Synthesis of Phenanthroindolizidine Alkaloids with an Acyloxy Group at the C3 Position and their Antitumor Activities and Toxicities

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Abstract: We previously reported that the phenanthroindolizidine alkaloid **3** and its derivatives had markedly potent *in vitro* cytotoxicity. However, they had low *in vivo* antitumor activities and high *in vivo* toxicities, which was a serious problem for further development. Based on the finding that antitumor activity and toxicity could be improved by acetylation of **3**, we synthesized new derivatives of **3**, which possessed various acyl groups, and evaluated their antitumor activities and toxicities. We found that derivatives with sterically less bulky acyl groups had improved antitumor activities.

Keywords: Acylation, Antitumor activity, Mortality, Phenanthroindolizidine alkaloid, Structure activity relationship, Toxicity.

INTRODUCTION

Phenanthroindolizidine alkaloids, which are isolated mainly from the plants of the *Asclepiadaceae* family, are known to possess various biological activities, including anti-arthritis [1], antifungal [2], anti-inflammatory [3], and antitumor [4] activities. In particular, their significant antitumor activities and unique mode of action make them attractive candidates for use as novel antitumor agents [4, 5]. Tylophorine (**1**) and tylocrebrine (**2**) are two representatives of this group of alkaloids (Fig. **1**) [6].

Compound **3**, which is derived from a butterfly (*Ideopsis similis*), has been reported to possess strong cytotoxicity [7]. Recently, we reported the synthesis of compound **3** and its derivatives and evaluated their cytotoxicities *in vitro* and their antitumor activities *in vivo* [8]. Almost all the synthesized derivatives of compound **3** exhibited strong *in vitro* cytotoxicities; however, their *in vivo* antitumor activities were not comparable to the remarkable cytotoxicities. In addition, it was observed that high-dose treatments with these compounds frequently caused fatal toxicity, leading to high mortality of the test mice. In our study, we defined toxicity as the incidence of mortality. In that study, compound **4** (3-acetylated **3**) was more active and less toxic than compound **3** *in vivo* [8]. Based on this finding, we hypothesized that more active and less toxic compounds could be realized by choosing an appropriate acyl group. To test this hypothesis, we synthesized various acylated derivatives of compound **3** and evaluated their antitumor activities and toxicities.

RESULTS AND DISCUSSION

Compound **3** possesses two hydroxyl groups (C3 and C14 positions); thus, we first explored the effect of acetylation of the hydroxyl group at the C14 position (compound **6**) and of both hydroxyl groups at the C3 and C14 positions (compound **5**). Compound **5** was synthesized by thorough acetylation of compound **3**, and hydrolysis of compound **5** under mild conditions afforded compound **6** (Scheme **1**). Evaluation of the cytotoxicities of compounds **3**, **4**, **5**, and **6** against three human cancer cell lines, A549 (lung), HT-29 (colorectal), and HCT116 (colorectal), revealed that they had almost comparable cytotoxicities (Table **1**). Subsequently, we assessed the *in vivo* antitumor activities and toxicities of these compounds using a murine Meth A sarcoma inoculated tumor model (Table **2**). Compound **4** was found to be the most promising compound. Therefore, we chose to acylate the hydroxyl group at the C3 position, and we synthesized various 3-acylated derivatives of compound **3** and evaluated their biological properties.

Synthesis of the acylated derivatives, compounds **7–20**, was achieved by treating compound **3** with appropriate acyl chlorides (Scheme **1**). The synthesized compounds were evaluated for their cytotoxicities (Table **3**). Except for the carbamate-type derivative (**20**), all the derivatives (**7–19**) exhibited similarly potent cytotoxicities. Subsequently, we evaluated the *in vivo* antitumor activities of all these compounds. The results of the *in vivo* assessment are summarized in Table **4**.

