

Supplementary information

4-Chlorocolchicine derivatives bearing a thiourea side chain at C-7 position as potent anticancer agents

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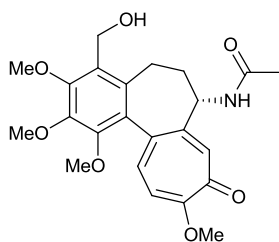
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General methods

All reagents were obtained from commercial sources and used without further purification. Optical rotation was determined using a JASCO DIP-360 digital polarimeter. ¹H-NMR spectra were recorded on a JEOL ALPHA-400 using CDCl₃ 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. ESI-MS data were obtained using a Waters 3100 Single Quadrupole LC/MS instrument equipped with an ESI source. HR-ESI-MS data were measured on an LCT-Premier XE time-of-flight instrument (Waters Corp.).

Synthesis of 4-(hydroxymethyl)colchicine (9)

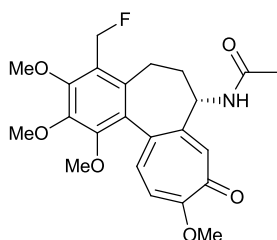


A mixture of 4-formylcolchicine (**8**) (100 mg, 0.23 mmol) and NaBH₄ (13 mg, 0.35 mmol) in MeOH (2.5 mL) was stirred at ambient temperature under argon atmosphere for 3 h. Acetone was added to the reaction mixture and the whole mixture was stirred for 30 min at ambient temperature. After adding water, the whole mixture extracted two times with CHCl₃. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and

concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **9** (74 mg, 0.17 mmol, 74% yield) as an off-white solid.

$[\alpha]_{\text{D}}^{25}$: -130.84 ° ($c = 0.425$, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.55 (1H, d, $J = 7.3$ Hz), 7.11 (1H, s), 7.09 (1H, d, $J = 10.7$ Hz), 7.02 (1H, d, $J = 10.7$ Hz), 4.87 (1H, t, $J = 5.1$ Hz), 4.54 (1H, dd, $J = 11.3, 4.4$ Hz), 4.47 (1H, dd, $J = 11.3, 5.9$ Hz), 4.31-4.24 (1H, m), 3.88 (3H, s), 3.87 (3H, s), 3.83 (3H, s), 3.50 (3H, s), 3.00 (1H, dd, $J = 12.6, 4.5$ Hz), 2.10-1.95 (2H, m), 1.85 (3H, s), 1.81-1.73 (1H, m). HR-ESI-MS: calcd for C₂₃H₂₈NO₇ [M+H]⁺, 430.1866; found, 430.1858.

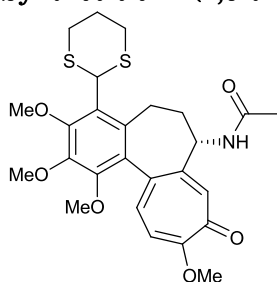
Synthesis of 4-(fluoromethyl)colchicine (**5**)



To a stirred solution of 4-(hydroxymethyl)colchicine (**9**) (30 mg, 0.070 mmol) in CH₂Cl₂ (1 mL) was added DAST (11 μL, 0.084 mmol) at 0°C under argon atmosphere. After stirring 2 h at 0 °C, the reaction mixture was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **5**. (19 mg, 0.044 mmol, 63% yield) as an off-white solid.

$[\alpha]_{\text{D}}^{25}$: -140.76 ° ($c = 0.157$, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.60 (1H, d, $J = 7.3$ Hz), 7.11 (1H, d, $J = 10.7$ Hz), 7.11 (1H, s), 7.03 (1H, d, $J = 10.7$ Hz), 5.49 (2H, d, $J = 48.5$ Hz), 4.28-4.21 (1H, m), 3.89 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.54 (3H, s), 2.93 (1H, dd, $J = 13.9, 4.6$ Hz), 2.13-1.94 (2H, m), 1.86-1.77 (1H, m), 1.85 (3H, s). HR-ESI-MS: calcd for C₂₃H₂₇FNO₆ [M+H]⁺, 432.1822; found, 432.1826.

Synthesis of 4-(1,3-dithian-2-yl)colchicine (**10**)



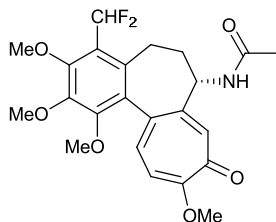
To a stirred solution of 4-formylcolchicine (**8**) (250 mg, 0.59 mmol) in CHCl₃ (6 mL) were added 1,3-propanedithiol (70 μL, 0.70 mmol) and iodine (15 mg, 0.059 mmol) at ambient temperature under argon atmosphere. After stirring overnight at ambient temperature, 10%Na₂SO₃ aq. and saturated NaHCO₃ aq. were added to the reaction mixture and the whole mixture was extracted two

times with CHCl₃. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **10** (224 mg, 0.43 mmol, 74% yield) as an amorphous solid.

$[\alpha]_{\text{D}}^{25}$: -94.32 ° ($c = 0.469$, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.58 (1H, d, $J = 7.3$ Hz), 7.08 (1H, s), 7.05 (1H, d, $J = 10.7$ Hz), 6.99 (1H, d, $J = 10.7$ Hz), 4.26-4.20 (1H, m),

3.94-3.89 (1H, m), 3.88 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.50 (3H, s), 3.18-3.09 (2H, m), 2.94-2.85 (2H, m), 2.19-2.01 (3H, m), 1.88-1.80 (2H, m), 1.86 (3H, s), 1.73-1.63 (1H, m). HR-ESI-MS: calcd for C₂₆H₃₂NO₆S₂ [M+H]⁺, 518.1671; found, 518.1719.

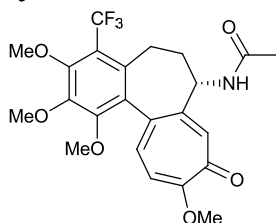
Synthesis of 4-(difluoromethyl)colchicine (6)



A solution of 4-(1,3-dithian-2-yl)colchicine (**10**) (50 mg, 0.097 mmol) in CH₂Cl₂ was added to a mixture of NIS (87 mg, 0.39 mmol) and HF-pyridine (HF: 65%, 119 mg, 3.9 mmol) in CH₂Cl₂ at -78 °C under argon atmosphere. The reaction mixture was stirred overnight and allowed to warm to ambient temperature during the course of the reaction. After adding 10% Na₂SO₃ aq., the mixture was stirred for 10 min at ambient temperature followed by addition of NaHCO₃ aq. The whole mixture was extracted with CHCl₃. The organic layer was washed with saturated NaHCO₃ aq, 0.1 mol/L HCl and then brine, dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was dissolved in MeOH (2 mL). NaBH₄ (2 mg, 0.048 mmol) was added to the solution, and the reaction mixture was stirred for 30 min at ambient temperature under argon atmosphere. The reaction was quenched with acetone followed by addition of CHCl₃. The whole mixture was washed with H₂O, dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **6** (30 mg, 0.067 mmol, 70% yield) as a white solid.

[α]_D²⁵: -31.24 ° (c = 0.120, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.62 (1H, d, *J* = 7.3 Hz), 7.25 (1H, t, *J* = 54.0 Hz), 7.11 (1H, d, *J* = 10.7 Hz), 7.10 (1H, s), 7.03 (1H, d, *J* = 10.7 Hz), 4.28-4.21 (1H, m), 3.90 (3H, s), 3.90 (3H, s), 3.89 (3H, s), 3.56 (3H, s), 3.11 (1H, dd, *J* = 13.7, 4.9 Hz), 2.17-2.08 (1H, m), 1.99-1.91 (1H, m), 1.87-1.80 (1H, m), 1.86 (3H, s). HR-ESI-MS: calcd for C₂₃H₂₆F₂NO₆ [M+H]⁺, 450.1728; found, 450.1736.

Synthesis of 4-(trifluoromethyl)colchicine(7)

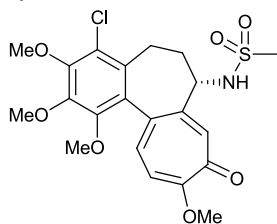


A mixture of 4-iodocolchicine (**11**) (50 mg, 0.095 mmol), CuI (9.1 mg, 0.048 mmol) and FSO₂CH₂COOMe (55 mg, 0.29 mmol) in NMP (1 mL) was stirred overnight at 120 °C under argon atmosphere in a sealed tube. Water was added to the mixture and the whole mixture was extracted two times with CHCl₃. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **7** (30 mg, 0.065 mmol, 68%

yield) as an off-white solid.

$[\alpha]_{\text{D}}^{25}$: -95.57° ($c = 0.212$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 8.62 (1H, d, $J = 7.3$ Hz), 7.11 (1H, d, $J = 11.1$ Hz), 7.09 (1H, s), 7.02 (1H, d, $J = 11.1$ Hz), 4.29-4.23 (1H, m), 3.90 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.57 (3H, s), 2.97 (1H, dd, $J = 14.7, 5.3$ Hz), 2.22-2.12 (1H, m), 1.99-1.89 (1H, m), 1.87 (3H, s), 1.86-1.77 (1H, m). HR-ESI-MS: calcd for $\text{C}_{23}\text{H}_{25}\text{F}_3\text{NO}_6$ $[\text{M}+\text{H}]^+$, 468.1634; found, 468.1634.

Synthesis of 4-chloro-*N*-(methanesulfonyl)deacetylcolchicine(13)

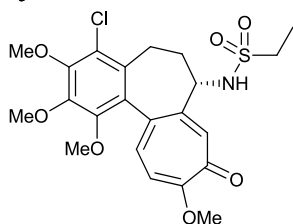


To a stirred solution of 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.070 mmol) and Et_3N (26 μL , 0.19 mmol) in CH_2Cl_2 (2 mL) was added MeSO_2Cl (12 μL , 0.15 mmol) at 0°C under argon atmosphere. After stirring 2 h at 0°C , the reaction was quenched with water. Then, the whole mixture was extracted

with CHCl_3 . The organic layer was washed with saturated NaHCO_3 aq, dried over MgSO_4 , filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography ($\text{CHCl}_3/\text{MeOH}$) to afford compound **13** (57 mg, 0.12 mmol, 95% yield) as a yellow solid.

$[\alpha]_{\text{D}}^{25}$: -79.11° ($c = 0.032$, MeOH). $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 7.77 (1H, s), 7.31 (1H, d, $J = 10.7$ Hz), 6.88 (1H, d, $J = 10.7$ Hz), 5.52 (1H, br-s), 4.36-4.30 (1H, m), 4.03 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.58 (3H, s), 3.27 (1H, dd, $J = 14.1, 5.4$ Hz), 2.89 (3H, s), 2.40-2.31 (1H, m), 2.15-2.12 (1H, m), 1.89-1.85 (1H, m). HR-ESI-MS: calcd for $\text{C}_{21}\text{H}_{25}^{35}\text{ClNO}_7\text{S}$ $[\text{M}+\text{H}]^+$, 470.1040; found, 470.1058; calcd for $\text{C}_{21}\text{H}_{25}^{37}\text{ClNO}_7\text{S}$ $[\text{M}+\text{H}+2]^+$, 472.1011; found, 472.1032.

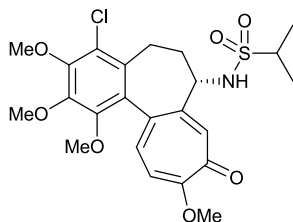
Synthesis of 4-chloro-*N*-(ethanesulfonyl)deacetylcolchicine (14)



Compound **14** was prepared from 4-chlorodeacetylcolchicine (**12**) (198 mg, 0.51 mmol) and EtSO_2Cl (72 μL , 0.73 mmol) using the same procedure as that described for compound **13**. Yellow solid (243 mg, 0.50 mmol, 99% yield).

$[\alpha]_{\text{D}}^{25}$: 113.46° ($c = 0.189$, MeOH). $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 7.70 (1H, s), 7.26 (1H, d, $J = 10.7$ Hz), 6.84 (1H, d, $J = 11.0$ Hz), 5.36 (1H, br-s), 4.33-4.27 (1H, m), 4.02 (3H, s), 3.99 (3H, s), 3.99 (3H, s), 3.58 (3H, s), 3.27 (1H, dd, $J = 13.8, 5.2$ Hz), 2.99-2.89 (2H, m), 2.36-2.31 (1H, m), 2.17-2.13 (1H, m), 1.91-1.83 (1H, m), 1.32 (3H, t, $J = 7.4$ Hz). HR-ESI-MS: calcd for $\text{C}_{22}\text{H}_{27}^{35}\text{ClNO}_7\text{S}$ $[\text{M}+\text{H}]^+$, 484.1197; found, 484.1221; calcd for $\text{C}_{22}\text{H}_{27}^{37}\text{ClNO}_7\text{S}$ $[\text{M}+\text{H}+2]^+$, 486.1167; found, 486.1211.

