Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 70 (2005) 627–639
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Structure activity relationships and quantitative structure activity relationships for the flavonoid-mediated inhibition of breast cancer resistance protein

Shuzhong Zhang [a], Xinning Yang [a], Robert A. Coburn [b], Marilyn E. Morris [a,*]

[a] *Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, 517 Hochstetter Hall, University at Buffalo, State University of New York, Amherst, NY 14260-1200, USA*
[b] *Department of Chemistry, University at Buffalo, State University of New York, Amherst, NY 14260-1200, USA*

Received 6 April 2005; accepted 4 May 2005

Abstract

Breast cancer resistance protein (BCRP) is a newly identified ABC transporter, which plays an important role in drug disposition and represents an additional mechanism for the development of MDR. Flavonoids, a major class of natural compounds widely present in foods and herbal products, have been shown to be BCRP inhibitors. The objective of the present study was to elucidate the SAR and derive a QSAR model for flavonoid–BCRP interaction. The EC_{50} values for increasing mitoxantrone accumulation in MCF-7 MX100 cells for 25 flavonoids, from five flavonoid subclasses, were determined in this study or obtained from our previous publication [Zhang S, Yang X, Morris ME. Combined effects of multiple flavonoids on breast cancer resistance protein (ABCG2)-mediated transport. Pharm Res 2004;21(7):1263–73], and ranged from 0.07 ± 0.02 μM to 183 ± 21.7 μM. We found that the presence of a 2,3-double bond in ring C, ring B attached at position 2, hydroxylation at position 5, lack of hydroxylation at position 3 and hydrophobic substitution at positions 6, 7, 8 or 4′, are important structural properties important for potent flavonoid–BCRP interaction. These structural requirements are similar but not identical to those for potent flavonoid–NBD2 (P-glycoprotein) interaction, indicating that inhibition of BCRP by flavonoids may involve, in part, the binding of flavonoids with the NBD of BCRP. In addition, a QSAR model consisting three structural descriptors was constructed, and both internally and externally validated, suggesting the model could be used to quantitatively predict BCRP inhibition activity of flavonoids. These findings should be useful for predicting BCRP inhibition activity of other untested flavonoids and for guiding the synthesis of potent BCRP inhibitors for potential clinical application.

Keywords: Breast cancer resistance protein; Flavonoids; Structure activity relationships; Quantitative structure activity relationships; Mitoxantrone transport; Membrane transport

1. Introduction

Flavonoids (Fig. 1) are a large class of polyphenolic compounds, which are ubiquitously present in the green plant world, and they are also integral components abundant in our common diet, such as vegetables, fruits and plant-derived beverages. Over 6500 flavonoids have been described and the average intake of total flavonoids from the Western diet was estimated to be 200 mg to 1 g per day [2]. A number of studies [3–6] have suggested that flavonoids may play a protective role in the prevention of cancer, coronary heart diseases, bone loss and many other age-related diseases. Due to these perceived health beneficial activities and their low toxicity [4,7], hundreds of herbal preparations containing flavonoids are available in the market as over-the-counter dietary supplements, and the consumption of these products are becoming more and more widespread, along with the burgeoning public interest in alternative medicine and in disease prevention. However, since these products are classified as dietary supplements and their marketing does not require FDA

Abbreviations: ABC, ATP binding cassette; BCRP, breast cancer resistance protein; FTC, fumitremorgin C; MDR, multidrug resistance; MRP1, multidrug resistance-associated protein 1; MRP, multidrug resistance-associated protein 2; MX, mitoxantrone; QSAR, quantitative structure activity relationship; SAR, structure activity relationship; NBD, nucleotide binding domain

* Corresponding author. Tel.: +1 716 645 2842x230; fax: +1 716 645 3693.
E-mail address: memorris@buffalo.edu (M.E. Morris).

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.05.017

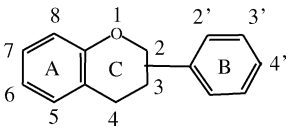

Fig. 1. Basic structure of flavonoids.

approval, the potential interactions of these herbal preparations with conventional drugs, in general, have not been carefully evaluated, leading to a serious concern about the safety of using these products. These concerns are relevant because significant or even life-threatening pharmacokinetic interactions of flavonoids or flavonoid-containing food/herbal products with conventional drugs have been observed in animals or patients [8–10]. Therefore, elucidating the interactions of flavonoids with molecular determinants that are important for drug disposition, such as drug transporters and drug metabolizing enzymes, as well as an understanding of the potential clinical consequences of these interactions, is very important.

One of the drug transporters for which flavonoid–drug interactions have been described is breast cancer resistance protein (BCRP, MXR, ABCP, ABCG2) [11,12], a newly identified membrane efflux transporter belonging to the ABC (ATP binding cassette) transporter superfamily [13–15]. This transporter extrudes its substrates out of the cells by using the energy derived from ATP hydrolysis. Since the transporter only has six transmembrane domains and one ATP binding site, distinct from other ABC proteins, which typically have a core structure of 12 transmembrane domains and two ATP binding sites, BCRP is considered an ABC half transporter and its transport activity may require the formation of a homodimer [16]. Many important drugs have been shown to be BCRP substrates including anthracyclines, topoisomerase I inhibitors, mitoxantrone (MX), methotrexate, flavopiridol, and nucleoside HIV reverse transcriptase inhibitors [17]. The expression of BCRP has been detected in a number of human tumors [18–21], and may represent an additional multidrug resistance (MDR) mechanism [22,23]. Therefore, potent inhibitors of BCRP may have the potential to reverse MDR in the treatment of cancer. High levels of BCRP are also present in excretory organs and tissues with barrier functions, such as liver canalicular membranes, the luminal surface of intestine, blood brain barrier, and human placenta [24–26]. This normal tissue localization profile indicates that BCRP may have an important role in drug disposition. This indication was subsequently confirmed by the observation that co-administration of GF120918 (a BCRP inhibitor) altered the disposition of topotecan (a BCRP substrate), including increase in bioavailability and decrease in biliary excretion, in mdr1a/1b (−/−) mice and in humans [27,28]. Therefore, BCRP inhibitors could potentially alter the pharmacokinetics of the drugs that are BCRP substrates, resulting in beneficial or adverse drug interactions.

We [11] and other investigators [12,29] have demonstrated that many naturally occurring flavonoids can inhibit BCRP. The EC_{50} values of the flavonoids chrysin, biochanin A and apigenin for BCRP inhibition (measured as the concentration of flavonoids for producing 50% of the maximal increase in MX (a BCRP substrate) accumulation in BCRP-overexpressing MCF-7 MX100 cells) were shown to be within the sub- or low micromolar range (0.39 ± 0.13, 1.62 ± 1.02 and 1.66 ± 0.55 μM, respectively) [1]. These EC_{50} values are likely much lower than the intestinal concentrations of these flavonoids after ingestion of food or herbal preparations [1]; thus, clinically relevant flavonoid–drug interactions may occur through a BCRP-mediated mechanism. In addition, flavonoids have little toxicity, and therefore, flavonoids with potent BCRP inhibition activities could be potentially used as reversal agents, or as the lead compounds for developing reversal agents, for BCRP-mediated MDR.

The objective of the present study was to elucidate the structure-activity relationship (SAR), and to derive a quantitative structure-activity relationship (QSAR) of flavonoid–BCRP interactions, in order to predict the BCRP inhibition activities of many other untested flavonoids, and to direct the synthesis of flavonoid compounds with higher potency for potential clinical application. To achieve this goal, a panel of 25 flavonoids, covering five flavonoid subclasses (flavones, isoflavones, chalcones, flavonols and flavanones), were selected and their EC_{50} values for BCRP inhibition were obtained from our previous report [1], or measured in the present study. The structural features of flavonoids important for BCRP inhibition were identified by comparing the EC_{50} values of flavonoids with and without a particular structural element. In addition, a QSAR model was constructed using a genetic algorithm coupled with multiple linear regression, and the model could be used to predict BCRP inhibition activities of flavonoids.

2. Materials and methods

2.1. Materials

MX and the flavonoid silybin were purchased from Sigma-Aldrich (St. Louis, MO). The other flavonoids were purchased from Indofine (Hillsborough, NJ). RPMI 1640, fetal bovine serum (FBS) and phosphate buffered saline (PBS) were purchased from Gibco BRL (Buffalo, NY). Human breast cancer MCF-7/sensitive and MCF-7 MX100 (MCF-7 cells selected with MX) and fumitremorgin C (FTC) were the kind gifts from Dr. Susan E. Bates (National Cancer Institute, Bethesda, MD). MCF-7 MX100 cells have been shown to over-express wild-type BCRP with no detectable expression of P-glycoprotein, MRP1, or MRP2 [1,11,30]; MCF-7/sensitive cells have no significant expression of any of these transporters [1,11].

2.2. Cell culture

MCF-7 cells (both sensitive and resistant) were cultured in 75 cm^2 flasks with RPMI 1640 culture media supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO_2/95% air. The culture media also contained 100 units/ml penicillin and 100 μg/ml streptomycin. For MCF-7 MX100 cells, the culture media also contained 100 nM of MX.

