Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 2219–2227

BIOORGANIC & MEDICINAL CHEMISTRY

Newly Synthesized Dihydropyridine Derivatives as Modulators of P-Glycoprotein-mediated Multidrug Resistance

Hirokazu Tanabe,[a] Shigeyuki Tasaka,[a,*] Hiromasa Ohmori,[a] Noriaki Gomi,[a] Yoshiyuki Sasaki,[a] Toshiki Machida,[a] Mayumi Iino,[a] Akira Kiue,[a] Seiji Naito[b] and Michihiko Kuwano[c]

[a]*Omiya Research Laboratory. Nikken Chemicals Co. Ltd., 1-346 Kitabukuro, Omiya, Saitama, 330, Japan*
[b]*Department of Urology, Kyushu University School of Medicine, 3-1-1, Maidashi, Higashi-ku. Fukuoka, 812, Japan*
[c]*Department of Biochemistry, Kyushu University School of Medicine, 3-1-1, Maidashi, Higashi-ku. Fukuoka, 812, Japan*

Received 24 June 1998; accepted 13 August 1998

Abstract—Newly synthesized 1,4-dihydropyridine derivatives possessing alkyl chains at the 4-position screened whether they could overcome P-glycoprotein-mediated multidrug resistance in cultured cancer cells and also leukemia-bearing animals. Of these derivatives, some could overcome drug resistance to doxorubicin and vincristine in multidrug resistant human cancer cell lines. Combined administration of vincristine and some of the derivatives significantly increased the life span of P-glycoprotein overexpressing multidrug-resistant P388 leukemia-bearing mice. The calcium antagonistic activities, an undesirable effects, were weaker than that of verapamil. These results suggested that the introduction of alkyl groups at the 4-position were effective for both overcoming multidrug resistance and reducing the calcium antagonistic activity.

Introduction

Development of drug resistance, both intrinsic drug resistance and acquired drug resistance, remains a clinical obstacle in the chemotherapy of many cancers.[1,2] Acquisition of the multidrug resistant (MDR) phenotype is correlated with enhanced expression of a membranous P-glycoprotein with a molecular weight of 170 KDa which is coded by a MDR-1 gene.[3–6] P-glycoprotein mediates the ATP-dependent cellular efflux of a variety of structurally and functionary diverse compounds across the plasma membrane.[3–6]

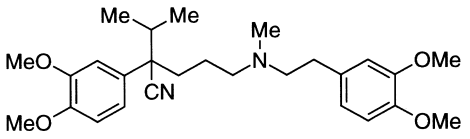
Strategies designed to reverse the multidrug resistance were actively sought by many laboratories.[7–13] Verapamil **1**, nicardipine **2** have been reported to successfully overcome multidrug resistance.[14] However, introduction of calcium antagonists in clinical use might pose a therapeutic problem because of their strong vasodilator activity. As for verapamil, several combination therapies with anticancer agents, such as vinca alkaloids or anthracyclines, have been tried but caused cardiovascular side effects.[14] The finding that the enantiomer of verapamil and nigludipine lacks calcium antagonistic activity but still has MDR reversal activity indicated that the MDR reversal activity is independent of the calcium antagonistic activity.[15,16] Consequently, a substance which has strong ability to overcome drug resistance but no calcium antagonistic activity would be of value in cancer chemotherapy (Fig. 1).

We have also reported that NIK-250 **3** which possess a heterocyclic ring at the 4-postion can overcome MDR and have moderate calcium antagonistic activity in vitro without optical resolution.[17–21] Further, we recently found that imidazothiazole derivatives could potentiate the MDR reversal activity without significant side effects observed for 1,4-dihidropyridine derivatives.[22]

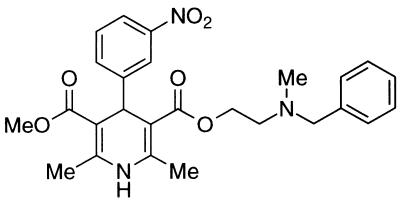
In this study, we have further examined newly synthesized 1,4-dihydropyridine derivatives possessing an alkyl chain at the 4-position to determine their ability to

Key words: Multidrug resistance; P-glycoprotein; P388 leukemia-bearing mice; calcium antagonistic activity; 1,4-dihydropyridine derivatives.
*Corresponding author. Tel.: 81 48 641 2334; fax: 81 48 641 5749.

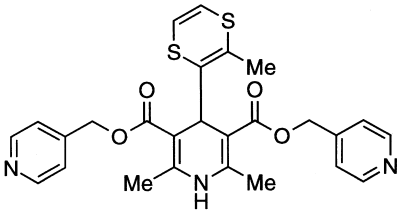
0968-0896/98/$ - see front matter
PII: S0968-0896(98)00170-9



Verapamil **1**



Nicardipine **2**



NIK-250 **3**

Figure 1.

overcome anti-cancer drug resistance in vitro, in vivo and calcium antagonistic activity in vitro.

Results and discussion

Chemistry

Dihydropyridine derivatives **4–14** listed in Table 1 were prepared by the variations of the Hantzch reaction[23–25] (method A, method B and method C) (Scheme 1).

Thus, refluxing the mixture of capronaldehyde, acetoacetic acid 3-(3-pyridyl) propyl ester and 3-aminocrotonic acid 3-(3-pyridyl) propyl ester in 2-propanol gave compound **4** in 29% yield (method A).

Refluxing the mixture of ammonia solution, phenyl acetaldehyde and 2 equiv. of acetoacetic acid 3-(3-pyridyl) propyl ester in 2-propanol gave compound **12** in 32% yield (method B).

