A Versatile Method to Determine the Cellular Bioavailability of Small-Molecule Inhibitors

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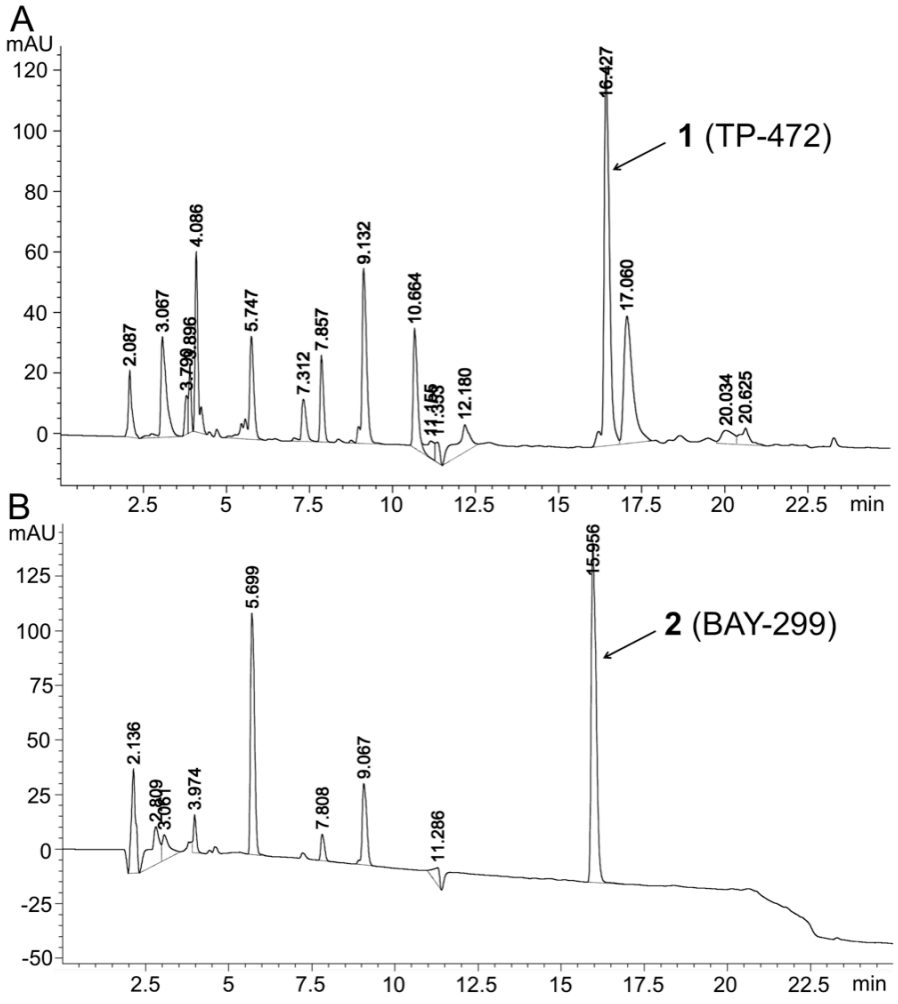
Supplemental Procedure.

MTs cell viability assay. MDA−MB−231 were seeded in the 96-well plates at 4×10^3 cells/well, maintained overnight at 37 °C, and incubated with **1** (TP-472), **2** (BAY-299), and **4** at various concentrations. Cell viability was monitored after 72 h using a freshly prepared mixture of 1 part phenazine methosulfate (PMS, Sigma) solution (0.92 mg/mL) and 19 parts 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTs, Promega) solution (2 mg/mL). Cells were incubated in 10 μL of this solution at 37 °C for 3 h, and A_{490} was measured. The effect of the compound is expressed as the concentration required to reduce A_{490} by 50% (IC_{50}) relative to DMSO-treated cells. Experiments were performed in triplicate.

