Cancer Chemother Pharmacol (2007) 60:789–797
DOI 10.1007/s00280-007-0426-7

ORIGINAL ARTICLE

Flavonoids inhibit breast cancer resistance protein-mediated drug resistance: transporter specificity and structure–activity relationship

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Received: 15 September 2006 / Accepted: 15 January 2007 / Published online: 8 March 2007

Abstract
Purpose ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-related protein 1 (MRP1), confer resistance to various anticancer agents. We previously reported that some flavonoids have BCRP-inhibitory activity. Here we show the reversal effects of an extensive panel of flavonoids upon BCRP-, P-gp-, and MRP1-mediated drug resistance.
Methods Reversal effects of flavonoids upon BCRP-, P-gp-, or MRP1-mediated drug resistance were examined in the *BCRP*- or *MDR1*-transduced human leukemia K562 cells or in the *MRP1*-transfected human epidermoid carcinoma KB-3-1 cells using cell growth inhibition assays. The IC_{50} values were determined from the growth inhibition curves. The RI_{50} values were then determined as the concentration of inhibitor that causes a twofold reduction of the IC_{50} in each transfectant. The reversal of BCRP activity was tested by measuring the fluorescence of intracellular topotecan.
Results The BCRP-inhibitory activity of 32 compounds was screened, and 20 were found to be active. Among these active compounds, 3′,4′,7-trimethoxyflavone showed the strongest anti-BCRP activity with RI_{50} values of 0.012 μM for SN-38 and 0.044 μM for mitoxantrone. We next examined the effects of a panel of 11 compounds on P-gp- and MRP1-mediated drug resistance. Two of the flavones, 3′,4′,7-trimethoxyflavone and acacetin, showed only low anti-P-gp activity, with the remainder displaying no suppressive effects against P-gp. None of the flavonoids that we tested inhibited MRP1.
Conclusion Our present results thus indicate that many flavonoids selectively inhibit BCRP only. Moreover, we examined the structure–BCRP inhibitory activity relationship from our current study.

Keywords BCRP/ABCG2 · P-glycoprotein/ABCB1 · MRP1/ABCC1 · Flavonoid · Growth inhibition assay

Abbreviations

ABC	ATP-binding cassette
BCRP	Breast cancer resistance protein
MDR	Multidrug resistance
P-gp	P-glycoprotein
MRP1	Multidrug resistance-related protein 1
SN-38	7-Ethyl-10-hydroxycamptothecin (the active metabolite of irinotecan)
VP-16	Etoposide

Introduction

Tumor cells often acquire multidrug resistance, characterized by cross-resistance to other structurally unrelated

Grant support: The Ministry of Education, Culture, Sports, Science, and Technology, and the Ministry of Health, Labor and Welfare, Japan.

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agents [1]. Multidrug-resistant cells express ABC transporters, such as the *MDR1* gene product P-glycoprotein (P-gp)/ABCB1, breast cancer resistance protein (BCRP)/ABCG2, and multidrug resistance-related protein 1 (MRP1)/ABCC1, that pump out various structurally unrelated anticancer agents in an ATP-dependent manner. In the 1980s, verapamil was firstly found to increase the intracellular concentration of anticancer agents in multidrug-resistant cells by binding P-gp and inhibiting the P-gp-mediated drug efflux [2, 3]. Subsequently, many P-gp inhibitors such as valspoder (PSC-833), dofequidar fumarate (MS-209), tariquidar (XR9576), and thiose-micarbazone derivative (NSC73306) have been developed that also interact with P-gp and reverse P-gp-mediated drug resistance [4–7]. Clinical trials using such P-gp inhibitors have shown an in vivo increase in the intracellular concentration of coadministered anticancer agents in P-gp-positive tumor cells [8]. However, phase III trials of these agents have not been successful and no significant survival benefit of P-gp inhibition has yet been achieved [9, 10]. Further clinical studies using new P-gp inhibitors and new combination treatment regimens have been devised however, and some are now ongoing.

