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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Referencia : Expediente No. 07-121967-00005-0000
Asunto : Solicitud de patente para: "Derivado de 3-aminopirrolidina tri-tetra-sustituido".
Solicitante : DAIICHI SANKYO COMPANY, LIMITED
Fecha de solicitud: 19 de Noviembre de 2007.

1. Yo, Luz Clemencia de PAÉZ, mayor de edad, domiciliada en Bogotá, D.C., identificada con la Cédula de ciudadanía N° 35.456.344 de Usaquén, obrando como apoderada del solicitante en el asunto antes identificado, de acuerdo con la sustitución de poder a mi otorgada por el Doctor Emilio Ferrero, me refiero al Oficio No. 11076 notificado por fijación en lista del 15 de Julio de 2011 y atentamente presento junto con este escrito el siguiente artículo:

- TSUZUKI YASUNORI ET AL: "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 "JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, US, vol.47, no.8, 8 April 2004 (2004-04-08), pages 2097-2109. XP002475250 ISSN:0022-2623 (retrieved on 2004-03-06).

2. Puesto que se han atendido a cabalidad las peticiones hechas por su Despacho sobre la presente solicitud de patente, atentamente solicito que se continúe su tramitación.

3. ANEXOS.

- Copia auténtica la sustitución del poder otorgado por el Doctor EMILIO FERRERO, a mi favor, para lo cual solicito me sea reconocida personería y así continuar con el trámite de este asunto en favor de la sociedad solicitante.

Señor Director,

Luz Clemencia de Paéz
LUZ CLEMENCIA DE PAEZ

C.C. N° 35.456.344 de Usaquén
T.P.A. No. 23.555

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E. S. D.

Poderdante : DAIICHI SANKYO COMPANY, LIMITED

Poder conferido el : 11/05/2007

Yo, EMILIO FERRERO, obrando como apoderado principal del poderdante arriba mencionado, mediante el presente escrito sustituyo el poder a mi otorgado a la doctora LUZ CLEMENCIA DE PAEZ, identificada con cédula de ciudadanía No. 35.456.344 y Tarjeta Profesional N°23.555, con todas las facultades que me fueron conferidas.

Hago la anterior sustitución conforme al artículo 66 del Código de Procedimiento Civil.

EMILIO FERRERO

•T.P.A. N° 31.929

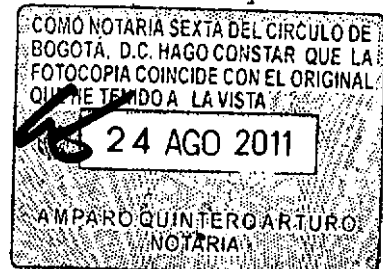
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Acepto:

LUZ CLEMENCIA DE PAEZ

T.P.A. N° 23.555

C.C. No. 35.456.344 de Usaquén



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Comparado(aron) *Emilio Ferrero*
Williamson + Luz Clemencia
Suarez de Paez

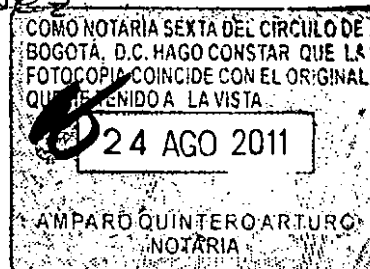
Quien(es) exhibió(aron) la(s) C.C.

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 que el contenido del mismo es cierto.

En constancia se firma esta diligencia.

Emilio Ferrero
Luz Clemencia Suarez de Paez



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
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Received October 3, 2003

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 (**271**) 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 **271**, 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 WIN57294⁷) and quinoben-zoxazine (A-62176⁸ and A-85226⁹). 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**,

