INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

2D QSAR Analysis on B-Ring Trifluromethylated chromenone analogues as anticancer agents

RB. Patil* and SD. Sawant

Sinhgad Technical Education Society's, Smt. Kashibai Navale College of Pharmacy, Kondhwa (Bk), Pune, Maharashtra, India.

ABSTRACT

Two dimensional quantitative structure activity relationship (2D-QSAR) study was performed on B-ring trifluoromethylated chromenone (flavonoid) analogues as anticancer agents. This study was performed with 25 compounds (data set) using random and manual data selection methods for the division of the data set into training and test set. Multiple linear regression analysis coupled with stepwise variable selection method was applied to derive QSAR models which were further validated for statistical significance. The most significant model has squared correlation coefficient (r^2), cross validated correlation coefficient (CV_ r^2) and predictive correlation coefficient (pred_r^2) 0.8271, 0.9985 and 0.827 respectively. The QSAR model indicates that the descriptors Kier Chi4 (path/cluster) index, Kier Chi4 (path) index, KAlpha3 index, Mp, IDDE, MWCO7, JGI5, TI1 and TPSA(tot) contributing 4.65%, 19.73%, 12.08%, 8.59%, 15.62%, 16.57%, 10.45%, 5.46% and 6.85% respectively. Negative coefficient value of Kier Chi4 (path/cluster) index and KAlpha3 index indicated that lower value leads to better inhibitory activity whereas higher value leads to decrease inhibitory activity whereas positive coefficient value of other descriptors indicated that higher value leads to good inhibitory activity while lower value leads to reduced inhibitory activity.

Keywords: 2D-QSAR, Chromenone, anticancer.

INTRODUCTION

Oxygen containing heterocycles are abundantly found in nature[1]. Chromenones are naturally occurring compounds possessing diverse biological and pharmacological activities. Many synthetic analogues of chromenones have been evaluated for their anticancer[2-4], anticonvulsant[5], angioprotective, antiallergic, antihistaminic[6], antimicrobial[7], antioxidant[8], anti-HIV[9]. Cancer is one of the most serious threats to human beings. In recent years, there has been a growing interest in search for anti-cancer substances with high efficacy, low toxicity and minimum side effects. Some flavonoids are reported to have carcinogenic benzo[α]pyrene metabolism inhibitory activity[10], cytotoxicity of TNF- α (tumor necrosis factor- α)[11] augmenting activity, tyrosinase inhibitory activity[12], aromatase inhibitory activity[13] and inhibition of estradiol induced DNA synthesis[14]. In the search for more effective and safer anticancer agents some attempts were made to synthesize flavonoids containing trifluoromethyl group as it is well known that the introduction of -CF_3 group into organic molecules often changes their physiological, physical and chemical properties without the introduction of extra steric hindrance. A-ring trifluoromethylated flavonoids[15] (7-methyl-8-trifluoromethyl-chrysin **1** and 6,8-ditrifluoromethyl-7acetoxychrysin **2,** Table 1) were reported to possess weak anticancer activity against SGC-7901 tumor cells. In order to enhance the biological activity B-ring trifluoromethylated flavonoids were synthesized by Xing Zheng et al[16] and were reported to possess considerably enhanced anticancer activity. Quantitative structure activity relationship (QSAR) is an accepted means for establishing quantitative relationship between biological activity and descriptors representing physicochemical properties of the compounds using statistical methods[17] and it helps to precisely predict the biological activities of newly designed analogues.[18] In a view to further refine and set precise structure activity relationship, we decided to establish quantitative relationship between physiochemical properties and biological activities of these reported B-ring trifluoromethylated flavonoids.

MATERIALS AND METHODS

QSAR analysis and statistical analysis were carried out by using various freeware available online[19 – 22] and some descriptors were computed by using TSAR 3.3 from Oxford Molecular Limited.