BCRP is a half-molecule ABC transporter with an NH_2-terminal ATP-binding site and COOH-terminal transmembrane domain [11–15]. Recently, we reported that BCRP forms homodimers via a disulfide bridge between Cys603, a residue on the third outer-membrane domain of the BCRP monomer [16, 17]. The homodimeric BCRP complex acts as an efflux pump for various anticancer agents including SN-38, mitoxantorone, and topotecan, and thus prevents the build up of high intracellular concentrations of such anticancer agents and decreases their cytotoxic effects. BCRP is reportedly also expressed in various normal human tissues and cells, such as the placenta, liver, brain, spinal code, adrenal gland, testes, prostate, uterus, kidney, heart, bone marrow, and small intestine [18]. Furthermore, BCRP is expressed in hematopoietic stem cells and is thought to be a stem cell marker [19]. We previously reported that estrone and 17β-estradiol inhibit BCRP-mediated drug transport and resistance. In addition, we have found from our studies that BCRP transports sulfated estrogens as physiologic substrates but not as free estrogens [20, 21]. We further demonstrated that some flavonoids, such as genistein and naringenin, diminished the function of BCRP as an efflux pump and reversed BCRP-mediated resistance to anticancer agents [22]. Flavopiridol, a flavonoid-derived antitumor agent, is a substrate of BCRP [23], and flavonoids and estrogenic compounds thus possess BCRP inhibitory properties.

In our current study, we screened a further panel of flavonoids possessing inhibitory activity for BCRP, including 29 flavonoids and 3 flavonoid-related compounds (total 32), by cell growth inhibition assay. We find that 20 of these compounds harbor inhibitory activity against BCRP. However, although two of the flavonoids that we tested induced a weak reversal of P-gp-mediated multidrug resistance, none of the other compounds displayed any inhibitory properties toward P-gp. Additionally, none of the flavonoids screened in this study were found to inhibit MRP1. We thus conclude that they selectively target BCRP only.

Materials and methods

Reagents

Flavonoid compounds were purchased from Funakoshi (Tokyo, Japan). Fumitremorgin C (FTC) was purchased from Alexis (San Diego, CA, USA). Anti-BCRP polyclonal antibody (3488) was raised by immunizing rabbits with a KLH-conjugated 20-mer peptide corresponding to the amino acid region 340–359 of the human BCRP protein [16]. The anti-P-gp monoclonal antibody (C219) was purchased from Zymed (South San Francisco, CA, USA), and the anti-MRP1 monoclonal antibody (MRPm6) was obtained from Nichirei (Tokyo, Japan).

Cells and cell culture

K562/BCRP and K562/MDR cells were established from human leukemia K562 cells in our laboratory [22], and grown in RPMI 1640 medium supplemented with 7% fetal bovine serum at 37°C in 5% CO_2. Human epidermoid carcinoma KB-3-1 cells were cultured in DMEM supplemented with 7% fetal bovine serum at 37°C in 5% CO_2. KB/MRP1 cells were established using the following procedures: KB-3-1 cells were transfected with the pCAL-MRP1 construct bearing the human *MRP1* cDNA insert by the use of the Mammalian Transfection Kit (Stratagene, La Jolla, CA, USA). This was followed by selection with increasing concentrations of etoposide (VP-16). The cells were subcloned, and the MRP1 expression levels of each clone was confirmed by western blotting with an anti-MRP1 monoclonal antibody. The western blotting procedure used has been described previously [16]. Subclone 14 showed the highest expression of MRP1 and these cells were thus further selected with increasing concentrations of doxorubicin for 4 weeks and designated as KB/MRP1 cells.

Growth inhibition assay

The effects of flavonoids on the sensitivity of cells to various cytotoxic agents were evaluated by measuring cell growth inhibition after incubation at 37°C for 5 days in the absence or presence of various concentrations of anticancer drugs in combination with the test compounds. Cell numbers were determined with a Coulter counter. The IC_{50} values (drug dose causing 50% inhibition of cell growth) were determined from the growth inhibition curves. The RI_{50} values were then determined as the concentration of inhibitor that causes a twofold reduction of the IC_{50} in each transfectant. RI_{50}^{-1}, the reciprocal value of RI_{50}, was also used as a reverse activity measurement of drug resistance.

Topotecan uptake

The intracellular accumulations of topotecan were determined by measuring the fluorescence spectrophotometrically. K562 or K562/BCRP cells (2×10^6 cells) were suspended in 1 ml of RPMI 1640 medium containing 0.5 μM topotecan and appropriate concentrations of the compounds. The cells were incubated at 37°C for 30 min, and washed with ice-cold PBS. The intracellular topotecan was extracted from the cells with 1 ml of ethanol. The intensity of topotecan fluorescence was measured using a fluorescent spectrophotometer.