2.3. MX accumulation studies

The accumulation studies were performed using flow cytometric analysis as described previously [11]. Briefly, cells grown in 75 cm^2 flasks with ~90% confluence were trypsinized and washed with FBS-free RPMI 1640 and resuspended in this medium with a cell density of $\sim 10^6$ cells/ml. The accumulation of MX was performed by incubating 1 ml of cells with various concentrations of the test compounds or the vehicle (0.1% DMSO) at 37 °C for 15 min, followed by addition of 3 μM of MX. FTC (10 μM), a specific BCRP inhibitor [31,32], was used as a positive control. After incubation for another 30 min, the MX accumulation was stopped by adding 3 ml of ice-cold phosphate buffered saline (PBS) and centrifugation. The cells were then washed twice with ice-cold PBS again and the intracellular level of MX was analyzed using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) equipped with a standard argon laser for 488 nm excitation, and 670 nm bandpass filter was used to detect MX fluorescence. Preliminary studies demonstrated that the fluorescence of all the tested flavonoids at this setting is negligible (Data not shown). The accumulation of MX was expressed as percent of the control (in the presence of 0.1% of DMSO).

2.4. Calculation of EC_{50} in accumulation studies

The EC_{50} values of the flavonoids for increasing MX accumulation in MCF-7 MX100 cells were obtained by fitting the fraction (F) of the maximal increase in MX accumulation by the following equation, using WinNonlin (Pharsight, Mountain View, CA) as previously described [1]

$$F = \frac{C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}$$

F was calculated as the ratio of the net increase of MX accumulation by the test compounds ($A - A_0$) to the maximal net increase represented by the net increase of MX accumulation in the presence of 10 μM FTC ($A_{ftc} - A_0$) measured in parallel. FTC at 10 μM concentration was shown to inhibit BCRP completely in MCF-7 MX100 cells [1]. A_{ftc}, A and A_0 are the MX accumulation in the presence of 10 μM FTC, the test compounds and 0.1% DMSO (control), respectively. C is the concentration of flavonoids; γ is a slope factor. The EC_{50} values are expressed as mean ± S.D. from three independent experiments and in each experiment, triplicate measurements were obtained for each sample.

2.5. QSAR analysis

2.5.1. Datasets

For the QSAR analysis, the whole dataset of 25 flavonoids was randomly divided into a train set and a test set before any analysis was performed. The train set had 19 flavonoids and the test set had six compounds. A total of 115 structural descriptors were calculated for these flavonoids, among which log P values were calculated using the online version of KowWin program developed by Syracuse Research Corporation (http://www.syrres.com/esc/est_kowdemo.htm) and the other 114 structural descriptors were calculated using the QSARis© version 1.2 software (SciVision-Academic Press, San Diego, CA). These structural descriptors include molecular connectivity Chi indices, Kappa shape indices, electrotopological state indices, information indices, subgraph count indices, molecular polarizability, weight and volume.

2.5.2. Genetic algorithm coupled with multiple linear regression

The selection of the independent variables (structural descriptors) in the QSAR model was accomplished by a genetic algorithm coupled with MLR using QSARis© version 1.2 software. A population of 32 subsets called "chromosomes" was randomly generated. Each chromosome was encoded by a binary string of digits called "genes", with the length equal to the total number of descriptors (115 in the present study). The values of "1" and "0" indicate the selection and non-selection of the corresponding structural descriptors, respectively. The fitness function was defined as 1/LOF, and LOF represents Friedman's lack of fit defined as follows (QSARis© version 1.2)

$$LOF = \frac{Rss \times p/N}{(1 - (d(p+1)/N))^2}$$

where Rss is the residual sum of squares; p the number of the selected independent variables, and N the total number of cases (compounds) in the train set; d a smoothing factor used to prevent overfitting, which was set to "2" in the present study. The genetic operation includes crossover and mutation. Two chromosomes were selected as the "parent" chromosomes from the population by the "tournament" selection method and crossed-over at a predefined probability of 0.5 to produce two "offsprings". The offspring chromosomes were subjected to mutation at a probability of 0.1. A new population of the same size (32 chromosomes) was then obtained by replacing the less fit chromosome(s) in the initial population with the fitter

Table 1
The effects of flavonoids on mitoxantrone (MX) accumulation in MCF-7/sensitive and MCF-7 MX100 cells

Flavonoid	Flavonoid concentration (μM)	MCF-7/sensitive	MCF-7 MX100	
		MX accumulation (% of control)	MX accumulation (% of control)	MX accumulation (% of the maximal accumulation)
Control		100 ± 6.97	100 ± 8.62	
5,7-Dimethoxyflavone	30	85.3 ± 7.16	314 ± 109***	89.6 ± 8.28
6,2′,3′-Trimethoxyflavanone	50	115 ± 12.5*	340 ± 80.0***	101 ± 19.2
7,8-Benzoflavone	5	141 ± 8.80***	435 ± 64.3***	100 ± 17.8
Baicalein	30	83.5 ± 5.91	300 ± 123***	84.9 ± 13.1
5,6,7-Trimethoxyflavone	30	100 ± 13.7	322 ± 98.4***	92.4 ± 6.70
7,8-Dihydroxyflavone	50	89.7 ± 8.75	313 ± 54.0***	78.5 ± 1.87
Chalcone	50	108 ± 32.6	495 ± 140***	111 ± 19.4
Flavanone	100	130 ± 8.41***	394 ± 23.0***	86.5 ± 9.25
Flavone	50	121 ± 9.52***	306 ± 60.7***	88.9 ± 9.02
Galangin	10	102 ± 6.76	386 ± 27.8***	95.0 ± 20.0
7-Hydroxyflavanone	50	108 ± 6.70	310 ± 60.2***	87.6 ± 7.23
2′-Hydroxy-α-naphthoflavone	5	98.0 ± 7.98	336 ± 18.8***	101 ± 17.4
6,4′-Dimethoxy-3-hydroxyflavone	5	95.7 ± 5.30	312 ± 22.4***	94.4 ± 18.0
Kaempferide	10	101 ± 4.47	373 ± 64.1***	86.4± 3.77
7-Methoxyflavanone	50	109 ± 14.3	345 ± 39.8***	80.4 ± 11.6
8-Methylflavone	5	111 ± 9.50	469 ± 120***	102 ± 10.9

MX accumulation in MCF-7/sensitive ($n = 3$) and MCF-7 MX100 ($n = 9$) cells was examined in the presence of the specified concentrations of flavonoids or 0.1% DMSO (control) as described in Section 2, and expressed as percent of the control. In addition, the MX accumulation in the MCF-7 MX100 cells in the presence of flavonoids was also normalized by the maximal accumulation represented as the MX accumulation in the presence of 10 μM FTC measured in parallel. Data are expressed as mean ± SD.

* $p < 0.05$, ANOVA followed by Dunnett test.

*** $p < 0.001$, ANOVA followed by Dunnett test.

offspring(s) and the creation of such a new population is referred as a ''generation''. The model optimization was accomplished after 7000 generations and the fitness of the population reached a plateau. The structural descriptors represented by the fittest chromosome in the final population were then selected as the independent variables for MLR over the train data set to construct a QSAR model. The co-linearity of the selected variables was checked and no significant correlations were found between any of these variables.

The performance of the constructed model was evaluated by crossvalidation using the ''leave-one-out'' method and by testing the predictability of the model applied to an external test dataset. In the ''leave-one-out'' method, the model was re-constructed over the train set with one compound excluded and the target properties of the removed compound was then predicted by the reconstructed model. This process was repeated until every compound in the train set was excluded once; the Q^2 value of the crossvalidation was computed by the following equation

$$Q^2 = 1 - \frac{\sum_i (y_i - y_{(i)})^2}{\sum_i (y_{(i)} - y_{\text{mean}})^2}$$

where y_i is the predicted value for the ith compound using the model constructed with the ith compound excluded; $y_{(i)}$ the observed value for the ith compound and y_{mean} the mean value of the dataset. A good model will have a Q^2 value close to unity.

