N-Alkylated 1,4-Dihydropyridines: New Agents to Overcome Multidrug Resistance

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New N-alkylated 1,4-dihydropyridine derivatives were synthesized and their ability to overcome multidrug resistance was examined in vincristine-resistant P388 cells (P388/VCR cells). Compounds that possessed an arylalkyl substituent on the dihydropyridine ring nitrogen were more potent than verapamil in potentiating the cytotoxicity of vincristine against P388/VCR cells. However, neither drug effectively enhanced the antitumor activity of vincristine in tumor-bearing mice. Introduction of basic nitrogen-containing substituents on the side chain of 1,4-dihydropyridines gave improved activity *in vitro* and *in vivo*. The piperazine derivative 12c and 12o were more than 10 times as potent as verapamil *in vitro*. Four compounds selected for *in vivo* testing showed superior antitumor activity in P388/VCR-bearing mice in combination with vincristine. The structure–activity relationships of the compounds are discussed.

Key words N-alkylated 1,4-dihydropyridine; multidrug resistance; verapamil; vincristine

One of the major problems in cancer chemotherapy is the development of multidrug resistance (MDR) during treatment.[1] When tumor cells become resistant to antitumor agents, such as vinca alkaloids or anthracyclines, they often also show resistance to other antitumor agents with different structures and mechanisms.[2] Riordan and Ling[3] have proved the involvement of a membrane-bound protein, P-glycoprotein, in MDR. This protein acts as an efflux pump for anticancer drugs.

In 1981, the calcium antagonist verapamil was introduced to overcome MDR by inhibiting outward transport of vincristine and adriamycin.[4] Since then, numerous types of compounds, including dihydropyridines,[5] isoquinolines,[6] and cyclosporin analogues,[7] have been introduced to overcome MDR. Among them, dihydropyridines have been studied most extensively, because of the analogy to the calcium antagonistic activity of verapamil.[5] As for verapamil, several combination therapies with antitumor agents, such as vinca alkaloids or anthracyclines, have been tried, but caused cardiovascular side effects because of the calcium antagonistic activity.[8] The finding that the enantiomer of verapamil lacks calcium-antagonistic activity but still possesses MDR reversal activity indicated that the former activity is independent of the latter.[9] To find an agent to overcome MDR with fewer side effects, we synthesized 1-alkylated1,4-dihydropyridine analogues because of their structural similarity to verapamil and also because 1-alkylation of 1,4-dihydropyridines is known to diminish calcium antagonistic activity. In this paper we describe the design and synthesis of a series of 1-alkylated 1,4-dihydropyridines, and their activity for overcoming MDR *in vitro* and *in vivo*.

Chemistry

The N-alkylated 1,4-dihydropyridine derivatives listed in Table 1 and 2 were prepared as shown in Chart 1

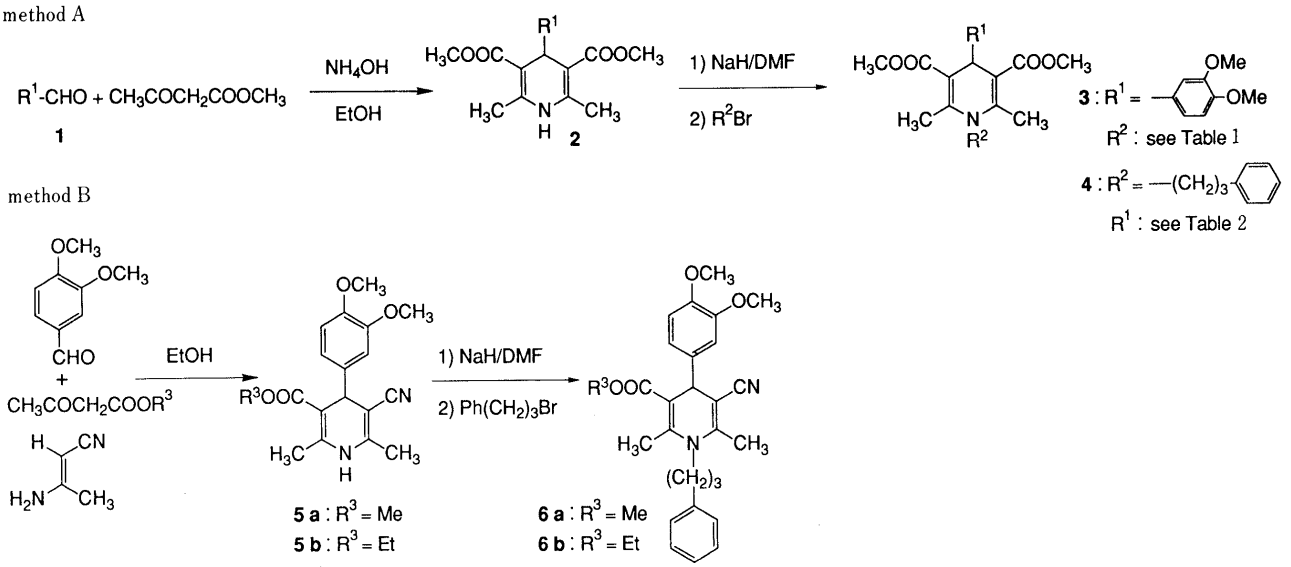

Chart 1. Synthesis of **3**, **4** and **6** Derivatives

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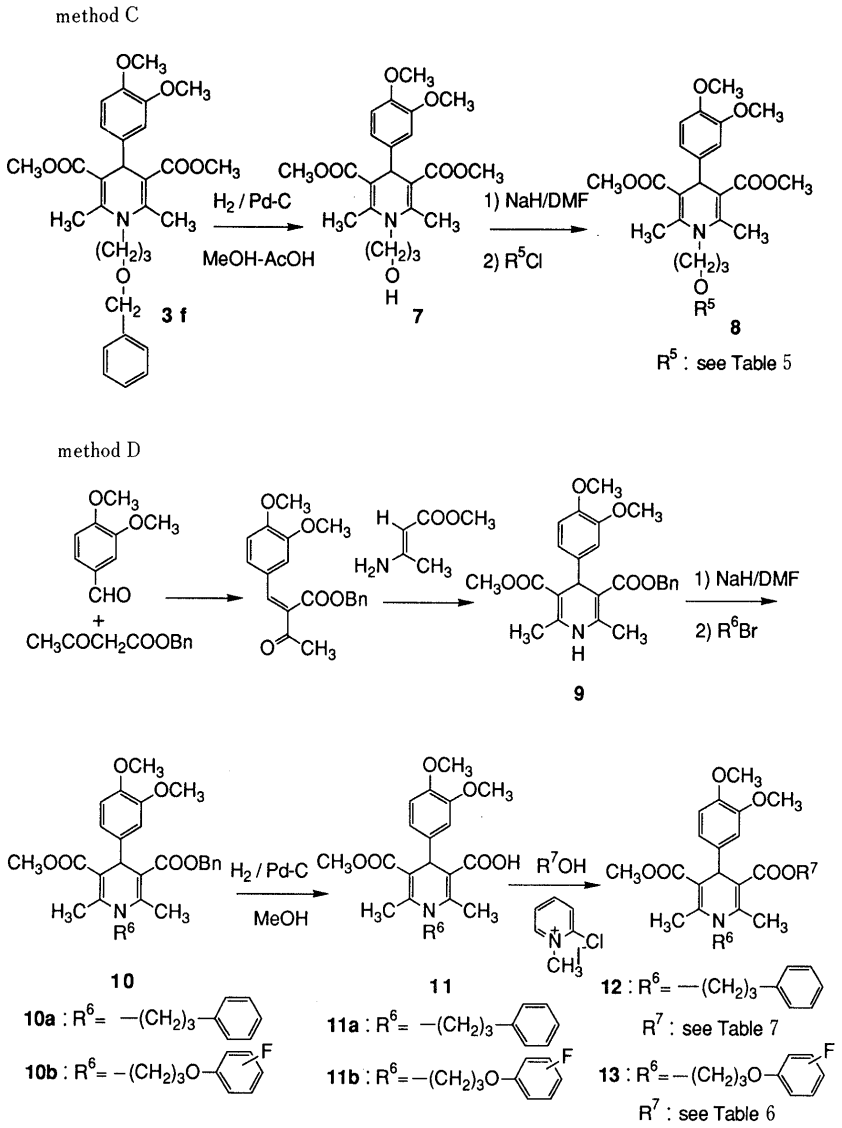


Chart 2. Synthesis of **8**, **11**, **12** and **13** Derivatives

(method A). The Hantzsch reaction of methyl acetoacetate with various aldehydes (**1**) gave the 1,4-dihydropyridine derivatives (**2**) in 30—62% yields. Compound **2** was then alkylated with alkyl halide in dimethylformamide to give **3** or **4** in 28—94% yields. Compounds **6a** and **6b** in Table 3 were prepared by using a modified Hantzsch reaction followed by alkylation with phenylpropylbromide in 60—68% yields (method B).

The N-alkylated 1,4-dihydropyridine derivatives listed in Table 5, 6 and 7 were prepared as shown in Chart 2. Compound **3f** was deprotected by catalytic hydrogenation on 10% Pd–C in MeOH–AcOH to give the alcohol (**7**) in 28% yields. Compound **7** was allowed to react with chloromethylpyridine or pyridinecarboxylic acid to give **8** in 23—90% yields (method C).

