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DRUG DESIGN

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SECTION A

1.

(i) Advantages of prodrugs are:

a. Enhancement of bioavailability
b. Improvement of stability or solubility properties
c. Decreased toxicity and adverse reaction
d. Increased site specificity
e. Increased duration of pharmacological effects
f. Alteration of pharmacokinetics

(ii) One school of thought views "lead" in 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
(b) Exploitation of Leads that requires the assessment, improvement and extension of the lead.

(iii) 'Biologically active prototype' is the development of a new drug molecule under the method of variation having the following advantages:

a. at least one new compound of known activity 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 e.Similar pharmacological technique for specific screening may be used effectively.

(iv) Two factors are:

(a) The smaller the expenditure of human and material resources involved evolving 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 drug.

(v) The principle of mixed moieties actually involves the conjunction of two or more different types of pharmacophoric moieties within a single molecule.

The development of ganglionic blocking agent-its development is based on the principle of mixed moieties. Here, the property of acetylcholine, an effective postganglionic parasympathetic stimulant and hexamethonium, a slightly postganglionic parasympathetic agent produce a high degree of ganglionic blockade.

(vi) The role of 'rigidity' in drug design is to improve potency of final drug molecule. 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.

(vii) A pharmacophore model can be proposed from the analysis of a set of biologically active ligands or from the analysis of enzyme or receptors active site structure preferably determined with abound ligand. Enzymes and receptors structures can be surveyed energetically by a program such as GRID to identify favourable interaction sites for a large variety of different functional group probes such as carbonyl group, an amine NH and an aromatic CH. The pharmacophore identification are based on pharmacophore based 3D database searching, conformational flexibility in 3D database searching, lead generation from 3D database pharmacophoric searches, complementary based 3D database searching.

(viii) Random screening is the old, traditional method of drug discovery in which drug molecules obtained from natural sources or synthetic origin are exposed to huge screening for testing their activities. This process is time consuming, costly and inappropriate method of screening, Here, maximum cases animal models are used for preliminary pharmacological screening.

(ix) Two methodologies in molecular modelling

a. Molecular mechanics
b. Quantum mechanics

(x) ALADDIN, CHEM DBS-3D, ISIS/3D UNITY

(xi) Typical 3D database recognised:

a. Complementary based 3D database
b. Conformational flexibility based 3D database
c. Pharmacophore based 3D database
d. Lead generation 3D database

(xii) The applications are as follows:

a. The method of Free and Wilson is based upon an additive mathematical model in which a particular substituent in a specific position is assumed to make an additive and constant contribution to the biological activity of a molecule in a series of chemically related molecules.
b. This method is preferred when nothing is known about the mode of action or when the physic-chemical properties of the substituent used are unknown. Best results with the Free-Wilson method are obtained in series with several positions available for substitutions and only if each substituent at any location is present in at least two compounds of the series.

SECTION B

2. The knowledge of pharmacodynamic processes and drug metabolism *in vivo* can be utilised to improve a wide variety of drug characteristics. The term prodrug was first introduced by Albert (1958) to describe compounds which undergo biotransformation prior to exhibiting their pharmacological effects. The reversibly modified compound usually inactive in itself is called a **prodrug** because it releases the active compound as a metabolite. Prodrugs could be divided into two classes:

(A) Carrier-linked prodrug- Becampicillin (B) Bioprecursurs- sulindac

Differences between Bioprecursors and Carrier Prodrugs

Characteristics	**Carrier Prodrugs**	**Bioprecursors**
Constitution	Active principles+carrier group	No carrier group
Bioactivation	Hydrolytic	oxidative or reductive
Catalysis	Chemical or enzymic	only enzymatic

Lipophilicity Strongly modified slightly modified

A. **Carrier–linked prodrugs** are formed from a temporary linkage of the active drug molecule with a transit moiety (promoiety) which is mostly lipophillic in nature. The linking of the bioactive substances to the carrier moiety can be either directly (bipartite prodrug) or through a spacer or connector group (link) (tripartite prodrug).

