Quantitative Structure−Activity Relationship of Flavonoid Analogues. 3. Inhibition of p56^{lck} Protein Tyrosine Kinase†

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Quantitative structure−activity relationship (QSAR) studies on 104 flavonoid derivatives as p56^{lck} protein tyrosine kinase (PTK) inhibitors were performed, using a large number of molecular descriptors calculated by CODESSA software. Multiple linear regression and orthogonalization of descriptors were applied to generate models for the prediction of biological activities for binding flavonoids to PTK. The obtained results demonstrate in detail the importance of electrostatic and quantum chemical descriptors for the interaction of flavonoids with the specific p56^{lck} enzymatic active site environment. In particular, the maximal total interaction for a C−O bond is the most important factor in regression. Use of orthogonalization in regression models provides a valuable improvement for the interpretative and predictive capacity of structure−activity relationships found.

1. INTRODUCTION

Protein kinases constitute a large and diverse family of enzymes that provide a central switching mechanism in cellular signal transduction pathways by catalyzing the transfer of the γ-phosphate of adenosine triphosphate (ATP) to a protein substrate. All protein kinases thus far characterized with regard to substrate specificity fall within one of two broad classes, serine/threonine-specific and tyrosine-specific. Although these enzymes differ in size, the substrate specificity, mechanism of activation, subunit composition, and subcellular localization, all, nevertheless, share a homologous catalytic core that has been conserved.[1]

Protein tyrosine kinases (PTKs) are important mediators of normal cellular signal transduction,[2] with PTKs being the intracellular effectors for many growth hormone receptors.[3] The discovery of activated protein−tyrosine kinases as the product of dominant viral-transforming genes (oncogene) first established the connection between protein−tyrosine phosphorylation and the cell transformation.[4] Abnormal activity of tyrosine kinases has been implicated in many cancers as well as atherosclerosis, psoriasis, and a large number of inflammatory responses.[5] The development of specific PTK inhibitors as pharmacological tools and potential antiproliferative agents has therefore become an active area of research.[6−8]

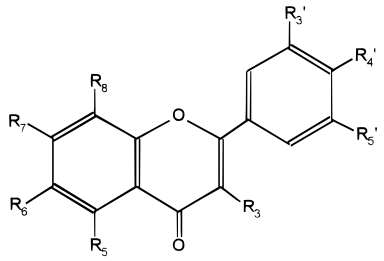

Figure 1. Molecular structure and numbering of substituents attached to the chromone moiety and phenyl ring (see also Table 1).

The flavonoids are a group of low molecular weight plant products, based on the parent compound, flavone (2-phenylchromone or 2-phenylbenzopyrone). A large number of natural and synthetic flavonoids have been tested for their PTK inhibitory activities. Kinetic analyses of the PTK inhibition indicated that flavonoids were competitive inhibitors with respect to the nucleotide ATP.[8−9] Various synthetic flavonoid analogues have been prepared[10−13] with the goal of the development of PTK inhibitors as chemotherapeutic agents.

A number of qualitative structure−activity studies, involving flavonoids as PTKs inhibitors, have been published,[11−15] but there is a lack of well-defined quantitative structure−activity relationships for this system. In our previous papers[16,17] we have used multiregression analysis and counterpropagation artificial neural network (CP-ANN) approaches to study the correlation of inhibitory activity of a large data set of 105 flavonoid as tyrosine kinase LCK p56 inhibitors,[11−13] using a limited set of classical and computed quantum chemical parameters. Experimental enzyme inhibitory activity for this series of natural and synthetic flavonoids at p56^{lck} lymphoid cell lineage-specific PTK of the Src family, which is overexpressed in several lymphomas, has been found by using both approaches to correlate with the same structural features: lypophilicity and the molecular size

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† Abbreviations: PTK, protein tyrosine kinase; ATP, adenosine triphosphate; MLR, multiple linear regression; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; WNSA, weighted negative surface area; HACA, hydrogen acceptor charged surface area; HASA, hydrogen acceptor surface area; TMSA, total molecular surface area.

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Table 1. Summary of Experimental and Calculated Biological Activities (Δ_i Is the Difference between Experimental and Calculated Biological Activity) of Flavone Derivatives along with Their Structures Used in QSAR Study

