Natural Product Sciences
20(1) : 1-6 (2014)

Evaluating the Regulation of P-glycoprotein by Phytochemicals Using Caco-2 Cell Permeability Assay System

Ran Joo Choi and Yeong Shik Kim*

Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Abstract – P-glycoprotein (P-gp) is a permeability glycoprotein also known as multidrug resistance protein 1 (MDR1). P-gp is an ATP-binding cassette (ABC) transporter that pumps various types of drugs out of cells. These transporters reduce the intracellular concentrations of drugs and disturb drug absorption. The Caco-2 cell permeability assay system is an effective *in vitro* system that predicts the intestinal absorption of drugs and the functions of enzymes and transporters. Rhodamine-123 (R-123) and digoxin are well-known P-gp substrates that have been used to determine the function of P-gp. Efflux of P-gp substrates by P-gp has been routinely evaluated. To date, a number of herbal medicines have been tested with Caco-2 cell permeability assay system to assess bioavailability. There are growing efforts to find phytochemicals that potentially regulate P-gp function. The Caco-2 cell permeability assay system is a primary strategy to search for candidates of P-gp inhibitors. In this mini review, we have summarized the P-gp modulation by herbal extracts, decoctions or single components from natural products using Caco-2 cell permeability assays. Many natural products are known to regulate P-gp and herbal medicines could be used in combination with conventional drugs to enhance bioavailability.
Keywords – P-glycoprotein, Caco-2 cells, Natural products

Introduction

Medications are most commonly administered orally. Thus, the bioavailability should be considered when developing new drugs to avoid the loss of efficacy due to the first pass effect in the intestine and liver.[1] Even commercially available drugs encounter bioavailability issues. For example, a number of research groups have investigated the chemotherapeutic agent paclitaxel to develop a delivery system to enhance the cellular absorption of this drug.[2,3] P-glycprotein (P-gp), which is encoded by the MDR1 gene, has an important role in drug resistance, and inhibiting P-gp could overcome some of the obstacles that are associated with oral medications.[4,5] Herbal medicines are generally considered safe to be used in combination with conventional drugs. However, herb-drug interactions may cause unwanted alterations of drug concentrations in the blood due to the modulation of P-gp by phytochemicals. Therefore, it is important to estimate the effects of natural products on P-gp in an effective *in vitro* strategy before oral administration. For this purpose, studies utilizing Caco-2 cell monolayer models to examine the modulation of P-gp by natural products will be briefly reviewed.

*Author for correspondence
Professor Yeong Shik Kim, Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 151-742, Korea
Tel: +82-2-880-2479; E-mail: kims@snu.ac.kr

P-glycoprotein

Multidrug resistance (MDR) is one of the primary reasons for the ineffectiveness of medication. P-gp is a 170 kDa membrane transporter that is realted to MDR.[6] P-gp belongs to a group of ATP-binding cassette (ABC)-transporters that use ATP energy to transport substances through cell membranes (Fig. 1). P-gp has a broad substrate specificity and decreases the cellular accumulation of several substrates in various tissues and organs. P-gp is known to be expressed in the apical membranes of the liver, kidney, intestine and blood-brain barrier.[7,8] A number of medicines used in cancer chemotherapy, immunosuppression, hypertension, allergy, infection, and inflammation are P-gp substrates, inhibitors or inducers.[9] ATP-binding domains are required for P-gp to transport drugs out of the cell. Etoposide, daunomycin, taxol, vinblastine and doxorubicin are known substrates for P-gp.[10] However, only a few P-gp inhibitors have been developed for clinical application because of their effects on drug metabolism.[11,12] Because P-gp can interrupt drug

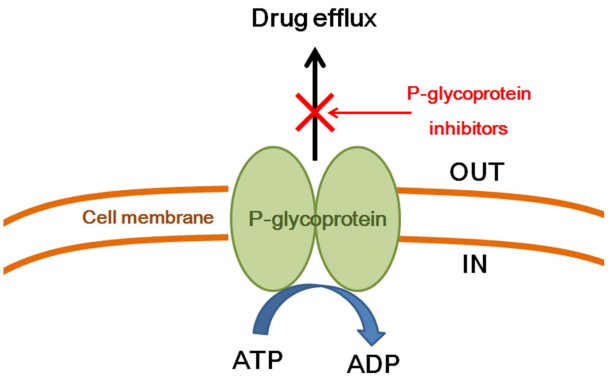

Fig. 1. P-glycoprotein (P-gp). P-gp transports drugs out of the cell, which is a process that requires the presence of ATP-binding domains. P-gp inhibitors block P-gp-mediated drug efflux.

