Original article DOI: 10.1515/aiht-2016-67-2779

Simple graph-theoretical model for flavonoid binding to P-glycoprotein

Ante Miličević and Nenad Raos

Institute for Medical Research and Occupational Health, Zagreb, Croatia

[Received in February 2016; CrossChecked in February 2016; Accepted in March 2016]

Three sets of flavonoid derivatives (N=32, 40, and 74) and logarithms of their dissociation constants (log K_d) that describe flavonoid affinity toward P-glycoprotein were modelled using six connectivity indices. The best results were obtained with the zero-order valence molecular connectivity index ($^0\chi^v$) for all three sets. Standard errors of the calibration models were around 0.3, and of the constants from the test sets even a little lower, 0.22 and 0.24. Despite using only one descriptor, our model proved better in internal (cross-validation) and especially in external (test set) statistics than much more demanding methods used in previous 3D QSAR modelling.

KEY WORDS: *connectivity indices; dissociation constant; flavonoids; molecular modelling; P-glycoprotein*

There is a rising interest in the research of bioflavonoids, secondary phenolic plant metabolites that have been evidenced as strong antioxidants (1-5) and therefore regarded as potential anti-cancer agents (6, 7). No less important are the research efforts to elucidate their metabolism and mode of action. Noteworthy are the measurements of their affinity to a variety of proteins such as human serum albumin (8), bovine serum albumin (9) or blood plasma (10), and milk proteins (11). Among them P-glycoprotein (P-gp), a 170 kDa transmembrane protein (12), has received particular attention because it expels hydrophobic compounds from the cell, which leads to resistance to cytostatic agents through a mechanism termed multidrug resistance (MDR) (13-15). The search for effective and safe P-gp inhibitors is in progress, and flavonoids seem very promising as MDR modulators. Investigations of flavonoid binding to P-glycoprotein using pharmacophore modelling (DISCOtech, CoMFA, CoIMFA, MIF) (16-18) have yielded a fair agreement with experimental findings and showed the dominance of steric and hydrophobic interactions (fields) in flavonoid binding to P-glycoprotein (17).

The aim of our study was, however, to develop a simpler but as reliable method with the same purpose, that is, to predict the dissociation constant, K_d, of P-glycoprotein/flavonoid complexes. The dissociation constant K_d could be modelled with only one molecular descriptor from the class of valence connectivity indices, just as the third-order valence connectivity index ($^3\chi^v$) was successfully applied to predict the stability of coordination compounds (19), especially of copper(II) chelates with peptides (20-22). Moreover, $^3\chi^v$ and other related graph-theoretical indices have been applied in quantitative structure activity relationship (QSAR) analysis (23-25), and particularly in the QSAR of 15 flavonoids isolated from Jerusalem thorn (*Paliurus spina-christi* Mill.) used in Croatian traditional herbal medicine (26). The authors have shown that the first-order valence connectivity index linearly correlated (r=0.993) with the hydrophobicity of flavonoids, *i.e.* with their octanol/water partition coefficient (log P) and Van der Waals volumes (r=0.999).

For this purpose, we used three sets of flavonoid derivatives and their dissociation constants (pK_d), which describe flavonoid affinity toward P-glycoprotein (16-18). The best fitting models and the best internal and external (cross-validation and test set) predictions for all sets were obtained with zero-order connectivity index ($^0\chi^v$).

METHODS

Calculation of topological indices

We calculated topological indices using the E-DRAGON program developed by R. Todeschini and co-workers, capable of yielding 119 topological indices in a single run along with many other molecular descriptors (27, 28). Connectivity matrices were constructed with the aid of the online SMILES Translator and Structure File Generator (29).

The zero-order valence molecular connectivity index, $^0\chi^v$, was defined as (23, 30-32):

$$^0\chi^v = \sum_{\text{vertex}} [\delta(i)]^{-0.5} \quad [1]$$

Correspondence to: Ante Miličević, Institute for Medical Research and Occupational Health, Ksaverska c. 2, P.O. Box 291, HR-10000 Zagreb, Croatia; E-mail: *antem@imi.hr*

where $\delta(i)$ denotes weight (valence values) of vertex (atom) i in a vertex-weighted molecular graph. The valence value, $\delta(i)$, of a vertex i is defined by:

$$\delta(i)=[Z^v(i) - H(i)]/[Z(i) - Z^v(i) - 1] \quad [2]$$

where $Z^v(i)$ is the number of valence electrons belonging to the atom corresponding to vertex i, $Z(i)$ is its atomic number, and $H(i)$ is the number of hydrogen atoms attached to it.

