

Supporting Information

Identification of a new heterocyclic scaffold for inhibitors of the polo-box domain of Polo-like kinase 1

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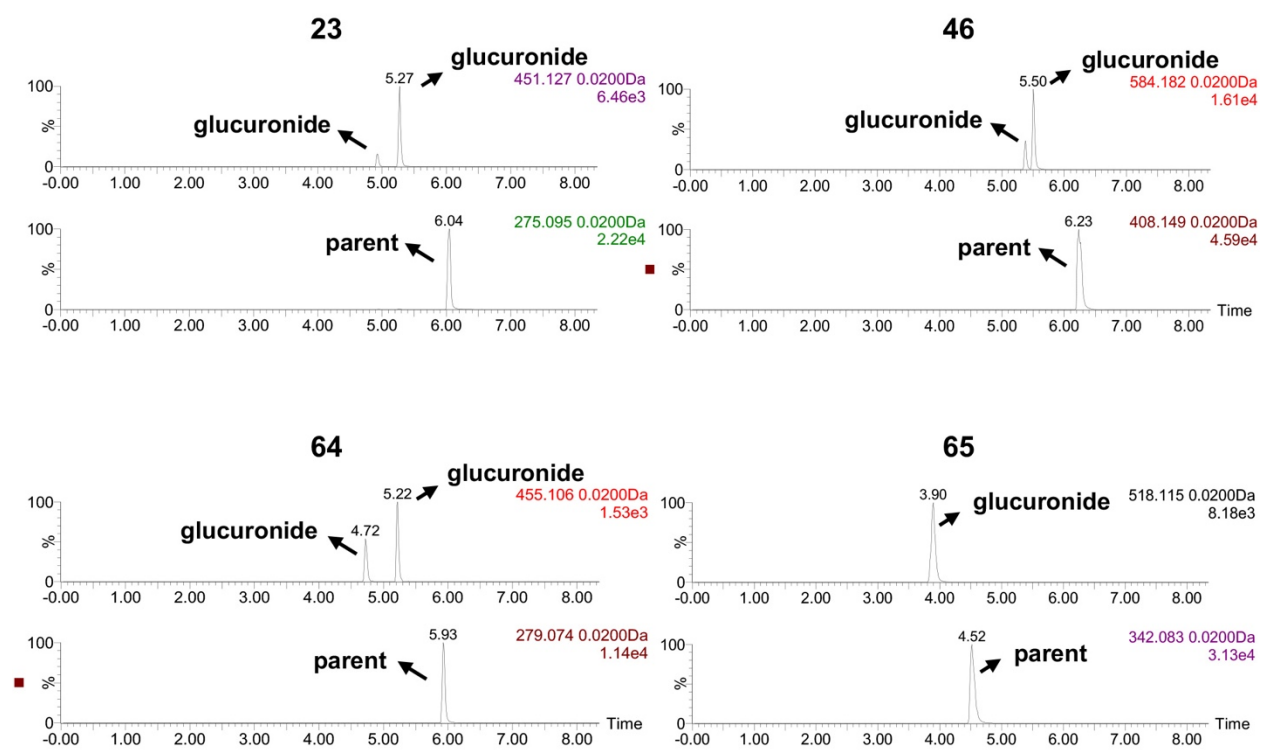
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3. HPLC purity, HRMS, and NMR data for selected tested analogs

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Supplementary Figures



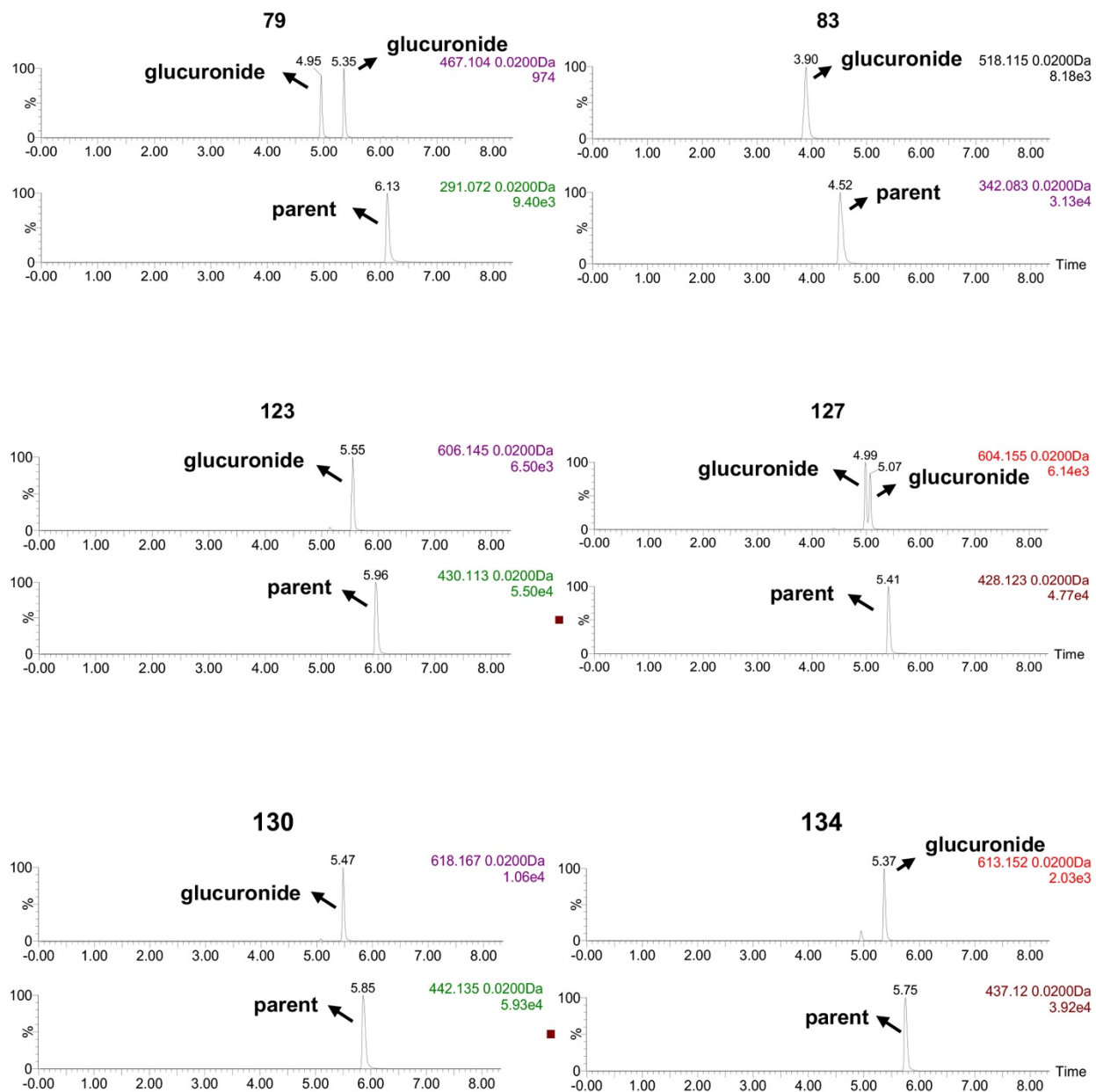
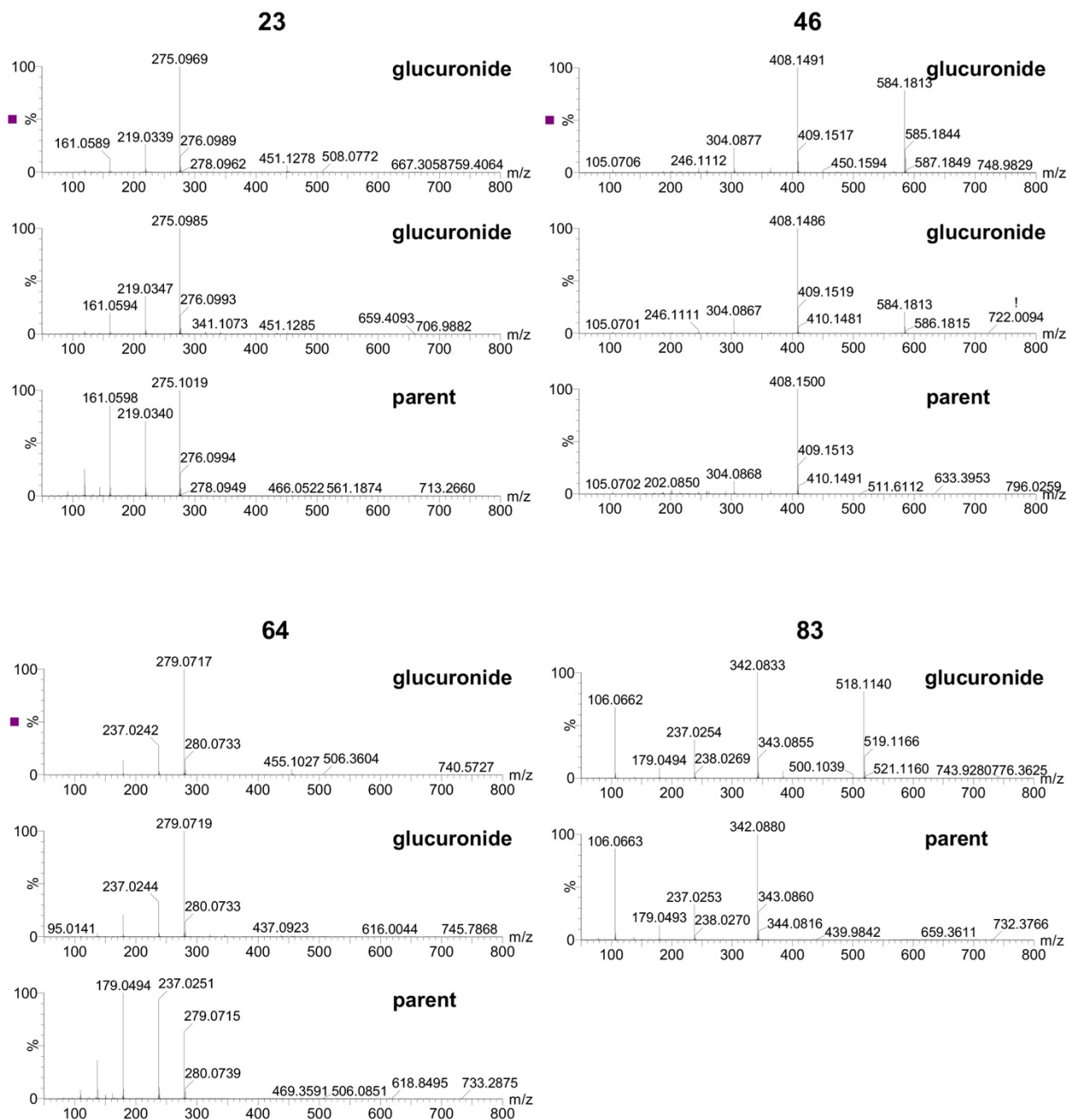


Figure S1. Extracted ion chromatograms of selected compounds and their glucuronides in MLM incubations with UDPGA after 60 min. Upper panels show extracted MS of glucuronidated product(s) and lower panels show extracted MS of parent.



