perhaps because it is easy to synthesize.

(2) The π and σ values are both positive by virtue of the fact that the chloro substituent is much more hydrophobic and electron-withdrawing than hydrogen-atom.

(3) The synthesized chloro-analogue is subjected to the biological activity measurements accordingly.

(4) Three situations may arise, namely : (*a*) analogue possessing less activity (L) ; (*b*) equal activity (E) ; and (*c*) more activity (M). Thus, the type of observed activity is solely the determining factor as to which '*branch*' of the Topliss scheme is to be adopted next.

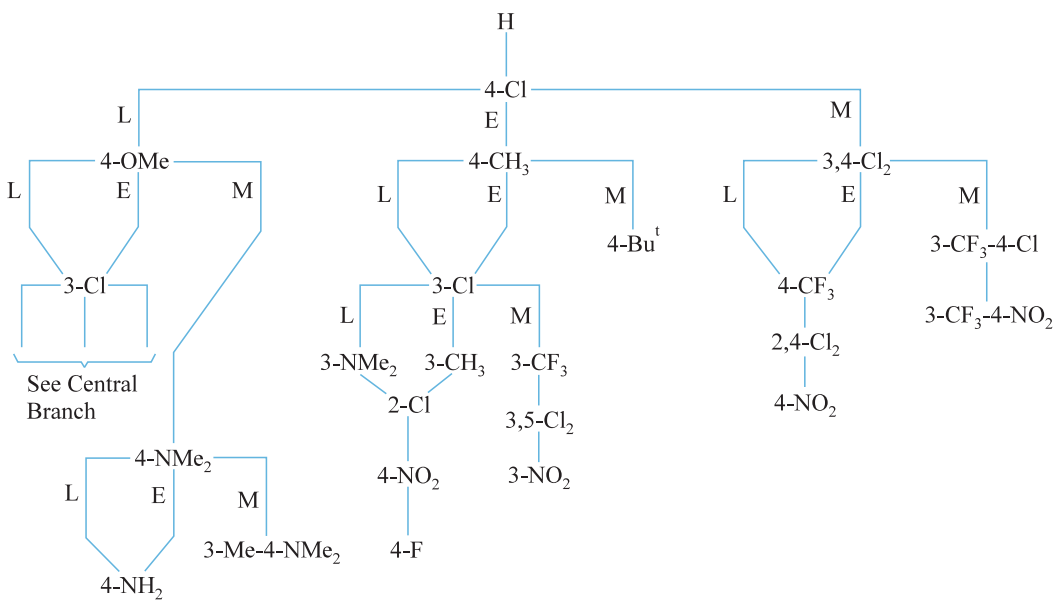


Fig. 2.7 : Topliss Scheme for Aromatic Substituents

(5) Further line of action towards the synthesis of structural analogues of 4-chloro aromatic substituents are entirely guided and based on the following *three* options, namely :

Biological Activity	Series Followed	Next Analogues Synthesized
(*a*) Increases	M-series	3, 4-Dichloro substituted derivatives
(*b*) Same profile	E-series	4-Methyl derivatives
(*c*) Decreases	L-series	4-Methoxy derivatives

(6) Let us consider the second analogous series which shows the same biological activity. The various situations that may arise are as follows :

(*i*) **4-Chloro derivative enhances the desired biological property :**

As the Cl-substituent exerts both positive π and σ values it evidently shows that either one or both of three characteristic features are quite critical and important to biological property. In case, both characteristic features are important, addition of the second Cl-moiety shall enhance the biological activity to the positive side furthermore. If it fails, there may exist either an excess hydrophobic character or an obstructive steric hindrance is exhibited. Thus, the situation demands further modification based on subsequent biological screening *vis-a-vis* the comparative importance and status of π as well as the steric features.

(*ii*) **4-Chloro derivative lowers the desired biological activity :**

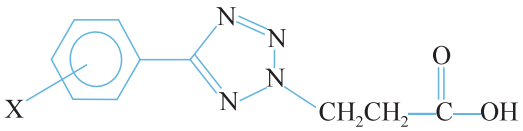
It gives a clue that either the location of the *para*-substituent is absolutely unsuitable sterically or the –ve π and/or σ values are prominently important with regard to the biological activity. It has been established that the reduced activity is solely attributed due to an unfavourable σ effect ; and hence, one may assign the *para*-methoxy moiety as the next probable substituent having a –ve σ factor. If by doing so there is an apparent improvement in activity, further alterations are accomplished with a view to ascertain the prevailing relative importance of the σ and π values. Now, if the above modifications *i.e.*, *para*-methoxy moiety fails to make any improvement in the activity, one may draw an inference that an undesired steric factor is playing the havoc, and the next possible entrance is of the *meta*-chloro group. However, further modifications of this functional group shall then be persued as depicted in the middle series of Fig. 2.8.

(*iii*) **4-Methyl derivative equals the desired biological activity :**

In this specific instance, the overall biological activity of the 4-chloro structural analogue exerts practically no change as compared to the **'lead compound'.** It might have emanated from the **'drug substance'** essentially looking for a negative π value and a positive σ value. As it is quite evident that the two said values attributed by the chloro moiety are apparently positive, the useful effect of the positive π value should have been nullified due to the detrimental influence of a positive σ value. Therefore, the most preferred substituent would be the *para*-methyl group, which adequately possesses positive π value and negative σ value. In case, it still exhibits no useful effect, one may draw a conclusion that there exists an unfavourable steric interaction prevailing at the *para*-position. Hence, the next preferable line of action would be the introduction of chloro group at the *meta*-position. However, any additional changes shall affect the values attributed by both π and σ factors.

The **Topliss scheme** has been thoroughly investigated, tested and above all validated by various researcher after evaluating their **structure-activity relationships (SARs)** for a host of **'drug substances'**.

Example : **Substituted phenyltetrazolylalkanoic acid :** A total of 28 structural analogues of **substituted phenyltetrazolylalkanoic acids** were synthesized in the laboratory and screened duly for their anti-inflammatory activities. Nevertheless, if the whole exercise would have been based on the **Topliss Scheme** only the first eight compounds (out of 28) should have yielded *three* most active compounds as given below :



Substituted Phenyltetrazolylalkanoic Acids

Sequence of Synthesis	X	Biological Activity (Observed)	Maximum Potency (Observed)
1	H	—	
2	*para*-Cl	L	
3	*para*-OCH_3	L	
4	*meta*-Cl	M	++++
5	*meta*-CF_3	L	++++
6	*meta*-Br	M	
7	*meta*-I	L	
8	3, 5—Cl_2	M	++++

L = Less Activity
M = More Activity
E = Equal Activity

[B] Fig. 2.9 designates the **Topliss Scheme** for the **Aliphatic** side-chains and adopted in the same vein and rationale as the aforementioned **'aromatic scheme'** (section 'A'). The present scheme is expanded exactly in the same fashion for the side functional moieties strategically linked to a variety of such functional groups as : **amine**, **amide** or **carbonyl**.

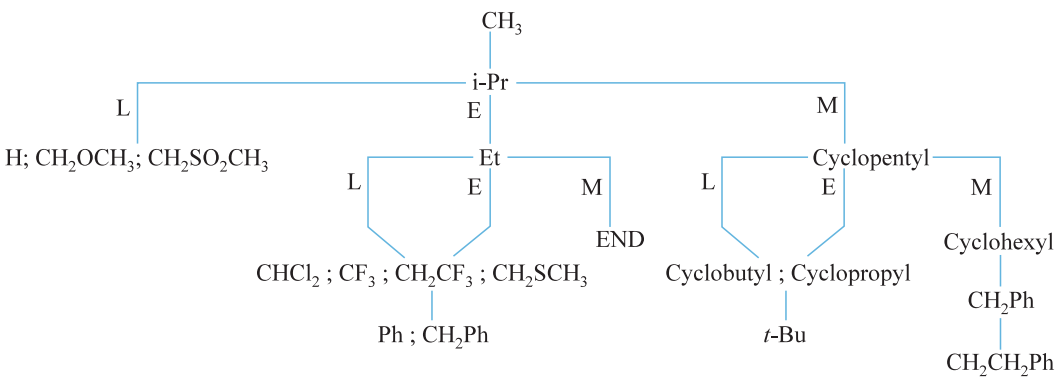


Fig. 2.9. Topliss Scheme for Aliphatic Side-chain Substituents.

Interestingly, the **Topliss Scheme** helps to make a clear cut distinction between the two pronounced physical characteristic features, namely : electronic effect, and hydrophobic effect, caused due to the various substituents ; and not the steric characteristic features. Perhaps that could be the possible line of thought judiciously utilized in the selection of appropriate substituents so as to reduce any steric differences. Let us have an assumption that the **'lead compound'** possesses a—CH_3 functional moiety.

A variety of typical situations may crop up during the said studies :

(*a*) **Rise in anti-inflammatory activity :** A cyclopentyl moiety is now utilized which provides a much larger π value, and simultaneously holds the steric influence to a bear minimum level. In case, a further rise in activity is observed one may institute more hydrophobic substituents. On the contrary if the activity fails to rise, there could be two possible reasons, namely : (*i*) optimum hydrophobicity has superseded ; and (*ii*) electronic effect (σ_1) has triggered action. Of course, an elaborated further study would reveal and ascertain the exact substituents to substantiate which of the two explanations stands valid.

(*b*) **Static anti-inflammatory activity :** The activity exerted by the isopropyl structural analogue almost remains the same as that of the methyl one. It could be explained most logically that both methyl and isopropyl moieties are actually located on either side of the **'hydrophobic optimum'**. Hence, an intermediate functional group *i.e.*, an *ethyl group*, possessing an intermediate π value, is employed as the next substituent (see Fig. 2.7). In case, it still registers practically no plausible or appreciable improvement in the activity profile, one may switch over to an electron-withdrawing moiety instead of an electron-donating moiety, having identical π values, as futuristic suggestive approach.

3. FACTORS GOVERNING ABILITY OF DRUGS TO REACH ACTIVE SITE

There are certain vital factors that govern the ability of a drug to reach the active site soon after its administration through various modes known to us. These factors essentially include **absorption, distribution, biotransformation (metabolism) and elimination**. However, in all these instances, the drug molecule has to cross a few biological membrane in one form or the other. These factors shall now be treated briefly with appropriate typical examples wherever necessary.

3.1. Absorption

Biological membranes play a vital role towards the absorption of a drug molecule. Soon after a drug is taken orally, it makes its way through the gastrointestinal tract, cross the various membranes and finally approach the site or cell where it exerts its desired pharmacological action.

It has been observed that a plethora of drug molecules normally cross biological membranes by passive diffusion from a region of high drug concentration (*viz* : gastrointestinal tract) to a region of low drug concentration (*viz* : blood). However, the rate of diffusion solely depends upon the magnitude of the concentration gradient (ΔC) across the biological membrane and may be represented by the following equation :

$$\text{Rate} = -K\Delta C = -K(C_{abs} - C_{bl}) \qquad ...(1)$$

where C_{abs} represents the concentration of drug at the absorption site and C_{bl} is the respective concentration present in the blood. The constant of proportionality K, is a complex constant which essentially includes the area and thickness of membrane, partition of drug molecule between aqueous phase and membrane and finally the **diffusion coefficient of the drug**. It may be assumed that the concentration of

drug in the blood is fairly negligible as compared to the concentration in the gastrointestinal lumen. Hence, equation (1) simplifies to

$$\text{Rate} = -KC_{abs} \qquad ...(2)$$

As one may observe from equation (2) that absorption by passive diffusion is nothing but a first-order process, hence the rate of drug absorption is directly proportional to the concentration of drug at the absorption site. In other words, the larger the concentration of drug, the faster is the rate of absorption. At any time after the administration of the drug, the percentage of the dose absorbed remains the same irrespective of the dose administered.

Lipid solubility of the drug is the determining factor for the penetration of cell membranes. Therefore, the passage of many drug molecules across the membranes of the skin, oral cavity, bile, tissue cells, kidneys, central nervous system and the gastrointestinal epithelium is very much related to the lipid solubility of the drug molecule.

3.2. Distribution

As soon as a drug finds its way into the blood stream, it tries to approach the site of biological action. Hence, the **distribution** of a drug is markedly influenced by such vital factors as tissue distribution and membrane penetration, which largely depends on the physico-chemical characteristics of the drug. For instance, the effect of the ultra-short acting barbiturate thiopental may be explained on its dissociation constant and lipid solubility. It is worthwhile to observe here that the duration of thiopental is not influenced by its rate of excretion or metabolism, but by its **rate of distribution**.

3.3. Metabolism (Biotransformation)

When a drug molecule gets converted into the body to an altogether different form, which may be either less or more active than the parent drug, the phenomenon is termed as **biotransformation**. Mostly the drug metabolism occurs in liver. In fact, a number of pathways are genuinely responsible for carrying out various diverse metabolism reactions in the body.

It may be pertinent to observe here that most of the metabolised products are usually more polar in character than the parent drug molecule. This increased polarity renders the metabolism less absorbable through the renal tubules and also makes it transient in the body.

A large number of **barbiturates** are metabolized by liver microsomes. **Isoniazid** is quickly metabolized in **Japanese race** to the extent of 86.7%, whereas approximately half of it (44.9%) in *American and* **Canadian whites.** This disparity is due to the genetic differences in the said races.

In a broader sense a plethora of metabolic processes which usually **detoxify** the foreign substances *in vivo*, such as : oxidation, reduction, hydrolysis, esterification or conjugation ; thereby rendering the **'drug substance'** normally more water-soluble, so as to enhance its excretion from the body. It has been duly observed that in a good number of cases a **'drug metabolite'** actually may serve as the *active compound*, almost showing identical biological activity to the original compound. Interestingly, after having undergone several **biotransformations**, the ultimate modified form of the drug is excreted finally.

Though liver is considered to be the **primary site** of **'detoxification'** ; however, many enzymatic degradation processes may also take place in the stomach, intestine, pancreas, and other locations in the body. Generally, the metabolic processes occurring in the liver may be conveniently categorized under the following **two** heads, namely :

(*a*) **Functional Group Changes :** Here, the **'drug substance'** undergoes functional group changes, for instance : side-chain or ring hydroxylation, reduction of nitrogroup, reduction, aldehyde oxidation, deamination or dealkylation, and

(*b*) **Conjugation :** In this instance, the **'drug substance'** undergoes conjugation whereby the metabolized product subsequently combines with various solubilizing groups, such as : **glycine** (an amino acid) or **glucuronic acid** (glucuronides) to result into the formation of excretable conjugates ultimately.

Hence, during the course of designing/development of a **'new target-drug-molecule'** the **'medicinal chemist'** must take into cognizance of such metabolic phenomena and modify the structure of the drug substance in question so as to alter the course in which it should have been metabolized otherwise.

3.4. Excretion

Excretion of drugs from their sites of action is of paramount importance and may be effectively carried out with the help of a number of processes, namely : renal excretion, biliary excretion, excretion through lungs and above all by **drug metabolism** (**biotransformation**).

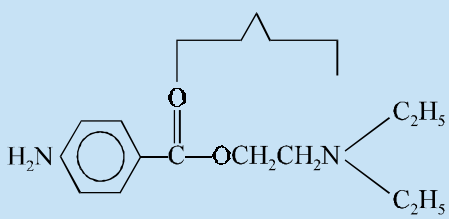
Drugs which are either water-soluble or get metabolized gradually are mostly eliminated through the kidneys by the aid of these three essential phenomena, *viz* : secretion, glomerular filtration and tubular reabsorption. For instance, **probenecid** considerably retards tubular secretion of **penicillin** thereby enhancing its duration of action appreciably.

Another aspect of excretion is the biliary excretion of drugs or its metabolites which essentially affects excretion of drugs by liver cells into the bile and subsequently into intestine. Invariably, a drug undergoes 'enterohepatic cycling', *i.e.,* instead of its elimination through the faeces it gains entry into the system through the intestines, *eg.*, **penicillin, fluorescein,** etc.

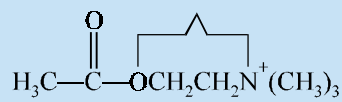
3.5. Intramolecular Distances and Biological Activity

The intramolecular distance is regarded as a structural feature of the drug molecule which falls within the regimen of physical property. It can be effectively measured either by X-ray or by electron diffraction measurements.

The intramolecular distance present in the grouping $-X\ CH_2CH_2N-$ between the nitrogen atom and *X* (where *X* = N, O etc.) that could be seen in a variety of medicinal compounds (*i* – *iii*) as stated below falls in the vicinity of 5A :

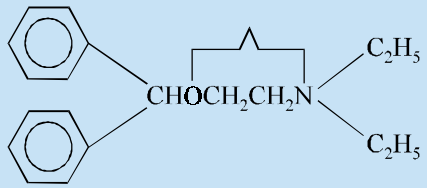


(*i*) Procaine
(local anaesthetic)



(*ii*) Acetylcholine
(parasympathomimetic)

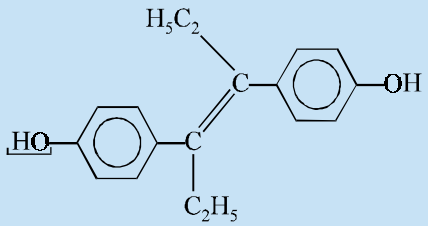
(*iii*)



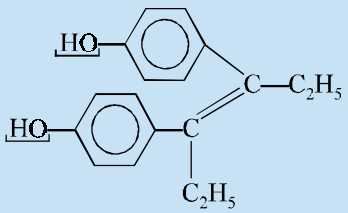
Diphenhydramine
(antihistaminic)

However, the distance between the two centres may alter based on the shape of the drug molecule.

Another interesting example can be observed in the two geometrical isomers of the artificial oestrogenic hormone, namely : *trans*-**diethylstilbesterol** and *cis*-**diethylstilbesterol.**



trans-Diethylstilbesterol
(Active)



cis-Diethylstilbesterol
(Inactive)

The distance between the two hydroxyl groups present in the ***trans*-diethylstilbesterol** is 14.5A which is being higher than the ***cis*-diethylstilbesterol** and this is actually responsible for the more potent oestrogenic activity of the former.

4. DISSOCIATION CONSTANTS

Usually the **dissociation constant** of week electrolytes is expressed by the **Henderson-Hasselbalch equations** as stated below :

$pKa = pH + \log [acid]/[conjugate\ base]$

For acids :

$pKa = pH + \log [undissociated\ acid]/[ionized\ acid]$

For bases :

$pKa = pH + \log [ionized\ base]/[undissociated\ base]$

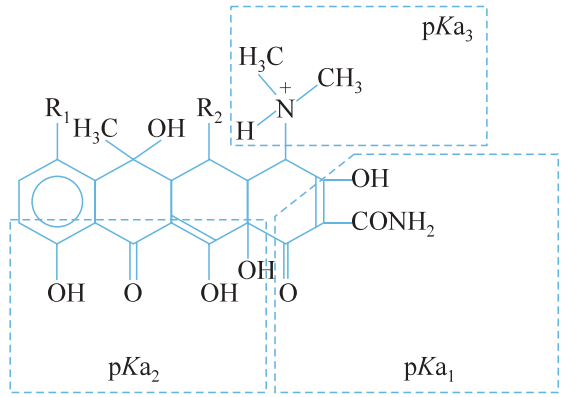
However, the dissociation constant of a weak base or acid may be conveniently determined by one of these several established methods, namely : ultraviolet or visible absorption spectroscopy, conductivity measurements and finally the potentiometric pH measurement.

It has been observed that most drugs exert their pharmacodynamic action either as undissociated molecules or as ionized molecules. These *two* different aspects shall be discussed briefly.

4.1. Drugs Exerting Action as Undissociated Molecules

In a large number of potent medicinal compounds the **dissociation constants** play a vital role for their respective **biological characteristics**.

The unusual structural groupings in the tetracyclines results *three* distinct acidity constants in aqueous solutions of the acid salts. The particular functional groups responsible for each of the thermodynamic p*K*a values has been determined by Lessen and co-workers as described below :



The approximate *p*Ka values for each of these groups in *four* commonly used **tetracyclines** are as shown below.

p*Ka* Values (of Hydrochlorides) in Aqueous Solutions at 25°C

S.No.	Names	pKa_1	pKa_2	pKa_3
1	**Tetracycline**	3.3	7.7	9.5
2	**Chlorotetracycline**	3.3	7.4	9.3
3	**Demeclocycline**	3.3	7.2	9.3
4	**Oxytetracycline**	3.3	7.3	9.1

Besides, the activity of several local anaesthetics, ***d*-tubucurarine** and **phenol** has also been proved to be related to their degree of ionization.

4.2. Drugs Exerting Action as Ionized Molecules

A plethora of medicinal compounds exert their pharmacodynamic action exclusively as their ionized molecules, *viz :* **acetylcholine**, **quaternary salts** as ganglionic blocking agents and muscle relaxants (discussed elsewhere in this book), and antiseptics.

5. ISOSTERISM AND BIO-ISOSTERISM

The constant endeavour toward newer and more potent biologically active compound has paved the way for research into more specific, more effective, structurally similar compounds either possessing same or opposite activity.

Langmuir* suggested that any two ions or molecules possessing essentially an identical number and arrangement of electrons must exhibit similar characteristics ; and all such pairs he named as **'isosteres'**

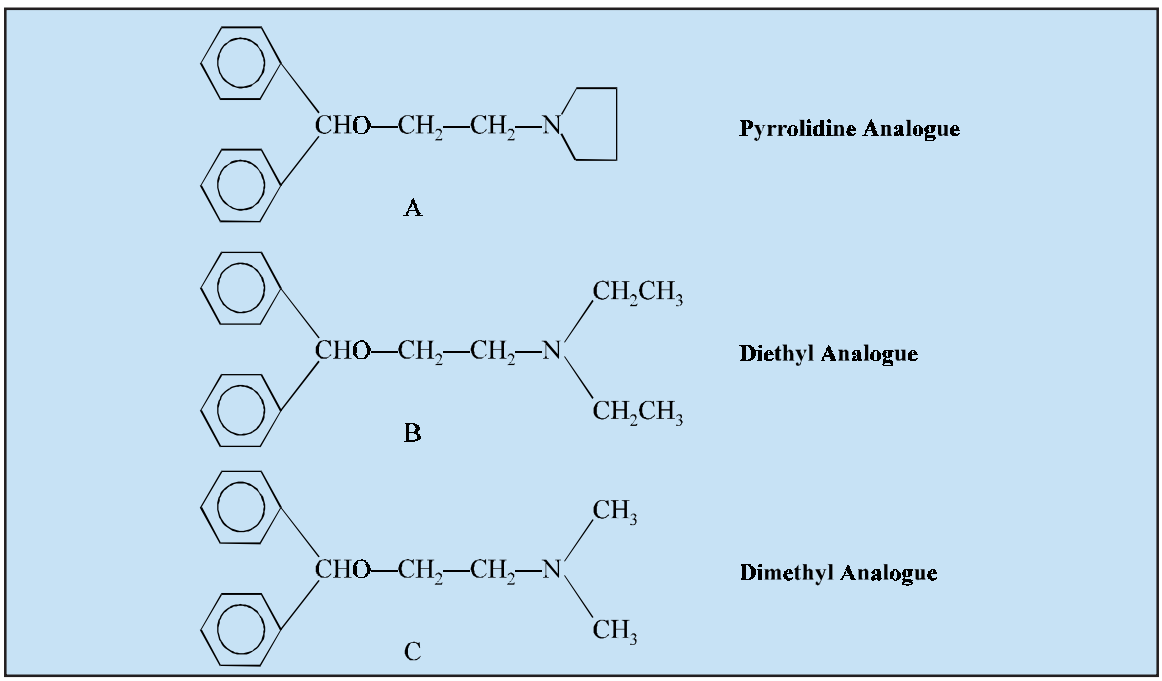
* I. Langmuir, *J. Am. Chem. Soc.*, **41**, 868, 1543, 1919.

e.g., CO and N ; CO_2 and N_2O ; and N_3^- and NCO^-. However, it is quite evident that such isosteres which are isoelectric in nature must show good similarity in properties.

Isosterism is of vital importance to a medicinal chemist because the biological characteristics of isosteres appear to be similar more frequently than their physical or chemical characteristics.

Keeping in view the numerous advantageous applications of **isosterism** in resolving biological problems effectively, Friedman* proposed the following definition of '**bio-isosterism**' — **the phenomenon by which compounds usually fit the broadest definition of isosteres and possess the same type of biological activity.**

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



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

It has been duly observed that it is more or less difficult to correlate the **biological properties** *vis-a-vis* **physico-chemical** properties inherited by specific individual atoms, functional groups or entire molecules by virtue of the glaring and established fact that a host of physical and chemical parameters are invovled simultaneously and are, therefore, extremely difficult to quantitate them justifiably. Besides, simpler relationships *e.g.*, **'isosterism'** invariably do not delay across the several varieties of biological systems that are often encountered with medicinal agents (*i.e.*, **drug substances**). In other words, a specific isoelectric replacement in one particular **biological system** (or a given **drug receptor**) may either work or fail to response in another.

---

* H.L. Friedman, **Influence of Isosteric Replacements upon Biological Activity,** National Academy of Sciences-National Research Council Publication No. 206, Washington, D.C., p. 295, 1951.

In order to expatiate further the terminology **'bioisosters'.** Burger* expanded the definition of Friedman to take into consideration the **biochemical views of the biological activity :**

"Bioisosteres are compounds or groups that essentially possess near equal molecular shapes and volumes, approximately the same distribution of electrons, and that exhibit similar physical characteristics, such as : hydro-phobicity. Bioisosteric compounds affect the same biochemically associated systems as agonists or antagonists and thereby produce biological properties that are more or less related to each other".**

Bioisosteres may be classified under **two** categories, namely :

(*a*) Classical Bioisosteres, and

(*b*) Nonclassical Bioisosteres.

5.1. Classical Bioisosteres

Functional moieties which either fulfil or satisfy the original conditionalities put forward by Langmuir** and Grimm*** are termed as **'classical bioisosteres'.** More explicitely, in animals the occurrence of several hormones, neurotransmitters etc., having almost idential structural features and above all similar biological activities may be classified as **bioisosteres.**

Example : **Insulins** isolated from various mammalian species are found to differ by a substantial quantum of **'aminoacid residues'** but surprisingly they do exert the same biological effects (*i.e.*, lowering of blood-sugar). However, if this did not occur ; the actual usage of **'insulin'** to treat, control and manage **diabetes** might had to wait for another half-a-century for the development and recognition of recombinant DNA technology to allow production of **human insulin******.

Actual applications of **'bioisosteres'** in the successful design of a specific given molecule interacting with a particular **'receptor'** in one glaring example, very often either fails or negates the biological characteristics in another environment (system). Therefore, it is pertinent to state at this juncture that the logical use of **biological replacement (classical or nonclassical)** in the design of a **'new target-drug molecule'** is solely and significantly dependent on the specific biological system under critical investigation. Hence, there are no predetermined, well-established, predictable hard and fast guidelines or laid-out generalized rules that may be useful to a **'medicinal chemist'** to affect biosteric replacement gainfully towards improved biological activity. The wisdom, intuition, skill, experience and creative imagination of a **'medicinal chemist'** contribute a major role to zero-down or pin-point or hit the bull's-eye to obtain the best possible results towards **'new target-drug'** molecules.

Table 2.1. Evidently shows the various **'classical bioisosteres'** with their appropriate examples :

---

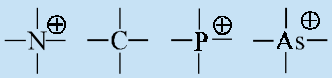
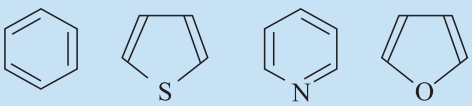
* Burger, A., **'Isosterism and Bioisosterism in Drug Design'**, *Progress in Drug Research*, **37,** 288–371, 1991.

** Langmuir, I. J., *Amer. Chem. Soc.*, ; **41** : 868 ; *ibid*, **41**, 1543, 1919.

*** Grimm, H.G., *Z. Elekrochemie.*, ; **31** : 474, 1925.

****A Danish recombinant-DNA-technology based firm has produced **'insulin'** from the yeast cells that almost meets all the stringent requirements of human insulin [**Humulin$^{(R)}$**].

Table 2.1 : Classical Bioisosteres*

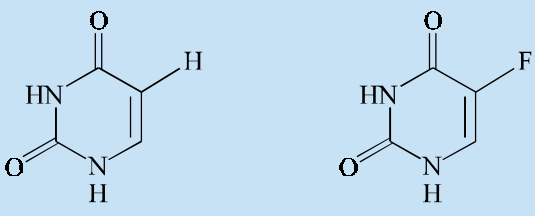
S.No.	Types of Classical Bioisosteres	Various suitable examples
1	**Monovalent atoms and groups**	F, H ; OH, NH ; F, OH, NH or CH_3 for H ; SH, OH ; Cl, Br, CF_3 ;
2	**Divalent bioisosteres**	– C = S, – C = O, – C = NH, – C = C – ,
3	**Trivalent atoms and groups**	 =, – N = ; – P =, – AS = ;
4	**Tetrasubstituted atoms**	
5	**Ring equivalents**	

* Groups within the row can replace each other conveniently.

Salient Features : Following are the various **salient features** of **'classical bioisosteres'** :

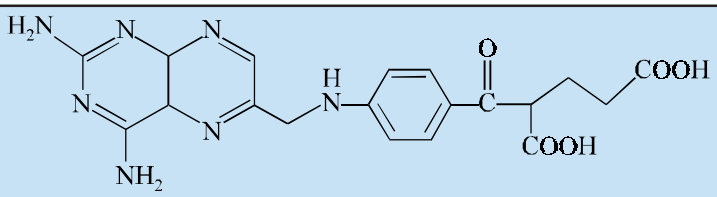
(1) **Hydrogen replaced by Fluorine :** It is regarded as one of the commonest monovalent isosteric replacements. Both H and F are fairly identical with their Van der Waal's radii being 1.2 Å and 1.35 Å respectively. **Fluorine** being the **most electronegative element** in the periodic table ; therefore, the augmentation in the biological profile of drugs containing F may be attributed to this specific characteristic.

Example : **[1] 5-Fluorouracil** from **uracil**, obtained by replacement of H with F gives rise to the formation of an extremely therapeutically potent **antineoplastic drug** :

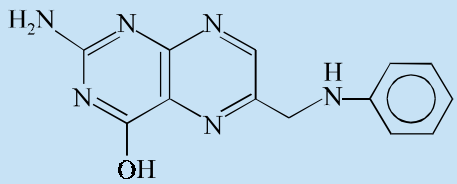


Uracil 5-Fluorouracil

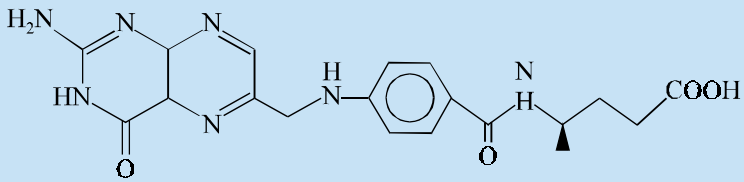
[2] Aminopterin (I) mimicks the tautomeric forms of folic acid (II), thereby giving rise to the formation of suitable H-bondings to the corresponding **enzyme active site,** as illustrated below :



Aminopterin (I)



Folic acid (*enol*-form) (II)



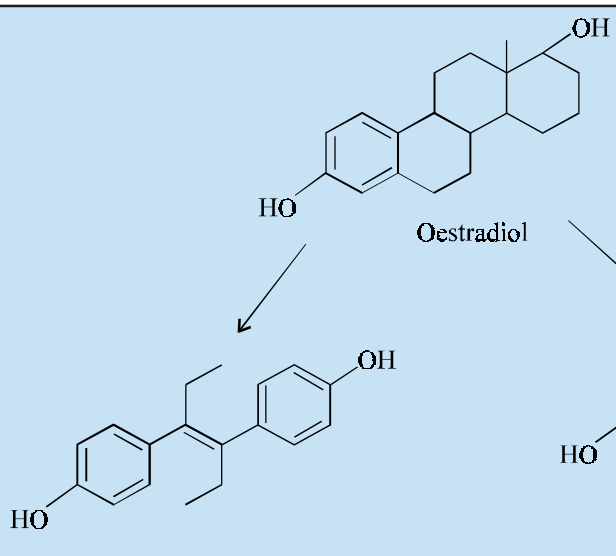
Folic acid (*keto*-form)

5.2. Nonclassical Bio-isosteres

Importantly, the **nonclassical bioisosteres** are precisely the replacements of functional groups not falling within the regimen by classical definitions. Although, several of these functional moieties practically just behave as one of the following characteristic specific features, such as :

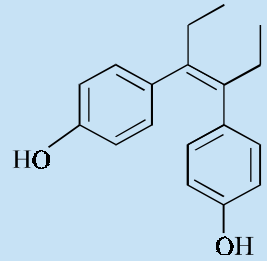
- Electronic proporties,
- Physicochemical property of the molecule,
- Spatial arrangements,
- Functional moiety critical for biological activity.

Examples : **[1] Non-cyclic analogues of oestradiol :**



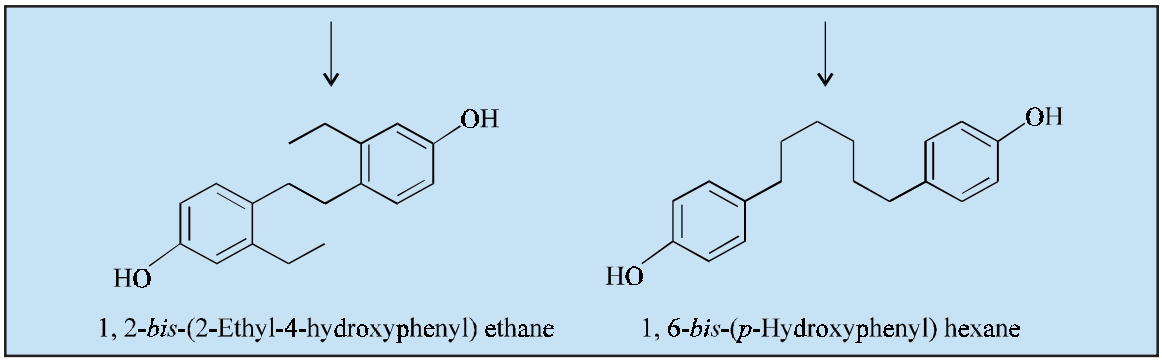
Oestradiol

trans-Diethylstilbesterol
(Active)



cis-Diethylstilbesterol
(Inactive)

(*Contd...*)

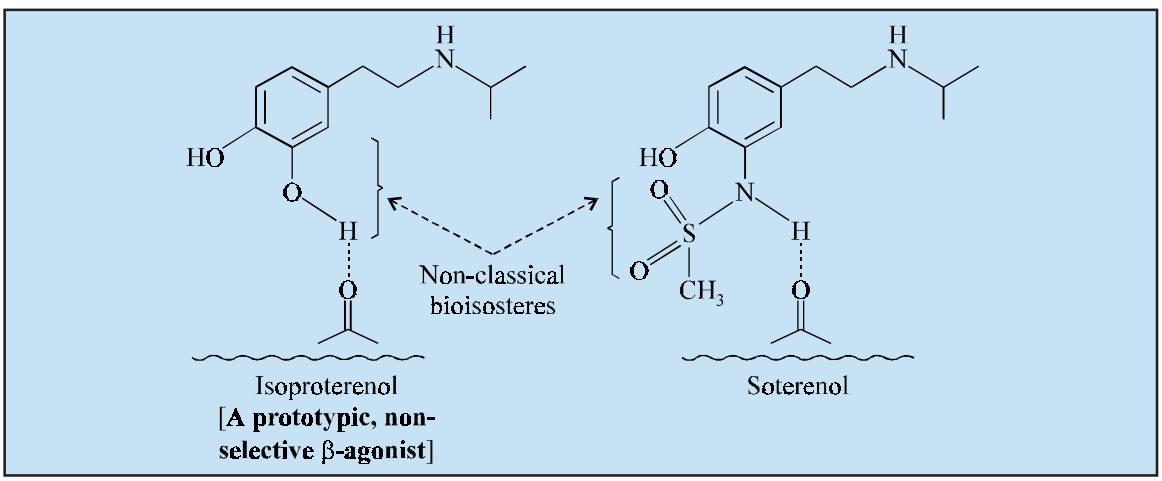


1, 2-*bis*-(2-Ethyl-4-hydroxyphenyl) ethane

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

The ***trans*-diethylstilbesterol**, the presence of two phenolic hydroxy functions very closely mimic the correct orientation of the phenolic and alcoholic functions present in the natural oestradiol*. However, it is *not* accomplished by the corresponding ***cis*-diethylstilbesterol**, besides some other more flexible analogues that practically show no activity at all**.

[2] Nonclassical replacement of a sulphonamide function for a phenol in catecholamines :



Isoproterenol
[**A prototypic, non-selective β-agonist**]

Soterenol

From the above typical example it is quite evident that —

(*i*) the steric factors exert a little influence upon the receptor binding in comparison to the **acidity** and **hydrogen bonding** potential of the incoming functional moiety positioned on the aromatic ring,

(*ii*) the p*K*a values (*i.e.*, dissociation constant) of the acidic proton present in the arylsulphonimide moiety of **soterenol** and the two phenolic hydroxyl functions in **isoprotenol** are almost equal to 10***.

*Dodds EC *et al. Nature*,**141** : 247, 1938

** Blanchard EW *et al. Endocrinology*, **32** : 307, 1943.

*** Baker BR. *J. Amer. Chem. Soc.*, **65 :** 1572, 1943.

(*iii*) the *two* aforesaid functional moieties are found to be weakly acidic in nature ; and hence, capable of :

- losing a proton
- interacting with the receptor as anions
- participating as H-bond donors at the receptor site
- as the above cited 'replacement' is NOT influenced to metabolism by *catechol O-methyl transferase* enzyme, thereby enhancing not only the span of action *in vivo* but also rendering the compound active orally.

6. STEREOCHEMISTRY AND DRUG ACTION

The **potential biological activity** (*i.e.*, **drug action**) of a **'targetted-drug molecule'** is solely dependent on its physicochemical characteristics essentially comprise of the nature and type of functional moieties ; and also the spatial arrangement of such groups in the molecule. Interestingly, the human body itself represents an asymmetric environment wherein the drug molecules interact with proteins and biological macromolecules (**receptors**). An elaborated study of the **3D-orientation** of the organic functional moieties present *in vivo* provides a substantial evidence about the most probable mechanism in the interaction existing between a specific **'drug substance'** and biological macromolecules. Hence, it is virtually important and necessary that the **decisive functional moieties** must be strategically located with respect to the exact spatial region encircling the **'targetted-drug molecule'** so as to enable the crucial and productive bonding interaction(s) particularly with the receptor (biological molecule), thereby potentially accomplishing the desired pharmacologic effect. It is, however, pertinent to state here that the right fitment of **correct 3D-orientation** of the functional moieties in a **'drug substance'** may ultimately result into the formation of an extremely viable and reasonably strong interaction with its receptor.

Stereochemistry is that branch of chemistry which deals with atoms in their space relationship, and the effect of such a relationship on the action and effects of the molecule.

Stereoisomers are compounds having the same number and kinds of atoms, the same configuration (arrangement) of bonds, but altogether different 3D-structures *i.e.*, they specifically differ in the **3D-arrangements of atoms in space**.

Stereoisomers may be further sub-divided into *two* types, namely : (*a*) **enantiomers** ; and (*b*) **diastereoisomers**.

6.1. Enantiomers

Enantiomers are isomers whose **3D-configuration** (arrangement) of atoms gives rise to the formation of **nonsuperimposable mirror images**.

These are also invariably termed as **chiral compounds, enantiomorphs or antipodes.** Furthermore, these compounds essentially possess identical physical as well as chemical characteristic features except for their inherent ability to rotate the plane of polarized light in just opposite directions with almost equal magnitude, quantum and extent.

Predominantly, when enantiomeric features are introduced strategically right into either a chiral environment or an asymmetric one, for instance : *the human body*, enantiomers shall evidently show marked and pronounced variant physical chemical properties thereby exhibiting appreciable and significant differences in their respective **'pharmacokinetic'** and **'pharmacodynamic'** behaviour.

Thus, the presence of **variant biological activities** based on their diverse enantiomeric features in a **'drug substance'** may lead to :

- adverse side effects,
- toxicity caused due to one of the isomers,
- exhibit appreciable differences in absorption *i.e.*, active transport,
- show significant variations in serum protein binding,
- extent/degree of metabolism,
- conversion into a toxic substance (impaired metabolism), and
- influence the metabolism of an altogether another drug.

6.2. Diastereoisomers

Diastereoisomers are all **stereoisomeric compounds which are not enantiomers**. In other words, the terminology **'distereoisomer'** essentially includes compounds containing both ring systems and double-bonds simultaneously. In apparent contrast to **'enantiomers'** diastereoisomers invariably display different physical and chemical characteristics, namely :

- chromatographic behaviour
- solubility
- melting point
- boiling point

Based on these glaring differences prevailing in their physical chemical properties one may effectively cause the separation of a mixture of **diastereoisomers** by the aid of established and standard chemical separation techniques, for instance :

- Crystallization
- Column chromatography.

Note : It may be pointed out at this juncture that 'enantiomers' cannot be separated by using any one of such methods unless either these are either converted to *diastereoisomers* or a chiral-environment is provided.

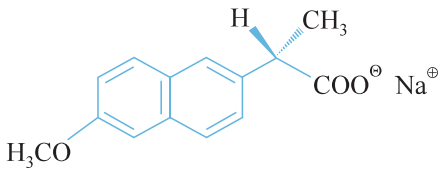
A few typical examples of **stereoisomers*** *viz.*, **enantiomers** and **diastereoisomers** are illustrated below :

---

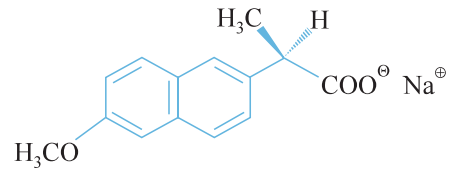
* **Stereoisomers :** The nomenclature of stereoisomers was postulated by Cahn, Ingold and Prelog (1956) and is commonly known as the *Sequence Rule System* (or CIP-system). Here, the atoms attached to a chiral centre (*i.e.*, asymmetric C-atom) are RANKED as per their atomic number according to the following laid-down norms : (*a*) Maximum (highest) priority is given to the atom with highest atomic number and subsequent atoms are ranked accordingly from highest to lowest ; (*b*) In a situation when a decision cannot be reached with respect to *'priority' i.e.*, 2-atoms having the same atomic number attached to the chiral centre, the process continues to the next atom until a decision could be arrived. The molecule is then viewed from the side opposite the lowest priority atom ; and the sequence of priority from highest to lowest is determined ; (*c*) In case, the sequence is to the right, or clockwise, the **chiral centre** is designated as the R absolute configuration ; when the priority sequence is to the left, or anticlockwise, the designation is S.

ENANTIOMERS

(*i*)

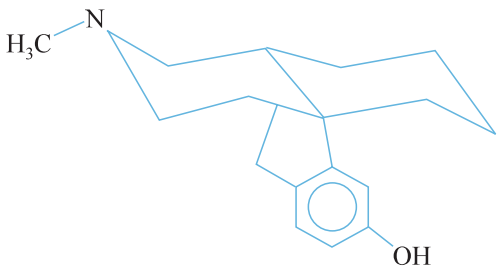


(S)–(+) Naproxen sodium
[2-Naphthaleneacetic acid, (+)-6-methoxy-α-methyl-, sodium salt]
Active as an analgesic, antipyretic and anti-inflammatory

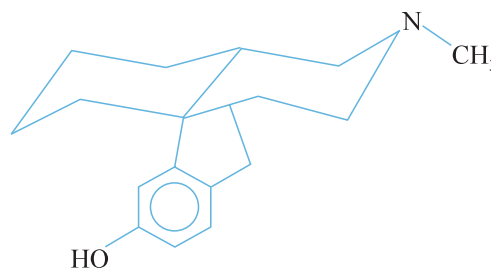


(R)–(–) Naproxen sodium
[Inactive]

(*ii*)



Levorphanol
[17-Methylmorphinan-3-ol]
A potent synthetic analgesic related to morphine



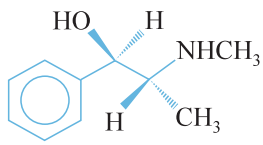
Dextrorphan
(Antitussive)

Example (*i*) shows that the priority sequence in **(S) – (+) naproxen sodium** is to the left ; and it exhibits activity as an antipyretic, analgesic and anti-inflammatory drug. In contrast, the **R – (–) naproxen** sodium is **inactive.**

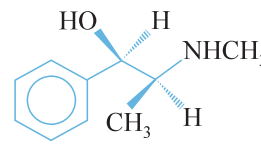
Example (*ii*) illustrates that levorphanol exhibits a potent analgesic activity, whereas the counterpart *i.e.,* **dextrorphan** exclusively shows an antitussive activity.

DIASTEREOISOMERS

(*i*)

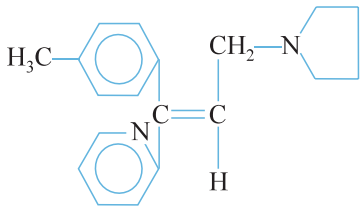


(–)–Pseudoephedrine
(Inactive)

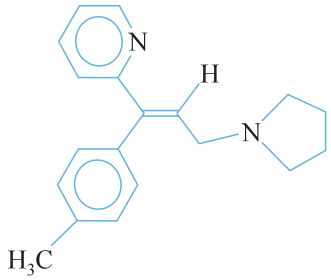


(–)–Ephedrine
[CNS-Stimulatory actions ; OTC-cold, allergy, and asthma remedies]

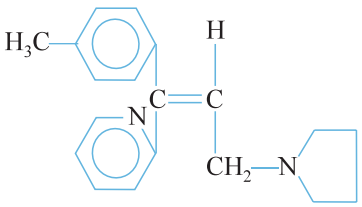
(*ii*)



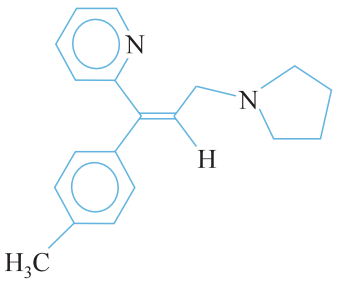
Or



(E)*–Tripolidine or *trans*-Tripolidine **[Active]**
(Antihistaminic activity, anticholinergic activity, and as in OTC cold and sinus preparations)



OR



(Z)*–Tripolidine or *cis*-Tripolidine **[Inactive]**
(About 1000 times less potent as the *trans*-or E-isomer as a H1-histamine antagonist)

In **Example (*i*)** *i.e.*, **(–)-pseudoephedrine** the two H-atoms are on the **opposite side of the plane of the ring** *i.e.*, one withbroken line is viewed as projecting beneath the ring plane (α-substituent) ; and the other with solid line is viewed as projecting above the ring plane. It does not exhibit any biological activity. However, in **(–)-Ephedrine** the said two H-atoms are located on the same side *i.e.*, beneath the ring plane ; and hence, it shows biological activities as stated above.

