

# Flavonoid compounds as reversal agents of the P-glycoprotein-mediated multidrug resistance: biology, chemistry and pharmacology

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**Abstract** Multidrug resistance (MDR) represents one of the major problems in pharmacotherapy of important diseases (e.g., cancer, epilepsy). Although many factors may contribute to the development of MDR phenotype, the increased expression and/or functional activity of P-glycoprotein (P-gp; active drug efflux transporter) across the cell membrane has been recognized as the main one. Therefore, a great attention has been given to the search of P-gp inhibitors as therapeutic agents to reverse the MDR mediated by P-gp. Since the chemical entities identified over the last three decades as potential P-gp inhibitors did not show suitable pharmacological properties, more recently herbal components, such as flavonoid compounds, have gained a great interest as safe P-gp inhibitors. The interest in flavonoids as P-gp inhibitors is increasing due to their potential favourable characteristics, including selectivity and non-

cytotoxic effects. Flavonoids integrate the third-generation non-pharmaceutical category of P-gp inhibitors, and some of them exhibited effects comparable to those of the classic P-gp inhibitors. In fact, some flavonoids found in foods and beverages of herbal origin appear to be quite promising to inhibit the P-gp-mediated drug efflux, indicating their potential value to enhance the systemic/cellular bioavailability of P-gp drug substrates when administrated in co-therapy. This review paper summarizes the current evidence of P-gp inhibitory effects produced by flavonoids, taking into account studies performed in cell-based in vitro models, in vivo animal models and clinical trials.

**Keywords** Flavonoids · P-glycoprotein inhibition · Drug efflux · Multidrug resistance

## Abbreviations

ABC	Adenosine triphosphate (ATP)-binding cassette
AUC	Area under the concentration–time curve
BCRP	Breast cancer resistance protein
Cl <sub>t</sub>	Total body clearance
C <sub>max</sub>	Peak concentration
CNS	Central nervous system
CYP	Cytochrome P450
IV	Intravenous
MDR	Multidrug resistance
MRP	Multidrug resistance-associated protein
NBD	Nucleotide-binding domain

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P-gp	P-glycoprotein
TMs	Transmembrane $\alpha$ -helix segments
TMD	Transmembrane domain

## Introduction

The multidrug resistance (MDR) was described for the first time in 1968 by Kessel and collaborators (Borska et al. 2010). MDR refers to the phenomenon of simultaneous resistance to various structurally and functionally unrelated drugs, and it may be developed in response to a specific drug or drug combination (Ling 1997; van der Kolk et al. 2000; Deeley et al. 2006). Moreover, it often encompasses agents to which individuals have not been previously exposed, and which may or may not share targets and mechanisms of action with those that elicited the development of drug resistance (Deeley et al. 2006). Actually, the resistance to drug treatment still represents one of the major problems in the pharmacotherapy of several diseases, including cancer, infectious diseases and brain disorders such as epilepsy (Brandt et al. 2006).

Up to date, one of the most important mechanisms implicated in MDR is the over-expression and/or increased activity of drug efflux transporters belonging to the adenosine triphosphate (ATP)-binding cassette (ABC) transporter superfamily (Girardin 2006; Chang 2007; Borska et al. 2010; Giacomini et al. 2010). Many of the ABC drug efflux transporters are primarily located in the cell membrane where they can extrude a variety of structurally diverse drugs, drug conjugates and metabolites, and other compounds from the inside of cells (Schinkel and Jonker 2012). In all mammalian species, these multidomain integral membrane proteins use energy of ATP hydrolysis to the translocation of solutes across cellular membranes against the concentration gradient (Löscher and Potschka 2005), reducing, consequently, the intracellular concentration of the substrates (Dantzig et al. 2003; Löscher and Potschka 2005; Schinkel and Jonker 2012). Indeed, drugs ability to cross cellular barriers and reach their therapeutic targets in appropriate concentrations is critical for their efficacy (Chan et al. 2004), and it may be considerably determined through the active efflux mediated by membrane proteins (Pérez-Tomás 2006). Thus, transmembrane efflux proteins can be major determinants of the pharmacokinetics, safety and efficacy profiles of

drugs (Giacomini et al. 2010). These membrane transporters are expressed in different cells and tissues such as lymphocytes, intestine, liver, kidney, testis, placenta, and central nervous system (CNS) and, therefore, they are critical to the disposition of many xenobiotics (Wang et al. 2002). In fact, the pharmacological properties of many drugs in current use are affected by these membrane transporters, which may determine the rate and extent of the absorption, distribution and elimination processes. Furthermore, the ABC transporters can extensively limit the drug penetration into specific cells and tissue compartments, restricting the access of drugs into important pharmacological sanctuaries such as brain, testis and fetus. Therefore, the clinical efficacy and toxicity of many drugs may be determined on a large extent by drug interactions with ABC transporters (Schinkel and Jonker 2012).

Although the family of mammalian ABC transporters is extensive and functionally highly diverse, the P-glycoprotein (P-gp), the MDR-associated proteins (MRPs) 1–5 (MRP1, MRP2, MRP3, MRP4 and MRP5), and the breast cancer resistance protein (BCRP) have demonstrated a clinical significant role in the transport of relevant drugs (Schinkel and Jonker 2012).

## P-glycoprotein

P-glycoprotein is certainly the most studied drug efflux membrane transporter of the ABC transporter superfamily (Schinkel and Jonker 2012). P-gp was originally discovered in 1976 by Juliano and Ling as a surface glycoprotein, which was over-expressed in drug resistant ovary cells mutants from Chinese hamster (Juliano and Ling 1976; Linnet and Ejning 2008; Miller et al. 2009; Zhang et al. 2012). The discovery of P-gp, more than 35 years ago, demonstrated that a single protein may confer resistance to a relatively large number of structurally diverse drugs with different mechanisms of action (Deeley et al. 2006). Consequently, the resistance conferred by P-gp efflux transporter is the best characterized process among the various putative mechanisms of MDR, and it is of considerable clinical importance (Wang et al. 2002; Löscher and Potschka 2005; Bansal et al. 2009). This transmembrane active efflux system has been found in numerous species (insects, fish, amphibians,

reptiles, birds and mammals), although there are differences among them (Löscher and Potschka 2002a; Fortuna et al. 2011).

In humans, P-gp is encoded by two MDR genes, MDR1 (ABCB1) and MDR3 (ABCB4), being the latter also designated MDR2; on the other hand, mice express *mdr1a* (*abcb1a*), *mdr1b* (*abcb1b*) and *mdr2* (*abcb4*) (Tandon et al. 2006; Hennessy and Spiers 2007; del Amo et al. 2009; Zhang et al. 2012). The human gene encoding for MDR associated to P-gp is MDR1 (ABCB1), while in mice are *mdr1a* (*abcb1a*) and *mdr1b* (*abcb1b*) (Fortuna et al. 2011). The combined tissue distribution of *mdr1a* and *mdr1b* transcripts in rodents roughly coincides with that of the MDR1 mRNA transcripts in humans (Kwan and Brodie 2005; Linnet and Ejsing 2008; del Amo et al. 2009). Hence, it is believed that those two genes in rodents perform the same functions of the human MDR1 (Löscher and Potschka 2002a; Chan et al. 2004; Kwan and Brodie 2005; Linnet and Ejsing 2008; Zhang et al. 2012). There is 82 % homology between human MDR1 and mouse *mdr1a*, and the corresponding value between human MDR1 and mouse *mdr1b* is 79 % (del Amo et al. 2009). The denomination P-gp usually refers to the protein encoded by MDR1, *mdr1a* and *mdr1b* and, therefore, it will be herein followed.

The human P-gp is predominantly and physiologically expressed at the apical/luminal membrane of polarized cells in several normal tissues with secretory (small intestine, liver, kidney, adrenal gland) and barrier functions (small intestine, blood–brain barrier, blood–testis barrier, blood–ovarian barrier and placenta) (Volk et al. 2004; Fromm 2004; Giacomini et al. 2010; Marquez and Van Bambeke 2011). Indeed, P-gp forms a functional barrier that protects the body by actively limiting the absorption and systemic distribution of xenobiotic compounds, and/or increasing their elimination together with xenobiotic-metabolizing enzymes (Löscher and Potschka 2002b; Dantzig et al. 2003; Volk et al. 2004; Fromm 2004; Kwan and Brodie 2005; del Amo et al. 2009). Thus, many xenobiotics are metabolized and/or excreted into bile, urine, and intestinal lumen, preventing their accumulation in body tissues (Fromm 2004; Tandon et al. 2006). In fact, the strategic physiological expression of P-gp in organs that play key roles in the processes of drug absorption and disposition suggest that P-gp has a relevant impact on the pharmacokinetics of its substrate molecules, including many clinically

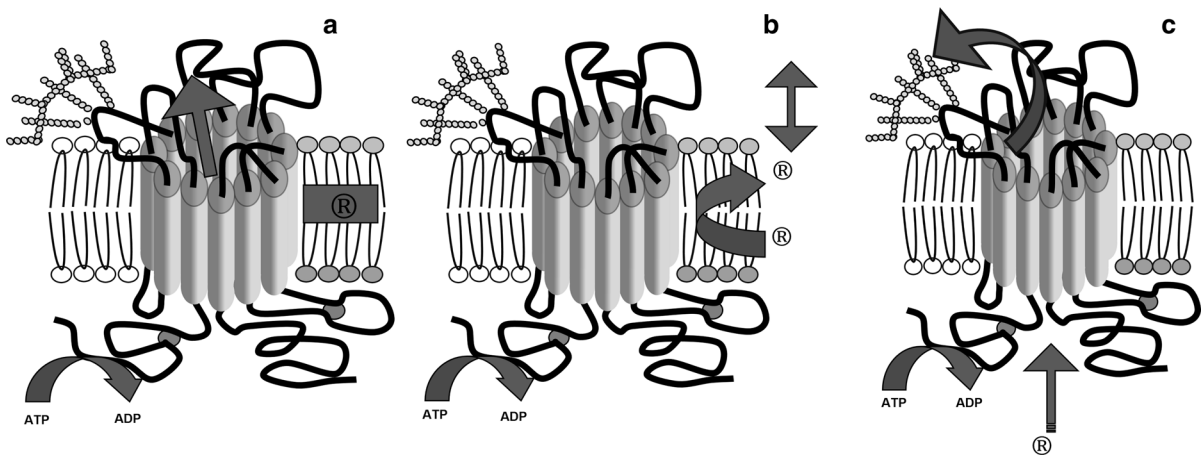
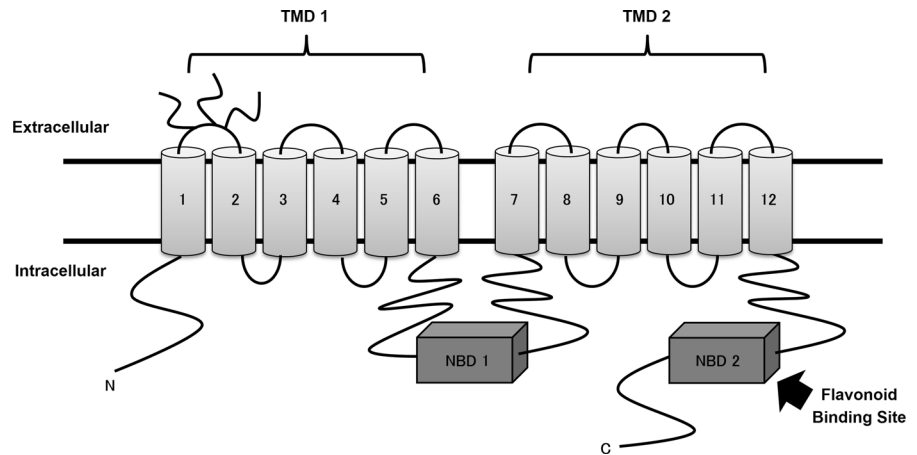
important therapeutic agents (Tandon et al. 2006; del Amo et al. 2009; Fortuna et al. 2011). Moreover, P-gp has also been associated to other physiological functions such as the transport of steroid hormones, transport of ions, and secretion of cytokines (del Amo et al. 2009). In addition, emerging evidence shows that P-gp expression is also changed at the level of blood–brain barrier during pathophysiology and/or therapy of CNS diseases such as epilepsy and stroke (Löscher and Potschka 2002b; Seegers et al. 2002; Kubota et al. 2006; Miller et al. 2009), as well as in multidrug resistant tumour cells (Zhang et al. 2012).

The human P-gp contains two homologous and symmetrical distinct halves, called NH<sub>2</sub>- and COOH-terminal halves (Fig. 1) (Yang et al. 2008; del Amo et al. 2009; Schinkel and Jonker 2012; Breier et al. 2013). Each half comprises a transmembrane domain (TMD) and a cytosolic nucleotide-binding domain (NBD) or ABC unit (de Wet et al. 2001; Barron et al. 2003; Linnet and Ejsing 2008; del Amo et al. 2009; Zhang et al. 2012; Breier et al. 2013). The two halves are joined by a 60 amino acid linker region (Ramakrishnan 2003) and each TDM is composed by six transmembrane  $\alpha$ -helix segments (TMs) (Di Pietro et al. 2002; Breier et al. 2013). The TMDs mediate the binding and efflux transport of P-gp substrates using energy derived from the hydrolysis of ATP at the NBD (Sheu et al. 2010). The NBDs are peripherally located at the cytoplasmic face of the cell membrane and they bind ATP and couple ATP hydrolysis with the drug transport process (Chan et al. 2009). The twelve TMs fold together to form a barrel-like structure that crosses the plasma membrane (Ramakrishnan 2003; Linnet and Ejsing 2008). The substrate specificity for P-gp is determined by the TM5, TM6, TM11 and TM12, which seem to be integrated to form a large drug binding pocket (Bellamy 1996; Breier et al. 2013). The drug recognition by P-gp, followed by ATP-binding and subsequent hydrolysis are essential to actively export relatively lipophilic substrates out of the lipid bilayer of the cell membrane to the extracellular medium (Tandon et al. 2006; Bansal et al. 2009; del Amo et al. 2009).

#### P-glycoprotein-mediated drug transport

Several models have been proposed to explain the mechanism of P-gp-mediated drug transport. The

**Fig. 1** Structure of P-glycoprotein (C, COOH; NBD, nucleotide-binding domain; N, NH<sub>2</sub>; TMD, transmembrane domain) (del Amo et al. 2009; Schinkel and Jonker 2012)



**Fig. 2** Models proposed to explain the P-glycoprotein function: the *pore* (a), the *flippase* (b) and the *hydrophobic vacuum cleaner* model (c). *ADP* Adenosine diphosphate; *ATP* Adenosine triphosphate; *R* Substrate (Varma 2003)

*pore*, the *flippase* and the *hydrophobic vacuum cleaner* are the most popular models of P-gp function (Fig. 2) (Krishna and Mayer 2000; Ramakrishnan 2003; Varma 2003; Hennessy and Spiers 2007; Bansal et al. 2009; del Amo et al. 2009).

According to the *pore* model, in the absence of the nucleotide, both TMDs form a single barrel with a central pore that opens to the extracellular surface and spans much of the membrane depth. On the contrary, upon binding of nucleotides, the TMDs reorganize into three compact domains that open the central pore along its length, allowing the direct access of hydrophobic drugs from the lipid bilayer to the central pore of the transporter (Fig. 2a) (Varma 2003; Bansal et al. 2009). P-gp has also been suggested to function as a *flippase* that flips the substrate from the inner leaflet of

the lipid bilayer to the outer leaflet of the plasma membrane, or directly to the extracellular environment against the concentration gradient (Fig. 2b) (Ramakrishnan 2003; Hennessy and Spiers 2007; Bansal et al. 2009; del Amo et al. 2009). The *flippase* model is based on the idea that after interaction of the substrate with the membrane, the substrate can access to the core of TMDs from the lipid bilayer and, then, P-gp flips the drug from the inner to the outer leaflet in an ATP hydrolysis-dependent manner (Ramakrishnan 2003). The assumptions of the substrates partition into the lipid phase prior to interacting with P-gp and the location of a P-gp binding pocket in the internal part of the plasma membrane are considered in the both models (Hennessy and Spiers 2007; del Amo et al. 2009). These characteristics may explain the

unusually broad substrate specificity of P-gp since the primary determinant for substrate specificity seems to be its ability to intercalate into the lipid bilayer appropriately, being the subsequent interaction with the substrate-binding site of secondary importance (Hennessy and Spiers 2007). Finally, the *hydrophobic vacuum cleaner* (Fig. 2c) combines the features of *pore* and *flippase* models (Varma 2003) and the designation of *hydrophobic* is due to the fact that the majority of P-gp substrates have been found to be hydrophobic compounds with a planar ring system, and often carrying a positive charge at physiological pH (Bansal et al. 2009). This model suggests that P-gp binds directly to the hydrophobic compounds embedded in the inner leaf of the plasma membrane and transports the substrate from the lipid bilayer to the extracellular medium through a protein channel (Krishna and Mayer 2000; Ramakrishnan 2003; Hennessy and Spiers 2007; Bansal et al. 2009; del Amo et al. 2009). Structural data indicate that the substrates could access to their binding sites through *gates* formed between TMs 5/8 and 2/11 (Hennessy and Spiers 2007). However, other mechanisms are also suggested particularly because uncharged hydrophilic molecules with planar ring systems (e.g. colchicine) are also effluxed out by P-gp. The change in electric potential and pH across membrane seems to contribute to that process (Bansal et al. 2009) as well as the conformational changes that P-gp undergoes when nucleotide binds to the intracellular NBDs (Varma 2003; Bansal et al. 2009).