The MTs cell viability assay result of **3** for triple negative breast cancer MDA-MB-231 cells have previously been reported.[24]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80
60
40
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4.086
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9.132
10.664
12.180
16.427
17.060
20.034
20.625
1 (TP-472)
2.5
5
7.5
10
12.5
15
17.5
20
22.5
min
B
mAU
125
100
75
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3.974
5.699
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9.067
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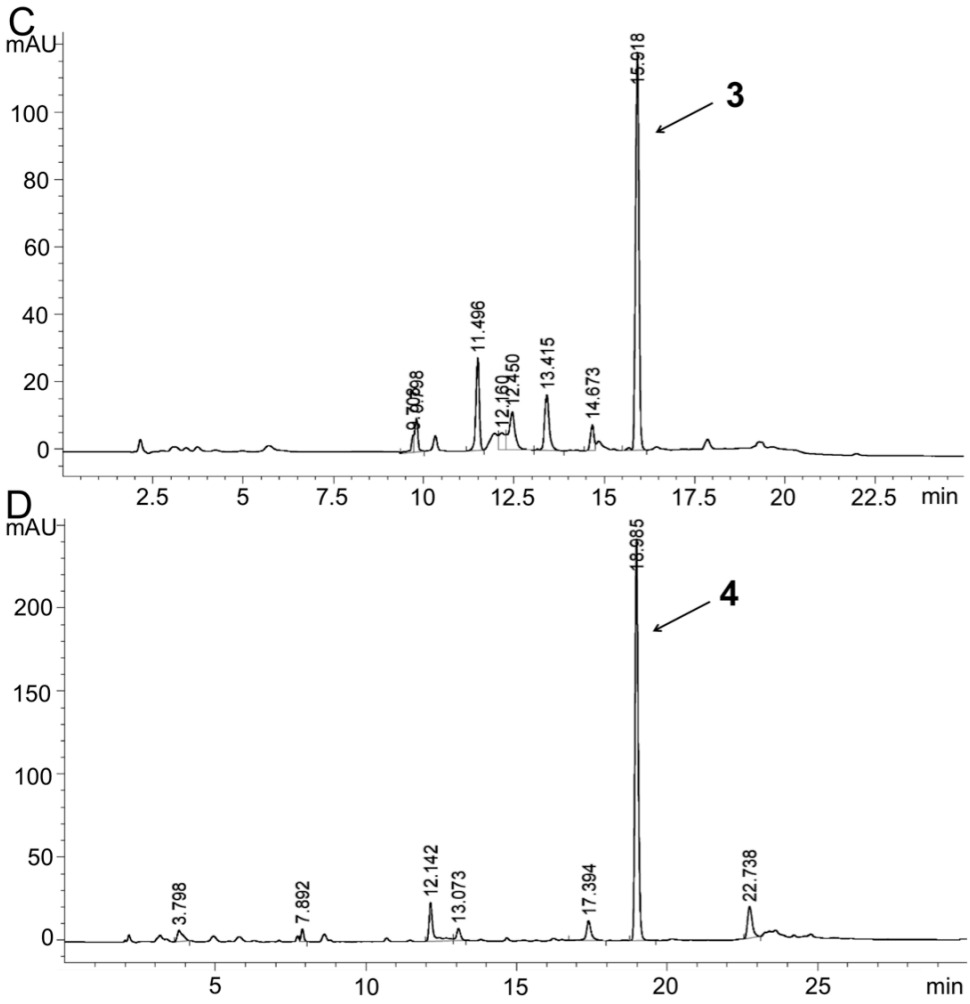
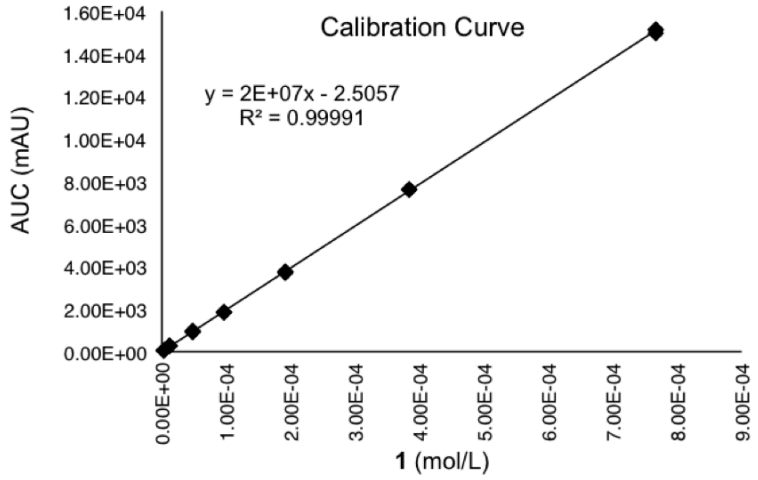


Figure S1. HPLC chromatograms of 1 (TP-472), 2 (BAY-299), **3**, and **4** in 5 mL DMEM with fetal bovine serum (FBS) (10% for **1** and **2**; 5% for **3** and **4**) at the starting time point.

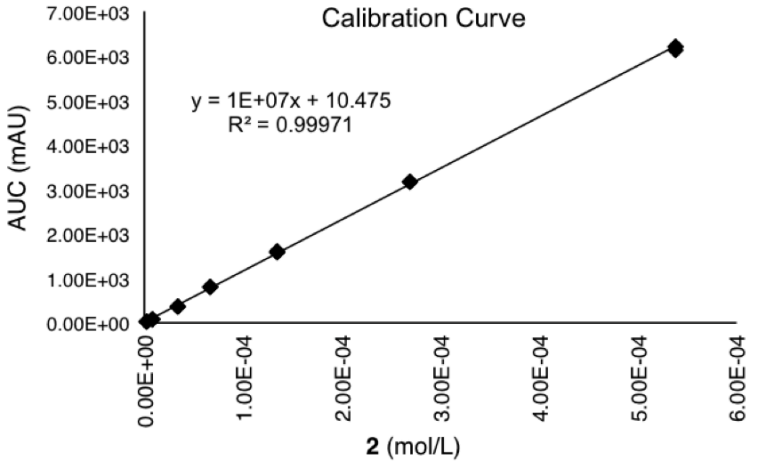
A

HPLC Injection (20 μL)	Concentration of **1** (mol/L)	AUC (mAU)
1	7.67×10^{-4}	1.51×10^{4}
2		1.51×10^{4}
3		1.50×10^{4}
1	3.84×10^{-4}	7.63×10^{3}
2		7.63×10^{3}
3		7.62×10^{3}
1	1.92×10^{-4}	3.74×10^{3}
2		3.74×10^{3}
3		3.71×10^{3}
1	9.59×10^{-5}	1.84×10^{3}
2		1.84×10^{3}
3		1.84×10^{3}
1	4.79×10^{-5}	9.47×10^{2}
2		9.55×10^{2}
3		9.45×10^{2}
1	1.20×10^{-5}	2.50×10^{2}
2		2.49×10^{2}
3		2.48×10^{2}
1	3.00×10^{-6}	7.82×10^{1}
2		6.64×10^{1}
3		1.85×10^{1}



