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Synthesis and structure–activity relationships of 3-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridines as novel antitumor agents

Yasunori Tsuzuki,* Kyoji Tomita, 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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Abstract—In order to obtain clinically useful antitumor agent, we have designed and synthesized various 3-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridines, and evaluated their cytotoxic activity. The series of novel 3-substituted derivatives synthesized in this study showed good antitumor activity against murine P388 leukemia. Particularly, the 3-formyl 1,8-naphthyridine displayed an antitumor activity equal to that of the 3-carboxy 1,8-naphthyridine against murine and human tumor cell lines as well as in vivo test for mouse leukemia. These results demonstrate that the carboxy group at the C-3 position of 1,8-naphthyridine ring is not essential for antitumor activity. In addition, the trend of cytotoxic activity for the 3-substituted 1,8-naphthyridines was different from that of antibacterial activity.

1. Introduction

DNA topoisomerases are a group of ubiquitous enzymes that are essential for cell survival and proliferation in both prokaryotic and eukaryotic organisms. These enzymes catalyze the interconversion of different topological forms of DNA through concerted sequential DNA breaking–passing–resealing processes.[1] The indispensable nature of these enzymes makes them target of choice for both potent antitumor and broad-spectrum antibacterial agents. While antitumor agents, such as etoposide, doxorubicin, and amsacrine target mammalian type II DNA topoisomerase,[2] quinolone antibacterials are potent broad-spectrum drugs that selectively target bacterial type II DNA topoisomerase.[3] Although some quinolone-related compounds have been considered to inhibit mammalian topoisomerase II and exhibit antitumor activity,[4] none of these compounds has, however, reached clinical trial as antitumor agent.

In a previous paper, we have reported that the 7-substituted 6-fluoro-l-(2-thiazolyl)-1,8-naphthyridine nucleus serves as a unique scaffold for finding new clinically useful quinolones.[5] Thus, the 7-(3-aminopyrrolidinyl)-l,4-dihydro-4-oxo-l-(2-thiazolyl)-l,8-naphthyridine-3-carboxylic acid (**1a**, Fig. 1) has been shown to have good antitumor activity in vitro against murine and human tumor cell lines as well as in vivo against mouse leukemia. Moreover, modifications at the C-6 and C-7 positions of **1a** led us to the 6-unsubstituted 7-(*trans*-3-methoxy-4-methylamino)pyrrolidine derivative **1b**, which possesses a more potent cytotoxic activity than **1a**.[6] The (*S*,*S*)-isomer of **1b** (AG-7352) is presently under development as a drug candidate.

In the course of our search for more potent antitumor agents, we focused our interest on modification of the carboxy group at the C-3 position of **1**. In the field of quinolone antibacterials, the carboxy group at the C-3

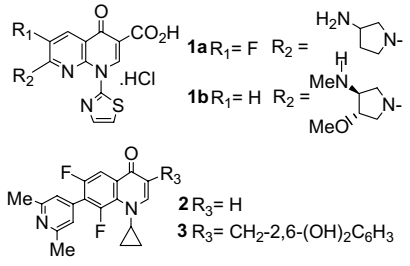

Figure 1. Structures of reference compounds.

* Corresponding author. Tel.: +81-6-6337-5900; fax: +81-6-6338-7656; e-mail: yasunori-tuduki@dainippon-pharm.co.jp

