Inhibitors of cancer cell multidrug resistance mediated by breast cancer resistance protein (BCRP/ABCG2)

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Breast cancer resistance protein (BCRP/ABCG2) belongs to the ATP-binding cassette (ABC) transporter superfamily. It is able to efflux a broad range of anti-cancer drugs through the cellular membrane, thus limiting their anti-proliferative effects. Due to its relatively recent discovery in 1998, and in contrast to the other ABC transporters P-glycoprotein (MDR1/ABCB1) and multidrug resistance-associated protein (MRP1/ABCC1), only a few BCRP inhibitors have been reported. This review summarizes the known classes of inhibitors that are either specific for BCRP or also inhibit the other multidrug resistance ABC transporters. Information is presented on structure–activity relationship aspects and how modulators may interact with BCRP. *Anti-Cancer Drugs* 17:239–243

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Introduction

The transporter ABCG2 was simultaneously discovered by three groups, and respectively named breast cancer resistance protein (BCRP [1]), ABC in placenta (ABCP [2]) and mitoxantrone-resistance protein (MXR [3]). This multidrug ATP-binding cassette (ABC) half-transporter has been studied in terms of both its common features with P-glycoprotein (P-gp) and multidrug-resistance protein (MRP)1, and its specific properties (for reviews, see [4,5]). The pattern of transported substrates highly overlaps with P-gp [6], which is obviously not the case for inhibitors. In addition, the effects of the hotspot mutation R482G/T, which are well known to change the pattern of transported substrates, have not been systematically characterized for the inhibitors. The few known inhibitors of ABCG2 may be classified in four categories: ABCG2-specific inhibitors (Fig. 1a), compounds also inhibiting P-gp and/or MRP1 (Fig. 1b), flavonoids and derivatives (Fig. 1c), and inhibitors of other targets that turned to be modulators and/or substrates of ABCG2 [tyrosine kinase inhibitors (TKIs) and HIV protease inhibitors) (Fig. 1d).

ABCG2-specific inhibitors

A few compounds behave as specific inhibitors towards ABCG2, (Fig. 1a), which means that they have not been reported to inhibit other ABC multidrug transporters. Fumitremorgin C (FTC), a mycotoxin from *Aspergillus fumigatus*, was reported first [7]. It chemosensitized S1-M1-3.2 and MCF-7/BCRP cells to mitoxantrone, doxorubicin and topotecan, promoted the accumulation of radioactive doxorubicin, and also efficiently inhibited ($IC_{50} = 1.3\,\mu mol/l$) the ATPase activity of insect cell membranes enriched with recombinant R482G mutant ABCG2 [8]. Its neurotoxicity, however, characterized by tremors and tetanic convulsions in mice, prevented any use in in-vivo experiments. A derivative with an open ring C, tryprostatin A, was obtained as a fungal diketopiperazine secondary metabolite. It reverted mitoxantrone resistance in a series of BRCP-overexpressing cells (gastric carcinoma EPG85-257RNOV, MCF-7/AdrVp and MCF-7/BCRP transfected clone 8) at concentrations of 10–50 μmol/l [9], which made it 10-fold less efficient than GF120918 – one of the most potent inhibitors (see next section).

Parallel solid-phase synthesis allowed the preparation of a library of less-toxic compounds as mixtures of 42 diastereoisomers of indolyl diketopiperazines differently substituted at C-3 and C-6 [10]. Two lead compounds, named Ko132 and Ko134, with a modified ring E and isobutyryl instead of 2,2-dimethylethylene at C-3, appeared 2- to 3-fold more efficient than FTC and similar to GF120918, with low cytotoxicity on mouse