In **Example (*ii*)** *i.e.*, **(Z)-Tripolidine** the two heterocyclic aromatic rings are strategically located on the same side of the double bond **(*cis*-configuration/Z-configuration) ;** and is found to be inactive. Interestingly, simply by swapping the said two rings on either sides of the double bond **(*trans*-configuration/E-configuration)** the new compound **(E)-tripolidine** shows potent pharmacological actions as mentioned above.

6.3. Stereochemistry and Biologic Activity

An intensive and extensive research carried out till date on **'drug-design'** has not only established but also paved the way in the specialized aspect of **'stereochemistry'** of the **'targetted-drug molecules'**. This particular approach has inspired the **'medicinal chemist'** to tailor-made such newer drug substance(s) in which the proper strategical positioning of various functional moieties are introduced (or inducted) so that they are capable of interacting optimally with either an **enzyme** or a **receptor.**

---

*__E and Z-isomer :__ When the two aromatic heterocyclic rings are on the same side of the molecule, having the double-bond, it is known as the *cis*-or **Z-isomer** (from the German **zusamer** or ''**together**'') ; when these are located on opposite sides the designation is *trans*- or **E -isomer**-(from the German **entagegen** or ''**opposite**'').

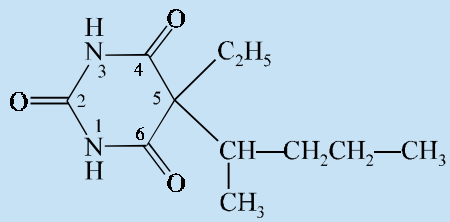
Interestingly, the following *five* different aspects of **stereochemistry** (*i.e.*, **types of isomeric drugs**) shall now be discussed in the sections that follows :

6.3.1. Positional Isomers (or Constitutional Isomers)

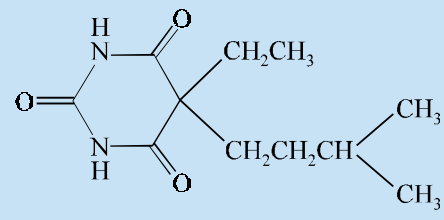
In this specific instance the compounds essentially possess the same **emperical formula** but the atoms of the molecule are rearranged in an altogether different order.

Examples :

(1) **Pentobarbital and Amobarbital :**



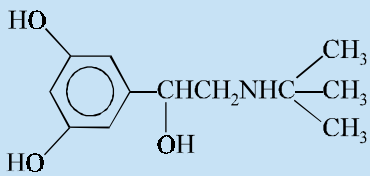
Pentobarbital (I)



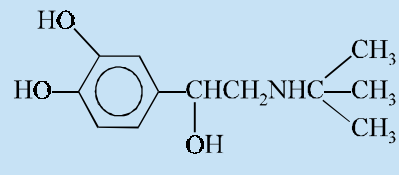
Amobarbital (II)

These positional isomers (I) and (II) belong to the barbiturate family. However, these **positional isomers** specifically differ only in the formation of the 5-carbon side chain attached to the C-5 position to the barbiturate ring system. Thus, compound (I) is a **short-acting barbiturate** ; whereas, compound (II) is an **intermediate-acting barbiturate.**

(2) **Terbutaline and N-*tert*-Butyl norepinephrine :**



Terbutaline (III)



N-*tert*-Butyl norepinephrine (IV)

The resorcinol residue in (III) has predominantly catered for as a biologically effective replacement of the catechol moiety present in (IV). Importantly, the resorcinol structural analogue (III), in a striking contrast to the catechol (IV), is **not** a substrate for **catechol-*O*-methyltransferase (COMT)**-an extremely important metabolic enzyme ; and hence, it possesses a marked and pronounced longer duration of action. In fact, **terbutaline** serves as a useful selective (**β_2-adrenergic stimulant** for the treatment of bronchial asthma and related physiological conditions (*administered orally*).

6.3.2. Geometrical Isomers

In **geometrical isomers** there exists a spatial arrangement of either atoms of functional groups in the carbon-carbon double bond locations, which has been duly expatiated earlier as under :

(*a*) section 5.2 *i.e.*, non-cyclic analogues of oestradiol, and

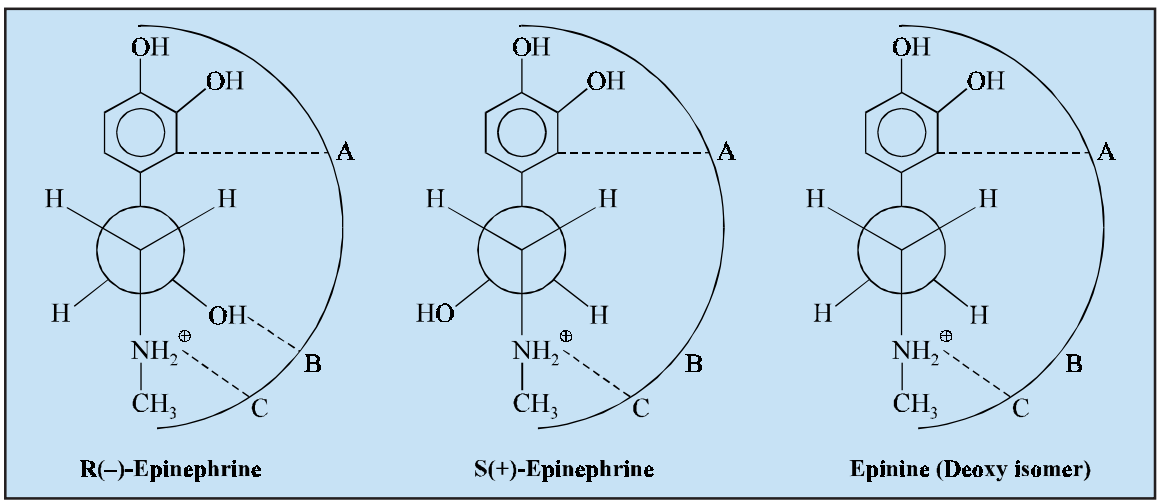
(*b*) section 6.2 *i.e.*, diastereoisomers example (*ii*).

6.3.3. Absolute Configuration

The terminogloy **'absolute configuration'** particularly refers to the arrangement of atoms in space of a chiral compound. It has been observed that there is a stark and distinct difference in specific biologic activity of the **optical isomers (enantiomers)** having the (R) and (S) configuration. A typical example of **Levorphanol** and **Dextrorphan** has already been discussed under Section 6.2 in this chapter.

6.3.4. Easson-Stedman Theory*

According to this theory put forward in 1974, the relative order of activity of the **'isomers'** *viz.*, **R(–) isomer epinephrine**, **S(+) isomer epinephrine** and **epinine deoxy isomer** on the **adrenergic receptors** are in the order of R > S ~ deoxy. Besides, the **R isomer** can bind to all the **three sites,** namely : (*i*) **catechol binding site 'A'** ; (*ii*) **hydroxy binding site 'B'** ; and (*iii*) **anionic binding site 'C'** as illustrated below ; whereas ; the **S isomer** and the **deoxy isomer,** that essentially exhibit practically identical biological activity, can exclusively bind to *two* of the sites.



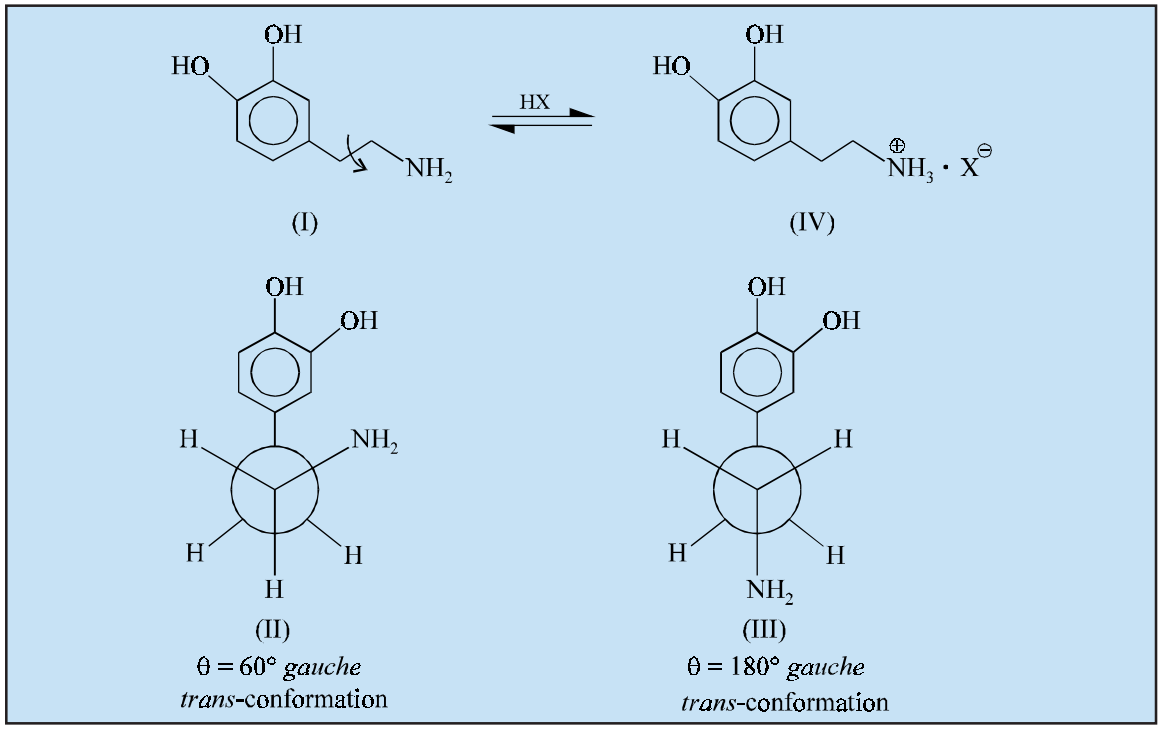
6.3.5. Conformationally Flexible to Conformationally Rigid Molecule

A vital, useful and latest strategy invariably employed and practised in **'drug design'** of **'newer targetted drug molecules'** by most of the **'medicinal chemists'** involves essentially of converting a rather **conformationally flexible molecule** into a **conformationally rigid molecule** so as to establish and find the **optimized conformation** that is required for binding to a drug receptor. This particular scientific and logical approach certainly helps in revealing certain cardinal aspects in **'drug design',** such as :

- in incorporating selectivity for receptors
- to minimise and eliminate undesired side effects
- to learn more with regard to spatial relationships of functional moieties for receptors.

Example : The aforesaid critical and important aspect may be expatiated with the aid of the following example of **DOPAMINE**, which enhances cardiac output by stimulating **β-receptors**.

*Patil, PN *et al*. *Pharmacol. Rev.* **26** : 232, 1974



In reality, **dopamine** (I) may exist in an **infinite number** of conformation about the **single** side-chain C—C bond. However, *two* such conformations, namely : (II) [θ = 60° *gauche*] and (III) [θ = 180° *gauche*], both having *trans*-conformation may show the maximum biological activity.

7. CHEMICAL PROPERTIES

Modern approaches to the design of bioactive molecules are usually based on the quantification of bioactivity as a function of a molecular structure. According to the concept of **receptor theory**, biological activity solely depends on the recognition of bioactive substrate by a **receptor site** followed by binding of the bioactive substrate to the receptor site. Realizing the ultimate dependence on configuration of a drug molecule one takes cognizance of the fact that steric effects of one type or another served as a major determining factor toward the **potency of bioactive drug**.

Following are some of the chemical parameters that have been put forward to buttress the above-mentioned facts. They are :

7.1. Molecule Negentropy

Molecule Negentropy is a summation of the negative information entropy calculated from the multiplicity and probability of equivalent sets of atoms in any selected **"pharmacea".**

7.2. Cammarata Correlation

It essentially establishes and also determines the prevailing relationship amongst electronic, hydrophobic and steric effects of a substituent and a change in biological effect.

Cammarate correlation may be expressed as :

$$\sigma\,An = \bar{a}\,\sigma + \bar{b}\,\pi + \bar{c}\,E_s + \bar{d}\,,$$

where $\bar{a}$, $\bar{b}$, $\bar{c}$ and $\bar{d}$, are constants and σ, π and E_S represent the **Hammett**, the **Hansch** and the **Taft constants**, respectively.

It is, however, pertinent to mention here that the biological transport characteristics of a drug molecule *i.e.*, the ability of diffusion of a drug across membranes, have been extensively studied by Hansch, Leo and Smith. These vital informations obviously help a medicinal chemist to a great extent in predicting the efficacy of biological activity in structurally related series of analogues.

Probable Questions for B. Pharm. Examinations

1. How do the newer disciplines like : Computer-aided physico-chemical methods, tracer techniques, genetic engineering, biotechnology and electron microscopy have evolved a new direction to expatiate the intricacies of drug interaction sequel to drug design ? Explain.

2. Effects on minor structural modifications of the hypoglycemic and cholinergic activities of the parent compound alter their duration of action. Give suitable examples to support your answer.

3. The biological activities of a drug molecule can be modified by—
(*a*) Meyer-Overton and Meyer-Hemmi Theory, and
(*b*) Ferguson's Theory.
Explain with typical examples.

4. The intramolecular distance present in the grouping—XCH_2CH_2N- between N-atom and X (where X = N, O etc.), which are present in procaine, acetylcholine and diphenylhydramine govern the biological activities. Explain.

5. Why the geometrical isomer *trans*-diethylstilbesterol exhibit higher oestrogenic activity than the *cis*-isoner ?

6. Discuss the specific role of absorption, distribution, excertion and biotransformation (*i.e.*, metabolism) to enable a 'drug' to reach the '*active site*'.

7. Explain Henderson-Hasselbalch equations with regard to dissociation constant of weak electrolytes.

8. Lessen *et al.* described the unusual structural grouping in tetracyclines which attribute three distinct acidity constants in aqueous medium of the acid salts. Explain.

9. Drugs exerting action as '*ionized molecules*' viz., muscle relaxants, ganglionic blocking agents vis-a-vis their pharmacodynamic action(s).

10. Give a comprehensive account of the importance of 'Isosterism' and 'Bio-isosterism' in drug design.

RECOMMENDED READINGS

1. A Cammarata and R L Stein *J Med. Chem-829*, 1968.

2. A. Leo, C Hansch and D Elkins *Chem. Rev.* **71** : 525, 1971.

3. C Hansch **Qualitative, Structure-Activity Relationship in Drug Design**,Vol. I, Ch. 2 (Ed. E J Ariens) Academic Press, New York, pp. 271-342, 1971.

4. **Foye's Principles of Medicinal Chemistry** (5th edn.), Lippincott Williams and Wilkins, New York, 2002.

5. Gennaro AR., **Remington ; The Science and Practice of Pharmacy**, Vol. I and II., Lippincott Williams and Wilkins, New York, 21st edn., 2005.

6. ME Wolff (*Ed.*), **Burger's Medicinal Chemistry and Drug Discovery**, 5th edn., John Wiley and Sons, New York, 1995.

7. RN Smith, C Hansch and M M Ames *J Pharm Sci*, **64**, 599, 1975.

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3

Molecular Modeling and Drug Design

Chapter

3

Molecular Modeling and Drug Design

1. INTRODUCTION

An eventful superb historical art of excellence is the remarkable tremendous advancement in the specific need-of-the-hour field of **molecular modeling**. The phenomenal success and world-wide recognition in **molecular modeling** is solely based upon the meticulously developed software so as to decepher and to visualize the complicated molecular structures with great ease and fervour. In the past two to three decades, the ability as well as scope to design, synthesize, characterize, and perform the biological evaluations of the **newer drug molecules** have paved the way for producing much safer, fast-acting, less toxic **drugs** to minimize the sufferings of the mankind.

The intimate relationship prevailing between **molecular modelling** and **drug design** may be safely categorized into *two* **predominant aspects**, *namely :*

(*a*) **Quantitative structure-activity problem :** that refers to the basic problems related to rationalization of **biological activity*** without the availability of well-defined 3D structural information(s) pertaining to the specific **receptor****, and

(*b*) **interactions between receptor-ligand complexes :** which particularly makes use of the pre-determined **3D structure***** of the ensuing **therapeutic target** with a view to design **novel drugs**.

In fact, the researchers over the years have duly modified, explored, and expanded '**molecular modeling**' into the following important aspects, namely :

- predicting and visualizing both 2D and 3D molecular structures,
- database storage of thousands of '*older*' and '*newer*' medicinal compounds and their respective characteristic properties,
- devising several highly sophisticated '*analytical tools*' for carrying out the acticulated characterization of various molecular properties, and
- '*atomic level*' simulation in the behavioural pattern of newly developed 'drug molecules'.

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*Pharmacological and microbiological activities studied separately.

****Receptor :** Both nuclear and transmembrane.

*****3D Structure : Three-dimensional structure.** *i.e.*, lying very much within **three axis**, perpendicular to each other, *viz.*, X-, Y-, and Z-axis.

In short, a plethora of extremely beneficial approaches and guidelines have been duly promulgated to the specific advantage of the **molecular modeling community** that are exclusively based upon the remarkable advances gained in the field of **molecular biology**, which serves essentially as the most pivotal centre towards the creation of the much-in-demand '**target proteins**' **abundantly***. However, one may meticulously derive most valuable informations which would not only be a guiding source with respect to the '**design**' but also help extensively towards the synthesis of potent novel therapeutic agents based upon the latest **molecular modeling techniques**. Besides, the copious volume of reviews** on **computer-aided drug design (CADD)** may certainly add on to the prevailing knowledge and wisdom pertaining to **molecular modeling.**

2. METHODOLOGIES : MOLECULAR MODELING

There are *two* different extensively studied methodologies that have not only put forward logical explanations but also expatiated the highly complicated cardinal factors that essentially govern the **molecular modeling** phenomenon, namely :

(*a*) Molecular mechanics, and

(*b*) Quantum mechanics.

The said *two* methodologies involved in **molecular modeling** shall now be discussed at length in the sections that follows :

2.1. Molecular Mechanics

In general, **molecular mechanics** visualizes a specific molecule as a conglomerate of atoms whose interactions may be explained judiciously by **Newtonian mechanics*****.

The various vital and important **salient features** with regard to the fundamental aspects of **molecualr mechanics** would not only elaborate the critical explanation(s) of the parametrization of the force field *vis-a-vis* the ensuing experimental data, but also the clarity of thoughts for significantly better understanding of the intricacies of '**drug design**', namely :

- Interactions taking place between atoms are classifed into *two* categores *viz.*, **bonded class**, and **unbonded class**.
- **bonded class :** essentially comprise of the **bonded atoms** assumed to possess a '***spring constant***' that specifically determines the precise '***energy of deformation***' provided by the ensuing **experimental bond lengths**.

Examples :

(*i*) **atoms with 1-3 interactions** *i.e.*, atoms that are directly linked to the same atoms get duly eliminated from the **Van der Waals list** ; and, therefore, do possess a specific energetic term relating the deviation distinctly from an ideal and conventional '**bond angle**'.

(*ii*) **atoms with 1-4 interactions** *i.e.*, they categorically define a **torsional relation** which is invariably characterised and parameterized depending solely upon the exact kinds of the *four* connected atoms responsible for defining the '**torsion angle**'*****.

*Crystallographic and NMR-spectroscopic studies to establish the 3D-structures have also stimulated such brain-storming investigative studies.

Kuntz ID : *Science*, **257 : 1078-1083, 1992 ; Navia *et al. Trends Pharmacol Sci.*, **14**, 189-195, 1993.

***Burkert U and Allinger NL : **Molecular Mechanics**, American Chemical Society, Washington DC, p339, 1982.

****Twisted angle between two bonds.

In short, the huge number of permutations and combinations of '**atoms types**' essentially require an equally large number of well-defined specifications (parameters) that need to be estimated precisely from the following means :

(*a*) *theoretical data* (or **quantum mechanics**), or

(*b*) *experimental data*.

Importantly, enough '**simplified force fields*** have been duly developed to describe various geometrical aspects (*i.e.*, arrangement with regard to space) in order to further refinement and exploration by means of **quantum mechanics**.

Figure 1 represents the '**electronic status**' for ***trans*-amide** and ***cis*-amide** configurations which are entirely different *i.e.*, they have altogether different **bond lengths** and **bond angles** ; and, therefore, different parameter sets may be employed judiciously for arriving at accurate and precise results. Jorgensen and Gao** (1988) demonstrated and proved the clear cut distinction between the said *two* conformational states using AMBER/OPLS tools.

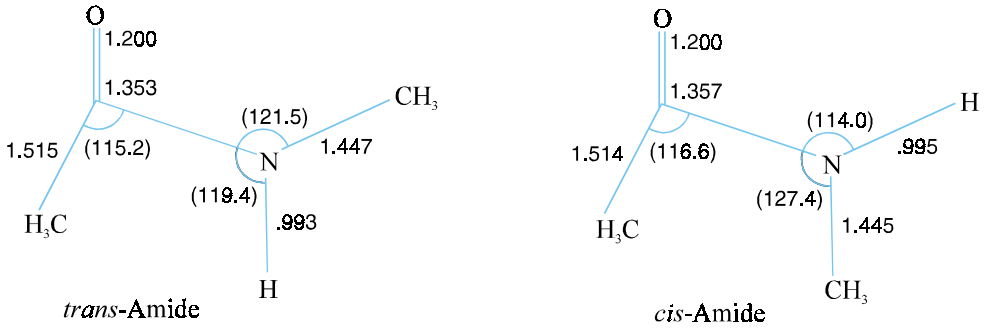

Fig. 3.1. Charge Distribution between *cis*- and *trans*-Isomers of Amide Bonds.

[Figures in *parenthesis* represent the respective 'bond angles', and the rest designate the 'bond length'.]

Interestingly, various other extremely important and rather difficult aspects of the **molecular mechanics** remain to be the **electrostatics**, for instance :

(*a*) **Dipole-dipole Interactions :** designated as r^{-3},—which essentially take place by virtue of the **nonsymmetric distribution** of electrons prevailing between the atoms of markedly **different dimension** and **electronegativity**.

(*b*) **Dispersive Interactions :** designated as r^{-6},—that are essentially caused due to the interaction of actually **induced dipoles** very much existing within the electron clouds because the molecules attain a definite close proximity to one another. Infact, r^{-6}, specifically give rise to the specific attractive segment pertaining to the nonbonded Van der Waals interaction(s).

(*c*) **Charge-dipole Interactions :** designated as, r^{-2},—that may be largely explained due to a definitive charge entity critically interacting with a permanent dipole which could be tackled conveniently and easily by taking into account the ensuing charge distinctly interacting with the two prevailing charges strategically located at the poles of the dipole.

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*Refer to the **torsional parameters** based exclusively upon the atoms at the end of a '**bond**'.

Jorgensen WL and Gao J : *J. Am. Chem. Soc.*, **110 : 4212-4216, 1988.

(*d*) **Charge-charge Interactions :** designated as, r^{-1},—which may be explained as the '**energy of interaction**' existing between two ensuing charges *eg*., q_1 and q_2. It may be expressed in the following expression commonly known as **Coulomb's Law :**

$$E = \frac{q_1 q_2}{4\pi\varepsilon r_{12}}$$

where, E = Energy of interaction

q_1 and q_2 = Two charge bearing entities

ε = Dielectric constant of the medium

r_{12} = Distance between the charges.

2.2. Quantum Mechanics (or Quantum Mechanical Methods)

Quantum mechanics present for acceptance the most elaborative and plausible description of a molecule's chemical behavioural pattern. It has been established beyond any reasonable doubt that a plethora of vital molecular characteristic features are only able to be reached by the help of **quantum mechanical methods** as they essentially and explicitely require a detailed description with regard to the **specific electronic structure** of the **molecule**. However, one may support the logical explanations solely based that practically all properties are dependent upon the electronic structure ; and, therefore, all calculations must be related intimately to these methodologies. Much to one's dismay the prevailing practical limitations with regard to the '**availability of computer time**' may drastically minimise the actual volume of these *investigative studies* merely to a small figure, nearly tens of atoms. Therefore, the ultimate calculations involving the **quantum mechanies** should be used most carefully and judicioulsy to solve such intricate problematic querries which may attract enough interest to support the relatively huge financial implications.

Importantly, at the expense of a **huge computational cost**, **quantum mechanics** makes available reasonably acceptable and precise information(s) with regard to *two* vital aspects, namely :

(*a*) **nuclear status** (or position) of a molecule ; and (*b*) **electronic distribution** of a molecule. Nevertheless, **quantum mechanics** essentially and predominantly plays *three* important roles related to the ever-expanding domain of '**drug-design**', such as :

- Approximattion of **charge in a molecule**,
- Characterization of ensuing **molecular electrostatic potentials**, and
- Parameterization for '**molecular mechanics**'

The applications of '**quantum mechanics**' in the field of *molecular mechanics* may be categorized into *three* vital groups, namely :

(*a*) Charge and electrostatics,

(*b*) Parameterization of force fields, and

(*c*) Chemical reaction(s) modeling and design of transition-state inhibitors.

These *three* different aspects of the applications of '**quantum mechanies**' in explaining molecular mechanics shall now be treated individually with appropriate examples wherever necessary :

2.2.1. Charge and Electrostatics

It has been well established that either ***ab initio*** or **semiemperical** quantum chemical modalities may be adopted effectively in order to determine precisely the prevailing charges in **molecular mechanics**. Importantly, the **quantum mechanics** or **quantum mechanical methods** are available abundantly for

carrying out the accurate calculations with regard to the actual **probability of the electron distributions** very much for **all electrons** in a molecule. Furthermore, these effective electron distributions subsequently undergo partitioning to produce almost an exact representations of the resulting overall **net atomic charges** borne by atoms duly present in a **molecule**. However, these **net atomic charges** may appear in different environments and modes*, such as :

(*i*) atom-centered monopole,

(*ii*) atom-centered dipole, and

(*iii*) atom-centered quadrupole.

Figure 3.2 illustrates the various means and ways towards most preferred localization of charge employed in different electrostatic models.

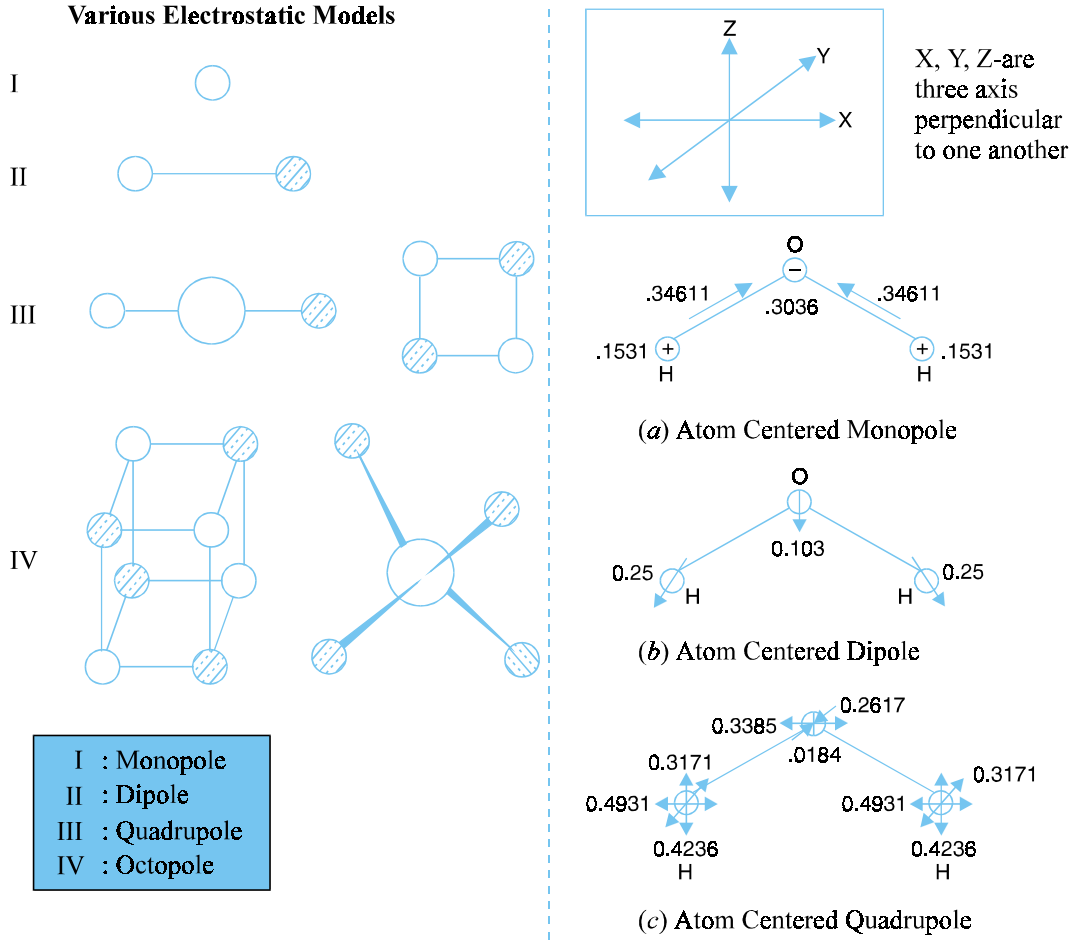

Fig. 3.2. Different Modes towards Most Preferered Localization of Charge Employed in Various Electrostatic Models.
(*a*) Atom centered monopole ; (*b*) Atom centered dipole ; (*c*) Atom centered quandrupole.
[Adapted from : Stone AJ *et al.*. *Mol. Phys.* 56 : 1047-64, 1985]

*Bayly CI *et al. J. Phys. Chem.*, **97** : 10269-280, 1993.

Explanations :

(*a*) **Atom Centered Monopole :** In fact, the one-centre charge* residing on an atom is being specifically assigned to that atom. Thus, one may obtain a rather poor representation of the prevailing electric field lying in the vicinity of the molecule which ultimately results solely due to the usage of **atom-centered monopole models**.

(*b*) **Atom Centered Dipole :** The precise and ultimate molecular recognition is exclusively dependent upon the ensuing **molecular electrostatic potential** (**MEP**) intimately surrounding the molecule which is duly formed by the actual *electronic* as well as *nuclear* distribution of charge. Williams** (1991) reported various methods in order to calculate accurately the '**charge models**' in the proper representation of MEP accomplished by *ab initio* methods. However, the most probable and correct choice between models is guided entirely upon one with a view to obtain **reproducible MEP values**.

(*c*) **Atom Centered Quadrupole :** The ultimate desire to attain reasonably accurate and reproducible MEP values may be accomplished by making use of an enhanced complexity of the model.

In conclusion, one may add that a **hydrid application** comprising of *modeling chemcial reactions* and *design of transition-state inhibitors* for which the **proposed reaction core** is modeled meticulously by the help of **quantum mechanics** and **molecular mechanics** would turn out to be the most **viable option**.

2.2.2. Parameterization of Force Fields

It is a well established fact that the '**molecular mechanics**' is absolutely empirical ; and, therefore, the parameters are derived exclusively *via* interactive evaluation of typical **computational results** *viz.*, (*a*) **Molecualr Geometry** (*i.e.*, **Bond Lengths, Bond Angles, Dihedrals**), and (*b*) **Heats of Formation**, in comparison to the various experimental values***. In actual practice, **parameterization** essentially makes use of the '**least-squares optimization**' based upon **consistent** a new terminology coined by Lifson) to account for the specific **force fields** wherein the various **intricate structures**, spectrum of **energies of formation**, and **vibrational spectra** all occur predominantly. Importantly, **crystallographic techniques** have adequately made available a substantial degree of **vital experimental data-base** derived from bond lengths, bond angles, and VDW parameters effectively. Dinur and Hagler (1991)**** succeeded in improvising altogether newer general sets of parameters derived solely from **quantum mechanical calculation**, particularly for such systems that lack sufficient experimental are scantily available. Tirado-Rivese and Jorgensen (1990)***** parameterized *via* fitting carefully the characteristic features of bulk liquids to **Monte Carlo simulations** to yield duly the AMBER/OPLS force field.

2.2.3. Chemical Reaction(s) Modeling and Design of Transition Inhibitors

It has been amply demonstrated that certain **enzymatic reactions** wherein chemical transformations do take place, one should specifically make use of the '**quantum chemical methods**' in order to deal effectively with ensuing electronic alternations in both **bond cessation** and **hybridization******.

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*As per the **Mulliken Population Analysis**.

Williams DE : In : Lipokwitz KB and Boyd DB (eds) : **Review of Computer Chemistry, VCH-Publishers, Inc., New York, pp 81-98, 1991.

***Lipkowitz KB and Boyd DB (eds.) : **Review of Computer Chemistry**, VCH Publishers, Inc., New York, 1991.

****Dinur U and Hagler AT, *Ibid*, pp. 99-164, 1991.

*****Tirado-Rivese J and Jorgensen WL, *J. Am. Chem. Soc.*, **112** : 2773-2781, 1990.

******Aqvist J and Warshel A : *Chem. Rev.*, **93** : 2523-2544, 1993.

Hence, the most plausible and viable option would be to affect hybridization judiciously ; and thereby the **reaction core** is quite often **modeled quantum mechanically** whereas the remaining by the aid of **molecular mechanics**. Andrews *et al.* (1984)* and Eksterowicz *et al.* (1993)** meticulously pioneered modeling of the particular **transition states** of prevailing enzymatic reactions to accomplish the design of the desired **transition-state inhibitors**.

3. KNOWN RECEPTOR SITES

The design of '**novel ligands**' specifically meant for the **therapeutic targets** has been duly accepted as a great and significant challenge by the **medicinal chemists** across the globe, wherein the 3D structure has been adequately substantiated and proved by the latest technological advancements in analytical instruments***, such as : NMR-Spectroscopy, X-Ray Crystallography. Interestingly, the accessebility of the ensuing coordinates duly present in almost all the atoms of the particular '**target site**' amply suggests not only the articulated application of **modeling of the site** but also the strategic interaction with the **prospective ligands****.

3.1. 3D Structure of Macromolecular Targets

In actual practice, the exploration and the intensive studies related to the **3D structure of macromolecular targets** may be regarded as the most vital and crucial requirement. The already mentioned *two* most prominent methods are :

(*a*) **NMR-Spectroscopy :** Both 2D and 3D nmr spectroscopy are used extensively and intensively for the determination of the macromolecular targets effectively. Though '**drug molecules**' are handled more efficaciously by these methods, whereas in the case of **proteins** not much success has been achieved due to *two* cardinal factors, namely : (*i*) poor solubility ; and (*ii*) bulky size of protein. However, the exorbitant cost of these *nmr* techniques render their usage significantly.

(*b*) **X-Ray Crystallography :** It is gaining prominence progressively which essentially comprises of *three* important aspects, namely : crystallization, data collection and storage, and computerized data analysis.

3.2. Structure-Based Drug Design (or Structure-Aided Drug Design)

The **structure-based drug design** or **structure-aided drug design** is indeed a well-known **multidisciplinary programme** which articulately and meticulously helps in the marvellous fusion of the ideas of **conventional (traditional) domain of medicinal chemistry** with the following ever-expanding sophisticated techniqes, for instance :

- NMR-Spectroscopy,
- X-Ray Crystallography,
- Molecular Modeling,
- Computational Chemistry,

*******Andrews PR and Winkler DA : In Jolles G and Wooldridge KRH (eds.) : **Drug Design : Fact or Fantasy**, Academic Press Inc., New York, pp. 145-174, 1984.

********Eksterowicz JE and Houk KN, *Chem. Rev.*, **93** : 2439-246, 1993.

- Small Molecular 3D-Database Search, and
- *ab initio* Design of Ligands.

It is, however, pertinent to state here that an adequate and **intuitive user interface** exclusively relies upon the inherent ability to produce, modify, and modulate effectively a good number of absolutely independent objects, for instance : **ligand**, and its **target**.

Nevertheless, with the advent of a host of **specific transformations** and **coordinate systems** amazingly give rise to a huge **cumulative graphical primitives** *via* an absolute imaginative creations of a scientist essentially comprising a '**ligand**' and its '**target**', namely :

(1) **Primitives** essentially required for the overall illustration are assigned separate coordinate systems *e.g.*, *points*, *polygons*, and *vectors* do represent particular shape(s).

(2) Resulting **primitives** are duly subjected to **transformation*** leading to the immediate next level of object space so as to construct bonds and atoms ultimately. Actually, one may make use of the **matrices** in order to accomplish the individual transformations on account of the fact that present-day high configuration computers are quite effective in solving the intricate problems.

(3) Each molecule is described in an elaborative manner by transforming the coordinates of its nuclei to their respective strategical position very much well within the molecules's '**object space**'. Thus, alterations with respect to the relative position of atoms *e.g.*, rotation of atoms about a **covalent bond** (*i.e.*, single bond) are duly achieved well within the '**object space**'.

(4) Each of the carefully designed molecules is duly **transformed**** into a **common 'world' coordinate system,** which eventually permits the individual molecules to be positioned strategically with respect to one another.

(5) The ultimate transformation to '**viewer space**' is obtained invariably when the *user* decides and selects to look at a particular desired orientation of the overall system. In fact, the *user* invariably mentions and defines this **specific transformation** while moving the viewed molecules with the help of the '**mouse**'.

(6) Finally, the **3D transformed 'world coordinates'** are carefully mapped to the corresponding **2D 'display coordinates'**.

3.3. Major Steps in Structure-Based Drug Design

The terminology **structure-based drug design** actually refers to the fundamental fact that exprimental structural data pertaining to the macromolecule of the drug-receptor complex is essentially involved in the modeling phenomenon explicitely.

Importantly, the choice of **CADD methods***** which may be applicable directly to drug design exclusively depends upon the availability of actual viable receptor information(s). In fact, the **structure-based drug design** proves to be of great help provided the ensuing **receptor structure** has been carefully and meticulously characterized by either high-resolution NMR-spectroscopy or X-ray

*__Transformation.__ is a mathematical procedure for altering the coordinates of all the vertices of the primitives immediately.

**Rotated or translated.

*__CADD-Methods :__ Computer-aided drug design methods.

crystallographic procedures.

One may consider the following major steps in the **structure-based drug design**, namely :

(*a*) Preparation of newer structure(s)

(*b*) Visualization of 3D structure of the target molecule using graphic tools.

(*c*) Active site detection

(*d*) Contact potential

(*e*) Ligand docking *e.g.*, potential energy function, conformational space of ligand, and notional freedom of the ligand.

3.4. Ligand Receptor Recognition

It is, however, pertinent to mention here that **steric*** complimentarily is an absolute necessity, but certainly not a sufficient evidence for ultimate recognition. It is indeed regarded to be a **second-order effect**, and do not represent a dominant one.

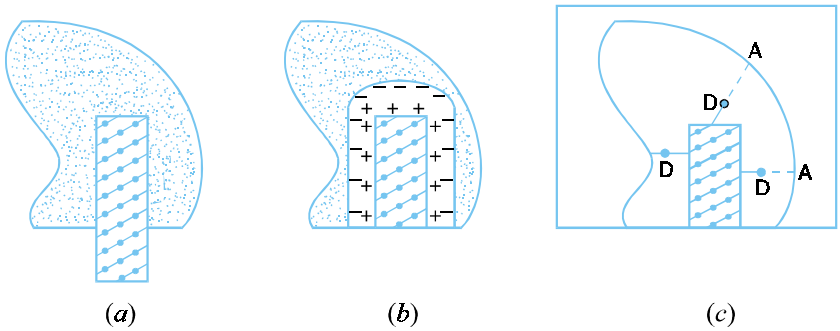


Fig. 3.3. Diagramatic Sketch of Complimentarity in Ligand Receptor Recognition (*a*) Illustrating shape complimentarity (*b*) Depicting electrostatic complimentarity (*c*) Showing hydrogen-bonding complimentarity.

Figure 3.3 (*a*, *b*, *c*) depicts the *three* recognized complimentarity** with regard to shape, electrostatic status, and hydrogen-bonding diagramatically.

The *three* aforesaid complimentarity (or similarity) may be legitimately confined to steric aspects (*i.e.*, shape), electrostatic status (*i.e.*, distribution of –ve and +ve charges within the ligand-receptor system), and finally the hydrogen-bonding (*i.e.*, hydrophobic characteristic feature of the system).

Salient Features : The various **salient features** with respect to the **ligand-receptor recognition** are as stated under :

(1) Dynamic uneasiness prevailing in the **ligand-receptor structures** together with the **conformational flexibility of the target and the drug is extremely vital and important**.

*Concerning the spatial arrangement of atoms in a chemical compound.

*Similarity.

(2) The resulting bound conformation of the '**drug molecule**' need not necesarily be having the **minimum energy conformation**.

(3) Presence of **water molecules*** at the '**active site**' also play a vital role ; and, therefore, must be taken into consideration positively.

(4) Presence of '**multiple binding sites**' strategically located in a '**ligand at the active site**' invariably poses an important subject for due cognizance.

3.5. Active Site for a Target Molecule

It is an usual practice to identify and subsequently find out the **active-site for a target molecule**, namely :

- Alpha-shape method
- Density difference method
- Filting circles, spheres, rectangles, squares etc.

Interestingly, each one of the aforesaid methods finally gives rise to a certain '**score**' rightly based upon the most befitting actual quantum of amino acid side chain(s) that occupy inside the '**active cavity**'. However, the available stored genetic modification data is found to be extremely beneficial in the accurate and precise location of the so called '**active site**'. It is worth while to state here that the ensuing '**active site**' (or '**envelope**') needs to be defined explicitely before the actual usage of any technique. One may come across a host of altogether **different binding sites** which really renders the actual selection process a little difficult and cumbersome. It has been established beyond any reasonable doubt that the **ligand binding site** may also be able to accomodate an **appreciable degree of dissimilarity** present in the ensuing **ligand structure**, as could be seen in **HIV protease**, designated as **HIV PR**, Figure : 4(*a*) and (*b*).

Salient Features : The **salient features** of **HIV PR** are as enumerated below :

(1) It is one of the several strategies that has been proposed to cure and arrest the AIDS phenomenon.

(2) It is considered to be absolutely necessary for the viral assembly and subsequent maturation.

(3) Scope of further meaningful **antiviral therapy** *via* two other enzymes *viz*., **HIV reverse transcriptase** and **HIV reverse integrase**, may be accomplished by using these structures skillfully.

(4) 3D structures of **HIV PR** and **reverse transcriptase** have been duly established, whereas the corresponding structure of the **integrase** is being investigated.

(5) Design of inhibitors for **HIV PR** has been a major domain of active and progressive research in a plethora of academic and pharmaceutical research laboratories.

(6) As **HIV PR** ascertained to be a member of the specific **aspartic proteinase family**, the legitimate progress has been accomplished in a relatively short span of time.

(7) Valuable information(s) derived *via* the **actual design of significantly small, potent, and bioavailable renin inhibitors** based upon the 3D structures of the ensuing **aspartic proteases** has virtually paved the way towards the tremendous task for designing inhibitors for the **HIV PR**.

*The '**water molecules**' very much trapped at the **active site**.

(8) **HIV PR** has ten binding pockets located strategically in the binding site, which ultimately cleaves eight different peptide sequences having wide structural variations. Importantly, a slight, specific, and significant modification in *one sequence only* may ultimately produce an **effective inhibitor**.

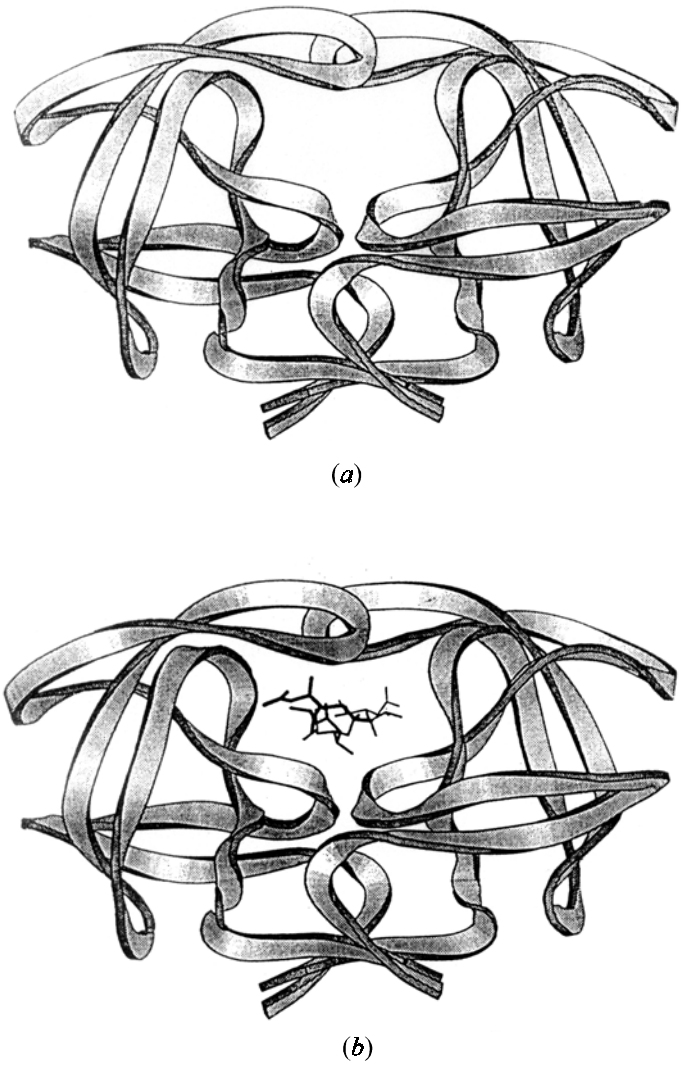


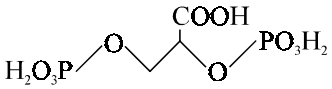
Fig. 3.4. Ribbon Representation of HIV-Protease Structure
(*a*) *HIV PR Enzyme* ; (*b*) HIV PR Enzyme Inhibitor Complexed
[Adapted From : Fitzgerald PMD *et al.J. Biol Chem.* 265, 14209, 1990]

Obviously, the most valuable qualitative information may be discerned by means of not-so-complicated screening of resulting complexes by making use of **advanced molecular graphics** ; and,

therefore, one may accomplish substantial improvement of the '**known ligands**' through meticulous investigative search for desired accessory binding interactions in terms of **ligand modification**. Evidently, this particular scientific approach ultimately yielded an exceptionally good dividend through wonderful designing of newer drug molecules at Burroughs-Wellcome Laboratories (UK), such as :

(*i*) **Dihydrofolate Reductase (DHFR) Antagonists** — giving an increased affinity, and

(*ii*) **2, 3-Diphosphoglycerate (DPG) Analogues** — regulating the O_2 binding to hemoglobin.