absorption after oral administration, great efforts have been made to discover compounds that can inhibit the drug efflux function of P-gp.[13]

P-glycoprotein inhibitors

P-gp inhibitors can be classified into three generations.[14,15] Most first-generation inhibitors are pharmacological chemicals that have been used to treat other diseases. These inhibitors include verapamil, cyclosporin A, vincristine, yohimbine and several calmodulin antagonists. These compounds have been identified as regulators of P-gp; however, these drugs have low specificity for P-gp, and some of these compounds are also P-gp substrates. Second-generation P-gp inhibitors have a higher affinity for P-gp and are more potent than first-generation inhibitors. Dexverapamil, dexniguldipine, valspodar (PSC 833) and biricodar (VX-710) are second-generation inhibitors. Unfortunately, these agents are substrates for cytochrome P450 3A4 (CYP3A4) and other drug transporters and cannot be used clinically.[16] Inhibition of CYP 3A4 may cause unwanted pharmacokinetic interactions that can lead to an unpredictable toxicity of drugs. Third-generation P-gp modulators overcome the limitations of first- and second-generation inhibitors by utilizing structure-activity relationships and combinatorial chemistry approaches. Tariquidar (anthranilamide derivative), LY335979 (cyclopropyldibenzosuberane) and R101933 (laniquidar) are novel P-gp inhibitors that have shown successful results in clinical trials. However, some of the third-generation inhibitors have not shown significant effects on P-gp inhibition in clinical trials, and it is necessary to search for more potent P-gp modulators.

Herbal medicines have emerged as a new category of P-gp modulators because of the rapid increase in herbal consumption. Natural molecules have benefits such as low toxicity and fewer pharmacokinetic effects. Herbs have long been used for medicinal purposes and as part of a normal diet. Therefore, herbal components are good candidates for modulating P-gp.

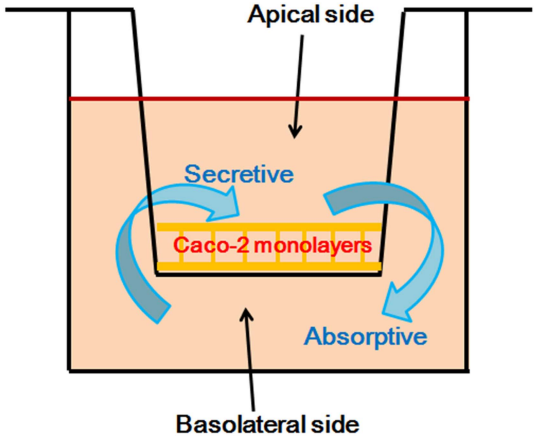

Fig. 2. A Caco-2 cell permeability assay system. Caco-2 cells are grown on the permeable filters. Caco-2 cells are cultured as confluent monolayers on the transwell insert within the well. Transport studies are performed by placing the compounds to be studied in the apical side (AP) and monitoring the amount of the test compound in the basolateral side (BL).

Caco-2 cell permeability assay

The Caco-2 cell line is a human intestinal epithelial cell line that spontaneously differentiates into monolayers and resembles enterocytes under conventional cell culture conditions.[17,18] After 21 days of cell culture, Caco-2 cell monolayers express various efflux transporters, brush border hydrolases, phase II enzymes and tight junctions.[19] Over the past few decades, the use of Caco-2 monolayers has been popular for predicting human intestinal absorption.[20,21] To evaluate the integrity of the monolayers, the transepithelial electrical resistance (TEER) value is measured, and a TEER value over 300 Ωcm^{-2} indicates an appropriate monolayer integrity for transport studies.[22] The apparent permeability coefficient (P_{app}) measured for drug candidates across Caco-2 cell monolayers has become a standard *in vitro* model for the prediction of the intestinal permeability. Caco-2 cells are grown on permeable filters and the cells are cultured as confluent monolayers on the transwell insert within the well. Transport studies are performed by placing the test compounds on the apical side (AP) of the cells and monitoring the amount of the compound on the basolateral side (BL) (Fig. 2).[23] Drugs that were efficiently absorbed had high permeability coefficients ($P_{app} > 1 \times 10^{-6}$ cm/s),

and drugs that were poorly absorbed had low permeability coefficients ($P_{app} < 1 \times 10^{-7}$ cm/s).[22]

