Design, Synthesis, and Biological Evaluation of 4-Quinoline Carboxylic Acids as Inhibitors of Dihydroorotate Dehydrogenase

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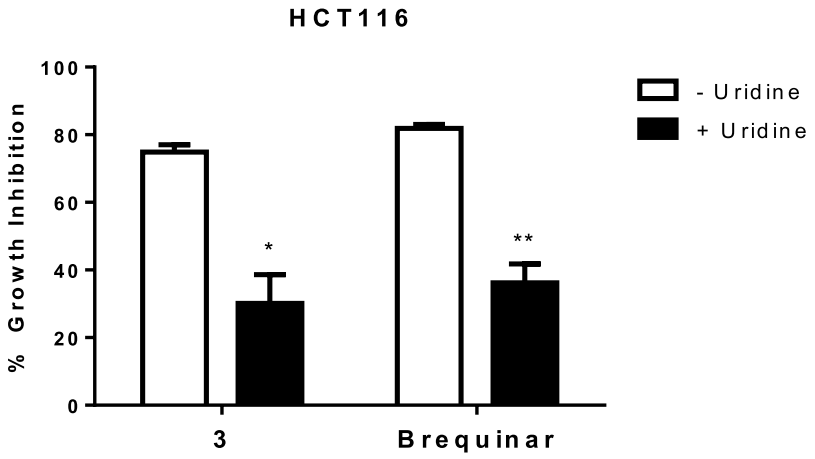
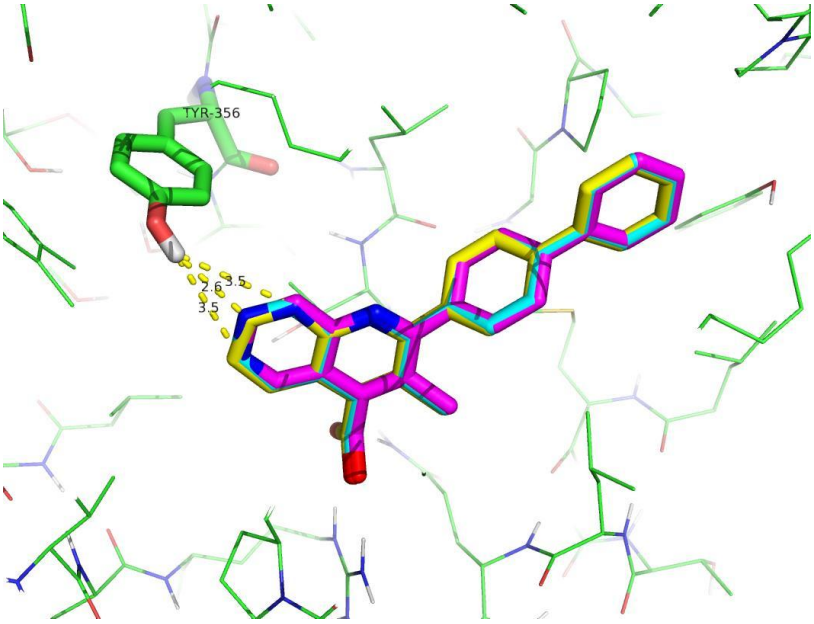
Supplemental Figures

Analysis Type by Cancer	Cancer vs. Normal		Cancer vs. Cancer			Cancer Subtype Analysis									Cancer vs. Baseline (DNA only)	Pathway and Drug		Outlier	
			Cancer Histology		Multi-cancer	Clinical Outcome	Metastasis vs. Primary	Molecular Subtype: Biomarker	Molecular Subtype: Mutation	Pathology Subtype: Grade	Pathology Subtype: Stage	Patient Treatment Response	Recurrence vs. Primary	Other		Drug Sensitivity	Perturbation		
Bladder Cancer																		2	2
Brain and CNS Cancer																		2	7
Breast Cancer																		6	8
Cervical Cancer																			
Colorectal Cancer	1																	5	6
Esophageal Cancer																			1
Gastric Cancer																		4	2
Head and Neck Cancer																		2	4
Kidney Cancer	1		1	1													1	3	4
Leukemia																		5	14
Liver Cancer		6	1	1														4	6
Lung Cancer																		2	4
Lymphoma	1																	4	7
Melanoma																		4	5
Myeloma																			9
Other Cancer																		4	10
Ovarian Cancer																		3	1
Pancreatic Cancer																		2	1
Prostate Cancer																		1	14
Sarcoma																		5	8
Significant Unique Analyses	3	6	2	2													1	51	101
Total Unique Analyses	464		743		255	622	50	254	634	211	462	100	27	118	243	942	291	964	

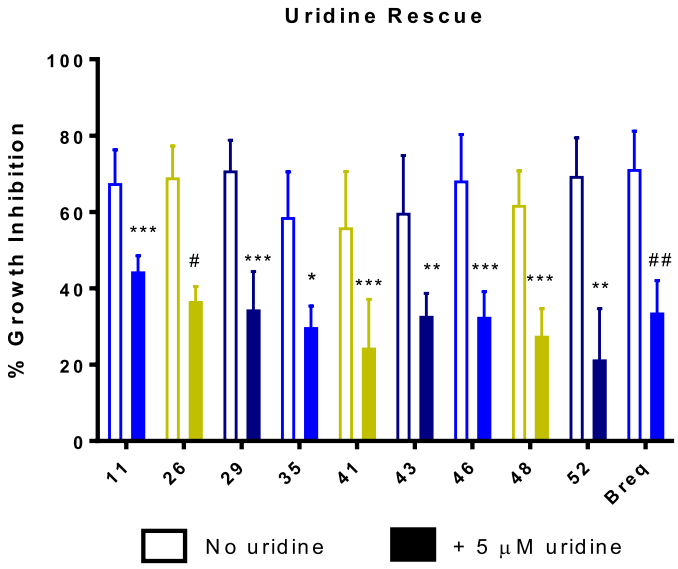
Supplemental Figure 1: Overexpression of DHODH in various cancers. This data was generated using the Oncomine database.[1-3]



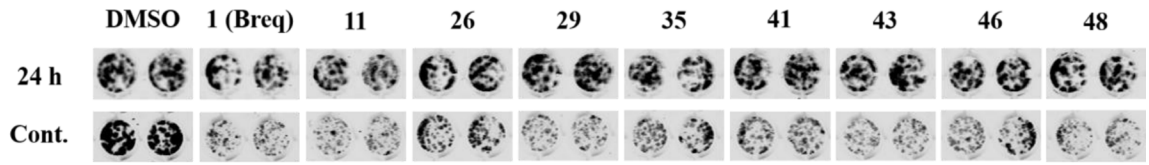
Supplemental Figure 2: Uridine supplementation rescues cell growth inhibition induced by lead compound 3. Compound 3 and brequinar were evaluated at twice their IC_{50} values. Antiproliferative effects were determined in media ± 5 μM uridine. Data are represented as the mean and standard deviation from three independent experiments. * $p < 0.001$, ** $p < 0.0005$



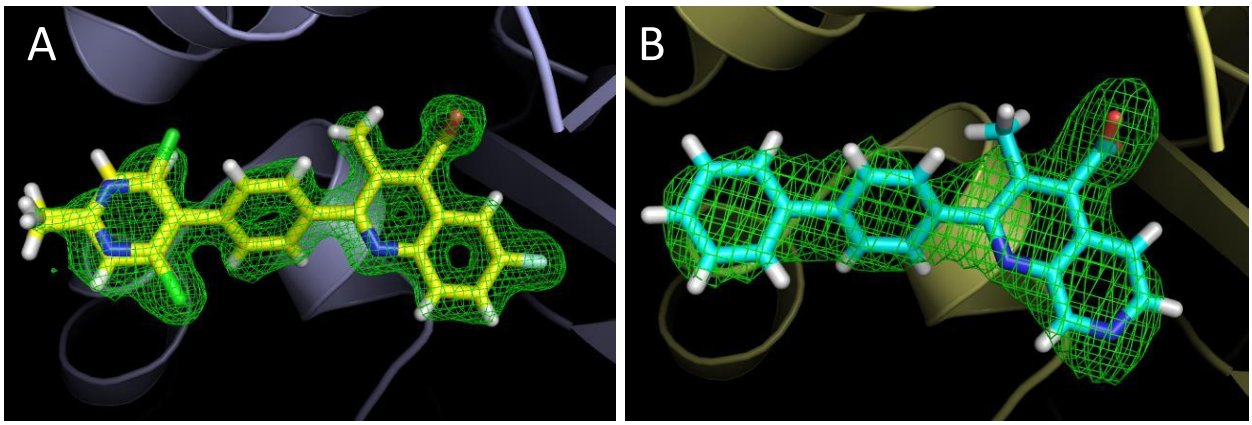
Supplemental Figure 3: **1,7-naphthyridines show optimal positioning to form a hydrogen bond with Y356.** DHODH (1D3G, green), **45** (yellow), **46** (cyan), and **47** (magenta).



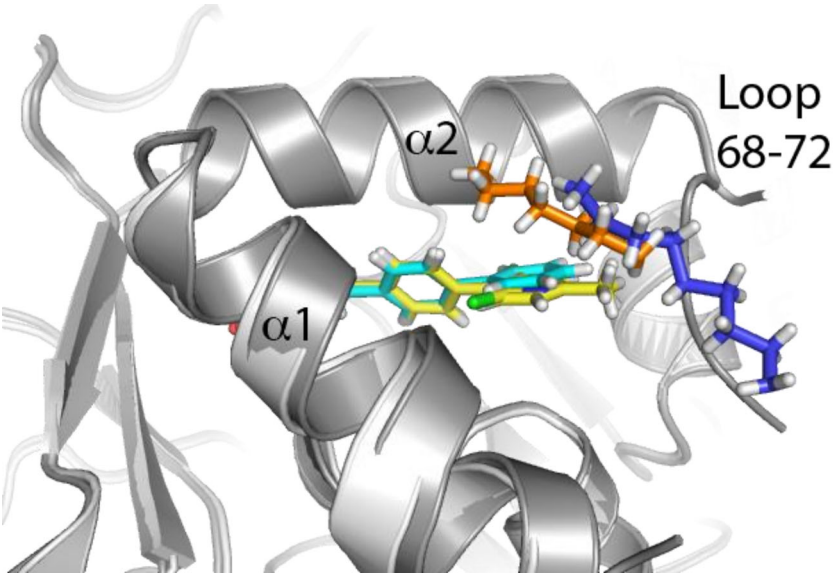
Supplemental Figure 4: Uridine supplementation rescues cell growth inhibition induced by select optimized analogs. Compounds were evaluated at twice their IC_{50} values. Antiproliferative effects were determined in media ± 5 μM uridine. Data are represented as the mean and standard deviation from at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, # $p = 0.0005$, ## $p \leq 0.0001$.



Supplemental Figure 5: **DHODH inhibitors are cytostatic.** HCT-116 cells were treated with 10 μM of the indicated analogs for either 24 hr or continuously for a period of seven days.



Supplemental Figure 6: Difference electron density (green mesh) corresponding to **43** (yellow, left) and **46** (cyan, right) contoured to 3σ.



Supplemental Figure 7: Overlay of DHODH structure with inhibitors **43** (polypeptide in light gray, inhibitor in yellow, Zwittergent aliphatic chain shown in orange) compared DHODH bound to **46** (polypeptide in dark gray, inhibitor in cyan, Zwittergent aliphatic chain shown in blue) showing differences in the loop near the binding site corresponding to residues 68-72 where peptide was able to be modeled. Also shown are modeled aliphatic chains associated with detergent packed against the 68-72 loop and inhibitors.

Supplemental Table 1: Molecular docking binding energies

Compound #	Affinity (kcal/mol)[a]	Predicted K_i
1D3G brequinar analog	-13.0	3.91E-11
13	-12.1	2.05E-10
14	-12.2	1.71E-10
16	-12.2	1.71E-10
22	-12.9	4.7E-11
26	-12.6	8.17E-11
28	-12.6	8.17E-11
32	-12.6	8.17E-11
45	-12.4	1.18E-10
46	-12.3	1.42E-10
47	-12.3	1.42E-10

[a]All binding energies shown are for the highest-ranking pose.

Supplemental Table 2: Crystallography Data Collection and Refinement Statistics

Data Collection	**DHODH:43**	**DHODH:46**
PDBID	6CJF	6CJG
Space Group	P1	$P3_221$
Unit Cell (Å)	a = 50.753 b= 51.528 c = 65.088 α = 89.15° β = 81.61° γ = 76.44°	a = b =90.098 c = 123.176 α = β = 90° γ = 120°
Wavelength (Å)	0.9786	0.9786
Resolution (Å)[1]	1.63 (1.63-1.66)	2.85 (2.85-2.90)
Rsym[2]	0.056 (0.429)	0.223 (1.075)
<I/□I>[3]	10 (2)	10 (2)
Completeness (%)[4]	97.2 (95.2)	100 (100)
Redundancy	4.0 (4.0)	16.2 (15.2)
Refinement		
Resolution (Å)	1.63	2.85
R-Factor [5]	0.163	0.170
Rfree [6]	0.188	0.206
Protein atoms	5303	2654
Compounds	2	1
Water Molecules	365	67
Unique Reflections	75537	13950
R.m.s.d.[7]		
Bonds	0.006	0.008
Angles	0.865	0.991
MolProbity Score[8]	1.44	1.54
Clash Score[8]	3.93	4.22
RSR[9]	0.10/0.09	0.16
RSCC[9]	0.93/0.93	0.97

[1]Statistics for highest resolution bin of reflections in parentheses.

[2]$R_{sym} = \Sigma_h\Sigma_j \mid I_{hj} - \langle I_h \rangle \mid / \Sigma_h\Sigma_j I_{hj}$, where I_{hj} is the intensity of observation j of reflection h and $\langle I_h \rangle$ is the mean intensity for multiply recorded reflections.

