

Theoretical Aspects of Rational Drug Design — An Overview[†]

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The major techniques of drug discovery processes for the past thirty years have been summarized. However, because of rapid advances in information technology and emergence of plethora of newer techniques, e.g., PCMM, UPGMA, MMG, FALS, MMFF, etc., this short review obviously does not give an exhaustive coverage. The paper summarizes different approaches of rational drug design methods with a primary focus on quantitative structure-activity relationship (QSAR) and molecular modelling studies. Apart from an overview of classical QSAR tools (Hansch approach, Fujita-Ban modification of Free Wilson model and topological schemes) and different mathematical methods of QSAR, different components of molecular modelling including techniques of computational chemistry (quantum and molecular mechanical approaches) are briefly discussed. Various receptor-dependent and receptor-independent 3-D QSAR methods and techniques like protein modelling, *de novo* ligand design and receptor mapping are also summarized. Some other trends in recent drug development process like mass ligand screening, recombinant DNA technology, peptidomimetics, oligonucleotide therapeutics, carbohydrate based drug design, and prodrug design are also mentioned.

Introduction

Exploration for lead and its exploitation have been the two mainstays of medicinal chemistry^{1,2}. As part of exploitation, principal aim of a medicinal chemist is to discover novel drugs with greater potency and reduced toxicity which may be achieved by molecular modification or tailoring of existing drugs, optimization of various lead compounds, isolation of active constituents from natural sources or syntheses of new series of compounds³. Identification of a lead compound for a particular activity is a real problem in drug design. Recognition of biochemical principles of drug action is a prerequisite for drug discovery process. A rational explanation of drug action is often limited by our ability to correlate the observed physiological effects with a reasonable hypoth-

esis or concept. Various structural, physicochemical, and biological parameters are used to correlate those with biological activity and the observed relations are used to predict activity of a new compound and this information is exploited to develop newer molecules of optimum activity. Such correlation may also help in exploring the mechanistic features of biological activity⁴.

Biological activity is an aftermath of various interactions of a bioactive substance at critical reaction site in the biological system that occurs simultaneously with complex series of events encompassing pharmacokinetics of the substance⁵. Drugs have varied structures, diverse biological activities, and multifarious modes of action. Despite the certainties of chemical structures of drugs and their biological activities, their mechanistic aspects are overshadowed by a great degree of uncertainties of the intervening steps between drug administration and response that make the drug response phenomenon a complicated multistep process⁶. Drug molecules have to confront the uncertainties of absorption, transport, metabolism, excretion and, above all, the 'random walk' to the critical reaction site and their subsequent adsorption and binding with the receptor. To circumvent the complexity, the biological aspect of the disease and / or drug action should be understood to the finest level, as far as possible. A *proper understand-*

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ing of the target site or process and influence of the structural or physicochemical attributes of drugs on the activity *is the basis of rational drug designing*. The various factors regulating pharmacokinetics and pharmacodynamics of a bioactive substance are considered for mechanistic interpretations of the activity. Once the factors are identified and their relationships with the intervening steps between drug administration and biological response are established, the process of drug design and tailoring and/or modifying structures of drugs becomes easier to be carried out⁴.

(A) Complexity of Biological System and Biological Activity

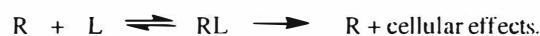
After administration into complex biological system, a drug undergoes processes like absorption, transportation ("random walk") to various compartments, including the critical reaction site and interaction with the active site. The drug also undergoes simultaneous biotransformation and elimination⁷. These processes encompassing pharmacokinetics and pharmacodynamics of the drug are regulated by the factors like solubility, partition coefficient, surface activity, degree of ionization, isosterism, interatomic distances between the functional groups and stereochemistry of the drug, apart from the biological factors⁸. Because of interplay of multifarious factors, a great degree of complexity and uncertainty, that overshadows the mechanistic aspects, prevails in drug response phenomenon.

Drug Receptor Interactions

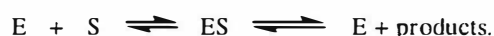
Drug molecules elicit response as a result of interaction with specific functional groups of macromolecular complex, located on cell surface or within the cells and having definite three dimensional geometry^{9, 10}. These macromolecular sites with which drugs bind and interact are termed as receptors. In a generic sense the term 'receptor' may be used to describe various recognition sites (including enzymes) at which drugs act¹¹.

For drug-receptor interaction, the drug binds to the receptor and alters nature of receptor interactions with its associated membrane components to effect a change in cellular and tissue function through transducer mechanisms. Both the ligand (L) and the receptor (R) are believed to undergo conformational changes but there is no chemical change of ligand from such interactions¹¹. Thus, the ligand is not directly involved in the conse-

quences of receptor activation, though it may take part in the subsequent feedback inhibition through changes in activity of intracellular messenger systems.



In case of enzyme (E)-substrate (S) interactions, substrate undergoes a catalytic change to a product or products. The product is then used in cellular events or alternatively can act as feedback modulators.



Compounds interacting with receptors have two properties¹²: (i) affinity or potency (the ability to recognize and bind to a receptor) and (ii) intrinsic activity or efficacy (ability of the ligand to activate the receptor and induce conformational changes to effect a change in cellular process via activation of transmembrane transductional mechanism, involving G-proteins or ion channel). Activity is defined in terms of affinity of the ligand (K_i or K_d), the reciprocal of association constant K_a .

Drugs having same affinity may have different degrees of efficacy^{12, 13}: (i) agonists have both affinity and maximal efficacy (e.g., morphine is a strong agonist of μ opioid receptors); (ii) competitive antagonists have affinity but no intrinsic activity (e.g., naloxone is an antagonist of μ opioid receptors); (iii) partial agonists have affinity and submaximal intrinsic activity (e.g., butorphanol is a partial agonist of μ opioid receptors); (iv) inverse agonists have affinity and negative intrinsic activity (e.g., dimethoxyethylcarbomethoxy- β -carboline is a benzodiazepine receptor inverse agonist).

