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Modeling of the Interaction of Flavanoids with GABA (A) Receptor Using PRECLAV (Property-Evaluation by Class Variables)

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ABSTRACT

Quantitative Structure-Activity Relationship (2*D-QSAR*) *models for binding affinity constants* (log K_i) *of* 78 *flavanoid ligands towards the benzodiazepine site of GABA* (*A*) *receptor complex were estimated using the PRECLAV* (*Property-Evaluation by Class Variables*) *program. The best MLR equation with nine PRECLAV descriptors has* $R^2 = 0.843$ *and* $R_C^2 = 0.782$. *Attempt is also made for obtaining* 2*D-QSAR model using NCSS software. The comparison of the results indicated that the PRECLAV method is very efficient in detecting structure-activity correlation with good predictive power.*

Keywords: *PRECLAV*, *NCSS*, *Regression Analysis*, *Cross-Validation*, *GABA*, *Flavanoids*

1. Introduction

During the last two decades quantitative structure-activity relationship (2D-QSAR) models have gained extensive recognition in drug design [1]. The widespread use of 2D-QSAR models come from the development of novel structural descriptors and statistical equations relating activity with chemical structure. The main hypothesis in the 2D-QSAR approach is that all biological activity of a chemical substance is statistically related to its molecular structure. The PRECLAV program uses the atom in the common skelton to compute bond and field (grid) descriptors [2,3]. The PRECLAV program computes five classes of structural descriptors: Constitutional, topological indices, molecular graph invariants, geometrical, quantum bond indices and field (grid) descriptors [2-4].

All molecules are aligned by superimposing the common atom before generating the multiple linear regression models; PRECLAV makes a descriptor selection by discarding those descriptors that are poorly correlated with the investigated activity.

During last decade more than 400 chemically unique flavonoids (phenyl-benzopyrans) have been isolated from vascular plants and many of them are used as tranquilizers in folkloric medicine. Such type of compounds are important constituents of the human diet, being derived largely from fruits and vegetables, nuts, seeds, stems and flowers and thus constitute one of the important classes of the metabolites. Some of the compounds from flavones family exhibit a potent *in vivo* anxiolitic activity, and do not involve unwanted side effect. As a result of this several attempts have been made to generate synthetic flavones derivatives with higher affinities for the GABA (A) receptor [5-10]. Subsequently, attempts were also made to establish quantitative structure-activity relationship so as to establish a 2D-QSAR model for inhibition of GABA (A) receptor that could serve as a guide for the rational design of further potent and selective inhibition having the flavones backbone [11-13]. One such attempt was recently made by Duchowiz and co workers [14-16]. They have proposed the best linear model for a set of 70 flavones and found that the best model involves four correlating descriptors with statistical quality given by $R^2 = 0.7174$, Se = 0.580, $R^2_{LOO} = 0.6757$, $S_{LOO} = 0.622$.

It was observed that out of several available software's such as COMFA [17], CORBA [18], OASIS [19], CODESSA [20], TSAR [21], PRECLAV [2,3], etc. The PRECLAV software is very efficient in detecting struc-

ture-activity relationship with good predictive power. This has prompted us to use PRECLAV program for investigating GABA (A) receptor binding and to compare the findings with those obtained using NCSS software.

2. Database and Modeling

The data base used as input by PRECLAV consists of 78 flavoniods presented in **Table 1** together with their log $K_i(\mu M)$ values [14]. The chemical structures were generated with Hyper Chem. [22], geometry optimization was performed with MOPAC [23] and the QSAR models were computed with PRECLAV [2,3]. MOPAC 7 output files are used by PRECLAV [2,3] program to compute PRECLAV descriptors for generating multiple linear regression models. Before such generation of the models PRECLAV software makes a descriptor selection by discarding those descriptors that are poorly correlated with the investigated activity. The following descriptors were generated in the present case:

3. Notations of the Structural Descriptors Generated by PRECLAV

1) MATS2p: Moran autocorrelation-lag 2/weighted by atomic polarizabilities (2-D autocorrelation indices) Dragon Descriptor.

2) OXX: presence of Oxygen. Maximum charge for O atom (at parabolic region) PRECLAV Descriptor.

3) NGS: area of negative charged surface/molecular surface area ratio (at parabolic region) PRECLAV Descriptor.

4) HBA: Capability to form.

5) Hydrogen bonded (function No. 1) (at parabolic region) PRECLAV Descriptor.

6) VLS: volume of circumscribed sphere (at parabolic region) PRECLAV Descriptor.

7) B05[O-B]: presence/absence of [O-B] at topological distance 05(2D binary fingerprint) Dragon Descriptor.

8) GVWAI-50: Ghose-Viswanadhan-Wendoloski drug-like index at 50% (molecular properties) Dragon Descriptor.

