Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Table S1. Molecular dataset of HDAC-8 inhibitors used for the development and validation of ligand-based pharmacophore models.

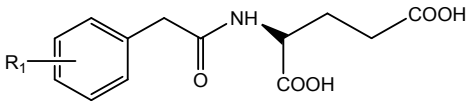
Cpd.	SMILES (Whole Molecule)	IC_{50} (nM)	pIC_{50}	Ref
01	CC(C)(C)OC(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)c1ccccc1)OCC(=O)NO	1980.00	5.703	1
02	ONC(=O)COc1ccc2c(c1)C[N+H2][C@@H](C2)C(=O)Nc1ccc(cc1)c1ccccc1	2210.00	5.656	1
03	CC(C)(C)OC(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccccc1)OCC(=O)NO	1290.00	5.889	1
04	ONC(=O)COc1ccc2c(c1)CN([C@@H](C2)C(=O)NCCc1ccccc1)C(=O)OCc1ccccc1	580.00	6.237	1
05	ONC(=O)COc1ccc2c(c1)C[N+H2][C@@H](C2)C(=O)Nc1cccc2ccccc12	1060.00	5.975	1
06	CC(C)(C)OC(=O)N1Cc2cc(ccc2C[C@H]1C(=O)NCCc1ccccc1)OCC(=O)NO	2670.00	5.573	1
07	CC(C)(C)OC(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1cccc2ccccc12)OCC(=O)NO	4250.00	5.372	1
08	Cc1cccc(c1)NC(=O)[C@@H]1Cc2ccc(cc2C[N+H2]1)OCC(=O)NO	3620.00	5.441	1
09	CC(C)(C)OC(=O)N1Cc2cc(ccc2C[C@H]1C(=O)NCc1ccccc1)OCC(=O)NO	3410.00	5.467	1
10	ONC(=O)COc1ccc2c(c1)C[N+H2][C@@H](C2)C(=O)Nc1ccccc1	8210.00	5.086	1
11	Cc1cccc(c1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)OC(C)(C)C)OCC(=O)NO	1770.00	5.752	1
12	Cc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)OC(C)(C)C)OCC(=O)NO	1650.00	5.783	1
13	Cc1ccccc1NC(=O)[C@@H]1Cc2ccc(cc2C[N+H2]1)OCC(=O)NO	4130.00	5.384	1
14	CC(C)(C)OC(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1cccc(c1)Cl)OCC(=O)NO	1170.00	5.932	1
15	ONC(=O)COc1ccc2c(c1)C[N+H2][C@@H](C2)C(=O)Nc1ccc(c(c1)Cl)F	3340.00	5.476	1
16	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)OC(C)(C)C)OCC(=O)NO	1000.00	6.000	1
17	Cc1ccccc1NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)OC(C)(C)C)OCC(=O)NO	4000.00	5.398	1
18	ONC(=O)COc1ccc2c(c1)CN[C@@H](C2)C(=O)NCc1ccccc1	5100.00	5.292	1
19	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2C[N+H2]1)OCC(=O)NO	5570.00	5.254	1
20	ONC(=O)COc1ccc2c(c1)C[N+H2][C@@H](C2)C(=O)Nc1ccc(cc1)F	3230.00	5.491	1
21	CC(C)(C)OC(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)F)OCC(=O)NO	2560.00	5.592	1
22	Cc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2C[N+H2]1)OCC(=O)NO	3820.00	5.418	1
23	ONC(=O)COc1ccc2c(c1)C[N+H2][C@@H](C2)C(=O)NCCc1ccccc1	4070.00	5.390	1
24	ONC(=O)COc1ccc2c(c1)C[N+H2][C@@H](C2)C(=O)Nc1cccc(c1)Cl	3200.00	5.495	1
25	Cc1ccc(c(c1)C)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)OC(C)(C)C)OCC(=O)NO	3780.00	5.423	1

26	CC(C)(C)OC(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(c(c1)Cl)F)OCC(=O)NO	1550.00	5.810	1
27	Cc1ccc(c(c1)C)NC(=O)[C@@H]1Cc2ccc(cc2C[N+H2]1)OCC(=O)NO	3390.00	5.470	1
28	CCCCCCNC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)OC(C)(C)C)OCC(=O)NO	4420.00	5.355	1
29	CC(C)(C)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)OC(C)(C)C)OCC(=O)NO	4580.00	5.339	1
30	CCCCCNC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)OC(C)(C)C)OCC(=O)NO	3020.00	5.520	1
31	CCCCCNC(=O)[C@@H]1Cc2ccc(cc2C[N+H2]1)OCC(=O)NO	3540.00	5.451	1
32	CCCCCCNC(=O)[C@@H]1Cc2ccc(cc2C[N+H2]1)OCC(=O)NO	5770.00	5.239	1
33	CC(C)(C)NC(=O)[C@@H]1Cc2ccc(cc2CN1)OCC(=O)NO	12170.00	4.915	1
34	COc1ccc(cc1)NC(=O)[C@H](Cc1ccc(cc1)OCC(=O)NO)NC(=O)Cc1ccccc1	63.00	7.201	2
35	COc1ccc(cc1)NC(=O)[C@H](Cc1ccc(cc1)OCC(=O)NO)NC(=O)CCc1ccccc1	90.00	7.046	2
36	ONC(=O)COc1ccc(cc1)C[C@H](NC(=O)OCc1ccccc1)C(=O)Nc1ccccc1	197.00	6.706	2
37	COc1ccc(cc1)NC(=O)[C@H](Cc1ccc(cc1)OCC(=O)NO)NCCCc1ccccc1	922.00	6.035	2
38	COc1ccc(cc1)NC(=O)[C@H](Cc1ccc(cc1)OCC(=O)NO)NC(=O)CCCc1ccccc1	86.00	7.066	2
39	CC(C)(C)OC(=O)N[C@@H](Cc1ccc(cc1)OCC(=O)NO)C(=O)Nc1ccccc1	227.00	6.644	2
40	COc1ccc(cc1)NC(=O)[C@H](Cc1ccc(cc1)OCC(=O)NO)NC(=O)c1ccccc1	87.00	7.060	2
41	COc1ccc(cc1)NC(=O)[C@H](Cc1ccc(cc1)OCC(=O)NO)NC(=O)OCc1ccccc1	120.00	6.921	2
42	COc1ccc(cc1)NC(=O)[C@H](Cc1ccc(cc1)OCC(=O)NO)[N+H2]CCc1ccccc1	660.00	6.180	2
43	ONC(=O)COc1ccc(cc1)C[C@H](NC(=O)OCc1ccccc1)C(=O)Nc1cccc(c1)Cl	213.00	6.672	2
44	COc1ccc(cc1)NC(=O)[C@H](Cc1ccc(cc1)OCC(=O)NO)NCc1ccccc1	1180.00	5.928	2
45	ONC(=O)COc1ccc(cc1)C[C@H](NC(=O)CCc1ccccc1)C(=O)Nc1ccccc1	151.00	6.821	2
46	[N+H3][C@@H](Cc1ccc(cc1)OCC(=O)NO)C(=O)Nc1ccccc1	467.00	6.331	2
47	CC(C)(C)OC(=O)N[C@@H](Cc1ccccc1)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	35.00	7.456	3
48	CC(C)(C)OC(=O)N[C@@H](Cc1c[nH]c2ccccc12)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	140.00	6.854	3
49	CC(C)(C)OC(=O)N[C@@H](Cc1ccc(cc1)O)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	21.00	7.678	3
50	N[C@@H](Cc1c[nH]c2ccccc12)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	690.00	6.161	3
51	[N+H3][C@@H](Cc1ccc(cc1)O)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	490.00	6.310	3
52	[N+H3][C@@H](Cc1ccccc1)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	840.00	6.076	3
53	ONC(=O)CCCCCCC(=O)Nc1ccccc1	1480.00	5.830	3
54	CC(C)(C)OC(=O)N1CCC[C@H]1C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	760.00	6.119	3
55	CC(C)C[C@H]([N+H3])C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	920.00	6.036	3

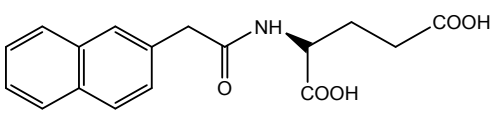
56	CC(C)(C)OC(=O)NCCCC[C@H](NC(=O)OC(C)(C)C)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	350.00	6.456	3
57	CC(C)(C)OC(=O)NCCCC(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	960.00	6.018	3
58	CC(C)(C)OC(=O)NCC(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1190.00	5.924	3
59	[N+H3][C@@H](CO)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	2520.00	5.599	3
60	[N+H3]CCCC(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	4120.00	5.385	3
61	CC(C)(C)OC(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1860.00	5.730	3
62	CC[C@H](C)[C@H]([N+H3])C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1070.00	5.971	3
63	CC(C)C[C@H](NC(=O)OC(C)(C)C)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	600.00	6.222	3
64	CC(C)[C@H](NC(=O)OC(C)(C)C)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	720.00	6.143	3
65	ONC(=O)CCCCCNC(=O)[C@@H]1CC2(CN1C(=O)C1CC[N+H2]CC1)SCCS2	2790.00	5.554	3
66	CC(C)(C)OC(=O)N[C@@H](CO)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	870.00	6.060	3
67	CC[C@H](C)[C@H](NC(=O)OC(C)(C)C)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1310.00	5.883	3
68	[N+H3]CCC(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1930.00	5.714	3
69	CC(C)(C)OC(=O)NCCC(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1900.00	5.721	3
70	C[C@H](NC(=O)OC(C)(C)C)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1150.00	5.939	3
71	C[C@H]([N+H3])C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	5900.00	5.229	3
72	[N+H3]CCCC[C@H]([N+H3])C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	4770.00	5.321	3
73	CC(C)(C)OC(=O)N1CCC(CC1)C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1440.00	5.842	3
74	ONC(=O)CCCCCNC(=O)[C@@H]1CC2(CN1C(=O)[C@@H]1CCC[N+H2]1)SCCS2	10280.00	4.988	3
75	[N+H3]CC(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1300.00	5.886	3
76	CC(C)[C@H]([N+H3])C(=O)N1CC2(C[C@H]1C(=O)NCCCCCC(=O)NO)SCCS2	1070.00	5.971	3
77	ONC(=O)CCCCCNC(=O)[C@@H]1CC2(CN1)SCCS2	2590.00	5.587	3
78	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@@H](N)Cc1ccccc1)OCC(=O)NO	368.00	6.434	4
79	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)Cc1ccccc1)OCC(=O)NO	141.00	6.851	4
80	CC[C@H](C)[C@H](NC(=O)[C@@H](NC(=O)OC(C)(C)C)C(C)C)C(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO	201.00	6.697	4
81	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)CNC(=O)OC(C)(C)C)OCC(=O)NO	514.00	6.289	4
82	CC[C@H](C)[C@H](NC(=O)[C@@H]1CCC[N+H2]1)C(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO	47.00	7.328	4
83	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@H](Cc1ccccc1)NC(=O)OC(C)(C)C)OCC(=O)NO	103.00	6.987	4
84	CC[C@H](C)[C@H](NC(=O)[C@@H]1CCCN1C(=O)OC(C)(C)C)C(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO	192.00	6.717	4
85	CC[C@H](C)[C@@H](CN1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO)NC(=O)OC(C)(C)C	263.00	6.580	4

86	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)c1ccccc1)OCC(=O)NO	164.00	6.785	4
87	CC[C@H](C)[C@H](NC(=O)OC(C)(C)C)C(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO	139.00	6.857	4
88	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@H](CC(C)C)NC(=O)OC(C)(C)C)OCC(=O)NO	163.00	6.788	4
89	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@@H](NC(=O)OC(C)(C)C)C(C)C)OCC(=O)NO	104.00	6.983	4
90	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)CCc1ccccc1)OCC(=O)NO	502.00	6.299	4
91	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@@H]1CCCN1C(=O)OC(C)(C)C)OCC(=O)NO	212.00	6.674	4
92	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1CCc1ccccc1)OCC(=O)NO	1020.00	5.991	4
93	CC[C@H](C)[C@@H](CN1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO)NC(=O)CC(C)(C)C	333.00	6.478	4
94	CC[C@H](C)[C@H](NC(=O)[C@@H]([N+H3])C(C)C)C(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO	68.00	7.167	4
95	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@H](Cc1ccc(cc1)O)NC(=O)OC(C)(C)C)OCC(=O)NO	175.00	6.757	4
96	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)CCCc1ccccc1)OCC(=O)NO	114.00	6.943	4
97	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1CCCc1ccccc1)OCC(=O)NO	1720.00	5.764	4
98	CC[C@H](C)[C@H](NC(=O)C1CC[N+H2]CC1)C(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO	1440.00	5.842	4
99	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@H](C)NC(=O)OC(C)(C)C)OCC(=O)NO	182.00	6.740	4
100	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@@H]([N+H3])Cc1ccc(cc1)O)OCC(=O)NO	634.00	6.198	4
101	ONC(=O)COc1ccc2c(c1)CN([C@@H](C2)C(=O)Nc1ccccc1)C(=O)CCc1ccccc1	759.00	6.120	4
102	ONC(=O)COc1ccc2c(c1)CN([C@@H](C2)C(=O)NCCc1ccccc1)C(=O)CCc1ccccc1	692.00	6.160	4
103	CC[C@H](C)[C@H](NC(=O)C1CCN(CC1)C(=O)OC(C)(C)C)C(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO	147.00	6.833	4
104	CC[C@H](C)[C@H]([N+H3])C(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO	1040.00	5.983	4
105	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)C[N+H3])OCC(=O)NO	2140.00	5.670	4
106	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@@H]1CCC[N+H2]1)OCC(=O)NO	481.00	6.318	4
107	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@H](C)[N+H3])OCC(=O)NO	1280.00	5.893	4
108	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@@H]([N+H3])C(C)C)OCC(=O)NO	1020.00	5.991	4
109	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1Cc1ccccc1)OCC(=O)NO	1920.00	5.717	4
110	COc1ccc(cc1)NC(=O)[C@@H]1Cc2ccc(cc2CN1C(=O)[C@@H]([N+H3])CC(C)C)OCC(=O)NO	675.00	6.171	4
111	CC[C@H](C)[C@H](NC(=O)CC(C)(C)C)C(=O)N1Cc2cc(ccc2C[C@H]1C(=O)Nc1ccc(cc1)OC)OCC(=O)NO	146.00	6.836	5

Table S2 Physical data of intermediate compounds (**I10-I18**)



I10-I17



I18

Compd.	R_1	M.P(^{0}C)	% Yield	Mol. Formula	Mol.wt.
I10	4-OCH_3	140-142	85.71	$C_{14}H_{17}O_6N$	295.29
I11	4-Cl	122-124	99.04	$C_{13}H_{14}O_5NCl$	299.71
I12	4-NO_2	80-82	87.49	$C_{13}H_{14}O_7N_2$	310.26
I13	4-Br	136-138	97.05	$C_{13}H_{14}O_5NBr$	344.16
I14	2-Cl	126-128	84.36	$C_{13}H_{14}O_5NCl$	299.71
I15	2-Br	150-152	93.33	$C_{13}H_{14}O_5NBr$	344.16
I16	2-F	134-136	83.25	$C_{13}H_{14}O_5NF$	283.25
I17	2,4-Cl_2	143-145	81.56	$C_{13}H_{13}O_5NCl2$	334.15
I18	-	178-180	98.2	$C_{17}H_{17}O_5N$	315.32

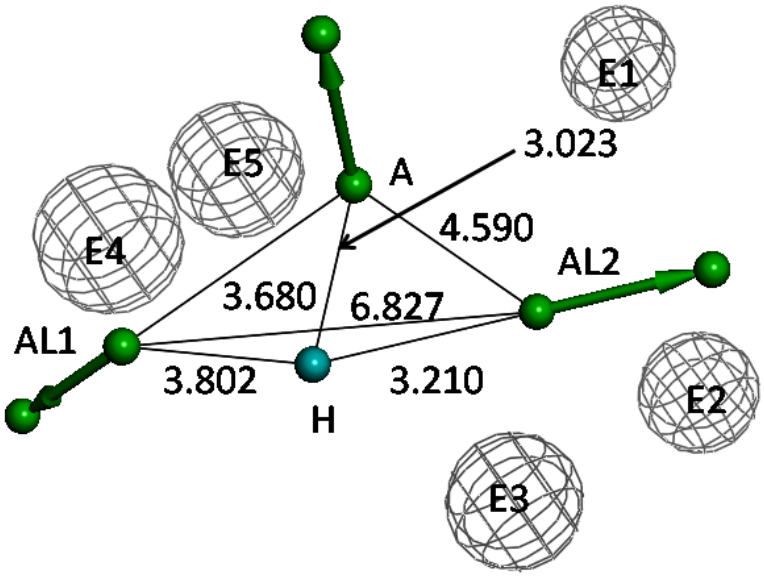


Figure S1. Interfeature distance constraints of Hypo2.

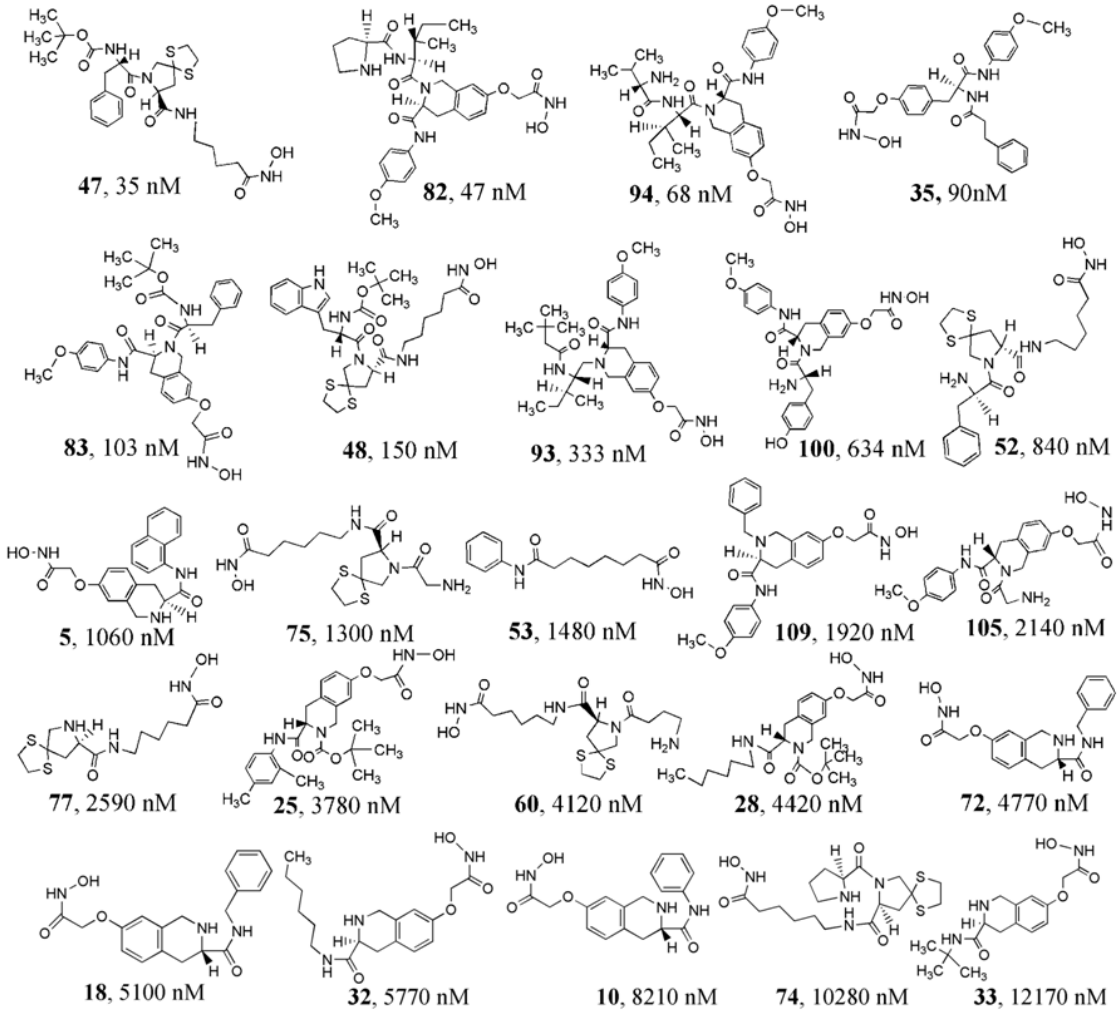


Figure S2. Diverse structures used for the development of ligand-based pharmacophore model.

