Breast cancer resistance protein (BCRP, ABCG2) is a recently identified member of the ATP-binding cassette family of cell surface transport proteins. This study was conducted to investigate the effect of a series of newly synthesized 1,4-dihydropyridines and pyridines, designed as potent P-glycoprotein inhibitors, on BCRP-mediated drug efflux both in vitro and in vivo. The effects of 25 synthesized dihydropyridines and corresponding pyridines along with 4 commercially available dihydropyridines (niguldipine, nicardipine, nifedipine, and nitrendipine) on the intracellular accumulation of the BCRP substrate mitoxantrone were evaluated in BCRP-expressing human breast cancer MCF-7/MX100 and human non-small cell lung cancer H460/MX20 cells. At a 2.5 μM concentration, 24 of 25 newly synthesized dihydropyridines and pyridines produced a significant increase of mitoxantrone accumulation in both cell lines. The most potent compound was able to enhance mitoxantrone accumulation approximately 4.5-fold, greater than that obtained with 10 μM fumitremorgin C, which is a specific BCRP inhibitor. The results from the two cell lines showed good correlation (r² = 0.71, p < 0.01). Niguldipine, nicardipine, and nitrendipine also demonstrated potent BCRP inhibition, whereas nifedipine had no effect. The effects of the dihydropyridine and pyridine compounds on mitoxantrone cytotoxicity paralleled their effects on mitoxantrone accumulation. Coadministration of a selected dihydropyridine compound, Im [DHP-014; 3-(3-(4,4-diphenylpiperidin-1-yl)propyl) 5-methyl 4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate] with toposetan, a good BCRP substrate and a moderate to poor P-glycoprotein substrate, resulted in significant increases in the systemic exposure and peak concentration of toposetan in Sprague-Dawley rats when oral toposetan (2 mg/kg) was combined with 20 mg/kg DHP-014. The observed increase of toposetan exposure provides proof-of-concept for in vivo inhibition of BCRP by these agents.
toxantrone. DHP-014 (compound I_m), a new dihydropyridine compound shown to be a potent BCRP and P-glycoprotein inhibitor in vitro, was selected to investigate its effect on topotecan pharmacokinetics in Sprague-Dawley rats. Topotecan is a good BCRP substrate (Jonker et al., 2000), as well as a moderate to poor P-glycoprotein substrate (Chen et al., 1991). Pronounced changes of topotecan bioavailability due to BCRP inhibition have been reported in mice (Jonker et al., 2000), humans (Kruijtzer et al., 2002), and rats (Zhang et al., 2005). Topotecan undergoes very limited metabolism in rats (Platzer et al., 1998) and humans (Rosing et al., 1997), making it a suitable model BCRP substrate for the investigation of BCRP-mediated drug-drug interactions in vivo, since interactions between inhibitors and metabolizing enzymes will not confound the results.

Materials and Methods

Compounds and Cell Lines. Mitoxantrone, nicardipine, nifedipine, niguldipine, and nitrendipine were purchased from Sigma (St. Louis, MO). Compounds I _a–I _m and II _a–II _m were synthesized in Dr. Robert Coburn’s laboratory, Department of Chemistry, University at Buffalo (Zhou et al., 2005). The chemical structures of these test compounds are shown in Fig. 1. Series I comprises 1,4-dihydropyridines and Series II comprises corresponding aromatized pyridines. Human breast cancer MCF-7-sensitive and MCF-7/MX100 human large cell lung carcinoma NCI-H460 and NCI-H460/MX20, and fumi-

<table>
<thead>
<tr>
<th>Series I Compound</th>
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<td>I _a</td>
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<td>I _o</td>
<td>3,4-OCH₂O-</td>
<td>II _o</td>
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Fig. 1. Chemical structures of dihydropyridines and pyridines.
tremorgin C were kind gifts from Dr. Susan E. Bates (National Cancer Institute, Bethesda, MD). Topotecan was purchased from ChemPacific (Baltimore, MD). Injectable anesthetics and suture materials were obtained from Henry Schein Inc. (Melville, NY). Acridine was purchased from Sigma.

