Synthesis and Structure−Activity Relationships of Novel 7-Substituted 1,4-Dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acids as Antitumor Agents. Part 1

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Received February 5, 2001

In an attempt to search for clinically useful antitumor agents, we have discovered that a series of 1,7-disubstituted-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids possessed moderate cytotoxic activity. We investigated the structure−activity relationships in this series of compounds by changing N-1 and C-7 positions and the core ring structure itself and evaluated the synthesized compounds against several murine and human tumor cell lines. These modifications led us to the following findings. (1) The 2-thiazolyl group at the N-1 position of the naphthyridine structure is the best substituent for antitumor activity. (2) Regarding core ring structure, the naphthyridine derivative is the most active followed by pyridopyrimidine analogue. (3) At the C-7 position, aminopyrrolidine derivatives are more effective than other amines or thioether derivatives. Finally, the *trans*-3-amino-4-methoxypyrrolidinyl derivative (**43j**) and the 3-amino-3-methylpyrrolidinyl derivative (**43f**) as well as 3-aminopyrrolidinyl derivative (AT-3639, **1**) were determined to be effective in in vitro and in vivo antitumor assays, and their activity was comparable to that of etoposide.

Introduction

Quinolone antibacterial agents have been known to inhibit DNA gyrase and topoisomerase IV, bacterial topoisomerase II enzymes.[1] DNA topoisomerase II enzymes are essential cellular enzymes that catalyze the double strand breakage of DNA to allow strand passage and thereby control the topology and conformation of DNA.[2] On the other hand, mammalian topoisomerase II is one of the targets of DNA-active antitumor agents, which include etoposide, doxorubicin, ellipticine, and amsacrine.[3] Since the mechanism of DNA synthesis between mammalian topoisomerase II and DNA gyrase/topoisomerase IV is similar, novel classes of quinolones possessing considerable inhibitory activity of mammalian topoisomerase II have recently been reported. All exemplified quinolones (Figure 1) A-65282,[4] CP-115,953,[5] and WIN57294[6] (1-cyclopropyl-6,8-difluoroquinolones) and A-62176[7] and A-85226[8] (quinobenzoxazines) were identified as potent antineoplastic agents. Isothiazoloquinolone A-65282 was nearly as potent as teniposide with topoisomerase II mediated DNA breakage activity.[4] The 7-hydroxyphenyl compound CP-115,953 was about 2 times more potent than etoposide at enhancing topoisomerase II mediated DNA cleavage.[5] It was also reported that the C-8 fluorine of CP-115,953 contributed to the potency against eukaryotic topoisomerase II. The 7-(2,6-dimethyl-4-pyridyl) derivative WIN57294 was shown to have an EC_{50} value of 7.6 μM in a DNA cleavage assay using HeLa topoisomerase II.[6] The quinobenzoxazines A-62176 and A-85226 demonstrated broad activity against human and murine tumor cell lines.[7,8] The fact that all these compounds showed topoisomerase II inhibitory activity has given rationality to quinolone-based drug design in the search for novel antitumor agents. No drug, however, has reached clinical trials so far to our knowledge.

Our goal for this study was to identify new quinolones that have potent antitumor activity and no cross-resistance with other agents. To find these clinically useful antitumor agents, a number of quinolones that have been prepared at our laboratories for antibacterial agents were screened and then 1,8-naphthyridine derivatives were found to display moderate cytotoxic activity against murine P388 leukemia. This finding prompted us to study analogues of the 1,8-naphthyridine compounds because their basic structures were novel as antitumor agents as far as we know. In light of this consideration, chemical modifications were carried out on the N-1 and C-7 positions in addition to conversion of the 1,8-naphthyridine ring itself to other similar ring systems. In this paper, we described details and discussions of structure−activity relationships (SARs) and antitumor activities that have been revealed during the study.

Chemistry

General synthetic routes of 7-(3-amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids **1** and **6**−**24** with various R_1 groups are illustrated in Scheme 1. Nicotinoyl acetate **2**[9] was converted to enaminoester **3** by reaction with ethyl orthoformate and acetic anhydride, followed by reaction with the primary amine R_1NH_2. 1,8-Naphthyridine **4** was obtained through base-assisted cyclization reaction of **3**. The coupling reaction of 3-aminopyrrolidine or 3-acetamidopyrrolidine to **4** was followed by acid hydrolysis of the ester and the acetyl group to give the

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10.1021/jm010057b CCC: $22.00
Published on Web 11/01/2002

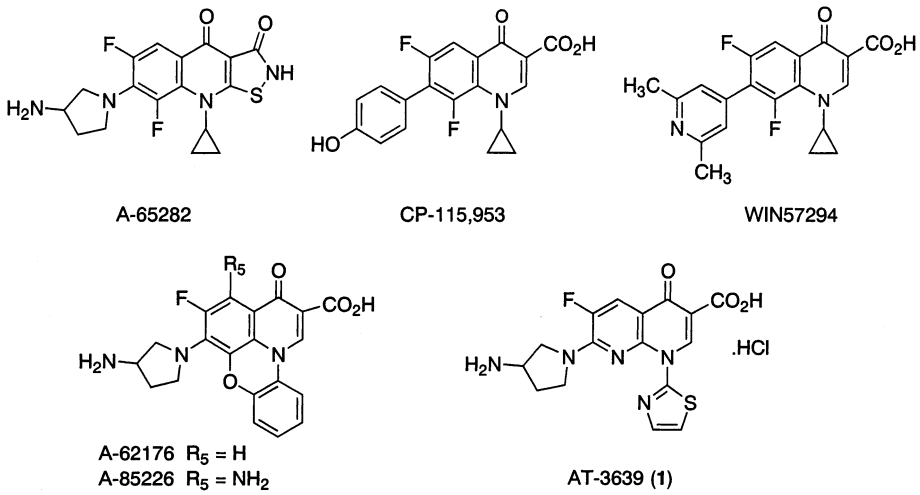
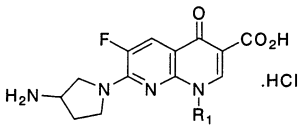


Figure 1. Structures of reference compounds and AT-3639.

Table 1. Physical Data and Cytotoxic Activity (IC_{50}) of 7-(3-Amino-1-pyrrolidinyl)-1,4-dihydro-4-oxonaphthyridine-3-carboxylic Acids **1** and **6**−**24** against Murine P388 Leukemia



compd	R_1	mp, °C	% yield[a]	IC_{50},[b] μg/mL
1 (AT-3639)	2-thiazolyl	286−288 dec	45	0.021
6	phenyl	292−295 dec	52	2.4
7	4-fluorophenyl	278−282 dec	68	2.1
8	2-pyridyl	283−288 dec	29	>10
9	cyclopropyl	290−295 dec	50	7.9
10	2-thienyl	288−291 dec	72	1.2
11	5-isoxazolyl	236−239	62	9.4
12	3-methyl-5-isoxazolyl	253−255 dec[c]	78	0.58
13	1,3,4-thiadiazol-2-yl	288−289	6	5.5
14	1-methylpyrazol-5-yl	271−273 dec	59	>10
15	(2-thiazolyl)methyl	236−239	66	>10
16	4-methylthiazol-2-yl	257−259 dec	82	0.051
17	5-methylthiazol-2-yl	265 dec	83	0.26
18	4,5-dimethylthiazol-2-yl	277 dec	70	0.18
19	4-*tert*-butylthiazol-2-yl	285 dec	47	0.88
20	4-phenylthiazol-2-yl	261 dec	74	0.052
21	5-chlorothiazol-2-yl	>300	49	0.094
22	5-bromothiazol-2-yl	293−297 dec	51	0.085
23	5-methoxythiazol-2-yl	267−269 dec	50	0.35
24	2-benzothiazolyl	273−275 dec	48	0.049
etoposide				0.0085
doxorubicin				0.0040
cisplatin				0.011

[a] Isolation yields based on **4**. [b] Concentration of agent to reduce cell viability by 50%. [c] Prepared as a free form.

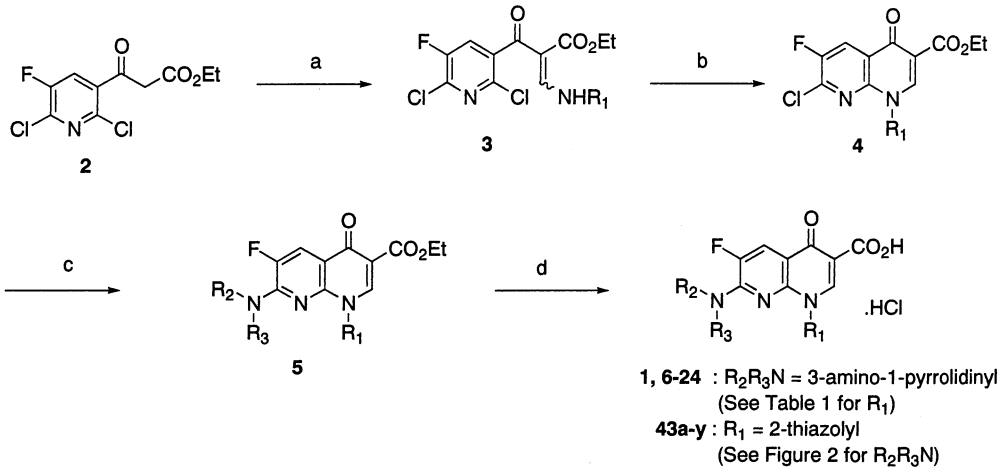
desired products **1** and **6**−**24**. The yields of **1** and **6**−**24** were calculated from **4** and are listed in Table 1 along with their in vitro cytotoxic activity data against P388 leukemia.

