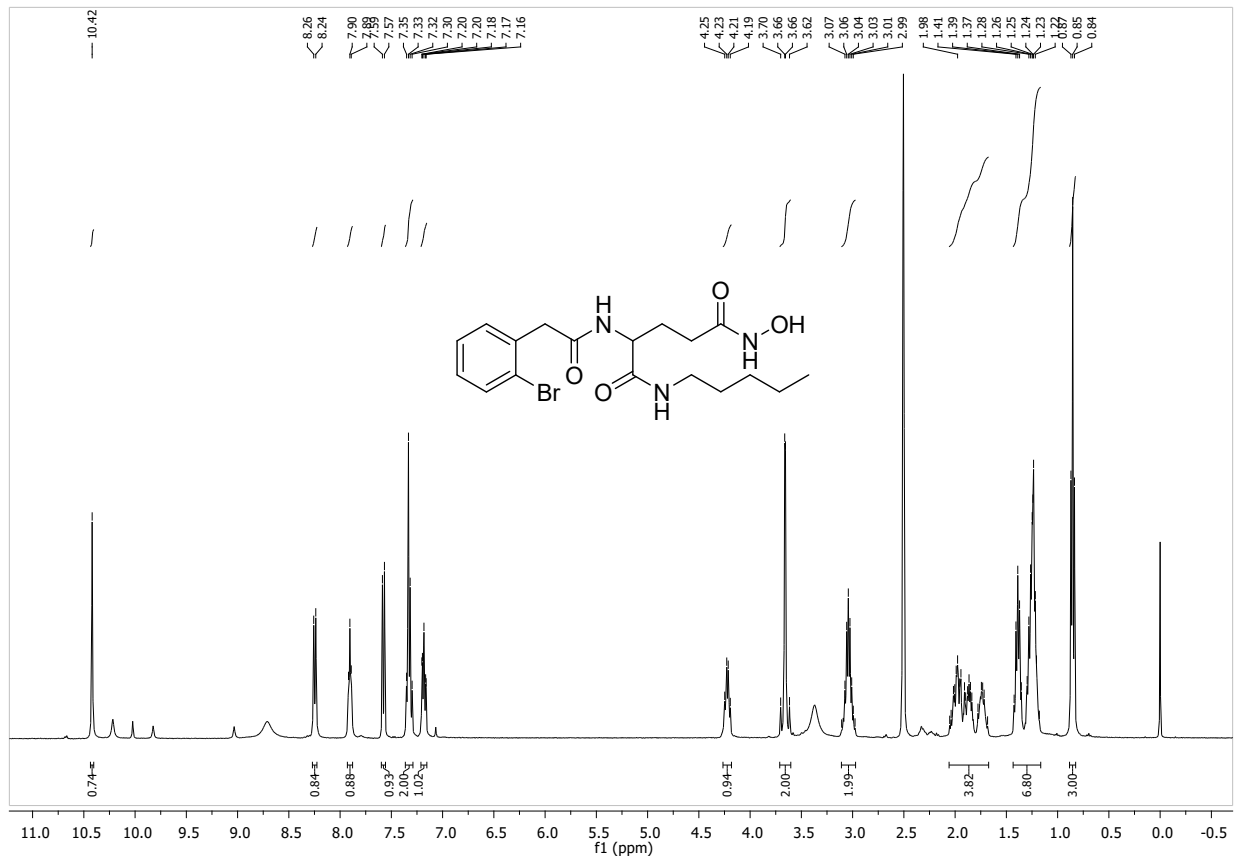**Supplementary File**

Quantitative activity-activity relationship (QAAR) driven design to develop hydroxamate derivatives of pentanoic acids as selective HDAC8 inhibitors: Synthesis, biological evaluation and binding mode of interaction studies

Sk. Abdul Amin[a], Prakruti Trivedi[b], Nilanjan Adhikari[a], Routholla Ganesh[b], Dhanya Vijayasarathi[b], Sanjib Das[a], Balaram Ghosh[b, *], Tarun Jha[a,*]

[a]*Natural Science Laboratory, Division of Medicinal and Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India.*
[b]*Epigenetic Research Laboratory, Department of Pharmacy, BITS-Pilani, Hyderabad Campus, Shamirpet, Hyderabad 500078, India.*
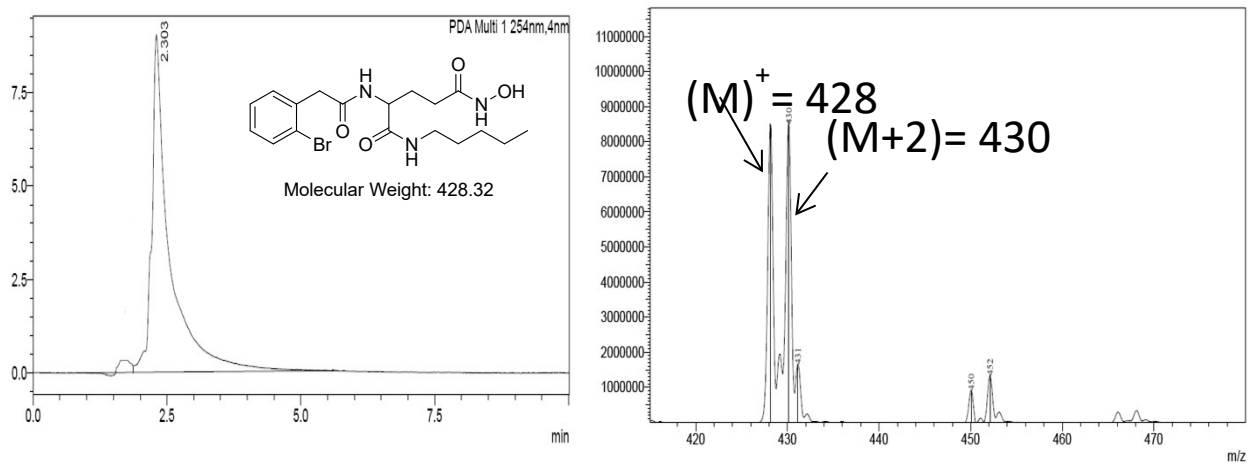
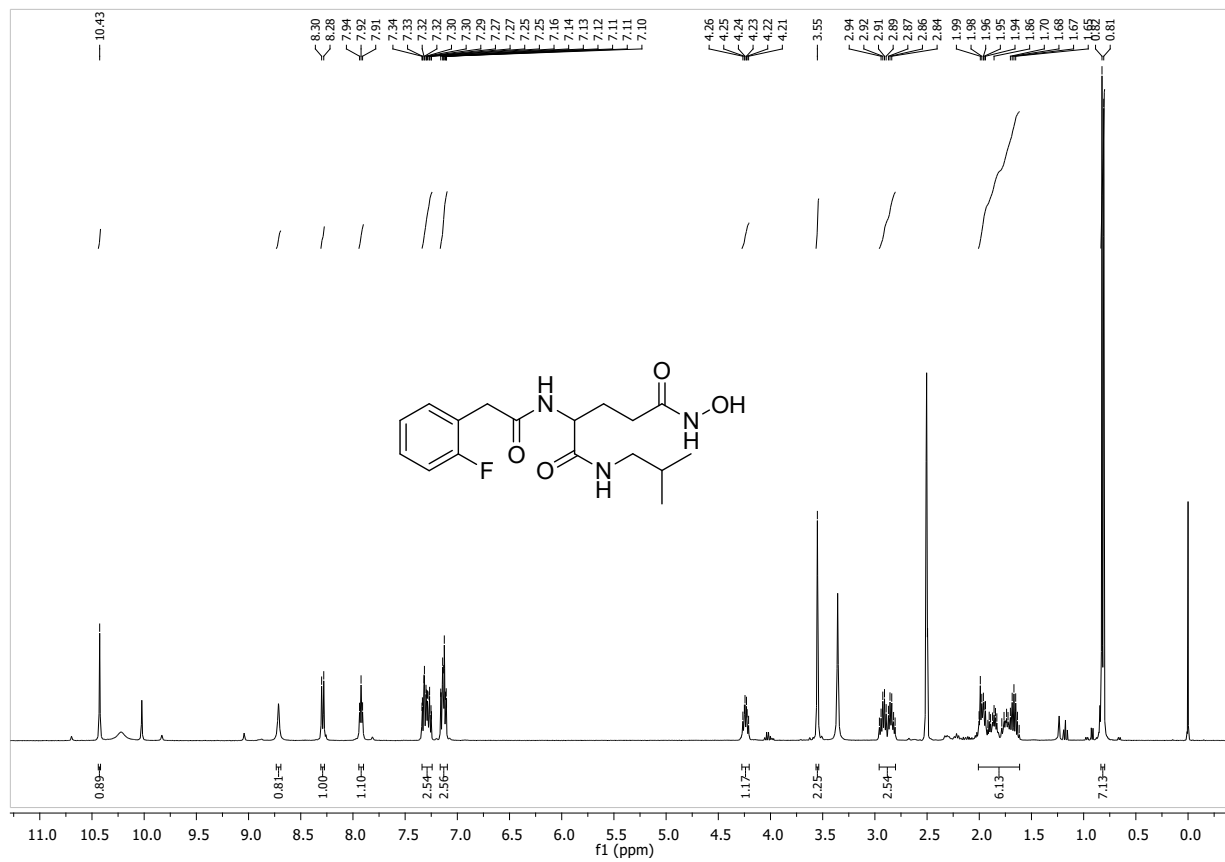*Corresponding author: balaram@hyderabad.bits-pilani.ac.in; tjupharm@yahoo.com.

Supplementary Figure S1. ^{1}H NMR of **7a**
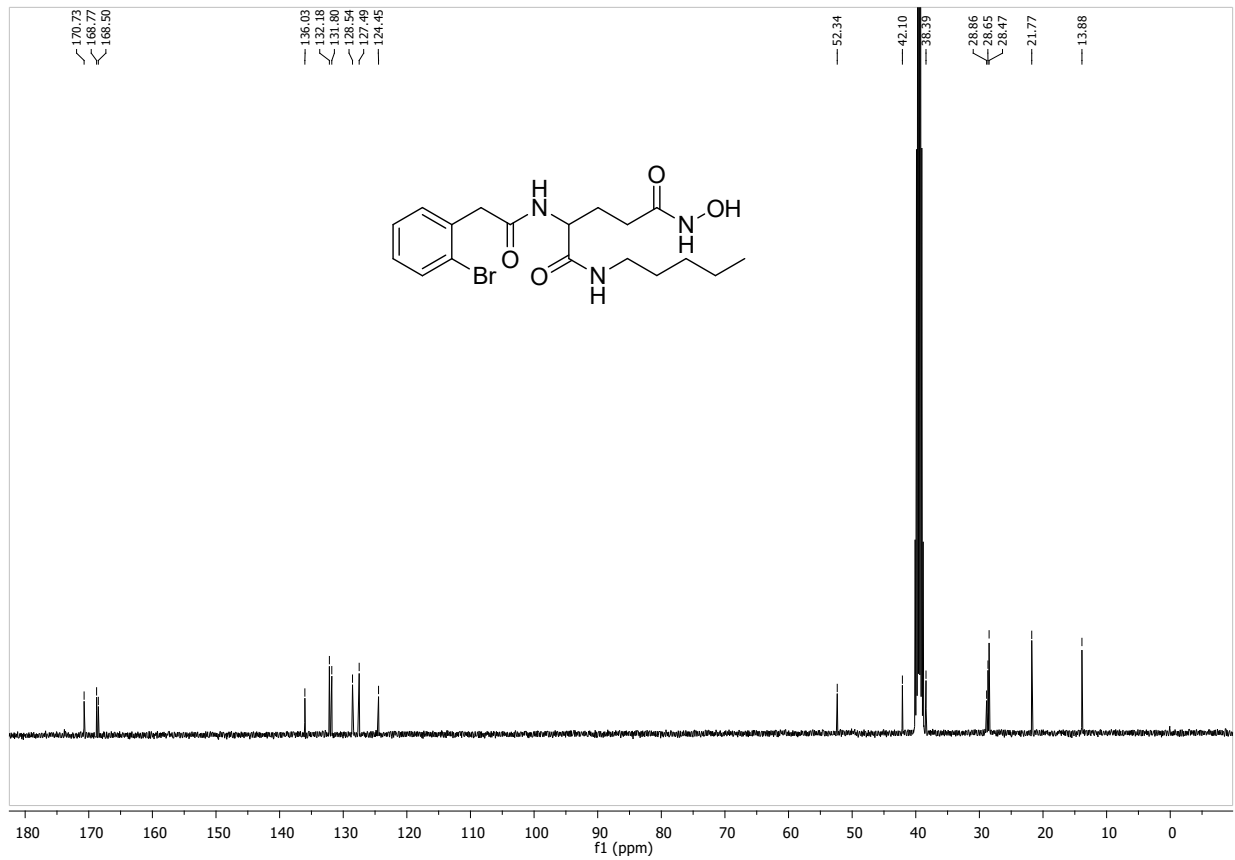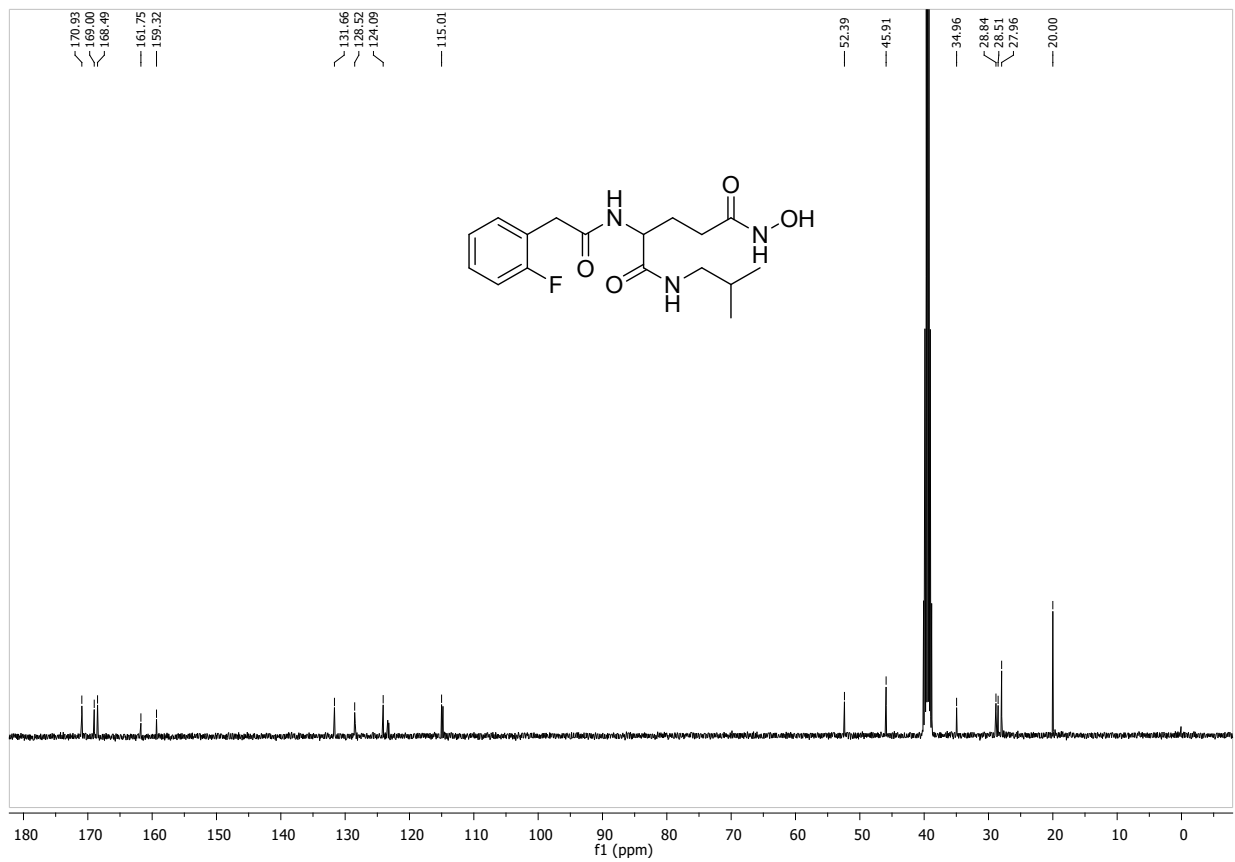
Supplementary Figure S2. ^{13}C NMR of **7a**