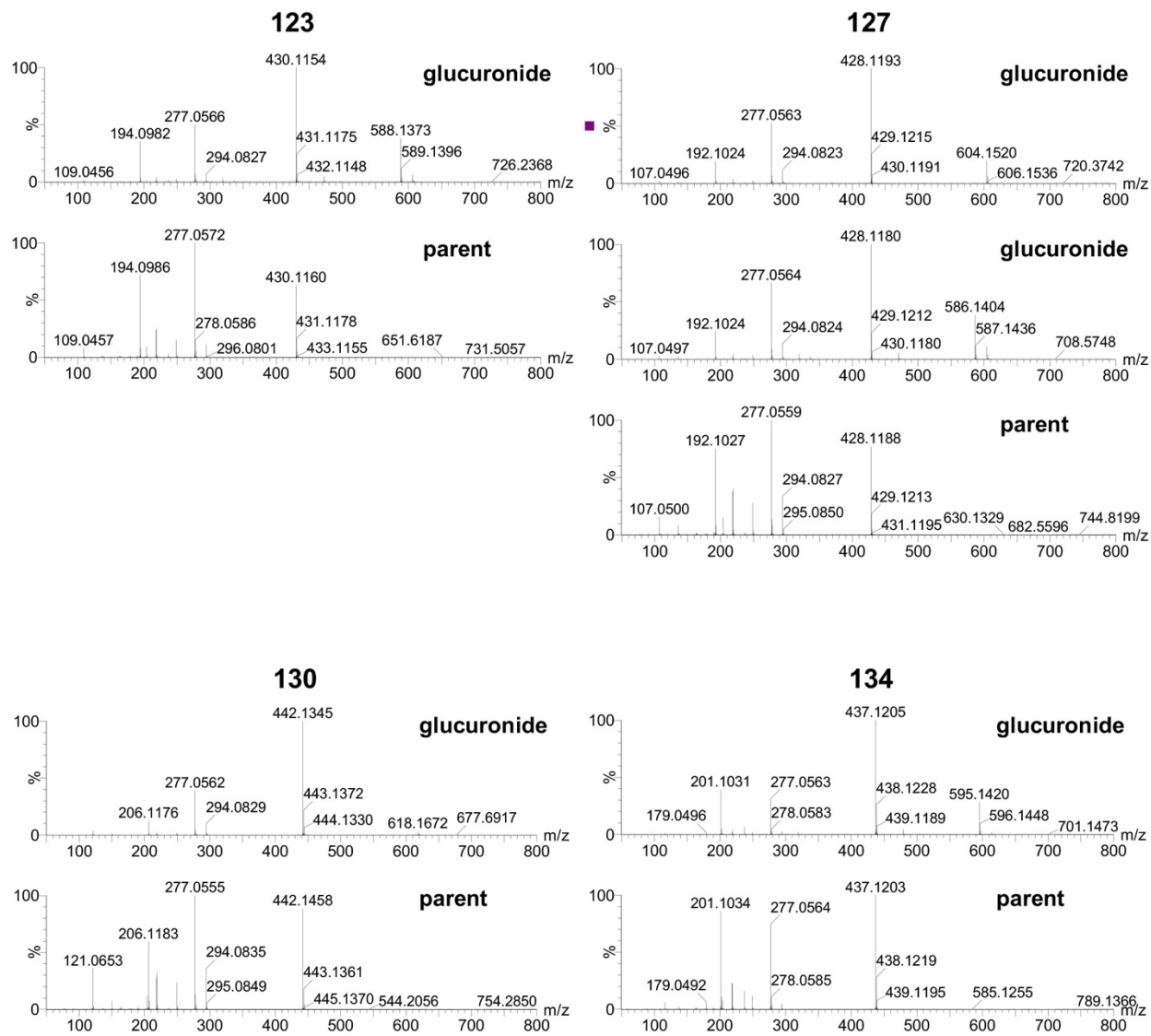


Figure S2. MS/MS spectrum of selected compounds and their glucuronides. Upper panel shows the MS/MS pattern of the glucuronide ion and lower panel shows the MS/MS pattern of the parent ion.

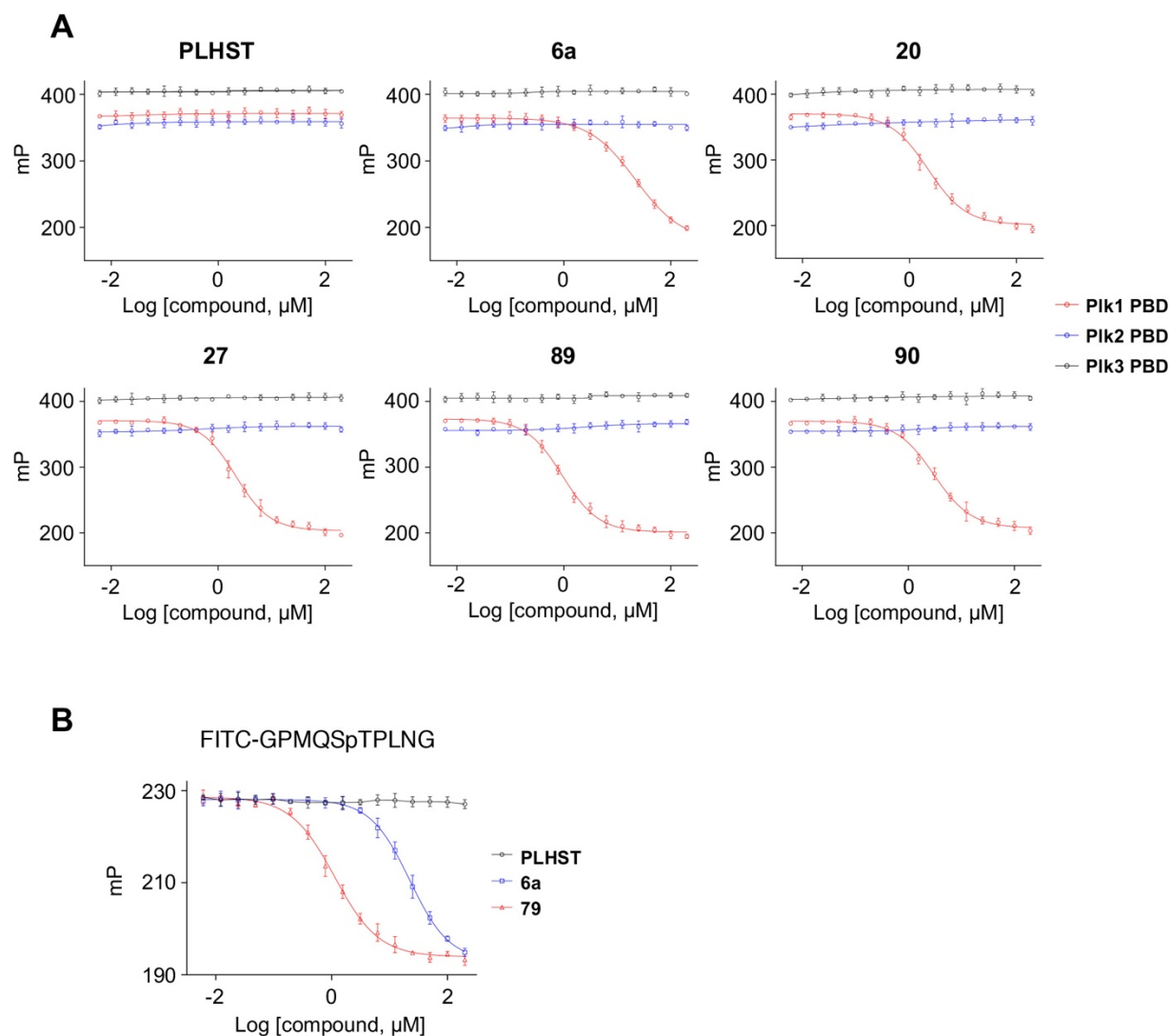


Figure S3. FP Comparative FP-based assays showing the ability of additional triazoloquinazolinone-derived compounds (**20**, **27**, **89**, and **90**) to specifically inhibit Plk1 PBD. (A) The FP assays shown here were performed currently with those in Figure 3 and therefore the data for control PLHST and **6a** are shown again. (B) To confirm anti-Plk1 PBD activity observed with the FITC-Ahx-DPPLHSpTAI-NH₂ ligand in (A), a second Plk1 PBD-binding ligand (FITC-Ahx-GPMQSpTPLNG-NH₂)²⁵ was used to carry out the assay. All the data shown in (A) and (B) are quantified from three independent experiments. Bars, mean \pm standard deviation.

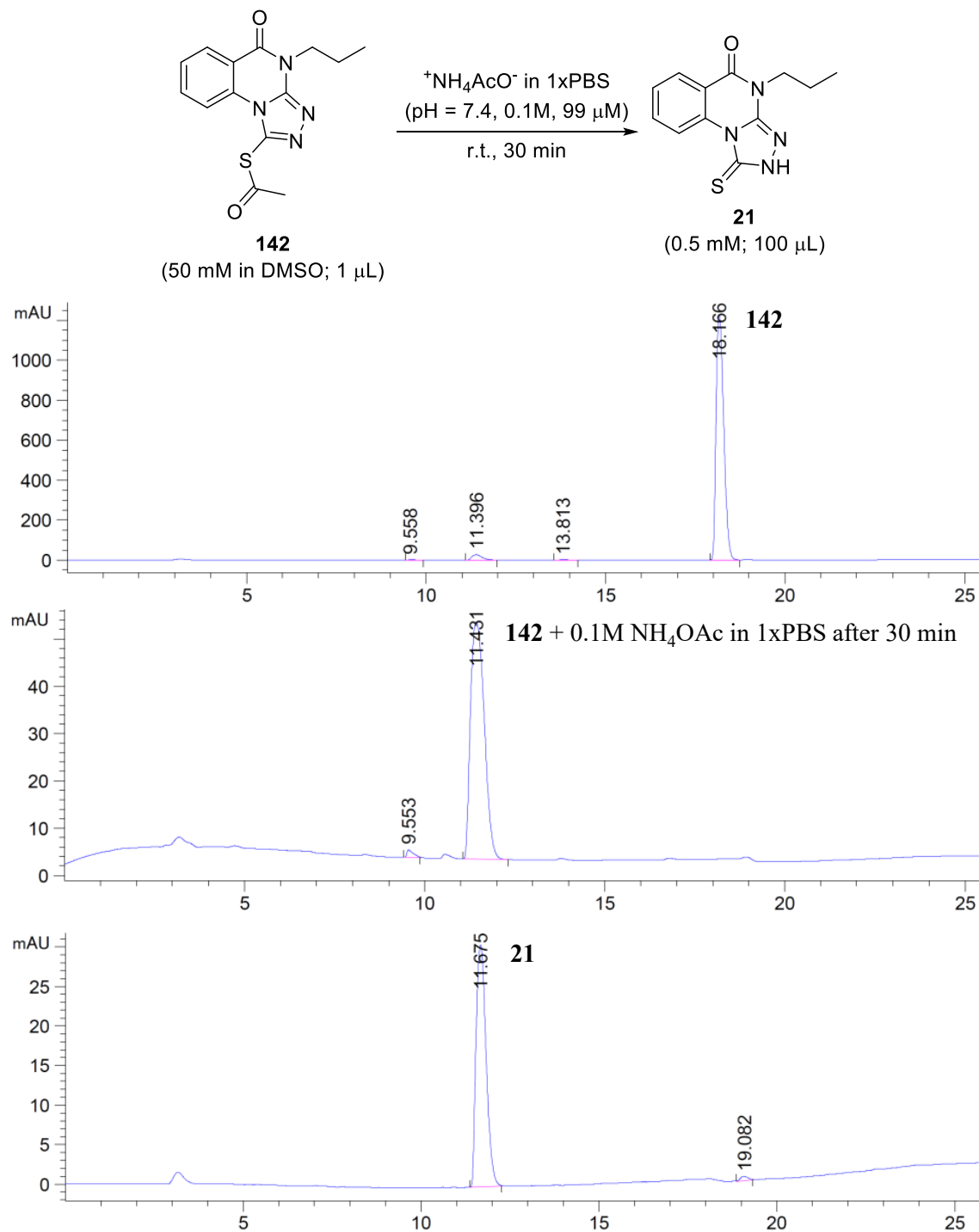


Figure S4. Acyl-transfer from **142**. (Upper Graph) HPLC chromatogram of **142**. (Middle Graph) HPLC chromatogram of compound **142** + 0.1M Ammonium acetate in 1xPBS after 30 min. (Lower Graph) HPLC chromatogram of **21**.