Data Set

In the present study a data set of B-ring trifluoromethylated flavonoid derivatives (25 molecules) has been taken from the literature[16] for QSAR studies. The reported IC_{50} values (μM) in in-vitro cytotoxicity test against SGC-7901 cell line have been changed to the logarithmic scale [log IC_{50}], for QSAR study. The structures of these derivatives with their reported IC_{50} and log IC_{50} values are given in Table-1. Structures were drawn using the 2D chemdraw ultra 11.0 application and converted to 3D structures. Structures were optimized by energy minimization and geometry optimization was done using Universal Force Field method with 10000 as maximum number of cycles, 0.01 as convergence criteria (root mean square gradient) and 1.0 as constant (medium's dielectric constant which is 1 for in vacuo optimization) in dielectric properties. The default values of 20.0 and 10.0 Kcal/mol were used for electrostatic and steric energy cutoff. The selected dataset were aligned by using most active molecules 5, 8, 12, 14,16 and 21. The aligned molecules are shown in fig.1

Descriptors used in the QSAR analysis

Numbers of physicochemical, topological, constitutional, alignment and atom type independent descriptors were calculated using www.vcclab.org interface, molinspiration.org interface and TSAR software after optimization or minimization of the energy of the data set molecules. Total 587 descriptors were computed and used in the statistical model development. By using various data reduction methods like Principal Componant Analysis (PCA) the descriptors contributing to the biological activity were only used in the development of statistically significant model. Some of the descriptors used while developing final models are given in table 2.

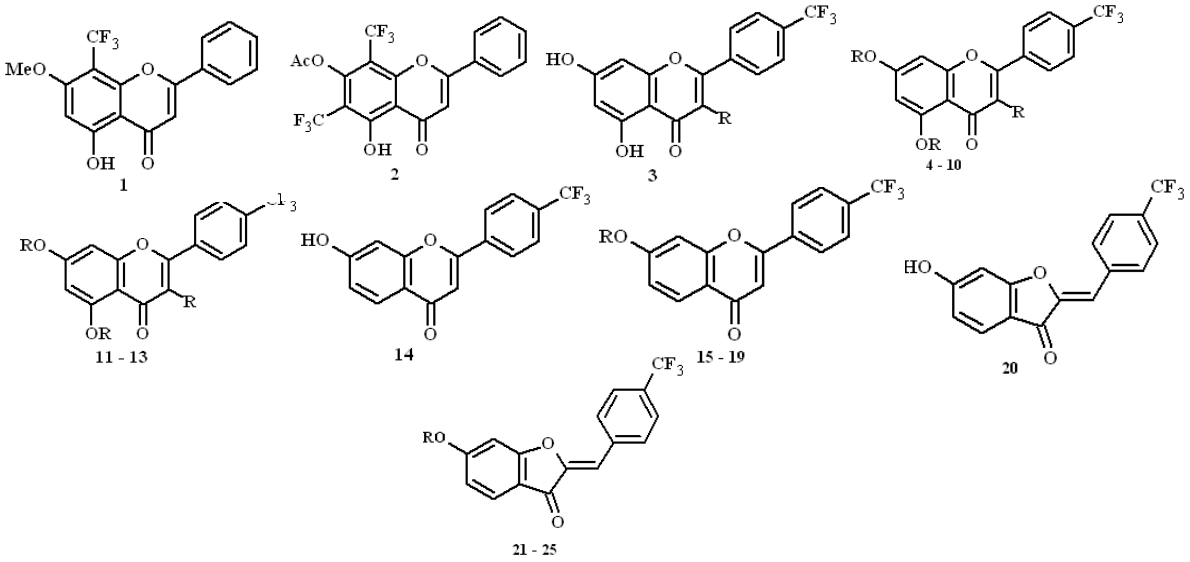
Data selection

The data generated was standardized by manually selecting data standardization by mean and standard deviation. Stepping was carried out during stepwise multiple regression analysis which include cross validation calculations. Stepping was carried out either by forward stepping method or by backward stepping method. Cross validation calculations were carried out by leave out one row method or leave out group of rows method. Two to ten random trials were carried out to arrive at the best model. The results of the cross validation were used to help detect overfitting and other potential problems with the final regression equation. Principal component analysis was carried out to help detect the descriptors contributing to the biological activity. Non contributing descriptors were eliminated while generating the final model. Thus from the set of initial 587 descriptors only 258 descriptors were chosen while generating final models.

RESULTS AND DISCUSSION

Selected data set of 25 B-ring triflоromethylated flavonoid derivatives was subjected to stepwise multiple regression analysis method for model building. Data set was manually subjected to cross validation analysis by leave out one row method or leave out group of rows method with different number of random trials. Thus in leave out one row cross validation method each row was automatically selected as test set data and in leave out group of rows alternate numbers of groups are randomly selected as test set data. Result of stepwise multiple regression analysis using random and manual data selection methods and leave out one row and leave out group of rows method with different random trials is shown in Table-3. The statistically significant models obtained are shown in Table-4. Model 1 generated from trial number 17 was found most significant. The contribution of each descriptor in model 1 is shown in Figure-2. Result of the observed and predicted biological activity for the compounds for the Model 1 is shown in Table- 5. The plot of observed vs. predicted activity for model 1 is shown in Figure 3. From the plot it can be seen that model is able to predict the activity quite well (all points are close to regression line).

