

Supporting Information

Natural Product Evodiamine with Borate Trigger Unit: Discovery of Potent Antitumor Agents against Colon Cancer

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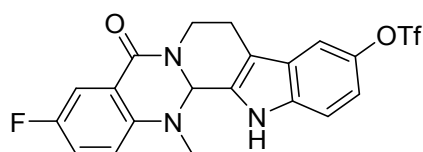
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1. General Information

All reagents and solvents are commercially available and applied without further purification. Solvent was dried using 4 Å molecular sieve. ^1H and ^{13}C NMR raw data were collected by a Bruker AMX500 (600MHz) spectrometer and spectrums were exported through MestReNova software. Deuterated solvent included $\text{DMSO-}d_6$, CDCl_3 and $\text{Acetone-}d_6$, tetramethylsilane used as internal standard. The chemical shifts (δ) are reported in parts per million (ppm) and the coupling constant (J) unit is Hz. High resolution mass spectrometry (HRMS) was obtained through an Agilent 6538 UHD Accurate-Mass ultra performance liquid chromatography quadrupole time-of-flight mass (UPLC-QTOF/MS) spectrometer. HPLC analyses were performed on an Agilent 1206, using water and methanol as mobile phase. Flash chromatography carried out on silica gel 100-200 mesh with newly configured eluent.

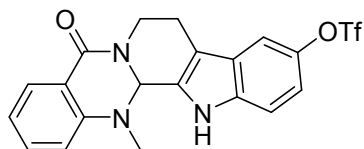
2. General experimental procedure for synthesis of borate derivatives



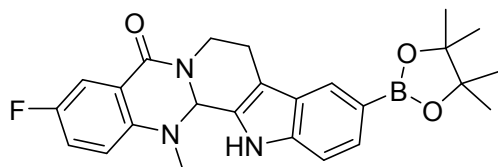
3-Fluoro-14-methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-10-yl trifluoromethanesulfonate (2a). Compound **1a** (500.0 mg, 1.52 mmol, 1.0 equiv) was dissolved in dichloromethane (DCM) (50 mL) under the protection of nitrogen at 0 °C. Then, pyridine (0.5 mL, 5.93 mmol, 4.0 equiv) was added dropwise to the solution. After stirring 10 min, trifluoromethanesulfonic anhydride (Tf_2O) (0.3 mL, 1.83 mmol, 1.2 equiv) was added slowly and further stirred for 2 h. After reaction, the solvent was removed under vacuum, and the residue was directly purified through flash column chromatography on silica gel (Petroleum ether/Ethyl acetate = 8: 1- 4: 1, v/v) to afford **2a** as a white solid (600.0 mg, yield 87%). ^1H NMR (600 MHz, $\text{DMSO-}d_6$): δ = 11.65 (s, 1H), 7.64 (d, J = 2.4 Hz, 1H), 7.54 (dd, J = 8.4, 3.0 Hz, 1H), 7.49 (d, J = 9.0 Hz, 1H), 7.39 (td, J = 8.4, 3.0 Hz, 1H), 7.20 (dd, J = 9.0, 4.8 Hz, 1H), 7.17 (dd, J = 9.0, 2.4 Hz, 1H), 6.14 (s, 1H), 4.65 - 4.58

(m, 1H), 3.22 (ddd, $J = 12.6, 10.2, 6.0$ Hz, 1H), 2.88 (q, $J = 10.2$ Hz, 2H), 2.69 (s, 3H). HRMS (ESI) calcd for $C_{20}H_{16}F_4N_3O_4S$ $[M + H]^+ = 470.0792$, found 470.0800.

The preparation of compound **2b** was similar to this procedure.

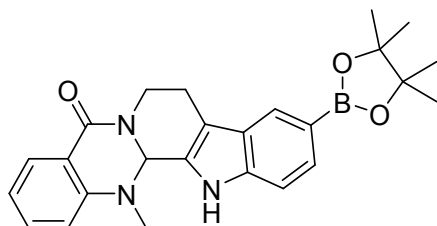


14-Methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-10-yl trifluoromethanesulfonate (2b). Pale yellow solid, yield 86%. 1H NMR (600 MHz, $DMSO-d_6$): $\delta = 11.52$ (s, 1H), 7.79 (dd, $J = 7.8, 1.2$ Hz, 1H), 7.61 (d, $J = 2.4$ Hz, 1H), 7.52 - 7.45 (m, 2H), 7.15 (dd, $J = 9.0, 2.4$ Hz, 1H), 7.06 (d, $J = 8.4$ Hz, 1H), 7.00 - 6.89 (m, 1H), 6.18 (s, 1H), 4.63 (dd, $J = 13.2, 4.8$ Hz, 1H), 3.22 (td, $J = 12.6, 4.8$ Hz, 1H), 2.96-2.87 (m, 4H), 2.82 (dd, $J = 15.6, 4.8$ Hz, 1H).

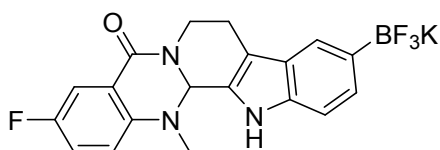


3-Fluoro-14-methyl-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-8,13,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-5(7H)-one (3a). Compound **2** (470.0 mg, 1.0 mmol, 1.0 equiv), bis(pinacolato)diboron (356.0 mg, 1.4 mmol, 1.2 equiv), $Pd(dppf)Cl_2$ (41.0 mg, 0.1 mmol, 0.1 equiv), KOAc (294.0 mg, 3.0 mmol, 3.0 equiv) were added in dry dioxane (30 mL). The resulting mixture was refluxed under nitrogen atmosphere for 8 h. After reaction, the solvent was removed under vacuum and the residue was directly purified by flash column chromatography on silica gel (Petroleum ether/Ethyl acetate = 8: 1- 4: 1, v/v) to afford **3a** as a pale yellow solid (400.0 mg, yield 85%). 1H NMR (600 MHz, $DMSO-d_6$): $\delta = 11.34$ (s, 1H), 7.89 (s, 1H), 7.54 (dd, $J = 9.0, 3.0$ Hz, 1H), 7.45 (d, $J = 7.8$ Hz, 1H), 7.39 (td, $J = 8.4, 3.0$ Hz, 1H), 7.36 (d, $J = 8.4$ Hz, 1H), 7.20 (dd, $J = 9.0, 4.8$ Hz, 1H), 6.10 (s, 1H), 4.63 (dd, $J = 13.2, 4.2$ Hz, 1H), 3.21 (td, $J = 12.6, 4.8$ Hz, 1H), 2.97-2.81 (m, 2H), 2.65 (s, 3H), 1.30 (s, 12H). ^{13}C NMR (150 MHz, $DMSO-d_6$) $\delta = 163.44, 157.71$ (d, $J_{c-f} = 237.3$

Hz), 146.53, 139.20, 134.85, 130.36, 128.39, 126.38, 126.02, 122.73 (d, $J_{c-f} = 7.05$ Hz), 122.45 (d, $J_{c-f} = 7.5$ Hz), 121.03 (d, $J_{c-f} = 22.8$ Hz), 113.86 (d, $J_{c-f} = 23.25$ Hz). 112.72, 111.62, 83.63, 69.60, 40.69, 37.15, 25.15, 19.88. HRMS (ESI) calcd for $C_{25}H_{28}BFN_3O_3$ $[M + H]^+ = 448.2202$, found 448.2210. The preparation of compound **3b** was similar to this procedure.

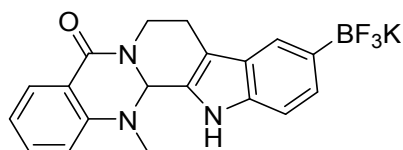


14-Methyl-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-8,13,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-5(7H)-one (3b). White solid, yield 93%. 1H NMR (600 MHz, $DMSO-d_6$): $\delta = 11.25$ (s, 1H), 7.88 (s, 1H), 7.82 (d, $J = 7.8$ Hz, 1H), 7.50 (t, $J = 7.8$ Hz, 1H), 7.46 (d, $J = 8.4$ Hz, 1H), 7.38 (d, $J = 8.4$ Hz, 1H), 7.08 (d, $J = 7.8$ Hz, 1H), 6.99 (t, $J = 7.8$ Hz, 1H), 6.16 (s, 1H), 4.66 (dd, $J = 12.6, 4.8$ Hz, 1H), 3.23 (td, $J = 12.6, 4.8$ Hz, 1H), 2.97 - 2.82 (m, 5H), 1.32 (s, 12H). ^{13}C NMR (150 MHz, $DMSO-d_6$): $\delta = 164.62, 149.27, 139.04, 133.90, 131.39, 128.46, 128.31, 126.26, 126.17, 120.89, 119.87, 118.14, 112.58, 111.62, 83.61, 70.13, 41.24, 40.55, 36.98, 25.22, 19.81$. HRMS (ESI) calcd for $C_{25}H_{29}BN_3O_3$ $[M + H]^+ = 430.2296$, found 430.2316.

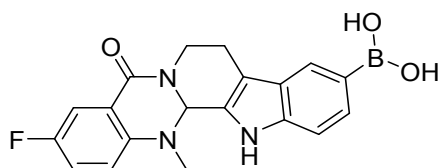


3-Fluoro-14-methyl-10-(trifluoro-boranyl)-8,13,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-5(7H)-one, potassium salt (4a). To a solution of aqueous potassium hydrogen fluoride (KHF_2) (0.2 mL, 4.5 M, 1.0 mmol, 5.7 equiv) in methanol (2 mL), compound **3** (100.0 mg, 0.2 mmol, 1.0 equiv) was added. The resulting mixture was stirred at room temperature for 0.5 h and concentrated in vacuo, then acetone (10 mL) was added and further heated to 57 °C for 0.5 h. After that, the

hot suspension was filtered immediately and the filtrate was concentrated in vacuum. The residue was recrystallized with DCM (10 mL) to afford the compound **4a** as a yellow solid (95.0 mg, yield 68%). which was used directly for the next step without further purification. The preparation of compound **4b** was similar to this procedure. ¹H NMR (600 MHz, Acetone-*d*₆) : δ = 9.05 (s, 1H), 6.93 (s, 1H), 6.87 (dd, *J* = 9.0, 3.0 Hz, 1H), 6.64 (d, *J* = 7.8 Hz, 1H), 6.52 (td, *J* = 8.4, 3.0 Hz, 1H), 6.44 (dd, *J* = 8.4, 4.8 Hz, 2H), 5.21 (s, 1H), 3.98 (ddd, *J* = 12.6, 4.2, 2.4 Hz, 1H), 2.45 (ddd, *J* = 15.6, 10.2, 6.0 Hz, 1H), 2.15 (dd, *J* = 4.8, 1.2 Hz, 1H), 2.06 (s, 4H).

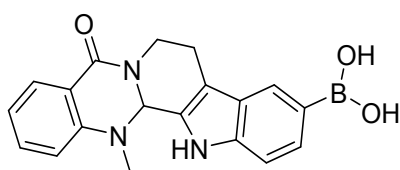


14-Methyl-10-(trifluoro-14-boranyl)-8,13,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-5(7H)-one, potassium salt (4b). Yellow solid, yield 68%. ¹H NMR (600 MHz, Acetone-*d*₆): δ = 9.78 (s, 1H), 7.94 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.67 (s, 1H), 7.46 (ddd, *J* = 8.4, 7.8, 1.8 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 7.05 - 7.00 (m, 1H), 5.98 (d, *J* = 1.4 Hz, 1H), 4.75 (ddd, *J* = 12.6, 5.4, 1.8 Hz, 1H), 3.18 (ddd, *J* = 12.6, 11.4, 4.8 Hz, 1H), 2.94 - 2.90 (m, 1H), 2.83 (s, 4H).

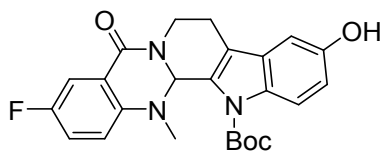


(3-Fluoro-14-methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-10-yl)boronic acid (5a). Trimethylsilyl chloride (TMS-Cl) (60 μ L, 0.44 mmol, 3.1 equiv) was slowly added to a suspension of compound **4a** (118.0 mg, 0.28 mmol, 1.0 equiv) in the mixture solvent of water (15 μ L, 0.83 mmol, 3.1 equiv) and acetonitrile (3 mL). The resulting mixture was stirred for 1 h at room temperature and diluted with ethyl acetate (10 mL). Then it was quenched with saturated sodium bicarbonate solution (0.3 mL), dried over sodium sulfate and concentrated under reduced pressure. The crude product was recrystallized with DCM (10 mL) to afford

the compound **5a** as a pale yellow solid (98.0 mg, yield 98%). ¹H NMR (600 MHz, DMSO-*d*₆): δ = 11.19 (s, 1H), 8.03 (s, 1H), 7.76 (s, 2H), 7.61 - 7.57 (m, 1H), 7.55 (dd, *J* = 8.4, 3.0 Hz, 1H), 7.39 (td, *J* = 9.0, 3.0 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.20 (dd, *J* = 9.0, 4.8 Hz, 1H), 6.09 (s, 1H), 4.71 - 4.59 (m, 1H), 3.22 (ddd, *J* = 12.6, 10.2, 6.0 Hz, 1H), 2.91 - 2.86 (m, 2H), 2.67 (s, 3H). HRMS (ESI) calcd for C₁₉H₁₈BFN₃O₃ [M + H]⁺ = 366.1420, found 366.1430. The preparation of compound **5b** was similar to this procedure.

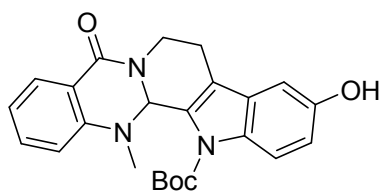


(14-Methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-10-yl)boronic acid (5b). Pale yellow solid, yield 60%. ¹H NMR (600 MHz, DMSO-*d*₆): δ = 11.06 (s, 1H), 7.99 (s, 1H), 7.79 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.76 (s, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.49 - 7.42 (m, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 6.96 (t, *J* = 7.2 Hz, 1H), 6.10 (s, 1H), 4.63 (dd, *J* = 12.0 4.8 Hz, 1H), 3.20 (td, *J* = 12.6, 4.2 Hz, 1H), 2.96 - 2.83 (m, 4H), 2.79 (dd, *J* = 15.6, 4.8 Hz, 1H). ¹³C NMR (150 MHz, DMSO-*d*₆) δ = 164.69, 149.30, 138.45, 133.89, 130.78, 128.46, 128.43, 126.10, 125.68, 120.79, 119.82, 118.02, 112.38, 111.02, 70.18, 41.30, 36.88, 19.99. HRMS (ESI) calcd for C₁₉H₁₉BN₃O₃ [M + H]⁻ = 348.1514, found 348.1529.