**Cell Culture.** Parental human breast cancer cell MCF/ls and human non-small cell lung cancer cell H460/ls were grown in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 5% heat-inactivated fetal bovine serum (Invitrogen). Resistant MCF-7/MX100 cells and H460/MX20 were cultured in the above-mentioned medium with addition of 100 nM and 20 nM mitoxantrone, respectively. All cells were incubated at 37°C in 5% CO₂/95% air.

**Animals.** Female Sprague-Dawley rats (220–260 g in body weight) were obtained from Harlan (Indianapolis, IN) and housed according to institutional guidelines. The rats were kept in a temperature-controlled environment with a 12-h light/dark cycle and given a standard diet with water ad libitum. Rats were fasted overnight before oral administration of drug. The animal protocol was approved by the Institutional Animal Use and Care Committee at the University at Buffalo.

**Western Blotting Assay.** Cells grown in 100 × 15 mm culture dishes were washed with phosphate-buffered saline and harvested using a cell scrapper. Total cell lysates were prepared by adding a lysis buffer (20 mM Tris, pH 7.5, 120 mM sodium chloride, 100 mM sodium fluoride, 1% octylphenoxypolyethoxy ethanol, 200 μM sodium orthovanadate, 50 mM β-glycerophosphate, 10 mM sodium pyrophosphate, 4 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine, 10 μg/ml leupeptin, and 10 μg/ml aprotonin) to the harvested cells and keeping on an ice bath for 30 min. Soluble extracts were obtained by centrifuging cell lysates at 13,000g for 20 min. The protein concentration of the supernatant was determined by the Bradford method (Bradford, 1976). Lysates were subjected to electrophoresis on 7.5% SDS-polyacrylamide gels and electroblotted onto nitrocellulose membranes (Invitrogen). Membranes were then blocked overnight at 4°C in Tris-buffered saline containing 0.2% (v/v) Tween 20 and 5% (w/v) fat-free dry milk (Bio-Rad, Hercules, CA) with a 1:750 dilution of the BXP-21 monoclonal antibody against BCRP (Maliepaard et al., 2001a). The blots were then incubated at room temperature for 1 h with a horseradish peroxidase-conjugated sheep anti-mouse IgG secondary antibody (Amersham Biosciences, Inc., Piscataway, NJ), followed by enhanced chemiluminescence (ECL) detection (Amersham Biosciences, Inc.).

**Mitoxantrone Cellular Accumulation Studies.** Mitoxantrone cellular accumulation studies with analysis by flow cytometry followed the protocols described by Minderman et al. (2002) and Zhang et al. (2004). Briefly, cells were incubated at a density of 1 × 10⁴ cells/ml for 30 min at 37° C in RPMI 1640 medium with 3 μM mitoxantrone. At the end of the 30-min incubation period, cells were washed once with ice-cold phosphate-buffered saline and then resuspended in ice-cold phosphate-buffered saline. An aliquot of cells was kept on ice until flow cytometry analysis for drug retention. Samples were analyzed on a FACScan flow cytometer (BD Biosciences, San Jose, CA) equipped with an argon laser for 488-nm excitation and with 530/30-nm band pass filters (FL1), 585/42 band pass (FL2), and 670 nm long pass (FL3) filters for emission collection. All flow-cytometric data were analyzed with the WinList software program (Verity Software House, Topsham, ME).

**Mitoxantrone Cytotoxicity Assay.** One hundred microliters of MCF-7/MX100 cells were seeded in 96-well plates at a density of 4000 cells per well. The cells were allowed to attach for 24 h at 37°C, following which, an additional 100 μl of medium was added to each well containing the desired final concentration of mitoxantrone (0.1 μM to 1000 μM) with or without the modulator. After a 6-h exposure to the drug, the cells were washed twice with sterile 1× phosphate-buffered saline, and fresh medium was added to each well. The cells were allowed to grow for 4 more days. After 4 days, the total protein was measured by a sulforhodamine B staining assay (Skehan et al., 1990). Briefly, 10% trichloroacetic acid was added to the cells for an hour on ice to fix cellular protein to the wells, rinsed five times with water, and allowed to air dry. Sulforhodamine B (0.4% w/v in 1% glacial acetic acid) was added to each well for 15 min and washed four times with 1% acetic acid. After drying the plates, protein-bound dye was solubilized in 10 nM Tris base and quantitated by measuring the absorbance at 570 nm.

