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Substituents at the C3' and C3'N positions are critical for taxanes to overcome acquired resistance of cancer cells to paclitaxel

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Abstract

We tested the role of substituents at the C3' and C3'N positions of the taxane molecule to identify taxane derivatives capable of overcoming acquired resistance to paclitaxel. Paclitaxel-resistant sublines SK-BR-3/PacR and MCF-7/PacR as well as the original paclitaxel-sensitive breast cancer cell lines SK-BR-3 and MCF-7 were used for testing. Increased expression of the ABCB1 transporter was found to be involved in the acquired resistance. We tested three groups of taxane derivatives: (1) phenyl group at both C3' and C3'N positions, (2) one phenyl at one of the C3' and C3'N positions and a non-aromatic group at the second position, (3) a non-aromatic group at both C3' and C3'N positions. We found that the presence of phenyl groups at both C3' and C3'N positions is associated with low capability of overcoming acquired paclitaxel resistance compared to taxanes containing at least one non-aromatic substituent at the C3' and C3'N positions. The

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Author's contributions

MJ carried out some western blot analyses, analyzed dose responses and significantly contributed to manuscript preparation, KB performed siRNAs experiments, PD carried out ATPase assay and helped with manuscript preparation, VNF carried out some western blot analyses, PK realized molecular docking studies, MJ carried out HPLC method, LW and XW were involved in the preparation of taxane derivatives, JV designed molecular docking studies, IO designed taxane derivatives and contributed to manuscript preparation and JK was main coordinator of the research and manuscript preparation. All authors read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interests.

increase in the ATPase activity of ABCB1 transporter after the application of taxanes from the first group was found to be somewhat higher than after the application of taxanes from the third group. Molecular docking studies demonstrated that the docking score was the lowest, i.e. the highest binding affinity, for taxanes from the first group. It was intermediate for taxanes from the second group, and the highest for taxanes from the third group. We can conclude that at least one non-aromatic group at the C3' and C3'N positions of the taxane structure, resulting in reduced affinity to the ABCB1 transporter, brings about high capability of taxane to overcome acquired resistance of breast cancer cells to paclitaxel, due to less efficient transport of the taxane compound out of the cancer cells.

Keywords

taxane derivatives; acquired resistance to paclitaxel; breast cancer cells; ABCB1 transporter; molecular docking

Introduction

Paclitaxel (Taxol®), a natural product from the bark of *Taxus brevifolia*, and the semisynthetic analog docetaxel (Taxotere®) from the needles of *Taxus baccata*, are taxanes used for the treatment of many solid cancers including breast, lung, prostate, ovarian and head and neck cancers (Chu et al., 2005, Markman, 2008, Nabholz and Gligorov, 2005, Yared and Tkaczuk, 2012). A third taxane in clinical use, cabazitaxel (Jevtana®), was approved by the Food and Drug Administration for treatment of metastatic castration-resistant prostate cancer in 2010 (Paller and Antonarakis, 2011).

The antitumor activity of taxanes is associated with their ability to bind to the β -tubulin subunit on the luminal side of the assembled microtubule (Parness and Horwitz 1981, Rao et al., 1995, Rao et al., 1999, Snyder et al., 2001). Taxane treatment causes a mitotic arrest due to the binding of taxanes to the mitotic spindle. After prolonged exposure to taxane, the mitochondrial pathway of apoptosis induction is often initiated (Jordan et al., 1996, Woods et al., 1995).

The effectiveness of taxane treatment is influenced by innate and/or acquired resistance of tumor cells. Several mechanisms of taxane resistance have been proposed (Murray et al., 2012). Taxane resistance involves drug efflux mediated by transporters of the ATP-binding cassette transporter (ABC) family that can be responsible for poor bioavailability of classical taxanes (Fojo et al., 2005). Mutations in the taxol-binding site of β -tubulin (Orr et al., 2003), alterations in β -tubulin isotype expression, especially β -III tubulin (Kavallaris et al., 1997), and the effect of microtubule associated proteins (MAPs), which can either affect tubulin dynamics or bind to the taxol-binding site, have all been observed in cell line models (Alli et al., 2007, Smoter et al., 2011, Sun et al., 2015).

To overcome resistance to classical taxanes, modified taxanes such as Cabazitaxel (Jevtana®) (de Bono et al., 2010) and Ortataxel (SB-T-101131, IDN-5109, BAY 59-8862) have been developed with the goal of overcoming P-glycoprotein-associated resistance (Geney et al., 2005).

Classical taxanes containing a baccatin III core (paclitaxel) or 10-deacetylbaaccatin (docetaxel) are esterified at the C13 position and thus they have a specific side chain at this position. The side chain contains two phenyl groups at the C3' and C3'N positions in paclitaxel or a phenyl group at C3' position and *tert*-butoxycarbonyl at the C3'N position in docetaxel. A series of second generation taxanes modified at the C2, C3', C3'N, C10 and C13 positions, have been synthesized and their potency to overcome resistance to taxanes mediated by P-glycoprotein (Pgp) or mutations in β -tubulin has been assessed in some cancer cell lines or in mouse models (Matesanz et al., 2014, Ojima et al., 1994, Ojima et al., 1996, Ojima et al., 2008). These structure-activity relationship studies have revealed that novel taxanes are extremely efficient in overcoming some mechanisms of the acquired resistance described above.

We have established paclitaxel-resistant breast cancer cell sublines, SK-BR-3/PacR and MCF-7/PacR, capable of long-term proliferation in a medium containing concentrations of paclitaxel that are death-inducing for original sensitive cell lines SK-BR-3 and MCF-7 (100 nM and 300 nM, respectively). Both resistant sublines overexpress multi-drug resistance transporters ABCB1 and ABCC3 (Němcová-Furstová et al., 2016). Moreover, both sublines also express other proteins which may be involved in resistance to paclitaxel (Pavlíková et al., 2014, Pavlíková et al., 2015). Our previous results have showed that novel taxanes with modified structures, especially at the C3' and C3'N positions (Jelínek et al., 2013, Kovář et al., 2009, Vobířilová et al., 2011), were able to overcome paclitaxel resistance and induced cell death in tested paclitaxel-resistant breast cancer sublines (Němcová-Furstová et al., 2016).

