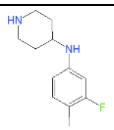
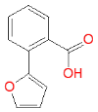
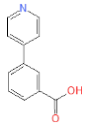
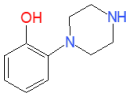
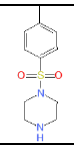
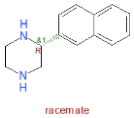
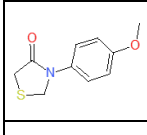
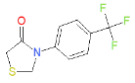
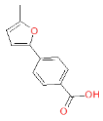
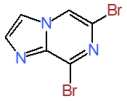
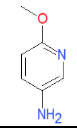
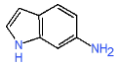
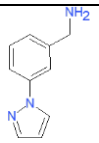
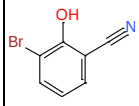
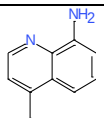
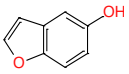
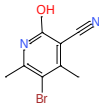
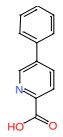
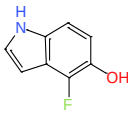
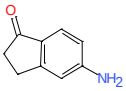
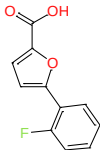
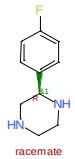
Supplementary Materials

Table S1. Fragment hits obtained from QM *Pf*DHFR screen, ΔT_m and inhibition results.

Structure	Fragment	Screen	ΔT_m (°C)	QM *Pf*DHFR inhibition at 500 µM (%)	QM *Pf*DHFR IC_{50} (mM)	WT *Pf*DHFR inhibition at 500 µM (%)	WT *Pf*DHFR IC_{50} (mM)	*Hs*DHFR inhibition at 500 µM (%)
	**002**	QM-NADPH	1.3±0.3	4.1±2.5	>2	32.4±2.2	0.7±0.04	1.7 ± 1.5
	**101**	QM-NADPH	ND	7.7±3.2		6.5±1.3		
	**136**	QM-NADPH	0.8±0.3	19.5±5.2	2.2±0.1			
	**205**	Both	1.33±0.3 (NADPH), 4.3±1.5 (Apo)	4.6±7.6	4.5±0.8	81.1±3.1	0.2±0.02	
	**218**	QM-NADPH	1.0±0	10.9±2.6	5.4±0.6	43.4±4.2	0.9±0.3	
	**273**	QM-NADPH	-0.8±0.6	9.9±6.3		20.4±5.5	4.3±0.3	
	**362**	QM-NADPH	-0.8±0.3	3.3±3.7		3.9±0.9		2.3 ± 2.0
	**363**	QM-NADPH	-1.3±0.3	2.1±2.1		1.1±4.6		
	**753**	QM-NADPH	-2.3±1.1	23.1±3.3		27.4±13.1	0.9±0.01	
	**935**	Both	-1.5±0.5 (NADPH), -1.8±1.5 (Apo)	1.3±4.4				

	**976**	QM-NADPH	n.d.	5.3±2.8				
	**1136**	Both	-0.75±0.9 (NADPH), 3.8±0.6(apo)	12.2±3.3	>2			
	**1150**	QM-NADPH	-1.8±0.6	10.2±5.8				
	**066**	QM	-1.5 ±0.9	17.8±9.4	0.9±0.1			5.4±5.4
	**163**	QM	1.8±0.3	16.3±4.4		2.3±9.3		
	**173**	QM	-1.2±1.3	12.2±2.0				
	**464**	QM	-2.2±0.8	41.7±12.9	0.6±0.02	27.5±6.8	0.6±0.04	17.3±8.7
	**626**	QM	-1.2±0.6	7.0±2.3	1.0±0.1			
	**1002**	QM	3.7±0.6	5.0±3.5	>2			
	**1078**	QM	1.2±0.3	12.1±7.9	0.9±0.02	6.8±2.0		14.5±4.5
	**1122**	QM	-1.8±0.8	2.6±8.3				
	**1158**	QM	-1.2±1.5	7.5±12.6	>2			

QM: quadruple mutant (N51I + C59R + S108N + I164L).

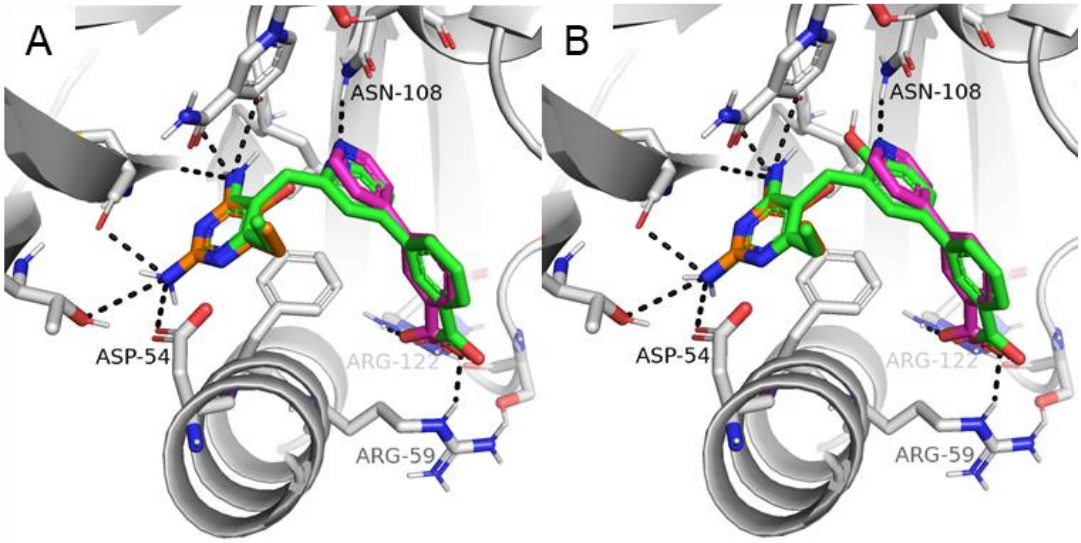


Figure S1. Molecular docking results and binding mode schematic representation for test 4 (**A**), test 5 (**B**) in the active site of QM *Pf*DHFR (PDB 4DP3). Molecular docking results for **L4** and **136** appear in orange and magenta, respectively, and polar contacts appear as black dashed lines.

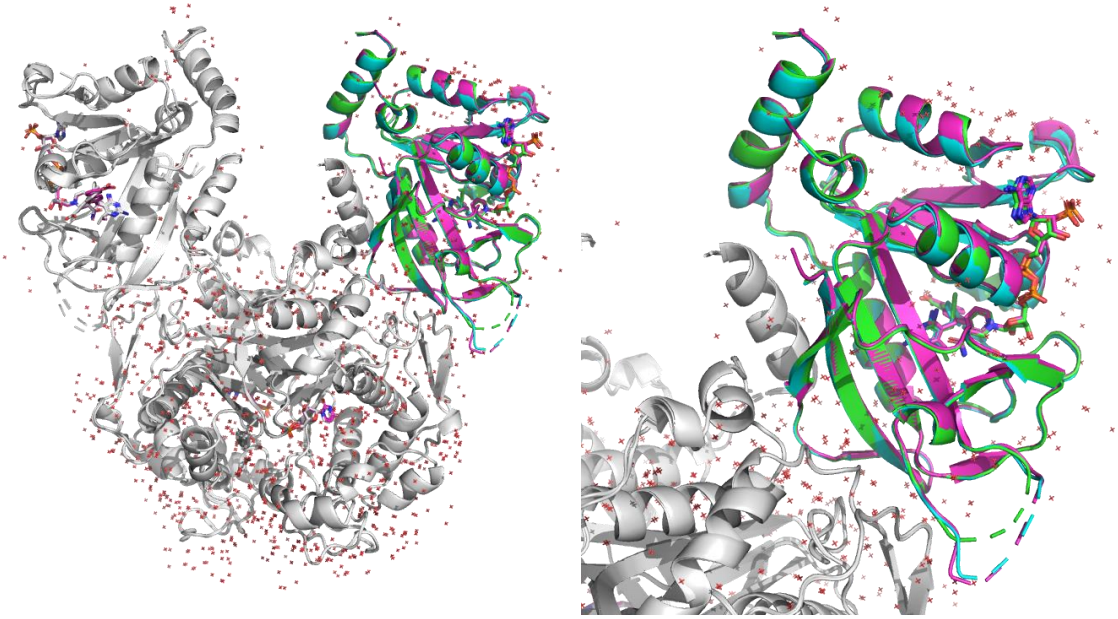


Figure S2. Superimposition of crystal structures of compounds **4** (magenta, PDB 8JFB), **6** (cyan, PDB 8JFC) and **8** (green, PDB 8JFD). *Pf*DHFR chain A appears in colour, *Pf*DHFR chain B and TS domains appear in grey for clarity.

Table S2. Data collection and refinement statistics of the ternary complexes of *Pf*DHFR-TS V1/S.

	4 (B21588)	**6 (B21591)**	**8 (B21594)**
PDB ID code	8JFB	8JFC	8JFD
Data collection			
wavelength (Å)	1.5418	1.5418	1.5418
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
cell parameters			
a, *b*, *c* (Å)	57.777, 156.233, 167.051	56.978, 155.757, 165.044	57.355, 156.500, 165.269
a, b, g (deg)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
resolution[a] (Å)	16.27-2.65 (2.75-2.65)	24.31-2.30 (2.40-2.30)	16.76-2.30 (2.40-2.30)
completeness (%)	93.15 (96.52)	99.1 (97.7)	98.9 (98.1)
redundancy	2.82 (2.40)	3.52 (2.76)	2.64 (2.04)
$<I/\sigma(I)>$	8.38 (2.05)	10.06 (2.70)	9.49 (2.26)
Wilson B-factor	34.14	24.95	27.48
R_{merge}[b]	0.1140 (0.3949)	0.0862 (0.2639)	0.0980 (0.3406)
Refinement			
R_{work}[c]	0.2118 (0.2614)	0.1876 (0.2330)	0.2024 (0.2526)
R_{free}[c]	0.2805 (0.3449)	0.2378 (0.2917)	0.2560 (0.3243)
Average B-factors (Å^2) (No. of Atoms)			
Chains A/B			
Protein	37.0(8691)/41.9(8599)	31.8(8650)/42.3(8678)	34.5(8615)/42.4(8647)
Inhibitor	33.3/62.8 (45)	40.6/65.7 (45)	23.7/49.3 (45)
NDP	52.3/81.6 (71)	35.2/92.4 (71)	35.9/73.2 (71)
UMP	59.5/59.7 (30)	33.3/36.3 (30)	37.4/45.7 (30)
GOL	-	23.2/30.0 (12)	28.1/30.1 (12)
Water	30.52 (285)	31.53 (642)	33.76 (620)
RMSD			
Bond lengths (Å)	0.008	0.008	0.008
Bond angles (°)	0.983	0.927	0.920
Ramachadran Plot			
favored (%)	94.39	96.63	96.62
allowed (%)	5.61	3.37	3.29
outlier (%)	0	0	0.10

[a]Values in parentheses are for the highest-resolution shell.

[b]$R_{merge} = \Sigma_{hkl}\Sigma_i|I_i(hkl) - \langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of anindividual reflection and $\langle I(hkl)\rangle$ is the mean intensity of symmetry-equivalent reflections.

[c]$R_{work} = \Sigma_{hkl}||F_{obs}| - |F_{calc}||/\Sigma_{hkl}|F_{obs}|$, where F_{obs} and $F_{calc}$are the observed and calculated structure-factor amplitudes, respectively. R_{free} was calculated in the same manner as R_{work} but using only a 5% unrefined subset of the reflection data.

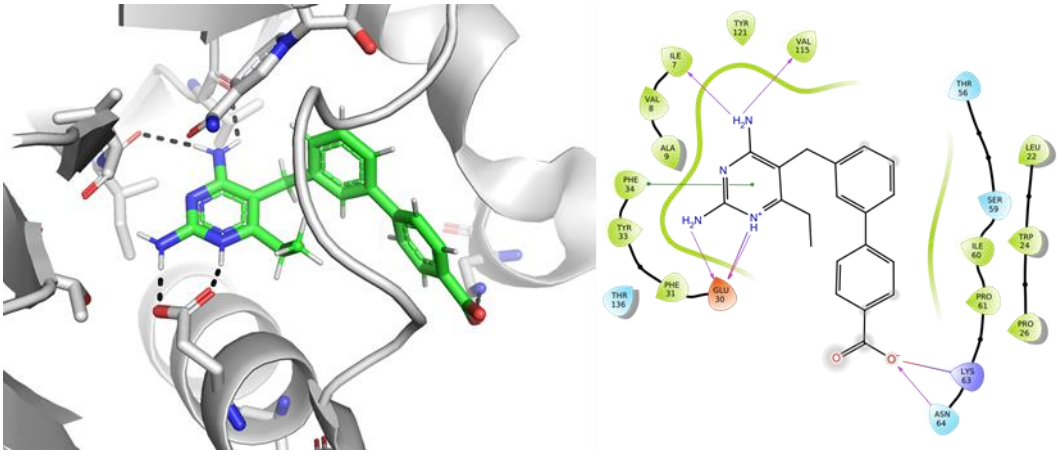


Figure S3. Molecular docking results and binding mode schematic representation for **6** in the active site of *Hs*DHFR (PDB 4DDR). Hydrogen bonds appear in pink, charge pairing interactions appear in purple, and π-π appear in green.