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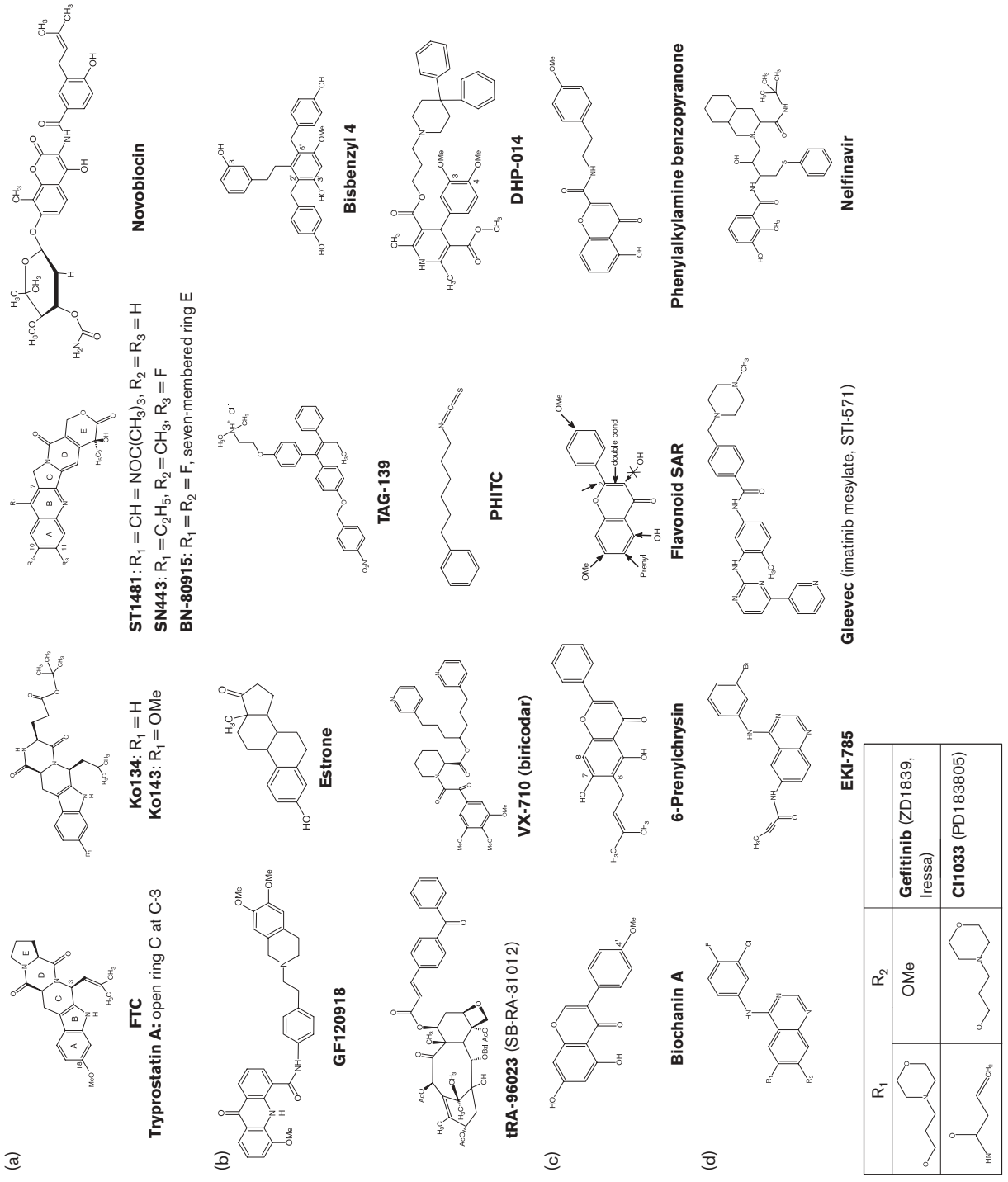
Fig. 1



Main ABCG2 inhibitors classified into four categories: (a) ABCG2-specific inhibitors, (b) inhibitors already known to inhibit P-gp and MRP1, (c) flavonoids and derivatives, and (d) TKIs and HIV protease inhibitors.

