

ABCG2 mediates differential resistance to SN-38 and homocamptothecins

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Abstract

One activity potentially limiting the efficacy of camptothecin anticancer agents is their cellular efflux by the ATP binding cassette half-transporter, ABCG2.

Homocamptothecins are novel anticancer drugs that inhibit topoisomerase I with a greater potency than camptothecins. Homocamptothecins differ from camptothecins by their E-ring, which is seven-membered instead of the six-membered ring of camptothecins. We report herein that, like camptothecins, homocamptothecin and its difluoro derivative BN80915 are substrates for ABCG2. However, the resistance of 3 selected cell lines overexpressing wild-type or mutant ABCG2 to homocamptothecin or BN80915 was less than resistance to SN-38, indicating that both the 7-membered E-ring present in homocamptothecin and the A and B ring modifications present in SN-38 are involved in substrate recognition by ABCG2. HEK-293 cells transfected with vectors encoding wild-type or mutant ABCG2 were found to be less resistant to both homocamptothecins than to SN-38. However, transfectants overexpressing mutant ABCG2 had relative resistance values for homocamptothecin and BN80915 4- to 14-fold higher than cells expressing wild-type ABCG2, suggesting that the gain of function resulting from mutation at amino acid 482, while not affecting SN-38, extends to the homocamptothecins. Resistance was reversed by the ABCG2 inhibitor fumitremorgin C. BN80915 was 17-fold more potent than SN-38 in wild-type ABCG2-transfected cells, suggesting that BN80915 has the potential to overcome ABCG2-related resistance to SN-38, the active metabolite of CPT-11 (irinotecan).

Camptothecins are among the most potent anticancer drugs introduced for cancer treatment in the recent years. Two semi-synthetic derivatives of camptothecin (CPT) are currently approved by the FDA: irinotecan for first line chemotherapy of colon cancers and topotecan for the treatment of refractory ovarian cancers. Both derivatives bear substitutions on the A and B rings of CPT (Fig. 1), which provide water solubility and enhanced potency against Top1 (Tanizawa et al., 1994), the selective target of these drugs (Hsiang et al., 1985) (Chen and Liu, 1994) (<http://discover.nci.nih.gov/pommier/topo1.htm>). Human carboxylesterase converts CPT-11 (irinotecan) to its active metabolite, SN-38 (Fig. 1).

One of the limitations of camptothecins is the presence of and the requirement for an alpha-hydroxylactone in the E-ring for Top1 inhibition and antitumor activity (Hsiang et al., 1989; Giovanella et al., 1989; Jaxel et al., 1989). Because of this alpha hydroxylactone, the E-ring can hydrolyze readily at physiological pH and generate carboxylate forms of camptothecins. Carboxylate derivatives are inactive or weakly active against Top1 (Hsiang et al., 1989; Jaxel et al., 1989). They also bind avidly to and are sequestered by human serum albumin, which shifts the equilibrium between lactone and carboxylate away from the active lactone (Burke and Mi, 1994). To overcome the E-ring instability, the group of Bigg and Lavergne (Lavergne et al., 1998) synthesized homocamptothecins, which differ from CPT by an E-ring containing an additional carbon, resulting in a beta-hydroxylactone and a seven-membered E-ring. This modification decreases the rates of hydrolysis and conversion to the carboxylate, and also prevents the reverse reaction; i.e. the conversion of the homocamptothecin carboxylate to

lactone (Lavergne et al., 1998; Lesueur-Ginot et al., 1999). Interestingly, homocamptothecins are more potent Top1 inhibitors than camptothecins and retain potent antitumor activity (Lavergne et al., 1998; Bailly et al., 1999; Lavergne et al., 2000; Larsen et al., 2001; Lansiaux et al., 2001). A difluoro homocamptothecin derivative, BN80915, whose Top1 and antitumor activity in model systems are greater than those of homocamptothecin (Larsen et al., 2001; Lansiaux et al., 2001), is being pursued for clinical development.

Another limitation of camptothecins is the resistance of cells overexpressing the ATP-binding cassette half-transporter, BCRP/ABCP/MXR/ABCG2 (Allikmets et al., 1998; Doyle et al., 1998; Miyake et al., 1999). ABCG2 mediates resistance to mitoxantrone as well as to the camptothecins topotecan, SN-38, and 9-aminocamptothecin (Brangi et al., 1999; Rajendra et al., 2003). Acquired mutations that replace the wild-type arginine with a glycine or a threonine at amino acid 482 in the ABCG2 gene alter the substrate specificity of the resulting protein. Cells overexpressing the mutant ABCG2 proteins exhibit a gain of function and add rhodamine 123 and the anthracyclines to the list of substrates (Robey et al., 2001; Honjo et al., 2001). ABCG2-mediated resistance is effectively inhibited by fumitremorgin C (FTC) (Rabindran et al., 1998). Topotecan has also been shown to be a P-glycoprotein substrate (Hoki et al., 1997).