We could not obtain compound **11** by method A and B. Thus, stirring the mixture of 2-octynal and 2 equiv. of 3-aminocrotonic acid 3-(3-pyridyl) propyl ester in acetic acid gave compound **11** in 22% yield (method C).

Evaluation of MDR activity and calcium antagonistic activity

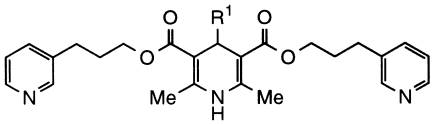
First, we determined the abilities of 1,4-dihydropyridine derivatives to overcome P-glycoprotein-mediated MDR in a cultured MDR cell line, KB/VJ300, derived from drug-sensitive parental KB cells.[26] KB/VJ300 cells used in this study were 20.4–52.8-fold resistant to doxorubicin (DXR) and 307–952-fold resistant to vincristine (VCR). The ability to overcome MDR was shown as the IC_{50} value and the relative resistance index determined by dividing the IC_{50} of an antitumor agent with 1 μg/mL of 1,4-dihydropyridine derivatives against KB/VJ300 cells by the IC_{50} against KB cells without addition of 1,4-dihydropyridine derivatives for each experiment (Table 2). We employed verapamil as a reference compound for which the calcium antagonistic action and the interaction with the P-glycoprotein are well known. We proposed these compounds to potentiate the cytotoxic action more than that of verapamil.

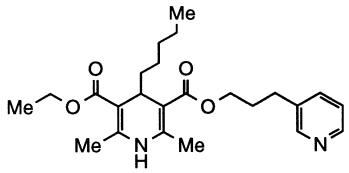
Compound **4** possessing a pentyl group and compound **5** possessing a octyl group at the 4-position were found to potentiate the cytotoxic action of DXR against KB/VJ300 cells. While compound **6** possessing a undecyl group did not potentiate the cytotoxic action in vitro and this result suggested that the chain length influenced MDR modulating activity. Introduction of a branched alkyl chain (**7–9**), a conjugated diene (**10**) and the triple bond (**11**) at the 4-position could potentiate the cytotoxic action similarly to compound **4** and seemed to be effective in vitro.

Many 1,4-dihydropyridine derivatives possessing an aromatic ring at the 4-position derived from antihypertensive drugs could potentiate cytotoxic action of anticancer agents.[11–13] However, compounds **12** and **13** showed a little potentiation of the cytotoxic effect of DXR.

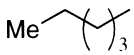
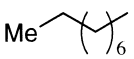
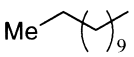
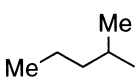
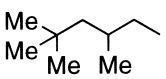
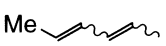
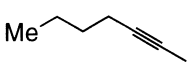
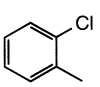
Compound **14** possessing ethyl ester at the 5-position which differs from compound **4** only at the 5-position was less active than compound **4** and this result indicated that the 3,5-bispyridyl propyl ester is one of the effective fragments.

Table 1. Yields and physical properties of dihydropyridine derivatives

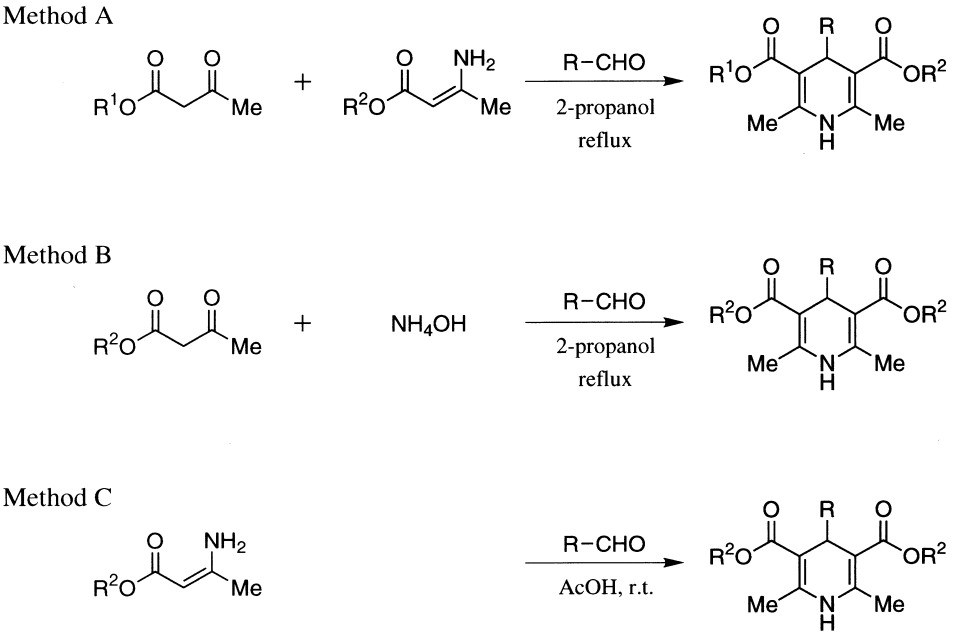




14

Compd	R^1	Yield (%) [method][a]	mp (°C)	Recryst. solvent	Formula[b]
4		28.7 (A)[c]	107–108	AcOEt	$C_{30}H_{39}N_3O_4$
5		48.7 (A)	76	Et_2O	$C_{33}H_{45}N_3O_4$
6		23.4 (A)	99	AcOEt	$C_{36}H_{51}N_3O_4$
7		50.2 (A)	107–108	AcOEt	$C_{28}H_{35}N_3O_4$
8		34.6 (A)[d]	83–85	Et_2O	$C_{30}H_{39}N_3O_4$
9		45.3 (A)	93–94	AcOEt	$C_{33}H_{45}N_3O_4$
10		34.3 (B)	155–157	AcOEt	$C_{30}H_{35}N_3O_4$
11		21.9 (C)	96–97	AcOEt	$C_{32}H_{39}N_3O_4$
12		32.4 (B)	127–128	AcOEt	$C_{31}H_{33}N_3O_4$
13		47.8 (A)	121–122	AcOEt	$C_{31}H_{32}ClN_3O_4$
14		23.0 (A)	Oil	—	$C_{24}H_{33}N_2O_4$[e]

