

Structure-activity Analysis of Taxane-based Broad-spectrum Multidrug Resistance Modulators

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Abstract. *Background:* Clinical drug resistance is frequently associated with overexpression of the multidrug resistance (MDR) proteins P-glycoprotein (Pgp), multidrug resistance protein (MRP-1) and breast cancer resistance protein (BCRP). Taxanes are substrates for Pgp and MRP-1, but not BCRP. Taxane-based reversal agents (tRAs) are non-cytotoxic MDR modulators previously examined for broad-spectrum modulation of Pgp, MRP-1 and BCRP. *Materials and Methods:* Modulation by tRAs was studied by flow cytometry and resistance to taxanes was studied in cytotoxicity assays in the parental HL60/wt, 8226/wt and MCF7/S, and the resistant HL60/ADR, 8226/Dox6, 8226/MR20 and MCF7 AdVp3000 cell lines. Amino acid sequence (BLAST) alignments were performed using ClustalW. *Results:* Structure-activity analysis demonstrated greatest alignment of BCRP with the transmembrane 7-12 region of Pgp and identified tRA side groups that contributed or were detrimental to modulation. *Conclusion:* Identification of tRA side groups contributing to modulation of Pgp, MRP-1 and BCRP will allow the design of a next generation of tRAs and will optimize their potential clinical applicability.

Resistance to chemotherapy drugs may be intrinsic or may be induced by treatment. Moreover, resistance can be to a specific agent or class of agents, such as the taxanes (1-3), or may be to multiple agents. Resistance to multiple agents,

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termed multidrug resistance (MDR) (4-8), frequently results from impaired drug retention caused by overexpression of members of the ATP-binding cassette (ABC) superfamily of transport proteins, which function as energy-dependent drug efflux pumps. Members include P-glycoprotein (Pgp), multidrug resistance protein (MRP-1) and breast cancer resistance protein (BCRP) (6). A variety of non-cytotoxic drugs, referred to as MDR modulators, block efflux mediated by these proteins. To design novel MDR modulators, we have focused on altering the structure of the taxanes, which are Pgp substrates. The cytotoxic taxane ortataxel (formerly IDN-5109, BAY 59-8862) and the noncytotoxic taxane-based reversal agent (tRA) 96023 (9) were shown to be broad-spectrum modulators: tRA 96023 modulated BCRP and ortataxel modulated MRP-1 and BCRP, in addition to Pgp (10). We subsequently screened twenty tRAs and identified four with activity against Pgp, MRP-1 and BCRP. Among these, tRA 98006 was the lead non-cytotoxic broad-spectrum modulator (11).

BCRP function is complex. BCRP is a half-transporter with six transmembrane domains that requires dimerization for function. Moreover, both wild-type (BCRP^{R482}) and mutant (BCRP^{R482T}) BCRP, with arginine and threonine in the amino acid 482 position, respectively, efflux mitoxantrone, but only BCRP^{R482T} effluxes anthracyclines (12). While the tRAs had strong activity against wild-type BCRP (BCRP^{R482}), they had no effect against mutant BCRP (BCRP^{R482T}). Thus amino acid 482 of BCRP appears to determine modulator, as well as substrate, specificity.

In this study, we sought to identify the structural determinants of the specificities of the tRAs. Specifically, we used multilinear regression to examine the relationships between tRA structures and modulation of each MDR protein. This information will be applied toward designing future tRA modulators with optimal broad-spectrum activity.

Materials and Methods

Cell lines. Cell lines included parental MCF7 breast cancer (13), HL60 myeloid leukemia (14) and 8226 myeloma human cells (15) and resistant 8226/Dox6 (15) (drug selected, Pgp and BCRP^{R482}), MCF7/R (16) (drug selected, Pgp and BCRP^{R482}), HL60/ADR (14) (drug selected, MRP-1), MCF7/MRP1-10 (17) (transfected, MRP-1), 8226/MR20 (15) (drug selected, BCRP^{R482}), and MCF7 AdVp3000 (18) (drug selected, BCRP^{R482T}) cells. Suspension cell lines were grown in RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco BRL), 2 mM L-glutamine, 20 U/mL penicillin and 20 µg/mL streptomycin (Gibco BRL), and attached cell lines in RPMI 1640 medium supplemented with 5% heat-inactivated FBS, 5% Nu-Serum, 10 mM HEPES and 2 mM L-glutamine. All cell lines were incubated at 37°C in 5% CO₂ buffered air.