Structurally, human P-gp is a 170 kDa transmembrane glycoprotein composed by 1,280 amino acids (Bellamy 1996; Schinkel 1999; Ramakrishnan 2003; Kwan and Brodie 2005; Linnet and Ejsing 2008). This well-known drug efflux transporter contains more than one substrate binding domain and extrudes a wide range of structurally and functionally unrelated compounds. P-gp is unique in its ability to recognize and transport a plethora of diverse substrates with variable chemical properties and pharmacological actions. Indeed, P-gp substrates include several clinically important drugs from different pharmacotherapeutic or pharmacological classes (Table 1) (Hennessy and Spiers 2007; Miller et al. 2009; Borska et al. 2010; Fortuna et al. 2011). However, the ability of P-gp to transport such enormous variety of chemical compounds is still poorly understood (Raub 2006). Moreover, it has been shown that drug–drug interactions

involving P-gp may decrease the therapeutic efficacy and/or potentiate toxic effects. This problem results from the large number of compounds that may interact with P-gp either as a substrate, inhibitor or inducer (Fortuna et al. 2011). Therefore, one of the key points that are increasingly considered in programs of drug discovery and development consists on testing new chemical entities as P-gp substrates. Indeed, drug candidates that are substrates of P-gp may be “victims” of drug–drug interactions where this transporter is inhibited or induced (Lin and Yamazaki 2003; Doran et al. 2005; Chang et al. 2006; Sugimoto et al. 2011; Palmeira et al. 2011). Hence, this type of studies are recommended and may be useful in the decision-making processes for optimization and selection of the most promissory drug candidates (Mizuno et al. 2003; Balyssac et al. 2005; Guidance for Industry, FDA 2006; Giacomini et al. 2010).

Furthermore, the understanding of the physiological role of P-gp as a natural detoxification mechanism in various human tissues (Lin 2003; Balyssac et al. 2005; Zhang et al. 2006; Chang et al. 2006) and the recognition of the involvement of P-gp in MDR (Matheny et al. 2001) have motivated the search of P-gp inducers and inhibitors with potential interest respectively in cases of poisoning and therapeutic inefficacy (Huisman et al. 2003; Dinis-Oliveira et al. 2006).

### P-glycoprotein inhibitors: an overview

The recognition that P-gp-mediated MDR is clinically important in several diseases has been followed by concerted efforts to search for therapeutically useful P-gp inhibitors in order to overcome that functional barrier and allow drug penetration into the target tissue (Tandon et al. 2006; Hennessy and Spiers 2007). The pharmacokinetics, efficacy and safety of P-gp substrates can be affected by the level of expression and functionality of P-gp, which can be modulated by inhibition and induction (Giacomini et al. 2010). If P-gp inhibitors or reversal agents become available, it might be possible, for example, to administer drugs targeted to the brain concomitantly with reversal agents, which would overcome the extrusion of P-gp substrates from the brain, increasing its concentration at the biophase and, consequently, the intended therapeutic effects (Ramakrishnan 2003).

**Table 1** Representative P-glycoprotein substrates

Therapeutic Class	Drugs
Analgesics	Asimadoline, morphine
Antibiotics	Erythromycin, gramicidin D, fluoroquinolones, tetracyclines, valinomycin
Antiemetics	Domperidone, ondansetron
Antiepileptics	Carbamazepine, felbamate, gabapentin, lamotrigine, phenobarbital, phenytoin, topiramate
Antigout agents	Colchicine
Anthelmintics	Abamectin, ivermectin
Antidepressants and Antipsychotics	Amirypiline, citalopram, doxepin, nortriptyline, paroxetine, risperidone, venlafaxine
Antidiarrheal agents	Loperamide
Antifungals	Itraconazole, ketoconazole
Anticancer drugs	Anthracyclines (bisanthrene, mitoxantrone), anthracyclines (daunorubicin, doxorubicin, epirubicin, idarubicin), camptothecins (irinotecan, topotecan), epipodophyllotoxins (etoposide, teniposide), miscellaneous (actinomycin D, amsacrine, colchicine, dactinomycin, imatinib, methotrexate, mithramycin, mitomycin C, tamoxifen, trimetrexate), taxanes (docetaxel, paclitaxel), vinca alkaloids (vinblastine, vincristine, vindesine, vinorelbine)
Antituberculous agents	Rifampicin
Beta blockers	bunitrolol, carvediol, celiprolol, talinolol
Calcium channel blocker	Diltiazem, felodipine, nicardipine, verapamil
Cardiac drugs	Digitoxin, digoxin, quinidine
Corticoids	Corticosterone, dexamethasone, hydrocortisone, methylprednisolone, triamcinolone
Histaminic H <sub>1</sub> -receptor antagonists	Fexofenadine, terfenadine
Histaminic H <sub>2</sub> -receptor antagonists	Cimetidine, Ranitidine
HIV protease inhibitors	Amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir
Hormones	Progesterone, testosterone, estradiol
Immunosuppressants	Cyclosporin A, sirolimus, tacrolimus
Lipid-lowering agents (Statins)	Atorvastatin, lovastatin
Proton pump inhibitors	Lansoprazole, omeprazole, pantoprazole
Sauna et al. (2001), Stouch and Gudmundsson (2002), Sun et al. (2003), Löscher and Poitschka (2005), Kwan and Brodie (2005), Balayssac et al. (2006), Hennessy and Spiers (2007), Bansal et al. (2009), Fortuna et al. (2011), Schinkel and Jonker (2012)	
HIV human immunodeficiency virus	

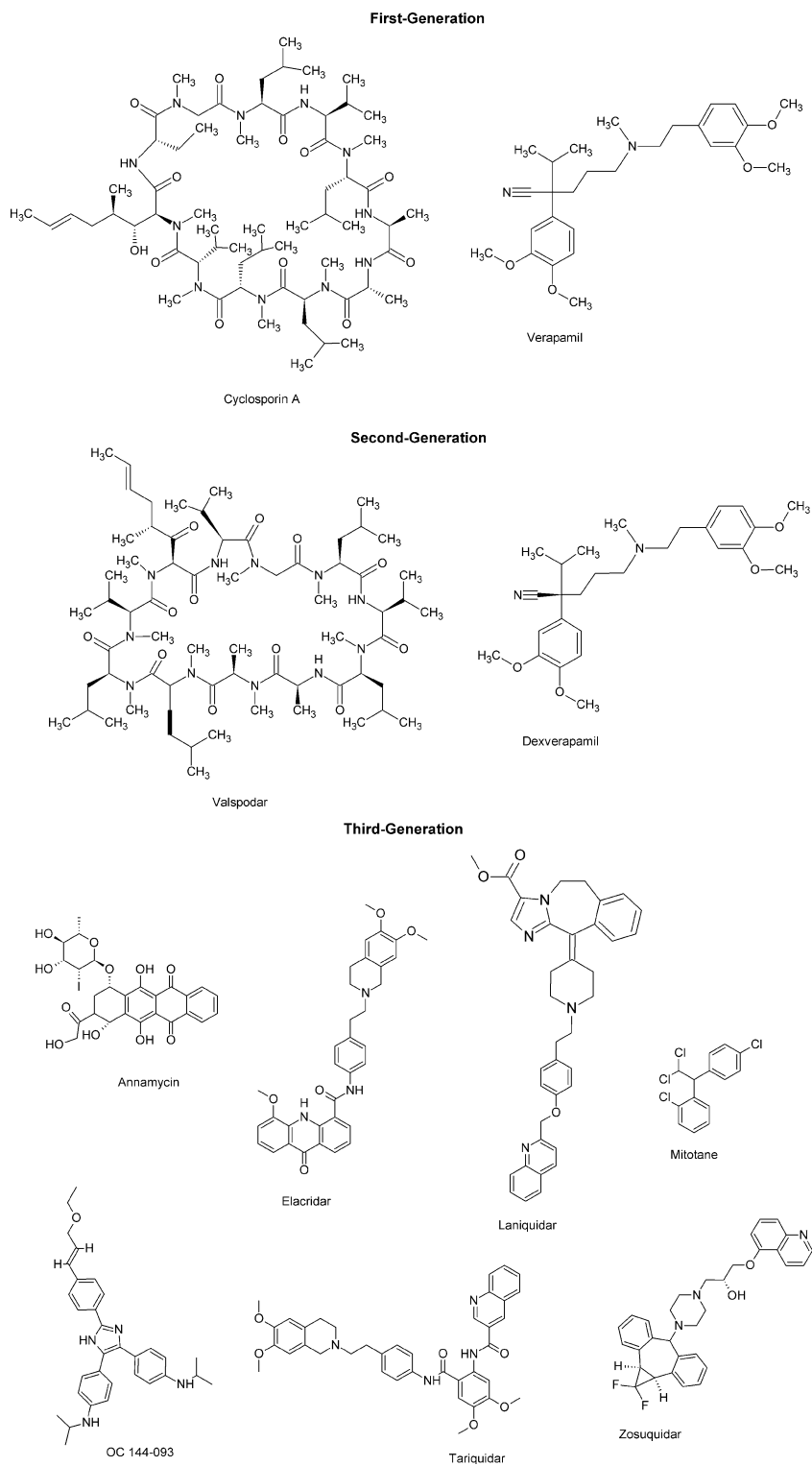
Although initial thoughts on the clinical application of P-gp inhibitors were focused on reversing MDR in chemo-resistant tumour cells that express significant amounts of P-gp, more recent insights indicated that such inhibitors might also be useful to modulate the general pharmacokinetic behaviour of drugs in the body (Schinkel and Jonker 2012). Therefore, P-gp inhibitors are currently in development not only to increase drug penetration into malignant tissues in order to optimize chemotherapy, but also to increase brain penetration of CNS-active drugs to circumvent the pharmacoresistance associated with several CNS diseases (e.g. epilepsy) (Kemper et al. 2003; Miller et al. 2009; Bansal et al. 2009; Coley 2010; Thomas and Coley 2013).

A P-gp inhibitor should have a high propensity for binding to P-gp without inhibiting other ABC transporters or interacting with cytochrome P450 (CYP) isoenzymes. Furthermore, it should be selectively targeted to P-gp in non-physiological tissues with a high affinity and individually adapted to the recipient's genotype. Thus, a non-toxic compound which does not have any pharmacological activity of its own is considered the ideal P-gp inhibitor (Bansal et al. 2009). P-gp inhibitors, even though structurally divergent, are responsible for decreased drug extrusion and reversal of cellular MDR (de Wet et al. 2001). Considering their physicochemical properties, P-gp inhibitors apparently are hydrophobic molecules, with a molecular weight ranging from 200 to almost 1900 Da (Chan et al. 2004; Fromm 2004; Schinkel and Jonker 2012), with a planar ring system and weak positive charge at physiologic pH (Schinkel 1999; Kwan and Brodie 2005; Miller et al. 2009; Giacomini et al. 2010; Zhang et al. 2012). Some investigators suggest a general pharmacophore with two or three hydrogen bond acceptor (or electron donor) groups in a fixed spatial separation (Sun et al. 2003; Chan et al. 2004; Kwan and Brodie 2005). The general features of a substrate/inhibitor include also a planar aromatic domain, as well as the existence of at least one tertiary basic nitrogen atom on the drug molecule in deprotonated state at neutral pH (Sun et al. 2003; Breier et al. 2013). Thus, based on their chronological development, specificity and affinity to the efflux transporter, the P-gp inhibitors are now categorized into three generations (Dantzig et al. 2003; Varma 2003; Bansal et al. 2009).

The first-generation of P-gp inhibitors are pharmacologically active and they are in clinical use for other

indications beyond the inhibition of P-gp (Dantzig et al. 2003; Varma 2003; Hennessy and Spiers 2007; Yang et al. 2008; Bansal et al. 2009; Potschka 2012). It includes calcium channel blockers such as verapamil (Fig. 3), anti-hypertensives like reserpine, quinidine and yohimbine, the immunosuppressant cyclosporin A (Fig. 3), anti-estrogens like tamoxifen and toremifene, and antiarrhythmic agents such as quinidine (Dantzig et al. 2003; Varma 2003; Hennessy and Spiers 2007; Bansal et al. 2009; Potschka 2012). However, these compounds have low potency and lack of selectivity for P-gp inhibition. Indeed, the clinical use of these agents at the concentrations necessary to inhibit P-gp is associated with severe toxicity (Krishna and Mayer 2000; Dantzig et al. 2003; Varma 2003; Chung et al. 2005; Hennessy and Spiers 2007; Bansal et al. 2009; Potschka 2012). For instance, the use of verapamil to inhibit P-gp leads to cardiotoxicity and the use of cyclosporin A induces an increase of hepatic, renal, myeloid, and neurological toxicity (Ramakrishnan 2003).

The second-generation of P-gp inhibitors was developed as derivatives of the first-generation compounds in order to retain the ability of the lead compound to inhibit P-gp, but decreasing the pharmacological activity and, consequently, the toxicity (Krishna and Mayer 2000; Dantzig et al. 2003; Varma 2003; Hennessy and Spiers 2007; Bansal et al. 2009; Potschka 2012). For example, the P-gp modulator valsopodar lacks the immunosuppressive effect of cyclosporin A, although it is a close structural analogue of this drug (Fig. 3). On the other hand, dexverapamil (*R*-isomer of verapamil; Fig. 3) retains the ability to inhibit P-gp, but lacks the cardiovascular effects of its precursor (verapamil) (Dantzig et al. 2003; Hennessy and Spiers 2007). Although these compounds have been developed to be less toxic, some of their characteristics still limit their clinical usefulness. Indeed, the affinity of these compounds towards P-gp was shown to be too low to produce significant P-gp inhibition *in vivo* at tolerable doses (Bansal et al. 2009). Additionally, first- and second-generation P-gp inhibitors also lack specificity as they inhibit two or more ABC transporters, which leads to complicated drug–drug interactions (Varma 2003; Hennessy and Spiers 2007). For instance, valsopodar inhibits not only P-gp but also MRP2 and the bile acid export protein (ABCB11) (Hennessy and Spiers 2007). Moreover, P-gp also has overlapping substrate specificity with



**Fig. 3** Chemical structure of some P-glycoprotein inhibitors belonging to the first-, second- and third-generations (PubChem Compound Database)



CYP3A. Therefore, numerous studies have demonstrated clinically important drug–drug interactions when a P-gp inhibitor is co-administered with another drug metabolised by CYP3A4; as example, valsopodar interferes with CYP enzyme activity thereby affecting clearance and systemic exposure of co-administered compounds (Hennessy and Spiers 2007; Potschka 2012). Thus, in spite of the early expected favourable contributions of the first- and second-generations of P-gp inhibitors, compounds have failed to yield the desired clinical benefit (Dantzig et al. 2003). Besides that, many first- and second-generation P-gp inhibitors are just competitive inhibitors, competing as substrates for the P-gp (Yang et al. 2008).

In contrast to first- and second-generation compounds, the third-generation of P-gp modulators has been designed to have an increased potency, inhibiting P-gp at concentrations in the nanomolar ranges, and to exhibit an increased specificity being simultaneously devoid of additional effects on drug metabolic enzymes. These include compounds as annamycin, elacridar, laniquidar, mitotane, OC 144-093, tariquidar and zosuquidar (Fig. 3) (Krishna and Mayer 2000; Dantzig et al. 2003; Hennessy and Spiers 2007; Potschka 2012). Bansal et al. (2009) presented a list of P-gp modulators belonging to different classes, which includes compounds that are inducers and inhibitors of P-gp. In the third-generation of P-gp inhibitors are included not only synthetic compounds, but also pharmaceutical excipients like polysorbates (Tween 20 or 80), herbs and fruits like curcumin or orange, and herbal constituents like flavonoids (Bansal et al. 2009). Among the herbal compounds, several new diterpene compounds isolated from *Euphorbia* spp. also showed to be potent MDR modulators acting specifically on P-gp transporter (Corea et al. 2009).

Competitive inhibitors, which are P-gp substrates themselves, and non-competitive inhibitors, are included in the three-generation of P-gp modulators. Non-competitive inhibitors can interfere with the activation and transport cycle of P-gp and induce alterations in the conformation of the transporter molecule which affect transport efficacy (Potschka 2012). Different mechanisms by which these inhibitors might interact with the transport proteins have been suggested (Brand et al. 2006). In general, P-gp can be inhibited by (a) blocking drug binding site either competitively, non-competitive or allosterically; (b) interfering with ATP hydrolysis; or (c) changing

integrity of cell membrane lipids (Varma 2003; Brand et al. 2006). However, most of the drugs inhibits P-gp function by blocking drug binding sites (Varma 2003). Furthermore, many of the inhibitors have their potential use reduced in clinical settings probably because they seem to be relatively nonspecific and participate in unwanted drug–drug interactions or interference with other physiological systems (Brand et al. 2006). Thereby, it is urgent the emergence of new agents with potential to reverse the MDR mediated by P-gp. These drug candidates should be potent, non-cytotoxic, and have minimal adverse effects (Hadjeri et al. 2003).

Over the last years, phytochemicals are gaining a great attention as P-gp modulators (Bansal et al. 2009), because, as potentially non-toxic compounds, they present characteristics of ideal P-gp inhibitors (Bansal et al. 2008, 2009). Herbal products are taken by about 10 % or more of the general population, and by 30–70 % of individuals with specific diseases (Chung et al. 2005). Hence, it has also been reported that some phytochemicals found in vegetables, fruits and some plants, which have, among others, anticancer, antiviral, and antioxidant properties, may modulate or inhibit P-gp activity and may have potential to be developed as MDR reversing agents (Chung et al. 2005). Accordingly, herbal constituents are considered good candidates for increasing the bioavailability and tissue-penetration (e.g. overpass the blood–brain barrier) or decreasing biliary excretion of P-gp substrates, reversing thus the MDR; although there are also safety concerns related to the phytochemical compounds, their continuous and long history of use in large amounts as a part of normal daily diet constitutes a favourable feature (Bansal et al. 2009).