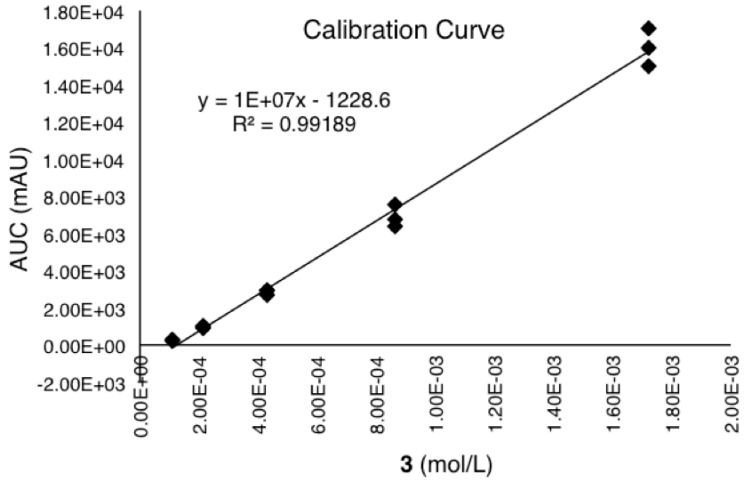
B

HPLC Injection (20 μL)	Concentration of **2** (mol/L)	AUC (mAU)
1	5.38×10^{-4}	6.21×10^{3}
2		6.21×10^{3}
3		6.13×10^{3}
1	2.69×10^{-4}	3.18×10^{3}
2		3.18×10^{3}
3		3.18×10^{3}
1	1.34×10^{-4}	1.58×10^{3}
2		1.60×10^{3}
3		1.58×10^{3}
1	6.72×10^{-5}	7.96×10^{2}
2		7.95×10^{2}
3		7.96×10^{2}
1	3.36×10^{-5}	3.71×10^{2}
2		3.68×10^{2}
3		3.71×10^{2}
1	8.40×10^{-6}	8.88×10^{1}
2		8.88×10^{1}
3		8.88×10^{1}
1	2.10×10^{-6}	2.09×10^{1}
2		2.08×10^{1}
3		2.03×10^{1}



C

HPLC Injection (10 μL)	Concentration of **3** (mol/L)	AUC (mAU)
1	1.72×10^{-3}	1.60×10^{4}
2		1.70×10^{4}
3		1.50×10^{4}
1	8.62×10^{-4}	6.37×10^{3}
2		6.76×10^{3}
3		7.54×10^{3}
1	4.31×10^{-4}	2.92×10^{3}
2		2.71×10^{3}
3		2.94×10^{3}
1	2.15×10^{-4}	1.03×10^{3}
2		9.55×10^{2}
3		9.10×10^{2}
1	1.08×10^{-4}	3.15×10^{2}
2		2.25×10^{2}



D

HPLC Injection (20 μL)	concentration of **4** (mol/L)	AUC (mAU)
1	1.27×10^{-3}	1.53×10^{4}
2		1.53×10^{4}
3		1.49×10^{4}
1	6.37×10^{-4}	7.25×10^{3}
2		7.15×10^{3}
3		7.27×10^{3}
1	3.19×10^{-4}	3.40×10^{3}
2		3.57×10^{3}
3		3.24×10^{3}
1	1.59×10^{-4}	1.55×10^{3}
2		1.54×10^{3}
3		1.61×10^{3}
1	7.97×10^{-5}	5.49×10^{2}
2		4.89×10^{2}
3		4.57×10^{2}
1	3.98×10^{-5}	1.57×10^{2}
2		1.34×10^{2}
3		1.28×10^{2}

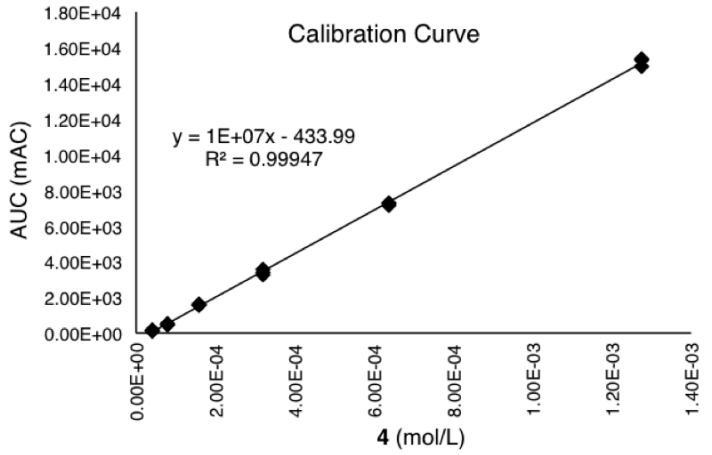


Figure S2. Determination of the calibration curves for **1**–**4**.