Drugs and modulators. Paclitaxel, docetaxel and ortataxel, provided by Dr. Iwao Ojima (Stony Brook, NY, USA), were solubilized in 100% DMSO to make stock solutions of 4 mM, 1 mM and 1mM, respectively. Mitoxantrone and daunorubicin, purchased from Sigma-Aldrich (St. Louis, MO, USA), were solubilized in phosphate-buffered saline (PBS) to make stock solutions of 1.933 mM and 10 mM, respectively. The tRAs, synthesized and provided for study by Dr. Iwao Ojima, were solubilized in 100% DMSO to make stock solutions ranging from 1 mM to 10 mM. As described below, twenty tRAs that decreased the IC₅₀ concentration of paclitaxel (Table I) were chosen for further study.

Drug efflux studies. tRA modulation of substrate retention was studied in cell lines overexpressing Pgp, MRP-1 and BCRP. Mitoxantrone is a substrate for all three MDR-associated transport proteins and daunorubicin is a substrate for Pgp, MRP-1 and BCRP^{R482T}. Cells were incubated at 1 x 10⁶/mL in RPMI 1640 medium with 3 µM mitoxantrone or daunorubicin for 30 minutes at 37°C, then washed with ice-cold PBS, and an aliquot of cells was resuspended in ice-cold PBS and placed at 4°C for analysis of uptake. The remaining cells were resuspended in medium with and without tRAs at a concentration of 10 µM. Efflux was allowed to occur at 37°C for 90 minutes; cells were then washed with ice-cold PBS and placed on ice until analysis. Experiments were performed in triplicate.

Flow cytometry. Cellular mitoxantrone and daunorubicin content were analyzed using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) equipped in standard fashion with an Argon laser for 488 nm excitation and 585/42 band-pass (FL2) and 670 long-pass (FL3) filters for emission collection, as previously described (15, 19).

Flow cytometry data analysis. Data were analyzed with WinList software (Verity Software House, Topsham, ME, USA). Distribution histograms of mean fluorescence intensity (MFI) following efflux in the presence and absence of modulator were compared by the Kolmogorov-Smirnov (KS) statistic, expressed as a D-value ranging from 0 (identical histograms) to 1.0 (no overlap in histograms). A D-value ≥ 0.2 indicates a significant separation of histograms (20).

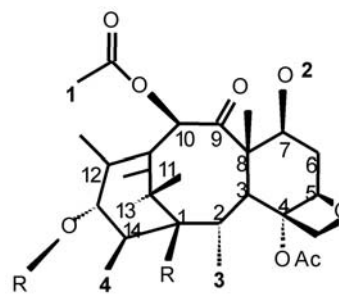


Figure 1. Variant chain assignments on tRA baccatin backbone. Variant position 1 was assigned to C-10, position 2 to C-7, position 3 to C-2 and position 4 to C-14 on the tRA baccatin backbone.

Cytotoxicity. To study cytotoxicity in the adherent cell lines, cells were seeded at 600-2000 cells/well (varying by cell line) in 96-well plates and incubated for 18-24 hours at 37°C to allow for attachment. Cells were incubated with drug at concentrations spanning a 5- to 6-log range, with and without modulators, for 72 hours, and analyzed for inhibition of growth, quantified with the Sulforhodamine B (SRB) dye-based assay, as described previously (21). Experiments were performed at least in quintuplicate.

To study cytotoxicity in suspension cell lines, cells were cultured in 24-well plates at 5 x 10⁴ cells/well or in 48-well plates at 2.5 x 10⁴ cells/well. Cells were incubated with drug at concentrations spanning a 5- to 6-log range, with and without modulators, for 96 hours. Cells in each well were counted with a Coulter counter (Coulter Electronics), as described previously (22-23), and total cell number/well was extrapolated with Excel. Experiments were performed at least in triplicate, with duplicates within each experiment.

