Supporting Information

Structure-based discovery of selective BRPF1 bromodomain inhibitors

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Table S1. X-ray data collection and refinement statistics for the structures of the BRPF1 bromodomain in complex with small molecules identified by virtual screening.

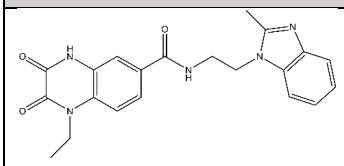
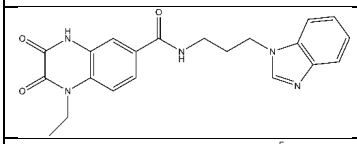
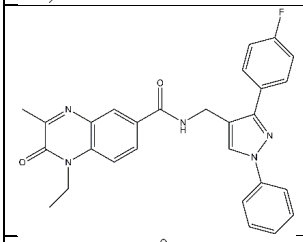
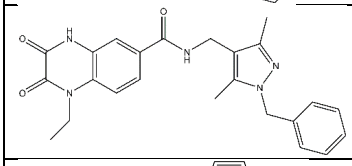
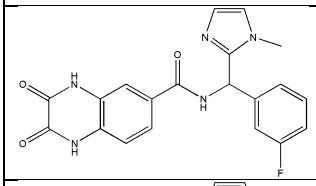
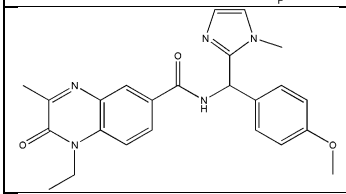
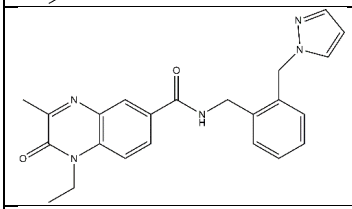
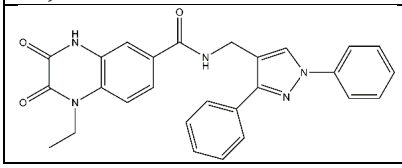
PDB ID	**5O5A**	**5O5F**	**5O55**	**5O4T**
Compound	**2**	**7**	**8**	**9**
Data Collection				
space group	$P3_221$	$P3_221$	$P3_221$	$P3_221$
Cell dimensions a, b, c (Å)	60.69, 60.69, 63.04	60.76, 60.76, 63.52	60.86, 60.86, 62.99	60.64, 60.64, 63.47
Cell dimensions α, β, γ (°)	90.00, 90.00, 120.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00
resolution (Å)	40.37 - 1.60	40.52 - 1.30	31.57 - 1.45	40.46 - 1.50
unique observations*	18144(900)	33637 (1591)	24259 (3462)	21891(1029)
completeness*	99.9 (100.0)	99.08 (96.04)	99.8 (99.0)	99.4(97.4)
redundancy*	10.6 (10.0)	7.3 (4.6)	9.4 (8.1)	13.2(12.9)
Rmerge*	0.043 (0.633)	0.022 (0.437)	0.031 (0.468)	0.090(0.642)
CC(1/2)	0.999 (0.879)	1.000 (0.802)	1.000 (0.999)	0.997(0.812)
I/σI*	25.5 (3.7)	37.3 (3.1)	31.2 (4.5)	16.9(3.0)
Refinement				
R_{work}/R_{free}*	0.189(0.229)/0.194(0.292)	0.199(0.233)/0.225(0.252)	0.196(0.208)/0.205(0.268)	0.182(0.247)/0.212(0.271)
r.m.s deviations bond (Å)	0.008	0.006	0.007	0.005
r.m.s deviations angles (°)	0.879	0.666	0.765	0.723
B-factors(P/L/O) (Å^2) **	37.5/41.9/45.5	20.7/27.0/34.1	30.6/32.0/41.7	28.9/35.3/39.5
Ramanchandran Favored	98.25	100.00	99.12	99.10
Ramanchandran Allowed	1.75	0.00	0.88	0.90
Ramanchandran Disallowed	0.00	0.00	0.00	0.00

PDB ID	**5OV8**	**5MWG**	**5MWH**	**5O4S**
Compound	**13**	**16**	**21**	**26**
Data Collection				
space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Cell dimensions a, b, c (Å)	48.19, 56.54, 48.60	48.60, 61.14, 48.60	60.69, 60.69, 63.04	48.61, 62.69, 48.82
Cell dimensions α, β, γ (°)	90.00, 102.41, 90.00	90.00, 101.63, 90.00	90.00, 90.00, 120.00	90.00, 101.81, 90.00
resolution (Å)	47.46 - 1.80	37.56 – 1.50	38.19 - 1.65	38.00 - 1.75
unique observations*	23018(1295)	42415(1960)	34911(1685)	29072(1578)
completeness*	97.1(93.1)	95.0(89.2)	99.5 (96.7)	99.9(99.4)
redundancy*	3.9(3.9)	6.8(3.5)	13.3(9.1)	13.2(12.9)
Rmerge*	0.040(0.220)	0.209(0.253)	0.135(0.816)	0.151(0.730)
CC(1/2)	0.998(0.946)	0.990(0.914)	0.998(0.864)	0.985(0.961)
I/σI*	18.7(4.3)	12.5(5.2)	21.6 (2.1)	22.6(4.1)
Refinement				
R_{work}/R_{free}*	0.196(0.230)/0.243(0.315)	0.224(0.256)/0.243(0.320)	0.186(0.295)/0.221(0.305)	0.178(0.254)/0.220(0.297)
r.m.s deviations bond (Å)	0.006	0.006	0.006	0.006
r.m.s deviations angles (°)	0.804	0.887	0.773	0.722
B-factors(P/L/O) (Å^2) **	26.9/29.7/36.7	24.9/28.0/35.0	37.7/40.2/45.8	35.8/38.0/42.8
Ramanchandran Favored	99.08	99.54	98.62	100.00
Ramanchandran Allowed	0.92	0.46	1.38	0.00
Ramanchandran Disallowed	0.00	0.00	0.00	0.00

PDB ID	**5MWZ**	**5OWA**	**6EKQ**	**5O5H**
Ligand	**36**	**42**	**42**	**43**
Data Collection				
space group	$P3_221$	$P2_1$	C2	$P3_221$
Cell dimensions a, b, c (Å)	60.86, 60.86, 62.99	34.89, 92.30, 81.22	71.21, 57.78, 70.45	61.03, 61.03, 63.79
Cell dimensions α, β, γ (°)	90.00, 90.00, 120.00	90.00, 101.22, 90.00	90.00, 108.66, 90.00	90.00, 90.00, 120.00
resolution (Å)	40.46 - 1.25	39.93 - 1.95	43.89 – 1.65	40.70 – 1.85
unique observations*	37830(1871)	34898(5200)	32602(1588)	12134(740)
completeness*	99.9(99.9)	94.9(96.5)	99.7(100.0)	100.0(100.0)
redundancy*	18.4(16.8)	3.3(3.4)	4.6(4.2)	18.6(19.2)
Rmerge*	0.056(0.452)	0.083(0.469)	0.046(0.345)	0.041(0.640)
CC(1/2)	0.999(0.957)	0.991(0.762)	0.999(0.879)	1.000(0.955)
I/σI*	31.4(7.9)	12.0(2.6)	18.0(3.4)	43.9(5.4)
Refinement				
R_{work}/R_{free}*	0.163(0.165)/0.180(0.186)	0.219(0.309)/0.267(0.393)	0.156(0.188)/0.181(0.226)	0.190(0.409)/0.234(0.460)
r.m.s deviations bond (Å)	0.004	0.007	0.009	0.008
r.m.s deviations angles (°)	0.768	1.205	1.003	1.098
B-factors(P/L/O) (Å^2) **	15.4/22.5/29.4	40.4/44.7/42.4	20.2/18.9/35.3	35.6/51.7/43.4
Ramanchandran Favored	99.11	98.86	100.00	100
Ramanchandran Allowed	0.89	0.92	0.00	0
Ramanchandran Disallowed	0.00	0.23	0.00	0

* Statistics for the highest resolution shell is shown in parentheses.
* * P/L/O indicate protein, ligand in the active site and solvent molecules, respectively.

Table S2. 2D structures of the 1-ethyl-2,3-dioxo-4H-quinoxaline and 1-ethyl-3-methyl-2-oxoquinoxaline derivatives that did not show binding at the highest concentration tested.

2D Structure	AlphaScreen (IC50, uM) on BRPF1
	>10
	>10
	>10
	>100
	>50
	>10
	>10
	>100

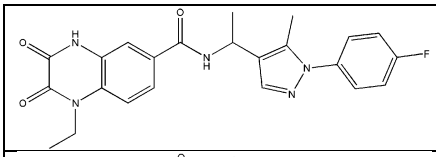
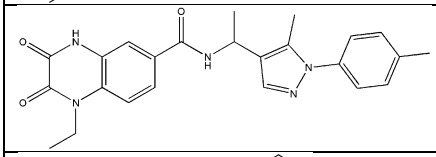
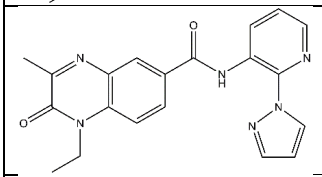
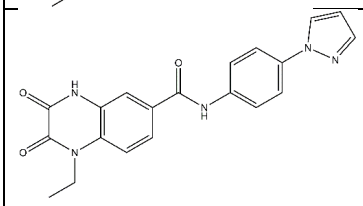
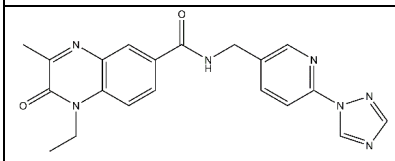
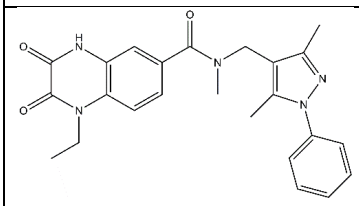
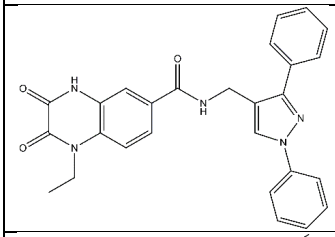
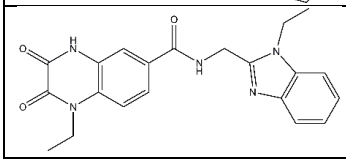
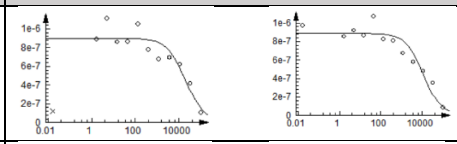
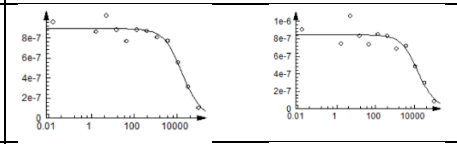
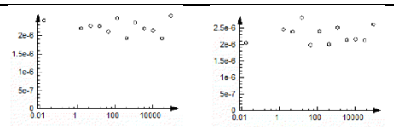
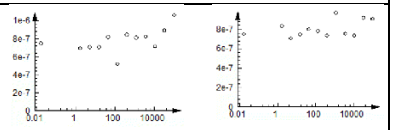
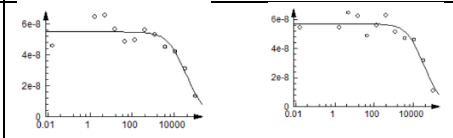
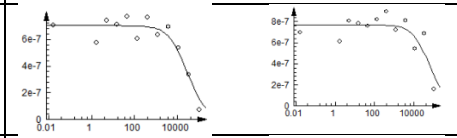
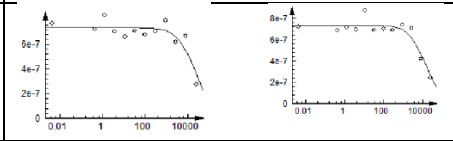
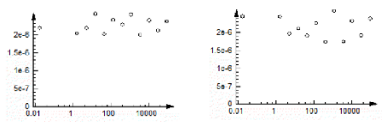
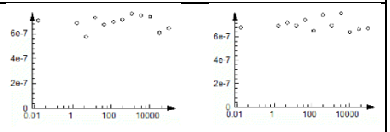
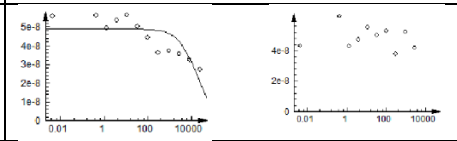
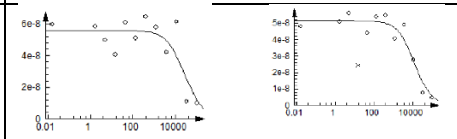
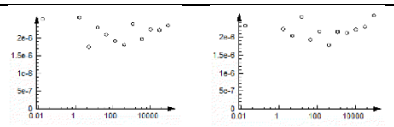
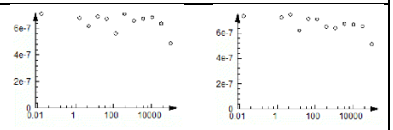
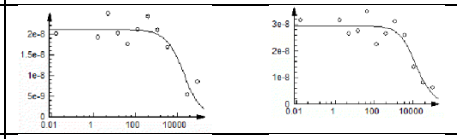
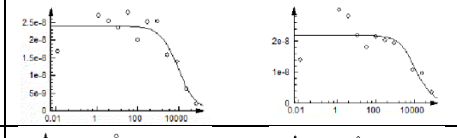
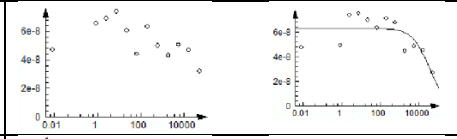
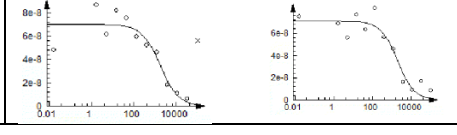
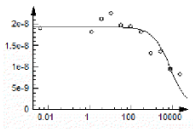
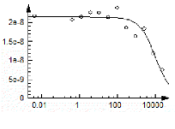
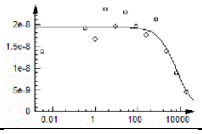
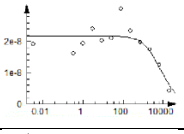
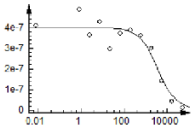
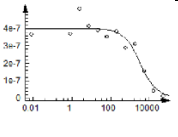
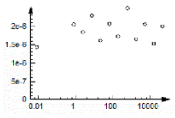
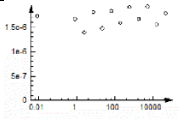
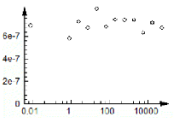
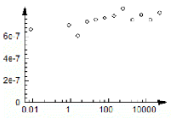
	>25
	>100
	>10
	>10
	>50
	>100
	>50
	>10

Table S3. BromoScan assay results for selected compounds on BRPF1, TRIM24 and BRD4(1) bromodomains. The assays were performed in duplicate.

Cpd	BRPF1	TRIM24	BRD4(1)
1			
2			
3			
4			
5			
6			
8			
13			
14			
20			
21			

28				
36				
42			 	 

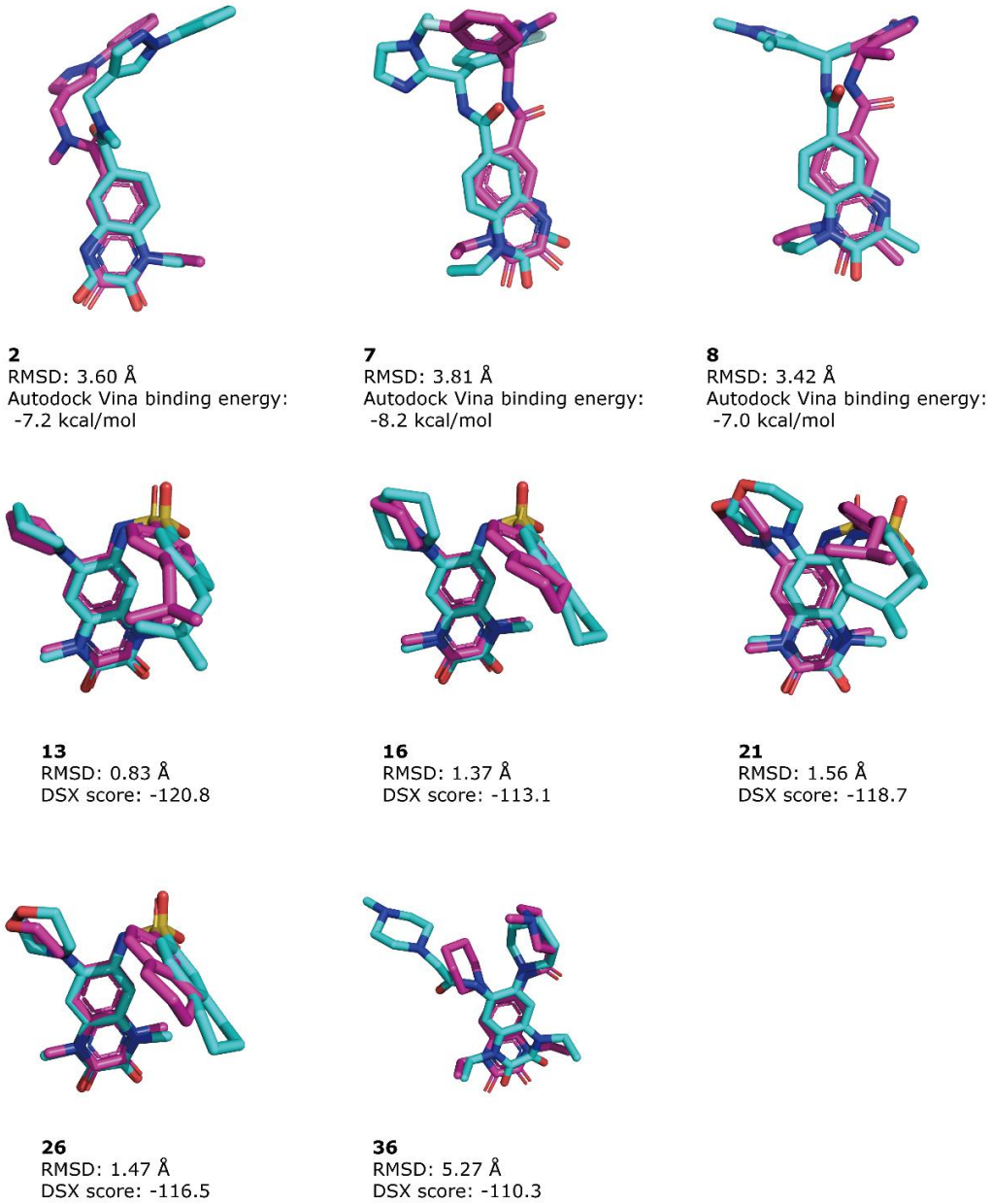


Fig. S1. Superimposition of the pose predicted by docking (cyan) and the corresponding binding mode in the crystal structures (magenta).

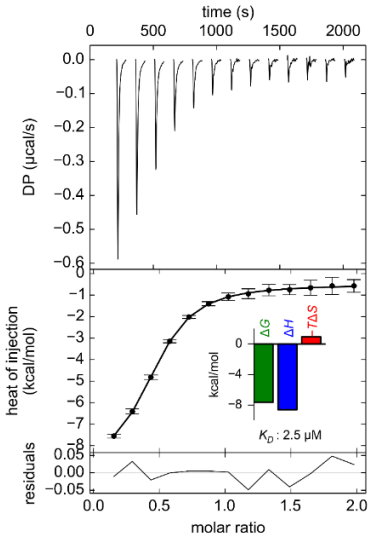
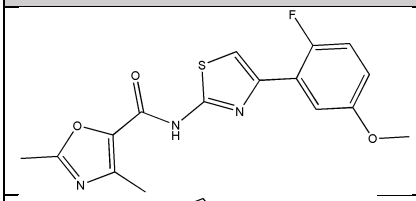
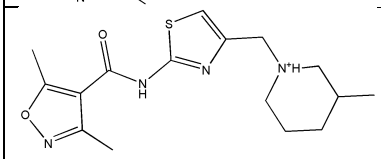
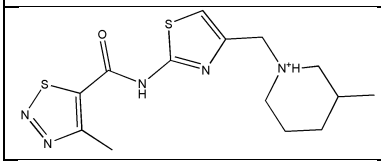
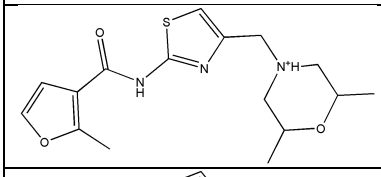
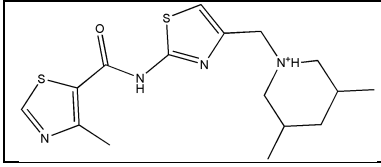
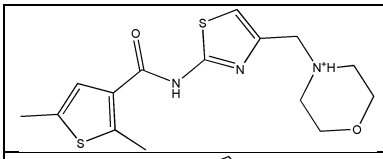
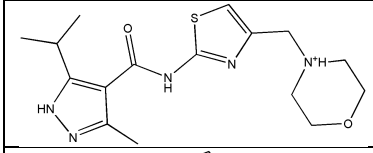
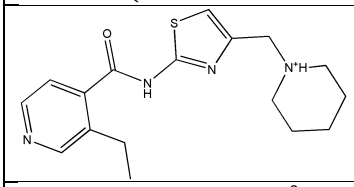
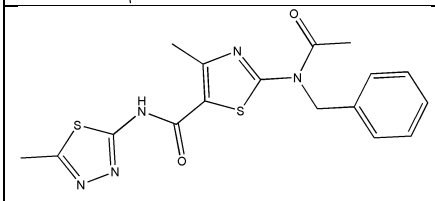
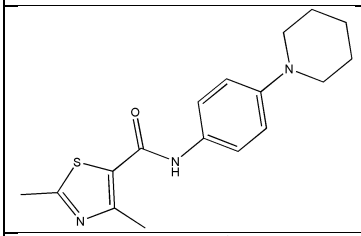
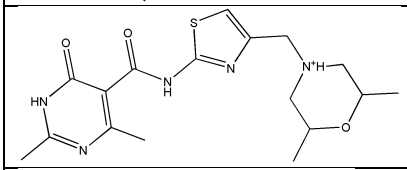
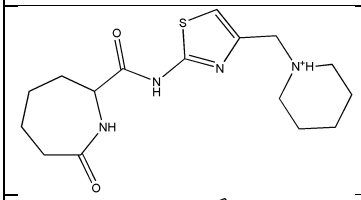
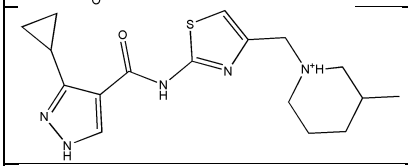
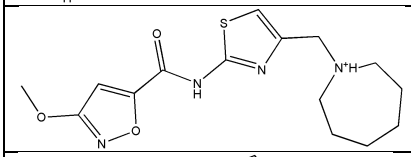
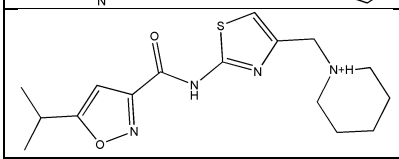


Fig. S2. Thermodynamic characterization of the BRPF1-**26** interaction by ITC. The Fig. shows thermographs (top), fit of integrated data (middle), and fit residuals (bottom).