Table 1: General structures of flavonoid derivatives and their biological activities



Comp.	R-	IC_{50} (µM)	log IC_{50}	Comp.	R-	IC_{50} (µM)	log IC_{50}
1	-	5.90	0.7708	14	-	17.75	1.2492
2	-	8.60	0.9345	15	$-CH_3$	8.28	0.9180
3	-	6.62	0.8208	16	$-CH_2-CH_3$	28.52	1.4551
4	$-CH_3$	4.37	0.6405	17	$-n-C_3H_7$	5.61	0.7490
5	$-CH_2-CH_3$	44.02	1.6436	18	-allyl	5.26	0.7210
6	$-n-C_3H_7$	2.70	0.4314	19	$-n-C_8H_{17}$	4.16	0.6191
7	$-CH_2C_6H_5$	5.00	0.6990	20	-	4.31	0.6345
8	$-n-C_7H_{15}$	30.83	1.4890	21	$-CH_3$	21.19	1.3261
9	$-n-C_8H_{17}$	18.06	1.2567	22	$-CH_2-CH_3$	15.60	1.1931
10	$-n-C_{10}H_{21}$	9.02	0.9552	23	-allyl	3.05	0.4843
11	$-CH_3$	10.08	1.0035	24	$-CH_2C_6H_5$	14.72	1.1679
12	$-CH_2-CH_3$	72.46	1.8601	25	$-n-C_7H_{15}$	5.35	0.7283
13	$-n-C_3H_7$	8.64	0.9365				

Table 2: Descriptors calculated for QSAR studies

Physicochemical Descriptors	Constitutional Descriptors	Topological descriptors	Other categories of Descriptors
Molecular Mass	Sv: sum of atomic van der Waals volumes (scaled on Carbon atom)		Walk and path counts
Molecular Surface area	Se: sum of atomic Sanderson electronegativities (scaled on Carbon atom)	ZM: Zagreb index	Connectivity indices
Molecular Volume	Sp: sum of atomic polarizabilities (scaled on Carbon atom)	Qindex: Quadratic index	Information indices
Log P	Ss: sum of Kier-Hall electrotopological states	VDA: average vertex distance degree	2D autocorrelations
Molecular Refractivity	Mv: mean atomic van der Waals volume (scaled on Carbon atom)	MSD: mean square distance index (Balaban)	Edge adjacency indices
Kier Chi (atom/ bond/path/path-cluster/cluster) index of order 0-6	Me:mean atomic Sanderson electronegativity (scaled on Carbon atom)	SMTI: Schultz Molecular Topological Index (MTI)	BCUT descriptors
Kier Chi V (atom/ bond/path/path-cluster/cluster) index of order 0-6	ARR: aromatic ratio	RHyDp reciprocal hyper-distance-path index	Topological charge indices
Kappa 1-3 index	nCIC: number of rings	Wap: all-path Wiener index	Eigenvalue-based indices
KAlpha 1-3 index	nDB: number of double bonds	ICR: radial centric information index	Randic molecular profiles
Shape flexibility index	nH: number of Hydrogen atoms	ZM: Zagreb index	Geometrical descriptors
Rotatable bonds	nC: number of Carbon atoms	Qindex: Quadratic index	Radial Distribution Function descriptors
Randic Topologic index	nR06: number of 6-membered rings	SNar: Narumi simple topological index (log)	3D-MoRSE descriptors
Wiener Topologic index	ATS: Broto-Moreau autocorrelation of a topological structure weighted by atomic masses	HNar: Narumi harmonic topological index	WHIM descriptors
Sum of E-state indices	MATS: Moran autocorrelation weighted by atomic masses	GNar: Narumi geometric topological index	GETAWAY descriptors
VAMP (total enery, electronic enery, Nuclear energy, surface area, mean polarizability)		Xt: Total structure connectivity index	Functional group counts
Ui: Unsaturation index		Dz: Pogliani index	Atom-centred fragments
Hy: hydrophilic factor		Ram: ramification index	Charge descriptors
MLOGP: Moriguchi octanol-water partition coeff. (logP)		Pol: polarity number	
ALOGP: Ghose-Crippen octanol-water partition coeff.			

