CODEN IEJMAT *Internet Electronic Journal of Molecular Design* **2006**, *5*, 1–12 ISSN 1538–6414
BioChem Press http://www.biochempress.com

Inter*net* Electronic Journal of Molecular Design

January 2006, Volume 5, Number 1, Pages 1–12

Editor: Ovidiu Ivanciuc

Special issue dedicated to Professor Danail Bonchev on the occasion of the 65th birthday

Structural Determinants of Flavones Interacting with the C–Terminal Nucleotide–Binding Domain as P–Glycoprotein Inhibitors

Yan Li,[1] Yonghua Wang,[2] Ling Yang,[2] Shuwei Zhang,[1] and Changhou Liu [1]

[1] School of Chemical Engineering, Dalian University of Technology, Dalian 116012, China
[2] Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

Received: December 1, 2005; Revised: December 13, 2005; Accepted: December 18, 2005; Published: January 31, 2006

Citation of the article:
Y. Li, Y. Wang, L. Yang, S. Zhang, and C. Liu, 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, http://www.biochempress.com.

Inter*net* Electronic Journal of Molecular Design

BioChem Press
http://www.biochempress.com

Structural Determinants of Flavones Interacting with the C–Terminal Nucleotide–Binding Domain as P–Glycoprotein Inhibitors

Yan Li,[1] Yonghua Wang,[2] Ling Yang,[2,]* Shuwei Zhang,[1] and Changhou Liu [1]

[1] School of Chemical Engineering, Dalian University of Technology, Dalian 116012, China
[2] Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

Received: December 1, 2005; Revised: December 13, 2005; Accepted: December 18, 2005; Published: January 31, 2006

Internet Electron. J. Mol. Des. **2006**, *5* (1), 1–12

Abstract

Motivation. To determine the structural features of flavones required for the reversal of P–glycoprotein (P–gp) mediated multidrug resistance, we studied the quantitative structure–activity relationships (QSAR) of a series of flavones specifically binding to the C–terminal nucleotide–binding domain of mouse P–gp.

Method. Pharmacophore modeling using DISCOtech and comparative molecular field analysis (CoMFA) methods were applied to the dataset to identify the pharmacophoric features as well as their 3D distribution.

Results. The proposed pharmacophore model including two hydrophobic and two hydrogen bond acceptor sites characterizes the necessary structural features of flavone inhibitors. CoMFA was also applied to the dataset, resulting in several 3D–QSAR models with good statistical indices ($R^2 > 0.9$ and $Q^2 > 0.5$).

Conclusions. All these models would be helpful for development of novel flavone–based P–gp inhibitors.

Keywords. P–glycoprotein (P–gp); flavone; pharmacophore; CoMFA; comparative molecular field analysis; QSAR; quantitative structure–activity relationships.

1 INTRODUCTION

The resistance of tumor cells to a broad array of chemically diverse cytostatic agents, a phenomenon termed multidrug resistance (MDR), remains a major obstacle to successful cancer chemotherapy [1]. One of the classical protection mechanisms of cancer cells involves an increased expression of drug efflux transport proteins like P–glycoprotein (P–gp) [2]. P–gp, a human MDR1 and MDR3 or rodent Mdr1a, Mdr1b and Mdr2–encoded product, is a 170 kDa transmembrane glycoprotein. P–gp belongs to the ATP–binding cassette transporter family and expels a wide variety of hydrophobic compounds from the cell, thus resulting in reduced intracellular drug

Dedicated on the occasion of the 65th birthday to Danail Bonchev.
* Correspondence author; E–mail: yling@dicp.ac.cn.

accumulations. The wide P–gp distribution in cancer tissues [3] including acute leukemias, breast, ovarian, head and neck tumors, and non–Hodgkins lymphoma has strongly suggested that the overexpression of P–gp plays a significant role in MDR phenotype. Therefore, the development of pharmacological agents to inhibit P–gp–mediated drug efflux is a promising way to overcome MDR, which leads much of research interests.

Up to now a wide panel of resistance modifying agents including channel blockers, calmodulin antagonists, immunosuppressants and protein kinase inhibitors [4] have been proven to be potent P–gp inhibitors. However, the clinical use of these modulators have been hampered, partially due to the unendurable toxic side effects resulting from the suprapharmacological doses used which are required to achieve an effective reversal of MDR. Some other drugs such as antiprogestin RU486 have satisfactory inhibitory effects on P–gp transport in vitro, whereas clinical use might be a risk due to their hormonal consequences [5]. Therefore, the search for safe and effective P–gp inhibitors with higher selectivity and potency has been in great demand.

Flavonoids are a group of polyphenolic compounds particularly abundant in fruits, vegetables, nuts, flowers, tea and wine, and constitute important components of normal human food with a few hundreds of milligrams daily intake in the human diet [6]. Flavonoids display a remarkable spectrum of biological activities including antioxidant, antiallergic, anti–inflammatory, antiviral as well as free–radical scavenging activities, and especially anticarcinogenic actions that are beneficial for human health [7]. Many, but not all, flavonoids have been demonstrated to inhibit the growth of various cancer cell lines in vitro and reduce tumor development in experimental animals [8,9]. A wide spectrum of flavonoids such as Biochanin A, morin, phloretin, silymarin, various flavonols and flavones have been confirmed to promote the concentration of intracellular anticancer drugs by active against P–gp functions, and the interaction mechanism involved at least in part a direct interaction [10,11,12]. Some synthesized flavonoids like *N*–benzylpiperazine ones displayed more potent MDR–modulating activity than the calcium–channel blocker verapamil, a standard MDR–reversing agent [13]. Therefore, flavonoids have been regarded as a new class of promising potential MDR modulators.

