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 2

Yasunori Tsuzuki,* Kyoji Tomita, Koh-ichiro Shibamori, Yuji Sato, Shigeki Kashimoto, and Katsumi Chiba

Chemistry Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33-94, Suita, Osaka 564-0053, Japan

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We have previously reported that a series of 7-substituted 6-fluoro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acids possess moderate cytotoxic activity. In a further attempt to find clinically useful antitumor agents, we investigated the structure−activity relationships (SARs) of a new series of compounds obtained by changing the C-6 position of the fluorine atom in addition to the C-5 and C-7 positions and evaluating their cytotoxic activity against several murine and human tumor cell lines. Our results showed that the 6-unsubstituted 1,8-naphthyridine structure had the most potent cytotoxic activity against murine P388 leukemia twice that of the 6-fluoro analogue. In addition, introduction of an amino group at the C-5 position did not have any substantial effect on the cytotoxic activity, while both the 5-chloro and 5-trifluoromethyl groups decreased the cytotoxic activity by 5- to 10-fold. Moreover, aminopyrrolidine derivatives at the C-7 position showed more potent cytotoxic activity than other amines or carbon derivatives. Among the 7-(3-aminopyrrolidinyl) derivatives, the *trans*-3-methoxy-4-methylaminopyrrolidinyl derivative (**27l**) was determined to have potent cytotoxic activity in both in vitro and in vivo assays and high water solubility. Finally, the (*S*,*S*)-isomer (AG-7352, **3**) of **27l**, with a cytotoxic activity against human tumor cell lines more potent than that of etoposide, was selected for further development.

Introduction

The properties of the quinolone class of antibacterials have been known for over 40 years.[1] Quinolones represent one of the first classes of antibacterial agents that act by inhibiting DNA gyrase and topoisomerase IV, bacterial topoisomerase II enzymes.[2] Clearly, the most significant modification of the quinolone antibacterials for potent activity has proven to be the introduction of a 6-fluoro substituent.[3] On the other hand, mammalian topoisomerase II, which possesses a mechanism of action similar to that of DNA gyrase/topoisomerase IV, has also been investigated, and accordingly, many topoisomerase II inhibitors including etoposide, doxorubicin, ellipticine, and amsacrine have been used in preclinical studies and clinical treatments.[4] Although novel classes of quinolones as mammalian topoisomerase II inhibitors have been reported since 1992, no compound reached clinical trial as an antitumor agent so far to our knowledge. Generally, quinolone topoisomerase II inhibitors are characterized by two basic structures (Figure 1), i.e., 6,8-difluoroquinolone (A-65282,[5] CP-115,953,[6] and WIN57294[7]) and quinobenzoxazine (A-62176[8] and A-85226[9]). These reported antitumor quinolones all have a fluorine atom at the C-6 position; however, the precise role of the fluorine atom at the C-6 position has never been clearly defined in the field of antitumor quinolones.

In a previous paper, we have reported that the 7-substituted 6-fluoro-1-(2-thiazolyl)-1,8-naphthyridine nucleus serves as a unique scaffold for finding new clinically useful quinolones that have potent antitumor activity and no cross-resistance with other agents.[10] Thus, the 7-(3-aminopyrrolidinyl)-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acid (**1**) and two of its close analogues have been shown to have good antitumor activity in vitro against murine and human tumor cell lines and in vivo test for mouse leukemia. Additionally, the pyridopyrimidine analogue (**2**) as another core structure has been demonstrated to be half as active as the 6-fluoro-1,8-naphthyridine (**1**). In the course of our search for more potent antitumor agents, we focused our interest on modifications at the C-6 position of **1** to examine whether the trend of the structure−activity relationships (SARs) for this position would be the same as that for the C-6 position of quinolone antibacterials. In the field of quinolone antibacterials, a fluorine atom of this position has been shown to be indispensable for enhanced antibacterial activity.[3] Thus, in this study, we investigated SARs of a new series of compounds obtained by changing substituents at the C-6 position of the 1,8-naphthyridine nucleus in addition to the C-5 and C-7 positions and evaluating their cytotoxic activity against several murine and human tumor cell lines. Our findings led us to a novel antitumor agent, AG-7352 (**3**), as a candidate for further development. In this paper, we present these findings and discuss the SARs and antitumor activity that have been revealed during the study.

Chemistry

To study the effect of various substituents at the C-6 position of the 1,8-naphthyridine ring, compounds **9**,

* To whom correspondence should be addressed. Phone: +81-6-6337-5900. Fax: +81-6-6338-7656. E-mail: yasunori-tuduki@dainippon-pharm.co.jp.

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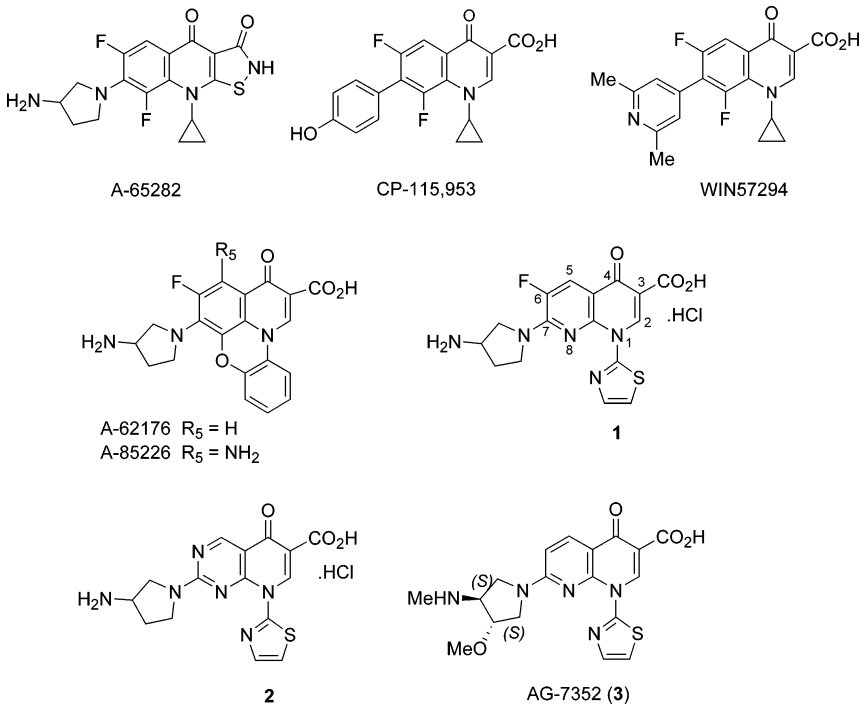
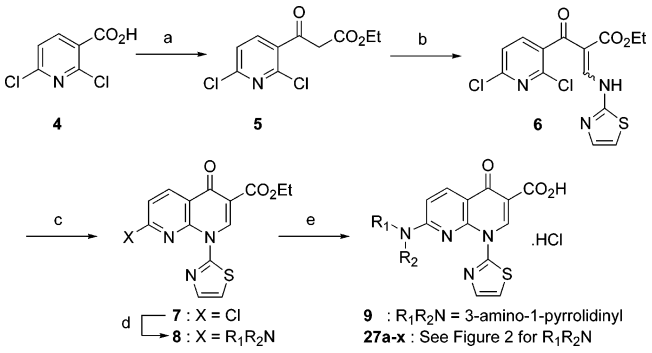


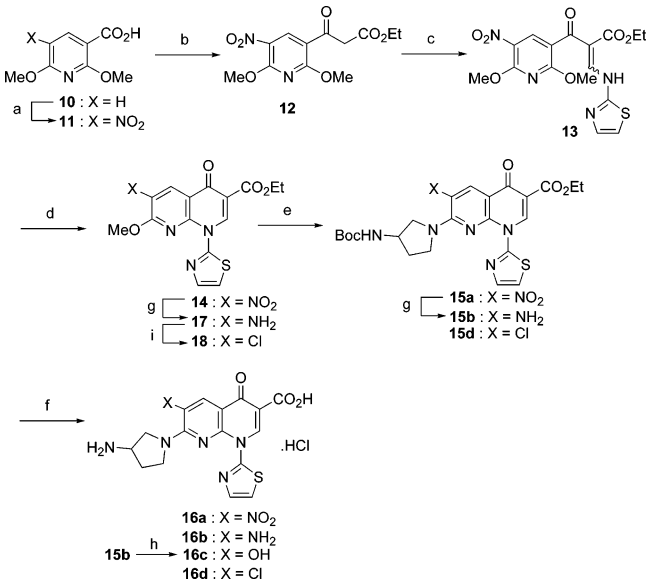
Figure 1. Structures of AG-7352 and reference compounds.

Scheme 1[a]



[a] (a) (1) CDI, (2) $EtOCOCH_2COOK$, $MgCl_2$, Et_3N; (b) (1) $(EtO)_3CH$, Ac_2O, (2) 2-aminothiazole; (c) K_2CO_3; (d) R_1R_2NH; (e) HCl.

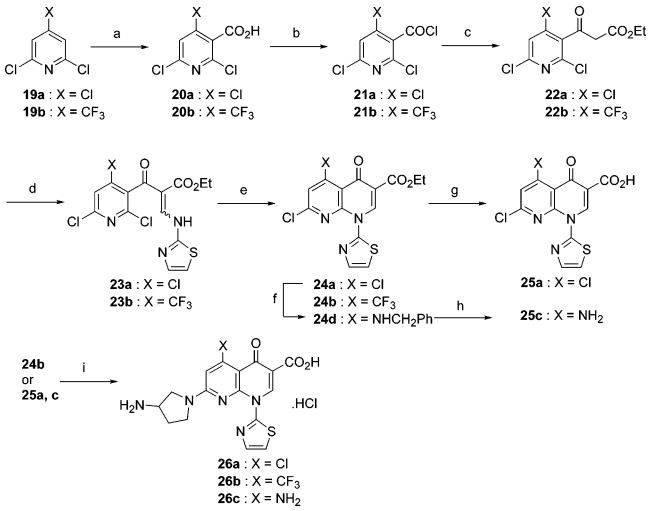
Scheme 2[a]



[a] (a) HNO_3, Ac_2O; (b) (1) CDI, (2) $EtOCOCH_2COOK$, $MgCl_2$, Et_3N; (c) (1) $(EtO)_3CH$, Ac_2O, (2) 2-aminothiazole; (d) K_2CO_3; (e) 3-(*N*-Boc-amino)pyrrolidine; (f) HCl−EtOH; (g) H_2/Ra−Ni; (h) HCl; (i) (1) $NaNO_2$−HCl, (2) CuCl.