embryo fibroblast T6400 and human ovarian carcinoma T8 lines selected on topotecan. In contrast, the C-18 demethoxy-FTC was much less active. Therefore, Ko143 was obtained from Ko134 by adding a methoxy at C-18, which increased its potency 4-fold, encompassing 2- to 3-fold that of GF120918 [11]. Very low concentrations of 5–10 nmol/l were recently reported to inhibit both ATPase activity of enriched insect cell membranes and Hoechst 33342 cell efflux, and to abolish resistance to mitoxantrone [12]. Its low cytotoxicity, with an IC_{50} 100-

to 1000-fold higher than for ABCG2 inhibition, made it promising for in-vivo assays. Indeed, Ko143 was not toxic in mice at 10–50 mg/kg oral doses; it inhibited gastrointestinal tract ABCG2 and increased the bioavailability of orally administered topotecan better than GF120918 [11].

Since camptothecins are substrates, it is not surprising that some of their derivatives are inhibitors. For example, ST1481, a 7-t-butoxyiminomethyl derivative, was not transported in mitoxantrone-resistant HT29 human colon carcinoma and displayed in-vivo activity through inhibition of tumor volume in xenografted mice [13]. Using PC-6/SN2-5H cells, a series of 14 new camptothecin analogs differently substituted at C-10 and C-11 showed that hydrophilic substituents produced transported substrates, whereas hydrophobic substituents produced good non-transported inhibitors, such as SN443 with methyl and fluoride groups [14]. Similar effects were produced by homocamptothecins, containing a seven-membered ring E [15]; interestingly, the best compound, BN-80915, was better transported by mutant (R482G/T) than wild-type (R482) ABCG2. Finally, novobiocin, a coumerycin derivative known as a prokaryotic enzyme gyrase inhibitor, was found to produce low-affinity effects, in the range 50–100 μmol/l: reversal of resistance to mitoxantrone, topotecan and SN-38 in various cell lines, and competitive inhibition of topotecan efflux [16].

Broad-spectrum MDR inhibitors

These inhibitors (Fig. 1b) were previously found to inhibit P-gp, and MRP1 in some cases, and might therefore be considered as multispecific blockers. GF120918 is a high-affinity third-generation inhibitor of P-gp. Its affinity is about 40-fold lower for ABCG2 since a concentration of 10 μmol/l was required to fully inhibit rhodamine-123 efflux from S1-M1-80 cells by flow cytometry, whereas 1 μmol/l induced cell growth sensitization to mitoxantrone and topotecan [17], as well as to a series of other camptothecins in resistant T8 and MX3 cell lines from human ovarian carcinoma IGROV1 [18]. GF120918 cytotoxicity appeared to be highly dependent on cell type, with IC_{50} values ranging from 1 to 30 μmol/l. In-vivo experiments in mice showed that GF120918 was able to increase oral bioavailability and fetal penetration of topotecan [19].

Estrogen agonists and antagonists were found to be low-affinity inhibitors. First, estrone and 17-β-estradiol were shown to chemosensitize K562/BCRP cells to SN-38 and topotecan, and to promote topotecan accumulation at a high concentration of 100 μmol/l [20]. Diethylstilbestrol was then reported as a better inhibitor of topotecan efflux, whereas tamoxifen and toremifene were less efficient. Chemical synthesis of a number of tamoxifen derivatives, however, led to TAG-139 as a relatively strong reverser of mitoxantrone resistance at 2 μmol/l, which made it about 5-fold better than estrone [21]. Very recently, natural stilbenoids from the tubers of the Orchidaceae *Bletilla striata* were found to be rather active, especially bisbenzyl 4 [3,3′-diOH-2′,6′-bis(*p*-OH-benzyl)-5-methoxybibenzyl], which reversed K562/BCRP resistance to SN-38 at 3 μmol/l [22].

