CHAPTER 2

Application of physicochemical and DFT based descriptors for QSAR Study of camptothecin derivatives

IN THIS CHAPTER-

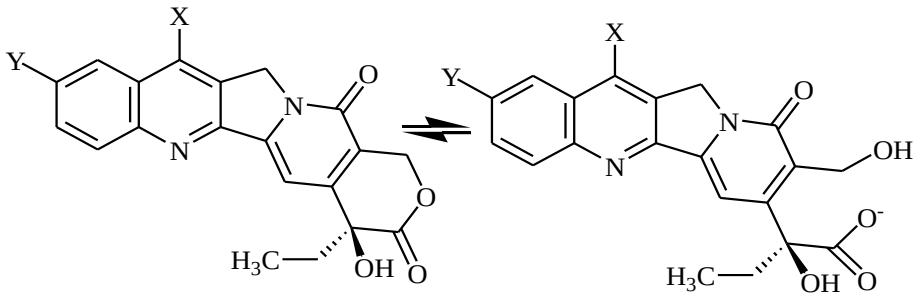
- INTRODUCTION
- THEORETICAL BACKGROUND
- QSAR MODELING

OUTLOOK-

- ✓ Two different sets of camptothecin analogues are investigated using DFT-based descriptors and effective regression models are obtained.
- ✓ A good correlation between the biological activity and the DFT based descriptors is obtained using MLR.
- ✓ The results reveal that DFT-based reactivity descriptors such as electrophilicity (ω), Fukui functions (f_k^+, f_k^-), energies of the frontier orbitals along with the physicochemical parameters (logP, hydration energy, molar refractivity and surface area) are very powerful in describing the cytotoxicity.

2.1 Introduction

Camptothecin (CPT) is a naturally occurring pentacyclic quinoline alkaloid that possess high cytotoxic activity against variety of cell lines.[1] CPT remains in an active lactone form and inactive hydrolyzed carboxylate form (Scheme 2.1) with a dynamic equilibrium between these two.[1] The active lactone is responsible for binding with DNA topoisomerase I complex, that is believed to be the site for its activity.[2] Initially, CPT is used as sodium salt of carboxylate and less effective due to its higher affinity towards human serum albumin under physiological pH condition.[3,4] Major limitations of the drug include poor solubility and hydrolysis under physiological conditions, avoiding its full clinical utilization. Because of above drawbacks and limitations, various derivatives have been synthesized, tested and represents an area of considerable interest. Although a large members of these family are currently in clinical trials, only two CPT derivatives, irinotecan and topotecan, are approved as drug. Irinotecan is used in metastasis of colorectal cancer in combination with other chemotherapeutic agents. Topotecan has been used for ovarian cancer, cervical cancer and small-cell lung cancer. These derivatives employ tertiary amine cations to improve solubility and subsequently improve lactone stability that inhibit topo I isomerase activity. Currently CPTs, notably topotecan [5,6], irinotecan[7-10], 9-aminocamptothecin[11,12], 9-nitrocamptothecin[13] and belotecan[14] are being explored for use as a late-stage therapy either alone or in combination therapies.



Lactone form Carboxylate form

Scheme 2.1 Interconversion between the lactone and carboxylic form of camptothecin

Various clinical trials as well as structure activity studies suggest that the intake of the E-ring lactone (Figure 2.1) and the 20(S)-configuration are essential for its maximum antitumor activity.[15] Furthermore, the activity of most of the compounds

with substituents at the 11- and 12-positions of the A-ring and substitutions in rings C and D are lesser than CPT itself, whereas activity of most of the compounds with substituent at the 9- and 10-positions of the A-ring are somewhat greater than CPT, and substitutions in position 7 of camptothecin are irrelative to steric clash (Figure 2.1 and 2.2). Wani et al. [16,17] has found that most of the compounds with substitutions at the 7-position are more potent against cancer than substitutions at other positions.