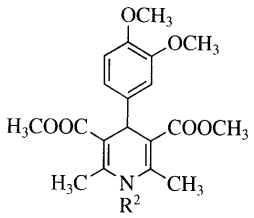
The reaction of 3,4-dimethoxybenzaldehyde with benzyl acetoacetic acid under reflux gave a benzylidene derivative, which was condensed with methyl aminocrotonate in EtOH to give **9** in 57% yields. Compound **9** was alkylated with alkyl halides to give **10** in 57—65% yields. The products (**10**) were deprotected by catalytic hydrogenation on 10% Pd–C in MeOH to give the carboxylic acids (**11**) in 48—70% yields. These products (**11**) was esterified with alcohol derivatives in the presence of 1-methyl-2-chloropyridinium iodide to give **12** or **13** in 23—63% yields (method D).

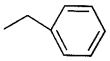
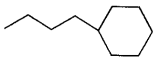
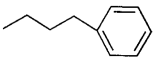
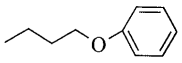
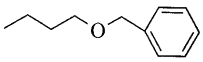
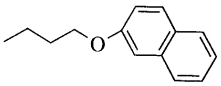
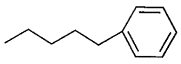
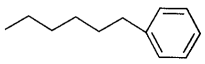
Results and Discussion

By structural analogy with verapamil, we designed the N-alkylated 1,4-dihydropyridines possessing aromatic substituents at the 4-position and arylalkyl substituents at the 1-position. First, the effect of the distance between the two aromatic rings was examined, and it was found that arylalkyl substituents at the 1-position enhanced the activity (Table 1). The unsubstituted dihydropyridine **3a** was far less active than verapamil, and compounds with aliphatic substituents (**3b** and **3d**) were also less active. The optimum length of the alkyl group at the 1-position was 3, 4 or 5 carbons, and compounds with an ether linkage in the alkyl chain showed similar potency (**3e**, **3f**, **4d**). A naphthyl group at the end of the aliphatic chain diminished the activity (**3g**).

Next, the effect of the substituent at the 4-position was examined. Table 2 summarizes the activity of compounds with various aromatic substituents at the 4-position and an optimized phenylpropyl group at the 1-position.

Table 1. Physical Properties and *in Vitro* Assays of N-Alkylated 1,4-Dihydropyridines



Compound No.	R^2	mp (°C)	Formula	HRMS for (M^+) *Anal.* Calcd (Found)			Activity to[a] overcome MDR	Cytotoxicity[b] (μg/ml)
				C	H	N		
3a	H	146—147	$C_{19}H_{23}NO_6$		361.1525 (361.1530)		0.11	>100
3b	CH_3	Oil	$C_{20}H_{25}NO_6$		376.1760[c] (376.1754)[c]		0.68	>100
3c		134—135	$C_{26}H_{29}NO_6$		451.1995 (451.1996)		1.31	>100
3d		Oil	$C_{28}H_{39}NO_6$		485.2777 (485.2793)		0.35	>100
4d		103—104	$C_{28}H_{33}NO_6$	70.12 (70.46	6.94 6.89	2.92 2.95)	3.0	>100
3e		107—108	$C_{28}H_{33}NO_7$	67.86 (67.73	6.71 6.65	2.83 2.84)	3.0	>100
3f		Oil	$C_{29}H_{35}NO_7$		510.2492[c] (510.2511)[c]		3.0	>100
3g		Oil	$C_{32}H_{35}NO_7$		545.2414 (545.2431)		1.62	>100
3h		107—108	$C_{29}H_{35}NO_6$	70.56 (70.55	7.15 7.06	2.84 2.83)	3.0	>100
3i		84—85	$C_{30}H_{37}NO_6$	70.98 (70.98	7.35 7.34	2.76 2.72)	2.5	>100

a) For biological methods, see Experimental. Activity to overcome MDR: IC_{50} (VCR + verapamil)/IC_{50} (VCR + test compound). *b*) The figures represent IC_{50} of test compounds in P388/S cells. *c*) For (MH^+).

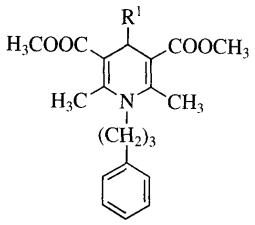
Aromatic groups at the 4-position proved to be indispensable for good activity, compared with **4a** and **4g**, and a naphthyl group was too bulky at this position (**4h**). A 3,4-dimethoxy group showed the best activity, and the activity of the compounds with related substituents revealed that the size and the electron density of the aromatic group were critical for the activity. These results revealed a structural similarity between the active compounds and verapamil in terms of the distances between the center nitrogen and the two aromatic groups, as well as the size of the aromatic group (Chart 3).

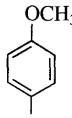
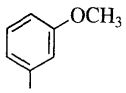
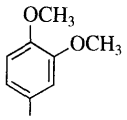
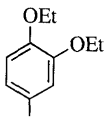
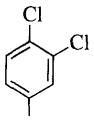
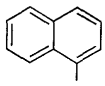
The effect of the substituents at the 3 and 5-positions was then examined (Table 3). The effect of structural changes in these positions was marginal, except in the carboxylic acid derivative **11a**. This is consistent with the fact that drugs recognized by P-glycoprotein are mostly basic.[10] The most potent compounds *in vitro*, **3e** and **3f**, were selected, and their potency for overcoming MDR was examined *in vivo* (Table 4). They showed little life-prolonging effect in P388/VCR-bearing mice when given in combination with vincristine, whereas verapamil, which was less effective *in vitro*, was moderately effective. Since the tested compounds were highly lipophilic, their low efficacy was thought to be partly due to their insolubility. A cationic and amphipathic character may be essential for agents that overcome MDR.[11] Since the nitrogen in the dihydropyridine ring is only weakly basic, we attempted to introduce another basic nitrogen into N-alkylated 1,4-dihydropyridines to obtain compounds with improved efficacy *in vitro* and *in vivo*.

Table 5 summarizes the activities of the compounds with a pyridine moiety in the 1-position. Most of the compounds showed low activity, and only **8c** showed potency equal to that of verapamil. It is noteworthy that these compounds are moderately cytotoxic by themselves. These results suggest that the 1-substituent should be hydrophobic and neutral.

Next, we introduced an amino group into the side chain of the 3-position of N-alkylated 1,4-dihydropyridines (Table 6). Among 16 compounds synthesized, **12c** and **12o**, which have an 4-alkylpiperidinopropyl ester, showed extremely high activity, being 14.1 times and 11.9 times respectively, as potent as verapamil. The pyridylpropyl esters were moderately potent, and the position of the substituents on the pyridine ring was critical (**12a**, **12b**). While N-alkylated 1,4-dihydropyridine derivatives having alkyl substituents on the 4-position of piperazine (**12c**, **12e**, **12i**, **12o**) were highly toxic, derivatives having aryl sub-

Table 2. Physical Properties and *in Vitro* Assays of N-Alkylated 1,4-Dihydropyridines



Compound No.	R^1	mp (°C)	Formula	HRMS for (M^+)		Activity to[a] overcome MDR	Cytotoxicity[b] (μg/ml)
				Calcd	Found		
4a		112—113	$C_{26}H_{29}NO_4$	419.2093	419.2097	1.0	>100
4b		116—117	$C_{27}H_{31}NO_5$	449.2202	449.2187	1.62	>100
4c		104—105	$C_{27}H_{31}NO_5$	449.2202	449.2184	1.62	>100
4d		103—104	$C_{28}H_{33}NO_6$	479.2308	479.2285	3.0	>100
4e		Oil	$C_{30}H_{37}NO_6$	507.2621	507.2645	2.63	>100
4f		98—99	$C_{26}H_{27}NO_4Cl_2$	487.1317	487.1337	0.84	>100
4g		70—71	$C_{26}H_{35}NO_4$	426.2637[c]	426.2637[c]	0.18	>100
4h		Oil	$C_{30}H_{31}NO_4$	469.2259	469.2259	0.81	>100

a) For biological methods, see Experimental. Activity to overcome MDR: IC_{50} (VCR + verapamil)/IC_{50} (VCR + test compound). *b*) The figures represent IC_{50} of test compounds in P388/S cells. *c*) For (MH^+).

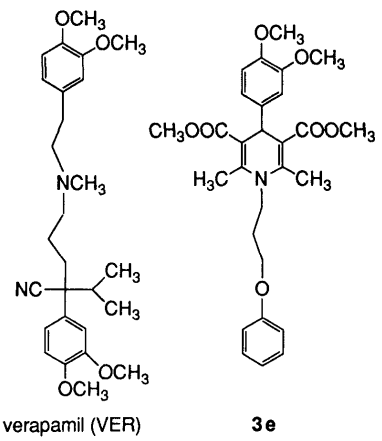


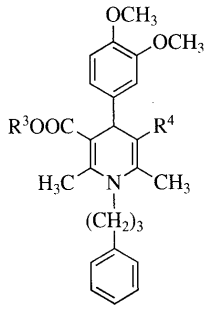
Chart 3. Structural Comparison between Verapamil and **3e**

stituents on the 4-position of piperazine (**12g**, **12h**) were less toxic. A strongly basic group at the 3-position of N-alkylated 1,4-dihydropyridines seems to enhance the inherent cytotoxicity.