The *in vivo* antitumor activities of these compounds were found to be strongly affected by the structure of the acyl moieties. Changing the acetyl group of compound **4** to the bulkier propionyl (**7**), isobutyryl (**8**), or pivaloyl (**9**) groups decreased the activities, while the succinate derivative (**10**) gave a better result than that of **3** (inhibition rates: 48.8% for **10** vs. 30.6% for **3**). We evaluated the pharmacokinetic properties of compound **10** and compound **3** *in vivo* to determine why compound **10** showed superior antitumor activity to that of compound **3**. However, within 0.083 h of administration, compound **10** converted to compound **3**, and the pharmacokinetic parameters of compound **3** and compound **3** that was derived from compound **10** revealed to be almost the same. Thus, the factor that caused the

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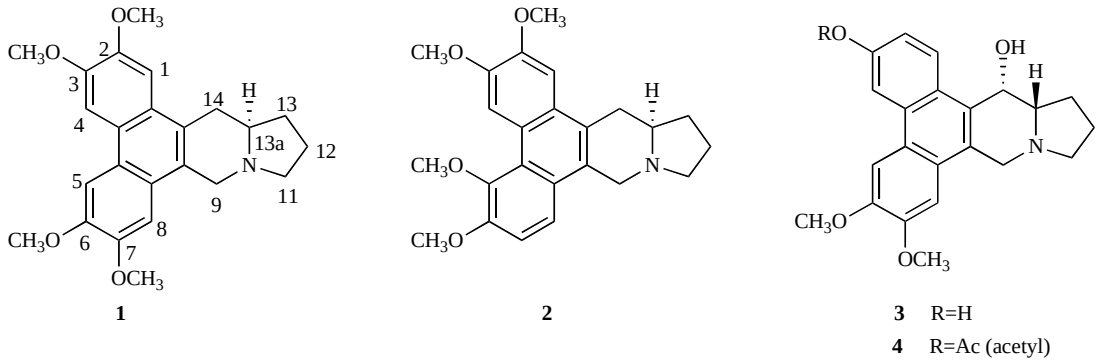
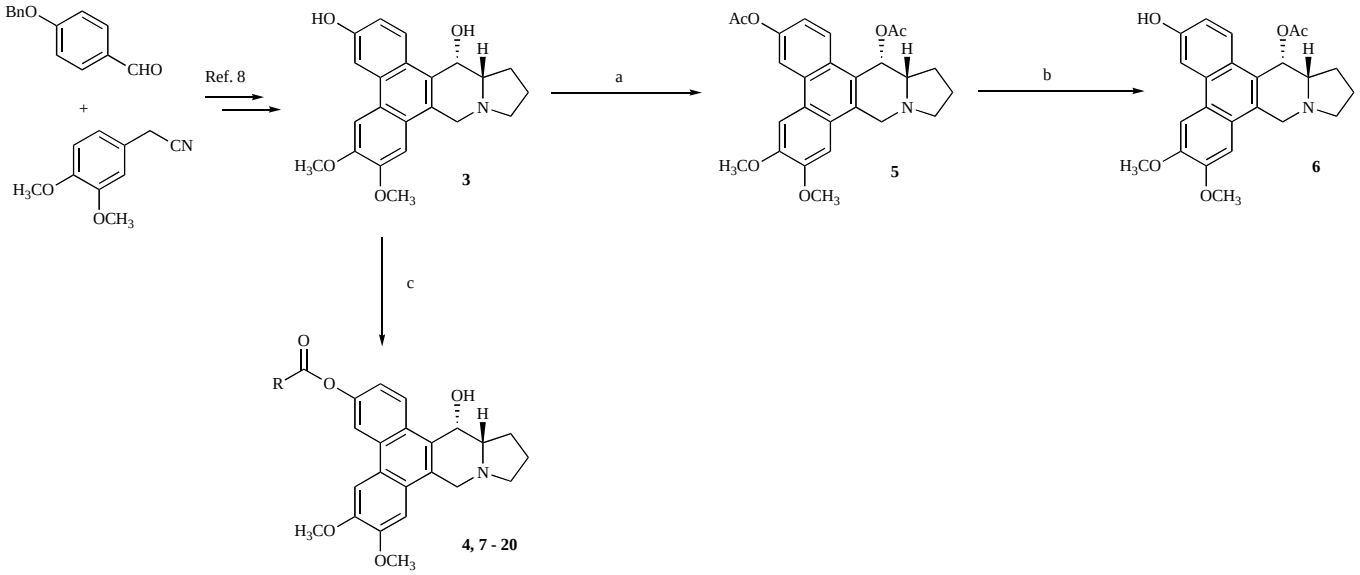
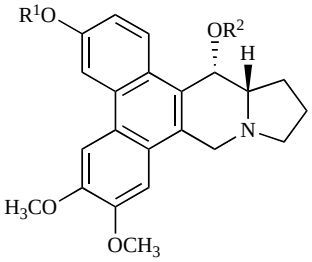


Fig. (1). Chemical structures of phenanthroindolizidine alkaloids.