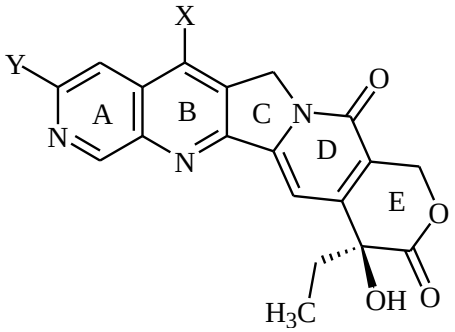


Figure 2.1 Structure of 7-X-10-Y-11 aza camptothecin

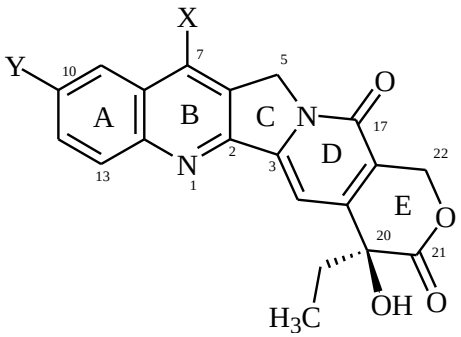


Figure 2.2 Structure of 7-X-10-Y-11camptothecin

Quantitative Structure-Activity Relationship (QSAR) basically deals with the relationship between the biological activities of a particular molecule and its structure.[18] Several physicochemical descriptors, such as hydrophobicity, topology, electronic parameters and steric effects have been used extensively in QSAR studies to estimate the biological property of drug candidate and frequently used in many disciplines, such as in drug design and environmental risk assessments.[19] There has been a number of QSAR studies evaluating the relationship between camptothecin structure and topoisomerase I inhibiting activity using various statistical methods such as multiple linear regression and genetic algorithm.[20,21]

In recent days, DFT based descriptors are gaining much interest in QSAR and QSTR studies for designing drug molecules.[22] However, these descriptors have not

been used in any of the previous QSAR study to investigate the cytotoxicity of camptothecins. In the present work, we introduce these descriptors for QSAR studies of camptothecins and their analogues. A series of aza-camptothecin analogue (Figure 2.1) containing fourteen molecules and a different series of ten camptothecin compounds (Figure 2.2) are studied and we have used a combination of DFT and molecular mechanics based descriptors to build some effective QSAR models for predicting the activity of camptothecin.

2.2 Theoretical Background

The schematic diagrams of aza-camptothecin and substituted camptothecins are shown in Figure 2.1 and Figure 2.2, respectively. All the twenty four compounds are fully optimized at DFT level using double numerical with polarization (DNP) basis set in combination with the BLYP functional.[23-25] The DNP basis set is equivalent to Gaussian 6-31G** basis set.[26-28] However, it is believed to be much more exact than a Gaussian basis set of the same size. The physio-chemical parameters, namely hydration energy (HE), molar refractivity (MR), logP, polarizability (Pol) and surface area (SA) are obtained from the MM+ computations using the HyperChem software.[29] The DFT calculations were performed with DMol^3 programme.[30]

2.2.1 QSAR calculation

Multiple regression analysis is used to generate the QSAR model equations. In all equations, R^2 is the squared correlation coefficient, R^2_{CV} is the leave-one-out (LOO) cross validated squared correlation coefficient, SE is the standard error of the estimates, F is the Fisher significance ratio, Q is the quality factor = R/SE and N is the number of cases used in the analysis.

2.3 Results and Discussion

The biological activity of thousand of camptothecin molecules has been studied in the recent years. Here, two potent sets of campothecins have been selected in order to build some effective regression model using the descriptors mentioned earlier against the 50% inhibitory concentration (pIC_{50}).

Our first QSAR analysis is based on a series of 7-X-10-Y-11 aza CPTs (Figure 2.1) synthesize and estimated for topoisomerase I (topo-I) targeting activity as well as its toxicities against different cell lines.[31] The list of studied camptothecin (CPT)

analogue with different substituents at 10-Y and 7-X positions of ring A and B, respectively along with their pIC_{50} values, are tabulated in Table 2.1

Table 2.1 List of different substituents along with their pIC_{50} values for 1st set

Compound	X	Y	pIC_{50}
1	CH_3	Br	7.55
2	C_2H_5	Br	6.91
3	CH3	CN	6.84
4	C_2H_5	CN	6.57
5	CH_3	CH_2NH_2	6.63
6	C_2H_5	CH_2NH_2	6.78
7	C_2H_5	$C(NH_2)NOH$	7.15
8	C_2H_5	$C(NH_2)NH$	7.48
9	CH_3	$C{\equiv}CCH_2NH_2$	6.3
10	C_2H_5	$C{\equiv}CCH_2NH_2$	5.97
11	C_2H_5	$C{\equiv}CCH_2N(CH_3)_2$	5.88
12	C_2H_5	$(CH_2)_3N(CH_3)_2$	5.83
13	C_2H_5	$COOC_2H_5$	6.02
14	C_2H_5	$CONH(CH_2)_2N(CH_3)_2$	5.03

The chemical hardness (η), chemical potential (μ), Fukui functions (f_k^+ and f_k^-), energy of lowest unoccupied orbital (E_{LUMO}), energy of next LUMO (E_{NL}) and electrophilicity (ω) values computed at BLYP/DNP level for the first series of fourteen CPT compounds are given in Table 2.2, along with the physical parameters such as hydration energy, logP, surface area and molar refractivity.