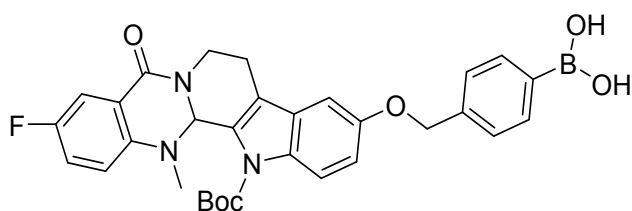


tert-Butyl 3-fluoro-10-hydroxy-14-methyl-5-oxo-7,8,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazoline-13(5H)-carboxylate (6a). Compound **1a** (500.0 mg, 1.48 mmol, 1.0 equiv), Boc₂O (970.0 mg, 4.45 mmol, 3.0 equiv) and dimethylaminopyridine (DMAP) (18.0 mg, 0.15 mmol, 0.1 equiv) were dissolved in tetrahydrofuran (THF) (20 mL). The reaction solution was stirred for 4 h at room temperature, half volume of the solvent was removed under vacuum, then K₂CO₃ (1.1

g, 7.41 mmol, 5.0 equiv) and methanol (10 mL) were added. The resulting mixture was further stirred for 4 h at room temperature. Subsequently, acetic acid (1 mL) was added to quench the reaction and directly purified through flash column chromatography on silica gel (Petroleum ether/Ethyl acetate = 8: 1- 4: 1, v/v) to afford **6a** as white solid (400.0 mg, yield 62%). ¹H NMR (600 MHz, DMSO-*d*₆): δ = 9.35 (s, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.59 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.41 (td, *J* = 8.6, 3.1 Hz, 1H), 7.26 (dd, *J* = 9.0, 4.6 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.87 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.15 (s, 1H), 4.61 (dd, *J* = 12.6, 4.2 Hz, 1H), 3.13 (td, *J* = 12.6, 3.6 Hz, 1H), 2.91 (m, *J* = 13.2 Hz, 1H), 2.80 - 2.63 (m, 1H), 2.36 (s, 3H), 1.45 (s, 9H). The preparation of compound **6b** was similar to this procedure.

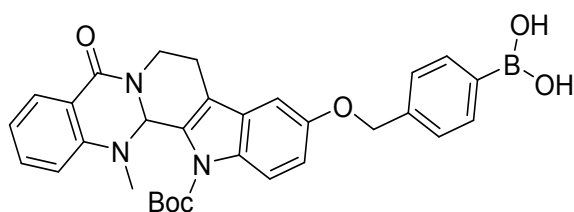


tert-Butyl 10-hydroxy-14-methyl-5-oxo-7,8,13b,14-tetrahydroindolo [2',3':3,4] pyrido[2,1-*b*]quinazoline-13(5*H*)-carboxylate (6b). White solid, yield 58%. ¹H NMR (600 MHz, DMSO-*d*₆): δ = 9.37 (s, 1H), 7.99 (d, *J* = 9.0 Hz, 1H), 7.89 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.51 (dd, *J* = 13.2, 4.8 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 7.09 (t, *J* = 7.8 Hz, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 6.87 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.14 (s, 1H), 4.60 (dd, *J* = 12.6, 4.8 Hz, 1H), 3.10 (td, *J* = 12.0, 3.6 Hz, 1H), 2.91 (d, *J* = 14.4 Hz, 1H), 2.79 - 2.65 (m, 1H), 2.44 (s, 3H), 1.44 (s, 9H). ¹³C NMR (150 MHz, DMSO-*d*₆): δ = 163.94, 154.17, 150.46, 149.46, 133.43, 130.50, 128.80, 128.66, 128.40, 121.89, 121.79, 121.63, 119.85, 116.40, 115.10, 104.21, 84.26, 68.28, 38.37, 35.10, 27.86, 20.59.



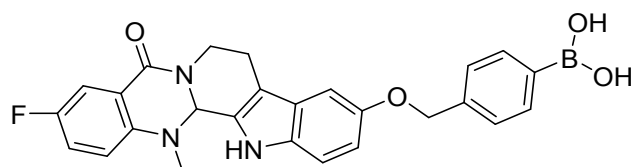
(4-(((13-(tert-Butoxycarbonyl)-3-fluoro-14-methyl-5-oxo-5,7,8,13,13b,14-hexahyd

roindolo[2',3':3,4]pyrido[2,1-b]quinazolin-10-yl)oxy)methyl)phenyl)boronic acid (7a). Compound **6a** (500.0 mg, 1.48 mmol, 1.0 equiv) was dissolved in dry dimethylformamide (DMF) (15 mL) and NaH (65.0 mg, 1.63 mmol, 1.1 equiv) was slowly added, then the mixture was stirred in an ice bath for 0.5 h. The solution of 4-bromomethylphenylboronic acid in dry DMF (3 mL) was added dropwise into the reaction and stirred at room temperature for 8 h. After the reaction, the pH was adjusted to 2 with 1 N HCl and then poured into ice-water (300 mL). The crude product was obtained after filtration and lyophilization, followed by the purification through flash column chromatography on silica gel (Petroleum ether/Ethyl acetate = 5: 1 - 1: 1, v/v) to afford **7a** as a white solid (170.0 mg, yield 79%). ¹H NMR (600 MHz, DMSO-*d*₆): δ = 8.06 (d, *J* = 9.0 Hz, 1H), 8.03 (s, 2H), 7.80 (d, *J* = 7.8 Hz, 2H), 7.58 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 2H), 7.40 (dd, *J* = 8.4, 3.0 Hz, 1H), 7.30 (d, *J* = 2.4 Hz, 1H), 7.26 (dd, *J* = 9.0, 4.8 Hz, 1H), 7.09 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.16 (s, 1H), 5.17 (s, 2H), 4.62 (dd, *J* = 12.0, 4.8 Hz, 1H), 3.17 - 3.09 (m, 1H), 2.99 (d, *J* = 13.8 Hz, 1H), 2.75 - 2.68 (m, 1H), 2.34 (s, 3H), 1.44 (s, 9H). ¹³C NMR (150 MHz, DMSO-*d*₆): δ = 162.82, 157.90 (d, *J*_{*c-f*} = 238.05 Hz), 155.37, 149.39, 147.19, 139.44, 134.69, 131.53, 128.62, 128.35, 127.00, 123.76 (d, *J*_{*c-f*} = 7.2 Hz), 123.49 (d, *J*_{*c-f*} = 7.5 Hz), 120.92 (d, *J*_{*c-f*} = 23.25 Hz), 120.84, 116.42, 115.48, 113.99 (d, *J*_{*c-f*} = 23.1 Hz), 103.69, 84.50, 70.17, 68.49, 40.55, 38.45, 35.68, 27.85, 20.56. HRMS (ESI) calcd for C₃₁H₃₂BFN₃O₆ [M + H]⁺ = 572.2363, found 572.2371. The preparation of compound **7b** was similar to this procedure.

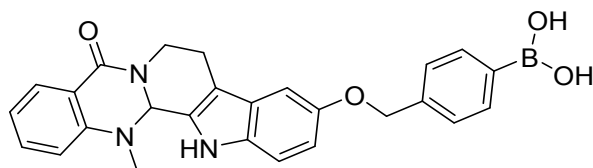


(4-(((13-(tert-Butoxycarbonyl)-14-methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-10-yl)oxy)methyl)phenyl)boronic acid (7b). White solid, yield 80%. ¹H NMR (600 MHz, DMSO-*d*₆): δ = 8.09 (d, *J* = 9.0 Hz, 1H), 8.04 (s, 2H), 7.90 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.56 - 7.48 (m,

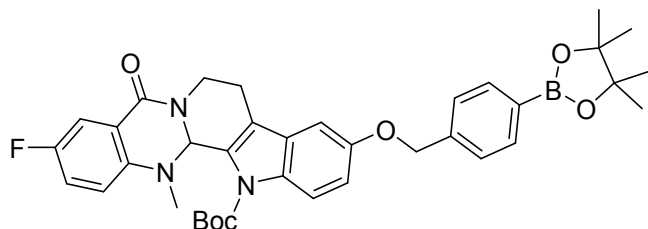
1H), 7.44 (d, $J = 7.8$ Hz, 2H), 7.32 (d, $J = 3.0$ Hz, 1H), 7.16 (d, $J = 7.8$ Hz, 1H), 7.13 - 7.06 (m, 2H), 6.17 (s, 1H), 5.19 (s, 2H), 4.63 (dd, $J = 11.4, 4.8$ Hz, 1H), 3.12 (td, $J = 12.6, 3.6$ Hz, 1H), 3.00 (d, $J = 13.8$ Hz, 1H), 2.78 - 2.68 (m, 1H), 2.44 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (150 MHz, DMSO- d_6) $\delta = 165.30, 156.75, 151.83, 150.78, 140.82, 136.07, 134.82, 132.83, 130.19, 130.14, 129.82, 128.38, 123.37, 123.24, 121.36, 117.88, 116.83, 105.06, 85.93, 71.53, 69.65, 56.74, 41.92, 39.77, 36.56, 29.22, 22.04$. HRMS (ESI) calcd for $\text{C}_{31}\text{H}_{33}\text{BN}_3\text{O}_6$ [$\text{M} + \text{H}$] $^- = 554.2457$, found 554.2469.



(4-(((3-Fluoro-14-methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-10-yl)oxy)methyl)phenyl)boronic acid (8a). Compound **7a** (500.0 mg, 1.48 mmol, 1.0 equiv) was dissolved in DCM (16 mL), followed by the addition of trifluoroacetic acid (TFA) (4 mL). The mixture was stirred at room temperature for 2 h, then purified directly through flash column chromatograph on silica gel (Petroleum ether/Ethyl acetate = 5: 1 - 1: 1, v/v) to afford **8a** as a pale yellow solid (77.0 mg, yield 63%). ^1H NMR (600 MHz, DMSO- d_6): $\delta = 10.99$ (s, 1H), 7.99 (s, 2H), 7.77 (d, $J = 7.8$ Hz, 2H), 7.51 (dd, $J = 9.0, 3.0$ Hz, 1H), 7.40 (d, $J = 8.4$ Hz, 2H), 7.35 (td, $J = 8.4, 3.1$ Hz, 1H), 7.24 (d, $J = 9.0$ Hz, 1H), 7.15 (dd, $J = 9.0, 4.2$ Hz, 1H), 7.07 (d, $J = 2.4$ Hz, 1H), 6.83 (dd, $J = 8.4, 2.4$ Hz, 1H), 6.03 (s, 1H), 5.08 (s, 2H), 4.63 - 4.54 (m, 1H), 3.17 (ddd, $J = 12.6, 10.8, 5.4$ Hz, 1H), 2.85 - 2.74 (m, 2H), 2.65 (s, 3H). ^{13}C NMR (150 MHz, DMSO- d_6): $\delta = 163.50, 157.57$ (d, $J_{c-f} = 237.3$ Hz), 152.87, 146.47, 140.01, 134.63, 132.34, 130.77, 126.90, 126.58, 122.48 (d, $J_{c-f} = 7.35$ Hz), 122.08 (d, $J_{c-f} = 7.35$ Hz), 121.01 (d, $J_{c-f} = 23.1$ Hz), 113.86 (d, $J_{c-f} = 23.25$ Hz), 113.15, 112.80, 111.88, 102.29, 70.28, 69.75, 40.84, 40.54, 37.06, 20.10. HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{22}\text{BFN}_3\text{O}_4$ [$\text{M} - \text{H}$] $^- = 470.1693$, found 470.1694. The preparation of compound **8b** was similar to this procedure.

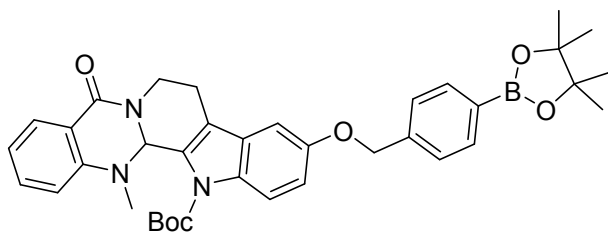


(4-(((14-Methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-10-yl)oxy)methyl)phenyl)boronic acid (8b). Pale yellow solid, yield 65%. ¹H NMR (600 MHz, DMSO-*d*₆): δ = 10.90 (s, 1H), 8.03 (s, 2H), 7.79 (d, *J* = 7.8 Hz, 2H), 7.79 (s, 1H), 7.49 - 7.45 (m, 1H), 7.42 (d, *J* = 7.8 Hz, 2H), 7.25 (d, *J* = 9.0 Hz, 1H), 7.07 (d, *J* = 2.4 Hz, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 6.95 (t, *J* = 7.2 Hz, 1H), 6.83 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.10 (s, 1H), 5.10 (s, 2H), 4.62 (dd, *J* = 12.6, 4.8 Hz, 1H), 3.23 - 3.11 (m, 1H), 2.93 - 2.83 (m, 4H), 2.74 (dd, *J* = 15.0, 4.2 Hz, 1H). ¹³C NMR (150 MHz, DMSO-*d*₆): δ = 166.05, 154.24, 150.57, 141.38, 136.00, 135.27, 133.54, 133.21, 129.83, 128.27, 128.12, 122.02, 121.02, 119.17, 114.39, 114.17, 113.13, 103.57, 71.65, 42.75, 38.25, 21.40. HRMS (ESI) calcd for C₂₆H₂₅BN₃O₄ [M + H]⁺ = 454.1933, found 454.1940.

