Synthesis and Antimetastatic Activity of 6-Trichloroacetamido and 6-Guanidino Analogues of Siastatin B

TAKAHIKO SATOH, YOSHIO NISHIMURA,*
SHINICHI KONDO and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

MASAYUKI AZETAKA, HARUMI FUKUYASU,
YUMIKO IIZUKA, SHOKICHI OHUCHI
and SEIJI SHIBAHARA

Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd.,
760 Morooka-cho, Kohoku-ku, Yokohama 222, Japan

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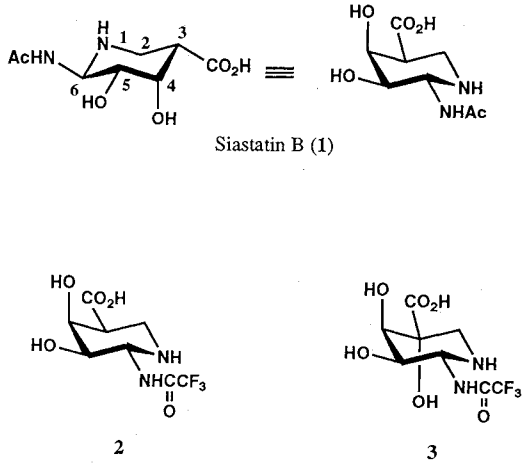
Current studies[1~5] have provided considerable evidence of increase of β-glucuronidase activity in human tumors and suggested that β-glucuronidase plays a key role in degradation of basement membranes during metastasis of the tumor cells, and showed that β-glucuronidase inhibitors inhibit tumor-cell metastasis.

In the course of our studies[6~16] of new drugs for tumor metastasis based on siastatin B (**1**),[17] we showed that synthetic (3*S*,4*S*,5*R*,6*R*)-6-(trifluoroacetamido)-4,5-dihydroxypiperidine-3-carboxylic acid (**2**) and (3*R*,4*R*,5*R*,6*R*)-6-(trifluoroacetamido)-3,4,5-trihydroxypiperidine-3-carboxylic acid (**3**) have the marked inhibitory activity against β-glucuronidase.[15] They were also found to show the inhibition of highly metastatic B16 variant (B16 BL6) and Lewis lung carcinoma (3LL) cell invasion through reconstituted basement membranes and the potent suppression of pulmonary metastasis of B16 BL6 and 3LL cells in mice.[15,16] These results prompted us to further modify the amide function at C-6 of **1** focused on the glucuronidase inhibition and suppression of lung metastasis of tumor cell. Here, we wish to report the syntheses of (3*S*,4*S*,5*R*,6*R*)-6-(trichloroacetamido) and (3*S*,4*S*,5*R*,6*R*)-6-guanidino-4,5-dihydroxypiperidine-3-carboxylic acids (**4** and **5**) having inhibitory activity for β-glucuronidase and pulmonary metastasis of B16 BL6.