In order to develop the QSAR models for predicting the cytotoxicity of camptothecins, the value of pIC_{50} is used as dependent variable in multiple regression. The equations are computed *via* multiple regression with two independent variables. In order to investigate predictive power of the developed models we calculate the R^2 values and finally we used the cross validation parameters to prove our finding. There is no significant correlation of individual parameters with the pIC_{50} values, however, the bivariate combinations of the parameters gives better results. The predicted QSAR models along with the inter-correlation values between the descriptors are shown in Table 2.3.

Table 2.2 Calculated values of all the selected descriptors for all compounds for 1st set

No	E_{LUMO} (au)	E_{NL} (au)	η (au)	μ (au)	ω (au)	HE (kcal mol^{-1})	logP	MR	Pol	SA
1	-0.1321	-0.1056	0.03903	-0.1712	0.37522	-9.47	5.67	49.81	39.94	478.94
2	-0.1314	-0.1053	0.03924	-0.1706	0.37095	-9.1	6.07	54.41	41.77	500.64
3	-0.1423	-0.1161	0.0373	-0.1796	0.43236	-13.89	4.88	47.73	39.16	490.98
4	-0.1412	-0.1156	0.03758	-0.1788	0.4253	-13.52	5.28	52.33	41	512.97
5	-0.121	-0.0959	0.04153	-0.1625	0.31801	-13.14	4.03	50.01	40.5	575.09
6	-0.1202	-0.0957	0.04173	-0.1619	0.31412	-12.72	4.43	54.61	42.33	496
7	-0.1253	-0.099	0.03187	-0.1571	0.38728	-22.86	4.76	52.18	43.9	502.42
8	-0.1313	-0.1056	0.03741	-0.1688	0.38058	-17.82	4.27	50.7	43.26	476.91
9	-0.1243	-0.0983	0.03936	-0.1637	0.34025	-13.91	4.34	58.58	43.06	583.55
10	-0.1239	-0.0984	0.03953	-0.1634	0.33781	-13.55	4.73	63.18	44.9	605.62
11	-0.1235	-0.0982	0.03263	-0.1561	0.37357	-7.41	5.5	73.25	48.57	707.91
12	-0.1196	-0.0949	0.03117	-0.1508	0.3646	-6.92	5.7	74.19	49.67	675.27
13	-0.1299	-0.1033	0.03946	-0.1693	0.3633	-10.52	7.15	59.69	45.37	586.75
14	-0.1278	-0.103	0.02911	-0.1569	0.42281	-10.44	6.57	74.64	51.11	681.16

Table 2.3 Results of MLR with different set of compounds using various descriptors using 1st set of compounds

No	QSAR equations	N	R^2	R^2_{CV}	SE	Auto correlation	Q	F
1	11.065(±0.754) − 15.631(±8.815) f^-_{C10} − 0.0748(±0.012) MR	14	0.805	0.769	0.338	0.285	2.654	22.64
2	11.447 (±0.748) − 10.60(±7.852) f^-_{C10} − 0.00854(±0.001) SA	14	0.829	0.799	0.316	0.211	2.881	26.66
3	12.462(±0.936) − 52.70(±18.536) f^+_{C10} − 0.0672(±0.009) MR	14	0.855	0.829	0.291	0.02	3.178	32.47
4	11.974(±0.969) − 28.757(±19.706) f^+_{C10} − 0.00775(±0.001) SA	14	0.833	0.803	0.312	0.001	2.925	27.43
5	7.25(±3.857) + 60.003(±63.040) f^+_{C7} − 0.00695(±0.001) SA	14	0.816	0.782	0.328	0.378	2.754	24.36
6	11.739(±1.109) – 33.769(±35.432) f^-_{H} − 0.0087(±0.002) SA	14	0.816	0.782	0.328	0.439	2.754	24.37
7	12.015(±1.187) – 35.40(±31.224) f^-_{LAC} − 0.00899(±0.002) SA	14	0.822	0.789	0.323	0.479	2.807	25.31
8	11.689 (±0.966)– 23.9663(±21.526) f^-_{OXY} − 0.0088(±0.001) SA	14	0.82	0.788	0.323	0.403	2.804	25.2
9	13.5365(±2.292) + 17.39265(±14.412) E_{LUMO} − 0.00856(±0.001) SA	14	0.827	0.792	0.317	0.231	2.869	26.38
10	13.5365 (±1.951) + 19.32654(±14.774) E_{NL} − 0.00856(±0.001) SA	14	0.824	0.796	0.32	0.252	2.837	25.74
11	11.74042 (±1.152)– 2.2256 (±2.460) ω – 0.00785 (±0.001) SA	14	0.814	0.781	0.329	0.004	2.742	24.14
12	11.25(±0.695) – 0.00728 (±0.001) SA – 0.1257(±0.103) logP	14	0.825	0.793	0.32	0.123	2.838	25.85

