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QSAR - Application in Drug Design

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Abstract

Earlier the process of drug research was confined to the empirical testing of a large number of compounds for a specific activity. The lead compound thus obtained was further altered based on one's synthetic abilities. Medicinal chemist was like a compulsive gambler who hoped that his next compound will be more active. This 'Edisonian' approach was too expensive and success rate was too low .Many attempts were made to make the process more rational. A landmark in this direction was the idea of QSAR proposed by Hansch and his coworkers. He converted the qualitative relationship like "chloro derivative is more active than bromo" to a quantitative structure activity relationship (QSAR). The paradigm of Hansch approach influenced every discipline of drug research. From that time, the medicinal chemistry is never the same again. Later, many more methods were introduced enlarging the scope of QSAR. It will also discuss briefly other methods of QSAR and their applications.

Key word: *QSAR model, Parameters, Log, CoMFA, 3D-QSAR, Drug design.*

1. Introduction

The purpose to describe the impact of Hansch analysis[1] on drug design, It will deal with the methodology and accomplishment of Hansch analysis. It will also discuss briefly other methods of QSAR and their applications. The purpose of this chapter is to describe the impact of Hansch analysis on drug design. It will deal with the methodology and accomplishment of Hansch analysis. It will also discuss briefly other methods of QSAR and their applications. Almost simultaneously, free and Wilson[2] proposed another QSAR model.

2. How to Quality the Chemical Structure ?

To make the structure activity relationship quantitative, both chemical structure and the biological activity must be quantify. Although biological activity can be measured in quantitative terms, it is not easy to quantify the chemical structure. So the crucial question is : how to quantify the chemical structure? The method used in QSAR to quantify the structure is to express it in terms of physico-chemical properties that can be measured easily and accurately like partition- coefficient, pk_a , spectral properties, steric characters etc. Most often, medicinal chemist works on a set of derivatives where only a part of the molecule varies and a large part remains constant. In such cases, one has to quantify only the substituents instead of the whole molecule.

One of the important contribution of Hansch and his coworkers is the development and standardization of a number of such substituent constants based on physico-chemical properties. Many others have also developed various substituent constants. Today a QSAR practitioner has a large number of such constants to choose using some of this these constants; one can also calculate the physico-chemical properties of the complete molecule. Some of the important and frequently used physico-chemical properties and the related substituent constants are brief described below.

2.1 Quantifying Structure in terms of Lipophilicity.

In the beginning of this century, *Overtone*[3] and *Meyer*[4] had demonstrated that

the narcotic activity is related to the partition coefficient between oil and water. Since then large number of reports have correlated various biological activities with the *partition coefficient*[5]. Thus a chemical structure can be expressed quantitatively in terms of partition coefficient *P* or log P. Hansch and Fujita recognized that partition coefficient is an additive constitutive property, that is, the partition coefficient of a molecule is the sum of the contributions from various parts of the molecule. It also means that, for example, if a methyl group is added to any molecule, the partition coefficient will increased to the same extent in all the molecules. By studying the partition coefficients of a large number of molecules, the contribution of each substitution was calculated. The hydrophobic parameter thus calculated for a substituent was called 'π'. It can be calculated as follows:

$$\pi_X = \log P_X - \log P_H$$

π_X is the hydrophobic constant of the substituent X . $\log P_X$ is the log partition coefficient of the molecule with the substitution X and $\log P_H$ is the log partition coefficient of the parent molecule that is, the unsubstituted molecule. For example,

$$\pi_{Cl} = \log P \text{ (Chlorobenzene)} - \log P \text{ (Benzene)}$$
$$= 2.84 - 2.13$$
$$= 0.71$$

Thus pi value for chloro substituent is 0.71. It means that the introduction of a chloro group will result in the increase of log P by 0.71. For example, the log P of dichlorobenzene will be

$$\text{Log P (dichlorobenzene)} = \log P \text{ (benzene)} + 2\,\pi_{Cl}$$
$$= 2.13 + 2\,(0.71) = 3.55$$

(experimental = 3.39)

Large number of substituent's have been studied and their π values are available in the literature[6]. Values for some of the important substituents are given in Table 1. Although many organic solvents can be used to measure the partition coefficient, Hansch and his group has standardized the method using n- octanol-water system. A second set of π values, named Pi-, was introduced by Fujita et al for substituents on phenols and anilines[7]. Later Nys and Rekker defined a new hydrophobic parameter called fragment constant 'f'. Log P is the sum of constituent 'f' values [8]. The fragment constants were further developed and revalue are not always possible due to various types of log P based on these value are not always possible due to various types of interactions within the molecule. However there is a computer program CLOGP that calculate log P from a set of empirical rules devised after examining thousand of compounds[9].

Apart from shake flask methods, partition coefficient can also be determined by HPLC with octanol coated columns[10]. Many have studied another R_m derived from thin layer chromatography as a measure of hydrophobicity[11]. These methods are faster compared to the traditional methods. Logarithm of micelle-water partition coefficient ($\log P_{mw}$) has been suggested as another simple parameter. It is measured by HPLC using micellar aqueous solution as mobile phase[12,13].

2.2 Quantifying Structure in terms of Electronic Parameters

In developing the hydrophobic constant, Crowin Hansch was highly influenced by the work of Prof. Hamnet. In 1935, Prof. L.P. Hammett developed an electric constant, now known as 'Hammett constant' for a substituent based on the P_{k_a} values[14]. These constants were very useful in predicting a number of reaction rates. His equation has been successfully applied in studying a very large of diverse reaction. The Hammett constant (σ, sigma) can be easily calculated by the equation:

$$\sigma X = (pK_a)_H - (pK_a)_X$$

σ X is the Hammett constant of the substituent *X*, $(pK_a)_H$ is the pk_a of the benzoic acid with the substituent *X* and $(pK_a)_H$ is the pk_a of benzoic acid, that is, the unsubstituted parent molecule. For example,

$$\sigma \text{ m - NO2} = (pK_a)_{\text{benzoic acid}} - (pK_a)_{\text{m - nitrobenzoic acid}}$$
$$= 4.17 - 3.45 = 0.72$$

