Modeling Of Half-Live Values By QSAR Using Computational Indices And Physico-Chemical Properties For B-Lactam Structure Containing Drugs

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Abstract: In current study forty-nine compounds of cephalosporin containing β-lactam type core structure containing drugs compounds were selected. The half-life ($t_{1/2}$) of these drug actions were searched out from various journal publications and other sources of chem-informatics. 2D & 3D chemical structures of these compounds were primed by by a software chem sketch and outputs were saved as a file in a format of mol file. Some 2D and 3D indices and physico-chemical properties were calculated by Dragon software. It was observed that Half-life ($t_{1/2}$) not strongly correlated with any one individual index out of selected indices and physico-chemical properties, so this is not possible to represent half-life of selected class of drug with simple linear regression obtained in single step regression analysis. Hence for developing QSAR equation/ model for prediction of half-life step wise multiple linear regression (MLR) analysis by means of forward selection method was carried through Microsoft excel software. For stepwise regression analysis excel notes for stepwise regression analysis was followed in which in each step independent variable (indices and physico-chemical properties) were filtered out/in on the basis of lowest P-value. it was observed that 3D-Morse, T(N-S), Harary index, orbital electro negativity at O-atom of carbonyl group, donar sites and density can predict half-life in much better way among others. There occurs a strong correlation (pearson's $r^2 = 0.887371$) of observed values with predicted values calculated by the regression equation model developed by current study.

Keywords: MLR, Stepwise multiple regression analysis, Half-life ($t_{1/2}$) of drugs, Cephalosporin, Dragon, Microsoft excel, Topological indices.

1. Introduction:

Computational Chemistry [1] enables applications of computer and computer operated calculations in chemistry for various purposes and targets. Most important scope of computational chemistry among others is QSAR and QSPR [2, 3] followed by designing of drug. QSAR i.e. Quantitative Structure Activity Relationship provides routes to correlate the effect of structure over activity in terms of mathematical descriptors [4, 5] which are known as topological indices [6,7]. A QSAR technique is a mathematical relationship between a biological activity of molecules/ molecular systems and its spatial, geometrical, stereo-chemical and chemical characteristics. QSAR aims to try to find consistent relationship between biological activity [8, 9] and molecular characteristics/properties, so that this developed relationship may routes provide rules which can be used to evaluate the activity [10] of new possible or hypothetical

compounds. QSAR is an applied series of mathematical models built to predict biological, physicochemical, pharmacological, toxicological as well as physical properties behavior of molecules based on their chemical structures. Drug designers employ QSAR and QSPR techniques to determine the possible potential of drugs to react with the human body/ animal body.

The prediction of biological effects of a chemical compound can be done by its molecular structure [11] which is based on the assumption that similar compounds have similar physical and biological properties. Principle involved in this referred to as Structure Activity Relationships (SAR), which is a powerful tool for predicting biological effects of a compound without the cost and minimum time associated. This also test by traditional whole-animal laboratory toxicology studies. Chemists generally have managed so far for synthesis and measure at least some of the properties of more than half and seven million different molecules. The data base containing this store of information can be drawn on by the research chemist, providing nearly instant knowledge about any molecule that has ever isolated. So, this is the need to use this data base [12] with knowledge for further utilization. For example, the data could be drawn on to predict the property of chemical substance before they are even synthesized that is before any molecule of that substance physically exists. By the invention of QSAR and QSPR this is a recognized fact that the biological activities of chemical entities can be expressed quantitatively by using their physico-chemical parameters [13]. The study of quantitative structure activity relationships (QSAR) has been proved a useful tool to design new molecules with modified and desirable activities. QSAR Techniques have been used extensively for rational drug design to predict and rationalize a drug activity out of a series of chemical entity belonging to same core structure. The augmentation of the idea and principles of QSAR to the pharmaco-kinetic data resulted in emergence of a new tool called Quantitative Structure Pharmacokinetic Relationship studies (QSPkR studies) [14]. QSPkR can be exercised best at the early stage of clinical trials for designing a better molecule with favorable pharmaco-kinetic activities and properties. For this purpose some pharmaceutical and pharmacological properties are: human intestinal absorption, oncogenicity (for tumors/cancers), plasma protein binding, water solubility, teratogenicity (congenital malformations in a developing fetus), volume of distribution, half time of elimination, absorption rate, blood brain barrier, neurotoxicity (central /peripheral nervous system, pK_a, bioavailability, mutagenicity (induce permanent and heritable changes in the genetic material i.e. DNA), and log P. The prospect of making such prediction has tantalized computational chemists since many decades. It is now becoming reality, thanks to novel methods and techniques that although still in early stages, can already claim a remarkable number of successes in surprisingly broad range of applications. The brain of new techniques is the topology of individual molecules. The different ways of interconnection among atoms in each molecules atom determine the ultimate architecture of the molecules. The main hypothesis in the QSAR/QSPR (quantitative structure-activity/property relationship) approach is that all activities/ properties (chemical, physical, physico-chemical as well as biological) of a chemical or material or drug are related statistically to its molecular structure/ characteristic [15]. Quantitative relationship generated from such studies help in hypothesizing important contributions of specific structural aspects or chemical interactions in modifying physico-chemical properties and biological activities and also in predicting properties and activities of new hypothetical/ imaginary novel molecules which are untested and not yet synthesized [16]. Mathematical descriptors of molecular structure, like various topological indices (TIs), have been widely utilized in qspr i.e. structure-property-activity relationship studies [17]. TIs are mathematical entities which encode molecular graphs composed of vertices and edges. Vertices correspond to the atoms and edges represents the bonds among different atoms. Two-dimensional descriptors take into account the internal atomic arrangement of molecules and encode it in numerical form (i.e. figure) information about molecular size, shape, branching, presence of heteroatoms and multiple bonds [18]. While three-dimensional descriptors take into account electronic configuration, charge distribution. HOMO, LUMO electronic configuration.

QSAR/QSPR generally takes the form of a linear equation [19, 20]:

$$\textbf{Physical Property/ Biological Activity} = \textbf{Constant} + (w_1 X_1) + (w_2 X_2) + (w_3 X_3) + \ldots + (w_n X_n)$$

Where, the parameters X_1, X_2, X_3, …… X_n are computed for each molecule in the series and the coefficients w_1, w_2, w_3, …… w_n are calculated by fitting variations in the parameters and the biological activity, this is the basic principle.

The QSAR equation is a linear single or multilinear regression model which relates variations in biological activity due to variations [20] in the molecular structure in terms of values of computed (or measured) properties [21] for a series of molecules. For efficient result, the training set i.e. the compounds selected to describe the chemical space of the experiments should be diverse. It is also observed in many synthesis drives the compounds are prepared which are structurally similar to the lead structure.

1.1 About Cephalosporin:

In the present study we shall use a series of cephalosporin [22] for QSAR & QSPR studies. General core structure of cephalosporin [23] is as follows:

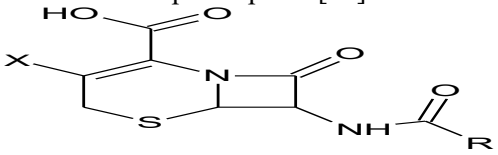


Figure (1): Structure of cephalosporin nucleus with β-lactam ring

Cephalosporin is a kind of drug which is derived from cephalosporin C [24, 25] which is an acid-stable molecule with antibacterial activity and is produced from 7-amino cephalosporanic acid. All bacterial cells have a cell wall that protects them. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls, which causes the walls to break down and eventually the bacteria die. Cephalosporins are bactericidal. They inhibit enzymes in the cell wall of susceptible bacteria, disrupting cell synthesis. All β-lactams bind to and inactivate enzymes required for bacterial cell wall synthesis. Cephalosporins are usually bactericidal against susceptible bacteria due to inhibiting mucopeptide synthesis in the cell wall resulting in a defective barrier and an osmotically unstable spheroplast. The exact mechanism for this effect has not been definitively determined, but beta-lactam antibiotics have been shown to bind to several enzymes like carboxypeptidases, transpeptidases, peptidases with in the bacterial cytoplasmic membrane that are involved with cell wall synthesis. The beta-lactam antibiotics have different affinities for these enzymes (penicillin-binding proteins, PBPs), which help to explain the differences in spectrums of activity of these drugs that are not explained by the influence of beta-lactamases.