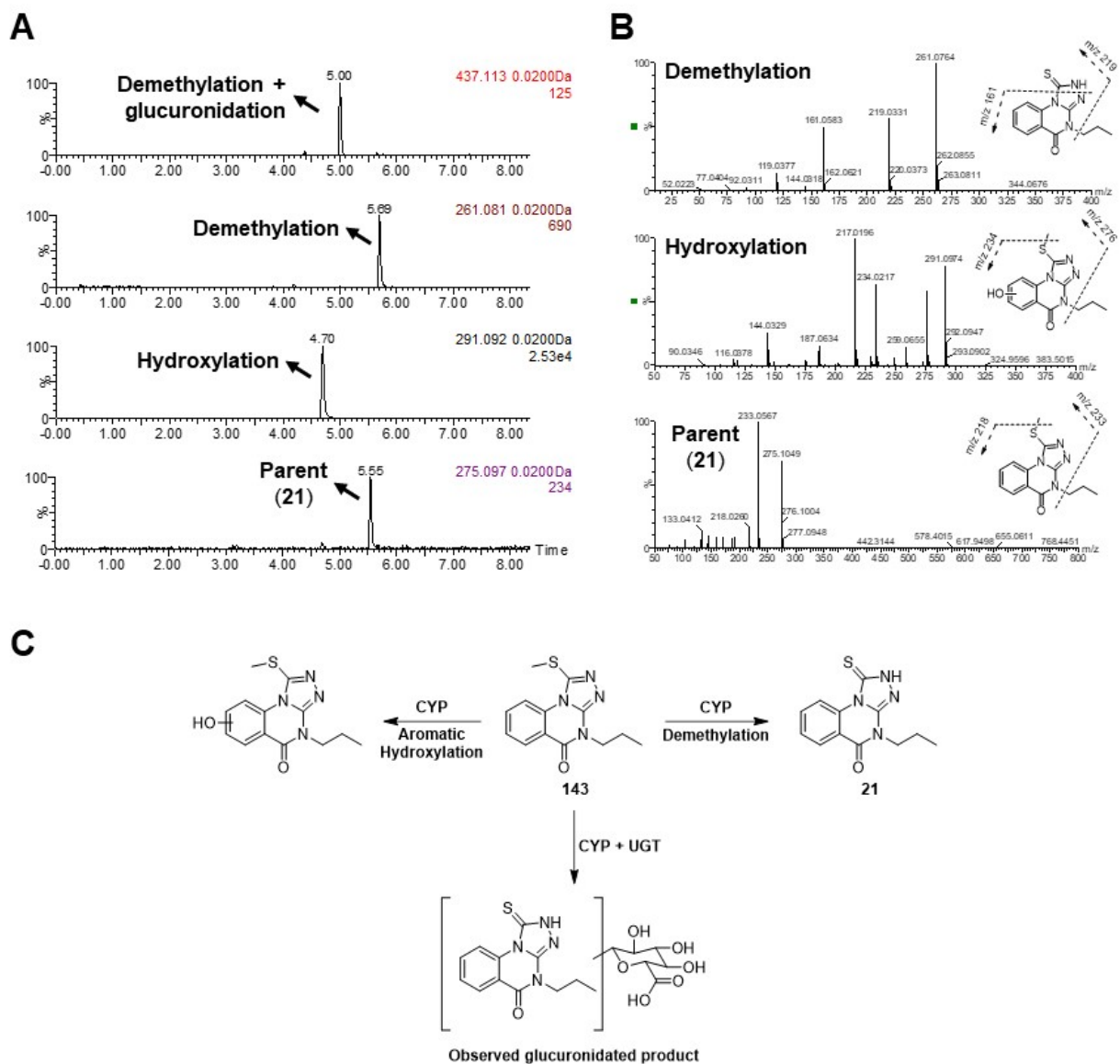


Figure S5. (A) Extracted ion chromatograms of **143** and its metabolites in MLM incubations with NADPH and UDPGA after 60 min. (B) MS/MS spectrum of **143**, its hydroxylated metabolite and its demethylated metabolite. (C) Proposed metabolic pathways of **143** in mice.

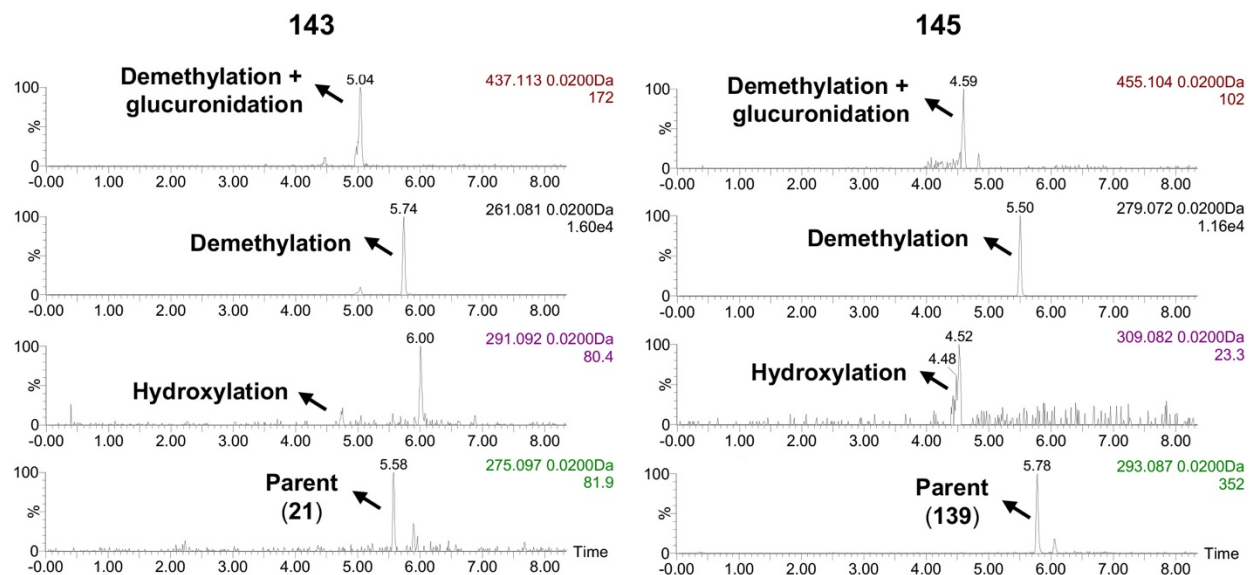


Figure S6. (A) Extracted ion chromatograms of **143** and its metabolites from MS after 20 mg/kg IP injection of C57BL/6 mice analyzed 4 hours post injection. (B) Extracted ion chromatograms of **145** and its metabolites from MS after 20 mg/kg IP injection of C57BL/6 mice analyzed 4 hours post injection.

Supplementary Tables

Table S1. Inactive *S*-alkylated compounds

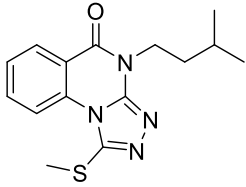
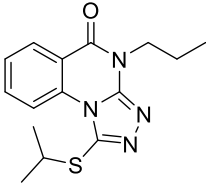
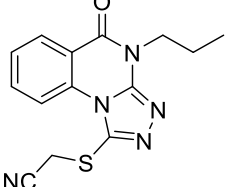
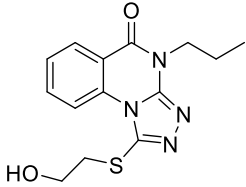
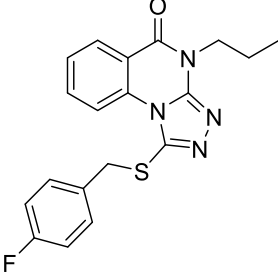
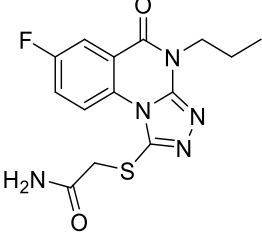
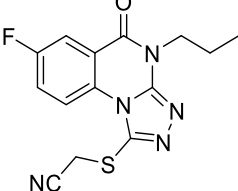
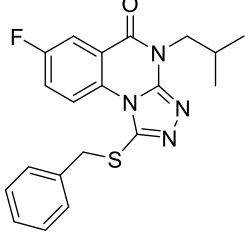
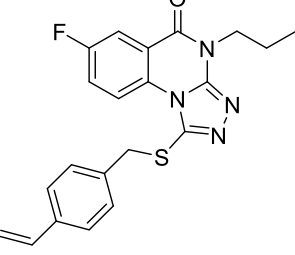
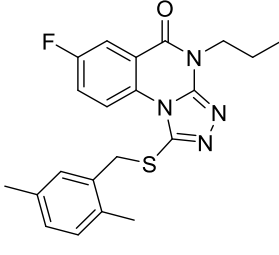
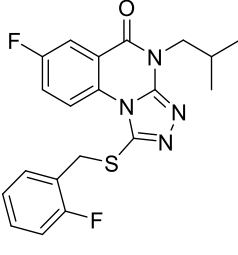
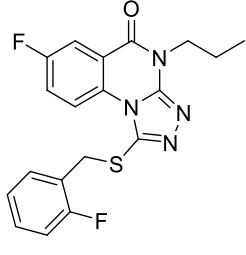
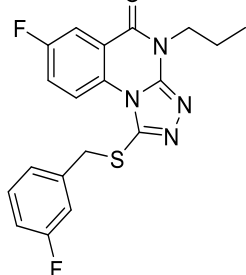
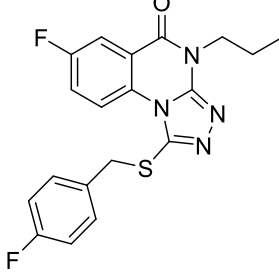
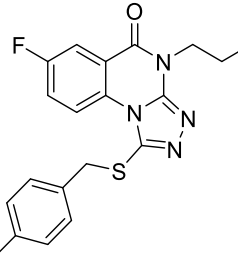
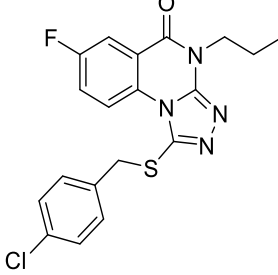
Inactives (ELISA IC ₅₀ >50 μM)			
			
