Synthesis of 6-Formyl-pyridine-2-carboxylate Derivatives and Their Telomerase Inhibitory Activities

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Abstract—Twenty-one pyridine-2-carboxylate derivatives were prepared by the coupling of 6-formyl-2-carboxylic acid with the corresponding phenol, thiophenol, and aniline, substituted with various functional groups. Among them, the 3,4-dichloro-thiophenol ester (**9p**) showed the highest in vitro telomerase inhibitory activity and quite significant in vivo tumor suppression activity.
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Telomeres are DNA-protein complexes at the ends of chromosomes, which play an essential protective role against DNA degradation and aberrant recombination during cell divisions.[1] They consist of tandem guanosine-rich nucleotide repeats, such as 5′-TTAGGG in humans.[2] They also act as a kind of marker for the number of cell divisions, which can control the cell number within the body. In normal somatic cells, the length of the telomere decreases by 50–200 nucleotides during each cell division.[3] After ca. 20 rounds of cell division, the telomere achieves a critical level, generating cellular senescence. However, male germ cells and 80–90% of tumor cells have infinite division capacities through the extension of telomeres by telomerase, which is one of the ribonucleoprotein reverse transcriptases.[4] Since telomerase is essential for the immortalization of tumor cells, it has become a new target for anticancer agents.[5]

Several telomerase inhibitors have been reported as candidates for new antitumor drugs. Oligonucleotide-type compounds,[6] porphyrins,[7] and cyclic polyoxazole[8] show potent telomerase inhibitory activities but their development as clinical drugs may be hindered by their high molecular weights. Smaller molecules, such as tricyclic amidoanthracene, acridine, and amidofluorenone derivatives,[9] pentacyclic acridinium salts,[10] and bisindole derivatives,[11] were introduced as moderate inhibitors. As part of our program to develop new antitumor drugs as small molecules, **1** (IC$_{50}$ = 1.0 μM) was chosen as a lead compound developed by Geron. Co. Ltd. as a telomerase inhibitor.[12] However, because the imine group in **1** is generally unstable against hydrolysis and the corresponding aldehyde, **2** (IC$_{50}$ = 7.0 μM) itself still preserves relatively high telomerase inhibitory activity, **2** was modified to develop more potent inhibitors. In this communication, we report the structure–activity relationship studies of 6-formyl-pyridine-2-carboxylate derivatives and their potential value as a new scaffold via an in vivo antitumor test.

First, we converted the thiobenzyl ether group of **2** to a carboxylate group, as **3**. The enhanced binding process between the carbonyl and the DNA nucleotide or amino residues in telomerase might increase the potency of the inhibitory activity. In addition, the modeling study

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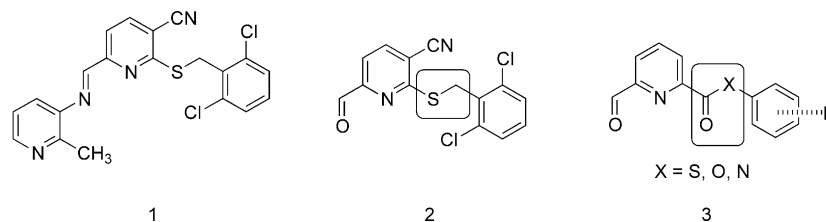
indicated that the conformations of **2** and **3** are quite similar (data not shown). Twenty-one 6-formyl-pyridine-2-carboxylate derivatives[13] were prepared from pyridine-2,6-dicarboxylic acid **4** in five steps (Scheme 1). The di-methylester formation of pyridine-2,6-dicarboxylic acid, followed by the mono-ester reduction with $NaBH_4$, gave the mono-alcohol **6**. The oxidation of **6** with MnO_2 and the following hydrolysis afforded the acid, **8**. Finally, the thioester, ester, and amide derivatives **9a–9u** were obtained by the coupling of **8** with the corresponding thiols (**9a–9s**), alcohol (**9t**), and amine (**9u**), respectively.

The telomerase inhibitory activities[14] of the prepared derivatives, **9a–9u**, along with that of **2**, are shown in Table 1. Most of the thioester derivatives showed comparable or higher activities than the reference compound, **2**. The *meta*-CH_3 and *meta*-CH_3O derivatives showed slightly higher activities than the *ortho*- and *para*-derivatives, respectively. Depending on the position substituted with the halide, quite wide variations in the activity were observed. The mono-chloro derivative series, *p*-Cl (**9h**, $IC_{50} = 33 \, \mu M$), shows 2-fold higher activity than both the *o*-Cl (**9l**, $IC_{50} = 84 \, \mu M$) and *m*-Cl