2, 3-Diphosphoglycerate (DPG)

3.6. Meaning of 'Site'

It has been adequately established that 3D structural information(s) with respect to a probable and potential '**therapeutic target**' in no way serves as a guarantee for the precise identification of the '**site of action**'* of the **substrate****, or **inhibitor**, till such time when one lays ones hands onto a **definite relevant complex** ultimately. In actual practice, various conformational changes invariably come into being in the course of realistic **binding of ligands to enzymes** which are eventually not registered in the 3D structure of the enzyme critically.

Examples :

(*i*) Major conformational changes as observed in **HIV protease** on getting bound to the respective inhibitor MVT-101***.

(*ii*) Alterations as seen in the '**domain orientation**' in the complex derived from an **anti-HIV peptide antibody** *vis-a-vis* the **peptide****.

(*iii*) In certain other **therapeutic targets**, one may observe the direct involvement of the specific **allosteric sites** in the control and regulation of binding phenomenon ; and, therefore, cannot be discerned vividly from the ensuing crystal structure easily at one's disposal. However, in this particular instance the usage of high-resolution NMR spectroscopy offers an extremely complementary approach ; besides, *three* **cardinal factors**, namely : (*a*) transfer ; (*b*) isotope-edited **Nuclear Overhauser Effects (NOEs)***** ; and (*c*) magic-angle spinning NMR effects, shall duly exert their actions upon the ensuing **solid samples** which would ultimately help in the proper identification of the residues belonging to the desired '**therapeutic target**' directly and intimately involved in the active receptor interaction******, as illustrated below in Figures 3.5 (*a*) and (*b*).

***Site of Action :** Cell receptors where a biological response is initiated.

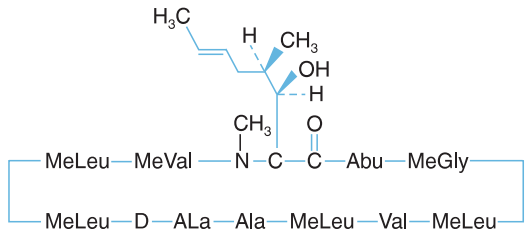
****Substrate :** The reactant in a chemical reaction that usually binds to an **enzyme active site**, and is ultimately converted to a product.

***Miller M *et. al.* : *Nature*, **337** : 576-79, 1989.

**** Stanfield RL *et al.* : *Structure*, **1** : 83-93, 1993.

*****NOEs :** They originate from dipole-dipole interactions between protons that are closer than 5Å, and thereby provide a mechanism for magnetization transfer between the signals corresponding to such protons in the H-NMR spectrum.

******Smith so : *Curr. Opin. Struct. Biol :* **3** : 755-59, 1993.



Cyclosporin A

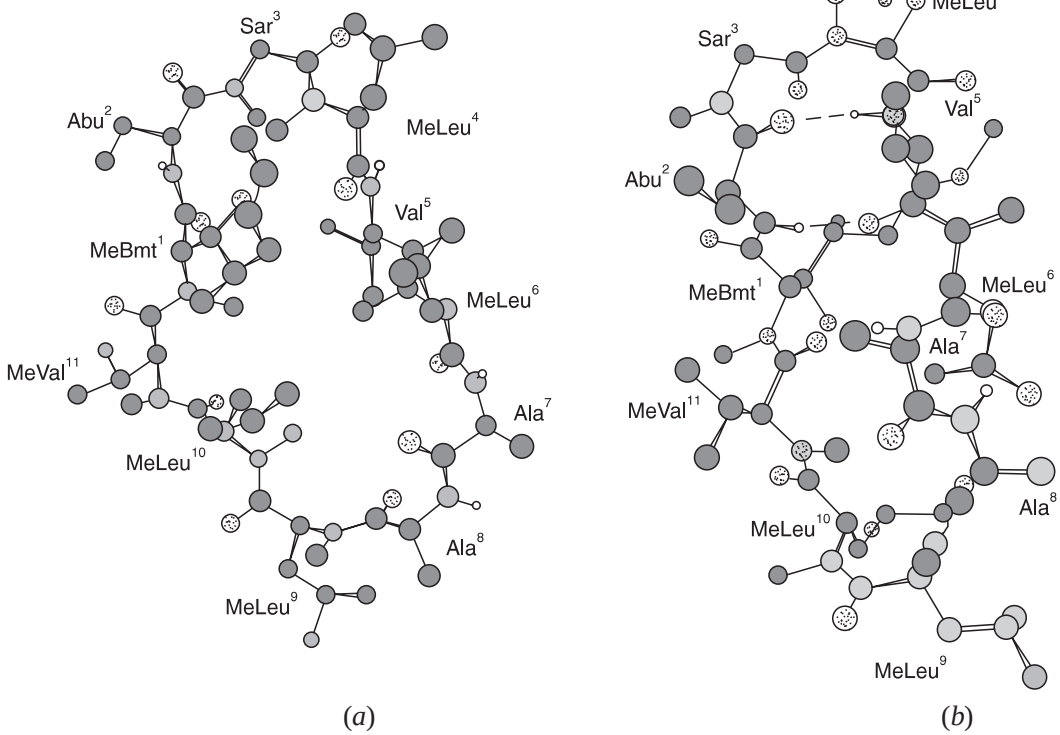


Fig. 3. 5. Illustration of Bound Conformation of Cyclosporin :
(*a*) As observed by NMR-Spectroscopy compared with solution conformation.
(*b*) Residues involved directly with interaction with Cyclosporin are depicted in bold.

3.7. Characterization of Site

The **characterization of site** may be classified into *two* groups, namely : (*a*) Hydrogen-bonding and other group binding sites ; and (*b*) Electrostatic and Hydrophobic Fields.

3.7.1. Hydrogen Bonding and Other Group Binding Sites

An extensive and intensive knowledge with respect to the optimal strategic location of the specific atoms or functional moieties, very much within the '**site**' may be able to afford rather informative

and valuable insight. In fact **GRID***, the most popular programme helps a group, or a '**probe atom**' in the exhaustive exploration of the prevailing **receptor site cavity** strategically present either in a **grid** or a **lattice** during the process of determining the **enthalpy**** of interaction. Importantly, one may obtain a precise **graphical representation** of the optimal locations for *group* or *atom* under investigation by the aid of a **3D contour map** duly produced from the **lattice of interaction energies**. **Comparative Molecular Field Analysis (CoMFA)** may also provide very much identical novel ideas derived carefully from the field mapping employed. In this manner, it is quite feasible to arrive at the so called '**ideal positions**' embodied duly either in the **H-bond donors or acceptors mapping phenomenon** to serve as a preamble to the '**ligand design**'.

3.7.2. Electrostatic and Hydrophobic Fields

The characteristic features of the '**active site**' as displayed by marked and pronounced **electrostatic and hydrophobic fields** go a long way in the conceptualization in the **design of ligands**. In a typical situation where one encounters with a **CAVITY***, the '**loci of filler atoms**' essentially required to pack the cavity is usually **computed** ; and, hence, such atoms present very much within the '**outermost layer**' of the *filler solid* are identified with great ease and convenience. In other words, these filler atoms do lie along the prevailing **cavity-pocket interface** ; and, therefore, are strategically located where they are duly represented by the electrostatic interactions taking place between the **pocket** and the **binding ligand**.

3.8. Design of Ligands

The **design of ligands** may be achieved efficaciously by *three* well established methodologies, namely :

(*a*) Visually assisted design,

(*b*) 3D-Databases, and

(*c*) *De NOVO* design

The *three* above cited techniques shall be treated individually in the sections that follows :

3.8.1. Visually Assisted Design

It becomes an absolute necessity, before emarking on a possible approach for **designing of ligands**, to initiate the very process of '**optimization of a lead**' to know and to assure as well whether modification is feasible at all or not. The **visually assisted design** specifically ascertains the clear cut availability of the **excess space** present in the **active site cavity** by examining the ligands directly. In this way, it may be quite beneficial for strategically locating a few **highly selected regions** wherein ligand modification may be accomplished suitably. However, this method may not be very much appropriate for complete characterization of the void space which prevails between the **ligand** and the **receptor** *i.e.*, **the ligand receptor gap region**.

In 1990, an **algorithm**** was duly put forward by Ho and Marshall so as to '**color code**' the cavity appearance by the help of the closest **atom-gap distance** between the **ligand receptor**. Once the

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***GRID :** A chart with horizontal and perpendicular lines for plotting curves.

****Enthalpy :** The heart content or chemical energy of a physical system.

*****Ho CMW *et al. J. Compt. Aided Mol. Des.*, **4** : 337-54, 1990.

******A formula of set of rules for solving a particular problem.

precise disatances existing between the ligand-receptor is determined by calculations virtually at each and every **cavity-pocket interface lattice points**, a user friendly **color-code mode** is applied to produce the desired and needed displays conveniently. Interestingly, this type of specific investigations emphasizes critically only such zones or segments which are not so densely packed ; and, therefore, indicate availability for necessary ligand modification.

3.8.2. 3D Databases

In the past several decades there have been an astronomical growth towards the meticulous potential investigative researches carried out on **3D chemical databses** to make a remarkable and noteworthy advancements in the prevailing process of **designing drugs** for both **hypothetical** as well as **known receptor sites**.

A few typical and recognized **3D databases** are as stated under :

(1) **Cambridge Structural Database (CSD)* :** Comprises of ~90,000 well-defined strucutres of small molecules.

(2) **Brookhaven Protein Databank (PBD)** :** Consists of relatively larger macromolecules related to the crystal coordinates of proteins.

(3) **3D Database from Chemical Abstracts*** :** Chemical structures totalling approximately 700,000, are duly generated through **CONCORD**.

In general, the major advantage of such databases is extremely important and viable in circumstances that essentially and explicitely explains the modalities of binding of a **specific ligand** and its **receptor** based on tis recognized functional moiety and known crystal structure of the complex under investigation****.

Improtantly, there are several highly specific and useful **database searching techniques** have been duly introduced into a plethora of most recent **database searching system**, namely :

(*a*) **CAVEAT :** Bartlett *et al.****** (1989) designed the database searching system so as to identify and detemine the ensuing **cyclic structures** which may be employed as the basis for designing **newer novel medicinal compounds**.

(*b*) **ALADDIN :** Abbott Laboratories, UK,****** (1989) introduced the database searching system whereby a medicinal chemist gains a legitimate access to search rather rapidly the wide-spectrum of structural databases for such chemical entities esentially comprising of various **substituent bonds** which categorically do satisfy and fulfil a particular existing **geometric relationship**.

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*Allen DH *et al. J. Chem. Inf. Comput. Sci.,* **31** : 187-204, 1991.

Abola EE *et al.* In : Glaeser PS (ed.) : **The Role of Data in Scientific Progrees, Elsevier Science Publishing Co. Inc, New York, 1985.

***Pearlman RS : *Chem. Des Auto. News*., **8** : 3-15, 1993.

****Sheridan RP *et al.* : *Proc. Natl. Acad. Sci*., USA : **86** : 8165-69 (1989).

***** Bartlett PA *et al.* : **Molecular Recognition : Chemical and Biological Problems** Royal Society of Chemistry, London (UK), pp 182-186, 1989.

******Van Drie JH *et al. J Comput. Aided Mol. Des*., **3**, 225-251, 1989.

(*c*) **3D SEARCH :** Lederle Laboratories, USA (1989)* designed an almost **identical geometric relationship** that predominantly prevailed amongst different types of **user-defined atomic components** which could be employed to *decepher* and to *retrieve* very much alike structures.

Examples :

(*i*) A researcher may effectively delineate such molecular characteristic features as : type of atom(s), bond angles, torsional limitations, and the like, in order to ensure the rapid possible retrieval of relevant '**drug molecules**'.

(*ii*) It also helps the retrieval of **ligand-receptor volume complementarity**.

(*d*) **MACCS-3D :** Introduced by Molecular Design Ltd. USA,** devised an elaborated **database searching system** expatiating the ensuing geometric relationships prevailing amongst a host of **user-specific atomic components** that could be judiciously exlpoited to retrieve nearly matching **molecular structures**.

(*e*) **CHEM-X :** The UK-based Chemical Design Ltd.,*** put forward a widely accepted databse searching system which aided the use to outline various molecular properties, for instance : bond angles, kind of atom, torsional restrictions etc., to make sure the actual retieval of newer drugs of interest.

(*f*) **UNITY-3DB :** The Tripos Associates Inc.,**** USA provided also a **database searching system** that would enable the **medicinal chemist** to have an easy access to the utility in designing '**novel compounds**' based upon the versatality of enormous available and considerable functional characteristic features of such entities.

3.8.3. 'Divide-and Rule' Concept in Design of Ligands

In the past two decades, several newer methodologies have been developed adequately which specifically make use of the very concept of **divide-and-rule** in the **design of ligands**. To accomplish this objective gainfully and effectively the '**active site**'***** is meticulously sub-divided further into various **subsites**, each of which is essentially bearing a number of vital and important **pharmacophoric moieties********. Subsequently, the '**chemical components**' that happen to complement to each specific **subsite** are now meticulously designed or suitably retrieved from the various **available databases**. The final shape of the '**designed molecule**' is duly accomplished by joining the selected, identified, and determined '**fragments**' to yield the **aggregate ligands** respectively. Interestingly, the major advantage of such an excellent concept being hte scope and manoeuvrability may be substantiated significantly *via* the combinatorial assembly of a plethora of available **subcomponents**.

DOCK-Programme : Desjaralis *et al.*******(1990) first and foremost introduced to utilize this novel concept and philosophy in the well-known programme termed as **DOCK**.

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*Sheridan RP *et al.* : *J. Chem. Inf. Compt. Sci.*, **29**, 255-260, 1989.

**Molecular Design Ltd. San Leandro California, USA.

***Chemical Design Ltd. Oxford, UK.

****UNITY-3DB Users Manual, Tripos Associates Inc., St. Louis, Mo., 1992.

***** **Active Site :** The cleft in the surface of an enzyme where a substrate pinds.

******Pharmacophoric Moieties :** The particular gorup or arrangement of atoms present in a molecule that gives the compound its medicinal activity.

*******Desjarlais RL *et al. Proe. Natl. Acad. Sci., USA,* **87 : 6644-48, 1990**.

Interestingly, the **DOCK-Programme** exclusively looks for the **3D-databases of ligands** ; and also determines, affirms, and establishes the different **potential binding modes** of any '**specific entity**' which will afford the most probable '**best-fit**' very much within a **target-receptor interface***. However, in this particular instance one would maintain solely only one single, static conformation pertaining to each individual '**database structure**'without any reference to the '**ligand-flexibility**' whatsoever.

LUDI-Programme ** :** The **LUDI-programme** particularly deals with a **receptor-volume of interest** that is meticulously scanned so as to strategically locate, identify and determine the various **subsites** whereupon either the **hydrophobic contact** or the **hydrogen-bonding** may be established with great fervour and confidence. Later on, one may selectively lay one's hand onto certain relatively smaller '**complementary molecules**' duly retrieved from a **database** ; and placed articulately and carefully within these subsites in order to maximize the ensuing **binding-energy**. Lastly, this particular phenomenon wraps up with the ultimate selection of different **bridging fragments** to hook upon the subsets of comparatively smaller molecules together in position.

FOUNDATION* : FOUNDATION—**programme hunts through the 3D-databses of various known and perceived chemical structures so as to help a researcher in finding a **user-defined querry** essentially made up of the coordinates of atoms and/or the corresponding bonds. Interestingly, in this particular programme one may have an access to almost **all possible structures** which prevalently comprise of any suitable combination of a **user-specified minimum** quantum of retrievable matching atoms and/or bonds.

SPLICE** ** :** The **SPLICE**-programme may specifically help in the generation of various desired permutations and combinations of hits automatically, *i.e.*, in a programmed manner. In fact, **SPLICE**, usually recognized as a **companion programme**, meticulously trims the molecules carefully retrieved from the database so as to have the **best-fit** very much within the **active-site** ; and, therefore, logically and gainfully aids in the combination of **ligand-receptor combinations** to a maximum level *via* overlapping of the ensuing bonds. In this manner, the ultimate addition of the available **bridging fragments** with those adequately obtained from the database remarkably assists the production of several **wonderful novel ligands** for future elaborated studies.

Docking a Flexible Ligand-Difficulties Encountered : A tremendous amount of reserarch has yielded a copious volume of databases with respect to the most articulated and difficult task of carrying out the **docking of a flexible ligand** particularly at the '**active site**' of macromolecules. Keeping in view the various steps involved usually in the **docking of a flexible ligand** one may come across a number of logical and plausible reasons, namely :

(*a*) Presence of multiple binding sites for a ligand,

(*b*) Inevitable difficulties encountered in scoring,

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*Kuntz ID *et al. J. Mol. Biol.*, **161**, 269, 1982.

Bohm HJ : *J. Comput. Aided Mol. Des.*, **6 : 593-606, 1992.

***Ho CMW and Marshall GR : *J. Comput. Aided Mol. Des.*, **7** : 3-22, 1993.

****Ho CMW and Marshall GR : *J. Comput. Aided Mol. Des.*, **7** : 623-647, 1993.

*****The most accurate and precise scores are invariably obtained from the fully free-energy calculation for ligand-protein interaction.

(*c*) Most commonly observed difficulties in the generation of precise force fields,*****

(*d*) Exceptionally huge computation time for the determination of comformational freedom in ligand, for instance : **62 billion conformations** may only be obtained from 10 rotatable bonds,*

(*e*) Observed rotational movement of ligand,

(*f*) Noticeable dynamic flexibility as displayed by the target molecules, and

(*g*) Unavoidable and inherent trapped water molecules.

Methodologies for Docking : There are several well-defined and generalized **methodologies for docking**, namely :

(*i*) Interactive graphics,

(*ii*) Docking by superimposition,

(*iii*) Energy-based docking programmes,

(*iv*) Builders, growers, and linkers,

(*v*) Flexible docking, and

(*vi*) Fragmentation approach.

These different aspects related to **methodologies for docking** shall now be treated individually in the sections that follows :

A. Interactive Graphics : The **interactive graphics** represent the most common as well as the simplest method for **docking**. The other end of complexicity essentially makes use of the rather **complete free-energy perturbation** thereby embracing the acceptable **molecular dynamics method**. Of these *two* ensuing extreme methods there exist a good number of such ligands that exclusively utilizes the most **sophisticated automated methodologies**.

Examples :

(*a*) For *analyzing* the **crystal structure** of a **target-drug molecule**, and

(*b*) For *docking* of a ligand at the active site.

In general, these methods do require prominently the wisdom, skill, and above all the much desired intuition inherited by a medicinal chemist to solve many intricate problems invariably encountered, of course, with the help of ever-expanding horizons of computational technique. However, these methodologies are not only give rise to purely qualitative results, but also are time-consuming and labour-intensive nature.

B. Docking by Superimposition : It has been established beyond any reasonable doubt that one may, with great ease and convenience, superimpose an altogehter **new ligand** derived meticulously from the 3D coordinate structures of the avilable ligand-bound protein upon the **prevailing ligand**. This may be considered as the *ab initio* position. In this manner one may skilfully utilize the specific **grid-based energy programmes** so as to determine the precise scores having different ligands.

Example : Holtz and Folkers** Exemplified the typical case of a **major histocompatibility complex (MHC) poptide** as depicted in Figures : 6. It broadly and vividly shows the simplified model of binding of a **nonapeptide** *i.e.*, having nine peptide linkages, to a Class I MHC-peptide as illustrated in HLA-B27.

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*Hypothetically, in case each minimization takes up at least 1 second, the complete and exhaustive calculation may require upto 2 millennium (*i.e.*, 2000 years).

Holtz HD and Folkers L : **Molecular Modeling—Basic Principles and Applications, Weinheim, New York, 1996.

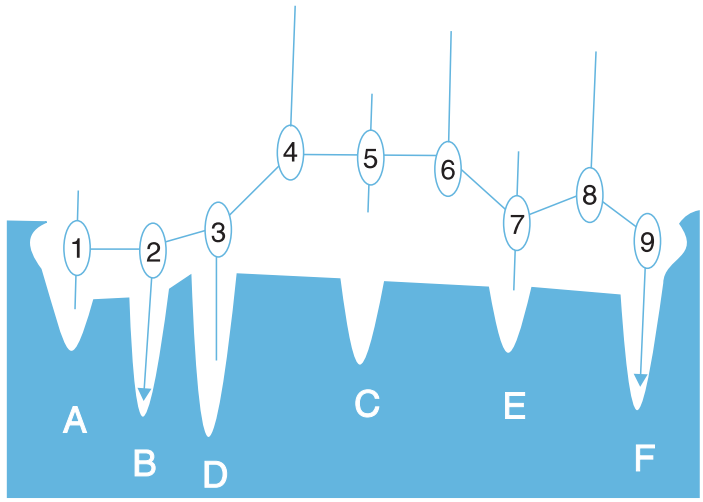

Fig. 3.6. Model of Monopeptide Binding to Class 1 MHC Peptide Exemplified by HLA-B27.

C. Energy-Based Docking Programs : There are quite a few well-known **docking programmes**, as discussed earlier, that essentially make use of a particular '**energy-grid**' with an assumption that body **ligand** and **target-molecule** happen to be absolutely '**rigid**' in nature. Importantly, the **energy-based docking programmes** may afford two important functionalities to the '**ligand**', namely : (*a*) **conformational flexibility** ; and (*b*) **motional flexibility**.

Examples :

(*i*) **DOCK 3.5 :** Kuntz *et al.** (1982) introduced a highly promising and effective means for the **3D databse search**. Infact, there exist quite a few well-known **modelling packages** *viz*., **CHEM**, **HYPERCHEM**, **LIGAND**, **MOE** etc., that essentially provide a plethora of such elaborated programmes to perform specifically the **energy-grid based docking**.

Applications : The various applications of **DOCK 3.5** are as follows :

(1) Capable of taking up only one **ligand** at a time.

(2) Prime aim is to lay hand upon the **starting model** of the proposed **ligand-receptor complex** that could be further modified and refined based on need and wisdom.

(3) Ligand may be taken on as '**rigid**' as well as '**flexible**'.

(4) Success rate of such studies is solely dependent upon the inputs provided by the various physicochemical studies.

(*ii*) **MHC-Peptide :** Holtz and Folkers** (1996) put forward the particular **coordinate system** whereby the Z-axis is duly projected along the length of the **acitve-site cleft**. In this instance, the different observable and registered translations all along the Z-axis, together with the rotations around the Z-axis are recorded duly over the **full-range**, but with various step sizes ranging between 3Å to 30Å. However, the native ligands of MHC are **peptides**.

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*Kuntz ID *et al. J. Mol. Biol.*, **161** : 269, 1982.

Holtz HD and Folkers L : **Molecular Modeling : Basic Principles and Applications, Weinheim, New York, 1996.

Example : An actual study using **CoMFA** and eight selected peptides related to the **helical superimposition segment** was performed meticulously. A chain of six specific amino acids *i.e.*, **Gly-Ile-Leu-Phe-Thr-Leu** was carefully docked by a manual process right inside the **peptide-binding zone** strategically located in the **binding-pocket of MHC peptide**. To achieve this objective the **electron-density map** was employed that critically maintained the volume under control so that the **'ligand'** should fit into it perfectly.

The *first residue*, **n-terminal of glycine**, shall duly fit in at a H-bonding distance with respect to the conserved residues like : Tyr 7, Tyr 59, and Tyr 171 as illustrated in Figure : 6. The *second residue*, **isoleucine**, has been proved to be one of the previously **detected and anchored residues** kept under protection from the corresponding **peptide ligand**. In Figure : 6 the six **binding pockets** are distinctly labeled from A to F.

D. Builders, Growers, and Linkers : In a broader sense, the **3D database searching methods** are obviously more precise and appropriate in comparison to several other techniques due to the fact that the existing overall available perceptive knowledge of **receptor-ligand recognition** is still not absolutely clear and perfect.

Alternatively, one may initially determine the most probable exact location wherein the moieties under investigation are adequately predicted by strategically establishing the precise position of the **H-bonding sites** *e.g.*, in **LUDI** or **multiple-bonding sites** *e.g.*, in **HOOK**, and ultimately linking them with the **best-fitting fragments** from the **database libraries**.

E. Flexible Docking : Flexible docking usually refers to a not-so-rigid kind of **virtual screening durg design technology** so as to evaluate specifically the binding of **ligands** to the **macromolecular targets**. In other words, it permits limited conformational flexibility amongst the **target** and **ligand molecules**.

It is, however, pertinent to state here that this type of suggestive action mode is proved to be quite good and effective for **peptide docking**, but it may turn out to be farily expensive proposition. Therefore, it is always advisible and wise to **prescreen the proposed ligand flexibility** in particular.

F. Fragmentation Approach : In actual practice, the **fragmentation approach** affords rather complete flexibility to the ligand ; and subsequently, bind them at the appropriate target site.

Example : CAVEAT* serves as a befitting example of this **fragmentation approach**.

Following are a few vital and important **Docking Programmes** commonly used in the design of newer drug molecules :

S. No.	Name of Programme	Involved Technique	Reference
1	**DOCK 3.5**	Obtaining comentary match *via* rigid-docking geometry.	Kuntz ID *et al. J. Mol. Biol.* **161** : 269, 1982.
2	**DOCK 4.0**	Rigid docking based upon fragmentation.	Ewing T and Kuntz ID : *J. Comput. Chem.*, **18**, 1175, 1997.

(*Contd.....*)

*Bartlett PA *et al.* : **Molecular Recognition : Chemical and Biological Problems**, Royal Society of Chemistry, London (UK), pp 182-186, 1989.

3	**GOLD**	Genetic **algorithm***.	Jones G *et al. : J. Mol. Biol.* **267**, 727, 1997.
4	**GROMOL**	Docking based on fragmentation.	Bohacek RS and McMartin C : *Curr. Opin. Chem. Biol.*, **1** : 157, 1997.
5	**HOOK**	Docking based on fragmentation.	Eisen MB *et al. Proteins*, 19, 1991.
6	**AUTODOCK**	Conceptualization of pharmacophor moieties.	Morris GM *et al. J. Comp. Aided Mol. Design*., **10**, 293, 1996.

3.8.4. *De Novo* Design

Interestingly, one may effectively combine the absolute and logical prediction of the '**binding phenomenon**' to a *receptor site* with the proposed **design of molecules**. To accomplish this, one could easily initiate the entire process using a set of **building blocks** together with a **known receptor ;** and, therefore, articulately align the complementary building blocks to the various available pockets in the receptor, with particular reference to such confirmations which maximize not only the H-bonding but also other interactions as well. In the event when such '**fragments**' are aligned adequately it is quite convenient and possible to connect the ensuing fragments with the **suitably-sized spacer fragments** to build up a **newer drug molecule** strategically positioned very much within the limits of the **receptor**. This approach, infact, is commonly termed as ***de novo* design** or **structure-based drug design**. Now, with the present day galloping progress achieved in the various models for binding to receptors, the ***de novo* design** is mustering tremendous world-wide popularity*.

In other words, the ***de novo* design** proves to be much more strong and robust as the **geometric foundations of the molecular sciences** are much firmer than the **thermodynamic ones**. In fact, it largely attributes a scientific and logical means to alter drastically the ensuing **side-effect profile** of the '**drug**' besides its usual physical and metabolic characteristic features.

3.9. Calculation of Affinity

The **calculation of affinity** may be categorized into the following *four* groups, namely :

(*a*) Components of bonding affinity,

(*b*) Binding energetics and comparisons,

(*c*) Simulations and the thermodynamic cycle, and

(*d*) Multiple binding modes.

These aforesaid variants observed in the **calculation of affinity** shall now be treated individually in the sections that follows :

Böhm JJ and Stahl M : Structure-based library design : Molecular Modelling Merges with Combinatorial Chemistry, *Curr. Opin. Chem. Biol.* **4 : 283-86, 2000.

3.9.1. Components of Bonding Affinity

The inherent capability for carrying out the effective calculation in the determination of the exact and precise affinity of the so called **prospective ligands** is solely dependent upon the 3D structure(s) of the **therapeutic target**. This in turn would certainly go a long way in establishing the desired prioritization of the **synthetic target(s)**. In doing so it would pave the way in favour of the quantitative aspect rather than the qualitative perspective in the observation of presence of a potential '**ligand**' strategically located at the **receptor site**. There exists a wide gap between the ability to circumvent such problem(s) in principle and the direct usage of **molecular mechanics**, to tackle such an intricate issue effectively. Perhaps this sort of difficulty may be overcome by exploitation of the '**free-energy of binding**' articulately tagged into a '**logical set of components**'.

Example : Vancomycin-peptide complex : Williams *et al.** (1992) made use of a **vancomycin-peptide complex** to demonstrate evidently the precise and exact evaluation of the different aspects of contributions to the ensuing **binding affinity** in an experimental measurement, as depicted in Figure 3.7.

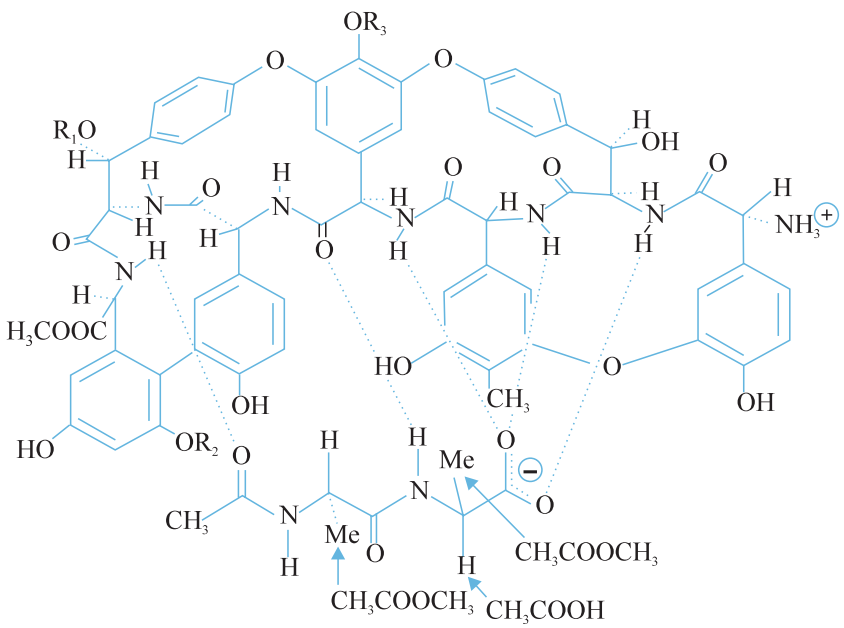


Fig. 3.7. Vancomycin-peptide Complex to Explore Components of Free Energy of Binding. [Adapted From : Williams DH : *Aldrichimica Acta*, 24 : 71 -81,1991.]

*(*a*) Williams *et al. J. Am. Chem. Soc*., **114** : 338-43, 1992.

(*b*) Williams *et al. J. Am. Chem. Soc*., **114** : 10690-697, 1992.

(*c*) Williams *et al. J. Am. Chem. Soc*., **114** : 10697-704,1992.

Explanation : In the **vancomycin-peptide complex** system the estimates as determined with regard to the exact contribution of the ensuing H-bonds to binding were found to be appreciably on the higher side *e.g.*, –24 kJ mol^{-1}, – 6 kcal mol^{-1}, in comparison to the ones duly obtained *via* actual experimental modes. To explain this relatively **higher binding affinity** giving rise to '**source of error**' is probably based on the assumption that there prevails virtually a substantial degree of loss of both internal as well as relative **entropy*** upon the binding phenomenon. The above plausible explanation expatiates an extent of entropy loss that may be incurred in the drug-ligand interaction specifically. Importantly, one may accomplish a rather more conventional view of the H-bond ranging between 0.5 to –2.0 kcal mol^{-1} in the above **vancomycin-peptide complex**.

Because **entropy** has a direct relationship with quite many fragments present intimately in the ensuing **binding-energy judgement** ; hence, inducted **simulations**** in the medium (*i.e.*, solvent) could be an absolute must be arrive at the following *two* cardinal objectives, namely :

(*a*) Qunatity precisely the extent to which the relative motions of the **ligand** (**vancomycin**) and **protein** (peptide) are duly quenched, and

(*b*) Restriction with respect to the observed '**degrees of freedom**' upon the prevailing **ligand-peptide complexation** phenomenon.

3.9.2. Binding Energetics and Comparisons

Lumry *et al.**** (1970) proposed a much simplified version of the well established correlation existing between ΔH (*i.e.*, the strain energy introduced by complexation, which otherwise refers to actual deformation in bond angles, bond lengths, and torsional angles from the solution states), and ΔG (*i.e.*, the interaction free energies between polar moieties) as a not-so-common characteristic feature of water as a **solvent**. However, it has been duly observed in the **cogeneric series** that the *two* important factors, such as : (*a*) **entropic effects** ; and (*b*) **desolvation energies**, shall be almost the same throughout all members of the series. However, the relatively large segment of '**complexation**' significantly rests upon the **total energetics of the complex**. One may also encounter a rather '**harmless change**' observed spontaneously in a substituent that would essentially initiate an altogether **different binding mode** wherein the particular **ligand** has undergone an effective reorientation. In fact, such a distinct and remarkable environmental alterations observed in the larger segment of **ligand interactions** would certainly result into **entropic** and **desolvation effects**.

3.9.3. Simulations and the Thermodynamic Cycle

Using a '**novel ligand**' one may conveniently calculate the **difference in affinity**, designated by ΔΔG, based upon the actual precise measurement of *two* vital parameters *viz.*, **thermodynamic cycle**, and **affinity of the ligand**, in a definite structure of an established **drug-receptor complex**.

Example :

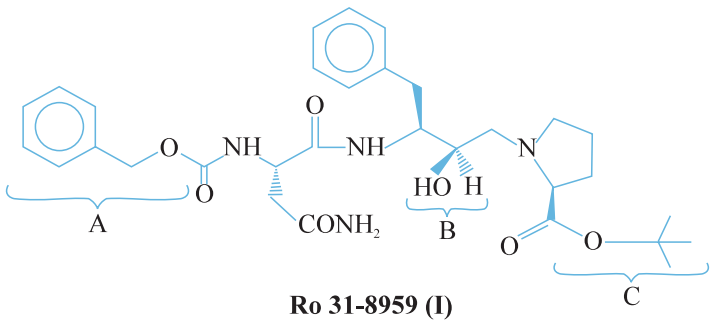
Differences in Binding of Two Stereoisomers of HIV-Proteases : Ro 31-8959 and JG-365 : Harte Jr. and Beveridge (1993)***** critically examined the actual prevailing difference in the bondage of the two stereoisomers pertaining to a **transition-state inhibitor of HIV protease**, such as : **Ro 31-8959 (I)** and **JG-365 (II)** as given below in Figure 3.8.

***Entropy :** The portion of energy within a system that cannot be used for mechanical work but is abundantly available for internal use exclusively.

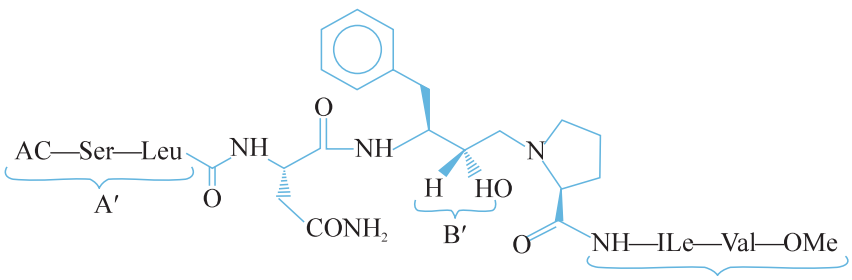
****Simulation :** Pretence to feel.

*****Lumry *et al. : Biopolymers*, **9** : 1125-27, 1970.

******Harte Jr WE and Beveridge DL : *J. Am. Chem. Soc.*, **115** : 3883-86, 1993.



Ro 31-8959 (I)



JG-365 (II)

Three Major Points of Difference Between I and II

Segment	I	II
A	Benzyl-oxy	—
A′	—	Ac-Ser-Leu
B	OH &H solid & dotted line.	
B′	—	H & OH solid & dotted line.
C	*tert*-butoxy	
C′		–NH-Ile-Val-Ome chain.

Fig. 3.8. Chemical Structures of Ro 31-8959 (I) and JG-365 (II).

In **Ro 31-8959** (**Ro** refers to **ROCHE′**-a renowned Swiss Pharma Co.) the segment A is rather a compact benzyl-oxy moiety, whereas in **JG-365** the corresponding A′ designates a chain of three amino acids *i.e.*, Ac-Ser-Leu. Further, in I (*i.e.*, **Ro 31-8959**) the chirality at B is represented by OH group with a solid line and H atom with a dotted line, whereas in II (*i.e.*, **JG-365**) the chirality at crucial transition-state, OH moiety, is reversed for optimal binding in the two analogues*. Lastly, in I, the portion C is a *tertiary*-butoxy function, and in II, the corresponding portion C′ is represented by an '**imine**' linked to two amino acids *viz.*, Ile-Val, and a methoxy terminal functional group. However, it is a usual practice to **cause minor perturbations**, as far as possible, to a given chemical structure for which one may probably encounter bear minimum chance for any **possible alterations in the binding mode**.

3.9.4. Multiple Binding Modes

Generally, **medicinal chemists** heavily bank upon the creation of **congeneric series** (*i.e.*, **structural analogous**) quite seriously and realistically that may ultimately prove to be the most useful construct in the design of **newer drug molecules**. To accomplish this serene objective one may have to take into consideration the specific orientation of the '**drug**' at the **active-site** which solely rests on **multifarious interactions** ; and, therefore, even a very slight perturbation in the body of the chemial structure may completely destablize the **most vital binding mode** towards another '**site**' (less active).

*A specific change in the **binding mode** could be predicted to expatiate this observation, which was subsequently confirmed by X-crystallographic studies.

Examples :

(*a*) Observed orientation of the C-terminal segment in the ROCHE HIV-protease inhibitor *i.e.*, **Ro 31-8959** (see Fig. 8) with **JG-365**.

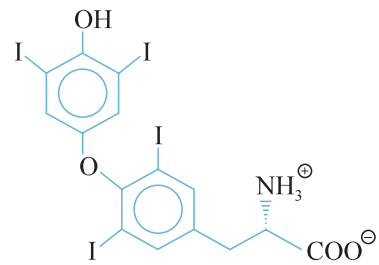
(*b*) Comprehensive X-ray crystallographic studies and analyses of the multifarious binding modes as illustrated with the following *two* drug molecules, namely :

(*i*) **Enkephalin** structural variants by an **Fab fragment***, and

(*ii*) **Thyroxine** congeners by **transthyretin**** (*i.e.*, a *transport protein*).

[Met] Enkephalin

[Leu] Enkephalin



L-Thyroxine

4. UNKNOWN RECEPTOR SITES

Interestingly, upto the recent past the '**receptor sites**' were considered to be more or less **unknown zones** by virtue of the fact that such **receptors** represented absolutely hypothetical (imaginary) macromolecules the existence of which was heavily relied upon the critically observed pharmacological experimental results. With the advent of enormous latest progresses accomplished in the field of '**Molecular Biology**' that ultimately influenced not only the wonderful '**cloning phenomenon**' but also the **adequate expression** of several such receptors whose very existences were duly assumed to be true as a basis for reasoning. Thus, the candid emergence of good many '**subtypes**' together with a substantial progress in an elaborated description of their 3D-structure(s) has yet to equip the '**medicinal chemist**' with the relevant and informative **atomic details** so as to enable designing of **novel medicinally potent compounds**. It is, however, pertinent to mention at this material time that in the absence of valid detailed information(s) with respect to the specific and precise 3D structure of the proposed receptor such conventionally computational-based methodologies, namely : **Monte Carlo Technique** and **Molecular Dynamics**, may not prove to be productive and result-oriented.

Salient Features : Following are some of the **Salient features** with regard to the exploration of **unknown receptor sites** :

(1) Deduce an **workable operational model** of the **receptor** which essentially provides a definitive, logical, and consistent explanation of the known data ; besides, gives rise to an **ideal productive**

*Edmundson AB *et al. : Philos. Trans. R. Soc. Lond. [Biol.]*, **323**, 495-509, 1989.

De La Paz P *et al.* : In : Beddell CR (ed.) : **The Design of Drugs in Macromolecular Targets, John Wiley & Sons, Inc., New York, pp. 119-172, 1992.

value while exploring newer drug molecules **for synthesis** and subsequent exhaustive **biological evaluation**.

(2) Pharmacophoric model meticulously obtained for **auxin** (*i.e.*, a plant hormone) to evolve ultimately *four* **novel groups of active medicinal compounds** by thoroughly screening a conglomerate 3D-database of structures.

(3) Receptor's existence may be established based upon the elaborated and comprehensive pharacological data, and certain low-resolution 3D design of the receptor-related to either the **active-site** or **binding-pocket** may be obtained carefully from the quantitative structure- activity relationship (QSAR) data.

The various important and vital aspects of the **unknown receptor sites** are as given under :

(*a*) Pharmacophore *Vs* binding-site models,

(*b*) Searching for similarity,

(*c*) Molecular comparisons, and

(*d*) Finding the common design.

All the above mentioned aspects shall now be treated individually in the sections that follows :

4.1. Pharmacophore *Vs* Binding-site Models

The **pharmacophore *Vs* binding-site models** may be further categorized under *four* groups which shall be treated briefly as under :

4.1.1. Pharmacophore Models

It has been observed that a majority of **drug substances**, due to their **in-built conformational freedom**, affords a **multifarious of 3D structures** to a specific receptor. In fact, this pre-determined pharmacophoric assumption may virtually lead to *two* altogether different phenomena, namely :

(*a*) The '**recognition of receptor**' could be determined by carrying out various chemical structural modification *vis-a-vis* biological screening of the different functional moieties present in the drug molecule(s). However, such investigative exercises would throw ample light with respect to the very fundamental nature of the functional moieties strategically positioned in the **receptor** which are exclusively responsible for affording the right type of linkage to the set of drugs.

(*b*) Proposed hypothesis with respect to close similarity existing either between the **pharmacophore*** (*i.e.*, *functional moieties*) duly located in various **structural analogues** (*i.e.*, *congeneric series*) of the drug molecule, or between the suggested **recognition site points** duly postulated to exist very much within the **receptor** *i.e.*, the *binding-site model*.

4.1.2. Binding-Site Models

There exists one singular major disadvantage in the '**pharmacophore models**' which refers to the most essential requirement for necessary overlapping of the various functional moieties in perfect alignment to the **pharmacophoric hypothesis**. It has been established beyond any reasonable doubt that :

***Pharmacophore :** It refers to the intellectual framework for utilizing the **quantitative structure-active relationship (QSAR)** data to extrapolate meticulously information with regard to the **receptor** *i.e.*, the ligand's intimate partner).

(*i*) molecules bearing such functional moieties which exhibit 3D similarity may interact with the same site, and

(*ii*) specific geometrically identical structures closely associated with one particular site is able to interact with almost equal fervour and affinity pertaining to a number of plausible structural orientations of the identical functional moieties.

Therefore, it has become almost necessary to take into consideration the converging point of approximately **equal** energetic configurations associated with either a H-bond donor or acceptor to circumvent the ensuing problem skillfully.

More restrictive assumption is the binding mode in a **ligand-receptor complex** which categorically designates the maximal position of the ligand in an assymetric force produced by the respective receptor which is duly exposed to the suitable entropic considerations and the ensuing perturbation due to solvation.

Less-restrictive assumption refers to the **receptor-binding site** which virtually remains almost fixed (static) in geometrical aspects on being getting bound to the series of congeners (*i.e.*, structural analogues) under critical investigative studies.

Interestingly, a comparison between the **specific pharmacophore** and the **binding-site hypotheses** vividly establishes the fact that the latter is definitely more acceptable and plausible physiochemically due to the fact that the ensuing **overlap of the functional moieties** in the process of binding to a receptor is found to be more restrictive than anticipating that the particular site does remain more or less fixed when getting bound to different ligands.

4.1.3. Molecular Extensions

The most fundamental aspects of **rigid-site points** assumed to be true and definitely more reasonable ; and, therefore, grossly applicable to the **enzymes** which have come into being to initiate catalysis of certain specific reactions. In order to accomplish this objective the most critical and vital functional moieties must be strategically placed in a particular 3D-arrangement to yield the **desired correct and conducive electronic environment for catalysis**. In reality, the molecular extension programme screens and evaluates the ensuing '**hypothesis**' by finding out precisely whether one or more such geometrical arrangements pertaining to the postulated functional moieties of site points are found to be common to the set of active medicinal entities. Thus, a distinct and prominent **candidate binding-site design** results due to a **geometrical arrangement of the receptor moieties** that may be evaluated ultimately for ascertaining their **predictive merit**.

Examples : Following are some of the typical examples that expatiates abundantly the concept of **molecular extensions** :

(*a*) **Active-site of angiotensin-converting enzyme (ACE)** : In an elaborated investigation, the **binding site model of ACE** was employed wherein the various available **active site components** were introduced as **integral segments of each synthesized compound** subjected to systematic analysis.

Hausin and Codding* (1990) meticulously extended the '**sulphhydryl component**' of **captopril** to incorporate gainfully a '**zinc**' suitably bound at the experimentally designed optimal **bond angle** and **bond length** for obtaining the **Zn-S complexes** as depicted in Figure 3.9.

*Hausin RJ and Codding PW : *J Med Chem*, **33** : 1940-47, 1993.

