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Modulation of drug cytotoxicity in wild-type and multidrug-resistant tumor cells by stereoisomeric series of C-20′-vinblastine congeners that lack antimicrotubule activity*

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Summary. Seven binary vinca alkaloid congeners were newly synthesized as the C14′ or C16′(20′) or C14′16′(20′) stereoisomers of C20′-modified VBL. These congeners lacked detectable antimicrotubule activity in assays of polymerization of purified microtubule protein and of mitotic arrest induction. The compounds modulated the cytotoxicity of VBL, VCR, and DOX in sarcoma and colon-tumor cell lines. In wild-type cell lines, each congener elicited a concentration-dependent enhancement of cytotoxicity that was drug- and cell-type-selective. For example, C20′-deoxy C14′16′20′-epi VBL sensitized sarcoma S180 cells 19-fold to DOX and 11-fold to VCR but had no effect on VBL cytotoxicity. In the rat colon-cancer cell lines there was preferential enhancement of VCR cytotoxicity by most congeners. In two MDR cell strains of S180, the modulation potency of each congener was independent of specific drug or of resistance level. As a result, the amount of modulator (concentration) required for reversal was proportional to the drug-resistance level. Such properties were not displayed by the monomeric vinca alkaloid modulator vindoline. The potency of drug modulation in both wild-type and MDR cell strains was dependent on the stereoisomeric form of the congener and its C20′-substituents.

Introduction

The occurrence of primary drug resistance and the associated phenomenon of acquired resistance to multiple drugs in relapsed cancer [21] are major determinants of treatment failure. Experimental efforts toward the therapeutic means of circumventing drug resistance have focused on a type of drug resistance acquired in various tumor cell lines after drug exposure. These MDR cell strains exhibit a broad cross-resistance to drugs of various structural classes, including anthracyclines, binary vinca alkaloids, and epipodophyllotoxins, and they show impaired cellular drug accumulation (for review, see [8]). Research has identified a variety of chemical modulators that enhance the cytotoxicity of anticancer drugs in MDR cell strains, beginning with the calcium antagonist verapamil [31]. Modulators are structurally diverse compounds that include reserpine [15], the cyclosporins [12, 20], quinacrine [13] and non-antitumor vinca alkaloids [14], and DOX analogs [16, 30]. Several modulators have been shown to inhibit the binding of drugs to a putative efflux pump, P-glycoprotein [1, 3], whose cellular content is elevated in MDR cell strains (see review in [23]).

The modulation of drug sensitivity in wild-type (parent) cell lines has been more problematic. Typically of leukemic origin, these wild-type cells have low or undetectable P-glycoprotein levels. More recently, wild-type cell lines derived from tumors of de novo drug resistance, such as renal carcinoma [19], have demonstrated P-glycoprotein expression and a sensitivity to modulator activity. However, due to reports of modulator action in wild-type [9] and so-called atypical MDR [25] cell lines that lack P-glycoprotein expression, this mechanism of modulator activity remains equivocal.

Of concern to the clinical application of modulators is inherent biologic activity and efficacy in tumor cell lines at high concentrations. Such limits were recognized as unacceptable cardiotoxicity in a phase I clinical trial of verapamil in cancer patients [22]. The future of modulator-based chemotherapy will require agents of high potency and of little or no inherent biologic action. The congener synthesis of phenothiazines [10], of dihydropyridines [28], and, more recently, of cyclosporin [17] has identified several candidates in this regard.

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Abbreviations: MDR, multidrug resistance; VBL, vinblastine; VCR, vincristine; DOX, doxorubicin; MTP, purified microtubule protein; MTX, methotrexate; 5-FU, 5-fluorouracil

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Table 1. Cell-culture systems

	IC_{50} (μM)		
	VBL	VCR	DOX
Wild-type cell lines:			
Rat colon adenocarcinoma, passages 60–85:			
RCC-2, undifferentiated	0.003	0.005	0.01
RCC-5, mucinous	0.009	0.020	0.05
Mouse sarcoma S180	0.008	0.03	0.1
	Relative resistance[a]		
Acquired-MDR cell strains:			
S180/A3	3	7	15
S180/A10	15	17	90