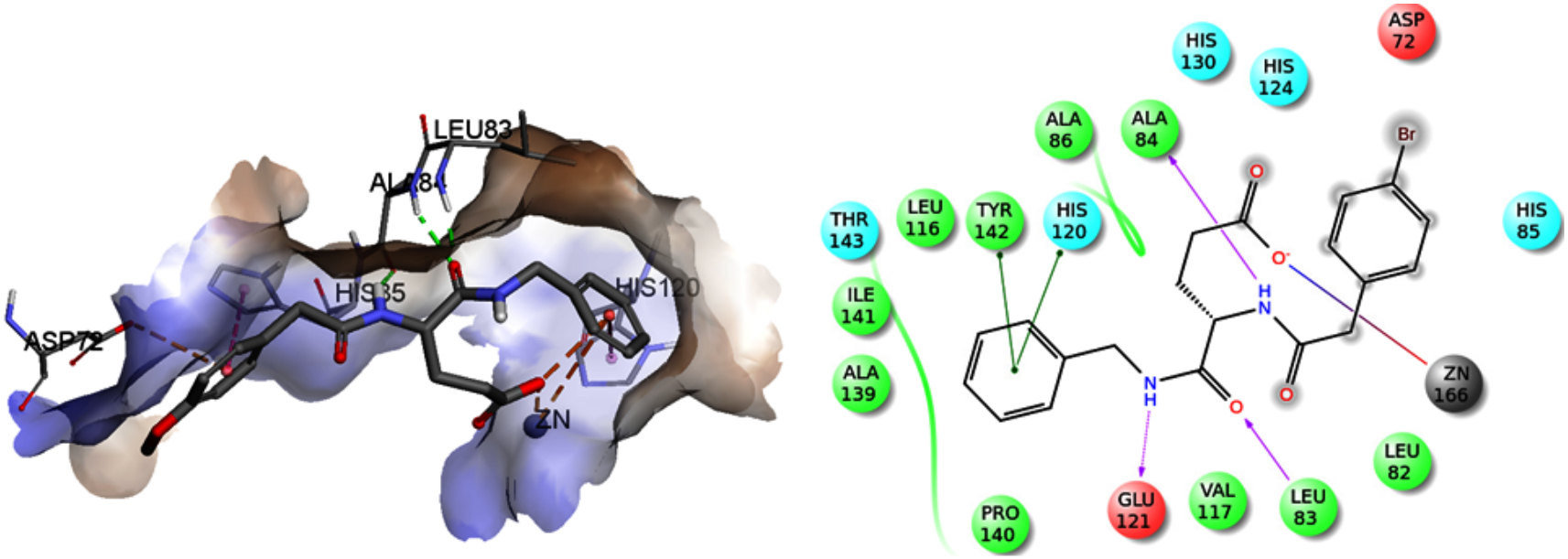


Figure S3. Best docking pose of compound **D11**; left side: three-dimensional (3D) representation with hydrophobic contour map, hydrophobic contour map is shown as higher to lower as brown to blue ( ); right side: 2D representation of the docking interactions, colours are as follows (charged (negative), charged (positive), hydrophobic, metal, polar, hydrogen bond (backbone), pi-pi interaction, salt bridge, solvent exposure)

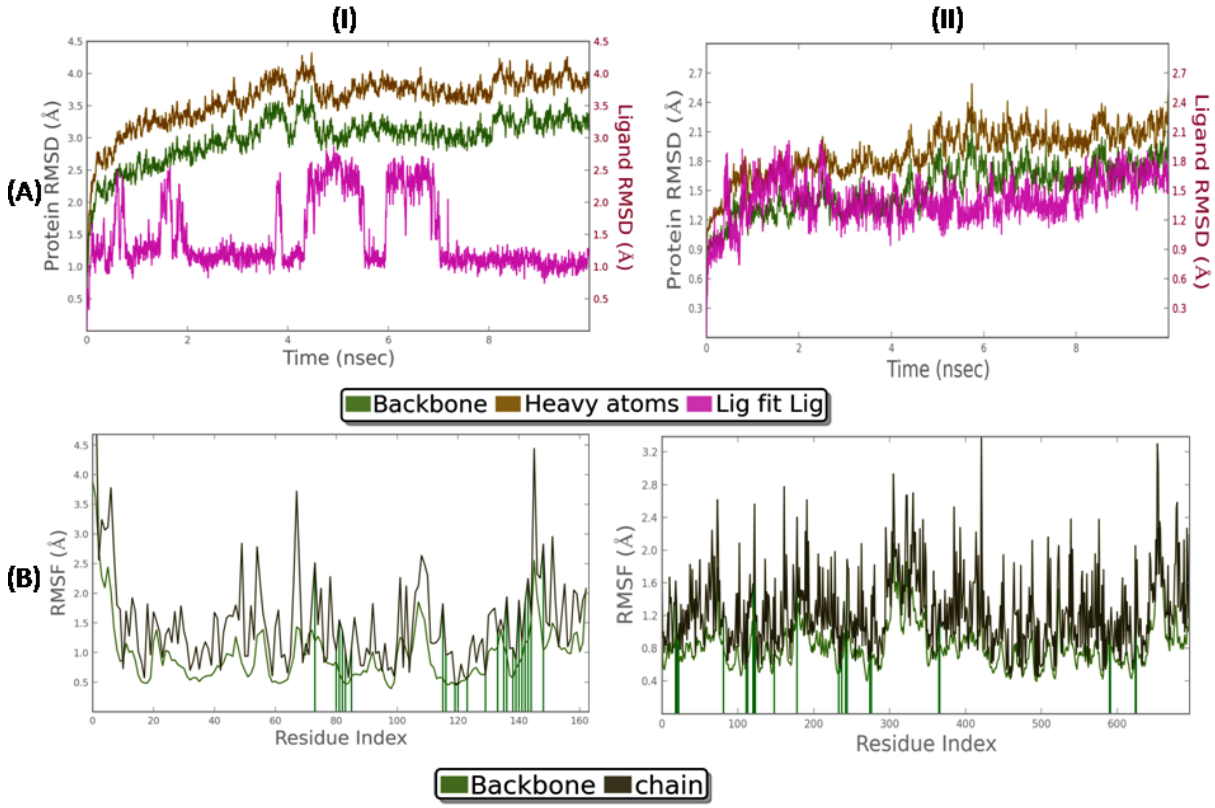


Figure S4 (A) Root mean square deviation (RMSD) and (B) root mean square fluctuation (RMSF) diagrams of (I) MMP-2: Cpd. **D33** (PDB:1HOV) and (II) HDAC-8: Cpd. D33 (PDB:1VKG) complexes.

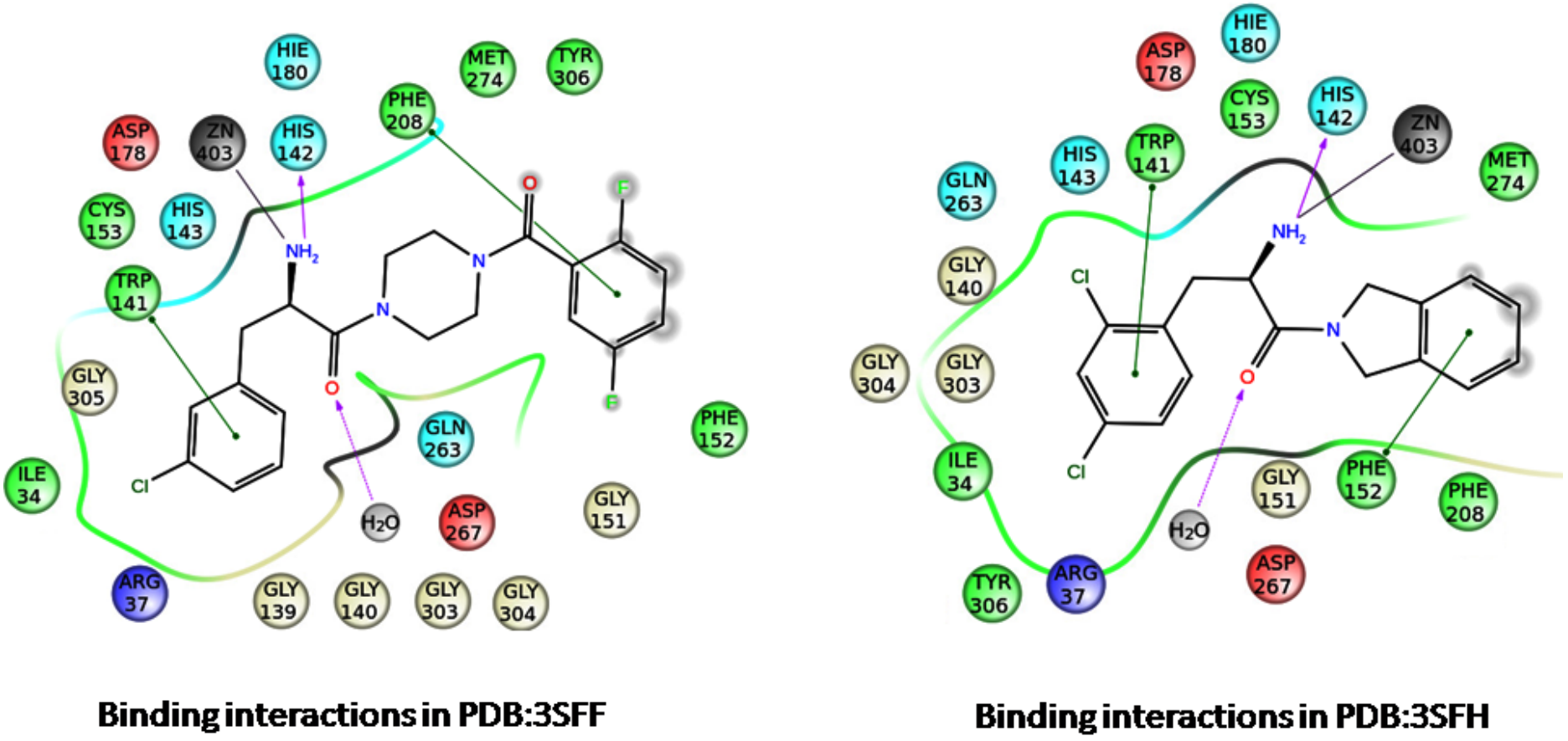


Figure S5 Binding interactions of the selective HDAC-8 inhibitors in complex with HDAC-8 (PDB IDs: 3SFF and 3SFH)[6].

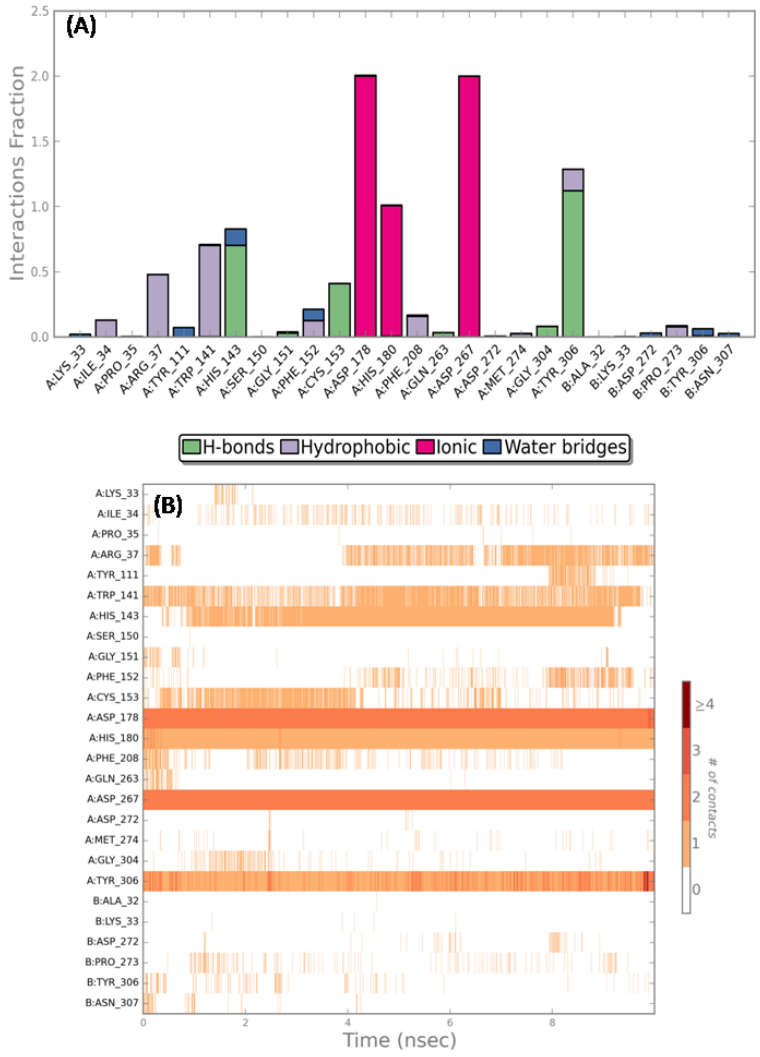


Figure S6 (A) The histogram interaction diagrams of HDAC-8:**D33** complex. (B) Timeline interaction diagram of 10 ns MD simulation run of HDAC-8:**D33** complex.