In order to determine whether homocamptothecins are ABCG2 substrates, we compared the cytotoxicity of homocamptothecin and its clinical difluoro derivative, BN80915, to CPT and to SN-38 in three ABCG2-overexpressing selected cell lines expressing either wild type (R482) or mutant (R482T, R482G) ABCG2. Cross-resistance

studies were also carried out with human embryonic kidney cells (HEK-293) stably transfected with either of the three ABCG2 proteins to exclude bias due to other potential mechanisms of resistance in the selected cell lines. We also examined the ability of FTC to reverse resistance to these compounds in the ABCG2 transfectants. Finally, we attempted to develop drug-resistant cell lines by selecting them in increasing concentrations of BN80915.

Materials and Methods

Cell Lines

The human colon carcinoma cell line S1 and its ABCG2-overexpressing S1-M1-80 subline were cultured in RPMI with the S1-M1-80 cells maintained in 80 μ M of mitoxantrone (Rabindran et al., 1998). The MCF-7 AdVp3000 subline was maintained in 3000 ng/ml adriamycin in the presence of 5 μ g/ml verapamil (Chen et al., 1990); the MX100 subline was maintained in 100 ng/ml mitoxantrone (Robey et al., 2001). Camptothecin resistance in the S1-M1-80, MCF-7 AdVp3000 and MCF-7 MX100 cell lines has previously been described (Brangi et al., 1999; Litman et al., 2000). The MCF-7 breast carcinoma cell line and its ABCG2-overexpressing sublines were grown in IMEM. HEK-293 cells were transfected with either empty pcDNA3.1 vector (Invitrogen, Carlsbad, CA) or pcDNA3.1 vector containing full-length ABCG2 encoding either an arginine, threonine or glycine at amino acid 482. Stable transfectants were maintained in Eagle's Minimum Essential Medium (ATCC, Manassas, VA) supplemented with 10% FCS, penicillin and streptomycin with G418 at a concentration of 2 mg/ml. The transfected cell lines have previously been described (Robey et al., 2003).

Top 1-Mediated DNA Cleavage Reactions

Human recombinant Top1 was purified from Baculovirus as described previously (Strumberg et al., 1999; Pourquier et al., 1999). The 161 bp fragment from pBluescript SK(-) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonuclease Pvu II and Hind III (New England Biolabs, Beverly, MA) in supplied NE buffer 2 (50 μ L reactions) for 1 h at 37°C, and separated by electrophoresis in a 1% agarose gel made in 1X TBE buffer. The 161 bp fragment was eluted from the gel slice using the QIAEX II kit (QIAGEN Inc., Valencia, CA). Approximately 200 ng of the fragment were 3'-end labeled at the Hind III site by fill-in reaction with [α -³²P]-dGTP and 0.5 mM dATP, dCTP, and dTTP, in React 2 buffer (50 mM Tris-HCl, pH 8.0, 100 mM MgCl₂, 50 mM NaCl) with 0.5 units of DNA polymerase I (Klenow fragment). Unincorporated ³²P-dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 161 bp 3'-end-labeled DNA fragment was collected. Aliquots (approximately 50,000 dpm/reaction) were incubated with Top1 at 25°C for the indicated times in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration). The samples (10 μ L) were mixed with 30 μ L of loading buffer (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue, pH 8.0). Aliquots were separated in a denaturing gel (16% polyacrylamide, 7 M urea). Gels were dried and visualized using a PhosphoImager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Cytotoxicity Assays

The assays performed were based on those of Skehan et al (Skehan et al., 1990). Cells were plated in 96-well plates at a density of 2000 cells/well and allowed to attach overnight at 37°C in 5% CO₂. Chemotherapy drugs with or without 5 μM of the ABCG2 inhibitor FTC were added at the desired concentrations and the cells were allowed to incubate for 96 hr. Subsequently, cells were fixed in 50% trichloroacetic acid. Plates were then stained with a sulforhodamine B solution (0.4% sulforhodamine B w/v in 1% acetic acid) and optical densities were read on a plate reader at an absorbance of 540 nm. Each concentration was tested in quadruplicate and controls were performed in replicates of eight.