In addition to the affinity of a receptor for its ligand, the response is also dependent on the number of receptors in a given tissue. Receptors, in general, are divided into two major groups: (i) G-protein coupled receptors and (ii) ligand gated ion channels. Other types include voltage sensitive ion channels.

Receptors subserve two essential functions: (i) recognition of the specific ligand and (ii) transduction of the signal into a response. Accordingly, a receptor has a ligand binding domain and an effector domain that undergoes conformational changes.

Drug molecules interact with the functional groups of receptors by utilizing various bonding forces (involved as these when simple molecules react) like ionic, hydrogen, ion-dipole, dipole-dipole, van der Waals and hydrophobic and occasionally covalent (e.g., anticancer

alkylating agents, irreversible enzyme inhibitors, etc.)¹⁴.

Drugs directly interacting with receptors exhibit structural (and stereo-) specificity and high potency, and specific antagonists are available or can be found. On the other hand, drugs altering solvent property do not need structural specificity and are required in large quantities and no specific antagonist may be found¹². In addition, drugs act by physical/chemical means and as anti-metabolites leading to production of nonfunctional or dysfunctional cellular components. These constitute non-receptor mediated drug action^{12, 14}.

Biological Activity : Factors Involved

In biological experiments, two factors viz., dose and response, are principally measured. Biological response depends on various factors related to drug, patient, formulation, etc. Only the drug related factors are discussed:

1 Drug Related Factors

To explore compounds of better activity and throw an insight into the intervening steps, biological activity of a congeneric series is often related to the structural variations and changes in physicochemical properties. Sometimes an indirect relationship is established with some other biological parameters like protein binding, serum level of various endogenous compounds and metabolites of drug substances and lipid peroxidation.

Influence of Structural Variations on Biological Activity

Biological activity depends on types and magnitude of interactions of a drug molecule with the active site. Therefore, structural features like electronic distribution, stereochemical property, and surface property of drug (and of receptor site) play significant role in the mediation of activity¹⁵. Depending upon the degree to which structural features influence biological action, drugs, in general, are classified into structurally nonspecific and specific categories.

1 Structurally Nonspecific Drugs—Biological effects of structurally nonspecific drugs are more closely related to their physical properties than with their chemical structures. Mere presence of structurally diverse compounds in tissue may lead to such effects⁹. General anaesthetics, volatile insecticides and certain bactericidals are generally classified under this category.

2 Structurally Specific Drugs—Most of the pharmacologically active drugs show structural specificity in action. The structural requirements for optimum receptor fit are complementary to the receptor geometry. Variations in structure of a congeneric series of drugs lead to changes in potency. Structural specificity is exhibited, both in cases of affinity of binding to the receptor site and intrinsic activity (ability to induce required conformational changes of the receptor site after binding)⁹.

Introduction of different substituents to the same ring system may give drugs of different pharmacological classes, e.g., different phenothiazines are used as antiparkinsonian (e.g., ethopropazine), antihistaminic (e.g., promethazine) and antipsychotic (e.g., chlorpromazine) agents. The degree and kind of effects of structural variation on biological activity vary in different groups of drugs : replacement of methyl group of tolbutamide (a short acting hypoglycemic) leads to chlorpropamide (a long acting antidiabetic) while replacement of *N*-methyl group of epinephrine (hypertensive) with *N*-isopropyl group gives isoproterenol (hypotensive). The spatial arrangements of atoms in three dimensional space, play major role in pharmacological properties, because many of the reactions of drug-receptor interactions are stereospecific. The orientation of different atoms in the drug molecule should be such that the different groups of the drug can optimally interact with receptor functionalities and induce necessary changes in receptor geometry. A high degree of stereospecificity (geometric / optical / conformational) is observed in some cases⁹, e.g., (i) *trans*-isomer of diethylstilbestrol has greater oestrogenic activity than the *cis*-form; (ii) (+) epinephrine is less active than (-) epinephrine for interaction with adrenoceptors; (iii) the probable pharmacophoric conformation of the tranquilizing drug 4-(4-hydroxypiperidino)-4'-fluorobutyrophenone is one having piperidine ring in chair form with axial hydroxy group. Stereoselectivity may also be important for selective metabolism, selective penetration through membrane, etc. The concept of isosterism is also important for understanding of the effects of structural variations on biological activity¹⁴.

Influence of Physicochemical Properties of Drugs on Biological Activity

Kinetics (ADME parameters) and dynamics (mechanism) of drug action are generally influenced by various

physicochemical parameters of drug substance¹⁻³, some of which are discussed subsequently.

(i) *Hydrophobicity* — Lipids being important constituents of all kinds of membranes, hydrophobicity of drug is an important parameter influencing absorption of drug from the site of administration and its partitioning to different compartments of the body (distribution pattern) and finally interaction with the receptor site which may have lipophilic area for hydrophobic interaction with the drug. However, optimum lipophilicity is required to maintain sufficient concentration of drug in extracellular fluid. Lipid solubility also plays an important role during elimination process and in determining half life of a drug. Hydrophobicity is mostly expressed in terms of partition coefficient ($\log P$) using n-octanol-water system. Various chromatographic parameters like R_M , $\log K'$, etc., also have been used instead of $\log P$.

(ii) *Electronic Influence* — Various electronic influences like dispersion forces, charge transfer interactions, electrostatic interactions, hydrogen bonding, polarization effects, and acid-base catalysis influence biological activity. Many interactions may occur through multiplicity of mechanisms.

(iii) *Steric Influence* — Various steric effects like intramolecular steric influences of substitutions on molecular properties, specific influence on the fitting to the receptor connected with the bulk and spatial arrangements of the substituents, conformational influence, and receptor requirement for specific steric configuration play an important role in drug action.