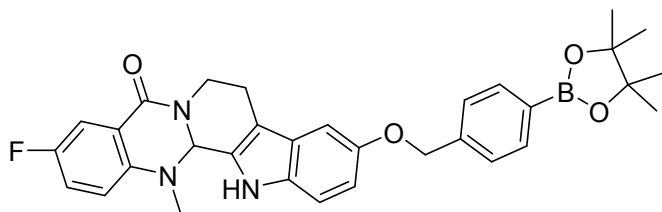


***tert*-Butyl 3-fluoro-14-methyl-5-oxo-10-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-7,8,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazoline-13(5*H*)-carboxylate (9a).** Compound **6a** (100.0 mg, 0.23 mmol, 1.0 equiv) was dissolved in dry DMF (15 mL) and NaH (10.0 mg, 0.25 mmol, 1.1 equiv) was added slowly, then the mixture was stirred in an ice bath for 0.5 h. The solution of 4-bromomethylphenylboronic acid pinacol ester (75.0 mg, 0.25 mmol, 1.1 equiv) in dry DMF (3 mL) was added dropwise into the reaction and stirred at room temperature for 8 h. Then, the reaction mixture was poured into ice water (100 mL). The crude products were acquired after filtration and purified directly through flash column chromatography on silica gel (Petroleum ether/Ethyl acetate = 10: 1, v/v) to afford **9a** as white solid (120.0 mg, 82% yield). ¹H NMR (600 MHz, DMSO-*d*₆): δ =

8.07 (d, $J = 9.0$ Hz, 1H), 7.69 (d, $J = 8.4$ Hz, 2H), 7.58 (dd, $J = 8.4, 3.0$ Hz, 1H), 7.48 (d, $J = 8.4$ Hz, 2H), 7.41 (td, $J = 8.4, 3.0$ Hz, 1H), 7.29 (d, $J = 2.4$ Hz, 1H), 7.26 (dd, $J = 8.4, 4.2$ Hz, 1H), 7.09 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.16 (s, 1H), 5.20 (s, 2H), 4.68 - 4.59 (m, 1H), 3.13 (td, $J = 12.6, 3.6$ Hz, 1H), 3.02 - 2.93 (m, 1H), 2.71 (ddd, $J = 15.6, 5.4, 3.6$ Hz, 1H), 2.34 (s, 3H), 1.44 (s, 9H), 1.28 (s, 12H). ^{13}C NMR (150 MHz, DMSO- d_6) $\delta = 162.81, 157.91$ (d, $J_{c-f} = 237.75$ Hz), 155.24, 149.38, 147.17, 141.10, 135.03, 131.57, 128.64, 128.35, 127.26, 123.76 (d, $J_{c-f} = 7.05$ Hz), 123.49 (d, $J_{c-f} = 7.2$ Hz), 120.91 (d, $J_{c-f} = 23.4$ Hz), 116.44, 115.45, 113.99 (d, $J_{c-f} = 23.1$ Hz), 103.73, 84.50, 84.13, 69.94, 68.48, 40.55, 38.44, 35.68, 27.84, 25.14, 20.55. The preparation of compound **9b** was similar to this procedure.

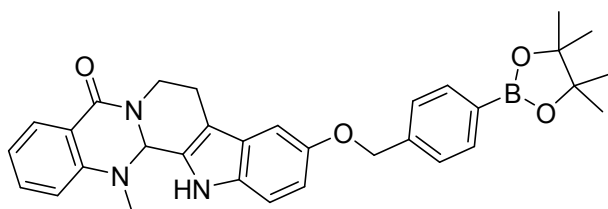


tert-Butyl 14-methyl-5-oxo-10-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-7,8,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-b]quinazoline-13(5H)-carboxylate (9b**)**. Pale yellow solid, yield 40%. ^1H NMR (600 MHz, DMSO- d_6) $\delta = 8.08$ (d, $J = 9.0$ Hz, 1H), 7.89 (dd, $J = 7.8, 1.2$ Hz, 1H), 7.70 (d, $J = 7.8$ Hz, 2H), 7.55 - 7.50 (m, 1H), 7.50 (d, $J = 7.8$ Hz, 2H), 7.31 (d, $J = 2.4$ Hz, 1H), 7.16 (d, $J = 8.4$ Hz, 1H), 7.10 (dt, $J = 9.0, 2.4$ Hz, 2H), 6.16 (s, 1H), 5.31 - 5.11 (m, 2H), 4.62 (dd, $J = 10.5, 4.8$ Hz, 1H), 3.11 (td, $J = 12.6, 3.6$ Hz, 1H), 3.00 (d, $J = 14.4$ Hz, 1H), 2.81 - 2.65 (m, 1H), 2.44 (s, 3H), 1.45 (s, 9H), 1.29 (s, 12H). ^{13}C NMR (150 MHz, DMSO- d_6) $\delta = 163.92, 155.25, 150.46, 149.40, 141.11, 135.04, 133.45, 131.50, 128.81, 128.45, 127.27, 122.00, 121.87, 120.00, 116.51, 115.44, 103.75, 84.57, 84.14, 69.94, 68.27, 40.55, 38.39, 35.19, 27.84, 25.15, 20.67$. HRMS (ESI) calcd for $\text{C}_{37}\text{H}_{43}\text{BN}_3\text{O}_6$ $[\text{M} + \text{H}]^+ = 636.3239$, found 636.3253.



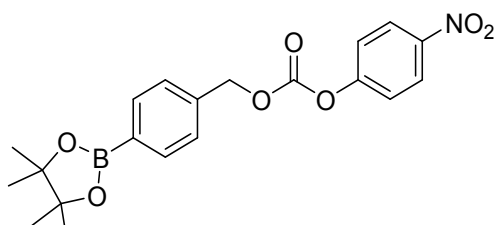
3-Fluoro-14-methyl-10-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-8,13,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-5(7H)-one

(10a). To a solution of compound **9a** (100.0mg, 0.15 mmol, 1.0 equiv) in DCM (20 mL), TFA (5 mL) was added slowly. Then the mixture was stirred at room temperature for 2 h. After reaction, the mixture was purified directly through flash column chromatograph on silica gel (Petroleum ether/Ethyl acetate = 8 : 1, v/v) to afford **10a** as a pale yellow solid (40.0 mg, yield 48%). ¹H NMR (600 MHz, DMSO-*d*₆): δ = 11.02 (s, 1H), 7.68 (d, *J* = 7.8 Hz, 2H), 7.52 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.37 (td, *J* = 8.4, 3.0 Hz, 1H), 7.25 (d, *J* = 9.0 Hz, 1H), 7.17 (dd, *J* = 9.0, 4.2 Hz, 1H), 7.08 (d, *J* = 2.4 Hz, 1H), 6.84 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.05 (s, 1H), 5.12 (s, 2H), 4.64 - 4.55 (m, 1H), 3.22 - 3.14 (m, 1H), 2.87 - 2.75 (m, 2H), 2.66 (s, 3H), 1.28 (s, 12H). ¹³C NMR (150 MHz, DMSO, TMS) δ = 163.52, 157.59 (d, *J*_{c-f} = 237.15 Hz), 152.75, 146.48, 141.69, 134.99, 132.38, 130.80, 127.17, 126.58, 122.50 (d, *J*_{c-f} = 7.05 Hz), 122.12 (d, *J*_{c-f} = 7.2 Hz), 121.03 (d, *J*_{c-f} = 22.95 Hz), 113.86 (d, *J*_{c-f} = 23.25 Hz), 113.12, 112.83, 111.89, 102.33, 84.11, 70.07, 69.74, 40.83, 37.07, 25.15, 20.10. HRMS (ESI) calcd for C₃₂H₃₄BFN₃O₄ [M + H]⁺ = 554.2621, found 554.2606. The preparation of compound **10b** was similar to this procedure.

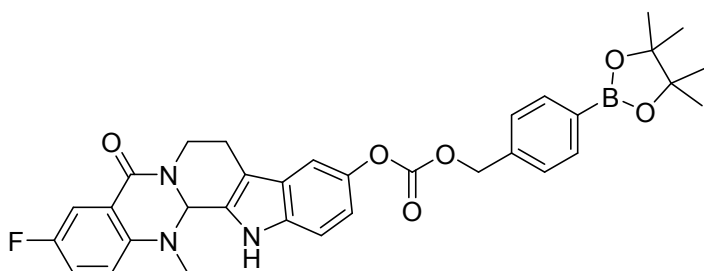


14-Methyl-10-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-8,13,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-5(7H)-one (10b). Pale yellow solid, yield 63%. ¹H NMR (600 MHz, DMSO-*d*₆) δ = 10.91 (s, 1H), 7.79 (dd,

$J = 7.8, 1.2$ Hz, 1H), 7.69 (d, $J = 7.8$ Hz, 2H), 7.47 (d, $J = 7.8$ Hz, 3H), 7.47 - 7.45 (m, 1H), 7.25 (d, $J = 9.0$ Hz, 1H), 7.06 (d, $J = 2.4$ Hz, 1H), 7.04 (d, $J = 7.8$ Hz, 1H), 6.95 (t, $J = 7.2$ Hz, 1H), 6.83 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.09 (s, 1H), 5.13 (s, 2H), 4.62 (dd, $J = 12.6, 4.8$ Hz, 1H), 3.18 (td, $J = 12.0, 3.6$ Hz, 1H), 2.91 - 2.80 (m, 4H), 2.73 (dd, $J = 15.0, 4.2$ Hz, 1H), 1.29 (s, 12H). ^{13}C NMR (150 MHz, DMSO- d_6) $\delta = 164.67, 152.74, 149.21, 141.68, 134.97, 133.90, 132.21, 131.86, 128.45, 127.17, 126.74, 120.67, 119.66, 117.82, 112.97, 112.81, 111.76, 102.26, 84.10, 70.26, 70.08, 41.36, 36.87, 31.62, 30.30, 25.14, 20.02$. HRMS (ESI) calcd for $\text{C}_{32}\text{H}_{35}\text{BN}_3\text{O}_4$ $[\text{M} + \text{H}]^+ = 536.2715$, found 536.2734.

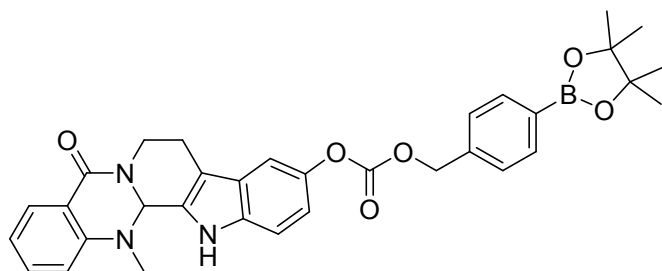


4-Nitrophenyl (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl) carbonate (12). Compound **11** (585.0 mg, 5.01 mmol, 1.0 equiv) and 4-nitrophenyl chloroformate (560.0 mg, 5.53 mmol, 1.1 equiv) were dissolved in dry THF (10 mL), followed by the addition of triethylamine (TEA) (0.7 mL, 10.02 mmol, 2 equiv) with dropwise. The mixture was stirred at room temperature for 6 h and then purified through flash column chromatograph on silica gel (Petroleum ether/Ethyl acetate = 10:1, v/v) to afford **12** as white solid (490.0 mg, yield 58%). ^1H NMR (600 MHz, CDCl_3): $\delta = 8.27 - 8.23$ (m, 2H), 7.84 (d, $J = 7.8$ Hz, 2H), 7.43 (d, $J = 7.8$ Hz, 2H), 7.38 - 7.34 (m, 2H), 5.30 (s, 2H), 1.34 (s, 12H).



3-Fluoro-14-methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-10-yl (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)

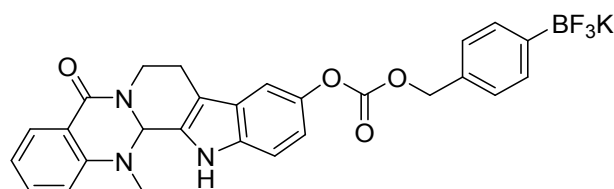
carbonate (13a). To a stirred solution of compound **1a** (310.0 mg, 9.23 mmol, 1.2 equiv) and compound **12** (300.0 mg, 7.69 mmol, 1.0 equiv) in THF (15 mL), K₂CO₃ (290.0 mg, 18.50 mmol, 2.4 equiv) was added. The mixture was refluxed at 70 °C for 10 h. Then, the solvent was evaporated and the residue was purified directly through flash column chromatograph on silica gel (Petroleum ether/Ethyl acetate = 8: 1-4: 1, v/v) to give **13a** as a white solid (280.0 mg, yield 57%). ¹H NMR (600 MHz, DMSO-*d*₆): δ = 11.34 (s, 1H), 7.71 (d, *J* = 7.8 Hz, 2H), 7.53 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.40 - 7.35 (m, 2H), 7.35 (d, *J* = 1.8 Hz, 1H), 7.19 (dd, *J* = 9.0, 4.8 Hz, 1H), 6.97 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.10 (s, 1H), 5.27 (s, 2H), 4.68 - 4.53 (m, 1H), 3.27 - 3.13 (m, 1H), 2.86 - 2.80 (m, 2H), 2.67 (s, 3H), 1.29 (s, 12H). ¹³C NMR (150 MHz, DMSO-*d*₆): δ = 163.47, 157.66 (d, *J*_{*c-f*} = 237.3 Hz), 154.30, 146.45, 144.67, 139.01, 135.11, 134.99, 131.92, 127.82, 126.26, 122.57 (d, *J*_{*c-f*} = 7.2 Hz), 122.27 (d, *J*_{*c-f*} = 7.5 Hz), 121.06 (d, *J*_{*c-f*} = 22.95 Hz), 116.21, 113.87 (d, *J*_{*c-f*} = 23.4 Hz), 112.57, 112.48, 110.73, 84.22, 69.75, 69.64, 40.75, 37.19, 25.14, 19.96. HRMS (ESI) calcd for C₃₃H₃₄BFN₃O₆ [M + H]⁺ = 598.2519, found 598.2546. The preparation of compound **13b** was similar to this procedure.



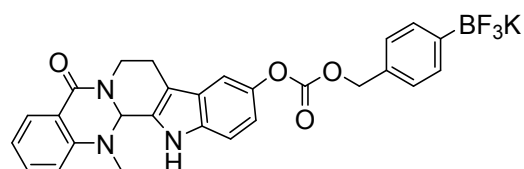
14-Methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-10-yl (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl) carbonate (13b).