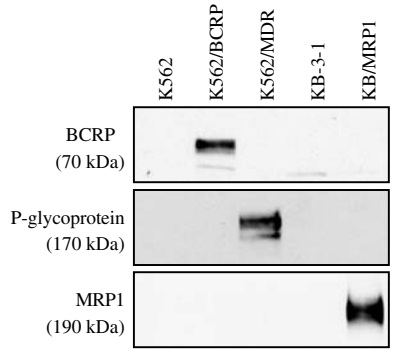


Fig. 1 Analysis of the expression levels of BCRP, P-gp, and MRP1 in stably transfected cells by western blot. Cell lysates (20 μg/lane) were resolved by SDS-PAGE and transferred onto nitrocellulose membranes. The expression levels of BCRP, P-gp, or MRP1 were then detected by incubation of the membrane with anti-BCRP polyclonal antibody (3488), anti-P-gp monoclonal antibody (C219), or anti-MRP1 monoclonal antibody (MRPm6), respectively

Table 1 Drug resistance of each resistant cells

Resistant protein	Resistant cells	Relative resistance factor			
		SN-38	VCR	Dox	VP-16
BCRP	K562/BCRP	21	ND	ND	ND
P-gp	K562/MDR	ND	160	ND	ND
MRP1	KB/MRP	2.6	25	8.8	12

Parental or resistant cells were cultured for 5 days with increasing concentrations of the indicated drugs. Cell numbers were counted with a Coulter counter, and IC_{50} was determined. Relative resistance factor is the ratio of IC_{50} for the resistant cells divided by that for the parental cells

VCR Vincristine, *Dox* doxorubicin, ND not determined

Results

To screen the various flavonoids under analysis, we used *MDR1*, *BCRP*, and *MRP1* cDNA transfectants. The expression of the ABC transporters in each transfectant was first confirmed by western blot. The parental cells, K562 or KB-3-1, did not express any of the three ABC transporters, whereas the K562/BCRP, K562/MDR, and KB/MRP1 cells expressed exogenous BCRP, P-gp, and MRP1, respectively (Fig. 1). These transfectants did not express any of other transporters (Fig. 1). We next examined the degree of resistance to various anticancer agents in the K562/BCRP, K562/MDR, and KB/MRP1 cells, compared with the corresponding parental cells. K562/BCRP cells showed a 21-fold higher resistance to SN-38 than K562 cells (Table 1). K562/MDR cells showed 160-fold higher resistance to vincristine than K562 cells (Table 1). In addition, the KB/MRP1 cells that we established in this study showed resistance to VP-16, doxorubicin, vincristine, and SN-38 (Table 1), which is similar to the cross-resistant patterns in other MRP-expressing cells [23–25].

The structures, symbols, and compound names of the flavonoids and their related agents used in this study are shown in Fig. 2. Each compound itself showed no or only marginal growth inhibitory effect on the cells in the concentrations used in this study (data not shown). We examined the effects of these compounds on SN-38 and mitoxantrone (MXR) resistances in K562/BCRP cells (Figs. 3, 4). The results shown in Fig. 3a indicate that 3′,4′,7-trimethoxyflavone (Fig. 2; 1-a) strongly suppressed BCRP-mediated multidrug resistance. This is evident from the growth inhibition curves of the K562/BCRP cells treated with this flavonoid at low concentrations (0.03 and 0.1 μM; closed triangle and lozenge, respectively) as they are well shifted to the left compared with the untreated cells (closed circle). The growth inhibition curve of K562/BCRP cells treated with 1 μM apigenin (Fig. 2;

A

1. Flavone	5	7	3'	4'	
1-a		OCH_3	OCH_3	OCH_3	3',4',7-trimethoxyflavone
1-b	OH	OH		OCH_3	Acacetin
1-c	OH	OH	OH	OCH_3	Diosmetin
1-d	OH	OH		OH	Apigenin
1-e	OH	OH			Chrysin
1-f	OH	OH	OH	OH	Luteolin
1-g	OH	OH	OH	G_3	Luteolin-4'-O-glucoside
1-h	OH	G_1			Rhoifolin
1-i	OH	G_2	OH	OCH_3	Diosmin

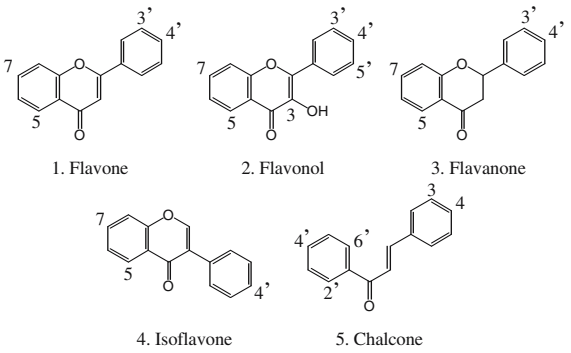
2. Flavonol	3	5	7	3'	4'	5'	
2-a		OH	OH		OCH_3		Kaempferide
2-b		OH	OH		OH		Kaempferol
2-c		OH	OH				Galangin
2-d		OH	OH	OH	OH	OH	Myricetin
2-e		OH	OH	OH	OH		Quercetin
2-f	G_3	OH	OH		OH		Kaempferol-3-O-glucoside
2-g		OH	G_1		OH		Kaempferol-7-O-nephesperidose
2-h			OH	OH	OH		Fisetin
2-i	G_4	OH	OH	OH	OH		Peltatoside
2-j		OH	OH	OCH_3	OH		Rutin