[3]Intensity signal-to-noise ratio.

[4]Completeness of the unique diffraction data.

[5]R-factor = $\Sigma_h \mid IF_oI - IF_cI \mid / \Sigma_h|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes for reflection h.

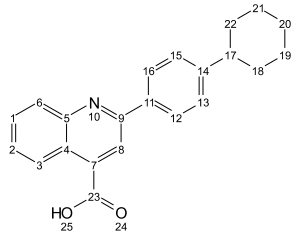
[6]R_{free} is calculated against a 5% random sampling of the reflections that were removed before structure refinement.

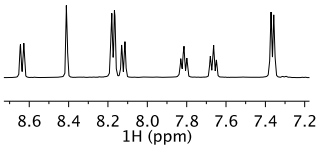
[7]Root mean square deviation of bond lengths and bond angles.

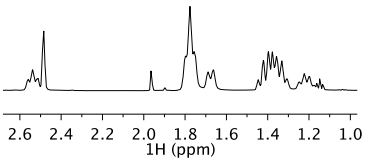
[8]Chen et al. (2010) MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallographica D66:12-21.

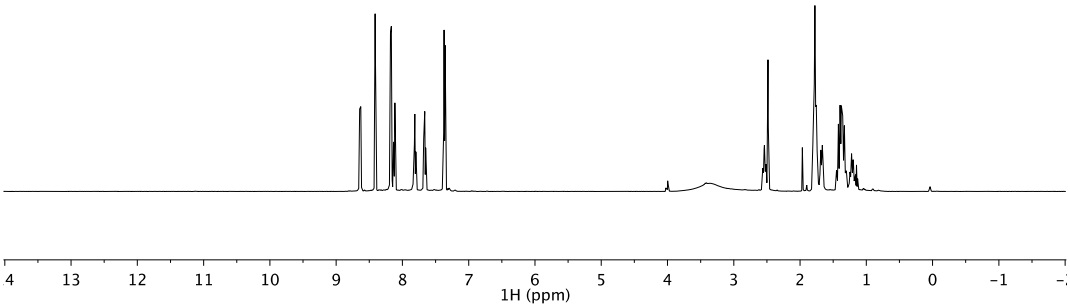
[9]wwPDB Validation Server.