Characteristics of a prodrug:

(i) A covalent linkage is between the drug molecule and the promoiety (carrier moiety)
(ii) The prodrug is inactive or less active than the parent drug
(iii) The prodrug is a reversible or bioreversible derivative of the drug
(iv) The promoiety released after the drug delivery must be non-toxic and should not have its own pharmacological activity.

The aim of prodrug development is in most cases to solve specific pharmaceutic or pharmacological problems. The main objectives of prodrugs are as follows:

g. Enhancement of bioavailability
h. Improvement of stability or solubility properties
i. Decreased toxicity and adverse reaction
j. Increased site specificity
k. Increased duration of pharmacological effects
l. Alteration of pharmacokinetics

Drug absorption, distribution, metabolism and excretion affect pharmacokinetics.

(i) Increase systemic availability:
Ampicillin possesses low lipophilicity due to the presence of an amino group in the side chain and is only 30 to 40% absorbed when taken by oral route. Altering the polarity of this antibiotic by esterifying the free carboxyl group results in compounds completely absorbed, that is, with greater bioavailability than the parent ampicillin. Similarly, such esters have been prepared to increase bioavailability.
(ii) Interference with transport characteristics:

The introduction of a hydrophilic disposable moiety can restrict a drug to the GIT and prevent its absorption. Such a type of drug is represented by the intestinal disinfectant succinyl sulfathiazole.

Novel peptide delivery systems have been investigated as prodrugs of 5-Fu. The prodrug of 5-Fu was well absorbed orally and produced sustained 5-Fu levels.

(iii) Decreased toxicity and adverse reaction:

Carboxyllic acids and phenols are sometimesxtoo toxic to be employed as such in clinical practice. Ester prodrugs of the acidic NSAID are devoid of gastric ulcerogenic activity. The presence of the thiol group in captopril is considered as one of the responsible factors for the adverse reaction of drugs. The masking of the SH group in alacepril which in vitro regenerates this group results in a less toxic and probably longer acting drug.

(iv) Improvement of taste:

Some oral drugs with markedly bitter taste may lead to poor compliance especially as paediatric syrup. Chloramphenicol for such purposes is now usually formulated as the inactive tasteless palmitate or cinnamate esters. The active parent drug is released from these compounds by esterase present in the small intestine.

(v) Increased duration of action:

The prodrug bitolteral which is the di-p-toluene ester of N-t-butyl noradrenaline has been shown in drugs to provide a longer duration of bronchodilator activity than the parent drug. Furthermore, the prodrug is preferentially distributed in lung tissues rather than plasma or heart so that the bronchodilator effect, following subsequent biotransformation of the prodrug, is not associated with undesirable cardiovascular effect and is slow and prolonged.

(vi) Sustained release prodrug system:

Sustained release products have become one of the major areas of pharmaceutical research. Steroid hormone palmitate and antimalarial ester insoluble salts (eg, cycloquanil pamoate) can deliver the active drugs for a long time, cycloguanil for several months. This can be a great convenience for the patients especially in areas with remote medical facilities.

B. Bioprecursors

The design of bioprecursors takes into account the common metabolic pathways of phase I, such as oxidation and reduction. The classical example is prontosil, which undergoes a reduction to sulphanilamide.

(i) Bioactivation

Hydroxylation of cyclophaosphamide (anticancer agent) followed by metabolic decomposition converts the prodrug to the cytotoxic phosphoramide mustard.

(ii) N-dealkylation

Many drugs are transformed into active metabolites by N-dealkylation. Methsuximide, an anti-epileptic drug is demethylated in the body to the active form and the metabolite has 700 fold greater concentrations than the parent drug in the plasma.

(iii) O-Dealkylation

The analgesic phenacetin acts in the form of its dealkylated derivative, p–acetaminophenol.

(iv) Oxidation

The 3-formyl derivative of the antimicrobial, norfloxacin has recently been shown to act as a prodrug in which the formyl group is oxidised to a carboxylate group generating the active drug norfloxacin.