3. Flavanone	5	7	3'	4'	
3-a	OH	OH	OH	OCH_3	Hesperetin
3-b	OH	G_1		OH	Naringenin-7-O-glucoside
3-c	OH	OH		OH	Naringenin
3-d	OH	OH	OH	OH	Eriodictyol

4. Isoflavone	5	7	4'	
4-a	OH	OH	OH	Genistein
4-b		OH	OH	Daidzein

5. Chalcone	3	4	2'	4'	6'	
5-a	OCH_3	OCH_3				3,4-dimethoxychalcone
5-b		OH	OH	OH	OH	Phloretin

B



C

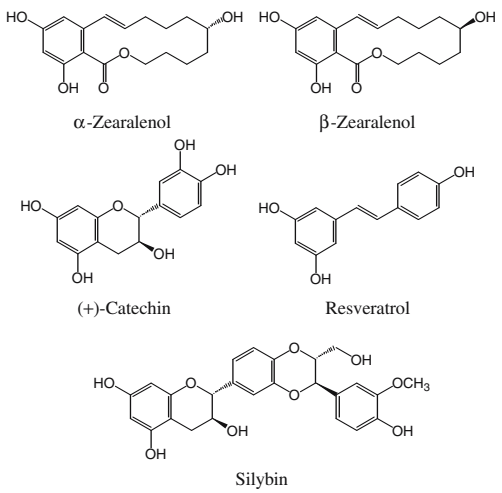


Fig. 2 Chemical structures of the flavonoids tested in the present study. **a** The structures of the flavones (9 compounds), flavonols (10 compounds), flavanones (4 compounds), isoflavones (2 compounds), and chalcones (2 compounds) screened in the present study. G_1, *O*-neohesperidoside; G_2, *O*-rutinoside; G_3, *O*-glucoside; G_4, *O*-arabinoglucoside. **b** Core structures of the five groups of compounds screened in the present study. **c** The flavonoid-related agents tested in the present study

1-d) (closed upward triangle) was also found to have shifted to the left compared with cells treated with 0.3 μM apigenin (closed downward triangle) or untreated (closed circle). This indicated that apigenin inhibits BCRP-mediated resistance to SN-38 and MXR (Fig. 3b). In contrast, diosmin (Fig. 2; 1-i) did not suppress BCRP-mediated drug resistance at any concentration (Fig. 3c). The reversal indices (RI_{50}) for SN-38 of 3′,4′,7-trimethoxyflavone, apigenin, and diosmin were measured as 0.012, 0.39, and >3 μM, respectively, and those for MXR were measured as 0.044, 0.62, and >3 μM, respectively. Other compounds were also examined using identical analyses, and the RI_{50} values for SN-38 were obtained from each growth inhibition curve. The RI_{50}^{-1} of the total panel of 32 compounds that we screened in this study are presented in Fig. 4, which shows that 20 of these 32 compounds can reverse BCRP-mediated SN-38 resistance. The lack of any reversal properties of the remaining 12 compounds was confirmed by treatments at the highest concentrations used in these experiments (data not shown). We also examined the reversal effects of a well-known BCRP inhibitor, FTC, as a positive control on BCRP-mediated drug resistances (Fig. 3d). As expected, FTC suppressed these resistances at the concentrations of 0.3 and 1 μM. The RI_{50} of FTC for SN-38 and that for MXR were measured as 0.24 and 0.23 μM, respectively.

