Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary information

Synthesis and biological evaluation of 4-chlorocolchicine derivatives as potent anticancer agents with broad effective dosage ranges

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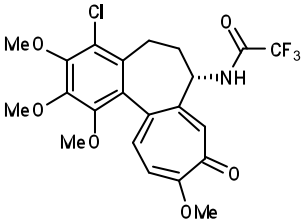
General methods

All reagents were obtained from commercial sources and used without further purification. Optical rotation was determined using a JASCO DIP-360 digital polarimeter. ^{1}H-NMR spectra were recorded on a JEOL ALPHA-400 using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. Chemical shifts are reported in ppm (δ scale) and all coupling constant (J) values are in Hz. HR-ESI-MS data were measured on an LCT-Premier XE time-of-flight instrument (Waters Corp.).

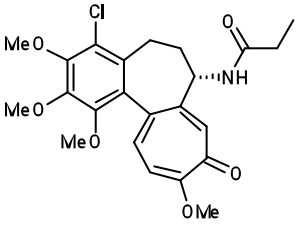
Synthesis of 4-chloro-*N*-(trifluoroacetyl)deacetylcolchicine (5)



A mixture of 4-chlorodeacetylcolchicine (**4**) (50 mg, 0.13 mmol) and TFAA (38 μL) in CH_2Cl_2 (6 mL) was stirred at 0 °C under argon atmosphere for 30 min. Saturated aqueous $NaHCO_3$ was added to the reaction mixture and the whole mixture was extracted two times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH) to afford compound **5** (56 mg, 0.12 mmol, 90% yield) as a white solid. $[\alpha]_D^{25}$: -114.4 ° (c = 0.250, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.64 (1H, br.s), 7.44 (1H,

s), 7.34 (1H, d, J = 10.5 Hz), 7.34 (1H, d, J = 11.0 Hz), 4.64-4.58 (1H, m), 4.03 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.63 (3H, s), 3.31 (1H, dd, J = 13.8, 5.7 Hz), 2.41-2.33 (1H, m), 2.20-2.17 (1H, m), 2.09-2.01 (1H, m). HR-ESI-MS: calcd for $C_{22}H_{22}{}^{35}ClF_3NO_6$ $[M+H]^+$, 488.1088; found, 488.1078; calcd for $C_{22}H_{22}{}^{37}ClF_3NO_6$ $[M+H+2]^+$, 490.1058; found, 490.1090.

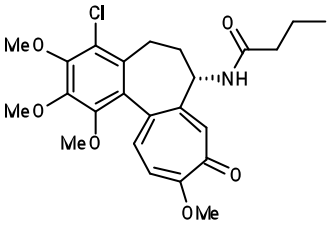
Synthesis of 4-chloro-*N*-propionyldeacetylcolchicine (6)



To a stirred solution of **4** (5.54 g, 14.1 mmol) in CH_2Cl_2 (200 mL) were added Et_3N (2.1 mL, 15.5 mmol) and propionyl chloride (1.23 mL, 14.1 mmol) under argon atmosphere at 0 °C. After stirring for 2 h at room temperature, the reaction was quenched by adding H_2O and the whole mixture was extracted four times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH) to afford compound **6** (6.33 g, 14.1 mmol, quant.) as an off-white solid.

$[\alpha]_D^{25}$: -119.5 ° (c = 0.997, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.49 (1H, s), 7.30 (1H, d, J = 10.6 Hz), 6.85 (1H, d, J = 10.8 Hz), 4.57 (1H, ddd, J = 12.0, 6.1, 6.1 Hz), 4.02 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.64 (3H, s), 3.24 (1H, dd, J = 13.5, 5.0 Hz), 2.34-2.23 (3H, overlapped), 2.15 (1H, ddd, J = 13.4, 13.4, 6.2 Hz), 1.80 (1H, ddd, J = 11.9, 11.9, 5.9 Hz), 1.10 (3H, dd, J = 7.6, 7.6 Hz). HR-ESI-MS: calcd for $C_{23}H_{27}{}^{35}ClNO_6$ $[M+H]^+$, 448.1527; found, 448.1509; calcd for $C_{23}H_{27}{}^{37}ClNO_6$ $[M+H+2]^+$, 450.1497; found, 450.1497.

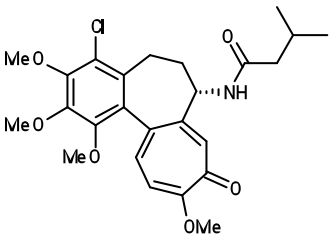
Synthesis of *N*-butyryl-4-chlorodeacetylcolchicine (7)



Compound **7** was prepared from compound **4** (50 mg, 0.13 mmol) and butyryl chloride (13 μL, 0.13 mmol) using the same procedure as that described for compound **6.** Amorphous solid (52 mg, 0.11 mmol, 88% yield).

$[\alpha]_D^{25}$: -128.6 ° (c = 0.391, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.52 (1H, s), 7.30 (1H, d, J = 10.7 Hz), 6.85 (1H, d, J = 10.7 Hz), 6.79 (1H, d, J = 6.8 Hz), 4.61-4.55 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.63 (3H, s), 3.25 (1H, dd, J = 12.8, 4.3 Hz), 2.25-2.23 (3H, m), 2.21-2.14 (1H, m), 1.81-1.77 (1H, m), 1.70-1.58 (3H, m), 0.92 (3H, t, J = 7.4 Hz). HR-ESI-MS: calcd for $C_{24}H_{29}{}^{35}ClNO_6$ $[M+H]^+$, 462.1683; found, 462.1696; calcd for $C_{24}H_{29}{}^{37}ClNO_6$ $[M+H+2]^+$, 464.1654; found, 464.1693.

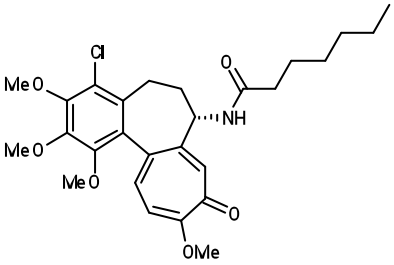
Synthesis of 4-chloro-*N*-isovaleryldeacetylcolchicine (8)



Compound **8** was prepared from compound **4** (50 mg, 0.13 mmol) and butyryl chloride (13 μL, 0.13 mmol) using the same procedure as that described for compound **6.** Amorphous solid (57 mg, 0.12 mmol, 94% yield).

$[\alpha]_D^{25}$: -145.1 ° (*c* = 0.300, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 7.47 (1H, s), 7.29 (1H, d, *J* = 10.7 Hz), 6.83 (1H, d, *J* = 10.7 Hz), 6.24 (1H, d, *J* = 7.3 Hz), 4.61-4.57 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.25 (1H, dd, *J* = 13.4, 4.4 Hz), 2.27-2.04 (5H, m), 1.77-1.74 (2H, m), 0.95 (3H, d, *J* = 6.3 Hz), 0.92 (3H, d, *J* = 6.1 Hz). HR-ESI-MS: calcd for $C_{25}H_{31}{}^{35}ClNO_6$ $[M+H]^+$, 476.1840; found, 476.1834; calcd for $C_{25}H_{31}{}^{37}ClNO_6$ $[M+H+2]^+$, 478.1810; found, 478.1840.

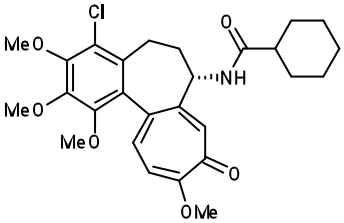
Synthesis of 4-chloro-*N*-heptanoyldeacetylcolchicine (9)



Compound **9** was prepared from compound **4** (50 mg, 0.13 mmol) and heptanoyl chloride (20 μL, 0.13 mmol) using the same procedure as that described for compound **6.** Amorphous solid (59 mg, 0.12 mmol, 92% yield).

$[\alpha]_D^{24}$: -136.4 ° (*c* = 0.460, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 7.48 (1H, s), 7.29 (1H, d, *J* = 10.7 Hz), 6.84 (1H, d, *J* = 10.7 Hz), 6.38 (1H, br-s), 4.58-4.55 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 2.26-2.11 (2H, m), 1.78-1.75 (1H, m), 1.62-1.54 (2H, m), 1.29-1.27 (8H, m), 0.88-0.84 (3H, m). HR-ESI-MS: calcd for $C_{27}H_{35}{}^{35}ClNO_6$ $[M+H]^+$, 504.2153; found, 504.2134; calcd for $C_{27}H_{35}{}^{37}ClNO_6$ $[M+H+2]^+$, 506.2123; found, 506.2112.

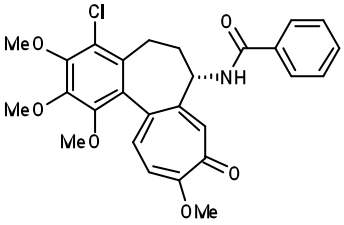
Synthesis of 4-chloro-*N*-(cyclohexanecarbonyl)deacetylcolchicine (10)



Compound **10** was prepared from compound **4** (50 mg, 0.13 mmol) and cyclohexanecarbonyl chloride (17 μL, 0.13 mmol) using the same procedure as that described for compound **6**. White solid (18 mg, 0.036 mmol, 28% yield).

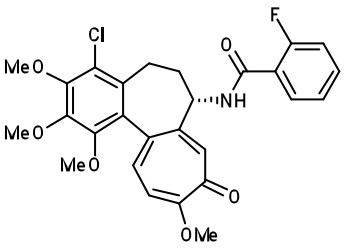
$[\alpha]_D^{25}$: -144.2 ° (*c* = 0.064, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* 7.47 (1H, s), 7.29 (1H, d, *J* = 10.7 Hz), 6.83 (1H, d, *J* = 10.7 Hz), 6.23 (1H, d, *J* = 6.6 Hz), 4.58-4.52 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.25 (1H, dd, *J* = 12.8, 4.5 Hz), 2.26-2.12 (3H, m), 1.94-1.90 (2H, m), 1.82-1.76 (1H, m), 1.66-1.63 (2H, m), 1.50-1.15 (5H, m). HR-ESI-MS: calcd for $C_{27}H_{33}{}^{35}ClNO_6$ $[M+H]^+$, 502.1996; found, 502.1990; calcd for $C_{27}H_{33}{}^{37}ClNO_6$ $[M+H+2]^+$, 504.1967; found, 504.1970.