Table S4. Analogues of compound **42** and their binding affinity measured by AlphaScreen.

2D Structure	Tanimoto coefficient with 42	AlphaScreen (IC50, uM)
	0.59	>200
	0.57	195
	0.58	240
	0.53	95.0 %Ctrl @100 uM
	0.62	95.1 %Ctrl @100 uM

	0.49	293
	0.51	93.9 %Ctrl @100 uM
	0.55	91.9 %Ctrl @100 uM
	0.43	82.1 %Ctrl @100 uM
	0.33	37.0 %Ctrl @100 uM
	0.421	>500
	0.441	>500
	0.533	515
	0.544	>200
	0.543	218

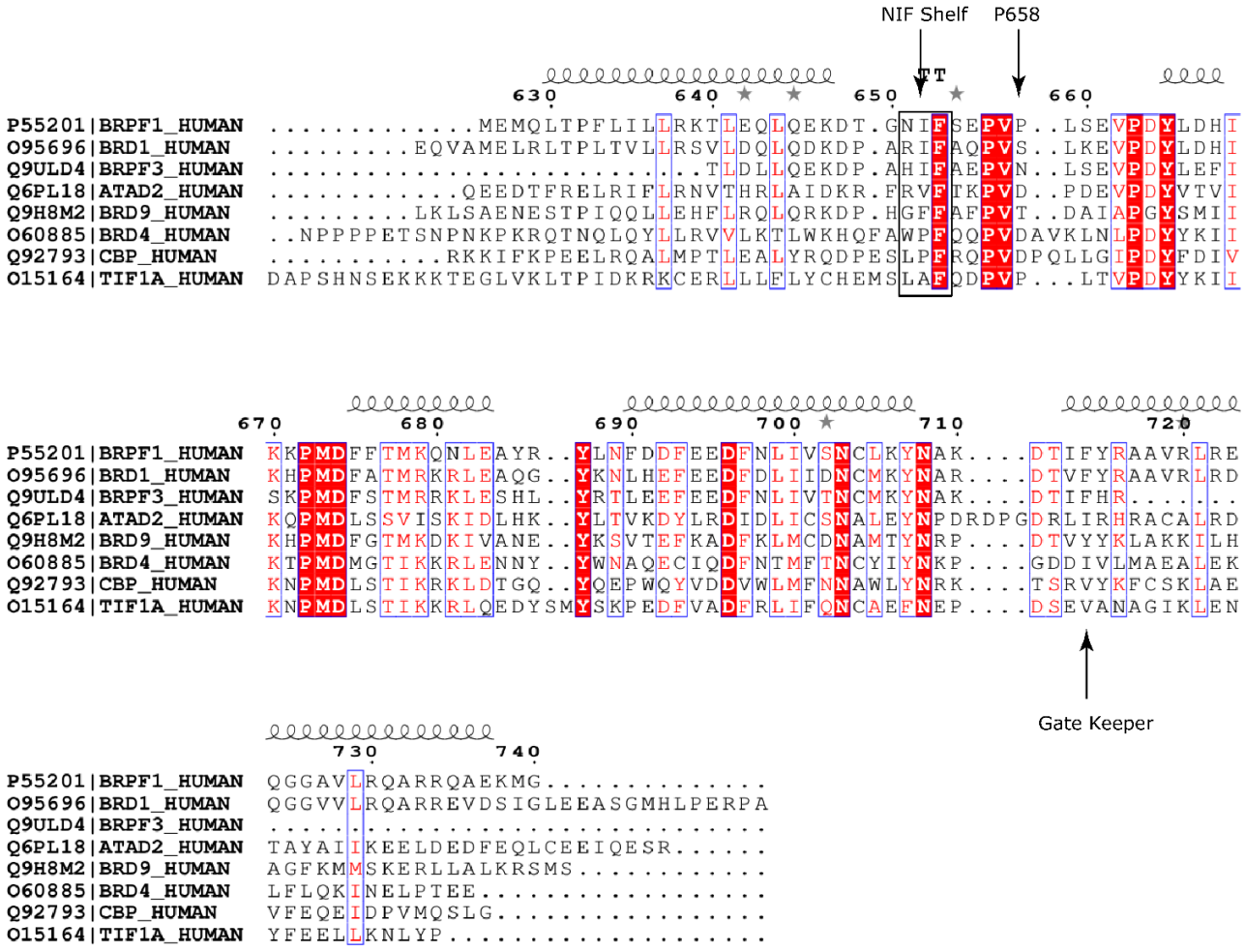


Fig. S3. Structure-based sequence alignment of the BRPF1 bromodomain with bromodomains of BRPF2 (BRD1), BRPF3, ATAD2, BRD9, BRD4(1), CREBBP (CBP) and TRIM24 (TIF1A). The sequence alignment was obtained with ESPript.[1]

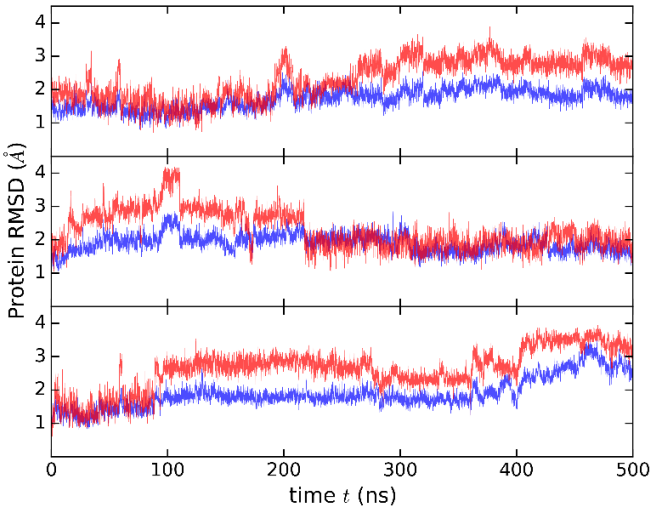


Fig. S4. Time series of RMSD from the X-ray structure along the three MD simulations of the BRPF1/**21** complex. RMSD time series for all Cα atoms (blue) and Cα atoms in the ZA loop segment 648-668(red) are shown.

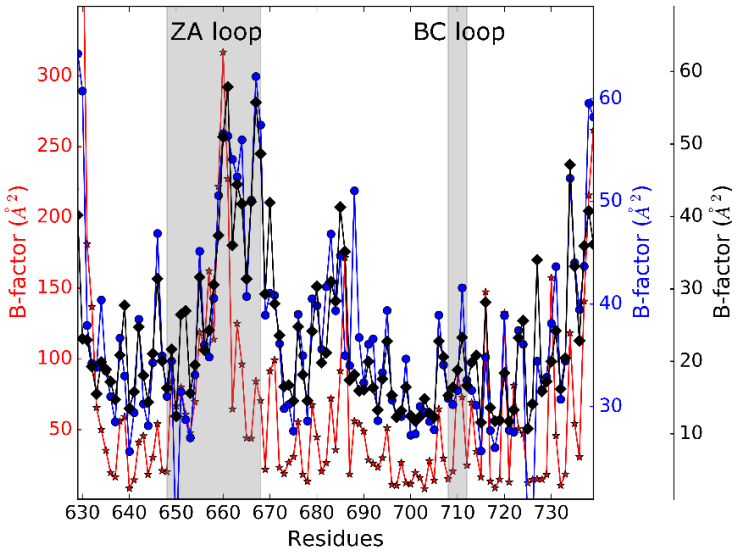
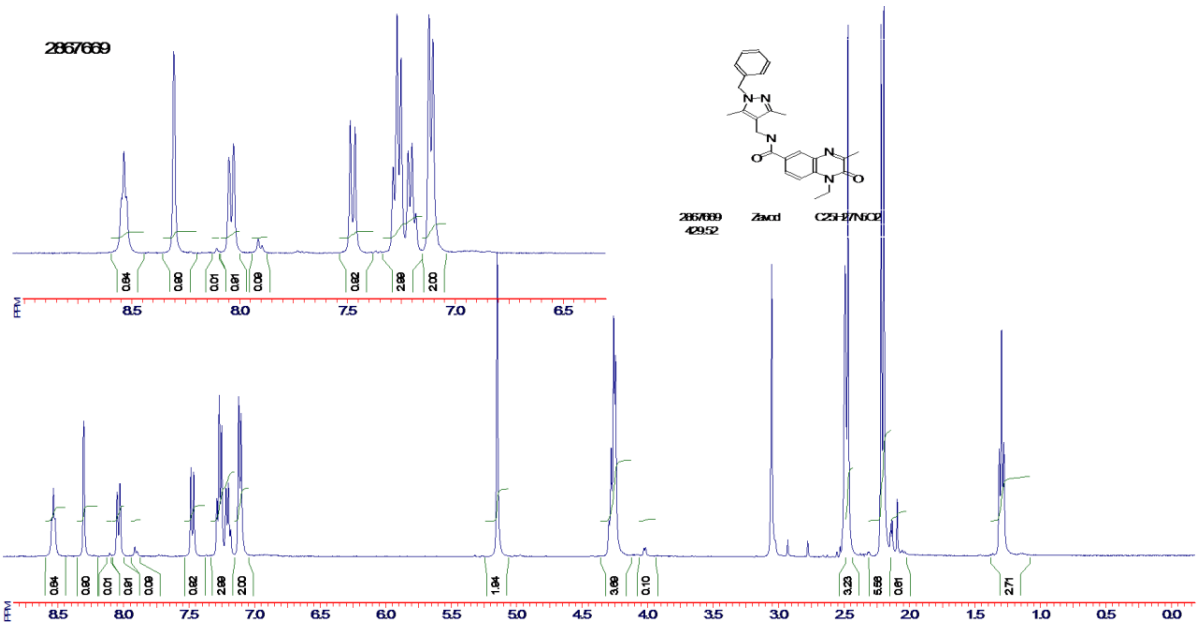


Fig. S5. Comparison of the B-factors calculated from the fluctuations of the atoms along the MD simulations of the BRPF1/**21** complex (red) and B-factors in X-ray crystals. The experimental B-factors are those of the crystal structure of the BRPF1/**21** complex (blue) and apo BRPF1 (PDB code 4LC2) (black). The ZA loop region (residues 648-668) and BC loop region (residues 708-712) are highlighted (grey vertical stripes). The function *gmx rmsf* in GROMACS was used to extract B-factors from the MD simulations.[2] B-factors were averaged over all non-hydrogen atoms for each residue.

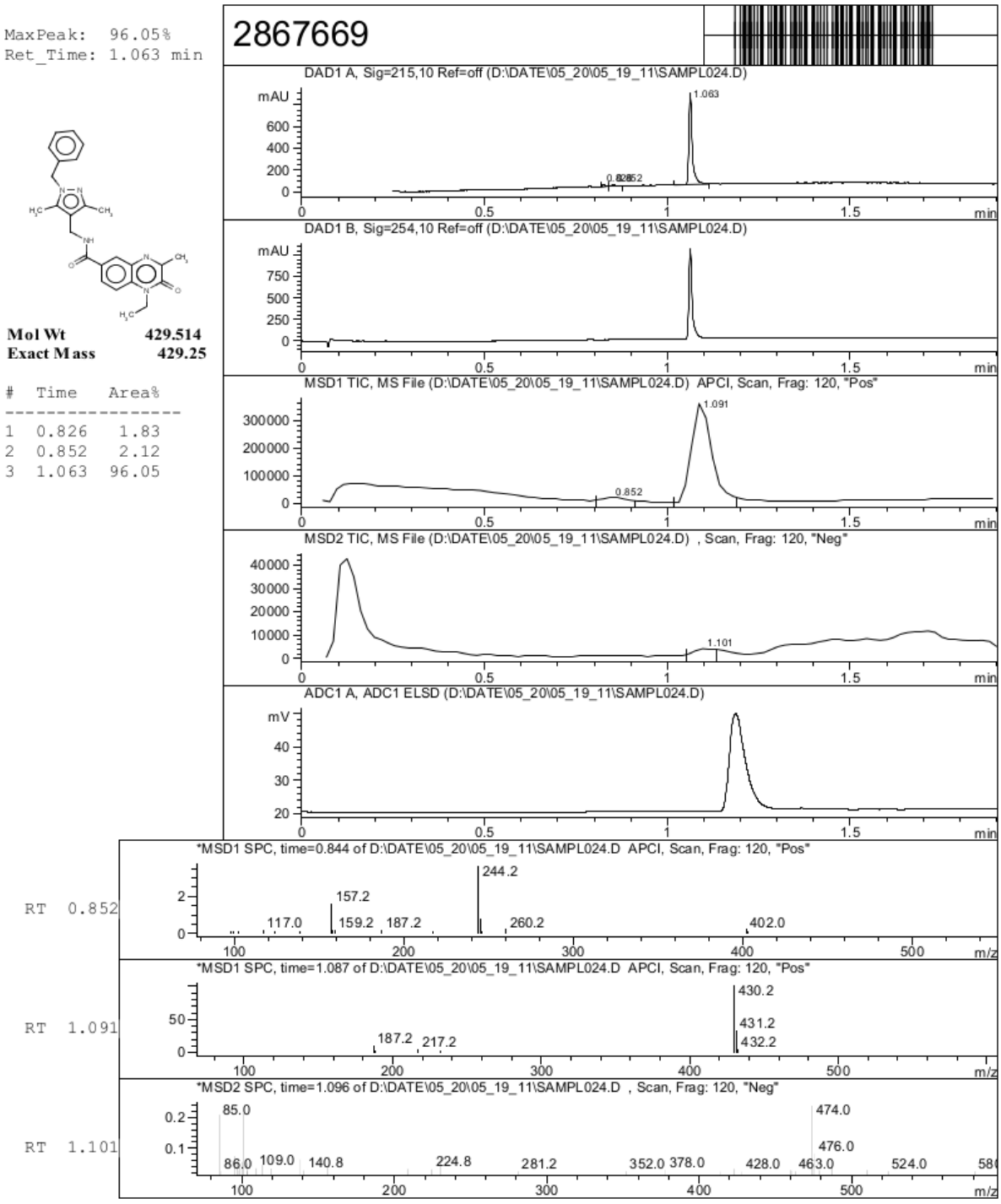
References:

[1] X. Robert, P. Gouet, Deciphering key features in protein structures with the new ENDscript server, Nucleic Acids Res. 42 (2014) W320-324.
[2] S. Pronk, S. Pall, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M.R. Shirts, J.C. Smith, P.M. Kasson, D. van der Spoel, B. Hess, E. Lindahl, GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit, Bioinformatics 29 (2013) 845-854.

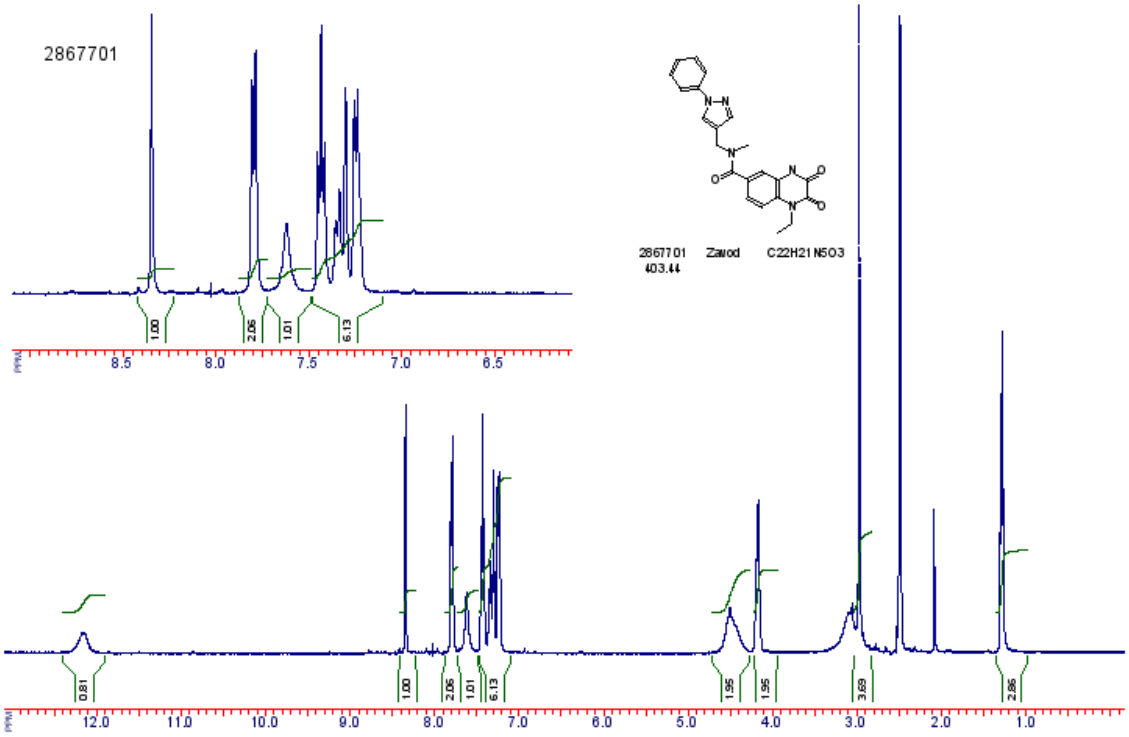
Proton NMR spectra and/or HPLC-MS analysis of compounds **1- 43** (except **9**).



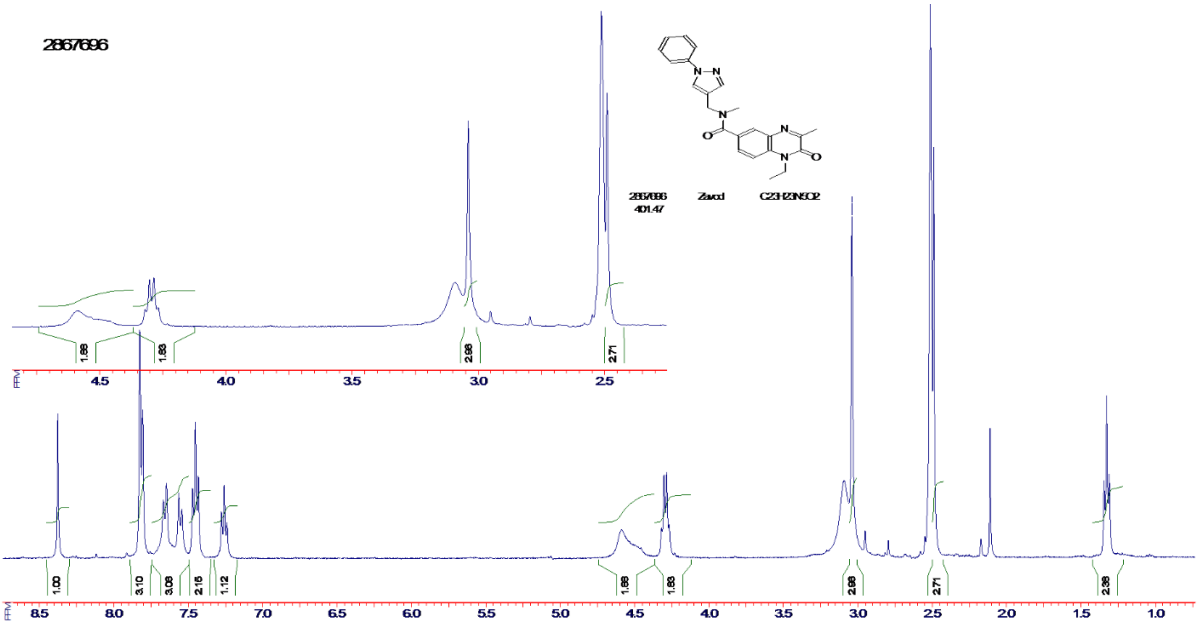
^{1}H-NMR spectra of compound **1**



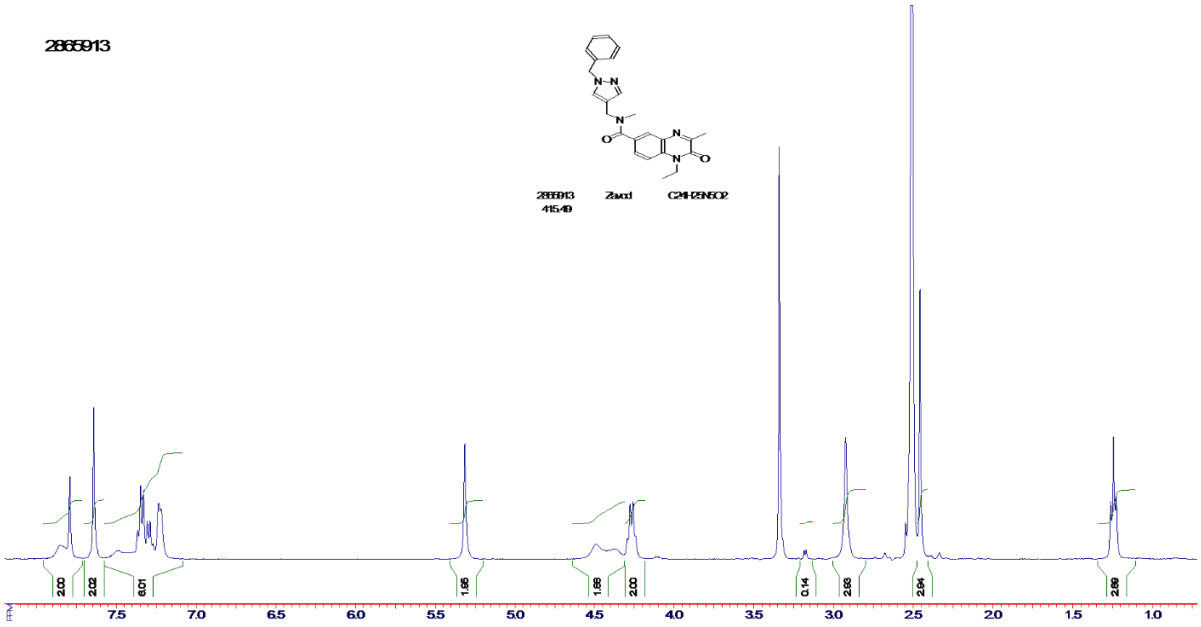
HPLC chromatogram and mass spectra of compound **1**