16a, **16b**, **16c**, and **16d**, which have H, NO_2, NH_2, OH, and Cl at this position, respectively, were designed. A synthetic route to the unsubstituted compound **9** is illustrated in Scheme 1. The 2,6-dichloronicotinic acid **4** was converted to the nicotinoyl acetate **5** with ethyl malonate potassium salt by means of 1,1′-carbonyldiimidazole (CDI). The nicotinoyl acetate **5** was treated with ethyl orthoformate and acetic anhydride, followed by reaction with 2-aminothiazole to produce the enaminoester **6** as a mixture of keto and enol forms. The 1,8-naphthyridine derivative **7** was obtained in good yield through base-assisted cyclization reaction of **6**. Coupling reaction of **7** with 3-aminopyrrolidine was followed by acid hydrolysis of the ester **8** formed to give the desired 1,8-naphthyridine-3-carboxylic acid **9**. Synthesis of the 6-nitro derivative **16a**, 6-amino derivative **16b**, and 6-hydroxy derivative **16c** is shown in Scheme 2. Because attempts to introduce directly a NO_2 sustitutent at the C-6 position of 1,8-naphthyridine ring of **7** were unsuccessful, we tried to prepare the 6-nitro derivative **16a** using the 2,6-dimethoxynicotinic acid **10** as starting material. Nitration of **10** by means of HNO_3 and acetic anhydride gave the 5-nitro compound **11**. In a manner similar to that described above, the naphthyridine ester **14** was prepared from the nicotinic acid **11** via the ketoester **12** and the enaminoester **13**. Reaction of compound **14** with 3-[*N*-*tert*-butyloxycarbonyl(Boc)-amino]pyrrolidine afforded the corresponding 6-nitro naphthyridine ester **15a**. Reduction of **15a** was effected by hydrogenation in the presence of Ra−Ni to give the amino derivative **15b**. Acid hydrolysis of both compounds **15a** and **15b** by HCl/EtOH afforded the desired compounds **16a** and **16b**, respectively. Acid hydrolysis of compound **15b** at 100 °C for 1 week by concentrated HCl furnished directly the 6-hydroxy derivative **16c**. Preparation of the 6-chloro derivative **16d** by direct

Scheme 3[a]



[a] (a) (1) *n*-BuLi, (2) CO_2; (b) $SOCl_2$; (c) $EtOCOCH_2COOH$, MeMgBr; (d) (1) $(EtO)_3CH$, Ac_2O, (2) 2-aminothiazole; (e) K_2CO_3; (f) benzylamine; (g) H_2SO_4−AcOH−H_2O; (h) (1) H_2SO_4, AcOH (2) H_2O; (i) (1) 3-aminopyrrolidine, (2) HCl.

chlorination at the C-6 position of the 1,8-naphthyridine ring of **7** was not successful. Therefore, the 6-chloro derivative **18** was obtained in good yield by Sandmeyer reaction of the 6-amino derivative **17**, prepared from the 6-nitro derivative **14**. Coupling reaction of compound **18** with 3-(*N*-Boc-amino)pyrrolidine in the presence of DBU followed by acid hydrolysis of the resultant **15d** afforded the desired compound **16d**.

To study the effects of various substituents at the C-5 position of the 1,8-naphthyridine ring, compounds **26a**, **26b**, and **26c**, which have Cl, CF_3, and NH_2 at this position, respectively, were synthesized by the route depicted in Scheme 3. Treatment of the 2,4,6-trichloropyridine **19a** and the 2,6-dichloro-4-trifluoromethylpyridine **19b** with *n*-BuLi at −78 °C followed by carboxylation of the resulting carbanion with CO_2 gave the nicotinic acids **20a** and **20b**, respectively. Reaction of the nicotinoyl chlorides **21a** and **21b**, prepared from **20a** and **20b** with $SOCl_2$, with ethyl hydrogen malonate and EtMgBr produced the ketoesters **22a** and **22b**. These compounds (**22a** and **22b**) were converted to the naphthyridine esters **24a** and **24b** via the enaminoesters **23a** and **23b** according to the procedure described for **7** from **5**. Coupling reaction of **24b** with 3-aminopyrrolidine was followed by acid hydrolysis to give the 5-trifluoromethyl derivative **26b**. Acid hydrolysis of **24a** by means of H_2SO_4−AcOH−H_2O afforded the ester **25a**. Reaction of the 5,7-dichloro ester **24a** with benzylamine in refluxing toluene gave only the 5-benzylamino derivative **24d** in good yield. Debenzylation of **24d** with H_2SO_4 was followed by acid hydrolysis to give the 5-amino derivative **25c**. The desired 5-chloro and 5-amino derivatives (**26a** and **26c**) were obtained by coupling reaction of the naphthyridine-3-carboxylic acids **25a** and **25c** with 3-aminopyrrolidine.

By a manner similar to that for the synthesis of **9**, various kinds of amines as shown Figure 2 were incorporated into the C-7 position of the ester **7** to provide the desired products **27a**−**x** after hydrolysis of the ester and the protecting group (Scheme 1). When diamines were used in the coupling reaction with **7**, one of the nitrogens was protected with an acetyl or Boc group in most cases. The overall yields of compounds **27a**−**x** were calculated from **7** and are listed in Table 3 along with their in vitro cytotoxic activity data against murine P388 leukemia cells.

The amines used in this study were purchased or prepared following procedures in the literature except for the new pyrrolidine derivatives **28**−**31**. The designed amine parts of **28**, **29**, **30**, and **31**, which afforded the corresponding substituents of the naphthyridinecarboxylic acids **27c**, **27f**, **27m**, and **27n**, respectively, were prepared by the methods shown in Scheme 4. The pyrrolidine derivative **32**[11] was treated with Boc_2O to give **33**, which was converted to **34** by reduction of the Boc group with sodium bis(2-methoxyethoxy)aluminum hydride and successive treatment with Boc_2O. Debenzylation of **34** was achieved by hydrogenation in the presence of Pd−C and produced the 3,3-disubstituted pyrrolidine **28**. N-Alkylation of the 3-(benzylamino)-propionitrile **35** with epibromohydrine followed by a ring-opening reaction of the epoxide using $NaNH_2$ produced the pyrrolidine derivative **36**. Treatment of **36** with thionyl chloride, followed by acid hydrolysis of the cyanide formed, gave the amide **37**, which was converted to the aminopyrrolidine **38** by Hofmann rearrangement and protection with Boc_2O. Transformation of **38** to the 3,4-disubstituted pyrrolidine **29** was achieved by removing the *N*-benzyl substituent via reductive cleavage with ammonium formate in the presence of Pd−C.[20] The pyrrolidine derivative **39**[22] was treated with acetic anhydride to give **40**, which was converted to **41** by reduction of the acetyl group with sodium bis(2-methoxyethoxy)aluminum hydride and successive treatment with Boc_2O. Removal of the benzyl group of **41** was achieved by hydrogenation to give the desired pyrrolidine derivative **30**. Addition of methanesulfenyl chloride to the double bond of the 3-pyrroline **42** and successive treatment with methylamine gave the 3,4-disubstituted pyrrolidine **43**. Acid-mediated removal of the Boc group completed the synthesis of **31** as a dihydrochloride.

The chiral pyrrolidine part of each isomer of **27l** was prepared by asymmetric synthesis using (+)-tartaric acid.[21] In addition, a large-scale preparation of the chiral pyrrolidines was achieved through resolution of the diastereoisomers.[22] The absolute configurations of the chiral pyrrolidines were determined as (*S*,*S*)/(*R*,*R*) by X-ray crystallographic analysis of (*S*,*S*)-3-methoxy-4-methylaminopyrrolidine.[22]

To study the cytotoxic activity of some analogues possessing a C−C bond at position 7, compounds **45a**−**e** were synthesized by the route depicted in Scheme 5. The overall yields of **45a**−**e** were calculated from **7** and are listed in Table 3 along with their in vitro cytotoxic activity data against murine P388 leukemia cells. A Stille-type palladium-catalyzed cross-coupling reaction of the 7-chloronaphthyridine **7** with stannyl compounds gave the 7-carbon derivatives **44a**−**d**. Compound **44d** was prepared by coupling reaction of **7** with 1-tri-*n*-butylstannyl-2-trimethylsilylacetylene, followed by deprotection of the silyl group. Finally, the acetylene derivative **44d** was subjected to 1,3-dipolar addition reaction with trimethylsilyldiazomethane to give the pyrazole derivative **44e**. Compounds **44a**−**e** were converted to the naphthyridine acids **45a**−**e** by acid or base hydrolysis of the ester.

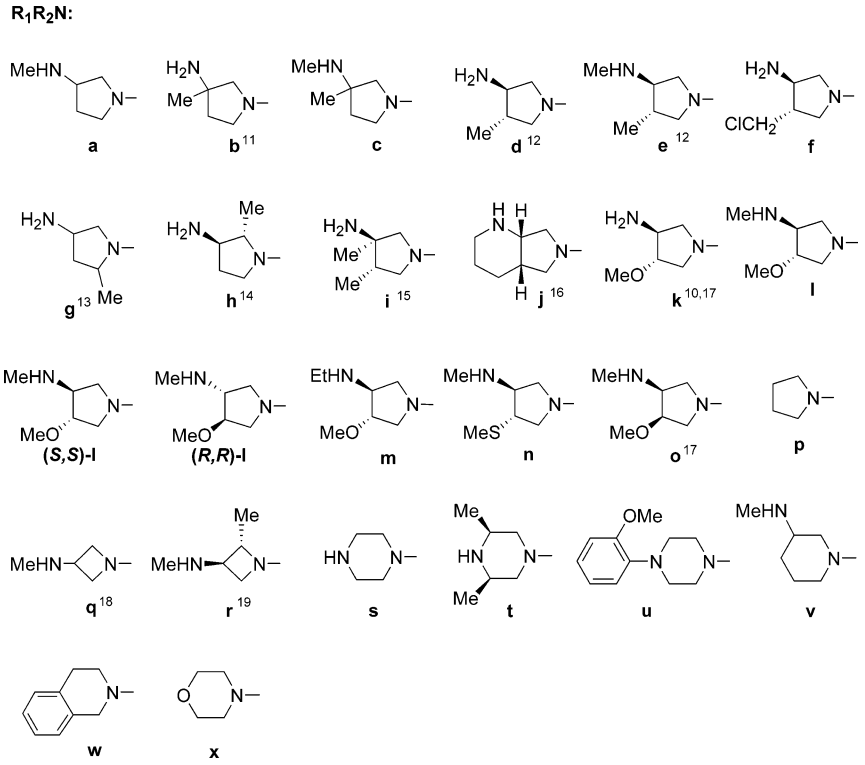
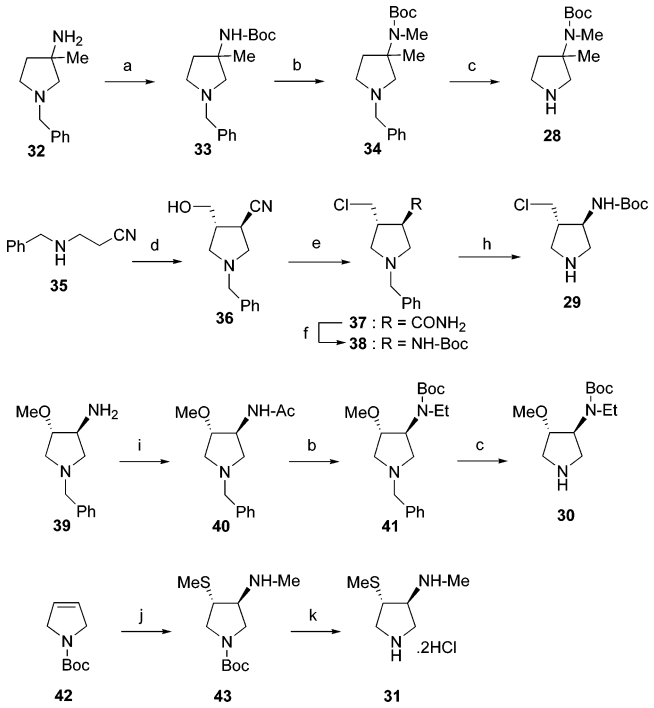


Figure 2. R_1R_2N groups used in this study.

Scheme 4[a]

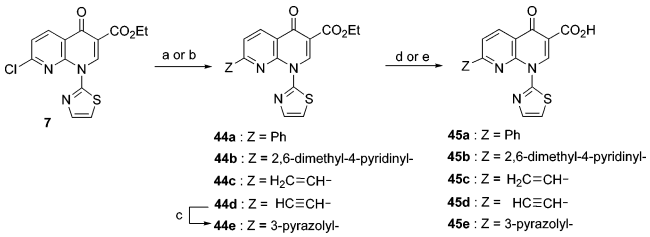


[a] (a) Boc_2O; (b) (1) $Na(OCH_2CH_2OMe)_2AlH_2$, (2) Boc_2O; (c) H_2/Pd−C; (d) (1) epibromohydrin, K_2CO_3, (2) $NaNH_2$; (e) (1) $SOCl_2$, (2) concentrated H_2SO_4; (f) (1) NaOCl−MeOH, (2) concentrated HCl, (3) Boc_2O; (h) ammonium formate, Pd−C; (i) Ac_2O; (j) (1) MeSCl, (2) $MeNH_2$; (k) 30% HCl−EtOH.