Our goal was to determine the requisite structure features for flavonoid that specifically interact with the C–terminal nucleotide–binding domain (NBD2, “2” refers to the C–terminal NBD site) of P–gp. Though according to structural difference there are several subtypes of flavonoids, our research only focuses on flavone type of flavonoids, with attempt to help design and screening of flavone–like P–gp inhibitors of greater affinity and selectivity. Since as yet no high–resolution P–gp structure is obtained, the aid of proper computer techniques is of importance.

2 MATERIALS AND METHODS

2.1 Dataset Building

A number of 32 flavones specifically binding to the NBD2 site of mouse P–gp [14] were used as dataset (supplementary materials). Their structure contains three conjugated rings, A and C being juxtaposed and B branched at position 2. The data of flavones directly binding to the NBD2 site by quenching of protein intrinsic fluorescence (K_D) were used (see column 3, Table 1) [14]. The inhibitory activity evaluation was performed using pK_D.

2.2 Molecular Modeling

All computations were performed on a Dell workstation running Linux RedHat 8.0 with Sybyl 6.92. The 2D structures of flavones were obtained from a commercially available MDL–ISIS database. Conformational search and energy minimization were carried out using simulated annealing molecular dynamics, from initial 700 to 200K for 1000 fs with the distance–dependent dielectric constant of 1.0 and time increment of 0.5 fs. Each conformer was minimized by the Powell conjugate gradient algorithm using a standard Tripos force field with an energy gradient limit set to 0.05 kcal/mol/Å. The minimization was terminated when the energy gradient convergence criterion of 0.001 kcal/mol was reached. Partial atomic charges were assigned to each atom with Gasteiger–Huckel method [15]. The hydrophobicity parameters (logP) were calculated based on the number and nature of fragments [16].

To identify the general pharmacophoric features the dataset was studied at first by Distance Comparisons technique (DISCOtech), a distance constraint pharmacophore building method.

2.2.1 Pharmacophore Modeling

A stochastic search routine was run to generate a maximum of 100 conformers for each molecule on the basis of maximum diversity to cover as many probable conformers as possible. 3–OH–flavone has the fewest rotating bonds and features in all molecules, and was selected as the reference compound [17]. For each conformation, the possible pharmacophoric elements were assigned. Seven (or fewer) conformations with maximum diversity selected for each molecule were then aligned to 3–OH–flavone. DISCO was initially run considering all the potential “feature” points, but additional runs with the specification of preserving only three possible features, *e.g.* hydrophobic, donor and acceptor atoms, were executed as well. The distances between the feature points in each flavone conformation were calculated and compared with those of the reference compound. The distance tolerance was set stepwise from 0.25 to 2.5 Å by 0.25 Å. If all the intramolecular distances of identical features between the reference conformation and the calculated conformations of other flavones were met within the tolerance, a valid pharmacophore model was established.

After this, these flavones were further studied by comparative molecular field analysis (CoMFA).

2.2.2 CoMFA Study

In CoMFA studies, the van der Walls and Columbic potentials representing steric and electrostatic fields respectively, were calculated using a Tripos standard force field at each lattice intersection. An sp^3 hybridized carbon with a +1 charge on a 2.0 Å regular spaced lattice extending roughly 4 Å from each side of the molecule [18] was used as probe atom. Values of the steric and electrostatic energies were truncated at 30.0 kcal/mol with the attenuation factor α set to 0.3. After all these calculations, a spreadsheet was generated and the correlation between various descriptors, including steric, electrostatic, hydrophobic, HB donor and acceptor, as well as the logP, was analyzed by partial least squares (PLS), a standard statistical method used successfully in many QSAR studies for rationalization of those structure features affecting the biological activity [19]. PLS relates a matrix of dependent variables Y, in this case the inhibitory properties (pK_D), to a matrix of descriptors X, here the logP parameter and various field descriptors. As long as the optimal number (OPN) of components was chosen that yielded the largest cross–validated Q^2 values, a final PLS analysis was performed using the chosen OPN with no cross–validation. This generated a fitted correlation of the entire training set. Presently, flavones were studied with 7 (**3**, **4**, **6**, **10**, **17**, **23** and **29**) selected as a test set and the remaining 25 ones as the training set. 8–Geranyl–chrysin with the lowest–energy conformation and the largest inhibitory effect was chosen as the template molecule for alignment.

3 RESULTS AND DISCUSSION

3.1 Pharmacophore Modeling

Although several pharmacophore models have proven useful for identifying the molecular features required for P–gp substrates [20,21,22] and inhibitors [23], a pharmacophore model interpreting the interaction mechanism of flavone specifically binding to the NBD2 site is still unavailable, which prompted us to build a 3D–pharmacophore model for this site as the first step. Presently, a pharmacophore model (Figure 1) was obtained with score of 2.10 and tolerance distance of 0.25 Å. This model is consisted of four pharmacophoric features, defined as two hydrophobic center sites (H) in rings A and B, and two HB acceptor atoms (AA) around the oxygen atom in ring C as well as in the C–4 carbonyl group respectively. Figure 2 shows the arrangement of these features, which is a tetrahedral combination of six distances found to be common to all flavones: H_1–AA_1, 2.00 ± 0.25 Å; H_2–AA_1, 3.72 ± 0.25 Å; H_1–AA_2, 3.71 ± 0.25 Å; H_2–AA_2, 6.14 ± 0.25 Å; H_1–H_2, 6.48 ± 0.25 Å; AA_1–AA_2, 4.01 ± 0.25 Å. The four sites of the distance constraints constitute the requisite elements of flavone inhibitors.