Equations 1-12 (Table 2.3) exhibit the applicability of DFT-based descriptors and physicochemical parameters for the QSAR study of camptothecins derivatives. The negative coefficients of surface area in all the predicted QSAR models and the negative coefficients of molar refractivity in Equations (1) and (3) bring out the steric effect of the substituent. These two equations signify that higher values of surface area and molar refractivity parameters will reduce the biological activity of camptothecin analogues. The electrophillic attack at a particular site of a system represents the site with maximum values of FF, f_k^- and supreme values of FF, f_k^+ is featured by the site for nucleophillic attack. The negative coefficients of f_k^- in Equations (1) and (2) explain that substitutions at C-10 position in this type camptothecins with the groups like -Br, -CN, $-CH_2NH_2$ etc will make this site less susceptible for the electrophillic attack and it has a negative effect on the inhibitory activity. Similarly, the negative coefficients of f_k^+ in Equations (3) and (4) predict that substitution at this position will also make this site less favorable for nucleophillic attack and any such substitution at this site will reduce the activity of these compounds. So, C-10 position with such analogue of camptothecin will not be a favorable position neither for an electrophillic nor for nucleophillic attack. Hence, C-10 position of the A ring will not contribute to the biological activity upon substitution. It has also been reported that only substitution with -OH or $-NH_2$ group at C-10 position of ring A could result in greatly enhanced human blood stabilities of CPT derivatives.[32] The positive coefficient of f_k^+ in Equation (5) indicates that substitution at C-7 position with the alkyl groups makes this site more susceptible for nucleophillc attack and an increase in value of f_k^+ at the C-7 position of the B-ring will substantially increased the DNA topo I inhibition of campthothecins. Substitution at 7 position of the B-ring has been also reported to be more effective, and depending on the nature of the substituent, water solubility of the CPT will vary.[33] In Equation (6) the negative coefficients associated with the f_k^- of hydrogen atom of 20-OH group in E-ring suggest that an increase in f_k^- at this position will have a negative influence on the activity of the camptothecins. Experimentally it has also been suggested that replacement of H-atom either by halogen or amino group results in significant reduction of activity of camptothecins [34,35] as this H-atom of C20-OH group is involved in hydrogen bonding with the topo I-

DNA complex.[36] In Equation (7) negative coefficients of f_k^- for lactone carbonyl implies that lactone carbonyl is not a favorable site for nucleophillic attack and thus any structural change that can lead to a decrease in f_k^- at this position will increase the anticancer activity of CPTs. Moreover, this is also in good agreement with experimental results which predicts that lactone carbonyl oxygen would serve as hydrogen bond acceptor.[37] Similar argument can also be given for the Equation (8) *i.e.* a decrease in f_k^- at lactone oxygen will significantly increase the activity of camptothecin. So, in agreement with experimental observation[38,39], our results predicts that substitution at C-10 position of A ring and C-7 position of B-ring with different substituents makes H-atom of 20-OH group, O-21 (lactone carbonyl) and O-(lactone oxygen) of the E-ring more active towards the interaction with topoI DNA-complex *via.* the formation of hydrogen bond or by serving as hydrogen bond acceptor. The negative coefficient of SA in Equations (6-8) explains the possibility of intramolecular H-bonding between H-atom of 20-OH group and O-21 (lactone carbonyl) atom.