1.2 Applicability of cephalosporins drugs:

Like other beta-lactam antibiotics, cephalosporins are generally considered to be more effective against actively growing bacteria. Cephalosporins are indicated for the treatment of bacterial infections caused by susceptible organisms. First generation cephalosporins are predominantly active against gram-positive bacteria, and successive generations have increased activity against gram-negative bacteria (often with reduced activity against gram-positive organisms). In brief, cephalosporin is a group of broad-spectrum antibiotics first isolated from the mediterranean fungus acremonium (Cephalosporium acremonium). They contain the beta-lactam moiety thia-azabicyclo-octenecarboxylic acid also called 7-aminocephalosporanic acid. Bacteria are classified in several ways. One of the ways is by their color after a particular chemical stain (gram stain) is applied. Some bacteria stain blue and are called gram-positive, others stain pink and are called gram-negative. Gram-negative bacteria have a unique outer membrane that prevents many drugs from penetrating them, making gram-negative bacteria generally more resistant to antibiotics than are gram-positive bacteria. Gram-negative bacteria are able to become resistant to antibiotics; gram-positive bacteria are usually slow to develop such resistance.

Cephalosporins are used to treat a wide variety of bacterial infections, such as respiratory tract infections (pneumonia, strep throat, tonsillitis, and bronchitis), skin infections and urinary tract infections. They are sometimes given with other antibiotics. Cephalosporins are also commonly used for surgical prophylaxis prevention of bacterial infection before, during, and after surgery. In open, laparoscopic or endoscopic surgery intravenous prophylaxis by cephalosporins had excellent efficacy. Because of the high susceptibility to infections, antibiotics are the most widely used drugs in newborns**.** Although side effects (like nausea, vomiting, etc.) are also linked with Cephalosporins.

In the structure of cephalosporin as given in figure 1, nature of group –R and –X affect activity as well as property of cephalosporin. Kinetic studies combined with absorption and nuclear magnetic resonance spectroscopy have shown the structure of the opening of the β-lactam ring of cephalosporyl. Cephalosporyl is the speices formed by the aminolysis of β-lactam ring of cephalosporin due to reaction with amino acid's amino group present in cell wall of bacterial cell wall. Opening of β-lactam ring takes place either in a concerted fashion or in some stages.

1.3 Aim:

The opening of the β-lactam ring leads to elimination of the group -X when this is configured as a leaving group. The process is well documented chemically and this property has been used as a strategy to obtain cephalosporins that can apply in a double action way. When the -X is conformed as the inactive form of the drug, the action of the β-lactam in the cephalosporin implies the release of the drug in situ. Due to this, beta-lactamases are also used as biological markers for the identification of pathogenic bacteria resistant to β-lactam antibiotics. Based upon the ability of the -X to act as a leaving group, cephalosporins can also be used as sensors to monitor processes or biological interactions. In cephalosporins with nucleophilic groups at -R autoaminolytic reactions may occur to yield the compound, in which the intramolecular opening of the β-lactam ring is followed by -X exclusion, when this side chain can act as a good leaving group. The inherent chemical reactivity of cephalosporins implies that the opening of the β-lactam ring by nucleophilic reagents generates an intermediate cephalosporyl which is chemically instable and that suffers multiple fragmentation reactions. Despite the structural similarities with penicillins, those cephalosporins that have a good -X leaving group undergo the process of expulsion when they conjugate to carrier proteins by opening of the β-lactam ring. For these cephalosporins the unstable dihydrothiazine moiety is enough to undergo further degradation processes. As a result, conjugation of cephalosporins by the β-lactam ring leads to loss of the -X side chain and to fractionation of the dihydrothiazine ring and this does not form part of the epitope presented in the hapten-carrier conjugate. Only the -R side chain and a fragment of the β-lactam ring remain bound to the carrier protein, constituting the epitope resulting from these conjugates. The presence of an -X side chain that may act as a good leaving group is closely related to enhanced reactivity of the β-lactam ring for nucleophilic attack. The effect of the -X side chain on the conjugation of the carrier protein can be interpreted only from a kinetic perspective, such that an increase in the capacity of the -X as a leaving group results in increased reactivity for the attack of nucleophiles to the β-lactam ring, increasing the facility and kinetics of the conjugation process. The cephalosporin nucleus can be modified to gain different properties.

A cephalosporin antibiotic has an excellent antibacterial activity and a low toxicity to mammals, and therefore it is an extremely effective medicine useful for the therapeutic treatment of bacterial infections in the mammals. In recent years, many cephalosporin derivatives having an aminothiazolylacetyl group at the 7-position of the cephem ring have been researched and developed, because they have a strong antibacterial activity and a stability to β-lactamase. The so-called third-generation cephalosporin antibiotics represented by cefotaxime and cefmenoxime have the aminothiazolylacetyl group at the 7-position and are characterized by a high antibacterial activity and wide antibacterial spectra, and so they have been practically used in many countries of the world. However, some compounds amongst the third-generation cephalosporin antibiotics such as cefotaxime and cefmenoxime are not satisfactory in points of their antibacterial activity to Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus which clinically provide some problems in the recent years. In particular, the methicillin-resistant Staphylococcus aureus brings about a serious bacterial infection, and nowadays it is desired to provide a novel cephalosporin antibiotic having an improved antibacterial activity to these resistant bacteria. Since the development of a new drug is a costly proposition, pharmaceutical companies have done very little research in the last decade. However, an alarming development has spurred a revived interest in the development of new antibiotics. It turns out that some of the disease-causing bacteria have mutated and developed a resistance to many of the standard antibiotics. This could have grave consequences on the world's public health unless new antibiotics are discovered or improvements are made on the ones that are available.

2. Literature review:

QSAR type study was originates from the field of toxicology. In this field Cros [26] in the year of 1863, during his Ph.D. research work, proposed a relationship between the toxicity and water solubility of primary alcohols. In the year of 1868, Crum-Brown [26] and Fraser postulated the relationship between chemical constitution and physiological action. Later, Richet (in 1893), Meyer (in 1899), and Overton (in 1901) [27] separately studied and hence discovered a linear correlation between lipophilicity (i.e. oil-water partition coefficients), and biological effects (e. g. toxicity and other narcotic effects). Hammett (in the year 1935, 1937) discovered and introduced a method to establish a quantitative relationship for substituent effects on reaction mechanisms through the use of an equation which took two parameters into consideration namely the 1st substituent constant and the 2nd reaction constant [28, 29]. By supporting the Hammet model, Taft proposed in 1956 an approach for separating polar, steric, and resonance effects of substituents in aliphatic compounds. Contributions from Hammett and Taft provide the basis for QSAR/QSPR development. by Hansch and Fujita [31] in 1964. A linear Hanch equation was developed which integrated hydrophobic parameters with Hammet's electronic constants. Hansch and Leo [32] gave an insightful account on development of QSAR/QSPR by publishing a book in 1995. Hansch applied the free energy approach, he used physicochemical properties and correlated with biological activity using regression analysis. The result of treatment was an equation which describes in a quantitative
manner the relationship between biological activities of compound with its chemical structure.