Flow Cytometry

For studies with the anti-ABCG2 antibody, 5D3 (Zhou et al., 2001), cells were trypsinized and resuspended in DPBS with 2% BSA to which was added phycoerythrin-conjugated 5D3 (eBioscience, San Diego, CA) or phycoerythrin-conjugated mouse IgG according to the manufacturer's instructions. The cells were incubated with antibody for 30 min at room temperature, washed twice with DPBS and kept in the dark until analyzed. Cells were analyzed on a FACSort flow cytometer equipped with a 488 nm argon laser. For all samples, at least 10,000 events were collected. Debris was eliminated

by gating on forward versus side scatter and dead cells were excluded based on propidium iodide staining.

Results

Topoisomerase 1 inhibition by BN80915

Figure 2 shows trapping of Top1 cleavage complexes by BN80915 in comparison with homocamptothecin. Both drugs were comparable in potency and no noticeable difference was observed in the distribution of drug-induced DNA cleavage sites.

To further compare BN80915 and homocamptothecin, the stability of Top1 cleavage complexes was assessed using a salt reversal assay. In this assay, addition of 0.35 M NaCl to the Top1 reactions inhibits Top1-mediated DNA cleavage without blocking Top1-mediated DNA religation, resulting in a shift of the cleavage-religation equilibrium toward religation. CPT is known to act as a Top1 poison by preventing the religation of Top1 cleavage complexes (Hsiang et al., 1985; Tanizawa et al., 1994) (<http://discover.nci.nih.gov/pommier/topo1.htm>). Figure 3 shows that reversal of Top1 cleavage at many sites was slower for BN80915 than for homocamptothecin and CPT. Therefore, BN80915 is more efficient than homocamptothecin and CPT in trapping Top1 cleavage complexes.

Relative Resistance of ABCG2-Overexpressing Selected Cell Lines to Homocamptothecins and SN-38

Table 1 summarizes the results obtained by cytotoxicity assays in the 5 cell lines for the four drugs tested. In agreement with previous results (Brangi et al., 1999), we found that resistance to SN-38 was high in all three drug-selected cell lines overexpressing either wild-type 482R (MCF-7 MX100) or mutant R482T (MCF-7 AdVp3000) or R482G (S1-M1-80) ABCG2. As previously reported (Brangi et al., 1999), all selected cell lines exhibited high levels of resistance to SN-38 (500-fold, 991-fold and 1485-fold in MCF-7/MX100, MCF-7/AdVp3000, and S1-M1-80 cells, respectively) and resistance to CPT was minimal (Table 1). Relative resistance (RR) values for homocamptothecin and BN80915 were comparable to that for SN-38 in MCF-7 AdVp3000 cells while MCF-7 MX100 and S1-M1-80 cells exhibited a marked reduction in RR values. Differences in IC_{50} values for resistant versus parental cell lines were significant in all cases ($p < 0.05$) except for one cell lines with BN80195 (MCF-7 MX100, $p = 0.12$) and one cell line for CPT (MCF-7 MX100, $p = 0.13$).

Relative Resistance of ABCG2-transfected cells to Homocamptothecins and SN-38 and Reversal by FTC

Since variations in RR values in the selected cell lines could be due to other mechanisms of resistance or differing levels of protein expression, we repeated the cytotoxicity assays with HEK-293 cells transfected with wild type (482R-2) or mutant (482G-2, 482T-10) ABCG2. The transfected cell lines have comparable expression of ABCG2 as evidenced by staining with the 5D3 antibody (Zhou et al., 2001), which recognizes an external epitope of ABCG2 (Figure 4A). Empty vector transfected cells do not express the

protein. Representative results of 4-day cytotoxicity assays with the drugs tested are shown in figure 4 and are summarized in Table 2A. In all cases, differences between IC_{50} values of empty vector transfected cells and ABCG2-transfected cells for each drug were significant, with $p < 0.05$.

In agreement with results obtained with the selected cell lines, the ABCG2 transfectants were highly resistant to SN-38 (129- to 175-fold), but not to CPT (1.8- to 1.9-fold). Cells transfected with wild-type ABCG2 were resistant to homocamptothecin and BN80915 but relative resistance values were 7 and 8, respectively, significantly less than that observed for SN-38 (134). Interestingly, cells transfected with a mutant R482G or R482G ABCG2 gene were 4- to 7-fold more resistant to homocamptothecin, and 7- to 14-fold more resistant to BN80915 than cells transfected with wild-type R482 ABCG2, suggesting that amino acid 482 affects the ability of ABCG2 to confer resistance to these compounds. Previous studies had demonstrated that in addition to the absence of anthracycline resistance and rhodamine transport, the wild-type R482 protein was less effective in transporting mitoxantrone compared to mutant (R482G, R482T) ABCG2 (Robey et al., 2003). SN-38, on the other hand, appeared to be equivalently transported. The studies presented here suggest that the gain of function mutation extends to homocamptothecin and its difluoro derivative BN80915.