Table 3: Result of stepwise multiple linear regression analysis

Trial	Stepwise Multiple linear regression								
	Cross validation method	Total sum of squares	s	F	r	r^2	CV_r^2	RSS	PRESS
1	LO each of 3 groups	2.3599	0.1877	17.311	0.8808	0.7758	0.1364	0.5288	2.03791
2	LO each of 3 groups	2.9732	0.2002	6.0137	0.9006	0.8111	-14.799	0.5614	46.975
3	LO each of 3 groups	3.8447	0.13860	18.612	0.9643	0.9300	-2.6445	0.26896	14.0125
4	LO each of 3 groups, 2 random trials	3.8447	0.1386	18.6126	0.9643	0.9300	-9.3658	0.2689	39.854
5	LO each of 3 groups	3.8447	0.138606	18.6126	0.964388	0.930044	-2.64458	0.268963	14.0125
6	LO each of 3 groups, 3 random trials	3.8447	0.138606	18.6126	0.964388	0.930044	-5.58402	0.268963	25.3139
7	LO each of 3 groups	2.9732	0.102443	22.6092	0.978593	0.957644	-5.99514	0.125935	20.7982
8	LO each of 3 groups, 2 random trials	2.9732	0.102443	22.6092	0.978593	0.978593	-13.5558	0.125935	0.125935
9	LO each of 4 groups	2.9732	0.102443	22.6092	0.978593	0.957644	-1.11797	0.125935	6.29724
10	LO each of 3 groups	2.9732	0.22595	3.85314	0.891037	0.793948	-76.0913	0.612642	229.211
11	LOO, 2 random trials	2.9732	0.102443	22.6092	0.978593	0.957644	-20.102	0.125935	62.7412
12	LOO	2.9732	0.102443	22.6092	0.978593	0.957644	-4.69974	0.125935	16.9467
13	LOO	2.9732	0.280014	7.96006	0.647945	0.419833	0.134313	1.72497	2.57389
14	LOO, 10 random trials	**2.9732**	0.185101	7.97541	**0.909476**	**0.827147**	**0.888275**	0.513934	**0.332185**
15	LOO, 10 random trials	**2.9731**	0.252849	4.75067	0.782903	**0.612937**	**0.625491**	1.15079	**1.11346**
16	LOO	**2.9732**	0.169451	9.83859	0.924737	**0.855139**	**0.299492**	0.430707	**2.08278**
17	LOO, 2 random trials	**2.9732**	0.185101	7.97541	**0.909476**	**0.827147**	**0.998521**	0.513934	**0.00439735**

S: standard error; F: a value derived from sum of squares and degrees of freedom; r: correlation coefficient; r^2: squared correlation coefficient, CV_r^2: cross validated correlation coefficient; RSS: Residual sum of squares; PRESS: Predictive sum of squares; LOO: Leaving out one row; LO: leaving out

Table 4: Statistically significant models generated

Model	Trial No.	Equation
1	**17**	Log IC_{50} = -0.13659 (KCV4PCI) + 0.5800 (KC4PI) - 0.3548 (Kα3I) + 0.2523 (Mp) + 0.4588 (IDDE) - 0.4870 (MWCO7) + 0.3070 (JGI5) + 0.1603 (TI1) + 0.20115773 (TPSA (tot))+ 0.9459 Cross validated leaving out one row randomly over 2 random trials; Correlation limit of 0.8 applied; 9 steps to generate final model
2	16	Log IC_{50} = -0.2283 (KCV4PCI) + 0.6053 (KC4PI) - 0.4384 (Kappa3 I) + 0.6016 (avg. molecular weight) + 0.3811 (IDDE) - 0.07215 (PW3) - 0.6284 (MWCO7) + 0.3451 (ATS7m) + 0.04055 (TPSA(tot)) + 0.9459 Cross validated leaving out one row randomly over 2 random trials; Correlation limit of 0.9 applied; 9 steps to generate; final model
3	15	Log IC_{50} = 0.9472 (Xu index) - 2.3787 (X4v) + 0.84356 (X5v) + 1.5253725(SOK) + 0.3601(BEHm8) + 1.4709 (VEv2) + 0.945912 Cross validated leaving out one row randomly over 10 random trials; Correlation limit of 1 applied; 6 steps to generate final model
4	**14**	Y = -0.1366 (KC4PCI) + 0.58003 (KC4PI) - 0.3548 (Kα3I) + 0.2523 (Mp) + 0.4588 (IDDE) - 0.4870 (MWCO7) + 0.3070 (JGI5) + 0.1603 (TI1) + 0.2011 (TPSA (tot)) + 0.945916 Cross validated leaving out one row randomly over 10 random trials; Correlation limit of 0.8 applied; 9 steps to generate final model