3. Biological half-life($t_{1/2}$):

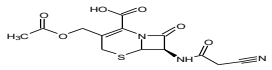
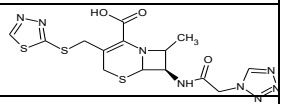
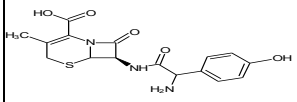
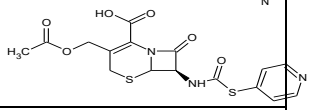
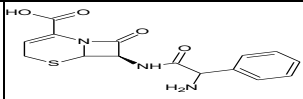
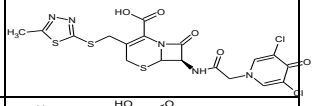
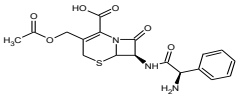
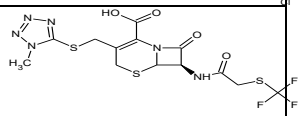
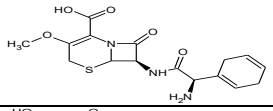
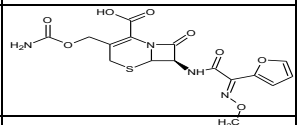
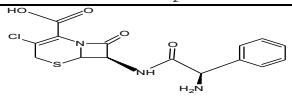
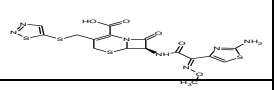
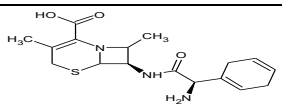
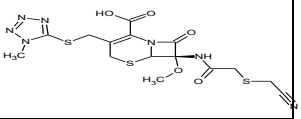
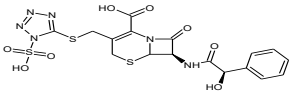
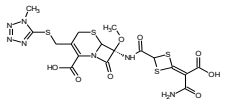
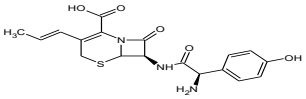
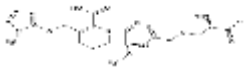
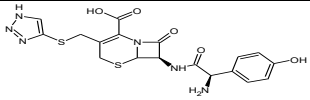
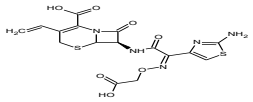
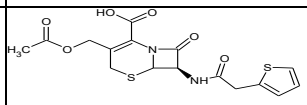
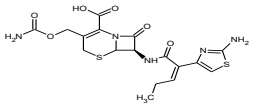
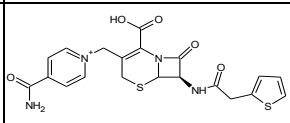
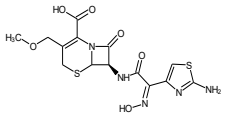
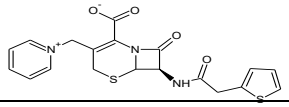
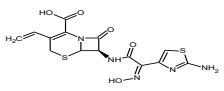
The biological half-life or elimination half life of a substance is the time it takes for a substance (drug, radioactive nuclide, or other) to lose half of its pharmacologic, physiologic, or radiologic activity [33], As per the MeSH definition, in a medical context, half-life may also describe the time it takes for the blood plasma concentration of a substance to halve (plasma half-life) of it's steady-state [34, 35]. The relationship between the biological and plasma half-lives of a substance can be complex, due to factors including accumulation in tissues, active metabolites, and receptor interactions.

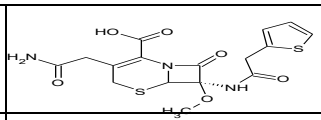
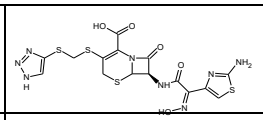
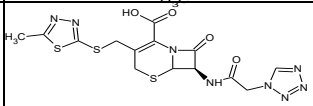
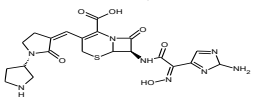
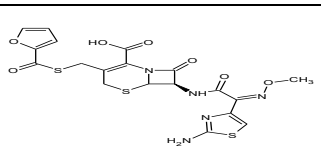
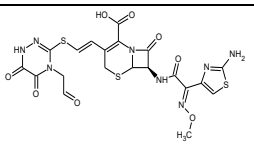
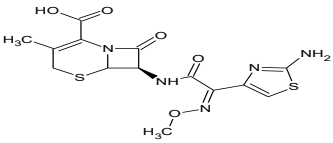
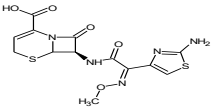
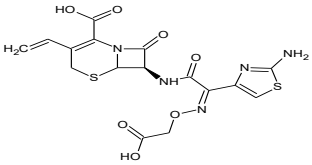
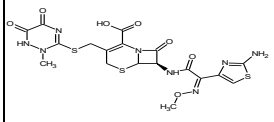
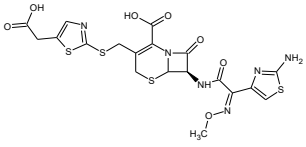
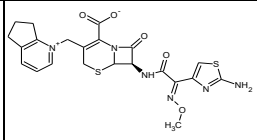
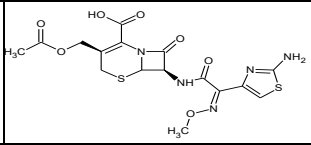
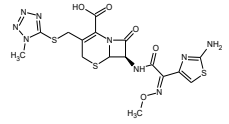
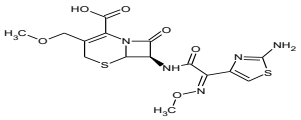
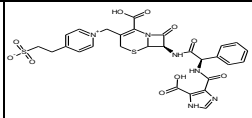
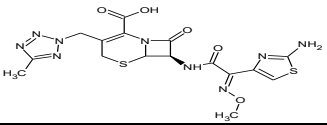
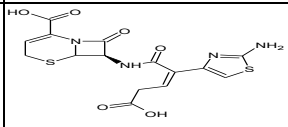
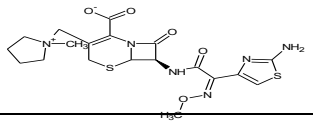
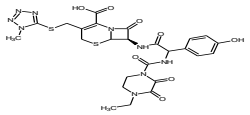
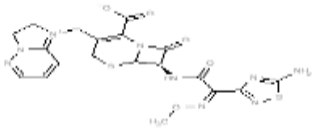
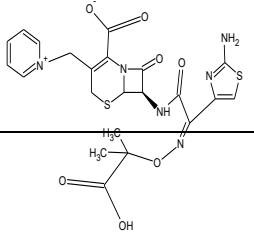
49 compounds of cephalosporin type β-lactam type core structure containing drugs compounds were selected. Half-life ($t_{1/2}$) of these drug actions were searched out from various journal publications and other sources of cheminformatics. Chemical structures (2D and 3D) of these compounds were prepared by chem sketch software and saved as a mol files. Some 2D and 3D indices and physico-chemical properties were calculated by Dragon software. It was observed that Half-life ($t_{1/2}$) not strongly correlated with any one individual selected indices and physico-chemical properties, hence for developing qsar equation for prediction of half-life a step wise multiple linear regression (MLR) analysis by means of forward selection was carried by Microsoft excel software. MLR was for half-life with other selected indices and physical properties. For stepwise regression analysis excel notes for stepwise regression analysis was followed in which in each step independent variable (indices and physico-chemical properties) were selected on the basis of lowest P-value and stepwise regression was carried out till the step when in the proceeding step no one regression's all the independent variables do not satisfies the condition $P < 0.05$ By following this method it was observed that 3D-Morse,T(N-S), Harary index, orbital electro negativity at O-atom of carbonyl group, donar sites and density can predict half-life in better way among others. There occurs a strong correlation (pearson's $r^2 = 0.887371$) between observed and predicted values calculated by the developed regression equation developed by this method. This method can be employed in various compounds for developing a regression equation for predicting the various pharmaco-chemical as well as physico-chemical properties.

4. Methods and Methodology:

Molecular structures of selected set of molecules were prepared by chem sketch software and stored as mol files. Topological descriptors were calculated by Dragon software; procedure of software application is shown by flow chart as shown in figure (2). Multilinear regression model for best presentation of half life and LD_{50} was developed by Microsoft excel software by forward/ backward selection method on the basis of inclusion/ extrusion criteria of lowest p-value, procedure of MS excel software application for MLR analysis is shown by flow chart as shown in figure (3). Values of Half-life for selected set of molecules were collected from reliable and authenticated sources of cheminformatics.

Table (1): Chemical structure of cephalosporin type β-lactam structure containing compounds as QSAR training set.

Training Set	Name	Structure of molecules	Training Set	Name	Structure of molecules
Cp 01	Cefacetrile		Cp 26	Ceftezole	
Cp 02	Cefadroxil		Cp 27	Cefapirine	
Cp 03	Cefalexin		Cp 28	Cefazedone	
Cp 04	Cefaloglycin		Cp 29	Cefazaflur	
Cp 05	Cefroxadine		Cp 30	Cefuroxime	
Cp 06	Cefaclore		Cp 31	Cefuzonam	
Cp 07	Cefradine		Cp 32	Cefmetazole	
Cp 08	Cefonicid		Cp 33	Cefotatam	
Cp 09	Cefprozil		Cp 34	Cefbuperazone	
Cp 10	Cefatrizine		Cp 35	Cefminoxime	
Cp 11	Cefalothin		Cp 36	Cefacapene	
Cp 12	Cefalonium		Cp 37	Cefdaloxime	
Cp 13	Cefaloridine		Cp 38	Cefdinir	

Cp 14	Cefoxitin		Cp 39	Cefmatilen	
Cp 15	Cefazoline		Cp 40	Ceftobiprole	
Cp 16	Cefditoren		Cp 41	Ceftiolene	
Cp 17	Cefatamet		Cp 42	Ceftizoxime	
Cp 18	Cefminoxime		Cp43	Ceftriaxone	
Cp19	Cefpodizime		Cp 44	Cefpirome	
Cp 20	Cefotaxime		Cp 35	Cefexime	
Cp 21	Cefpodoxime		Cp 46	Cefpimizole	
Cp 22	Cefteram		Cp 47	Ceftibuten	
Cp 23	Cefepime		Cp 48	Cefoperazone	
Cp 24	Cefozopran		Cp 49	Ceftazidime	