We then examined the effects of the compounds on the BCRP-mediated efflux of topotecan. The intracellular topotecan in K562/BCRP cells was threefold lower than that in K562 cells (Fig. 5). 3′,4′,7-Trimethoxyflavone

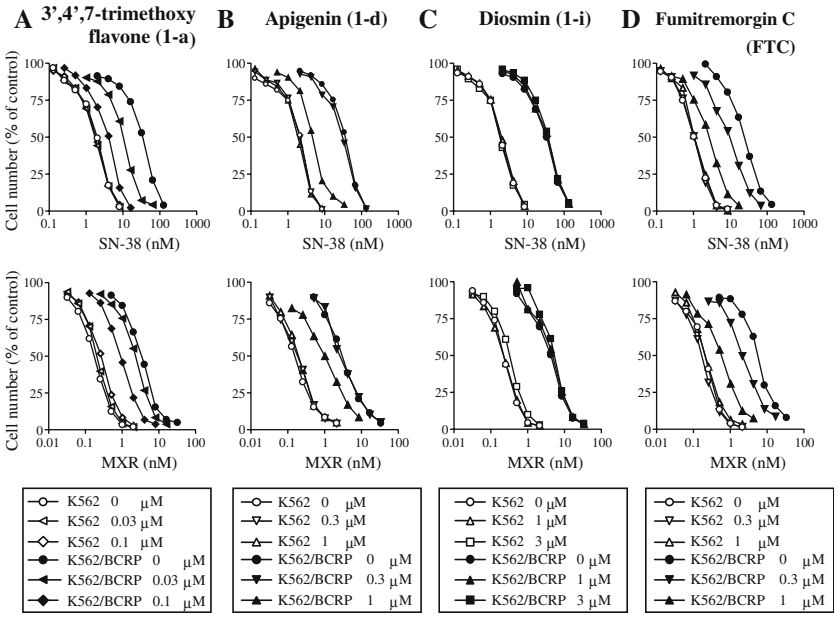


Fig. 3 Reversal effects of flavonoids on BCRP-mediated anticancer drug resistance. K562 (*open symbols*) and K562/BCRP (*closed symbols*) cells were cultured for 5 days in the absence (*circle*) or presence of 0.03 μM (*leftward triangle*), 0.1 μM (*lozenge*), 0.3 μM (*downward triangle*), 1 μM (*upward triangle*), or 3 μM (*square*) of the indicated compounds under increasing concentrations of SN-38. Cell numbers were determined with using a Coulter counter. **a** Effects of 3′,4′,7-trimethoxyflavone (*1-a*) on the sensitivity to SN-38 in K562 and K562/BCRP cells. **b** Effects of Apigenin (*1-d*) on the sensitivity to SN-38 in K562 and K562/BCRP cells. **c** Effects of Diosmin (*1-i*) on the sensitivity to SN-38 in K562 and K562/BCRP cells. **d** Effects of Fumitremorgin C on the sensitivity to SN-38 in K562 and K562/BCRP cells. Data points are the measurements of the mean ± SD from triplicate determinations

(1-a) at 1 μM increased the intracellular topotecan in K562/BCRP cells to a similar level as that in K562 cells (Fig. 5). Apigenin (1-d) at 10 μM also increased the intracellular accumulation of topotecan in K562/BCRP cells (Fig. 5). Diosmin (1-i) treatment did not alter the intracellular levels of topotecan (Fig. 5). These results clearly indicate that 3′,4′,7-trimethoxyflavone (1-a) and apigenin (1-d) increase the intracellular concentrations of BCRP substrate anticancer agents, but diosmin (1-i) does not.

We next examined whether any of the compounds showing reversal effects against BCRP-mediated drug resistance showed any cross-reactivity against other ABC transporter-mediated drug resistance pathways. As shown in Fig. 6a and b (left panels), 3′,4′,7-trimethoxyflavone (1-a) weakly suppresses P-gp- but not MRP1-mediated drug resistance. In addition, apigenin (1-d) and diosmin (1-i) do not reverse either P-gp- or MRP1-mediated drug resistance (Fig. 6a, b, middle and right panels). Among the representative 11 compounds that we chose to analyze in this experiment from the 32 compound panel, only two (1-a and -b) in fact inhibited P-gp-mediated drug resistance and none suppressed MRP1-mediated drug resistance (Table 2). These compounds also did not show growth inhibitory effects against K562 and KB-3-1 cells at the highest concentrations used in these experiments (data not shown). These data indicate that many flavonoids are select inhibitors of BCRP only.

Discussion

Estrogens such as estrone and 17β-estradiol have been found to contain inhibitory activity against the BCRP-mediated multidrug resistance pathways [20]. In addition, sulfated estrogens are found to be physiological substrates of BCRP, suggesting that they compete with anticancer agents on efflux from cells [21]. Synthesized estrogen antagonists and agonists have also been demonstrated to reverse drug resistance and have structural similarities that can directly inhibit BCRP function and/or reduce its expression levels [26]. It is noteworthy also that some flavonoids have structures that somewhat resemble the estrogens and display weak estrogenic activity [27]. Significantly, we and others have now shown that flavonoids possess anti-BCRP activity [22, 28–31].