[a] (A), (B) and (C), respectively, correspond to methods A, B and C.
[b] Compounds **4–13** were analyzed for C, H, N. Analytical results obtained for these three elements were within 0.3% of the calculated values for the formula shown.
[c] The yield of compound **4** was 34.0% when employing method B.
[d] The yield of compound **8** was 43.7% when employing method B.
[e] The structure was established by ^1H NMR and ^{13}C NMR.



Scheme 1.

We next evaluated the reversal activity of compounds **4**, **8–11**, for the cytotoxicity of VCR against KB/VJ300 cells. The relative resistance indices of these derivatives were 4.6- to 6.4-fold smaller compared with verapamil (Table 2).

These compounds thus seemed to modulate MDR because these derivatives could reverse the cytotoxic activity of both DXR and VCR.

We further evaluated the calcium antagonistic activity, an undesirable effect, and the results are summarized in Table 2. The IC_{50} value of a longer alkyl chain at the 4-position (compounds **5**, **6**) indicated weak action. Compound **8** indicated the most strong activity among our compounds but this activity was about a 3.5-fold higher concentration compared with verapamil.

The relative resistance indices of compounds **8** and **9** were close to each other, but their IC_{50} values of the calcium antagonistic activity were entirely different and this results suggested compound **9** was beneficial for both MDR reversal activity and reducing calcium antagonistic activity.

Compounds **12** and **13** might have a calcium antagonistic activity more potent than any other derivatives because they possess an aromatic ring at the 4-position such as other calcium antagonistic agents. However the calcium antagonistic activity of compounds **12** and **13** were moderately low. Compound **14** possessing an ethyl ester at the 5-position activated the calcium antagonism more than compound **4** possessing a 3-pyridyl propyl ester. These results suggested that an alkyl chain at the 4-position and 3,5-bispyridyl propyl ester of 1,4-dihydropyridine were the effective fragments to reverse MDR and reduce the calcium antagonistic activity.

Based on the results of the in vitro and calcium antagonistic activities, a combination therapy of VCR with these five derivatives in P388/VCR-bearing mice was screened (Table 3). Each derivative was administered ip at a dose of 100 mg/kg/day with 100 μg/kg/day of VCR for five days in P388/VCR-bearing mice. The life-prolonging effects of the combination of each derivative with VCR are shown in Table 3, as the T/C(%) value (life-prolonging rate) and T/V(%) value (effect for overcoming resistance).

Most of them showed a good life-prolonging effect, especially the life-prolonging effect of compounds **4**, **8**, **9** and **11** with an alkyl group at the 4-position well reflected in a good relationship to the vitro results. These activities appeared to be more potent than that of NIK-250 **3** reported in the same kind of experiments by A. Kiue et al.[19] On the other hand, the effect of compound **10** possessing the conjugated diene did not reflect in the in vitro activity.

Table 2. Reversal of the cyotoxicity of antitumor agents in KB/VJ300 cell line and calcium antagonistic activity

Compd	Cell line	Cytotoxicity[a]		Calcium antagonistic activity[d] $IC_{50}(10^{-6}\,M)$
		Ic_{50}[b] (resistance indices[c]) (ng/ml)		
		DXR	VCR	
None	KB	3.2–4.7(1.0)	0.42–1.37(1.0)	—
None	KB/VJ300	96–248(20.4–52.8)	390–496(307–952)	—
4		6.3(2.0)	4.9(3.6)	4.6
5		18(3.8)	—	> 30.0[e]
6		88(23.2)	—	> 30.0[e]
7		44(3.4)	—	5.9
8		8.3(1.8)	1.8(4.3)	2.1
9		8.4(2.6)	1.9(3.5)	> 30.0[e]
10		6.4(1.6)	1.4(2.6)	2.2
11		7.0(1.8)	3.6(2.0)	10.5
12		17.5(5.0)	—	11.7
13		18.5(5.3)	—	7.5
14		8.0(4.9)	—	2.3
(R)-(+)-Verapamil		19(4.4)	43(102)	0.6
3 (NIK2-250)		34(7.2)	51(92)	3.1

[a] Cytotoxicity of each antitumor agent was expressed as IC_{50} and resistant rate as follows.

[b] IC_{50} was concentration of antitumor agents necessary to inhibit the growth of the cells by 50% after five days culture in the presence or absence of compounds (1 μg/mL).

[c] Resistance index $= \frac{IC_{50} \text{ of antitumor agent with compound in KB/VJ300}}{IC_{50} \text{ of antitumor agent alone in KB for each experiment}}$.

[d] Calcium antagonistic activity was expressed as IC_{50}. IC_{50} was concentration of compounds necessary to inhibit to high potassium (50 mM) depolarization-induced contraction in isolated rat rectum.

[e] IC_{50} of these compounds could not be determined over 30×10^{-6} M.

We have previously reported that NIK-250 **3** and other 1,4-dihydropyridine derivatives, possessing heterocyclic ring at the 4-position, have potent MDR reversing activity.[19] Compound **9** could potentiate more MDR reversing activity and less calcium antagonistic activity than that of the previous compounds.

Further studies will be required to explore the possibility of applying our compounds to clinical use.