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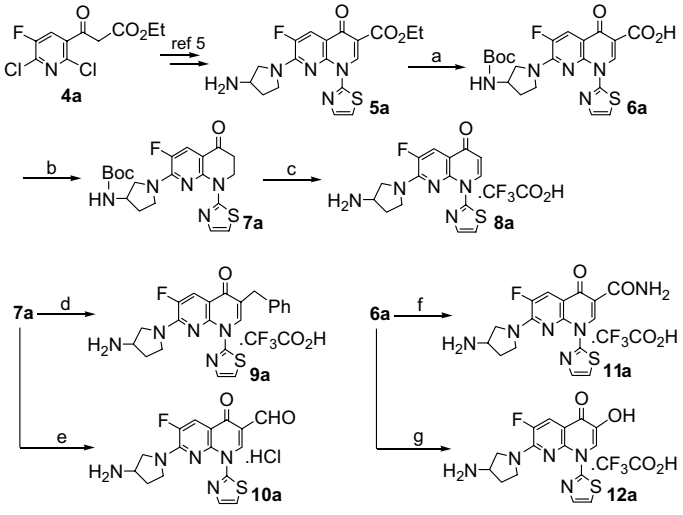
position in both the quinolone and naphthyridone series has been accepted as an indispensable functional moiety for antibacterial activity.[7] On the other hand, in the field of antitumor quinolones, the precise role of the carboxy group at the C-3 position has not been clearly defined; it has only been reported that the 3-descarboxy **2** and the 3-(2,6-dihydroxybenzyl) **3** quinolones (Fig. 1) exhibit antitumor activity.[4c,f] Thus, in this study, we synthesized various 1,8-naphthyridines modified at the C-3 position of **1a**,**b** and evaluated their cytotoxic activity against murine P388 leukemia. Moreover, we assayed the synthesized compounds for their antibacterial activity against both Gram-positive and Gram-negative bacteria to examine whether the trend of cytotoxic activity for this position (C-3) is the same as that of antibacterial activity.

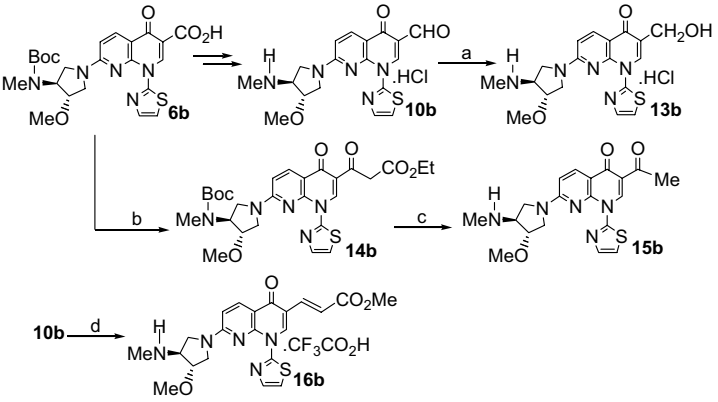
2. Chemistry

To study the effect of various substituents at the C-3 position of the 1,8-naphthyridine ring, compounds **5a**, **8a**–**12a**, and **10b**, **13b**, **15b**, **16b**, which have ethoxycarbonyl, hydro, benzyl, formyl, carbamoyl, hydroxyl, hydroxymethyl, acetyl, and 2-(methoxycarbonyl)vinyl groups at this position, respectively, were designed. A synthetic route to these analogs of **1a** is illustrated in Scheme 1. The 3-ethoxycarbonyl-1,8-naphthyridone **5a** was readily prepared in 56% yield for four steps from the nicotinoyl acetate **4a**.[5] Protection of **5a** with Boc_2O followed by base hydrolysis of the ester gave the 3-carboxy-l,8-naphthyridone **6a**. Reduction of **6a** using $NaBH_4$ along with decarboxylation afforded the desired 1,2,3,4-tetrahydro derivative **7a**. Oxidation of **7a** using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) followed by removal of Boc group afforded the 3-unsubstituted-1,8-naphthyridone **8a** as trifluoroacetic acid (TFA) salt. Aldol condensation of **7a** with benzaldehyde followed by removal of Boc group provided the 3-benzyl-1,8-naphthyridone **9a**. Treatment of **7a** with *n*-BuLi at −78 °C, followed by formylation of the resulting carbanion with ethyl formate gave the 3-formyl 1,2,3,4-tetrahydro-1,8-naphtyridone.[8] Without purification of this 3-formyl derivative, the 2,3-double bond was reintroduced by oxidation with DDQ to give the 3-formyl-1,8-naphthyridone, which after removal of Boc group gave the desired compound **10a**. Esterification of the 3-carboxy-1,8-naphtyridone **6a** using ethyl chloroformate in the presence of Et_3N followed by reaction of the activated ester with NH_3 and removal of Boc group gave the 3-carbamoyl-1,8-naphthyridone **11a**. Oxidation of **6a** using KO_2 followed by removal of Boc group afforded the 3-hydroxy-naphthyridone **12a**. The 3-hydroxy compound was presumably obtained through epoxidation of the 2,3-double bond,[9] ring-opening reaction of the epoxide and decarboxylation.