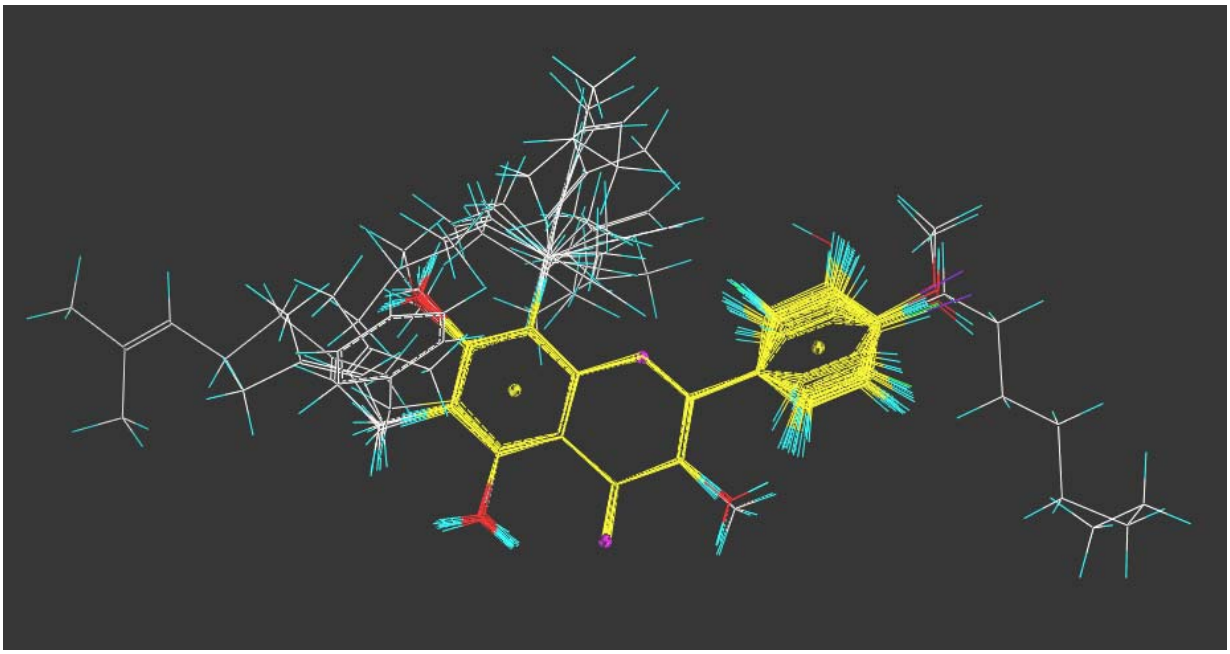

Figure 1. A stereoscopic view of the pharmacophore model of flavones derived from the DISCO technique. The stick frame representation of all individual flavones is shown in their overlapping conformations. There are four pharmacophore feature points, *i.e*., two hydrophobic center sites (shown by the yellow dummy center points in rings A and B) and two HB acceptor atom sites around the oxygen atom in ring C and in the C–4–carbonyl group respectively (shown by the red dummy atoms). The specific distances between these sites are given in Figure 2.

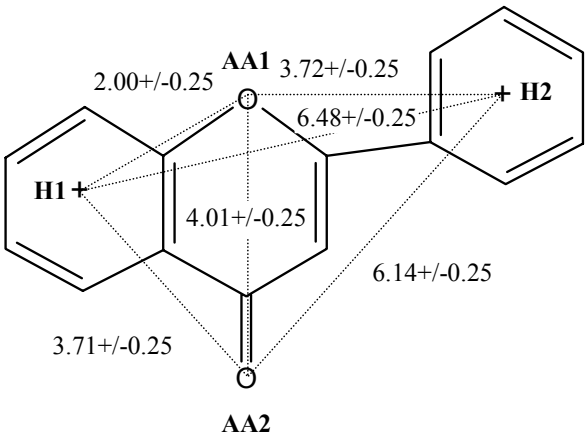

Figure 2. Schematic pharmacophore of flavones. Pharmacophore feature points include two H sites (+ points in rings A and B) and two AA sites (points around the oxygen atoms in ring C and in the C–4–carbonyl group respectively). They are annotated as follows: AA, HB acceptor atom, H, hydrophobic center. The distance between these sites are shown beside each straight line with angstrom unit.

The features in our model, the aromatic rings A and B and the HB acceptor sites, naturally forms π–bond–hydrogen bond–π–bond (π–HB–π) interactions, which supports the speculation of Suzuki's [24] that π–HB–π interactions are requisite features for P–gp inhibitors. They studied the modulating activity of quinolines, a kind of P–gp inhibitors with a hydrophobic moiety composed of two separated aryl rings linked only by one C–C single bond in structure, and found that the presence and deviation of the two aryl rings from planarity in the hydrophobic moiety is essential for effective reversal of tumor cell MDR. Because in their experiments quinolines with two phenyl

rings, each of which deviated greatly from planar (*e.g.*, by 80°, 56° or 43°), demonstrated greater activity than more nearly planar configurations (*e.g.*, by 1° or 5°), due to the role of the π–HB–π interactions produced between the HB donors of P–gp (set between the two separated aryl rings) and the hydrophobic moieties. Besides, though we don't know if the binding mechanism of quinolines is the same as that of flavones with P–gp due to lack of related information, our model is close to theirs in the distance constraints of pharmacophoric features. In our model the distances between each centroid of the phenyl ring and one HB acceptor site of AA_2 are about 3.71 and 6.14 Å respectively. Thus the distance between the hydrophobic moiety (the midpoint between ring A and ring B centers) and AA_2 is approximately 5 Å, which is consistent with Suzuki's conclusion that the distance between the HB acceptor and hydrophobic moieties is at least about 5 Å required for P–gp inhibitors of high activity. We therefore assume that the pharmacophore features presented by our model are necessary for general P–gp modulators. However due to the difference of quinolines and flavones, our model has another HB acceptor site which remains further study.