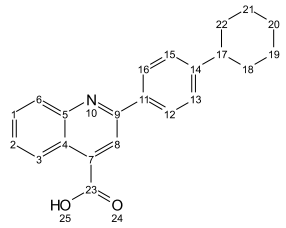
Copies of NMR Spectra







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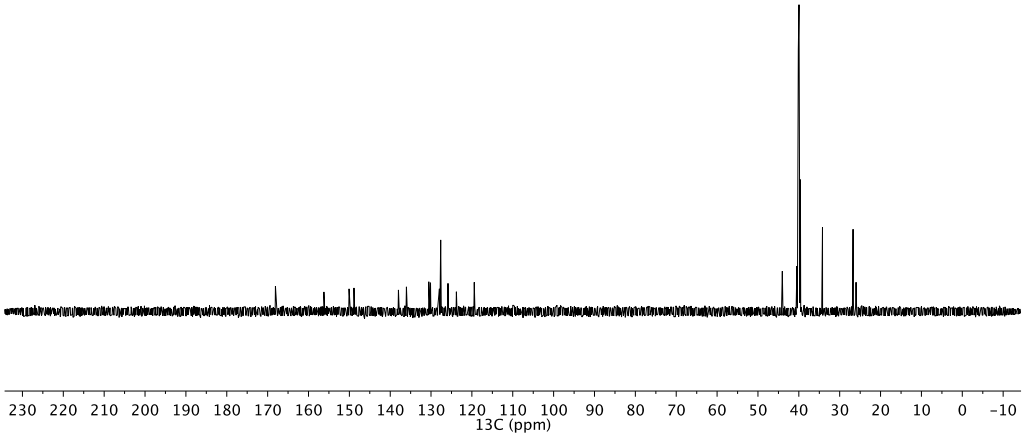
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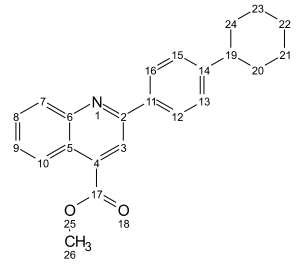
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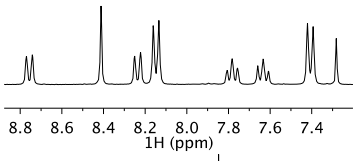
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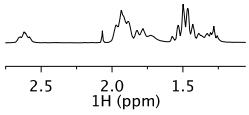
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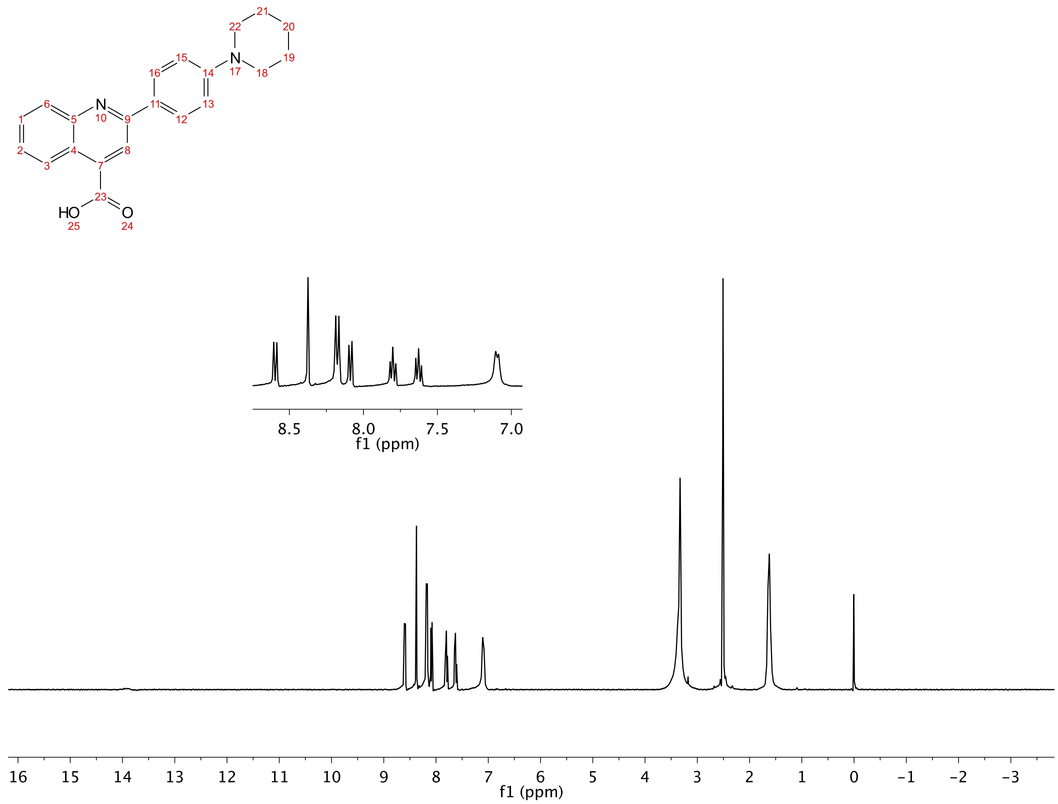
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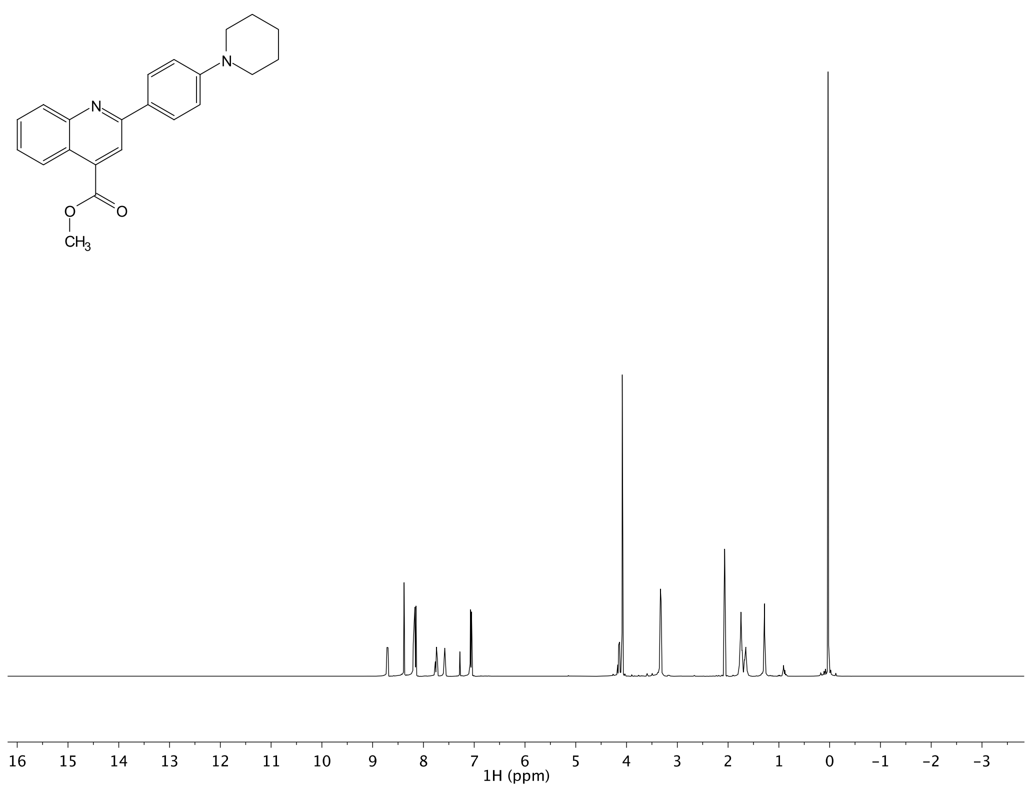
f1 (ppm)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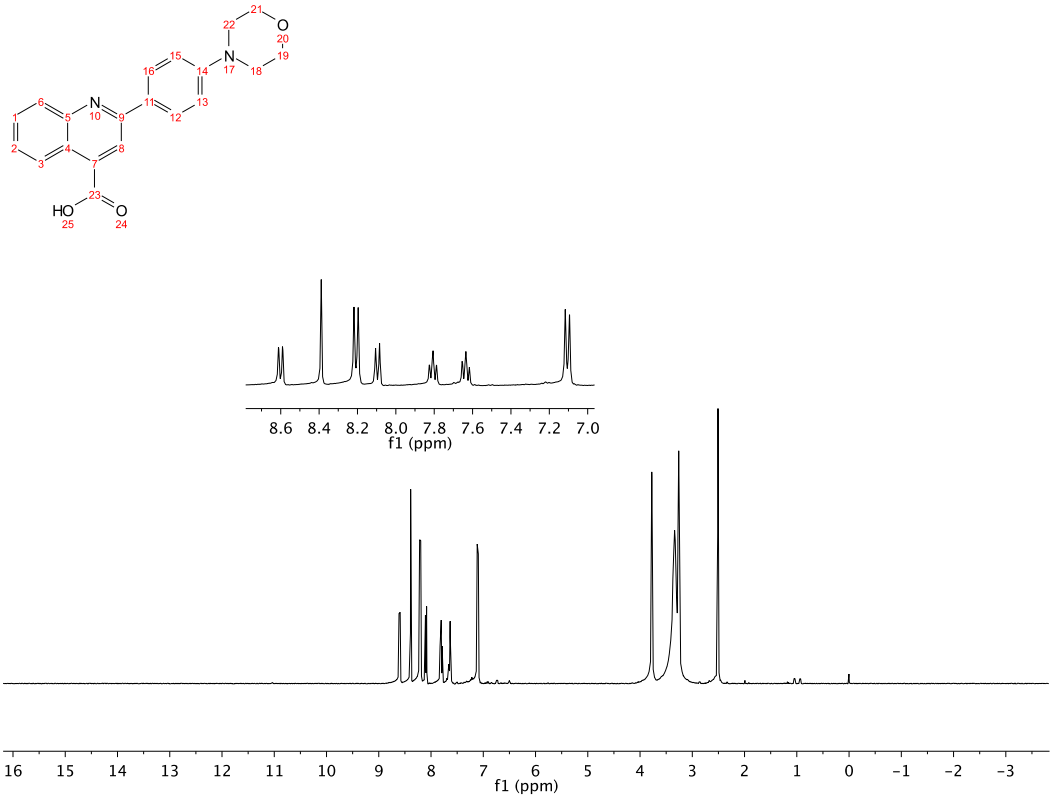
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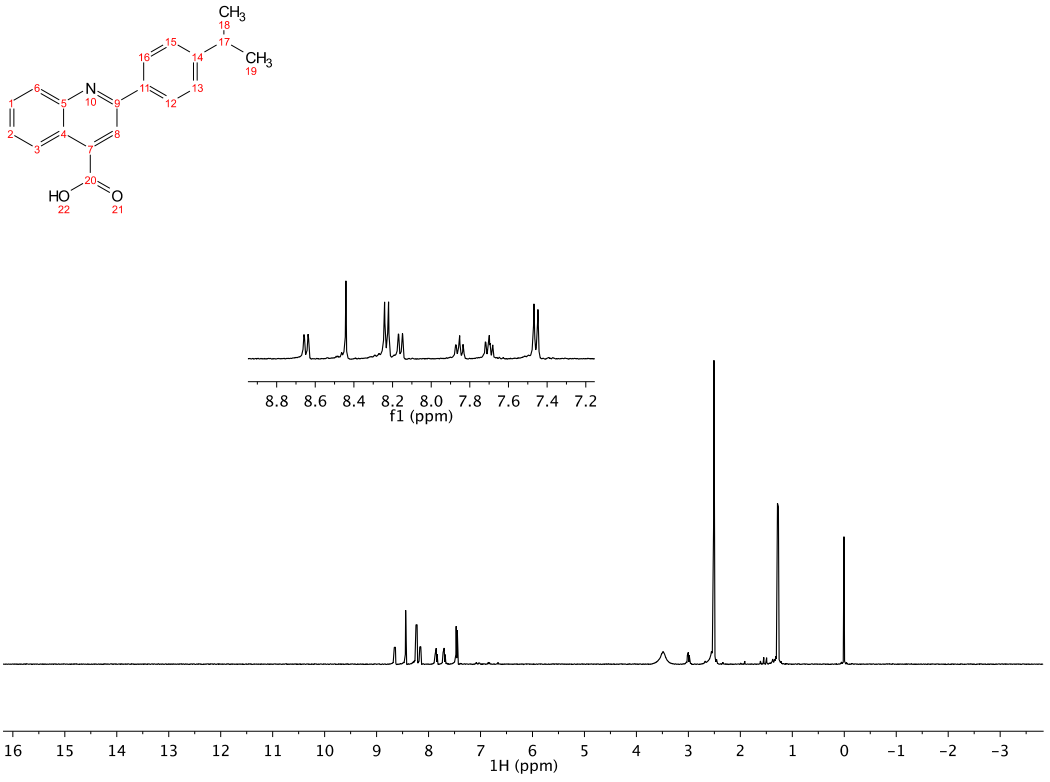
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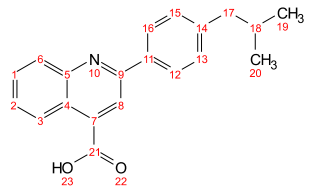