**Mitoxantrone Interaction Study in Rats.** Topotecan was freshly prepared in 5% d-glucose for oral dosing. Three groups of four animals each were given 2 mg/kg topotecan orally, alone or coadministered with DHP-014 (10 mg/kg or 20 mg/kg) by intraperitoneal injection. Blood samples (150 μl) taken from the jugular vein cannula were collected in heparinized tubes prior to drug administration and at 2, 7, 15, 30, 60, 120, 240, 480, and 720 min after dosing. Plasma samples were obtained by centrifugation at 1000g for 10 min and analyzed by HPLC (Chen and Balthasar, 2002; Zhang et al., 2005). The HPLC assay utilized a mobile phase of methanol/10 mM potassium phosphate (KH₂PO₄) (25:75, v/v) containing 2% triethylamine with a pH of 3.72 and a flow rate of 1.0 ml/min. For sample preparation, 40 μl of plasma was mixed with 4 μl of acridine (1.5 μg/ml as the internal standard), 120 μl of methanol, and 40 μl of 100 mM phosphoric acid. The mixture was then centrifuged at 2000g for 8 min. A clear supernatant was collected, and 100 μl of the sample solution was injected into the HPLC system. Topotecan was detected with excitation and emission wavelengths set at 361 and 527 nm, respectively. The lower limit of quantitation of this method was 0.02 ng. Standard curves are linear over the concentration range of 1 to 500 ng/ml. The intraand interday variabilities were 9.48% and 14.2%, respectively.

**Data Analysis.** For mitoxantrone accumulation studies, mitoxantrone fluorescence in modulator-treated cells and vehicle (0.1% DMSO)-treated cells were compared. Statistical significance was determined using a one-way analysis of variance followed by Dunnett’s post hoc test. Differences were considered to be significant when p < 0.05. In the concentration-dependent study, the EC₅₀ values of compounds for increasing mitoxantrone accumulation in MCF-7/MX100 and H460/MX20 cells were obtained by fitting the fraction of maximal increase (F) by eq. 1 using the computer program WinNonlin (Pharsight, Mountain View, CA).

\[ F = \frac{C}{EC_{50} + C} \]  \hspace{1cm} (1)

where C is the concentration of the test compound. \( \gamma \) represents the curve fitting coefficient. F was calculated as the ratio of the net increase of mitoxantrone accumulation in the presence of the test compound (\( A_i - A_0 \)) to the maximal net increase, in this study, represented by the net increase of mitoxantrone accumulation in the presence of 40 μM nicardipine (\( A_{nicardipine} - A_0 \)). \( A_0, A_i, \) and \( A_{nicardipine} \) are the mitoxantrone accumulation in the presence of the vehicle control (0.1% DMSO), the test compound, and 40 μM nicardipine, respectively.

In mitoxantrone cytotoxicity studies, the IC₅₀ values were obtained by fitting the growth inhibition data to an inhibitory sigmoidal model of a Hill Equation (eq. 2 using the computer program WinNonlin (Pharsight, Mountain View, CA).

\[ SF = 1 - \frac{I_{max} \times C}{IC_{50} + C} \]  \hspace{1cm} (2)

SF represents the survival fraction of cells after treatment. \( I_{max} \) is the maximal percentage of inhibition and, in the present study, was fixed as 1. IC₅₀ is the concentration of mitoxantrone that causes 50% inhibition of cell growth. \( \gamma \) represents the curve-fitting coefficient, or Hill coefficient.