Results and Discussion

In the field of quinolone antibacterials, introduction of a fluorine atom into the C-6 position opened new era of fluoroquinolone antibactrerials. It has also been shown that the optimum substituent at the C-6 position for antibacterial activity was a fluorine atom in both quinolone and naphthyridone series.[3] On the other hand, in the field of antitumoral quinolones, the precise role of the fluorine atom of the C-6 position has never been clearly defined, although reported potent antitumoral quinolones shown in Figure 1 had the fluorine atom of the C-6 position in all cases. We have previously reported that the 6-fluoro-1,8-naphthyridone series have good antitumor activity both in vitro and in vivo.[10] Moreover, the pyridopyrimidine analogue (**2**), which has very weak antibacterial activity, has been demonstrated to be half as antitumor-active as 6-fluoro-1,8-naphthyridine (**1**). In a further attempt to find clinically useful antitumor agents, we investigated SARs of a new series of compounds obtained by changing substituents at the C-6 position of the 1,8-naphthyridine nucleus in addition to the C-5 and C-7 positions and evaluating their cytotoxic activity against several murine and human tumor cell lines.

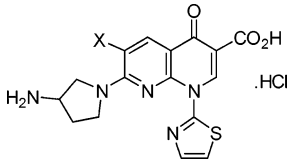
Scheme 5[a]



[a] (a) Z-Sn(*n*-Bu)$_3$ or Z-SnMe$_3$, $PdCl_2(PPh_3)_2$; (b) (1) 1-tri-*n*-butylstannyl-2-trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, (2) KF; (c) $TMSCH_2N_2$; (d) HCl; (e) NaOH.

As shown in Table 1, the 6-hydrogen compound **9** had a cytotoxic activity (IC_{50}) against murine P388 leukemia cells twice that of the 6-fluorine counterpart (**1**). This finding indicates a significant difference from what has been reported in the literature; i.e., potent antitumor quinolones have in all cases a 6-fluorine atom.[5−9] With

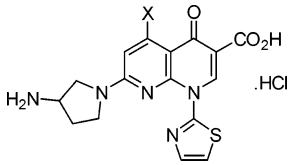
Table 1. Cytotoxic Activity (IC_{50}) of C-6 Substituted Compounds against Murine P388 Leukemia Cells



compd	X	IC_{50},[a] μg/mL
1	F	0.021
9	H	0.010
16a	NO_2	2.29
16b	NH_2	1.01
16c	OH	4.31
16d	Cl	0.227

[a] Concentration of agent that reduces cell viability by 50%.

Table 2. Cytotoxic Activity (IC_{50}) of C-5 Substituted Compounds against Murine P388 Leukemia Cells



compd	X	IC_{50},[a] μg/mL
9	H	0.010
26a	Cl	0.049
26b	CF_3	0.098
26c	NH_2	0.0081

[a] Concentration of agent that reduces cell viability by 50%.

regard to other substituents, the 6-NO_2, 6-NH_2, and 6-OH compounds **16a**−**c** were far less active than the 6-F counterpart (**1**). In addition, the 6-Cl compound **16d**, which like compound **1** carries a halogen atom, exhibited a cytotoxic activity against murine P388 leukemia 10-fold less than that of compound **1**. Generally, in this group of substituents, the cytotoxic activity order was as follows: H > F > Cl > NH_2 > NO_2 > OH. On the other hand, the antibacterial activity order was as follows: F > Cl > H > NH_2 > NO_2 = OH, for example, against *Staphylococcus aureus* 209P JC-1 (MIC_{50} = 0.39, 1.56, 3.13, 50, >100, and >100 μg/mL, respectively) and *Escherichia coli* NIHJ JC-2 (MIC_{50} = 0.025, 0.2, 0.39, 1.56, >100, and >100 μg/mL, respectively). These results indicate that SAR for the C-6 position of 1,8-naphthyridones against cytotoxic activity is different from that of antibacterial activity.

Since the 6-H compound **9** had a cytotoxic activity twice that of the 6-F counterpart (**1**), further modifications at the C-5 and C-7 positions of the 1,8-naphthyridine nucleus were carried out using the 6-unsubstituted 1,8-naphthyridone series. As shown in Table 2, both the chloro and trifluoromethyl groups (compounds **26a**,**b**) decreased the cytotoxic activity by 5- to 10-fold compared to the parent compound **9**. However, introduction of an amino group (compound **26c**) had no substantial effect on the activity of **9**. With respect to the 5-unsubtituted **9** and the 5-amino **26c** activity, the 1,8-naphthyridine structure showed a SAR trend similar to that reported for the quinobenzoxazines (A-62176 and A-85226).[8,9] In addition, the degree of activity change among the C-5 modified compounds was smaller than that among the C-6 modified compounds.

On the basis of the results of C-6 modifications, many kinds of cyclic amines were introduced into the C-7 position of the 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridone-3-carboxylic acid and tested for their cytotoxic activity. Data for the synthesized compounds **27a**−**x** are summarized in Table 3, along with those for reference drugs, i.e., etoposide, doxorubicin, and cisplatin. In general, most pyrrolidine derivatives with or without an amine group showed good cytotoxic activity. The pyrrolidine itself (**27p**) exhibited an activity 6-fold less than the 3-aminopyrrolidine (**9**), which had the same activity as cisplatin with an IC_{50} value of 0.011 μg/mL.

Table 3. Physical Data and Cytotoxic Activity (IC_{50}) of 1-(2-Thiazolyl)naphthyridine-3-carboxylic Acids **27** and **45** against Murine P388 Leukemia Cells

compd	mp, °C	% yield[a]	IC_{50},[b] μg/mL
9	260−264 dec	72	0.010
27a	280−282 dec	91	0.010
27b	243−246	57	0.019
27c	297−299	77	0.018
27d	269−271 dec	75	0.0096
27e	279−282 dec	68	0.012
27f	255−258 dec	92	0.024
27g	259−262 dec	33	0.11
27h	296−299 dec	52	0.094
27i	245−248	38	0.020
27j	292−295 dec	50	0.017
27k	275−278 dec	64	0.018
27l	270−273	73	0.010
(*S*,*S*)-**27l**	278−282 dec	83	0.0075
(*R*,*R*)-**27l**	278−282 dec	74	0.021
27m	294−296 dec	38	0.025
27n	271−272	82	0.020
27o	263−269 dec	43	0.020
27p	>300[c]	68	0.066
27q	259−260 dec[c]	78	0.024
27r	275−277 dec[c]	50	0.041
27s	290−293 dec	53	0.057
27t	290−295	95	0.081
27u	263−265	62	>1
27v	297−299 dec	71	0.52
27w	298−300 dec[c]	41	>10
27x	>300[c]	70	0.12
45a	227−230 dec[c]	41	6.33
45b	272−275 dec	29	7.37
45c	284−288 dec[c]	62	>1
45d	>300[c]	36	2.3
45e	>300[c]	22	4.34
etoposide			0.0085
doxorubicin			0.004
cisplatin			0.011

[a] Isolation yield based on **7**. [b] Concentration of agent that reduces cell viability by 50%. [c] Prepared as a free form.

With regard to introduction of a methyl group on the pyrrolidine ring, compound **27b**, possessing a 3-methyl group at the geminal position of the 3-amino group of the parent compound **9**, had slightly less activity than **9**, while compound **27d**, possessing a 4-methyl group at the vicinal position, showed an activity almost similar to that of **9**. Dimethylation in the two positions resulted in a little decreased activity (compound **27i**). Similarly, introduction of a 2-methyl group (**27g**) or 5-methyl group (**27h**) on the pyrrolidine ring resulted in much decreased activity. Among the methylated compounds of 3-aminopyrrolidine, the cytotoxic activity order was as follows: 4-methyl **27d** > 3-methyl **27b** = 3,4-dimethyl **27i** > 2-methyl **27h** = 5-methyl **27g**. Introduction of a chlorine atom into the methyl group of **27d** furnished the vicinal chloromethyl derivative **27f**, which had a cytotoxic activity almost half that of **27d**. On the other

hand, the bicyclic aminopyrrolidine (**27j**) was slightly less active than the monocylclic aminopyrrolidine (**27d** or **27e**).

Introduction of a methoxy group on the pyrrolidine ring of **9** produced a slight decrease in cytotoxic activity with compound **27k** having an IC_{50} value of 0.018 μg/mL. Methylation of the amino group of **9** resulted in no substantial effect on the activity (**9** vs **27a**, **27b** vs **27c**, and **27d** vs **27e**) or slightly increased the activity (**27k** vs **27l**). Replacement of the *N*-methyl group of **27l** with an *N*-ethyl group, giving **27m**, had a negative effect on the activity. Also, replacement of the methoxy group of **27l** with a methylthio group, giving **27n**, resulted in a decrease in the activity. With regard to the stereochemistry, the trans isomer **27l** was twice as active as the cis isomer **27o**.

To compare the effect of ring size on the cytotoxic activity, azetidine with a four-member ring and piperidine with a six-member ring were investigated. The 3-methylaminoazetidine (**27q**) had slightly less activity than the 3-methylaminopyrrolidine (**27a**). Introduction of a 2-methyl group (**27r**) on this azetidine produced a decrease in the cytotoxic activity. On the other hand, the 3-methylaminopiperidine (**27v**) had much less activity than **27a**. The piperazines (**27s** and **27t**) with a cyclic diamine showed decreased cytotoxic activity (IC_{50} values of 0.057 and 0.081 μg/mL, respectively). Introduction of a 2-methoxyphenyl group on the piperazine, giving **27u**, deteriorated the cytotoxic activity. In comparison with other rings, the 1,2,3,4- tetrahydroisoquinoline (**27w**) was inactive, and the morpholine (**27x**) had weak activity.

Next, analogues of the pyrrolidine derivatives possessing a C−C bond at the C-7 position were investigated. A phenyl, 2,6-dimethyl-4-pyridinyl, vinyl, ethynyl, or pyrazolyl group introduced at the C-7 position (**45a**−**e**) regrettably resulted in much decreased cytotoxic activity compared to the pyrrolidine derivatives (Table 3). With respect to the effect of C−C bond at the C-7 position, the 1,8-naphthyridine structure obviously showed a SAR trend dissimilar to that of the reported 6,8-difluoroquinolones (CP-115,953, WIN57294).[6,7]

An overall evaluation of substituents at the C-7 position of the 1,8-naphthyridine nucleus showed that the 3-aminopyrrolidine derivatives have potent cytotoxic activity with IC_{50} values similar to that of cisplatin. With regard to substitution at the C-7 position, the 6-unsubstituted 1,8-naphthyridine series generally showed a SAR trend similar to that of the 6-fluoro-1,8-naphthyridine series. Thus, various 3-aminopyrrolidine derivatives with specific substituents were found to have potent in vitro cytotoxic activity with only small differences in IC_{50} values.

From the results above, selected compounds were tested for their in vivo antitumor activity using mice implanted with P388 leukemia cells. Data for the selected compounds **9**, **27a**−**e**, **27k**, and **27l** are summarized in Table 4, along with their water solubility (pH 7.2 buffer). The in vivo test consisted of intraperitoneal (ip) implantation of tumor cells, followed 1 day and 5 days later by ip treatment with each test compound at doses of 3.13, 12.5, and 50 mg/kg. The end point for response to treatment was taken as the relative life span expressed as median survival time of treated mice (*T*) over that of untreated control mice (*C*) (*T*/*C* (%); see Experimental Section). As shown in Table 4, all test compounds were active even at the lowest dose of 3.13 mg/kg; however, four compounds **9**, **27b**, **27c**, and **27e** were considered to cause some degree of toxicity at the highest dose of 50 mg/kg. The remaining compounds **27a**, **27d**, **27k**, and **27l** displayed potent cytotoxic activity at the highest dose of 50 mg/kg with an efficacy at all doses comparable to that of etoposide. Regarding the solubility of these 1,8-naphthyridines, while compounds **9**, **27a**−**e**, and **27k** had very low water solubility, compound **27l** was highly soluble in water, demonstrating a solubility in pH 7.2 buffer of 20.1 mg/mL. Thus, the sole combination of a methoxy and methylamino group in the trans position of **27l** gave remarkably high solubility. Considering an infusion drug as a preferable formulation, this result encouraged us to proceed to the next step of our investigation with compound **27l**.

