Effects of Isoprenoid Analogues of *SDB*-Ethylenediamine on Multidrug Resistant Tumor Cells Alone and in Combination with Chemotherapeutic Drugs

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Received August 3, 2001

Multidrug resistance (MDR) mediated by P-glycoprotein (Pgp) remains the major obstacle for successful treatment of cancer. Inhibition of Pgp transport is important for higher efficacy of anticancer drugs. Lipophilic cationogenic amines with at least one tertiary N atom, such as verapamil, are classical PgP-blocking agents. In a search for novel accessible compounds potent against MDR tumor cells, we synthesized a series of arylalkylamines that contain isoprenoid side chains of different length. Two out of seven new analogues of the known *N*,*N*′-bis(3,4-dimethoxybenzyl)-*N*-solanesylethylenediamine (*SDB*-ethylenediamine), namely, compounds with C_{10} and C_{15} side chains, at low micromolar concentrations were preferentially toxic for several mammalian tumor cell lines that acquired MDR during prolonged drug selection. Moreover, at noncytotoxic concentrations, these compounds potently sensitized MDR cells to Pgp substrates vinblastine and adriamycin. We conclude that these analogues of *SDB*-ethylenediamine may have dual therapeutic advantage because (i) they are preferentially toxic for MDR cells when administered alone and (ii) they potentiate the cytotoxicity of Pgp-transported anticancer drugs.

Introduction

Multidrug resistance (MDR) of tumor cells remains a major obstacle for successful chemotherapy of cancer patients.[1−6] This type of resistance is associated with an increased efflux of antineoplastic drugs out of the cells and subsequent decrease of intracellular concentration of the drug.[2] Active extrusion of drugs is mediated by several molecular transporters; among them, P-glycoprotein (Pgp), a 140−170 kDa adenosine 5′-triphosphate (ATP)-dependent transmembrane pump, is the most widely studied.[1,3] Pgp is capable of effluxing a broad variety of compounds of different chemical structure, thereby rendering cells resistant to many agents. Consequently, blocking of Pgp-mediated transport can sensitize tumor cells to chemotherapeutic drugs. The combination of Pgp-reversing agents with conventional anticancer drugs resulted in an improved therapeutic response, at least in some types of malignancies.[4−6]

Generally, MDR-reversing compounds interfere with Pgp function by the depleting intracellular ATP pool or by interacting with the pump itself. Most agents of the second type, e.g., verapamil (VRP),[7−9] are cationogenic lipophilic amines with at least one secondary or tertiary N atom.[10,11] Among those amines, two synthetic isoprenoids, *N*-(*p*-methylbenzyl)decaprenylamine[12−14] and *N*,*N*′-bis(3,4-dimethoxybenzyl)-*N*-solanesylethylenediamine (*SDB*-ethylenediamine, **1**),[14−20] were found to potently overcome the MDR phenotype in cell culture as well as in in vivo tumor models. In particular, diamine **1** became the subject of synthetic[17,19−21] and biomedical[14−20,22−27] studies. Photoaffinity labeling experiments showed that **1**, like VRP, can directly interact with Pgp and inhibit the binding of Pgp-transported agents to the pump, thus blocking drug efflux.[16,17] Because of structural similarities between **1** and VRP (i.e., a tertiary amino group, two benzene rings connected by an aliphatic chain, and a hydrocarbon side chain), one may suggest that the isoprenoid chain of **1** is necessary for effective blocking of Pgp. Neither of the two constituents of **1**, *N*,*N*′-bis(3,4-dimethoxybenzyl)-ethylenediamine (**2**), and solanesol (SolOH) (Figure 1) sensitized the MDR human hepatoma PLC/COL or Chinese hamster V79/ADM sublines to anticancer drugs.[23,24] Plausibly, among many conformations of intact **1**, only one or only a few can be important for its MDR-reversing activity. Therefore, a search for optimal structure of isoprenoid amines to circumvent the MDR phenotype may have a significant clinical potential.

With the exception of a few synthetic long-chained (C_{45}−C_{50}) isoprenoid amines that display synergistic effects in combination with some anticancer drugs,[12,19] no data have been reported on the cytotoxicity and MDR-reversing effect of other isoprenoid analogues of **1**, in particular, of those with shorter isoprenoid chains. Considering the putative role of the length and conformation of isoprenoid chains in interaction with plasma membrane proteins, as well as greater commercial availability of linear C_{10}, C_{15}, and C_{20} isoprenoids as

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10.1021/jm011010t CCC: $22.00
Published on Web 10/26/2002

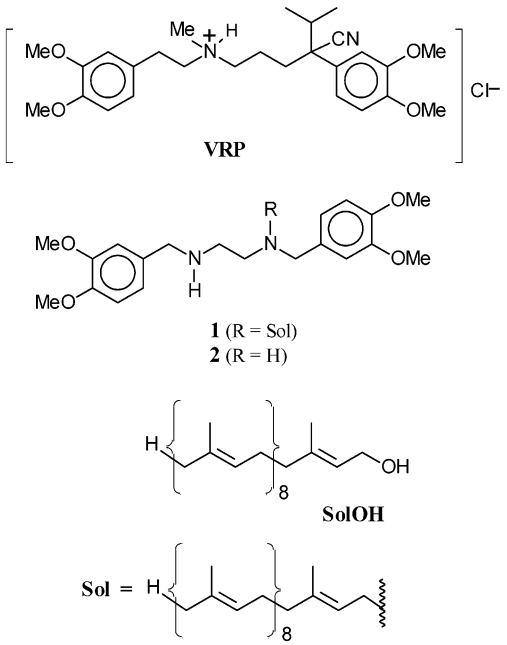


Figure 1. Structures of MDR-reversing amines, VRP, and *SDB*-ethylenediamine (**1**) and biologically inert constituents of **1**.

compared with higher polymer homologues, this lack of information is surprising. We report here the synthesis and biological characteristics (i.e., cytotoxicity and MDR-reversing activity) of several close analogues of **1** (Figure 2). These analogues have one (subseries A, **3**−**5**) or two (subseries B, **1a**, **4a**, and **5a**) side chains. We provide evidence that the compounds with relatively short C_{10} and C_{15} isoprenoid chains efficiently killed different mammalian MDR tumor cells in culture. Moreover, these compounds synergized with conventional chemotherapeutic drugs, thereby circumventing pleiotropic drug resistance.

Results

Chemistry. Our interest was originally focused on the impact of isoprenoid side chains (or, more generally, of conformationally flexible large lipophilic groups) on cytotoxicity and MDR-reversing activity of **1** and its congeners. For the initial modification of **1**, we were primarily interested in the properties of different short chain analogues of this compound. No modification of the pharmacogenic template was undertaken. The synthesis of novel compounds is shown in Scheme 1. Diamine **1** and its *N*,*N*′-bis-alkylated analogue **1a** were obtained from diamine **2**[17] and solanesyl bromide (**6**) with 85 and 12% yield, respectively (Scheme 1, reaction ii). Low molecular isoprenoid analogues **3**−**5** were prepared by alkylating **2** via a slightly modified procedure previously used for the synthesis of **1**.[17,21] Allylic halides **7**−**9** used in these alkylations contained 2*E* and 2*Z* stereoisomers in a roughly 3:1 ratio; the same stereomeric ratio was found in the specimens **3**−**5** (Scheme 1). Because *N*,*N*′-bis-alkylated products **1a** and **3a**−**5a** were also the subject of our study and their separation from **1** and **3**−**5** was easy, no attempts at selective mono- or bis-alkylation of **2** were made. Taking advantage of low selectivity of alkylation (Scheme 1, reaction iii), we obtained both types of isoprenoid amines