To study the activity of varied core structure, compounds **25**−**29** having other heterocyclic systems were designed. Scheme 2 depicts a route for the synthesis of pyrido[2,3-*d*]pyrimidine **25**. A ketoester **31**, prepared from pyrimidinecarbonyl chloride **30**,[10] was converted to 7-(methylthio)ester **32** and then to 7-(methanesulfonyl)ester **33** by oxidation. The coupling reaction of **33** to 3-aminopyrrolidine and subsequent hydrolysis afforded the product **25**. Pyrido[2,3-*c*]pyridazine **26** was prepared from the ketoester **2** in four steps as shown in Scheme 3. Thus, the condensation reaction of **2** with 2-diazothiazole, which was prepared by diazotization of 2-aminothiazole, followed by cyclization reaction proceeded to give ester **36** in low yield. The synthesis of 1,6-naphthyridine **27** and quinolones **28** and **29** is summarized in Scheme 4. These compounds were prepared in good yield according to a method similar to the preparation of 1,8-naphthyridines **6**−**24**.

Various kinds of amines (Figure 2) were incorporated into the C-7 position of the 1-(2-thiazolyl)-1,8-naphthyridine ester **4** (R_1 = 2-thiazolyl) to provide the desired products **43a**−**y** after hydrolysis of the ester and the protecting group (Scheme 1). When diamines were used in the coupling reaction with ester **4**, one of the nitrogens was protected with the acetyl or the *tert*-butyloxycarbonyl (Boc) group in most cases. The 7-aminoethylthio derivative **59** and the 7-hydroxy derivative **60** were also prepared from **4** (Scheme 5). The yields of **43a**−**y**, **59**, and **60** were calculated from **4** and listed in Table 3 along with their in vitro cytotoxic activity data against P388 leukemia.

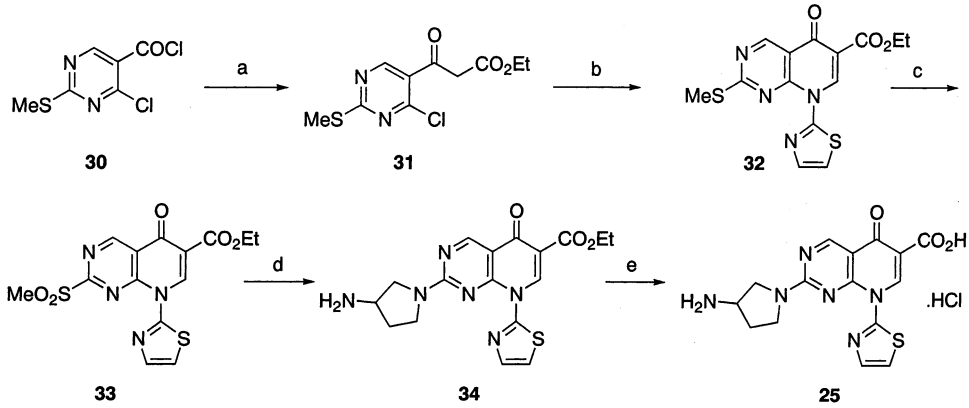
The amines used in this study were purchased or prepared following procedures in the literature except for **44**−**46**. The amine parts of **44**−**46**, which afforded the corresponding substituents of naphthyridonecarboxylic acids (*R*,*R*)-**43j** and (*S*,*S*)-**43j**, **43m**, **43p**, respectively, were prepared by the methods shown in Schemes 6−8. *trans*-3-Amino-1-benzyl-4-methoxypyrrolidine **47** was prepared from 3-pyrroline in several steps. With a procedure similar to that reported for *cis*-3-amino-1-Boc-4-methoxypyrrolidine,[14,20] the optical resolution of **47** was achieved using (+)- or (−)-tartaric acid as a resolving agent.[21] Each isomer of **47** was debenzylated to give (+)/(−)-**44**, whose absolute configurations were determined as (*R*,*R*)/(*S*,*S*), respectively, by comparing the derivative from (+)-**44** with that prepared by asymmetric synthesis using (+)-tartaric acid.[22] Hydantoin-spiropyrrolidine **48**[23] was hydrolyzed to the amino acid **49**, which was converted to amino alcohol **51** via ester **50**. Boc protection of the amino group of **51** followed by debenzylation gave 3,3-disubstituted pyrrolidine **45**. The synthesis of bicyclodiamine **46** was started from N-propargylation of acetal **53**.[24] After conversion of the acetal group into an aldehyde, compound **55** was subjected to an intramolecular 1,3-dipolar cycloaddition reaction to afford the desired ring system **56**. Acid-

Scheme 1[a]



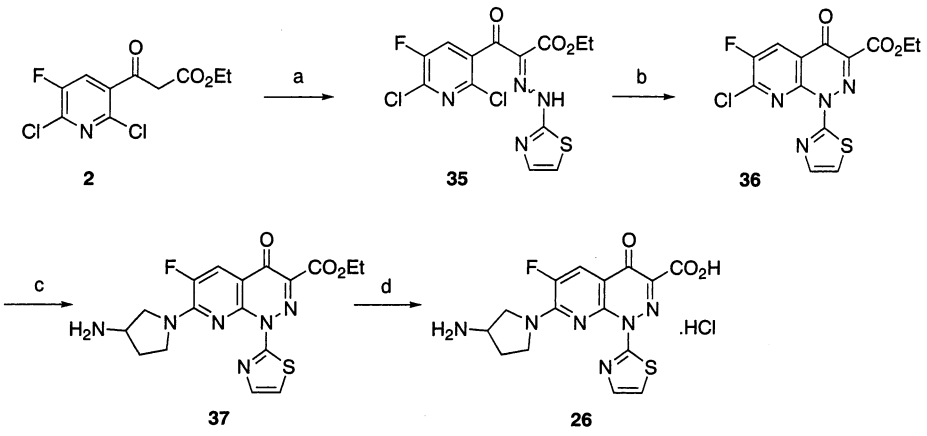
[a] (a) (1) $(EtO)_3CH$, Ac_2O, (2) R_1NH_2; (b) K_2CO_3 or *t*-BuOK; (c) R_2R_3NH; (d) HCl.

Scheme 2[a]



[a] (a) $EtO_2CCH_2CO_2H$, MeMgBr; (b) (1) $(EtO)_3CH$, Ac_2O, (2) 2-aminothiazole, (3) K_2CO_3; (c) MCPBA; (d) 3-aminopyrrolidine; (e) (1) 1 N NaOH, (2) HCl.

Scheme 3[a]



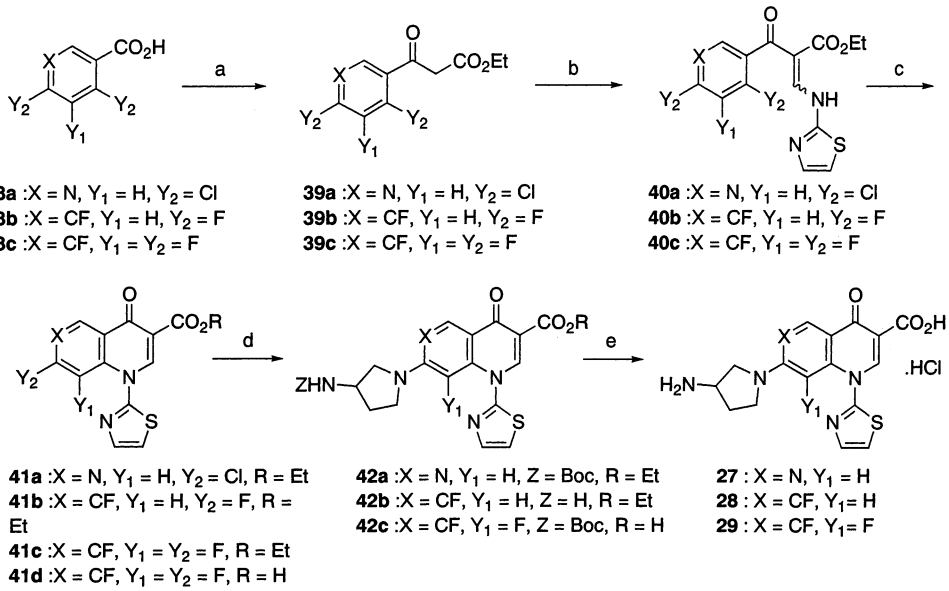
[a] (a) 2-Aminothiazole, $NaNO_2$; (b) K_2CO_3; (c) 3-aminopyrrolidine; (d) 10% HCl.

mediated removal of the ethoxycarbonyl group completed the synthesis of **46**.

Results and Discussion

In the course of our search for novel antitumor agents, we first screened a number of quinolones that have been prepared in the field of antibacterial agents at our laboratories. Then, 7-aminopyrrolidinyl derivatives of 1,8-naphthyridine having a 1-(4-fluorophenyl) or 1-cyclopropyl substituent (**7** or **9**) were determined as moderate cytotoxic compounds against murine P388 leukemia with IC_{50} values of 2.1 or 7.9 mg/mL, respectively. In a comparison of the N-1 substituent between an aliphatic group and an aromatic one, the aliphatic cyclopropyl group **9** was less potent than the aromatic group **7**. This indicates a significant difference from the 6,8-difluoroquinoline structure in which a cyclopropyl group is reported to be the most potent substituent for

Scheme 4[a]



[a] (a) (1) CDI, (2) $EtO_2CCH_2CO_2H$, MeMgBr; (b) (1) $(EtO)_3CH$, Ac_2O, (2) 2-aminothiazole; (c) K_2CO_3; (d) 3-(Boc-amino)pyrrolidine or 3-aminopyrrolidine; (e) 10% HCl.