Cells were incubated for 3 days in the absence/presence of each drug and population growth was enumerated by an electronic particle counter. The IC_{50} value was determined from plots of the percentage of control growth versus drug concentration

[a] Calculated by dividing the IC_{50} value for each drug in the MDR cell line by the IC_{50} value for the parental cell line

In recent years, we have developed new methodologies for the stereoselective synthesis of the vinca binary alkaloids [18]. Using unique, cytotoxic congeners of VBL at the C20′-position, we accomplished the first significant breakthrough in the structure/function relationship of this drug with respect to its antimicrotubule actions [5, 7]. In the present paper, we report on a series of stereoisomers of C20′-modified VBL congeners that modulate both intrinsic and acquired drug resistance and *lack* antimicrotubule actions.

Materials and methods

Cell culture. The murine sarcoma cell line S180 and two MDR cell strains, A3 and A10, selected by DOX treatment ([29; courtesy of Dr. T. Tritton, Department of Pharmacology, University of Vermont) were maintained in Dulbecco's modified MEM medium (K. C. Biologicals, Lexena, Kan.) supplemented with 10% heat-inactivated (57° C, 20 min) horse serum (Hazelton Biologicals, Lexena, Kan.). The P-glycoprotein content of the S180/MDR A3 and A10 cell strains is proportional to the overall level of MDR, whereas the wild-type cell line is devoid of activity, as determined by Western blots of plasma membranes with the C219 antibody (courtesy of Dr. V. Ling; Bhushan and Tritton, personal communication). The rat colon-adenocarcinoma cell lines RCC-2 and RCC-5 [4, 6] were maintained in Ham's F12 medium supplemented with 5% heat-inactivated fetal bovine serum (Hyclone, Sterile Systems, Logan, Utah) at passage levels 60–85. The population-doubling times of all cell lines were 18–24 h. The S180/MDR cell strains were grown in DOX-free medium for 48 h prior to their experimental use. Cells were not exposed to antibiotics during maintenance or experimental culture. Routine mycoplasma tests were negative.

Growth-inhibition studies. For all assays, mid-logarithmic populations were seeded either into tubes for suspension culture of S180 cell lines (1×10^5 cells/ml) or into 24-well plates for monolayer culture of rat colon-cancer cell lines (2×10^4 cells/ml per well). The latter cell lines were plated at 16 h prior to treatment to permit attachment to the substratum. The population growth of control and treated cultures was determined after 72 h incubation by cell enumeration with an electronic particle counter (Coulter counter model ZB1; Coulter Electronics, Hialeah, Fla.). The modulation of drug sensitivity in *wild-type* cell lines was evaluated by adding the congeners to cell cultures just prior (~6–8 min) to the addition of drug. The action of congeners in *MDR* cell lines was determined by an assay of reversal of drug resistance to parental sensitivity, whereby each MDR cell strain was treated with a concentration range of congener and with the parental IC_{70} of each drug.

Antimicrotubule activities. Microtubule protein (MTP) was purified from 100 g bovine calf brain by two cycles of depolymerization/polymerization as described by Shelanski et al. [26]. The final pellet was resuspended in 4 M glycerol in assembly buffer [0.1 M morpholinoethanesulfonate (MES), 1.0 mM ethylene glycol-bis(aminoethyl ether)tetraacetate (EGTA), and 0.5 mM $MgCl_2$; pH 6.4] to a protein concentration of 10–15 mg/ml and was stored at –70° C. We monitored the effects of VBL and its congeners on MTP assembly into microtubules by the forward light-scattering procedure of Gaskin et al. [11]. MTP (1.0–1.2 mg/ml) was exposed to 10^{-7}–10^{-4} M VBL or congener, and assembly was initiated by the addition of 1 mM guanosine 5′-triphosphate (GTP) and warming to 37° C in a microprocessor-equipped spectrophotometer (Beckman DU 70, Beckman Instruments) operated at 350 nm. An aliquot of each sample at the steady-state condition was evaluated for microtubule morphology and quantity using transmission electron microscopy as previously described [5].

