# Structure–Activity Relationships of Diamines, Dicarboxamides, and Disulfonamides on Vinblastine Accumulation in P388/ADR Cells

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Received December 22, 1993; accepted March 18, 1994

**Diamines, dicarboxamides, and disulfonamides that have terminal benzene, methyl- or chloro-substituted benzene rings were synthesized and evaluated for the activity of [$^3$H]vinblastine accumulation in multidrug-resistant P388/ADR cells. The efficacy of these compounds was generally in the order of dicarboxamides < diamines < disulfonamides. *N*-Methylated diamine and disulfonamide compounds having terminal methyl- or chloro-substituted benzene rings in their structure also showed rather potent efficacy. From these findings, we synthesized a novel disulfonamide compound, 1,2,3,4,5,6-hexahydro-2,5-bis(*p*-toluenesulfonyl)benzo[2,5]diazocine (22). Compound 22 suppressed the efflux of vinblastine from P388/ADR cells and increased its intracellular accumulation, while it barely increased the vinblastine accumulation in sensitive cells (P388/S). Compound 22 significantly potentiated the growth-inhibitory effects of vinblastine, vincristine, colchicine and Adriamycin against P388/ADR cells *in vitro*.**

**Keywords** multidrug resistance; disulfonamide; vinblastine; Adriamycin; P388 cell

Multidrug resistance (MDR) is a major obstacle in cancer chemotherapy. In MDR cells, the efflux of certain kinds of antitumor drugs such as vinblastine (VBL) and Adriamycin (ADR) is enhanced, and the intracellular concentration of these drugs is lowered. These phenomena are mainly due to the result of the overexpression of P-glycoprotein in the plasma membrane in MDR cells.[1,2]

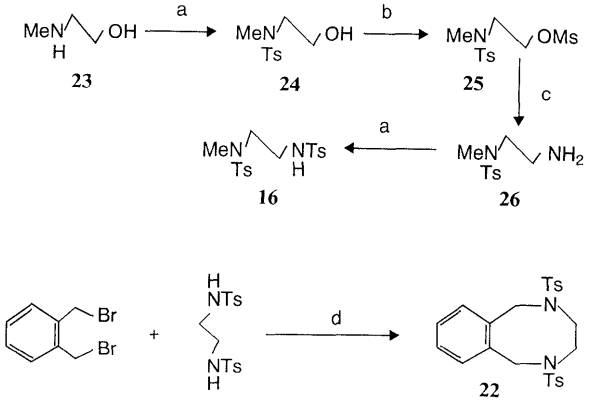
Along with many researchers, we have reported on compounds that inhibit drug efflux and increase the intracellular accumulation of VBL and ADR: verapamil,[3] phenothiazines,[4] dihydropyridines,[5] *Rauwolfia* alkaloids,[6–8] cyclosporines,[9] *N*-solanesyl-*N*,*N*′-bis(3,4-dimethoxybenzyl)ethylenediamine,[10,11] isoquinolinesulfonamides,[12–14] and staurosporine derivatives.[15] Most of these drugs share a common structural feature of aromatic rings and amino groups located between the rings. A few studies have looked at the structure–activity relationships of the basic compounds which have these structural factors, however.

In this study, to obtain potent MDR modulators we synthesized a series of diamine, dicarboxamide, and disulfonamide compounds with various aryl groups at both termini and examined the effect on VBL accumulation in MDR mouse leukemia P388/ADR cells.

Compounds, except for **7**, **10**—**13**, **16**, **18**—**21**, and **22** were synthesized by the general procedure. The novel diamine (**7**) and dicarboxamides (**10**—**13**) were synthesized by the reactions of ethylenediamine with 4-chlorobenzyl or arenecarbonyl chloride. Disulfonamides (**18**—**21**) were obtained by the reactions of ethylenediamine with 4-chlorobenzenesulfonyl chloride, or the alkylation of *N*,*N*′-bis(*p*-toluenesulfonyl)alkanediamine by methyl iodide.