P_{app} values are calculated by the following equation: $P_{app} = (dQ/dt) \times (1/A) \times (1/C_0)$, where dQ/dt (mol transported/sec) is the permeability rate of compounds across Caco-2 cell monolayers, A (cm^2) represents the diffusional surface area of the insert, and C_0 (M) denotes the initial concentration of loaded solutes.

P-glycoprotein modulation by natural products

The combined use of herbal medicines and drugs can affect the efficacy and the side effects of drugs by affecting pharmacokinetics properties such as the absorption, metabolism, distribution and excretion of the drugs.[24,25] Therefore, it is critical to search for a compound that inhibits the function of P-gp, without impacting the efficacy of the drug. The Caco-2 cell permeability assay is efficient in determining the effects of natural products on P-glycoprotein. Thus, we systematically investigated the herbs that have been studied via the Caco-2 cell permeability assay to determine P-gp regulation.

Grapefruit juice is known to alter the bioavailability of some medications. More than 20 drugs have been discovered to interact with grapefruit juice by decreasing CYP3A4 in the intestinal wall. Thus, the intake of grapefruit juice with medicines may increase the cumulative concentration of the drugs.[26] P-gp, which is also expressed in the intestinal mucosa, is another primary factor in the oral bioavailability of drugs that is affected by grapefruit juice. Flavonoids such as naringin, naringenin and 6',7'-dihydroxybergamottin are abundant in grapefruit juice and were thought to play a major role in P-gp modulation. Caco-2 cells were used to determine the effects of the major components of grapefruit juice on the transport of drugs by P-gp. Grapefruit juice extracts significantly decreased the transport of vinblastine and saquinavir, two well-known P-gp substrates, through Caco-2 monolayers. However, the inhibitory effects of flavonoids on P-gp were mild compared to those of grapefruit juice extract.[27,28] These results suggest that the flavonoids in grapefruit juice are not the main components that increase drug concentrations by P-gp inhibition.

Aged garlic extract is another popular food source that could affect P-gp transport. *In vitro* investigations have been conducted to test effects of aged garlic extract on P-gp transport and drug absorption. Berginc *et al*., performed two types of permeability assay models utilizing the rat jejunum and Caco-2 cell monolayers in the presence of aged garlic extract. An efflux study of a P-gp substrate (Rhodamine-123 (R-123)) and an MRP-2 substrate (2,4-dinitrophenyl-S-glutathione) revealed that aged garlic extract significantly increased P-gp and MRP-2 activity in the rat jejunum. Interestingly, only the MRP-2 substance efflux was increased by aged garlic extract in the Caco-2 cell permeability assay.[29] Moreover, Caco-2 cell monolayers were prepared in chambers to observe the pharmacokinetic interactions between aged garlic extract and cardiovascular, antidiabetic and antiviral drugs. In an investigation with R-123, hydrophilic sulfur compounds increased R-123 efflux which is mediated by P-gp.[30] Although aged garlic extract increased the effects of the multidrug resistance-related transporters P-gp and MRP-2 in the rat intestine and Caco-2 cell monolayers, combining P-gp and MRP-2 substrates showed different results because some drugs have distinct pharmacokinetic characteristics that alter P-gp-mediated transport in the liver and liver enzyme ability. Overall, the intake of aged garlic extract could increase the activity of P-gp and MRP-2, which could reduce drug absorption and drug concentrations in blood.