The zero-order connectivity index is the first member of the family of valence connectivity indices. The valence connectivity indices of higher orders ($^1\chi^v$, $^2\chi^v$, $^3\chi^v$, etc.) are taking into account paths, more precisely, neighbouring vertices (atoms) making up those paths. For example, $^3\chi^v$ is taking into account all paths of the length 3, that is, three consecutive chemical bonds in a vertex-weighted molecular graph.

$$^3\chi^v=\sum_{path}[\delta(i)\,\delta(j)\,\delta(k)\,\delta(l)]^{-0.5} \quad [3]$$

Regression calculations

Regression calculations, including the leave-one-out procedure (LOO) of cross validation, were done using the CROMRsel program (33). The standard error of the cross-validation estimate was defined as:

$$S.E._{cv} = \sqrt{\sum_i \frac{\Delta X_i^2}{N-1}} \quad [4]$$

where ΔX and N denote cv residuals and the number of reference points, respectively.

RESULTS

The first set consisted of 32 flavonoids (marked *a* in Table 1). It was further divided into the training (N=25) and test set (N=7) (16). Regressions on the full (N=32) and the training set (N=25) yielded similar statistics (Table 2, Figure 1), with the standard errors very close to those of the test set (SE_{test}=0.22).

The second set included 42 flavones (marked *b* in Table 1). Two compounds were excluded from the set: **34** because of the very different structure and **42** because it was the same as **23**. The new set (N=40) was also divided into the training (N=31) and test set (N=9) (17). Regressions on the full (N=40) and the training set (N=31) of flavones yielded similar statistics (Table 2, Figure 2), and the standard error of prediction of the test set (SE_{test}=0.24) was even lower.

The third set (marked *c* in Table 1) consisted of 78 flavonoids, including calcone (compounds **1**-**22**), flavone (compounds **23**-**64**), and aurone derivatives (compounds **65**-**78**) (18). We excluded the derivatives of dehydrosylibin and xanthone (18) and compounds **14**, **34**, and **67** because of their unrelated structure and flavone **42** because of the same structure as flavone **23**.

Regression on the new set of 74 flavonoid derivatives yielded R^2=0.790 and SE=0.37 (Table 2, Figure 3). The regressions on separate groups of compounds gave similar standard errors (N=21, R^2=0.861, SE=0.26 for calcones and N=40, R^2=0.900, SE=0.30 for flavones). The results for aurones however were much worse: N=13, R^2=0.073, SE=0.59, but they lie around the same regression line as calcones and flavones.

DISCUSSION

The first set was previously investigated by Li et al. (16) using pharmacophore modelling and comparative molecular field analysis (CoMFA). They built three models for the training set (N=25) with steric, electrostatic, and both steric and electrostatic descriptors (standard CoMFA).

The steric model yielded worse results (R^2=0.951, R^2_{cv}=0.764, SE=0.200) than the electrostatic model (R^2=0.987, R^2_{cv}=0.789, SE=0.105). The standard model was in between (R^2=0.980, R^2_{cv}=0.716, SE=0.131), but it also gave the best predictions for the test set (N=7). The SE_{test} was 0.35, 0.30, and 0.24, for the steric, electrostatic, and standard CoMFA model, respectively.

Our model yielded worse fit statistics (R^2=0.918, SE=0.24) but better cross-validated statistics (R^2_{cv}=0.905, SE_{cv}=0.26), and the best predictions for the test set of all CoMFA models (SE_{test}=0.22).

The second set (41 flavonoids without compound **35**) was previously investigated by Kothandan et al. (17) using ligand-based and receptor-guided alignment molecular docking and 3D-QSAR. On the training set (N=32) the best CoMFA and CoMSIA models for ligand-based alignment yielded R^2=0.951, R^2_{cv}=0.747, SE=0.21 and R^2=0.936, R^2_{cv}=0.810, SE=0.25, respectively. For receptor-guided alignment the models yielded R^2=0.976, R^2_{cv}=0.712, SE=0.16 and R^2=0.987, R^2_{cv}=0.805, SE=0.12, for CoMFA and CoMSIA respectively. But these models gave the SE of predictions of the constants from the test set (N=9) in the range from 0.42 to 0.54.