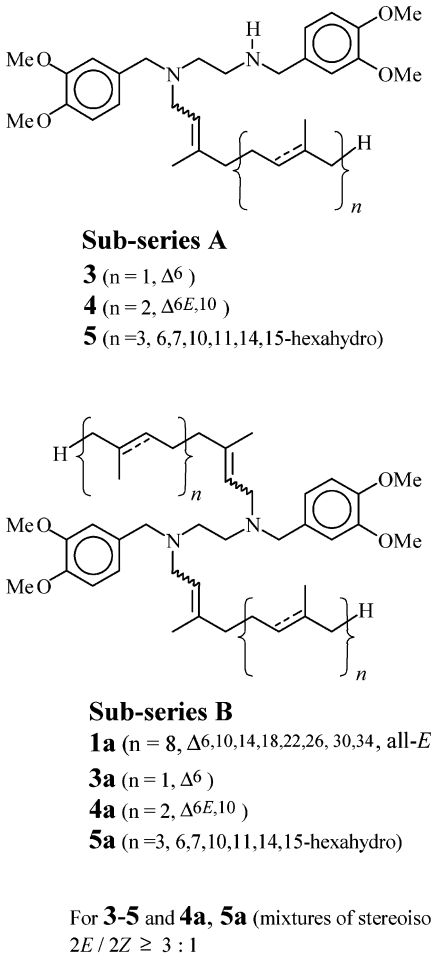


Figure 2. Novel *N*-monosubstituted (subseries A) and *N*,*N*′-disubstituted (subseries B) analogues of *SDB*-ethylenediamine (**1**) derived from diamine **2**. Compounds of the type A (**3**−**5**) contain a single isoprenoid chain attached to one of the nitrogen atoms. Analogues of the type B (**1a** and **3a**−**5a**) contain two isoprenoid side chains of equal size attached to each of the nitrogen atoms in the molecule of the parental diamine **2**.

1, **3**−**5** and **1a**, **4a**, and **5a** in acceptable yields from a single chemical operation. It is noteworthy that when alkylation of **2** was performed with C_{10}-chloride **7**, the reaction yielded monoalkylated diamine **3** as almost a single product, whereas the reactions of **2** with bromides **6**, **8**, and **9** were less selective. In the reaction of **2** with **6**, the selectivity of Suga's procedure[28] was substantially higher (**1**:**1a** ∼7:1) than the selectivity of conventional methods.[17,21]

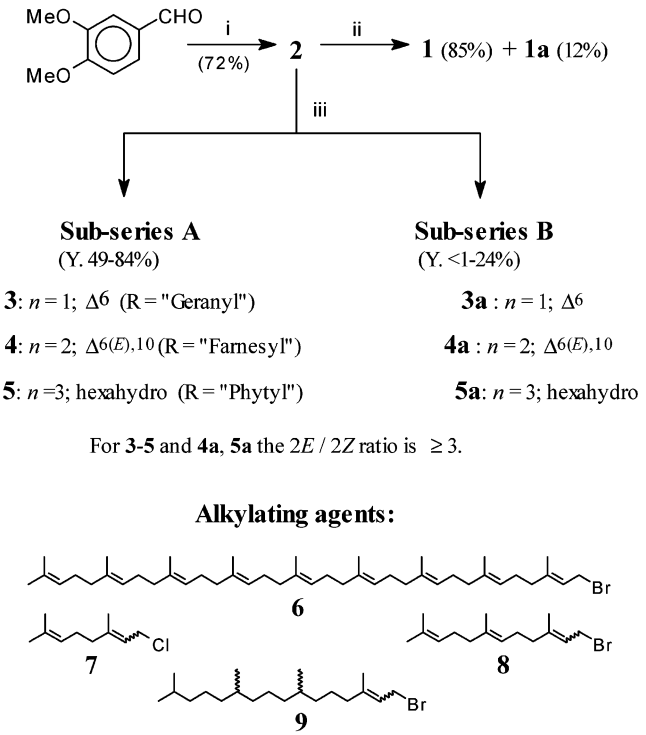
All novel analogues of *SDB*-diamine were obtained as hydrophobic waxes or viscous gums. Free bases were characterized by their R_f values and ^{1}H NMR and MS-FAB (mass spectrometry fast atom bombardment) spectra (Table 1). For biological testing, free bases were converted into dihydrochlorides **1**·2HCl, **3**·2HCl, **5**·2HCl, **1a**·2HCl, **4a**·2HCl, and **5a**·2HCl that appeared to be poorly soluble in water and only sparingly soluble in a dimethyl sulfoxide (DMSO)−H_2O mixture (see Experimental Section). The solubility in DMSO−H_2O tended to increase with the decrease of the number and length of isoprenoid chains in the molecule. For the compounds of subseries B, the solubility was below 0.5 mM even at 10% of DMSO in water, whereas for dihydrochlorides of **3**−**5** it was above 1 mM at 2%

Table 1. Chemical Characteristics of *SDB*-Ethylenediamine (**1**), Its Close Analogues (**2**−**5** and **1a**−**5a**), and Their Dihydrochlorides

	chemical parameters					
compds	method of synthesis[a]	yield (%)	R_f[b]	molecular formula	elemental analysis[c]	molecular ion (MS-FAB)[c]
				Subseries A		
1	ii, iii	85 (ii), 43 (iii)	0.27	$C_{65}H_{100}N_2O_4$		973
1·2HCl				$C_{65}H_{102}Cl_2N_2O_4$	C, 74.25; H, 9.94	
3	iii	84	0.11	$C_{30}H_{44}N_2O_4$		496
3·2HCl				$C_{30}H_{46}Cl_2N_2O_4$	C, 63.09; H, 8.21	
4	iii	49	0.25	$C_{35}H_{52}N_2O_4$		564
4·2HCl				$C_{35}H_{54}Cl_2N_2O_4$	C, 65.73; H, 8.68	
5	iii	54	0.22	$C_{40}H_{66}N_2O_4$		624 (M − CH_3)
5·2HCl				$C_{40}H_{68}Cl_2N_2O_4$	C, 67.29; H, 9.72	
				Subseries B		
1a	ii, iii	12 (ii), 19 (iii)	0.67	$C_{110}H_{172}N_2O_4$		1586
1a·2HCl				$C_{110}H_{174}Cl_2N_2O_4$	N, 1.83	
3a	iii	<1	0.53	$C_{40}H_{60}N_2O_4$		633
4a	iii	19	0.60	$C_{50}H_{76}N_2O_4$		769
4a·2HCl				$C_{50}H_{78}Cl_2N_2O_4$	C, 71.06; H, 9.54	
5a	iii	14	0.51	$C_{60}H_{104}N_2O_4$		902 (M − CH_3)
5a·2HCl				$C_{60}H_{106}Cl_2N_2O_4$	N, 2.69	

[a] Relates to stages (ii) or (iii) in Scheme 1. [b] On Silufol sheets using AcOEt−MeOH (4:1, v/v) as the eluent. [c] Corresponds to calculated values with 0.1−0.5% accuracy. [d] Using a Cratos 50-TS instrument at 8000 eV (see Experimental Section for details); the *m*/*z* values are given.

Scheme 1. Synthesis of *SDB*-Ethylenediamine (**1**) and Its Close Analogues[a]



[a] Reagents and conditions: (i) $(H_2NCH_2)_2$, $NaBH_4$, MeOH, room temperature, 8 h. (ii) $Li^+[C_{10}H_8]^-$ under Ar, room temperature, 1 h, and then **6** (1.04 equiv), THF, room temperature, 15 h. (iii) Compounds **7**−**9** (0.5 equiv), THF−Et_2O (1:1), room temperature, 2 h (for **2** + **6** → **1** + **1a** in Et_2O alone).