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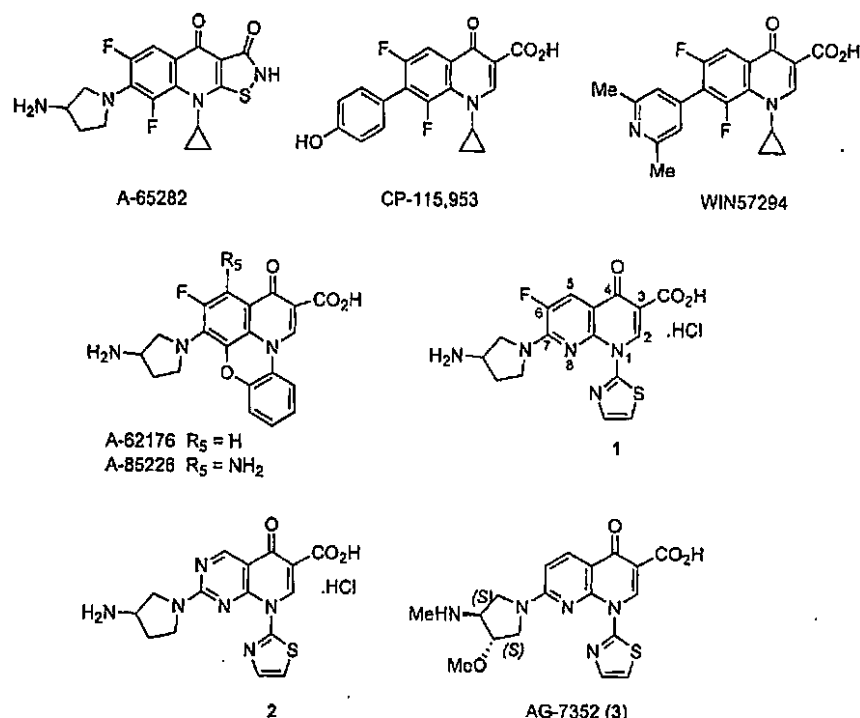
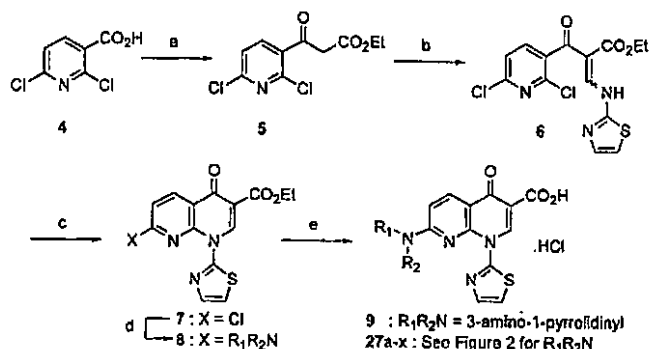
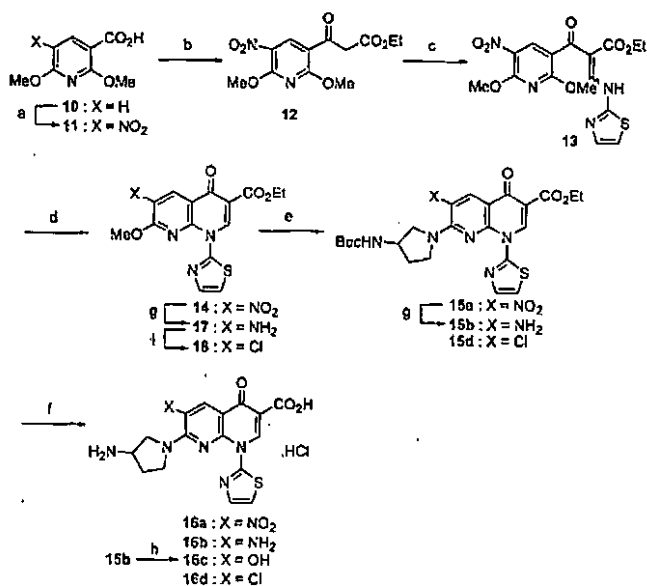