Scheme 1. Reagents and conditions: (a) Et_3N, DMAP (4-dimethylaminopyridine), Ac_2O, CH_2Cl_2; (b) $NaHCO_3$, CH_3OH, THF, H_2O; (c) Et_3N, DMAP, acyl chloride, CH_2Cl_2.

Table 1. Cytotoxicities of the Synthesized Compounds

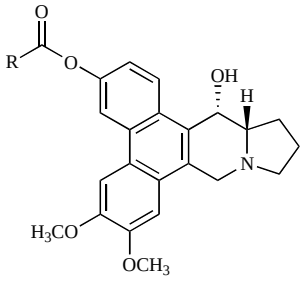


Compound	R^1	R^2	IC_{50} (nM)		
			A549	HT-29	HCT116
3	H	H	2.7	2.9	1.3
4	Ac	H	3.2	3.0	2.4
5	Ac	Ac	17.9	31.9	4.7
6	H	Ac	6.4	36.4	4.5

Table 2. ***In Vivo*** **Antitumor Activities of Compounds 3-6**

Compound	Inhibition rate (%)	Total dose (mg/kg)	Mortality
3	30.6	25	0/5
4	54.9	25	0/5
5	12.2	50	0/5
6	11.2	50	0/5
CPT-11	78.3	100	0/5

Table 3. Cytotoxicities of the Derivatives with an Acyloxy Group at the C3 Position



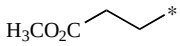
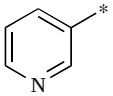
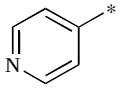
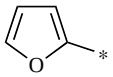
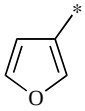
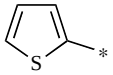
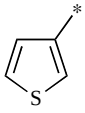
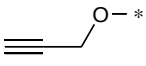
Compound	R	IC_{50} (nM)		
		A549	HT-29	HCT116
7	CH_3CH_2	2.2	2.7	0.7
8	$(CH_3)_2CH$	2.7	2.8	0.9
9	$(CH_3)_3C$	2.6	2.9	1.7
10		2.8	2.8	1.2
11		3.0	2.9	1.1
12		0.4	0.6	0.3
13		2.8	3.0	1.1
14		3.2	3.2	2.9
15		3.2	3.1	2.5
16		2.8	2.8	1.8
17	CH_3O	3.2	3.2	2.1
18	CH_3CH_2O	2.3	2.9	0.7
19		3.2	3.3	2.3
20	$(CH_3)_2N$	44.9	71.0	45.8

Table 4. ***In Vivo*** **Antitumor Activities and Toxicities of the Derivatives with an Acyloxy Group at the C3 Position**

Compound	Inhibition rate (%)	Total dose (mg/kg)	Mortality
7	24.0	25	0/5
	-	50	5/5
8	0.8	25	0/5
	-	50	5/5
9	4.4	25	0/5
	-	50	5/5
10	48.8	25	0/5
11	18.1	25	0/5
12	12.8	25	0/5
13	53.0	50	0/5
	-	100	5/5
14	35.0	25	0/5
15	24.8	50	0/5
	-	100	5/5
16	-2.0	25	0/5
17	54.2	25	0/5
18	32.4	25	0/5
19	24.7	50	0/5
20	3.0	50	0/5

improvement in the antitumor activity following introduction of this acyl group is not clear at this point. Introduction of hetero aromatic acyl groups (**11**–**16**) resulted in poor to good activities (inhibition rates: −2.0% to 53.0%). Among these compounds, the 2-furyl derivative (**13**) afforded the highest antitumor activity. Carbonate-type derivatives (**17**–**19**), especially methyl carbonate (**17**), also exhibited good activities, while the carbamate-type derivative (**20**) resulted in poor activity. There seemed to be a subtle tendency of the compounds that possessed sterically less bulky acyl groups to exhibit better antitumor activities. Higher dose treatments with these derivatives caused high toxicities, thus leading to high mortality of test mice in almost all cases (Table **4**). This finding indicated that acylation of the C3 hydroxyl group contributed little to the mitigation of toxicities. Introduction of a bulky acyl group can lead to considerable loss of antitumor activity, whereas the *in vivo* toxicity was not affected by this structural change. Thus, the causes of "antitumor activity" and "toxicity" might be different. This suggests that antitumor activity and toxicity could be independently influenced if compounds are designed appropriately.

