Design, Synthesis and Biological Evaluation of Novel Quinazolin-4(3H)-one Based Histone Deacetylase 6 (HDAC6) Inhibitors for Anticancer Activity

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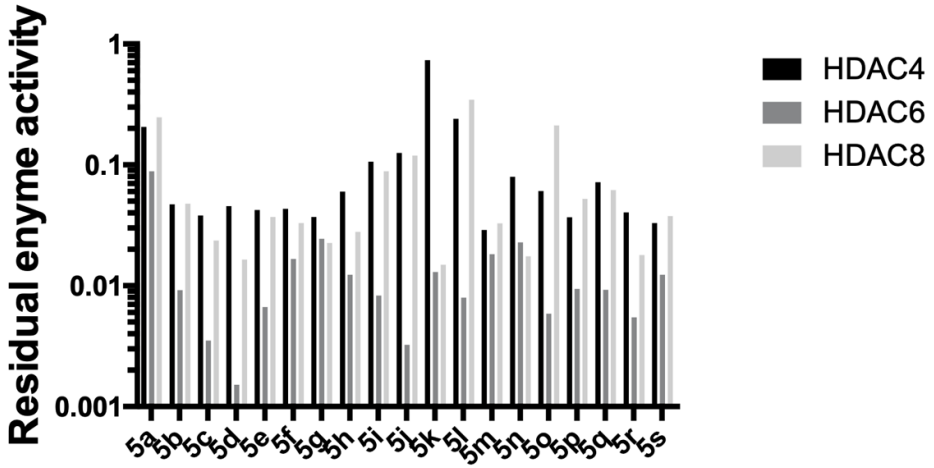


Figure S1. Shown is the residual enzyme activity of HDAC4, 6 and 8. in the presence of 35 µM compounds measured in duplicate **(Table S1).** A residual activity of 1 means no inhibition.

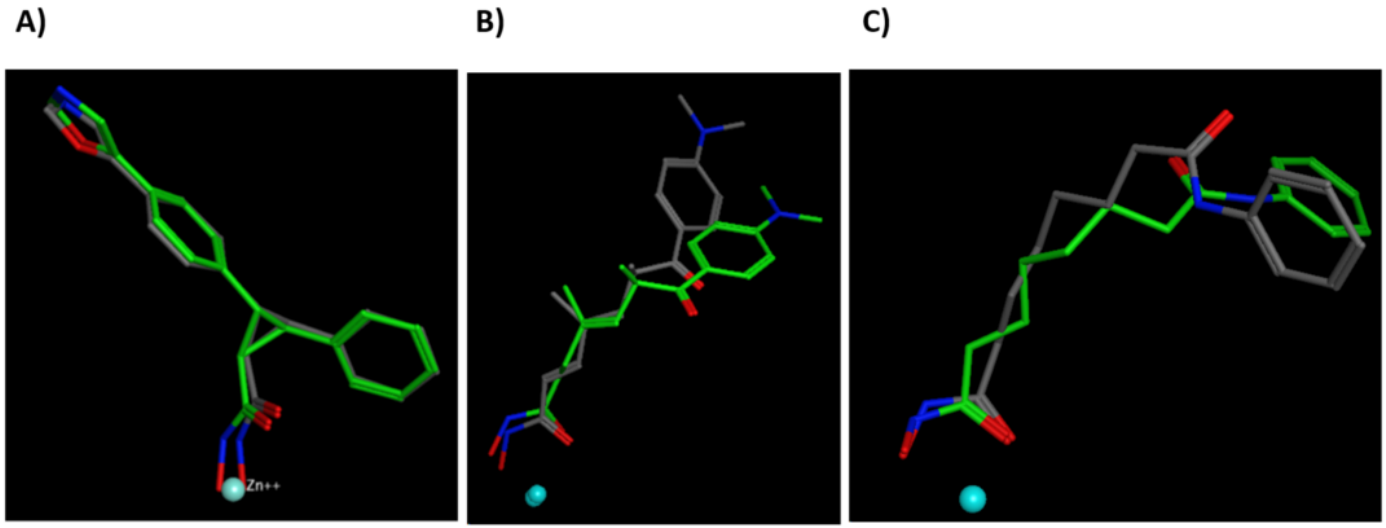


Figure S2. (A) Redocking of ligand into the crystal structure of the binding pocket of HDAC4 (PDB-ID: 4CBY) shows perfect overlap between the docked ligand (gray) and the X-ray binding pose (green). **(B)** Redocking into HDAC6 (PDB-ID: 5EDU) shows very good agreement in the lower part of the binding pocket, but considerable deviation between the head group, which protrude freely into solution. C) Redocking of ligand into the crystal structure of the binding pocket of HDAC8 (PDB-ID: 1T69) shows very good overlap between the docked ligand (grey) and the X-ray binding pose (green) in the lower part of the binding pocket. The phenyl head group, which protrudes into free solution, shows more deviation.

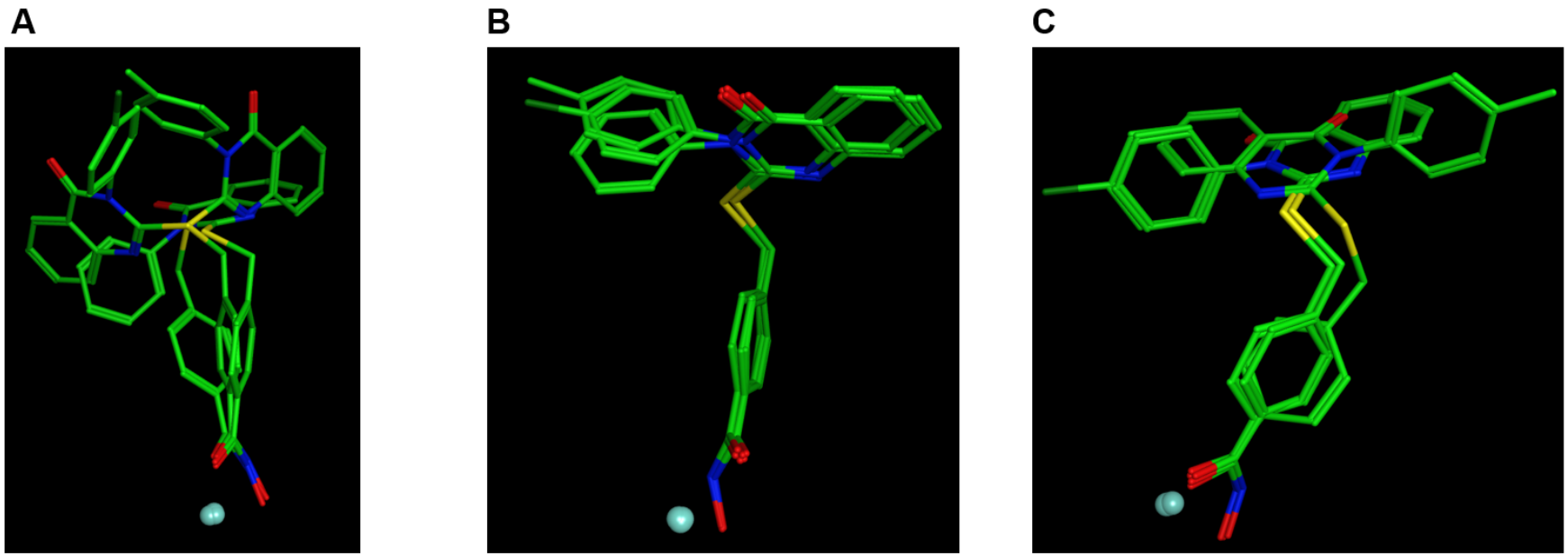


Figure S3. Overlay of energy-minimized docking poses of compounds 5a-c within the binding pockets of (A) HDAC4 (PDB-ID: 4CBY), (B) HDAC6 (PDB-ID: 5EDU) and (C) HDAC8 (PDB-ID: 1T69). Cyan beads represent the catalytic zinc ions.

Table S1. List of every residual activity, which are shown in **Figure S2.**

Compound	HDAC4		HDAC6		HDAC8	
	0.19440		0.07799		0.21513	
5a	4	0.216831	2	0.098287	9	0.28015
	0.04589		0.01336		0.04544	
5b	4	0.048698	9	0.005031	5	0.049903
	0.04022		0.00305		0.02607	
5c	3	0.036002	9	0.003964	8	0.021322
	0.04621		0.00040			
5d	7	0.044928	9	0.002628	0.01771	0.015115
			0.00318		0.03825	
5e	0.04467	0.03974	9	0.010169	7	0.035911
	0.04257		0.01007			
5f	6	0.043961	2	0.023193	0.03638	0.029747
			0.01627		0.02059	
5g	0.0293	0.04467	7	0.03263	7	0.024671
	0.05507		0.01931		0.02733	
5h	8	0.064905	5	0.005343	7	0.028617
	0.10096		0.01326		0.08827	
5i	1	0.111852	1	0.003372	4	0.088338
	0.13189		0.00490		0.12205	
5j	4	0.119843	1	0.001594	9	0.116172

	0.74214		0.01439			
5k	1	0.730283	2	0.011548	0.0168	0.013068
	0.26132		0.00896			
5l	9	0.218989	3	0.006991	0.3384	0.351155
			0.02154		0.03032	
5m	0.02669	0.031105	5	0.015049	3	0.035229
	0.08050		0.01933		0.01769	
5n	1	0.078535	6	0.026349	6	0.017333
	0.05436		0.00408		0.22121	
50	9	0.067515	3	0.007638	8	0.203344
	0.04412		0.01080		0.05361	
5p	2	0.029526	5	0.007993	4	0.050969
	0.07318		0.00799		0.06387	
5q	6	0.07077	3	0.010514	4	0.059778
	0.03864		0.00598		0.01810	
5r	5	0.041963	9	0.004998	1	0.017675
	0.03542					
5s	2	0.030621	0.00739	0.017279	0.04359	0.031965

Table S2. *In silico* predicted physico-chemical parameters of the titled compounds

