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Tariquidar-Related Chalcones and Ketones as ABCG2 Modulators

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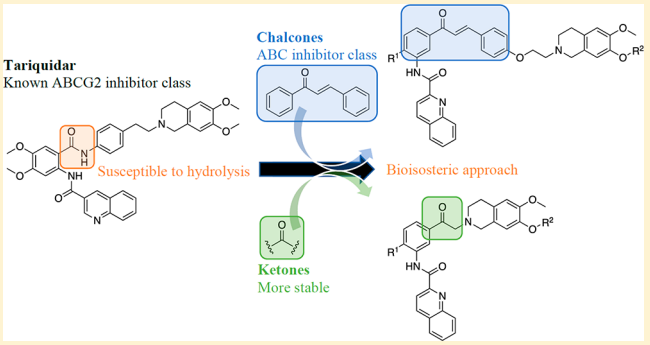
Supporting Information

ABSTRACT: ABC transporters, including ABCG2, play a vital role in defending the human body against the vast range of xenobiotics. Even though this is beneficial for human health, these protein transporters have been implicated in the emerging resistance of cancer cells to a variety of structurally and functionally diverse anticancer drugs. In order to investigate their role in resistance, potent and selective ABCG2 modulators have been described in the literature. A leading class of modulators are the tariquidar analogues; however, their susceptibility to hydrolysis limits their applicable use. To overcome this, we synthesized a novel series of chalcone- and ketone-based compounds inspired by reported tariquidar analogues. Compounds were characterized and evaluated for their ABCG2 modulatory activity and ABC transporter selectivity. When compared to transporters ABCB1 and ABCC1, the chalcone-based compounds exhibited selectivity for ABCG2, while the ketone-based compounds showed only a slight preference for ABCG2. From the former series, chalcone **16d** (UR-DP48) displayed similar activity to the reference fumitremorgin C, both producing comparable maximal effects. The compound exhibited marked antiproliferative activity, while cytotoxicity was less pronounced for the most active compound **17f** from the ketone series. Chalcone-containing tariquidar analogues are promising modulators to aid in functional investigations of ABCG2 transporters.



KEYWORDS: *Tariquidar, chalcone, ketone, ABCG2, modulator*

Cytotoxic drugs are well established in the treatment of many cancer types. In order to improve the effectiveness of treatment, multiple cytotoxic drugs with different mechanisms of action are administered simultaneously. However, emerging resistance of cells, known as multidrug resistance (MDR), has become an increasing issue for effective cancer treatment.[1] A well-established mechanism of resistance is associated with an overexpression of ATP-binding cassette (ABC) transporters, including P-glycoprotein (P-gp/ABCB1), multidrug resistance protein 1 (MRP1/ABCC1), and breast cancer resistance protein (BCRP/ABCG2). Several anticancer drugs are substrates of these proteins and are exported from the tumor cells, which prevents the drugs from exerting their intracellular cytotoxic effects.[2,3] Strategies to increase the efficacy of cancer chemotherapy involve the inhibition or modulation of such cellular efflux pumps. Even though the role of these pumps in drug resistance has been investigated for decades,[4−6] ABCG2 transporters have only recently received increasing attention with a desire to further understand their structure,[7,8] function,[9,10] and molecular pharmacology.[11]

A class of compounds that are able to interact with ABC transporters are chalcones. These chemical scaffolds are present in various biomolecules and are synthetically accessible.[12] Chalcone-based compounds exert a variety of biological effects including anticancer, anti-inflammatory, chemopreventive, antioxidant, and antimicrobial effects.[13] Compounds, containing this scaffold, have been developed to inhibit the transport activity of ABCcassette proteins, such as P-gp. Further developments have resulted in compounds that selectively engage with ABCG2.[14]

Structural investigations of chalcones have shown that the methoxy and hydroxy substituents at positions 2′ and 4′ on ring A (see Figure 1; general chalcone structure and compound **2**) are crucial for activity.[12,13] Recent structure−activity relationship studies[14] revealed that the 2′-OH-4′,6′-dimethoxyphenyl group (A-ring) could be replaced by either a 2′-naphthyl group or a 3′,4′-methylenedioxyphenyl moiety. It was found that at least two methoxy groups on the B-ring are necessary for optimal inhibition. Additionally, substitution at positions 3, 4, and 5 (B-ring) improved cytotoxicity, while the presence of a large O-benzyl substituent at position 4 and a 2′-naphthyl substituent (A-ring) decreased cytotoxicity.[14] Re-