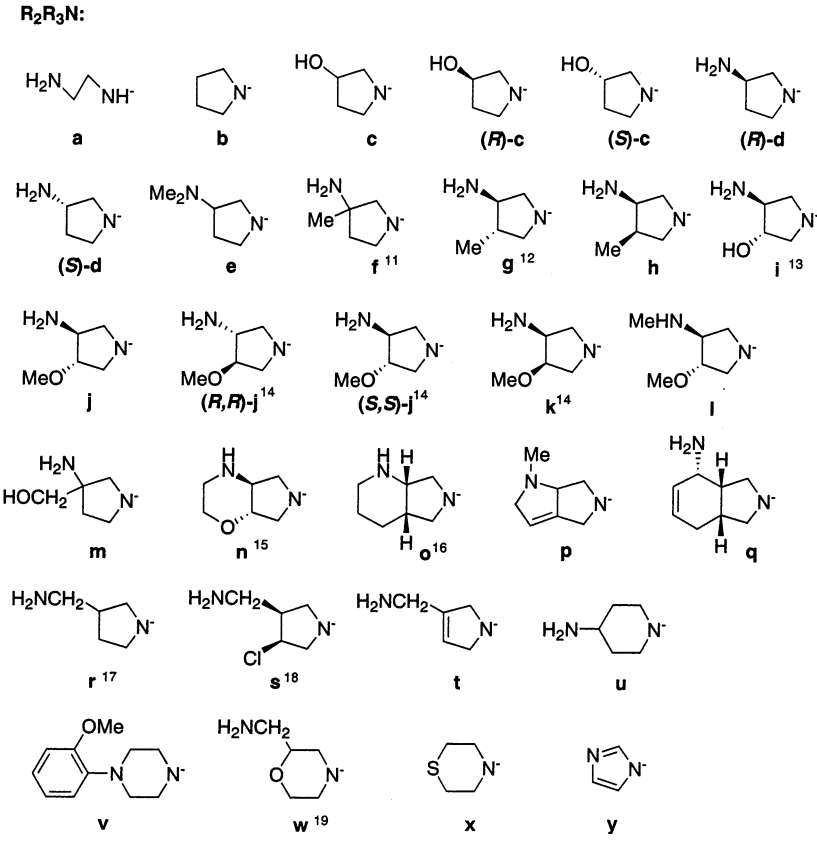
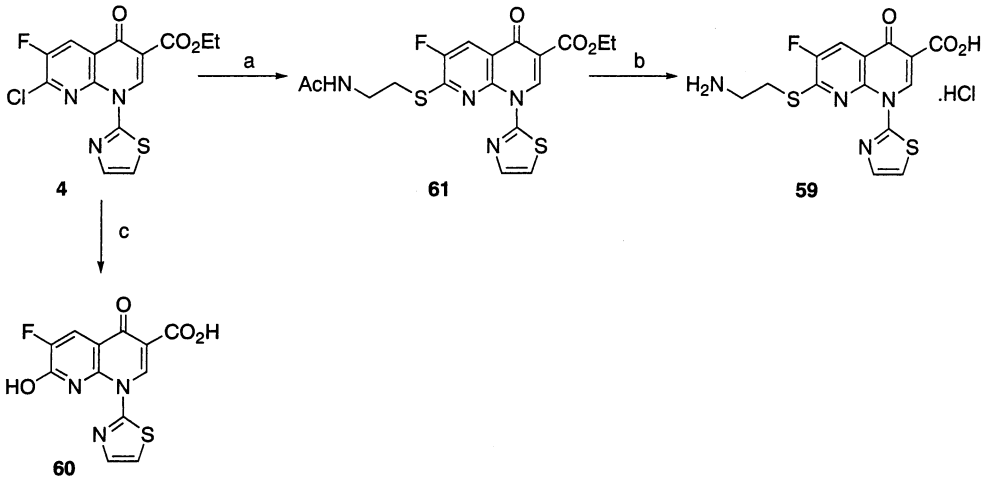


Figure 2. R_2R_3N groups used for this study.

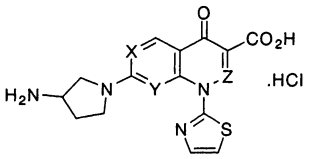
cytotoxic activity.[6,25] Next, we focused our attention on the derivatives having an N-1 aromatic group and evaluated the cytotoxity of their related compounds. Thereby, we found that some heteroaromatic derivatives were more effective than substituted or unsubstituted phenyl groups. Among them, the 2-thiazolyl ring showed considerable cytotoxic activity. The relationship between activity and the aromatic ring is shown in decreasing order: 2-thiazolyl **1** > 2-benzothiazolyl **24** > 3-methyl-5-isoxazolyl **12** > 2-thienyl **10** > phenyl **6** = 4-fluorophenyl **7** > 1,3,4-thiadiazol-2-yl **13** > 5-isoxazolyl **11**. 2-Pyridyl **8** as well as 5-pyrazolyl **14** had no activity in this assay. Regrettably, 2-thiazolylmethyl derivative **15**, arylaliphatic type, was inactive. Since 2-imidazolyl and 2-thiazolidinyl analogues, the closest to the 2-thiazolyl analogue, were not included in our libraries, we attempted to prepare them. The corresponding ester **4**, however, was not obtained in both cases because intramolecular cyclization of compound **3** occurred at the imidazole or thiazolidine ring nitrogen atom with the

Scheme 5[a]



[a] (a) (1) 2-Aminoethanethiol, (2) Ac_2O; (b) 20% HCl; (c) (1) 1 N NaOH, (2) AcOH.

Table 2. Cytotoxic Activity (IC_{50}) of AT-3639 Analogues against Murine P388 Leukemia



compd	X	Y	Z	IC_{50},[a] μg/mL
1	CF	N	CH	0.021
25	N	N	CH	0.047
26	CF	N	N	>10
27	N	CH	CH	>1
28	CF	CH	CH	>10
29	CF	CF	CH	55

[a] Concentration of agent to reduce cell viability by 50%.

carbonyl of the ethyl ester. In addition, the 2-oxazolyl analogue was not obtained because we could not prepare 2-aminooxazole as a starting material. Since we could not examine these analogues, we finally concluded that 2-thiazolyl derivatives were the most potent among our quinolones libraries and had potential to develop as an antitumor agent.

To investigate the substituent effect on the thiazole ring, we prepared analogues having a variety of substituents on the thiazole ring. With regard to the methyl group, 4-substituted **16** was more active than 5-substituted **17**, while middle activity was observed in the case of 4,5-disubstituted **18**. At position 4, a phenyl group maintained the activity of the methyl group but a *tert*-butyl group decreased the activity (compare **16**, **19**, and **20**). A halogen atom at position 5 (compounds **21** and **22**) retained almost the same activity, but a methoxy group at the same position weakened the activity more than 16 times (compound **23**). The effect of different substituents on the thiazole ring is summarized in the following decreasing order: unsubstituted **1** > 4-methyl **16** = 4-phenyl **20** > 5-bromo **22** = 5-chloro **21** > 4,5-dimethyl **18** > 5-methyl **17** > 5-methoxy **23** > 4-*tert*-butyl **19**. It is clear that unsubstituted 2-thiazolyl group is the most potent substituent as to the N-1 position.

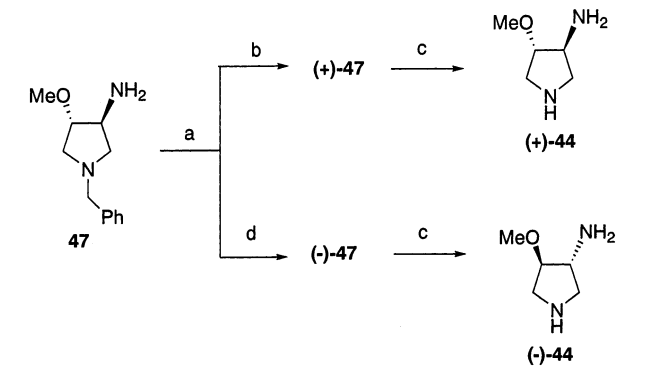
It was particularly interesting to change the core structure to other ring systems because only 6,8-difluoroquinolone and quinobenzoxazine have been reported to be active against tumor. Five ring systems having the 1-(2-thiazolyl) group and the 7-(3-aminopyrrolidinyl) group in common were thus synthesized. Table 2 shows evaluation results for these compounds (**25**–**29**). Among them, only pyridopyrimidine (**25**) showed a good activity with an IC_{50} value of 0.047 μg/mL. On the other hand, pyridopyridazine (**26**) and 1,6-naphthyridine (**27**) were far less active. Although the basic structures of **28** and **29** are similar to those of the

Table 3. Physical Data and Cytotoxic Activity (IC_{50}) of 1-(2-Thiazolyl)naphthyridine-3-carboxylic Acids **43** and **59** and of **60** against Murine P388 Leukemia

compd	mp, °C	% yield[a]	IC_{50},[b] μg/mL
1	286–288 dec	45	0.021
43a	270–275 dec	14	>10
43b	>300	88	0.08
43c	297–300 dec[c]	70	0.036
(*R*)-**43c**	291–293 dec[c]	62	0.065
(*S*)-**43c**	291–293 dec[c]	61	0.02
(*R*)-**43d**	297–300 dec	90	0.016
(*S*)-**43d**	>300	91	0.028
43e	284–286 dec	74	0.057
43f	284–286 dec	78	0.026
43g	283–287 dec	67	0.011
43h	255–260 dec	58	0.057
43i	263–269 dec	49	2.09
43j	258–264 dec	72	0.038
(*R*,*R*)-**43j**	255–261 dec	60	0.068
(*S*,*S*)-**43j**	254–260 dec	58	0.023
43k	259–264 dec	61	0.084
43l	260–265 dec	69	0.018
43m	255–260 dec	58	0.084
43n	259–265 dec	71	>1
43o	294–300 dec	70	0.304
43p	256–258	31	0.038
43q	266–270 dec	23	0.15
43r	210–211	44	0.101
43s	263–266 dec	26	0.156
43t	235–238 dec	33[d]	0.084
43u	268–273 dec	36	1.63
43v	203-210 dec	85	>1
43w	265–269 dec	87	1.73
43x	275–285 dec[c]	79	1.74
43y	292–296 dec[c]	76	>10
59	247–251 dec	20	>10
60	261–264 dec[c]	73	>10
etoposide			0.0085
doxorubicin			0.004
cisplatin			0.011

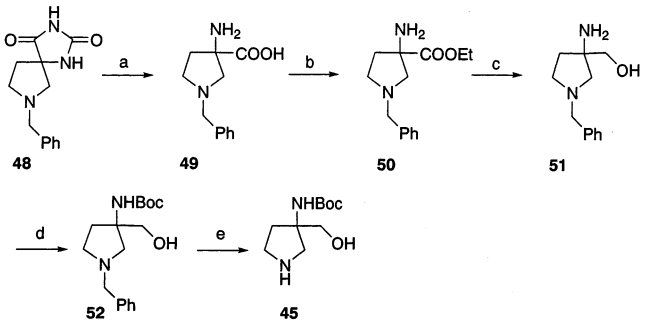
[a] Isolation yields based on **4**. [b] Concentration of agent to reduce cell viability by 50%. [c] Prepared as a free form. [d] Obtained by treatment of **43s** with 10% NaOH at 80 °C.