no.	substituents	log (1/IC_{50})[a]	eq 1	Δ_1	eq 2	Δ_2	eq 3	Δ_3	eq 4	Δ_4	series[b]
					Series 1						
1	5,7-OH,4′-NH_2	5.13	4.55	0.58	4.18	0.95	4.48	0.65	4.52	0.61	1
2	3,5,7,3′,4′-OH	4.88	4.91	−0.03	4.32	0.56	5.32	−0.44	5.25	−0.37	1
3	3,7,3′,4′-OH	4.86	4.25	0.61	3.99	0.87	4.67	0.19	4.38	0.48	1
4	5,7,4′-OH	4.83	4.57	0.26	4.30	0.53	4.75	0.08	4.76	0.07	1
5	5,4′-OH	4.80	4.42	0.38	4.26	0.54	4.59	0.21	4.54	0.26	1
6	6,3′-OH	4.80	3.69	1.11	4.02	0.78	4.46	0.34	4.35	0.45	1
7	6-OH,5,7,4′-NH_2	4.74	4.09	0.65	3.60	1.14	4.45	0.29	4.24	0.50	1
8	5,7-OH	4.71	4.55	0.16	4.26	0.45	4.47	0.24	4.39	0.32	1
9	4′-OH,3′,5′-OCH_3	4.57	3.38	1.19	3.70	0.87	3.73	0.84	3.57	1.00	1
10	5,7,3′,4′-OH	4.46	4.53	−0.07	4.40	0.06	4.67	−0.21	4.76	−0.30	1
11	7,3′-OH	4.41	3.85	0.56	4.12	0.29	4.09	0.32	3.88	0.53	1
12	6-OH,5,7,3′-NH_2	4.34	4.10	0.24	3.73	0.61	4.46	−0.12	4.26	0.08	1
13	6-OMe,8,3′-NH_2	4.25	3.46	0.79	3.75	0.50	3.76	0.49	3.66	0.59	1
14	6-OH,3′,4′,5′-OCH_3	4.22	3.26	0.96	3.30	0.92	3.76	0.46	3.56	0.66	1
15	3,5,7,4′-OH,3′,5′-OCH_3	4.16	4.53	−0.37	3.87	0.29	4.22	−0.06	4.18	−0.02	1
16	3,5,7,3′,5′-OH	4.00	4.08	−0.08	4.10	−0.10	4.13	−0.13	4.11	−0.11	1
17	6,4′-NH_2	3.99	3.70	0.29	3.60	0.39	4.13	−0.14	3.97	0.02	1
18	6,8,4′-NH_2	3.97	3.65	0.32	3.48	0.49	3.99	−0.02	3.80	0.17	1
19	6-OH,8,4′-NH_2	3.93	3.60	0.33	3.83	0.10	3.84	0.09	3.72	0.21	1
20	6,4′-OH	3.93	3.63	0.30	3.90	0.03	3.92	0.01	3.80	0.13	1
21	7,8,4′-OH,3′,5′-OCH_3	3.92	3.35	0.57	3.68	0.24	3.79	0.13	3.67	0.25	1
22	6-OH,4′-OR	3.92	3.32	0.60	3.59	0.33	3.59	0.33	3.85	0.07	1
23	8,4′-NH_2	3.91	3.67	0.24	3.57	0.34	3.86	0.05	3.63	0.28	1
24	6,4′-OH,3′,5′-OCH_3	3.89	3.42	0.47	3.63	0.26	3.89	0.00	3.78	0.11	1
25	7-OH,4′-NH_2	3.86	3.78	0.08	3.94	−0.08	3.95	−0.09	3.90	−0.04	1
26	7-OH,6,4′-NH_2	3.85	3.68	0.17	3.83	0.02	3.56	0.29	3.45	0.40	1
27	7,4′-OH	3.78	3.74	0.04	3.92	−0.14	3.98	−0.20	3.89	−0.11	1
28	7,8,3′OH	3.75	3.58	0.17	4.15	−0.40	3.79	−0.04	3.64	0.11	1
29	6,3′-NH_2	3.70	3.69	0.01	3.69	0.01	4.04	−0.34	3.90	−0.20	1
30	4′-NH_2	3.68	3.71	−0.03	3.79	−0.11	3.90	−0.22	3.74	−0.06	1
31	5-OH,6,4′-NH_2	3.65	4.39	−0.74	3.73	−0.08	4.35	−0.70	4.10	−0.45	1
32	3,5,7-OH	3.53	3.73	−0.20	4.19	−0.66	3.58	−0.05	3.44	0.09	1
33	5,4′-OH,7-OCH_3	3.55	3.29	0.26	4.22	−0.67	3.45	0.10	3.45	0.10	1
34	5,3′-OH	3.50	3.25	0.25	4.09	−0.59	3.68	−0.18	3.53	−0.03	1
35	7,8-OH	3.50	3.63	−0.13	3.99	−0.49	3.84	−0.34	3.67	−0.17	1
36	5-OH,8,4′-NH_2	3.49	3.28	0.21	3.73	−0.24	3.41	0.08	3.25	0.24	1
37	7-OH,8,4′-NH_2	3.48	3.70	−0.22	3.76	−0.28	3.92	−0.44	3.90	−0.42	1
38	7-OH	3.47	3.70	−0.23	4.00	−0.53	3.92	−0.45	3.77	−0.30	1
39	6-OH,3′,5′-OCH_3,4′-OR[c]	3.43	3.02	0.41	3.45	−0.02	3.55	−0.12	3.54	−0.11	1
40	6-OCH_3,8,4′-NH_2	3.42	3.50	−0.08	3.62	−0.20	3.82	−0.40	3.71	−0.29	1
41	7,8-OH,3′,4′,5′-OCH_3	3.40	3.26	0.14	3.43	−0.03	3.75	−0.35	3.55	−0.15	1
42	3-$COOCH_3$,4′-OH	3.36	3.03	0.33	3.45	−0.09	3.08	0.28	3.20	0.16	1
43	4′-OH	3.30	3.65	−0.35	3.92	−0.62	3.83	−0.53	3.65	−0.35	1
44	7-OH,6,3′-NH_2	3.30	3.67	−0.37	3.89	−0.59	3.66	−0.36	3.54	−0.24	1
45	7-OH,6,8,4′-NH_2	3.12	3.54	−0.42	3.60	−0.48	3.39	−0.27	3.22	−0.10	1
46	3-$COOCH_3$,4′-NH_2	3.09	3.01	0.08	3.31	−0.22	3.00	0.09	3.14	−0.05	1
47	7-OH,4′-OR[c]	3.01	3.40	−0.39	3.45	−0.44	3.10	−0.09	3.04	−0.03	1
48	3-COOH,7-OCH_3,4′-OH	2.99	2.98	0.01	3.58	−0.59	3.11	−0.12	3.32	−0.33	1
49	7,4′-OH,3′,5′-OCH_3	2.90	3.52	−0.62	3.77	−0.87	3.94	−1.04	3.88	−0.98	1
50	7-OH,3′,5′-OCH_3,4′-OR[c]	2.82	3.16	−0.34	3.42	−0.60	3.10	−0.28	2.81	0.01	1
51	7-OH,6,8,4′-NO_2	2.81	2.44	0.37	2.33	0.48	2.28	0.53	2.41	0.40	1
52	3-COOH,4′-OH	2.80	3.01	−0.21	3.61	−0.81	3.04	−0.24	3.11	−0.31	1
53	5-OCH_3,8,4′-NH_2	2.79	3.23	−0.44	3.54	−0.75	3.09	−0.30	2.91	−0.12	1
54	7-OH,8,4′-NO_2	2.73	2.95	−0.22	2.74	−0.01	3.03	−0.30	2.95	−0.22	1
55	3-$COOCH_3$, 3′,4′,5′-OCH_3	2.70	2.54	0.16	2.68	0.02	2.82	−0.12	2.68	0.02	1
56	3-$COOCH_3$, 3′,5′-OCH_3	2.70	2.64	0.06	2.99	−0.29	2.81	−0.11	2.75	−0.05	1
57	3-$COOCH_3$, 3′,4′-OCH_3	2.70	2.73	−0.03	2.94	−0.24	2.88	−0.18	2.83	−0.13	1
58	3-$COOCH_3$,4′-OBn[d]	2.70	2.65	0.05	2.47	0.23	2.81	−0.11	2.62	0.08	1
59	3-$COOCH_3$,7-OCH_3,4′-OBn[d]	2.70	2.49	0.21	2.45	0.25	2.78	−0.08	2.56	0.14	1
60	3-$COOCH_3$,6-OCH_3,4′-OBn[d]	2.70	2.47	0.23	2.42	0.28	2.79	−0.09	2.62	0.08	1
61	3-COOH,4′-Br	2.70	2.81	−0.11	2.98	−0.28	2.82	−0.12	3.02	−0.32	1
62	3-COOH,4′-NO_2	2.70	2.57	0.13	3.01	−0.31	2.64	0.06	2.52	0.18	1
63	3-COOH,7-OCH_3,4′-NO_2	2.70	2.64	0.06	2.99	−0.29	2.82	−0.12	2.70	0.00	1
64	3-COOH,6-OCH_3,4′-NO_2	2.70	2.62	0.08	3.01	−0.31	2.91	−0.21	2.77	−0.07	1
65	3-COOH,5,7-OH,4′-NO_2	2.70	3.44	−0.74	3.40	−0.70	3.01	−0.31	2.93	−0.23	1
66	4′-NO_2	2.70	3.38	−0.68	3.15	−0.45	3.58	−0.88	3.08	−0.38	1
67	6,4′-NO_2	2.70	2.80	−0.10	2.55	0.15	2.66	0.04	2.43	0.27	1
68	8,4′-NO_2	2.70	2.84	−0.14	2.53	0.17	2.85	−0.15	2.57	0.13	1
69	6-OCH_3,8,4′-NO_2	2.70	2.65	0.05	2.62	0.08	2.89	−0.19	2.73	−0.03	1
70	5-OCH_3,8,4′-NO_2	2.70	2.40	0.30	2.53	0.17	2.44	0.26	2.27	0.43	1