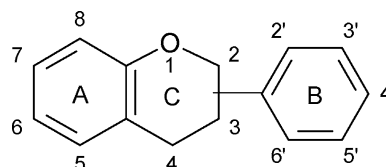
## Flavonoids

For a long time, flavonoids have been consumed in order to improve health status of humans (Bansal et al. 2009); indeed, the medicinal use of natural products, such as extracts of plants, is now a common practice worldwide (Aszalos 2008). Since several flavonoids share some of the optimal properties of an ideal P-gp inhibitor, the interest on testing whether they inhibit P-gp has significantly increased during the search for selective and non-cytotoxic P-gp inhibitors (Brand et al. 2006; Bansal et al. 2009). Currently, they are

considered good drug candidates (or lead compounds) as modulators of MDR due to their inhibitory activities on P-gp function and because of their physiological safety (Kitagawa et al. 2005).

Flavonoids consist on a large group of polyphenolic herbal constituents, which integrate the third-generation, non-pharmaceutical category of P-gp inhibitors (Ross and Kasum 2002; Brand et al. 2006; Bansal et al. 2009). Some of these compounds produced effects that are found to be comparable to those of the classic P-gp inhibitors, verapamil and cyclosporin A (Fig. 3) (Bansal et al. 2009). Actually, flavonoids and/or their metabolites are considered interesting modulators or substrates of intestinal membrane bound transport proteins, including P-gp (Brand et al. 2006; Chan et al. 2009).

The flavonoids are herbal secondary metabolites (Kitagawa 2006; Shohai et al. 2011; Thilakarathna and Rupasinghe 2013; Romano et al. 2013). According to Kitagawa (2006) and Sheu et al. (2010), flavonoids comprise more than 4,000 known compounds, resulting from the various combinations of multiple hydroxyl and methoxyl group substituents of the basic flavonoid skeleton (Fig. 4). Moon et al. (2006) reported more than 8000 compounds with a flavonoid structure. Although occasionally they are found, in nature, as aglycones, most of the flavonoids are attached to sugars (glycosides) (Ross and Kasum 2002; Morris and Zhang 2006). Many of the flavonoids have a very limited bioavailability because of high intestinal metabolism or poor absorption in vivo (Tsuji et al. 2013). In humans and animals, these compounds are absorbed from the gastrointestinal tract and are excreted in the urine and faeces, either unchanged or as flavonoid metabolites (Cook and Samman 1996; Morris and Zhang 2006; Brand et al. 2006). In the small intestine and liver, dietary flavonoids are substrates for phase I and phase II metabolizing enzymes (Wasowski and Marder 2012). Upon ingestion, and before oral absorption, flavonoid glycosides are deglycosylated, by lactase phlorizin hydrolase or cytosolic  $\beta$ -glucosidase. The compounds that are not absorbed in the intestine will reach the colon, where the enzymes of the gut microflora degrade flavonoids to simple phenolic acids, that may undergo absorption and further hepatic metabolism (Spencer 2007; Vauzour et al. 2008; Thilakarathna and Rupasinghe 2013). The absorbed aglycone is metabolized into glucuronide-, sulphate-

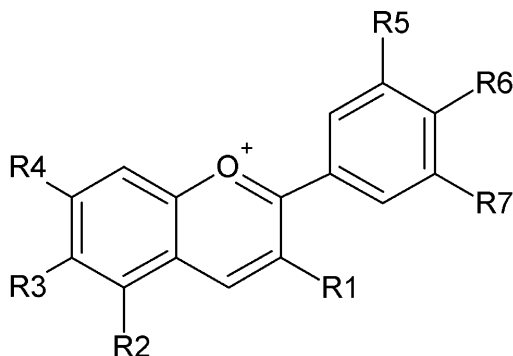


**Fig. 4** Basic chemical structure of flavonoids (Moon et al. 2006; Vauzour et al. 2008; Crozier et al. 2009)

and methoxylated conjugates (Brand et al. 2006; Spencer 2007; Vauzour et al. 2008; Bansal et al. 2009; Jäger and Saaby 2011; Wasowski and Marder 2012).

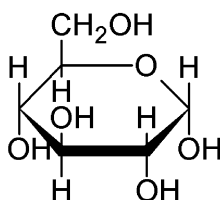
### Chemistry

The term flavonoid is used to describe herbal pigments mostly derived from benzo- $\gamma$ -pyrone (rings A and C; Fig. 4), with an assortment of basic structures (Hendrich 2006). These compounds are found in all vascular plants and have low molecular weights (Wasowski and Marder 2012; Romano et al. 2013). The basic structure of flavonoids consists of two benzene moieties (rings A and B, Fig. 4), which are linked in the middle through a heterocyclic pyran or pyrone with a double bond (ring C; Fig. 4) (Di Pietro et al. 2002; Ross and Kasum 2002; Shohai et al. 2011; Wasowski and Marder 2012; Tsuji et al. 2013). Hence, the flavonoids can be divided into several classes depending on their structure (Kitagawa 2006; Jäger and Saaby 2011), namely the oxidation status of ring C, the hydroxylation pattern of the ring structure and the substituent located on the 3rd position (Morris and Zhang 2006; Spencer 2007; Bansal et al. 2009; Alvarez et al. 2010; Tsuji et al. 2013). Taking into account the chemical nature of each molecule and the positions of moieties substituting rings A, B, and C, the flavonoids are divided into six major subclasses that are particularly well known and characterized and they correspond to the main dietary groups of flavonoids (Ross and Kasum 2002; Spencer 2007). These subclasses include the anthocyanidines (Fig. 5); the flavanols or catechins, in which the 2,3-bond is reduced, contain an additional hydroxyl substituent at position 3 of the ring C, and the carbonyl at position 4 is deleted (Fig. 6); the flavanones, where the 2,3-bond is reduced, losing electron conjugation and ring planarity (Fig. 7); the flavones (Fig. 8); the flavonols, which contain an additional hydroxyl substituent at the position 3 of the



	R1	R2	R3	R4	R5	R6	R7
<b>Aurantidin</b>	H	OH	OH	OH	H	OH	H
<b>Cyanidin</b>	OH	OH	H	OH	OH	OH	H
<b>Delphinidin</b>	OH	OH	H	OH	OH	OH	OH
<b>Diosmetinidin</b>	H	OH	H	OH	OH	OCH <sub>3</sub>	H
<b>Europinidin</b>	OH	OCH <sub>3</sub>	H	OH	OCH <sub>3</sub>	OH	OH
<b>Fisetinidin</b>	OH	H	H	OH	OH	OH	H
<b>Kuromanin</b>	O-Glu	OH	H	OH	OH	OH	H
<b>Luteolinidin</b>	H	OH	H	OH	OH	OH	H
<b>Malvidin</b>	OH	OH	H	OH	OCH <sub>3</sub>	OH	OCH <sub>3</sub>
<b>Peonidin</b>	OH	OH	H	OH	OCH <sub>3</sub>	OH	H
<b>Pelargonidin</b>	OH	OH	H	OH	H	OH	H
<b>Petunidin</b>	OH	OH	H	OH	OCH <sub>3</sub>	OH	OH
<b>Quercetagetinidin</b>	OH	OH	OH	OH	OH	OH	H
<b>Rosinidin</b>	OH	OH	H	OCH <sub>3</sub>	OCH <sub>3</sub>	OH	H

#### Substituents



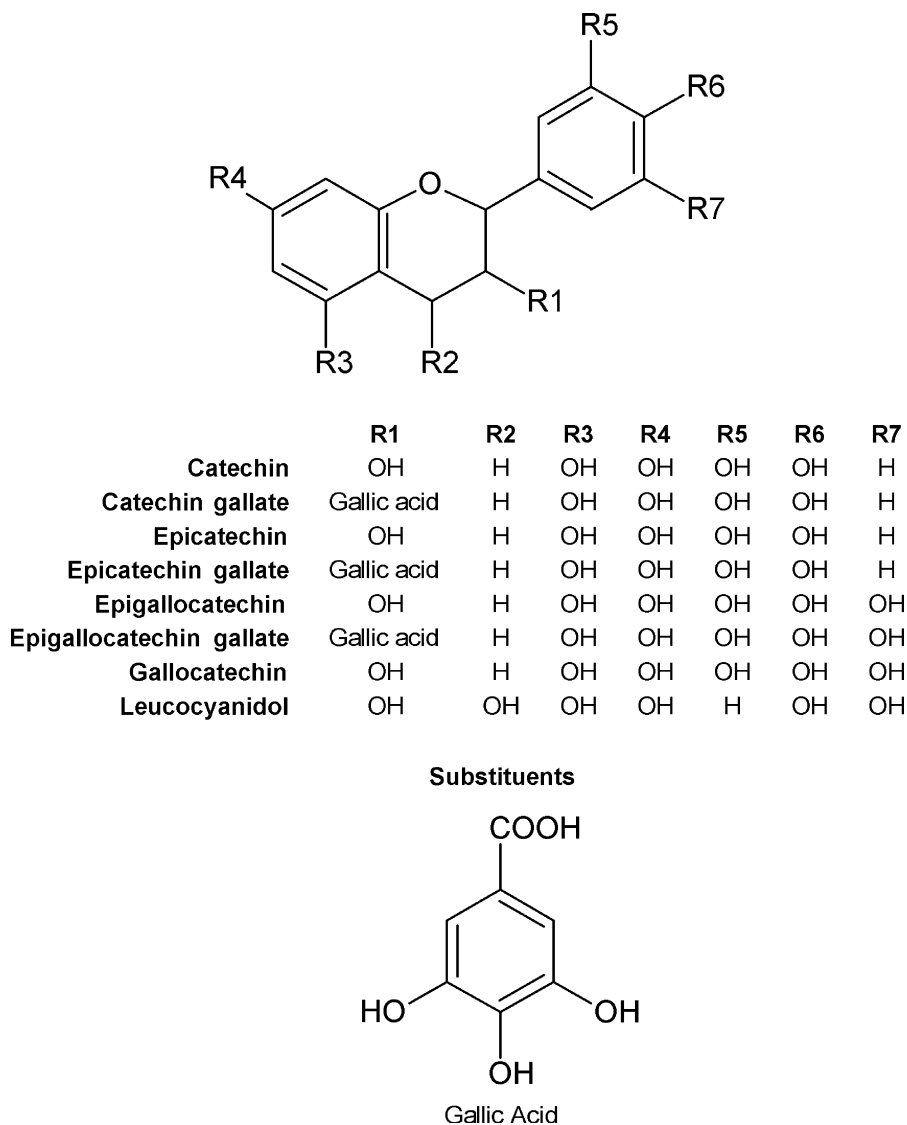
Glu = Glucose

**Fig. 5** Chemical structures of the compounds belonging to the anthocyanidines subgroup of flavonoids and their glycosides (anthocyanins) (PubChem Compound Database; Toki et al.

2008; Vauzour et al. 2008; Shohai et al. 2011; Jäger and Saaby 2011; Kellenberger et al. 2011)

ring C and where the 4',5'-bond is reduced (Fig. 9); and the isoflavones, where the ring B is branched at position 3 of the ring C (Fig. 10) (Ross and Kasum 2002; Deferme and Augustijns 2003; Hendrich 2006;

Kitagawa 2006; Moon et al. 2006; Spencer 2007; Crozier et al. 2009; Sheu et al. 2010; Thilakarathna and Rupasinghe 2013). Indeed, flavonoids and isoflavonoids (e.g. isoflavones) are distinguished by the



**Fig. 6** Chemical structures of the compounds belonging to the flavanols or catechins subgroup of flavonoids (PubChem Compound Database; Cook and Samman 1996; Vauzour

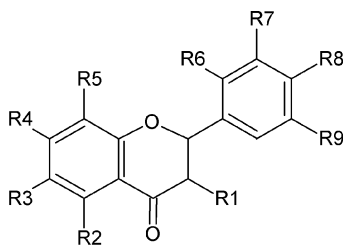
et al. 2008; Crozier et al. 2009; Shohai et al. 2011; Jäger and Saaby 2011; Du et al. 2012)

position at which the ring B is attached into the benzo- $\gamma$ -pyrone core of the molecule. Thus, in flavonoids the ring B is bound at position 2 of ring C, whereas in isoflavonoids the ring B is bound at position 3 of ring C (Figs. 5, 6, 7, 8, 9, 10) (Hendrich 2006). Minor dietary flavonoids include dihydroflavonols, flavan-3,4-diols, coumarins, chalcones, dihydrochalcones, and aurones (Crozier et al. 2009). Although a modified number system is used for chalcones and isoflavones derivatives, the individual carbon atoms are identified with ordinary numerals for the ring A and the ring C, and

“primed numerals” for the ring B (Fig. 4) (Tapas et al. 2008; Wasowski and Marder 2012).

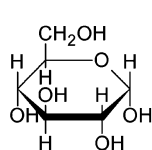
#### Structure–activity relationship

Specifically, there are structural requirements necessary for the inhibitory effects of flavonoids on P-gp function (Kitagawa et al. 2005). The structure–activity relationships for flavonoid–P-gp interaction have been extensively studied mainly evaluating the binding affinity of different flavonoids with mouse NBD2

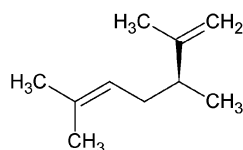


	R1	R2	R3	R4	R5	R6	R7	R8	R9
Butin	H	H	H	OH	H	H	H	OH	OH
Butrin	H	H	H	O-Glu	H	H	H	OH	O-Glu
Eriocitrin	H	OH	H	O-Glu-Rh	H	H	OH	OH	H
Eriodictyol	H	OH	H	OH	H	H	OH	OH	H
Hesperidin	H	OH	H	O-Glu-Rh	H	H	OH	OCH <sub>3</sub>	H
Hesperetin	H	OH	H	OH	H	H	H	OCH <sub>3</sub>	H
Homoeriodictyol	H	OH	H	OH	H	H	OCH <sub>3</sub>	OH	H
Isomonospermoside	H	H	H	OH	H	H	H	OH	O-Glu
Isosakuranetin	H	OH	H	OH	H	H	H	OCH <sub>3</sub>	H
Liquiritigenin	H	H	H	OH	H	H	H	OH	H
Lonchocarpol A	H	OH	Prenyl group	OH	Prenyl group	H	H	OH	H
Naringenin	H	OH	H	OH	H	H	H	OH	H
Naringin	H	OH	H	O-Glu-Rh	H	H	H	OH	H
Neohesperidin	H	OH	H	O-Glu-Rh	H	H	OH	OCH <sub>3</sub>	H
Pinocembrin	H	OH	H	OH	H	H	H	H	H
Ponciretin	H	OH	H	OH	H	H	H	OCH <sub>3</sub>	H
Poncirin	H	OH	H	O-Glu-Rh	H	H	H	OCH <sub>3</sub>	H
Sakuranetin	H	OH	H	OCH <sub>3</sub>	H	H	H	OH	H
Sakuranin	H	O-Glu	H	OCH <sub>3</sub>	H	H	H	OH	H
Sophoraflavanone G	H	OH	H	OH	Lavandulyl	OH	H	OH	H
Sterubin	H	OH	H	OCH <sub>3</sub>	H	H	OH	OH	H
Taxifolin	OH	OH	H	OH	H	H	OH	OH	H

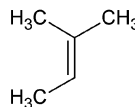
## Substituents



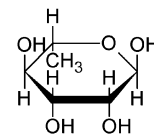
Glu = Glucose



Lavandulyl



Prenyl group



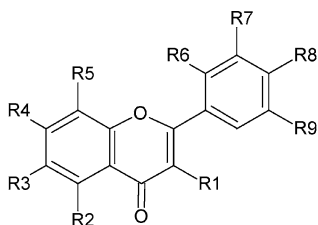
Rh = Rhamnose = 6-deoxyl-L-mannose

**Fig. 7** Chemical structures of the compounds belonging to the flavanones subgroup of flavonoids and their glycosides (PubChem Compound Database; Horowitz and Gentili 1960; Wagner et al. 1969; Sacco and Maffei 1997; Rakwal et al.