KCV4: Kier ChiV4 (path/cluster) index; KC4PI: Kier Chi4 (path) index; Kα3I: KAlpha3 index; Mp: mean atomic polarizability (scaled on Carbon atom); IDDE: mean information content on the distance degree equality; MWCO7: molecular walk count of order 07; JGI5: mean topological charge index of order5; TI1: first mohar index; TPSA(tot): total polar surface area; PW3: path/walk 3 - Randic shape index; ATS7m: Broto-Moreau autocorrelation of a topological structure - lag 7 / weighted by atomic masses; X4v: valence connectivity index chi-4; X5v: valence connectivity index chi-5; SOK: Kier symmetry index; BEHm8: highest eigenvalue n. 8 of Burden matrix / weighted by atomic masses; VEv2: average eigenvector coefficient sum from van der Waals weighted distance matrix

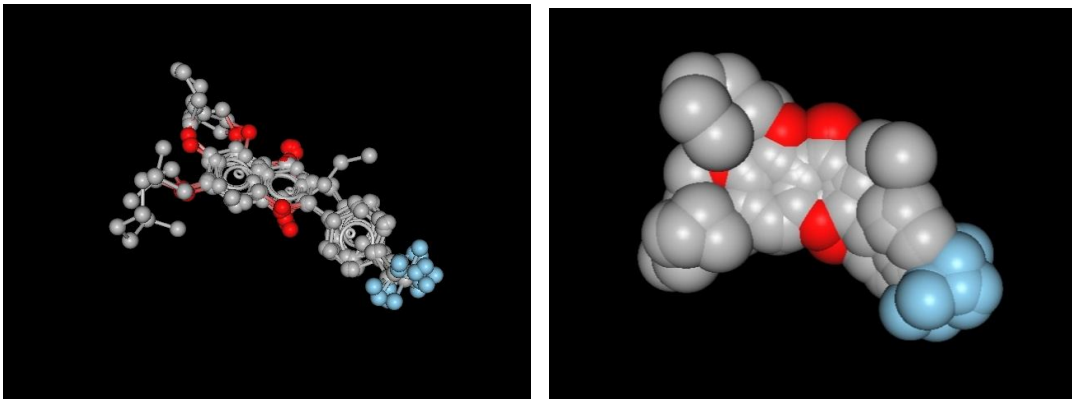
Interpretation of the Model 1 (most significant)

Among the four significant models generated (Table-4), model 1 is the most significant one as it is having the highest cross validated correlation coefficient value (CV_r^2 : 0.9985). The regression model (Model 1) has standard error (s: 0.1851) which explains predictive ability with standard error of 0.1851 units. The squared correlation coefficient (r^2: 0.8271) is in close agreement with cross validated correlation coefficient which explains the better the predictive power. The residual sum of squares (RSS: 0.5139) is the