Conclusion

We synthesized new 1,4-dihydropyridine derivatives possessing an alkyl chain at the 4-position and found that the alkyl chains were one of the effective substituents to potentiate the MDR reversing activity. Some compounds effectively reduced calcium antagonistic activity compared with verapamil and there appeared to be no correlation between the MDR reversal activity and reduction of the calcium antagonism. Based on our results, the most suitable compound among synthesized new 1,4-dihydropyridine derivatives seemed to be 2,6-dimethy-4-(2,4,4-trimethlypentyl)-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl) propyl] ester (compound **9**).

Experimental

Biological assays

Cell line and drugs.[26] The MDR subline KB/VJ300 derived from human epidermoid cancer KB cells was employed in this study. Cells were grown in a monolayer on MEM (Nissui Seiyaku Co., Tokyo, Japan) containing 10% fetal bovine serum (Kanto Chemicals, Tokyo, Japan) and 2 mM L-glutamine (Gibco BRL, Tokyo, Japan). Vincristine sulfate (VCR) was obtained from Wako Chemical Industries, Ltd. (Osaka, Japan) in vitro and Shionogi & Co., Ltd. (Osaka, Japan) in vivo. Doxorubicin hydrochloride (DXR) and verapamil hydrochloride were obtained from Wako Chemical Industries, Ltd.

Cell survival by colony formation.[26] In brief, 300–500 cells were plated in 35 mm dishes in the absence of any drugs. DXR or VCR was added 24 h later with or without dihydropyridine derivatives, and the colonies which appeared were scored by tripane blue staining after incubation for seven days at 37 °C. Solutions of drugs were freshly prepared before use in dimethyl sulfoxide. Relative resistance was determined by dividing the IC_{50} (concentration of drug which reduced the cloning

Table 3. The life prolonging effect against P388/VCR-bearing mice

Compd	Survival time (days)			T/C (%)[a]	T/V (%)[b]
	Control	VCR alone	Combination		
4	10.3	10.5	17.7	172**	161*
8	10.0	10.7	17.2	172**	165*
9	10.7	10.9	15.8	148**	148**
10	10.7	10.9	12.0	112	110
11	10.7	10.9	14.5	136**	133**
3 (NIK-250)	10.7	10.9	12.8	120	117

The control group consisted of 12 to 18 mice, the VCR alone group consisted of 6 to 12 mice and the group treated with VCR and compound consisted of 5 or 6 mice. Asterisks indicate a significant difference from the respective result with the same dose of VCR alone group by Student's and Cochran's *t*-tests: *$p < 0.05$, **$p < 0.01$.

[a] $\mathrm{T/C}\,(\%) = \frac{\text{Survival days of VCR and test compound}}{\text{Survival days of control}} \times 100.$

[b] $\mathrm{T/V}\,(\%) = \frac{\text{Survival days of VCR and test compound}}{\text{Survival days of VCR alone}} \times 100.$

efficiency to 50% of control without drugs) of KB/VJ300 with or without dihydropyridine derivatives by the IC_{50} of KB cells without dihydropyridine derivatives.

Evaluation of antitumor activity.[19] Six to eight-week-old female $CD2F_1$ (BALB/C×DBA/2) mice were obtained from Charles River Japan, Inc, (Tokyo, Japan). $CD2F_1$, mice were inoculated ip with 0.2 mL of diluted ascites fluid containing 10^6 P388/VCR cells on Day 0. VCR and 1,4-dihydropyridine derivatives were given ip daily from Day 1 after tumor inoculation for five days. Antitumor activity was evaluated by the mean of survival days for each group and also expressed by the T/C and T/V values (percentage). The data of mean survival days were analyzed by the two-tailed Student's *t*-test and the two-tailed Cochran's *t*-test if the difference in distribution between the two groups to be compared is significant ($p < 0.05$) by the *F* test.

Evaluation of calcium antagonism.[19] Nine to 14-week-old male Wistar rats were obtained from CLEA Japan, Inc, (Tokyo, Japan). Calcium antagonistic activity of 1,4-dihydropyridine derivatives was tested by the inhibitory effect on high potassium depolarization-induced concentration (K^+-contracture) in isolated rat aorta. An approximately 2 mm long ring specimen was taken from the thoracic artery of the rat and mounted in an organ bath filled with Krebs-bicarbonate solution at 37 °C aerated with 95% O_2–5% CO_2. The specimen was stabilized for 60 min, and a potassium chloride solution was then added to the organ bath to give a final concentration of 50 mM. Verapamil or 1,4-dihydropyridine derivatives of the present invention were cumulatively added from 1×10^{-10} to 1×10^{-4} M when the contraction reaction occurring at that time reached equilibrium. The results are shown as the 50% inhibitory concentration (the IC_{50} value) of hypercalcemic concentration.

Chemistry

Melting points are determined using Yamamoto melting point apparatus (Mp–21) and uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL-EX 400 instrument. Elemental analyses were performed by the Sumika Research Laboratory.

The starting materials were prepared as follows.

Acetoacetic acid 3-(3-pyridyl)propyl ester. A mixture of 3-(3-pyridyl)propanol (1.37 g, 10.0 mmol) and diketen (0.84 g, 10.0 mmol) in 10 ml of THF were stirred at 0 °C for 5 h. The mixture was evaporated under reduced presser and the residue was purified by chromatography on florisil to give 1.73 g (78.3%) of acetoacetic acid 3-(3-pyridyl)propyl ester as pale yellow liquid. ^{1}H NMR ($CDCl_3$) δ 1.99 (m, 2H), 2.27 (s, 3H), 2.70 (t, $J = 7.4$ Hz, 2H), 3.46 (s, 2H), 4.17 (t, $J = 6.4$ Hz, 2H), 7.22 (dd, $J = 4.4$, 7.8 Hz, 1H), 7.51 (d, $J = 7.8$ Hz, 1H), 8.45 (m, 2H).