The ABCG2 inhibitor FTC was used to evaluate whether inhibition of ABCG2-mediated transport could affect resistance to homocamptothecins in the cell lines tested. Table 2B shows that 5 μ M FTC sensitized all three ABCG2-transfected cell lines to SN-38, homocamptothecin and BN80915, and reduced resistance to very low levels compared to results obtained in the absence of FTC. Dose modifying factor (DMF)

values were calculated for each cell line by dividing the IC_{50} for each drug in the absence of FTC by the IC_{50} in the presence of 5 μ M FTC (Table 1B). IC_{50} values for each drug in the presence of FTC were not significantly different between empty vector transfected cells and ABCG2-transfected cells ($p>0.15$).

Cells selected in BN80915 do not overexpress ABCG2

Attempts were made to generate BN80915-resistant cell lines by selecting MCF-7 breast cancer cells or SF295 glioblastoma cells in increasing concentrations of the drug.

Previous studies have shown that incubating cells with SN-38 results in resistant cells that overexpress ABCG2 as a resistance mechanism (Kawabata et al., 2001). Resistant cells were generated by stepwise increase in exposure to BN80195. The resistant cell lines we subsequently examined were maintained in 100 nM BN80195, approximately 50-fold higher than the IC_{50} value for MCF-7 cells.

To test for surface expression of ABCG2, cells were incubated with phycoerythrin-labeled negative control antibody (solid line) or anti-ABCG2 antibody 5D3 (dotted line) according to the manufacturers instructions (figure 5). In MCF-7 parental cells, a small but detectable level of ABCG2 is observed as evidenced by the 5D3 histogram (dotted line) being shifted slightly to the left of the negative control histogram (solid line). The difference between the negative control and ABCG2 histograms is nearly identical in the BN80915 selected cells, showing no increase of ABCG2 expression in the selected cells. In the SF295 cells, no detectable amount of ABCG2 is observed on the surface of these cells as the negative control and ABCG2

histograms are overlapping. No ABCG2 expression is observed in the SF295 cells selected in BN80915 (figure 5). Interestingly, we have previously observed that these cell lines readily overexpress ABCG2 after selection with mitoxantrone (Robey et al., 2001). High levels of ABCG2 are detected in the MCF-7 MX100 cells and are shown as a positive control. These results suggest BN80915 does not readily increase expression of ABCG2 in tumors.

Discussion

The present study extends previous results with camptothecin analogs to include homocamptothecin and difluorocamptothecin (BN80915) as substrates for ABCG2. Three drug-selected cell lines overexpressing wild-type (R482) or mutant (R482G, R482T) ABCG2 as well as HEK-293 cells expressing wild-type or mutant ABCG2 were found to be resistant to homocamptothecin and BN80915. However, the difluorocamptothecin was found to be approximately 16-fold more potent than SN-38 in wild-type ABCG2 transfected cells. Resistance to all camptothecin compounds was reversed by the ABCG2 antagonist FTC.

Camptothecins were among the first drugs identified as substrates for ABCG2. Camptothecins have also been shown to select for ABCG2-mediated drug resistance (Kawabata et al., 2001; Nakatomi et al., 2001) whereas, to the best of our knowledge, there is no cell line described as selected by camptothecins for P-glycoprotein/MDR1- or MRP-mediated resistance. Hence, ABCG2 appears to be the major transporter for camptothecins and thus potentially a major factor in drug resistance. Interestingly, camptothecin resistance in yeast is also mediated by the ATP binding cassette transmembrane transport proteins PDR5, YOR1, and SNQ2 (Reid et al., 1997).

Our data again show that CPT is a poorer substrate for ABCG2 than SN-38, the active metabolite of irinotecan (CPT-11). This observation is consistent with a previous structure-activity study indicating that camptothecin derivatives with potential for glucuronidation are better ABCG2 substrates than CPT itself (Brangi et al., 1999).

Because these derivatives bear substitutions on the A- and B-rings of camptothecins (see Fig. 1 for SN-38), it appears that that ABCG2 recognizes the A- and B-ring portion of the camptothecins. The present finding that resistance to homocamptothecins in ABCG2-transfected cells was markedly higher than to CPT suggests that the E-ring also contributes to recognition of CPT molecules by the ABCG2 transporter. The finding that BN80915 resistance was comparable to homocamptothecin resistance in HEK-293 cells transfected with wild-type ABCG2, suggests that the two fluoro substitutions on the A-ring do not interfere with the binding of the compound to ABCG2.