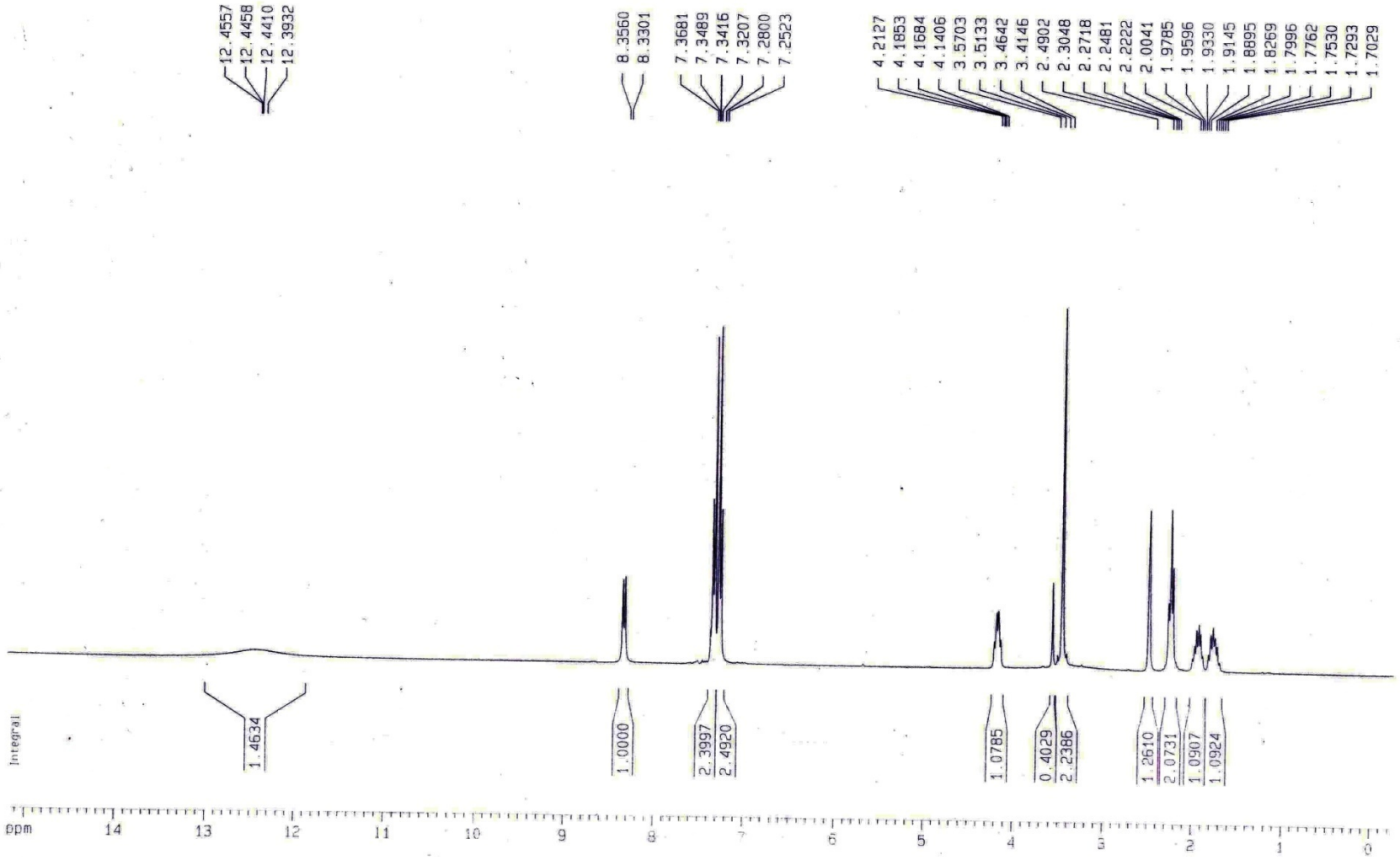


Figure S7 ^{1}H NMR spectra of **I10**

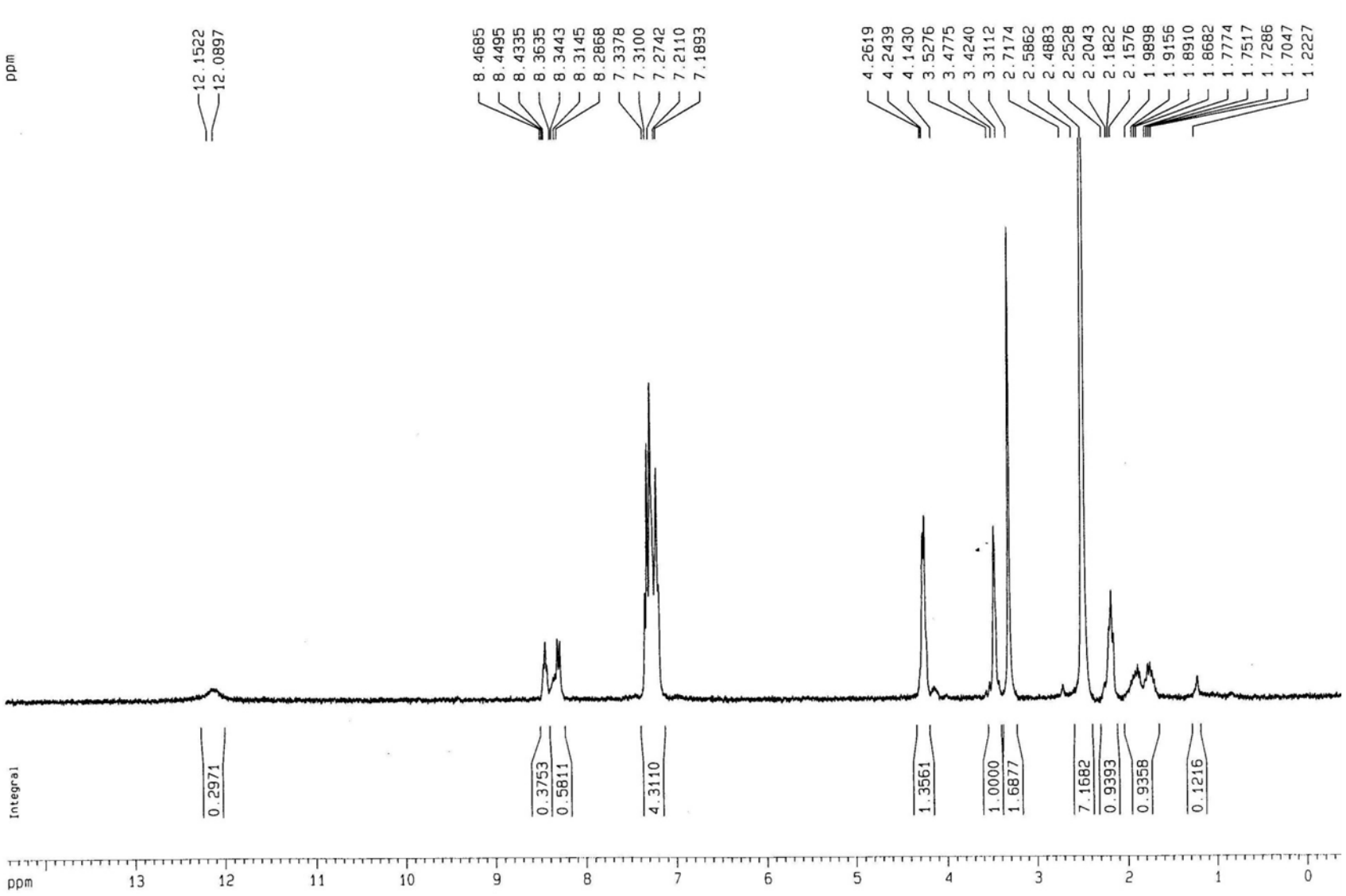


Figure S8 ^{1}H NMR spectra of **D1**

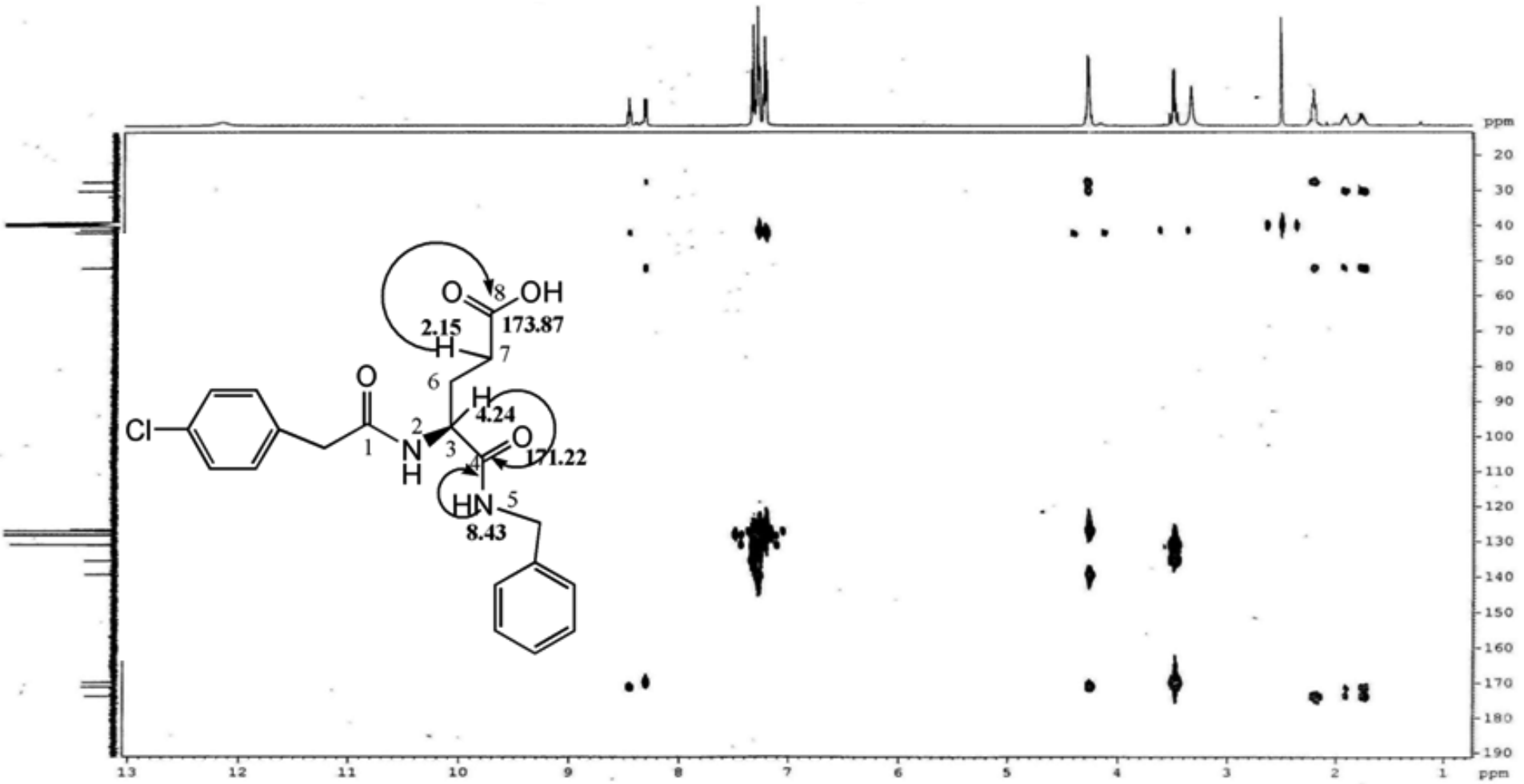


Figure S9a. HMBC spectrum of **D1**

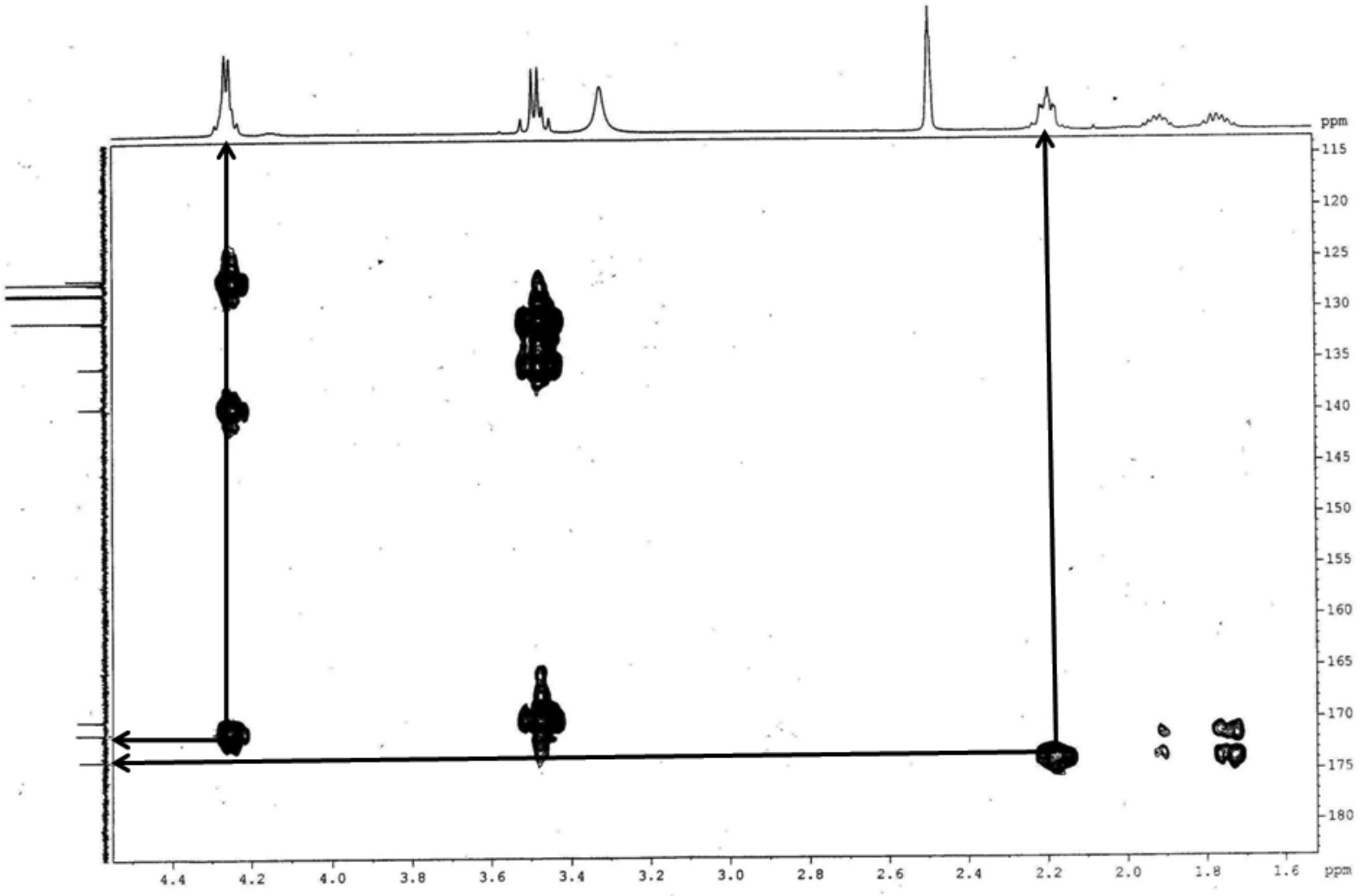


Figure S9b. HMBC spectrum of **D1** (Magnified)

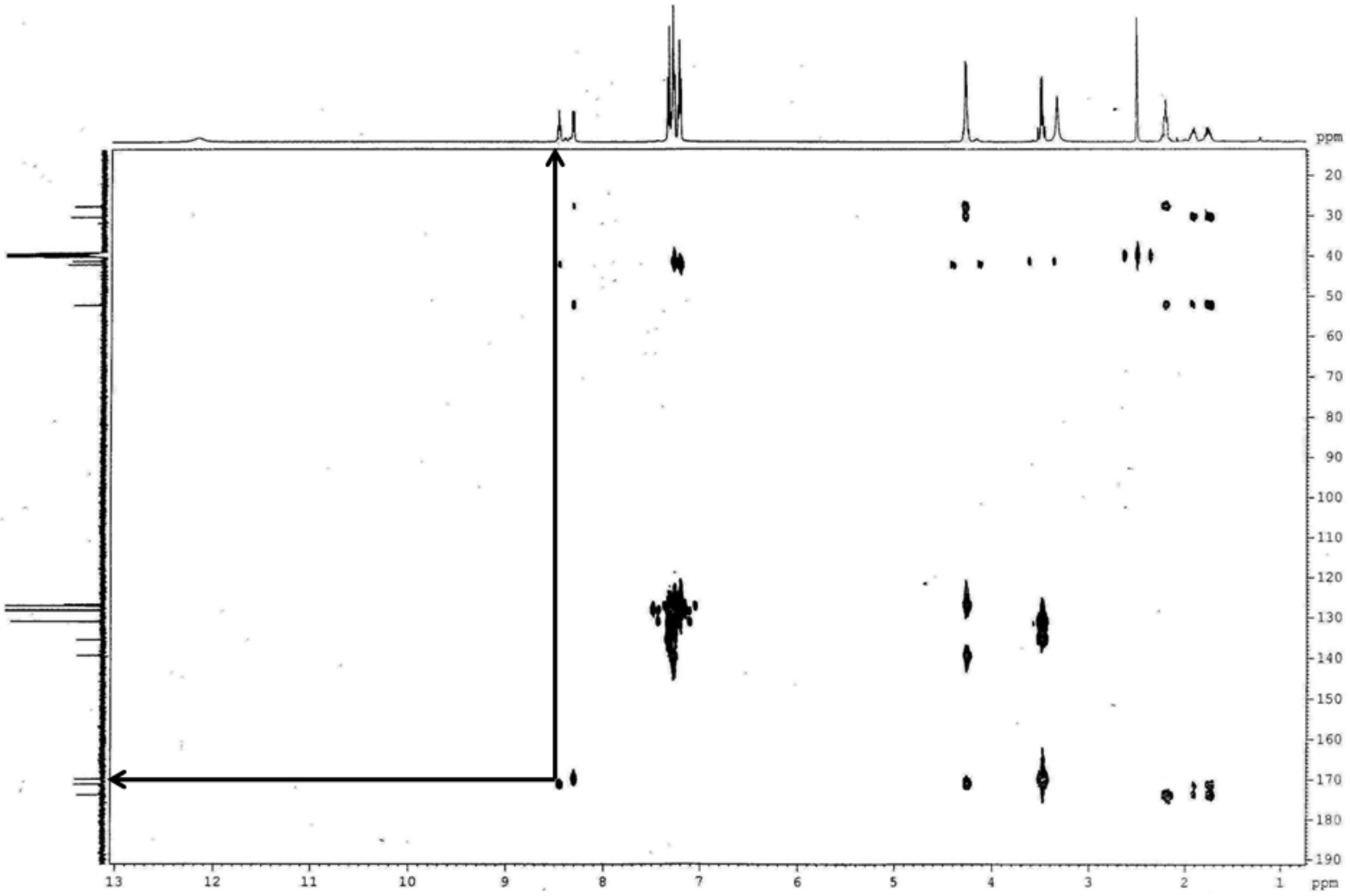


Figure S9c. HMBC spectrum of **D1** (Magnified)

Text S1: Development and validation of ligand- and structure-based pharmacophore models for HDAC-8

1. Ligand based pharmacophore mapping of HDAC-8 inhibitors

Experimentally reported HDAC-8 inhibitors (n=111)[1-5] (Table S1) were used for the development and validation of ligand-based pharmacophore model. The structures of these inhibitors were prepared by *Prepare ligand for QSAR* tool of Discovery Studio (DS)[7], where the duplicate structures were removed, pH of these molecules was set to 7.4. The structures were minimized by Smart Minimizer algorithm (Max steps: 10000, RMS gradient: 0.01) applying CHARMm as the forcefield and *Distance dependent dielectric* as the implicit solvent model. A training set (n_{Tr} = 24) (Figure S2) was for Hypogen model development[8] and the remaining compounds (n_{Ts} = 87) were used as the test set. The feature mapping technique was used for initial selection of the features. After optimizations, the final model was developed with hydrogen bond acceptor (Max: 2, Min: 0), hydrophobic (Max: 3, Min:0), ring aromatic (Max: 2, Min:0), hydrophobic aromatic (Max:2, Min:0) and zinc binding feature (Max:1, Min:1). The uncertainty value and minimum interfeature distance were optimized at 1.5 and 2.5 respectively. Ten hypotheses were developed by Hypogen of Discovery Studio (DS)[7] with fixed, null and configuration costs of 74.61, 289.04 and 16.68 respectively. Important statistical qualities of these models (LigPharm1-10) are listed in Table TS1.

Table TS1. Statistical results of Hypogen models.

Model No.	Total Cost	Cost diff[a]	RMSD[b]	Error cost	Correlation	Features[c]
LigPharm01	**99.38**	**189.66**	**1.432**	**81.41**	**0.945**	**$AH_{Ar}Z$**
LigPharm 02	102.77	186.27	1.524	84.69	0.938	ARZ
LigPharm 03	105.4	183.64	1.595	87.33	0.932	ARZ
LigPharm 04	105.45	183.59	1.608	87.16	0.935	ARZ
LigPharm 05	105.57	183.47	1.597	87.41	0.932	$AH_{Ar}Z$
LigPharm 06	113.63	175.41	1.801	97.74	0.912	HRZ
LigPharm 07	113.91	175.13	1.809	96.07	0.911	HRZ
LigPharm 08	114.05	174.99	1.808	96.05	0.911	AHZ
LigPharm 09	115.47	173.57	1.845	97.66	0.908	AHZ
LigPharm 10	115.71	173.33	1.848	97.78	0.907	AHZ

[a]Cost difference, [b]Root mean square deviation, [c]A: hydrogen bond acceptor, H_{Ar}: hydrogen bond acceptor aromatic, H: hydrophobic, R: ring aromatic, Z: zinc binding feature

The LigPharm01 model was developed with the most promising statistical results (correlation of 0.945, RMSD of 1.432 and cost difference of 189.66)[9] and it contained three pharmacophore features – hydrogen bond acceptor (A), hydrophobic aromatic (H_{Ar}) and zinc

binding (Z). The Fischer randomization test[9] was performed with 95% confidence interval and is repeated in Table TS2. The average correlation coefficient (0.783) and total cost values (163.65) of the randomized models (Table TS2) indicate that the current model followed the null hypothesis and was not developed by chance.

Table TS2. Results of Fischer randomization test of LigPharm01.

Model	Correlation	Cost
LigPharm01	0.946	99.381
Random1	0.848	140.568
Random2	0.828	145.658
Random3	0.686	197.752
Random4	0.871	143.372
Random5	0.617	215.481
Random6	0.913	116.184
Random7	0.845	140.267
Random8	0.825	149.926
Random9	0.808	153.488
Random10	0.734	180.597
Random11	0.742	180.609
Random12	0.693	193.529
Random13	0.751	173.392
Random14	0.674	199.867
Random15	0.797	162.681
Random16	0.841	142.195
Random17	0.768	173.631
Random18	0.831	148.517
Random19	0.811	151.582
Average	0.783	163.647

The LigPharm01 was used to screen the test set molecules (n_{Ts} = 87) and the correlation coefficient (R^2) between the observed and the predicted activities was found to be 0.642 which was higher than other pharmacophore models (LigPharm02-10). Five compounds (Cpds. **37**, **46**, **56**, **78** and **81**) showed high error values (>7.0) and thus, treated as outliers. After removing these compounds, the correlation (R^2) was increased to 0.706 (R^2_{Pred} = 0.502). Furthermore, the predictability of this model was found to be the higher than other developed hypotheses (LigPharm02-10). Therefore, the LigPharm01 was selected as the best pharmacophore model for HDAC-8 inhibition. The activity plot, the mappings of the most (Cpd. **49**) and the least (Cpd. **33**) active compounds in the dataset are depicted in Figure TS1.

