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ABCB1 transports Seliciclib (R-Roscovitine), a cyclin-dependent kinase inhibitor

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Running Title: Seliciclib is a selective ABCB1 substrate

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Abbreviations

ABCB1: ATP-binding cassette transporter B1, also known as P-glycoprotein (P-gp) and multidrug resistance protein 1 (MDR1)

ABCC1: ATP-binding cassette transporter C1 also known as MRP1 (Multidrug resistance associated protein 1)

ABCC2: ATP-binding cassette transporter C2 also known as MRP2 (Multidrug resistance associated protein 2)

ABCG2: ATP-binding cassette transporter G2 also known as BCRP (Breast cancer resistance protein)

BBB: blood-brain-barrier

Calcein AM: calcein-acetoxy-methylester

CDK: cyclin-dependent kinase

HL-60: Human promyelocytic leukemia cells

MDCKII: Madin-Darby canine kidney strain II cells

NMQ: N-methyl quinidine

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Abstract

Seliciclib, a cyclin-dependent kinase inhibitor is a promising candidate to treat a variety of cancers. Pharmacokinetic studies have shown high oral bioavailability but limited brain exposure to the drug. This study shows that seliciclib is a high affinity substrate of ABCB1 as it activates the ATPase activity of the transporter with an EC_{50} of 4.2 μM and shows vectorial transport in MDCKII-MDR1 cells yielding an efflux ratio of 8. This interaction may be behind the drug's limited penetration of the blood-brain barrier. ABCB1 overexpression on the other hand does not confer resistance to the drug in the models tested. These findings should be considered when designing treatment strategies utilizing seliciclib.

Introduction

Seliciclib (R-roscovitine, CYC202), a 2, 6, 9-trisubstituted purine analog is a second generation cyclin-dependent kinase (CDK) inhibitor (Meijer et al., 2006). It arrests cellular proliferation and induces apoptosis through molecular interactions with the heterodimers of CDKs and cyclins. It is a potent inhibitor of the human CDK2/cyclin E, CDK1/cyclin B, CDK7/cyclin H and CDK9/cyclin T1 (Meijer et al., 2006; Okyar et al., 2008). Seliciclib binds to the ATP binding site of the respective kinases in a competitive fashion as shown in structural and kinetic studies (De Azevedo et al., 1997). A few other enzymes such as calmodulin-dependent kinase isoforms (CaM kinase 2), CK1α, CK1δ/ε, DYRK1A, EPHB2, ERK1, ERK2, FAK and IRAK4 were also inhibited at micromolar concentrations (Meijer et al., 2006). In addition, seliciclib also bound pyridoxal kinase (PDXK) and reduced the level of pyridoxal phosphate in human erythrocytes (Bach et al., 2005). Seliciclib has displayed activity against human non-small-cell lung, colon, breast and prostate cancer cell lines in xenografted mouse models as well as against mouse Glasgow osteosarcoma (Meijer et al., 2006; Iurisci et al., 2006). Phase I and II clinical trials have shown adequate drug tolerability (Benson et al., 2007) and recently revealed high activity in patients with chronic lymphocytic leukemia or nasopharyngeal carcinoma (Weingrill et al., 2007; Hsieh et al., 2009).

Limited information exists on the resistance of cancer cells to seliciclib. One study in chronic lymphocytic leukemia (CLL) in vitro models showed that CD40 stimulation up-regulated

antiapoptotic Bcl-xL, A1/Bfl-1, and Mcl-1 proteins, and afforded resistance to seliciclib among several agents in various pharmacologic classes (Hallaert et al., 2008).

Although seliciclib has been shown to inhibit ABCB1-mediated transport of Rhodamine 123 (Bachmeier and Miller., 2005) the nature of seliciclib – ABCB1 interaction, namely substrate vs. inhibitor is unknown as yet. The potential role of the MDR transporter ABCB1, in seliciclib resistance has not been evaluated either. A further indication of the possible role of ABCB1 in seliciclib transport stems from the observation of its limited brain uptake, which was estimated as 25% in adult rats (Vita et al., 2005).

In this study we show that seliciclib is a selective substrate of ABCB1 and discuss how this seliciclib - ABCB1 interaction may affect seliciclib disposition.

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Materials and Methods

Chemicals

Seliciclib (R-Roscovitine (2-(1-ethyl-2-hydroxyethylamino)-6-benzylamino-9-isoprpopylpurine)) powder was kindly provided by the Institute de Chimie Organique, Université René Descartes, Paris, France (Hervé Galons)."

The LY335979 compound was synthesized as described (Barnett et al., 2004). ^{3}H-NMQ is from Dr. Csaba Tömböly (Biological Research Center, Hungary), Ko134 was from Solvo Biotechnology (Szeged, Hungary). Advanced RPMI 1640 [Gibco, 12 633-012], was from Csertex Ltd. (Budapest, Hungary). Fetal bovine serum (Lonza, DE14-802F), Dulbecco's Modified Eagle's Medium (DMEM, Lonza, 12-708F) penicillin-streptomycin (Lonza, 09-757F) were purchased from Biocenter Kft. (Szeged, Hungary). The mouse anti-ABCB1 monoclonal antibody C-219 (Abcam, ab3364) was purchased from Biomarker Kft (Budapest, Hungary) and the anti-mouse IgG-HRP secondary antibody, a horseradish peroxidase-conjugated species-specific whole antibody (Sigma-Aldrich, 4416IML) was from Sigma Hungary (Budapest, Hungary). The Western Lightning Plus-ECL (Perkin-Elmer, NEL103001EA) was from Per-Form Hungary Kft (Budapest, Hungary). CalceinAM (Molecular Probes, C3100) was purchased from Invitrogen Hungary (Budapest, Hungary), Verapamil (V4629), Hoechst33342 (B2261) and other chemicals were from Sigma Hungary (Budapest, Hungary).