The amino acid at position 482 has been previously shown to impact substrate specificity with mutation from arginine to glycine, threonine, or methionine adding the capacity to transport anthracyclines and rhodamine 123 (Allen et al., 2002; Ozvegy et al., 2002). Further, our studies with ABCG2 transfectants indicate that mitoxantrone is more effectively transported by the mutant protein as well (Robey et al., 2003). The present study shows that the amino acid at position 482 also affected the ability of ABCG2 to confer resistance to the camptothecins tested. Resistance to homocamptothecin and BN80915 was highest in cells transfected with mutant (R482G, R482T) compared to wild-type (R482) ABCG2. These data would suggest that while homocamptothecin and BN80915 appear less susceptible to ABCG2-mediated resistance, their effectiveness could be reduced by tumors acquiring mutations in ABCG2 at amino acid 482.

However, no such mutations have been described clinically, nor have changes at this position been observed in SNP analyses of the ABCG2 gene (Imai et al., 2002; Honjo et al., 2002; Zamber et al., 2003).

From a clinical standpoint, it is not clearly established to what extent ABCG2 contributes to resistance to camptothecins. However, studies have implicated ABCG2 in decreasing the oral absorption of the camptothecins. Bioavailability studies in knockout mice in which the murine ortholog for the human MDR-1 gene has been deleted show increased topotecan absorption after exposure to GF120918, an inhibitor of both MDR-1 and ABCG2 (Jonker et al., 2000). Similar findings were reported in a clinical study in which patients were treated with oral topotecan and GF120198, thereby increasing the oral bioavailability of topotecan (Kruijtzter et al., 2002). While these results do not indicate that ABCG2 has a role in clinical drug resistance, they do show that levels of ABCG2 found in normal tissues can modulate drug retention. It is notable that BN80915, the difluoro derivative of homocamptothecin selected for clinical development, was found to be more active than SN-38 (the active metabolite of irinotecan) in ABCG2-transfected cells. These studies suggest that the circumvention of ABCG2, represented in BN80195, has the potential to bring dual activity to the clinic: efficacy in ABCG2 expressing cancers and oral bioavailability. Further studies will be needed to confirm these activities.

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Figure Legends

Figure 1. Drug structures

Figure 2. Induction of Top1 cleavage by BN80915. Reactions were performed for 20 min at 25°C using 3'-end labeled pSK DNA as a substrate. Drug concentrations were: 0.1 μ M (lanes 3 & 8), 0.3 μ M (lanes 4 & 9), 1 μ M (lanes 5 & 10), 3 μ M (lanes 6 & 11), and 10 μ M (lanes 7 & 12). Reactions were stopped by adding 0.5% SDS final concentration. C: control DNA; T: DNA reacted with Top1 without drug. Numbers to the right correspond to the genomic positions of Top1 cleavage sites (23).

Figure 3. Stability of Top1 cleavage sites induced by BN80915 in comparison with homocamptothecin (hCPT) and camptothecin (CPT). After 20 min incubation with 1 μ M drug, 0.35 M NaCl (final concentration) was added to induce the reversal of Top1 cleavage complexes. Aliquots were taken and reactions were stopped at various time points: 0: immediately before NaCl addition (lanes 2, 8, 14), 1 min (lanes 3, 9, 15), 3 min (lanes 4, 10, 16), 10 min (lanes 5, 11, 17), 20 min (lanes 6, 12, 18), and 40 min (lanes 7, 13, 19). T: DNA reacted with Top1 without drug.

Figure 4. Resistance of ABCG2-transfected cells to camptothecins and homocamptothecins. **A.** HEK-293 cells transfected with empty vector (pcDNA3-

10) or ABCG2 (482G-2, 482R-2, 482T-2) were incubated with phycoerythrin-labeled negative control antibody (solid line) or anti-ABCG2 antibody 5D3 (dashed line) according to the manufacturer's instructions. **B.** Four day cytotoxicity assays were performed on HEK-293 cells transfected with empty vector (filled squares) or wild-type (R482, triangles) or mutant (R482G, circles; R482T, hatched squares) ABCG2 with SN-38, camptothecin (CPT), homocamptothecin (hCPT), or BN80915. Representative results are shown.

Figure 5. Selection with BN80195 does not upregulate ABCG2. Resistant cell lines were incubated with phycoerythrin-labeled negative control antibody (solid line) or anti-ABCG2 antibody 5D3 according to the manufacturers instructions. MCF-7 MX100 cells are shown as a positive control.

Table 1: Relative resistance (RR) to camptothecin and homocamptothecin derivatives in selected cell lines overexpressing ABCG2.