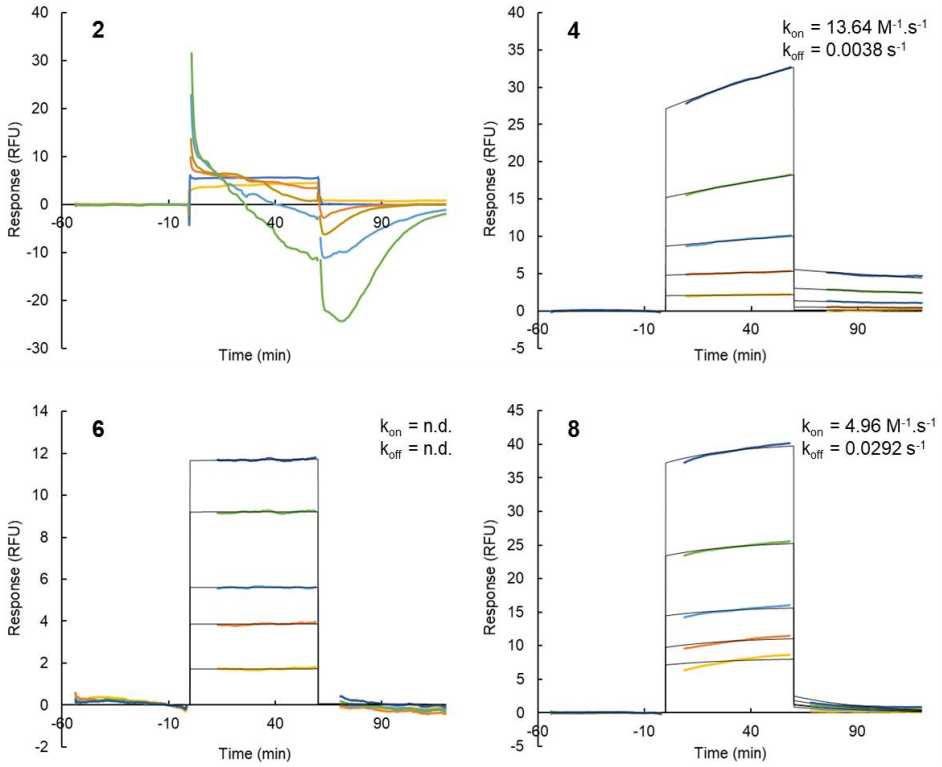


Figure S4. SPR profiles for **2**, **4**, **6** and **8** binding to QM *Pf*DHFR. Raw data appears as colour lines, curve fitting appears as thin black lines. n.d.: not determined.

^{1}H NMR in DMSO-*d*$_6$

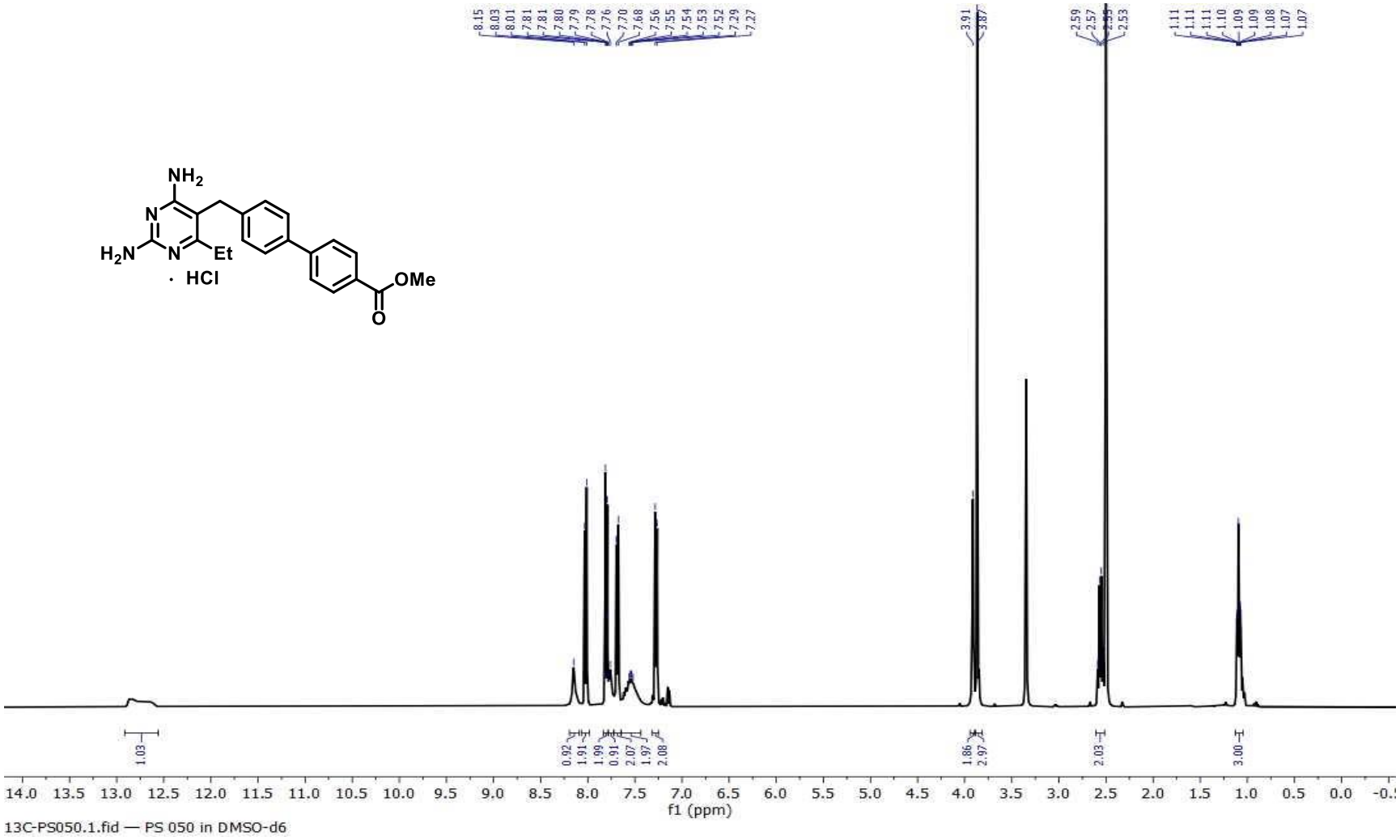


Figure S5. ^{1}H NMR spectra of compound 1-HCl.

^{13}C NMR in DMSO-d_6

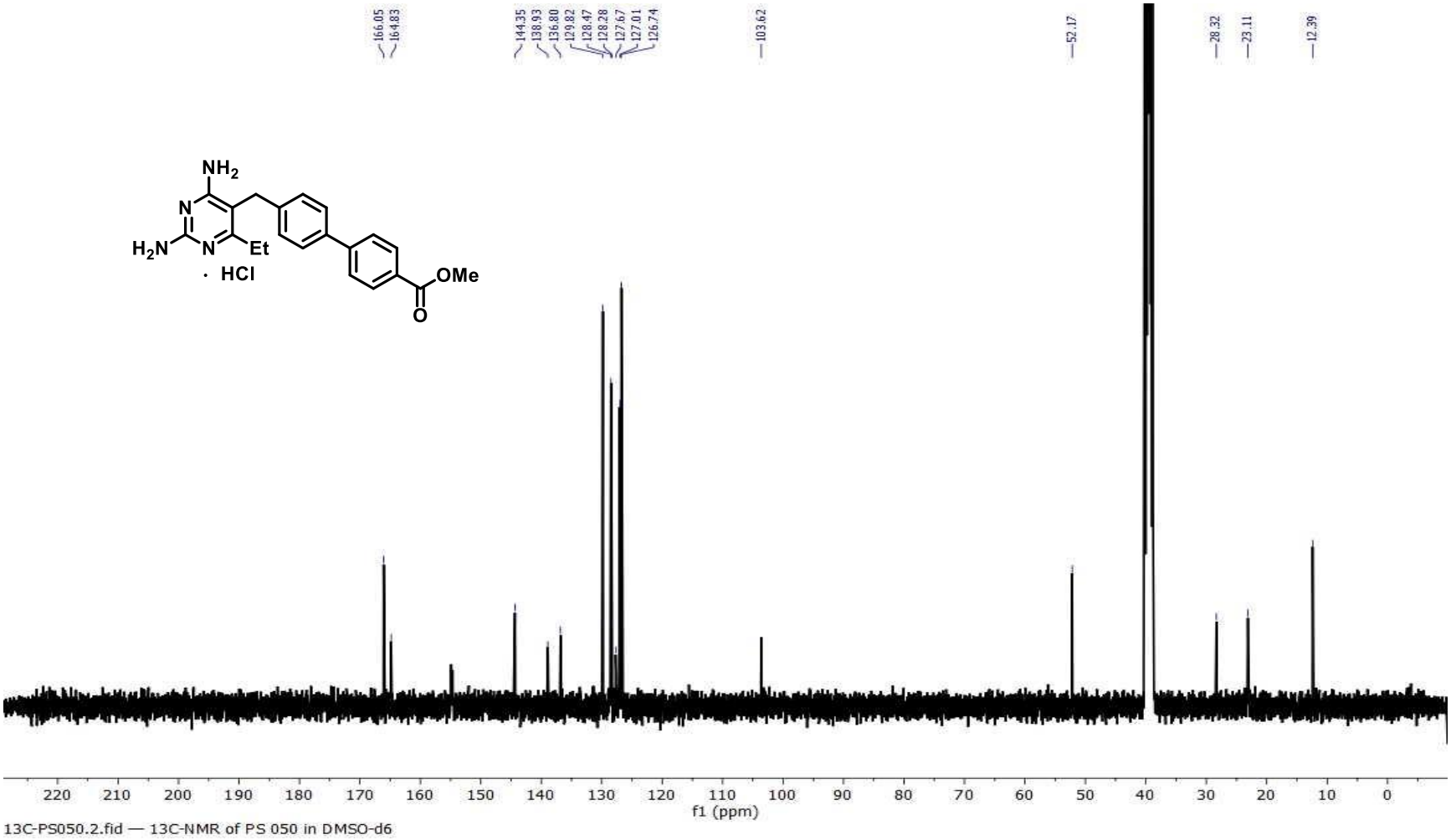
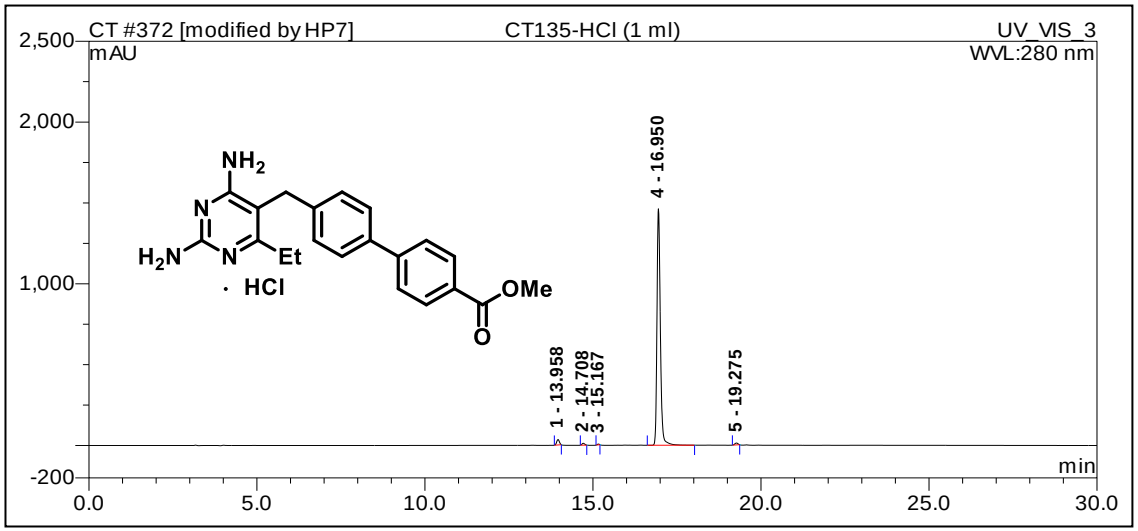


Figure S6. ^{13}C NMR spectra of compound 1-HCl.



372 CT135-HCl (1 ml)

Sample Name:	**CT135-HCl (1 ml)**	*Injection Volume:*	**2.0**
Vial Number:	**RD3**	*Channel:*	**UV_VIS_3**
Sample Type:	**unknown**	*Wavelength:*	**280.0**
Control Program:	**15-60_30min_AB_flow 0-6_column B (1-2_6-1)**	*Bandwidth:*	**1**
Quantif. Method:	**Standard CALIBRATION**	*Dilution Factor:*	**1.0000**
Recording Time:	**12/8/2021 18:16**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount ng	Type
1	13.96	na	33.427	3.130	1.77	n.a.	BMB*
2	14.71	na	11.534	1.058	0.60	n.a.	BMB*
3	15.17	na	4.409	0.304	0.17	n.a.	BMB*
4	16.95	na	1461.851	170.811	96.75	n.a.	BMB
5	19.28	na	11.540	1.250	0.71	n.a.	BMB*
Total:			1522.761	176.553	100.00	0.000	

Figure S7. HPLC profile of compound 1-HCl.

