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Research article

Quantitative structure property relationship studies for predicting plasma protein binding properties of Cephalosporins

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Abstract

Cephalosporins are of key clinical importance for treatment of bacterial infections. In the past decade, many cephalosporins have been synthesized and evaluated for antibacterial activity. These cephalosporins, with broad spectra of activity and high stability against various β-lactamases such as cefixime, cefteram pivoxil, and cefpodoxime proxetil, have been developed and introduced in clinical practice. Efforts to synthesize more compounds for better activity are still on. It is very important that the antibiotic has favorable pharmacokinetic properties [absorption, distribution, metabolism, excretion (ADME)]. Hence, predicting pharmacokinetic parameters, of a new molecule, in an early stage of drug design, is of as high importance as the activity of the compound. With rapid advances in computation power of machines and availability of experimental data, these ADME properties can now be better predicted by using suitable computational methods. In present study, an attempt has been made to derive quantitative relationships between structure of cephalosporins and one of the important pharmacokinetic property, serum plasma protein binding.

Key words: Cephalosporins, ADME, QSPR.

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1. Introduction

A cherished goal of chemists for generations has been to create molecules with specific properties. Finding new drugs, in particular, is an important part of the new initiatives in health care. However, it is an extremely challenging process due to the complexities involved [1]. Traditionally, a combination of serendipity and empiricism has been the basis of new drug discovery. Trial and error synthesis of compounds and their random screening for activity have proved to be both time-consuming and uneconomical. Further, therapeutic effects and hazards to health are assessed using a series of experimental and *in-vivo* tests. However, usage of animal models is often subject to ethical (and financial) considerations. Therefore, alternative methods have been under development to reduce the requirement of animals in testing [2].

Structure-based design, spurred by significant pitfalls of the traditional methods and rapid advances in molecular structure determination and computational resources, were tested as a means of generating new pharmaceuticals [3, 4] and for predicting their properties prior to synthesis [5].

The structural formula of an organic compound, in principle, contains coded within it all the information which predetermines the chemical, biological, and physical properties of that compound. If we can understand how a molecular structure brings about a particular effect in a biological system, we have a key to unlocking the relationship and using that information to our advantage. Formal development of these relationships on this premise proved to be the foundation for the development of predictive models. If we took a series of chemicals and attempted to form a quantitative relationship between the biological effects (i.e. bioactivity) and the chemistry (i.e. structure) of each of the chemicals, then it would be possible to form a quantitative structure–activity relationship or QSAR [6, 7].

Quantitative structure–property relationships (QSPRs), are mathematical models that attempt to relate the structure-derived features of a compound to its biological or physicochemical activity. Similarly, quantitative structure–toxicity relationship (QSTR) or quantitative structure–pharmacokinetic relationship (QSPR) is used when the modeling applies on toxicological or pharmacokinetic systems. QSAR (also QSPR, QSTR, and QSPR) works on the assumption that structurally similar compounds have similar activities. Therefore, these methods have predictive and diagnostic abilities. They can be used to predict the biological activity (e.g., IC50) or class (e.g., inhibitor versus non-inhibitors) of compounds before the actual biological testing. They can also be used in the analysis of structural characteristics that can give rise to the properties of interest.

The explosive development of computer technology and methodologies to calculate molecular properties increasingly made it possible to use computer techniques to aid the drug discovery process. The use of computer techniques in this context is often called computer-aided drug design (CADD), but since the development of drug involves a large number of steps in addition to the development of a high affinity ligand a more appropriate name computer-aided ligand design (CALD) has also been proposed [8].

2. Materials and Methods

The present study was undertaken with an objective to establish quantitative-structure pharmacokinetic relationships (QSPR) of prognostic relevance in the β-lactam (Cephalosporins) series of drugs. The reason to select β-lactam series of drugs was because such correlations are developed for very few drugs. Further, very few reports on QSPR were available for this series of drugs and that too involving only small sets of drugs and few descriptors. Thus, quantitative relationships between structural descriptors of cephalosporin molecules and serum protein binding (PB) were evaluated.