Cell line	Residue	SN-38 (IC ₅₀)	RR	CPT (IC ₅₀)	RR	hCPT (IC ₅₀)	RR	BN80915 (IC ₅₀)	RR
MCF-7	R	9±2	-	11±5	-	3.7±3.7	-	2±0.1	-
MCF-7 AdVp3000	T	8916±156*	991	312±75*	28	3583±1800*	968	1750±760*	875
MCF-7 MX100	R	4500±707*	500	187±209	17	663±488*	179	127±123	64
S1	R	1.1±0.6	-	4±1	-	2.4±2.4	-	1±0.04	-
S1-M1-80	G	1633±706*	148 5	32±9*	8	243±50*	101	73±15*	73

IC₅₀ values are in nM. Relative resistance values were obtained by dividing the IC₅₀ value of the ABCG2-overexpressing cell lines by the IC₅₀ value of the respective parental cell lines. Values obtained were from at least four separate experiments. Values marked with an * indicate statistical difference from parental value with p<0.05.

Table 2A: Cross resistance profile of HEK-293 cells transfected with wild-type or mutant ABCG2 protein

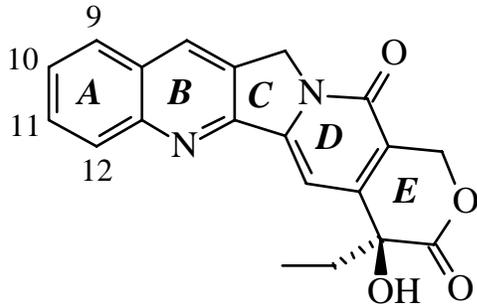
Cell line	SN-38 (IC ₅₀)	RR	CPT (IC ₅₀)	RR	hCPT (IC ₅₀)	RR	BN80915 (IC ₅₀)	RR
PcDNA3-10	0.7±0.5	-	3.8±2.2	-	1.2±0.4	-	0.16±0.13	-
482T-10	90±18	129	7.3±2.0	1.9	36±18	30	9.0±1.1	56
482R-2	94±48	134	7.0±1.6	1.8	8±4.2	6.7	1.3±0.5	8.1
482G-2	123±51	175	7.4±2.2	1.9	57±11	47.5	18±3.9	112

IC₅₀ values are in nM. Relative resistance values (RR) were obtained by dividing the IC₅₀ value of the ABCG2-transfected cells (482T-10, 482R-2, 482G-2) by the IC₅₀ value of empty vector-transfected cells (pcDNA3-10). Values obtained were from at least six separate experiments. All ABCG2-transfected cell lines had IC₅₀ values that were statistically different from empty vector control with p<0.05.

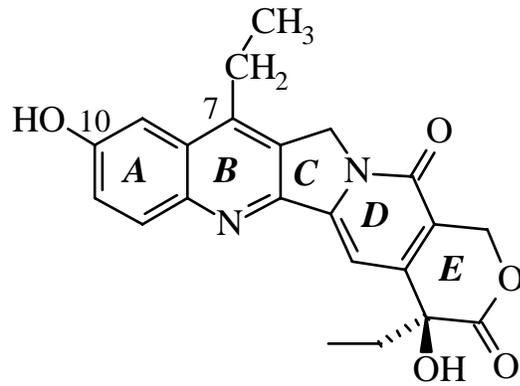
Table 2B: Dose modifying factor (DMF) values for camptothecin and homocamptothecin derivatives in selected cell lines.

Cell line	SN-38+ FTC (IC ₅₀)	DMF	CPT+ FTC (IC ₅₀)	DMF	hCPT+ FTC (IC ₅₀)	DMF	BN80915+ FTC (IC ₅₀)	DMF
PcDNA3-10	1.8±1.8	0.38	5.3±2.3	0.71	0.8±0.1	1.3	0.15±0.13	1.1
482T-10	0.86±0.11	100	6.7±1.1	1.1	1±0.1	36	0.13±0.06	69
482R-2	0.9±0.1	104	6.3±0.6	1.1	1±0.1	8	0.13±0.06	10
482G-2	0.7±0.26	175	5.7±1.1	1.3	0.97±0.1	58	0.72±0.11	25

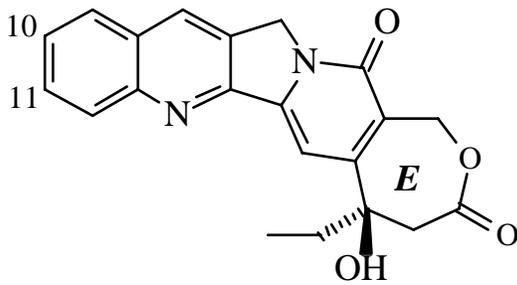
IC₅₀ values are in nM. DMF values were calculated by dividing the IC₅₀ value for each drug in the absence of FTC by the IC₅₀ value in the presence of 5 μM FTC. Experiments were performed 3 times. Reversal of resistance was statistically significant for all ABCG2-transfected cell lines (p<0.05), except for 482R-2 cells with camptothecin (p=0.1).