Scheme 6[a]



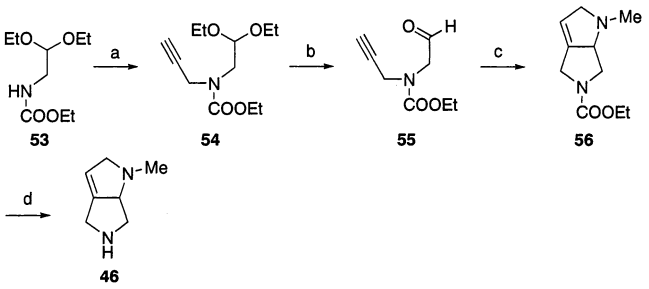
[a] (a) (+)-Tartaric acid; (b) K_2CO_3; (c) H_2/Pd−C; (d) (1) K_2CO_3, (2) (−)-tartaric acid, (3) K_2CO_3.

Scheme 7[a]



[a] (a) *c*-HCl, AcOH; (b) HCl, EtOH; (c) $NaAlH_2(OCH2CH2OMe)_2$; (d) Boc_2O; (e) H_2/Pd−C.

Scheme 8[a]



[a] (a) Propargyl bromide, KOH, TEBAC, toluene; (b) HCO_2H; (c) sarcosine, toluene; (d) *c*-HCl.

reported quinolines (Figure 1), these two compounds were almost inactive. This result implies that a nitrogen atom at position 8 plays an important role in good activity. It was also shown that a carbon atom at position 2 was essential for cytotoxic activity because replacement of this carbon with nitrogen eradicated the activity (compare **1** vs **26**). Since 1,8-naphthyridine (**1**) was twice as active as pyridopyrimidine (**25**), further study was carried out using 1,8-naphthyridine as a core structure.

To study SARs of substituents at the C-7 position, many kinds of amines and diamines along with 2-aminoethylthio and hydroxyl groups were introduced into position 7 of 6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acid. Evaluation of cytotoxic activity revealed a distinct tendency of these compounds (Table 3). A nitrogen atom attached to C-7 induced good activity. However, neither a sulfur (**59**) nor an oxygen (**60**) atom rendered the activity. Although acyclic diamine **43a** and imidazole **43y** have a C−N bond at position 7, they were exceptionally devoid of activity.

Most pyrrolidine derivatives with or without fused rings showed good activity, whereas six-membered cyclic amines (**43u**−**x**) had a much decreased activity compared to pyrrolidine derivatives. Pyrrolidine itself (**43b**) exhibited an activity 4 times less than 3-aminopyrrolidine (**1**), which showed half the potency of cisplatin. The hydroxyl group was another potency-enhancing group, since 3-hydroxypyrrolidine (**43c**) showed almost the same activity as **1** with an IC_{50} of 0.036 μg/mL. Coexistence of the amino and hydroxyl groups (e.g., **43i**), however, resulted in a deterioration of the activity.

Introduction of a methyl group at the geminal position (compound **43f**) of the 3-amino group in compound **1** had no substantial effect on the activity. The effect of a methyl group at the vicinal position, however, varied with its stereochemistry; the trans isomer **43g** was twice as active as **1**, but the cis isomer **43h** was about 3 times less active than **1**. In fact, **43g** possessed potency comparable to that of cisplatin with an IC_{50} value of 0.011 μg/mL. The same tendency was observed when both isomers of 3-amino-4-methoxypyrrolidine were compared. The trans isomer **43j** was more active than the cis isomer **43k**, and the potency of **43j** was comparable to that of **43c**. As mentioned before, **43i** has weak activity but its O-methylation caused a dramatic 55 times increase in activity to give **43j**. Monomethylation (giving **43l**) of the amino group in **43j** doubled the activity, while dimethylation (giving **43e**) of **1** resulted in a decrease of activity. Introduction of a hydroxyl group in the methyl group of **43f** furnished the *gem*-hydroxymethyl derivative **43m** whose activity was one-third that of **43f**.

An overall evaluation of the racemic compounds showed that *trans*-3-amino-4-methylpyrrolidine derivative **43g** was the most active, displaying an IC_{50} value similar to that of etoposide or cisplatin. 3-Aminopyrrolidine (**1**) and 3-hydroxypyrrolidine (**43c**) derivatives, 3-amino-3-methylpyrrolidine derivative **43f**, *trans*-3-amino-4-methoxypyrrolidine derivative **43j**, and its *N*-methyl analogue **43l** also exhibited good cytotoxic activity. Among these compounds, the potency decreased in the following order: **43g** > **43l** = **1** = **43f** > **43c** > **43j**.

The effect of chirality on the pyrrolidine ring was also investigated, and the following results were obtained (compare (*R*)-**43c**/(*S*)-**43c**, (*R*)-**43d**/(*S*)-**43d**, (*R*,*R*)-**43j**/(*S*,*S*)-**43j**). The activity of (***R***)-3-hydroxypyrrolidine (***R***)-**43c** was about 3-fold weaker than that of the *S* isomer (*S*)-**43c**. The (***R***)-3-aminopyrrolidine (*R*)-**43d** was twice as potent as the *S* isomer (*S*)-**43d**. The isomer (*S*,*S*)-**43j**, whose amino group has the same configuration as that of (*R*)-**43d**, was about 3-fold more active than the isomer (*R*,*R*)-**43j**. Since the difference in activity between each enantiomer was from 2- to 3-fold, the configuration of the substituent at position 3 of the pyrrolidine ring hardly influenced the activity.

3-Aminomethylpyrrolidines (**43r**, **43s**) and 3-aminomethylpyrroline (**43t**) retained activity to some extend but were less active than 3-aminopyrrolidine (**1**). Among the pyrrolidine-containing bicyclodiamines, only **43p** was as potent as 3-hydroxypyrrolidine (**43c**) and *trans*-

Table 4. In Vivo Antitumor Activity of Selected Compounds against Murine P388 Leukemia[a]

compd	dose, mg/kg	*T*/*C*,[b] %
1	3.13	150
	12.5	213
	50	>375
43c	3.13	138
	12.5	175
	50	225
43e	3.13	138
	12.5	175
	50	225
43f	3.13	138
	12.5	213
	50	>375
43g	3.13	138
	12.5	175
	50	238
43j	3.13	138
	12.5	238
	50	>375
43l	3.13	175
	12.5	275
	50	125
etoposide	3.13	175
	12.5	250
	50	>375
doxorubicin	3.13	235
	6.25	293
cisplatin	3.13	239
	6.25	290

[a] See Experimental Section. [b] (Median survival time of treated mice)/(median survival time of controls) × 100.

Table 5. Cytotoxic Activity of AT-3639 (**1**) and **43j** against Human Tumor Cell Lines

	IC_{50},[a] μg/mL					
compd	G-361 melanoma	AZ-521 stomach	HT-29 colon	A-427 lung	SK-OV-3 ovary	SCaBER bladder
1	0.39	0.35	1.2	0.22	0.93	0.34
43j	0.4	0.58	2.1	0.42	1.1	0.78
etoposide	0.28	0.080	1.3	0.095	0.95	0.31

[a] Concentration of agent to reduce cell viability by 50%.

3-amino-4-methoxypyrrolidine (**43j**), while others (**43n**, **43o**, and **43q)** were inactive or had moderate activity.

Some selected compounds were tested for in vivo antitumor activity in mice bearing P388 leukemia (Table 4). The in vivo test consisted of intraperitoneal (ip) implantation of tumor cells followed 1 and 5 days later by ip drug treatment with the response endpoint being the relative life span of treated mice (*T*) over that of untreated control mice (*C*) expressed as *T*/*C*% (see Experimental Section). All compounds tested were considered to be active even at a dose of 3.13 mg/kg, since they surpassed the 125 *T*/*C*% limit. Compound **43l** was the most potent at 3.13 mg/kg, but it showed some toxicity at the highest dose of 50 mg/kg. Compounds **1**, **43f**, and **43j** displayed good activity similar to that of etoposide.

AT-3639 (**1**) and **43j** were subjected to assay against various human tumor cells (Table 5). In general, both compounds displayed good cytotoxic activity in the assay; their activity was similar or slightly less than that of the reference drug etoposide.

When we measured the inhibitory activities of compound **1** against topoisomerase II mediated cleavage activities,[26] compound **1** did not inhibit topoisomerase II mediated DNA cleavage. That is, the IC_{50} value of **1** was >200 μg/mL, whereas that of etoposide was 12.5 μg/mL. The result implies that there is a mechanistic difference between **1** and etoposide. Compound **1** also seems to differ from other quinolones that are reported inhibitors of topoisomerase II mediated DNA cleavage activity. Further investigation should be done to clarify the naphthyridine derivatives.

In conclusion, novel types of antitumor agent, 6-fluoro-1,4-dihydro-4-oxo-7-(substituted pyrrolidinyl)-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acids, were determined. In the course of the SAR study of this series of compounds, the following findings were obtained. (1) The 2-thiazolyl group is optimal as a N-1 substituent. Introduction of any substituent on the thiazole ring generally reduces the activity. (2) As a core structure having a 1-(2-thiazolyl) group, 6-fluoro-1,8-naphthyridine-3-carboxylic acid is the most active followed by pyrido[2,3-*d*]pyrimidine-6-carboxylic acid. However, 6,8-difluoroquinoline-3-carboxylic acid is far less potent. (3) As a C-7 substituent, many kinds of pyrrolidine derivatives are tolerable. Among them, 3-aminopyrrolidine, which may have other groups on the ring, is best.