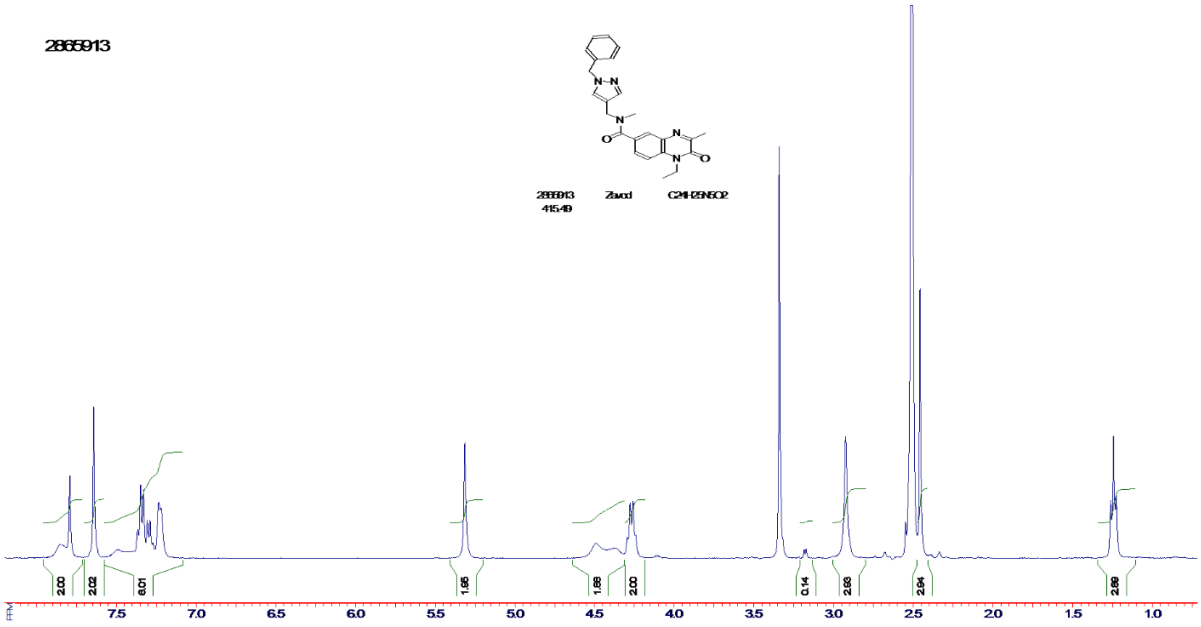
^{1}H-NMR spectra of compound **2**



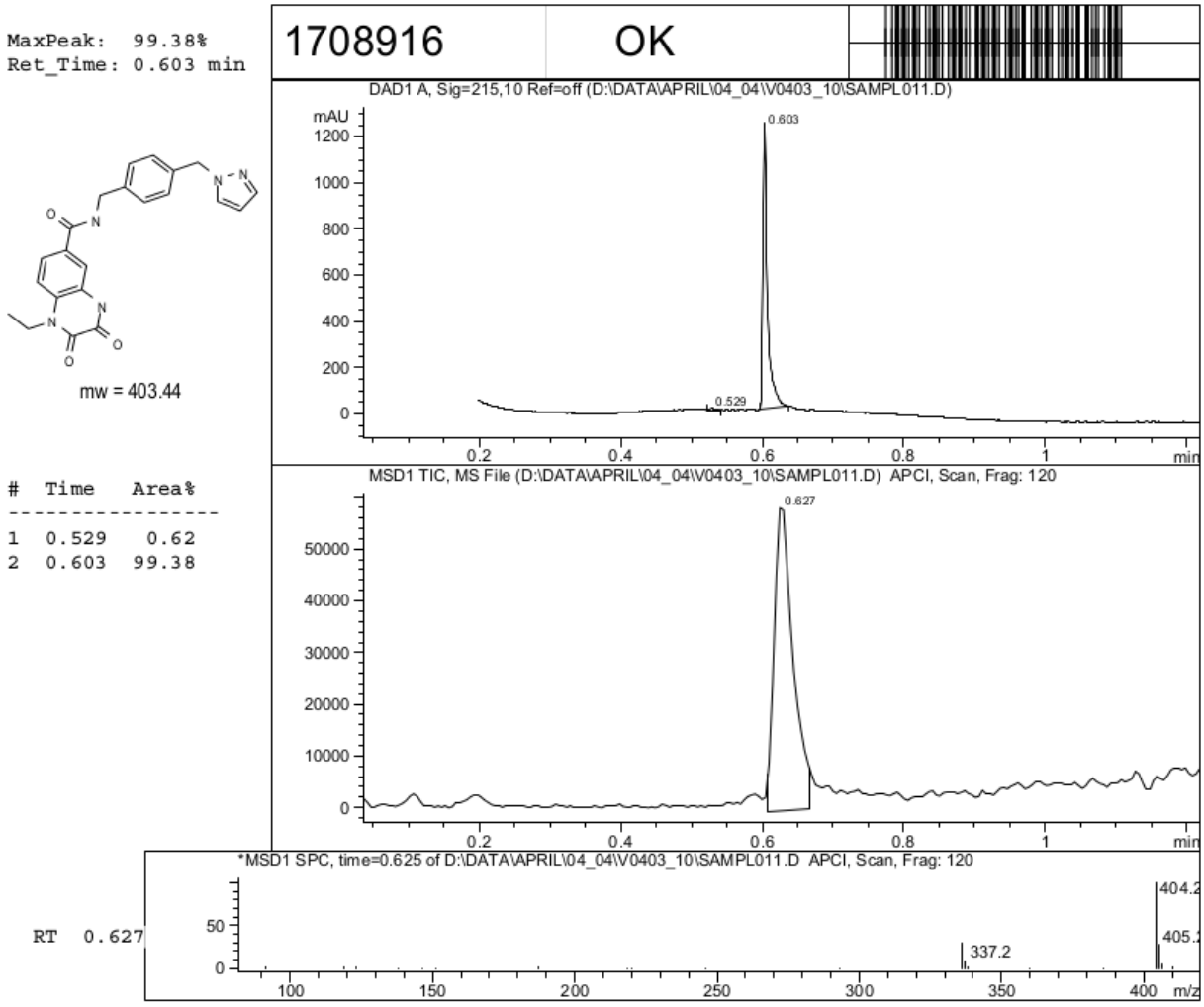
^{1}H-NMR spectra of compound **3**



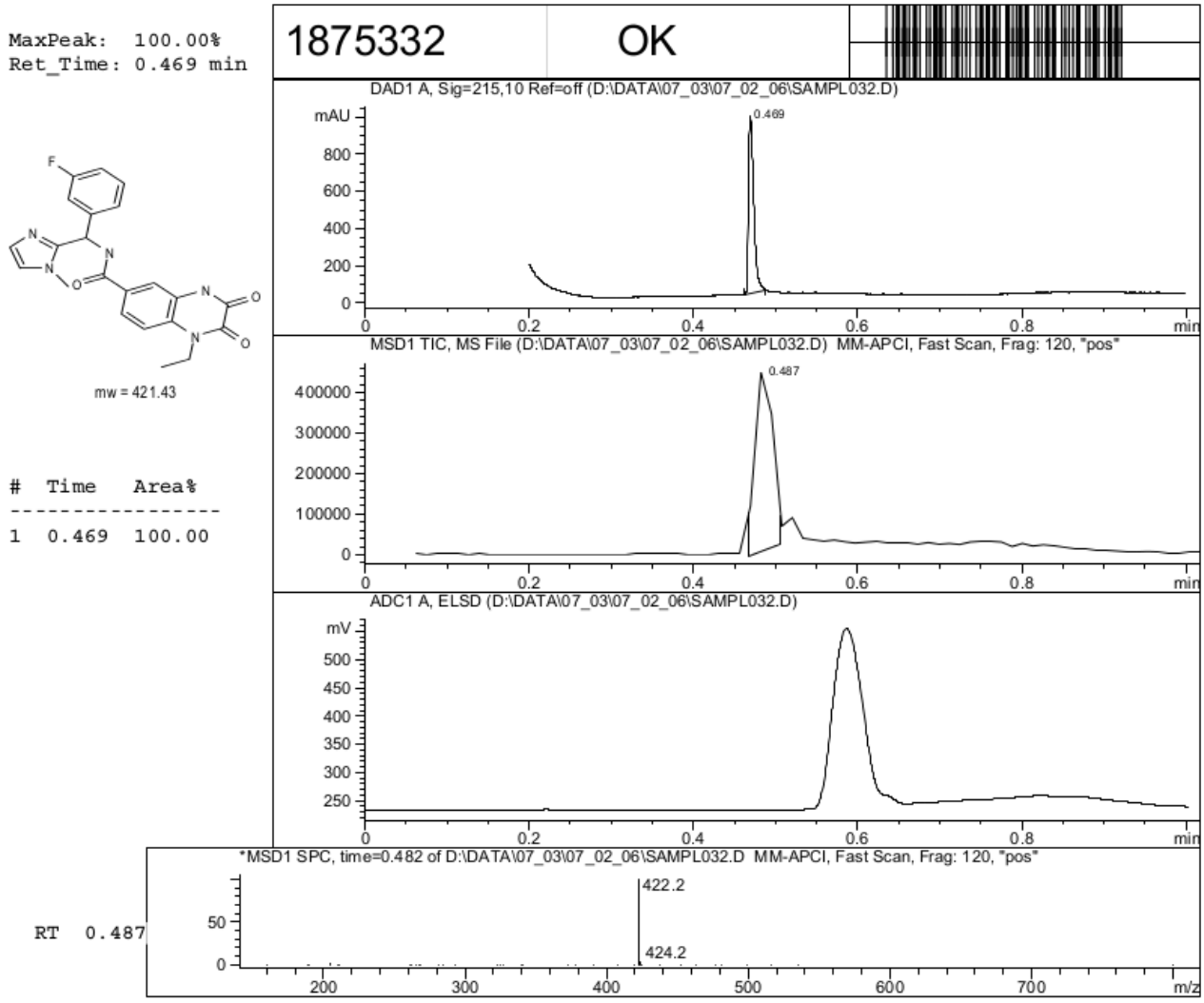
^{1}H-NMR spectra of compound **4**



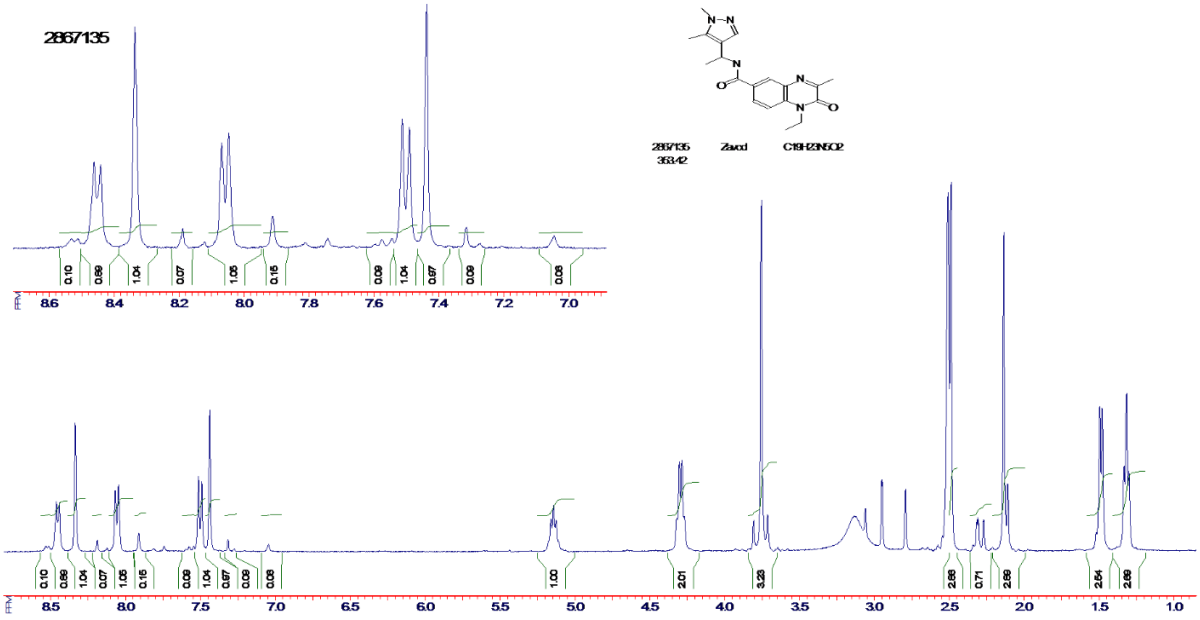
^{1}H-NMR spectra of compound **5**



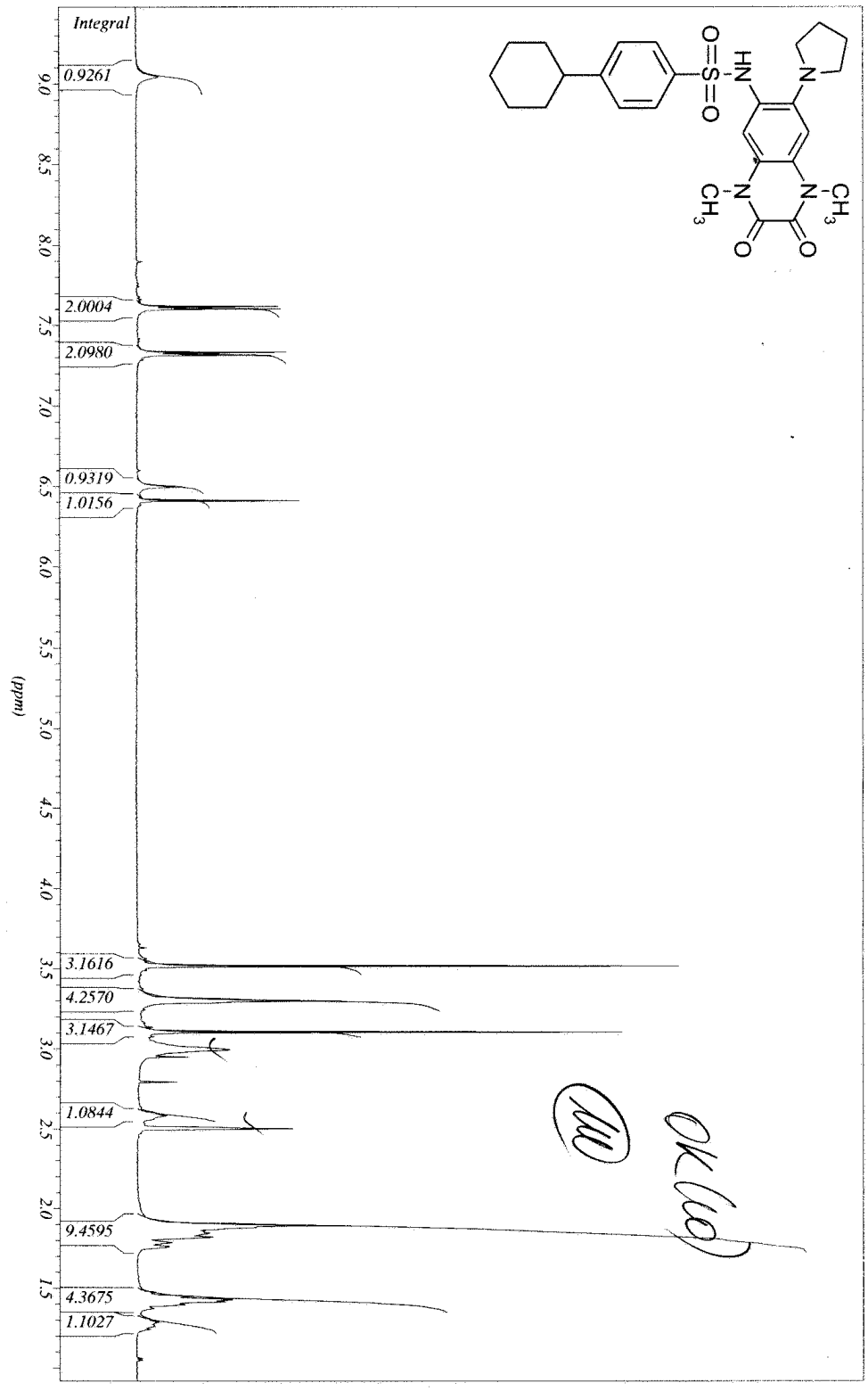
HPLC chromatogram and mass spectra of compound **6**



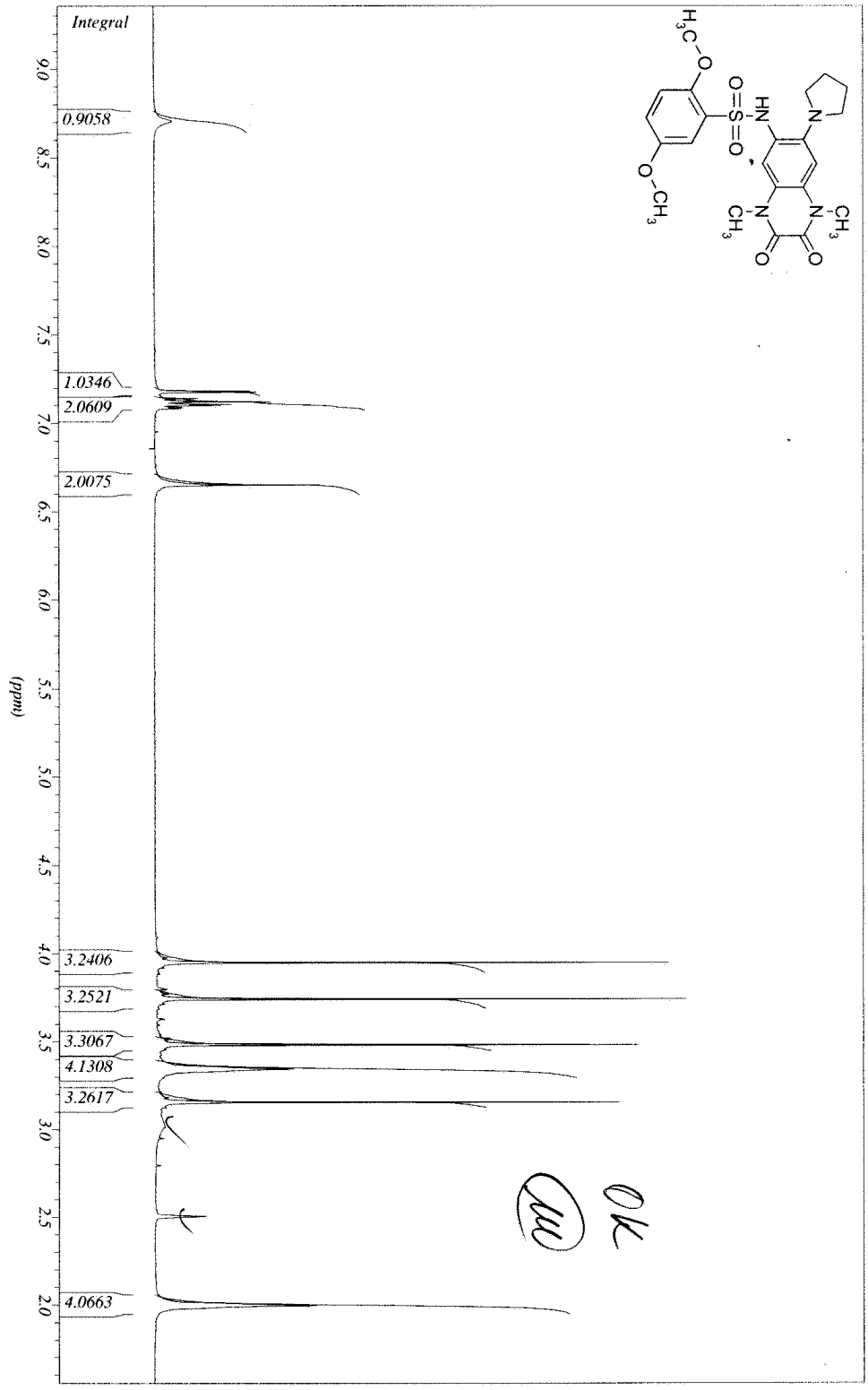
HPLC chromatogram and mass spectra of compound **7**