A positive value indicates electron withdrawing effect and a negative value indicates electron releasing effect (eg: methyl = -0.14). For substituents on the aliphatic chain, another parameter σ^*(sigma star) is used. The major drawback of sigma values in correlation studies is that they do not differ to a great extent from one another since they are located along a scale that is only 1.8 logarithmic units from one end to the other. Moreover the second decimal place is not very reliable because sigma value are obtained by subtracting two logarithms of which one or both of the two number is likely to be insure in the second decimal[15]. The sigma constants are also position specific that is, the constant for a substituent at para position is different from the Meta position. For example, the value for chloro group at para position is 0.23 and meta position is 0.47. A separate set of sigma values for para substitution was introduced depending on the resonance interactions where a

position center (σ_p^+) or a negative center (σ_p^-) is generated. There was no need for such separate values for meta substituents. The availablility of so many sigma values results in selection problem. Many times, it is difficult to decide which sigma value is appropriate. Several attempts were made to separate resonance effect from the sigma values. Swain and Lupton established another set of substituent constant F and R for field - inductive and resonance effect respectively[16]. The advantage of F and R constants is that they are position independent, that is, there are no separate values for Meta or Para position unlike sigma values. The separation of sigma to F and R is also criticized because by using two parameters instead of one, there is a loss of degree of freedom. This loss in degree of freedom may be serious if the sample size is small. However all these parameters sigma F and R are widely used in QSAR studies. Some of these values for important substituents are given in Table 1.

Many parameters based on quantum chemical calculations such as energies of highest occupied molecular orbitals (HOMO) and lowest unoccupied molecular orbitals (LUMO) have also been extensively used as electronic parameters. Further, many spectroscopic characteristics like chemical shift in NMR, IR frequencies etc also have been used in correlation studies.

2.3 Steric Parameters

Taft introduced the steric parameter, Es, which was calculated using rate of ester hydrolysis. But the method could not be used to determine the values for many substituents whose esters were not stable. Kuffer and Hansch utilized the relationship between Es and the van der waal radii, which was first recognised by Charton and developed a general method to calculate the Es value of large number of substituents[6]. Verloop developed a multidimensional steric parameter using a computer program based on the standard values of van der wall radii, bond lengths and bond angles[17]. These parameters are labeled as B_1, B_2, B_3, B_4 and L. L is the length of the substituent and B_1 to B_4 are width parameters where B_1 is the minimum and B_4 is the maximum width. Moriguchi developed van der waal volume V_w as another steric parameter which is easy to calculated [18]. Some of the common parameters used are given in Table 1 for important substituents.

2.4 Molar Refractivity (MR)

Molar refractivity is an additive constitutive property of a compound which can be calculated easily.[6] It is connected with the molar volume. But it is not purely a steric parameter. It also reflects drug receptor dispersion interactions. It is generally assumed that a positive coefficient with an MR term in a correlation equation suggests a binding action via dispersion forces. A negative coefficient with MR has been assumed to reflect steric hindrance[6]. It has been found that MR and log P are highly correlated in a homologous series. But, if the series is designed to include different types of substituents, the MR and log P are not correlated and can give useful information. Table 1 gives MR value for some important substituents.

2.5 Molecular Connectivity

Petroleum chemists have frequently used calculations based on branching in molecules to predict many physical properties like boiling point, viscosity etc. This approach was quantified in the form of matrices. Kier has extensively used these methodologies in QSAR[19]. The main advantage of this method is that these values can be calculated easily and no physical properties have to be measured. An example of molecular connectivity calculation is given in Fig.1. Large number of correlation have been reported between various indices of molecular connectivity and physical and biological properties.[17] But one fundamental problem with the concept of connectivity is that while it is designed to parametrize molecular shape in a more sophisticated manner it falls short of consideration of the three-dimensional array of atoms in space. It is this topography rather than topology which is probably essential at the molecular level[20]. It also does not give any information to medicinal chemist for further design unlike other parameters. Recently Kier and has coworkers have developed a new parameter called electrotopological state index from graph theory. This combines both Electronic and topological characters.[21,22]

3. Regression Analysis (Hansch Analysis):-

Relationship between biological activity and physic-chemical parameter can be expressed through linear equations of the type:

$$Y = a + bx \qquad \text{……..(1)}$$

Where Y is the biological activity, a is the intercept and x is the Physico-chemical parameter and b is the slope or regression coefficient. For QSAR studies, the equation 1 can be appropriately modified as,

$$\log (1/C) = a + b\ (\log P) \qquad \text{……(2)}$$

Here, biological activity is expressed as *log (1/C)* where *C* is the molar concentration of the compound which gives specific response say IC50, LD50 etc. It is essential to express the concentration in logarithmic scale because the right side of the equation contains parameters which are derived from logarithmic scale like log P, Pi, sigma, Es etc.

Hansch observed that the relationship between partition coefficient and activity is not always linear. A liner relationship is observed only when the range of partition coefficient studied is small. Activity cannot increase indefinitely with the increase in partition coefficient. After a certain limit, there will be a decrease in the activity. Such a non linear relationship can be expressed as a parabolic equation.

$$\log (1/C) = a + b (\log P) + c(\log p)^2 \quad \ldots\ldots(3)$$

Statistically better equations were derived with a parabolic model compared to linear model. This model was further improved by Kubinyi who suggested a bilinear model,

$$\log(1/C) = a + b (\log P) + c \log (BP + 1) \quad \ldots.(4)$$

Here, B is a nonlinear term to be derived by a step wise process or by other mathematical methods.[23]

Another important contribution of Hansch is the recognition that biological activity will not depend on a single physico-chemical parameter but on many. Hence a generalized equation was suggested.

$$\log(1/C) = a + b\pi + c\sigma + dEs \quad \ldots\ldots\ldots\ldots(5)$$

Here, π, σ and Es are Hanch hydrophobic constant, hammett constant and Tafts steric parameter respectively. The number of parameters in the equation can be increased or decreased. The coefficients "b, c, d" and the intercept "a" are to be calculated using multiple regression analysis. The equation (5) can also have a parabolic term as.

$$\text{Log } (1/C) = a + b\pi + c\pi^2 + d\sigma + eEs \quad \ldots\ldots(6)$$

3.1 Application of Statistics

The significance of the equation is tested by using a number of statistical parameters. Most frequently used statistical parameters are correlation coefficient(r), standard deviation from regression (s) and F-test. In addition to the above, r^2 statistics and t-test are also used. r^2 statistics give the fraction of the total variance in the data explained by the regression and t – test gives the significance of the coefficient.