(v) Reduction

The NSAID sulondac is reduced in vivo to the active form. An unusual sulfone reduction has been proposed recently to explain the anti-leukemic activities.

(vi) Cyclization reaction

A number of aminophenone analogs of triazolobenzodiazepines have been considered as open chain analogs. Of these benzodiazepines actually function as their bioprecursors, because in vivo they cyclise giving triazolobenzo-diazepines as anxiolytic agents.

(vii) Selective bioactivation:

The insecticide, malathion (acetylcholinesterases inhibitor), is desulfurized selectively to the toxic malaoxon, but only by insect and not mammalian enzymes. Malathion is therefore relatively non-toxic to mammals.

3. Molecular hybridisation: This is 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 principale, whereby he exploited the beneficial properties of phenols and carboxylic acids possessing potent antibacterial characteristics features into the design of newer drug molecules with better and improved pharmacological activities by means of simple esterification.

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: Streptoniazid a) Antibacterial agent: A molecule of streptomycin and a molecule of isoniazid by means of a strong double bond between C and N with the elimation of a mole of water. The hyberdised molecule exhibits a significant potentiated antibacterial and tuberculosstatic agent.

b) Antitussive Expectorant drug: Guaicyl phenyl cinchoninate, amole each of cincophen and guaiacol gets hyberdised by forming an ester-linkage and losing a mole of water. The few product shows an improved antitussive and expectorant activity.

c) Antipyretic –analgesic agents: Quinine acetylsalicylate: Hybridisation takes place between a mole of acetylsalicylic acid and quinine to lose mole of water and the resulting hybridised product potentiates the antimicrobial activity along with substantial antipyretic –analgesic activity.

Rigidity and flexibility *vs* Drug design:

It has been observed beyond any responsible 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 of time two different situations may usually crop up, namely:

a) Increased rigidity: that may ultimately lead to improved potencies
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 targeted drug molecules.

Increased rigidity: There are a plethora of drug molecules which are inherently flexible in nature ie they can assume a wide range of shapes. Of these structural variants quite a few are absolutely not so favourably acceptable for reaction at a specific receptor site. Therefore the design of 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 targeted-drug molecules may be subjected to vigorous and critical examinations by the aid of several sophisticated latest physicochemical analytical devices, such as MASS, NMR, FTIR, ORD.

Examples: Structural analogues of acetylcholine , 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 referring drug:

It has been observed that the intraatomic distance between ‘O’ and ‘N’ atoms for the cis isomer ranged between 2.5-2.9Å, whereas, between the corresponding Trans isomers varied between 2.9-3.7Å. 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

S no	Drugs	Intra-atomic distance 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

A=Modified ACh with 5 membered ring

B= Modified ACh with 6 membered ring

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 isomer of A and B did not show any important in their cholinergic activities.

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 particularly negligible capacity to have them rearranged to a more favoured conformation that may ultimately give rise to enhanced bioactivity.

4. Quantum mechanics present for acceptance the most elaborate 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 reached by the help of quantum mechanical methods as they essentially and explicitly require a detailed description with regard to the specific electronic structure of the molecule. Therefore, the ultimate calculations involving the quantum mechanics should be used most carefully and judiciously to solve such intricate problematic quarries 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 which 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: i) Approximation of charge in a molecule, ii) Characterization of ensuring molecular electronic potentials, and iii) Parameterization for 'quantum mechanics'. The applications of 'quantum mechanics' in the field of molecular mechanics may be categorized into three vital groups, namely: 1) Charge and electrostatics, 2) Parameterization of force fields, and 3) Chemical reaction modelling and design of transition-state inhibitors. These three different aspects of the applications of 'quantum mechanics' in explaining molecular mechanics shall now be treated individually with appropriate example wherever necessary.

Charge and electrostatics: It has been well established that either *ab intio* or semiemperical quantum chemical modalities may be adopted effectively in order to determine precisely the prevailing charges in molecular mechanics. Importantly, the quantum mechanics 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 exact representations of the resulting overall net atomic charges borne by atoms duly present in molecule. However, these net atomic charges may appear in different environments and modes such as: i) atom-cantered monopole, ii) atom-cantered dipole and iii) atom-cantered quadruple.