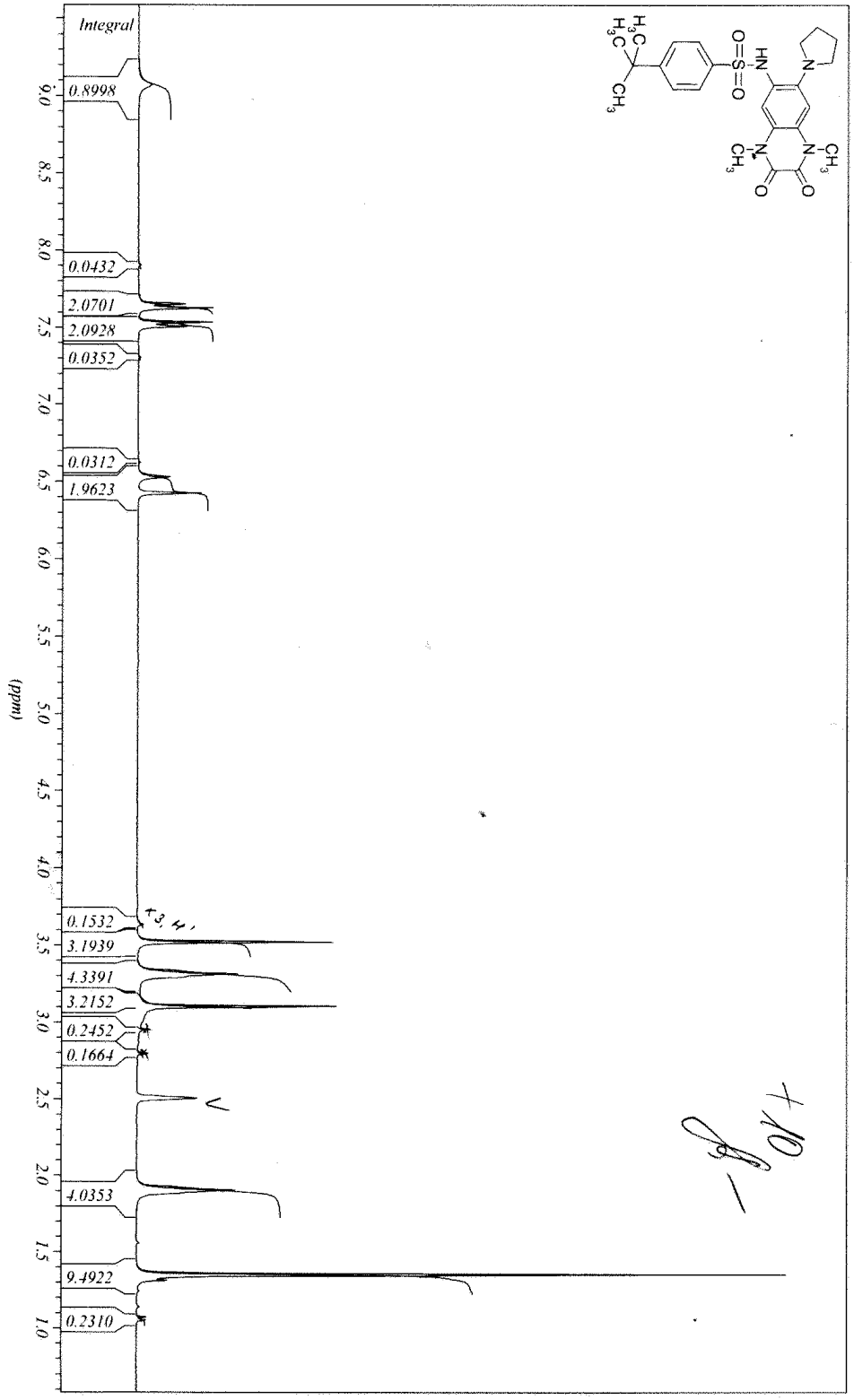
^{1}H-NMR spectra of compound **8**



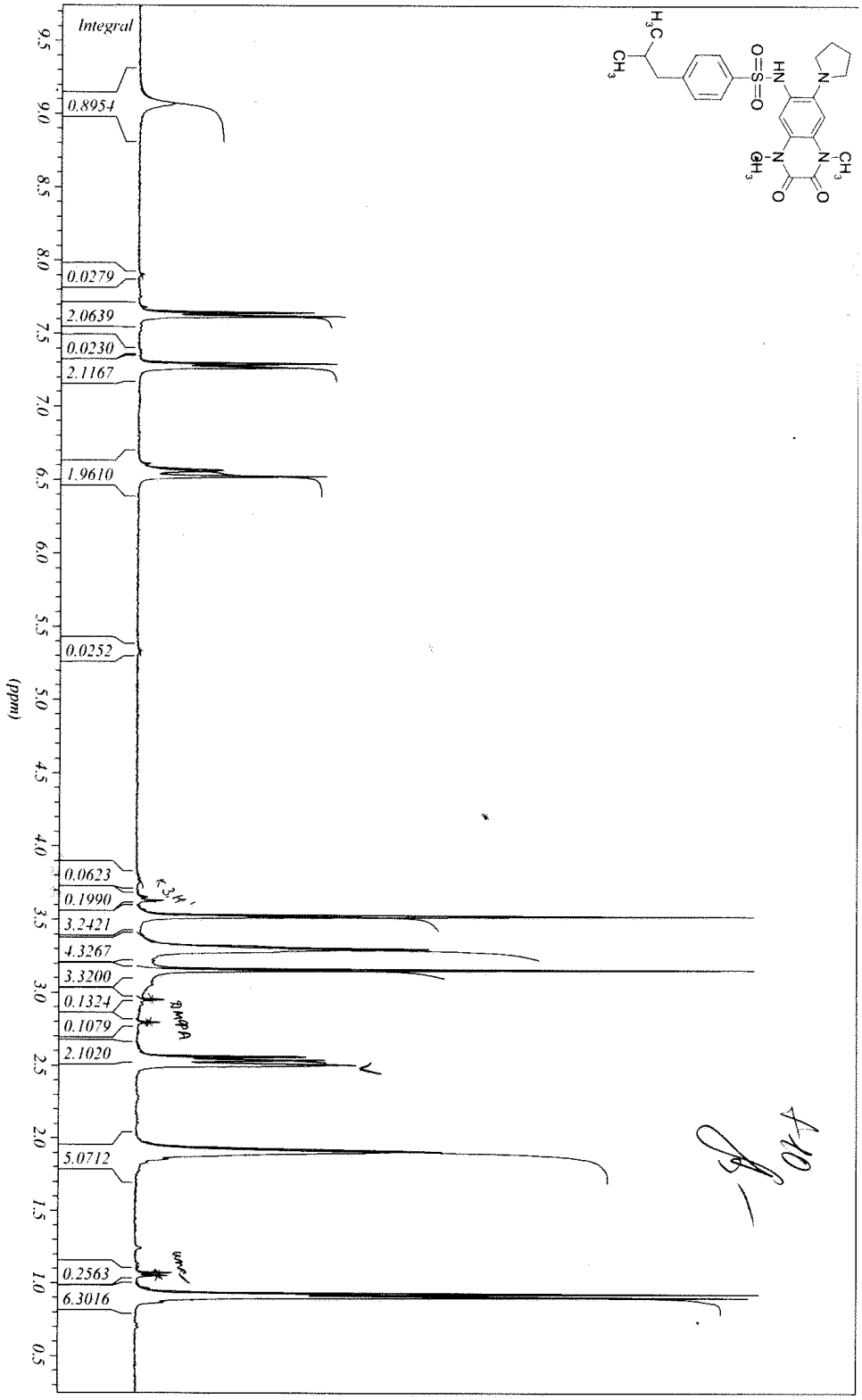
^{1}H-NMR spectra of compound **10**



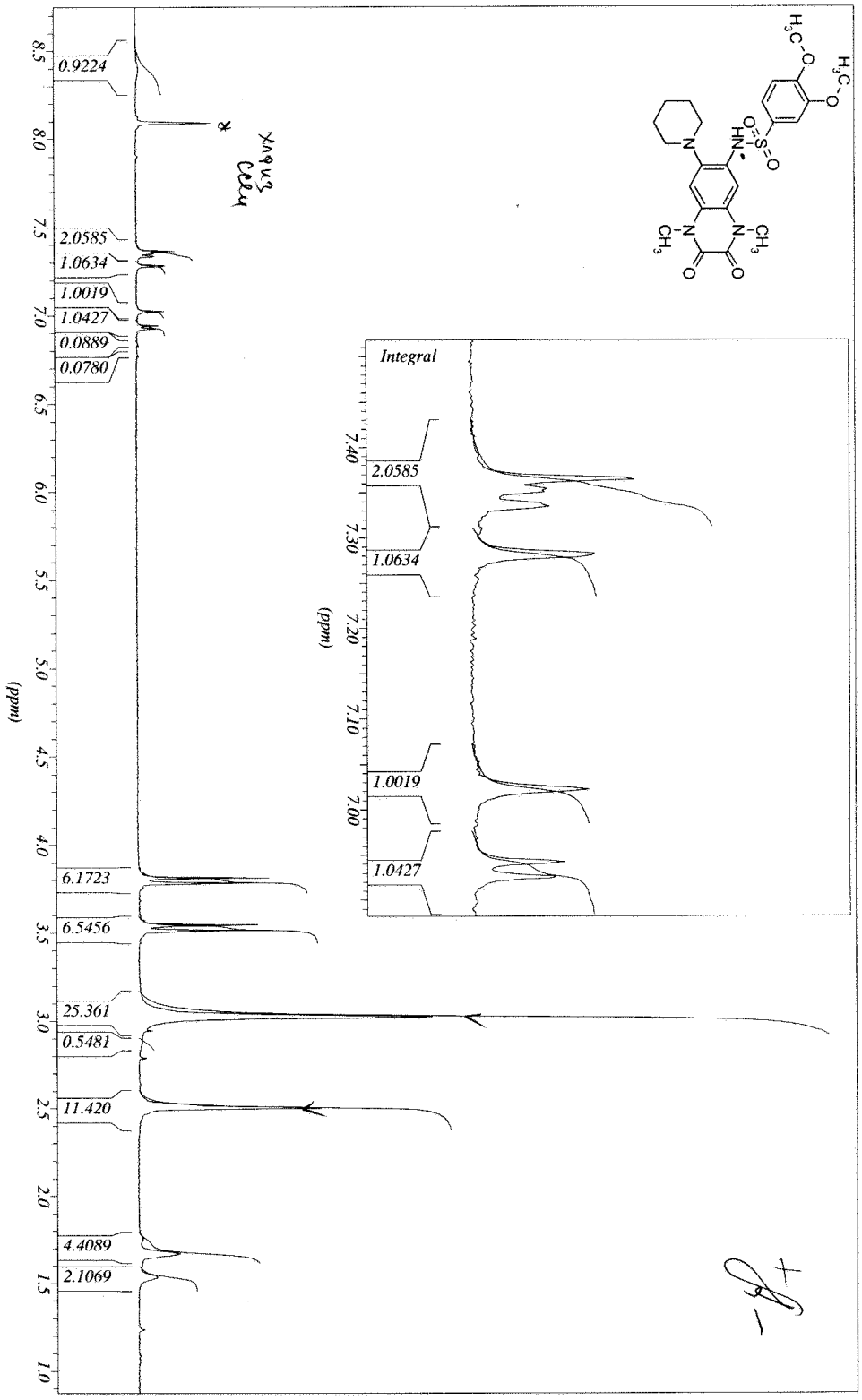
^{1}H-NMR spectra of compound **11**



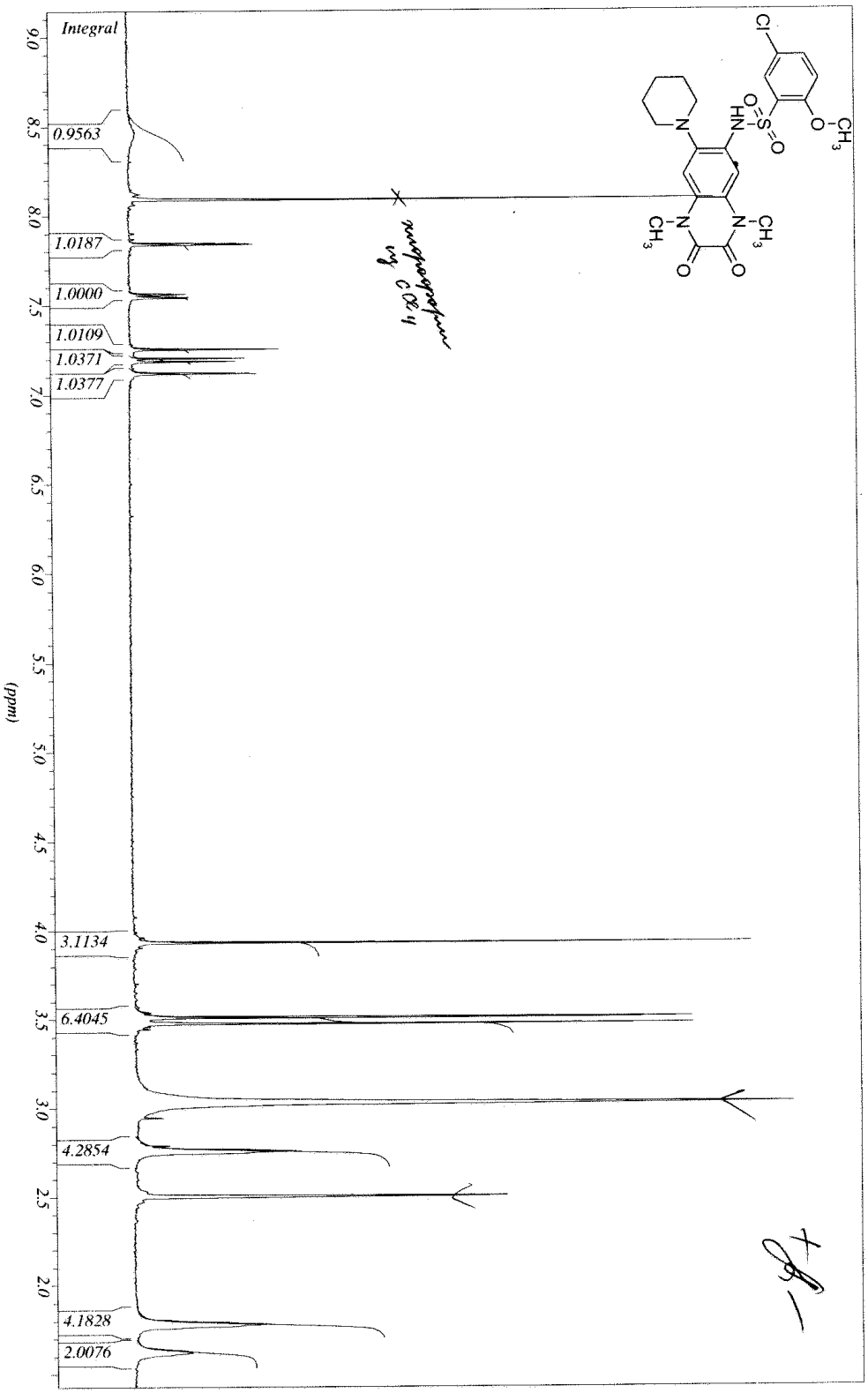
[1]H-NMR spectra of compound **12**



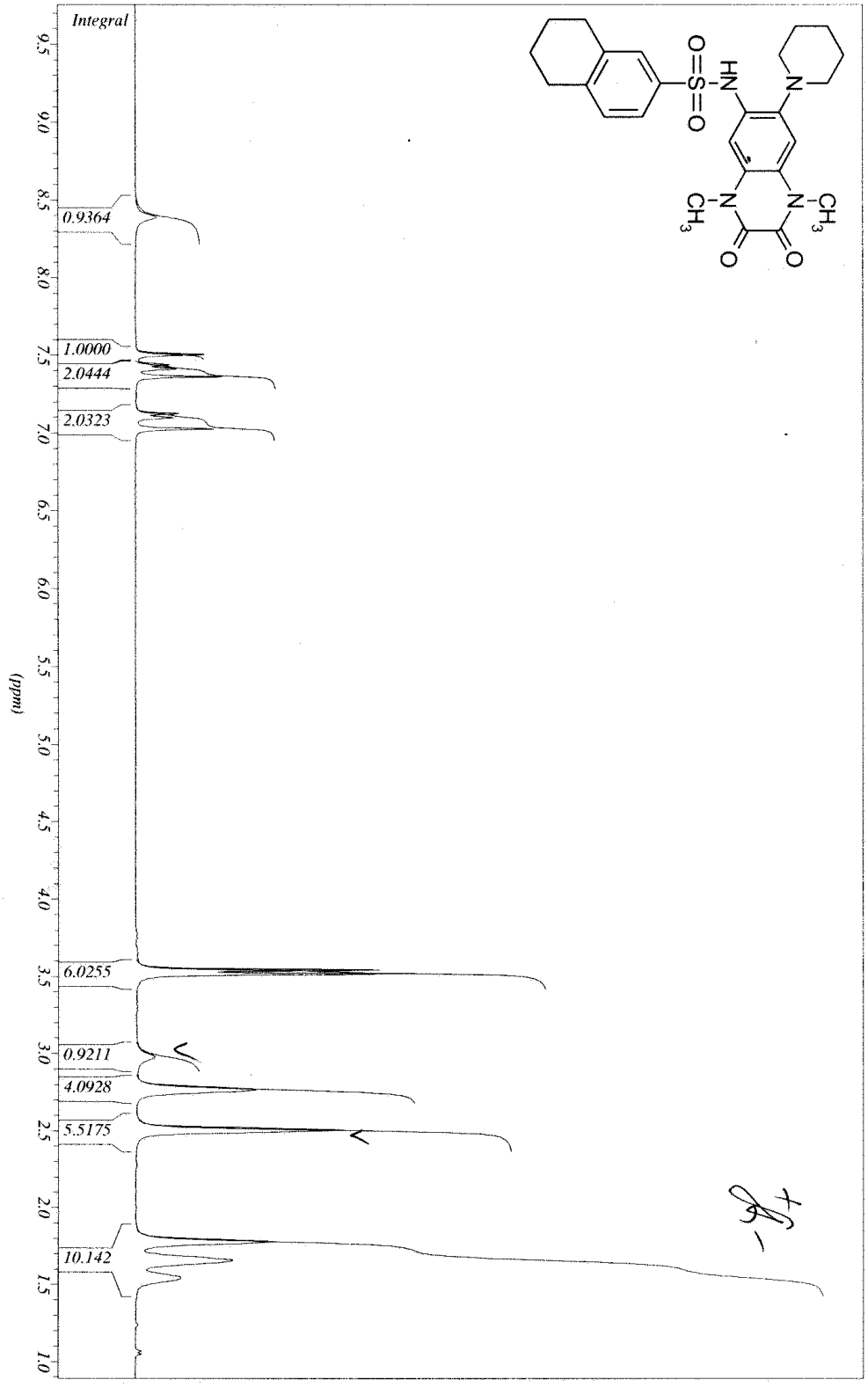
^{1}H-NMR spectra of compound **13**



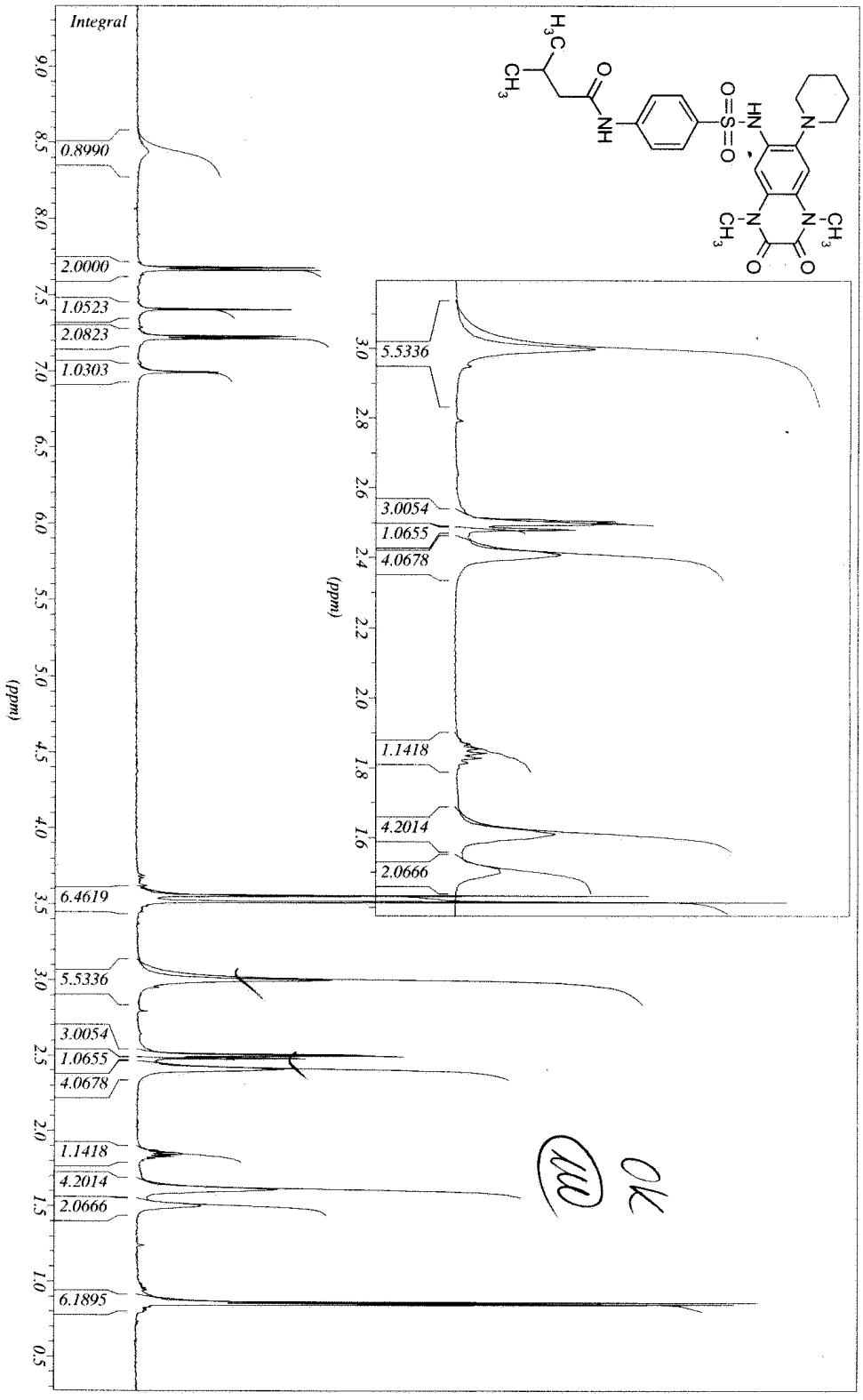
[1]H-NMR spectra of compound **14**



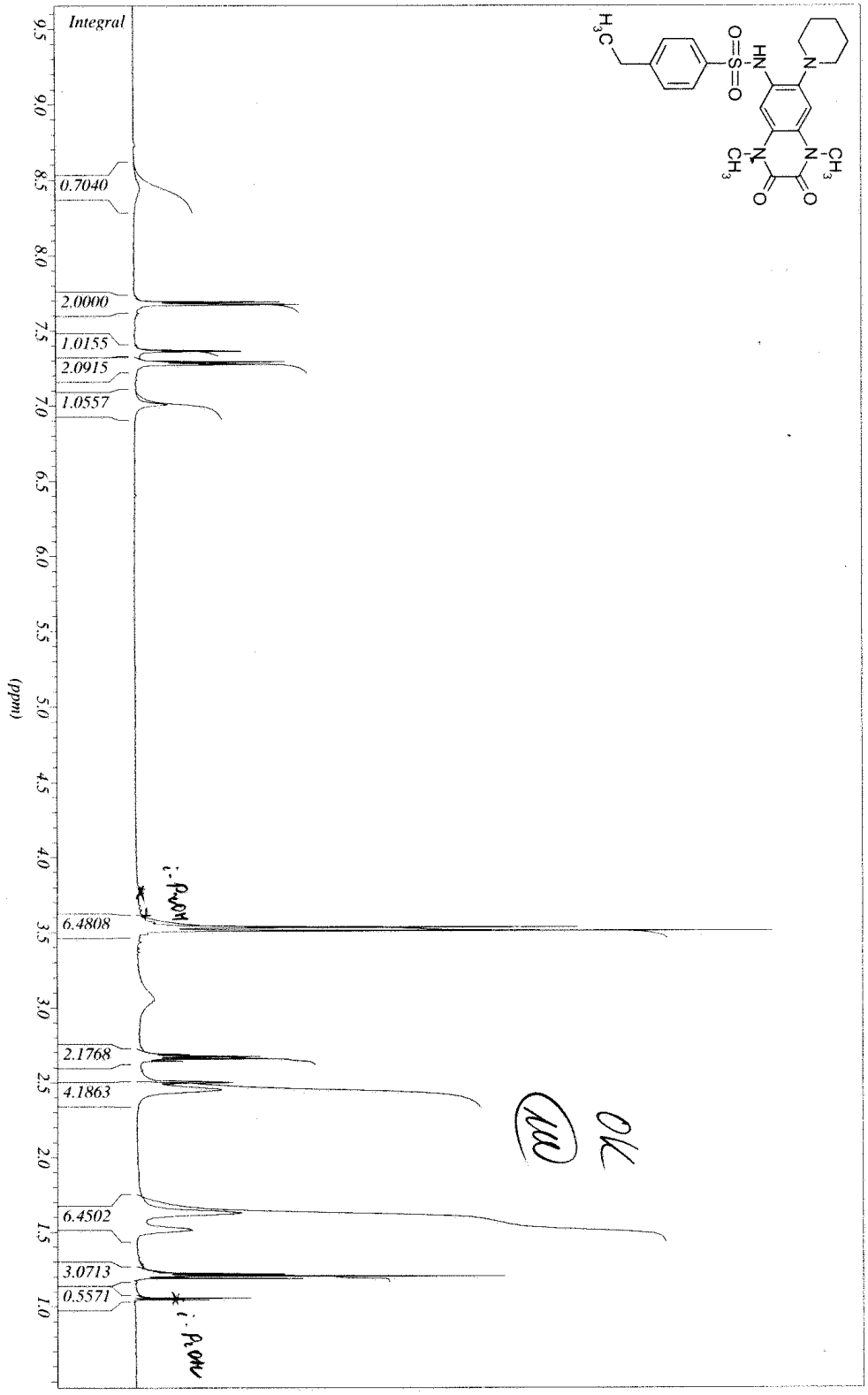