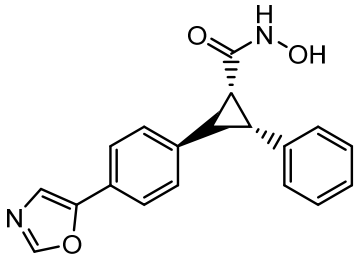
Entry	mol MW	Donor HB	Accpt HB	Log Po/w	Rule of Five	TPSA	ESOL Class	GI absorption
5a	437.9	2	4	2.96	0	109.52	Moderately soluble	High
5b	403.45	2	4	3.04	0	109.52	Moderately soluble	High
5c	417.48	2	4	2.87	0	109.52	Moderately soluble	High
5d	431.51	2	4	3.26	0	109.52	Moderately soluble	High
5e	482.35	2	4	3.01	0	109.52	Moderately soluble	High
5f	431.51	2	4	2.7	0	109.52	Moderately soluble	High
5g	417.48	2	4	3.34	0	109.52	Moderately soluble	High
5h	437.9	2	4	3.41	0	109.52	Moderately soluble	High
5i	421.44	2	5	3.05	0	109.52	Moderately soluble	High
5j	437.9	2	4	3.43	0	109.52	Moderately soluble	High
5k	451.93	2	4	3.25	0	109.52	Moderately soluble	High
5l	451.93	2	4	3.12	0	109.52	Moderately soluble	High
5m	455.89	2	5	3.19	0	109.52	Moderately soluble	High
5n	472.34	2	4	2.52	0	109.52	Moderately soluble	High
5o	465.95	2	4	3.21	0	109.52	Poorly soluble	High
5p	417.48	2	4	2.79	0	109.52	Moderately soluble	High
5q	435.47	2	5	3.25	0	109.52	Moderately soluble	High
5r	431.51	2	4	2.56	0	109.52	Moderately soluble	High
5s	451.93	2	4	2.68	0	109.52	Moderately soluble	High

Description: Lipinski rule of five – Number of violations of Lipinski's rule of five. The rules are: mol MW - 130.0 – 725.0, log Po/w - –2.0 – 6.5, donor HB ≤ 5, accept HB ≤10 and maximum 4 violations. **Log Po/w**:Log of partition coefficient; **TPSA**: Topological polar surface area:TPSA < 140 is essential for good absorption; **ESOL:** Estimated aqueous solubility in mg/mL; **GI:** Gastrointestinal.

Table S3. Docking scores (GBVI/WSA dG) and IC_{50}-values against HDAC4, 6 and 8. The PDB-Id's used for docking are indicated in brackets.

Compound	HDAC4 (PDB:4CBY)	HDAC6 (PDB:5EDU)	HDAC8 (PDB:1T69)	HDAC4 IC_{50} (nM)	HDAC6 IC_{50} (nM)	HDAC8 IC_{50} (nM)
5a	-11.5	-13.1	-12.0	4500	5600	24000
5b	-11.5	-13.1	-12.1	2300	150	1400
5c	-11.6	-13.1	-12.0	720	580	2300
Cpd 31 (4CBY)	-13.2	-	-	20*	-	360*
Trichostatin A (5EDU)	-	-13.7	-	3300	17	360
SAHA (1T69)	-	-	-12.0	27000	86	5300
Tubastatin	-	-	-	4700	160	2700

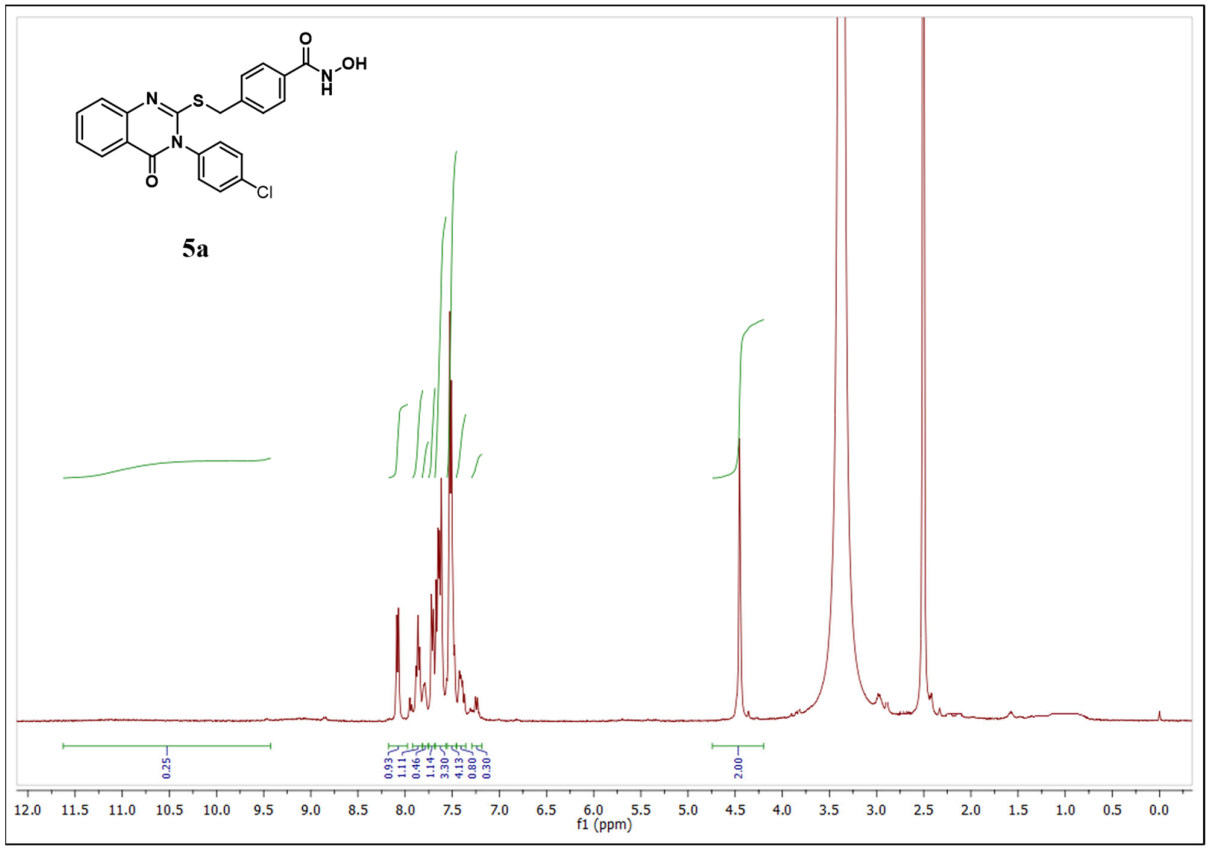
*Values are from Bürli et al's work [1]



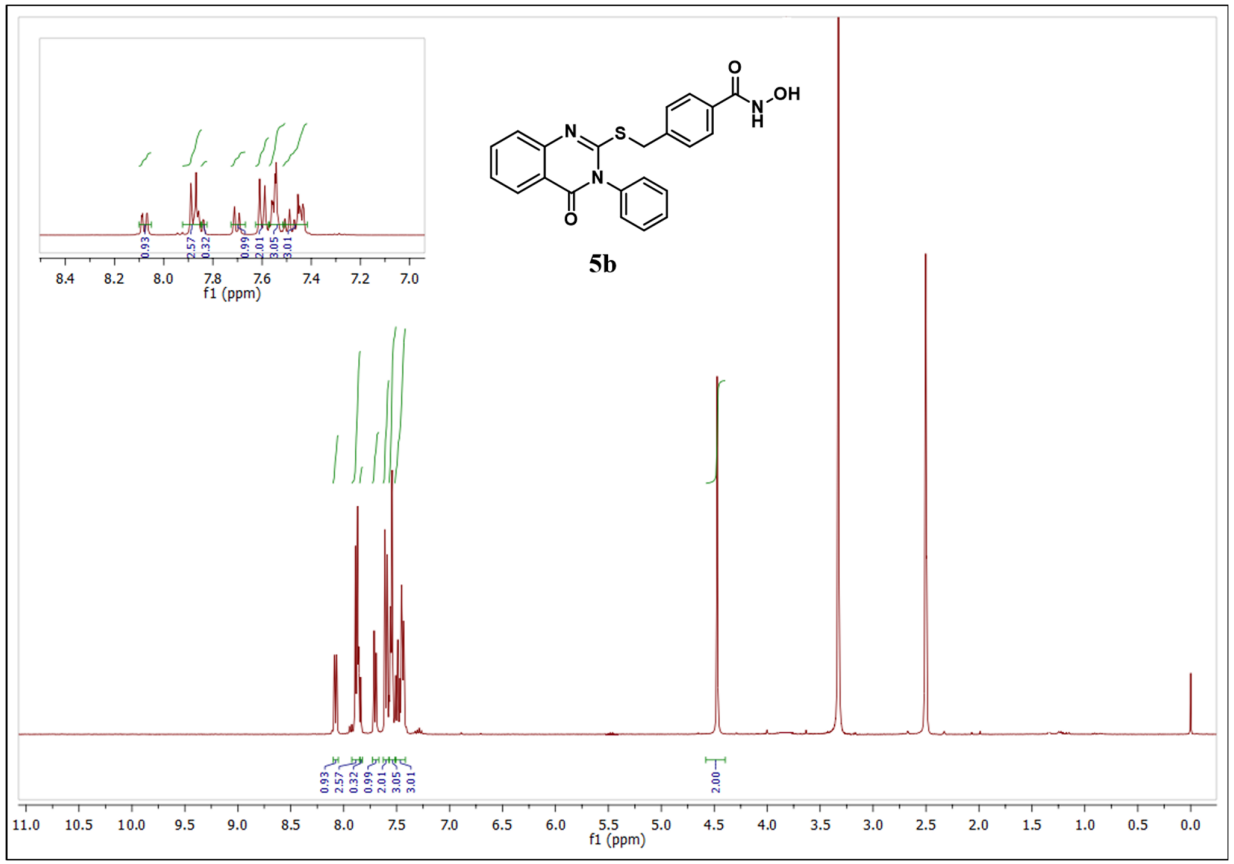
Structure of **Cpd 31**

1. Bürli, R. W., Luckhurst, C. A., Aziz, O., Matthews, K. L., Yates, D., Lyons, K. A., Dominguez, C. (2013). Design, synthesis, and biological evaluation of potent and selective class IIa histone deacetylase (HDAC) inhibitors as a potential therapy for Huntington's disease. *Journal of Medicinal Chemistry*, *56*(24), 9934-9954.