Molecular Weight: 428.32

PDA Multi 1 254nm,4nm

2.303

$(M)^+ = 428$

$(M+2) = 430$

Supplementary Figure S3. LC/MS of **7a**

Supplementary Figure S4. ^{1}H NMR of **7b**

Supplementary Figure S5. ^{13}C NMR of **7b**
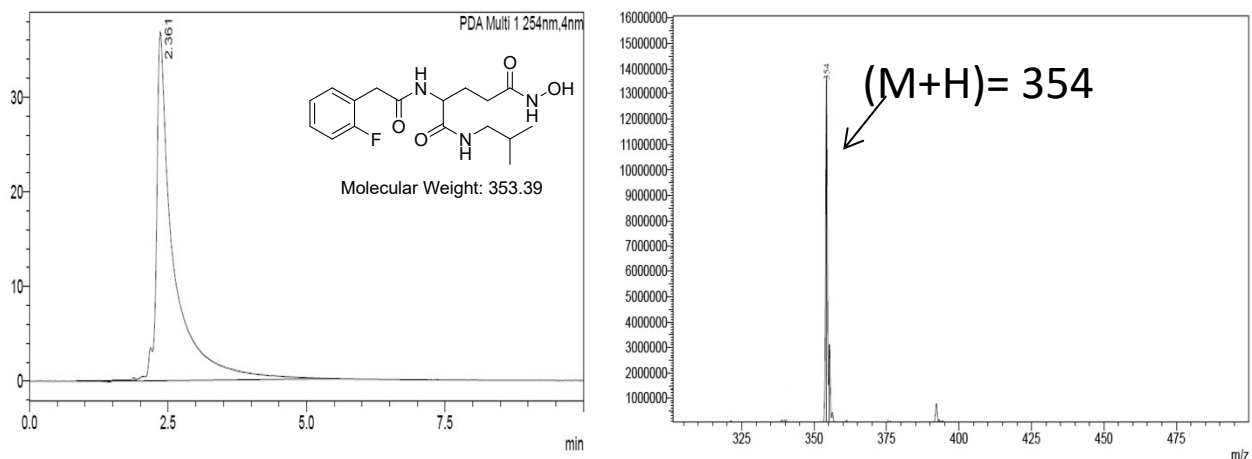
Supplementary Figure S6. LC/MS of **7b**
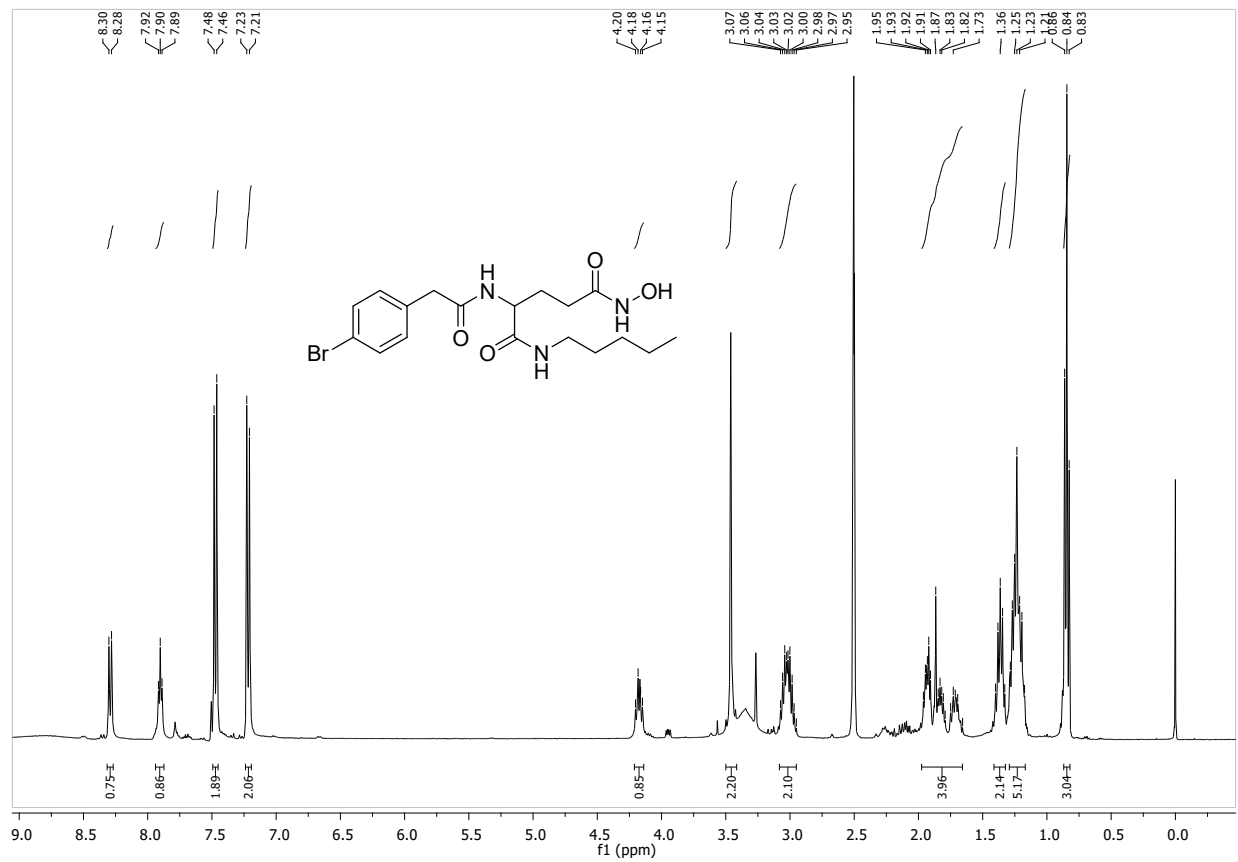
Supplementary Figure S7. ^{1}H NMR of **7c**
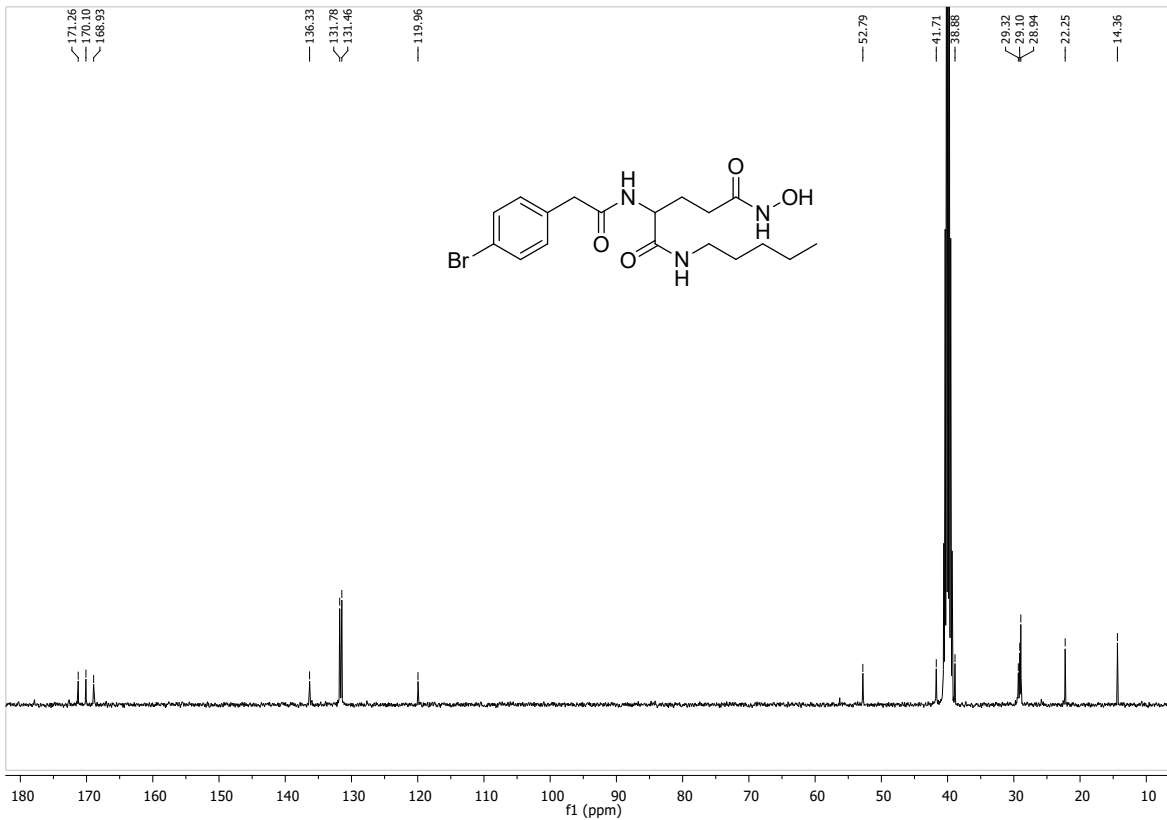
Supplementary Figure S8. ^{13}C NMR **7c**
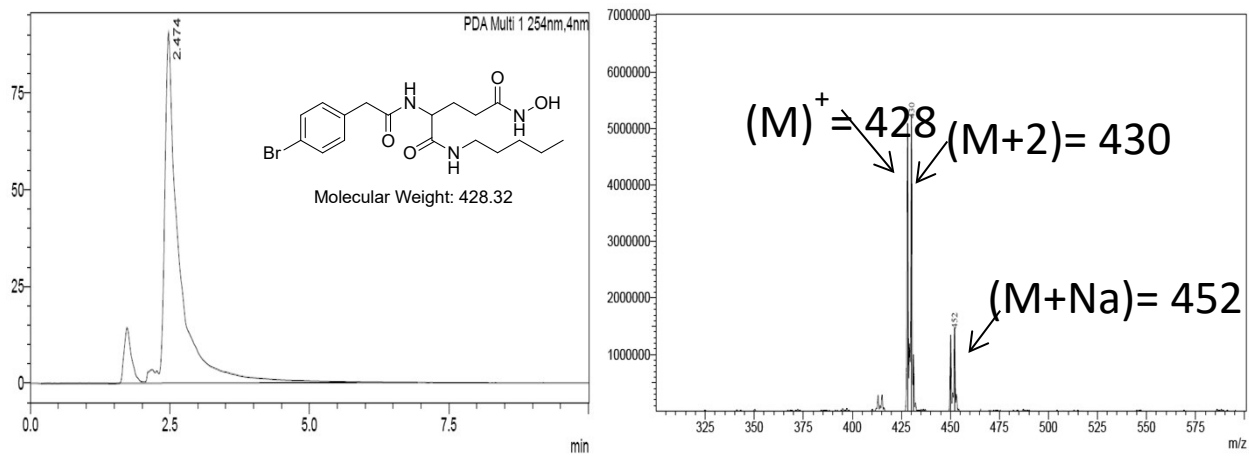
Supplementary Figure S9. LC/MS of **7c**

