Med Chem Res (2014) 23:1–15
DOI 10.1007/s00044-013-0612-6

MEDICINAL CHEMISTRY RESEARCH

REVIEW ARTICLE

Impact of ABC transporters, glutathione conjugates in MDR and their modulation by flavonoids: an overview

Vinay Kumar Gupta · Yashika Bhalla · Vikas Jaitak

Received: 18 February 2013 / Accepted: 2 May 2013 / Published online: 16 May 2013

Abstract Overexpression of ATP-binding cassette (ABC) transporter and glutathione conjugates results in efflux of cytotoxic agent from tumor cells leading to multidrug resistance (MDR). Many MDR inhibitors have been identified but none of them have been proven clinically valuable without side effects. Efforts are continue to develop an ideal MDR inhibitor. Recently, herbal modulation of ABC transporter and glutathione conjugates by flavonoids is emerging as popular therapy in MDR. In this review, we have covered structure, function of different ABC transporters and glutathione-mediated MRP overexpression. This review also focuses on the problems with existing MDR inhibitors, modulation of ABC transporter and glutathione-*S*-transferase by flavonoids.

Keywords ABC transporters · Anticancer · Glutathione-conjugated transporter · Flavonoids · MDR inhibitors

Introduction

Cancer chemotherapy is critically affected by different obnoxious factors like efflux transporter specificity, poor solubility, narrow therapeutic index, etc. Apart from this severe toxicity of anticancer drug is also an unruly in cancer chemotherapy. The emergence of drug resistance in many cases makes the currently available chemotherapeutic agents ineffective (Fojo and Bates, 2003). MDR is the resistance of a tumor cell population against drugs differing in chemical structure and cellular target. MDR may very well be "intrinsic", whenever illness is actually refractory to be able to chemotherapy with analysis or even "acquired", once the pill gets to be insensitive right after the remedy (Krishna and Mayer, 2000). MDR is in part mediated by the overexpression of plasma membrane transporters, such as P-glycoprotein (P-gp, MDR1 or ABCB1), MDR-associated proteins (MRP1 or ABCC1, and MRP2 or ABCC2), or breast cancer resistant protein (BCRP or ABCG2) (Ishikawa, 1992). Among various obstacles, discovery of the membrane transporter P-gp was a breakthrough in understanding the MDR phenotype of cancer cells (Juliano and Ling, 1976). Efflux transporter especially P-gp transporter is involved in multidrug resistance, has received huge reflection in both cancer research and pharmaceutical field which belongs to the ABC transporter protein (Higgins, 1992). Other important enzymes responsible for clinical multidrug resistance are glutathione-*S*-transferase (GSTs), especially of the π class (Stavrovskaya, 2000). Proteins involved in development of MDR and their respective substrates have been summarized in Table 1. Flavonoids are a large group of polyphenolic antioxidants found in fruits and vegetables. In foods, flavonoids appear as β-glycosides as well as aglycones and methoxylated flavonoids. Upon ingestion, flavonoids get metabolized into glucuronide, sulphated and methoxylated conjugates (Ader *et al.*, 2000; Day *et al.*, 2000, 2001; Mullen *et al.*, 2002). Flavonoids and flavonoid-rich extracts have been implicated as beneficial agents in a multitude of disease states most commonly in cancer, cardiovascular disease, and neurodegenerative disorders (Havsteen, 2002; Rice-Evans, 2001; Spencer *et al.*, 2004). Interestingly, it has become clear over the last few years that the bioactive forms of flavonoids in vivo are not

V. K. Gupta · Y. Bhalla · V. Jaitak (✉)
Centre for Chemical and Pharmaceutical Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda 151001, India
e-mail: vikasjaitak@gmail.com

necessarily the natural phytochemical forms, for example, the aglycones or their various glycosides, but possibly also conjugates and metabolites arising from these upon absorption (Spencer *et al.*, 2004). Many evidences indicated that flavonoids interact with ABC transporter and modulate MDR in tumors. The dual effects, i.e. modulation of ABC transporters, glutathione conjugates and antitumor activity of these herbal derivatives, may synergistically act in cancer chemotherapy. Thus, the unique property of reversal of multidrug resistance of these compounds might help protect against MDR tumors.

Structure and function of protein involved in MDR

P-glycoprotein (P-gp)

P-gp is the efflux pump discovered in 1976 due to its expression in various types of MDR tumors (Juliano and Ling, 1976). It is encoded by MDR1 gene in humans and the mdr1-a, mdr1-b genes in rodents. P-gp is found to be present in the biliary canalicular surface of hepatocytes, luminal surface of cells of jejunum and colon, apical surface of proximal tubular cells of kidney, endothelial cells of blood brain barrier, apical membrane of fetal-membrane barrier function in placenta and in other tissues like lungs, adrenals, prostrate, skin, spleen, heart and skeletal muscle (Ambudkar *et al*., 1999).

P-gp is overexpressed in many intrinsically resistant tumors (leukemias, lymphomas, adult, childhood sarcomas and neuroblastomas) and those that acquire resistance during chemotherapy treatment (Krishna and Mayer, 2000). Advances in biotechnological field proved the presence of P-gp not only in tumor cells but also in a wide variety of normal tissues where they have a role to play in absorption, distribution, metabolism and excretion. P-gp mediated efflux affects each step which a drug comes across during its stay in the body. It influences absorption through intestinal carriers, which expel drug molecules back into the lumen; distribution, by preventing drug entry into tissues like brain; metabolism, as it acts synergistically with cytochrome P450 3A4 (CYP 3A); excretion, by affecting both biliary and renal tubular function (Lin, 2003). In this way, P-gp acts as a barrier which prevents entry of xenobiotics in the body and expelled out them once they have entered and protect the cell or tissue from cytotoxic substances, keep toxic substances in blood circulation (Ambudkar *et al.*, 1999). P-gp is an adenosine triphosphatase (ATPase), energy-dependent membrane bound protein belonging to members of ABC transporters (Loo and Clarke, 2005). It consists of 1,280 amino acids and is expressed as a single chain containing two homologous portions of equal length, each containing six transmembrane domains and two ATP-binding regions separated by a flexible linker polypeptide region. P-gp has amino and carboxyl terminals (Fig. 1) (Kimura *et al.*, 2004).

Initially, it was believed that N-terminal ATP-binding domain contains all residues necessary to hydrolyze ATP without interacting with the C-terminal ATP-binding domain (Kimura *et al.*, 2004). But now, it is believed that both the amino and carboxyl terminal ATP sites can hydrolyze ATP. However, there is no evidence that ATP can be hydrolyzed simultaneously by both sites (Tandon *et al.*, 2006). ATP-binding domain(s) located in the cytosol side are also known as nucleotide-binding folds (NBFs); they transfer the energy to transport the substrates across the membranes. Each ATP-binding domain contains three regions: Walker A, B, and signature C motifs. Highly conserved Lys residue within the walker A motif of histidine permease is directly involved with the binding of ATP and a highly conserved Asp residue within the walker B motif serves to bind the Mg^{2+} ion. Human P-gp requires both (Mg^{2+})-ATP-binding and hydrolysis to function as a drug transporter (Tombline *et al.*, 2004). It has also been proposed that Mg^{2+} may play a role in stabilizing the ATP-binding site. Signature C motifs probably participate to accelerate ATP hydrolysis via chemical transition state interaction and are also suggested to be involved in the

Table 1 Proteins involved in MDR and the anti-cancer drugs affected by their upregulation

Sr. No.	Name of proteins	Systematic name	Name of anticancer drugs	References
1.	P-glycoprotein	ABCB1	Doxorubicin, daunorubicin, epirubicin, etoposide, paclitaxel, docetaxel, vincristine, vinblastine, Vinorelbine, vindesine, bisantrene, mitoxantrone, colchicine	Sawicka *et al.* (2004)
2.	MRP1	ABCC1	Vincristine, daunorubicin, doxorubicin, etoposide	Teodori *et al.* (2002)
3.	MRP2	ABCC2	Methotrexate, etoposide, cisplatin, vinca alkaloids	Sparreboom *et al.* (2003)
4.	BCRP	ABCG2	Mitoxantrone, bisantrene, camptothecin, daunorubicin, doxorubicin, epirubicin, topotecan, CPT-11 (irinotecan)	Leonessa and Clarke (2003)
5.	GSTs	GSTP1(π class)	Chloroethylnitrosoureas, cisplatin, thiotepa, anthracyclines, phosphanides, acrolein, melphalan, cyclophosphamide	O'brien and Tew (1996); Townsend and Tew (2003)

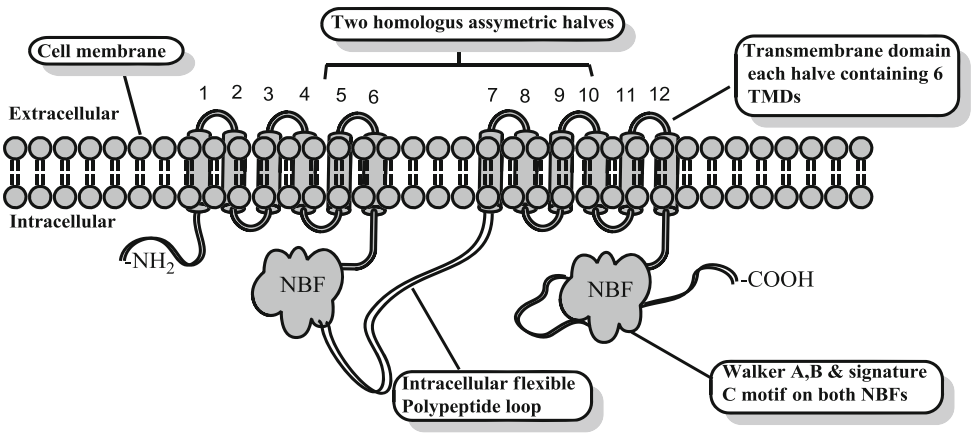

Fig. 1 Structure of P-gp transporter

transduction of the energy of ATP hydrolysis to the conformational changes in the membrane integral domains required for translocation of the substrate (Tandon *et al.*, 2006). Unlike the ATP-binding sites that are restricted to Walker A motifs of ATP-binding domains, many substrate binding sites have been identified throughout the transmembrane domain (TMDs) of P-gp. The major drug-binding sites reside in or near TM6 and TM12. In addition to this, TM1, TM4, TM10, and TM11 have drug-binding sites. Amino acids in TM1 are involved in the formation of a binding pocket that plays a role in determining the suitable substrate size for P-gp, whereas glycine residues in TM 2 and 3 are important in determining substrate specificity. The close proximity of TM2/TM11 and TM5/TM8 indicates that these regions between the two halves must enclose the drug-binding pocket at the cytoplasmic side of P-gp. They may form the "hinges" required for conformational changes during the transport cycle (Ambudkar *et al.*, 2006; Tandon *et al.*, 2006). In addition to the TMDs, intracellular loops and even ATP-binding domains have drug-binding sites.