Measuring the transport of digoxin across Caco-2 cell monolayers can be used to evaluate the effects of natural molecules on P-gp. Djuv and Nilsen made efforts to establish a quality control setup for essential Caco-2 cell characteristics in P-gp activity. *Aloe vera* juice was tested on Caco-2 cell monolayers with digoxin treatment. The methodology of this study was typical of Caco-2 cell assays, and the results were expressed as the P_{app} efflux ratio, calculated by $P_{app\ B\text{-}A} / P_{app\ A\text{-}B}$, of digoxin transport across Caco-2 cell monolayers. *Aloe vera* juice showed no significant effect on the transport of digoxin in either the apical to basolateral (A-B) or basolateral to apical (B-A) transport.[31] *Aloe vera* juice did not influence on P-gp-mediated drug transport, and the effects of *Aloe vera* juice on P-gp substrates drug interactions are likely negligible.

Alkaloids belong to naturally occurring chemical groups that commonly include nitrogen atoms. Because alkaloids are structurally diverse, a number of researchers have been interested in the pharmacological effects of this group. Furthermore, the intestinal transport of alkaloids has become an area of great interest. *Aconitum* species (Ranunculaceae) have been used as analgesic, anti-inflammatory and hypotensive agents in Asian countries.[32] However, bioactive alkaloids found in the *Aconitum* species are too toxic to be used in medication. To determine the proper concentrations of these alkaloids, intestinal absorption was investigated by using a Caco-2 cell monolayer model. Aconitine, mesaconitine and

hypaconitine and diterpenoid alkaloids, which are present in aconite tubers were tested. These alkaloid compounds showed high permeability through Caco-2 monolayers. Additionally, these alkaloids were influential in P-gp-mediated transport, as confirmed by a digoxin transport study. Aconitine, mesaconitine and hypaconitine significantly reduced P-gp-mediated digoxin efflux in the Caco-2 cell monolayer model.[33] This investigation provided insight for further clinical trials by revealing that co-treatment of aconitine, mesaconitine, hypaconitine or aconite extract with P-gp substrates may cause involuntary toxicity or higher drug concentrations in the blood.

In contrast, one alkaloid exhibited opposite results. Berberine is an isoquinoline alkaloid found in the *Berberis* species, *Phellodendron amurense*, *Coptis chinensis* and many other traditional medicines. Berberine has various pharmacological activities against diabetes, cancer, HIV and other diseases.[34-36] Maeng *et al*., studied the effects of a 10 day treatment of berberine on the P-gp-mediated transport of [^{3}H]Daunomycin in Caco-2 cell permeability models. Berberine did not change the A-B absorption of daunomycin, but significantly increased the B-A flux, indicating the release of daunomycin pumped by P-gp transporters.[37] This study revealed that berberine can up-regulate P-gp in gastrointestinal epithelial cells and may decrease the intestinal absorption of drugs like daunomycin.

Aconite alkaloids and berberine are also P-gp substrates. Tryptanthrin is an indole quinazoline alkaloid isolated from *Strobilanthes cusia,* which has P-gp inhibitory effects but is not a P-gp substrate.[38] Transport studies in Caco-2 cell monolayers were conducted to evaluate the involvement of tryptanthrin on P-gp and MRP-2. The P-gp inhibitor verapamil, the MRP-2 inhibitor glibenclamide and tryptanthrin were applied to a Caco-2 cell permeability system. Neither verapamil nor glibenclamide changed the efflux transport of tryptanthrin indicating that tryptanthrin is not a substrate of P-gp or MRP-2. Here, the P-gp-mediated transport of digoxin and the MRP-2-mediated transport of pravastatin sodium were examined with the treatment of tryptanthrin. Tryptanthrin was absorbed through Caco-2 cell monolayers; however, the transport was not mediated by the multidrug resistance transporters P-gp and MRP-2, but by passive diffusion. Although tryptanthrin is not a substrate of P-gp or MRP-2, it is a novel inhibitor of P-gp and MRP-2.