Synthesis of *N*-benzoyl-4-chlorodeacetylcolchicine (11)



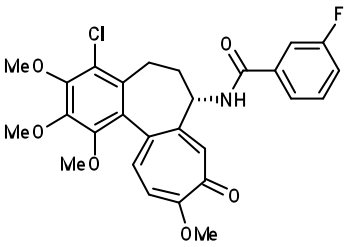
Compound **11** was prepared from compound **4** (150 mg, 0.38 mmol) and benzoyl chloride (44 μL, 0.38 mmol) using the same procedure as that described for compound **6.** Amorphous solid (146 mg, 0.29 mmol, 77% yield). $[\alpha]_D^{25}$: -53.3 ° (*c* = 0.324, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.80-7.79 (2H, m), 7.63 (1H, s), 7.49-7.47 (1H, m), 7.41-7.37 (2H, m), 7.35 (1H, d, *J* = 10.7 Hz), 6.93 (1H, br-s), 6.88 (1H, d, *J* = 11.0 Hz), 4.80-4.78 (1H, m), 4.01 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.70 (3H, s), 3.32 (1H, dd, *J* = 13.5, 5.2 Hz), 2.37-2.32 (1H, m), 2.25-2.22 (1H, m), 1.98-1.94 (1H, m). HR-ESI-MS: calcd for $C_{27}H_{27}{}^{35}ClNO_6$ $[M+H]^+$, 496.1527; found, 496.1517; calcd for $C_{27}H_{27}{}^{37}ClNO_6$ $[M+H+2]^+$, 498.1497; found, 498.1507.

Synthesis of 4-chloro-*N*-(2-fluorobenzoyl)deacetylcolchicine (12)



Compound **12** was prepared from compound **4** (50 mg, 0.13 mmol) and 2-fluorobenzoyl chloride (15 μL, 0.13 mmol) using the same procedure as that described for compound **6**. Yellow-brown solid (46 mg, 0.090 mmol, 70% yield). $[\alpha]_D^{24}$: -54.2 ° (*c* = 0.137, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.96-7.94 (1H, m), 7.55 (1H, s), 7.52-7.48 (1H, m), 7.33 (1H, d, *J* = 10.7 Hz), 7.26-7.14 (4H, m), 6.85 (1H, d, *J* = 10.7 Hz), 4.79-4.73 (1H, d, *J* = 10.7 Hz), 4.01 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.70 (3H, s), 3.32 (1H, dd, *J* = 13.5, 5.2 Hz), 2.42-2.32 (1H, m), 2.27-2.21 (1H, m), 1.95-1.87 (1H, m). HR-ESI-MS: calcd for $C_{27}H_{26}{}^{35}ClFNO_6$ $[M+H]^+$, 514.1433; found, 514.1430; calcd for $C_{27}H_{26}{}^{37}ClFNO_6$ $[M+H+2]^+$, 516.1403; found, 516.1418.

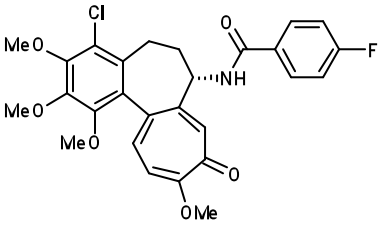
Synthesis of 4-chloro-*N*-(3-fluorobenzoyl)deacetylcolchicine (13)



Compound **13** was prepared from compound **4** (50 mg, 0.13 mmol) and 3-fluorobenzoyl chloride (15 μL, 0.13 mmol) using the same procedure as that described for compound **6**. Red-brown solid (21 mg, 0.041 mmol, 32% yield). $[\alpha]_D^{24}$: -17.9 ° (*c* = 0.078, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.82 (1H, s), 7.66 (1H, d, *J* = 7.6 Hz), 7.55-7.53 (2H, m), 7.47 (1H, d, *J* = 10.7 Hz), 7.35-7.33 (1H, m), 7.15-7.13 (1H, m), 6.99 (1H, d, *J* = 11.0 Hz), 4.82-4.77 (1H, m), 4.05 (3H, s), 4.01 (3H, s), 3.99 (3H, s), 3.70 (3H, s), 3.32 (1H,

dd, *J* = 13.1, 5.7 Hz), 2.41-2.32 (1H, m), 2.29-2.19 (1H, m), 2.16-2.08 (1H, m). HR-ESI-MS: calcd for $C_{27}H_{26}{}^{35}ClFNO_6$ $[M+H]^+$, 514.1433; found, 514.1442; calcd for $C_{27}H_{26}{}^{37}ClFNO_6$ $[M+H+2]^+$, 516.1403; found, 516.1436.

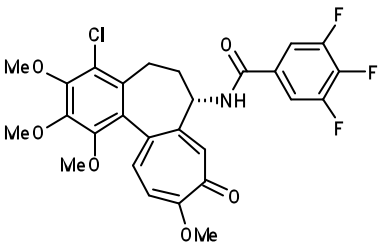
Synthesis of 4-chloro-*N*-(4-fluorobenzoyl)deacetylcolchicine (14)



Compound **14** was prepared from compound **4** (50 mg, 0.13 mmol) and 4-fluorobenzoyl chloride (15 μL, 0.13 mmol) using the same procedure as that described for compound **6**. Amorphous solid (45 mg, 0.088 mmol, 68% yield).

$[\alpha]_D^{25}$: -66.9 ° (*c* = 0.063, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.83-7.79 (2H, m), 7.57 (1H, s), 7.36 (1H, br-s), 7.34 (1H, d, *J* = 10.7 Hz), 7.01-6.99 (2H, m), 6.88 (1H, d, *J* = 11.0 Hz), 4.78-4.72 (1H, m), 4.02 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.70 (3H, s), 3.31 (1H, dd, *J* = 13.4, 5.4 Hz), 2.37-2.33 (1H, m), 2.24-2.20 (1H, m), 1.97-1.94 (1H, m). HR-ESI-MS: calcd for $C_{27}H_{26}{}^{35}ClFNO_6$ $[M+H]^+$, 514.1433; found, 514.1440; calcd for $C_{27}H_{26}{}^{37}ClFNO_6$ $[M+H+2]^+$, 516.1403; found, 516.1421.

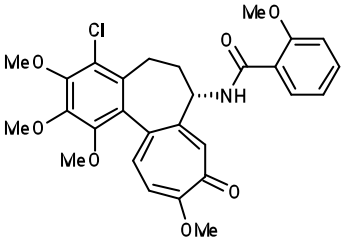
Synthesis of 4-chloro-*N*-(3,4,5-trifluorobenzoyl)deacetylcolchicine (15)



Compound **15** was prepared from compound **4** (50 mg, 0.13 mmol) and 3,4,5-trifluorobenzoyl chloride (17 μL, 0.13 mmol) using the same procedure as that described for compound **6**. Red-brown solid (38 mg, 0.069 mmol, 54% yield).

$[\alpha]_D^{25}$: -52.3 ° (*c* = 0.078, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.26 (1H, br-s), 7.65 (1H, s), 7.49-7.47 (2H, m), 7.40 (1H, d, *J* = 10.7 Hz), 6.95 (1H, d, *J* = 11.0 Hz), 4.78-4.72 (1H, m), 4.05 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.68 (3H, s), 3.31 (1H, dd, *J* = 13.5, 5.6 Hz), 2.39-2.35 (1H, m), 2.20-2.15 (2H, m). HR-ESI-MS: calcd for $C_{27}H_{24}{}^{35}ClF_3NO_6$ $[M+H]^+$, 550.1244; found, 550.1237; calcd for $C_{27}H_{24}{}^{37}ClF_3NO_6$ $[M+H+2]^+$, 552.1215; found, 552.1190.

Synthesis of 4-chloro-*N*-(2-methoxybenzoyl)deacetylcolchicine (16)

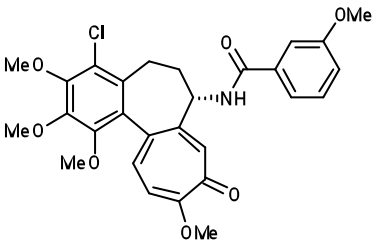


Compound **16** was prepared from compound **4** (50 mg, 0.13 mmol) and 2-methoxybenzoyl chloride (17 μL, 0.13 mmol) using the same procedure as that described for compound **6**. Pale yellow solid (49 mg, 0.093 mmol, 73% yield).

$[\alpha]_D^{25}$: -64.6 ° (*c* = 0.110, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$):

δ 8.35 (1H, d, J = 6.6 Hz), 8.01 (1H, dd, J = 7.7, 1.8 Hz), 7.51 (1H, s), 7.48-7.44 (1H, m), 7.28 (1H, d, J = 10.7 Hz), 7.04-7.00 (2H, m), 6.80 (1H, d, J = 10.7 Hz), 4.79-4.73 (1H, m), 4.05 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, J = 13.2, 4.6 Hz), 2.40-2.31 (1H, m), 2.27-2.23 (1H, m), 1.89-1.85 (1H, m). ESI-MS *m/z*: 526 $[M+H]^+$, 528 $[M+H+2]^+$. HR-ESI-MS: calcd for $C_{28}H_{29}{}^{35}ClNO_7$ $[M+H]^+$, 526.1633; found, 526.1623; calcd for $C_{28}H_{29}{}^{37}ClNO_7$ $[M+H+2]^+$, 528.1603; found, 528.1541.

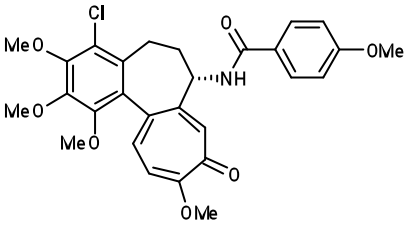
Synthesis of 4-chloro-*N*-(3-methoxybenzoyl)deacetylcolchicine (17)



Compound **17** was prepared from compound **4** (50 mg, 0.13 mmol) and 3-methoxybenzoyl chloride (17 μL, 0.13 mmol) using the same procedure as that described for compound **6**. Amorphous solid (53 mg, 0.101 mmol, 79% yield).

$[\alpha]_D^{25}$: -209.4 ° (*c* = 0.087, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.54 (1H, s), 7.34-7.31 (3H, m), 7.27 (1H, d, J = 10.7 Hz), 7.02-7.00 (1H, m), 6.96 (1H, d, J = 5.4 Hz), 6.85 (1H, d, J = 10.7 Hz), 4.01 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.80 (3H, s), 3.70 (3H, s), 3.31 (1H, dd, J = 13.3, 5.0 Hz), 2.39-2.30 (1H, m), 2.25-2.21 (1H, m), 1.95-1.89 (1H, m). HR-ESI-MS: calcd for $C_{28}H_{29}{}^{35}ClNO_7$ $[M+H]^+$, 526.1633; found, 526.1649; calcd for $C_{28}H_{29}{}^{37}ClNO_7$ $[M+H+2]^+$, 528.1603; found, 528.1633.

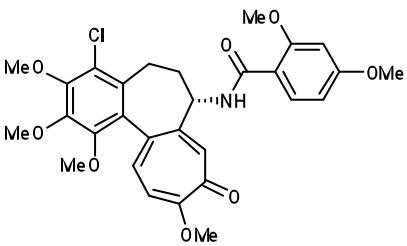
Synthesis of 4-chloro-*N*-(4-methoxybenzoyl)deacetylcolchicine (18)



Compound **18** was prepared from compound **4** (50 mg, 0.13 mmol) and 4-methoxybenzoyl chloride (26 mg, 0.13 mmol) using the same procedure as that described for compound **6**. Amorphous solid (56 mg, 0.11 mmol, 83% yield).