The preparation of the unsymmetrical disulfonamide (**16**) is shown in Chart 1. 2-Methylaminoethanol (**23**) was treated with *p*-toluenesulfonyl chloride to give the alcohol (**24**). Conversion of **24** to the mesylate (**25**) and displacement of **25** with ammonia yielded the amino compound (**26**). Finally, reaction of **26** with *p*-toluenesulfonyl chloride gave **16**.

Table I shows the structures of diamines, dicarboxamides, and disulfonamides and the efficacy of each compound at 10 $\mu$M on the VBL accumulation in P388/ADR cells. In the case of diamines (**1**—**7**), compounds **1**, **2**, **4** and **5** having terminal benzene rings increased the VBL accumulation 2 to 3-fold over that of untreated cells. The elongation of methylene chain between the benzene ring and *N*-atom only slightly influenced the efficacy. Compound **6** with chloro-substituted benzene rings increased the intracellular VBL level over 5-fold. The introduction of the methyl group at *N*-atom (**3** and **7**) increased the VBL level more than the parental compounds **2** and **6**. Dicarboxamides (**8**—**13**) were generally less effective than diamines, although the efficacy was increased by the introduction of the methyl- or chloro-group to the benzene rings and the methylation of *N*-atom. These results coincided with the hypothesis by Klopman *et al.*[16] that the carboxamide bond reduced the efficacy of a compound as a MDR modulator. In the case of disulfona-




a) TsCl, $Et_3N$ b) MsCl, pyridine c) $NH_3$, EtOH d) Na, *n*-BuOH

Chart 1

TABLE I. Chemical Structures and Effect on VBL Uptake in P388/ADR Cells of Diamines, Dicarboxamides, and Disulfonamides

$$R^1\text{–}N\text{–}(CH_2)_n\text{–}\overset{R^2}{\underset{|}{N}}\text{–}\overset{R^3}{\underset{|}{}}\text{–}R^1$$

| Compd. No. | $n$ | $R^1$ | $R^2$ | $R^3$ | VBL uptake (% of control[a]) |
|---|---|---|---|---|---|
| Diamine | | | | | |
| **1** | 2 | $C_6H_5$ | H | H | 221 |
| **2** | 2 | $C_6H_5CH_2$ | H | H | 224 |
| **3** | 2 | $C_6H_5CH_2$ | Me | Me | 329 |
| **4** | 2 | $C_6H_5CH_2CH_2$ | H | H | 211 |
| **5** | 2 | $C_6H_5CH_2CH_2CH_2$ | H | H | 298 |
| **6** | 2 | 4-$ClC_6H_4CH_2$ | H | H | 573 |
| **7** | 2 | 4-$ClC_6H_4CH_2$ | Me | Me | 735 |
| Dicarboxamide | | | | | |
| **8** | 2 | $C_6H_5CO$ | H | H | 105 |
| **9** | 2 | $C_6H_5CO$ | Me | Me | 120 |
| **10** | 2 | 4-$MeC_6H_4CO$ | H | H | 155 |
| **11** | 2 | 4-$MeC_6H_4CO$ | Me | Me | 242 |
| **12** | 2 | 4-$ClC_6H_4CO$ | H | H | 249 |
| **13** | 2 | 4-$ClC_6H_4CO$ | Me | Me | 548 |
| Disulfonamide | | | | | |
| **14** | 2 | $C_6H_5SO_2$ | H | H | 139 |
| **15** | 2 | 4-$MeC_6H_4SO_2$ | H | H | 265 |
| **16** | 2 | 4-$MeC_6H_4SO_2$ | H | Me | 490 |
| **17** | 2 | 4-$MeC_6H_4SO_2$ | Me | Me | 777 |
| **18** | 3 | 4-$MeC_6H_4SO_2$ | Me | Me | 515 |
| **19** | 4 | 4-$MeC_6H_4SO_2$ | Me | Me | 555 |
| **20** | 2 | 4-$ClC_6H_4SO_2$ | H | H | 750 |
| **21** | 2 | 4-$ClC_6H_4SO_2$ | Me | Me | 978 |

a) VBL uptake in the presence of 10 μM compound.