variance in the residuals which is not accounted in the regression method. The predictive sum of square values (PRESS: 0.004397) for the Model 1 are much smaller than other models and also it is much smaller than total sum of square value (2.9732) which explains Model 1 generated is reasonable. The predictive squared coefficient (pred_r^2) for the model is 0.827 which explains true predictive ability of model. Various descriptors contributing in Model 1 are Kier Chi4 (path/cluster) index [This is the molecular connectivity index developed by Hall and Kier that reflect the atom identities, bonding environments and number of bonding hydrogen and of order 4 and of path/cluster type], Kier Chi4 (path) index [molecular connectivity index of order 4 and of path type], KAlpha3 index [This is molecule shape index based on the assumption that the shape of a molecule is a function of the number of atoms and their bonding relationship and KAlpha3 index signifies contribution of each atom to the overall shape of a molecule based on a comparison with a Carbon sp^3 atom], Mp [This is a constitutional descriptor which signifies mean atomic polarizability (scaled on Carbon atom)], IDDE [It is one of the information indices and signifies mean information content on the distance degree equality], MWCO7[This is molecular walk and path count and signifies molecular walk count of order 07], JGI5[This is topological charge index and signifies mean topological charge index of order 5], TI1[This is a topological descriptor and signifies first Mohar index] and TPSA(Tot)[This is a measure of total polar surface area] and these descriptors contribute 4.65%, 19.73%, 12.08%, 8.59%, 15.62%, 16.57%, 10.45%, 5.46% and 6.85% respectively. In the QSAR model 1, the negative coefficient value of Kier Chi4 (path/cluster) index [This is the molecular connectivity index developed by Hall and Kier that reflect the atom identities, bonding environments and number of bonding hydrogen and of order 4 and of path/cluster type] on the biological activity indicated that lower value leads to better inhibitory activity whereas higher value leads to decreased activity. Positive coefficient value of Kier Chi4 (path) index [molecular connectivity index of order 4 and of path type] on the biological activity indicated that higher values leads to good activity while lower value leads to reduced activity. Negative coefficient value of KAlpha3 index [This is molecule shape index based on the assumption that the shape of a molecule is a function of the number of atoms and their bonding relationship and KAlpha3 index signifies contribution of each atom to the overall shape of a molecule based on a comparison with a Carbon sp^3 atom] and MWCO7[This is molecular walk and path count and signifies molecular walk count of order 07] show inverse relation to biological activity where as positive coefficient value of other descriptors viz. Mp [This is a constitutional descriptor which signifies mean atomic polarizability (scaled on Carbon atom)], IDDE [It is one of the information indices and signifies mean information content on the distance degree equality], , JGI5[This is topological charge index and signifies mean topological charge index of order 5], TI1[This is a topological descriptor and signifies first Mohar index] and TPSA(Tot)[This is a measure of total polar surface area] show direct relationship with biological activity. Thus molecular connectivity, molecule shape as a function of the number of atoms, distance parameter, molecular walk and path count and mean topological charge parameter are decisive in conferring anticancer activity to the compounds.

Table 5: Observed and predicted activity for Model 1

Compound	Observed activity	Predicted activity
1	0.7708	0.90647
2	0.6345	0.62908
3	1.3261	1.3801
4	1.1931	1.0654
5	0.4843	0.338
6	1.1679	0.88895
7	0.7284	0.5853
8	0.9345	0.92375
9	0.8209	0.77168
10	0.6405	0.83205
11	0.6042	0.63329
12	0.4314	0.47789
13	0.699	0.81986
14	1.489	1.5002
15	1.2567	1.3507
16	0.9552	0.92402
17	1.0035	1.1879
18	1.86	1.6648
19	0.9365	0.75327
20	1.2492	1.1623
21	0.918	1.0184
22	1.4551	1.2199
23	0.749	1.037
24	0.721	0.84512
25	0.6191	0.73246



a) b)

Fig. 1: Aligned molecules a) ball and stick model b) Space fill model

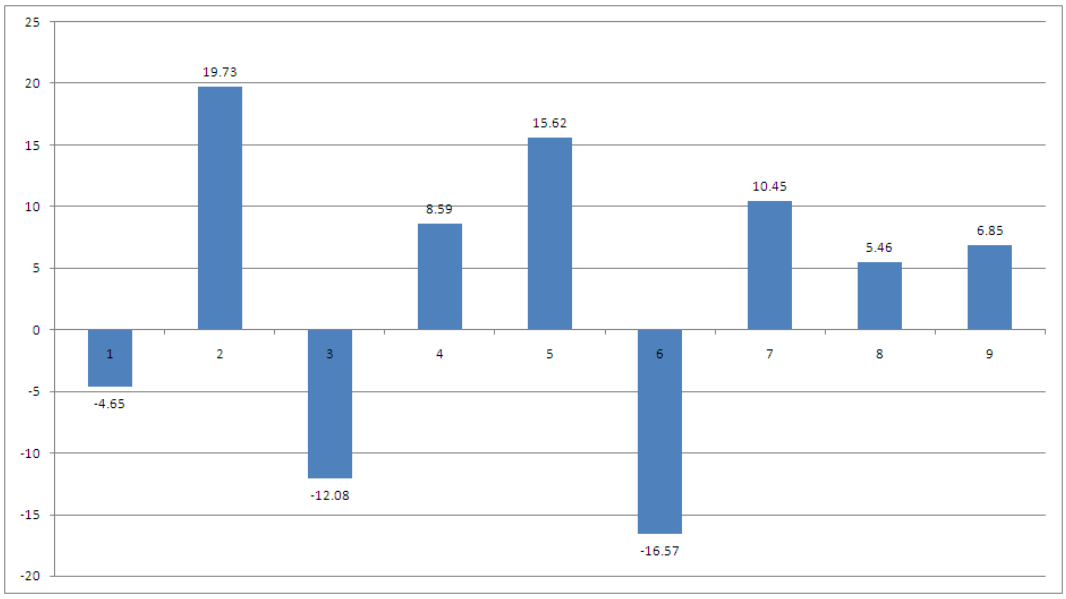


Fig. 2: Contribution chart for model 1 showing contribution of different descriptors