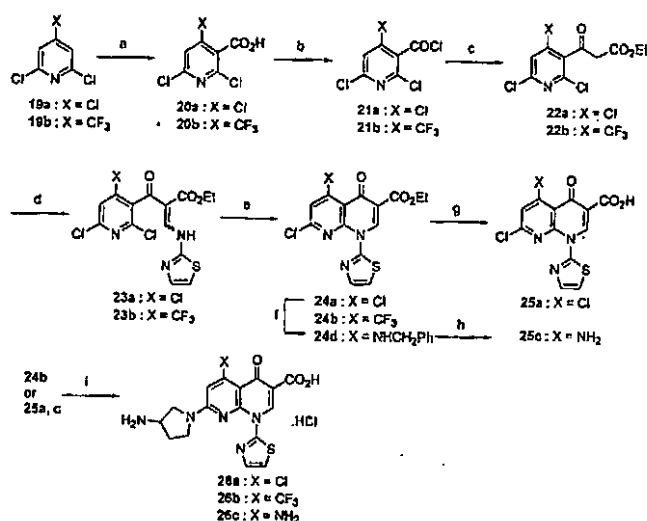
Figure 1. Structures of AG-7352 and reference compounds.

Scheme 1^a

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 substituent 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

Scheme 2^a

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) CO₂; (b) SOCl₂; (c) EtOCOCH₂COOH, MeMgBr; (d) (1) (EtO)₃CH, Ac₂O, (2) 2-aminothiazole; (e) K₂CO₃; (f) benzylamine; (g) H₂SO₄-AcOH-H₂O; (h) (1) H₂SO₄, AcOH (2) H₂O; (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, CF₃, and NH₂ 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-(trifluoromethyl)pyridine 19b with *n*-BuLi at -78 °C followed by carboxylation of the resulting carbanion with CO₂ gave the nicotinic acids 20a and 20b, respectively. Reaction of the nicotinoyl chlorides 21a and 21b, prepared from 20a and 20b with SOCl₂, 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 enaminoketones 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 H₂SO₄-AcOH-H₂O 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 H₂SO₄ 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.

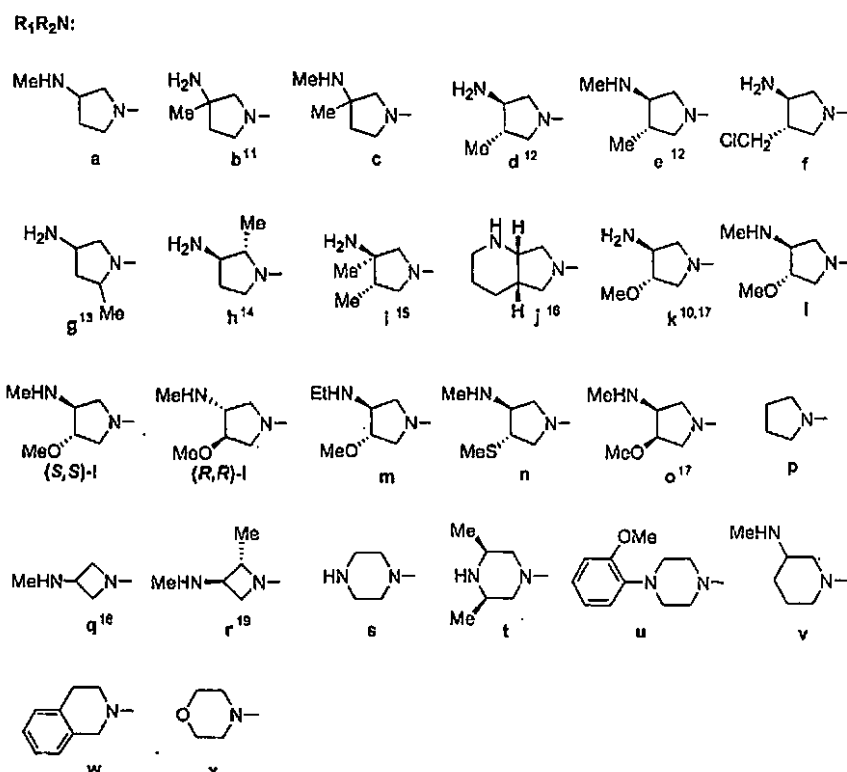
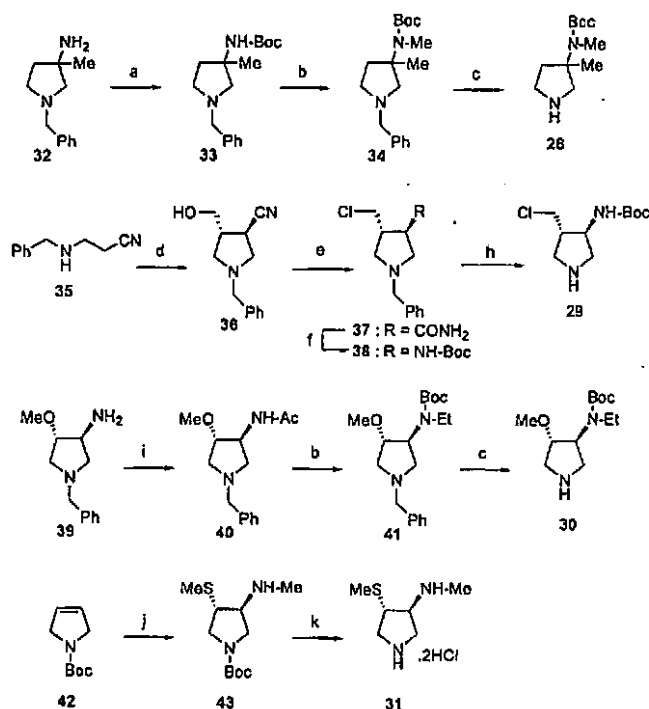
By a manner similar to that for the synthesis of 9, various kinds of amines as shown Figure 2 were incorporated into the C-7 position of the ester 7 to provide the desired products 27a-x after hydrolysis of the ester and the protecting group (Scheme 1). When diamines were used in the coupling reaction 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 32¹¹ was treated with Boc₂O 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 Boc₂O. 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 NaNH₂ 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 Boc₂O. 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 39²² 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 Boc₂O. Removal of the benzyl group of 41 was achieved by hydrogenation to give the desired pyrrolidine derivative 30. Addition of methanesulfonyl 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