Influence of Interactions of Drug with Non-target Sites in Biological System

The therapeutic action (targeted biological activity) of a drug is evoked by the action of the drug on the receptor site, but the course of the action is often regulated by various actions of the drug on non-target sites in the body, i.e., biological action of a drug is influenced by its effects on various constituents of biosystem that are not directly linked with the pharmacodynamics of the drug. These side reactions may modulate the pharmacological action of the drug and/or contribute to the toxicity. Some important biological parameters are *protein binding*^{12, 14, 16}, *tissue binding*^{12, 14}, *interactions with membrane lipids*^{17, 18} (including drug induced lipid peroxidation^{19, 38}), etc.

2 Patient Related Factors

Various patient related factors like age, sex, body size, species and race, genetics and physiological variables (gastrointestinal physiology, pathological states like liver and kidney disease, congestive heart failure, thyroid disease) modulate drug action.

3 Formulation Variables

Mode of drug administration, drug release profile, drug-excipient interaction, manufacturing process variables, etc., may modify drug action.

4 Other Factors

Other factors include environmental conditions, drug-drug interaction, etc., which may modulate drug action.

(B) The Drug Development Process

The process of drug development is time consuming and costly affair that can no longer be satisfied by classical and empirical mode of research. In the late 1980s and early 1990s, it required approx. 150 - 250 million dollars and about 12 to 15 y to bring a drug to market³⁹. The chance of discovery of a new agent has diminished to 1 in 10000 and the situation is even more unfavourable with anticancer and antiviral agents³. Splinks claimed that in a purely randomized search some 4×10^8 compounds would have to be investigated before a therapeutically applicable drug could be discovered³.

The time and cost requirement of drug development process are due to thoroughness and caution prescribed. The various stages of classical drug development process are as follows⁴⁰:

(i) Synthesis of compounds and their initial screening for pharmacological activity: thousands of compounds are synthesized and subjected to *in vitro* and *in vivo* pharmacological screening in search of the best candidates for the subsequent step.

(ii) The requisite preclinical animal studies of the selected compounds (about dozens) for both short-term and long-term toxicity.

(iii) Phase I clinical trials in healthy volunteers of the few compounds (two or three) selected from step (ii).

(iv) Phase II clinical trials in limited cohort of patients with target disease: some of the compounds under clinical trial are usually eliminated from further consideration when unforeseen side effects occur.

(v) Phase III clinical trial in broad population of affected patients.

After stringent criteria have been satisfactorily met in each of the above steps, FDA approval is given so that a compound can be marketed. A significant cost built into the drug development process is the expense of synthesis and testing of the unsuccessful drug candidates. To reduce the cost and time requirement, the probability of obtaining potential and prospective agents should be increased.

(C) Theoretical Aspects of Rational Drug Design (RDD)

Earlier, drugs were designed by systemic modification of chemical precursors using standard tools of medicinal chemistry. But, the approach of Edisonian research for synthesizing organic molecules with an objective of obtaining medicinal compounds with desired biological activities is not effective in the present days, in view of heavier demands to be met by the new molecules. Random synthesis is quite time consuming and expensive, and also failure rate is often high in this approach, as it does not adequately and properly utilise the information obtainable from the compounds already synthesized or available³. As in the process, the prospective drug substances have to cross long and rigid methodologies of tests and should satisfy all the requirements, which makes the probability of success very little. Thus, pharmaceutical research and drug discovery involves a gamble at a very high stake. The rational approach of drug designing is, therefore, a natural choice to enhance probability of success as well as to minimize labour, time, and cost. The quantitative aspects of the biological activity and the mathematical relationships existing between the biological activity (BA), chemical structure (C) and physicochemical properties (P) must be understood for the rational drug design (RDD).

Quantitative Structure-Activity Relationship (QSAR) Studies

This non-experimental part of drug design encompassing study of both structure-activity and structure-property relations in broad sense is an intellectual exercise of assembling, manipulating and examining data obtained from physical, chemical and biological experiments and correlating these to biological activity. Biological activity of a drug depends on the types and magnitude of in-

teractions between the receptor and the drug molecule³. Various structural attributes of the drug molecule like electronic distribution, steric feature, etc., are the determining factors regulating the interactions. All Quantitative Structure-Activity Relationship (QSAR) studies are based on the notion that BA is function of C and/or P.

$$BA = f(C, P) \quad \dots(1)$$

The goals of QSAR studies include better understanding of the modes of actions, prediction of new analogs with better activity, and optimization of the lead compound to reduce toxicity and increase selectivity.

The knowledge of biological system, various factors regulating physiological processes and those contributing to pathological states, a thorough examination of molecular structures of drugs and their properties and unearthing of the factors modulating biological activity of drugs are required to find out the biochemical rationale of drug action. Such understanding helps to develop more effective drugs in a scientific way potentially reducing the cost of drug discovery, time, and manpower requirement³ (Table 1).

1 Activity-Property Relationship Studies

The first quantitative correlation of biological activity was made with physical property rather than the structure¹⁵. Probably the reason was that the concept of structure was ill defined till 1929 when the symmetric structure of benzene was confirmed by X ray studies. In 1901, Meyer and Overton drew attention to the significance of lipid solubility as a determinant of biological activity. They showed that narcotic effect of a wide variety of compounds could be correlated with their partition coefficient. In 1939, Ferguson showed that parameters like relative solubility obtained by applying simple thermodynamic principles could also be used for correlation with narcotic or depressant effects. This is known as Ferguson principle.

In 1940, Hammett⁶³ showed that chemical reactivity of *meta*- and *para*-substituted benzenes derivatives could be correlated by the following equation^{64, 65}:

$$\text{Log } (K_x/K_H) = \rho\sigma \quad \dots(2)$$

In Eq. (2), K_x and K_H are equilibrium constants for the substituted and parent compounds, σ is the electronic contribution of the substituent, and ρ is the parameter representing the specific reaction type.