A synthetic route to the analogs of **1b** is illustrated in Scheme 2. By a similar manner to that for the synthesis of **10a**, the 3-formyl analog **10b** was readily prepared in 22% yield for four steps from the 3-carboxy-1,8-naphthyridone **6b**.[6] Selective reduction of the aldehyde **10b** to alcohol using $NaBH_4$ in the presence of $CeCl_3$ in water, followed by crystallization gave the 3-hydroxymethyl-1,8-naphthyridone **13b** as HC1 salt. Esterification of the carboxylic acid **6b** using benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (BOP) reagent[10] in the presence of Et_3N followed by reaction of the activated ester with potassium ethyl malonate in the presence of $MgCl_2$ and Et_3N gave the 3-(2-ethoxycarbonylacetyl)-1,8-naphthyridone **14b**. Decarboxylation of this β-keto ester derivative (**14b**), followed by crystallization gave the desired 3-acetyl-1,8-naphthyridone **15b** as free base. Reaction of the 3-formyl derivative **10b** with Horner–Emmons reagent, followed by crystallization afforded the 3-(*E*)-(2-methoxycarbonylethenyl)-1,8-naphthyridone **16b** as TFA salt.



Scheme 1. Reagents and conditions: (a) (1) Boc_2O, Et_3N, CH_2Cl_2, 94% (2) 1 N NaOH, EtOH, 95%; (b) $NaBH_4$, MeOH, 0 °C to rt, 53%; (c) (1) DDQ, 1,4-dioxane, 39% (2) CF_3CO_2H, CH_2Cl_2, 91%; (d) (1) PhCHO, NaOH, EtOH, 68% (2) CF_3CO_2H, CH_2Cl_2, 91%; (e) (1) *n*-BuLi, HCO_2Et, THF, −78 °C to rt (2) DDQ, 1,4-dioxane, 51% for two steps (3) 10% HCl, 81%; (f) (1) (i) $ClCO_2Et$, Et_3N, CH_2Cl_2, (ii) NH_3/EtOH, 54% (2) CF_3CO_2H, CH_2Cl_2, 88%; (g) (1) KO_2, EtOH–H_2O, 62% (2) CF_3CO_2H, CH_2Cl_2, 96%.



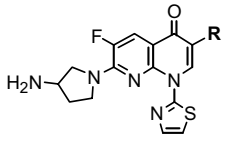
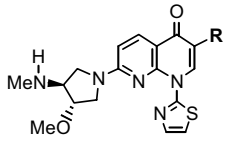
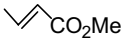
Scheme 2. Reagents and conditions: (a) (i) $NaBH_4$, $CeCl_3$, H_2O (ii) HCl/EtOH, 63%; (b) (i) BOP reagent, Et_3N, $CHCl_3$, rt, (ii) $MgCl_2$, Et_3N, potassium ethyl malonate, 29%; (c) (i) 10% HCl (ii) $NaHCO_3$, 71%; (d) (i) trimethyl phosphonoacetate, NaH, THF, (ii) CF_3CO_2H, EtOH, 73%.