Some flavonoids have been reported to interact with and competitively inhibit ABC transporters, including P-gp, MRP1, MRP2, and cystic fibrosis transmembrane conductance regulator [32–37]. In this regard,

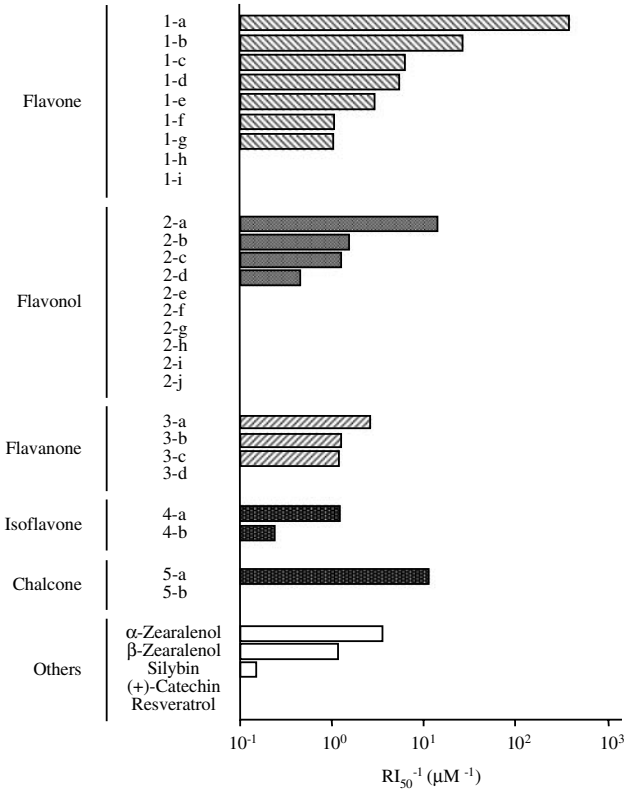


Fig. 4 Inhibitory effects of flavonoids on BCRP-mediated SN-38 resistance. K562 and K562/BCRP cells were cultured for 5 days in the absence or presence of the appropriate concentrations of flavonoids with increasing concentrations of SN-38. Cell numbers were determined with a Coulter counter. RI_{50} values were obtained graphically from the IC_{50} values of the K562 and K562/BCRP cells as described in Materials and methods. RI_{50}^{-1}, the reciprocal value of RI_{50}, was used to show the extent of drug resistance reversal of the compounds. These data are averaged from triplicate experiments

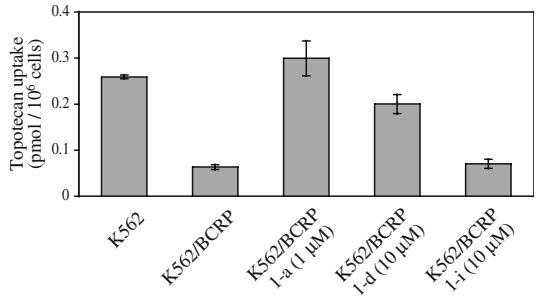


Fig. 5 Effect of flavonoids on topotecan uptake in K562/BCRP cells. K562 and K562/BCRP cells were incubated with 0.5 μM topotecan in the absence or presence of flavonoids at indicated concentrations at 37°C for 30 min. The intracellular topotecan was extracted from the cells with 1 ml of ethanol. The fluorescence intensity of topotecan was measured using a fluorescence spectrophotometer. Each *vertical bar* represents the mean ± SD of the measurements from triplicate determinations

genistein, naringenin, acacetin, kaempferol, quercetin, and flavopiridol have shown reversal effects against BCRP-mediated drug resistance in the previous studies from our laboratory and from other groups [22, 28, 38]. We therefore set out to screen additional flavonoids that may also reverse the effects of BCRP-mediated multidrug resistance in our present study. Of the 32 compounds that we tested, including the above flavonoids except flavopiridol, 20 compounds showed reversal effect for the resistance (Fig. 4). Of interest is that 3′,4′,7-trimethoxyflavone (1-a) has stronger inhibitory activity against BCRP than acacetin (1-b) that has been the strongest BCRP inhibitor in the flavonoids demonstrated in the previous study (Fig. 4). Almost all of the flavones, including 3′,4′,7-trimethoxyflavone, acacetin, diosmin, apigenin, chrysin, luteolin, luteolin-4′-*O*-glucoside, show BCRP-reversing activity (Figs. 3, 4). Moreover, many of the flavones, isoflavones, and chalcones tested in this study also reverse BCRP-mediated drug resistance, but 50% of the flavonols examined had no impact upon BCRP (Fig. 4).

