Supporting Information (SI)

Simple Structural Modifications Converting a *Bona fide* MDM2 PROTAC Degrader into a Molecular Glue Molecule: A Cautionary Tale in the Design of PROTAC Degraders

Jiuling Yang,[†,‡,∥,⊥] Yangbing Li,[‡,§,∥,⊥] Angelo Aguilar,[‡,∥,⊥] Zhaomin Liu,[‡,∥] Chao-Yie Yang,[‡,∥] and Shaomeng Wang*[,†,‡,§,∥]

[†]Department of Pharmacology, [‡]Department of Internal Medicine, [§]Department of Medicinal Chemistry, and [∥]Rogel Cancer Center, University of Michigan, Ann Arbor, Michigan 48109, United States.

Contents of Supporting Information:

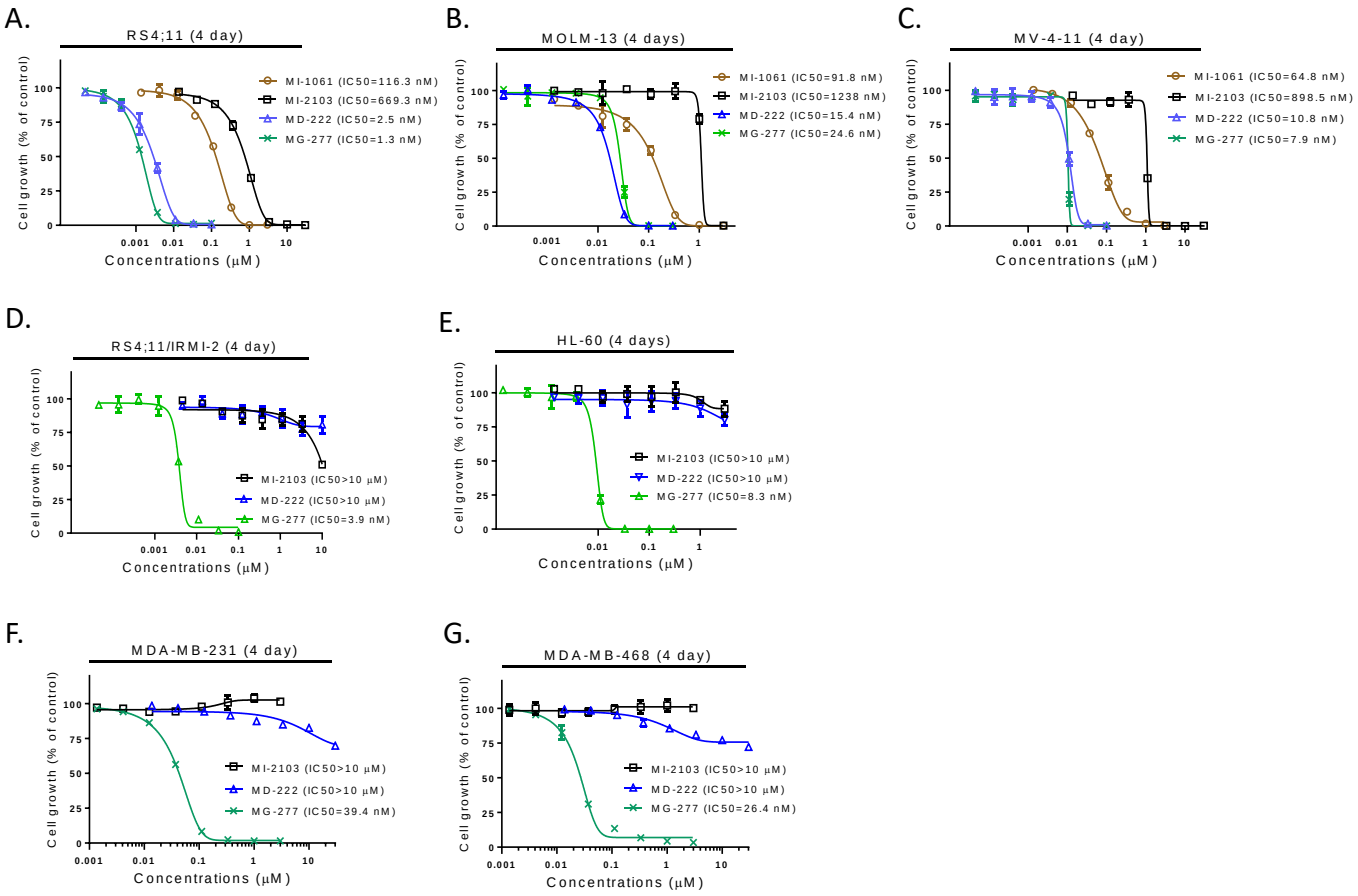


Figure S1. The cell growth inhibitory activity of MG-277 is p53-independent. (A-C) Cell growth inhibitory activity of the *bona fide* MDM2 degrader, MD-222, the new putative MDM2 degrader MG-277, and MDM2 inhibitors, MI-1061 and MI-2103, in three p53 wild-type leukemia cell lines, RS4;11, MV-4-11 and MOLM-13. (D-G) Cell growth inhibitory activity of MD-222, MG-277, and MDM2 inhibitor, MI-2103 in a p53 mutant cell line, RS4;11/IRMI-2 (D), a leukemia cell line with deleted p53, HL-60 (E), and two p53 mutant breast cancer cell lines, MDA-MB-231 (F) and MDA-MB-468 (G). Each cell line was treated with the indicated compounds at increasing concentrations for 4 days and then cell growth inhibition was determined by a 4-day cell viability/WST assay.