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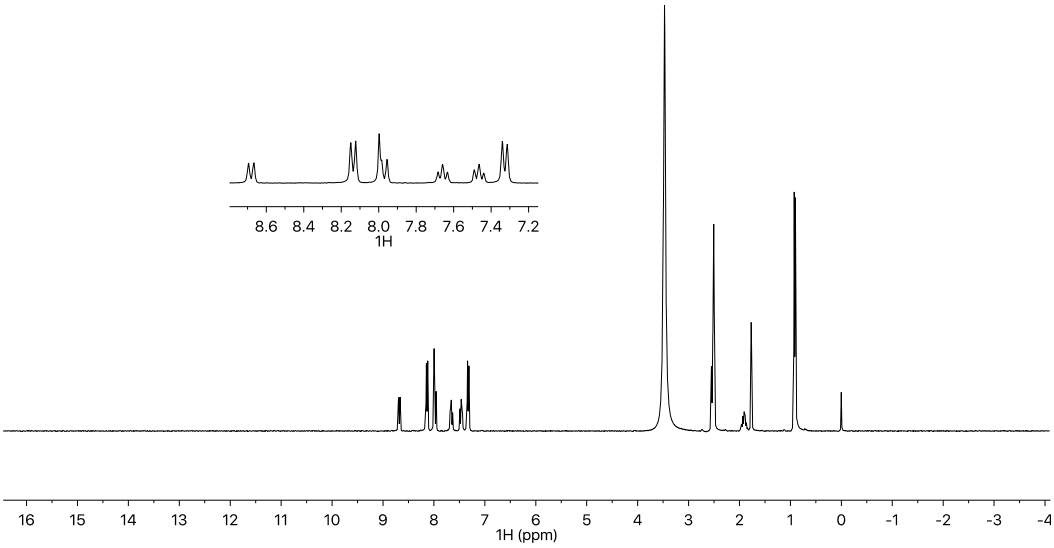
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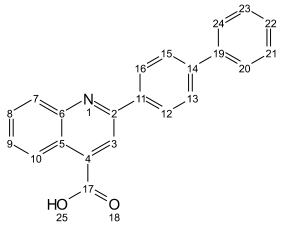
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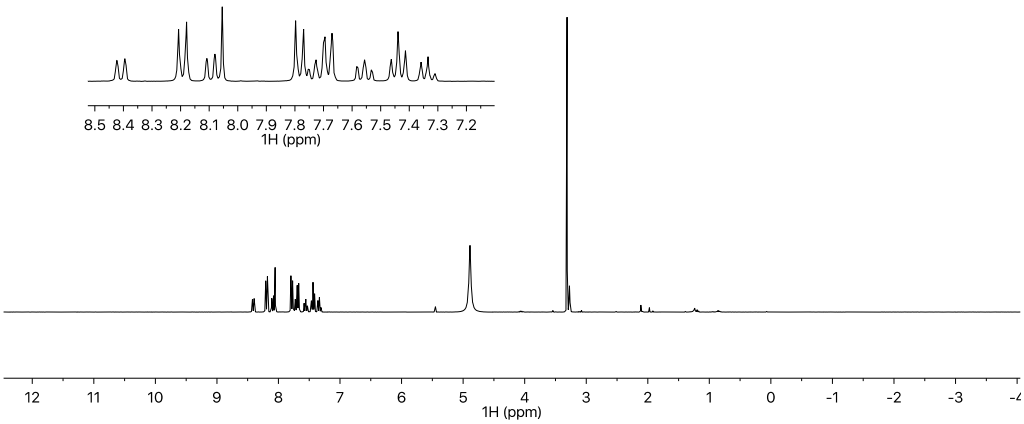
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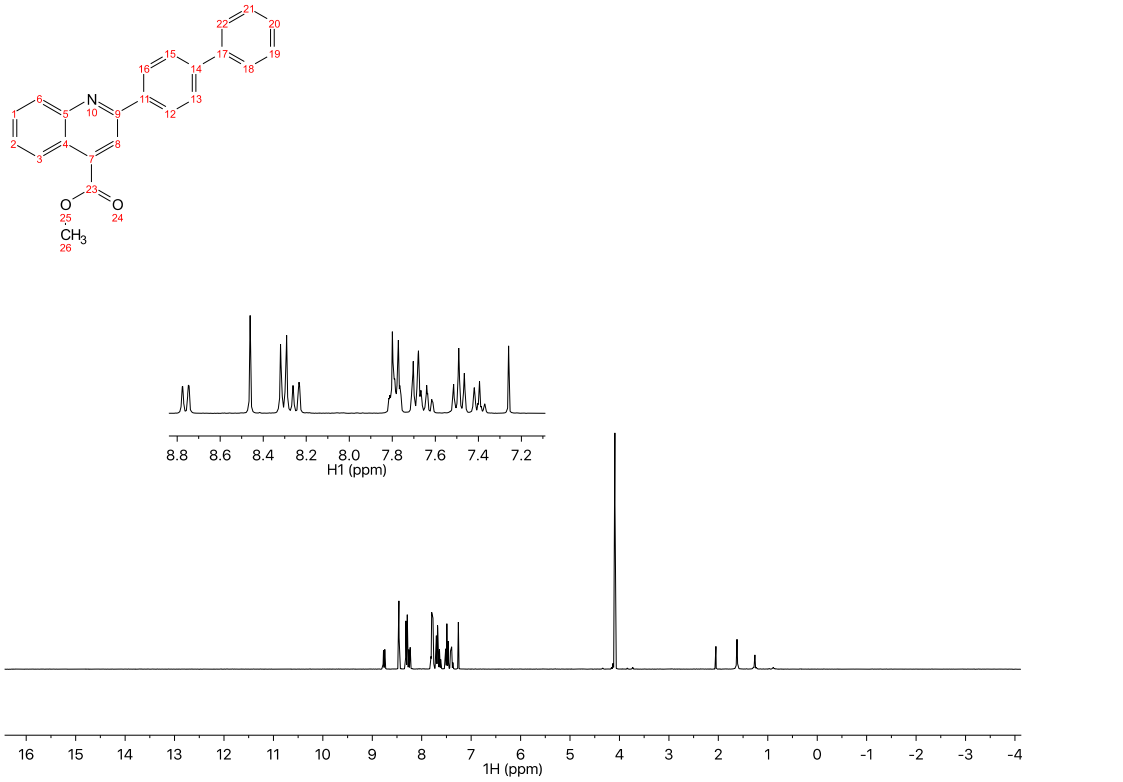
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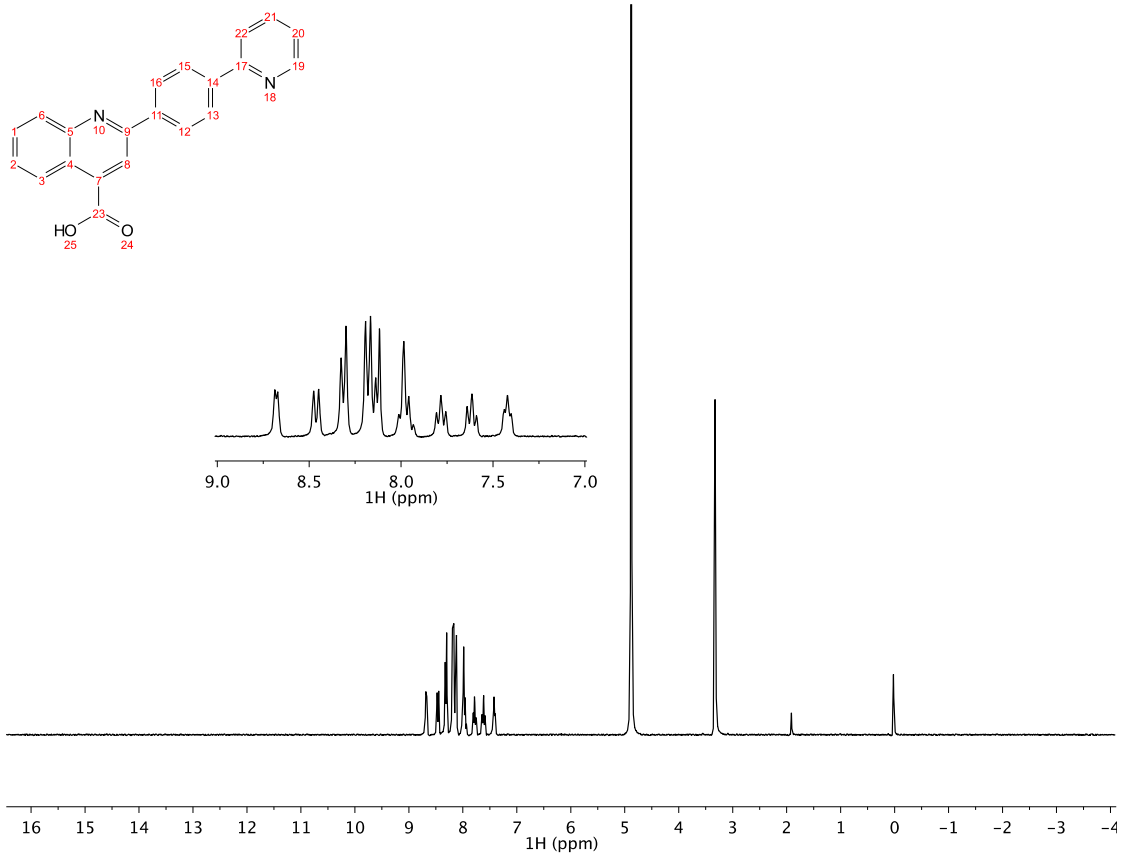
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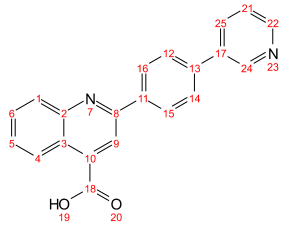
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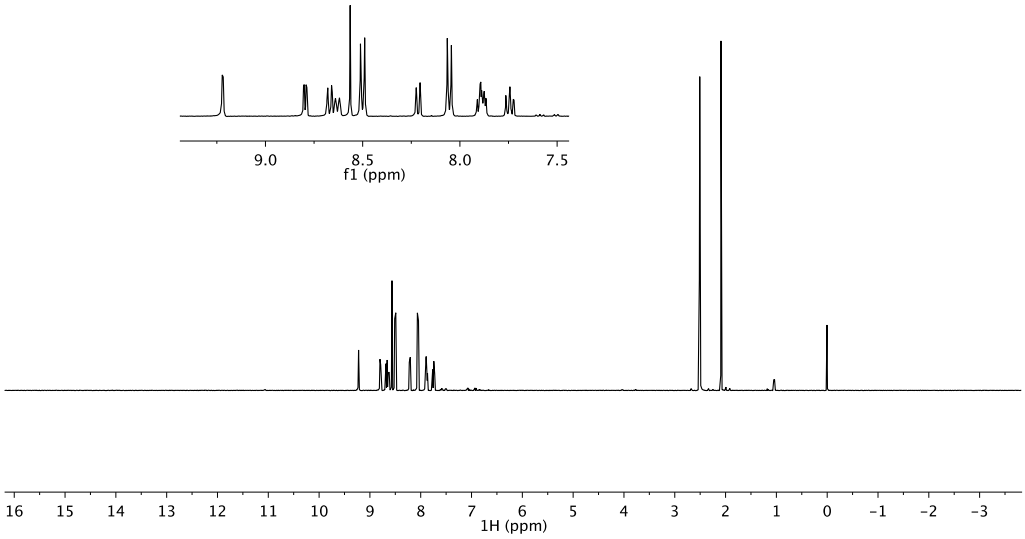
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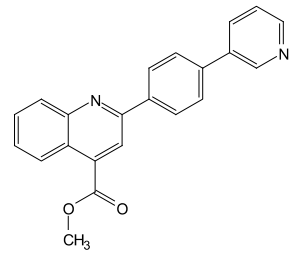
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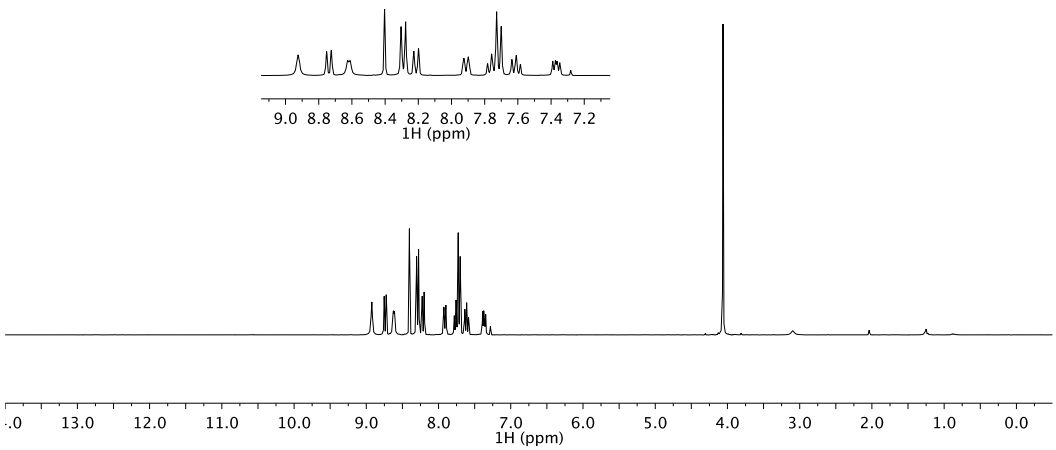
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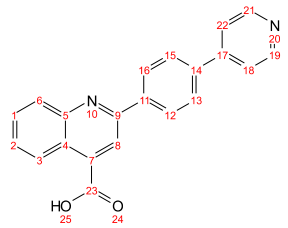
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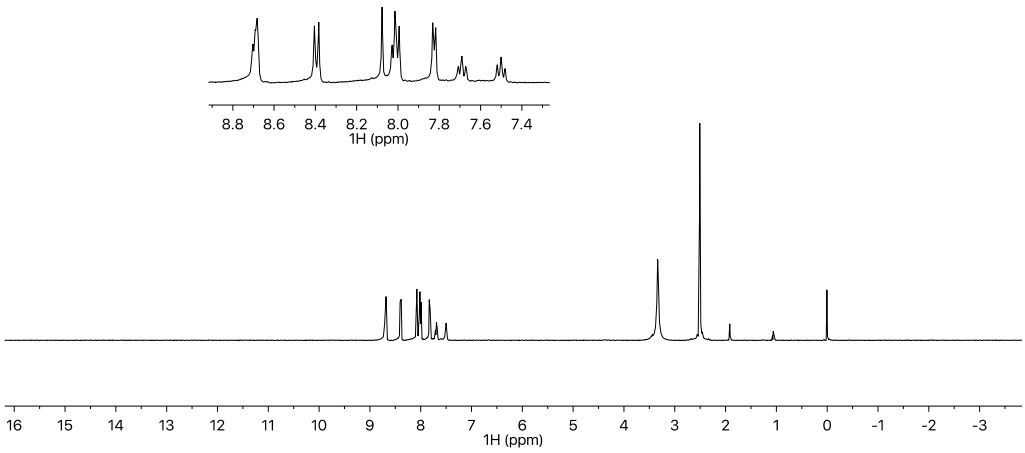