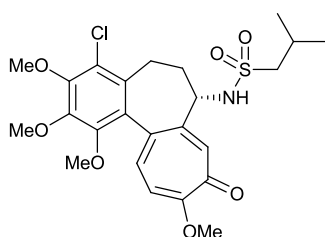
Synthesis of 4-chloro-*N*-(propane-2-sulfonyl)deacetylcolchicine (15)



Compound **15** was prepared from 4-chlorodeacetylcolchicine (**12**) (50 mg, 0.13 mmol) and *i*-PrSO₂Cl (17 μL, 0.15 mmol) using the same procedure as that described for compound **13**. Pale yellow solid (6 mg, 0.012 mmol, 9% yield).

$[\alpha]_{\text{D}}^{25}$: -104.47 ° ($c = 0.013$, MeOH). ¹H-NMR (400 MHz, CDCl₃): δ 7.65 (1H, s), 7.26-7.23 (1H, m), 6.81 (1H, d, $J = 11.0$ Hz), 5.08 (1H, br-s), 4.30-4.24 (1H, m), 4.01 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.57 (3H, s), 3.26 (1H, dd, $J = 13.5, 5.2$ Hz), 3.04-2.97 (1H, m), 2.38-2.33 (1H, m), 2.22-2.11 (1H, m), 1.88-1.83 (1H, m), 1.32 (3H, d, $J = 6.8$ Hz), 1.27 (3H, d, $J = 6.8$ Hz). HR-ESI-MS: calcd for C₂₃H₂₉³⁵ClNO₇S [M+H]⁺, 498.1353; found, 498.1387; calcd for C₂₃H₂₉³⁷ClNO₇S [M+H+2]⁺, 500.1324; found, 500.1371.

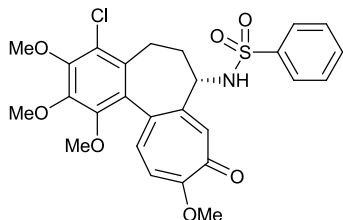
Synthesis of 4-chloro-*N*-(2-methylpropane-1-sulfonyl)deacetylcolchicine (16)



Compound **16** was prepared from 4-chlorodeacetylcolchicine (**12**) (50 mg, 0.13 mmol) and 2-methylpropane-1-sulfonyl chloride (12 μL, 0.15 mmol) using the same procedure as that described for compound **13**. White solid (56 mg, 0.11 mmol, 85% yield).

$[\alpha]_{\text{D}}^{25}$: -129.61 ° ($c = 0.228$, MeOH). ¹H-NMR (400 MHz, CDCl₃): δ 7.70 (1H, s), 7.26 (1H, d, $J = 10.7$ Hz), 6.84 (1H, d, $J = 10.7$ Hz), 5.18 (1H, d, $J = 5.1$ Hz), 4.33-4.27 (1H, m), 4.02 (3H, s), 3.99 (3H, s), 3.99 (3H, s), 3.57 (3H, s), 3.26 (1H, dd, $J = 14.0, 5.0$ Hz), 2.88 (1H, dd, $J = 14.0, 6.0$ Hz), 2.74 (1H, dd, $J = 14.0, 7.0$ Hz), 2.38-2.29 (1H, m), 2.25-2.10 (2H, m), 1.88-1.82 (1H, m), 1.03 (3H, d, $J = 6.8$ Hz), 1.00 (3H, d, $J = 6.8$ Hz). HR-ESI-MS: calcd for C₂₄H₃₁³⁵ClNO₇S [M+H]⁺, 512.1510; found, 512.1517; calcd for C₂₄H₃₁³⁷ClNO₇S [M+H+2]⁺, 514.1480; found, 514.1517.

Synthesis of *N*-benzenesulfonyl-4-chlorodeacetylcolchicine (17)

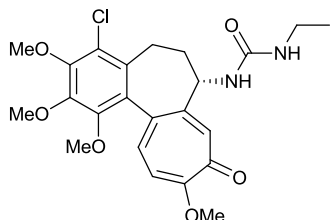


Compound **17** was prepared from 4-chlorodeacetylcolchicine (**12**) (50 mg, 0.13 mmol) and PhSO₂Cl (20 μL, 0.15 mmol) using the same procedure as that described for compound **13**. White solid (30 mg, 0.056 mmol, 44% yield).

$[\alpha]_{\text{D}}^{25}$: -179.37 ° ($c = 0.134$, MeOH). ¹H-NMR (400 MHz, CDCl₃): δ 7.65-7.63 (2H, m), 7.47 (1H, t, $J = 7.4$ Hz), 7.37 (2H, t, $J = 7.6$ Hz), 7.28 (1H, s), 7.17 (1H, d, $J = 10.5$ Hz), 6.72 (1H, d, $J = 11.0$ Hz), 5.41 (1H, d, $J = 7.8$ Hz), 4.16-4.10 (1H, m), 4.00 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.62 (3H, s), 3.17 (1H, dd, $J = 13.2, 4.6$ Hz),

2.18-2.03 (2H, m), 1.74-1.71 (1H, m). HR-ESI-MS: calcd for $C_{26}H_{27}^{35}ClNO_7S$ $[M+H]^+$, 532.1197; found, 532.1132; calcd for $C_{26}H_{27}^{37}ClNO_7S$ $[M+H+2]^+$, 534.1167; found, 534.1107.

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

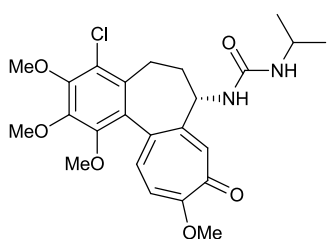


A solution of 4-chlorodeacetylcolchicine (**12**) (50 mg, 0.13 mmol) and ethyl isocyanate (20 μ L, 0.26 mmol) in MeOH-H₂O (2:1, 1.5 mL) was stirred for 90 min at 0 °C. The reaction mixture was extracted two times with CHCl₃. The combined organic layers were washed with brine, dried over

MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **18** (36 mg, 0.078 mmol, 61% yield) as a pale yellow solid.

$[\alpha]_D^{25}$: -45.84 ° (c = 0.094, MeOH). ¹H-NMR (400 MHz, CDCl₃): δ 7.79 (1H, s), 7.36 (1H, d, J = 10.6 Hz), 6.92 (1H, d, J = 10.6 Hz), 6.67 (1H, br-s), 4.54-4.49 (1H, m), 4.03 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.63 (3H, s), 3.24-3.15 (2H, m), 3.10-3.06 (1H, m), 2.30-2.27 (1H, m), 2.14-2.07 (1H, m), 1.72-1.65 (1H, m), 1.04 (3H, t, J = 7.2 Hz). HR-ESI-MS: calcd for $C_{23}H_{28}^{35}ClN_2O_6$ $[M+H]^+$, 463.1636; found, 463.1666; calcd for $C_{23}H_{28}^{37}ClN_2O_6$ $[M+H+2]^+$, 465.1606; found, 465.1636.

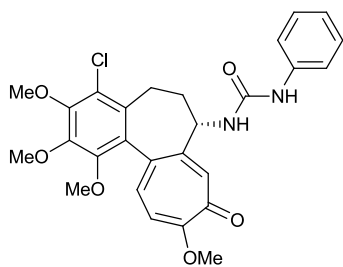
Synthesis of 4-chloro-*N*-(isopropylcarbamoyl)deacetylcolchicine (19)



Compound **19** was prepared from 4-chlorodeacetylcolchicine (**12**) (50 mg, 0.13 mmol) and isopropyl isocyanate (25 μ L, 0.26 mmol) using the same procedure as that described for compound **18**. Amorphous solid (59 mg, 0.12 mmol, 97% yield).

$[\alpha]_D^{25}$: -72.78 ° (c = 0.264, MeOH). ¹H-NMR (400 MHz, CDCl₃): δ 7.94 (1H, s), 7.46 (1H, d, J = 10.9 Hz), 7.02 (1H, d, J = 10.9 Hz), 6.28 (1H, br-s), 4.57-4.53 (1H, m), 4.06 (3H, s), 3.99 (3H, s), 3.97 (3H, s), 3.84-3.80 (1H, m), 3.62 (3H, s), 3.23 (1H, dd, J = 13.5, 4.9 Hz), 2.34-2.28 (1H, m), 2.12-2.05 (1H, m), 1.79-1.76 (1H, m), 1.08 (3H, d, J = 6.3 Hz), 1.05 (3H, d, J = 6.6 Hz). HR-ESI-MS: calcd for $C_{24}H_{29}^{35}ClN_2O_6$ $[M+H]^+$, 477.1792; found, 477.1838; calcd for $C_{24}H_{29}^{37}ClN_2O_6$ $[M+H+2]^+$, 479.1763; found, 479.1815.

Synthesis of 4-chloro-*N*-(phenylcarbamoyl)deacetylcolchicine (**20**)

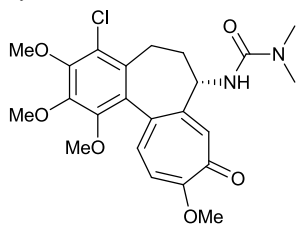


A solution of 4-chlorodeacetylcolchicine (**12**) (200 mg, 0.51 mmol) and phenyl isocyanate (110 μ l, 1.02 mmol) in CH_2Cl_2 (4 mL) was stirred for 2 h at 0 °C. The reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography ($\text{CHCl}_3/\text{MeOH}$) to afford compound **20** (168 mg, 0.33 mmol,

65% yield) as an amorphous solid.

$[\alpha]_{\text{D}}^{25}$: -52.32 ° ($c = 0.052$, MeOH). $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 8.19 (1H, s), 7.48 (1H, d, $J = 10.7$ Hz), 7.29-7.26 (2H, m), 7.16-7.14 (2H, m), 7.07 (1H, d, $J = 8.8$ Hz), 7.03 (1H, d, $J = 11.2$ Hz), 6.92-6.90 (1H, m), 4.73-4.67 (1H, m), 4.03 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.65 (3H, s), 3.22 (1H, dd, $J = 13.5, 5.2$ Hz), 2.43-2.33 (1H, m), 2.12-2.04 (1H, m), 1.84-1.80 (1H, m). HR-ESI-MS: calcd for $\text{C}_{27}\text{H}_{28}^{35}\text{ClN}_2\text{O}_6$ $[\text{M}+\text{H}]^+$, 511.1636; found, 511.1549; calcd for $\text{C}_{27}\text{H}_{28}^{37}\text{ClN}_2\text{O}_6$ $[\text{M}+\text{H}+2]^+$, 513.1606; found, 513.1595.

Synthesis of 4-chloro-*N*-(dimethylcarbamoyl)deacetylcolchicine (**21**)

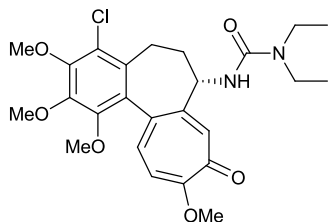


To a stirred solution of 4-chlorodeacetylcolchicine (**12**) (50 mg, 0.13 mmol) and Et_3N (26 μ L, 0.19 mmol) in CH_2Cl_2 (2 mL) was added Me_2NCOCl (14 μ L, 0.15 mmol) at ambient temperature under argon atmosphere. After stirring 4 h under reflux, Et_3N (52 μ L, 0.38 mmol) and Me_2NCOCl (28 μ L, 0.38 mmol) were

added. Then, the reaction mixture was further refluxed overnight. After adding saturated NaHCO_3 aq, the whole mixture was extracted with CHCl_3 . The organic layer was dried over MgSO_4 , filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography ($\text{CHCl}_3/\text{MeOH}$) to afford compound **21** (58 mg, 0.13 mmol, 98% yield) as a pale yellow solid.

$[\alpha]_{\text{D}}^{25}$: -43.28 ° ($c = 0.298$, MeOH). $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 7.58 (1H, s), 7.29 (1H, d, $J = 10.7$ Hz), 6.83 (1H, d, $J = 10.7$ Hz), 5.03 (1H, d, $J = 5.9$ Hz), 4.51-4.45 (1H, m), 4.00 (3H, s), 3.98 (3H, s), 3.95 (3H, s), 3.64 (3H, s), 3.25 (1H, dd, $J = 13.1, 4.3$ Hz), 2.92 (6H, s), 2.31-2.22 (1H, m), 2.18-2.14 (1H, m), 1.77-1.74 (1H, m). HR-ESI-MS: calcd for $\text{C}_{23}\text{H}_{28}^{35}\text{ClN}_2\text{O}_6$ $[\text{M}+\text{H}]^+$, 163.1636; found, 163.1640; calcd for $\text{C}_{23}\text{H}_{28}^{37}\text{ClN}_2\text{O}_6$ $[\text{M}+\text{H}+2]^+$, 165.1606; found, 165.1614.