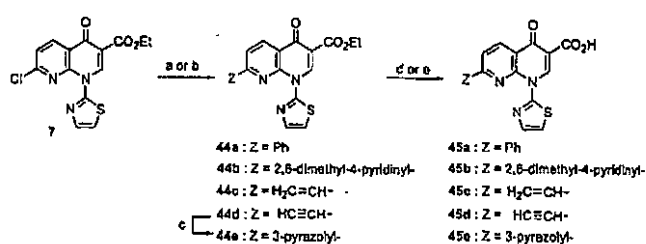
To study the cytotoxic activity of some analogues possessing a C-C bond at position 7, compounds 45a-e were synthesized by the route depicted in Scheme 5. The overall yields of 45a-e were calculated from 7 and are listed in Table 3 along with their *in vitro* cytotoxic activity data against murine P388 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 44c. 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 ; (g) ammonium formate, $Pd-C$; (h) Ac_2O ; (i) (1) $MeSCl$, (2) $MeNH_2$; (j) 30% $HCl-EtOH$.

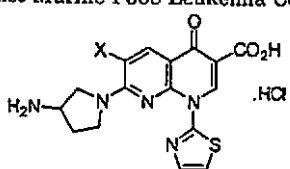
Results and Discussion

In the field of quinolone antibacterials, introduction of a fluorine atom into the C-6 position opened new era of fluoroquinolone antibacterials. It has also been shown that the optimum substituent at the C-6 position for antibacterial activity was a fluorine atom in both

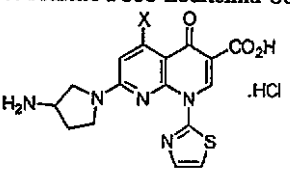
Scheme 5^a

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.