Since hydrophobic nature of the 1-arylalkyl group was essential for the activity, the potency of fluorine-containing compounds with increased hydrophobicity was examined (Table 7). Compound **13a**, with a *p*-fluorophenoxypropyl group, was twice as potent as its phenylpropyl counterpart, **12h**, without enhanced cytotoxicity. However in other cases, the activity was equal to or lower than those of the phenylpropyl counterparts.

The effects of compound **12a** compared with verapamil on the growth-inhibitiory activity of vincristine on P388 and P388/VCR cells was examined in detail. The IC_{50} value of vincristine against P388/VCR cells was 12 times higher than that against P388/S cells, as shown in Fig. 1. The addition of 1 μg/ml verapamil to vincristine potentiates

Table 3. Physical Properties and *in Vitro* Assays of N-Alkylated 1,4-Dihydropyridines



Compound No.	R^3	R^4	mp (°c)	Formula	HRMS for (M^+) Calcd	HRMS for (M^+) Found	Activity to[a] overcome MDR	Cytotoxicity[b] (μg/ml)
6a	CH_3	CN	Oil	$C_{27}H_{30}N_2O_4$	447.2284[c]	447.2281[c]	2.63	>100
6b	Et	CN	Oil	$C_{28}H_{32}N_2O_4$	460.2362	460.2371	2.63	>100
11a	H	$COOCH_3$	90—91	$C_{27}H_{31}NO_6$	466.2230[c]	466.2222[c]	0.08	>100

a) For biological methods, see Experimental. Activity to overcome MDR: IC_{50} (VCR + verapamil)/IC_{50} (VCR + test compound). *b*) The figures represent IC_{50} of test compounds in P388/S cells. *c*) For (MH^+).

Table 4. Antitumor Activities of N-Alkylated 1,4-Dihydropyridines Combined with Vincristine in P388/VCR-Bearing Mice

Compound No.	75 mg/kg		100 mg/kg		200 mg/kg	
	T/C (%)	*T/V* (%)	*T/C* (%)	*T/V* (%)	*T/C* (%)	*T/V* (%)
Verapamil	135	124	139	127	105	96
3e	109	100	105	96	107	98
3f	107	107	104	104	122	122

$$T/C\ (\%): \frac{\text{mean survival time of treated mice}}{\text{mean survival time of untreated mice}} \times 100.$$

$$T/V\ (\%): \frac{\text{mean survival time of treated mice}}{\text{mean survival time of mice treated with VCR alone}} \times 100.$$

the cytotoxicity of vincristine against P388/VCR cells to almost the same level as against P388/S cells. One μg/ml of **12a** made P388/VCR cells 66 times more sensitive to vincristine (**12a** is not cytotoxic by itself at this concentration). The EC_{50} value for **12a** depended on the concentration of vincristine. The EC_{50} values of 0.8, 0.4, and 0.3 μg/ml were obtained at vincristine concentrations of 0.3, 1.0, and 3.0 ng/ml, respectively (Fig. 2).

We synthesized compound **12a** in a racemic mixture, so it was of interest to know the activity of each enantiomer in connection with the structure–activity relationships. Each enantiomer was prepared by optical resolution of intermediate **11a** and esterification. The two were found to have similar activity and cytotoxicity [activity to overcome MDR as compared to verapamil; (+)-**12a**: 6.6, (−)-**12a**: 6.5/cytotoxicity; (+)-**12a**: 50 μg/ml, (−)-**12a**: 50 μg/ml]. From this result, we assumed that the amino group in the side chain at the 3-position of the N-alkylated 1,4-dihydropyridines might enhance the activity by controlling the physicochemical properties of the compounds. From the *in vitro* results, 5 compounds were selected and their activity to overcome MDR when combined with vincristine was examined on P388/VCR cells *in vivo*. The results are summarized in Table 8.

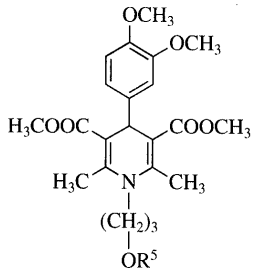
The most potent compound *in vitro*, **12c**, did not prolong life in mice. At a dose of over 100 mg/kg toxic death was observed, which might be related to cytotoxicity *in vitro*. Compounds **12a**, **12h**, **12p**, and **13a** markedly prolonged life in tumor-bearing mice [*T/C* (%); 126, 146, 130, 144 at a dose of 100 mg/kg respectively]. At a dose of 200 mg/kg, **12h** and **12p** did not show efficacy, probably owing to their own toxicity.

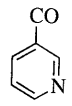
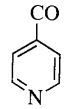
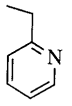
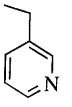
We have synthesized N-alkylated 1,4-dihydropyridine derivatives and found that some of the 3-(4-arylpiperidinopropyloxycarbonyl) and 3-(2-pyridylpropyloxycarbonyl) derivatives have good *in vivo* efficacy. Since these compounds have little calcium-antagonistic activity, which may be associated with 1,4-dihydropyridine derivatives, further evaluation is warranted. Studies on their efficacy against other tumor cells, both *in vitro* and *in vivo*, and on their pharmacological and toxicological profiles are in progress.

Experimental

All the melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. NMR spectra in $CDCl_3$ solution were recorded on a Varian EM-390 spectrometer with tetramethylsilane as the internal standard. Mass spectra (MS) were measured on JEOL JMS-DX300 (FD) and JEOL JMS-HX110/HX110 (HRMS) instruments. Five percent Pd–C (PH) was purchased from Kawaken Fine Chemicals Co., Ltd.

Table 5. Physical Properties and *in Vitro* Assays of N-Alkylated 1,4-Dihydropyridines



Compound No.	R^5	mp (°C)	Formula	HRMS for (M^+)		Activity to[a] overcome MDR	Cytotoxicity[b] (μg/ml)
				Calcd	Found		
8a		Oil	$C_{28}H_{32}N_2O_8$	524.2159	524.2130	0.4	50.0
8b		Oil	$C_{28}H_{32}N_2O_8$	524.2159	524.2133	0.7	50.0
8c		Oil	$C_{28}H_{34}N_2O_7$	511.2444[c]	511.2426[c]	1.0	50.0
8d		Oil	$C_{28}H_{34}N_2O_7$	511.2444[c]	511.2417[c]	0.3	51.0

a) For biological methods, see Experimental. Activity to overcome MDR: IC_{50} (VCR + verapamil)/IC_{50} (VCR + test compound). *b*) The figures represent IC_{50} of test compounds in P388/S cells. *c*) For (MH^+).

General Procedure for the Preparation of Dihydropyridine Derivatives 3a—3i, 4a—4h All the derivatives, **3a—3i, 4a—4h**, were prepared in the same manner described below for **4d**. Their spectral data are summarized in Table 9.

Synthesis of 1,4-Dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-(3-phenylpropyl)-3,5-pyridinedicarboxylic Acid 3,5-Dimethyl Ester (4d) A solution of 3,4-dimethoxybenzaldehyde (8.08 g, 0.098 mol), methyl acetoacetate (11.2 g, 0.098 mol) and 30% NH_3/H_2O (5.5 ml) in ethanol (40 ml) was refluxed for 4 h with continuous removal of water using a Dean–Stark apparatus. The mixture was evaporated *in vacuo*, and the residue was dissolved in CH_2Cl_2 and the solution was washed with saturated NaCl solution. The organic extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was crystallized from methanol to give **3a** (5.5 g, 31%) as yellow crystals. A solution of **3a** (5.0 g, 13.8 mmol) in dry dimethylformamide (DMF, 20 ml) was added to a suspension of 60% NaH (660 mg,washed with hexane) in dry DMF (60 ml) at room temperature with stirring. Then, phenylpropylbromide (2.1 ml) was added, and the mixture was heated at 100 °C for 1 h. After cooling, the mixture was poured into ice-water and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2) to give **4d** (4.52 g, 68.3%).

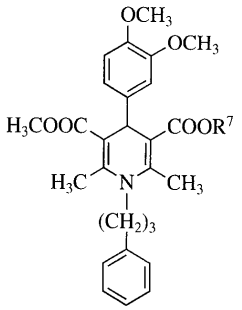
Synthesis of 1,4-Dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-(3-phenylpropyl)-3-cyano-5-pyridinecarboxylic Acid 5-Methyl Ester (6a) A solution of 3,4-dimethoxybenzaldehyde (10.0 g, 0.060 mol), methyl acetoacetate (6.98 g), and aminocrotononitrile (5.0 g) in EtOH (50 ml) was refluxed for 12 h. After cooling, the mixture was concentrated *in vacuo*, and the residue was crystallized from AcOEt to give 1,4-dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-3-cyano-5-pyridinecarboxylic acid 5-methyl ester (**5a**) (9.5 g, 48.3%) as yellow crystals, mp 171—172 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.10 (3H, s), 2.37 (3H, s), 3.59 (3H, s), 3.84 (3H, s), 3.88 (3H, s), 4.58 (1H, s), 5.75 (1H, br s), 6.70—6.83 (3H, m). MS *m/z*: 328 (M^+). HRMS *m/z*: (M^+) Calcd for $C_{18}H_{20}N_2O_4$: 328.1423. Found: 328.1399.