Table 1 — Representative examples of correct predictions from QSARs

Types of compounds	Biological activity	Ref.
Benzothiadiazines	Antihypertensive	41 - 43
Clonidine analogs	Antihypertensive	44
β -Carbolines	Inhibition of monoamine oxidase	45
Sulphonamides	Inhibition of carbonic anhydrase	46, 47
Carbamoylpiperidines	Inhibition of cholinesterase	48, 49
Pyrazoles	Antivirals	50
Nitrosoureas	Cytostatic	51
Mytomyins	Cytostatic	52
Sulfonamides	Antibacterial	53, 54
Erythromycins	Antibacterial	55
Quinoxaline 1,4-dioxides	Antibacterial	56
Promazines	Neuroleptic	57
Benzothiepine derivatives	Neuroleptic	58
Thyroxine analogs	Thyroxine	59
Azapurine-6-ones	Immunosuppressives	60
Triazines	Inhibition of dihydrofolate reductase	61
Adenosine analogs	Antihypertensives	62
Trimethoprim analogs	Antibacterial	62

By analogy with Hammett equation, in 1963, Hansch proposed⁵,

$$\text{Log } (P_x/P_H) = \rho\pi \quad \dots(3)$$

In Eq. 3, P_x and P_H stand for partition coefficients for the substituted and parent compounds respectively, π is lipophilicity contribution of the substituent and ρ has a value of 1 for *n*-octanol-water system.

Hansch model is one of the most successfully applied methods in the field of QSAR and RDD. It was developed, based on the following postulates⁶⁴:

- (i) Drug reaches near the receptor site by "random walk", i.e., crossing various lipid barriers by passive diffusion process.
- (ii) Drug binds with the receptor (critical reaction site) forming a complex.
- (iii) The drug-receptor complex may undergo chemical reaction or conformational changes for the desired activity.

- (iv) The drugs in a congeneric series act through same mechanism of action.

A generalised equation of Hansch model may be represented as,

$$\text{Log } 1/c = k_1\pi - k_2\pi^2 + k_3\sigma + k_4 \quad \dots(4)$$

The k terms in Eq. (4) are constants. All variable terms appearing in the equation may not be necessary or any additional term may be included in a particular case. The term π is a measure of hydrophobic binding energy with the receptor. However, if hydrophobicity plays a part in drug transport then the sensitivity of the receptor for hydrophobic binding cannot be revealed from Eq. (4). For the transport of a drug through extracellular fluid across the biomembranes, it should possess optimum lipophilicity; thus π usually shows parabolic relationship with biological activity *in vivo*. The π^2 term is not necessary *in vitro* except if hydrophobic binding site of restricted size or to limiting solubility is present.

Although parabolic relationship of biological activity with lipophilicity parameter has been widely used, in many cases activity is better described by a bilinear model¹⁵. Bilinear model equations of McFarland are :

$$\text{Log } 1/c = a \log P - b \log(\beta P + 1) + c. \quad \dots(5)$$

$$\text{Log } 1/c = a \pi - b \log(\beta 10^\pi + 1) + c. \quad \dots(6)$$

Apart from $\log P$ and π , various other parameters like chromatographic retention time, etc. have been used as hydrophobicity parameters¹⁻³ in QSAR studies (Table 2).

Hansch model is a linear free energy related (LFER) model. It considers that each substituent has a specific

influence on the equilibrium and rate constants of a reaction via changes in electron density and steric effects at the reaction centre and this can be described in terms of linear free energy relationships that do not follow immediately from the law of thermodynamics (extrathermodynamic relations). Apart from various electronic substituent constants¹⁻³ (Table 2) like Hammett σ , σ^+ , σ^- , etc., various experimental quantities expressing intermolecular forces like dipole moment (μ), ionization potential (I), polarizability (P_E), and also various quantum chemical parameters (like energies of highest occupied and lowest unoccupied molecular orbitals, electron density, electrophilic and nucleophilic localization energies, electrophilic and nucleophilic

Table 2— The most important physicochemical parameters used in regressional QSAR¹

Hydrophobicity parameters	Electronic parameters	Steric parameters
1 CSA (Cavity surface area)	1 σ_m, σ_p (Hammett constants)	1 E_s (Tafts steric substituent constant)
2 $\log P$ (P = partition coefficient, mostly in n-Octanol- water system)	2 σ^0, σ^1 (“normal” substituent constants)	2 E_s^c (Hancocks steric substituent constant corrected for hyperconjugation)
3 $-\log c_s$ (c_s = solubility in water)	3 $\sigma_I, \sigma_I^+, \mathfrak{S}, F, S$ (Inductive substituent constants)	3 ν (Chartons steric substituent const)
4 R_M ($R_M = \log [1/R_f - 1]$)	4 σ_L (Localized substituent constant)	4 V_w (Van der Waals volume)
5 π ($\pi = \log P_x - \log P_H$)	5 σ^* (Polar substituent constant)	5 MR (Molar refractivity)
6 ΔR_M ($\Delta R_M = [R_M]_x - [R_M]_H$)	6 σ^+, σ^- (Enhanced substituent constants)	6 V (Molar volume)
7 f ($\log P = \sum af$)	7 $\sigma_R, \sigma_R^+, \sigma_R^-, \sigma_R^{\cdot}, \mathfrak{R}, R, P, \Delta\sigma_R^+, \Delta\sigma_R^-$ (Resonance substituent constants)	7 χ (Molecular connectivity)
8 F ($F = \delta V$, δ is solubility parameter)	8 σ_D (Delocalized substituent constant)	8 $L, B_1 - B_4$ (Verloops STERIMOL parameter)
9 $\log k'$ (k' = retention index from HPLC)	9 σ_H (Enthalpic substituent constant)	9 MSD (Minimal steric difference)
10 $\log K_b$ (K_b = affinity constant for binding to a suitable protein)	10 σ_s (Entropic substituent constant)	10 MTD (Minimal topological difference)
	11 E_R (Radical parameter)	11 V_0, S_0, L_0 (Molecular shape descriptors)
	12 $C_T \chi$ (Charge transfer constants)	

superdelocalizability) have been used as electronic parameters³.

The various steric parameters¹⁻³ which are commonly used in Hansch analysis include molar refractivity (*MR*), Taft steric parameters (*Es*), van der Waals volume (*V_w*), etc. (Table 2).

This model has considerable predictive value and diagnostic potential and it gives an insight into mode of action and location of the receptor site⁶⁶.