3. Results

3.1. MX accumulation in MCF-7/sensitive and MCF-7 MX100 cells

In order to test that the flavonoids selected for this study are BCRP inhibitors, and to ensure that the increase of MX accumulation in the BCRP-overexpressing MCF-7 MX100 cells by the flavonoids can be ascribed to the inhibition of BCRP by these compounds, we first evaluated the effects of the flavonoids on MX accumulation in both MCF-7/sensitive and MCF-7 MX100 cells. The flavonoids daidzein and silybin had already been shown [11] to be BCRP inhibitors, and produce significant increases in MX accumulation in MCF-7 MX100 cells with no significant effects in MCF-7/sensitive cells. As shown in Table 1, all the tested flavonoids increased the MX accumulation by more than 300% compared with the control in MCF-7 MX100 cells, and the tested concentration for each flavonoid was able to produce about 80% or more of the maximal MX accumulation (the MX accumulation in the presence of 10 μM FTC measured in parallel) in these resistant cells. Most of the tested flavonoids had no significant effects on MX accumulation in MCF-7/sensitive cells when tested at the same concentrations (Table 1). Although flavonoids 6,2′,3′-trimethoxyflavanone, 7,8-benzoflavone, flavanone and flavone produced slight but statistically significant increases in MX accumulation in MCF-7/sensitive cells, the extent of these increases was much lower than their effects in MCF-7 MX100 cells (Table 1) (MCF-7/sensitive

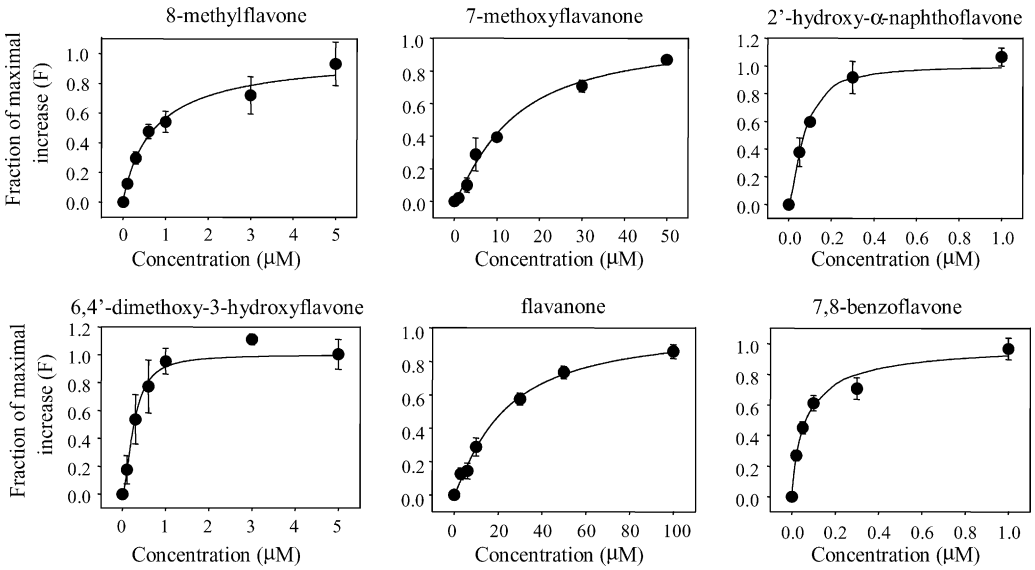

Fig. 2. Dose-response curves of the flavonoids for increasing mitoxantrone (MX) accumulation in MCF-7 MX100 cells. The accumulation of MX in MCF-7 MX100 cells in the presence of 0.1% DMSO (control), various concentrations of the test compounds, and 10 μM FTC was determined, and the increase of MX accumulation by the test compounds was calculated as described in Section 2. Presented are typical dose-response curves of some flavonoids for increasing MX accumulation. The circles represent the observed data and the solid lines represent the predicted data by fitting a Hill equation as described in Section 2. Data are expressed as mean ± S.D. from a typical triplicate experiment. Three separate experiments were conducted.

versus MCF-7 MX100: 115 ± 12.5% versus 340 ± 80.0% for 6,2′,3′-trimethoxyflavanone, 141 ± 8.80% versus 435 ± 64.3% for 7,8-benzoflavone, 130 ± 8.41% versus 394 ± 23.0% for flavanone and 121 ± 9.52% versus 306 ± 60.7% for flavone). In summary, all the tested flavonoids produced substantial increases in MX accumulation in BCRP-overexpressing MCF-7 MX100 cells with no or minimal effects in MCF-7/sensitive cells, which express negligible BCRP, indicating that the tested flavonoids are BCRP inhibitors and the increase of MX accumulation in MCF-7 MX100 cells by these flavonoids can be attributed to the inhibition of BCRP.

3.2. EC_{50} values for increasing MX accumulation in MCF-7 MX100 cells

To carry out the SAR and QSAR analysis, a measure of the potency of the flavonoids for inhibiting BCRP is required. In the present study, the potency for inhibiting BCRP was represented by the EC_{50} values of the flavonoids for increasing MX accumulation in the BCRP-overexpressing MCF-7 MX100 cells. For accurate estimation of these EC_{50} values, the concentration range examined for each flavonoid was carefully selected (Fig. 2) by performing preliminary studies, and the estimated EC_{50} values are listed in Table 2. The EC_{50} values of flavonoids apigenin, biochanin A, chrysin, genistein, kaempferol, hesperetin and naringenin for increasing MX accumulation in MCF-7 MX100 cells had been obtained in a previous study [1] and are also listed in this table. As we can

Table 2
The EC_{50} values of flavonoids for increasing mitoxantrone (MX) accumulation in MCF-7 MX100 cells

Flavonoids	EC_{50} (μM)	pEC_{50}
Apigenin*	1.66 ± 0.55	5.78
Chrysin*	0.39 ± 0.13	6.41
Flavone	3.99 ± 1.78	5.4
Galangin	1.21 ± 0.27	5.92
Kaempferol*	6.04 ± 0.09	5.22
5,7-Dimethoxyflavone	1.41 ± 0.26	5.85
7,8-Benzoflavone	0.07 ± 0.02	7.14
Trimethoxyflavanone	5.67 ± 4.74	5.25
Baicalein	1.21 ± 0.35	5.92
5,6,7-Trimethoxyflavone	1.09 ± 0.41	5.96
7,8-Dihydroxyflavone	20.1 ± 4.30	4.7
6,4′-Dimethoxy-3-hydroxy-flavone	0.45 ± 0.25	6.35
2′-Hydroxy-α-naphthoflavone	0.09 ± 0.05	7.05
Kaempferide	1.02 ± 0.33	5.99
8-Methylflavone	0.61 ± 0.15	6.21
Biochanin A*	1.62 ± 1.02	5.79
Daidzein	57.3 ± 15.8	4.24
Genistein*	14.9 ± 2.69	4.83
Flavanone	24.6 ± 5.21	4.6
Hesperetin*	12.4 ± 2.21	4.91
6,2′,3′-7-Hydroxyflavanone	14.2 ± 1.48	4.85
Silybin	183 ± 21.7	3.74
7-Methoxyflavanone	16.3 ± 4.25	4.79
Naringenin*	32.0 ± 3.22	4.49
Chalcone	11.7 ± 2.17	4.93

MX accumulation in the presence of different concentrations of each flavonoid or 10 μM FTC was evaluated and the EC_{50} values of the flavonoids for increasing MX accumulation was calculated as described in Section 2. Data are expressed as mean ± S.D., $n = 3$ independent experiments and in each experiment, triplicate measurements were performed for each flavonoid. pEC_{50} is defined as "$-\log (EC_{50} \times 10^{-6})$".

* Data were obtained from our previous study [1].

Table 3
Summary of the structure-activity relationships of flavonoid–BCRP interactions

Structural elements	BCRP inhibition activity
2,3-Double bond in ring C	Increase
Hydroxylation at:	
Position 3	Decrease
Position 5	Increase
Position 6	Decrease
Position 7	Increase moderately
Position 8	Decrease
Position 2′	No effect
Position 4′	Decrease
Methylation of the hydroxyl group at:	
Position 5	Decrease
Position 6	Increase
Position 7	No effect
Position 4′	Increase
Substitution of a methyl group or addition of a benzene ring to position 7 or 8	Increase
B-ring attached at position 3 (isoflavones)	Decrease

see, these EC_{50} values ranged from 0.07 ± 0.02 μM for 7,8-benzoflavone to 183 ± 21.7 μM for silybin, demonstrating a 2614-fold difference in the BCRP inhibition activities of these flavonoids.

3.3. Structure-activity analysis

The influence of some structural elements of flavonoids on BCRP inhibition activity was elucidated by comparing the EC_{50} values of flavonoids possessing or lacking a particular structural element. These structure-activity relationships are summarized in Table 3.