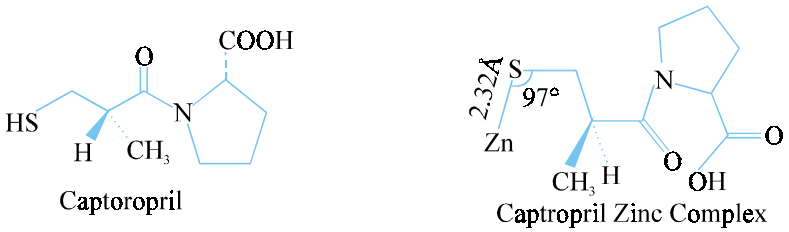
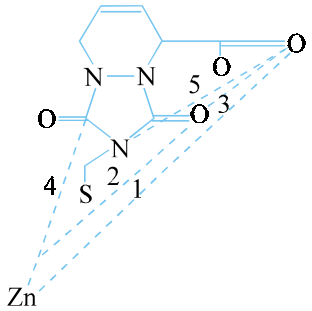


Fig. 3.9. Plausible Extension of Sulphhydryl Moiety in Captropril to Include the Postulated Active-Site Zinc-Employing Bond Angle and Bond Length. [Adapted From : Hausing and Codding, *J, Med Chem*, 33 : 1940-47, 1993]

(*b*) **Application of oreintation map (OMAP) for analysis of ACE inhibitors :** About two decades ago, the **orientation map* (OMAP)** came into being which was entirely based upon the actual distances between the **binding-site points**.

Example : The presence of the **Zn-atom** *vis-a-vis* the careful introduction of relatively more **degrees of torsional freedom** so as to contain effectively the most probable strategical positioning of the **Zn-atom** relative to **ACE inhibitors** *viz.*, **captopril****, as illustrated in Figure 3.10.



Interatomic Distances Between
1 : Zn and O
2 : Zn and O
3 : O and O
4 : Zn and N
5 : N and O

Fig. 3.10. Distance employed in 5D-OMAP for the Analysis of ACE-Inhibitors. [After : Marshall GR *et al.* 1979]

4.1.4. Activity *Vs* Affinity

It has been amply demonstrated that the very presence of the **most appropriate and suitable design** into a proposed '**drug molecule**' is not a sufficient evidence to ensure **positive biological activity**.

Example : Legitimate, viable, and noticeable competition occurring with a specific receptor looking for the available '**occupied space**' by other suitable segments of the '**drug molecule**' may inhibit binding phenomenon predominantly, and preclude activity distinguishably.

Cardinal Parameters for Exhibiting Biological Activity : The *three* major and prominent **cardinal parameters for exhibiting apparent biological activity** are as given below :

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*__OMAP :__ It refers to the multidimensional representation of the interatomic distances between pharmacophoreic moieties.

Marshall GR *et al.* : In : EC Olsen and RE Christofffersen (eds) : **Computer Assisted Drug Design, American Chemical Society, Washington DC., pp 205-226, 1979.

(*a*) '**Drug Molecule**' should by all means be stable metabolically and thereby capable of transport to the particular site for necessary and desired drug-receptor interaction (obviously the **inactive molecules** lack bio-availability appreciably),

(*b*) '**Drug Molecule**' should be in a position to readily adapt itself to such a conformation that would categorically warrant the very presence of both **binding-site** and **pharmacophoric** model quite intimately complementary to that of the **receptor**, and

(*c*) '**Drug Molecule**' in any case should not compete with the '**receptor**' at all with respect to space specifically while displaying the **binding-site** as well as **pharmacophoric** design.

Important Features : Following are certain **important features** to expatiate activity *Vs* affinity :

(1) All the *three* aforesaid conditions *i.e.*, (*a*) through (*c*) essentially belong to **3D-QSAR***, and the usage of **variant CoMFA**** upon the ACE inhibitors.

(2) The parameter (*c*) above aids in determining the precise location of the available **receptor-occupied space** with respect to the particular **pharmacophore** (*i.e.*, **actual receptor mapping**)***.

4.2. Searching for Similarity

The **searching for similarity** also establishes the exact location(s) of the unknown receptor sites. This may be accomplished judiciously by carrying out the investigative studies under the following *two* categories :

4.2.1. Simple Comparisons

In order to have a thorough understanding of the molecular formation and subsequent recognition one may have to penetrate one's insight deep into the very minute prevailing differences in the ensuing '**drug molecules**'. In fact, such **simple comparisons** may be achieved in *two* ways, namely :

(*a*) comparisons which are **absolutely independent** with respect to the **position** as well as **orientation** of the molecule, and

(*b*) comparisons that are **exclusively dependent** upon a known **pre-determined structural frame of reference**.

Generally, the simple comparisons take action about properties that are independent of a **reference frame**.

Examples : A few typical examples are as stated under :

(1) Certain **physical parameters** *e.g.*, bond length, valence angle, torsion angle, and interatomic distance are found to be independent with regard to orientation.

(2) **Distance matrix** made up of the pair of interatomic distances is a convenient and easier mode of drawing a molecular structure which is evidently constant to both translation and rotation of the molecule. However, distance matrix records the particular alterations related to internal degrees of freedom. The conformationl flexibility of the molecule is caused on account of the ensuing variations observed for a given interatomic distance.

4.2.2. Visualization of Molecular Properties

Medicinal chemists now enjoy the privilege of having a clearent visual displays of physical and chemical characteristic features along with the 3D molecular structures of compounds. In a broad per-

*Marshall GR and Cramer RD : *Trends Pharmacol. Sci.*, **9** : 285-89, 1988.

**Cramer RD and Wold SB : US Pat. 5,025, 388, 1991.

***Sufrin JR *et al.* : *Mol. Pharmacol.*, **19**, 307-313, 1981.

spective such vivid displays throw enough light with respect to such molecular properties as : electronic charge distribution pattern, internal energy, and hydrophobicity.

Marshall and Naylor*(1990) critically observed a plethora of divergent characteristic properties that have been studied meticulously in this fashion with a view to gain an insight in the actual molecular structure *vis-a-vis* recognition in several congeners.

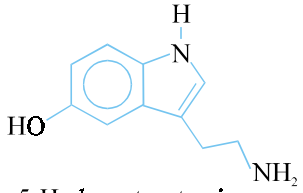
Importance of Electrostatic Potential : Electrostatic potential is one of the most vital and beneficial molecular properties ; and, therefore, enjoys a good number of influences in **molecular modeling in drug design**, for instance :

(1) Distribution of ensuing '**electrostatic charge**' evidently gives rise to a corresponding *electrostatic potential* prevailing in the adjoining space which specifically designates the overall potential of the '**drug molecule**' that eventually interacts with an electrostatic charge available at that material point.

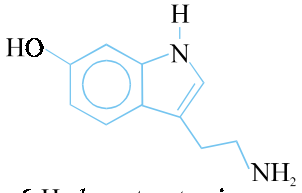
(2) Nevertheless, the **electrostatic potential** helps largely in the **molecular reactive behaviour** with regard to correct prediction and precise analysis.

(3) **Electrostatic potential** serves predominantly as an indicator of the *particular regions* or *specific sites* of a '**drug molecule**' either from which an **incoming nucleophile or electrophile** is first and foremost **repelled** or to which it is **attracted ultimately**.

(4) Weinstein *et al.*** (1981) demonstrated successfully the orientation between the two chemical entities **5-Hydroxytryptamine** and **6-Hydroxytryptamine** that was critically based upon the alignment due to an **electrostatically derived orientation vector**.



5-Hydroxytryptamine



6-Hydroxytryptamine

(5) Cohen*** (1985) evolved an altogether new methodology due to which the prevailing **steric field** in the vicinity of a '**drug molecule**' may be shown vividly on a *graphics monitor* in the shape of a beautiful **3D isopotential contour map** to expatiate the findings from **molecular modeling in drug design**.

4.3. Molecular Comparisons

Various means and ways of **molecular comparisons** have been duly recognized as stated under :

- **superimposition** of the proposed **drug molecules** in the same **reference frame**,
- **orientation** of the investigative **drug molecules** in the same **reference frame**,
- strategical placement of an atom in the **drug molecule** located at the centre of the **coordinate frame** along with other atoms carefully placed in line with the **coordinate axes**,

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*Marshall GR and Naylor CB : In : Ramsden CA (Ed.) : **Quantitative Drug Design**. Vol. 4. **Comprehensive Medicinal Chemistry**, Pergamon Press, Oxford, UK, pp. 1-766, 1990.

Weinstein *et al.* : *Ann. NY Acad. Sci.*, **367 : 434-448, 1981.

***Cohen NC : In : Testa B (Ed.) : **Advances in Drug Reasearch**, Academic Press Inc., New York, pp. 40-144, 1985.

• proposed **drug molecules** that would fit well into the coordinate frame/axes may serve as the **standard orientation**,

• comparison between two **molecules** having distinct geometric similarities with respect to the placement of H-bonded atoms,

• method of **least-squares fitting** for certain highlighted atoms do afford high degree of selectivity with respect to the orientations in the **drug molecules** *vis-a-vis* the predetermined conformations, and

• well-defined fitment of **structural analogues** (congeners) bearing **atom to atom linkage** have been determined between the ensuing members of congeners.

Besides, the **molecular comparisons** also embrace several critical and vital aspects to expatiate molecular modeling in drug design, such as : volume mapping, locus maps, vector maps and conformational mimicry, directionality, and field effects.

5. PREDICTIVE ADME*

In the recent past a tremendous aggresive thrust has been observed in the enormous development of computer-based **adsorption, distribution, metabolism, and elimination (ADME)** of molecular models. Interestingly, a plethora of **predictive ADME molecular models** are heavily dependent upon the extensive and intensive application of **QSAR****. In short, one may have a significant and appreciable insight into the design of **chemical libraries for an elaborative biological evaluation** that could be entirely based upon the ensuing spatial arrangements and descriptors which prove to be absolutely essential and necessary for various **drug-like molecules** still under detailed investigative procedures.

The **pharmaceutical scientiests** of today are adequately equipped with highly advanced and most sophisticated methodologies based upon several latest **molecular modeling software** that would certainly and legitimately help them to attain perfection in the modification of the various structural characteristic features of a '**potential-drug candidate**' **in silico**. In true sense, such **predictions** with regard to the **physicochemical properties** of the **potential-drug candidate** prior to the actual laboratory synthesis invariably prove to be of immense help and guidance to the on-going, time-consuming, and money-churning research undertakings.

Computer-based techniques do offer enough strength and power to accomplish difficult and intricate problems with appreciable convenience. Thus, it is quite evident that the **computer-generated molecular models** (*i.e.*, of '**newer drugs**') should be accurate and precise enough to muster **enough confidence** amalgamated with a reasonably **high-degree of success rate***** amongst its users (*i.e.*, **medicinal chemists**). In other words, one ought to get the procedural steps duly validated, irresective of the wisdom and intellectual calibre of **CADD**, with respect to the **known-drug substances** so as to restore and gain confidence in a plethora of circumstances when similar techniques shall be applied to the **unknown-drug substances** *i.e.*, the newly designed molecules. Thus, the stark reality in terms of the distinct apparent differences between a **computer-generated model** and **reality of a known-drug** must always be borne in mind while making use of **computer simulations** in **drug-design**.

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*__ADME :__ Refers to absorption, distribution, metabolism, and elimination of a drug substance *in vivo*.

**__QSAR :__ Quantitative structure-activity relationship.

***__Success Rate :__ It may be assessed by a close comparison of the computer results with the experimental results.

Nevertheless, the fundamental objective of **computer assisted drug design (CADD)** is to generate, and subsequently understand meticulously the most complex and intricate prevailing relationships at the molecular level between a skilfully designed **drug-like molecule** and a **disease-producing target** (*i.e.*, a **macromolecule**) in order to enable a medicinal chemist to make a fairly reliable and trustworthy prediction to increase molecular interactions with utmost accuracy.

There are a plethora of very critical and most vital **pharmacokinetic** characteristic properties so as to obtain a highly specific and effective therapeutic drug substance. Lipinski *et. al.* (1997)* postulated that the *three* **major physical variables** *viz*., **potency**, **solubility** and **permeability** may be carefully adapted to increase the overall activity of **potential oral drug** substances **predominantly**. They also observed that relatively poor permeation (*i.e.*, absorption) is commonly attributed by the following characteristic features either inducted alone or more than one right into the proposed drug molecule :

- Plus **five** H-bond donors,
- Plus **ten** H-bond acceptors,
- More than 500 molecular weight, and
- More than **five** computed 'log P' (hydrophobicity) values.

Singh *et al.* (2003)** put forward a more latest predictive model (design) for the **cytochrome P-450 (CYP) 3A4** metabolism. This method exclusively rests upon the **primary lateral sclerosis (PLS)**, however, one of the descriptors is totally based on acute myocardial infarction (AMI)-calculated H-atom abstraction process.

In fact, there are several important assumptions, namely :

(1) **CYP-3A4 :** its greater susceptibility is a determining factor of the electronic atmosphere surrounding the specific H-atom undergoing abstraction phenomenon,

(2) **Abstraction** of the particular H-atom designates the '**rate-determining step**', and

(3) '**Drug**' undergoing the process of metabolism enjoys almost a free access in the '**active-site**', of the specific enzyme till such time the '**most active H-atom**' is avilable abundantly.

AMI-H-atom Abstraction : The AMI-calculations essentially makes use of a procedure to explain the fact that '**unpaired electrons**' are involved, which eventually interacted on a series of known drug substances. It may be modified duly according to the availability of **chemical descriptors**.

By the year 2020, there lies a tremendous scope for the phenomenal advancement and increment of both **toxicity predictions** and **in-silico characteristic feature predictions**. The **dependability**, **versatility**, and **reliability** of the **predictive ADME** procedures and methodologies would overwhelmingly incorporate and legitimately include its dire and intimate presence in practically each and every initial molecular modeling drug-design process rather than at a stage when the drug has already conceived literally.

In short, **CADD** has already acclaimed enormous qualified success in the recent past, and intend to accomplish still greater peak in the years yet to come.

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*Lipinski CA *et al.* : *Adv. Drug Delivery Rev*., **23** : 3, 1997.

Singh SB *et al.* : *J Med Chem*, **46 : 1330, 2003.

6. REVERSE DESIGNING

Interestingly, **reverse designing** has come into being by virtue of the introduction of *two* extremely important scientific discoveries, namely : (*a*) **High Throughput Screening**, and (*b*) **Combinatorial Chemistry**.

6.1. High Throughput Screening

Biological testing procedures may be adopted profusely and automated meticulously in an extremely innovated process known widely as **high throughput screening**, that could be able to carry out the normal, rapid, and precise testing scores of newly designed chemical structures all and sundry at a time. It is, however, pertinent to mention here that in several occasions it is absolutely feasible as well as possible to make use of the enormous fruitful advantages of various well-known **gene-cloning methodologies**. In this manner, one has to first and foremost **clone the desired receptor**, and subsequently measure the **binding phenomenon** of the **newly synthesized drug molecules** to the corresponding **cloned receptor**.

6.2. Combinatorial Chemistry

Even with the advent of a plethora of such widely accepted and practised methodologies as : Superb statistical methods, traditional synthetic techniques, and time-tested biological screening procedures, invariably prove to be very expensive and sometimes trun out to be non-productive in nature at the end. In fact, the tremendous stress and strain of this sort of cumbersome testing procedures ultimately led to the enterprising technique commonly known as **combinatorial chemistry**. Interestingly, it overwhelmingly makes use of comprehensive and extended libraries of chemical functional moieties which specifically interact either with a '**base molecule**' or with a '**parent molecule**' in a highly systematic small quantum of well-defined purely synthetic stepwise procedures.

Baum and Borman* (1996) postulated that '**combinatorial chemistry**' refers to a particular sophisticated method of minimizing the effective cost of drug discovery whereby the following *three* cardinal objectives may be accomplished with utmost satisfaction and fruitful results, namely :

(*a*) to determine altogether '**new leads**'

(*b*) to find newer '**prototype drug molecules**', and

(*c*) to **refine and optimize the QSAR**.

Salient Features : The various *salient features* of '**combinatorial chemistry**' are as enumerated under :

(1) **Chemical diversity of products :** Useful libraries of '**reactive**' chemical functional moieties invariably give rise to the **chemical diversity of products** which shall be duly screened for respective biological activity.

(2) Chemistry involved is not only graceful and stylish but also comparatively simple, whereby a few '**same reactions**' could suffice in yielding thousands of **drug molecules** in a specific congeneric series.

*Baum R and Borman S : *Chem. Engg. News.*, **74** : 28, 1996.

(3) Invariably, one makes use of the **solid-state synthetic techniques** to allow the **desired growth of drug molecules** upon **polymer support**.

(4) The '**chemical reactions**' involved in (2) and (3) above must fulfil *three* vital and important criteria, such as : (*i*) clean reaction, (*ii*) reproducible reaction, and (*iii*) high yielding reaction.

(5) '**Robotics**' have been employed profusely to cut down the '**effective cost of synthesis**' drastically.

In a rather broader perspective towards the ever increasing and eternal (never-ending) search for newer '**drug molecules**' *i.e.*, chemical entities that essentially requires specific and noteworthy biological characterstic feature do require *two* kinds of approaches, for instance :

(*a*) **Rational Designing :** It is considered to be the most '**popular technique**' by virtue of the fact that it bears a **direct relationship** along with a **methodical stepwise development** of the creative genius and wisdom of a **medicinal chemist** to explore and exploit the **Lock-and Key Model** with respect to the **ligand-receptor docking**.

Rational designing usually encounters the following *three* obstacles and hinderances, such as :

(*i*) **limitations*.** *e.g.*, conformational flexibilities for both ligand and receptor,

(*ii*) **conformers**.** *i.e.*, binding with higher-energy conformers.

(*iii*) **active conformers***.** *i.e.*, influence of salt and water concentration upon the active conformers.

However, all the aforesaid *three* obstacles have been duly taken care of and adequately attended to, with an aim to design a '**drug**' that could mimick vividly the same in an ***in vitro* model**.

(*b*) **Reverse Designing.** Essentially involves the grouping together and searching**** of functionally and structurallly identical chemical entities***** by making use of common and biologically effective motif, termed as **pharmacophore********, which is specifically found either in the **corporate** or **commercial** database. Importantly, at every articulated step carried out meticulously in the **intricate discovery phenomenon** one has to heavily depend upon the manipulative skill(s) related to **CADD**, which provides an extra mileage plus meritorious advantage in **data-processing** into several vital and relevant informations for future analysis in **drug design**.

The elaborated and comprehensive '**Flow Chart**' has been dipicted in Figure 3.11 that evidently depicts the *two* above cited processes *viz.*, **rational designing** and **reverse designing** together with the latest well-known ***in silico* techniques** (**in box**) that are employed very commonly in various processes associated with **drug design**.

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*Jorgensen WL., *Science*, **254** : 954, 1991.

Oshiro CM *et al.* : *J Comput Aided Mol Des.*, **9 : 113, 1995.

***Wlodek ST *et al.* : *Protein Sc.*, **7**, 573, 1998.

****Giller VJ and Johnsen AP : In : **Designing Bioactive Molecules : Three Dimensional Techniques and Applications**, Martin YC and Willen P (Eds.) : **AM. Chem. Soc**, Washington DC, 1997.

*****Martin YC, *J. Med Chem.*, **35** : 2145, 1992.

******Schneider G *et al.* : *Angew Chem Int Ed Engl.* **38**, 2894, 1999.

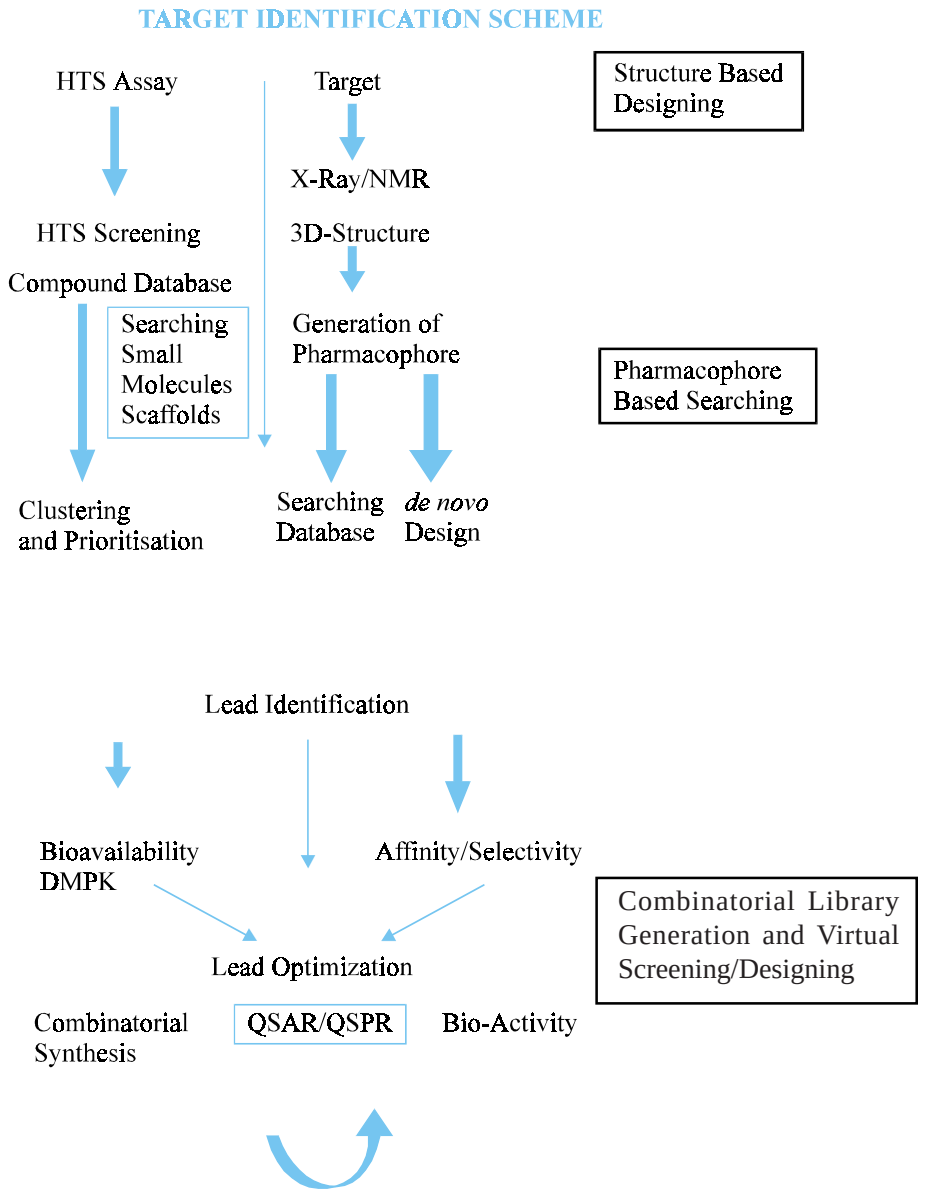


Fig. 3.11. Flow Chart of Rational Designing and Reverse Designing plus *in silico* Techniques Used in Drug Designing Method

[In Silico Techniques are shown in Boxes *viz,* structure-based designing ; Pharmacophore-based designing ; and combinatorial Library generation and virtual screening/designing]

7. CADD METHODS : COMPARISON FOR DETERMINING RELATIVE BINDING AFFINITES OF COX-2 INHIBITORS

Non-steroidal anti-inflammatory drugs (NSAIDs) is known to constitute an altogether chemically diverse class (as shown in the left-hand side box) in Figure : 12, but they all do possess the inherent and qualified capability to inhibit the enzyme **cyclodeoxygenase** (**COX**). Interestingly, the resulting inhibition of **prostaglandin (PG) synthesis** is largely and categorically responsible for their observed therapeutic effects. It is, however, important to state here that the unfortunate inhibition of the prostaglandin (PG) synthesis caused particularly in the '**gastric mucosa**' most abundantly gives rise to **gastrointestinal damage**, thus producing dyspepsia, gastritis, and nausea. **COX** invariably exists in the tissue as a constitutive isoform known as **COX-1** ; however, at particualr sites of inflammation the presence of **cytokins** do stimulate the critical induction of a second isoform (**COX-2**). Therefore, the ultimate inhibition of **COX-2** is strongly believed to be entirely responsible for affording the **anti-inflammatory actions of NSAIDs** ; whereas, the corresponding inhibition of **COX-1** is solely responsible for their respective **gastrointestinal toxicity**.

Recently, certain selective **COX-2 inhibitors** *e.g.*, **Celecoxib**, **Rofecoxib, Parecoxib**, and **Valdecoxib** have been introduced that usually possess identical efficacy to **non-selective COX-inhibitors**, but they are virtually successful in **lowering upto 50%** the incidence of **bleeding**, obstruction, and **gastric perforation** efficaciously*.

Computational assessment of the specific binding affinity of enzyme inhibitors just before the actual synthesis essentially serves as an extremely important CADD paradigms. **Free-energy perturbation technique** is found to be the most accurate and precise means of determining relative binding affinities between the two inhibitors. Keeping in view the inherent complexity togehter with the apparent computation-intensive nature, practical applications are significantly constrained and restricted to the specific analysis of only the structurally-related inhibitors. Besides, there exists an ample legitimate scope for such methodologies which categorically enable rapid and precise assessment of a huge number of distinct structurally-unrelated drug molecules in an accurate manner justifiably.

Thus, one may fairly compare the **free-energy perturbation technique** with the **molecular mechanics methods** in order to have a correct assessment of the various advantages associated with the estimation of the relative binding affinities of **COX-2, inhibitors**.

*Neal MJ : **Medical Pharmacology-At a Glance**, Blackwell Publishing (New Age International Publishers), New Delhi, 5th edn., 2006.

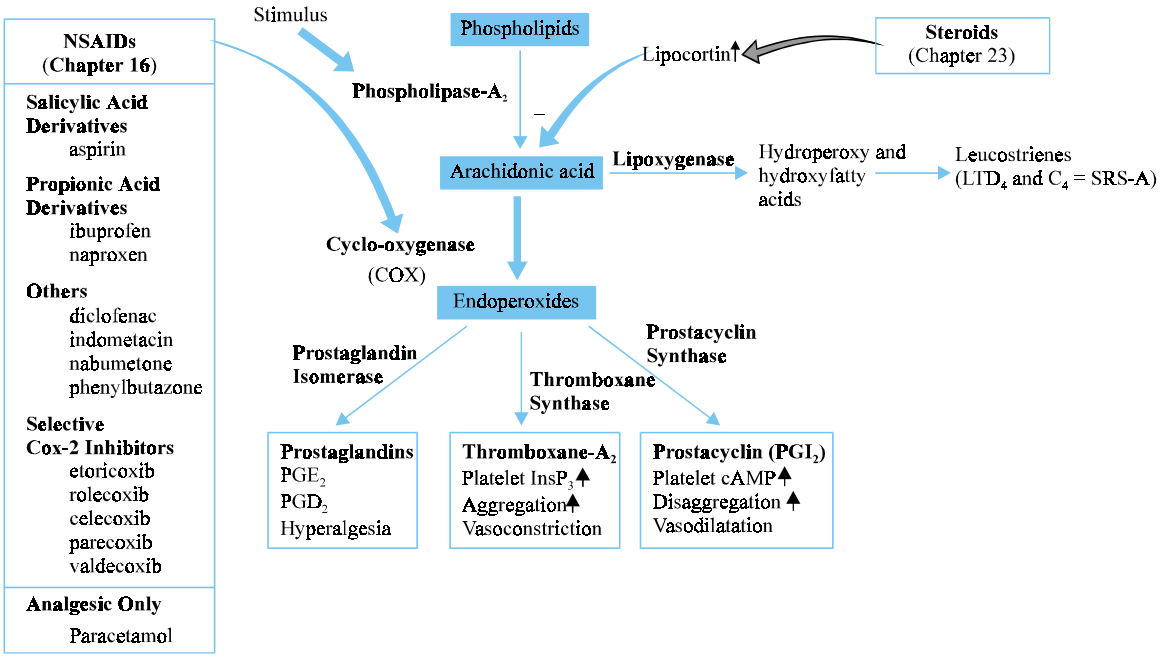
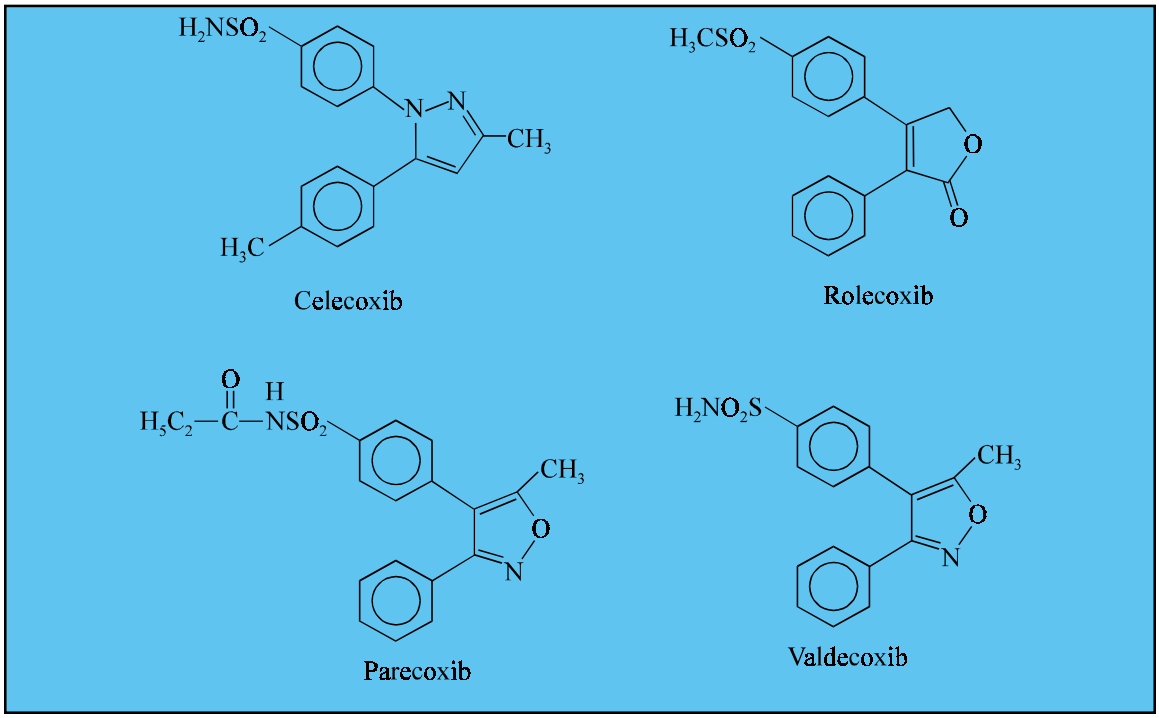


Fig. 3.12. NSAIDs as Selective COX-2, Inhibitors

The structures of *four* potent **COX-2 inhibitors** are given below :



The recent developments* of **COX-2** specific NSAIDs has remarkably reduced and almost eliminated the plethora of side effects intimately associated with the traditional NSAIDs. In fact, several natural products have been duly isolated, purified, and identified as precisely selective **COX-2** inhibitors**. Now, with the availability of **COX-2 based NSAIDs** in the market, it has become rather important to strike a bonafide comparison of CADD methods for determining the relative binding affinities of **COX-2 inhibitors**.

CADD-Strategic Approaches : Kurumbail *et al.**** (1996) employed high-resolution X-ray crystallographic structure of various **COX-2 inhibitors** (**drugs**) with a view to explore the possible interactions of certain potential ligands with the **binding-site residues** and ultimately design a host of newer structural analogues. Two major techniques were duly utilized in the successful design of **COX-2 inhibitors**, namely :

(*a*) Graphical visualization of the '**investigative ligand**' strategically located in the **binding-site cavity**, and

(*b*) Determination (by calculation) of the ensuing **relative** binding affinities employing **specific molecular dynamic simulations** in associaton with the **free-energy perturbation** (FEP) approach.*****

The following Figure 3.13 illustrates a typical '**flowchart**' used by drug-discovery researchers by making uses of a variety of known **CADD approaches**.

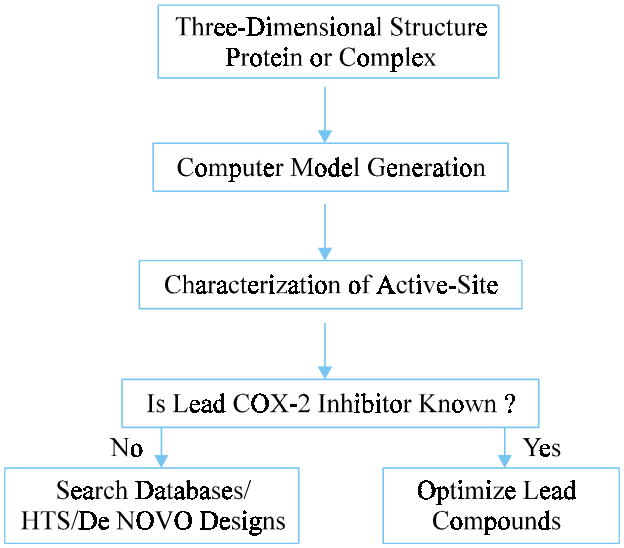


Fig. 3.13. Flowchart for Computer Aided Drug Design [CADD].

*Mardini IA and FitzGerald GA, *Mol. Interr.*, **1** : 30, 2001.

Madhavan Reddy C *et al.* : *Biochem. Biophys. Res. Comun.*, **277 : 599, 2000.

***Kurumbail RG *et al.* : *Nature*, **384** : 644, 1996.

****Reddy MR *et al.* : **Reviews in Computational Chemistry**, Wiley & Sons, New York, 2000.

Method : The various steps involved in the calculation of relative binding affinities of **COX-2 inhibitors** employing **CADD techniques** are :

(1) Generation of a **working computational design** based on the available X-ray crystallographic data.

(2) The aforesaid Step-1 may be conveniently accomplished by adequate development of **molecular mechanies parameters** for :

- non-standard residues,
- assigning protonation states of histidines, and
- orientation of carbonyl ($-\overset{\overset{O}{\|}}{C}-$) and amide ($-\overset{\overset{O}{\|}}{C}-NH_2$) moieties present in aspartate and glutamate residues based solely on adjoining donor/and, or acceptor moieties.

(3) Characterization of the **active-site** is duly substantiated by a host of available and recognized **visualization tools**.

Example : Both **hydrophilic** and **hydrophobic** segments located in the **active-site** are precisely and rapidly identified by determining the '**electrostatic potential**' at various **surface-grid points** or by **graphical analysis**.

(4) Valuable informations obtained *via* graphical analysis of the **active-site** helps formation of **newer lead design**, and **critical optimization of the new lead *via* the analog design**.

Optimization of Lead Compounds : A typical flowchart used for the desired optimization of '**lead compounds**' with **CADD techniques** is depicted in Figure 3.14.

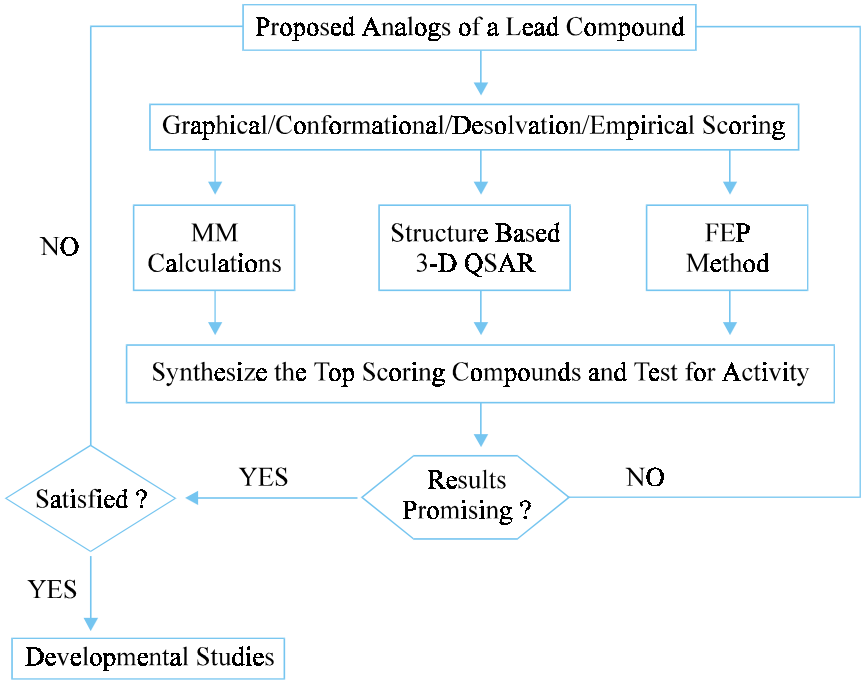


Fig. 3.14. Flowchart for the Optimization of a Lead inhibitor.
[Adapted From : Amaravani M *et al. Ind. J Chem.*, 45A : 174-181, 2006.]

Importantly, one may discard a relatively bigger percentage of the proposed analogues by critically determining their anticipated binding affinities solely dependent upon various **analytical profile**, such as : **docking***, **empirical scoring**, **graphical analysis**, and **conformational analysis**. However, the remaining short-listed and selected **structural analogues** are duly subjected to various methodologies based upon their priorities as follows :

(*a*) **FEP-determinations**.** to provide accurate and precise predictions, but prove to be quite expensive due to exhorbitant computational costs involved,

(*b*) **Molecular Mechanic Determinations***.** to produce relatively faster qualitative predictions.

(*c*) **Regression Methods**.** to induct interaction variants and characteristic features of ligands which ultimately give rise to semi-quantitative predictions ; and thus prove to be comparatively much faster than the corresponding **FEP determinations**.

Importantly, the sequence of FEP and MM techniques are used in the determination of **relative binding affinities** of the **investigative COX-2 inhibitors** meticulously. Obviously, the compounds having the maximum score are duly synthesized and evaluated for the **relative binding affinity profile**. However, it is always advisible to repeat the above process repeatedly, of course, in an interactive manner till one arrives at certain **definite potential drug candidates**. Such '**drug molecules**' having desired biological profile need to be identified for further studies exhaustively.

Thermodynamic Cycle-Perturbation (TCP) Approach*** :** Pearlman *et al.* (2001) advocated the **TCP approach** whereby it would enable one to compute the relative changes associated with **binding free energy**. Importantly, the **TCP method** involves as a necessary consequence the making of **non-physical routes** thereby joining together the anticipated **initial and final states**. In actual practice, the **TCP approach** makes it possible to determine (by calculation) the ensuing relative alterations taking place in *two* observed fronts, namely :

(*a*) **Solvation free-energy.** designated as $\mathbf{\Delta\Delta G_{sol}}$ and

(*b*) **Binding free-energy.** represented as $\mathbf{\Delta\Delta G_{bind}}$

In fact, there two physical parameters essentially occur between two two intimately **related drug molecules**; and, therefore, may be obtained by judicious and careful computationally simulating the '**mutation**' of one molecule to the other. Thus, the **relative observed solvation free-energy ($\Delta\Delta G_{sol}$)** change existing between the two substrates is adequately computed employing the '**solvation cycles**' as illustrated in Figure 3.15, which may be expresed by the following equation :

$$\Delta G_3 - \Delta G_4 = \Delta G_{aq} - \Delta G_{gas} = \Delta\Delta G_{sol} \qquad ...(a)$$

Likewise, the corresponding **relative free-energy of binding (ΔΔbind)** is accomplished by determining the prevailing difference in the *two* distinct affinites, namely :

(*i*) affinity of the **ligand for the protein** ($\mathbf{\Delta G_{com}}$), and

(*ii*) affinity of the **ligand for water** ($\mathbf{\Delta G_{aq}}$).

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*Kurtz ID *at al.* : *Ac. Chem. Res*., **27 :** 117, 1994.

Reddy MR and Erion MD : *J Am Chem Soc*., **123 : 6246, 2001.

***Reddy MR *et al.* : **QSAR in Drug Design**, Vol. 2, Kluwer Academic Publishers, New York, p. 85, 1998.

****Holloway K *et al. J Med Chem*., **38** : 305, 1995.

*****Reddy MR *et al.* : **Reviews in Computational Chemistry**, Wilay & Sons, New York, 2000.

These two aforesaid affinities *viz.*, ΔG_{com} and ΔG_{aq} are computed easily by making use of the '**binding cycles**' as depicted in Figure : 15, which may be expressed by the following equation :

$$\Delta\Delta G_{bind} = \Delta G_{com} - \Delta G_{aq} = - k_{\beta}\, T \ln (k_2/k_1) \qquad ...(b)$$

where, k_1 and k_2 = Experimentally measured binding constants with reference to reactions involving S1 and S2 inhibitors respectively; and having corresponding free energy differences as ΔG_1 and ΔG_2,

k_{β} = Boltzmann Constant,

T = Absolute Temperature.

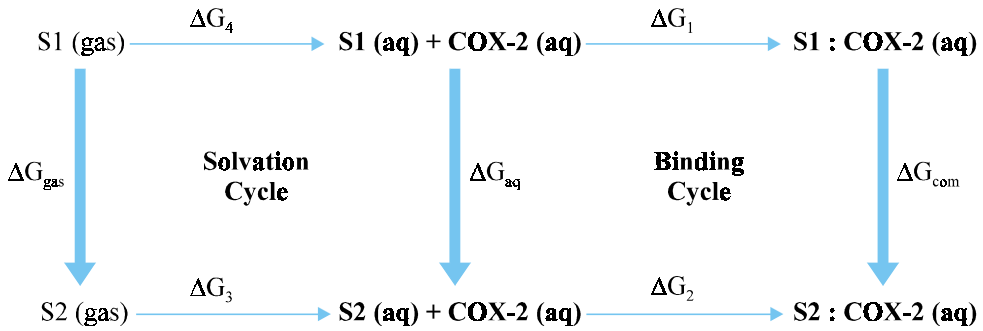

Fig. 3.15. Thermodynamic Cycle-Perturbation (TCP) Approch for PEP Calculations.

From Figure : 15, the actual free enregy change for the conversion of S1 and S2 is meticulously computed by carrying out the perturbation in the Hamiltonian of **reactant state** (*i.e.*, initial state) S1 into that of the **product state** (*i.e.*, final state) S2. In actual practice, such a transformation is invariably achieved *via* articulated parameterization of the variuos terms and conditions that essentially constitute the ensuing **interaction potentials** of the prevailing system. It is also accompanied with a distinct and noticeable **change of state variable** which specifically maps onto the desired reactant and product stales in a situation where the '**variable**' stands at 0 and 1 respectively.

Generalized Conclusions. From a careful comparison between the **experimantal** and **calculated** (*i.e.*, **practical** and **theoretical**) **relative binding affinites** with regard to the structurally identical inhibitors to **COX-2** one may draw the following '**generalized conclusions**' without any reservations, namely :

(1) **Free-energy perturbation (FEP) method** proves to be more accurate and precise but offers *two* serious **limitations**, such as :

(*a*) Inherent relative complexity, and

(*b*) High computation-intensive nature.

(2) Absolute dire need for innovations techniques which may enable quick and judicious assessment of a fairly good number of particular '**structurally-unrelated drug molecules**' in a reasonably precise manner.

(3) Qualitative estimation of the **relative binding free energy differences** between two **specific COX-2 inhibitors** may be accomplished from the various energy components duly determined by carefully carrying out the **molecular mechanics calculations** in both **complex states** as well as **specific solvent**.

(4) Logical improvement and enhanced accuracy in these '**qualitative estimation techniques**' may be obtained with the utmost ease and fervour if one essentially adopts the following steps and directives intimately, such as :

(*a*) Usage of **Quantum Mechanics (QM)** and/or **Molecular Mechanies (MM)** techniques* for the desired and anticipated minimizations,

(*b*) **Variables** that are vital and important for the **binding process** are absolutely necessary, such as : **entropy**,

(*c*) Appreciable **procedural improvement** with respect to '**docking**' and '**scoring**', and

(*d*) **Energy variables** obtained duly *via* average **molecular dynamics simulations**.

It may, however, be added at this point in time that all efforts must be geared before carying out the actual synthesis and biochemical testing procedures meant for newer structural analogues. Sequential application of **molecular mechanics (MM)** based techniques invariably employed for the exclusive **qualitative determination** of relative binding affinities of the **COX-2 enzyme inhibitors** ; and subsequently followed by **free-energy perturbation (FEP)** simulations solely designed for **quantitative determinations** of the comparatively more useful and extremely promising '**drug candidates**'.

Probable Questions for B. Pharm. Examinations

1. (*a*) What do you mean by **Molecular Modeling ? What are two major aspects of** Molecular Modeling ? Explain.

   (*b*) Discuss briefly **Molecular Mechanics** and **Quantum Mechanics** the two methodologies associated with **Molecualr Modeling**.

2. Describe the following aspects with regard to the **Known Receptor Sites** with suitable explanations/examples :

   (*a*) 3D structure of Macromolecular Targets

   (*b*) Structure-Based rug esign

   (*c*) Major Steps in Structure Based Drug Design

   (*d*) Ligand Receptor Recognition

   (*e*) Active Site for a Target Molecule.

3. Give a comprehensice account on the **Characterization of Site** in Molecular Modeling. Give suitable examples to support your answer.

4. What do you understand by **'Design of Ligands'** ? Discuss the following aspects in an elaborated manner :

   (*a*) Visually assisted design

   (*b*) 3D-Database

   (*c*) *De Novo* design.

5. (*a*) Give a brief account on the 'Divide and Rule' Concept in Design of Ligands.

   (*b*) Describe the various methodologies for DOCKING.

---

*Treatment of protein and solvent employing **molecular mechanies (MM)**, and COX-2 inhibitors using **quantum mechanics (QM)**.

6. Discuss the following two categories with regard to **calculation of affinity** :
   (*a*) Binding energetics and comparisons
   (*b*) Multiple binding modes.
7. Write short notes on any **THREE** of the following :
   (*i*) Components of bonding affinity
   (*ii*) Simulations and the thermodynamic cycle
   (*iii*) Ligand-receptor recognition.
8. Write a comprehensive essay on the **Unknown Receptor Sites** with special emphasic upon the following aspects :
   (*a*) Pharmacophore *Vs* bindign site models
   (*b*) Molecular comparisons
   (*c*) Searching for similarity.
9. Give a detailed account on the **'Predictive ADME'**. Expatiate your answer with appropriate explanations/examples.
10. What do you understand by the term **'Reverse Designing'** ? Discuss its various aspects with suitable examples.
11. Exaplain **Computer Aided Drug Design (CADD) methods** *vis-a-vis* determining the relative binding affinities of COX-2 inhibitors.

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4

General Anaesthetics

Chapter

4

General Anaesthetics

1. INTRODUCTION

General anaesthetics are a group of drugs that produce loss of consciousness ; and, therefore, loss of all sensation. The absolute loss of sensation is termed as **anaesthesia** (derived from the Greek word meaning insensitivity or lack of feeling). General anaesthetics bring about descending depression of the central nervous system ; starting with the cerebral cortex, the basal ganglia, the cerebellum and finally the spinal cord.

These drugs are used in surgical operations to induce unconsciousness ; and, therefore, abolish the sensation of pain.