mides (**14**—**21**), the compound having methyl- or chloro-substituted benzene rings tended to increase the VBL accumulation more than the unsubstituted compound (**14**). The introduction of the methyl group at *N*-atom further potentiated the efficacy. The results of **18** and **19** indicated that elongation of the methylene chain between the two *N*-atoms did not increase the efficacy.

From these results, the ethylenedisulfonamide structure might be an effective factor for the compounds that are expected to increase the VBL accumulation in MDR cells. Based on this evidence we synthesized compound **22** by the reaction of *N*,*N*′-bis(*p*-toluenesulfonyl)ethylenediamine with, α,α′-dibromo-*o*-xylene in the presence of sodium in *n*-butanol (Chart 1) and examined the efficacy on VBL accumulation in P388/S and P388/ADR cells (Fig. 1). Compound **22** increased the VBL accumulation in P388/ADR cells in a dose-dependent and potent manner, but had only slight effect in P388/S cells. It potently inhibited the VBL efflux from P388/ADR cells, and the activity in these cells was more potent than that of verapamil which has been shown to reverse MDR.[3] These results indicated that **22** increased the VBL accumulation through the inhibition of VBL efflux from P388/ADR cells, probably by inhibiting P-glycoprotein function.

We then examined the effect of **22** on the growth inhibitory effects of VBL, vincristine, colchicine and ADR in P388/S and P388/ADR cells (Table II). A non-cytotoxic concentration of **22** (5 μM) markedly increased the effect of these agents in P388/ADR cells. The combined effect was also observed in P388/S cells, but was recognized to

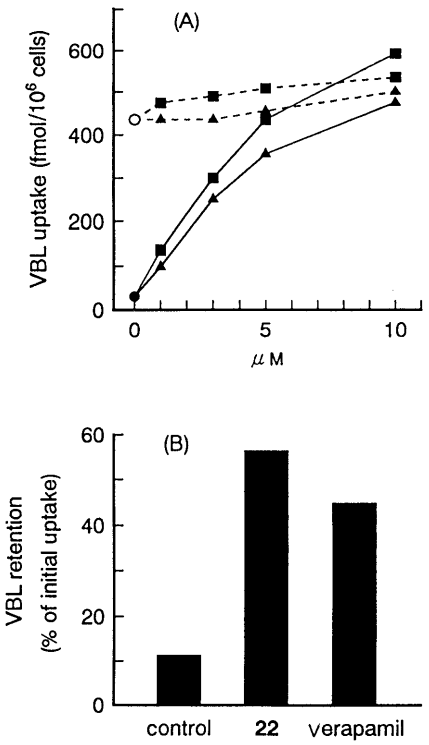



Fig. 1. Effect of Compound **22** and Verapamil on Accumulation and Efflux of VBL

(A) Cells were suspended in 20 mM Hepes-buffered RPMI-1640 medium (pH 7.4) and incubated with 37 kBq [$^3$H]VBL in the presence (closed symbols) or absence (open symbol) of each drug for 30 min at 37 °C. Solid lines indicate P388/ADR cells and dotted lines indicate P388/S cells. ■: **22**, ▲: verapamil.

(B) P388/ADR cells were preincubated with 74 kBq [$^3$H]VBL in glucose-deprived Hanks' medium containing 10 mM $NaN_3$, and washed with chilled PBS. The cells were suspended in 25 mM Hepes-buffered RPMI-1640 medium (pH 7.4) supplemented with 10% fetal calf serum and incubated for 30 min at 37 °C with or without a compound. Concentration of each compound was 10 μM. Results are the mean of two determinations in triplicate.