^{1}H-NMR spectra of compound **15**



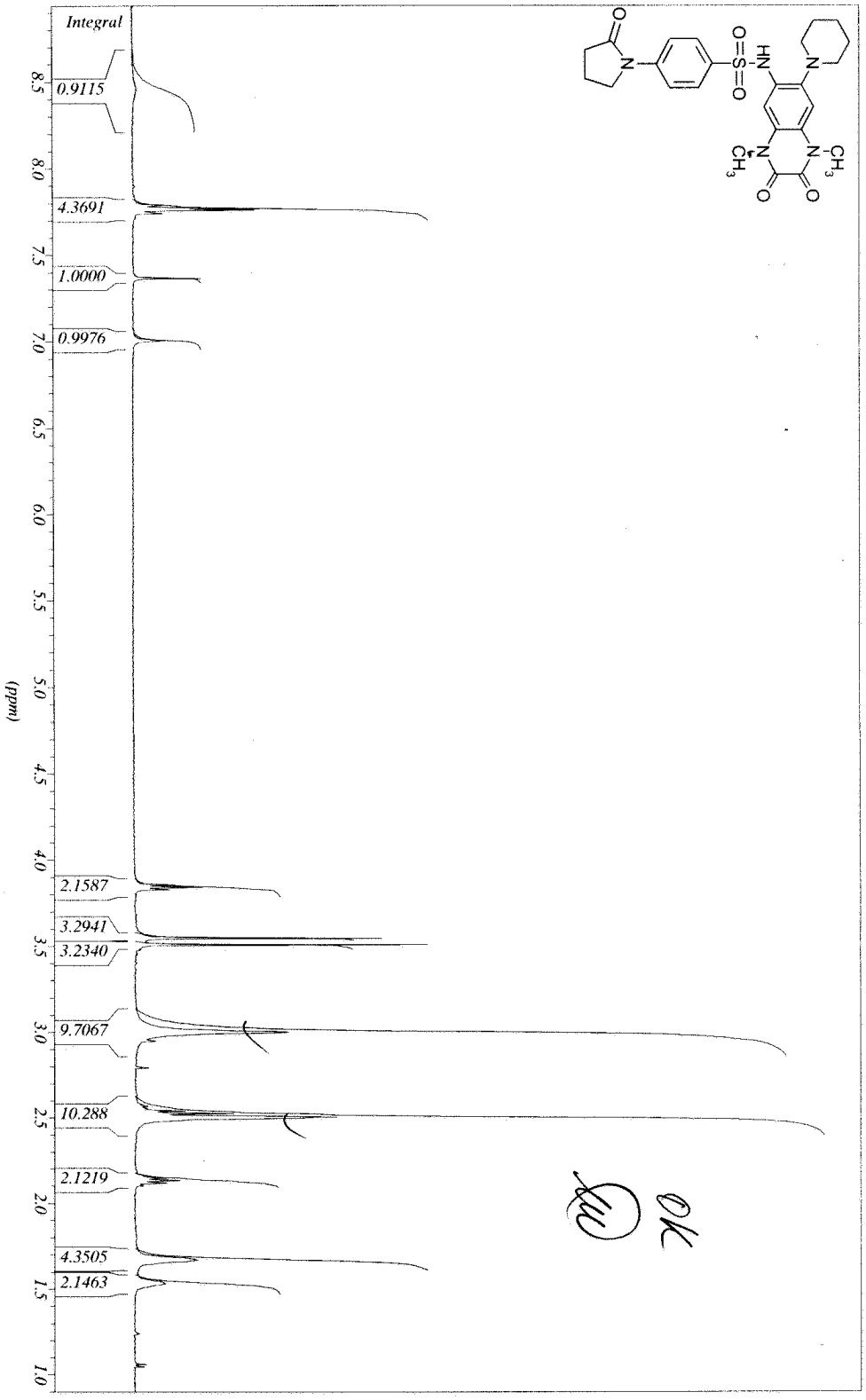
^{1}H-NMR spectra of compound **16**



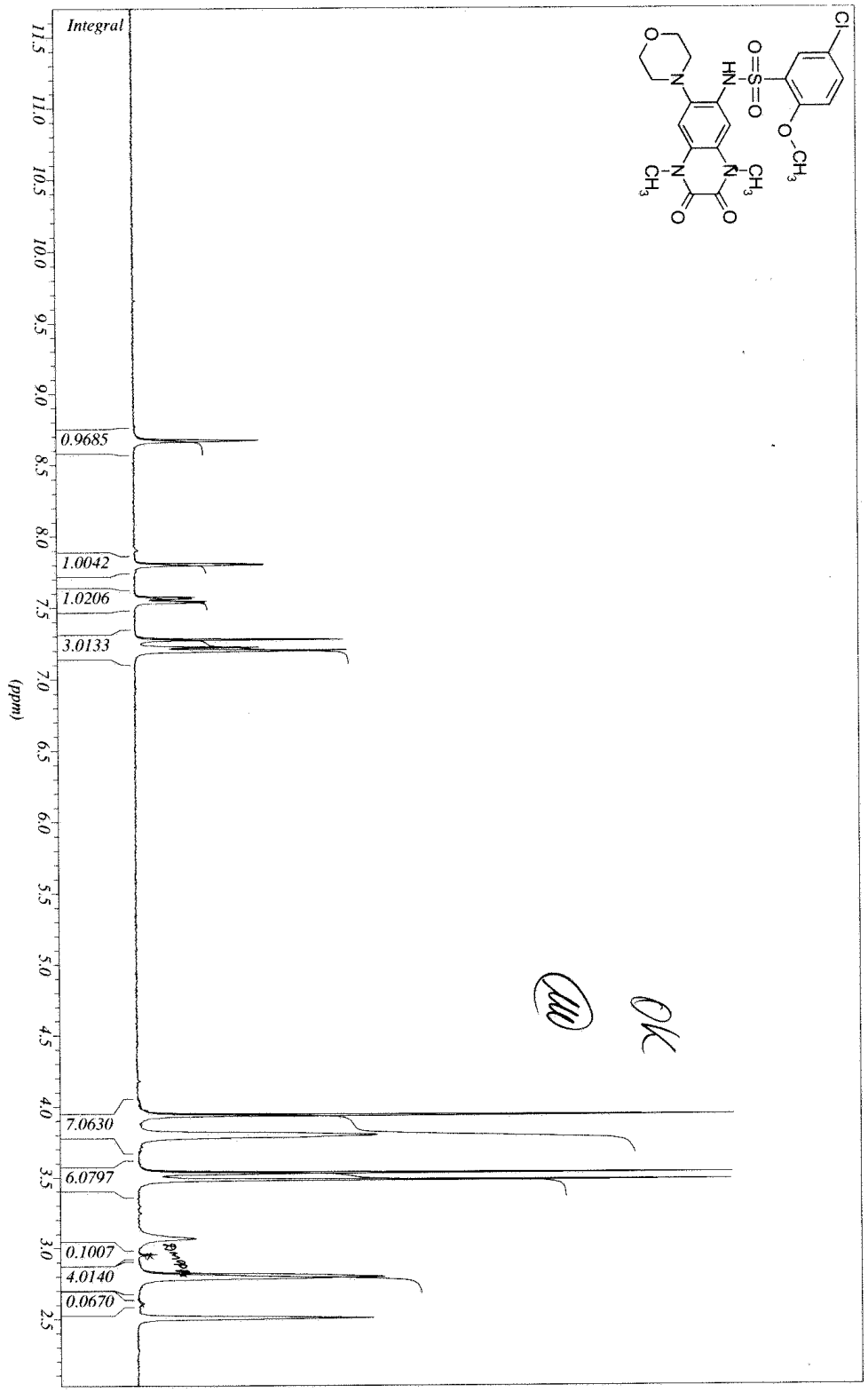
[1]H-NMR spectra of compound **17**



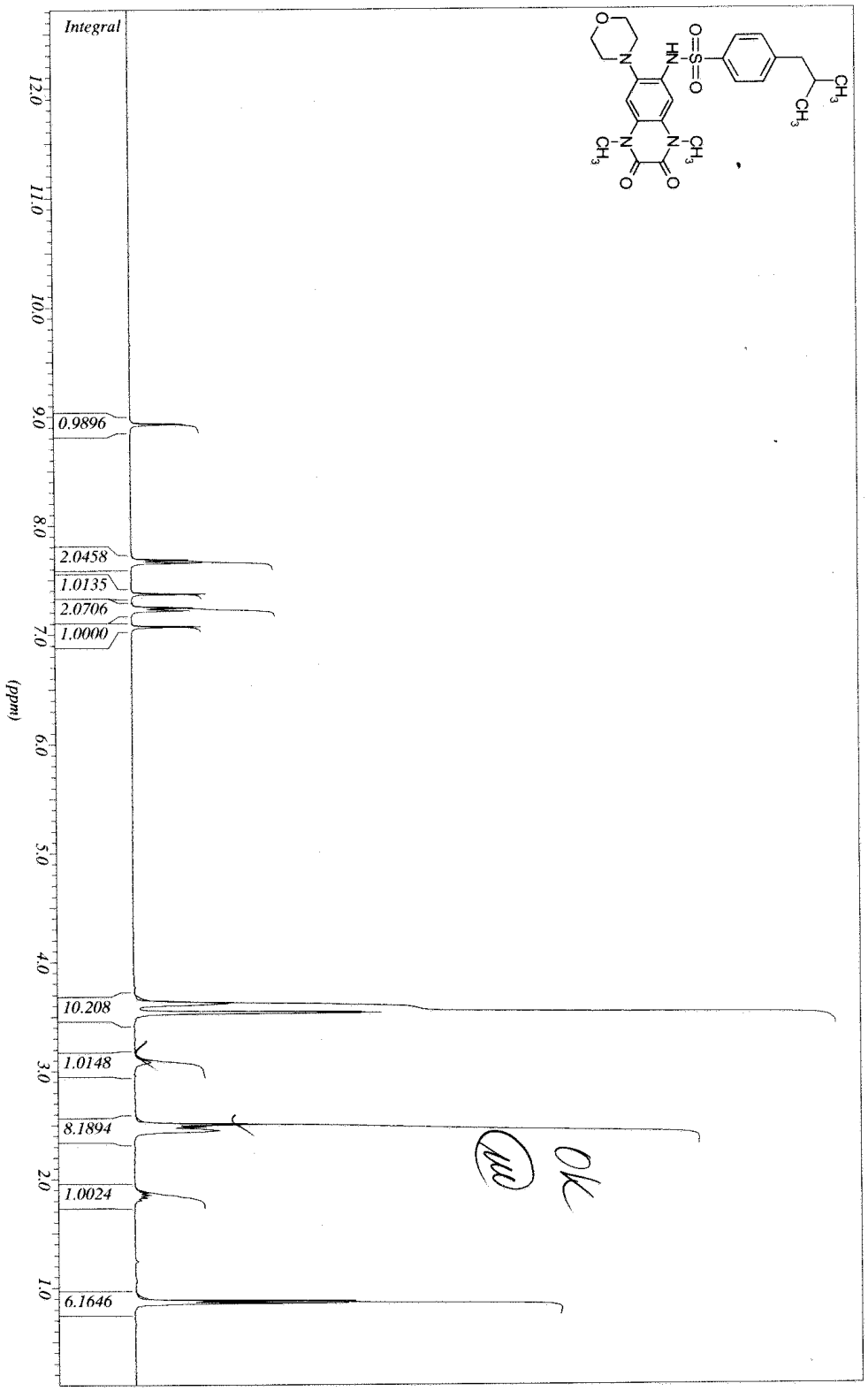
^{1}H-NMR spectra of compound **18**



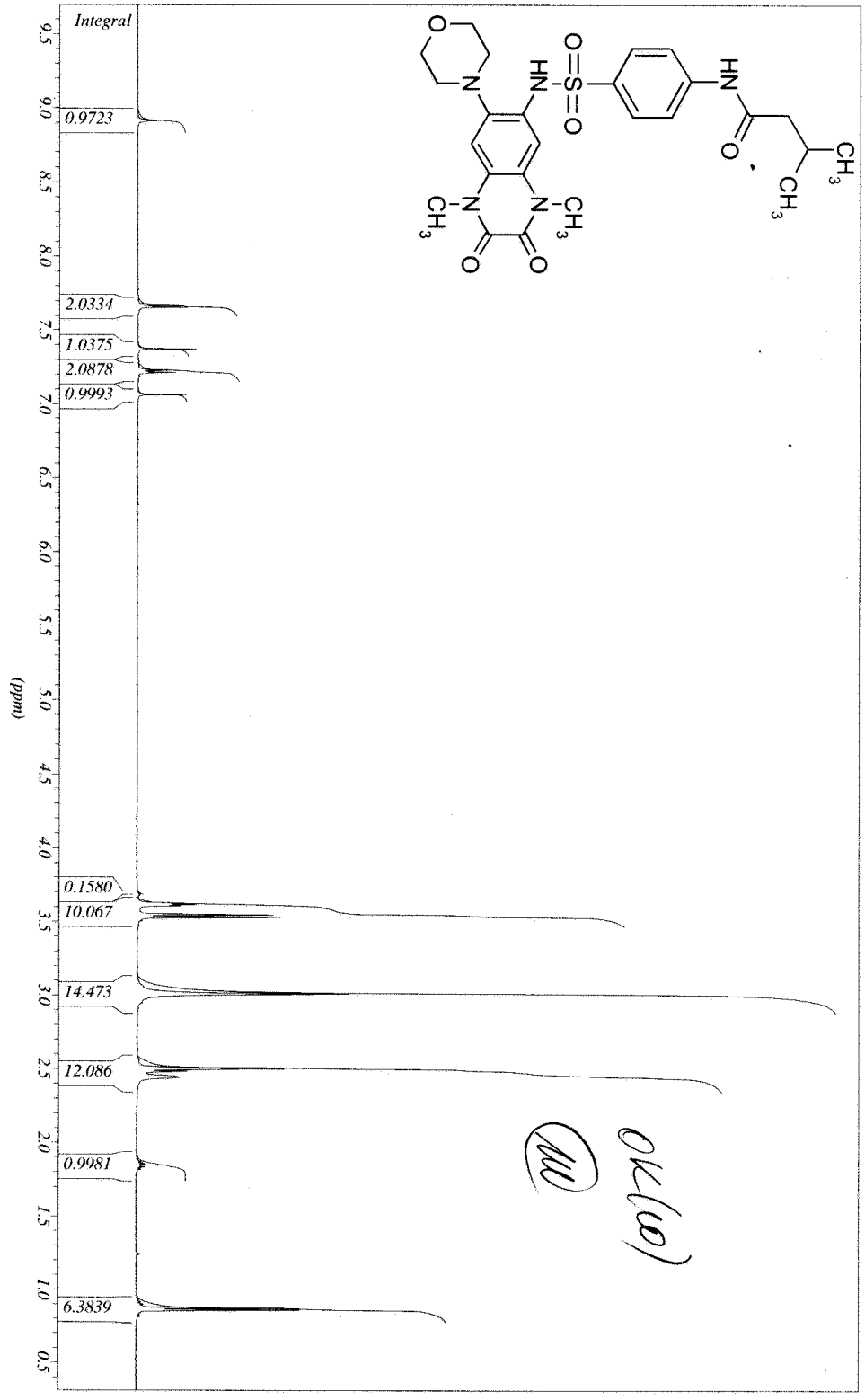
^{1}H-NMR spectra of compound **19**



^{1}H-NMR spectra of compound **20**



^{1}H-NMR spectra of compound **21**



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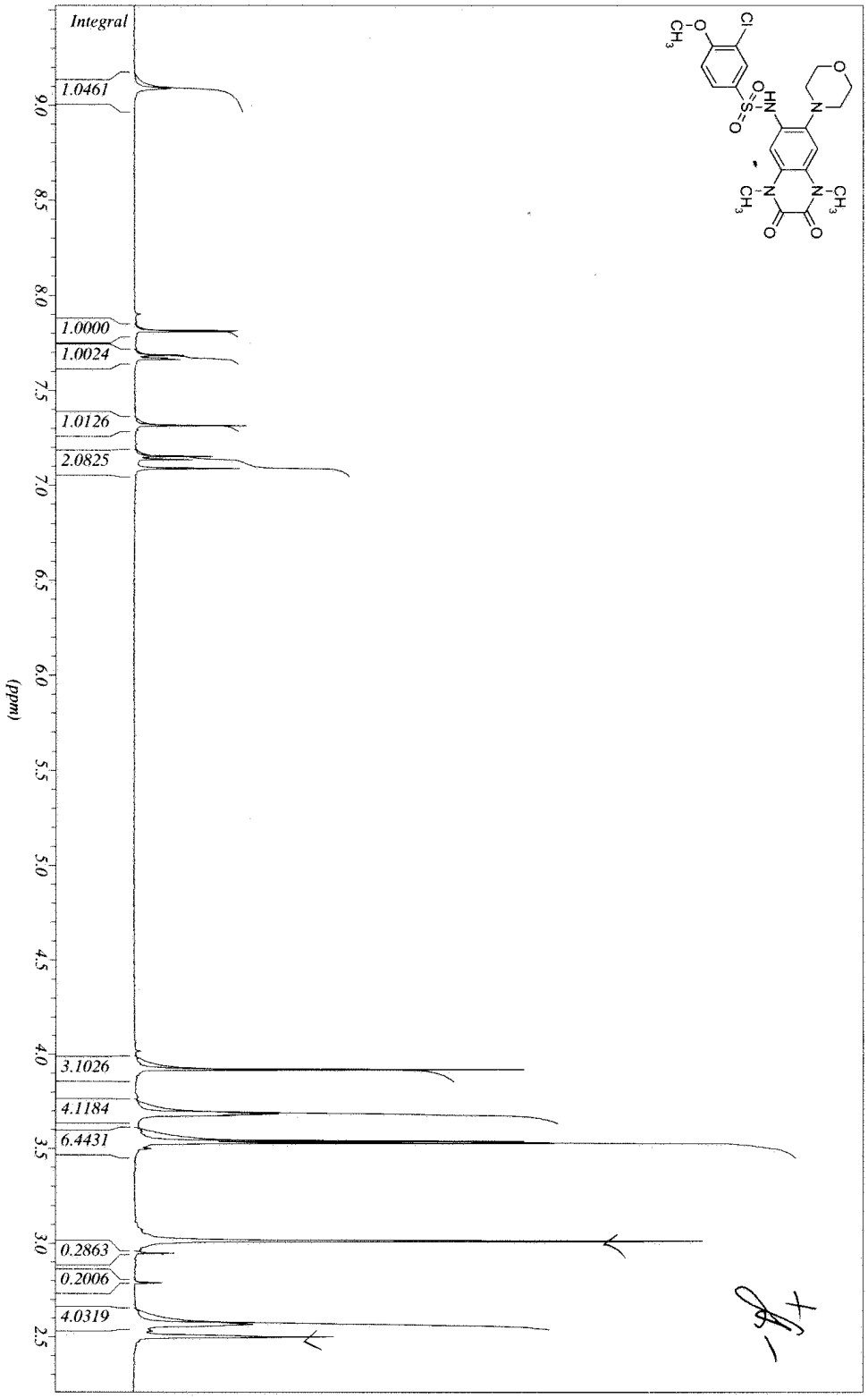