(**9m**, $IC_{50} = 79 \, \mu M$). The *p*-Cl (**9h**, $IC_{50} = 33 \, \mu M$) is a better substituent than *p*-F (**9g**, $IC_{50} = 64 \, \mu M$) and *p*-Br (**9i**, $IC_{50} = 56 \, \mu M$). This tendency is consistent with that of the di-halide cases (**9p**, $IC_{50} = 24 \, \mu M$; **9s**, $IC_{50} = 69 \, \mu M$). The number of chlorides on the aromatic ring is not directly related to the activity. The best result was obtained with the 3,4-dichloro derivative **9p** ($IC_{50} = 24 \, \mu M$). The conversion of the thioester to the ester (**9t**, $IC_{50} > 100 \, \mu M$) and the amide (**9u**, $IC_{50} > 100 \, \mu M$) abolished the activity, implying that the more hydrogen bonding abilities of O and NH, relatively to S or the more tautomerism of ester and amide, relatively to thioester led to unfavorable binding with the enzyme.

Before the in vivo antitumor activity test with the best derivative, **9p**, we needed to confirm that **9p** is not associated with cytotoxicity, which is a typical mechanism of most anticancer agents, such as camptothecin. The in vitro cytotoxicity[15] of **9p** was evaluated against several cancer cell lines. As shown in Table 2, neither of **9p** nor **2** showed significant cytotoxicity relative to camptothecin, suggesting that their antitumor mechanism would be different from cytotoxicity. An in vivo
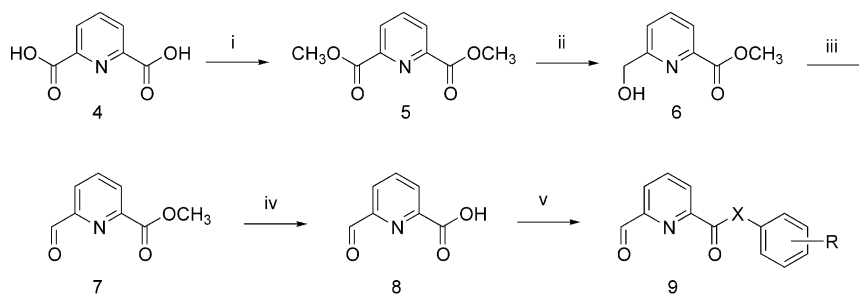
9a R = 2-CH_3, X = S	9l R = 2-Cl, X = S
9b R = 3-CH_3, X = S	9m R = 3-Cl, X = S
9c R = 4-CH_3, X = S	9n R = 2,5-Cl_2, X = S
9d R = 2-OCH_3, X = S	9o R = 2,6-Cl_2, X = S
9e R = 3-OCH_3, X = S	9p R = 3,4-Cl_2, X = S
9f R = 4-OCH_3, X = S	9q R = 3,5-Cl_2, X = S
9g R = 4-F, X = S	9r R = 2,4,6-Cl_3, X = S
9h R = 4-Cl, X = S	9s R = 3,4-F_2, X = S
9i R = 4-Br, X = S	9t R = 3,4-Cl_2, X = O
9j R = 4-NO_2, X = S	9u R = 3,4-Cl_2, X = N
9k R = 4-NHAc, X = S	

Scheme 1. Reagents and conditions: (i) $SOCl_2$, CH_3OH, reflux, 3 h, 100%; (ii) $NaBH_4$, CH_3OH, rt, 4 h, 85%; (iii) MnO_2, acetone, 74%; (iv) KOH, $THF/H_2O = 1{:}1$, 72%; (v) HOBT, EDC, Et_3N, the corresponding thiophenol, phenol or aniline derivatives, CH_2Cl_2, 50–80%.

Table 1. Telomerase inhibitory activities of 2-pyridine-carboxylate derivatives
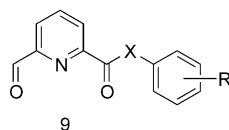
Compd	R	X	Telomerase activity[a] (IC$_{50}$, μM)	Compd	R	X	Telomerase activity[a] (IC$_{50}$, μM)
9a	2-CH$_3$	S	100<	**9l**	2-Cl	S	84
9b	3-CH$_3$	S	76	**9m**	3-Cl	S	79
9c	4-CH$_3$	S	100<	**9n**	2,5-Cl$_2$	S	68
9d	2-OCH$_3$	S	100<	**9o**	2,6-Cl$_2$	S	79
9e	3-OCH$_3$	S	76	**9p**	3,4-Cl$_2$	S	24
9f	4-OCH$_3$	S	91	**9q**	3,5-Cl$_2$	S	64
9g	4-F	S	64	**9r**	2,4,6-Cl$_3$	S	50
9h	4-Cl	S	33	**9s**	3,4-F$_2$	S	69
9i	4-Br	S	56	**9t**	3,4-Cl$_2$	O	100<
9j	4-NO$_2$	S	75	**9u**	3,4-Cl$_2$	N	100<
9k	4-NHAc	S	100<	**2**	—	—	77[b]