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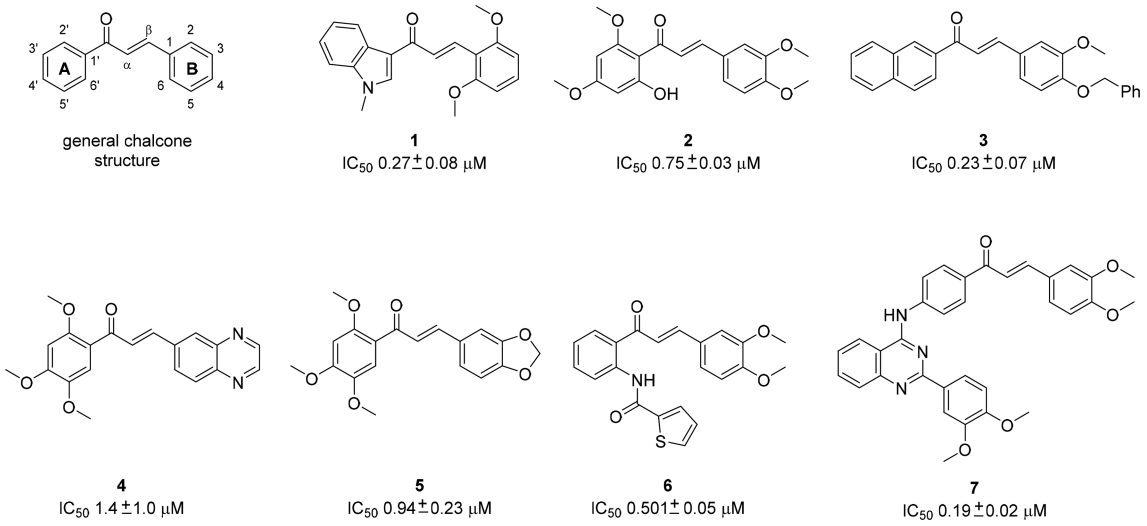


Figure 1. General chalcone structure and chalcones, recently reported as ABCG2modulators.[15−19]

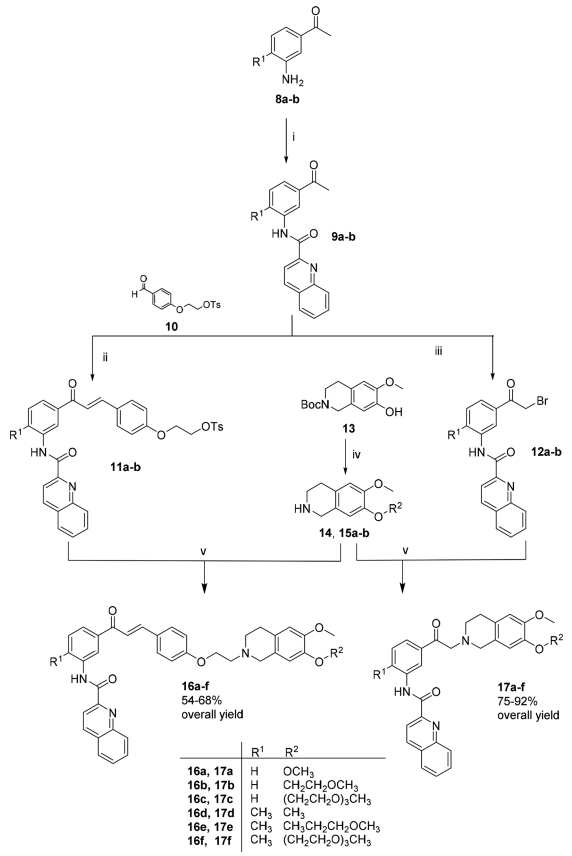
cently published chalcone-based ABCG2 modulators are shown in Figure 1.[15,16]