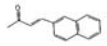
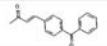
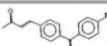
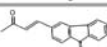
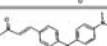
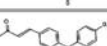
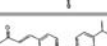
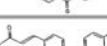

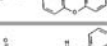
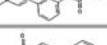
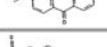
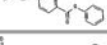



Drugs from frozen stock solutions were diluted in RPMI 1640 with 2% HEPES to achieve desired concentrations. Final DMSO concentrations were less than 0.1%. Each tRA was studied at 0.1, 1 and 10 µM.

Cytotoxicity data analysis. IC₅₀'s, or the drug concentrations required to inhibit control growth by 50%, were determined using the Datalog and Gplate Microsoft FORTRAN software program developed by Dr. William Greco at Roswell Park Cancer Institute. Briefly, data were fitted using the Sigmoid-Emax concentration-effect model with nonlinear regression, weighted by the reciprocal of the square of the predicted response (24). This software uses the Marquardt algorithm (25) as adapted by Nash for the nonlinear regression (26). Modulators were assessed for cell growth inhibition at three concentrations.

The IC₅₀'s of mitoxantrone in the absence and presence of each tRA (0.1, 1 and 10 µM) were compared in each cell line by calculating the resistance-modifying factor (RMF) as the ratio: (IC₅₀ drug) / (IC₅₀ drug + tRA). Thus, an RMF > 1 indicates enhanced drug sensitivity in the presence of tRA, an RMF = 1 indicates no effect and an RMF < 1 indicates an antagonistic effect; the greater the RMF, the more significant the effect.

Structure-activity analysis. Quantitative structure-activity analysis was performed for the tRAs with the QSAR-PC: PAR computer program from Microsoft Corporation (Redmond, WA, USA).

Table I. Variant designations and chemical structures. Corresponding letters/designations and chemical structures for the various sidechains in their respective positions.

Designation	Variant Number	Side Chain
Me	α	Me
Meo	1	Meo
A	2	
B	2	
C	2	
D	2	
E	2	
F	2	
G	2	
H	2	
I	2	
J	2	
K	2	
L	2	
M	2	
OBz	3	
OBz-ketone	3	
OH	4	OH
Cyclic Carbonate	4	

Briefly, physicochemical-activity relationships were calculated using multilinear regression equations. Experimental values for modulation by tRAs were analyzed in concert with various substituents of the tRAs to establish contributing and subtracting variant side chains.

Results

BCRP homology to Pgp. As BCRP does not efflux any of the taxanes (10), we sought to identify a mechanism by which tRA might modulate BCRP. Utilizing the amino acid sequences of human Pgp and BCRP identified on the

Entrez-Protein website, alignments were performed of the entire BCRP sequence to either Pgp amino acids 51-348 or 709-992 [transmembrane (tm) regions 1-6 or 7-12]. In collaborative work with Dr. Donald Gruol, mapping of diverse substrate binding sites within Pgp by mutational analysis demonstrated paclitaxel binding in both tm regions 1-6 and 7-12 (27). Using tRA 96023 as a mutational selecting agent, since it has only the baccatin backbone structure of the paclitaxel molecule, taxane binding sites were identified in tm 7-12. Further work using the C-13 sidechain of ortataxel as the mutational selecting agent identified taxane-binding sites in the tm 1-6 region. These data strongly suggest that the C-13 sidechain of paclitaxel (or docetaxel) binds in the first "half" of Pgp, while the baccatin backbone binds in the second "half" of Pgp, and binding of both regions is required for active transport. The amino acid alignments of BCRP to Pgp tm1-6 or Pgp tm 7-12 demonstrated more homology of BCRP to Pgp tm 7-12, of the order of 48% versus 27%. Thus BCRP contains more sites homologous to the baccatin-binding portion of the Pgp molecule, which is the structure on which the tRAs are based, providing a working hypothesis for the mechanism of tRA modulation of BCRP-mediated efflux.

Structure-activity relationships of tRAs. Using the QSAR program to analyze the modulation data previously reported (11), we examined the side groups of the tRAs that contributed to or were detrimental to modulation. We assigned four variance groups (Figure 1). Group 1 was either a methyl or an O-methyl group; group 2 contained the most variation, with 13 different side groups; group 3 was either an O-benzyl or an O-benzyl-ketone group; and group 4 was either a hydroxy group or a cyclic carbonate group linking to C-1 (Table I).