3.3.1. The 2,3-double bond in ring C

The important impact of the 2,3-double bond in ring C for the flavonoid–BCRP interaction was demonstrated in Fig. 3A. The EC_{50} value of flavanone (24.6 ± 5.21 μM) was shown to be 6.17-fold higher ($p < 0.01$) than that of flavone (3.99 ± 1.78 μM), and the EC_{50} value of naringenin (32.0 ± 3.22 μM) was shown to be 19.3-fold higher ($p < 0.001$) than that of apigenin (1.66 ± 0.55 μM). The only structural difference between these two pairs of compounds is that flavone and apigenin both have a 2,3-double bond in ring C but their counterparts, flavanone and naringenin, do not, indicating that the presence of the 2,3-double bond in the C ring of flavonoids is critical for potent BCRP inhibition. Consistent with this finding is the observation that among all the tested flavonoids, flavanones (the double bond in ring C is saturated), in general, have lower pEC_{50} values (equivalent to higher EC_{50}) than flavones (including flavonols) (Fig. 3B), and the mean EC_{50} value of all the tested flavanones (15.3 ± 2.87 μM, calculated as the average of the EC_{50} values of 7-hydroxyflavanone and 7-methoxyflavanone, $n = 6$ compounds) is 13.3-fold higher

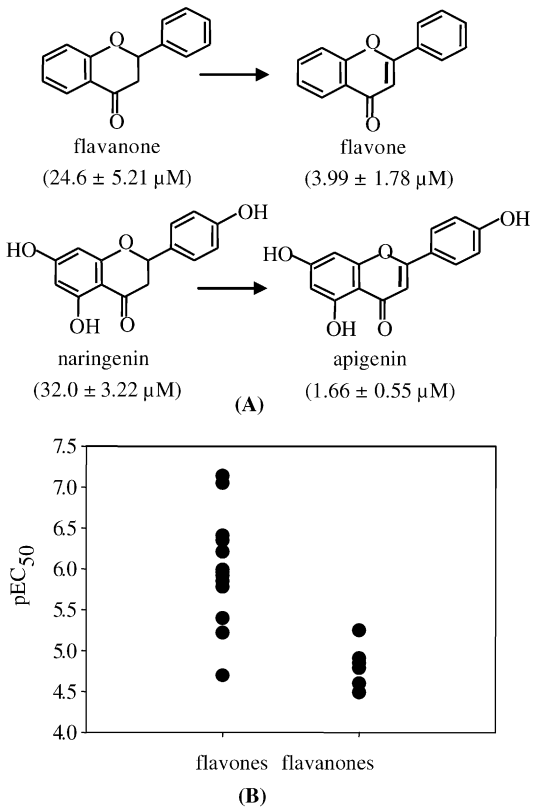

Fig. 3. Effect of the 2,3-double bond on BCRP inhibition activity. (A) comparison of the structures and EC_{50} values (in parentheses) with and without the 2,3-double bond. (B) comparison of the pEC_{50} ($-\log(EC_{50} \times 10^{-6})$) values of all the six test flavanones (without the 2,3-double bond) with those of all the 14 tested flavones (including flavonols, with the 2,3-double bond). The mean EC_{50} value of the flavones (including flavonols) was 1.15 ± 0.38 μM, much lower ($p < 0.001$) than that of the flavanones (15.3 ± 2.87 μM).

($p < 0.01$) than that of the flavones (1.15 ± 0.38 μM, calculated as the average of the EC_{50} values of baicalein and 5,6,7-trimethoxyflavone, $n = 14$ compounds).

3.3.2. Hydroxylation

The effects of flavonoid hydroxylation on BCRP inhibition activity are demonstrated in Fig. 4, and these effects appear to be variable depending on the positions where the hydroxyl groups are attached. As shown in Fig. 4A, attaching a hydroxyl group at position 4′ of the flavonoids resulted in an average 4.63-fold decrease in BCRP inhibition activity since the EC_{50} of apigenin (1.66 ± 0.55 μM) is 4.26-fold higher ($p < 0.05$) than that of chrysin (0.39 ± 0.13 μM), and the EC_{50} of kaempferol (6.04 ± 0.09 μM) was 4.99-fold higher ($p < 0.001$) than that of galangin (1.21 ± 0.27 μM). Similarly, hydroxylation at position 3 of the flavonoids also decreased BCRP inhibition activity by an average of 3.37-fold (Fig. 4B), as demonstrated by the 3.64-fold higher EC_{50} value ($p < 0.001$) of kaempferol (6.04 ± 0.09 μM) in comparison to that of apigenin (1.66 ± 0.55 μM), and the 3.10-fold higher EC_{50} value ($p < 0.01$) of galangin

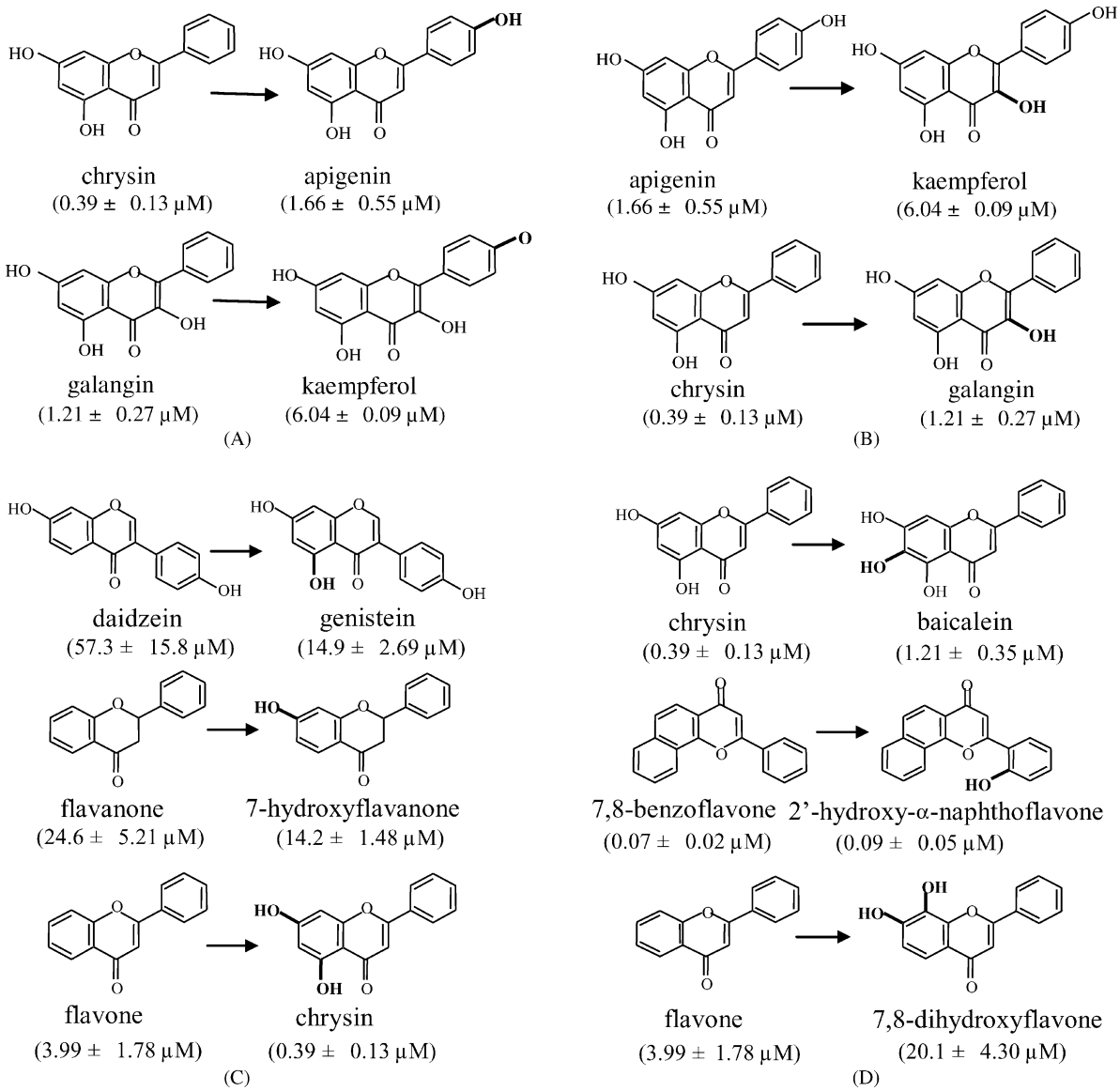

Fig. 4. Effects of hydroxylation on BCRP inhibition activity. Shown in this figure are the effects of structural modification by (A) 4′-hydroxylation, (B) 3-hydroxylation, (C) 5-hydroxylation and/or 7-hydroxylation, and (D) 6-hydroxylation, 2′-hydroxylation or 7,8-hydroxylation on BCRP inhibition activity. The EC_{50} values are presented in parentheses.