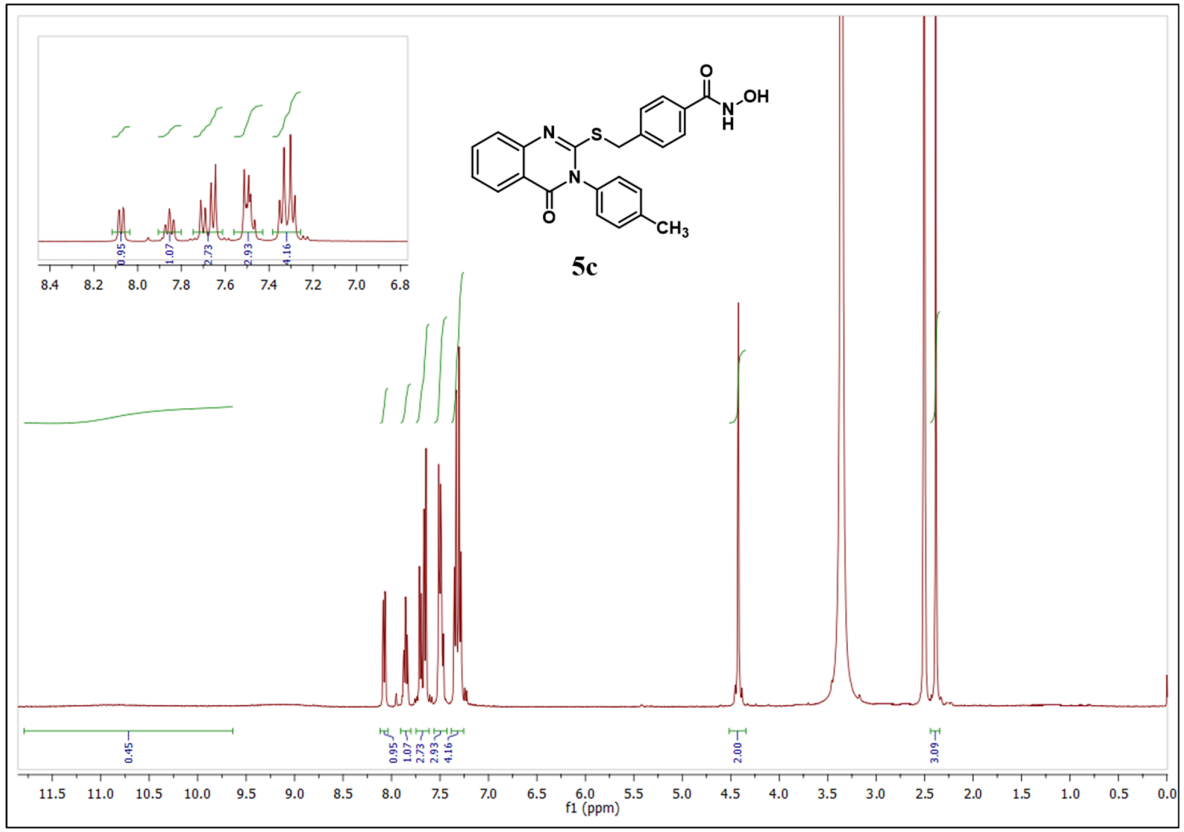
[1]H NMR spectras of final compounds (5a-5s):



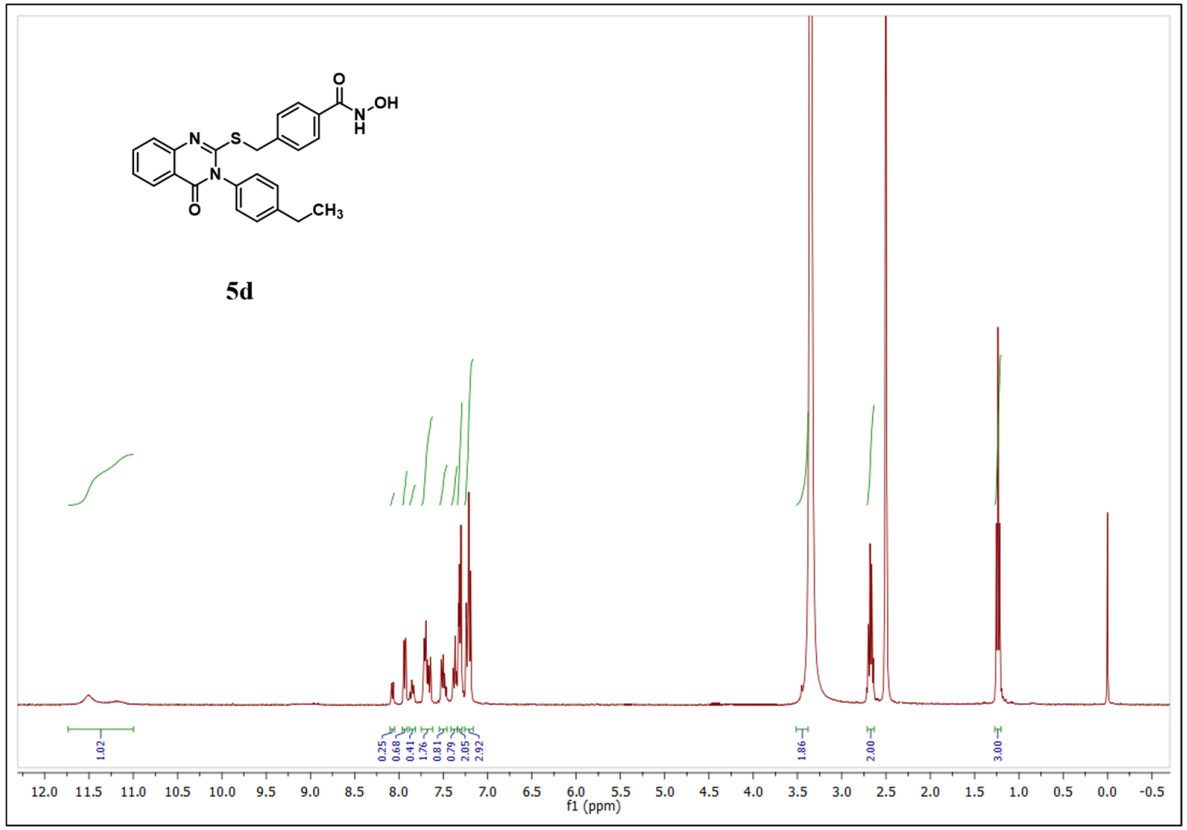
[1]H NMR spectrum of compound **5a (**DMSO-d_6 400 MHz)



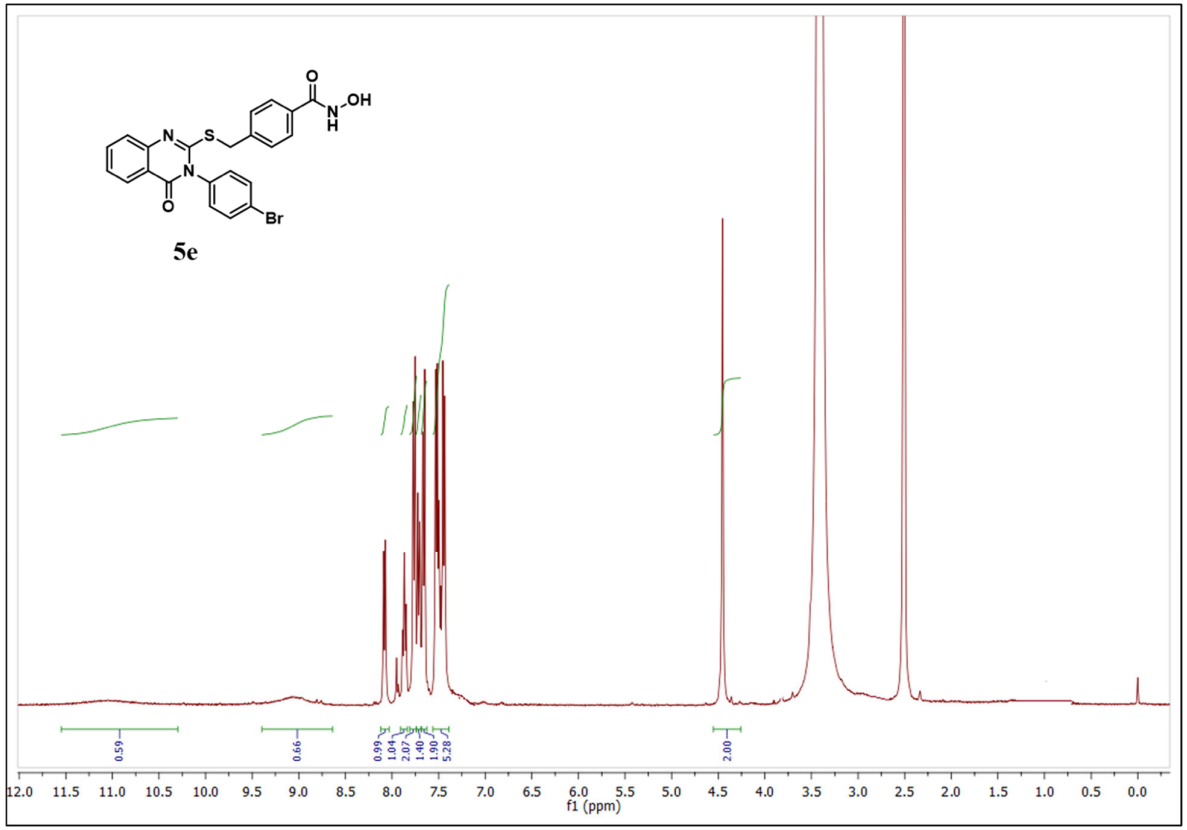
^{1}H NMR spectrum of compound **5b (**DMSO-*d*$_6$ 400 MHz)