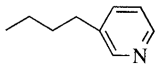
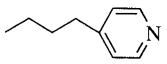
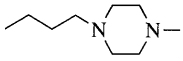
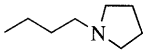
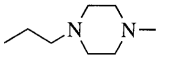
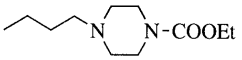
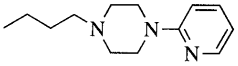
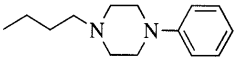
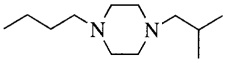
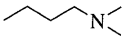
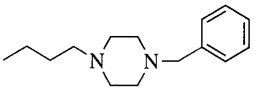
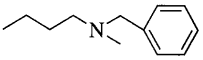
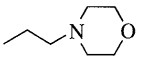
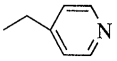
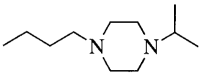
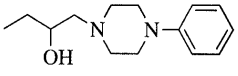
A solution of **5a** (3.0 g, 9.15 mmol) in dry DMF (20 ml) was added to a suspension of 60% NaH (400 mg, washed with hexane) in dry DMF (70 ml) at room temperature, with stirring. Then, phenylpropyl bromide (1.5 ml, 9.8 mmol) was added, and the mixture was heated at 100 °C for 1 h. After cooling, the mixture was poured into ice-water and extracted with CH_2Cl_2.The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2) to give **6a** (2.80 g, 68%). Compound **6b** was synthesized similarly. Spectral data of these compounds are summarized in Table 9.

Synthesis of 1,4-Dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-[3-propyl-(3-pyridinecarboxylic ester)]-3,5-pyridinedicarboxylic Acid 3,5-dimethyl Ester (8a) 1,4-Dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-(3-benzyloxypropyl)-3,5-pyridinedicarboxylic acid 3,5-dimethyl ester (**3f**) (4.98 g, 9.7 mmol) was treated under a hydrogen atmosphere with 10% Pd–C (500 mg) in AcOH (18 ml) and MeOH (18 ml) for 6 h. The reaction mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOH : hexane = 2 : 1) to give 1,4-dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-(3-hydroxypropyl)-3,5-pyridinedicarboxylic acid 3,5-dimethyl ester (**7**) (1.01 g, 28%). ^{1}H-NMR ($CDCl_3$) δ: 1.54 (2H, br), 2.50 (6H, s), 3.28 (2H, br), 3.74 (6H, s), 3.79 (2H, t, J = 6.6 Hz), 3.81 (6H, s), 6.64 (1H, dd, J = 1.5, 8.4 Hz), 6.70 (1H, J = 8.4 Hz), 6.80 (1H, d, J = 1.5Hz). MS *m/z*: 419 (M^+). HRMS *m/z*: (M^+) Calcd for $C_{22}H_{29}N_1O_7$: 419.1944. Found: 419.1957. Compound **7** (125 mg, 0.3 mmol), dimethylaminopyridine (6.1 mg, 0.05 mmol) and pyridine-3-carboxylic acid (61.6 mg, 0.5 mmol) were dissolved in DMF (2 ml). Then 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (104 mg, 0.6 mmol) was added and the mixture was stirred at room temperature for 2 h, poured into water and extracted with AcOEt. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt : hexane) to give **8a** (141 mg, 90%). Compound **8b** was synthesized similarly. Spectral data of these compounds are summarized in Table 9.

Synthesis of 1,4-Dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-[3-(2-pyridinemethyloxy)propyl]-3,5-pyridinedicarboxylic Acid 3,5-Dimeth-

Table 6. Physical Properties and *in Vitro* Assays of N-Alkylated 1,4-Dihydropyridines



Compound No.	R^7	mp (°C)	Formula	HRMS for (MH^+)		Activity to[a] overcome MDR	Cytotoxicity[b] (μg/ml)
				Calcd	Found		
12a		Oil	$C_{35}H_{40}N_2O_6$	585.2965	585.2980	6.5	50.0
12b		Oil	$C_{35}H_{40}N_2O_6$	585.2965	585.2990	3.3	9.5
12c		Oil	$C_{35}H_{47}N_3O_6$	606.3547	606.3453	14.1	5.5
12d		Oil	$C_{34}H_{44}N_2O_6$	577.3278	577.3306	5.1	6.2
12e		Oil	$C_{34}H_{45}N_3O_6$	592.3387	592.3404	4.1	6.1
12f		Oil	$C_{37}H_{49}N_3O_8$	664.3598	664.3850	7.0	13.0
12g		Oil	$C_{39}H_{48}N_4O_6$	669.3652	669.3667	6.6	60.0
12h		Oil	$C_{40}H_{49}N_3O_6$	668.3700	668.3704	4.8	100
12i		Oil	$C_{38}H_{53}N_3O_6$	648.4013	648.4039	6.3	9.5
12j		Oil	$C_{32}H_{42}N_2O_6$	551.3121	551.3121	5.1	6.0
12k		Oil	$C_{41}H_{51}N_3O_6$	682.3856	682.3882	6.0	10.5
12l		Oil	$C_{37}H_{44}N_2O_6$	613.3263	613.3278	1.8	60.0
12m		Oil	$C_{33}H_{42}N_2O_7$	579.3070	579.3058	1.2	20.0
12n		Oil	$C_{33}H_{36}N_2O_6$	557.2652	557.2653	1.2	8.0
12o		Oil	$C_{37}H_{51}N_3O_6$	634.3856	634.3853	11.9	5.8
12p		Oil	$C_{40}H_{49}N_3O_7$	684.3649	684.3640	6.3	42.0

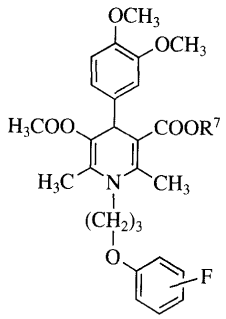
a) For biological methods, see Experimental. Activity to overcome MDR: IC_{50} (VCR + verapamil)/IC_{50} (VCR + test compound). *b*) The figures represent IC_{50} of test compounds in P388/S cells.

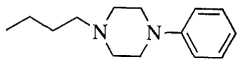
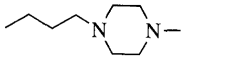
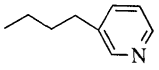
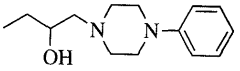
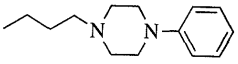
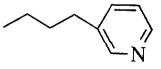
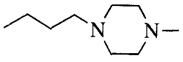
yl Ester (8c) A solution of **7** (63 mg, 0.15 mmol) in dry DMF (1 ml) was added to a suspension of 60% NaH (27.5 mg, washed with hexane) in dry DMF (0.4 ml) at 0 °C, with stirring. 2-Chloromethylpyridine (32.8 mg, 0.2 mmol) was added and the mixture was heated at 100 °C for 2 h. After cooling, the mixture was poured into ice-water and extracted with AcOEt. The extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt : hexane = 2 : 1) to give **8c** (18.1 mg, 23.6%). Compound **8d** was synthesized similarly. Spectral data of these compounds are summarized in Table 9.

General Procedure for the Preparation of 12a—12p All derivatives, **12a—12p**, were prepared in the same manner as described below for **12h**. Their spectral data are summarized in Table 10.

Synthesis of 1,4-Dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-(3-phenylpropyl)-3,5-pyridinedicarboxylic Acid 3-Methyl-5-[3-(4-phenylpiperazionopropyl]ester (12h) 3,4-Dimethoxybenzaldehyde (10.0 g, 0.060 mol), benzyl acetoacetate (11.6 g, 0.060 mol), piperidine (2 ml) and acetic acid (2 ml) in benzene (50 ml) were heated under reflux for 2 h. Then, methyl aminocrotonate (6.9 g, 0.060 mol) and triethylamine (3 ml) were added and the mixture was heated under reflux for 3 h. After cooling,

Table 7. Physical Properties and *in Vitro* Assays of N-Alkylated, 1,4-Dihydropyridines



Compound No.	Position of F	R^7	mp (°C)	Formula	HRMS for (MH^+)		Activity to[a] overcome MDR	Cytotoxicity[b] (μg/ml)
					Calcd	Found		
13a	*p*		Oil	$C_{40}H_{48}N_3O_7F$	702.3555	702.3536	10.0	100
13b	*p*		Oil	$C_{35}H_{46}N_3O_7F$	640.3398	640.3378	7.3	5.7
13c	*p*		Oil	$C_{35}H_{39}N_2O_7F$	619.2820	619.2808	1.1	17.0
13d	*p*		Oil	$C_{40}H_{48}N_3O_8F$	718.3504	718.3489	7.7	50.0
13e	*p*		Oil	$C_{39}H_{47}N_4O_7F$	703.3507	703.3532	7.7	70.0
13f	*m*		Oil	$C_{35}H_{39}N_2O_7F$	619.2820	619.2827	3.0	27.0
13g	*m*		Oil	$C_{35}H_{46}N_3O_7F$	640.3398	640.3386	4.4	5.9

a) For biological methods, see Experimental. Activity to overcome MDR: IC_{50} (VCR + verapamil)/IC_{50} (VCR + test compound). *b*) The figures represent IC_{50} of test compounds in P388/S cells.