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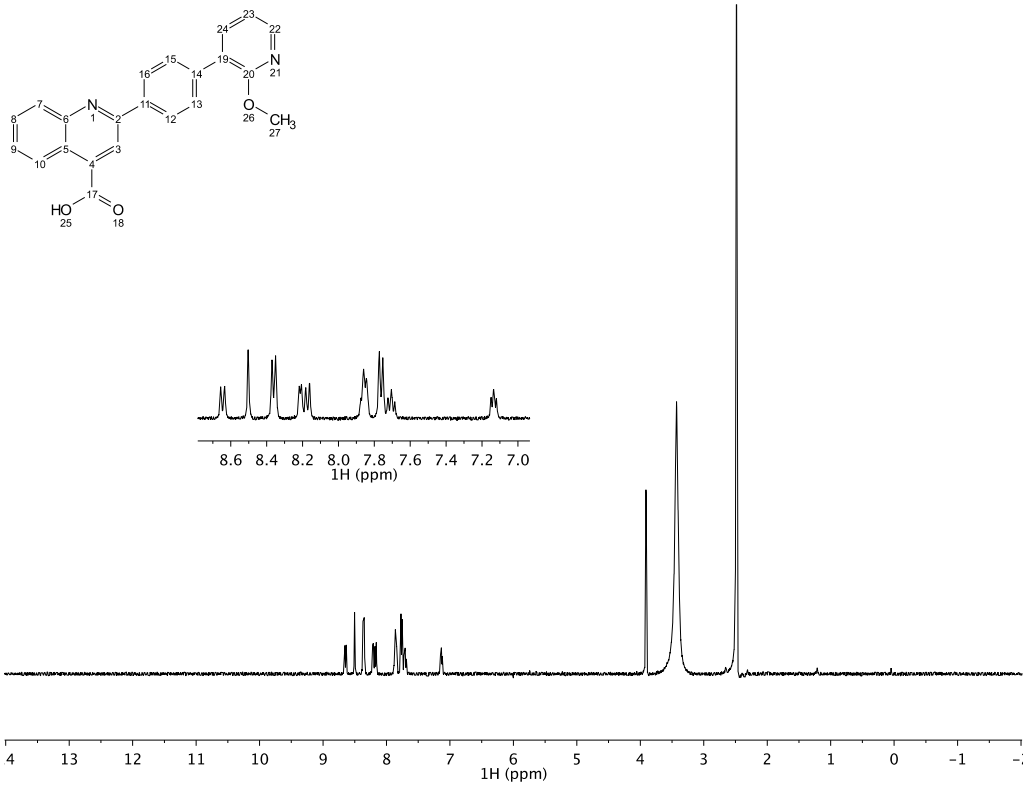
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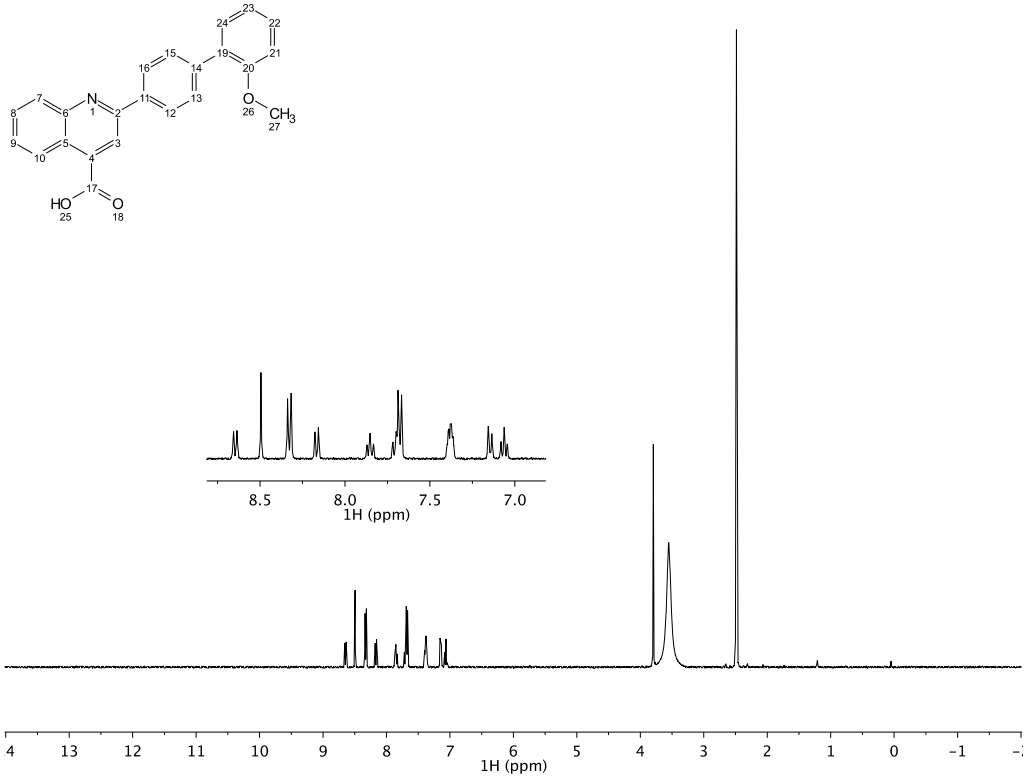
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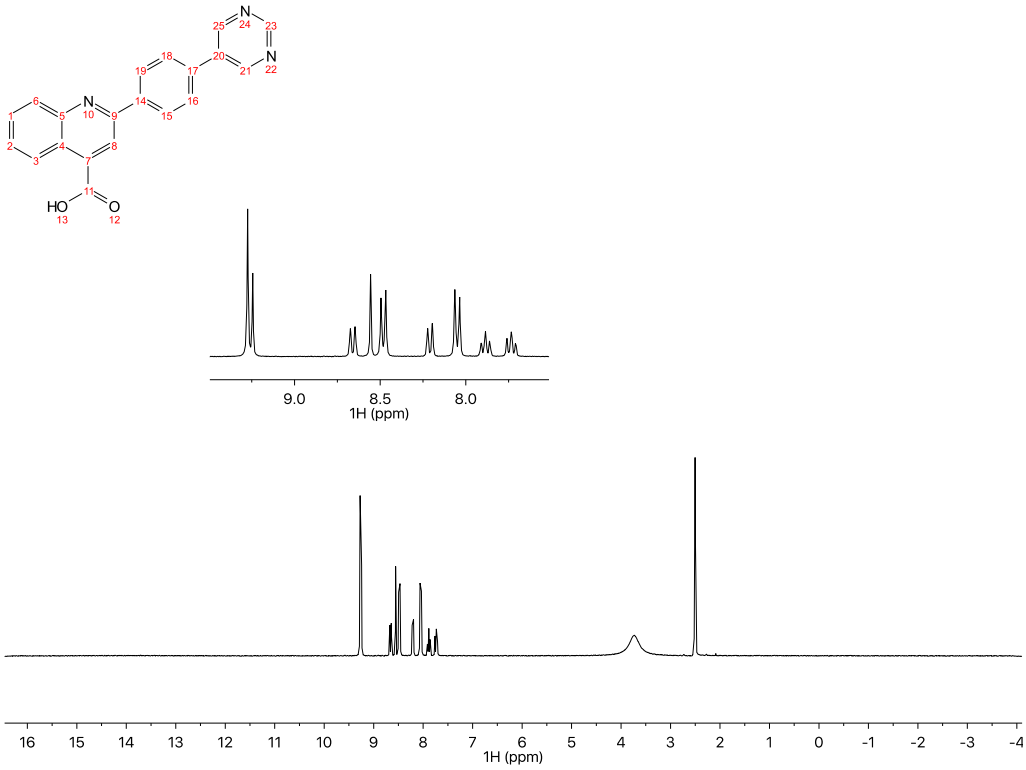
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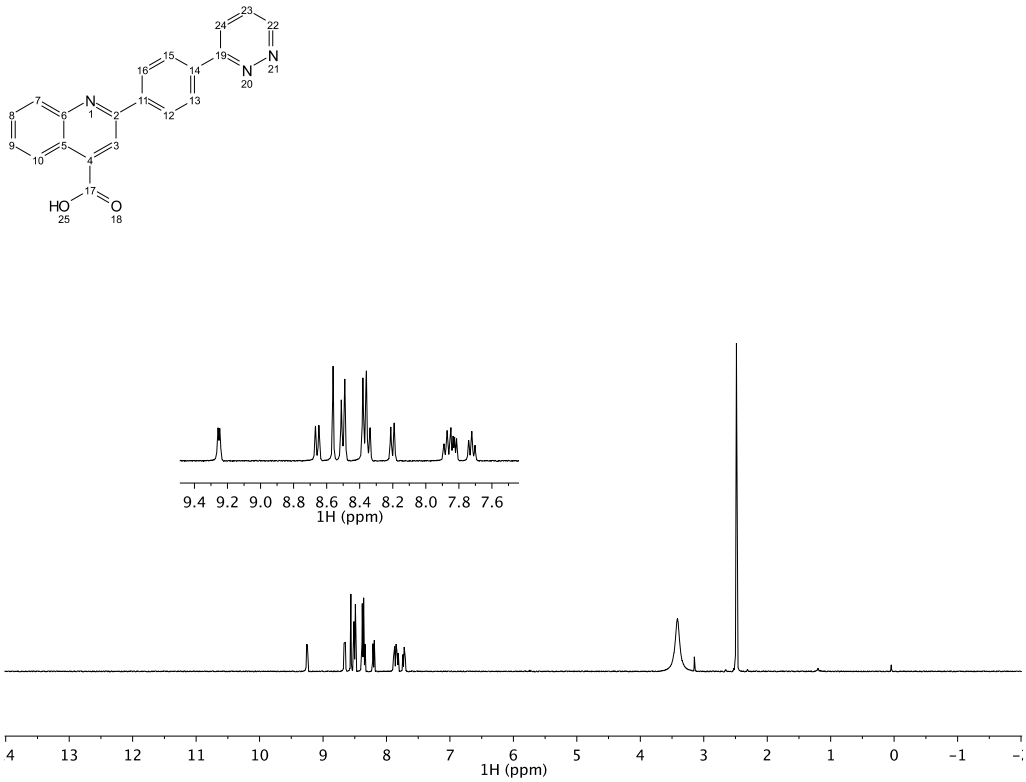
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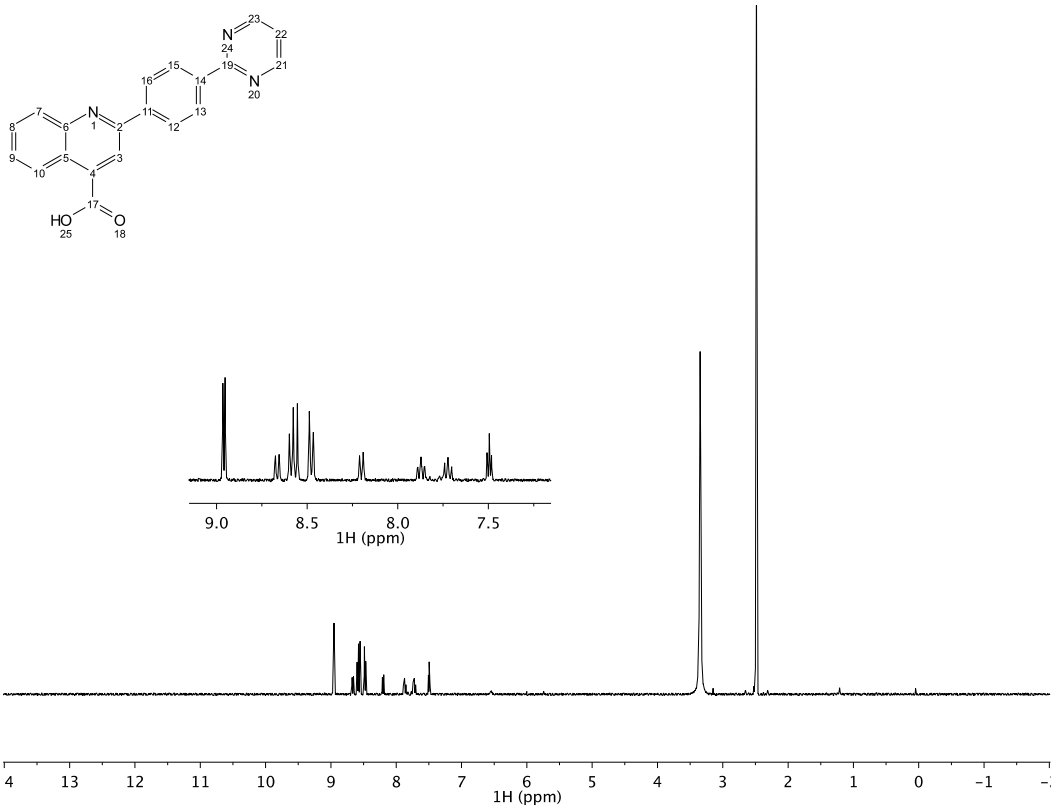
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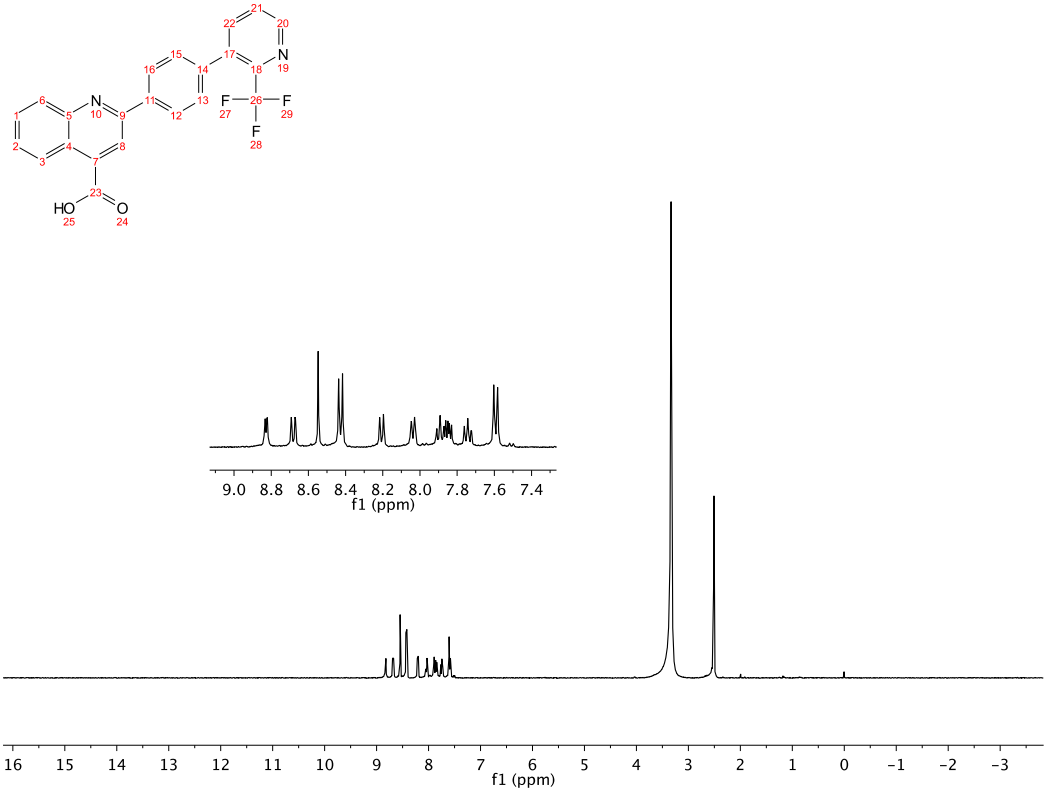
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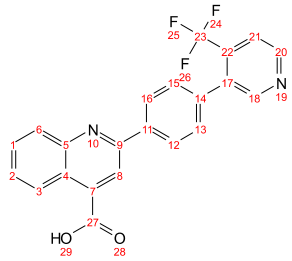
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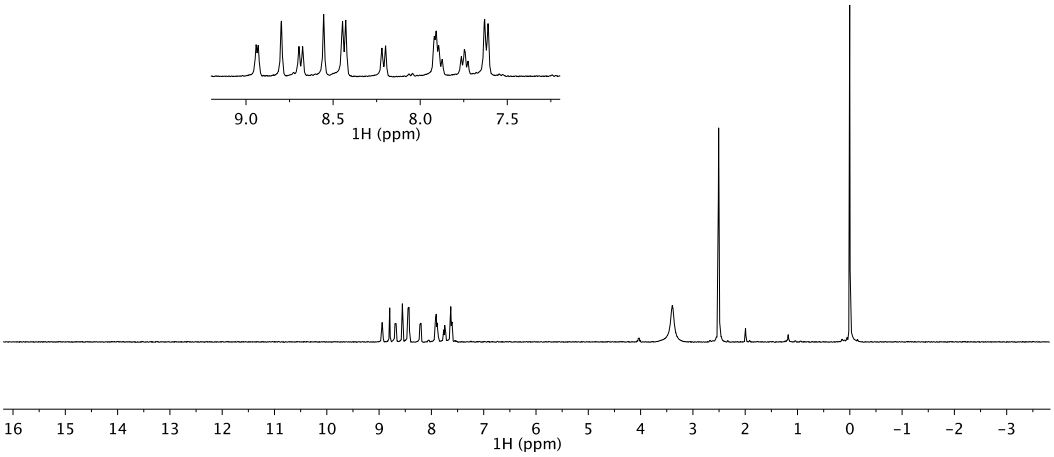
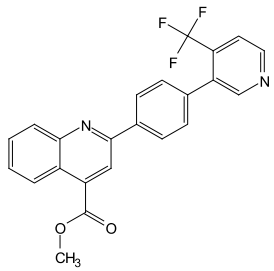
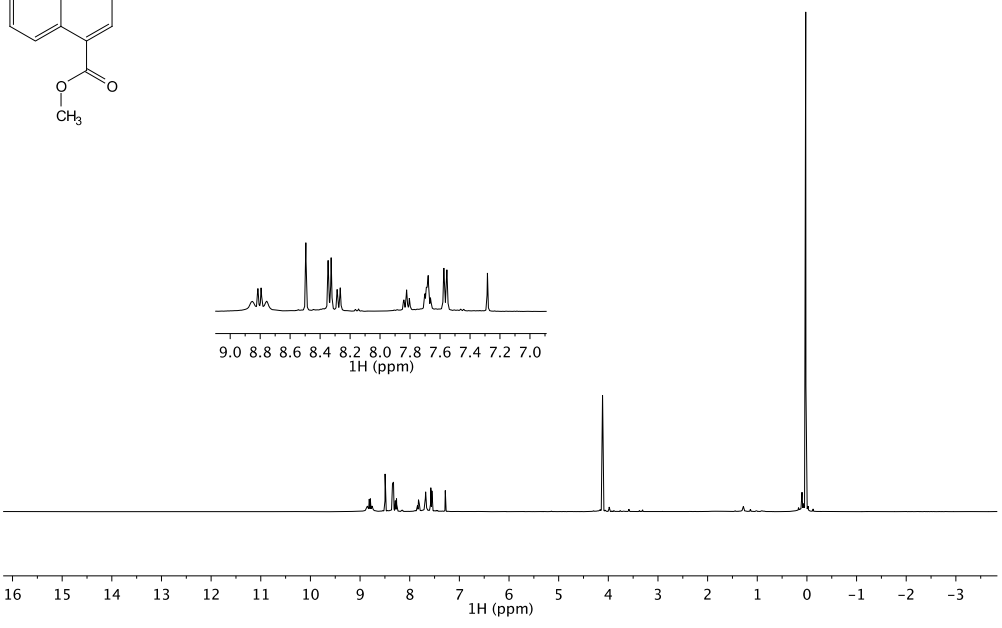
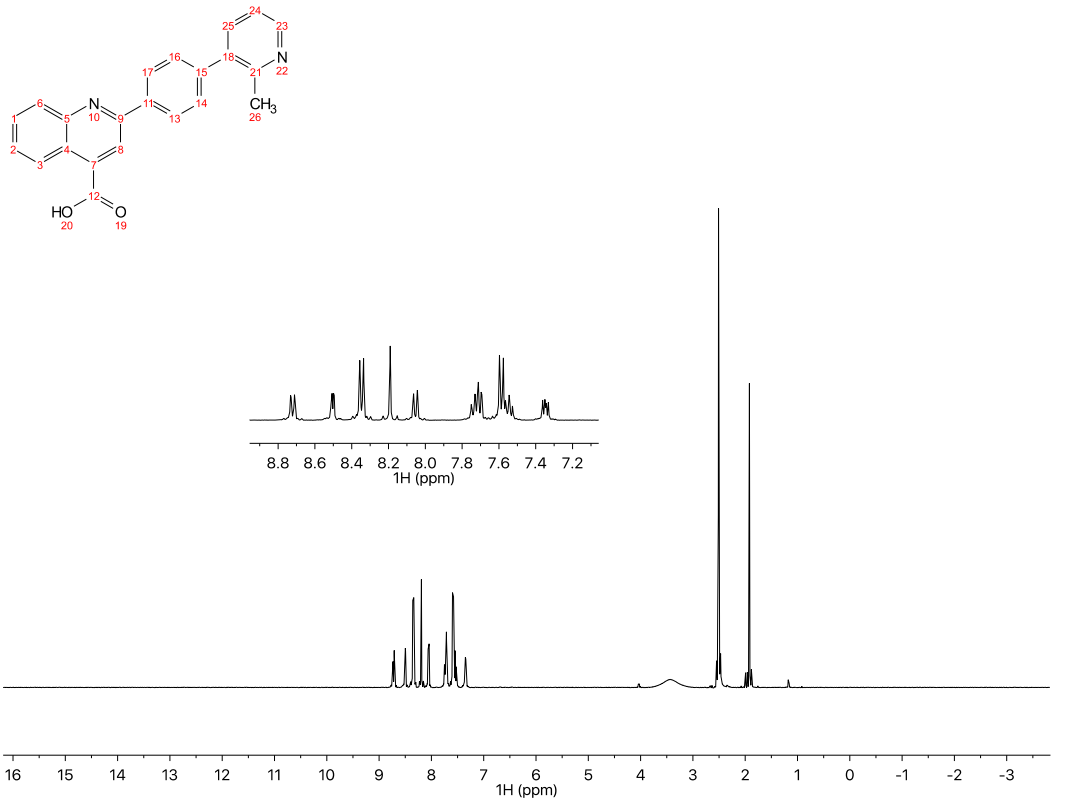
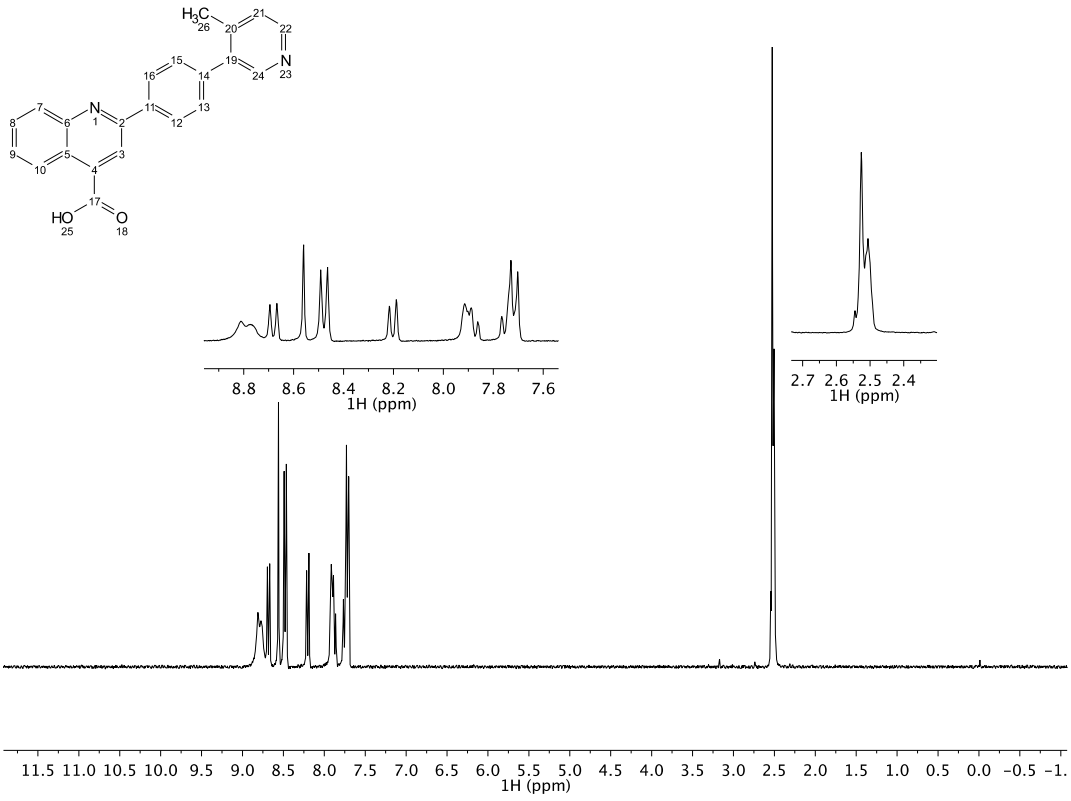
F
F
N
N
HO
O