3.2 Methodology and Precautions

Today, simple linear regression analysis can be performed using calculators. But for more complex problems use of computer is essential. Before performing the regression analysis certain precautions are be considered.

3.2.1 Biological activity

The biological activity data must be obtained at different doses. The log dose response must be converted to ED_{50} or ED_{30} etc. The dose in mg/kg should also be converted to moles/kg for comparison. If the activity is calculated based on one single dose the data must be converted to log it units. The following calculation can be used.

$$B_A = (M_W/d) \log (P/100 - P)$$

B_A is biological activity, M_W is molecular weight of the compound, d is the dose in mg/kg and P is the percent activity obtained dose d. form the above equation B_A can be directly used for QSAR studies. However it must be noted that biological activity data from a single dose is less accurate than the ED50 etc. which are obtained from multiple doses.

3.2.2 Number of independent variables

In a multi regression analysis, it must be noted that good correlation can be obtained by including large number of parameters. If number of compounds are two, than one gets correlation coefficient = 1. The number of parameters to be included depends on the number of compounds we are analyzing. It has been shown that for sample size less than 20, when the number of variable is about one fifth of the number of compounds, one is likely to get good correlation which is only a chance correlation and not a true correlation. If the maximum chance r^2 tolerated is 0.5, then in order to use five parameters one should have 20 compounds. For 10 parameters one need 28 compounds. This is generally called Topliss limit. As a general rule, about five compounds are needed for one parameter. But this general rule can be disobeyed if other statistical values like F-test, t- test and confidence intervals of regression coefficients are favorable. One can also appreciate the importance of number of compounds from F-test. One can get high correlation even when the numbers of compounds are small. However, in such case F-test will not be significant. The value of 'n' is utilized in calculating F-test but not in calculation 'r'. Similarly, f-test will not be significant

when more parameters are used in the equation since it reduces the degree of freedom.

3.2.3 Interco relation matrix

In multiple regression analysis it is essential to studies inter correlation between the variables. This is given in the formation of matrix. for example,

Table 1. Interco relation matrix

	x	σ	L	B1
x	1	0.12	0.21	0.89
σ			0.73	0.31
L			1	0.92
B1				1

This gives the correlation coefficient between the variables .This matrix helps in the selection of variable for multiple regression equations. The variables selected should not have high inter correlation. The variables which are orthogonal, that is, low inter correlation are to be selected for combinations.

3.2.4 Other considerations

A good equation is one where minimum number of parameters or variables are included. The tendency to get higher correlation will sometime results in over fitting the data. In the first step it is essential to include all the compounds. From the regression equation the biological activity for each compound can be calculated. By comparing the calculated and experimental value one can find the difference, the residual. This residual helps in finding out an outlier. This outlier can be detected in the next step. Many times, an explanation can be found for outlier, like rapid metabolism or a different mechanism of action. Hansch called outliers as "blessings in disguise".

4. Free-Wilson Model

Free-Wilson proposed a model, which is also called de novo model, which says that the biological activity of a compound is the sum of the contributions of all the substituents and the parent moiety. It assumes that the contributions of the substituents and the parent moiety are constant and independent. The model is expressed as follows.

$$B_{A_1} = \Sigma\, a_j\, X_{ij} + \mu$$

BA is biological activity, X_j is the j^{th} substituent with a value of 1 if present and zero if not. a_j is the contribution of j^{th} substituent to BA and μ is the average activity. The model is simple and easy to calculate. There is no need for any physico-chemical parameters. Fujita and Ban suggested two modifications. BA was converted to log(1/C) or an equivalent terms like in Hansch analysis. Also μ is the activity of unsubstituted parent molecule instead of average activity. Thus a becomes the intercept. Today Fujita-Ban modified model has almost replaced the original Free-Wilson mode. Kubinyi has combined Hansch and Free-Wilson model as mixed approach. But a general criticism of Free-Wilson method is that it is too simplified and does not after any great advantage to medicinal chemist. The assumption that the substituents are independent is not always correct. Another limitation is that the information obtained cannot be used for other substituents which were not present in the original set. The main advantage of Free-Wilson method is that it can be incorporated into Hansch analysis by using an indicator variable (I). Many times, the chemist by intuition recognizes the importance of a substituent. In such cases an indicator variable (I) can be used in Hansch equation where I = 1 if a substituent is present, and I = zero if absent.

5. Other Statistical Model:-

5.1 Discriminant Analysis

In many cases, the biological activity can be expressed only in qualitative or semi qualitative terms like active, highly active, inactive etc. Discriminant analysis is statistical method by which one can predict the group to which it belongs (active or inactive) from a set of physico chemical parameters like π, σ, Es etc. An advantage in this method is that inactive compounds can also be included in the analysis and several activities can be considered at the same time. Fig 2 describes the basic principle of the discriminant analysis.

5.2 Cluster Analysis

Cluster analysis is a method to study relationship between compounds based on their physical or biological properties. Clusters are formed on the basis of various distances between compounds in a space formed by the physical or biological properties. Analysis is performed in a sequential manner, reducing the number of clusters at each step. Cluster analysis has been used in drug design to

group substituents that have the most similarity when various combinations of physico chemical parameters are included.[6]

5.3 Principal Component Analysis (PCA)

Often a series of compounds have to be evaluated in several biological tests as in the case of antimicrobial screening where activity has to be tested against several bacteria or fungi, or screening of CNS activity etc. In such cases it is not possible to use normal regression analysis because of its limitation to use only one dependent variable at a time. In such cases principal component analysis is very useful. From a set of observed intercorrelated variables few principal components (PC) are derived. These Pcs are orthogonal vectors formed by linear combination of the original variables and are assumed to represent basic properties of these variables. PCA has been extensively used in drug design.