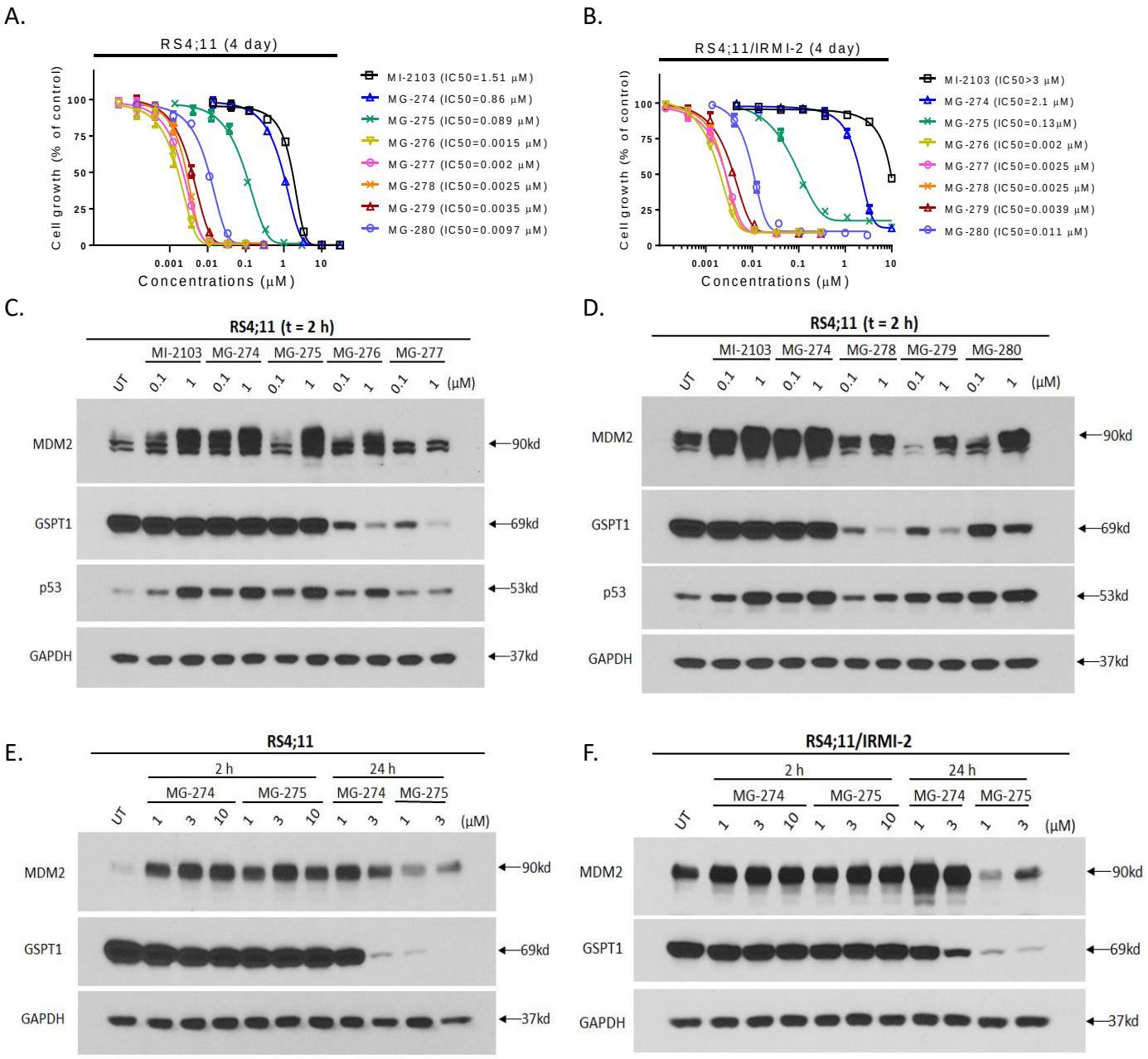


Figure S2. Different analogues of MG-277 inhibit cell growth and degrade GSPT1. (A-B) Cell growth inhibitory activity of the new putative MDM2 degraders by a 4-day cell viability assay in RS4;11 and IRMI-2 cells. (C-D) Western blotting for MDM2, GSPT1 and p53 protein levels after treatment with the new series of putative MDM2 degraders for 2 h in RS4;11 cells. (E-F) Western blotting or MDM2 and GSPT1 protein levels after treatment with MG-274 and MG-275 for 2h or 24 h in RS4;11 (E) and IRMI-2 (F) cells.