CONCLUSION

We synthesized various 3-acylated derivatives of **3** to create more active and less toxic phenanthroindolizidine alkaloids. As a result, antitumor activity was improved to some extent when sterically less bulky acyl groups were introduced. However, the improvement in toxicity was only marginal and high mortality was observed when higher dose treatments were administered. Another approach to the synthesis of more active and less toxic phenanthroindolizidine alkaloids is now underway.

EXPERIMENTAL SECTION

All reagents were obtained from commercial sources and used without further purification. ^{1}H-NMR spectra were recorded in DMSO-d_6 on a JEOL ALPHA-400 using TMS as an internal standard and chemical shifts are expressed as δ ppm. HR-ESI-MS data were measured on a LCT-premier XE (Waters Corp.) time-of-flight instrument.

Procedure for the Synthesis of Compound 3

Compound **3** was synthesized according to the Lit. [8].

General Procedure for the Synthesis of Compounds 4, 7 – 20

To a suspension of **3** (0.12 mmol) in CH_2Cl_2 (1.0 mL) was added Et_3N (0.36 mmol), DMAP (0.01 mmol), and appropriate acyl chloride (0.26 mmol) at 0 ºC. After stirring for 6 h at room temperature, the reaction mixture was purified by flash column chromatography ($CHCl_3$/ MeOH) to yield **4**, **7** - **20**.

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl acetate (4)

Yield: 21.0%, white powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.78-1.92 (3H, m), 2.15-2.25 (1H, m), 2.30-3.05 (2H, m), 2.36 (3H, s), 3.25-3.40 (1H, m), 3.48-3.63 (1H, m), 3.94 (3H, m), 4.01 (3H, m), 4.59-5.05 (3H, m), 7.26 (1H, s), 7.35 (1H, dd, *J* = 2.2, 9.0 Hz), 8.08 (1H, s), 8.32 (1H, d, *J* = 9.0 Hz), 8.48 (1H, d, *J* = 2.2 Hz); HRMS (ESI) calcd for $C_{24}H_{26}NO_5$ $[M+H]^+$, 408.1811. Found: 408.1850; $[\alpha]_D^{27}$+102.72 (c = 0.02, $CHCl_3$)

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl propionate (7)

Yield: 90.2%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.20 (3H, t, *J* = 7.4Hz), 1.78-1.92 (3H, m), 2.15-2.25 (1H, m), 2.30-2.45 (2H, m), 2.70 (2H, q, *J* = 7.4Hz), 3.33-3.40 (1H, m), 3.48 (1H, d, *J* = 15.9Hz), 3.93 (3H, s), 4.00 (3H, s), 4.57 (1H, d, *J* = 15.9Hz), 4.75 (1H, d, *J* = 9.8Hz), 4.96 (1H, dd, *J* = 2.0, 9.8Hz), 7.34 (1H, dd, *J* = 2.4, 9.2Hz), 7.23 (1H, s), 8.01 (1H, s), 8.32 (1H, d, *J* = 9.2Hz), 8.45 (1H, d, *J* = 2.4Hz); HRMS (ESI) calcd for $C_{25}H_{28}NO_5$ $[M+H]^+$, 422.1967. Found: 422.1974; $[\alpha]_D^{27}$+113.48 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl isobutyrate (8)

Yield: 93.1%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.32 (6H, d, *J* = 7.0Hz), 1.75-1.92 (3H, m), 2.10-2.26 (1H, m), 2.32-2.49 (2H, m), 2.91 (1H, heptet, *J* = 7.0Hz), 3.33-3.40 (1H, m), 3.50 (1H, d, *J* = 15.6Hz), 3.93 (3H, s), 4.01 (3H, s), 4.59 (1H, d, *J* = 15.6Hz), 4.75 (1H, d, *J* = 9.8Hz), 4.97 (1H, dd, *J* = 2.1, 9.8Hz), 7.25 (1H, s), 7.32 (1H, dd, *J* = 2.2, 9.0Hz), 8.08 (1H, s), 8.33 (1H, d, *J* = 9.0Hz), 8.43 (1H, d, *J* = 2.2Hz); HRMS (ESI) calcd for $C_{26}H_{30}NO_5$ $[M+H]^+$, 436.2124. Found: 436.2129; $[\alpha]_D^{29}$+ 92.78 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl pivalate (9)