Chemicals. The various stereoisomers of C20′-modified VBL congeners were synthesized in our laboratory as the free bases using recently developed protocols [18]. Vindoline (free base) was obtained from Omni Chem (Louvain-La-Neuve, Belgium). All compounds were dissolved in dimethylsulfoxide (DMSO) at a concentration of 0.01 M just prior to their use. The maximal DMSO concentration in cell cultures was 0.1%, which affected neither cell growth nor drug cytotoxicity. We purchased VCR sulfate solution, VBL sulfate, and DOX HCl from Adria Laboratories (Columbus, Ohio).

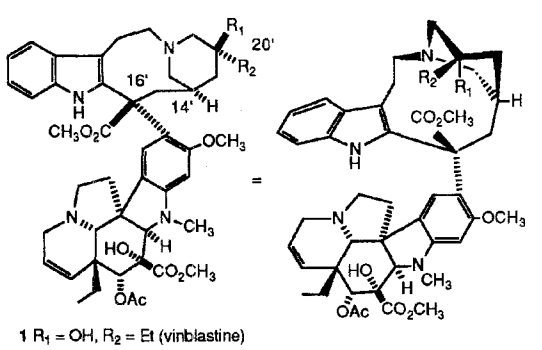
Results

Three wild-type tumor cell lines and two MDR cell strains were used in this work (Table 1). The rat colon-adenocarcinoma cell line RCC-5 is 3–5 times more resistant to natural-product drugs than is the RCC-2 cell line. Neither cell line was exposed to or selected by drug treatment. The S180/MDR cell strains exhibit from 3- to 90-fold resistance to natural-product drugs as compared with the wild-type S180 cell line.

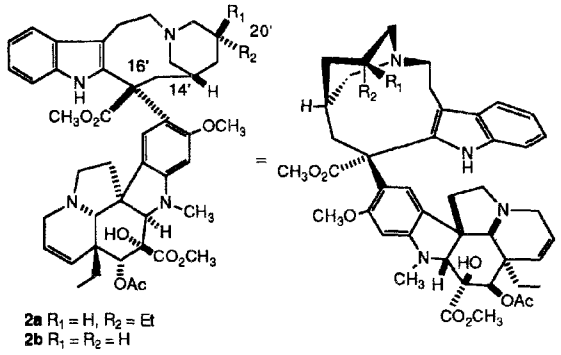
The synthetic compounds used in this study were C20′-modified (R1, R2) VBL congeners in a series of three stereoisomers: **2**, C14′(20′); **3**, C16′; and **4**, C14′, 16′ (20′) as shown in Fig. 1. Also included in the study were vincovaline (**4d**, C14′16′20′-epi VBL, a very rare, only once-isolated natural product, synthesized for this work), and the monomer **5**, vindoline, the "lower"-half moiety of VBL and a known modulator of MDR in several systems [16, 32]. All congeners were weakly cytotoxic, their IC_{50} values being ~1000-fold that of VBL itself (data not presented).

Modulation of intrinsic drug sensitivity

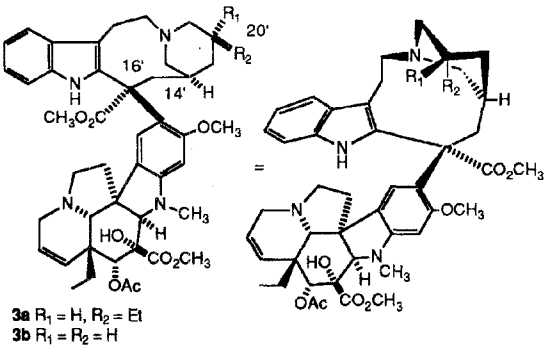
The dose-response curve illustrated in Fig. 2 shows the progressive sensitization of the wild-type S180 cells to DOX in the presence of increasing concentrations of **4a**, C-20′-deoxy C14′16′20′-epi VBL. When the modulation of VCR, DOX, and VBL cytotoxicity in S180 cells was compared for this congener (Fig. 3), drug selectivity of modulation was observed. Respective 11- and 18-fold en-