The first step in drug efflux is drug recognition by P-gp followed by ATP-binding and hydrolysis. The major drug-binding sites reside in or near TM6, TM12, TM1, TM4, TM10, and TM11. There is the formation of a binding pocket which plays a role in determining the suitable substrate drug size for P-gp and, therefore, substrate specificity. The energy released in this process is utilized to efflux substrate outside the cell membrane through central pore. Two ATP molecules are hydrolyzed for the transport of every substrate molecule, one in the transport of substrate and the other in causing conformational changes to reset the pump for the next catalytic cycle (Fig. 2) (Ambudkar *et al.*, 2006). There are many hypothesis regarding efflux mechanism of P-gp transporter; however, exact site of substrate and protein interaction is not clear (Ambudkar *et al.*, 2006). The most popular model includes pore model, flip-pas model and hydrophobic vacuum cleaner (Tandon *et al.*, 2006). Among these models, hydrophobic vacuum cleaner has drawn huge attention according to which P-gp recognizes the substrate embedded in the inner layer of plasma membrane and transports it through a protein channel. P-gp undergoes conformational changes on binding of nucleotide to the intracellular nucleotide-binding domains (Rosenberg *et al.*, 2003). According to "pore model", there is a major reorganization of the transmembrane domains throughout the entire depth of the membrane on binding of nucleotide. This restructuring opens the central "pore" along its length in a manner that allows access of hydrophobic drugs (or, substrates) directly from the lipid bi-layer to the central pore of the transporter (Sauna *et al.*, 2001). Further, the second catalytic cycle starts by hydrolysis of another molecule of ATP and released energy is utilized to reset the protein to its original confirmation, where it again binds with substrate and nucleotide to initiate the next cycle (Varma *et al.*, 2003).

In normal cell, due to selective distribution of P-gp at the drug entry and exit ports, it is speculated that P-gp could play a major physiological role in absorption, distribution and excretion of xenobiotics. Therefore, overall P-gp functions as a biochemical barrier for entry of xenobiotics and expels them from the organs into the systemic circulation (Fardel *et al.*, 1996).

Numerous studies and research have been performed regarding expression of P-gp in solid tumors and hematological malignancies. It has been seen that P-gp expression is usually high and constitutive in tumors. As it participates in physiological phenomenon in normal cell, protect cells from harmful substances and expelled out toxic substances which have entered earlier. But in cancerous cell, this P-gp transporter expelled out cytotoxic drugs from cytoplasm resulting in development of resistance. Actually expression of P-gp in tumors is moderate but its expression increases only when chemotherapy is started especially in case of breast tumors, acute myeloid leukemias, lymphomas and myelomas (Drach *et al.*, 1995). In this way, this P-gp acts as a barrier in cancer chemotherapy. P-gp positivity in some cancers is associated with increased levels of other

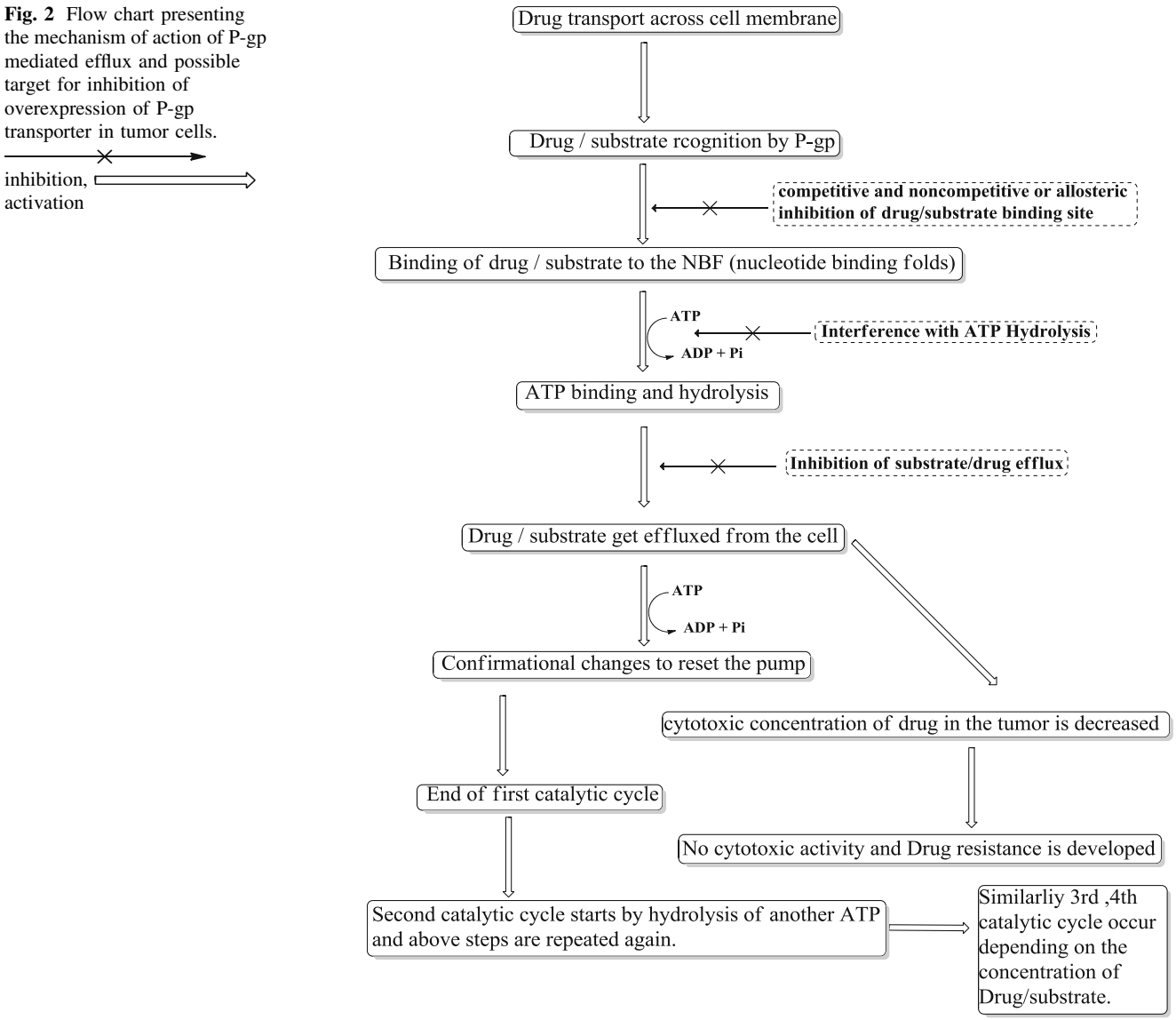

Fig. 2 Flow chart presenting the mechanism of action of P-gp mediated efflux and possible target for inhibition of overexpression of P-gp transporter in tumor cells. inhibition, activation

drug resistance markers such as multidrug resistance-associated protein (MRP) and glutathione-*S*-transferase (π class) expression (Abolhoda *et al.*, 1999). A strong correlation of increased levels of MDR1 expression with relapse has been evidenced in pediatric soft tissue sarcomas, neuroblastoma and acute lymphoblastic leukemia (Thomas and Coley, 2003).

Multidrug resistance protein (MRP)

For a long time, P-gp was believed to be the only protein capable of conferring MDR in mammalian tumor cells. However, several reports on human tumor cell lines displaying MDR in the absence of P-gp overexpression, together with studies that failed to detect P-gp in a variety of human tumors pointed to the existence of other MDR conferring proteins (Ozben, 2006). Apart from P-gp, other ABC transporters now known to be involved in multiple drug resistance in humans include the family of multidrug resistance-associated proteins (MRP1-7 or ABCC1-7) (Litman *et al.*, 2001). Two additional members, MRP8 (ABCC11) and MRP9 (ABCC12) have been reported more recently (Bera *et al.*, 2001, 2002). The multidrug resistance proteins (MRP1 and MRP2) are 190 kDa membrane glycoproteins that are unidirectional, ATP-driven, export pumps, with an amino acid identity of 49 % in humans (Keppler *et al.*, 1998).

This ABC transporter is similar in structure to P-gp in that they possess two ATP-binding sites. In addition to the 12 transmembrane domains, they also contain an additional 5 transmembrane domains at the amino terminal end (Fig. 3) (Altenberg, 2004). Other transporters with a similar structure include MRP2, MRP3 and MRP6. Both MRP1 and MRP2 are important in xenobiotic detoxification as well as in the defense against oxidative stress,

because of their role in glutathione disulfide export (Rosenberg *et al*., 2001). Unlike P-gp that targets and transports hydrophobic drugs, MRP proteins can transport hydrophilic molecules and even organic anions (Jedlitschky *et al*., 1996). Although both P-gp and MRP belong to the ATP-binding cassette (ABC) superfamily, their primary structures are quite dissimilar, sharing only approximately 15 % amino acid homology. These two classes of proteins were originally considered to play a similar role in the development of multidrug resistance, leading to the exclusion from the cell of a similar range of chemotherapeutic agents. However, it seems that MRP, unlike P-gp, can specifically transport a range of glutathione conjugates, suggesting a dichotomy in function of the two types of transporter. Thus, the MRP1 gene product contributes to cellular glutathione *S*-conjugate efflux and protects against oxidative stress inducing quinines. MRP1 is the primary folate efflux route, and it plays a functional role in the maintenance of cellular folate homeostasis (Assaraf *et al*., 2003). MRP2, also called canalicular MRP (cMRP) or canalicular multispecific organic anion transporter (cMOAT), accepts a diverse range of substrates, including glutathione, glucuronide, and sulfate conjugates of many xenobiotics (Gerk and Vore, 2002). In addition, MRP2 transports a range of anticancer drugs, leukotrienes, glutathione, toxins, and heavy metals. MRP2 is regulated at several levels, including membrane retrieval and reinsertion, translation, and transcription. The expression and activity of MRP2 are altered by certain drugs and disease states (Adachi *et al*., 2002).