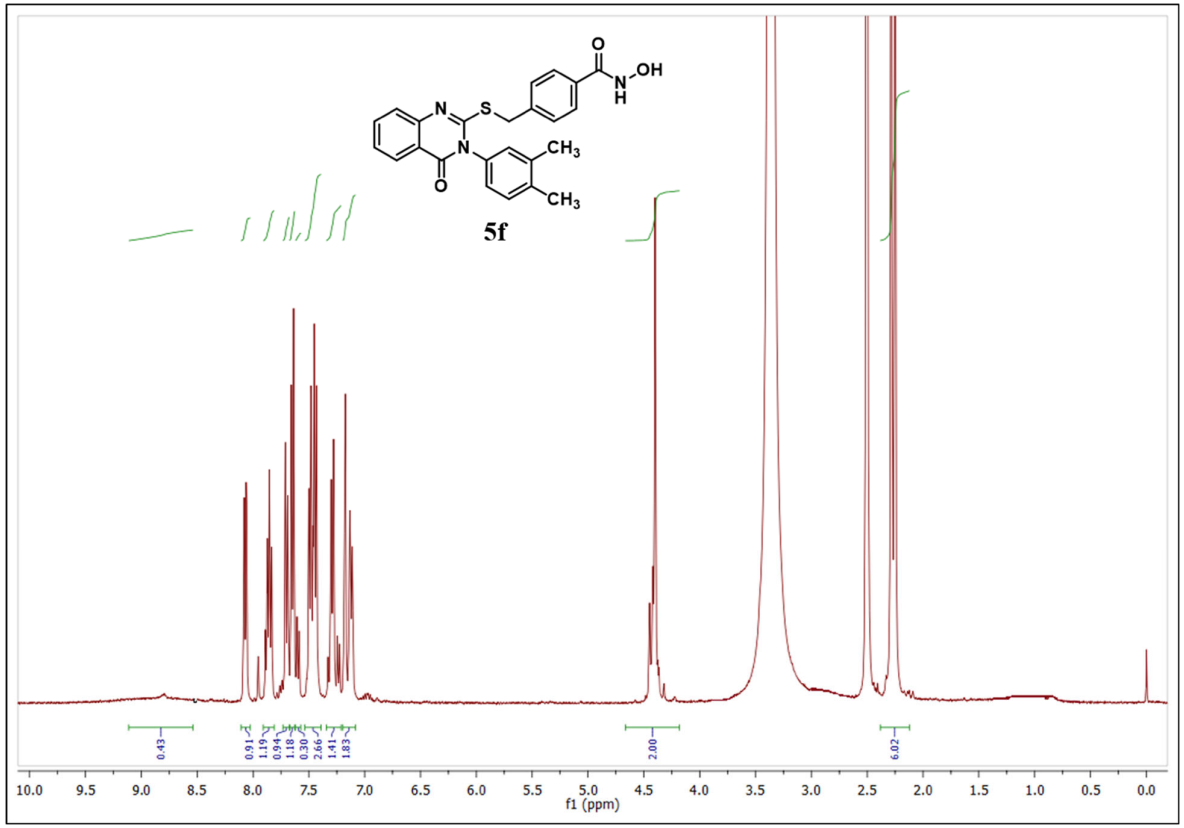
^{1}H NMR spectrum of compound **5c** (DMSO-*d*$_6$ 400 MHz)



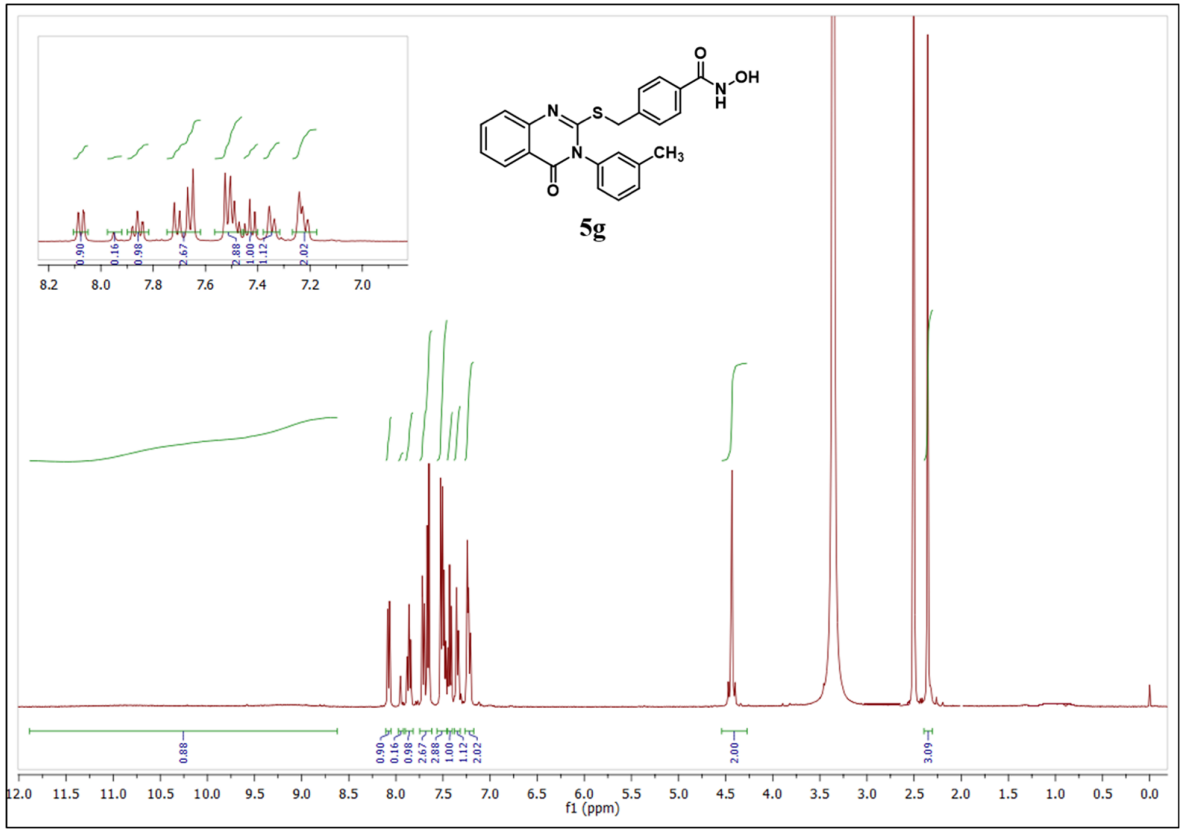
^{1}H NMR spectrum of compound **5d (**DMSO-*d*$_6$ 400 MHz)



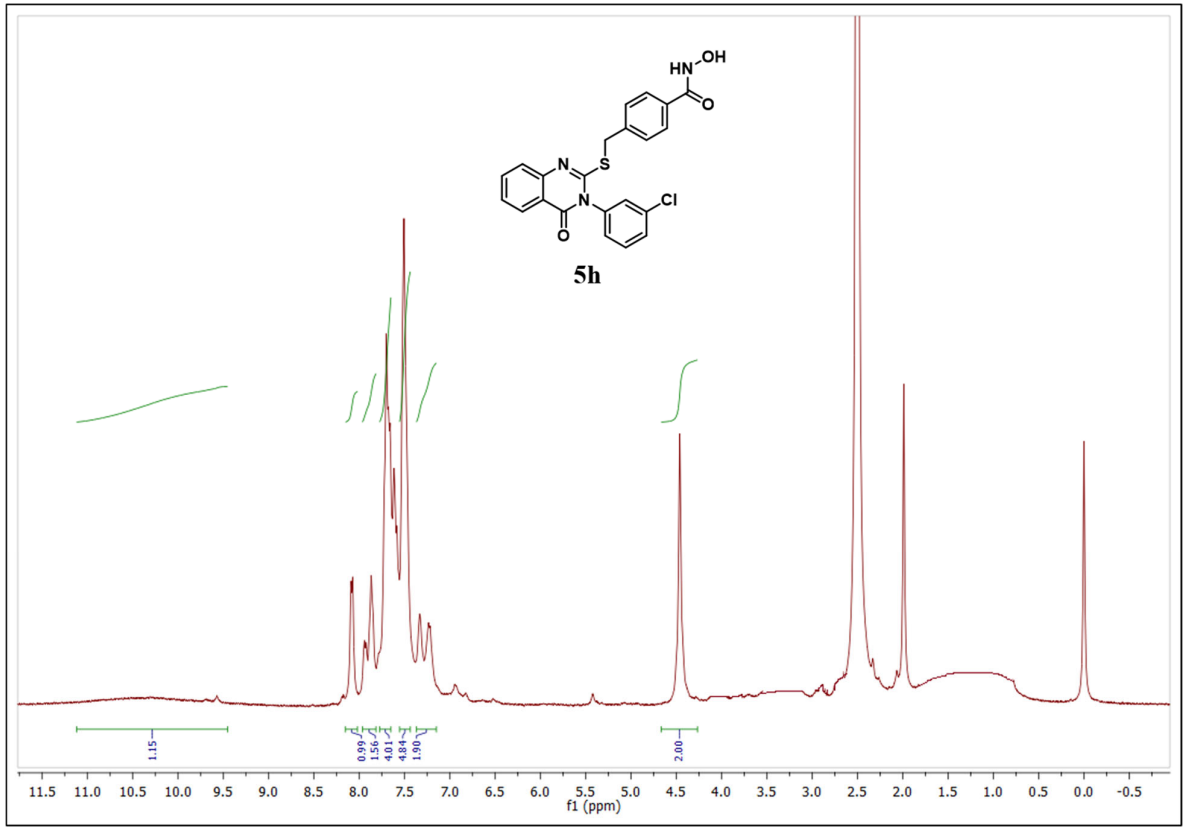
^{1}H NMR spectrum of compound **5e (**DMSO-*d*$_6$ 400 MHz)