The pharmacokinetic parameters of topotecan were obtained by noncompartamental analysis using WinNonlin version 2.1 (Pharsight). The area under the plasma concentration-time curves (AUC) was calculated using the trapezoidal method. Analysis of variance was applied to assess the statistical significance of pharmacokinetic parameters of topotecan among different dosing regimens. Differences were considered to be statistically significant when p < 0.05.

**Results**

**Expression of BCRP in H460/MX20 and MCF-7/MX100 Cells.** Total cell lysate was prepared from H460/wt, H460/MX20, MCF-7/wt, and MCF-7/MX100 cells and subjected to an immunoblotting assay for BCRP expression. The results shown in Fig. 2 demonstrate that BCRP was expressed in the mitoxantrone-resistant H460/MX20 and MCF-7/MX100 cells but was undetectable in the drug-sensitive parental cells when using the same amount of lysate protein per lane. Since dihydropyridines and pyridines are known as P-glycoprotein

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and MRP1 inhibitors, to rule out the possible contributions of these two transporters. Western blotting assays for P-glycoprotein and MRP1 expression were also performed using the same protein samples. No MRP1 or P-glycoprotein protein bands were detected in these cell lines (Zhang et al., 2004).

**Mitoxantrone Retention Studies.** Figures 3 and 4 show the effect of the modulators on mitoxantrone retention in BCRP-overexpressing H460/MX20 and MCF-7/MX100 cells, respectively. To exclude the possibility that the dihydropyridine/pyridine compounds may contribute to the fluorescence intensity, H460/MX20 and MCF-7/MX100 cells were incubated in medium with a 10 μM concentration of each test compound. No test compound-associated fluorescence was detected with the FACScan instrument settings used to detect mitoxantrone (data not shown). The 30-min accumulation of 3 μM mitoxantrone in MCF-7/adr cells was measured in the presence of 2.5 μM synthesized dihydropyridines and pyridines. Fumitremorgin C (10 μM) served as the positive control in these experiments. As shown in Fig. 3, all synthesized dihydropyridine compounds demonstrated a significant increase of intracellular mitoxantrone concentration at p < 0.001 or p < 0.05 except for compound I\(_h\) (no significant difference versus control). All of the corresponding pyridine analogs also significantly increased the intracellular mitoxantrone concentrations in the drug-resistant breast cancer cells.

Similar results were obtained in MCF-7/MX100 cells (Fig. 4). All of the synthesized dihydropyridines except for compound I\(_h\) and all of the pyridine compounds demonstrated a significant increase of intracellular mitoxantrone concentration. None of the synthesized compounds significantly affected mitoxantrone accumulation in the parental MCF-7 and H460 cells (data not shown).

Figure 5 represents the relationship between mitoxantrone accumulation in the two BCRP-overexpressing cell lines after treatment with modulators. A statistically significant correlation for the inhibition of mitoxantrone efflux by the modulators in MCF-7/MX100 and H460/MX20 cells was observed (r\(^2\) = 0.71, p < 0.01).

Four commercially available dihydropyridines, nicardipine, nifedipine, niguldipine, and nitrendipine, along with compounds I\(_m\) and I\(_o\), two compounds selected from our new series of compounds due to their potent effect on modulating mitoxantrone retention in resistant cells, were investigated for their concentration-dependent effects on mitoxantrone accumulation in MCF-7/MX100 and H460/MX20 cells (Fig. 6). Nicardipine, niguldipine, nitrendipine, compound I\(_m\), and compound I\(_o\) elicited concentration-dependent increases in mitoxantrone retention in two resistant cell lines. No significant effect of nifedipine was observed at concentrations ranging from 1 to 20 μM. The maximal accumulation was based on that observed with nicardipine at concentrations of 10, 20, and 40 μM, and was similar to that observed at the 40 μM concentrations of niguldipine, nitrendipine, compound I\(_m\), and compound I\(_o\), although compound I\(_o\) at a 40 μM concentration demonstrated somewhat higher values in H460/MX20 cells. It should be noted that the maximal accumulation values were approximately 2-fold higher than those observed with 10 μM FTC. The estimated EC\(_{50}\) values of nicardipine, niguldipine, nitrendipine, compound I\(_m\), and compound I\(_o\) for increasing mitoxantrone accumulation in MCF-7/MX100 and H460/MX20 cells are summarized in Table 1.