Examples: a) *atom-cantered monopole*: In fact, the one-centre charge on an atom is being specifically assigned to the 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 usages of atom-cantered monopole.

b) *atom-cantered dipole*: The precise and ultimate molecular recognition is exclusively depended upon the ensuring 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 guide entirely upon one with a view to obtain reproducible MEP values.

c) *atom-cantered quadruple*: The ultimate desire to attain reasonably accurate and reproducible MEP values may be accomplished by making use of an enhanced complexity of the model.

Parameterization of force fields: It is a well established fact that the molecular mechanics is absolutely essential and therefore the parameters are divided exclusively via interactive evaluation to typical computational results *viz* a) Molecular geometry (*i.e.* Bond length, Bond angle, Dihedrals) and b) Heats of formation, in comparison to the various experimental values.

Importantly, crystallographic techniques have adequately made available a substantial degree of vital experimental database derived from bond length, 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 system that lack sufficient experimental are scantily available. Trado-Rivese and Zorgensen

(1990) parameterized via fitting carefully the characteristics figures of bulk liquids to MonteCarlo simulations to yield duly the AMBER/OPLS force field.

Chemical modelling and design of transition inhibitors: It has-been amply demonetised that certain enzymatic reactions where in chemical transformation do take place, one should specifically make use of the "quantum chemical methods" in order to deal effectively with ensuing electronic alterations in both bond cessation and hybridization. Hence the most viable option would be to effect hybridisation judiciously and there by the reaction core is quit often model quantum mechanically where as the remaining by aid of the molecular mechanics. Andrews et al. and Eksterowicz etal meticulously pioneered modelling of the particular transition states of prevailing enzymatic reactions to accomplice the design of the desired transition states inhibitors.

5.a. Besides π and log p (for a molecule) other parameters that describe lipophilicity of the drug molecule include, partition coefficient R_M value, molecular connectivity index and van der Waals volume V_w. In 1965, Boyce and Miliborrow suggested the use of R_M value from reversed-phase thin layer chromatography as alternative lipophilicityparameter in QSAR $R_M = \log(1/Rf - 1)$. However $R_M$values cannot be regarded as true equilibrium parameters. Usually silica gel palates are impregnated with liquid paraffin, silicone oil, ethyl oleate or n-octanol as stationary phases.While mobile phases may consist of mixtures of polar solvents like methanol, ethanol or acetone with water or aqueous buffer solutions. Many advantages are associated with the use of $R_M$values instead of use of partition coefficients , These include 1) Only minute quantity is required. 2) Since the impurity donoteffectRf values, the compounds need not to be pure. 3) Since the determination of Rf value is much quicker and less tedious process, a number of compounds can be investigated simultaneously on the same plate. 4) No specific quantitative analytical method is involved in the spot localization. 5) Rf value can be calculated for both very polar and very lipophilic substance with equal ease by using a wide range of solvent mixtures. 6) log P value cannot be calculated in following cases: a) labile substances may decompose under experimental conditions. b) Mutual electrical interactions may occur between the substituents. The most prominent disadvantages of chromatographic method are:

1) The sensitivity of Rf values to the experimental conditions. 2) Different stationary and mobile phases make it impossible to combine different seats of R_f values due to lack of uniformity. 3)Chromatographic behavior of a drug is not identical to the drug

partitioning in a biological system. 4) fine separation of the small spots between a relatively narrow range of $R_f$value (0.2-0.8) is needed. Hence the selection of suitable solvent system becomes some times problematic. 5) Large changes due to ionization or H-bonding may cause departure from linearity.