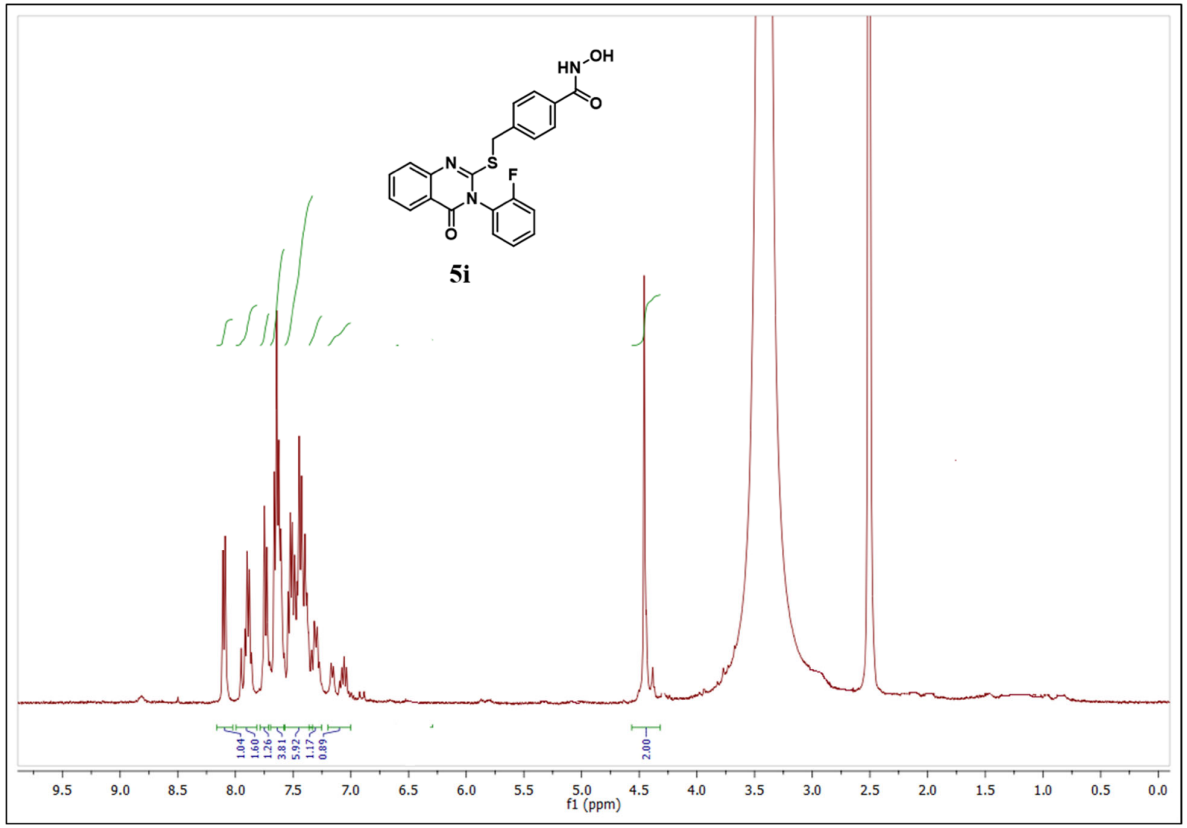
^{1}H NMR spectrum of compound **5f (**DMSO-*d*$_6$ 400 MHz)



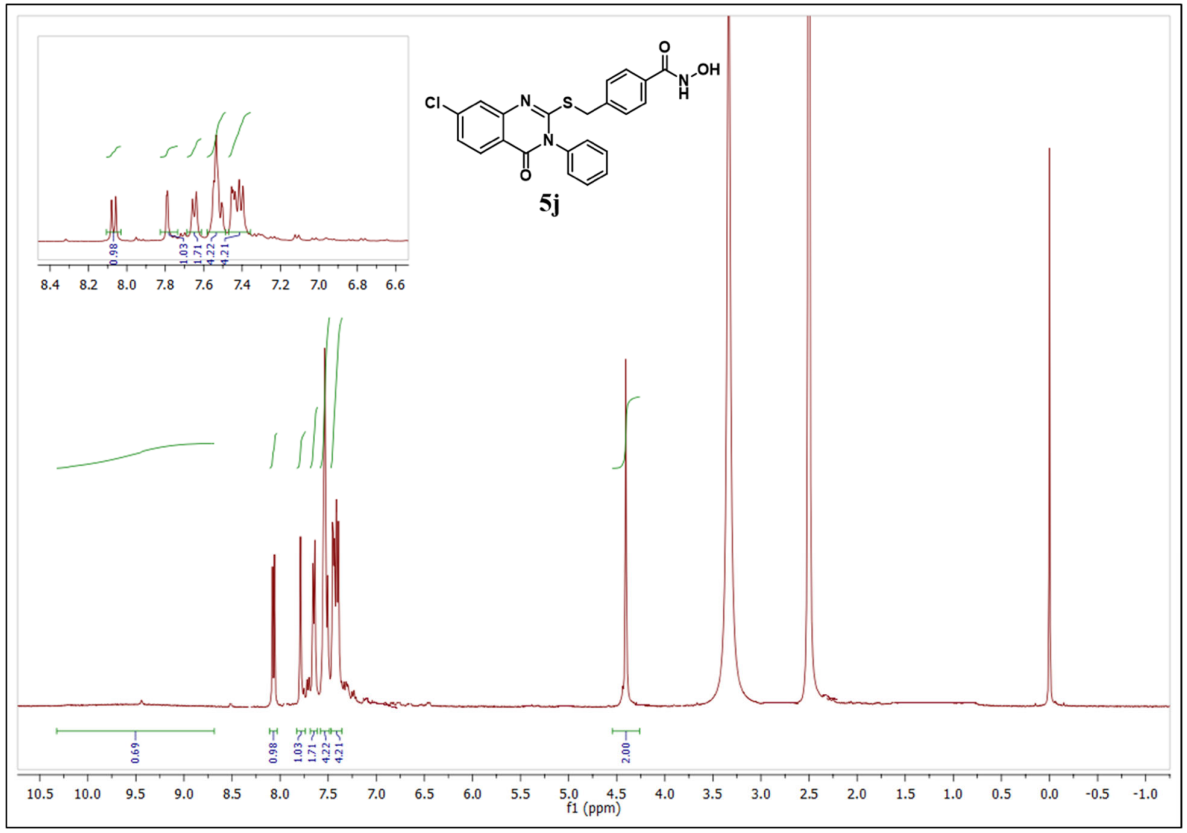
^{1}H NMR spectrum of compound **5g (**DMSO-*d*$_6$ 400 MHz)



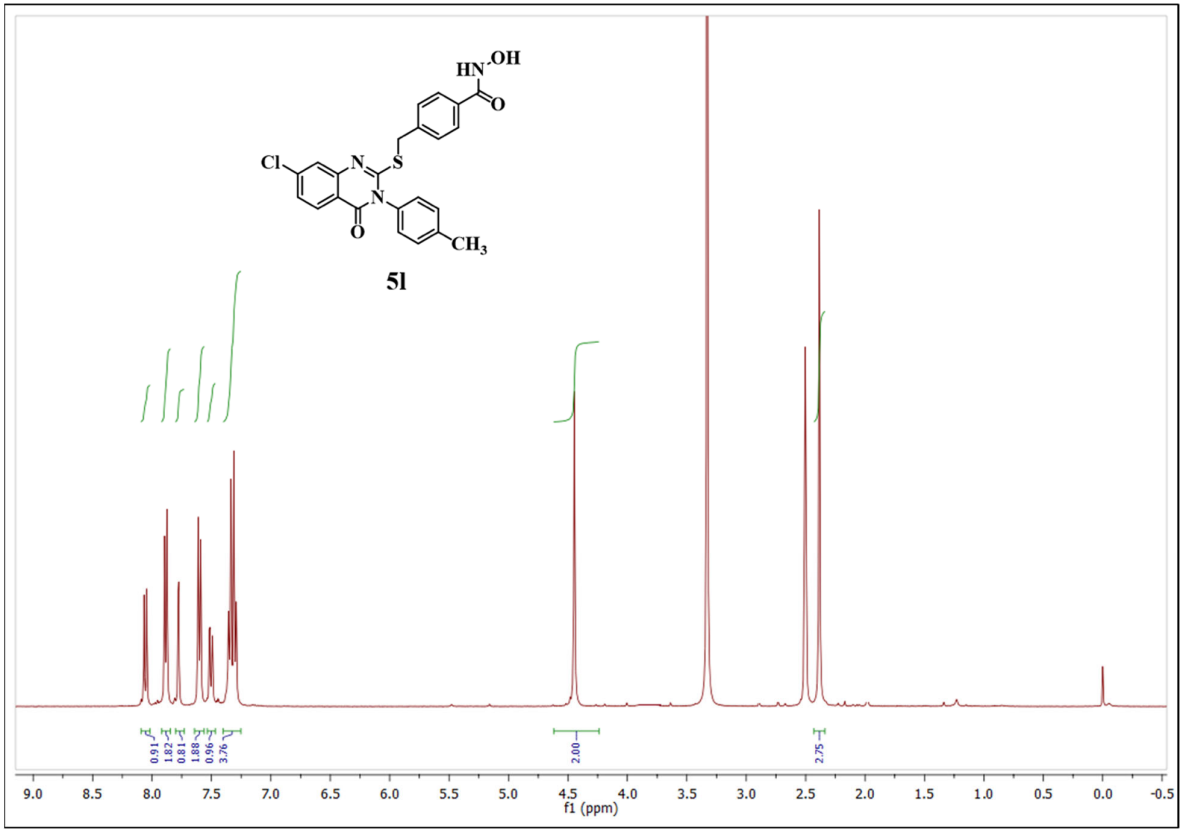
^{1}H NMR spectrum of compound **5h (**DMSO-*d*$_{6}$ 400 MHz)