In the present study we tested the role of substituents at the C3' and C3'N positions of the taxane structure to assess the capability of taxanes to overcome acquired resistance to paclitaxel in SK-BR-3 and MCF-7 cancer cells. We found that taxanes with phenyl groups at the C3' and at C3'N positions are able to induce apoptosis in paclitaxel-resistant cells only at very high concentrations. However, taxanes with a nonaromatic group instead of a phenyl group at least at either the C3' or C3'N position are able to overcome acquired resistance to paclitaxel. These taxanes induce apoptosis at significantly lower concentrations. These acquired resistance to paclitaxel results from a dramatically increased expression of the ABCB1 (Pgp) transporter. Mechanism of the capability of taxanes to overcome this acquired resistance is based on lower affinity to ABCB1 transporter and thus lower export from resistant cells.

Materials and Methods

Materials

The following primary and secondary antibodies were used for the detection of proteins: *anti*-ABCB1 (#12683, dilution 1:1000) from Cell Signaling Technology (Danvers, MA, USA), *anti*-actin (AC-40, A3853) from Sigma-Aldrich, HRP-linked goat *anti*-mouse (sc-2005, dilution 1:6000) and HRP-linked goat *anti*-rabbit (sc-2004, dilution 1:6000) antibody from Santa Cruz (Santa Cruz, CA, USA).

Paclitaxel and docetaxel were purchased from Sigma-Aldrich (St. Louis, MO, USA), deacetyl paclitaxel from Santa Cruz (Santa Cruz, CA, USA). Acetyl docetaxel, SB-T-0035, SB-T-1102, SB-T-1211, SB-T-1212N1, SB-T-1214 and SB-T-1216 were synthesized at the Institute of Chemical Biology and Drug Discovery (Stony Brook, NY, USA), (Ojima et al., 1996, Ojima et al., 1997). Taxanes were dissolved in DMSO (tissue culture quality) to obtain 10 mM stock solution.

Cells and culture conditions

Human breast carcinoma cell lines MCF-7 and SK-BR-3 were obtained from the National Cancer Institute (Frederick, MD, USA) and the American Type Culture Collection (ATCC) (Manassas, VA, USA), respectively. The cells were maintained at 37 °C in a humidified atmosphere of 5% CO₂ in air in RMPI-1640 based culture medium containing extra l-glutamine (300 µg/ml), sodium pyruvate (110 µg/ml), HEPES (15 mM), penicillin (100 U/ml) and streptomycin (100 µg/ml), and supplemented with 10% heat-inactivated fetal bovine serum.

Taxane-resistant cell sublines were maintained as long-term culture in a taxane-containing medium. Concentrations were as follows: 100 nM paclitaxel for the SK-BR-3/PacR cell subline, 300 nM paclitaxel for the MCF7/PacR cell subline, 300 nM SB-T-0035 for the MCF7/SB-T-0035R cell subline.

Assessment of cell growth and survival

Cells were harvested and seeded into 96-well plate at a density of 2×10^4 cells per well in 100 µl of culture medium. After 24h pre-incubation period allowing cells to attach, the culture medium was replaced by a culture medium without taxane (control) or with a medium containing tested taxane at desired concentrations. The number of living cells was determined after 96 hours of incubation using a hemocytometer after staining with trypan blue.

Establishment of MCF7 subline resistant to SB-T-0035

The MCF7 cell subline resistant to the effect of SB-T-0035, referred to as MCF7/SB-T-0035R, was established in a similar way as the paclitaxel-resistant SK-BR-3/PacR and MCF7/PacR sublines (N mcová-Furstová et al., 2016). The MCF7/SB-T-0035R subline was established by gradual adaptation of the original cell line to increasing concentrations of SB-T-0035. The starting concentration of SB-T-0035 was 1 nM. SB-T-0035 concentration then was increased as follows: 1 nM → 3 nM → 5nM → 10 nM → 20 nM → 30 nM → 50 nM → 70 nM → 100 nM → 300 nM. Cells were maintained at a particular SB-T-0035 concentration for approximately 10 passages or until they displayed, more or less, standard growth and survival. The final concentration of SB-T-0035 achieved was 300 nM. Long-term growth and survival of MCF7/SB-T-0035R in 300 nM SB-T-0035 was similar to cells without SB-T-0035. On the other hand, most of the original MCF-7 cells exposed to 300 nM SB-T-0035 died within 96 h.

Preparation of cell lysates

Cells were harvested and seeded to 60mm Petri dish at the density 1.5×10^6 cells in 5 ml of culture medium. After a 24h pre-incubation period allowing cells to attach, the culture medium was replaced with taxane-free medium or taxane-containing medium. After each particular period of incubation, cells were harvested by trypsinization, washed three-times with ice-cold PBS and centrifuged at 500 g for 10 minutes. Cell pellets were frozen at 80 °C for 1 hour and then dissolved in the RIPA lysis buffer containing protease and phosphatase inhibitors (described in detail in Jelínek et al., 2015). Protein concentrations were determined using the BCA method (Pierce BCA Protein Assay Kit, Thermofisher Scientific, MA, USA).

Western blot analysis

Protein samples (20 µg) were mixed with sample buffer (0.125 M Tris/HCl pH 6.8, 10% glycerol, 4% SDS, 0.25 M DTT) and heated for 5 min at 100 °C. For detection of the ABCB1 transporter, samples were heated for 5 minutes at 37 °C. Samples were separated in 10% acrylamide gels using protein electrophoresis (Bio-Rad, Hercules, CA). Proteins separated by SDS-PAGE were blotted onto 0.2 µm nitrocellulose membrane PROTRAN BA 83 (Whatman-Schleicher and Schuell, Maidstone, UK) for 3 h at 0.25 A, using a MiniProtean II blotting apparatus (Bio-Rad). The membrane was blocked with 5% BSA in TBS for 20 min and incubated with the primary antibody at 4 °C overnight. After incubation, the membrane was washed three times (5–10 min) with TBS containing 0.1% Tween-20. Then the membrane was incubated for 1–2 h with the corresponding horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Afterwards, the membrane was washed (as described above) and the chemiluminescence signal was detected using the Supersignal reagents from Pierce (Thermo Fisher Scientific, MA, USA) and a CCD camera (Carestream).

Silencing by siRNA

ABCB1 knock-down was performed similarly to a protocol already reported (Němcová-Furstová et al., 2016). The following siRNA were used: ABCB1 specific siRNA (catalog no: 4427037, ID: s10419, Life Technologies) and nonspecific siRNA (catalog no.: AM4635, Life Technologies) as a negative control.

The siRNA transfection mixture was prepared using INTERFERin (PolyPlus-Transfection, Illkirch, France) according to manufacturer` instructions. In the transfection mixture, ABCB1 or nonspecific siRNAs were diluted with the Opti-MEM® Reduced Serum Medium to a final concentration of 5 nM of siRNA in the culture medium together with INTERFERin transfection reagent at a 1:250 dilution.