Horace Wells, a Hartford dentist first and foremost demonstrated the usage of nitrous oxide **('laughing gas')** as an effective surgical anaesthetic in 1844. However, its application as a **'general anaesthetic'** resurfaced in mid 1860's when it was dispensed in steel cylinders as an admixture with oxygen. Interestingly, nitrous oxide finds its application even today, particularly in combination with other anaesthetic and analgesic agents.

Later on, William Morton — a Boston dentist demonstrated the anaesthetic actions of diethyl ether in 1846 at the historical **"Ether Dome"** located at the Massachusetts General Hospital. In actual practice, the usage of diethyl ether followed by cyclopropane were withdrawn completely for being highly toxic amalgamated with equally dangerous physical properties, such as : flammable and explosive.

The anaesthetic agents that have gained cognizance today are invariably hydrocarbons and ethers with halogen (F, Br, Cl) substitution.

2. CLASSIFICATION

The **general anaesthetics** may be divided into *three* groups based solely on the method of administration. These are : **inhalation anaesthetics ; intravenous anaesthetics ;** and **basal anaesthetics.**

The above *three* categories of **general anaesthetics** shall be discussed here with appropriate examples.

2.1. Inhalation Anaesthetics

Inhalation anaesthetics could be either volatile liquids or gases and they are administered through inhalation process. As few typical examples are discussed below :

A. **Ether USAN, Anaesthetic Ether BAN,**

$CH_3CH_2—O—CH_2CH_3$

Ethane, 1, 1′-oxybis- ; Ethyl ether ; Diethyl ether ; Sulphuric ether ; U.S.P., B.P., Eur. P., Int. P., Ind. P.,

Synthesis

Method-I (From Alcohol) :

$$C_2H_5OH + H_2SO_4 \longrightarrow C_2H_5HSO_4 + H_2O$$

Ethanol — Ethyl sulphuric acid

$$C_2H_5HSO_4 + C_2H_2OH \longrightarrow (C_2H_5)_2O + H_2SO_4$$

Ether

It may be prepared by the interaction of alcohol with sulphuric acid between 130—137°C commonly termed as the **etherifying temperature.**

Method-II (From Ethylene) :

$$H_2C = CH_2 + H_2SO_4 \longrightarrow C_2H_5HSO_4$$

Ethylene — Ethyl sulphuric acid

$$C_2H_5HSO_4 + C_2H_5OH \longrightarrow (C_2H_5)_2O + H_2SO_4$$

Ether

Ethylene reacts with sulphuric acid to form ethyl sulphuric acid which on subsequent treatment with ethanol results into the formation of **ether**.

It still continues to be employed as an anaesthetic for producing insensitivity to pain in surgical trauma. It has a broad spectrum of usefulness besides its acclaimed potency for very painful surgical conditions and for being relatively benign with regard to the metabolic processes of the body.

Dose : *By inhalation as required.*

B. Ethyl Chloride BAN, USAN,

$C_2H_5—Cl$

Ethane, chloro- ; Chloroethane ; Monochloroethane ; Kelene ; B.P., U.S.P., Int. P., Ind. P., Ethyl Chloride$^{(R)}$ (Bengue' U.K.) ;

Synthesis

$$C_2H_5OH + NaCl + H_2SO_4 \longrightarrow C_2H_5Cl + NaHSO_4 + H_2O$$

Ethanol — Ethyl chloride

Ethyl chloride is prepared conveniently by distilling together a mixture of alcohol, sodium chloride and sulphuric acid.

Used in the past as a general anaesthetic by inhalation, particularly for minor operations, it is also employed as a local anaesthetic by **'freezing'.** *It is no longer used as a general anaesthetic because of its damage to the liver and serious disturbances of the cardiac rhythm.*

Dose : *Topical, as spray on intact skin.*

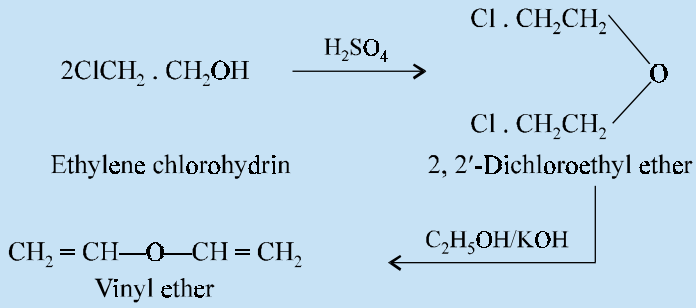
C. Vinyl Ether BAN, USAN,

$CH_2 = CO - O - CH = CH_2$

Ethene, 1, 1′-oxybis- ; Vinyl ether ; Divinyl oxide ; B.P., U.S.P., Int. P., Ind. P.,

Vinesthene(R) (May and Baker) ; Vinydan(R) (Byk Gulden) ;

Synthesis

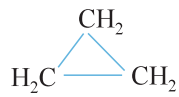


It is prepared by treating ethylene chlorohydrin with sulphuric acid to obtain 2, 2′-dichloroethyl ether, which on subsequent treatment with alkaline ethanol yields **vinyl ether.**

A **volatile anaesthetic** administered by inhalation, it is 4 times as potent as **ether.** Its prolonged inhalation for long operations is rather harmful because of the risk of liver necrosis.

Dose : *By inhalation as required.*

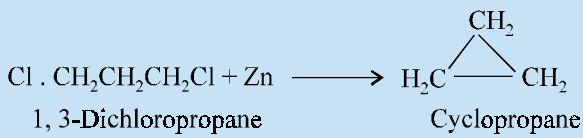
D. Cyclopropane INN, BAN, USAN,



Trimethylene ; B.P., U.S.P., Ind. P.,

Cyclopropane(R) (CI Pharmaceuticals U.K.) ;

Synthesis



It may be prepared by the action of zinc metal (or sodium or magnesium) on 1, 3-dichloropropane.

Cyclopropane is one of the most potent anaesthetics administered by inhalation with the help of a brass regulator. The chief merits of this anaesthetics over others are due to its non-irritant nature and rapid recovery from anaesthesia. Its demerits include : **depressant effects on respiration, tendency to induce cardiac arrhythmias and to enhance haemorrhage.**

Dose : *By inhalation as required.*

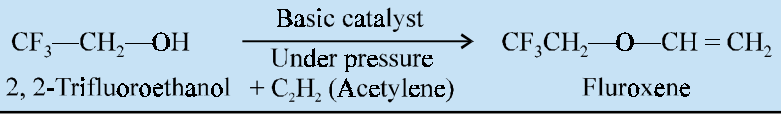
E : Fluroxene INN, USAN,

$CF_3CH_2 — O — CH = CH_2$

2, 2, 2-Trifluoroethyl vinyl ether ; Ethene, (2, 2, 2-trifluoroethoxy)- ; N.F. XIV ;

Fluoromar(R) (Anaquest) ;

Synthesis

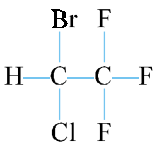


2, 2, 2-Trifluoroethanol undergoes addition to acetylene in the presence of basic catalyst under moderate pressure to yield **fluroxene.**

It is a comparatively highly volatile pleasant-smelling anaesthetic employed most frequently for procedures requiring either the first or upper second plane of anaesthesia, *e.g.*, cardiac, dental, obstetric, orthopedic and certain types of urologic and gynaecologic surgery. Being a good analgesic it brings recovery rapidly. Postoperative nausea and vomiting are uncommon.

Dose : *By inhalation as required.*

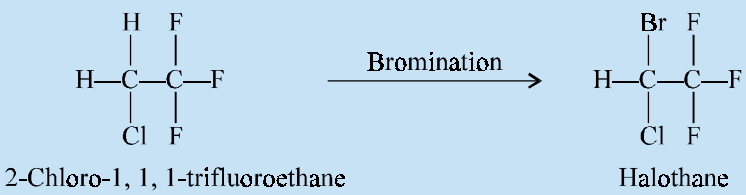
F. Halothane INN, BAN, USAN,



2-Brome-2-chloro-1, 1, 1-trifluoroethane ; Ethane, 2-bromo-2-chloro-1, 1, 1-trifluoro-; B.P., U.S.P., Eur. P.,

Fluothane(R) (Ayerst) ; Halothane(R) (May and Baker) ;

Synthesis



Bromination of 2-chloro-1, 1, 1-trifluoroethane yields halothane which is isolated from the reaction product by fractional distillation.

It is a relatively safe potent volatile anaesthetic administered by inhalation. It is twice as potent as chloroform and 4 times that of ether. It may produce any depth of anaesthesia without causing hypoxia. Being a non-irritant, its inherent hypotensive effect retards capillary bleeding and renders a comparatively bloodless field.

Dose : *By inhalation as required.*

G. Methoxyflurane INN, BAN, USAN,

$CHCl_2CF_2—O—CH_3$

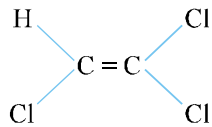
2, 2-Dichloro-1, 1-difluoroethyl methyl ether ; Ethane, 2, 2-dichloro-1, 1, 1-difluoro-1-methoxy-; B.P., B.P.C., U.S.P., N.F. ;

Penthrane(R) (Abbott) ;

It is one of the most potent anaesthetic agents frequently used in practice today. In fact, it is employed to cause comparatively light anaesthesia with deep analgesic and muscle relaxation, features which make it convenient for short surgical operations, *e.g.*, obstetrics.

Dose : *By inhalation as required.*

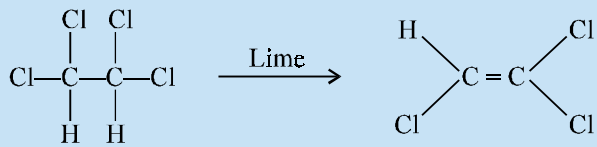
H. Trichloroethylene INN, BAN,



Ethene, trichloro-; B.P., U.S.P. 1970 ; Eur. P., N.F. XIV, Int. P., Ind. P.,

Trilene(R) (Ayerst) ; Trimar(R) (Ohio Medical)

Synthesis



Sym-tetrachloro ethane Trichloroethylene

It may be prepared by the careful abstraction of the elements of hydrogen chloride from *sym*-trichloroethane with the aid of lime.

It may be used sporadically as a weak volatile anaesthetic administered by inhalation. It possesses an excellent analgesic property but is wanting miserably as a muscle relaxant. It is frequently employed in short surgical operations where a mild anaesthesia having a potent analgesia is desired, as in obstetrics.

Dose : *By inhalation as required.*

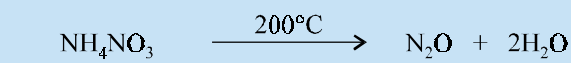
I. Nitrous Oxide BAN, USAN,

N_2O

Nitrogen oxide ; Dinitrogen monoxide ; Laughing gas ; B.P., U.S.P., Eur. P. Int. P., Ind. P.,

Entomox(R) (BOC Medishield U.K.) (N_2O : O_2 : : 1 : 1)

Synthesis



Ammonium nitrate Nitrous oxide

It may be prepared by heating ammonium nitrate up to 200°C.

It is the weakest but the safest inhalation general anaesthetic. It is usually administered in conjunction with other potent inhalation anaesthetics, such as **methoxyflurane** and **halothane**. However,

its anaesthetic regimen may be further broadened by the incorporation of neuromuscular blocking agents whereby the muscle relaxant characteristics are increased to a considerable extent. Some patients often get an attack of hysteria and for this reason it is invariably termed as '**laughing gas**'. It is an inhalation anaesthesia of choice in dental surgery by dint of its ability of the rapid recovery.

Dose : *By inhalation as required.*

J. Chloroform BAN, USAN,

$CHCl_3$

Trichloromethane ; Methane, trichloro-; Chloroformum pro Narcosi ; B.P., N.F., Int. P., Ind. P.,

Synthesis

$CaOCl_2 + H_2O \longrightarrow Ca(OH)_2 + Cl_2$
Bleaching powder — Slaked lime

$C_2H_5 - OH + Cl_2 \longrightarrow CH_3CHO + 2HCl$
Ethanol — Acetaldehyde

$CH_3CHO + Cl_2 \longrightarrow Cl_3C.CHO + 3HCl$
Tri-chloroacetaldehyde

$2Cl_3C.CHO + Ca(OH)_2 \longrightarrow CHCl_3 + (HCOO)_2Ca$
Chloroform

It may be prepared from bleaching powder and ethanol after a series of chemical reactions as shown above.

It is a potent anaesthetic administered by inhalation. It has both reasonably good muscle relaxant and analgesic properties. It is no longer used because of its liver and kidney toxicity.

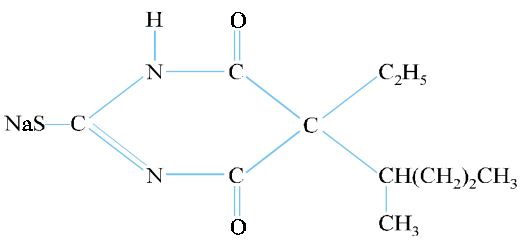
Dose : *By inhalation as required.*

2.2. Intravenous Anaesthetics

Intravenous anaesthetics usually cause unconsciousness when administered parenterally. However, the duration of action can be safely monitored depending on the amount of drug administered.

A few such potent intravenous anaesthetics shall be discussed here.

A. Thiopental Sodium INN, USAN, Thiopentone Sodium BAN,



Sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate ; U.S.P., B.P., Eur. P., Int. P., Ind. P.,
Pentothal Sodium(R) (Abbott) ;

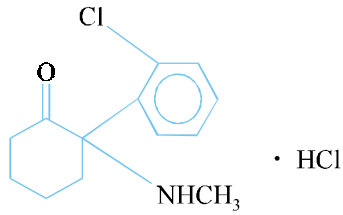
Synthesis

It has been described in the chapter on sedatives and hypnotics.

It belongs to the category of ultra-short-acting barbiturates which are usually administered intravenously for the production of complete anaesthesia of a short duration. It is also used as a basal anaesthesia.

Dose : *100 to 500 mg intravenously.*

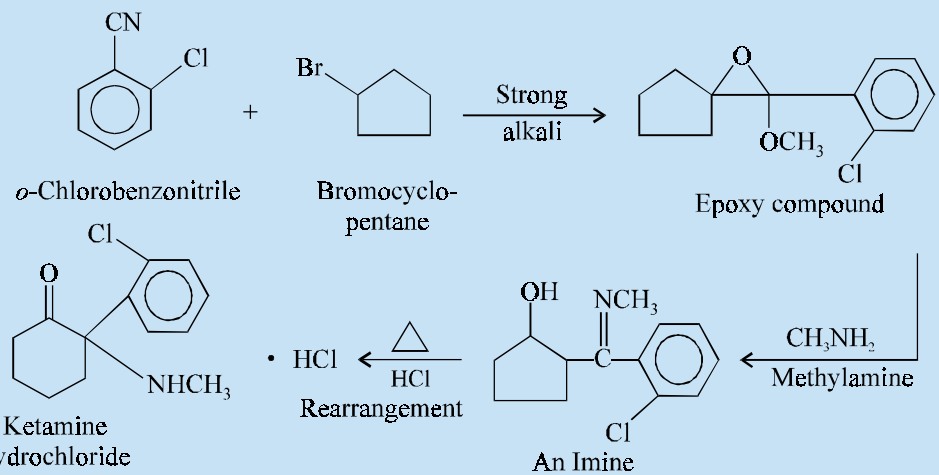
B. Ketamine Hydrochloride INN, BAN, USAN,



(±)-2-(*o*-Chlorophenyl)-2-(methylamino) cyclohexanone hydrochloride ; Cyclohexanone, 2-(2-chlorophenyl)-2-(methylamino)-, hydrochloride ; U.S.P., N.F.

Ketalar(R) (Parke-Davis) ; Ketaject(R) (Bristol).

Synthesis



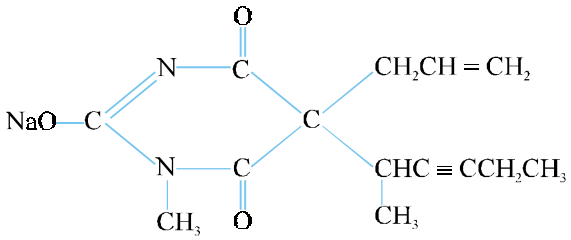
It is prepared first by the interaction of *o*-chlorobenzonitrile and bromo-cyclopentane in the presence of strong alkali to yield an epoxy compound.

Secondly, the resulting epoxy compound on treatment with methylamine forms an imine which undergoes molecular rearrangement upon heating in the presence of hydrochloric acid to yield **ketamine hydrochloride.**

It is a rapid-acting general anaesthetic drug which causes anaesthesia accompanied by deep analgesia, slightly modified skeletal muscle tone and appreciable cardiovascular and respiratory stimulation. Of course, it is an intravenous anaesthetic agent of choice for surgical operations of short duration, but **with additional doses it may effect anaesthesia for a span of 6 hours or even longer.**

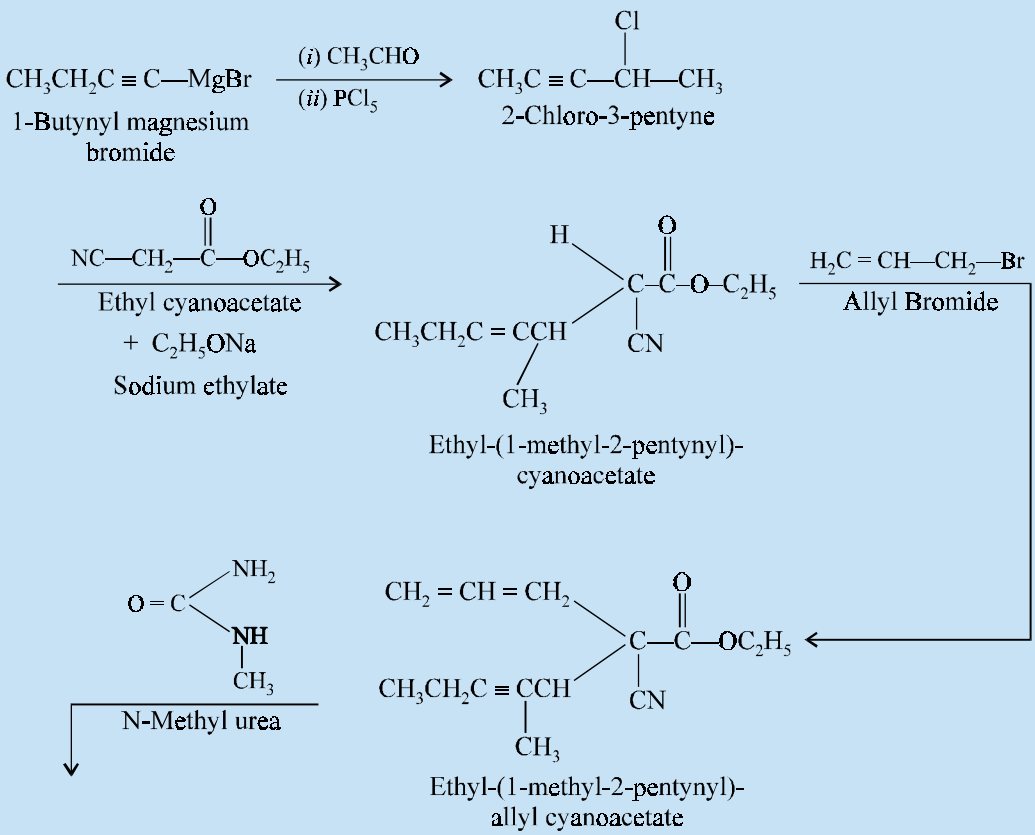
Dose : *Induction, intravenous 1 to 4.5 mg/kg.*

C. Methohexital Sodium USAN, Methohexitone Sodium BAN,



Sodium 5 allyl-1-methyl-5-(1-methyl-2-pentynyl) barbiturate ; 2, 4, 6 (1H, 3H, 5H)-Pyrimidinetrione, 1-methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-, (±)- , monosodium salt ; U.S.P., Brevital Sodium(R) (Lilly).

Synthesis



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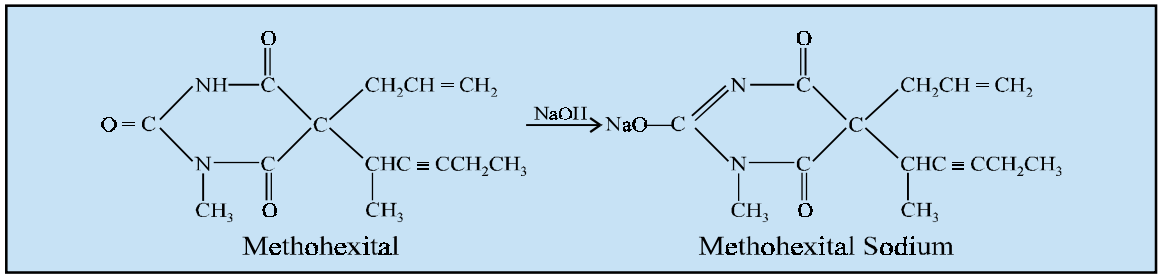
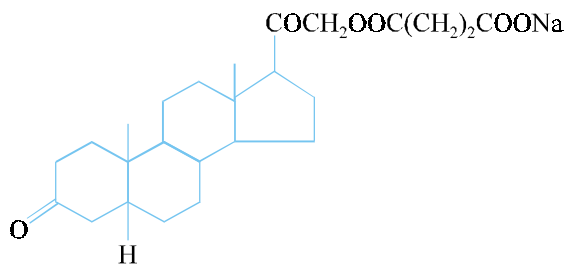


Grignardization of 1-butynyl magnesium bromide with acetaldehyde and subsequent treatment of the resulting alcohol with PCl_5 yields 2-chloro-3-pentyne. Now, ethyl (1-methyl-2-pentynyl)-cyanoacetate is obtained therefrom by its condensation with ethyl cyanoacetate in the presence of sodium ethylate. Further condensation of the resulting product with allyl bromide gives rise to ethyl-(1-methyl-2-pentynyl) allylcyanoacetate. Condensation with N-methyl urea and subsequent neutralization with NaOH produces **methohexital sodium.**

It is used for the induction of anaesthesia through the intravenous administration. It has two advantages over thiopental sodium ; *first*, being its less affinity towards fatty tissues and *secondly*, its greater potency. Its onset of action is quite rapid comparable to thiopental sodium while its recovery is more rapid. For these reasons this intravenous anaesthetic is specifically useful for short surgical operation, such as : oral surgery, gynaecologic investigation, genitourinary procedures and eletroconvulsive therapy.

Dose : *5 to 12ml of 1% solution, at the rate of 1ml every 5 seconds ; usual intravenous administration ; maintenance 2 to 4 ml every 4 to 7 min.*

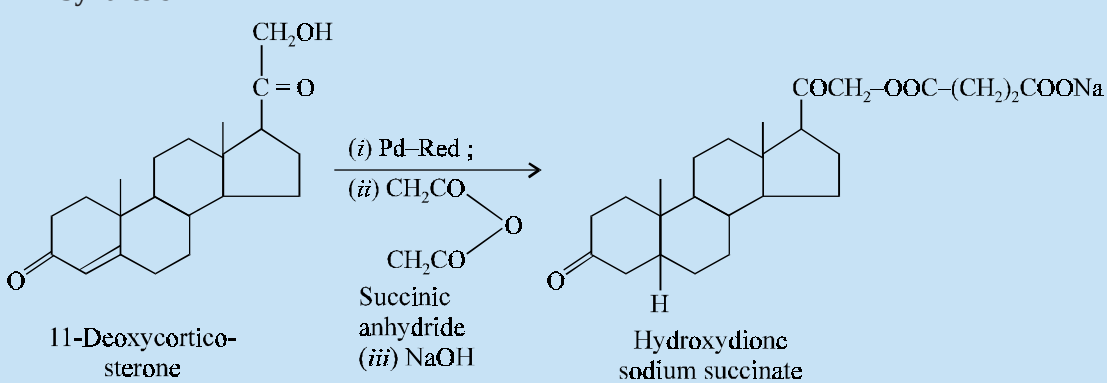
D. Hydroxydione Sodium Succinate INN, BAN,



2l-Hydroxy-5-pregnane-3, 2O-dione-21(sodium succinate) ;

Viadril(R) (Pfizer)

Synthesis

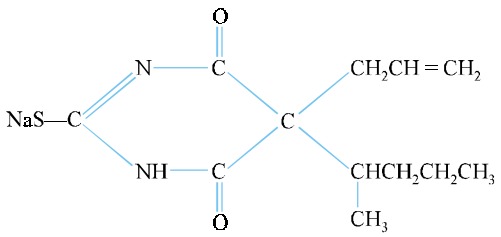


It may be prepared *first* by the reduction of 11-deoxycorticosterone in the presence of palladium, *secondly* by treatment with succinic anhydride and *thirdly* the formation of its sodium salt from sodium hydroxide.

It is a steroidal drug previously administered by intravenous route for the induction of anaesthesia. **Its toxic effects range from causing respiratory depression, hypotension and venous irritation.**

Dose : *0.5 to 1.5g.*

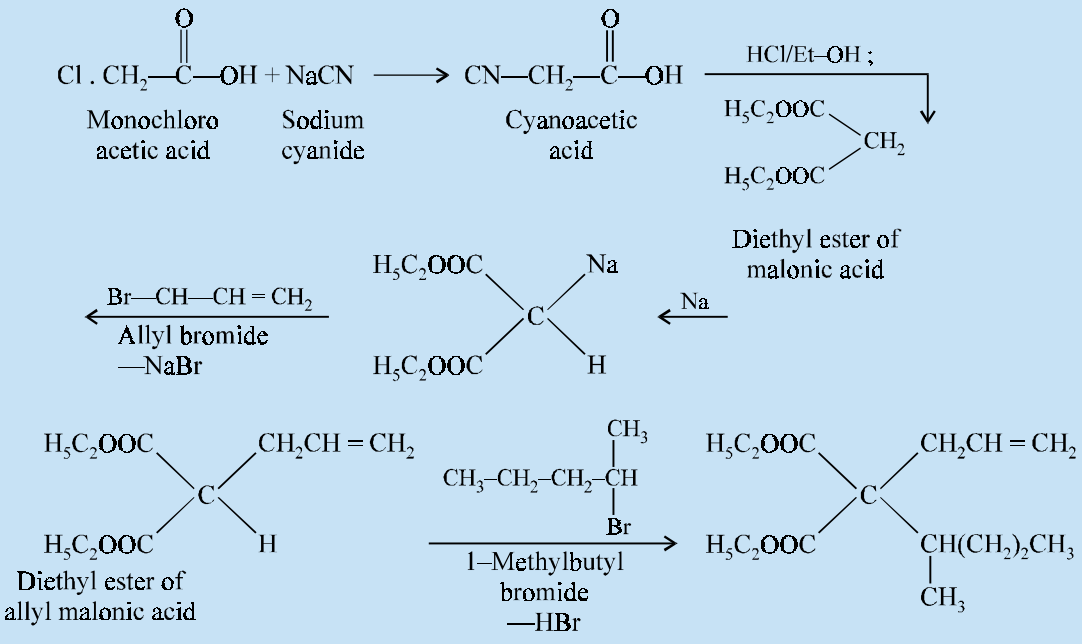
E. Thiamylal Sodium USAN, Sodium Thiamylal BAN,



Sodium 5-allyl-5-(1-methylbutyl)-2-thiobarbiturate ; 4, 6-(1H, 5H)-Pyrimidine-dione, dihydro-5-(1-methylbutyl)-5-(2-propenyl)-2-thioxo-, monosodium salt ; U.S.P.,

Surital(R) (Parke-Davis) ;

Synthesis



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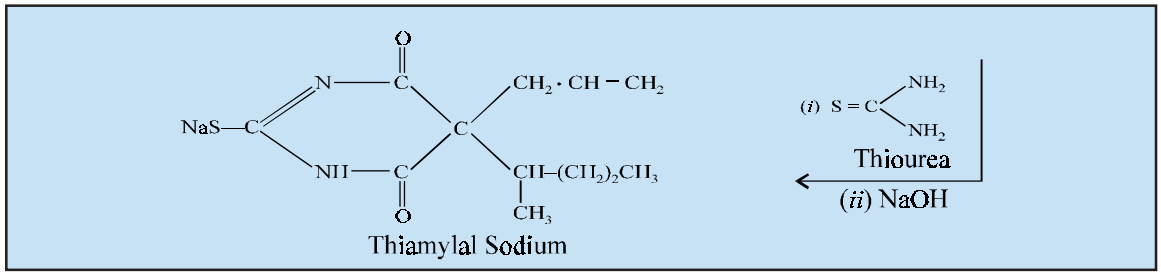


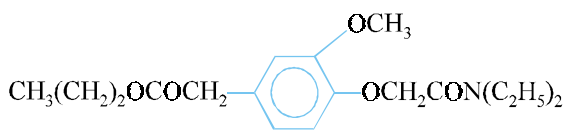
Thiamylal Sodium

The cyanoacetic acid obtained from monochloroacetic acid and sodium cyanide, is treated with hydrochloric acid and ethanol to yield the diethyl ester of malonic acid. The ester, in absolute ethanol, is reacted with the stoichiometric proportion of metallic sodium so as to replace only one active hydrogen of the methylene (CH_2) group. Thereupon, a slight excess of the calculated amount of allyl bromide is added. The second replaceable hydrogen is abstracted with 1-methyl butyl bormide and the resulting product is made to react with a theoretical amount of thiourea to yield thiamylal. The free acid thus obtained is conveniently transformed into the official sodium salt by neutralization with a stoichiometric proportion of sodium hydroxide (1 : 1).

It is an ultra-short acting barbiturate mainly used for intravenous anaesthesia in conditions of comparatively short-duration. It is also effective for the termination of convulsions of unknown origin.

Dose : *Usual, intravenous, 3 to 6 ml of 2.5% solution at the rate of 1 ml every 5 seconds ; maintenance dose being 0.5 to 1 ml as per requirement.*

F. Propanidid INN, BAN, USAN,



Propyl {4-[(diethylcarbamoyl)-methoxy]-3 methoxyphenyl} acetate ; Benzene-acetic acid, 4-[2-(diethylamino)-2-oxoethyl]-3-methoxy-, propyl ester ; B.P.,

Epontol$^{(R)}$ (Bayer) :

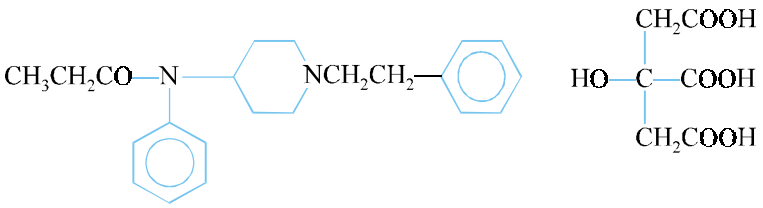
It is an ultra-short acting anaesthetic which is administered intravenously either for producing complete anaesthesia of short duration or for induction of general anaesthesia. It is relatively less potent than thiopentone. It possesses neither analgesic nor muscle relaxant activities.

Dose : *5 to 10 mg per kg body weight, normally administered as a 5% solution.*

2.3. Basal Anaesthetics

Basal anaesthetics are agents which induce a state of unconsciousness but *the depth of unconsciousness is not enough for surgical procedures.* They are often used to induce basal anaesthesia before the administration of inhalation anaesthetics. They are also used for repeated short procedures in children like the changing of painful dressings. **Basal anaesthetics offer three cardinal merit points, namely : devoid of mental distress, pleasant induction and lesser respiratory irritation.** They are often administered through the rectum. Few deserve mention.

A. Fentanyl Citrate INN, BAN, USAN,

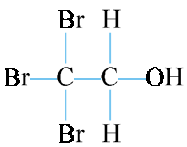


N-(1-Phenethyl-4-piperidyl) propionanilide citrate (1 : 1) ; Propanamide, N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-, 2-hydroxy-1, 2, 3-propanetricarboxylate (1 : 1) ; B.P., U.S.P., Sublimaze(R) (Janssen) ; Innovar(R) (McNeil) ;

It is employed basically as an analgesic for the control of pain associated with all kinds of surgery. It may also be used an an adjunct to all drugs commonly employed for regional and general anaesthesia. It is one of the components in **'Fentanyl citrate and Droperidol Injection'** which is used as premedication for anaesthesia and also as an supplement for induction and maintenance of anaesthesia.

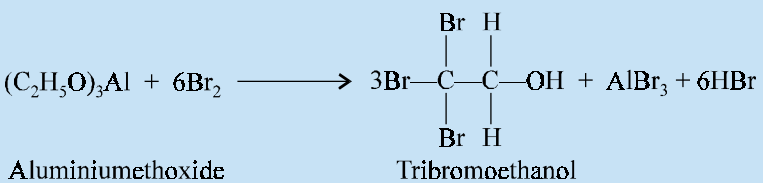
Dose : *Usual, intramuscular, 0.05 to 0.1 mg 30 to 60 minutes before operation.*

B. Tribromoethanol USAN, Tribromoethyl Alcohol BAN,



2, 2, 2-Tribromoethanol ; Tribromoethyl alcohol ; B.P. 1953, Int. P., N.F. XIII ; Avertin(R) (Winthrop) ;

Synthesis

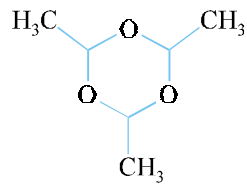


It is prepared by the interaction of a solution of bromine with aluminium ethoxide or preferably aluminium isopropoxide.

It is a basal anaesthetic agent of choice which is administered through rectum in the form of its solution. The main advantage of such an anaesthesia being its pleasant induction amalgamated with lack of irritating vapours.

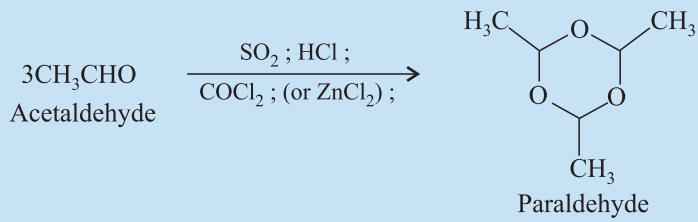
Dose : *Usual, rectal, 60 to 80 mg/kg body weight, not more than 8 g for woman and 10 g for man.*

C. Paraldehyde BAN, USAN,



2, 4, 6-Trimethyl-s-trioxane ; 1, 3, 5-Trioxane, 2, 4, 6-trimethyl-; Paracetaldehyde ;
The trimer of acetaldehyde ; B.P., U.S.P., Eur. P., Ind. P.,
Paral$^{(R)}$ (O'Neal, Jones and Feldman) ;

Synthesis



It may be prepared by treating acetaldehyde with small amounts of sulphur dioxide, hydrochloric acid, zinc chloride or carbonyl chloride, when almost complete conversion is caused. The resulting liquid is freezed and then distilling the crystallized substance under reduced pressure yields the pure **paraldehyde**.

It is one of the oldest and best hypnotics having anti-convulsant effects. It is sometimes used as an obstetrical analgesic, in which situation large doses are administered, usually through rectum.

Dose : *Adult, oral, sedative 5 to 10 ml ; as hypnotic 10 to 30 ml ; Intra-muscular sedative, 5 ml ; hypnotic 10 ml.*

3. MODE OF ACTION OF GENERAL ANAESTHETICS

General anaesthetics have been in use for more than a century, but unfortunately so far no exact mechanism of action has been put forward. Of course, a few theories, namely ; lipid, physical, biochemical, miscellaneous, **Meyer-Overton, minimum alveolar concentration (MAC)**, stereochemical effects and ion-channel and protein receptor theories have been advocated from time to time in support of the mode of action of the general anaesthetics. These will be discussed briefly in this context.

3.1. Lipid Theory

There exists a direct relationship between the anaesthetic activity of an agent and its lipid solubility. It offers a reasonably acceptable correlation between anaesthetic activity (pharmacologic) of a compound and its oil/water or oil/gas partition coefficient (physical). This hypothesis rightly advocates that the site of action of anaesthetics is usually hydrophobic in nature. It may be anticipated that *the greater the lipid solubility of an anaesthetic agent the higher would be its potency.*

3.2. Physical Theory

An increase in the strength of the Van der Waals correlation factors exerts a positive improvement of anaesthetic potency. Likewise, the size of anaesthetic molecules is a determining factor for their ability to reach the site of action. Another school of thought suggests that anaesthesia commences when the critical space within the membrane is charged with the anaesthetic molecules.

3.3. Biochemical Theory

It has been observed that barbiturates normally interfere with the creation of high-energy products by decoupling oxidative phosphorylation. There also exists ample evidence to prove that the cerebral oxygen consumption gets decreased considerably in a human being treated with a variety of anaesthetic agents. This may be expatiated by the fact that **anaesthesia invariably retards the central nervous system activity thereby resulting in a diminished oxygen intake.**

3.4. Miscellaneous Theory

According to one concept the decrease in surface tension caused by an anaesthetic is directly related to its potency. Another possible explanation may suggest a relationship between the anaesthetic action and the function and structure of membrane. However, many of these theories converge to a point indicating that the lipid portion of membranes is the site of anaesthesia.

A good deal of factual information has been accumulated in connection with physical properties together with biochemical and physiological processes of anaesthetic agents, but unfortunately not a single theory proved and substantiated by experimental facts of anaesthesia is known.

3.5. Meyer-Overton Theory

Meyer and Overton put forward that the potency of a drug substance as an anaesthetic exhibited a direct relationship to its ability to attain lipid solubility, or **oil-gas-partition coefficient***. They have studied various *membrane-like lipids*, octanol and olive oil to establish and determine the lipid-soluble properties of the *'volatile anaesthetics'* that existed at that time. However, it has been observed critically the drug substances having reasonably high lipid solubility essentially needed appreciably lower concentrations [*i.e.*, lower **Minimum Alveolar Concentration (MAC)**] to cause anaesthesia. It was suggested further that the possible interaction between the hydrophobic section of the membrane and the anaesthetic molecules afforted an apparent distortion of the former very close to the channels that particularly conducted Na^+ ions. Thus, the membrane has been subjected to **squeeze** and **bloat** in onto the corresponding channel to bring about *two* distinct biological actions, namely :

(*a*) interference with *sodium conductance*, and

(*b*) interference with usual *neuronal depolarization.*

In short, the recent development in the field of **protein : drug interactions** has more or less challenged this theory squarely.

3.6. Minimum Alveolar Concentration (MAC)

MAC may be defined as — **"the concentration at 1 atmosphere of anaesthetic in the alveoli required to produce immobility in 50% of adult patients being subjected to a surgical procedure."** It has been duly observed that a further increase to 30% MAC (*i.e.*, 1.3 MAC) invariably affords apparent *immobility in 99% subjects.*

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* Meyer HH., *J. Am. Med. Assoc.*, 1906, **26 :** 1499–1502 ; Overton E., *Jena : Gustav Fischer*, 1901.

Mechanism : Probably at equilibrium, the prevailing concentration of a volatile anaesthetic in the alveoli is equal to that in the brain ; and consequently this particular concentration in the brain that very intimately exhibits the concentration at the site responsible for the anaesthetic activities. Therefore, the MAC of a volatile anaesthetic is most frequently employed as a reliable **'yardstick'** to ascertain the exact potency of an individual general anaesthetic agent. Table-1 depicts the **MAC values** of several gaseous and volatile anaesthetics commonly put into practice nowadays.

Table 1. MACs Partition Coefficients and Metabolism of Volatile Anaesthetics

Volatile Anaesthetics	MAC (% of 1 ATM)*		Partition Coefficients(At 37°C**)		Metabolism
	Without N_2O	with N_2O(%)	Oil/Gas	Blood/Gas	(%)
Halothane	0.77	0.29 (66)	224	2.3	20
Isoflurane	1.15	0.50 (70)	90.8	1.4	0.17
Sevoflurane	1.71	0.66 (64)	53.4	0.60	4–6
Nitrous oxide	104	—	1.4	0.47	None

Interestingly, when these general (volatile) anaesthetics are employed in *combination*, the MACs for the **Inhaled Anaesthetics are Additive.**

Example : The intensity as well as depth accomplished with 0.5 MAC of **enflurane** together with 0.5 MAC of **nitrous oxide** is almost equivalent to what is produced by 1 MAC of either of the said two agents employed alone. The major advantage of such a combination being that a patient is not exposed to excessive quantum of any one of the individual agents, which in other words drastically minimises the probable risk of **adverse reactions,** if any.

3.7. Stereochemical Effects

It is pertinent to observe here that a number of volatile anaesthetics *viz*., halothane, isoflurane, enflurane and the like essentially contain in each of them an **asymmetric carbon atom** (*i.e.*, a **chiral centre**) ; therefore, may invariably occur both as (+)-or (–)-enantiomers. It has been a common practice to make use of these volatile anaesthetics as their racemates commercially ; however, another school of thought devised a mean to establish and determine the anaesthetic characteristics of individual enantiomers.

Salient Features : The following are some of the **salient features** of such investigations, namely :

(1) Lysco *et al****. (1994) reported that (+)-isoflurane (MAC 1.06%) is approximately 50% more potent as an anaesthetic in the rat than its corresponding (–)-isoflurane compound (MAC 1.62%).

(2) However, in another study by Graf *et al.***** (1994) it was revealed beyond any reasonable doubt that the potency of the individual enantiomers to cause depression of mycardial activity was determined to be almost identical.

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* **MAC** is the minimum alveolar concentration, expressed as volume %, which is essentially needed to cause immobility in 50% of middle-aged human beings.

** Stoelting RK., **'Pharmacology and Physiology of Anaesthetic Practice',** 3rd, edn., Lippincott Williams and Wilkins, Philadelphia, 1999.

*** Lysco GS *et al. Eur. J. Pharmacol.*, **263,** 25-29 (1994).

****Graf BM *et al. Anaesthisiology.*, **81,** 129-136 (1994).

In short, the above findings in (1) and (2) evidently drive out attention to a rather more intricate and complex mechanism responsible for such distinct and apparent variations in their activities ; and that is the **protein-anaesthetic interactions.***

3.8. Ion Channel and Protein Receptor Hypotheses

Importantly, a relatively more recent intensive studies have not only established but also helped in determining the cardinal effects of a host of volatile anaesthetics on a good number of protein receptors very much within the realm of **central nervous system (CNS)**. The various characteristic features which critically and overwhelmingly support the possibility of an important and a vital interaction with a protein essentially include are, namely :

(*a*) Steep dose-response curves observed,

(*b*) Stereochemical requirements of different volatile anaesthetics,

(*c*) Observations with regard to enhanced molecular weight *vis-a-vis* lipid solubility profile of a general anaesthetic may eventually either decrease or negate absolutely the desired anaesthetic activity, and

(*d*) Revelations that the presence of particular **ion channels** and **nerotransmitter receptor** systems are a vital need and basic requirements for a plethora of the noticeable activities of the volatile anaesthetics.

Mechanism : The most **pivotal theme** to explain the actual mechanism of action of volatile (general) anaesthetics logically and legitimately involves the interaction of the anaesthetics with the receptors which critically regulate the performance of the ion-channels, such as : K^+, Cl^- ; or with the ion-channel in a direct fashion (*e.g.*, Na^+).

4. MECHANISM OF ACTION OF GENERAL ANAESTHETICS

The probable mechanism of action of certain **general anaesthetics** dealt with in this chapter are enumerated as under :

4.1. Ethyl Chloride

An extremely volatile liquid with an agreeable pleasant odour. When sprayed on the skin, it evaporates so rapidly that the tissue is *cooled* immediately. By virtue of this characteristic property, the skin gets anaesthetized ; and hence, used in minor surgery for very short durations.

4.2. Vinyl ether

An anaesthetic agent which has become virtually obsolete because it is explosive or highly inflammable in the concentration needed to cause anaesthesia.

4.3. Cyclopropane

It also enjoyed some popularity earlier, but has to be abandoned because of its highly explosive nature just like diethyl ether.

4.4. Fluroxene

It is a fluorinated unsaturated ethereal compound showing a rapid on-set of action by inhalation.

*Sidebotham DA, and Schug SA., *Clin. Exp. Pharmacol. Physiol.,* **24,** 126-130, 1997.

4.5. Halothane

It is a noninflammable, nonexplosive fluorinated volatile anaesthetic which is invariably mixed with either air or oxygen. Importantly, the presence of the C and halogen bonds generously contributes to its noninflammability nature. It was so designed to accomplish certain characteristic properties, namely : (*a*) **chemical stability ;** (*b*) **exert an intermediate blood solubility ;** and (*c*) **appreciable anaesthetic potency.** Nevertheless, it is the only useful general anaesthetic having a bromine atom, which is exclusively responsible for its enhanced potency. Likewise, the presence of three strategically positioned in **halothane** is believed to increase its inherent potency, volatility and chemical stability of the hydrocarbon structure to a considerable extent.

Salient Features :

(*i*) It exhibits a rapid onset of action followed by rapid recovery from the induced anaesthetic effect in *two* different situations ; *first*, when used alone with high potency ; and *secondly*, when used in combination along with nitrous oxide.

(*ii*) A large number of metals, with the exception of chromium (Cr), nickel (Ni) and titanium (Ti), are very quickly tarnished (to lose lustre) by it.

(*iii*) Though it is comparatively stable ; however, it undergoes **spontaneous oxidative decomposition** resulting into the formation of hydrobromic acid (HBr), hydrochloric acid (HCl), and phosgene ($COCl_2$). Therefore, it is specifically dispensed in dark-amber coloured glass containers with the addition of **'thymol'** as a preservative to reduce the chances of oxidation.

(*iv*) It has observed duly that nearly 20% of an administered dosage of **'halothane'** gets metabolized that ultimately is responsible for the enhanced observed hepatotoxicity.

4.6. Methoxyflurane

A **general anaesthetic** usually administered by inhalation for surgical procedures of relatively short duration. Its renal toxicity prevents its being used for prolonged anaesthesia.

4.7. Trichloroethylene

It is mostly used as an analgesic and anaesthetic agent to supplement the action of nitrous oxide. It should not be used with **epinephrine**.

4.8. Nitrous Oxide

The tasteless, odourless and colourless and sweet smelling N_2O **('laughing gas')** having **minimum alveolar concentration (MAC)** value almost exceeding 105% is observed to be **incapable** of inducing surgical anaesthesia if administered alone. It has already been established for N_2O to have an **MAC value** ranging between 105–140% ; and, therefore, is **not** able to succeed in accomplishing in **'surgical anaesthesia'** under conditions prevailing at **standard barometric pressure.** Interestingly, Bert in 1879 demonstrated that an **MAC** more than 100% could be achieved by employing an admixture of 85% N_2O with O_2 at 1.2 atmospheres in a pressurized vessel, which would provide an **MAC** fairly sufficient for causing **'surgical anaesthesia'.**

In fact, N_2O is usually employed alone as an anaesthetic agent during some special localized **'dental procedures'** only. However, most frequently N_2O is utilized with other volatile anaesthetics to cause a sufficient desirable depth of anaesthesia essentially required for various **'surgical procedures'.**

Mechanisms :

Importantly, to date no definite mechanism(s) have been put forward to explain how nitrous oxide exerts its anaesthetic activity.