A new class of potent and selective ABCG2 inhibitors are the tariquidar analogues.[17−19] These compounds were developed to investigate the functional role of ABCG2; however, their susceptibility to hydrolysis limits their use in biochemical and biological studies. In search for more stable analogues, we propose a bioisosteric approach for developing new tariquidar-like compounds. Two series of compounds were explored in this study−chalcones and ketones. The design of the former series involved incorporation of tariquidar's key structural features necessary for ABCG2 inhibition. The latter series was designed to have a reduced molecular weight and more drug-like properties by replacing the 1,4-ethoxystyryl moiety by a methylene group. We hypothesized that this may reduce the compound toxicity. All compounds were evaluated for ABCG2, ABCB1, and ABCC1 modulatory activity by fluorescence based assays (an increase in cell-associated fluorescence is indicative of a decrease in transporter-mediated efflux) as well as for cytotoxicity.

As shown in Scheme 1, amines **12a−b** were prepared from commercially available acetophenone via an electrophilic aromatic substitution, followed by a Bechamp reduction.[20] The amides **9a−b** were obtained by reaction with quinaldic acid, *p*-toluenesulfonyl chloride (Ts-Cl), and the amines (**8a−b**). Compound **10** was prepared according to known procedures.[21]

A Claisen−Schmidt condensation of the ketones **9a−b** with the aldehyde **10** resulted in the desired protected chalcones **11a−b**, while compounds **12a−-b** were obtained by a bromination with *N*-bromosuccinimide. Tetrahydroisoquinoline **14** was obtained commercially as a hydrochloride salt. The Boc-protected tetrahydroisoquinoline **13** was synthesized as previously described.[18] This compound was reacted with 2-methoxyethyl 4-methylbenzenesulfonate or 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate and subsequently deprotected to produce the desired compounds as trifluoroacetic salts (**15a−b**). Finally, a nucleophilic substitution between the tosylated compounds (**11a−b**) or brominated compounds (**12a−b**) and the respective tetrahydroisoquinolines (**14**, **15a−b**) led to the target compounds

Scheme 1. Synthesis of Compounds 16−17a−f[a]



[a]Reagents and conditions: (i) Quinaldic acid, Ts-Cl, TEA, 50 °C, overnight; (ii) NaOH 30%, rt, 24 h; (iii) NBS, methanol, reflux, 2 h; (iv) (a) 2-methoxyethyl 4-methylbenzenesulfonate or 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate, KOH, THF, N_2, 6 h; (b) DCM, trifluoroacetic acid, rt, overnight; (v) **15a−b** or **16a−b**, K_2CO_3, acetonitrile, 90 °C, overnight.

(**16a−f** and **17a−f**, Scheme 1) in moderate to good yields (54−92%, Scheme 1). All synthesized compounds were characterized by nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and high resolution mass spectrometry

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(HR-MS), and the purity was confirmed by analytical high performance liquid chromatography (HPLC).

The synthesized compounds **16a−f** and **17a−f** and the reference compounds fumitremorgin C (FTC) and tariquidar were investigated in a Hoechst 33342 microplate assay[22] using ABCG2-overexpressing MCF-7/Topo cells. Relative IC_{50} values,[23] i.e. the concentration corresponding to the response midway between the lower and the upper plateau of a full concentration−response curve, relative to the maximal response achieved by each individual compound, were calculated according to a four parameter curve fit (GraphPad Prism 7). Relative IC_{50} values are summarized in Table 1. Concentration response curves of selected compounds obtained in the Hoechst 33342 assay are shown in Figure 2.

Table 1. ABCG2 Inhibitory Activity in Comparison to Reference Compounds, Chalcones 16a−f, and Ketones 17a−f

	ABCG2[a]		
compd	IC_{50} (μM)[b]	I_{max} (%)[c]	clogP[d]
FTC	0.731 ± 0.092	100	1.23
Tariquidar	0.526 ± 0.085	69 ± 5	6.38
16a	4.08 ± 0.29	79 ± 4	6.51
16b	2.13 ± 0.47	72 ± 8	6.18
16c	2.83 ± 0.30	81 ± 5	5.46
16d	0.88 ± 0.02	86 ± 10	6.97
16e	1.55 ± 0.36	97 ± 3	6.64
16f	2.72 ± 0.54	111 ± 4	5.92
17a	6.73 ± 1.02	77 ± 1	4.45
17b	14.39 ± 11.8	55 ± 2	4.12
17c	1.13 ± 0.27	47 ± 1	3.40
17d	8.76 ± 1.59	77 ± 1	4.91
17e	23.24 ± 4.36	79 ± 1	4.58
17f	6.65 ± 1.27	86 ± 2	3.86