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

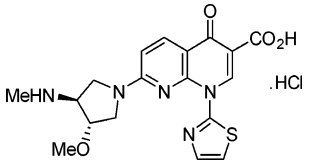
compd	dose, mg/kg	*T*/*C*,[b] %	solubility,[c] mg/mL
9	3.13	188	0.0069
	12.5	275	
	50	125	
27a	3.13	200	0.0080
	12.5	300	
	50	>375	
27b	3.13	150	0.0038
	12.5	213	
	50	138	
27c	3.13	163	0.0072
	12.5	225	
	50	113	
27d	3.13	175	0.017
	12.5	200	
	50	288	
27e	3.13	188	0.0062
	12.5	288	
	50	75	
27k	3.13	163	0.047
	12.5	238	
	50	338	
27l	3.13	200	20.1
	12.5	250	
	50	>375	
etoposide	3.13	175	
	12.5	250	
	50	>375	

[a] See Experimental Section. [b] (Median survival time of treated mice)/(median survival time of controls) × 100. [c] Solubility measured in a pH 7.2 phosphate buffer.

Both optical isomers of the racemic compound **27l** were synthesized and evaluated for their in vitro and in vivo cytotoxic activity and solubility in water. The in vitro cytotoxic activity of the isomer (*S*,*S*)-**27l** was 3-fold that of the corresponding (*R*,*R*)-**27l** and was superior to that of cisplatin (IC_{50} = 0.0075 vs 0.021 μg/mL and IC_{50} = 0.0075 vs 0.011 μg/mL, respectively; Table 3). As for the in vivo cytotoxic activity, the (*S*,*S*)-isomer of **27l** was more effective than the (*R*,*R*)-isomer at all tested doses (Table 5). This in vivo activity of (*S*,*S*)-**27l** is comparable to that of cisplatin, inferior to that of doxorubicin, and superior to that of etoposide. Regarding water solubility in pH 7.2 buffer, both isomers showed almost the same solubility as the racemic **27l**.

At this point we considered (*S*,*S*)-**27l** as one of the promising antitumor agents and examined the physicochemical properties of some of its salts and a free base.

Table 5. In Vivo Antitumor Activity of Compound **27l** and Its Optical Isomers against Murine P388 Leukemia Cells[a]



compd	dose, mg/kg	*T/C*,[b] %	solubility,[c] mg/mL
27l	0.78	150	20.1
	1.56	163	
	3.13	200	
	6.25	225	
	12.5	250	
	25	300	
(*S*,*S*)-**27l**	0.78	150	23.7
	1.56	188	
	3.13	225	
	6.25	275	
	12.5	>375	
	25	>375	
(*R*,*R*)-**27l**	0.78	125	19.8
	1.56	125	
	3.13	163	
	6.25	188	
	12.5	238	
	25	300	
etoposide	0.78	138	
	1.56	150	
	3.13	175	
	6.25	200	
	12.5	250	
	25	>375	
doxorubicin	0.78	173	
	1.56	228	
	3.13	235	
	6.25	293	
cisplatin	0.78	154	
	1.56	186	
	3.13	239	
	6.25	290	

[a] See Experimental Section. [b] (Median survival time of treated mice)/(median survival time of controls) × 100. [c] Solubility measured in a pH 7.2 phosphate buffer.

This led us to select the free base **3** (Figure 1) as a candidate for development. Compound **3** was subjected to assay against various types of human tumor cells. In general, compound **3** displayed good cytotoxic activity with an efficacy clearly higher than that of the reference drug etoposide (Table 6). Detailed in vivo antitumor efficacy of **3** will be reported elsewhere.

Additionally we have measured the inhibitory activities of **3** against topoisomerase II relaxation and cleavage assays.[23] Compound **3** exhibited potent inhibitory effect in topoisomerase II relaxation ($IC_{50} = 3.2$ μg /mL), whereas it did not induce topoisomerase II cleavage ($IC_{50} > 1000$ μg/mL).[24] The IC_{50} values of etoposide were >1000 and 1 μg /mL against topoisomerase II relaxation and cleavage, respectively. The result implies that there is a mechanistic difference between **3** and etoposide.

Conclusions

We have designed and synthesized a novel type of antitumor agent, 7-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acids. In the course of the SAR study of this series of compounds, the following findings were obtained. First, the 6-unsubstituted 1,8-naphthyridine **9** had an activity twice that of the 6-fluoro-1,8-naphthyridine **1**, indicating a significant difference from conventional antitumor quinoline structures in which the 6-fluorine atom has been shown to be the most potent substituent for cytotoxic activity. Second, introduction of an amino group (compound **26c**) at the C-5 position did not have any substantial effect on the cytotoxic activity, while both the 5-chloro and 5-trifluoromethyl groups (compounds **26a**,**b**) decreased the cytotoxic activity by 5- to 10-fold compared to the parent compound **9**. Third, overall evaluation of C-7 substituents showed that various 3-aminopyrrolidine derivatives have potent in vitro cytotoxic activity against murine P388 leukemia cells and that this activity is similar to that of cisplatin.

As for the in vivo test, the 3-aminopyrrolidine derivatives **27a**, **27d**, **27k**, and **27l**, having the best combination of substituents, displayed good cytotoxic activity with an efficacy comparable to that of etoposide. Of these compounds, only **27l** had remarkably high water solubility. Synthesis and in vitro/in vivo evaluation of both optical isomers of the racemic compound **27l** revealed that (*S*,*S*)-**27l** has great cytotoxic activity against murine and human tumor cell lines with high water solubility. Finally the free base **3** of (*S*,*S*)-**27l**, with its preferable physicochemical properties, was selected as a drug candidate for further development.

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 sprctra 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, N, and S.

3-Aminopyrrolidine, pyrrolidine, piperazine, 2,6-dimethylpiperazine, 1-(2-methoxyphenyl)piperazine, 1,2,3,4-tetrahydroisoquinoline, morpholine, and 2-aminothiazole were purchased from commercial suppliers.

Ethyl 2,6-Dichloronicotinoylacetate (5). To a solution of of 2,6-dichloronicotinic acid **4** (401 g, 2.10 mol) in THF−CH_3CN (1:1, 1.4 L) was added CDI (379 g, 2.30 mol). The resulting mixture was stirred at room temperature for 2.5 h. This crude imidazolide solution was used without purification in the next step. To a solution of ethyl malonate potassium salt (376 g, 2.20 mol) in CH_3CN (3.3 L) was added dropwise $MgCl_2$ (297 g, 3.10 mol) and Et_3N (880 mL, 6.30 mol) under ice-cooling. After the mixture was stirred at room temperature for 5 h, the imidazolide prepared in the above was added. The reaction mixture was stirred at room temperature for 15 h, poured into ice−water, acidifed to pH 5−6 with concentrated HCl, and then extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated to dryness to give 513 g (93%) of **5** as a mixture of keto and enol forms, bp 135−140 °C/2 mmHg. MS (*m*/*z*): 262 (MH^+). IR (neat) cm^{-1}: 1736. ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, 3 H × 1/2, J = 7 Hz), 1.35 (t, 3 H × 1/2, J = 7 Hz), 4.09 (s, 2 H × 1/2), 4.20 (q, 2 H × 1/2, J = 7 Hz), 4.30 (d, 1 H × 1/2, J = 7 Hz), 5.73 (s, 1 H × 1/2), 7.36 (d, 1 H × 1/2, J = 8.5 Hz), 7.40 (d, 1 H × 1/2, J = 8.5 Hz), 7.92 (d, 1 H × 1/2, J = 8.5 Hz), 7.98 (d, 1 H × 1/2, J = 8.5 Hz), 12.54 (s, 1 H × 1/2).

Ethyl 2-(2,6-Dichloro-3-nicotinoyl)-3-(2-thiazolylamino)acrylate (6). A mixture of **5** (44.9 g, 171 mmol), ethyl orthoformate (37.7 g, 255 mmol), and acetic anhydride (43.8 g, 429 mmol) 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 residue was diluted with *i*-Pr_2O (500 mL), and

Table 6. Cytotoxic Activity of Compound **3** against Human Tumor Cell Lines

	IC_{50},[a] μg/mL					
compd	HL-60 leukemia	Hs746T stomach	PANC-1 pancreas	SBC-3 lung	SK-OV-3 ovary	SCaBER bladder
3	0.0498	0.194	0.388	0.0319	0.266	0.121
etoposide	0.150	3.29	1.60	0.0676	1.06	0.276

[a] Concentration of agent that reduces cell viability by 50%. Each value is the mean of at least two independent experiments.

then 2-aminothiazole (20.0 g, 200 mmol) was added under ice-cooling. After the mixture was stirred at room temperature for 5 h, the resulting precipitates were collected by filtration, washed with *i*-Pr_2O, and then dried to give 52.8 g (83%) of **6** as a mixture of keto and enol forms, mp 119−122 °C. MS (*m*/*z*): 373 (MH^+). IR (KBr) cm^{-1}: 1700, 1624. ^1H NMR ($CDCl_3$) *δ*: 0.94 (t, 3 H × 1/3, *J* = 7 Hz), 1.13 (t, 3 H × 2/3, *J* = 7 Hz), 4.08 (q, 2 H × 1/3, *J* = 7 Hz), 4.13 (q, 2 H × 2/3, *J* = 7 Hz), 7.02 (d, 1 H × 1/3, *J* = 3.8 Hz), 7.08 (d, 1 H × 2/3, *J* = 3.7 Hz), 7.34 (d, 1 H, *J* = 8 Hz), 7.48 (d, 1 H × 1/3, *J* = 3.8 Hz), 7.53 (d, 1 H × 2/3, *J* = 3.7 Hz), 7.58 (d, 1 H × 2/3, *J* = 8 Hz), 7.68 (d, 1 H × 1/3, *J* = 8 Hz), 8.81 (d, 1 H × 1/3, *J* = 13 Hz), 8.94 (d, 1 H × 2/3, *J* = 13 Hz), 11.62 (d, 1 H × 1/3, *J* = 13 Hz), 12.80 (d, 1 H × 2/3, *J* = 13 Hz). Anal. ($C_{14}H_{11}Cl_2N_3O_3S$) C, H, Cl, N, S.

Ethyl 7-Chloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (7). To a solution of **6** (51.7 g, 139 mmol) in dioxane (310 mL) was added K_2CO_3 (21.4 g, 155 mmol) at room temperature. The reaction mixture was heated at 60 °C for 1 h and diluted with ice−water. The resulting precipitates were collected by filtration, washed with water, and then dried to afford a crude product, which was recrystallized from a mixture of $CHCl_3$ and *i*-Pr_2O to give 44.8 g (96%) of **7**, mp 176−177 °C. MS (*m*/*z*): 336 (MH^+). IR (KBr) cm^{-1}: 1724, 1632. ^1H NMR ($CDCl_3$) *δ*: 1.43 (t, 3 H, *J* = 6.5 Hz), 4.45 (q, 2 H, *J* = 6.5 Hz), 7.38 (d, 1H, *J* = 3.5 Hz), 7.52 (d, 1 H, *J* = 8.5 Hz), 7.75 (d, 1H, *J* = 3.5 Hz), 8.78 (d, 1 H, *J* = 8.5 Hz), 10.00 (s, 1 H). Anal. ($C_{14}H_{10}ClN_3O_3S$) C, H, Cl, N, S.