DMSO in water. These data are in contrast with good solubility of VRP in water (7 g/100 g, that is, 140 mM).[29]

To determine the hydrophilicity of dihydrochlorides of **1** and **3**, their partition coefficients between ethyl acetate, a volatile solvent of nearly the same lipophilicity as 1-octanol,[30] and aqueous phosphate buffers (0.1 m, pH 6.4 and pH 4.0) were determined (Table 2). As expected for ionizable compounds, these coefficients were pH-dependent. For such solutes, the apparent partition or distribution coefficient (log D) is an adequate characteristic.[31,32] Table 2 shows that at pH 6.4 (where monocation forms can appear) and at pH 4.0 (which favors the dication form) the measured values of log D for the dihydrochloride of **3** were ∼1.2 and 1.9 log units lower than in the case of **1**·2HCl. Actually, the C_{org}/C_{water} ratio at pH 6.4 was 2.45 for the dihydrochloride of **3** and 42.5 for the dihydrochloride of **1**, while the C_{org}/C_{water} ratio at pH 4.0 was 0.38 for **3**·2HCl and 35.7 for **1**·2HCl. Thus, the concentration of what is presumably the dication form of **1** in the mildly acidic aqueous phase is approximately 2 orders of magnitude lower than that of **3**·2HCl.

Table 2. Distribution Coefficients of **1**·2HCl and **3**·2HCl in AcOEt−H_2O System and Calculated Partition Coefficients (Clog P) for **1**−**3**

	Log D		
compds	pH 6.4	pH 4.0	Clog P[a]
1·2HCl	1.628 ± 0.02 (n = 2)	1.552 ± 0.06 (n = 3)	**1**:5.587
3·2HCl	0.389 ± 0.03 (n = 3)	−0.426 ± 0.03 (n = 3)	**3**:4.506
2·2HCl	not determined	not determined	**2**:3.782

[a] Using CHEM 3D ULTRA, version 6.0, for 1-octanol/water system.

These data are qualitatively similar to the values of ClogP (for 1-octanol/water) of compounds **1** (∼5.59) and **3** (∼4.51). For their common template, molecule **2**, Clog P is ∼3.78.

Biological Testing. To assess pharmacological potency of our novel compounds, we investigated their cytotoxicity and ability to reverse the MDR phenotype in cultured mammalian tumor cell lines.

Characterization of the MDR Phenotype in Drug-Selected Syrian Hamster Fibroblast Cells. The HET-SR-2SC-LNM Syrian hamster embryo fibroblast cell line is a highly malignant variant obtained after infection of embryos with the Rous sarcoma virus followed by in vivo selection for the ability to produce metastases.[33,34] Furthermore, this cell line is intrinsically resistant to colchicine (CLC), a Pgp-transported drug.[35] To establish an isogenic subline with a Pgp-mediated MDR, HET-SR-2SC-LNM cells were selected for resistance to increasing concentrations of CLC.[34,36] The resulting 2SC/20 subline (constantly maintained at 20 μg/mL CLC) was resistant to CLC as well as to Pgp-

Table 3. MDR Phenotype in 2SC/20 Subline

	IC_{50} (nM)[a] (IR)[b]					intracellular drug uptake			
						[^{3}H]VBL (pmol/mg protein)		ADM (pmol/30 min)	
cell line	CLC	VBL	ADM	AraC	BLEO	−VRP	+VRP	−VRP	+VRP
HET-SR-2SC-LNM	25 ± 2	18 ± 8	52 ± 11	772 ± 383	519 ± 160	22 ± 1	29 ± 1[c]	235 ± 36	310 ± 14
2SC/20	500 ± 12 (20)	246 ± 25 (12)	620 ± 130 (12)	1250 ± 250 (1.6)	517 ± 248 (1.0)	10 ± 2	23 ± 2[c]	132 ± 16	289 ± 20[c]

[a] IC_{50} calculated as the concentration that caused 50% inhibition of growth of respective cell line after 72 h of drug exposure (by MTT test; see Experimental Section for details) and expressed as mean ± SE of three experiments. [b] Index of resistance (IR) for each drug was calculated as mean IC_{50} for 2SC/20 subline divided by mean IC_{50} for HET-SR-2SC-LNM cells. For uptake experiments, [^{3}H]VBL (10 nM) or ADM (10 μM) were added to the cells with or without 5 μM VRP for 30 min. The values represent mean ± SE of four experiments.[c] $p < 0.01$ as compared with uptake of respective drug (VBL or ADM) without VRP (−VRP group).

Table 4. Toxicity of VRP and Dihydrochlorides of **1**−**4** for HET-SR-2SC-LNM and 2SC/20 Cell[a]

	IC_{50} (μM)				
cell line	VRP	**1**	**3**	**4**	**2**
HET-SR-2SC-LNM	48 ± 3	80 ± 6	15 ± 2[b]	35 ± 1[b]	520 ± 33
2SC/20	58 ± 0.4	20 ± 2[b]	10 ± 1[c]	13 ± 2[b,d]	470 ± 60

[a] Cells were treated with indicated compounds for 72 h. Cell viability was determined in an MTT test. Data are mean ± SE of three experiments. [b] $p < 0.001$ as compared with IC_{50} of **1** for HET-SR-2SC-LNM cells. [c] $p < 0.01$. [d] $p < 0.05$ as compared with IC_{50} of **1** for 2SC/20 cells.

Table 5. Toxicity of VRP and Dihydrochlorides of **1** and **3** for Parental and Multidrug Transporter-Expressing Cell Lines[a]

	multidrug	IC_{50} (μM)		
cell line	transporter	VRP	**1**	**3**
McA RH 7777		150 ± 20	65 ± 5	55 ± 5
McA RH 7777/0.4	Pgp	470 ± 60	55 ± 4	20 ± 5[b]
mS		66 ± 7	86 ± 12	40 ± 8
mS/0.5	Pgp	230 ± 14	45 ± 7[c]	18 ± 4[c]
K562		100 ± 5	250 ± 8	125 ± 1
K562i/S9	Pgp	100 ± 3	250 ± 6	125 ± 4
COR/L23P		80 ± 3	>200	25 ± 1
COR/L23R	MRP	80 ± 4	>200	13 ± 1[b]

[a] Cells were treated with indicated compounds for 72 h. Cell viability was assessed in an MTT test (see Experimental Section). [b] $p < 0.01$. [c] $p < 0.05$ as compared with respective parental cell line.

transported drugs vinblastine (VBL) and adriamycin (ADM) (resistance factors 20, 12, and 12, respectively) as compared with the parental HET-SR-2SC-LNM cell line (Table 3). However, both the parental- and the CLC-selected cells were similarly sensitive to 1-β-D-arabinofuranosylcytidine (ARA C) and bleomycin (BLEO), agents that normally do not participate in the MDR phenotype (Table 3).[37] The 2SC/20 cells overexpress Pgp as determined by Western blotting.[36] Furthermore, the Pgp-blocking drug VRP markedly increased intracellular accumulation of [^{3}H]VBL and ADM in 2SC/20 cells (Table 3). Interestingly, VRP also increased [^{3}H]VBL uptake by HET-SR-2SC-LNM cells to a moderate but statistically significant extent ($p < 0.01$ as compared with the −VRP group). These data indicate that 2SC/20 cells express "typical" Pgp-mediated MDR. Together, HET-SR-2SC-LNM and 2SC/20 cell lines are adequate models to assess the potency of **1** and its novel isoprenoid analogues for highly malignant cells that demonstrate intrinsic (i.e., prior to drug selection) as well as acquired (i.e., associated with selection procedure) resistance to several anticancer drugs.