5.4 Comparative Molecular field Analysis (CoMFA)

The comparative molecular field Analysis method (CoMFA) is one of the most appealing and successful method development in the recent time. As a means to determine the three dimensional quantitative structure activity relationship (3D-QSAR). CoMFA is very rapidly advancing since its introduction in 1988 by Cramer et al. It looks at molecule in 3D from the viewpoint of the receptor and describes the magnitude and directional preference of electronic and staeric interaction. It gives a graphical representation of the results of the analysis as 3D grid which represent regions where steric bulk detracts or contributes to the activity as well as contours displaying regions where positive or negative charge favorable contributes to activity. These data and representations can be used to guide further synthesis and developed hypotheses for ligand – receptor interaction. It has produced very good correlation with enzyme and receptor binding site. Recently an excellent review on 3D – Method of QSAR has appeared.

6. Application of QSAR

Since the landmark publication of Hansch analysis there is an explosion of literature describing its application in various areas of medicinal chemistry. Apart from its predictive abilities, QSAR has helped in understanding the mechanism of action of large number of drugs.

6.1 Information from the intercept values

Useful information can be obtained from the intercepts in QSAR equation. Intercept represents the activity of the unsubstituted compound in a series. The activity increases or decreases depending on the substition which is reflected in the slope or regression coefficient. In a regression equation if the intercept is very high and slope is low, it indicates that the basic nucleus or the parent compound has high activity and the contribution of the substituents is not significant. It also means that further variation of that position may not result in large change in the activity. Thus intercept is a measure of intrinsic activity. For example alcohol and carbamates inhibit bacterial luminescence as per equation 7 and 8 respectively.

Alcohols : $\log(1/C) = 1.17 (\log P) + 0.22$ …(7)

$n = 8 \quad r = 0.998 \quad s = 0.100$

Carbamates : $\log(1/C) = 1.07 (\log P) + 1.36$. .(8)

$n = 7 \quad r = 0.985 \quad s = 0.248$

Both equation have similar slope and correlation coefficients. But their intercept are 0.22 for alcohols and 1.36 for carbamets. From these values it is clear that the cabamates are more activity than the alcohols. Similarly, the esters and alkyl carbamets were studied for their narcotic action on tadpoles to obtain the equation 9 and 10.

Esters : $\log(1/C) = 1.09 (\log P) + 0.69$ …..(9)
$n = 4 \quad r = 0.994 \quad s = 0.093$

Alkyl carbonates :
$\log(1/C) = 1.34 (\log P) + 1.61$ ….(10)

$n = 5 \quad r = 0.985 \quad s = 0.192$

The intercept values are 0.69 for esters and 1.61 for carbamates. Thus carbamates are more active than the esters.

A close parallel was observed between the antibacterial activities and hemolytic action for a series of compounds. Due to similarities in their intercept value, it was suggested that the mechanism of action of these antibacterial agent is through the lysis of the membrane. Further the average intercepts for many antifungal data sets were similar to those of the antibacterial agent that perturb membranes. Thus QSAR equation suggested that these antifungal agents also act through membrane perturbation. Many bis (arylamino) pyrimidines inhibit the growth of bacterial and fungi. These compounds act by interfering with pyrimidine metabolism and not by

membrane action. The QSAR studies showed that the intercept for these pyrimidines is very high compared to other antifungal agents that act through membrane mechanism.

The above example show the importance of intercept values in comparing different series of molecules for a specific activity.

6.2 Importance of log P_0 concept

The idea of optimum partition coefficient log P_o based on the parabolic relationship between partition coefficient and the biological activity is an important concept in drug design. log Po can be easily calculated from the parabolic equation.

$$\log(1/C) = a + b(\log P) + c(\log P)^2$$

$$\log P0 = b/2(C)$$

For example,

$$\log(1/C) = 1.93 + 1.58(\log P) - 0.444(\log P)^2$$

$$\log P_0 = 1.58/2(0.44) = 1.8$$

$$\log(1/C) = 1.35 + 2.38(\log P) - 0.53(\log P)^2$$

$$\log P_0 = 2.38/2(0.53) = 2.25$$

Hanch had suggested that $\log P_0$ is the ideal lipophilicity for drug to pass the blood brain barrier. For various CNS activity the following equation were obtained.

Analgesic activity of Hydroxycodenone esters :

$$\log(BA) = -1.75 + 2.19(\log P) - 0.34(\log P)^2 \quad ..(11)$$

n = 13, r = 0.960, s = 0.307, log P0 = 3.23

General anesthetic activity of ethers :

$$\log(1/C) = 21.16 + 1.038(\log P) - 0.22(\log P)^2 \quad \text{…….(12)}$$

n = 26, r = 0.966, s = 0.101, log P0 = 2.35

Anticonvulsant activity (barbiturates, benzodiazepines etc) :

$$\log(1/C) = 4.14 + 0.63 + 0.85(\log P) - 0.30(\log P)^2 \quad \text{…..(13)}$$

n = 12, r= 0.915, s = 0.227, log P0 = 1.42

Hallucinogenic activity of Phenylethylamines :

$$\log(1/C) = -3.17 + 3.15(\log P) - 0.50(\log P)^2 \quad \text{…….(14)}$$

n = 26, r = 0.790, s = 0.41, log P0 = 3.14

In the case of barbiturates and other hypnotics the $\log P_0$ was about 2.0. The logP value of many CNS drug was around 2.0. For example, cholrodiazepoxide = 2.44, diphenylhydantoin = 2.47, diazepam =2.82, etc. These results show that for a compound to be CNS activity, it must have $\log P_0$ around 2.0. This information is very useful in designing a new CNS activity compound.

The concept of $\log P_0$ has also given useful information about compounds with activities other than CNS.

Many triazines were studies for their antibacterial activity against both S. aureus and E. coli.