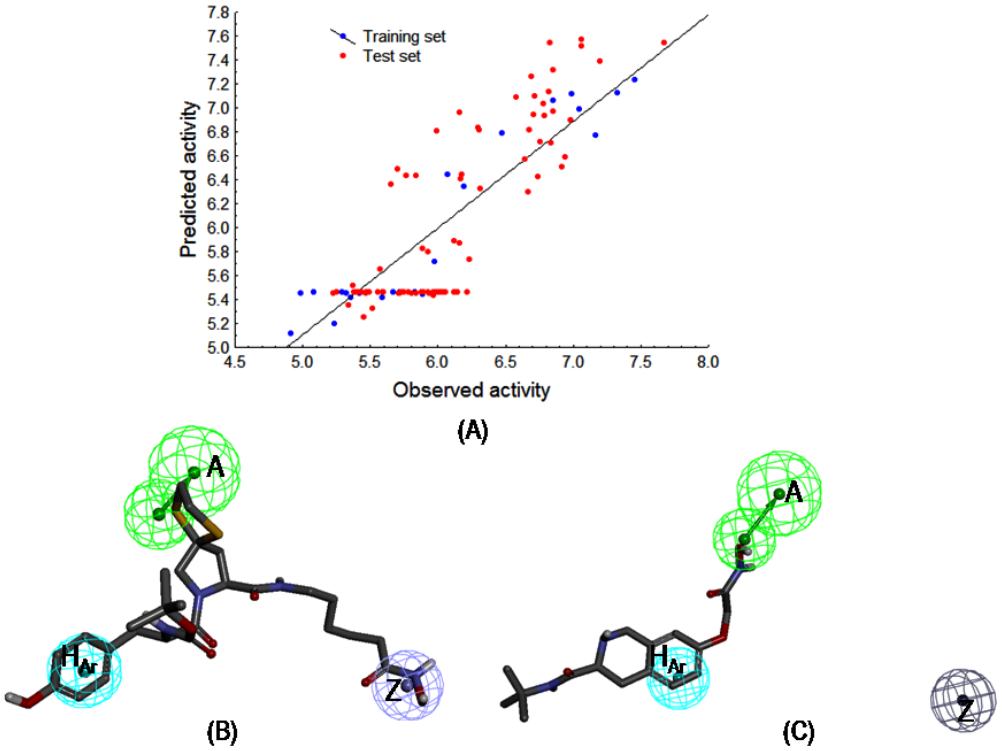


Figure TS1. (A) The observed vs predicted activity plot of LigPharm01, (B) mapping of the best active compound (Cpd. **33**) in LigPharm01, (C) mapping of the least active compound (Cpd. **49**) in LigPharm01.

2. Structure based pharmacophore mapping of HDAC-8 inhibitors

Out of different X-ray crystal structures of HDAC-8 proteins available in the Protein Data Bank[10] (accessed on 10th May, 2014), 18 structures with resolution less than 2.5 Å were considered for the structure-based pharmacophore model development. These protein structures were prepared by *Prepare Protein* tool of DS[7]. The prepared proteins were subjected to the structure-based pharamacophore generation by the help of *Receptor ligand pharmacophore generation* tool in DS[7]. For each protein structure, 10 hypotheses were generated and were ranked on the basis of the selectivity score[9]. All developed pharmacophore models were validated by a decoy set (n_{ds} = 10,748) collected from Directory of Useful Decoys-Enhanced (DUD-E) dataset (http://dude.docking.org, accessed on 12th June, 2014)[11]. The decoy set contained 234 active and 10,514 inactive molecules. The *Receptor ligand pharmacophore generation* tool is unable to generate zinc binding feature like Hypogen tool. Zinc binding feature (Z) is not only one of the most crucial features for HDAC-8 inhibition but its presence in the pharmacophore model considerably reduces the number of false positives and improved validation statistics for each hypothesis. Therefore, after developing the structure-based hypotheses, models with zinc binding group associated

features were selected and these features (mostly donor) were replaced with Z features. These models were validated by a decoy set of DUD-E database to select most suitable structure-based pharmacophore model. It was observed that 1VKG protein[12] developed the most promising structure-based pharmacophore comparing with other proteins. Out of ten pharmacophores generated by this protein-ligand complex, four appeared with donor features associated with the zinc binding hydroxamic acid group of the bound ligand [**CRA-19156**, 5-(4-methyl-benzoylamino)-biphenyl-3,4'-dicarboxylic acid -3- dimethylamide-4'-hydroxamide]. After modification of the pharmacophore with Z feature, the decoy set was screened with these models with the help of *Ligand pharmacophore mapping* protocol of DS[7], setting maximum omitted feature value as 0 (i.e., ligands that fit all pharmacophore features will only be passed). The pharmacophore hits were analyzed by different statistical parameters, like selectivity score, sensitivity, specificity, accuracy and 'goodness of hit' (GH) score[13]. The statistical results of the structure-based pharmacophore models are shown in Table TS3.

Table TS3. Statistical results of the structure-based pharmacophore models.

Model No.	**TA**	**TI**	**TP**	**TN**	**FP**	**FN**	**Select[a]**	**Sn[b]**	**Sp[c]**	**Acc[d]**	**GH[e]**	Features
StrPharm01	234	10514	36	10481	33	198	2.474	0.154	0.997	0.978	0.429	ZHHR
StrPharm02	**234**	**10514**	**115**	10469	**45**	**119**	**2.384**	**0.491**	**0.996**	**0.985**	**0.659**	**ZHRR**
StrPharm03	234	10514	70	10491	23	164	2.245	0.299	0.997	0.982	0.639	ZHRR
StrPharm04	234	10514	123	10457	57	111	1.819	0.526	0.991	0.984	0.644	ZHHR

[a]Selectivity score, [b]sensitivity, [c] specificity, [d]accuracy, [e]Goodness of hit (GH) score

The models showed the importance of three features - hydrophobic (H), zinc binder (Z) and ring aromatic (R). Except StrPharm01, all hypotheses showed goodness of hit (GH) scores of more than 0.60. However, on the basis of selectivity score, accuracy and GH score, StrPharm02 hypothesis appeared to be the most promising pharmacophore model. It showed accuracy of 0.985 and GH score of 0.659. Therefore, this pharmacophore model was selected as the best structure-based pharmacophore model. The interfeature distance constraints of StrPharm02 are shown in Figure 1 (main text).

This pharmacophore model suggests that minimum two aromatic rings a zinc binding group are required for the HDAC-8 inhibition. To understand the importance of the features of this hypothesis, each feature was removed and the decoy set validation was repeated. It was found that removal of R_1 reduced the GH score to 0.510 whereas deletion of R_2 reduced the value to 0.527. Therefore, both these aromatic features are equally important. Deletion of Z feature completely abolished the selectivity of the pharmacophore and reduced the GH score to

0.084. However, removal of H feature had minimum effect on the GH score as the modified score was 0.600. Therefore, it may be inferred that the H is the least and Z is the most important pharmacophore feature of StrPharm02 model.

Text S2: Spectral analyses data of the synthesized molecules

4(S)-5-[(2-methylpropyl)amino]-4-(2-(4-methoxyphenyl)acetamido)-5-oxopentanoic acid (D2)

Yield: 58.3%. MP 138-140^{o}C. MS (ESI Postive) m/z [M+Na^{+}] 373.21.^{1}H NMR (DMSO-d6, 300 mHz, ppm) δ12.09 (s, 1H, COOH), δ10.14 (s, 1H, CONH), δ 8.40 (d, 1H, CONH, J=8.09), δ 6.85 (m, 5H, Benzene),δ 4.24 (m, 1H, CH), δ 3.68 (s, 3H, OCH_3), δ 3.56 (s, 2H, CH_2), δ 2.84 (m, 2H, CH_2), δ 2.20 (m, 2H, CH_2), δ 1.80 and 1.68 (m, 2H, CH_2), δ 1.63 (m, H, CH) δ 0.79 (m, 6H, $2CH_3$).IR (KBr, cm-^{1}):3320 (N-Hstr of CONH), 3054 (aromatic =C-H str), 2935 (assym. aliphatic -C-H str), 2895 (sym. aliphatic -C–H str), 1724(C=O str COOH), 1654 (C=O str of CONH), 1529(N-H deformation), 1443 (aliphatic -C-H deformation), 1388, 1224 (C-O str and O-H bending of COOH).

4(S)-5-(Butylamino)-4-(2-(4-chlorophenyl)acetamido)-5-oxopentanoic acid (D3)

Yield: 37.8%. MP 138-140 ^{o}C. MS (ESI): 377.20 (M+Na). ^{1}HNMR(300MHz, DMSO-D_6, δ ppm): δ 12.07 (s, 1H, COOH), δ 8.43 (m, 1H, CONH), δ 8.28 (d, 1H, CONH, J=8.28), δ 7.18 (m, 5H, Benzene), δ 4.20 (m, 1H-2, CH), δ 3.55 (s, 2H, CH_2), δ 3.02 (m, 2H, CH_2), δ 2.18 (m, 2H, CH_2), δ 1.81 and 1.65 (m, 2H, CH_2), δ 1.32 (m, 2H, CH_2), δ 1.23 (m, 2H, CH_2), δ 0.84 (m, 3H, CH_3). IR (KBr, cm-^{1}):3309 (N-Hstr of CONH), 3063 (aromatic =C-H str), 2942 (assym. aliphatic -C-H str), 2877 (sym. aliphatic -C–H str), 1727 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -C-H deformation), 1411, 1261 (C-O str and O-H bending of COOH), 966 (O-H out of plane deformation of COOH).

4(S)-5--[(2-methylpropyl)amino]-4-(2-(4-chlorophenyl)acetamido)-5-oxopentanoic acid (D4)

Yield: 42.7%. MP 148-150^{o}C.MS (ESI): 377.18 (M+Na^{+}), ^{1}HNMR(300MHz, DMSO-D_6, δ ppm): δ 12.08 (s, 1H, COOH), δ 8.43 (m, 1H, CONH), δ 8.28 (d, 1H, CONH, J=8.24), δ 7.16 (m, 5H, Benzene), δ 4.22 (m, 1H-2, CH), δ 3.57 (s, 2H, CH_2), δ 2.82 (m, 2H, CH_2), δ 2.19 (m, 2H, CH_2),δ 1.85 and 1.68 (m, 2H, CH_2), δ1.63 (m, 1H, CH) δ0.79, (d, 6H, $2CH_3$). IR (KBr, cm-^{1}): 3309 (N-Hstr of CONH), 3063 (aromatic =C-H str), 2943 (assym. aliphatic -C-

H str), 2878 (sym. aliphatic -C–H str), 1723 (C=O str COOH), 1649 (C=O str of CONH), 1610 (aromatic C=C str), 1542 (N-H deformation), 1436 (aliphatic -C-H deformation), 1412, 1262 (C-O str and O-H bending of COOH), 968 (O-H out of plane deformation of COOH).

4(S)-5-(Benzylamino)-4-(2-(4-chlorophenyl)acetamido)-5-oxopentanoic acid (D5)

Yield: 49.5%. MP 158-160^{o}C.MS (ESI): 411.12 ($M+Na^{+}$), 413.11 ($M+Na^{+}+2H^{+}$), 414.12 ($M+Na^{+}+3H^{+}$). ^{1}HNMR(300MHz, DMSO-$D_{6,}$ δ ppm): δ 12.08 (s, 1H, COOH), δ 8.43 (m, 1H, CONH), δ 8.28 (d, 1H, CONH, J=8.31), δ 7.18 (m, 9H, Benzene), δ 4.24 (m, 1H, CH), δ 4.14 (m, 2H, CH_2), δ 3.42 (s, 2H, CH_2), δ 2.15 (m, 2H, CH_2), δ 1.70 (m, 2H-3).IR (KBr, cm-^{1}):3309 (N-Hstr of CONH), 3063 (aromatic =C-H str), 2942 (assym. aliphatic -C-H str), 2877 (sym. aliphatic -C–H str), 1727 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -C-H deformation), 1411, 1261 (C-O str and O-H bending of COOH), 966 (O-H out of plane deformation of COOH).

4(S)-5-(Hexylamino)-4-(2-(4-chlorophenyl)acetamido)-5-oxopentanoic acid (D6)

Yield: 33.9%. MP 130-132^{o}C.MS (ESI): 405.20 ($M+Na^{+}$). ^{1}HNMR(300MHz, DMSO-$D_{6,}$ δ ppm): δ 12.07 (s, 1H, COOH), δ 8.42 (m, 1H, CONH), δ 8.28 (d, 1H, CONH, J=8.35), δ 7.16 (m, 9H, Benzene), δ 4.14 (m, 1H-2, CH), δ 3.32 (s, 2H, CH_2), δ 2.93 (m, 2H, CH_2), δ 2.07 (m, 2H-4, CH_2), δ 1.81 and 1.67 (m, 2H-3, CH_2), δ 1.31 (m, 2H, CH_2), δ 1.21 (m, 6H, $CH_2CH_2CH_2$), δ 0.81 (m, 3H-6", CH_3).IR (KBr, cm-^{1}):3309 (N-Hstr of CONH), 3060 (aromatic =C-H str), 2941 (assym. aliphatic -C-H str), 2883 (sym. aliphatic -C–H str), 1727 (C=O str COOH), 1646 (C=O str of CONH), 1604 (aromatic C=C str), 1536 (N-H deformation), 1439 (aliphatic -C-H deformation), 1416, 1258 (C-O str and O-H bending of COOH), 976 (O-H out of plane deformation of COOH).

4(S)-5-(Pentylamino)-4-(2-(4-nitrophenyl) acetamido)-5-oxopentanoic acid (D7)

Yield: 33.9%. MP 164-166^{o}C.MS (ESI): 380.19 ($M+H^{+}$), 402.17 ($M+Na^{+}$), 403.17 ($M+Na^{+}+H^{+}$),418.14 ($M+K^{+}$). ^{1}HNMR (300MHz, DMSO-$D_{6,}$ δ ppm): δ 12.32 (s, 1H, COOH), δ 8.20 (d, 1H, CONH-2, J=8.04), δ 8.13 (m, 2H, Benzene), δ 7.77 (m, 1H, CONH), δ 7.50 (m, 2H, Benzene), δ 4.15 (m, 1H, CH), δ 3.64 (m, 2H, CH_2), δ 2.97 (m, 2H, CH_2), δ 2.13 (m, 2H, CH_2), δ 1.71 (m, 2H, CH_2), δ 1.32 (m, 2H, CH_2), δ 1.24 (m, 2H, CH_2), δ 1.15 (m, 2H, CH_2), δ 0.79 (m, 3H, CH_3). IR (KBr, cm-^{1}): 3313 (N-H str of CONH), 3108 (aromatic =C-H str), 2953 (assym. aliphatic –CH_3 str), 2930 (assym. aliphatic -CH_2 str), 2870 (sym. aliphatic –CH_3str), 1724 (C=O str COOH), 1650 (C=O str of CONH), 1606 (aromatic

C=C str), 1551 (N-H deformation of CONH), 1518 (aromatic C-NO_2 str), 1498 (aliphatic -CH_2 deformation), 1442 (aliphatic –CH_3 deformation), 1414,1230 (C-O str and O-H bending of COOH), 1019 (aromatic =C-H in plane defrormation), 937 (O-H out of plane deformation of COOH).

4(S)-5-[(2, 2-dimethylethyl) amino]-4-(2-(4-nitrophenyl) acetamido)-5-oxopentanoic acid (D8)

Yield: 32.6%. MP 160-162^{o}C.MS (ESI): 388.20 (M+Na^+). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): MS (ESI): 380.19 (M+H^+), 402.17 (M+Na^+), 403.17 (M+Na^++H^+),418.14 (M+K^+). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.32 (s, 1H, COOH), δ 8.21 (d, 1H, CONH-2, J=8.04), δ 8.13 (m, 2H, Benzene), δ 7.68 (m, 1H, CONH), δ 7.53 (m, 2H, Benzene), δ 4.16 (m, 1H, CH), δ 3.64 (m, 2H, CH_2), δ 2.18 (m, 2H, CH_2), δ 1.74 (m, 2H, CH_2), δ 1.35 (m, 9H, 3CH_3). IR (KBr, cm-^{1}): 3310 (N-H str of CONH), 3107 (aromatic =C-H str), 2954 (assym. aliphatic –CH_3 str), 2940 (assym. aliphatic -CH_2 str), 2874 (sym. aliphatic –CH_3 str), 1712 (C=O str COOH), 1655 (C=O str of CONH), 1620 (aromatic C=C str), 1552 (N-H deformation of CONH), 1511 (aromatic C-NO_2 str), 1441 (aliphatic –CH_3 deformation), 1414,1230 (C-O str and O-H bending of COOH), 937 (O-H out of plane deformation of COOH).

4(S)-5-[(1-Methylethyl)amino]-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D9)

Yield: 32.3%. MP 184-186^{o}C.MS (ESI): 386.21 (M+Na^+H^+), 408.10 (M+Na^++2H^+). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.10 (s, 1H, COOH), δ 8.20 (d, 1H, CONH, J=8.20), δ 7.75 (m, 1H, CONH), δ 7.44 (m, 2H, Benzene), δ 7.18 (m, 2H, Benzene), 4.19 (m, 1H, CH), δ 3.90 (m, 1H, CH), δ 3.48 (s, 2H, CH_2), δ 2.14 (m, 2H, CH_2), δ 1.73 (m, 2H-3, CH_2), δ 0.98 (d, 6H, 2CH_3).IR (KBr, cm-^{1}): 3292 (N-H str of CONH), 3097 (aromatic =C-H str), 2955 (assym. aliphatic –CH_3 str), 2928 (assym. aliphatic -CH_2 str), 2856 (sym. aliphatic –CH_3 str), 1712 (C=O str COOH), 1634 (C=O str of CONH), 1592 (aromatic C=C str), 1546 (N-H deformation of CONH), 1488 (aliphatic -CH_2 deformation), 1435 (aliphatic –CH_3 deformation), 1408,1228 (C-O str and O-H bending of COOH), 1071 (aromatic C-Br str), 1013 (aromatic =C-H in plane defrormation), 949 (O-H out of plane deformation of COOH).