Toxicity of *SDB*-Ethylenediamine Analogues for MDR Tumor Cells. We were interested in the ability of our novel compounds to completely execute their cytotoxic effect. For this purpose, we used prolonged (up to 72 h) treatments and assessed late events of cell death such as formazan conversion by mitochondrial dehydrogenases (MTT test)[38] or loss of the plasma membrane integrity (Trypan blue exclusion test). The cytotoxicity of newly synthesized analogues of **1** to HET-SR-2SC-LNM and 2SC/20 cells was compared with the effects of three reference compounds: VRP, dihydrochloride of **1**, and dihydrochloride of the parental diamine **2**. Compound **1** and its short chain analogues **3** and **4** (all tested as the corresponding dihydrochlorides) were toxic to HET-SR-2SC-LNM and 2SC/20 cells at micromolar concentrations (Table 4). Most importantly, **1**, **3**, and **4** were even more toxic for the 2SC/20 subline than for HET-SR-2SC-LNM cells (indices of resistance are 4, 1.5, and 2.7, respectively; see Experimental Section), whereas 2SC/20 cells were more resistant to VRP than HET-SR-2SC-LNM cells (Table 4). The range of cytotoxicity for the 2SC/20 subline was **3** > **4** > **1** > VRP, indicating that **1** and its analogues **3** and **4** could be more potent than VRP in killing MDR tumor cells. Moreover, both **3** and **4** were significantly more potent than **1** for HET-SR-2SC-LNM cells ($p < 0.001$; see Table 3). In agreement with previous data,[22] diamine **2** demonstrated much poorer cytotoxicity (Table 4). Its derivatives with two side chains also demonstrated much poorer cytotoxicity (**1a**, **4a**, and **5a**); for these compounds, the IC_{50} values were in the submillimolar range (data not shown).

We sought to expand the therapeutic potential of novel compounds by testing the toxicity of **3**, the most accessible analogue, for other tumor cell lines that express MDR transporters. Drug-selected mammalian tumor cell lines of different species and tissue origin were used as models (Table 5). The compound **3** proved to be more toxic for Pgp positive rat hepatoma McA RH 7777/0.4 subline[39] than to its parental Pgp negative counterpart McA RH 7777 (Table 5). In contrast, McA RH 7777/0.4 cells were ∼3-fold more resistant to VRP than McA RH 7777 cells. *SDB*-Ethylenediamine **1** was similarly toxic for McA RH 7777 and McA RH 7777/0.4 cells. Furthermore, **1** and **3** were more potent against the Pgp-expressing human melanoma mS/0.5 subline[40] as compared with the wild-type Pgp negative mS cell line (Table 5). In contrast, the mS/0.5 subline showed cross-resistance to VRP. The compound **3** was more toxic for the MDR-related protein (MRP) positive, Pgp negative COR/L23R lung carcinoma cells[41] than to the parental COR/L23P counterparts (Table 5). Thus, the monogeranyl derivative **3** could be efficient for a broader panel of drug transporter-expressing tumor cells that are otherwise resistant to several chemotherapeutics.

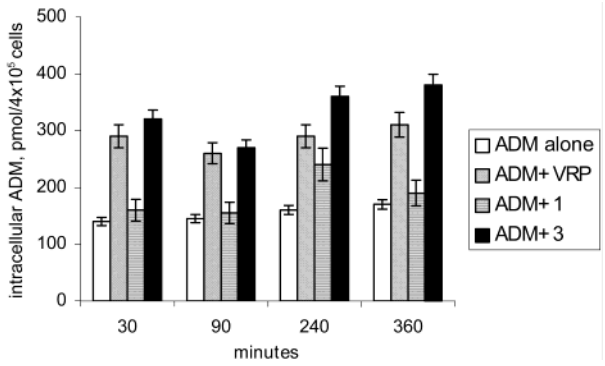


Figure 3. Potentiation of ADM accumulation by VRP, **1**·2HCl, and **3**·2HCl. The 2SC/20 cells were treated with 10 μM ADM for indicated times in the presence or absence of VRP and dihydrochlorides of **1** or **3** (5 μM each). Intracellular ADM concentration was determined as described in the Experimental Section. Each treatment was performed in triplicate. One representative experiment out of three (with essentially similar results) is shown.

Drug-selected cells presumably accumulate various changes in addition to up-regulation of multidrug transporters. To discriminate between Pgp expression per se and other selection-associated mechanism(s) that render cells sensitive to the analogues of **1**, we compared the toxicity of these analogues for K562 and K562i/S9 cells. The latter subline expresses exogenous Pgp without drug selection.[42,43] Table 5 shows that there was no preferential toxicity of VRP, **1**, and **3** for K562i/S9 cells as compared to K562 cells, suggesting that it is drug selection (but not merely Pgp expression) that confers higher sensitivity of cells to our novel compounds. Taken together, our observations demonstrate that the analogues of **1** with shorter isoprenoid chains not only retain toxicity for the parental tumor cells but also can be even more potent than **1** in killing cells selected for Pgp- and MRP-mediated MDR.

***SDB*-Ethylenediamine and Its Novel Analogue 3 Increase ADM Uptake by Pgp-Expressing Cells.** We next studied the ability of **1** and its congeners to reverse MDR in tumor cells. Two activities were tested as follows: (i) potentiation of the intracellular accumulation of anticancer drugs and (ii) increased cell death after coincubation with chemotherapeutic drugs. To address the first activity, we studied the uptake of ADM in the presence or absence of **1** or **3**. VRP was used as a reference agent. As shown in Figure 3, **1** and **3** augmented the accumulation of ADM in 2SC/20 cells, although the rate of uptake was different in the presence of **1** and **3**. The effect of **1** was detectable only when the cells were coincubated with this compound and ADM for 4 h; however, after an additional 2 h with **1**, the intracellular amount of ADM decreased. In contrast, **3** and VRP markedly potentiated ADM accumulation within 30 min (Figure 3). These results indicate that **1** and its short chain isoprenoid congeners can reverse MDR by increasing intracellular content of the Pgp-transported drug(s).

Drug Resistance Reversing Activity of *SDB*-Ethylenediamine Analogues. We further investigated the capability of **1** and its close analogues to overcome intrinsic (HET-SR-2SC-LNM cells) and acquired (2SC/20 cells) drug resistance. Cell survival was studied after both continuous coexposure of *SDB*-ethylenediamine analogues (at low micromolar concentrations) with anticancer drugs for 72 h as well as cotreatment with *SDB*-ethylenediamine analogues plus anticancer drugs for 24 h followed by cell washing and further incubation in drug-free medium for an additional 48 h. In the experiments with a 72 h coexposure, geranyl derivative **3** and farnesyl derivative **4** (5 μM each) sensitized 2SC/20 cells to Pgp-transported drugs VBL and ADM as potently as VRP (Table 5). Furthermore, **3** and **4** were more efficient than VRP in sensitizing HET-SR-2SC-LNM cells to VBL and ADM (Table 6). Compounds **1** and **2** did not influence the survival of the wild-type Syrian hamster embryo fibroblasts treated with VBL or ADM. Neither VRP nor **1** or **3** sensitized 2SC/20 cells to ARA C or BLEO (not shown), drugs that are not transported by Pgp. These data indicate that a continuous (72 h) coexposure of **3** and **4** (at low micromolar concentrations) with Pgp-transported chemotherapeutics can reverse both intrinsic and acquired Pgp-mediated drug resistance with an efficacy similar or higher than that of **1** or VRP.