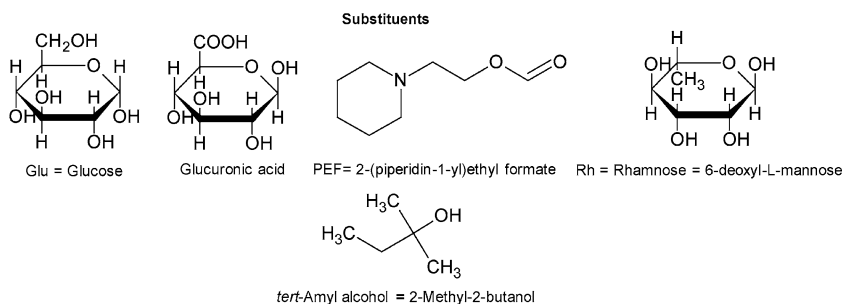
2000; Ley et al. 2005; Ogawa et al. 2006; Miyake et al. 2007; Chokchaisiri et al. 2009; Sheu et al. 2010; Shohai et al. 2011; Jäger and Saaby 2011; Tran et al. 2011; Wesolowska 2011)

(Morris and Zhang 2006). The presence of the 5-hydroxyl group, the 3-hydroxyl group, and the 2,3-double bond appears to be important for potent flavonoid-NBD2 interaction and are required for the P-gp inhibitory activity (Figs. 1, 11) (Conseil et al. 1998; Boumendjel et al. 2002; Hendrich 2006; Morris and Zhang 2006; Bansal et al. 2009). Isoflavonoids have lower P-gp interaction activity due to the different position where the ring B is branched (Boumendjel et al. 2002; Morris and Zhang 2006). Boumendjel et al. (2002) evaluate the binding affinity toward the NBD2

of P-gp of a set of naturally occurring flavones, isoflavones, flavonols, flavanones and chalcones. The structure–activity relationship analysis of flavonoids–P-gp concluded that flavonols, chalcones and flavones are the most active and the binding affinities are lower for flavanones and isoflavones (Boumendjel et al. 2002; Sheu et al. 2010). On the other hand, Sheu et al. (2010) conducted a study aiming to establish the qualitative and quantitative structure–activity relationships of flavonoids modulation effects on P-gp, using the human colorectal adenocarcinoma cells (HCT15)

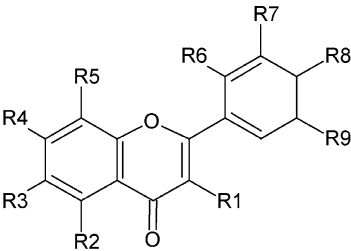


	R1	R2	R3	R4	R5	R6	R7	R8	R9
Acacetin	H	OH	H	OH	H	H	H	OCH <sub>3</sub>	H
Apigenin	H	OH	H	OH	H	H	H	OH	H
Apigenin	H	OH	H	O-Glu	H	H	H	OH	H
Baicalin	H	OH	OH	O- Glucuronic acid	H	H	H	H	H
Baicalein	H	OH	OH	OH	H	H	H	H	H
Bayin	H	H	H	OH	Glu	H	H	OH	H
Chrysin	H	OH	H	OH	H	H	H	H	H
Chrysoeriol	H	OH	H	OH	H	H	OCH <sub>3</sub>	OH	H
Cirsiliol	H	OH	H	OCH <sub>3</sub>	H	H	OH	OH	H
Cirsimaritin	H	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	H	OH	H
Diosmetin	H	OH	H	OH	H	H	OH	OCH <sub>3</sub>	H
Diosmin (synthetic)	H	OH	H	O-Glu-Rh	H	OH	OH	OH	H
Eupafolin	H	OH	OCH <sub>3</sub>	OH	H	H	OH	OH	H
Eupatilin	H	OH	OCH <sub>3</sub>	OH	H	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H
Flavoxate (synthetic)	CH <sub>3</sub>	H	H	H	PEF	H	H	H	H
Gardenin A (demethylated)	H	OH	OH	OH	OH	H	OH	OH	OH
Gardenin D	H	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	OH	OCH <sub>3</sub>	H
Genkwanin	H	OH	H	OCH <sub>3</sub>	H	H	H	OH	H
Hispidulin	H	OH	H	OH	H	H	H	OCH <sub>3</sub>	H
Hypolactin	H	OH	H	OH	OH	H	OH	OH	H
Isoorientin	H	OH	Glu	OH	H	H	OH	OH	H
Luteolin	H	OH	H	OH	H	H	OH	OH	H
Luteolin 7-glucoside	H	OH	H	O-Glu	H	H	OH	OH	H
Nobiletin	H	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H
Orientin	H	OH	H	OH	Glu	H	OH	OH	H
Oroxylin A	H	OH	OCH <sub>3</sub>	OH	H	H	H	H	H
Pedalitin	H	OH	H	OCH <sub>3</sub>	H	H	OH	OH	H
Rhoifolin	H	OH	H	O-Glu-Rh	H	H	H	OH	H
Scutellarein	H	OH	OH	OH	H	H	H	OH	H
Sideritoflavone	H	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	OH	OH	H
Sinensetin	H	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H
Tangeretin	H	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>	H
Techtochrysin	H	OH	H	H	OCH <sub>3</sub>	H	H	H	H
Vicenin-2	H	OH	H	Glu	Glu	H	H	OH	H
Vitexin	H	OH	H	OH	Glu	H	H	OH	H
Wogonin	H	OH	H	OH	OCH <sub>3</sub>	H	H	H	H
Xanthomicrol	H	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	OH	H
8-Methoxycirisilicol	H	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	OCH <sub>3</sub>	OH	H



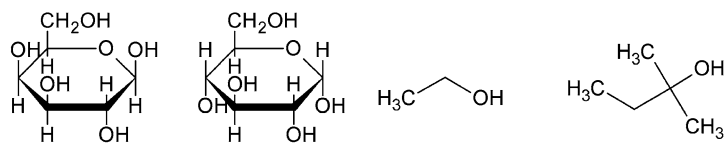
**Fig. 8** Chemical structures of the compounds belonging to the flavones subgroup of flavonoids and their glycosides (PubChem Compound Database; Cook and Samman 1996; Kim et al. 2004; Tapas et al. 2008; Crozier et al. 2009; Kim et al. 2010;

Shohai et al. 2011; Rao et al. 2011; Tran et al. 2011; Wasowski and Marder 2012; Moreno Escobosa et al. 2012; Lam et al. 2012; Kumar et al. 2013)



	R1	R2	R3	R4	R5	R6	R7	R8	R9
Amurensin	OH	OH	H	O-Glu	tert-amyl alcohol	H	H	OH	H
Azalein	O-Rh	OCH <sub>3</sub>	H	OH	H	H	OH	OH	H
Azaleatin	OH	OCH <sub>3</sub>	H	OH	H	H	OH	OH	H
Datiscetin	OH	OH	H	OH	OH	H	H	H	H
Fisetin	OH	H	H	OH	H	H	OH	OH	H
Galangin	OH	OH	H	OH	H	H	H	H	H
Gossypetin	OH	OH	H	OH	OH	H	OH	OH	H
Hyperoside	O-Gal	OH	H	OH	H	H	OH	OH	H
Isoquercetrin	O-Glu	OH	H	OH	H	H	OH	OH	H
Isorhamnetin	OH	OH	H	OH	H	H	OCH <sub>3</sub>	OH	H
Kaempferide	OH	OH	H	OH	H	H	H	OCH <sub>3</sub>	H
Kaempferitrin	O-Rh	OH	H	O-Rh	H	H	H	OH	H
Kaempferol	OH	OH	H	OH	H	H	H	OH	H
Morin	OH	OH	H	OH	H	OH	H	OH	H
Myricitin	OH	OH	H	OH	H	H	OH	OH	OH
Myricitrin	O-Rh	OH	H	OH	H	H	OH	OH	OH
Natsudaoidain	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>	OCH <sub>3</sub>
Quercetagetin	OH	OH	OH	OH	H	H	OH	OH	H
Quercetin	OH	OH	H	OH	H	H	OH	OH	H
Quercitrin	O-Rh	OH	H	OH	H	H	OH	OH	H
Pachypodol	OCH <sub>3</sub>	OH	H	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>	OH	H
Rhamnetin	OH	OH	H	OCH <sub>3</sub>	H	H	OH	OH	H
Rhamnazin	OH	OH	H	OCH <sub>3</sub>	H	H	H	OH	OCH <sub>3</sub>
Robinetin	OH	H	H	OH	H	H	OH	OH	OH
Robinin	O-Rh-Glu	OH	H	O-Rh	H	H	OH	OH	H
Rutin	O-Rh-Glu	OH	H	OH	H	H	OH	OH	H
Spiraeoside	OH	OH	H	OH	H	H	H	O-Glu	OH
Spirenoside	OH	OH	H	OH	H	H	OH	O-Glu	H
Tamarixetin	OH	OH	H	OH	H	H	OH	OCH <sub>3</sub>	H
Troxeutin	O-Rh-Glu	OH	H	O-He	H	H	O-He	O-He	H
Vitexcarpin	OCH <sub>3</sub>	OH	OCH	OCH <sub>3</sub>	H	H	OH	OCH <sub>3</sub>	H

## Substituents



Gal = Galactose

Glu = Glucose

He = Hydroxyethyl

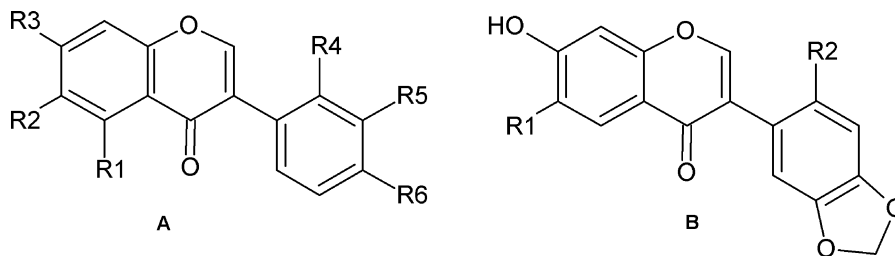
tert-amyl alcohol = 2-Methyl-2-butanol

**Fig. 9** Chemical structures of the compounds belonging to the flavonols subgroup of flavonoids and their glycosides (PubChem Compound Database; Harborne 1962; Cook and Samman 1996; Raj 2001; Hasegawa and Shirato 2002; Boumendjel et al. 2002; Tapas et al. 2008; Ali et al. 2008;

Matsui et al. 2009; Zhou et al. 2009; Crozier et al. 2009; Sheu et al. 2010; Shohai et al. 2011; Jäger and Saaby 2011; Tran et al. 2011; Wasowski and Marder 2012; Alonso-Castro et al. 2013; Yang et al. 2013; Makino et al. 2013)

as an in vitro model and fexofenadine as a P-gp substrate probe. Accordingly, the flavonoid compounds with hydroxyl groups attached at the 5- and

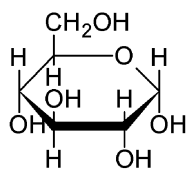
7-positions of the ring A and with the total number of hydroxyl groups, at an optimal of three, possess a high inhibitory effect on P-gp activity (Fig. 11). However,



	R1	R2	R3	R4	R5	R6
<b>A</b>						
Afrormosin	H	OCH <sub>3</sub>	OH	H	H	OCH <sub>3</sub>
Biochanin A	OH	H	OH	H	H	OCH <sub>3</sub>
Calycosin	H	H	OH	H	OH	OCH <sub>3</sub>
Castanin	H	OCH <sub>3</sub>	OH	H	H	OCH <sub>3</sub>
Daidzein	H	H	OH	H	H	OH
Daidzin	H	H	O-Glu	H	H	OH
Formononetin	H	H	OH	H	H	OCH <sub>3</sub>
Genistein	OH	H	OH	H	H	OH
Genistin	OH	H	O-Glu	H	H	OH
Glycitein	H	OCH <sub>3</sub>	OH	H	H	OH
Odoratin	H	OCH <sub>3</sub>	OH	H	OH	OCH <sub>3</sub>
Ononin	H	H	O-Glu	H	H	OCH <sub>3</sub>
Sophoraisoflavone A	OH	H	OH	#	#	OH
Sissotrin	OH	H	O-Glu	H	H	OCH <sub>3</sub>
Tectorigenin	OH	OCH <sub>3</sub>	OH	H	H	OH
<b>B</b>						
Cuneatin	H	OCH <sub>3</sub>	--	--	--	--
Fujikinetin	OCH <sub>3</sub>	H	--	--	--	--
Pseudobaptogenin	H	H	--	--	--	--

# - pyran ring between positions 2' (R4) and 3' (R5)

#### Substituents



Glu = Glucose

**Fig. 10** Chemical structures of the compounds belonging to the isoflavones subgroup of flavonoids and their glycosides (PubChem Compound Database; Keung and Vallee 1993;

Khan et al. 2000; Tapas et al. 2008; Chokchaisiri et al. 2009; Sheu et al. 2010; Tran et al. 2011; Wesołowska 2011; Zhang et al. 2011; Zou et al. 2012; Shajib et al. 2012)

flavonoids with four hydroxyl groups such as kaempferol (3,5,7,4'-tetrahydroxyflavone; Fig. 9) and fisetin (3,7,3',4'-tetrahydroxyflavone; Fig. 9) have not shown this high inhibitory effect on P-gp activity, whereas flavonoids with a greater number of hydroxyl groups

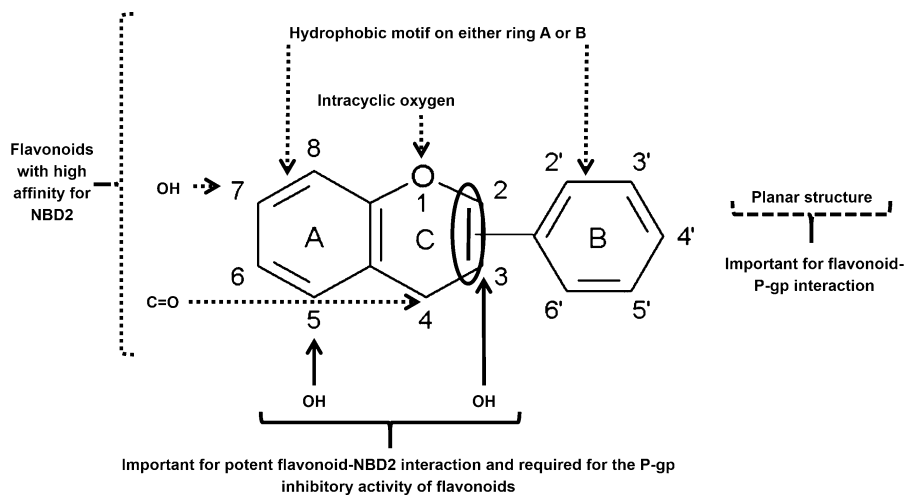
such as quercetin (3,5,7,3',4'-pentahydroxyflavone; Fig. 9) and taxifolin (3,5,7,3',4'-pentahydroxyflavone; Fig. 7), were able to enhance P-gp activity (Sheu et al. 2010). Wesołowska (2011) besides stating that flavonoids should possess hydroxyl groups at



positions 3 and 5 of ring A and the 2,3-double bond to exhibit high affinity for NBD2 of P-gp, also described the importance of the carbonyl group at position 4 and a hydrophobic motif on either ring A or B (Fig. 11) (Wesołowska 2011). The planar structure of flavonoids also seems to be important for their interaction with P-gp (Fig. 11). As a result, for flavanones, which lack the double-bond between the 2- and 3-position in ring C, the stereoscopic relationship of the ring B with other rings (A and C) is different from that of flavones (with the double-bond in that position) (Figs. 7, 8). This double bond confers a special structure on flavonoid molecules that are largely planar so that they may readily intercalate between the hydrophobic amino acid residues of P-gp (Kitagawa et al. 2005).

Over the years, flavonoids have been subjected to various chemical modifications in order to obtain improved chemical entities as P-gp inhibitors. In general, it was found that chemical modifications that increased hydrophobicity of the flavonoid molecules, such as prenylation, geranylation, or a linear sequence of two isoprenyl residues, significantly increased the modulatory activity of P-gp (Wesołowska 2011). Therefore, in some cases, prenylated flavonoids bind with high affinity to P-gp and show an increased inhibitory potency (Di Pietro et al. 2002; Hendrich 2006). In this context, it was demonstrated that the introduction of alkoxy groups at position 4 of chalcones resulted in compounds much more active, being

recognised that the binding affinity increases as a function of the chain length (Bois et al. 1999); on the other side, it was reported that halogenated chalcones exhibited high-affinity binding to P-gp and that the increase in binding affinity is related to the increase in hydrophobicity of the halogenate substituent (Bois et al. 1998). The intracyclic oxygen may be also required, since, when it was replaced by a nitrogen atom, the P-gp inhibitory activity dramatically reduced (Fig. 11) (Kitagawa et al. 2005). The hydroxyl groups in polyphenols, such as those in the gallic acid moiety of tea catechins and alkyl gallates may be important in polar interactions with P-gp, possibly at the ATP-binding site. Accordingly, the hydrophobic moiety of polyphenols may be important for interaction at the steroid-interacting hydrophobic sequence of P-gp (Kitagawa 2006). Thus, the hydrophobicity of flavonoid compounds is a critical parameter for binding affinity towards NBD2 and consequently for their inhibitory effects on P-gp-mediated efflux of substrates (Di Pietro et al. 2002; Kitagawa et al. 2005; Kitagawa 2006). Thereby, the P-gp inhibitory activity of flavonoids increases as their molecular hydrophobicity increases (Kitagawa et al. 2005). Additionally, Wesołowska (2011) underlined the role of the hydrophobicity of ring substituents because the compounds containing geranyl groups are more active than those substituted by halogen atoms, which are followed by flavonoids with methoxy and hydroxyl groups. On the



**Fig. 11** Structure-activity relationship of flavonoids (NBD, nucleotide-binding domain; P-gp, P-glycoprotein) (Boumendjel et al. 2002; Kitagawa et al. 2005; Hendrich 2006; Morris and

Zhang 2006; Bansal et al. 2009; Sheu et al. 2010; Wesołowska 2011)

other hand, the results of a virtual screening of flavonoids as P-gp inhibitory agents indicated that glutamine-47, tyrosine-53, serine-83, isoleucine-87, glycine-100 and arginine-154 of the P-gp molecule are determinant residues for binding interactions as they establish strong hydrogen bonding with flavonoid molecules (Udaya Kumar et al. 2011). Udaya Kumar et al. (2011) also referred that for the stability of the complex an important role is played by hydrogen binding interactions.

Although detailed studies have been performed on the interaction of flavonoids with NBD2, a similar binding site certainly also exists on NBD1 since flavonoids compete with ATP. Indeed, Trompier et al. (2003) reported that a series of flavonoids bind to both NBDs (1 and 2) of the human MRP1, being stated that isoprenylation of flavonoid molecules leads to a preferential binding to NBD1. Thus, it is expected that additional binding(s) is(are) also found within the TMDs' drug-binding sites, as described for MRP1 (Trompier et al. 2003).