Cell lines

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The chronic myeloid leukaemia cell line, K562 and it's ABCB1 overexpressing variant K562-MDR were received as kind gifts from Prof. Balazs Sarkadi (National Blood Transfusion Service, Hungary), MDCKII-MDR1, PLB985-BCRP (Kis et al., 2009) and parental cells were kindly provided by Dr Katalin Német (National Blood Transfusion Service, Hungary). Cells were maintained in Advanced RPMI 1640 except MDCKII and MDCKII-MDR1 cells which were in Dulbecco's Modified Eagle's Medium supplemented with 1 g/L glucose and 1% non-essential amino acids. All media were supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 µg/ml penicillin-streptomycin and were grown under standard conditions (5% CO_2, 37°C).

ATPase activity

ABC transporter overexpressing membrane preparations show vanadate-sensitive ATPase activity that is modulated by interacting compounds. ATPase activity was measured as described previously (von Richter et al., 2009). Briefly, the rate of ATP hydrolysis was determined by measuring the liberation of inorganic phosphate using the PREDEASY ATPase kits for ABCB1, ABCC1, ABCC2 and ABCG2-HAM were from SOLVO Biotechnology (Hungary) according to the manufacturer's instructions. Membrane vesicles were incubated with various concentrations of test drugs with or without 1.2 mM sodium orthovanadate. ATPase activities were determined as the difference of inorganic phosphate liberation measured in the presence and absence of 1.2 mM sodium orthovanadate, an inhibitor of ABC efflux pumps. Results are presented as vanadate sensitive ATPase activites.

Vesicular Transport assay

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The interaction of seliciclib with the transporter was detected as the modulation of the initial rate of NMQ transport into the membrane vesicles. The vesicular transport assay was performed using the PREDIVEZ Kit (Solvo Biotechnology, Hungary) for human ABCB1 (SB-MDR1/P-gp-PREDIVEZ™-VT kit) according to the manufacturer's recommendations. Briefly, membrane fractions containing inside-out membrane vesicles were incubated in a 96 well plate in the presence or absence of ATP using ^{3}H-NMQ as probe substrate. The transport was stopped by addition of cold washing buffer and consecutive rapid filtration through Millipore B-glass fiber filters of a 96-well filter plate (Millipore). After washing five-times with 200 µl ice-cold wash buffer the filters were dried and the retained radioactivity was measured in scintillation cocktail (Packard UltimaGold; Perkin-Elmer) using a Wallac MicroBeta TriLux liquid scintillation analyzer.

Hoechst assay

Hoechst 33342 intercalates DNA yielding a fluorescent product that can be detected. The presence of BCRP in the cell membrane strongly reduces Hoechst 33342 accumulation. Inhibitors of BCRP produce an increased rate of accumulation. The Hoechst assay was performed as described earlier (Kis et al., 2009). Briefly, accumulation of Hoechst 33342 dye was measured in a fluorometer (Fluoroskan Ascent Type 374) at 350 nm (excitation) and 460 nm (emission) by using PLB985-BCRP cells. The cells were preincubated at 37°C in 1x Hank's solution with drugs for 30 minutes. The Hoechst dye was added in 50 µl, at a final concentration of 12.5 µM. The fluorescence intensities were recorded for 15 minutes. The positive control measurements to determine 100% inhibition were carried out in the presence of 400 nM Ko134, a specific BCRP inhibitor (Allen et al., 2002).

Calcein assay

Calcein AM penetrates plasma membrane by passive diffusion. Intracellularly calcein AM is hydrolyzed by endogenous esterases, yielding a fluorescent product, calcein that can be detected. The presence of ABCB1 in the cell membrane strongly reduces calcein accumulation. Inhibitors of ABCB1 produce an increased rate of accumulation. The Calcein assay was done as described earlier (von Richter et al., 2009). Briefly, accumulation of the calcein dye was measured in a fluorimeter (Fluoroskan Ascent Type 374) at 485 nm (excitation) and 538 nm (emission) by using K562MDR cells. Cells (80,000 per well) were incubated in 100 µl Hank's balanced salt solution (HBSS) in the presence of the test compound or positive control for 15 minutes. After the incubation calcein AM in 100 µl HBSS was added at a final concentration of 0.25 µM. Fluorescence intensities were recorded for 8 minutes. The positive control measurements to determine 100% inhibition were carried out in the presence of 60 µM verapamil.

Western blotting

The proteins were separated using a 10% polyacrylamide gel and transferred to polyvinylidene difluoride membrane (Immobilon-P; Millipore, Bedford, MA) at 350 mA in a transfer buffer composed of 25 mM Tris, 192 mM glycine, and 15% (v/v) methanol, pH 8.3. The membrane was treated with blocking buffer (5% nonfat dry milk powder and 0.5% bovine serum albumin in phosphate-buffered saline with 0.05% Tween 20) for 2 h at room temperature. The membrane was then incubated with the primary antibody, a mouse anti-ABCB1 monoclonal antibody C-219,

diluted 1:3000 in blocking buffer for 2 h at room temperature. The membrane was washed for 3-times 10 min with phosphate-buffered saline/0.05% Tween 20 at room temperature. It was then incubated with the secondary antibody, anti-mouse IgG-HRP, a horseradish peroxidase-conjugated species-specific whole antibody diluted 1:5000 in blocking buffer for 1 h at room temperature. The membrane was subsequently washed as described above, and immunoreactive bands were visualized with ECL.

MDCKII monolayer assay

Transport assays across MDCKII-WT and MDCKII-MDR1 cells were done as described (10). Cells were seeded on Millicell® 24 (Millipore, Carrigtwohil, Ireland) devices according the manufacturer's instructions. Seliciclib (5 µM) was added w/o an ABCB1 inhibitor, LY335979 (1µM) to the medium at either the basolateral or apical compartment. Samples were taken from the receptor chamber at 15, 30, 60 and 120 min. Concentrations of seliciclib were determined using an Agilent1100-Series HPLC equipped with mass selective detector (MSD) Quad VL System (Agilent, Germany). Samples from the 60-min point were used for the apparent permeability coefficient (P_{app}) calculations.

Cytotoxicity assay

Cytotoxicity assays were performed by seeding HL60 (50,000 cell/well), HL60-MDR1 (50,000 cell/well), K562 (50,000 cell/well), K562MDR1 (50,000 cell/well), MDCKII-WT (1,000 cells/well) and MDCKII-MDR1 (1,000 cells/well) in 96-well plates containing the culture medium (200 µl/well). After 24 hours, drugs were prediluted in medium and added to the cells at different concentrations as shown in the Figures. The cells were further incubated with the drug

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in a humidified tissue-culture chamber (37°C, 5% CO_2) for 96 hours. Surviving cells were detected by the MTS method (www.promega.com). IC_{50} values were calculated from dose-response curves (i.e. cell survival vs. drug concentration) obtained in triplicate experiments.