			
			
			

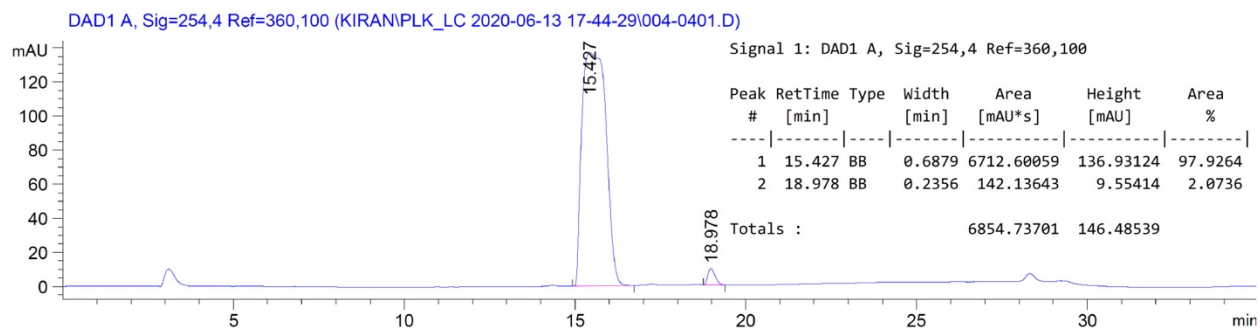
Table S2. Multi species microsomal and cytosol stability of selected compounds

Cmpd	<i>t</i>_{1/2} Human LM (min)	<i>t</i>_{1/2} Mouse LM (min)	<i>t</i>_{1/2} Rat LM (min)
21	>120	>120	>120
64	>120	>120	119.71
68	>120	>120	106.37
69	>120	88.35	>120
80	50.83	105.09	>120
130	>120	78.19	54.81
134	>120	>120	>120
143	16.80	3.77	9.41
144	14.45	10.58	26.36
	<i>t</i>_{1/2} Human cytosol (min)	<i>t</i>_{1/2} Mouse cytosol (min)	<i>t</i>_{1/2} Rat cytosol (min)
21	>120	>120	>120
64	>120	>120	>120
68	>120	>120	>120
69	118.48	97.58	>120
80	>120	>120	>120
130	>120	>120	>120
134	>120	103.84	>120
143	>120	>120	>120
144	>120	>120	>120

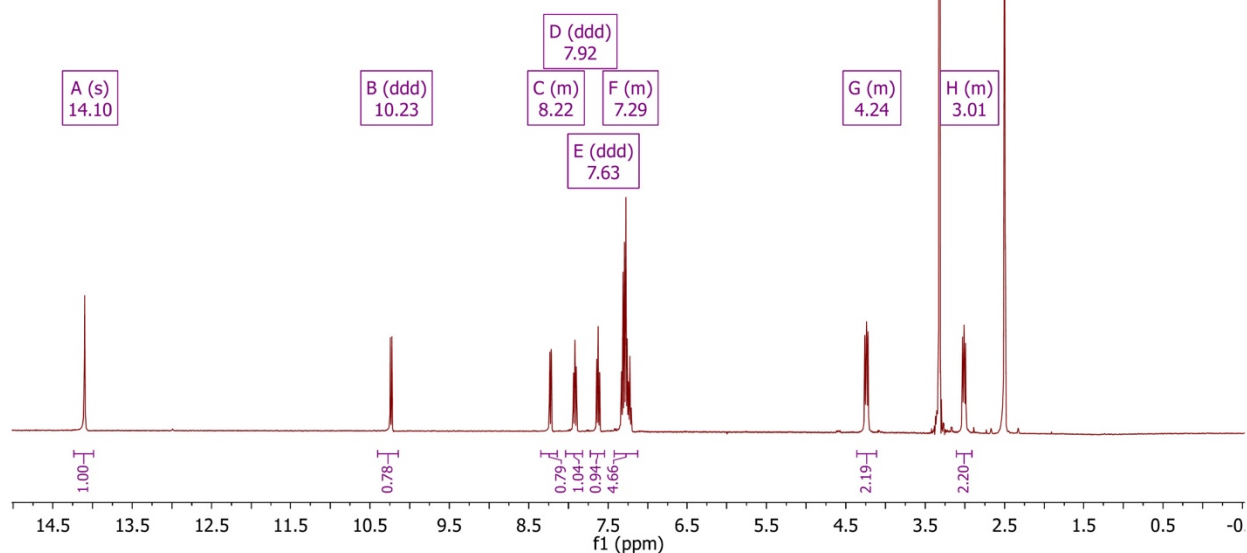
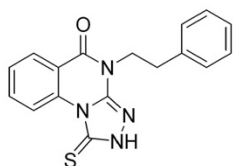
Table S3. In vitro and in vivo stability of selected prodrugs in mice

Cmpd	<i>t</i>_{1/2} in vitro (min)	<i>t</i>_{1/2} in vivo (min)
143	11.37 ± 3.49	28.74 ± 8.30
145	21.80 ± 6.03	51.26 ± 11.23

HPLC purity, NMR and HRMS for compound 7

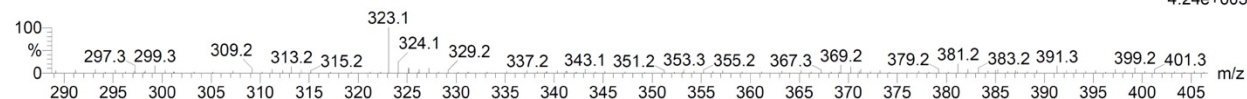


¹H NMR (400 MHz, DMSO-d₆) δ 14.10 (s, 1H), 10.23 (ddd, J=8.5, 1.1, 0.5 Hz, 1H), 8.35–8.14 (m, 1H), 7.92 (ddd, J=8.5, 7.3, 1.7 Hz, 1H), 7.63 (ddd, J=7.9, 7.4, 1.1 Hz, 1H), 7.42–7.13 (m, 5H), 4.36–4.11 (m, 2H), 3.11–2.91 (m, 2H).



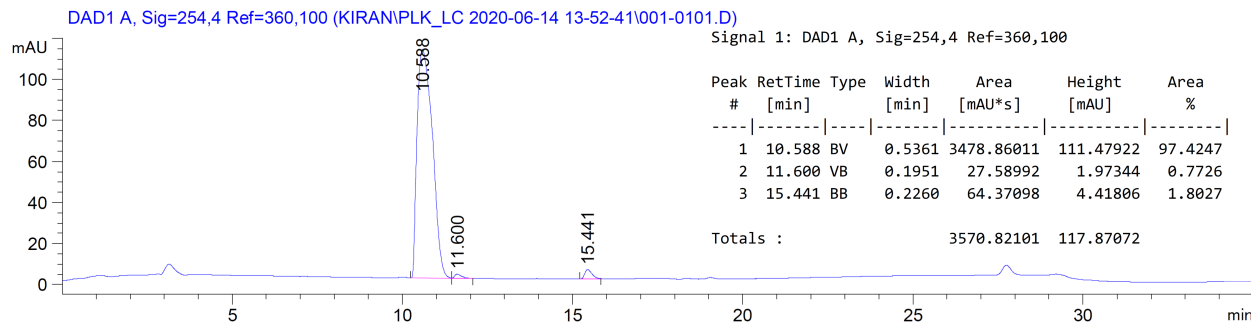
kst-28jul17-mrs-5308 170 (3.144) Cn (Cen,5, 50.00, Ar); Sm (SG, 3x5.00); Sb (12.5.00)

TOF MS ES+
4.24e+003

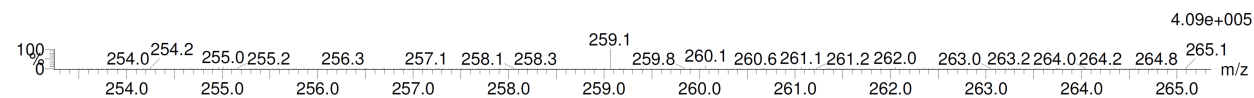
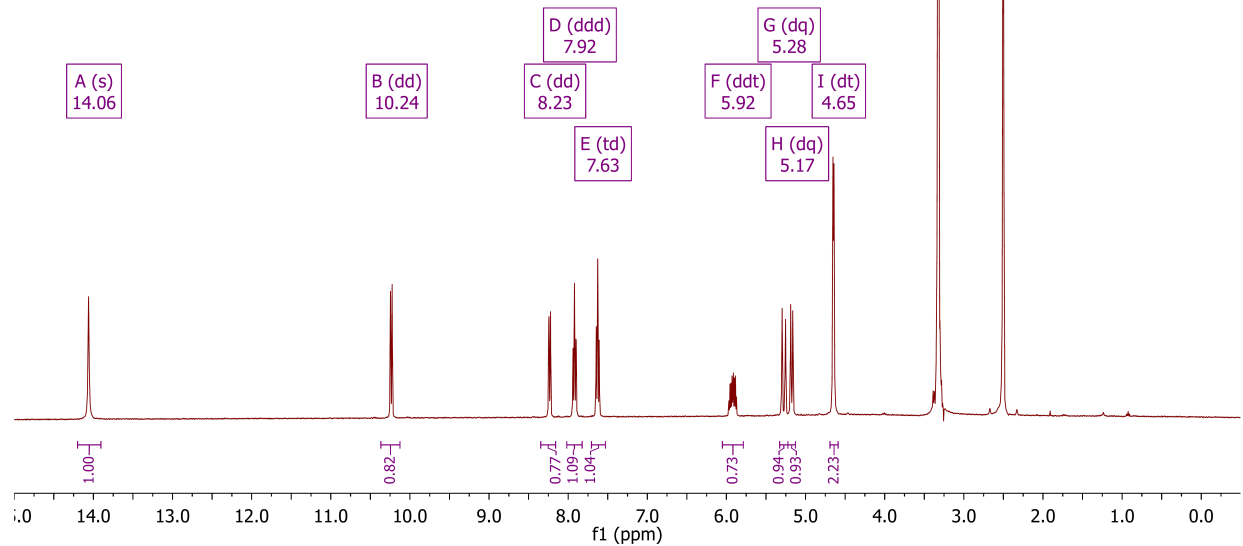
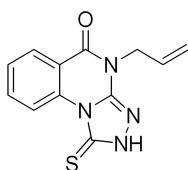


Minimum: -2.0
Maximum: 10.0 10.0 1000.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
323.0973	323.0967	0.6	1.9	12.5	122.7	C17 H15 N4 O 32S
	323.1025	-5.2	-16.1	3.5	222.7	C10 H19 N4 O6 32S