The work was divided into following three phases:

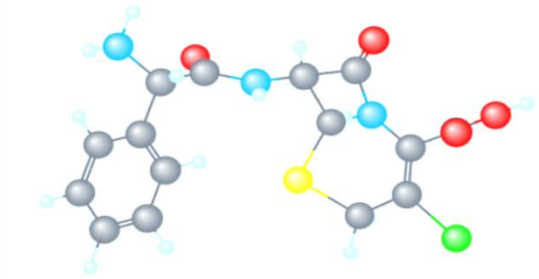
1. Computation of molecular descriptors
2. Compilation of pharmacokinetic data
3. Development of meaningful correlations

Computation of molecular descriptors

It is well known fact that the structure of drug molecules is expressed quantitatively in terms of its physicochemical descriptors,

which are lipophilic, electronic and steric in nature. The physicochemical descriptors govern the biological activity of the compounds.

PUBCHEM database contains 2D and 3D minimized structures of large number of drugs and other molecules. 3D structures of 39 cephalosporins selected for the study were downloaded from the database and used as such for correlation studies. Sample 3D structure of one of the cephalosporin, Cefaclor, used in the study is given in figure 1. Structures of 39 cephalosporins in molfile format were used as input for computation of descriptors.

S No.	Molecule	3 D Structure
1	Cefaclor	

Here

= Hydrogen	= Carbon	= Oxygen	= Nitrogen
= Sulfur	= Chlorine		

*Source: PubChem Compound

Figure 1. Sample 3D structures of one cephalosporin used in the study

We used two software, namely, QikProp and CODESSA to calculate the descriptors.

QikProp, an application in Maestro version 10.4.018 which in turn is a part of Schrödinger Suite release 2015-4, was used for this work. This suite of applications is used to predict physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs.

CODESSA version 3.2.13 was used in our work. This software integrates all necessary mathematical and computational tools to calculate a large variety of molecular descriptors (up to 400, depending on input files) on the basis of the 3D geometrical and/or quantum-chemical structural input of chemical compounds. Within the framework of the CODESSA program, a variety of statistical techniques are also available for structure-property correlation and for the analysis of the experimental data in combination with the calculated molecular descriptors.

MOL files were used as input to the software by selecting the command Project→Import structures. All the molfiles were selected and imported into QikProp. Descriptors were calculated by using commands Application→QikProp and pressing run. QikProp calculates all the descriptors and creates a project

table. The data was imported into CODESSA by using command "Add CODESSA input file", which is a standard CSV format containing all the requisite information like molfiles, descriptor values and property values.
CODESSA calculates additional descriptors for each of cephalosporin. In this case, more than 200 descriptors were calculated using QikProp and CODESSA.

Compilation of pharmacokinetic (serum protein binding) data

The reported values of serum protein binding of cephalosporins in humans were taken from literature [9-15]. Most reviewers, while compiling pharmacokinetic data for a series of drugs, take the mean value as the value for the pharmacokinetic parameter. On similar lines, pharmacokinetic data for all the drugs were compiled and the arithmetic mean was taken for the correlation studies. The mean values of these values for all cephalosporins used in study are compiled in Table 1.

Table 1. Plasma Protein Binding Values of selected Cephalosporins

#	Drug	P_b (%)	#	Drug	P_b (%)
1	Cefaclor	37.33	2	Cefotiam	40
3	Cefadroxil	15	4	Cefoxitin	68.33
5	Cefamandole	74.67	6	Cefpiramide	96
7	Cefamandole nafate	75	8	Cefpodoxime	27.5
9	Cefatrizine	59	10	Cefprozil	45
11	Cefazaflur	65	12	Cefroxadine	10
13	Cefazedone	95	14	Cefsulodin	30
15	Cefazolin	83.67	16	Ceftazidime	16.33
17	Cefdinir	65	18	Ceftezole	42.5
19	Cefditoren	88	20	Ceftizoxime	108
21	Cefepime	20	22	Ceftriaxone	89.67
23	Cefetamet	30	24	Cefuroxime	36.67
25	Cefixime	63	26	Cephacetrile	35
27	Cefmenoxime	60	28	Cephalexin	13.33
29	Cefmetazole	85	30	Cephaloglycin	25
31	Cefonicid	98	32	Cephaloridine	20
33	Cefoperazone	90	34	Cephalothin	66.67
35	Ceforanide	81.67	36	Cephapirin	46.67
37	Cefotaxime	37.67	38	Cephradine	11.67
39	Cefotetan	90			