Data analysis

All assays were run in duplicates unless indicated otherwise. The Calcein and the Hoechst assays were analyzed using the slope of the curve determined without inhibitors (Rbase), the slope of the curve in the presence of the inhibitor (Rmax), and the slope of the curve determined for any drug at the given drug concentration (Rdrug). The inhibition (%) of dye extrusion can be represented with the following formula:

$$Inhibiton\ (\%) = \frac{Rdrug - Rbase}{R\,max - Rbase} * 100$$

IC50 values were derived from these curves.

For calculation of the apparent permeability coefficient (P_{app}) the following equation was used:

$$P_{app} = \frac{dQ}{dT} \times \frac{1}{A \times C_0}$$

where dQ is the amount of test and dT is the incubation time. C_0 is the initial concentration of the compound in the donor compartment and A is the membrane surface area in cm^2 (standard: 0.7). Efflux ratio is given as the $P_{app\ B\text{-}A}$ / $P_{app\ A\text{-}B}$ apparent permeability ratio.

For curve fitting and IC50 calculations GraphPad PRISM 4.0 software was used (GraphPad Software Inc., CA). Statistical analysis was done using unpaired t test.

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Results

To test interactions of seliciclib with efflux transporters present in the blood-brain barrier ATPase assays were carried out using Sf9 membranes overexpressing ABCB1, ABCC1, ABCC2 and ABCG2. Seliciclib activated the vanadate sensitive ATPase activity of ABCB1 with an EC_{50} value of 4.2 μM (Table 1) to the level observed in the presence of verapamil, the positive control (Fig 1A). ABCG2 ATPase was not activated by seliciclib (Fig 1D). However, the activated ABCG2 as well as basal vanadate sensitive ABCG2 ATPase was inhibited by seliciclib (Fig 1D), albeit at a suprapharmacological concentration range (Table 1). ABCC1 only showed inhibition at very high concentrations (Fig 1B) while ABCC2 did not interact at all (Fig 1C).

To confirm interactions with ABCB1 vesicular transport assay using NMQ as a probe and calcein assay was carried out. Both assays showed interaction (Fig 2 A-B) with IC50 values of 35.5 μM and 11.5 μM respectively (Table 1). ABCG2 inhibition by seliciclib was also confirmed in Hoechst assay (Fig 2C) with an IC50 of 38 μM (Table 1).

Activation of the ABCB1 ATPase by seliciclib indicated that the drug is a transported substrate of ABCB1. To confirm the transport MDCKII-MDR1 cells were used (Fig 3). MDCKII-MDR1 cells greatly overexpress human MDR1 (Fig 3A). Basal calcein fluorescence of MDCKII-MDR1 cells was much lower than basal calcein fluorescence of control cells but reached about the same level in the presence of LY335979 (Fig 3B), an ABCB1 specific inhibitor (Dantzig et al., 2003). In addition, in MDCKII-MDR1 cells unlike in control cells (Fig 3C) permeability of seliciclib was much greater in the B-A direction than in the A-B direction, resulting in an efflux ratio of

around 8 in MDCKII-MDR1 cells. In the presence of LY335979, the observed efflux ratio in the MDCKII-MDR1 cells was 1.2 (Fig 3D).

We have tested if ABCB1 overexpression would result in resistance to seliciclib using HL60-MDR1, K562-MDR and MDCKII-MDR1 and control cells. We have found that overexpression of MDR1 did not confer resistance to seliciclib as IC_{50} values in cytotoxicity tests were not significantly different for MDCKII-WT and MDCKII-MDR1 cells (4.9 ± 1.3 μM vs 7.1 ±1.6 μM respectively) and addition of LY335979 did not affect susceptibility to seliciclib of either cell line (Fig 4A). In contrast, marked difference was observed in susceptibility to doxorubicin (Fig 4B) and paclitaxel (Fig 4C). No statistically significant difference was observed in IC_{50} values for seliciclib in K562 and K562-MDR (45.9 ±5.94 μM vs 47.1 ±33.5 μM respectively) as well as in HL60 and HL60-MDR1 (12.6 ±4.6 μM vs 17.5 ± 7.5 μM respectively) cells either.

Discussion

While seliciclib was taken-up in the brain of rat pups via simple equilibrium diffusion, the brain/plasma AUC ratio was only 25% in adult rat brain (Vita et al., 2005, Sallam et al., 2008). This finding is in line with prior published data on the maturation of the blood brain barrier, in which ABC transporters play an essential role. In the current study, we tested if efflux transporters, thought to limit brain exposure to drugs would play a role in limiting seliciclib brain exposure. It is commonly accepted that ABCB1/Abcb1a and ABCG2/Abcg2 are expressed in the luminal membrane of brain microcapillary endothelial cells (Roberts et al., 2008) and limit penetration of substrate drugs (Enokizono et al., 2007). Barrier function of ABCC1/Abcc1 is controversial as it was shown to localize in the luminal membrane in human (Nies et al., 2004) and more recently found in the luminal membrane in rat brain microcapillary endothelial cells (Roberts et al., 2008). On the contrary, it is generally accepted that Abcc1 is expressed in the basolateral membrane of murine choroid plexus epithelial cells (Roberts et al., 2008) and protects brain from toxic stimuli (Wijnholds et al., 2000). Function of ABCC2/Abcc2 in the blood-brain barrier is even more controversial. The conclusion from multiple studies suggests that ABCC2/Abcc2 may not play a substantial role in the blood-brain barrier under normal conditions but may get up-regulated and, thus, limit brain penetration of substrate drugs under pathological conditions (Hoffmann et al., 2006).