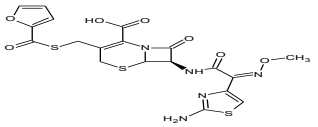
Cp 25	Ceftiofur				

Table (2):

Molecular Set	Half Life ($t_{1/2}$) (in minutes)	References	Molecular Set	Half Life ($t_{1/2}$) (in minutes)	References
C_1	72	[36, 37]	C_{26}	90	[42]
C_2	70	[36, 37]	C_{27}	120	[42]
C_3	72	[36, 37]	C_{28}	102	[45]
C_4	90	[41]	C_{29}	102	[45]
C_5	57	[41]	C_{30}	720	[46]
C_6	48	[41]	C_{31}	96	[46]
C_7	54	[41]	C_{32}	132	[41]
C_8	270	[37]	C_{33}	60	[41]
C_9	78	[36]	C_{34}	76	[41]
C_{10}	102	[36]	C_{35}	78	[47]
C_{11}	30	[36]	C_{36}	150	[12, 13]
C_{12}	110	[21, 22]	C_{37}	63	[12, 13]
C_{13}	84	[21, 22]	C_{38}	120	[42]
C_{14}	42	[21, 22]	C_{39}	116	[42]
C_{15}	108	[21, 22]	C_{40}	163	[42]
C_{16}	56	[21, 22]	C_{41}	150	[50]
C_{17}	30	[21, 22]	C_{42}	84	[50]
C_{18}	108	[21, 22]	C_{43}	348	[51]
C_{19}	120	[21, 22]	C_{44}	114	[51]
C_{20}	60	[21, 22]	C_{45}	180	[11, 51]
C_{21}	72	[21, 22]	C_{46}	110	[11, 51]
C_{22}	60	[43]	C_{47}	150	[13]
C_{23}	180	[43]	C_{48}	96	[54]
C_{24}	97.3	[44]	C_{49}	84	[54]
C_{25}	37	[44]			

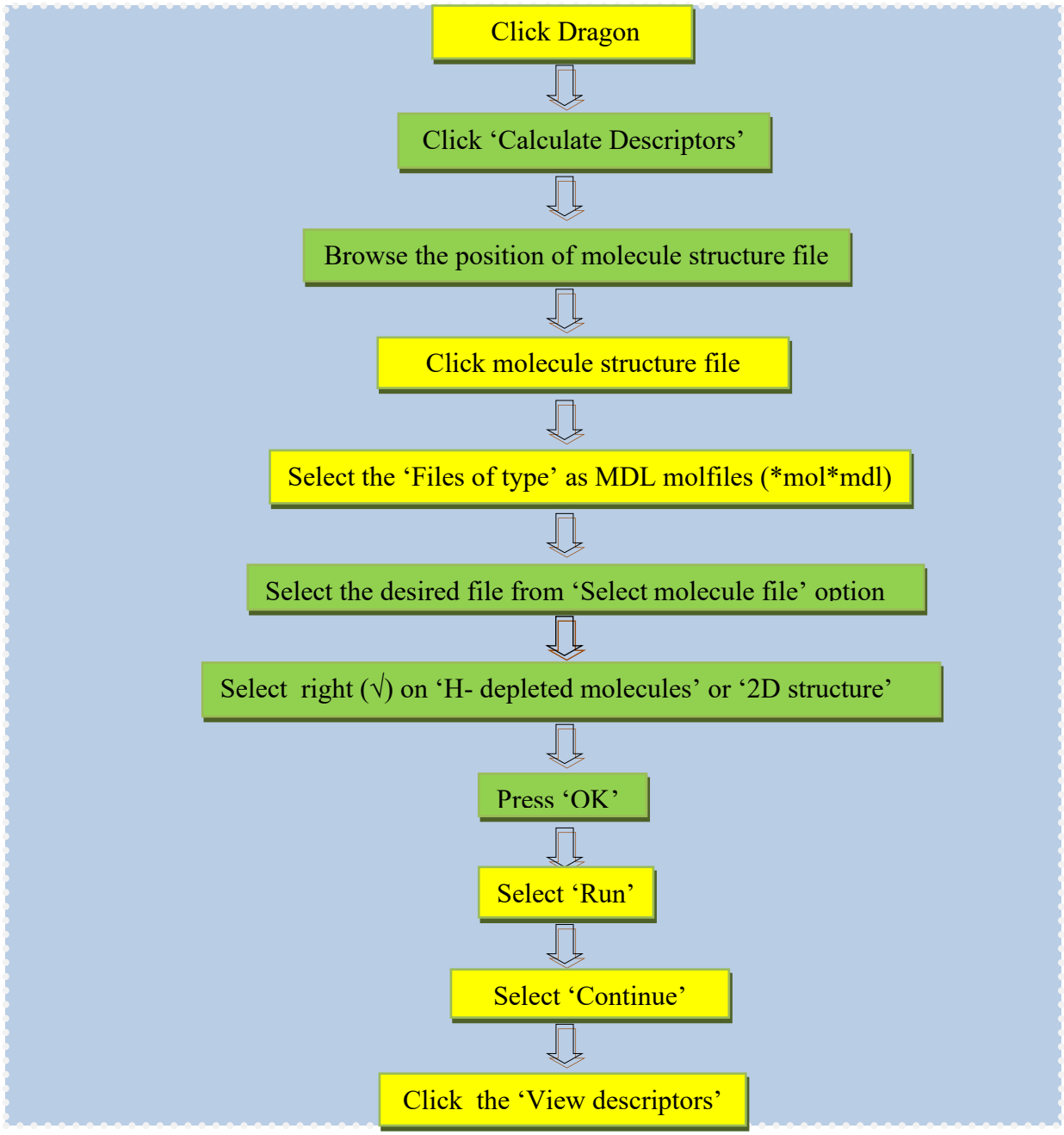


Figure (2): Flow diagram showing the steps to calculation of descriptors by Dragon software