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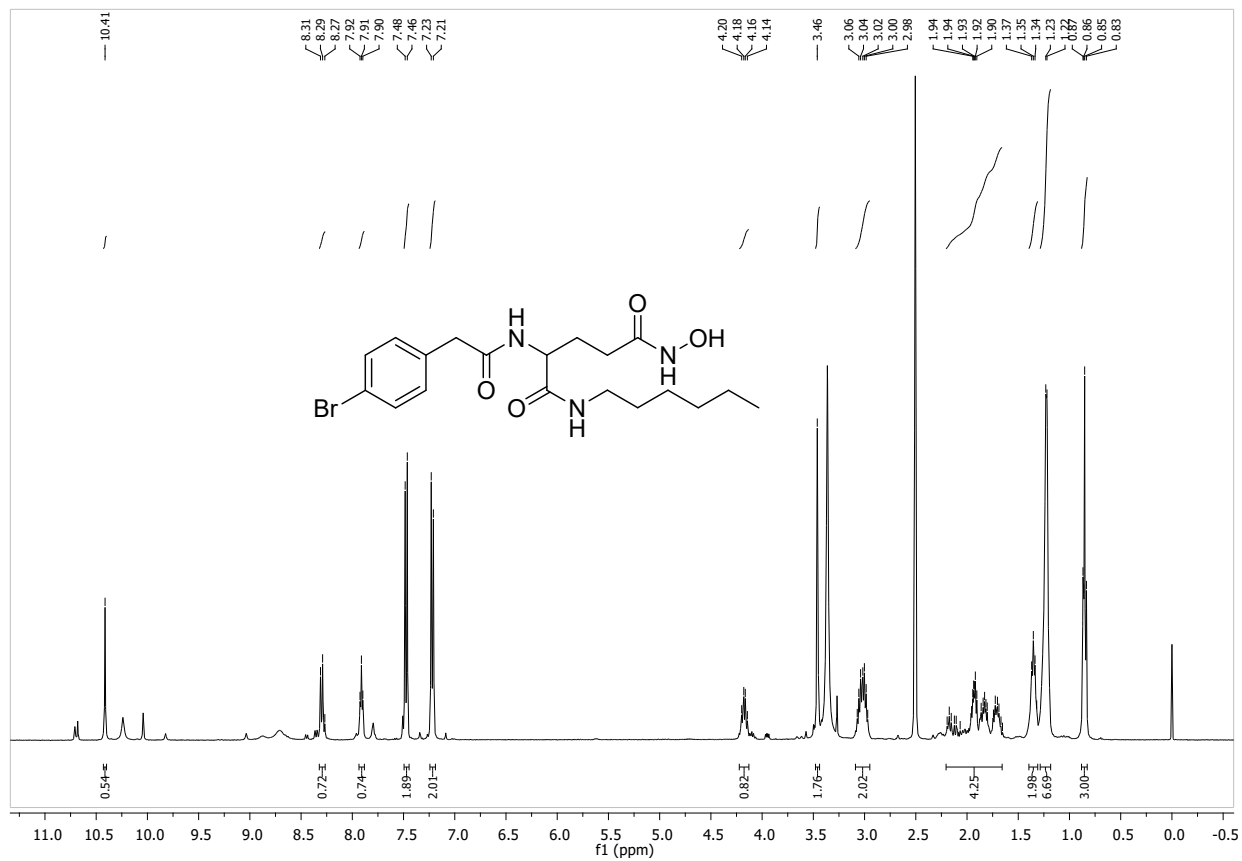
Supplementary Figure S10. ^{1}H NMR of **7d**
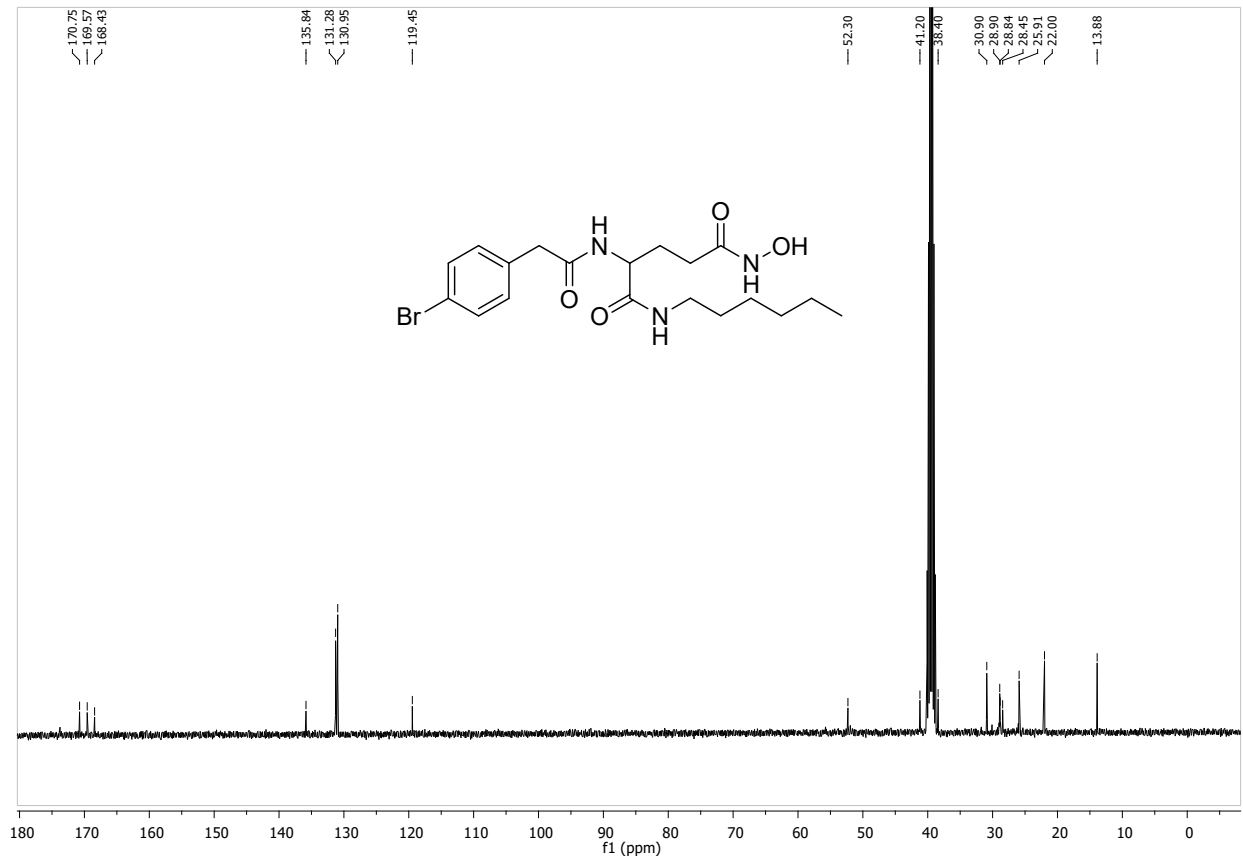
Supplementary Figure S11. ^{13}C NMR of **7d**
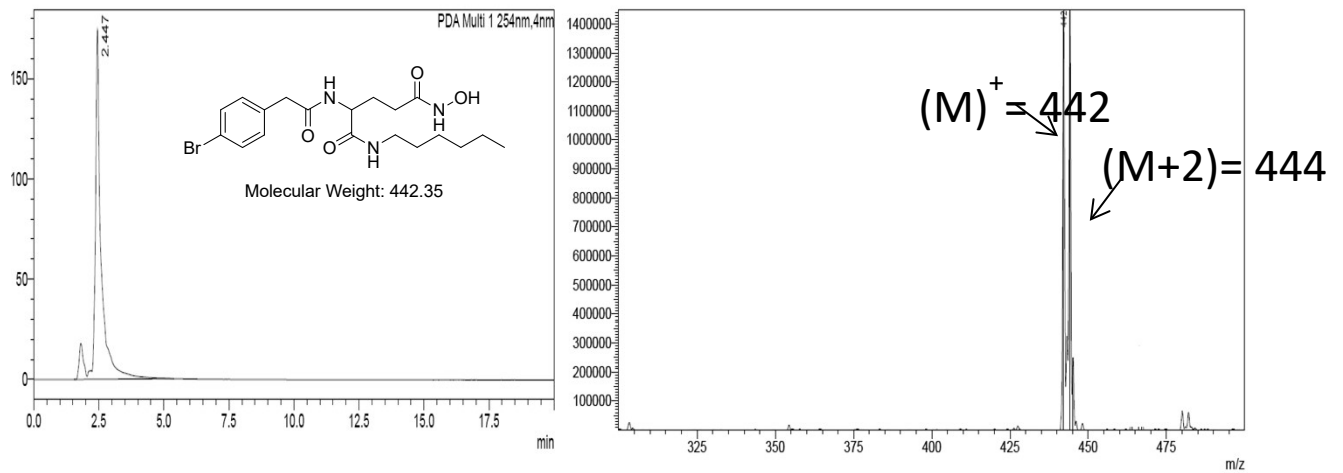
Supplementary Figure S12. LC/MS of **7d**
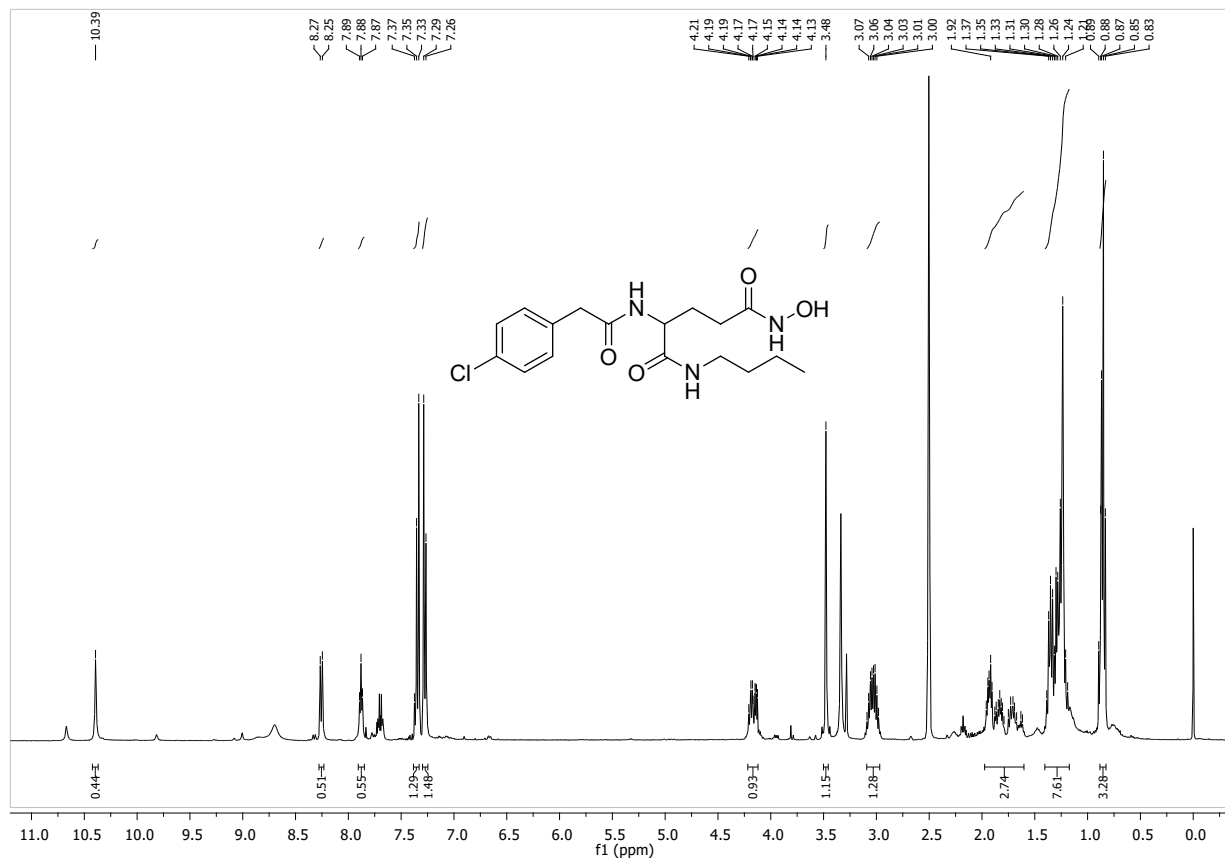
Supplementary Figure S13. ^{1}H NMR of **7e**
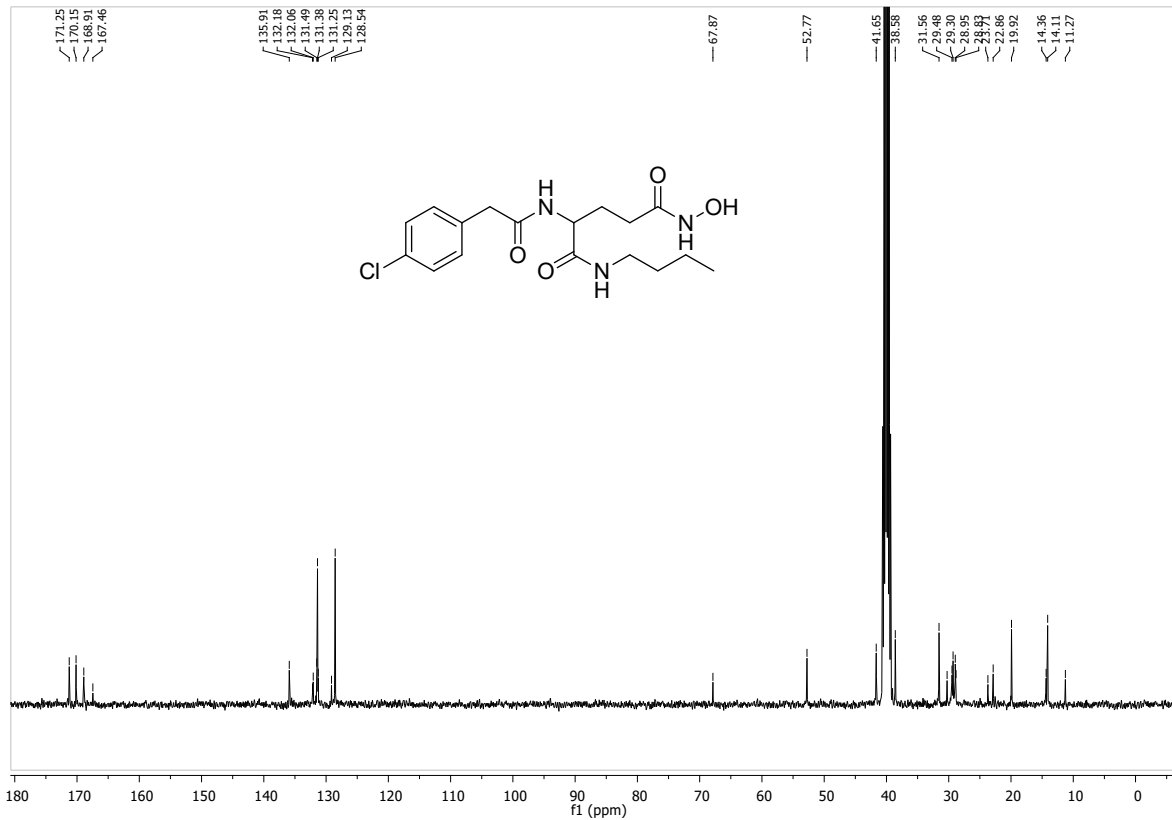
Supplementary Figure S14. ^{13}C NMR of **7e**
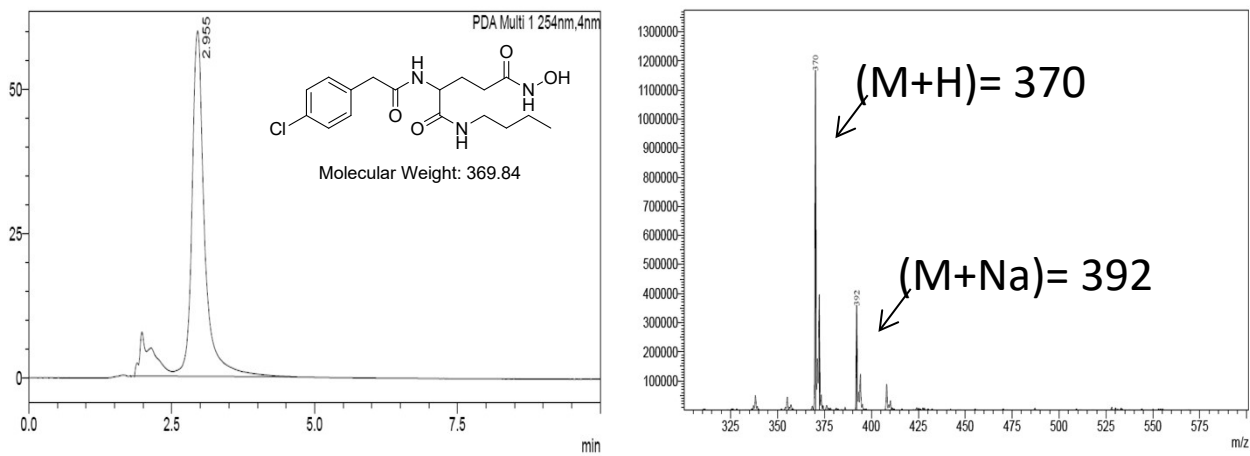
Supplementary Figure S15. LC/MS of **7e**