About the research on P–gp binding sites, recently Ekins and Pajeva independently proposed two pharmacophore models for the verapamil binding site using diverse sets of inhibitors. Despite that the verapamil binding site is possibly independent of the NBD2 site, we compared our results with theirs. Ekins [23] suggested the presence of at least four distinct groupings of features composed of two hydrophobic domains at the extremes of the figure, along with one HB acceptor and one ring aromatic feature both near one of the hydrophobic domains are necessary for P–gp recognition. Our model agrees with Ekins' on emphasizing the importance of the hydrophobic regions and HB acceptor points as necessary elements for P–gp–inhibitor interaction. However, the two pharmacophores are different in the inter–site distances of pharmacophoric points. Pajeva [20] put forward another pharmacophore that is composed of two hydrophobic centers H_1 and H_2 around the centers of the aromatic rings, three HB acceptors A_1, A_2, A_D, and one HB donor points D_A. This model agrees with ours in two hydrophobic regions and two hydrogen acceptor atoms. Whereas, the two models are also different in both the number of HB acceptor atoms and inter–site distances. Though to date we are still lack of sufficient information about the 3D–structure of NBD site, our model together with the ones mentioned above, to some extent, indicate that the three pharmacophoric features of two hydrophobic regions and one HB acceptor atom are the basic structural features for general P–gp inhibitor. And the closer a new flavone molecule met with our model, the greater the possibility it has to be a potent P–gp inhibitor.

3.2 CoMFA Studies

A common limitation to pharmacophore model is its failure to report on steric and electrostatic functionalities that result in short and long range ligand–protein interactions respectively. This limitation also exists in several computation methods commonly used in QSAR studies. Based on a Bayesian–regularized neural network (BRNN), Wang *et al*. [25] successfully built a model

correlating the inhibitory activities of flavonoids with their structures with high predictability. For comparison, they also built another model based on back–propagation neural network (BPNN). These models are very helpful for screening novel flavonoid P–gp inhibitors. Whereas, both BRNN– and BPNN–derived models are invisible, actually, they are "black boxes" which are difficult in demonstrating the specific 3D–distributions of the molecular descriptors. The application of CoMFA and its derived form, the comparative molecular similarity indices analysis (CoMSIA) techniques in QSAR studies, however, have achieved success in overcoming this limitation and are more intuitively helpful for chemists to understand the SAR of modulators, such as the pharmacophore feature model built for propafenone–type MDR–modulators [26] and the SAR studies of dihydro–β–agarofuran sesquiterpenes as P–gp inhibitors [27]. Therefore, we extended our model by correlating variability in binding affinity to variations in molecular structure by implementing CoMFA techniques.

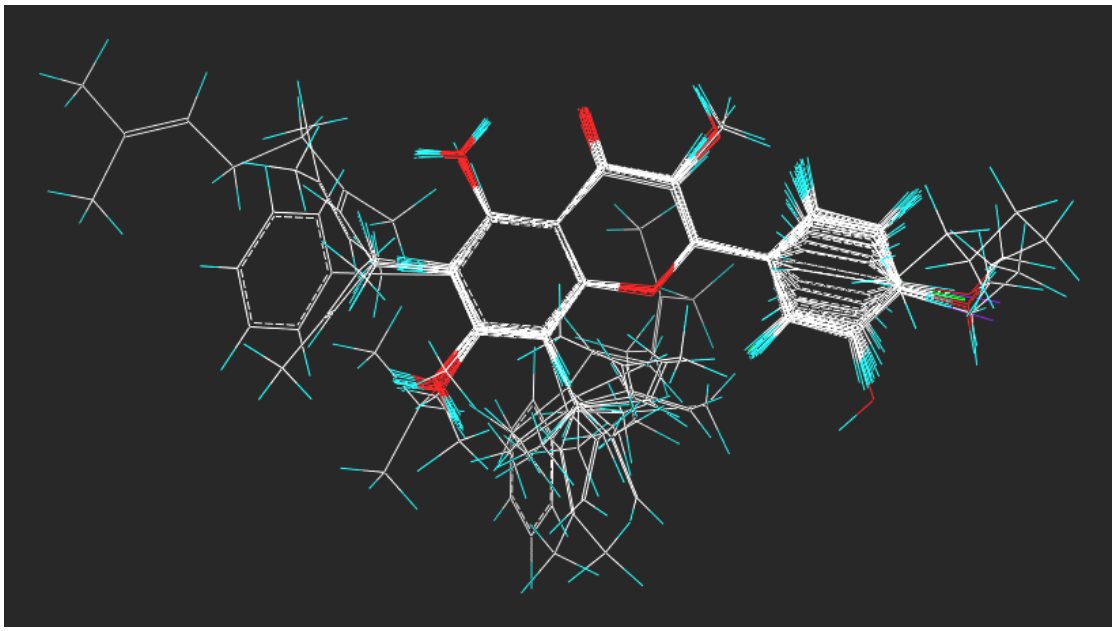

Figure 3. Stereoview of the flavone inhibitors superimposed.

Based on proper superimposition of flavones (Figure 3) three models with steric descriptors, electrostatic descriptors and CoMFA standard (std) (including both steric and electrostatic) descriptors were generated respectively. The statistical results are that for the steric model cross–validated Q^2 = 0.764, non–cross–validated R^2 = 0.951, SEE = 0.200 and F = 97.586, for the electrostatic model Q^2 = 0.789, R^2 = 0.987, SEE = 0.105 and F = 291.573, and for the std model Q^2 = 0.716, R^2 = 0.980, SEE = 0.131 and F = 188.133 were obtained respectively, indicating strong relationship between the flavone structure and inhibitory activity. The predictivity of the models was also proven when they were validated by the test set, where the steric, the electrostatic and the std models achieved an SEP of 0.354, 0.299 and 0.242 respectively. Table 1 shows the actual, calculated and residual pK_D values of these models.