[a]Hoechst 33342 microplate assay using ABCG2-overexpressing MCF-7/Topo cells. [b]Relative *IC*$_{50}$ value[23] *calculated using GraphPad Prism 7 four parameter curve fitting and presented as* mean values ± SEM from three independent experiments performed in triplicate. [c]Maximal inhibitory effects (%) are expressed as inhibition caused by the highest concentration (100 μM) of the compound, relative to the inhibitory effect caused by 10 μM FTC (100% inhibition). [d]Calculated values using ACD/Laboratories I-Lab 2.0 ilab.acdlabs, Algorithm Version: v5.0.0.184.

All chalcones (**16a−f**) exhibited maximal inhibitory activity between 72 and 111% (Table 1), indicating an ABCG2 related decrease in Hoechst 33342 efflux. Compounds with a methyl substituent at the amino chalcone core (**16d−f**) were superior in potency and maximal inhibitory effect than compounds bearing a hydrogen atom (**16a−c**). This suggests an importance in the substitution pattern at this particular position. Compound **16d** (**UR-DP48**) showed similar potency to the reference compound FTC (Figure 2). Moreover, the maximal inhibitory effects of these compounds (**16d−f**, 86%, 97%, and 111%, respectively) were higher than the effects of tariquidar and even surpassed that of FTC. The insertion of ethylene and triethylene glycol chains (R^2 in the structure, Scheme 1) at the tetrahydroisoquinoline moiety in compounds **16a−c** had no effect on the IC_{50} or I_{max} values (Table 1). In contrast, the maximal inhibitory effect achieved by the compounds **16d−f** increased from 86 to 111% (Table 1).

The partition coefficient (log P) is a measure of the differential solubility of a compound in a hydrophobic solvent (octanol) and a hydrophilic solvent (water). The logarithm of these two values enables compounds to be ranked in terms of hydrophilicity (or hydrophobicity).[24] As shown in Table 1, compounds bearing a methyl substituent at the amino chalcone core (**16d−f**) exhibited a higher calculated partition coefficient, and so, were more lipophilic, in comparison to analogs with hydrogen as a substituent at the same position (**16a−c**). These compounds also had the highest values for the maximal inhibition of Hoechst 33342 efflux. Comparing compounds **16a** and **16d**, the maximal inhibition for the most lipophilic compound **16d** (86%) was higher and the IC_{50} value was almost 5 times lower (0.88 μM, Table 1). Chalcones **16e** and **16f** showed a considerable increase in maximal inhibitory effect (97% and 111%) in comparison to compound **16b** and **16c** (72% and 81%). With the highest partition coefficient (log P) among all synthesized compounds, chalcone **16d** (**UR-DP48**) was the most potent inhibitor in the series, with similar activity to the reference compound (FTC).

By contrast, all ketones (**17a−f**) exhibited lower modulatory activities (47−86% maximal inhibition of Hoechst 33342 efflux) in comparison to the chalcone series (**16a−f**). The synthesized ketones were found to have lower partition coefficients compared to the chalcones (3.40−4.91 vs 5.46−6.97), which could explain the reduction in ABCG2 modulation (Table 1). As ABCG2 is located in the cell membrane, compound lipophilicity may be essential for modulation. In addition, recently published cryo-EM structural studies suggest that compounds can access one binding pocket of ABCG2 via a hydrophobic membrane entrance from the lipid bilayer.[8] This reflects the importance of lipophilicity, which should be considered when designing ABCG2 modulators. In accordance to this hypothesis, the two most