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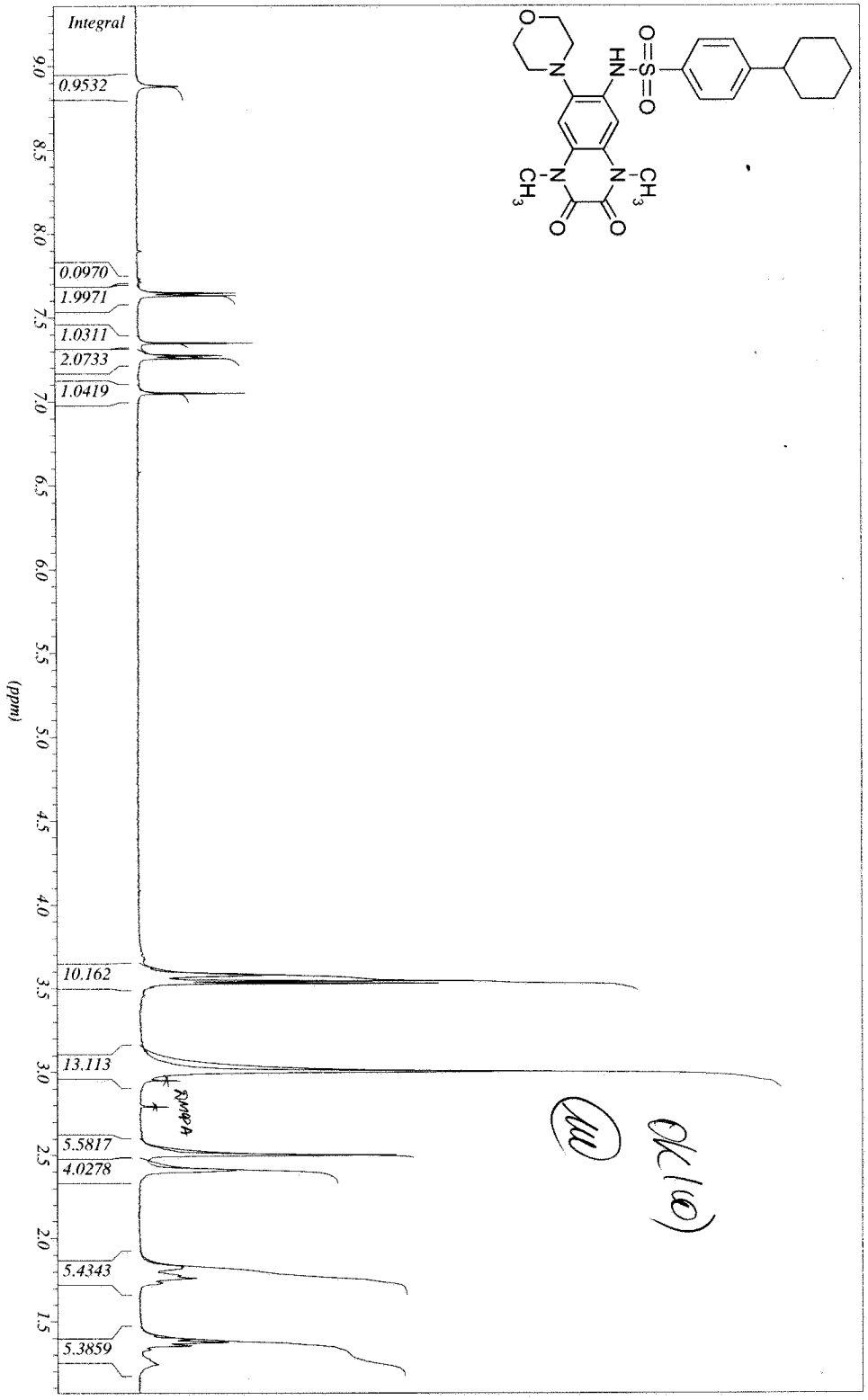
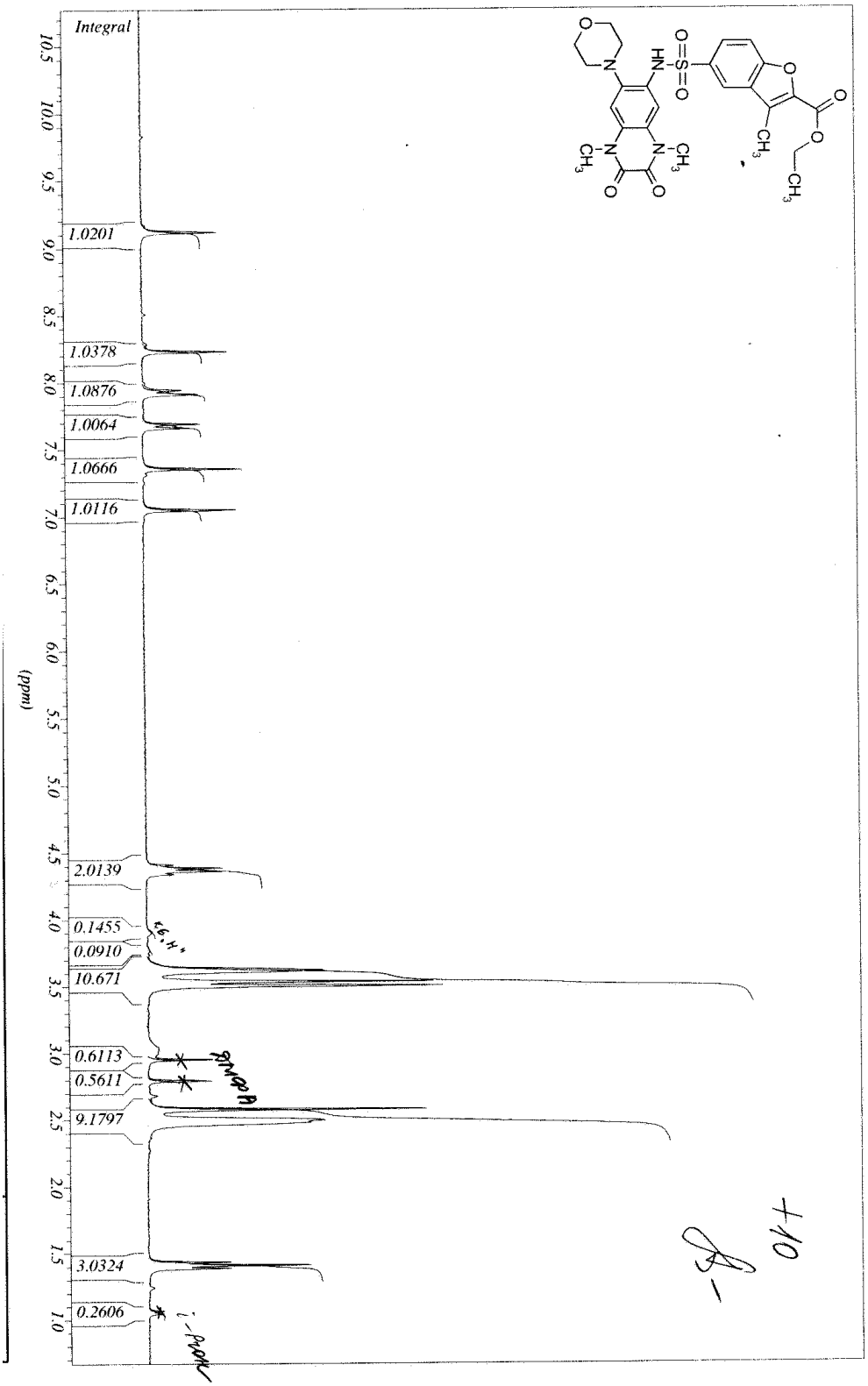
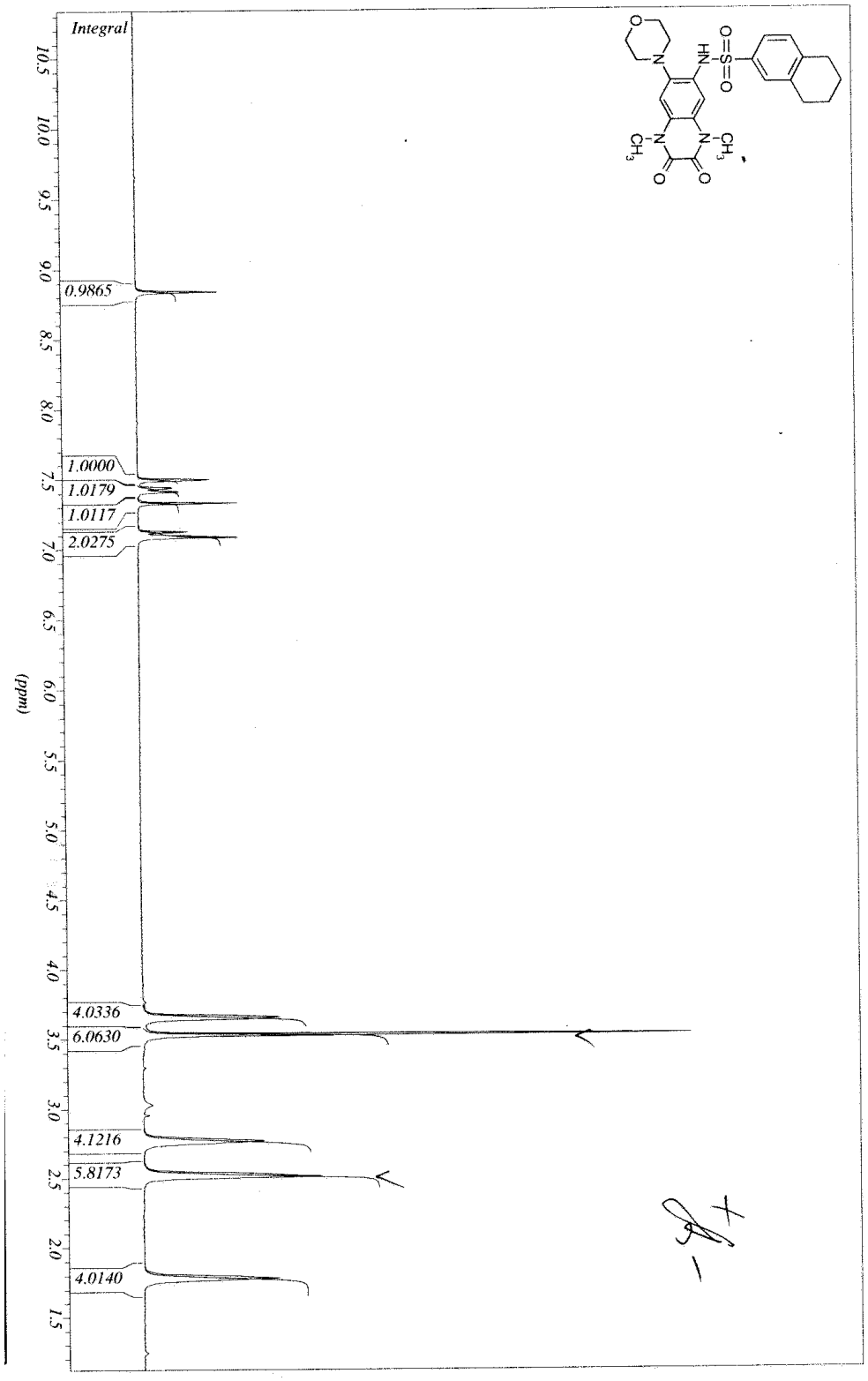