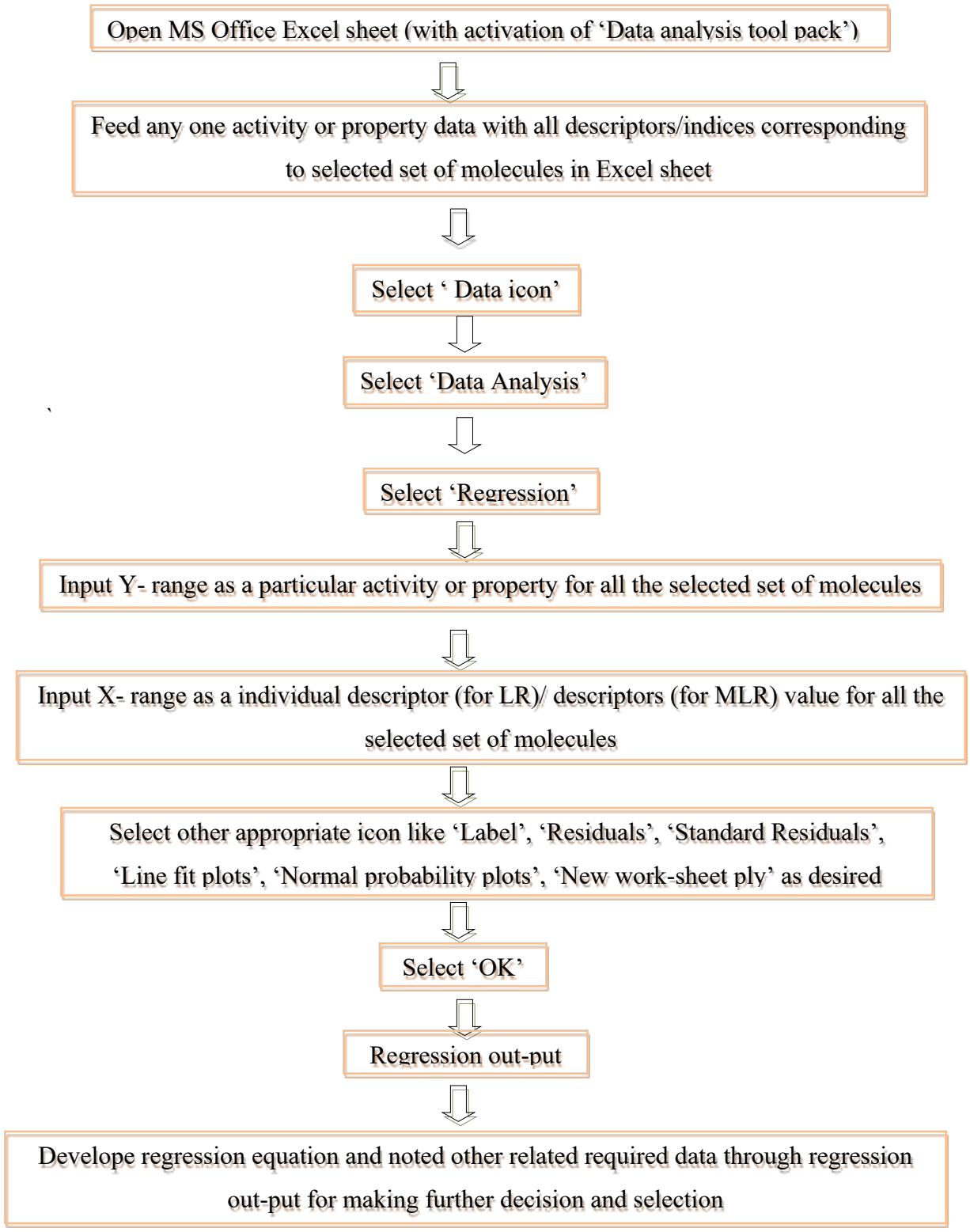


Figure (3) : Brief sketch of various major steps for regression analysis through MS Office Excel

5. Data processing and data analysis:

Table (3): Step-1: For selection of first parameter index.

S. No.	Regression expressions	Index	P-value of each independent variable	Significance–F	R^2-Adjusted	F-ratio	SE
1	$t_{1/2}$ = 0.02(±0.01) W +66.07	W	0.0927	0.0927	0.039	0.093	102.96
2	$t_{1/2}$ =11.73(±5.67) χ1 - 58.83	χ1	0.0440	0.0440	0.064	4.283	101.61
3	$t_{1/2}$ = - 201.99(±83.54) W' + 410.13	J	0.0195	0.0195	0.092	5.846	100.01
4	$t_{1/2}$ = 0.01(±0.01) W' + 57.34	W'	0.0677	0.0677	0.049	3.497	102.40
5	$t_{1/2}$ = 2.67(±1.23) H - 44.13	H	0.0355	0.0355	0.071	4.687	101.21
6	$t_{1/2}$ = 0.31(±0.17) T(N-N) + 83.69	T(N-N)	0.0671	0.0672	0.049	3.511	102.38

Step-2: For selection of second parameter index.

S. No.	Regression expressions	Index	P-value of each independent variable	Significance–F	R^2-Adjusted	SE
1	$t_{1/2}$ = - 225.98(±44.46) $(EN_{orb.})^{*O=C<}$ + 0.04 (±0.01)W + 2915.47	$(EN_{orb.})^{*O=C<}$	6.65 X10^{-6}	8.72 X 10^{-6}	0.37121	83.28
		W	0.00082			
2	$t_{1/2}$ = - 245.397(±42.44) $(EN_{orb.})^{*O=C<}$ + 21.25 (±4.66) χ1 + 2935.49	$(EN_{orb.})^{*O=C<}$	6.13X10^{-7}	4.7 X 10^{-7}	0.44619	78.16
		χ1	3.78 X10^{-5}			
3	$t_{1/2}$ = - 200.06(±42.81) $(EN_{orb.})^{*O=C<}$ - 251.48 (±70.34) J + 3038.43	$(EN_{orb.})^{*O=C<}$	2.61 X10^{-5}	8.88 X 10^{-6}	0.37072	83.32
		J	0.000836			
4	$t_{1/2}$ = - 225.29(±43.87) $(EN_{orb.})^{*O=C<}$ + 0.02 (±0.01) W' - 2896.43	$(EN_{orb.})^{*O=C<}$	5.57 X10^{-6}	5.7 X 10^{-6}	0.38273	82.52
		W'	0.000522			
5	$t_{1/2}$ = - 241.95(±42.11) $(EN_{orb.})^{*O=C<}$ + 4.63 (±1.010621)H + 2930.711	$(EN_{orb.})^{*O=C<}$	6.97 X10^{-7}	4.44 X 10^{-7}	0.44754	78.06
		H	3.57 X10^{-5}			

Step-3: For selection of third parameter index.

S. No.	Regression expressions	Index	P-value of each independent variable	Significance–F	R^2-Adjusted	SE
1	$t_{1/2}$ = -297.60(±36.02) $(EN_{orb.})^{*O=C<}$ + 349.94 (±59.82)ρ +0.02 (±0.01) W + 3227.86	$(EN_{orb.})^{*O=C<}$	1.44 X10^{-10}	1.49 X 10^{-10}	0.635	63.46
		ρ	5.22 X10^{-7}			
		W	0.000642			
2	$t_{1/2}$= -305.07(±34.68) $(EN_{orb.})^{*O=C<}$+ 324.86 (±58.23) ρ + 16.19 (±3.73) χ1 + 3196.58	$(EN_{orb.})^{*O=C<}$	2.47 X10^{-11}	2.15 X 10^{-11}	0.665	60.76
		ρ	1.31 X10^{-6}			
		χ1	8.13 X10^{-5}			
3	$t_{1/2}$= -267.08 (±37.79) $(EN_{orb.})^{*O=C<}$ + 329.04 (±68.24) ρ + 143.07 (±61.97) J + 3152.37	$(EN_{orb.})^{*O=C<}$	8.11 X10^{-9}	4.17 X 10^{-10}	0.576	68.40
		ρ	1.66 X10^{-5}			
		J	0.025607			
4	$t_{1/2}$ = - 296.42 (±35.56) $(EN_{orb.})^{*O=C<}$ + 347.23 (±59.33)ρ + 0.02 (±0.01)W' + 3209.44	$(EN_{orb.})^{*O=C<}$	1.13 X10^{-10}	9.79 X 10^{-11}	0.642	62.87

Step-4
For selection of 4th parameter index.

S. No.	Regression expressions	Index	P-value of each independent variable	Significance–F	R^2-Adjusted	SE
1	$t_{1/2}$ = -302.96(±32.73) $(EN_{orb.})^{*O=C<}$ + 272.85 (±59.19) ρ + 13.02 (±3.99) W + 2939.49	$(EN_{orb.})^{*O=C<}$	6.83 X10^{-12}	8.19 X 10^{-12}	0.699	57.58
		ρ	3.46 X10^{-5}			
		H	0.002121			
		W	0.019486			
2	$t_{1/2}$= - 299.84(±36.45) $(EN_{orb.})^{*O=C<}$ + 325.23 (±58.72) ρ + 6.98 (±13.70) H - 15.90 (±63.13) χ1 + 3188.82	$(EN_{orb.})^{*O=C<}$	1.92 X10^{-10}	1.19 X 10^{-10}	0.659	61.27
		ρ	1.6 X10^{-6}			
		H	0.613146			
		χ1	0.802309			
3	$t_{1/2}$= - 309.19(±35.65) $(EN_{orb.})^{*O=C<}$ + 338.11 (±60.81) ρ + 4.19 (±1.17) H + 61.71 (±79.54) J + 3125.94	$(EN_{orb.})^{*O=C<}$	4.46 X10^{-11}	9.18 X 10^{-11}	0.664	60.90

Step-5: For selection of 5th parameter index.

S. No.	Regression expressions	Index	P-value of each independent variable	Significance–F	R^2-Adjusted	SE
1	$t_{1/2}$ = - 78.051(±32.95) $(EN_{orb.})^{*O=C<}$ + 393.03 (±76.29) ρ - 9.97 (±14.10) H - 0.56 (±0.24) T(N-S) -0.06(±0.023) W + 2454.17	$(EN_{orb.})^{*O=C<}$	$1.14 X10^{-10}$	3.84 X 10^{-12}	0.727	54.86
		ρ	$6.16 X10^{-6}$			
		H	0.002169			
		T(N-S)	0.024064			
		W	0.039284			
2	$t_{1/2}$ = - 79.89(±34.64) $(EN_{orb.})^{*O=C<}$ + 477.81 (±77.07) ρ - 9.97 (±14.10) H - 0.77 (±0.27) T(N-S) + 67.77(±65.85) χ1 + 2492.81	$(EN_{orb.})^{*O=C<}$	$3.65 X10^{-10}$	1.88 X 10^{-11}	0.706	56.98

Step-6: For selection of 6th parameter index.