Yield: 80.6%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.39 (9H, s), 1.75-1.88 (3H, m), 2.10-2.26 (1H, m), 2.32-2.49 (2H, m), 3.33-3.40 (1H, m), 3.50 (1H, d, *J* = 15.6Hz), 3.94 (3H, s), 4.02 (3H, s), 4.59 (1H, d, *J* = 15.6Hz), 4.75 (1H, d, *J* = 9.8Hz), 4.97 (1H, dd, *J* = 2.1, 9.8Hz), 7.25 (1H, s), 7.30 (1H, dd, *J* = 2.2, 9.0Hz), 8.08 (1H, s), 8.33 (1H, d, *J* = 9.0Hz), 8.39 (1H, d, *J* = 2.2Hz); HRMS (ESI) calcd for $C_{27}H_{32}NO_5$ $[M+H]^+$, 450.2280. Found: 450.2284; $[\alpha]_D^{30}$+89.72 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl methyl succinate (10)

Yield: 81.4%, yellow powder

^{1}H NMR (400 MHz, DMSO-d_6) δ:1.80-1.92 (3H, m), 2.14-2.29 (1H, m), 2.32-2.49 (2H, m), 2.74 (2H, t, *J* = 6.6Hz), 2.95 (2H, t, *J* = 6.6Hz), 3.33-3.40 (1H, m), 3.49 (1H, d, *J* = 16.1Hz), 3.65 (3H, s), 3.94 (3H, s), 4.01 (3H, s), 4.58 (1H, d, *J* = 16.1Hz), 4.76 (1H, d, *J* = 9.5Hz), 4.97 (1H, d, *J* = 9.5Hz), 7.24 (1H, s), 7.32 (1H, dd, *J* = 2.0, 9.3Hz), 8.11 (1H, s), 8.33 (1H, d, *J* = 9.3Hz), 8.44 (1H, d, *J* = 2.0Hz); HRMS (ESI) calcd for $C_{27}H_{30}NO_7$ $[M+H]^+$, 480.2022. Found: 480.2054; $[\alpha]^{30}{}_D$ +77.88 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl nicotinate (11)

Yield: 85.0%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.80-1.92 (3H, m), 2.14-2.29 (1H, m), 2.32-2.49 (2H, m), 3.33-3.40 (1H, m), 3.50 (1H, d, *J* = 15.6Hz), 3.94 (3H, s), 3.99 (3H, s), 4.60 (1H, d, *J* = 15.6Hz), 4.80 (1H, d, *J* = 10.0Hz), 5.00 (1H, dd, *J* = 2.2, 10.0Hz), 7.26 (1H, s), 7.54 (1H, dd, *J* = 2.4, 9.0Hz), 7.70 (1H, ddd, *J* = 1.0, 4.9, 8.2Hz), 8.11 (1H, s), 8.39 (1H, d, *J* = 9.0Hz), 8.57 (1H, ddd, *J* = 1.7, 2.2, 7.8Hz), 8.71 (1H, d, *J* = 2.4Hz), 8.93 (1H, dd, *J* = 1.7, 4.9Hz), 9.36 (1H, dd, *J* = 1.0, 2.2Hz); HRMS (ESI) calcd for $C_{28}H_{27}N_2O_5$ $[M+H]^+$, 471.1920. Found: 471.1926; $[\alpha]_D^{29}$+ 64.28 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl isonicotinate (12)

Yield: 82.9%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.80-1.92 (3H, m), 2.14-2.29 (1H, m), 2.32-2.49 (2H, m), 3.33-3.40 (1H, m), 3.52 (1H, d, *J* = 15.6Hz), 3.95 (3H, s), 3.98 (3H, s), 4.61 (1H, d, *J* = 15.6Hz), 4.91 (1H, d, *J* = 10.0Hz), 5.00 (1H, dd, *J* = 2.1, 10.0Hz), 7.27 (1H, s), 7.55 (1H, dd, *J* = 2.2, 9.0Hz), 8.04-8.14 (3H, m), 8.50 (1H, d,*J* = 9.0Hz), 8.71 (1H, d, *J* = 2.2Hz), 8.90-8.97 (2H, m); HRMS (ESI) calcd for $C_{28}H_{27}N_2O_5$ $[M+H]^+$, 471.1920. Found: 471.1917; $[\alpha]_D^{27}$+68.68 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl furan-2-carboxylate (13)