$[\alpha]_D^{25}$: -37.2 ° (*c* = 0.148, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.75 (2H, d, J = 8.5 Hz), 7.54 (1H, s), 7.31 (1H, d, J = 10.7 Hz), 6.87 (2H, d, J = 9.0 Hz), 6.85 (1H, br-s), 6.84 (1H, d, J = 11.0 Hz), 4.78-4.72 (1H, m), 4.00 (3H, s), 4.00 (3H, s), 3.97 (3H, s), 3.83 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, J = 13.3, 4.5 Hz), 2.38-2.29 (1H, m), 2.25-2.21 (1H, m), 1.92-1.89 (1H, m). HR-ESI-MS: calcd for $C_{28}H_{29}{}^{35}ClNO_7$ $[M+H]^+$, 526.1633; found, 526.1649; calcd for $C_{28}H_{29}{}^{37}ClNO_7$ $[M+H+2]^+$, 528.1603; found, 528.1602.

Synthesis of 4-chloro-*N*-(2,4-dimethoxybenzoyl)deacetylcolchicine (19)

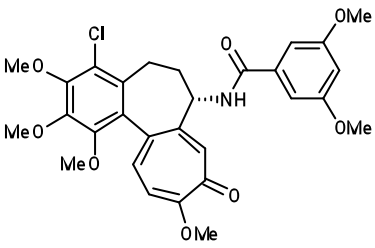


Compound **19** was prepared from compound **4** (50 mg, 0.13 mmol) and 2,4-dimethoxybenzoyl chloride (26 mg,

0.13 mmol) using the same procedure as that described for compound **6**. White solid (33 mg, 0.059 mmol, 46% yield).

$[\alpha]_D^{26}$: -23.8 ° (*c* = 0.147, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 8.29 (1H, d, *J* = 6.3 Hz), 7.98 (1H, d, *J* = 8.8 Hz), 7.64 (1H, s), 7.34 (1H, d, *J* = 10.7 Hz), 6.85 (1H, d, *J* = 10.7 Hz), 6.54 (1H, dd, *J* = 8.7, 2.1 Hz), 6.51 (1H, d, *J* = 2.0 Hz), 4.78-4.71 (1H, m), 4.02 (3H, s), 4.00 (3H, s), 4.00 (3H, s), 3.97 (3H, s), 3.85 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, *J* = 13.2, 5.1 Hz), 2.40-2.31 (1H, m), 2.26-2.18 (1H, m), 1.93-1.84 (1H, m). HR-ESI-MS: calcd for $C_{29}H_{31}{}^{35}ClNO_8$ $[M+H]^+$, 556.1738; found, 556.1747; calcd for $C_{29}H_{31}{}^{37}ClNO_8$ $[M+H+2]^+$, 558.1709; found, 558.1755.

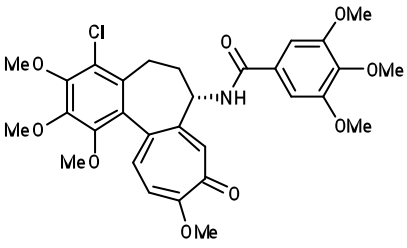
Synthesis of 4-chloro-*N*-(3,5-dimethoxybenzoyl)deacetylcolchicine (20)



Compound **20** was prepared from compound **4** (50 mg, 0.13 mmol) and 3,5-dimethoxybenzoyl chloride (26 mg, 0.13 mmol) using the same procedure as that described for compound **6**. White solid (47 mg, 0.085 mmol, 66% yield).

$[\alpha]_D^{26}$: -44.0 ° (*c* = 0.219, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.65 (1H, s), 7.38 (1H, d, *J* = 10.7 Hz), 7.01 (1H, d, *J* = 7.1 Hz), 6.93 (2H, s), 6.90 (1H, d, *J* = 10.7 Hz), 6.56 (1H, s), 4.77-4.71 (1H, m), 4.02 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.79 (6H, s), 3.69 (3H, s), 3.31 (1H, dd, *J* = 13.3, 5.2 Hz), 2.40-2.30 (1H, m), 2.25-2.17 (1H, m), 2.02-1.95 (1H, m). HR-ESI-MS: calcd for $C_{29}H_{31}{}^{35}ClNO_8$ $[M+H]^+$, 556.1738; found, 556.1727; calcd for $C_{29}H_{31}{}^{37}ClNO_8$ $[M+H+2]^+$, 558.1709; found, 558.1723.

Synthesis of 4-chloro-*N*-(3,4,5-trimethoxybenzoyl)deacetylcolchicine (21)

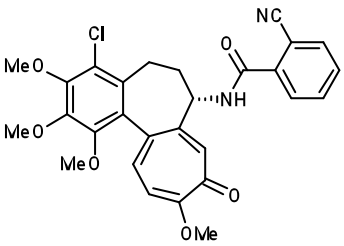


Compound **21** was prepared from compound **4** (50 mg, 0.13 mmol) and 3,4,5-trimethoxybenzoyl chloride (30 mg, 0.13 mmol) using the same procedure as that described for compound **6**. Yellow-brown solid (68 mg, 0.12 mmol, 91% yield).

$[\alpha]_D^{25}$: -41.5 ° (*c* = 0.084, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, s), 7.63 (1H, br-s), 7.40 (1H, d, *J* = 10.7 Hz), 7.07 (2H, s), 6.93 (1H, d, *J* = 11.0 Hz), 4.79-4.72 (1H, m), 4.03 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.84 (3H, s), 3.81 (3H, s), 3.81 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, *J* = 13.5, 5.5 Hz), 2.42-2.33 (1H, m), 2.22-2.16 (1H, m), 2.09-2.03 (1H, m). HR-ESI-MS: calcd for $C_{30}H_{33}{}^{35}ClNO_9$ $[M+H]^+$, 586.1844; found, 556.1727; calcd for $C_{30}H_{33}{}^{37}ClNO_9$ $[M+H+2]^+$, 588.1814; found,

588.1821.

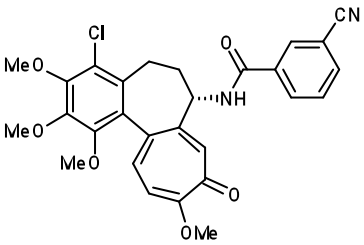
Synthesis of 4-chloro-*N*-(2-cyanobenzoyl)deacetylcolchicine (22)



To a stirred solution of 2-cyanobenzoic acid (23 mg, 0.15 mmol) in DMF (1 mL) were added EDCI (29 mg, 0.15 mmol) and HOBt (21 mg, 0.15 mmol) under argon atmosphere at 0 °C. After stirring for 30 min at 0 °C, compound **4** (50 mg, 0.13 mmol) was added to the mixture. The resulting mixture was further stirred at ambient temperature for 3 h. The reaction was quenched by adding H_2O and the whole mixture was extracted two times with AcOEt. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and then brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH) to afford compound **22** (41 mg, 0.079 mmol, 62% yield) as a yellow-brown solid.

$[\alpha]_D^{25}$: -129.1 ° (*c* = 0.338, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.96 (1H, d, *J* = 7.8 Hz), 7.72 (1H, d, *J* = 6.6 Hz), 7.64-7.53 (3H, m), 7.46 (1H, br-s), 7.35 (1H, d, *J* = 10.5 Hz), 6.89 (1H, d, *J* = 11.0 Hz), 4.86-4.80 (1H, m), 4.02 (3H, s), 4.01 (3H, s), 4.00 (3H, s), 3.70 (3H, s), 3.32 (1H, dd, *J* = 13.5, 5.5 Hz), 2.47-2.38 (1H, m), 2.26-2.18 (1H, m), 2.03-1.95 (1H, m). ESI-MS *m*/*z*: 521 $[M+H]^+$, 523 $[M+H+2]^+$. HR-ESI-MS: calcd for $C_{28}H_{26}{}^{35}ClN_2O_6$ $[M+H]^+$, 521.1479; found, 521.1479; calcd for $C_{28}H_{26}{}^{37}ClN_2O_6$ $[M+H+2]^+$, 523.1450; found, 523.1472.

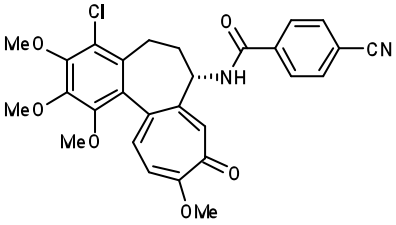
Synthesis of 4-chloro-*N*-(3-cyanobenzoyl)deacetylcolchicine (23)



Compound **23** was prepared from compound **4** (50 mg, 0.13 mmol) and 3-cyanobenzoyl chloride (21 mg, 0.13 mmol) using the same procedure as that described for compound **6**. Light yellow solid (44 mg, 0.084 mmol, 66% yield).

$[\alpha]_D^{25}$: -58.6 ° (*c* = 0.251, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.33 (1H, br-s), 8.14 (1H, s), 7.98 (1H, d, *J* = 8.1 Hz), 7.67 (2H, s), 7.66-7.65 (2H, m), 7.42-7.40 (1H, m), 7.41 (1H, d, *J* = 11.0 Hz), 6.95 (1H, d, *J* = 10.7 Hz), 4.81-4.75 (1H, m), 4.03 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.71 (3H, s), 3.33 (1H, dd, *J* = 12.7, 5.4 Hz), 2.41-2.35 (1H, m), 2.26-2.15 (2H, m). HR-ESI-MS: calcd for $C_{28}H_{26}{}^{35}ClN_2O_6$ $[M+H]^+$, 521.1479; found, 521.1464; calcd for $C_{28}H_{26}{}^{37}ClN_2O_6$ $[M+H+2]^+$, 523.1450; found, 523.1428.

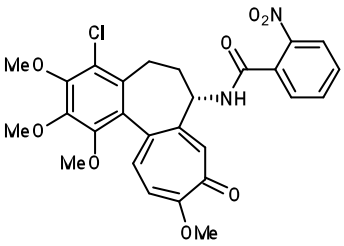
Synthesis of 4-chloro-*N*-(4-cyanobenzoyl)deacetylcolchicine (24)



Compound **24** was prepared from compound **4** (50 mg, 0.13 mmol) and 4-cyanobenzoyl chloride (21 mg, 0.13 mmol) using the same procedure as that described for compound **6**. White solid (51 mg, 0.098 mmol, 77% yield).