4(S)-5-(Pentylamino)-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D10)

Yield: 27.8%. MP 150-152^{o}C.MS (ESI): 436.20 (M+Na). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.12 (s, 1H, COOH), δ 8.20 (d, 1H, CONH, J=8.13), δ 7.75 (m, 1H, CONH), δ

7.44-7.47 (m, 2H, Benzene), δ 7.18-7.21(m, 2H, Benzene), δ 4.15 (m, 1H, CH), δ 3.64 (m, 2H, CH_2), δ 2.90 (m, 2H, CH_2), δ 2.14 (m, 2H, CH_2), δ 1.70 (m, 2H, CH_2), δ 1.30 (m, 2H, CH_2), δ 1.27 (m, 2H, CH_2), δ 1.16 (m, 2H, CH_2), δ 0.80 (m, 3H, CH_3). IR (KBr, cm^{-1}): 3292 (N-H str of CONH), 3097 (aromatic =C-H str), 2950 (assym. aliphatic –CH_3 str), 2930 (assym. aliphatic -CH_2 str), 2856 (sym. aliphatic –CH_3str), 1718 (C=O str COOH), 1644 (C=O str of CONH), 1592 (aromatic C=C str), 1546 (N-H deformation of CONH), 1408,1228 (C-O str and O-H bending of COOH), 1076 (aromatic C-Br str), 1010 (aromatic =C-H in plane defrormation), 952 (O-H out of plane deformation of COOH).

4(S)-5-(Benzylamino)-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D11)

Yield: 33.4%. MP 144-146^oC.MS (ESI): 456.10 (M+Na), δδ 12.12 (s, 1H, COOH), δ 8.20 (d, 1H, CONH, J=8.13), δ 7.78 (m, 1H, CONH), δ 6.94 (m, 9H, Benzene),δ 4.24 (m, 1H, CH), δ 4.14 (m, 2H, CH_2), δ 3.42 (s, 2H, CH_2), δ 2.15 (m, 2H, CH_2), δ 1.70 (m, 2H, CH_2). IR (KBr, cm^{-1}): 3309 (N-Hstr of CONH), 3063 (aromatic =C-H str), 2942 (assym. aliphatic -C-H str), 2877 (sym. aliphatic -C–H str), 1727 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -C-H deformation), 1411, 1261 (C-O str and O-H bending of COOH), 1076 (aromatic C-Br str).

4(S)-5-(Hexylamino)-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D12)

Yield: 32.1%. MP 152-154^oC. MS (ESI): 451.06 ($M+Na^+H^+$), 452.06 ($M+Na^++2H^+$). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.11-12.24 (s, 1H, COOH), δ 8.20-8.34 (d, 1H, CONH-2, J=8.13), δ 7.75 (m, 1H, CONH-1), δ 7.44 (m, 2H, Benzene), δ 7.18 (m, 2H, Benzene), δ 4.14 (m, 1H-2, CH), δ 3.30 (s, 2H, CH_2), δ 2.95 (m, 2H, CH_2), δ 2.07 (m, 2H, CH_2), δ 1.68 (m, 2H, CH_2), δ 1.31 (m, 2H, CH_2), δ 1.21 (m, 6H, $CH_2CH_2CH_2$), δ 0.81 (m, 3H, CH_3). IR (KBr, cm^{-1}): 3292 (N-H str of CONH), 3097 (aromatic =C-H str), 2955 (assym. aliphatic –CH_3 str), 2928 (assym. aliphatic -CH_2 str), 2856 (sym. aliphatic –CH_3str), 1712 (C=O str COOH), 1634 (C=O str of CONH), 1592 (aromatic C=C str), 1546 (N-H deformation of CONH), 1488 (aliphatic -CH_2 deformation), 1435 (aliphatic –CH_3 deformation), 1408,1228 (C-O str and O-H bending of COOH), 1071 (aromatic C-Br str), 1013 (aromatic =C-H in plane defrormation), 949 (O-H out of plane deformation of COOH.

4(S)-5-[(2-Methylpropyl)amino]-4-(2-(2-chlorophenyl) acetamido)-5-oxopentanoic acid (D13)

Yield: 35.6%. MP 188-190°C.MS (ESI): 377.09 ($M+Na^+$), 379.09 ($M+Na^++2H^+$), 399.07 ($M+2Na^+$). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.11 (s, 1H, COOH), δ 8.20 (d, 1H, CONH, J=8.01), δ 7.86 (m, 1H, CONH), δ 7.23 (m, 4H, Benzene), δ 4.22 (m, 1H, CH), δ 3.57 (s, 2H, CH_2), δ 2.82 (m, 2H, CH_2), δ 2.19 (m, 2H, CH_2), δ 1.63 (m, 2H, CH_2), δ 0.79, (d, 7H, $CH(CH_3)_2$). IR (KBr, cm^{-1}): 3312 (N-H str of CONH), 3235 (O-H str of COOH), 3034 (aromatic =C-H str), 2962 (assym.aliphatic –CH_3 str), 2931 (assym.aliphatic-CH_2 str), 2873 (sym. aliphatic –CH_3str),1725 (C=O str COOH), 1650 (C=O str of CONH), 1610 (aromatic C=C str), 1545 (N-H deformation of CONH), 1499 (aliphatic -CH_2 deformation), 1447 (aliphatic –CH_3 deformation), 1414,1243 (C-O str and O-H bending of COOH), 1387 (aliphatic –CH deformation), 1069 (aromatic C-Cl str), 1018 (aromatic =C-H in plane defrormation), 953 (O-H out of plane deformation of COOH.

4(S)-5-(Benzylamino)-4-(2-(2-chlorophenyl) acetamido)-5-oxopentanoic acid (D14)

Yield: 31.6%. MP 194-196°C.MS (ESI): 411.10 ($M+Na^+$), ^{1}HNMR(300MHz, DMSO-D_6, δ ppm): δ 12.11 (s, 1H, COOH), δ 8.20 (d, 1H, CONH, J=8.01), δ 7.86 (m, 1H, CONH), δ 7.01 (m, 9H, Benzene),δ 4.21 (m, 1H, CH), δ 4.12 (m, 2H, CH_2), δ 3.35 (s, 2H, CH_2), δ 2.05 (m, 2H, CH_2), δ 1.72 (m, 2H-3).IR (KBr, cm^{-1}):3309 (N-H str of CONH), 3063 (aromatic =C-H str), 2942 (assym. aliphatic -C-H str), 2877 (sym. aliphatic -C–H str), 1727 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -C-H deformation), 1411, 1261 (C-O str and O-H bending of COOH), 966 (O-H out of plane deformation of COOH).

4(S)-5-[(1-Methylethyl)amino]-4-(2-(2-chlorophenyl) acetamido)-5-oxopentanoic acid (D15)

Yield: 25.1%. MP 201-210°C.MS (ESI): 363.10 ($M+Na^+$), ^{1}HNMR(300MHz, DMSO-D_6, δ ppm): δ 12.11 (s, 1H, COOH), δ 8.20 (d, 1H, CONH, J=8.01), δ 7.86 (m, 1H, CONH), δ 7.01 (m, 9H, Benzene),4.14 (m, 1H, CH), δ 3.92 (m, 1H, CH), δ 3.46 (s, 2H, CH_2), δ 2.14 (m, 2H, CH_2), δ 1.71 (m, 2H-3, CH_2), δ 0.95 (d, 6H, $2CH_3$). IR (KBr, cm^{-1}):3308 (N-Hstr of CONH), 3059 (aromatic =C-H str), 2944 (assym. aliphatic -C-H str), 2878 (sym. aliphatic -C–H str), 1720 (C=O str COOH), 1645 (C=O str of CONH), 1614 (aromatic C=C str), 1540 (N-H deformation), 1436 (aliphatic -C-H deformation), 1410, 1265 (C-O str and O-H bending of COOH), 956 (O-H out of plane deformation of COOH).

4(S)-5-(Pentylamino)-4-(2-(2-chlorophenyl) acetamido)-5-oxopentanoic acid (D16)

Yield: 34.9%. MP 190-192^{o}C. MS (ESI): 390.20 ($M+Na^{+}$), 368.10 ($M+H^{+}$), ^{1}HNMR(300MHz, DMSO-$D_{6,}$ δ ppm): δ 12.11 (s, 1H, COOH), δ 8.20 (d, 1H, CONH, J=8.01), δ 7.86 (m, 1H, CONH), δ 7.01 (m, 5H, Benzene),δ 4.16 (m, 1H, CH), δ 3.65 (m, 2H, CH_2), δ 2.94 (m, 2H, CH_2), δ 2.10 (m, 2H, CH_2), δ 1.73 (m, 2H, CH_2), δ 1.32 (m, 2H, CH_2), δ 1.25 (m, 2H, CH_2), δ 1.16 (m, 2H, CH_2), δ 0.85 (m, 3H, CH_3).IR (KBr, cm-^{1}):3308 (N-Hstr of CONH), 3062 (aromatic =C-H str), 2946 (assym. aliphatic -C-H str), 2875 (sym. aliphatic -C–H str), 1729 (C=O str COOH), 1648 (C=O str of CONH), 1614 (aromatic C=C str), 1545 (N-H deformation), 1434 (aliphatic -C-H deformation), 1421, 1246 (C-O str and O-H bending of COOH), 958 (O-H out of plane deformation of COOH), 806, 750, 696, 651 (aromatic –C-H out of plane deformation).

4(S)-5-[(1-Methylethyl)amino]-4-(2-(2-bromophenyl) acetamido)-5-oxopentanoic acid (D17)

Yield: 42.7%. MP 218-220^{o}C.MS (ESI): 408.10 ($M+Na^{+}$). ^{1}HNMR (300MHz, DMSO-$D_{6,}$ δ ppm): δ 12.10 (s, 1H, COOH), δ 8.21 (s, 1H, CONH), δ 7.86 (d, 1H, CONH, J=7.78), δ 7.10 (m, 5H, Benzene), δ 4.19 (m, 1H, CH), δ 3.46 (s, 2H, CH_2), δ 2.13 (m, 2H, CH_2), δ 1.71 (m, 2H-3, CH_2), δ 0.94 (d, 6H, $2CH_3$).IR (KBr, cm-^{1}): 3320 (N-H str of CONH), 3287 (O-H str of COOH), 3072 (aromatic =C-H str), 2946 (assym.aliphatic –CH_3 str), 2933 (assym. aliphatic -CH_2 str), 2870 (sym. aliphatic –CH_3 str), 1726 (C=O str COOH), 1658 (C=O str of CONH), 1601 (aromatic C=C str), 1541 (N-H deformation of CONH), 1498 (aliphatic -CH_2 deformation), 1447 (aliphatic –CH_3 deformation), 1414,1245 (C-O str and O-H bending of COOH), 1075 (aromatic C-Br str), 1027 (aromatic =C-H in plane defrormation), 947 (O-H out of plane deformation of COOH).

4(S)-5-[(2-Methylpropyl)amino]-4-(2-(2-bromophenyl) acetamido)-5-oxopentanoic acid (D18)

Yield: 43.0%. MP 196-198^{o}C.MS (ESI): 422.10 ($M+Na^{+}$). ^{1}HNMR (300MHz, DMSO-$D_{6,}$ δ ppm): δ 12.08 (s, 1H, COOH), δ 8.23 (s, 1H, CONH), δ 7.86 (d, 1H, CONH, J=7.78), δ 6.99 (m, 5H, Benzene), δ 4.16 (m, 1H, CH), δ 3.54 (s, 2H, CH_2), δ 2.80 (m, 2H, CH_2), δ 2.10 (m, 2H, CH_2), δ 1.65 (m, 2H, CH_2), δ 0.80 (d, 7H, $CH(CH_3)_2$).IR (KBr, cm-^{1}): 3314 (N-H str of CONH), 3074 (aromatic =C-H str), 2946 (assym.aliphatic –CH_3 str), 2931 (assym. aliphatic -CH_2 str), 2870 (sym. aliphatic –CH_3str), 1724 (C=O str COOH), 1659 (C=O str of CONH), 1601 (aromatic C=C str), 1543 (N-H deformation of CONH), 1498 (aliphatic -CH_2

deformation), 1447 (aliphatic –CH_3 deformation), 1078 (aromatic C-Br str), 1022 (aromatic =C-H in plane defrormation), 949 (O-H out of plane deformation of COOH).

4(S)-5-(Pentylamino)-4-(2-(2-bromophenyl) acetamido)-5-oxopentanoic acid (D19)

Yield: 41.4%. MP 190-192^{o}C.MS (ESI): 414.1 ($M+H^+$). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.10 (s, 1H, COOH), δ 8.21 (s, 1H, CONH), δ 7.86 (d, 1H, CONH, J=7.78), δ 7.10 (m, 5H, Benzene), δ 4.14 (m, 1H, CH), δ 3.61 (m, 2H, CH_2), δ 2.92 (m, 2H, CH_2), δ 2.14 (m, 2H, CH_2), δ 1.70 (m, 2H, CH_2), δ 1.34 (m, 2H, CH_2), δ 1.24 (m, 2H, CH_2), δ 1.16 (m, 2H, CH_2), δ 0.80 (m, 3H, CH_3). IR (KBr, cm-^{1}): 3320 (N-H str of CONH), 3300 (O-H str of COOH), 3072 (aromatic =C-H str), 2946 (assym.aliphatic –CH_3 str), 2933 (assym. aliphatic -CH_2 str), 2870 (sym. aliphatic –CH_3str),1726 (C=O str COOH), 1658 (C=O str of CONH), 1601 (aromatic C=C str), 1541 (N-H deformation of CONH), 1498 (aliphatic -CH_2 deformation), 1444 (aliphatic –CH_3 deformation), 1414,1245 (C-O str and O-H bending of COOH), 1075 (aromatic C-Brstr), 1027 (aromatic =C-H in plane deformation), 947 (O-H out of plane deformation of COOH).

4(S)-5-(Phenylamino)-4-(2-(2-bromophenyl) acetamido)-5-oxopentanoic acid (D20)

Yield: 30.9%. MP 216-218^{o}C.MS (ESI): 443.04 ($M+Na^+$+H^+), 444.04 ($M+Na^+$+$2H^+$).^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.16 (s, 1H, COOH), δ 10.02 (s, 1H, CONH), δ 8.38 (d, 1H, CONH, J=7.74), δ 7.01 (m, 9H, Benzene), δ 4.43 (m, 1H, CH), δ 3.67 (s, 2H, CH_2), δ 2.26 (m, 2H, CH_2), δ 1.87 (m, 2H, CH_2). IR (KBr, cm-^{1}): 3320 (N-H str of CONH), 3287 (O-H str of COOH), 3072 (aromatic =C-H str), 2946 (assym.aliphatic –CH_3 str), 2933 (assym. aliphatic -CH_2 str), 2870 (sym. aliphatic –CH_3str),1726 (C=O str COOH), 1658 (C=O str of CONH), 1601 (aromatic C=C str), 1541 (N-H deformation of CONH), 1498 (aliphatic -CH_2 deformation), 1447 (aliphatic –CH_3 deformation), 1414,1245 (C-O str and O-H bending of COOH), 1075 (aromatic C-Brstr), 1027 (aromatic =C-H in plane defrormation), 947 (O-H out of plane deformation of COOH), 912,848,799,761,737,712,690,651 (aromatic –C-H out of plane deformation).

4(S)-5-(Butylamino)-4-(2-(2-fluorophenyl) acetamido)-5-oxopentanoic acid (D21)

Yield: 39.9%. MP 188-190^{o}C.MS (ESI): 361.15 ($M+Na^+$), 362.15 ($M+Na^+$+H^+). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.11 (s, 1H, COOH), δ 8.20 (d, 1H, CONH-2, J=8.07), δ 7.85 (m, 1H, CONH-1), δ 7.10 (m, 5H, Benzene), δ 4.19 (m, 1H, CH), δ 3.55 (s, 2H, CH_2), δ 3.02 (m, 2H, CH_2), δ 2.18 (m, 2H, CH_2), δ 1.32 (m, 2H, CH_2), δ 1.22-1.29 (m, 2H, CH_2), δ

0.84 (m, 3H, CH_3). IR (KBr, cm-^{1}): 3309 (N-H str of CONH), 3105 (aromatic =C-H str), 2961 (assym. aliphatic –CH_3 str), 2932 (assym. aliphatic -CH_2 str), 2874 (sym. aliphatic –CH_3str),1727 (C=O str COOH), 1648 (C=O str of CONH), 1612 (aromatic C=C str), 1544 (N-H deformation of CONH), 1496 (aliphatic -CH_2 deformation), 1446 (aliphatic –CH_3 deformation), 1415,1231 (C-O str and O-H bending of COOH), 1172 (aromatic C-F str), 1033 (aromatic =C-H in plane defrormation), 955 (O-H out of plane deformation of COOH), 955,924,848,800,760,712,647 (aromatic –C-H out of plane deformation).

4(S)-5-(Pentylamino)-4-(2-(2-fluorophenyl) acetamido)-5-oxopentanoic acid (D22)

Yield: 39.5%. MP 180-182^{o}C.MS (ESI): 375.1 (M+Na^{+}), ^{1}HNMR (300MHz, DMSO-$D_{6,}$ δ ppm): δ 12.10 (s, 1H, COOH), δ 8.20 (d, 1H, CONH-2, J=8.07), δ 7.85 (m, 1H, CONH-1), δ 7.10 (m, 5H, Benzene), δ 4.17 (m, 1H, CH), δ 3.65 (m, 2H, CH_2), δ 2.91 (m, 2H, CH_2), δ 2.14 (m, 2H, CH_2), δ 1.69 (m, 2H, CH_2), δ 1.32 (m, 2H, CH_2), δ 1.23 (m, 2H, CH_2), δ 1.13 (m, 2H, CH_2), δ 0.79 (m, 3H, CH_3).IR (KBr, cm-^{1}): 3319 (N-H str of CONH), 3065 (aromatic =C-H str), 2968 (assym. aliphatic –CH_3 str), 2931 (assym. aliphatic -CH_2 str), 2876 (sym. aliphatic –CH_3 str),1729 (C=O str COOH), 1649 (C=O str of CONH), 1610 (aromatic C=C str), 1541 (N-H deformation of CONH), 1497 (aliphatic -CH_2 deformation), 1446 (aliphatic –CH_3 deformation), 1413,1233 (C-O str and O-H bending of COOH), 1172 (aromatic C-F str), 1033 (aromatic =C-H in plane defrormation), 955 (O-H out of plane deformation of COOH).

4(S)-5-(Phenylamino)-4-(2-(2-fluorophenyl) acetamido)-5-oxopentanoic acid (D23)

Yield: 55.1%. MP 213-215^{o}C.MS (ESI): 381.23 (M+Na^{+}), 397.25 (M+K^{+}).^{1}HNMR (300MHz, DMSO-$D_{6,}$ δ ppm): δ10.06 (s, 1H, CONH-1), δ8.43 (d, 1H, CONH-2, J=7.74), δ7.06- (m, 9H, Benzene), δ4.43 (m, 1H, CH), δ3.58 (s, 2H, CH_2), δ2.27 (m, 2H, CH_2), δ1.88 (m, 2H, CH_2), IR (KBr, cm-^{1}):3320 (N-Hstr of CONH), 3072 (aromatic =C-H str), 2933 (assym. aliphatic -C-H str), 2870 (sym. aliphatic -C–H str), 1726 (C=O str COOH), 1601 (aromatic C=C str), 1541 (N-H deformation),1447 (aliphatic -C-H deformation), 1414, 1245 (C-O str and O-H bending of COOH)

4(S)-5-(Benzylamino)-4-(2-(2, 4-dichlorophenyl)acetamido)-5-oxopentanoic acid (D24)

Yield: 55.2%. MP 181-183^{o}C.MS (ESI): 434.1 (M+Na^{+}), 413.11 (M+Na^{+}+2H^{+}), 414.12 (M+Na^{+}+3H^{+}). ^{1}HNMR(300MHz, DMSO-$D_{6,}$ δ ppm): δ 8.41 (m, 1H, CONH), δ 8.10 (d, 1H, CONH, J=7,971), δ 7.211 (m, 8H, Benzene), δ 4.26 (m, 3H, CH and CH_2), δ 3.31 (s, 2H,

CH_2), δ 2.20 (m, 2H, CH_2), δ 1.73 (m, 2H-3). IR (KBr, cm-^{1}):3308 (N-H str of CONH), 3063 (aromatic =C-H str), 2942 (assym. aliphatic -C-H str), 2877 (sym. aliphatic -C–H str), 1723 (C=O str COOH), 1645 (C=O str of CONH), 1604 (aromatic C=C str), 1541 (N-H deformation), 1438 (aliphatic -C-H deformation), 1411, 1260 (C-O str and O-H bending of COOH), 968 (O-H out of plane deformation of COOH).