Compounds **1**, **43c**, **43e**, **43f**, **43g**, **43j**, and **43l**, which have the best combination of substituents, displayed good activity against murine tumor cells in vitro as well as in vivo tests in mouse P388 leukemia models. Of these compounds, the effect of **1**, **43f**, and **43j** was comparable to that of etoposide. Compounds **1** and **43j** also demonstrated good activity against several human tumor cell lines, indicating the possibility that these compounds can act as antitumor agents for human. Further development of this series of compounds will be reported in the next paper.

Experimental Section

Chemistry. All melting points were determined on a Yanagimoto micro-melting-point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrophotometer. ^{1}H NMR spectra were taken at 200 MHz on a Varian Gemini-200 spectrometer. Chemical shifts are expressed in ppm (δ) with tetramethylsilane as an internal standard. Mass spectra were obtained on a Hitachi M-1000 or Hitachi M-80B spectrometer. The spectral data for all compounds were consistent with the assigned structures. All compounds that were stable solids were analyzed for C, H, Cl, F, and N.

3-Aminopyrrolidine, 3-dimethylaminopyrrolidine, 1-(2-methoxyphenyl)piperazine, thiomorpholine, imidazole, and 2-aminothiazole derivatives except for 2-amino-5-methoxythiazole were purchased from commercial suppliers. 3-Hydroxypyrrolidine was prepared from the corresponding 1-benzyl derivative by hydrogenation on 5% Pd/C and used without further purification.

Ethyl 2-(2,6-Dichloro-3-fluoro-5-nicotinoyl)-3-(2-thiazolylamino)acrylate (3, R_1 = 2-Thiazolyl). A mixture composed of 2.8 g (10 mmol) of ethyl 2,6-dichloro-5-fluoronicotinoylacetate **2**, 2.2 g (14.9 mmol) of $CH(OEt)_3$, and 2.5 g (24.5 mmol) of Ac_2O was heated to reflux for 1 h at 140 °C, during which period the resulting AcOEt was distilled off under atmospheric pressure. After concentration under reduced pressure, the obtained residue was diluted with 5 mL of EtOH, then 1.2 g (12 mmol) of 2-aminothiazole was added. After the mixture was stirred at room temperature for 3 h, the resulting precipitates were collected by filtration, washed with *n*-hexane, and dried to give 3.5 g (90%) of **3** (R_1 = 2-thiazolyl).

Ethyl 7-Chloro-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (4, R_1 = 2-Thiazolyl). To a solution of 3.5 g (9.0 mmol) of **3** (R_1 = 2-thiazolyl) in 5 mL of dioxane was added 1.1 g (9.8 mmol) of *tert*-BuOK

under ice-cooling. The reaction mixture was heated at 50 °C for 2 h and diluted with ice−water. The resulting precipitates were collected by filtration, washed with water, and dried to give 2.0 g (63%) of **4** (R_1 = 2-thiazolyl).

Ethyl 7-(3-Amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (5, R_1 = 2-Thiazolyl, R_2R_3N = 3-Amino-1-pyrrolidinyl). To a suspension of 50 g (0.14 mol) of **4** (R_1 = 2-thiazolyl) in 1 L of CH_3CN was added 36.5 g (0.42 mol) of 3-aminopyrrolidine. The reaction mixture was stirred at room temperature for 1 h. After ice-cooling, the resulting precipitates were collected by filtration, washed with CH_3CN and a mixture of CH_3CN and EtOH, successively, and dried to give 55.4 g (98%) of **5** (R_1 = 2-thiazolyl, R_2R_3N = 3-amino-1-pyrrolidinyl), mp 218−220 °C (dec). IR (KBr) cm^{-1}: 1728, 1638. ^1H NMR (DMSO-d_6) δ: 1.31 (t, 3 H, J = 7.0 Hz), 1.40−2.40 (m, 2 H), 3.40−4.00 (m, 5 H), 4.80 (q, 2 H, J = 7.0 Hz), 7.68 (d, 1 H, J = 3.5 Hz), 7.76 (d, 1 H, J = 3.5 Hz), 7.83 (d, 1 H, J = 13 Hz), 9.54 (s, 1H).

7-(3-Amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (1). A suspension of 27.0 g (67.0 mmol) of **5** in 540 mL of 20% HCl was heated to reflux for 6 h. After ice-cooling, the resulting precipitates were collected by filtration, washed with 0.5 N HCl and EtOH successively, and dried to give 25.2 g (91%) of **1**, mp 286−288 °C (dec). MS (*m*/*z*): 376 (MH^+). IR (KBr) cm^{-1}: 1727, 1635. ^1H NMR (NaOD/D_2O) δ: 1.30−3.8 (m, 7 H), 6.99 (d, 1 H, J = 3.5 Hz), 7.23 (d, 1 H, J = 13 Hz), 7.30 (d, 1 H, J = 3.5 Hz), 8.90 (s, 1H).

7-(3-Amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-4-oxo-1-phenyl-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (6). Compound **6** was prepared from **2** and aniline. 3-Acetamidopyrrolidine and $NaHCO_3$ were used instead of 3-aminopyrrolidine in the coupling reaction at C-7, mp 292−295 °C (dec). IR (KBr) cm^{-1}: 1720, 1625.

7-(3-Amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-1-(3-methyl-5-isoxazolyl)-4-oxo-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (12). Compound **12** was prepared from **2** and 5-amino-3-methylisoxazole. Et_3N was added in the coupling reaction at C-7, mp 253−255 °C (dec). MS (*m*/*z*): 390 (MH^+). IR (KBr) cm^{-1}: 1636, 1458.

According to the similar procedure for **1**, **6**, and **12**, compounds **7**, **8**, **10**, **11**, **13**, **14**, and **16**−**24** were prepared.

7-(3-Amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (9). Compound **9** was prepared by a coupling reaction of 3-aminopyrrolidine and 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, which was derived from **4** (R_1 = cyclopropyl), mp 280−285 °C (dec).

7-(3-Amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)methyl-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (15). To a solution composed of 4.0 g (13.5 mmol) of ethyl 7-ethylthio-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate, 5.3 g (20.2 mmol) of PPh_3, 3.2 g (16.5 mmol) of diethyl azodicarboxylate (90%), and 80 mL of THF was added a THF (2 mL) solution of 1.7 g (14.9 mmol) of 2-hydroxymethylthiazole. The reaction mixture was stirred at room temperature for 1 h and concentrated under reduced pressure. The obtained residue was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$/MeOH (19:1) and recrystallized from AcOEt to give 5.2 g (98%) of ethyl 7-ethylthio-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)methyl-1,8-naphthyridine-3-carboxylate, mp 148−149 °C. MS (*m*/*z*): 394 (MH^+). IR (KBr) cm^{-1}: 1728, 1612. ^1H NMR ($CDCl_3$) δ: 1.35 (t, 3 H, J = 7 Hz), 1.40 (t, 3 H, J = 7 Hz), 3.23 (q, 2 H, J = 7 Hz), 4.40 (q, 2 H, J = 7 Hz), 5.85 (s, 2 H), 7.33 (d, 1 H, J = 3 Hz), 7.77 (d, 1 H, J = 3 Hz), 8.19 (d, 1 H, J = 9 Hz), 8.73 (s, 1 H).

To a solution of 5.2 g (13.2 mmol) of the above compound in 210 mL of CH_2Cl_2 was added 6.8 g (27.6 mmol) of 3-chloroperoxybenzoic acid (MCPBA, 70%). The reaction mixture was stirred at room temperature overnight, then diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$/MeOH (50:1) to give 3.3 g (59%) of ethyl 7-ethanesulfonyl-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)methyl-1,8-naphthyridine-3-carboxylate.

A mixture composed of 1.15 g (2.7 mmol) of the above compound and 0.70 g (8.1 mmol) of 3-aminopyrrolidine in 30 mL of CH_3CN was heated to reflux for 15 min. The reaction mixture was concentrated under reduced pressure, and the obtained residue was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was recrystallized from CH_3CN to give 1.1 g (96%) of ethyl 7-(3-amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)methyl-1,8-naphthyridine-3-carboxylate, mp 165−166 °C. MS (*m*/*z*): 418 (MH^+). IR (KBr) cm^{-1}: 1718, 1631. ^1H NMR ($CDCl_3$) δ: 1.39 (t, 3 H, J = 7 Hz), 1.70−1.90 (m, 1 H), 2.08−2.26 (m, 1 H), 3.45−3.58 (m, 1 H), 3.70−4.05 (m, 4 H), 4.39 (q, 2 H, J = 7 Hz), 5.73 (s, 2 H), 7.30 (d, 1 H, J = 3 Hz), 7.76 (d, 1 H, J = 3 Hz), 8.07 (d, 1 H, J = 13 Hz), 8.59 (s, 1 H).

A suspension of 1.1 g (2.6 mmol) of the above compound in 9 mL of 10% HCl and 1 mL of EtOH was heated to reflux for 1 h. The reaction mixture was concentrated under reduced pressure and triturated with EtOH. The resulting precipitates were collected by filtration, washed with EtOH, and dried to give 0.69 g (60%) of **15**, mp 268−269 °C. MS (*m*/*z*): 390 (MH^+). IR (KBr) cm^{-1}: 1716, 1630.

Ethyl 3-(4-Chloro-2-methylthiopyrimidin-5-yl)-3-oxopropionate (31). To a solution of 12.3 g (92.8 mmol) of ethyl hydrogen malonate in 80 mL of THF was added dropwise 64 mL (192 mmol) of 3 M MeMgBr in Et_2O under ice-cooling. After the mixture was stirred for 20 min, THF (100 mL) solution of 8.6 g (38.3 mmol) of **30**[10] was added dropwise. After being stirred at room temperature for 2 h, the reaction mixture was poured into ice−water and acidified to pH 5−6 with concentrated HCl and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$ to give 8.0 g (76%) of **31**. MS (*m*/*z*): 275 (MH^+). IR (neat) cm^{-1}: 1743.