$[\alpha]_D^{26}$: -48.6 ° (*c* = 0.366, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.45 (1H, d, *J* = 5.9 Hz), 7.93 (2H, d, *J* = 8.3 Hz), 7.71 (1H, s), 7.52 (2H, d, *J* = 8.1 Hz), 7.44 (1H, d, *J* = 11.0 Hz), 6.98 (1H, d, *J* = 11.0 Hz), 4.81-4.75 (1H, m), 4.06 (3H, s), 4.02 (3H, s), 3.99 (3H, s), 3.72 (3H, s), 3.31 (1H, dd, *J* = 13.2, 5.6 Hz), 2.44-2.35 (1H, m), 2.24-2.17 (1H, m), 2.16-2.06 (1H, m). HR-ESI-MS: calcd for $C_{28}H_{26}{}^{35}ClN_2O_6$ $[M+H]^+$, 521.1479; found, 521.1472; calcd for $C_{28}H_{26}{}^{37}ClN_2O_6$ $[M+H+2]^+$, 523.1450; found, 523.1439.

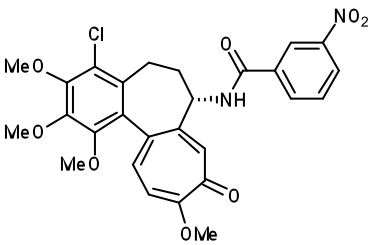
Synthesis of 4-chloro-*N*-(2-nitrobenzoyl)deacetylcolchicine (25)



Compound **25** was prepared from compound **4** (50 mg, 0.13 mmol) and 2-nitrobenzoic acid (26 mg, 0.15 mmol) using the same procedure as that described for compound **22**. White solid (23 mg, 0.043 mmol, 33% yield).

$[\alpha]_D^{26}$: -127.2 ° (*c* = 0.157, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.04 (1H, d, *J* = 7.8 Hz), 7.71-7.63 (3H, m), 7.58-7.56 (1H, m), 7.35 (1H, d, *J* = 10.2 Hz), 6.88 (1H, d, *J* = 10.7 Hz), 6.86 (1H, br-s), 4.85-4.79 (1H, m), 4.02 (3H, s), 4.02 (3H, s), 4.02 (3H, s), 3.66 (3H, s), 3.31 (1H, dd, *J* = 13.7, 5.4 Hz), 2.44-2.34 (1H, m), 2.22-2.18 (1H, m), 1.95-1.81 (1H, m). HR-ESI-MS: calcd for $C_{27}H_{26}{}^{35}ClN_2O_8$ $[M+H]^+$, 541.1378; found, 541.1356; calcd for $C_{27}H_{26}{}^{37}ClN_2O_8$ $[M+H+2]^+$, 543.1348; found, 543.1362.

Synthesis of 4-chloro-*N*-(3-nitrobenzoyl)deacetylcolchicine (26)

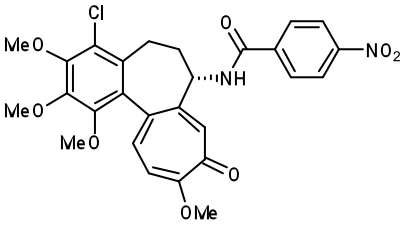


Compound **26** was prepared from compound **4** (50 mg, 0.13 mmol) and 3-nitrobenzoyl chloride (27 mg, 0.13 mmol) using the same procedure as that described for compound **6**. Yellow solid (59 mg, 0.11 mmol, 85% yield).

$[\alpha]_D^{26}$: -49.7 ° (*c* = 0.507, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.90 (1H, d, *J* = 6.1 Hz), 8.57 (1H, s), 8.16 (1H, d, *J* = 8.1 Hz), 7.96 (1H, d, *J* = 7.6 Hz), 7.89 (1H, s), 7.47 (1H, d, *J* = 10.7 Hz), 7.34 (1H, t, *J* = 7.9 Hz), 7.03 (1H, d, *J* = 11.0 Hz), 4.83-4.77 (1H, m), 4.07 (3H, s), 4.02 (3H, s), 3.98 (3H,

s), 3.70 (3H, s), 3.33 (1H, dd, J = 13.4, 5.6 Hz), 2.47-2.38 (1H, m), 2.34-2.29 (1H, m), 2.27-2.17 (1H, m). HR-ESI-MS: calcd for $C_{27}H_{26}{}^{35}ClN_2O_8$ $[M+H]^+$, 541.1378; found, 541.1421; calcd for $C_{27}H_{26}{}^{37}ClN_2O_8$ $[M+H+2]^+$, 543.1348; found, 543.1346.

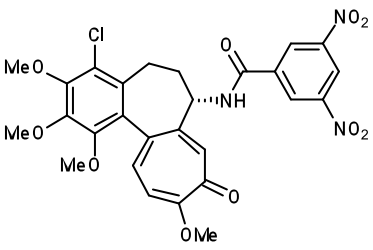
Synthesis of 4-chloro-*N*-(4-nitrobenzoyl)deacetylcolchicine (27)



Compound **27** was prepared from compound **4** (50 mg, 0.13 mmol) and 4-nitrobenzoyl chloride (24 mg, 0.13 mmol) using the same procedure as that described for compound **6**. White solid (53 mg, 0.10 mmol, 77% yield).

$[\alpha]_D^{26}$: -47.6 ° (c = 0.283, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.31 (1H, d, J = 8.3 Hz), 8.07-8.02 (2H, m), 7.95 (2H, d, J = 8.5 Hz), 7.64 (1H, s), 7.41 (1H, d, J = 10.7 Hz), 6.95 (1H, d, J = 10.7 Hz), 4.80-4.74 (1H, m), 4.05 (3H, s), 4.02 (3H, s), 3.99 (3H, s), 3.72 (3H, s), 3.32 (1H, dd, J = 13.5, 5.5 Hz), 2.45-2.36 (1H, m), 2.22-2.19 (1H, m), 2.11-2.04 (1H, m). HR-ESI-MS: calcd for $C_{27}H_{26}{}^{35}ClN_2O_8$ $[M+H]^+$, 541.1378; found, 541.1393; calcd for $C_{27}H_{26}{}^{37}ClN_2O_8$ $[M+H+2]^+$, 543.1348; found, 543.1349.

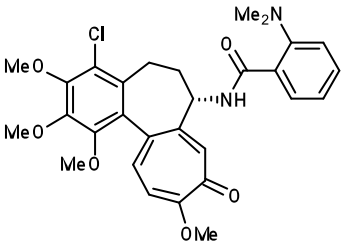
Synthesis of 4-chloro-*N*-(3,5-dinitrobenzoyl)deacetylcolchicine (28)



Compound **28** was prepared from compound **4** (50 mg, 0.13 mmol) and 3,5-dinitrobenzoyl chloride (30 mg, 0.13 mmol) using the same procedure as that described for compound **6**. Yellow solid (47 mg, 0.080 mmol, 63% yield).

$[\alpha]_D^{26}$: -34.4 ° (c = 0.375, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.91 (1H, d, J = 5.9 Hz), 8.95 (1H, t, J = 1.8 Hz), 8.63 (2H, s), 7.94 (1H, s), 7.54 (1H, d, J = 10.7 Hz), 7.14 (1H, d, J = 11.0 Hz), 4.78-4.72 (1H, m), 4.12 (3H, s), 4.03 (3H, s), 3.98 (3H, s), 3.67 (3H, s), 3.37 (1H, dd, J = 12.2, 2.9 Hz), 2.49-2.42 (2H, m), 2.28-2.19 (1H, m). HR-ESI-MS: calcd for $C_{27}H_{25}{}^{35}ClN_3O_{10}$ $[M+H]^+$, 586.1228; found, 586.1213; calcd for $C_{27}H_{25}{}^{35}ClN_3O_{10}$ $[M+H+2]^+$, 588.1199; found, 588.1182.

Synthesis of 4-chloro-*N*-(2-(dimethylamino)benzoyl)deacetylcolchicine (29)

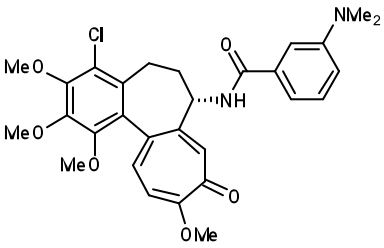


Compound **29** was prepared from compound **4** (50 mg, 0.13 mmol) and 2-(dimethylamino)benzoic acid (25 mg, 0.15 mmol) using the same procedure as that described for compound **22**. White solid (24 mg, 0.045 mmol, 35% yield).

$[\alpha]_D^{26}$: -59.0 ° (c = 0.119, MeOH). ^{1}H-NMR (400 MHz,

DMSO-*d*6): δ 9.54 (1H, d, *J* = 7.3 Hz), 7.42-7.38 (2H, m), 7.33 (1H, s), 7.17-7.15 (2H, m), 7.06-6.99 (2H, m), 4.52-4.46 (1H, m), 3.94 (3H, s), 3.90 (3H, s), 3.89 (3H, s), 3.60 (3H, s), 3.15 (1H, dd, *J* = 12.9, 3.7 Hz), 2.70 (6H, s), 2.17-2.14 (1H, m), 2.10-1.98 (2H, m). HR-ESI-MS: calcd for $C_{29}H_{32}{}^{35}ClN_2O_6$ $[M+H]^+$, 539.1949; found, 539.1949; calcd for $C_{29}H_{32}{}^{37}ClN_2O_6$ $[M+H+2]^+$, 541.1919; found, 541.1932.

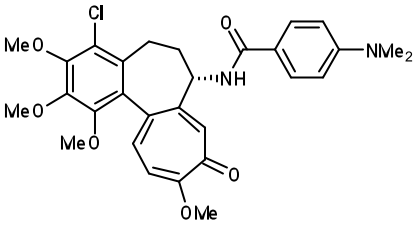
Synthesis of 4-chloro-*N*-(3-(dimethylamino)benzoyl)deacetylcolchicine (30)



Compound **30** was prepared from compound **4** (50 mg, 0.13 mmol) and 3-(dimethylamino)benzoic acid (25 mg, 0.15 mmol) using the same procedure as that described for compound **22**. Yellow solid (37 mg, 0.069 mmol, 54% yield).

$[\alpha]_D^{24}$: -54.5 ° (*c* = 0.112, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*6): δ 8.96 (1H, d, *J* = 7.3 Hz), 7.28 (1H, t, *J* = 7.9 Hz), 7.20-7.17 (3H, m), 7.13 (1H, d, *J* = 2.0 Hz), 7.05 (1H, d, *J* = 11.2 Hz), 6.89 (1H, dd, *J* = 8.1, 2.4 Hz), 4.51-4.45 (1H, m), 3.94 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.60 (3H, s), 3.16 (1H, dd, *J* = 12.1, 5.5 Hz), 2.92 (6H, s), 2.21-2.10 (2H, m), 2.07-2.03 (1H, m). HR-ESI-MS: calcd for $C_{29}H_{32}{}^{35}ClN_2O_6$ $[M+H]^+$, 539.1949; found, 539.1940; calcd for $C_{29}H_{32}{}^{37}ClN_2O_6$ $[M+H+2]^+$, 541.1919; found, 541.1937.