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Supplementary Figure S16. ^{1}H NMR of **7f**
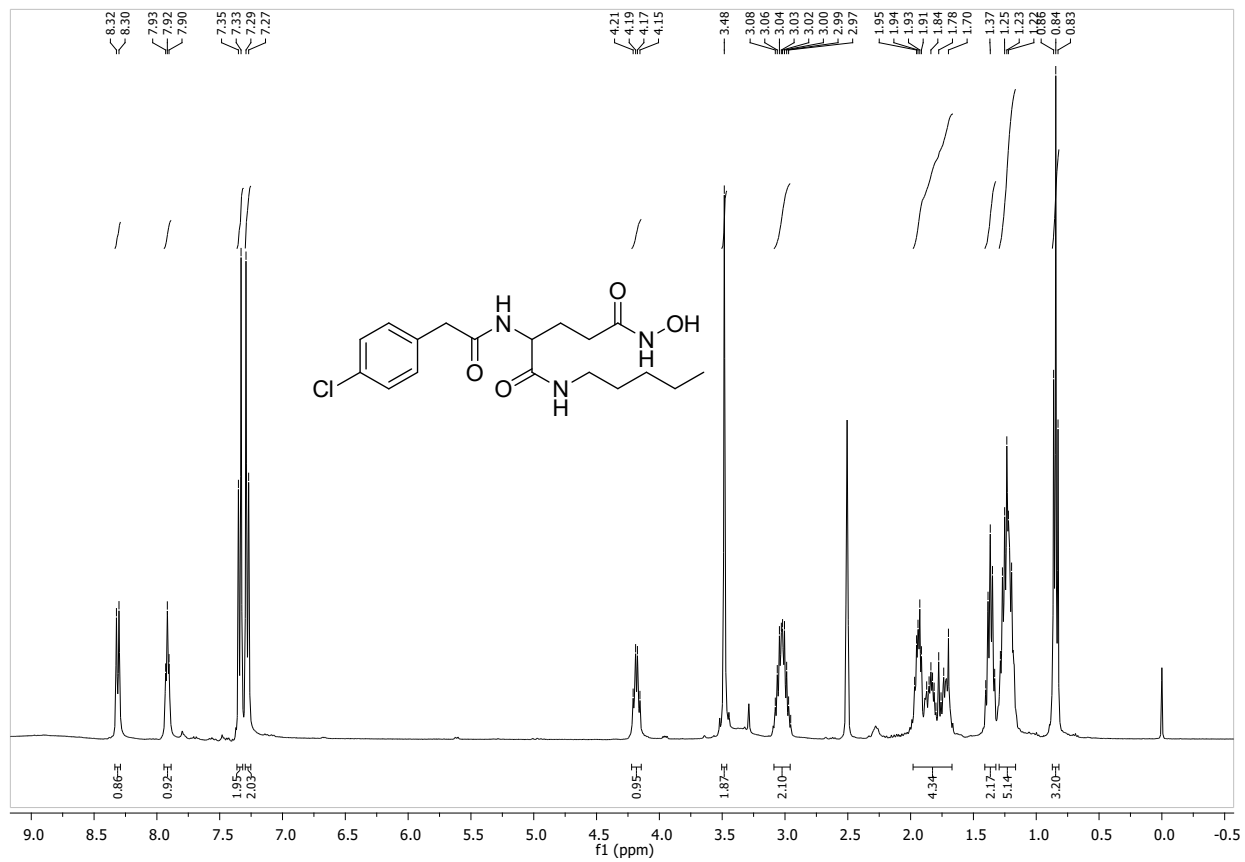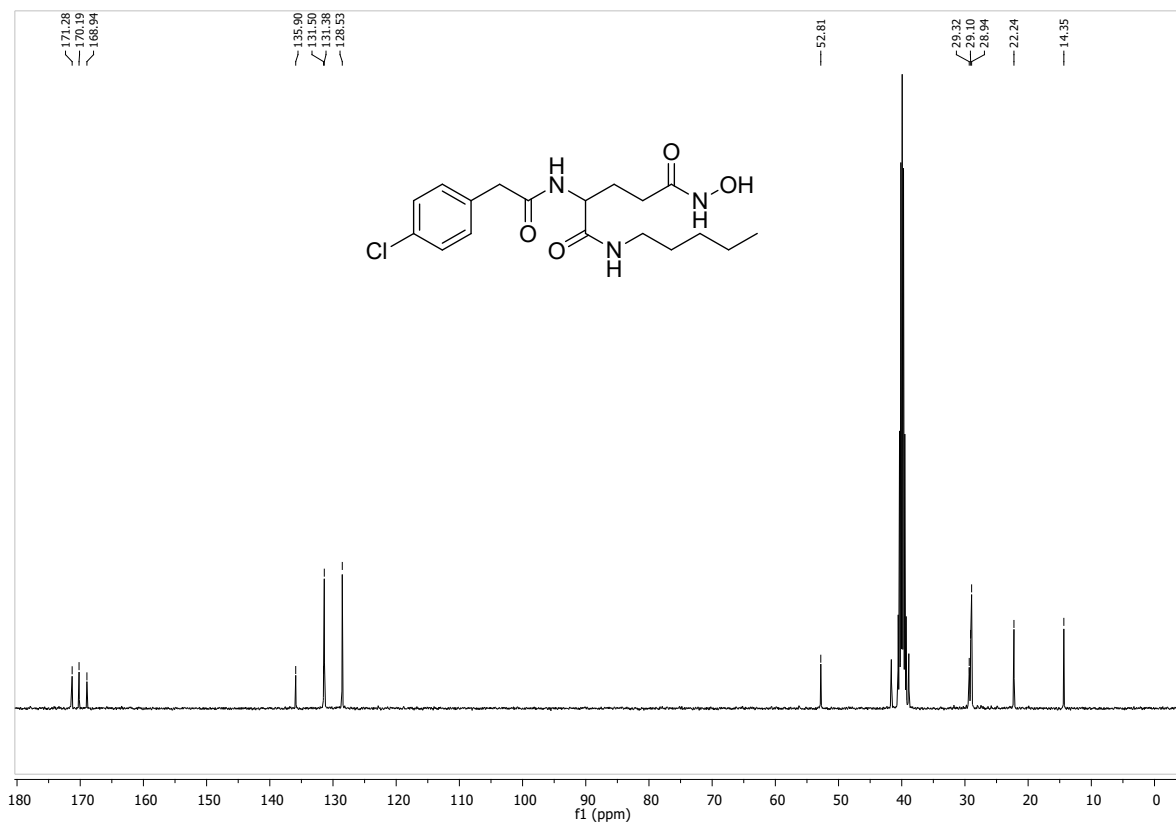
Supplementary Figure S17. ^{13}C NMR of **7f**
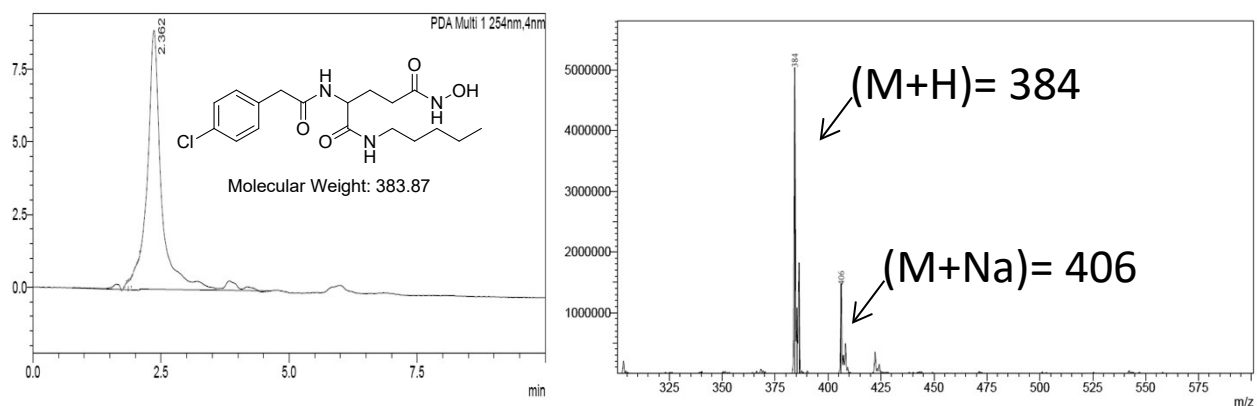
Supplementary Figure S18. LC/MS of **7f**
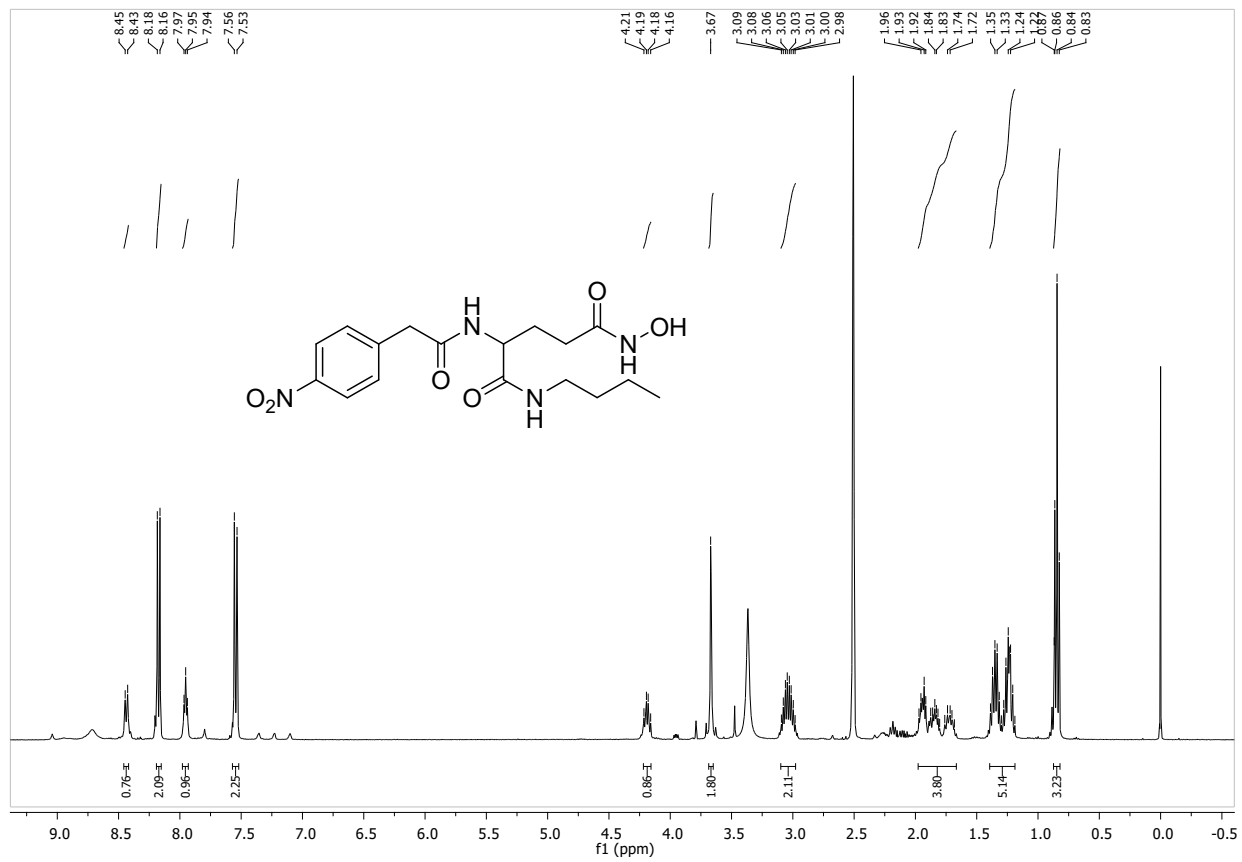
Supplementary Figure S19. ^{1}H NMR of **7g**
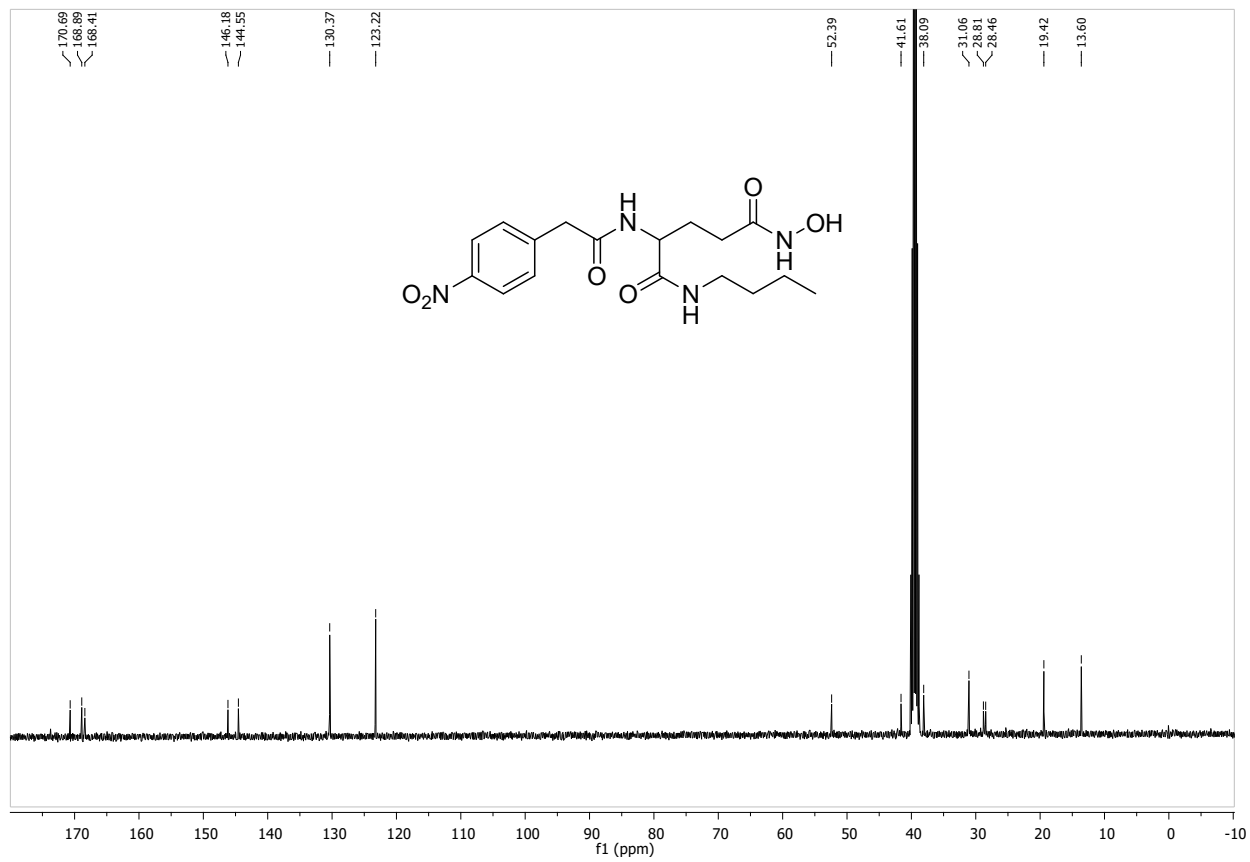
Supplementary Figure S20. ^{13}C NMR of **7g**
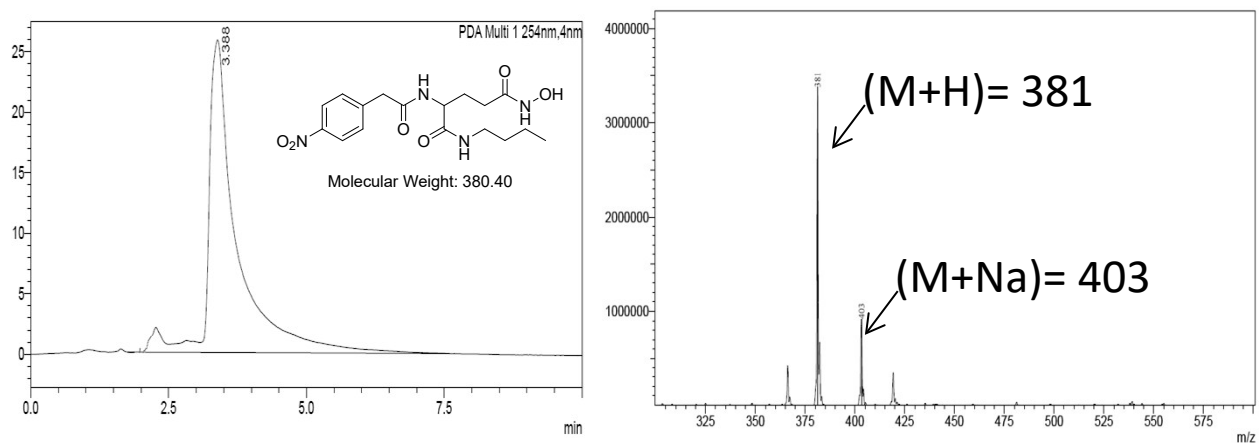
Supplementary Figure S21. LC/MS of **7g**
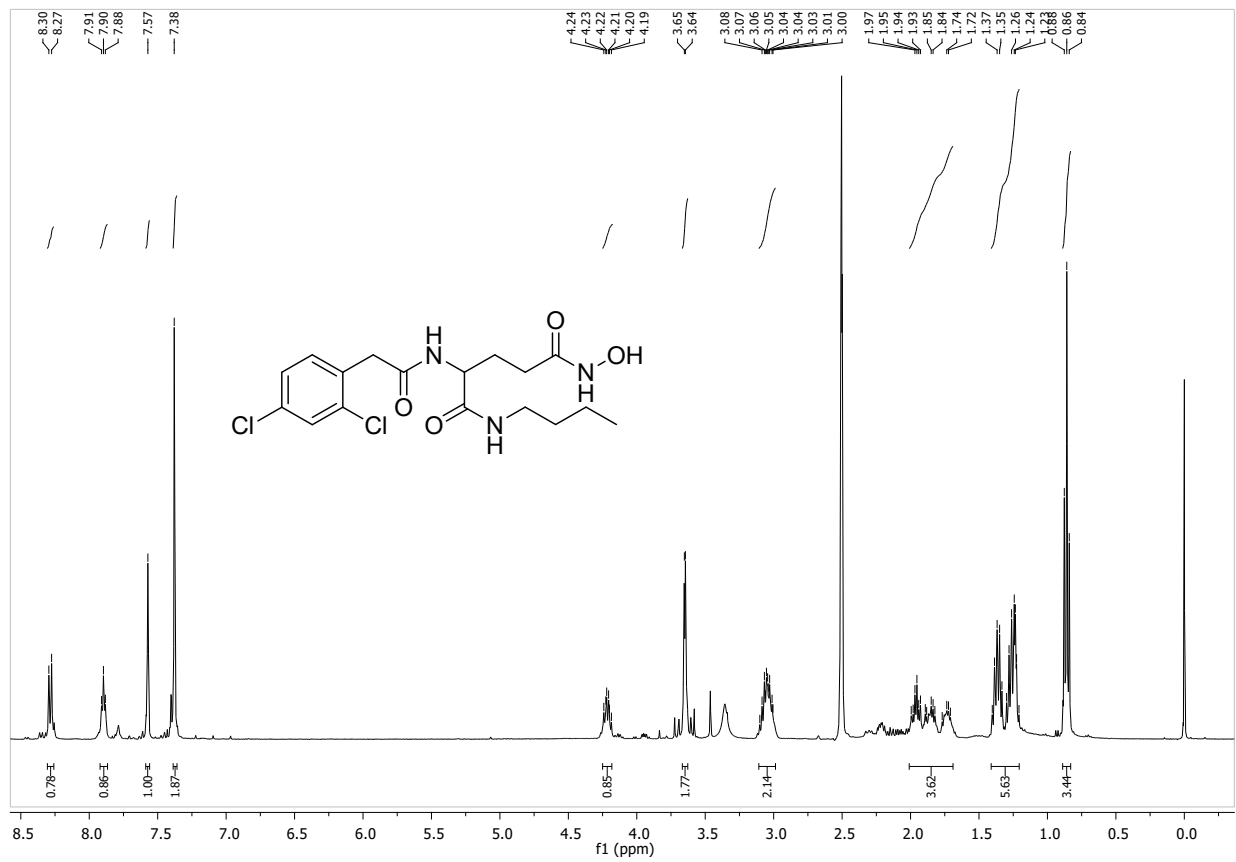
Supplementary Figure S22. ^{1}H NMR of **7h**
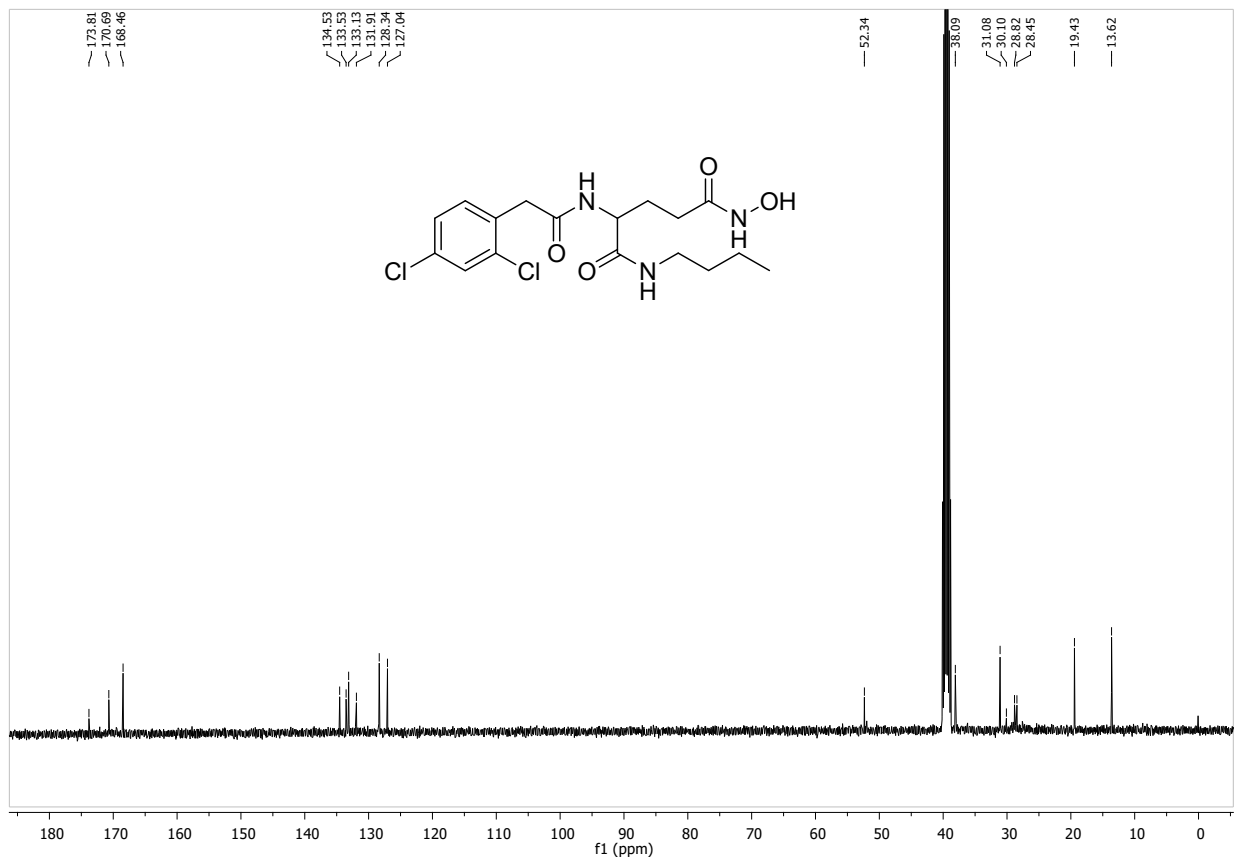
Supplementary Figure S23. ^{13}C NMR of **7h**