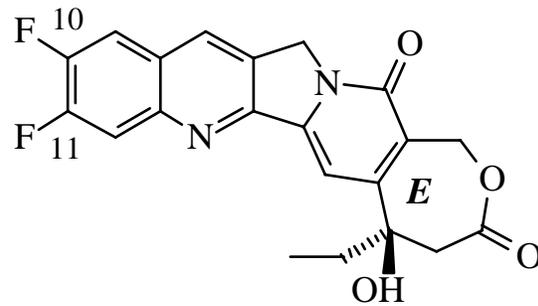
Camptothecin (CPT)



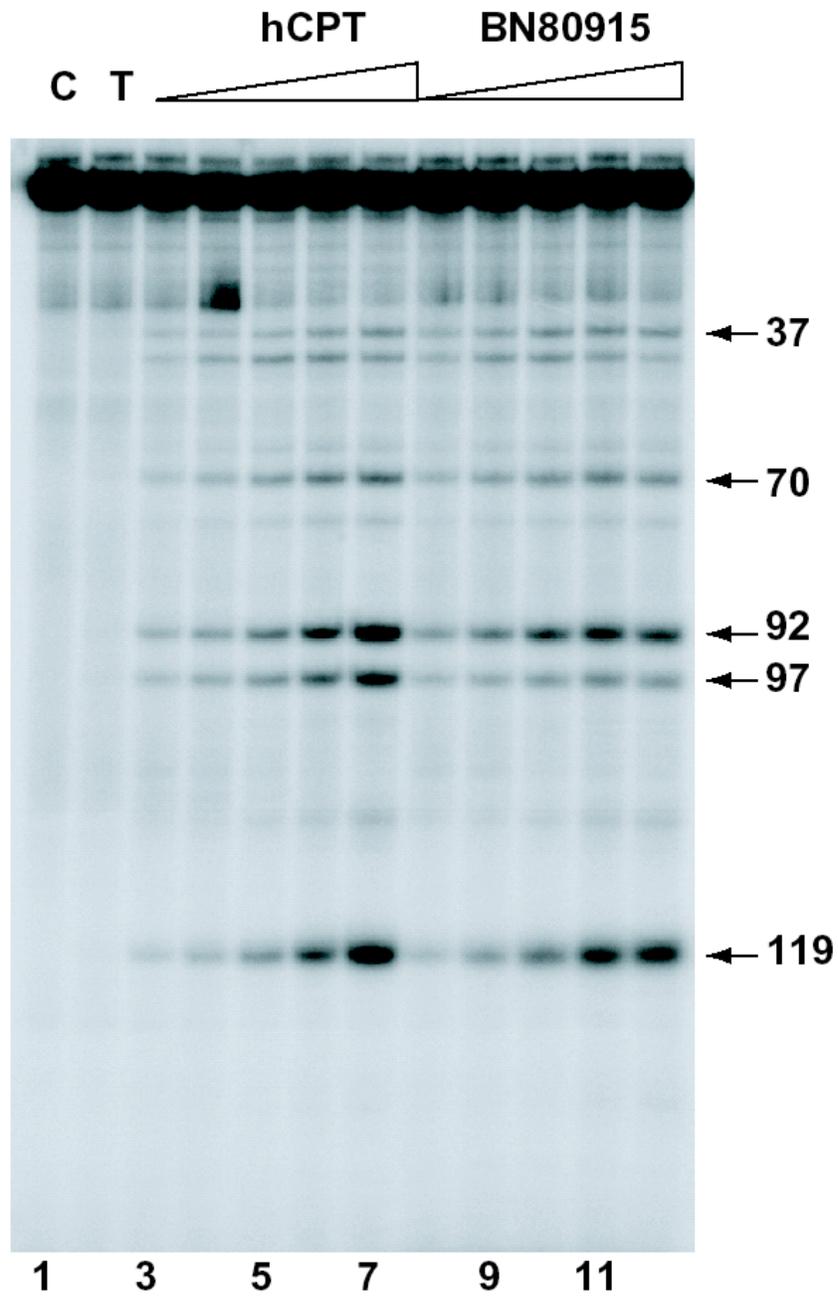
SN-38