1 R_1 = OH, R_2 = Et (vinblastine)



2a R_1 = H, R_2 = Et
2b R_1 = R_2 = H



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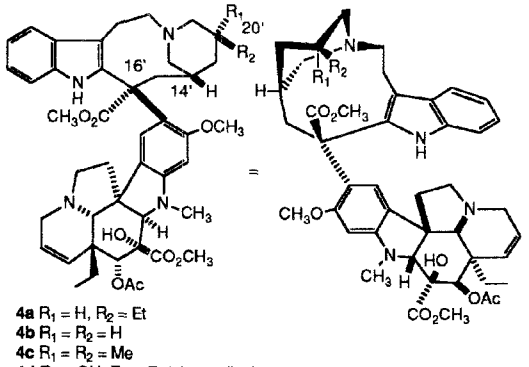
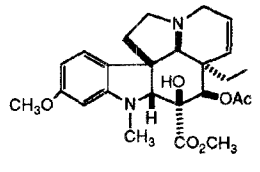
3b R_1 = R_2 = H



4a R_1 = H, R_2 = Et
4b R_1 = R_2 = H
4c R_1 = R_2 = Me
4d R_1 = OH, R_2 = Et (vincovaline)



5 (vindoline)

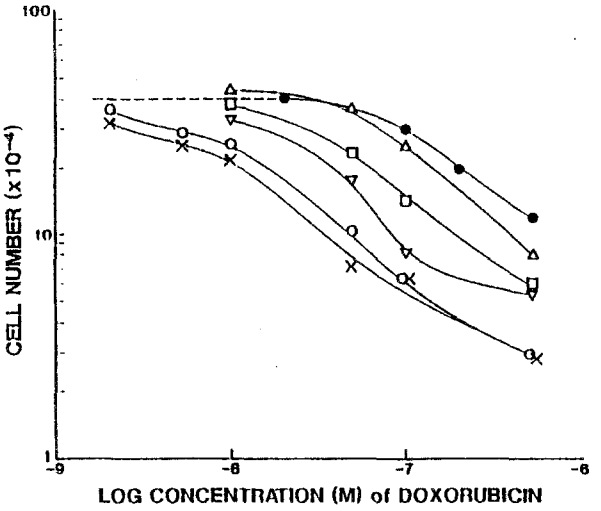


Fig. 2. Modulation of DOX sensitivity in parental S180 cells by congener 4a, C-20′-deoxy C14′16′20′-epi VBL. Population growth was determined after a 72-h incubation in the absence (●) or presence of 1 (△), 5 (□), 10 (▽), 20 (○), or 50 μM (×) of the congener. Each value is the mean (SEM, ≤10%) for 2–3 separate experiments performed with duplicate samples

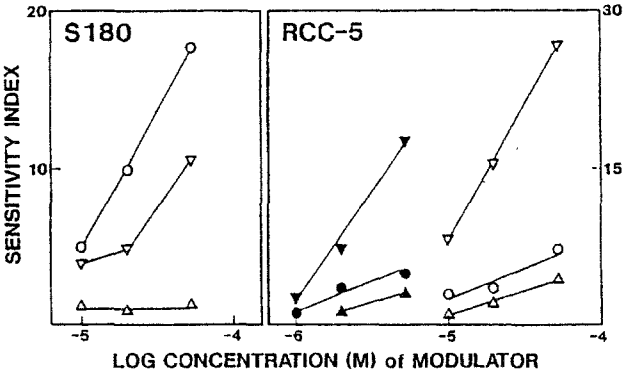


Fig. 3. Potency of VBL congeners as modulators of wild-type cell sensitivity to VBL (*triangles*), VCR (*inverted triangles*), or DOX (*circles*). S180 cells were treated with 4a, C-20′-deoxy C14′16′20′-epi VBL; RCC-5 cells were treated with 4b, C-20′-deoxy deethyl C14′16′-epi VBL (*open symbols*), or 4c, C-20′-dimethyl, C20′-deoxy deethyl C14′16′-epi VBL (*closed symbols*). The sensitization index was calculated as the IC_{50} value for the drug alone divided by the IC_{50} value for the [drug + modulator] combination.

hancement of VCR and DOX cytotoxicity was achieved, but there was no effect on cell sensitivity to VBL. In the RCC-5 cell line (Fig. 3), the selectivity of modulation followed the order of VCR > DOX > VBL for the congeners **4b**, C20′-deoxy deethyl C14′16′-epi VBL, and **4c**, its C20′-dimethyl derivative. A ~10-fold difference was noted between these compounds in the potency of modulation.