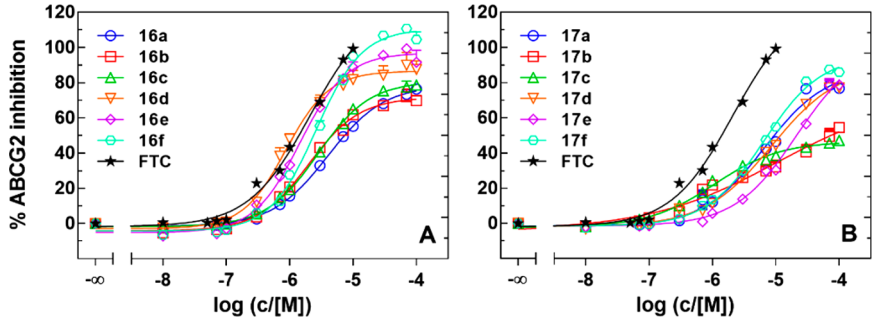

Figure 2. Concentration dependent inhibition of ABCG2 mediated Hoechst 33342 efflux by chalcones **16a−f** (A) and ketones **17a−f** (B) using MCF-7/Topo cells; inhibition relative to 10 μM FTC (100%).

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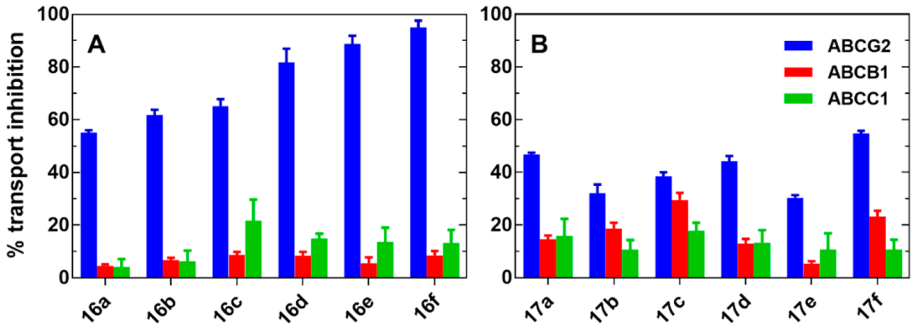

Figure 3. Comparison of ABC transporter mediated efflux inhibition by 10 μM of **16a–f** (A) and **17a–f** (B) on MCF-7/Topo cells (ABCG2; blue), KbV1 cells (ABCB1; red), and MDCKII/MRP1 cell (ABCC1; green); the accumulation of Hoechst 33342 (ABCG2) and calcein (ABCB1 and ABCC1) was measured relative to 10 μM FTC (ABCG2), 10 μM tariquidar (ABCB1), and 30 μM reversan, respectively (100%).

hydrophilic compounds in the ketone series (**17b** and **17c**) showed the lowest maximal inhibitory effect. Interestingly, compound **17f**, which also had a low calculated log P value, showed the highest maximal inhibition in the Hoechst 33342 accumulation assay. This may emphasize the importance of a methyl group at the R^1 position in the central phenyl core.

In summary, the results of the Hoechst assay illustrated that the synthesized chalcones are more potent and efficacious in ABCG2 modulation than the ketones. Additionally, similar potencies were observed for chalcone **16d** (IC_{50} 0.880 μM) and the previously reported ABCG2 modulators **2** (IC_{50} 0.75 μM), **4** (IC_{50} 1.4 μM), **5** (IC_{50} 0.94 μM), and **6** (IC_{50} 0.501 μM) described in Figure 1.

Compound modulatory activities for ABCB1 and ABCC1 were screened in a calcein accumulation assay, using tariquidar (10 μM) and reversan (30 μM) as reference compounds. Tariquidar has been reported as a potent and specific inhibitor of ABCB1 (P-gp); therefore, it is commonly used as a reference compound for this transporter inhibition assay.[25,26] Reversan is a potent ABCC1 modulator.[27−29] Screening was conducted at a fixed concentration of 1 μM and 10 μM using Kb-V1 (ABCB1) and MDCKII (ABCC1) cell lines (Figure 3). Concentration−response curves were not constructed, as none of the compounds showed more than 25% inhibition of calcein-AM efflux (ABCB1 and ABCC1) at 10 μM. The results from the calcein accumulation assay suggested that the chalcones (**16a–f**) and ketones (**17a–f**) had a low inhibitory activity against ABCB1 and ABCC1 (values below 25%, Figure 3A, red and green bar respectively).