Assessment of ABC transporter ATPase activity

The effect of tested taxanes on P-glycoprotein ATPase activity was assessed using the Pgp-Glo™ Assay System with a P-glycoprotein kit (Promega, CA, USA) according to the manufacturer`s protocol. Briefly, membranes (25 µg) containing human recombinant P-glycoprotein were incubated with verapamil (positive control), sodium orthovanadate (inhibitor of ATPase activity), tested compound (paclitaxel, SB-T-1216, SB-T-0035) or Pgp-

Glo buffer (as a control) with non-limiting concentration of ATP in Nunc™ F96 MicroWell™ (ThermoFisher Scientific, MA, USA) white plate for 120 minutes at 37 °C. Afterwards, ATP-detection reagent, containing recombinant Ultra-Glo™ Luciferase and luciferin substrate, was added to the samples and luciferase activity signal was determined using a TECAN Infinite M200 Pro luminometer (TECAN, Männedorf, Switzerland).

Assesment of intracellular taxane level

Cells were harvested and lysed by buffer containing 8µg/µl digitonin (Sigma Aldrich) (see Jelínek et al., 2015).

Samples were prepared by mixing 200 µl of acetonitrile (ACN) with 100 µl of cell lysate. After centrifugation, 50 µl of the supernatant was injected onto HPLC system equipped with a column Ascentis Express C18 (150 × 4.6 mm, 5 µm, Supelco, Bellefonte, PA, USA). Our method was based on principles of previously published work (Kim et al., 2005). As a mobile phase, mixture of deionized water and ACN was used. Flow rate was 1 ml/min, maintaining first 5 min ACN concentration of 34% and increasing by linear gradient to ACN concentration of 61% by 23rd min. It was followed by purifying gradient with ACN (99%) from 24th min to 28th min and finished by the decrease of ACN concentration back to 34%.

For UV detection, the wave length $\lambda=228$ nm was used, retention time was 17.82 min for paclitaxel, 17.87 min for SB-T-0035 and 20.85 min for SB-T-1216. The areas of peaks of individual taxanes were finally compared for tested cell lines to determine relative taxane level.

Molecular modeling studies

The human ABCB1 sequence was retrieved from the UniProt database with the accession ID: P08183. An ABCB1 homology model was constructed using the Swiss-Model automated homology model server (Biasini et al., 2014). The sequence identity of the target and template protein was 87.36%. This value indicated that the P-glycoprotein (PDB: 4Q9I) was a good model for use as a template (Szewczyk et al., 2015). The homology model of ABCB1 was further utilized for the docking studies.

The protein was prepared using the Protein Preparation Wizard in Schrodinger following a standard protocol described elsewhere (Sastry et al., 2013). Hydrogen atoms were added and minimization was done using OPLS_2005 force field. This ABCB1-homology model was further used to predict the potential small-molecule binding site using the SiteMap (SiteMap, version 3.3, Schrodinger, LCC, New York, 2014). This module in Schrodinger employs three stages of calculation-finding of appropriate sites, mapping the sites and evaluating the sites for possible binding region of ligand molecules. The receptor grid was generated around the predicted binding site with a scaling factor of 1 Å and a partial cutoff charge of 0.25 Å. Ligand docking was confined to the enclosing box of 20 Å to the selected binding site. The compounds were prepared using the LigPrep module (LigPrep, version 3.2, Schrodinger, LCC, New York, 2014) and possible ionization states were generated for neutral pH. The docking calculations were carried out using the Glide Extra Precision (Glide XP) mode with default parameters (Glide, version 6.5, Schrodinger, LCC, New York, 2014), followed by post-docking minimization to maintain the accuracy of the binding complex. The 10 most

favorable docking poses for each ligand were selected for structural analysis. The mean docking scores, and hydrogen bond amino acids were provided for each protein-ligand complex.

Statistical analysis

Statistical significance of differences was determined using the Student's t-test. $P < 0.05$ and $P < 0.01$ were considered statistically significant at the 5% and 1% levels, respectively.

Results

Three groups of tested taxanes

In this study we tested three groups of taxanes. The first group, including classical paclitaxel, is represented by taxanes containing phenyl groups at both the C3' (R_1) and C3'N (R_2) positions, i.e. deacetyl paclitaxel, paclitaxel, and SB-T-0035. Taxanes from this group have different chemical groups at the C10 (R_3) position (Fig. 1).

The second group contains taxanes with a phenyl at either the C3' (R_1) or C3'N (R_2) positions and with a non-aromatic group at the second position, i.e. *tert*-butoxycarbonyl at the C3'N position or 2-methylpropenyl at the C3' position. This group of taxanes includes classical docetaxel, acetyl docetaxel, and SB-T-1212N1. The analogs of this group have two different types of substituents at the C10 (R_3) position (Fig. 1).

The third group is represented by taxanes bearing a non-aromatic 2-methylpropenyl group at the C3' (R_1) position and a non-aromatic *tert*-butoxycarbonyl group at the C3'N (R_2) position. This group of taxanes is represented by SB-T-1211, SB-T-1102, SB-T-1216, and SB-T-1214. Taxanes of this group have different substituents at the C10 (R_3) position (Fig. 1).

Characterization of paclitaxel-sensitive and paclitaxel-resistant counterpart cells

We established, from original paclitaxel-sensitive SK-BR-3 and MCF-7 cell lines, paclitaxel-resistant counterpart cell sublines SK-BR-3/PacR and MCF-7/PacR via adaptation to gradually increasing concentrations of paclitaxel. The established paclitaxel-resistant SK-BR-3/PacR and MCF-7/PacR cells display long-term survival and proliferation in a culture medium with 100 nM paclitaxel (SK-BR-3/PacR) or 300 nM paclitaxel (MCF-7/PacR). Application of these concentrations of paclitaxel results in cell death in most cells of the original lines within 36 h (Němcová-Furstová et al., 2016).

Activation of executioner caspase-3 and caspase-7 was not detected in SK-BR-3/PacR cells after 100 nM paclitaxel application, while activation of these caspases in original sensitive SK-BR-3 was clearly seen. Similar data were obtained for caspase-7 in MCF-7/PacR *versus* original MCF-7 cells. There is no functional caspase-3 in MCF-7 cells (Němcová-Furstová et al., 2016).