The cellular mechanisms of P-gp inhibition have been investigated with anthraquinones from rhubarb in Caco-2 transport studies. It was reported that the extract of *R. palmatum* L. inhibited MDR function in the HeLa cell line, but this compound was not a specific substrate of P-gp.[39] Five major anthraquinone derivatives from rhubarb extract were tested with R-123 transport in Caco-2 cell monolayers and emodin was found to be the most potent P-gp inhibitor. This study verified that emodin could inhibit P-gp expression by COX-2 inhibition through additional mechanisms in Caco-2 cells. That is, emodin reduced the expression of COX-2 and the phosphorylated forms of the MAPK family (pJNK1, pERK1/2 and p-p38 via the AP-1 pathway.[40]

Because multidrug resistance often occurs in chemotherapy, researchers have conducted studies to overcome the obstacles of chemotherapeutic agents with low oral bioavailability. 20(s)-Ginsenoside Rg3 could enhance the oral absorption of anti-cancer drugs by P-gp inhibition. Yang *et al*., found that 20(s)-ginsenoside Rg3 effectively blocked the P-gp-mediated drug efflux of paclitaxel.[41] In Caco-2 cells, 20(s)-ginsenoside Rg3 increased the absorption rate and decreased secretion of paclitaxel in the Caco-2 cell permeability model. An *in vivo* study was performed to confirm the P-gp inhibitory properties of 20(s)-ginsenoside Rg3, and the results showed that this compound could enhance the efficacy of paclitaxel when the drugs were co-administered *p.o.* or *i.v.*. This investigation is a good example of using the Caco-2 cell permeability assay system as an effective way to select potent P-gp inhibitors *in vivo*. A similar approach was conducted with the chemotherapeutic agent irinotecan (CPT-11), a semi-synthetic derivate of camptothecin. Irinotecan is known to be converted into the metabolite 7-ethyl-10-hydroxy camptothecin (SN-38) *in vivo*. The excessive biliary secretion of SN-38 causes side effects.[42] P-gp activity can divert SN-38 to targeted tissues, alleviating these side effects.[43] Hence, the modulation of P-gp can increase the bioavailability of orally administered irinotecan. Five herbal compounds were selected to be co-administered with irinotecan *in vitro* and *in vivo*. In a Caco-2 cell monolayer study using R-123, very little irinotecan was absorbed. Instead, irinotecan was pumped out by P-gp, indicating an efflux ratio of more than 15. In the presence of quercetin, the $P_{app\ B\text{-}A}$ of irinotecan decreased and the $P_{app\ A\text{-}B}$ increased, resulting in a lower efflux ratio of 3.2. Quercetin and irinotecan were co-administered *i.v.* or *p.o.* to investigate the pharmacokinetics of irinotecan and SN-38. The results indicated that quercetin had little effect on the pharmacokinetic parameters of irinotecan and SN-38 but significantly improved the oral absorption of irinotecan.[44]

Herbal remedies can also regulate P-gp. Natto K2, *Agaricus*, mistletoe, noni juice, green tea and garlic are herbal remedies that have been used in the treatment of

Table 1. Modulation of P-glycoprotein (P-gp) by natural products in Caco-2 cell permeability assay system

Natural products	Compounds	P-gp modulation	References
Grapefruit juice	Naringin, naringenin, 6',7'-dihydroxybergamottin	Inhibition	27, 28
Aged garlic		Upregulation	29, 30
Aloe vera juice		No effect	31
Aconitum	Aconitine, mesaconitine, hypaconitine	Inhibition	33
	Berberine	Upregulation	37
Strobilanthes cusia	Tryptanthrin	Inhibition	38
Rheum palmatum L.	Emodin	Inhibition	39, 40
	20(s)-Ginsenoside Rg3	Inhibition	41
	Quercetin	Inhibition	44
Herbal remedy	Natto K2, *Agaricus*, mistletoe, noni juice, green tea, garlic	Inhibition	47

cancer patients.[45,46] Herbal remedies are often used in conjunction with medications with the expectation of synergistic effects. The Caco-2 cell transport assay is one of the best ways to estimate herb-drug interactions *in vitro.* Digoxin transport across the Caco-2 cell monolayers was affected by green tea, mistletoe, *Agaricus* and Natto K2, but was unchanged by noni juice and garlic.[47] The IC_{50} values of those herbs were lower than that of verapamil against the P-gp transport of digoxin in Caco-2 cell monolayers. Likewise, the *in vitro* evaluation of herb-drug interactions by utilizing Caco-2 permeability assays can suggest clinical indications for herbal remedies.