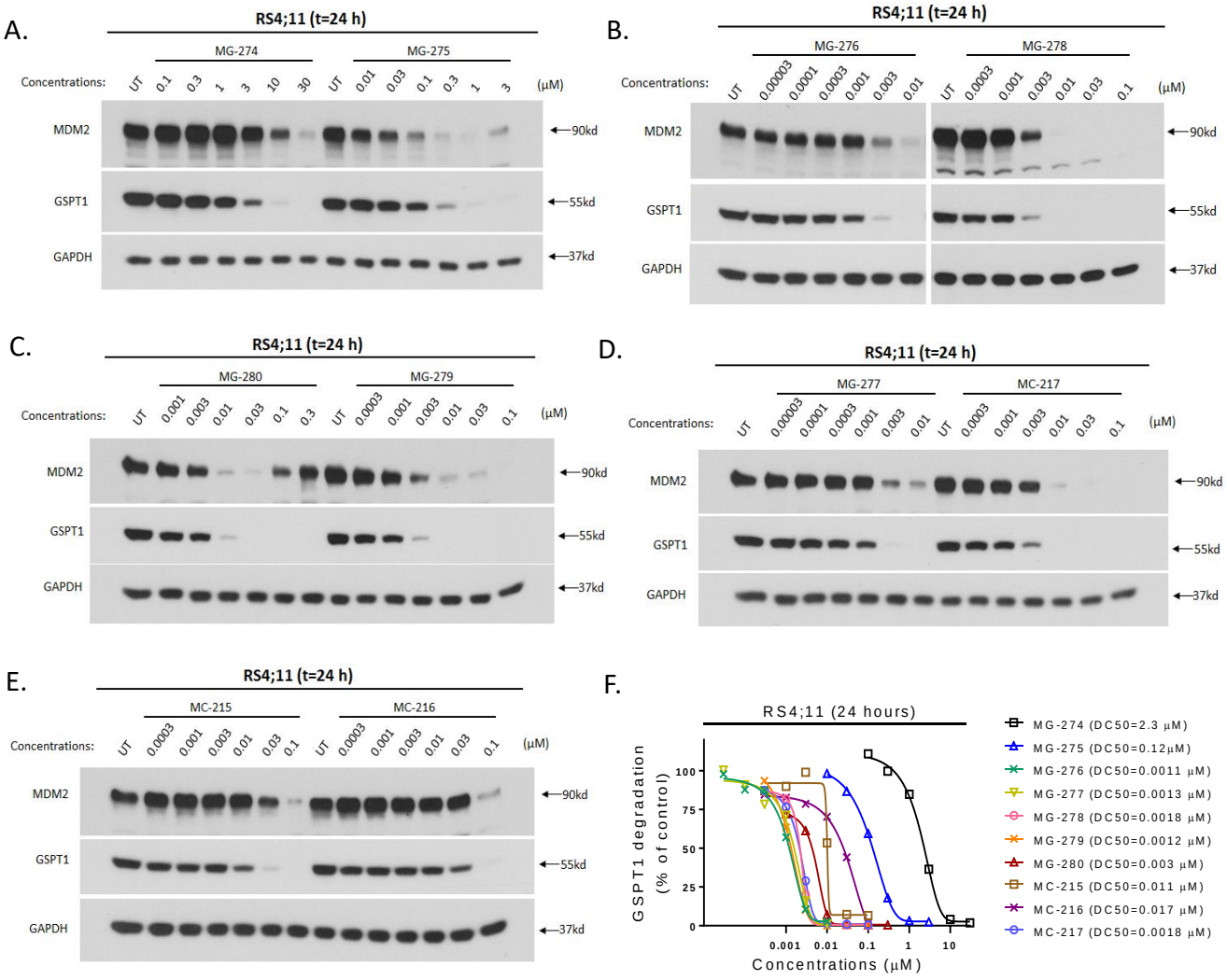
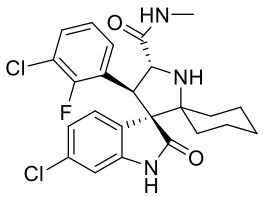


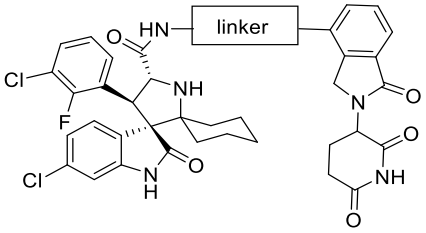
Figure S3. Potency in GSPT1 degradation by the different analogues of MG-277 in RS4;11 cell line. (A-E) Western blotting for MDM2 and GSPT1 protein levels after 24 h treatment with the indicated phthalimide conjugate degraders in RS4;11 cell line. (F) GSPT1 protein levels were quantified by using an imager and were normalized over the GAPDH loading control. The half maximal degradation concentration (DC_{50}) of GSPT1 was then calculated.

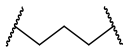
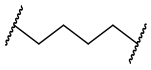
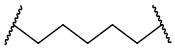
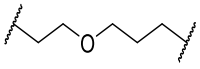
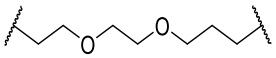
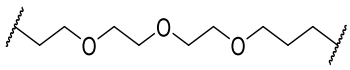
Table S1.

Correlation between the GSPT1 degradation DC_{50} of the compounds in RS4;11 cells and the corresponding IC_{50} in RS4;11 cell line and p53 mutant RS4;11/IRMI-2 cell line.