O-methylation at variant position 1 contributes to tRA modulation of Pgp-mediated efflux of daunorubicin, while slightly detracting from Pgp-mediated efflux of mitoxantrone (Figure 2A vs. 2B). O-methylation also contributes positively to modulation of MRP-1- and BCRP-mediated efflux of mitoxantrone, as well as MRP-1-mediated efflux of daunorubicin (Figure 2C-E). Variant position 2 contains the greatest number of side chains – thirteen (Table I). With regard to Pgp modulation of efflux of both mitoxantrone and daunorubicin, side chain J, was a contributing factor, while side chains A and M detracted from modulation (Figure A-B). For MRP-1, the patterns of contribution and detriment are similar for modulation of efflux of both mitoxantrone and daunorubicin: groups E, F, J and K were all contributory, with E contributing the most to modulation and groups B, G, H, I and M subtracted from modulation of MRP-1, with groups H and M having the largest detrimental effects (Figure 2C-D). For BCRP activity, the effects are

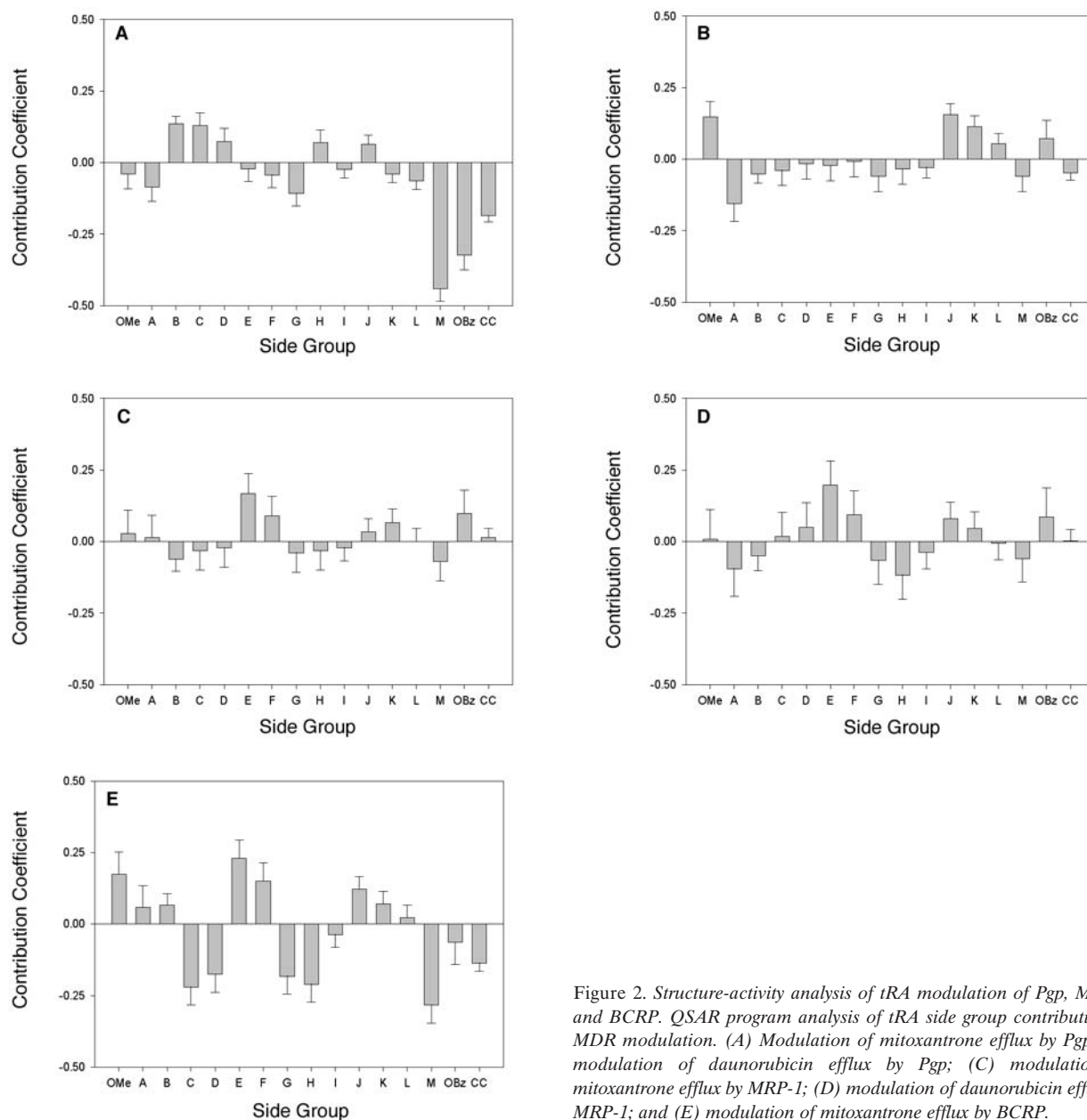


Figure 2. Structure-activity analysis of tRA modulation of Pgp, MRP-1 and BCRP. QSAR program analysis of tRA side group contribution to MDR modulation. (A) Modulation of mitoxantrone efflux by Pgp; (B) modulation of daunorubicin efflux by Pgp; (C) modulation of mitoxantrone efflux by MRP-1; (D) modulation of daunorubicin efflux by MRP-1; and (E) modulation of mitoxantrone efflux by BCRP.

striking. Groups E and F contribute strongly, while groups J, K and L contribute moderately. Side groups C, D, G, H and M, respectively, strongly detract from tRA modulation of mitoxantrone efflux (Figure 2E). At variant position 3, an O-benzyl group has a mixed contribution with respect to Pgp modulation, contributing to daunorubicin efflux and detracting from mitoxantrone efflux (Figure 2A vs. 2B). For tRA activity against MRP-1, an O-benzyl contributes to modulation of both mitoxantrone and daunorubicin efflux (Figure 2C-D).