The four oxygen atoms in the D/E ring of the camptothecins will behave as hydrogen bond acceptor during its bonding with the topoI-DNA complex and the mechanism involves the nucleophilic attack at this site. In this type of interaction E_{LUMO} and E_{NL} play an important role. The lower values of these parameters will make the system stable by increasing the capacity of the molecules to accept electrons from DNA. We found that coefficient of E_{NL} and E_{LUMO} in Equations (9) and (10) are positive suggesting that lower value of E_{NL} and E_{LUMO} will highly favour the intermolecular interaction between the topo I-DNA complex and camptothecin molecule and enhances the cytotoxity of the compounds. Moreover, the negative coefficients of SA suggest the low surface area favors the intermolecular interaction between the molecule and the topo I-DNA complex. The coefficients of the other independent variables *viz.* ω and logP in Equations (11) and (12) are also found to be negative. Negative coefficients of these variables suggest that decreasing the value of ω and logP will enhance the inhibitory action of camptothecins.

Secondly, we made our observation on a series of ten camptothecin compounds of the type 7-X-10-Y-CPT (Figure 2.2). This type of camptothecins is reported to be active towards HL-60 human leukemia cell.[40] The pIC_{50} values of the various substituted CPTs are presented in Table 2.4 and the calculated values of the various

descriptors are given in Table 2.5. Based on the predicted descriptors, seven QSAR models have been developed which are quite different from the previous QSAR models, shown in Table 2.6 with their statistical values.

Table 2.4 List of different substituents along with their pIC_{50} values for 2nd set

Compound	X	Y	pIC_{50}
1	CH_3	H	6.93
2	CH_3CH_2	H	7.11
3	$(CH_2)_2CH_3$	H	6.73
4	$(CH_2)_3CH_3$	H	6.41
5	CH_3	OH	8.28
6	CH_3CH_2	OH	8.43
7	$(CH_2)_2CH_3$	OH	8.45
8	$(CH_2)_3CH_3$	OH	8.96
9	H	OCH_3	6.51
10	CH_3	OCH_3	7.18

Table 2.5 Calculated values of all the selected descriptors for all compounds of 2nd set

No	E_{NL} (au)	η (au)	μ (au)	ω (au)	HE (kcal mol^{-1})	logP	MR	Pol	SA
1	-0.0856	0.043	-0.157	0.288	-8.58	5.89	42.93	38.02	445.62
2	-0.0854	0.043	-0.156	0.284	-8.16	6.28	47.53	39.85	467.18
3	-0.0851	0.043	-0.156	0.282	-7.76	6.68	52.13	41.69	506.12
4	-0.0846	0.043	-0.156	0.28	-7.43	7.08	56.73	43.52	535.86
5	-0.0852	0.042	-0.153	0.277	-15.1	5.71	44.31	38.66	461.8
6	-0.0847	0.042	-0.152	0.274	-14.7	6.11	48.91	40.49	482.53
7	-0.0843	0.042	-0.152	0.272	-14.3	6.51	53.51	42.33	524.68
8	-0.084	0.042	-0.152	0.271	-13.9	6.9	58.11	44.16	563.59
9	-0.0855	0.042	-0.154	0.284	-11.1	5.33	44.74	38.66	481.18
10	-0.0823	0.042	-0.151	0.271	-10.1	5.64	49.35	40.49	507.42

Table 2.6 Results of MLR with different set of compounds using various descriptors using 2nd set of molecules

No	QSAR equations	N	R^2	R^2_{CV}	SE	F	Auto correlation	Q
1	7.090 (±1.059) – 157.847(±60.335) f^-_{C7} – 0.301(±0.041) HE	10	0.885	0.852	0.359	26.935	0.123	2.62046
2	8.636 (±1.607) – 0.268(±0.038) HE – 77.971 (±29.661) f^+_{C7}	10	0.886	0.853	0.358	27.08	0.002	2.62926
3	11.841(±5.280) – 0.215(±0.059) HE – 494.905 (±357.599) f^+_{LAC}	10	0.821	0.770	0.447	16.099	0.348	2.02705
4	10.233 (±2.812) – 0.235 (±0.0450)HE – 905.661 (±443.036) f^+_{OXY}	10	0.858	0.817	0.4	21.075	0.096	2.31571
5	16.540 (±10.092) −40.883(±34.423) ω −0.210 (±0.067)HE	10	0.811	0.757	0.461	14.989	0.442	1.95348
6	2.353 (±1.465)+ 0.045(±0.027) MR −0.263 (±0.046)HE	10	0.835	0.788	0.43	17.694	0.00003	2.12508
7	−0.019(±2.797) – 0.261(±0.0457) HE + 0.113(±0.068) Pol	10	0.837	0.791	0.427	18.032	0.0007	2.14257