(1.21 ± 0.27 μM) in comparison to that of chrysin (0.39 ± 0.13 μM) (Fig. 4B). Additionally, hydroxylation at position 6 may also decrease BCRP inhibition activity because the EC_{50} of baicalein (1.21 ± 0.35 μM) was shown to be 3.10-fold higher ($p < 0.05$) than that of chrysin (0.39 ± 0.13 μM), which only differs structurally from the latter by an additional hydroxyl group at position 6 (Fig. 4D). Instead of the aforementioned depressing effects of hydroxylation on BCRP inhibition activity, attachment of hydroxyl groups to both position 5 and position 7 increased BCRP inhibition activity (Fig. 4C). As shown in Fig. 4C, the EC_{50} of genistein (14.9 ± 2.69 μM) decreased by 3.86-fold ($p < 0.05$) by hydroxylation at position 5 in comparison to that of daidzein (57.3 ± 15.8 μM), and the EC_{50} of 7-hydroxyflavanone (14.2 ± 1.48 μM) decreased by 1.73-fold ($p < 0.05$) by hydroxylation at position 7 in comparison to that of flavanone (24.6 ± 5.21 μM). Furthermore, simultaneous hydroxylation at both positions 5 and 7, as seen in chrysin (EC_{50}: 0.39 ± 0.13 μM), decreased EC_{50} value by 10.2-fold ($p < 0.05$) in comparison to that of flavone (3.99 ± 1.78 μM) (Fig. 4C), and the level of this decrease in EC_{50} (10.2-fold) is higher than the decreases by either hydroxylation at position 5 alone (3.86-fold) or hydroxylation at position 7 alone (1.73-fold). This provides additional support for the enhancing effects of hydroxylation at position 5, or 7 on BCRP inhibition activity. Hydroxylation at position 2′ seems to have no significant effect, since the EC_{50} of 7,8-benzoflavone (0.07 ± 0.02 μM) was not significantly different from that of 2′-hydroxy-α-naphthoflavone (0.09 ± 0.05 μM), which only structurally differs from the former by possessing a hydroxyl group at position

2′ (Fig. 4D). Lastly, hydroxylation at position 8 may decrease BCRP inhibition activity because the presence of hydroxyl groups at both position 7 and 8 in the flavonoid 7,8-dihydroxyflavone (EC_{50}: 20.1 ± 4.30 μM) increased the EC_{50} by 5.04-fold ($p < 0.01$) when compared with that of flavone (3.99 ± 1.78 μM) (Fig. 4D); however, hydroxylation at position 7, as aforementioned, was expected to decrease the EC_{50} value instead of increasing it. Therefore, the elevated EC_{50} of 7,8-dihydroflavone compared with that of flavone can be most likely attributed to the presence of the hydroxyl group at position 8.

3.3.3. O-Methylation (methoxylation)

The effects of O-methylation (methylation of hydroxyl groups) on flavonoid BCRP inhibition activity were also analyzed in this study. As shown in (Fig. 5A), methylation of the hydroxyl group at position 4′ as in biochanin A (EC_{50}: 1.62 ± 1.02 μM) and in kaempferide (EC_{50}: 1.02 ± 0.33 μM) decreased the EC_{50} by 9.20-fold ($p < 0.01$) and 5.92-fold ($p < 0.001$) when compared with genistein (EC_{50}: 14.9 ± 2.69 μM) and kaempferol (EC_{50}: 6.04 ± 0.09 μM), respectively, indicating that methylation of the hydroxyl group at position 4′ can substantially increase BCRP inhibition activity; however, methylation of the hydroxyl group at position 7 may have no significant effect, since 7-methoxyflavanone and 7-hydroxyflavanone were shown to have very similar EC_{50} values (16.3 ± 4.25 μM and 14.2 ± 1.48 μM, respectively, $p > 0.05$) (Fig. 5B). Simultaneous methylation of the hydroxyl groups at both position 5 and 7 as in 5,7-dimethoxyflavone (EC_{50}: 1.41 ± 0.26 μM) resulted in 3.61-fold lower EC_{50} value ($p < 0.01$) when compared with that of flavone (3.99 ± 1.78 μM); however, simultaneous methylation of the hydroxyl groups at position 5, 6 and 7 as in 5,6,7-trimethoxyflavone did not change the EC_{50} value (1.21 ± 0.35 μM, $p > 0.05$) significantly when compared with that of baicalein (1.09 ± 0.41 μM) (Fig. 5B). Since methylation of the hydroxyl group at position 7 appears to have no significant effect on BCRP inhibition activity, the increased EC_{50} of 5,7-dimethoxyflavone in comparison of flavone, therefore, is most likely due to the methylation of the hydroxyl group at position 5. Similarly, because methylation of the hydroxyl groups at both position 5 and 7 are expected to lower the BCRP inhibition activity, the insignificant alteration in the EC_{50} value produced by simultaneous methylation of hydroxyl groups at position 5, 6 and 7 (5,6,7-trimethoxyflavone versus baicalein) can be explained as follows: methylation of the hydroxyl group at position 6 decreased EC_{50}, which compensated for the increase of EC_{50} by the simultaneous methylation of hydroxyl groups at position 5 and 7, leading to no significant change. Therefore, methylation of the hydroxyl group at position 5 may decrease BCRP inhibition activity; however, methylation of the hydroxyl group at position 6 may increase BCRP inhibition activity.

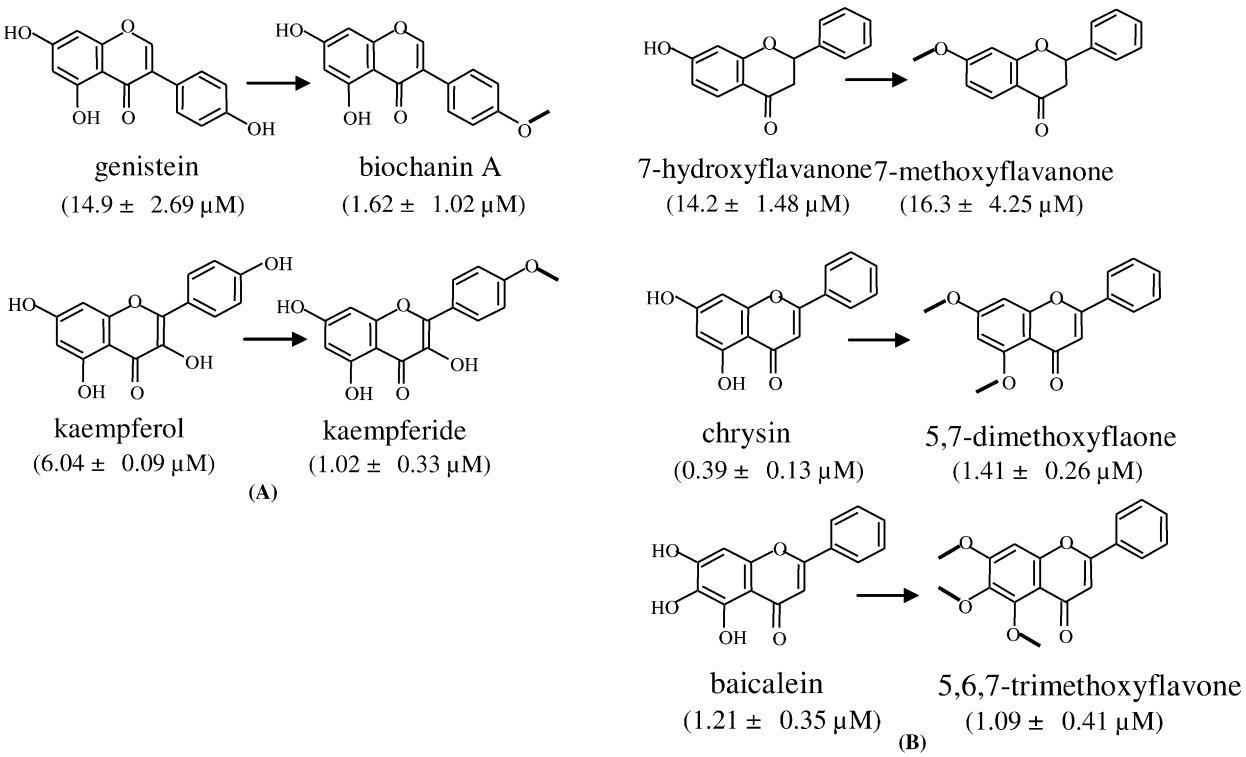

Fig. 5. Effects of methylation of the hydroxyl groups attached to the flavonoids on BCRP inhibition activity. Shown in the figure are the effects of O-methylation of hydroxyl groups at position 4′ (A) and at position 7, at positions 5 and 7, or at positions 5, 6 and 7 (B) on BCRP inhibition activity. The EC_{50} values are presented in parentheses.

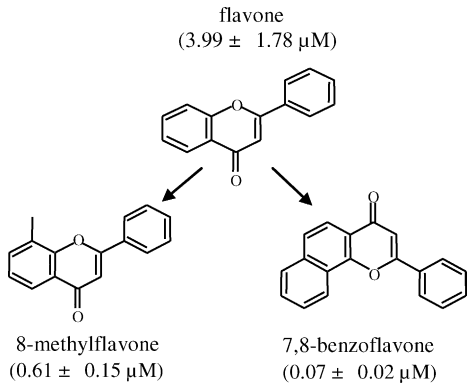

Fig. 6. Effects of adding hydrophobic groups to flavonoids at position 7 or 8 on BCRP inhibition activity. Addition of a methyl group to position 8 of flavone, as in 8-methylflavone, decreased EC_{50} value by 6.51-fold ($p < 0.05$); addition of a benzene ring at position 7 and 8 of flavone, as in 7,8-benzoflavone, decreased EC_{50} value by 54.9-fold ($p < 0.05$).