9) B08[C-O]: presence/absence of [C-O] at topological distance 08. (2D b inaryfingerprint) Dragon Descriptor.

10) HTm: H total index/weighted by atomic masses (GETAWAY descriptors).

These descriptors are chosen on the basis of their quality (Q) and were used to generate the best MLR (Multilinear regression) model.

Finally, the leave-one-out (LOO) cross-validation procedure is applied to each and every MLR equation in order to estimate the prediction power of the proposed QSAR equations. The predictive ability of a QSAR equation is estimated with the LOO Pearson and Rank (Kendall) correlation coefficients R^2_{cv} and $R^2_{Kendall}$. The equation with the highest predictive power is considered to be the one with the highest value for the product $R^2_{cv} \times R^2_{Kendall}$. This QSAR model can further be used to predict the activity of novel, not yet tested compounds (Drugs).

In the present study for modeling log K_i of 78 compounds initially we have used 400 PRECLAV and 1457 DRAGON descriptors. The number of excluded near constant descriptors being 89, while the number of significant descriptors is 174. One by one outliers is removed from calibration set so that final 2D-QSAR model is obtained.

4. Results and Discussion

After computing the structural descriptors for the 78 flavones (**Table 1**) PRECLAV performs the descriptors solution and generation of best QSAR equation. Because it is important to have a reference for the evaluation of MLR model, we give here correlations prediction/property of the aforementioned most valuable predictors MATS2P, 0.444175: OXX, 0.2524; NGS, 0.1232; HBA, 0.1232; VLS, 0.112; BO5[O-Br], 0.1035; GVWAI-50, 0.0886; BO8[C-O], 0.0836; HT_m, 0.0607.

During the PRECLAV MLR analysis, we observed that the equation with highest value of the $R^2_{cv} \times R^2_{Kendall}$ is the 7-parametric models and that this model also has the highest predictive power and is as follows.

$$\log K_i = -2.5590 - 14.7642\ \text{MATS2P} + 0.8940\ \text{OXX} + 0.5971\ \text{NGS} - 1.0633\ \text{HBA} + 1.0633\ \text{XNC} + 0.0656\ \text{HTm} + 4.3878\ \text{R2U}.$$

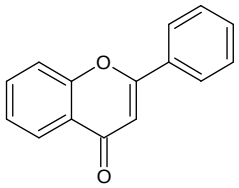
$$N = 78,\ R^2 = 0.7150,\ F = 24.447,\ R^2_{kendal} = 0.6424,\ R^2_{cv} = 0.6459,\ R_{kcv} = 0.6063,\ Q = 0.5879.$$

A detailed regression analysis of this model using PRECLAV software indicated that there are seven compounds which are (67, 59, 40, 25, 38, 46, and 64) acting as outliers. These compounds are, therefore, removed for obtaining an appropriate model.

Also, note that in the above model the coefficient of MATS2P, HBA, and GVWAI-50 are negative indicating that log K_i increases with decrease in the magnitude of these predictors. Both Pearson and Kendall LOO coefficients are high showing that the equation can provide reliable predictions. Furthermore, despite the larger number of structural descriptors in the above equation there is no evidence of over fitting, as indicated by high values of R^2_{cv} and R^2_{Kendal}.

It is worth mentioning that one by one removal of compounds 67, 59, 40, 25, 38, 36, and 64 acting as outliers resulted into both changes in the number of descriptors as well as the regression quality. The statistical

Table 1. List of 78 flavoniods and their observed log K_i values.