Synthesis of 4-chloro-*N*-(diethylcarbamoyl)deacetylcolchicine (**22**)

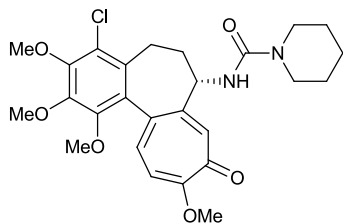


A solution of 4-chlorodeacetylcolchicine (**12**) (301 mg, 0.77 mmol), Et₃N (215 μL, 1.54 mmol) and Et₂NCOCl (146 μL, 1.54 mmol) in CH₂Cl₂ (6 mL) was refluxed overnight under argon atmosphere with stirring. After cooling to room temperature, CHCl₃ and saturated NaHCO₃ aq were added.

The organic layer was taken, washed with brine, dried over MgSO₄, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **21** (168 mg, 0.34 mmol, 44% yield) as a yellow solid.

$[\alpha]_{\text{D}}^{25}$: -20.00 ° ($c = 0.050$, MeOH). ¹H-NMR (400 MHz, CDCl₃): δ 7.60 (1H, s), 7.31 (1H, d, $J = 10.7$ Hz), 6.84 (1H, d, $J = 10.7$ Hz), 4.85 (1H, d, $J = 6.3$ Hz), 4.55-4.49 (1H, m), 4.01 (3H, s), 3.98 (3H, s), 3.96 (3H, s), 3.64 (3H, s), 3.36-3.32 (2H, m), 3.28-3.19 (3H, m), 2.28-2.23 (1H, m), 2.17-2.14 (1H, m), 1.76-1.74 (1H, m), 1.14 (6H, t, $J = 7.1$ Hz). HR-ESI-MS: calcd for C₂₅H₃₂³⁵ClN₂O₆ [M+H]⁺, 491.1949; found, 491.1982; calcd for C₂₅H₃₂³⁷ClN₂O₆ [M+H+2]⁺, 493.1919; found, 493.1946.

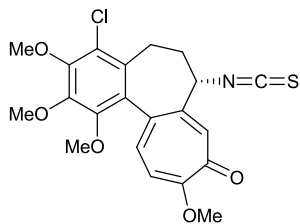
Synthesis of 4-chloro-*N*-(piperidine-1-carbonyl)deacetylcolchicine (**23**)



Compound **23** was prepared from 4-chlorodeacetylcolchicine (**12**) (50 mg, 0.13 mmol) and piperidine-1-carbonyl chloride (80 μL, 0.64 mmol) using the same procedure as that described for compound **22**. Yellow solid (36 mg, 0.072 mmol, 56% yield).

$[\alpha]_{\text{D}}^{25}$: -45.51 ° ($c = 0.145$, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 7.18 (1H, s), 7.11 (1H, d, $J = 10.7$ Hz), 7.02 (1H, d, $J = 11.0$ Hz), 7.00 (1H, d, $J = 7.1$ Hz), 4.20-4.17 (1H, m), 3.91 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.53 (3H, s), 3.29-3.28 (4H, m), 3.10 (1H, dd, $J = 13.5, 4.3$ Hz), 2.09-2.06 (1H, m), 2.00-1.89 (2H, m), 1.56-1.50 (2H, m), 1.41-1.37 (4H, m). HR-ESI-MS: calcd for C₂₆H₃₂³⁵ClN₂O₆ [M+H]⁺, 503.1949; found, 503.1952; calcd for C₂₆H₃₂³⁷ClN₂O₆ [M+H+2]⁺, 505.1919; found, 505.1948.

Synthesis of 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (64)

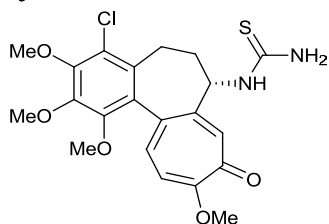


A mixture of 4-chlorodeacetylcolchicine (**12**) (400 mg, 1.02 mmol), thiophosgene (93 μ L, 1.22 mmol) and Et₃N (355 μ L, 2.55 mmol) in CH₂Cl₂ (10 mL) was stirred 2h at 0 °C under argon atmosphere. 10% NaHSO₄ aq was added to the reaction mixture and the whole mixture was extracted two times with

CHCl₃. The combined organic layers were washed with saturated NaHCO₃ aq and brine, dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **64** (443 mg, 1.02 mmol, quant.) as an amorphous solid.

$[\alpha]_D^{25}$: -81.11 ° (c = 0.233, MeOH). ¹H-NMR(400MHz, DMSO-*d*₆): δ 7.24 (1H, s), 7.17 (1H, d, J = 10.7 Hz), 7.10 (1H, d, J = 10.7 Hz), 4.92-4.87 (1H, m), 3.92 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.59 (3H, s), 3.11 (1H, dd, J = 12.8, 6.0 Hz), 2.46-2.39 (1H, m), 2.20-2.08 (2H, m). HR-ESI-MS: calcd for C₂₁H₂₁³⁵ClNO₅S [M+H]⁺, 434.0829; found, 434.0819; calcd for C₂₁H₂₁³⁷ClNO₅S [M+H+2]⁺, 436.0799; found, 436.0788.

Synthesis of 4-chloro-N-carbamothioyldeacetylcolchicine (24)

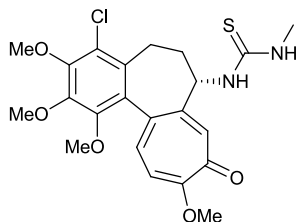


To a stirred solution of 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (26 mg, 0.060 mmol) in 1,4-dioxane (1 mL) was added NH₃ (0.5 mol/L in 1,4-dioxane, 240 μ L, 0.12 mmol) at ambient temperature under argon atmosphere. After stirring overnight, the reaction mixture

was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **24** (16 mg, 0.035 mmol, 45% yield) as a pale yellow solid.

$[\alpha]_D^{25}$: -104.97 ° (c = 0.072, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.29 (1H, d, J = 7.6 Hz), 7.14 (1H, d, J = 11.0 Hz), 7.05 (1H, s), 7.03 (1H, d, J = 11.0 Hz), 4.70-4.63 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.59 (3H, s), 3.11 (1H, dd, J = 12.4, 4.1 Hz), 2.17-2.01 (2H, m), 1.89-1.81 (1H, m). HR-ESI-MS: calcd for C₂₁H₂₄³⁵ClN₂O₅S [M+H]⁺, 451.1094; found, 451.1065; calcd for C₂₁H₂₄³⁷ClN₂O₅S [M+H+2]⁺, 453.1065; found, 453.1064.

Synthesis of 4-chloro-*N*-(methylcarbamothioyl)deacetylcolchicine (**25**)

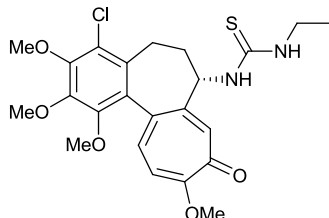


Compound **25** was prepared from 4-chlorodeacetylcolchicine (**12**) (63 mg, 0.16 mmol) and methylamine hydrochloride (22 mg, 0.32 mmol) using the same procedure as that described for compound **32**. Yellow solid (58 mg, 0.12 mmol, 78% yield).

$[\alpha]_D^{25}$: -95.53° ($c = 0.112$, MeOH). $^1\text{H-NMR}$ (400 MHz,

DMSO- d_6): δ 8.09 (1H, br-s), 7.46 (1H, br-s), 7.14 (1H, d, $J = 10.7$ Hz), 7.07 (1H, s), 7.03 (1H, d, $J = 10.7$ Hz), 4.82-4.73 (1H, m), 3.91 (3H, s), 3.88 (6H, s), 3.60 (3H, s), 3.14-3.09 (1H, m), 2.82 (3H, d, $J = 4.4$ Hz), 2.16-1.85 (3H, m). HR-ESI-MS: calcd for $\text{C}_{22}\text{H}_{26}^{35}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 465.1251; found, 465.1207; calcd for $\text{C}_{22}\text{H}_{26}^{37}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 467.1221; found, 467.1202.

Synthesis of 4-chloro-*N*-(ethylcarbamothioyl)deacetylcolchicine (**26**)

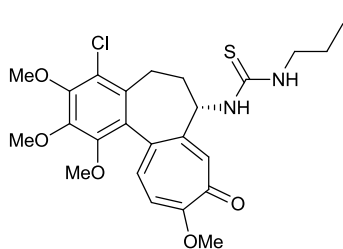


A solution of 4-chlorodeacetylcolchicine (**12**) (50 mg, 0.13 mmol) and ethyl isothiocyanate (22 μL , 0.26 mmol) in MeOH- H_2O (2:1, 1.5 mL) was stirred for 90 min at 0°C . The reaction mixture was extracted two times with CHCl_3 . The combined organic layers were washed with brine, dried over

MgSO_4 , filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography ($\text{CHCl}_3/\text{MeOH}$) to afford compound **26** (30 mg, 0.063 mmol, 49% yield) as a yellow solid.

$[\alpha]_D^{25}$: -97.12° ($c = 0.202$, MeOH). $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 8.44 (1H, br-s), 7.84 (1H, s), 7.50 (1H, dd, $J = 10.7$ Hz), 7.04 (1H, d, $J = 11.0$ Hz), 5.07-5.01 (1H, m), 4.07 (3H, s), 4.00 (3H, s), 3.99 (3H, s), 3.74 (3H, s), 3.63-3.56 (1H, m), 3.38-3.33 (1H, m), 3.25 (1H, dd, $J = 13.7, 5.4$ Hz), 2.49-2.40 (1H, m), 2.13-2.10 (1H, m), 1.96-1.89 (1H, m), 1.11 (3H, t, $J = 7.2$ Hz). HR-ESI-MS: calcd for $\text{C}_{23}\text{H}_{28}^{35}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 479.1407; found, 479.1410; calcd for $\text{C}_{23}\text{H}_{28}^{37}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 481.1378; found, 481.1365.

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



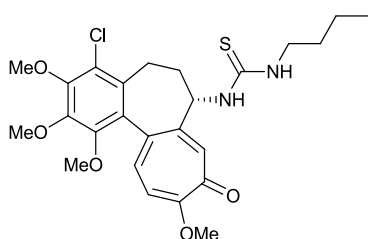
Compound **27** was prepared from 4-chlorodeacetylcolchicine (**12**) (63 mg, 0.16 mmol) and propylamine (26 μL , 0.32 mmol) using the same procedure as that described for compound **32**. Yellow solid (54 mg, 0.11 mmol, 69% yield).

$[\alpha]_D^{25}$: -88.98° ($c = 0.251$, MeOH). $^1\text{H-NMR}$ (400 MHz,

DMSO- d_6): δ 7.97 (1H, br-s), 7.54 (1H, br-s), 7.14 (1H, d, $J = 10.7$ Hz), 7.06 (1H, s), 7.03 (1H, d, $J = 10.7$ Hz), 4.81-4.71 (1H, m), 3.91 (3H, s), 3.88 (6H,

s), 3.60 (3H, s), 3.30-3.21 (2H, m), 3.12 (1H, dd, $J = 12.9, 5.1$ Hz), 2.17-1.81 (3H, m), 1.51-1.42 (2H, m), 0.84 (3H, t, $J = 7.4$ Hz). HR-ESI-MS: calcd for $C_{24}H_{30}^{35}ClN_2O_5S$ $[M+H]^+$, 493.1564; found, 493.1559; calcd for $C_{24}H_{30}^{37}ClN_2O_5S$ $[M+H+2]^+$, 495.1534; found, 495.1614.

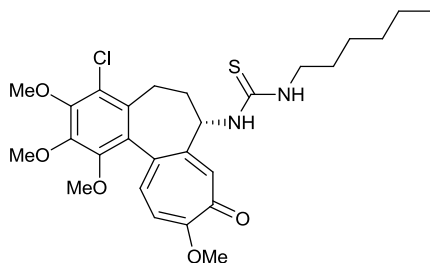
Synthesis of 4-chloro-*N*-(butylcarbamothioyl)deacetylcolchicine (28)



Compound **28** was prepared from 4-chlorodeacetylcolchicine (**12**) (64 mg, 0.16 mmol) and butylamine (32 μ L, 0.32 mmol) using the same procedure as that described for compound **32**. Yellow solid (55 mg, 0.11 mmol, 68% yield).