We found that the expression of ABCB1 (PgP) and ABCC3/MRP3 transporters are significantly upregulated in both resistant sublines SK-BR-3/PacR and MCF-7/PacR (Fig. 2A). Employing ABCB1 silencing by a specific siRNA, we tested whether the

overexpression of ABCB1 was responsible for developed resistance to paclitaxel. Silencing of the ABCB1 expression to nearly undetectable level resulted in a significant decrease in the number of surviving cells 96 h after paclitaxel application in both resistant sublines. It was a decrease to about 70% of the number of control cells (without paclitaxel) for SK-BR-3/PacR cells and about 20% for MCF-7/PacR cells (Fig. 2B). Such differing effects of ABCB1 silencing on the number of surviving SK-BR-3/PacR cells and MCF-7/PacR cells after paclitaxel application could simply reflect differing dependence of the resistance of these sublines on ABCB1 transporter.

Effect of tested taxanes on growth and survival of paclitaxel-sensitive and paclitaxel-resistant cells

We assessed the effect of tested taxanes on growth and survival of paclitaxel-sensitive and corresponding paclitaxel-resistant cells. Taxane concentrations 10–300 nM for SK-BR-3 cells and 3–3000 nM for MCF-7 cells were used.

Data for the first group (phenyl groups at both C3' and C3'N positions) of tested taxanes are shown in Fig. 3. Data for the second group (phenyl at either C3' or C3'N position and a non-aromatic substituent at the other position) of taxanes are shown in Fig. 4. Data for the third group (non-aromatic substituents at both C3' and C3'N positions) are in Fig. 5.

The results are summarized in Table 1. The C_0 values (taxane concentration for which there is no increase or decrease in the number of cells of the inoculum after 96-hour incubation) concerning individual tested taxanes for sensitive and resistant SK-BR-3 as well as MCF-7 cells are presented. Furthermore, fold increase of the C_0 value for resistant cells compared with the C_0 value for sensitive cells is also presented. In the first group of taxanes, the increase in the C_0 value for resistant cells was between $8.6 \times$ and more than $13.6 \times$ in the case of SK-BR-3 cells and between $26 \times$ and $132 \times$ in the case of MCF-7 cells. In the second group, the increase in the C_0 value was between $0.5 \times$ and $4.5 \times$ for SK-BR-3 cells and between $8.0 \times$ and $14.1 \times$ for MCF-7 cells. As to the third group, the increase in the C_0 value was between $1.6 \times$ and $3.5 \times$ for SK-BR-3 cells and between $2.8 \times$ and $5.9 \times$ for MCF-7 cells. We can see that the C_0 increase in the first group of taxanes was noticeably higher when compared with the C_0 increase in the second and third group of taxanes for both SK-BR-3 and MCF-7 cells.

Establishment of MCF-7 subline resistant to SB-T-0035

Like in the case of established paclitaxel-resistant counterpart cell sublines, we established SB-T-0035-resistant cell subline MCF-7/SB-T-0035R, from the original SB-T-0035-sensitive MCF-7 cell line, by adaptation to gradually increasing concentrations of SB-T-0035.

Cells of the established SB-T-0035-resistant subline MCF-7/SB-T-0035R display long-term survival and proliferation in a medium containing 300 nM SB-T-0035. Cells of the original sensitive MCF-7 line cultured with such SB-T-0035 concentration die nearly completely within 96 h (see Fig. 7).

Characterization of SB-T-0035-resistant cells

As with the paclitaxel-resistant sublines, we found that the expression of ABCB1 (PgP) transporter is significantly upregulated in the SB-T-0035-resistant subline of MCF-7 cells (Fig. 6A).

Again, we tested whether the overexpression of ABCB1 was responsible for the developed resistance using ABCB1 silencing. Silencing of ABCB1 expression by a specific siRNA to suitable level led to a significant decrease in the number of surviving cells 96 h after SB-T-0035 application in SB-T-0035-resistant cells. It was a decrease to about 30% of the number of control cells (without SB-T-0035) (Fig. 6B).

Effect of tested taxanes on growth and survival of SB-T-0035-resistant cells

We also assessed the effect of some of the tested taxanes on growth and survival of SB-T-0035-sensitive and SB-T-0035-resistant MCF-7 cells (see previous two sections) over a range of concentrations (3–3000 nM).

Data for paclitaxel and SB-T-0035 (the first group of taxanes with phenyl groups at both C3' and C3'N positions) and SB-T-1216 (the third group of taxanes with non-aromatic substituents at both C3' and C3'N positions) are in Fig. 7. The results are summarized in Table 2. The C_0 values concerning individual tested taxanes for sensitive and resistant MCF-7 cells are presented. Fold increase of the C_0 value for resistant cells compared with the C_0 value for sensitive cells is also shown. The increase in the C_0 value for resistant MCF-7 cells was $95 \times$ and $133 \times$ for paclitaxel and SB-T-0035 (the first group of taxane derivatives), respectively, and $2.0 \times$ for SB-T-1216 (the third group of taxanes). Again, we can see that the C_0 increase in the first group of taxanes was significantly higher compared with the C_0 increase in the third group of taxanes.

Effect of tested taxanes on ATPase activity of ABCB1 transporter

We assessed the effect of some of the tested taxanes (300 nM) on the ATPase activity of the ABCB1 (PgP) transporter employing a non-cellular system, i.e. membranes containing human recombinant ABCB1 (see “Materials and Methods”).

We found that all three tested taxanes (paclitaxel, SB-T-0035, and SB-T-1216) increased basal activity of the ATPase. However, the increase in the ATPase activity was 1.7–2.1 times higher for taxanes from the first group (paclitaxel, SB-T-0035) than for the taxane from the third group (SB-T-1216). The difference was not found to be statistically significant. When comparing the increase in the basal ATPase activity after the application of tested taxanes and the application of verapamil (plus control), the increase after the application of taxanes was 2.8–5.8 times lower than after verapamil application (Fig. 8).

Intracellular level of tested taxanes after their application in paclitaxel-resistant and SB-T-0035-resistant cells

Using a HPLC system (see “Materials and Methods”), we assessed intracellular level of some of tested taxanes after their application (300 nM) in sensitive, paclitaxel-resistant and SB-T-0035-resistant MCF-7 cells. We supposed that intracellular level of particular taxane

after the incubation of the cells with this taxane reflects the efficacy of taxane transport out of the cells.

We found that both taxanes from the first group (paclitaxel, SB-T-0035) were transported out of paclitaxel-resistant as well as SB-T-0035-resistant MCF-7 cell very efficiently.