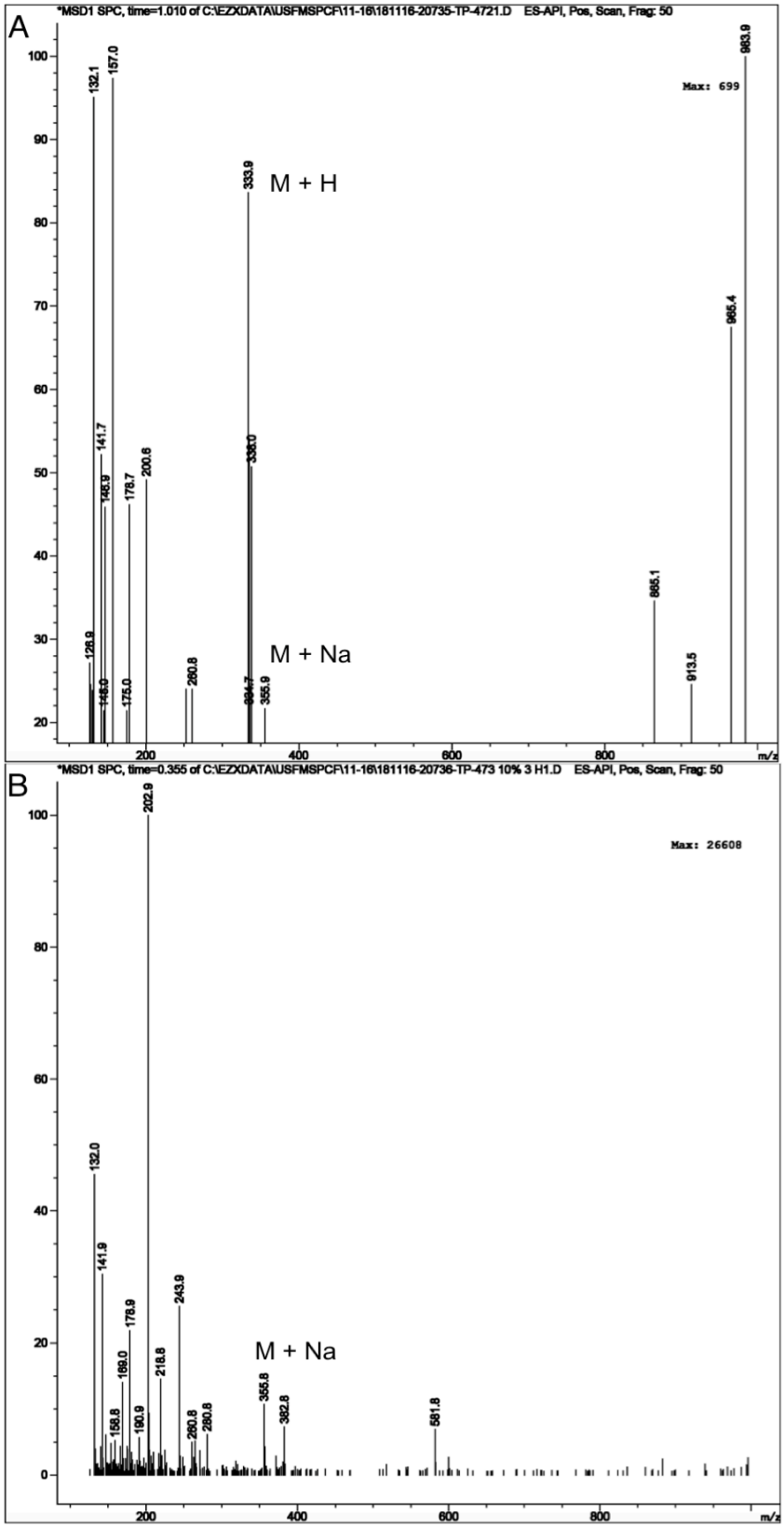


Figure S3. Mass spectrometry (MS) data for the HPLC peaks in Figure 3A. (A) pure **1**. (B) MDA-MB-231 cell samples after the treatment with **1** for 24 h.