3.3.4. Substitution of hydrophobic groups to position 7 or 8

As shown in Fig. 6, addition of a methyl group to position 8 as in 8-methylflavone and addition of an extra aromatic ring to position 7 and 8 as in 7,8-benzoflavone (see Fig. 6 for the structure) decreased the EC_{50} (0.61 ± 0.15 μM and 0.07 ± 0.02 μM for 8-methyflavone and 7,8-benzoflavone, respectively) by 6.51-fold ($p < 0.05$) and 54.9-fold ($p < 0.05$), respectively, when compared with that of flavone (3.99 ± 1.78 μM). This finding suggests that substitution of hydrophobic groups at position 7 or 8 may dramatically increase BCRP inhibition activity.

3.3.5. Flavones versus isoflavones

As shown in Fig. 7, the EC_{50} of genistein (14.9 ± 2.69 μM), which is an isoflavone, was 8.98-fold higher ($p < 0.01$) than that of apigenin (1.66 ± 0.55 μM), indicating that isoflavones, in which the B ring is attached at position 3 instead of position 2, have lower BCRP inhibition activity than flavones with similar structures.

3.4. QSAR model

A QSAR model consisting of three structure descriptors (Table 4) was constructed as follows

$$pEC_{50} = 1.156 \times \log P + 0.891 \times \text{SdssC\_acnt} - 0.176 \times \text{Dy} + 0.480, \qquad (R^2 = 0.852,\ p < 0.0001)$$

The pEC_{50} values predicted by the model correlated very well with the observed pEC_{50} values for the train data set with correlation coefficient (R^2) equal to 0.852 (Fig. 8A). The "leave-one-out" cross validation produced a Q^2 value equal to 0.784, which is close to the R^2 value (0.852) and close to unity, indicating the constructed model performs stably. In addition, application of the model to an external test data set consisting of six flavonoids demonstrated that

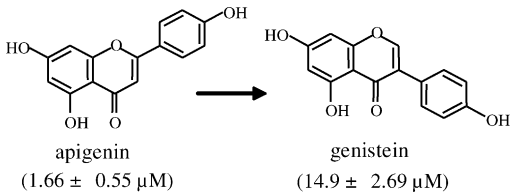

Fig. 7. Different BCRP inhibition activity between flavones and isoflavones. The isoflavone genistein, which has the B-ring present at position 3, had a significantly higher EC_{50} value ($p < 0.01$) than the corresponding flavone apigenin, which has the B-ring present at position 2.

Table 4
The structural descriptors included in the QSAR model

Symbol	Descriptor
log P	Calculated octanol–water partition coefficient
SdssC_acnt	Count of all =C< groups in a molecule
Dy	Moment of the displacement between the center-of-mass and the center-of-dipole along the inertial *Y*-axis

the model-predicted pEC_{50} values correlated well with the observed pEC_{50} values for the test data set as well ($p < 0.01$) with the R^2 equal to 0.922 (Fig. 8B), indicating that the constructed model is valid. Furthermore, as can been seen from Fig. 8C, the constructed model seems to predict the pEC_{50} values for all the five flavonoid subclasses reasonably well, since no subclasses are substantially skewed from the prediction line (the solid straight line in Fig. 8C). In summary, the constructed QSAR model could be used to predict the BCRP-inhibition activities of flavonoid compounds.

4. Discussion

In the past two decades, there has been a resurgence of scientific interest in flavonoids due to the association of a variety of health promoting activities with this class of naturally occurring compounds and the increasing human exposure. Numerous studies have indicated that flavonoids have anti-oxidant, anti-carcinogenic, anti-viral, anti-inflammatory and anti-estrogenic (estrogenic) activities [4,7]. These multiple activities and the structural diversity render this class of compounds a rich source for identifying lead compounds with targeted pharmacological properties, and both dietary and synthetic flavonoids have been subjected to clinical trials for prevention of post-menopausal symptoms or anticancer activities [33,34]. As such, a number of studies have been conducted to elucidate the structural requirements of flavonoids for their biological activities [35,36], in order to predict the potency of these compounds with regards to the targeted activity and to direct the synthesis of more potent analogues.

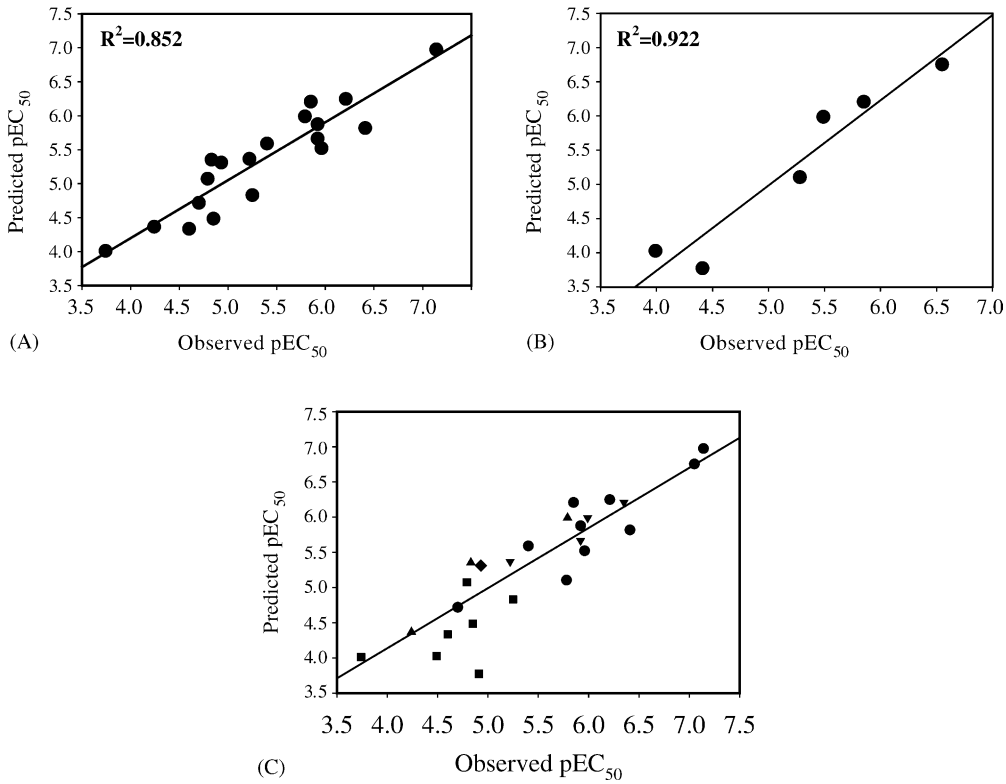

Fig. 8. Performance of the constructed QSAR model. (A) presents the correlation of the model-predicted pEC50 with the observed pEC50 for the train data set; (B) presents the correlation of the model-predicted pEC50 with the observed pEC50 for the test data set; the circles represent the locations (observed pEC50, predicted pEC50) of individual compounds and the solid straight lines are the regression lines. (C) presents the relationship between the model-predicted pEC50 values with the observed values for the whole data set (including both train and test data sets). The symbols represent the locations (observed pEC50, predicted pEC50) of flavonoids from different subclasses: (●) flavones; (■) flavanones; (▲) isoflavones; (◆) chalcones and (▼) flavonols; the solid straight line is the regression line.

The objective of the present study was to understand the SAR and QSAR of flavonoid–BCRP interactions. To achieve this goal, a valid measure of potency for flavonoid–BCRP interactions was required, and was represented by the EC_{50} values of the flavonoids for increasing MX accumulation in MCF-7 MX100 cells in the present study. The validity of this approach was confirmed by the observation that all the tested flavonoids can produce more than a 300% increase of MX accumulation (80% or more of the maximal accumulation) in MCF-7 MX100 cells at specified concentrations with no or marginal effects in MCF-7/sensitive cells. MCF-7 MX100 cells have been shown to overexpress BCRP, but MCF-7/sensitive cells have no or very little BCRP expression by Western blot analysis [11]. In addition, both MCF-7/sensitive and MCF-7 MX100 cells have no detectable P-glycoprotein, MRP1 or MRP2 expression [1,11]. Therefore, the increase of MX (a known BCRP substrate) accumulation in MCF-7 MX100 cells by the flavonoids can be ascribed to the inhibition of BCRP in these cells, and thus, the EC_{50} values of flavonoids for increasing MX accumulation in MCF-7 MX100 cells should be a valid measure of potency for flavonoid–BCRP interactions. Flavonoids 6,2′,3′-trimethoxyflavanone (50 μM), 7,8-benzoflavone (5 μM), flavone (50 μM) and flavanone (100 μM) did produce slight but statistically significant increases in MX accumulation in MCF-7/sensitive cells. The exact reason(s) for these increases is currently unknown, but could be due to the presence of a very low level of BCRP in these sensitive cells because BCRP mRNA was indeed detected, although very low, in these cells by Northern blot analysis [32] and the BCRP proteins in these cells were also detectable by flow cytometry [21].