Finally, we addressed the ability of **1** and its congeners to circumvent Pgp-mediated MDR in the course of a shorter (24 h) coexposure with anticancer drugs. As exemplified in Figure 4, VRP, **1**, and **3** (all at 5 μM) were not toxic for 2C/20 cells when administered alone for 24 h followed by withdrawal and further incubation for an additional 48 h. ADM in combination with VRP or **3**

Table 6. Sensitization to Pgp-Transported Drugs by VRP and Dihydrochlorides of **1**, **3**, and **4**

	HET-SR-2SC-LNM		2SC/20	
compd	VBL	ADM	VBL	ADM
VRP	4 ± 0.3^a	2 ± 0.1	11 ± 2	11 ± 1
1	1 ± 0.4	2 ± 0.1	4 ± 0.3	6 ± 0.3
3	8 ± 1^b	2 ± 0.04	11 ± 0.4^b	12 ± 0.4^b
4	6 ± 2^c	2 ± 0.1	12 ± 0.3^b	12 ± 0.3^b

[a] Index of sensitization (IS) was calculated as mean IC_{50} for cells incubated with VBL or ADM alone divided by mean IC_{50} for cells incubated with one of these drugs plus VRP or **1**, **3**, or **4** (5 μM each). [b] $p < 0.001$. [c] $p = 0.05$ as compared with IS by **1** for VBL or ADM in respective cell line. The experiments were repeated four times with <10% error.

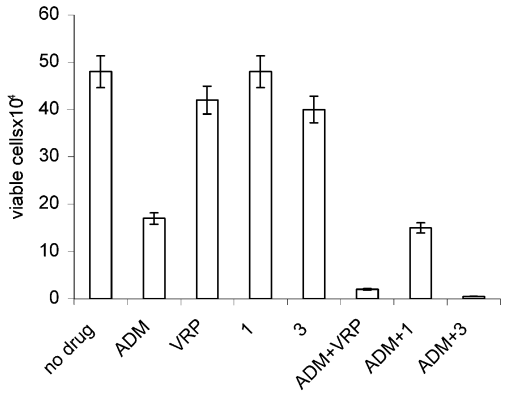


Figure 4. Effects of dihydrochlorides of **1** and **3** on ADM-induced death in 2SC/20 cells. Cells were treated with 10 μM ADM alone (open bars) or with ADM + VRP (oblique hatching), ADM + **1** (horizontal hatching), or ADM + **3** (closed bars) for 24 h followed by washing with PBS and incubation in drug free medium for an additional 48 h. Cell viability was determined by Trypan blue exclusion. VRP, **1**, and **3** were at 5 μM each. Data are mean ± SE of three independent experiments.

potently killed cells whereas **1** did not potentiate cell death by ADM (Figure 4). This lack of synergistic activity of **1** cannot be explained by insufficient intracellular concentration of ADM because even by 4 h of coincubation of **1** and ADM the level of intracellular ADM was similar to that attained by combinations of VRP + ADM or **3** + ADM (see Figure 3). It is tempting to speculate that cotreatment of cells with **1** and ADM somehow alters the intracellular distribution of the latter drug, so ADM does not reach its targets. Whatever the mechanism, the results establish the advantage of **3** over **1** in synergy with conventional chemotherapeutics.

Discussion and Conclusions

Our results show that the modification of isoprenoid chains in *SDB*-ethylenediamine **1** yielded readily accessible compounds with higher antitumor activity against various tumor cell lines than the parental agent. Two major biological characteristics of the novel congeners are particularly important. First, these novel analogues were preferentially toxic for several MDR tumor cells. Second, our novel compounds sensitized MDR cells to conventional chemotherapeutic drugs. The latter effect is due to the ability of novel compounds to increase the intracellular uptake of chemotherapeutic drugs that could be otherwise effluxed by multidrug transporters.

Out of seven novel analogues of *SDB*-ethylenediamine, the compounds **3** and **4** with relatively short isoprenoid chains (C_{10} and C_{15}, respectively) proved to be the most efficient both when used in a single agent treatment and in circumventing MDR when combined with conventional chemotherapeutic drugs. The compounds with longer or more saturated side chains were less potent, suggesting that the length of isoprenoid side chains and the extent of its saturation can be limiting factors for pharmacological activity of this group of antineoplastic drugs. Taking into consideration that the analogues of **1** with shortened isoprenoid side chains were synthesized using linalool (for **3** and **3a**) and nerolidol (for **4** and **4a**) that are large-scale manufactured chemicals, these MDR-reversing compounds warrant further investigation as potentially useful antineoplastic agents.

Interestingly, **1** and **3**, whose hydrocarbon chains are longer and conformationally more flexible than the isopropyl moiety of VRP, were more potent than VRP for some of tested cell lines. This fact implies that the specific features of the hydrocarbon side chain can markedly influence the toxicity of Pgp blockers. The role of structural and conformational modifications in pharmacological function of hydrocarbon chains has since been long-recognized in other areas of medicinal chemistry, e.g., for cannabinoids and ceramide analogues.[44,45]

The time course of biological effects and the range of active concentrations for **3**, **4**, and VRP did not vary dramatically. Indeed, **3** and VRP showed good synergism as determined by the kinetics of ADM uptake by MDR cells and viability analyses (Figure 3). In contrast, the increase of ADM uptake by the prototypic compound **1** was slower (Figure 3); moreover, **1** did not potentiate ADM toxicity for 2SC/20 cells even after a 24 h coexposure (Figure 4). These data suggest that use of MDR-reversing agents to increase the uptake of the anticancer drug is necessary but may not be sufficient for cell killing.

The reason(s) for differential effects of **1** and **3** on kinetics of intracellular ADM accumulation and potentiation of ADM toxicity is unknown. For **1**, the necessity of prolonged treatment of cells for effective sensitization may be partly due to high hydrophobicity and low solubility of **1** in aqueous media. While VRP can form true molecular solutions in water up to $C = 140$ mM, the surfactant-like **1** may exist in water as an equilibrium between molecular and micellar (colloidal) solutions. The micellar fraction of **1** would serve as a pool of free molecules (cations) capable of interfering with Pgp. If the effective concentration of molecular **1** in aqueous medium is low, the rate of this interaction would be low too. Because the side chain in **3** is much shorter, this compound should be relatively hydrophilic. Consequently, the equilibrium between true and micellar solutions would favor the molecular interaction of **3** with Pgp. This speculation is in agreement with experimentally found log D values for dihydrochlorides of **1** and **3**.

These considerations might explain the differential time course of the effects of **1** and **3** on intracellular ADM concentration (Figure 3). The amount of ADM in cells treated with ADM + **1** was low and transient, peaking at 240 min and then declining by 360 min whereas the MDR-reversing effect of **3** was fast (by 30 min of coexposure with ADM) and sustained for at least 360 min. One explanation for this difference is an equilibrium between molecular and colloidal forms of **1** in water (see above), so the capability of **1** to interfere with Pgp transport should be a function of time. Alternatively (or in addition to), **1** could alter the interaction of ADM with its intracellular targets, allowing for easier washing of ADM out of the cell. Or else **1** can influence compartmentalization of ADM. Most importantly, **3**, which is more hydrophilic due to shorter hydrocarbon chains, increased ADM accumulation in a faster and more stable manner than **1** (Figure 3). If this fact is due to the predominance of true molecular form, then the major principle of chemical modification of **1**, i.e., shortening its isoprene units for higher pharmacological potency, is validated.

Considering the role of the isoprenoid side chain in MDR-circumventing activity, it is worth noting that monophytyl derivative **5** was inactive. Possibly, the reduction of three double bonds in the acyclic C_{20} isoprenoid causes a modification of the shape (the bulkiness) and an opportunity to establish an electronic interaction (e.g., $\pi-\pi$ interaction) with the target. Reduced biological activity after saturation of double bonds has been demonstrated earlier.[44,45]

Clinical use of MDR-reversing drugs is often limited by their side effects such as toxicity for normal tissues.[4,46] It remains to be elucidated whether close analogues of **1** retain the in vivo effects of their prototype. Significantly, for cultured cells, **3**·2HCl and **4**·2HCl were potent at concentrations that were lower than toxic doses of **1**·2HCl or VRP (Tables 4 and 5). Moreover, low micromolar concentrations of **3**·2HCl and **4**·2HCl were sufficient for MDR reversal. These findings

make the compounds **3** and **4** attractive candidates for further studies.