TABLE II. Combined Effects of 5 μM Compound **22** with VBL, Vincristine, Colchicine and ADR

| | $IC_{50}$ values (nM) | | | |
|---|---|---|---|---|
| | P388/S | | P388/ADR | |
| | − | + | − | + |
| VBL | 6.0 | 0.9 | 27 | 1.7 |
| Vincristine | 7.6 | 2.1 | 174 | 4.2 |
| Colchicine | 19 | 11 | 560 | 78 |
| ADR | 120 | 85 | 2600 | 280 |

Cells were incubated with various concentrations of antitumor agents in the presence or absence of 5 μM compound **22** for 72 h. (−) indicates $IC_{50}$ values of antitumor agent alone and (+) indicates $IC_{50}$ values in the presence of **22**.

be less. These results suggest that **22** reverses the drug resistance in P388/ADR cells by increasing drug accumulation. The reason for the slight combination effect in P388/S cells is not understood.

In conclusion, from the structure–activity relationships of diamines, dicarboxamides, and disulfonamides for VBL accumulation in MDR cells, we found that ethylenedisulfonamide could be a better structure for MDR modulation and that an ethylenedisulfonamide compound, **22**, was a potent MDR modulator.

## Experimental

Melting points were measured on Yanagimoto micro melting point hot stage apparatus and were uncorrected. Infrared (IR) spectra were determined with a Hitachi 270-30 spectrometer and mass spectra (MS) were measured with a JEOL DX-300 instrument. $^1$H-NMR spectra were recorded on a JEOL PMX-60 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard unless otherwise stated; spectral assignments were confirmed by spin decoupling experiments and, in the case of NH protons, by exchange with $D_2O$. Microanalyses were performed in the Microanalytical Laboratory of this school by Miss Yakubo.

***N,N'*-Bis(4-chlorobenzyl)-*N,N'*-dimethylethylenediamine (7)** A mixture of *N,N'*-dimethylethylenediamine dihydrochloride (0.628 g, 5 mmol), 4-chlorobenzyl chloride (1.6 g, 10 mmol), and potassium carbonate (2.0 g) in EtOH (30 ml) was stirred at 110—120 °C in a sealed tube for 7 h. The solution was concentrated, and water was added. The mixture was extracted with $CH_2Cl_2$, and the extracts were dried and evaporated *in vacuo*. The residue was chromatographed on alumina using $CH_2Cl_2$ as eluent to give (**7**) as a viscous oil. 66% yield, viscous oil, [dihydrochloride, mp 250—252 °C, colorless prisms (from $H_2O$)]. High-resolution MS *m/z*: $M^+$ Calcd for $C_{18}H_{22}Cl_2N_2$: 337.1160, Found: 337.1161. IR (neat): 1492 cm$^{-1}$. $^1$H-NMR $\delta$: 2.13 (6H, s, N–Me), 2.50 (4H, s, N–$CH_2$), 3.43 (4H, s, Ar–$CH_2$), 7.24 (8H, s, Ar–H).

***N,N'*-Bis(arenecarbonyl)ethylenediamine (10, 11, 12, 13)** General Procedure: To a mixture of the corresponding ethylenediamine (4.0 mmol) and triethylamine (20 mmol) in $CH_2Cl_2$ (20 ml) was added arenecarbonyl chloride (8.0 mmol). The reaction mixture was stirred at room temperature for 12 h. Water was added, and the mixture was extracted with $CH_2Cl_2$. The extract was washed with satd. NaCl, dried and evaporated *in vacuo* to dicarboxamides.