While some theories have been advocated which probably suggest the **'irreversible oxidation'** of the cobalt atom in vitamin B_{12} by the help of N_2O that may ultimately render the inactivation of certain specific enzymes* dependent on Vit B_{12} having resultant deviations from its normal course.

4.9. Chloroform :

It has been established that the addition of halogens to the hydrocarbon backbone not only enhances potency but also retards flammability to a great extent. **Chloroform** ($CHCl_3$) is a very potent anaesthetic agent having appreciable analgesic and neuromuscular relaxing activity. It is a known **'carcinogen'** and proved to be both **hepatotoxic** and **nephrotoxic ;** besides, causing severe adverse circulatory effects, for instance : **arrythmias** and **hypotension.** Hence, by virtue of its absolutely unacceptable therapeutic index it is no longer used as a volatile anaesthesia.

4.10. Thiopental Sodium

Thiopental sodium belonging to the class of **ultrashort-acting barbiturates** (*e.g.,* **thiopental**) are mostly employed IV to cause a rapid on set of unconsciousness for both surgical and basal anaesthesia. Importantly, it may be used first and foremost to cause anaesthesia, which subsequently should be adequately subtained as well as maintained in the course of a surgical operative procedure with the aid of a general anaesthetic.

Mechanism : Barbiturates, in general, afford a marked decrease in the specific functional activities in the brain. They are found to increase considerably the **GABAergic inhibitory response**, by categorically influencing conductance at the **chloride channel** (just like the **benzodiazepines**). It has been observed that at a relatively higher doses, these may cause potentiation of the existing **$GABA_A$–mediated chloride ion conductance,** thereby strengthening the bondage between **GABA** and **benzodiazepine.**

Another school of thought suggests the following mechanisms of **barbiturates**, such as : (*a*) uncoupling of oxidative phosphorylation ; (*b*) prevention of the electron-transport system ; and (*c*) inhibition of the prevailing cerebral carbonic-anhydrase activity ; occurring all at relatively higher concentrations. Barbiturates are also found to induce liver microsomal enzymes which may invariably lead to an enhanced rate of biotransformation of a host of other commonly employed drugs. They also exert an appreciable affect on the transport of sugars *in vivo*.

4.11. Ketamine hydrochloride

It is an extremely potent fast-acting anaesthetic agent and having comparatively short duration of action (10–25 minutes).

Mechanism :

(*i*) It does not relax skeletal muscles ; and hence, it may be employed safely in such cases of short-duration wherein muscle-relaxation is not needed at all.

(*ii*) Cessation of the acute action is caused mostly due to its redistribution from the brain into other tissues of the body.

(*iii*) It has been observed that a plethora of **'metabolites'** invariably occur on account of the formation of the **glucoronide conjugate** and **metabolism in the liver.**

(*iv*) **Norketamine,** a metabolite is generated *via* the action of **cytochrome P450.** Interestingly, this particular **demethylated structural analogue** does retain an appreciable activity at

*__Methionine synthetase__ and __thymidylate synthetase__ are necessary in the synthetic pathways thereby leading to the production of **myelin** and **thymidine** respectively.

the site of the N-methyl-D-aspartate (NDMA) receptor, which may eventually be responsible for attributing towards the longer duration of action of this anaesthetic drug. Norketamine gets converted to its corresponding hydroxylated metabolites which upon further conjugation form certain metabolites that gets eliminated through the kidney.

(*v*) **Ketamine** is believed to act very much alike the *phencyclidine* **(PCP)** that essentially serves as an antagonist very much within the *cationic channel* of the **NDMA-receptor complex*.** By preventing the flow of cations through the cationic channel, it evidently checks neuronal activation that is usually needed for holding the conscious state.

(*vi*) It is largely able to sustain and produce a **'dissociative'** anaesthesia, that is particularly characterized by electraencephalogram (EEG) alterations thereby showing a marked and pronounced dissociation occurring between the **thermocortical** and **limbic systems**.**

(*vii*) **Ketamine's** analgesic activity could be due to an interaction either with an **opioid receptor** or a **sigma receptor** (which is relatively not-so-well understood).

4.12. Hydroxydione sodium succinate

It is basically a **steroidal drug** used intravenously as an anaesthetic agent. It has, however, no hormonal activity.

4.13. Thiamylal Sodium

Thiamylal is a highly **hydrophobic thiobarbiturate** having its structural features very much related to **thiopental**. Besides, its biological activities are almost identical to **thiopental**. After IV administration, unconsciousness is induced within a span of a few seconds only, while complete recovery of consciousness occurs within 30 minutes. Therefore, it is mostly used effectively in short surgical procedures.

4.14. Fentanyl citrate

Fentanyl citrate is a potent narcotic analgesic with rapid onset and short duration of action when administered parenterally. It shows a profile of pharmacological action quite similar to **morphine**, but with two glaring exceptions, namely : (*a*) does not cause emesis ; and (*b*) releases histamine. After IV administration, the peak analgesia seems to occur within a span of 3–5 minutes and lasts for 30–60 minutes. Interestingly, it is employed primarily as an analgesic for the acute control and management of pain associated with all types of surgery. It also finds its enormous application as a supplement to all such agents that are invariably used either for general and regional anaesthesia.

Advantage : The administration of **'fentanyl'** (*i.e.*, the base) *via* a **transdermal patch** exhibits a much slower onset (8 to 12 hours) and significantly longer duration of action (more than 72 hours) ; and, therefore , quite frequently is employed to manage chronic pain that essentially requires an **'opiate analgesic'.**

4.15. Paraldehyde

It is one of the **'oldest sedatives and hypnotic'** which gets absorbed very quickly after oral administration and helps to induce sleep within 10–15 minutes after a 4-to 8-mL dose. Its application has been resticted in patients with a history of asthma or other pulmonary diseases because it gets

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*Yamamura T *et al. Anesthesiology,* **72** : 704—710 (1990)

Reich DL and Silvay G., *Can. J. Anaesth*, **36 : 186–197 (1989).

partially excreted through the lungs thereby imparting an odour to the exhaled air that produces undesirable irritation.

Mechanism : Paraldehyde mostly gets detoxified by the liver (70 to 80%) and 11 to 28% is excreted by the lungs. However, only a negligible small extent is excreted through the urine.

Probable Questions for B. Pharm. Examinations

1. What is the importance of 'Inhalation Anaesthetics' over 'Intravenous' and 'Basal' Anaesthetics ? How would you synthesize the following :

(*a*) Ethyl Chloride (*b*) Cyclopropane (*c*) Fluroxene (*d*) Halothane (*e*) Trichloroethylene

2. What are intravenous anaesthetics ? Discuss the synthesis of the following :

(*i*) Thiopental Sodium (*ii*) Ketamine Hydrochloride (*iii*) Methahexital Sodium

(*iv*) Hydroxydione sodium succinate (*v*) Thiamylal Sodium.

3. What are the merits and demerits of '*Basal Anaesthetics*' ? Describe the synthesis of the following :

(*i*) Fentanyl citrate (*ii*) Tribromoethanol (*iii*) Paraldehyde.

4. Give a brief account of the following theories put forward to explain the '*mode of action*' of general anaesthetics :

(*a*) Lipid Theory (*b*) Physical Theory (*c*) Biochemical Theory (*d*) Miscellaneous Theory.

5. How would you classify the '*General Anaesthetics*' ? Give the structure, chemical name and uses of one potent drug from each class.

6. Give the synthesis of a '*General Anaesthetic*' having a steroidal nucleus.

7. Name the **three** modified varsions of '*barbiturates*' that are used abundantly as intravenous anaesthetics.

8. Discuss three fluorinated compounds employed mostly as inhalation anaesthetics.

RECOMMENDED READINGS

1. Alex Gringauz, **'Introduction to Medicinal Chemistry',** Wiley-VCH, New York, 1997.

2. DLednicer and LA Mitscher **The Organic Chemistry of Drug Synthesis** John Wiley and Sons New York, (1995).

3. EI Eger II **Anaesthetic Uptake and Action** Williams and Wilkins Co. Baltimore, (1974).

4. Graham L. Patrick, **'An Introduction to Medicinal Chemistry',** Oxford University Press, New York, 2nd, edn, 2002.

5. HG Mautner and HC Clemson **Hypnotics and Sedatives.** In ; M.E. Wolff (*Ed.*) **Burger's Medicinal Chemistry and Drug Discovery** (5th edn), Wiley & Sons. lnc., New York, (1995).

6. LS Goodman and A Gilman **The Pharmacological Basis of Therapeutics** (9th edn) Macmillan Co. London, (1995).

7. RR Macintosh and FB Bannister **Essentials of General Anaesthesia,** Blackwell Scientific Publication, London, (1947).

8. TC Daniels and EC Jorgensen **General Anaesthetics.** In : **Textbook of Organic Medicinal and Pharmaceutical Chemistry** *(Eds)* (CO Wilson), O Gisvold and RF Doerge) JB Lippincott Philadelphia, (1977).

9. VJ Collins **Principles of Anesthesiology**, Philadelphia, Lea and Febiger (1966).

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5

Local Anaesthetics

Chapter

5

Local Anaesthetics

1. INTRODUCTION

Local anaesthetics are drugs which reversibly prevent the generation and the propagation of active potentials in all excitable membranes including nerve fibres by stabilizing the membrane. They achieve this by preventing the transient increase in sodium permeability of the excitable membrane. Generally, small diameter cells are more sensitive to their action than larger diameter cells. Thus conduction in nerve fibres is more readily blocked than conduction in muscle fibres. Also usually **the smaller diameter nerve fibres are more susceptible to the action of local anaesthetics than the larger diameter ones.** For example, on a mixed sensory fibre, local anaesthetic will abolish conduction on the fibre conveying the sensation of pain first. This will be followed by block of the fibre responsible for the sensations of cold, warmth, touch and deep pressure in that order. This is in accordance with the fibre diameter. It is important to mention here that **fibre diameter though very important is not always the only factor that determines the relative sensitivity of the nerve fibres to the action of the local anaesthetics.**

It is, however, pertinent to mention here that both **'local anaesthetics'** and **'general anaesthetics'** essentially afford anaesthesia by blocking nerve conductance in motor neurons as well as sensory neurons. The ultimate blockade of nerve conduction causes not only a *loss of pain sensation* but also affects *impairment of motor functions*. Interestingly, the anaesthesia produced by the **'local anaesthetic** does not necessarily cause complete loss of either consciousness or significant impairment of important central functions. It is, however, believed that **'local anaesthetics'** normally exert their action by blocking nerve conductance after having bondage to selective site(s) of the Na-channels in the particular excitable membranes. In this manner, the passage of Na through the pores gets reduced significantly and hence cause direct interference with the action potentials. Evidently, a local anesthetic helps in drastically minimising the excitability of the nerve membranes with touching the resting potentials. In short, local anaesthetics neither **interact with the pain receptors nor affect the biosynthesis of pain mediators.**

Local anaesthetics are used to abolish the sensation of pain in a restricted area of the body and for minor surgical operations when loss of consciousness is not desirable. The area is determined by the site and the technique of administration of the anaesthetic agent. The main uses are as follows :

(*a*) **Surface or Topical Anaesthesia**

The **local anaesthetic** is applied to the mucous membrane, *.e.g.,* conjunctiva, larynx, throat, damaged skin surface, etc.

(*b*) **Infiltration Anaesthesia**

The **drug** is injected subcutaneously to paralyse the sensory nerve endings around the area to be rendered insensitive, *e.g.*, an area to be incised or for tooth extraction.

(*c*) **Nerve Block Anaesthesia**

The **local anaesthetic** is injected as close as possible to the nerve trunk supplying the specific area to be anaesthetised. This blocks conduction in both sensory and motor fibres and minor operations on the limb are possible.

(*d*) **Spinal Anaesthesia**

The **drug** is injected into the subarachnoid space, *i.e.*, into the cerebrospinal fluid, to paralyse the roots of the spinal nerves. This method is used to induce anaesthesia for abdominal or pelvic surgical operations.

Saddle block is a variation of spinal anaesthesia where the injection is made into the lower part of the subarachnoid space. The **drug** normally settles in the lower part of the dural space. It is used in obstetrics and for surgery in the perineal region.

(*e*) **Epidural Anaesthesia**

This is a special type of nerve block anaesthesia in which the drug is injected into the epidural space. It is technically a more difficult procedure. The roots of the spinal nerves are anaesthetized.

(*f*) **Caudal Anaesthesia**

This is smaller to **epidural anaesthesia** where the injection is made through *sacral hiatus* into the vertebral canal which contains the *cauda equina.* It is used for operations on the pelvic viscera.

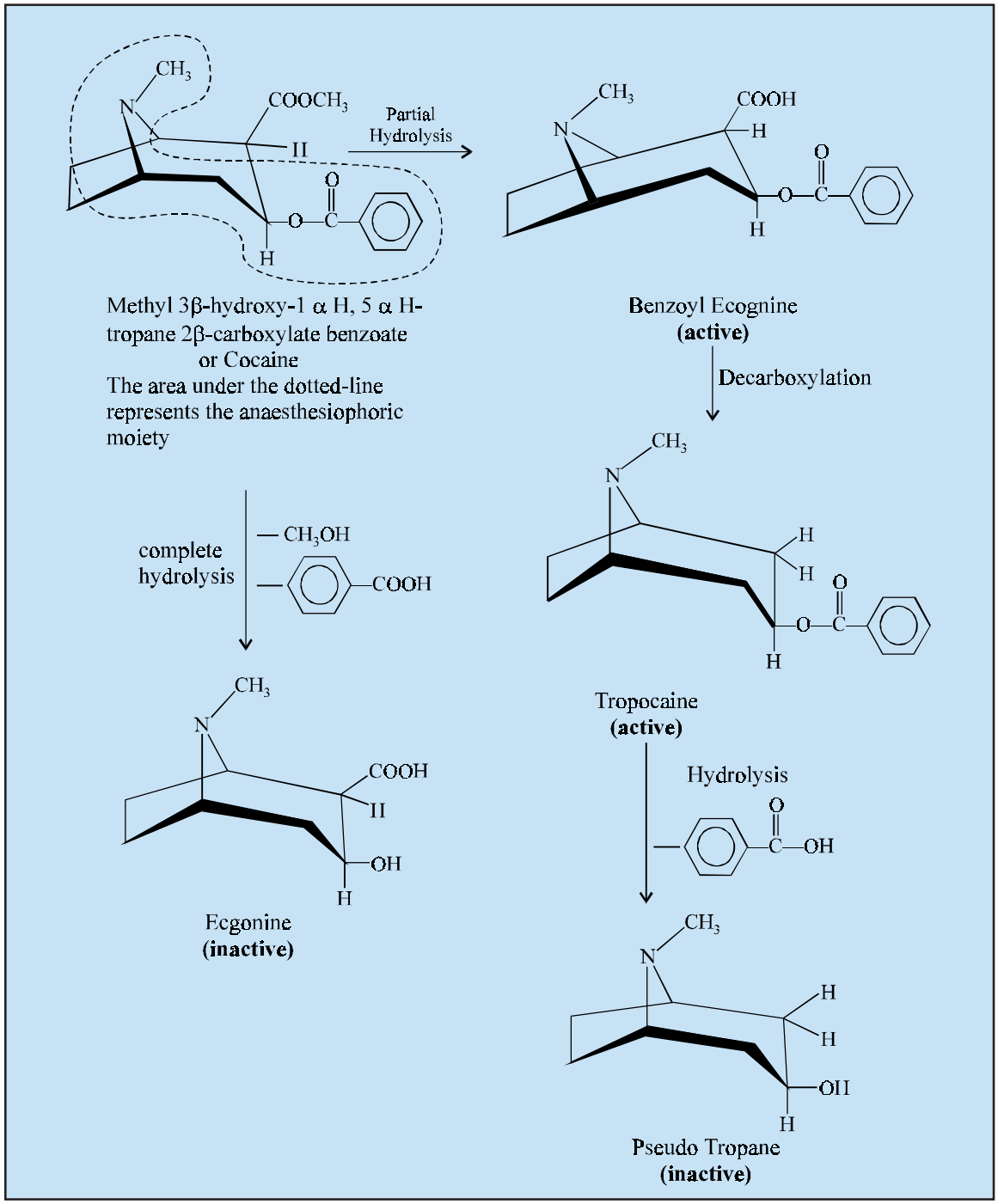
The **first local anaesthetic** to be used was **cocaine** as alkaloid isolated from the leaves of *Erythroxylon coca (Erythroxylum coca).* Carl Koller,[1] an Australian ophthalmologist in 1884, made an epoch making observation that **cocaine hydrochloride** causes anaesthesia in the eye. A follow-up by Willstätter and Müller[2] towards an elaborated elucidation of the structure of cocaine ultimately paved the way to the synthesis of a large number of compounds exhibiting **local anaesthetic** characteristics.

The scheme shown as under, represents the formation of **active benzoyl ecgnine** and **tropocaine** from **cocaine** and **inactive ecgnine** and **pseudo tropane** therefrom.

The portion of the cocaine molecule enclosed by a dotted line in the above scheme represents the **'anaesthesiophoric moiety'** and may be designed by the general structure : Ar—COO—$(CH_2)_n$ NR_1R_2. The next step in the advance of the knowledge of anaesthesia was the recognition that the presence of a basic nitrogen atom in the esterified alcohol was highly desirable. It permitted the formation of the neutral salt, solutions of which could be injected conveniently and it might also influence the anaesthetic activity effectively.

[1]C. Koller, *Wien. Med. Bl. 7,* 1224 (1884) ; C. Koller, *Lancet,* 2, 990 (1884).

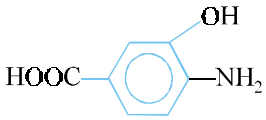
[2]R. Willstatter and W. Muller, *Chem. Ber.* **31,** 2655 (1898).



Einhorn generalised that **all aromatic esters** possess the ability to cause **anaesthesia** and hence he prepared *two* types of esters, namely :

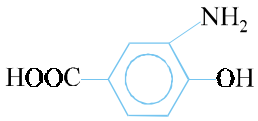
(*a*) **Methyl ester of *p*-amino-*m*-hydroxy benzoic acid**

i.e.,



(*b*) **Methyl ester of *m*-amino-*p*-hydroxy benzoic acid**

i.e.,



This generalization eventually led to the preparation of a large number of analogous esters. It was, however, observed that esters of higher alkyl groups and those with the normal chains are most active. During the period 1904 to 1909, several esters of basic alcohols with benzoic acid were prepared, *viz.*, **amylocaine** (1904), **procaine** (1906) and **orthoform** (1909).

2. CLASSIFICATION

The **local anaesthetics** may be classified on the basis of their **'chemical structures'** as described below :

2.1. The Esters

The earlier observations made by Einhorn really stimulated research towards the synthesis of a number of **benzoic acid esters which exhibited significant local anaesthetic properties**. A few important esters are described below :

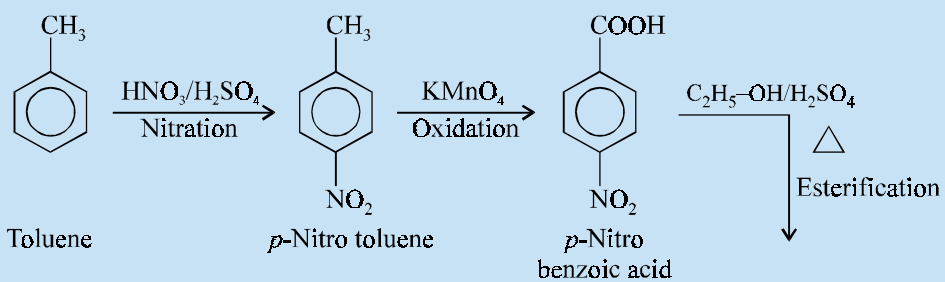
Examples : Ethyl-*p*-amino benzoate ; Butamben ; Orthocaine ; Procaine Hydrochloride ; Tetracaine Hydrochloride ; Butacaine Sulfate ; Cyclomethycaine Sulphate ; Proxymetacaine Hydrochloride ; Propoxycaine Hydrochloride ; Hexylcaine Hydrochloride, etc.

A. Ethyl p-aminobenzoate INN, Benzocaine BAN, USAN,

Benzoic acid, 4-amino-, ethyl ester ; Ethyl Aminobenzoate ; B.P., U.S.P., Eur. P., Int. P., N.F., Ind. P.,

Americaine(R) (American Critical Care) :

Synthesis



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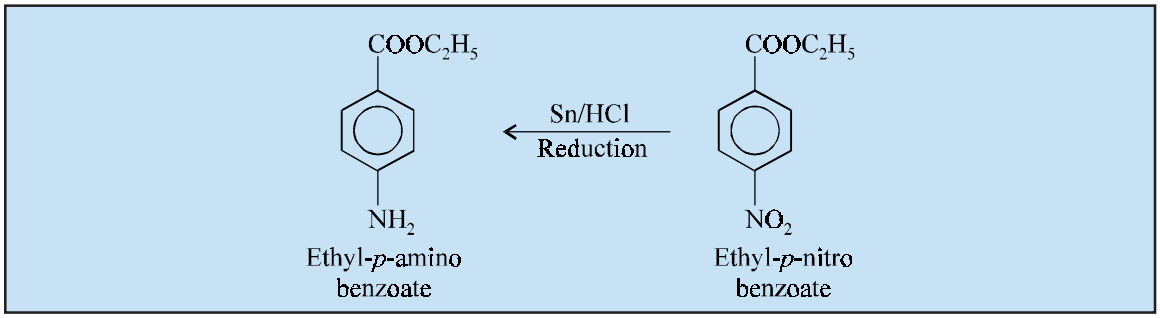
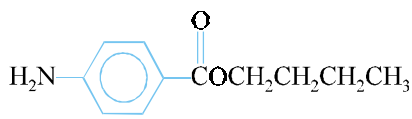


p-Nitrobenzoic acid, may be prepared by nitration of toluene and oxidation of the resulting *p*-nitrotoluene, is esterified to the corresponding ethyl ester by heating with absolute ethanol and a few drops of sulphuric acid. The resulting ethyl *p*-nitrobenzoate is reduced with tin and hydrochloric acid to give the official compound.

Being insoluble it is generally used as an ointment to get rid of the pain caused due to wounds, ulcers and mucous surfaces. Its **anaesthetic action** is usually displayed for the period it remains in contact with the skin or mucosal surface. Hence, it forms an important ingredient in various types of creams, ointments, powders, lozenges and aerosol sprays so as to relieve the pain of naked surfaces and severely inflamed mucous membranes.

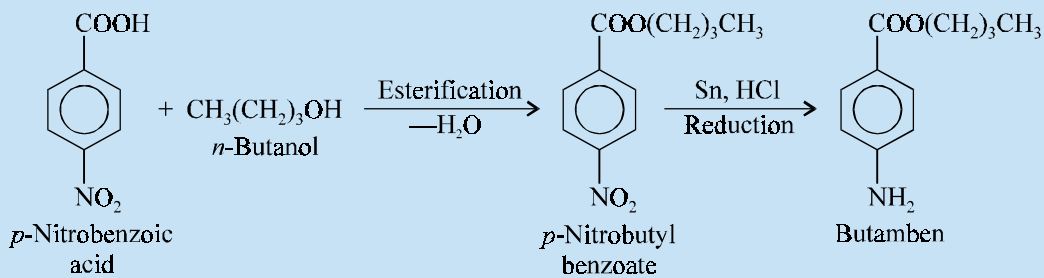
Dose : *Topical, 1 to 20% in ointment, cream, aerosol for skin.*

B. Butamben USAN, Butyl Aminobenzoate BAN,



Butyl *p*-aminobenzoate ; Benzoic acid, 4-amino-, butyl ester ; U.S.P., N.F. XIII ;
Butesin(R) (Abbott).

Synthesis

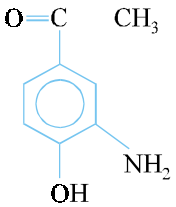


It may be prepared by the esterification of *p*-nitrobenzoic acid with *n*-butanol, then reducing the nitro group with tin and hydrochloric acid.

It is a **local anaesthetic** of relatively low solubility and used in a similar manner to benzocaine. It has been reported to be more efficacious than its corresponding ethyl ester when applied to intact mucous membranes.

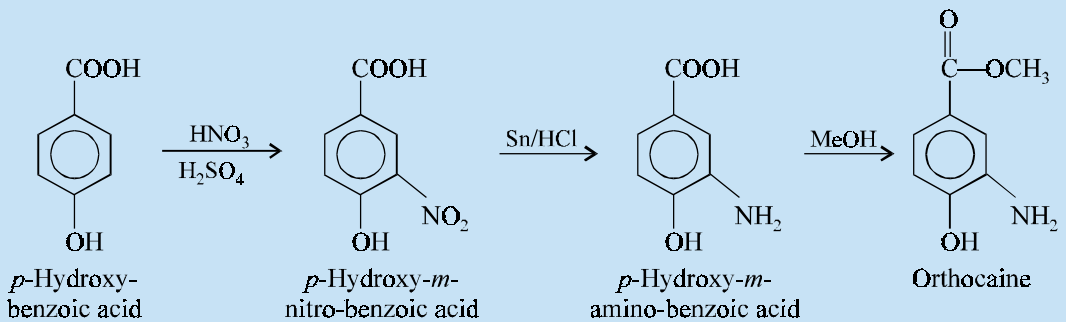
Dose : *Topical, 1 to 2% in conjunction with other local anaesthetics in creams, ointments, sprays and suppositories.*

C. Orthocaine BAN,



Methyl 3-amino-4-hydroxybenzoate ; B.P. 1953

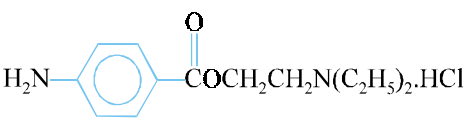
Synthesis



p-Hydroxy-*m*-aminobenzoic acid is obtained by the nitration and reduction of *p*-Hydroxy benzoic acid, which on esterification with methanol yields **orthocaine**.

It is used for surface anaesthesia, but now it is obsolete due to its *irritation and necrosis effects.*

D. Procaine Hydrochloride BAN, USAN,



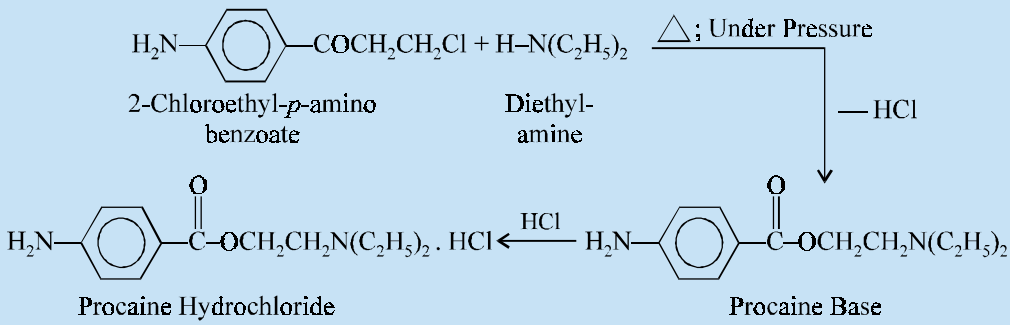
2-(Diethylamino) ethyl-*p*-aminobenzoate hydrochloride ; Benzoic acid, 4-amino-, 2-(diethylamino) ethyl ester, monohydrochloride ; Ethocaine hydrochloride ; Allocaine ; Syncaine ; B.P. U.S.P., Eur. P. Int. P., Ind. P.,

Novocaine(R) (Sterling) ;

Synthesis

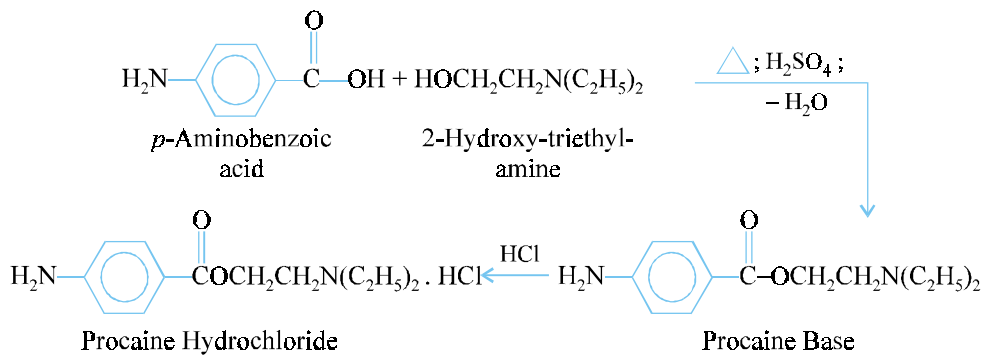
It may be prepared by any one of the following *three* methods :

Method-1 : From 2-Chloroethyl p-amino benzoate :



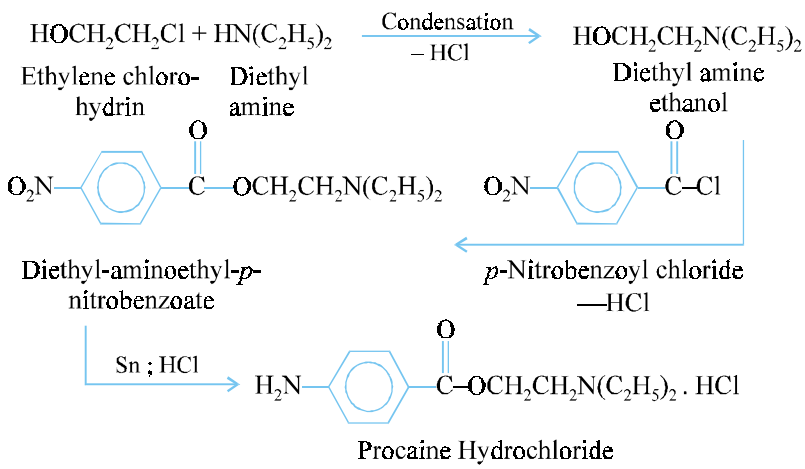
Procaine base may be prepared by the interaction of 2-chloro ethyl-*p*-amino benzoate and diethylamine at an elevated temperature under pressure. The base is converted into its hydrochloride subsequently.

Method-II : From p-Aminobenzoic Acid :



The **procaine** base is obtained by the dehydration of molecule of *p*-amino benzoic acid and 2-hydroxy triethyl amine, which on treatment with hydrochloric acid yields the official compound.

Method-III : From Ethylene Chlorohydrin :

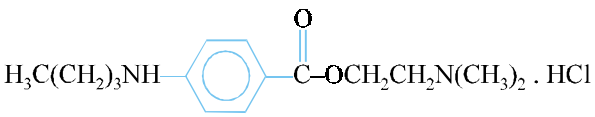


Condensation of a molecule each of ethylene chlorohydrin and diethyl amine yields diethyl amino ethanol, which on treatment with a mole of *p*-nitrobenzoyl chloride gives rise to diethyl amino ethyl-*p*-nitrobenzoate. This on reduction with tin and hydrochloric acid yields the procaine hydrochloride.

It is one of the least toxic and most commonly used local anaesthetics. The salient features for its wide popularity may be attributed due to its lack of local irritation, minimal systemic toxicity, longer duration of action, and low cost. It can be effectively used for causing anaesthesia by infiltration, nerve block, epidural block or spinal anaesthesia. In usual practice it is used in a solution containing adrenaline (1:50,000) which exerts and modifies the local anaesthetic activity through retarded absorption, and the duration of action is considerably prolonged.

Dose : *Usual, infiltration, 50 ml of a 0.5% solution ; usual, peripheral nerve block, 25 ml of a 1 or 2% solution ; usual, epidural, 25 ml of a 1.5% solution.*

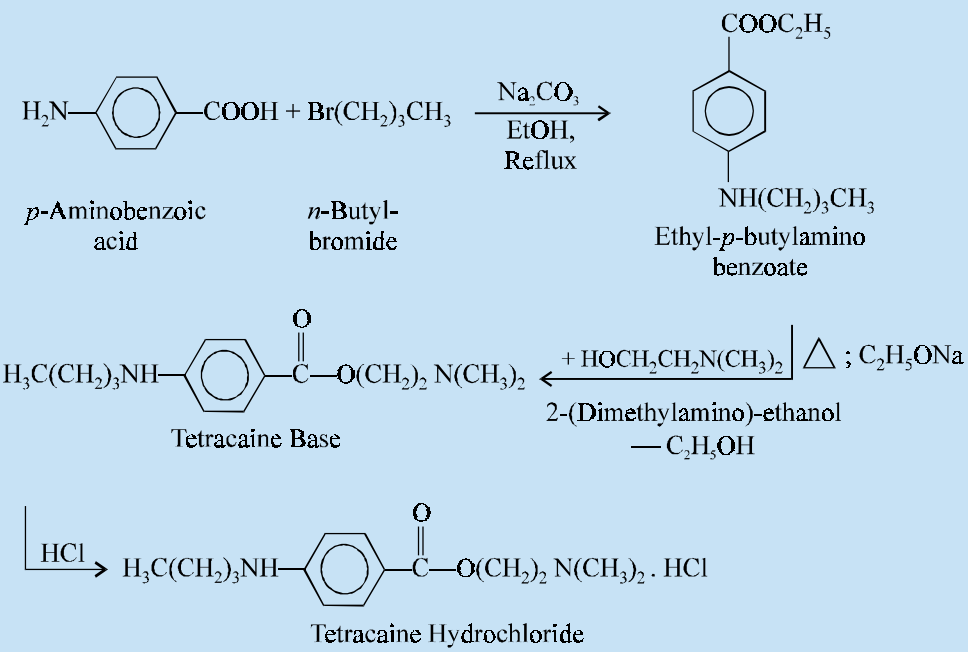
E. Tetracaine Hydrochloride INN, USAN, Amethocaine Hydrochloride BAN,



2-(Dimethylamino)-ethyl-*p*-(butylamino) benzoate monohydrochloride ; Benzoic acid, 4-(butylamino)-, 2-(dimethylamino) ethyl ester, monohydrochloride ; Tetracaine Hydrochloride U.S.P., Eur. P., Amethocaine Hydrochloride B.P., Int. P., Ind. P.,

Pontocaine Hydrochloride(R) (Sterling) ; Anethaine(R) (Farley, U.K.) ;

Synthesis

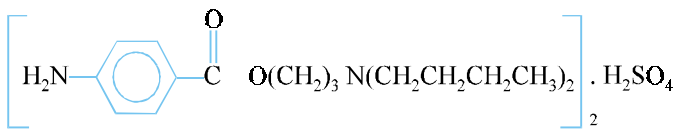


Butylation of *p*-aminobenzoic acid with *n*-butyl bromide under reflux in ethanolic solution and in the presence of sodium carbonate yields ethyl-*p*-butylamino benzoate. This is then caused to undergo transesterification by heating a solution of it in 2-(dimethylamino) ethanol in the presence of sodium ethoxide in such a manner that the liberated ethanol is continuously removed from the reaction mixture by distillation. The tetracaine base is dissolved in benzene and hydrogen chloride is passed through the solution to obtain the corresponding monohydrochloride salt.

It is an **all-purpose local anaesthetic drug** used frequently in surface infiltration, block, caudal and spinal anaesthesia. It is reported to be 10 times more toxic and potent than procaine, whereas its duration of action is twice than that of procaine.

Dose : *Usual, subarachnoid 0.5 to 2 ml as a 0.5% solution ; topically, 0.1 ml of a 0.5% solution to the conjunctiva.*

F. Butacaine Sulfate USAN, Butacaine Sulphate BAN, Butacaine INN,



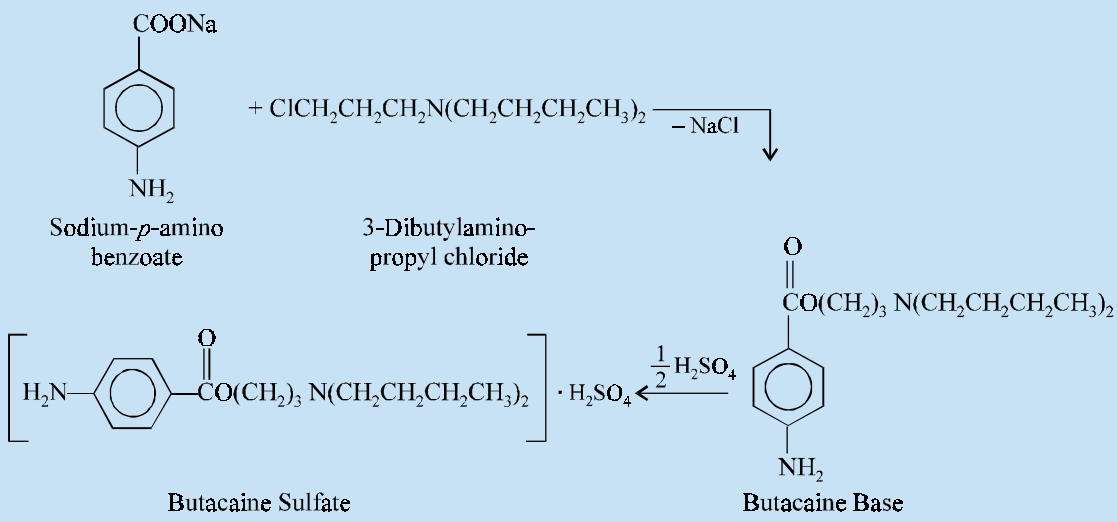
3-(Dibutylamino)-1-propanol-*p*-aminobenzoate (ester) sulfate (2 : 1) ; 1-Propanol, 3-(dibutylamino)-, 4-amino benzoate (ester) sulfate (salt) (2 : 1) ; U.S.P., B.P.C. 1968, Ind. P.,

Butyn Sulphate(R) (Abbott)

Synthesis

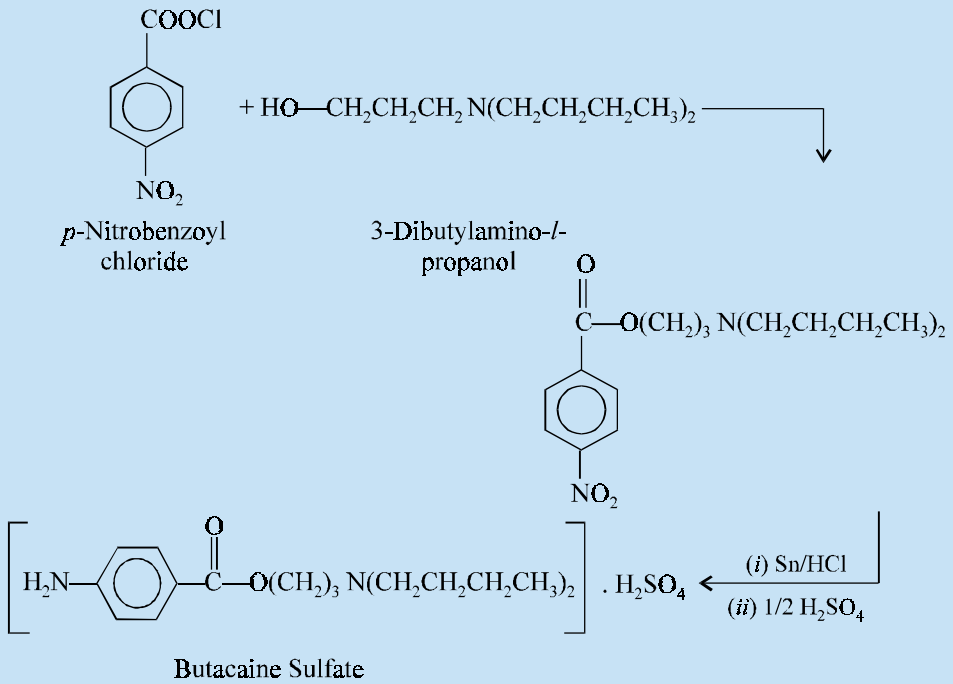
It may be prepared from either of the following *two* methods :

Method-I : From 3-Dibutylaminopropyl chloride :



Butacaine base may be prepared by the interaction of a mole each of 3-dibutylaminopropyl chloride with sodium *p*-amino benzoate, which is then treated with a half-molar quantity of sulphuric acid to obtain the official compound.

Method-II : From 3-(Dibutylamino)-1-Propanol :

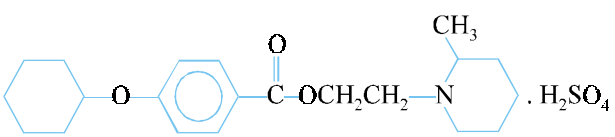


3-(Dibutylamino)-1-propanol is coupled with *p*-nitrobenzoyl chloride and the corresponding nitro group is subsequently reduced to amino by treatment with tin and hydrochloric acid. The resulting **butacaine base** is made to react with a half-molar quantity of sulphuric acid.

It is a surface anaesthetic having effects similar to those of cocaine, but it exhibits more *rapid onset of action followed by a prolonged action.*

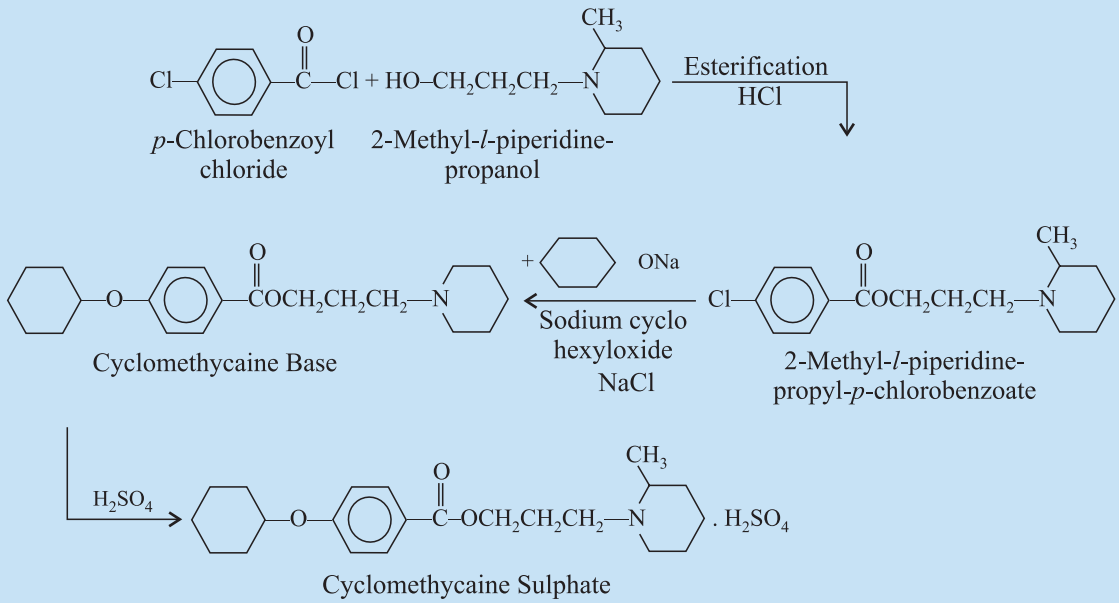
Dose : *Several instillations of a 2% solution about 3 minutes apart allow most surgical procedures.*

G. Cyclomethycaine Sulphate BAN, Cyclomethycaine Sulfate USAN, Cyclomethycaine INN,



3-(2-Methylpiperidino)-propyl *p*-(cyclohexyloxy) benzoate sulfate (1 : 1) ; Benzoic acid, 4-(cyclohexyloxy)- , 3-(2-methyl-1-piperidinyl) propyl ester sulfate (1 : 1) ; B.P., U.S.P., N.F., Surfacaine(R) (Lilly)

Synthesis

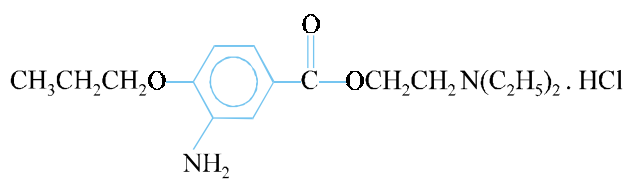


p-Chlorobenzoyl chloride undergoes esterification with 2-methyl-1-piperidinepropanol with the elimination of a mole of hydrogen chloride. This on treatment with sodium cyclohexyl oxide yields the **cyclomethycaine base** which on further treatment with sulphuric acid gives the official compound.

It is extensively used as an effective topical anaesthetic in thermal and chemical burns ; in dermatological lesions, sunburn and skin abrasions ; in urology, gynaecology, obstetrics and anaesthetic procedures.

Dose : *Topical, 0.25 to 1.0% in suitable form.*

H. Proxymetacaine Hydrochloride BAN, Proxymetacaine INN, Proparacaine Hydrochloride USAN,

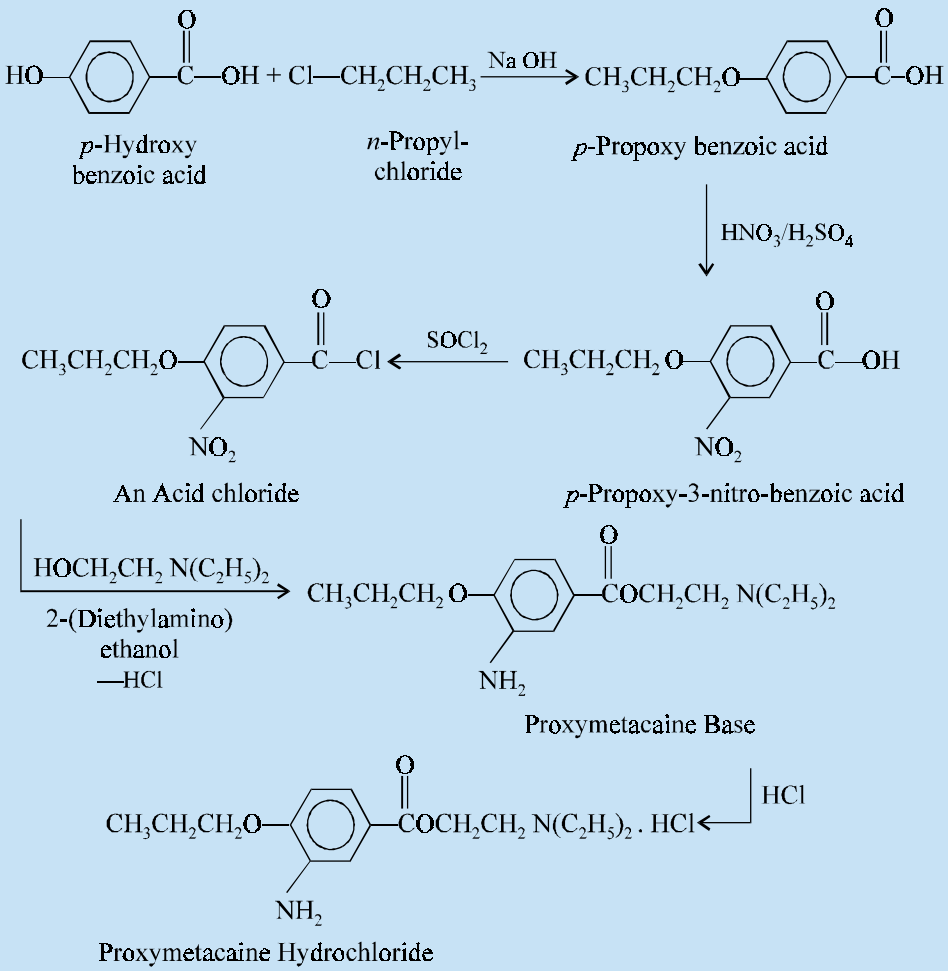


2-(Diethylamino) ethyl 3-amino-4-propoxybenzoate monohydrochloride ; Benzoic acid, 3-amino-4-propoxy-, 2-(diethylamino)-ethyl ester, monohydrochloride ;

Proxymetacaine Hydrochloride B.P.C. (1973) ; Proparacaine Hydrochloride U.S.P.