White solid, yield 83%. ¹H NMR (600 MHz, DMSO-*d*₆): δ = 11.22 (s, 1H), 7.79 (d, *J* = 7.2 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 2H), 7.36 (d, *J* = 9.0 Hz, 1H), 7.32 (d, *J* = 1.8 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.947 - 6.978 (m, 2H), 6.14 (s, 1H), 5.28 (s, 2H), 4.62 (dd, *J* = 12.6, 5.4 Hz, 1H), 3.20 (td, *J* = 12.6, 4.8 Hz, 1H), 2.92 - 2.84 (m, 4H), 2.76 (dd, *J* = 15.0, 4.2 Hz, 1H), 1.30 (s, 12H). ¹³C NMR (150 MHz, DMSO-*d*₆): δ = 166.02, 155.65, 150.55, 146.03, 140.39,

136.48, 136.17, 135.32, 134.35, 129.84, 129.19, 127.79, 122.19, 121.08, 119.35, 117.46, 113.94, 113.72, 111.98, 85.60, 71.53, 71.10, 42.66, 41.93, 38.39, 26.52, 21.25. HRMS (ESI) calcd for C₃₃H₃₅BN₃O₆ [M + H]⁺ = 580.2613, found 580.2635.

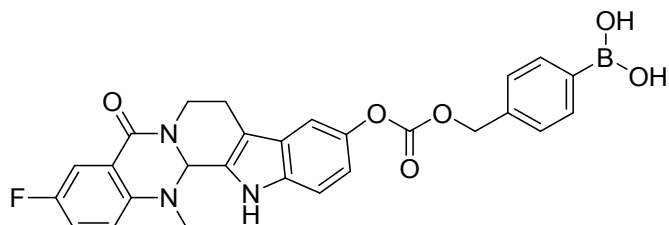


3-Fluoro-14-methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo [2',3':3,4]pyrido [2,1-b]quinazolin-10-yl (4-(trifluoro- λ^4 -boranyl)benzyl) carbonate, potassium salt (14a). Aqueous KHF₂ (210 μ L, 4.5 M, 0.95 mmol, 5.7 equiv) was added to a suspension of compound **13a** (100.0 mg, 0.17 mmol, 1.0 equiv) in methanol (2 mL). The resulting mixture was stirred at room temperature for 0.5 h, then solvent was removed under vacuum, acetone (10 mL) was added and heated to 57 °C for 0.5 h. The hot suspension was filtered immediately, the filtrate was concentrated under vacuum. Compound **14a** was obtained through recrystallization from DCM (10 mL) as a yellow solid (140.0 mg, yield 74%). ¹H NMR (600 MHz, Acetone-*d*₆): δ = 10.49 (s, 1H), 7.63 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.38 (d, *J* = 2.4 Hz, 1H), 7.30 (td, *J* = 8.6, 3.1 Hz, 1H), 7.25 - 7.21 (m, 3H), 7.01 (dd, *J* = 8.4, 2.4 Hz, 2H), 6.06 (s, 1H), 5.21 (s, 2H), 4.77 (dt, *J* = 7.2, 4.2 Hz, 1H), 3.26 (dt, *J* = 12.6, 7.8 Hz, 1H), 2.96 - 2.91 (m, 2H), 2.65 (s, 3H). The preparation of compound **14b** was similar to this procedure.

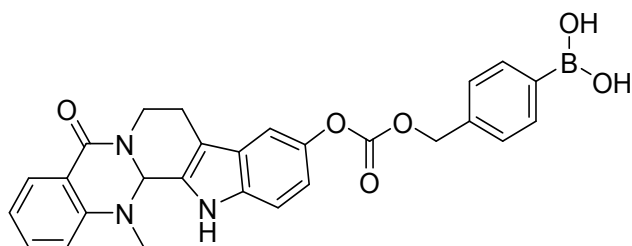


14-Methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-10-yl (4-(trifluoro- λ^4 -boranyl)benzyl) carbonate, potassium salt (14b). Yellow solid, yield 83%. ¹H NMR (600 MHz, Acetone-*d*₆): δ = 10.48 (s, 1H), 7.95 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.56 (d, *J* = 7.8 Hz, 2H), 7.51 - 7.47 (m, 1H), 7.43 (d, *J* = 8.4 Hz,

1H), 7.37 (d, $J = 1.8$ Hz, 1H), 7.24 (d, $J = 7.8$ Hz, 2H), 7.10 (d, $J = 8.4$ Hz, 1H), 7.07 - 7.03 (m, 1H), 6.99 (dd, $J = 8.7, 2.3$ Hz, 1H), 6.08 (s, 1H), 5.21 (s, 2H), 4.79 (ddd, $J = 12.6, 5.4, 1.2$ Hz, 1H), 3.25 (ddd, $J = 12.6, 10.2, 4.2$ Hz, 1H), 3.01 - 2.98 (m, 1H), 2.89 (dd, $J = 15.6, 4.2$ Hz, 1H), 2.84 (s, 3H).

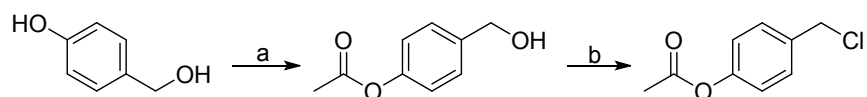


(4-((((3-Fluoro-14-methyl-5-oxo-4a,5,7,8,13,13b,14,14a-octahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-10yl)oxy)carbonyl)oxy)methyl)phenyl)boronic acid (15a). TMS-Cl (60 μ L, 0.44 mmol, 3.1 equiv) was slowly added to a suspension of compound **14** (118.0 mg, 0.28 mmol 1.0 equiv) in the mixture solvent of water (15 μ L, 0.83 mmol, 3.1 equiv) and acetonitrile (3 mL). Then, the resulting mixture was stirred at room temperature for 1 h. Then it was quenched with saturated sodium bicarbonate solution (0.3 mL) and dried over with sodium sulfate. After filtration, the filtrate was concentrated under vacuum and the residue was recrystallized from DCM (10 mL) to afford the compound **15a** as a white solid (76.0 mg, yield 63%). ^1H NMR (600 MHz, DMSO- d_6): $\delta = 11.31$ (s, 1H), 8.05 (s, 2H), 7.80 (d, $J = 8.1$ Hz, 2H), 7.51 (dd, $J = 8.9, 3.1$ Hz, 1H), 7.38 (d, $J = 8.1$ Hz, 2H), 7.35 (dd, $J = 8.7, 3.1$ Hz, 2H), 7.33 (d, $J = 2.3$ Hz, 1H), 7.17 (dd, $J = 8.9, 4.5$ Hz, 1H), 6.95 (dd, $J = 8.7, 2.4$ Hz, 1H), 6.08 (s, 1H), 5.24 (s, 2H), 4.65 - 4.54 (m, 1H), 3.22 - 3.14 (m, 2H), 2.88 - 2.76 (m, 1H), 2.66 (s, 3H). ^{13}C NMR (150 MHz, DMSO- d_6): $\delta = 163.49, 157.65$ (d, $J_{c-f} = 237.3$ Hz), 154.35, 146.45, 144.68, 137.39, 134.97, 134.77, 131.91, 127.54, 126.27, 122.54 (d, $J_{c-f} = 6.9$ Hz), 122.27 (d, $J_{c-f} = 7.5$ Hz), 121.07 (d, $J_{c-f} = 23.25$ Hz), 116.24, 113.87 (d, $J_{c-f} = 23.4$ Hz), 112.57, 112.48, 110.75, 70.00, 69.64, 40.76, 40.54, 37.19, 19.96. HRMS (ESI) calcd for $\text{C}_{27}\text{H}_{22}\text{BFN}_3\text{O}_6$ [$\text{M} - \text{H}$] $^- = 514.1591$, found 514.1586. The preparation of compound **15b** was similar to this procedure.

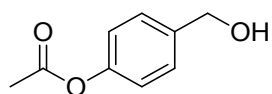


4-((((14-Methyl-5-oxo-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-10-yl)oxy)carbonyloxy)methyl)phenyl)boronic acid (15b). White solid, yield 68%. ^1H NMR (600 MHz, $\text{DMSO-}d_6$): δ = 11.20 (s, 1H), 8.07 (s, 2H), 7.82 (d, J = 7.7 Hz, 1H), 7.78 (d, J = 7.2 Hz, 1H), 7.49 - 7.27 (m, 6H), 7.03 (d, J = 8.0 Hz, 1H), 6.94 (d, J = 7.6 Hz, 2H), 6.11 (s, 1H), 5.24 (s, 2H), 4.61 (dd, J = 12.5, 5.1 Hz, 1H), 3.19 (dd, J = 12.0, 4.5 Hz, 1H), 2.86 (s, 4H), 2.74 (d, J = 12.5 Hz, 1H). ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ = 164.67, 154.34, 149.19, 144.68, 137.40, 134.79, 134.61, 133.94, 132.97, 128.48, 127.55, 126.43, 120.82, 119.71, 117.98, 116.12, 112.58, 112.36, 110.62, 70.17, 70.00, 41.30, 37.01, 19.89. HRMS (ESI) calcd for $\text{C}_{27}\text{H}_{25}\text{BN}_3\text{O}_6$ [$\text{M} + \text{H}$] $^+$ = 498.1831, found 498.1853.

3. The synthesis of 4-(chloromethyl)phenyl acetate

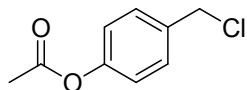


^aReagent and Conditions: (a) Acetyl chloride, TEA, 0 °C, 2 h, yield 70%; (b) Dichlorosulfoxide, DMF, rt, 2 h, yield 43%.



4-(Hydroxymethyl)phenyl acetate. 4-(Hydroxymethyl)phenol (1.0g, 8.06 mmol 1.0 equiv) and TEA (0.69g, 8.87 mmol 1.1 equiv, 630 μL) were dissolved in DCM (25 mL). Under ice bath, acetyl chloride (0.89g, 8.87 mmol, 1.1 equiv, 1.2 mL) was slowly added to above resulting mixture. Then, the solvent was evaporated and the residue was purified directly through flash column chromatograph on silica gel (Petroleum ether/Ethyl acetate = 5: 1, v/v) to give 4-(hydroxymethyl)phenyl acetate as a white solid (914.0 mg, yield 70%). ^1H NMR (300 MHz, DMSO) : δ = 7.39 - 7.33

(m, 2H), 7.11 - 7.04 (m, 2H), 5.26 (t, $J = 5.76$ Hz, 1H), 4.52 (d, $J = 5.76$ Hz, 2H), 2.26 (s, 3H).



4-(Chloromethyl)phenyl acetate.

4-(Hydroxymethyl)phenyl acetate (457.0 mg, 2.75 mmol 1.0 equiv) was dissolved in dichlorosulfoxide (5 mL). Subsequently, DMF (20.1 mg, 0.275 mmol, 0.1 equiv, 21 μ L) was dropped slowly to the mixture. After stirred for 2 h at room temperature, the solvent was evaporated and the residue was purified directly through flash column chromatograph on silica gel (Petroleum ether/Ethyl acetate = 15: 1, v/v) to give 4-(chloromethyl)phenyl acetate as a white solid (220.0 mg, yield 43%). ^1H NMR (600 MHz, DMSO) : $\delta = 7.54 - 7.46$ (m, 2H), 7.21 - 7.14 (m, 2H), 4.79 (s, 2H), 2.29 (s, 3H). ^{13}C NMR (150 MHz, DMSO) : $\delta = 169.60, 150.81, 135.67, 130.54, 122.52, 45.98, 21.28$. HRMS (ESI) calcd for $\text{C}_9\text{H}_9\text{ClNaO}_2$ $[\text{M} + \text{Na}]^+ = 207.0183$, found 207.0187.

4. *In vitro* cytotoxicity assay

Antiproliferative activity of compounds were tested against three cell lines (A549, HCT116 and MCF-7). Cells were cultured with mixed medium, which included DMEM medium (Hyclone) or McCoy's 5A medium (Gibco), fetal calf serum (Gibco) and Penicillin-Streptomycin Solution. HCT116 cells were cultured with McCoy's 5A medium, A549 cells and MCF-7 cells were cultured with DMEM (Hyclone). The cells were cultured at 37 °C in a humidified incubator with 5% CO_2 . The three cell lines were purchased from the Chinese Academy of Sciences cell bank.

The cells in logarithmic phase were seeded in 96-well plates at a density of 7×10^3 cells per well and incubated in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h. Compounds at different concentrations were prepared through triple dilutions and then were added into triplicate wells with a volume of 100 μ L per well. After cells were incubated with compounds for 72 h, CCK8 Kit diluted to 10 times with DMEM

medium was added to each well at a volume of 100 μ L per well. Then the plates were incubated for additional 0.5 h. The absorbance (OD) was read on a WellscanMK-2 microplate reader (Labsystems) at 450 nm. The concentration causing 50% inhibition of cell growth (IC_{50}) was calculated by the Logit method using Graphd Prism. All assays were repeated three times.

Table S1 *In vitro* antitumor activity of 4-(chloromethyl)phenyl acetate and 1a (IC_{50} , μ M)

Compound	HCT116	MCF-7	A549
4-(chloromethyl)phenyl acetate	>30	>30	18
1a	0.010	0.066	0.012

5. Apoptosis detection and cell-cycle arrest assay

HCT116 cells (4×10^5 cells/well) were seeded in six-well plates. After 24 h, cells were treated with compounds at different concentrations (0.4 μ M, 0.2 μ M, 0.05 μ M) for 48 h. Cells were collected by trypsinization and washed once with cold phosphatic buffer solution (PBS) (1.5 mL). After centrifugation and removal of the supernatants, cells were resuspended in cold PBS (300 μ L), then Binding Buffer (30 μ L), AnnexinV-FITC (2 μ L) and Propidium Iodide (2 μ L) (Becton, Dickinson and Company, catalog numbers: 556547) were added into each tube and incubated at room temperature for 15 min in the dark. The stained cells were tested through a flow cytometer (BD Accuri C6). The test results were analyzed by FLOW JO software.

HCT116 cells (4×10^5 cells/well) were seeded in six-well plates. After 24 h, cells were treated with compounds at different concentrations (0.4 μ M, 0.2 μ M, 0.05 μ M) for 24 h. Cells were collected by trypsinization, washed with cold PBS once and resuspended in DNA staining solution (300 μ L) and permeabilization solution (3 μ L) [MultiSciences (Lianke) Biotech Co. Ltd., catalog numbers: CCS012], then incubated for 15 min at room temperature in the dark. The stained cells were tested through a flow cytometer (BD Accuri C6). For each sample, about 1×10^4 cells were tested. The test results analyzed by Mod Fid software.