On a similar training set (N=31; we excluded compounds **34** and **42** but kept **35**) our model gave worse fit statistics (R^2=0.885, SE=0.32), but again better cross-validated statistics (R^2_{cv}=0.870, SE_{cv}=0.34) and far better test set (N=9; the same as in reference 17) predictions (SE_{test}=0.24).

The third set originally consisted of 89 flavonoids, but Boccard et al. (18) obtained an acceptable model for 83 compounds (after omitting compounds **13**, **14**, **68**, **71**, **72**, and **78** in reference 3). Using the 3D-QSAR and statistical tools [principal component analysis (PCA) and partial least-squares (PLS) regression], their model yielded R^2=0.76 and R^2_{cv}=0.71.

Our model was built on a smaller set (N=74) and yielded both better fit (R^2=0.790, SE=0.37) and cross-validated

Table 1 *pK_d of flavonoid complexes with P-glycoprotein, SMILES formula, and $^0\chi^v$ index of flavonoids*

No.*	SMILES formula	Set(s)	pK_d	$^0\chi^v$
1	O=C(/C=C/C1=CC=CC=C1)C2=C(O)C=C(O)C=C2O	c	5.34	2.555
2	O=C(/C=C/C1=CC=C(O)C=C1)C2=C(O)C=C(O)C=C2O	c	5.32	2.648
3	O=C(/C=C/C1=CC=C(OC)C=C1)C2=C(O)C=C(O)C=C2O	c	5.64	2.871
4	O=C(/C=C/C1=CC=C(F)C=C1)C2=C(O)C=C(O)C=C2O	c	5.44	2.625
5	O=C(/C=C/C1=CC=C(Cl)C=C1)C2=C(O)C=C(O)C=C2O	c	5.89	2.877
6	O=C(/C=C/C1=CC=C(Br)C=C1)C2=C(O)C=C(O)C=C2O	c	6.24	3.154
7	O=C(/C=C/C1=CC=C(I)C=C1)C2=C(O)C=C(O)C=C2O	c	6.60	3.344
8	O=C(/C=C/C1=CC=C(CC)C=C1)C2=C(O)C=C(O)C=C2O	c	5.68	3.143
9	O=C(/C=C/C1=CC=C(CCC)C=C1)C2=C(O)C=C(O)C=C2O	c	6.00	3.273
10	O=C(/C=C/C1=CC=C(CCCCCC)C=C1)C2=C(O)C=C(O)C=C2O	c	6.57	4.054
11	O=C(/C=C/C1=CC=C(C2CCCCC2)C=C1)C3=C(O)C=C(O)C=C3O	c	6.28	4.768
12	O=C(/C=C/C1=CC=C(CCCCCCCCC)C=C1)C2=C(O)C=C(O)C=C2O	c	7.70	4.554
13	O=C(/C=C/C1=CC=C(CCCCCCCCCCC)C=C1)C2=C(O)C=C(O)C=C2O	c	7.22	5.054
14	O=C(/C=C/C1=CC=C(CCCCCCCCCCCCCCC)C=C1)C2=C(O)C=C(O)C=C2O	c	4.85	6.054
15	O=C(/C=C/C1=CC=C(C/C=C(C)/C)C=C1)C2=C(O)C=C(O)C=C2O	c	6.28	3.481
16	O=C(/C=C/C1=CC(O)=C(O)C=C1)C2=C(O)C=C(O)C(C/C=C(C)\C)=C2O	c	6.36	3.749
17	O=C(/C=C/C1=CC=CC=C1)C2=C(O)C=CC=C2	c	5.05	2.396
18	O=C(/C=C/C1=CC=CC=C1)C2=C(O)C=CC(C(C)(C=C)C)=C2	c	6.36	3.743
19	O=C(/C=C/C1=CC=C(O)C=C1)C2=C(O)C=CC=C2	c	4.96	2.49
20	O=C(/C=C/C1=CC(C/C=C(C)/C)=CC=C1)C2=C(O)C=CC=C2	c	6.28	3.294
21	O=C(/C=C/C1=CC=C(OC)C=C1)C2=C(O)C=CC=C2	c	5.74	2.712
22	O=C(/C=C/C1=CC(C/C=C(C)/C)=C(OC)C=C1)C2=C(O)C=CC=C2	c	6.57	3.631