Alcaine(R) (Alcon) ; Ophthaine(R) (Squibb) ; Ophthetic(R) (Allergan)

Synthesis

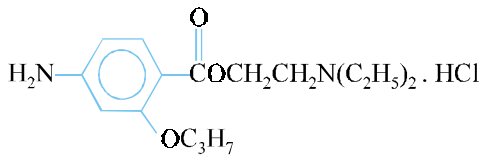


p-Propoxybenzoic acid is obtained by the interaction of *p*-hydroxy benzoic acid and *n*-propyl chloride in an alkaline medium, which on nitration yields the corresponding 3-nitro analogue. Subsequent treatment with thionyl chloride yields an acid chloride which is then coupled with 2-(diethylamino) ethanol yields the **proxymetacaine base**. This on reaction with an equimolar quantity of HCl gives the official compound.

It is a **potent surface anaesthetic** mainly used in ophthalmology and induces no initial irritation. Because of its rapid onset of action it is useful for most occular procedures that require topical anaesthesia such as tonometry, removal of foreign particles, gonioscopy and various short operative procedures which may involve the conjunctiva and cornea. It has also been reported to be employed frequently as a surface anaesthesia in glaucoma surgery and in cataract operations.

Dose : *Topical, 0.05 ml of a 0.5% solution to the conjunctiva.*

I. **Propoxycaine Hydrochloride BAN**, **USAN**, **Propoxycaine INN**,



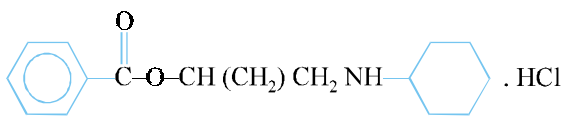
2-(Diethylamino) ethyl 4-amino-2-propoxybenzoate monohydrochloride ; Benzoic acid, 4-amino-2-propoxy-, 2-(diethylamino) ethyl ester, monohydrochloride ; U.S.P., N.F.,

Blockain(R) (Breon) ; Ravocaine Hydrochloride(R) (Cook Waite)

Its **local anaesthetic potency is reported to be 7 or 8 times more than that of procaine**. It is mainly used for infiltration and nerve block anaesthesia.

Dose : *Usual, 2 to 5 ml of a 0.5% solution.*

J. **Hexylcaine Hydrochloride USAN**, **Hexylcaine INN**,



1-(Cyclohexylamino)-2-propanol benzoate (ester) hydrochloride ; 2-Propanol, 1-(cyclohexylamino)-, benzoate (ester), hydrochloride ; U.S.P., N.F.,

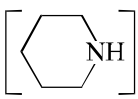
Cyclaine(R) (MSD)

It is regarded as an **all-purpose soluble local anaesthetic agent**. The onset and duration of action is almost similar to that of lignocaine.

Dose : *For infiltration anaesthesia 1% ; for nerve block anaesthesia 1 and 2% solution ; and for topical application to skin and mucous membranes 1 to 5%.*

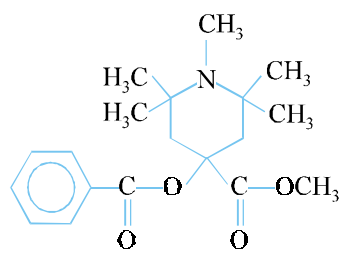
2.2. Piperidine or Tropane Derivatives

The members of this particular group of compounds essentially contain the piperidine nucleus



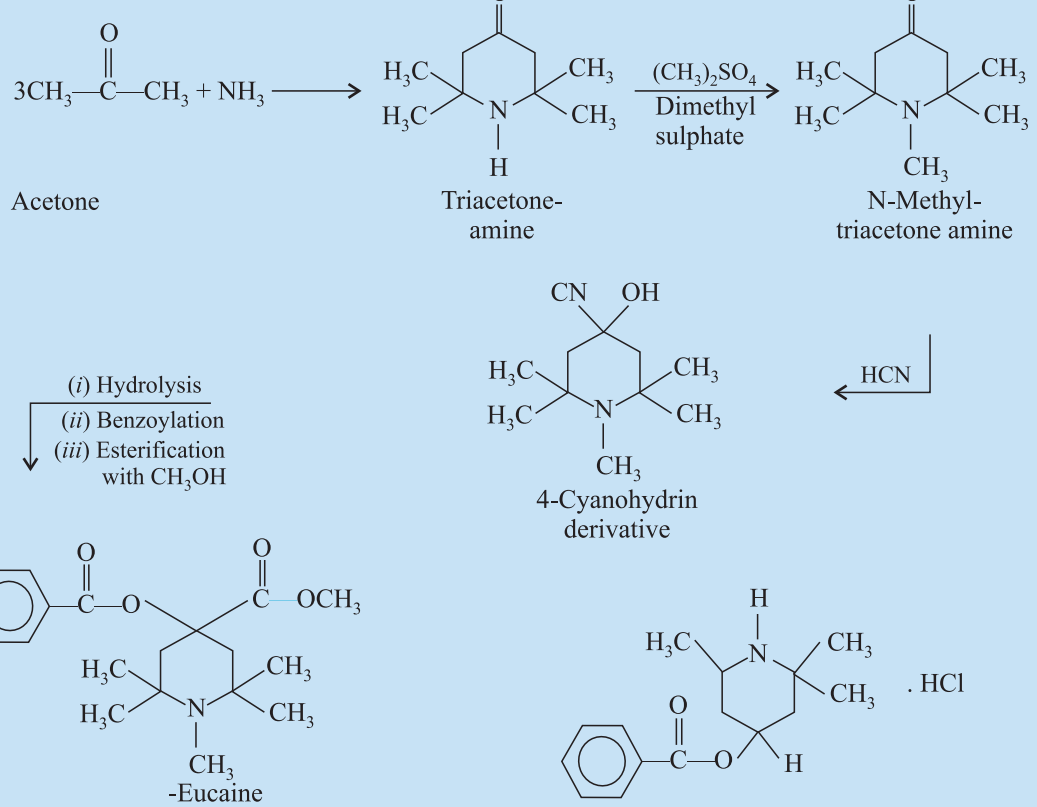
. **Examples : α-Eucaine ; Benzamine Hydrochloride ; Euphthalmin**

A. α-Eucaine



2, 2, 6, 6-Tetramethyl-4-benzoxy-4-methyl carboxylate-N-methyl piperidine.

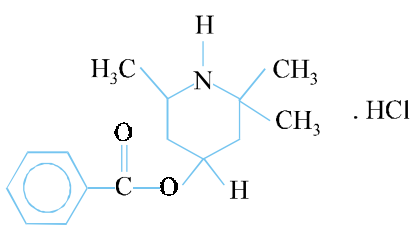
Synthesis



Triacetoneamine is first prepared by the condensation of three moles of acetone with one mole of ammonia. This on methylation with dimethyl sulphate yields the corresponding N-methyl triacetoneamine which on treatment with hydrocyanic acid gives cyanohydrin analogue. Finally, when the resulting product is subjected to hydrolysis, followed by benzoylation and esterification with methanol yields **α-eucaine.**

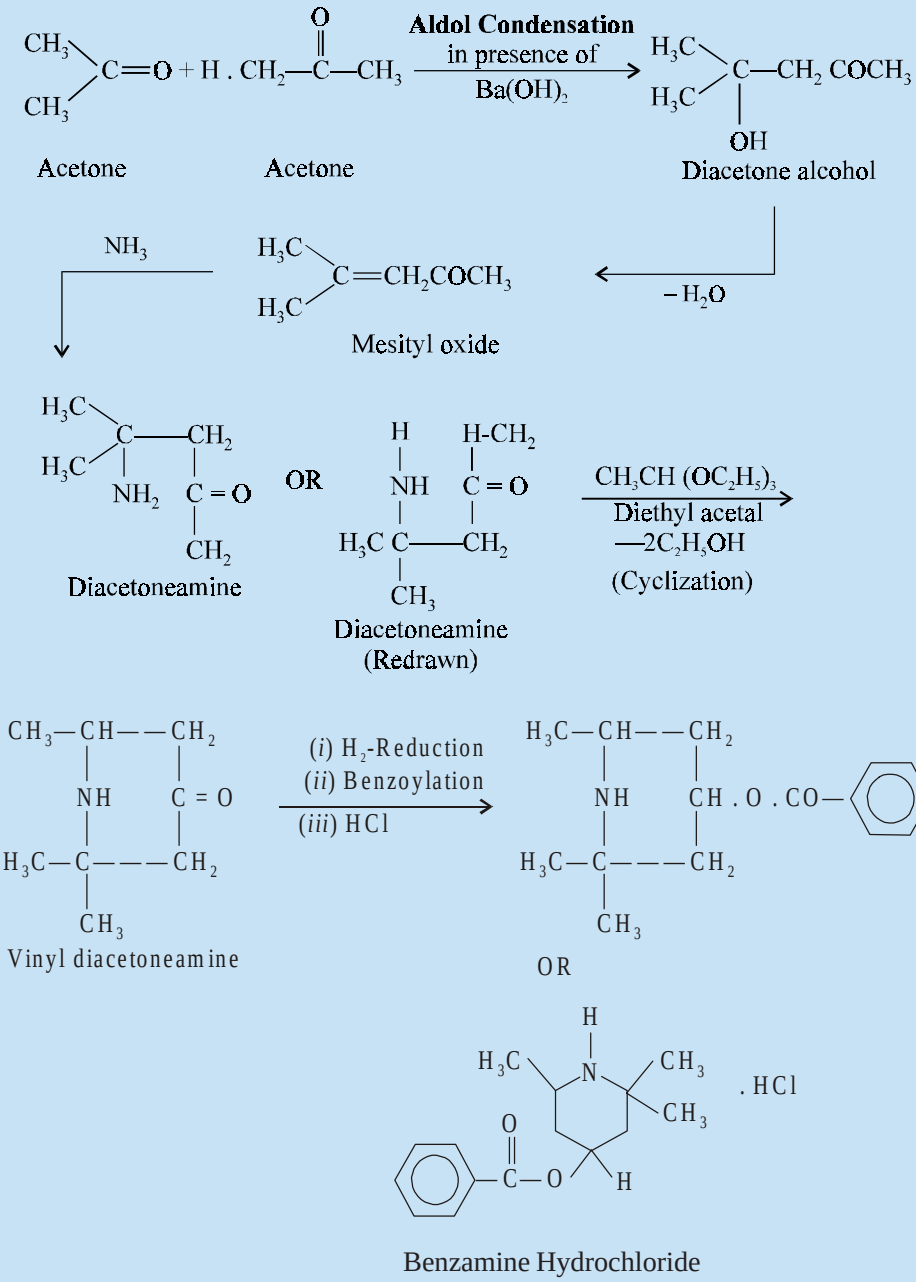
It is comparatively less toxic than cocaine, but is more painful and irritant than the later and hence ; it has been replaced by **β-eucaine.**

B. Benzamine Hydrochloride BAN



2, 2, 6-Trimethyl-4-piperidyl benzoate hydrochloride ; 2, 2, 6-Trimethyl-4-benzoxy piperidine hydrochloride ; β-Eucaine ; B.P.C. 1954 ;

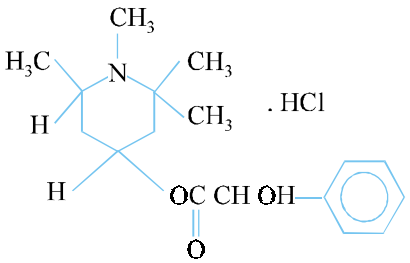
Synthesis



Diacetone alcohol is obtained by the **Aldol condensation** of two moles of acetone in the presence of barium hydroxide. On dehydration diacetone alcohol yields mesityl oxide, which upon amination gives diacetoneamine. This on treatment with diethyl acetal undergoes cyclization with the elimination of two moles of ethanol to give vinyl diacetoneamine. The cyclized product on reduction followed by benzoylation and treatment with hydrogen chloride yields **benzamine hydrochloride.**

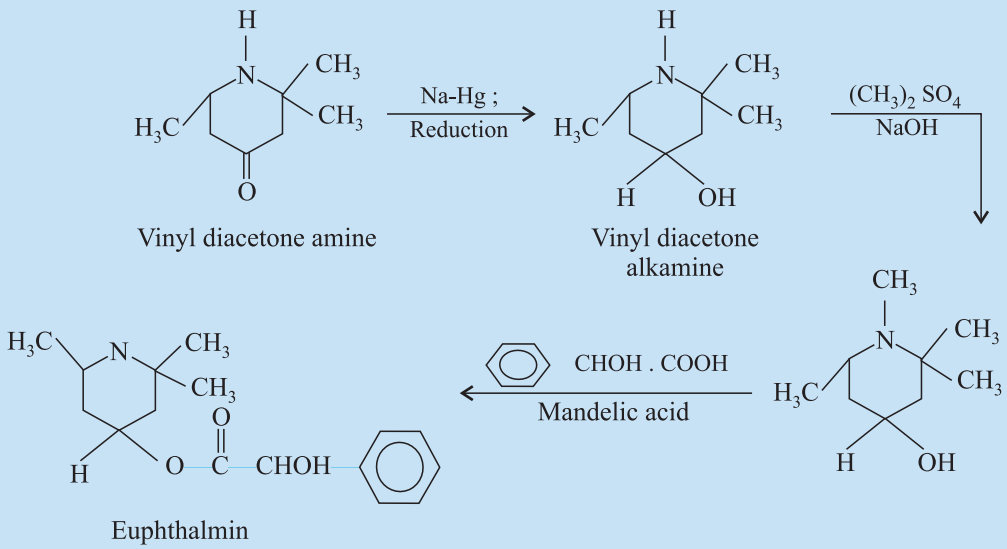
It is a local anaesthetic formerly used for surface anaesthesia. Its anaesthetic property is fairly comparable to that of cocaine.

C. Euphthalmin



2, 2, 6-Trimethyl-4-N-methyl piperidyl mandeloate ;

Synthesis



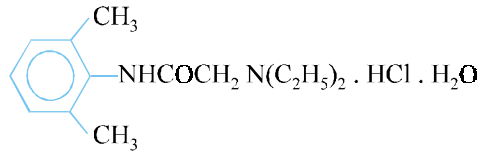
Vinyl diacetoneamine is prepared by the same method as described for benzamine hydrochloride above, which on reduction with (sodium-amalgam) yields vinyl diacetone alkamine. This on treatment with dimethylsulphate and sodium hydroxide yields the N-methyl derivative. The resulting product on treatment with mandelic acid undergoes esterification to yield **euphthalmin**.

2.3. The Amides

The earlier usage of acetanilide and **methylacetanilide (exalgin)** in the therapeutic armamentarium as antipyretic and analgesic drugs strongly advocate the idea and belief that when the $COCH_3$ moiety of these simple amides is further extended to the $COCH_2NR_2NR_2$, the resulting compounds offer remarkable local anaesthetic properties. This, in fact, led to synthesis of a large number of compounds of the amide type and a few classical examples are described below.

Examples : Lignocaine Hydrochloride ; Prilocaine Hydrochloride ; Mepivacaine Hydrochloride ; Bupivacaine Hydrochloride ; Pyrrocaine Hydrochloride ; Diperodon.

A. Lignocaine Hydrochloride BAN, Lidocaine INN, Lidocaine Hydrochloride USAN.

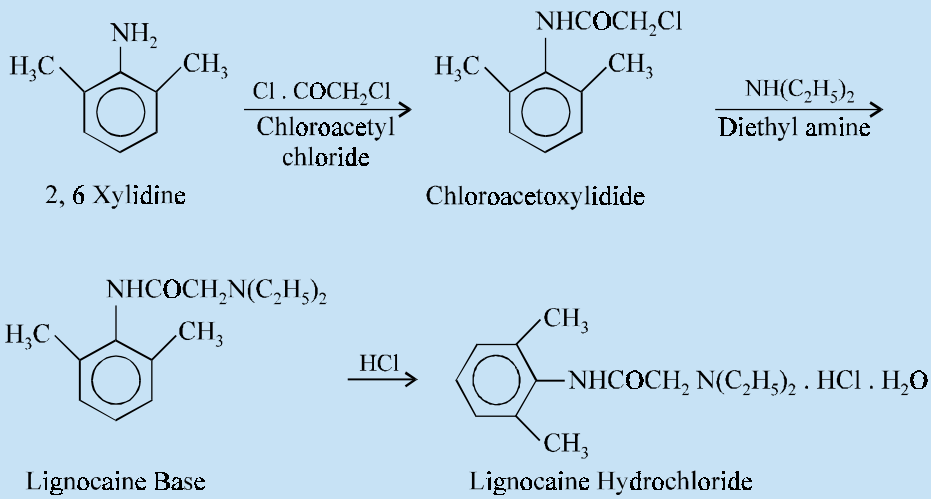


2-(Diethylamino)-2′, 6′-acetoxylidide monohydrochloride monohydrate ; Acetamide, 2-(diethylamino)-N-(2, 6-dimethyl-phenyl-, monohydrochloride, monohydrate ;

Lidocaine Hydrochloride U.S.P., Eur. P., Lignocaine Hydrochloride B.P., Int. P., Ind. P.

Xylocaine(R) (Astra) ; Dolicaine(R) (Reid-Provident) ;

Synthesis

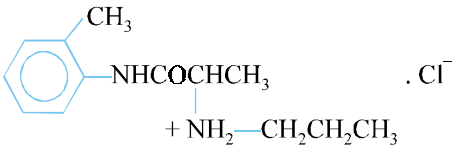


Chloroacetoxylidide is prepared by the interaction of 2, 6-xylidine with chloroacetyl chloride which on treatment with diethylamine yields the lignocaine base. This when treated with an equimolar quantity of hydrochloric acid gives the respective **lignocaine hydrochloride.**

It is a potent local anaesthetic and is reported to be twice as active as **procaine hyrdrochloride** in the same concentration. A 0.5% solution is toxic, *but at 2% its toxicity is increased to 50% than that of* **procaine hydrochloride**.

Dose : *Usual, infiltration, 50 ml of a 0.5% solution ; Usual, peripheral nerve block, 25 ml of a 1.5% solution, usual epidural 15 to 25 ml of a 1.5% solution ; Topical, up to 250 mg as a 2-4% solution or as a 2% jelly to mucous membranes.*

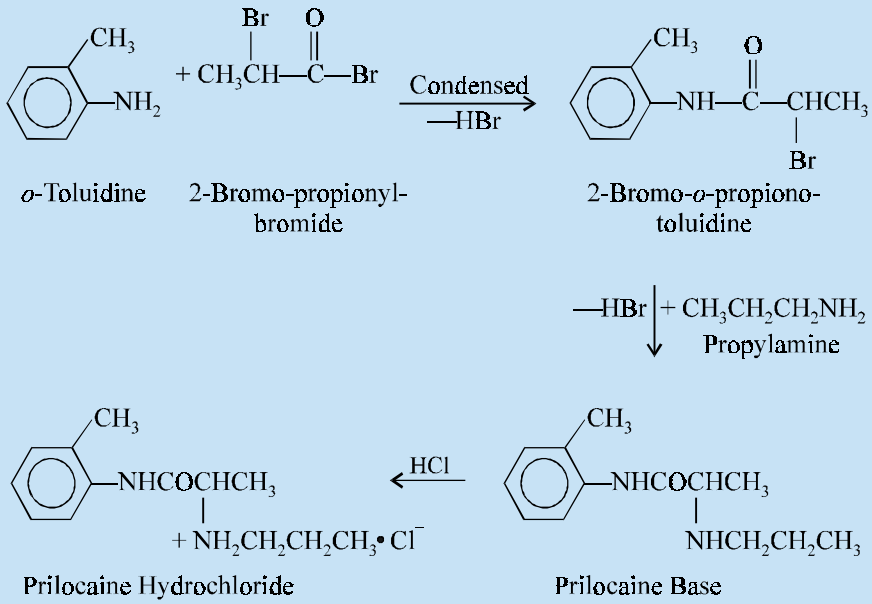
B. Prilocaine Hydrochloride BAN, USAN, Prilocaine INN,



2-(Propylamino)-*o*-propionotoluidine monohydrochloride ; Propanamide, N-(2-methylphenyl)-2-propylamino-, monohydrochloride ; B.P., U.S.P., N.F.,

Citanest Hydrochloride(R) (Astra)

Synthesis

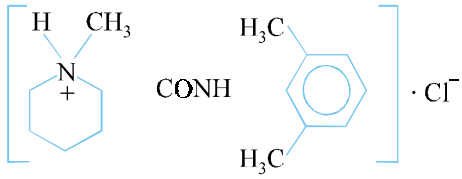


2-Bromo-*o*-propiono toluidine may be prepared by the condensation of a mole each of *o*-toluidine and 2-bromopropionyl bromide with the elimination of a mole of hydrogen bromide. This on further condensation with one mole of propyl amine yields the **prilocaine base** which on reaction with an equimolar amount of hydrochloric acid gives the official compound.

It is a local anaesthetic of the amide type which is employed for surface, infiltration and nerve block anaesthesia. Its duration of action is in between the **shorter-acting lidocaine** and **longer-acting mepivacaine**. It possesses less vaso-dilator activity than **lidocaine** and hence may be used without adrenaline. Therefore, solutions of **prilocaine hydrochloride** are specifically beneficial for such patients who cannot tolerate vasopressor agents ; patients having cardiovascular disorders, diabetes, hypertension and thyrotoxicosis.

Dose : *Usual, therapeutic nerve block, 3 to 5 ml of a 1 or 2% solution ; infiltration, 20 to 30 ml of a 1 or 2% solution ; peridural, caudal, regional, 15 to 20 ml of a 3% solution ; infiltration and nerve block, 0.5 to 5 ml of a 4% solution.*

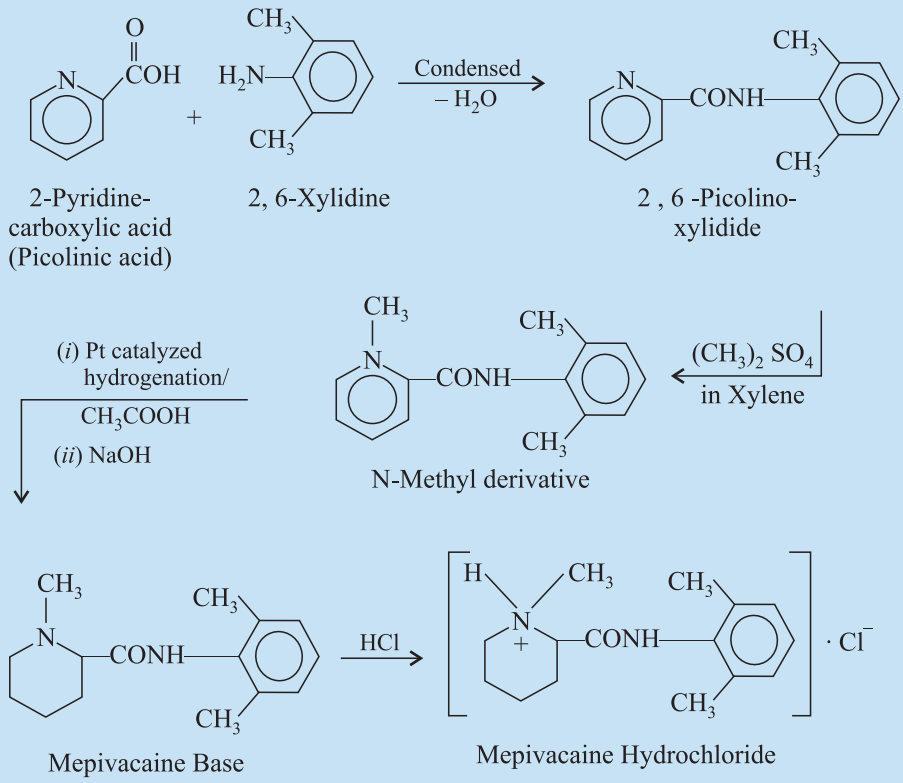
C. Mepivacaine Hydrochloride BAN, USAN, Mepivacaine INN,



1-Methyl-2′, 6′-pipecoloxylidide monohydrochloride ; 2-Piperidinecarboxamide, N-(2, 6-dimethylphenyl)-1-methyl-, monohydrochloride ; U.S.P.

Carbocaine Hydrochloride(R) (Winthrop) ; Polocaine(R) (Astra) ;

Synthesis

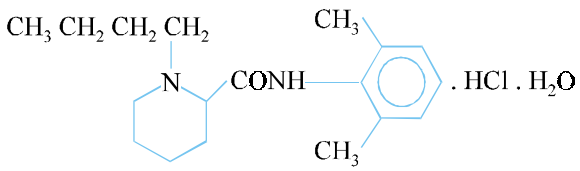


2′, 6′-Picolinoxylidide is prepared by the condensation of picolinic acid (2-pyridinecarboxylic acid) with 2, 6-xylidine which on methylation with dimethyl sulphate in xylene yields the corresponding N-methyl derivative. This when subjected to platinum-catalysed hydrogenation in active acetic acid followed by alkalinization yields **mepivacaine base**, which is then dissolved in an inert solvent and made to react with an equimolar amount of hydrochloric acid.

It is a local anaesthetic used for infiltration, peridural, nerve block, and caudal anaesthesia. It is found to be twice as potent as **procaine**. It has been reported that its duration of action is significantly longer than that of lidocaine, even without adrenaline. Hence, it is of particular importance in subjects showing contraindication to **adrenaline**.

Dose : *Infiltration and nerve block, 20 ml of 1 or 2% solution is sterile saline ; Caudal and peridural, 15 to 30 ml of 1%, 10 to 25 ml of 1.5% or 10 to 20 ml of a 2% solution in modified Ringer's solution.*

D. Bupivacaine Hydrochloride BAN, USAN, Bupivacaine INN,



(±)-1-Butyl-2′, 6′-pipecoloxylidide monohydrochloride monohydrate ; 2-Piperidinecarbonxamide, 1-butyl-N (2, 6-dimethyl-phenyl)-, monohydrochloride monohydrate ; B.P., U.S.P.

Marcaine(R) (Sterling) ; Sensorcaine(R) (Astra)

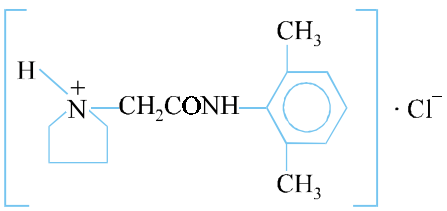
Synthesis

It may be prepared by adopting the same course of reactions as stated in for the synthesis of **mepivacaine, hydrochloride** except employing butyl bromide instead of dimethyl sulphate for the N-alkylation.

It is a **long-acting local anaesthetic of the amide type**, similar to **mepivacaine** and **lidocaine** but about four times more potent. The effects of **bupivacaine** last longer to **lidocaine hydrochloride**. It is mainly employed for regional nerve block, specifically epidural block, when a prolonged effect is required.

Dose : *Regional nerve block, 0.25 to 0.5% solution ; Lumbar epidural block, 15 to 20 ml of 0.25 to 0.5% solution ; Caudal block, 15 to 40 ml of 0.2% solution.*

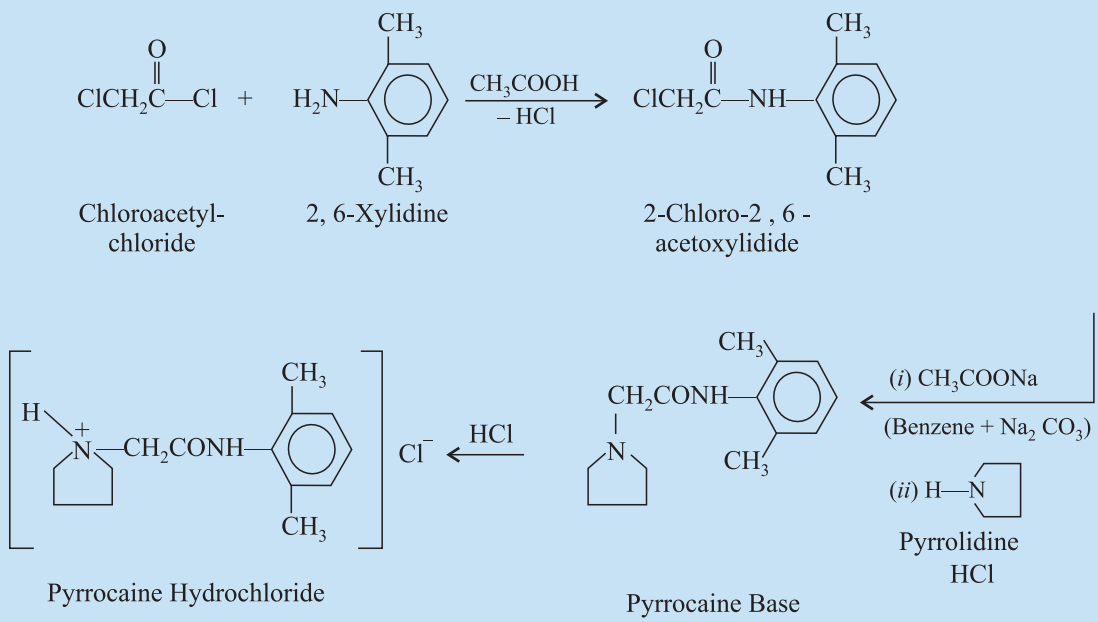
E. Pyrrocaine Hydrochloride BAN, Pyrrocaine INN, USAN,



1-Pyrrolidinoaceto-2′, 6′-xylidide monohydrochloride ; 1-Pyrrolidineacetamide, N-(2-6-dimethylphenyl)- ; N.F.,

Endocaine Hydrochloride(R) (Endo)

Synthesis

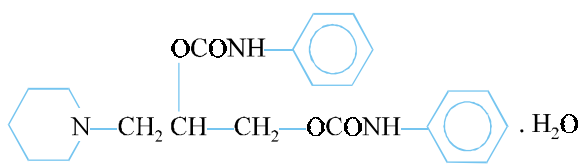


2-Chloro-2′, 6′-acetoxylidide is prepared by treating a solution of 2, 6-xylidine in glacial acetic acid with chloroacetyl chloride which is precipitated with sodium acetate, dissolved in benzene containing suspended sodium carbonate. This on treatment with pyrrolidine yields the **pyrrocaine base** which on reacting with an equimolar quantity of hydrochloric acid produces the **pyrrocaine hydrochloride**.

It is used in dentistry for infiltration and block anaesthesia. Its duration of action as well as potency is almost similar to that of lidocaine.

Dose : *Usual, infiltration, 1 ml of a 2% solution ; Nerve block, 1.5 to 2 ml of a 2% solution.*

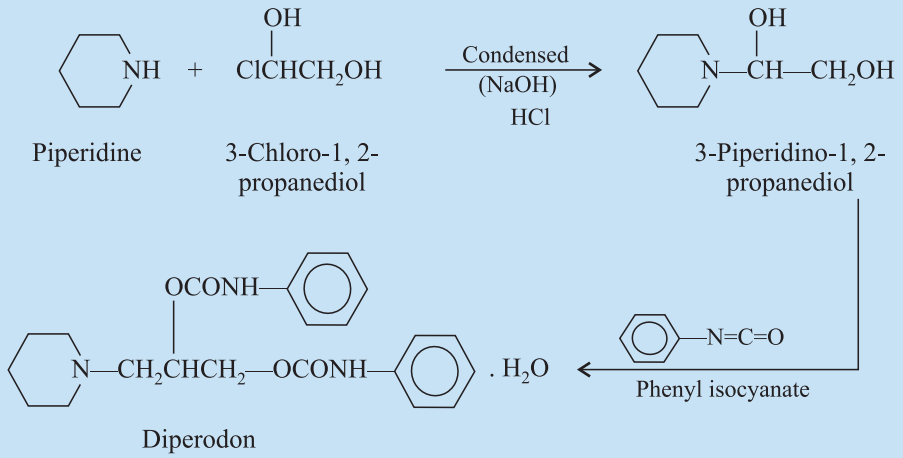
F. Diperodon INN, BAN, USAN,



3-Piperidino-1, 2-propanediol carbanilate (ester), monohydrate ; 1, 2-Propanediol, 3-(1-piperidinyl)-, *bis*-(phenyl-carbamate) (ester), monohydrate ; U.S.P., N.F.,

Diothane(R) (Merrell)

Synthesis



3-Piperidino-1, 2-propanediol is prepared by the condensation of piperidine and 3-chloro-1, 2-propanediol in an alkaline medium which is caused to undergo addition to phenylisocyanate to give diperodon.

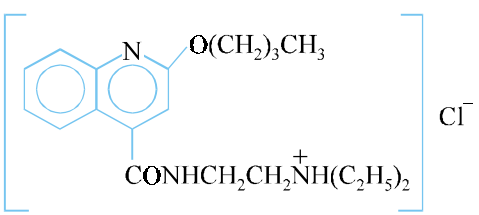
It is used as a potent **surface anaesthesia**.

Dose : *Topical, 0.5 to 1% solution, to the mucous membranes.*

2.4. The Quinoline and Iso-quinoline Analogues

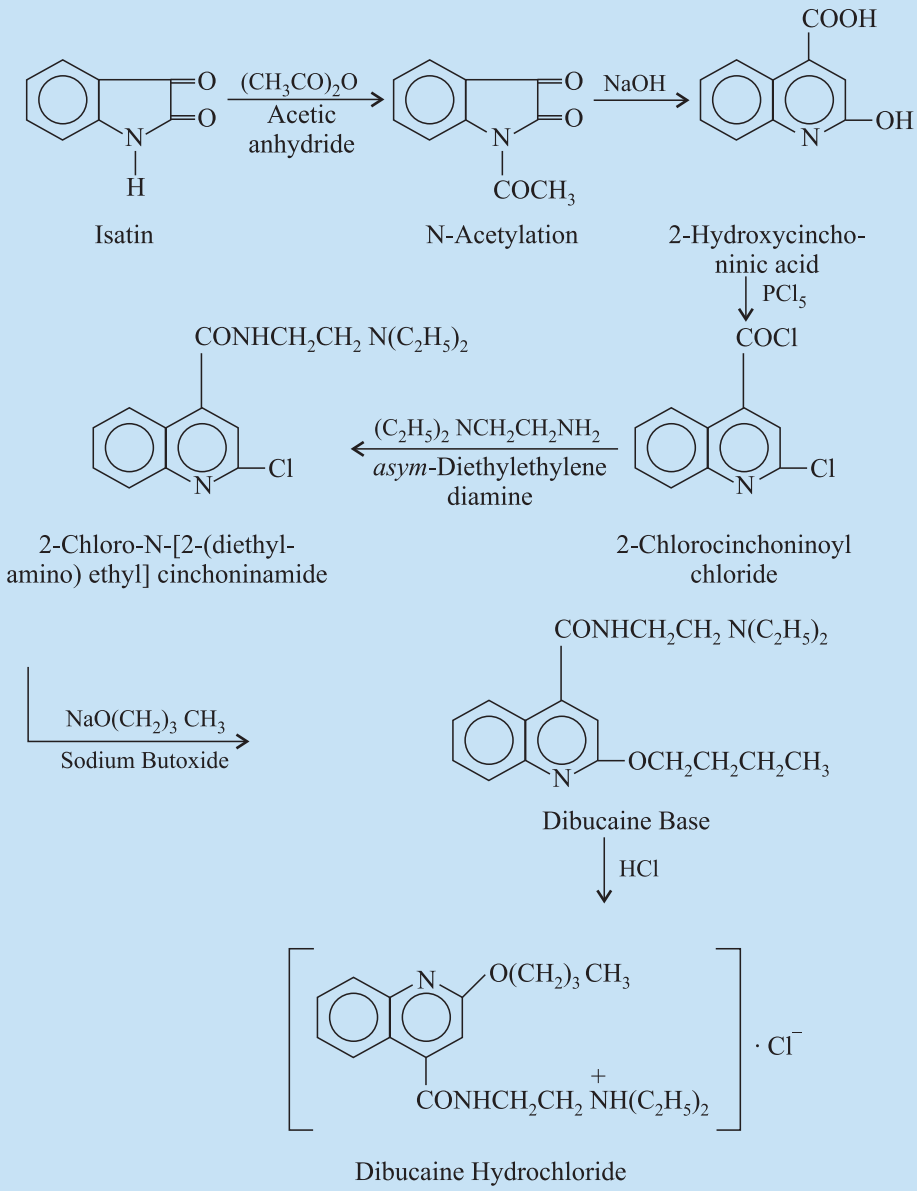
In an attempt to search for **more potent** and **better tolerated local anaesthetics**, it has been observed that the amides of quinoline derivatives are more potent than common **local anaesthetics** and the most important member in this class is **dibucaine hydrochloride.** Besides, another member which essentially contains an **iso-quinoline nucleus** but without an amide moiety, namely, **dimethisoquine hydrochloride** is reported to be one of the most potent local anaesthetics. These *two* members will be described below :

A. Dibucaine Hydrochloride USAN, Cinchocaine INN, Cinchocaine Hydrochloride BAN,



2-Butoxy-N-[2-(diethylamino)-ethyl]-, monohydrochloride ; 4-Quinolinecarboxamide, 2-butoxy-N-[2-diethylamino)-ethyl]-, monohydrochloride ; U.S.P., N.F., Cinchocaine Hydrochloride B.P., Nupercaine Hydrochloride(R) (Ciba-Geigy)

Synthesis



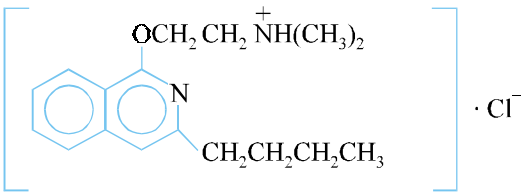
N-Acetylation of **isatin** with acetic anhydride yields N-acetylation which undergoes intra molecular rearrangement with alkali to the quinoline compound 2-hydroxycinchoninic acid under the name **Pfitzinger Reaction.** The diamino group is now introduced under controlled condition at room temperature so as to avoid reaction with relatively less reactive 2-chloro substituent.

The subsequent treatment with sodium butoxide yields the **dibucaine base** which is then dissolved in a suitable organic solvent and precipitated by bubbling hydrogen chloride through the liquid.

It is one of the most toxic, most potent and longest acting of the frequently used local anaesthetics. It may be used as infiltration, surface, epidural and spinal anaesthesia. It finds its use in dentistry. Its anaesthetic activity is similar to those of procaine or cocaine when injected. However, it is several times more potent than procaine when injected subcutaneously and about 5 times more toxic than **cocaine** when injected intravenously.

Dose : *Subarachnoid, 0.5 to 2 ml of 0.5% solution ; usual, 1.5 ml of a 0.5% solution.*

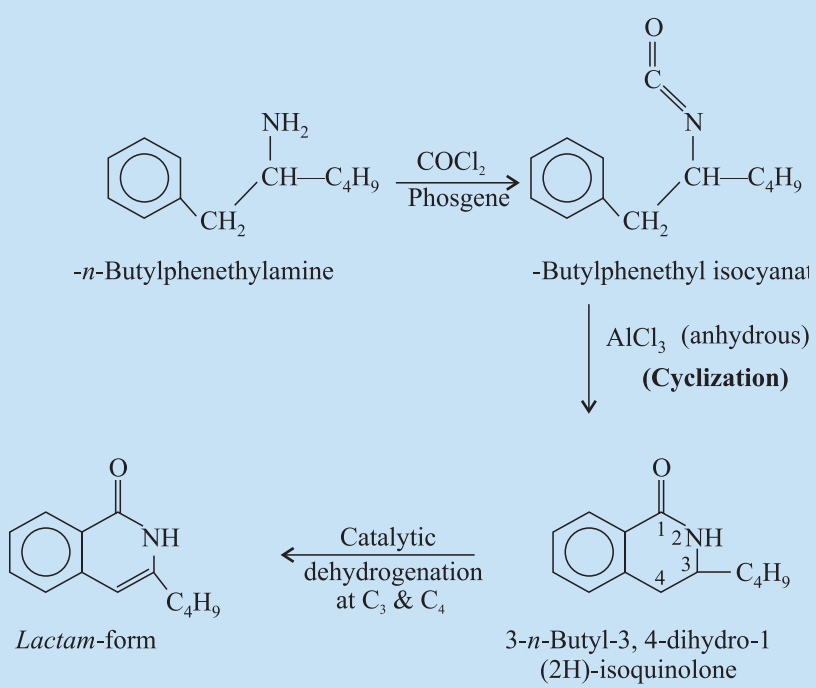
B. Dimethisoquin Hydrochloride BAN, USAN, Quinisocaine INN,



3-Butyl-1-[2-(dimethylamino)-ethoxy] isoquinoline monohydrochloride ; Ethanamine, 2-[(3-butyl-1-isoquinolinyl) oxy]-N, N-dimethyl-, monohydrochloride ; U.S.P., N.F.,

Quotane(R) (SK & F)

Synthesis



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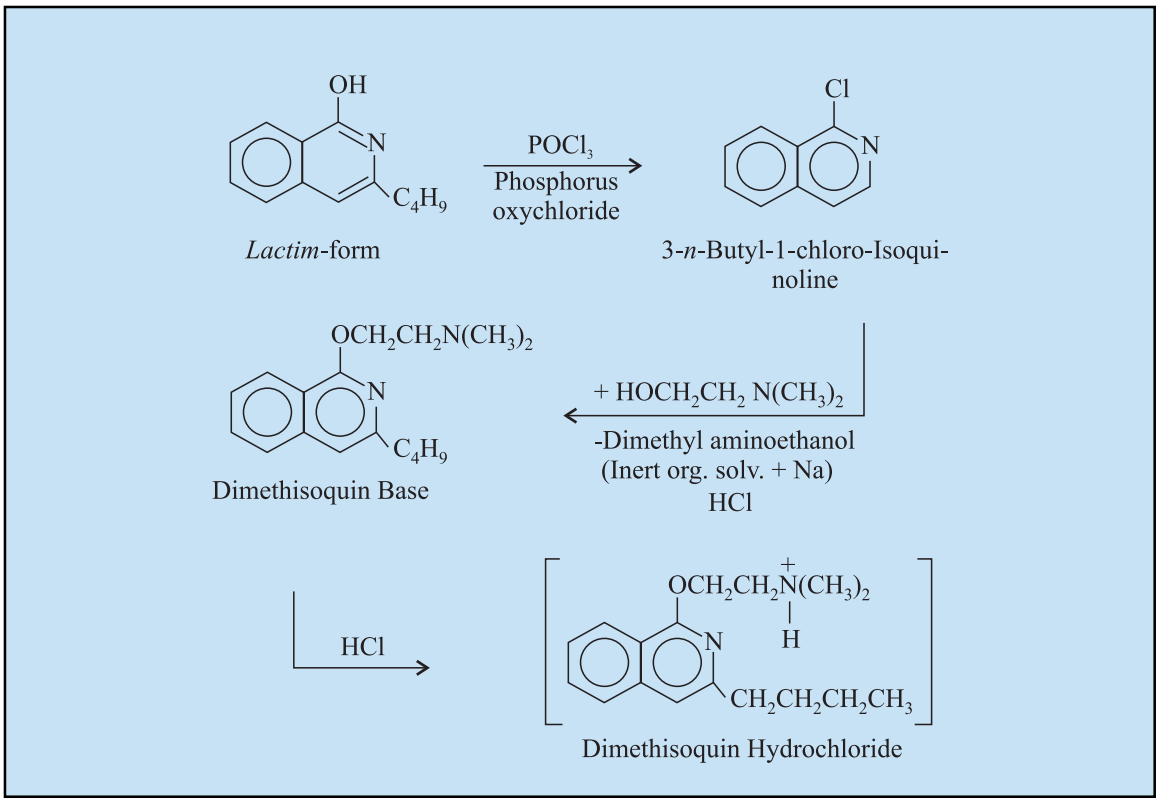
CHAPTER 5



α-Butylphenethyl isocyanate is prepared by treating α-*n*-butylphenethylamine with phosgene in an appropriate organic solvent which upon treatment with anhydrous aluminium chloride undergoes cyclization to yield 3-*n*-butyl-3, 4-dihydro-1 (2H)-isoquinolone. This on catalytic dehydrogenation at C_3 and C_4 and the subsequent conversion to the *Lactim*-form, which is then reacted with phosphorus oxychloride to yield 3-*n*-butyl-1-chloroisoquinoline. The resulting product when dissolved in an inert organic solvent in the presence of sodium metal and is reacted with β-dimethylaminoethanol it produces the **dimethisoquin base**. The crude base may be purified by distillation under reduced pressure, dissolved in an appropriate organic solvent, and treated with an equimolar amount of hydrogen chloride to yield the official compound.

It is a **surface anaesthetic** and has been used as a lotion or ointment in a concentration of 0.5% for the relief of irritation, itching, burning or pain in dermatoses, including mild sunburn and nonspecific pruritus. It is reported to be less toxic than dibucaine but more toxic than procaine.