Ethyl 5,8-Dihydro-2-methylthio-5-oxo-8-(2-thiazolyl)-pyrido[2,3-*d*]pyrimidine-6-carboxylate (32). A mixture composed of 7.95 g (30 mmol) of **31**, 6.8 g (45.9 mmol) of $CH(OEt)_3$, and 7.76 g (76.0 mmol) of Ac_2O was heated to reflux at 130 °C for 1 h, during which period the resulting AcOEt was distilled off under atmospheric pressure. After concentration under reduced pressure, the obtained residue was diluted with 50 mL of *i*-Pr_2O, and 3.28 g (32.8 mmol) of 2-aminothiazole was added. After the mixture was stirred at room temperature overnight, the resulting precipitates were collected by filtration and washed with *i*-Pr_2O to give 6.4 g (55%) of ethyl 2-[(4-chloro-2-methylthiopyrimidin-5-yl)carbonyl]-3-(2-thiazolylamino)acryrate. To a solution of 6.4 g (18.2 mmol) of the above compound in 70 mL of dioxane was added 2.72 g (19.7 mmol) of K_2CO_3 under ice-cooling. After being stirred for 5 h, the reaction mixture was diluted with 100 mL of ice−water and neutralized with 10% HCl. The resulting precipitates were collected by filtration, washed with water, dioxane, and *i*-Pr_2O successively, and dried to give 6.0 g (95%) of **32**, mp 183−184 °C. MS (*m*/*z*): 349 (MH^+). IR (KBr) cm^{-1}: 1736.

Ethyl 5,8-Dihydro-2-methanesulfonyl-5-oxo-8-(2-thiazolyl)pyrido[2,3-*d*]pyrimidine-6-carboxylate (33). To a solution of 5.99 g (17.2 mmol) of **32** in 450 mL of CH_2Cl_2 was added 9.30 g (43.1 mmol) of 80% MCPBA under ice-cooling. After being stirred for 15 h at room temperature, the reaction mixture was washed with aqueous $Na_2S_2O_3$ and aqueous $NaHCO_3$ successively. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was recrystallized from a mixture of AcOEt and *i*-Pr_2O to give 4.48 g (69%) of **33**, mp 185−187 °C. MS (*m*/*z*): 381 (MH^+). IR (KBr) cm^{-1}: 1741.

Ethyl 2-(3-Amino-1-pyrrolidinyl)-5,8-dihydro-5-oxo-8-(2-thiazolyl)pyrido[2,3-*d*]pyrimidine-6-carboxylate (34).

To a suspension of 2.02 g (5.32 mmol) of **33** in 20 mL of CH_3CN was added dropwise 1.15 g (13.4 mmol) of 3-aminopyrrolidine. The resulting mixture was stirred at room temperature for 30 min. After concentration under reduced pressure, the obtained residue was diluted with $CHCl_3$ and washed with aqueous $NaHCO_3$.. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$/MeOH (50:1) to give 1.34 g (65%) of **34**, mp 228−230 °C. MS (*m*/*z*): 387 (MH^+). IR (KBr) cm^{-1}: 1732.

2-(3-Amino-1-pyrrolidinyl)-5,8-dihydro-5-oxo-8-(2-thiazolyl)pyrido[2,3-*d*]pyrimidine-6-carboxylic Acid Hydrochrolide (25). A solution of 0.44 g (1.14 mmol) of **34** and 2 mL (2 mmol) of 1 N NaOH and 0.2 mL of EtOH was heated at 50 °C for 10 min, and then 2 mL of 20% HCl was added under ice-cooling. The resulting precipitates were collected by filtration and washed with EtOH, then dried to give 0.24 g (59%) of **25**, mp 288−291 °C. MS (*m*/*z*): 359 (MH^+). IR (KBr) cm^{-1}: 1775, 1727, 1619. ^1H NMR (DMSO-d_6) δ: 2.13−2.50 (m, 2 H), 3.75−4.18 (m, 5 H), 7.87 (dd, 1 H, J = 2.5, 3.5 Hz), 7.91 (s, 1 H), 8.2−8.6 (br, 3 H), 9.32 (d, 1 H, J = 2.5 Hz), 9.81 (d, 1 H, J = 3.5 Hz), 13.7−14.5 (br, 1 H).

Ethyl 3-Aza-2-(2,6-dichloro-3-fluoro-5-nicotinoyl)-3-(2-thiazolylamino)acrylate (35). To a solution of 2.7 g (27 mmol) of 2-aminothiazole in 14 mL of 20% HCl was added an aqueous solution (7 mL) of 2.0 g (29 mmol) of $NaNO_2$ under ice-cooling. After being stirred for 10 min, the reaction mixture was added dropwise over 15 min to a mixture composed of 5.0 g (17.9 mmol) of **2**, 7.2 g (87.8 mmol) of AcONa, and 108 mL of EtOH/$CHCl_3$/water (1:1:1). This reaction mixture was stirred for 2 h at room temperature, then extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$/MeOH (50:1) to give 1.4 g (20%) of **35**.

Ethyl 2-Aza-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (36). To a solution of 1.4 g (3.6 mmol) of **35** in 30 mL of CH_3CN was added 0.6 g (4.35 mmol) of K_2CO_3 under ice-cooling. The resulting mixture was heated to reflux for 1 h. After concentration under reduced pressure, the obtained residue was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$/MeOH (50:1) and recrystallized from AcOEt to give 0.59 g (46%) of **36**, mp 115−116 °C.

Ethyl 7-(3-Amino-1-pyrrolidinyl)-2-aza-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (37). To a solution of 0.6 g (1.69 mmol) of **36** and 0.34 g (3.37 mmol) of Et_3N in 10 mL of CH_3CN was added 0.15 g (1.74 mmol) of 3-aminopyrrolidine. The resulting mixture was stirred at room temperature for 10 min. The resulting precipitates were collected by filtration, washed with CH_3CN, and dried to give 0.59 g (86%) of **37**, mp 251−252 °C (dec). MS (*m*/*z*): 405 (MH^+). IR (KBr) cm^{-1}: 1728, 1626, 1459. ^1H NMR (DMSO-d_6) δ: 1.32 (t, 3 H, J = 7 Hz), 2.0−2.3 (m, 2 H), 3.2−3.4 (m, 2 H), 3.7−4.1 (m, 3 H), 4.36 (q, 2 H, J = 7 Hz), 7.6−7.8 (br, 2 H), 7.84 (d, 1 H, J = 3.5 Hz), 7.89 (d, 1 H, J = 3.5 Hz), 7.98 (d, 1 H, J = 13 Hz).

7-(3-Amino-1-pyrrolidinyl)-2-aza-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (26). A mixture of 0.52 g (1.29 mmol) of **37** and 10% HCl was heated at 70 °C for 1 h. After ice-cooling, the resulting precipitates were collected by filtration, washed with 0.5 N HCl and EtOH, and dried to give 0.45 g (84%) of **26**, mp 274−275 °C (dec). IR (KBr) cm^{-1}: 1728, 1631, 1460. ^1H NMR (NaOD/D_2O) δ: 1.5−1.8 (m, 1 H), 2.0−2.2 (m, 1 H), 2.7−3.9 (m, 5 H), 7.14 (d, 1 H, J = 3.5 Hz), 7.35 (d, 1 H, J = 13 Hz), 7.45 (d, 1 H, J = 3.5 Hz).

Ethyl 2,4-Dichloro-5-nicotinoylacetate (39a). To a solution of 15 g (78.1 mmol) of **38a** in 100 mL of THF was added 15.2 g (93.8 mmol) of 1,1′-carbonylbis-1*H*-imidazole (CDI). The resulting mixture was heated at 60 °C for 2 h. This crude imidazolide solution was used without purification in the next step. To a solution of 10.3 g (78.0 mmol) of ethyl hydrogen malonate in 75 mL of THF was added dropwise 53.4 mL (160 mmol) of 3 M MeMgBr in Et_2O under ice-cooling. After being stirred for 20 min, the imidazolide prepared in the above was added. The reaction mixture was stirred at 60 °C for 1.5 h, poured into ice−water, and acidifed to pH 5−6 with concentrated HCl, then extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$ to give 18.2 g (89%) of **39a**.

Ethyl 2-(4,6-Dichloro-3-nicotinoyl)-3-(2-thiazolylamino)acrylate (40a). A mixture composed of 18.2 g (69.5 mmol) of **39a**, 17.3 g (104 mmol) of $CH(OEt)_3$, and 16.4 g (164 mmol) of Ac_2O was heated to reflux for 1.5 h at 130 °C, during which period the resulting AcOEt was distilled off under atmospheric pressure. After concentration under reduced pressure, the obtained residue was diluted with *i*-Pr_2O and 6.9 g (69 mmol) of 2-aminothiazole was added. After the mixture was stirred at room temperature, the resulting precipitates were collected by filtration and washed with *i*-Pr_2O to give 19.0 g (74%) of **40a**.

Ethyl 7-Chloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,6-naphthyridine-3-carboxylate (41a). To a solution of 19.0 g (51.1 mmol) of **40a** in 50 mL of AcOEt was added 8.5 g (61.6 mmol) of K_2CO_3 under ice-cooling. After being stirred at 60 °C for 1 h, the reaction mixture was diluted with ice−water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$/MeOH (50:1) and recrystallized from AcOEt to give 8.8 g (51%) of **41a**.

Ethyl 7-(3-*N*-*tert*-Butoxycarbonylamino-1-pyrrolidinyl)-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,6-naphthyridine-3-carboxylate (42a). To a solution of 0.5 g (1.49 mmol) of **41a** and 0.38 g (3.76 mmol) of Et_3N in 30 mL of CH_3CN was added 0.3 g (1.61 mmol) of 3-(*N*-*tert*-butoxycarbonylamino)pyrrolidine. The resulting mixture was heated to reflux for 4 h. After concentration, the resulting residue was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$/MeOH (19:1) and recrystallized from AcOEt to give 0.69 g (95%) of **42a**, mp 182−183 °C. MS (*m*/*z*): 486 (MH^+). IR (KBr) cm^{-1}: 1730, 1704, 1621. ^1H NMR (DMSO-d_6) δ: 1.39 (t, 3 H, J = 7 Hz), 1.44 (s, 9 H), 1.9−2.1 (m, 1 H), 2.2−2.4 (m, 1 H), 3.3−3.4 (m, 1 H), 3.5−3.8 (m, 3 H), 4.3−4.4 (m, 1 H), 4.38 (q, 2 H, J = 7 Hz), 4.6−4.8 (br, 1 H), 6.03 (s, 1 H), 7.57 (d, 1 H, J = 3.5 Hz), 7.87 (d, 1 H, J = 3.5 Hz), 8.49 (s, 1 H), 9.22 (s, 1 H).