3-Aminocrotonic acid 3-(3-pyridyl)propyl ester. Ammonia gas was introduced into acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol) in 20 mL of 2-propanol at 0 °C for 7 h and stood one week at room temperature. The mixture was evaporated under reduced presser and the residue was purified by chromatography on florisil to give 1.56 g (70.9%) of 3-aminocrotonic acid 3-(3-pyridyl)propyl ester as pale yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H), 1.97 (m, 2H), 2.71 (t, $J = 7.8$ Hz, 2H), 4.08 (t, $J = 6.4$ Hz, 2H), 4.55 (s, 2H), 7.21 (dd, $J = 4.4$, 7.8 Hz, 1H), 7.51 (d, $J = 7.8$ Hz, 1H), 8.44 (d, $J = 4.4$ Hz, 1H), 8.47 (s, 1H).

2,6-Dimethyl-4-(1-pentyl)-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (4). A mixture of acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol), 3-aminocrotonic acid 3-(3-pyridyl)propyl ester (2.20 g, 10.0 mmol) and capronaldehyde (1.20 g, 12.0 mmol) in 10 mL of 2-propanol was refluxed for 7 h. The mixture was evaporated under reduced pressure and the residue was then dissolved in 1 N hydrochloric acid and washed with AcOEt. The water phase was made alkaline with 10% sodium hydroxide solution and extracted with AcOEt. The organic phase was washed with water, dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica gel and recrystallized from AcOEt to give 1.45 g (28.7%) of **4**.

mp 107–108 °C. Anal. calcd for $C_{30}H_{39}N_3O_4$: C, 71.26; H, 7.77; N, 8.31. Found: C, 71.23; H, 7.65; N, 8.27. ^{1}H NMR ($CDCl_3$) δ 0.84 (t, J = 6.8 Hz, 3H), 1.21 (m, 6H), 1.35 (m, 2H), 2.00 (m, 4H), 2.31 (s, 6H), 2.73 (m, 4H), 4.00 (t, J = 5.2 Hz, 1H), 4.16 (m, 4H), 5.89 (s, 1H), 7.18 (dd, J = 4.8, 7.6 Hz, 2H), 7.48 (d, J = 7.6 Hz, 2H), 8.43 (dd, J = 1.6, 4.8 Hz, 2H), 8.45 (d, J = 1.6 Hz, 2H). ^{13}C NMR ($CDCl_3$); δ 14.1, 19.4, 22.7, 24.6, 29.5, 30.2, 32.2, 33.0, 37.0, 62.5, 103.0, 123.3, 135.7, 136.5, 145.1, 147.5, 149.9, 167.9.

2,6-Dimethyl-4-(1-octyl)-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (5). In a similar manner, **5** was prepared from acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol), 3-aminocrotonic acid 3-(3-pyridyl)propyl ester (2.20 g, 10.0 mmol) and nonyl aldehyde (1.70 g, 12.0 mmol) and recrystallized from Et_2O to yield 2.67 g (48.7%) of **5**. mp 76 °C. Anal. calcd for $C_{33}H_{45}N_3O_4$: C, 72.36; H, 8.28; N, 7.67. Found: C, 72.36; H, 8.13; N, 7.68. ^{1}H NMR ($CDCl_3$) δ 0.84 (t, J = 6.4 Hz, 3H), 1.21 (m, 12H), 1.35 (m, 2H). 2.01 (m, 4H), 2.31 (s, 6H), 2.73 (m, 4H), 4.00 (t, J = 6.0 Hz, 1H), 4.15 (m, 4H), 5.88 (s, 1H), 7.18 (dd, J = 4.8, 7.6 Hz, 2H), 7.48 (d, J = 7.6 Hz, 2H). 8.43 (dd, J = 1.6, 4.8 Hz, 2H), 8.45 (d, J = 1.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 14.1, 19.4, 22.6, 25.0, 29.3, 29.5, 29.7, 30.1, 30.2, 31.8, 33.0, 37.0, 62.5, 103.0, 123.3, 135.7, 136.5, 145.1, 147.5, 149.9, 167.9.

2,6-Dimethyl-4-(1-undecyl)-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (6). In a similar manner, **6** was prepared from acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol), 3-aminocrotonic acid 3-(3-pyridyl)propyl ester (2.20 g, 10.0 mmol) and dodecyl aldehyde (2.21 g, 12.0 mmol) and recrystallized from AcOEt to yield 1.38 g (23.4%) of **6**. mp 99 °C. Anal. calcd for $C_{36}H_{51}N_3O_4$: C, 73.31; H, 8.72; N, 7.12. Found: C, 73.22; H, 8.55; N, 7.06. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 6.8 Hz, 3H), 1.20 (m, 18H), 1.35 (m, 2H), 2.00 (m, 4H), 2.31 (s, 6H), 2.73 (m, 4H), 4.00 (t, J = 6.0 Hz, 1H), 4.16 (m, 4H), 5.97 (s, 1H), 7.18 (dd, J = 4.8, 7.6 Hz, 2H), 7.48 (d, J = 7.6 Hz, 2H), 8.43 (dd, J = 1.6, 4.8 Hz, 2H), 8.45 (d, J = 1.6 Hz, 2H). ^{13}C NMR ($CDCL_3$); δ 14.1, 19.4, 22.7, 25.1, 29.4, 29.6, 29.6, 29.7, 29.8, 29.8, 30.1, 30.2, 31.9, 33.1, 37.1, 62.5, 103.1, 123.4, 135.8, 136.6, 145.2, 147.6, 149.9, 167.9.