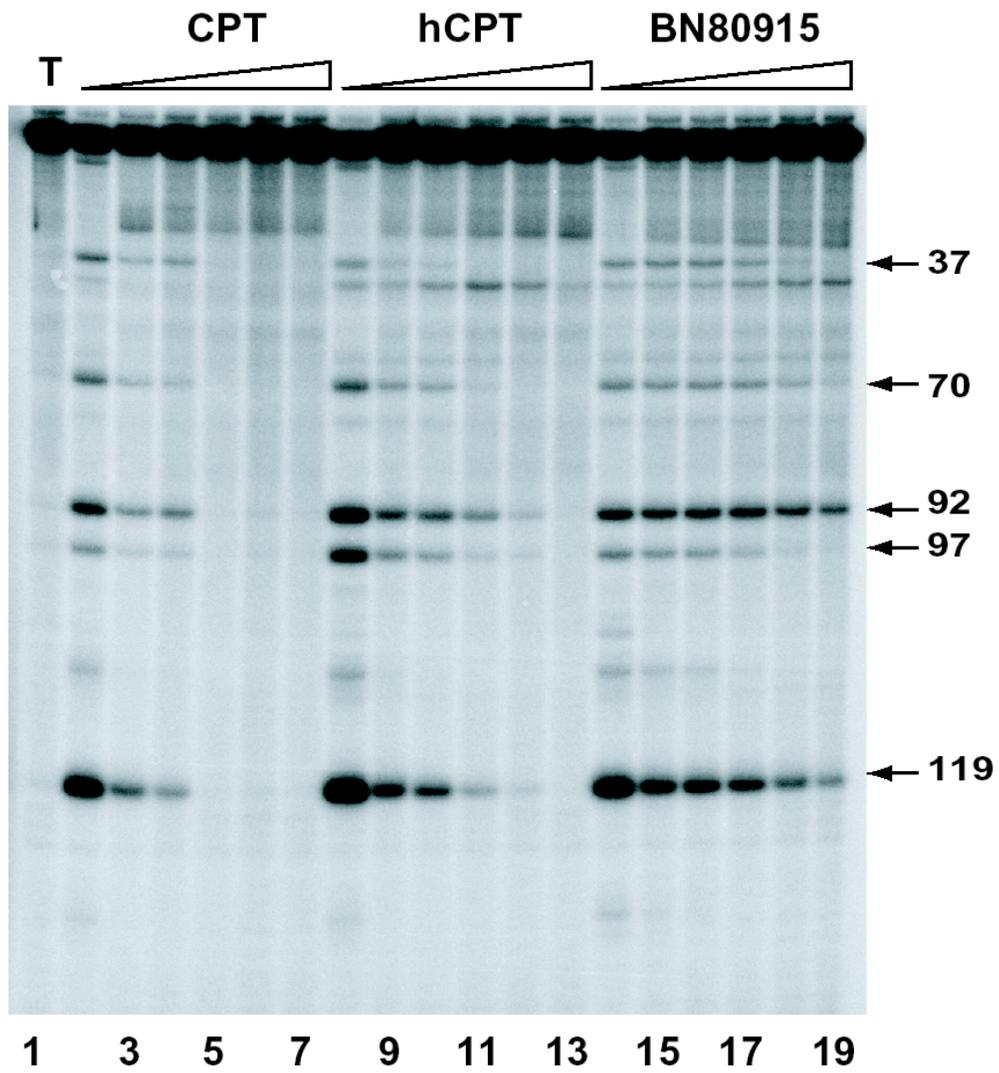


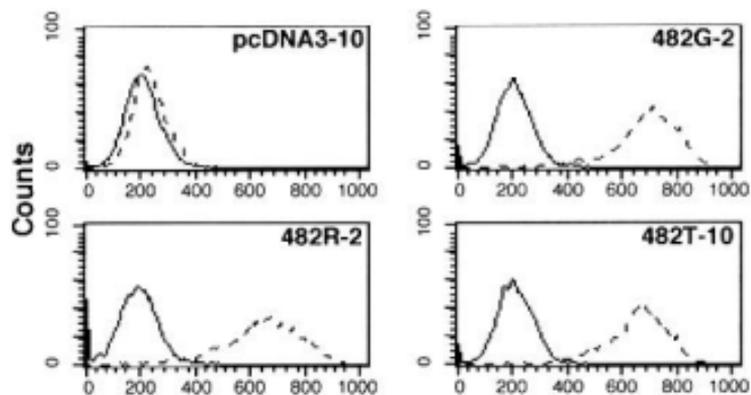
Homocamptothecin (hCPT)



BN-80915





A.**B.**