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	$IC_{50},^a$ $\mu\text{g/mL}$
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	$IC_{50},^a$ $\mu\text{g/mL}$
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: $\text{H} > \text{F} > \text{Cl} > \text{NH}_2 > \text{NO}_2 > \text{OH}$. On the other hand, the antibacterial activity order was as follows: $\text{F} > \text{Cl} > \text{H} > \text{NH}_2 > \text{NO}_2 = \text{OH}$, for example, against *Staphylococcus aureus* 209P JC-1 ($\text{MIC}_{50} = 0.39, 1.56, 3.13, 50, >100$, and >100 $\mu\text{g/mL}$, respectively) and *Escherichia coli* NIHJ JC-2 ($\text{MIC}_{50} = 0.025, 0.2, 0.39, 1.56, >100$, and >100 $\mu\text{g/mL}$, 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-unsubstituted 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

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	$IC_{50},^b$ $\mu\text{g/mL}$
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.

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 $\mu\text{g/mL}$.

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 monocyclic 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 μ g/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 μ g/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

Table 4. *In Vivo* Antitumor Activity of Selected Compounds against Murine P388 Leukemia Cells^a

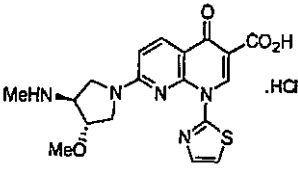
compd	dose, mg/kg	<i>T/C</i> , ^b %	solubility, ^c mg/mL
9	3.13	188	0.0069
	12.5	275	
	50	125	
27a	3.13	200	0.0080
	12.5	300	
	50	>375	
27b	3.13	150	0.0038
	12.5	213	
	50	138	
27c	3.13	163	0.0072
	12.5	225	
	50	113	
27d	3.13	175	0.017
	12.5	200	
	50	288	
27e	3.13	188	0.0062
	12.5	288	
	50	75	
27k	3.13	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) $\times$ 100. ^c Solubility measured in a pH 7.2 phosphate buffer.

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.

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 μ g/mL and IC_{50} = 0.0075 vs 0.011 μ g/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 271 and Its Optical Isomers against Murine P388 Leukemia Cells^a


compd	dose, mg/kg	T/C, ^b %	solubility, ^c mg/mL
271	0.78	150	20.1
	1.56	163	
	3.13	200	
	6.25	225	
	12.5	250	
	25	300	
(S,S)-271	0.78	150	23.7
	1.56	188	
	3.13	225	
	6.25	275	
	12.5	>375	
	25	>375	
(R,R)-271	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) $\times$ 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 ($IC_{50} = 3.2 \mu\text{g/mL}$), whereas it did not induce topoisomerase II cleavage ($IC_{50} > 1000 \mu\text{g/mL}$).²⁴ The IC_{50} values of etoposide were > 1000 and $1 \mu\text{g/mL}$ 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 struc-

tures 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 271 had remarkably high water solubility. Synthesis and in vitro/in vivo evaluation of both optical isomers of the racemic compound 271 revealed that (S,S)-271 has great cytotoxic activity against murine and human tumor cell lines with high water solubility. Finally the free base 3 of (S,S)-271, 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. ¹H 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, 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 2,6-dichloronicotinic acid 4 (401 g, 2.10 mol) in THF-CH₃CN (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 imidazolid solution was used without purification in the next step. To a solution of ethyl malonate potassium salt (376 g, 2.20 mol) in CH₃CN (3.3 L) was added dropwise MgCl₂ (297 g, 3.10 mol) and Et₃N (880 mL, 6.30 mol) under ice-cooling. After the mixture was stirred at room temperature for 5 h, the imidazolid prepared in the above was added. The reaction mixture was stirred at room temperature for 15 h, poured into ice-water, acidified to pH 5–6 with concentrated HCl, and then extracted with AcOEt. The organic layer was dried over Na₂SO₄ 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. ¹H NMR (CDCl₃) δ : 1.25 (t, 3 H $\times$ 1/2, $J = 7$ Hz), 1.35 (t, 3 H $\times$ 1/2, $J = 7$ Hz), 4.09 (s, 2 H $\times$ 1/2), 4.20 (q, 2 H $\times$ 1/2, $J = 7$ Hz), 4.30 (d, 1 H $\times$ 1/2, $J = 7$ Hz), 5.73 (s, 1 H $\times$ 1/2), 7.36 (d, 1 H $\times$ 1/2, $J = 8.5$ Hz), 7.40 (d, 1 H $\times$ 1/2, $J = 8.5$ Hz), 7.92 (d, 1 H $\times$ 1/2, $J = 8.5$ Hz), 7.98 (d, 1 H $\times$ 1/2, $J = 8.5$ Hz), 12.54 (s, 1 H $\times$ 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*-Pr₂O (500 mL), and