Ethyl 7-(3-Amino-1-pyrrolidinyl)-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (8). To a suspension of **7** (2.04 g, 6.10 mmol) in CH_3CN (90 mL) was added 3-aminopyrrolidine (1.56 g, 18.4 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 1.5 h. The resulting precipitates were collected by filtration, and recrystallized from $CHCl_3$−MeOH to give 1.89 g (81%) of **8**, mp 219−221 °C. MS (*m*/*z*): 386 (MH^+). IR (KBr) cm^{-1}: 1727, 1635. ^1H NMR (DMSO-d_6) *δ*: 1.30 (t, 3 H, *J* = 7 Hz), 1.75−1.95 (m, 1 H), 2.03−2.25 (m, 1 H), 3.10−4.00 (m, 7 H), 4.28 (q, 2 H, *J* = 7 Hz), 6.72 (d, 1H, *J* = 9 Hz), 7.72 (d, 1H, *J* = 3.5 Hz), 7.79 (d, 1H, *J* = 3.5 Hz), 8.21 (d, 1 H, *J* = 9 Hz), 9.63 (s, 1 H). Anal. ($C_{18}H_{19}N_5O_3S$·0.2HCl) C, H, Cl, N, S.

7-(3-Amino-1-pyrrolidinyl)-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (9). A suspension of **8** (1.02 g, 2.65 mmol) in 10% HCl (15 mL) was heated to reflux for 5 h. After the mixture was ice-cooled, the resulting precipitates were collected by filtration, washed with 0.5 N HCl and EtOH successively, and dried to give 930 mg (89%) of **9**, mp 260−264 °C (dec). MS (*m*/*z*): 358 (MH^+). IR(KBr) cm^{-1}: 1727, 1631. ^1H NMR (DMSO-d_6) *δ*: 2.12−4.15 (m, 7 H), 6.99 (d, 1 H, *J* = 9 Hz), 7.90 (m, 2 H), 8.40 (d, 1 H, *J* = 9 Hz), 9.85 (s, 1 H), 14.1 (br s, 1 H). Anal. ($C_{16}H_{15}N_5O_3S$·HCl·0.5H_2O) C, H, Cl, N, S.

2,6-Dimethoxy-5-nitronicotinic Acid (11). To a mixture of concentrated HNO_3 (5 mL) and acetic anhydride (60 mL) was added dropwise 2,6-dimethoxynicotinic acid **10** (8.71 g, 47.6 mmol) under ice-cooling. The reaction mixture was stirred at the same temperature for 3 h and then at room temperature for 4 h. The resulting precipitates were collected by filtration, washed with *i*-PrOH and *i*-Pr_2O successively, and dried to give 6.95 g (64%) of **11**, mp 227−230 °C. ^1H NMR (DMSO-d_6) *δ*: 4.03 (s, 3 H), 4.08 (s, 3 H), 8.71 (s, 1 H), 10.3 (br s, 1 H).

Ethyl 2,6-Dimethoxy-5-nitronicotinoylacetate (12). To a solution of **11** (18.5 g, 81.3 mmol) in THF (150 mL) was added CDI (14.8 g, 90.9 mmol). The resulting mixture was heated at 70 °C for 0.5 h. This crude imidazolide solution was used without purification in the next step. To a suspension of ethyl malonate potassium salt (18.6 g, 109 mmol) and $MgCl_2$ (11.3 g, 118 mmol) in AcOEt (250 mL) was added dropwise Et_3N (28.0 g, 277 mmol) under ice-cooling. After the mixture was stirred for 2.5 h at room temperature, the imidazolide prepared above was added. The reaction mixture was stirred at 70 °C for 1.5 h, poured into ice−water, and acidified to pH 5−6 with concentrated HCl. The resulting precipitates were collected by filtration and washed with AcOEt to give 8.6 g of **12**. The filtrate was extracted with AcOEt. 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 11.3 g of **12**. Each product was combined to give 19.9 g (82%) of **12**. MS (*m*/*z*): 299 (MH^+). ^1H NMR (DMSO-d_6) *δ*: 1.19 (t, 3 H, *J* = 7 Hz), 4.00 (s, 2 H), 4.10 (s, 3 H), 4.11 (q, 2 H, *J* = 7 Hz), 4.15 (s, 3 H), 8.78 (s, 1 H).

Ethyl 2-(2,6-Dimethoxy-5-nitro-3-nicotinoyl)-3-(2-thiazolylamino)acrylate (13). Following the procedure for **6**, **13** was prepared from **12** in 74% yield, mp 166−170 °C. MS (*m*/*z*): 377 (MH^+ − MeOH). IR (KBr) cm^{-1}: 1729, 1603. ^1H NMR ($CDCl_3$) *δ*: 1.01 (t, 3 H × 1/4, *J* = 7 Hz), 1.21 (t, 3 H × 3/4, *J* = 7 Hz), 4.00 (s, 3 H × 3/4), 4.02 (s, 3 H × 1/4), 4.08−4.21 (m, 2 H), 4.14 (s, 3 H × 1/4), 4.16 (s, 3 H × 3/4), 6.95 (d, 1 H × 1/4, *J* = 4 Hz), 7.02 (d, 1 H × 3/4, *J* = 4 Hz), 7.48 (d, 1 H × 3/4, *J* = 4 Hz), 7.78 (d, 1 H × 1/4, *J* = 4 Hz), 8.49 (d, 1 H × 1/4, *J* = 13 Hz), 8.55 (s, 1 H × 3/4), 8.70 (s, 1 H × 1/4), 8.75 (d, 1 H × 3/4, *J* = 13 Hz), 11.20 (d, 1 H × 1/4, *J* = 13 Hz), 12.50 (d, 1 H × 3/4, *J* = 13 Hz).

Ethyl 1,4-Dihydro-7-methoxy-6-nitro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (14). To a solution of **13** (2.00 g, 4.90 mmol) in dioxane (90 mL) was added K_2CO_3 (1.00 g, 7.50 mmol) under ice-cooling. The reaction mixture was heated at 60 °C for 3 h and then diluted with ice−water and 20% HCl. The resulting precipitates were collected by filtration, washed with water, EtOH, and *i*-Pr_2O successively, and dried to give 1.70 g (90%) of **14**, mp 208−210 °C. MS (*m*/*z*): 377 (MH^+). IR (KBr) cm^{-1}: 1739, 1648. ^1H NMR (DMSO-d_6) *δ*: 1.31 (t, 3 H, *J* = 7 Hz), 4.30 (s, 3 H), 4.33 (q, 2 H, *J* = 7 Hz), 7.85 (d, 1 H, *J* = 4 Hz), 7.88 (d, 1 H, *J* = 4 Hz), 9.08 (s, 1 H), 9.69 (s, 1 H).

Ethyl 7-[3-(*N*-*tert*-Butoxycarbonylamino)-1-pyrrolidinyl]-1,4-dihydro-6-nitro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (15a). To a solution of 3-(*N*-Boc-amino)pyrrolidine (5.90 g, 31.8 mmol) in DMF (150 mL) were added **14** (10.3 g, 27.3 mmol) and K_2CO_3 (4.70 g, 34.1 mmol). The reaction mixture was stirred at 60 °C for 2 h and cooled to 0 °C. The resulting mixture was diluted with water and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was recrystallized from AcOEt−EtOH to give 11.4 g (79%) of **15a**, mp 204−205 °C. MS (*m*/*z*): 531 (MH^+). IR (KBr) cm^{-1}: 1714, 1627. ^1H NMR (DMSO-d_6) *δ*: 1.32 (t, 3 H, *J* = 7 Hz), 1.38 (s, 9 H), 2.0−2.5 (m, 2 H), 3.4−4.2 (m, 5 H), 4.30 (q, 2 H, *J* = 7 Hz), 7.24 (m, 1H), 7.80 (d, 1 H, *J* = 3.5 Hz), 7.84 (d, 1 H, *J* = 3.5 Hz), 8.78 (s, 1 H), 9.65 (s, 1 H). Anal. ($C_{23}H_{26}N_6O_7S$) C, H, N, S.

7-(3-Amino-1-pyrrolidinyl)-1,4-dihydro-6-nitro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (16a). A suspension of **15a** (0.16 g, 0.30 mmol), 20% HCl (15 mL), and EtOH (1 mL) was heated at 100 °C for 3 h and cooled to 0 °C. The resulting precipitates were collected by filtration, washed with 10% HCl and EtOH successively, and then dried to give 60 mg (46%) of **16a**, mp 255−257 °C (dec). MS (*m*/*z*): 403 (MH^+). IR (KBr) cm^{-1}: 1734, 1634. ^1H NMR (DMSO-d_6) *δ*: 2.1−2.5 (m, 2 H), 3.4−4.2 (m, 5 H), 7.90

(d, 1 H, J = 3.5 Hz), 7.92 (d, 1 H, J = 3.5 Hz), 8.98 (s, 1 H), 9.83 (s, 1 H), 14.0 (br s, 1 H). Anal. ($C_{16}H_{14}N_6O_5S$·HCl·0.25H_2O) C, H, Cl, N, S.

Ethyl 6-Amino-7-[3-(*N*-*tert*-butoxycarbonylamino)-1-pyrrolidinyl]-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (15b). To a solution of **15a** (1.00 g, 1.90 mmol) in EtOH−dioxane (2:1, 150 mL) was added Raney nickel, which was washed with EtOH before use, under ice-cooling. The resulting mixture was heated at 50 °C for 2.5 h in an atmosphere of hydrogen gas. The resulting precipitates were collected by filtration and dissolved in DMF under heating. The catalyst was removed by filtration, and most of the solvent was removed from the filtrate under reduced pressure. The resulting precipitates were collected by filtration and washed with EtOH to give 0.81 g (85%) of **15b**, mp 270−272 °C (dec). MS (*m*/*z*): 501 (MH^+). IR (KBr) cm^{-1}: 3342, 1731, 1682, 1621. ^1H NMR (DMSO-d_6) δ: 1.31 (t, 3 H, J = 7 Hz), 1.41 (s, 9 H), 1.9−2.3 (m, 2 H), 3.6−4.2 (m, 5 H), 4.26 (q, 2 H, J = 7 Hz), 5.10 (br s, 2 H), 7.1−7.2 (m, 1H), 7.58 (s, 1 H), 7.71 (d, 1 H, J = 3.5 Hz), 7.79 (d, 1 H, J = 3.5 Hz), 9.51 (s, 1 H). Anal. ($C_{23}H_{28}N_6O_7S$) C, H, N, S.

6-Amino-7-(3-amino-1-pyrrolidinyl)-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (16b). Following the procedure for **16a**, **16b** was prepared from **15b** in 76% yield, mp >300 °C. MS (*m*/*z*): 373 (M^+). IR (KBr) cm^{-1}: 1700, 1617. ^1H NMR (DMSO-d_6) δ: 2.0−2.5 (m, 2 H), 3.8−4.3 (m, 5 H), 5.4 (br s, 2 H), 7.68 (s, 1 H), 7.85 (d, 1 H, J = 2.5 Hz), 7.89 (d, 1 H, J = 2.5 Hz), 8.3 (br s, 3 H), 9.68 (s, 1 H), 15.3 (br s, 1 H). Anal. ($C_{16}H_{16}N_6O_3S$·HCl) C, H, Cl, N, S.

7-(3-Amino-1-pyrrolidinyl)-1,4-dihydro-6-hydroxy-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (16c). A suspension of **15b** (0.42 g, 0.84 mmol) in 20% HCl (40 mL) was stirred at 100 °C for 1 week and cooled to 0 °C. The resulting precipitates were collected by filtration, dissolved in 5 mL of 1 N NaOH, and treated with active carbon to remove insoluble materials. To this filtrate was added 20% HCl (20 mL), and the resulting precipitates were collected by filtration and washed with 10% HCl and EtOH successively to give 50 mg (17%) of **16c**, mp 275−279 °C (dec). MS (*m*/*z*): 374 (MH^+). IR (KBr) cm^{-1}: 3429, 1716, 1623. ^1H NMR (DMSO-d_6) δ: 2.0−2.5(m, 2 H), 3.9−4.3 (m, 5 H), 7.63 (s, 1 H), 7.83 (d, 1 H, J = 2.5 Hz), 7.88 (d, 1 H, J = 2.5 Hz), 9.68 (s, 1 H). Anal. ($C_{16}H_{15}N_5O_4S$·HCl·0.5H_2O) C, H, Cl, N, S.