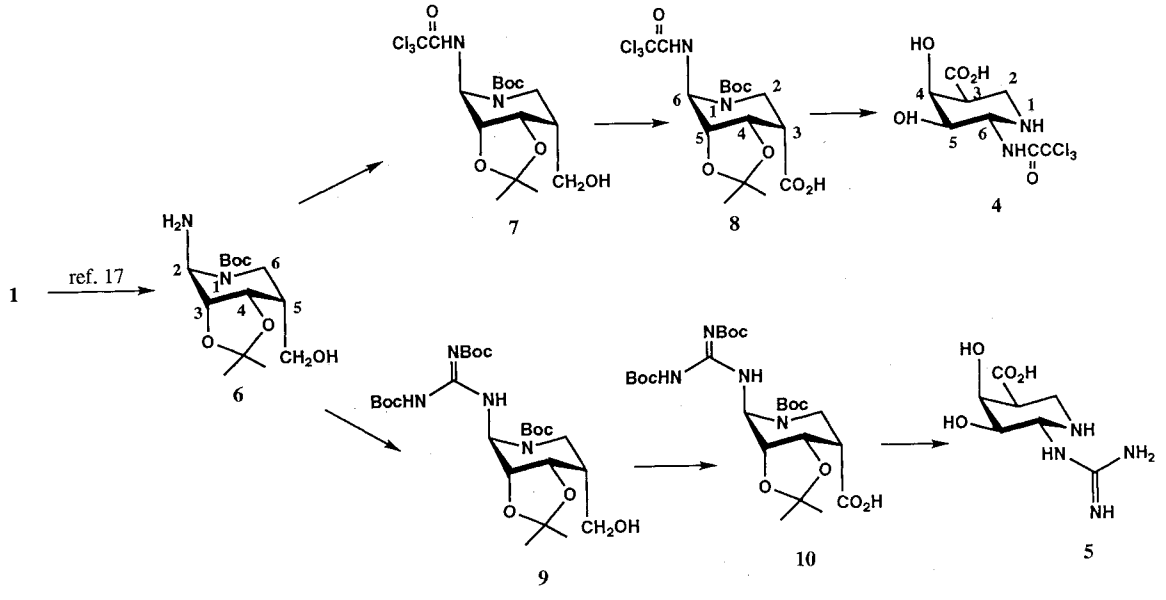
Chemistry

The potent inhibition of **2** and **3** for β-glucuronidase suggest that the trifluoroacetamide function may play an important role in the strong binding of 1-*N*-iminosugar to β-glucuronidase. In order to examine the neighboring participation of amide group of **1** in β-glucuronidase inhibition, replacement of acetamide function by trichloroacetamide and guanidino ones are next undertaken.

Fig. 1. Structures of siastatin B (**1**) and its 6-trifluoroacetamido analogues **2** and **3**.



Scheme 1. Synthesis of 6-trichloroacetamido and 6-guanidino analogues of siastatin B.



The starting (2*S*,3*R*,4*S*,5*R*)-2-amino-*N*-(*tert*-butoxycarbonyl)-5-hydroxymethyl-3,4-*O*-isopropylidene-3,4-piperidinediol (**6**) was easily obtained from **1** by the method developed by us.[18] Trichloroacetylation of the amino-alcohol **6** with trichloroacetyl chloride gave the trichloroacetamide **7** in a good yield. Oxidation of the hydroxymethyl group to the carboxylic acid was best achieved by ruthenium tetraoxide catalyzed oxidation in a solvent system of $CH_3CN/CCl_4/H_2O$ developed by SHARPLESS *et al.*[19] Thus obtained compound **8** was smoothly converted into **4** by removal of the protecting groups with acid. On the other hand, treatment of **6** with *N*,*N'*-di-(*tert*-butoxycarbonyl)thiourea in the presence of mercury (II) chloride[20] afforded the guanidino compound **9** in an excellent yield. Compound **9** was straightforwardly transformed into **5** by the similar method described above.

Biological Activities

As expected, both **4** and **5** inhibited β-glucuronidase from bovine liver shown in Table 1. Compound **4** as well as **5**, also showed weak inhibitory activity against β-glucosidase (almonds), while they did not inhibit any other glycosidases (bakers yeast α-glucosidase, Jack beans α-mannosidase, snail β-mannosidase, *E. coli* α- and β-galactosidase, bovine kidney β-*N*-acetylglucosaminidase, chicken liver α-*N*-acetylgalactosidase; $IC_{50} > 100$ μg/ml). Lung colonization after intravenous transplantation of the highly metastatic variants of B16 melanoma (B16 BL6)[21,22] was suppressed dose-dependently by *in vivo* pretreatment with **4** and **5** as shown in Table 2. Compounds **4** and **5** had no significant effects on cell growth at the concentrations used in this study (data not shown). Tables 1 and 2 show that the inhibitory activity for B16 BL6 metastasis is proportional to the inhibitory activity against β-glucuronidase. On the other hand, NAKAJIMA *et al.*[3,23,24] proved that heparanase (endo-β-glucuronidase) activity correlates with the lung colonization abilities of murine B16 melanoma cells by extracellular matrix degradation. These facts suggest that **4** and **5** as glucuronidase inhibitors inhibit extracellular matrix degradation and/or modify cell-surface glycoconjugate of B16 BL6 cells, resulting in the inhibition observed of experimental pulmonary metastasis of B16 BL6. These facts indicate that the antimetastatic effect of **4** and **5** may be due to its antiinvasive rather than antiproliferative activities. The present study promise a way to inhibit tumor metastasis through a better understanding of biochemical processes. Further investigation of β-glucuronidase inhibitors for inhibition of tumor metastasis is in progress.

Table 1. IC_{50} (μM) of siastatin B (**1**), and its analogues **4** and **5** against glycosidases.

Compound	β-Glucosidase (almond)	β-Glucuronidase (bovine liver)
1	>440	16
4	137	4.7
5	96	4.1

Experimental

General Methods

Melting points were determined with a Yanagimoto apparatus and were uncorrected. IR spectra were determined on a Hitachi Model 260-10 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. ^1H NMR spectra were recorded with a JEOL JNM EX270 spectrometer. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane as an internal standard. MS spectra were taken by a JEOL JMS-SX102 in the FAB mode.