The EC_{50} values of the 25 flavonoids included in this study for increasing MX accumulation in MCF-7 MX100 cells ranged from 0.07 ± 0.02 μM for 7,8-benzoflavone to 183 ± 21.7 μM for silybin, demonstrating a 2614-fold difference in the ability of these compounds to inhibit BCRP. The most potent flavonoids 7,8-benzoflavone and 2′-hydroxy-α-naphthoflavone have EC_{50} values lower than 0.1 μM, indicating that these flavonoids are more potent than the well-known potent BCRP inhibitor FTC, which was shown to have an EC_{50} value of 0.215 ± 0.008 μM [1]. Therefore, these compounds could be used as potent BCRP inhibitors, or as the lead compounds for developing more potent BCRP inhibitors for potential clinical application. The huge variation in the EC_{50} values of these flavonoids indicates that some structural features of flavonoids must be critical for flavonoid–BCRP interactions,

and a careful SAR analysis would be, therefore, very interesting and worthwhile.

By comparing the EC_{50} values of the flavonoids possessing or lacking a particular structural element, we have elucidated the effects of some structural properties of flavonoids on BCRP inhibition activity. The presence of a 2,3-double bond in ring C, as in flavone and apigenin, increased BCRP inhibition activity by 6.17- and 19.3-fold in comparison to flavanone and naringenin, respectively, indicating that the presence of the 2,3-double bond may be critical for inhibiting BCRP. Because flavone and apigenin have essentially a planar backbone structure due to the conjugation of π electrons in the presence of a 2,3-double bond and the carbonyl group at position 4, in contrast to a non-planar conformation of flavanone and naringenin lacking this double bond, it is very likely that a planar conformation may be beneficial for the binding of flavonoids to the binding site(s) on BCRP. This notion is also supported by the observation that among all the tested flavonoids, flavanones (nonplanar structure), in general, have lower BCRP inhibition activities than flavones (planar structure). In addition, the enhancing effects of the 2,3-double bond on BCRP inhibition appear to depend on other flavonoid substitutions as well, because the double bond in flavone (no substitution) increased BCRP inhibition activity by 6.17-fold, while it increased BCRP inhibition activity by 19.3-fold in apigenin (5,7,4′-hydroxylation). A plausible explanation for this interesting observation is that the conversion of a nonplanar conformation to a planar backbone with the presence of a 2,3-double bond could alter the orientation of other flavonoid substituents for interacting with BCRP, and therefore, results in additional changes in BCRP inhibition activity. This explanation is also consistent with the important roles of the flavonoid substitutions in determining BCRP inhibition activity, which will be discussed later.

Hydroxylation represents a predominant flavonoid substitution, and the effects of hydroxylation on BCRP inhibition activity were shown to be variable depending on the positions of hydroxylation. Hydroxylation at position 4′, position 3, position 6 or 8 decreases BCRP inhibition activity, indicating that substitution of this polar group at these positions is detrimental for BCRP inhibition and the interaction of these sites of flavonoids with BCRP might be mainly through hydrophobic interactions. Consistent with this hypothesis is that increasing local hydrophobicity by O-methylation (demonstrated for position 6 and 4′) or methylation (demonstrated for position 8) substantially increased BCRP inhibition activity. In addition, the local hydrophobicity at or close to position 8 appears to correlate with BCRP inhibition activity, since the EC_{50} values of flavone, 8-methylflavone and 7,8-benzoflavone are 3.99 ± 1.78 μM, 0.61 ± 0.15 μM and 0.07 ± 0.02 μM, respectively, inversely correlate with their local hydrophobicity at position 8 (–H, $–CH_3$ and benzene ring, respectively). This latter observation provided additional support for the beneficial effects of high local hydrophobicity at position 8 on BCRP interactions. In contrast to the detrimental effects of hydroxylation at positions 3, 6, 8 and 4′ on the flavonoid–BCRP interactions, hydroxylation at position 5 increased BCRP inhibition activity. This potentiating effect on BCRP inhibition may be due to the formation of a hydrogen bond between the 5-hydroxyl group and the flavonoid BCRP binding site. Consistently, O-methylation of this hydroxyl group appears to decrease BCRP inhibition activity substantially, suggesting that elimination of the oxygen-bonded hydrogen atom prevented the formation of the hydrogen bond, and thus, decreased the interaction of position 5 with BCRP binding site. Hydroxylation at position 7 was also shown to increase BCRP inhibition activity, indicating that there may be also a hydrogen bond formation between 7-hydroxyl group and the BCRP binding site; however, the formation of a hydrogen bond between position 7 and BCRP binding site may be not as important as the hydrogen bond between position 5 and BCRP binding site, and this hydrogen bond interaction could be replaced by strong hydrophobic interactions. This hypothesis is supported by the data demonstrating that hydroxylation at position 7 increased BCRP inhibition activity to a lesser extent (1.73-fold compared with 3.86-fold by hydroxylation at position 5) and attachment of a hydrophobic benzene ring, at position 7 and 8 (7,8-benzoflavone) resulted in a very high BCRP inhibition activity (EC_{50}: 0.07 ± 0.02 μM). In addition, O-methylation of the 7-hydroxyl group did not significantly alter the BCRP inhibition activity, possibly since the formation of a hydrogen bond between position 7 and the BCRP binding site is not essential, as discussed above, or that the 7-hydroxyl group acts as a hydrogen bond acceptor, and therefore O-methylation does not affect the hydrogen bond formation. Lastly, we also demonstrated that isoflavonoids, which have B ring present at position 3, may have lower BCRP inhibition activity than flavonoids with a B ring present at position 2.

It is interesting to point out that the SAR for flavonoid–BCRP interactions demonstrated in the present study share considerable similarities with that established for flavonoid–P-glycoprotein interactions [35,37,38]. For example, the presence of a 2,3-double bond in ring C, a B-ring present at position 2 instead of position 3 (isoflavonoids), hydroxylation at position 5 and hydrophobic substitution at position 8 and 4′, which are all structural features important for potent flavonoid–BCRP interactions, have also been shown to be important for potent interaction of flavonoids with the C-terminal nucleotide binding domain (NBD2) of mouse P-glycoprotein [35,37,38]. These similar structural requirements may indicate that the interaction of flavonoids with BCRP may share a similar mechanism(s) with flavonoid–P-glycoprotein interaction; more specifically, because the similar structural requirements for flavonoid BCRP inhibition and for flavonoid-NBD2 (P-glycoprotein) binding, it is reasonable to speculate that

flavonoids may inhibit BCRP by, at least partly, binding to the nucleotide binding domain (NBD) of this transporter. On the other hand, the SAR for flavonoid-BCRP and flavonoid–P-glycoprotein interactions is by no means identical. A notable difference is that 3-hydroxylation was shown to increase flavonoid–P-glycoprotein interaction and O-methylation of this hydroxyl group markedly decreased the interaction [38], in contrast to a negative effect of this hydroxyl group on flavonoid–BCRP interaction. In addition, although hydrophobic substitution at position 4′ increased both flavonoid-P-glycoprotein and flavonoid–BCRP interactions, hydroxylation, as well as methoxylation at this position appears to have no significant effects on flavonoid–P-glycoprotein interactions [37]; however, hydroxylation at the same position markedly decreased the flavonoid–BCRP interaction and O-methylation essentially reversed the effect of the hydroxylation. Furthermore, hydroxylation at position 7 did not produce much effect on flavonoid–P-glycoprotein interaction [38], but it did moderately increase the flavonoid–BCRP interaction (1.73-fold). All these differences in the SAR for flavonoid-BCRP and flavonoid–NBD2 (glycoprotein) interactions may reflect the distinct structural requirements for binding to NBD(s) of BCRP and P-glycoprotein, or may be due to the involvement of other interaction mechanism(s) other than binding to the NBD of BCRP for flavonoid BCRP inhibition.

In addition to the SAR analysis, we also constructed a QSAR model for the quantitative prediction of flavonoid-BCRP inhibition activity. The independent variables in the QSAR model were selected from a large number of calculated topological descriptors, log P and a limited number of 3D descriptors (molecular volume and surface area) by a genetic algorithm. This approach has been already employed in a number of QSAR studies [39,40] and was shown to be able to generate models superior or comparable [39,40] to those constructed by 3D QSAR analysis such as CoMFA (Comparative Molecular Field Analysis), while avoiding ambiguities in the selection of the most likely molecular conformation and molecular alignment inherent in CoMFA process. The model predicted data correlated well ($R^2 = 0.852$) with the experimental data over the entire train data set, and internal validation (leave-one-out cross validation) indicates that the model performs stably ($Q^2 = 0.784$). Since an acceptable Q^2 value in a leave-one-out cross validation is only a necessary but not a sufficient condition for a model to have high predictive power [41], the predictability of the model was further tested on an external test data set consisting of six compounds. It was found that the model predicted data also correlated well with the experimental data over the entire test data set ($R^2 = 0.922$). Therefore, the constructed QSAR model may be suitable for predicting the BCRP inhibition activity of flavonoids. Interestingly, based on the QSAR model, log P makes a positive contribution to the BCRP inhibition activity, which is consistent with our SAR analysis, where we demonstrated that increasing local hydrophobicity at position 6, 7, 8 or 4′ could increase BCRP inhibition activity. Unfortunately, further connections of the other two structural descriptors in the QSAR model (SdssC acnt and Dy) with the SAR are difficult, mainly because of a lack of detailed interpretation of those descriptors. However, such a detailed interpretation may be unnecessary for a QSAR model to be used mainly for prediction instead of for drug design.