7-(3-Amino-1-pyrrolidinyl)-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,6-naphthyridine-3-carboxylic Acid Hydrochloride (27). A mixture of 0.68 g (1.4 mmol) of **42a** and 7 mL of 10% HCl was heated at 70 °C for 4 h. After concentration under reduced pressure, EtOH was added to the residue. The resulting precipitates were collected by filtration, washed with EtOH, and dried to give 0.45 g (78%) of **27**, mp 280−282 °C (dec). MS (*m*/*z*): 358 (MH^+). IR (KBr) cm^{-1}: 1728, 1626, 1458. ^1H NMR (DMSO-d_6) δ: 2.0−2.2 (m, 1 H), 2.2−2.4 (m, 1 H), 3.4−3.8 (m, 4 H), 3.8−4.0 (m, 1 H), 5.97 (s, 1 H), 8.60 (d, 1 H, J = 3.5 Hz), 8.12 (d, 1 H, J = 3.5 Hz), 8.2−8.4 (br, 1 H), 8.83 (s, 1 H), 9.15 (s, 1 H).

Ethyl 2,4,5-Trifluorobenzoyl-3-(2-thiazolylamino)acrylate (40b). Following the procedure for **40a**, **40b** was prepared from **39b** in 93% yield.

Ethyl 6,7-Difluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-quinoline-3-carboxylate (41b). To a solution of 10.3 g (29.0 mmol) of **40b** in 50 mL of dioxane was added 4.4 g (32.0 mmol) of K_2CO_3 under ice-cooling. After being stirred at 50 °C for 2 h, the reaction mixture was diluted with ice−water. The resulting precipitates were collected by filtration, washed with *i*-Pr_2O, and recrystallized from $CHCl_3$/*i*-Pr_2O to give 2.2 g (22%) of **41b**, mp 187−189 °C. MS (*m*/*z*): 337 (MH^+). IR (KBr) cm^{-1}: 1700, 1647.

Ethyl 7-(3-Amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)quinoline-3-carboxylate (42b). To a solution of 1.1 g (3.2 mmol) of **41b** and 0.82 g (8.1 mmol) of

Et_3N in 50 mL of CH_3CN was added 0.31 g (3.6 mmol) of 3-aminopyrrolidine. The resulting mixture was heated at 50 °C for 1.5 h. After concentration under reduced pressure, the resulting precipitates were recrystallized from a mixture of $CHCl_3$/MeOH/AcOEt to give 0.90 g (70%) of **42b**, mp 203−207 °C. MS (*m*/*z*): 403 (MH^+). IR (KBr) cm^{-1}: 1734, 1632.

7-(3-Amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)quinoline-3-carboxylic Acid Hydrochloride (28). A mixture of 0.49 g (1.2 mmol) of **42b** and 12 mL of 20% HCl was heated at 110 °C for 3 h. The resulting precipitates were collected by filtration, washed with EtOH and *i*-Pr_2O successively, and dried to give 0.31 g (63%) of **28**, mp 260 °C (dec). MS (*m*/*z*): 375 (MH^+). IR (KBr) cm^{-1}: 1754, 1627.

Ethyl 6,7,8-Trifluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-quinoline-3-carboxylate (41c). Following the procedure for **32**, **41c** was prepared from **39c** in 75% yield, mp 166−167 °C.

6,7,8-Trifluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)quinoline-3-carboxylic Acid (41d). A solution of 6.1 g (17.2 mmol) of **41c** in 60 mL of H_2O/H_2SO_4/AcOH (6:1:8) was heated to reflux for 1.5 h. The resulting mixture was diluted with ice−water. The resulting precipitates were collected by filtration and washed with water to give 5.2 g (93%) of **41d**, mp 204−205 °C.

7-(3-*N*-*tert*-Butoxycarbonylamino-1-pyrrolidinyl)-6,8-difluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)quinoline-3-carboxylic Acid (42c). To a mixture of 0.40 g (1.23 mmol) of **41d** and 0.35 g (3.47 mmol) of Et_3N in 20 mL of CH_3CN was added 0.24 g (1.29 mmol) of 3-(*N*-*tert*-butoxycarbonylamino)pyrrolidine. The mixture was heated to reflux for 1.5 h. After concentration under reduced pressure, the resulting precipitates were diluted with water and extracted with $CHCl_3$. The organic layer was dried and concentrated to dryness to afford a crude product, which was recrystallized from AcOEt to give 0.40 g (66%) of **42c**, mp 224−225 °C. IR (KBr) cm^{-1}: 1716, 1468. ^1H NMR ($CDCl_3$) δ: 1.43 (s, 9 H), 1.80−2.00 (m, 1 H), 2.00−2.20 (m, 1 H), 3.40−3.60 (m, 1 H), 3.60−4.00 (m, 3 H), 4.20−4.30 (br, 1 H), 4.70−4.80 (br, 1 H), 7.51 (d, 1 H, J = 3.5 Hz), 7.77 (d, 1 H, J = 3.5 Hz), 7.85 (dd, 1 H, J = 1.5, 13 Hz), 8.51 (s, 1 H), 14.2 (s, 1 H).

7-(3-Amino-1-pyrrolidinyl)-6,8-difluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)quinoline-3-carboxylic Acid Hydrochloride (29). A mixture of 0.35 g (0.71 mmol) of **42c** in 12 mL of 10% HCl and 2 mL of EtOH was heated to reflux for 1 h. Most of the solvent was removed in vacuo, and EtOH was added. The resulting precipitates were collected by filtration, washed with 0.5 N HCl and EtOH, successively, and dried to give 0.17 g (56%) of **29**, mp 205−207 °C. IR (KBr) cm^{-1}: 1724, 1627.

6-Fluoro-1,4-dihydro-7-(3-hydroxy-1-pyrrolidinyl)-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (43c). To a solution of 3.92 g (45 mmol) of 3-pyrrolidinol in 60 mL of CH_3CN was added 5.30 g (15 mmol) of **4** (R_1 = 2-thiazolyl). The resulting mixture was stirred at room temperature for 1.5 h. After ice-cooling, the resulting precipitates were collected by filtration, washed with *i*-Pr_2O, and then dried to give 7.60 g (quantitative) of ethyl 6-fluoro-1,4-dihydro-7-(3-hydroxy-1-pyrrolidinyl)-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate, mp 242−245 °C. A mixture of 2.0 g (5.0 mmol) of the above compound and 25 mL of 20% HCl was heated to reflux for 3.5 h. After cooling, the resulting precipitates were collected by filtration, washed with water, EtOH, and *i*-Pr_2O successively, and then dried to give 1.31 g (70%) of **43c**, mp 297−300 °C (dec). MS (*m*/*z*): 377 (MH^+). IR (KBr) cm^{-1}: 1715, 1634.

According to the similar procedure for **43c**, compounds **43a**−**s**,**u**−**y** were prepared.

7-(2-Aminoethylthio)-6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (59). To a solution of 1.85 g (24.0 mmol) of 2-aminoethanethiol in 200 mL of CH_3CN was added 2.83 g (8.0 mmol) of **4** (R_1 = 2-thiazolyl). The reaction mixture was stirred at room temperature for 4 h, and the resulting precipitates were collected by filtration and washed with EtOH. This product was dissolved with $CHCl_3$, and then 0.75 mL (8.0 mmol) of Ac_2O and 1.1 mL (8.0 mmol) of Et_3N were added. The reaction mixture was stirred at room temperature overnight, and insoluble materials were removed by filtration. The resulting precipitates in the filtrate were collected by filtration to give 0.90 g (2.1 mmol) of **61**, mp 232−235 °C. MS (*m*/*z*): 437 (MH^+).

A solution of 0.85 g (2.02 mmol) of **61** in 20% HCl was heated at 130 °C for 9 h. After cooling, the resulting precipitates were collected by filtration and washed with EtOH and *i*-Pr_2O successively to give 0.61 g (1.52 mmol) of **59**, mp 247−251 °C (dec). MS (*m*/*z*): 367 (MH^+). IR (KBr) cm^{-1}: 1740.

6-Fluoro-1,4-dihydro-7-hydroxy-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid (60). A mixture of 1.00 g (2.83 mmol) of **4** (R_1 = 2-thiazolyl) and 10 mL (10 mmol) of 1 N NaOH was heated at 85 °C for 6.5 h. After cooling, the resulting precipitates were collected by filtration. The product was purified from 5% NaOH/AcOH to give 0.69 g (2.07 mmol) of **60**, mp 261−264 °C (dec). IR (KBr) cm^{-1}: 1684.

(+)-(3*S*,4*S*)-3-Amino-1-benzyl-4-methoxypyrroridine (+)-44 and (−)-(3*R*,4*R*)-3-Amino-1-benzyl-4-methoxypyrroridine (−)-44. An amount of 22.4 g (109 mmol) of *trans*-3-amino-1-benzyl-4-methoxypyrroridine **47** and an amount of 19.6 g (131 mmol) of L-(+)-tartaric acid were dissolved in 350 mL of MeOH, and the solution stood at room temperature for 7 h. The resulting precipitates were collected by filtration and recrystallized from MeOH/water to give 14.1 g (74%) of (+)-(3*S*,4*S*)-3-amino-1-benzyl-4-methoxypyrroridine l-(+)-tartarate salt (+)-**47**, mp 206−208 °C (dec). $[\alpha]^{29}_D$ +33.0° (*c* 1.00, H_2O). Anal. Calcd. for $C_{12}H_{18}N_2O\cdot{}^3/_2C_4H_6O_6$: C, 50.11; H, 6.31; N, 6.49. Found: C. 49.85; H, 6.26; N, 6.27.