2 Structure-Activity Relationship Studies

Free Wilson Model

Free and Wilson⁶⁷ model is a true structure-activity relationship model. It is *de novo* mathematical model that finds out contributions of various substituents and the parent ring to the biological activity through regression method. Its limitation is that it cannot predict contribution of any substituent that is absent in the original data set.

Fujita-Ban modification of Free-Wilson model is now commonly used instead of the original method⁶⁸. An arbitrary reference compound is chosen and activity contributions of various structural features are found out in relation to that present in the reference compound⁶⁹.

Topological Schemes

These are based on graph theoretic approach and mostly deal with hydrogen suppressed graphs⁷⁰. Topological consideration includes number and types of atoms and bonds, interatomic connections (adjacency count), paths, branching, molecular size, shape, functionality, etc⁷¹.

Among the various topological schemes, *molecular connectivity indices (MCI)*⁷²⁻⁷⁹ of Kier and Hall is most successful. These indices encode various structural features of molecules that are obtainable from two dimensional representation of molecular structures and have been successfully correlated with various physicochemical and biological parameters.

TAU scheme of Pal *et al.*⁸⁰⁻⁸⁵ offers some advantages over MCI from the point of view of diagnostic features of these indices and presence of scope of its application for molecules with higher complexity.

*Molecular negentropy*⁸⁶, a global index calculated based on the information theory of Shannon and Weaver⁸⁷ applied on total molecular graph, has also been used in structure-activity correlations.

Kier and Hall, have formulated, more recently, two other schemes applying the basic concept of topological schemes. One of these is Kappa shape index⁸⁸, an index for molecular shape, and the other is electrotopological state atom index^{89, 90}, which has been claimed to have power to identify important atoms or fragments necessary for a particular biological activity⁹¹.

Other Substructural Approaches

Other methods involving structural parameters include⁹²:

- (i) Cramer's substructural analysis.
- (ii) Statistical-Heuristic method for automated search of drugs for screening.
- (iii) The logico-structural approach.
- (iv) Heuristic approach to topological pharmacophores.

3 Mathematical Methods of QSAR

Multiple Regression by the Method of Least Squares³

This is the most widely employed mathematical method in classical QSAR (extrathermodynamic approach, Free-Wilson model and topological methods).

Pattern Recognition Technique⁹³

The number of variables in pattern recognition technique is much higher than Hansch analysis. A training set (50 - 70 per cent of data) is chosen to derive a quantitative model for the prediction of the rest of the data.

Discriminant Analysis⁹⁴

It separates objects with different properties e.g., active and inactive compounds, by deriving a function of other features (e.g., different physicochemical properties) which gives the best separation of the individual classes. A training set is used and the quality of fit is checked with the help of a test set. COMPACT (computer optimized molecular parametric analysis of chemical toxicity) is a discriminant analysis approach to predict carcinogenicity and other forms of toxicity.

Cluster Analysis⁹⁵

It separates and groups objects according to their distances in multidimensional space. Cluster significance

Table 3 — Representative examples of successful applications of molecular modelling

Types of compounds	Biological activity	Ref.
Ro 46-6240	Thrombin inhibition	103
BCX-34	Purine nucleoside phosphorylase inhibition	104
Thymitaq (AG337)	Thymidylate synthetase inhibition	105
Trusopt (MK-507)	Carbonic anhydrase inhibition	106 - 108
Tolrestat	Aldose reductase inhibition	109
Ritonavir	HIV-1 protease inhibition	110
DX-9065a	Factor Xa inhibition	111
Marimastat	Matrix metalloproteinase inhibition	112

Table 4 — List of selected popular software packages for molecular modelling⁴⁰

Molecular mechanics	<i>Ab initio</i> quantum mechanics	Semiempiric quantum
AMBER	GAUSSIAN	MOPAC
CHARMm	GAMESS	AMPAC
Discover MM2 / MM3 SYBIL	HONDO	PCILO

analysis is a graphical method to look at the clustering of the active compounds in a space that is made up of various physicochemical parameters.

Principal Component Analysis⁹⁶

It reduces multidimensional data matrices of physicochemical properties to fewer orthogonal dimensions. It shares many features with factor analysis⁹⁷.

K-nearest Neighbour Method⁹⁸

It is based on the consideration of distances between objects in feature space.

Adaptive Least Square (ALS) Method⁹⁹

It is a modification of discriminant analysis that separates several activity classes by a discriminant function.

Partial Least Square (PLS) method¹⁰⁰

It is a principal component like method where hundreds or even thousands of variables can be correlated with one or several dependent variables. Often perfect correlations are obtained due to large number of X variables. A cross-validation procedure (PRESS) must be used to select the model having highest predictive value.

Artificial Neural Networks^{101, 102}

It is derived from a simplified concept of brain in which a number of nodes, called processing elements or neurones, are interconnected in a network like structure. This method is able to perform nonlinear mapping of physicochemical parameters to a corresponding biological activity implicitly.

Molecular Modeling and 3D QSAR Methods

In recent few years, drug research has witnessed explosive growth of the field of molecular modelling and computer aided drug design (CADD). Nowadays, it is a critical component of RDD⁴⁰ (Table 3). Molecular modeling is a visual interface between the computer and the scientists and it attempts to rationalize the behaviour and activity of bioactive agents. Its components are⁴⁰ :

(i) *Molecular Graphics* - It represents drug molecules and associated molecular properties in a visual way.

(ii) *Computational Chemistry* — It involves simulation of atomic or molecular properties of compounds of medicinal interest through equations and solving these through computer by either molecular mechanic or quantum mechanical approach. Table 4 lists some of the popular modelling packages.

(iii) *Statistical Modeling* — It encompasses the QSAR and QSPR studies.

(iv) *Molecular Data and Information Management* — It includes compilation of databases of properties and synthesis strategies of a large number of compounds, capable of being searched by a user according to his need.

It is anticipated that molecular modelling will play the major role in future drug design process.