In summary, the structural properties of flavonoids important for inhibiting BCRP have been identified by comparing the EC_{50} values of the flavonoids for increasing MX accumulation in MCF-7 MX100 cells overexpressing BCRP. The structural requirements for potent flavonoid-BCRP, such as the presence of a 2,3-double bond in ring C, ring B attached at position 2, hydroxylation at position 5, lack of hydroxylation at position 3 and hydrophobic substitution at positions 6, 7, 8 or 4′, were shown to be similar, but not identical to those for flavonoid–P-glycoprotein interaction. In addition, a QSAR model for flavonoid–BCRP interaction was also constructed. Both internal and external validations indicate that the model could be used to predict BCRP inhibition activity of other untested flavonoids. These findings should be very useful for developing potent BCRP inhibitors from flavonoid compounds (e.g. 7,8-benzoflavone) for potential clinical application.

Acknowledgements

Financial support for this study was provided by grants from the Susan G. Komen Breast Cancer Foundation and Pfizer Global Research and Development. SZ was the recipient of a graduate fellowship from Pfizer Global Research and Development.

References

[1] Zhang S, Yang X, Morris ME. Combined effects of multiple flavonoids on breast cancer resistance protein (ABCG2)-mediated transport. Pharm Res 2004;21(7):1263–73.
[2] Harborne JB, Williams CA. Advances in flavonoid research since. Phytochemistry 2000;55(6):481–504.
[3] Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993;342(8878):1007–11.
[4] Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther 2002;96(2–3):67–202.
[5] Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, Day NE. Dietary effects on breast-cancer risk in Singapore. Lancet 1991;337(8751):1197–200.
[6] Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman Jr JW. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr 1998;68(6 Suppl):1375S–9S.
[7] Middleton Jr E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52(4):673–751.

[8] Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ, et al. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. Clin Pharmacol Ther 2002;71(1):11–20.
[9] Hsiu SL, Hou YC, Wang YH, Tsao CW, Su SF, Chao PD. Quercetin significantly decreased cyclosporin oral bioavailability in pigs and rats. Life Sci 2002;72(3):227–35.
[10] Ruschitzka F, Meier PJ, Turina M, Luscher TF, Noll G. Acute heart transplant rejection due to Saint John's wort. Lancet 2000;355(9203): 548–9.
[11] Zhang S, Yang X, Morris ME. Flavonoids are inhibitors of breast cancer resistance protein (ABCG2)-mediated transport. Mol Pharmacol 2004;65(5):1208–16.
[12] Cooray HC, Janvilisri T, van Veen HW, Hladky SB, Barrand MA. Interaction of the breast cancer resistance protein with plant polyphenols. Biochem Biophys Res Commun 2004;317(1):269–75.
[13] Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res 1998;58(23):5337–9.
[14] Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci USA 1998;95(26):15665–70.
[15] Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, et al. Molecular cloning of cDNAs, which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res 1999;59(1):8–13.
[16] Litman T, Druley TE, Stein WD, Bates SE. From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. Cell Mol Life Sci 2001;58(7):931–59.
[17] Doyle LA, Ross DD. Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). Oncogene 2003;22(47): 7340–58.
[18] Ross DD, Karp JE, Chen TT, Doyle LA. Expression of breast cancer resistance protein in blast cells from patients with acute leukemia. Blood 2000;96(1):365–8.
[19] Kanzaki A, Toi M, Nakayama K, Bando H, Mutoh M, Uchida T, et al. Expression of multidrug resistance-related transporters in human breast carcinoma. Jpn J Cancer Res 2001;92(4):452–8.
[20] Sargent JM, Williamson CJ, Maliepaard M, Elgie AW, Scheper RJ, Taylor CG. Breast cancer resistance protein expression and resistance to daunorubicin in blast cells from patients with acute myeloid leukaemia. Br J Haematol 2001;115(2):257–62.
[21] van der Kolk DM, Vellenga E, Scheffer GL, Muller M, Bates SE, Scheper RJ, et al. Expression and activity of breast cancer resistance protein (BCRP) in de novo and relapsed acute myeloid leukemia. Blood 2002;99(10):3763–70.
[22] Steinbach D, Sell W, Voigt A, Hermann J, Zintl F, Sauerbrey A. BCRP gene expression is associated with a poor response to remission induction therapy in childhood acute myeloid leukemia. Leukemia 2002;16(8):1443–7.
[23] Friedrich RE, Punke C, Reymann A. Expression of multi-drug resistance genes (mdr1, mrp1, bcrp) in primary oral squamous cell carcinoma. In Vivo 2004;18(2):133–47.
[24] Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, et al. Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res 2001;61(8):3458–64.
[25] Cooray HC, Blackmore CG, Maskell L, Barrand MA. Localisation of breast cancer resistance protein in microvessel endothelium of human brain. Neuroreport 2002;13(16):2059–63.
[26] Cisternino S, Mercier C, Bourasset F, Roux F, Scherrmann JM. Expression, up-regulation, and transport activity of the multidrug-resistance protein Abcg2 at the mouse blood–brain barrier. Cancer Res 2004;64(9):3296–301.
[27] Jonker JW, Smit JW, Brinkhuis RF, Maliepaard M, Beijnen JH, Schellens JH, et al. Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. J Natl Cancer Inst 2000;92(20):1651–6.
[28] Kruijtzer CM, Beijnen JH, Rosing H, ten Bokkel Huinink WW, Schot M, Jewell RC, et al. Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. J Clin Oncol 2002;20(13):2943–50.
[29] Imai Y, Tsukahara S, Asada S, Sugimoto Y. Phytoestrogens/flavonoids reverse breast cancer resistance protein/ABCG2-mediated multidrug resistance. Cancer Res 2004;64(12):4346–52.
[30] Robey RW, Steadman K, Polgar O, Morisaki K, Blayney M, Mistry P, et al. Pheophorbide a is a specific probe for ABCG2 function and inhibition. Cancer Res 2004;64(4):1242–6.
[31] Rabindran SK, He H, Singh M, Brown E, Collins KI, Annable T, et al. Reversal of a novel multidrug resistance mechanism in human colon carcinoma cells by fumitremorgin C. Cancer Res 1998; 58(24):5850–8.
[32] Robey RW, Honjo Y, van de Laar A, Miyake K, Regis JT, Litman T, et al. A functional assay for detection of the mitoxantrone resistance protein, MXR (ABCG2). Biochim Biophys Acta 2001;1512(2): 171–82.
[33] Nikander E, Kilkkinen A, Metsa-Heikkila M, Adlercreutz H, Pietinen P, Tiitinen A, et al. A randomized placebo-controlled crossover trial with phytoestrogens in treatment of menopause in breast cancer patients. Obstet Gynecol 2003;101(6):1213–20.
[34] Aklilu M, Kindler HL, Donehower RC, Mani S, Vokes EE. Phase II study of flavopiridol in patients with advanced colorectal cancer. Ann Oncol 2003;14(8):1270–3.
[35] Boumendjel A, Di Pietro A, Dumontet C, Barron D. Recent advances in the discovery of flavonoids and analogs with high-affinity binding to P-glycoprotein responsible for cancer cell multidrug resistance. Med Res Rev 2002;22(5):512–29.
[36] van Zanden JJ, Geraets L, Wortelboer HM, van Bladeren PJ, Rietjens IM, Cnubben NH. Structural requirements for the flavonoid-mediated modulation of glutathione S-transferase P1-1 and GS-X pump activity in MCF7 breast cancer cells. Biochem Pharmacol 2004;67(8): 1607–17.
[37] Boumendjel A, Bois F, Beney C, Mariotte AM, Conseil G, Di Pietro A. B-ring substituted 5,7-dihydroxyflavonols with high-affinity binding to P-glycoprotein responsible for cell multidrug resistance. Bioorg Med Chem Lett 2001;11(1):75–7.
[38] Conseil G, Baubichon-Cortay H, Dayan G, Jault JM, Barron D, Di Pietro A. Flavonoids: a class of modulators with bifunctional interactions at vicinal ATP- and steroid-binding sites on mouse P-glycoprotein. Proc Natl Acad Sci USA 1998;95(17):9831–6.
[39] Hoffman B, Cho SJ, Zheng W, Wyrick S, Nichols DE, Mailman RB, et al. Quantitative structure-activity relationship modeling of dopamine D(1) antagonists using comparative molecular field analysis, genetic algorithms-partial least-squares, and K nearest neighbor methods. J Med Chem 1999;42(17):3217–26.
[40] Wanchana S, Yamashita F, Hashida M. QSAR analysis of the inhibition of recombinant CYP 3A4 activity by structurally diverse compounds using a genetic algorithm-combined partial least squares method. Pharm Res 2003;20(9):1401–8.
[41] Golbraikh A, Tropsha A. Beware of q2! J Mol Graph Model 2002; 20(4):269–76.