Most strikingly, at variant position 4, a cyclic carbonate is detrimental to activity against both Pgp and BCRP, and neutral with regard to tRA activity in modulating MRP-1 (Figure 2A-E). For broad-spectrum modulation tRA, the introduction of a methoxy group at variant position 1 and side chain J at variant position 2 contribute to modulation, whereas side chains G and M at variant position 2 and cyclic carbonate at variant position 4, although neutral with regard to MRP-1 modulation, were detrimental to broad-spectrum modulation.

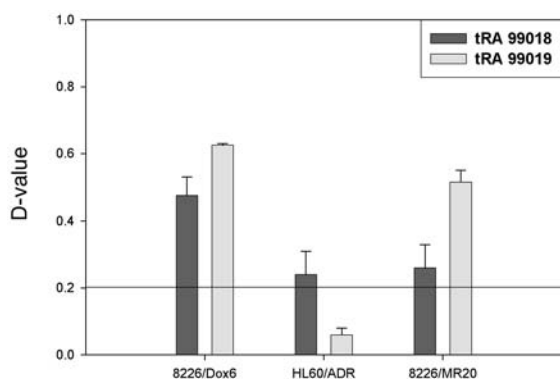


Figure 3. Modulation of mitoxantrone retention by tRA 99018 and tRA 99019. Examination of the role of cyclic carbonate (cc) in enhancing mitoxantrone retention. Uptake (30 minute) and efflux (90 minute) in both the absence and presence of 10 μ M tRA 99018 and tRA 99019 were studied in 8226/Dox6 myeloma (Pgp), HL60/ADR myeloid leukemia (MRP-1) and 8226/MR20 myeloma (BCRP^{R482}) cell lines. D-values compare efflux histograms in the absence and in the presence of tRA. tRA 99018 contains the cc, while tRA 99019 lacks it.

Removal of cyclic carbonate enhances tRA activity against Pgp and BCRP. As the cyclic carbonate (cc) at variant position 4 was clearly detrimental to the activity of the tRAs against Pgp and, especially, BCRP, we examined the effect of removing the cc from a previously identified broad-spectrum modulator, tRA 99018 (11). The structure resulting from removal of the cc is tRA 99019. Enhancement of retention of mitoxantrone by 10 μ M tRA 99018 and tRA 99019 was measured by flow cytometry (Figure 3). Removal of the cc resulted in loss of modulation in the MRP-1-overexpressing HL60/ADR cell line, reducing the tRA 99018 D-value from 0.24 to 0.06. However, consistent with the structure-activity analysis, removal of the cc enhanced activity against both Pgp and BCRP, with D-values increasing from 0.46 to 0.63 and from 0.34 to 0.52, respectively.