#### Sources of flavonoids

The flavonoids are among the most ubiquitous groups of polyphenolic compounds found in foods and beverages of herbal origin (Boumendjel et al. 2002; Kitagawa et al. 2005; Badhan and Penny 2006; Brand et al. 2006; Wesołowska et al. 2009; Chan et al. 2009). These compounds, diverse in chemical structure and characteristics, are found in high levels in fruits, vegetables, nuts, stems, flowers, red wine, soybeans and tea (Cook and Samman 1996; Deferme and Augustijns 2003; Badhan and Penny 2006; Brand et al. 2006; Aszalos 2008; Chan et al. 2009). The anthocyanidines are mainly found in berries, cherries, grapes, red cabbage and red wine; the flavanols or catechins in apples, black tea, chocolate, bilberry, cocoa, grapes, hawthorn, motherwort, red wine and tea; the flavanones in tomato and citrus fruits and juices; the flavones in celery, honey, parsley, peppers, propolis, sweet red and thyme; the flavonols in apples, berries, broccoli, cherries, kale, leeks, lettuce, onions red wine, tea and tomato; and, finally, the isoflavones can be found in legumes like peas, red clover and soy (Moon et al. 2006; Alvarez et al. 2010; Shohai et al. 2011; Thilakarathna and Rupasinghe 2013; Toh et al. 2013).

Apart from food components, flavonoids are also present in medicinal herbs or dietary supplements

including silibin, which is extracted from milk thistle, *Silybum marianum*, *Alpina officinarum*, *Soy Isoflavones*, *Ginkgo Biloba* or *Hypericum perforatum* (Hendrich 2006; Moon et al. 2006; Sheu et al. 2010). Although in the usual human diet, the intake level of these compounds can be up to several hundred milligrams a day (Wang et al. 2005; Badhan and Penny 2006; Brand et al. 2006), in people consuming over-the-counter botanical and dietary supplements the intake may be considerably higher (Lohner et al. 2007).

#### Biological properties and flavonoid–P-glycoprotein interactions

Flavonoids are water-soluble pigments and play diverse functions in the plant kingdom including defence, growth, development, photosensitization, ultraviolet protection, auxin transport inhibition, allelopathy and flower colouring (Buer et al. 2010; Shohai et al. 2011; Jäger and Saaby 2011). In 1936, Rusznyak and Szent-Gyorgyi reported the first observation regarding the biological activities of flavonoids (Ross and Kasum 2002). As integral constituents of the diet, they may exert a wide range of beneficial effects on human health in a multitude of disease states including cancer, cardiovascular diseases, neurodegenerative disorders and osteoporosis (Boumendjel et al. 2002; Badhan and Penny 2006; Morris and Zhang 2006; Brand et al. 2006; Wesołowska et al. 2009; Chan et al. 2009; Wasowski and Marder 2012). Moreover, there are recent evidence of an inverse relationship between dietary flavonoid intake and cardiovascular disease mortality and its risk factors (Toh et al. 2013). These broad spectrum of biological activities also include anti-inflammatory, antimicrobial, antiviral, anti-proliferative, pro-apoptotic, free-radical scavenging, antioxidant and hormonal effects (Boumendjel et al. 2002; Deferme and Augustijns 2003; Hendrich 2006; Badhan and Penny 2006; Brand et al. 2006; Wesołowska et al. 2009; Chan et al. 2009; Buer et al. 2010; Wasowski and Marder 2012). However, Choi et al. (2011) also referred that the protective defence mechanism against oxidants can be overcome by the inadvertent overproduction of reactive oxygen species. Interactions with key enzymes, signalling cascades involving cytokines and transcription factors, or antioxidant systems, may originate the health-promoting effects of flavonoids (Buer et al. 2010; Wasowski

and Marder 2012). Flavonoids have also attracted interest as new chemical entities with activity on the CNS. These actions can result from their binding to the benzodiazepine site on the  $\gamma$ -aminobutyric acid receptor type A (GABA<sub>A</sub> receptor) resulting in sedation, anxiolytic or anti-convulsive effects, or from their action as inhibitors of monoamine oxidase A or B, working as anti-depressants or antiparkinson agents (Romano et al. 2013). However, although some of these activities have been demonstrated by Fernández et al. (2005), the depressant effects were not observed in rodents.

Flavonoids produce their modulating effects by a number of possible mechanisms (Chan et al. 2009). Some of them are good inhibitors of a variety of ATP binding proteins including protein kinases such as cyclic adenosine monophosphate dependent protein kinase, protein kinase C, serine/threonine kinases, tyrosine kinase, topoisomerase II, and myosin. They also inhibit various membrane ATPases, such as mitochondrial H<sup>+</sup>-ATPase, Na<sup>+</sup>/K<sup>+</sup>-ATPase, Ca<sup>2+</sup>-ATPase, and H<sup>+</sup>/K<sup>+</sup>-ATPase (Di Pietro et al. 2002; Boumendjel et al. 2002; Chan et al. 2009). Moreover, the activities of many other enzymes are also inhibited by flavonoids, for instance  $\beta$ -glucuronidase, lipoxygenase, cyclooxygenase, inducible nitric oxide synthase, monoxygenase, thyroid peroxidase, xanthine oxidase, mitochondrial succinoxidase and nicotinamide adenine dinucleotide-oxidase, phosphodiesterase and phospholipase A2 (Moon et al. 2006).

Although the molecular mechanisms particularly underlying flavonoid–P-gp interactions are not clear, several hypotheses have been proposed (Chan et al. 2009; Go et al. 2009; Cassidy and Setzer 2010). Among the proposed mechanisms are the following: (a) flavonoids modulate P-gp by interacting with the vicinal ATP-binding site and the steroid binding site; (b) flavonoids act as substrate and may interact with P-gp directly either by competitive binding to the substrate-binding site or by binding to other drug-binding sites and changing the P-gp conformation; and (c) flavonoids may bind to the allosteric site or other binding sites (Morris and Zhang 2006; Lohner et al. 2007; Go et al. 2009; Alvarez et al. 2010). Thus, due to the complexity of interactions between P-gp and its modulators, and also because the interactions of flavonoids with P-gp may be different for specific flavonoids, the elucidation of the exact interaction mechanism between these polyphenolic compounds

and P-gp is difficult (Zhang and Morris 2003a; Go et al. 2009). Therefore, it has been shown that flavonoids, such as flavonols (e.g., quercetin; Fig. 9), flavanones (e.g., naringenin; Fig. 7), isoflavones (e.g., genistein; Fig. 10) and glycosylated flavonol derivatives (e.g., rutin; Fig. 9), directly interact with the ATP-binding site and the vicinal steroid binding site, leading to the inhibition of P-gp function (Morris and Zhang 2006; Lohner et al. 2007; Chen et al. 2010). Others flavonoids compounds, like epicatechin (Fig. 6) from green tea, activate P-gp by a heterotropic allosteric mechanism (Lohner et al. 2007). In addition, Morris and Zhang (2006) reported that flavonoids may also directly bind to the C-terminal NBD2 of the purified recombinant mouse P-gp; for instance chrysin (Fig. 8), quercetin (Fig. 9) and kaempferol (Fig. 9) directly bound to the NBD2 cytosolic domain of mouse P-gp (Chan et al. 2009). According to Badhan and Penny (2006) flavonoids have also shown to interact with NBDs of ATPase transporters. The inhibition of the ATPase activity and direct binding of flavonoids to the C-terminal NBD2 of murine Abcb1 has been demonstrated using saturation transfer difference-nuclear magnetic resonance spectroscopy (Badhan and Penny 2006).

Thus, the use of flavonoids as potential modulators of P-gp-mediated drug efflux is highly attractive and NBDs represent potential targets for therapeutic intervention (Badhan and Penny 2006). Flavonoids bind to NBD of P-gp by overlapping simultaneously both binding sites of the cytosolic domains (Trompier et al. 2003; Kitagawa 2006). As a result, these compounds have been suggested to be modulators with bifunctional interactions at vicinal ATP-binding sites and steroid-interacting regions of the P-gp, which are expected to be in close proximity to the ATP-binding site, within a cytosolic domain of P-gp (Conseil et al. 1998; Kitagawa et al. 2005; Kitagawa 2006; Morris and Zhang 2006; Tran et al. 2011). In such situation, the hydrophobic ring B would bind to the steroid-binding region, whereas rings A and C would bind to the ATP pocket (Kitagawa et al. 2005). The results presented by Sheu et al. (2010) confirm that flavonoids have bifunctional interactions in their rings A and C at the ATP-binding site, and the hydrophobic ring B at the steroid-interacting hydrophobic sequence of P-gp. Thus, flavonoids may induce their binding affinity towards NBD2 of P-gp through their ability to mimic the adenine moiety of ATP (Di

Pietro et al. 2002; Boumendjel et al. 2002; Kitagawa 2006). However, it is also postulated that these compounds may also interact with P-gp by other mechanisms once opposite effects on P-gp ATPase activity have been observed for different flavonoids (Morris and Zhang 2006).

Although chalcones, flavones and flavonols have demonstrated to possess MDR reversing activity through their high affinity to bind P-gp, the isoflavones have been reported as inactive as modulators of the P-gp functioning (Hadjeri et al. 2003). Overall, the concentrations of flavonoids required to produce a significant modulation of P-gp activity seem to be 10  $\mu\text{M}$  or higher. These concentrations appear to be easily achievable in the intestine after food ingestion and, especially, in the course of dietary supplementation (Morris and Zhang 2006; Bansal et al. 2009).

Indeed, many of flavonoid glycosides may not potently interact with P-gp. However, the aglycones released from these glycosides could be present in intestine at high enough concentrations to inhibit intestinal P-gp, resulting in potential drug interactions (Morris and Zhang 2006; Bansal et al. 2009). Nevertheless, except for the more potent flavonoids, the concentration of flavonoid aglycones in the systemic circulation may not be high enough for the occurrence of significant P-gp interactions in other tissues (Morris and Zhang 2006). Moreover, because the main metabolites of flavonoids (glucuronides and sulphate conjugates) are organic anions, these may not interact with P-gp. Hence, even after regular supplementation the systemic inhibition of P-gp by flavonoids or their metabolites may be, in general, insignificant. However, a significant interaction could occur after administration of an extremely high dose, especially by intravenous (IV) injection (Morris and Zhang 2006; Bansal et al. 2009). In that way, flavonoid modulators also have limitations besides their moderate activity as P-gp inhibitors. Thus, they have a broad spectrum of biological activities including anti-estrogenic activity and the inhibition of other ATPases (Chan et al. 2009); they can also inhibit the absorption of some nutrients, change the pharmacokinetics of certain drugs, and affect neurobehavioral development (Wang et al. 2007).

#### Interactions of flavonoids with P-glycoprotein

Currently, with the resurgence of the use of medicinal herbs in the western world, the combined use of

modern and traditional therapies is increasingly becoming more common (Pathak and Udupa 2010). Although many ingredients in the dietary supplements are not thoroughly studied, a significant number of people regularly consume them in the hope of improving their health and prevent or treat specific diseases (Peng et al. 2006). However, either harmful or beneficial food–drug interactions can occur, particularly due to changes in drug bioavailability. Therefore, the interactions of dietary supplements with drugs may be exploited as a way to improve the pharmacokinetic properties of the co-administered drug (Peng et al. 2006; Bansal et al. 2008; Pathak and Udupa 2010). In the process of drug discovery and development, several drug candidates are found to be potent, safe, selective and affordable, but often do not have a favourable pharmacokinetic profile. Thus, the co-administration of another drug or molecule may improve the drug's pharmacokinetic properties. In this way a promising approach to overcome the P-gp-mediated drug resistance can be the co-administration of safe dietary phytochemicals that are inhibitors of known drug transporters or metabolizing enzymes which are determinants for drug biodisposition (Peng et al. 2006). This strategy can result in a more cost-effective therapy, by saving additional amounts of drugs due to the optimization of drug delivery (Amin 2013).

The resistance against a variety of chemically unrelated drugs may be due to the overexpression of P-gp in the plasma membrane (Breier et al. 2013). The dietary flavonoids have gained a great attention in respect of the discovery of effective P-gp inhibitors owing to their various health promoting effects and favourable safety profiles. Indeed, because some of these compounds appear to inhibit P-gp-mediated drug efflux, they have potential value to enhance the cellular availability of diverse drugs (Go et al. 2009). Hence, through an effect on the ATPase activity, the flavonoids can modulate ABC transporters by inhibition (e.g., genistein and quercetin) or induction (e.g., glabridin) (Alvarez et al. 2010).

Many prototypic inhibitors and inducers affect both CYP3A4 and P-gp and thus many drug interactions caused by these inhibitors and inducers involve these two systems. Nevertheless, although the P-gp participates in drug efflux phenomena affecting absorption, distribution and elimination of numerous drugs, the CYP enzymes are only involved in drug metabolism (Lin 2003). Due to their beneficial pharmacological

activities and their additional ability to modulate both CYP3A4 and P-gp, flavonoids have attracted much attention in recent years (Hsiu et al. 2002; Fang et al. 2005; Choi and Li 2005; Rao et al. 2007; Cho et al. 2011; Li et al. 2011). To eliminate foreign compounds, P-gp co-localizes with CYP3A4 in the polarized epithelial cells of excretory organs, such as the liver, kidney and intestine (Li et al. 2011). Since CYP3A4 and P-gp share a substantial overlap in substrate specificity, their function can modulate the bioavailability of many drugs, affecting their pre-systemic metabolism (Choi and Burm 2006; Choi and Kang 2008; Li et al. 2009, 2011) and promoting the circulation of P-gp substrates between the lumen and epithelial cells. These facts prolong the drug exposure to CYP3A4, reducing its systemic absorption (Choi and Li 2005; Choi and Burm 2006; Choi and Kang 2008). Thus, it seems important to understand the actions of flavonoids not only on the function of P-gp, but also at the P-gp expression levels (Lohner et al. 2007).

Actually, the effects of flavonoids compounds on the level of P-gp expression and/or P-gp activity in cells and tissues may globally change the pharmacokinetic parameters of many drugs. Obviously, this can lead to modifications in drug bioavailability, as well as in biodisposition and toxicity (Chieli et al. 2009). However, many phytochemicals have poor bioavailability and show limited efficacy in vivo (Go et al. 2009). With the great interest in herbal components as alternative medicines, more preclinical and clinical investigation of interactions between herbal constituents and drugs needs to be performed to prevent potential adverse reactions, or to utilize those interactions for therapeutic benefit (Piao and Choi 2008a; Shin et al. 2009; Pathak and Udupa 2010; Singh et al. 2012). However, there is far less information on the pharmacokinetic interactions between herbal products and drugs (Shin et al. 2009; Pathak and Udupa 2010; Singh et al. 2012) and several efforts are currently being expended to identify natural phytocompounds that modulate P-gp and metabolic enzymes (Shin et al. 2009; Cho et al. 2011).

Due to the popular use of flavonoid-containing dietary supplements, flavonoid–drug interactions have been increasingly reported (Singh et al. 2012). Therefore, the discovery of promising compounds for P-gp inhibition among flavonoids still continuous (Go et al. 2009). The literature cites various examples of

flavonoids as inhibitors of P-gp transport, which affect the bioavailability and uptake of many drugs. These include in vitro *studies* on the effect of flavonoids on intracellular accumulation, efflux or transport of P-gp substrates using cells over-expressing P-gp, or a variety of animal and clinical studies (Bansal et al. 2009). Then, several in vitro, in vivo non-clinical and clinical assays performed with different flavonoids are presented below, constituting a summary of the literature data and a starting point for new studies.

#### Cell-based in vitro models

Over the years, a great diversity of flavonoids compounds has been tested in in vitro conditions, particularly in cell-based models, aiming to assess their effects on the expression levels and functional activity of P-gp (Table 2). In many studies, molecular biology assays prior to functional tests were performed either as confirmatory tests to effectively verify the presence of P-gp in the cells, or as quantization assays to determine the effects of the flavonoids on the expression levels of P-gp.

In order to evaluate the potential effect that a flavonoid may have in the P-gp activity (as inhibitor or inducer), at least one P-gp probe substrate must be used in the in vitro functional assays which include accumulation/efflux and/or transport assays. However, the selection of the adequate P-gp probe substrate is not easy, especially because they may interact with other transport proteins and metabolizing enzymes. Thus, according to the Food and Drug Administration draft guidance for drug interaction studies, an in vitro P-gp probe substrate should be selective for the P-gp transporter, exhibit low to moderate passive membrane permeability through the cell monolayer, not suffer significant metabolism, be commercially available, and be suitable to be also used as an in vivo P-gp probe substrate (Guidance for Industry, FDA, 2006).