Here in this set we have found a good correlation of hydration energy with the cytotoxictiy rather than the surface area in the first case, as shown in Table 2.6. In all the equations, we have found that the coefficient of hydration energy is negative which suggest that an increase in hydration energy will diminished the biological activity of such camptothecins. In the Equations (1) and (2) it is found that the coefficient of f_k^- and f_k^+ are negative, thus, substitution at 7-position of B-ring with alkyl group makes this site less susceptible for nucleophillic or electrophillic attack and an increase in their values will have negative effect on the biological activity of the compounds.[33] Similarly, the negative coeffecients of f_k^+ in Equations (3) and (4) indicates that substitution at 10-position of the A-ring with electron rich group makes the lactone oxygen and the oxygen atom of the 20-OH group in E-ring less prone to nucleophillic attack. Hence, a decrease in their values will significantly improve the inhibitatory activity.[32] The negative coefficients of ω in Equation (5) suggest that decrease of its value will enhance the activity of the compounds. Molar refractivity of compound is dependent on the volume and the polarizability. So, the positive coefficient of MR and polarizability in Equations (6) and (7) brings out a less steric effect of the substituents.

In order to investigate the relative importance of the variable appeared in the final models obtained by multiple linear regression analysis (MLR), the P-values using the F statistics for each equations are compared. The P-value reflects the importance of variable in multiple regression. A regression model or a QSAR descriptor is significant only if its P-value is <0.05. It has been observed that in all cases the P-value of surface area (SA) is <0.05 for the first set of aza compounds and for the second set of substituted CPTs the P value for hydration energy (HE) is found to be less than 0.05, which has been shown in Table 2.7 and 2.8. On the other hand, the P-values for the other molecular descriptors are found to be more than 0.05 and hence contribute less to QSAR model in determining the cytotoxicity of the studied camptothecins. Thus, depending on different types of substitution or structural modification different descriptor will have different influence on the biological activity of the compounds. Our results demonstrate that the surface area parameter is very much prominent for the first set of camptothecin, whereas, hydration energy becomes a dominant factor in the analysis of second set. The correlation plot between experimental and calculated pIC_{50} for the best fit models are shown in Figure 2.3 and

2.4. The developed model revels that besides the physiochemical parameters, the calculated DFT based descriptors such as Fukui function, electrophilicity, energy of the lowest unoccupied molecular orbital can also be effective in the predicting the cytotoxicity of camptothecins.

Table 2.7 P-values for each independent variables used in the studied model for the 1st set

Equation No	Independent variable		P-values	
	X_1	X_2	X_1	X_2
1	f^-_{C10}	MR	0.104	4.8×10^{-5}
2	f^-_{C10}	SA	0.204	2.3×10^{-5}
3	f^+_{C10}	MR	0.016	7.7×10^{-6}
4	f^+_{C10}	SA	0.172	1.7×10^{-5}
5	f^+_{C7}	SA	0.362	0.0005
6	f^-_{H}	SA	0.361	0.0001
7	f^-_{LAC}	SA	0.281	0.0001
8	f^-_{OXY}	SA	0.289	7.5×10^{-5}
9	E_{LUMO}	SA	0.253	3.3×10^{-5}
10	E_{NL}	SA	0.217	2.6×10^{-5}
11	ω	SA	0.319	2.5×10^{-5}
12	LogP	SA	0.247	6.7×10^{-5}

Table 2.8 P-values for each independent variables used in the studied model for 2nd set

Equation No	Independent variable		P-values	
	X_1	X_2	X_1	X_2
1	f^-_{C7}	HE	0.035	0.0001
2	f^+_{C7}	HE	0.034	0.0002
3	f^+_{LAC}	HE	0.209	0.0008
4	f^+_{OXY}	HE	0.080	0.0012
5	ω	HE	0.274	0.0162
6	MR	HE	0.148	0.0007
7	Pol	HE	0.138	0.0007