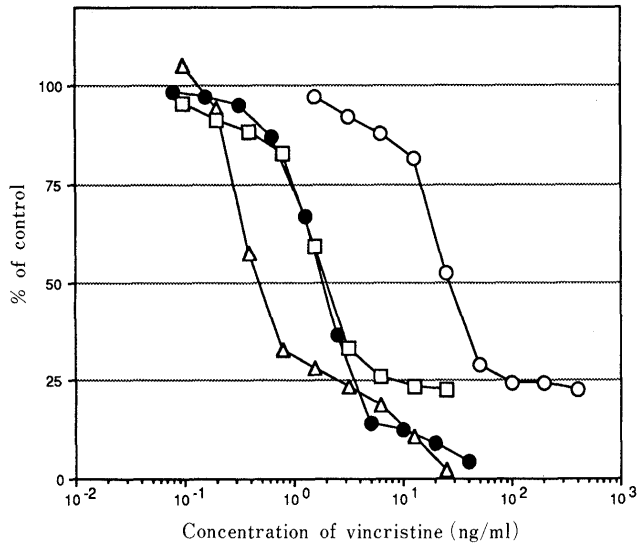


Fig. 1. Reversing Effect of **12a** on VCR Resistance of P388/VCR Cells *in Vitro*

P388/VCR cells were cultured with various concentrations of VCR in the absence or presence of 1.0 μg/ml of **12a** or verapamil. P388/S cells (●) were cultured without **12a** or verapamil. —●—, P388/S; —○—, P388/V; —△—, +**12a** (1.0 μg/ml); —□—, +VER (1.0 μg/ml).

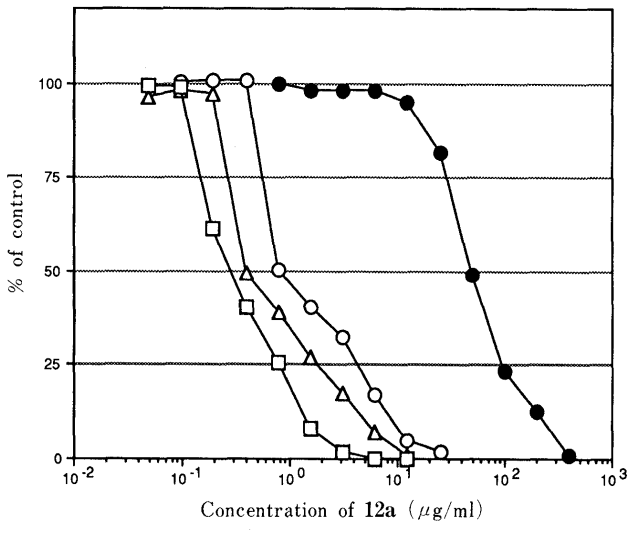


Fig. 2. Dose-Dependency Curve for **12a**

P388/VCR cells were treated with **12a** at the indicated concentrations in the absence or presence of vincristine. Concentrations of vincristine were 0 (●), 0.3 (○), 1.0 (△) and 3.0 (□) ng/ml.

the mixture was concentrated and purified by silica gel column chromatography (AcOEt : hexane = 1 : 2) to give 1,4-dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-3,5-pyridinedicarboxylic acid 3-methyl-5-benzylester (**9**) (15.0 g, 57.2%). ^{1}H-NMR ($CDCl_3$) δ: 2.32 (3H, s), 2.35 (3H, s), 3.64 (3H, s), 3.67 (3H, s), 3.80 (3H, s), 4.98—5.18 (2H, m), 6.71 (2H, t, J = 8.4 Hz), 6.81 (1H, br s), 7.20—7.28 (5H, m). MS m/z: 437 (M^+). HRMS m/z: (M^+) Calcd for $C_{25}H_{27}N_1O_6$: 437.1838. Found: 437.1841.

A solution of **9** (13.0 g, 0.029 mol) in dry DMF (30 ml) was added to a suspension of 60% NaH (1.3 g, washed with hexane) in dry DMF (100 ml) at room temperature. Phenylpropylbromide (5.2 ml, 0.035 mol) was then added and the mixture was heated at 100 °C for 1 h. After

Table 8. Antitumor Activities of N-Alkylated 1,4-Dihydropyridines Combined with Vincristine in P388/VCR-Bearing Mice

Compound No.	50 mg/kg		100 mg/kg		200 mg/kg	
	T/C (%)	*T/V* (%)	*T/C* (%)	*T/V* (%)	*T/C* (%)	*T/V* (%)
12a	115	115	126	126	133	133
12c	97	103	—*a)*	—*a)*	—*a)*	—*a)*
12h	126	126	146	146	109	109
12p	120	120	130	130	78	78
13a	128	128	144	144	102	102

T/C (%): $\frac{\text{mean survival time of treated mice}}{\text{mean survival time of untreated mice}} \times 100.$

T/V (%): $\frac{\text{mean survival time of treated mice}}{\text{mean survival time of mice treated with VCR alone}} \times 100.$

a) All mice died because of toxicity.