Analyzing Table 2, there are flavonoids that stand out as being simultaneously inducers and inhibitors of P-gp. For example, the flavanol epigallocatechin gallate (Fig. 6) appeared to be a strong inducer of P-gp expression in Caco-2 cells when used at a concentration of 10  $\mu\text{M}$  (Lohner et al. 2007); however, its uses at concentrations levels in the order of 100  $\mu\text{M}$  inhibited P-gp function in the same cell model (Jodoin

**Table 2** An overview of the effects of flavonoids on P-glycoprotein (P-gp) evaluated by cell-based in vitro studies

Flavonoids	Cell models	Flavonoid concentration ( $\mu\text{M}$ )	P-gp expression assays		P-gp functional assays		References	
			Western blot (no significant effect)	Western blot (no significant effect)	Type of assays	Probe substrates		Methods
<i>Chalcones</i>								
Phloretin	MCF-7/ADR, MDA435/LCC6WT	50	Western blot	Western blot (no significant effect)	A	[ $^3\text{H}$ ]Daunomycin	Liquid scintillation counting	Inhibition Zhang and Morris (2003a)
<i>Flavanolols</i>								
Silibinin	MCF-7/ADR	10–100	–	–	A	Rhodamine 123	Spectrofluorometry	Inhibition Lee and Choi (2010)
Silymarin	Caco-2	50–150 7–300 150 100	Western blot	Western blot	A T A A/E	[ $^3\text{H}$ ]Digoxin [ $^3\text{H}$ ]vinblastine [ $^3\text{H}$ ]Daunomycin	Liquid scintillation counting Liquid scintillation counting	Inhibition Zhang and Morris (2003b) Inhibition Chung et al. (2005)
<i>Flavanols</i>								
Catechin	MCF-7/ADR, MDA435/LCC6WT, MDA435/LCCM	50	Western blot	Western blot (no significant effect)	A	[ $^3\text{H}$ ]Daunomycin	Liquid scintillation counting	Inhibition Zhang and Morris (2003a)
	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction- $\uparrow$ mRNA/ $\uparrow$ protein)	Western blot, flow cytometry, RT-PCR (induction- $\uparrow$ mRNA/ $\uparrow$ protein)	–	–	–	– Lohner et al. (2007)
	CH $^{\text{R}}$ C5	100	Western blot	Western blot	A/E	Rhodamine 123	Spectrofluorometry	No effect Jodoin et al. (2002)
	CH $^{\text{R}}$ C5	100	Western blot	Western blot	A/E	Rhodamine 123	Spectrofluorometry	Inhibition Jodoin et al. (2002)
	CH $^{\text{R}}$ C5	100	Western blot	Western blot	A/E	Rhodamine 123	Spectrofluorometry	No effect Jodoin et al. (2002)
	KB-C2	100	Western blot	Western blot	A/E	Daunorubicin	Spectrofluorometry	No effect Kitagawa et al. (2004)
	CH $^{\text{R}}$ C5	300	Western blot	Western blot	A/E	Rhodamine 123	Spectrofluorometry	Inhibition Jodoin et al. (2002)
	KB-C2	100	–	–	A/E	Daunorubicin	Spectrofluorometry	Inhibition Kitagawa et al. (2004)
	CH $^{\text{R}}$ C5	100	Western blot	Western blot	A/E	Rhodamine 123	Spectrofluorometry	No effect Jodoin et al. (2002)
	KB-C2	200 100	–	–	A/E	Daunorubicin Rhodamine 123	Spectrofluorometry Spectrofluorometry	Inhibition Kitagawa et al. (2004)
	CH $^{\text{R}}$ C5	100	Western blot	Western blot	A/E	Rhodamine 123	Spectrofluorometry	No significant effect
<i>Epigallocatechin gallate</i>	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction- $\uparrow$ mRNA/ $\uparrow$ protein)	Western blot, flow cytometry, RT-PCR (induction- $\uparrow$ mRNA/ $\uparrow$ protein)	–	–	–	– Lohner et al. (2007)
	Caco-2	100	Western blot	Western blot	A/E	[ $^3\text{H}$ ]vinblastine	Liquid scintillation counting	Inhibition Jodoin et al. (2002)
	CH $^{\text{R}}$ C5	100	Western blot	Western blot	A/E	Rhodamine 123	Spectrofluorometry	Inhibition Jodoin et al. (2002)
	KB-C2	25 0–100	Western blot	Western blot	A/E	Daunorubicin Rhodamine 123	Spectrofluorometry Spectrofluorometry, FACS flow cytometry	Inhibition Kitagawa et al. (2004)

**Table 2** continued

Flavonoids	Cell models	Flavonoid concentration (μM)	P-gp expression assays		P-gp functional assays		References		
			Western blot, flow cytometry, RT-PCR (induction-↑ mRNA/↑ protein)	Western blot, flow cytometry, RT-PCR (induction-↑ protein)	Type of assays	Probe substrates		Methods	Effects
Taxifolin	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction-↑ mRNA/↑ protein)	Western blot, flow cytometry, RT-PCR (induction-↑ protein)	-	-	-	Lohner et al. (2007)	
<i>Flavanones</i>									
Eriodictyol	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction-↑ protein)	Western blot, flow cytometry, RT-PCR (induction-↑ protein)	-	-	-	Lohner et al. (2007)	
Hesperetin	Caco-2	250	-	-	T	[ <sup>3</sup> H]Talimolol	Liquid scintillation counting	Inhibition?	Ofer et al. (2005)
Naringenin	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction-↑ protein)	Western blot, flow cytometry, RT-PCR (induction-↑ protein)	-	-	-	-	Lohner et al. (2007)
	Caco-2	10–500	-	-	T	Talinolol	HPLC-FLD	Inhibition	Castro et al. (2007)
	Caco-2	855	-	-	T	[ <sup>3</sup> H]Vinblastine	Liquid scintillation counting	Inhibition	Takanaga et al. (1998)
	CRIR12, G185 KB-C2	-	-	-	T	Daurorubicin	FACS flow cytometry	No effect	Wang et al. (2001)
	Caco-2	100	-	-	A/E	Daurorubicin	Spectrofluorometry	No effect	Kitagawa et al. (2005)
Naringin	Caco-2	250	-	-	T	[ <sup>3</sup> H]Talimolol	Liquid scintillation counting	Inhibition?	Ofer et al. (2005)
	Caco-2	100–2,500	-	-	T	Talinolol	HPLC-FLD	Inhibition	Castro et al. (2007)
	CRIR12, G185 HK-2	643	-	-	T	[ <sup>3</sup> H]Vinblastine	Liquid scintillation counting	Slightly inhibition	Takanaga et al. (1998)
	Caco-2	-	-	-	T	Daurorubicin	FACS flow cytometry	No effect	Wang et al. (2001)
	HK-2	10–100	Western blot, RT-PCR (inhibition-↓ mRNA)	Western blot, RT-PCR (inhibition-↓ mRNA)	A	Calcein-AM	Spectrofluorometry	Inhibition	Romiti et al. (2004)
<i>Flavones</i>									
Apigenin	Caco-2	10	Western blot, flow cytometry, RT-PCR (no effect)	Western blot, flow cytometry, RT-PCR (no effect)	-	-	-	-	Lohner et al. (2007)
Baicalein	KB-C2	0–200	-	-	A/E	Daurorubicin	Spectrofluorometry	Inhibition	Kitagawa et al. (2005)
	MCF-7/ADR	1–10	-	-	A	Rhodamine 123	Spectrofluorometry	Inhibition	Shin et al. (2009); Cho et al. (2011); Li et al. (2011)
Chrysin	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction-↑ mRNA/↑ protein)	Western blot, flow cytometry, RT-PCR (induction-↑ mRNA/↑ protein)	-	-	-	-	Lohner et al. (2007)
5,7-dimethoxyflavone	LLC-GA5-COL150	1–300	Western blot	Western blot	A	Rhodamine 123	Spectrofluorometry	Inhibition	Patansethanon et al. (2007)
Diosmin	Caco-2	10–100 0–50	-	-	A T	Rhodamine 123 Digoxin	Spectrofluorometry LC-MS/MS	Inhibition Inhibition	Yoo et al. (2007)

Table 2 continued

Flavonoids	Cell models	Flavonoid concentration ( $\mu\text{M}$ )	P-gp expression assays	P-gp functional assays		References	
				Type of assays	Effects		
Flavone	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction- $\uparrow$ mRNA/ $\uparrow$ protein)	-	-	Lohner et al. (2007)	
3,5,6,7,8,3',4'-Heptamethoxyflavone	Caco-2	0.5–50	-	T	Talinolol	Inhibition	Mertens-Talcott et al. (2007)
	Caco-2	1–100	-	A	[ $^3\text{H}$ ]vinblastine	Inhibition	Takanaga et al. (2000)
Nobiletin	LLC-GA5-COL300	20	-	-	-	Inhibition	-
	K562/ADR	1–1000	Western blot	-	[ $^3\text{H}$ ]vincristine	Inhibition	Ikegawa et al. (2000)
Oroxylin A	Caco-2	0.5–50	-	T	Talinolol	Inhibition	Mertens-Talcott et al. (2007)
	Caco-2	1–100	-	A	[ $^3\text{H}$ ]vinblastine	Inhibition	Takanaga et al. (2000)
3,5,7,3',4'-pentamethoxyflavone	LLC-GA5-COL300	20	-	-	-	Inhibition	-
	K562/ADR	1–1000	Western blot	A	[ $^3\text{H}$ ]vincristine	Inhibition	Ikegawa et al. (2000)
Oroxylin A	LLC-GA5-COL150	1–300	Western blot	A	Daunorubicin	Inhibition	Patanasethanont et al. (2007)
	NCI/ADR-RES	0.01–40	Western blot	A	Rhodamine 123	Inhibition	Go et al. (2009)
Sinensetin	MCF-7/ADR	10–20	Western blot	A	Calectin-AM	Inhibition	-
	Caco-2	30–90	Western blot, RT-PCR (inhibition- $\downarrow$ mRNA/ $\downarrow$ protein)	A	Paclitaxel	Inhibition	Zhu et al. (2013)
Tangeritin	Caco-2	0.5–50	-	T	Talinolol	Inhibition	Mertens-Talcott et al. (2007)
	Caco-2	0.5–50	-	T	Talinolol	Inhibition	Mertens-Talcott et al. (2007)
7,3',4'-trimethoxyflavone	Caco-2	1–100	-	A	[ $^3\text{H}$ ]vinblastine	Inhibition	Takanaga et al. (2000)
	LLC-GA5-COL300	20	-	-	-	Inhibition	-
Fisetin	K562/ADR	1–1,000	Western blot	A	[ $^3\text{H}$ ]vincristine	Inhibition	Ikegawa et al. (2000)
	Caco-2	50–400	-	T	Paclitaxel	Inhibition	Jeong and Choi (2007)
-	KB-C2	0–200	-	A/E	Daunorubicin	Inhibition	Kitagawa et al. (2005)



**Table 2** continued

Flavonoids	Cell models	Flavonoid concentration (μM)	P-gp expression assays		P-gp functional assays		References	
			Flavonoid concentration (μM)	Assay type	Type of assays	Methods		Effects
Isoquercetin	Caco-2	100	–	–	T	[ <sup>3</sup> H]Talinolol	Liquid scintillation counting	Inhibition? Ofer et al. (2005)
	KB-C2	0–200	–	–	A/E	Daurorubicin Rhodamine 123	Spectrofluorometry Spectrofluorometry, FACS flow cytometry	No effect No effect Kitagawa et al. (2005)
Morin	KB-C2	0–200	–	–	A/E	Daurorubicin	Spectrofluorometry	Inhibition Kitagawa et al. (2005)
	MCF-7/ADR, MDA435/LCC6WT, MDA435/LCCM	50	Western blot (no significant effect)	–	A	[ <sup>3</sup> H]Daunomycin	Liquid scintillation counting	Inhibition Zhang and Morris (2003a)
Myricetin	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction-↑mRNA/protein)	–	–	–	–	– Lohner et al. (2007)
	KB-C2	0–200	–	–	A/E	Daurorubicin	Spectrofluorometry	Inhibition Kitagawa et al. (2005)
Kaempferol	MCF-7/ADR	3–30	–	–	A	Rhodamine 123	Spectrofluorometry	Inhibition Choi et al. (2010)
	Caco-2	30	–	–	T	[ <sup>3</sup> H]Talinolol	Liquid scintillation counting	Inhibition? Ofer et al. (2005)
	CRIR12, G185	–	–	–	T	Daurorubicin	FACS flow cytometry	No effect Wang et al. (2001)
	HK-2	10–100	Western blot, RT-PCR (inhibition-↓mRNA)	–	A/E	Calcein-AM	Spectrofluorometry	Inhibition Romiti et al. (2004)
Kaempferol-3,4-O-dimethyl ether	KB-C2	0–200	–	–	A/E	Daurorubicin Rhodamine 123	Spectrofluorometry Spectrofluorometry, FACS flow cytometry	Inhibition Inhibition Kitagawa et al. (2005)
	KB-V1	10–200	Western blot (no effect)	–	A/E	Rhodamine 123	Flow cytometry	Inhibition Khantamat et al. (2004)
	MFC-7/ADR	100	Western blot	–	A/E	[ <sup>3</sup> H]Daunomycin	Liquid scintillation counting	Inhibition Chung et al. (2007)
	MFC-7/ADR	100	Western blot	–	A/E	[ <sup>3</sup> H]Daunomycin	Liquid scintillation counting	Inhibition Chung et al. (2007)
Kaempferol-3-O-methyl ether	MFC-7/ADR	100	Western blot	–	A/E	[ <sup>3</sup> H]Daunomycin	Liquid scintillation counting	Inhibition Chung et al. (2007)

Table 2 continued

Flavonoids	Cell models	Flavonoid concentration ( $\mu\text{M}$ )	P-gp expression assays	P-gp functional assays		References	
				Type of assays	Methods		Effects
Quercetin	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction- $\uparrow$ mRNA/ $\uparrow$ protein)	–	–	Lohner et al. (2007)	
	Caco-2	20	–	T	Liquid scintillation counting	Inhibition? Ofer et al. (2005)	
	HK-2	0.5–200	Western blot	A	Calcein-AM	Spectrofluorometry	Chieli et al. (2009)
	CRIR12	0–250	–	T	Rhodamine 123 Daunorubicin	FACS flow cytometry	Slightly inhibition Wang et al. (2001)
	EPP85-181RDB	3–12	Western blot, immunocytochemical analysis (inhibition)	A	Calcein-AM	Spectrofluorometry	Inhibition Borska et al. (2010)
	KB-C2	0–200 100	–	A/E	Daunorubicin Rhodamine 123	Spectrofluorometry Spectrofluorometry, FACS flow cytometry	Inhibition Inhibition Kitagawa et al. (2005)
	MBEC4	5–50	ELISA (biphasic effect)	A/E	$[^3\text{H}]$ vincristine	Liquid scintillation counting	Biphasic effect Mitsunaga et al. (2000)
	MCF-7/ADR	1–10	Immunocytofluorimetric assay (inhibition-10 $\mu\text{M}$ )	E	Rhodamine 123	FACS flow cytometry	Inhibition Scambia et al. (1994)
	MCF-7/ADR	1–10	–	A	Rhodamine 123	Spectrofluorometry	Inhibition Choi et al. (2011)
	Caco-2	100	–	T	$[^3\text{H}]$ Talinolol	Liquid scintillation counting	Inhibition? Ofer et al. (2005)
	MCF-7/ADR	1–10	Immunocytofluorimetric assay	E	Rhodamine 123	FACS flow cytometry	Inhibition Scambia et al. (1994)
	3',4',7-trimethoxyquercetin	MCF-7/ADR	1–10	Immunocytofluorimetric assay	E	Rhodamine 123	FACS flow cytometry
KB-C2		100	–	A/E	Daunorubicin	Spectrofluorometry	No effect Kitagawa et al. (2005)
KB-C2		100	–	A/E	Rhodamine 123	Spectrofluorometry, FACS flow cytometry	No effect
<i>Isoflavones</i>							

**Table 2** continued

Flavonoids	Cell models	Flavonoid concentration (µM)	P-gp expression assays	P-gp functional assays			References
				Probe substrates	Methods	Effects	
			Type of assays				
Biochanin A	Caco-2	50, 100, 150	Western blot	[ <sup>3</sup> H]Digoxin	Liquid scintillation counting	Inhibition	Zhang and Morris (2003b)
		20, 50, 100, 150		[ <sup>3</sup> H]Digoxin		Inhibition	
Daidzein	MCF-7/ADR	150	–	[ <sup>3</sup> H]Vinblastine	Liquid scintillation counting	Inhibition	Chung et al. (2005)
		100		[ <sup>3</sup> H]Daunomycin		Inhibition	
Genistein	MCF-7/ADR	10, 50	–	Mitoxantrone	FACS flow cytometry	Biphasic effect	An and Morris (2010)
		50		Western blot (no significant effect)	[ <sup>3</sup> H]Daunomycin	Liquid scintillation counting	
Daidzein	MCF-7/ADR, MDA435/LCC6WT, MDA435/LCC6MDR1	10	Western blot, flow cytometry, RT-PCR (induction-↑mRNA/↑protein)	–	–	–	Lohner et al. (2007)
Genistein	Caco-2	10	Western blot, flow cytometry, RT-PCR (induction-↑mRNA/↑protein)	–	–	–	Lohner et al. (2007)
	BC19/3, MCF-7/NP, BALB/c-3T3-1000	200	Western blot, RT-PCR	A/E	Daunorubicin Rhodamine 123	Inhibition Inhibition	Castro and Altenberg (1997)

A accumulation studies, E efflux studies, ELISA enzyme-linked immunosorbent assay, FACS fluorescence-activated cell sorting, FLD fluorescent detection, HPLC high-performance liquid chromatography, LC-MS/MS liquid chromatography–tandem mass spectrometry, mRNA messenger ribonucleic acid, RT-PCR real-time polymerase chain reaction, T transport studies, UV ultraviolet detection

? Probably

↓ Decrease

↑ Increase

et al. 2002). An and Morris (2010) and Mitsunaga et al. (2000) also reported a biphasic effect of biochanin A (Fig. 10) and quercetin (Fig. 9) in the P-gp function. Mitsunaga et al. (2000) concluded that low concentrations of quercetin indirectly activate the efflux transport of [ $^3\text{H}$ ]vincristine by enhancing the phosphorylation, and thus the activity of P-gp, whereas high concentrations of quercetin lead to P-gp inhibition. Thus, the uptake of [ $^3\text{H}$ ]vincristine by MBEC4 cells in the steady-state was decreased by 10  $\mu\text{M}$  quercetin, but increased by 50 mM quercetin (Mitsunaga et al. 2000). Likewise, Scambia et al. (1994) reported that 10  $\mu\text{M}$  of quercetin reduces the expression of the immunoreactive P-gp in MCF-7/ADR-resistant cells as evaluated by flow cytometry. In the study performed by An and Morris (2010), the intracellular fluorescence of mitoxantrone, a P-gp substrate, decreased when co-incubated with 10  $\mu\text{M}$  of biochanin A while there was no significant difference in mitoxantrone cell accumulation in the presence of 50  $\mu\text{M}$  biochanin A. These results indicate that biochanin A may induce the P-gp activity at lower concentrations, and, hence, decreasing the intracellular concentration of mitoxantrone. Actually, several studies summarized in Table 2 revealed that the inhibitory effects of various flavonoids on P-gp function were concentration-dependent (e.g., diosmin, fisetin, naringin, silymarin, tangeritin) (Romiti et al. 2004; Kitagawa et al. 2005; Castro et al. 2007; Mertens-Talcott et al. 2007; Yoo et al. 2007). The increase in P-gp expression levels caused by some flavonoids such as catechin (Fig. 6), naringenin (Fig. 7) or chrysin (Fig. 8) in *in vitro* intestinal epithelial cells may represent an adaptation or defence mechanism limiting the entry of lipophilic xenobiotics into the organism (Lohner et al. 2007). In this context, through intracellular drug accumulation studies, it was demonstrated that the *C*-isoprenylation of chrysin enhances the binding affinity toward P-gp. Actually, the 8-(3,3-dimethylallyl)chrysin was described to be more efficient than cyclosporin A in the inhibition of P-gp in leukemic cells (Comte et al. 2001).