To compare the individual influences of steric and electrostatic fields on P–gp–flavone interactions, the CoMFA std model was studied in detail. Figure 4 shows the actual vs. calculated inhibitory activities scatter graph (pK_D values) of this model.

Table 1. The Actual, Calculated and Residual pK_D Values of CoMFA Models

No	Flavone	$K_D \times 10^6$ (mol/l)	pK_D						
			Actual	Std model		Steric model		Electrostatic model	
				Calc.	Res.	Calc.	Res.	Calc.	Res.
1	7–OH–flavone	34.9	4.457	4.776	–0.319	4.936	–0.479	4.629	–0.172
2	Chrysin	8.9	5.051	5.001	0.050	4.999	0.052	4.997	0.054
3	6–Methyl–chrysin	3.1	5.509	5.264	0.245	5.233	0.276	5.300	0.209
4	Tectochrysin	6.3	5.201	5.212	–0.011	5.414	–0.213	5.580	–0.379
5	Apigenin	10.1	4.996	4.917	0.079	5.082	–0.086	4.810	0.186
6	3',4'–Difluoro–chrysin	6.3	5.201	5.120	0.081	4.875	0.326	5.218	–0.017
7	4'–Iodo–chrysin	2.2	5.658	5.827	–0.169	5.725	–0.067	5.774	–0.116
8	7–O–Isopropyl–chrysin	1.3	5.886	5.837	0.049	5.719	0.167	5.845	0.041
9	6–Benzyl–chrysin	0.34	6.469	6.597	–0.128	6.754	–0.285	6.502	–0.033
10	8–Benzyl–chrysin	0.99	6.004	6.435	–0.431	6.518	–0.514	6.277	–0.273
11	6–Prenyl–chrysin	0.30	6.523	6.406	0.117	6.477	0.046	6.637	–0.114
12	8–DMA–chrysin	0.20	6.699	6.606	0.093	6.529	0.170	6.673	0.026
13	8–Prenyl–chrysin	0.28	6.553	6.457	0.096	6.543	0.010	6.501	0.052
14	6–Geranyl–chrysin	0.045	7.347	7.322	0.025	7.467	–0.120	7.223	0.124
15	8–Geranyl–chrysin	0.025	7.602	7.603	–0.001	7.429	0.173	7.622	–0.020
16	8–DMA–apigenin	0.7	6.155	6.192	–0.037	6.089	0.066	6.163	–0.008
17	3–OH–flavone	10.1	4.996	5.115	–0.119	5.149	–0.153	5.030	–0.034
18	Galangin	5.3	5.276	5.393	–0.117	5.355	–0.079	5.236	0.040
19	Kaempferol	6.7	5.174	5.024	0.150	5.145	0.029	4.994	0.180
20	Kaempferide	4.5	5.347	5.376	–0.029	5.454	–0.107	5.393	–0.046
21	Quercetin	7.0	5.155	5.198	–0.043	5.040	0.115	5.171	–0.016
22	8–DMA–kaempferide	0.20	6.699	6.792	–0.093	6.727	–0.028	6.794	–0.095
23	8–DMA–galangin	0.45	6.347	6.376	–0.029	6.126	0.221	6.295	0.052
24	6–Prenyl–galangin	0.21	6.678	6.702	–0.024	6.721	–0.043	6.587	0.091
25	8–Prenyl–galangin	0.22	6.658	6.596	0.062	6.717	–0.059	6.647	0.011
26	4'–Fluoro–galangin	6.8	5.167	5.266	–0.099	5.136	0.031	5.333	–0.166
27	2',4'–Dichloro–galangin	4.0	5.398	5.238	0.160	5.073	0.325	5.448	–0.050
28	4'–Iodo–galangin	1.1	5.959	5.811	0.148	5.534	0.425	5.859	0.100
29	4'–n–C_8H_{17}–galangin	0.06	7.222	6.847	0.375	6.664	0.558	7.821	–0.599
30	3–Methyl–galangin	8.9	5.051	4.930	0.121	5.240	–0.189	5.073	–0.022
31	8–DMA,3,7–dimethyl–galangin	0.15	6.824	6.920	–0.096	6.808	0.016	6.842	–0.018
32	6,7–Dimethyl–chrysin	1.3	5.886	5.884	0.002	5.967	–0.081	5.913	–0.027

The relative field contributions in the CoMFA models are shown in Table 2. Clearly, a common tendency could be observed that the hydrophobicity of flavones does contribute to their interaction with P–gp, with LogP accounts for no less than 23.5% in all models (23.5%, 35.0% and 24.4% for steric, electrostatic and std models respectively). The importance of hydrophobicity to flavone inhibitors can be clearly demonstrated by the fact that the chrysin analogs with geranyl substituents (the logP of geranyl is about 2.538 (data not shown, which was calculated based on the number and nature of fragments) are more active than those with prenyl ones (the logP of prenyl is about 1.444, data not shown) in the dataset. Comte *et al*. [28] also demonstrated that the increase of hydrophobicity of chrysin by alkylation of certain substituents was correlated with an increase in

affinity for in vitro binding to P–gp cytosolic domain. Table 2 also indicates the greater influence of the steric field than electrostatic field according to their contributions in the CoMFA std model (43.0% : 32.6%).