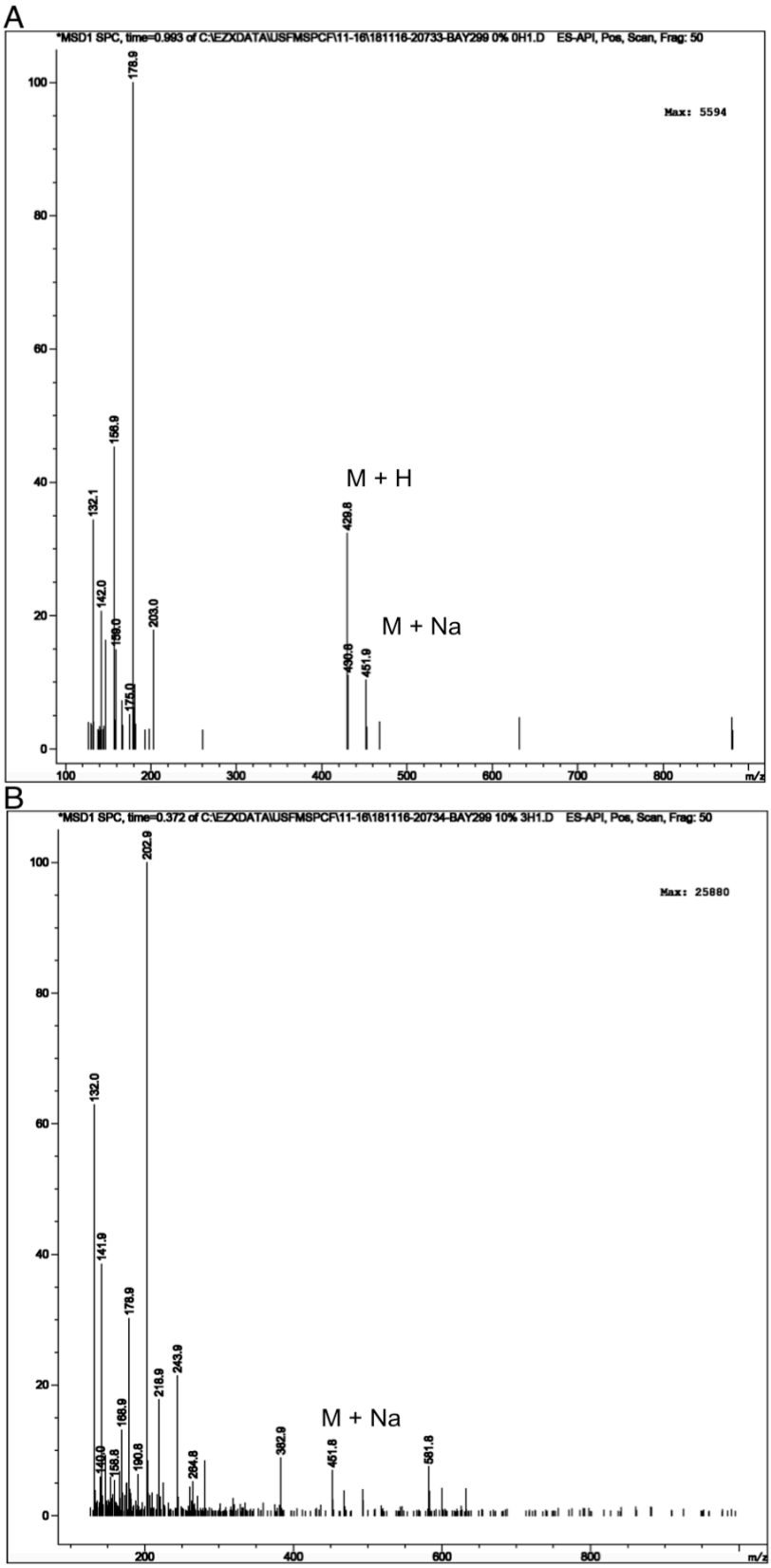


Figure S4. Mass spectrometry (MS) data for the HPLC peaks in Figure 3B. (A) pure **2**. (B) MDA-MB-231 cell samples after the treatment with **2** for 24 h.

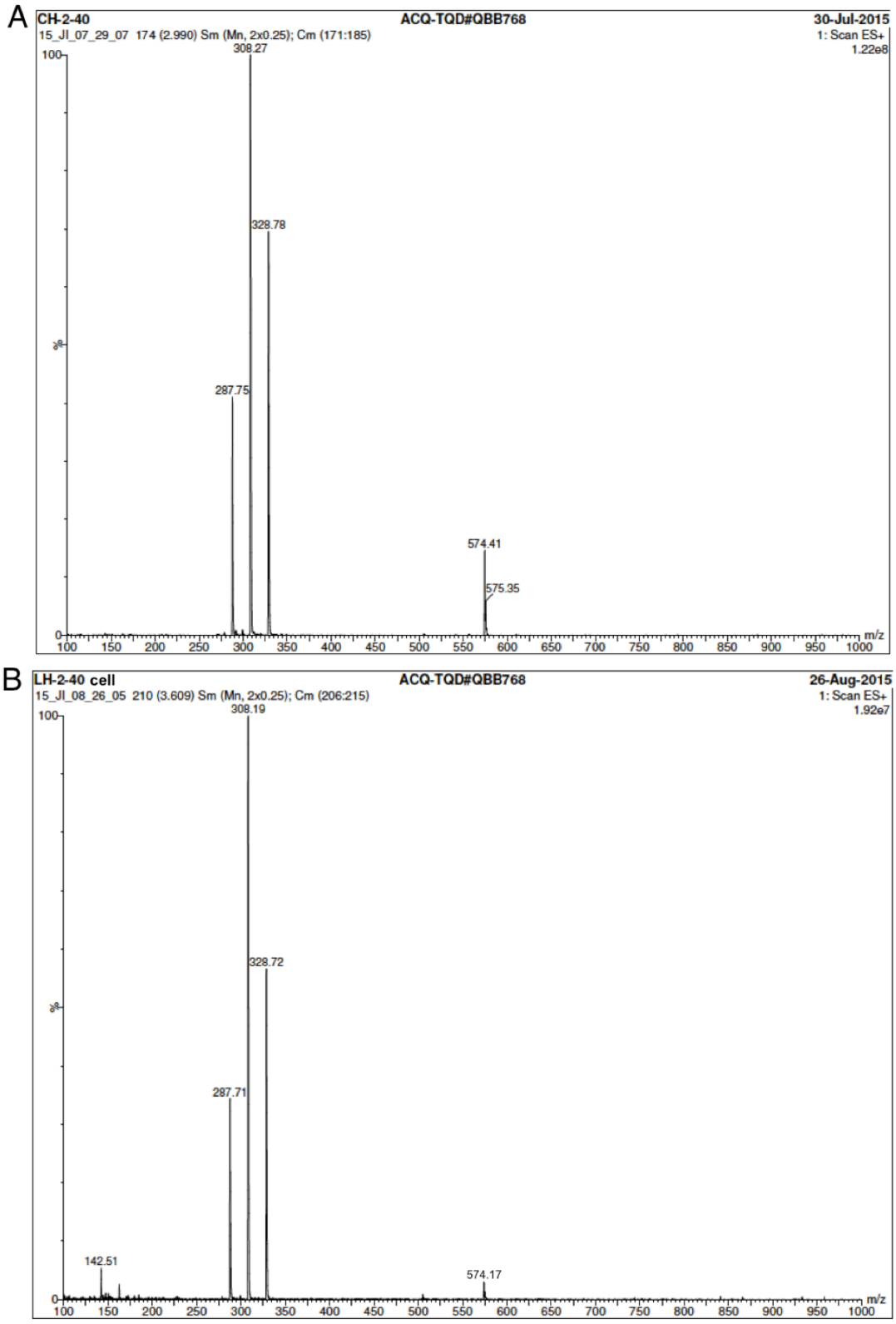


Figure S5. Mass spectrometry (MS) data for the HPLC peaks in Figure 3C. (A) pure **3**. (B) MDA-MB-231 cell samples after the treatment with **3** for 24 h.