$[\alpha]_D^{25}$: -95.34° ($c = 0.236$, MeOH). 1H -NMR (400 MHz, DMSO- d_6): δ 7.96 (1H, br-s), 7.51 (1H, br-s), 7.14 (1H, d, $J = 10.5$ Hz), 7.05 (1H, s), 7.03 (1H, d, $J = 10.5$ Hz), 4.80-4.70 (1H, m), 3.91 (3H, s), 3.88 (6H, s), 3.60 (3H, s), 3.42-3.35 (1H, m), 3.30-3.26 (1H, m), 3.12 (1H, dd, $J = 12.6, 5.0$ Hz), 2.17-1.81 (3H, m), 1.47-1.40 (2H, m), 1.30-1.24 (2H, m), 0.86 (3H, t, $J = 7.3$ Hz). HR-ESI-MS: calcd for $C_{25}H_{32}^{35}ClN_2O_5S$ $[M+H]^+$, 507.1720; found, 507.1777; calcd for $C_{25}H_{32}^{37}ClN_2O_5S$ $[M+H+2]^+$, 509.1691; found, 509.1759.

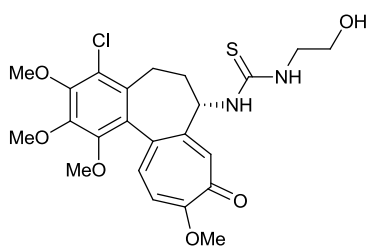
Synthesis of 4-chloro-*N*-(hexylcarbamothioyl)deacetylcolchicine (29)



Compound **29** was prepared from 4-chlorodeacetylcolchicine (**12**) (62 mg, 0.16 mmol) and *n*-hexylamine (42 μ L, 0.32 mmol) using the same procedure as that described for compound **32**. Yellow solid (54 mg, 0.10 mmol, 63% yield).

$[\alpha]_D^{25}$: -90.24° ($c = 0.232$, MeOH). 1H -NMR (400 MHz, DMSO- d_6): δ 7.98 (1H, br-s), 7.51 (1H, br-s), 7.14 (1H, d, $J = 10.7$ Hz), 7.05-7.01 (2H, m), 4.81-4.69 (1H, m), 3.91 (3H, s), 3.88 (6H, s), 3.60 (3H, s), 3.31-3.23 (2H, m), 3.14-3.09 (1H, m), 2.17-1.80 (3H, m), 1.30-1.21 (8H, m), 0.85 (3H, t, $J = 6.8$ Hz). HR-ESI-MS: calcd for $C_{27}H_{36}^{35}ClN_2O_5S$ $[M+H]^+$, 535.2033; found, 535.2028; calcd for $C_{27}H_{36}^{37}ClN_2O_5S$ $[M+H+2]^+$, 537.2004; found, 537.2000.

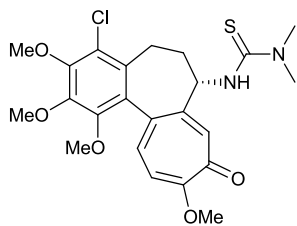
Synthesis of 4-chloro-*N*-(hydroxyethylcarbamothioyl)deacetylcolchicine (**30**)



Compound **30** was prepared from 4-chlorodeacetylcolchicine (**12**) (62 mg, 0.16 mmol) and hydroxyethylamine (19 μ L, 0.32 mmol) using the same procedure as that described for compound **32**. Yellow solid (15 mg, 0.030 mmol, 19% yield).

$[\alpha]_D^{25}$: -85.83° ($c = 0.200$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 8.19 (1H, br-s), 7.60 (1H, br-s), 7.15 (1H, d, $J = 10.5$ Hz), 7.05-7.02 (2H, m), 4.82 (1H, s), 4.75 (1H, s), 3.91 (3H, s), 3.89 (6H, s), 3.60 (3H, s), 3.49-3.42 (3H, m), 3.39-3.35 (1H, m), 3.14-3.09 (1H, m), 2.17-2.03 (2H, m), 1.89-1.77 (1H, m). HR-ESI-MS: calcd for $\text{C}_{23}\text{H}_{28}^{35}\text{ClN}_2\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$, 495.1357; found, 495.1364; calcd for $\text{C}_{23}\text{H}_{28}^{37}\text{ClN}_2\text{O}_6\text{S}$ $[\text{M}+\text{H}+2]^+$, 497.1327; found, 497.1322.

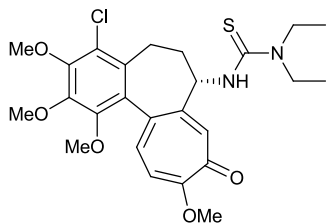
Synthesis of 4-chloro-*N*-(dimethylcarbamothioyl)deacetylcolchicine (**31**)



Compound **31** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and dimethylamine hydrochloride using the same procedure as that described for compound **32**. Pale yellow solid (37 mg, 0.077 mmol, quant.).

$[\alpha]_D^{25}$: -52.10° ($c = 0.119$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 7.63 (1H, d, $J = 7.1$ Hz), 7.15 (1H, d, $J = 11.0$ Hz), 7.09 (1H, s), 7.02 (1H, d, $J = 11.0$ Hz), 4.91-4.85 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.62 (3H, s), 3.20 (6H, s), 3.12 (1H, dd, $J = 13.2, 5.1$ Hz), 2.26-2.18 (1H, m), 2.16-2.08 (1H, m), 2.04-1.95 (1H, m). HR-ESI-MS: calcd for $\text{C}_{23}\text{H}_{28}^{35}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 479.1407; found, 479.1403; calcd for $\text{C}_{23}\text{H}_{28}^{37}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 481.1378; found, 481.1453.

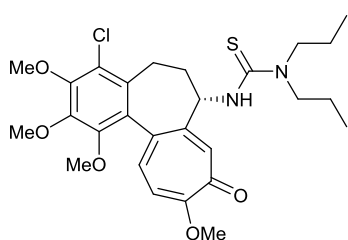
Synthesis of 4-chloro-*N*-(diethylcarbamothioyl)deacetylcolchicine (**32**)



To a stirred solution of 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.077 mmol) and Et_3N (26 μ L, 0.18 mmol) in CH_2Cl_2 (1.5 mL) was added thiophosgene (6.1 μ L, 0.080 mmol) at 0°C under argon atmosphere. After stirring 2 h at 0°C , Et_2NH (16 μ L, 0.15 mmol) was added. Then the reaction mixture was stirred overnight and allowed to warm to ambient temperature during the course of the reaction. The reaction was quenched with 10% citric acid. After diluting with AcOEt, the organic layer was taken, dried over Na_2SO_4 , filtered, and evaporated *in vacuo*. The residue was purified by silica gel flash column chromatography ($\text{CHCl}_3/\text{MeOH}$) to afford compound **32** (33 mg, 0.065 mmol, 85% yield) as an off-white solid.

$[\alpha]_{\text{D}}^{25}$: -38.54° ($c = 0.472$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 7.54 (1H, d, $J = 6.8\text{ Hz}$), 7.15 (1H, d, $J = 11.0\text{ Hz}$), 7.08 (1H, s), 7.03 (1H, d, $J = 11.0\text{ Hz}$), 5.02-4.95 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.72-3.64 (4H, m), 3.62 (3H, s), 3.12 (1H, dd, $J = 13.5, 5.5\text{ Hz}$), 2.33-2.24 (1H, m), 2.15-2.07 (1H, m), 2.02-1.95 (1H, m), 1.09 (6H, t, $J = 7.0\text{ Hz}$). HR-ESI-MS: calcd for $\text{C}_{25}\text{H}_{32}^{35}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 507.1720; found, 507.1714; calcd for $\text{C}_{25}\text{H}_{32}^{37}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 509.1691; found, 509.1718.

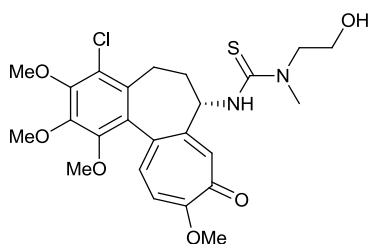
Synthesis of 4-chloro-*N*-(di-*n*-propylcarbamothioyl)deacetylcolchicine (33)



Compound **33** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and di-*n*-propylamine using the same procedure as that described for compound **32**. Off-white solid (41 mg, 0.075 mmol, 98% yield).

$[\alpha]_{\text{D}}^{25}$: -45.49° ($c = 0.220$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 7.50 (1H, d, $J = 7.1\text{ Hz}$), 7.14 (1H, d, $J = 10.7\text{ Hz}$), 7.09 (1H, s), 7.02 (1H, d, $J = 10.7\text{ Hz}$), 5.01-4.94 (1H, m), 3.91 (3H, s), 3.88 (6H, s), 3.69-3.61 (2H, m), 3.62 (3H, s), 3.54-3.46 (2H, m), 3.13 (1H, dd, $J = 13.3, 5.2\text{ Hz}$), 2.31-2.23 (1H, m), 2.16-2.07 (1H, m), 2.01-1.93 (1H, m), 1.60-1.50 (4H, m), 0.83 (6H, t, $J = 7.3\text{ Hz}$). HR-ESI-MS: calcd for $\text{C}_{27}\text{H}_{36}^{35}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 535.2033; found, 535.2015; calcd for $\text{C}_{27}\text{H}_{36}^{37}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 537.2004; found, 537.2060.

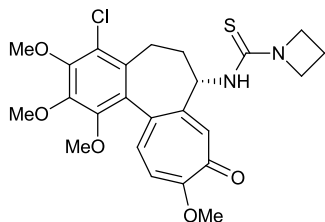
Synthesis of 4-chloro-*N*-(*N*²-(2-hydroxyethyl)methylcarbamothioyl)deacetylcolchicine (34)



Compound **34** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and 2-(methylamino)ethanol using the same procedure as that described for compound **32**. Pale yellow solid (36 mg, 0.071 mmol, 92% yield).

$[\alpha]_{\text{D}}^{25}$: -50.35° ($c = 0.228$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 7.75 (1H, d, $J = 6.3\text{ Hz}$), 7.15 (1H, d, $J = 10.7\text{ Hz}$), 7.08 (1H, s), 7.03 (1H, d, $J = 11.0\text{ Hz}$), 4.98 (1H, br-s), 4.89-4.83 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.86-3.64 (2H, m), 3.62 (3H, s), 3.59-3.54 (2H, m), 3.21 (3H, s), 3.12 (1H, dd, $J = 12.6, 5.2\text{ Hz}$), 2.21-1.97 (3H, m). HR-ESI-MS: calcd for $\text{C}_{24}\text{H}_{30}^{35}\text{ClN}_2\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$, 509.1513; found, 509.1327; calcd for $\text{C}_{24}\text{H}_{30}^{37}\text{ClN}_2\text{O}_6\text{S}$ $[\text{M}+\text{H}+2]^+$, 511.1484; found, 511.1308.

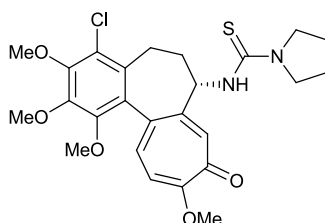
Synthesis of *N*-(azetidine-1-carbonothioyl)-4-chlorodeacetylcolchicine (**35**)



Compound **35** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and azetidine using the same procedure as that described for compound **32**. Off-white solid (7 mg, 0.014 mmol, 18% yield).

$[\alpha]_{\text{D}}^{25}$: -17.42° ($c = 0.092$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 7.85 (1H, d, $J = 7.3$ Hz), 7.14 (1H, d, $J = 11.0$ Hz), 7.08 (1H, s), 7.03 (1H, d, $J = 11.0$ Hz), 4.80-4.73 (1H, m), 4.08-3.90 (4H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.60 (3H, s), 3.12 (1H, dd, $J = 12.6, 6.2$ Hz), 2.22-2.06 (4H, m), 2.02-1.92 (1H, m). HR-ESI-MS: calcd for $\text{C}_{24}\text{H}_{28}^{35}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 491.1407; found, 491.1491; calcd for $\text{C}_{24}\text{H}_{28}^{37}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 493.1378; found, 493.1458.

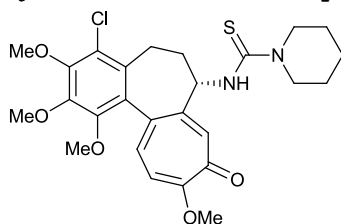
Synthesis of 4-chloro-*N*-(pyrrolidine-1-carbonothioyl)deacetylcolchicine (**36**)



Compound **36** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and pyrrolidine using the same procedure as that described for compound **32**. Pale yellow solid (28 mg, 0.055 mmol, 72% yield).