Intracellular level of both taxanes in resistant cells represented only about 5% or less of the level in control sensitive MCF-7 cells. However, intracellular level of the taxane from the third group (SB-T-1216) in resistant cells represented about 40–50% of the level in sensitive cells. It showed that transport of SB-T-1216 out of both types of resistant cells was much less efficient (Fig. 9).

Molecular docking of tested taxanes to the ABCB1 transporter

We performed molecular docking of some of tested taxanes to the human ABCB1 (PgP) transporter (Fig. 10) using Schrodinger docking suite (see “Materials and Methods”).

To investigate the binding affinities computationally, deacetyl paclitaxel, paclitaxel, SB-T-0035, SB-T-1212N1, acetyl docetaxel and SB-T-1216 were docked into the active site of the homology model of the ABCB1 protein. The obtained docking scores were divided into three groups based on the respective substitution of the side chains in the ligand molecules. The first group of taxanes included deacetyl paclitaxel (-10.77 ± 0.21), paclitaxel (-10.12 ± 0.19) and SB-T-0035 (-10.02 ± 0.15) and exhibited the lowest docking scores, i.e. highest predicted binding affinity. This probably reflects the importance of the two phenyl groups at the C3' and C3'N positions in their structures. The second group contained SB-T-1212N1 (-9.89 ± 0.07) and acetyl docetaxel (-9.49 ± 0.18). These taxanes, lacking one phenyl group at either C'3 or C3'N position in their structures, had worse docking scores compared to taxanes of the first group. The last taxane SB-T-1216 (-8.15 ± 0.25), from the third group, bearing no phenyl group at the C'3 and C3'N positions, had a docking score reflecting the lowest predicted binding affinity compared to the other taxanes. The docking predictions are summarized in Table 3.

These results demonstrated that the docking energy score reflecting the predicted free energies of individual taxanes to the ABCB1 transporter, was the lowest (the highest predicted free energy) for taxanes from the first group (deacetyl paclitaxel, paclitaxel, SB-T-0035), i.e. from -10.02 to -10.77 . The taxanes from the second group (acetyl docetaxel, SB-T-1212N1) had lower predicted free energy compared to taxanes from the first group, i.e. from -9.49 to -9.89 . SB-T-1216 from the third group had the lowest predicted free energy to ABCB1, i.e. -8.15 (Table 3.).

Schemes showing molecular interactions of individual taxanes with the ABCB1 transporter at their binding sites are shown in Fig. 11.

Discussion

Previously we have established paclitaxel-resistant sublines of the original sensitive breast cancer cell lines SK-BR-3 and MCF-7 by adaptation to gradually increasing concentrations of paclitaxel (N mcová-Furstová et al., 2016). Interestingly, we found that both paclitaxel-

resistant sublines maintained their sensitivity to taxane SB-T-1216 (Ojima et al., 1996) that was similar to the sensitivity of the original sensitive lines. Furthermore, despite repeated efforts, we were unable to establish variants of the original cell lines resistant to SB-T-1216 (N mcová-Furstová et al., 2016). These findings together with results of subsequent pilot experiment with other taxanes led us to hypothesize that substituents at the C3' and C3'N positions of the taxane structure are critical with regard to capability of overcoming resistance to paclitaxel.

We have decided to test this hypothesis. As an experimental model, we used our previously established paclitaxel-resistant sublines SK-BR-3/PacR and MCF-7/PacR *versus* original paclitaxel-sensitive lines SK-BR-3 and MCF-7. Resistant sublines are capable of long-term survival and near-normal proliferation in a medium with such concentrations of paclitaxel (100 nM for SK-BR-3/PacR and 300 nM for MCF-7/PacR) in which most cells of the original sensitive lines die within 96 h (N mcová-Furstová et al., 2016). Later, within the framework of this study, we established SB-T-0035-resistant subline from the original sensitive MCF-7 cell line using similar approach as used for paclitaxel-resistant sublines (see "Results"). Taxane SB-T-0035 has the same substituents at the C3' and C3'N positions as paclitaxel. We used the model of established SB-T-0035-resistant MCF-7 cells *versus* the original sensitive MCF-7 cells in our experiments in order to confirm results regarding paclitaxel-resistant *versus* paclitaxel-sensitive cells.

Previously, we also found that both paclitaxel-resistant sublines significantly upregulated the expression of ABCB1 (PgP) or ABCC3 (MRP3) transporters which was detected by western blot analysis. Furthermore, effective silencing of ABCB1 expression employing a specific siRNA significantly decreased resistance to paclitaxel in the paclitaxel-resistant cells. It suggests the important role for ABCB1 in mechanisms of paclitaxel resistance (N mcová-Furstová et al., 2016). In this study, we also found that ABCB1 expression in SB-T-0035-resistant sublines of MCF-7 cells is significantly upregulated. Together with this, we confirmed that effective silencing of ABCB1 expression by a specific siRNA results in a significant decrease in resistance to SB-T-0035 in SB-T-0035-resistant cells (see Fig. 6). Upregulation of ABCB1 and ABCC3 expression in taxane-resistant variants of breast cancer cells has been demonstrated by several authors (Ajabnoor et al., 2012, Hembruff et al., 2008, O'Brien et al., 2008, Reed et al., 2010, Shi et al., 2014, Wang et al., 2014). However, there are papers indicating that ABCB1 upregulation is not required for the development of paclitaxel resistance in breast cancer cells (Kars et al., 2006, Kenicer et al., 2014).

Data from our pilot experiments pointed to the possibility that only the presence of phenyl groups at both C3' and C3'N positions are responsible for low capability of taxane to overcome acquired resistance to paclitaxel as well as a high probability of developing resistance to this taxane. When there is a nonaromatic group at one or both C3' and C3'N positions, such taxane has higher capability of overcoming resistance to paclitaxel and there is low probability of developing resistance to this taxane. In further studies we tested this hypothesis. In order to do that we compared three groups of taxanes. Taxanes of the first group have phenyl groups at both C3' and C3'N positions. Taxanes of the second group have a phenyl group at either C3' or C3'N position and a non-aromatic group at the other position. The third group involves taxanes with non-aromatic groups at both C3' and C3'N

positions (see Fig. 1). The role of substituents at the C3' and C3'N positions of the taxane structure in resistance of tumor cells has been previously studied (Ojima et al., 1994, Ojima et al., 1996, Ojima et al., 1997, Ojima et al., 2000). However, these studies were not focused on acquired resistance to paclitaxel.