1 Computational Chemistry

Molecular properties like potential energy of a compound in a particular conformation, electron density at each atom, molecular volume and shape, etc. are computed and the factors that contribute to the biological activity are predicted or explained. The computations are done by molecular mechanical or quantum mechanical methods.

Molecular Mechanical Methods¹³

In this approach, each atom is treated as a mass proportional to its atomic mass and each of its bonds is treated as an analog of a mechanical spring with a force constant. The total potential energy of a molecule is assumed to be composed of bonded (stretching, bending and torsional) and non-bonded (van der Waals steric and coulombic) interactions.

$$E_{\text{total}} = \sum E_{\text{stretch}} + \sum E_{\text{bend}} + \sum E_{\text{torsion}} + \sum E_{\text{vdW}} + \sum E_{\text{coulomb}} \quad \dots (7)$$

Apart from the above mentioned forms of energies, total energy also includes hydrophobic energy, hydrogen bond energy, libration energy, and looping energy^{14a}. Molecular mechanical methods are not concerned with properties and distribution of electrons, thus these are not suitable for computing characters that depend on the movement of electrons. Quantum mechanical methods should be used for computation of electron density at various atoms and energies of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). However, an intuitive and judicious combination of the two methods seem to be an ideal choice¹⁴.

Quantum Mechanical Methods¹³

Quantum mechanical approach is the most difficult and the ultimate one⁷¹. It decodes the complete structural information and gives description at the atomic and

electronic levels. Because of its complexity of calculations, quantum mechanical methods are limited to simple systems and the approximation known as molecular orbital (MO) theory is mostly used for practical purposes¹⁵. Electronic aspects of structure in terms of electron location and energy are generated by computation. In MO theory, each MO (ψ) is represented as linear combination of atomic orbitals (LCAO).

$$\psi_i = c_{i1}\psi_1 + c_{i2}\psi_2 + \dots + c_{in}\psi_n \quad \dots (8)$$

In Eq. (8), the c terms represent contributions of atomic orbital to molecular orbital.

The values of c^2 is a measure of probability of location of electrons at region of space in the molecular orbital. Various properties like charge density, dipole moment and ionization energy may be calculated from structure based calculations. Ionization energy is an important parameter and its solution for each MO in a molecule gives series of energy levels reflecting electron donating or accepting capacity of the molecule.

Quantum mechanical methods use Schrodinger equation of molecular orbital theory that gives exact analytical solutions only for simplest systems like hydrogen atom. Various semiempirical methods like MNDO (modified neglect of differential overlap) and PCIO (perturbative configuration interaction using localized orbitals) have been used. These methods neglect interactions among nonvalence orbitals. The quantum mechanical calculations give an insight into geometry of HOMO and LUMO that in turn leads to better understanding of possible orientation of the transition state during reaction. These calculations also provide information on electron density distribution and magnetic properties at different parts of a molecule, energy of a system in a definite configuration, and help in tracing reaction pathway and also various thermodynamic data like heat of formation, etc.

Prediction of preferred conformation of molecules based on MO theory is now an active area of theoretical research. Preferred conformation is a function of interactions of atoms within the molecule. Attainment of minimum energy conformation which is resultant of attractions and repulsions among the atoms is the driving force of conformational changes. **Minimum energy conformation** is function of bond angles, bond lengths and torsional angles and it can be obtained by varying the parameters and calculating total energy as a sum of orbital energies.

Minimum Energy Conformation (MEC)¹¹³

Determination of minimum energy conformation, which is one of the most powerful features of molecular modelling, is most often performed using molecular mechanics, but the quantum mechanical methods can also be used at the cost of increased computer time. A minimization process begins with an assigned starting geometry and calculates the steric or potential energy of the molecule at that geometry. The positions of the atoms are then adjusted in a systemic way to lower energy of each atom and then energy of the entire molecule is computed. If the energy of the new geometry is less than the starting energy, then the new geometry is adopted as the starting geometry. The process is repeated until no further reduction in energy occurs. One of the disadvantage is that the calculations always find the nearest MEC to the starting geometry which may not be the global one. If enough numbers of starting points are chosen then the global minimum may be found. For a command with a large numbers of rotatable bonds, the process becomes impossible with the present day computer technology. An alternative approach is molecular dynamics which is based on simulation of molecular motion by solving Newton's equations of motion for each atom and changing position and increasing velocity of each atom, using a small time increment. This is a powerful tool for dissecting the molecular nature of the reaction phenomenon and details of the force contributions to the behaviour of the system.

A combination of quantum mechanical and classical mechanical methods, **Iterative Self-Consistent Partition of Energy Method and Molecular Orbital (ISC-PEM-MO) formalism**, for the derivation of minimum energy conformation-cum-preferred conformation (MEC-PC) was reported by Ghose *et al.*¹¹⁴. This starts with anti-configuration of a compound of which electron density and charges on atoms are calculated by quantum mechanical method. The charges are subsequently utilised for the calculation of electrostatic energy, one of the important components of classical mechanics, and ultimately total energy (composed of bond angle deformation energy, torsional energy, van der Waals non-bonded interaction energy, etc.^{114a}) is computed. These calculations are repeated at 5° interval till the original or equivalent configuration is reached and MEC-PC is found out.

2 3-D QSAR Methods

3-D QSAR methods have two basic components, one is an explicit computational relation relating biological

activity to 2-D and 3-D molecular properties and the other is a graphic representation of 3-D information packaged in the computational structure-activity relationship¹¹⁶. Thus many approaches to receptor mapping and pharmacophore design cannot be placed under 3-D QSAR methods, according to this definition, because in these cases no explicit mathematical relationships are generated. QSAR analysis has almost exclusively been used when molecular geometry of the common receptor is unknown. If the receptor geometry is known then intermolecular docking is usually performed.

Receptor Independent 3-D QSAR Methods

(i) *Comparative Molecular Field Analysis (CoMFA)*¹¹⁷ — CoMFA is most often employed receptor independent 3-D QSAR approach. The development of CoMFA is based on the concept that biological activity is sensitive to spatially localized differences in molecular field intensities. The three major phases of CoMFA are : (a) set up CoMFA data table composed of biological activity and structural parameters, each recording intensity of a particular type of interaction of a particular point in space with a probe atom of specified charge and steric properties for each of the compound in the list; (b) application of partial least square (PLS) method and cross validation with PRESS, and (c) representation and analysis.