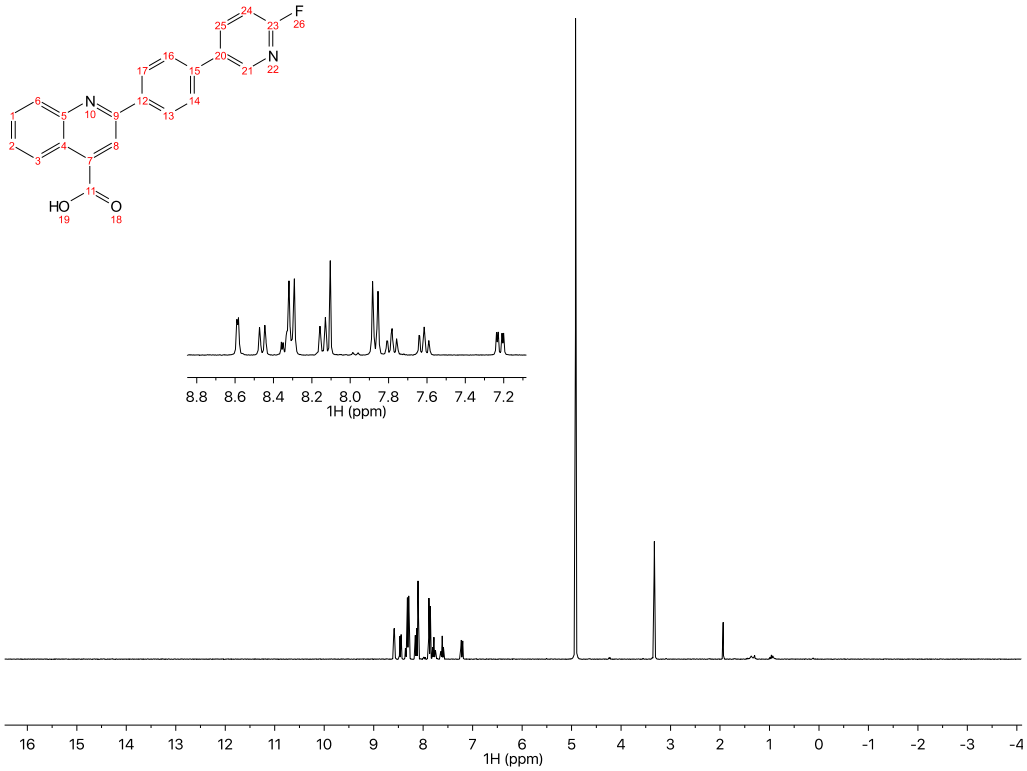

9.0
8.5
8.0
7.5
1H (ppm)
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3
1H (ppm)

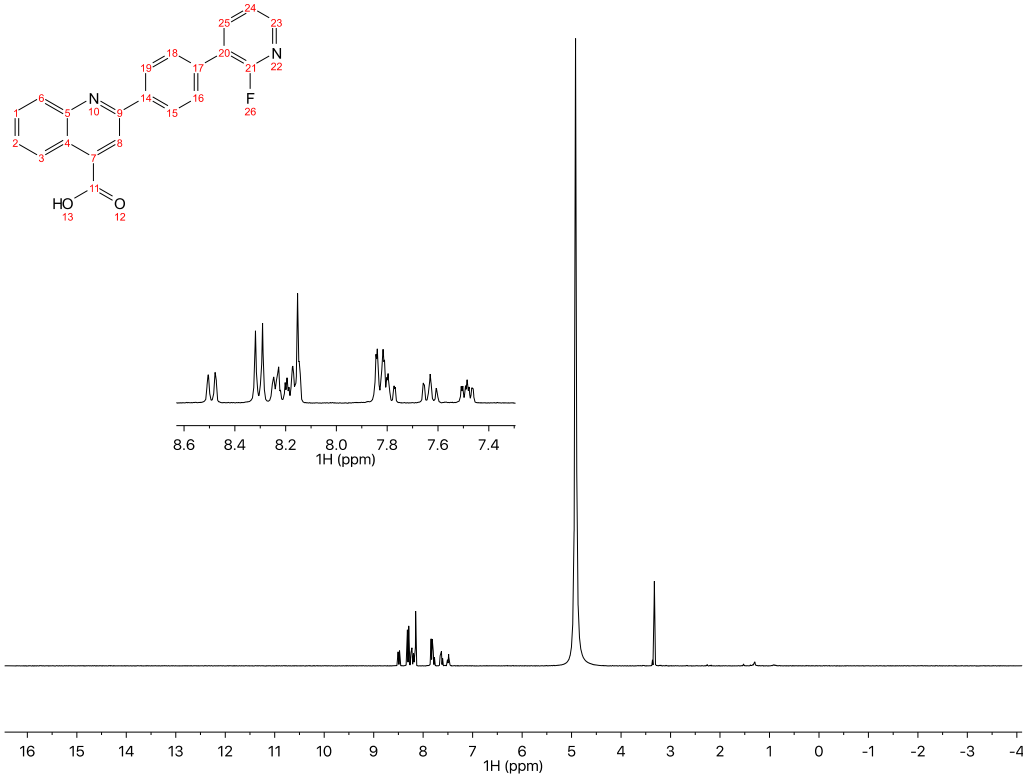

F
F
F
N
N
O
O
CH3

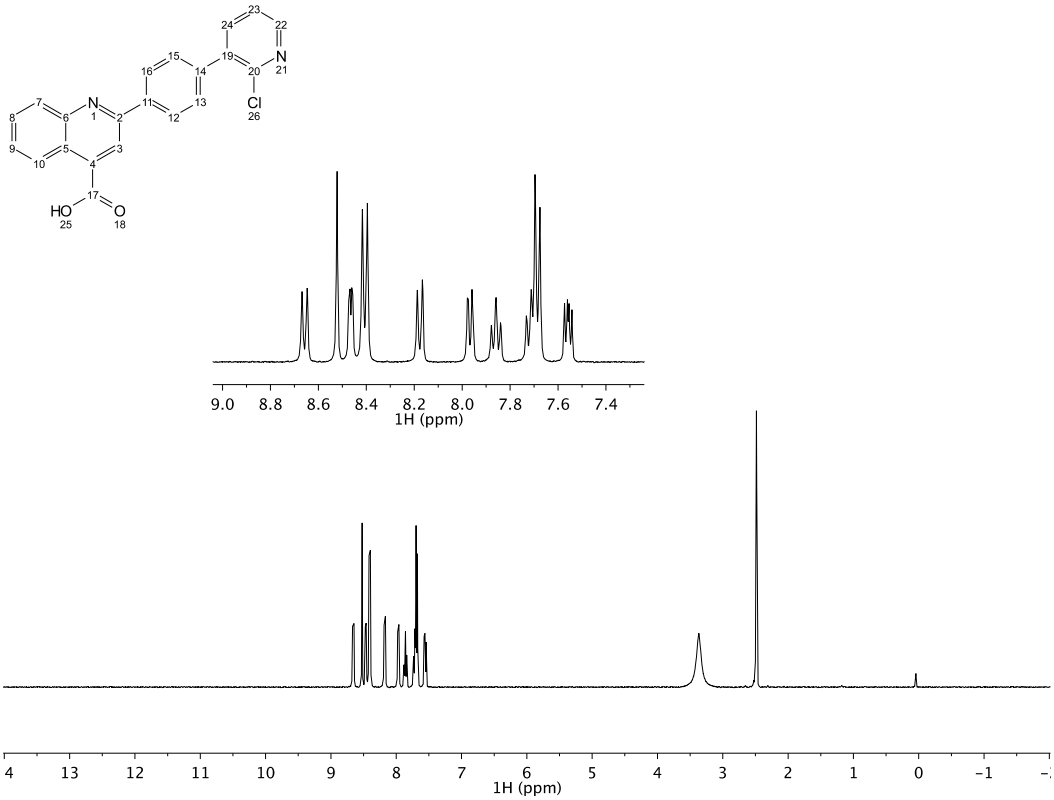

9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0
1H (ppm)
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3
1H (ppm)

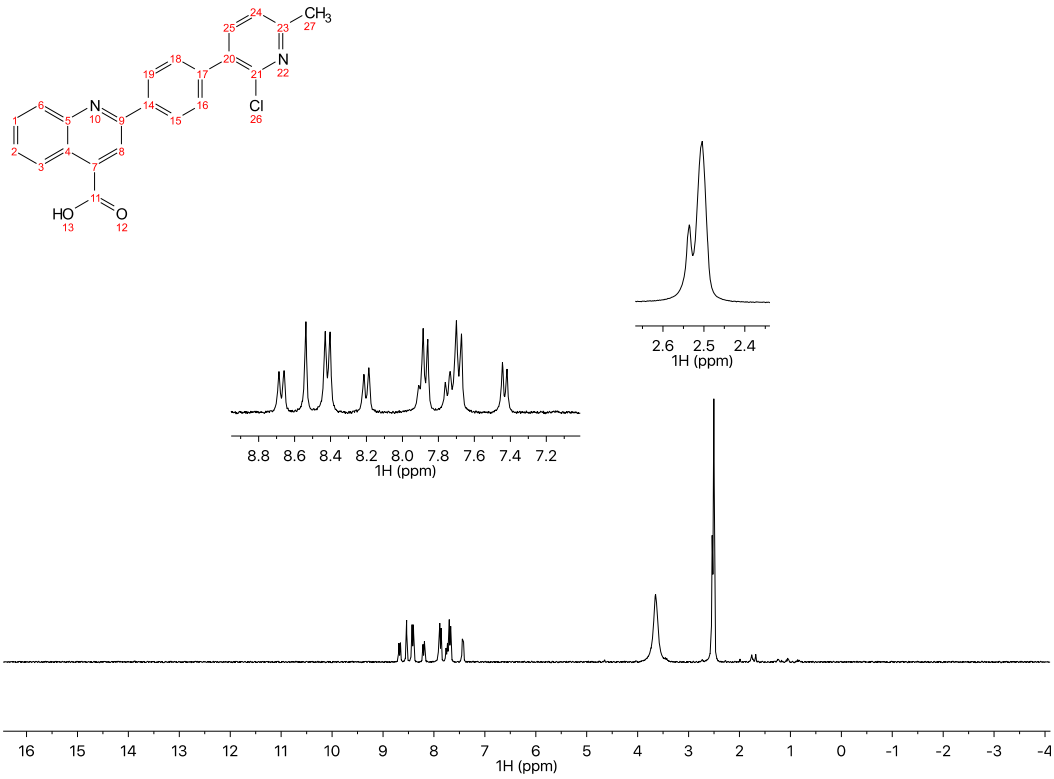

8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2
1H (ppm)
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
1H (ppm)

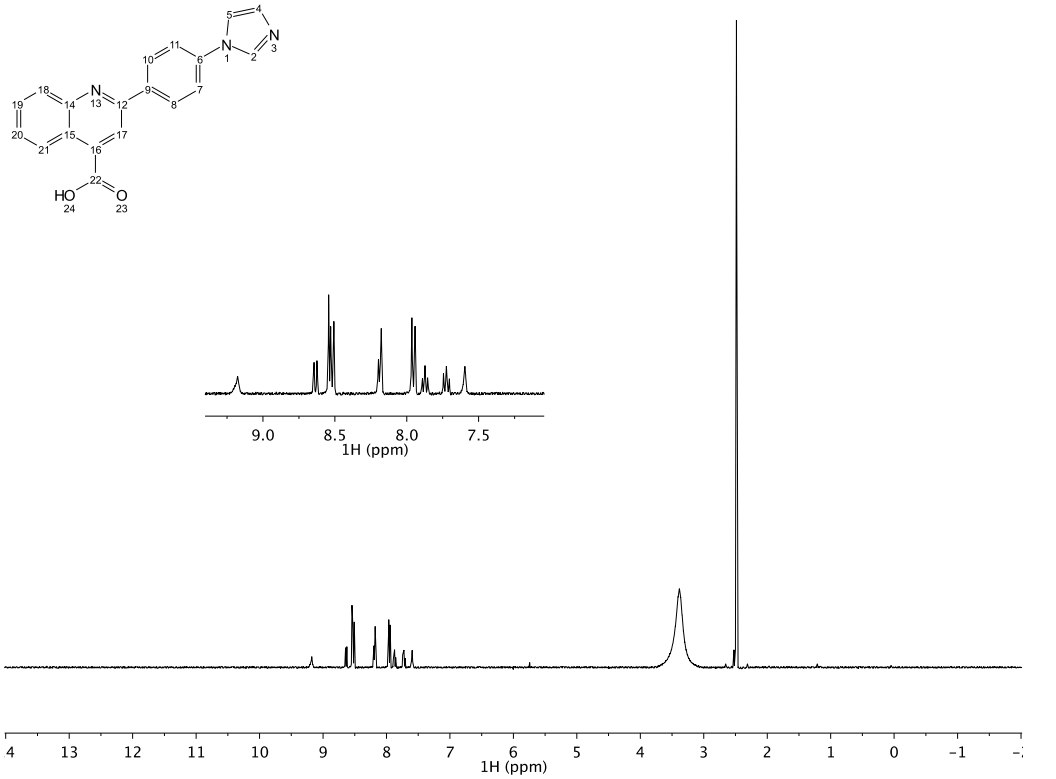

8.8 8.6 8.4 8.2 8.0 7.8 7.6
1H (ppm)
2.7 2.6 2.5 2.4
1H (ppm)
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.
1H (ppm)