HPLC purity, NMR and HRMS for compound **22**

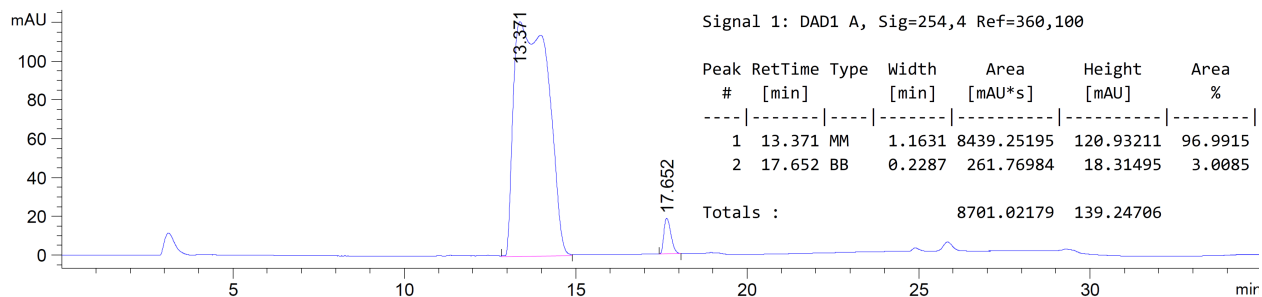
¹H NMR (400 MHz, DMSO-*d*₆) δ 14.06 (s, 1H), 10.24 (dd, *J* = 8.6, 1.1 Hz, 1H), 8.23 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.92 (ddd, *J* = 8.7, 7.3, 1.7 Hz, 1H), 7.63 (td, *J* = 7.6, 1.1 Hz, 1H), 5.92 (ddt, *J* = 17.3, 10.4, 5.2 Hz, 1H), 5.28 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.17 (dq, *J* = 10.4, 1.4 Hz, 1H), 4.65 (dt, *J* = 5.4, 1.7 Hz, 2H).



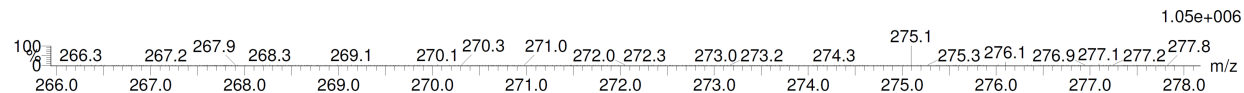
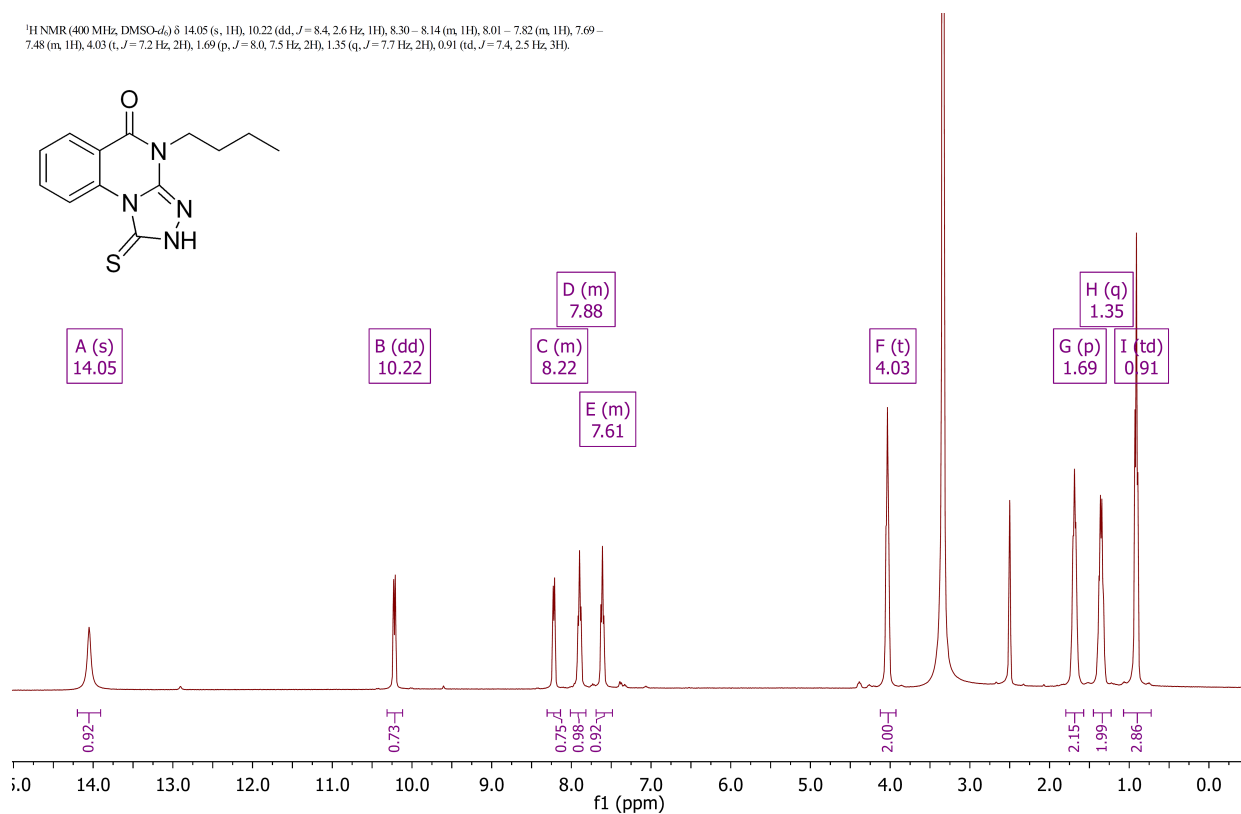
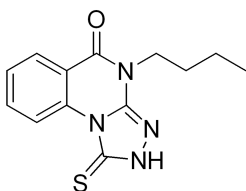
Minimum:									
Maximum:		5.0	5.0	100.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula	
259.0650	259.0654	-0.4	-1.5	9.5	570.1	n/a	n/a	C12 H11 N4 O 32S	

HPLC purity, NMR and HRMS for compound **23**

DAD1 A, Sig=254,4 Ref=360,100 (KIRAN\PLK_LC 2020-06-18 18-52-35\PLK000003.D)

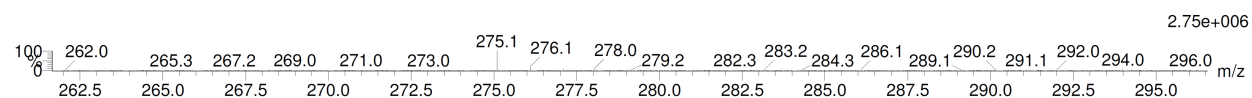
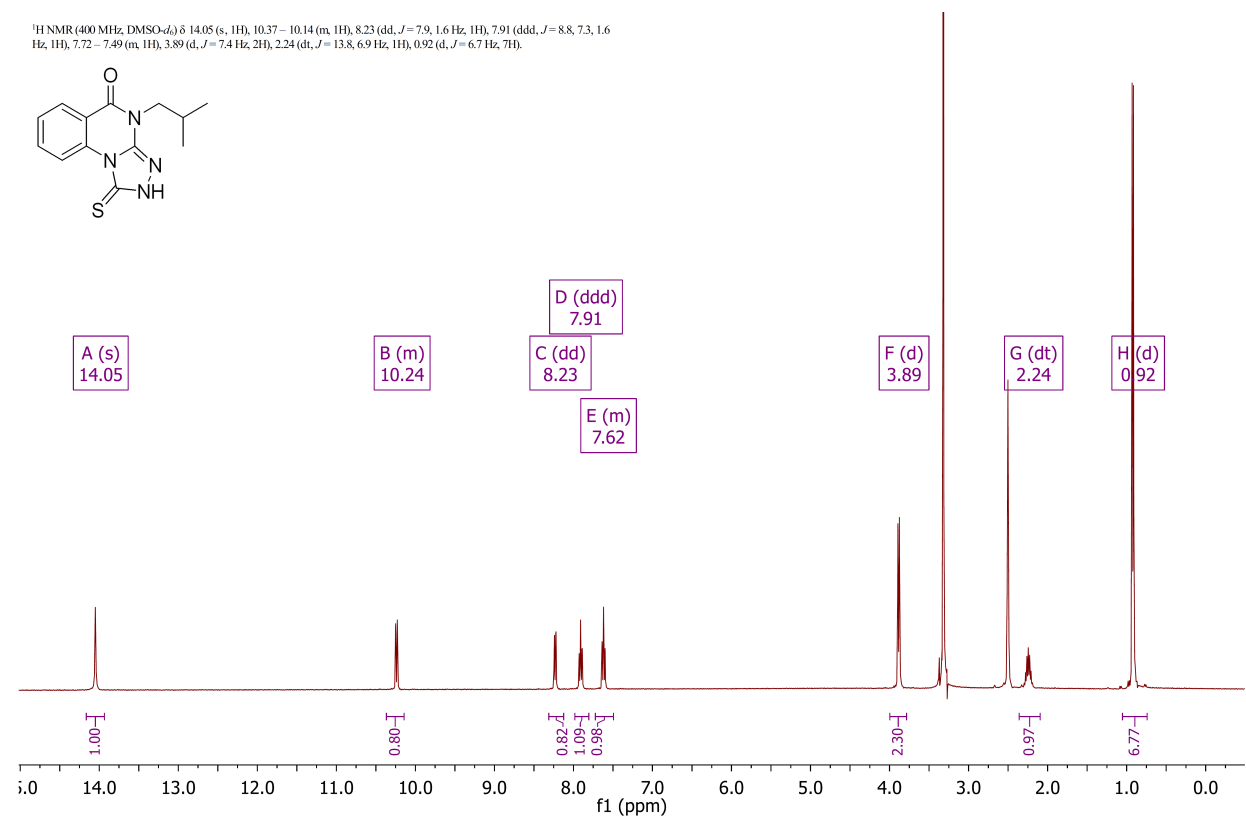
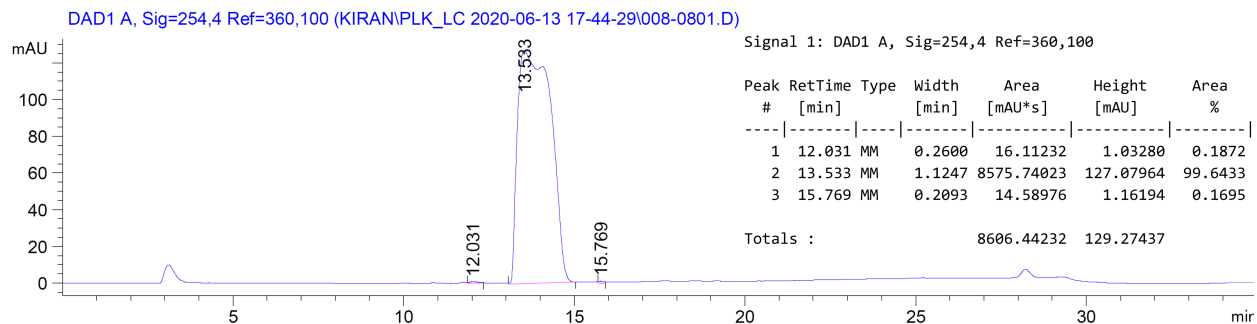


¹H NMR (400 MHz, DMSO-d₆) δ 14.05 (s, 1H), 10.22 (dd, *J* = 8.4, 2.6 Hz, 1H), 8.30 – 8.14 (m, 1H), 8.01 – 7.82 (m, 1H), 7.69 – 7.48 (m, 1H), 4.03 (t, *J* = 7.2 Hz, 2H), 1.69 (p, *J* = 8.0, 7.5 Hz, 2H), 1.35 (q, *J* = 7.7 Hz, 2H), 0.91 (td, *J* = 7.4, 2.5 Hz, 3H).