Molecular Weight: 404.29

$(M)^+ = 404$
$(M+2) = 406$
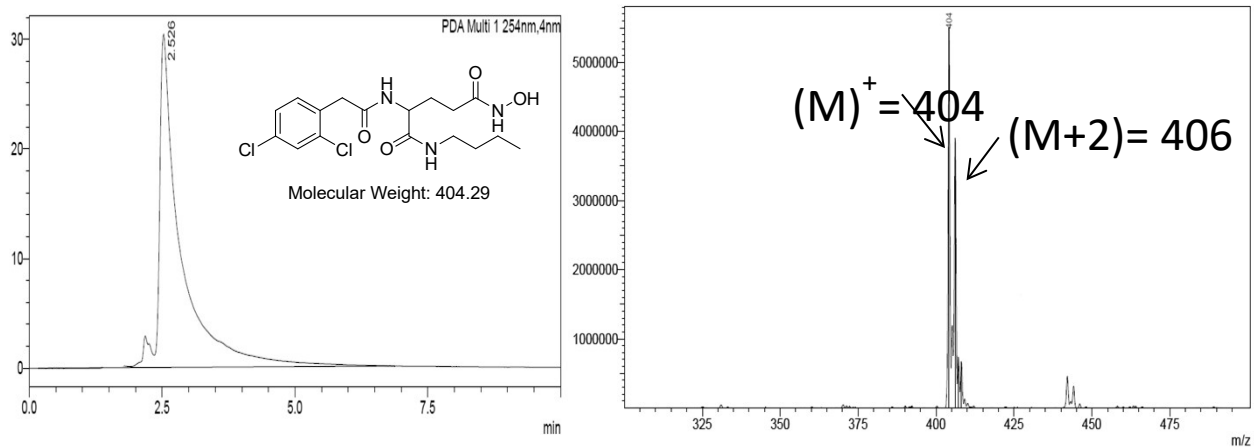
Supplementary Figure S24. LC/MS of **7h**
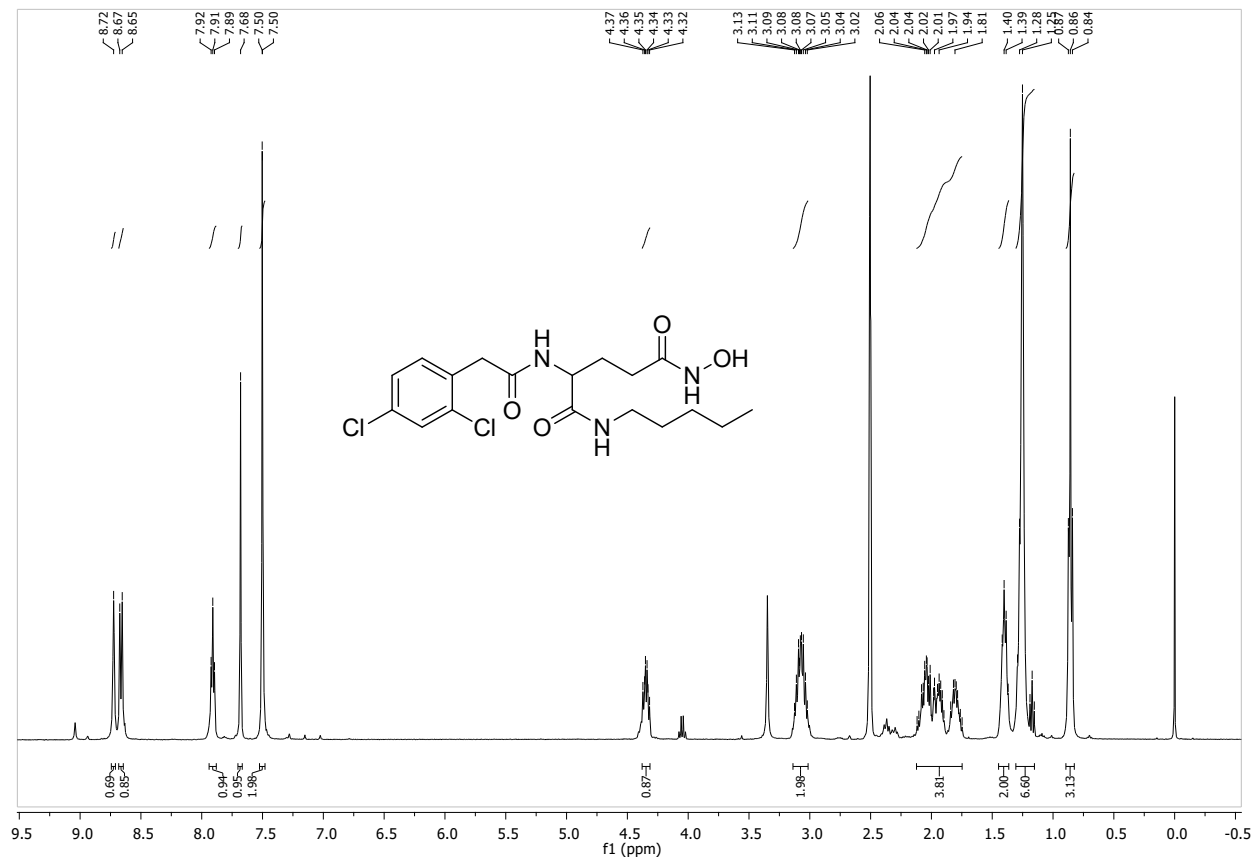
Supplementary Figure S25. ^{1}H NMR of **7i**
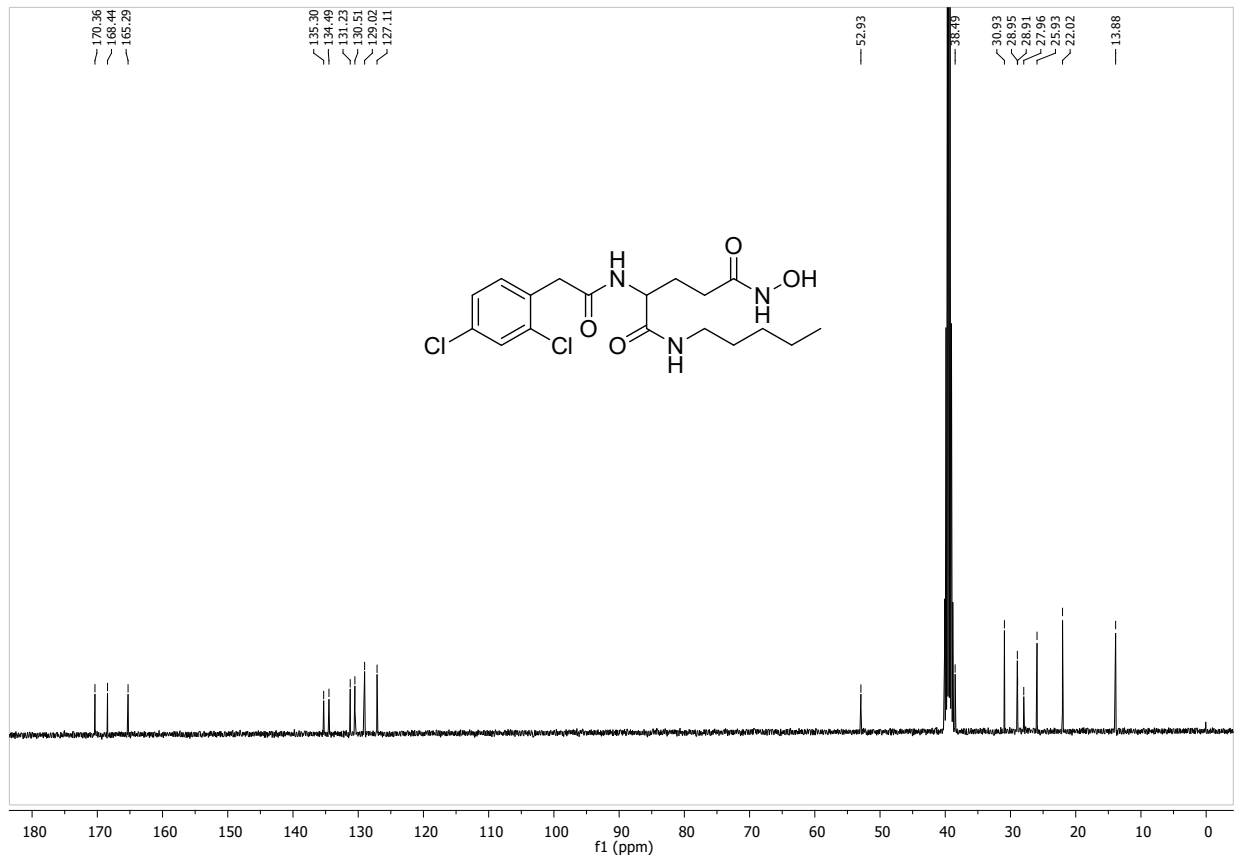
Supplementary Figure S26. ^{13}C NMR of **7i**
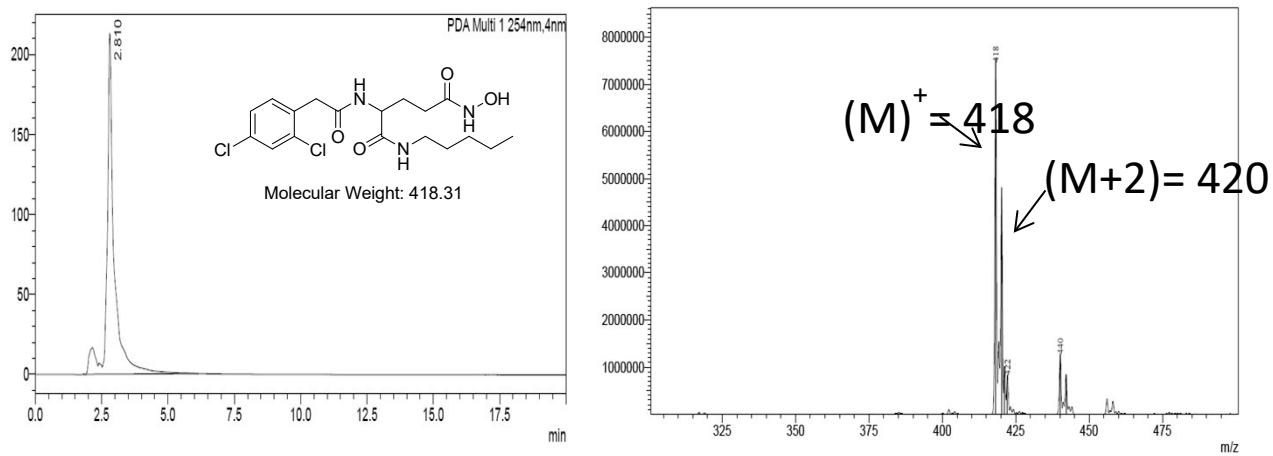
Supplementary Figure S27. LC/MS of **7i**
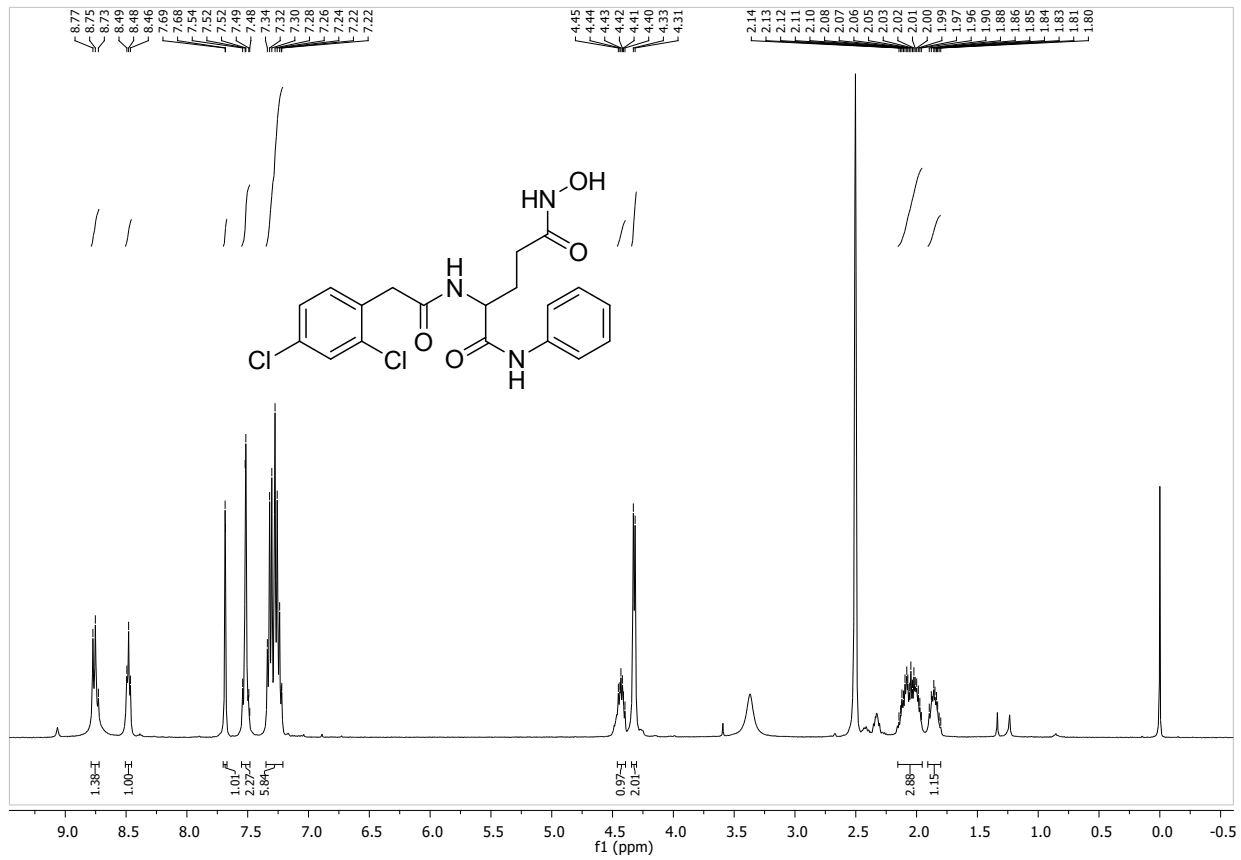
Supplementary Figure S28. ^{1}H NMR of **7j**