Ethyl 6-Amino-1,4-dihydro-7-methoxy-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (17). Following the procedure for **15b**, **17** was prepared from **14** in 61% yield, mp 272−275 °C (dec). MS (*m*/*z*): 347 (MH^+) . IR (KBr) cm^{-1}: 3313, 1727, 1624. ^1H NMR (DMSO-d_6) δ: 1.32 (t, 3 H, J = 7 Hz), 4.23 (s, 3 H), 4.28 (q, 2 H, J = 7 Hz), 7.78 (d, 1H, J = 3.5 Hz), 7.82 (d, 1 H, J = 3.5 Hz), 8.47 (s, 1 H), 9.62 (s, 1 H).

Ethyl 6-Chloro-1,4-dihydro-7-methoxy-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (18). To a solution of **17** (0.31 g, 0.90 mmol) in concentrated HCl (5 mL) was added a solution of $NaNO_2$ (0.12 g, 1.7 mmol) in water (2 mL) under ice-cooling. After being stirred for 10 min, the reaction mixture was added dropwise over 15 min to a mixture of CuCl (90 mg, 0.91 mmol) and concentrated HCl (2 mL). After being stirred at 60 °C for 10 min, the resulting mixture was alkalized to pH 8 with 20% NaOH 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 $CHCl_3$−EtOH to give 0.27 g (82%) of **18**, mp 219−221 °C. MS (*m*/*z*): 366 (MH^+). IR (KBr) cm^{-1}: 1723, 1615. ^1H NMR (DMSO-d_6) δ: 1.30 (t, 3 H, J = 7 Hz), 4.18 (s, 3 H), 4.28 (q, 2 H, J = 7 Hz), 7.65 (s, 1 H), 7.76 (d, 1H, J = 3.5 Hz), 7.81 (d, 1 H, J = 3.5 Hz), 9.59 (s, 1 H).

Ethyl 7-[3-(*N*-*tert*-Butoxycarbonylamino)-1-pyrrolidinyl]-6-chloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (15d). To a suspension of **18** (0.21 g, 0.58 mmol) in CH_3CN (15 mL) were added 3-(*N*-Boc-amino)pyrrolidine (0.16 g, 0.86 mmol) and DBU (0.22 g, 1.45 mmol). After being stirred at 70 °C for 3 days, the reaction mixture was concentrated under reduced pressure, and then the obtained residue was chromatographed on silica gel with $CHCl_3$−MeOH (100:1) to give 70 mg (23%) of **15d**, mp 105−110 °C. MS (*m*/*z*): 520 (MH^+). IR (KBr) cm^{-1}: 1719, 1625. ^1H NMR ($CDCl_3$) δ: 1.41 (t, 3 H, J = 7 Hz), 1.48 (s, 9 H), 1.9−2.5 (m, 2 H), 3.8−4.3 (m, 5 H), 4.41 (q, 2 H, J = 7 Hz), 4.8 (m, 1 H), 7.32 (d, 1 H, J = 3.5 Hz), 7.70 (d, 1 H, J = 3.5 Hz), 8.49 (s, 1 H), 9.20 (s, 1 H).

7-(3-Amino-1-pyrrolidinyl)-6-chloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (16d). Following the procedure for **16a**, **16d** was prepared from **15d** in 73% yield, mp 288−291 °C (dec). MS (*m*/*z*): 391 (M^+). IR (KBr) cm^{-1}: 1734, 1626. ^1H NMR (DMSO-d_6) δ: 2.0−2.5 (m, 2 H), 3.9−4.3 (m, 5 H), 7.88 (s, 2 H), 8.40 (s, 1 H), 9.71 (s, 1 H). Anal. ($C_{16}H_{14}ClN_5O_3S$·HCl·0.5EtOH) C, H, Cl, N, S.

2,4,6-Trichloronicotinoic Acid (20a). To a solution of **19a** (5.50 g, 30.4 mmol) in anhydrous THF (55 mL) was added of *n*-BuLi (1.6 M in hexane, 20 mL, 32.0 mmol) at −78 °C under a nitrogen atmosphere. After the mixture was stirred at the same temperature for 1 h, excess amount of dry ice was added to the reaction mixture. The whole was stirred at the same temperature for 1 h, warmed to 0 °C, acidified with diluted 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 washed with *i*-Pr_2O to give 6.5 g (75%) of **20a**, mp 138−141 °C. IR (KBr) cm^{-1}: 1715.

2,4,6-Trichloronicotinoyl Chloride (21a). A mixture of **20a** (6.50 g, 28.9 mmol) and $SOCl_2$ (25 mL) was heated to reflux for 3 h and cooled to room temperature. After excess $SOCl_2$ was removed under reduced pressure, the crude product was distilled under reduced pressure to give 6.60 g (93%) of **21a**, bp 93−95 °C/1 mmHg. IR (neat) cm^{-1}: 1791.

Ethyl 2,4,6-Trichloronicotinoylacetate (22a). To a solution of ethyl hydrogen malonate (3.60 g, 27.3 mmol) in anhydrous THF (30 mL) was added dropwise MeMgBr (3 M in Et_2O, 19 mL, 57.0 mmol) under ice-cooling. After being stirred at room temperature for 1 h, a solution of **21a** (6.60 g, 26.9 mmol) in anhydrous THF (30 mL) was added dropwise at the same temperature. After the mixture was stirred at 60 °C for 1.5 h, most of the solvent was removed under reduced pressure. The resulting residue was poured into ice−water, acidified to pH 5−6 with concentrated HCl, 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 distilled under reduced pressure to give 4.80 g (59%) of **22a**, bp 160−162 °C/2 mmHg. MS (*m*/*z*): 296 (MH^+). IR (neat) cm^{-1}: 1746. ^1H NMR ($CDCl_3$) δ: 1.26 (t, 3 H × 1/2, J = 7 Hz), 1.34 (t, 3 H × 1/2, J = 7 Hz), 3.92 (s, 2 H × 1/2), 4.22 (q, 2 H × 1/2, J = 7 Hz), 4.30 (d, 1 H × 1/2, J = 7 Hz), 5.28 (s, 1 H × 1/2), 7.40 (s, 1 H × 1/2), 7.42 (s, 1 H × 1/2), 12.23 (s, 1 H × 1/2).

Ethyl 2-(2,4,6-Trichloro-3-nicotinoyl)-3-(2-thiazolylamino)acrylate (23a). Following the procedure for **7**, **23a** was prepared from **22a** in 58% yield, mp 126−127 °C. MS (*m*/*z*): 406 (MH^+). IR (KBr) cm^{-1}: 1691, 1636.

Ethyl 5,7-Dichloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (24a). To a suspension of **23a** (3.80 g, 9.35 mmol) in AcOEt (40 mL) was added K_2CO_3 (1.5 g, 10.9 mmol) under ice-cooling. The reaction mixture was heated at 60 °C for 1 h, and most of the solvent was removed under reduced pressure. The resulting residue was treated with ice−water and 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 (200:1) and recrystallized from $CHCl_3$ to give 2.50 g (72%) of **24a**, mp 226−227 °C. MS (*m*/*z*): 370 (MH^+). IR (KBr) cm^{-1}: 1737, 1692.

5,7-Dichloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid (25a). A solution of **24a** (0.45 g, 1.22 mmol) in H_2SO_4−AcOH−H_2O (1:8:6, 4.5 mL) was heated to reflux for 15 h and cooled to room temperature. The reaction mixture was diluted with ice−water. The resulting precipitates were collected by filtration and washed with water to give 0.35 g (84%) of **25a**, mp 264−266 °C. MS (*m*/*z*): 342 (MH^+). IR (KBr) cm^{-1}: 1729. ^1H NMR (DMSO-d_6) δ: 7.92 (s, 2H), 8.12 (s, 1H), 9.82 (s, 1H).

7-(3-Amino-1-pyrrolidinyl)-5-chloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (26a). A mixture of **25a** (0.35 g, 1.02 mmol), 3-(*N*-Boc-amino)pyrrolidine (0.23 g, 1.24 mmol), Et_3N (0.31 g, 3.07 mmol), and CH_3CN (10 mL) was heated to reflux for 15 h. Most of the solvent was removed under reduced pressure, and then the resulting residual solid was washed with AcOEt and dried to give 0.45 g (89%) of 7-[3-(*N*-Boc-amino)-1-pyrrolidinyl]-5-chloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acid, mp >300 °C. MS (*m*/*z*): 492 (MH^+). IR (KBr) cm^{-1}: 1732, 1677.

A mixture of the above compound (0.45 g, 0.92 mmol) and 20% HCl (10 mL) was heated to reflux for 1.5 h. The resulting precipitates were collected by filtration, washed with 0.5 N HCl and EtOH successively, and then dried to give 0.32 g (89%) of **26a**, mp >300 °C. MS (*m*/*z*): 392 (MH^+). IR (KBr) cm^{-1}: 1726, 1626. ^1H NMR (DMSO-d_6) δ: 2.1−2.3 (m, 1H), 2.3−2.5 (m, 1 H), 3.4−4.0 (m, 5 H), 7.01 (s, 1 H), 7.80 (d, 1 H, J = 3.5 Hz), 7.85 (d, 1 H, J = 3.5 Hz), 8.2 (br s, 3 H), 9.75 (s, 1 H), 14.3 (br s, 1 H). Anal. ($C_{16}H_{14}ClN_5O_3S\cdot HCl\cdot 0.75H_2O\cdot 0.1EtOH$) C, H, Cl, N, S.

2,6-Dichloro-4-trifluoromethylnicotinoyl Chloride (21b). Following the procedure for **21a**, **21b** was prepared from **19b** in 59% yield via **20b**, bp 77−78 °C/2 mmHg. IR (neat) cm^{-1}: 1797.

Ethyl 2,6-Dichloro-4-trifluoromethylnicotinoylacetate (22b). Following the procedure for **22a**, **22b** was prepared from **21b** in 33% yield. MS (*m*/*z*): 330 (MH^+). IR (neat) cm^{-1}: 1744, 1721.

Ethyl 2-(2,6-Dichloro-4-trifluoromethyl-3-nicotinoyl)-3-(2-thiazolylamino)acrylate (23b). Following the procedure for **7**, **23b** was prepared from **22b** in 31% yield. MS (*m*/*z*): 440 (MH^+). IR (neat) cm^{-1}: 1713.

Ethyl 7-Chloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-5-trifluoromethyl-1,8-naphthyridine-3-carboxylate (24b). Following the procedure for **24a**, **24b** was prepared from **23b** in 82% yield, mp 184−185 °C. IR (KBr) cm^{-1}: 1736, 1703.

7-(3-Amino-1-pyrrolidinyl)-1,4-dihydro-4-oxo-1-(2-thiazolyl)-5-trifluoromethyl-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (26b). Following the procedure for **9**, 0.14 g (96%) of **26b** was prepared from **24b**, mp 291−292 °C (dec). MS (*m*/*z*): 426 (MH^+). IR (KBr) cm^{-1}: 1746, 1631. ^1H NMR ($NaOD/D_2O$) δ: 1.6−1.9 (m, 1H), 2.0−2.3 (m, 1 H), 2.7−2.8 (m, 1 H), 2.9−3.1 (m, 1 H), 3.1−3.3 (m, 1 H), 3.3−3.6 (m, 2 H), 6.52 (d, 1 H, J = 7.0 Hz). Anal. ($C_{17}H_{14}F_3N_5O_3S\cdot HCl\cdot 0.25H_2O$) C, H, Cl, F, N, S.