The maximal degree of drug sensitization achieved by all congeners in the S180 and RCC cell lines at their nontoxic concentration limit is compiled in Table 2. In

Fig. 1. Planar and conformational (approximations of computer-generated energy minimizations) structures of the binary stereoisomers of VBL congeners examined in this study

Table 2. Sensitization of wild-type cells by stereoisomers of C20′-vinblastine congeners

Stereoisomer	Cell line	Drug[a]			Modulator concentration (μM)
		DOX	VBL	VCR	
2, C14′-epi:					
2a, C20′-deoxy VBL	S180	4.0	1.4	7.0	40
	RCC-2	2.1	1.8	7.3	10
	RCC-5	6.4	1.6	3.5	10
2b, C20′-deoxy deethyl VBL	S180	4.2	1.0	2.0	20
	RCC-2	1.0	1.0	2.0	10
	RCC-5	2.2	2.0	6.0	10
3, C16′20′-epi:					
3a, C20′-deoxy VBL	S180	5.0	1.0	3.0	20
	RCC-2	1.9	1.2	4.0	5
	RCC-5	4.0	1.9	13	5
3b, C20′-deoxy deethyl VBL[b]	S180	5.0	1.0	3.0	50
	RCC-2	1.0	1.0	3.1	10
	RCC-5	4.0	1.4	5.1	10
4, C14′16′(20′)-epi:					
4a, C20′-deoxy VBL	S180	19	1.0	11	50
	RCC-2	1.4	1.8	2.8	2
	RCC-5	3.0	2.0	3.6	2
4b, C20′-deoxy deethyl VBL	S180	5.5	2.3	2.7	50
	RCC-2	2.0	1.0	2.7	20
	RCC-5	7.8	5.0	28	50
4c, C20′-dimethyl, C20′-deoxy deethyl VBL	S180	2.3	1.3	3.5	5
	RCC-2	1.0	2.3	8.6	5
	RCC-5	2.5	3.3	18	5
4d, VBL (vincovaline)	S180	3.7	1.6	3.5	10
	RCC-2	1.0	1.7	3.8	10
	RCC-5	3.5	5.0	12	10
5, Vindoline (monomer)	S180	7.0	4.0	7.0	20
	RCC-2	0.9	4.0	12	20
	RCC-5	5.7	5.0	20	20

Sarcoma S180 cells and rat colon-adenocarcinoma cell lines RCC-2 and RCC-5 were incubated with various concentrations of each drug and with the maximal nontoxic concentration of each vinca alkaloid, or the solvent limit (50 μM), for 3 days. Population growth was enumerated by an electronic particle counter

[a] The sensitization values were calculated as the IC_{50} value for the drug alone divided by the IC_{50} value for the [drug + modulator] combination. Data represent the mean values (± SD ≤15%) for 2–4 separate experiments

[b] Single experiment

general, modulator activity was poor toward VBL in all cell lines. The most active modulator in the sarcoma cell line was **4a**, C20′-deoxy C14′16′20′-epi VBL, at 50 μM. Its potency (magnitude of sensitization per unit concentration) was similar to that of other congeners whose dose was limited by higher inherent cytotoxicity, for example, congeners **3a**, C20′-deoxy C16′20′-epi VBL, and **4d**, vincovaline. In the RCC cell lines, a modulator of clearly superior potency for VCR sensitization was **4c**, C20′-dimethyl, C20′-deoxy deethyl C14′16′-epi VBL. The vinca alkaloids tested in this study did not modulate cell sensitivity to the antimetabolites MTX or 5-FU (data not presented).

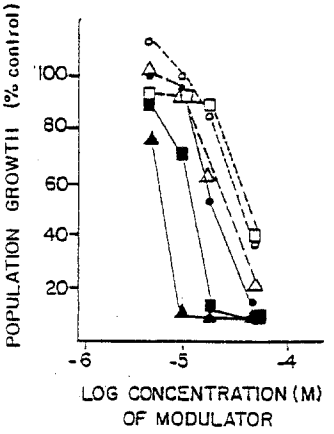


Fig. 4. Reversal of MDR by 4a, C20′-deoxy C14′16′20′-epi VBL, in A3 (*closed symbols*) and A10 (*open symbols*) cells. Population growth was determined at 72 h after treatment of each cell strain with various concentrations (5–50 μM) of the modulator and with the *parental* IC_{70} of VBL (*triangles*), VCR (*squares*), and DOX (*circles*)