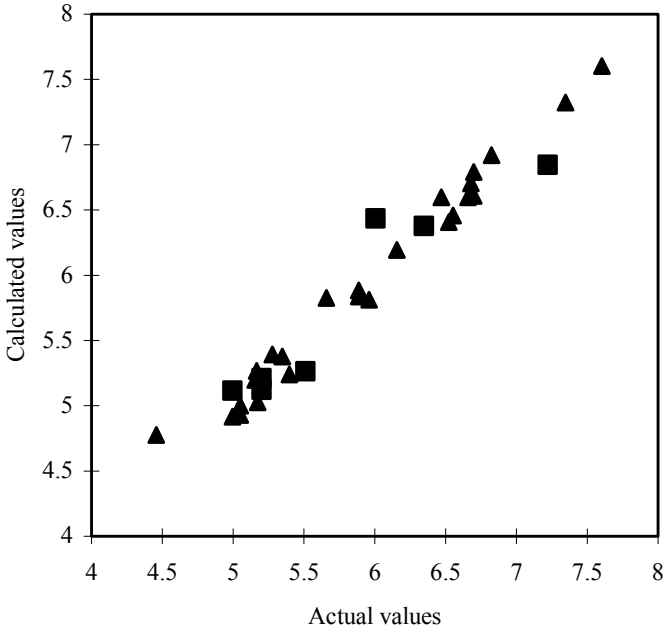

Figure 4. Scatter graph of actual versus calculated pK_D values in CoMFA std model. ▲: training set molecules; ■: test set molecules.

Table 2. Relative Field Contribution of CoMFA Models.

CoMFA descriptors	Relative contributions		
	Steric field	Electrostatic field	LogP
Steric	76.5%	–	23.5%
Electrostatic	–	65.0%	35.0%
Std	43.0%	32.6%	24.4%

Figure 5 depicts the steric and electrostatic (transparent) contour maps based on the CoMFA std model. In expression for steric interaction, the green and yellow contours describe the favorable and unfavorable bulk groups, respectively. Sterically favored green regions are found near the C–8 and C–6 positions of ring A, where all active compounds have geranyl, prenyl or DMA (dimethylallyl) substituents. The region near the C–4' of ring B is also sterically favored, which agrees with the fact that the activity sequence: compounds no. 29 > 28 > 26 corresponds to the size sequence of substituents at C–4': C_8H_{17} > I > F. Sterically unfavored yellow regions occupy almost all of the molecular regions, especially covering both the up and down sides of rings A and B which can be explained by the π–π conjugation effects above and below the two rings make the flavones a stable hydrophobic structure. It can also be seen from Figure 5 that the only regions which seem not very sensitive to the impact of any steric hindrances might be around ring C and part of ring A, which remains to be studied further.