**Mitoxantrone Cytotoxicity Studies.** Nicardipine, niguldipine, nitrendipine, compound I\(_m\), and compound I\(_o\) restored the sensitivity of resistant MCF-7/MX100 cells to mitoxantrone cytotoxicity in a concentration-dependent manner. As shown in Table 2, the IC\(_{50}\) values of mitoxantrone in MCF-7/MX100 were significantly reduced by treatment with compounds I\(_m\) and I\(_o\) at concentrations ranging from 1 to 40 μM. Nicardipine demonstrated the most potent effect on mitoxantrone cytotoxicity compared with the other commercially available dihydropyridines. At 2.5 μM, nicardipine potentiated mitoxantrone cytotoxicity by nearly 2-fold compared with the vehicle control, an
effect comparable to that of the positive control fumitremorgin C at 10 μM. However, nifedipine showed no significant effect on mitoxantrone cytotoxicity in BCRP-overexpressing MCF-7/MX100 cells.

**Coadministration of Topotecan with DHP-014 (Compound Ia) in Rats.** Topotecan was administered to rats (2 mg/kg orally) either alone or with DHP-014 (10 or 20 mg/kg i.p.). DHP-014 was selected for further study based on the following criteria: good inhibitory activity for BCRP, negligible calcium channel binding activity, and predicted low systemic clearance based on quantitative structure-pharmacokinetic relationship analysis (Zhou et al., 2003, 2005). The plasma concentration-time profiles of topotecan are given in Fig. 7 and the pharmacokinetic parameters are summarized in Table 3. The area under the curve (AUC) and peak concentration (C_max) of topotecan were significantly increased (p < 0.05) by coadministration with DHP-014 at a dose of 20 mg/kg, whereas the oral clearance (CL/F) of topotecan was markedly decreased (p < 0.05) compared to topotecan given alone. No statistically significant differences of topotecan terminal half-life (t½) were observed among the three treatments.

**Discussion**

ABC transporters play important roles in the absorption, distribution, and elimination of many substrate drugs, including anticancer drugs, and in the multidrug resistance observed in tumor cells. The development of novel agents to inhibit ABC transporters for clinical application thus represents an active area of research, with P-glycoprotein being the most extensively studied ABC transporter (Lin and Yamazaki, 2003).

BCRP is a recently identified ABC transporter and is highly expressed in the placenta (Maliepaard et al., 2001a), with lesser expression in brain, prostate, small intestine, testis, ovary, colon, and liver (Doyle et al., 1998). Several substances have been reported as BCRP inhibitors: GF120918 (de Bruin et al., 1999), the mycotoxin fumitremorgin C (Rabindran et al., 2000), its derivatives demethoxyfumitremorgin C (van Loevezijn et al., 2001), Ko132, Ko134, and Ko143 (Allen et al., 2002), the HER tyrosine kinase inhibitor CI1033 (Erlichman et al., 2001), experimental camptothecin analogs (Maliepaard et al., 2001b; Perego et al., 2001), and estrogens such as estrone and 17β-estradiol (Imai et al., 2002).