[a]The telomeric repeat amplification protocol (TRAP) assay was performed by a modification of the published method.[14] The telomerase products were resolved by 10% nondenaturing polyacrylamide gel electrophoresis, and were visualized by staining with SYBR Green (Molecular Probes, USA). Signal intensity was quantified with a LAS-1000 Plus Image analyzer (Fuji Film, Japan).
[b]The 10-fold lower potency in the IC$_{50}$ value, as compared to Geron's data (7.0 μM), might be due to the less sensitive detection method for the telomerase product in the TRAP assay.
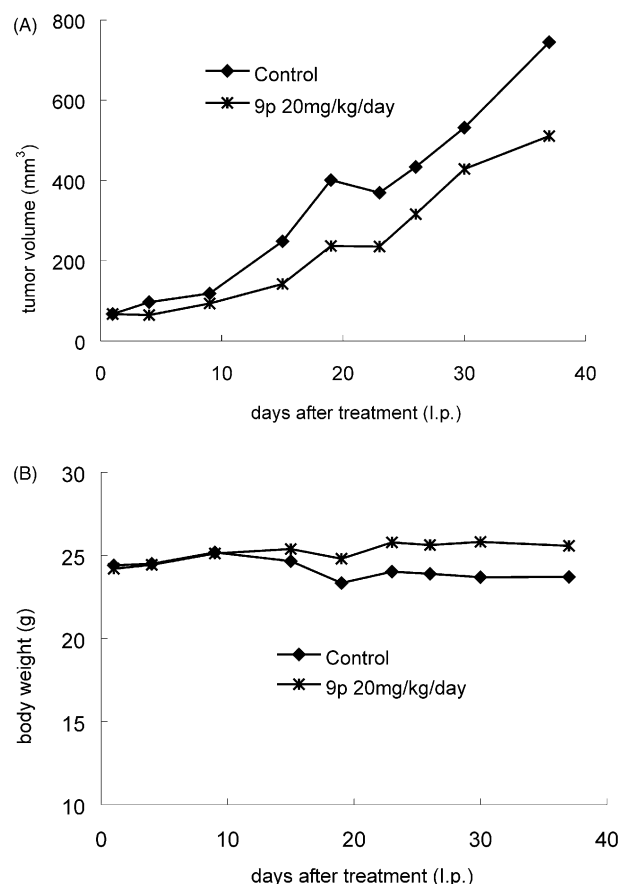
Table 2. In vitro cytotoxicity of **9p** using human cancer cell lines

Compd	Cytotoxicity[a] (IC$_{50}$, μM)				
	HT-29	Caki-2	A549	HEC-1-B	HL-60
Camptothecin	1.40	3.96	2.53	8.64	0.066
2	113.28	300<	300<	175.09	26.73
9p	33.09	300<	56.93	56.44	12.97

[a]In vitro antiproliferative activities of the analogues against five tumor cell lines (HT-29, human colon cancer; Caki-2, human renal cancer; A549, human lung cancer; HEC-1-B, human endometrial cancer; HL-60, human leukemia cancer) were measured by an SRB assay[15] after 3 days of incubation, and are expressed as the doses required to inhibit the growth of 50% of the cells cultivated (IC$_{50}$, μM).

Figure 1. Antitumor activity of **9p** in CX-1 human clone-bearing athymic nude mice: (A) tumor volume change; (B) body weight change.

antitumor activity assay[16] in CX-1 human clone-bearing athymic nude mice after the subcutaneous administration of **9p** was performed (Fig. 1). A clear tumor-suppressing effect, by 30% in tumor volume at the dose of 20 mg/kg/day, was observed after 37 days. Notably, there was no significant loss of body weight, which is normally observed with cytotoxic antitumor agents. These results suggest that the tumor suppression effect is associated with telomerase inhibitory activity, not cytotoxicity.

In conclusion, twenty-one pyridine-2-carboxylate derivatives were prepared. Among them, the 3,4-dichloro-thiophenol ester (**9p**) showed the highest in vitro telomerase inhibitory activity and quite significant in vivo tumor suppression activity. These cumulative findings show that the 6-formyl-pyridine-2-carboxylic thioester would be a useful scaffold for the development of new anticancer agents, and prove that telomerase inhibitors can be novel and efficient antitumor drugs without the side effects emanating from the non-selec-

tivity between the normal somatic cells and cancer cells. Further analyses of the structure–activity relationship are now underway, replacing the pyridine ring with another heterocyclic ring system.