**10**: 59% yield, mp 239—240 °C, colorless prisms (from AcOEt–MeOH). IR (KBr): 3312 (N–H), 1634 (C=O) cm$^{-1}$. $^1$H-NMR $\delta$: 2.40 (6H, s, Ar-Me), 3.60—3.70 (4H, m, N–$CH_2$), 7.23 (2H, d, $J$=8 Hz, Ar-H), 7.30 (2H, br s, N–H), 7.70 (2H, d, $J$=8 Hz, Ar-H). *Anal.* Calcd for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 72.84; H, 6.89; N, 9.47.

**11**: 69% yield, mp 152—153 °C, colorless prisms (from $CH_2Cl_2$–isopropyl ether). IR (KBr): 1616 (C=O) cm$^{-1}$. $^1$H-NMR $\delta$: 2.35 (6H, s, Ar-Me), 3.07 (6H, s, N–Me), 3.80 (4H, br s, N–$CH_2$), 7.20—7.40 (8H, m, Ar-H). *Anal.* Calcd for $C_{20}H_{24}N_2O_2$: C, 74.05; H, 7.46; N, 8.63. Found: C, 73.97, H, 7.47; N, 8.55.

**12**: 79% yield, mp 269—270 °C, colorless prisms from dimethyl sulfoxide (DMSO)). IR (KBr): 3284 (N–H), 1634 (C=O) cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 3.40—3.60 (4H, m, N–$CH_2$), 7.63 (4H, d, $J$=8 Hz, Ar-H), 8.00 (4H, d, $J$=8 Hz, Ar-H), 8.70 (2H, br s, N–H). *Anal.* Calcd for $C_{16}H_{14}Cl_2N_2O_2$: C, 56.99; H, 4.18; N, 8.31. Found: C, 57.09; H, 4.04; N, 8.32.

**13**: 84% yield, mp 196—197 °C, colorless prisms (from $CH_2Cl_2$–isopropyl ether). IR (KBr): 1626 (C=O) cm$^{-1}$. $^1$H-NMR $\delta$: 3.07 (6H, br s, N–Me), 3.87 (4H, s, N–$CH_2$), 7.40 (8H, s, Ar-H). *Anal.* Calcd for $C_{18}H_{18}C_{12}N_2O_2$: C, 59.19; H, 4.97; N, 7.67. Found: C, 59.27; H, 5.01; N, 7.78.

***N,N'*-Dimethyl-*N,N'*-bis(arenesulfonyl)ethylenediamine (18, 19, 21)** General Procedure: To a mixture of bis(arenesulfonyl)alkanediamine (5 mmol), MeOH (20 ml) and 32% sodium hydroxide solution (4 ml) cooled in an ice-bath, methyl iodide (6 mmol) was added during 2 h, and after a further hour of stirring at room temperature, reaction was completed by 4 h of heating under reflux. After the solvent was evaporated *in vacuo*, water was added. The mixture was extracted with $CH_2Cl_2$, and the extract was washed with satd. NaCl, dried, and evaporated *in vacuo*. The residue was chromatographed on alumina using $CH_2Cl_2$ as an eluent to give disulfonamides.

**18**: 65% yield, mp 112—113 °C, colorless prisms (from $CH_2Cl_2$–isopropyl ether). IR (KBr): 1598, 1338 cm$^{-1}$. $^1$H-NMR $\delta$: 1.93 (2H, q, $J$=8 Hz, C–$CH_2$–C), 2.47 (6H, s, Ar-Me), 2.77 (6H, s, N–Me), 3.10 (4H, t, $J$=8 Hz, N–$CH_2$), 7.40 (4H, d, $J$=8 Hz, Ar–H), 7.80 (4H, d, $J$=8 Hz, Ar-H). *Anal.* Calcd for $C_{19}H_{28}N_2O_4S_2$: C, 55.32; H, 6.84; N, 6.79. Found: C, 55.66; H, 6.65; N, 6.82.