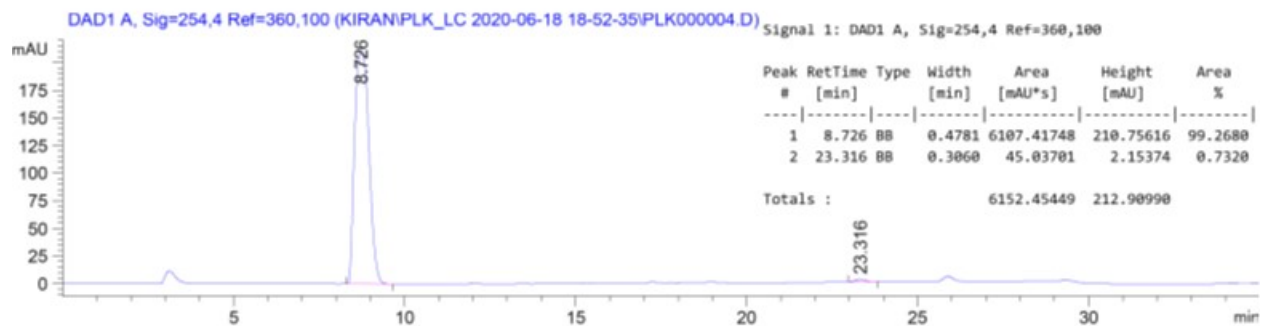


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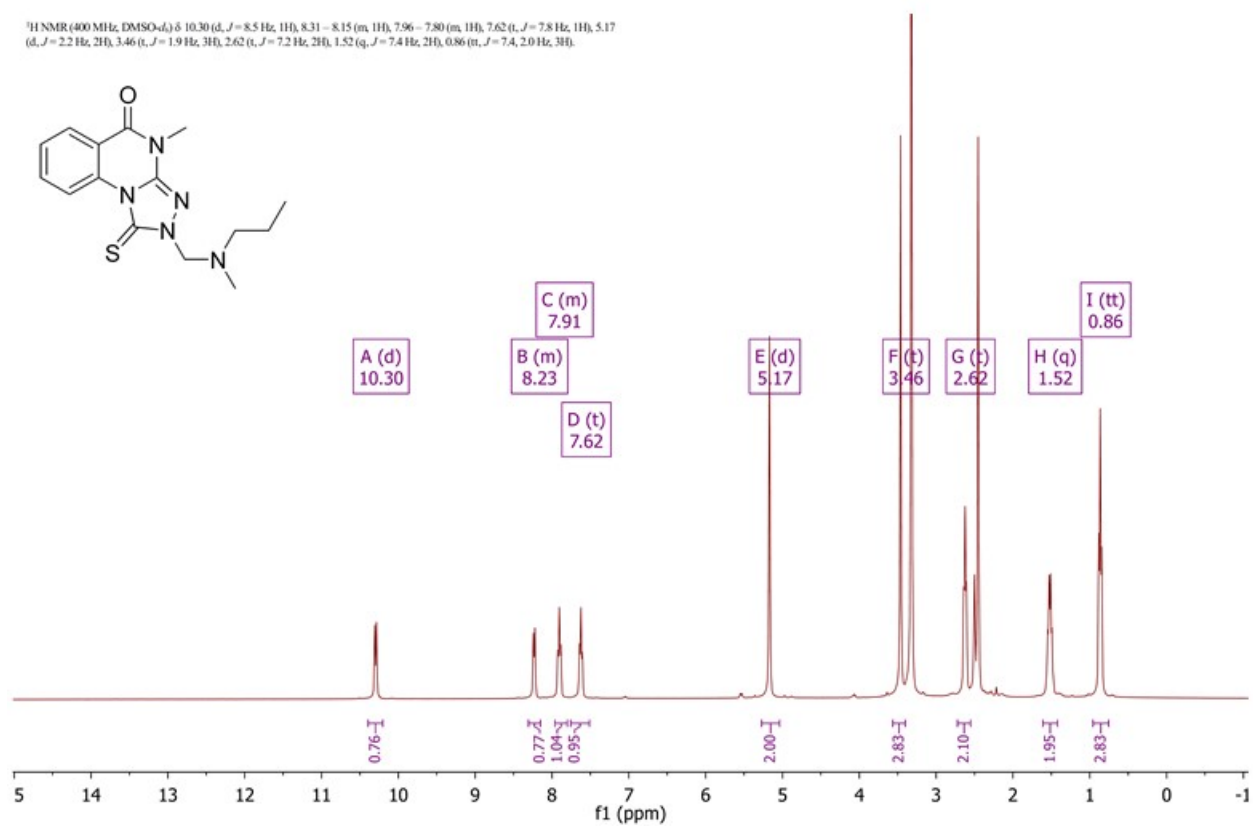
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
275.0966	275.0967	-0.1	-0.4	8.5	532.8	0.565	56.86	C13 H15 N4 O 32S
	275.0926	4.0	14.5	4.5	533.0	0.841	43.14	C8 H15 N6 O3 32S

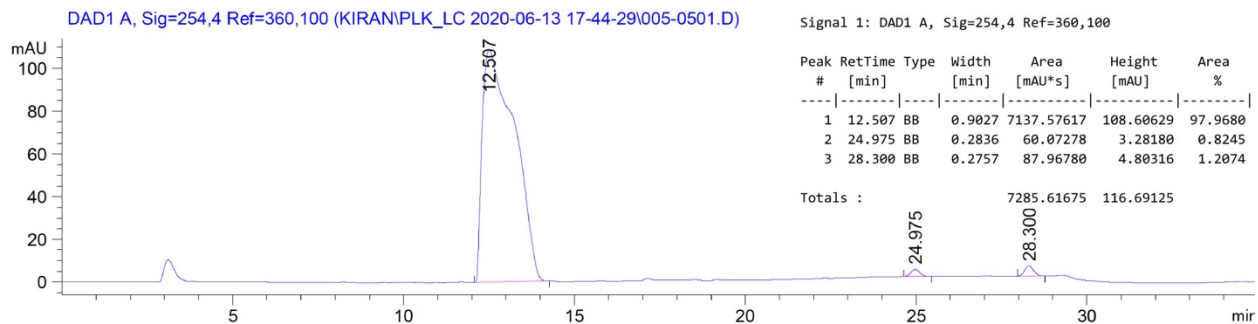
HPLC purity, NMR and HRMS for compound **26**

Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
275.0970	275.0967	0.3	1.1	8.5	677.3	n/a	n/a	C13 H15 N4 O 32S

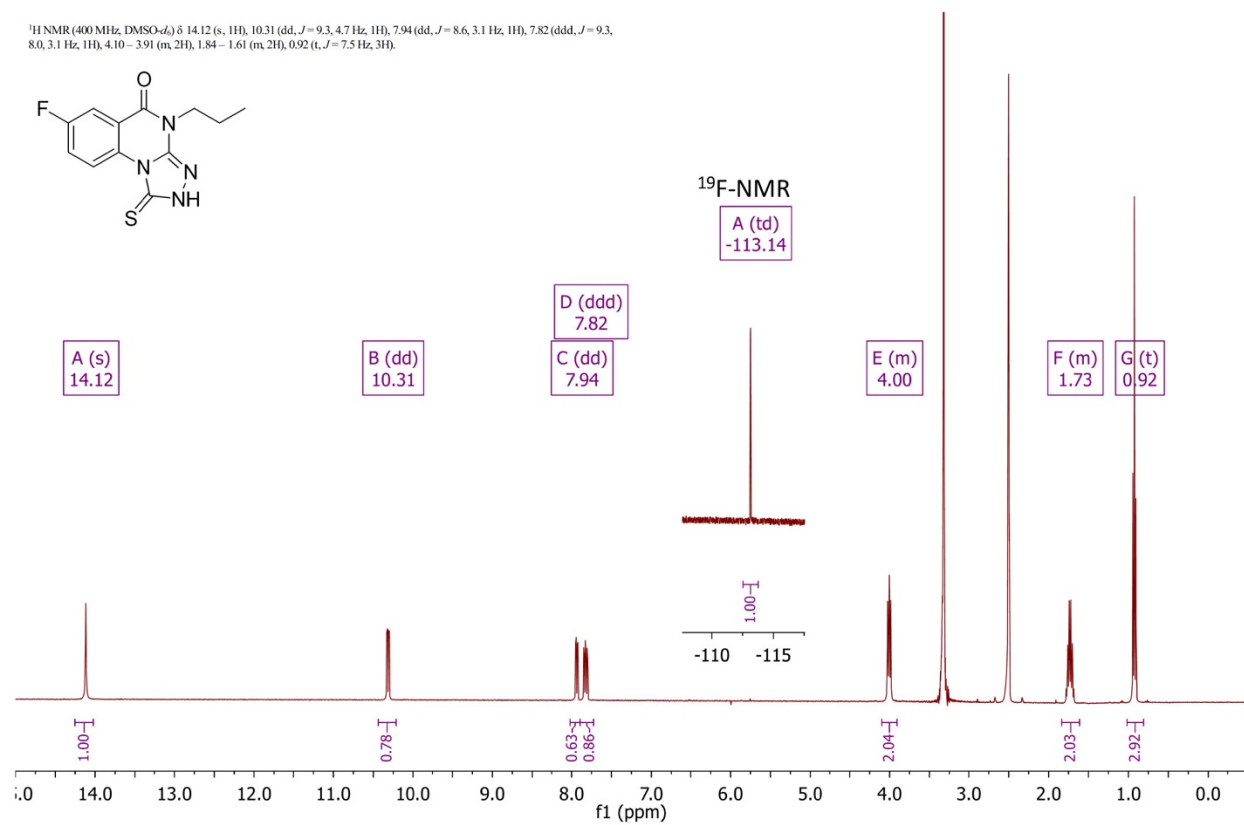
HPLC purity and NMR for compound **36**

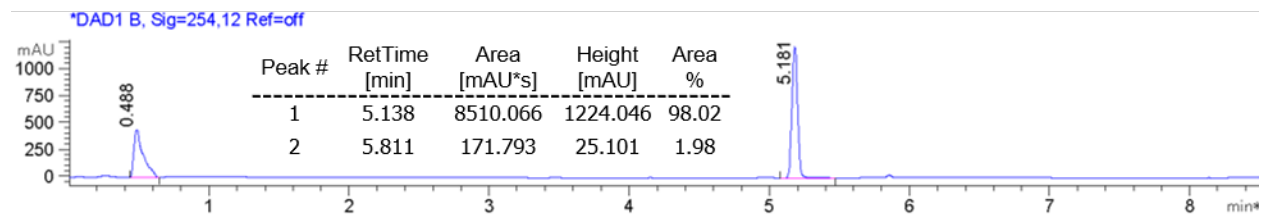
¹H NMR (400 MHz, DMSO-d₆) δ 10.30 (d, *J* = 8.5 Hz, 1H), 8.31 – 8.15 (m, 1H), 7.96 – 7.80 (m, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 5.17 (d, *J* = 2.2 Hz, 2H), 3.46 (t, *J* = 1.9 Hz, 3H), 2.62 (t, *J* = 7.2 Hz, 2H), 1.52 (q, *J* = 7.4 Hz, 2H), 0.86 (t, *J* = 7.4, 2.0 Hz, 3H).