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

compd	IC ₅₀ , ^a µg/mL					
	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
stoptoside	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*-Pr₂O, 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 (CDCl₃) δ: 0.94 (t, 3 H, *J* = 7 Hz), 1.13 (t, 3 H, *J* = 7 Hz), 4.08 (q, 2 H, *J* = 7 Hz), 4.13 (q, 2 H, *J* = 7 Hz), 7.02 (d, 1 H, *J* = 3.8 Hz), 7.08 (d, 1 H, *J* = 3.7 Hz), 7.34 (d, 1 H, *J* = 8 Hz), 7.48 (d, 1 H, *J* = 3.8 Hz), 7.53 (d, 1 H, *J* = 3.7 Hz), 7.58 (d, 1 H, *J* = 8 Hz), 7.68 (d, 1 H, *J* = 8 Hz), 8.81 (d, 1 H, *J* = 13 Hz), 8.94 (d, 1 H, *J* = 2/3, *J* = 13 Hz), 11.62 (d, 1 H, *J* = 13 Hz), 12.80 (d, 1 H, *J* = 2/3, *J* = 13 Hz). Anal. (C₁₄H₁₁Cl₂N₃O₅S) 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 K₂CO₃ (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 CHCl₃ and *i*-Pr₂O 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 (CDCl₃) δ: 1.43 (t, 3 H, *J* = 6.5 Hz), 4.45 (q, 2 H, *J* = 6.5 Hz), 7.38 (d, 1 H, *J* = 3.5 Hz), 7.52 (d, 1 H, *J* = 8.5 Hz), 7.75 (d, 1 H, *J* = 3.5 Hz), 8.78 (d, 1 H, *J* = 8.5 Hz), 10.00 (s, 1 H). Anal. (C₁₄H₁₀ClN₃O₅S) 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 CH₃CN (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 CHCl₃-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, 1 H, *J* = 9 Hz), 7.72 (d, 1 H, *J* = 3.5 Hz), 7.79 (d, 1 H, *J* = 3.5 Hz), 8.21 (d, 1 H, *J* = 9 Hz), 9.63 (s, 1 H). Anal. (C₁₈H₁₉N₅O₅S·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. (C₁₆H₁₅N₅O₅S·HCl·0.5H₂O) C, H, Cl, N, S.