Breast cancer resistant protein (BCRP)

Breast cancer resistant protein is also a type of ABC transporter super family and identified from drug selected human breast cancer cells (MCF-7) (Doyle *et al*., 1998), human colon carcinoma cells (S1-M1-80) (Miyake *et al*., 1999) and human placenta (Allikmets *et al*., 1998). It has been reported that the BCRP is also responsible for drug resistance against various anticancer agents and plays an important function in drug disposition (Ejendal and Hrycyna, 2002). Molecular characterization revealed that BCRP consists of 655 amino acids with a molecular mass of 72.1 kDa (Ejendal and Hrycyna, 2002). In contrast to P-gp, which has 12 transmembrane domains and two ATP-binding sites, BCRP is a half-ABC transporter and contains only six transmembrane domains and one ATP-binding site (Fig. 4) (Allikmets *et al*., 1998; Mao, 2005). The contribution of BCRP to clinical MDR is receiving extensive investigation, and some association has been reported (Steinbach *et al*., 2002). Significant and variable expressions of BCRP have been detected in human tumors, such as acute myeloid leukemia and breast cancer (Maliepaard *et al*., 2001; Ross *et al*., 2000). Overexpression of BCRP has been shown to cause cross-resistance to doxorubicin, topotecan, SN38, mitoxantrone, methotrexate and flavopiridol, as well as to nucleoside human immunodeficiency virus reverse transcriptase inhibitors such as zidovudine and lamivudine (Maliepaard *et al*., 2001).

Glutathione conjugates transporter (GST)

One complex system of proteins involved in MDR is the glutathione-related biotransformation system. The GSTs comprise a family of dimeric phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to a wide variety of endogenous and exogenous electrophilic compounds. GSTs are divided into two distinct super-families: the membrane-bound microsomal and cytosolic family. Cytosolic GSTs are highly polymorphic and can be divided into six classes which share ~30 % sequence identity, and are designated by Greek letters α, μ, ω, π, θ and ζ. Several GSTs can conjugate GSH to anti-cancer DNA alkylating agents like busulfan, melphalan, chlorambucil, thiotepa and other anticancer drugs, thereby detoxifying these drugs (Shen *et al*., 1997; Tew and Gaté, 2001; van Bladeren and van Ommen, 1991). The overexpression in tumors of GSTs, especially of GSTP1-1, is considered as a possible mechanism of tumor cell drug resistance (Salinas and Wong, 1999; Suzuki *et al*., 2005; Zhou *et al*., 2005).

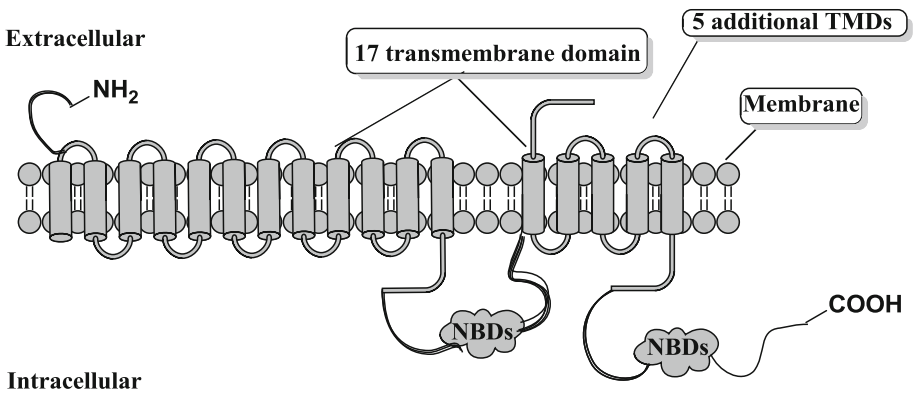

Fig. 3 Structure of multidrug resistance protein 1 (MRP1)

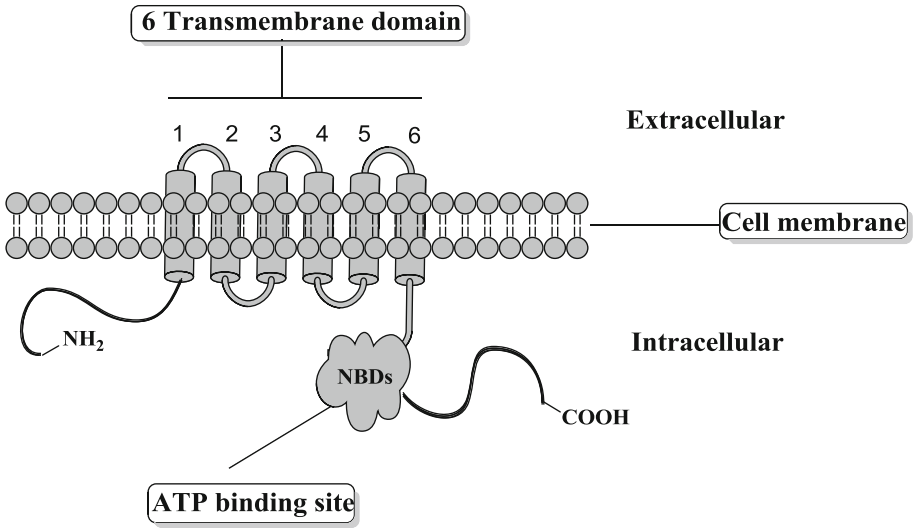

Fig. 4 Structure of BCRP

Glutathione-*S*-transferase P1-1 is a homodimeric enzyme. Each subunit contains one binding site for GSH (G-site) and another for the hydrophobic substrate (H-site), and cysteine residues located at positions 14, 47, 101 and 169 (Morrow *et al.*, 1998). It is conceivable that GSTs serve two distinct roles in the development of drug resistance: (1) via direct detoxification as well as (2) acting as an inhibitor of the MAP kinase pathway (pi class only). The link between GSTs and the MAP kinase pathway provides a rationale as to why in many cases the drugs used to select for resistance are neither subject to conjugation with GSH, nor substrates for GSTs (Hayes and Pulford, 1995). The contribution of any GSTs to drug resistance is likely to vary with cell type and drug, as well as with the expression profile of other enzymes and transporters. However, it is still widely accepted that the GSTs can contribute directly to drug resistance in some cell types via their catalytic activity, so inhibitors of GSTs catalytic activity are considered as a potential therapeutic tool. Historically, GSTs were named according to their ability to catalyze the nucleophilic addition or substitution of glutathione (GSH; γ-glutamyl-cysteinyl-glycine) at electrophilic centers in a wide range of xenobiotic electrophilic substrates (Fig. 5) (Lehane *et al.*, 2011).

The typical GST-catalyzed reactions include Michael-type addition, nucleophilic aromatic substitution, nucleophilic addition to epoxides, cis–trans double bond isomerization, positional double bond isomerization, and peroxide reduction (Hayes and Pulford, 1995).

Development of MDR reversal agents or chemosensitizers

The first report of the pharmacological reversal of MDR came in 1980 (Tsuruo *et al.*, 1981). The compounds that would reverse resistance against anticancer drugs are called MDR inhibitors, MDR modulators, MDR reversal agents or chemosensitizers. They may modulate more than one transporter (Choi, 2005; Liscovitch and Lavie, 2002). Although a few distinct ways of minimizing multidrug resistance are achievable, the most crucial cellular mechanism involving resistance may be the greater detoxification and/or efflux of anticancer medications by means of biotransformation enzymes and proteins (Fojo and Bates, 2003). One strategy to overcome transporter-mediated drug resistance relies on the identification of inhibitors of these enzymes and transporters. These compounds should be relatively non-cytotoxic allowing high levels to be administered and maintained without encountering toxicity. A lot of the acknowledged inhibitors tend to be relatively non-specific and also may, therefore, have to put up with undesirable drug-drug interaction or interference together with various other physiological systems decreasing their utilization clinically, as observed for several analyzed P-gp inhibitors (Borst and Elferink, 2002; Tan *et al.*, 2000). The hunt for relatively non-cytotoxic GST/MRP inhibitors is really a strategy which could give encouraging results (Tan *et al.*, 2000).

First-generation MDR inhibitors

Inhibiting P-gp and other ABC transporters has been extensively studied for more than two decades (Liscovitch and Lavie, 2002; Thomas and Coley, 2003). Many agents of diverse structure and function that modulate MDR have been identified, including calcium channel blockers (e.g. verapamil), calmodulin antagonists, steroidal agents, protein kinase C inhibitors, immunosuppressive drugs (e.g. cyclosporine A), antibiotics (e.g. erythromycin), antimalarials (e.g. quinine), psychotropic phenothiazines, indole