4(S)-5-(Hexylamino)-4-(2-(2, 4-dichlorophenyl)acetamido)-5-oxopentanoic acid (D25)

Yield: 64.8%. MP 145-147^{o}C.MS (ESI): 430.1 (M+H$^+$), 405.2 (M+Na$^+$). ^{1}HNMR(300MHz, DMSO-D_6, δ ppm): δ 12.06 (s, 1H, COOH), δ 8.40 (m, 1H, CONH), δ 8.22 (d, 1H, CONH, J=8.31), δ 7.104 (m, 3H, Benzene), δ 4.20 (m, 1H, CH), δ 3.56 (m, 2H, CH_2), δ 2.96 (m, 2H, CH_2), δ 2.13 (m, 2H, CH_2), δ 1.70 (m, 2H, CH_2), δ 1.36 (m, 2H, CH_2), δ 1.15 (m, 6H, 3CH_2), δ 0.78 (m, 3H, CH_3).IR (KBr, cm-^{1}):3315 (N-Hstr of CONH), 3064 (aromatic =C-H str), 2940 (assym. aliphatic -C-H str), 2874 (sym. aliphatic -C–H str), 1723 (C=O str COOH), 1645 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -CH deformation), 1417, 1269 (C-O str and O-H bending of COOH), 974 (O-H out of plane deformation of COOH).

4(S)-5-[(2, 2-dimethylethyl) amino]-4-(2-(naphthalen-1-yl)acetamido)-5-oxopentanoic acid (D26)

Yield: 32.7%. MP 178-180^{o}C. MS (ESI): 379.10 (M+Na$^+$), ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.20 (s, 1H, COOH), δ 8.31 (d, 1H, CONH, J=7.34), δ 8.10 (d, 1H, CONH), δ 7.41 (m, 7H, Naphthalene), δ 4.20 (m, 1H, CH), δ 3.96 (m, 1H, CH), δ 3.78 (s, 2H, CH_2), δ 2.10 (m, 2H, CH_2), δ 1.69 (m, 2H, CH_2), δ 1.29 (m, 9H, 3CH_3). IR (KBr, cm-^{1}): 3284 (N-H str of CONH), 3159 (O-H str of COOH), 3069 (aromatic =C-H str), 2971 (assym.aliphatic –CH_3 str), 2933 (assym. aliphatic -CH_2 str), 2874 (sym. aliphatic –CH_3 str), 1714 (C=O str COOH), 1632 (C=O str of CONH), 1599 (aromatic C=C str), 1545 (N-H deformation of CONH), 1446 (aliphatic –CH_3 deformation), 1410,1227 (C-O str and O-H bending of COOH), 1386 (aliphatic –CH deformation), 1017 (aromatic =C-H in plane defrormation), 958 (O-H out of plane deformation of COOH), 924,856,784,733,663,609 (aromatic –C-H out of plane deformation).

4(S)-5-(i-propylamino)-4-(2-(naphthalen-1-yl)acetamido)-5-oxopentanoic acid (D27)

MS (ESI): 378.98 (M+Na$^+$), 380.00 (M+Na$^+$+H$^+$). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.24 (s, 1H, COOH), δ 8.31 (d, 1H, CONH, J=7.34), δ 8.06 (d, 1H, CONH), δ 7.41 (m, 7H,

Naphthalene), δ 4.19 (m, 1H, CH), δ 3.94 (m, 1H, CH), δ 3.77 (s, 2H, CH_2), δ 2.14 (m, 2H, CH_2), δ 1.73 (m, 2H, CH_2), δ 0.98 (d, 6H, $2CH_3$). IR (KBr, cm^{-1}): 3286 (N-H str of CONH), 3159 (O-H str of COOH), 3069 (aromatic =C-H str), 2971 (assym.aliphatic $–CH_3$ str), 2933 (assym. aliphatic $-CH_2$ str), 2874 (sym. aliphatic $–CH_3$ str), 1714 (C=O str COOH), 1632 (C=O str of CONH), 1599 (aromatic C=C str), 1545 (N-H deformation of CONH), 1446 (aliphatic $–CH_3$ deformation), 1410,1227 (C-O str and O-H bending of COOH), 1386 (aliphatic –CH deformation), 1017 (aromatic =C-H in plane defrormation), 958 (O-H out of plane deformation of COOH), 924,856,784,733,663,609 (aromatic –C-H out of plane deformation).

4(S)-5-(Benzylamino)-4-(2-(naphthalen-1-yl) acetamido)-5-oxopentanoic acid (D28)

Yield: 54.2%. MP 178-180.MS (ESI): 404.4 ($M+H^+$). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.10 (s, 1H, COOH), δ 8.26 (d, 1H, CONH, J=7.37), δ 8.10 (d, 1H, CONH), δ 7.01 (m, 12H, naphthalene and benzene), δ 4.36 (m, 1H, CH), δ 4.30 (m, 2H, CH_2), δ 3.58 (s, 2H, CH_2), δ 2.16 (m, 2H, CH_2), δ 1.85 and 1.74 (m, 2H, CH_2).IR (KBr, cm^{-1}): 3069 (aromatic =C-H str), 2971 (assym.aliphatic $–CH_3$ str), 2933 (assym. aliphatic $-CH_2$ str), 2874 (sym. aliphatic $–CH_3$ str), 1714 (C=O str COOH), 1632 (C=O str of CONH), 1599 (aromatic C=C str), 1545 (N-H deformation of CONH), 1511 (aliphatic $-CH_2$ deformation), 1445 (aliphatic $–CH_3$ deformation), 1389 (aliphatic –CH deformation), 1017 (aromatic =C-H in plane defrormation), 958 (O-H out of plane deformation of COOH), 924,856,784,733,663,609 (aromatic –C-H out of plane deformation).

4(S)-5-(Hexylamino)-4-(2-(naphthalen-1-yl) acetamido)-5-oxopentanoic acid (D29)

Yield: 30.3%. MP 138-140.MS (ESI): 421.1 ($M+H^+$). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm): δ 12.15 (s, 1H, COOH), δ 8.20 (d, 1H, CONH, J=7.40), δ 8.10 (d, 1H, CONH), δ 7.14 (m, 7H, naphthalene), δ 4.30 (m, 1H, CH), δ 4.17 (m, 2H, CH_2), δ 3.76 (s, 2H, CH_2), δ 2.20 (m, 2H, CH_2), δ 1.86 and 1.75 (m, 2H, CH_2).IR (KBr, cm^{-1}): 3284 (N-H str of CONH), 3159 (O-H str of COOH), 3069 (aromatic =C-H str), 2971 (assym. aliphatic $–CH_3$ str), 2933 (assym. aliphatic $-CH_2$ str), 2874 (sym. aliphatic $–CH_3$ str), 1714 (C=O str COOH), 1632 (C=O str of CONH), 1599 (aromatic C=C str), 1545 (N-H deformation of CONH), 1446 (aliphatic $–CH_3$ deformation), 1410,1227 (C-O str and O-H bending of COOH), 1368 (aliphatic –CH deformation), 1019 (aromatic =C-H in plane defrormation), 958 (O-H out of plane deformation of COOH), 924,856,784,733,663,609 (aromatic –C-H out of plane deformation).

4(S)-5-(4-Chlorobenzylamino)-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D30)

Yield: 41.1%. MP 165-167^{o}C.MS (ESI): 490.10 ($M+Na^{+}$), ^{1}HNMR (300MHz, DMSO-$D_{6,}$ δ ppm):δ12.14 (s, 1H, COOH), δ 8.33 (m, 1H, CONH), δ 8.25 (d, 1H, CONH, J=7.92), δ 7.44 (m, 2H, Benzene), δ 7.20 (m, 2H, Benzene), δ 7.05 (m, 2H, Benzene), δ 6.95 (m, 2H, Benzene), δ 4.30 (m, 1H, CH), δ 4.20 (s, 2H, CH_2), δ 3.72 (m, 3H, CH_3), δ 3.42 (m, 2H,CH_2), δ 2.16 (m, 2H, CH_2), δ 1.70 (m, 2H, CH_2).IR (KBr, cm-^{1}): 3319 (N-Hstr of CONH), 3061 (aromatic =C-H str), 2946 (assym. aliphatic -C-H str), 2873 (sym. aliphatic -C–H str), 1727 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -C-H deformation), 1418, 1262 (C-O str and O-H bending of COOH), 966 (O-H out of plane deformation of COOH), 806, 750, 696, 651 (aromatic –C-H out of plane deformation).

4(S)-5-(4-Fluorobenzylamino)-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D31)

Yield: 29.5%. MP 153-155^{o}C.MS (ESI): 474.10 ($M+Na^{+}$), ^{1}HNMR (300MHz, DMSO-$D_{6,}$ δ ppm):δ12.16 (s, 1H, COOH), δ 8.32 (m, 1H, CONH), δ 8.24 (d, 1H, CONH, J=7.85), δ 7.43 (m, 2H, Benzene), δ 7.21 (m, 2H, Benzene), δ 7.06 (m, 2H, Benzene), δ 6.85 (m, 2H, Benzene), δ 4.32 (m, 1H, CH), δ 4.21 (s, 2H, CH_2), δ 3.70 (m, 3H, CH_3), δ 3.46 (m, 2H,CH_2), δ 2.18 (m, 2H, CH_2), δ 1.75 (m, 2H, CH_2).IR (KBr, cm-^{1}): 3309 (N-Hstr of CONH), 3063 (aromatic =C-H str), 2942 (assym. aliphatic -C-H str), 2877 (sym. aliphatic -C–H str), 1729 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -C-H deformation), 1423, 1256 (C-O str and O-H bending of COOH), 966 (O-H out of plane deformation of COOH), 806, 750, 696, 651 (aromatic –C-H out of plane deformation).

4(S)-5-(3, 4-Dichlorobenzylamino)-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D32)

Yield: 27.8%. MP 185-187^{o}C MS (ESI): 525.2 ($M+Na^{+}$), ^{1}HNMR (300MHz, DMSO-$D_{6,}$ δ ppm):δ12.10 (s, 1H, COOH), δ 8.30 (m, 1H, CONH), δ 8.21 (d, 1H, CONH, J=7.92), δ 7.32 (m, 2H, Benzene), δ 6.85 (m, 5H, Benzene), δ 4.32 (m, 1H, CH), δ 4.23 (s, 2H, CH_2), δ 3.71 (m, 3H, CH_3), δ 3.48 (m, 2H,CH_2), δ 2.18 (m, 2H, CH_2), δ 1.78 (m, 2H, CH_2).IR (KBr, cm-^{1}): 3065 (aromatic =C-H str), 2912 (assym. aliphatic -C-H str), 2865 (sym. aliphatic -C–H

str), 1732 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -C-H deformation), 966 (O-H out of plane deformation of COOH), 806, 750, 696, 651 (aromatic –C-H out of plane deformation).

4(S)-5-(4-Nitrobenzylamino)-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D33)

MS (ESI): 501.2 ($M+Na^+$), ^{1}HNMR (300MHz, DMSO-D_6, δ ppm):δ12.10 (s, 1H, COOH), δ 8.30 (m, 1H, CONH), δ 8.21 (d, 1H, CONH, J=7.92), δ 8.05 (m, 2H, Benzene), δ 7.30 (m, 4H, Benzene),δ 6.85 (m, 2H, Benzene),δ 4.32 (m, 1H, CH), δ 4.21 (s, 2H, CH_2), δ 3.70 (m, 3H, CH_3), δ 3.48 (m, 2H,CH_2), δ 2.12 (m, 2H, CH_2), δ 1.69 (m, 2H, CH_2).IR (KBr, cm-^{1}): 3310 (N-Hstr of CONH), 3060 (aromatic =C-H str), 2952 (assym. aliphatic -C-H str), 2867 (sym. aliphatic -C–H str), 1723 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1441 (aliphatic -C-H deformation), 966 (O-H out of plane deformation of COOH), 806, 750, 696, 651 (aromatic –C-H out of plane deformation).

4(S)-5-(4-Methoxybenzylamino)-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D34)

Yield: 28.3%. MP 198-200^{o}C MS (ESI): 490.1 ($M+Na^+$). ^{1}HNMR (300MHz, DMSO-D_6, δ ppm):δ 12.09 (s, 1H, COOH), δ 8.33 (m, 1H, CONH), δ 8.25 (d, 1H, CONH, J=7.92), δ 7.44 (m, 2H, Benzene), δ 7.18 (m, 2H, Benzene), δ 7.10 (m, 2H, Benzene), δ 6.82 (m, 2H, Benzene), δ 4.23 (m, 1H, CH), δ 4.16 (s, 2H, CH_2), δ 3.70 (m, 3H, CH_3), δ 3.40 (m, 2H,CH_2), δ 2.14 (m, 2H, CH_2), δ 1.69 (m, 2H, CH_2). IR (KBr, cm-^{1}): 3316 (N-Hstr of CONH), 3063 (aromatic =C-H str), 2946 (assym. aliphatic -C-H str), 2877 (sym. aliphatic -C–H str), 1725 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -C-H deformation), 1414, 1266 (C-O str and O-H bending of COOH), 966 (O-H out of plane deformation of COOH), 806, 750, 696, 651 (aromatic –C-H out of plane deformation).

4(S)-5-(2-Chlorobenzylamino)-4-(2-(4-bromophenyl) acetamido)-5-oxopentanoic acid (D35)

Yield: 32.5%. MP 135-137^{o}C MS (ESI): 490.2 ($M+Na^+$), ^{1}HNMR (300MHz, DMSO-D_6, δ ppm):δ12.10 (s, 1H, COOH), δ 8.33 (m, 1H, CONH), δ 8.24 (d, 1H, CONH, J=7.92), δ 7.36 (m, 2H, Benzene), δ 6.85 (m, 6H, Benzene), δ 4.46 (m, 1H, CH), δ 4.30 (s, 2H, CH_2), δ 3.70

(m, 3H, CH_3), δ 3.32 (m, 2H,CH_2), δ 2.16 (m, 2H, CH_2), δ 1.70 (m, 2H, CH_2).IR (KBr, cm-[1]): 3308 (N-Hstr of CONH), 3061 (aromatic =C-H str), 2940 (assym. aliphatic -C-H str), 2873 (sym. aliphatic -C–H str), 1727 (C=O str COOH), 1647 (C=O str of CONH), 1604 (aromatic C=C str), 1542 (N-H deformation), 1439 (aliphatic -C-H deformation), 1416, 1266 (C-O str and O-H bending of COOH), 966 (O-H out of plane deformation of COOH), 806, 750, 696, 651 (aromatic –C-H out of plane deformation).

The HMBC spectra of **D23** are presented in Figure TS2. Cross peaks were found between N5-H (10.06) and C4 (171.62), N3-H (4.43) and C4 (171.62) as well as C7-H (2.30) and C8 (174.21). No cross-peak was observed between N5-H (10.06) and C8 (174.21). It confirms that the conformations of the aromatic amine derivatives remain consistent to the proposed conformations. In addition, [1]NMR spectra of some synthesized final compounds are presented in Figures TS3-TS12.