Our data clearly show that seliciclib is a selective substrate of ABCB1 as it activates the ABCB1 ATPase (Fig 1A) and it shows an ABCB1-dependent vectorial transport in the MDCKII-MDR1 cells (Fig 3C-D). Seliciclib in Phase I clinical trials reached a plasma concentration of about 10

μM, 90% of which is protein bound (Benson et al., 2007). This value is approximately one log below the EC50 of 4.2 μM for the seliciclib – ABCB1 interaction, a concentration range where transporters exert significant effect on transcellular permeability of their substrates (Shirasaka et al., 2008). Interestingly, the AUC_{brain} over AUC_{plasma} value of seliciclib was around 1 in the day 12 old rat pups while it was about 0.2 in adult rats (Iurisci et al., 2006, Vita et al., 2005). BBB matures at 3-4 weeks postnatal and it was hypothesized that immature BBB in pups might account for this difference (Sallam et al., 2008). No data are available comparing Abcb1a expression in brain microvascular endothelial cells in rat pups and in adult rats. In cerebellum, however, Abcb1a expression is lower at 11 days than in adult animals (de Zwart et al., 2008). Therefore, it is conceivable that Abcb1a is responsible for lower brain exposure in adult rats. Data demonstrating that seliciclib inhibits Abcb1-mediated rhodamine 123 transport in bovine brain microvascular endothelial cells substantiate this hypothesis (Bachmeier and Miller., 2005).

ABCB1 is also responsible for multidrug resistance to substrate drugs (Szakacs et al., 2004). We have not observed ABCB1-mediated resistance to seliciclib-induced cell killing in three different cell lines K562-MDR1 (Ye et al., 2009), HL-60MDR1 (Rohlff et al., 1993) and MDCKII-MDR1 (Fig 3 B-D) that display significant ABCB1-dependent resistance to substrate drugs. Seliciclib is a moderate – to high passive permeability compound based on the permeability data of about 5×10^{-5} cm/s (Fig 3D), high bioavailability (Benson et al., 2007), the log P value of 3.244 (Meijer et al., 2006) as well as the reasonable agreement between the membrane and cellular IC50 values (35.5 μM vs. 11.5 μM). Therefore, the lack of the protective effect of ABCB1 against cell death inflicted by seliciclib may simply be explained by the passive permeability of the drug. Interestingly, in MCF7 cells seliciclib displays greater cytotoxicity in the ABCB1 overexpressing

cells than in the control MCF7 line (Cappellini et al., 2009). This class of compounds has been termed as MDR1-inverse as ABCB1 sensitizes cells to cytotoxicity by these compounds (Szakács et al., 2004). Thus, ABCB1 may play a dual role in HL60-MDR1, K562-MDR1 as well as MDCKII-MDR1 cells by reducing intracellular drug concentration and at the same time sensitizing cells to seliciclib. The two opposing effects may effectively cancel out each other in the cell lines used in this study.

Seliciclib also interacts with ABCG2 (Fig 1-2). The interaction does not result in the activation of the ATPase, therefore seliciclib is a likely inhibitor of the transporter. The observed IC50 values correlate with the low affinity observed for inhibition of ABCG2-mediated hematoporphyrin transport (An et al., 2009). The clinical free drug concentrations under current schedules (Weingrill et al., 2007) are way below the IC50 values for seliciclib-mediated ABCG2 inhibition rendering this interaction as unlikely clinically relevant.

In conclusion, seliciclib is a high affinity, selective ABCB1 substrate. This interaction is likely to affect disposition of the drug. ABCB1 overexpression on the other hand does not confer resistance to seliciclib, making the drug a favorable candidate to treat ABCB1 transporter overexpressing tumors.

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References

Allen JD, van Loevezijn A, Lakhai JM, van der Valk M, van Tellingen O, Reid G, Schellens JH, Koomen GJ, Schinkel AH. Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. Mol Cancer Ther. 2002 Apr;1(6):417-25.

An R, Hagiya Y, Tamura A, Li S, Saito H, Tokushima D, Ishikawa T. Cellular phototoxicity evoked through the inhibition of human ABC transporter ABCG2 by cyclin-dependent kinase inhibitors in vitro. Pharm Res 2009; 26 (2): 449-45

Bach S, Knockaert M, Reinhardt J, Lozach O, Schmitt S, Baratte B, Koken M, Coburn SP, Tang L, Jiang T, Liang DC, Galons H, Dierick JF, Pinna LA, Meggio F, Totzke F, Schächtele C, Lerman AS, Carnero A, Wan Y, Gray N, Meijer L. Roscovitine targets protein kinases and pyridoxal kinase. J Biol Chem. 2005 Sep 2:280(35):31208-19

Bachmeier CJ and Miller DW. A fluorometric screening assay for drug efflux transporter activity in the blood-brain barrier. Pharm Res 2005; 22:113-121.

Barnett C.J., Huff B, Kobierski M.E., Letourneau M., and Wilson T.M. Stereochemistry of C-6 nucleophilic displacements on 1,1-Difluorocyclopropyldibenzosuberanyl substrates. An Improved synthesis of multidrug resistance modulator LY335979 Trihydrochloride. J Org Chem 2004; 69:7653–7660.

Benson C, White J, De Bono J, O'Donnell A, Raynaud F, Cruickshank C, McGrath H, Walton M, Workman P, Kaye S, Cassidy J, Gianella-Borradori A, Judson I and Twelves C. A phase I trial of the selective oral cyclin-dependent kinase inhibitor seliciclib (CYC202; R-Roscovitine), administered twice daily for 7 days every 21 days. Br J Cancer 2007; 96:29-37.

Cappellini A, Chiarini F, Ognibene A, McCubrey JA and Martelli AM. The cyclin-dependent kinase inhibitor roscovitine and the nucleoside analog sangivamycin induce apoptosis in caspase-3 deficient breast cancer cells independent of caspase mediated P-glycoprotein cleavage: implications for therapy of drug resistant breast cancers. Cell Cycle 2009; 8:1421-1425.

Dantzig AH, de Alwis DP, Burgess M. Considerations in the design and development of transport inhibitors as adjuncts to drug therapy. Adv Drug Deliv Rev. 2003 Jan 21;55(1):133-50.