^{1}H NMR in DMSO-*d*$_6$

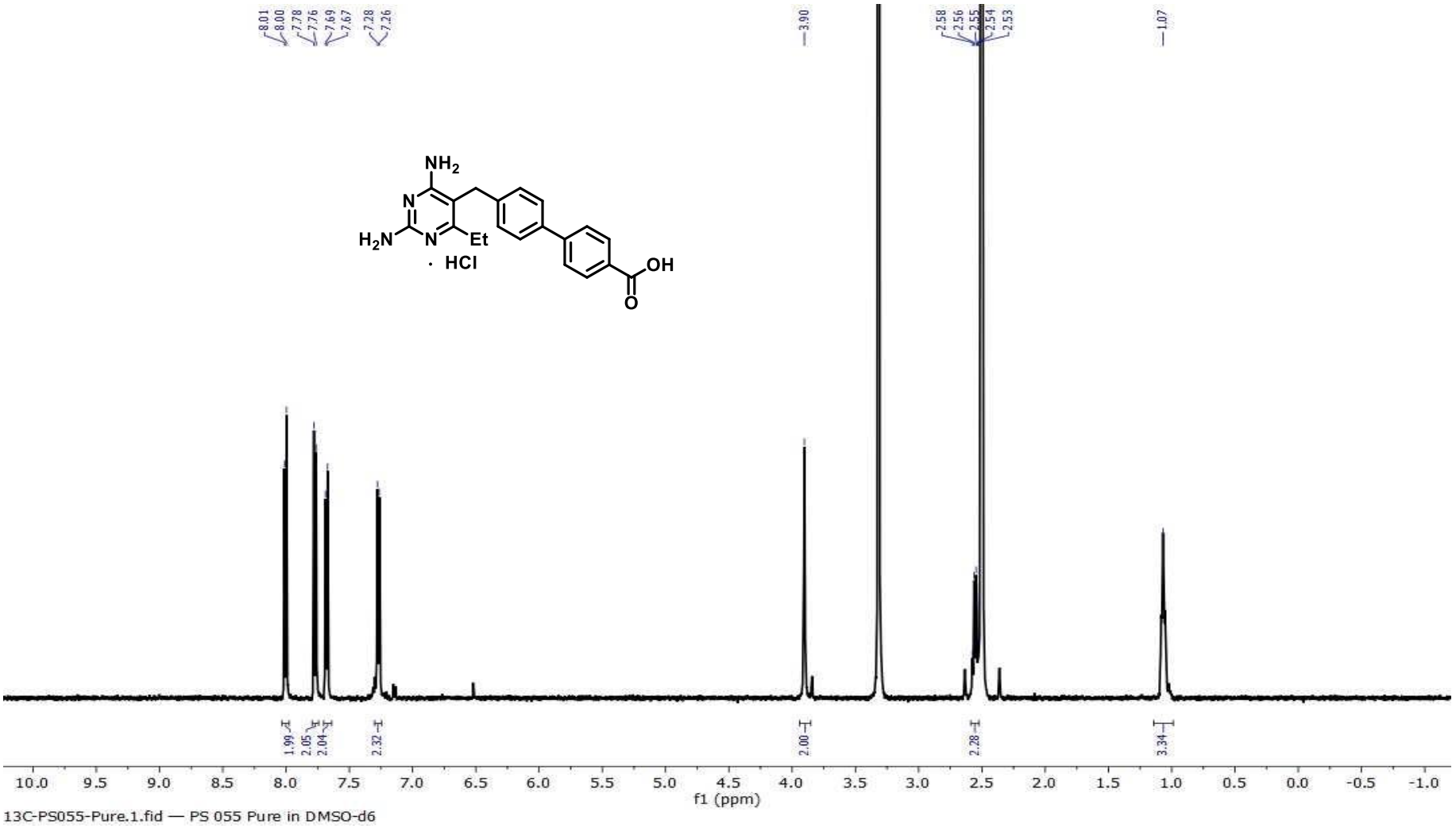


Figure S8. ^{1}H NMR spectra of compound 2-HCl.

^{13}C NMR in DMSO-d_6

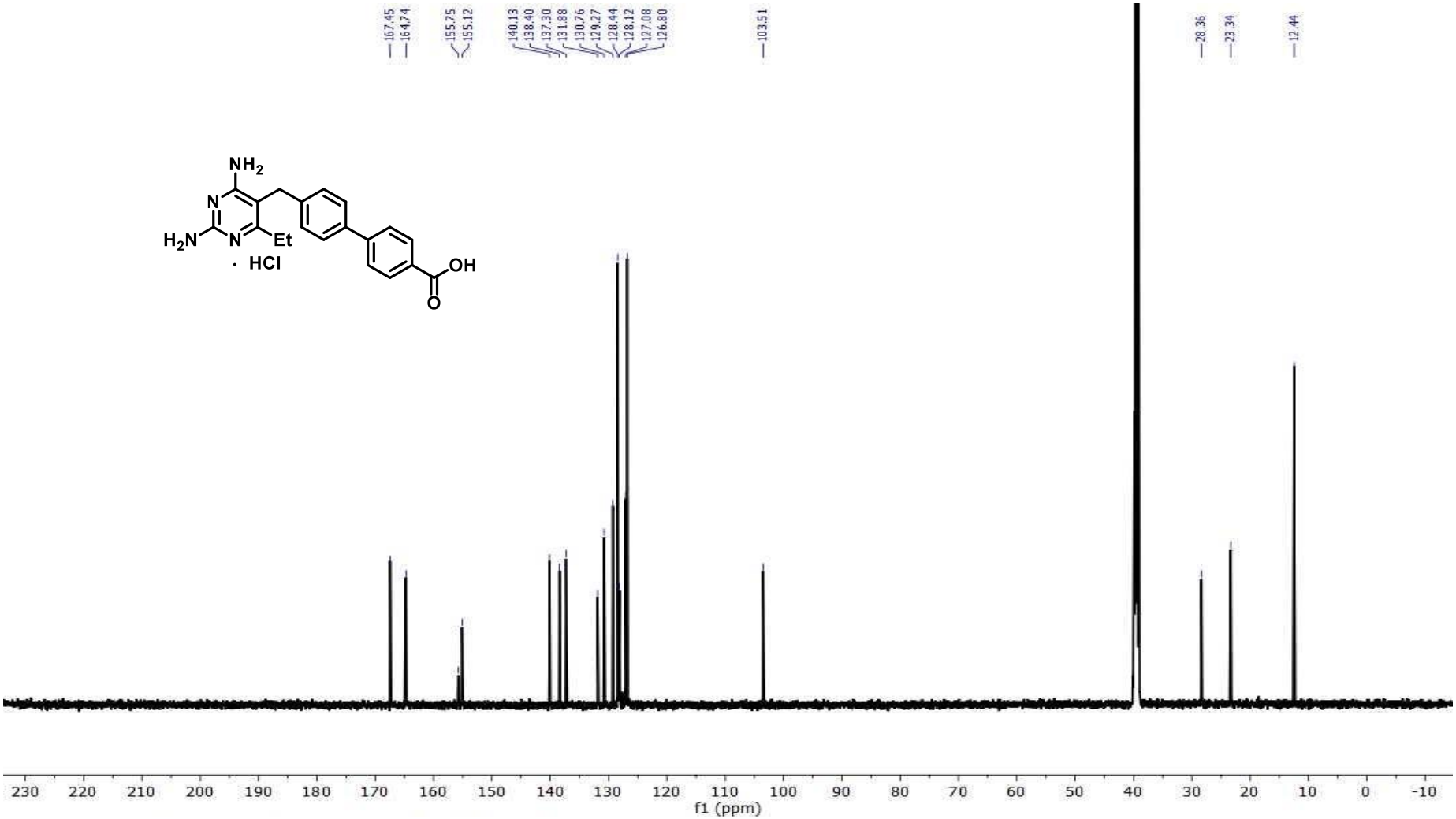
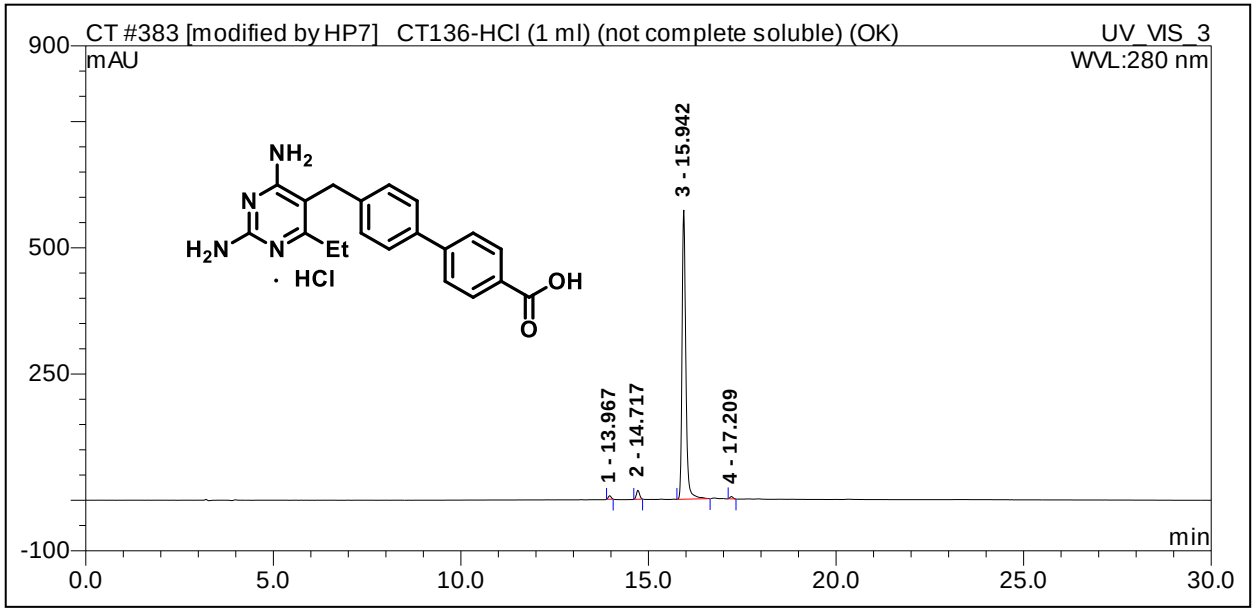


Figure S9. ^{13}C NMR spectra of compound 2-HCl.



383 CT136-HCl (1 ml) (not complete soluble) (OK)

Sample Name:	**CT136-HCl (1 ml) (not complete soluble) (OK)**	*Injection Volume:*	**1.0**
Vial Number:	**GA8**	*Channel:*	**UV_VIS_3**
Sample Type:	**unknown**	*Wavelength:*	**280.0**
Control Program:	**15-60_30min_AB_flow 0-6_column B (1-2_6-1)**	*Bandwidth:*	**1**
Quantif. Method:	**Standard CALIBRATION**	*Dilution Factor:*	**1.0000**
Recording Time:	**12/9/2021 17:47**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount ng	Type
1	13.97	na	6.753	0.602	0.87	n.a.	BMB*
2	14.72	na	17.547	1.718	2.47	n.a.	BMB*
3	15.94	na	572.206	66.668	96.04	n.a.	BMB
4	17.21	na	4.520	0.431	0.62	n.a.	BMB*
Total:			601.027	69.419	100.00	0.000	

Figure S10. HPLC profile of compound 2-HCl.

^{1}H NMR in DMSO-d_6

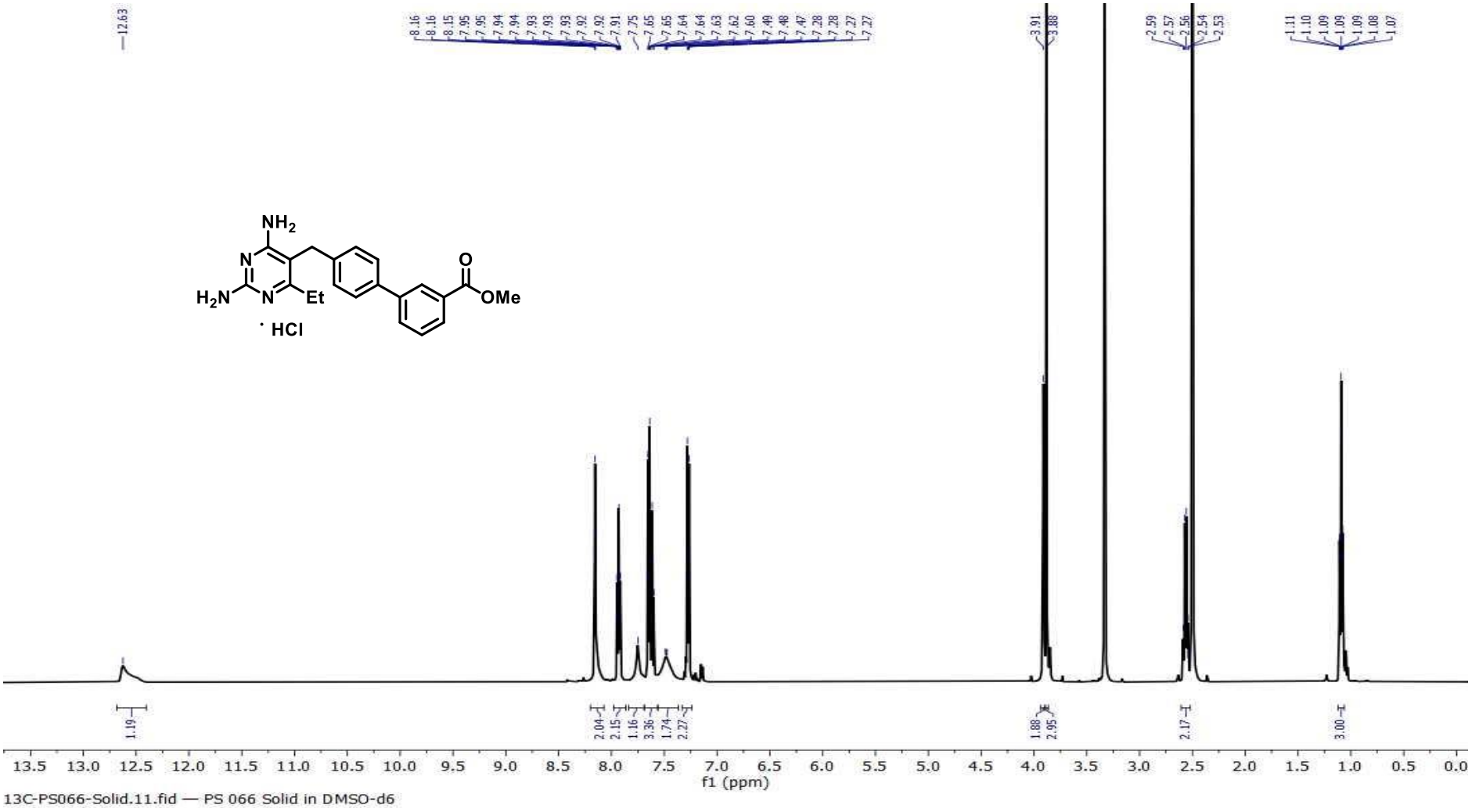


Figure S11. ^{1}H NMR spectra of compound 3-HCl.