HPLC purity, NMR and HRMS for compound **64**

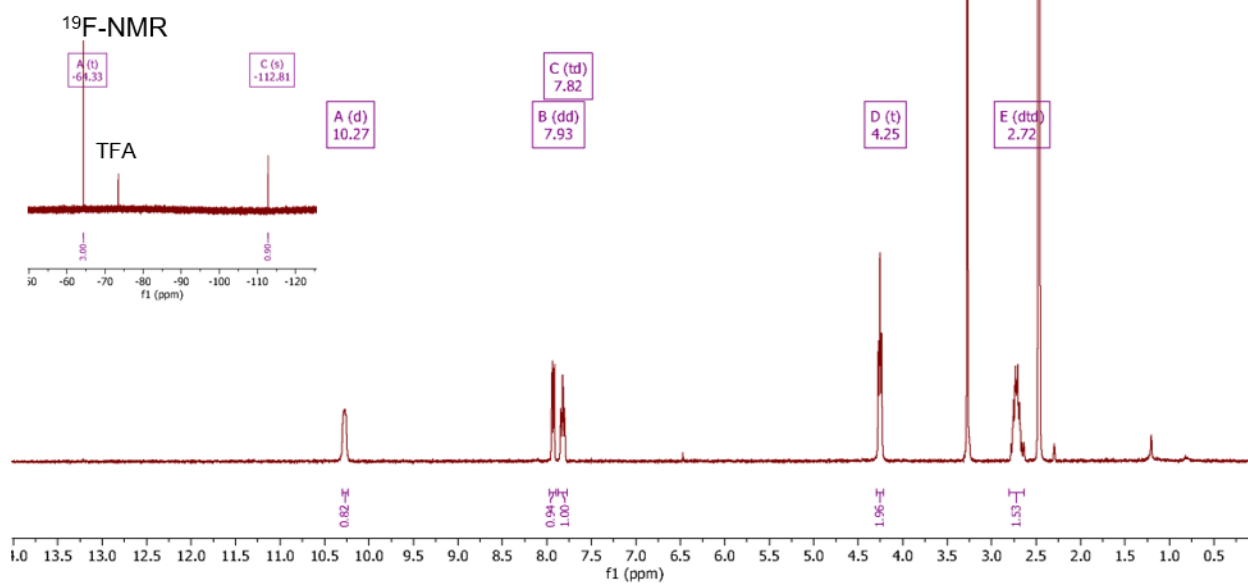
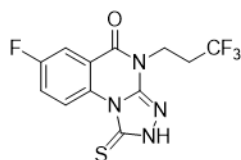
¹H NMR (400 MHz, DMSO-d₆) δ 14.12 (s, 1H), 10.31 (dd, *J* = 9.3, 4.7 Hz, 1H), 7.94 (dd, *J* = 8.6, 3.1 Hz, 1H), 7.82 (ddd, *J* = 9.3, 8.0, 3.1 Hz, 1H), 4.10 – 3.91 (m, 2H), 1.84 – 1.61 (m, 2H), 0.92 (t, *J* = 7.5 Hz, 3H).

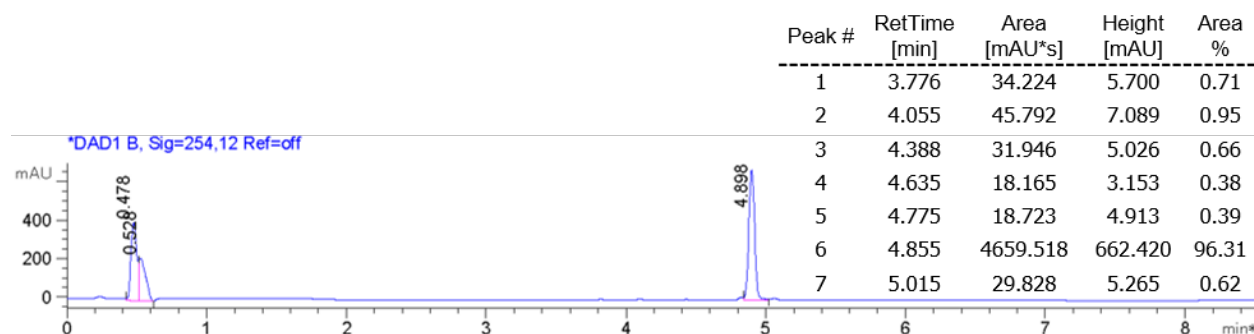


HPLC purity and NMR for compound **65**

cna002-074_PROTON_01

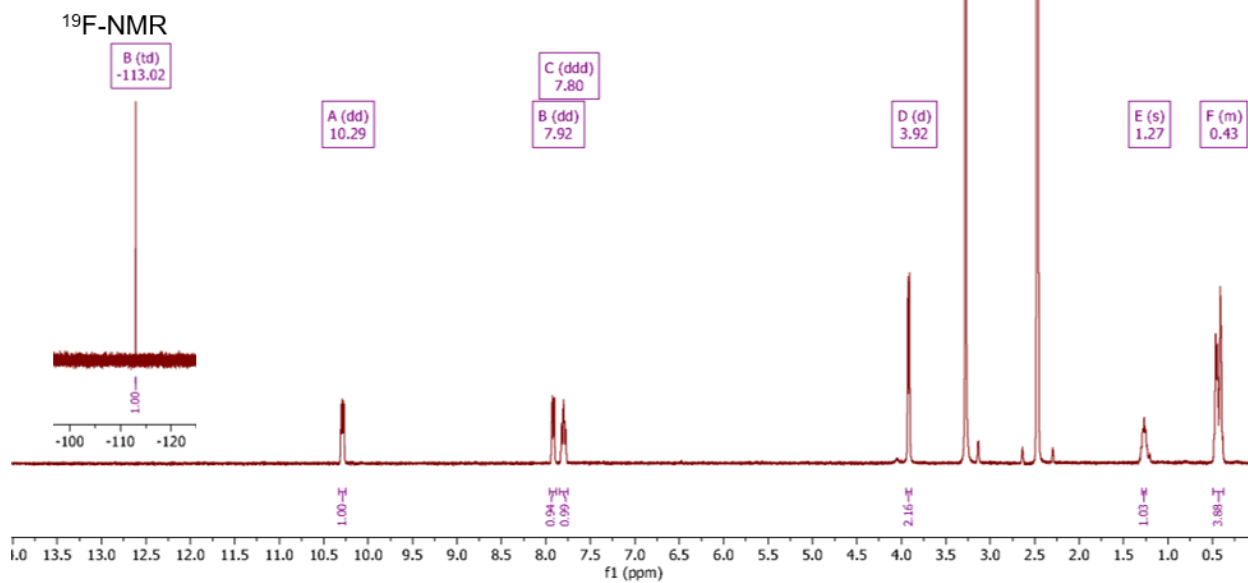
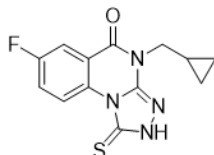
¹H NMR (400 MHz, dms_o) δ 10.27 (d, *J* = 8.4 Hz, 1H), 7.93 (dd, *J* = 8.6, 3.0 Hz, 1H), 7.82 (td, *J* = 9.3, 8.7, 3.1 Hz, 1H), 4.25 (t, *J* = 7.3 Hz, 2H), 2.72 (dtd, *J* = 18.6, 11.3, 7.7 Hz, 2H).

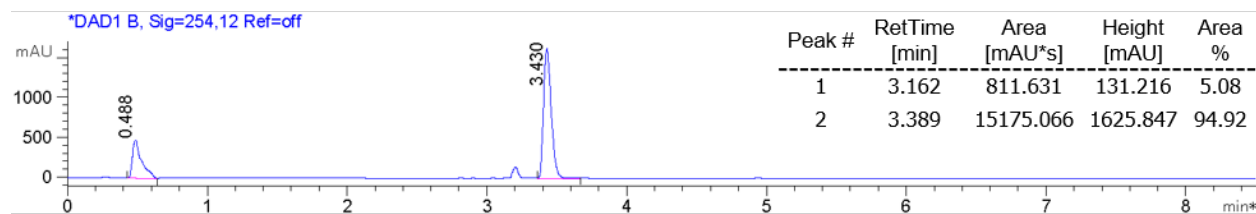


HPLC purity and NMR for compound **79**

CNA003-054_PROTON_01

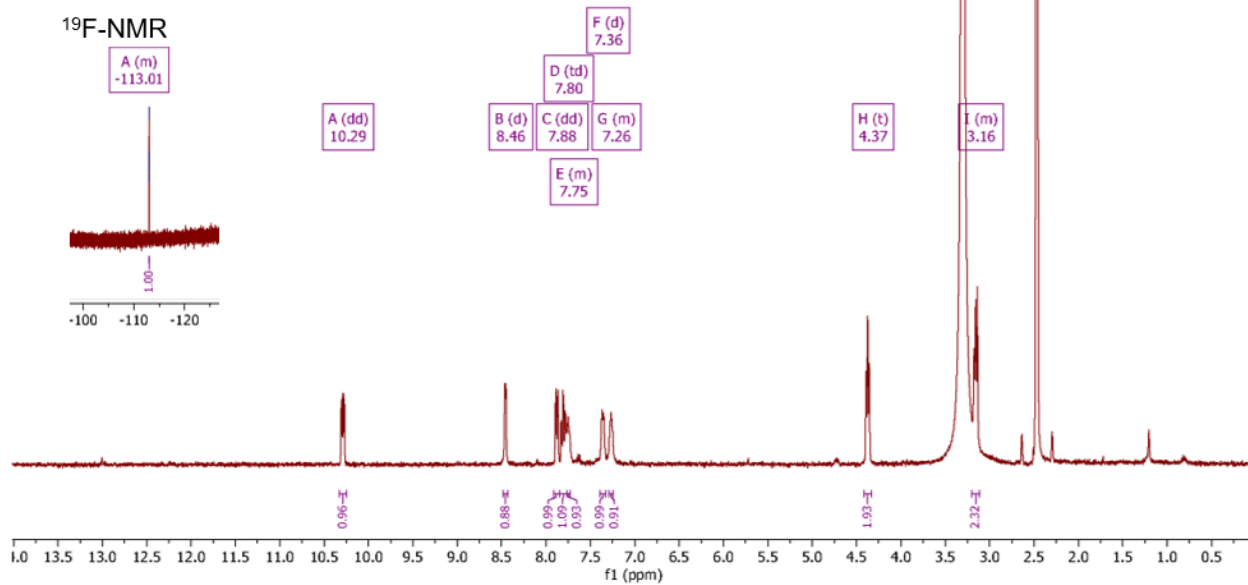
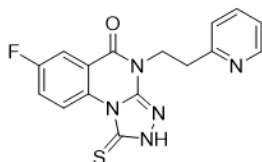
^1H NMR (400 MHz, $\text{dms}\text{-}d_6$) δ 10.29 (dd, $J=9.3, 4.7$ Hz, 1H), 7.92 (dd, $J=8.6, 3.1$ Hz, 1H), 7.80 (ddd, $J=9.2, 7.9, 3.1$ Hz, 1H), 3.92 (d, $J=7.1$ Hz, 2H), 1.27 (s, 1H), 0.49–0.37 (m, 4H).