$[\alpha]_{\text{D}}^{25}$: -25.57° ($c = 0.078$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 7.55 (1H, d, $J = 7.3$ Hz), 7.15 (1H, d, $J = 10.7$ Hz), 7.12 (1H, s), 7.03 (1H, d, $J = 10.7$ Hz), 4.94-4.87 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.61 (3H, s), 3.58-3.45 (4H, m), 3.12 (1H, dd, $J = 13.4, 5.1$ Hz), 2.25-1.84 (7H, m). HR-ESI-MS: calcd for $\text{C}_{25}\text{H}_{30}^{35}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 505.1564; found, 505.1608; calcd for $\text{C}_{25}\text{H}_{30}^{37}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 507.1534; found, 507.1632.

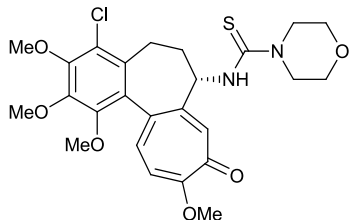
Synthesis of 4-chloro-*N*-(piperidine-1-carbonothioyl)deacetylcolchicine (**37**)



Compound **37** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and piperidine using the same procedure as that described for compound **32**. Off-white solid (28 mg, 0.054 mmol, 70% yield).

$[\alpha]_{\text{D}}^{25}$: -30.97° ($c = 0.090$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 7.85 (1H, d, $J = 7.1$ Hz), 7.15 (1H, d, $J = 10.7$ Hz), 7.06 (1H, s), 7.03 (1H, d, $J = 10.7$ Hz), 4.96-4.90 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.86-3.76 (4H, m), 3.63 (3H, s), 3.12 (1H, dd, $J = 13.1, 5.2$ Hz), 2.23-1.96 (3H, m), 1.64-1.58 (2H, m), 1.50-1.44 (4H, m). HR-ESI-MS: calcd for $\text{C}_{26}\text{H}_{32}^{35}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 519.1720; found, 519.1714; calcd for $\text{C}_{26}\text{H}_{32}^{37}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 521.1691; found, 521.1689.

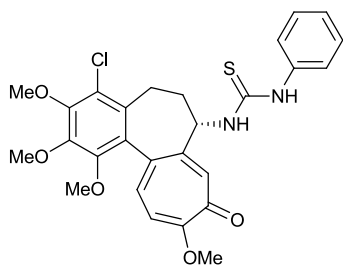
Synthesis of 4-chloro-*N*-(morpholine-4-carbonylthio)deacetylcolchicine (38)



Compound **38** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and morpholine using the same procedure as that described for compound **32**. Off-white solid (28 mg, 0.054 mmol, 70% yield).

$[\alpha]_D^{25}$: -50.76° ($c = 0.118$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 8.03 (1H, d, $J = 7.1$ Hz), 7.16 (1H, d, $J = 10.7$ Hz), 7.05 (1H, s), 7.03 (1H, d, $J = 10.7$ Hz), 4.92-4.86 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.87-3.75 (4H, m), 3.62 (3H, s), 3.59 (4H, t, $J = 4.8$ Hz), 3.12 (1H, dd, $J = 12.2, 5.1$ Hz), 2.21-1.99 (3H, m). HR-ESI-MS: calcd for $\text{C}_{25}\text{H}_{30}^{35}\text{ClN}_2\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$, 521.1513; found, 521.1523; calcd for $\text{C}_{25}\text{H}_{30}^{37}\text{ClN}_2\text{O}_6\text{S}$ $[\text{M}+\text{H}+2]^+$, 523.1484; found, 523.1518.

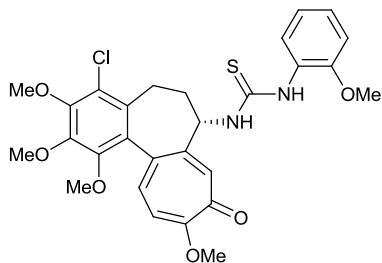
Synthesis of 4-chloro-*N*-(phenylcarbonylthio)deacetylcolchicine (39)



Compound **39** was prepared from 4-chlorodeacetylcolchicine (**12**) (50 mg, 0.13 mmol) and phenyl isothiocyanate (30 μL , 0.26 mmol) using the same procedure as that described for compound **26**. Pale yellow solid (50 mg, 0.095 mmol, 74% yield).

$[\alpha]_D^{25}$: -84.76° ($c = 0.140$, MeOH). $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 8.85 (1H, br-s), 8.01 (1H, br-s), 7.86 (1H, d, $J = 7.3$ Hz), 7.50 (1H, d, $J = 10.2$ Hz), 7.36 (1H, d, $J = 7.6$ Hz), 7.29-7.27 (2H, m), 7.15 (1H, t, $J = 7.2$ Hz), 7.02 (1H, d, $J = 11.2$ Hz), 5.13-5.07 (1H, m), 4.04 (3H, s), 3.99 (3H, s), 3.99 (3H, s), 3.72 (3H, s), 3.20 (1H, dd, $J = 13.8, 5.7$ Hz), 2.45-2.35 (1H, m), 2.04-1.98 (1H, m), 1.94-1.86 (1H, m). HR-ESI-MS: calcd for $\text{C}_{27}\text{H}_{28}^{35}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 527.1407; found, 527.1257; calcd for $\text{C}_{27}\text{H}_{28}^{37}\text{ClN}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 529.1378; found, 529.1252.

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

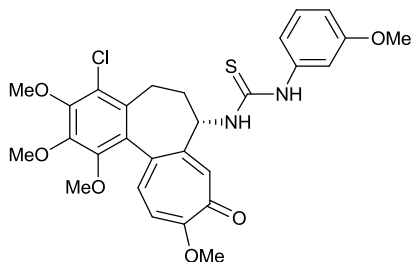


Compound **40** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and *o*-anisidine using the same procedure as that described for compound **45**. Off-white solid (14 mg, 0.025 mmol, 33% yield).

$[\alpha]_D^{25}$: -62.49° ($c = 0.066$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 9.17 (1H, s), 8.61 (1H, d, $J = 7.1$ Hz), 7.92 (1H, dd, $J = 7.8$ Hz, 1.4 Hz), 7.16 (1H, d, $J = 10.5$ Hz), 7.13-7.09 (2H, m), 7.06-7.02 (2H, m), 6.86 (1H, td, $J = 7.8, 1.4$ Hz), 4.84-4.78 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.86 (3H, s), 3.62 (3H, s), 3.18-3.10 (1H, m),

2.20-2.09 (2H, m), 1.99-1.91 (1H, m). HR-ESI-MS: calcd for $C_{28}H_{30}^{35}ClN_2O_6S$ $[M+H]^+$, 557.1513; found, 557.1429; calcd for $C_{28}H_{30}^{37}ClN_2O_6S$ $[M+H+2]^+$, 559.1484; found, 559.1428.

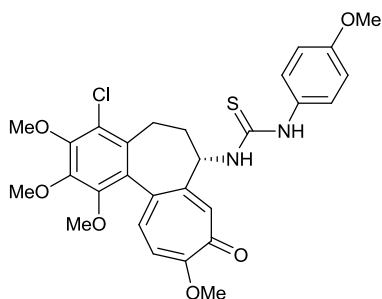
Synthesis of 4-chloro-*N*-(3-methoxyphenylcarbamothioyl)deacetylcolchicine (41)



Compound **41** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and *m*-anisidine using the same procedure as that described for compound **45**. Off-white solid (39 mg, 0.070 mmol, 91% yield).

$[\alpha]_D^{25}$: -74.42° ($c = 0.258$, MeOH). 1H -NMR (400 MHz, DMSO- d_6): δ 9.72 (1H, s), 8.44 (1H, d, $J = 7.1$ Hz), 7.22-7.11 (4H, m), 7.04 (1H, d, $J = 11.2$ Hz), 6.95 (1H, dd, $J = 8.3, 1.5$ Hz), 6.66 (1H, ddd, $J = 8.3, 2.4, 0.7$ Hz), 4.83-4.76 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.71 (3H, s), 3.62 (3H, s), 3.16-3.10 (1H, m), 2.19-2.10 (2H, m), 2.04-1.98 (1H, m). HR-ESI-MS: calcd for $C_{28}H_{30}^{35}ClN_2O_6S$ $[M+H]^+$, 557.1513; found, 557.1515; calcd for $C_{28}H_{30}^{37}ClN_2O_6S$ $[M+H+2]^+$, 559.1484; found, 559.1506.

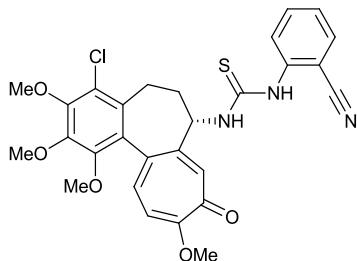
Synthesis of 4-chloro-*N*-((4-methoxyphenyl)carbamothioyl)deacetylcolchicine (42)



Compound **42** was prepared from 4-chlorodeacetylcolchicine (**12**) (62 mg, 0.16 mmol) and *p*-anisidine (39 mg, 0.32 mmol) using the same procedure as that described for compound **32**. Yellow solid (64 mg, 0.12 mmol, 72% yield).

$[\alpha]_D^{25}$: -51.66° ($c = 0.211$, MeOH). 1H -NMR (400 MHz, DMSO- d_6): δ 9.50 (1H, s), 8.20 (1H, d, $J = 7.3$ Hz), 7.27 (2H, d, $J = 8.8$ Hz), 7.15 (1H, d, $J = 10.5$ Hz), 7.11 (1H, s), 7.03 (1H, d, $J = 10.5$ Hz), 6.88 (2H, d, $J = 8.8$ Hz), 4.83-4.77 (1H, m), 3.91 (3H, s), 3.89 (6H, s), 3.73 (3H, s), 3.61 (3H, s), 3.15-3.10 (1H, m), 2.16-1.96 (3H, m). HR-ESI-MS: calcd for $C_{28}H_{30}^{35}ClN_2O_6S$ $[M+H]^+$, 557.1513; found, 557.1516; calcd for $C_{28}H_{30}^{37}ClN_2O_6S$ $[M+H+2]^+$, 559.1484; found, 559.1501.

Synthesis of 4-chloro-*N*-(2-cyanophenylcarbamothioyl)deacetylcolchicine (**43**)

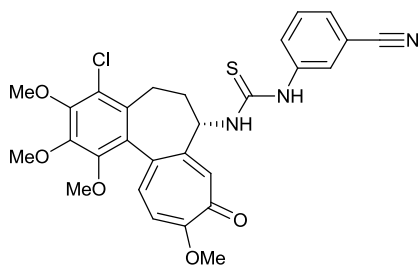


A solution of 2-cyanoaniline (14 mg, 0.11 mmol) and thiophosgene (8.8 μ L, 0.11 mmol) in CH_2Cl_2 - H_2O (1:1, 3 mL) was stirred for 3 h at room temperature under argon atmosphere. After adding a solution of 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.077 mmol) in CH_2Cl_2 (0.5 mL) and a solution of Na_2CO_3 (12 mg, 0.11 mmol) in H_2O (0.5

mL), the reaction mixture was stirred overnight and allowed to warm to ambient temperature during the course of the reaction. Brine was added to the reaction mixture and the whole mixture was extracted two times with CHCl_3 . The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography ($\text{CHCl}_3/\text{MeOH}$) to afford compound **43** (19 mg, 0.035 mmol, 45% yield) as an off-white solid.

$[\alpha]_{\text{D}}^{25}$: -96.20° ($c = 0.089$, MeOH). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 9.77 (1H, s), 8.87 (1H, d, $J = 7.1$ Hz), 7.79 (1H, dd, $J = 7.7, 1.3$ Hz), 7.66-7.57 (2H, m), 7.35 (1H, td, $J = 7.7, 1.3$ Hz), 7.16 (1H, d, $J = 10.7$ Hz), 7.14 (1H, s), 7.04 (1H, d, $J = 10.7$ Hz), 4.83-4.77 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.60 (3H, s), 3.19-3.10 (1H, m), 2.17-1.98 (3H, m). HR-ESI-MS: calcd for $\text{C}_{28}\text{H}_{27}^{35}\text{ClN}_3\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 552.1360; found, 552.1365; calcd for $\text{C}_{28}\text{H}_{27}^{37}\text{ClN}_3\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 554.1330; found, 554.1353.

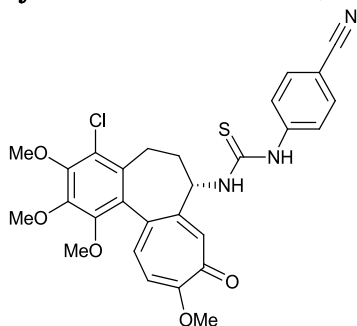
Synthesis of 4-chloro-*N*-(3-cyanophenylcarbamothioyl)deacetylcolchicine (**44**)



Compound **44** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.077 mmol) and 3-cyanoaniline using the same procedure as that described for compound **45**. Amorphous solid (42 mg, 0.077 mmol, quant.).