**19**: 80% yield, mp 129—130 °C, colorless prisms (from $CH_2Cl_2$–isopropyl ether). IR (KBr): 1608, 1382 cm$^{-1}$. $^1$H-NMR $\delta$: 1.50—1.80 (4H, m, C–$CH_2$–C), 2.43 (6H, s, Ar-Me), 2.70 (6H, s, N–Me), 2.90—3.20 (4H, m, N–$CH_2$), 7.40 (4H, d, $J$=8 Hz, Ar-H), 7.80 (4H, d, $J$=8 Hz, Ar-H). *Anal.* Calcd for $C_{20}H_{30}N_2O_4S_2$: C, 56.31; H, 7.03; N, 6.57. Found: C, 56.42; H, 6.93; N, 6.62.

**21**: 81% yield, mp 169—170 °C, colorless prisms (from $CH_2Cl_2$–isopropyl ether). IR (KBr): 1586, 1344 cm$^{-1}$. $^1$H-NMR $\delta$: 2.83 (6H, s, N–Me), 3.30 (4H, s, N–$CH_2$), 7.53 (4H, d, $J$=8 Hz, Ar-H), 7.80 (4H, d, $J$=8 Hz, Ar-H). *Anal.* Calcd for $C_{16}H_{18}C_{12}N_2O_4S_2$: C, 43.94; H, 4.15; N, 6.41. Found: C, 44.02; H, 4.20; N, 6.50.

***N,N'*-Bis(4-chlorobenzenesulfonyl)ethylenediamine (20)** To a mixture of ethylenediamine (4.0 mmol) and triethylamine (20 mmol) in $CH_2Cl_2$ (20 ml) was added 4-chlorobenzenesulfonyl chloride (8.0 mmol). The reaction mixture was stirred at room temperature for 12 h. Water was added, and the mixture was extracted with $CH_2Cl_2$. The extract was washed with satd. NaCl, dried, and evaporated *in vacuo* to disulfonamides. 65% yield, mp 229—230 °C, colorless prisms (from MeOH). IR (KBr): 3292 (N–H), 1588, 1336 cm$^{-1}$. $^1$H-NMR $\delta$: 3.33 (4H, br s, N–$CH_2$), 7.70—8.00 (8H, m, Ar-H). *Anal.* Calcd for $C_{14}H_{14}C_{12}N_2O_4S_2$: C, 41.08; H, 3.45; N, 6.84. Found: C, 41.14; H, 3.40; N, 6.92.

***N*-Methyl-*N,N'*-bis(*p*-toluenesulfonyl)ethylenediamine (16)** To a stirred, cooled mixture of **23** (15.02 g, 0.2 mol) and triethylamine (20.24 g, 0.2 mol) in tetrahydrofuran (THF) (100 ml) was added *p*-toluenesulfonyl chloride (38.13 g, 0.2 mol) in several portions. The reaction mixture was stirred at 0—5 °C for 4 h, water was added, and the mixture was made acidic (pH 2) with 2 N HCl. The mixture was extracted with EtOAc, and the extracts were dried and evaporated *in vacuo*. The residue was chromatographed on alumina using $CH_2Cl_2$ as an eluent to give *N*-methyl-*N*-*p*-toluenesulfonylaminoethanol (**24**) as a viscous oil. 92% yield, high-resolution MS *m/z*: $M^+$ Calcd for $C_{10}H_{15}NO_3S$: 229.0783. Found: 229.0778. IR (neat): 3544, 1336, 1160 cm$^{-1}$. $^1$H-NMR $\delta$: 2.40 (3H, s, Ar-Me), 2.80 (3H, s, N–Me), 3.17 (4H, t, $J$=6 Hz, N–$CH_2$), 3.70 (4H, t, $J$=6 Hz, O–$CH_2$), 7.36 (2H, d, $J$=8 Hz, Ar-H), 7.76 (2H, d, $J$=8 Hz, Ar-H).