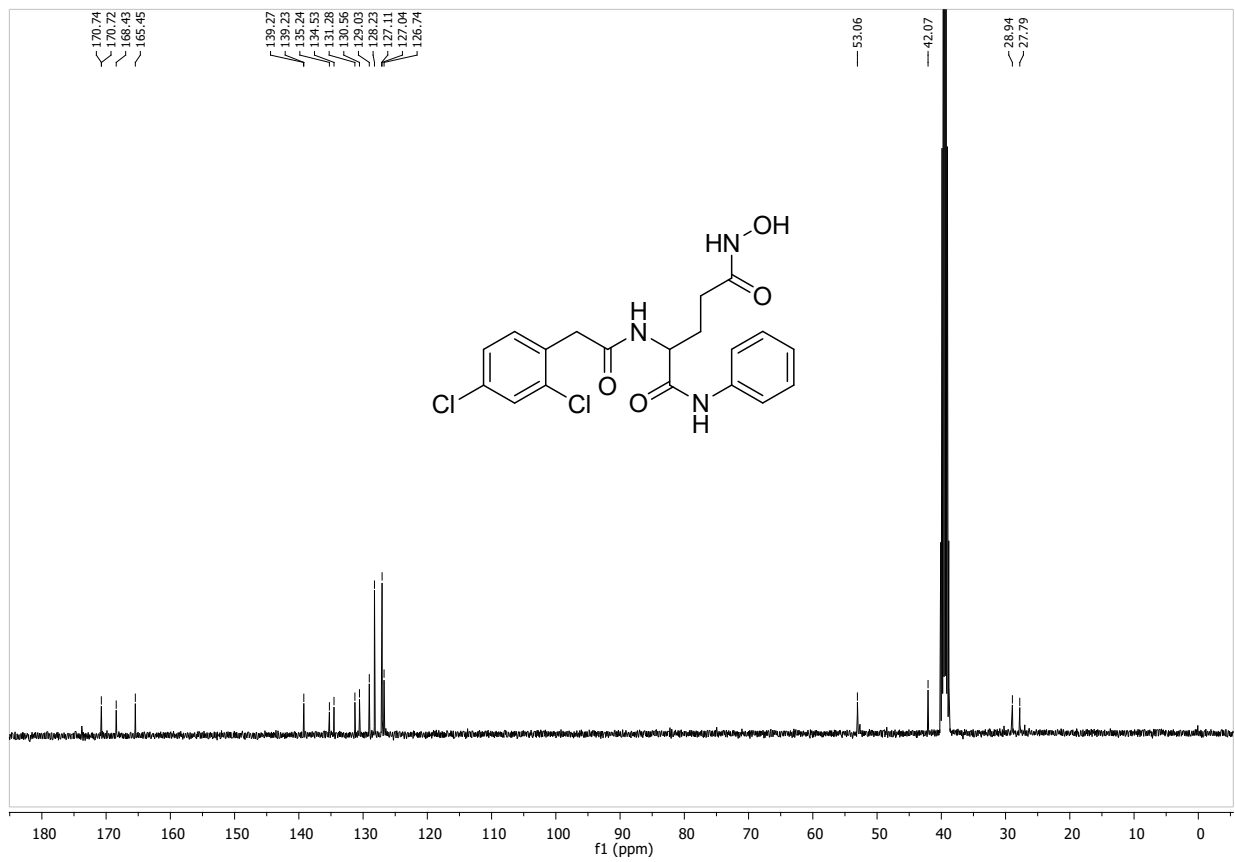
Supplementary Figure S29. ^{13}C NMR of **7j**
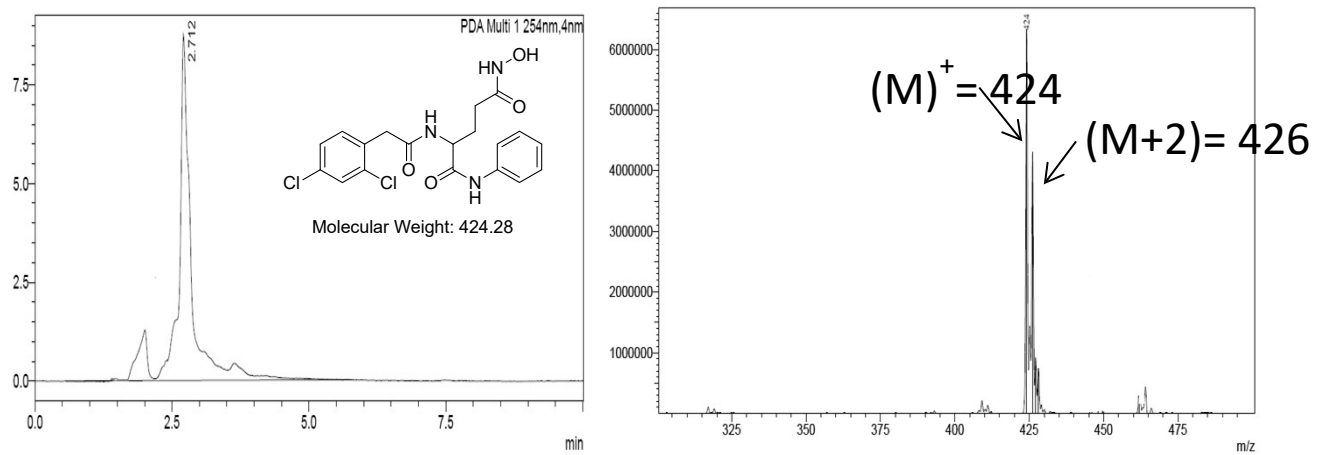
Supplementary Figure S30. LC/MS of **7j**
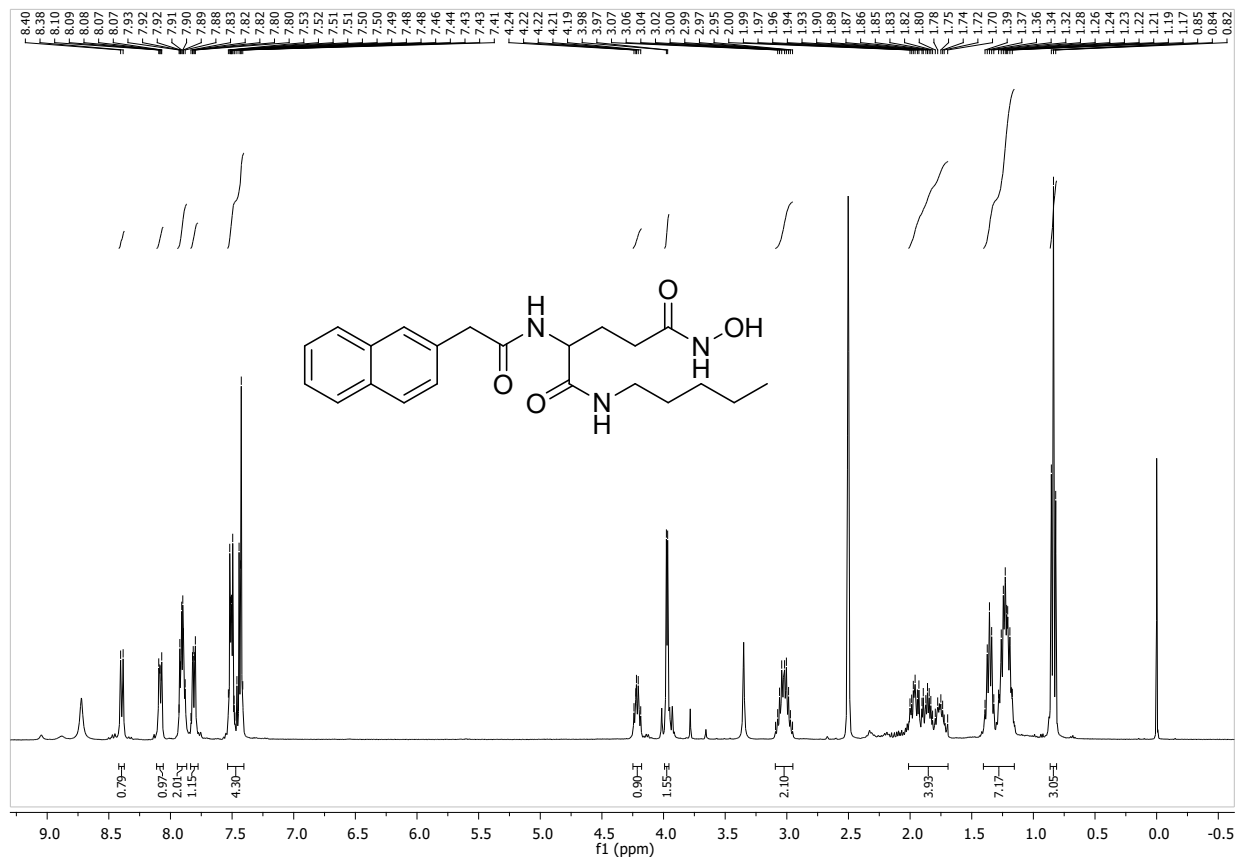
Supplementary Figure S31. ^{1}H NMR of **7k**
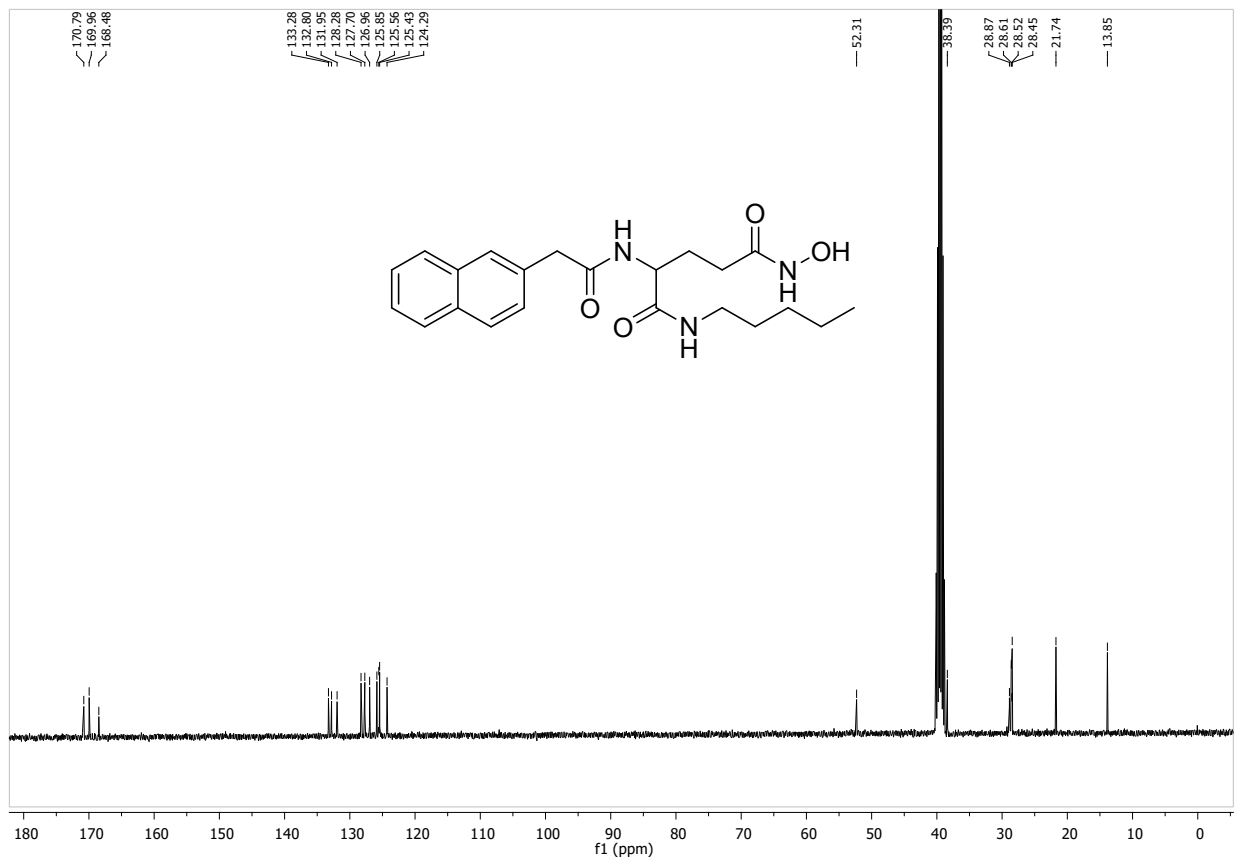
Supplementary Figure S32. ^{13}C NMR of **7k**
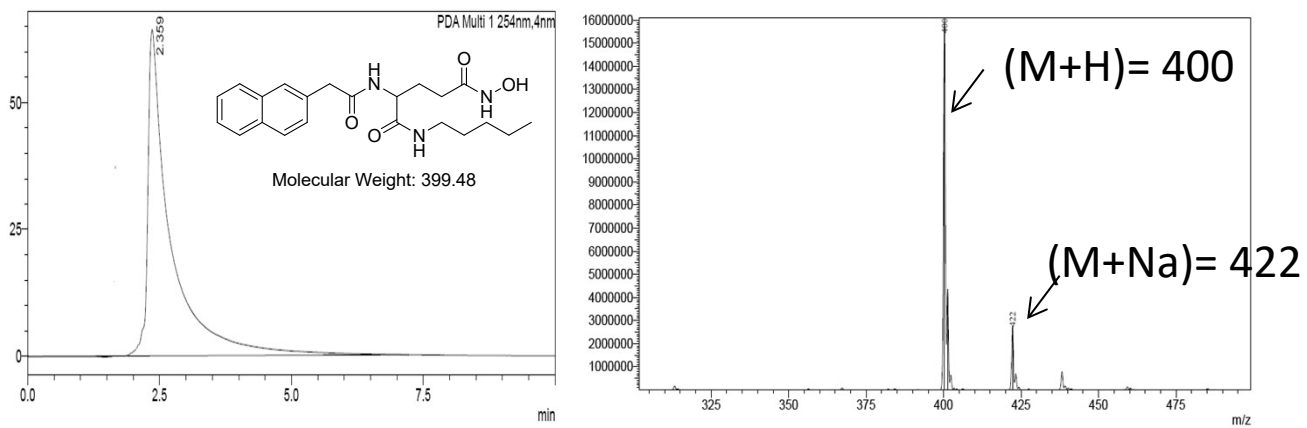
Supplementary Figure S33. LC/MS of **7k**
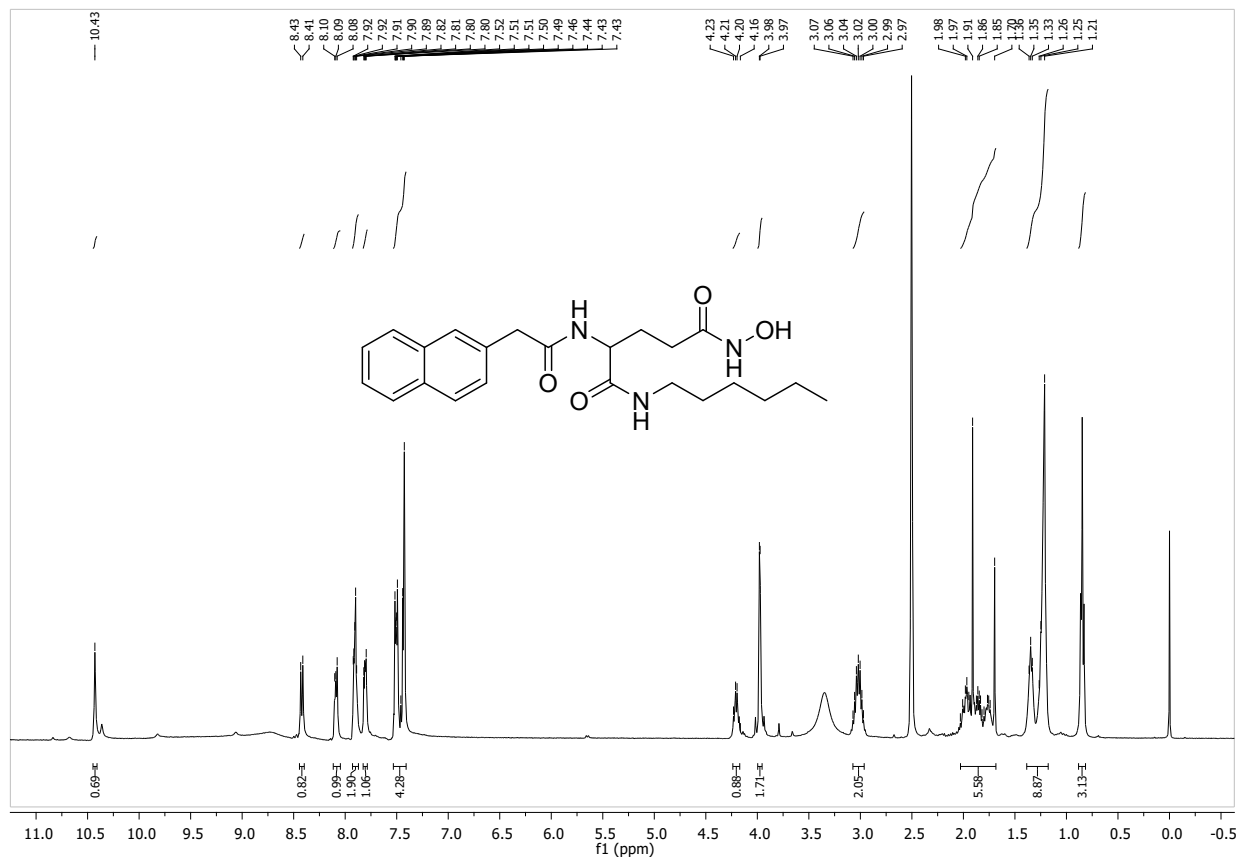
Supplementary Figure S34. ^{1}H NMR of **7l**