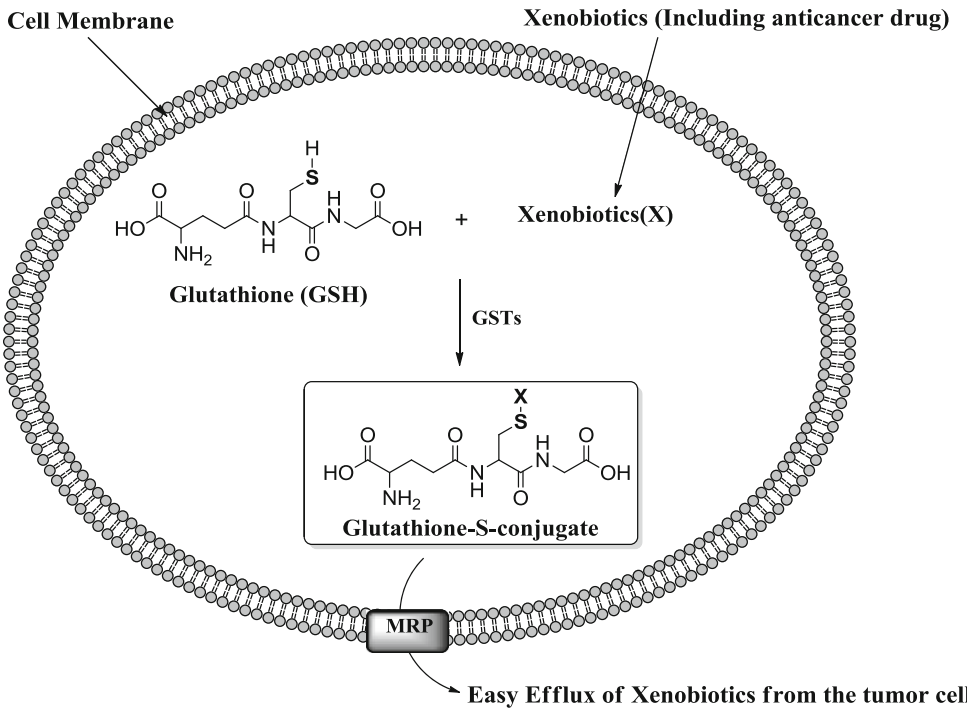

Fig. 5 Diagram representing glutathione conjugation to xenobiotics via GSTs and efflux through MRP

alkaloids (e.g. fluphenazine and reserpine), steroid hormones, anti-steroids (e.g., progesterone and tamoxifen), detergents and surfactants (Ferry *et al.*, 1996; Thomas and Coley, 2003; Wu *et al.*, 2003). First-generation MDR drugs had other pharmacological activities and were not specifically developed for inhibiting MDR. Their affinity was low for ABC transporters and necessitated the use of high doses, resulting in unacceptable high toxicity which limited their application (Ferry *et al.*, 1996; Krishna and Mayer, 2000; Thomas and Coley, 2003). Clinical trials with first-generation MDR drugs failed for various reasons, often due to side effects (Ferry *et al.*, 1996; Krishna and Mayer, 2000; Liscovitch and Lavie, 2002; Thomas and Coley, 2003). Many of the first-generation chemosensitizers were themselves substrates for ABC transporters and competed with the cytotoxic drugs for efflux by the MDR pumps. Therefore, high serum concentrations of the chemosensitizers were needed to produce sufficient intracellular concentrations (Ambudkar *et al.*, 1999). These limitations prompted the development of new chemosensitizers that are more potent, less toxic and selective for the P-gp and other ABC transporters (Krishna and Mayer, 2000; Thomas and Coley, 2003).

Second-generation MDR inhibitors

Second-generation chemosensitizers were designed to reduce the side effects of the first-generation drugs. Second-generation MDR modulators have a better pharmacologic profile than the first-generation compounds; still they retain some characteristics that limit their clinical usefulness. Co-administration of a MDR modulator usually elevates plasma concentrations of an anticancer drug by interfering with its clearance or inhibiting its metabolism and excretion, thus leading to unacceptable toxicity that necessitates chemotherapy dose reductions in clinical trials down to pharmacologically ineffective levels (Liscovitch and Lavie, 2002). The affinity of second-generation MDR drugs towards ABC transporters was too low to produce significant inhibition of MDR in vivo at tolerable doses (Ferry *et al.*, 1996). The majority of the anticancer medications are substrates both for ABC transporter proteins and for the cytochrome P450 isoenzyme 3A4. Most of the second-generation MDR chemosensitizers are also substrates for cytochrome P450 3A4 and metabolized by means of this enzyme (Thomas and Coley, 2003). Competition between anticancer agents and MDR modulators intended for cytochrome P450 3A4 activity may result throughout unpredictable pharmacokinetic connections. Co-administration of a MDR drug may perhaps significantly elevate plasma concentrations of anticancer drug simply by interfering with their clearance (e.g. via biliary elimination) or by metabolism (e.g. via the cytochrome P450 system), resulting in dose reductions down to pharmacologically ineffective levels (Liscovitch and Lavie, 2002). The pharmacokinetic interactions concerning chemosensitizers along with cytotoxic agent tend to be capricious, decreasing the dose of any cytotoxic agent may perhaps cause under- or over-dosing in patients (Fischer *et al.*, 1998; Gottesman *et al.*, 2002; Thomas and Coley, 2003).

Third-generation MDR inhibitors

Third-generation molecules have been developed to overcome the limitations of the second-generation MDR modulators (Krishna and Mayer, 2000; Thomas and Coley, 2003). They are not metabolized by cytochrome P450 3A4 and they do not alter the plasma pharmacokinetics of anticancer drugs (Table 2). Third-generation agents specifically and potently inhibit P-gp and do not inhibit other ABC transporters (Thomas and Coley, 2003). None of the third-generation agents tested so far have caused clinically relevant alterations in the pharmacokinetics of the co-administered anticancer drugs. Because of their specificity for P-gp transporters and lack of interaction with cytochrome P450 3A4, third-generation P-gp inhibitors offer significant improvements in chemotherapy without a need for chemotherapy dose reductions (Thomas and Coley, 2003).

A non-immunosuppressive cyclosporin D derivative (PSC-833; Valspodar; Novartis AG) was the first of these drugs to be studied (Liscovitch and Lavie, 2002). Unfortunately, further research with PSC-833 revealed pharmacokinetic interactions with several anticancer drugs and inhibition of non-MDR-related transporters (Liscovitch and Lavie, 2002). One of the most promising third-generation P-gp inhibitors is an anthranilamide derivative tariquidar (XR9576) which is developed by NCI/Xenova/QLT Company (Liscovitch and Lavie, 2002). In phase I and II studies with paclitaxel and vinorelbine in ovarian cancer, tariquidar gave successful results and phase III trials have been initiated with tariquidar in patients with non-small cell Lung cancer (Liscovitch and Lavie, 2002). Vertex Pharmaceuticals Inc. developed a pipecolinate analog, VX-710 (biricodar, Incel) which is a high-affinity P-gp and MRP inhibitor. VX-710 has no pharmacokinetic interactions with doxorubicin and is undergoing trials in solid tumors (Liscovitch and Lavie, 2002). Laniquidar (R101933; NCI/EORTC Inc.) and the substituted diarylimidazole ONT-093 (Ontogen Inc.) are among the third-generation P-gp inhibitors. They have high potency and specificity for the P-gp transporter despite having diverse chemical structures and origins (Newman *et al.*, 2000; Thomas and Coley, 2003; van Zuylen *et al.*, 2000). R101933 and ONT-093 were shown to inhibit P-gp pump with no effect on the pharmacokinetics of docetaxel and paclitaxel. The cyclopropyldibenzosuberane modulator LY335979 developed by Eli Lilly Inc. was shown to competitively inhibit the binding of vinblastine to P-gp (Liscovitch and Lavie, 2002; Thomas and Coley, 2003). LY335979 showed no significant pharmacokinetic interactions with doxorubicin, etoposide, daunorubicin, vincristine, or paclitaxel in both solid and hematologic malignancies (Dantzig *et al.*, 1999; Starling *et al.*, 1997; Thomas and Coley, 2003). To increase life span of cancer patients, clinical trials with above drugs are still going on. Several other MDR modulators have been demonstrated to renovate chemotherapeutic sensitivity in cancer cells and tissues in vitro and in vivo, but none of them has found to be used clinically so for (Crowley *et al.*, 2010; Nobili *et al.*, 2011). Since most of the third-generation compound have failed in phase III clinical trials. Recently, compounds belonging to class of pharmaceutical excipients like PEG esters, polysorbates, tocopherol esters, polymers, amphiphilic diblock co-polymers came into existence as MDR modulators (Rege *et al.*, 2002; Regev *et al.*, 2007). Co-formulations of pharmaceutical excipients with MDR inhibitors significantly improved bioavailability and duration of action (Regev *et al.*, 2007). Although these "inert" pharmaceutical excipients appear to be a better choice from formulation perspective, they suffer from limitations of causing undesirable alterations in drug disposition resulting in serious adverse reactions (Buggins *et al.*, 2007).

Another class which is gaining rapid attention as MDR inhibitors consists of herbal components (Deferme *et al.*, 2002; Zhou *et al.*, 2004). The safety of herbs is ensured by the continuous and long history of usage in large amounts as a part of normal daily diet (Yáñez *et al.*, 2013). They are considered as perfect candidates for bioavailability enhancement, tissue-penetration (e.g. blood brain barrier), decreasing biliary excretion and multidrug resistance modulating agents. The effects of flavonoids on cellular accumulation, herbs interaction, transport or bioavailability of various anti-cancer drugs have been discussed (Bansal *et al.*, 2009).

Table 2 List of drugs which are in clinical trials for MDR

Category	Name of the drug	Developed by	References
Cyclosporine D derivative	Valspodar (PSC-833)	Novartis AG	Liscovitch and Lavie (2002)
Anthranilamide derivative	Tariquidar (XR-9576)	NCI/Xenova/QLT	Liscovitch and Lavie (2002)
Pipecolinate analog	Biricodar, Incel (VX-710)	Vertex Pharmaceutical Inc.	Liscovitch and Lavie (2002)
Diarylimidazole	ONT-093	Ontagen Inc.	Liscovitch and Lavie (2002)
Cyclopropyldibenzosuberane	LY335979	Eli-Lilly Inc.	Liscovitch and Lavie (2002); Thomas and Coley (2003)
–	Elacridar (GF-120918)	GlaxosmithKline	Liscovitch and Lavie (2002)

Role of flavonoids in modulation of MDR

Flavonoids are a group of more than 4,000 polyphenolic compounds that occur naturally in foods of plant origin. These compounds possess a common phenylbenzopyrone structure and they are categorized according to the saturation level and opening of the central pyran ring, mainly into flavones, flavanols, isoflavones, flavonols, flavanones, and flavanonols (Hasrat *et al.*, 1997; Holiman *et al.*, 1996). Flavonoids have probably existed in the plant kingdom for over one billion years. They are present in practically all dietary plants, like fruits and vegetables (Yáñez *et al.*, 2013).