S. aureus:

$$\log(1/C) = 1.05\pi - 0.1\pi^2 + 2.68 \quad \text{……..(15)}$$

n = 52, r = 0.916, s = 0.47, π_0 = 5.23

E. coli :

$$\log(1/C) = 0.74\pi - 0.07\pi^2 + 2.38 \quad \text{…..(16)}$$

n = 49, r = 0.852, s = 0.47, π_0 = 5.15

Based on the measured log P for one member of the series $\log P_0$ =5.8 was obtained against both S. aureus and E. coli. These results show that triazines do not offer scope for development as useful antibacterial agents. To achieve useful potency log P would have to be so high that the compounds will be strongly bond to serum in vivo. This was also demonstrated by the fact that none of them were active against infected mice. Antibacterial activity from 21 sources against seven gram positive and four gram negative bacteria were studied. From the regression equation it was found that log P0 =6 for structurally specific drugs that damage the cell membrane of gram positive bacteria and $\log P_0$ = 4 for such activity against gram negative bacteria. This suggested that micelle formation is not importance because, in that case $\log P_0$ would have been related to this type of compound rather than the organism. Another interaction information revealed from $\log P_0$ value is the fact that different tumors may show different lipophilicity. For example in the case of N – nitrosoureas against ascitic tumors such as L 1210, the $\log P_0$ obtained was about 0 (zero), where as solid tumors such Walker 256 needs more lipophilic drug with a $\log P_0$ about 2.0. Absorption of drugs through gastrointestinal tract or buccal cavity or skin is also influenced to a great extent by the optimum lipophilicity. It was found that favorable range for gastrointestinal absorption appears to be 0.5 to 2.0

and for buccal absorption it is about 4 to 5.5. For dermal absorption, favorable range extends about 2.0.

These are some of the examples of the application of the $\log P_0$ concept both in designing a new molecule and in understanding the mechanism of action.

6.3 Bioisosterism

Since many years the concept of biososterism has been applied, sometime successfully, in drug design. But the concept remains qualitative and intuitive. With the advent of QSAR, one can now quantify the similarities. These can also be classified according to the physico- chemical properties. Now it is possible to replace one group with other having similar properties both qualitatively and quantitatively. One of the most interesting application of biososterism based on modern physico-chemical concepts is the discovery of cyanoguanidine as the bioisostere of thiourea, which resulted in the development of H2 antagonists. Two derivatives (metiamide and burmamide) with a thiourea [NHC(S)NHR] group in the side chain under went extensive clinical trials. But because of the side effects they were abandoned. The side effects were attributed to thiourea group. Hence efforts were made to replace thiourea with another bioisosteric group. A guanidine isoster, where C=S is replaced with C=NH, resulted in increased basicity and reduced activity. To decrease the basicity, an electron withdrawing group like -NO2, -CN were introduced into the guanido group. Out of them the cyanoguanidine group –NH(C=NCN)-NHR was found to be an ideal isoster for thiourea –NH(C=S) -NHR with reduced basicity and increased activity resulting in the famous drug cimetidine. These groups are bioisoster with respect to their partition coefficient, dipole moment, tautomeric form and other physico-chemical properties. It is interesting to note that another clinically useful analog, ranitidine has a different side chain where thiourea is replaced with –NH(C=CHNO$_2$) -NHR group. The logic remains same.

6.4 Information on receptor site

Our knowledge and understanding of the receptor sites has enhanced significantly from QSAR studies. One of the most widely studied system is the inhibition of dihydro folate reductanse(DHFR) by benzyl pyrimidines and other compounds. For a series of benzyl pyrimidines (Trimethoprim type) the following QSAR equation were obtained for the inhibition of DHFR from bovine liver and from E.coli.

Liver DHFR :

$$\log (1/C) = 0.621\,\pi_3 + 0.33\,\Sigma\sigma + 4.99 \quad \ldots\ldots\ldots\ldots\ldots\ldots(17)$$

$$n = 23, \quad r = 0.931, \quad s = 0.416$$

E. coli DHFR :

$$\log (1/C) = 1.38\,MR^{1}_{3,5} + 0.82\,MR^{1}_{4} + 5.77 \quad \ldots\ldots\ldots\ldots..(18)$$

$$n = 23, \quad r = 0.918, \quad s = 0.25$$

In the equation 17, the electronic terms accounts for less than 10% of the data variance and is thus only of marginal importance. It was therefore concluded that the inhibition of mammalian enzyme is related to the hydrophobicity. In the case of bacterial enzyme the situation is different. The hydrophobic effects are absent and the inhibition mainly depends on the bulk of the substituent. In this equation MR^1 represents MR value but only up to 0.79. For higher values of MR, the value used was 0.79. It was based on the assumption that only a certain fraction of larger substituents in the 3 and 4 position content the enzyme so that inhibition will not change with a substituent bulk above a certain size defined by MR = 0.79. These studies suggest that a molecule with a hydrophilic substituent at 3 positions will have lower activity against mammalian enzyme (hence low toxicity), and a molecule with optimum steric bulk at 3, 4 and 5 positions will have high activity against bacterial enzyme. This may explain the high potency and low toxicity of trimethoprim where a hydrophilic –OMe is present at 3 position.

Studies from QSAR on Quinazolines has given a crude map of binding site of dehydrofolate on DHFR. In all correlation equation a hydrophobic parameter for the substituent at 5 positions of quinazolines appears with a large positive coefficient so the region of space into which 5 substituents would project is characterized as hydrophobic. Such a hydrophobic pocket is inferred from the QSAR for both mammalian and bacterial DHFR. Studies with triazines have provided evidence that this hydrophobic pocket is larger in bacterial enzyme than the mammalian enzyme. A similar study has been conducted for papain. QSAR and x- ray studies suggested that the amide part of the substarate binds with the hydrophobic cleft of the enzyme. This was further confirmed by the synthesis of new analogs.

Result from QSAR studies were used to formulate a working hypotheses for centrally acting α- receptor agonists. Broadly it show that the receptor accepts electrons for the donor protonated drug. In the case of clonidine type, an electron deficient phenyl is required and one side of the phenyl dominates the steric requirements.

A useful picture of β- receptor is emerging from QSAR studies. Steric factors were found to be useful in differentiating β-1 and β-2 effects.

In the case of progesterone receptors it was found that polar groups reduces the binding but nonpolar groups increased or decreased the binding depending on whether the nonpolar group is present in the hydrophobic pocket of the receptor that appear to be near the 6α, 11β, 16α, 17α, 17β, 18 and 21 position of the rabbit progesterone receptor.

Thus QSAR studies can give very useful information about the receptor or enzyme surfaces. This information can be utilized in drug design. Many attempts have been made to combine QSAR with X-ray analysis. Such attempts are complimentary to each other and can be very valuable.