The role of the cyclic carbonate in tRA activity was further examined in cytotoxicity studies. The cell lines studied in the flow cytometry experiments were treated with mitoxantrone in the absence and presence of tRA (0.1, 1 and 10 μ M) for 96 hours (Table II). tRA 99019 had no activity in the MRP-1-overexpressing HL60/ADR cell line, consistent with the retention data (Figure 3, Table II). In the Pgp-overexpressing 8226/Dox6 cell line, tRA 99019 enhanced cytotoxicity more than tRA 99018 at all concentrations; RMFs were in the range of 1.6-6.3 with tRA 99018 and 1.8-9.7 with tRA 99019 (Table II). Similarly, in the BCRP-overexpressing 8226/MR20 cell line, tRA 99019 had a greater effect on cytotoxicity than tRA 99018, with RMFs from 2.6-13.7 and 1.6-6.2, respectively (Table II).

Table II. Resistance modifying factors (RMFs) of tRA 99018 and tRA 99019. Results are expressed as Resistance Modifying Factors for Pgp-overexpressing 8226/Dox6, MRP-1-overexpressing HL60/ADR, and BCRP-overexpressing 8226/MR20 cells. Cells were treated with mitoxantrone in the absence or presence of tRAs 99018 or 99019 at 0.1 μ M, 1 μ M and 10 μ M for 96 hours. Experimental values are representative of at least triplicate experiments.

	tRA 99018			tRA 99019		
	0.1 μ M	1 μ M	10 μ M	0.1 μ M	1 μ M	10 μ M
8226/Dox6	1.64	3.89	6.24	1.8	7.38	9.69
HL60/ADR	0.99	0.9	0.99	1.13	0.89	1.3
8226/MR20	1.62	3.07	6.15	2.6	13.1	13.7

Discussion

In this study we sought to understand the mechanism by which tRAs mediate broad-spectrum modulation and the structure-activity relationships of the tRAs. Homology studies between Pgp and BCRP lead to an understanding of BCRP modulation by tRAs. We previously examined a library of tRAs for activity in modulating Pgp, MRP-1 and BCRP (11) and used that information to assess the contribution of each portion of the tRA to modulation, in order to optimize the design of future agents.

Pgp effluxes members of the taxane family, including paclitaxel and docetaxel (2). MRP-1 also effluxes taxanes, but to a much lesser extent than Pgp (28). In contrast, BCRP, in both its wild-type and mutant forms, does not efflux taxanes (29). The relative resistance ratios of cell lines overexpressing these three MDR proteins to the various taxanes (10) reflect important information with respect to the mechanisms of tRA modulation. Pgp confers high levels of resistance to paclitaxel and docetaxel and mutational studies have identified several paclitaxel binding sites in both tm regions of the protein (27, 30). MRP-1 confers a low level of resistance to the taxanes, suggesting weak binding/interaction between the drug and the protein. This weak interaction is maintained in the tRAs: fewer agents work against MRP-1 than against Pgp and the degree of modulation is also less.

BCRP modulation by tRAs raises an interesting question: if BCRP does not efflux taxanes, why are the tRAs strong modulators of the protein? To explain this phenomenon, we examined the amino acids making up the protein and compared them to both "halves" of Pgp – tm 1-6 and tm 7-12. From the mutational studies mentioned above, there is strong evidence for paclitaxel binding to Pgp in both tm regions 1-6 and 7-12 (27, 30). The β -tubulin binding portion of paclitaxel, the C-13 sidechain, seems to bind in the tm 1-

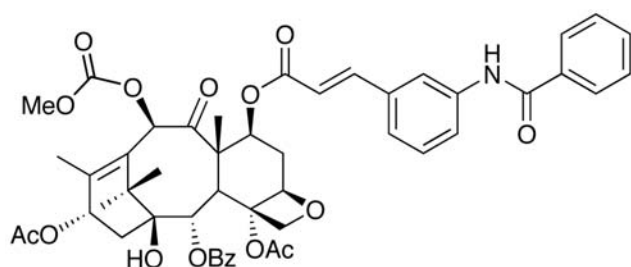


Figure 4. Structure of proposed second generation broad-spectrum tRA. Based on QSAR analysis, a structure for a second generation agent is proposed to optimize broad-spectrum modulation by the tRAs.