We have previously reported that our new series of dihydropyridines and pyridines were synthesized based on the structural modification of dextriniguldipine (Zhou et al., 2005). Most compounds from this series have been shown to be potent P-glycoprotein inhibitors, with little or no calcium channel blocking activity (Zhou et al., 2005). Moderate effects on MRP1-mediated drug efflux were also observed (unpublished data). In the present study, we have demonstrated that these compounds can effectively increase the intracellular accumulation of mitoxantrone in BCRP-overexpressing mitoxantrone-resistant cell lines. To the best of our knowledge, effects of dihydropyridines on BCRP have not been previously reported. Four commercially available dihydropyridines, nicardipine, nigeludipine, nifedipine, and nitrendipine, were also evaluated for their effects on BCRP-mediated drug transport. All compounds except nifedipine demonstrated a BCRP modulation effect by enhancing intracellular mitoxantrone accumulation and sensitizing resistant cells to mitoxantrone cytotox-
icity in a concentration-dependent manner. In the mitoxantrone accumulation studies in H460/MX20 cells, compound IIb exhibited more than a 4-fold increase of drug accumulation in BCRP-expressing cells at the concentration of 2.5 \( \mu \)M. Compound Ia also showed a nearly 4-fold enhancement of mitoxantrone accumulation at the same concentration. These increases are larger than that observed with the positive control, 10 \( \mu \)M fumitremorgin C. Compounds Ia and IIb were selected for the mitoxantrone cytotoxicity study in MCF-7/MX100

FIG. 6. Concentration-dependent effects on mitoxantrone accumulation in H460/MX20 (■) cells and MCF-7/MX100 (□) cells. The 30-min accumulation of mitoxantrone in MCF-7/MX100 cells and H460/MX20 cells in the presence of various concentrations (1–40 \( \mu \)M) of test compounds (nicardipine, nifedipine, niguldipine, nitrendipine, Ia, and IIb) or the vehicle control (0.1% DMSO) was performed as described under Materials and Methods. FTC was used as a positive control at a concentration of 10 \( \mu \)M. Data are expressed as mean ± S.D. (n = 9). The absolute values of mean mitoxantrone fluorescence intensity in the vehicle (0.1% DMSO)-treated H460/MX20 cells and MCF-7/MX100 cells (controls) were 5.9 ± 0.6 (n = 9) and 4.3 ± 0.5 (n = 9), respectively. *, *p < 0.05; ***, *p < 0.001 compared with the vehicle control.
cells, and they were able to substantially reduce the IC₅₀ values of mitoxantrone in a concentration-dependent manner. Since H460/MX20 and MCF-7/MX100 cells do not express P-glycoprotein or MRP1, and the dihydropyridines/pyridines do not affect transport of mitoxantrone in the parental cell line, our results suggest that the effects of the dihydropyridines/pyridines are mediated through BCRP.

Our in vivo studies with topotecan confirmed the results of the in vitro studies. Topotecan is a BCRP substrate that also demonstrates moderate to weak affinity for P-glycoprotein. Moderate to large systemic exposure of oral topotecan approximately 7-fold in mdr1a/1b(-/-) knockout and wild-type mice when topotecan was administered intravenously in combination with intravenous topotecan in mdr1a/1b(-/-) mice decreased the plasma clearance and hepatobiliary excretion of topotecan (Jonker et al., 2000). Recently, Zhang et al. (2005) demonstrated for the first time that the dihydropyridine derivative dexniguldipine can inhibit P-glycoprotein-mediated drug extrusion through a direct interaction with P-glycoprotein (Borchers et al., 2002). Dihydropyridines and pyridines may act on BCRP in a similar manner because these compounds have a planar, multiring structure, like mitoxantrone; thus, they may compete with mitoxantrone for binding sites on BCRP. Pascaud et al. (1998) characterized the modulation of P-glycoprotein ATPase activity by five dihydropyridines: nicardipine, nimodipine, nitrendipine, nifedipine, and azidipine. They reported that P-glycoprotein ATPase was activated by approximately 2-fold in the presence of 3 to 4 μM nicardipine. Nifedipine had no effect on P-glycoprotein ATPase. In the present study, nicardipine was the most potent BCRP inhibitor among the four commercially available dihydropyridines, whereas nifedipine showed no inhibitory effect on BCRP-mediated mitoxantrone efflux. It is possible that nicardipine and other dihydropyridines may interact with BCRP by a similar mechanism, i.e., activation of BCRP ATPase activity.