6. Top1/ Top2 activity inhibitory activity assay

Assay buffer (2 μL per sample), bull serum albumin (BSA) (2 μL per sample) and water (15 μL per sample) were mixed and took out mixture (19 μL) into first tube. A calculated amount of Top1 enzyme (0.025 μL , 20 U/ μL , per sample) (TaKaRa Biotechnology Co., Ltd., Dalian) was added to the remaining mixture and gently vortexed then the mixture was dispensed into a suitable number of tubes 19 μL per tube. The first tube and second tube were added of DMSO (0.5 μL), the third tube was added of CPT (0.5 μL), the remaining tubes were added of the test compounds (0.5 μL). After vortex, supercoiled pBR322 (0.5 μL , 0.5 $\mu\text{g}/\mu\text{L}$) were added into each tube. Assay mixtures were incubated for 15 min at 37 °C and stopped by addition of 10 \times loading buffer (0.9% sodium dodecyl sulfate (SDS), 0.05% bromophenol blue, and 50% glycerol) (2.2 μL). Electrophoresis was carried out in a agarose gel (contain 1% agarose and 99% TAE) in TAE buffer (Tris-acetate-EDTA) at 85 V for 1.5 h. Gels were stained with propidium iodide (YeaRed Nucleic Acid Gel Stain 10,000 \times in Water) for 1 h and the DNA band was visualized under UV light and photographed with Gel Doc Ez imager (Bio-Rad Laboratories Ltd.).

Assay buffer (3 μL per sample), adenosine triphosphate (ATP) (2 μL per sample) water (23.5 μL per sample) and pBR322 DNA (0.4 μL , 0.5 $\mu\text{g}/\mu\text{L}$, per sample) were mixed, the mixture was added to appropriate number of tubes 27.9 μL per tube. The first one and second one were added of DMSO (0.6 μL), the third tube was added of etoposide (0.6 μL), the remaining tubes were added of the test compounds (0.6 μL). After vortex, calculated amount of Top2 enzyme (0.02 μL , 10 U/ μL , per sample) were added to all tubes except the first one. Assay mixtures were incubated for 30 min at 37 °C and stopped by addition of 10 \times loading buffer (0.9% sodium dodecyl sulfate (SDS), 0.05% bromophenol blue, and 50% glycerol) (3.3 μL). Electrophoresis was carried out in a agarose gel (contain 1% agarose and 99% TAE) in TAE buffer (Tris-acetate-EDTA) at 85 V for 1.5 h. Gels were stained with propidium iodide (YeaRed Nucleic Acid Gel Stain 10,000 \times in Water) for 1 h and the DNA band was visualized under UV light and photographed with Gel Doc Ez imager (Bio-Rad Laboratories Ltd.).

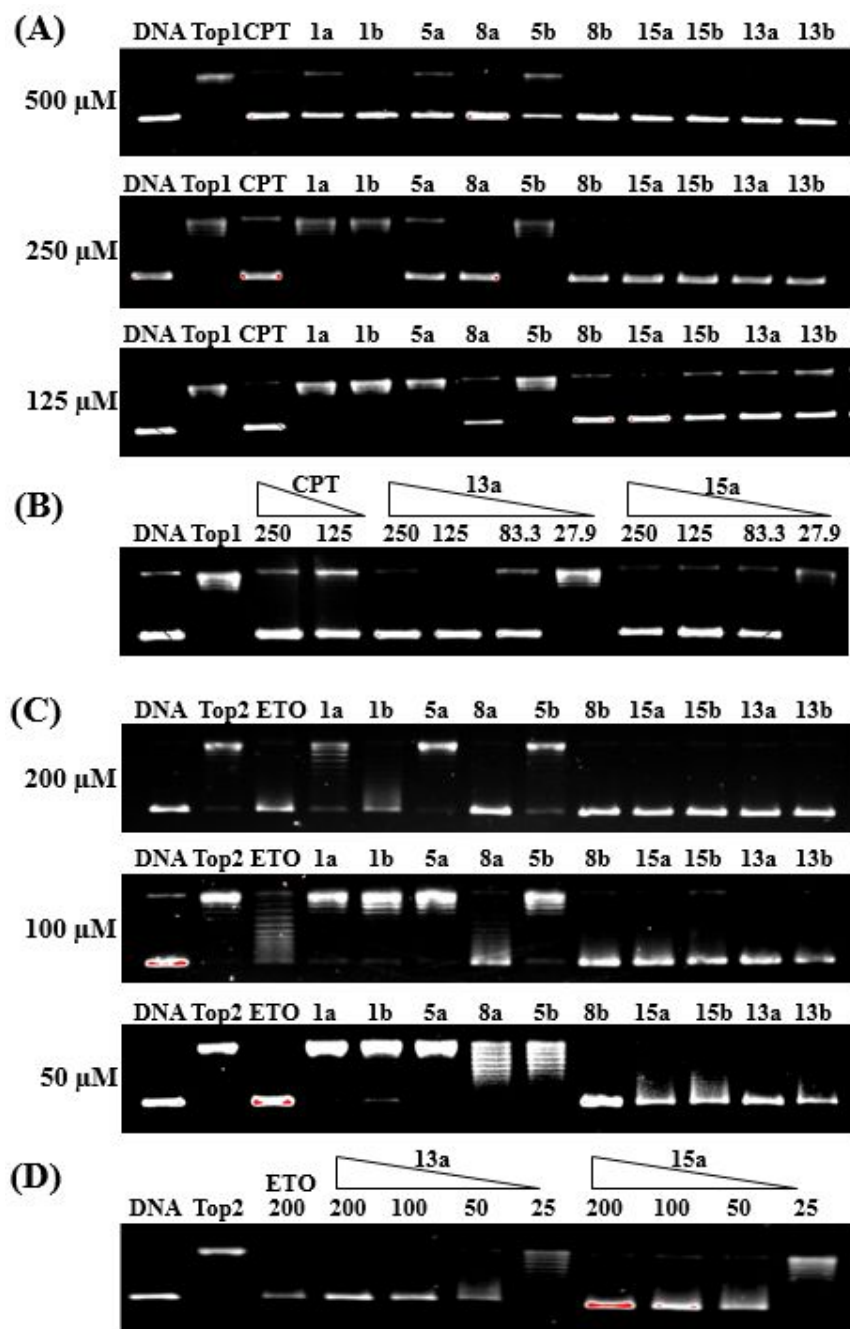


Figure 3. (A) Top1 inhibitory activity assay of evodiamine borate derivatives. Inhibition of Top1 relaxation activity at 500 μM, 250 μM, 125 μM respectively. Lane 1, pBR322 DNA; Lane 2, pBR322 DNA + Top1; Lane 3, pBR322 DNA + Top1 + CPT; Lane 4, pBR322 DNA + Top1 + **1a**; Lane 5, pBR322 DNA + Top1 + **1b**; Lane 6-13, pBR322 DNA + Top1 + derivatives (**5a**, **8a**, **5b**, **8b**, **15a**, **15b**, **13a**, **13b**). (B) Top1 inhibitory activity assay of compounds **13a** and **15a** ranging from 27.9 to 250 μM: Lane 1, pBR322 DNA; Lane 2, pBR322 DNA + Top1; Lane 3-4, pBR322 DNA + Top1 + CPT (250, 125 μM); Lane 5-11, pBR322 DNA + Top1 + **13a** (250,

125, 83.3 and 27.9 μM); Lane 12-18, pBR322 DNA + Top1 + **15a** (250, 125, 83.3 and 27.9 μM). (C) Top2 inhibitory activity assay of derivatives: Inhibition of Top2 relaxation activity at 200 μM , 100 μM , 50 μM respectively. Lane 1, pBR322 DNA; Lane 2, pBR322 DNA + Top2; Lane 3, pBR322 DNA + Top2 + **ETO**; Lane 4, pBR322 DNA + Top2 + **1a**; Lane 5, pBR322 DNA + Top2 + **1b**; Lane 6-13, pBR322 DNA + Top2 + derivatives (**5a**, **8a**, **5b**, **8b**, **15a**, **15b**, **13a**, **13b**). (D) Top2 inhibitory activity assay of compounds **13a** and **15a** ranging from 25 to 200 μM : Inhibition of Top2 relaxation activity ranging from 25 μM to 200 μM : Lane 1, pBR322 DNA; Lane 2, pBR322 DNA + Top2; Lane 3, pBR322 DNA + Top2 + **ETO**; Lane 4-9, pBR322 DNA + Top2 + **13a** (200, 100, 50, and 25 μM); Lane 10-15, pBR322 DNA + Top2 + **15a** (200, 100, 50 and 25 μM).

7. The binding modes of selected compounds with Top1 and Top2

Selected compounds were docked into the active site of Top1 (PDB code: 1T8I) and Top2 α (PDB code: 5GWK) by a procedure similar to that described previously. Molecular docking was achieved with Maestro 10.2 and the figures were generated using Pymol (<http://www.pymol.org/>).

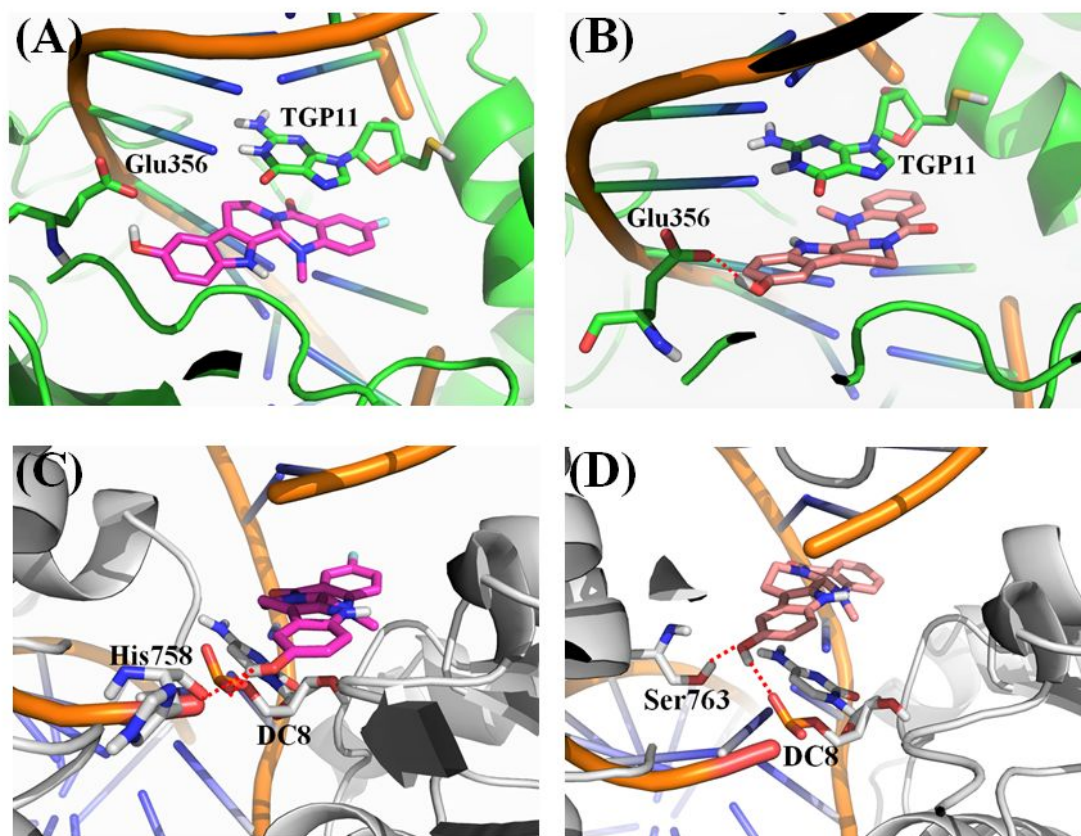


Figure S1. The proposed binding modes for compounds **1a** (A, C) and **1b** (B, D) with Top1-DNA complex (PDB code: 1T8I) and ATP-binding domain of Top2 α (PDB code: 5GWK). The figures were generated using Pymol (<http://www.pymol.org/>).

8. Determination of *in vitro* metabolic stability in mice liver microsomes

The metabolic stability was tested according to the literature.¹ Liver microsomal incubations were conducted in triplicate. Incubation mixtures (0.75 mg/mL microsome protein, pH 7.4 of 100 mM potassium phosphate buffer, 1 μ M compounds in acetonitrile) were first shaken for pre-incubation at 37 °C. The reaction was initiated by adding NADPH (6 mM, 5 mg/mL) to obtain concentration of 1 mM NADPH in the mixture. For metabolic stability studies, aliquots of the incubation sample mixture were collected at 0, 5, 15, 30 and 45 min. After collection of samples, the reaction was terminated with acetonitrile containing the internal standard (200 ng/mL Tolbutamide). The mixture was then centrifuged to remove the protein and the supernatant was subsequently applied to LC-MS/MS analysis. Positive control samples were prepared as described above, except the test compound was replaced with the known substrate ketanserin.

Table S2 The mice microsomal stability of compounds 13a, 15a and 1a.

Compound	$t_{1/2}$ (minute)	Cl_{int} (mL/min/kg)
ketanserin	23.79	229.42
13a	8.36	653.16
15a	0.74	7395.17
1a	3.69	1479.84

9. Tubulin polymerization inhibitory activity assay

The tubulin polymerization assay was performed according to the literature.² A purified brain tubulin polymerization kit was purchased from Cytoskeleton (BK006P, Denver, CO). The final assay buffer, which contained 0.1 M MES (pH 6.6), 10 mM MgCl₂, 1 mM GTP, 1 mM EGTA, and 3.4 M glycerol, was cooled to 0 °C. Compounds was added at different concentrations, and the reaction was initiated by the addition of tubulin to a final concentration of 10 mM with warming to 37 °C. Combretastatin A4 (CA4) was used as a positive control under similar experimental conditions. The optical density at 340 nm was measured at 1 min intervals for 15~30 min with a BioTek's Synergy 4 multifunction microplate reader, and the values was used to calculate the IC₅₀ values.