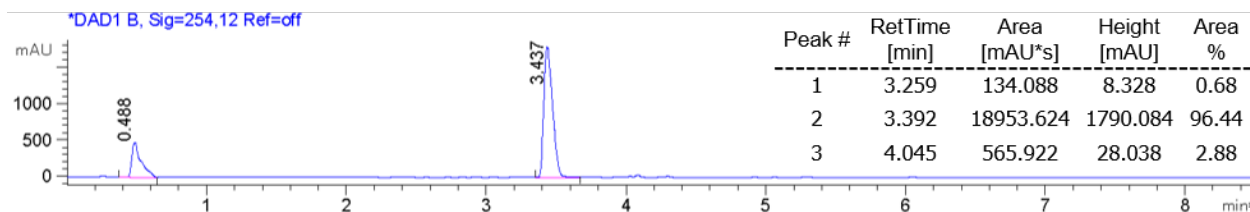


HPLC purity and NMR for compound **83**

cna002-068_PROTON_01

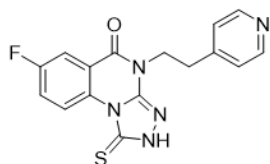
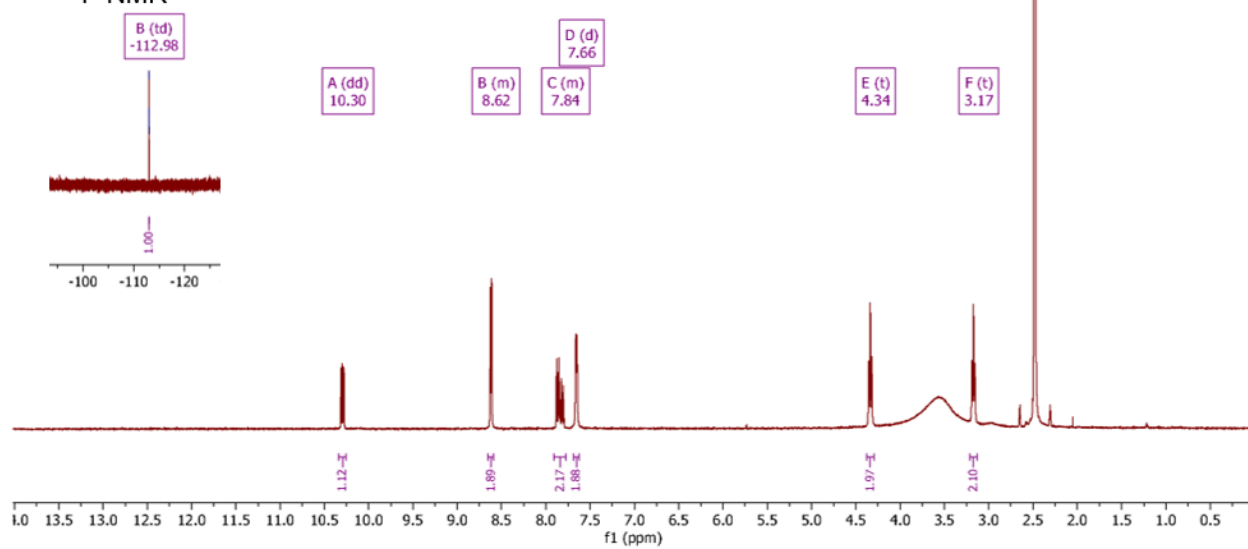
¹H NMR (400 MHz, dms_o) δ 10.29 (dd, *J* = 9.3, 4.7 Hz, 1H), 8.46 (d, *J* = 4.9 Hz, 1H), 7.88 (dd, *J* = 8.6, 3.1 Hz, 1H), 7.80 (td, *J* = 8.6, 3.1 Hz, 1H), 7.76–7.73 (m, 1H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.29–7.24 (m, 1H), 4.37 (t, *J* = 7.6 Hz, 2H), 3.20–3.11 (m, 2H).

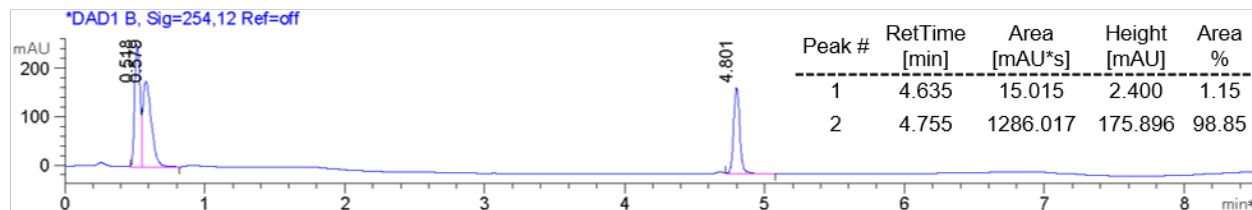


HPLC purity and NMR for compound **85**

cna002-078_PROTON_01

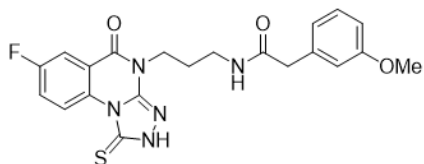
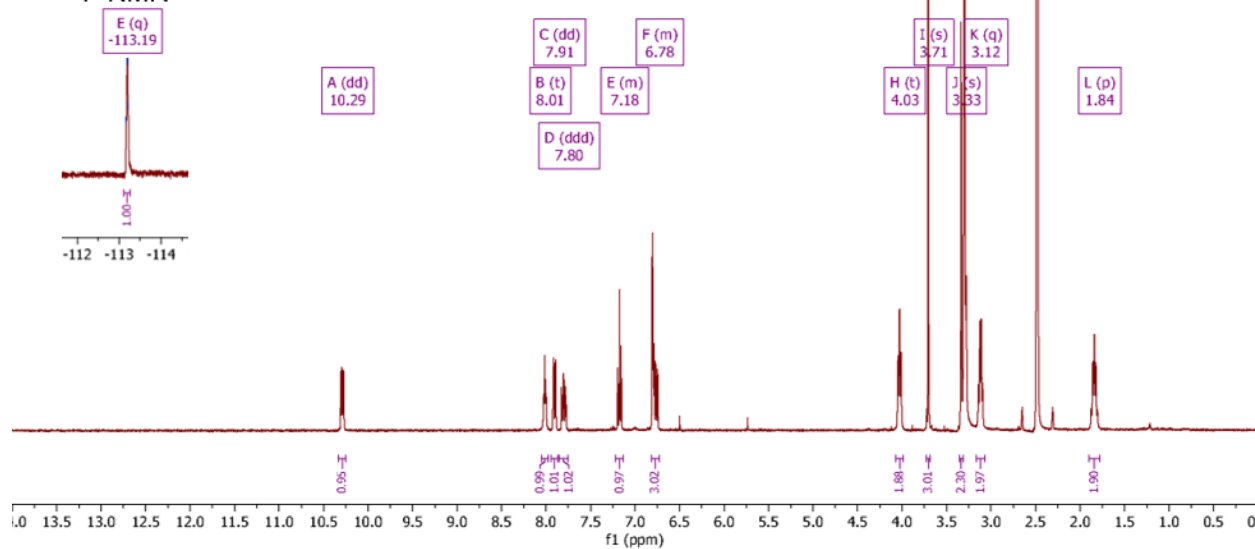
¹H NMR (400 MHz, dms_o) δ 10.30 (dd, *J* = 9.3, 4.7 Hz, 1H), 8.65 – 8.59 (m, 2H), 7.91 – 7.77 (m, 2H), 7.66 (d, *J* = 5.5 Hz, 2H), 4.34 (t, *J* = 7.1 Hz, 2H), 3.17 (t, *J* = 7.1 Hz, 2H).

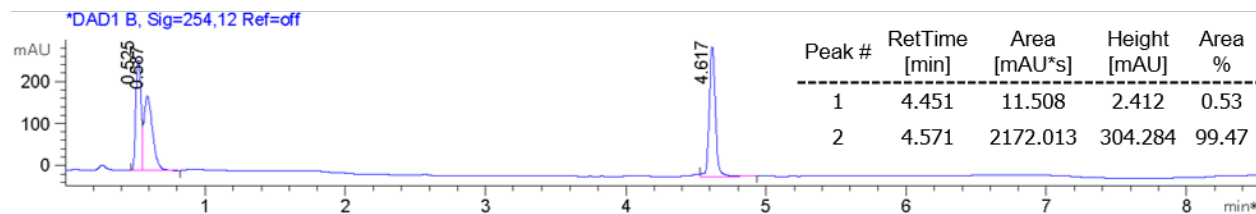
¹⁹F-NMR

HPLC purity and NMR for compound **129**

CNA007-058_PROTON_01

¹H NMR (400 MHz, DMSO-d₆) δ 10.29 (dd, *J* = 9.3, 4.7 Hz, 1H), 8.01 (t, *J* = 5.7 Hz, 1H), 7.91 (dd, *J* = 8.6, 3.1 Hz, 1H), 7.80 (ddd, *J* = 9.3, 7.9, 3.1 Hz, 1H), 7.22 – 7.13 (m, 1H), 6.82 – 6.73 (m, 3H), 4.03 (t, *J* = 7.3 Hz, 2H), 3.71 (s, 3H), 3.33 (s, 2H), 3.12 (q, *J* = 6.7 Hz, 2H), 1.84 (p, *J* = 7.2 Hz, 2H).

¹⁹F-NMR

HPLC purity and NMR for compound **134**

CNA007-062_PROTON_01

¹H NMR (400 MHz, dms_o) δ 10.29 (dd, *J* = 9.3, 4.7 Hz, 1H), 8.16 (t, *J* = 5.6 Hz, 1H), 7.91 (dd, *J* = 8.6, 3.1 Hz, 1H), 7.80 (ddd, *J* = 9.3, 8.0, 3.1 Hz, 1H), 7.79 – 7.72 (m, 2H), 7.47 – 7.40 (m, 2H), 4.02 (t, *J* = 7.3 Hz, 2H), 3.50 (s, 2H), 3.18 – 3.09 (m, 2H), 1.84 (p, *J* = 7.1 Hz, 2H).