Modulation of acquired MDR

The stereoisomers of C20′-modified VBL congeners produced a concentration-dependent sensitization of the MDR cell strains to the *wild-type* IC_{70} of VBL, VCR, or DOX, as exemplified in Fig. 4 for **4a**, C20′-deoxy C14′16′20′-epi VBL. Table 3 displays the minimal concentration of modulator required to reverse drug resistance (70% growth inhibition in coincubations with each drug) as calculated from such cytotoxicity curves. The C20′-deoxy VBL congener in all three stereoisomeric forms was capable of reversing the complete MDR profile in both cell strains, representing up to a 90-fold sensitization to drug (DOX). Monomeric vinca alkaloid **5**, vindoline, was also a potent modulator of MDR in this cell system. On the other hand, **2b**, **3b**, and **4b**, the stereoisomers of C20′-deoxy deethyl VBL; **4c**, the C20′-dimethyl derivative of C20′-deoxy deethyl C14′16′-epi VBL; and **4d**, vincovaline, were limited to activity toward VBL and VCR resistance.

There was direct proportionality between the amount (concentration) of *binary* vinca alkaloid required for reversal and the cellular drug-resistance level for all drugs in both MDR cell strains (Fig. 5). In contrast, monomeric vinca alkaloid **5**, vindoline, exhibited a single threshold concentration of ~9 μM for reversal of the 3- to 17-fold range of drug-resistance levels (Fig. 5, insert).

Antimicrotubule activities

The effects of the various stereoisomers of C-20′ VBL congeners on the polymerization of microtubules from isolated MTP and on the mitotic indices of cultured tumor cells are presented in Table 4. The C20′-VBL congeners with the natural (VBL-like) stereochemistry are antimicrotubule agents in both assays; the activity of C20′-deoxy VBL is nearly indistinguishable from that of VBL itself, and the C20′-deoxy deethyl congener is ~10 times less potent than VBL [7, 18]. The **2**, C14′-; **3**, C16′(20′)-; and **4**, C14′16′(20′)-epimers of these congeners as well as

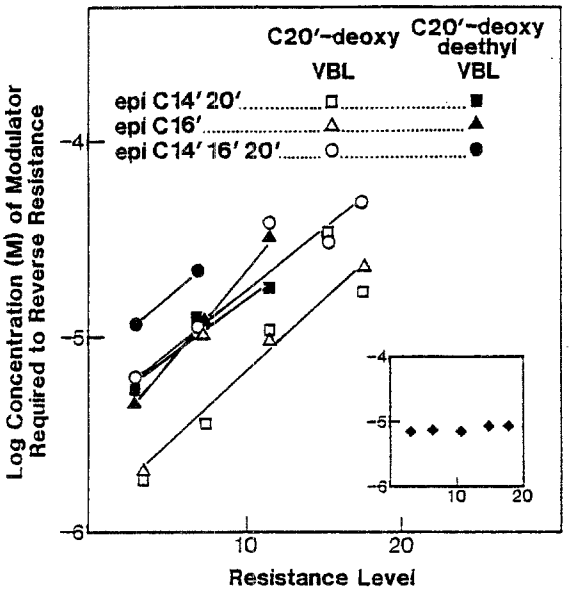


Fig. 5. Relationship between the drug-resistance levels of S180/MDR A3 and A10 cells and the minimal concentration of each congener required for the reversal of drug resistance. *Inset:* Vindoline