2,6-Dimethyl-4-isopropyl-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (7). In a similar manner, **7** was prepared from acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol), 3-aminocrotonic acid 3-(3-pyridyl)propyl ester (2.20 g, 10.0 mmol) and isobutyraldehyde (0.86 g, 12.0 mmol) and recrystallized from AcOEt to yield 2.40 g (50.2%) of **7**. mp 107–108 °C. Anal. calcd for $C_{28}H_{35}N_3O_4$: C, 70.42; H, 7.39; N, 8.80. Found: C, 70.39; H, 7.32; N, 8.83. ^{1}H NMR ($CDCl_3$) δ 0.79 (d, J = 6.8 Hz, 6H), 1.64 (m, 1H), 1.99 (m, 4H), 2.33 (s, 6H), 2.73 (m, 4H), 4.00 (d, J = 5.6 Hz, 1H), 4.16 (m, 4H), 5.99 (s, 1H), 7.14 (dd, J = 4.8, 7.6 Hz, 2H), 7.48 (d, J = 7.6 Hz, 2H), 8.43 (dd, J = 1.6, 4.8 Hz, 2H), 8.45 (d, J = 1.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 18.6, 19.3, 29.5, 30.2, 35.6, 38.8, 62.5, 101.5, 123.3, 135.7, 136.6, 145.1, 147.5, 149.8, 168.4.

2,6-Dimethyl-4-[1-(1-methyl)butyl]-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (8). In a similar manner, **8** was prepared from acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol), 3-aminocrotonic acid 3-(3-pyridyl)propyl ester (2.20 g, 10.0 mmol) and 2-methyl pentanal (1.20 g, 12.0 mmol) and recrystallized from Et_2O to yield 1.75 g (34.6%) of **8**. mp 83–85 °C. Anal. calcd for $C_{30}H_{39}N_3O_4$: C, 71.26; H, 7.77; N, 8.31. Found: C, 71.23; H, 7.65; N, 8.27. ^{1}H NMR ($CDCl_3$) δ 0.75 (d, J = 6.8 Hz, 3H), 0.82 (t, J = 6.8 Hz, 3H), 1.00 (m, 1H), 1.18–1.32 (m, 3H), 1.45 (m, 1H), 2.00 (m, 4H), 2.32 (s, 3H), 2.33 (s, 3H), 2.73 (m, 4H), 4.07 (d, J = 4.8 Hz, 1H), 4.16 (m, 4H), 5.95 (s, 1H), 7.19 (dd, J = 4.8, 7.6 Hz, 2H), 7.48 (d, J = 7.6 Hz, 2H), 8.43 (d, J = 4.8 Hz, 2H), 8.44 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 14.5, 15.1, 18.3, 19.3, 19.4, 20.6, 29.5, 29.6, 29.9, 30.2, 34.9, 38.1, 40.9, 62.6, 101.1, 101.9, 123.3, 123.4, 135.7, 135.8, 136.5, 136.6, 144.9, 145.1, 147.5, 147.5, 149.8, 149.8, 168.3, 168.7.

2,6-Dimethyl-4-[1-(2,4,4-trimethylpentyl)]-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (9). In a similar manner, **9** was prepared from acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol), 3-aminocrotonic acid 3-(3-pyridyl)propyl ester (2.20 g, 10.0 mmol) and 3,5,5-trimethyl hexanal (1.69 g, 12.0 mmol) and recrystallized from AcOEt to yield 2.48 g (45.3%) of **9**. mp 93–94 °C. Anal. calcd for $C_{33}H_{45}N_3O_4$: C, 72.36; H, 8.28, N, 7.67. Found: C, 72.20; H, 8.22; N, 7.64. ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 9H), 1.00 (d, J = 6.4 Hz, 3H), 1.03 (m, 3H), 1.41 (m, 2H), 2.00 (m, 4H), 2.31 (s, 3H), 2.32 (s, 3H), 2.73 (m, 4H), 4.00 (d, J = 4.8 Hz, 0.5H), 4.01 (d, J = 4.8 Hz, 0.5H), 4.17 (m, 4H), 5.78 (s, 1H), 7.18 (m, 2H), 7.47 (m, 2H), 8.43 (m, 2H), 8.44 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 19.3, 19.4, 22.6, 24.9, 29.5, 29.5, 29.8, 30.1, 30.2, 30.8, 31.1, 46.6, 52.2, 62.6, 62.7, 103.3, 104.1, 123.3, 123.3, 135.7, 135.8, 136.5, 136.5, 145.0, 145.1, 147.5, 147.5, 149.8, 149.8, 167.3, 168.3.

4-(2-Chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (13). In a similar manner, **13** was prepared from acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol), 3-aminocrotonic acid 3-(3-pyridyl)propyl ester (2.20 g, 10.0 mmol) and 2-chlorobenzaldehyde (1.69 g, 12.0 mmol) and recrystallized from AcOEt to yield 2.61 g (47.8%) of **13**. mp 121–122 °C. Anal. calcd for $C_{31}H_{32}ClN_3O_4$: C,

68.19; H, 5.91; N, 7.70. Found: C, 68.07; H, 5.95; N, 7.61. ^{1}H NMR ($CDCl_3$) δ 1.93 (m, 4H), 2.37 (s, 6H), 2.51 (m, 4H), 4.09 (m, 4H), 5.41 (s, 1H), 6.12 (s, 1H), 7.03 (td, J = 1.6, 7.6 Hz, 1H), 7.14 (t, J = 7.6 Hz, 1H), 7.17 (dd, J = 4.8, 7.2 Hz, 2H), 7.22 (d, J = 7.6 Hz, 1H), 7.41 (d, J = 7.2 Hz, 2H), 7.42 (dd, J = 1.6, 7.6 Hz, 1H), 8.34 (d, J = 1.6 Hz, 2H), 8.41 (dd, J = 1.6, 4.8 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 19.6, 29.4, 30.0, 37.2, 63.0, 103.8, 123.3, 127.1, 127.5, 129.3, 131.4, 132.2, 135.8, 136.8, 144.4, 145.9, 147.4, 149.9, 167.5.