$[\alpha]_{\text{D}}^{25}$: -84.31° ($c = 0.102$, MeOH). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 9.95 (1H, s), 8.73 (1H, d, $J = 6.8$ Hz), 8.03 (1H, s), 7.72 (1H, d, $J = 7.6$ Hz), 7.54-7.47 (2H, m), 7.17 (1H, d, $J = 10.7$ Hz), 7.12 (1H, s), 7.05 (1H, d, $J = 10.7$ Hz), 4.80-4.74 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.62 (3H, s), 3.20-3.11 (1H, m), 2.21-2.12 (2H, m), 2.04-1.98 (1H, m). HR-ESI-MS: calcd for $\text{C}_{28}\text{H}_{27}^{35}\text{ClN}_3\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 552.1360; found, 552.1364; calcd for $\text{C}_{28}\text{H}_{27}^{37}\text{ClN}_3\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 554.1330; found, 554.1354.

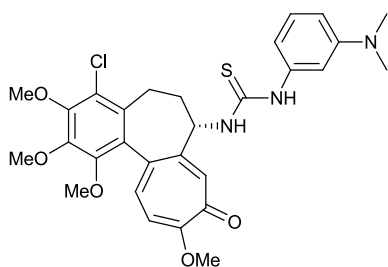
Synthesis of 4-chloro-*N*-(4-cyanophenylcarbamothioyl)deacetylcolchicine (**45**)



A solution of 4-cyanoaniline (14 mg, 0.11 mmol), thiophosgene (8.8 μ l, 0.11 mmol) and Et₃N (32 μ l, 0.11 mmol) in CH₂Cl₂ (1.5 mL) was stirred for 2 h at 0 °C under argon atmosphere. After adding 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.077 mmol), the reaction mixture was stirred overnight and allowed to warm to ambient temperature during the course of the reaction. The

resulting mixture was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **45** (42 mg, 0.077 mmol, quant.) as an off-white solid. $[\alpha]_D^{25}$: -91.09 ° ($c = 0.206$, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 10.12 (1H, s), 8.83 (1H, d, $J = 7.1$ Hz), 7.76-7.71 (4H, m), 7.17 (1H, d, $J = 10.7$ Hz), 7.12 (1H, s), 7.05 (1H, d, $J = 10.7$ Hz), 4.77-4.71 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.63 (3H, s), 3.20-3.11 (1H, m), 2.22-1.98 (3H, m). HR-ESI-MS: calcd for C₂₈H₂₇³⁵ClN₃O₅S [M+H]⁺, 552.1360; found, 552.1370; calcd for C₂₈H₂₇³⁷ClN₃O₅S [M+H+2]⁺, 554.1330; found, 554.1359.

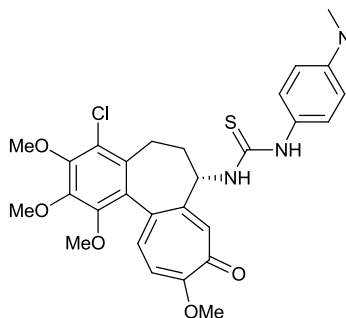
Synthesis of 4-chloro-*N*-(3-(dimethylamino)phenylcarbamothioyl)deacetylcolchicine (**46**)



Compound **46** was prepared from 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.078 mmol) and *N,N*-dimethyl-*m*-phenylenediamine hydrochloride using the same procedure as that described for compound **32**. Amorphous solid (7 mg, 0.014 mmol, 18% yield).

$[\alpha]_D^{25}$: -12.71 ° ($c = 0.157$, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 9.60 (1H, s), 8.29 (1H, d, $J = 7.1$ Hz), 7.16 (1H, d, $J = 10.9$ Hz), 7.11 (1H, s), 7.10 (1H, t, $J = 8.1$ Hz), 7.03 (1H, d, $J = 10.9$ Hz), 6.91 (1H, t, $J = 2.0$ Hz), 6.66 (1H, dd, $J = 8.1, 1.6$ Hz), 6.48 (1H, dd, $J = 8.1, 2.4$ Hz), 4.84-4.78 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.62 (3H, s), 3.17-3.08 (1H, m), 2.86 (6H, s), 2.18-1.99 (3H, m). HR-ESI-MS: calcd for C₂₉H₃₃³⁵ClN₃O₅S [M+H]⁺, 570.1829; found, 570.1815; calcd for C₂₉H₃₃³⁷ClN₃O₅S [M+H+2]⁺, 572.1800; found, 572.1799.

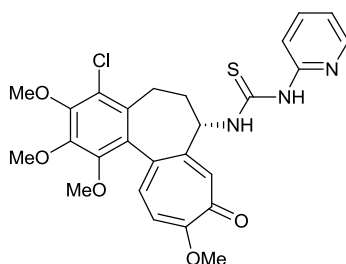
Synthesis of 4-chloro-*N*-(4-(dimethylamino)phenylcarbamothioyl)deacetylcolchicine (47)



Compound **47** was prepared from 4-chloro-deacetylcolchicine (**12**) (50 mg, 0.13 mmol) and 4-(dimethylamino)phenyl isothiocyanate (43 mg, 0.26 mmol) using the same procedure as that described for compound **26**. Yellow solid (46 mg, 0.081 mmol, 63% yield).

$[\alpha]_{\text{D}}^{25}$: -10.77° ($c = 0.204$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 9.40 (1H, s), 8.02 (1H, d, $J = 6.6$ Hz), 7.15 (1H, d, $J = 10.7$ Hz), 7.14 (2H, d, $J = 8.3$ Hz), 7.10 (1H, s), 7.03 (1H, d, $J = 11.0$ Hz), 6.68 (2H, d, $J = 8.8$ Hz), 4.84-4.79 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.61 (3H, s), 3.11 (1H, dd, $J = 12.8, 5.5$ Hz), 2.87 (6H, s), 2.13-2.09 (2H, m), 2.03-1.98 (1H, m). HR-ESI-MS: calcd for $\text{C}_{29}\text{H}_{33}^{35}\text{ClN}_3\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 570.1829; found, 570.1864; calcd for $\text{C}_{29}\text{H}_{33}^{37}\text{ClN}_3\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 572.1800; found, 572.1852.

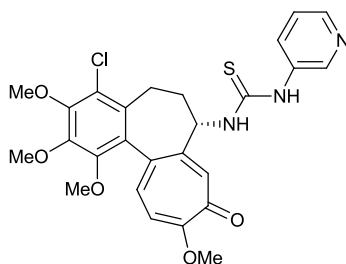
Synthesis of 4-chloro-*N*-(pyridin-2-ylcarbamothioyl)deacetylcolchicine (48)



Compound **48** was prepared from 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (30 mg, 0.069 mmol) and 2-aminopyridine (7 mg, 0.076 mmol) using the same procedure as that described for compound **59**. Off-white solid (17 mg, 0.033 mmol, 47% yield).

$[\alpha]_{\text{D}}^{25}$: $+158.33^\circ$ ($c = 0.108$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 12.37 (1H, d, $J = 6.8$ Hz), 10.85 (1H, s), 8.34-8.32 (1H, m), 7.86-7.81 (1H, m), 7.22-7.18 (2H, m), 7.14-7.11 (1H, m), 7.07 (1H, d, $J = 10.7$ Hz), 7.00 (1H, s), 4.87-4.80 (1H, m), 3.93 (3H, s), 3.90 (6H, s), 3.63 (3H, s), 3.20-3.15 (1H, m), 2.31-2.09 (3H, m). HR-ESI-MS: calcd for $\text{C}_{26}\text{H}_{27}^{35}\text{ClN}_3\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 528.1360; found, 528.1315; calcd for $\text{C}_{26}\text{H}_{27}^{37}\text{ClN}_3\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 530.1330; found, 530.1295.

Synthesis of 4-chloro-*N*-(3-pyridylcarbamothioyl)deacetylcolchicine (49)

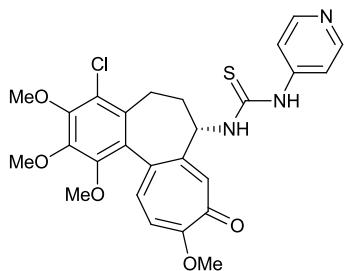


Compound **49** was prepared from 4-chloro-deacetylcolchicine (**12**) (50 mg, 0.13 mmol) and 3-pyridyl isothiocyanate (29 μL , 0.26 mmol) using the same procedure as that described for compound **26**. Pale yellow solid (51 mg, 0.097 mmol, 76% yield).

$[\alpha]_{\text{D}}^{25}$: -127.75° ($c = 0.227$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 9.79 (1H, s), 8.67 (1H, d, $J = 6.8$ Hz), 8.57 (1H, d, $J = 2.2$ Hz), 8.28 (1H, d, J

= 3.7 Hz), 7.94 (1H, d, J = 8.1 Hz), 7.32 (1H, dd, J = 8.2, 4.8 Hz), 7.17 (1H, d, J = 10.7 Hz), 7.14 (1H, s), 7.05 (1H, d, J = 11.0 Hz), 4.81-4.75 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.61 (3H, s), 3.17-3.12 (1H, m), 2.19-2.13 (2H, m), 2.06-1.99 (1H, m). HR-ESI-MS: calcd for $C_{26}H_{27}^{35}ClN_3O_5S$ $[M+H]^+$, 528.1360; found, 528.1376; calcd for $C_{26}H_{27}^{37}ClN_3O_5S$ $[M+H+2]^+$, 530.1330; found, 530.1366.

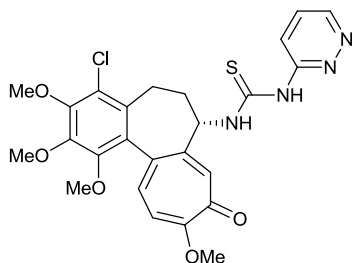
Synthesis of 4-chloro-*N*-(pyridin-4-ylcarbamothioyl)deacetylcolchicine (50)



Compound **50** was prepared from 4-chloro-deacetylcolchicine (**12**) (30 mg, 0.077 mmol) and 4-aminopyridine (11 mg, 0.11 mmol) using the same procedure as that described for compound **32**. Yellow solid (25 mg, 0.048 mmol, 63% yield).

$[\alpha]_D^{25}$: -114.24 ° (c = 0.128, MeOH). 1H -NMR (400 MHz, DMSO- d_6): δ 10.07 (1H, s), 8.89 (1H, d, J = 6.8 Hz), 8.37 (2H, d, J = 5.6 Hz), 7.59 (2H, d, J = 5.6 Hz), 7.18 (1H, d, J = 10.7 Hz), 7.11 (1H, s), 7.05 (1H, d, J = 10.7 Hz), 4.76-4.69 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.63 (3H, s), 3.18-3.13 (1H, m), 2.22-2.12 (2H, m), 2.06-1.97 (1H, m). HR-ESI-MS: calcd for $C_{26}H_{27}^{35}ClN_3O_5S$ $[M+H]^+$, 528.1360; found, 528.1323; calcd for $C_{26}H_{27}^{37}ClN_3O_5S$ $[M+H+2]^+$, 530.1330; found, 530.1307.

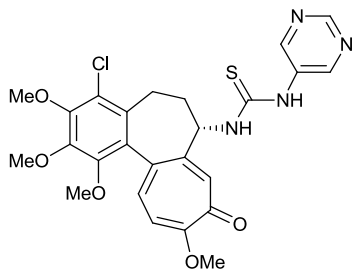
Synthesis of 4-chloro-*N*-(pyridazin-3-ylcarbamothioyl)deacetylcolchicine (51)



Compound **51** was prepared from 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (30 mg, 0.069 mmol) and 3-aminopyridazine (7 mg, 0.076 mmol) using the same procedure as that described for compound **59**. Off-white solid (20 mg, 0.037 mmol, 54% yield).

$[\alpha]_D^{25}$: +83.98 ° (c = 0.077, MeOH). 1H -NMR (400 MHz, DMSO- d_6): δ 12.15 (1H, d, J = 6.8 Hz), 11.07 (1H, s), 8.97 (1H, d, J = 4.6 Hz), 7.74 (1H, dd, J = 9.0, 4.6 Hz), 7.54 (1H, d, J = 9.0 Hz), 7.21 (1H, d, J = 10.7 Hz), 7.08 (1H, d, J = 10.7 Hz), 7.00 (1H, s), 4.87-4.81 (1H, m), 3.93 (3H, s), 3.90 (6H, s), 3.64 (3H, s), 3.19 (1H, dd, J = 12.4, 3.4 Hz), 2.35-2.19 (2H, m), 2.13-2.05 (1H, m). HR-ESI-MS: calcd for $C_{25}H_{26}^{35}ClN_4O_5S$ $[M+H]^+$, 529.1312; found, 529.1375; calcd for $C_{25}H_{26}^{37}ClN_4O_5S$ $[M+H+2]^+$, 531.1283; found, 531.1337.