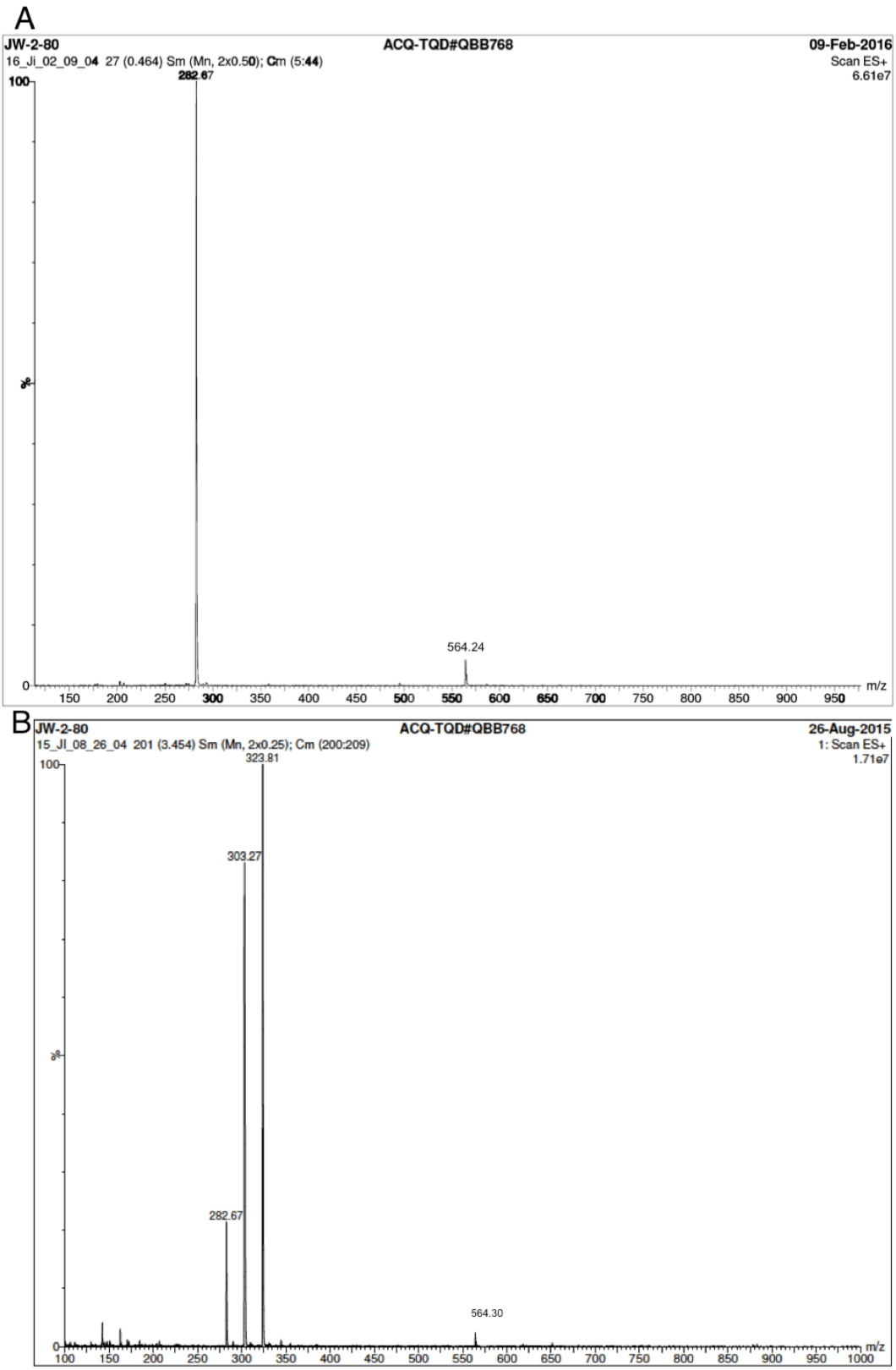


Figure S6. Mass spectrometry (MS) data for the HPLC peaks in Figure 3D. (A) pure **4**. (B) MDA-MB-231 cell samples after the treatment with **4** for 24 h.

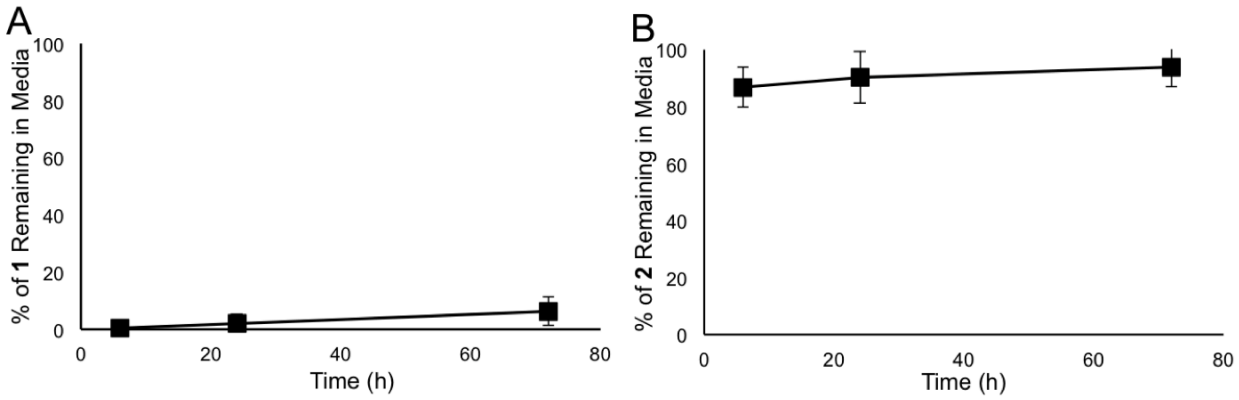
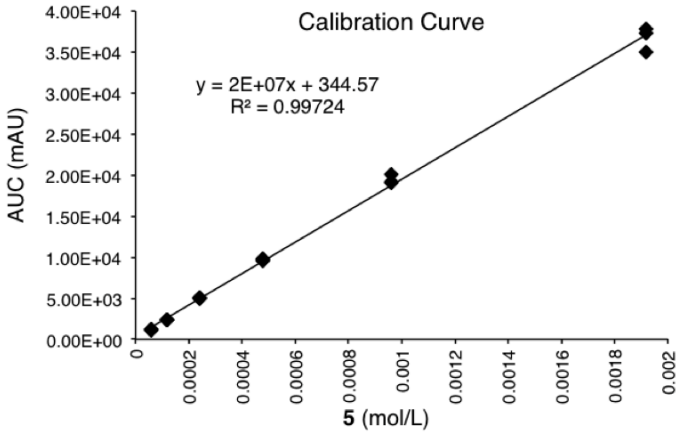