All the filtrates were combined, and the solvent was distilled off under reduced pressure. The residue was treated with saturated aqueous NaCl, and the mixture was made alkaline with K_2CO_3 and then extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over Na_2SO_4, and then concentrated to dryness. The resultant residue and 6.73 g (45 mmol) of d-(−)-tartaric acid were dissolved in 180 mL of MeOH, and this solution stood at room temperature for 7 h. The resulting precipitates were collected by filtration and recrystallized from MeOH/water to give 14.1 g (74%) of (−)-(3*R*,4*R*)-3-amino-1-benzyl-4-methoxypyrroridine d-(−)-tartarate salt (−)-**47**, mp 207−209 °C (dec). $[\alpha]^{29}_D$ −33.4° (*c* 1.02, H_2O). Anal. Calcd for $C_{12}H_{18}N_2O\cdot{}^3/_2C_4H_6O_6$: C, 50.11; H, 6.31; N, 6.49. Found: C, 50.35; H, 6.32; N, 6.47.

A mixture of 3.65 g (9.53 mmol) of (+)-**47** in saturated aqueous NaCl was neutralized with K_2CO_3 and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated to dryness to give 1.23 g (63%) of (+)-**44**, $[\alpha]^{27}_D$ +32.2° (*c* 1.05, MeOH). According to the same procedure described above, 2.57 g (13.5 mmol) of (−)-**47** gave 1.01 g (36%) of (+)-**44**, $[\alpha]^{27}_D$ −32.7° (*c* 1.02, MeOH).

3-Amino-1-benzyl-3-carboxypyrrolidine (49). A mixture composed of 195 g (1.00 mol) of **48**,[23] 1.0 L of concentrated HCl, 200 mL of AcOH, and 60 mL of 47% HBr was heated to reflux for 4 days and concentrated under reduced pressure. The resultant residue was diluted with 300 mL of water, and insoluble materials were removed by filtration. To the filtrate was added 300 mL of water and 200 mL of concentrated NH_4-OH. The resulting precipitates were collected by filtration, washed with water, and dried to give 161 g (73%) of **49**.

3-Amino-1-benzyl-3-ethoxycarbonylpyrrolidine (50). To a solution of 62 g (0.28 mol) of **49** in 165 mL of EtOH was added 130 mL of 35% HCl in EtOH. The reaction mixture was heated to reflux for 2 h and then concentrated under reduced pressure. The resultant residue was treated with water and made alkaline with concentrated NH_4OH. The resulting solution was extracted with $CHCl_3$, dried over K_2CO_3, and then concentrated to dryness to give 65.3 g (93%) of **50**.

3-Amino-1-benzyl-3-hydroxymethylpyrrolidine (51). To a mixture of 360 mL (1.26 mol) of sodium bis(2-methoxyethoxy)aluminum hydride (70% in toluene) and 800 mL of toluene was added dropwise 101 g (0.41 mol) of **50** in 300 mL

of toluene under water-cooling. The reaction mixture was heated to reflux for 3 h and then treated with 20 mL of EtOH and 20 mL of water under ice-cooling. Aqueous NaOH and NaCl were added. The resulting solution was extracted with $CHCl_3$, dried over K_2CO_3, and concentrated to dryness to give 81.7 g (98%) of **51**.

1-Benzyl-3-*N*-*tert*-butoxycarbonylamino-3-hydroxymethylpyrrolidine (52). To a solution of 1.70 g (8.25 mmol) of **51** in CH_2Cl_2 was added 1.73 g (7.93 mmol) of Boc_2O under ice-cooling. The reaction mixture was stirred at 0 °C for 2 h and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$ to give 2.00 g (82%) of **52**. ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9 H), 1.82−2.17 (m, 2 H), 2.55−2.85 (m, 4 H), 3.61 (d, 2 H, J = 3 Hz), 3.67 (d, 1 H, J = 10 Hz), 3.77 (d, 1 H, J = 10 Hz), 4.80−5.00 (br, 1 H), 7.20−7.35 (m, 5 H).

3-*N*-*tert*-Butoxycarbonylamino-3-hydroxymethylpyrrolidine (45). To a solution of 2.0 g (6.5 mmol) of **52** in 20 mL of EtOH was added 0.30 g of 5% Pd/C under ice-cooling. The resulting mixture was heated at 50 °C for 3.5 h in an atmosphere of hydrogen gas. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the crude product of **45**.

Ethoxy *N*-(2,2-diethoxyethyl)-*N*-prop-2-ynylcarboxamide (54). To a mixture composed of 41 g (0.20 mol) of **53**,[24] 44 g (0.78 mol) of KOH, and 0.7 g (3 mmol) of benzyltriethylammonium chloride in 200 mL of toluene was added dropwise 25 g (0.21 mol) of 3-bromopropyne. The resulting mixture was stirred at room temperature overnight. Insoluble materials were removed by filtration, and the filtrate was washed with saturated brine, dried over K_2CO_3, and then concentrated under reduced pressure to give 45 g (95%) of **54**, bp 93−102 °C/2 mmHg. MS (*m*/*z*): 244 (MH^+). IR (neat) cm^{-1}: 1707, 3255.

Ethoxy *N*-(2-Oxoethyl)-*N*-prop-2-ynylcarboxamide (55). A solution of 45 g (0.19mol) of **54** in 100 mL (1.9 mol) of 88% formic acid was heated at 100 °C for 1 h, poured into ice, and extracted with CH_2Cl_2. The organic layer was washed with aqueous $NaHCO_3$, dried over Na_2SO_4, and then concentrated under reduced pressure to give 21.6 g (68%) of **55**, bp 80−85 °C/2 mmHg. MS (*m*/*z*): 170 (MH^+). IR (neat) cm^{-1}: 1703, 3280.

Ethyl 6-Methyl-1,2,3,5,6,6a-hexahydro-2,6-diazapentalene-2-carboxylate (56). A solution of 46.6 g (0.28 mol) of **55** and 24.5 g (0.28 mol) of *N*-methylglycine in 1.5 L of toluene was heated to reflux for 2 days and concentrated under reduced pressure to give 15.6 g (29%) of **56**, bp 95−100 °C/2 mmHg. MS (*m*/*z*): 197 (MH^+). IR (neat) cm^{-1}: 1703.

1,2,4,5,6,6a-Hexahydro-1-methyl-1,5-diazapentalene (46). A solution of 13.5 g (68 mmol) of **56** in 80 mL of concentrated HCl was heated to reflux for 2 days. After concentration, the resulting residue was diluted with water and made alkaline with aqueous NaOH and extracted with $CHCl_3$. The organic layer was dried over K_2CO_3 and concentrated under reduced pressure to give 4.5 g (53%) of **46**, bp 37−43 °C/1.5 mmHg. MS (*m*/*z*): 125 (MH^+). IR (neat) cm^{-1}: 1666, 3306. ^{1}H NMR ($CDCl_3$) δ: 2.47 (s, 3 H), 2.55 (dd, 1 H, J = 8, 10 Hz), 3.14 (dd, 1 H, J = 6, 10 Hz), 3.39−3.48 (m, 2 H), 3.50−3.76 (m, 2 H), 3.92−4.10 (m, 1 H), 5.44 (br, 1 H).

Conventional Antitumor Drugs. Etoposide and cisplatin were purchased from Nippon Kayaku Co., Ltd. (Tokyo, Japan), and doxorubicin was obtained from Kyowa Hakko Co., Ltd. (Tokyo, Japan).

Cell Culture. The following cell lines were used: murine P388 leukemic cells, human G-361 melanoma, human AZ-521 stomach carcinoma, human HT-29 colon adenocarcinoma, human A-427 lung carcinoma, human SK-OV-3 ovary adenocarcinoma, and human SCaBER bladder squamous carcinoma.

P388 cells were cultured in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, and 50 units/mL penicillin, and 50 μg/mL streptomycin. G-361 and HT-29 cells were cultured in McCoy's 5A medium supplemented with 10% FBS. AZ-521 cells were grown in EMEM supplemented with 10% FBS, 2 mM glutamine, and nonessential amino acids (NEAA). A-427 and SCaBER cells were maintained in EMEM supplemented with 10% FBS, 2 mM glutamine, NEAA, and 1 mM sodium pyruvate. SK-OV-3 were maintained in McCoy's 5A medium supplemented with 15% FBS.

In Vitro Assay. Cells ($(1-2) \times 10^4$ cells/mL) were put into wells of a 96-well microtiter plate in the amount of 0.1 mL/well, preincubated for 24 h except for P388 cells, and incubated with various concentrations of a test compound in the 5% CO_2 incubator at 37 °C for 72 h. After the culturing, 0.02 mL of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was put into each well, and the cells were cultured for further 4 h. The medium was removed by suction, and 0.2 mL of DMSO was put into each well to dissolve the formed formazan. The absorbance was measured by Multiskan Bichromatic (Labsystems, main wavelength 570 nm, subwavelength 690 nm). The IC_{50} was defined as the drug concentration needed to produce a 50% reduction of absorbance relative to the control.

In Vivo Assay. Into BDF_1 mice, 0.1 mL of diluted ascites fluid containing 10^6 P388 leukemic cells was transplanted intraperitoneally. Test compounds were suspended in 0.4% CMC (carboxymethyl cellulose), and conventional antitumor drugs were dissolved and diluted with distilled water and administered ip on days 1 and 5 after tumor implantation. Seven mice were used for each experimental group. Antitumor activities were evaluated by determining the *T*/*C* (%), which is

$$\frac{\text{median survival time of treated group}}{\text{median survival time of control group}} \times 100$$

Acknowledgment. We are grateful to Drs. Y. Sekine and T. Karasawa for their encouragement throughout this work. Thanks are also due to members of the Department of Physico-Chemical Analysis for elemental analyses and spectral measurements.

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JM010057B