MI-2103



Compound No.	Linker	IC_{50} (μM) in cell growth inhibition		DC_{50} (μM) of GSPT1 degradation
		RS4;11	IRMI-2	RS4;11
MG-274	No linker	0.76±0.096	2.58±0.45	2.3
MG-275		0.13±0.039	0.18±0.045	0.12
MG-276		0.0025±0.00091	0.0026±0.00052	0.0011
MG-277		0.0035±0.0013	0.0034±0.00069	0.0013
MG-278		0.0036±0.00091	0.0035±0.00085	0.0018
MG-279		0.0049±0.0012	0.0049±0.00078	0.0012
MG-280		0.014±0.0037	0.014±0.0026	0.003

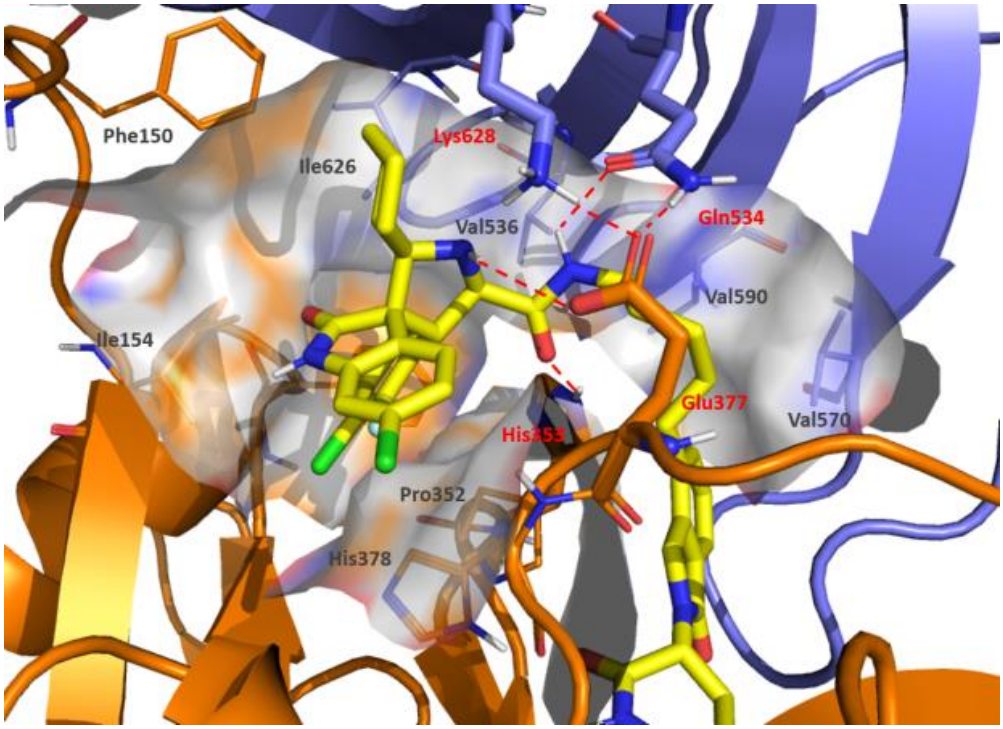


Figure S4. Detailed illustration of the predicted binding mode of MG-277 (yellow) to the cereblon (orange) – GSPT1 (blue) complex. Residues in both proteins involved in hydrophobic packing are labeled with dark grey and shown with surface; residues participated in hydrogen bonding are labeled in red. Interactions between lenalidomide and cereblon are the same as in the crystal structure, and are not shown in this figure.