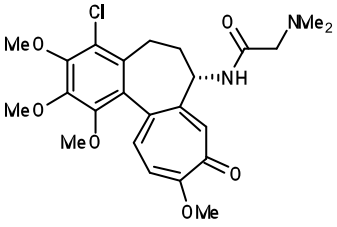
Synthesis of 4-chloro-*N*-(4-(dimethylamino)benzoyl)deacetylcolchicine (31)



Compound **31** was prepared from compound **4** (50 mg, 0.13 mmol) and 4-(dimethylamino)benzoic acid (25 mg, 0.15 mmol) using the same procedure as that described for compound **22**. Light yellow solid (38 mg, 0.070 mmol, 55% yield).

$[\alpha]_D^{24}$: +10.4 ° (*c* = 0.413, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.71 (2H, d, *J* = 8.8 Hz), 7.50 (1H, s), 7.28 (1H, d, *J* = 10.7 Hz), 6.83 (1H, d, *J* = 7.8 Hz), 6.80 (1H, d, *J* = 10.7 Hz), 6.73 (2H, d, *J* = 8.3 Hz), 4.78-4.72 (1H, m), 4.00 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.70 (3H, s), 3.30 (1H, dd, *J* = 12.9, 4.1 Hz), 3.01 (6H, s), 2.32-2.23 (2H, m), 1.95-1.88 (1H, m). HR-ESI-MS: calcd for $C_{29}H_{32}{}^{35}ClN_2O_6$ $[M+H]^+$, 539.1949; found, 539.1956; calcd for $C_{29}H_{32}{}^{37}ClN_2O_6$ $[M+H+2]^+$, 541.1919; found, 541.1945.

Synthesis of 4-chloro-*N*-(dimethylaminoacetyl)deacetylcolchicine (32)

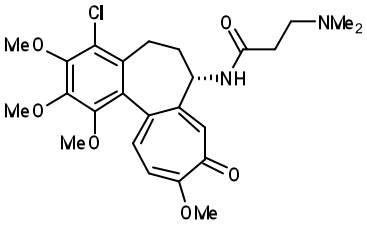


Compound **32** was prepared from compound **4** (130 mg, 0.33 mmol) and *N*,*N*-dimethylglycine (41 mg, 0.40 mmol) using

the same procedure as that described for compound **22**. Yellow solid (107 mg, 0.22 mmol, 68% yield).

$[\alpha]_D^{25}$: -117.9 ◦ (*c* = 0.168, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 8.58 (1H, d, *J* = 7.3 Hz), 7.16 (1H, s), 7.12 (1H, d, *J* = 10.7 Hz), 7.03 (1H, d, *J* = 10.7 Hz), 4.31-4.28 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.10 (1H, dd, *J* = 13.2, 5.6 Hz), 2.89 (2H, d, *J* = 8.3 Hz), 2.21 (6H, s), 2.10-2.06 (1H, m), 1.93-1.92 (1H, m). HR-ESI-MS: calcd for $C_{24}H_{30}{}^{35}ClN_2O_6$ $[M+H]^+$, 477.1792; found, 477.1782; calcd for $C_{24}H_{30}{}^{37}ClN_2O_6$ $[M+H+2]^+$, 479.1763; found, 479.1757.

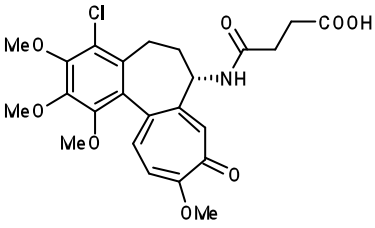
Synthesis of 4-chloro-*N*-(3-(dimethylamino)propionyl)deacetylcolchicine (33)



Compound **33** was prepared from compound **4** (50 mg, 0.13 mmol) and 3-dimethylpropionic acid hydrochloride (39 mg, 0.26 mmol) using the same procedure as that described for compound **22**. Off-white solid (27 mg, 0.055 mmol, 43% yield).

$[\alpha]_D^{24}$: -129.3 ◦ (*c* = 0.121, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 8.64 (1H, d, 7.1 Hz), 7.13 (1H, s), 7.12 (1H, d, *J* = 11.0 Hz), 7.02 (1H, d, *J* = 11.0 Hz), 4.26-4.20 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.11 (1H, dd, *J* = 13.4, 5.1 Hz), 2.43-2.37 (2H, m), 2.31-2.27 (2H, m), 2.11 (3H, s), 2.10-2.08 (1H, m), 1.99-1.90 (1H, m), 1.86-1.78 (1H, m). HR-ESI-MS: calcd for $C_{25}H_{32}{}^{35}ClN_2O_6$ $[M+H]^+$, 491.1949; found, 491.1931; calcd for $C_{25}H_{32}{}^{37}ClN_2O_6$ $[M+H+2]^+$, 493.1919; found, 493.1919.

Synthesis of *N*-(3-carboxypropionyl)-4-chlorodeacetylcolchicine (34)

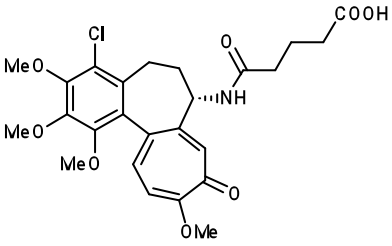


A solution of compound **4** (80 mg, 0.20 mmol), *N*-methylmorpholine (88 mL, 1.0 mmol), and succinic anhydride (24 mg, 0.22 mmol) in DMSO was stirred at ambient temperature for 1 h under argon atmosphere. To the reaction mixture was added saturated aqueous citric acid and the whole mixture was extracted two times with AcOEt. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH) to afford compound **34** (60 mg, 0.12 mmol, 60% yield) as a light yellow solid.

$[\alpha]_D^{25}$: -125.6 ◦ (*c* = 0.158, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 8.75 (1H, d, *J* = 7.1 Hz), 7.16 (1H, s), 7.13 (1H, d, *J* = 10.5 Hz), 7.03 (1H, d, *J* = 11.0 Hz), 4.25-4.19 (1H, m),

3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.10 (1H, dd, J = 13.4, 5.4 Hz), 2.35 (2H, t, J = 6.5 Hz), 2.26 (2H, t, J = 6.6 Hz), 2.12-2.08 (1H, m), 1.97-1.92 (1H, m), 1.87-1.78 (1H, m). HR-ESI-MS: calcd for $C_{24}H_{27}{}^{35}ClNO_8$ $[M+H]^+$, 492.1425; found, 492.1436; calcd for $C_{24}H_{27}{}^{37}ClNO_8$ $[M+H+2]^+$, 494.1396; found, 494.1397.

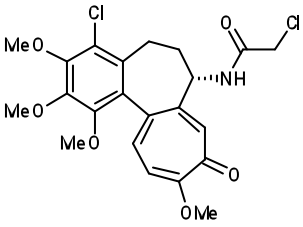
Synthesis of *N*-(4-carboxybutyryl)-4-chlorodeacetylcolchicine (35)



Compound **35** was prepared from compound **4** (100 mg, 0.26 mmol) and glutaric anhydride (32 mg, 0.28 mmol) using the same procedure as that described for compound **34**. Light yellow solid (112 mg, 0.24 mmol, 95% yield).

$[\alpha]_D^{25}$: -124.5 ° (c = 0.381, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.87 (1H, s), 7.77 (1H, br-s), 7.44 (1H, d, J = 10.7 Hz), 7.02 (1H, d, J = 10.5 Hz), 4.58-4.52 (1H, m), 4.04 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.60 (3H, s), 3.27 (1H, dd, J = 13.1, 4.8 Hz), 2.53-2.34 (6H, m), 2.26-2.23 (1H, m), 2.18-2.10 (1H, m), 2.01-2.00 (1H, m). HR-ESI-MS: calcd for $C_{25}H_{29}{}^{35}ClNO_8$ $[M+H]^+$, 506.1582; found, 506.1560; calcd for $C_{25}H_{29}{}^{37}ClNO_8$ $[M+H+2]^+$, 508.1552; found, 508.1545.

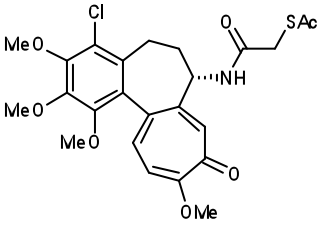
Synthesis of *N*-chloroacetyl-4-chlorodeacetylcolchicine (46)



Compound **46** was prepared from compound **4** (24 mg, 0.061 mmol) and chloroacetyl chloride (5.8 μL, 0.073 mmol) using the same procedure as that described for compound **6**. Yellow oil (31 mg, 0.061 mmol, quant.).

$[\alpha]_D^{13}$: -129.2 ° (c = 0.05, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.57 (1H, br-d, J = 6.8 Hz), 7.40 (1H, s), 7.28 (1H, d, J = 10.7 Hz), 6.84 (1H, d, J = 10.7 Hz), 4.55 (1H, ddd, J = 12.1, 6.2, 6.2 Hz), 4.09 (1H, d, J = 14.9 Hz), 4.02 (1H, d, J = 14.9 Hz), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.28 (1H, dd, J = 13.2, 4.4 Hz), 2.29 (1H, dddd, J = 12.1, 12.1, 6.0, 6.0 Hz), 2.19 (1H, ddd, J = 13.1, 13.1, 5.9 Hz), 1.89 (1H, ddd, J = 11.6, 11.6, 5.0 Hz). HR-ESI-MS: calcd for $C_{22}H_{24}{}^{35}Cl_2NO_6$ $[M+H]^+$, 468.0981; found, 468.0979; calcd for $C_{22}H_{24}{}^{35}Cl^{37}ClNO_6$ $[M+H+2]^+$, 470.0951; found, 470.0981; calcd for $C_{22}H_{24}{}^{37}Cl_2NO_6$ $[M+H+4]^+$, 472.0922; found, 472.0947.

Synthesis of 4-chloro-*N*-(thioacetoxyacetyl)deacetylcolchicine (47)

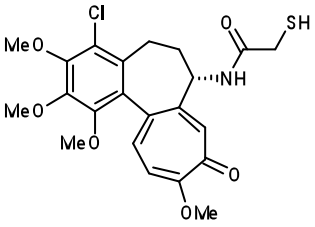


To a stirred solution of compound **46** (50 mg, 0.11 mmol) in acetone (10 mL) was added potassium thioacetate (75 μg, 0.66 mmol) under argon atmosphere at 0 °C and the reaction

mixture was stirred for 90 min at 65 °C. The reaction mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH) to afford compound **47** (46 mg, 0.090 mmol, 82%yield) as a yellow oil.