P-gp inhibition by flavonoids

Flavonoid interacts with P-gp via blockade of the drug-binding site either competitive, non-competitive or allosterically (Tandon *et al.*, 2006), interference with the ATP hydrolysis process (Sauna and Ambudkar, 2000; Sauna *et al.*, 2001), alteration in integrity of cell membrane lipids (Regev *et al.*, 1999) or decrease in P-gp expression (Fig. 2). SAR studies revealed that 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. SAR studies of chalcones, flavones, and flavonols conclude that a hydroxyl group on position 5 (position 6′ in chalcones) is important. 5-OH methylation leads to slightly less active compounds. The hydroxyl loses its acidic properties because of the chelating effect induced by the adjacent carbonyl group and, therefore, does not affect the activity which can be decreased by the presence of acidic groups. Hydroxylation on position 7 (position 4′ in chalcones) was deleterious for activity, probably due to the acidic group influence, whereas methoxylation was slightly beneficial. The 2,3 double bond (the α,β-double bond in chalcones) and the carbonyl group are also essential for MDR modulation (Boumendjel *et al.*, 2002b; Comte *et al.*, 2001; Conseil *et al.*, 1998; Hadjeri *et al.*, 2003).

In addition, isoflavonoids with ring B branched at position 3 instead of 2 have lower P-gp interaction activity (Boumendjel *et al.*, 2002a). Genistein (an isoflavone) has been proved inactive against P-gp mediated MDR (Versantvoort *et al.*, 1993). But Castro *et al.* challenged this conclusion (Castro and Altenberg, 1997). There are some controversy regarding modulation of P-gp by natural flavonoids polyphenols, such as kaempferol, galangin and quercetin. It was reported that kaempferol, galangin and quercetin stimulated P-gp efflux of 7,12-dimethylbenz(a)anthracene, a carcinogen known to induce mammary tumors in animals (Morris and Zhang, 2006; Phang *et al.*, 1993).

In contrast, it was also demonstrated that quercetin inhibited P-gp transport of adriamycin dose dependently in MCF-7/Adr cells, an adriamycin resistant human tumor cell line (Scambia *et al.*, 1994). Quercetin inhibited P-gp mediated transport of the fluorescent probe Hoechst 33342, at least in part by inhibiting P-gp ATPase activity (Shapiro and Ling, 1997). On the basis of these facts, some scientists have synthesized and evaluated the MDR modulating activity of flavonoid derivatives containing an *N*-benzylpiperazine side chain. These new derivatives were designed with the aim of introducing basic nitrogen and increasing their lipophilic properties (Ferté *et al.*, 1999). Apart from this, EGCG has been found to improve the activity of the anticancer drug doxorubicin by inhibiting the P-gp efflux pump activity in a murine model for chemoresistant HCC and reducing the expression of multidrug resistance (MDR)1 protein (Stagos *et al.*, 2012). Ethyl acetate fraction of *Gynura procumbens* has been also found to prevent chemoresistance through inhibition of MDR1 expression on MCF-7 breast cancer cell line and sensitizes the cells to doxorubicin (Nurulita *et al.*, 2012). It has been also reported that Naringin, tangeritin obtained from *Citrus* plant have been found to reverse multidrug resistance and caused induction of apoptosis in human colon cancer (Wesołowska *et al.*, 2012). Some studies revealed that the flavonolignan, (−)-hydnocarpin, potentiates vincristine cytotoxicity in 697 cells and can re-sensitize resistant 697-R cells to vincristine treatment (Bueno Pérez *et al.*, 2012; Gupta *et al.*, 2011).

Inhibition of MRP by flavonoids

The first review of MRP1 inhibitors was published by Norman (1998). Many reports describe the interaction of flavonoids with MRP1 and MRP2, showing an inhibition effect on transporter activity (Table 3). Several flavonoids have been tested for MRP inhibitory activity and some of them have been found to be very potent. The synthetic compound flavopiridol, which has been extensively studied for its antiproliferative effect through inhibition of cyclin-dependent kinases (Sedlacek *et al.*, 1996), has then been introduced as an MRP1 inhibitor (Hooijberg *et al.*, 1999).

In 1993, it has been reported that genistein (4′,5,7-trihydroxyisoflavone) can inhibit the efflux of daunorubicin on MRP1-overexpressing small cells from human lung cancer (Versantvoort *et al.*, 1994). VX-710, agosterol A, PAK-104P, verapamil, cyclosporin A, certain flavonoids, RU486, budesonide are non-specific inhibitors, LY475776 and LY402913 are the GSH (glutathione)-dependent highly specific inhibitors, while MK571, ONO-1078, glibenclamide, some GSH conjugates are the relatively specific inhibitors of MRP1 (Haimeur *et al.*, 2004; Lee, 2004). In vitro, it was demonstrated that some flavonols such as myricetin and robinetin are able to inhibit calcein efflux mediated by MRP2 in MDCKII cells transfected with this

Table 3 MRP1 and MRP2 inhibition by flavonoids

Pump	Substrate	Concentration	Model	Most potent inhibitors	References
MRP1	Daunomycin, vinblastine	100 μM	Panc-1 cells	Morin > kaempferol > quercetin > genistein	Borst and Elferink (2002); Nguyen *et al.* (2003)
MRP1	17β-estradiol	1–100 μM	Vesicles	Apigenin > kaempferol > naringenin > quercetin > myricetin	Borst *et al.* (2000)
MRP1	LTC4	1–100 μM	Vesicles	Kaempferol > apigenin > quercetin > myricetin > naringenin	Borst *et al.* (2000)
MRP2	Calcein	0.1–50 μM	MDCKII cells	Robinetin > myricetin	Van Zanden (2005)
MRP1	Calcein	0.1–50 μM	MDCKII cells	Diosmetin > chrysoeriol > tamarixetin > tetra-methoxyflavone > robinetin > iso-rhamnetin > kaempferol > myricetin > quercetin > luteolin	Van Zanden (2005)

transporter (Zhang *et al.*, 2007). In addition, a significant increase in apical to basolateral transport as well as cellular accumulation of ochratoxin A, a food-borne mycotoxin, in Caco-2 cells, was observed upon co-incubation with chrysin, quercetin, genistein, biochanin A or resveratrol, all at concentrations that can be expected in the gastrointestinal tract (Sergent *et al.*, 2005). It has been hypothesized that polyphenols may exert this effect through competitive inhibition of MRP2 (Sergent *et al.*, 2005). Recently, the major phase II metabolites of quercetin have been identified as potent MRP2 inhibitors, improving the potential use of quercetin as MRP2 inhibitor (Van Zanden *et al.*, 2007). These observations are important because flavonoid metabolites in the intestinal mucosa may reach a concentration that can significantly inhibit transporters in several body tissues (Aszalos, 2008). In addition, it has been reported that the interaction of MRP2 with quercetin glucuronides is dependent on the position and nature of substitution (Williamson *et al.*, 2007). All these results support the hypothesis that flavonoid supplementation could affect the biliary or renal excretion of MRP2 substrates. In this respect, the hyperbilirubinemia inducible by the presence of flavopiridol and its conjugates is very relevant and is probably due to the inhibition of biliary excretion of conjugated bilirubin, an MRP2 substrate (Jäger *et al.*, 2003). For inhibition of MRP1, a quantitative structure–activity relationship (QSAR) was obtained that indicates three structural characteristics to be of major importance for MRP1 inhibition by flavonoids: the total number of methoxylated moieties, the total number of hydroxyl groups and the dihedral angle between the B and C ring. While for MRP2 inhibition, flavonol B ring pyrogallol moiety is important (Van Zanden *et al.*, 2005).

Inhibition of BCRP by flavonoids

About 20 flavonoids, representing all the chemical subclasses of flavonoids, have been tested on BCRP-mediated transport and demonstrated that the flavonoids apigenin, biochanin A, chrysin, genistein, hesperetin, kaempferol, naringenin and silymarin all produced a more than threefold increase in mitoxantrone accumulation in the BCRP-overexpressing cells (MCF-7 MX100 and NCI-H460 MX20), with no or minimal effects on mitoxantrone accumulation in the corresponding BCRP-negative cell lines (MCF-7 sensitive and NCI-H460) (Zhang *et al.*, 2004). The flavonoids daidzein, fisetin, phloretin, quercetin, and silibin produced at more than twofold increase in the mitoxantrone accumulation in the BCRP-overexpressing cells, with no or only slight effects in the BCRP-negative counterparts (MCF-7 sensitive and NCI-H460), suggesting that these flavonoids are also BCRP inhibitors (Zhang *et al.*, 2004). Concentration-dependent effects of flavonoids (apigenin, biochanin A, chrysin, genistein, and kaempferol) have been performed in both MCF-7 MX100 and NCI-H460 MX20 cells; it was observed high BCRP inhibition when tested at 50 μM concentration (Zhang *et al.*, 2004). Genistein and naringenin have also been found to diminish the function of BCRP as an efflux pump and reverse BCRP-mediated resistance to anticancer agents in BCRP-overexpressing cells (Imai *et al.*, 2004; Takahata *et al.*, 2008). In addition, genistein and daidzein reversed BCRP-mediated transport of the fluoroquinolone enrofloxacin, a BCRP substrate flavones were found more efficient than flavonols, isoflavones, and flavanones (Pulido *et al.*, 2006). With the help of structure–activity studies and rational screening facilitated by the use of ABCG2-transfected cell lines, with a high drug efflux capacity, 6-prenylchrysin and tectochrysin have been found as potent and specific inhibitors of breast cancer resistance protein ABCG2 (Ahmed-Belkacem *et al.*, 2005). Chrysin at the oral dose of 200 mg/kg significantly increased bioavailability and decreased the apparent oral clearance of nitrofurantoin, a BCRP substrate, in rats (Wang and Morris, 2007). In contrast, at the lower chrysin dose of 50 mg/kg, only a small effect in the AUC was observed after intra venous administration. No significant interaction between BCRP and chrysin was observed after oral administration

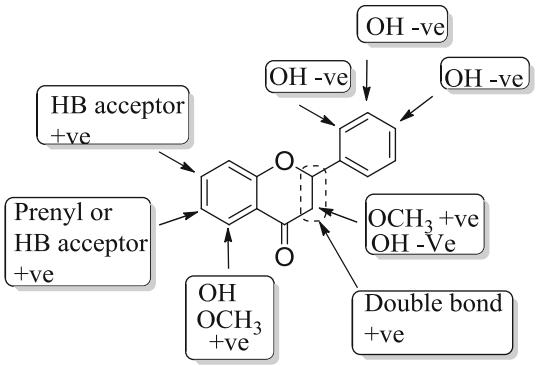

Fig. 6 SAR studies of flavonoids with respect to BCRP modulation

at that dose. One explanation for this lack of interaction might be due to the poor bioavailability of chrysin or to the extensive metabolism of chrysin in the intestine (Wang and Morris, 2007). SAR studies of flavonoids for BCRP inhibition suggest that hydroxylation of ring B is not favorable while hydrogen bond acceptor group at position 6,7 and 2,3-double bond is necessary for activity. Methoxylation at positions 3 and 5 is favorable, whereas hydroxylation at position 3 diminishes modulation activity (Fig. 6) (Pick *et al.*, 2011).