The effect of individual taxanes from the three mentioned groups on tested cells was assessed by the C_0 value (see "Results"). Then the degree of resistance of paclitaxel-resistant cells to individual taxanes was assessed by the increase of the C_0 value of resistant cells when compared with the C_0 value of corresponding sensitive cells (see Table 1). In the third group of taxanes with non-aromatic groups at both C3' and C3'N positions, the increase in the C_0 value of resistant cells was 1.6 – 3.5 times for SK-BR-3 cells and 2.8 – 5.9 times for MCF-7 cells. As to the second group of taxanes with one phenyl and one non-aromatic group at the C3' and C3'N positions respectively, the increase in the C_0 value for resistant cells was similar or somewhat higher. It was 0.5 – 4.5 times for SK-BR-3 cells and 8.0 – 14.1 times for MCF-7 cells. Presented unexpected increase (only 0.5 times) in the C_0 value for acetyl docetaxel and resistant SK-BR-3 cells (see Table 2) may represent just a stochastic fluctuation. However, in the first group of taxanes with phenyl groups at both C3' and C3'N positions, the increase in the C_0 value was noticeable. It was 8.6 – 13.6 times for SK-BR-3 cells and 26 – 132 times for MCF-7 cells. The data show that the increase in the C_0 value for resistant cells is significantly higher for taxanes of the first group compared with the increase in the C_0 value for taxanes of the second and third group. Thus, these results confirm our hypothesis that phenyl groups at both C3' and C3'N positions of taxane is associated with low capability of taxane to overcome acquired paclitaxel resistance compared with taxanes containing at least one non-aromatic substituent at the C3' and C3'N positions. To the best of our knowledge, this is the first direct confirmation of such fact (see Ojima et al., 1994, Ojima et al., 1996, Ojima et al., 1998, Ojima et al., 2008). Our finding is strongly supported by the data obtained with SB-T-0035-resistant cells (see Table 2). Similarly to paclitaxel, SB-T-0035 has phenyl groups at both C3' and C3'N positions.

As with paclitaxel-resistant SK-BR-3 and MCF-7 cells (N mcová-Furstová et al., 2016), we detected significant upregulation of the expression of ABCB1 (PgP) transporter in SB-T-0035-resistant MCF-7 cells (see Fig. 6A). It points at the possibility that upregulation of ABCB1 transporter can play an important role in acquired resistance to paclitaxel, as it was shown by several groups previously (Aldonza et al., 2016, Kathawala et al., 2015, Reed et al., 2010), and also to SB-T-0035. The possibility was confirmed by our experiments with paclitaxel-resistant and SB-T-0035-resistant cells by inhibiting ABCB1 expression using a specific siRNAs (N mcová-Furstová et al., 2016, see Fig. 6B).

Increased level of ABCB1 in cells resistant to docetaxel and cabazitaxel (taxanes belonging to the second group of taxanes) (Duran et al., 2014, Li et al., 2014, Ojima et al., 1996, Ojima et al., 1998) and transport of novel taxane BMS-275,183 (taxane belonging to the third group of taxanes) by ABCB1 were described previously (Marchetti et al., 2014). The ability of taxanes SB-T-1212 and SB-T-1213 (with non-aromatic groups at C3' and C3'N positions) to overcome established P-glycoprotein-related resistance was also described (Ferlini et al., 2000, Ojima et al., 1996). Thus the hydrophobicity of molecules, which was highest for

taxanes from the first group of taxanes, seems to be a key feature of molecules to be transported by ABCB1 (Liu et al., 2013, Ojima et al., 1998,).

The visual inspection of the docking poses of deacetyl paclitaxel, paclitaxel and SB-T-0035, from the first group of taxanes, acetyl docetaxel and SB-T-1212N1, from the second group of taxanes, and SB-T-1216 from the third group of taxanes, provided almost identical interactions with few exceptions in the binding conformation/orientation of the compounds to the active site of the ABCB1 protein (see Fig. 11). The 5 Å surrounding region of the bound compound was mostly occupied by hydrophobic amino acid residues. The two phenyl groups at the C3' and C3'N positions are buried into the hydrophobic cleft of the ABCB1 protein, indicating a tight binding of deacetyl paclitaxel, paclitaxel, SB-T-0035, acetyl docetaxel and SB-T-1212N1. Docking energy scores of taxanes of all three taxane groups reflect the importance of substitutions at the C3' and C3'N positions of the C13 side chain of taxanes. The importance of hydrophobic interactions was documented previously. Phe 339 of ABCB1 molecule was found to form a gate for entry of paclitaxel into its binding site, while several other Phe residues (Phe71, Phe332, Phe728) helped paclitaxel to be stabilized in the binding site of ABCB1 (Zhang et al., 2015).

Further, hydrogen bonding interaction was predicted between the protein-ligand complexes. The hydrogen bond involving the polar carboxyl group of Ser337 is maintained by the oxygen atom (=O) near the NH of all reported taxanes and it helps to keep the same orientation and position of the phenyl group in deacetyl paclitaxel, paclitaxel, SB-T-0035 and SB-T-1212N1. On the other hand, acetyl docetaxel and SB-T-1216 lack the phenyl group near the oxygen atom (=O), which results in the slight change in the orientation of the binding pose. Moreover, the interaction of Gln347 was observed with the carbonyl oxygen atom, which is connected to the phenyl group at the C2 position of deacetyl paclitaxel, SB-T-0035, acetyl docetaxel and SB-T-1212N1. For so far unknown reason, this interaction is not present in complexes with paclitaxel and SB-T-1216. Additionally, the interaction of side chain of the Gln946 was predicted in the baccatin core of all tested taxanes.

Docking scores reflecting predicted free energy of taxanes to ABCB1 transporter correspond to the three groups of tested taxanes (see Table 3). Taxanes from the first group (two phenyl groups at the C3' and C3'N positions) have the highest predicted free energy, taxanes from the second group (one phenyl and one non-aromatic substituent at the C3' and C3'N positions) have intermediate predicted free energy, and taxane from the third group (two non-aromatic substituents at the C3' and C3'N positions) has the lowest predicted free energy. Lower predicted free energy means less effective transport out of cancer cell and *vice versa*. These data are in agreement with our data concerning the efficacy of taxane transport out of cells (see Figure 9). The higher capability of taxanes, with at least one non-aromatic group at the C3' and C3'N positions, to overcome acquired resistance to paclitaxel as well as reduced probability of developing resistance is very likely based on their less effective transport out of cancer cell. The affinity of taxane to the ABCB1 transporter was reported to be strongly affected by the substituent at the position C10 previously (Ferlini et al., 2000). However, authors did not use the human ABCB1 transporter in their molecular docking study. We did not confirm that finding in our study when comparing taxanes from the first group.