Compd. No	Compound	Obs. log K_i
1	6-F-luoro-3'-methoxyflavone	0.398
2	6-Bromo-3'-mcthoxyflavone	–0.215
3	6-Chloro-4'-methoxyflavonc	0.097
4	6-Bromo-4'-methoxyflavone	0.322
5	6-Chloro-2'-fluoroflavone	–0.380
6	6-Bromo-2'-fluoroflavone	–0.424
7	6,3'-Difluoroflavone	–0.036
8	6-Chloro-3'-fluoroflavone	–0.9.2
9	6-Bromo-3'-fluoroflavone	–1.377
10	4'-Fluoroflavonc	0.556
11	6.4'-Difluoroflavone	0.398
12	6-Chloro-4'-fluoroflavone	–0.742
13	3'-Chloroflavone	–0.212
14	6,3'-Dichloroflavone	–1.638
15	3'-Bromoflavone	–0.384
16	6-Fluoro-3'-bromoflavone	–0.627
17	6-Chloro-3'-bromoflavone	–1.638
18	6,3'-Dibromollavone	–1.721
19	6-Bromo-4'-nitroflavone	–0.699
20	6-Bromoflavone	–1.155
21	6-Chloroflavone	–0.785
22	6-Nitroflavone	–0.561
23	6-Methoxyllavone	–0.066
24	6-Fluoroflavone	0.653
25	6-Bromo-3'-nitroflavone	–3.000
26	6-Methyl-3'-nitroflavone	–2.252
27	6-Chloro-3'-nitroflavone	–2.097
28	6-3'-Dinitroflavone	–1.585
29	6-Fluoro-3'-nitroflavone	–0.745
30	3'-Nitroflavonc	–0.545
31	6-Methyl-3'-bromoflavone	–1.886
32	6-Nitro-3'-bromoflavone	–1.602
33	6-Hydroxy-3'-bromoflavone	0.000
34	6-Methoxy-3'-bromoflavone	0.000
35	6-3'-Dimethylflavone	–0.682
36	3'-Methylflavone	1.000
37	5,2'-Dihydroxy-6,7,8,6'-tetramethoxy flavone	–0.444
38	5,7,2'-Trihydroxy-6,8-dimethoxyflavone	–2.215
39	2'-Hydroxy-a-napthoflavone	–1.569
40	6.2'-Dihydroxyflavone	–1.469
41	5,7,2'-Trihydroxy-b.-methoxyflavone	–1.420
42	5,7,2'-Trihydroxyflavone	–1.125
43	2'-Hydroxyflavone	–0.678
44	5.7-Dihydroxy-6.8-dimethoxyflavone	–0.699
45	7,2'-Dihydroxyflavone	–0.252
46	5,7-Dihydroxy-6-methoxyflavone	–0.051
47	5.7-Dihydroxy-8-methoxyflavone	0.182
48	6-F Hydroxyflavone	0.422
49	7-Hydroxyflavone	0.623
50	5,6,7-Trihydroxyflavone	0.747
51	6-Hydroxy-2'-mcthoxytlavone	0.976
52	2'-Methoxyflavone	1.508
53	2'-Amino-6-methoxyflavone	0.544
54	Flavone	0.000
55	5,7-Dihydroxyflavone	0.477
56	5,3',4'-Trihydroxy-6,7-dimethoxyflavone	2.301
57	5,4'-Dihydroxy-6,7-dimcthoxyflavone	1.362
58	5,7,4'-Trihydroxy-6-mcthoxyflavone	0.000
59	5,7-Dihydroxy-2'-chloroflavone	0.903
60	5,7-Dihydroxy-2'-fluoroflavone	0.903
61	5,7-Dihydroxy-6,8-dibromoflavone	–0.155
62	5,7,4'-Trihydroxyflavone	0.602
63	3,5,7,4'-Tetrahydroxytlavone	1.969
64	5-Hydroxy-7-methoxyllavone	1.699
65	5,7-Dihydroxy-6,8-diiodollavone	0.000
66	6-Fluoro,3'-hydroxyflavone	0.400
67	6-Chloro,3'-hydroxyflavone	–0.070
68	6-Bromo,3'-hydroxyflavone	–0.220
69	6-Bromo-2'-nitrotlavone	–0.680
70	6-Nitro-4'-bromollavone	–1.600
71	3'-Methoxyflavone	0.380
72	6-Chloro-3'-methoxyflavone	–0.072
73	3'-Fluoroflavone	0.550
74	6-Bromo-4'-fluoroflavone	–0.939
75	6-Fluoro-3'-chlorollavone	–0.701
76	6-Bromo-3'-chlorollavone	–1.770
77	6-Methylflavone	–0.903
78	6-Bromo-3'-methylflavone	–0.812

detail of this model is given below:

$$\log K_i = -2.9314 - 16.6179\ MATS2P + 0.8709O\ XX + 0.4905\ NGS - 1.8923\ HBA + 0.3671\ VLS - 0.34610.5135\ BO5[O\text{-}Br] - 0.3461\ GVWAI\text{-}50 + 0.7031\ BO8[C\text{-}O] + 0.0489\ HT_m$$

$$N = 71,\ R^2 = 0.8098,\ F = 29.333,\ R^2_{kendal} = 0.7095,\ R^2_{cv} = 0.7494,\ R_{kcv} = 0.6732,\ Q = 0.6544$$

We observed that the quality and predictive power of the earlier model is considerably improved after deletion of outliers. Furthermore, the physical significance of the involved parameters is the same as before.

We have also used PRECLAV descriptors for obtaining the best 2D-QSAR model using NCSS software. Variable selection for multiple regression analysis has demonstrated the occurrence of best regression model with nine correlating parameters using variable selection analysis (**Table 2**). These parameters are the same as those were used in the aforementioned PRECLAV QSAR modeling. However, unlike PRECLAV, the NCSS programs clearly demonstrate successive arrival of 9-para- metric model. (**Table 2**). Among the regression results the best one-, two-, three-, four-, five-, six-, seven-, eight- and nine-parametric models were selected and are given in **Table 3**.

In these models, the correlation coefficient, R^2, is a measure of the fit of the model. F, the Fisher test value, reflects the ratio of the variance explained by the model and the variance due to the error in the model. Higher values of F-test indicate the significance of the model.