Table 9. NMR Spectral Data

Compound No.	^{1}H-NMR ($CDCl_3$) δ (ppm)
3a	2.33 (6H, s), 3.66 (6H, s), 3.81 (3H, s), 3.83 (3H, s), 4.96 (1H, s), 5.73 (1H, br s), 6.70—6.82 (2H, m), 6.87 (1H, d, J=1.5 Hz)
3b	2.48 (6H, s), 3.19 (3H, s), 3.72 (6H, s), 3.82 (6H, s), 5.09 (1H, s), 6.38 (1H, dd, J=1.8, 8.1 Hz), 6.71 (1H, d, J=8.1 Hz), 6.79 (1H, d, J=1.8 Hz)
3c	2.44 (6H, s), 3.73 (6H, s), 3.78 (3H, s), 3.85 (3H, s), 4.86 (2H, s), 5.17 (1H, s), 6.58—6.76 (2H, m), 6.79 (1H, s), 6.94 (2H, br s), 7.16—7.32 (3H, m)
3d	0.73—1.68 (19H, unresolved), 2.42 (6H, s), 3.57 (2H, t, J=7.5 Hz), 3.70 (6H, s), 3.78 (3H, s), 3.79 (3H, s), 5.09 (1H, s), 6.62—6.79 (3H, m), 6.79 (2H, s)
3e	1.74 (2H, m), 2.48 (6H, s), 3.39 (2H, t, J=5.4 Hz), 3.74 (6H, s), 3.78 (3H, s), 3.79 (3H, s), 3.88 (2H, t, J=6.6 Hz), 5.13 (1H, s), 6.60—6.79 (3H, m), 6.79 (1H, s), 6.92 (2H t, J=6.3 Hz), 7.21—7.27 (2H, m)
3f	1.55—1.63 (2H, m), 2.48 (6H, s), 3.05 (2H, t, J=5.7 Hz), 3.73 (6H, s), 3.79 (3H, s), 3.80 (3H, s), 4.30 (2H, s), 5.12 (1H, s), 6.63—6.80 (2H, m), 6.80 (1H, s), 7.23—7.34 (5H, m)
3g	1.75—1.84 (2H, m), 2.52 (6H, s), 3/43 (2H, br s), 3.72 (9H, s), 3.81 (3H, s), 3.93 (2H, t, J=6.3 Hz), 5.14 (1H, s), 6.56—6.78 (4H, m), 6.82 (1H, s), 7.05 (1H, d, J=7.8 Hz), 7.28—7.36 (1H, m), 7.40—7.48 (1H, m), 7.68—7.78 (3H, m)
3h	1.02—1.21 (2H, m), 1.30—1.41 (2H, m), 1.44—1.54 (2H, m), 2.44 (6H, s), 2.49 (2H, t, J=4.8 Hz), 3.58 (2H, t, J=7.5 Hz), 3.70 (6H, s), 3.77 (3H, s), 3.82 (3H, s), 5.09 (1H, s), 6.65—6.71 (2H, m), 6.80 (1H, s), 7.17—7.26 (3H, m), 7.13 (2H, d, J=7.5 Hz)
3i	1.81—1.95 (2H, m), 2.16 (6H, s), 2.64 (2H, t, J=7.8 Hz), 3.51 (2H, t, J=7.8 Hz), 3.58 (3H, s), 3.83 (3H, s), 4.21 (1H, s), 6.72—6.76 (2H, m), 6.83 (1H, d, J=8.7 Hz), 7.16 (2H, d, J=7.2 Hz), 7.23—7.35 (3H, m)
4a	1.64 (2H, m), 2.36 (2H, t, J=7.2 Hz), 2.39 (6H, s), 3.58 (2H, t, J=7.2 Hz), 3.72 (6H, s), 5.16 (1H, s), 6.99 (2H, d, J=8.0 Hz), 7.10—7.29 (8H, m)
4b	1.61—1.71 (2H, m), 2.39 (6H, s), 2.41 (2H, t, J=7.8 Hz), 3.59 (2H, t, J=7.8 Hz), 3.70 (6H, s), 3.71 (3H, s), 5.08 (1H, s), 6.70 (2H, d, J=6.6 Hz), 7.01 (2H, d, J=8.4 Hz), 7.07 (2H, d, J=6.6 Hz), 7.18—7.26 (3H, m)
4c	1.62—1.69 (2H, m), 2.38 (2H, t, J=7.8 Hz), 2.40 (6H, s), 3.59 (2H, t, J=7.5 Hz), 3.69 (3H, s), 3.72 (6H, s), 5.16 (1H, s), 6.64 (1H, d, J=8.1 Hz), 6.74 (1H, s), 6.76 (1H, d, J=8.1 Hz), 7.01 (2H, d, J=6.9 Hz), 7.11 (1H, t, J=8.1 Hz), 7.18—7.28 (3H, m)
4d	1.66—1.72 (2H, m), 2.39 (6H, s), 2.42 (2H, t, J=7.5 Hz), 3.61 (2H, t, J=7.8 Hz), 3.71 (6H, s), 3.74 (3H, s), 3.75 (3H, s), 5.09 (1H, s), 6.66 (2H, s), 6.78 (1H, s), 7.03 (2H, d, J=6.9 Hz), 7.18—7.28 (3H, m)
4e	1.29—1.41 (6H, m), 1.61—1.71 (2H, m), 2.38 (6H, s), 3.59 (2H, t, J=7.5 Hz), 3.92 (6H, s), 3.93—4.02 (4H, m), 5.07 (1H, s), 6.60—6.69 (2H, m), 6.76 (1H, s), 7.01 (1H, d, J=8.7 Hz), 7.15—7.35 (3H, m)
4f	1.62—1.75 (2H, m), 2.40 (6H, s), 2.46 (2H, t, J=7.8 Hz), 3.61 (2H, t, J=7.8 Hz), 3.72 (6H, s), 5.09 (1H, s), 7.03 (3H, d, J=9.9 Hz), 7.18—7.31 (5H, m)
4g	0.80—1.20 (5H, m), 1.49—1.72 (6H, m), 1.80—1.92 (2H, m), 2.33 (6H, s), 2.65 (2H, t, J=7.5 Hz), 3.60 (2H, t, J=7.5 Hz), 3.71 (6H, s), 3.80 (1H, d, J=8.4 Hz), 7.15—7.35 (5H, m)
4h	1.60—1.69 (2H, m), 2.32 (2H, t, J=9.0 Hz), 2.43 (6H, s), 3.62 (2H, t, J=9.0 Hz), 3.73 (6H, s), 5.33 (1H, s), 6.81—6.84 (2H, m), 7.13—7.17 (3H, m), 7.36—7.42 (3H, m), 7.49 (1H, s), 7.64—7.75 (3H, m)
6a	1.80—1.86 (2H, m), 2.10 (3H, s), 2.42 (3H, s), 2.57 (2H, t, J=7.8 Hz), 3.44—3.55 (2H, m), 3.64 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 4.59 (1H, s), 6.68 (1H, d, J=9.0 Hz), 6.74 (2H, d, J=9.0 Hz), 7.10 (2H, d, J=8.7 Hz), 7.18—7.32 (3H, m)
6b	1.16 (3H, t, J=6.9 Hz), 1.70—1.95 (2H, m), 2.11 (3H, s), 2.41 (3H, s), 2.58 (2H, t, J=7.8 Hz), 3.70—3.78 (2H, m), 3.76 (3H, s), 3.80 (3H, s), 4.03—4.08 (2H, m), 4.60 (1H, s), 6.62—6.77 (3H, m), 7.08—7.29 (5H, m)
8a	1.72—1.83 (2H, m), 2.52 (6H, s), 3.75 (3H, s), 3.79 (3H, s), 3.83 (3H, s), 3.98—4.05 (2H, m), 5.13 (1H, s), 6.62 (1H, d, J=11.4 Hz), 6.67 (1H, d, J=11.4 Hz), 6.81 (1H, s), 7.41 (1H, dd, J=5.1, 8.1 Hz), 8.24 (1H, m), 8.79 (1H, m), 9.17 (1H, s)
8b	2.51 (6H, s), 3.71—3.79 (2H, m), 3.76 (3H, s), 3.78 (3H, s), 3.81 (3H, s), 3.95—4.03 (2H, m), 5.14 (1H, s), 6.25 (1H, d, J=8.4 Hz), 6.69 (1H, d, J=8.4 Hz), 6.80 (1H, s), 7.77 (1H, d, J=6.0 Hz), 8.79 (1H, d, J=6.0 Hz)
8c	1.60—1.78 (2H, m), 2.50 (6H, s), 3.18—3.27 (2H, m), 3.67—3.89 (2H, m), 3.73 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 4.48 (2H, s), 5.12 (1H, s), 6.62—6.72 (2H, m), 6.81 (1H, s), 7.20 (1H, m), 7.31 (1H, m), 7.70 (1H, m), 8.57 (1H, br s)
8d	1.58—1.64 (2H, m), 2.47 (6H, s), 3.00—3.11 (2H, m), 3.78—3.88 (2H, m), 3.76 (3H, s), 3.77 (3H, s), 3.79 (3H, s), 4.28 (2H, s), 5.12 (1H, s), 6.61 (1H, d, J=8.2 Hz), 6.69 (1H, d, J=8.2 Hz), 6.79 (1H, m), 7.22—7.31 (1H, m), 7.52—7.60 (1H, m), 8.50—8.57 (1H, m)
11a	1.16 (3H, t, J=6.9 Hz), 1.70—1.95 (2H, m), 2.11 (3H, s), 2.41 (3H, s), 2.58 (2H, t, J=7.8 Hz), 3.70—3.78 (2H, m), 3.76 (3H, s), 3.80 (3H, s), 4.03—4.08 (2H, m), 4.60 (1H, s), 6.62—6.77 (3H, m), 7.08—7.29 (5H, m)