6 pocket, and the baccatin backbone portion of the drug in the tm 7-12 pocket. Binding of both regions is required for active transport. A multi-sequence alignment of BCRP with either the tm 1-6 region or the tm 7-12 region of Pgp showed greater homology to the latter region, which is where the baccatin backbone is believed to bind. Since BCRP is a "half-transporter" likely to homodimerize, it does not mediate efflux of taxanes; however tRAs contain the backbone structure and are likely to bind BCRP but not be transported, thereby effectively blocking function of the protein.

With a working hypothesis for the mechanism of modulation of tRAs, we sought to analyze the structure-activity relationship for each of the MDR proteins. We were able to identify portions of the agent that contributed or were detrimental to modulation of each protein, as well as to broad-spectrum activity. From the analysis, the optimal next generation broad-spectrum modulator is tRA 99019 with a methoxy group at variant position 1 (Figure 4), which remains to be synthesized and tested.

The pros and cons of MDR modulation are often debated (31). Modulation is likely to be beneficial to patients whose cancer cells express MDR proteins, but an optimal approach might be to identify these patients prior to treatment. Some clinical trials of MDR modulation have demonstrated toxicity without significant benefit to patients (32), while others have shown clinical efficacy (33). Decreasing the dosage of chemotherapy drugs has been necessary in combination in many modulation trials because of pharmacokinetic interactions (34). While the blood-brain and blood-testis barriers may also be susceptible to the effects of MDR modulators and toxicity to "protected" areas of the body is therefore a concern (35), the efficacy of treatment of cancers, such as gliomas, may be enhanced by modulation (36-38). In this study, we sought to understand the mechanism of broad-spectrum MDR modulation by tRAs, in order to optimize the activity of future agents.

Acknowledgements

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References

- Schiff PB: Taxol stabilized microtubules in mouse fibroblast cells. *Proc Natl Acad Sci USA* 77: 1561-1565, 1980.
- Horwitz SB: Taxol: mechanisms of action and resistance. *Ann NY Acad Sci* 466: 733-744, 1986.
- Schibler MJ: Taxol-dependent mutants of chinese hamster ovary cells with alterations in alpha- and beta-tubulin. *J Cell Biol* 102: 1522-1531, 1986.
- Kaye S: Tumour cell resistance to anthracyclines: a review. *Can Chem Pharm* 14: 96-103, 1985.
- Kasimir-Bauer S: Impact of the expression of P glycoprotein, the multidrug resistance-related protein, bcl-2, mutant p53, and heat shock protein 27 on response to induction therapy and long-term survival in patients with *de novo* acute myeloid leukemia. *Exp Hematol* 30: 1302-1308, 2002.
- Dean M: The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res* 11: 1156-1166, 2001.
- Dano K: Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. *Biochim Biophys Acta* 323: 466-483, 1973.
- Dano K: Cross resistance between vinca alkaloids and anthracyclines in Ehrlich ascites tumor *in vivo*. *Cancer Chem Rep* 56: 701-708, 1972.
- Vredenburg MR: Effects of orally active taxanes on P-glycoprotein modulation and colon and breast carcinoma drug resistance. *J Natl Can Inst* 93: 1234-1245, 2001.
- Minderman H: Multidrug resistance (MDR) modulation by the taxane derivatives IDN-5109 and tRA96023: Effects on P-glycoprotein (Pgp)-, multidrug resistance protein (MRP-1)-, and breast cancer resistance protein (BCRP)-mediated drug transport. *Proc Am Assoc Can Res* 43: 950, 2002.
- Brooks TA: Taxane-based reversal agents modulate drug resistance mediated by P-glycoprotein, multidrug resistance protein, and breast cancer resistance protein. *Mol Can Ther* in press, 2003.
- Honjo Y: Acquired mutations in the MXR/BCRP/ABCP gene alter substrate specificity in MXR/BCRP/ABCP-overexpressing cells. *Cancer Res* 61: 6635-6639, 2001.
- Soule HD: A human cell line from a pleural effusion derived from a breast carcinoma. *J Natl Cancer Inst* 51: 1409-1416, 1973.
- Gollapudi S: Lack of reversal of daunorubicin resistance in HL60/AR cells by cyclosporin A. *Anticancer Res* 12: 2127-2132, 1992.