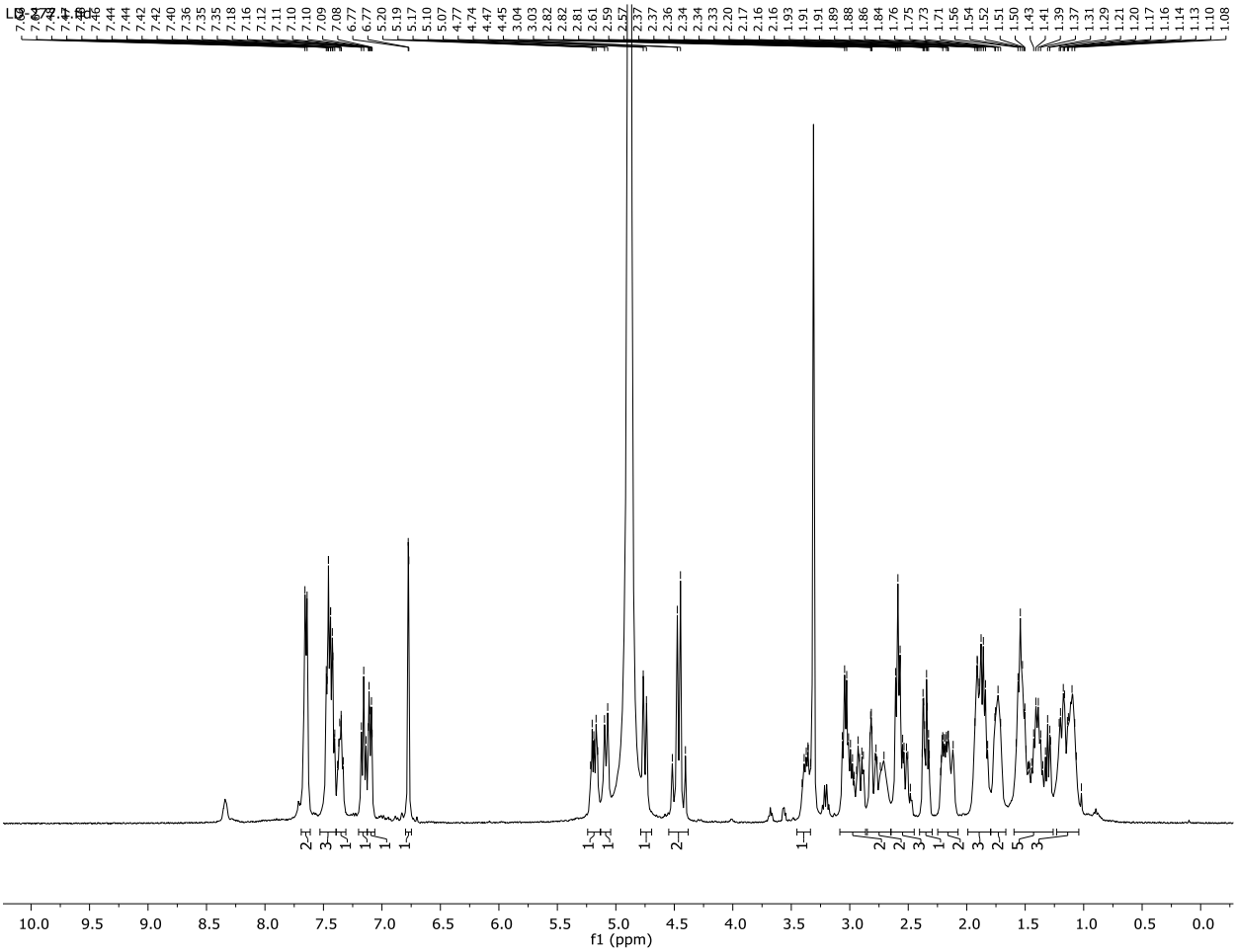