We can summarize that the high capability of a taxane to overcome acquired resistance of breast cancer cells to paclitaxel and the low probability of developing resistance to this taxane correspond to the type of substituents at the C3' and C3'N positions of the taxane molecule. At least one non-aromatic group at these positions means high capability to overcome acquired resistance and low probability to develop resistance to the taxane. On the other hand, the presence of phenyl groups at both C3' and C3'N positions leads to low capability of overcoming acquired resistance and high probability of developing resistance. These findings are related to the function of the ABCB1 transporter which plays a crucial role in acquired resistance to taxanes.

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Abbreviations

ABC	ATP-binding cassette
ABCB1/MRP1	ATP-binding cassette B1/multidrug resistance protein 1
ABCC3/MRP3	ATP-binding cassette C3/multidrug resistance protein 3
MAPs	microtubule associated proteins
Pgp	P-glycoprotein

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Highlights pro Toxicology and Applied Pharmacology

- Taxane C3' and C3'N substituents determine whether taxane overcomes acquired resistance.
- At least one non-aromatic substituent at these positions produces capability of overcoming acquired resistance.
- Transport of taxanes with such substituent out of cancer cells seems to be less effective.

A **B**

GROUP OF TAXANES	TAXANE	C3' (R ₁)	C3'N (R ₂)	C10 (R ₃)
1	DEACETYL PACLITAXEL	phenyl	phenyl	- H
	PACLITAXEL	phenyl	phenyl	- CO-CH ₃
	SB-T-0035	phenyl	phenyl	- CO-N(CH ₃) ₂
2	DOCETAXEL	phenyl	- O-C(CH ₃) ₃	- H
	ACETYL DOCETAXEL	phenyl	- O-C(CH ₃) ₃	- CO-CH ₃
	SB-T-1212N1	- CH=C(CH ₃) ₂	Phenyl	- CO-CH ₃
3	SB-T-1211	- CH=C(CH ₃) ₂	- O-C(CH ₃) ₃	- H
	SB-T-1102	- CH-CH(CH ₃) ₂	- O-C(CH ₃) ₃	- CO-CH ₃
	SB-T-1216	- CH=C(CH ₃) ₂	- O-C(CH ₃) ₃	- CO-N(CH ₃) ₂
	SB-T-1214	- CH=C(CH ₃) ₂	- O-C(CH ₃) ₃	- CO-◻

- CH=C(CH₃)₂: 2-methylpropenyl, -CH-CH(CH₃)₂: 2-methylpropyl, -O-C(CH₃)₃: *tert*-butoxycarbonyl, -CO-CH₃: acetyl, -CO-N(CH₃)₂: dimethylcarbamoyl, -CO-◻: cyclopropanecarboxyl

Fig. 1.

A) Chemical structure of taxanes. R₁, R₂ and R₃ represent substituents at the C3', C3'N and C10 positions, respectively. B) Groups of tested taxanes. Substituents at the positions C3' (substituent R₁), C3'N (substituent R₂) and C10 (substituent R₃) are described.

Fig. 2.

(A) The level of ABCB1 and ABCC3 transporters in paclitaxel-sensitive (sen) and paclitaxe-resistant (res) SK-BR-3 and MCF-7 cells. (B) The effect of ABCB1 silencing on the growth and survival of paclitaxel-resistant SK-BR-3 and MCF-7 cells after paclitaxel treatment. (A) After 24 h of incubation with paclitaxel (100 nM for SK-BR-3 and 300 nM for MCF-7) the levels of ABC transporters were determined using western blot analysis and relevant antibodies (see “Materials and Methods”). Actin levels were used to confirm equal protein loading. The data shown were obtained in one representative experiment of three independent experiments. Western blot quantification by densitometry is shown. Data are presented as the mean of relative density $\pm$ SEM. * $P < 0.05$ when comparing the density in sensitive and resistant cells. (B) The cells were prepared as described in “Materials and

Methods” and seeded at 20×10^3 cells/100 μ l of medium per well. The relative number of living sensitive cells, resistant cells (no siRNA), resistant cells treated with non-specific siRNA (ns siRNA) and resistant cells treated with an ABCB1 specific siRNA (ABCB1 siRNA) was determined after 96 h of incubation without paclitaxel (control cells) or with paclitaxel (100 nM for SK-BR-3 and 300 nM for MCF-7). Each column represents the mean of 4 separate culture $\pm$ SEM. ** $P < 0.01$ when comparing the effect in cells without paclitaxel and treated with paclitaxel. ++ $P < 0.01$ when comparing the effect in ns siRNA-treated and ABCB1 siRNA-treated cells after paclitaxel application. The data shown were obtained in one representative experiment of three independent experiments. The effect of non-specific siRNA (ns siRNA) and specific siRNA (ABCB1 siRNA) on ABCB1 expression in paclitaxel-resistant SK-BR-3 and MCF-7 cells is also shown. Actin levels were used to confirm equal protein loading.

A SK-BR-3 **B MCF-7** **Fig. 3.**

Effect of deacetyl paclitaxel, paclitaxel and SB-T-0035 on the growth and survival of paclitaxel-sensitive (sen) and paclitaxel-resistant (res) (A) SK-BR-3 cells (10–300 nM taxane) and (B) MCF-7 cells (3–3000 nM taxane). The number of cells of the inoculum (20×10^3 cells/100 μ l of medium per well) is shown as a dotted line. The number of living cells was determined after 96 h of incubation (see “Materials and Methods”). Each column represents the mean of three separate experiments $\pm$ SEM.

A SK-BR-3 **B MCF-7** **Fig. 4.**

Effect of docetaxel, acetyl docetaxel and SB-T-1212N1 on the growth and survival of paclitaxel-sensitive (sen) and paclitaxel-resistant (res) (A) SK-BR-3 cells (10–300 nM taxane) and (B) MCF-7 cells (3–3000 nM taxane). The number of cells of the inoculum (20×10^3 cells/100 μ l of medium per well) is shown as a dotted line. The number of living cells was determined after 96 h of incubation (see “Materials and Methods”). Each column represents the mean of three separate experiments $\pm$ SEM.

Fig. 5.

Effect of SB-T-1211, SB-T-1102, SB-T-1216 and SB-T-1214 on the growth and survival of paclitaxel-sensitive (sen) and paclitaxel-resistant (res) (A) SK-BR-3 cells (10–300 nM taxane) and (B) MCF-7 cells (3–3000 nM taxane). The number of cells of the inoculum (20×10^3 cells/100 μ l of medium per well) is shown as a dotted line. The number of living cells was determined after 96 h of incubation (see “Materials and Methods”). Each column represents the mean of three separate experiments $\pm$ SEM.