The *in vitro* results shown by Ofer et al. (2005) also demonstrated that the flavonoids hesperetin, naringin (Fig. 7), isoquercetin, kaempferol, quercetin and spiraeoside (Fig. 9) bear the ability to interfere with processes of secretory intestinal transport. These effects may occur due to the interaction of flavonoids with P-gp, but apparently not via competition at the

talinolol (P-gp probe substrate) binding site of P-gp as demonstrated by the radioligand binding assay, where none of the tested flavonoids showed to induce the displacement of talinolol. Another mode of interaction may be the inhibition of members of the organic cation transporters-family, which are located at the basolateral membrane of intestinal epithelial cells. These organic cation transporters are mainly located in the basolateral membranes of kidney and liver and usually mediate the uptake of compounds with a transiently or permanently positive net charge into the cells of these excretory organs (Ofer et al. 2005).

In some cases, the use of distinct *in vitro* systems and/or assays yielded slightly different results. Therefore, it is important to test flavonoid compounds in different cell lines and using different assays in order to increase the confidence in their classification as inhibitors or inducers of P-gp. The inhibition of P-gp activity by many flavonoids and the increased cell accumulation of many drugs (P-gp substrates) suggests the possibility of important changes in absorption/bioavailability of such drugs by the co-administration of these compounds (Zhang and Morris 2003b). In this context, despite the valuable evidences often obtained from these *in vitro* studies on the modulation of P-gp by flavonoids, more conclusive results are only achieved through interaction studies conducted in *in vivo* conditions (Choi et al. 2010, 2011a).

#### *In vivo* animal models

Table 3 summarizes the main features regarding the *in vivo* studies conducted to assess the effects of the oral administration of flavonoids in the bioavailability of different drugs (P-gp substrates) administered orally and/or intravenously. Indeed, it has been reported that pharmacokinetic interaction between herbal constituents and drugs may be mediated by CYP450 metabolizing enzymes and also by drug transporters such as P-gp (Cho et al. 2009). Based on the broad overlap in their substrate specificities as well as their co-localization in the small intestine (the primary site of absorption for orally administered drugs), CYP3A4 and P-gp have been recognized as a concerted barrier to drug intestinal absorption (Shin et al. 2009). Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically in pre-systemic metabolism, limiting the absorption of drugs which are substrates of both

**Table 3** An overview of the effects of flavonoids on the pharmacokinetic parameters of some drugs (P-glycoprotein substrates) evaluated by non-clinical in vivo studies

Flavonoids	Animal model	Drug, route, dose (mg kg <sup>-1</sup> )	Flavonoid dose (mg kg <sup>-1</sup> )	Effects on pharmacokinetic parameters	References
<i>Flavanonols</i>					
Silibinin	Male Sprague-Dawley rats	Paclitaxel, PO, 40	0.5 <sup>#</sup> 2.5–10 <sup>#</sup>	→T <sub>max</sub> , ↓V <sub>d</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB ↓T <sub>max</sub> , ↓V <sub>d</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB ↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑t <sub>1/2</sub> , ↑RB	Lee and Choi (2010)
Silymarin	Male Sprague-Dawley rats	Paclitaxel, IV, 4 Paclitaxel (Taxol®) PO, 25 Paclitaxel (microemulsion) PO, 25	0.5–10 <sup>#</sup> 10–20 <sup>†</sup> 10 <sup>†</sup> 15–20 <sup>†</sup>	↑t <sub>1/2</sub> , ↓V <sub>ss</sub> , ↑C <sub>max</sub> , ↑F, ↑AUC <sub>0–∞</sub> , ↓CL <sub>t</sub> /F, ↑T <sub>max</sub> , ↑RB ↓t <sub>1/2</sub> , ↓V <sub>ss</sub> , ↓C <sub>max</sub> , ↓F, ↑AUC <sub>0–∞</sub> , ↓CL <sub>t</sub> /F, ↑T <sub>max</sub> , ↓RB ↓t <sub>1/2</sub> , ↓V <sub>ss</sub> , ↑C <sub>max</sub> , ↑F, ↑AUC <sub>0–∞</sub> , ↓CL <sub>t</sub> /F, ↑T <sub>max</sub> , ↑RB	Park et al. (2012)
<i>Flavonols</i>					
Epigallocatechin	Male Sprague-Dawley rats	Diltiazem, PO, 15	1–12 <sup>#</sup>	↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑t <sub>1/2</sub> , →T <sub>max</sub> , ↑F, ↑RB	Li and Choi (2008)
Hesperidin	Male Sprague-Dawley rats	Verapamil, PO, 9	3–10 <sup>†</sup>	↓CL <sub>t</sub> , ↓K <sub>el</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑T <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB →T <sub>max</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB	Piao and Choi (2008a) Choi et al. (2009)
	Male Sprague-Dawley rats	Diltiazem, PO, 15	1 <sup>#</sup> 5–15 <sup>#</sup>	↓T <sub>max</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB	
Morin	Female Sprague-Dawley rats	Docetaxel, PO, 20	15 <sup>#</sup>	↓T <sub>max</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑t <sub>1/2</sub> , ↑C <sub>max</sub> , ↑F	Yang et al. (2011)
	Male Sprague-Dawley rats	Docetaxel, IV, 4	15 <sup>#</sup>	↓AUC <sub>0–∞</sub> , ↓t <sub>1/2</sub> , ↓MRT, ↓V <sub>ss</sub> , ↑CL <sub>t</sub> , ↑CL <sub>R</sub> , ↓CL <sub>NR</sub>	
	Male Sprague-Dawley rats	Tamoxifen, PO, 10	3–10 <sup>#</sup>	↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB	Shin et al. (2008)
	Male Sprague-Dawley rats	Diltiazem, PO, 15	1.5–7.5 <sup>†</sup> 15 <sup>†</sup>	↓C <sub>max</sub> , →T <sub>max</sub> , ↓t <sub>1/2</sub> , ↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑K <sub>el</sub> , ↑F, ↑RB ↓C <sub>max</sub> , →T <sub>max</sub> , ↓t <sub>1/2</sub> , ↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑K <sub>el</sub> , ↑F, ↑RB →T <sub>max</sub> , ↑AUC <sub>0–∞</sub> , ↑t <sub>1/2</sub> , ↓C <sub>max</sub> , ↑F	Choi and Han (2005)
	Male Sprague-Dawley rats	Paclitaxel, PO, 6 Paclitaxel, IV, 2	5–15 <sup>#</sup> 5 <sup>#</sup> 15 <sup>#</sup>	↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑t <sub>1/2</sub> , ↓V <sub>ss</sub> ↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑t <sub>1/2</sub> , →V <sub>ss</sub> ↓CL <sub>t</sub> , ↓t <sub>1/2</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑K <sub>el</sub> , ↑RB	Li et al. (2007)
	Male Sprague-Dawley rats	Nicardipine, PO, 12	1.5 <sup>#</sup> 7.5 <sup>#</sup>	↓CL <sub>t</sub> , →t <sub>1/2</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , →K <sub>el</sub> , ↑RB ↓CL <sub>t</sub> , ↑t <sub>1/2</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↓K <sub>el</sub> , ↑RB	Piao and Choi (2008b)
	Male Sprague-Dawley rats	Nicardipine, IV, 4	15 <sup>#</sup>	↓CL <sub>t</sub> , ↑t <sub>1/2</sub> , ↑AUC <sub>0–∞</sub> , ↓K <sub>el</sub>	
	Male Sprague-Dawley rats	Paclitaxel, PO, 30	7.5–15 <sup>#</sup> 3.3 <sup>#</sup> 10 <sup>#</sup>	↓T <sub>max</sub> , ↓CL <sub>t</sub> , ↓V <sub>d</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , →t <sub>1/2</sub> , ↑F, ↑RB ↓T <sub>max</sub> , ↓CL <sub>t</sub> , ↓V <sub>d</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB ↓CL <sub>t</sub> , ↓V <sub>d</sub> , ↓t <sub>1/2</sub> , ↑AUC <sub>0–∞</sub> , ↑MRT	Choi et al. (2006)
	Male New Zealand white rabbits	Paclitaxel, IV, 3 Nimodipine, PO, 15	3.3–10 <sup>#</sup> 2 <sup>#</sup>	↓K <sub>el</sub> , ↑AUC <sub>0–24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB ↓K <sub>el</sub> , ↑AUC <sub>0–24</sub> , ↑C <sub>max</sub> , ↑t <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB ↓K <sub>el</sub> , ↑AUC <sub>0–24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB	Choi and Burm (2006)
	Male Wistar rats	Nimodipine, PO, 15 Talinolol, PO, 10	10–20 <sup>#</sup> 2–20 <sup>†</sup> 0.1 <sup>#</sup> 2.5 <sup>#</sup> 5 <sup>#</sup>	↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↓T <sub>max</sub> , ↓t <sub>1/2</sub> , ↑F ↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↑T <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F ↓CL <sub>t</sub> , ↑AUC <sub>0–∞</sub> , ↑C <sub>max</sub> , ↓T <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F ↓AUC <sub>0–∞</sub> , ↑t <sub>1/2</sub> , ↓CL <sub>t</sub>	Pathak and Udupa (2010)
	Yorkshire pigs	Digoxin, PO, 0.02	5 <sup>#</sup> 40 <sup>†</sup>	↑T <sub>max</sub> , ↓MRT, ↑AUC <sub>0–t</sub> , ↑AUMC <sub>0–t</sub> , ↑C <sub>max</sub>	Wang et al. (2004)

Table 3 continued

Flavonoids	Animal model	Drug, route, dose (mg kg <sup>-1</sup> )	Flavonoid dose (PO; mg kg <sup>-1</sup> )	Effects on pharmacokinetic parameters	References
Naringenin	Male Sprague-Dawley rats	Talinolol, PO, 10	0.7 <sup>†</sup>	↑AUC <sub>0-6</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑T <sub>max</sub> , →T <sub>1/2</sub>	de Castro et al. (2008)
Naringin	Female Sprague-Dawley rats	Tamoxifen, PO, 10	1.5–10 <sup>#</sup>	↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Choi and Kang (2008)
	Male New Zealand rabbit	Verapamil, PO, 9	1.5–15 <sup>#</sup>	↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑K <sub>d</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Yeum and Choi (2006)
	Male Sprague-Dawley rats	Paclitaxel, IV, 3	3.3–10 <sup>#</sup>	↓CL <sub>r</sub> , ↑AUC <sub>0-24</sub> , ↑T <sub>1/2</sub> , ↓MRT, ↑V <sub>ss</sub>	Lim and Choi (2006)
	Male Sprague-Dawley rats	Talinolol, PO, 10	2.4 <sup>†</sup>	↑AUC <sub>0-6</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑T <sub>max</sub> , ↑T <sub>1/2</sub>	de Castro et al. (2008)
			9.4 <sup>†</sup>	↑AUC <sub>0-6</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑T <sub>1/2</sub>	
<i>Flavones</i>					
Baicalein	Male Sprague-Dawley rats	Doxorubicin, PO, 50	0.3–6 <sup>#</sup>	↓CL <sub>r</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Shin et al. (2009)
		Doxorubicin, IV, 10		↓CL <sub>r</sub> , ↑AUC <sub>0-24</sub> , ↑T <sub>1/2</sub>	
	Male Sprague-Dawley rats	Tamoxifen, PO, 10	0.5–10 <sup>#</sup>	↓CL <sub>r</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Li et al. (2011)
	Male Sprague-Dawley rats	Irinotecan, PO, 12	0.4–8 <sup>#</sup>	↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Cho et al. (2011)
		Irinotecan, IV, 3		↓CL <sub>r</sub> , ↑AUC <sub>0-24</sub> , ↑T <sub>1/2</sub> , ↑RB	
Flavone	Male Sprague-Dawley rats	Paclitaxel, PO, 40	2–20 <sup>#</sup>	↓T <sub>max</sub> , ↓K <sub>d</sub> , ↑K <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Choi et al. (2004a)
Oroxylin A	Male Sprague-Dawley rats	Paclitaxel, PO, 15	30 <sup>†</sup>	↑AUC <sub>0-24</sub> , →T <sub>max</sub> , ↑C <sub>max</sub> , ↑RB	Go et al. (2009)
<i>Flavonols</i>					
Kampferol	Male Sprague-Dawley rats	Etoposide, PO, 6	1–12 <sup>#</sup>	↓CL <sub>r</sub> , ↓K <sub>d</sub> , ↑AUC <sub>0-24</sub> , →T <sub>max</sub> , ↑C <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Li et al. (2009)
		Etoposide, IV, 2		↓CL <sub>r</sub> , ↓K <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↑T <sub>1/2</sub> , ↑RB	
	Male Sprague-Dawley rats	Tamoxifen, PO, 10	2.5 <sup>#</sup>	↓T <sub>1/2</sub> , ↓T <sub>max</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑F	Piao et al. (2008)
			10 <sup>#</sup>	↓T <sub>1/2</sub> , ↑T <sub>max</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑F	
Myricetin	Male Sprague-Dawley rats	Doxorubicin, PO, 40	0.4–10 <sup>#</sup>	↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Choi et al. (2011b)
	Male Sprague-Dawley rats	Losartan, PO, 9	0.4 <sup>#</sup>	↓V <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Choi et al. (2010)
			2–8 <sup>#</sup>	↓V <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	
	Female Sprague-Dawley rats	Tamoxifen, PO, 10	2–15 <sup>†</sup>	↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑K <sub>d</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Shin et al. (2006)
	Female Wistar rats	Irinotecan, PO, 80	20 <sup>#</sup>	↓T <sub>max</sub> , ↓CL <sub>r</sub> , ↓K <sub>d</sub> , ↓V <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑MRT, ↑T <sub>1/2</sub> , ↑AUC <sub>0-24</sub> , ↑F, ↑RB	Bansal et al. (2008)
				↓CL <sub>r</sub> , ↓V <sub>d</sub> , ↓C <sub>0</sub> , ↑AUC <sub>0-24</sub> , ↑MRT, ↑K <sub>d</sub> , ↑RB	
	Male Sprague-Dawley rats	Cyclosporine, PO, 10	50 <sup>†</sup>	↓AUC <sub>0-24</sub> , ↓C <sub>max</sub>	Hsiu et al. (2002)
	Male Sprague-Dawley rats	Etoposide, PO, 9	1 <sup>†</sup>	↓CL <sub>r</sub> , ↓K <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↓C <sub>max</sub> , ↑T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Li and Choi (2009)
			5–15 <sup>†</sup>	↓CL <sub>r</sub> , ↓K <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	
			1–15 <sup>†</sup>	↓CL <sub>r</sub> , ↓K <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↑T <sub>1/2</sub>	
	Male Sprague-Dawley rats	Etoposide, IV, 3	2–20 <sup>#</sup>	↓T <sub>max</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑T <sub>1/2</sub> , ↑MRT, ↑F, ↑RB	Choi et al. (2004b)
	Male Sprague-Dawley rats	Paclitaxel, PO, 40	0.6–15 <sup>†</sup>	↓V <sub>ss</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Choi et al. (2011a)
	Male New Zealand white rabbits	Doxorubicin, PO, 50	2–20 <sup>#</sup>	↓K <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , →T <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	Choi and Li (2005)
		Diltiazem, PO, 15	2–10 <sup>†</sup>	↓K <sub>d</sub> , ↑AUC <sub>0-24</sub> , ↑C <sub>max</sub> , ↑T <sub>1/2</sub> , ↑F, ↑RB	
			20 <sup>†</sup>	↓K <sub>d</sub> , ↓AUC <sub>0-24</sub> , ↓C <sub>max</sub> , ↑T <sub>1/2</sub> , ↓F, ↓RB	
	Male Yorkshire pigs	Cyclosporine, PO, 10	50 <sup>†</sup>	↓C <sub>max</sub> , ↓AUC <sub>0-24</sub> , ↓AUC <sub>0-3</sub> , ↓AUC <sub>3-24</sub>	Hsiu et al. (2002)