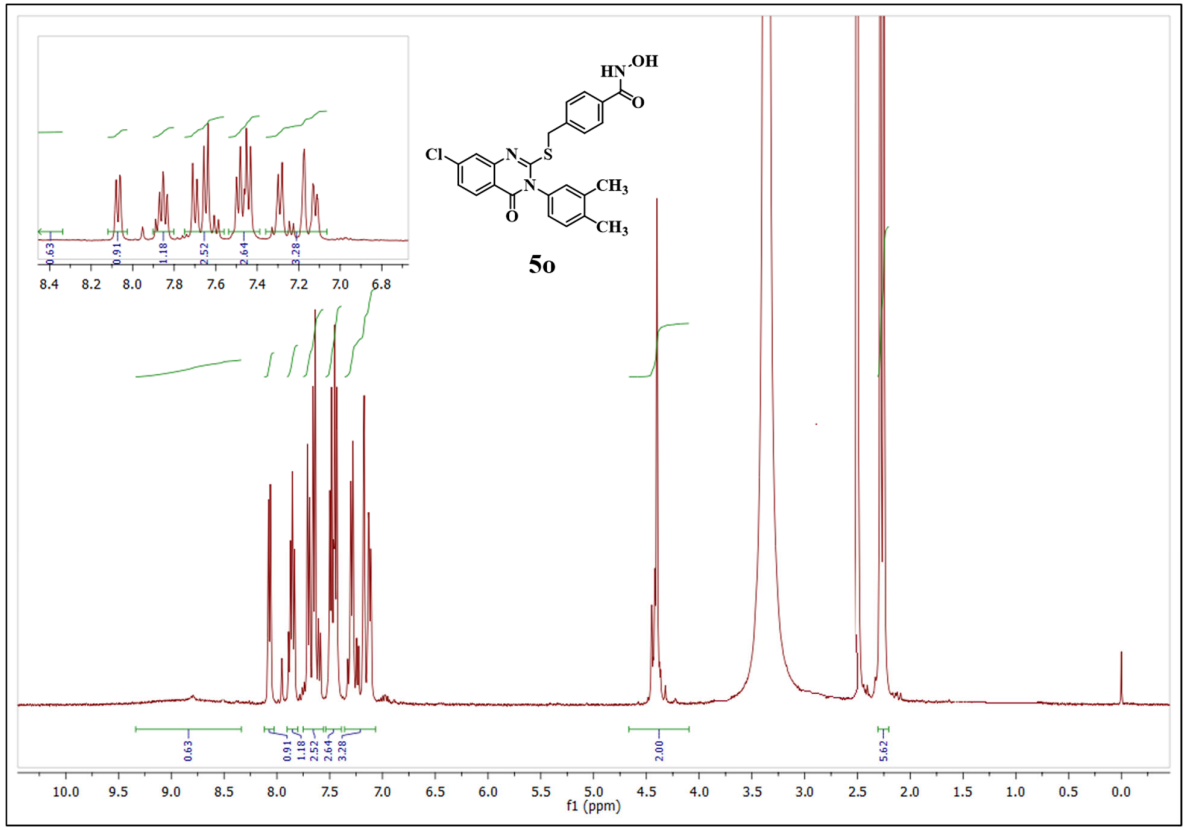
^{1}H NMR spectrum of compound **5i (**DMSO-*d*$_6$ 400 MHz)



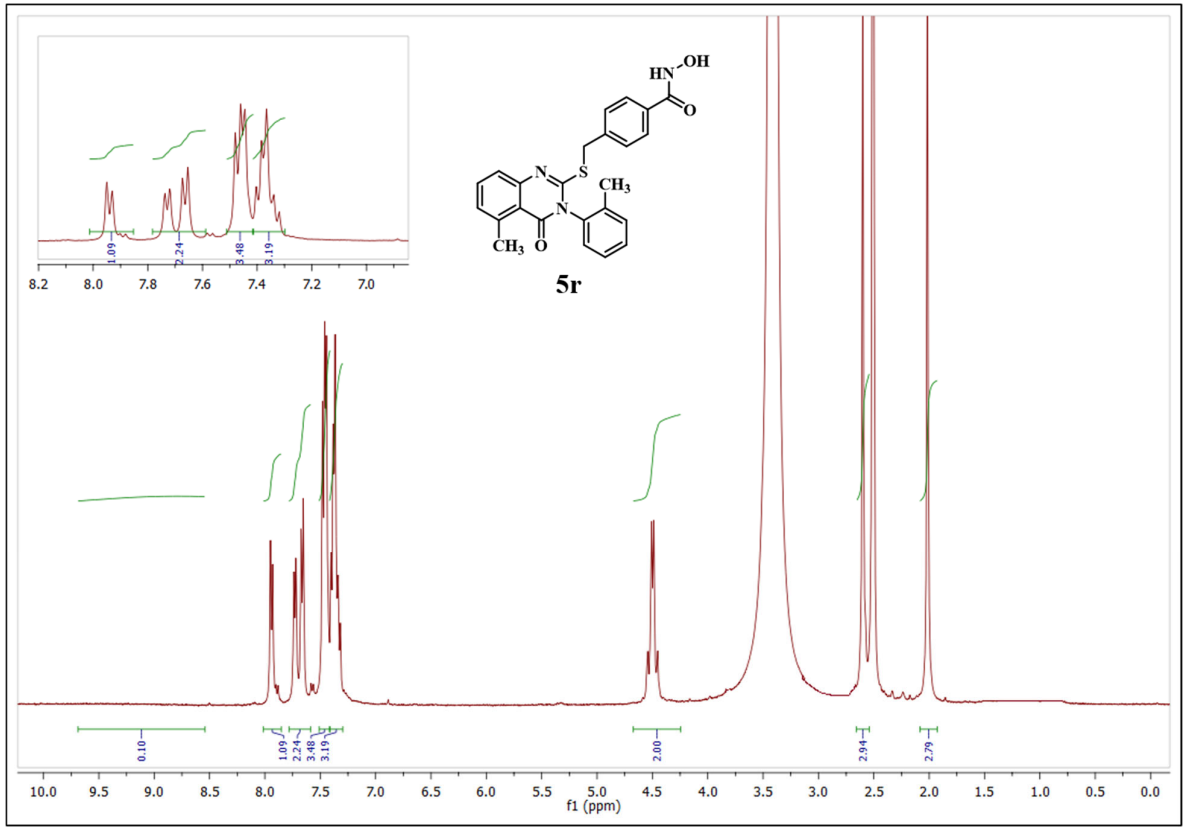
^{1}H NMR spectrum of compound **5j (**DMSO-*d*$_6$ 400 MHz)



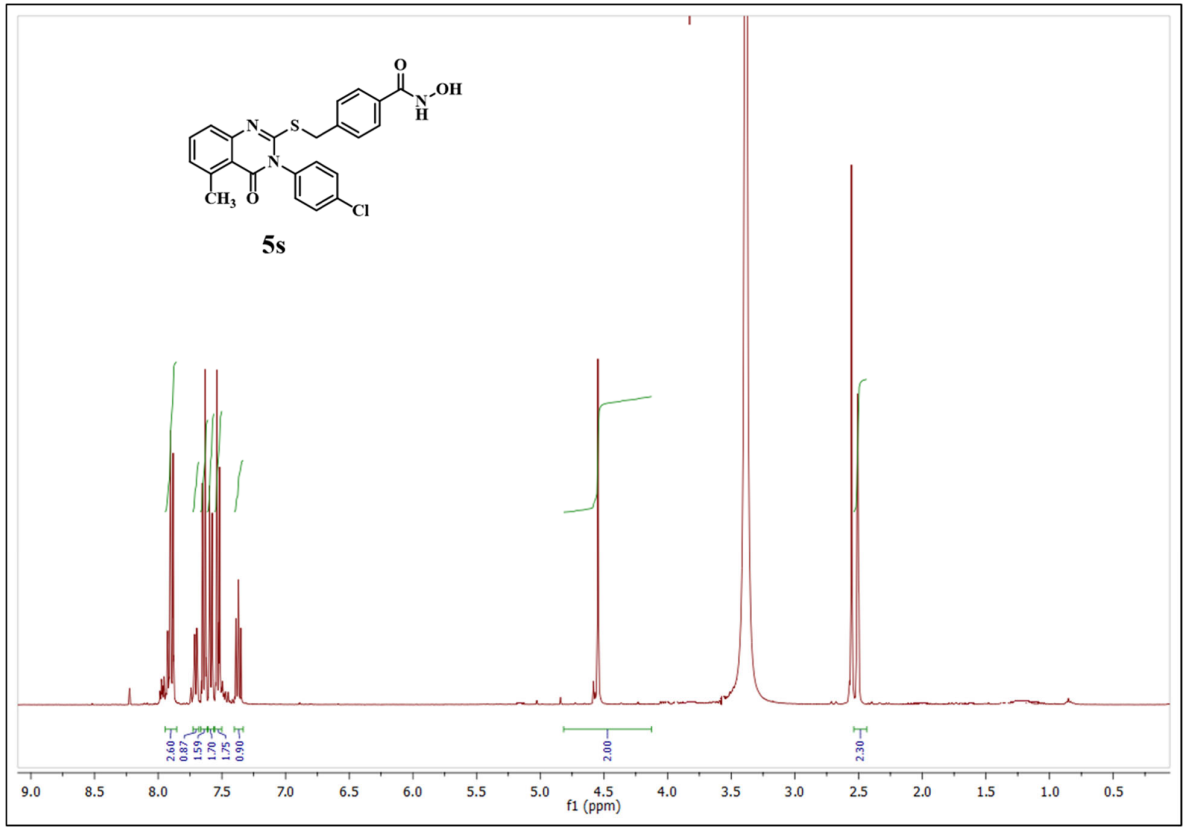
^{1}H NMR spectrum of compound **5l (**DMSO-d_6 400 MHz)



^{1}H NMR spectrum of compound **5o (**DMSO-*d*$_6$ 400 MHz)