In the present study, we used VRP, the classical MDR-reversing drug structurally related to **1**, as a major reference agent for MDR-reversing effects of our novel compounds. Although a detailed comparison of the potency of novel derivatives of **1** with other Pgp blockers was not the major goal of this work, the efficient doses of our compounds can be matched with the reported potency of other MDR-modulating drugs. The leading agent **3** reversed MDR at concentrations (5 μM, Table 6) similar to those used in the experiments with SDZ PSC 833, a nonimmunosuppressive cyclosporin currently in clinical trials.[47] These results are encouraging in terms of the possibility to synthesize relatively inexpensive and potent agents for MDR circumvention.

Importantly, dihydrochlorides of **1**, **3**, and **4** were more toxic to cells selected for MDR than to their wild-type counterparts, whereas no difference was found for the cells that express Pgp without selection (Table 6). Preferential toxicity against MDR cells has been demonstrated for Pgp blockers of different chemical structure.[48] This implies that Pgp is important for the physiology of resistant cells, and its inhibition is clinically relevant. Regardless of the fact that in drug-selected cells, mechanisms additional to drug transporters can cooperate to ensure cell survival, selection for MDR can confer sensitivity to compounds such as short chain isoprenoid analogues of **1**. The phenomenon of collateral sensitivity, "the price paid by the cell" for adaptation to the toxic environment, indicates that the functionality of some death pathways is preserved even after prolonged drug exposure.[49,50] The agents that are preferentially toxic for MDR cells may be valuable tools for dissecting mechanisms of apoptosis and/or necrosis in drug resistant cancer. Directed targeting of these mechanisms should improve clinical outcome in patients with chemotherapy resistant malignancies.

Experimental Section

***N,N′*-Bis(3,4-dimethoxybenzyl)ethylenediamine (2).** The mp 102−104 °C (acetone) was obtained according to ref 17 (Scheme 1). Solanesyl bromide (**6**) was prepared from crystalline SolOH (mp 38−41 °C, acetone at −10 °C) according to a stereoselective procedure.[51] The same method was used for converting racemic isophytol (a technical grade product purified by preparative thin-layer chromatography (TLC) on SiO_2) into phytyl bromide (**9**) and for converting (6*E*)-nerolidol[52] into (2*E*/*Z*,6*E*)-farnesyl bromide (**8**). "Geranyl" chloride (**7**) was obtained from linalool and concentrated HCl by analogy with the preparation of farnesyl chlorides from nerolidols.[51] Chemical purity of all synthesized compounds was controlled by TLC on Silufol sheets using AcOEt−MeOH (4:1, v/v) for developing, by ^{1}H NMR spectroscopy ($CDCl_3$, Bruker AM-300 instrument), and by FAB mass spectrometry (Cratos 50-TS instrument, 8000 eV, xenon as a reagent gas, glycerol as a solvent; in the case of **1a**, a mixture of glycerol with thioglycerol was used).

***SDB*-Ethylenediamine (1) and *N,N′*-Bis(3,4-dimethoxybenzyl)-*N,N′*-disolanesylethylenediamine (1a).** To a solution of naphthalene (100 mg, 0.78 mM) in absolute tetrahydrofuran (THF, 2 mL), lithium cuttings (10 mg, 1.1 mM) were added at ∼20 °C under the atmosphere of argon, and the mixture was magnetically agitated for 1 h. Then, diamine **2** (450 mg, 1.25 mM) was slowly added, the stirring was continued for 2 h, and the resulting amide was gradually quenched with bromide **6** (900 mg, 1.3 mM). The reaction mixture was agitated for 1 h at ∼20 °C, left overnight, decomposed with MeOH, diluted with water, and extracted with Et_2O. The ethereal layer was washed with water, dried (Na_2SO_4), and concentrated in vacuo to leave a brownish oil that was fractionated by flash column chromatography on SiO_2 (30 g, 40−100 μm) using a heptane−AcOEt gradient (0→25% AcOEt) as the eluent. Less polar fractions afforded compound **1a** (238 mg, 12%) as a yellowish viscous oil. TLC: R_f = 0.67. FAB-MS: $[M]^+$ = 1586 ($C_{110}H_{172}N_2O_4$). ^{1}H NMR: 1.6−1.62 (m, 54 H, Me groups); 1.7 (s, 6H, Me groups); 1.9−2.2 (m, 64 H, allylic CH_2 groups); 2.58 (br s, 4 H, N−CH_2CH_2−N moiety); 3.08 (d, 4 H, J = 6 Hz, two NCH_2CH=C); 3.49 (br s, 4 H, two NCH_2Ar); 3,85 (s, 6H, two OMe); 3.87 (s, 6 H, two OMe); 5.12−5.14 (overlap of two broad triplets with J = 6 Hz, 16 H, internal CH_2CH=C); 5.30 (br t, 2 H, J = 6 H, two NCH_2CH=C), 6.7−6.95 (m, 6 H, Ar−H). Further elution yielded diamine **1** as a viscous oil (1.034 g, 85%). TLC: R_f = 0.27. FAB-MS: $[M]^+$ = 973 ($C_{65}H_{100}N_2O_4$). ^{1}H NMR: 1.6 (br s, 27 H, Me-groups); 1.70 (s, 3 H); 1.95−2.15 (m, 32 H, allylic CH_2 groups); 2.62 and 2.70 (dt, 4 H, NCH_2CH_2N'); 3.08 (d, 2 H, J = 6 Hz, NCH_2CH=C); 3.50 (s, 2 H, NCH_2Ar); 3.7 (s, 2 H, N′CH_2Ar); 3.8 (br s, ^{1}H, NH); 3.86 (s, 6 H, two OMe); 3.88 (s, 6 H, two OMe); 5.1 (br t, 8 H, CH_2CH=C); 5.3 (br t, 1 H, J = 6 Hz, NCH_2CH=C); 6.7−6.95 (m, 6 H, Ar−H).

Synthesis of Compounds 4, 5 and 4a, 5a. General Procedure.[17,21] To a magnetically stirred solution of diamine **2** (720 mg, 2 mM) in dry THF−Et_2O (4:1.5 mL), a solution of an isoprenoid bromide **8** or **9** (1 mM) in dry Et_2O (2 mL) was added at ∼20 °C within 45 min, and the stirring was continued for an additional 3 h. The reaction mass was diluted with water (5 mL) and extracted with $CHCl_3$ (2 × 5 mL). The extract was thoroughly washed with a saturated aqueous solution of Na_2CO_3 and water, dried (Na_2SO_4), and concentrated (25−30 °C, 40 Torr) to the permanent weight of the residue. Flash column chromatography on SiO_2 using heptane−AcOEt (20→100%) and then AcOEt−MeOH (5→25%) gradients as eluents afforded first bis-alkylated diamines (**4a** or **5a**) and subsequently the corresponding monoalkylated diamines (**4** or **5**). Finally, the excess of starting diamine **2** (mp 102−103 °C) was eluted. All products were isolated as free bases. Prolonged chromatography resulted in somewhat darker specimens, although no contaminants were revealed by routine ^{1}H NMR control.