Table 1 (Continued)

no.	substituents	log (1/IC$_{50}$)[a]	eq 1	Δ_1	eq 2	Δ_2	eq 3	Δ_3	eq 4	Δ_4	series[b]
					Series 1						
71	7-OH,4′-OBn[d]	2.70	3.57	−0.87	3.17	−0.47	3.98	−1.28	3.63	−0.93	2
72	7,8,3′,4′,5′-OCH$_3$	2.70	3.04	−0.34	2.52	0.18	3.67	−0.97	3.08	−0.38	2
73	7,8-OH,3′,5′-OCH$_3$,4′-OR[c]	2.70	3.06	−0.36	3.38	−0.68	3.07	−0.37	2.80	−0.10	2
74	7,8-OAc,3′,5′-OCH,4′-OR[c]	2.70	1.96	0.74	2.63	0.07	2.17	0.53	1.67	1.03	2
75	6,3′,4′,5′-OCH$_3$	2.70	3.26	−0.56	2.86	−0.16	3.88	−1.18	3.43	−0.73	2
76	7-OH,3′,4′,5′-OCH$_3$	2.70	3.33	−0.63	3.43	−0.73	3.78	−1.08	3.60	−0.90	2
77	7-OAc,3′,5′-OCH$_3$,4′-OH	2.70	3.18	−0.48	3.53	−0.83	3.63	−0.93	3.61	−0.91	2
78	7-OAc,3′,5′-OCH$_3$,4′-OR[c]	2.70	3.01	−0.31	3.22	−0.52	3.46	−0.76	3.27	−0.57	2
79	7,3′,4′,5′-OCH$_3$	2.70	3.18	−0.48	2.97	−0.27	3.63	−0.93	3.24	−0.54	2
80	3′,4′,5′-OCH$_3$	2.70	3.34	−0.64	3.26	−0.56	3.76	−1.06	3.42	−0.72	2
81	5-OH,4′-OBn[d]	2.70	4.18	−1.48	3.18	−0.48	4.36	−1.66	4.15	−1.45	2
82	3-COOCH$_3$,4′-OCH$_3$	2.70	2.83	−0.13	3.12	−0.42	2.88	−0.18	2.92	−0.22	2
83	3-COOCH$_3$,4′-Br	2.70	2.77	−0.07	2.79	−0.09	2.83	−0.13	3.00	−0.30	2
84	3-COOCH$_3$,4′-NO$_2$	2.70	2.61	0.09	2.81	−0.11	2.76	−0.06	2.46	0.24	2
85	3-COOCH$_3$,7-OCH$_3$,4′-NO$_2$	2.70	2.53	0.17	2.68	0.02	2.86	−0.16	2.61	0.09	2
86	3-COOCH$_3$,6-OCH$_3$,4′-NO$_2$	2.70	2.41	0.29	2.70	0.00	2.71	−0.01	2.63	0.07	2
87	3-COOCH$_3$,5,7-OBn[d],4′-NO$_2$	2.70	1.40	1.30	1.44	1.26	1.79	0.91	1.09	1.61	2
88	3-COOH, 3′,4′,5′-OCH$_3$	2.70	2.58	0.12	3.04	−0.34	2.83	−0.13	2.83	−0.13	2
89	3-COOH, 3′,5′-OCH$_3$	2.70	2.73	−0.03	3.34	−0.64	2.87	−0.17	3.00	−0.30	2
90	3-COOH, 3′,4′-OCH$_3$	2.70	2.74	−0.04	3.29	−0.59	2.91	−0.21	3.04	−0.34	2
91	3-COOH,4′-OCH$_3$	2.70	2.97	−0.27	3.45	−0.75	3.05	−0.35	3.21	−0.51	2
92	3-COOCH$_3$,7-OCH$_3$,4′-OH	2.70	2.85	−0.15	3.30	−0.60	2.99	−0.29	3.08	−0.38	2
93	3-COOCH$_3$,6-OCH$_3$,4′-OH	2.70	2.78	−0.08	3.29	−0.59	2.94	−0.24	3.08	−0.38	2
94	3-COOCH$_3$,7-OCH$_3$,4′-NHAc	2.70	2.69	0.01	2.96	−0.26	2.81	−0.11	2.97	−0.27	2
95	3-COOCH$_3$,6-OCH$_3$,4′-NHAc	2.70	2.60	0.10	2.98	−0.28	2.80	−0.10	2.93	−0.23	2
96	7-OH,4′-NO$_2$	2.70	3.35	−0.65	3.33	−0.63	3.63	−0.93	3.29	−0.59	2
97	6-OH,4′-NO$_2$	2.70	3.41	−0.71	3.35	−0.65	3.74	−1.04	3.72	−1.02	2
98	5,7-OH,4′-NO$_2$	2.70	4.21	−1.51	3.58	−0.88	4.22	−1.52	3.97	−1.27	2
99	7-OH,6,4′-NO$_2$	2.70	2.91	−0.21	2.84	−0.14	2.77	−0.07	2.76	−0.06	2
100	5-OH,8,4′-NO$_2$	2.70	2.57	0.13	2.74	−0.04	2.60	0.10	2.51	0.19	2
101	6,8,4′-NO$_2$	2.70	2.48	0.22	2.15	0.55	2.33	0.37	2.11	0.59	2
102	6-OH,8,4′-NO$_2$	2.70	2.81	−0.11	2.81	−0.11	2.94	−0.24	2.78	−0.08	2
103	5-OH,6,4′-NO$_2$	2.70	2.47	0.23	2.74	−0.04	2.43	0.27	2.33	0.37	2
104	6-OH,5,7,4′-NO$_2$	2.70	2.32	0.38	2.30	0.40	2.77	−0.07	2.76	−0.06	2