- 15 Minderman H: Flow cytometric analysis of breast cancer resistance protein expression and function. *Cytometry* 48: 59-65, 2002.
- 16 Fairchild CR: Isolation of amplified and overexpressed DNA sequences from adriamycin-resistant human breast cancer cells. *Can Res* 47: 5141-5148, 1987.
- 17 Paumi CM: Multidrug resistance protein (MRP) 1 and MRP3 attenuate cytotoxic and transactivating effects of the cyclopentenone prostaglandin, 15-deoxy- Δ (12,14) prostaglandin J(2) in MCF7 breast cancer cells. *Biochem* 42: 5429-5437, 2003.
- 18 Doyle LA: A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA* 95: 15665-15670, 1998.
- 19 Speth PA: Quantitation of anthracyclines in human hematopoietic cell subpopulations by flow cytometry correlated with high pressure liquid chromatography. *Cytometry* 6: 143-150, 1985.
- 20 Young IT: Proof without prejudice: use of the Kolmogorov-Smirnov test for the analysis of histograms from flow systems and other sources. *J Histochem Cytochem* 25: 935-941, 1977.
- 21 Skehan P: New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 82: 1107-1112, 1990.
- 22 Attallah AM: A simple highly sensitive methods for the determination of cell viability using an electronic particle analyzer, Coulter counter. *J Immunol Methods* 41: 155-162, 1981.
- 23 Attallah AM: ADCC analysis with the Coulter counter: a highly sensitive, ultramicro assay suitable for clinical and experimental application. *J Immunol Methods* 43: 121-129, 1981.
- 24 Holford NH: Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. *Clin Pharmacokinet* 6: 429-453, 1981.
- 25 Marquardt DW: An algorithm for least squares estimation of nonlinear parameters. *J Soc Ind Appl Math* 11: 431-441, 1963.
- 26 Nash, JC: Compact Numerical Method for Computers: Linear Algebra and Function Minimization. New York: John Wiley & Sons, 1979.
- 27 Gruol DJ: The use of a novel taxane-based P-glycoprotein inhibitor to identify mutations that alter the interaction of the protein with paclitaxel. *Mol Pharmacol* 60: 104-113, 2001.
- 28 Borst P: The multidrug resistance protein family. *Biochim Biophys Acta* 1461: 347-357, 1999.
- 29 Litman T: From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. *Cell Mol Life Sci* 58: 931-959, 2001.
- 30 Gruol DJ: Evidence for the locations of distinct steroid and vinca alkaloid interaction domains within the murine mdr1b P-Glycoprotein. *Molec Pharm* 62: 1238-1248, 2002.
- 31 Larson RA: Is modulation of multidrug resistance a viable strategy for acute myeloid leukemia? *Leukemia* 17: 488-491, 2003.
- 32 Baer MR: Phase 3 study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia: Cancer and Leukemia Group B Study 9720. *Blood* 100: 1224-1232, 2002.
- 33 List AF: Benefit of cyclosporine modulation of drug resistance in patients with poor-risk acute myeloid leukemia: a Southwest Oncology Group study. *Blood* 98: 3212-3220, 2001.
- 34 Tan B: Multidrug resistance transporters and modulation. *Curr Opin Oncol* 12: 450-458, 2000.
- 35 Litman, T: From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. *Cell Mol Life Sci* 58: 931-959, 2001.
- 36 Hendrikse NH: A new *in vivo* method to study P-glycoprotein transport in tumors and the blood-brain barrier. *Can Res* 59: 2411-2416, 1999.
- 37 Declèves X: Molecular and functional MDR1-Pgp and MRPs expression in human glioblastoma multiforme cell lines. *Int J Cancer* 98: 173-180, 2002.
- 38 Serra M: Value of P-glycoprotein and clinicopathologic factors as the basis for new treatment strategies in high-grade osteosarcoma of the extremities. *J Clin Onc* 21: 536-542, 2003.

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