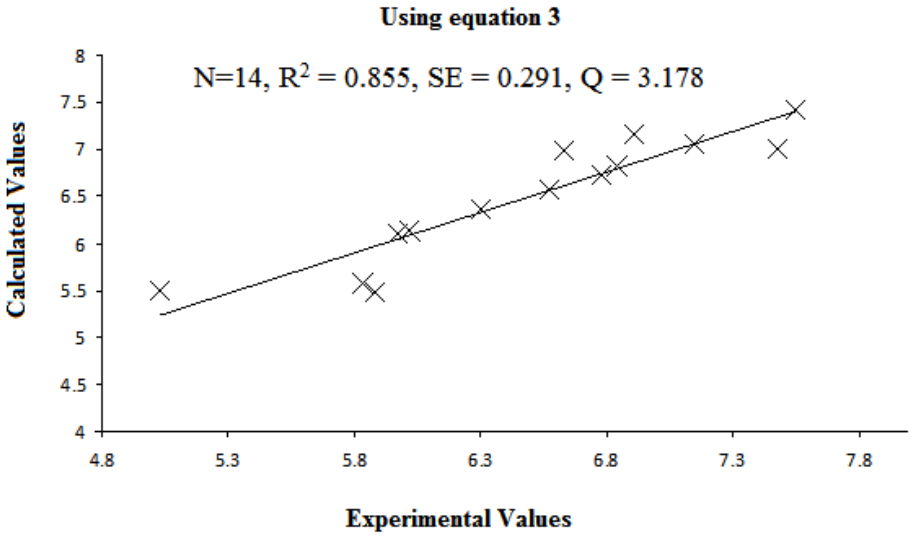


Figure 2.3 Correlation plot between experimental and calculated values of pIC_{50} for the 1st set of compounds (best fit model)

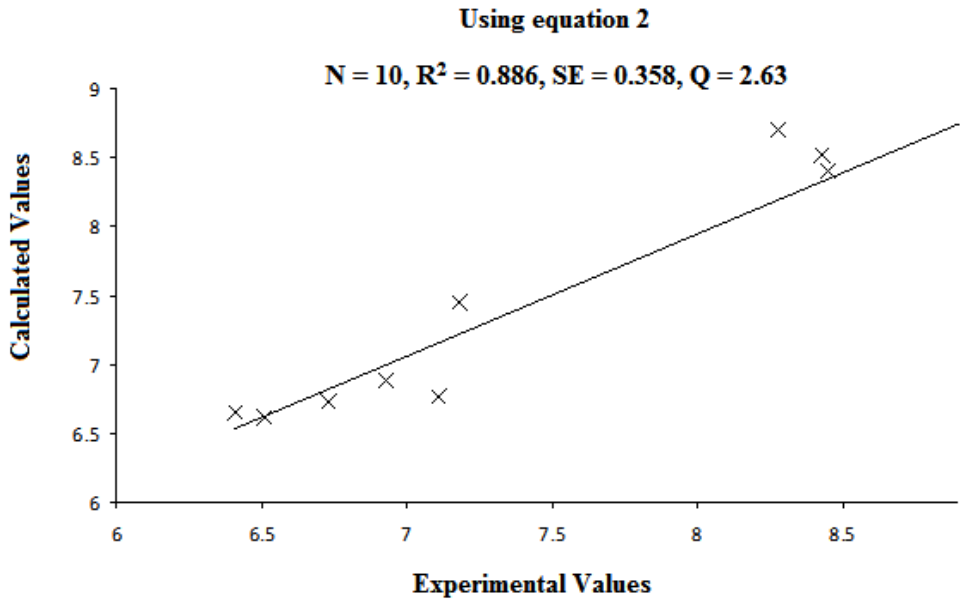


Figure 2.4 Correlation plot between experimental and calculated values of pIC_{50} for the 2st set of compounds (best fit model)

2.4 Conclusion

QSAR modeling plays an important role in drug design. Here, two different sets of camptothecin analogues are investigated by DFT-based descriptors and good QSAR models are obtained. The analysis of the QSAR models based on the CPT analogues suggest that DFT based descriptors such as Fukui function (FF), electrophilicity (ω)

are equally important as the others physiological parameters namely hydration energy and surface area in describing the activity of camptothecins. Our QSAR models derived from the present study may be helpful for designing some new analogues and for predicting its activity which in turn help to understand the mode of action.

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