To a stirred, cooled (0 °C) mixture of **24** (42 g, 0.21 mol) and pyridine (41.5 g, 0.53 mol) in THF (100 ml) was added a solution of methanesulfonyl chloride (60.14 g, 0.53 mol) in THF (40 ml). The reaction mixture was stirred at room temperature for 12 h, cold water was added, and the mixture was made acidic (pH 2) with 2 N HCl. The mixture was extracted with EtOAc, and the extracts were dried and evaporated *in vacuo*. The residue was chromatographed on alumina using $CH_2Cl_2$ as an eluent to give *N*-methyl-*N*-*p*-toluenesulfonylaminoethyl methanesulfonate (**25**). 85% yield, mp 75—76 °C, colorless prisms (from $CH_2Cl_2$–isopropyl ether). IR (neat): 1600, 1346, 1176 cm$^{-1}$. $^1$H-NMR $\delta$: 2.40 (3H, s, Ar-Me), 2.80 (3H, s, N–Me), 3.02 (3H, s, O–Me), 3.40 (2H, t, $J$=6 Hz, N–$CH_2$), 4.36 (2H, t, $J$=6 Hz, O–Me), 7.36 (2H, d, $J$=8 Hz, Ar-H), 7.60 (2H, d, $J$=8 Hz, Ar-H). *Anal.* Calcd for $C_{11}H_{17}NO_5S_2$: C, 43.01; H, 5.57; N, 4.55. Found: C, 42.98; H, 5.57; N, 4.56.

A mixture of **25** (1.55 g, 5.6 mmol) and 28% ammonia water (10 ml) in EtOH (50 ml) was heated at 80—100 °C in a sealed tube for 13 h. The solution was concentrated, water was added, and the mixture was made alkaline with 2 N NaOH. The mixture was extracted with $CH_2Cl_2$, and the extracts were dried and evaporated *in vacuo*. The residue was chromatographed on alumina using $CH_2Cl_2$ as eluent to give *N*-methyl-*N*-*p*-toluenesulfonylethylenediamine (**26**) as a viscous oil. 50% yield, high-resolution MS *m/z*: $M^+$ Calcd for $C_{10}H_{17}N_2O_2S$: 229.1011, Found: 229.0984. IR (neat): 1600, 1336, 1158 cm$^{-1}$. $^1$H-NMR: 2.67 (2H, br s, N–H), 2.46 (3H, s, Ar-Me), 2.76 (3H, s, N–Me), 2.80—3.20 (4H, m, N–$CH_2$), 7.40 (2H, d, $J$=8 Hz, Ar-H), 7.80 (2H, d, $J$=8 Hz, Ar-H).

To a stirred, cooled mixture of **26** (0.4 g, 1.7 mmol) and triethylamine (0.353 g, 3.5 mmol) in $CH_2Cl_2$ (5 ml) was added *p*-toluenesulfonyl chloride (0.33 g, 1.7 mmol) in several portions. The reaction mixture was stirred at room temperature for 48 h, water was added, the mixture was extracted with $CH_2Cl_2$, and the extract was washed with water, dried, and evaporated *in vacuo* to give *N*-methyl-*N,N'*-bis(*p*-toluenesulfonyl)-ethylenediamine (**16**) as colorless needles. 91% yield, mp 99—100 °C, colorless prisms (from $CH_2Cl_2$–isopropyl ether). IR(KBr): 3312 (N–H) cm$^{-1}$. $^1$H-NMR $\delta$: 2.40 (6H, s, Ar-Me), 2.67 (3H, s, N–Me), 3.01—3.23 (4H, br s, N–$CH_2$), 5.50 (1H, br s, N–H), 7.33 (8H, d, $J$=8 Hz, Ar-H), 7.70 (4H, d, $J$=8 Hz, Ar-H), 7.83 (4H, d, $J$=8 Hz, Ar-H). *Anal.* Calcd for $C_{17}H_{22}N_2O_4S_2$: C, 53.38; H, 5.80; N, 7.32. Found: C, 53.36; H, 5.84; N, 7.40.