^{13}C NMR in DMSO-d_6

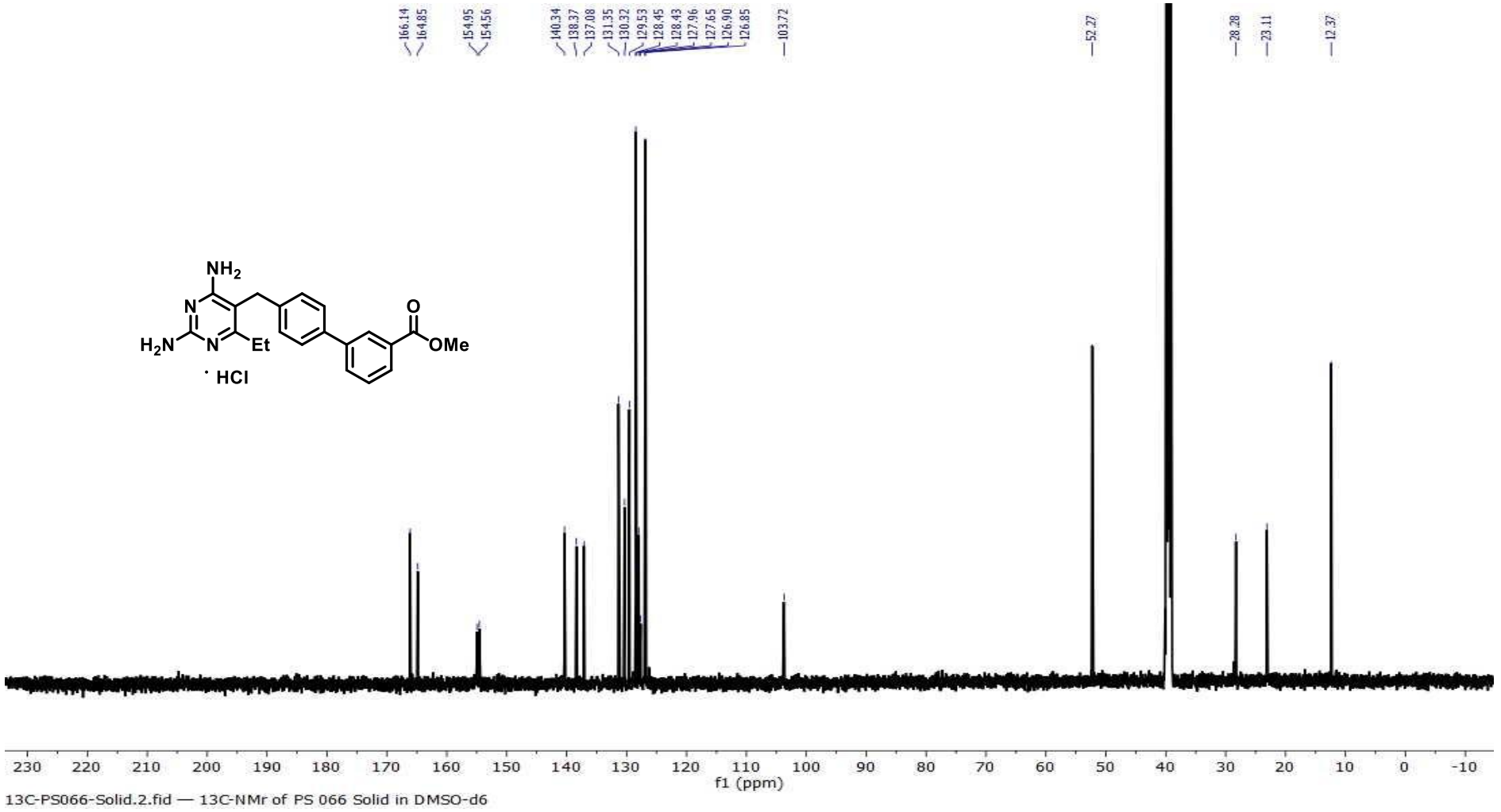
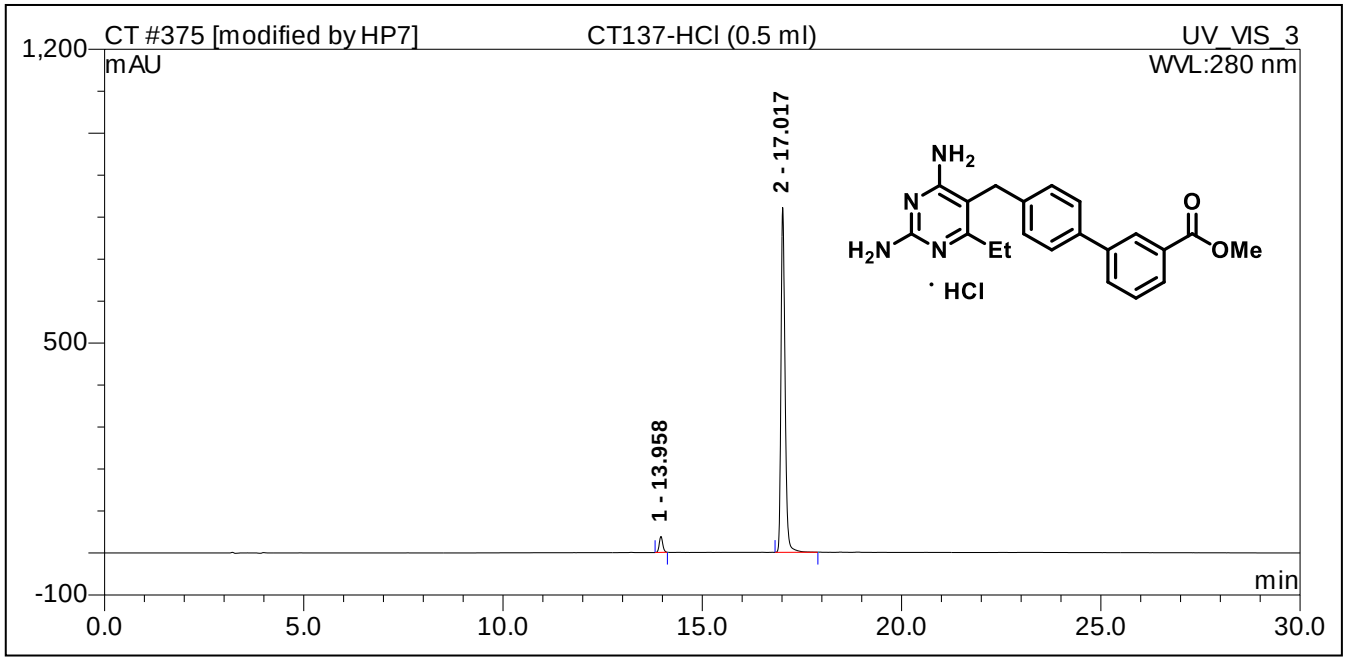


Figure S12. ^{13}C NMR spectra of compound 3-HCl.



375 CT137-HCl (0.5 ml)

Sample Name:	**CT137-HCl (0.5 ml)**	*Injection Volume:*	**1.0**
Vial Number:	**RD4**	*Channel:*	**UV_VIS_3**
Sample Type:	**unknown**	*Wavelength:*	**280.0**
Control Program:	**15-60_30min_AB_flow 0-6_column B (1-2_6-1)**	*Bandwidth:*	**1**
Quantif. Method:	**Standard CALIBRATION**	*Dilution Factor:*	**1.0000**
Recording Time:	**12/8/2021 18:52**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**

No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount	Type
	min		mAU	mAU*min	%	ng	
1	13.96	na	37.904	3.823	3.78	n.a.	BMB*
2	17.02	na	821.642	97.314	96.22	n.a.	BMB
Total:			859.546	101.137	100.00	0.000	

Figure S13. HPLC profile of compound 3-HCl.

^{1}H NMR in DMSO-*d*$_6$

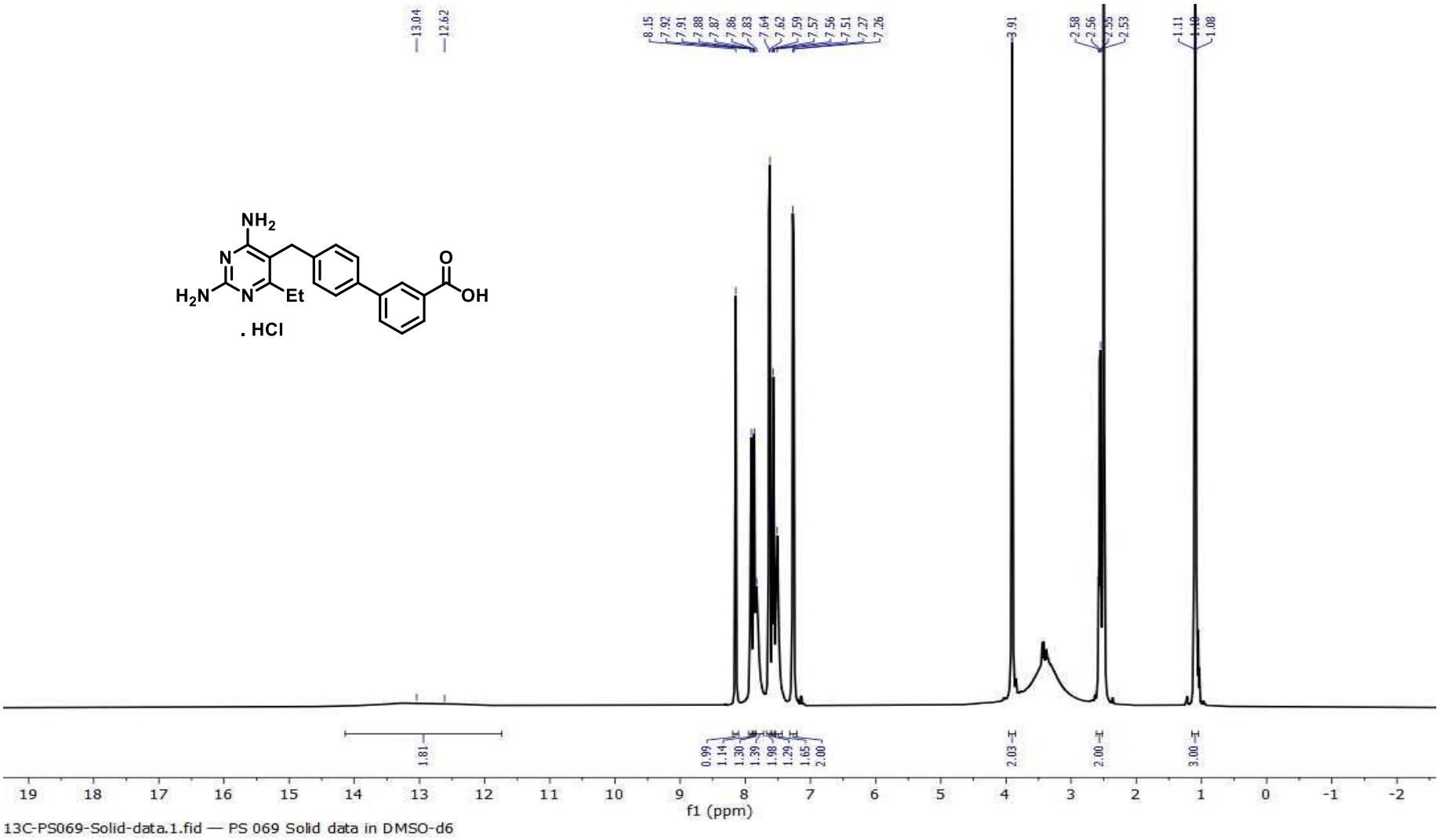


Figure S14. ^{1}H NMR spectra of compound 4-HCl.