6.5 Importance in Drug Research

One of the important objectives of QSAR is to get useful information for the synthesis of more active or less toxic compounds. QSAR has correctly predicted the activity of large number of compounds before their synthesis. Example of correct predictions which was verified experimentally is increasing every day. Franke has listed around eighty such examples. It is reasonable to assume that many more examples are with the pharmaceutical companies which are not revealed for obvious purposes. Many times, QSAR analysis has helped in terminating the synthesis.

Colchicine is a potent inhibitor of cell division and has a potential as an anti-cancer drug. But its extreme toxicity has limited its use. Several modification have been made and the QSAR study resulted in equation 19.

$$\log (1/C) = 0.67(\log P) - 0.19(\log P)^2 + 1.77\mathbf{I} + 4.13 \quad \text{……..(19)}$$

n = 16 r = 0.927 s = 0.499

I is the indicator variable for the presence of the group $-COCH_3$ at 10 position. On the basis of the above equation, twelve new compounds were designed, synthesis and tested. Out of them nine were found to be active. Inclusion of these compounds resulted in equation 20

$$\log (1/C) = 0.58(\log P) - 0.20(\log P)^2 + 1.72\,\mathbf{I} + 4.22 \quad \text{…….……(20)}$$

n = 25, r = 0.883, s = 0.546

It is interesting to note that both equation 19 and 20 are similar in many respects. Toxicity studies on the same data resulted in equation 21.

$$\log (1/LD_{50}) = 0.40(\log P) - 0.14(\log P)^2 + 1.24\,\mathbf{I} + 3.65 \quad \text{……..(21)}$$

n = 26, r = 0.790, s = 0.564

The equation 21 is very similar to equation 20 indicating a strong correlation between the activity and the toxicity. Based on these results, the author decided to abandon further variation in this series. This is one of the examples where QSAR correctly predicted the activity and also gave the clue that activity and the toxicity are related hence further synthesis may be terminated.

Another example of successful prediction of potency is in the case of erythromycin esters. The antibacterial activity of the ester resulted in the equation 22.

$$\log (1/C) = 1.26 + 1.33(\log P) - 0.269(\log P)^2 + 0.894\,E_s^4 + 0.799E_s^{11} \quad \text{……..(22)}$$

n = 32, r = 0.965, s = 0.13

This equation correctly predicted the potency of 33 additional analogs. When these new analogs were included in QSAR, the equation obtained was similar to equation 22. The establishment of this regression equation with an optimum log P and negative steric effects helped to take the decision that synthesis of further analogs must be terminated.

There are many examples where further synthesis was discontinued because QSAR analysis suggested that toxicity and activity are correlated and cannot be separated. In the case of aromatic nitrogen mustards, QSAR suggested that electronic parameter is important for the activity (equation 23).

$$\log (1/C) = -0.26\,\pi_r - 1.62\,\sigma_r + 3.66 \quad \text{..(23)}$$

n = 9, r = 0.840, s = 0.45

Presence of electron donating group results in increased in activity. Unfortunately, the toxicity parallels the activity of QSAR of the toxicity data resulted in the equation.

$$\log (1/LD_{50}) = -0.27\,\pi_r - 1.58\,\sigma_r + 3.48 \quad \text{..(24)}$$

n = 9, r = 0.93, s = 0.270

In the equation 24 also electronic parameter is very significant. Both equation 24 and 23 are highly comparable. Thus any effort to increase the activity will result in the increase in the toxicity. Similar results were obtained with another class of anticancer agents, bis(1-aziridinyl) phosphinyl carbamates.

Equation 25 and 26 are QSAR analysis of their activity against Walker 256 tumor in rats and toxicity respectively.

$$\log (1/C) = 2.74\sigma^{*}_{R} + 3.34 \quad \ldots(25)$$
$$n = 10, \quad r = 0.949, \quad s = 0.260$$
$$\log (1/LD_{50}) = 1.73\,\sigma^{*}R + 3.03 \quad ...(26)$$
$$n = 10, \quad r = 0.77, \quad s = 0.39$$

The activity and the toxicity are highly correlated in terms of electronic parameter.

Anthracyclines are another class of important clinically useful anticancer drugs. But their use is limited to their cardiac toxicity. In an attempt to use QSAR to separate anticancer activity and cardiotoxicity, Fink et al derived a quantitative structure selectivity relationship (QSSR).From the data against B-16 melanoma in mouse equation 27 was derived where C is the molar concentration giving a 25% increase in life span in the test group.

$$\log (1/C) = -0.41 \log P + 0.481\,\mathbf{I_0} + 0.81\,\mathbf{I_1} + 6.57 \quad \ldots\ldots\ldots(27)$$
$$n = 23, \quad r = 0.870, \quad s = 0.290$$

The indicator variable $\mathbf{I_0}$ refers to the presence of 4-OH group and $\mathbf{I_1}$ refers to the presence of a lipophilic hydrazone at position 9.
Cumulative cardiotoxicity is given by equation 28.

$$\log (1/C) = -0.30 \log P + 1.01\,\mathbf{I_0} + 0.69\,\mathbf{I_1} + 0.74\,\mathbf{I_2} + 4.82 \quad \ldots\ldots(28)$$
$$n = 21, \quad r = 0.930, \quad s = 0.180$$

It is similar to equation 27 but with additional indicator variable $\mathbf{I_2}$ which refers to tertiary amino group. From these relationship the effect of lipophilicity on both activity is almost equivalent and form these data it appears that the separation of activity and cardiotoxicity may not be feasible.

In the case or anti-inflammatory agents, QSAR analysis suggested that in a series, maximum activity has been reached and thus further analogs need not be prepared. In case of nitroimidazole, the fear of carcinogenic potential has encouraged many to search for non nitro derivatives. But QSAR studies, correlating half- wave reduction potential with activity, has clearly demonstrated that such efforts are futile. In case of diphenhydramine analogs QSAR study showed a negative contribution from partition coefficient for the antihistaminic activity. This implies that increased hydrophilicity results in enhanced activity. It opens up the possibility of discovering a good antihistaminic agent free from CNS side effects, since increased hydrophilicity will prevent CNS entry.