23	O=C1C2=CC=CC=C2OC(C3=CC=CC=C3)=C1O	a[t], b[t], c	5.00	2.81
24	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC=CC=C3)=C1O	a, b, c	5.23	2.971
25	O=C1C2=C(C=C(C(C(C)(C)C=C)=C2OC(C3=CC=CC=C3)=C1O)O)O	a[t], b, c	6.35	4.292
26	O=C1C2=C(O)C(C/C=C(C)/C)=C(O)C=C2OC(C3=CC=CC=C3)=C1O	a, b[t], c	6.68	3.966
27	O=C1C2=C(O)C=C(O)C(C/C=C(C)/C)=C2OC(C3=CC=CC=C3)=C1O	a, b, c	6.66	3.952
28	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC=C(O)C=C3)=C1O	a, b, c	5.17	3.065
29	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC=C(OC)C=C3)=C1O	a, b, c	5.35	3.288
30	O=C1C2=C(C=C(C(C(C)(C)C=C)=C2OC(C3=CC=C(C=C3)OC)=C1O)O)O	a, b, c	6.70	4.608
31	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC=C(F)C=C3)=C1O	a, b, c	5.17	3.042
32	O=C1C2=C(O)C=C(O)C=C2OC(C3=C(Cl)C=C(Cl)C=C3)=C1O	a, b, c	5.40	3.659
33	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC=C(I)C=C3)=C1O	a, b[t], c	5.96	3.761
34	O=C1C2=C(O)C=C(O)C=C2OC(C(C3=CC=CC=C3)C4=CC=CC=C4)=C1O	b, c	5.70	4.49
35	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC=C(CCCCCCCC)C=C3)=C1O	a[t], b, c	7.22	4.97
36	O=C1C2=C(C=C(C=C2OC(C3=CC=CC=C3)=C1OC)O)O	a, b, c	5.05	3.158
37	O=C1C2=C(C=C(C(C(C)(C)C=C)=C2OC(C3=CC=CC=C3)=C1OC)OC)O	a, b, c	6.82	4.683
38	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC(O)=C(O)C=C3)=C1O	a, b, c	5.15	3.173
39	O=C1C2=C(C=C(C=C2OC=C1C3=CC=C(C=C3)O)O)O	b, c	4.58	3.02
40	O=C1C2=CC=CC=C2OC(C3=CC=CC=C3)=C1	b[t], c	4.47	2.693
41	O=C1C2=CC=C(O)C=C2OC(C3=CC=CC=C3)=C1	a, b, c	4.46	2.771
42	O=C1C2=CC=CC=C2OC(C3=CC=CC=C3)=C1O	b, c	5.00	2.81
43	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC=CC=C3)=C1	a, b[t], c	5.05	2.853
44	O=C1C2=C(O)C(C)=C(O)C=C2OC(C3=CC=CC=C3)=C1	a[t], b, c	5.51	3.277
45	O=C1C2=C(O)C=C(OC)C=C2OC(C3=CC=CC=C3)=C1	a[t], b, c	5.20	3.077
46	O=C1C2=C(O)C(C)=C(OC)C=C2OC(C3=CC=CC=C3)=C1	a, b, c	5.89	3.477
47	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC=C(O)C=C3)=C1	a, b[t], c	5.00	2.946
48	O=C1C2=C(O)C=C(O)C=C2OC(C3=CC(F)=C(F)C=C3)=C1	a[t], b, c	5.2	2.999
49	O=C1C2=C(C=C(C=C2OC(C3=CC=C(C=C3)I)=C1)O)O	a, b, c	5.66	3.643
50	O=C1C2=C(O)C=C(OC(C)C)C=C2OC(C3=CC=CC=C3)=C1	a, b, c	6.00	3.213
51	O=C1C2=C(O)C(C(C)C)=C(O)C=C2OC(C3=CC=CC=C3)=C1	b, c	6.68	3.646
52	O=C1C2=C(O)C(C(C)C)=C(OC(C)C)C=C2OC(C3=CC=CC=C3)=C1	b, c	6.55	3.993
53	O=C1C2=C(C(C(C)C)=C(C(C(C)C)=C2OC(C3=CC=CC=C3)=C1)OC(C)C)O	b[t], c	7.48	4.74