A perusal of **Table 3** shows that using NCSS software statistically allowed models start pouring with two- and higher-parametric modeling. The regression parameters and quality of these models are given below:

Table 2. Variable selection for multiple regression using NCSS.

Model No	R^2	R^2-Change	Names
1	0.4175	0.4175	MATS2p
2	0.5359	0.1183	MATS2p, OXX
3	0.5940	0.0581	MATS2p, OXX, HTm
4	0.6605	0.0665	MATS2p, OXX, HBA, HTm
5	0.7140	0.0535	MATS2p, OXX, NGS, HBA, HTm
6	0.7462	0.0323	MATS2p, OXX, NGS, HBA, B08[C-O], HTm
7	0.7714	0.0252	MATS2p, OXX, NGS, HBA, B05[O-Br], B08[C-O], HTm
8	0.7929	0.0215	MATS2p, OXX, NGS, HBA, VLS, B05[O-Br], B08[C-O], HTm
9	0.8098	0.0169	MATS2p, OXX, NGS, HBA, VLS, B05[O-Br], GVWAI-50, B08[C-O], HTm

Table 3. Quality of regression and predictive potential for one to nine variable models.

Model No	R^2	R^2_A	CV	F	Q	PRESS/SSY	R^2_{CV}	S_{PRESS}	PSE
1	0.4175	0.4091	–2.2701	49.464	–0.2846	1.3950	–0.3950	0.7260	0.7157
2	0.5359	0.5222	–2.0413	39.255	–0.3586	0.8661	0.1339	0.6528	0.6389
3	0.5940	0.5758	–1.9234	32.675	–0.4007	0.6835	0.3165	0.6151	0.5975
4	0.6605	0.6399	–1.7722	32.095	–0.4586	0.5141	0.4859	0.5668	0.5464
5	0.7140	0.6920	–1.6391	32.448	–0.5155	0.4006	0.5994	0.5242	0.5015
6	0.7462	0.7225	–1.5558	31.368	–0.5552	0.3401	0.6599	0.4976	0.4724
7	0.7714	0.7460	–1.4483	30.372	–0.6064	0.2963	0.7037	0.4760	0.4484
8	0.7929	0.7662	–1.4280	29.672	–0.6236	0.2612	0.7388	0.4567	0.4268
9	0.8098	0.7818	–1.3797	28.860	–0.6522	0.2349	0.7651	0.4412	0.4090

Two-variable model

$$\log K_i = -0.6987 - 7.0909\ (\pm 1.1003)\ \text{MATS2p} + 0.6765\ (\pm 0.1625)\ \text{OXX}$$

$$N = 71,\ R^2 = 0.5339,\ R_A^2 = 0.5222,\ CV = -2.0413,\ F = 39.255$$

The positive coefficient of the parameter OXX indicates that presence of Oxygen Maximum charge for O atom (at parabolic region) is favourable for the exhibition of the activity.

Three-variable model

$$\log K_i = -1.2772 - 9.4417\ (\pm 1.2848)\ \text{MATS2p} + 0.7375\ (\pm 0.1544)\ \text{OXX} + 0.0296\ (\pm 0.0095)\ \text{HTm}$$

$$N = 71,\ R^2 = 0.5940,\ R_A^2 = 0.5758,\ CV = -1.9234,\ F = 32.675$$

Here the coefficients of both the parameters OXX and HTm are positive meaning thereby that presence of Oxygen Maximum charge for O atom (at parabolic region) as well as H total index/weighted by atomic masses are favourable for the exhibition of the activity.

Four-variable model

$$\log K_i = -2.1460 - 13.1294\ (\pm 1.5666)\ \text{MATS2p} + 0.9464\ (\pm 0.1536)\ \text{OXX} + 0.0471\ (\pm 0.0101)\ \text{HTm} - 1.1215\ (\pm 0.3120)\ \text{HBA}$$

$$N = 71,\ R^2 = 0.6605,\ R_A^2 = 0.6399,\ CV = -1.7722,\ F = 32.095$$

Here also the parameters OXX and HTm have positive coefficients meaning thereby that presence of Oxygen Maximum charge for O atom (at parabolic region) as well as H total index/weighted by atomic masses are favourable for the exhibition of the activity

Five-variable model

$$\log K_i = -2.0375 - 13.1086\ (\pm 1.4489)\ \text{MATS2p} + 0.9128\ (\pm 0.1424)\ \text{OXX} + 0.0499\ (\pm 0.0093)\ \text{HTm} - 1.2511\ (\pm 0.291\ \text{HTm}\ 0)\ \text{HBA} + 0.6149\ (\pm 0.1763)\ \text{NGS}$$

$$N = 71,\ R^2 = 0.7140,\ R_A^2 = 0.6920,\ CV = -1.6391,\ F = 32.448$$

In this model, in addition to the two parameters OXX and HTm the third parameter NGS has positive coefficient. This means that in addition to presence of Oxygen Maximum charge for O atom (at parabolic region) as well as H total index/weighted by atomic masses, the area of negative charged surface/molecular surface area ratio (at parabolic region) is also favourable for the exhibition of the activity.