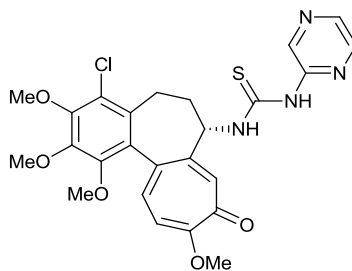
Synthesis of 4-chloro-*N*-(pyrimidin-5-ylcarbamothioyl)deacetylcolchicine (**52**)



Compound **52** was prepared from 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (30 mg, 0.069 mmol) and 5-aminopyrimidine (8 mg, 0.083 mmol) using the same procedure as that described for compound **59**. Yellow solid (18 mg, 0.034 mmol, 49% yield).

$[\alpha]_D^{25}$: -130.95° ($c = 0.160$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 9.83 (1H, s), 8.89 (1H, d, $J = 7.1$ Hz), 8.88 (1H, s), 8.87 (2H, s), 7.17 (1H, d, $J = 10.7$ Hz), 7.13 (1H, s), 7.05 (1H, d, $J = 10.7$ Hz), 4.81-4.74 (1H, m), 3.91 (3H, s), 3.89 (3H, s), 3.89 (3H, s), 3.61 (3H, s), 3.20-3.11 (1H, m), 2.23-2.11 (2H, m), 2.05-1.98 (1H, m). HR-ESI-MS: calcd for $\text{C}_{25}\text{H}_{26}^{35}\text{ClN}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 529.1312; found, 529.1310; calcd for $\text{C}_{25}\text{H}_{26}^{37}\text{ClN}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 531.1283; found, 531.1276.

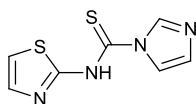
Synthesis of 4-chloro-*N*-(pyrazin-2-ylcarbamothioyl)deacetylcolchicine (**53**)



Compound **53** was prepared from 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (30 mg, 0.069 mmol) and 2-aminopyrazine (10 mg, 0.10 mmol) using the same procedure as that described for compound **59**. Off-white solid (14 mg, 0.026 mmol, 38% yield).

$[\alpha]_D^{25}$: $+148.25^\circ$ ($c = 0.069$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 11.51 (1H, d, $J = 6.8$ Hz), 11.18 (1H, s), 8.59 (1H, d, $J = 1.2$ Hz), 8.34 (1H, dd, $J = 2.7, 1.2$ Hz), 8.32 (1H, d, $J = 2.7$ Hz), 7.20 (1H, d, $J = 10.7$ Hz), 7.07 (1H, d, $J = 10.7$ Hz), 7.00 (1H, s), 4.84-4.78 (1H, m), 3.93 (3H, s), 3.89 (6H, s), 3.63 (3H, s), 3.20-3.15 (1H, m), 2.30-2.12 (3H, m). HR-ESI-MS: calcd for $\text{C}_{25}\text{H}_{26}^{35}\text{ClN}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 529.1312; found, 529.1284; calcd for $\text{C}_{25}\text{H}_{26}^{37}\text{ClN}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 531.1283; found, 531.1236.

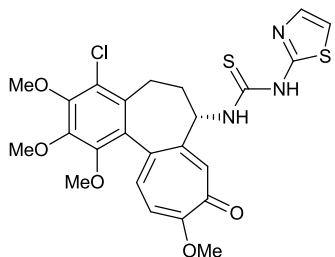
Synthesis of *N*-(thiazol-2-yl)-1*H*-imidazole-1-carbothioamide



To a stirred solution of 2-aminothiazole (1.00 g, 9.99 mmol) in MeCN (10 mL) was added 1,1'-thiocarbonyldiimidazole (1.78 g, 9.99 mmol) at ambient temperature under argon atmosphere. After stirring 2 h at 40 °C, the mixture was cooled to 0 °C. The precipitate was collected by filtration, washed with cold MeCN and *n*-hexane, and dried in reduced pressure to afford the desired material (1.37 g, 6.49 mmol, 65% yield) as a pale yellow solid.

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 8.64 (1H, s), 7.95 (1H, t, $J = 1.2$ Hz), 7.75 (1H, d, $J = 4.4$ Hz), 7.31 (1H, dd, $J = 4.1$ Hz), 7.07 (1H, s). ESI-MS m/z : 211 $[\text{M}+\text{H}]^+$.

Synthesis of 4-chloro-*N*-(thiazol-2-ylcarbamothioyl)deacetylcolchicine (**54**)

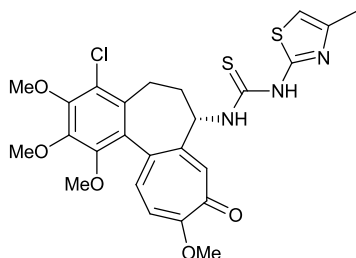


To a stirred solution of 4-chlorodeacetylcolchicine (**12**) (30 mg, 0.077 mmol) in MeCN (1.5 mL) was added *N*-(thiazol-2-yl)-1*H*-imidazole-1-carbothioamide (18 mg, 0.084 mmol) at room temperature under argon atmosphere. The solution was heated under reflux for 1 h with stirring. After cooling to room temperature, 10% NaHSO₄ aq. was

added to the reaction mixture and the whole mixture was extracted two times with AcOEt. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **54**. (38 mg, 0.071 mmol, 93% yield) as an off-white solid.

$[\alpha]_D^{25}$: +28.38 ° ($c = 0.250$, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 7.44 (1H, br-s), 7.20-7.01 (4H, m), 4.76-4.69 (1H, m), 3.93 (3H, s), 3.89 (6H, s), 3.64 (3H, s), 3.20-3.13 (1H, m), 2.23-1.98 (3H, m). HR-ESI-MS: calcd for C₂₄H₂₅³⁵ClN₃O₅S₂ [M+H]⁺, 534.0924; found, 534.1058; calcd for C₂₄H₂₅³⁷ClN₃O₅S₂ [M+H+2]⁺, 536.0895; found, 536.1025.

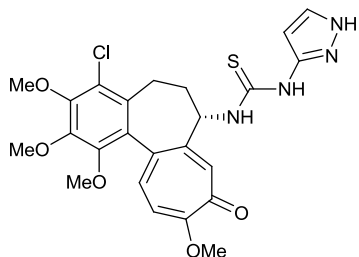
Synthesis of 4-chloro-*N*-(4-methylthiazol-2-yl)carbamothioyl)deacetylcolchicine (**55**)



Compound **55** was prepared from 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (30 mg, 0.069 mmol) and 2-amino-4-methylthiazole (16 mg, 0.14 mmol) using the same procedure as that described for compound **59**. Yellow solid (25 mg, 0.046 mmol, 66% yield).

$[\alpha]_D^{25}$: +38.97 ° ($c = 0.136$, MeOH). ¹H-NMR (400 MHz, DMSO-*d*₆): δ 7.18 (1H, d, $J = 10.5$ Hz), 7.08-7.00 (2H, m), 6.69 (1H, br-s), 4.76-4.69 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.64 (3H, s), 3.19-3.13 (1H, m), 2.27-2.00 (3H, m), 2.24 (3H, br-s). HR-ESI-MS: calcd for C₂₅H₂₇³⁵ClN₃O₅S₂ [M+H]⁺, 548.1081; found, 548.1034; calcd for C₂₅H₂₇³⁷ClN₃O₅S₂ [M+H+2]⁺, 550.1051; found, 550.0988.

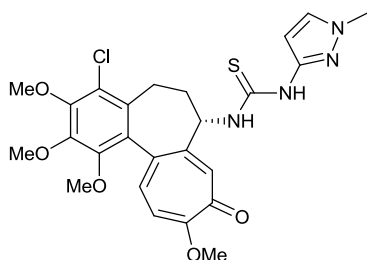
Synthesis of 4-chloro-*N*-(pyrazol-3-ylcarbamothioyl)deacetylcolchicine (**56**)



Compound **56** was prepared from 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (30 mg, 0.069 mmol) and 3-aminopyrazole (11 mg, 0.14 mmol) using the same procedure as that described for compound **59**. Yellow solid (26 mg, 0.050 mmol, 72% yield).

$[\alpha]_{\text{D}}^{25}$: +73.95 ° ($c = 0.119$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 12.64 (1H, s), 10.66 (1H, s), 10.39 (1H, br-s), 7.73 (1H, t, $J = 2.0$ Hz), 7.18 (1H, d, $J = 10.7$ Hz), 7.05 (1H, d, $J = 10.7$ Hz), 6.98 (1H, s), 5.96 (1H, s), 4.88-4.81 (1H, m), 3.93 (3H, s), 3.90 (3H, s), 3.89 (3H, s), 3.63 (3H, s), 3.19-3.14 (1H, m), 2.30-2.16 (2H, m), 2.00-1.90 (1H, m). HR-ESI-MS: calcd for $\text{C}_{24}\text{H}_{26}^{35}\text{ClN}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 517.1312; found, 517.1301; calcd for $\text{C}_{24}\text{H}_{26}^{37}\text{ClN}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 519.1283; found, 519.1272.

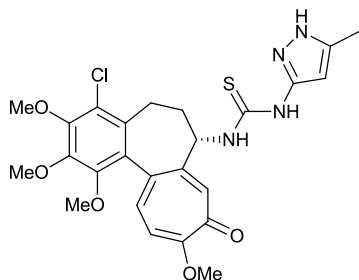
Synthesis of 4-chloro-*N*-((1-methylpyrazol-3-yl)carbamothioyl)deacetylcolchicine (**57**)



Compound **57** was prepared from 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (30 mg, 0.069 mmol) and 3-amino-1-methylpyrazole (13 mg, 0.14 mmol) using the same procedure as that described for compound **59**. Yellow solid (32 mg, 0.060 mmol, 96% yield).

$[\alpha]_{\text{D}}^{25}$: +139.62 ° ($c = 0.106$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 10.61 (1H, s), 10.20 (1H, br-s), 7.66 (1H, d, $J = 2.2$ Hz), 7.18 (1H, d, $J = 10.7$ Hz), 7.05 (1H, d, $J = 10.7$ Hz), 6.98 (1H, s), 5.92 (1H, s), 4.83-4.76 (1H, m), 3.92 (3H, s), 3.90 (6H, s), 3.82 (3H, s), 3.63 (3H, s), 3.19-3.14 (1H, m), 2.29-2.16 (2H, m), 2.05-1.98 (1H, m). HR-ESI-MS: calcd for $\text{C}_{25}\text{H}_{28}^{35}\text{ClN}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 531.1469; found, 531.1487; calcd for $\text{C}_{25}\text{H}_{28}^{37}\text{ClN}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 533.1439; found, 533.1485.

Synthesis of 4-chloro-*N*-((5-methylpyrazol-3-yl)carbamothioyl)deacetylcolchicine (**58**)

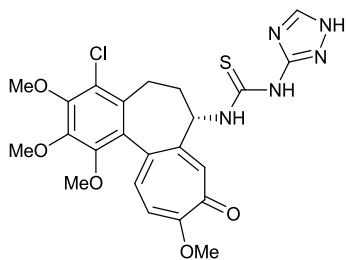


Compound **58** was prepared from 4-chloro-deacetylcolchicine (**12**) (62 mg, 0.16 mmol) and 3-amino-5-methylpyrazole (23 mg, 0.24 mmol) using the same procedure as that described for compound **45**. Yellow solid (73 mg, 0.14 mmol, 86% yield).

$[\alpha]_{\text{D}}^{25}$: +78.89 ° ($c = 0.199$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 12.36 (1H, s), 10.59 (1H, br-s), 10.46 (1H, br-s), 7.18 (1H, d, $J = 10.7$ Hz), 7.06 (1H, d, $J = 10.7$ Hz), 6.96 (1H, s), 5.73 (1H, s), 4.86-4.80 (1H, m), 3.92 (3H, s), 3.89

(6H, s), 3.62 (3H, s), 3.18-3.08 (1H, m), 2.27-2.16 (5H, m), 1.96-1.90 (1H, m). HR-ESI-MS: calcd for $C_{25}H_{28}^{35}ClN_4O_5S$ $[M+H]^+$, 531.1469; found, 531.1613; calcd for $C_{25}H_{28}^{37}ClN_4O_5S$ $[M+H+2]^+$, 533.1439; found, 533.1595.