(ii) *Molecular Shape Analysis (MSA)*¹¹⁸ — The common overlap steric volume (COSV) between a pair of superimposed molecules can be used as a global measure of molecular shape similarity. The goals of MSA are to identify the biologically relevant conformation without knowledge of receptor geometry and then in a quantitative fashion explain the activity of a series of analogs using structure-activity table.

(iii) *Molecular Similarity Matrices*¹¹⁹ — This approach is similar to CoMFA and assumes that the alignments and conformations used in the analysis are correct ones. It is based on comparing each molecule in the training set with each other. Various indices used in the construction of similarity matrices are Carbo index, Meyer shape index, Hodgkin index, and Spearman rank correlation coefficient.

(iv) *Distance Geometry*¹²⁰ — The use of interatomic distances as representative of molecular shape has also shown success in 3-D QSAR. The four important methods of 3-D QSAR based on distance geometry are Ensemble distance geometry, site pocket model, REMOTEDISC and Veronoi site modelling.

(v) *Hypothetical Active-site Lattice Model (HASL)*¹²¹ - It is related to both CoMFA and MSA. The two aims of the HASL approach are the prediction of activities of untested compounds and identification of substructures influencing observed activities.

(vi) *Genetically Evolved Receptor Model*¹²² — The objective of this method is to produce atomic level models of receptor site based on a trial set of ligands.

(vii) *Quantitative Binding Site Models (COMPASS)*¹²³ — It is based on structure-only-type properties and predicts the bioactive conformation, alignment, and binding affinity of a series of ligands in an automated procedure.

Receptor Dependent 3-D QSAR Methods

(i) *Receptor Dependent MSA*¹²⁴ — The drawbacks in this technique include not considering the isolated ligand and the receptor in their respective lowest energy conformation. But it considers all types of internal energy changes except solvent reorganization.

(ii) *3-D QSAR Based on Intermolecular Contribution to Binding Energy*¹²⁵ — Models of ligands are built using X-ray crystallographic structures of the target proteins and minimizing the energy of the ligands. The interaction energies between ligand and receptor are then correlated with the biological activity data.

(iii) *COMBINE Analysis*¹²⁶ — This may be considered as a receptor dependent version of CoMFA. Here, different regions of receptor serve as probes for elucidation of major interaction sites.

Protein Modeling

The target sites of many drugs being proteins, insights into three-dimensional structures of proteins are of paramount importance for the process of drug design and understanding mode of drug action at molecular level. The structure of proteins may be determined by X-ray crystallography or indirect spectroscopic techniques like NMR. Determination of amino acid sequence of a protein may be done by automated sequencing procedure or by reading corresponding genetic code by biotechnological procedure. The rate of structure determination is 50-fold higher than rate of structure elucidation. For determination of 3-D structure of a protein, molecular environment present at the time of folding is considered in addition to amino acid sequencing. Comparative protein modelling is the most reliable technique available so far. Currently, large

databases of known protein structures is available, and software packages assist in the prediction of unknown protein structure by comparing its sequence to those of known structures. The various steps of comparative modelling are¹²⁷ :

- (i) Identification of at least one suitable template structure and sequence alignment.
- (ii) Coordinate generation.
- (iii) Model optimization.

Automated protein modelling server SWISS-MODEL is reachable on the Worldwide Web (WWW) and may be used by the user.

De Novo Ligand Design

This is a design of novel chemical structures capable of interacting receptors with known structures. Various classes of ligand design methods are¹²⁸ :

- (i) Methods that analyze active site — Such methods determine which kind of atoms and functional groups are best able to interact with the active site.
- (ii) Methods that dock whole molecule - These methods take each proposed ligand, one at a time, and attempt to position it in the active site of the receptor or match it to a pharmacophore model.
- (iii) Methods that connect molecular fragments or atoms together to produce a ligand :
 - (a) Site-point connection methods — These determine desirable locations of individual atoms and then place suitable fragments at that location.
 - (b) Fragment connection methods — These start with previously positioned fragments and find 'linkers' to connect those fragments without moving them.
 - (c) Sequential build-up methods — These construct a ligand atom by atom, or fragment by fragment.
 - (d) Random connection methods — These are a special class of techniques combining some of the features of the methods listed under (a) to (c).

Some *de novo* drug design methods are DOCK (1982), GRID (1985), CAVEAT (1989) AUTODOCK (1990), HINT (1991), LUDI (1992), SPROUT (1993),

NEWLEAD (1993), PRO-LIGAND (1995) and CONCERTS (1995). Some examples of recent successes in structure based drug design are : carbonic anhydrase II, HIV-1 protease, influenza sialidase, etc.

Receptor Mapping and Pharmacophore Search

For understanding of structure-activity relations at the receptor level, a direct study of the forces and properties involved in drug receptor interactions is necessary. The term receptor mapping refers to various methods employed to evaluate the structure of a receptor (binding site) by regarding it as complimentary to the drugs fitting the receptor. Drug receptor interactions proceed through three steps : (i) recognition of the right features of the compound by the receptor, (ii) binding of the drug compound with receptor, and (iii) specific perturbation of the three-dimensional receptor structure. A pharmacophore is a certain pattern of elements (e.g., atoms), the presence of which in drug is necessary and sufficient condition for the production of a stimulus at the receptor under consideration. There are three types of pharmacophores : (i) recognition pharmacophore, (ii) affinity pharmacophore, and (iii) intrinsic pharmacophore³.