**Table 3** continued

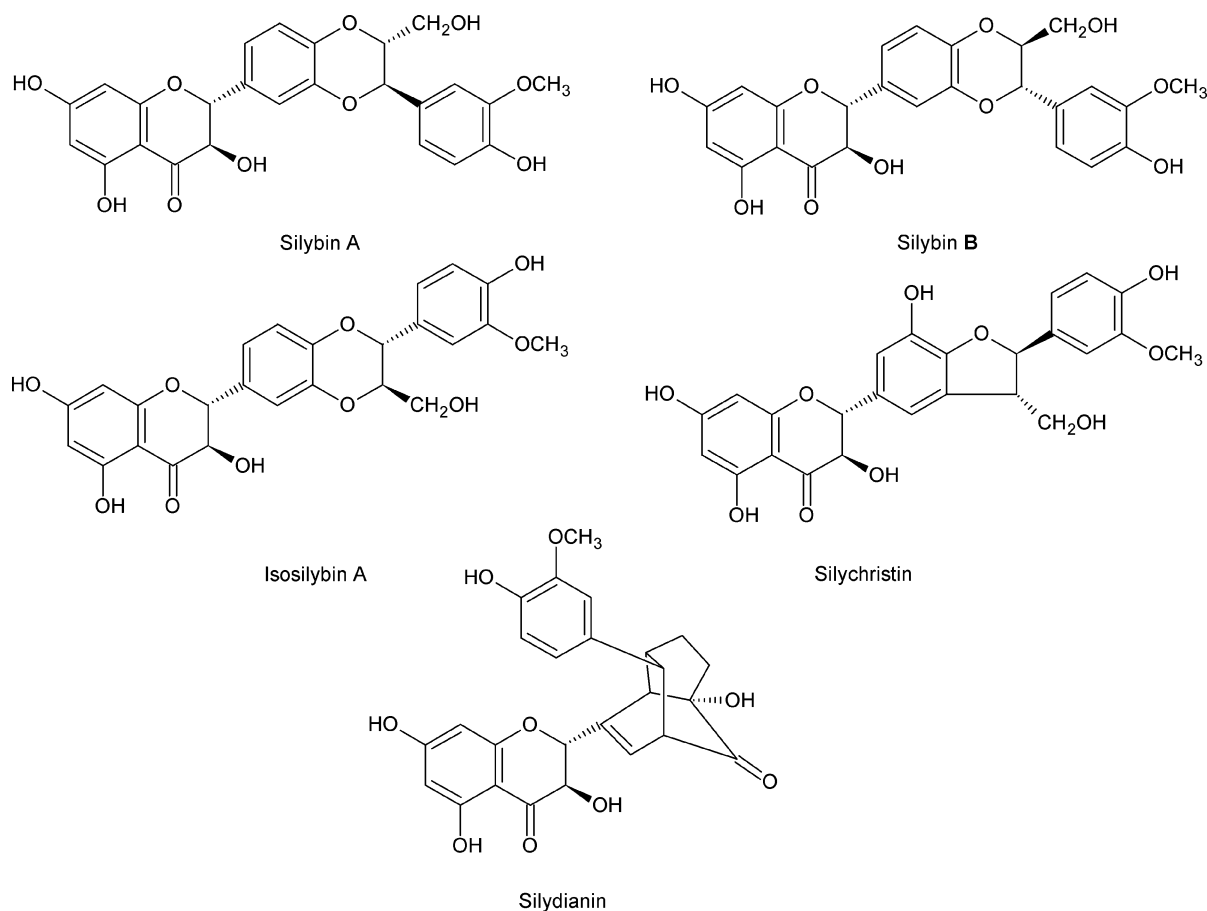
Flavonoids	Animal model	Drug, route, dose (mg kg <sup>-1</sup> )	Flavonoid dose (PO; mg kg <sup>-1</sup> )	Effects on pharmacokinetic parameters	References
<i>Isoflavones</i>					
Biochanin A	Female Sprague-Dawley rats	Tamoxifen, PO, 10	100 <sup>#</sup>	↓AUC <sub>0-∞</sub> , ↓C <sub>max</sub> , ↓T <sub>max</sub> , ↓t <sub>1/2</sub> , ↓F	Singh et al. (2012)
	Male Sprague-Dawley rats	Digoxin, PO, 20	100 <sup>#</sup>	↓t <sub>1/2</sub> , ↑AUC <sub>0-∞</sub> , ↑C <sub>max</sub> , ↓T <sub>max</sub> , ↓MRT, ↑F	Peng et al. (2006)
		Fexofenadine, PO, 20		↓t <sub>1/2</sub> , ↓AUC <sub>0-∞</sub> , ↓C <sub>max</sub> , ↑T <sub>max</sub> , ↑MRT, ↓F,	
		Paclitaxel, PO, 20		↓K <sub>e</sub> , ↓t <sub>1/2</sub> , ↑AUC <sub>0-∞</sub> , ↑C <sub>max</sub> , ↑T <sub>max</sub> , ↑MRT, ↑F	
Genistein	Male Sprague-Dawley rats	Paclitaxel, PO, 30	3.3–10 <sup>#</sup>	↓Cl <sub>t</sub> , ↓T <sub>max</sub> , ↓K <sub>el</sub> , ↑AUC <sub>0-∞</sub> , ↑C <sub>max</sub> , ↑t <sub>1/2</sub> , ↑F, ↑RB	Li and Choi (2007)
		Paclitaxel, IV, 3		↓Cl <sub>t</sub> , ↓K <sub>el</sub> , ↑AUC <sub>0-∞</sub> , ↑t <sub>1/2</sub> , ↑RB	

AUC area under the concentration–time curve, AUC<sub>0-t</sub>, AUC from 0 to t, AUC<sub>0-∞</sub>, AUC from 0 h to infinity, AUMC<sub>0-t</sub> area under the moment curve from time zero to the last point, C<sub>max</sub> peak concentration, CL<sub>NR</sub> non-renal clearance, CL<sub>R</sub> renal clearance, CL<sub>T</sub> total body clearance, CL/F oral clearance, C<sub>0</sub> initial plasma concentration of drugs obtained by back-extrapolation to y-axis, F absolute bioavailability, IV intravenous, k<sub>e</sub> absorption rate constant, K<sub>e</sub> elimination rate constant, MAT mean absorption time, MRT mean residence time, t<sub>max</sub> time to reach C<sub>max</sub>, t<sub>1/2</sub> terminal elimination half-life, V<sub>d</sub> apparent volume of distribution at steady-state, V<sub>d</sub> apparent volume of distribution

<sup>#</sup> Pre-treatment with the flavonoid compound  
 † Co-administration of the drug and flavonoid  
 ↓, Decrease; ↑, increase; →, maintain

proteins (Piao et al. 2008; Cho et al. 2009; Li et al. 2011). Therefore, dual inhibitors against CYP3A4 and P-gp can have a great impact on the bioavailability of many drugs (Shin et al. 2009). Bearing this in mind, several studies using flavonoids have been performed in order to develop P-gp inhibitors that reduce the drug efflux in the intestine and, hence, enhance oral bioavailability of P-gp substrate drugs (Park et al. 2012). The dosage of drug should be also concerned in case of the increased drug toxicity following the combination with flavonoids (Li et al. 2009). In most studies included in the Table 3, the pre-treatment of the animals with a flavonoid (10–30 min before drug administration) is sufficient to significantly increase the oral drug bioavailability. For instance, the oral bioavailability of diltiazem was increased in male Sprague-Dawley rats submitted to a pre-treatment with the flavanone hesperidin (Fig. 7). Li et al. (2011) also describe the higher bioavailability of tamoxifen in male Sprague-Dawley rats pre-treatment with the flavone baicalein (Fig. 8). According to the authors, this increase might be mainly due to the flavonoid-mediated inhibition of CYP3A-mediated metabolism and P-gp efflux (Choi et al. 2004a, 2006; Yeum and Choi 2006; Li et al. 2007, 2009; Shin et al. 2008; Piao and Choi 2008a; Li and Choi 2008; Choi and Kang 2008; Cho et al. 2009, 2011; Shin et al. 2009; Lee and Choi 2010)

In addition, some studies also compared the effect of the flavonoid pre-treatment on the bioavailability of a drug administered either orally or intravenously; as expected, in the majority of these studies the impact of the flavonoid pre-treatment on pharmacokinetics of the drug administered intravenously was less significant than after oral administration (Choi et al. 2006; Li et al. 2007, 2009; Piao and Choi 2008a; Bansal et al. 2008; Shin et al. 2009; Pathak and Udupa 2010; Lee and Choi 2010; Cho et al. 2011). Accordingly, the enhanced oral drug exposure in the presence of the flavonoid together with insignificant changes on drug pharmacokinetics after IV administration, could be mainly due to the increased intestinal absorption via flavonoid-mediated P-gp inhibition rather than the reduced elimination of the drug (Choi et al. 2006; Lee and Choi 2010). As shown in Table 3, the study performed by Li and Choi (2007), in which genistein (Fig. 10) was pre-administered at a dose of 10 mg kg<sup>-1</sup>, the area under the concentration–time curve (AUC) significantly increased and the total body



**Fig. 12** Some constituents of the flavonoid silymarin (Gazák et al. 2007; Katiyar et al. 2008)

clearance ( $CL_t$ ) was reduced for IV paclitaxel. Hence, since genistein is not only subjects to CYP2C8 and CYP3A4 mediated metabolism, but also provides inhibition of P-gp, BCRP and MRP2 efflux function, the decreased  $CL_t$  and increased AUC of etoposide suggests that the metabolism and excretion of paclitaxel in the liver and kidney might be inhibited. This study is consistent with the results reported by Lim and Choi (2006) where a dose of  $10 \text{ mg kg}^{-1}$  of naringin (Fig. 7), a dual inhibitor of CYP3A and P-gp, significantly increased the AUC and reduced the  $CL_t$  of paclitaxel administered by IV route to rats. According to the authors, the significantly greater value of AUC could be due mainly to an inhibition of metabolism of paclitaxel via CYP3A1/2 by oral naringin, but the inhibition of hepatic P-gp by oral naringin could also contribute (Lim and Choi 2006).

In other studies, the animals were not pre-treated with the flavonoid before the administration of drug,

but a simultaneous co-administration was performed. The results obtained after oral and/or IV administration of the drug are similar to the aforementioned (Choi and Han 2005; Shin et al. 2006; Piao and Choi 2008b; de Castro et al. 2008; Li and Choi 2009; Go et al. 2009). In other words, the oral drug bioavailability significantly increased after co-administration of the flavonoid, but the same was not true when the drug was administered intravenously. However, the results reported by Choi and Burm (2006) and Choi and Li (2005) disagree with this theory. Indeed, in male New Zealand white rabbits pre-treated with the flavanone morin (Fig. 7), the oral bioavailability of nimodipine was significantly increased, but the same did not occur when the drug was simultaneously co-administered with the flavonoid. Accordingly, the morin can interact with nimodipine in the gastrointestinal lumen forming a flavonoid-drug complex that is not absorbed, or the absorption of morin in the



gastrointestinal mucosa is early enough to inhibit nimodipine-CYP3A4 metabolizing enzyme and P-gp efflux pump by a pre-treatment period of 30 min before nimodipine administration (Choi and Burm 2006). The same results were obtained by Choi and Li (2005) wherein the bioavailability of diltiazem in rabbits pre-treated with quercetin (Fig. 9) is significantly increased, but not in the rabbits co-administered with quercetin. The co-administration of naringenin (Fig. 7) with talinolol in male Sprague-Dawley rats also did not result in a significant increase of the oral drug bioavailability (de Castro et al. 2008).

As biochanin A (Fig. 10) is an inhibitor of CYP3A and P-gp and tamoxifen a well-known substrate of CYP3A4/P-gp, after their co-administration an increase of the oral bioavailability of tamoxifen was expected; however this was not observed, and a significant decrease in the relative bioavailability of tamoxifen after the co-administration of the flavonoid was reported by Singh et al. (2012). This isoflavone also caused a significant decrease in oral bioavailability of fexodenadine which is not metabolized by CYP450 isoenzymes at a large extent (Peng et al. 2006). Similarly, Hsiu et al. (2002) reported the decrease in the oral bioavailability of cyclosporin when co-administered with quercetin (Fig. 9) in male Sprague-Dawley rats and in male Yorkshire pigs. Finally, Park et al. (2012) studied the effect of the co-administration of silymarin [a flavonoid composed mainly by silibinin (90 %) and small amounts of silibinin stereoisomers as isosilybin A, silydianin, and silychristin (Fig. 12)] on oral bioavailability of paxlitaxel, and the results obtained demonstrated an increased oral bioavailability of the drug (a P-gp substrate). Actually, all the previously reported results suggest that the concurrent use of flavonoids or flavonoids-containing dietary supplement or herbs with medications whose absorption and metabolism are mediated by P-gp and/or CYP3A4 should be given special (Hsiu et al. 2002).

### Clinical studies

Up to date there are few clinical studies published in literature documenting the effect of a particular flavonoid on the pharmacokinetics of a given drug. Two of those studies were developed by Rao et al. (2007) and Kim et al. (2009) testing the flavonoids silymarin (Fig. 7) and quercetin (Fig. 5) respectively.

Rao et al. (2007) investigated the effect of silymarin pre-treatment on the pharmacokinetics of ranitidine in twelve healthy male human volunteers (age range 19–26 years, weight 50–68 kg). The study design involved the administration of 150 mg ranitidine to the volunteers, either alone, or after a 7 days pre-treatment period with thrice-daily dose of 140 mg silymarin, after an overnight fast. The washout period between each ranitidine treatment was 7 days and the serum levels of the drug were determined by high-performance liquid chromatography with ultraviolet detection. The results showed that the pharmacokinetics of ranitidine was not influenced by silymarin. The plasma peak concentration ( $C_{max}$ ), the plasma AUC from zero to 12 h ( $AUC_{0-12}$ ), the plasma AUC from zero to infinity ( $AUC_{0-\infty}$ ), elimination rate constant ( $K_{el}$ ), terminal elimination half-life ( $T_{1/2el}$ ) and time to reach  $C_{max}$  ( $T_{max}$ ) were not significantly altered after pre-treatment with silymarin. However, the area under the first moment curve (AUMC) and the mean residence time (MRT) showed a significant increase after the silymarin pre-treatment. Indeed, despite ranitidine is a substrate of CYP3A4 and P-gp, and silymarin a flavonoid compound that has potential to influence drug biodisposition by decreasing the metabolic activity of CYP3A4 and by inhibiting P-gp function, important changes were not evidenced in the majority of the pharmacokinetic parameters of ranitidine. Rao et al. (2007) suggested that silymarin was so poorly absorbed after oral administration that its blood concentrations were too low to cause an appreciable effect on CYP3A4 or P-gp.

In turn, Kim et al. (2009) developed a study aimed at evaluating whether quercetin exhibited any inhibitory effect on P-gp-mediated drug disposition in humans using fexofenadine as a P-gp probe substrate. In this study, twelve healthy male subjects (age range 24–31 years, weight 67–82 kg) were treated for 7 days with 500 mg quercetin or placebo three times daily. Subsequently, a single dose of 60 mg fexofenadine was administered orally on day 7. Afterwards, the fexofenadine drug concentrations in plasma and urine samples were quantified using high-performance liquid chromatography coupled to fluorescence detection. After a pharmacokinetic analysis of the concentration–time profiles, a significant increase in the mean plasma concentrations of fexofenadine after quercetin treatment was registered; more specifically, the AUC of plasma fexofenadine was increased by 55 % and the

$C_{\max}$  was elevated by 68 % during the quercetin treatment. Nevertheless, no differences were observed in the renal clearance or terminal elimination half-life ( $t_{1/2}$ ) of fexofenadine between placebo and quercetin phases, but the oral clearance of the drug was significantly decreased (37 %) under quercetin treatment. The results of this clinical study showed that the short-term use of quercetin elevated the plasma concentrations of fexofenadine and it may occur due to the inhibition of P-gp-mediated drug efflux (Kim et al. 2009). Hence, more clinical trials are needed to reliably assess the effects of flavonoids in modulating P-gp function in humans.

## Conclusion

Over the last years the strategies for drug discovery and development have changed considerably and new features are taken into account by pharmaceutical industry in making decision process (Mizuno et al. 2003). The therapeutic failure due to the development of MDR is a growing concern in the clinical practice. As a result, due to the recognition that P-gp-mediated drug efflux is one of the major factors contributing for MDR in several diseases (Wang et al. 2002; Löscher and Potschka 2005; Hennessy and Spiers 2007; Bansal et al. 2009), the investigation of the role of this drug transporter on the biodisposition of new drug candidates has been emphasized as an integral component of the processes of drug discovery, drug development and clinical therapy (Sun et al. 2004). Moreover, the advances in molecular biology have allowed to increase the knowledge on the structure, localization and functional role of P-gp, as well the subjacent drug efflux mechanisms (Varma 2003).

Because of the broad substrate specificity and the ubiquitous distribution of P-gp through tissues frequently involved in absorption, distribution and elimination, the modulation of the activity of this transporter may be responsible by substantial changes in the drug disposition of P-gp substrates (Matheny et al. 2001; Chang et al. 2006). The realization of the importance of drug efflux transporters in the course of disease processes and their treatments, led to the rapid identification of P-gp substrates and/or inhibitors (Chang et al. 2006), and promoted the search of selective P-gp inhibitors as add-on therapy in order to overcome the MDR phenotype (Dantzig et al. 2003).

However, the selective P-gp inhibition is a complex and difficult task and much work still remaining to optimize this strategy. Nevertheless, the growing understanding of P-gp efflux mechanisms, the development of improved screening methods, and the increasing number of structure–activity relationship studies will provide new opportunities not only to enhance the bioavailability of life saving drugs, but also to improve their pharmacokinetics, tissue distribution and cell uptake (Varma 2003). Even though, there are particular criteria that should be satisfied in order to find clinically promising P-gp inhibitors (e.g. specificity for P-gp, lack of interference with CYP450 enzymes) (Dantzig et al. 2003).

The herbal products are increasingly used worldwide for medicinal purposes and there has been a growing interest in assessing the modulating effects of dietary constituents on P-gp function (Sun et al. 2004). Among these, flavonoids appear to have optimal properties as potential inhibitors of the P-gp multidrug transporter (Ross and Kasum 2002; Brand et al. 2006; Schinkel and Jonker 2012). This evidence is supported by many in vitro and in vivo assays, as well as by a study in humans. Accordingly, it is essential to perform more and better studies, in order to recognize and select which flavonoids are the best candidates to enhance the cellular availability of diverse drugs and, thus, their pharmacological potential. This therapeutic approach can be extremely important for the treatment of important diseases, such as epilepsy and cancer, for which the existing treatments are often ineffective, particularly due to the MDR phenomena (Dantzig et al. 2003; Varma 2003; Brandt et al. 2006; Lazarowski et al. 2007; Zhang et al. 2012).

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**Conflict of interest** The authors have declared no conflict of interest.

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