Development of meaningful correlations

Only significant descriptors calculated by QikProp and CODESSA were taken in the correlation studies. Insignificant or intercorrelated descriptors were skipped. Correlation studies were carried out by CODESSA.
Selection criteria and steps used for "Best Multilinear Regression" in CODESSA is shown as following:

- Maximum number of descriptors, started from 1 and then taken up to depending on the number of molecules selected. Drug molecules: Descriptor ratio was taken as 6:1, which implies that not more than one descriptor per 6 molecules in a series was used for developing correlations. For example, if there were 21 molecules for a particular property, maximum number of descriptors used for developing regression equations was kept at 3. Similarly for a series having 40 molecules, maximum number of descriptors was 6.
- Maximum number of correlations per number of descriptor were kept as 5
- Correlation improvement cut-off was kept as 0.01
- Maximum r^2 for orthogonal descriptor was kept as 0.5
- If missing property value, then the selection was made to skip structure

"Best Multilinear Regression" routine tests a large number of correlations as each descriptor type is analyzed for correlations individually for the selected pharmacokinetic property.

3. Results and Discussion

Correlations for serum protein binding with structural descriptors are discussed below:
Serum protein binding data was available for 39 cephalosporins, thus these cephalosporins were taken for the present study out of 45 selected cephalosporins. Thus, correlations were attempted keeping the number of maximum descriptors to 6 thereby limiting the drug: descriptor ration to 6:1. LOO and y-scramble tests were also performed. The best correlations obtained with serum protein binding (PB) for cepahlosporins are given in below Table 2. The table lists equations starting from 1 descriptor equation up to an equation with maximum number of descriptors that can be used as mentioned above.
With the probability of reporting a large number of such correlations for each property, it was considered necessary to change the format of these correlations into an equation format. The validity of the equation and the relative importance of the different parameters used can be judged by four statistical criteria; namely coefficient of determination R^2, Cross validated R^2 (Q^2), Fisher's F value, and R^2 Rand which is the maximum R^2 obtained after randomizing the property values and finding correlations with descriptors again. The larger value of *F* indicates higher probability of QSPR equation being significant. These methods provide correlation coefficient (*r*), standard deviation (*s*), and ratio between variance of calculated and observed activates (*F*). Depending upon the values of these statistical parameters, the significance of each equation was evaluated.

Goodness of correlations and types of descriptors involved

Constitutional and electrostatic descriptors resulted in statistically significant correlations. Reasonably high values of R^2 and Q^2 were obtained (Equations. 1-6, Table 2). Excellent correlations of serum protein binding were obtained in the series when more than 4 descriptors were used (Equations. 5-6, Table 2). The max. R^2 (~0.79) and Q^2 (~0.62) for equation 5 and max. R^2 (~0.82) and Q^2 (~0.54) for equation 6 indicate the importance of these descriptors in describing the serum protein binding of cephalosporins. It is notable that the R^2 RAND for all the equations is lesser than the R^2, which indicates that the equations obtained are not chance correlations and hence can be used for prediction purposes.

As it would be too voluminous to give details of each of the equations obtained, details of only the best correlation for each property in a series are given. The correlation matrix of descriptors used in Equation 5 is given in the following Table 3. **Correlation matrix for selected descriptors of Equation 5,**.

The correlation matrix indicates that none of the descriptors used in the correlation are orthogonal with the other descriptors.

The MLR regression coefficie*nts* for individual descriptors used in Equation 5 are given in Table 4. The plots of observed versus predicted serum protein binding values obtained given below in Figure 2.