Acknowledgements

References and Notes

1. Raymond, E.; Sun, D.; Chen, S. F.; Windle, B.; von Hoff, D. D. *Curr. Opin. Biotech.* **1996**, 7, 583.

2. (a) Blackburn, E. H. *Nature* **1991**, *350*, 569. (b) Meyne, J.; Ratliff, R. L.; Moyzis, R. K. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7049.

3. Kipling, D. *The Telomere*; Oxford University Press: Oxford, 1995.

4. (a) Morin, G. B. *Cell* **1989**, *59*, 521. (b) Feng, J.; Funk, W.; Wang, S.-S.; Weinrich, S. L.; Avilion, A. A.; Chiu, C.-P.; Adams, R. R.; Chang, E.; Allsopp, R. C.; Yu, J.; Le, S.; West, M. D.; Harley, C. B.; Andrews, W. H.; Greider, C. W.; Villeponteau, B. *Science* **1995**, *269*, 1236. (c) Meyerson, M.; Counter, C. M.; Eaton, E. N.; Ellison, L. W.; Steiner, P.; Caddle, S. D.; Ziaugra, L.; Beijersbergen, R. L.; Davidoff, M. J.; Liu, Q.; Bacchetti, S.; Haber, D. A.; Weinberg, R. A. *Cell* **1997**, *90*, 785.

5. (a) Hamilton, S. E.; Corey, D. R. *Chem. Biol.* **1996**, *3*, 863. (b) Parkinson, E. K. *J. Cancer* **1996**, *73*, 1.

6. (a) Neidle, S.; Kelland, L. R. *Anti-Cancer Drug Des.* **1999**, *14*, 341. (b) Lichtsteiner, S. P.; Lebkowski, J. S.; Vasserot, A. P. *Ann. N.Y. Acad. Sci.* **1999**, *886*, 1.

7. Wheelhouse, R.; Sun, D.; Han, H.; Han, F. X.; Hurley, L. H. *J. Am. Chem. Soc.* **1998**, *120*, 3261.

8. Shin-ya, K.; Wierzba, K.; Matsuo, K.; Ohtani, T.; Yamada, Y.; Furihata, K.; Hayakawa, Y.; Seto, H. *J. Am. Chem. Soc.* **2001**, *123*, 1262.

9. (a) Perry, P. J.; Gowan, S. M.; Reszka, A. P.; Polucci, P.; Jenkins, T. C.; Kelland, L. R.; Neidle, S. *J. Med. Chem.* **1998**, *41*, 3253. (b) Harrison, R. J.; Gowan, S. M.; Kelland, L. R.; Neidle, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2463. (c) Perry, P. J.; Read, M. A.; Davies, R. T.; Gowan, S. M.; Reszka, A. P.; Wood, A. A.; Kelland, L. R.; Neidle, S. *J. Med. Chem.* **1999**, *42*, 2679.

10. Heald, R. A.; Modi, C.; Cookson, J. C.; Hutchinson, I.; Laughton, C. A.; Gowan, S. M.; Kelland, L. R.; Stevens, M. F. G. *J. Med. Chem.* **2002**, *45*, 590.

11. Sasaki, S.; Ehara, T.; Sakata, I.; Fujino, Y.; Harada, N.; Kimura, J.; Nakamura, H.; Maeda, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 583.

12. (a) Geron, Co. Ltd. US Patent No. 5,767,278. (b) Geron, Co. Ltd. US Patent No. 5,770,613.

13. All compounds gave satisfactory spectroscopic data consistent with the proposed structures.

14. Kim, N. W.; Piatyszek, M. A.; Prowse, K. R.; Harley, C. B.; West, M. D.; Ho, P. L. C.; Caviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W. *Science* **1994**, *266*, 2011.

15. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenny, S.; Boyd, M. R. *J. Med. Natl. Canc. Inst.* **1990**, *82*, 1107.

16. Yasutoshi, K.; Tomio, F.; Masashi, A.; Kensuke, M. *Cancer Chemother. Pharmacol.* **1991**, *28*, 192. CX-1 was transplanted and maintained by serial subcutaneous transplantation in athymic nude mice. CX-1 tumors were resected aseptically from the donor mice, and the prepared tumor mass of 50 mg was implanted sc into the right axillary region by 16-gauge trocar. When the tumor size was 100–200 mm^3, mice were randomized into treatment groups (five nude mice in each group), and **9p** (20 mg/kg/day) was treated via ip for 37 days. No mortality was observed after 37 days. Tumor volume was calculated from two-dimensional measurements using the following equation: Tumor volume (mm^3) = (length × width^2) × 0.5. The tumors were weighed on the day that the experiments finished. The effects by treatments were represented by the following equation: Inhibition Rate, IR(%) = (1−TWt/TWc) × 100. Where TWt is mean tumor weight (volume) value obtained for the treated group and TWc is mean tumor weight (volume) value obtained for the control group.