Table 3. Modulation of MDR by stereoisomers of C20′-vinblastine congeners

Stereoisomer	Concentration of congener (μM) for reversal of two levels of resistance to drugs					
	Dox		VBL		VCR	
	15	90	3	11	7	17
2, C14′-epi:						
2a, C20′-deoxy VBL	35	50[b]	1.5	10	3.5	17
2b, C20′-deoxy deethyl VBL	50[b]	NR	5.2	18	11	50[b]
3, C16′20′-epi:						
3a, C20′-deoxy VBL[c]	20[a]	40	1.7	10	10	20
3b, C20′-deoxy deethyl VBL	50[b]	NR	5.0	34	10	50[b]
4, C14′16′(20′)-epi:						
4a, C20′-deoxy VBL	30	50	6.0	40	10	50
4b, C20′-deoxy deethyl VBL	20[a]	NR	11	20[a]	19	20[a]
4c, C20′-dimethyl, C20′-deoxy deethyl VBL	NR	NR	9.0	NR	6.5	NR
4d, VBL (vincovaline)	50[b]	50[b]	20	60	20	60
5, Vindoline (monomer)	11	40[b]	9.0	8.0	9.0	10

S180/MDR cell strains A3 and A10 provided the low and higher resistance levels to each drug, respectively. Cells were incubated with the *parental* IC_{70} of each drug and various concentrations of each compound for 3 days. Population growth was enumerated by an electronic particle counter. The minimal concentration of modulator required to inhibit growth by 70% in coincubations (i.e., to restore parental sensitivity to each drug) was determined from plots of the percentage of control growth versus the modulator concentration of each drug. Data represent average values for 2–3 separate experiments ± SD ≤20%. NR, No response

[a] Significant (>50%) reversal of drug resistance at the maximal nontoxic concentration of modulator

[b] Partial (20%–50%) reversal of drug resistance at the maximal nontoxic concentration limit of modulator

[c] Single experiment

Table 4. Antimicrotubule activities of C20′-VBL congeners and their stereoisomers

Compound	Microtubule assembly[a]			Mitotic index[b] (%)
	Conc. (μM)	Polymerization rate (%)	Steady-state assembly (%)	
None	–	100	100	1.0
VBL	0.5	14	40	65
C20′-deoxy VBL	0.5	11	12	71
C20′-deoxy deethyl VBL	2.0	19	60	70
2, C14′-epi:				
2b, C20′-deoxy deethyl VBL	100	77	100	ND
3, C16′20′-epi:				
3a, C20′-deoxy VBL	100	94	100	ND
3b, C20′-deoxy deethyl VBL	25	100	100	ND
4, C14′16′(20′)-epi:				
4a, C20′-deoxy VBL	50	75	90	0.8
4b, C20′-deoxy deethyl VBL	20	111	107	2.7
4c, C20′-dimethyl, C20′-deoxy deethyl VBL	100	83	98	ND
4d, VBL (vincovaline)	100	80	100	ND
5, Vindoline (monomer)	50	96	85	ND

[a] Microtubule protein (MTP) was purified from bovine calf brain by two cycles of depolymerization and polymerization [24]. The effect of the vinca alkaloids on the polymerization of MTP (8–10 μM) in assembly buffer (0.1 M MES, 1 mM EGTA, 0.5 mM $MgCl_2$ and 1.0 mM GTP; pH 6.4) was measured by turbidimetric assay [11] and is presented as a percentage of the control value. Data are from a single, typical experiment

[b] The mitotic index of populations of rat colon-adenocarcinoma cell line RCC-2 was measured after a 21-h incubation with VBL or congener at a concentration 10-fold that of the IC_{70} value. The trypsinized RCC-2 cells (and washes) were centrifuged at 175 *g* and 4° C for 5 min, and the pellets were resuspended in 0.75 M KCl prior to incubation for 13 min at 37° C. After centrifugation, the cell pellet was resuspended in fresh fixative consisting of 3 : 1 (v/v) methanol : acetic acid, washed twice in the fixative, and then dropped onto wet glass slides. The specimens were stained with 2% Giemsa in water for 4 min and then scored. At least 400 nuclei were counted, and the percentage for the mitotic index was calculated as the number of metaphases divided by the total number of nuclei counted × 100. Values are from a single, typical experiment. ND, Not done

4d, vincovaline, and **5**, vindoline, do not inhibit microtubule polymerization from MTP nor do they elicit mitotic arrest in cultured cells at high, growth-inhibitory concentrations. In addition, **4a**, C20′-deoxy vincovaline, and **5**, vindoline, did not affect the antimicrotubule activities of VBL with MTP in coincubations (data not presented).