No.*	SMILES formula	Set(s)	pK_d	$^0\chi^v$
54	O=C1C2=C(O)C(CC3=CC=CC=C3)=C(O)C=C2OC(C4=CC=CC=C4)=C1	a, b, c	6.47	4.357
55	O=C1C2=C(C=C(C(CC3=CC=CC=C3)=C2OC(C4=CC=CC=C4)=C1)O)O	a^t, b^t, c	6.00	4.343
56	O=C1C2=C(O)C(CC3=CC=CC=C3)=C(O)C(CC4=CC=CC=C4)=C2OC(C5=CC=CC=C5)=C1	b, c	7.44	5.822
57	O=C1C2=C(O)C=C(OCC3=CC=CC=C3)C=C2OC(C4=CC=CC=C4)=C1	b, c	7.17	4.075
58	O=C1C2=C(O)C(C/C=C(C)/C)=C(O)C=C2OC(C3=CC=CC=C3)=C1	a, b, c	6.52	3.847
59	O=C1C2=C(O)C=C(O)C(C(C=C)(C)C)=C2OC(C3=CC=CC=C3)=C1	a, b, c	6.7.	4.173
60	O=C1C2=C(O)C=C(O)C(C/C=C(C)/C)=C2OC(C3=CC=CC=C3)=C1	a, b, c	6.55	3.833
61	O=C1C2=C(O)C(C/C=C(C)\C)=C(O)C(C/C=C(C)/C)=C2OC(C3=CC=CC=C3)=C1	b, c	7.82	4.803
62	O=C1C2=C(O)C(C/C=C(C)/CC/C=C(C)/C)=C(O)C=C2OC(C3=CC=CC=C3)=C1	a, b, c	7.35	4.879
63	O=C1C2=C(O)C=C(O)C(C/C=C(C)/CC/C=C(C)/C)=C2OC(C3=CC=CC=C3)=C1	a, b, c	7.60	4.865
64	O=C1C2=C(O)C=C(O)C(C(C)(C)C=C)=C2OC(C3=CC=C(O)C=C3)=C1	a, b^t, c	6.15	4.267
65 (76)	O=C1C2=C(OC)C=C(OC)C=C2O/C1=C\C3=CC=C(C#N)C=C3	c	4.70	3.503
66 (77)	O=C1C2=C(OC)C=C(OC)C=C2O/C1=C\C3=CC=C(N(C)C)C=C3	c	5.59	3.873
67 (78)	O=C1C2=C(OC)C=C(OC)C=C2O/C1=C\C3=CC(OC)=C(OC)C=C3OC	c	4.04	4.188
68 (79)	O=C1C2=C(O)C=C(OC)C=C2O/C1=C\C3=CC=CC=C3	c	5.88	3.059
69 (80)	O=C1C2=C(O)C=C(OC)C=C2O/C1=C\C3=CC=C(F)C=C3	c	5.57	3.129
70 (81)	O=C1C2=C(O)C=C(OC)C=C2O/C1=C\C3=CC=C(Cl)C=C3	c	6.34	3.381
71 (82)	O=C1C2=C(O)C=C(OC)C=C2O/C1=C\C3=CC=C(Br)C=C3	c	6.82	3.658
72 (83)	O=C1C2=C(O)C=C(OC)C=C2O/C1=C\C3=CC=C(I)C=C3	c	6.59	3.848
73 (84)	O=C1C2=C(O)C=C(OC)C=C2O/C1=C\C3=CC=C(CN)C=C3	c	5.54	3.475
74 (85)	O=C1C2=C(OC)C=C(OC)C=C2O/C1=C\C3=CC=CC=C3	c	5.15	3.263
75 (86)	O=C1C2=C(OC)C=C(OC)C=C2O/C1=C\C3=CC=C(F)C=C3	c	5.54	3.334
76 (87)	O=C1C2=C(OC)C=C(OC)C=C2O/C1=C\C3=CC=C(Cl)C=C3	c	6.00	3.586
77 (88)	O=C1C2=C(OC)C=C(OC)C=C2O/C1=C\C3=CC=C(Br)C=C3	c	6.09	3.862
78 (89)	O=C1C2=C(OC)C=C(OC)C=C2O/C1=C\C3=CC=C(I)C=C3	c	6.27	4.053

**Numbers in parentheses correspond to notation in Ref 18.*
^{t}*Test set*

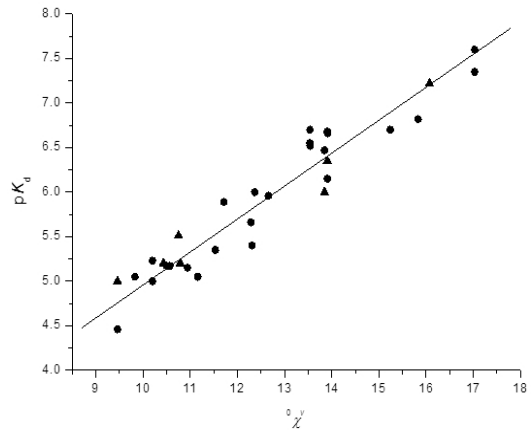

Figure 1 *Linear dependence of the* pK_d *of flavonoid complexes with P-glycoprotein (set a) on the* $^0\chi^v$ *index of flavonoids (*R^2*=0.918, SE=0.24,* SE_{cv}*=0.26,* SE_{test}*=0.22). Triangles denote predicted values of the* pK_d *of the test set compounds (*N*=7) from the calibration model made on the training set (circles,* N*=25)*