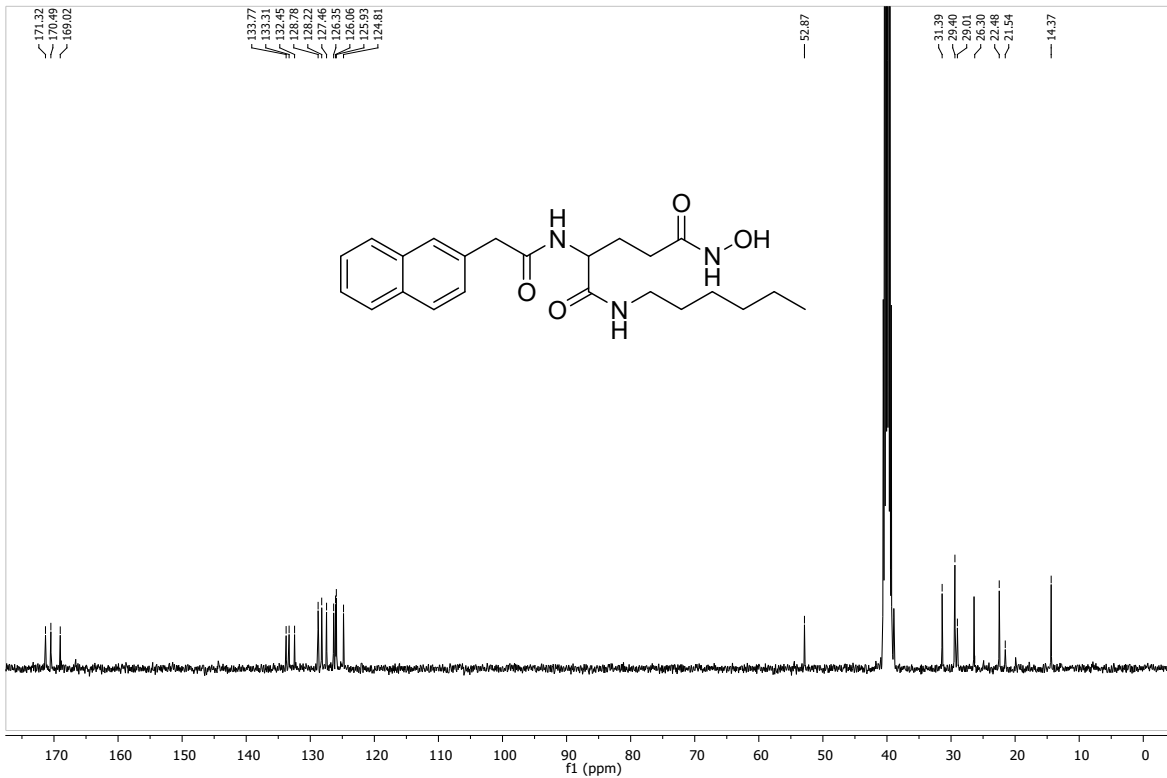
Supplementary Figure S35. ^{13}C NMR of **7l**
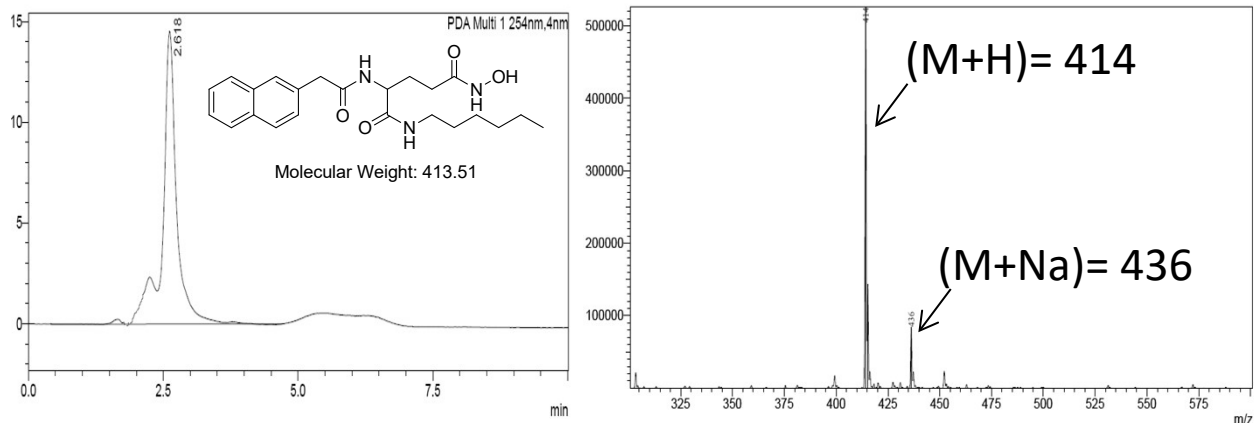
Supplementary Figure S36. LC/MS of **7l**
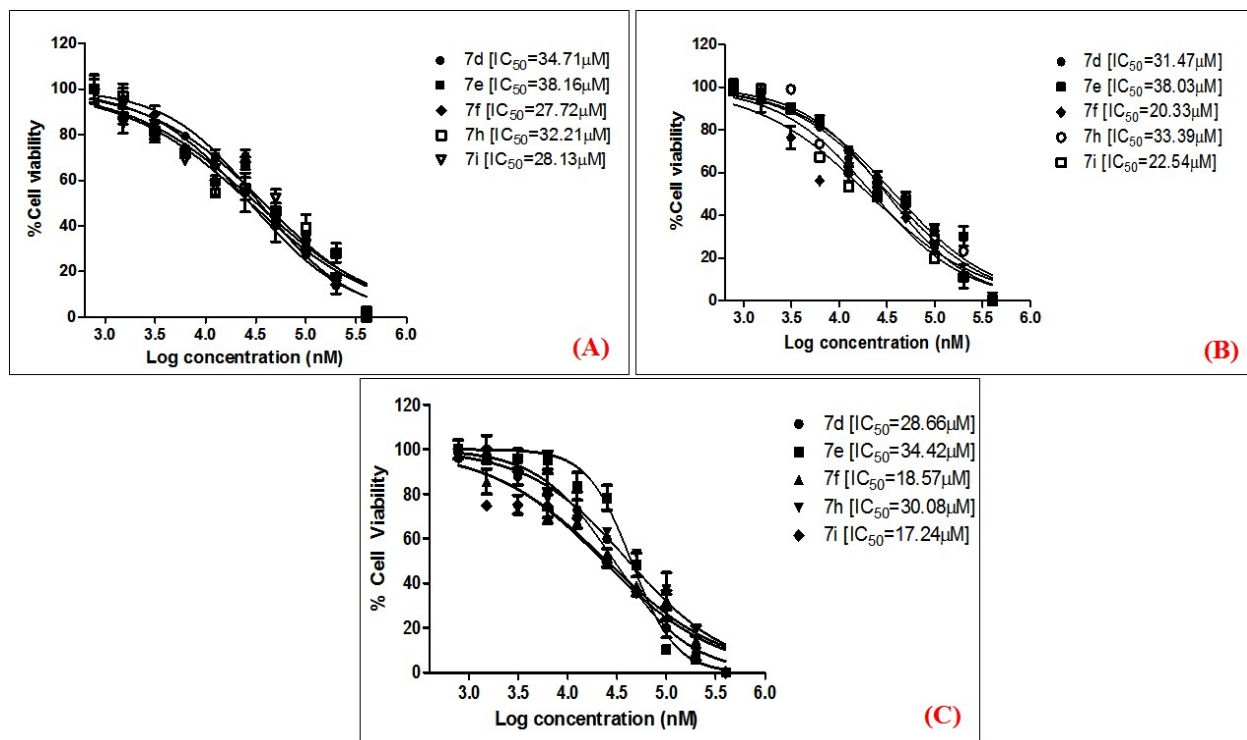
Figure S37. Dose response curve of **7d, 7e, 7f, 7h** and **7i** on (A) B16F10, (B) A549 and (C) Jurkat E6.1 cell lines. These compounds were tested in 10 different doses and incubated for 72 hours. Cell viability was measured using MTT reagent. Data represents mean $\pm$ SD (n=3).