Dearden reported the QSAR studies on the anti-inflammatory activity of aspirin series. The equation 29 was suggested for the anti-inflammatory activity by rat paw oedema assay.

$$\log (1/LD50) = 1.03(\log P) - 0.20(\log P)^2 - 0.05L_4 - 0.24 + B_{2(4)} + 2.29 \quad \ldots..(29)$$
$$n = 28, \quad r = 0.966, \quad s = 0.113, \quad \log P_0 = 2.6$$

In this equation L and B_2 are Verloop steric parameters for substitutions at 4 positions.

Rainsford derived equation 30 for the ulcerogenic activity of aspirins.

$$\log (1/C_{10}) = 1.21 (\log P) - 0.30(\log P)^2 - 0.76\,\sigma + 0.08 \quad \ldots\ldots\ldots..(30)$$
$$n = 10, \quad r = 0.613, \quad s = 0.708, \quad \log P_0 = 2.0$$

Although Rainsford equation is statistically not very good with small sample number, it is interesting to note that apart from log P parameter, there is an electronic parameter which is absent in equation 29. Thus, ulcerogenicity has a preference for electron donating substituents. From these equations one can draw a conclusion that electron withdrawing substituent, lipophilic enough to produce a log P for the molecule around 2.6, should produce the maximum separation between the anti-inflammatory activity and ulcerogenicity.

In the above examples useful information were gleaned from QSAR equation. Topliss suggested an empirical scheme for the selection of substituents for the synthesis. This scheme is based on the physico- chemical parameters like hydrophobic, electronic and steric parameters. In this scheme, each compound is synthesized and tested. Based on the comparative activity with the previous compound, the scheme suggested the next substituents to be selected for the synthesis. Then that compound will be synthesized and tested. The scheme suggest further substituents for the next synthesis. The scheme is called Topliss scheme. There are two schemes, one for aromatic substituents and another for aliphatic. For example, in the case of aromatic compounds, the first analog to be made is para- chloro derivative. If it is more active than the parent, then the hydrophobic or electronic effect or both may be contributing to the activity. In that case next compound to be synthesized is 3, 4 – dichloro derivative. If it is more potent than para- chloro then the third compound to synthesized should be 3 -CF_3, 4 –Cl derivative. On the other hand if the first

compound para chloro is less active then the scheme suggests para methoxy derivative. If a para- chloro derivative is equally active as parent molecule then the scheme suggests para methyl derivatives. In this way the scheme guides the synthesis every stage. The study shows that in the case of anti-malarial activity of Quinones about 295 compounds were synthesized and tested and using Topliss scheme an equipotent analog would have been reached by synthesizing only 5 compounds. In the case of β – adrenergic activity of phenyl ethyl amines, only 3 compounds were sufficient through Topliss tree whereas 9 compounds were synthesized. About 19 compounds were synthesized for the antibacterial activity of pyridyl-anilines where only 5 compounds would have been sufficient to obtain most active derivative through topless scheme.

Topliss further modified his scheme where initially only 5 compounds are to be synthesized. Based on their order of activity more specific compounds can be synthesized using his modification scheme.

6.5.1 Success story of PCA inhibitors

Developed of anti-allergic pyranen-amines by Cramer at smith Kline and French Laboratories is one of the finest examples of the application of QSAR methodology. The study began with 19 compounds. These compounds were selected based on Topliss scheme. However, analogs based on Topliss scheme did not produce any significant increase in the activity. Hence, many other analogs which were bioisosters were synthesized. These analogs showed similar activities. Out of all these compounds the most active one was the 4-OH derivative. QSAR analysis of the data resulted in a modest equation 31

$$pI_{50} = -0.72 - 0.14\Sigma\pi - 1.35 \quad \text{.....(31)}$$
$$n = 19, \quad r = 0.701, \quad s = 0.47$$

Here pI_{50} is the activity of passive cutaneous anaphylaxis (PCA) response in rats. The equation suggests that activity increases with the reduction in partition coefficient and electronically neutral substituent ($\sigma = \Phi$). Based on the equation, additional compound with variety of hydrophilic substituents were prepared and tested. The activity increased about six times compared to the earlier series. QSAR with these new compounds confirmed the earlier observation about hydrophilicity and electrnically neutral substituents. It further refined the information about H – bounding and an indicator variable for 4 – acyl group. It also suggested that increasing the size of substituents at meta position will enhance the activity. Based on this information, further synthesis was carefully planned. Synthesis of certain analogs required considerable synthetic effort. Finally all the compounds were subjected to QSAR (about 100 compounds). The potent compound found was about 1000 times more potent than a member in the original series. The activity predicted by QSAR was 2.3 and the actual activity was 3.0. Several features of the pyranen-amine development illustrates the power of QSAR. The original series of 19 compounds was already considered as sufficient SAR. But QSAR suggested the hypothesis that increase in hydrophilicity results in increase in activity, which resulted in about 80 compounds with higher activity than the original set. Finally, some of the compounds synthesized would not have been attempted without a QSAR guideline since these were unusual and their synthesis was too difficult.

6.5.2 From QSAR to Clinics

Many compounds developed using QSAR methodology have reached clinical significance. Muzolimine is a potent, high ceiling, potassium sparing diuretics. A simplified topliss scheme suggested $^{+}\pi,^{+}\sigma$ contribution. This resulted in the selection of 3,4-dichloro analog. A new cardiotonic agent, Ar-L115 was developed using a novel QSAR based method that makes use of the theory of sets. Since cardiotonic activity is seen in imidazoles, calcium complexing agents etc., these two features were selected and many subsets were defined. Optimization of the lead and use of cluster analysis resulted in the discovery of Ar-L115. In the design of acridines as potential anticancer agent more than seven hundred derivatives have been synthesized studied by Cain and coworkers at the cancer chemotherapy Research Laboratory in New Zealand. These compounds have been extensively studied using QSAR methods. About 509 compounds were included in deriving a QSAR equation, which contained about 13 variables giving a correlation coefficient of 0.878. QSAR studies on the toxicity of acridines (n=643, r = 0.771) suggested that the anti-cancer activity is related to the toxicity. One of the analog from this group m- AMSA showed promising results and has reached clinical stage. RS- 51288 is a potent, selective non depressive β- blocker which did well in clinical trials was developed with the aid of QSAR. RS-51288 has a unique side chain. Short, bulky, moderately lipophilic group was suggested by the QSAR analysis of the adenylate cyclase data. This resulted in the search which ended with the unique side chain of endobicyclo h- exylethyl. However, in clinics, its potency was not sufficient for commercial development. QSAR of 2 – Phenyl – 8 – azapurin- 6- ones as anti-allergic agents suggested an

ortho substituent with small size, good hydrogen bonding property. The 2- propoxy derivative resulted in clinically active compound which can be given as aerosol. The discovery of cimetidine based on the modern concept of bioisosterism and the story of pyranenamines where potency increased by about 1000 times has been already mentioned.