$[\alpha]_D^{15}$: -211.7 ° (*c* = 0.02, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.29 (1H, br-d, *J* = 3.5 Hz), 7.28 (1H, s), 7.24 (1H, d, *J* = 10.6 Hz), 6.80 (1H, d, *J* = 10.8 Hz), 4.49 (1H, ddd, *J* = 12.2, 6.1, 6.1 Hz), 4.00 (3H, s), 3.98 (3H, s), 3.97 (3H, s), 3.62 (1H, dd, *J* = 14.5, 1.8 Hz), 3.60 (3H, s), 3.54 (1H, dd, *J* = 14.4, 2.5 Hz), 3.25 (1H, dd, *J* = 12.7, 3.9 Hz), 2.45 (3H, s), 2.22 (1H, dddd, *J* = 11.0 11.0, 5.9, 5.9 Hz), 2.16 (1H, ddd, *J* = 10.7, 10.7, 4.8 Hz), 1.78 (1H, ddd, *J* = 11.0, 11.0, 4.9 Hz). HR-ESI-MS: calcd for $C_{24}H_{27}{}^{35}ClNO_7S$ $[M+H]^+$, 508.1197; found, 508.1198; calcd for $C_{24}H_{27}{}^{37}ClNO_7S$ $[M+H+2]^+$, 510.1167; found, 510.1167.

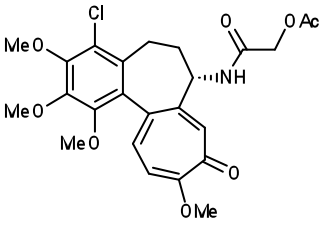
Synthesis of 4-chloro-*N*-(mercaptoacetyl)deacetylcolchicine (36)



To a stirred solution of compound **47** (10 mg, 0.020 mmol) in MeOH (4.5 mL) was added NaOMe (1 M in MeOH, 30 μL, 0.030 mmol) under argon atmosphere at 0 °C. After stirring for 40 minutes at ambient temperature, the reaction was quenched with AcOH (3 drops). Then, the reaction mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH) to afford compound **36** (9.5 mg, 0.020 mmol, quant.) as a yellow oil.

$[\alpha]_D^{16}$: -173.7 ° (*c* = 0.02, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.67 (1H, br-d, *J* = 7.0 Hz), 7.40 (1H, s), 7.28 (1H, d, *J* = 10.4 Hz), 6.83 (1H, d, *J* = 10.8 Hz), 4.53 (1H, ddd, *J* = 12.1, 6.2, 6.2 Hz), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.32-3.20 (3H, overlapped), 2.27 (1H, dddd, *J* = 12.0 12.0, 5.9, 5.9 Hz), 2.19 (1H, ddd, *J* = 13.0, 13.0, 5.9 Hz), 2.02 (1H, dd, *J* = 9.0, 9.0 Hz), 1.84 (1H, ddd, *J* = 11.5, 11.5, 4.9 Hz). HR-ESI-MS: calcd for $C_{22}H_{25}{}^{35}ClNO_6S$ $[M+H]^+$, 466.1091; found, 466.1093; calcd for $C_{22}H_{25}{}^{37}ClNO_6S$ $[M+H+2]^+$, 468.1062; found, 468.1107.

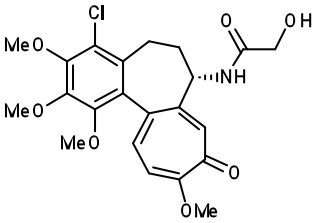
Synthesis of *N*-acetoxyacetyl-4-chlorodeacetylcolchicine (48)



Compound **48** was prepared from compound **4** (0.15 g, 0.38 mmol) and acetoxyacetyl chloride (45.3 μL, 0.42 mmol) using the same procedure as that described for compound **6**. Off-white solid (0.14 g, 0.24 mmol, 76% yield).

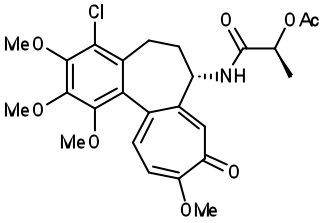
$[\alpha]_D^{25}$: -143.1 ° (*c* = 0.227, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.45 (1H, s), 7.31 (1H, d, *J* = 10.7 Hz), 7.12 (1H, d, *J* = 7.1 Hz), 6.86 (1H, d, *J* = 10.7 Hz), 4.63-4.58 (1H, m), 4.60 (1H, d, *J* = 15.7 Hz), 4.54 (1H, d, *J* = 15.7 Hz), 4.02 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.30-3.25 (1H, m), 2.32-2.14 (2H, m), 2.18 (3H, s), 1.90-1.83 (1H, m). HR-ESI-MS: calcd for $C_{24}H_{27}{}^{35}ClNO_8$ $[M+H]^+$, 492.1425; found, 492.1429; calcd for $C_{24}H_{27}{}^{37}ClNO_8$ $[M+H+2]^+$, 494.1396; found, 494.1408.

Synthesis of *N*-acetoxyacetyl-4-chlorodeacetylcolchicine (37)



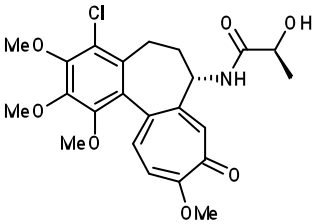
Compound **37** was prepared from compound **48** (0.16 g, 0.33 mmol) using the same procedure as that described for compound **36**. Off-white solid (0.15 g, 0.33 mmol, quant.). $[\alpha]_D^{25}$: -123.9 ° (*c* = 1.002, MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.68 (1H, s), 7.56 (1H, d, *J* = 7.3 Hz), 7.34 (1H, d, *J* = 10.7 Hz), 6.90 (1H, d, *J* = 10.7 Hz), 4.66-4.59 (1H, m), 4.20 (1H, d, *J* = 16.3 Hz), 4.04 (1H, d, *J* = 16.3 Hz), 4.02 (3H, s), 3.99 (3H, s), 3.98 (3H, s), 3.62 (3H, s), 3.31-3.26 (1H, m), 2.33-2.14 (2H, m), 1.94-1.87 (1H, m). HR-ESI-MS: calcd for $C_{22}H_{25}{}^{35}ClNO_7$ $[M+H]^+$, 450.1320; found, 450.1360; calcd for $C_{22}H_{25}{}^{37}ClNO_7$ $[M+H+2]^+$, 452.1290; found, 452.1357.

Synthesis of (S)-*N*-(2-acetoxypropionyl)-4-chlorodeacetylcolchicine (49)



Compound **49** was prepared from compound **4** (0.10 g, 0.26 mmol) and (S)-2-acetoxypropionyl chloride (36 μL, 0.28 mmol) using the same procedure as that described for compound **6**. Off-white solid (0.12 g, 0.24 mmol, 95% yield). $[\alpha]_D^{25}$: -159.2 ° (*c* = 0.197, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 8.80 (1H, d, *J* = 7.3 Hz), 7.14 (1H, d, *J* = 10.7 Hz), 7.06 (1H, s), 7.03 (1H, d, *J* = 10.7 Hz), 4.93 (1H, q, *J* = 6.7 Hz), 4.27-4.21 (1H, m), 3.91 (3H, s), 3.89 (6H, s), 3.53 (3H, s), 3.13 (1H, dd, *J* = 12.3, 3.8 Hz), 2.16-2.08 (1H, m), 2.02 (3H, s), 1.99-1.90 (2H, m), 1.33 (3H, d, *J* = 6.7 Hz). HR-ESI-MS: calcd for $C_{25}H_{29}{}^{35}ClNO_8$ $[M+H]^+$, 506.1582; found, 506.1582; calcd for $C_{25}H_{29}{}^{37}ClNO_8$ $[M+H+2]^+$, 508.1552; found, 508.1594.

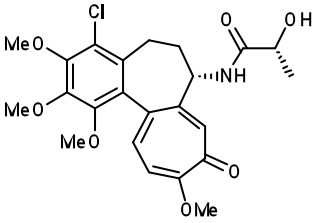
Synthesis of (S)-4-chloro-*N*-(2-hydroxypropionyl)deacetylcolchicine (38)



Compound **38** was prepared from compound **49** (0.12 g, 0.25 mmol) using the same procedure as that described for compound **36**. Off-white solid (0.11 g, 0.23 mmol, 94% yield). $[\alpha]_D^{25}$: -136.1 ° (*c* = 0.212, MeOH). ^{1}H-NMR (400 MHz,

DMSO-*d*6): δ 8.50 (1H, d, *J* = 7.6 Hz), 7.16 (1H, s), 7.12 (1H, d, *J* = 10.7 Hz), 7.02 (1H, d, *J* = 10.7 Hz), 5.58 (1H, d, *J* = 5.4 Hz), 4.29-4.22 (1H, m), 4.02 (1H, m), 3.91 (3H, s), 3.88 (6H, s), 3.53 (3H, s), 3.10 (1H, dd, *J* = 12.4, 6.1 Hz), 2.14-2.06 (2H, m), 1.93-1.84 (1H, m), 1.17 (3H, d, *J* = 6.8 Hz). HR-ESI-MS: calcd for $C_{23}H_{27}{}^{35}ClNO_7$ $[M+H]^+$, 464.1476; found, 464.1482; calcd for $C_{23}H_{27}{}^{37}ClNO_7$ $[M+H+2]^+$, 466.1447; found, 466.1487.

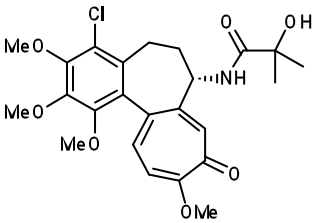
Synthesis of (R)-4-chloro-*N*-(2-hydroxypropionyl)deacetylcolchicine (39)



To a stirred solution of compound **38** (100 mg, 0.22 mmol), AcOH (15 μL, 0.26 mmol) and PPh_3 (0.17 g, 0.66 mmol) in toluene (4.3 mL) was added dropwise DEAD (2.2 mol/L in toluene, 0.30 mL, 0.66 mmol) at 0 °C under argon atmosphere. After stirring overnight at ambient temperature, the reaction mixture was purified by silica gel flash column chromatography ($CHCl_3$/MeOH) to afford compound **50**. Compound **39** was prepared from compound **50** using the same procedure as that described for compound **36**. Off-white solid (64 mg, 0.14 mmol, 64% yield in 2 steps).

$[\alpha]_D^{25}$: -125.5 ° (*c* = 0.209, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*6): δ 8.48 (1H, d, *J* = 7.6 Hz), 7.15 (1H, s), 7.12 (1H, d, *J* = 10.7 Hz), 7.02 (1H, d, *J* = 10.7 Hz), 5.52 (1H, d, *J* = 5.1 Hz), 4.27-4.20 (1H, m), 4.00-3.93 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.54 (3H, s), 3.10 (1H, dd, *J* = 12.7, 5.9 Hz), 2.15-2.04 (2H, m), 1.95-1.85 (1H, m), 1.17 (3H, d, *J* = 6.6 Hz). HR-ESI-MS: calcd for $C_{23}H_{27}{}^{35}ClNO_7$ $[M+H]^+$, 464.1476; found, 464.1492; calcd for $C_{23}H_{27}{}^{37}ClNO_7$ $[M+H+2]^+$, 466.1447; found, 466.1470.