5.b. Craig plot is a simple graphical plot of π versus σ or any such two parameters to guide the selection of next substituent. In other word, Craig Plotare two dimensional plot of one parameter against another. The plot are divided into four sections corresponding to the positive and negative values of the parameters. They are used in conjugation with an already established Hansch equation for a series of related aromatic compounds to select the aromatic substituents that are likely to produce highly active analogues. For example, suppose Hansch analysis carried out on a series of aromatic compounds yield the Hansch equation'

Log I/c=2.67 π-2.56σ + 3.92

To obtain high value for the activity it is necessary to pick up a substituents with a positive π and a negative σ value, than substituent should be taken from the lower right hand equation of the plot.

Advantages of Craig Plot:

1) The plot shows clearly that there is no overall relationship between π and σ
2) It is possible to tell the glance which substituent have positive π and σ parameters, which substituents have negative π and σ parameters.
3) It is easy to see which substituents have similar π values.
4) It is use full in planning which substituents should be used to derive the nmost accurate equation involving π and σ.

However it is emphasized that the use of Craig Plot does not guarantee that the resultant analogues will be more active than the lead because the parameter used may not be relevant to the mechanism by which the analogies act.

6. Mathematics has a quit impressive record of biomedical applications. To mention just a few growth and propagation of tumors, computational neuroscience, design of implantable devices and drug delivery mechanisms, genetics, computerized tomography, expert systems, clinical analysis, and epidemiology. This application which goes by the name of molecular topology is still young, its origin dating from the 1970, when LB Kier and LH Hall and other

researchers started using indices based on graph theory to study some physicochemical properties of organic compounds, like formation heat and boiling temperature, they found that those properties can be expressed as linear combinations of a few such indices. The application of molecular topology to the pharmacological research was only a matter of time, the pioneering work being done in mid 1980s and the first paper appearing at the begening of 1990s, whatever the field the interest on molecular topology is clear: predicting with confidence some specific activity of a molecule saves time and money. Although the applications of molecular topology are manifold, we will focus on the pharmacological ones become of their novelty value and social impact. Basically molecular topology builds on the somewhat surprising correlations existing between a given physical, chemical or biological property of a substance and the corresponding molecular characterization provided by some numerical descriptors generically called topological indices. So to speak, these indices encapsulate structural information at the molecular level which is pertinent to the property in question. Other subsequently proposed ideas, called topological charges indicates, incorporate also physicochemical information in the form of the number of valence electrons. To illustrate the modus operandi of molecular topology. Suppose that a new drug with a specific activity is sought, once an optimal suite of topological indices has been selected with the aid of known active molecules, a classification function is produced to distinguish between active and inactive molecules. This classification between active and inactive molecules. This classification function is then used to filter potentially active candidates from a chemical data base. If the data base contained naturally occurring molecules and the activity of the selected molecule was unknown before, the result is the discovery of a new drug. If the data base contained the synthetic molecules, we are dealing with the inverse task, design of new drugs. Last but not the least. The predicted activity of the candidates is put of test in vivo or in vitro. Beside the molecular technology there are other technologies for molecular design, but they are not so straightforward nor are they always applicable. In particular, in the case of drug design exnovo i.e. design of entirely new drug, these techniques, unlike molecular topology, require information on the biological receptor. It is worth highlighting that graph theory is a fine instance of pure mathematics that has found a variety of applications in the course of time, graph theory became in the twentieth century an essential tool in any area of science and technology where connectivity plays a role. Think for instance of the optimization of communication and transport networks, the design of electrical circuits, the synchronization of interacting oscillators with different topologies, the analysis of social network, etc. interestingly enough it was Cayley who pointed out the

correspondence between certain chemical constituents and graph technologies. molecular topology deals with application of graph theory to the description of molecular structure. To fix the basic concepts and the notation, let us recall that a graph G is a set of points, called vertices. Along with a set of link, called edges, joining some pairs of vertices..the set of vertices will be denoted by V=V(G) and the set of edges by E=E(G). formally a graph is an ordered pair of sets G=(V, E) where E is a subset of unordered pairs of V, We consider only finite graphs, that is graphs with a finite number of vertices and ages. In this case G denotes the order or number of vertices of G, which can be thought to be numbered in some convenient way. We say that two vertices are adjacent if they are joined by an edge 'e', in which we write $e=e_{ij}=e_{ji}$ alternatively we say that i, j, ϵ, V are the endvertices of e_{ij}, ϵ, E. further more, the number of adjacent varieties to a given vertex j ϵV will be called the degree of I and denoted by deg. A path P is a graph of the form