De Azevedo WF, Leclerc S, Meijer L, Havlicek L, Strnad M, Kim SH. Inhibition of cyclin-dependent kinases by purine analogues: crystal structure of human cdk2 complexed with roscovitine. Eur J Biochem. 1997 Jan 15;243(1-2):518-26.

de Zwart L, Scholten M, Monbaliu JG, Annaert PP, Van Houdt JM, Van den Wyngaert I, De Schaepdrijver LM, Bailey GP, Coogan TP, Coussement WC, Mannens GS. The ontogeny of drug metabolizing enzymes and transporters in the rat. Reprod Toxicol. 2008; 26:220-30.

Enokizono J, Kusuhara H and Sugiyama Y. Effect of breast cancer resistance protein (Bcrp/Abcg2) on the disposition of phytoestrogens. Mol Pharmacol 2007; 72:967-975.

Hallaert DY, Jaspers A, van Noesel CJ, van Oers MH, Kater AP and Eldering E. c-Abl kinase inhibitors overcome CD40-mediated drug resistance in CLL: implications for therapeutic targeting of chemoresistant niches. Blood 2008; 112:5141-5149.

Hoffmann K, Gastens AM, Volk HA and Loscher .Expression of the multidrug transporter MRP2 in the blood-brain barrier after pilocarpine-induced seizures in rats. Epilepsy Res 2006; 69:1-14.

Hsieh WS, Soo R, Peh BK, Loh T, Dong D, Soh D, Wong LS, Green S, Chiao J, Cui CY, Lai YF, Lee SC, Mow B, Soong R, Salto-Tellez M, Goh BC. Pharmacodynamic effects of seliciclib, an orally administered cell cycle modulator, in undifferentiated nasopharyngeal cancer. Clin Cancer Res 2009; 15; 1435-1442.

Iurisci I, Filipski E, Reinhardt J, Bach S, Gianella-Borradori A, Iacobelli S, Meijer L, Lévi F. Improved tumor control through circadian clock induction by seliciclib a cyclin-dependent kinase inhibitor. Cancer Res 2006; 66: 10720-8.

Kis E, Nagy T, Jani M, Molnar E, Janossy J, Ujhellyi O, Nemet K, Heredi-Szabo K and Krajcsi P. Leflunomide and its metabolite A771726 are high affinity substrates of BCRP: implications for drug resistance. Ann Rheum Dis 2009; 68:1201-1207.

Meijer L, Bettayeb K, Galons H. (R)-Roscovitine (CYC202, Seliciclib). In: Inhibitors of cyclin-dependent kinases as anti-tumor agents. Smith P.J&Yue E.W. (Ed.) CRC Press Taylor&Francis, Boca Raton FL, 2006, p.187-225.

Nies AT, Jedlitschky G, Konig J, Herold-Mende C, Steiner HH, Schmitt HP and Keppler D (2004) Expression and immunolocalization of the multidrug resistance proteins, MRP1-MRP6 (ABCC1-ABCC6), in human brain. Neuroscience 129:349-360.

Okyar A, Lévi F. Circadian clock control of cell cycle pathways: Relevance for cancer chronotherapeutics. In: Trends in Cell Cycle Research, K. Yoshida (Ed.), Research Signpost, Kerala- India, 2008, p.293-317.

Roberts LM, Black DS, Raman C, Woodford K, Zhou M, Haggerty JE, Yan AT, Cwirla SE and Grindstaff KK. Subcellular localization of transporters along the rat blood-brain barrier and blood-cerebral-spinal fluid barrier by in vivo biotinylation. Neuroscience 2008; 155:423-438.

Rohlff C, Safa B, Rahman A, Cho-Chung YS, Klecker RW, Glazer RI. Reversal of resistance to adriamycin by 8-chloro-cyclic AMP in adriamycin-resistant HL-60 leukemia cells is associated with reduction of type I cyclic AMP-dependent protein kinase and cyclic AMP response element-binding protein DNA-binding activities. Mol Pharmacol. 1993 Mar;43(3):372-9.

Sallam H, Jimenez P, Song H, Vita M, Cedazo-Minguez A and Hassan. Age-dependent pharmacokinetics and effect of roscovitine on Cdk5 and Erk1/2 in the rat brain. Pharmacol Res 2008; 58:32-37.

Shirasaka Y, Sakane T and Yamashita S. Effect of P-glycoprotein expression levels on the concentration-dependent permeability of drugs to the cell membrane. J Pharm Sci 2008;97:553-565.

Szakacs G, Annereau JP, Lababidi S, Shankavaram U, Arciello A, Bussey KJ, Reinhold W, Guo Y, Kruh GD, Reimers M, Weinstein JN and Gottesman MM. Predicting drug sensitivity and resistance: profiling ABC transporter genes in cancer cells. Cancer Cell 2004; 6:129-137.

Vita M, Abdel-Rehim M, Olofsson S, Hassan Z, Meurling L, Siden A, Siden M, Pettersson T and Hassan M. Tissue distribution, pharmacokinetics and identification of roscovitine metabolites in rat. Eur J Pharm Sci 2005; 25:91-103.

von Richter O, Glavinas H, Krajcsi P, Liehner S, Siewert B and Zech K. A novel screening strategy to identify ABCB1 substrates and inhibitors. Naunyn Schmiedebergs Arch Pharmacol 2009; 379:11-26.

Weingrill E, Wölfler A, Strunk D, Linkesch W, Sill H, Liebmann PM. Roscovitine in B-chronic lymphocytic leukemia cells: high apoptosis-inducing efficacy and synergism with alemtuzumab independent of the patients' pretreatment status. Haematologica 2007; 92:1286-1288

Wijnholds J, deLange EC, Scheffer GL, van den Berg DJ, Mol CA, van der Valk M, Schinkel AH, Scheper RJ, Breimer DD and Borst P. Multidrug resistance protein 1 protects the choroid plexus epithelium and contributes to the blood-cerebrospinal fluid barrier. J Clin Invest 2000; 105:279-285.

Ye X, Liu T, Gong Y, Zheng B, Meng W, Leng Y. Lentivirus-mediated RNA interference reversing the drug-resistance in MDR1 single-factor resistant cell line K562/MDR1. Leuk Res. 2009 Aug;33(8):1114-9. Epub 2008 Nov 26.