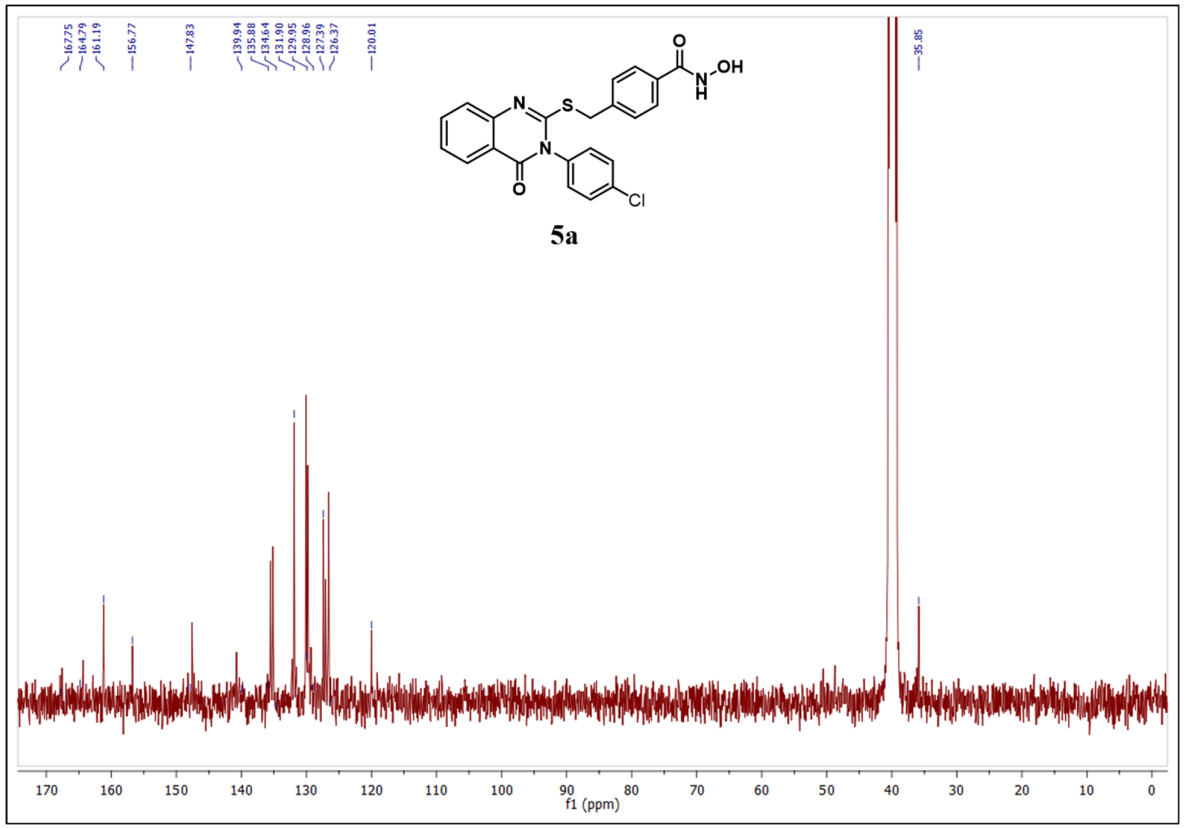


^{1}H NMR spectrum of compound **5r (**DMSO-*d*$_6$ 400 MHz)

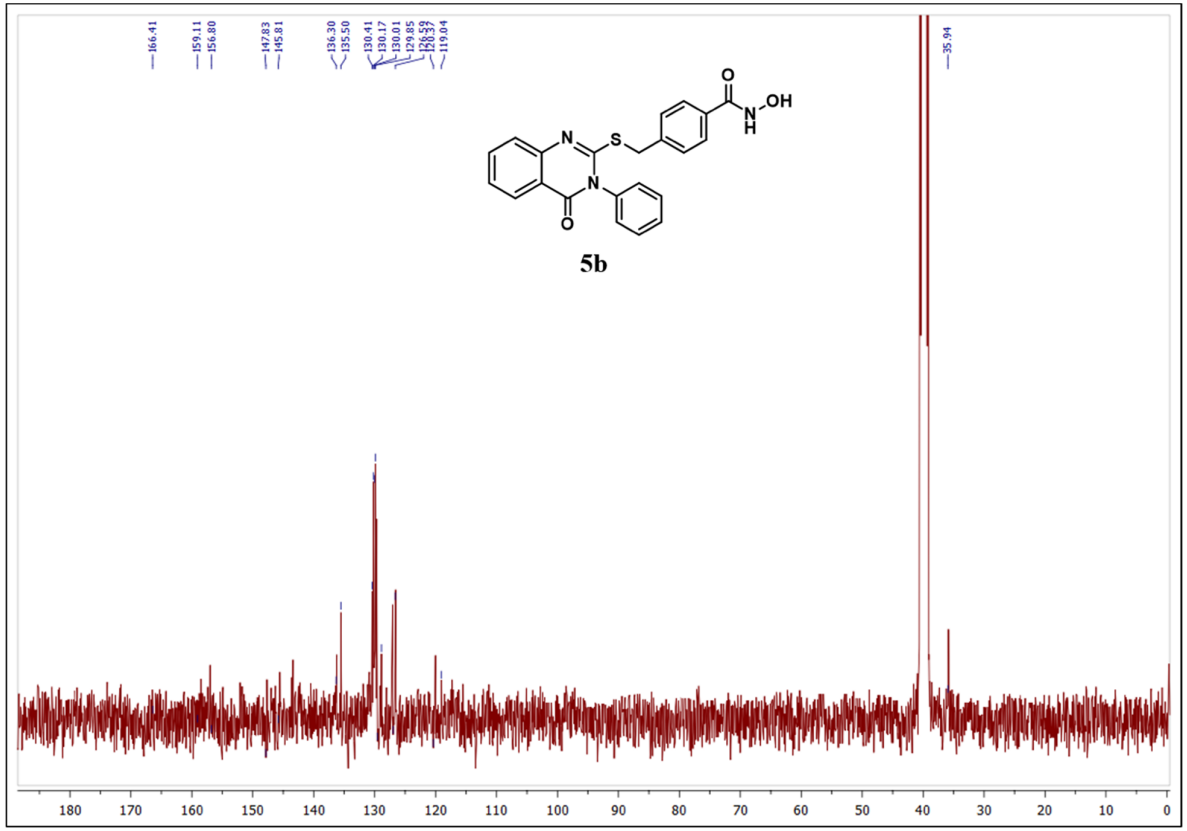


^{1}H NMR spectrum of compound **5s (**DMSO-*d*$_6$ 400 MHz)

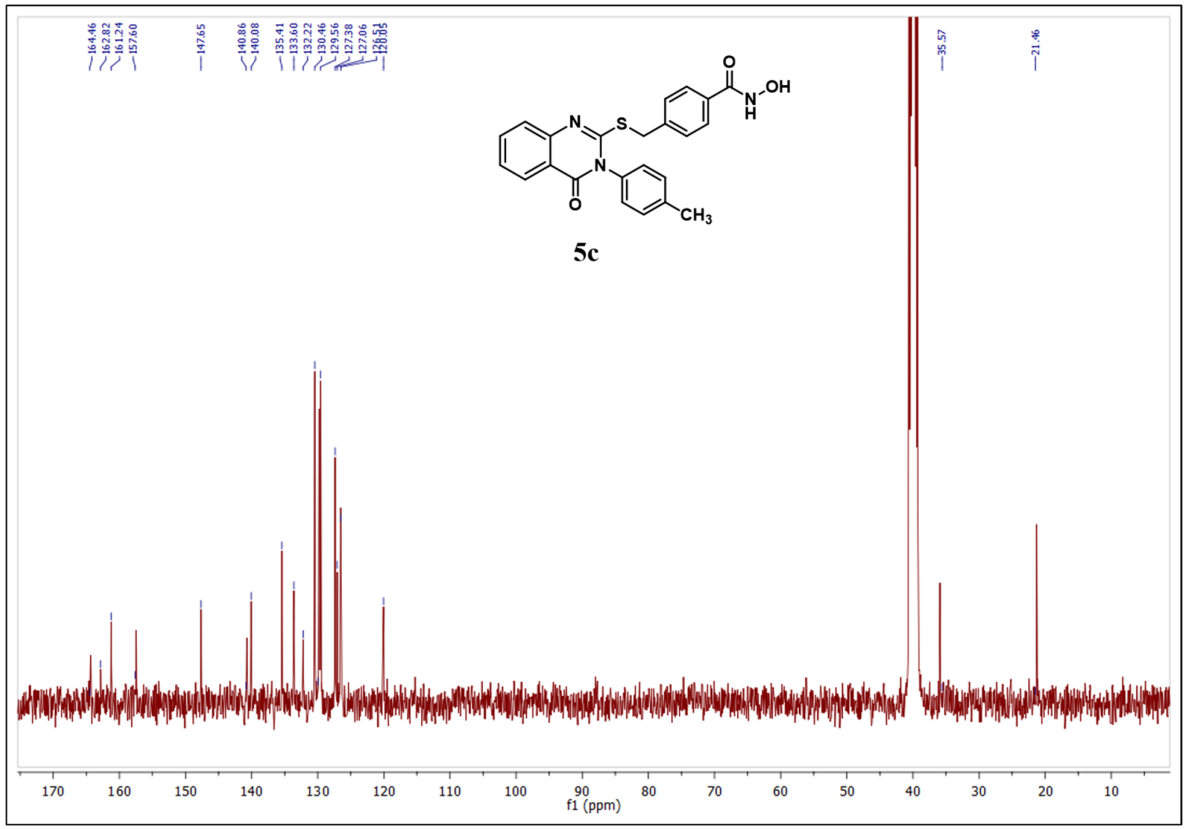
^{13}C NMR spectras of final compounds (5a-5s):