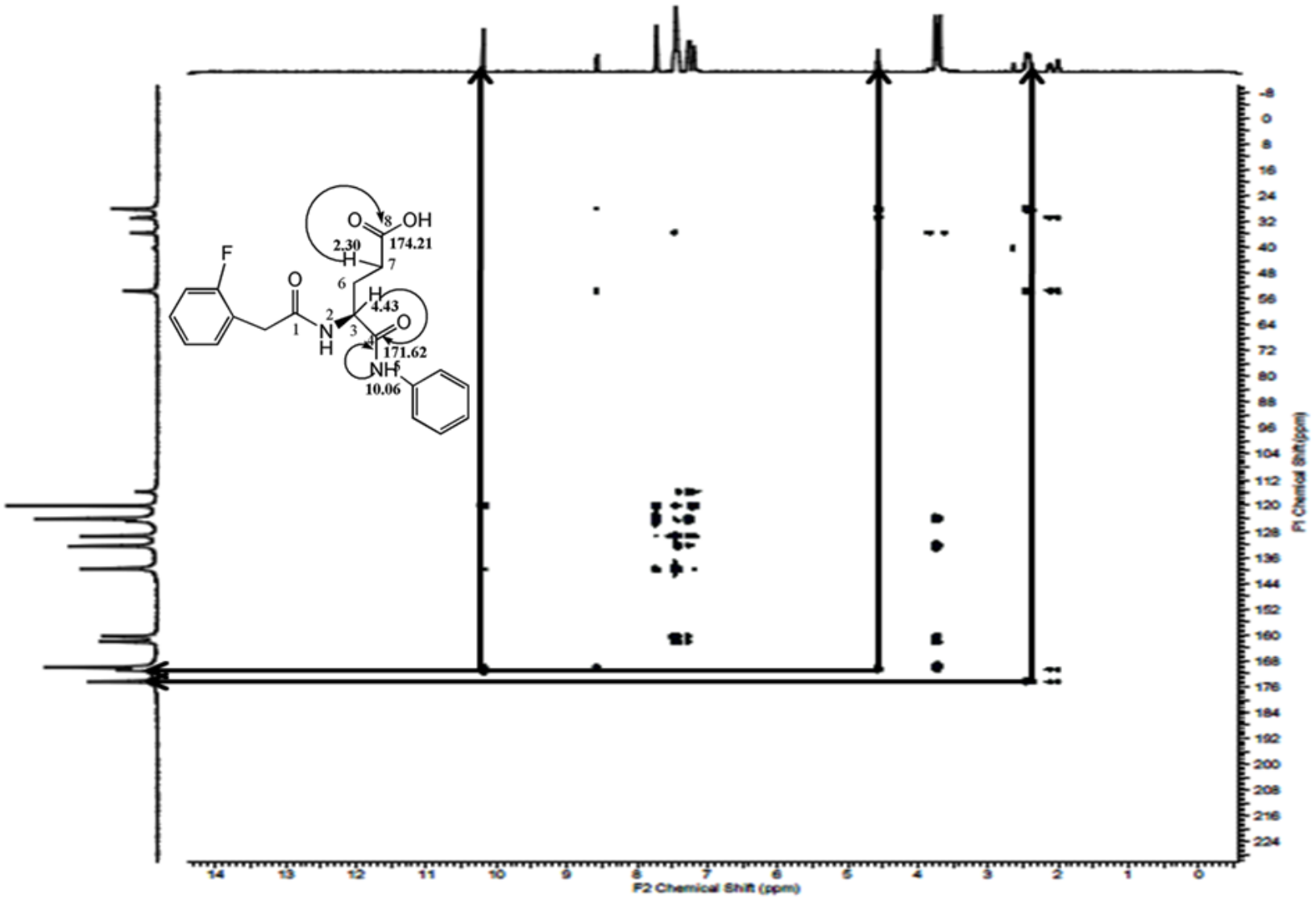


Figure TS2. HMBC spectrum of Cpd. **D23**

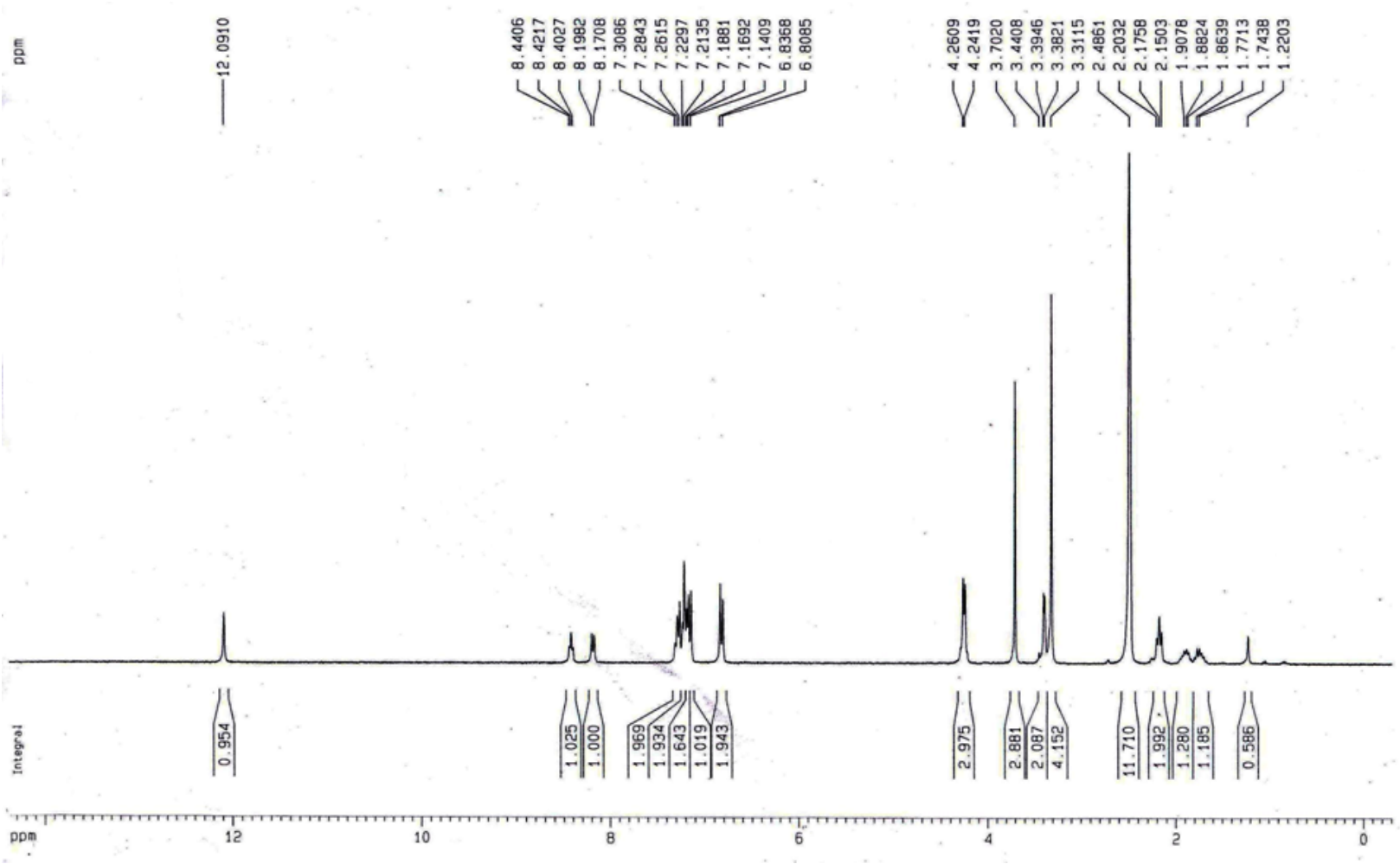


Figure TS3 ^{1}H NMR spectra of **D5**

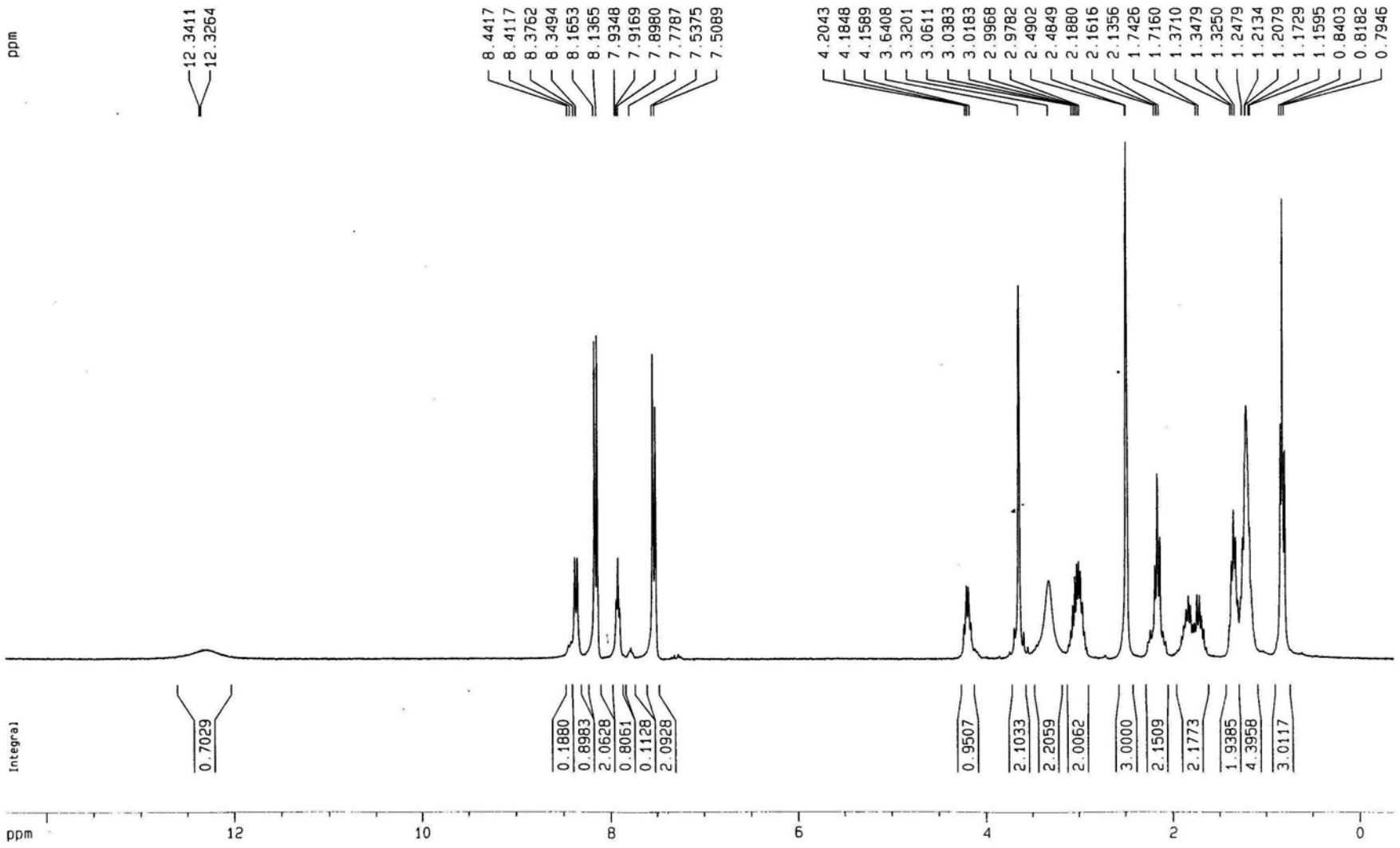


Figure TS4 ^{1}H NMR spectra of **D7**

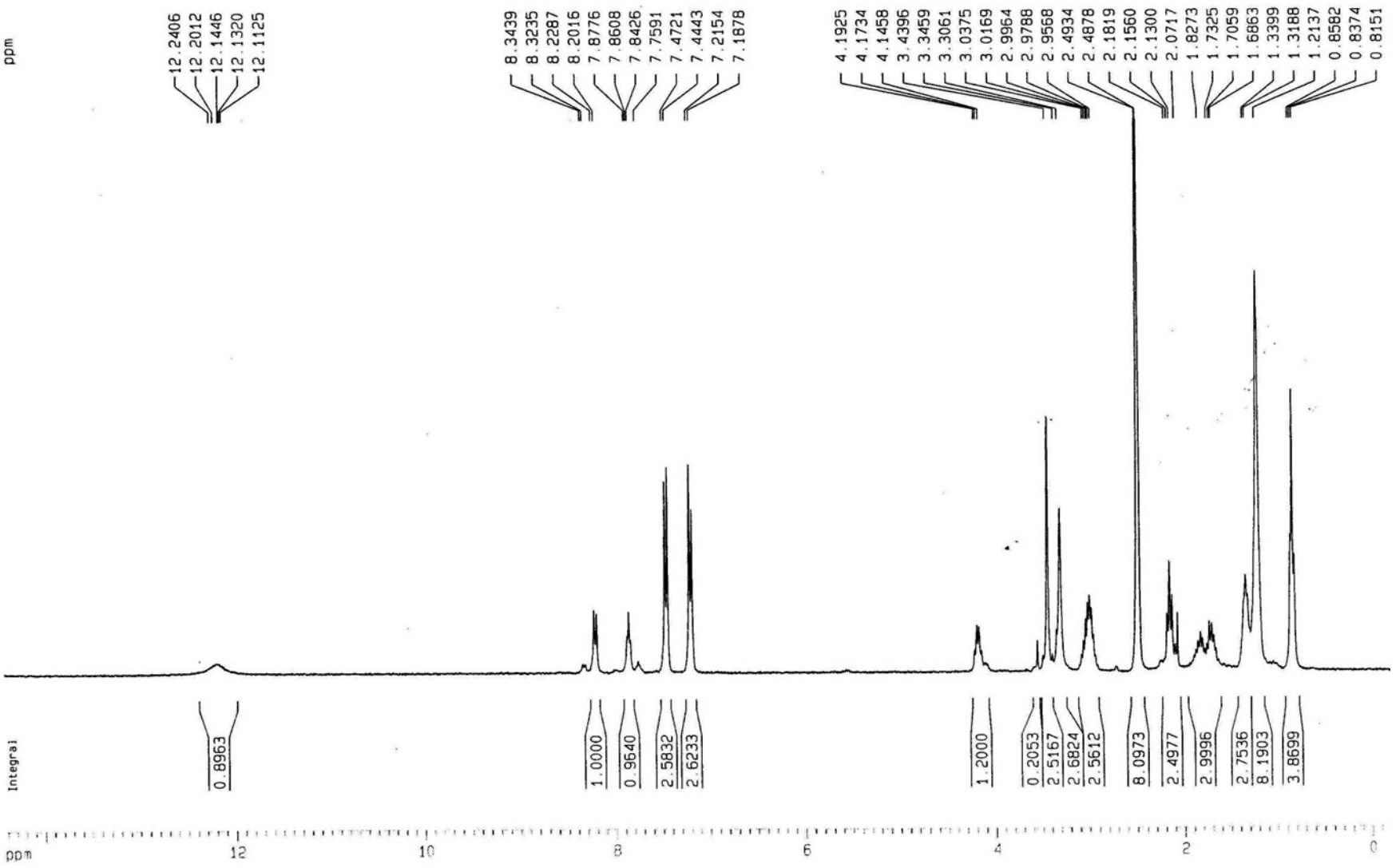


Figure TS5 ^{1}H NMR spectra of **D12**

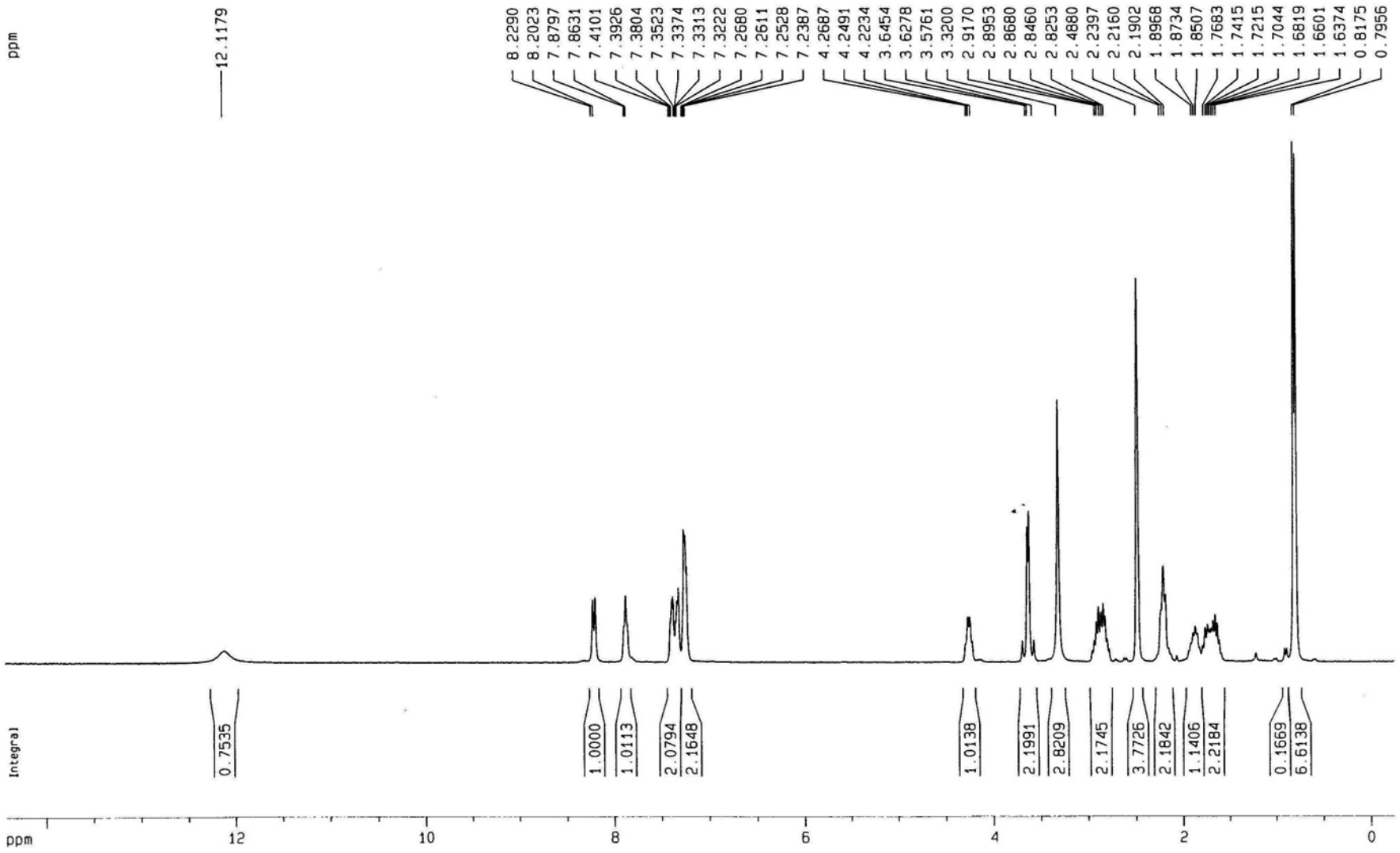


Figure TS6 ^{1}H NMR spectra of **D13**

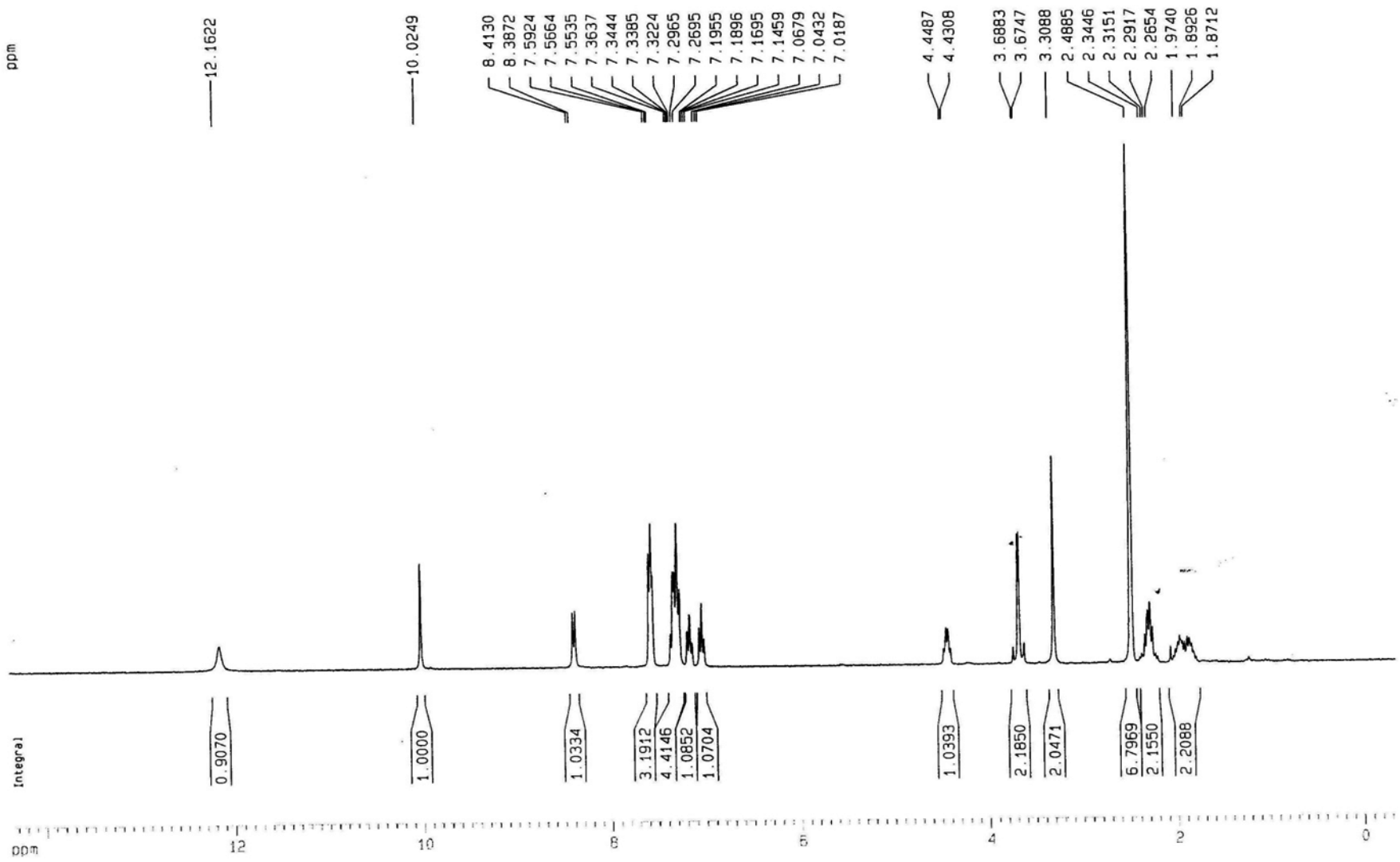


Figure TS7 ^{1}H NMR spectra of **D20**