Six-variable model

$$\log K_i = -2.7781 - 14.5970\ (\pm 1.4709)\ \text{MATS2p} + 0.8088\ (\pm 0.1400)\ \text{OXX} + 0.0557\ (\pm 0.0091)\ \text{HTm} - 1.7353\ (\pm 0.3242)\ \text{HBA} + 0.5118\ (\pm 0.1712)\ \text{NGS} + 0.4724\ (\pm 0.1656)\ \text{B08[C-O]}$$

$$N = 71,\ R^2 = 0.7462,\ R_A^2 = 0.7225,\ CV = -1.5558,\ F = 31.368$$

We observe that in this model, in addition to the aforementioned three parameters a fourth parameter viz. B08 [C-O] has positive coefficient clearly meaning thereby that presence of Oxygen Maximum charge for O atom (at parabolic region) as well as H total index/weighted by atomic masses, the area of negative charged surface/ molecular surface area ratio (at parabolic region), the presence/absence of C-O at topological distance 08. (2D binary fingerprint) also favours the exhibition of the activity.

Seven-variable model

$$\log K_i = -3.0056 - 16.0272\ (\pm 1.5082)\ \text{MATS2p} + 0.8461\ (\pm 0.1347)\ \text{OXX} + 0.0509\ (\pm 0.0089)\ \text{HTm} - 1.8204\ (\pm 0.3118)\ \text{HBA} + 0.5333\ (\pm 0.1640)\ \text{NGS} + 0.5286\ (\pm 0.1598)\ \text{B08[C-O]} + 0.4638\ (\pm 0.1761)\ \text{BO5[O-Br]}$$

$$N = 71,\ R^2 = 0.7714,\ R_A^2 = 0.7460,\ CV = -1.4883,\ F = 30.372$$

In this model, in addition to the positive coefficients of the aforementioned four parameters, the fifth parameter namely BO5[O-Br] also has positive coefficient. It means that in addition to presence of Oxygen Maximum charge for O atom (at parabolic region) as well as H total index/weighted by atomic masses, the area of negative charged surface/molecular surface area ratio (at parabolic region), the presence/absence of C-O at topological distance 08. (2D binary fingerprint), the presence/absence of O-B at topological distance 05 (2D binary fingerprint) also favours the exhibition of the activity.

Eight-variable model

$$\log K_i = -2.7789 - 15.4338\ (\pm 1.4659)\ \text{MATS2p} + 0.8315\ (\pm 0.1294)\ \text{OXX} + 0.0505\ (\pm 0.0085)\ \text{HTm} - 1.7761\ (\pm 0.2996)\ \text{HBA} + 0.5435\ (\pm 0.1574)\ \text{NGS} + 0.4955\ (\pm 0.1539)\ \text{B08[C-O]} + 0.4533\ (\pm 0.1690)\ \text{BO5[O-Br]} + 0.4045\ (\pm 0.1595)\ \text{VLS}$$

$$N = 71,\ R^2 = 0.7929,\ R_A^2 = 0.7662,\ CV = -1.4280,\ F = 29.672$$

Here, we observe that in addition to the positive coefficients of the above mentioned five parameters, the six parameter namely VLS also has positive coefficient. This clearly means that in addition to the presence of Oxygen Maximum charge for O atom (at parabolic region) as well as H total index/weighted by atomic masses, the area of negative charged surface/molecular surface area ratio (at parabolic region), the presence/absence of [C-O] at topological distance 08. (2D binary fingerprint), the presence/absence of [O-B] at topological distance 05 (2D binary fingerprint), volume of circumscribed sphere (at parabolic region) also favours the exhibition of the activity.

Nine-variable model

$$\log K_i = -2.9314 - 16.6178\ (\pm 1.5048)\ \text{MATS2p} + 0.8709\ (\pm 0.1261)\ \text{OXX} + 0.0489\ (\pm 0.0083)\ \text{HTm} - 1.8923\ (\pm 0.2938)\ \text{HBA} + 0.4905\ (\pm 0.1538)\ \text{NGS} + 0.7031\ (\pm 0.1734)\ \text{B08[C-O]} + 0.5135\ (\pm 0.1653)\ \text{BO5[O-Br]} + 0.3672\ (\pm 0.1549)\ \text{VLS} - 0.3461\ (\pm 0.1486)\ \text{GVWAI-50}$$

$$N = 71,\ R^2 = 0.8098,\ R^2_A = 0.7818,\ CV = -1.3797,\ F = 28.860$$

We observe that in this 9-parametric model the aforementioned six correlating parameters have positive coefficients .This means that their physical significance in this model is the same as that of the 8-parametric model discussed above.