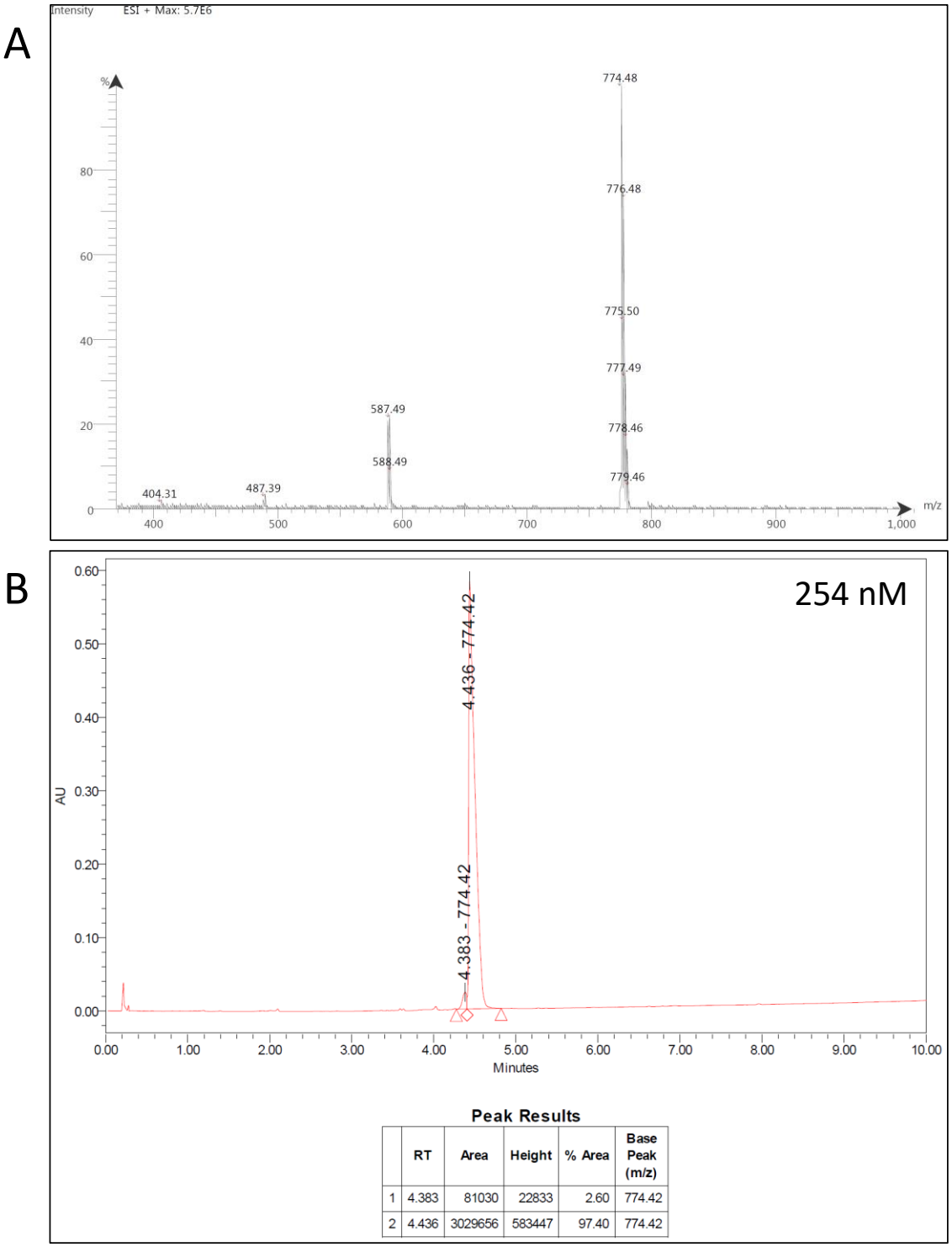