Yield: 66.5%, white powder

^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.76-1.91 (3H, m), 2.10-2.29 (1H, m), 2.32-2.49 (2H, m), 3.33-3.40 (1H, m), 3.49 (1H, d, *J* = 16.0Hz), 3.94 (3H, s), 3.99 (3H, s), 4.57 (1H, d, *J* = 16.0Hz), 4.81 (1H, d, *J* = 9.8Hz), 4.98 (1H, d, *J* = 9.8Hz), 6.84 (1H, dd, *J* = 3.5, 1.8 Hz), 7.24 (1H, s), 7.48 (1H, dd, *J* = 9.0, 2.4 Hz), 7.65 (1H, dd, *J* = 3.5, 0.7 Hz), 8.11 (1H, s), 8.15 (1H, dd, *J* = 1.8, 0.7 Hz), 8.37 (1H, d, *J* = 9.0 Hz), 8.66 (1H, d, *J* = 2.4 Hz); HRMS (ESI) calcd for $C_{27}H_{26}NO_6$ $[M+H]^+$, 460.1760. Found: 460.1754; $[\alpha]^{26}{}_D$ +46.93 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl furan-3-carboxylate (14)

Yield: 43.6%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.80-1.92 (3H, m), 2.14-2.29 (1H, m), 2.32-2.49 (2H, m), 3.33-3.40 (1H, m), 3.51 (1H, d, *J* = 15.6Hz), 3.94 (3H, s), 3.99 (3H, s), 4.61 (1H, d, *J* = 15.6Hz), 4.77 (1H, d, *J* = 9.8Hz), 5.01 (1H, dd, *J*

= 2.0, 9.8Hz), 7.00-7.04 (1H, m), 7.26 (1H, s), 7.45 (1H, dd, *J* = 2.2, 9.0Hz), 7.92-7.98 (1H, m), 8.11 (1H, s), 8.36 (1H, d, *J* = 9.0Hz), 8.62 (1H, d, *J* = 2.2Hz), 8.70-8.73 (1H, m); HRMS (ESI) calcd for $C_{27}H_{26}NO_6$ $[M+H]^+$, 460.1760. Found: 460.1768; $[\alpha]_D^{26}$+ 58.45 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl thiophene-2-carboxylate (15)

Yield: 79.2%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.80-1.92 (3H, m), 2.14-2.29 (1H, m), 2.32-2.49 (2H, m), 3.33-3.40 (1H, m), 3.52 (1H, d, *J* = 15.9Hz), 3.94 (3H, s), 3.99 (3H, s), 4.61 (1H, d, *J* = 15.9Hz), 4.79 (1H, d, *J* = 9.8Hz), 5.00 (1H, d, *J* = 9.8Hz), 7.26 (1H, s), 7.35 (1H, dd, *J* = 3.7, 4.9Hz), 7.49 (1H, dd, *J* = 2.2, 9.0Hz), 8.11 (1H, dd, *J* = 1.2, 3.7Hz), 8.13 (1H, s), 8.13 (1H, dd, *J* = 1.2, 3.7Hz), 8.37 (1H, d, *J* = 9.0Hz), 8.67 (1H, d, *J* = 2.2Hz); HRMS (ESI) calcd for $C_{27}H_{26}NO_5S$ $[M+H]^+$, 476.1532. Found: 476.1542; $[\alpha]_D^{28}$+52.89 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl thiophene-3-carboxylate (16)

Yield: 42.1%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.76-1.92 (3H, m), 2.10-2.29 (1H, m), 2.35-2.49 (2H, m), 3.33-3.40 (1H, m), 3.52 (1H, d, *J* = 15.9Hz), 3.95 (3H, s), 3.99 (3H, s), 4.63 (1H, d, *J* = 15.9Hz), 4.76 (1H, d, *J* = 9.8Hz), 5.01 (1H, dd, *J* = 2.0, 9.8Hz), 7.28 (1H, s), 7.47 (1H, dd, *J* = 2.2, 9.0Hz), 7.69 (1H, dd, *J* = 1.2, 5.1Hz), 7.78 (1H, dd, *J* = 3.0, 5.1Hz), 8.12 (1H, s), 8.37 (1H, d, *J* = 9.0Hz), 8.64 (1H, d, *J* = 2.2Hz), 8.69 (1H, dd, *J* = 1.2, 3.0Hz); HRMS (ESI) calcd for $C_{27}H_{26}NO_5S$ $[M+H]^+$, 476.1532. Found: 476.1531; $[\alpha]_D^{29}$+53.65 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl methyl carbonate (17)