[a] Experimental biological activity IC$_{50}$ is the molar concentration of the flavonoid necessary to give half-maximal inhibition as compared to the control assay carried out in the absence of inhibitor, but in the presence of DMSO carrier. [b] Series 1 contains 70 compounds (54 active and 16 inactive), and series 2 contains 34 compounds (all inactive). [c] R denotes *tert*-butyldimethylsilyl. [d] Bn denotes benzyl.

of the entire molecule (π, MR, and total surface density area) and local electronic properties (σ and $\sigma_{3',4'}$). These results showed that the chromone moiety of the flavonoid structure appears to be a mixed region for hydrophobic (π) and electronic interactions ($\sum\sigma_{3-8}$), while the phenyl ring moiety, especially the substituents at the 3′ and 4′ position, are involved in electronic interactions with the enzyme.

Flavones were studied also as inhibitors of cyclic AMP (cAMP) phosphodiesterase[18] by using QSAR approach. The models were generated using the topological (zero-order valence connectivity index $^0\chi^v$, number of the paths of length $10p_{10}$, and topological resonance energy per electron TRE-(PE)) and electronic descriptors. QSAR studies on a limited set of 19 polyhydroxylated and polymethoxylated flavones as xanthine oxidase inhibitors[19] have also been performed. These results indicate that flavones act as donors in the charge-transfer mechanism of their interaction with the enzyme and that the anion at the C-7 hydroxyl is the most active form in solution. To further explore the effect of substituents in the 2-phenyl moiety on the xanthine oxidase inhibitory activity, these authors synthesized and tested a new series of 7-hydroxyflavones carrying a wide and balanced variety of substituents (π, σ_p) at the 4′ position. The correlation found with quantum chemical polarizabilities and solvent accessible surface area found in the latter work was interpreted as the existence of $\pi-\pi$ stacking interactions with an aromatic amino acidic residue of the enzyme.

Clearly, a variety of molecular descriptors have been shown to correlate with bioactivity of flavonoids in different enzymatic targets. In a comprehensive study of the PTK system we have used a very large descriptor set (more than 500 topological, geometrical, constitutional, electrostatic, and quantum-chemical descriptors) in order to be able to compare the predictive ability of descriptors from the same and from different descriptor groups. Further, we have shown that orthogonalization provides a means for improving the clarity of the interpretation of interactions of the inhibitor−enzyme target.

2. MATERIALS AND METHODS

The biological assay data used in our study are results of in vitro tests for inhibitory activity against protein−tyrosine kinase p56^{lck}, a lymphoid cell lineage-specific PTK of the Src family which is overexpressed in several lymphomas.[20,21] Molecular structure and numbering of the substituents in the series of natural and synthetic flavonoid derivatives are represented in Figure 1, and structural data of the series along with their inhibitory data are summarized in Table 1.

For a group of 104 flavonoids values for log(1/IC$_{50}$) were determined in range from 2.7 to 5.13. Experimental biological activity IC$_{50}$ is defined as the molar concentration of the flavonoid necessary to give half-maximal inhibition as compared to the control assay carried out in the absence of

Table 2. Specification (t, Topological; e, Electrostatic; g, Geometric, q, Quantum-Chemical; c, Constitutional) of Descriptors Used and Corresponding Correlation Coefficients for One-Parameter Correlations

descriptor	definition	r^2
$E_{tot.}$(CO)	[q]max total interaction for a C−O bond	0.6379
$HASA2_R$	[e]HASA-2/TMSA ratio	0.4889
${}^nR^C_{av}$	[q]av nucleophilic reactn index for a C-atom	0.2778
G^2	[c]gravitation index (all bonds)	0.2771
${}^1\chi$	[t]Randic index (order 1)	0.2739
${}^2\kappa$	[t]Kier shape index (order 2)	0.2594
q^C_{max}	[e]max partial charge for a C-atom	0.2589
μ_{hybr}	[q]total hybridization composition of the molecular dipole	0.2051
AC_{MO}	[q]max antibonding contribution of MO	0.2049
GAP	[q]HOMO−LUMO energy gap	0.2020
I_C	[g]moment of inertia C	0.1977
${}^0BIC_a$	[t]av bonding information content (order 0)	0.1633
${}^2CIC_a$	[t]av complementary information content (order 2)	0.1503
${}^nR^C_{min}$	[q]min nucleophilic reacn index for a C-atom	0.1159
WNSA3	[e]WNSA-3 Weighted PNSA (PNSA3*TMSA/1000)	0.1155
${}^1CIC$	[t]Complementary information content (order 1)	0.0860
E_c(CC)	[q]min Coulombic interaction for a C−C bond	0.0827
E_{ee}(CC)	[q]max e−e repulsion for a C−C bond	0.0552
RN_H	[c]rel no. of H-atoms	0.0327
RN_O	[c]rel no. of O-atoms	0.0284
RPCG	[q]rel partial charge	0.0277

inhibitor, but in the presence of dimethyl sulfoxide (DMSO) carrier. Inactive flavonoids were automatically assigned the value $\log(1/IC_{50}) = 2.7$ ($IC_{50} = 2000\ \mu M$).

The orientation of the phenyl ring with respect to the chromone moiety of the flavonoid structure requested prior determination of the minimum energy conformation for rotations about the C(2)−C(1′) bond. We have used the semiempirical MNDO method available in the Spartan[22] program. Optimized atomic coordinates served us as an input data for the CODESSA program, where calculation and selection of descriptors for quantitative structure−activity relationships were carried out.