From our present results, we propose a structure–activity relationship for BCRP inhibition by flavonoids (Fig. 7). We postulate that: (a) The double bond between position 2 and 3 of the C-ring is associated with high inhibitory activity against BCRP (Fig. 7a). As an example of this, apigenin (1-b) shows stronger suppressive activity toward BCRP than naringenin (3-c), and luteolin (1-f) is also more potent in this regard than eriodictyol (3-d) (Figs. 2, 4). (b) The 4′-*O*-methoxylation of the B-ring or the 4′-hydroxylation of the B-ring is also associated with more potent BCRP inhibition (Fig. 7b, c). In the former instance, hesperetin (3-a) has stronger BCRP inhibitory activity than eriodictyol (3-d), diosmetin (1-c) is more strongly inhibitory than luteolin (1-f), acacetin (1-b) is more potent than apigenin (1-d), and kaempferide (2-a) is a better inhibitor of BCRP than kaempferol (2-b) (Figs. 2, 4). In the case of 4′-hydroxylation of the B-ring, apigenin (1-d) showed slightly stronger BCRP suppression than chrysin (1-e), and kaempferol (2-b) is marginally more potent than galangin (2-c) (Figs. 2, 4). (c) The 3-hydroxylation of the C-ring or 3′-hydroxylation of the B-ring reduce BCRP inhibitory activity (Fig. 7d, e). In the former case, we mentioned our present results with galangin (2-c) compared with chrysin (1-e), kaempferol (2-b) compared with apigenin (1-d), kaempferide (2-a) compared with acacetin (1-b), and quercetin (2-e) compared with luteolin (1-f) (Figs. 2, 4). In the latter instance, our available examples include, luteolin (1-f) versus apigenin (1-d), eriodictyol (3-d) versus naringenin (3-c), and quercetin (2-e) versus kaempferol (2-b) (Figs. 2, 4). It is noteworthy also that in our present study, some glycosilated flavonoids showed anti-BCRP