^{13}C NMR in DMSO-d_6

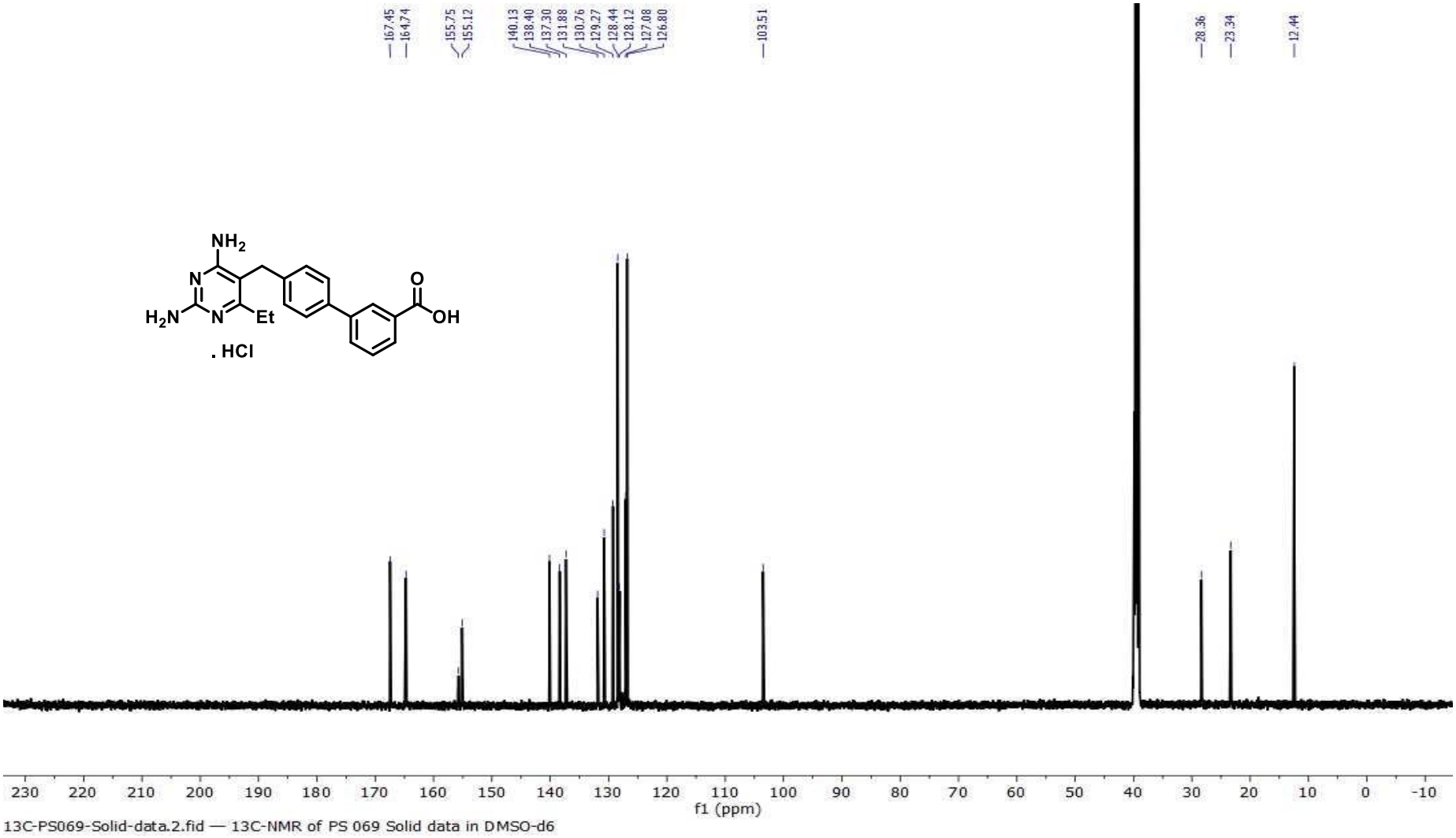
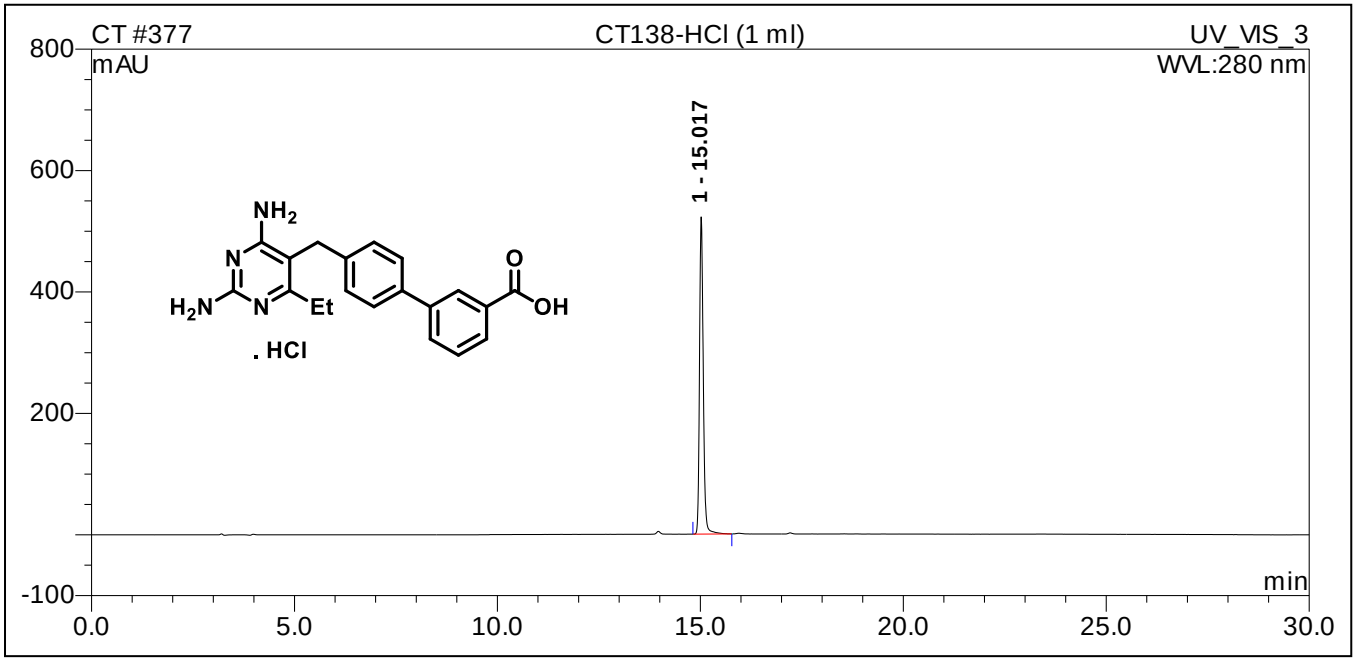


Figure S15. ^{13}C NMR spectra of compound 4-HCl.



377 CT138-HCl (1 ml)

Sample Name:	**CT138-HCl (1 ml)**	*Injection Volume:*	**1.0**
Vial Number:	**RD5**	*Channel:*	**UV_VIS_3**
Sample Type:	**unknown**	*Wavelength:*	**280.0**
Control Program:	**15-60_30min_AB_flow 0-6_column B (1-2_6-1)**	*Bandwidth:*	**1**
Quantif. Method:	**Standard CALIBRATION**	*Dilution Factor:*	**1.0000**
Recording Time:	**12/9/2021 15:59**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**

No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount	Type
	min		mAU	mAU*min	%	ng	
1	15.02	na	522.387	56.121	100.00	n.a.	BMB
Total:			522.387	56.121	100.00	0.000	

Figure S16. HPLC profile of compound 4-HCl.

^{1}H NMR in DMSO-*d*$_6$

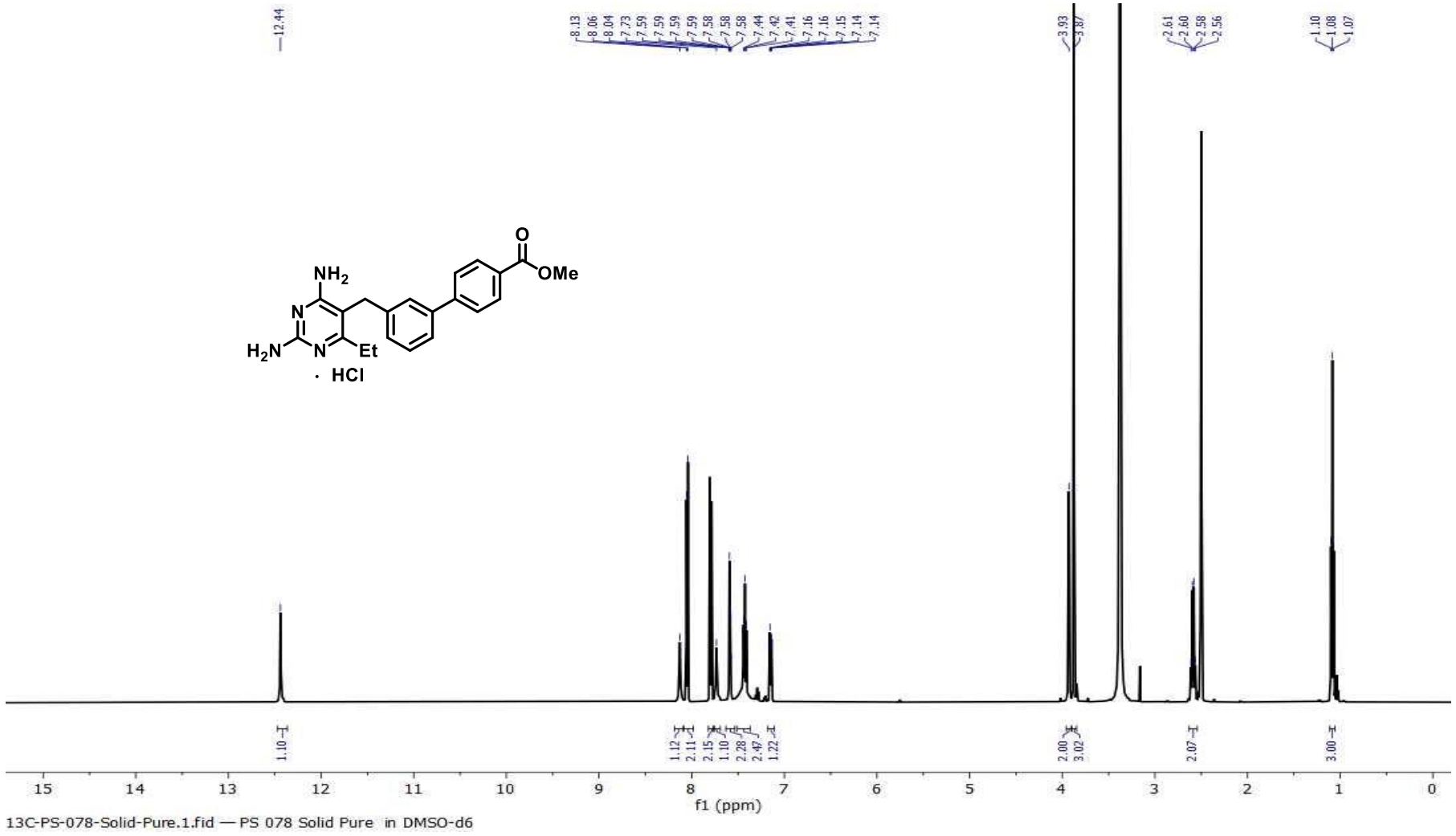


Figure S17. ^{1}H NMR spectra of compound 5-HCl.