Ethyl 5-Benzylamino-7-chloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (24d). A mixture of **24a** (0.50 g, 1.35 mmol), benzylamine (0.14 g, 1.31 mmol), Et_3N (0.28 g, 2.78 mmol), and toluene (15 mL) was heated to reflux for 30 min. After most of the solvent was removed under reduced pressure, the resulting residue was recrystallized from AcOEt to give 0.51 g (86%) of **24d**, mp 141−143 °C. MS (*m*/*z*): 441 (MH^+). IR (KBr) cm^{-1}: 1733, 1635. ^1H NMR ($CDCl_3$) δ: 1.42 (t, 3 H, J = 7 Hz), 4.41 (q, 2 H, J = 7 Hz), 4.49 (d, 1H, J = 6.5 Hz), 6.47 (s, 1 H), 7.31 (d, 1 H, J = 3.5 Hz), 7.32−7.40 (m, 5 H), 7.70 (d, 1 H, J = 3.5 Hz), 9.87 (s, 1 H), 11.2−11.7 (m, 1 H).

5-Amino-7-chloro-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid (25c). A mixture of **24d** (500 mg, 1.14 mmol), H_2SO_4 (2 mL), and AcOH (8 mL) was heated at 100 °C for 5 h and cooled to 0 °C. To the reaction mixture was added H_2O (8 mL), and the mixture was heated at 100 °C for 1 h. After the mixture was ice-cooled, the resulting precipitates were collected by filtration, washed with H_2O, and dried to give 350 mg (98%) of **25c**, mp 264−265 °C. MS (*m*/*z*): 322 (M^+). IR (KBr) cm^{-1}: 1727, 1636.

5-Amino-7-(3-amino-1-pyrrolidinyl)-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride (26c). Following the procedure for **26a**, **26c** was prepared from **25c** in 47% yield, mp 261−263 °C (dec). MS (*m*/*z*): 373 (MH^+). IR (KBr) cm^{-1}: 1712, 1628. ^1H NMR (DMSO-d_6) δ: 2.1−2.3 (m, 1H), 2.3−2.5 (m, 1 H), 3.5−4.1 (m, 5 H), 5.64 (s, 1 H), 7.74 (d, 1 H, J = 3.5 Hz), 7.81 (d, 1 H, J = 3.5 Hz), 8.5 (br s, 3 H), 9.68 (s, 1 H). Anal. ($C_{16}H_{16}N_6O_3S\cdot HCl$) C, H, Cl, N, S.

1,4-Dihydro-7-[(*S*,*S*)-3-methoxy-4-methylamino-1-pyrrolidinyl]-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid Hydrochloride ((*S*,*S*)-27l). To a solution of (*S*,*S*)-3-methoxy-4-methylaminopyrrolidine di-*p*-toluenesulfonic acid[21,22] (85.0 g, 179 mmol) in CH_3CN (700 mL) was added Et_3N (100 mL, 719 mmol) at 0 °C. After the mixture was stirred for 15 min, **7** (50.44 g, 150 mmol) was added. The mixture was stirred for 1 day at room temperature, and then the resultant precipitates were collected by filtration and washed with CH_3CN to give 52.4 g (87%) of ethyl 1,4-dihydro-7-[(*S*,*S*)-3-methoxy-4-methylamino-1-pyrrolidinyl]-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate, mp 184−185 °C. $[\alpha]^{28}_D$ −16.9° (*c* 0.50, $CHCl_3$). MS (*m*/*z*): 430 (MH^+). IR (KBr) cm^{-1}: 3350, 1730, 1630. ^1H NMR ($CDCl_3$) δ: 1.42 (t, 3 H, J = 6.8 Hz), 2.54 (s, 3 H), 3.44 (s, 3 H), 3.6−4.2 (m, 6 H), 4.41 (q, 2 H, J = 6.8 Hz), 6.51 (d, 1 H, J = 8.7 Hz), 7.27 (d, 1H, J = 3.5 Hz), 7.69 (d, 1H, J = 3.5 Hz), 8.46 (d, 1 H, J = 8.7 Hz), 9.80 (s, 1 H). Anal. Calcd for $C_{20}H_{23}N_5O_4S$: C, 55.93; H, 5.40; N, 16.31; S, 7.47. Found: C, 55.99; H, 5.33; N, 16.25; S, 7.28.

A mixture of the above compound (28.0 g, 65.3 mmol), 0.5 N NaOH (170 mL, 85 mmol), and EtOH (10 mL) was stirred at room temperature for 1 day. The reaction mixture was acidified with 10% HCl. The resulting precipitates were collected by filtration, washed with 0.5 N HCl and i-Pr_2O successively, and dried to give 10.9 g (97%) of (*S*,*S*)-**27l**, mp 278−284 °C (dec). $[\alpha]^{27}_D$ +50.4° (*c* 1.00, 1 N NaOH). MS (*m*/*z*): 402 (MH^+). IR (KBr) cm^{-1}: 1738, 1640. ^1H NMR (DMSO-d_6) δ: 2.71 (s, 3 H), 3.42 (s, 3 H), 3.5−4.4 (m, 6 H), 7.03 (d, 1 H, J = 9 Hz), 7.84 (d, 1H, J = 3.5 Hz), 7.90 (d, 1H, J = 3.5 Hz), 8.45 (d, 1 H, J = 9 Hz), 9.3 (br s, 2 H), 9.85 (s, 1 H). Anal. ($C_{18}H_{19}N_5O_4S\cdot HCl\cdot 0.25H_2O$) C, H, Cl, N, S.

According to a similar procedure for **9** and (*S*,*S*)-**27l**, compounds **27a**−**x** and (*R*,*R*)-**27l** were prepared.

1-Benzyl-3-(*N*-*tert*-butoxycarbonyl)methylamino-3-methylpyrrolidine (34). To a solution of 3-amino-1-benzyl-3-methylpyrrolidine[11] **32** (20.0 g, 105 mmol) in CH_2Cl_2 (200 mL) was added Boc_2O (25.2 g, 116 mmol) under ice-cooling. The reaction mixture was stirred at room temperature for 2 h and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with hexane−AcOEt (10:1) to give 28.3 g (93%) of **33**. MS (*m*/*z*): 291 (MH^+). IR (neat) cm^{-1}: 3356, 1716, 1697.

To a solution of the above compound **33** (11.4 g, 39 mmol) in toluene (150 mL) was added dropwise sodium bis(2-methoxyethoxy)aluminum hydride (70% in toluene, 50 mL, 173 mmol) under ice-cooling. The reaction mixture was heated to reflux for 2 h and then treated with EtOH and water under ice-cooling. Insoluble material was removed by filtration, and the filtrate was extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated to dryness. The resulting residue was diluted with CH_2Cl_2 (100 mL), and Boc_2O (8.6 g, 39.4 mmol) in CH_2Cl_2 (5 mL) was added under ice-cooling. The reaction mixture was stirred at room temperature for 2 h and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with hexane−AcOEt (4:1) to give 7.5 g (63%) of **34**. MS (*m*/*z*): 305 (MH^+). IR (neat) cm^{-1}: 1697.

3-(*N*-*tert*-Butoxycarbonyl)methylamino-3-methylpyrrolidine (28). To a solution of **34** (7.50 g, 24.7 mmol) in EtOH (35 mL) was added 10% Pd−C (0.83 g) under ice-cooling. The resulting mixture was heated at 50 °C for 4 h in an atmosphere of hydrogen gas. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give the product **28**. MS (*m*/*z*): 215 (MH^+). IR (neat) cm^{-1}: 3337, 1697. This material was used without purification in the next step.

***trans*-1-Benzyl-3-cyano-4-hydroxymethylpyrrolidine (36).** A mixture of 3-(benzylamino)propionitrile **35** (50.0 g, 0.31 mmol), K_2CO_3 (86.0 g, 0.62 mmol), and epibromohydrine (27 mL, 0.32 mmol) in DMF (400 mL) was heated at 90 °C for 1 h, during which period epibromohydrine was added twice [27 mL (0.32 mmol) after 20 min and 30 mL (0.35 mmol) after 40 min]. After the mixture was ice-cooled, insoluble material was

filtered off. The filtrate was concentrated under reduced pressure and dissolved in toluene (500 mL) and DMSO (200 mL). To the mixture was added sodium amide (25.0 g, 0.64 mmol), and the mixture was heated at 65 °C for 10 min, diluted with ice−water, and extracted with toluene. The organic layer was washed with water and 3% AcOH successively, dried over $MgSO_4$, and concentrated under reduced pressure to afford a crude product, which was chromatographed on silica gel with $CHCl_3$ to give 9.0 g (13%) of **36**. IR (neat) cm^{-1}: 3417, 2241.

***trans*-1-Benzyl-4-chloromethylpyrrolidine-3-carboxamide (37).** A mixture of **36** (9.0 g, 42 mmol) and $SOCl_2$ (7.6 mL, 104 mmol) in $CHCl_3$ (120 mL) was heated to reflux for 4 h. The reaction mixture was treated with ice−water, alkalized with 20% NaOH, and extracted with $CHCl_3$. The organic layer was washed with saturated brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford a crude product, which was chromatographed on silica gel with hexane−AcOEt (3:1) to give 8.0 g (82%) of *trans*-1-benzyl-3-chloromethyl-4-cyanopyrrolidine. MS (*m*/*z*): 234 (M^+). IR (neat) cm^{-1}: 2250.

To a solution of the above compound (8.0 g, 34 mmol) in toluene (20 mL) was added a solution of concentrated H_2SO_4 (18 mL). The reaction mixture was stirred at room temperature for 40 min and then heated at 60 °C for 30 min. After being ice-cooled, the solution was alkalized with 50% NaOH and extracted with AcOEt. The organic layer was washed with saturated brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford a crude product, which was recrystallized from hexane−AcOEt to give 7.5 g (87%) of **37**, mp 98−99 °C. MS (*m*/*z*): 252 (M^+). IR (KBr) cm^{-1}: 3400, 3200, 1650, 1620.

***trans*-1-Benzyl-3-*N*-*tert*-butoxycarbonylamino-4-chloromethylpyrrolidine (38).** To a solution of **37** (7.0 g, 27.7 mmol) in MeOH (150 mL) was added dropwise NaOCl (8.5% solution, 26.7 g, 30.5 mmol) under ice-cooling. The reaction mixture was stirred at room temperature for 30 min and then heated at 65 °C for 1.5 h. Most of the solvent was concentrated under reduced pressure, and the obtained residue was extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford a crude product of 8.0 g of *trans*-1-benzyl-3-chloromethyl-4-methoxycarbonyaminopyrrolidine. MS (*m*/*z*): 282 (M^+). IR (neat) cm^{-1}: 3310, 1720.

A mixture of the above compound (8.0 g) and concentrated HCl (50 mL) was heated under reflux for 8 h and cooled to 0 °C. The reaction mixture was diluted with water, and activated carbon was added. After filtration, the filtrate was alkalized with 50% NaOH and extracted with AcOEt. The organic layer was washed with saturated brine, dried over $MgSO_4$, and concentrated under reduced pressure. After the obtained residue was dissolved in $CHCl_3$ (50 mL), to the solution was added a solution of Boc_2O (4.4 g, 20 mmol) in $CHCl_3$ (5 mL) under ice-cooling. The reaction mixture was stirred at room temperature for 3 h and then concentrated under reduced pressure to afford a crude product, which was chromatographed on silica gel with $CHCl_3$ to give 4.8 g (53% from **37**) of **38**, mp 87−88 °C. MS (*m*/*z*): 324 (M^+). IR (KBr) cm^{-1}: 3210, 1694.

***trans*-3-*N*-*tert*-Butoxycarbonylamino-4-chloromethylpyrrolidine (29).** To a solution of **38** (1.03 g, 3.17 mmol), 5% Pd−C (0.24 g) and AcOH (0.70 mL, 12.2 mmol) in EtOH (30 mL) was added ammonium formate (0.97 g, 15.4 mmol) under ice-cooling. The reaction mixture was stirred at room temperature for 1 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford a crude product of **29**. This material was used without purification in the next step.