***N,N′*-Bis(3,4-dimethoxybenzyl)-*N*-(2*E*/*Z*,6*E*)-farnesylethylenediamine (4).** Yield, 141 mg (50%). A brownish, glassy gum. R_f 0.25. FAB-MS: $[M]^+$ =564 ($C_{35}H_{52}N_2O_4$). ^{1}H NMR: 1.60−1.62 (overlap of three singlets, 9 H, Me groups); 1.68 (s, 12-H_3); 1.95−2.12 (m, 8 H, allylic CH_2); 2.62−2.70 (dt, 4 H, J∼6 Hz, N−CH_2CH_2−N′ moiety); 3.08 (d, 2 H, NCH_2-CH=); 3.5 and 3.66 (two s, 4 H, $ArCH_2N$ and $ArCH_2N'$); 3.82 (br s, 1 H, NH); 3.88 and 3.90 (two s, 6 H + 6 H, OMe groups); 5.1 (br t, 2 H, CH_2CH=); 5.31 (br t, 1 H, NCH_2CH=); 6.75−6.95 (m, 6 H, Ar−H).

***N,N′*-Bis(3,4-dimethoxybenzyl)-*N,N′*-di-(2*E*/*Z*,6*E*)-farnesylethylenediamine (4a).** Yield, 36.5 mg (19%). A brownish solid foam. R_f 0.60. FAB-MS: $[M + 1]^+$ =769 ($C_{50}H_{76}N_2O_4$). ^{1}H NMR: 1.61−1.63 (overlapping singlets, 18 H, internal Me); 1.69 (s, 6 H, terminal Me); 1.93−2.12 (m, 16 H, =CCH_2); 2.59 (m, 4 H, N−CH_2CH_2−N); 3.07 (d, 4 H, J = 6.5 Hz, NCH_2CH=); 3,49 (br s, 4 H, NCH_2Ar); 3.85 and 3.87 (s + s, 12 H, OMe); 5.12 (br t, 4 H, CH_2CH=); 5.3 (br t, 2 H, NCH_2CH=); 6.73−6.96 (m, 6 H, Ar−H).

***N,N′*-Bis(3,4-dimethoxybenzyl)-*N*-(2*E*/*Z*)-phytylethylenediamine (5).** Yield, 344 mg (54%). A brownish gum with R_f 0.22. FAB-MS: 624 ([M − CH_3] ion. ^{1}H NMR: 0.9−1.12 (overlapping doublets, 12 H, CH-*Me*); 1.24−1.53 (m, 17 H, CH_2 and CH); 1.62 >1.66 (two s, 3 H, Me-C=); 1.97 (m, 2 H, allylic CH_2); 2.46−2.6 (m, 4 H, N−CH_2CH_2−N′); 2.97 (d, 2 H, J = 6 Hz, NCH_2CH=); 3.42 (s, 2 H, $ArCH_2N$); 3.50 (s, 2 H, $ArCH_2N'$); 3.83 and 3.87(s + s, 12 H, OMe); 5.23 (t, 2 H, NCH_2CH=); 6.75−6.97 (m, 6 H, Ar−H).

***N,N′*-Bis(3,4-dimethoxybenzyl)-*N,N′*-di-(2*E*/*Z*)-phytylethylenediamine (5a).** Yield, 130 mg (14.2%). A brownish gum with R_f 0.51. FAB-MS: 902 ([M − CH_3] ion. ^{1}H NMR: 0.9−1.1 (m, 24 H, Me); 1.25−1.55 (m, 34 H, CH_2 and CH); 1.63 > 1.67 (d, 4 H, J = 1.5 Hz, Me-C=); 2.5 (m, 4 H, N−CH_2CH_2−N′); 2.9−3.05 (br d, J∼6 Hz, NCH_2CH=);

3.45 (s, 4 H, $ArCH_2N$); 3.83−3.86 (s + s, 12 H, OMe); 5.3 (t, 2 H, NCH_2CH=); 6.8−7.0 (m, 6 H, Ar−H).

The reaction of diamine **2** with solanesyl bromide **6** in pure Et_2O (∼20 °C, 45 min + 3 h) proceeded similarly to give compounds **1** and **1a** with yields 43 and 12%, respectively.

***N,N′*-Bis(3,4-dimethoxybenzyl)-*N,N′*-(2*E*/*Z*)-geranylethylenediamine (3).** A solution of diamine **2** (720 mg, 2 mM) in dry THF (4 mL) and a solution of (2*E*/*Z*)-geranyl chloride (173 mg, 1 mM) in dry Et_2O (2 mL) were mixed and refluxed for 4 h. The reaction mixture was diluted with water (5 mL) and extracted with $CHCl_3$ (2 × 5 mL). The extract was washed with an aqueous solution of Na_2CO_3 (until gas production stopped) and water, dried (Na_2SO_4), and concentrated in vacuo. The column chromatography on SiO_2 using the AcOEt−MeOH (0→25%) gradient as an eluent gave 6 mg of a yellowish oil with R_f 0.53 that was tentatively identified as the compound **3a** (yield < 1%). Further elution afforded the compound **3** as a yellowish gum with R_f 0.11. Yield, 417 mg (84%). FAB-MS: $[M]^+ = 496$ ($C_{30}H_{44}N_2O_4$). ^{1}H NMR: 1.60 (s, 6 H, internal Me groups); 1.68 (s, 3 H, 8-H3); 1.95−2.15 (m, 4 H, allylic CH_2); 2.62 and 2.7 (t, 4 H, $N-CH_2CH_2-N'$); 3.08 (d, J = 6 Hz, NCH_2-CH=); 3.5 and 3.66 (s + s, 4 H, NCH_2Ar and $N'CH_2Ar$); 3.83 (br s, 1 H, NH); 3.87−3.88 (s, 12 H, OMe); 5.10 (br t, 1 H, J = 6 Hz, CH=); 5.3 (br t, 1 H, J = 6.5 Hz, NCH_2CH=); 6.75−6.95 (m, 6 H, Ar−H).

Transformation of Free Bases into Corresponding Dihydrochlorides. General Procedure. In contrast to diamine **2**, its isoprenylated derivatives were insoluble in water as free bases. To increase the solubility of compounds **1**, **1a**, **3**, **4**, **4a**, **5**, and **5a** in DMSO−water, their dihydrochlorides were prepared by adding an anhydrous ethereal solution of a base to a saturated solution of dried gaseous HCl in dry Et_2O at 5−10 °C. This was followed by either collecting the resulting precipitates on a porous (4−5.5 mm) Pyrex funnel or by evaporating the solutions in vacuo. The specimens thus obtained were dried for 4 h in a rotary evaporator at 45−50 °C/5 Torr (oil bath) and kept over $CaCl_2$ in a desiccator. Dihydrochlorides of **1**, **1a** (mp ∼40 °C), **3** (mp ∼110 °C, decomposed; creamy-white solid from cold EtOH), and **4a** (mp ∼104 °C, decomposed) were yellowish, paraffin-like substances. Dihydrochlorides of **4** and **5** were brownish solid foams with imprecise melting temperatures. The solubility of dihydrochlorides (mol/L) in DMSO−H_2O at 20 °C was determined nephelometrically by diluting their stock solutions in DMSO with distilled water until faint turbidity appeared. The results are shown below.

DMSO:H_2O ratio (v/v) in the solvent	base in [base·2HCl]						
	1a	**5a**	**4a**	**1**	**5**	**4**	**3**
10:90	2.5×10^{-5}	3×10^{-4}	5×10^{-4}	6×10^{-4}	nd	nd	nd
2:98	nd[a]	nd	nd	nd	3×10^{-3}	$>1.6\times10^{-3}$	1.8×10^{-3}

[a] nd, not determined.