**1,2,3,4,5,6-Hexahydro-2,5-bis(*p*-toluenesulfonyl)benzo[2,5]diazocine (22)** Na (2.1 g, 0.04 mol) was added to a stirred suspension of *N,N'*-bis(*p*-toluenesulfonyl)ethylenediamine (15.0 g, 0.04 mol) in *n*-BuOH (500 ml). The reaction mixture was refluxed for 1 h, and then a solution of α,α'-dibromo-*o*-xylene (10.6 g, 0.04 mol) in *n*-BuOH (100 ml) was slowly added, and stirring was continued for 24 h. The resulting yellowish brown

colored solid was collected by filtration and washed with *n*-BuOH. Recrystallization from *n*-BuOH afforded **22** as colorless needles. 45% yield, mp 184—186 °C.

$^1$H-NMR ($CDCl_3$) $\delta$: 2.40 (6H, s, $CH_3 \times 2$), 3.40 (4H, s, $C_1$-, $C_6$-H), 4.46 (4H, s, $C_3$-, $C_4$-H), 7.26, 7.61 (total 12H, each d, $J=8$ Hz, Ph). MS *m/z*: 470 ($M^+$). *Anal.* Calcd for $C_{24}H_{26}N_2O_4S_2$: C, 61.25; H, 5.57; N, 5.95. Found: C, 61.38; H, 5.57; N, 5.77.

**Cells and Culture** Parent mouse leukemia P388 cells (P388/S) and ADR-resistant P388 cells (P388/ADR) were generously provided by the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan. Cells were passaged weekly in the abdominal cavities of female BALB/c × DAB/2 ($CDF_1$) mice (Nippon SLC, Hamamatsu, Japan). To assess the effects on P388/S and P388/ADR cells *in vitro*, cells were suspended at the density of $10^5$ cells/ml in RPMI-1640 medium supplemented with 10% fetal calf serum, 20 $\mu$M $\beta$-mercaptoethanol, and 100 $\mu$g/ml kanamycin (G-medium). Two hundred $\mu$l of the cell suspension was seeded in each well of 96-well plastic dishes. A test compound was dissolved in DMSO, and the final concentration of DMSO was 0.25% (v/v). The effects of drugs on cell growth were evaluated after consecutive culture for 48 h by MTT assay.[17]

**VBL Accumulation and Efflux** Accumulation experiment: Cells ($10^6$) were suspended in 1 ml of 20 mM Hepes-buffered G-medium (pH 7.4) and incubated with 37 kBq [$^3$H]VBL in the presence or absence of a test compound at 37 °C for 30 min. The final concentration of DMSO was 1%. After the incubation, the cells were chilled on ice and collected by centrifugation (2000 rpm × 5 min) at 2 °C. The cells were washed twice with chilled phosphate-buffered saline (PBS, pH 7.4), and their VBL content was measured by determining the radioactivity after solubilization with NaOH and neutralization with acetic acid.

Efflux Experiment: Cells were loaded with 20 nM [$^3$H]VBL (74 kBq) by incubation in glucose-deprived Hanks' solution (pH 7.4) containing 10 mM $NaN_3$. They were washed once with chilled PBS and incubated without or with a compound in 20 mM Hepes-buffered G-medium (pH 7.4). After incubation of the cells for 30 min at 30 °C, the radioactivity remaining in them was measured as above. The results were expressed by the percentage of VBL remaining in the cells to initially loaded intracellular VBL.

**Materials** [$^3$H]VBL (374 GBq/mmol) was purchased from Amersham International U.K. The antitumor drugs used were VBL, vincristine (Shionogi & Co., Osaka, Japan), ADR (Kyowa Hakko Kogyo Co., Tokyo, Japan). Colchicine was obtained from Nakarai Chemicals, Kyoto, Japan.

**Acknowledgment** This study was supported in part by the Hokkoku Foundation for Cancer Research.

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