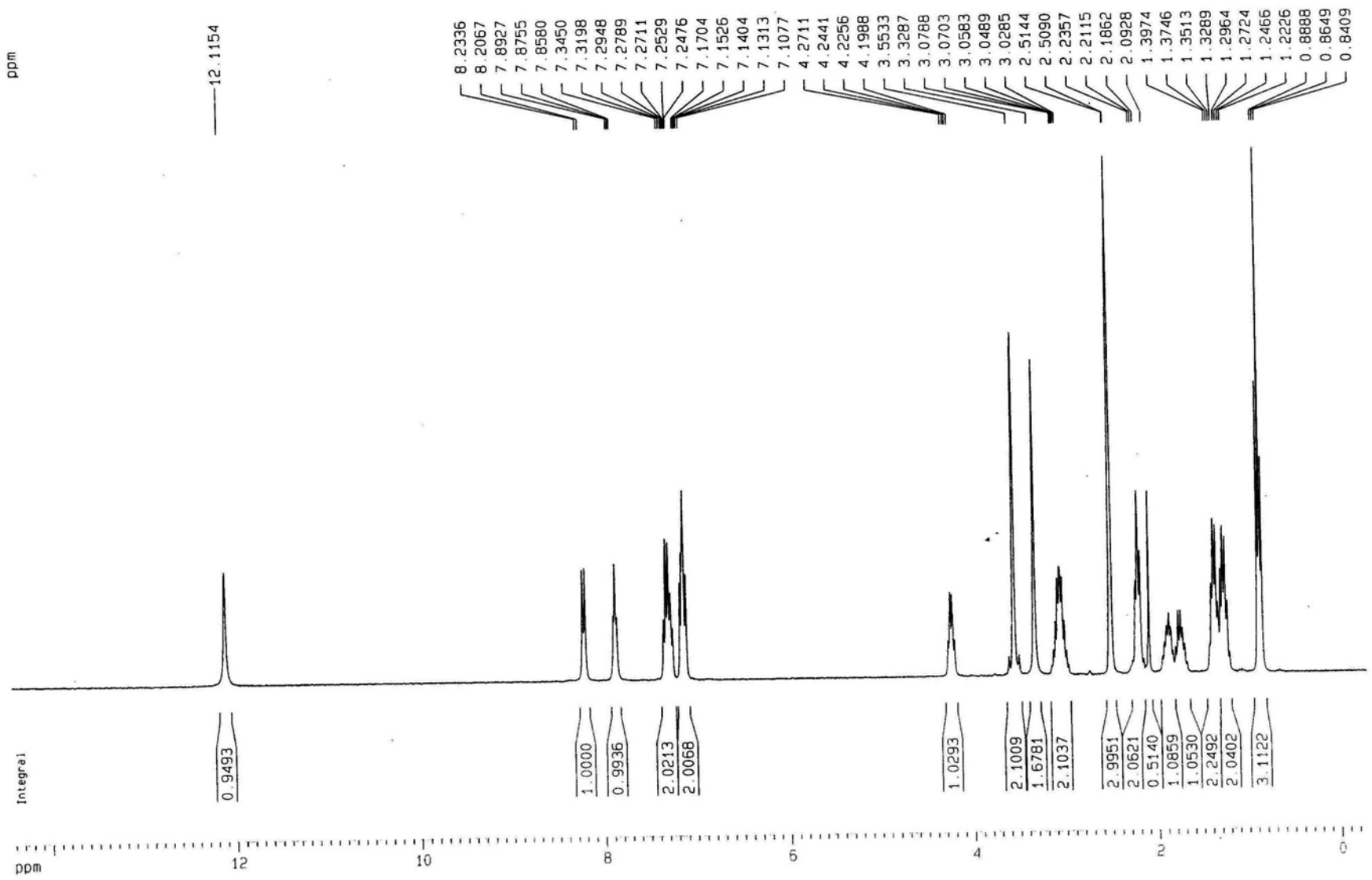


Figure TS8 ^{1}H NMR spectra of **D21**

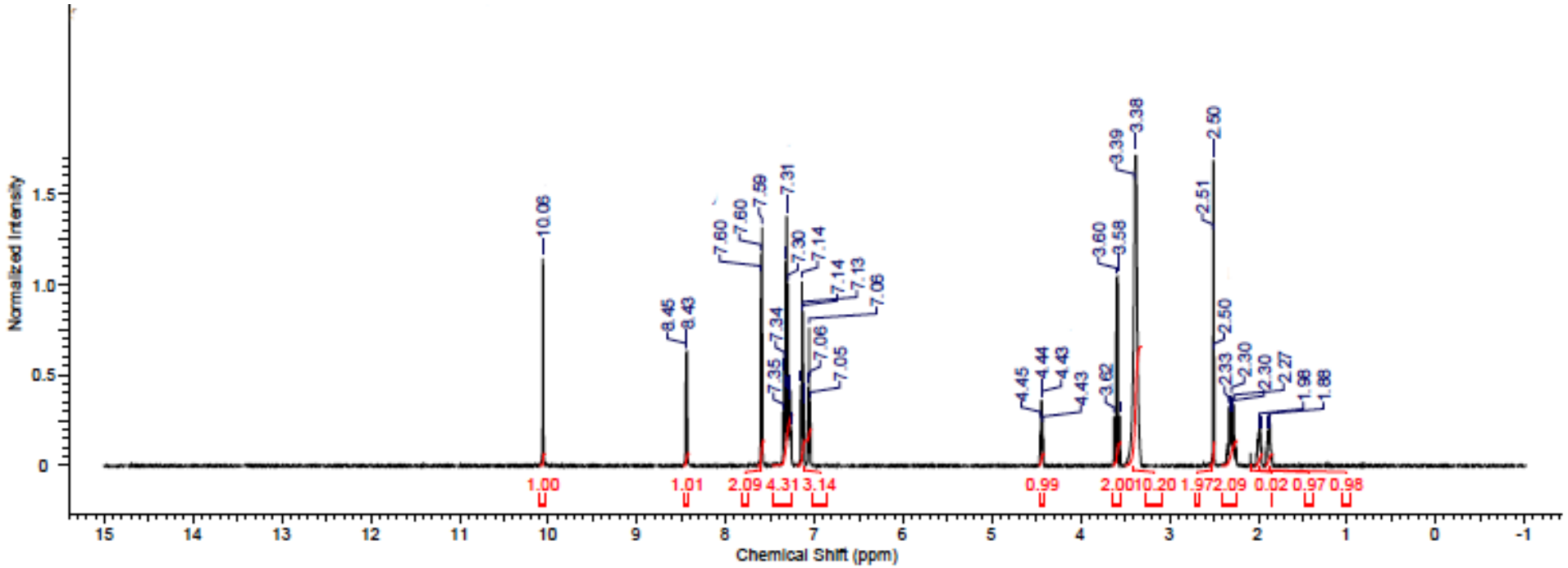


Figure TS9 ^{1}H NMR spectra of **D23**

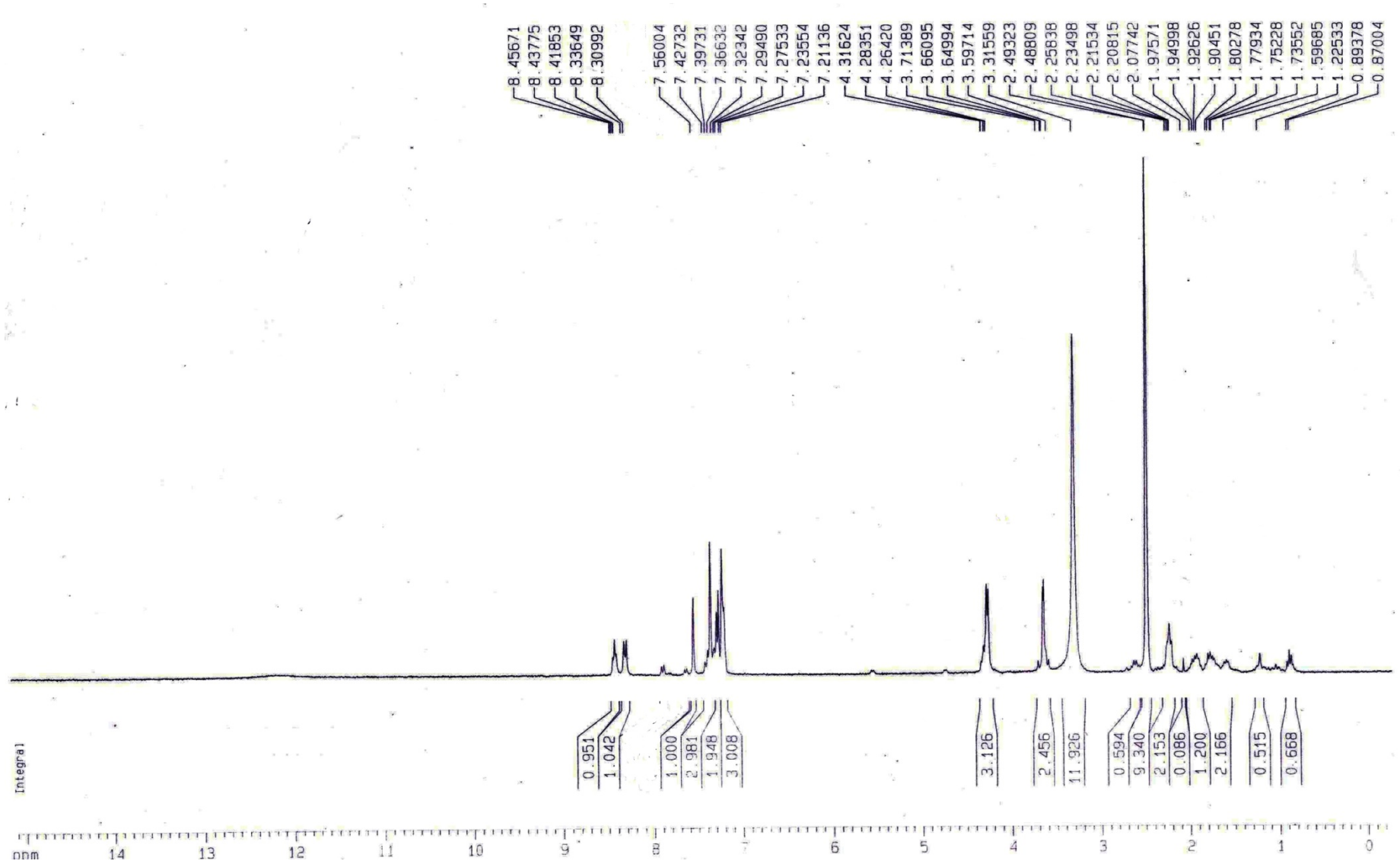


Figure TS10 ^{1}H NMR spectra of **D24**

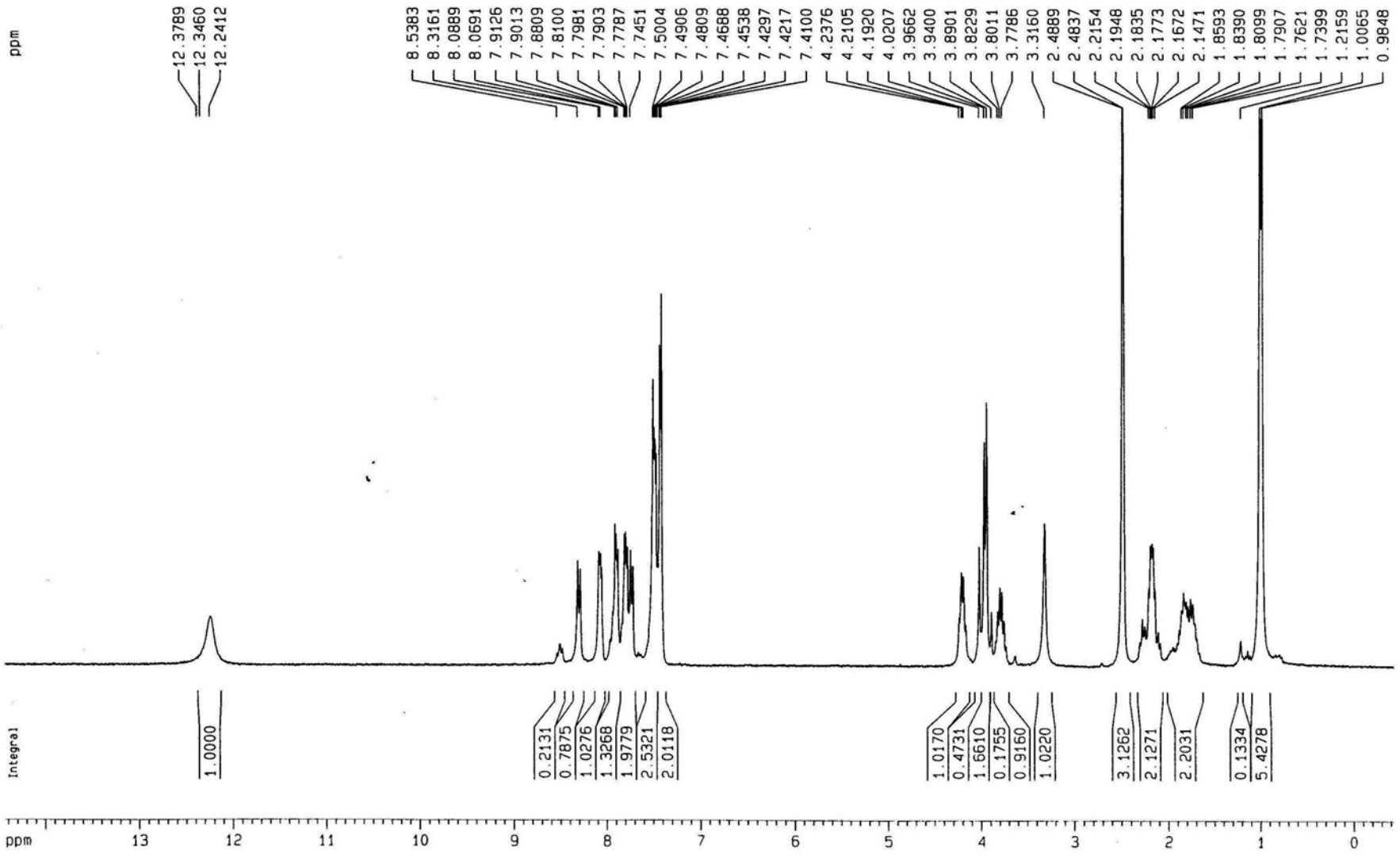


Figure TS11 ^{1}H NMR spectra of **D27**

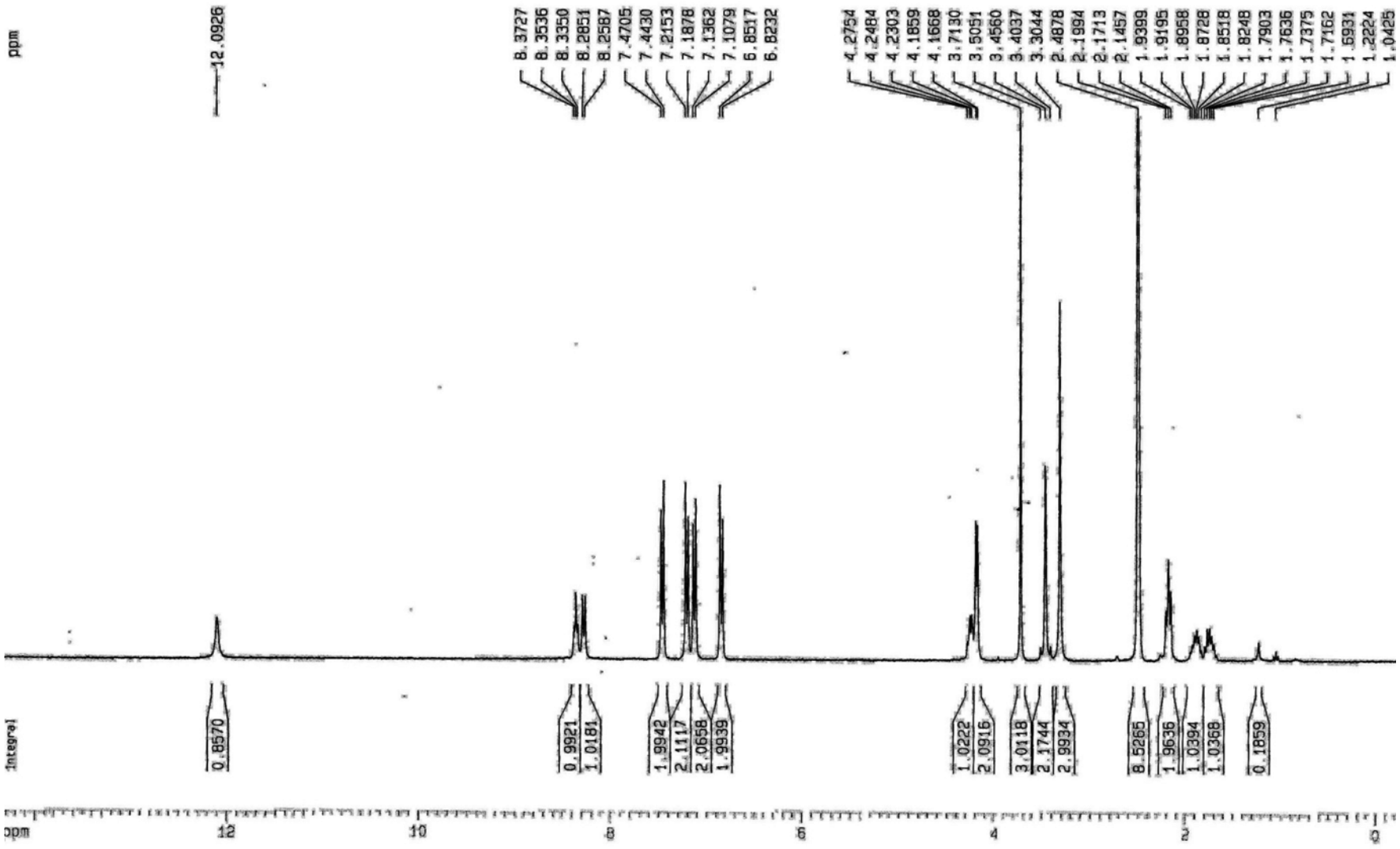


Figure TS12 ^{1}H NMR spectra of **D34**

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