Table S3 α -Tubulin inhibitory activity

Compound	α -tubulin IC ₅₀ (μ M)
13a	> 100
13b	> 100
15a	25.7 \pm 2.2
15b	> 100
CA4	1.0 \pm 0.2

10. cLogP prediction

The cLogP of compounds **13**, **15** and **1** were predicted by website:

<http://www.scfbioitd.res.in/software/drugdesign/lipinski.jsp>

Table S4 The cLogP value of evodiamine analogues

Compound	cLogP
13a	5.93
13b	5.22
15a	2.59
15b	0.84
1a	3.16
1b	2.58

11. Relative drug release by hydrogen peroxide

Test compounds were prepared in DMSO at concentration of 10 mM. Compounds (10 μ L), PBS and hydrogen peroxide (50 mM or 10 mM) were mixed to give a final volume of 100 μ L. The mixture was incubated at 37 $^{\circ}$ C for 8 h and then quenched with equal sodium thiosulfate solution (50 mM or 10 mM). After filtration with 0.22 μ m filter membrane, the solution was directly analyzed by HPLC (Eclipse Plus C18, 5 μ m, 4.62.1 \times 250 mm).

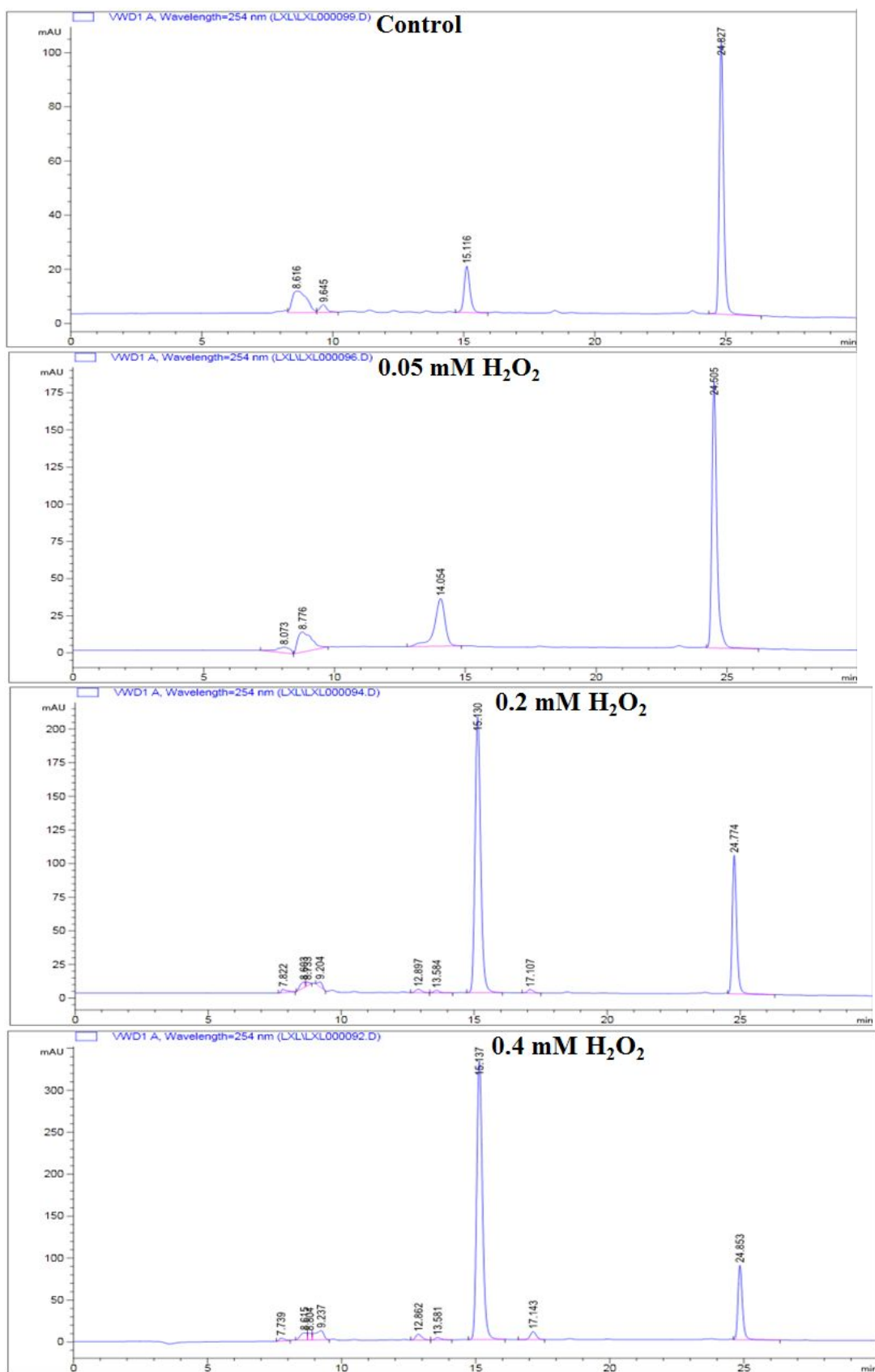


Figure S2. Relative drug release of compound **15a** determined by HPLC

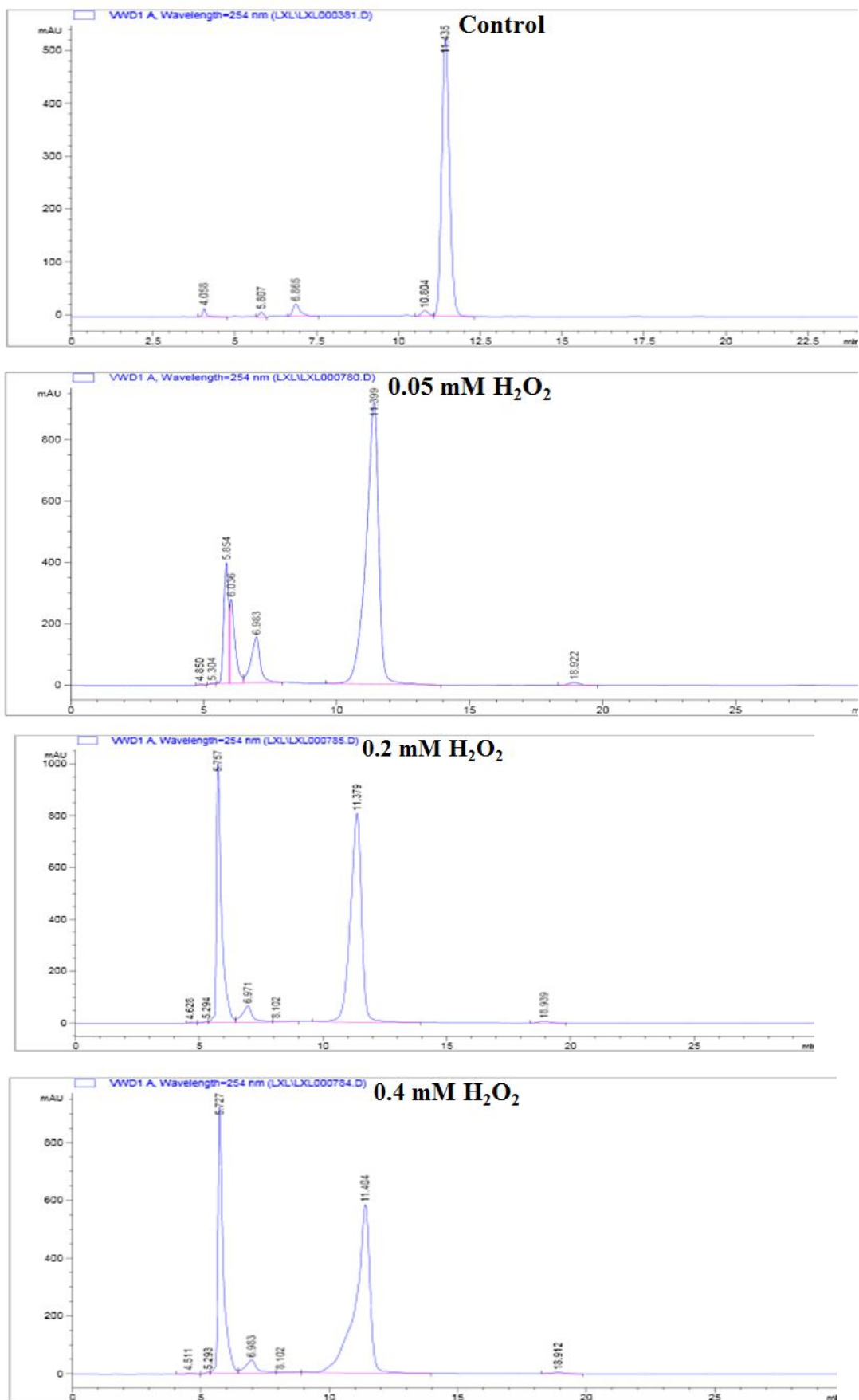


Figure S3. Relative drug release of compound **13a** determined by HPLC

Table S5. Relative drug release rate by H₂O₂ and elution conditions

Compound	H ₂ O ₂ (%)	H ₂ O ₂ (%)	H ₂ O ₂ (%)	Elution conditions
	0.05 mM	0.2 mM	0.4 mM	
5a	10.1	19.7	25.4	methanol/water = 70: 30, 0.25 mL/min
5b	6.3	23.7	52.1	methanol/water = 70: 30, 0.25 mL/min
13a	11.6	35.6	36.4	methanol/water = 90: 10, 0.5 mL/min
15a	20.8	72.0	82.8	methanol/water = 70: 30, 0.3 mL/min
15b	18.6	57.8	83.2	methanol/water = 70: 30, 0.3 mL/min

12. Relative drug release by HCT116 cells

HCT116 cells (1×10^6 cells/well) were seeded in six-well plates. After 24 h, cells were treated with compounds at 0.2 mM for 8 h. Culture medium was collected and extracted with ethyl acetate (1 mL). After centrifugation, the supernatant (100 μ L) was diluted with methanol (100 μ L). The mixture was filtrated with 0.22 μ m filter membrane, and then directly analyzed on an Agilent UPLC-QTOF/MS system. Chromatographic separations were achieved by using a Waters XBridge® BEH C18 2.5 μ m column (2.1 \times 100 mm). Elution conditions were at flow rate 0.4 mL/min with gradient elution (water with 0.1% formic acid)/(acetonitrile with 0.1% formic acid) = 95: 5 for 13 min, (water with 0.1% formic acid)/(acetonitrile with 0.1% formic acid) = 5: 95 from 13 min to 15 min).

Total Ion Chromatography (TIC) was measured by chromatography-mass spectrometry to test the total ions of various mass-to-charge ratios and changes over time. Diode array detector (DAD) was used to detect the UV absorption chromatography of compounds under 254 nm.

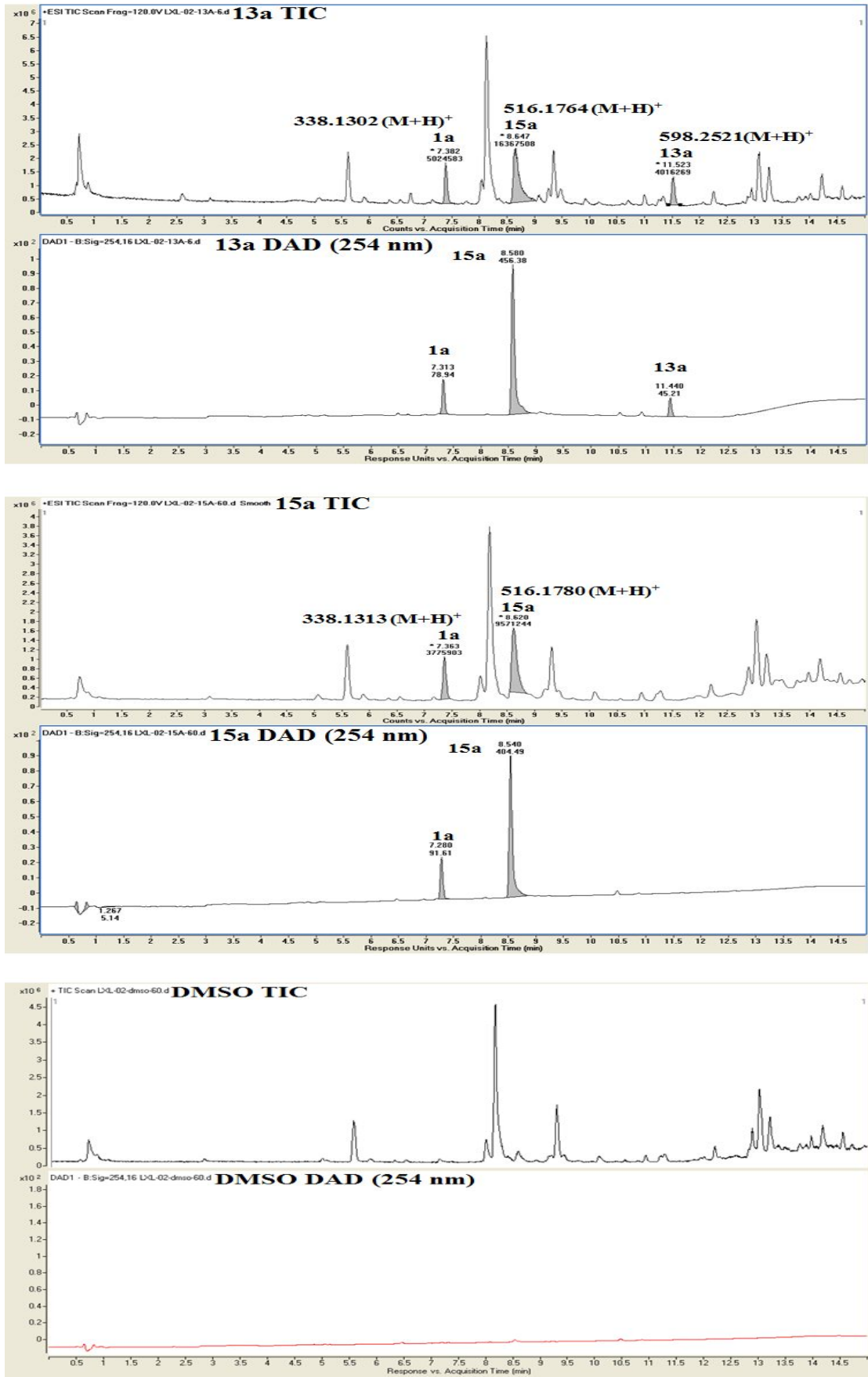


Figure S4. Relative drug release of compound 13a and 15a determined by UPLC-

QTOF/MS after incubation with HCT116 cells for 8 h.