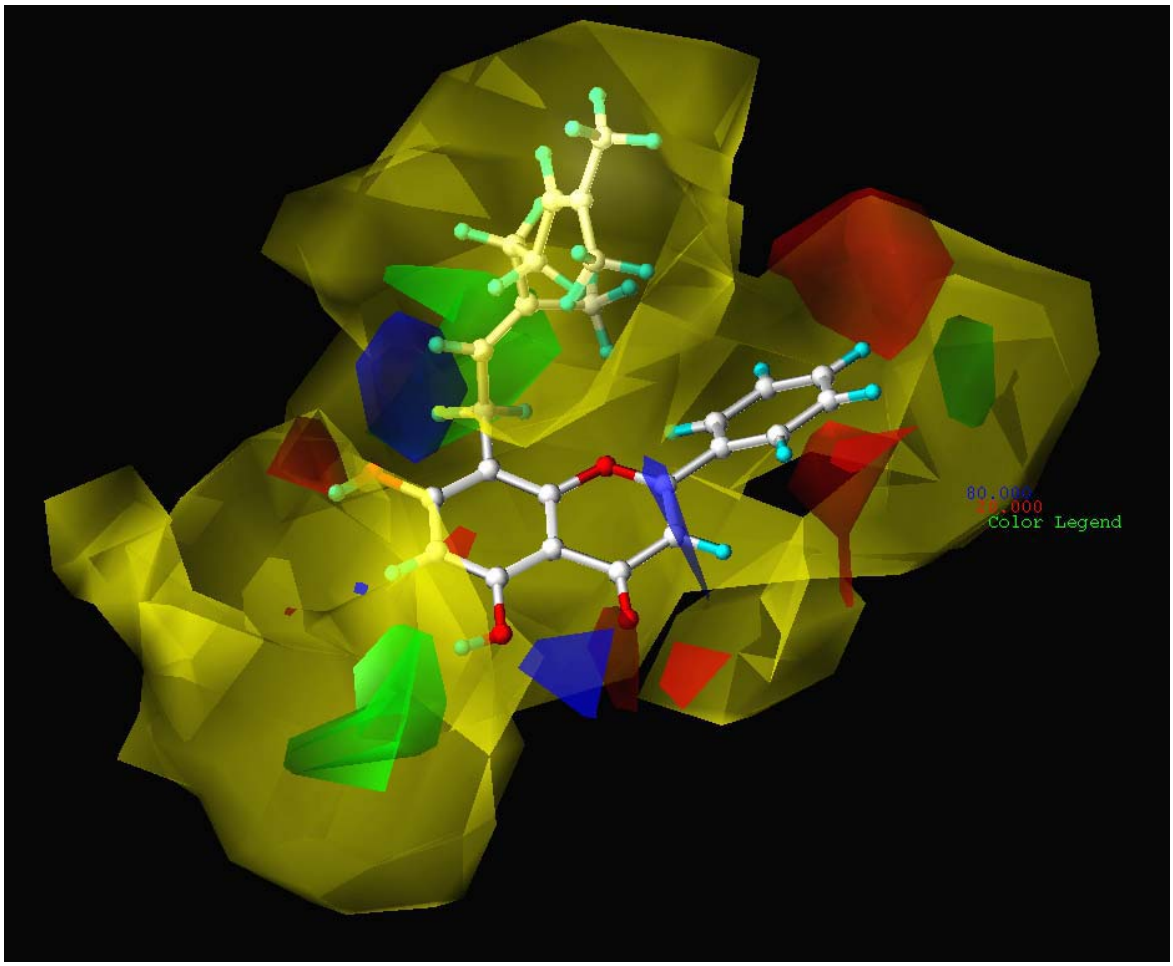

Figure 5. The steric and electrostatic contour plots of CoMFA std model. Green contours indicate regions where bulky groups increase activity, whereas yellow contours indicate regions where bulky groups decrease activity. Blue contours indicate regions where positive–charged groups increase activity, whereas red contours indicate regions where negative–charged groups increase activity.

In expression for electrostatic interaction, the blue contours indicate regions where positive–charged groups increase activity and red contours indicate regions where negative–charged groups increase activity. In Figure 5, negative charge favored red regions were found near the carbonyl oxygen atom (of the C–4) which is a pharmacophoric point, and the C–5 where all active flavones contain an electronegative oxygen atom in the form of a hydroxyl group. Electrostatically favored blue or red regions are also found near the C–3, 6 and 7, indicating that these regions are very sensitive to electrostatic groups. In addition, an extra negative charge favored area is observed occupying the C–4', which supports the fact that 8–DMA–kaempferol exhibits more activity than its unsubstituted analogue 8–DMA–galangin.

4 CONCLUSIONS

Our work provided a detailed QSAR study on flavone inhibitors specifically binding to the NBD2 site of P–gp from 3D spaces. The pharmacophore model including two hydrophobic center

sites and one HB acceptor site constitutes the requisite structural criteria for flavone modulators. The CoMFA models with proper predictability further present chemically intuitive representations of the spatial characteristics of the molecules. All these models have extended the understanding of flavone structure–activity relationship, and are useful for rational design and screening of novel flavone–based P–gp inhibitors.

Acknowledgment

This work was finally supported by the 973 program (No.2003CCA03400), the 863 program (No.2003AA223061), and Cooperation Scientific Research Fund supported by Dalian University of Technology and Dalian Institute of Chemical Physics.

5 REFERENCES

[1] S. M. Simon and M. Schindler, Cell biological mechanisms of multidrug resistance in tumors. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3497–3504.
[2] M. M. Gottesman and I. Pastan, Biochemistry of multidrug resistance mediated by the multidrug transporter, *Annu. Rev. Biochem.* **1993**, *62*, 385–427.
[3] C. Cordon–Cardo, J. P. O'Brian, J. Boccia, D. Casals, J. R. Bertino, M. R. Melamed, Expression of the multidrug resistance gene product (P–glycoprotein) in human and tumor tissues, *J. Histochem.Cytochem.* **1990**, *38*, 1277–1287.
[4] C. R. Leveille–Webster and I. M. Arias, The biology of the P–glycoprotein, *J. Membr. Biol.* **1995**, *143*, 89–102.
[5] V. Lecureur, O. Fardel, A. Guillouzo, The antiprogestatin drug RU486 potentiates doxorubicin cytotoxicity in multidrug resistant cells through inhibition of P–glycoprotein function, *FEBS Lett.* **1994**, *355*, 187–191.
[6] P. C. H. Hollman and M. B. Katan, Dietary flavonoids: intake, health effects and bioavailability, *Food Chem. Toxicol.* **1999**, *37*, 937–942.
[7] E. Middleton, Jr., C. Kandaswami, T. C. Theoharides, The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer, *Pharmacol. Rev.* **2000**, *52*, 673–751.
[8] J. W. Critchfield, C. J. Welsh, J. M. Phang, G. C. Yeh, Modulation of adriamycin accumulation and efflux by flavonoids in HCT–15 colon cells: activation of P–glycoprotein as a putative mechanism, *Biochem. Pharmacol.* **1994**, *48*, 1437–1445.
[9] C. H. Versantvoort, G. J. Schuurhuis, H. M. Pinedo, C. A. Eekman, C. M. Kuiper, J. Lankelma, H. J. Broxterman, Genistein modulates the decreased drug accumulation in non–P–glycoprotein mediated multidrug resistant tumor cells, *Br. J. Cancer* **1993**, *68*, 939–946.
[10] S. Zhang, M. E. Morris, Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on P–glycoprotein–mediated transport, *J. Pharmacol. Exp. Theor.* **2003**, *304*, 1258–1267.
[11] E. Chieli, N. Romiti, F. Cervelli, R. Tongiani, Effects of flavonols on P–glycoprotein activity in cultured rat hepatocytes, *Life Sci.* **1995**, *57*, 1741–1751.
[12] A. F. Castro and G. A. Altenberg, Inhibition of drug transport by genistein in multidrug–resistant cells expressing P–glycoprotein, *Biochem. Pharmacol.* **1997**, *53*, 89–93.
[13] J. Ferté, J.–M. Kühnel, J. Chapuis, Y. Rolland; G. Lewin, M. A. Schwaller, Flavonoid–related modulators of multidrug resistance: synthesis, pharmacological activity, and structure–activity relationships, *J. Med. Chem.* **1999**, *42*, 478–489.
[14] A. Di Pietro, G. Conseil, J. M. Pérez–Victoria, G. Dayan, H. Baubichon–Cortay, D. Trompier, E. Steinfels, J.–M. Jault, H. de Wet, M. Maitrejean, G. Comte, A. Boumendjel, A.–M. Mariotte, C. Dumontet, D. B. McIntosh, A. Goffeau, S. Castanys, F. Gamarro, D. Barron, Modulation by flavonoids of cell multidrug resistance mediated by P–glycoprotein and related ABC transporters, *Cell. Mol. Life Sci.* **2002**, *59*, 307–322.
[15] J. Gasteiger, H. Saller, Calculation of the charge distribution in conjugated systems by a quantification of the resonance concept, *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 687–689.
[16] C. Hansch and A. J. Leo, *Substituent constants for correlation analysis in chemistry and biology*, John Wiley & Sons, New York, USA, 1979.
[17] Y. C. Martin, M. G. Bures, E. A. Danaher, J. DeLazzer, I. Lico, P. A. Pavlik, A fast new approach to pharmacophore mapping and its application to dopaminergic and benzodiazepine agonists, *J. Comput. Aided Mol. Des.* **1993**, *7*, 83–102.