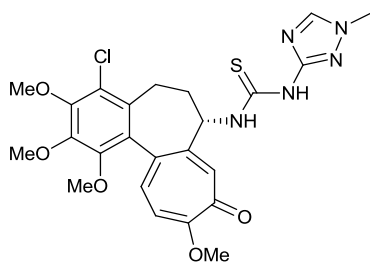
Synthesis of 4-chloro-*N*-((1,2,4-triazol-3-yl)carbamothioyl)deacetylcolchicine (59)



To a stirred solution of 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (72 mg, 0.17 mmol) in DMF (1 mL) was added 3-amino-1,2,4-triazole (17 mg, 0.20 mmol) at ambient temperature under argon atmosphere. The mixture was stirred for 2 h at 80 °C. After cooling to room temperature, $CHCl_3$ was added. The whole mixture was

washed with $NaHCO_3$ aq., dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue purified by silica gel flash column chromatography ($CHCl_3/MeOH$) to afford compound **59** (27 mg, 0.053 mmol, 31% yield) as a yellow solid. $[\alpha]_D^{25}$: -77.02 ° ($c = 0.082$, MeOH). 1H -NMR (400 MHz, $DMSO-d_6$): δ 10.49 (1H, br-s), 8.10 (2H, br-s), 7.72 (1H, br-s), 7.19 (1H, d, $J = 10.7$ Hz), 7.09 (1H, s), 7.06 (1H, d, $J = 10.7$ Hz), 4.80-4.78 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.65 (3H, s), 3.17-3.13 (1H, m), 2.55-2.53 (1H, m), 2.21-2.03 (2H, m). HR-ESI-MS: calcd for $C_{23}H_{25}^{35}ClN_5O_5S$ $[M+H]^+$, 518.1265; found, 518.1307; calcd for $C_{23}H_{25}^{37}ClN_5O_5S$ $[M+H+2]^+$, 520.1235; found, 520.1282.

Synthesis of 4-chloro-*N*-((1-methyl-1*H*-1,2,4-triazol-3-yl)carbamothioyl)deacetylcolchicine (60)

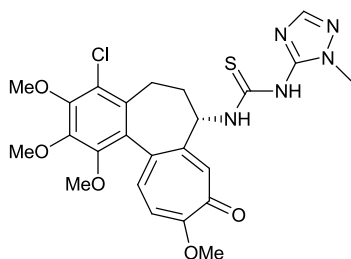


Compound **60** was prepared from 4-chloro-7-isothiocyanato-7-de(acetamide)colchicine (**64**) (54 mg, 0.12 mmol) and 3-amino-1-methyl-1*H*-1,2,4-triazole (15 mg, 0.14 mmol) using the same procedure as that described for compound **59**. Yellow solid (42 mg, 0.079 mmol, 66% yield).

$[\alpha]_D^{25}$: +53.60 ° ($c = 0.216$, MeOH). 1H -NMR (400 MHz, $DMSO-d_6$): δ 11.17 (1H, s), 10.42 (1H, d, $J = 6.6$ Hz), 8.50 (1H, s), 7.19 (1H, d, $J = 10.7$ Hz), 7.06 (1H, d, $J = 10.7$ Hz), 6.95 (1H, s), 4.85-4.79 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.85 (3H, s), 3.63 (3H, s), 3.17-3.15 (1H, m), 2.26-2.19 (2H, m), 2.05-2.00 (1H, m). HR-ESI-MS: calcd for $C_{24}H_{27}^{35}ClN_5O_5S$ $[M+H]^+$, 532.1421; found, 532.1516; calcd for $C_{24}H_{27}^{37}ClN_5O_5S$ $[M+H+2]^+$, 534.1392; found, 534.1474.

Synthesis of 4-chloro-*N*-((1-methyl-1*H*-1,2,4-triazol-5-yl)carbamothioyl)deacetylcolchi

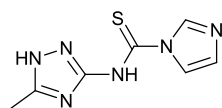
-cine (**61**)



Compound **61** was prepared from 4-chloro-7-
isothiocyanato-7-de(acetamide)colchicine (**64**) (81 mg, 0.18
mmol) and 5-amino-1-methyl-1*H*-1,2,4-triazole (22 mg,
0.22 mmol) using the same procedure as that described for
compound **59**. Yellow solid (42 mg, 0.079 mmol, 66% yield).
[α]_D²⁵: -49.09 ° (*c* = 0.265, MeOH). ¹H-NMR (400 MHz,

DMSO-*d*₆): δ 10.96 (1H, s), 10.74 (1H, d, *J* = 6.8 Hz), 7.91 (1H, s), 7.19 (1H, d, *J* = 10.7 Hz), 7.06 (1H, d, *J* = 10.7 Hz), 6.99 (1H, s), 4.82-4.75 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.77 (3H, s), 3.63 (3H, s), 3.20-3.17 (1H, m), 2.24-2.19 (2H, m), 2.08-2.01 (1H, m). HR-ESI-MS: calcd for C₂₄H₂₇³⁵ClN₅O₅S [M+H]⁺, 532.1421; found, 532.1477; calcd for C₂₄H₂₇³⁷ClN₅O₅S [M+H+2]⁺, 534.1392; found, 534.1448.

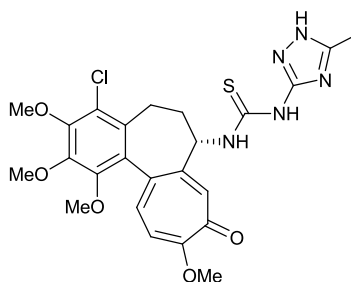
Synthesis of *N*-(5-methyl-1,2,4-triazol-3-yl)-1*H*-imidazole-1-carbothioamide



To a stirred solution of 3-amino-5-methyl-1,2,4-triazole (265 mg, 2.7 mmol) in MeCN (2.7 mL) was added 1,1'-thiocarbonyldiimidazole (481 mg, 2.7 mmol) at ambient temperature under argon atmosphere. After stirring for 2h, the precipitate was collected by filtration, washed with cold MeCN, and dried under reduced pressure to afford the desired material (377 mg, 1.8 mmol, 67% yield) as a yellow solid.

¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.41 (1H, t, *J* = 1.1 Hz), 8.26 (2H, br s), 7.83 (1H, t, *J* = 1.5 Hz), 7.05 (1H, dd, *J* = 1.5, 1.1 Hz), 2.12 (3H, s). ESI-MS *m/z*: 209 [M+H]⁺.

Synthesis of 4-chloro-*N*-((5-methyl-1,2,4-triazol-3-yl)carbamothioyl)deacetylcolchicine (**62**)

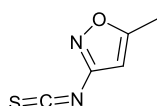


To a stirred solution of 4-chlorodeacetylcolchicine (**12**) (61 mg, 0.16 mmol) in DMF (2 mL) was added *N*-(5-methyl-1,2,4-triazol-3-yl)-1*H*-imidazole-1-carbothioamide (36 mg, 0.17 mmol) at room temperature under argon atmosphere. The solution was stirred for 2 h at 60 °C. After cooling to room temperature, Water was added

to the reaction mixture and the whole mixture was extracted two times with AcOEt. The combined organic layers were washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH) to afford compound **62**. (46 mg, 0.088 mmol, 55% yield) as a yellow solid.

$[\alpha]_D^{25}$: -64.44° ($c = 0.225$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 10.32 (1H, d, $J = 7.3$ Hz), 8.06 (2H, br-s), 7.19 (1H, d, $J = 10.7$ Hz), 7.07-7.04 (2H, m), 4.82-4.76 (1H, m), 3.92 (3H, s), 3.89 (6H, s), 3.64 (3H, s), 3.15 (1H, dd, $J = 12.9, 5.4$ Hz), 2.60-2.53 (1H, m), 2.20 (3H, s), 2.18-1.99 (2H, m). HR-ESI-MS: calcd for $\text{C}_{24}\text{H}_{27}^{35}\text{ClN}_5\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$, 532.1421; found, 532.1414; calcd for $\text{C}_{24}\text{H}_{27}^{37}\text{ClN}_5\text{O}_5\text{S}$ $[\text{M}+\text{H}+2]^+$, 534.1392; found, 534.1376.

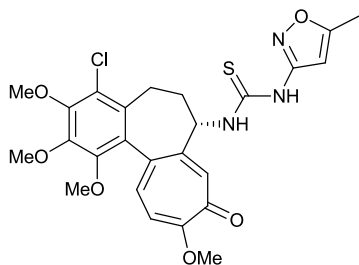
Synthesis of 3-isothiocyanato-5-methylisoxazole



A solution of 3-amino-5-methylisoxazole (326 mg, 3.3 mmol), thiophosgene (278 μL , 3.6 mmol) and Na_2CO_3 aq. (769 mg in 4 mL, 7.3 mmol) in CH_2Cl_2 (6.5 mL) was stirred for 3h at room temperature. Brine was added to the reaction mixture and the whole mixture was extracted two times with CHCl_3 . The combined organic layers were dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography ($\text{CHCl}_3/\text{MeOH}$) to afford the title compound (255 mg, 1.8 mmol, 55% yield) as a yellow oil.

$^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 5.97 (1H, t, $J = 0.9$ Hz), 2.43 (3H, t, $J = 0.9$ Hz). ESI-MS m/z : 141 $[\text{M}+\text{H}]^+$.

Synthesis of 4-chloro-*N*-((5-methylisoxazol-3-yl)carbamothioyl)deacetylcolchicine (63)



Compound **63** was prepared from 4-chlorodeacetylcolchicine (**12**) (75 mg, 0.19 mmol) and 3-isothiocyanato-5-methylisoxazole (32 mg, 0.23 mmol) using the same procedure as that described for compound **20**. Yellow solid (98 mg, 0.15 mmol, 80% yield).

$[\alpha]_D^{25}$: -108.11° ($c = 0.244$, MeOH). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 9.20 (1H, s), 7.17 (1H, d, $J = 10.5$ Hz), 7.13 (1H, s), 7.05 (1H, d, $J = 10.5$ Hz), 4.25-4.14 (1H, m), 3.93 (3H, s), 3.88 (6H, s), 3.54 (3H, s), 3.15 (1H, dd, $J = 12.8, 4.8$ Hz), 2.28-2.12 (2H, m), 1.93 (3H, s), 1.91-1.83 (1H, m). HR-ESI-MS: calcd for $\text{C}_{25}\text{H}_{27}^{35}\text{ClN}_3\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$, 532.1309; found, 532.1328; calcd for $\text{C}_{25}\text{H}_{27}^{37}\text{ClN}_3\text{O}_6\text{S}$ $[\text{M}+\text{H}+2]^+$, 534.1280; found, 534.1323.

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 (IC₅₀) 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).

Tubulin polymerization assay. *In vitro* tubulin polymerization assays were done using a Tubulin Polymerization Assay Kit (Cytoskeleton, inc.). Lyophilized porcine microtubule protein was resuspended in G-PEM buffer (80 mM PIPES, pH 6.9, 0.5 mM EGTA, 2 mM MgCl₂, 1 mM GTP, 10% glycerol) to a final concentration of 4 mg/mL. Tubulin polymerization was monitored spectrophotometrically by the change in absorbance at 340 nm. The absorbance was measured at 1-min intervals for 45 min using SpectraMax

(Molecular device). The IC_{50} for tubulin polymerization was defined as the concentration of compound that inhibited tubulin assembly by 50% compared to solvent-treated control at 5 min.

***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 $55 \pm 10\%$, 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 mm³ (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 (mm³) = $1/2 \times \text{long diameter} \times \text{short diameter} \times \text{short diameter}$ (B).

IR (%) = $(1 - \text{mean tumor weight in the tested group} / \text{mean tumor weight in the control group}) \times 100$ (C).

Docking simulation

The modules in Maestro (the unified interface for all Schrödinger software) were used by default settings to search stable conformations of 4-chlorocolchicines in the colchicine binding site of tubulin. Firstly, to prepare the docking receptor, the α, β -tubulin complex merged with the DAMA-colchicine and GTPs (PDB code 1SA0, chain A and B) was processed using the Protein Preparation module in Maestro. The module assigned ionization states and hydrogens in the complex structure, and energy-minimized the hydrogens in the OPLS 2005 force field.¹ Secondly, the Receptor Grid Generation module was used to create the receptor grid where 4-chlorocolchicines were docked. The grid box and center were set to default by using the DAMA-colchicine molecule in the

β -tubulin. The van der Waals scaling of the receptor was 1.0. Thirdly, the 3D structure of each 4-chlorocolchicine derivative was generated by the Ligprep 2.4 module, which produced a single low-energy and geometrically reasonable conformer using the OPLS 2005 force field. Finally, the Glide 5.6 module was used to dock the derivative (ligand) to the colchicine binding pocket.² As a first step in the Glide SP docking mode, many new ligand conformations were generated through torsional variations of the each ligand 3D structure. For each conformer with default 0.8 van der Waals scaling, the Glide module performed an exhaustive search of possible positions and orientations over the receptor grid previously defined, and the energetically stable poses were taken through minimization in the OPLS 2005 force field. They were further evaluated and re-ranked using the Glide score, and top 25 poses for each ligand were outputted.

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