Inhibition of GSTs (GSTP1-1) by flavonoid

The inhibition of human glutathione-*S*-transferase P1-1 (GSTP1-1) by the flavonoid quercetin has been investigated. The results showed a time and concentration-dependent inhibition of GSTP1-1 by quercetin (Van Zanden *et al.*, 2003). Quercetin is one of the natural polyphenols, which are important constituents of fruits, vegetables, nuts, red wine and tea (Harborne and Williams, 2000). GSTP1-1 activity is completely inhibited upon 1 h incubation with 100 μM quercetin or 2 h incubation with 25 μM quercetin, whereas 1 and 10 μM quercetin inhibit GSTP1-1 activity to a significant extent reaching a maximum of 25 and 42 % inhibition, respectively, after 2 h. Co-incubation with tyrosinase greatly enhances the rate of inactivation, whereas co-incubation with ascorbic acid or glutathione prevents this inhibition. Addition of glutathione upon complete inactivation of GSTP1-1 partially restores the activity. Inhibition studies with the GSTP1-1 mutants C47S, C101S and the double mutant C47S/C101S showed that cysteine 47 is the key residue in the interaction between quercetin and GSTP-1. Quinone-type oxidation products of quercetin likely act as specific active site inhibitors of GSTP1-1 by binding to cysteine 47 (Van Zanden *et al.*, 2003). Apart from this many more flavonoids have been tested for GSTP1-1 inhibitory activity, especially galangin appeared to be able to inhibit GSTP1-1 activity with an IC 50 value of 14.4 μM (Van Zanden *et al.*, 2004). Apple flavonoids also have been tested for their modulation activity of phase II metabolizing enzymes and inhibition of growth of human colon cancer cells HT29 (Veeriah *et al.*, 2006). It has been reported in literature that the inhibitory potency of different flavonoids for GST activity depends exclusively on the pattern of hydroxylation and number of hydroxyl groups in the ring B. Especially, pyrogallol-type catechins with 3-OH group esterified with gallic acid showed strong potential to inhibit GST in vitro (Boušová *et al.*, 2012). Inhibitory and excitatory effects of other flavonoids have been also reviewed (Boušová and Skálová, 2012). Recently, anthocyanidine has been tested on different drug metabolizing enzyme and reported as weak GSTs inhibitor (Szotáková *et al.*, 2013).

Conclusions

Multidrug resistance is a major problem which makes cancer chemotherapy ineffective. Classical ABC transporter involved in MDR is P-gp; apart from this, other ABC transporters like MRP and BCRP have been found to be involved in MDR. On the basis of structure and function of each transporter, several MDR inhibitors have been developed but none of them have been proven clinically so far, this embolden interest in flavonoids as ideal MDR inhibitors because they do not produce any side effects, but still research is in progress to identify an appropriate MDR inhibitors.

Acknowledgments Authors are grateful to Prof. P. Ramarao, Dean Academic and Centre Coordinator of Centre for Chemical and Pharmaceutical Sciences for guiding and positive feedback during the course of the work. Authors are also thankful to Vice-Chancellor for providing necessary facilities at Central University of Punjab, Bathinda.

References

Abolhoda A, Wilson AE, Ross H, Danenberg PV, Burt M, Scotto KW (1999) Rapid activation of MDR1 gene expression in human metastatic sarcoma after in vivo exposure to doxorubicin. Clin Cancer Res 5:3352–3356

Adachi M, Reid G, Schuetz JD (2002) Therapeutic and biological importance of getting nucleotides out of cells: a case for the ABC transporters, MRP4 and 5. Adv Drug Deliv Rev 54:1333–1342

Ader P, Wessmann A, Wolffram S (2000) Bioavailability and metabolism of the flavonol quercetin in the pig. Free Radic Biol Med 28:1056–1067

Ahmed-Belkacem A, Pozza A, Muñoz-Martínez F, Bates SE, Castanys S, Gamarro F, Pietro Di, APérez-Victoria JM (2005) Flavonoid structure-activity studies identify 6-prenylchrysin and tectochrysin as potent and specific inhibitors of breast cancer resistance protein ABCG2. Cancer Res 65:4852–4860

Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M (1998) A human placenta-specific ATP-binding cassette gene

(ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res 58:5337–5339
Altenberg GA (2004) Structure of multidrug-resistance proteins of the ATP-binding cassette (ABC) superfamily. Curr Med Chem 4:53–62
Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter 1. Annu Rev Pharmacol 39:361–398
Ambudkar SV, Kim IW, Sauna ZE (2006) The power of the pump: mechanisms of action of P-glycoprotein (ABCB1). Eur J Pharm Sci 27:392–400
Assaraf YG, Rothem L, Hooijberg JH, Stark M, Ifergan I, Kathmann I, Dijkmans BAC, Peters GJ, Jansen G (2003) Loss of multidrug resistance protein 1 expression and folate efflux activity results in a highly concentrative folate transport in human leukemia cells. J Biol Chem 278:6680–6686
Aszalos A (2008) Role of ATP-binding cassette (ABC) transporters in interactions between natural products and drugs. Curr Drug Metab 9:1010–1018
Bansal T, Jaggi M, Khar R, Talegaonkar S (2009) Emerging significance of flavonoids as P-glycoprotein inhibitors in cancer chemotherapy. J Pharm Pharm Sci 12:46–78
Bera TK, Lee S, Salvatore G, Lee B, Pastan I (2001) MRP8, a new member of ABC transporter superfamily, identified by EST database mining and gene prediction program, is highly expressed in breast cancer. Mol Med 7:509–516
Bera TK, Iavarone C, Kumar V, Lee S, Lee B, Pastan I (2002) MRP9, an unusual truncated member of the ABC transporter superfamily, is highly expressed in breast cancer. Proc Natl Acad Sci 99:6997–7002
Borst P, Elferink RO (2002) Mammalian ABC transporters in health and disease. Ann Rev Biochem 71:537–592
Borst P, Evers R, Kool M, Wijnholds J (2000) A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 92:1295–1302
Boumendjel A, Beney C, Deka N, Mariotte AM, Lawson MA, Trompier D, Baubichon-Cortay H, Pietro AD (2002a) 4-Hydroxy-6-methoxyaurones with high-affinity binding to cytosolic domain of P-glycoprotein. Chem Pharm Bull 50:854–856
Boumendjel A, Di Pietro A, Dumontet C, Barron D (2002b) Recent advances in the discovery of flavonoids and analogs with high-affinity binding to P-glycoprotein responsible for cancer cell multidrug resistance. Med Res Rev 22:512–529
Boušová I, Skálová L (2012) Inhibition and induction of glutathione *S*-transferases by flavonoids: possible pharmacological and toxicological consequences. Drug Metabol Rev 44:267–286
Boušová I, Hájek J, Dršata J, Skálová L (2012) Naturally occurring flavonoids as inhibitors of purified cytosolic glutathione *S*-transferase. Xenobiotica 42:872–879
Bueno Pérez L, Pan L, Sass E, Gupta SV, Lehman A, Kinghorn ADLucas DM (2012) Potentiating effect of the flavonolignan (−)-hydnocarpin in combination with vincristine in a sensitive and P-gp-expressing acute lymphoblastic leukemia cell line. Phytother Res. doi:10.1002/ptr.4903
Buggins TR, Dickinson PA, Taylor G (2007) The effects of pharmaceutical excipients on drug disposition. Adv Drug Deliv Rev 59:1482–1503
Castro AF, Altenberg GA (1997) Inhibition of drug transport by genistein in multidrug-resistant cells expressing P-glycoprotein. Biochem Pharmacol 53:89–93
Choi CH (2005) ABC transporters as multidrug resistance mechanisms and the development of chemosensitizers for their reversal. Cancer Cell Int 5:30
Comte G, Daskiewicz JB, Bayet C, Conseil G, Viornery-Vanier A, Dumontet C, Di Pietro A, Barron D (2001) C-Isoprenylation of flavonoids enhances binding affinity toward P-glycoprotein and modulation of cancer cell chemoresistance. J Med Chem 44:763–768
Conseil G, Baubichon-Cortay H, Dayan G, Jault JM, Barron D, Di Pietro A (1998) Flavonoids: a class of modulators with bifunctional interactions at vicinal ATP-and steroid-binding sites on mouse P-glycoprotein. Proc Natl Acad Sci 95:9831–9836
Crowley E, McDevitt CA, Callaghan R (2010) Generating inhibitors of P-glycoprotein: where to, now. Methods Mol Biol 596:405–432
Dantzig A, Shepard R, Law K, Tabas L, Pratt S, Gillespie J, Binkley S, Kuhfeld M, Starling J, Wrighton S (1999) Selectivity of the multidrug resistance modulator, LY335979, for P-glycoprotein and effect on cytochrome P-450 activities. J Pharmacol Exp Ther 290:854–862
Day AJ, Bao Y, Morgan MRA, Williamson G (2000) Conjugation position of quercetin glucuronides and effect on biological activity. Free Radic Biol Med 29:1234–1243
Day AJ, Mellon F, Barron D, Sarrazin G, Morgan MRA, Williamson G (2001) Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. Free Radic Res 35:941–952
Deferme S, Gelder J, Augustijns P (2002) Inhibitory effect of fruit extracts on P-glycoprotein related efflux carriers: an in-vitro screening. J Pharm Pharmacol 54:1213–1219
Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, Ross DD (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci 95:15665–15670
Drach D, Zhao S, Drach J, Andreeff M (1995) Low incidence of MDR1 expression in acute promyelocytic leukaemia. Brit J Haematol 90:369–374
Ejendal KFK, Hrycyna CA (2002) Multidrug resistance and cancer: the role of the human ABC transporter ABCG2. Curr Protein Pept Sci 3:503–511
Fardel O, Lecureur V, Guillouzo A (1996) The P-glycoprotein multidrug transporter. Gen Pharmacol Vasc Syst 27:1283–1291
Ferry D, Traunecker H, Kerr D (1996) Clinical trials of P-glycoprotein reversal in solid tumours. Eur J Cancer 32:1070–1081
Ferté J, Kühnel JM, Chapuis G, Rolland Y, Lewin G, Schwaller MA (1999) Flavonoid-related modulators of multidrug resistance: synthesis, pharmacological activity, and structure-activity relationships. J Med Chem 42:478–489
Fischer V, Rodríguez-Gascón A, Heitz F, Tynes R, Hauck C, Cohen D, Vickers AEM (1998) The multidrug resistance modulator valspodar (PSC 833) is metabolized by human cytochrome P450 3A. Drug Metab Dispos 26:802–811
Fojo T, Bates S (2003) Strategies for reversing drug resistance. Oncogene 22:7512–7523
Gerk PM, Vore M (2002) Regulation of expression of the multidrug resistance-associated protein 2 (MRP2) and its role in drug disposition. J Pharmacol Exp Ther 302:407–415
Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2:48–58
Gupta SV, Sass EJ, Davis ME, Edwards RB, Lozanski G, Heerema NA, Lehman A, Zhang X, Jarjoura D, Byrd JC (2011) Resistance to the translation initiation inhibitor silvestrol is mediated by ABCB1/P-glycoprotein overexpression in acute lymphoblastic leukemia cells. AAPS J 13:357–364
Hadjeri M, Barbier M, Ronot X, Mariotte AM, Boumendjel A, Boutonnat J (2003) Modulation of P-glycoprotein-mediated multidrug resistance by flavonoid derivatives and analogues. J Med Chem 46:2125–2131