Discussion

Modulation of intrinsic drug resistance

The potency of the binary vinca alkaloid congeners and of the monomer vindoline as modulators of drug cytotoxicity

in S180 cells reflected the rank order of inherent drug resistance, i.e., DOX > VCR > VBL. Also, the modulators were usually more potent in the RCC-5 cell line than in its more drug-sensitive correlate, RCC-2. Although P-glycoprotein is not detected in the wild-type cell lines by Western blot assay (see Materials and methods), a low level of P-glycoprotein may be present in these cells. However, the magnitude of drug sensitization achievable by our modulators (>10-fold for DOX and/or VCR) in these cell lines suggests that other mechanisms are involved. Structural aspects of activity among the binary vinca alkaloid modulators also point to this conclusion (see below).

Modulation of acquired MDR

Using two MDR cell strains and three natural-product drugs, we possessed a total of six different levels of resistance for the evaluation of our compounds. We found unequivocally that the modulation *potency* of each binary vinca alkaloid congener was independent of drug-resistance level, specific drug, or MDR cell strain; that is, a given concentration of congener elicited a similar degree of sensitization to any and all drugs among the cell strains. Consequently, the *amount* (concentration) of modulator required for reversal was proportional to drug-resistance level. Since the P-glycoprotein content of the A3 and A10 cell strains is proportional to their degree of drug resistance (see Materials and methods), these data are consistent with a competitive interaction between congener and drug for efflux from the cell. In preliminary studies, we found a 3.5-fold increase in VBL accumulation in a human MDR cell strain by **4a**, C20′-deoxy C14′16′20′-epi VBL, at 10 μM (Keilhauer, personal communication).

On the other hand, by virtue of restoring wild-type sensitivity to all drugs at a single concentration (~9 μM), the *monomeric* vinca alkaloid vindoline was most potent toward the drug (DOX) or cell line (A10) of highest resistance. This property is shared by the modulators verapamil [2, 7] and cepharanthine [27] in human MDR cell strains. These properties would be consistent with a modulator that inhibited the overall function of the pump, not necessarily by direct interaction between the modulator and the drug.

General conclusions about structure/activity

That the binary vinca alkaloid drugs VBL and VCR are potent antimicrotubule agents raised the possibility that their congeners could modulate drug cytotoxicity through a direct interaction at this intracellular target. However, our results (Table 4) show that this is not the case; the various stereoisomers of C20′-modified VBL did not inhibit microtubule assembly nor did they elicit mitotic arrest in tumor cells at cytotoxic concentrations. The lack of known biologic activity for the binary vinca alkaloid modulators, in their own right, addresses the clinical limitations of other modulators, such as the unacceptable cardiac effects reported for the calcium channel blocker verapamil in phase I trials [22].

In *wild-type* cells, the new modulators generally showed more potency toward rat colon-adenocarcinoma RCC cells than toward sarcoma S180 cells. Overall, the potency of stereoisomers followed the order type 4 ≥ type 3 >> type 2 (see Fig. 1), with the notation that the loss of the C20′-ethyl substituent reduced modulator potency in all cases. Vindoline and vincovaline showed similar overall potency for modulation of VCR and DOX cytotoxicity. In MDR cell strains, the relative potency followed the order type 2 ≥ type 3 > type 4. Again, the C20′-deoxy deethyl VBL congeners were of lower overall potency.

Therefore, the relative sensitization potency of the type 4 and type 2 stereoisomeric series (see Fig. 1) were reversed in wild-type and MDR cells. Since a possible fit for binding sites for these diastereomeric series must have drastically different requirements, a common receptor would be highly unlikely. These considerations suggest that different mechanisms underlie modulator action in wild-type versus MDR cells. Considering the MDR results alone, it is remarkable that modulation is found with compounds that are diastereomerically very different. These results indicate that a simple pharmacophore model, such as that proposed by Pearce et al. [24] on the basis of yohimbine-like compounds, may not be predictive for other MDR modulators. It is noteworthy that the potentiation of activity by C20′-alkyl substitution, which we had found for antimicrotubule activities and cytotoxicity in congeners of VBL with the natural stereochemistry [5, 7], was also observed in the present study. Thus, relatively minor structural changes produce more significant alterations in modulation activity than do some of the major changes in the overall shape of these molecules, whereas the VBL-like shape is an absolute requirement for antimicrotubule activity.

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