Figure S7. Time-dependence of the percent of the β-catenin/BCL9 inhibitors remaining the DMEM medium. Inhibitors **3** (A) and **4** (B) were incubated over a period of 72 h with the initial concentration of 2 and 20 μM. Each set of data is expressed as mean ± standard deviation (SD) (n = 3).

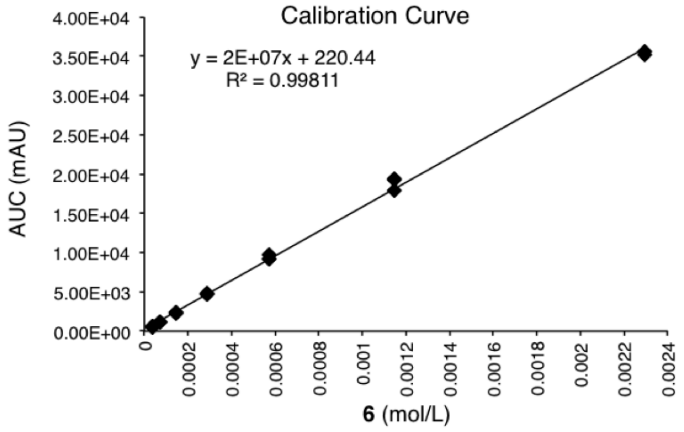
A

HPLC Injection (10 μL)	Concentration of **5** (mol/L)	AUC (mAU)
1	1.92×10^{-3}	3.78×10^{4}
2		3.50×10^{4}
3		3.72×10^{4}
1	9.59×10^{-4}	2.01×10^{4}
2		1.92×10^{4}
3		1.90×10^{4}
1	4.79×10^{-4}	9.82×10^{3}
2		9.52×10^{3}
3		9.69×10^{3}
1	2.40×10^{-4}	4.97×10^{3}
2		5.03×10^{3}
3		5.02×10^{3}
1	1.20×10^{-4}	2.32×10^{3}
2		2.33×10^{3}
3		2.32×10^{3}
1	5.99×10^{-5}	1.23×10^{3}
2		1.24×10^{3}
3		1.14×10^{3}



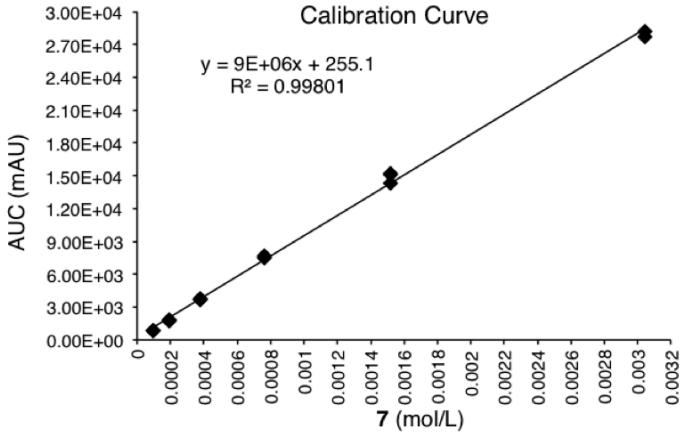
B

HPLC Injection (10 μL)	Concentration of **6** (mol/L)	AUC (mAU)
1	2.29×10^{-3}	3.52×10^{4}
2		3.56×10^{4}
1	1.15×10^{-3}	1.94×10^{4}
2		1.79×10^{4}
3		1.92×10^{4}
1	5.74×10^{-4}	9.74×10^{3}
2		9.22×10^{3}
3		9.26×10^{3}
1	2.87×10^{-4}	4.73×10^{3}
2		4.71×10^{3}
3		4.77×10^{3}
1	1.43×10^{-4}	2.28×10^{3}
2		2.34×10^{3}
3		2.37×10^{3}
1	7.17×10^{-5}	1.13×10^{3}
2		1.13×10^{3}
3		1.09×10^{3}
1	3.59×10^{-5}	5.47×10^{2}
2		5.60×10^{2}
3		5.43×10^{2}



C

HPLC Injection (10 μL)	Concentration of **7** (mol/L)	AUC (mAU)
1	3.04×10^{-3}	2.77×10^{4}
2		2.82×10^{4}
1	1.52×10^{-3}	1.51×10^{4}
2		1.43×10^{4}
3		1.52×10^{4}
1	7.61×10^{-4}	7.71×10^{3}
2		7.46×10^{3}
3		7.59×10^{3}
1	3.80×10^{-4}	3.71×10^{3}
2		3.73×10^{3}
3		3.78×10^{3}
1	1.90×10^{-4}	1.76×10^{3}
2		1.80×10^{3}
3		1.84×10^{3}
1	9.51×10^{-5}	8.82×10^{2}
2		8.77×10^{2}
3		8.91×10^{2}