The extrapolation of drug-transporter interactions from animals to humans should be done cautiously due to potential species differences in the expression and activity of these transporters. Whereas Jonker et al. (2000) have demonstrated a substantial increase in AUC and a decreased plasma clearance in mdr1a/1b(-/-) knockout and wild-type mice when topotecan was administered intravenously in combination with oral GF120918, the effect was less pronounced in humans than in mice (Kruijtzer et al., 2002). One possible explanation for this difference is that the expression of BCRP in kidneys is higher in rats than in mice (Kruijtzer et al., 2002). We speculate that the observed increase of the systemic exposure levels of topotecan is due to inhibition of BCRP in rat small intestine, increasing the amount of topotecan entering the circulation. Inhibition of topotecan metabolism by DHP-014 is unlikely to contribute significantly to the increased systemic availability of topotecan because topotecan metabolism in rat liver is low (Platzer et al., 1998).

P-glycoprotein inhibition by DHP-014 will also contribute to the enhanced topotecan systemic exposure observed in this investigation. P-glycoprotein is expressed in the apical membrane of intestinal epithelial cells and is associated with a decreased bioavailability for several drugs (Lin and Yamazaki, 2003). DHP-014 is a potent P-glycoprotein inhibitor, capable of substantially enhancing the intracellular accumulation of vinblastine in P-glycoprotein-overexpressing human breast cancer MCF-7/adr cells (Zhou et al., 2005). However, topotecan is a moderate to weak P-glycoprotein substrate (Chen et al., 1991); therefore, the effect of P-glycoprotein inhibition on the bioavailability of topotecan is likely to be less important than that of BCRP inhibition.

The mechanism underlying the interaction of dihydropyridines and pyridines with BCRP has not been elucidated. It has been reported that the dihydropyridine derivative dexniguldipine can inhibit P-glycoprotein-mediated drug extrusion through a direct interaction with P-glycoprotein (Borchers et al., 2002). Dihydropyridines and pyridines may act on BCRP in a similar manner because these compounds have a planar, multiring structure, like mitoxantrone; thus, they may compete with mitoxantrone for binding sites on BCRP. Pascaud et al. (1998) characterized the modulation of P-glycoprotein ATPase activity by five dihydropyridines: nicardipine, nimodipine, nitrendipine, nifedipine, and azidipine. They reported that P-glycoprotein ATPase was activated by approximately 2-fold in the presence of 3 to 4 μM nicardipine. Nifedipine had no effect on P-glycoprotein ATPase. In the present study, nicardipine was the most potent BCRP inhibitor among the four commercially available dihydropyridines, whereas nifedipine showed no inhibitory effect on BCRP-mediated mitoxantrone efflux. It is possible that nicardipine and other dihydropyridines may interact with BCRP by a similar mechanism, i.e., activation of BCRP ATPase activity.

The extrapolation of drug-transporter interactions from animals to humans should be done cautiously due to potential species differences in the expression and activity of these transporters. Whereas Jonker et al. (2000) have demonstrated a substantial increase in AUC and a decreased plasma clearance in mdr1a/1b(-/-) knockout and wild-type mice when topotecan was administered intravenously in combination with oral GF120918, the effect was less pronounced in humans than in mice (Kruijtzer et al., 2002). One possible explanation for this difference is that the expression of BCRP in kidneys is higher in rats than in mice (Kruijtzer et al., 2002). We speculate that the observed increase of the systemic exposure levels of topotecan is due to inhibition of BCRP in rat small intestine, increasing the amount of topotecan entering the circulation. Inhibition of topotecan metabolism by DHP-014 is unlikely to contribute significantly to the increased systemic availability of topotecan because topotecan metabolism in rat liver is low (Platzer et al., 1998).

P-glycoprotein inhibition by DHP-014 will also contribute to the enhanced topotecan systemic exposure observed in this investigation. P-glycoprotein is expressed in the apical membrane of intestinal epithelial cells and is associated with a decreased bioavailability for several drugs (Lin and Yamazaki, 2003). DHP-014 is a potent P-glycoprotein inhibitor, capable of substantially enhancing the intracellular accumulation of vinblastine in P-glycoprotein-overexpressing human breast cancer MCF-7/adr cells (Zhou et al., 2005). However, topotecan is a moderate to weak P-glycoprotein substrate (Chen et al., 1991); therefore, the effect of P-glycoprotein inhibition on the bioavailability of topotecan is likely to be less important than that of BCRP inhibition.