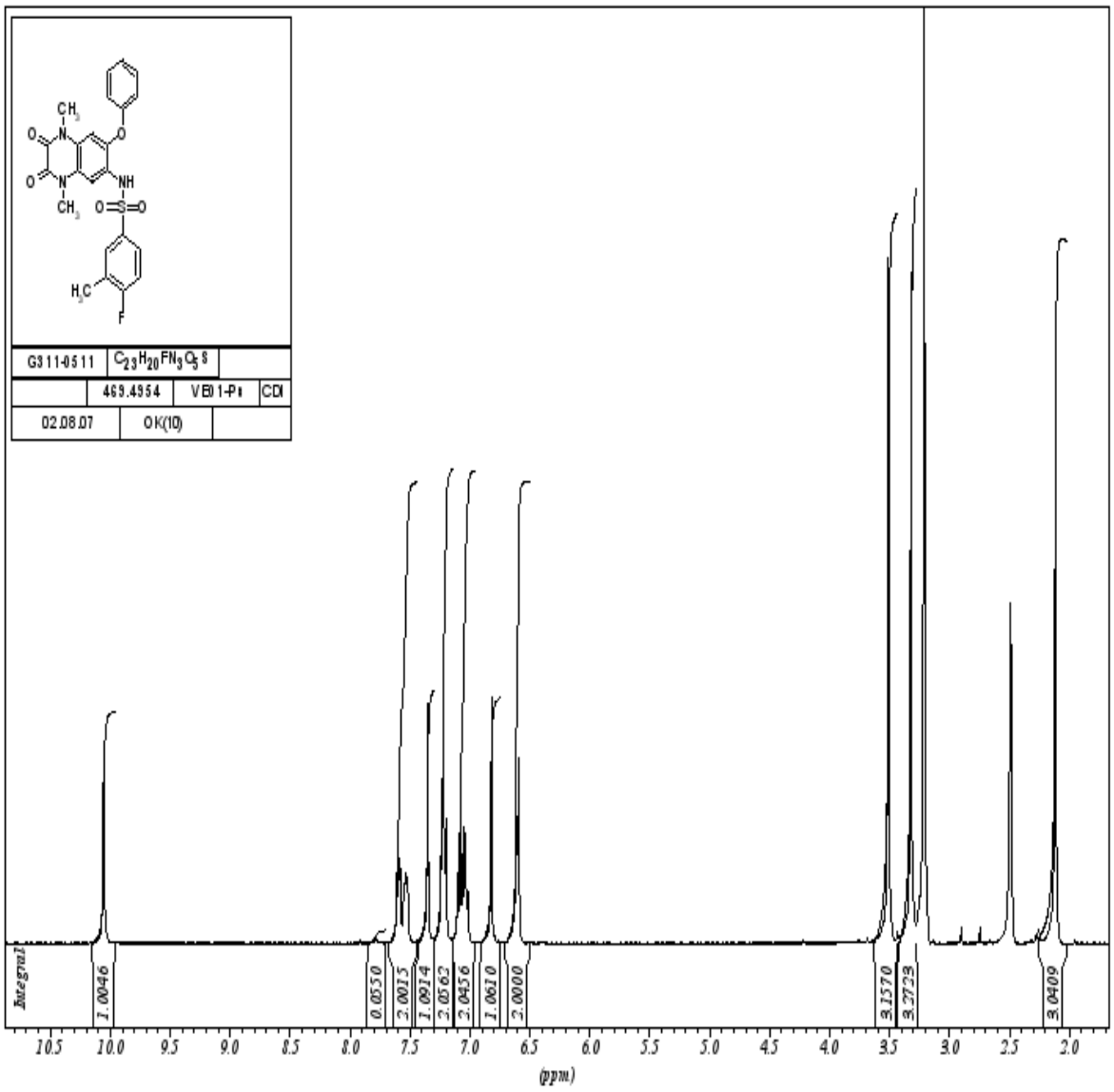
^{1}H-NMR spectra of compound **24**



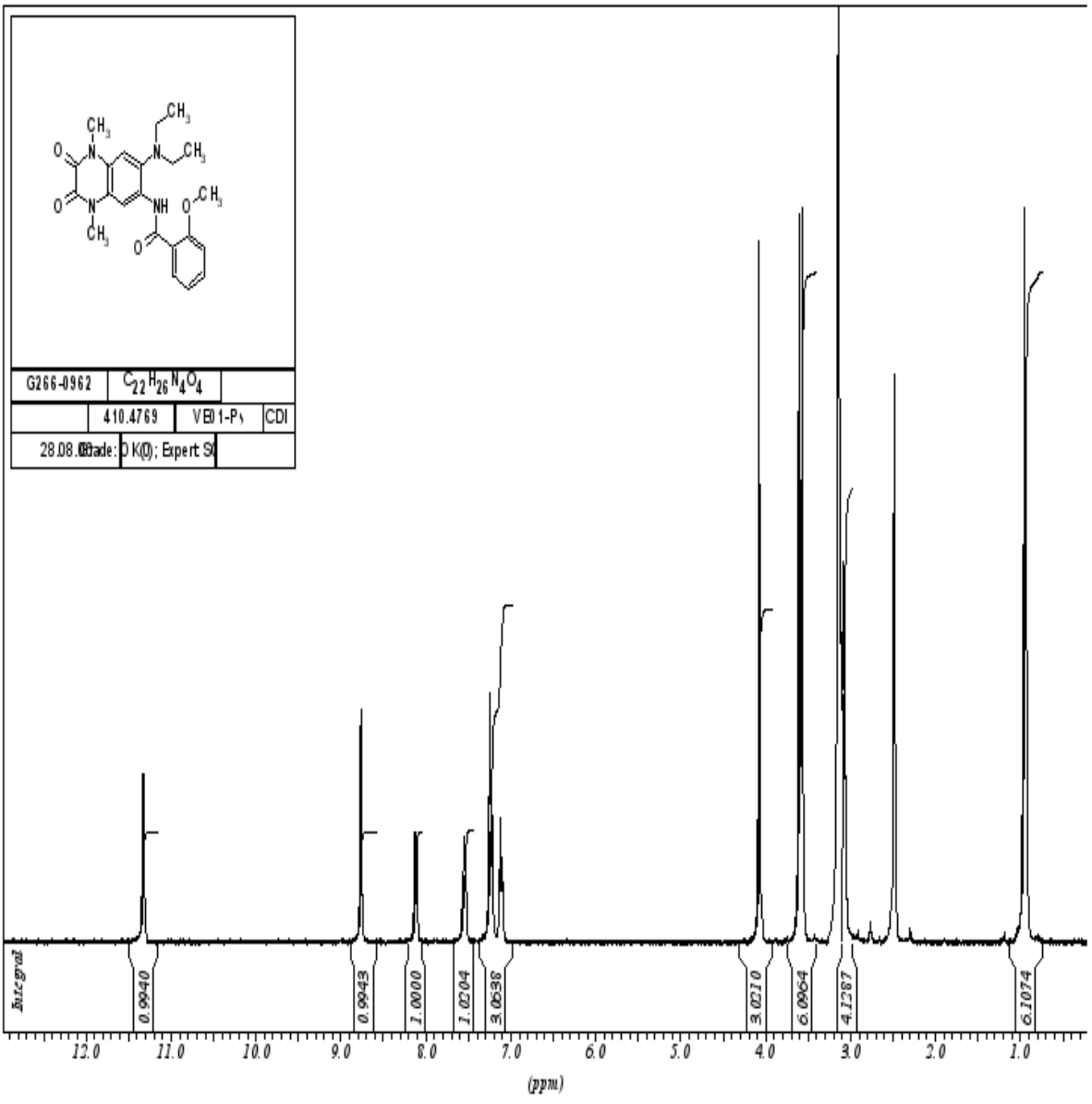
[1]H-NMR spectra of compound **25**



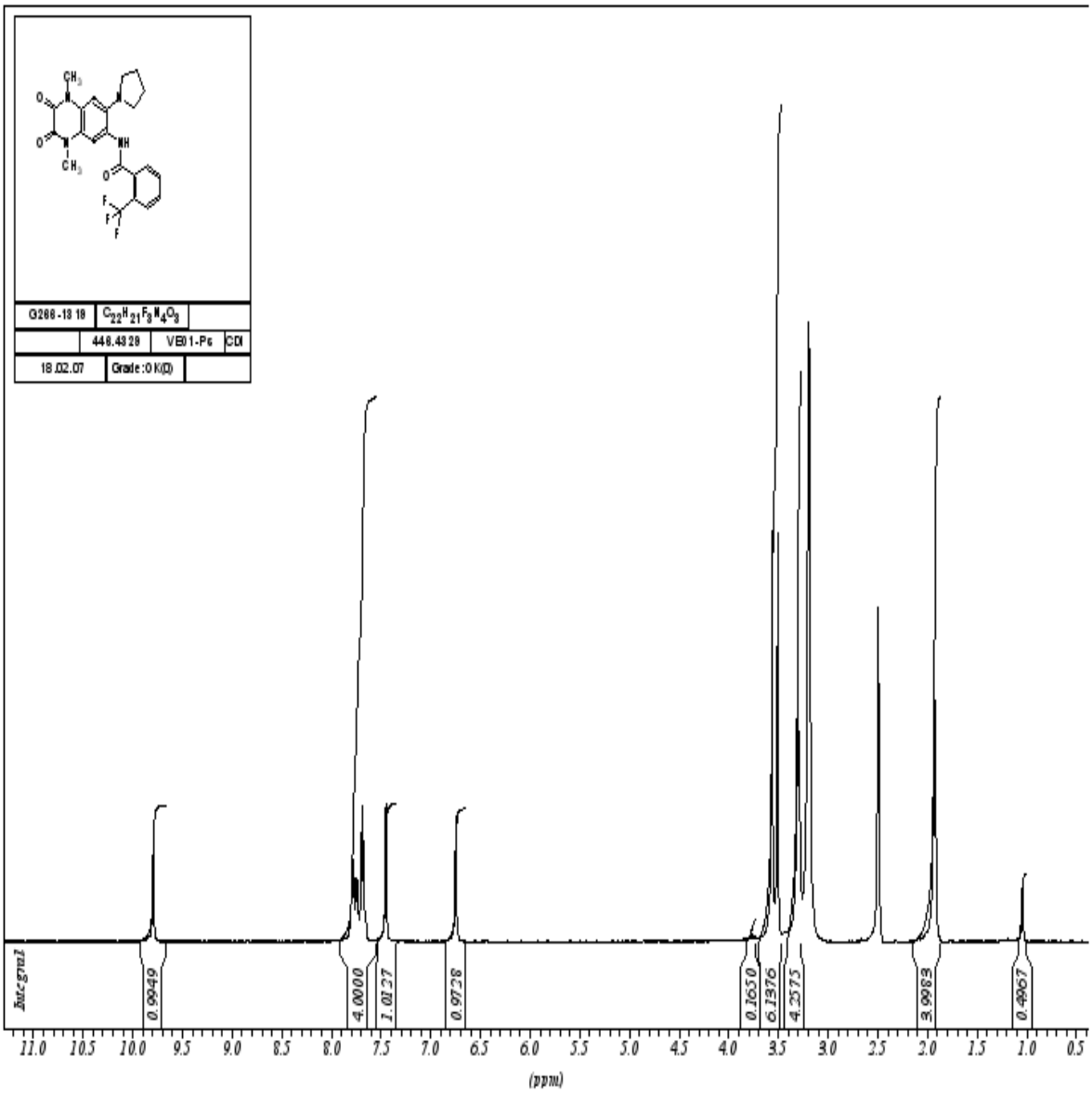
^{1}H-NMR spectra of compound **26**



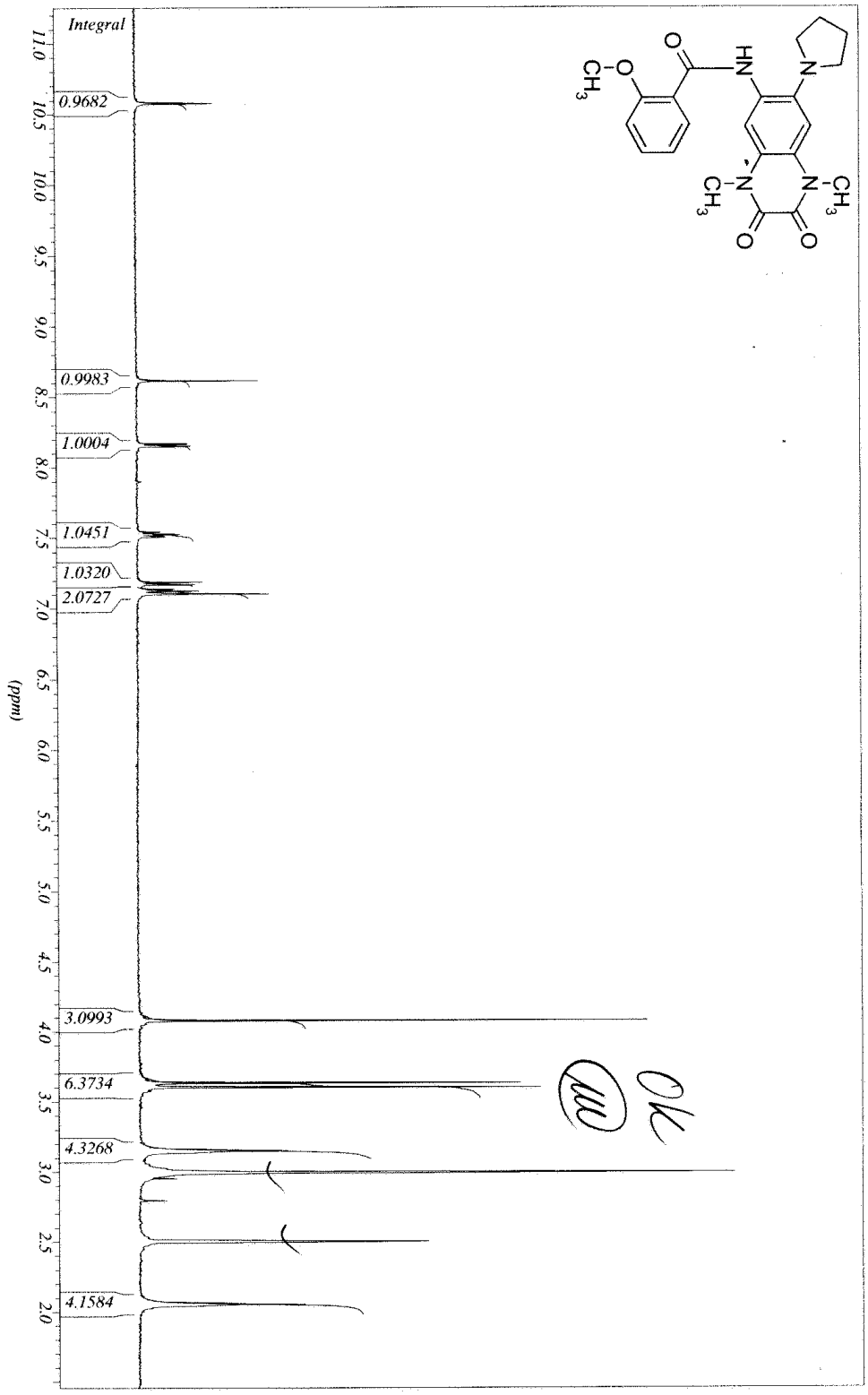
^{1}H-NMR spectra of compound **27**



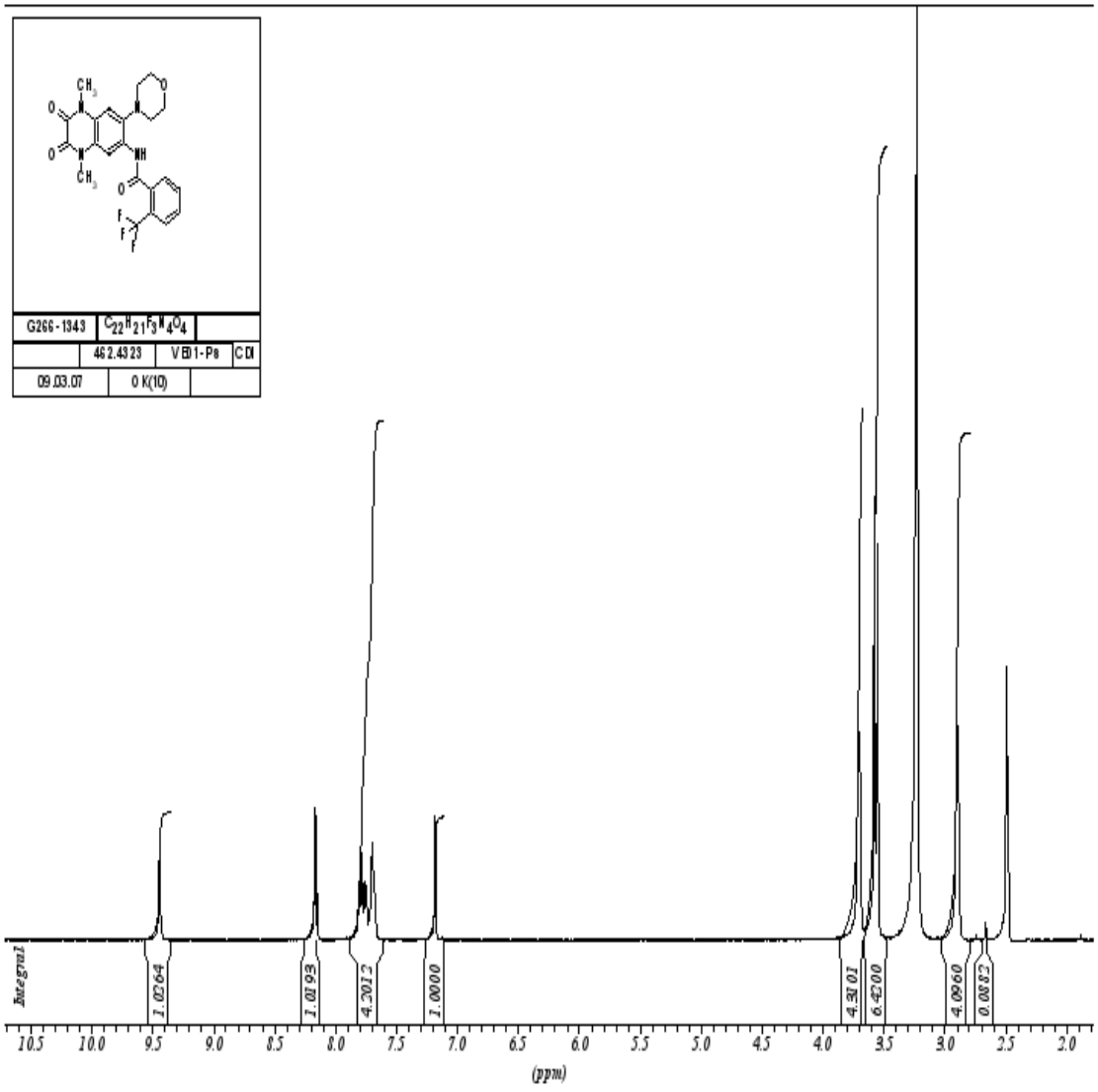
^{1}H-NMR spectra of compound **28**



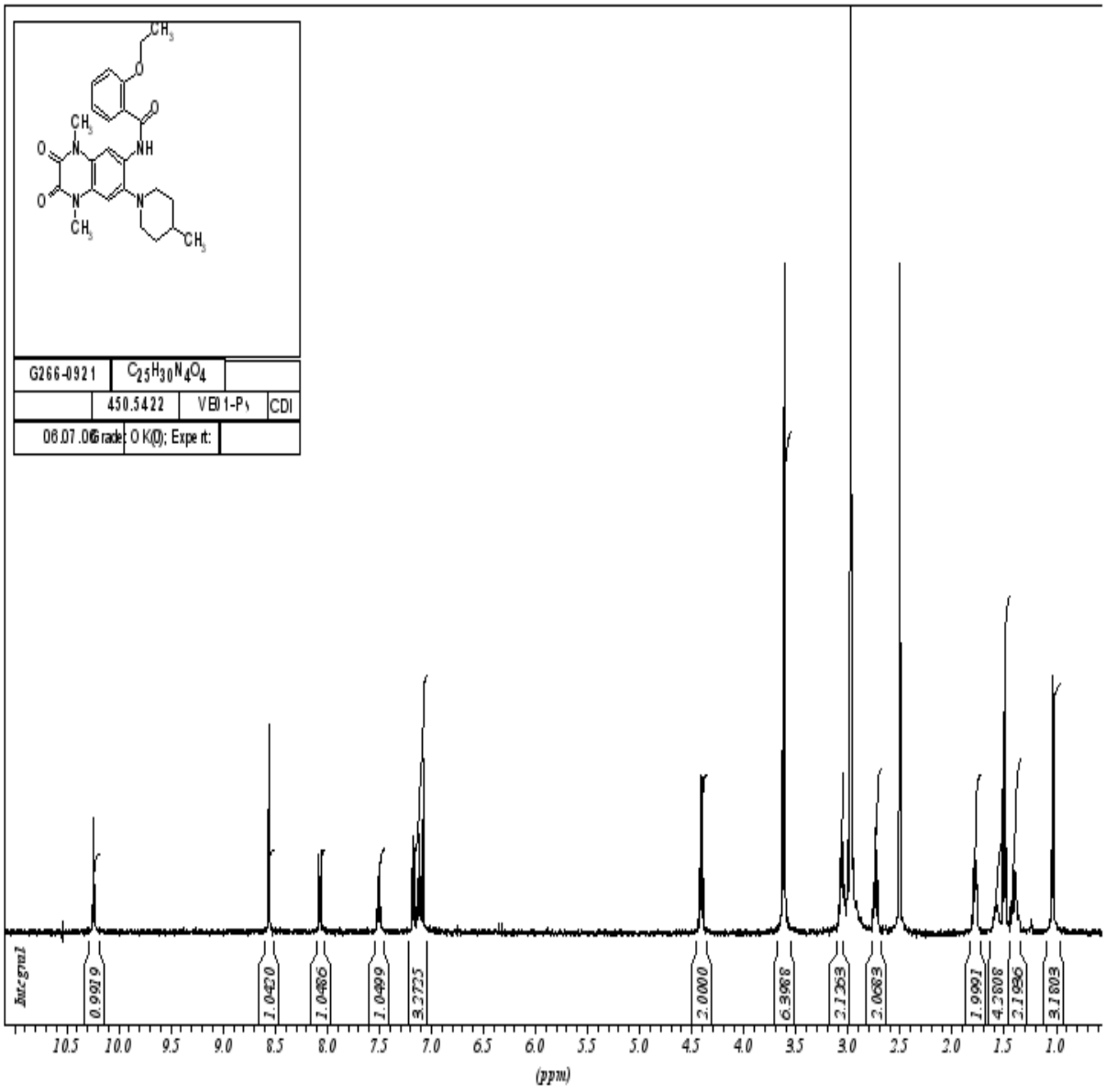
^{1}H-NMR spectra of compound **29**



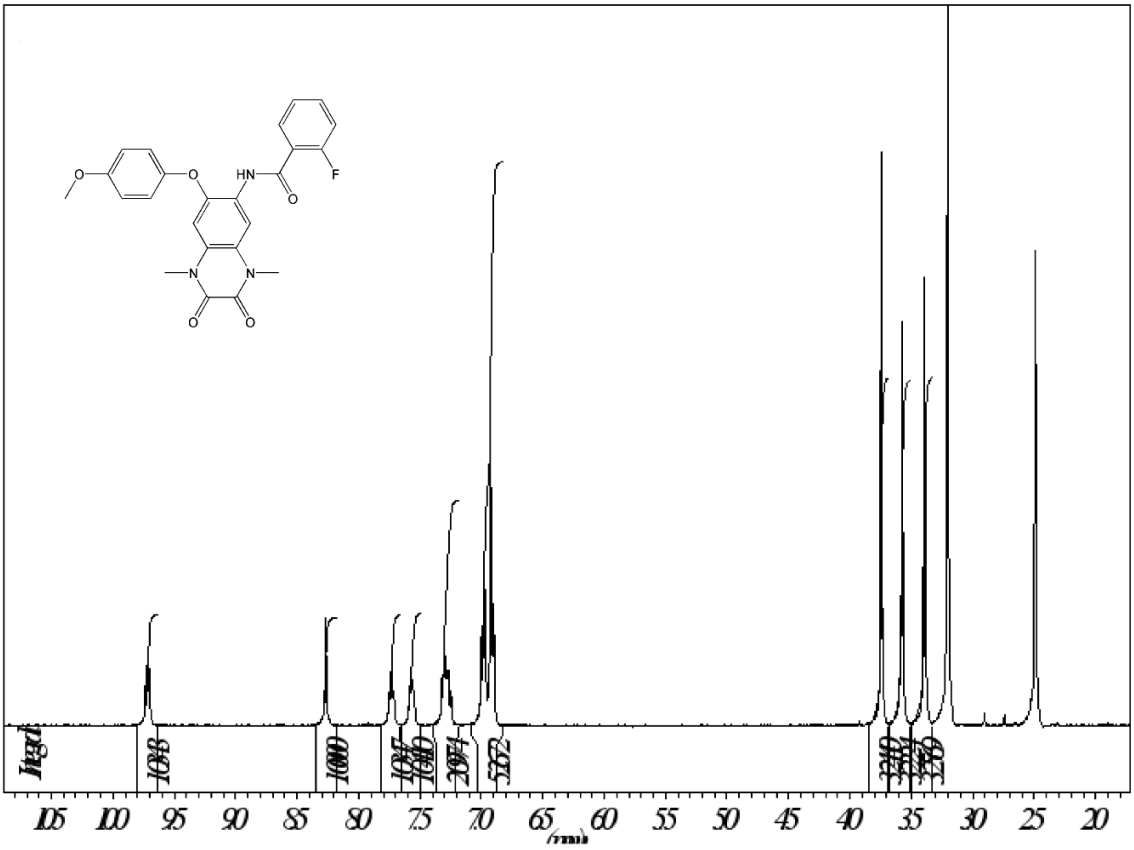
^{1}H-NMR spectra of compound **30**



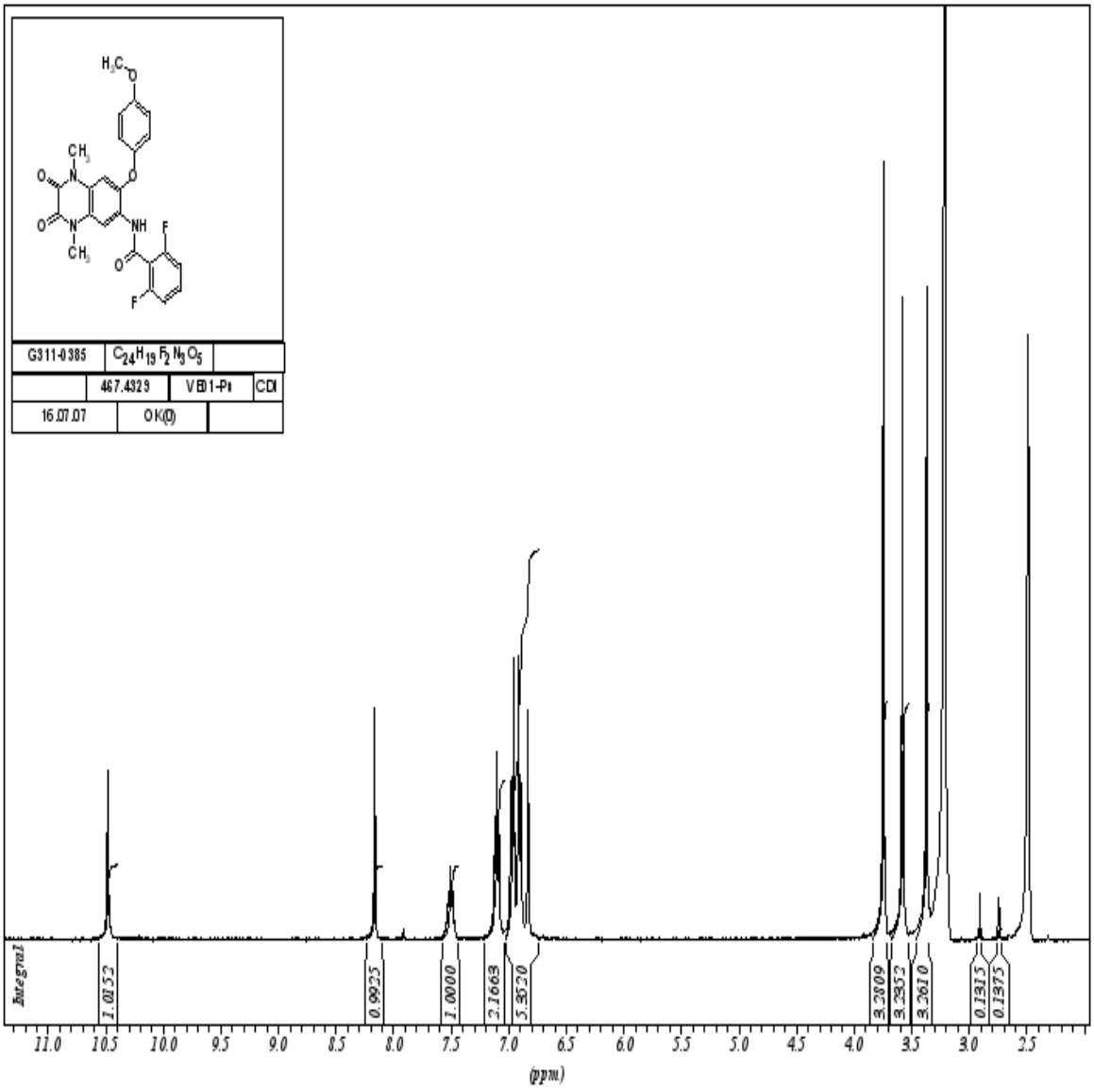
^{1}H-NMR spectra of compound **31**



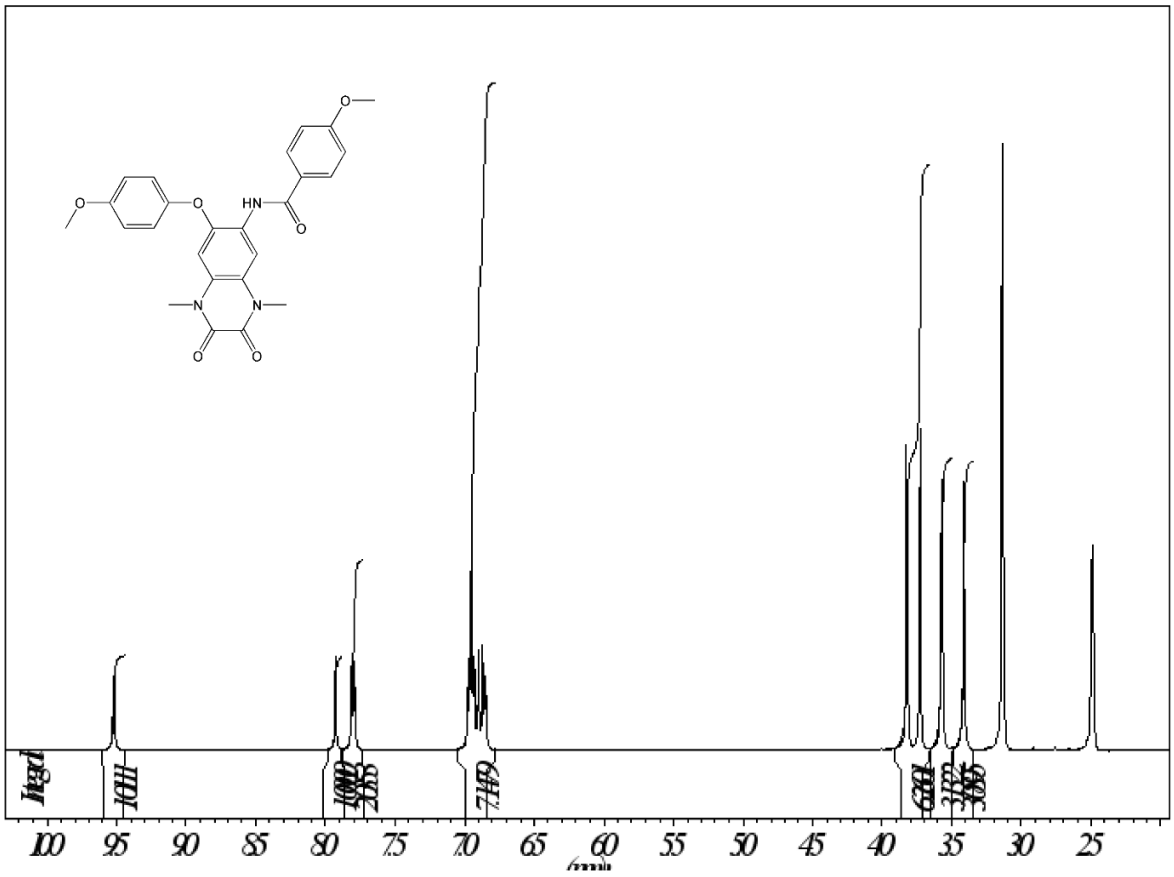
^{1}H-NMR spectra of compound **32**



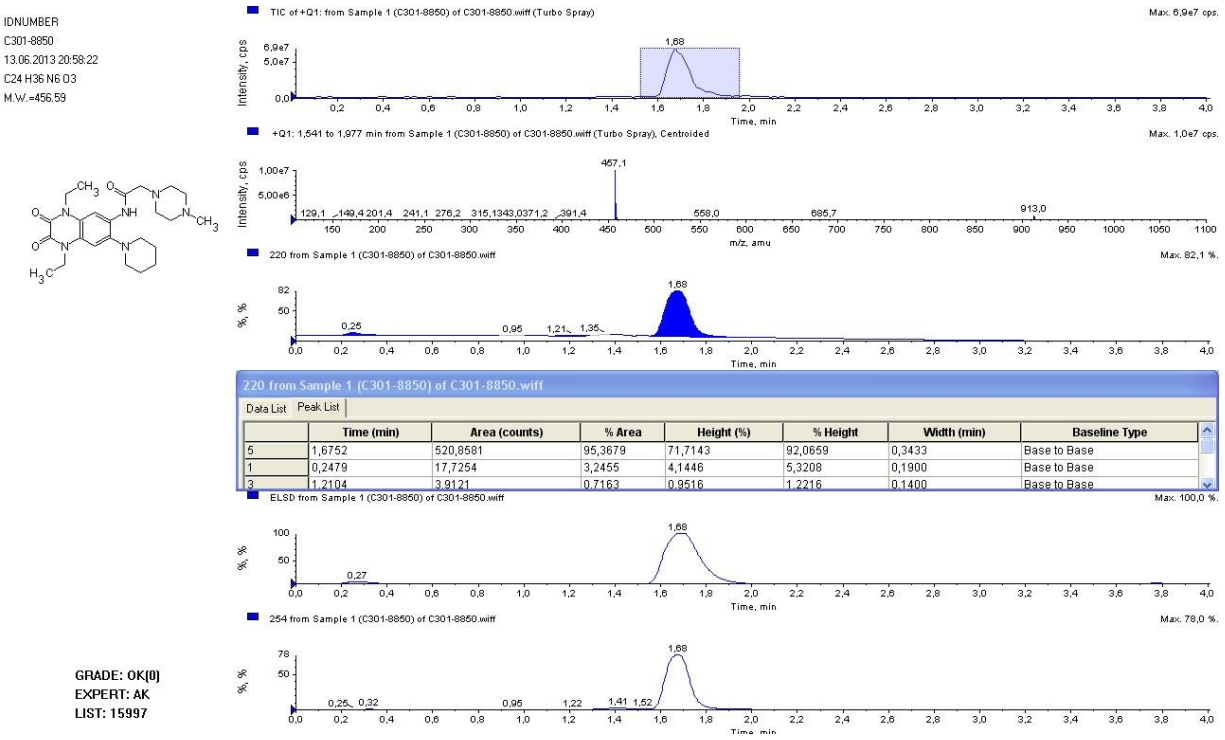
^{3}C-NMR spectra of compound **33**



^{1}H-NMR spectra of compound **34**

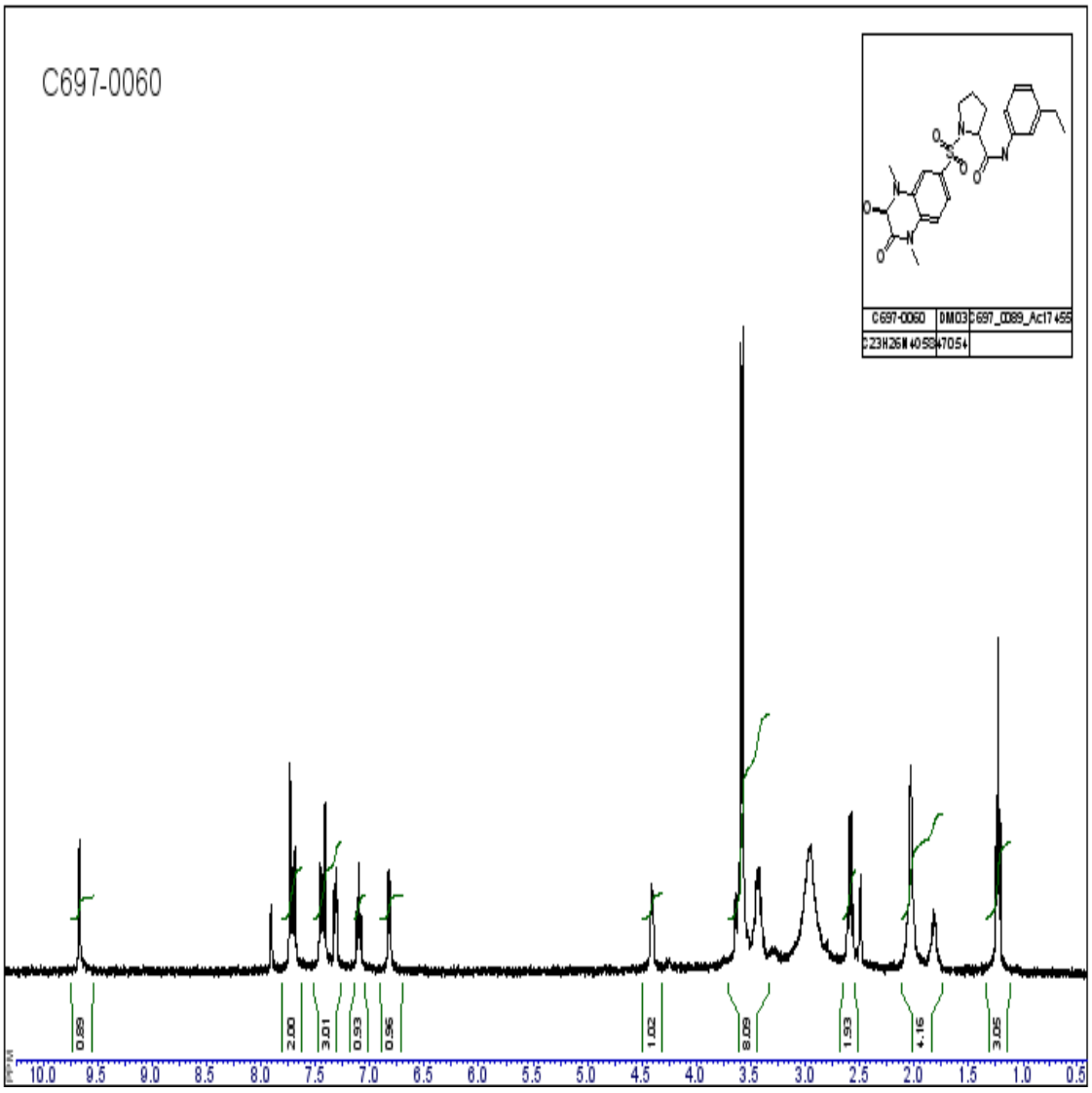