^{13}C NMR in DMSO-d_6

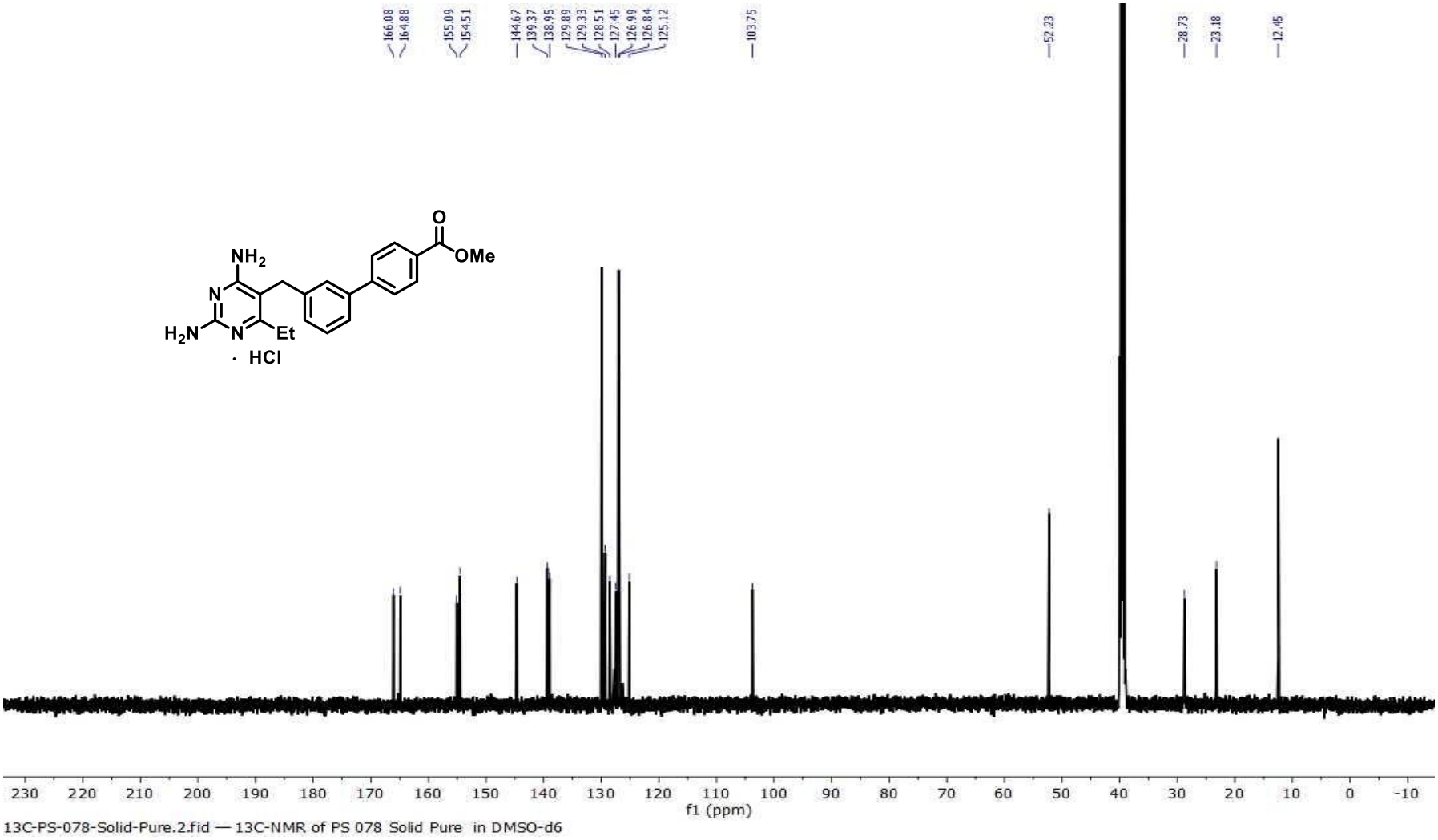
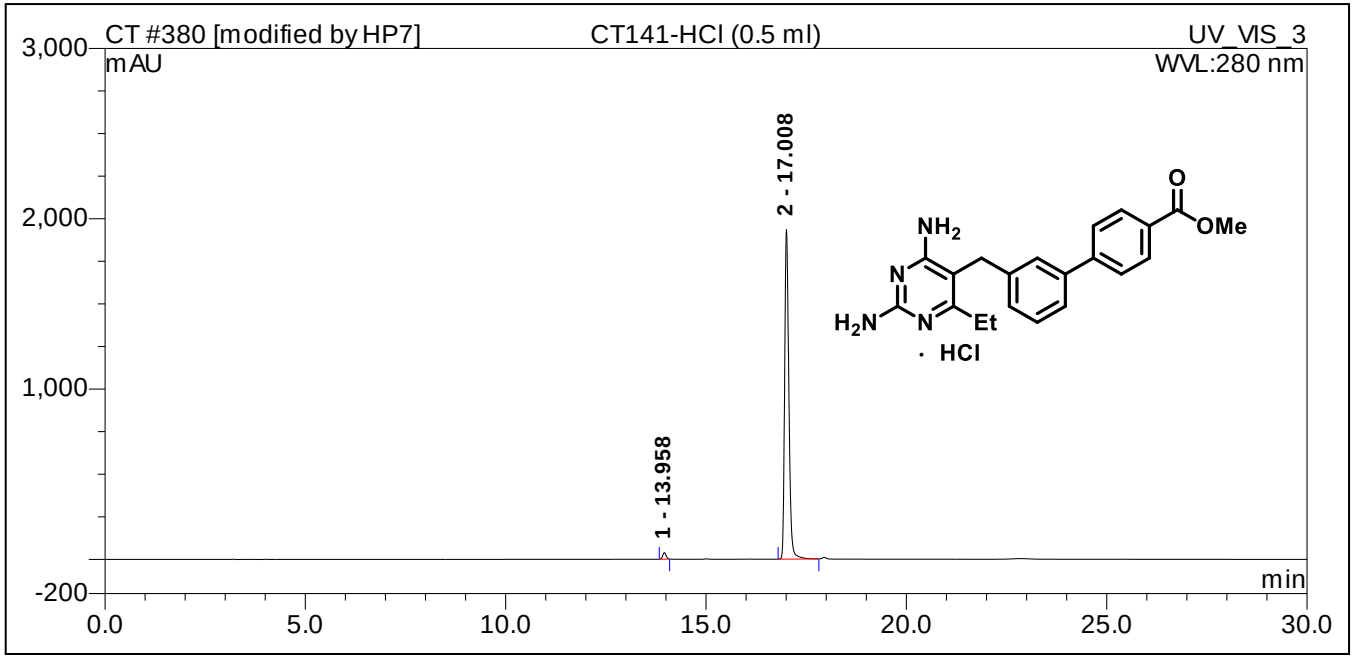


Figure S18. ^{13}C NMR spectra of compound 5-HCl.



380 CT141-HCl (0.5 ml)

Sample Name:	**CT141-HCl (0.5 ml)**	*Injection Volume:*	**1.0**
Vial Number:	**RD8**	*Channel:*	**UV_VIS_3**
Sample Type:	**unknown**	*Wavelength:*	**280.0**
Control Program:	**15-60_30min_AB_flow 0-6_column B (1-2_6-1)**	*Bandwidth:*	**1**
Quantif. Method:	**Standard CALIBRATION**	*Dilution Factor:*	**1.0000**
Recording Time:	**12/8/2021 21:17**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount ng	Type
1	13.96	na	38.211	3.790	1.59	n.a.	BMB*
2	17.01	na	1934.245	234.896	98.41	n.a.	BMB
Total:			1972.456	238.686	100.00	0.000	

Figure S19. HPLC profile of compound 5-HCl.

^{1}H NMR in DMSO-*d*$_6$

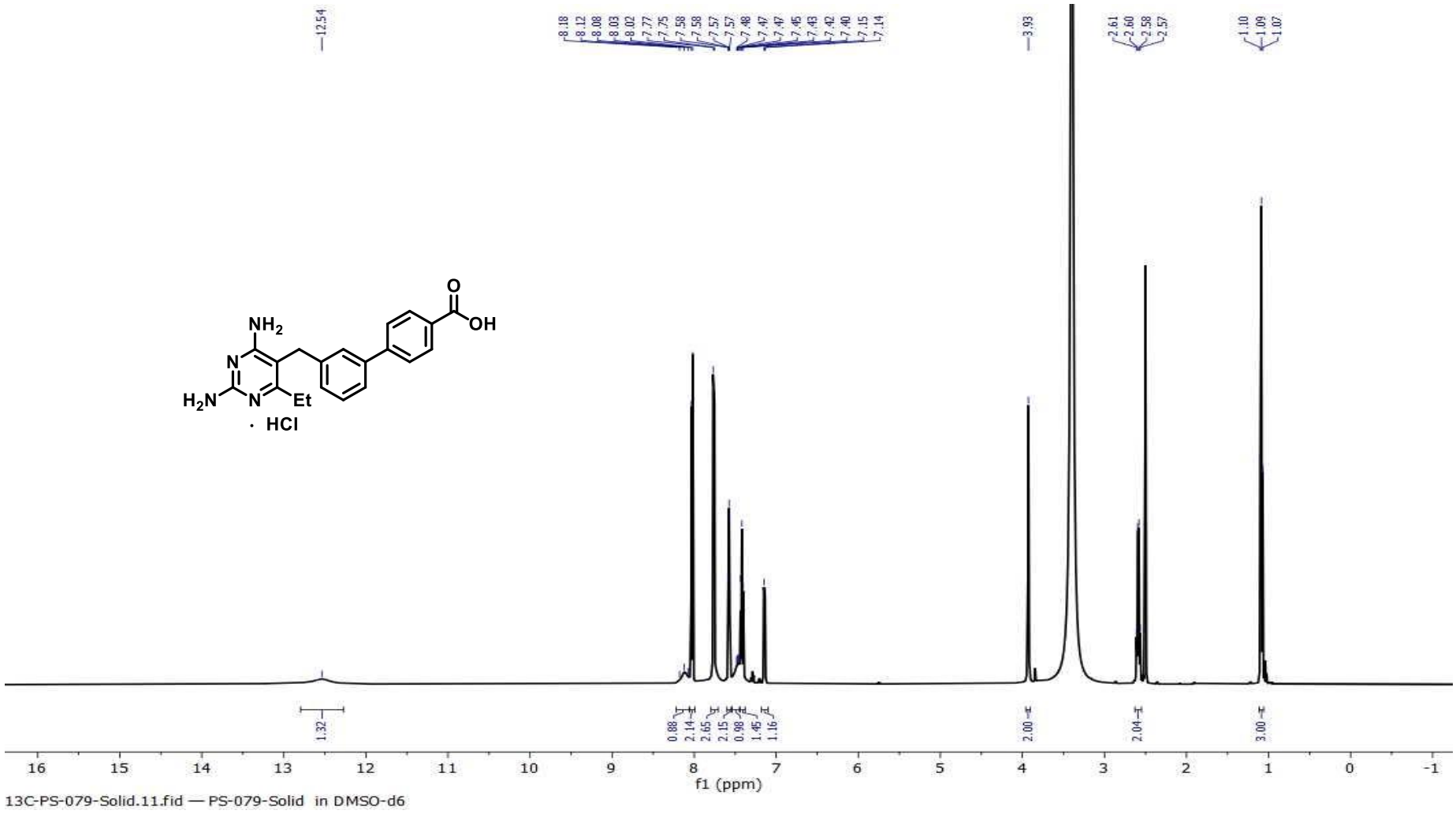


Figure S20. ^{1}H NMR spectra of compound 6-HCl.

^{13}C NMR in DMSO-d_6

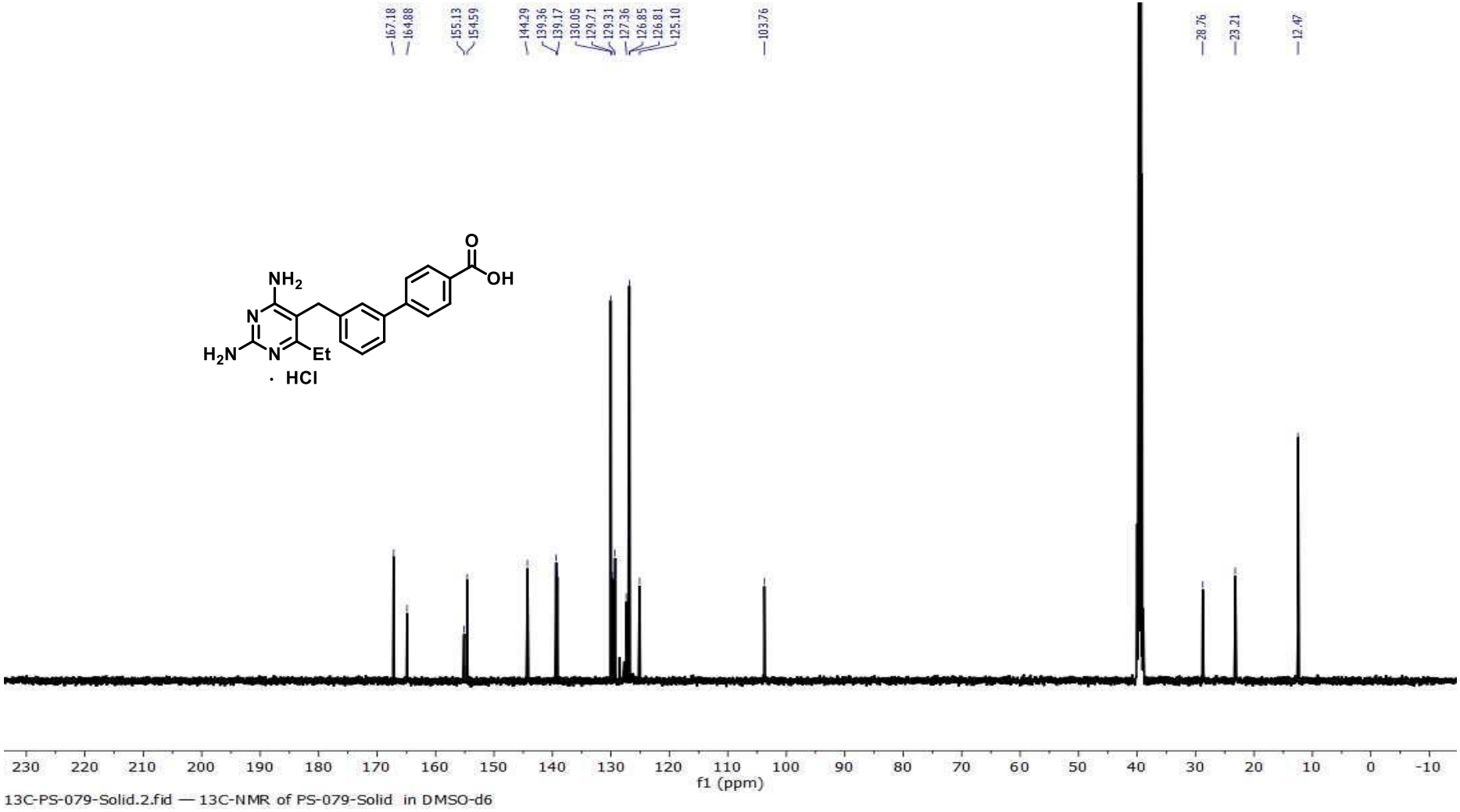
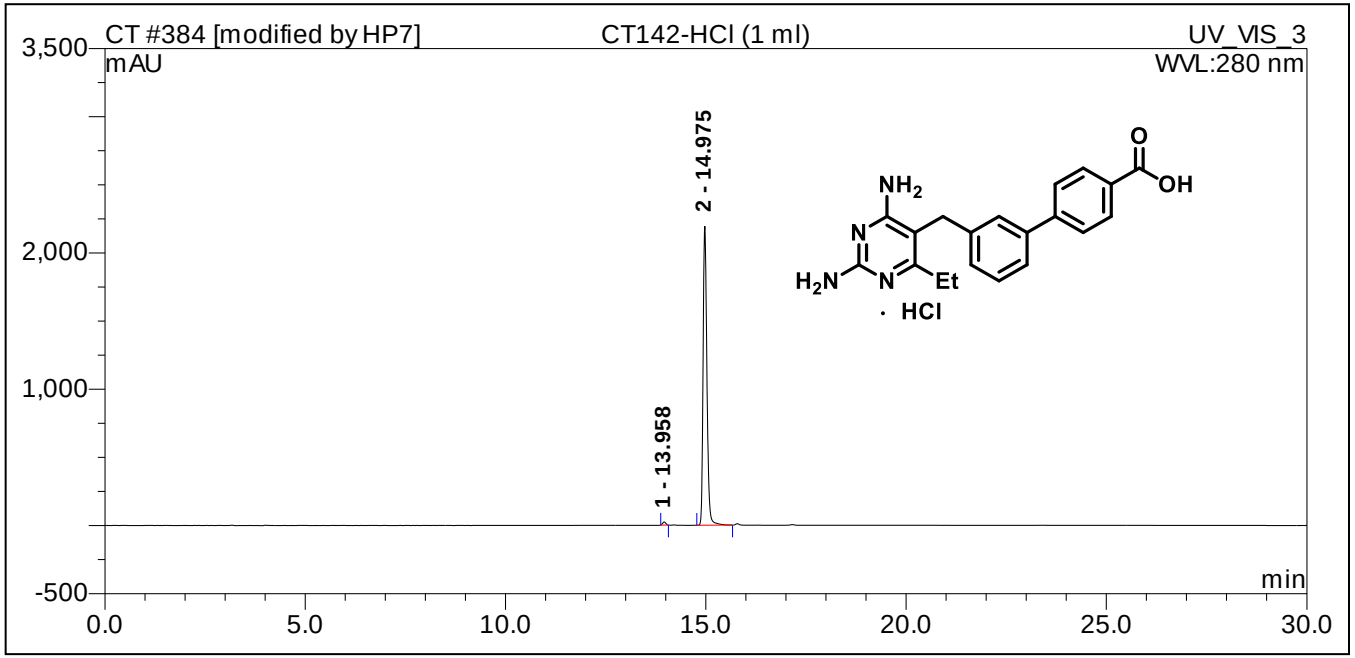