2,6-Dimethoxy-5-nitronicotinic Acid (11). To a mixture of concentrated HNO₃ (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*-Pr₂O 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 imidazolidine solution was used without purification in the next step. To a suspension of ethyl malonate potassium salt (18.6 g, 109 mmol) and MgCl₂ (11.3 g, 118 mmol) in AcOEt (250 mL) was added dropwise Et₃N (28.0 g, 277 mmol) under ice-cooling. After the mixture was stirred for 2.5 h at room temperature, the imidazolidine 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 Na₂SO₄ and concentrated to dryness to afford a crude product, which was recrystallized from a mixture of AcOEt and *i*-Pr₂O 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 (CDCl₃) δ: 1.01 (t, 3 H, *J* = 7 Hz), 1.21 (t, 3 H, *J* = 3/4, *J* = 7 Hz), 4.00 (s, 3 H, *J* = 3/4), 4.02 (s, 3 H, *J* = 1/4), 4.08–4.21 (m, 2 H), 4.14 (s, 3 H, *J* = 1/4), 4.16 (s, 3 H, *J* = 3/4), 6.95 (d, 1 H, *J* = 4 Hz), 7.02 (d, 1 H, *J* = 3/4, *J* = 4 Hz), 7.48 (d, 1 H, *J* = 3/4, *J* = 4 Hz), 7.78 (d, 1 H, *J* = 1/4, *J* = 4 Hz), 8.49 (d, 1 H, *J* = 1/4, *J* = 13 Hz), 8.55 (s, 1 H, *J* = 3/4), 8.70 (s, 1 H, *J* = 1/4), 8.75 (d, 1 H, *J* = 3/4, *J* = 13 Hz), 11.20 (d, 1 H, *J* = 1/4, *J* = 13 Hz), 12.50 (d, 1 H, *J* = 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 K₂CO₃ (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*-Pr₂O 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 K₂CO₃ (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 Na₂SO₄ 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, 1 H), 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. (C₂₅H₂₈N₆O₇S) 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. ($C_{18}H_{14}N_6O_5S \cdot HCl \cdot 0.25H_2O$) 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. ($C_{28}H_{28}N_6O_5S$) 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. ($C_{18}H_{16}N_6O_5S \cdot HCl$) 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. ($C_{18}H_{16}N_6O_5S \cdot HCl \cdot 0.5H_2O$) 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, 1 H, $J = 3.5$ 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 NaNO₂ (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 alkalinized to pH 8 with 20% NaOH and extracted with CHCl₃. The organic layer was dried over Na₂SO₄ and concentrated to dryness to afford a crude product, which was recrystallized from CHCl₃-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, 1 H, $J = 3.5$ 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 CH₃CN (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 CHCl₃-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 (CDCl₃) 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. ($C_{18}H_{14}ClN_6O_5S \cdot HCl \cdot 0.5EtOH$) C, H, Cl, N, S.

2,4,6-Trichloronicotinic 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 Na₂SO₄ and concentrated to dryness to afford a crude product, which was washed with *i*-Pr₂O 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 SOCl₂ (25 mL) was heated to reflux for 3 h and cooled to room temperature. After excess SOCl₂ 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 Et₂O, 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 CHCl₃. The organic layer was dried over Na₂SO₄ 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 (CDCl₃) δ : 1.26 (t, 3 H $\times$ 1/2, $J = 7$ Hz), 1.34 (t, 3 H $\times$ 1/2, $J = 7$ Hz), 3.92 (s, 2 H $\times$ 1/2), 4.22 (q, 2 H $\times$ 1/2, $J = 7$ Hz), 4.30 (d, 1 H $\times$ 1/2, $J = 7$ Hz), 5.28 (s, 1 H $\times$ 1/2), 7.40 (s, 1 H $\times$ 1/2), 7.42 (s, 1 H $\times$ 1/2), 12.23 (s, 1 H $\times$ 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 K₂CO₃ (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 CHCl₃. The organic layer was dried over Na₂SO₄ and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with CHCl₃-MeOH (200:1) and recrystallized from CHCl₃ 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 H₂SO₄-AcOH-H₂O (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), Et₃N (0.31 g, 3.07 mmol), and CH₃CN (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, 1H), 3.4–4.0 (m, 5H), 7.01 (s, 1H), 7.80 (d, 1H, *J* = 3.5 Hz), 7.85 (d, 1H, *J* = 3.5 Hz), 8.2 (br s, 3H), 9.75 (s, 1H), 14.3 (br s, 1H). Anal. (C₁₆H₁₄ClN₅O₃S·HCl·0.75H₂O·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/D₂O) δ: 1.6–1.9 (m, 1H), 2.0–2.3 (m, 1H), 2.7–2.8 (m, 1H), 2.9–3.1 (m, 1H), 3.1–3.3 (m, 1H), 3.3–3.6 (m, 2H), 6.52 (d, 1H, *J* = 7.0 Hz). Anal. (C₁₇H₁₄F₃N₅O₃S·HCl·0.25H₂O) 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), Et₃N (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 (CDCl₃) δ: 1.42 (t, 3H, *J* = 7 Hz), 4.41 (q, 2H, *J* = 7 Hz), 4.49 (d, 1H, *J* = 6.5 Hz), 6.47 (s, 1H), 7.31 (d, 1H, *J* = 3.5 Hz), 7.32–7.40 (m, 5H), 7.70 (d, 1H, *J* = 3.5 Hz), 9.87 (s, 1H), 11.2–11.7 (m, 1H).