Conclusion

This review summarized the P-gp modulation by herbal extracts, decoctions or single components from natural products using Caco-2 cell permeability assay system (Table 1). It has been clearly demonstrated that the Caco-2 cell permeability assay system is a simple and effective way to predict P-gp modulation by natural molecules. More in depth investigations such as *in vivo* animal tests are required to clarify that natural products-derived P-gp modulators really alter the bioavailability of drugs which are pumped out by P-gp.

Acknowledgement

This work was supported by a grant from the Korean Food and Drug Administration (09112KFDA817 and 12172KFDA989) for Studies on the Identification of the Efficacy of Biologically Active Components from Oriental Herbal Medicines and the MRC grant funded by the Korean government (National Research Foundation of Korea) (No. 2009-93146).

References

(1) Han, M.; Fang, X. L. *Acta Pharmacol. Sin.* **2006**, *27*, 499-505.
(2) Iqbal, J.; Sarti, F.; Perera, G.; Bernkop-Schnurch, A. *Biomaterials* **2011**, *32*, 170-175.
(3) Roger, E.; Lagarce, F.; Garcion, E.; Benoit, J. P. *Eur. J. Pharm. Sci.* **2010**, *40*, 422-429.
(4) Ling, V.; Thompson, L. H. *J. Cell. Physiol.* **1974**, *83*, 103-116.
(5) Gros, P.; Croop, J.; Housman, D. *Cell* **1986**, *47*, 371-380.
(6) Juliano, R. L.; Ling, V. *Biochim. Biophys. Acta* **1976**, *455*, 152-162.
(7) Thiebaut, F.; Tsuruo, T.; Hamada, H.; Gottesman, M. M.; Pastan, I.; Willingham, M. C. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 7735-7738.
(8) Cordon-Cardo, C.; O'Brien, J. P.; Casals, D.; Rittman-Grauer, L.; Biedler, J. L.; Melamed, M. R.; Bertino, J. R. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 695-698.
(9) Kim, R. B. *Drug Metab. Rev.* **2002**, *34*, 47-54.
(10) Sorrentino, B. P. *Nat. Rev. Cancer* **2002**, *2*, 431-441.
(11) Ferry, D. R. *Int. J. Clin. Pharmacol. Ther.* **1998**, *36*, 29-40.
(12) Dantzig, A. H.; Law, K. L.; Cao, J.; Starling, J. J. *Curr. Med. Chem.* **2001**, *8*, 39-50.
(13) Ford, J. M. *Eur J Cancer* **1996**, *32A*, 991-1001.
(14) Varma, M. V.; Ashokraj, Y.; Dey, C. S.; Panchagnula, R. *Pharmacol. Res.* **2003**, *48*, 347-59.
(15) Bansal, T.; Jaggi, M.; Khar, R. K.; Talegaonkar, S. *J. Pharm. Pharm. Sci.* **2009**, *12*, 46-78.
(16) Liscovitch, M.; Lavie, Y. *IDrugs* **2002**, *5*, 349-355.
(17) Fogh, J.; Fogh, J. M.; Orfeo, T. *J. Natl. Cancer Inst.* **1977**, *59*, 221-226.
(18) Chantret, I.; Barbat, A.; Dussaulx, E.; Brattain, M. G.; Zweibaum, A. *Cancer Res.* **1988**, *48*, 1936-1942.
(19) Artursson, P. *J. Pharm. Sci.* **1990**, *79*, 476-482.
(20) Artursson, P.; Karlsson, J. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 880-885.
(21) Chiou, W. L.; Barve, A. *Pharm. Res.* **1998**, *15*, 1792-1795.
(22) Yee, S. *Pharm. Res.* **1997**, *14*, 763-766.
(23) Li, A. P. *Drug Discov. Today* **2001**, *6*, 357-366.
(24) Fugh-Berman, A.; Ernst, E. *Br. J. Clin. Pharmacol.* **2001**, *52*, 587-595.
(25) Hu, Z.; Yang, X.; Ho, P. C.; Chan, S. Y.; Heng, P. W.; Chan, E.; Duan, W.; Koh, H. L.; Zhou, S. *Drugs* **2005**, *65*, 1239-1282.
(26) Bailey, D. G.; Malcolm, J.; Arnold, O.; Spence, J. D. *Br. J. Clin. Pharmacol.* **1998**, *46*, 101-110.
(27) Takanaga, H.; Ohnishi, A.; Matsuo, H.; Sawada, Y. *Biol. Pharm. Bull.* **1998**, *21*, 1062-1066.
(28) Eagling, V. A.; Profit, L.; Back, D. J. *Br. J. Clin. Pharmacol.* **1999**,