The aforementioned 9-variable model is, therefore, the most appropriate model and is subjected to Ridge regression [25] for investigating the existence or otherwise of any co-linearity defect. The Ridge parameters, namely VIF (variance inflation factor), T (Tolerance), CN (Condition number), have been calculated and presented in **Table 4**. We observed that VIF (variance inflation factor)values are much smaller than the allowed range of 10. Also, that condition number for for the correlating parameters all are much lower than 100 and the tolerance are <1. These observations therefore, suggest that no co-linearity defect is present in the proposed model.

Relative performance of PRECLAV and NCSS software

In order to further investigate the relative performance of both PRECLAV as well as NCSS software we have calculated (estimated) log K_i values for the 9-parametric models using both softwares and compared them with the experimental values of log K_i (**Table 5**). This is demonstrated in **Figures 1** and **2** indicating that quality of the model obtain from both PRECLAV and NCSS software is more or less same. log K_i values are much closer to the experimental values in case of PRECLAV software. From the study made herein we cannot definitely say as to which software is superior. Both have their own merits and demerits. However, the number of good points are more in PRECLAV software as compared to NCSS software. From the results obtained we conclude that there are some good or bad points in both the software and that overall PRECLAV software yields better statistics compared to NCSS software. The comparison of the performance of this software is demonstrated as below:

Comparison of results obtained using PRECLAV and NCSS software.

PRECLAV	NCSS
1) Overall the best model is proposed	1) Recommends obtaining of the best model in succession which need to be confirmed by their means
2) Predicts and removes the outliers one by one during the regression so that the final model does not have any outlier	2) This is not possible
3) Performance cross-validation	3) Not possible
4) Selects most significant descriptors by quality	4) Selection of descriptors is not based on quality
5) Most valuable descriptors set is generated	5) Not possible
6) Correlation of predictor/activity is possible	6) Not possible
7) Yields inter correlation of predictors	7) Not possible
8) Makes estimated and observed values in calibration set	8) Yes it is also possible in NCSS
9) Analysis virtual fragments is possible	9) Not possible
10) Standard deviation of coefficients are not measured	10) We can estimate standard error of the coefficient of the correlating parameters
11) Ridge statistics is not possible	11) We can make ridge analysis and then investigate co- linearity defect
12) Estimate qulity Q of the model	12) Not possible

It is worth mentioning that one of the important features of PRECLAV software is the analysis of virtual fragments. The software has indicated that for the set of 78 molecules analyzed here 30 virtual fragments are present out of which 9 fragments are significant. These most significant virtual fragments by correlation of "The Mass percent" and "Property values" are given in **Table 6**. This **Table 6** demonstrates that large mass percent of CO, $C_9H_4O_4$, $C_6H_5O_7$, C_6H_4O, and $C_8H_5O_4$ increases logK_i values while the large mass percent at C_8H_4O, NO_2, Br and C_6H_4 decreases the log K_i values. These observations, therefore, be taken care of while synthesizing new flavones with better log K_i values.

In order to confirm our findings we have compared the estimated values of the activities (log K_i) with the experimental ones (log K_i) (**Table 5**). This has further been demonstrated in **Figures 1** and **2**. Also, we have obtained Ridge traces as shown in **Figures 3** and **4**. From **Figures 1** and **2** as well as **Table 5**, we observed that the estimated activities (log K_i) are very close to the experimental activities (log K_i). Similarly, **Figures 3** and **4** indicates absence of any co-linearity defect.

Table 4. Ridge parameters for the most significant model.

Variable	VIF	T	λ_i	CN
MATS2p	4.4014	0.2272	3.367143	1.00
OXX	1.4179	0.7053	1.446224	2.33
NGS	1.1562	0.8649	0.957829	3.52
HBA	2.9861	0.3349	0.919456	3.66
VLS	1.0819	0.9243	0.771579	4.36
B05[O-Br]	1.9536	0.5119	0.643925	5.23
GVWAI-50	1.8986	0.5267	0.460259	7.32
B08[CO]	2.5441	0.3931	0.316425	10.64
HTm	2.3977	0.4171	0.117160	28.74

Table 5. Comparison of log K_i values estimated using PRECLAV and NCSS Software.