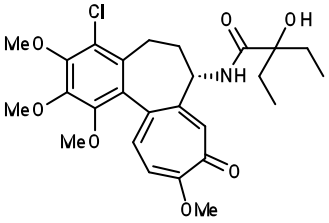
Synthesis of 4-chloro-*N*-(2-hydroxy-2-methylpropionyl)deacetylcolchicine (40)



Compound **40** was prepared from compound **4** (30 mg, 0.077 mmol) and 2-hydroxy-2-methylpropionic acid (10 mg, 0.092 mmol) using the same procedure as that described for compound **22**. Off-white solid (37 mg, 0.077 mmol, quant.).

$[\alpha]_D^{25}$: -140.9 ° (*c* = 0.171, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*6): δ 8.37 (1H, d, *J* = 7.3 Hz), 7.17 (1H, s), 7.12 (1H, d, *J* = 10.7 Hz), 7.02 (1H, d, *J* = 10.7 Hz), 5.49 (1H, s), 4.24-4.17 (1H, m), 3.92 (3H, s), 3.88 (6H, s), 3.54 (3H, s), 3.10 (1H, dd, *J* = 12.2, 5.4 Hz), 2.18-2.06 (2H, m), 1.94-1.85 (1H, m), 1.21 (3H, s), 1.20 (3H, s). HR-ESI-MS: calcd for $C_{24}H_{29}{}^{35}ClNO_7$ $[M+H]^+$, 478.1633; found, 478.1666; calcd for $C_{24}H_{29}{}^{37}ClNO_7$ $[M+H+2]^+$, 480.1603; found, 480.1607.

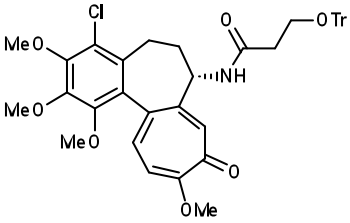
Synthesis of 4-chloro-*N*-(2-ethyl-2-hydroxybutyryl)deacetylcolchicine (41)



Compound **41** was prepared from compound **4** (50 mg, 0.13 mmol) and 2-ethyl-2-hydroxybutyric acid (20 mg, 0.16 mmol) using the same procedure as that described for compound **22**. White solid (37 mg, 0.075 mmol, 59% yield).

$[\alpha]_D^{25}$: -166.5 ° (*c* = 0.254, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 8.32 (1H ,d, *J* = 7.8 Hz), 7.22 (1H, s), 7.11 (1H, d, *J* = 10.5 Hz), 7.01 (1H, d, *J* = 11.0 Hz), 4.97 (1H, s), 4.33-4.27 (1H, m), 3.92 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.55 (3H, s), 3.10 (1H, dd, *J* = 12.9, 5.4 Hz), 2.20-2.06 (2H, m), 1.90-1.82 (1H, m), 1.67-1.54 (2H, m), 1.47-1.38 (2H, m), 0.76-0.69 (6H, m). HR-ESI-MS: calcd for $C_{26}H_{33}{}^{35}ClNO_7$ $[M+H]^+$, 506.1946; found, 506.1957; calcd for $C_{26}H_{33}{}^{37}ClNO_7$ $[M+H+2]^+$, 508.1916; found, 508.1867.

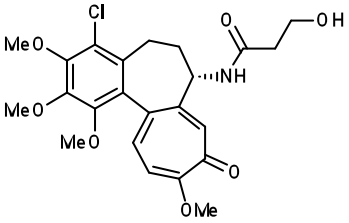
Synthesis of 4-chloro-*N*-(3-(triphenylmethyloxy)propionyl)deacetylcolchicine (44)



Compound **44** was prepared from compound **4** (70 mg, 0.18 mmol) and 3-(triphenylmethyloxy)propionic acid (65 mg, 0.20 mmol) using the same procedure as that described for compound **22**. Off-white solid (118 mg, 0.17 mmol, 94% yield).

$[\alpha]_D^{25}$: -120.4 ° (*c* = 0.222, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 8.78 (1H, d, *J* = 7.3 Hz), 7.34-7.20 (16H, m), 7.13 (1H, d, *J* = 10.7 Hz), 7.03 (1H, d, *J* = 10.7 Hz), 4.35-4.28 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.54 (3H, s), 3.18-3.09 (2H, m), 3.05-2.99 (1H, m), 2.53-2.39 (2H, m), 2.18-2.09 (1H, m), 2.02-1.83 (2H, m). HR-ESI-MS: calcd for $C_{42}H_{41}{}^{35}ClNO_7$ $[M+H]^+$, 706.2572; found, 706.2534; calcd for $C_{42}H_{41}{}^{37}ClNO_7$ $[M+H+2]^+$, 708.2542; found, 708.2211.

Synthesis of 4-chloro-*N*-(3-hydroxypropionyl)deacetylcolchicine (42)

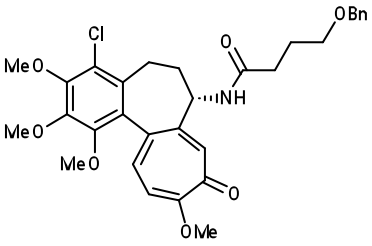


A solution of compound **44** (95 mg, 0.14 mmol) and *p*-TsOH·H$_2$O (5 mg, 0.028 mmol) in MeOH was stirred at ambient temperature for 5 h. After evaporation of the reaction mixture, the residue was purified by silica gel flash column chromatography ($CHCl_3$/MeOH) to afford compound **42** (54 mg, 0.12 mmol, 86% yield) as a pale yellow solid.

$[\alpha]_D^{25}$: -138.0 ° (*c* = 0.280, MeOH). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 8.59 (1H, d, *J* = 7.1 Hz), 7.13 (2H, s), 7.12 (2H, d, *J* = 10.7 Hz), 7.02 (1H, d, *J* = 10.7 Hz), 4.54 (1H, t, *J* = 5.2

Hz), 4.28-4.22 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.57-3.52 (2H, m), 3.53 (3H, s), 3.10 (1H, dd, J = 13.7, 5.1 Hz), 2.35-2.23 (2H, m), 2.15-2.06 (1H, m), 1.99-1.80 (2H, m). HR-ESI-MS: calcd for $C_{23}H_{27}{}^{35}ClNO_7$ $[M+H]^+$, 464.1476; found, 464.1491; calcd for $C_{23}H_{27}{}^{37}ClNO_7$ $[M+H+2]^+$, 466.1447; found, 466.1482.

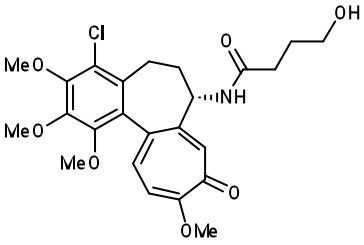
Synthesis of 4-chloro-*N*-(4-(benzyloxy)butyryl)deacetylcolchicine (45)



Compound **45** was prepared from compound **4** (50 mg, 0.13 mmol) and 4-(benzyloxy)butyric acid (27 μL, 0.26 mmol) using the same procedure as that described for compound **22**. Pale yellow solid (36 mg, 0.063 mmol, 50% yield).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.61 (1H, d, J = 7.1 Hz), 7.34-7.30 (4H, m), 7.28-7.24 (1H, m), 7.13 (1H, d, J = 10.5 Hz), 7.12 (1H, s), 7.03 (1H, d, J = 11.0 Hz), 4.46 (1H, d, J = 12.0 Hz), 4.41 (1H, d, J = 12.0 Hz), 4.26-4.20 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.37 (2H, t, J = 6.5 Hz), 3.10 (1H, dd, J = 13.4, 5.1 Hz), 2.23 (2H, t, J = 6.8 Hz), 2.14-2.06 (1H, m), 1.98-1.88 (1H, m), 1.86-1.78 (1H, m), 1.75-1.68 (2H, m). ESI-MS m/z: 568 $[M+H]^+$, 570 $[M+H+2]^+$.

Synthesis of 4-chloro-*N*-(4-hydroxybutyryl)deacetylcolchicine (43)



To a stirred solution of compound **45** (41 mg, 0.072 mmol) in CH_2Cl_2 (0.5 mL) was added BBr_3 (1 mol/L in CH_2Cl_2, 72 μL, 0.072 mmol) at -78 °C under argon atmosphere. After stirring for 30 min at -78 °C, the reaction was quenched with saturated aqueous $NaHCO_3$. The mixture was extracted two times with $CHCl_3$. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/MeOH) to afford compound **43** (14 mg, 0.029 mmol, 41% yield) as a white solid. $[\alpha]_D^{25}$: -137.8 ° (c = 0.078, MeOH). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.59 (1H, d, J = 7.1 Hz), 7.13 (1H, d, J = 10.7 Hz), 7.11 (1H, s), 7.03 (1H, d, J = 10.7 Hz), 4.46 (1H, t, J = 5.1 Hz), 4.26-4.20 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.34-3.30 (2H, m), 3.10 (1H, dd, J = 13.5, 5.5 Hz), 2.17 (2H, t, J = 7.6 Hz), 2.13-2.06 (1H, m), 1.99-1.90 (1H, m), 1.87-1.79 (1H, m), 1.62-1.54 (2H, m). ESI-MS m/z: 478 $[M+H]^+$, 480 $[M+H+2]^+$. HR-ESI-MS: calcd for $C_{24}H_{29}{}^{35}ClNO_7$ $[M+H]^+$, 478.1633; found, 478.1642; calcd for $C_{24}H_{28}{}^{37}ClNO_7$ $[M+H+2]^+$, 480.1603; found, 480.1632.

In vitro assay

Cell culture. Human lung cancer cell line A549 and human colorectal cancer cell lines, HT-29 and HCT116, were obtained from American Type Culture Collection (ATCC, USA). The cancer cell lines were continuously cultured at 37 °C under 5% CO_2 atmosphere in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin (10% FBS/DMEM).

Cytotoxicity evaluation. A 50 μL volume (1000 cells) of an exponentially growing cell suspension was seeded into a 96-well microtiter plate and 50 μL of each drug at various concentrations was added 24 h after the seeding of the tumor cells. After incubation for 96 h at 37 °C, 10 μL of TetraColor ONE (Seikagaku Biobusiness Corporation, Tokyo, Japan) was added to each well and the plates were incubated further for 1 h at 37 °C. After incubation, optical density was measured at 450 nm with a microplate reader (Spectramax 384 Plus, Molecular Devices, CA), and the concentration causing 50% inhibition of cell proliferation (IC_{50}) was calculated by linear regression analysis of the linear portion of the growth curves.