Figure S21. ^{13}C NMR spectra of compound 6-HCl.



384 CT142-HCl (1 ml)

Sample Name:	**CT142-HCl (1 ml)**	*Injection Volume:*	**2.0**
Vial Number:	**RE1**	*Channel:*	**UV_VIS_3**
Sample Type:	**unknown**	*Wavelength:*	**280.0**
Control Program:	**15-60_30min_AB_flow 0-6_column B (1-2_6-1)**	*Bandwidth:*	**1**
Quantif. Method:	**Standard CALIBRATION**	*Dilution Factor:*	**1.0000**
Recording Time:	**12/8/2021 21:53**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.01**	*Sample Amount:*	**1.0000**

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount ng	Type
1	13.96	na	22.608	2.065	0.84	n.a.	BMB*
2	14.98	na	2193.459	242.824	99.16	n.a.	BMB
Total:			2216.067	244.889	100.00	0.000	

Figure S22. HPLC profile of compound 6-HCl.

^{1}H NMR in DMSO-d_6

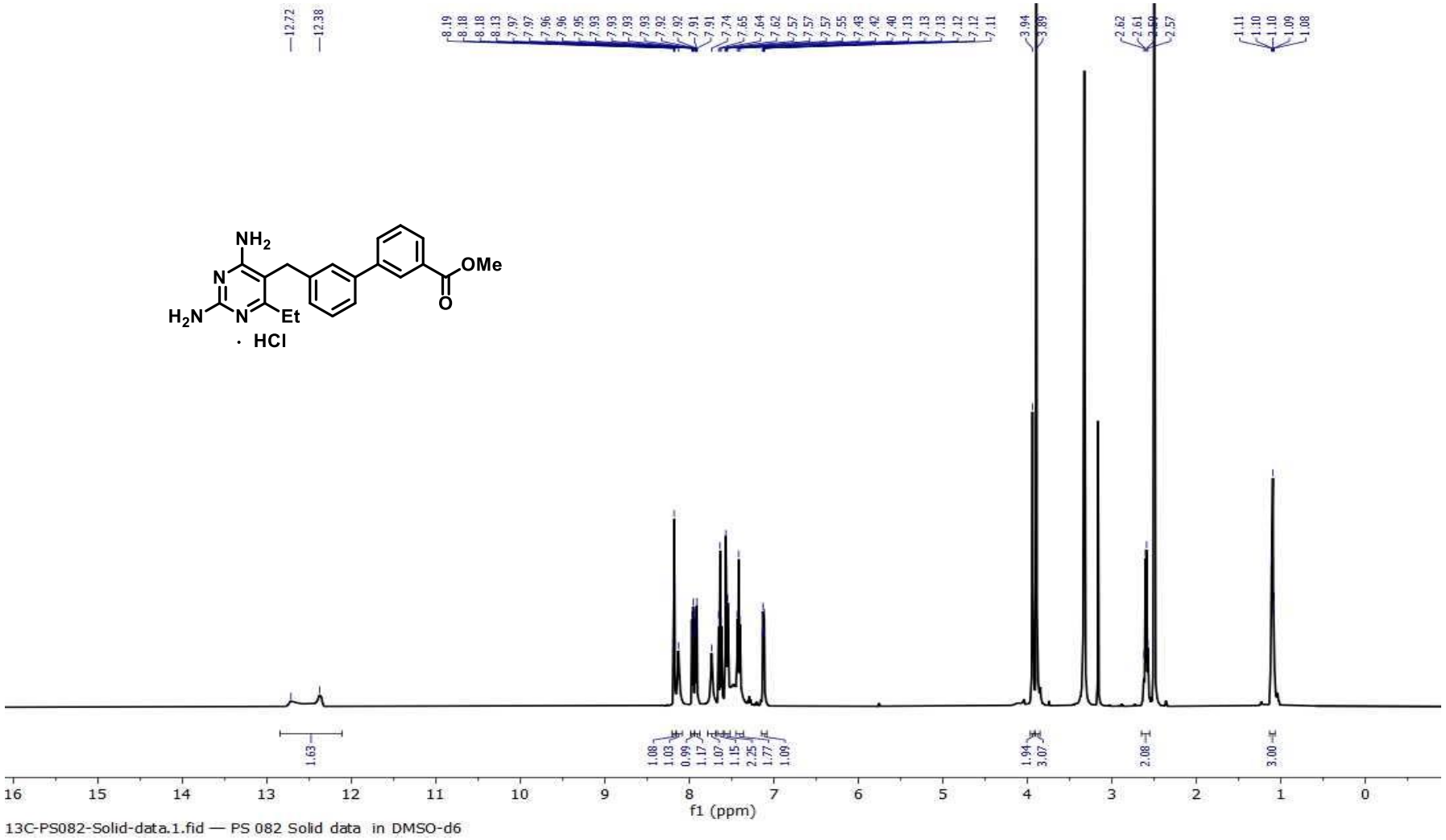


Figure S23. ^{1}H NMR spectra of compound 7-HCl.

^{13}C NMR in DMSO-d_6

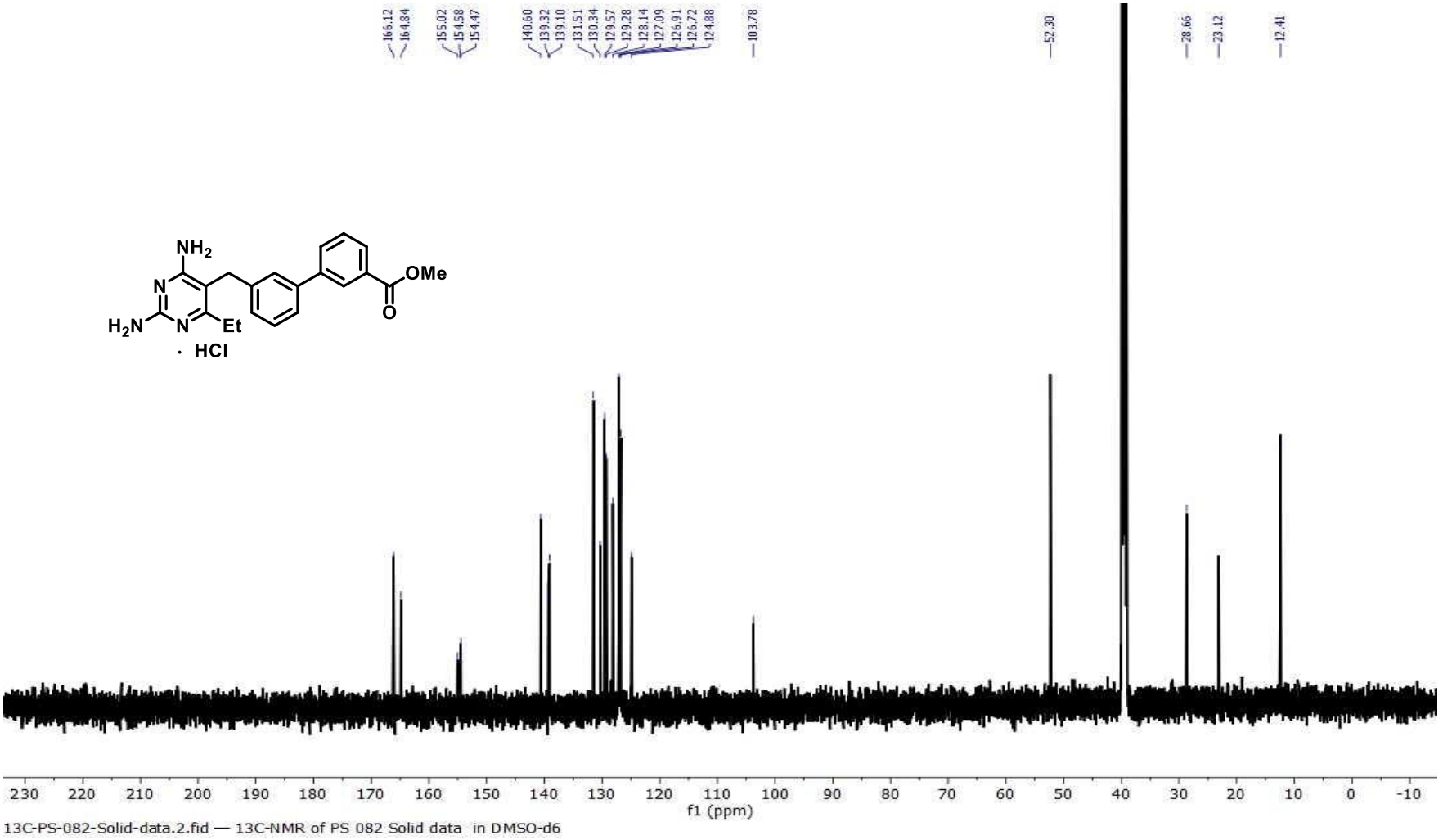
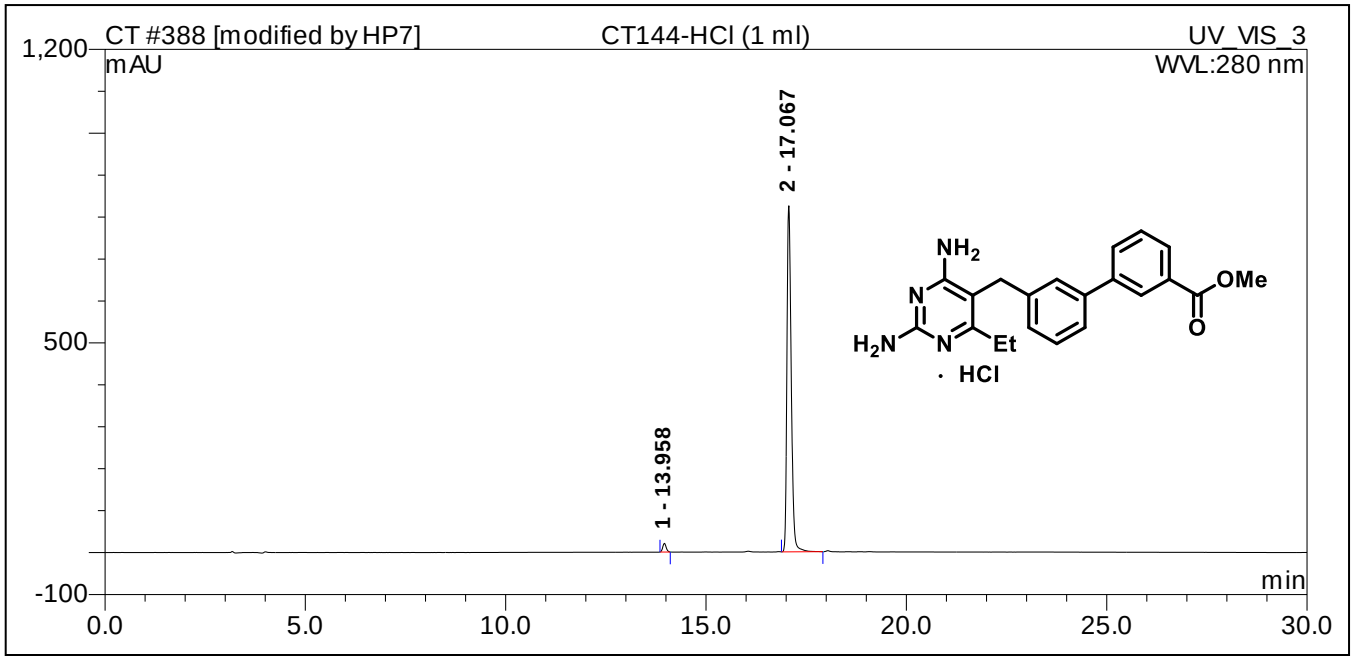


Figure S24. ^{13}C NMR spectra of compound 7-HCl.