Using PRECLAV				Using NCSS	
Compd. No	Obs. log Ki	Est. log K_i	Residual	Est. log Ki	Residual
1	0.398	0.501	–0.103	0.501	–0.103
2	–0.215	–0.373	0.158	–0.373	0.158
3	0.097	0.228	–0.131	0.228	–0.131
4	0.322	0.084	0.238	0.084	0.238
5	–0.380	–0.898	0.518	–0.898	0.518
6	–0.424	0.067	–0.491	0.067	–0.491
7	–0.036	0.067	–0.103	0.067	–0.103
8	–0.932	–0.855	–0.077	–0.855	–0.077
9	–1.377	–1.013	–0.364	–1.013	–0.364
10	0.556	–0.118	0.674	–0.118	0.674
11	0.398	–0.042	0.44	–0.042	0.440
12	–0.742	–0.743	0.001	–0.743	0.001
13	–0.212	–0.76	0.548	–0.760	0.548
14	–1.638	–1.446	–0.192	–1.446	–0.192
15	–0.384	–0.641	0.257	–0.641	0.257
16	–0.627	–0.267	–0.36	–0.267	–0.360
17	–1.638	–1.302	–0.336	–1.302	–0.336
18	–1.721	–1.208	–0.513	–1.208	–0.513
19	–0.699	–0.828	0.129	–0.828	0.129

20	–1.155	–1.285	0.13	–1.285	0.130
21	–0.785	–1.056	0.271	–1.056	0.271
22	–0.561	–0.992	0.431	–0.992	0.431
23	–0.066	0.535	–0.601	0.535	–0.601
24	0.653	0.256	0.397	0.256	0.397
25	Outlier				
26	–2.252	–1.941	–0.311	–1.941	–0.311
27	–2.097	–1.751	–0.346	–1.751	–0.346
28	–1.585	–1.868	0.283	–1.868	0.283
29	–0.745	–0.463	–0.282	–0.463	–0.282
30	–0.545	–1.07	0.525	–1.070	0.525
31	–1.886	–1.285	–0.601	–1.285	–0.601
32	–1.602	–0.92	–0.682	–0.920	–0.682
33	0.000	–0.015	0.015	–0.015	0.015
34	0.000	–0.414	0.414	–0.414	0.414
35	–0.682	–0.631	–0.051	–0.631	–0.051
36	Outlier				
37	–0.444	–0.485	0.485	–0.485	0.041
38	Outlier				
39	–1.569	–1.114	–0.455	–1.114	–0.455
40	Outlier				
41	–1.420	–0.757	–0.663	–0.757	–0.663
42	–1.125	–0.293	–0.832	–0.293	–0.832
43	–0.678	0.178	–0.856	0.178	–0.856
44	–0.699	–0.976	0.277	–0.976	0.277
45	–0.252	0.148	–0.4	0.148	–0.400
46	–0.051	0.131	–0.182	0.131	–0.182
47	0.182	0.004	0.178	0.004	0.178
48	0.422	0.948	–0.526	0.948	–0.526
49	0.623	0.736	–0.113	0.736	–0.113
50	0.747	0.838	–0.091	0.838	–0.091
51	0.976	0.986	–0.01	0.986	–0.010
52	1.508	0.93	0.578	0.930	0.578
53	0.544	0.228	0.316	0.228	0.316
54	0.000	–0.096	0.096	–0.096	0.096
55	0.477	0.805	–0.328	0.805	–0.328

56	2.301	1.944	0.357	1.944	0.357
57	1.362	2.045	–0.683	2.045	–0.683
58	0.000	–0.638	0.638	–0.638	0.638
59	Outlier				
60	0.903	0.401	0.502	0.401	0.502
61	–0.155	–0.79	0.635	–0.790	0.635
62	0.602	0.062	0.54	0.062	0.540
63	1.969	1.588	0.381	1.587	0.382
64	Outlier				
65	0.000	–0.039	0.039	–0.039	0.039
66	0.400	0.162	0.238	0.162	0.238
67	Outlier				
68	–0.220	–0.865	0.645	–0.865	0.645
69	–0.680	–1.133	0.453	–1.133	0.453
70	–1.600	–1.395	–0.205	–1.395	–0.205
71	0.380	0.688	–0.308	0.688	–0.308
72	–0.072	–0.173	0.101	–0.173	0.101
73	0.550	0.064	0.486	0.064	0.486
74	–0.939	–1.003	0.064	–1.003	0.064
75	–0.701	–0.313	–0.388	–0.313	–0.388
76	–1.770	–1.131	–0.639	–1.131	–0.639
77	–0.903	–0.776	–0.127	–0.776	–0.127
78	–0.812	–1.17	0.358	–1.170	0.358

Table 6. List of virtual fragments.