S. No.	Regression expressions	Index	P-value of each independent variable	Significance–F	R^2-Adjusted	F-ratio	SE
1	$t_{1/2}$ = - 263.94(±33.06) $(EN_{orb.})^{*O=C<}$ + 399.44 (±74.46) ρ - 11.36 (±3.76) H - 0.74 (±0.25) T(N-S) - 0.13 (±0.07) $(Mor_{1u})^{wox}$ - 0.04 (±0.03) W + 2369.41	$(EN_{orb.})^{*O=C<}$	$5.91 X10^{-10}$	4.61 X 10^{-12}	0.74069	23.851	53.48
		ρ	$3.24 X10^{-6}$				
		H	0.004237				
		T(N-S)	0.005632				
		$(Mor_{1u})^{wox}$	0.078634				
		W	0.192497				

No, further parameter can be introduced after sixth i.e. at seventh for developing the significant model. Since the regression equations obtained by stepwise regression analysis do not correspond to the situation in which all the parameters contain P-values less than 0.05.

6. Result and conclusion:

Finally, the developed regression equations by stepwise regression analysis for expression of half-life ($t_{1/2}$) can be given as follows:

$t_{1/2}$ = - 234.714(±33.39813) $(E.N._{orb.})^{*O=C<}$ + 367.7906(±74.00061) ρ + 7.247449(±1.159888) H - 0.82582(±1.159888) T(N-S) - 0.2595(±0.077381) $(Mor_{01u})^{wox}$ + 17.08953(±7.880375) $(Sites)^{Donar}$ + 2209.521

→ **Reg. equation no. (I)**

Statistical characteristics of the developed model is as follows:

	N	R^2-Adjusted	R^2	Pearson's r	F-ratio	Overall significance-F	SE	PRESS
	49	0.75706	0.787	0.8874	25.930	$1.21X10^{-12}$	51.767	112551.6

Predicted value related to observed value by the following equation:
$(t_{1/2})_{pred.} = 0.787\,(t_{1/2})_{obs.} + 24.34$

Figure (4) : Graph showing the relationship between observed and predicted $t_{1/2}$ values

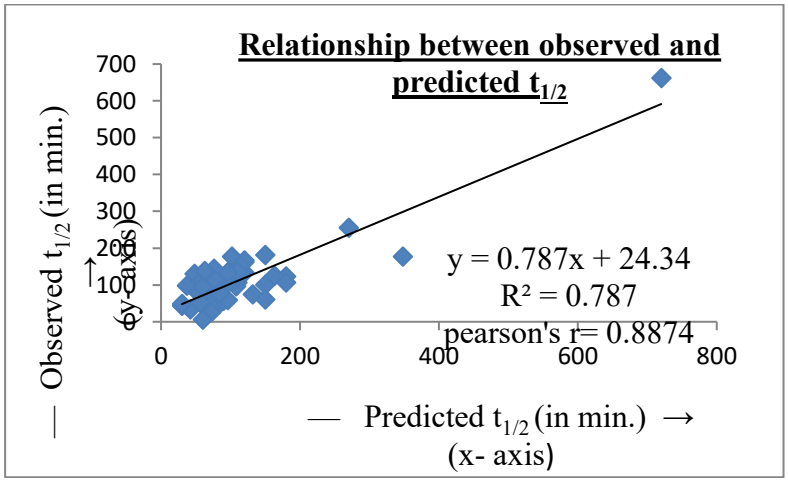


Table 4 : Summary of stepwise multiple regression for developing a model equation for half-life prediction.

S. No.	Step no.	Developed MLR equation	R^2	PRESS	SE
1	Step-1	$t_{1/2}$ = 1.740283 + 2376.009 - 177.021(±47.33028) $(EN_{orb.})^{*O=C<}$ ± 93.18	0.229	408032.519	93.18
2	Step-2	$t_{1/2}$ = 2844.912 - 267.337(±39.53261) $(EN_{orb.})^{*O=C<}$ + 386.2 023(±66.5 162) ρ ± 71.55	0.555	235468.586	71.55
3	Step-3	$t_{1/2}$ = - 302.554(±34.45529) $(EN_{orb.})^{*O=C<}$ + 324.8313(±58.0 8864) ρ + 3.532014(±0.808889) H ± 60.62	0.688	165392.445	60.62
4	Step-4	$t_{1/2}$ = 2591.266 - 273.995(±34.18516) $(EN_{orb.})^{*O=C<}$ + 453.8 172(± 73.50967) ρ + 4.513608(±0.847833) H - 0.64119(±0 .244501) T(N-S) ± 57.02	0.730	143035.704	57.02

5	Step-5	$t_{1/2}$ = 2422.48 - 257.689(±33.00895) $(EN_{orb.})^{*O=C<}$ + 435.34 8(±6 9.95445) ρ + 6.631199(±1.171959) H - 0.83756(±0.24 4541) T(N-S) - 0.16591(±0.066938) $(Mor_{1u})^{wox}$ ± 53.95	0.764	125154.472	53.95
6	Step-6	$t_{1/2}$ = 2209.521 - 234.714(±33.39813) $(E.N._{orb.})^{*O=C<}$ + 367 .7906 (±74.00061) ρ + 7.247449(±1.159888) H - 0.82582(± 1.159888) T(N-S) - 0.2595(±0.077381) $(Mor_{01u})^{wox}$ + 17.08 953(±7.880375) $(Sites)^{Donar}$ ± 51.767	0.787	112551.611	51.77
7	Step-7 (Final)	No variable can be added satisfactory since in this step each regression equation contains one or more independent variable parameter's p-value > 0.05	-	-	-

However, different regression equations can also be developed for the prediction of half life by selecting the variable index at each step which are less significant but satisfying the situation P-value < 0.05. Although the regression equations developed by this way give less correlation between observed and predicted properties. For example in above stepwise multilinear regression analysis $(Mor_{01u})^{wor}$ {P =0.001199}is the second most variable index than $(E.N._{orb.})^{*O=C<}$ {P-value = 0.0004990} which can be so if we select the $(Mor_{1u})^{wor}$ in first step then add the other significant variable indices by stepwise regression analysis on the basis of lowest P-values then on further taking in consideration Vanderwaal 3D surfacearea $(3D\text{-}Surfacearea)_{V.W.}$ developed regression equation will be as follows:

$t_{1/2}$ = 2118.176586 + 0.175467 $(Mor_{1u})^{wor}$ - 241.892850 $(E.N._{orb.})^{*O=C<}$ + 510.850229 - 0.918213 T(N-S) + 19.816819 X_1 - 0.270681 $(3D\text{-}Surfacearea)_{V.W.}$ + 51.889606

→ Reg. equation no. (II)

Statistical parameters of the developed regression equation –II is as follows:

	n	R^2-Adjusted	R^2	Pearson's r	F-ratio	Overall significance-F	SE	PRESS
	49	0.75590	0.786	0.8868	25.774	$1.34X10^{-12}$	51.89	113086.309

Predicted value related to observed value by the following equation:
$(t_{1/2})_{pred.}$ = 0.786 $(t_{1/2})_{obs.}$ + 24.45

Table (5) : Comparative data from eq. (III) for the parental and proposed molecules to predict LD50.

S. No.	Name of molecule	(+) ive terms		(-) ive terms					Inference
		$(N_C)^R$	$(N_S)^R$	$\sum$ (MoRSE	{	$(Mor_{1en})^{wox}$	T(N-N)	$(S_s)^{wox}$	
1	Cefalexin, Cp 03 (Parantal molecule)	7	0	2435.05	-1809.3	739.94	12	63.17	$(LD_{50})_2 > LD_{50})_1$
2	Proposed molecule, P_2	6	1	2413.37	-186.18	713.12	3	62.58	

LD_{50} =56942.163460- 1503.322364(±185.441755) (Mor1en)wox –

1062.353812 (±152.969426)
– 862.2505917 (±149.059912){ } + 5070.884 388(±1164.085224)
(NC)R – 1729.418221(±452.404134) (Ss)wox + 12529.92919(±3432.907573) (NS)R + 29.1140

(±14.75841) T(N-N) ± 7190

→ **Reg. equation no. (III)**

Figure (5). : Graphical presentation of observed v/s predicted $t_{1/2}$ values relationship