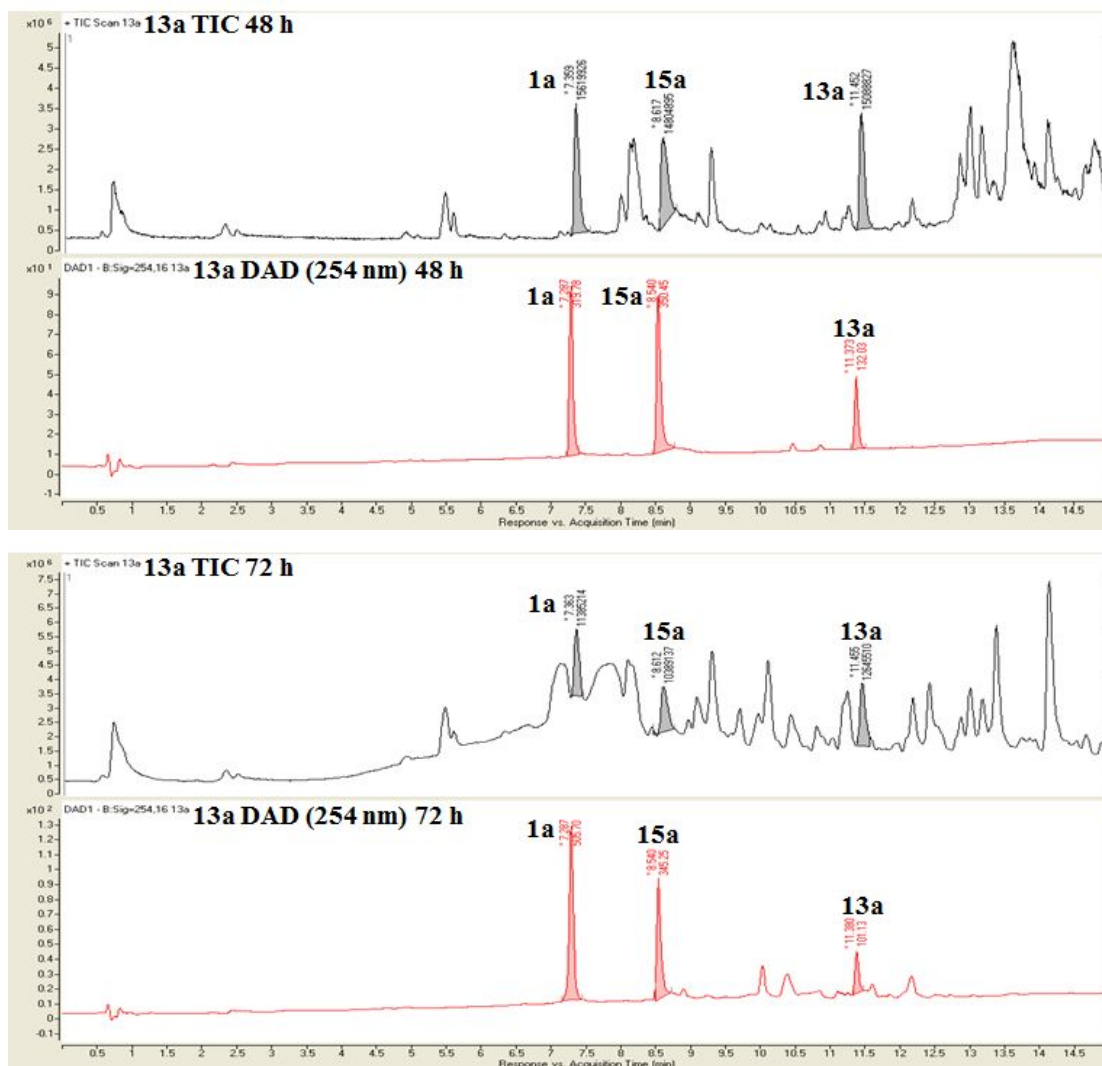


Figure S5. Relative drug time-dependent release of compound 13a determined by UPLC-QTOF/MS after incubation with HCT116 cells for 48 h and 72 h.

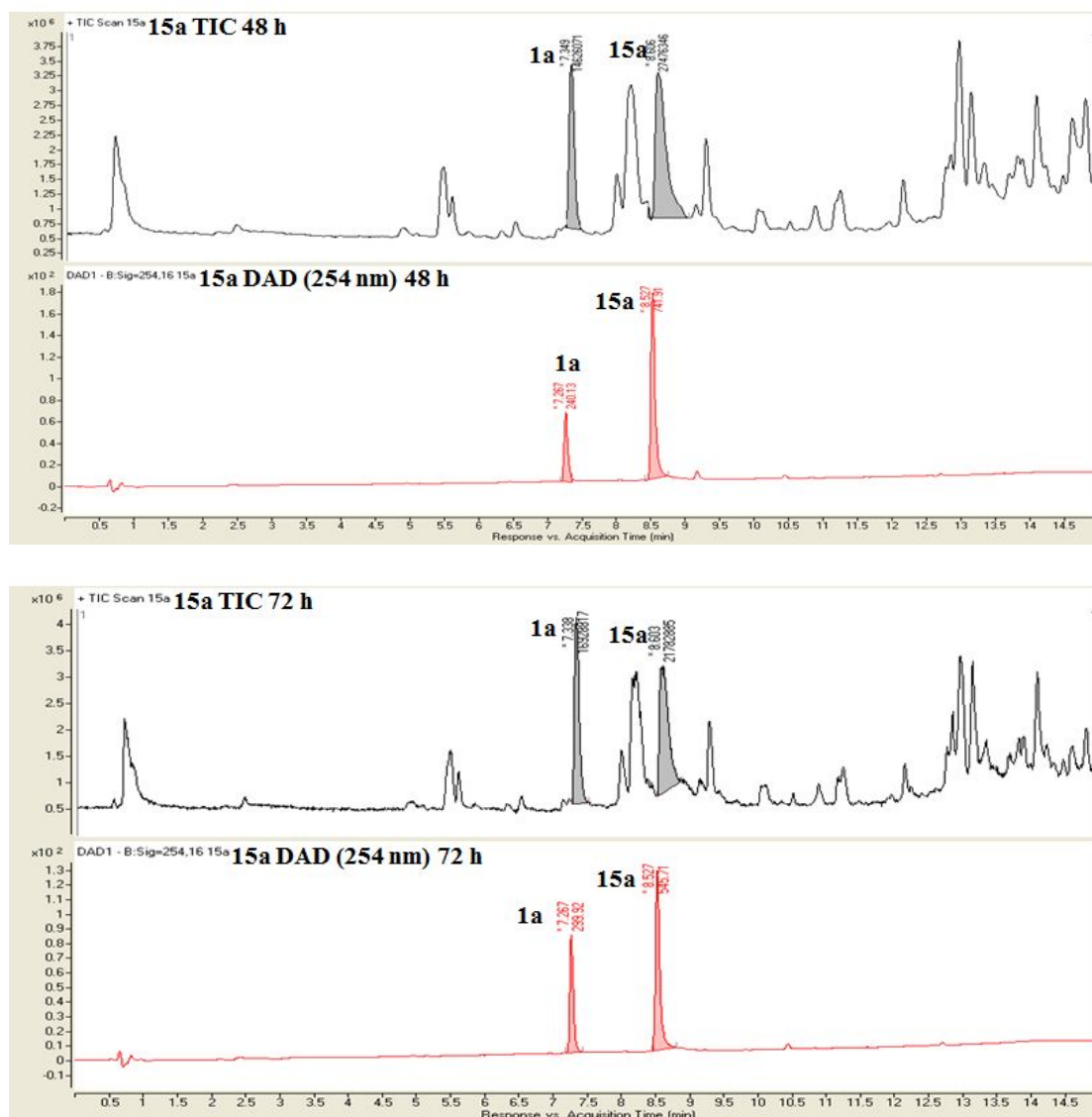


Figure S6. Relative drug time-dependent release of compound **15a** determined by UPLC- QTOF/MS after incubation with HCT116 cells for 48 h and 72 h.

Compound **13a** (retention time: 11.4 min) was first converted to compound **15a** (43.7%) and then transformed to compound **1a** (39.9%) for 48 h (**Figure S5**). Compound **15a** (retention time: 8.5 min) was directly converted into compound **1a** (retention time: 7.3 min) with the relative drug release rate of 24.5% for 48 h (**Figure S6**).

Compound **13a** (retention time: 11.4 min) was first converted to compound **15a** (36.3%) and then transformed to compound **1a** (53.1%) for 72 h (**Figure S5**). In contrast, compound **15a** (retention time: 8.5 min) was directly converted into compound **1a** (retention time: 7.3 min) with the relative drug release rate of 35.5% for

72 h (Figure S6).

13. *In vivo* antitumor activity

The female nude mice were purchased from Shanghai Jihui Experimental Animal Breeding Co., Ltd. The cultured HCT116 cells were collected, counted and resuspended in cold PBS, then cells were transplanted to the right forelimbs of the female nude mice, 5×10^6 cells/mouse. After 12 days, the solid tumors grown to about 100 mm^3 , the mice were randomly divided into four groups consisting of 6 mice/group. The mice were injected compounds by intraperitoneal administration once on day or twice on day, mice weight and tumor volume were recorded every two days. Tumor volumes were calculated from formula: $TV = 1/2 \times a \times b^2$, where “a” is the tumor length and “b” is the width and both “a” and “b” were measured by a vernier caliper. Post treatment mice were sacrificed on 22 days, and tumors were removed for further analysis.

The experimental procedures and the animal use and care protocols were approved by the Committee on Ethics of Biomedicine, Second Military Medical University.

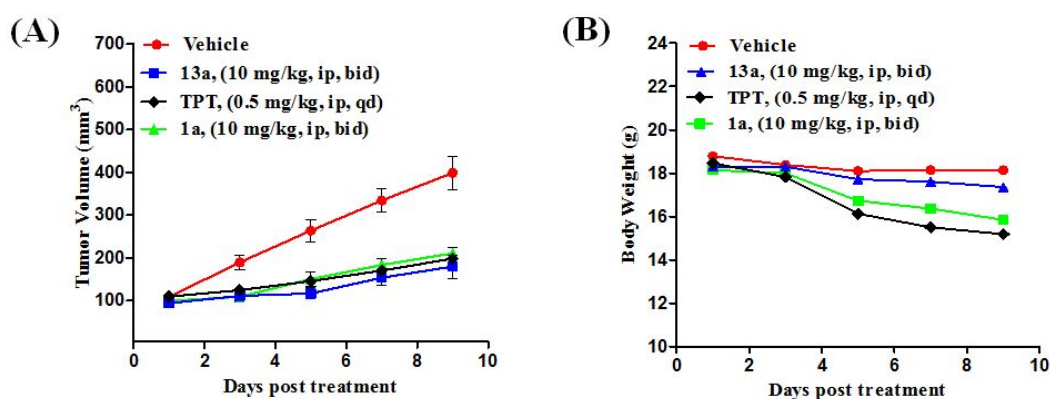
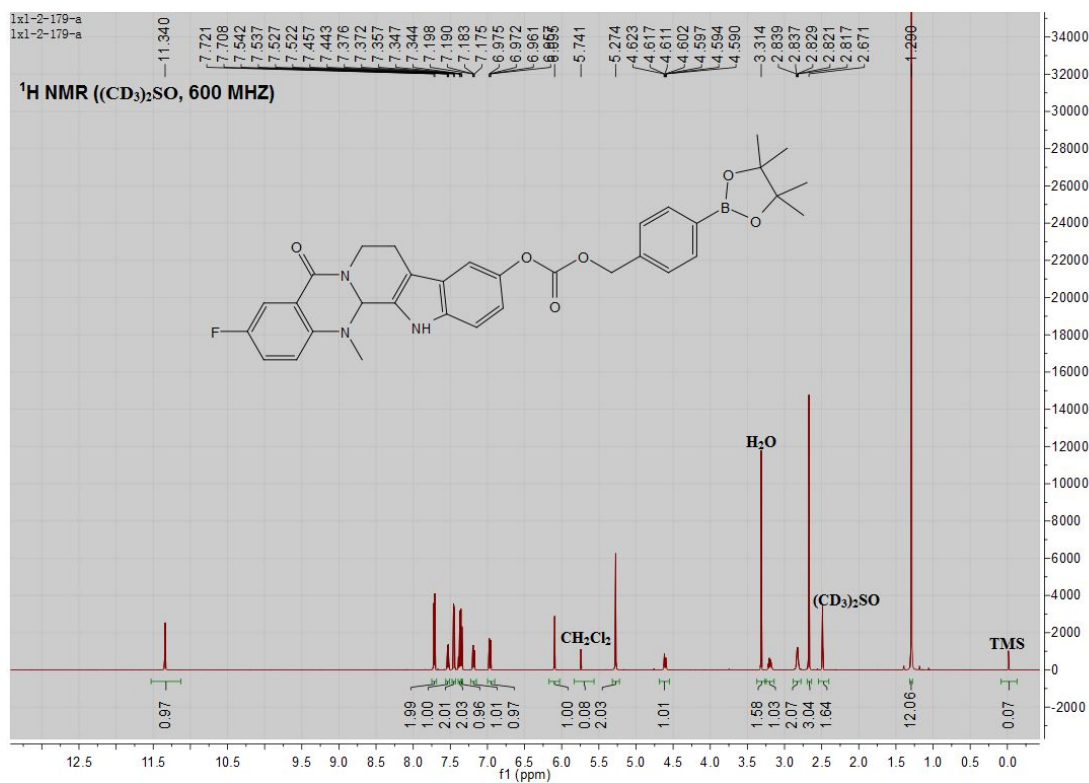