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), H₂SO₄ (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 H₂O (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 H₂O, 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, 1H), 3.5–4.1 (m, 5H), 5.64 (s, 1H), 7.74 (d, 1H, *J* = 3.5 Hz), 7.81 (d, 1H, *J* =

3.5 Hz), 8.5 (br s, 3H), 9.68 (s, 1H). Anal. (C₁₆H₁₆N₆O₃S·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*)-271). To a solution of (*S,S*)-3-methoxy-4-methylaminopyrrolidine di-*p*-toluenesulfonic acid^{21,22} (85.0 g, 179 mmol) in CH₃CN (700 mL) was added Et₃N (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 CH₃CN 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. [α]_D²⁵ –16.9° (c 0.50, CHCl₃). MS (*m/z*): 430 (MH⁺). IR (KBr) cm⁻¹: 3350, 1730, 1630. ¹H NMR (CDCl₃) δ: 1.42 (t, 3H, *J* = 6.8 Hz), 2.54 (s, 3H), 3.44 (s, 3H), 3.6–4.2 (m, 6H), 4.41 (q, 2H, *J* = 6.8 Hz), 6.51 (d, 1H, *J* = 8.7 Hz), 7.27 (d, 1H, *J* = 3.5 Hz), 7.69 (d, 1H, *J* = 3.5 Hz), 8.46 (d, 1H, *J* = 8.7 Hz), 9.80 (s, 1H). Anal. Calcd for C₂₀H₂₂N₅O₄S: 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*-Pr₂O successively, and dried to give 10.9 g (97%) of (*S,S*)-271, mp 278–284 °C (dec). [α]_D²⁷ +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, 3H), 3.42 (s, 3H), 3.5–4.4 (m, 6H), 7.03 (d, 1H, *J* = 9 Hz), 7.84 (d, 1H, *J* = 3.5 Hz), 7.90 (d, 1H, *J* = 3.5 Hz), 8.45 (d, 1H, *J* = 9 Hz), 9.3 (br s, 2H), 9.85 (s, 1H). Anal. (C₁₈H₁₉N₅O₄S·HCl·0.25H₂O) C, H, Cl, N, S.

According to a similar procedure for 9 and (*S,S*)-271, compounds 27a–x and (*R,R*)-271 were prepared.

1-Benzyl-3-(*N*-tert-butoxycarbonyl)methylamino-3-methylpyrrolidine (34). To a solution of 3-amino-1-benzyl-3-methylpyrrolidine¹¹ 32 (20.0 g, 105 mmol) in CH₂Cl₂ (200 mL) was added Boc₂O (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 Na₂SO₄ and concentrated to dryness. The resulting residue was diluted with CH₂Cl₂ (100 mL), and Boc₂O (8.6 g, 39.4 mmol) in CH₂Cl₂ (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), K₂CO₃ (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-methoxypyrrolidine²² 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*-Pr₂O, 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 ($\text{DMSO}-d_6$) 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*-Pr₂O, 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 ($\text{DMSO}-d_6$) 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. ($\text{C}_{18}\text{H}_{12}\text{N}_4\text{O}_3\text{S}\cdot 0.5\text{H}_2\text{O}$) 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 ($\text{DMSO}-d_6$) 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. ($\text{C}_{14}\text{H}_7\text{N}_3\text{O}_3\text{S} \cdot 0.25\text{H}_2\text{O}$) 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 ($\text{DMSO}-d_6$) 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*-Pr₂O 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 ($\text{DMSO}-d_6$) 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. ($\text{C}_{15}\text{H}_9\text{N}_5\text{O}_3\text{S} \cdot 0.25\text{H}_2\text{O}$) 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 $\mu\text{g/mL}$ 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 ($(1-2) \times 10^4$ 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 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 BDF₁ 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) $\times 100$.

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