D

HPLC Injection (10 μL)	Concentration of **8** (mol/L)	AUC (mAU)
1	3.14×10^{-3}	2.22×10^{4}
2		2.17×10^{4}
3		2.28×10^{4}
1	1.57×10^{-3}	1.17×10^{4}
2		1.12×10^{4}
3		1.05×10^{4}
1	7.85×10^{-4}	5.61×10^{3}
2		5.54×10^{3}
3		5.92×10^{3}
1	3.93×10^{-4}	2.89×10^{3}
2		2.87×10^{3}
3		2.97×10^{3}
1	1.96×10^{-4}	1.42×10^{3}
2		1.37×10^{3}
3		1.46×10^{3}
1	9.82×10^{-5}	7.30×10^{3}
2		9.49×10^{3}
3		7.14×10^{3}

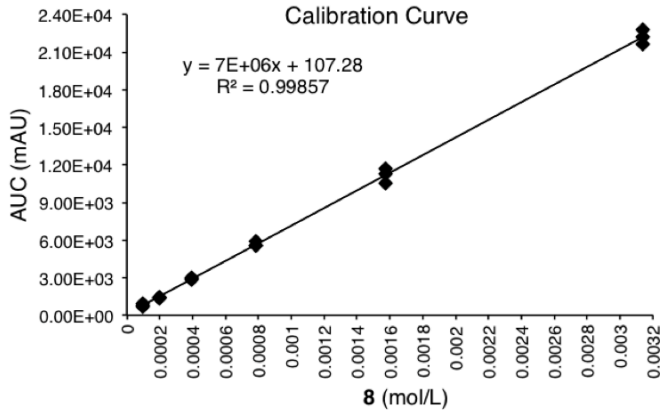
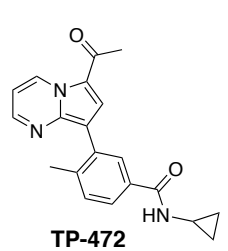
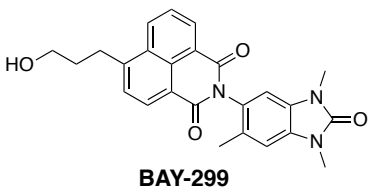
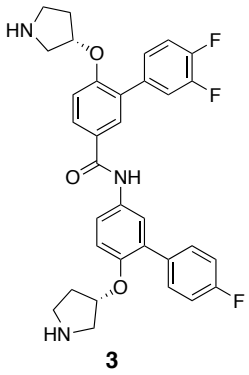


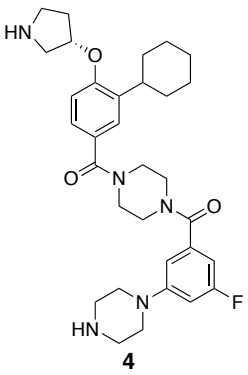
Figure S8. Determination of the calibration curves for **5**–**8**. (A) Areas under curve (AUCs) of the UV absorption and the different concentrations of pure **5** for HPLC analyses. The calibration curve and the calibration equation of **5**. (B) Areas under curve (AUCs) of the UV absorption and the different concentrations of pure **6** for HPLC analyses. The calibration curve and the calibration equation of **6**. (C) Areas under curve (AUCs) of the UV absorption and the different concentrations of pure **7** for HPLC analyses. The calibration curve and the calibration equation of **7**. (D) Areas under curve (AUCs) of the UV absorption and the different concentrations of pure **8** for HPLC analyses. The calibration curve and the calibration equation of **8**.

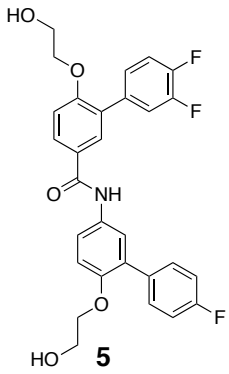
Table S1. Calculated physicochemical properties of **1**–**8**.

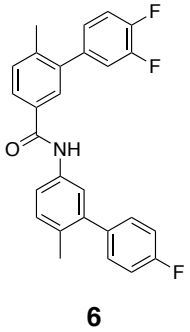


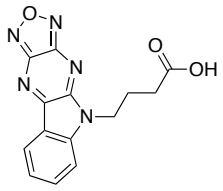


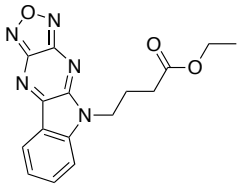












	Physical Properties							
Compound	MW[a]	HBD[b]	HBA[c]	tPSA[d]	rotatable bond	charge	cLogP	$logD_{pH = 7.0}$[e]
1	333	1	3	61.8	6	0	2.48	not calculated
2	429	1	4	81.2	8	0	2.55	not calculated
3	573	3	5	71.6	8	+2	5.08	1.21
4	563	2	5	77.2	6	+2	3.96	0.63
5	523	3	5	88.0	12	0	4.14	1.70
6	431	1	1	29.1	4	0	7.25	7.17
7	297	1	7	99.2	4	–1	2.98	0.34
8	325	0	7	88.2	6	0	3.94	3.96

[a] molecular weight.
[b] number of hydrogen bond acceptors.
[c] number of hydrogen bond donors.
[d] topological polar surface area ($Å^2$).
[e] logD was calculated by ACD/logD.[41]

Supplementary Reference:

(41) Liao, C.; Nicklaus, M. C. Comparison of nine programs predicting pK_a values of pharmaceutical substances. *J. Chem. Inf. Model.* **2009**, *49*, 2801–2812.