Enzyme Inhibition Assay

α-Glucosidase (yeast)[25] and β-glucuronidase (bovine liver)[26] assays were evaluated by methods described in references.

Table 2. Inhibitory effect of **4** and **5** on the experimental metastasis of B16 BL6 cells in mice.

Compound	Dose (μM)	No. of cells (ml)	No. of lung metastasis (Mean±SD)	(Range)	Inhibition of metastasis (%)
None	—	1.3×10^7	90.0 ± 33.3	142-53	0
4	9	1.2×10^7	91.0 ± 33.4	147-64	0
	31	1.4×10^7	66.6 ± 17.5	82-41	26
	93	1.0×10^7	63.4 ± 18.6	93-45	29.6
	156	1.2×10^7	29.4 ± 20.4	55-3	67.3*
5	14	1.3×10^7	69.8 ± 11.1	79-51	22.4
	46	1.3×10^7	36.8 ± 7.0	45-27	59.1*
	137	1.1×10^7	23.2 ± 10.1	37-10	74.2*
	229	1.2×10^7	11.6 ± 7.2	19-0	87.1**

* and **, $P<0.01$ and $P<0.001$, respectively.

Experimental Metastasis Assay[21,22]

The highly metastatic melanoma B16 (B16 BL6) cells (3×10^5 cells) were cultured in DULBECCO's modified EAGLE's medium supplemented with fetal bovine serum under 5% CO_2 at 37°C for 24 hours. Cells were incubated with (or without) each test compound under the same condition for 72 hours. After treatment with 0.05% trypsin and 0.02% EDTA soln, a cell suspension containing $1.0\sim1.4\times10^7$ cells in 1 ml of divalent cation-free DULBECCO's phosphate-buffered saline was prepared. Cells in 0.1 ml were injected intravenously into the tail vein of each mouse (male BDF_1, 7 weeks old). Fourteen days later, after tumor cell implantation, the mice were autopsied. The number of pulmonaty tumor nodules was counted. Inhibition (%) of metastasis was calculated from the ratio of tumor nodules in treated and control experiments.

(2*S*,3*R*,4*S*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-2-(trichloroacetamido)-5-hydroxymethyl-3,4-*O*-isopropylidene-3,4-piperidinediol (**7**)

To a soln of **6** (124 mg) in dichloromethane (2.5 ml) were added pyridine (0.13 ml) and trichloroacetyl chloride (0.1 ml) at 0°C, and the mixture was stirred at the same temperature for 10 minutes. After dilution with chloroform, the soln was washed with satd aq $NaHCO_3$ soln and H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a pale yellow solid. The solid was dissolved in a satd methanolic K_2CO_3 soln (3 ml). After being stirred at room temperature for 10 minutes, evaporation of the solvent gave a solid. The solid was dissolved in chloroform, and the soln was washed with satd aq NH_4Cl soln, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave a solid, which was subjected to column chromatography on silica gel. Elution with a mixture of toluene - acetone (5 : 1) gave **7** (162 mg, 88%) as a colorless foam: $[\alpha]_D^{25}$ +16.8° (*c* 3.8, MeOH); IR ($CHCl_3$) 3420, 2980, 2930, 2900, 1720, 1700, 1490, 1460, 1395, 1385, 1370, 1360, 1325, 1280 (sh), 1260, 1170, 1140, 1115, 1070, 1060, 1005 cm^{-1}; ^1H NMR (CD_3OD, 40°C) δ 1.36 (3H, s, isopropylidene), 1.48 (12H, s, isopropylidene and $NCOOC(CH_3)_3$), 1.95 (1H, t, $J=5.6$ Hz, –OH), 2.01 (1H, broad m, 5-H), 3.16 (1H, t, $J=12.2$ Hz, 6-Hax), 3.59 (1H, dd, $J=4.4$ and 12.2 Hz, 6-Heq), 3.79 (2H, t, $J=5.6$ Hz, $-CH_2OH$), 4.58 (1H, dd, $J=2.4$ and 7.3 Hz, 3-H), 4.62 (1H, dd, $J=2.0$ and 7.3 Hz, 4-H), 5.73 (1H, broad s, 2-H) and 6.81 (1H, broad s, –NHCO–). *Anal.* Calcd for $C_{16}H_{25}Cl_3N_2O_6$: C 42.92, H 5.63, N 6.26. Found: C 42.37, H 5.61, N 6.14.