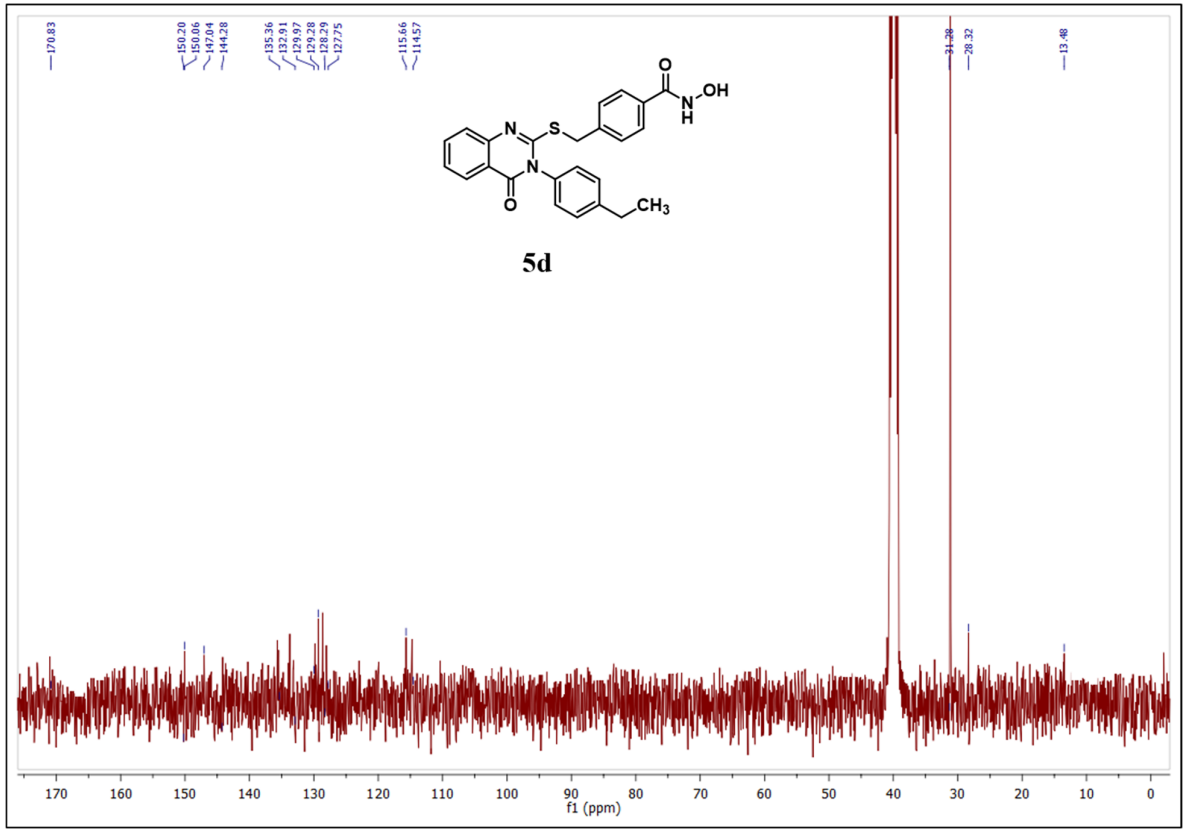
^{13}C NMR spectrum of compound **5a** (DMSO-d_6 101 MHz)



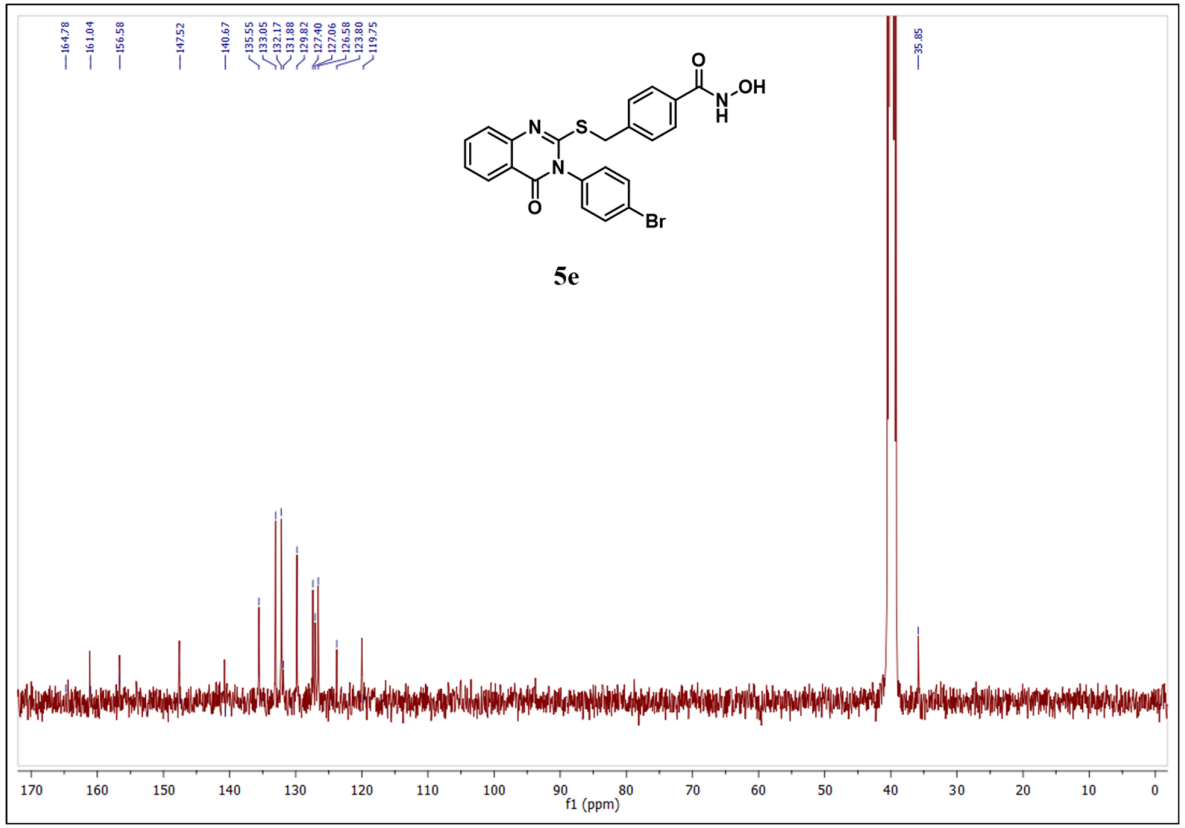
^{13}C NMR spectrum of compound **5b** (DMSO-d_6 101 MHz)



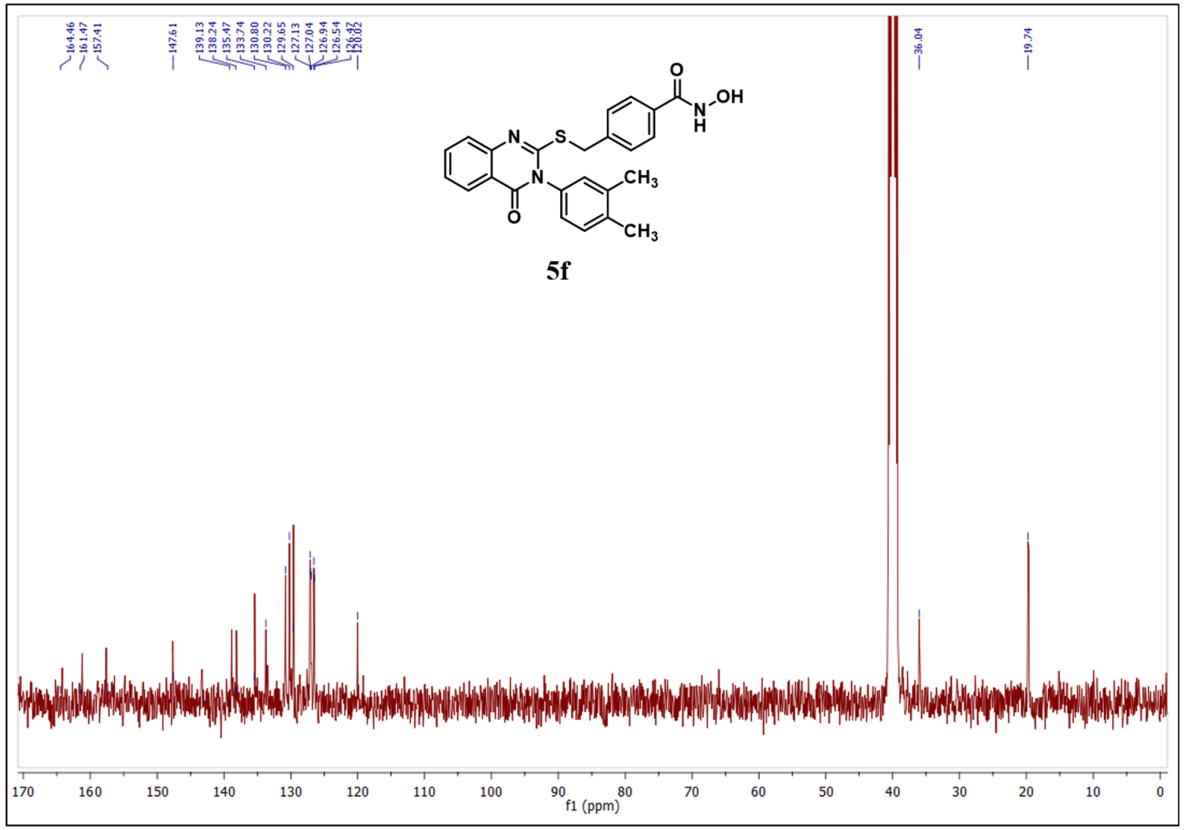
^{13}C NMR spectrum of compound **5c** (DMSO-*d*$_6$ 101 MHz)



^{13}C NMR spectrum of compound **5d** (DMSO-*d*$_6$ 101 MHz)



^{13}C NMR spectrum of compound **5e** (DMSO-*d*$_6$ 101 MHz)



^{13}C NMR spectrum of compound **5f** (DMSO-*d*$_6$ 101 MHz)