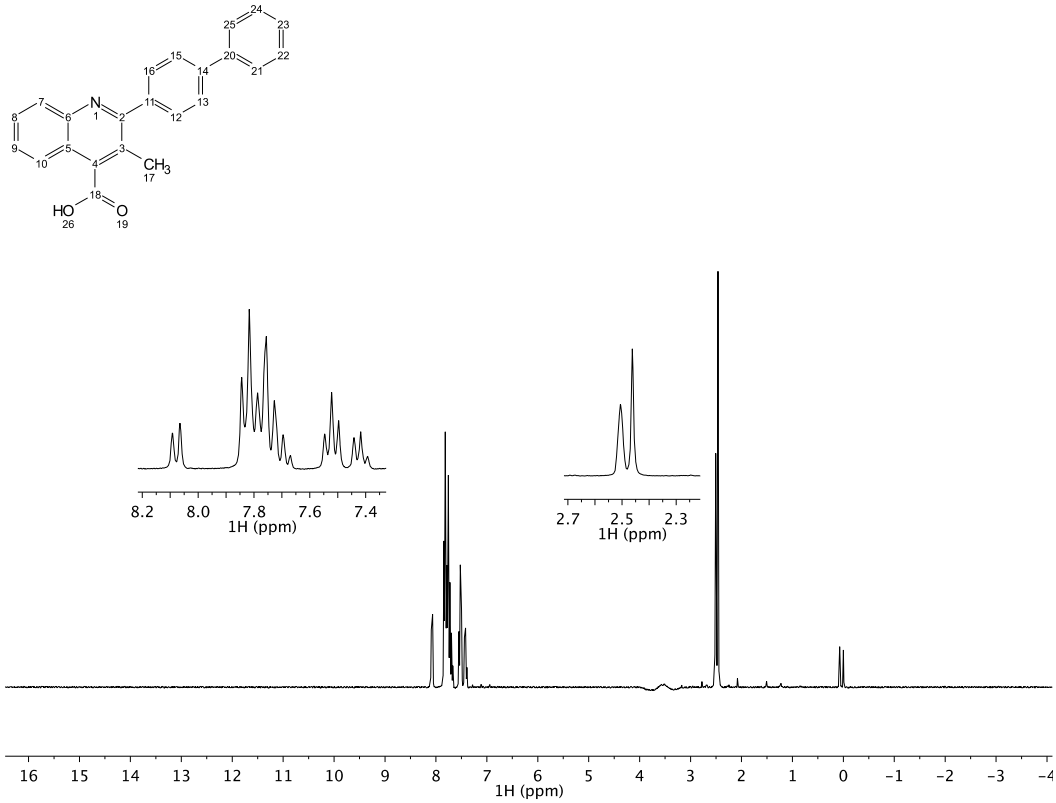

F
N
N
HO
O
8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2
1H (ppm)
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
1H (ppm)

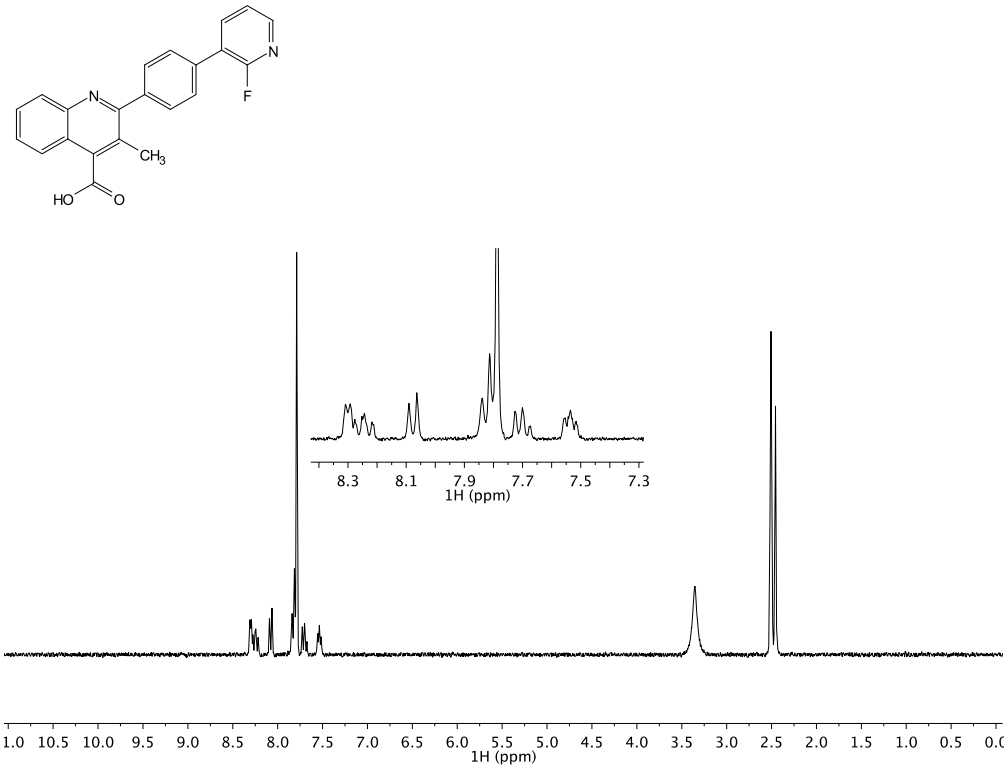

F
N
N
HO
O
8.6 8.4 8.2 8.0 7.8 7.6 7.4
1H (ppm)
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
1H (ppm)

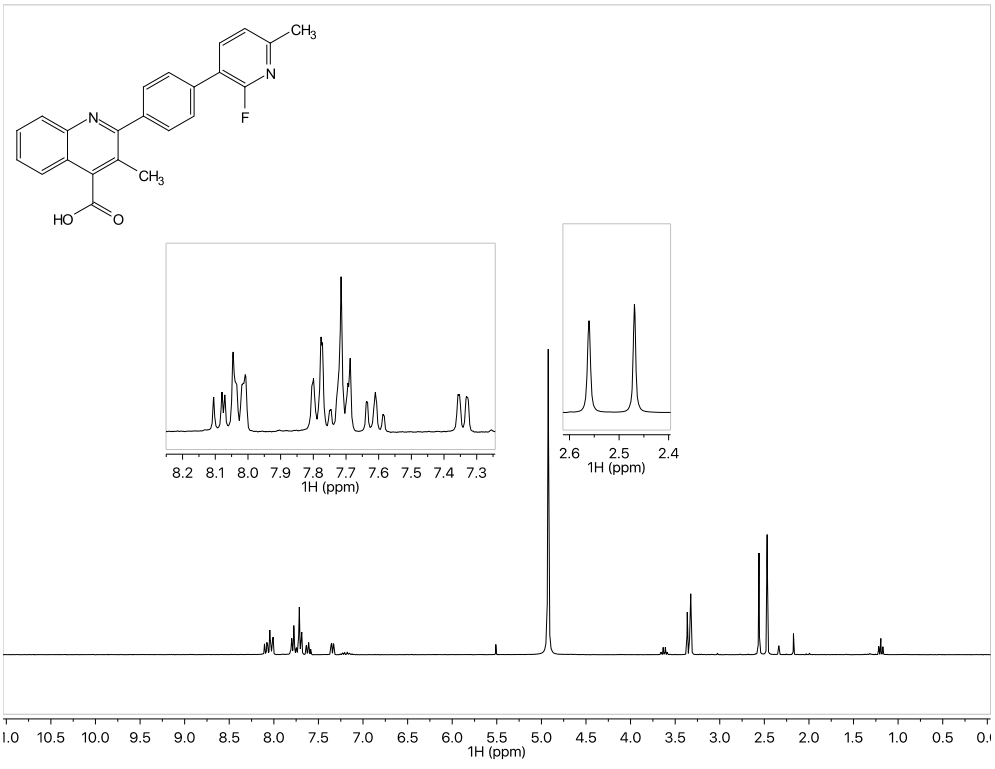

1H (ppm)
1H (ppm)

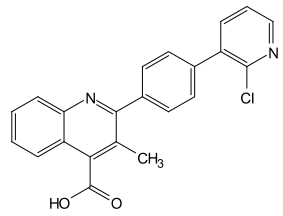

1H (ppm)
1H (ppm)
1H (ppm)

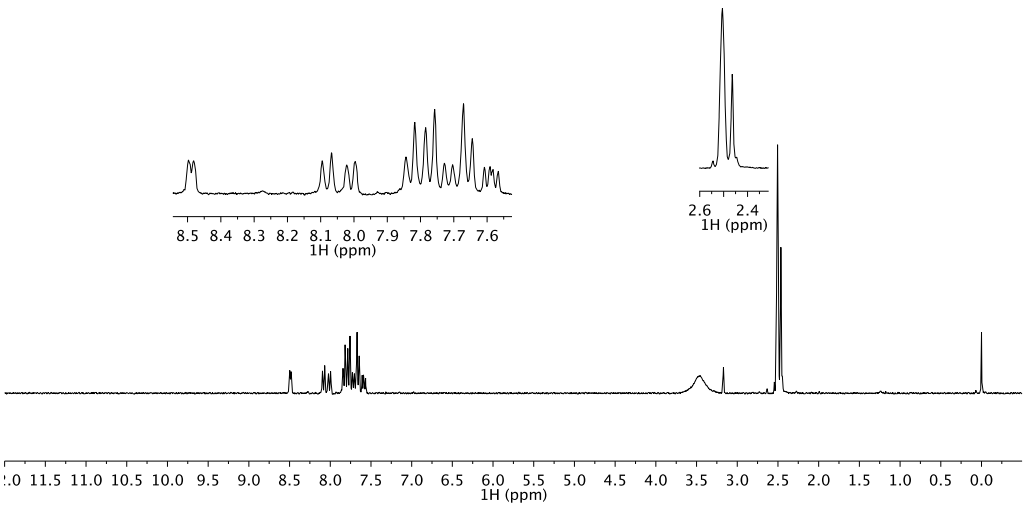

9.0
8.5
8.0
7.5
1H (ppm)
4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
1H (ppm)

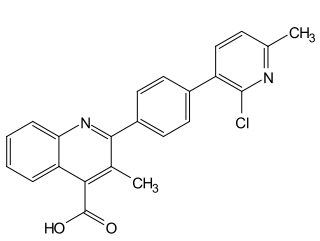

8.2
8.0
7.8
7.6
7.4
1H (ppm)
2.7
2.5
2.3
1H (ppm)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
−3
−4
1H (ppm)


N
F
CH3
HO
O
8.3 8.1 7.9 7.7 7.5 7.3
1H (ppm)
1.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1H (ppm)

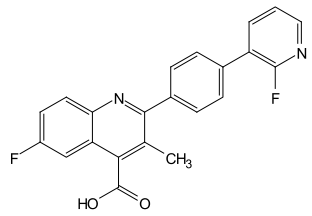

CH3
N
F
CH3
HO
O
8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3
1H (ppm)
2.6 2.5 2.4
1H (ppm)
1.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1H (ppm)

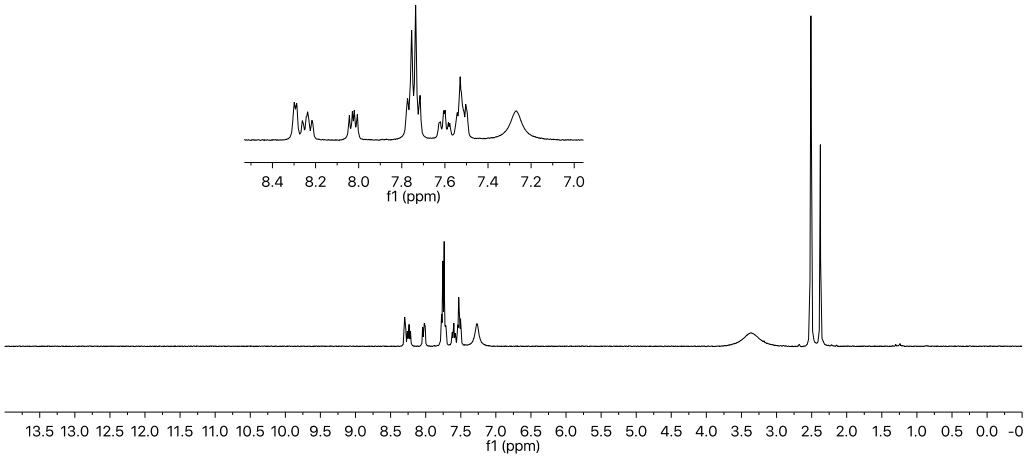

N
Cl
CH3
HO
O

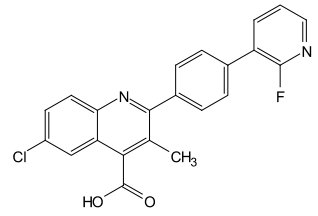

8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6
1H (ppm)
2.6 2.4
1H (ppm)
1H (ppm)