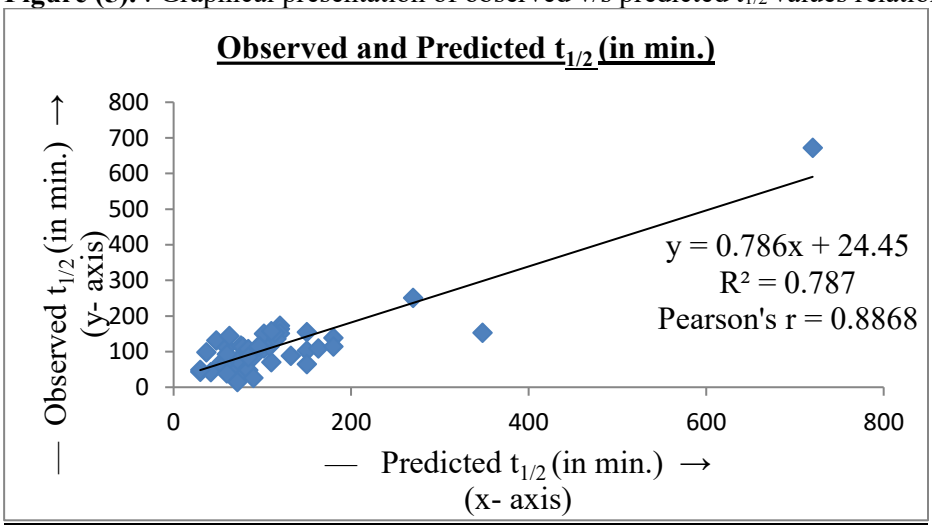


Table 6 : Comparative view of observed and predicted half-life values for selected set molecules

Training Set	Observed $t_{1/2}$ (in min.)	Predicted $t_{1/2}$ (in min.) (By reg.eq.-I)	Predicted $t_{1/2}$ (in min.) (By reg.eq.-II)	Training Set	Observed $t_{1/2}$ (in min.)	Predicted $t_{1/2}$ (in min.) (By reg.eq.-I)	Predicted $t_{1/2}$ (in min.) (By reg.eq.-II)
CEP 01	72	36.04635	74.11319	CEP 26	90	56.68097	26.22529
CEP 02	70	78.05619	63.5276	CEP 27	120	166.5298	163.9474
CEP 03	72	74.88881	76.43083	CEP 28	102	130.2729	123.6299
CEP 04	90	99.85242	85.7492	CEP 29	102	177.4294	150.3766
CEP 05	57	79.6345	68.67895	CEP 30	720	661.8094	672.3388
CEP 06	48	130.4495	131.4921	CEP 31	96	58.88123	115.3782
CEP 07	54	76.92604	69.30705	CEP 32	132	74.15138	88.36809
CEP 08	270	254.9774	250.6555	CEP 33	60	95.05159	92.47106
CEP 09	78	53.41482	33.05949	CEP 34	76	143.9427	116.8941

CEP 10	102	145.497	109.2514	CEP 35	78	111.9106	113.7995
CEP 11	30	42.95307	43.3617	CEP 36	150	100.928	102.4207
CEP 12	110	106.5235	70.08716	CEP 37	63	137.8821	143.7832
CEP 13	84	49.06379	47.82667	CEP 38	120	133.7392	150.7195
CEP 14	42	33.4197	42.30536	CEP 39	116	126.9523	135.7034
CEP 15	108	95.82758	119.1494	CEP 40	163	124.9778	109.3363
CEP 16	56	51.48907	62.1214	CEP 41	150	181.7009	154.5024
CEP 17	30	48.26569	48.93385	CEP 42	84	79.33951	106.7302
CEP 18	108	116.3488	114.6877	CEP 43	348	176.8667	153.1415
CEP 19	120	161.8239	172.803	CEP 44	114	150.9591	152.7802
CEP 20	60	113.6245	112.8815	CEP 45	180	107.0088	114.1765
CEP 21	72	25.68611	14.54226	CEP 46	110	135.9653	157.6749
CEP 22	60	5.858002	39.29796	CEP 47	150	60.27104	65.04475
CEP 23	180	122.7582	138.9752	CEP 48	96	132.4059	115.6255
CEP 24	97.3	107.0967	114.4256	CEP 49	84	77.55926	84.81219
CEP 25	37	97.6025	97.75585				

7. Molecular Modeling based on Half Life –

For prediction of half-life, the developed regression equations given in Table (I). The regression model proved itself by means of that there occurs strong correlation between predicted and observed half lives when applied over selected series of molecules (viz. C_1 to C_{49}) The value of $R^2 = 0.786$ (pearson's r = 0.887) and 0.787 (pearson's r = 0.887) respectively for reg. eq. (I), (II) and (III) Standard error reaches till 51.77 and 51.89 respectively. Predicted residual squares sum to reach 1125516.6 and 113086.3 respectively. Both models are significant in the sense that their over all significance are 1.21 X 10^{-12} and 1.34 X 10^{-12} The predicted and observed values by both reg. eq. are shown in Table (5) and correlation graph between above two are shown previous The distribution of selected indices in derived reg. eq. with half life can be given by following distribution curves:

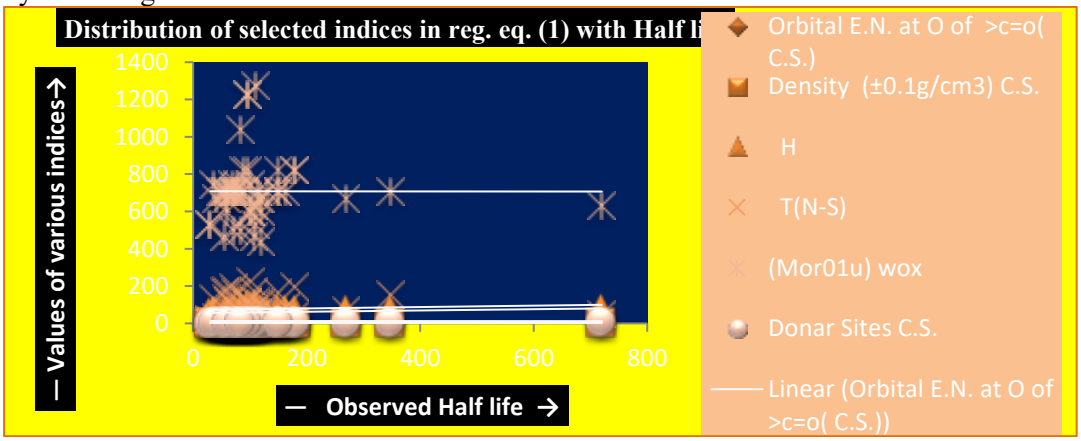


Figure 6 : Distribution of selected indices in reg. eq. (1) with Half life

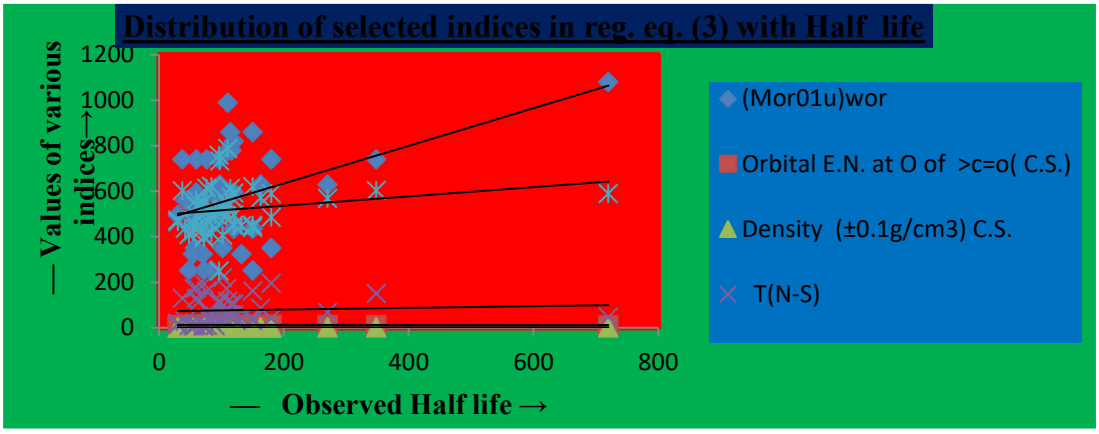


Figure 6: Distribution of selected indices in reg. eq. (II) with Half life

For a good cephalosporin drug low value of $t_{1/2}$ is desirable. So, with the help of developed reg. eq. (I) and (II), someone can design a new molecule of drug with improved (i.e. low $t_{1/2}$). If reg. eq. (I) is considered then most dominant indices are $(E.N._{orb.})^{*O=C<}$ and ρ (since their coefficients are comparatively more than others. Collectively in reg. eq. (I)

- the $(-)_{ive}$ participation terms are $(E.N._{orb.})^{*O=C<}$, T(N-S), $(Mor_{01u})^{wox}$ and
- the $(+)_{ive}$ terms are ρ, H, $(Sites)^{Donar}$