***trans*-1-Benzyl-3-(*N*-*tert*-butoxycarbonyl)ethylamino-4-methoxypyrrolidine (41).** To a solution of *trans*-3-amino-1-benzyl-4-methoxypyrrolidine[22] **39** (1.02 g, 4.95 mmol) in CH_2Cl_2 (5 mL) was added acetic anhydride (5 mL, 52.9 mmol) under ice-cooling. The reaction mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure to afford a crude product, which was chromatographed on silica gel with $CHCl_3$−MeOH (50:1) to give 1.30 g of **40**.

To a solution of the above compound **40** (1.30 g, 4.95 mmol) in toluene (15 mL) was added dropwise sodium bis(2-methoxyethoxy)aluminum hydride (70% in toluene, 3.60 mL, 12.5 mmol) under ice-cooling. The reaction mixture was heated at 90 °C for 1 day and then treated with water under ice-cooling. Insoluble material was filtered off. The filtrate was extracted with AcOEt, 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 0.54 g (46%) of *trans*-1-benzyl-3-(ethylamino)-4-methoxypyrrolidine. MS (*m*/*z*): 235 (MH^+).

To a solution of the above compound (0.54 g, 2.3 mmol) in MeOH (5 mL) was added Boc_2O (0.75 g, 3.44 mmol) under ice-cooling. The reaction mixture was stirred at room temperature for 7 h and concentrated under reduced pressure. The resulting residue was chromatographed on silica gel with $CHCl_3$−MeOH (100:1) to give 0.69 g (90%) of **41**. MS (*m*/*z*): 335 (MH^+).

***trans*-3-(*N*-*tert*-Butoxycarbonyl)ethylamino-4-methoxypyrrolidine (30).** Following the procedure for **28**, **30** was prepared from **41**.

***trans*-1-*N*-*tert*-Butoxycarbonyl-3-methylamino-4-methylthiopyrrolidine (43).** To a solution of 1-Boc-pyrroline **42** (47.3 g, 0.28 mol) in CH_2Cl_2 (130 mL) was added dropwise a solution of MeSCl (purity of 80%, 35.0 g, 0.42 mol) in CH_2Cl_2 (40 mL) under ice-cooling. The reaction mixture was stirred at room temperature for 1 day and concentrated under reduced pressure. To the resulting residue were added THF (270 mL) and $MeNH_2$ (40% aqueous solution, 240 mL, 2.8 mol) at room temperature. The reaction mixture was heated at 70 °C for 15 h, treated with water under ice-cooling, and then extracted with CH_2Cl_2. 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 53.1 g (77%) of **43**. MS (*m*/*z*): 247 (MH^+). IR (neat) cm^{-1}: 3322, 1693.

***trans*-3-Methylamino-4-methylthiopyrrolidine Dihydrochloride (31).** To a solution of **43** (24.7 g, 0.10 mol) in EtOH (200 mL) was added 30% HCl−EtOH (42 mL). The reaction mixture was stirred at room temperature for 1 day. The resulting precipitates were collected by filtration, washed with *i*-Pr_2O, and then dried to give 20.3 g (92%) of **31**, mp 193−194 °C. MS (*m*/*z*): 147 (MH^+). IR (KBr) cm^{-1}: 3300.

Ethyl 1,4-Dihydro-4-oxo-7-phenyl-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (44a). To a mixture of **7** (1.00 g, 3.0 mmol) in toluene (40 mL) were added bis(triphenylphosphine)palladium dichloride (0.23 g, 0.33 mmol) and trimethyl(phenyl)tin (0.80 g, 3.3 mmol). The resulting mixture was heated to reflux for 3 h. After ice-cooling, the reaction mixture was poured into water and extracted with toluene. 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 EtOH to give 0.74 g (66%) of **44a**, mp 175−178 °C. MS (*m*/*z*): 378 (MH^+). IR (KBr) cm^{-1}: 1731, 1699. ^1H NMR (DMSO-d_6) δ: 1.33 (t, 3 H, J = 7.0 Hz), 4.33 (q, 2 H, J = 7.0 Hz), 7.50−7.70 (m, 3 H), 7.83 (d, 1 H, J = 3.5 Hz), 7.87 (d,1 H, J = 3.5 Hz), 8.26−8.35 (m, 3 H), 8.72 (d, 1 H, J = 8.0 Hz), 9.85 (s, 1 H).

1,4-Dihydro-4-oxo-7-phenyl-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid (45a). A mixture of **44a** (0.54 g, 1.4 mmol), 20% HCl (55 mL), and EtOH (10 mL) was heated at 100 °C for 10 h and cooled to 0 °C. The resulting precipitates were collected by filtration and washed with water and EtOH to afford a crude product, which was dissolved with NH_4OH and then acidified with 0.5 N HCl. The resulting precipitates were collected by filtration, washed with water, EtOH, and *i*-Pr_2O, successively, and dried to give 0.31 g (62%) of **45a**, mp 227−230 °C (dec). MS (*m*/*z*): 350 (MH^+). IR (KBr) cm^{-1}: 1739, 1615. ^1H NMR (DMSO-d_6) δ: 7.60−7.70 (m, 3 H), 7.93 (s, 2 H), 8.30−8.35 (m, 2 H), 8.39 (d, 1 H, J = 8.0 Hz), 8.86 (d, 1 H, J = 8.0 Hz), 10.00 (s, 1 H). Anal. ($C_{18}H_{11}N_3O_3S\cdot0.5H_2O$) C, H, N, S.

According to the similar procedure for **45a**, compounds **45b**,**c** were prepared.

Ethyl 1,4-Dihydro-7-ethynyl-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (44d). To a solution of **7** (3.01 g, 8.97 mmol) in toluene (110 mL) were added bis(triphenylphosphine)palladium dichloride (0.70 g, 1.0 mmol) and 1-tri-*n*-butylstannyl-2-trimethylsilylacetylene (3.94 g, 10.2 mmol). The resulting mixture was heated to reflux for 5 h. After ice-cooling, the reaction mixture was poured into water and extracted with toluene. 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 $CHCl_3$ and recrystallized from EtOH to give 2.49 g (70%) of ethyl 1,4-dihydro-4-oxo-1-(2-thiazolyl)-7-(2-trimethylsilylethynyl)-1,8-naphthyridine-3-carboxylate. MS (*m*/*z*): 698 (MH^+). ^1H NMR ($CDCl_3$) δ: 0.32 (s, 9 H), 1.42 (t, 3 H, J = 7.0 Hz), 4.45 (q, 2 H, J = 7.0 Hz), 7.37 (d, 1 H, J = 3.5 Hz), 7.61 (d, 1 H, J = 8.0 Hz), 7.72 (d, 1 H, J = 3.5 Hz), 8.78 (d, 1 H, J = 8.0 Hz), 10.05 (s, 1 H).

A mixture of the above compound (2.04 g, 6.28 mmol), KF (1.05 g, 18.1 mmol), dioxane (50 mL), and EtOH (10 mL) was stirred at room temperature for 3 h. The reaction mixture concentrated to dryness, poured into 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 $CHCl_3$ to give 1.10 g (54%) of **44d**, mp 228−231 °C (dec). MS (*m*/*z*): 326 (MH^+). ^1H NMR ($CDCl_3$) δ: 1.41 (t, 3 H, J = 7.0 Hz), 3.33 (s, 1 H), 4.42 (q, 2 H, J = 7.0 Hz), 7.36 (d, 1 H, J = 3.5 Hz), 7.66 (d, 1 H, J = 8.0 Hz), 7.73 (d, 1 H, J = 3.5 Hz), 8.80 (d, 1 H, J = 8.0 Hz), 10.06 (s, 1 H).

1,4-Dihydro-7-ethynyl-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid (45d). Following the procedure for **45a**, **45d** was prepared from **44d** in 47% yield, mp >300 °C. MS (*m*/*z*): 298 (MH^+). IR (KBr) cm^{-1}: 1737, 1615. ^1H NMR (DMSO-d_6) δ: 3.34 (s, 1 H), 7.85 (d, 1 H, J = 3.7 Hz), 7.88 (d, 1 H, J = 3.7 Hz), 7.93 (d, 1 H, J = 8.2 Hz), 8.81 (d, 1 H, J = 8.2 Hz), 9.98 (s, 1 H). Anal. ($C_{14}H_7N_3O_3S\cdot0.25H_2O$) C, H, N, S.

Ethyl 1,4-Dihydro-4-oxo-7-(3-pyrazolyl)-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylate (44e). To a solution of **44d** (0.34 g, 1.05 mmol) in $CHCl_3$ (20 mL) was added dropwise trimethylsilyldiazomethane (2 M in hexane, 1.05 mL, 2.10 mmol) at room temperature. After the mixture was stirred at 70 °C for 5 h, most of the solvent was concentrated to dryness to afford a crude product, which was recrystallized from EtOH to give 0.31 g (80%) of **44e**, mp 285−287 °C (dec). MS (*m*/*z*): 368 (MH^+). ^1H NMR (DMSO-d_6) δ: 1.33 (t, 3 H, J = 7.2 Hz), 4.31 (q, 2 H, J = 7.2 Hz), 7.22 (d, 1 H, J = 1.9 Hz), 7.78 (d, 1 H, J = 3.5 Hz), 7.84 (d, 1 H, J = 3.5 Hz), 7.73 (d, 1 H, J = 3.5 Hz), 8.01 (d, 1 H, J = 1.9 Hz), 8.25 (d, 1 H, J = 8.0 Hz), 8.68 (d, 1 H, J = 8.0 Hz), 9.87 (s, 1 H), 13.5 (br s, 1 H).

1,4-Dihydro-4-oxo-7-(3-pyrazolyl)-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic Acid (45e). A mixture of **44e** (0.18 g, 0.49 mmol), 0.5 N NaOH (2.83 mL, 1.42 mmol), and EtOH (0.2 mL) was stirred at room temperature for 1 day. The reaction mixture was acidified with 10% HCl. The resulting precipitates were collected by filtration, washed with water, EtOH, and *i*-Pr_2O successively, and then dried to give 0.12 g (73%) of **45e**, mp >300 °C. MS (*m*/*z*): 340 (MH^+). IR (KBr) cm^{-1}: 1731, 1616. ^1H NMR (DMSO-d_6) δ: 7.28 (d, 1 H, J = 1.9 Hz), 7.88 (d, 1 H, J = 3.5 Hz), 7.91 (d, 1 H, J = 3.5 Hz), 7.73 (d, 1 H, J = 3.5 Hz), 8.04 (d, 1 H, J = 1.9 Hz), 8.36 (d, 1 H, J = 8.0 Hz), 8.82 (d, 1 H, J = 8.0 Hz), 10.03 (s, 1 H), 13.6 (br s, 1 H). Anal. ($C_{15}H_9N_5O_3S\cdot0.25H_2O$) C, H, N, S.

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 leukemia, human HL-60 leukemia, human Hs746T stomach carcinoma, human PANC-1 pancreas carcinoma, human SBC-3 lung tumor, human SKOV3 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) and 50 units/mL of penicillin and 50 μg/mL of streptomycin. HL-60 cells were grown in RPMI 1640 with 20% FBS. Hs746T cells and PANC-1 cells were maintained in Dulbecco's modification of Eagle's medium with 10% FBS. SBC-3 cells were cultured in RPMI 1640 with 10% FBS. SKOV3 cells were grown in RPMI 1640 with 10% heat-inactivated FBS. SCaBER cells were maintained in EMEM supplemented with 10% FBS and nonessential amino acids.

In Vitro Assay. Cells ((1−2) × 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 in each well, and the cells were cultured for a further 4 h. The medium was removed by suction, and 0.2 mL of DMSO was put in 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 (median survival time of treated group)/(median survival time of control group) × 100.

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