Figure S5. NMR Spectra of MG-277; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.65 (d, J = 7.3 Hz, 2H), 7.53 – 7.38 (m, 3H), 7.40 – 7.31 (m, 1H), 7.16 (t, J = 8.0 Hz, 1H), 7.10 (dt, J = 8.2, 2.4 Hz, 1H), 6.77 (d, J = 2.0 Hz, 1H), 5.19 (dt, J = 13.3, 5.5 Hz, 1H), 5.08 (d, J = 11.0 Hz, 1H), 4.75 (d, J = 11.0 Hz, 1H), 4.55 – 4.38 (m, 2H), 3.45 – 3.33 (m, 1H), 3.08 – 2.86 (m, 2H), 2.85 – 2.65 (m, 2H), 2.65 – 2.45 (m, 3H), 2.40 – 2.30 (m, 1H), 2.25 – 2.07 (m, 2H), 1.99 – 1.80 (m, 3H), 1.79 – 1.67 (m, 2H), 1.60 – 1.26 (m, 5H), 1.23 – 1.04 (m, 3H).



	RT	Area	Height	% Area	Base Peak (m/z)
1	4.383	81030	22833	2.60	774.42
2	4.436	3029656	583447	97.40	774.42

Figure S6. (A.) Mass Spectrum of MG-277 LC-MS (ESI) m/z $(M+H)^+$ calcd for $C_{41}H_{43}Cl_2FN_5O_5^+$ = 774.26; Found 774.42**; (B.)** UPLC spectra at 254 nm showing purity >95%