Table 2. Correlations of Protein Binding in the series of Cephalosporins

	Equation	M	N	R^2	Q^2	F-Value	R^2 RAND
1.	PB = 1351.659*Average Zefirov Charge for a N Atom + 156.997	1	39	0.4495	0.4032	30.2105	0.3986
2.	PB = 0.318*NSASA-1, Zefirov - 179.149*Average Valence for a N Atom + 495.983	2	39	0.6123	0.5339	28.4325	0.4549
3.	PB = 0.373*NSASA-1, Zefirov - 194.677*Average Valence for a N Atom + 6653.126*Maximum Bond Length - 11620.449	3	39	0.7004	0.5009	27.2722	0.4546
4.	PB = 3405.017*Average Bond Length for a C-O Bond - 647.698*Net Zefirov Charge of All C Atoms - 197.354*Uniform-Mass, Center of Mass, X - 3904.678	4	39	0.7426	0.5304	24.5221	0.3384
5.	PB = 0.295*NSASA-1, Zefirov - 176.289*Average Valence for a N Atom + 176.363*Average Valence - 168.45*Center of Mass, Z + 5379.612*Maximum Bond Length - 9762.544	5	39	0.7899	0.6164	24.8073	0.4789
6.	PB = 0.329*NSASA-1, Zefirov - 391.292*Average Valence for a N Atom - 10624.959*Maximum Bond Length for a H-N Bond - 803.326*Average Bond Length for a C-C Bond + 171.46*Minimum Bond Length for a O Atom + 12500.639*Maximum Bond Length for a C-C Bond - 6489.243	6	39	0.8228	0.5426	24.7673	0.4991

Table 3. Correlation matrix for selected descriptors of Equation 5,Table 2

	NSASA-1, Zefirov	Average Valence for a N Atom	Average Valence	Center of Mass, Z	Maximum Bond Length
NSASA-1, Zefirov	1.0000				
Average Valence for a N Atom	0.1238	1.0000			
Average Valence	0.5457	0.2618	1.0000		
Center of Mass, Z	0.0900	0.3093	0.4595	1.0000	
Maximum Bond Length	-0.3589	0.0761	-0.0123	-0.0023	1.0000

Table 4. MLR regression coefficients and t-values for PB in Cephalosporins

Descriptor Name	Coeff.	T	p(t)	SE
Intercept	-9762.5440	-2.9327	0.006067	3328.8645
NSASA-1, Zefirov	0.2952	5.9752	1.04E-06	0.0494
Average Valence for a N Atom	-176.2893	-5.2252	9.49E-06	33.7382
Average Valence	176.3634	2.7857	8.78E-03	63.3095
Center of Mass, Z	-168.4497	-3.5180	0.001291	47.8828
Maximum Bond Length	5379.6115	2.9175	0.006305	1843.8894

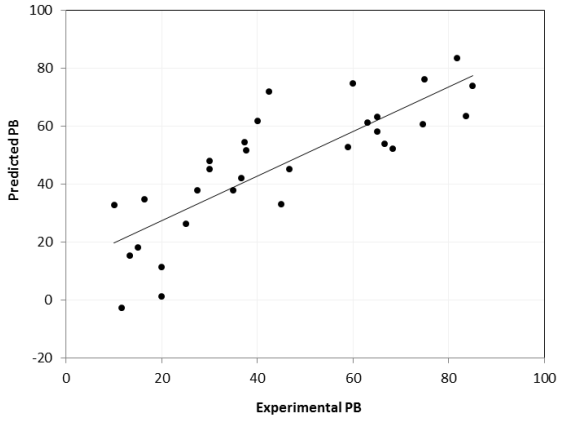


Figure 2. Plot of experimental vs predicted PB

In literature, serum protein binding of drugs has primarily been correlated to lipophilicity [16-22]. The relation of graph theoretical descriptors [23], connectivity indices and pKa [24], steric parameters [25], and electronic descriptors [22] have also been studied. Our findings are also similar as these reports. Hydrophobicity (HumanAbsorption) descriptors did not correlate well in cephalosporins, however, all other types of descriptors namely constitutional, topological and electrostatic were part of the final correlations.

Conclusion

Structure-pharmacokinetic relationships were established for Protein binding in cephalosporins. Excellent correlations of serum protein binding were obtained in the cephalosporin series when more than 4 descriptors were used. High R^2 (~0.82) and Q^2 (~0.54) values indicate the importance of these descriptors in describing the serum protein binding of cephalosporins. The correlation matrix also indicated that none of the descriptors used in the correlation are orthogonal with the other descriptors. Also, lesser R^2 RAND values in comparison to R^2 indicates that the equations obtained are not chance correlations.

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