(3*S*,4*S*,5*R*,6*R*)-*N*-(*tert*-Butoxycarbonyl)-6-(trichloroacetamido)-4,5-dihydroxy-4,5-*O*-isopropylidenepiperidine-3-carboxylic Acid (**8**)

To a soln of **7** (152 mg) in a mixture of CCl_4 (2 ml) and CH_3CN (2 ml) were added a soln of $NaIO_4$ (218 mg) in water (3 ml) and RuO_2 (8 mg), and the mixture was vigorously stirred at room temperature for 30 minutes. The phases were separated. The aq phase was extracted three times with EtOAc. To the combined organic extracts was added 2-propanol (0.5 ml), and the mixture was stirred at room temperature for 1 hour. After filtration off the precipitates, the filtrate was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a solid, which was chromatographed on silica gel. Elution with a mixture of $CHCl_3$ - MeOH - concd aq ammonia (50 : 10 : 1) gave an amorph solid of **8** (119 mg, 76%): $[\alpha]_D^{24}$ +3.6° (*c* 0.87, MeOH); IR ($CHCl_3$) 3425, 2980, 2940, 1720, 1695, 1515, 1485, 1465, 1400, 1375, 1350, 1330, 1260, 1220, 1175, 1105, 1070, 1000 cm^{-1}; ^1H NMR (CD_3OD, 40°C) δ 1.35 and 1.41 (3H, each s, isopropylidene), 1.48 (9H, s, $-NCOOC(CH_3)_3$), 3.06 (1H, ddd, $J=2.0$, 4.9 and 12.5 Hz, 3-H), 3.44 (1H, t, $J=12.5$ Hz, 2-Hax), 3.66 (1H, dd, $J=4.9$ and 12.5 Hz, 2-Heq), 4.53 (1H, dd, $J=2.0$ and 7.3 Hz, 5-H), 4.84 (1H, dd, $J=2.0$, 7.3 Hz, 4-H) and 5.76 (1H, d, $J=2.0$ Hz, 6-H). *Anal.* Calcd for $C_{16}H_{23}Cl_3N_2O_7$: C 41.62, H 5.02, N 6.07. Found: C 41.40, H 5.24, N 6.24.

(3*S*,4*S*,5*R*,6*R*)-4,5-Dihydroxy-6-(trichloroacetamido)-piperidine-3-carboxylic Acid (**4**)

Compound **8** (32 mg) was dissolved in 4 M hydrogen chloride in dioxane (1 ml), and the mixture was stirred at room temperature overnight. The resulting precipitates were collected by centrifugation and washed with Et_2O to give a colorless amorph solid of **4** as its hydrochloride (25 mg, 100%): $[\alpha]_D^{26}$ +31° (*c* 0.7, H_2O); IR (KBr) 3500, 3400, 2990, 2925, 2890 (sh), 2870, 1735, 1730, 1530, 1460, 1430, 1410, 1315, 1290, 1230, 1170, 1110, 1050, 1020 cm^{-1}; ^1H NMR (D_2O) δ 3.15 (1H, ddd, $J=2.4$, 8.3 and 9.8 Hz, 3-H), 3.55 and 3.56 (each 1H, d, $J=8.3$ and 9.8 Hz, 2-H), 4.22 (1H, dd, $J=2.4$ and 10.3 Hz, 5-H), 4.62 (1H, t, $J=2.4$ Hz, 4-H) and 5.16 (1H, d, $J=10.3$ Hz, 6-H); FAB-MS (positive) *m/z* 320 $(M+H)^+$, 286, 160, 142, 110, 75 and 57.

(2*S*,3*R*,4*S*,5*R*)-*N*-(*tert*-Butoxycarbonyl)-2-[*N*,*N*'-di-(*tert*-butoxycarbonyl)]guanidino-3-hydroxymethyl-3,4-*O*-isopropylidene-3,4-piperidinediol (**9**)

To a soln of **6** (109 mg) in DMF (2 ml) were added triethylamine (0.2 ml), *N*,*N*'-di-(*tert*-butoxycarbonyl)-thiourea (199 mg) and mercury (II) chloride (196 mg) at 0°C, and the mixture was stirred at 0°C for 2 hours. After addition a large amount of EtOAc, the resulting precipitates were removed by centrifugation. The precipitates were washed with EtOAc. The supernatant and washings were combined, and the soln was washed with water and a satd aq NaCl soln, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave a solid, which was subjected on column chromatography of silica gel. Elution with a mixture of $CHCl_3$ and MeOH (100 : 1) gave an amorph solid of **9** (184 mg, 94%): $[\alpha]_D^{24}$ +45.6° (*c* 0.83, $CHCl_3$); IR ($CHCl_3$) 3310, 2980, 2940, 1790, 1720, 1700, 1620, 1560, 1480, 1460, 1400, 1375, 1330, 1290, 1255, 1235, 1210 (sh), 1170 (sh), 1160, 1120, 1060, 1030, 1005 cm^{-1}; ^1H NMR ($CDCl_3$, 40°C) δ 1.34