Fig. 6.

(A) The level of ABCB1 transporter in SB-T-0035-sensitive (sen) and SB-T-0035-resistant (res) MCF-7 cells. (B) The effect of ABCB1 silencing on the growth and survival of SB-T-0035-resistant MCF-7 cells after SB-T-0035 treatment. (A) After 24 h of incubation with 300 nM SB-T-0035 the level of ABCB1 transporter was determined using western blot analysis and relevant antibody (see “Materials and Methods”). Actin levels were used to confirm equal protein loading. The data shown were obtained in one representative experiment of two independent experiments. (B) The cells were prepared as described in “Materials and Methods” and seeded at 20×10^3 cells/100 μ l of medium per well. The relative number of living sensitive cells, resistant cells (no siRNA), resistant cells treated with nonspecific siRNA (ns siRNA) and resistant cells treated with an ABCB1 specific siRNA (ABCB1 siRNA) was determined after 96 h of incubation without SB-T-0035 (control cells) or with 300 nM SB-T-0035. Each column represents the mean of 4 separate culture $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ when comparing the effect in cells without SB-T-0035

and treated with SB-T-0035. +++ $P < 0.001$ when comparing the effect in ns siRNA-treated and ABCB1 siRNA-treated cells after SB-T-0035 application. The data shown were obtained in one representative experiment of three independent experiments. The effect of non-specific siRNA (ns siRNA) and specific siRNA (ABCB1 siRNA) on ABCB1 expression in SB-T-0035-resistant MCF-7 cells is also shown. Actin levels were used to confirm equal protein loading.

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Fig. 7.

Effect of paclitaxel, SB-T-0035 and SB-T-1216 (3–3000 nM) on the growth and survival of SB-T-0035-sensitive (sen) and SB-T-0035-resistant (res) MCF-7 cells. The number of cells of the inoculum (20×10^3 cells/100 μ l of medium per well) is shown as a dotted line. The number of living cells was determined after 96 h of incubation (see “Materials and Methods”). Each column represents the mean of three separate experiments $\pm$ SEM.

Fig. 8.

Effect of paclitaxel, SB-T-0035 and SB-T-1216 (300 nM) on the ATPase activity of ABCB1 transporter. To assess the ATPase activity of ABCB1 (PgP) transporter, commercial Pgp-Glo™ Assay System with P-glycoprotein kit (Promega) using membranes with human recombinant P-glycoprotein was used (see “Materials and Methods”). The ATPase activity is expressed as relative light units (RLU). The ATPase activity after verapamil application was used as a positive control and after sodium orthovanadate application as a negative control. Basal ATPase activity of ABCB1 transporter (without taxane, verapamil or vanadate application) is shown. Each column represents the mean of two independent experiments $\pm$ SEM.

Fig. 9.

Level of paclitaxel, SB-T-0035 and SB-T-1216 after their application in sensitive MCF-7 cells, paclitaxel-resistant MCF-7/PacR cells and SB-T-0035-resistant MCF-7/SB-T-0035R cells. After 24 h of incubation with 300 nM concentration of tested taxane relative taxane levels were assessed using a HPLC system (see “Materials and Methods”). Levels of individual taxanes in sensitive MCF-7 cells were used as controls (100%). Each column represents mean of two independent experiments $\pm$ SEM.

Fig. 10.
Structure of the human ABCB1 transporter with bound deacetyl paclitaxel.

Fig. 11.

Visualization of representative docking poses of tested taxanes (deacetyl paclitaxel, paclitaxel, SB-T-0035, acetyl docetaxel, SBT-1212N1, and SB-T-1216) into the ABCB1 binding site. The hydrogen bonding interactions are shown as green dotted lines.

Table 1

Comparison of the effect of tested taxanes on the growth and survival of paclitaxel-sensitive (SEN) and paclitaxel-resistant (RES) SK-BR-3 and MCF-7 cells.

TAXANE	SK-BR-3			MCF-7		
	C ₀ /SEN (nM)	C ₀ /RES (nM)	INCREASE (x)	C ₀ /SEN (nM)	C ₀ /RES (nM)	INCREASE (x)
DEACETYL PAC.	22	> 300	> 13.6	138	3600	26.1
PACLITAXEL	29	250	8.6	27	2500	92.6
SB-T-0035	23	260	11.3	19	2500	132
DOCETAXEL	22	98	4.5	25	200	8.0
ACETYL DOC.	52	26	0.5	17	240	14.1
SB-T-1212N1	12	27	2.2	18	230	12.8
SB-T-1211	20	70	3.5	29	170	5.9
SB-T-1102	22	45	2.0	25	84	3.4
SB-T-1216	32	72	2.2	51	141	2.8
SB-T-1214	41	67	1.6	< 30	114	> 3.8

C₀ represents taxane concentration (nM) for which there is not any increase or any decrease of the number of cells of the inoculum (20×10^3 cells/100 μ l of medium per well) after 96 h of incubation. Fold increase (x) of C₀ value for resistant cells in comparison with C₀ value for sensitive cells is shown.

Table 2

Comparison of the effect of tested taxanes on the growth and survival of SB-T-0035-sensitive (SEN) and SB-T-0035-resistant (RES) MCF-7 cells.

TAXANE	MCF-7		
	C ₀ /SEN (nM)	C ₀ /RES (nM)	INCREASE (x)
PACLITAXEL	20	1900	95
SB-T-0035	18	2400	133
SB-T-1216	66	135	2.0

C₀ represents taxane concentration (nM) for which there is not any increase or any decrease of the number of cells of the inoculum (20×10^3 cells/100 μ l of medium per well) after 96 h of incubation. Fold increase (x) of C₀ value for resistant cells in comparison with C₀ value for sensitive cells is shown.

Table 3

The docking score and hydrogen bond interactions of the ABCB1 transporter with tested taxanes. The values are based on the average score of top ten conformations.

Taxane	Docking conformations	Score (kcal/mol)	Hydrogen bond
DEACETYL PACLITAXEL	10	-10.77±0.21	S 337, Q 347, Q 946
PACLITAXEL	10	-10.12±0.19	S 337, Q 946
SB-T-0035	10	-10.02±0.15	S 337, Q 347, Q 946
SB-T-1212N1	10	-9.89±0.07	S 337, Q 347, Q 946
ACETYL DOCETAXEL	10	-9.49±0.18	S 337, Q 347, Q 946
SB-T-1216	10	-8.15±0.25	S 337, Q 946