Yield: 79.2%, white powder

^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.80-1.92 (3H, m), 2.14-2.29 (1H, m), 2.32-2.49 (2H, m), 3.33-3.40 (1H, m), 3.50 (1H, d, *J* = 16.1Hz), 3.88 (3H, s), 3.94 (3H, s), 4.01 (3H, s), 4.58 (1H, d, *J* = 16.1Hz), 4.80 (1H, d, *J* = 9.5Hz), 4.97 (1H, d, *J* = 9.5Hz), 7.24 (1H, s), 7.45 (1H, dd, *J* = 2.0, 9.3Hz), 8.11 (1H, s), 8.34 (1H, d, *J* = 9.3Hz), 8.63 (1H, d, *J* = 2.0Hz); HRMS (ESI) calcd for $C_{24}H_{26}NO_6$ $[M+H]^+$, 424.1760. Found: 424.1763; $[\alpha]^{26}{}_D$ +118.53 (c = 0.1, $CHCl_3$).

ethyl ((13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl) carbonate (18)

Yield: 52.3%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.33 (3H, t, *J* = 7.1Hz), 1.79-1.89 (3H, m), 2.13-2.27 (1H, m), 2.30-2.48 (2H, m), 3.26-3.35 (1H, m), 3.50 (1H, d, *J* = 15.6Hz), 3.94 (3H, s), 4.01 (3H, s), 4.30 (2H, q, *J* = 7.1Hz), 4.59 (1H, d, *J* = 15.6Hz), 4.76 (1H, d, *J* = 9.8Hz), 4.97 (1H, dd, *J* = 2.2, 9.8Hz), 7.25 (1H, s), 7.45 (1H, dd, *J* = 2.4, 9.0Hz), 8.11 (1H, s), 8.33 (1H, d, *J* = 9.0Hz), 8.62 (1H, d, *J* = 2.4Hz); HRMS (ESI) calcd for $C_{25}H_{28}NO_6$ $[M+H]^+$, 438.1917. Found: 438.1915; $[\alpha]_D^{28}$+90.54 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl prop-2-yn-1-yl carbonate (19)

Yield: 75.1%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.78-1.90 (3H, m), 2.12-2.27 (1H, m), 2.34-2.50 (2H, m), 3.27-3.34 (1H, m), 3.41 (1H, d, *J* = 15.5Hz), 3.75 (1H, t, *J* = 2.4Hz), 3.95 (3H, s), 4.01 (3H, s), 4.62 (1H, d, *J* = 15.5Hz), 4.76 (1H, d, *J* = 9.8Hz), 4.95 (2H, d, *J* = 2.4Hz), 4.98 (1H, dd, *J* = 2.2, 9.8Hz), 7.27 (1H, s), 7.46 (1H, dd, *J* = 2.4, 9.0Hz), 8.12 (1H, s), 8.35 (1H, d, *J* = 9.0Hz), 8.66 (1H, d, *J* = 2.4Hz); HRMS (ESI) calcd for $C_{26}H_{26}NO_6$ $[M+H]^+$, 448.1760. Found: 448.1769; $[\alpha]_D^{28}$+91.68 (c = 0.1, $CHCl_3$).

(13aS,14S)-14-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-3-yl dimethylcarbamate (20)

Yield: 86.8%, yellow powder

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.75-1.92 (3H, m), 2.14-2.29 (1H, m), 2.32-2.49 (2H, m), 2.96 (3H, s), 3.14 (3H, s), 3.33-3.40 (1H, m), 3.51 (1H, d, *J* = 15.6Hz), 3.94 (3H, s), 4.01 (3H, s), 4.62 (1H, d, *J* = 15.6Hz), 4.72 (1H, d, *J* = 9.8Hz), 4.97 (1H, dd, *J* = 2.0, 9.8Hz), 7.27 (1H, s), 7.33 (1H, dd, *J* = 2.2, 9.0Hz), 8.08 (1H, s), 8.29 (1H, d, *J* = 9.0Hz), 8.44 (1H, d, *J* = 2.2Hz); HRMS (ESI) calcd for $C_{25}H_{29}N_2O_5$ $[M+H]^+$, 437.2076. Found: 437.2077; $[\alpha]_D^{27}$+ 74.72 (c = 0.1, $CHCl_3$).