2,6-Dimethyl-4-(1-pentyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-[3-(3-pyridyl)propyl] ester-5-ethyl ester (14). In a similar manner, **14** was prepared from acetoacetic acid ethyl ester (1.30 g, 10.0 mmol), 3-aminocrotonic acid 3-(3-pyridyl)propyl ester (2.20 g, 10.0 mmol) and capronaldehyde (1.20 g, 12.0 mmol) to yield 0.95 g (23.0%) of **14** as pale yellow liquid. ^{1}H NMR ($CDCl_3$) δ 0.83 (t, J = 6.8 Hz, 3H), 1.22 (m, 6H), 1.29 (t, J = 7.6 Hz, 3H), 1.32 (m, 2H), 2.01 (m, 2H), 2.30 (s, 6H), 2.73 (m, 2H), 3.98 (t, J = 6.0 Hz, 1H), 4.17 (m, 4H), 5.89 (s, 1H), 7.21 (dd, J = 4.8, 7.6 Hz, 1H), 7.53 (d, J = 7.6 Hz, 1H), 8.46 (dd, J = 1.6, 4.8 Hz, 1H), 8.47 (d, J = 1.6 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 14.1, 14.4, 19.3, 19.4, 22.7, 24.6, 29.5, 30.2, 32.1, 32.9, 36.9, 59.6, 62.4, 102.9, 103.4, 123.4, 135.9, 136.7, 144.6, 145.3, 147.5, 149.8, 167.9, 168.1.

2,6-Dimethyl-4-(1-penta-1,3-dienyl)-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (10). A mixture of acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol), 2,4-hexadienal (0.58 g, 6.00 mmol) and 2 mL of concentrated ammonia solution was refluxed in 10 mL of 2-propanol for 7 h. The mixture was evaporated under reduced pressure and the residue was then dissolved in 1 N hydrochloric acid and washed with AcOEt. The water phase was made alkaline with 10% sodium hydroxide solution and extracted with AcOEt. The organic phase was washed with water, dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica gel and recrystallized from AcOEt to give 0.86 g (34.3%) of **10**. mp 155–157 °C. Anal. calcd for $C_{30}H_{35}N_3O_4$: C, 71.83; H, 7.03; N, 8.38. Found: C, 71.73; H, 7.04; N, 8.37. Compound **10** was isomers mixture, so a main product's ^{1}H NMR was assiged as follows. ^{1}H NMR ($CDCl_3$) δ 1.68 (d, J = 7.6 Hz, 3H), 1.98 (m, 4H), 2.32 (s, 3H), 2.33 (s, 3H), 2.71 (m, 4H), 4.10 (m, 2H), 4.21 (m, 2H), 4.52 (d, J = 6.4 Hz, 1H), 5.55 (dd, J = 7.2, 14.4 Hz, 1H), 5.72 (s, 1H), 5.73 (dd, J = 6.4, 11.6 Hz, 1H), 5.88–6.02 (m, 2H), 7.17 (dd, J = 5.2, 8.0 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 8.43 (m, 4H).

2,6-Dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (12). In a similar manner, **12** was prepared from acetoacetic acid 3-(3-pyridyl)propyl ester (2.21 g, 10.0 mmol) and benzaldehyde (0.64 g, 0.60 mmol) and 2 mL of concentrated ammonia solution and recrystallized from AcOEt to yield 0.83 g (32.4%) of **12**. mp 127–128 °C. Anal. calcd for $C_{31}H_{33}N_3O_4$: C, 72.78; H, 6.50; N, 8.21. Found: C, 72.60; H, 6.51; N, 8.16. ^{1}H NMR ($CDCl_3$) δ 1.92 (m, 4H), 2.36 (s, 6H), 2.56 (m, 4H), 4.05 (m, 2H), 4.11 (m, 2H), 5.06 (s, 1H), 6.19 (s, 1H), 7.13 (t, J = 7.2 Hz, 1H), 7.16 (dd, J = 4.8, 7.6 Hz, 2H), 7.22 (t, J = 7.2 Hz, 2H), 7.32 (d, J = 7.2 Hz, 2H), 7.37 (d, J = 7.6 Hz, 2H), 8.34 (d, J = 1.6 Hz, 2H), 8.42 (dd, J = 1.6, 4.8 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 19.6, 29.3, 30.1, 39.5, 62.6, 103.8, 123.3, 126.3, 127.8, 128.1, 135.8, 136.6, 144.5, 147.4, 147.6, 149.8, 167.4.