Footnotes

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Figure legends

Figure 1. Vanadate-sensitive ATPase activity of (A) MDR1-Sf9, (B) MRP1- Sf9, (C) MRP2- Sf9 and (D) BCRP-HAM-Sf9 membrane preparations in the presence of seliciclib in activation (solid lines) and inhibition (dotted lines) experiments. Significant differences (*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) between the basal activity of the transporter in the absence and presence of seliciclib in the case of the activation data and the differences between the activated transporter in the absence and presence of seliciclib in the case of inhibition data are indicated.

Figure 2. Inhibition of (A) calcein AM efflux from K562MDR cells, (B) ATP-dependent N-methyl-quinidine transport into MDR1-M inside-out vesicles and (C) Hoechst 33342 dye efflux from PLB985-BCRP cell by seliciclib.

Figure 3. Characterization of MDCKII-MDR1 cells in western blotting (A), in calcein assay (B) as well as demonstration of vectrorial transport of seliciclib in monolayer efflux assays in MDCKII-MDR1 cells (C-D). Effect of ABCB1 on the apparent permeability of seliciclib determined by bidirectional transport across MDCKII-MDR1 and control monolayers in the presence or absence of the specific ABCB1 inhibitor LY335979 is shown (Fig 3C-D). Significant differences (*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) are indicated.

Figure 4. Effect of ABCB1 overexpression on cytotoxicity of substrate drugs. MDCKII-MDR1 and control cells were treated with seliciclib, doxorubicin and paclitaxel at concentrations

indicated in the figure in the absence and presence of LY335979. Dose response curves are shown.

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Table 1. IC50 and EC50 values of roscovitine transport.

ABC transporters	Assays	EC_{50}(μM)	IC_{50}(μM)
MDR1	ATPase assay	4.2	-
	Vesicular transport assay	-	35.5
	Calcein assay	-	11.5
BCRP	ATPase assay	-	63
	Hoechst assay	-	38

Figure 1

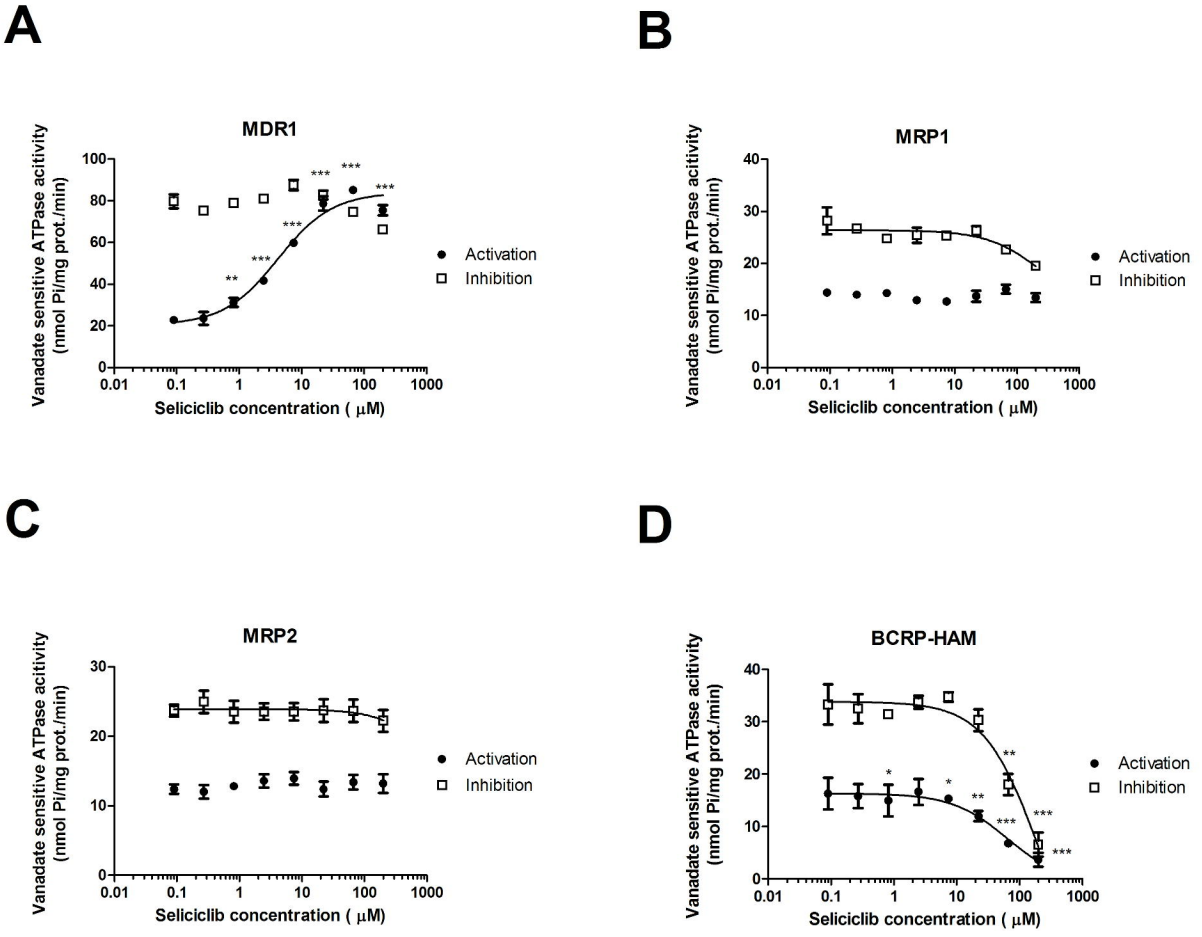

A
MDR1
Vanadate sensitive ATPase acitivity (nmol Pi/mg prot./min)
Seliciclib concentration (μM)
Activation
Inhibition
B
MRP1
Vanadate sensitive ATPase acitivity (nmol Pi/mg prot./min)
Seliciclib concentration (μM)
Activation
Inhibition
C
MRP2
Vanadate sensitive ATPase acitivity (nmol Pi/mg prot./min)
Seliciclib concentration (μM)
Activation
Inhibition
D
BCRP-HAM
Vanadate sensitive ATPase acitivity (nmol Pi/mg prot./min)
Seliciclib concentration (μM)
Activation
Inhibition