Synthetic taxane derivatives, such as ortataxel (BAY59-8862, IDN-5109) and non-toxic tRA-96023 (SB-RA-31012), appeared to be less potent than FTC to inhibit mitoxantrone efflux from 8226/MR20 cells. Interestingly, however, they were able to inhibit only wild-type (R482) ABCG2, but not the mutant (R482T) transporter [23], as this was also the case with the third-generation P-gp inhibitor VX-710 (biricodar, Incel) [24]. Organic isothiocyanates are dietary compounds known as weak inhibitors of both P-gp and MRP1. They have been found to increase mitoxantrone accumulation at 10–30 μmol/l and to sensitize cell growth to mitoxantrone, with 6-phenyl isothiocyanate (PHITC) being the most efficient (IC_{50} around 5 μmol/l) [25]. Very recently, various calcium channel blockers have been investigated. Dipyridamole, nicardipine, nitrendipine and nimodipine inhibited mitoxantrone efflux, whereas verapamil did not produce any effect [26]. The best inhibitor was nicardipine with an IC_{50} around 5 μmol/l; dipyridamole was transported in an FTC-sensitive manner, whereas nitrendipine was not. Analysis of a series of dihydropyridine and pyridine derivatives showed that nearly all compounds were efficient, except for a carboxylate-charged one and for commercially available nifedipine [27]. Some compounds appeared better than FTC at 2.5 μmol/l for mitoxantrone accumulation and chemosensitization, the most efficient one being DHP-014, a 3,4-dimethoxy dihydropyridine derivative of niguldipine, possibly by competition towards mitoxantrone. The same preference for nicardipine over nifedipine as in P-gp might suggest a similar interaction mechanism. In-vivo experiments in rats showed that co-administration of DHP-014 with topotecan increased systemic exposure and peak concentration of orally administered topotecan. In contrast, no definitive conclusion could be drawn for cyclosporin A, for which contradictory results have been reported. A recent study concluded that cyclosporin A was neither an inhibitor nor a substrate for ABCG2 in MCF-7 drug-selected or HeLa-transfected cells, and did not inhibit ATPase activity of wild-type ABCG2-enriched membranes [28]. Cyclosporin A was considered, however, as a broad-spectrum inhibitor on the basis of its 3-fold induced increase in mitoxantrone accumulation and cell growth sensitization [29], and was reported as a potent ATPase inhibitor of recombinant R482T mutant ABCG2 in insect cell membranes [8].

Flavonoids and derivatives

Flavonoids (Fig. 1c) are also known inhibitors of P-gp and MRP1, but the structure–activity relationships are clearly different for the three types of multidrug ABC transpor-

ters. A first interaction of ABCG2 with various classes of plant polyphenols was reported at a high concentration of 30 μmol/l [30]. More extensive studies with drug-selected human MCF-7/MX100 and H460MX20 cells indicated that the two most efficient compounds, chrysin and biochanin A, were able to promote mitoxantrone accumulation at 10–50 μmol/l and reversal of resistance to mitoxantrone at 2.5 μmol/l [31]. Surprisingly, different classes of flavonoids, such as flavones (acacetin), flavonols (kaempferol), isoflavones (genistein), flavanones (naringenin) and some glycosylated derivatives, were all found to revert resistance to SN-38 in ABCG2-transfected cells, while genistein and naringenin increased topotecan accumulation, and genistein was itself transported [32]. We have established structure–activity relationships of inhibitors of mitoxantrone efflux in ABCG2-transfected HEK-293 cells. A first comparison between 3-OH containing flavonoids indicated the following efficiency: flavones (apigenin) > flavonols (galangin) > isoflavones (genistein) > flavanones (naringenin) [33]. Analysis of a number of differently substituted flavones led to the following substituent effects: 6-prenyl > 6-1,1-dimethylallyl/8-prenyl > 6,8-digeranyl > 7-methoxy > 7-OH. In addition, the importance of hydrophobic substitution at position 4′ was shown by the much higher efficiency of biochanin A over genistein [34]. The best compound, 6-prenylchrysin, exhibited an IC_{50} value of 0.3 μmol/l, similar to GF120918 [33]. Interestingly, the R482T hotspot mutation altered the impact of prenylation on the inhibitory potency; tectochrysin being the best compound with an IC_{50} of 1.9 μmol/l. In addition, both chrysin derivatives were specific for ABCG2, due to minimal interaction with P-gp and MRP1. Their relatively low cytotoxicity and efficient sensitization of cell growth to mitoxantrone made these compounds promising for future potential use in clinical trials. A very recent study with a series of benzopyranones (chromones) on human carcinoma HCT116/BCRP cells identified phenylalkylamine derivatives as the most efficient at 10 μmol/l, with higher inhibition than FTC at low concentration [35].