2,6-Dimethyl-4-(1-hept-1-ynyl)-1,4-dihydropyridine-3,5-dicarboxylic acid bis [3-(3-pyridyl)propyl] ester (11). A mixture of 3-aminocrotonic acid 3-(3-pyridyl)propyl ester (2.20 g, 10.0 mmol) and 2-octynaldehyde (0.74 g, 6.00 mmol) was stirred at room temperature in 10 mL of acetic acid for 24 h. The mixture was neutralized by 10% sodium bicarbonate solution and then extracted with AcOEt. The organic phase was washed with water, dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica gel and recrystallized from AcOEt to give 0.58 g (21.9%) of **11**. mp 96–97 °C. Anal. calcd for $C_{32}H_{39}N_3O_4$: C, 72.56; H, 7.42; N, 7.93. Found: C, 72.47; H, 7.37; N, 7.93. ^{1}H NMR ($CDCl_3$) δ 0.78 (t, J = 6.8 Hz, 3H), 1.15 (m, 4H), 1.37 (m, 2H). 2.02 (m, 2H), 2.06 (m, 4H), 2.33 (s, 6H), 2.79 (m, 4H), 4.11 (m, 2H), 4.25 (m, 2H), 4.81 (s, 1H), 5.93 (s, 1H), 7.18 (dd, J = 4.8, 7.2 Hz, 2H), 7.51 (d, J = 7.2 Hz, 2H), 8.43 (dd, J = 1.6, 4.8 Hz, 2H), 8.47 (d, J = 1.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 13.9, 18.9, 19.2, 22.0, 26.3, 28.6, 29.3, 30.2, 31.0, 62.4, 79.5, 83.9, 100.3, 123.3, 135.9, 136.7, 145.3, 147.4, 149.9, 167.0.

References

1. Davis, H. L.; Davis, T. E. *Cancer Treat. Rep.* **1979**, *63*, 809.
2. Pastan, I.; Gottesman, M. M. *N. Engl. J. Med.* **1987**, *316*, 1388.
3. Endicott, J. A.; Ling, V. *Ann. Rev. Biochem.* **1989**, *58*, 137.
4. Kartner, N.; Riordan, J. R.; Ling, V. *Science* **1983**, *221*, 1285.
5. Ueda, K.; Cornwell, M. M.; Gottesman, M. M.; Pastan, I.; Roninson, I. B.; Ling, V.; Riordan, J. R. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 956.
6. Gottesman, M. M.; Pastan, I. *Ann. Rev. Biochem.* **1993**, *62*, 385.
7. Akiyama, S.; Shiraishi, N.; Kuratomi, Y.; Nakagawa, M.; Kuwano, M. *J. Natl. Cancer Inst.* **1986**, *76*, 839.
8. Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1981**, *41*, 1967.
9. Rogan, A. M.; Hamilton, T. C.; Young, R. C. *Science* **1984**, *224*, 994.

10. Ince, P.; Appleton, D. R.; Finney, K. J.; Sunter, J. P.; Watson, A. J. *Br. J. Cancer* **1986**, *53*, 137.

11. Shinoda, H.; Inaba, M.; Tsuruo, T. *Cancer Res.* **1989**, *49*, 1722.

12. Tsuruo, T.; Iida, H.; Nojiri, M.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1983**, *43*, 2905.

13. Dodic, N.; Dumaitre, B.; Daugan, A.; Pianetti, P. *J. Med. Chem.* **1995**, *38*, 2418.

14. Fisher, G. A.; Sikic, B. I. *Drug Resistance in Clinical Oncology and Hemalology* **1995**, *9*, 363.

15. Pommerenke, E. W.; Mattern, J.; Traugott, U.; Volm, M. *Arzneim-Forsch. Drug. Res.* **1991**, *41*, 855.

16. Hofmann, J.; Gekeler. V.; Ise, W.; Noller, A.; Mitterdorfer, J.; Hofer, S.; Utz, I.; Gotwald. M.; Boer, R.; Glossmann, H.; Grunicke, H. H. *Biochem. Phann.* **1994**, *49*. 603.

17. Kiue, A.; Sano, T.; Naito, A.; Inada, H.; Suzuki, K.; Okumura, M.; Kikuchi, J.; Sato, S.; Takano, H.; Kohno, K.; Kuwano, M. *Jpn. J. Cancer Res.* **1990**, *81*, 1057.

18. Watanabe, Y.; Takano, H.; Kiue, A.; Kohno, K.; Kuwano, M. *Anti-Cancer Drug Design* **1991**, *6*, 47.

19. Kiue, A.; Sano, T.; Suzuki, K.; Inada, H.; Okumura, M.; Kikuchi, J.; Sato, S.; Kohono, K.; Kuwano, M. *Cancer Res.* **1990**, *50*, 310.

20. Kiue, A.; Sano, T.; Naito, A.; Okumura, M.; Kohono, K.; Kuawno, M. *Br. J. Cancer* **1991**, *64*, 221.

21. Nogae, I.; Kohno, K.; Kikuchi, J.; Kuwano, M.; Akiyama, S.; Kiue, A.; Suzuki, K.; Yoshida, Y.; Cornwell, M. M.; Pastan, I.; Gottesman, M. M. *Biochem. Pharm.* **1989**, *38*, 519.

22. Tasaka, S.; Tanabe, H.; Sasaki, Y.; Machida, T.; Iino, M.; Kiue, A.; Naito, S.; Kuwano, M. *J. Heterocyclic Chem.* **1997**, *34*, 1763.

23. Meguro, K.; Aizawa, M.; Sohda, T.; Kawamatu, Y.; Nagaoka, A. *Chem. Pharm. Bull.* **1985**, *33*, 3787.

24. Cozzi, P.; Carganico, G.; Fusar, D.; Grossoni, M.; Menichincheri, M.; Pinciroli V.; Tonani, R.; Vaghi, F.; Salvati, P. *J. Med. Chem.* **1993**, *36*, 2964.

25. Loev, B.; Goodman, M. M.; Snader, K. M.; Tedeschi, R.; Macko, E. *J. Med. Chem.* **1974**, *17*, 956.

26. Kohno, K.; Kikuchi, J.; Sato, S. *Jpn. J. Cancer Res.* **1988**, *79*, 1238.