$V(P)=\{i_o, i_v \ldots, i_j\}$ $E(P)=\{ e_{ij} =e_{ji} \ldots. e_{ij} =e_{ji}\} \ldots(1)$

7. 'Divide and rule' concept in design of legends: In the past two decades, several newer methodologies had 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 pharmacoforic moieties. Subsequently the chemical components that happened to complement to each specific subsite are now meticulously designed or suitable retrieved from the various available databases. The final shape of the deigned molecule is duly accomplished by joining the selected, identified and determined fragments to yield the agreed legends respectively. Interestingly the major advantage of such an excellent concept being the scope and manoeavrability may be substantiated sufficiently via the combinatorial assembly of a plethora of available subcomponents.

DOCK Programme:

Desjaralis etal (1990) first and foremost introduced to utilise this novel concept and philosophy in the well known program termed as **DOCK**

Interestingly, the DOCK program exclusively looks for the 3D database 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: This programme particularly diles with a receptor volume of interest 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 confident.

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

SPLICE- Programme: This programme may specifically help in the generation of various desired permuations and combinations of hits automatically, i.e., in a programmed manner.in fact, SPLIC uslally recognised as a companion programme which logically and gainfully aids in the combination of ligand-recepter combination to a maximum level via overlapping of the ensuing bonds.

Docking a Flexible Ligand-Difficulties Encountered: A tremendous amount of research 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 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,
c) Most commonly observed difficulties in the generation of precise force fields,
d) Exceptionally huge computation time for the determination of conformational freedom in ligand, for instance.
e) Observed rotational movement of ligand.
f) Noticeable dynamic flexability as displayed by the target molecules.

g) Unavoidable and inherent trapped warer molecules.

Methodologies for Doking: 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 following:

A. **Interactive Graphics:** This represents 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.

   Examples: a)For analyzing the crystal structure of a target-drug molecule
   b)For docking of a ligand at the active site.

   In general these methods do require prominently the wisdom.

B. **Docking by Superimposition:** It has been established beyond any reasonable doubt that one may with great ease and convenience, superimpose an altogether new ligand derived meticulously from the 3D coordinate structures of the available ligand-bond protein upon the prevailing ligand.

   Example: a) HoltZ and Folkers Exemplified the typical case of a major histocompatibility complex (MHC).

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.

   Example: a)DOCK 3.5: KUNTZ *et. al*.(1982) introduced a highly promising and effective means for the 3D database search

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.

E. **Flexible Docking:** This usually refers to a not so rigid kind of virtual screening drug design technology so as to evaluate specifically the binding of ligands to the macromolecular targets.

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, the important Docking Programmes commonly used in the design of newer molecules are DOCK3.5, DOCK4 etc.

8. QSAR equation are very much related to physiochemical parameters. Again, biological activities of most of the drugs are related to combination of physiochemical properties. Various methods are used to draw the QSAR model:

A. Hansch Analysis: This is the most popular mathematical approach to QSAR introduced by Corwin Hansch. It is based on the fact that the drug action could be divided into two stages:

i) transport of drug to its site of action

ii) the binding of drug to the target site

Each of these stages dependent on the chemical and physical properties of the drug and its target site. In Hansch analysis these properties are described by the parameters which correlate the biological activity. The most commonly used

Physicochemical parameters for Hansch analysis are log p, π, σ and steric parameters as practically all the parameters used in Hansch analysis are linear free energy related (i.e., derived from equilibrium constant) so it is known as “linear free energy approach” or “extra thermodynamic approach”.

If the hydrophobicity values are limited to a small range then the equation will be linear as follows:

Log (1/c) =k_1 log p +$k_2\sigma$ +k_3E_3 +k_4 , where k_1, k_2, k_3 are constant obtained by least square procedure, c is the molar concentration that produce certain biological action.