CODESSA[23] (COmprehensive DEscriptors for Structural and Statistical Analysis) is a multipurpose program for developing quantitative structure−activity or structure−property relationships designed for an MS/Windows environment. CODESSA provides various methods for statistical analysis of experimental data, such as (multiple) linear and nonlinear regression, principal component analysis (PCA), nonlinear iterative partial least squares (NIPALS), and target transformation (TT), etc. A set of 540 molecular descriptors derived from geometrical and quantum-chemical structures of the 104 flavonoid molecules was computed in our study as available in this program. All descriptors were divided into five groups: constitutional, topological, geometric, electrostatic, and quantum-chemical.[23,24]

Constitutional descriptors reflect only the molecular composition of the compound such as the number of atoms, the number of bonds, and the molecular weight, etc.

Topological descriptors describe the atomic connectivity in a molecule. This group of descriptors contains various indices that reflect structural features such as size, symmetry, branching, shape, and cyclicity. The information content index and its derivatives (order 0−2) are based on the Shannon information theory.

Geometric descriptors are calculated from 3D atomic coordinates of the molecule. These descriptors comprise moments of inertia, shadow indices, molecular volume, molecular surface area, and gravitation indices.

Electrostatic descriptors reflect characteristics of the charge distribution in the molecule. The empirical partial charges are calculated by the Zefirov method. Minimum and maximum partial charges in the molecule and, for particular types of atoms, the polarity parameter and charged partial surface area descriptors belong to this group of descriptors.

Quantum-chemical descriptors add important information to the conventional descriptors. They are divided into three subgroups: charge distribution-related descriptors, valency-related descriptors, and quantum mechanical energy-related descriptors.

MLR analysis was applied for developing QSAR models. For selection of descriptors we used the heuristic method[23] as available in CODESSA. First, descriptors with missing or constant values for the set of structures are discarded from the original set. Further selection of descriptors is accomplished on the basis of the statistical parameters: r^2, F-test, and t-test for the one-parameter equations with the descriptors. The default values which were kept constant throughout the calculations were set as follows: the descriptor is eliminated if it does not meet the criteria for a one-parameter equation: the F-test's value is below 1.0, the squared correlation coefficent is less than r^2_{min}, and the parameter's t-value is less than t_1 ($r^2_{min} = 0.001$; $t_1 = 0.01$). Default value for the intercorrelation of descriptors was set to $r^2_{max} = 0.99$.

After the preselection of descriptors, MLR models are developed in a stepwise procedure. Thus, descriptors and correlations are ranked according to the values of the F-test and the correlation coefficient. Starting with the top descriptor from the list, two-parameter correlations are calculated. In the following steps new descriptors are added one-by-one until the preselected number of descriptors in the model is achieved. The final result is a list of the 10 best models according to the values of the F-test and correlation coefficient.

Orthogonalization of Descriptors. For statistical improvement of obtained models, we have used the Randic method for orthogonalization of descriptors.[25] The concept of the orthogonalization of descriptors is similar to constructing a basis of orthogonal vectors in linear algebra. Orthogonal $\delta_1-\delta_n$ are derived from nonorthogonal molecular descriptors D_1-D_n in a stepwise procedure. Initially we select descriptor

Table 3. Selection of Five Best Descriptors from Each Group of Descriptors According to Correlation Coefficients for One-Parameter Correlations

		r^2	F
	Topological Descriptors		
$^1\chi$	Randic index (order 1)	0.2739	25.6487
$^2\kappa$	Kier shape index (order 2)	0.2594	23.8182
^0IC_a	av information content (order 0)	0.2487	22.5050
$^0\chi$	Randic index (order 0)	0.2405	21.5321
W	Wiener index	0.2348	20.8670
	Constitutional Descriptors		
G^2	gravitation index (all bonds)	0.2771	26.0676
MW	molecular weight	0.2519	22.8972
N_{db}	no. of double bonds	0.2164	18.7809
RN_{db}	rel no. of double bonds	0.1649	13.4272
N_C	no. of C-atoms	0.1478	11.7931
	Geometric Descriptors		
I_C	moment of inertia C	0.1977	16.7611
I_A	moment of inertia A	0.1926	16.2253
MV	molecular volume	0.1784	14.7691
S_2	YZ shadow	0.1658	13.5179
I_B	moment of inertia B	0.1456	11.5889
	Electrostatic Descriptors		
$HASA2_R$	HASA-2/TMSA ratio	0.4888	65.0112
$HACA2_R$	HACA-2/TMSA ratio	0.4753	61.5984
$HASA1_R$	HASA-1/TMSA ratio	0.4673	59.6554
HASA2	HASA-2	0.4498	55.5915
HACA2	HACA-2	0.4382	53.0297
	Quantum-Chemical Descriptors		
$E_{tot.}(CO)$	max total interaction for a C−O bond	0.6379	119.7885
$E_{nn}(CO)$	max n−n repulsion for a C−O bond	0.6101	106.4178
$E_r(CO)$	max resonance energy for a C−O bond	0.5819	94.6332
BO^O_{min}	min (>0.1) bond order of a O− atom	0.5089	70.4671
$E_{ne}(CO)$	max n−e attraction for a C−O bond	0.5073	70.0104

D_1 as the first orthogonal descriptor δ_1. The next step is construction of the second orthogonal descriptor δ_2. To make D_2 orthogonal to δ_1, we make a simple linear regression between these two descriptors, where δ_1 is independent and D_2 a dependent variable. Next, we calculate a new D_2(calc) and then subtract these values from the original D_2. The residual, the difference between D_2 and D_2(calc), represents our new orthogonal descriptor δ_2. We resume this procedure until all descriptors are orthogonal. Finally, we apply the MLR method to develop regression models for orthogonal descriptors.

Due to many inactive compounds (50 out of 104) we rearranged the original set of 104 flavonoids into two groups. Data from series 1 (54 active and 16 inactive flavonoids) in Table 1 were used for developing regression models, while the remaining 34 inactive compounds from series 2 were used only in the prediction of biological activities.

3. RESULTS AND DISCUSSION

In Table 2 only a selection of descriptors used in our study is presented. They are sorted by descending value of correlation coefficient of one-parameter correlations.

In the first step of regression analysis we calculated the five best one-parameter correlations inside each group of descriptors. All correlations were sorted by type of descriptor and values of correlation coefficient and F-test.