and 1.45 (3H, each s, isopropylidene), 1.45, 1.47 and 1.49 (9H, each s, $-NCOOC(CH_3)_3$), 2.08 (1H, m, 5-H), 3.21 (1H, t, $J=12.2$ Hz, 6-Hax), 3.50 (1H, dd, $J=4.4$ and 12.2 Hz, 6-Heq), 3.7~3.9 (2H, m, $-CH_2OH$), 4.56 (2H, broad s, 3-H and 4-H), 6.19 (1H, broad d, $J=6.4$ Hz, 2-H), 8.35 (1H, broad d, $J=6.4$ Hz, $-C(NCOOC(CH_3)_3)NH-$) and 11.37 (1H, broad s, $-NH(COOC(CH_3)_3)$); FAB-MS (positive) m/z 545 $(M+H)^+$, 186, 148 and 57.

(3*S*,4*S*,5*R*,6*S*)-*N*-(*tert*-Butoxycarbonyl)-6-[*N*,*N'*-di(*tert*-butoxycarbonyl)]guanidino-4,5-dihydroxy-4,5-*O*-isopropylidenepiperidine-3-carboxylic Acid (**10**)

The procedures used for the preparation of **10** were similar to those used for the preparation of **8** from **7**; the yields of amorph solid of **10** was 69%: $[\alpha]_D^{25}$ +41.3° (*c* 0.67, MeOH); IR (KBr) 3420, 3310, 2980, 2940, 1720, 1645, 1620, 1560, 1480, 1465, 1400, 1380, 1330, 1290, 1260, 1235, 1220, 1170, 1130, 1070, 1035, 1000 cm^{-1}; ^1H NMR (CD_3OD, 40°C) δ 1.35 and 1.41 (3H, each s, isopropylidene), 1.46 and 1.50 (27H, each s, $-NCOOC(CH_3)_3\times3$), 2.84 (1H, ddd, $J=2.4$, 4.6 and 12.7 Hz, 3-H), 3.46 (1H, broad t, $J=12.7$ Hz, 2-Hax), 3.68 (1H, dd, $J=4.6$ and 12.7 Hz, 2-Heq), 4.61 (1H, dd, $J=2.0$ and 7.3 Hz, 5-H), 4.86 (1H, dd, $J=2.4$ and 7.3 Hz, 4-H) and 6.04 (1H, d, $J=2.0$ Hz, 6-H); FAB-MS (positive) m/z 559 $(M+H)^+$, 200, 148, 104 and 57.

(3*S*,4*S*,5*R*,6*R*)-6-Guanidino-4,5-dihydroxypiperidine-3-carboxylic Acid (**5**)

To a soln of **10** in dichloromethane (0.6 ml) was added trifluoroacetic acid (0.6 ml), and the mixture was stirred at room temperature for 8 hours. Evaporation of the solvent gave a solid, which was subjected to column chromatography on Diaion HP20 resin. Elution with water gave an oil, which was solidified by removal of water by azeotropic evaporation with MeOH. The solid was dissolved in a small amount of MeOH (50 μl), and to the soln were added a soln of 4 M hydrogen in dioxane (1 ml) and Et_2O (5 ml) under vigorously stirring. The resulting precipitates were collected by centrifugation and washed with Et_2O to give a colorless amorph solid of **5** as its hydrochloride (19 mg, 94%): $[\alpha]_D^{26}$ +26.6° (*c* 0.39, H_2O); IR (KBr) 3400, 1720, 1690, 1640 (sh), 1400, 1345, 1285 (sh), 1230 (sh), 1185, 1145, 1100, 1030, 1010 cm^{-1}; ^1H NMR (D_2O) δ 3.10 (1H, dt, $J=2.2$ and 8.8 Hz, 3-H), 3.54 (2H, d, $J=8.8$ Hz, 2-H), 4.01 (1H, dd, $J=2.2$ and 9.8 Hz, 5-H), 4.60 (1H, t, $J=2.2$ Hz, 4-H) and 5.04 (1H, d, $J=9.8$ Hz, 6-H); FAB-MS (positive) 219 $(M+H)^+$, 185, 152, 110, 93, 75, 60 and 57.

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