The mechanism underlying the interaction of dihydropyridines and pyridines with BCRP has not been elucidated. It has been reported that the dihydropyridine derivative dexniguldipine can inhibit P-glycoprotein-mediated drug extrusion through a direct interaction with P-glycoprotein (Borchers et al., 2002). Dihydropyridines and pyridines may act on BCRP in a similar manner because these compounds have a planar, multiring structure, like mitoxantrone; thus, they may compete with mitoxantrone for binding sites on BCRP. Pascaud et al. (1998) characterized the modulation of P-glycoprotein ATPase activity by five dihydropyridines: nicardipine, nimodipine, nitrendipine, nifedipine, and azidipine. They reported that P-glycoprotein ATPase was activated by approximately 2-fold in the presence of 3 to 4 μM nicardipine. Nifedipine had no effect on P-glycoprotein ATPase. In the present study, nicardipine was the most potent BCRP inhibitor among the four commercially available dihydropyridines, whereas nifedipine showed no inhibitory effect on BCRP-mediated mitoxantrone efflux. It is possible that nicardipine and other dihydropyridines may interact with BCRP by a similar mechanism, i.e., activation of BCRP ATPase activity.
Institute, Bethesda, MD) for providing MCF-7 and H460 cell lines. All these compounds were synthesized based on the structure optimization of dexniguldipine to maximize P-glycoprotein binding. The plasma concentration-time profile of topotecan after an oral dose of 2 mg/kg in Sprague-Dawley rats alone (1) or in combination with 10 mg/kg DHP-014 (2) or 20 mg/kg DHP-014 (3), administered min before topotecan, was determined by HPLC as described under Materials and Methods. Data are expressed as mean ± S.D. For all three treatment groups, n = 4 animals.

TABLE 3
Pharmacokinetic parameters of topotecan in rat plasma after oral administration alone (2 mg/kg) or in combination with DHP-014

<table>
<thead>
<tr>
<th>Treatment</th>
<th>With DHP-014</th>
<th>Without DHP-014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topotecan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (ng·min/mL)</td>
<td>48.619 ± 14.908*</td>
<td>25.995 ± 6.316</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>160.4 ± 34.5</td>
<td>93.3 ± 10.3</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>130 ± 4.8</td>
<td>139 ± 3.5</td>
</tr>
<tr>
<td>Clearance/F</td>
<td>41 ± 12.6*</td>
<td>76.5 ± 20.6</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with the treatment group that was given 2 mg/kg topotecan alone.

and mice than in humans (Tanaka et al., 2004), although other possibilities, such as differences in topotecan/GFI20918 affinity for human versus murine BCRP, may also be important.

In summary, the present study indicates that most of the dihydropyridines and pyridines in our new series of compounds reverse resistance against mitoxantrone in MCF-7/MX100 and H460/MX20 cell lines. All these compounds were synthesized based on the structure optimization of dexniguldipine to maximize P-glycoprotein binding affinity and decrease calcium channel binding activity. Therefore, these dihydropyridines/pyridines may be promising agents for clinical application due to their potent inhibition of both BCRP and P-glycoprotein. The commercially available dihydropyridines, nicardipine, nifedipine, and nitrendipine were also demonstrated to be potent BCRP inhibitors; nifedipine, on the other hand, did not show any significant effect on BCRP function. This study represents the first report that dihydropyridines and pyridines are potent inhibitors of BCRP.

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References


Zhang S, Wang X, Sagawa K, and Morris ME (2005) Flavonoids chrysirin and benzoflavone, potent breast cancer resistance protein inhibitors, have no significant effect on topotecan pharmacokinetics in rats or Mdr1a/1b (−/−) mice. *Drug Metab Dispos* **33**:341–348.


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