3. Results and discussion

In the course of our search for novel antitumor agents, we have previously reported that 1,8-naphthyridones with 1-(2-thiazolyl) substituent have good antitumor activity both in vitro and in vivo assays.[5,6] In further research in this area, we synthesized, in this study, various 3-substituted 1,8-naphthyridones and evaluated their cytotoxic activity against murine P388 leukemia. The synthesized compounds and their cytotoxic activity are summarized in Table 1, along with data for the reference drugs etoposide and cisplatin. The lead compound **1a** with an IC_{50} value of 0.021 μg/mL exhibited a cytotoxic activity 2-fold less than that of cisplatin, and the 3-ester **5a** exhibited a cytotoxic activity 6-fold less than that of **1a**. The 3-unsubstituted analog **8a** had moderate cytotoxic activity, and the 3-benzyl analog **9a** had a cytotoxic activity 2.5-fold more potent than that of **8a**. The SARs trend of the 3-unsubstituted **8a** and the 3-benzyl **9a** was similar to that reported for the 6,8-difluoroquinolone analogs (**2** and **3**).[4c,f] The 3-formyl analog **10a**, a reduced derivative of **1a**, showed a cytotoxic activity similar to that of **1a**. In contrast, the 3-amide analog **11a** showed a cytotoxic activity 10-fold less than that of **1a**. Additionally, the 3-hydroxy analog **12a** had moderate cytotoxic activity similar to that of the 3-unsubstituted analog **8a**.

Next, we investigated SARs of 3-substituted analogs of compound **1b**, which has been shown to possess a

Table 1. Cytotoxic activity and antibacterial activity of C-3 substituted 1,8-naphthyridones

	Compd[a]	R	Cytotoxic activity (IC_{50},[b] μg/mL)	Antibacterial activity (MIC[c] μg/mL)	
			Murine lymphocytic leukemia P388	*S. aureus* 209 JC-1	*E. coli* NIHJ JC-2
	**1a**[d]	CO_2H	0.0211	0.39	0.025
	5a	CO_2Et	0.136	50	12.5
	8a[e]	H	0.175	12.5	12.5
	9a[e]	CH_2Ph	0.0718	50	100
	10a[d]	CHO	0.0212	6.25	3.13
	11a[e]	$CONH_2$	0.269	>100	>100
	12a[e]	OH	0.168	50	0.78
	**1b**[d]	CO_2H	0.0104	3.13	1.56
	10b[d]	CHO	0.0309	12.5	100
	13b[d]	CH_2OH	0.0551	25	>100
	15b	COMe	0.0593	6.25	>100
	16b[e]		0.0947	>100	>100
	Etoposide		0.0085	NT[f]	NT[f]
	Cisplatin		0.0110	NT[f]	NT[f]

[a] ^1H NMR, IR, and MS were consistent with the assigned structures of all new compounds. C, H, N, F, and S elemental analyses were obtained for all new targets and most intermediates and were within ±0.4% of the theoretical values.
[b] Concentration of agent to reduce cell viability by 50%.
[c] Minimum inhibitory concentration.
[d] HCl salt.
[e] TFA salt.
[f] NT, not tested.

cytotoxic activity twice that of the lead compound **1a**. Since the 3-formyl analog **10a** showed a cytotoxic activity similar to that of **1a**, the cytotoxic activity of the 3-formyl **10b**, 3-hydroxymethyl **13b**, and 3-ketone **15b** 1,8-naphthyridones with an oxygen atom at the α-position was evaluated. The 3-formyl analog **10b** exhibited a slightly decreased cytotoxic activity as compared to **10a**, and the 3-hydroxymethyl analog **13b** had less cytotoxic activity than **10b**. Also, the 3-ketone analog **15b** resulted in a decreased cytotoxic activity as compared to compound **10b**. Even the 3-vinyl analog **16b** possessing a 2-methoxycarbonylethenyl group had moderate cytotoxic activity. Thus, the series of novel 3-substituted derivatives synthesized in this study showed good antitumor activity against murine leukemia P388. Particularly, replacement of the 3-carboxy group with the 3-formyl group resulted in no substantial effect on the cytotoxic activity (**1a** vs **10a**), or only slightly decreased the cytotoxicity (**1b** vs **10b**).