Table S1. List of molecules considered for the modeling study

Comp[a]	Smiles	IC$_{50}$ (nM)		SEL[b]	Set
		HDAC8	HDAC3		
A1	N(C(=O)c1cc(cc(c1)N=[N+]=[N-])CN=[N+]=[N-])c1ccc(c2onc(C(=O)NCCCCCCC(=O)NO)c2)cc1	651	45	0	Train
A2	C(CCCCCC(=O)NO)NC(=O)c1noc(c1)c1ccc(cc1)N=[N+]=[N-]	707	73	0	Test
A3	C(CCCCCC(=O)NO)C(=O)Nc1cnn(c1)Cc1ccccc1	76	44	0	Test
A4	C(CCCCCC(=O)NO)C(=O)Nc1cnn(c1)Cc1ccc(cc1)[N+](=O)[O-]	82	59	0	Train
A5	C(CCCCCC(=O)NO)C(=O)Nc1cnn(c1)Cc1ccc(cc1)N=[N+]=[N-]	28	22	0	Train
A6	C(CCCCCC(=O)NO)C(=O)Nc1cnn(c1)Cc1ccc(cc1)NC(=O)OC(C)(C)C	147	191	1	Train
A7	C(CCCCCC(=O)NO)C(=O)Nc1cnn(c1)Cc1ccc(cc1)NC(=O)c1cc(cc(c1)N=[N+]=[N-])CN=[N+]=[N-]	487	432	0	Test
A8	C(CCCCCC(=O)NO)C(=O)Nc1cnn(c1)Cc1cc(cc(c1)N=[N+]=[N-])CN=[N+]=[N-]	17	128	1	Train
A9	C(=O)(c1ccc(cc1)CN C(C(=O)Nc1ccccc1)CCCC)NO	954	6,680	1	Train
A10	c1cc(ccc1NC(=O)CCCCCCC(=O)NO)c1cnnn1Cc1ccc(cc1)F	2,010	16	0	Test
A11	c1cc(ccc1NC(=O)CCCCCCC(=O)NO)c1cnnn1c1ccccc1	518	12	0	Train
A12	c1cc(ccc1NC(=O)CCCCCCC(=O)NO)c1cnnn1CC(O)c1ccc(c1)Br	737	15	0	Test
A13	c1c(cccc1NC(=O)CCCCCCC(=O)NO)c1cnnn1Cc1ccccc1	433	4.5	0	Train
A14	c1c(cccc1NC(=O)CCCCCCC(=O)NO)c1cnnn1Cc1ccc(cc1)F	529	6	0	Test
A15	c1c(cccc1NC(=O)CCCCCCC(=O)NO)c1cnnn1c1ccccc1	942	8	0	Train
A16	c1c(cccc1NC(=O)CCCCCCC(=O)NO)c1cnnn1CC(c1cccc(c1)Br)O	406	4.2	0	Train
A17	c1(onc(c1)COCCCCC(=O)NO)c1ccccc1	3,320	12.9	0	Test
A18	c1(onc(c1)C(=O)NCCCCC(=O)NO)c1ccccc1	3,580	158	0	Train
A19	c1(onc(c1)C(=O)NCCCCC(=O)NO)c1cccc(c1)NC(=O)OC(C)(C)C	5,810	84	0	Train
A20	c1(onc(c1)C(=O)NCCCCCCC(=O)NO)c1cccc(c1)N	938	4	0	Train
A21	c1(onc(c1)C(=O)NCCCCC(=O)NO)c1cccc(c1)N	3,430	144	0	Test
A22	c1(onc(c1)C(=O)NCCCCCCC(=O)NO)c1ccc(cc1)NC(=O)OC(C)(C)C	6,851	0	0	Train
A23	c1(onc(c1)C(=O)NCCCCC(=O)NO)c1ccc(cc1)NC(=O)OC(C)(C)C	3,650	115	0	Train
A24	c1(onc(c1)C(=O)NCCCCCCC(=O)NO)c1ccc(cc1)N	3,458	10	0	Train
A25	c1(onc(c1)C(=O)NCCCCC(=O)NO)c1ccc(cc1)N	2,830	66	0	Train
A26	c1(onc(c1)C(=O)NCCCCCCC(=O)NO)c1ccc(cc1)NC(=O)C	4,900	18	0	Train
A27	c1c(ccc(c1)NC(=O)CCCCCCC(=O)NO)OCc1cc(cc(c1)N=[N+]=[N-])CN=[N+]=[N-]	7,340	1,480	0	Train
A28	c1ccccc1NC(=O)CCCCCCC(=O)NO	2,290	17	0	Train
A29	c1cc(ccc1C(=O)/C(=C/C(C)/C=C/C(=O)NO)/C)N(C)C	1,380	2	0	Test
A30	c1c(cc(cc1)NC(=O)CCCCCCC(=O)NO)c1nnn(c1)c1ccc(	12,000	18	0	Train

	cc1)F				
A31	c1c(cc(cc1)NC(=O)CCCCCCC(=O)NO)c1nnn(c1)c1ccc(cc1)C(F)(F)F	5,220	3	0	Train
A32	c1c(cc(cc1)NC(=O)CCCCCCC(=O)NO)c1nnn(c1)c1cc(cc(c1)CO)CO	1,320	3	0	Train
A33	c1c(cc(cc1)NC(=O)CCCCCCC(=O)NO)c1nnn(c1)C1CCCCC1	1,190	4	0	Train
A34	c1c(cc(cc1)NC(=O)CCCCCCC(=O)NO)n1nnc(c1)c1ccccc1	4,180	3	0	Train
A35	c1c(cc(cc1)NC(=O)CCCCCCC(=O)NO)n1nnc(c1)C1CCCCC1	2,750	5	0	Train
A36	c1cc(ccc1NC(=O)CCCCCCC(=O)NO)c1cn(nn1)Cc1ccc(cc1)F	797	14	0	Train
A37	c1cc(ccc1NC(=O)CCCCCCC(=O)NO)c1cn(nn1)c1ccccc1	1,970	8	0	Train
A38	c1cc(ccc1NC(=O)CCCCCCC(=O)NO)c1cn(nn1)CC(O)c1cccc(c1)Br	3,130	25	0	Train
A39	c1c(cccc1NC(=O)CCCCCCC(=O)NO)c1cn(nn1)Cc1ccccc1	1,990	8	0	Train
A40	c1c(cccc1NC(=O)CCCCCCC(=O)NO)c1cn(nn1)Cc1ccc(cc1)F	552	4	0	Train
A41	c1c(cccc1NC(=O)CCCCCCC(=O)NO)c1cn(nn1)c1ccccc1	2,780	7	0	Test
A42	c1c(cccc1NC(=O)CCCCCCC(=O)NO)c1cn(nn1)CC(O)c1cccc(c1)Br	440	3	0	Test
A43	c1cc(ccc1NC(=O)CCCCCCC(=O)NO)c1cnnn1Cc1ccccc1	790	9.7	0	Train
A44	C(=O)(CCCCCC[NH2+]Cc1ccccc1)NO	1,800	430	0	Train
A45	C(=O)(CCCC[NH2+]Cc1ccccc1)NO	10,000	22,000	1	Train
A46	C(=O)(CCCCCC[NH+](Cc1ccccc1)Cc1ccccc1)NO	4,000	1,100	0	Train
A47	C(=O)(CCCC[NH+](Cc1ccccc1)Cc1ccccc1)NO	1,900	1,400	0	Train
A48	C(=O)(CC[NH+](Cc1ccccc1)Cc1ccccc1)NO	1,400	52,000	1	Train
A49	C(=O)(CCCCCC[NH+]1Cc2c(CC1)cccc2)NO	1,900	97	0	Train
A50	C(=O)(CC[NH+]1Cc2c(CC1)cccc2)NO	19,000	98,000	1	Test
A51	C(=O)(CCCC[NH+]1C(c2c(CC1)cccc2)c1ccccc1)NO	44	810	1	Train
A52	C(=O)(CC[NH+]1C(c2c(CC1)cccc2)c1ccccc1)NO	950	10,000	1	Train
A53	C(C[NH+]1C(c2c(CC1)cccc2)c1cccc(c1)OC)C(=O)NO	110	34,000	1	Train
A54	C(=O)(CC[NH+]1C(c2c(CC1)cccc2)c1ccc(cc1)C(F)(F)F)NO	190	27,000	1	Test
A55	C(=O)(CC[NH+]1C(c2c(CC1)cccc2)C1CCCCC1)NO	6,700	75,000	1	Test
A56	C(=O)(CC[NH+]1C(c2c(CC1)cccc2)c1ccc(cc1)C(C)(C)C)NO	480	29,000	1	Train
A57	C(=O)(CC[NH+]1C(c2c(CC1)cccc2)c1cccc2ccccc12)NO	390	3,400	1	Train
A58	C(=O)(CC[NH+]1C(c2c(CC1)cccc2)c1ccc(cc1)c1ccccc1)NO	55	38,000	1	Train
A59	c1c(cc2c(c1)ccn2Cc1ccc(cc1)OC)C(=O)N[O-]	6.6	7,100	1	Train

aCompound number; bRange of HDAC8 selectivity

28

Table S2. Correlation matrix among biological activity and selected parameters of Eq. (1)

	nAtomLAC	PubchemFP357	PubchemFP300	PubchemFP2	GATS6v	PubchemFP528	GATS3s	PubchemFP713	BA(8/3)
nAtomLAC	1	0.39	0.73	0.25	-0.46	-0.56	0.62	-0.6	-0.77
PubchemFP357		1	0.44	-0.18	-0.43	-0.33	0.58	-0.22	-0.67
PubchemFP300			1	0	-0.27	-0.83	0.51	-0.31	-0.42
PubchemFP2				1	0.17	0.2	0.24	-0.31	-0.37
GATS6v					1	0.07	-0.32	0.4	0.51
PubchemFP528						1	-0.45	0.02	0.3
GATS3s							1	-0.4	-0.65
PubchemFP713								1	0.56
BA(8/3)									1

Table S3. *t*-Value and *p*-Value for Eq. (1)

	$t(36)$	*p*-level
Intercept	-2.274	0.029
nAtomLAC	-6.693	0.000
PubchemFP357	-13.182	0.000
PubchemFP300	8.014	0.000
PubchemFP2	-9.345	0.000
GATS6v	4.422	0.000
PubchemFP528	5.721	0.000
GATS3s	3.187	0.003
PubchemFP713	2.549	0.015

Table S4. R^2 and Q^2 values after several Y-randomization tests for Eq. (1)

MODEL TYPE	*R*	R^2	$Q^2_{(LOO)}$
Original	**0.978**	**0.956**	**0.932**
Random 1	0.619	0.383	-0.097
Random 2	0.616	0.380	-0.145
Random 3	0.477	0.228	-0.676
Random 4	0.490	0.240	-0.143
Random 5	0.492	0.242	-0.209
Random 6	0.451	0.203	-0.095
Random 7	0.329	0.108	-0.470
Random 8	0.419	0.176	-0.900
Random 9	0.520	0.271	-0.283
Random 10	0.522	0.273	-0.293

Random 11	0.486	0.236	-0.351
Random 12	0.307	0.094	-0.446
Random 13	0.298	0.089	-0.912
Random 14	0.379	0.144	-0.311
Random 15	0.431	0.186	-0.660
Random 16	0.493	0.243	0.002
Random 17	0.341	0.117	-0.322
Random 18	0.441	0.194	-0.507
Random 19	0.432	0.187	-0.327
Random 20	0.387	0.150	-0.354
Random 21	0.454	0.206	-0.538
Random 22	0.416	0.173	-0.392
Random 23	0.362	0.131	-0.801
Random 24	0.365	0.133	-0.301
Random 25	0.295	0.087	-0.351
Random 26	0.353	0.124	-0.335
Random 27	0.262	0.069	-0.360
Random 28	0.425	0.181	-0.287
Random 29	0.415	0.173	-0.252
Random 30	0.345	0.119	-0.399
Random 31	0.292	0.086	-0.674
Random 32	0.423	0.179	-0.222
Random 33	0.288	0.083	-0.437
Random 34	0.352	0.124	-0.232
Random 35	0.266	0.071	-0.759
Random 36	0.453	0.205	-0.264
Random 37	0.244	0.059	-1.515
Random 38	0.583	0.340	-0.046
Random 39	0.396	0.157	-0.277
Random 40	0.489	0.239	-0.175
Random 41	0.454	0.206	-0.542
Random 42	0.537	0.288	-0.152
Random 43	0.415	0.172	-0.427
Random 44	0.428	0.183	-0.638
Random 45	0.501	0.251	-1.405
Random 46	0.387	0.150	-0.398
Random 47	0.454	0.206	-0.150
Random 48	0.354	0.126	-0.409
Random 49	0.425	0.181	-0.448
Random 50	0.399	0.159	-0.508

Table S5. The descriptors and predicted activity of designed inhibitors

Cpd[a]	nAtomLAC	PubchemFP357	PubchemFP300	PubchemFP2	GATS6v	PubchemFP528	GATS3s	PubchemFP713	MLR	SVM
7a	5	0	0	1	0.846	1	0.909	0	SEL	SEL
7b	5	0	0	1	0.977	1	0.931	0	SEL	SEL
7c	5	0	0	1	0.882	1	0.911	0	SEL	SEL
7d	6	0	0	1	0.898	1	0.893	0	SEL	SEL
7e	5	0	0	1	0.937	1	0.934	0	SEL	SEL
7f	5	0	0	1	0.887	1	0.912	0	SEL	SEL
7g	5	0	0	1	0.964	1	0.778	0	SEL	SEL
7h	5	0	0	1	0.925	1	0.932	0	SEL	SEL
7i	5	0	0	1	0.875	1	0.911	0	SEL	SEL
7j	5	0	0	1	0.952	1	0.994	0	SEL	SEL
7k	5	0	0	1	0.895	1	0.896	1	NS	SEL
7l	6	0	0	1	0.909	1	0.880	1	NS	SEL

[a]Compound number; SEL, HDAC8 selective; NS, non-selective