1 : Kier Chi4 (path/cluster) index 2: Kier Chi4 (path) index 3: KAlpha3 index
4: Mp 5: IDDE 6: MWCO7 7: JGI5 8: TI1 9: TPSA(Tot)
(Contributions shown are in %)

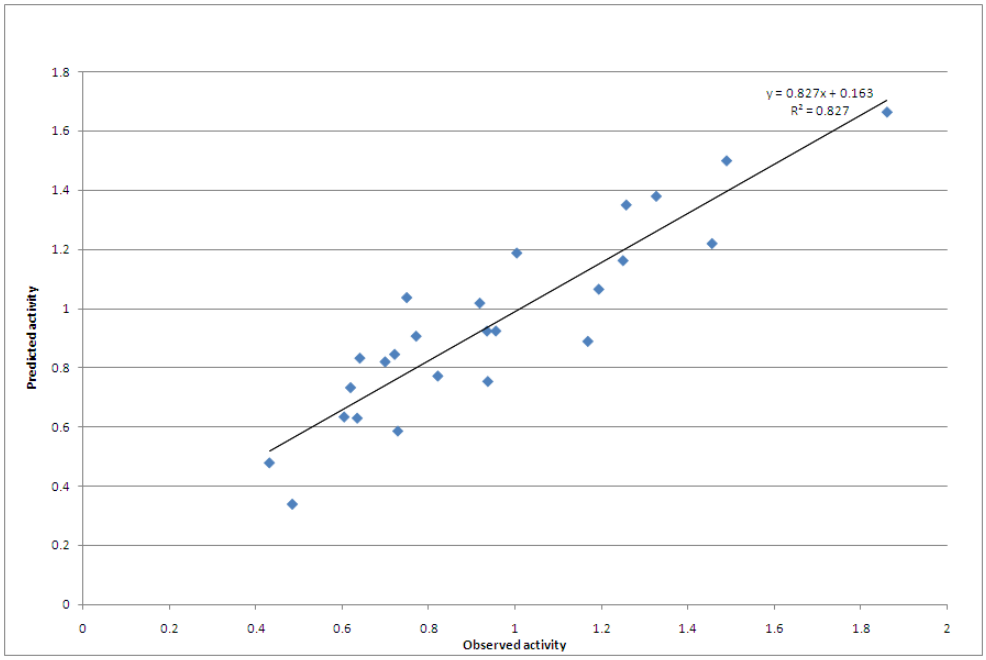


Fig. 3: Graph between actual and predicted biological activity for Model 1

CONCLUSION

Two dimensional quantitative structure activity relationship (2D-QSAR) study by means of stepwise multiple linear regression method was performed on a series of B-ring trifluoromethylated flavonoids derivatives as anticancer agents. Statistically significant QSAR models were generated. Among them most significant model (Model 1) has squared correlation coefficient (r^2), cross validated correlation coefficient (CV_r^2) and predictive squared correlation coefficient ($pred\_r^2$) are 0.8271, 0.9985 and 0.827 respectively. The QSAR model indicates that the descriptors Kier Chi4 (path/cluster)index, Kier Chi4 (path)index, KAlpha3 index, Mp, IDDE, MWCO7, JGI5, TI1 and TPSA(tot) contributing to anticancer activity. The negative coefficient value of Kier Chi4 (path/cluster)index, KAlpha3 index and MWCO7 on the biological activity indicated that lower value leads to better anticancer activity whereas higher value leads to decrease activity. Positive coefficient value of Kier Chi4 (path) index, Mp, IDDE, JGI5, TI1 and TPSA(tot) indicates that higher value leads to better anticancer activity whereas lower value leads to decrease activity.

ACKNOWLEDGEMENT

Authors express their gratitude to Prof. M. N. Navale, Founder President, Sinhgad Technical Education Society, Pune for providing the necessary facilities during the course of this research work.