S. No	Fragment	Specimen Molecule	Correlation	F
1	C_8H_4O	1	–0.4294	174.1
2	NO_2	19	–0.4006	147.1
3	Br	2	–0.3603	114.9
4	CO	1	0.3422	102.1
5	$C_9H_4O_4$	56	0.3405	100.9
6	C_6H_4	5	–0.3177	86.4
7	$C_6H_5O_2$	56	0.2928	72.2
8	C_6H_4O	1	0.2592	55.5
9	$C_8H_5O_4$	63	0.2559	54.0

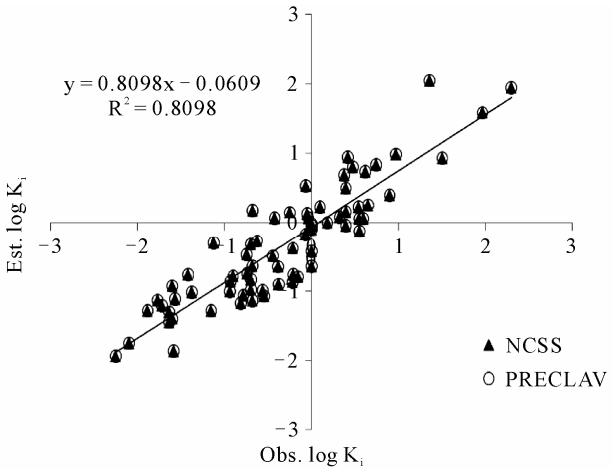


Figure 1. Correlation between observed and estimated log K_i using 9-parametric model both from PRECLAV and NCSS softwares.

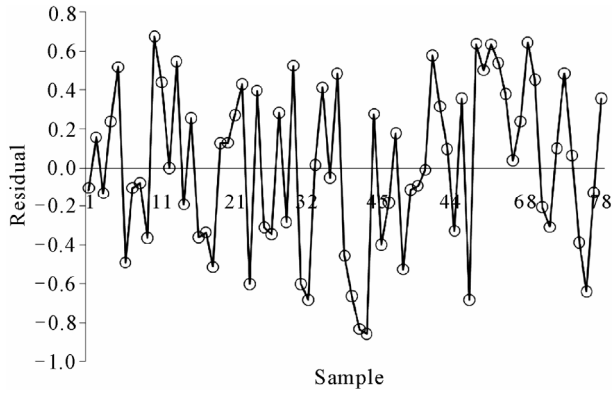


Figure 2. Residual plot for log K_i using 9-parametric model both from PRECLAV and NCSS softwares.

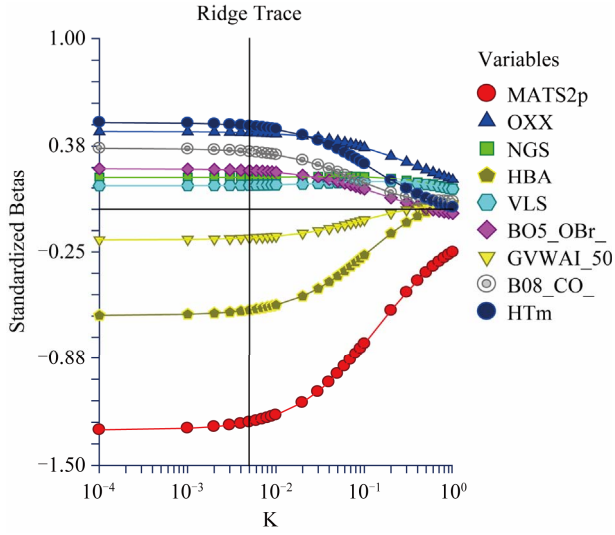


Figure 3. Ridge plot.

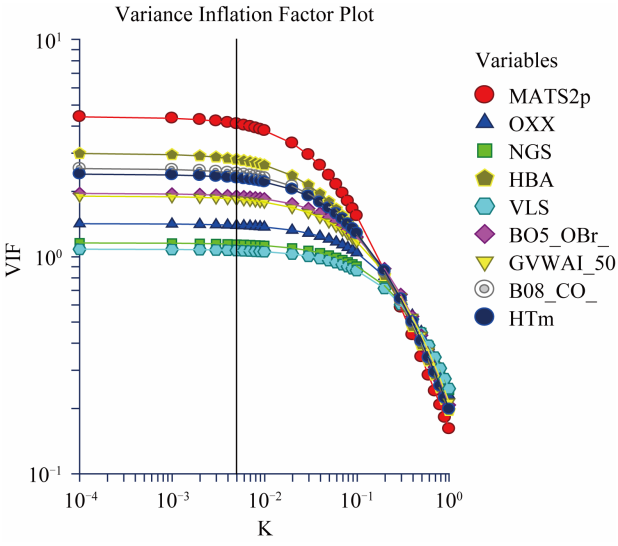


Figure 4. Ridge plot.

5. Conclusions

From the results and discussion made above we conclude that the PRECLAV software generates and proposes the overall best model and that there is no need of performing successive or stepwise regression to arrive at the best model. Such regressions are needed in NCSS software for obtaining the best model. Furthermore, while using PRECLAV software there is no need to perform model validation separately. Finally, PRECLAV software proposes virtual fragment which increases or decreases the biological activity. From the comparison made above we conclude that the PRECLAV software is the best for future 2D-QSAR study.

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