For deriving an insight into pharmacophoric requirements, it is necessary to determine the three-dimensional structures of drug substances by experimental (e.g., X-ray and neutron diffraction in solid state, NMR spectra in solution state and electron diffraction and microwave spectroscopy for gaseous molecules) or theoretical (conformational analysis by quantum or classical mechanical methods) studies³. Some methods for pharmacophore search are :

- (i) Static pharmacophores and receptor mapping by model interaction calculations¹²⁹
- (ii) Molecular electrostatic potential (interaction pharmacophore)¹³⁰
- (iii) Molecular matching and superimposition.¹³¹
- (iv) Active analog approach¹³²
- (v) Transition state analogs¹³³
- (vi) Steric and electrostatic alignment (SEAL)¹³⁴
- (vii) Monte Carlo search procedure¹³⁵
- (viii) Genetic algorithm (GA)¹³⁶
- (ix) "Hypermolecule" approach¹³⁷.

(D) Other Trends in Recent Drug Development Process

Mass Ligand Screening

Identification of lead compounds is a prerequisite for introduction of new therapeutics to improve quality of life which is the primary objective of pharmaceutical industries. Mass ligand screening has emerged as a tool for discovery of new lead compounds. There are many potential sources of new chemicals that provide leads for new drugs including existing chemical libraries, as well as natural sources. A more recent strategy for lead compound identification is the approach of molecular diversity which can utilize the best of natural products and synthetic approaches to lead identification. Recent advances in biological screening procedure make it possible to screen hundreds of thousands of compounds in a relatively short period. The *radioligand binding assay*¹³⁸ technique offers a simple method to determine ability of a test compound to interact with a targeted receptor.

Various types of chemical libraries include corporate library (physical collection of all the compounds that have been synthesized, characterized and catalogued), natural product library and novel / combinatorial library (physical collection of vials containing compounds that are thought to have been synthesized during a single combinatorial experiment). Moreover, pharmacophore based ligand libraries and diversity based ligand libraries are available. With the help of *combinatorial chemistry*¹³⁹, chemists are now able to synthesize thousands of compounds in weeks to months. The development of these methods has been enhanced by the development of solid-phase chemistry technique.

Recently developed computational technologies like clustering, docking, and three dimensional searching are applied on the data bases to lower the cost of screening. *High throughput screening*¹⁴⁰ of the archives has resulted in the discovery of potent lead compounds.

Recombinant DNA Technology

The importance of molecular genetics to provide unique and valuable tools for drug discovery is being exploited. Recombinant DNA technology^{141, 142}, an integrated part of present drug discovery process, is providing new targets for drug action. Molecular genetics uncovers molecular etiology of a disease state, gains access to the disease relevant target enzyme/receptor and identifies proteins/macromolecules as drugs or drug targets and produce them in meaningful quantities. The

biotechnologically derived therapeutics are usually large extracellular proteins destined to be injectables for use in either chronic replacement of therapies or in acute or chronic situations for treatment of life threatening diseases. Some of the examples of these are human insulin (approved for diabetes in 1982), human growth hormone (approved for growth hormone deficiency in 1985), tissue-type plasminogen activator (approved for myocardial infarction in 1987), interferon- α (approved for hairy cell leukaemia in 1986), and Haemophilus B (approved for influenza B in 1988).

Peptidomimetics¹⁴³

Peptidomimetics are modified structures of naturally occurring bioactive peptides (hormones/enzyme inhibitors/growth promoters or regulators/neurotransmitters/immunomodulators) that imitate natural molecules and are believed to enhance desirable properties and avoid undesirable properties of native molecules. Many analogs have exhibited improved pharmacological and pharmacokinetic properties including increased solubility, absorption, metabolic stability and bioactivity, and decreased toxicity. Exploration of the binding conformation is one of the most important task involved in the process to obtain potent and selective therapeutic agents as bioactive peptides must adopt a specific conformation for binding to the acceptor site. The transformation of a peptide to completely non-peptidic molecule (retaining pharmacophore and required three-dimensional array) is an attractive approach to the development of therapeutic agents from native peptides. Some examples of peptidomimetics are cyclic dermorphin analogs, cyclic somatostatin analogs and cyclic vasopressin analogs.

Oligonucleotide Therapeutics¹⁴⁴

Recently, therapeutic applications of oligonucleotides designed for interaction with nucleic acid receptors and also non-nucleic acid receptors have drawn considerable attention and interest. The growth of libraries of oligonucleotide analogs is greatly facilitating the exploration of these as potential candidates for therapeutic purposes. The first generation antisense drugs (phosphorothioates) are examples of prospective oligonucleotides for therapeutic use.

Carbohydrate Based Drug Design¹⁴⁵

Carbohydrates are critical in the operation of fundamental biological process of cellular recognition.

Specific interaction of many biopolymers is mediated by complex carbohydrates, instead of proteins or oligonucleotides. Carbohydrates have potential for greater complexity on a unit basis in comparison to polypeptides or oligonucleotides. Carbohydrates provide signals for protein or lectin targeting and cell-cell interactions, and serve as receptors for binding toxins, viruses, and hormones. They are ideal carrier for biological specificity. Complex oligosaccharides and polysaccharides being modulators of important physiological processes, research on carbohydrates has a promising future in the drug discovery process. Several carbohydrate drugs (antibiotics, nucleoside antivirals, anticancers, and cardiac glycosides) are already in use. Many carbohydrates of current interest are glycoconjugates: glycolipids, proteoglycans, and glycoproteins. Some approaches of carbohydrate based therapeutics are inhibition of enzymes for carbohydrate biosynthesis and catabolism, immunomodulation, carbohydrate based cell-cell interaction, etc.

Prodrug Design

Prodrugs undergo biotransformation prior to exhibiting their pharmacological effects. These are mostly concerned with optimization of drug delivery. They may offer advantages from the viewpoints of improved organoleptic property, enhanced stability, better bioavailability and pharmacokinetic profile, desired release profile, less toxicity and also tissue targeting¹⁴⁶⁻¹⁴⁸. Some examples of different types of prodrugs are esters of hydroxy or carboxy group containing drugs (e.g., chloramphenicol palmitate), Mannich bases, macromolecular prodrug (liposomes), derivatives of peptides, peptide esters of drugs, amine prodrug, and lipidic peptides.

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