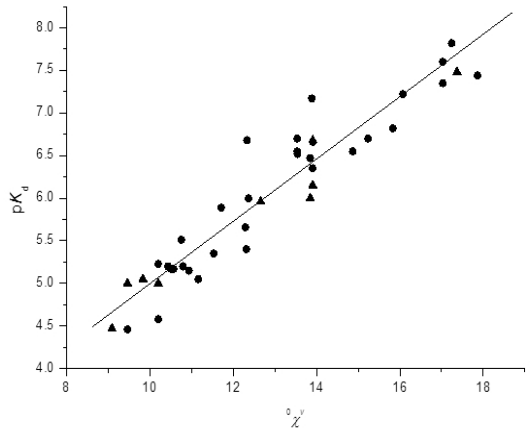

Figure 2 *Linear dependence of the* pK_d *of flavone complexes with P-glycoprotein (set b) on the* $^0\chi^v$ *index of flavones (*R^2*=0.885, SE=0.32,* SE_{cv}*=0.34,* SE_{test}*=0.24). Triangles denote predicted values of the* pK_d *of the test set compounds (*N*=9) from the calibration model made on the training set (circles,* N*=31)*

Table 2 *Regression models for the estimation of the pK_d of flavonoid complexes with P-glycoprotein*

Set	*N*	Regression coefficients		R^2	SE	SE_{cv}
		a_1(SE)	b(SE)			
a full	32	0.358(19)	1.42(24)	0.921	0.23	0.25
a training	25	0.370(23)	1.26(30)	0.918	0.24	0.26
b full	40	0.360(19)	1.40(26)	0.900	0.30	0.32
b training	31	0.366(25)	1.34(33)	0.885	0.32	0.34
c full	74	0. 331(20)	1.82(26)	0.790	0.37	0.38

statistics (R^2_{cv}=0.781, SE_{cv}=0.38). Other valence connectivity indices ($^1\chi^v$, $^2\chi^v$, $^3\chi^v$, $^4\chi^v$, and $^5\chi^v$) yielded R^2 in the range 0.542-0.787, and SE 0.37-0.55.

CONCLUSION

Our model for the prediction of dissociation constants of the flavonoid-P-glycoprotein system (K_d) gives results comparable to much more demanding 3D QSAR - CoMFA and CoMSIA models. It may have fared worse in fitting the data but gave better internal (cross-validation) and external predictions (for test sets). In this most important aspect in modelling, our model surpasses CoMFA and CoMSIA.

Our results also show that the model is stable ($SE \approx SE_{cv} \approx SE_{test}$ for the same set), yielding consistent results, regardless of the grouping of flavonoid derivatives (for not too big structural diversity).

The comparison of our model (Set 1, Table 2; *N*=32) with the CoMFA models gave standard errors of 0.99, 0.96, and 0.99 log K_d units for the electrostatic, steric, and standard model, respectively. These standard errors are much bigger than those obtained by comparison between the CoMFA models: 0.27, 0.15, and 0.21 for the comparison between steric and electrostatic, steric and standard, and electrostatic and standard models, respectively. Therefore, our model gives quite different predictions than the CoMFA models despite similar general agreement with the experiment (SE=0.24 and 0.13, for $^0\chi^v$ and the standard CoMFA model, respectively). A much bigger difference in standard error between $^0\chi^v$ and each of the CoMFA models, as well as the difference in standard errors between the CoMFA models suggest that the $^0\chi^v$ model is not an approximation of the CoMFA models but is the model in its own right.