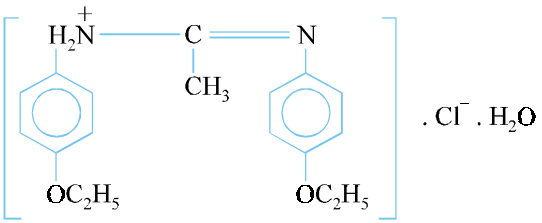
Dose : *Topical, to the skin, as a 0.5% ointment or lotion 2 to 4 times daily.*

2.5. Miscellaneous Type

There are a few medicinal compounds which have proved to be potent **local anaesthetics** and could not be accommodated conveniently into any one of the previous categories discussed, are grouped together under this heading.

Examples : Phenacaine Hydrochloride ; **Pramoxine Hydrochloride** ; **Eugenol etc**.

A. Phenacaine Hydrochloride BAN, USAN, Phenacaine INN,

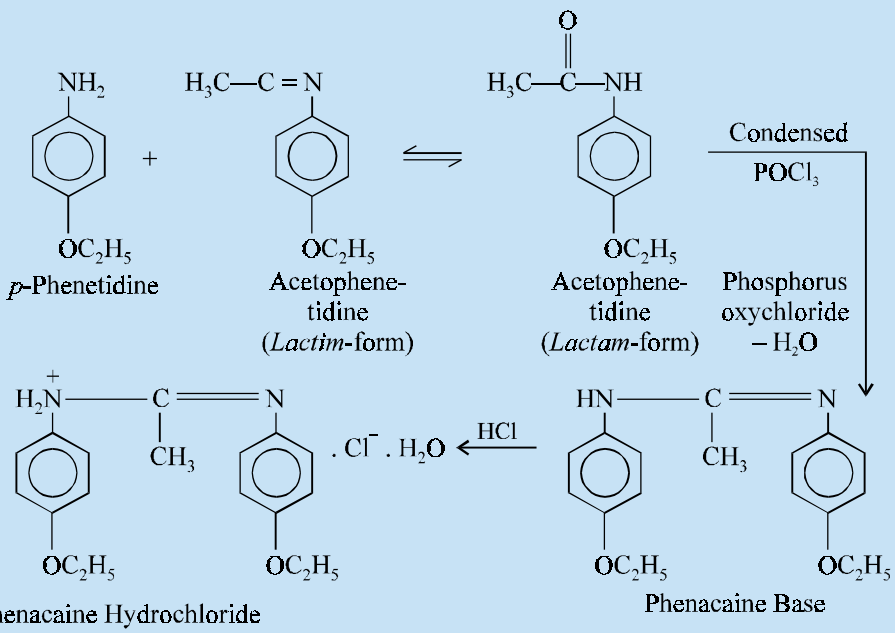


N,N-bis(*p*-ethoxyphenyl) acetamidine monohydrochloride monohydrate ; Ethanimidamide N, N′-*bis* (4-ethoxyphenol)-, monohydrochloride, monohydrate ;

U.S.P., N.F.,

Holocaine Hydrochloride(R) (Abbott)

Synthesis

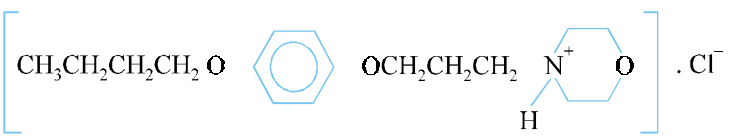


Condensation of *para*-phenetidine and acetophenetidine in *Lactim*-form in the presence of phosphorus oxychloride yields phenacaine base with the elimination of a molecule of water, which on treatment with an equimolar quantity of hydrochloric acid gives the official compound.

It is one of the **oldest synthetic local anaesthetics**. It is chiefly employed as a 1% solution for effecting local anaesthesia of the eye.

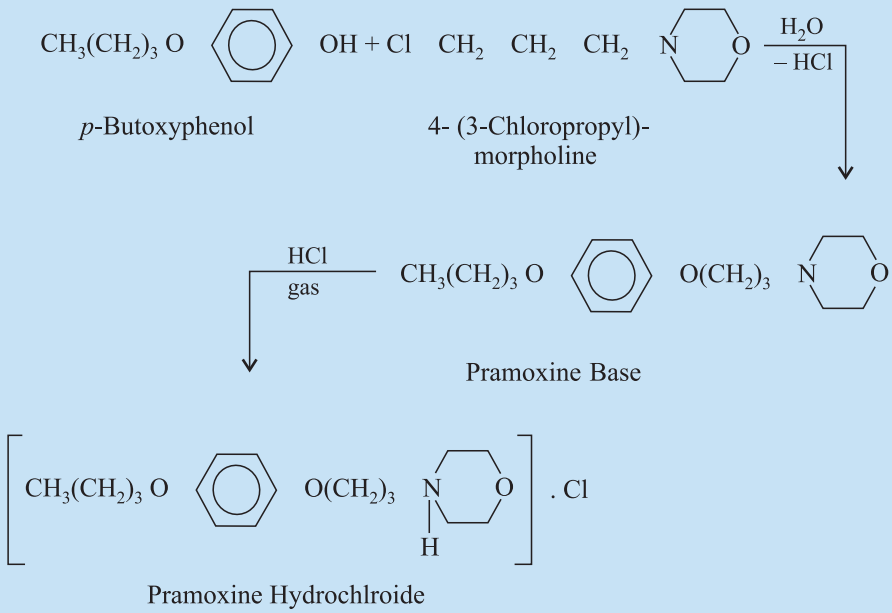
Dose : *To the conjuctiva as 1-2% ointment or as a 1% solution.*

B. Pramoxine Hydrochloride BAN, USAN, Pramocaine INN,



4-[3-(*p*-Butoxyphenoxy) propyl] morpholine hydrochloride ; Morpholine,
4-[3-(4-butoxyphenoxy) propyl]-, hydrochloride ; U.S.P., N.F.,
Tronothane(R) (Abbott)

Synthesis

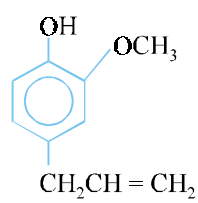


The condensation of *p*-buoxyphenol and 4-(3-chloro-propyl)-morpholine is carried out by refluxing them together in an aqueous medium. On cooling the reaction mixture the pramoxine base is *first* extracted with benzene, *secondly* purified by distillation under reduced pressure, *thirdly* dissolved in an appropriate organic solvent, and *fourthly* converted to the corresponding hydrochloride by means of a stream of hydrogen chloride.

It is a surface anaesthetic which possesses very low degree of toxicity and sensitization. It may be applied locally in a 1% strength to soothen pain in hemorrhoids and rectal surgery, itching dermatoses, some intubation procedures, anogenital pruritus and moderate burns and sunburn.

Dose : *Topical as a 1% jelly or cream every 3 to 4 hours.*

C. Eugenol BAN, USAN,



4-Allyl-2-methoxyphenol ; Phenol, 2-methoxy-4-(2-propenyl)- ; Synthetic Clove Oil, B.P., U.S.P.

It is used frequently in dentistry as an obtundent for hypersensitive dentine, caries or exposed pulp, as mild rubefacient in dentifrices and as a temporary anodyne dental filling.

Dose : *Topical, in dental protectives.*

3. CHEMICAL CONSIDERATIONS OF LOCAL ANAESTHETIC DRUG SUBSTANCES

The presence of the **'anaesthesiophoric moiety'** in **cocaine**, discovered in the year 1880, employed profusely as a vital local anaesthetic in various surgical procedures by virtue of its ability to cause anaesthesia by **blocking nerve conductance,** ultimately paved the way for the synthesis of thousands of new compounds known as **'local anaesthetics'.** However, about twenty such compounds have gained recognition and congnizance as local anaesthetics in the therapeutic armamentarium.

A good number of purely synthesized structural analogues belonging to the different chemical classifications of such compounds have been adequately dealt with under Section 2.1 through 2.5 in this chapter.

It is pertinent to state that (under Section 3) the **structure activity relationships (SARs)** of certain compounds with reasonably plausible explanations wherever applicable to understand the mechanism of action more explicitely and vividly.

Broadly speaking, it has been observed critically amongst all the known **'local anaesthetics',** which are invariably employed in clinical practice, that there exists no clear-cut and obvious structure activity relationship in them. Besides, a plethora of these clinically prevalent and useful **local anaesthetics** are importantly **'tertiary amines',** such as : **Butacaine, Bupivacaine, Dibucaine, Lidocaine, Mepivacaine, Prilocaine, Pramoxine, Proxymetacaine, Tetracaine** etc.

Interestingly, the dissociation constant, *p*K*a* values, of these stated local anaesthetics usually range between 7.0 and 9.0, for instance : **Bupivacaine (8.1) ; Lidocaine (7.8) ; Mepivacaine (7.6) ; Proxymetacaine (9.1) ; Tetracaine (8.4) ; Prilocaine (7.9) ; Dibucaine (8.8) ; Pramoxine (7.1).**

It is worthwhile to mention at this juncture that the above stated purely synthetic **'local anaesthetics'** evidently display their biological activities on account of their ability of the binding between the corresponding **onium ions** and a **selective site** very much existing within the **sodium channels**. Therefore, it is absolutely necessary to acquire the following *two* vital and characteristic features in the **'design'** of a **local anaesthetic** so as to retain maximum therapeutic activity :

(*a*) Alteration in the lipoidal solubility (*i.e.*, *p*K*a*) of the '**drug**' ;

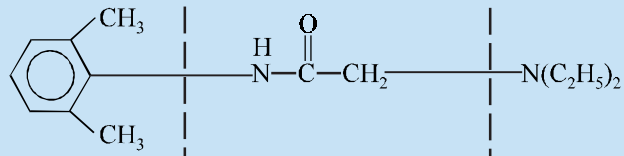
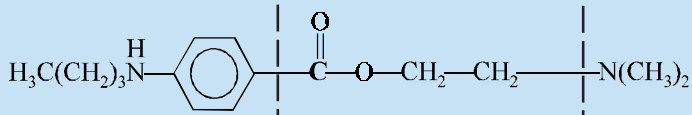
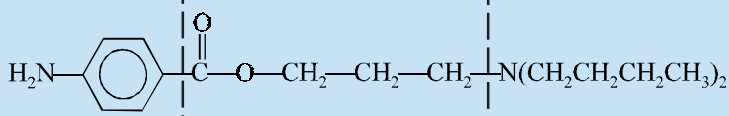
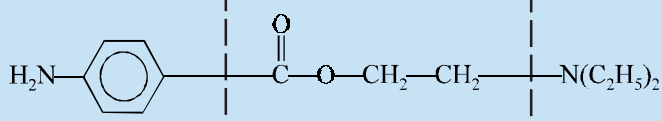
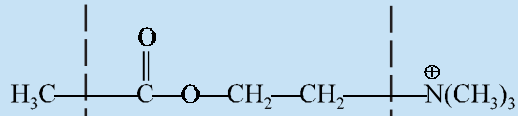
(*b*) Effect on the ability of a **'drug substance'** to first reach and then get bound to the **hypothetical receptor site(s).**

3.1. Löfgren's Classification

Bean *et al.*(1983)* assumed it to be true as a logical basis for reasoning the structure activity relationships amongst the known **'local anaesthetics'** as per the following specific structural characteristic features put forward under **Löfgren's classification** as illustrated below in Table 4.1.

*Bean BP *et al. J. Gen. Physiol.*, **81** : 613–642, 1983.

Table. 4.1. Local Anaesthetics and Cholinergic Agent : A Comparison

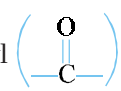
Name	Lipophilic Entity	Intermediate Chain	Hydrophilic Entity
Lidocaine			
Tetracaine			
Butacaine			
Procaine			
Acetylcholine			

From Table 4.1. one may evidently observe that a entire molecule of a **'local anaesthetic'** has been judiciously divided into *three* distinct compartments/zones, otherwise termed as : **lipophilic entity, intermediate chain** and **hydrophilic entity.** However these *three* zones have been clearly illustrated in a few typical examples *e.g.,* **Lidocaine, Tetracaine, Butacaine, Procaine** and a cholinergic agent **Acetylcholine.**

It will be now worthwhile to elaborate and discuss the structure-activity relationship of certain specific examples of **'local anaesthetics'** *vis-a-vis* the aforesaid *three* separate zones imbeded into the drug molecule.

3.1.1. Lipophilic Entity

The various **salient features** essentially associated with the lipophilic portion (entity) and the anaesthetic activity are as given under :

(*a*) the presence of an **aryl function** attached directly to a carbonyl  moiety, such as :

amino-ester series ;

Examples : Tetracaine ; Butacaine ; Procaine ; etc.,

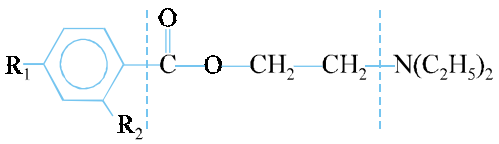
(*b*) the presence of a **2, 6-dimethylphenyl function** usually linked to a carbonyl moiety by means of an imino (—NH—) group, for instance : **amino-amide series ;**

Examples : Lidocaine ; Pyrocaine ; etc.

Thus, the amino-ester and the amino-amide series attribute a highly lipophilic property to the **'drug molecule'** ; and is believed to afford a substantial contribution towards the binding of **local anaesthetics** particularly to the channel-receptor proteins. In other words, whatever structural modifications are intended to be carried out in this particular zone of the molecule, it would certainly reflect directly upon the physical and chemical characteristics thereby causing an appreciable alteration in its local anaesthetic profile ultimately.

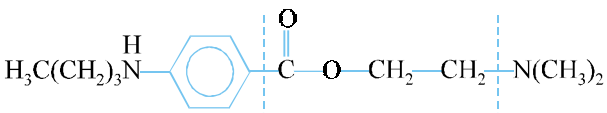
(*c*) the effect of **electron-donating moieties in the amino-ester series** are quite vital and significant *i.e.*, present in both or *para*- or *ortho*-positions.

Examples : (*i*) **Amino (—NH_2) Function.** *e.g.*, **Procaine, Propoxycaine,** and **Chloroprocaine :**

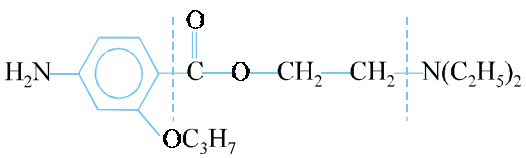


R_1 = – NH_2 ; R_2 = – H : Procaine ;
R_1 = – NH_2 ; R_2 = – OC_3H_7 Propoxycaine ;
R_1 = – NH_2 ; R_2 = – Cl ; Chloroprocaine ;

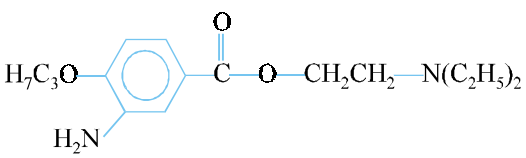
(*ii*) **Alkylamino (RHN) Function :** *e.g.,* Tetracaine ;



(*iii*) **Alkoxy (RO) Function : *e.g.*, Propoxycaine ; Proparacaine ;**

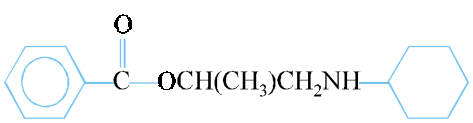


Propoxycaine

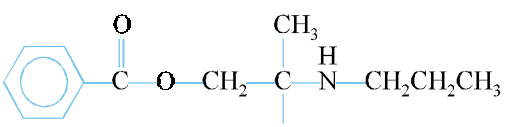


Proparacaine

Note : All these three functional moieties being electron-donating in nature do help in profusely contributing electron density to the π-clouds of electrons present in the aromatic ring by such effects as — 'resonance' and 'inductive', thereby increasing ultimately the local anaesthetic potency in comparison to the non-substituted structural analogues, namely : Hexylcaine ; Meprylcaine ;



Hexylcaine

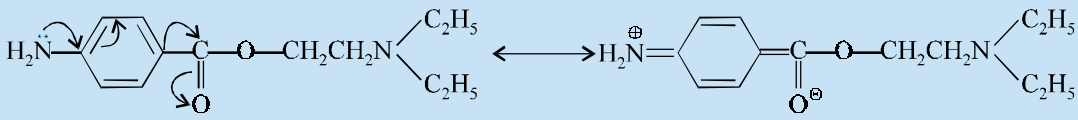


Meprylcaine

(*d*) the **'Resonance Effects'** do play a predominant role in explaining specifically the influence of various substituents of the aromatic portion of the molecule upon the **'local anaesthetic'** actions.

Examples : Possible resonance structures of **procaine**, **lidocaine** and **tetracaine** are as describe below :

(*i*) **Procaine :**



(Possible resonance form of procaine) I (Protonated onium ion of procaine) II

The above two structures, I and II, are two resonance forms of procaine, neither of which actually represents the 'drug'. In fact, a **'hybrid structure',** wherein the carbonyl moiety attains a partially ionic (not totally as in II), would perhaps be a more probable and correct representation. The aforesaid hypothesis may be further substantiated by varying the anaesthetic potency of structrural variants *vis-a-vis* the altered nature of the respective *para* substituents on the benzene ring ; and relate it to the **bond order** of the *'ester carbonyl'* by adequately measuring the corresponding observed **IR-stretching frequency,** as summarized in Table 4.2 below :

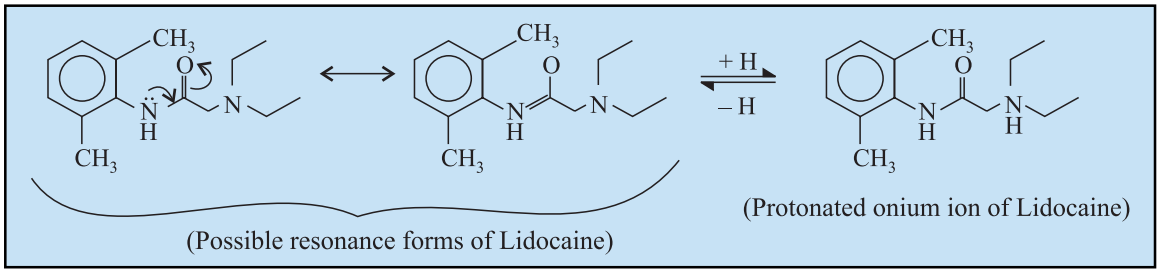
Table 4.2. Relationship of Local Anaesthetic Profile and Bond Order

S.No.	R-Substituent	IR (C = 0) cm^{-1}	ED_{50} (m mol/100 mL)*
1	H_3CO —	1.708	0.060
2	H_5C_2O —	1.708	0.012
3	H_2N —	1.711	0.075
4	HO —	1.714	0.125
5	O_2N —	1.731	0.740

From Table 4.2., it may be observed that both **amino** and **alkoxy** functions are regarded as **'electron donors'** by virtue of their resonance characteristic ; and, therefore, increase the dipolar (*i.e.*, ionic) nature of the carbonyl (C = 0) moiety. Further, *para*-substituents do exert a marked and pronounced electron-withdrawing effect in the carbonyl function having more double-bond character and ultimately resulting in *less intense* local anaesthetic activity.

* Galinsky AM *et al. J. Med. Chem.*, **6** : 320, 1963.

(*ii*) **Lidocaine :**



(Possible resonance forms of Lidocaine)

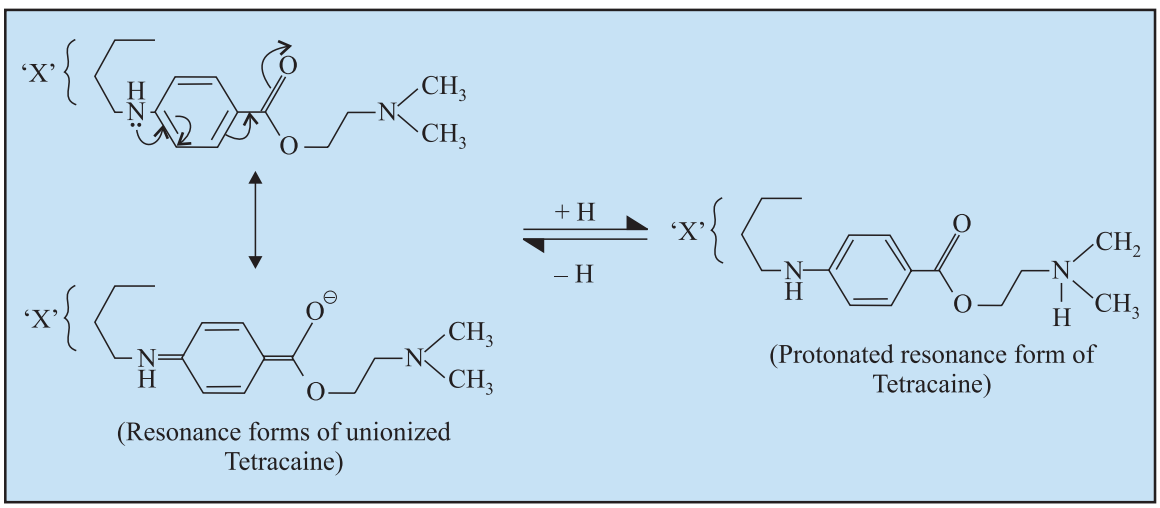
(Protonated onium ion of Lidocaine)

In this particular instance *i.e.*, amino-amides, lidocaine analogues, the very strategically positioned O, O′-dimethyl functions are meant to afford adequate protection from amide hydrolysis to ascertain a predicted and desirable duration of action.

In the same vein, one may derive logical conclusions to only justify but also rationalize the possible enhancement in the duration of action of **propoxycaine** by the presence of ***ortho*-propoxy moiety.**

However, in another instance *i.e.*, **chloroprocaine,** the observed relatively shorter duration of action, in comparison to procaine, may be evidently expatiated by the *inductive effect* due to the presence of *ortho*-chloro function, which might help in pulling out the density of electron away from the carbonyl group, thereby rendering it more prone to **nucleophilic attack** by the **plasma esterases.**

(*iii*) **Tetracaine :**



(Resonance forms of unionized Tetracaine)

(Protonated resonance form of Tetracaine)

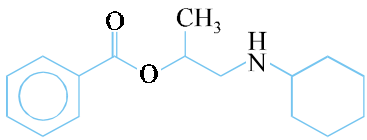
Tetracaine is found to be 50-times more active than **procaine**. However, this phenomenal enhancement in potency may not be explained and proved experimentally by virtue of the presence of the overwhelming surge in lipid-solubility attributed by the *n*-butyl moiety marked 'X'. Logistically, the marked and pronounced potentiation of **local anaesthetic** profile offered by tetracaine may be attributed to the distinct electron-releasing activity of the said *n*-butyl function *via* the inductive effect, that preferentially increases the prevailing electron density of the *para*-amino moiety, which subsequently enhances the creation of the **'resonance form'** readily available for enabling to get bound to the viable receptor proteins.

3.1.2. Intermediate Chain

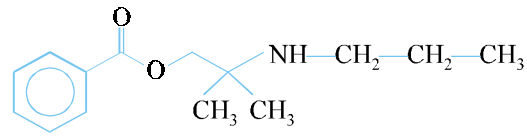
The intermediate chain essentially comprise of a **'distinct short alkylene chain',** made up of 1-3 C-atoms hooked on to the aromatic ring *via* a plethora of organic functional moieties. It has been established that the **'intermediate chain'** categorically serve as a *'determinant factor'* to decide the **'chemical stability of the drug molecule'.** In addition to this, it also exerts its marked and pronounced influence on the duration of action and relative toxicity of the drug substance. It has been duly observed that invariably both the **amino carbamates** and the **amino amides** exert a much higher resistance to the **'metabolic inactivation'** particularly in comparison to the corresponding **'amino esters',** which ascertains importantly a longer-acting local anaesthetic criterion.

A few other equally **vital characteristic features** are, namely :

(*a*) induction of small alkyl moieties in the vicinity of the ester function *e.g.*, **hexylcaine** and **meprylcaine.** Both these compounds check and prevent amide and ester hydrolysis thereby increasing the duration of action appreciably.

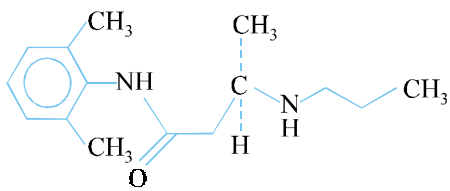


Hexylcaine (pKa = 9.3)

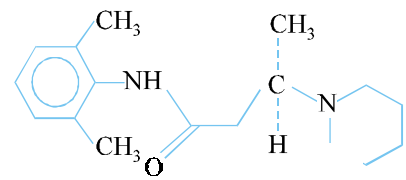


Meprylcaine (pKa = 7.8)

(*b*) induction of amide function in the close range of simple alkyl amino or branched alkyl amino functions located in the viccinity of an amide moiety *e.g.*, **prilocaine** and **etidocaine.** Interestingly, these two compounds hinder ester hydrolysis thereby enhancing the duration of action significantly.



Prilocaine (pKa = 7.9)



Etidocaine (pKa = 7.9)

Salient Features : The **salient features** are as follows :

(*i*) Increasing the length of the alkylene chain in the **lidocaine** structural analogues from one to two (increases pKa from 7.7. to 9.9) ; and from two to three (increases pKa from 9.9 to 9.5)

(*ii*) Enhancement of the **'intermediate chain'** (Fig. 4.1) drastically lowers the potency of local anaesthetics due to the reduction of **onium ions** specifically under the prevailing physiologic conditions. Nevertheless, the onium ions are essentially utilized in promoting the binding to the **'channel receptor'** efficiently and effectively.

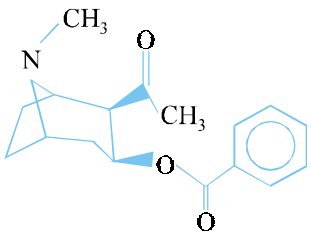
3.1.3. Hydrophilic Entity

If one examines the most potent and commonly used **'local anaesthetics'** in the therapeutic armamentarium it may be abundantly clear that they essentially possess a **tertiary alkylamine** moiety which rapidly converts the **'base'** into the corresponding water-soluble salts with the various mineral acids. Obviously, the **'basic entity'** is frequently regarded as the **hydrophilic entity** of the drug molecule.

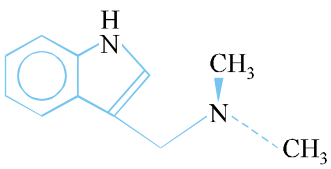
However, it has been adequately proved both logically and scientifically, that the **voltage-activated sodium channel** along with the **most probable mechanism of action discussed earlier,** one may safely infer and suggest that the onium ions generated by protonation of the tertiary amine function are urgently required in carrying out the binding phenomenon with the **'receptors'**.

4. BENZOIC ACID AND ANILINE ANALOGUES WITH POTENTIAL LOCAL ANAESTHETIC PROFILE

It fact, the *'esters'* of benzoic acid derivatives are derived from **cocaine,** while the *'amides'* of aniline derivatives are obtained from **isogramine.**



Cocaine

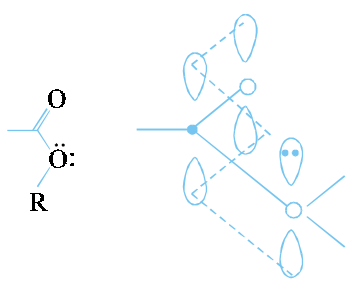


Isogramine

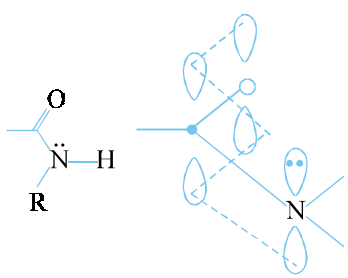
The following general arrangement holds good with regard to the chemical structures of **'esters'** and **'amides'** :

LIPOPHILIC CENTRE — ESTER OR AMIDE FUNCTION — CONNECTING BRIDGE — HYROPHILIC CENTRE

It is, however, pertinent to mention here that the **'ester'** as well as the **N-substituted functional moieties** are nothing but **bioisosteres** as illustrated below ; and it further expatiates and justifies the strategical presence of such groups in almost identical locations in the various **tailor-made structurally designed local anaesthetics**.



An 'Ester'



An 'Amide'

Salient Features : The various salient features influencing the functional moieties *vis-a-vis* the lipophilic and hydrophilic characterstics of the **'local anaesthetics'** are enumerated as under :

(1) A heterocyclic-ring system or a carboxylic moiety invariably gives rise to a distinct and prominent ***'lipophilic centre'***,

(2) A secondary or tertiary amine that could be either cyclic or an open-chain analogue usually affords a marked and pronounced **'hydrophilic centre'**,

(3) A **'hydrophilic centre'** may be suitably linked to either an amide function or an ester moiety either by S, N, O-atoms or apporpriately by a short hydrocarbon unit. However, it has been observed that the latter (*i.e.*, short hydrocarbon unit) happens to be the most preferred choice in majority of **synthesized local anaesthetics**,

(4) Evidently, the **lipophilicity** of a local anaesthetic is exclusively attributed by the embedded **'lipophilic centre'**,

(5) The **inducted lipophilicity of a local anaesthetic** solely depends on its capability to penetrate right into the cell membrane of the axon,

(6) The water-solubility characteristics are predominantly provided by the corresponding hydrophilic centre of the molecule. Undoubtedly, this constitutes a cardinal factor in the transportation of the **'drug substance'** (*i.e.*, **local anaesthetic**) to the membrane and once slipping inside the cell, subsequently moves on to the desired receptor site. Besides, hydrophilicity also aids towards the binding of the **'drug molecule'** ultimately to the receptor.

(7) An ideal **'local anaesthetic'** characteristic is best accomplished by **balancing the lipophlilic and hydrophilic centres**. In case the lipophilic centre is prevailing as the dominant structure, the ensuing anaesthetic action of the **'drug substance'** is poor by virtue of the fact that it is able to penetrate the lipoidal membrane of the axon, while its expected solubility in both the intracellular and extracellular fluids remains equally poor. In another situation, when the hydrophilic centre is found to be predominant in the structure, the ensuing anaesthetic action of the drug is weak in nature on account of its poor membrane penetration ability.

(8) The **dissociation constant**, ***p*K*a***, values, of various **'local anaesthetic'** drugs have been employed as an important and critical measure of their extent of **'ionization'** ; and, therefore, serves as a means of evaluating their lipophilic/hydrophilic ratio. Broadly speaking, the *p*K*a* values of quite a few therapeutically potent **'local anaesthetic'** drugs essentially fall within the range 7.5 to 9.5.

(9) Based on the above findings the *p*K*a* values essentially give rise to the following *two* distinct situations, such as :

(*a*) **Local anaesthetics having pKa values less than 8.0** — are observed to be not so adequately ionizable at the prevailing physiological pH in order to exert their effective influence in causing anaesthesia even though they are capable of penetrating the axon*, and

(*b*) **Local anaesthetics having pKa values more than 9.5** — are found to be practically fully ionized at the prevailing physiological pH ; as a result these 'local anaesthetic' drugs exert a significant less effective activity by virtue of the fact that they experience an obvious difficulty in penetrating the cell membrane.

10. The partition coefficient characteristic of '**local anaesthetic**' drugs, having identical structural features, exhibit an enhancement in activity corresponding to an enhancement in the partition coefficient values, unless and until a maximum activity is accomplished. Once the peak activity is reached, the activity starts decreasing progressively even though the partition coefficient gets enhanced appreciably.

*A process of a neuron that conducts impulses away from the cell body.

11. A comprehensive study* of the homologous series obtained meticulously by substituting the **aromatic ring** of '**local anaesthetics**' by such substituents as : alkyl, alkyloxy ; and alkyl amino moieties evidently displayed that the partition coefficients of the members of a series enhanced according to an enhancement in the actual number of methylene (—CH_2—) functions present in the substituent of that specific series. However, the maximum activity in a homologous series was observed with a C_4 to C_6 methylene chain. Likewise, the increase in the number of C-atoms of the substituents in the **'hydrophilic centre'** exhibited not only an enhancement of the partition coefficient but also an increase in activity.

    Example : The introduction of such functional moieties as : **diethyl amino ; piperidino ;** and **pyrrolidino** etc., — gave rise to newer products having almost identical degree of activity. Importantly, the **morpholino** function resulted in lowering the activity considerably.**

12. It is vehemently assumed that the **'local anaesthetics'** get bound to various tissue and plasma proteins by means of *three* vital physical forces, namely : **Van der Waals forces ; dipole-dipole attractions ;** and **electrostatic forces** as illustrated below in Fig. 5.1.

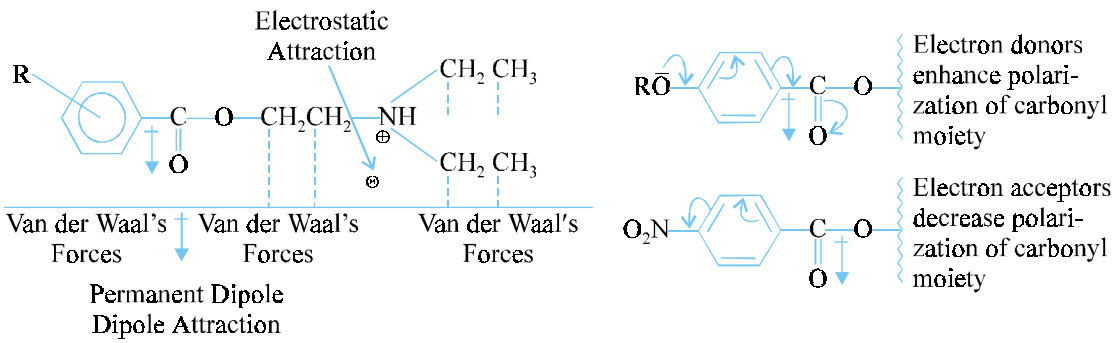


Fig. 5.1. A diagramatic sketch of the binding phenomenon of an ester-type local anaesthetic drug to a receptor site by various physical forces.

[Adapted from Buchi J and Perlia, X : In Ariens EJ (ed.) Drug Design, Academic Press, New York, Volume III, p-243, 1972]

Observations : The various important observations derived from Fig. 4.1 are as follows :

(*i*) The activity of benzoic acid-based '**local anaesthetic**' drug gets improved when the aromatic lipophilic centre essentially possesses electron-donor substituents (*e.g.*, O^-, COO^-, CHR_2, CH_2R, CH_3) ; and consequently gets reduced with electron-acceptor substituents (*e.g.*, NO_2 ; COR, CN, NH_3^+, COOR, Cl, Br, I, F, SH).

(*ii*) From (*i*) above one may safely conclude that the electron donor functional moieties enhances the binding capability of the '**local anaesthetic**' agent to the receptor site, whereas the electron acceptor functional moieties help in lowering this binding phenomenon significantly.

*Buchi J. and Perlia X., **'Local Anaesthetics Encyclopedia of Pharmacology and Therapeutics',** Section 8, Vol. I., Pergamon, New York, 39, 1971.

Ariens E.J. (ed.) **'Drug Design', Academic Press, New York, Vol. III, p-243, 1972.

Buchi and Perlia put forward a plausible explanation for lowering the binding phenomenon on account of an electron acceptor engaged in the withdrawl of electrons from the carbon of carbonyl moiety that eventually retards the polarization ability of this moiety. Furthermore, it also consequently reduces the actual strength of the dipole moment of the carbonyl group, which ultimately weakens its dipole-dipole attraction with the receptor.

(13) It has been proved with substantial evidence that **'local anaesthetics'** essentially containing **'amide'** functional moities show a tendency to afford a reasonably stronger bondage with the receptor site.

Examples : (*a*) Tucker *et. al.** proved that approximately 95% of **'bupivacaine'** (see Section 4.2.3.D) usually get bound to plasma and tissue proteins in comparison to 55% for **'prilocaine'** (see Section 4.2.3.B.).

(*b*) Tucker and Mather** further demonstrated that the relatively longer-acting and distinctly more potent **'local anaesthetics'** are more intimately and extensively get bound to the plasma proteins. Nevertheless, it may not be the only critical factor that controls the potency.

5. MODE OF ACTION OF SOME SELECTED LOCAL ANAESTHETICS

The specific **mode of action** of certain **local anaesthetics**, already discussed under Section 4.2, are enumerated below :

5.1. Amethocaine (or Tetracaine)

The onset of action is found to be rather slow but it is of relatively longer duration. It is normally hydrolyzed by plasma esterases to ***para*-aminobenzoic acid (PABA)** together with some other metabolites. Due to its inherent high level of toxicity it is invariably being employed for several restricted **'topical applications',** for instance : bronchoscopy, ENT-related surgical procedures, and local treatment of hemorrhoids.

5.2. Benzocaine

It is found to possess both low potency and low systemic toxicity. It is mostly employed as a **local topical anaesthetic** in conjunction with other similar agents ; though some of these mixtures may give rise to undesired allergic manifestations. Besides, benzocaine is also employed as a possible **sulphonamide antagonist.**

5.3. Bupivacaine

It is generally employed in solution form either alone or in combination with adrenaline (a vasodilator). It exhibits upto 95% ability to cause plasma protein binding. Because it exerts a minimal nerve motor block, hence it is specifically suitable for some surgical operations. The drug is largely metabolized in the liver ; and its metabolites are chiefly excreted in the urine.

5.4. Butacaine Sulphate

Its solution finds usage as a **topical local anaesthesia** in dentistry. It has also been employed in several ear and nose drops for the relief of pain along with other drugs.

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*Wilson and Gisvold's : **Text book of Organic Medicinal and Pharmaceutical Chemistry,** Delgado J.N. and Remers W.A., Lippincott-Raven, New York, 10th edn, p-647, 1998.

Tucker G.T. and Mather L.E., *Br. J. Anaesthesia,* **47, 213, 1975.

5.5. Dibucaine Hydrochloride (or Cinchocaine)

Its onset of action is found to be rapid, and action is of long duration. The overall extent of anaesthetic action is almost one fourth to that of **cocaine**. It has an apparent half-life of 11 hr, and gets metabolized mostly in the liver. It has been observed that the **'amide portion'** of the drug does not get hydrolyzed even to a small extent in the serum. Therefore, the prevailing sluggish rate of metabolism is perhaps responsible for giving rise to a relatively high degree of systemic toxicity.

5.6. Diperodon

Diperodon hydrochloride is usually used which is found to be slightly soluble in water ; however, its solubility may be enhanced by adding NaCl-solution. Importantly, even the traces of alkali will precipitate the free-base, and, therefore, it is always preferred that its aqueous solutions must be utilized as soon as possible.

5.7. Lidocaine (or Lignocaine)

It is one of the most widely used **local anaesthetics** having a plasma-protein binding ability of approximately 64% at therapeutic drug concentrations. **Lidocaine** is reported to penetrate the placenta ; however, fetal-plasma binding is only about half that found in maternal plasma. It is also found to cause depressive action on the cardiovascular system ; and perhaps based on this characteristic feature it is invariably employed IV for the control and management of cardiac arrythmias. Lidocaine is largely metabolized in the liver by the help of a plethora of **'metabolic pathways'** particularly using oxidases of the mixed function type. It has been duly observed that careful modification of the pH of lignocaine solutions by means of $NaHCO_3$ appreciably retards the noted discomfort caused in patients when the local anaesthetic is administered by infiltration anaesthesia.

5.8. Mepivacaine

Tullar resolved **mepivacaine** and demonstrated that the **(+)-isomer had an S configuration which displayed a significant long-acting profile**. It is found to get bound to the plasma proteins to a considerable extent, upto 78%, and exerts identical activities comparable to lidocaine. Its onset of action is rather sluggish and slow ; however, the **'local anaesthetic'** activity lasts much longer as compared to **lidocaine**. Admixtures with known vasoconstrictors usually prolong the therapeutic action. **Mepivacaine** is invariably metabolized in the liver, and less than 10% gets excreted unchanged in the urine. However, some other metabolites are normally excreted by the kidney and the bile, whereas relatively smaller amounts of the latter metabolites are found to be excreted through the faecal wastes.

In obstetrics, the maternal plasma concentration varies between 2.9–6.9 mcg.mL^{-1}, whereas the umblical vein concentration ranges between 1.9–4.9 mcg.mL^{-1}. Thus, the faetus is only exposed to 60–70% of that available in maternal plasma. It is found to have a $t_{1/2}$ of 1.9 hour, aVd of 1.2 Lkg^{-1}, and a partition coefficient of 12.1.

5.9. Pramoxine

It is mostly employed as a **topical anaesthetic** for the relief of insect bites, hemmoroids, and minor wounds. As the drug shows a stinging and burning sensation, hence it must not be used for the eyes, nose and throat at all. The local anaesthetic action commences in 3–5 minutes ; its potency is fairly comparable to that of **benzocaine** and is not adequate to abolish the gag reflex. It is also indicated in a 1% (*w*/*v*) solution for the rapid relief of pain in rectal surgery, episiotomies, anogenital pruritus, itching dermatoses and minor burns.

5.10. Prilocaine Hydrochloride

It has a plasma-protein binding of 55% and also having anaesthetic activity very much identical to that of cocaine. It has an onset of action rather slow, but the duration of action is almost comparable to that of lidocaine. It has been observed that **prilocaine hydrochloride** is slightly less toxic than **lidocaine ;** however, large doses of approximately 800 mg or more may cause severe methemoglobinemia*. It may pass across the placenta in due course and thus can cause methemoglobinemia in the *faetus.* It gets mostly metabolized in the liver, but also a portion in the kidney. Interestingly, one of the metabolites happens to be **2-methylaniline,** which seems to get subsequently metabolized to such compounds that are responsible for causing methemoglobinemia. Thus, 2-methyl aniline and other metabolites are duly exereted in the urine. A combination of **prilocaine** and **lignocaine** gives rise to an **eutectic mixture** (**eutectic point**) having *mp* below-either compounds, and is **used exclusively for the preparation of topical-dosage forms**.

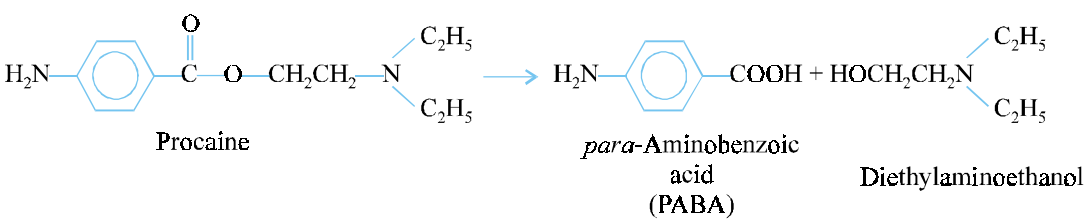
Approximately 55% of **prilocaine** is bound to plasma protein. After 600 mg of the drug, peak-plasma levels are accomplished in 20 minutes, at which time span plasma levels average 4 mcg mL^{-1}, the same dose with **epinephrine** also attains peak at 20 minute, at which time plasma levels average 2 mcg mL^{-1}. As a result of this, prilocaine is mostly used without **epinephrine**. Therefore, it is specifically useful for such patients who cannot tolerate vasopressor agents, such as : patients having diabetes, hypertension, thyrotoxicosis, or other cardiovascular disorders.

5.11. Procaine Hydrochloride

It is largely hydrolyzed in the plasma by plasma cholinesterase to produce **PABA** (this particularly prevents the action of sulphonamides) and diethylaminoethanol. Both these metabolites are exereted in the urine, the former being partially in the form of conjugates. Diethylaminoethanol is normally metabolized in the liver upto 70% approximately. It has been duly observed that procaine specifically prolongs the action of certain drugs by the subsequent formation of their corresponding salts which ultimately gets decomposed slowly to release the drug.

Example : IM injection of **procaine** retards the absorption of **Penicillin-G,** thereby prolonging its action significantly.

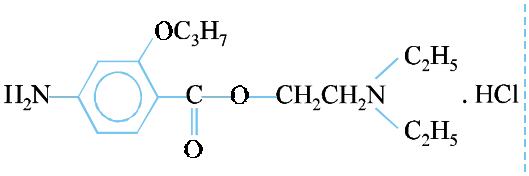
As stated earlier, the drug is affected by esterases ; and since the spinal-fluid virtually contains little or no esterase ; therefore, when given by this route of administration it remains active till such time it gradually gets absorbed into the general circulation.



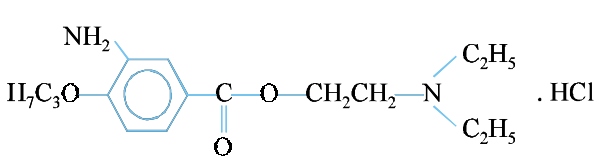
5.12. Propoxycaine Hydrochloride

It is nothing but a **structural isomer of proparacaine** ; and also being less toxic but slightly having lower potency than **proparacaine.**

*The clinical condition in which more than 1% of haemoglobin in blood has been oxidized to the ferric (Fe^{3+}) form. The principal system is cyanosis because the oxidized haemoglobin is incapable of transporting oxygen.



Propoxycaine Hydrochloride
[Less toxic ; Less potent than proparacaine]



Proparacaine Hydrochloride
(An *isomer* of Propoxycaine)
[More toxic ; More potent than propoxycaine]

Probable Questions for B. Pharm. Examinations

1. Differentiate between the '*Local anaesthetics*' and the '*General Anaesthetics*'. Is it necessary to include local anaesthetics as adjuncts in antiseptic creams used in severe burns and painful skin abrasions ? Explain with typical examples.

2. The '*anaesthesiophoric moiety*' present in local anaesthetics is essentially derived from COCAINE. Explain.

3. Einhorn's generalization of aromatic esters gave rise to :

(*a*) Methyl ester of *p*-amino-*m*-hydroxy benzoic acid,

(*b*) Methyl ester of *m*-amino-*p*-hydroxy benzoic acid

This ultimately led to the synthesis of certain potent local anaesthetics. Explain with suitable examples.

4. Explain how *PROCAINE* can be synthesized from :

(*a*) 2-Chloroethyl *p*-amino benzoate

(*b*) *p*-Aminobenzoic acid

(*c*) Ethylene chlorohydrin.

5. Justify why propoxycaine hydrochloride is eight times more potent than procaine hydrochloride.

6. Discuss '*tropane derivatives*' as potent surface anaesthetic agents. Give examples and synthesis of any one compound selected by you.

7. '*Amides*' constitute an important category of local anaesthetic. Describe mepvacaine hydrochloride.

8. Discuss the synthesis of a quinoline analogue *i.e.*, dibucaine hydrochloride from isatin.

9. Dimethisoquin hydrochloride [Quotane® (SK & F)] an iso-quinoline analogue may be synthesized from alpha-*n*-butylphenethyl amine. Explain.

10. How would you synthesize pramoxine hdyrochloride ? Explain its applications.

RECOMMENDED READINGS

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