So, to decrease $t_{1/2,}$ the nature of –X and –R group should be designed in such a way that on introduction of these groups on core structure of cephalosporin results in increase of $(E.N._{orb.})^{*O=C<}$ specially. Increase in some other indices like T(N-S), $(Mor_{01u})^{wox}$ also desirable to reduce $t_{1/2}$ but T(N-S), $(Mor_{01u})^{wox}$ can do this to only small extent because their coefficients are very small than the first index i.e. $(E.N._{orb.})^{*O=C<}$ By using reg. eq. (I) all the available molecular structure can be modified to show low half life but during modification toxicity must not be increased i.e. LD_{50} must not be decreased. For example Cefalexin (molecular set- C_3) can be modified to proposed molecule, P_1, whose data employed in reg. eq. no. (I) are given as follows:

Table (7) : Comparative data of eq. (I) for parental and proposed molecule to predict improved $t_{1/2}$

S. No.	Name of molecule	(+) ive terms			(-) ive terms			Inference
		P	$(Sites)^{Donar}$	H	$(E.N._{orb.})^{*O=C}$	$(Mor_{1u})^{wox}$	T(N-S)	
1	Cefalexin Cp 03 (Parantal molecule)	1.54	4	42.423	12.30	703	9	$(t_{1/2})_2 < (t_{1/2})_1$
2	Proposed molecule, P_1	1.52	2	42.996	12.34	741	9	

Table (8) : Comparative data of eq. (III) for parental and proposed molecule to predict LD_{50}

S	Name	(+) ive terms	(-) ive terms	I n f e

	of molecule	$(N_C)^R$	$(N_S)^R$	$\sum_{u,am,v,en,p}(MoRSE)w$	$\{\sum_{u,am,v,en,p}(MoRSE$	$(Mor_{1en})^{wox}$	T(N-N)	$(S_s)^{wox}$	
1	Cefalexin, Cp 03 (Parantal molecule)	7	0	2435.05	-1809.3	739.94	12	63.17	$(LD_{50})_2 > LD_{50})_1$
2	Proposed molecule, P_1	6	1	2531.47	-1024.7	783.14	3	62.25	

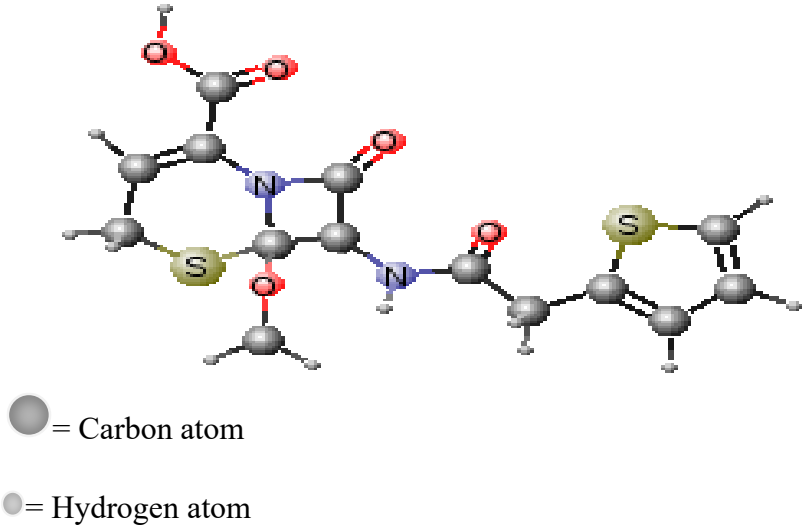


Figure (6): Proposed molecule, P_1 designed and developed through reg. eq. (I)

Characteristics of proposed molecule, P_1: This molecule is expected to show less half-life than parental molecule Cefalexin. Since the values of various variables indices included in reg. eq. (I) are designed to show less value of half-life. This molecule is suggested for showing less half-life than the parental cefalexin molecule. This can be inferenced due to following facts: Since, all the + ive factors and negative factors included in reg. eq. (I) are less and more respectively for P_1 molecule than parental cefalexin [as shown in Table (6)]. P_1 molecule is also expected to show low toxicity i.e. more LD_{50} than parental cefalexin. Since, near about all the + ive factors and negative factors included in reg. eq. (I) are more and less respectively for P_1 molecule than parental cefalexin [as shown in Table (6)]. Although due to decrease in carbon atom in P_1 than parental force to reduce LD_{50} but increase in Sulphur atom in P_1 than parental overcome this and other reduction, since, $(N_S)^R$ has 10 times stronger prediction power than $(N_C)^R$ and more than others indices. Particulars of this proposed molecule, P_1 are as follows: Molecular formula: $C_{14}H_{14}N_2O_5S_2$, Molecular weight: 354.40, IUPAC name of molecule- (6S,7R)-6-methoxy-8-oxo-7-[(thiophen-2-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Value of molar refractivity is 85.95 cm^3, which enable this compound to satisfy the one condition of 'Lipinski's rule of five's extension' as showing suitability for application through oral dose. Molar volume = 223.4 cm^3, Parachore value is 668.1 cm^3, Surface tension = 79.9 dyne /cm. Value of polarizability 34.07 x 10^{24} It's expected half-life will be less than it's parental compound cefalexin. So, this proposed molecule is suggested for further practical study.

Comparative data of eq. (II) for parental and proposed molecule to predict improved $t_{1/2}$

Table (9):

S. No.	Name of molecule	(+) ive terms			(-) ive ter`ms			Inference
		P	$^{1}X_{l}$	$(Mor_{1u})^{wor}$	$(E.N_{\bullet orb.})^{*O=C}$	$(3D\text{-}surfacearea)^{V.W.}$	T(N-S)	
1	Cefalexin, Cp $_{03(Parental\ molecule)}$	1.54	11.003	253	12.30	396.58	9	$(t_{1/2})_2 <$ $(t_{1/2})_1$
2	Proposed molecule, P_2	1.53	10.441	666	12.67	461.45	8	

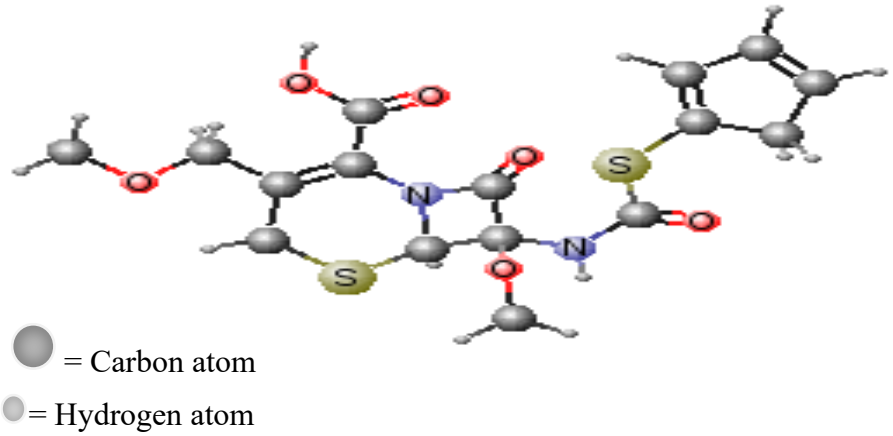


Figure (7) : Proposed molecule, P_2 derived from. eq. (II)

Characteristics of proposed molecule, P_2: This molecule is suggested for showing less half-life than the parental cefalexin molecule. Since, all the + ive factors and negative factors included in reg. eq. (II) are mostly less and more respectively for P_2 molecule than parental cefalexin. However the opposite effects of one index are overcome by other indices having more predictive power similar in case of the P_1 molecule. P_2 molecule is also expected to show low toxicity i.e. more LD_{50} than parental cefalexin. Since, mostly all the + ive factors and negative factors included in reg. eq. (III) are more and less respectively for P_2 molecule than parental cefalexin [as shown in Table (7)]. However, decrease in LD_{50} due to reduction in carbon atom in P_2 overcome by increase in LD_{50} by addition of Sulphur atom which contributes in $(N_S)^R$ having 10 times more predictive power than$(N_c)^R$. Particulars of this molecule are as follows: IUPAC name: (7R)-7-{[(cyclopenta-1,3-dien-1-ylsulfanyl)carbonyl] amino}-7-methoxy-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, Molecular weight: 398.45, Molecular formula: $C_{16}H_{18}N_2O_6S_2$, Molar refractivity: 97.96 cm^3, Molar volume: 258.8 cm^3, Density: 1.53g/ cm^3.

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