Procedure for the Synthesis of Compound 5

(13aS,14S)-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinoline-3,14-diyl diacetate (5)

To a suspension of **3** (90 mg, 0.25 mmol) in CH_2Cl_2 (3.0 mL) was added Et_3N (1.4 mL, 10 mmol), DMAP (3 mg, 0.03 mmol), and Ac_2O (0.95 mL, 10 mmol) at 0 ºC. After stirring for 6 h at room temperature, the reaction mixture was purified by flash column chromatography ($CHCl_3$/ MeOH) to yield **5** (47 mg, 41.9% yield) as a yellow powder.

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.48-1.77 (2H, m), 1.86-2.12 (2H, m), 2.16 (3H, s), 2.41 (3H, s), 2.40-2.52 (1H, m), 2.67-2.79 (1H, m), 3.50-3.58 (1H, m), 3.66 (1H, d, *J* = 15.4Hz), 4.07 (3H, s), 4.12 (3H, s), 4.81 (1H, d, *J* = 15.4Hz), 6.73 (1H, brs), 7.23 (1H, s), 7.31 (1H, dd, *J* = 2.2, 9.0Hz), 7.89 (1H, s), 7.95 (1H, d, *J* = 9.0Hz), 8.21 (1H, d, *J*= 2.2Hz); HRMS (ESI) calcd for $C_{26}H_{28}NO_6$ $[M+H]^+$, 450.1917. Found: 450.1912; $[\alpha]_D^{29}$+156.90 (c = 0.1, $CHCl_3$).

Procedure for the Synthesis of Compound 6

(13aS,14S)-3-hydroxy-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]pyrrolo[1,2-b]isoquinolin-14-yl acetate (6)

To a solution of **5** (114 mg, 0.26 mmol) in MeOH (2.0 mL), THF (tetrahydrofuran) (2.0 mL), and H_2O (2.0 mL) was added $NaHCO_3$ (24 mg, 0.29 mmol) at 0 ºC. After stirring for 12 h at room temperature, the reaction mixture

was poured into brine, extracted with AcOEt, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography ($CHCl_3$/ MeOH) to yield **6** (83 mg, 78.4% yield) as a yellow powder.

^{1}HNMR (400 MHz, DMSO-d_6) δ: 1.43-1.55 (1H, m), 1.74-1.94 (3H, m), 2.04 (3H, s), 2.30-2.40 (1H, m), 2.60-2.69 (1H, m), 3.10-3.40 (1H, m), 3.53 (1H, d, *J* = 15.7Hz), 3.94 (3H, s), 4.00 (3H, s), 4.67 (1H, d, *J* = 15.7Hz), 6.50 (1H, brs), 7.08 (1H, dd, *J* = 2.4, 8.8Hz), 7.27 (1H, s), 7.62 (1H, d, *J* = 8.8Hz), 7.95 (1H, s), 7.99 (1H, d, *J* = 2.4Hz); HRMS (ESI) calcd for $C_{24}H_{26}NO_5$ $[M+H]^+$, 408.1811. Found: 408.1836; $[\alpha]_D^{28}$+172.68 (c = 0.1, CH_3OH : $CHCl_3$ = 1 : 1).

In Vitro Cytotoxicity Assay

Cell viability was assayed in a 96-well plate using TetraColor ONE (Seikagaku Corp., Tokyo, Japan), according to the manufacturer's protocol. Briefly, exponentially growing A549, HT-29, or HCT116 cells were seeded in 96-well plates at a density of 10^3 cells/well. The next day, serially diluted compounds were added to each plate. After 96 h of incubation, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt was added to each well and the plates were incubated at 37°C for 1 h. Absorbance was measured at 450 nm using SPECTRAMax PLUS384 (Molecular Devices, Sunnyvale, CA, USA). The half maximal inhibitory concentration (IC_{50}) was defined as the concentration of a compound that inhibited cell growth by 50% with the solvent-treated cells as control.

In Vivo Antitumor Activity on Meth A in BALB/c Mice

Meth A cells (sarcoma, 2.5×10^5 cells/mouse) were inoculated subcutaneously into 7-week-old male BALB/c mice (5/group), and samples were injected intravenously on days 1, 5, and 9. All samples were administered as their hydrochlorides. The vehicle was saline. The tumors were excised and weighed on day 21 after tumor inoculation. The inhibition rate (%) was calculated using the following formula: [1 − (mean tumor weight of tested mice)/(mean tumor weight of control mice] × 100

In vivo antitumor experiment was performed according to our internal and ethics committee regulations.

CONFLICT OF INTEREST

Declared none.

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