^{3}C-NMR spectra of compound **35**

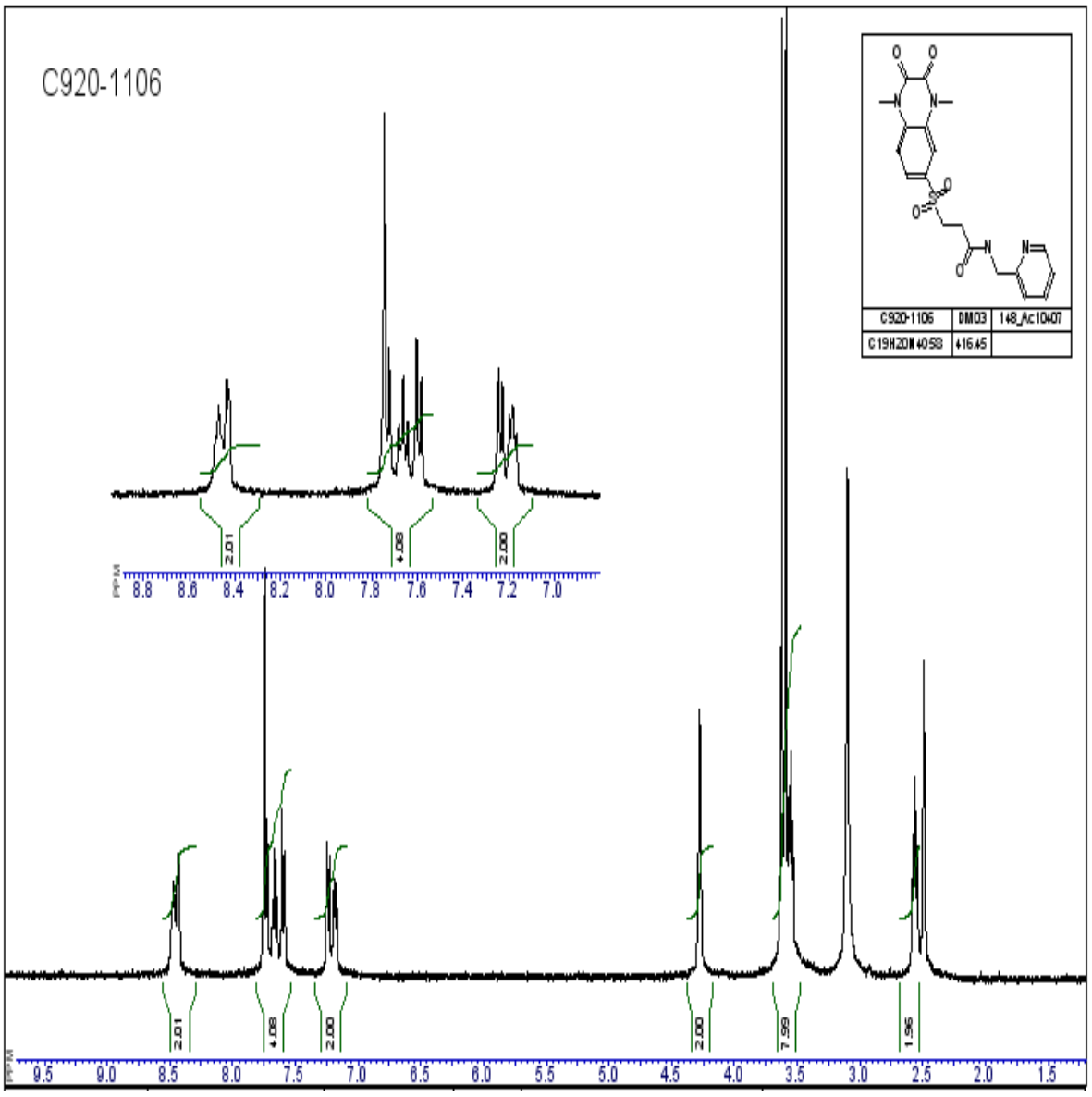


	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
5	1,6752	520,8581	95,3679	71,7143	92,0659	0,3433	Base to Base
1	0,2479	17,7254	3,2455	4,1446	5,3208	0,1900	Base to Base
3	1,2104	3,9121	0,7163	0,9516	1,2216	0,1400	Base to Base

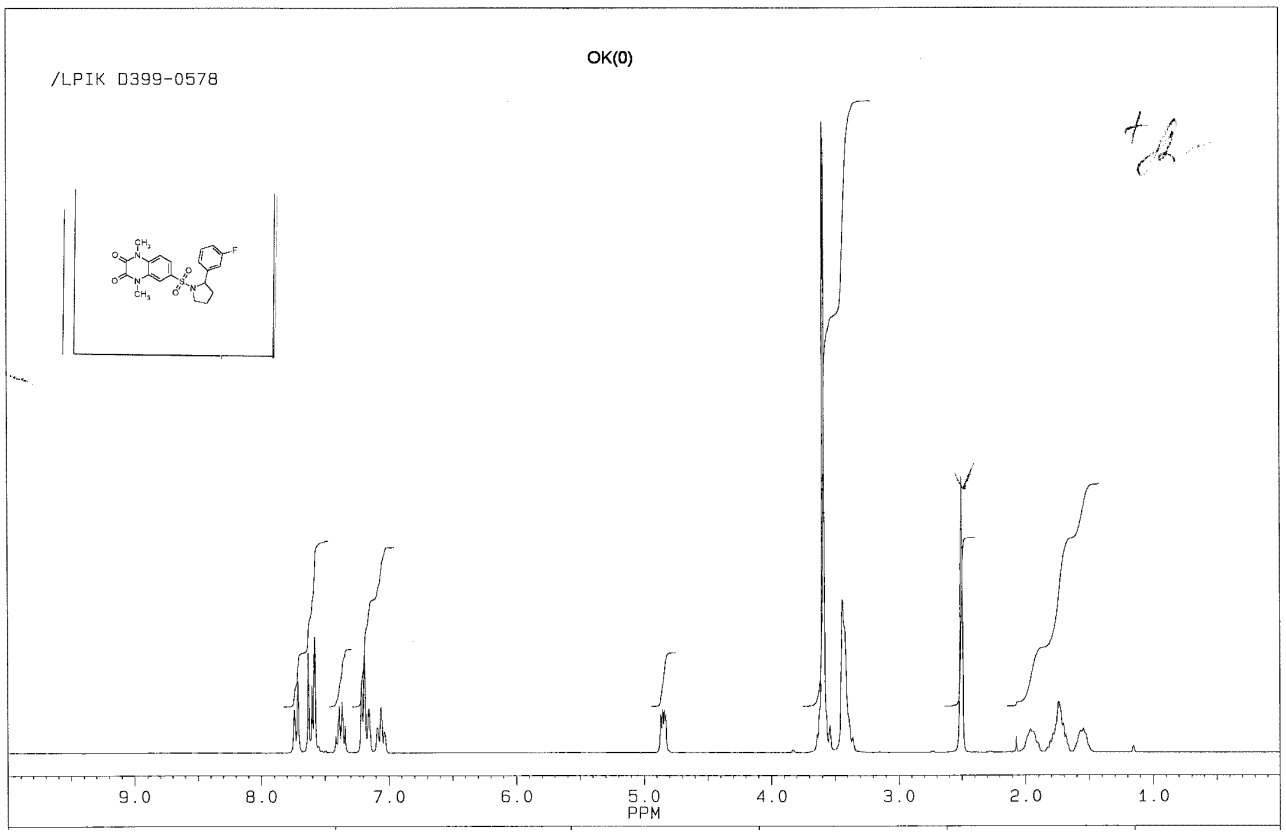
HPLC chromatogram and mass spectra of compound **36**



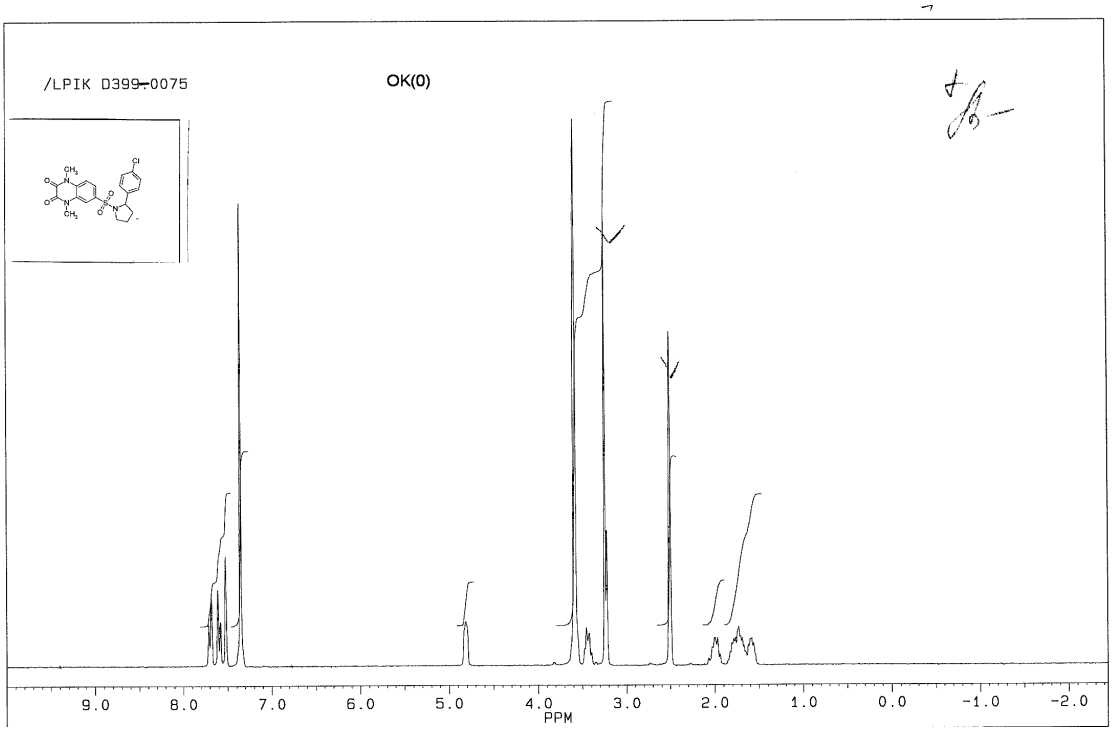
^{1}H-NMR spectra of compound **37**



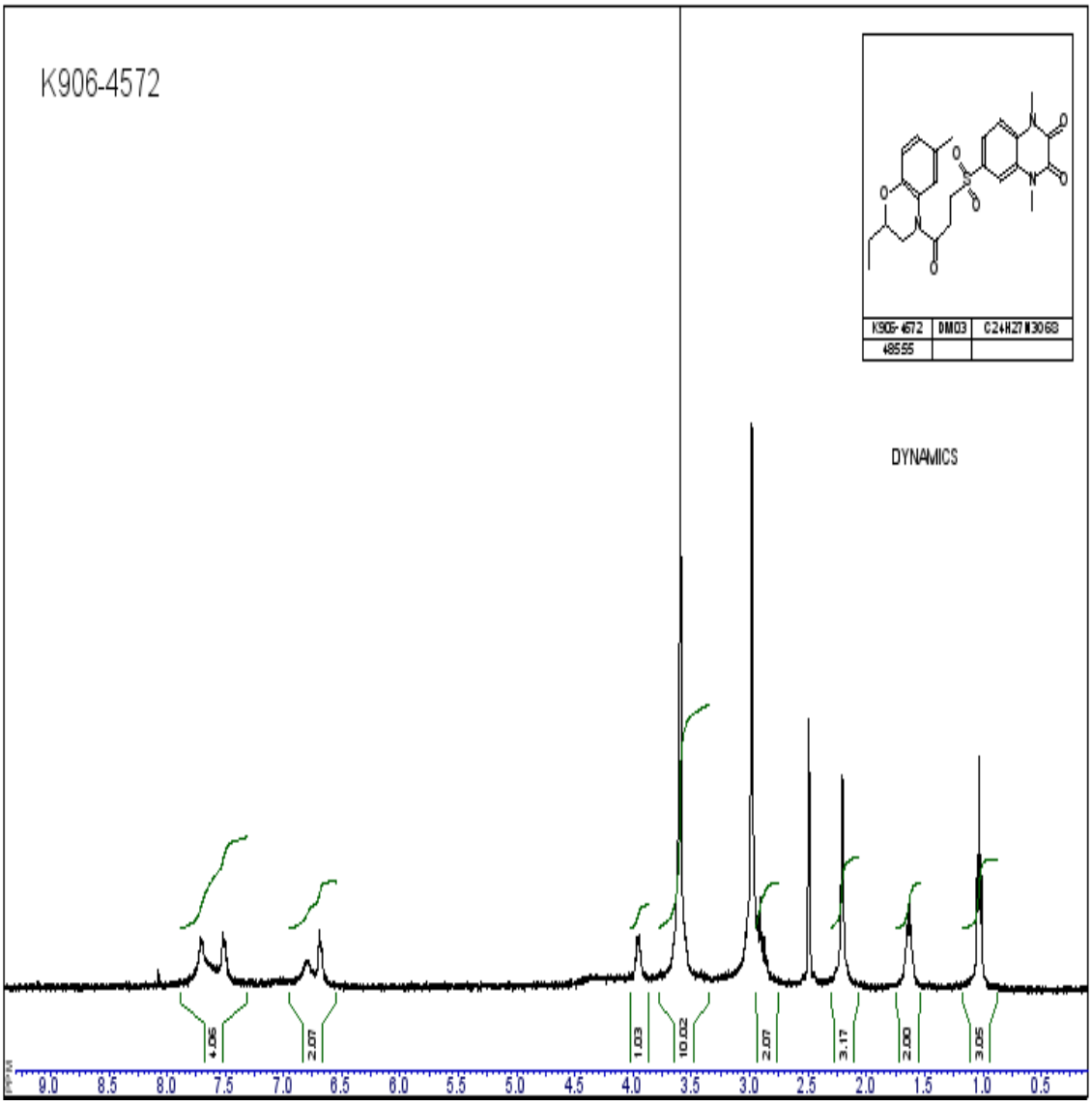
[1]H-NMR spectra of compound **38**



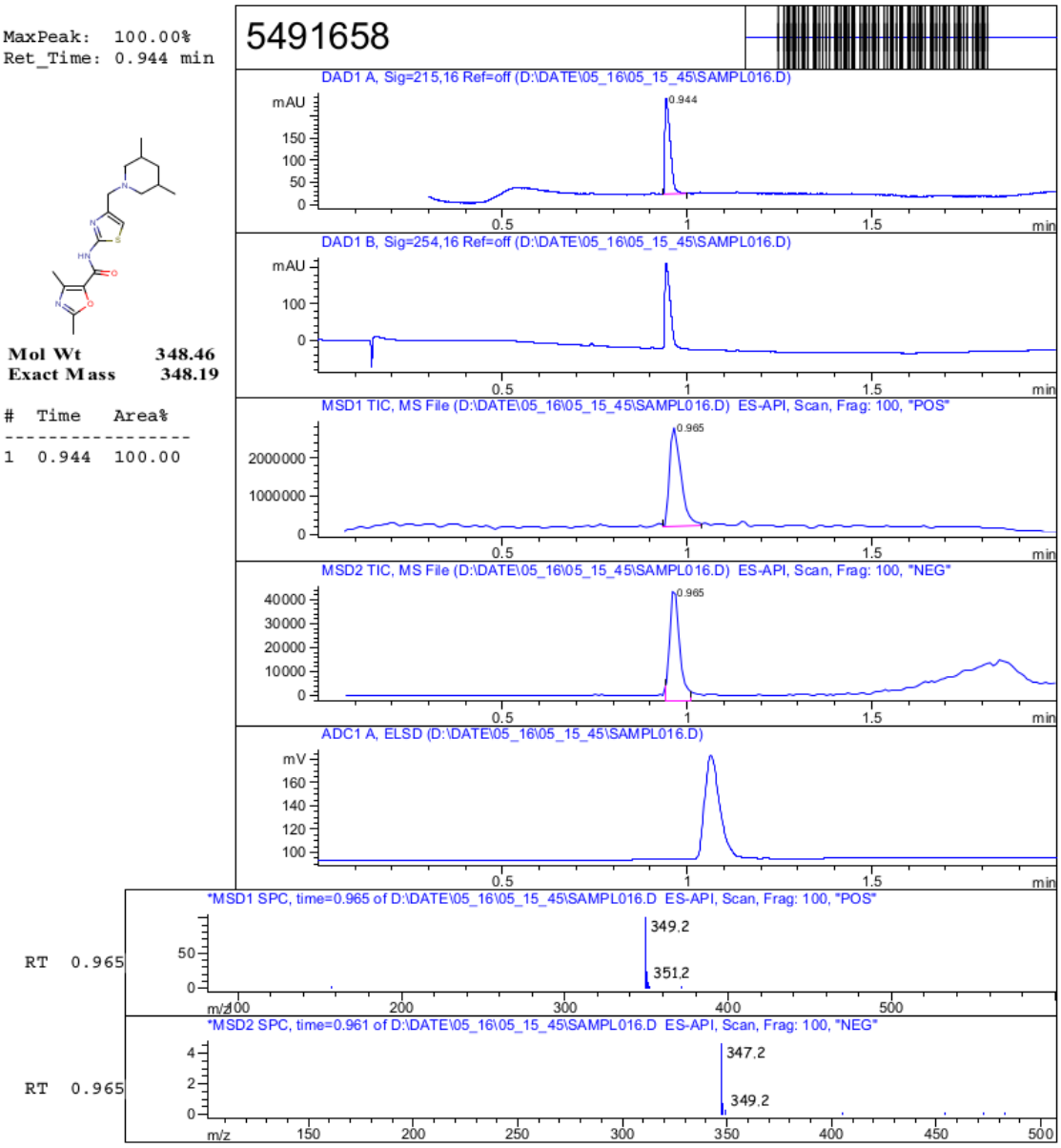
^{1}H-NMR spectra of compound **39**



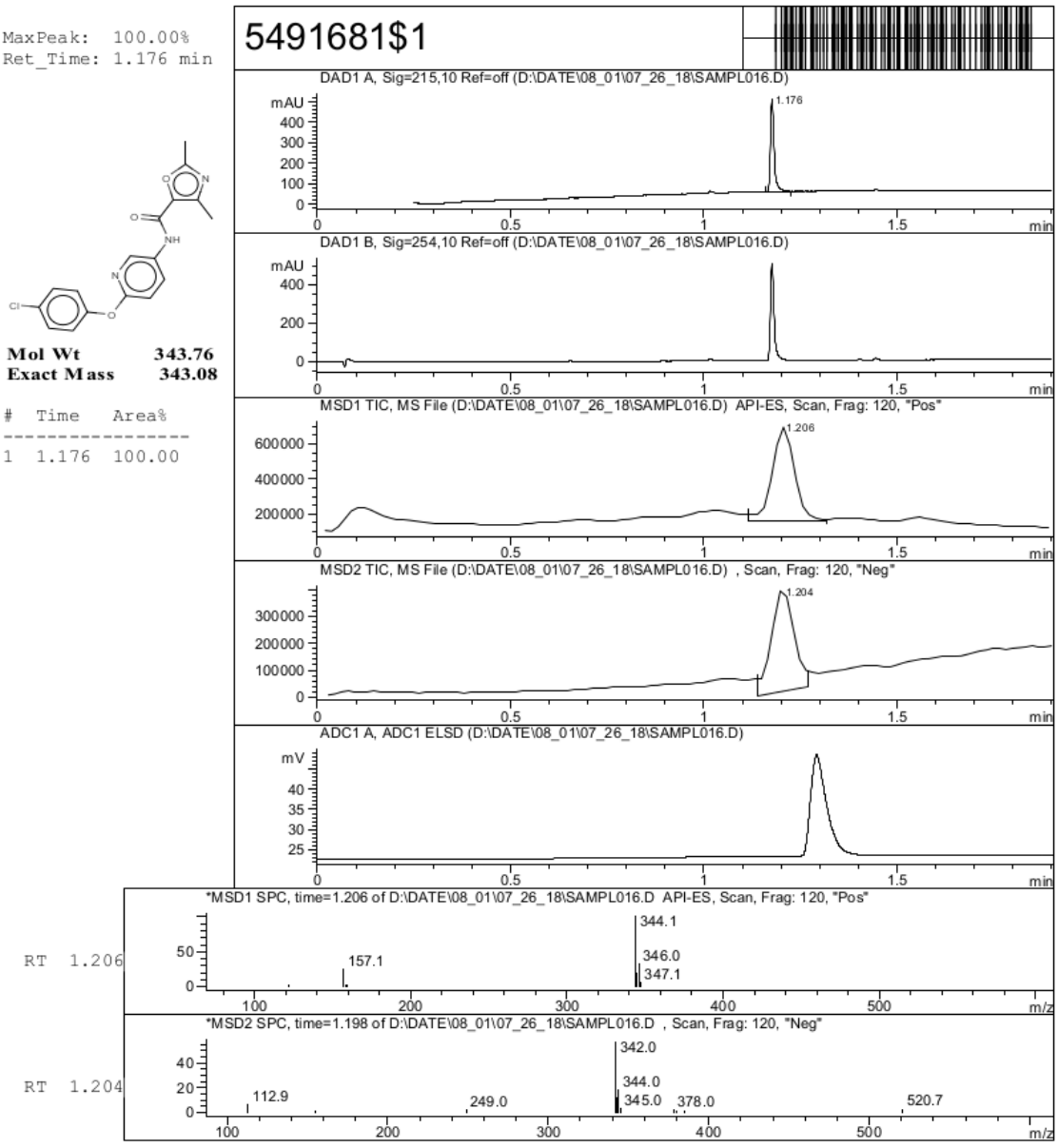
^{1}H-NMR spectra of compound **40**



^{1}H-NMR spectra of compound **41**



HPLC chromatogram and mass spectra of compound **42**



HPLC chromatogram and mass spectra of compound **43**