Table 10. NMR Spectral Data

Compound No.	^{1}H-NMR ($CDCl_3$) δ (ppm)
12a	1.64—1.78 (2H, m), 1.88—1.99 (2H, m), 2.37 (3H, s), 2.43 (3H, s), 2.40—2.53 (2H, m), 2.58 (2H, t, J=8.1 Hz), 3.61 (2H, t, J=8.1 Hz), 3.70 (3H, s), 3.72 (3H, s), 3.73 (3H, s), 4.05—4.12 (2H, m), 5.14 (1H, s), 6.68 (2H, s), 6.79 (1H, s), 7.01 (2H, d, J=8.1 Hz), 7.12—7.28 (4H, m), 7.35 (1H, d, J=9.3 Hz), 8.39 (2H, m)
12b	1.66—1.78 (2H, m), 2.37 (3H, s), 2.43 (3H, s), 2.45 (2H, t, J=7.5 Hz), 2.58 (2H, t, J=7.5 Hz), 3.62 (2H, t, J=7.5 Hz), 3.72 (3H, s), 3.73 (3H, s), 3.75 (3H, s), 4.04—4.20 (2H, m), 5.14 (1H, s), 6.63—6.71 (2H, m), 6.79 (1H, s), 6.98—7.05 (3H, m), 7.15—7.32 (4H, m), 8.46 (2H, br s)
12c	1.67—1.84 (2H, m), 2.33 (3H, s), 2.35 (3H, s), 2.33—2.46 (8H, m), 3.58—3.63 (2H, t, J=7.8 Hz), 3.71 (3H, s), 3.74 (3H, s), 3.76 (3H, s), 4.13—4.18 (2H, m), 5.09 (1H, br s), 7.03 (2H, d, J=6.9 Hz), 7.18 (3H, m)
12d	1.50—1.76 (4H, m), 2.39 (3H, s), 2.41 (3H, s), 2.44 (2H, t, J=5.4 Hz), 2.60—2.72 (2H, m), 3.61 (2H, t, J=6.6 Hz), 3.71 (3H, s), 3.74 (3H, s), 3.76 (3H, s), 4.22—4.32 (2H, m), 5.09 (1H, s), 6.66 (2H, s), 6.78 (1H, s), 7.02 (2H, d, J=6.9 Hz), 7.14—7.30 (3H, m)
12e	1.64—1.76 (2H, m), 2.30 (3H, s), 2.38 (3H, s), 2.40 (3H, s), 2.42—2.62 (8H, m), 2.62—2.70 (2H, m), 3.61 (2H, t, J=8.1 Hz), 3.72 (3H, s), 3.75 (3H, s), 3.77 (3H, s), 4.15—4.28 (4H, m), 5.09 (1H, s), 6.66 (2H, s), 6.77 (1H, s), 7.02 (2H, d, J=7.5 Hz), 7.11 (1H, s), 7.14—7.30 (3H, m)
12f	1.25 (3H, t, J=6.9 Hz), 1.76—1.87 (2H, m), 1.63—1.75 (2H, m), 2.27—2.50 (8H, m), 2.40 (3H, s), 2.44 (3H, s), 3.55—3.68 (2H, m), 3.72 (3H, s), 3.74 (3H, s), 3.76 (3H, s), 4.08—4.22 (4H, m), 5.08 (1H, s), 6.65 (2H, s), 6.77 (1H, s), 7.03 (2H, d, J=6.6 Hz), 7.14—7.32 (3H, m)
12g	1.64—1.77 (2H, m), 1.80—1.92 (2H, m), 2.33—2.44 (4H, m), 2.35 (3H, s), 2.38 (3H, s), 2.45—2.54 (4H, m), 3.45—3.56 (4H, m), 3.56—3.66 (2H, m), 3.69 (3H, s), 3.71 (3H, s), 3.72 (3H, s), 4.08—4.27 (2H, m), 5.09 (1H, s), 6.56—6.83 (2H, m), 6.63 (2H, s), 6.78 (1H, s), 7.03 (2H, d, J=6.6 Hz), 7.12—7.31 (3H, m), 7.41—7.49 (1H, m), 8.16 (1H, d, J=4.8 Hz)
12h	1.65—1.79 (2H, m), 1.82—1.92 (2H, m), 2.41 (6H, s), 2.54 (2H, t, J=5.1 Hz), 3.17 (2H, t, J=5.1 Hz), 3.62 (2H, t, J=7.2 Hz), 3.72 (3H, s), 3.75 (3H, s), 3.76 (3H, s), 4.10—4.27 (2H, m), 5.10 (1H, s), 6.66 (2H, s), 6.78 (1H, s), 6. (1H, d, J=7.2 Hz), 6.89 (1H, d, J=8.1 Hz), 7.02 (1H, d, J=8.1 Hz), 7.16—7.31 (7H, m)
12i	0.90 (6H, d, J=6.6 Hz), 1.63—1.85 (4H, m), 1.90—2.01 (1H, m), 20.2—2.08 (2H, m), 2.29—2.58 (12H, m), 2.36—2.39 (6H, m), 3.55—3.63 (2H, m), 3.71 (3H, s), 3.74 (3H, s), 3.75 (3H, s), 4.08—4.22 (2H, m), 5.09 (1H, s), 6.66 (2H, s), 6.77 (1H, s), 7.02 (2H, d, J=8.7 Hz), 7.15—7.38 (3H, m)
12j	1.62—1.88 (4H, m), 2.18 (6H, s), 2.18—2.28 (21H, m), 2.34—2.44 (2H, m), 2.39 (6H, s), 3.56—3.69 (2H, m), 3.74 (3H, s), 3.76 (3H, s), 3.77 (3H, s), 4.12—4.22 (2H, m), 5.09 (1H, s), 6.66 (2H, s), 6.78 (1H, s), 7.01 (2H, d, J=8.1 Hz), 7.14—7.32 (3H, m)
12k	2.30—2.76 (12H, m), 3.52—3.68 (4H, m), 3.54 (3H, s), 3.71 (3H, s), 3.75 (3H, s), 4.08—4.28 (2H, m), 5.08 (1H, s), 6.15 (1H, t, J=6.9 Hz), 6.55 (1H, d, J=12.0 Hz), 6.77 (1H, s), 7.02 (1H, d, J=7.2 Hz), 7.19—7.35 (7H, m)
12l	1.63—1.79 (2H, m), 2.25 (3H, unresolved), 2.39 (6H, s), 2.68—2.78 (2H, m), 2.39—2.50 (2H, m), 3.54—3.76 (4H, m), 3.66 (3H, s), 3.72 (3H, s), 3.74 (3H, s), 4.22—4.30 (2H, m), 5.14 (1H, s), 6.59—6.72 (2H, m), 6.78 (1H, s), 7.02 (2H, d, J=8.1 Hz), 7.12—7.36 (8H, m)
12m	1.62—1.77 (2H, m), 2.40—2.52 (6H, m), 2.38 (3H, s), 2.41 (3H, s), 2.63 (2H, t, J=6.0 Hz), 3.56—3.68 (6H, m), 3.72 (3H, s), 3.76 (3H, s), 3.77 (3H, s), 4.15—4.32 (2H, m), 5.09 (1H, s), 6.66 (2H, s), 6.77 (1H, s), 7.02 (2H, d, J=7.2 Hz), 7.14—7.29 (3H, m)
12n	1.65—1.72 (2H, m), 1.93 (3H, s), 2.42—2.52 (2H, m), 2.49 (3H, s), 3.52—3.72 (2H, m), 3.69 (3H, s), 3.74 (3H, s), 3.77 (3H, s), 4.89 (1H, s), 5.07—5.27 (2H, m), 6.60—6.70 (2H, m), 6.76 (1H, s), 7.00—7.13 (3H, m), 7.15—7.38 (5H, m), 8.51 (1H, d, J=6.0 Hz)
12o	1.05 (6H, d, J=6.6 Hz), 1.63—1.78 (2H, m), 1.78—1.89 (2H, m), 1.91—2.00 (1H, m), 2.38 (3H, s), 2.39 (3H, s), 2.31—2.60 (8H, m), 2.61—2.72 (2H, m), 3.52—3.63 (2H, m), 3.71 (3H, s), 3.76 (3H, s), 3.78 (3H, s), 4.09—4.21 (2H, m), 5.09 (1H, s), 6.66 (2H, s), 6.77 (1H, s), 7.02 (2H, d, J=8.40 Hz), 7.14—7.33 (3H, m)
12p	1.63—1.79 (2H, m), 2.38 (3H, s), 2.43 (3H, s), 2.41—2.59 (4H, m), 2.68—2.80 (2H, m), 3.10—3.21 (4H, m), 3.62 (2H, t, J=7.5 Hz), 3.72 (3H, s), 3.71—3.82 (6H, unresolved), 5.11 (1H, s), 6.68 (2H, s), 6.81 (1H, s), 6.90 (2H, d, J=9.9 Hz), 7.02 (2H, d, J=7.8 Hz), 7.18—7.29 (6H, m)

cooling, the mixture was poured into ice-water and extracted with dichloromethane. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt : hexane = 1 : 2) to give 1,4-dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-(3-phenylpropyl)-3,5-pyridinedicarboxylic acid 3-methyl-5-benzylester (**10a**) (10.5 g, 65.2%). ^{1}H-NMR ($CDCl_3$) δ: 1.70—1.77 (2H, m), 2.39—2.44 (2H, m), 2.40 (6H, s), 3.59—3.65 (2H, m), 3.61 (3H, s), 3.70 (3H, s), 3.75 (3H, s), 5.10—5.26 (2H, m), 5.14 (1H, br s), 6.63 (2H, s), 6.73 (1H, s), 7.01—7.04 (2H, m), 7.18—7.30 (8H, m). MS *m/z*: 555 (M^+). HRMS *m/z*: (M^+) Calcd for $C_{34}H_{37}N_1O_6$: 555.2621. Found: 555.2625.

Compound **10a** (7.7 g, 13.8 mmol) was treated with 5% Pd–C (PH) (700 mg) in MeOH (80 ml) under a hydrogen atmosphere for 2 h at room temperature. The mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt : hexane = 1 : 1) to give 1,4-dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-(3-phenylpropyl)-3,5-pyridinedicarboxylic acid 3-methylester (**11a**) (4.5 g, 70%).

Compound **11a** (285 mg, 0.61 mmol), triethylamine (0.20 ml, 1.5 mmol) and 1-phenyl-4-(3-hydroxypropyl)piperazine (174 mg, 0.8 mmol) were dissolved in CH_2Cl_2 (6 ml). 1-Methyl-2-chloropyridinium iodide (172 mg, 0.67 mmol) was added, and the mixture was stirred for 3 h at room temperature, then poured into water and extracted with AcOEt. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt : hexane = 1 : 1) to give **12h** (168 mg, 41.3%).

General Procedure for the Preparation of 13a—13g All derivatives, **13a—13g**, were prepared in the same manner as described below for **13a**. Their spectral data are summarized in Table 9.

Synthesis of 1,4-Dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-[3-(4-fluorophenyloxy)propyl]-3,5-pyridinedicarboxylic Acid 3-Methyl-5-[3-(4-phenylpiperazino)propyl] Ester (13a) A solution of **9** (17.9 g, 0.041 mol) in dry DMF (20 ml) was added to a suspension of 60% NaH (1.97 g, washed with hexane) in dry DMF (100 ml) at room temperature. 3-(4-Fluorophenoxy)propylbromide (9.59 g, 0.041 mmol) was then added, and the mixture was heated at 100 °C for 4 h. After cooling, the mixture was poured into ice-water and extracted with dichloromethane. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt : hexane = 1 : 3) to give 1,4-dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-[3-(4-fluorophenoxy)propyl]-3,5-pyridinedicarboxylic acid 3-methyl-5-benzylester (**10b**) (6.9 g, 29%).