To evaluate the chemical stability, all synthesized chalcones and ketones were incubated in assay medium (DMEM supplemented with 10% fetal calf serum) at 37 °C. Aliquots were then analyzed by HPLC over a period of 24 h. Products of cleavage and degradation of compounds were not detected (see the Supporting Information, pp 62−63), indicating that all compounds were not affected by the components present in the assay medium.

Finally, the effect of the modulators **16d** and **17f** on the proliferation of ABCG2-overexpressing MCF-7/Topo cells was investigated by means of a kinetic chemosensitivity assay (Figure 4).[30] The cytostatic drug vinblastine was used as a positive control.

Compound **16d** showed a concentration dependent cytostatic effect on proliferating MCF-7/Topo cells, which may be due to the reactive α−β-unsaturated ketone core.[31] In accordance with the working hypothesis, cytotoxicity was

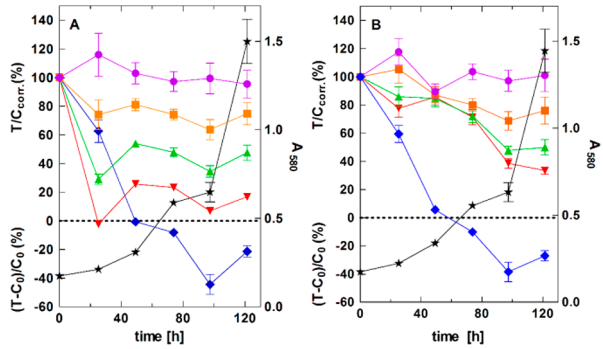

Figure 4. Concentration dependent effect of **16d** (**UR-DP48**) (A) and **17f** (B) on proliferating MCF-7/Topo cells upon long-term exposure; 100 nM (purple circles); 1 μM (orange squares); 3 μM (green triangles); 10 μM (red inverted triangles); positive control: 100 nM vinblastine (blue diamonds); growth curve of vehicle (DMSO) treated cells (negative control) (black stars).

reduced in the case of ketone **17f**, which is lacking the Michael acceptor system.

In summary, new types of tariquidar-related chalcone and ketone derivatives were synthesized and investigated for their inhibitory effect and selectivity toward ABCG2. All chalcones were selective for ABCG2 inhibition. Compounds bearing a methyl substituent at the A ring (**16d–f**) were most active and potent (86%, 97%, and 111%, respectively). Compound **16d** showed similar potency to the reference compound (FTC) with an IC_{50} value of 0.88 μM in the Hoechst assay and a maximal inhibitory effect of 86%. The compounds of the ketone series showed none or only very small preference for ABCG2 over ABCB1 and ABCC1. Compounds **17d–f** surpassed the maximal inhibitory effect of tariquidar although requiring higher concentrations. The difference in ABCG2 modulation and selectivity when comparing the two series of compounds indicated that the lipophilicity plays a critical role. The chemosensitivity assay demonstrated antiproliferative activity of the synthesized chalcones, whereas cytotoxicity was less pronounced in the ketone series. With further optimization, chalcone **16d**, displaying pronounced ABCG2 modulating potential with a submicromolar IC_{50} value, is a promising lead to aid in future investigations of ABCG2 to further understand its role in cancer drug resistance.

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■ ASSOCIATED CONTENT

Supporting Information

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Chemical synthesis, characterization of target compounds; and protocols of biological assays (PDF)

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Notes

The authors declare no competing financial interest.
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■ ABBREVIATIONS

ABCB1,ATP-binding cassette transporter, subfamily B, member 1; ABCC1,ATP-binding cassette transporter, subfamily C, member 1; ABCG2,ATP-binding cassette transporter, subfamily G, member 2; BCRP,breast cancer resistance protein (= ABCG2); IC_{50},concentration of inhibitor required to give 50% inhibition of activity; MDR,multidrug resistance; MRP1,multidrug resistance associated protein 1 (= ABCC1); p-gp,pglycoprotein (= ABCB1); SEM,standard error of the mean.

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