Haimeur A, Conseil G, Deeley R, Cole S (2004) The MRP-related and BCRP/ABCG2 multidrug resistance proteins: biology, substrate specificity and regulation. Curr Drug Metab 5:21–53

Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. Phytochemistry 55:481–504

Hasrat J, Bruyne TD, Backer JP, Vauquelin G, Vlietinck A (1997) Cirsimarin and cirsimaritin, flavonoids of *Microtea debilis* (Phytolaccaceae) with adenosine antagonistic properties in rats: leads for new therapeutics in acute renal failure. J Pharm Pharmacol 49:1150–1156

Havsteen BH (2002) The biochemistry and medical significance of the flavonoids. Pharmacol Ther 96:67–202

Hayes JD, Pulford DJ (1995) The glutathione-*S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance part II. Crit Rev Biochem Mol 30:521–600

Higgins CF (1992) ABC transporters: from microorganisms to man. Ann Rev Cell Biol 8:67–113

Holiman PCH, Hertog MGL, Katan MB (1996) Analysis and health effects of flavonoids. Food Chem 57:43–46

Hooijberg J, Broxterman H, Scheffer G, Vrasdonk C, Heijn M, De Jong M, Scheper R, Lankelma J, Pinedo H (1999) Potent interaction of flavopiridol with MRP1. Brit J cancer 81:269–276

Imai Y, Tsukahara S, Asada SSugimoto Y (2004) Phytoestrogens/flavonoids reverse breast cancer resistance protein/ABCG2-mediated multidrug resistance. Cancer Res 64:4346–4352

Ishikawa T (1992) The ATP-dependent glutathione S-conjugate export pump. Trends Biochem Sci 17:463–468

Jäger W, Gehring E, Hagenauer B, Aust S, Senderowicz A, Thalhammer T (2003) Biliary excretion of flavopiridol and its glucuronides in the isolated perfused rat liver: role of multidrug resistance protein 2 (Mrp2). Life Sci 73:2841–2854

Jedlitschky G, Leier I, Buchholz U, Barnouin K, Kurz G, Keppler D (1996) Transport of glutathione, glucuronate, and sulfate conjugates by the MRP gene-encoded conjugate export pump. Cancer Res 56:988–994

Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 455:152–162

Keppler D, Leier I, Jedlitschky G, König J (1998) ATP-dependent transport of glutathione S-conjugates by the multidrug resistance protein MRP1 and its apical isoform MRP2. Chem Biol Interact 111:153–161

Kimura Y, Matsuo M, Takahashi K, Saeki T, Kioka N, Amachi T, Ueda K (2004) ATP hydrolysis-dependent multidrug efflux transporter: MDR1/P-glycoprotein. Curr Drug Metab 5:1–10

Krishna R, Mayer LD (2000) Multidrug resistance (MDR) in cancer: mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur J Pharm Sci 11:265–283

Lee CH (2004) Reversing agents for ATP-binding cassette (ABC) transporters: application in modulating multidrug resistance (MDR). Curr Med Chem 4:43–52

Lehane AM, McDevitt CA, Kirk K, Fidock DA (2011) Degrees of chloroquine resistance in Plasmodium—is the redox system involved? Int J Parasitol Drugs Drug Resist 2:47–57

Leonessa F, Clarke R (2003) ATP binding cassette transporters and drug resistance in breast cancer. Endocr Relat Cancer 10:43–73

Lin JH (2003) Drug-drug interaction mediated by inhibition and induction of P-glycoprotein. Adv Drug Deliv Rev 55:53–81

Liscovitch M, Lavie Y (2002) Cancer multidrug resistance: a review of recent drug discovery research. Idrugs 5:349–355

Litman T, Druley T, Stein W, Bates S (2001) From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. Cell Mol Life Sci 58:931–959

Loo T, Clarke D (2005) Recent progress in understanding the mechanism of P-glycoprotein-mediated drug efflux. J Membr Biol 206:173–185

Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg ACLM, Schinkel AH, van de Vijver MJ, Scheper RJ, Schellens JHM (2001) Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res 61:3458–3464

Mao Q (2005) Role of the breast cancer resistance protein (ABCG2) in drug transport. AAPS J 7:118–133

Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, Brangi M, Greenberger L, Dean M, Fojo T (1999) Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells. Cancer Res 59:8–13

Morris ME, Zhang S (2006) Flavonoid–drug interactions: effects of flavonoids on ABC transporters. Life Sci 78:2116–2130

Morrow CS, Smitherman PK, Townsend AJ (1998) Combined expression of multidrug resistance protein (MRP) and glutathione *S*-transferase P1-1 (GSTP1-1) in MCF7 cells and high level resistance to the cytotoxicities of ethacrynic acid but not oxazaphosphorines or cisplatin. Biochem Pharmacol 56:1013–1021

Mullen W, Graf BA, Caldwell ST, Hartley RC, Duthie GG, Edwards CA, Michael E, Crozier A (2002) Determination of flavonol metabolites in plasma and tissues of rats by HPLC-radiocounting and tandem mass spectrometry following oral ingestion of [2-14C] quercetin-4′-glucoside. J Agric Food Chem 50:6902–6909

Newman MJ, Rodarte JC, Benbatoul KD, Romano SJ, Zhang C, Krane S, Moran EJ, Uyeda RT, Dixon R, Guns ES (2000) Discovery and characterization of OC144-093, a novel inhibitor of P-glycoprotein-mediated multidrug resistance. Cancer Res 60:2964–2972

Nguyen H, Zhang S, Morris ME (2003) Effect of flavonoids on MRP1-mediated transport in Panc-1 cells. J Pharm Sci 92:250–257

Nobili S, Landini I, Mazzei T, Mini E (2011) Overcoming tumor multidrug resistance using drugs able to evade P-glycoprotein or to exploit its expression. Med Res Rev 32:1220–1262

Norman BH (1998) Inhibitors of MRP1-mediated multidrug resistance. Drug Future 23:1001–1014

Nurulita NA, Meiyanto E, Matsuda E, Kawaichi M (2012) *Gynura procumbens* prevents chemoresistance through inhibition MDR1 expression on MCF-7 breast cancer cell line and sensitizes the cells to doxorubicin. Indones J Biotech 17:51–60

O'brien M, Tew K (1996) Glutathione and related enzymes in multidrug resistance. Eur J Cancer 32:967–978

Ozben T (2006) Mechanisms and strategies to overcome multiple drug resistance in cancer. FEBS Lett 580:2903–2909

Phang JM, Poore CM, Lopaczynska J, Yeh GC (1993) Flavonol-stimulated efflux of 7,12-dimethylbenz(a)anthracene in multidrug-resistant breast cancer cells. Cancer Res 53:5977–5981

Pick A, Müller H, Mayer R, Haenisch B, Pajeva IK, Weigt M, Bönisch H, Müller CE, Wiese M (2011) Structure–activity relationships of flavonoids as inhibitors of breast cancer resistance protein (BCRP). Bioorg Med Chem 19:2090–2102

Pulido MM, Molina AJ, Merino G, Mendoza G, Prieto JG, Alvarez AI (2006) Interaction of enrofloxacin with breast cancer resistance protein (BCRP/ABCG2): influence of flavonoids and role in milk secretion in sheep. J Vet Pharmacol Ther 29:279–287

Rege BD, Kao JPY, Polli JE (2002) Effects of nonionic surfactants on membrane transporters in Caco-2 cell monolayers. Eur J Pharm Sci 16:237–246

Regev R, Assaraf YG, Eytan GD (1999) Membrane fluidization by ether, other anesthetics, and certain agents abolishes