48, 543-552.
(29) Berginc, K.; Zakelj, S.; Ursic, D.; Kristl, A. *Biol. Pharm. Bull.* **2009**, *32*, 694-699.
(30) Berginc, K.; Zakelj, S.; Kristl, A. *Eur. J. Nutr.* **2010**, *49*, 373-384.
(31) Djuv, A.; Nilsen, O. G. *Phytother. Res.* **2008**, *22*, 1623-1628.
(32) Singhuber, J.; Zhu, M.; Prinz, S.; Kopp, B. *J. Ethnopharmacol.* **2009**, *126*, 18-30.
(33) Li, N.; Tsao, R.; Sui, Z.; Ma, J.; Liu, Z.; Liu, Z. *Planta Med.* **2012**, *78*, 692-697.
(34) Dong, H.; Wang, N.; Zhao, L.; Lu, F. *Evid. Based Complement. Alternat. Med.* **2012**, *2012*, 591654.
(35) Bodiwala, H. S.; Sabde, S.; Mitra, D.; Bhutani, K. K.; Singh, I. P. *Eur. J. Med. Chem.* **2011**, *46*, 1045-1049.
(36) Katiyar, S. K.; Meeran, S. M.; Katiyar, N.; Akhtar, S. *Mol. Carcinog.* **2009**, *48*, 24-37.
(37) Maeng, H. J.; Yoo, H. J.; Kim, I. W.; Song, I. S.; Chung, S. J.; Shim, C. K. *J. Pharm. Sci.* **2002**, *91*, 2614-2621.
(38) Zhu, X. G.; Zhang, X. L.; Ma, G.; Yan, J. K.; Wang, H. H.; Yang, Q. *J. Pharm. Pharm. Sci.* **2011**, *14*, 325-335.
(39) Takara, K.; Horibe, S.; Obata, Y.; Yoshikawa, E.; Ohnishi, N.; Yokoyama, T. *Biol. Pharm. Bull.* **2005**, *28*, 138-142.
(40) Choi, R. J.; Ngoc, T. M.; Bae, K.; Cho, H. J.; Kim, D. D.; Chun, J.; Khan, S.; Kim, Y. S. *Eur. J. Pharm. Sci.* **2013**, *48*, 272-281.
(41) Yang, L. Q.; Wang, B.; Gan, H.; Fu, S. T.; Zhu, X. X.; Wu, Z. N.; Zhan, D. W.; Gu, R. L.; Dou, G. F.; Meng, Z. Y. *Biopharm. Drug Dispos.* **2012**, *33*, 425-436.
(42) Mathijssen, R. H.; van Alphen, R. J.; Verweij, J.; Loos, W. J.; Nooter, K.; Stoter, G.; Sparreboom, A. *Clin. Cancer Res.* **2001**, *7*, 2182-2194.
(43) Itoh, T.; Itagaki, S.; Sumi, Y.; Hirano, T.; Takemoto, I.; Iseki, K. *Cancer Chemother. Pharmacol.* **2005**, *55*, 420-424.
(44) Bansal, T.; Awasthi, A.; Jaggi, M.; Khar, R. K.; Talegaonkar, S. *Life Sci.* **2008**, *83*, 250-259.
(45) Boon, H.; Stewart, M.; Kennard, M. A.; Gray, R.; Sawka, C.; Brown, J. B.; McWilliam, C.; Gavin, A.; Baron, R. A.; Aaron, D.; Haines-Kamka, T. *J. Clin. Oncol.* **2000**, *18*, 2515-2521.
(46) Boon, H. S.; Olatunde, F.; Zick, S. M. *BMC Womens Health* **2007**, *7*, 4.
(47) Engdal, S.; Nilsen, O. G. *Xenobiotica* **2008**, *38*, 559-573.

Received June 6, 2013
Revised July 13, 2013
Accepted November 26, 2013