The dihydrochloride of parental diamine **2** was soluble in water, DMSO, and EtOH. When stored in sealed tubes in a refrigerator at −15 °C for 6−8 months, dihydrochlorides of **1**, **1a**, **3**, **4**, and **4a** slowly decayed with the formation of artifacts detectable by TLC and ^{1}H NMR (diminished intensity of signals attributed to =CH at δ ∼5.2 and allylic methyl groups at δ ∼1.6−1.7), which may be due to isomerization or polymerization induced by traces of HCl. After 2 years of storage, the decay was nearly complete (TLC and ^{1}H NMR data).

Determination of the Distribution Coefficients. The distribution coefficients of dihydrochlorides of **1** and **3** between ethyl acetate and two aqueous phases were determined by the shake-flask method. The aqueous phases consisted of (i) 0.1 M phosphate buffer prepared from Na_2HPO_4 and NaH_2PO_4 in distilled water, pH 6.4, and (ii) 0.1 M phosphate buffer made from Na_2HPO_4 and H_3PO_4 in distilled water, pH 4.0. The aqueous and organic phases were saturated with each other. Ten milliliters of a 1 mm solution of dihydrochlorides of **1** or **3** in AcOEt (washed to neutrality and redistilled before use) was mixed with an equal volume of buffer and shaken on a reciprocal shaker for 1 h at room temperature. The mixture was left for 12 h in a separation funnel to attain a clean exfoliation. The two phases were carefully separated, and the organic phase was transferred into a calibrated flask and concentrated to permanent weight at 45−50 °C (bath)/3 Torr. The weight of the residue was determined using an OHAUS Explorer balance (readability 0.1 mg). The mass of the solute in the organic layer was calculated as $m_{AcOEt} = w - c$, where w is the weight of the residue and c is the standard weight of inorganic contaminant(s) from the aqueous buffer (0.2 mg at pH 6.4 and 0.3 mg at pH 4.0). The content of dihydrochlorides of **1** or **3** in the aqueous phase was calculated as $m_{water} = m_0 - m_{AcOEt}$, where m_0 is the mass of the analyzed specimen.

Cell Lines and Drugs. The HET-SR-2SC-LNM Syrian hamster embryo fibroblast cell line (a gift of G. I. Deichman, Moscow, Russia) and its isogenic Pgp positive variant 2SC/20 (refs 33 and 34) were propagated in Dulbecco's modified Eagle's medium (Gibco BRL, Grand Island, NY) supplemented with 5% fetal bovine serum (Gibco), 2 mm L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C, 5% CO_2 in a humidified atmosphere. Other cell models included human melanoma cell line mS, its MDR variant mS/0.5, and rat hepatoma McA RH 7777 and its MDR subline McA 7777/0.4. The mS/0.5 and McA 7777/0.4 variants were obtained by us after a stepwise selection of respective wild-type cell lines for Pgp-mediated MDR.[39,40] In addition, we used K562 human leukemia cell line (American Type Culture Collection, Manassas, VA) and its subline K562/i-S9. The latter variant expresses Pgp after infection of K562 cells with a retrovirus carrying full-length human *MDR*1 cDNA followed by flow cytometry-based sorting of Pgp positive cells (gift of I. B. Roninson, Chicago).[42,43] The K562/i-S9 cells have not been selected with any drug. For studies of Pgp-unrelated drug resistance, we used human lung carcinoma cell line COR L23/P (parental) and its ADR-selected subline COR L23/R (an MRP but not Pgp-mediated resistance[41] (provided by P. Twentyman, Cambridge, U.K.). All nonfibroblast cell lines were maintained in RPMI-1640 supplemented as above. The selective agent was withdrawn from cultures for 2 days prior to the experiments. The cells were routinely tested and found free from mycoplasma. The cultures in the logarithmic phase of growth were used in all experiments.

Dihydrochloride of *SDB*-ethylenediamine and dihydrochlorides of **1a**, **2**−**5**, **4a**, **5a**, and VRP (Sigma Chemical Co., St. Louis, MO) were dissolved in DMSO. ARA C (Upjohn, Belgium), BLEO (Nippon Kayaki Co., Ltd., Japan), VBL (G. Richter, Hungary), and ADM (Bristol Myers Squibb Pharmaceuticals, Princeton, NJ) were reconstituted in deionized water. All chemicals were prepared as 100−1000× stock solutions immediately before the experiments. When the cells were treated with the compounds dissolved in DMSO, the final concentration of the solvent in the medium was 0.1%. In our preliminary experiments, this dose of DMSO did not influence the activity of Pgp or MRP as compared with untreated cells. This dose also did not cause any discernible cytotoxicity, growth arrest, or morphological changes in cells within the time frame of experiments (not shown). [^{3}H]VBL (specific activity, 2−10 Ci/mM) was purchased from Amersham, U.K.

Cytotoxicity Assays. The toxicity of chemotherapeutic drugs and novel compounds was determined in a MTT test[38] or by direct count of cells after drug exposure. For the MTT test, cells ((3−4) × 10^3 in 100 μL of culture medium) were plated into a 96 well plate (Becton Dickinson, Franklin Lakes, NJ). After 16 h, cells were either mock-treated (vehicle control) or treated with increasing concentrations of VBL, ADM, BLEO, or ARA C (in duplicate) in the absence or presence of **1**·2HCl or its close analogues for 72 h. In the experiments with leukemia cells and lung carcinoma cells, the initial cell densities were 3 × 10^4 and 5 × 10^3 in 200 μL of culture medium, respectively. After the drug exposure was completed, 20 μL of aqueous MTT solution (Sigma; 2 mg/mL) was added into each well for an additional 3 h. Formazan was dissolved

in acidified DMSO, and the absorbency at $\lambda = 540$ nm was measured on a Flow Multiscan plate reader (LKB, Sweden). In some experiments, cells (5×10^4 in 1 mL of medium) were treated for 24 h with ADM in the absence or presence of tested isoprenoid analogue, washed with ice-cold phosphate-buffered saline (PBS), pH 7.4, and further incubated in drug-free medium for 48 h. The cells were detached from plastic using 1 mM ethylenediaminetetraacetic acid disodium salt in PBS and counted in a hemocytometer, and the percentage of viable cells (by Trypan blue exclusion) was calculated.

Drug Uptake Studies. The effects of **1**·2HCl and its isoprenoid analogues on intracellular accumulation of VBL or ADM were determined in the in vitro drug uptake assay.[36] HET-SR-2SC-LNM or 2SC/20 cells (5×10^5 per well in a 24 well plate) were treated with [^{3}H]VBL (0.037 MBq, 10 nM) in 1 mL of culture medium in the absence or presence of **1**·2HCl or its analogues for 30 min at 37 °C, 5% CO_2. Cells were then washed three times with PBS and lysed in 0.5 N NaOH overnight, and radioactivity was counted on a β-counter (LKB, Sweden). Data were normalized by total protein content as determined by the Lowry method.[53] For ADM uptake, cells were incubated with 10 μM ADM for 30−360 min, then lysed in 1 mL of 0.3 N HCl in 50% ethanol, and centrifuged (100*g*, 30 min). The fluorescence of supernatants was assayed on a Specord M-40 spectrophotometer (excitation at $\lambda = 470$ nm, emission at $\lambda = 585$ nm). All treatments were performed in triplicate. Statistical analysis was performed using Student's *t*-test. Data are expressed as mean ± SE of 3−4 experiments.

Acknowledgment. We are grateful to G. I. Deichman (N. Blokhin Cancer Center, Moscow, Russia), I. B. Roninson (University of Illinois, Chicago, U.S.A.), and P. R. Twentyman (MRC Centre, Cambridge, U.K.) for generous gifts of cell cultures. This study was supported by Grants 96-15-97461 and 00-15-97347 of the Federal Program of Supporting the Leading National Scientific Schools and 99-04-48617 of the Russian Foundation for Basic Research.

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JM011010T