^{1}H-NMR ($CDCl_3$) δ: 1.68—1.77 (2H, m), 2.49 (6H, s), 3.25—3.39 (2H, m), 3.61 (3H, s), 3.72 (3H, s), 3.77 (3H, s), 3.85—3.89 (2H, m), 5.15 (1H, d, J=12.0 Hz), 5.21 (1H, s), 5.26 (1H, d, J=12.0 Hz), 6.56—6.66 (5H, m), 6.73 (1H, s), 6.89—6.94 (3H, m), 7.25—7.30 (3H, m). MS *m/z*: 589 (M^+). Compound **10b** (6.9 g, 11.7 mmol) was treated with 5% Pd–C (PH) (1.4 g) in MeOH (100 ml) under a hydrogen atmosphere for 2 h

Table 11. NMR Spectral Data

Compound No.	^{1}H-NMR ($CDCl_3$) δ (ppm)
13a	1.71 (2H, dd, J=5.1, 6.0 Hz), 1.85 (2H, dd, J=6.3, 7.5 Hz), 2.39 (2H, t, J=7.5 Hz), 2.47 (3H, s), 2.48 (3H, s), 2.51 (4H, m), 3.14 (4H, m), 3.28 (2H, t, J=5.5 Hz), 3.72 (3H, s), 3.75 (3H, s), 3.77 (3H, s), 3.85 (2H, t, J=6.5 Hz), 4.20 (2H, dd, J=5.7, 6.1 Hz), 5.13 (1H, s), 6.56 (2H, m), 6.65 (2H, m), 6.77 (1H, d, J=1.5 Hz), 6.82 (1H, t, J=7.4 Hz), 6.90 (4H, m), 7.23 (2H, m)
13b	1.73 (2H, m), 1.83 (2H, m), 2.29 (3H, s), 2.38 (2H, t, J=7.8 Hz), 2.40—2.50 (8H, m), 2.48 (3H, s), 2.49 (3H, s), 3.32 (2H, t, J=5.4 Hz), 3.74 (3H, s), 3.77 (3H, s), 3.79 (3H, s), 3.87 (2H, t, J=6.6 Hz), 4.18 (2H, m), 5.13 (1H, s), 6.58 (1H, dd, J=4.5, 9.0 Hz), 6.58 (1H, m), 6.63 (1H, d, J=1.8 Hz), 6.67 (1H, d, J=8.4 Hz), 6.77 (1H, d, J=1.8 Hz), 6.92 (2H, m)
13c	1.74 (2H, m), 1.96 (2H, m), 2.49 (3H, s), 2.52 (3H, s), 2.63 (2H, t, J=7.2 Hz), 3.34 (2H, t, J=5.4 Hz), 3.75 (3H, s), 3.79 (3H, s), 3.88 (2H, t, J=6.6 Hz), 4.15 (2H, m), 5.19 (1H, s), 6.59 (1H, dd, J=4.2, 9.3 Hz), 6.59 (1H, m), 6.67 (2H, m), 6.79 (1H, s), 6.92 (2H, t, J=8.7 Hz), 7.17 (1H, dd, J=4.5, 7.8 Hz), 7.39 (1H, d, J=7.8 Hz), 8.39 (1H, s), 8.43 (1H, d, J=3.6 Hz)
13d	1.75 (2H, t, J=6.0 Hz), 2.39 (2H, m), 2.48 (3H, s), 2.51 (3H, s), 2.74 (2H, m), 3.10—3.24 (6H, m), 3.35 (2H, m), 3.74 (3H, s), 3.79 (6H, s), 3.88 (2H, t, J=6.6 Hz), 3.96 (1H, m), 4.10 (1H, dd, J=5.4, 11.4 Hz), 4.19 (1H, d, J=4.8 Hz), 4.33 (1H, dd, J=4.2, 11.4 Hz), 5.15 (1H, s), 6.60 (2H, m), 6.66 (1H, s), 6.68 (1H, d, J=8.1 Hz), 6.80—6.98 (6H, m), 7.26 (2H, m)
13e	1.73 (2H, m), 1.87 (2H, m), 2.44—2.52 (4H, m), 2.41 (2H, t, J=7.2 Hz), 2.48 (2H, m), 2.48 (3H, s), 2.50 (3H, s), 3.32 (2H, t, J=5.4 Hz), 3.51 (4H, t, J=5.1 Hz), 3.74 (3H, s), 3.78 (3H, s), 3.79 (3H, s), 3.87 (2H, t, J=6.6 Hz), 6.56—6.66 (5H, m), 6.67 (1H, d, J=8.1 Hz), 6.78 (1H, d, J=1.8 Hz), 6.93 (2H, m), 7.46 (1H, ddd, J=2.1, 7.2, 15.6 Hz), 8.18 (1H, m)
13f	1.74 (2H, m), 1.96 (2H, m), 2.49 (3H, s), 2.52 (3H, s), 2.63 (2H, t, J=7.2 Hz), 3.35 (2H, t, J=5.7 Hz), 3.76 (3H, s), 3.79 (3H, s), 3.88 (2H, t, J=5.7 Hz), 3.76 (3H, s), 3.79 (3H, s), 3.88 (2H, t, J=5.7 Hz), 4.16 (2H, m), 5.20 (1H, s), 6.37 (1H, ddd, J=2.1, 4.8, 10.8 Hz), 6.64 (2H, m), 6.71 (1H, d, J=8.1 Hz), 6.79 (1H, d, J=1.5 Hz), 7.18 (2H, m), 7.21 (1H, s), 7.40 (1H, d, J=7.8 Hz), 8.39 (1H, s), 8.43 (1H, d, J=1.5 Hz)
13g	1.73 (2H, m), 1.84 (2H, m), 2.48 (3H, s), 2.39 (2H, t, J=7.2 Hz), 2.49 (3H, s), 2.42—2.56 (8H, m), 3.33 (2H, t, J=5.4 Hz), 3.74 (3H, s), 3.78 (3H, s), 3.80 (3H, s), 3.87 (2H, t, J=6.6 Hz), 4.18 (2H, m), 5.13 (1H, s), 6.37 (1H, ddd, J=2.4, 4.5, 10.8 Hz), 6.43 (1H, dd, J=2.1, 8.4 Hz), 6.64 (2H, m), 6.69 (1H, d, J=8.1 Hz), 6.77 (1H, d, J=1.8 Hz), 7.17 (1H, ddd, J=7.2, 8.4, 8.4 Hz)

at room temperature. The mixture was filtered through Celite and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt : hexane = 1 : 1) to give 1,4-dihydro-4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1-[3-(4-fluorophenoxy)propyl]-3,5-pyridinedicarboxylic acid 3-methylester (**11b**) (3.8 g, 65%). ^{1}H-NMR ($CDCl_3$) δ: 1.69—1.77 (2H, m), 2.51 (6H, s), 3.27—3.35 (2H, m), 3.70 (3H, s), 3.72 (3H, s), 3.74 (3H, s), 4.09—4.16 (2H, m), 5.17 (1H, s), 6.55—6.69 (4H, m), 6.81—6.95 (3H, m). MS m/z: 499 (M^+).

Compound **11b** (3.8 g, 7.6 mmol), triethylamine (4.0 ml, 30 mmol) and 1-phenyl-4-(3-hydroxypropyl)piperazine (5.3 g, 22.8 mmol) were dissolved in CH_2Cl_2 (60 ml). 1-Methyl-2-chloropyridinium iodide (2.13 g, 8.36 mmol) was added, and the mixture was stirred for 3 h at room temperature, then poured into water and extracted with AcOEt. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt : hexane = 1 : 1) to give **13a** (2.95 g, 55%).

Biological Methods. Cell Culture and Drug Treatment P388/S and P388/VCR cell lines were supplied by the National Cancer Institute, NIH, Bethesda, MD and were stored frozen. P388/S and P388/VCR ascites cells were harvested from tumor-bearing $CD2F_1$ mice and maintained in plastic dishes in RPMI-1640 supplemented with 5% fetal bovine serum and 5 μM 2-mercaptoethanol. For *in vitro* treatment, tumor cells were seeded in 0.1 ml of culture medium/well in 96-well plates to a final cell density of 1×10^5 cells/ml. The cells were treated with various concentrations of vincristine, test compounds, or both and incubated in a CO_2 incubator at 37 °C for 48 h. The number of viable cells was estimated using a tetrazolium dye reduction assay (MTT assay).[12] Potency of the tested compounds to overcome MDR is expressed in terms of the ratio between IC_{50} values of vincristine for P388/VCR cells in the presence of test compounds and in the presence of verapamil. Cytotoxicity of the test compounds was expressed in terms of IC_{50} values for P388/S cells in the absence of vincristine.

Evaluation of Antitumor Activity Female BALB/c X DBA/2 ($CD2F_1$) mice weighing 20—23 g were purchased from Charles River Japan, Inc., Tokyo, Japan. Diluted ascites fluid (0.1 ml) containing 10^6 P388/VCR cells was transplanted i.p. into $CD2F_1$ mice. Test compounds and vincristine (0.1 mg/kg) were dissolved in 0.9% NaCl solution containing 0.1% carboxymethyl cellulose and administrated i.p. daily for 5 d, starting on the day after tumor inoculation. Antitumor activity was expressed in terms of the T/C value (%), the mean survival time of treated mice divided by the mean survival time of untreated mice, as well as the T/V value (%), the mean survival time of treated mice divided by the mean survival time of mice treated with vincristine alone. Five mice were used for each experimental group.

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