REFERENCES

1. Maegawa H, Obata T and Shibata T. A new antidiabetic agent (JTT-501) rapidly stimulates glucose disposal rates by enhancing insulin signal transduction in skeletal muscle. Diabetologia. 1999;42(2):151-159.
2. Garg HG and Singh PP. New compounds: Potential antidiabetics IV: 1-(2,4-dinitrophenyl)-3,5-diphenyl-4-arylazopyrazoles and 1-carbamoyl-3,5-diphenyl-4-arylazopyrazoles. J Pharm Sci. 1970;59(6):876–877.
3. Lee HW, Kim BY and Ahn JB. Molcular design, synthesis, and hypoglycemic and hypolipidemic activities of novel pyrimidine derivatives having thiazolidinedione. Eur J Med Chem. 2005;40(9):862-874.
4. Sofia RD, Diamantis W and Ludwig BJ. Comparative anti-inflammatory, analgesic, and antipyretic activities of 7-chloro-3,3a-dihydro-2-methyl-2H,9H-isoxazolo-(3,2-b)(1,3)-benzoxazin-9-one and 5-

chlorosalicylic acid in rats. J Pharm Sci. 1975;64(8):1321-1324.
5. Farghaly AM, Soliman FS and El Semary MM. Polysubstituted pyrazoles, Part 4: Synthesis, antimicrobial and antiinflammatory activity of some pyrazoles. Pharmazie. 2001;56(1):28-32.
6. Bennett GB, Mason RB and Alden LJ. Synthesis and antiinflammatory activity of trisubstituted pyrimidines and triazines. J Med Chem. 1978;21(7):623-628.
7. Lee YS and Kim BH. Heterocyclic nucleoside analogues: design and synthesis of antiviral, modified nucleosides containing isoxazole heterocycles. Bioorg Med Chem Lett. 2002;12(10):1395-1397.
8. Pancic F, Steinberg BA and Diana GD. Antiviral activity of Win 41258-3, a pyrazole compound, against herpes simplex virus in mouse genital infection and in guinea pig skin infection. Antimicrob Agents Chemother. 1981;19(3):470–476.
9. Holy A, Votruba I and Masojidkova M. 6-[2-(Phosphonomethoxy) alkoxy] pyrimidines with antiviral activity. J Med Chem. 2002;45(9):1918-1929.
10. Liu YL, Ho DK and Cassady JM. Isolation of potential cancer chemopreventive agents from Eriodictyon californicum. J Nat Prod. 1992;55:357-363.
11. Hebtemariam S. Flavonoids as inhibitors or enhancers of the cytotoxicity of tumor necrosis factor-alpha in L-929 tumor cells. J Nat Prod. 1997;60:775-778.
12. Kubo I, Kinst-Hori I, Chaudhuri SK, Kubo Y and Sanchez Y. Flavonols from Heterotheca inuloides: tyrosinase inhibitory activity and structural criteria. Bioorg Med Chem. 2000;8:1749.
13. Suresh CT, Leena S, Sari M and Risto SJ. Inhibition of 17-beta-Estradiol Formation by Isoflavonoids and Flavonoids in Cultured JEG-3 Cells: Search for Aromatase-Targeting Dietary Compounds. J Med Food. 1999;2:235.
14. Wang CF and Kurzer MS. Effects of phytoestrogens on DNA synthesis in MCF-7 cells in the presence of estradiol or growth factors. Nutr Cancer. 1998;31:90-100.
15. Xing Z, Wei-Dong M and Yang-Yan X. Synthesis and Anticancer Effect of Chrysin Derivatives. Bioorg Med Chem Lett. 2003;13:881–884.
16. Xing Z, Wei-Dong M and Yang-Yan X. Synthesis and anticancer effect of B-ring trifluoromethylated flavonoids. Bioorg Med Chem Lett. 2003;13:3423-3427.
17. Kiralj R and Ferreira MMC. Basic validation procedures for regression models in QSAR and QSPR studies: theory and applications. J Braz Chem Soc. 2009;20:770-787.
18. de Melo EB, Martins JPA, Jorge TCM and Ferreira MMC. Mutivariate QSAR study on the antimutagenic activity of flavonoids against 3-NFA on Salmonella typhimurium TA98. Eur J Med Chem. 2010;45:4562-4569.
19. http://www.vcclab.org
20. Tetko IV, Gasteiger J, Todeschini R, Mauri A, Livingstone D, Ertl P, Palyulin VA, Radchenko EV, Zefirov NS, Makarenko AS, Tanchuk VY and Prokopenko VV. Virtual computational chemistry laboratory - design and description. J Comput Aid Mol Des. 2005;19:453-63.
21. http://www.graphpad.com (Graphpad InStat 3 trial version)
22. http://www.molinspiration.org