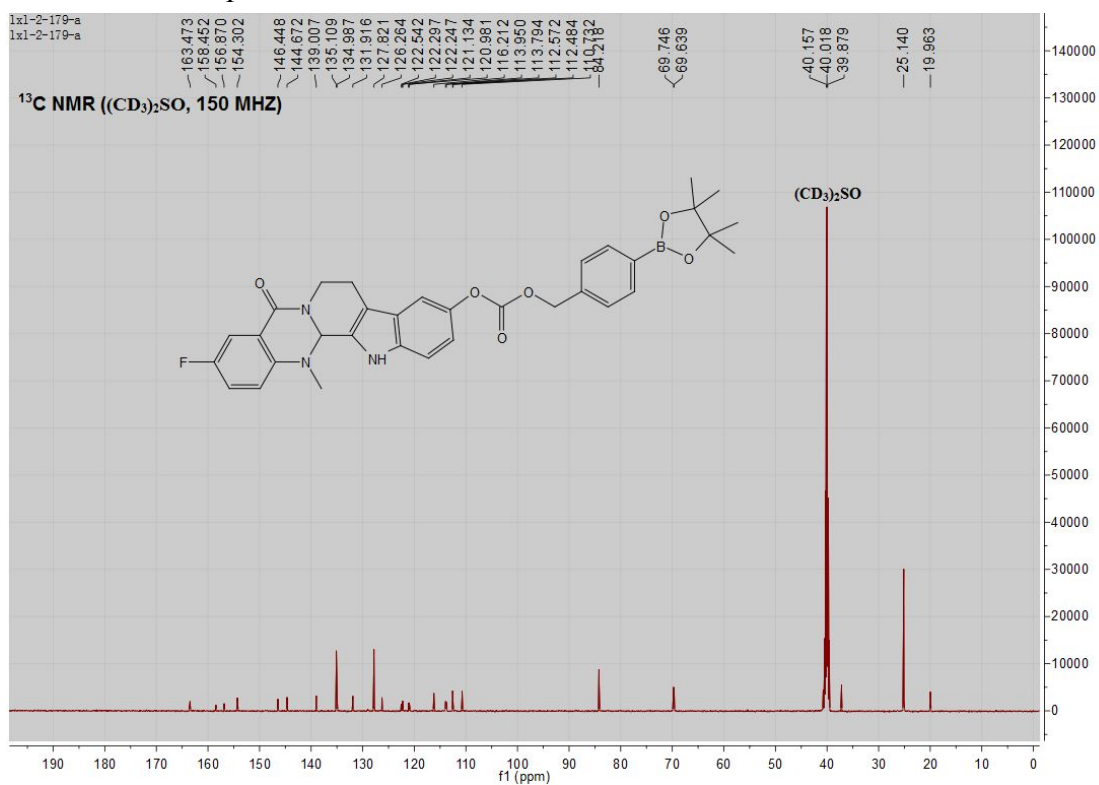
Figure S7. Antitumor efficacy of compounds **1a**, **13a** and TPT in the xenograft model for 9 days. (A) The efficacy of compounds **1a**, **13a** in the HCT116 xenograft model. (B) The change of body weight for the nude mice that were treated with compounds **1a**, **13a** and TPT.

14. ¹H NMR and ¹³C NMR spectrums

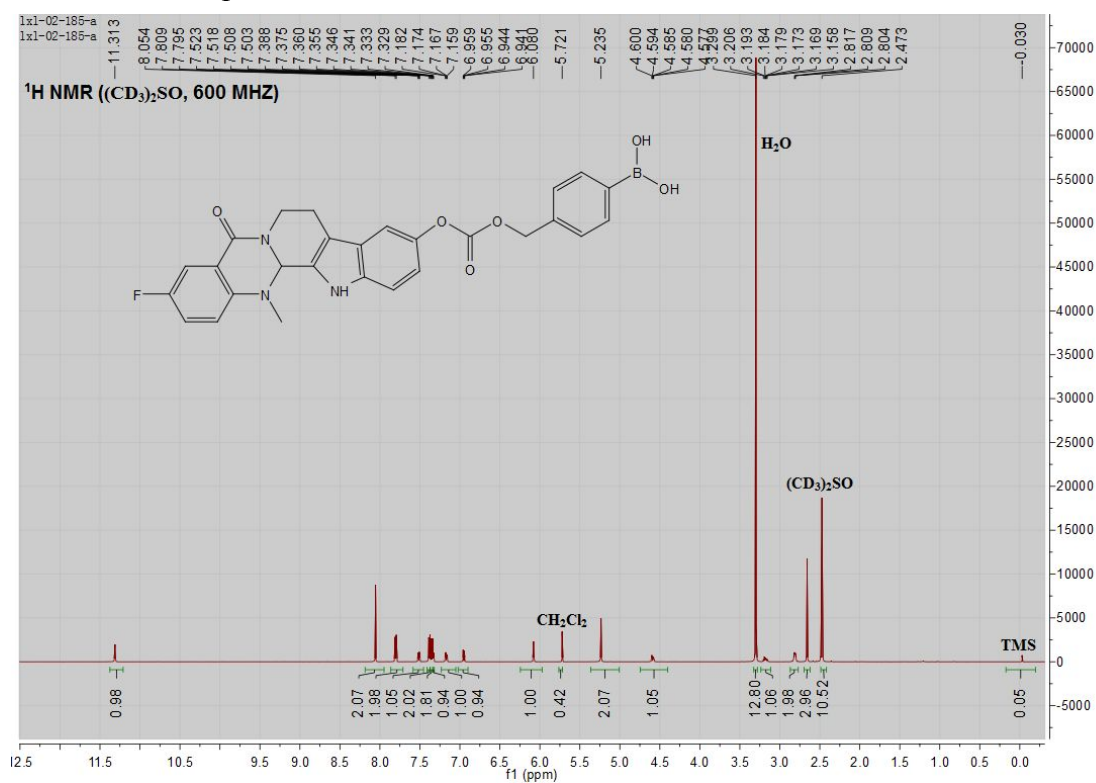
¹H NMR of compound **13a**.



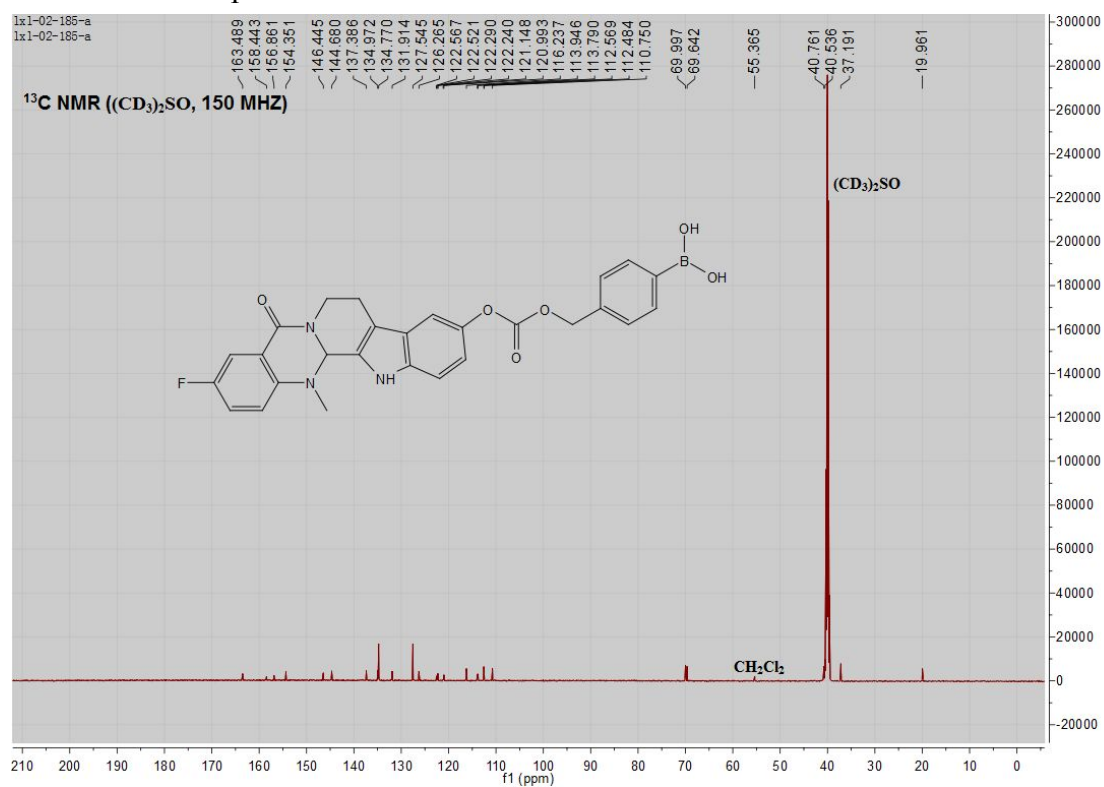
¹³C NMR of compound **13a**.



¹H NMR of compound **15a**.

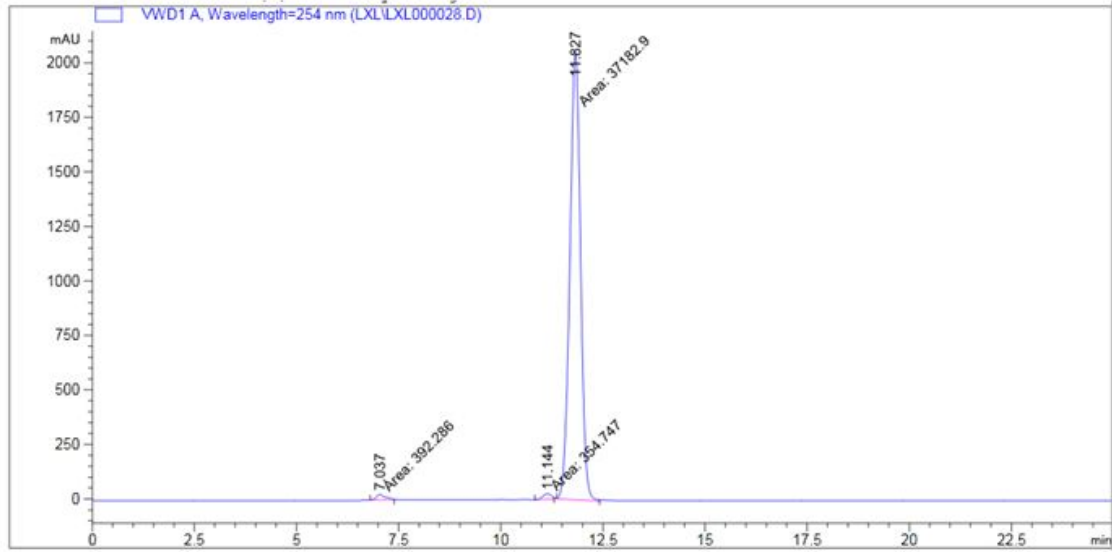


¹³C NMR of compound **15a**.



15. HPLC purity of representative compounds

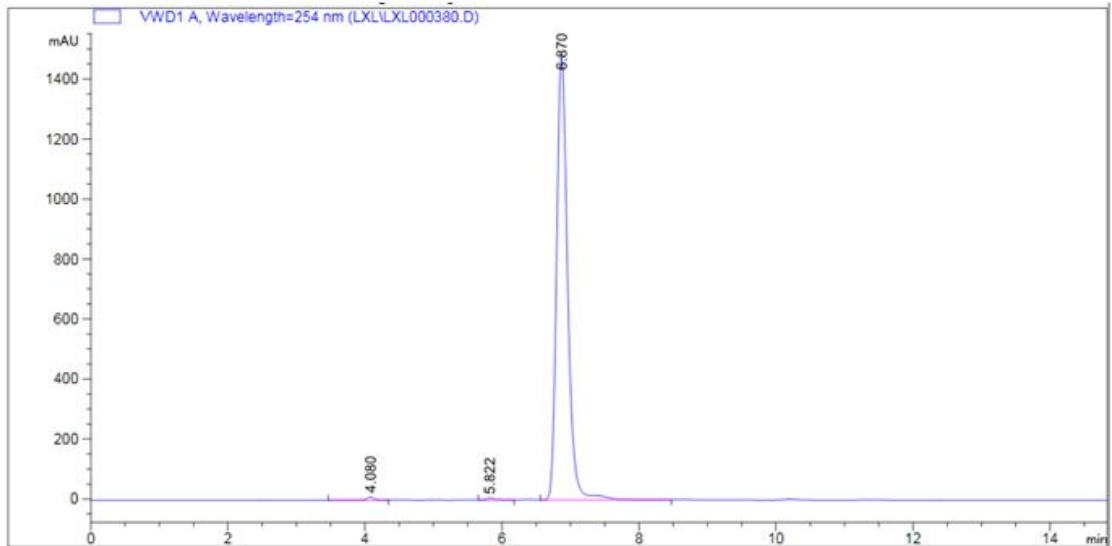
HPLC of compound 13a.



Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.037	MM	0.2598	392.28619	25.16863	1.0342
2	11.144	MM	0.2411	354.74719	24.52522	0.9353
3	11.827	MM	0.3028	3.71829e4	2046.78564	98.0305

HPLC of compound 15a.



Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.080	BV	0.0989	76.49403	11.36674	0.4586
2	5.822	VB	0.1533	54.97726	5.41901	0.3296
3	6.870	VV	0.1711	1.65483e4	1481.12280	99.2118

16. Certificate of STR analysis

CBTCCAS

Certificate of STR Analysis

1.Sample

HCT 116

2.2.Methods

The genomic DNA was purified with Purelink@ Genomic DNA Kits in our Bank.

The DNA sample was analysed in Beijing Microread GeneticsCo.,Ltd.

The sample was amplified with Goldeneye™20A STR Complex Amplification Kit.

The profiles STR loci and Amelogenin gene were characterized on ABI 3100 Type Genetic Analysis Instrument.

3.Results

D5S818	10,11
D13S317	10,12
D7S820	11,12
D16S539	11,13
vWA	17,22
TH01	8,9
Amelogenin	X,Y
TPOX	8,9
CSF1PO	7,10

The above results were consistent with the DNA profiles reported by ATCC, and DSMZ, and indicated no other human cell lines contamination.



Cell Bank,
Type Culture Collection,
Chinese Academy of Sciences
(CBTCCAS)

2013/7/5

17. Reference

1. Huang, Y.; Chen, S.; Wu, S.; Dong, G.; Sheng, C., Evodiamine-inspired dual inhibitors of histone deacetylase 1 (HDAC1) and topoisomerase 2 (TOP2) with potent antitumor activity. *Acta Pharm. Sin. B.* **2019**, <https://doi.org/10.1016/j.apsb.2019.11.011>.