As we will show below (Figure 3), five descriptors appear to be sufficient for a successful regression model.

On the basis of obtained results, we have assumed that most of the information about the structure−activity relationship for our series of flavonoids is contained in the group of electrostatic and quantum-chemical descriptors (with correlation coefficients in the range $r^2 = 0.44-0.64$), while geometric, constitutional, and topological descriptors give us the least information (with correlation coefficients in the range $r^2 = 0.14-0.28$) on binding flavonoids to the enzyme $p56^{lck}$. However, considering the simplicity and speed of the calculation of topological and constitutional descriptors (e.g. the Randic index (order 1) has $r^2 = 0.27$, and the gravitation index (all bonds), $r^2 = 0.28$) are a valuable addition to the arsenal of QSAR tools, in particular in connection to the modern combinatorial chemistry/HTS approach.

Table 4. Correlation Matrices for Descriptors Used in Equations 1−4

Equation 1

	$E_{tot.}(CO)$	$HASA2_R$	G^2	I_C	$^1\chi$
$E_{tot.}(CO)$	1.0000				
$HASA2_R$	−0.7423	1.0000			
G^2	0.3805	−0.5399	1.0000		
I_C	−0.2933	0.4784	−0.9341	1.0000	

Equation 2

	$^0BIC_a$	$^2CIC_a$	RN_H	RN_O	$^2\kappa$
$^0BIC_a$	1.0000				
$^2CIC_a$	−0.2870	1.0000			
RN_H	−0.4491	0.4833	1.0000		
RN_O	0.4992	−0.2614	−0.7408	1.0000	
$^2\kappa$	0.2375	0.6648	0.2423	0.2139	1.0000

Equation 3

	$E_{tot.}(CO)$	$E_{ee}(CC)$	$^nR^C_{av}$	RPCG	$E_c(CC)$
$E_{tot.}(CO)$	1.0000				
$E_{ee}(CC)$	0.0558	1.0000			
$^nR^C_{av}$	−0.4126	−0.1077	1.0000		
RPCG	0.2022	0.4735	−0.2465	1.0000	
$E_{cc}(CC)$	−0.0521	0.0116	0.6012	−0.1621	1.0000

Equation 4

	$E_{tot.}(CO)$	$E_{ee}(CC)$	^1CIC	AC_{MO}	WNSA3
$E_{tot.}(CO)$	1.0000				
$E_{ee}(CC)$	0.0558	1.0000			
^1CIC	0.1743	0.3203	1.0000		
AC_{MO}	−0.3302	0.3876	0.1566	1.0000	
WNSA3	−0.1706	0.5411	0.0181	0.7826	1.0000
$^1\chi$	0.3806	−0.5643	0.9828	−0.9091	1.0000

Next, the best descriptor of each group of descriptors was selected and the following regression model is obtained.

$$\log(1/IC_{50}) = -(3.58 \pm 0.60)E_{tot}(CO) + (6.12 \pm 6.31)HASA2_R - (7.31 \pm 9.65) \times 10^{-4}G^2 - (28.76 \pm 123.74)I_C + (3.48 \pm 19) \times 10^{-2}\,{}^1\chi + (100.65 \pm 16.18) \quad (1)$$

$$n = 70 \qquad r^2 = 0.7003 \qquad s^2 = 0.1679 \qquad F = 29.91$$

We proceeded with the determination of MLR models using the heuristic approach. With respect to the content of the information all descriptors were rearranged into two subsets. The first subset consisted of geometric, constitutional, and topological descriptors, while the second subset involved descriptors with information about electrostatic interactions (electrostatic and quantum-chemical descriptors). For both subsets heuristic optimization for five parameters was performed.

For the first subset the model shown in eq 2 was developed. Interestingly, no geometric descriptors are present

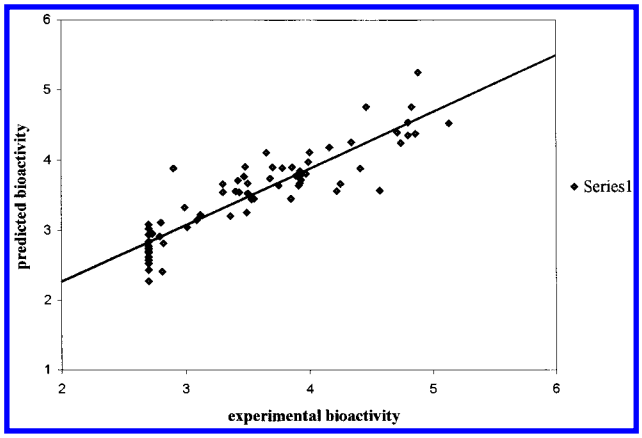

Figure 2. Correlation between experimental and predicted biological activity of flavonoids for eq 4.

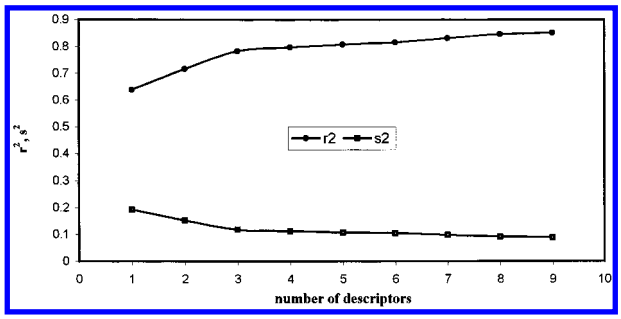

Figure 3. Influence of the number of descriptors on the correlation coefficient (r^2) and standard deviation (s^2) of the regression models.

in this model obtained by heuristic approach. The correlation coefficient for eq 2 is much lower than the correlation coefficient for eq 1. Thus, we can conclude that topological and constitutional descriptors alone are insufficient for a successful regression model.

$$\log(1/IC_{50}) = -(11.00 \pm 3.60)^0BIC_a - (1.06 \pm 0.40)^2CIC_a + (9.88 \pm 2.31)RN_H + (7.97 \pm 2.54)RN_O - (0.28 \pm 0.11)^2\kappa + (5.33 \pm 1.69) \quad (2)$$

$$n = 70 \qquad r^2 = 0.5659 \qquad s^2 = 0.2433 \qquad F = 16.69$$

Equation 3, derived from the group of electrostatic and quantum-chemical descriptors, provides a still better statistical description of flavonoid binding to the p56lck PTK enzyme, which is not surprising, if we consider the results of one-parameter correlations (Table 3). As shown in Table

$$\log(1/IC_{50}) = -(4.12 \pm 0.37)E_{tot.}(CO) + (0.36 \pm 0.07)E_{ee}(CC) + (190.19 \pm 97.09)^nR^C_{av} - (1.88 \pm 0.98)RPCG + (0.44 \pm 0.25)E_c(CC) + (66.21 \pm 13.26) \quad (3)$$

$$n = 70 \qquad r^2 = 0.7997 \qquad s^2 = 0.1122 \qquad F = 51.10$$

4, the highest correlation of descriptors in eq 3 is between $^nR^C_{av}$ and E_c(CC) ($^nR^C_{av}$ is the average nucleophilic reactivity index for a C-atom; E_c(CC) is the minimum Coulombic interaction for a C−C bond), with the corresponding correlation coefficient $r = 0.6012$, which points to a considerable proportion of orthogonality for all of the descriptors in eq 3.