Metabolic stability in mouse microsomes. BALB/c mouse hepatic microsomes were purchased from XenoTech, LLC (Lenexa, KS). The reaction mixture (0.5 mL) containing 0.25 mg/mL microsomal protein and 1 μmol/L test compound in 100 mmol/L phosphate buffer (pH 7.4) was preincubated for 5 min at 37 °C, and the reaction was started by adding 30 μL of NADPH-regenerating system solution (BD Gentest, Woburn, MA). The reaction mixture (50 μL) was sampled and the reaction was terminated by adding 150 μL of 1% formic acid at 0, 5, 10, 15, 30, and 60 min. All incubations were done in triplicate. The test compound in the reaction mixture was measured by LC-MS/MS using a Scherzo SM-C18 column (150 × 2.0 mm ID., Imtakt, Japan). The column temperature and the flow rate were 40 °C and 0.35 mL/min, respectively. Mobile phases A and B were acetonitrile and 2 mmol/L ammonium formate (pH 3.3), respectively. The gradient elution was as follows: mobile phase A was linearly increased from 15% to 95% over a period of 4 minutes, kept at 95% for the next 2 minutes, and then equilibrated at 15% for 5 minutes. Metabolic stability was calculated by the following formula (A).

Metabolic stability (mL/min/mg) = slope of a semi-logarithmic plot of test compound concentration / microsomal protein concentration (0.25 mg/mL) (A).

In vivo assay

Animals. Inbred specific-pathogen-free 5-week-old male BALB/C nude mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The mice were kept in plastic cages and allowed free access to water and a standard diet (MF, Oriental Yeast Industry Co., Tokyo, Japan). Temperature and humidity were kept at 24±1 °C and 55±10%, respectively. In vivo antitumor experiments were performed according to our internal and ethics committee regulations.

Antitumor experiments. After transplanting HCT116 cells (2×10^6 cells/mouse) subcutaneously to the inguinal region of nude mice, the mice were grouped (3~5 mice/group) on the day when the estimated tumor volume calculated by the following formula (B) reached approximately 300 mm^3 (Day 1). The derivatives were administered intravenously three times on days 1, 5, and 9. As negative control, 5% glucose/Tween 80/propylene glycol (85/10/5) was administered following the same administration schedule as that of the derivatives. The tumors were excised on Day 22 and tumor growth inhibition rate (IR (%), formula (C)) was calculated from the tumor weights.

Estimated tumor volume (mm^3) = 1/2 × long diameter × short diameter × short diameter (B).

IR (%) = (1 – mean tumor weight in the tested group / mean tumor weight in the control group) × 100 (C).

Docking simulation

Glide 5.6 and Prime 2.2 modules were used in the default settings to search stable conformations of 4-chlorocolchicine derivatives in the colchicine binding site of tubulin, where the receptor residues moved concomitantly during the docking (induced fit docking).[1)] The receptor complex merged with DAMA-colchicine and GTP (PDB code 1SA0, chains A and B) was processed by using the protein preparation module of Schrödinger to optimize the complex in the OPLS 2005 force field. The grid was automatically calculated to cover the entire colchicine binding site. Each 4-chlorocolchicine derivative, whose 3D structure was previously generated by Ligprep 2.4 module, was taken for the initial Glide SP docking using a soft potential (0.5 van der Waals scaling of ligands and receptor). The derived docking poses were then refined by using the Prime module. Residues within 5.0 Å of the ligand poses were minimized in order to form suitable conformations of the poses at the active site of the receptor. Glide SP redocking of each protein–ligand complex was performed.

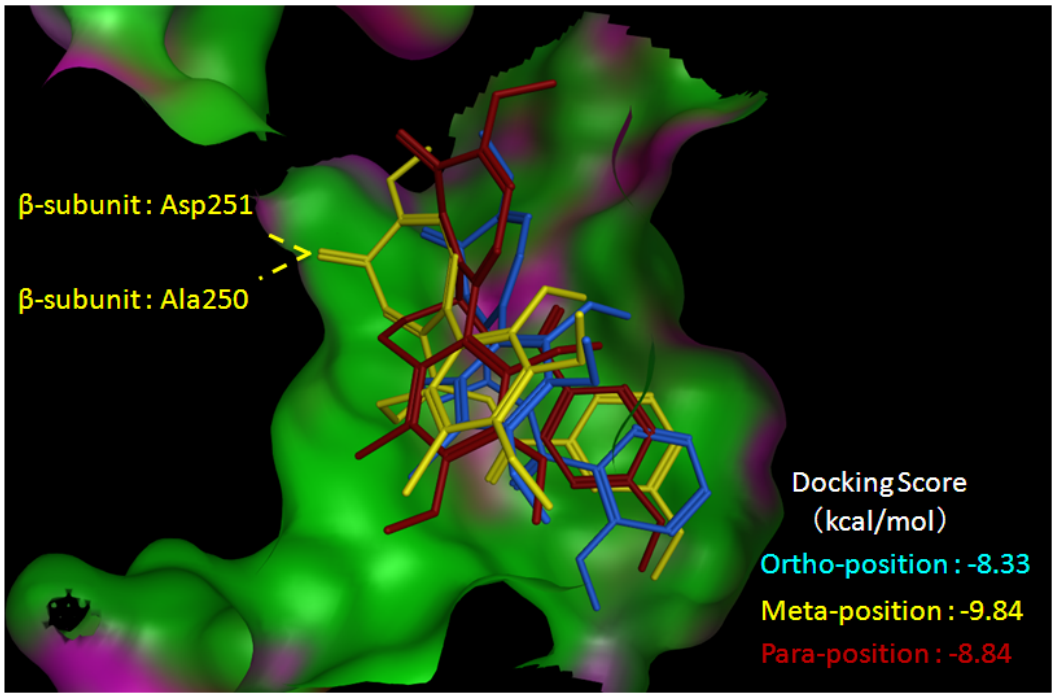


Fig. S-1 Docking modes of compounds **16** (blue), **17** (yellow), and **18** (red) at the colchicine binding site of tubulin (green : hydrophobic regions, pink : polar regions).

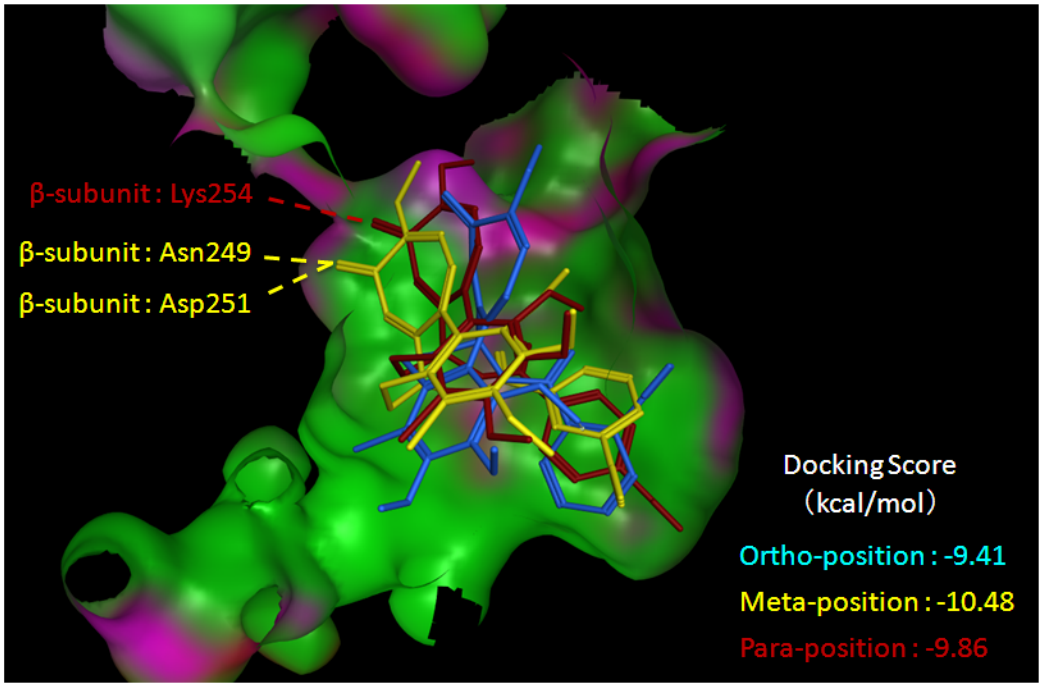


Fig. S-2 Docking modes of compounds **22** (blue), **23** (yellow), and **24** (red) at the

colchicine binding site of tubulin(green : hydrophobic regions, pink : polar regions).

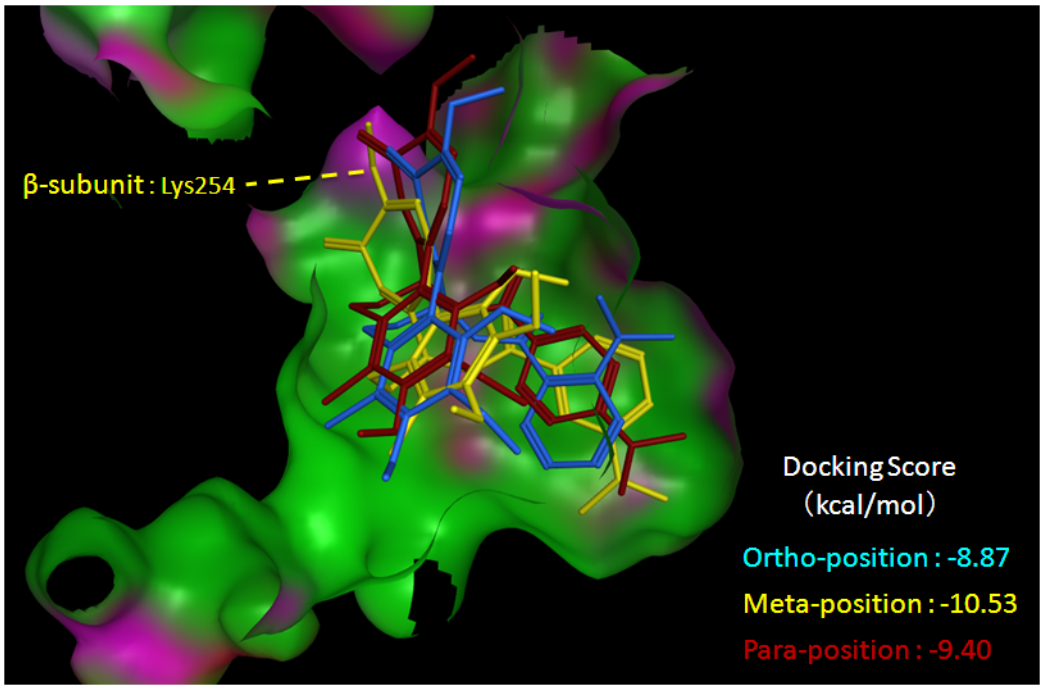


Fig. S-3 Docking modes of compounds **29** (blue), **30** (yellow), and **31** (red) at the colchicine binding-site of tubulin(green : hydrophobic regions, pink : polar regions).

Reference

1. W. Sherman, T. Day, M. P. Jacobson, R. A. Friesner and R. Farid, *J. Med. Chem.*, 2006, **49**, 534–553.