Other types of inhibitors

Some inhibitors (Fig. 1d) of various cellular targets have been found to be substrates or inhibitors of multidrug ABC transporters such as ABCG2, leading to cellular resistance against treatments using these inhibitors. Recent examples have been illustrated with TKIs and HIV protease inhibitors. Up to four TKIs have been reported to interact with ABCG2 (for a recent review, see [36]). CI1033 (PD183805), which inhibits HER tyrosine kinase, was first reported to promote SN-38 accumulation in both T98G glioblastoma and HCT8 colorectal carcinoma cell lines [37]. Iressa (gefitinib, ZD1839) specifically inhibits epidermal growth factor receptor and is clinically active on non-small cell lung cancer. In human K562/BCRP and colon HT-29 lines, as well as P388/BCRP, Iressa inhibited the transport of estrone 3-sulfate, with a submicromolar IC_{50}, and increased survival of mice with xenografted tumors [38], as well as oral bioavailability of irinotecan and SN-38 in normal mice [39]. Submicromolar concentrations of Iressa inhibited both Hoechst 33342 and topotecan efflux, and sensitized cell growth to mitoxantrone, camptothecins and doxorubicin [12,40]. Iressa stimulation of ABCG2 ATPase activity together with ABCG2 prevention of Iressa effects strongly suggest that Iressa is indeed pumped out by ABCG2 [41]. Iressa transport/binding appears to be dependent on natural ABCG2 polymorphisms such as Q141K [36]. EKI-785, an irreversible inhibitor, was found to behave similarly to Iressa as a high-affinity transported and inhibitory compound towards ABCG2 [12]. Gleevec (imatinib mesylate, STI-571) inhibits specifically Bcr–Abl (a fusion product of Philadelphia chromosome), activated c-Kit kinases and platelet-derived growth factor receptor tyrosine kinase, and is clinically used for treatment of myelogenous leukemia and gastrointestinal stromal tumors. In human osteosarcoma Saos2 ABCG2#4 cells, Gleevec potently reversed resistance to camptothecins (IC_{50} = 0.17 μmol/l) and increased topotecan accumulation [42]. In contrast to the initial conclusion that Gleevec was not transported, resistance was indeed observed and assumed to be due to Gleevec transport, competitive to mitoxantrone and sensitive to Ko143 [43].

Finally, HIV protease inhibitors, previously shown to interact with P-gp and MRP1, have been reported as weak ligands of ABCG2. In transfected HEK-293 cells, ritonavir, sequinavir and, especially, nelfinavir inhibited mitoxantrone efflux, with IC_{50} values in the range 12–20 μmol/l, whereas indinavir and amprenavir were inefficient [44]. The interaction was altered 2-fold by the R482T/G hotspot mutations.

Conclusion

Several interesting ABCG2 inhibitors have been put forward. Some are specific and highly potent, such as Ko143, which inhibits not only drug transport, but also ATPase activity. Others may be considered as polyspecific inhibitors, such as GF120918, which inhibits P-gp even better. Flavonoids such as hydrophobic derivatives of chrysin constitute a promising family of natural compounds, especially since they are present in the diet and in nutritive supplements. TKIs are high-affinity ligands that behave as both inhibitors and transported substrates, suggesting that ABCG2 plays an important role, much more than P-gp, in clinical resistance to anticancer treatments using TKIs. The mechanism of interaction of these inhibitors is, however, essentially unknown. Purification of the transporter in sufficient amounts would allow us to measure and characterize the direct binding of these inhibitors, and to initiate structural investigations and characterization of confor-

mational changes associated with drug binding and transport or, on the contrary, with their inhibition.

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