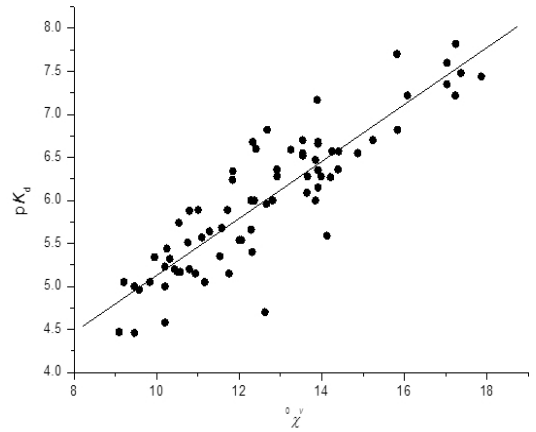

Figure 3 *Linear dependence of the pK_d of flavonoid complexes with P-glycoprotein (set c) on the $^0\chi^v$ index of flavonoids (*R^2*=0.790, SE=0.37, SE_{cv}=0.38)*

Our model, as well as models based on topological indices in general, is essentially holistic, which means that it fits all the relevant interactions in a molecule equally well.

Acknowledgement

This work was supported by the Croatian Ministry of Science, Technology, Education and Sport.

REFERENCES

1. Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol 1995;33:1061-80. doi: 10.1016/0278-6915(95)00077-1
2. Scott BC, Butler J, Halliwell B, Aruoma OI. Evaluation of the antioxidant actions of ferulic acid and catechins. Free Radic Res Commun 1993;19:241-53. PMID: 7507456
3. Teixeira S, Siquet C, Alves C, Boal I, Marques MP, Borges F, Lima JLFC, Reis S, Structure-property studies on the antioxidant activity of flavonoids present in diet. Free Radic Biol Medic 2005;39:1099-108. doi: 10.1016/j.freeradbiomed.2005.05.028
4. Cohen SD, Kennedy JA. Plant metabolism and the environment: Implications for managing phenolics. Crit Rev Food Sci Nutr 2010;50:620-43. doi: 10.1080/10408390802603441
5. Vinson JA. Flavonoids in foods as *in vitro* and *in vivo* antioxidants. Adv Exp Med Biol 1998;439:151-64. doi: 10.1007/978-1-4615-5335-9_11
6. Johnson J, de Mejia EG. Dietary factors and pancreatic cancer: The role of food bioactive compounds. Mol Nutr Food Res 2011;55:58-73. doi: 10.1002/mnfr.201000420
7. Perron NR, Brumaghin JL. A review of the antioxidant mechanisms of polyphenol Compounds related to iron binding. Cell Biochem Biophys 2009;53:75-100. doi: 10.1007/s12013-009-9043-x
8. Xiao J, Chen T, Cao H, Chen L, Yang F. Molecular property-affinity relationship of flavonoids and flavonoids for HSA *in vitro*. Mol Nutr Food Res 2011;55:310-7. doi: 10.1002/mnfr.201000208
9. Shi J, Cao H. Molecular structure-affinity relationship of dietary flavonoids for bovine serum albumin. Rev Bras