The molecule which are too hydrophilic or too lipophillic will not be able to cross the lipophillic or hydrophilic barriers respectively. Therefore, the p value is spread over a large range, then the equation will be parabolic and given as:

Log(1/c)= $k_1(\log p)^2$ + k_2 log p+$k_3\sigma$ +k4ξ_5 +k_5

The constant k1-k5 are obtained by least sqare method. Not all the parameters are necessarily significant in a QSAR model for biological activity. To derive an extra thermodynamic equation following rules are formulated by Hansch:

a. Selection of independent variables. A wide range of different parameters like log p, π, σ, MR, steric parameters etc should be tried. The parameters selected for the 'best equation' should be essentially independent i.e., the intercorrelation coefficient should not be larger than 0.6-0.7.
b. All the reasonable parameters must be validated by appropriate statistical procedure i.e., either by stepwise regression analysis or cross validation. The best equation is normally one with lower standard deviation and higher F value.
c. If all the equations are equal then one should accept the simplest one
d. Number of terms or variables should be at least 5 or 6 data point per variable to avoid chance correlations.
e. It is important to have a model which is consistent with known physical organic and biomedical chemistry of the process under consideration.

B. Free Wilson Model:

The presuppositions made by Bruice et al during study of thyroxine analogs were extended further by Free and Wilson in a more generalised form with a hypothesis that groups make linear contributions either positive or negative to the basic skeleton. The method of Free and Wilson is based upon an additive mathematical model in which a particular substituent in a specific position is assumed to make an additive and constant contribution to the biological activity of a molecule in a series of chemically related molecules. This method is based on the assumption that the introduction of a particular substituent at a particular molecular position always leads to a quantitatively similar effect on biological potency of the whole molecule, as expressed by the equation.

Log BA= contribution of unsubstituted parent compound + contribution of corresponding substituent = $\mu + \Sigma aij$, where i is the number of the position at which substitution occurs and j is the number of the substituents at that position while μ is the overall average.

This method is preferred when nothing is known about the mode action or when the physic-chemical properties of the substituent used are unknown. The principle of Free-Wilson method can be illustrated with the example of acetylenic carbamates having antitumour activity.

BA=f(R) + f(R1) + f(R3) + μ, where μ is the biological activity of unsubstituted acetylenic carbamate.

Advantages of Free-Wilson approach:

a. It is simple, fast and cheap method where no substitution constants like pi, sigma, Es etc were considered.
b. The greater the complexicity of the structure, the larger is the number of possible substituents at desired positions. Hence, the efficiency of this method is high
c. At each position, the contribution of each substituent can clearly be identified. The substituents which can or cannot fulfil the principle of additivity can be recognised.
d. The Free-Wilson method is effective especially when substituent constants are not available.

Disadvantages of Free-Wilson approach:

a. A prediction of activity increments outside the substituents used in the data set by extrapolation is rather impossible.
b. The assumed independence of the influence of substituents on the total activity is often not seen in practice.

To overcome above mentioned disadvantages, based on the Free-Wilson additivity approach, two other modifications were derived, namely the Cammarata model and the Fujita-Ban method.

C. Mixed Approach

Hansch analysis and Free Wilson model differ in their application, but they are closely related. Mixed approach of this with indicator variable offers the advantages of both, Hansch and Free-Wilson analysis and widens their applicability.

The mixed approach can be written as:

Log $1/c = \Sigma aij + \Sigma k_j \phi j + k$

Where kj represent the coefficient of different physicochemical parameters; Σaij is the free – Wilson part for the substituent and $\phi j = \pi$, σ and Es contribution of the parent skeleton.

Today the mixed approach is the most powerful tool for the quantitative description of large and structurally diverse data sets.

D. Other Quantitative Models:

The Cluster Significance Analysis (CSA), Discriminant Analysis, Minimal Topological Difference (MTD) method, Molecular Orbital Method, Principal Component Analysis (PCA), The Topless Decision Tree, Craig Plot.