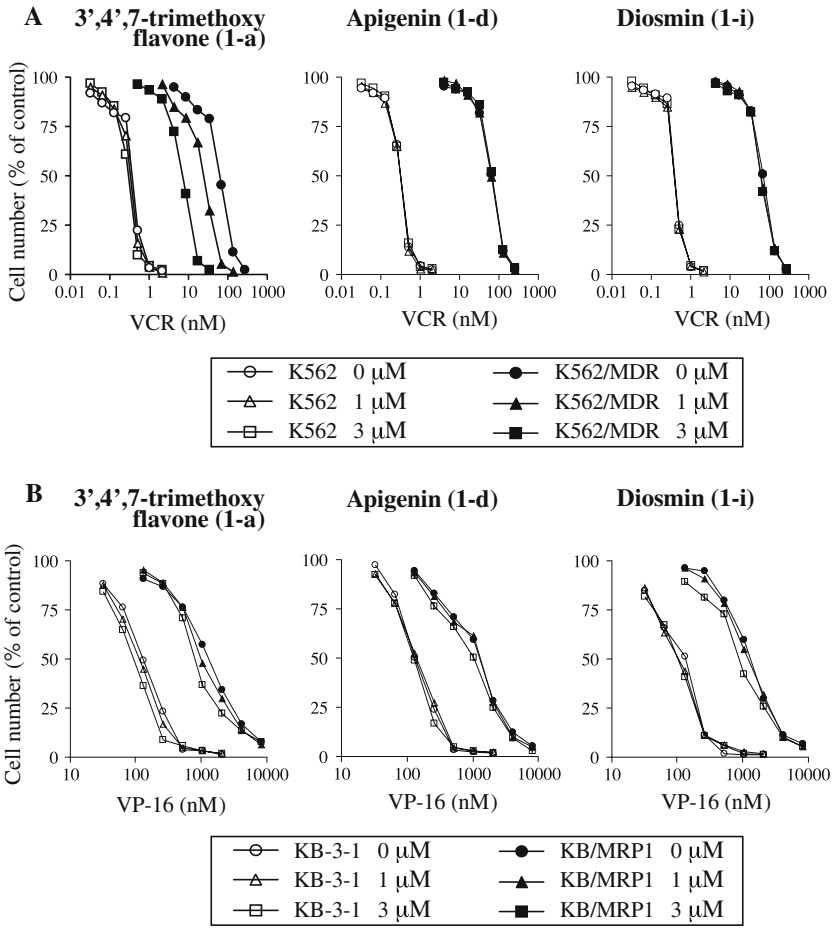


Fig. 6 Reversal effects of flavonoids on P-gp- or MRP1-mediated anticancer drug resistance. Parental cells (*open symbols*) and transfected cells (*closed symbols*) were cultured for 5 days in the absence (*circle*) or presence of 1 μM (*triangle*), or 3 μM (*square*) of the specific compounds indicated under increasing concentrations of anticancer agents. Cell numbers were determined using a Coulter counter. **a** Effects of flavonoid treatment upon the sensitivity to vincristine (VCR) in K562 and K562/MDR cells. **b** Effects of flavonoid treatment on the sensitivity to VP-16 in KB-3-1 and KB/MRP1 cells. Data points are measurements of the mean ± SD from triplicate determinations

Table 2 Reversal of BCRP-, P-gp-, and/or MRP1-mediated anticancer drug resistance by flavonoids

	RI_{50} (μM)		
	BCRP	P-gp	MRP1
1. Flavone			
1-a	0.012	0.68	>3
1-b	0.11	1.5	>3
1-d	0.39	>3	>3
1-e	0.67	>3	>3
1-f	1.5	>3	>3
1-h	>3	>3	>3
1-i	>3	>3	>3
2. Flavonol			
2-a	0.17	>3	>3
2-b	1.1	>3	>3
2-g	>3	>3	>3
2-h	>3	>3	>3

Parental or resistant cells were cultured for 5 days with increasing concentrations of anticancer drugs together with or without flavonoids. Cell numbers were counted with a Coulter counter, and RI_{50} was determined

activity. Glycosilated flavonoids may be useful for clinical practice because they are soluble in water and we would predict that both they and/or water-soluble derivatives of flavonoids will be developed as BCRP inhibitors in the future.

Flavonoids are safe nutrients, being the most abundant polyphenolic compounds present in the human diet in fruits, vegetables, and plant-derived beverages. It has been reported that a human adult normally assimilates 200–300 mg of flavonoids per day in their diet [39]. For example, 100 g of soybean contains 100–200 mg of isoflavones comprising genistein, daidzein, glycitein, and their corresponding glycosides. In the case of an intake of 50 mg of genistein, the peak plasma concentration of this compound was reported to reach a level of approximately 1 μM in healthy premenopausal women [40]. Therefore, flavonoids contained in foods can be considered to have a positive effect on the pharmacokinetics of anticancer agents. Hence, dietary controls will be necessary for patients undergoing cancer chemotherapy and it will also be important that the peak plasma concentrations of anticancer agents are continually monitored in these individuals.

In conclusion, we have shown that flavonoids selectively reverse BCRP-mediated drug resistance, that these compounds may be useful as BCRP inhibitors,

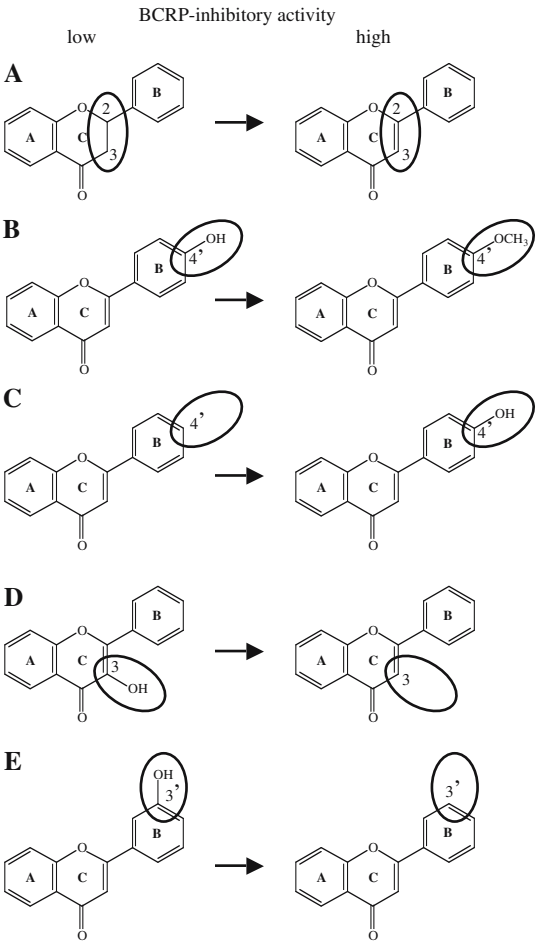


Fig. 7 Structure–activity relationship of BCRP inhibition by flavonoids

and that they are likely to bring clinical benefits via more effective and safer cancer chemotherapy treatments.

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