Table 5. *t*-Values of Regression Coefficients in QSAR Models

eq 1		eq 2		eq 4	
descriptor	*t*-test	descriptor	*t*-test	descriptor	*t*-test
$E_{tot.}$(CO)	−5.927	$E_{tot.}$(CO)	−11.179	$E_{tot.}$(CO)	11.484
$HASA2_R$	0.969	E_{ee}(CC)	5.461	E_{ee}(CC)	5.328
G^2	−0.757	$^nR^C_{av}$	1.959	^{1}CIC	−5.285
I_C	−0.232	RPCG	−1.917	AC_{MO}	2.870
$^1\chi$	0.184	E_{cc}(CC)	1.777	WNSA3	−1.940

The best regression model (eq 4, Figure 2) was obtained using heuristic optimization for a group of all descriptors, but the correlation coefficient does not improve significantly compared to eq 3. This is yet another indicator that electrostatic interactions are strongly involved in inhibitory binding. Correlation between experimental and predicted biological activity of flavonoids for eq 4 is presented in Figure 2.

$$\log(1/IC_{50}) = -(4.02 \pm 0.35)E_{tot.}(CO) + (0.39 \pm 0.07)E_{ee}(CC) - (1.03 \pm 0.196) \times 10^{-2}\,{}^1CIC + (1.34 \pm 0.47)AC_{MO} - (1.48 \pm 0.764) \times 10^{-2}\,WNSA3 + (65.33 \pm 12.42)$$

$$n = 70 \qquad r^2 = 0.8079 \qquad s^2 = 0.1077 \qquad F = 53.82$$

On comparison of results of MLR analysis in eqs 1, 3, and 4 with values of the *t*-test, which reflects the significance of the parameter within a particular model (Table 5), we can assume that $E_{tot.}$(CO), the maximal total interaction for a C−O bond, is the most important parameter in these regression models.

The obtained results suggest that the carbonyl group in the chromone moiety of flavone inhibitor plays a critical role in inhibitory binding and that hydrogen bonds are very likely involved in the binding mechanism.

Among all the descriptors in eqs 1−4 only ^{1}CIC (the topological index of complementary information content, which expresses the internal flexibility of the molecule) does not belong to a group of electrostatic and quantum-chemical descriptors. Therefore, the condition of correct spatial charge distribution which is dependent on the internal flexibility as well as the electrostatic properties of inhibitor molecules in the series is essential for binding flavonoid derivatives on PTK.

Orthogonalization of Descriptors. To demonstrate the differences between the use of nonorthogonal and orthogonal descriptors in the development of a QSAR model, we applied heuristic optimization to generate the 10 best *n*-parameter ($n = 1, 2, ..., 9$) regression models. Statistical parameters r^2, s^2, and *F*-test were used for evaluation of the quality of the obtained models. In Table 6 only the best *n*-parameters ($n = 1, 2, ..., 9$) regression models are presented. For six-, seven-, and nine-parameter models better results were achieved, but due to difficult interpretation those models were not used in the orthogonalization process.

As shown in Figure 3, we notice that the plot of the value of correlation coefficients r^2_i in dependence of the number of descriptors used in the model reaches a plateau at five parameters used in the model, and the introduction of a new descriptor to the regression model does not significantly improve the value of the correlation coefficient. For a five-

Table 6. Regression Coefficients in QSAR Models for Nonorthogonal (D_i)[a] and Orthogonal Descriptors (δ_i)

D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9[b]	constant	r^2	s^2	F
−4.5638									126.023	0.6379	0.1910	119.79
−4.6532	0.2873								92.1450	0.7162	0.1519	84.54
−4.4055	0.3756	−0.0086							74.9132	0.7828	0.1180	79.29
−4.1133	0.3221	−0.0090	0.6580						75.2805	0.7966	0.1122	63.63
−4.0152	0.3894	−0.0103	1.3421	−0.0148					65.3268	0.8079	0.1077	53.82
−3.6315	0.3653	−0.0102	1.6873	−0.0206	−5.2746				58.9875	0.8160	0.1048	46.56
−2.9315	0.2919	−0.0096	2.1542	−0.0232	−7.8513	−0.5552			55.2169	0.8319	0.0972	43.83
−2.9427	0.2628	−0.0108	2.5140	−0.0240	−9.8996	−0.7361	−0.1818		61.8899	0.8454	0.0909	41.70
−2.9065	0.2631	−0.0105	2.5011	−0.0244	−9.6976	−0.7223	−0.2035	5929.0	60.6852	0.8521	0.0884	38.40
δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7	δ_8	δ_9[b]	constant	r^2	s^2	F
−4.5638									126.023	0.6379	0.1910	119.79
−4.5638	0.2873								126.023	0.7162	0.1519	84.54
−4.5638	0.2873	−0.0086							126.023	0.7828	0.1180	79.29
−4.5638	0.2873	−0.0086	0.6580						126.023	0.7966	0.1122	63.63
−4.5638	0.2873	−0.0086	0.6580	−0.0148					126.023	0.8079	0.1077	53.82
−4.5638	0.2873	−0.0086	0.6580	−0.0148	−5.2746				126.023	0.8160	0.1048	46.56
−4.5638	0.2873	−0.0086	0.6580	−0.0148	−5.2746	−0.5552			126.023	0.8319	0.0972	43.83
−4.5638	0.2873	−0.0086	0.6580	−0.0148	−5.2746	−0.5552	−0.1818		126.023	0.8454	0.0909	41.70
−4.5638	0.2873	−0.0086	0.6580	−0.0148	−5.2746	−0.5552	−0.1818	5929.0	126.023	0.8521	0.0884	38.40

[a] D_1, $E_{tot.}$(CO); D_2, E_{ee}(CC); D_3, ^{1}CIC; D_4, AC_{MO}; D_5, WNSA3; D_6, q^C_{max}; D_7, GAP; D_8, μ_{hybr}; D_9, $^nR^C_{min}$. [b] Values for D_9 (average nucleophilic reaction index for a C-atom) are between 10^{-5} and 10^{-9} for molecules in the series.