Figure 2

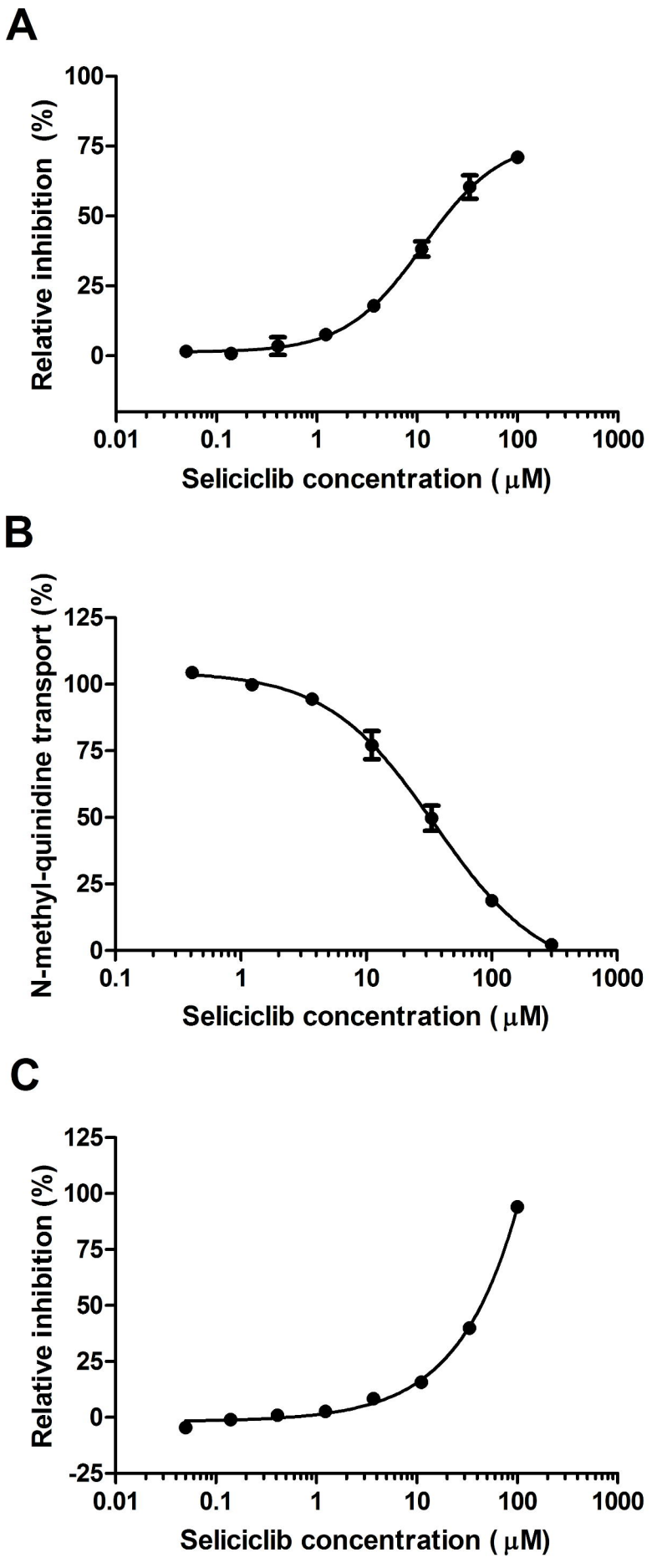

A
Relative inhibition (%)
100
75
50
25
0
0.01
0.1
1
10
100
1000
Seliciclib concentration (μM)
B
N-methyl-quinidine transport (%)
125
100
75
50
25
0
0.1
1
10
100
1000
Seliciclib concentration (μM)
C
Relative inhibition (%)
125
100
75
50
25
0
-25
0.01
0.1
1
10
100
1000
Seliciclib concentration (μM)