6.6 applications of Free – Wilson model

Free – Wilson method is almost like performing QSAR analysis using only indicator variables and without any physico – chemical parameter. Although the limitations of such method is obvious. Free- Wilson method has been used in many cases to obtain useful information. The antimicrobial activity of Quinoxaline 1,4-dioxider was analyzed by Free – Wilson method. The analysis was very useful in predicting a more potent analog that otherwise would not been made. Although all the compounds with $-COCH_3$ substitution showed only modest activity, the Free- Wilson analysis revealed unexpectedly large contribution. The final compound synthesis using this information was not only potent in vitro but showed potent in vivo activity. In another application analgesic activity of semi-synthetic opoid narcotics, morphinan – 6 – ones analysed by Free – Wilson method. The analysed showed that if phenolic and nonphenolic members of this series act on the receptor, they must bind at different sites or act in alternative mechanism. In the case of erythromycin esters, Free-Wilson method was more useful than Hansch method. Firstly the type substituents were various combinations of OH, OCOH, OCOCH3 , OCOC2 H5. Hence Free – Wilson method was an ideal choice. For Hanch type analysis broader variation in the substituents would better. The Free Wilson analysis resulted in an equation with r^2 of 0.986 and s of 0.072. The same data was also subjected Hansch analysis whish resulted in an equation with $r^2 = 0.945$ and s= 0.127. Because s is larger than that from Free- Wilson equation, it was concluded that the the physicochemical properties do reflect the activity properly. Free – Wilson method has also been applied to peptides. Since the only variation is the individual amino-acid and one do not come across many types of substituent. Free – Wilson method is suitable for the analysis of peptide. Here, if one assumes that the contribution of each amino acid the activity is not influenced by any other amino-acid, then using free – Wilson analysis, the individual contributions of the amino-acid can be quantified. The method has been applied to ACTH related peptides, bradykinin potentiating peptides and other peptides.

6.7 Quantitative Structure Pharmacokinetic Relationship (QSAR)

Influence of physic chemical properties in the absorption, distribution, metabolism and excretion (ADME) has been widely studied using QSAR methodologies. It is known since long time that absorption, penetration into the brain and metabolism by liver increase with increasing log P, whereas urinary excretion decreases with increasing log P. However, QSAR analysis has shown many deviations from the linear relationships. The optimum partition coefficient for gastric, buccal and dermal absorption are given in section 6.2. Lipophilicity is also important for protein binding. However, structural features influences protein binding. Thus a plot of binding constant (human serum albumin) against log P for various types of drugs does not give a single regression line but several parallel lines. Many studies have shown the relationship between the metabolism of compounds through microsomal system and physicochemical properties.

6.8 Quantitative Structure Toxicity Relationship (QSTR)

Currently, QSAR methods are increasingly applied in the area of toxicology and ecological sciences. Several attempts have been made to predict mutagenicity of the compounds using QSAR. For example, Hansch and his group studied aromatic and hetero-aromatic amines for their mutagenicity using Ames test. The study showed that the hydrophobicity is the most important determinant of the relative mutagenicity and other factors like steric and electronic parameters are of secondary importance, while the size of the aromatic ring system has small or no effect on the mutagenicty. The same group extended their work to nitro compounds obtain a bilinear equation. QSAR was also useful in studying carcinogenicity and mutagenicity of various amines. A significant correlation between mutagenic and carcinogenic potencies was observed. In the case of quinolines, mutagenic potential was correlated with log P and electronic parameters. It was suggested that 2 – position of the quinoline is the site for metabolic activation. Lipophilicity was found to influence the bio-concentration of pesticides in food chain. DDT and other chlorinated hydrocarbons which are lipophilic are frequently involved in the environmental toxicity, whereas, more polar phosphates are considered relatively safe. However, Leptophos has been implicated in the human neurotoxicity. It has a log P = 6.31 which is slightly above that of DDT (log = 6.19). In many series of compounds, the toxicity data like LD-50 has been found to correlates with the physico – chemical

properties. Many such examples are given in section 6.5.

7 Conclusions

This chapter is a brief introduction to QSAR, especially Hansch analysis and its application in drug design. The physicochemical parameter and the methodology have been described only to the extent which helps in appreciation the application of QSAR. There is a cadency to look at QSAR only as a predictive tool but the important of QSAR is much beyond its predictive potential, frequently the predictions based on QSAR fails. The reasons for the failure can be understood if one appreciates the limitations of the technique. The major inputs of QSAR are biological activity and physic chemical parameters. There are many problems in measuring the biological activity in an accurate manner. The animal data often has a large standard error. While testing large number of compounds the number of animals used will be a limitation, especially when it has to be tested at many doses. It is equally difficult to express a three dimensional compound in terms of physic chemical properties. Stereochemistry is a major challenge to QSAR. Several attempts have been made to study chiral drugs by QSAR but with varying results. In the last two decades QSAR has played a major role in developing medicinal chemistry into a more rational science. It ha changed the whole paradigm of medicinal chemistry. It brought computer literacy to the subject which greatly helped in assimilating more sophisticated computer aided drug design techniques like molecular graphics, 3D QSAR, multivariate analysis etc. its importance will be appreciated more and more in the years to come. The affair has just started.

Limitation

1. Measuring the biological activity in accurate.
2. For listing the biological acting large animal using.
3. Difficult to express 3 D component in term of physic – chemical properties.
4. Chiral drug study may be difficult.

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