Table 7. Relative Standard Deviations of Regression Coefficients in Nonorthogonal Models (i) and Orthogonal Models (ii)

(i) Nonorthogonal Model[a]

	RSD_{D_1}	RSD_{D_2}	RSD_{D_3}	RSD_{D_4}	RSD_{D_5}	RSD_{D_6}	RSD_{D_7}	RSD_{D_8}	RSD_{D_9}	RSD_C
1	0.0914									0.0888
2	0.0801	0.2326								0.1380
3	0.0756	0.1653	0.2223							0.1582
4	0.0859	0.2039	0.2076	0.4765						0.1535
5	0.0871	0.1877	0.1892	0.3484	0.5155					0.1902
6	0.1141	0.2012	0.1890	0.2999	0.4030	0.5996				0.2175
7	0.1681	0.2639	0.1949	0.2432	0.3473	0.4113	0.4128			0.2256
8	0.1619	0.2874	0.1745	0.2108	0.3254	0.3279	0.3194	0.4328		0.2001

(ii) Orthogonal Model

	RSD_{δ_1}	RSD_{δ_2}	RSD_{δ_3}	RSD_{δ_4}	RSD_{δ_5}	RSD_{δ_6}	RSD_{δ_7}	RSD_{δ_8}	RSD_{δ_9}	RSD_C
1	0.0914									0.0888
2	0.0815	0.2326								0.0792
3	0.0718	0.2050	0.2223							0.0698
4	0.0700	0.1999	0.2168	0.4765						0.0681
5	0.0686	0.1958	0.2123	0.4667	0.5155					0.0667
6	0.0677	0.1931	0.2094	0.4603	0.5085	0.5996				0.0658
7	0.0652	0.1861	0.2018	0.4435	0.4899	0.5777	0.4128			0.0634
8	0.0630	0.1799	0.1951	0.4287	0.4736	0.5585	0.3991	0.4328		0.0613
9	0.0622	0.1774	0.1924	0.4229	0.4672	0.5509	0.3936	0.4269	0.6085	0.0604

[a] See footnote *a* of Table 6 for definitions of D_i.

parameter model, the correlation coefficient equals $r^2_5 = 0.8079$, and for a nine-parameter model, the correlation coefficient improves to the value $r^2_9 = 0.8521$. Thus, we have employed only five-parameter models to predict biological activity.

Comparison of the regression equations derived from nonorthogonal and from orthogonal descriptors (Table 6) is instructive. As is well-known[25] orthogonalization of descriptors does not alter the values of the correlation coefficients (r^2) and standard deviations (s^2). However, as seen from Table 6, introduction of a new descriptor to nonorthogonal models introduces fluctuation of the coefficients in the regression equation, while the corresponding coefficients in orthogonal models remain constant. Use of orthogonal descriptors also decreases the standard deviation of the coefficients in the regression equation regression with each new descriptor added in the regression model. Despite the obvious advantages of the orthogonalized MRA (multivariate regression analysis), it is somewhat disappointing to see how little attention use of orthogonal descriptors has received in MRA, although recently orthogonalization of descriptors has been combined with the use of CODESSA.[26−28]

In Table 7 we show the relative standard deviations of regression coefficients (Figure 4). Observe first that in the case of orthogonalized descriptors at each successive step of regression introduction of a new descriptor decreases the relative standard deviation of all the descriptors already used. This is generally not the case with the relative standard deviation of regression coefficients in the nonorthogonalized model. It appears however that when the coefficient is almost constant (as is the case with D_3) or when the magnitude of the coefficient increases (as is the case with D_4 and D_6 in

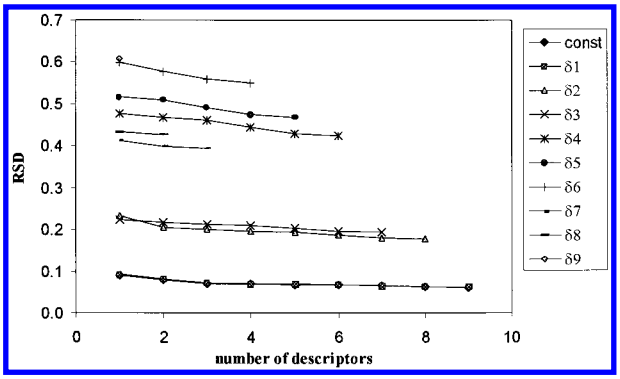

Figure 4. Influence of the number of descriptors on the relative standard deviation (RSD) of the regression coefficients in orthogonal regression models.

our example), there is also a decrease in the relative standard error. Hence, the relative standard deviation for δ_1 in the one-parameter regression model (Table 7) equals $RSD_{\delta 1} = 0.0914$, while in the nine-parameter regression model it decreases to $RSD_{\delta 1} = 0.0622$.

Therefore the stability and constancy of regression coefficients in orthogonal models enable interpretation of obtained models—a significant advantage for the use of developed QSAR models.

4. CONCLUSIONS

We have used a series of 104 flavonoids, inhibitors of enzyme p56lck PTK, to determine QSAR for their inhibitory activity as a function of a set of 540 molecular descriptors. The heuristic choice of descriptors revealed the most important properties of the inhibitors for binding to the enzyme active site: the maximal total interaction of the CO bond of the chromone ring, internal flexibility of the molecules as expressed by CIC (the topological index of complementary information content) and various descriptors connected with electrostatic properties of the inhibitors: the average nucleophilic reactivity, the minimum Coulombic interaction (eq 3), or the maximal antibonding contribution and weighted negative surface area (eq 4). Use of orthogonalization of descriptors introduced stability of coefficients of the regression models as well as a decrease of the relative standard deviation of regression coefficients with progressive introduction of more descriptors into the model. This has effectively provided convergence of the regression models with respect to the number of descriptors.

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