Figure 3

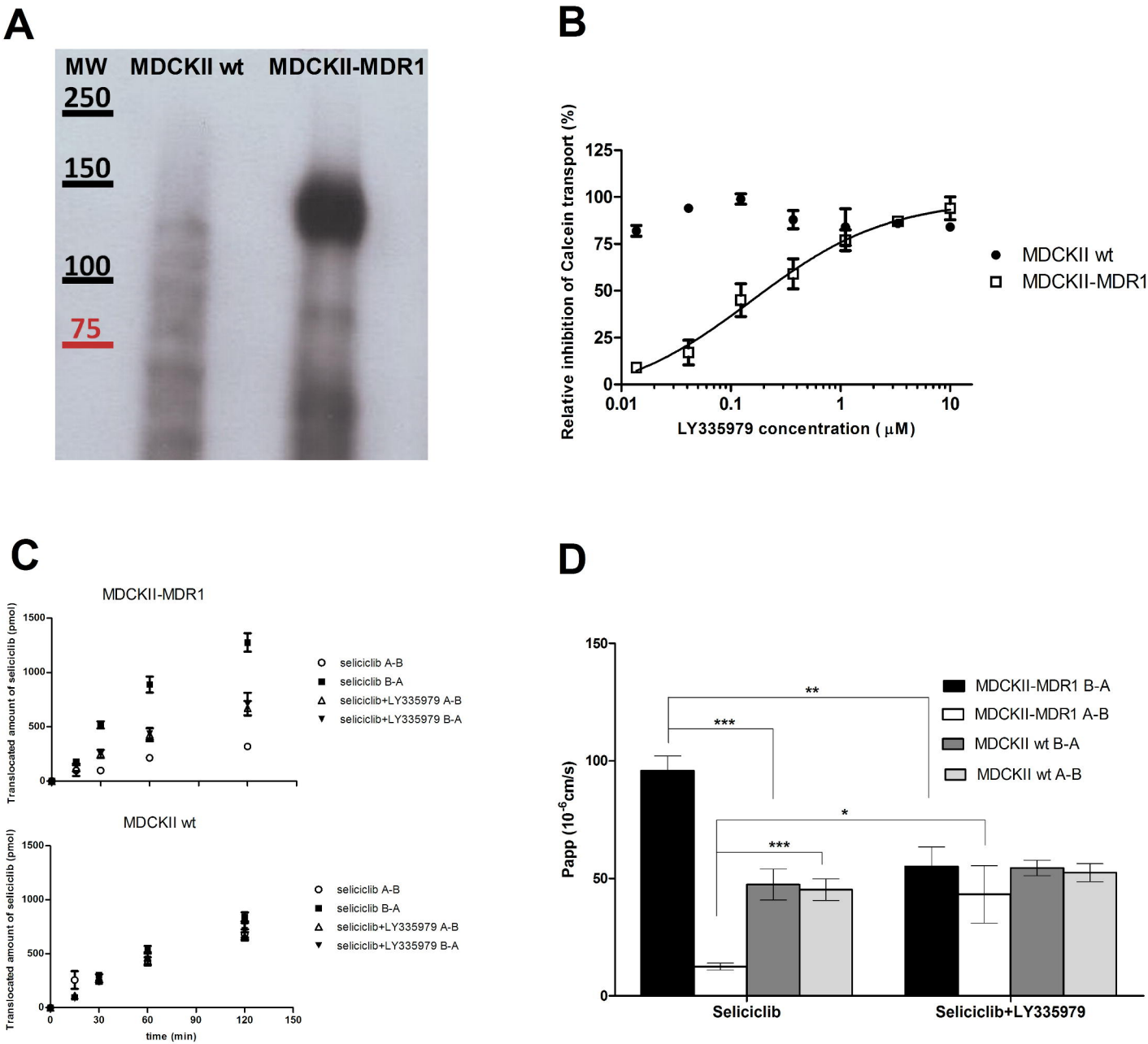
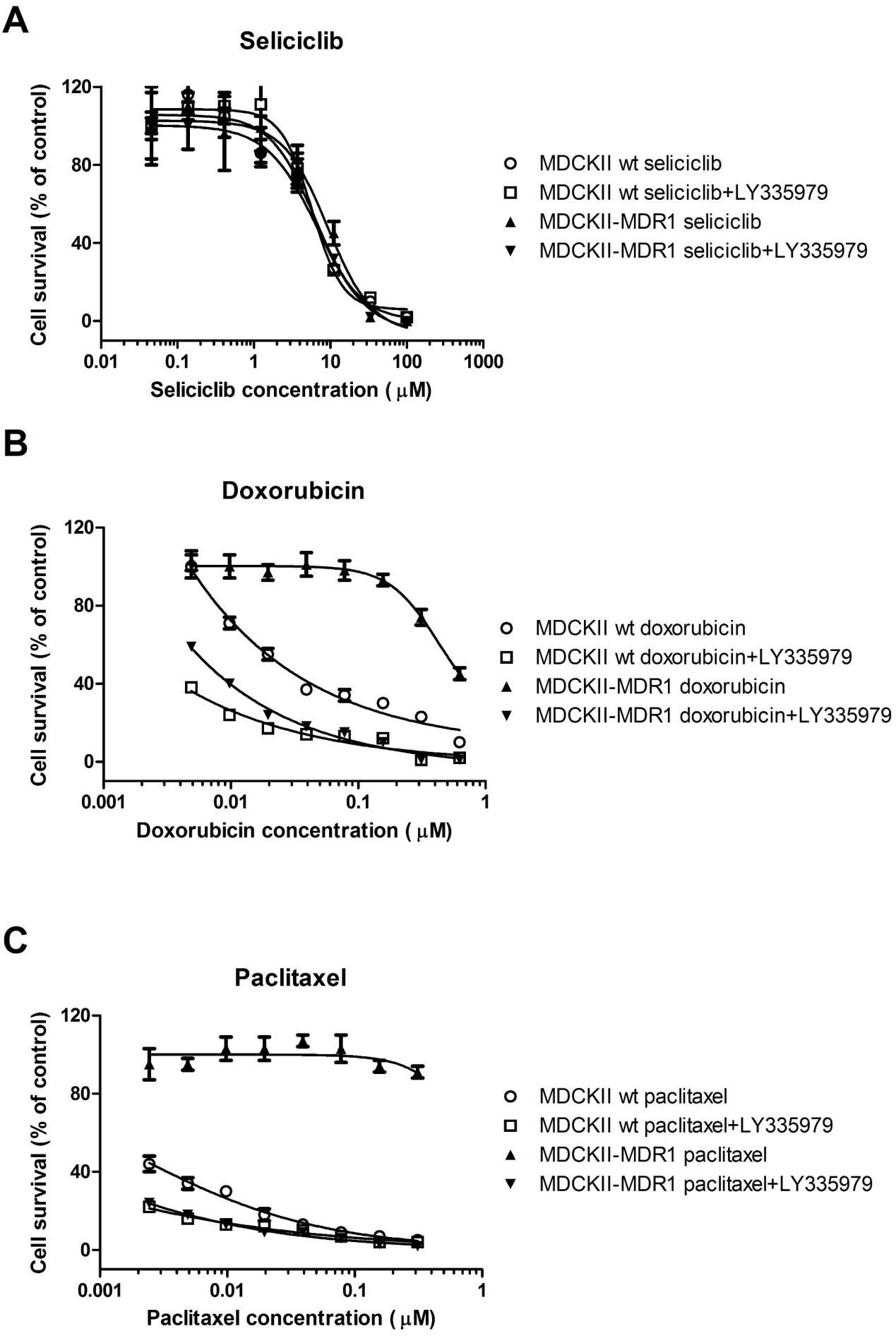


Figure 4


A
Seliciclib
Cell survival (% of control)
120
80
40
0
0.01
0.1
1
10
100
1000
Seliciclib concentration (μM)
MDCKII wt seliciclib
MDCKII wt seliciclib+LY335979
MDCKII-MDR1 seliciclib
MDCKII-MDR1 seliciclib+LY335979
B
Doxorubicin
Cell survival (% of control)
120
80
40
0
0.001
0.01
0.1
1
Doxorubicin concentration (μM)
MDCKII wt doxorubicin
MDCKII wt doxorubicin+LY335979
MDCKII-MDR1 doxorubicin
MDCKII-MDR1 doxorubicin+LY335979
C
Paclitaxel
Cell survival (% of control)
120
80
40
0
0.001
0.01
0.1
1
Paclitaxel concentration (μM)
MDCKII wt paclitaxel
MDCKII wt paclitaxel+LY335979
MDCKII-MDR1 paclitaxel
MDCKII-MDR1 paclitaxel+LY335979