Farmacogn 2011;21:594-600. doi: 10.1590/S0102-695X2011005000118
10. Xiao J, Cao H, Chen T, Yang F, Liu C, Xu X. Molecular property-binding affinity relationship of flavonoids for common rat plasma proteins *in vitro*. Biochemie 2011;93:134-40. doi: 10.1016/j.biochi.2010.08.013
11. Xiao J, Mao F, Yang F, Zhao Y, Zhang C, Yamamoto K. Interaction of dietary polyphenols with bovine milk proteins: Molecular structure-affinity relationship and influencing bioactivity aspects. Mol Nutr Food Res 2011;55:1637-45. doi: 10.1002/mnfr.201100280
12. Leveille-Webster CR, Arias IM. The biology of the P-glycoproteins. J Membr Biol 1995;143:89-102. doi: 10.1007/BF00234655
13. Simon SM, Schindler M. Cell biological mechanisms of multidrug resistance in tumors. Proc Natl Acad Sci USA 1994;91:3497-504. PMID: 7909602
14. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 1993;62:385-427. doi: 10.1146/annurev.bi.62.070193.002125
15. Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem 1990;38:1277-87. PMID: 1974900
16. Li Y, Wang Y, Yang L, Zhang S, Liu C. Structural determinants of flavones interacting with the C-terminal nucleotide-binding domain as P-glycoprotein inhibitors. Internet Electron J Mol Des 2006;5:1-12.
17. Kothandan G, Gadhe CG, Madhaven T, Choi CH, Cho SJ. Docking and 3D-QSAR (quantitative structure activity relationship) studies of flavones, the potent inhibitors of p-glycoprotein targeting the nucleotide binding domain. Eur J Med Chem 2011;46:4078-88. doi: 10.1016/j.ejmech.2011.06.008
18. Boccard J, Bajot F, Di Pietro A, Rudaz S, Boumendjel A, Nicolle E, Carrupt P-A. A 3D linear solvation energy model to quantify the affinity of flavonoid derivatives toward P-glycoprotein. Eur J Pharm Sci 2009;36:254-64. doi: 10.1016/j.ejps.2008.09.009
19. Raos N, Miličević A. Estimation of stability constants of coordination compounds using models based on topological indices. Arh Hig Rada Toksikol 2009;60:123-8. doi: 10.2478/10004-1254-60-2009-1923
20. Miličević A, Raos N. Prediction of stability constants for copper(II) binding to tetrapeptides containing histidyl residue with graph-theoretical method. Int J Chem Model 2014;6:301-9.
21. Miličević A, Raos N. Graph-theoretical modelling of stability constants of copper(II) complexes with tripeptides containing glycine, glutamic acid, and histidine. Bull Chem Soc Jpn 2015;88:490-5. doi: 10.1246/bcsj.20140358
22. Miličević A, Raos N. Modelling of copper(II) binding to pentapeptides related to atrial natriuretic factor using the $^{3}\chi^{v}$ connectivity index. Arh Hig Rada Toksikol 2015;66:165-70. doi: 10.1515/aiht-2015-66-2631
23. Kier LB, Hall LH. Molecular Connectivity in Chemistry and Drug Research. New York: Academic Press; 1976.
24. Hall LH, Kier LB. The relation of molecular connectivity to molecular volume and biological activity. Eur J Med Chem 1981;16:399-407.
25. Miličević A, Nikolić S, Trinajstić N. Toxicity of aliphatic ethers: A comparative study. Mol Diversity 2006;10:95-9. doi: 10.1007/s11030-005-9006-0
26. Medić-Šarić M, Maleš Ž, Šarić S, Brantner A. Quantitative modeling of flavonoid glycosides isolated from *Paliurus spina-christi* Mill. Croat Chem Acta 1996;69:1603-16.
27. Tetko IV, Gasteiger J, Todeschini R, Mauri A, Livingstone D, Ertl P, Palyulin VA, Radchenko EV, Zefirov NS, Makarenko AS, Tanchuk VY, Prokopenko VV. Virtual computational chemistry laboratory-design and description. J Comput Aided Mol Des 2005;19:453-63. doi: 10.1007/s10822-005-8694-y
28. Virtual Computational Chemistry Laboratory [display 14 March 2016]. Available at http://www.vcclab.org
29. Enhanced NCI Database Browser 2.2 [displayed 14 March 2016]. Available at http://cactus.nci.nih.gov/ncidb2.2/
30. Kier LB, Hall LH. Molecular connectivity VII: Specific treatment to heteroatoms. J Pharm Sci 1976;65:1806-9. doi: 10.1002/jps.2600651228
31. Kier LB, Hall LH. Molecular Connectivity in Structure-Activity Analysis. New York: Willey; 1986.
32. Randić M. On history of the Randic index and emerging hostility toward chemical graph theory. MATCH Commun Math Comput Chem 2008;59:5-124.
33. Lučić B, Trinajstić N. Multivariate regression outperforms several robust architectures of neural networks in QSAR modeling. J Chem Inf Comput Sci 1999;39:121-32. doi: 10.1021/ci980090f

Jednostavan graf-teorijski model vezivanja flavonoida za P-glikoprotein

Upotrebom indeksâ povezanosti modelirani su logaritmi konstanti disocijacije ($\log K_d$) triju skupova flavonoidnih derivata (N=32, 40 i 74); K_d opisuje afinitet flavonoida prema P-glikoproteinu. Najbolji su rezultati postignuti na svim trima skupovima upotrebom valencijskoga molekularnog indeksa povezanosti nultoga reda ($^{0}\chi^{v}$). Standardne su pogreške modela za kalibraciju oko 0,3, a one za konstante iz seta za provjeru malo su niže - 0,22 i 0,24. Unatoč upotrebi samo jednoga deskriptora, naš se model pokazao boljim u pogledu interne provjere (unakrsna validacija), a posebice u pogledu eksterne provjere (prema skupu za provjeru) od puno zahtjevnijih metoda (3D QSAR) korištenih za modeliranje toga sistema.

KLJUČNE RIJEČI: *indeksi povezanosti; konstanta disocijacije; molekularno modeliranje*