P-glycoprotein ATPase activity and modulates efflux from multidrug-resistant cells. Eur J Biochem 259:18–24
Regev R, Katzir H, Yeheskely-Hayon D, Eytan GD (2007) Modulation of P-glycoprotein-mediated multidrug resistance by acceleration of passive drug permeation across the plasma membrane. FEBS J 274:6204–6214
Rice-Evans C (2001) Flavonoid antioxidants. Curr Med Chem 8:797–807
Rosenberg MF, Mao Q, Holzenburg A, Ford RC, Deeley RG, Cole SPC (2001) The structure of the multidrug resistance protein 1 (MRP1/ABCC1). J Biol Chem 276:16076–16082
Rosenberg MF, Kamis AB, Callaghan R, Higgins CF, Ford RC (2003) Three-dimensional structures of the mammalian multidrug resistance P-glycoprotein demonstrate major conformational changes in the transmembrane domains upon nucleotide binding. J Biol Chem 278:8294–8299
Ross DD, Karp JE, Chen TT, Doyle LA (2000) Expression of breast cancer resistance protein in blast cells from patients with acute leukemia. Blood 96:365–368
Salinas AE, Wong MG (1999) Glutathione *S*-transferases—a review. Curr Med Chem 6:279–310
Sauna ZE, Ambudkar SV (2000) Evidence for a requirement for ATP hydrolysis at two distinct steps during a single turnover of the catalytic cycle of human P-glycoprotein. Proc Natl Acad Sci 97:2515–2520
Sauna ZE, Smith MM, Müller M, Kerr KM, Ambudkar SV (2001) The mechanism of action of multidrug-resistance-linked P-glycoprotein. J Bioenerg Biomembr 33:481–491
Sawicka M, Kalinowska M, Skierski J, Lewandowski W (2004) A review of selected anti-tumour therapeutic agents and reasons for multidrug resistance occurrence. J Pharm Pharmacol 56:1067–1081
Scambia G, Ranelletti F, Panici PB, Vincenzo R, Bonanno G, Ferrandina G, Piantelli M, Bussa S, Rumi C, Cianfriglia M (1994) Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast-cancer cell line: P-glycoprotein as a possible target. Cancer Chemother Pharmacol 34:459–464
Sedlacek H, Czech J, Naik R, Kaur G, Worland P, Losiewicz M, Parker B, Carlson B, Smith A, Senderowicz A (1996) Flavopiridol (L86 8275; NSC 649890), a new kinase inhibitor for tumor therapy. Int J Oncol 9:1143–1168
Sergent T, Garsou S, Schaut A, Saeger SD, Pussemier L, Peteghem CV, Larondelle Y, Schneider YJ (2005) Differential modulation of ochratoxin A absorption across Caco-2 cells by dietary polyphenols, used at realistic intestinal concentrations. Toxicol Lett 159:60–70
Shapiro AB, Ling V (1997) Effect of quercetin on Hoechst 33342 transport by purified and reconstituted P-glycoprotein. Biochem Pharmacol 53:587–596
Shen H, Kauvar L, Tew KD (1997) Importance of glutathione and associated enzymes in drug response. Oncol Res 9:295–302
Sparreboom A, Danesi R, Ando Y, Chan J, Figg WD (2003) Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. Drug Resist Update 6:71–84
Spencer JPE, Abd El Mohsen MM, Rice-Evans C (2004) Cellular uptake and metabolism of flavonoids and their metabolites: implications for their bioactivity. Arch Biochem Biophys 423:148–161
Stagos D, Amougias G, Matakos A, Spyrou A, Tsatsakis A, Kouretas D (2012) Chemoprevention of liver cancer by plant polyphenols. Food Chem Toxicol 50:2155–2170
Starling JJ, Shepard RL, Cao J, Law KL, Norman BH, Kroin JS, Ehlhardt WJ, Baughman TM, Winter MA, Bell MG (1997) Pharmacological characterization of LY335979: a potent cyclopropyldibenzosuberane modulator of P-glycoprotein. Adv Enz Reg 37:335–347
Stavrovskaya A (2000) Cellular mechanisms of multidrug resistance of tumor cells. Biochem Biokhim 65:95–106
Steinbach D, Sell W, Voigt A, Hermann J, Zintl F, Sauerbrey A (2002) BCRP gene expression is associated with a poor response to remission induction therapy in childhood acute myeloid leukemia. Leukemia 16:1443–1447
Suzuki T, Takagi Y, Osanai H, Li L, Takeuchi M, Katoh Y, Kobayashi M, Yamamoto M (2005) Pi class glutathione *S*-transferase genes are regulated by Nrf 2 through an evolutionarily conserved regulatory element in zebrafish. Biochem J 388:65–73
Szotáková B, Bártíková H, Hlavácová J, Boušová I, Skálová L (2013) Inhibitory effect of anthocyanidins on hepatic glutathione *S*-transferase, UDP-glucuronosyltransferase and carbonyl reductase activities in rat and human. Xenobiotica. doi:10.3109/00498254.2012.756557
Takahata T, Ookawa K, Suto K, Tanaka M, Yano H, Nakashima O, Kojiro M, Tamura Y, Tateishi T, Sakata Y (2008) Chemosensitivity determinants of irinotecan hydrochloride in hepatocellular carcinoma cell lines. Basic Clin Pharmacol Toxicol 102:399–407
Tan B, Piwnica-Worms D, Ratner L (2000) Multidrug resistance transporters and modulation. Curr Opin Oncol 12:450–458
Tandon VR, Kapoor B, Bano G, Gupta S, Gillani Z, Kour D (2006) P-glycoprotein: pharmacological relevance. Indian J Pharmacol 38:13–24
Teodori E, Dei S, Scapecchi S, Gualtieri F (2002) The medicinal chemistry of multidrug resistance (MDR) reversing drugs. II. Farmaco 57:385–415
Tew KD, Gaté L (2001) Glutathione-*S*-transferases as emerging therapeutic targets. Expert Opin Ther Target 5:477–489
Thomas H, Coley HM (2003) Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting P-glycoprotein. Cancer Control 10:159
Tombline G, Bartholomew L, Gimi K, Tyndall GA, Senior AE (2004) Synergy between conserved ABC signature Ser residues in P-glycoprotein catalysis. J Biol Chem 279:5363–5373
Townsend DM, Tew KD (2003) The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene 22:7369–7375
Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y (1981) Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res 41:1967–1972
van Bladeren PJ, Van Ommen B (1991) The inhibition of glutathione-S-transferases: mechanisms, toxic consequences and therapeutic benefits. Pharmacol Ther 51:35–46
Van Zanden JJ (2005) Modulation of multidrug resistance by flavonoids. Inhibitors of glutathione conjugation and MRP-mediated transport. Dissertation, Wageningen University, Wageningen, The Netherlands
Van Zanden JJ, Ben Hamman O, Van Iersel MLPS, Boeren S, Cnubben NHP, Lo Bello M, Vervoort J, van Bladeren PJ, Rietjens IMCM (2003) Inhibition of human glutathione *S*-transferase P1-1 by the flavonoid quercetin. Chem-Biol Interact 145:139–148
Van Zanden JJ, Geraets L, Wortelboer HM, Bladeren PJ, Rietjens IMCM, Cnubben NHP (2004) Structural requirements for the flavonoid-mediated modulation of glutathione-S-transferase P1-1 and GS-X pump activity in MCF7 breast cancer cells. Biochem Pharmacol 67:1607–1617
Van Zanden JJ, Wortelboer HM, Bijlsma S, Punt A, Usta M, Bladeren PJv, Rietjens IM, Cnubben NH (2005) Quantitative structure activity relationship studies on the flavonoid mediated inhibition of multidrug resistance proteins 1 and 2. Biochem Pharmacol 69:699–708

Van Zanden JJ, van der Woude H, Vaessen J, Usta M, Wortelboer HM, Cnubben NHP, Rietjens IMCM (2007) The effect of quercetin phase II metabolism on its MRP1 and MRP2 inhibiting potential. Biochem Pharmacol 74:345–351

Van Zuylen L, Nooter K, Sparreboom A, Verweij J (2000) Development of multidrug-resistance convertors: sense or non-sense? Invest New Drug 18:205–220

Varma MVS, Ashokraj Y, Dey CS, Panchagnula R (2003) P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. Pharmacol Res 48:347–359

Veeriah S, Kautenburger T, Habermann N, Sauer J, Dietrich H, Will F, Pool-Zobel BL (2006) Apple flavonoids inhibit growth of HT29 human colon cancer cells and modulate expression of genes involved in the biotransformation of xenobiotics. Mol Carcinog 45:164–174

Versantvoort C, Schuurhuis G, Pinedo H, Eekman C, Kuiper C, Lankelma J, Broxterman H (1993) Genistein modulates the decreased drug accumulation in non-P-glycoprotein mediated multidrug resistant tumour cells. Brit J Cancer 68:939–946

Versantvoort CHM, Broxterman HJ, Lankelma J, Pinedo HM (1994) Competitive inhibition by genistein and ATP dependence of daunorubicin transport in intact MRP overexpressing human small cell lung cancer cells. Biochem Pharmacol 48:1129–1136

Wang X, Morris ME (2007) Effects of the flavonoid chrysin on nitrofurantoin pharmacokinetics in rats: potential involvement of ABCG2. Drug Metab Dispos 35:268–274

Wesołowska O, Wiśniewski J, Środa-Pomianek K, Bielawska-Pohl A, Paprocka M, Duś D, Nl Duarte, Ferreira M-JU, Michalak K (2012) Multidrug resistance reversal and apoptosis induction in human colon cancer cells by some flavonoids present in citrus plants. J Nat Prod 75:1896–1902

Williamson G, Aeberli I, Miguet L, Zhang Z, Sanchez MB, Crespy V, Barron D, Needs P, Kroon PA, Glavinas H (2007) Interaction of positional isomers of quercetin glucuronides with the transporter ABCC2 (cMOAT, MRP2). Drug Metab Dispos 35:1262–1268

Wu H, Hait WN, Yang JM (2003) Small interfering RNA-induced suppression of MDR1 (P-glycoprotein) restores sensitivity to multidrug-resistant cancer cells. Cancer Res 63:1515–1519

Yáñez JA, Remsberg CM, Takemoto JK, Vega-Villa KR, Andrews PK, Sayre CL, Martinez SE, Davies NM (2013) Polyphenols and flavonoids: an overview. Flavonoid pharmacokinetics: methods of analysis, preclinical and clinical pharmacokinetics, safety, and toxicology. Wiley, New Jersey, pp 1–69

Zhang S, Yang X, Morris ME (2004) Flavonoids are inhibitors of breast cancer resistance protein (ABCG2)-mediated transport. Mol Pharmacol 65:1208–1216

Zhang L, Lin G, Kovács B, Jani M, Krajcsi P, Zuo Z (2007) Mechanistic study on the intestinal absorption and disposition of baicalein. Eur J Pharm Sci 31:221–231

Zhou S, Lim LY, Chowbay B (2004) Herbal modulation of P-glycoprotein. Drug Metab Rev 36:57–104

Zhou L, Jing Y, Styblo M, Chen Z, Waxman S (2005) Glutathione-S-transferase π inhibits As_2O_3-induced apoptosis in lymphoma cells: involvement of hydrogen peroxide catabolism. Blood 105:1198–1203