388 CT144-HCl (1 ml)

Sample Name:	**CT144-HCl (1 ml)**	*Injection Volume:*	**2.0**
Vial Number:	**RE3**	*Channel:*	**UV_VIS_3**
Sample Type:	**unknown**	*Wavelength:*	**280.0**
Control Program:	**15-60_30min_AB_flow 0-6_column B (1-2_6-1)**	*Bandwidth:*	**1**
Quantif. Method:	**Standard CALIBRATION**	*Dilution Factor:*	**1.0000**
Recording Time:	**12/8/2021 23:06**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount ng	Type
1	13.96	na	20.513	2.037	1.94	n.a.	BMB*
2	17.07	na	825.330	102.974	98.06	n.a.	BMB
Total:			845.843	105.011	100.00	0.000	

Figure S25. HPLC profile of compound 7-HCl.

^{1}H NMR in DMSO-*d*$_6$

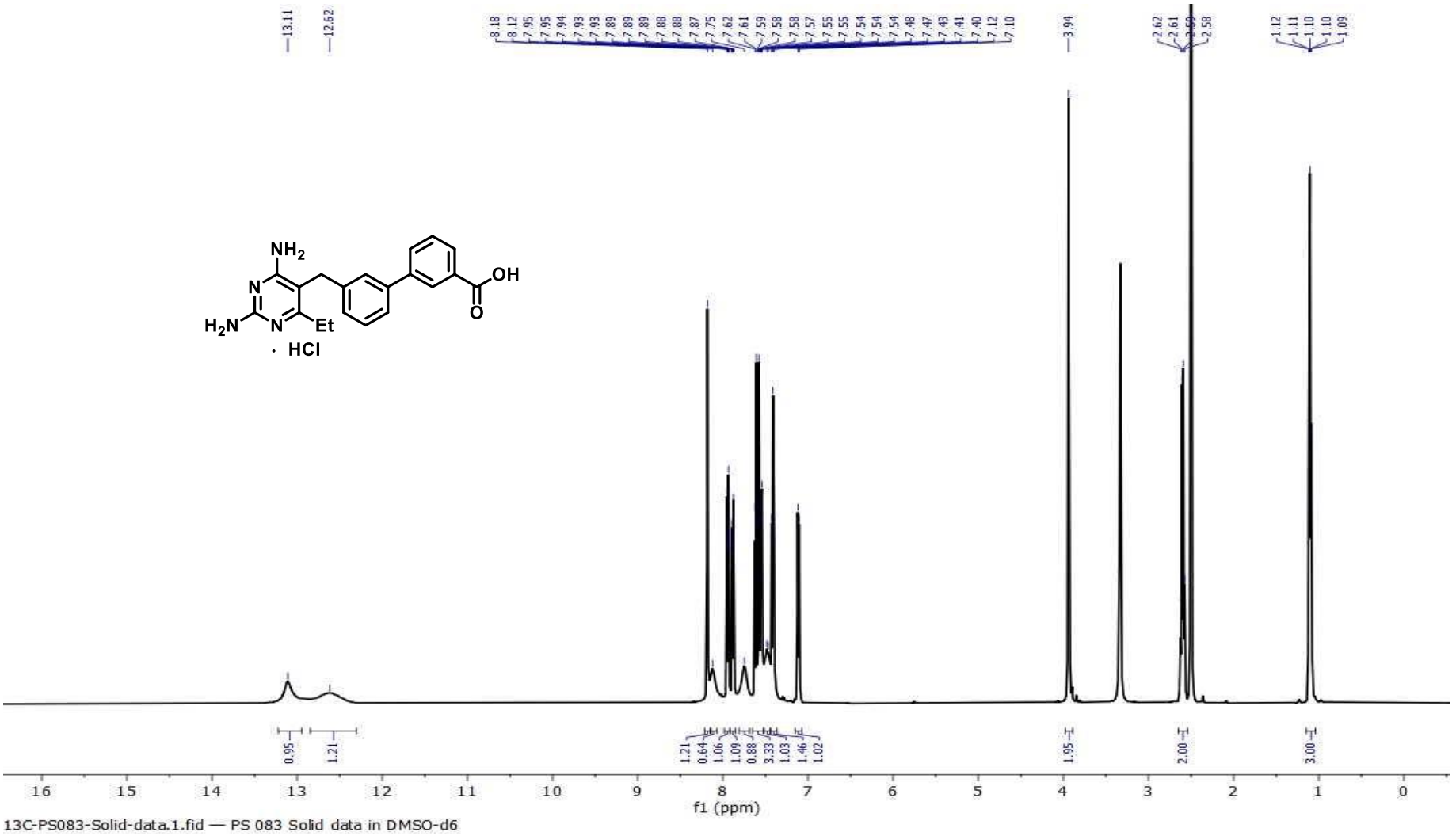


Figure S26. ^{1}H NMR spectra of compound 8-HCl.

^{13}C NMR in DMSO-d_6

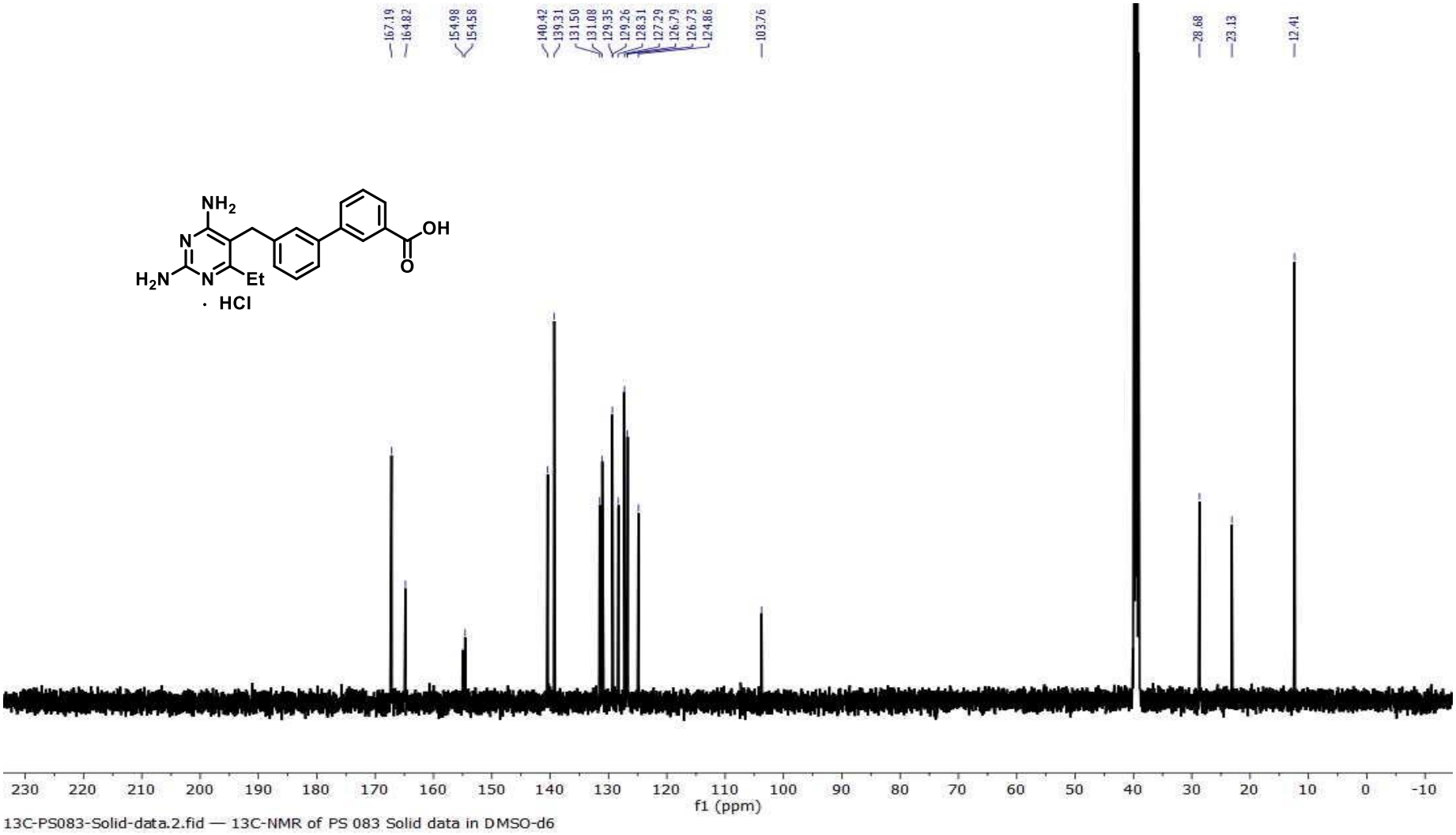
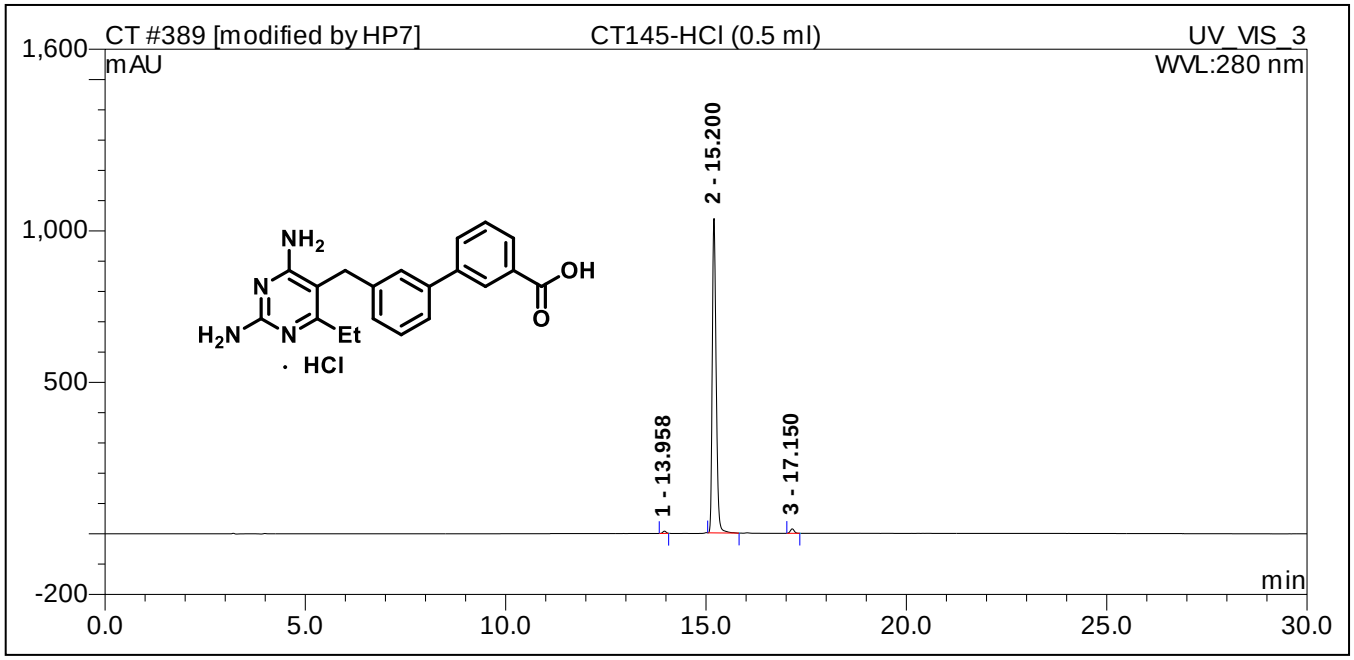


Figure S27. ^{13}C NMR spectra of compound 8-HCl.



389 CT145-HCl (0.5 ml)

Sample Name:	**CT145-HCl (0.5 ml)**	*Injection Volume:*	**1.0**
Vial Number:	**RE4**	*Channel:*	**UV_VIS_3**
Sample Type:	**unknown**	*Wavelength:*	**280.0**
Control Program:	**15-60_30min_AB_flow 0-6_column B (1-2_6-1)**	*Bandwidth:*	**1**
Quantif. Method:	**Standard CALIBRATION**	*Dilution Factor:*	**1.0000**
Recording Time:	**12/8/2021 23:42**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount ng	Type
1	13.96	na	7.565	0.720	0.60	n.a.	BMB*
2	15.20	na	1037.876	116.663	97.99	n.a.	BMB
3	17.15	na	14.746	1.674	1.41	n.a.	BMB*
Total:			1060.187	119.058	100.00	0.000	

Figure S28. HPLC profile of compound 8-HCl.