The 3-substituted 1,8-naphthyridines were next assayed for their antibacterial activity against representatives of Gram-positive bacteria (*Staphylococcus aureus* 209P JC-1) and Gram-negative bacteria (*Escherichia coli* NIHJ JC-2) to examine whether the trend of cytotoxic activity for this position (C-3) is the same as that of antibacterial activity. As shown in Table 1, the 3-substituted 6-fluoro-1,8-naphthyridines **5a**, **8**–**12a** were far less active than **1a** against both Gram-positive and Gram-negative bacteria. In particular, the 3-formyl **10a** showed an antibacterial activity 16- to 125-fold less than that of the 3-carboxy **1a** even though compound **10a** had a cytotoxic activity similar to that of **1a**. Other 6-unsubstituted 1,8-naphthyridines **10b**, **13b**, **15b**, **16b** also had much less antibacterial activity than **1b**. In general, the decrease in antibacterial activity among the C-3 modified compounds was more significant than that in cytotoxic activity. This result demonstrates that the carboxy group at 3-position of 1,8-naphthyridone is necessary for antibacterial activity, while the 3-carboxy group is not essential for antitumor activity.

The 3-formyl 1,8-naphthyridines were next tested for their in vivo antitumor activity using mice implanted with P388 leukemia cells. Data for **10a**,**b** are summarized in Table 2, along with those for the 3-carboxy derivatives **1a**,**b** and reference drugs. The in vivo test consisted of intraperitoneal (i.p.) implantation of tumor cells, followed 1 day and 5 days later by i.p. treatment with each test-compound at doses of 3.13, 12.5, and 50 mg/kg. 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%).[5] The 3-carboxy analogs **1a**,**b** showed an in vivo antitumor activity equal to that of etoposide against murine P388 leukemia. Similarly, the 3-formyl analogs **10a**,**b** displayed potent in vivo antitumor activity with good efficacy at all doses. This efficacy was approximately similar to that of the corresponding 3-carboxy analogs **1a**,**b**.

Table 2. In vivo antitumor activity of **1a**, **10a**, **1b**, and **10b** against murine P388 leukemia[a]

Compd	Dose, mg/kg	T/C,[b] %
1a	3.13	150
	12.5	213
	50	>375
10a	3.13	150
	12.5	213
	50	>375
1b	3.13	200
	12.5	250
	50	>375
10b	3.13	188
	12.5	250
	50	325
Etoposide	3.13	175
	12.5	250
	50	>375

[a] See Ref. 5.
[b] (Median survival time of treated mice)/(median survival time of controls) × 100.

Finally, we evaluated the cytotoxic activity of the 3-formyl 1,8-naphythyridine **10b** against various types of human tumor cells. In general, compound **10b** displayed good cytotoxic activity with an efficacy equal to that of the 3-carboxy-1,8-naphythyridine **1b** (Table 3). In addition, compounds **10b** displayed a moderate in vivo antitumor activity against various human tumors implanted in nude mice (data not shown). However, considering an infusion drug as a preferable formulation, compound **10b** had regrettably a low water-solubility (0.069 mg/mL, pH 7.2 buffer).[11] Although further studies on compounds **10b** may be hampered, the knowledge obtained from this study could be applicable to the design of more useful antitumor agents.

In conclusion, we have designed and synthesized various 3-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridines in order to obtain clinically useful antitumor agents. The series of novel 3-substituted derivatives synthesized in this study showed good cytotoxic activity against murine P388 leukemia, but significantly decreased antibacterial activity against Gram-positive

Table 3. Cytotoxic activity of 3-formyl 1,8-naphythyridine **10b** against human tumor cell lines

Compd	IC_{50}, μg/mL[a]						
	A-427 lung	AZ-521 stomach	MKN45 stomach	HMV-2 melanoma	C-33A cervix	WiDr colon	KB nasopha
1b	0.0962	0.11	0.145	0.209	0.185	0.607	0.099
10b	0.0749	0.0307	0.144	0.214	0.161	0.264	0.179
Etoposide	0.095	0.080	0.49	0.298	0.084	1.63	0.201

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

and Gram-negative bacteria. These findings highlight a discrepancy between antitumor SARs and antibacterial SARs. The 3-formyl-1,8-naphthyridines displayed antitumor activity equal to that of 3-carboxy 1,8-naphthyridines against murine tumor cell lines as well as in vivo test for mouse leukemia. Moreover, the 3-formyl-1,8-naphthyridine **10b** displayed good activity against several human tumor cell lines. These results demonstrate that the carboxy group at the C-3 position of 1,8-naphthyridine ring is not essential for antitumor activity.

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