[18] M. Clark, R. D. Cramer III, N. Van Opdenbosch, Validation of the general purpose Tripos 5.2 force field, *J. Comput. Chem.* **1989**, *10*, 982–1012.
[19] A. Espinosa–Mansilla, M. I. Acedo Valenzuela, A. Munoz de la Pena, F. Salinas, C. F. Canada, Comparative study of partial least squares and a modification of hybrid linear analysis calibration in the simultaneous spectrophotometric determination of rifampicin, pyrazinamide and isoniazid, *Anal. Chim. Acta* **2001**, *427*, 129–136.
[20] I. K. Pajeva and M. Wiese. Pharmacophore model of drugs involved in P–glycoprotein multidrug resistance: explanation of structural variety (hypothesis), J. Med. Chem. **2002**, *45*, 5671–5686.
[21] I. K. Pajeva, C. Globisch, M. Wiese, Structure–Function relationships of multidrug resistance P–glycoprotein, *J. Med. Chem.* **2004**, *47*, 2523–2533.
[22] C. R. Yates, C. Chang, J. D. Kearbey, K. Yasuda, E. G. Schuetz, D. D. Miller, J. T. Dalton, P. W. Swaan, Structural determinants of P–glycoprotein–mediated transport of glucocorticoids, *Pharm. Res.* **2003**, *20*, 1794–1803.
[23] S. Ekins, R. B. Kim, B. F. Leake, A. H. Dantzig, E.G. Schuetz, L. B. Lan, K. Yasuda, R. L. Shepard, M. A. Winter, J. D. Schuetz, J. H. Wikel, S. A. Wrighton, Application of three–dimensional quantitative structure–activity relationships of P–glycoprotein inhibitors and substrates, *Mol. Pharmacol.* **2002**, *61*, 974–981.
[24] T. Suzuki, N. Fukazawa, K. San–nohe, W. Sato, O. Yano, T. Tsuruo, Structure–activity relationship of newly synthesized quinoline derivatives for reversal of multidrug resistance in cancer, *J. Med. Chem.* **1997**, *40*, 2047–2052.
[25] Y. H. Wang, Y. Li, S. L. Yang, L. Yang, An in silico approach for screening flavonoids as P–glycoprotein inhibitors based on a Bayesian–regularized neural network, *J. Comput.–Aided Mol. Des.* **2005**, *19*, 137–147.
[26] T. Langer, M. Eder, R. D. Hoffmann, P. Chiba, G. F. Ecker, Lead identification for modulators of multidrug resistance based on in silico screening with a pharmacophoric feature model, *Arch. Pharm.* (*Weinheim*). **2004**, *337*, 317–327.
[27] F. Cortes–Selva, M. Campillo, C. P. Reyes, I. A. Jimenez, S. Castanys, I. L. Bazzocchi, L. Pardo, F. Gamarro, A.G. Ravelo. SAR studies of dihydro–beta–agarofuran sesquiterpenes as inhibitors of the multidrug–resistance phenotype in a Leishmania tropica line overexpressing a P–glycoprotein–like transporter, *J. Med. Chem.* **2004**, *47*, 576–587.
[28] G. Comte, J. B. Daskiewicz, C. Bayet, G. Conseil, A. Viornery–Vanier, C. Dumontet, A. Di Pietro, D. Barron, *C*–Isoprenylation of Flavonoids Enhances Binding Affinity toward P–Glycoprotein and Modulation of Cancer Cell Chemoresistance, *J. Med. Chem.* **2001**, *44*, 763–768.

Biographies

Yan Li is a graduate student seeking for a Ph.D degree in Dalian University of Technology (DUT) under the supervision of Prof. Changhou Liu and Prof. Shuwei Zhang, with major research direction on QSAR of P–glycoprotein–related multidrug resistance (MDR) modulators. Doing research financially supported by Cooperation Scientific Research Fund supported by DUT and Dalian Institute of Chemical Physics (DICP), therefore she is also studying in DICP under supervision of Prof. Ling Yang.

Yonghua Wang is a graduate student seeking for a Ph.D degree in DICP, with major research direction of computer–aided molecular design and prediction of eADME (early absorption, distribution, metabolism and excretion).

Prof. Ling Yang is a pharmacological researcher in tradition Chinese medicine. After obtaining a Ph.D degree in Japan, he became a director of laboratory of pharmaceutical research discovery in DICP, with major research direction of eADME predictions of traditional Chinese medicines.

Prof. Changhou Liu (Ph.D) is a scientist in inorganic chemical technology of DUT, possessing many years of experience in taking specific inorganic chemical engineering projects and all kinds of catalysts.

Prof. Shuwei Zhang (Ph.D) is a researcher in inorganic chemical technology of DUT, also possessing many years of computational experience in chemical engineering projects with various chemical plants. He has developed a RPS software with national patents, aiding the design of new chemical plant, which has been successfully applied in many plants.