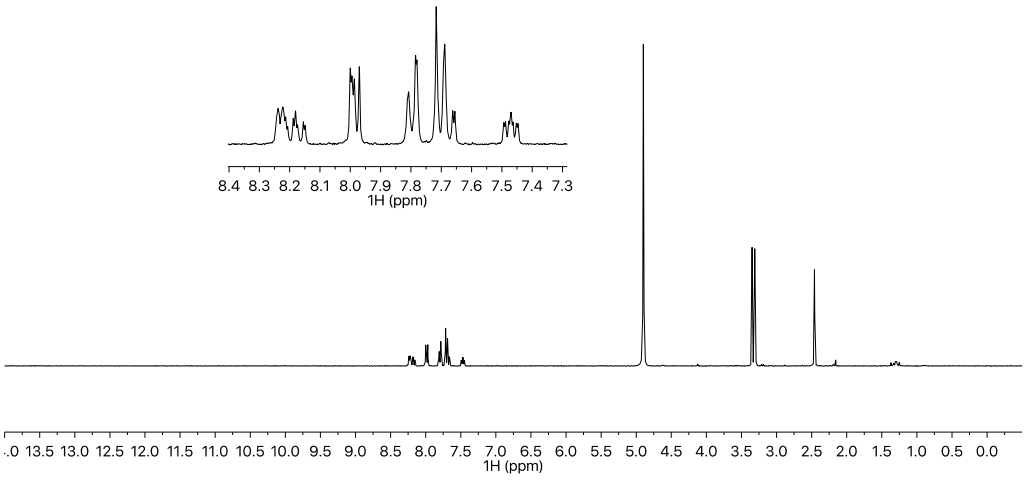

CH3
N
Cl
N
CH3
HO
O

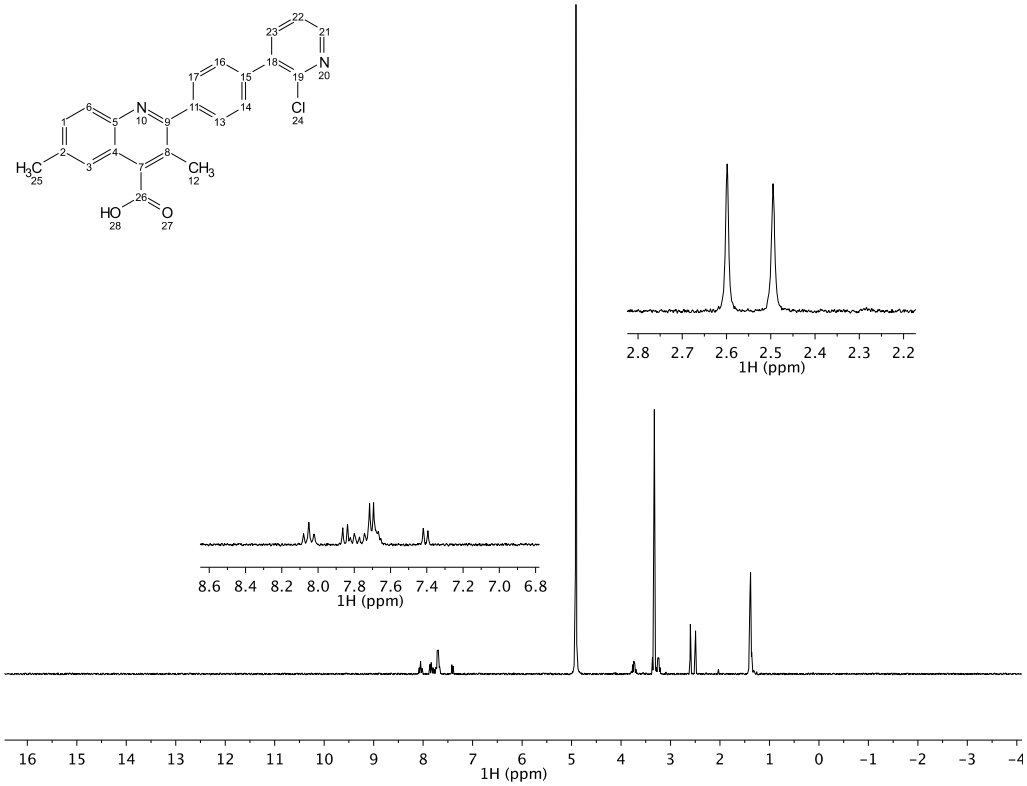
8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3
1H (ppm)
2.6 2.5
1H (ppm)
1H (ppm)

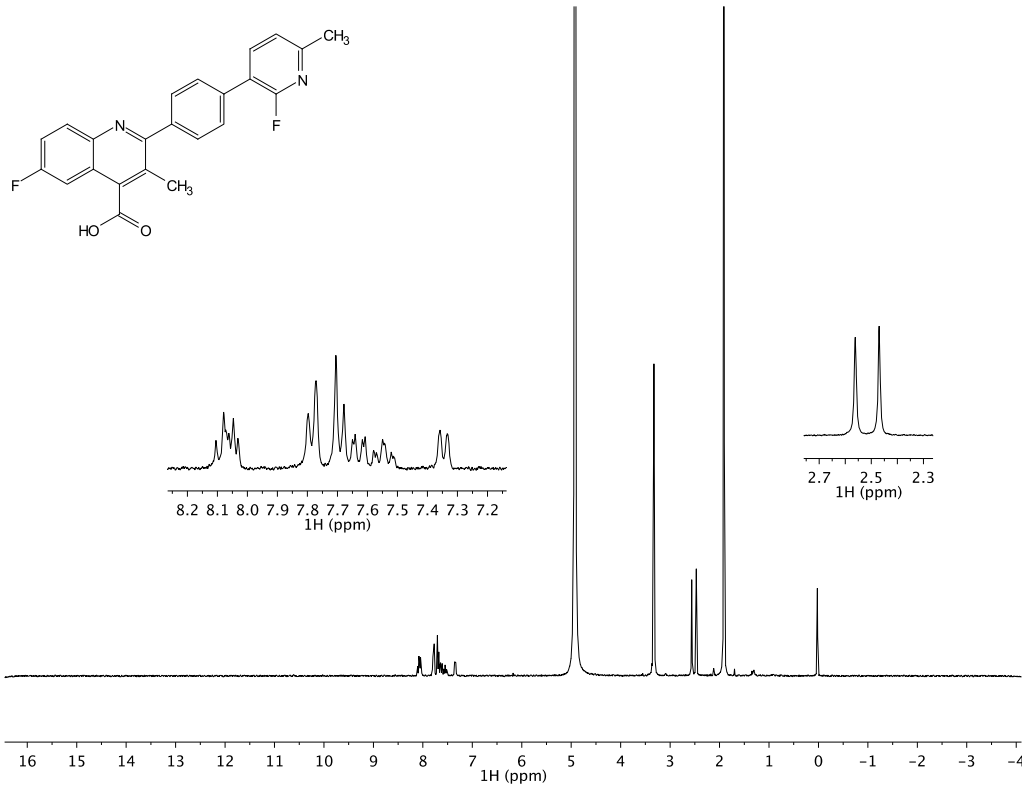

N
F
F
CH3
HO
O

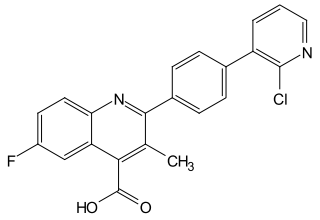

8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0
f1 (ppm)
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0
f1 (ppm)

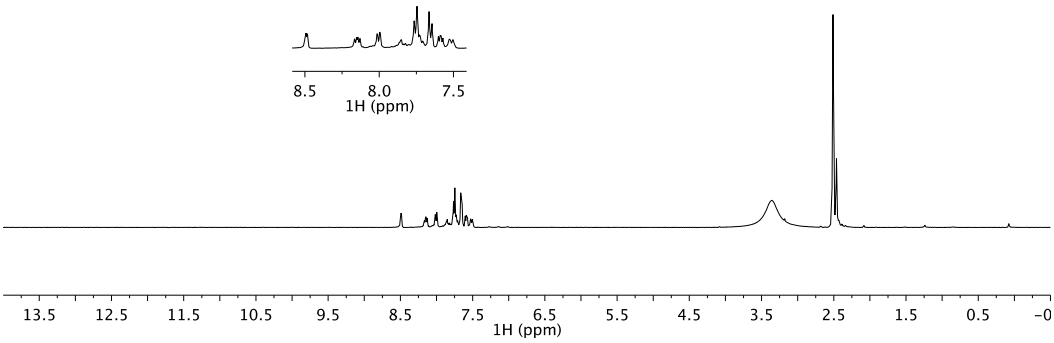

N
F
Cl
CH3
HO
O

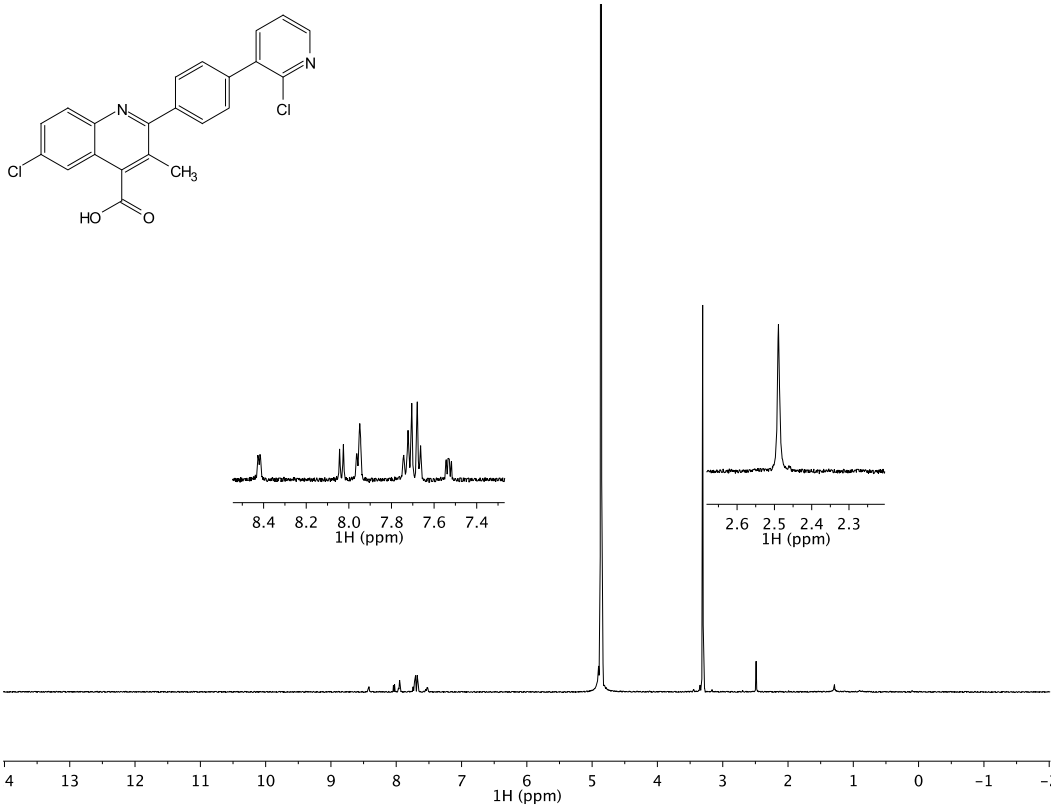

8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3
1H (ppm)
.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)

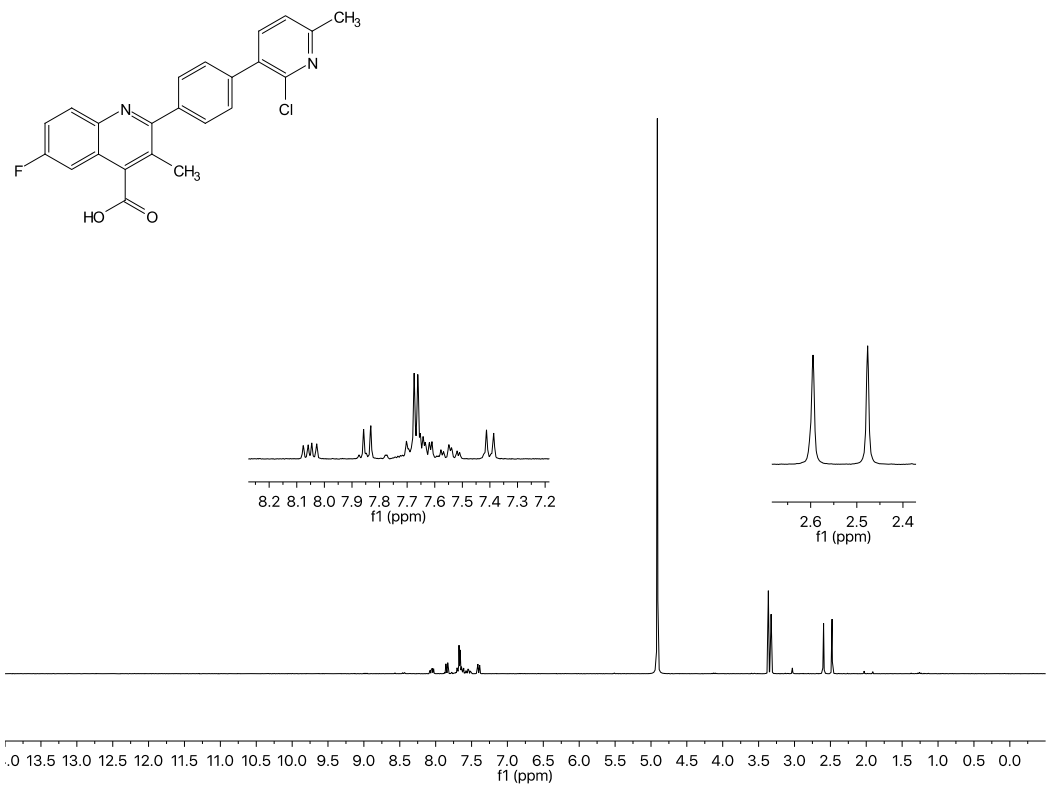

1H (ppm)

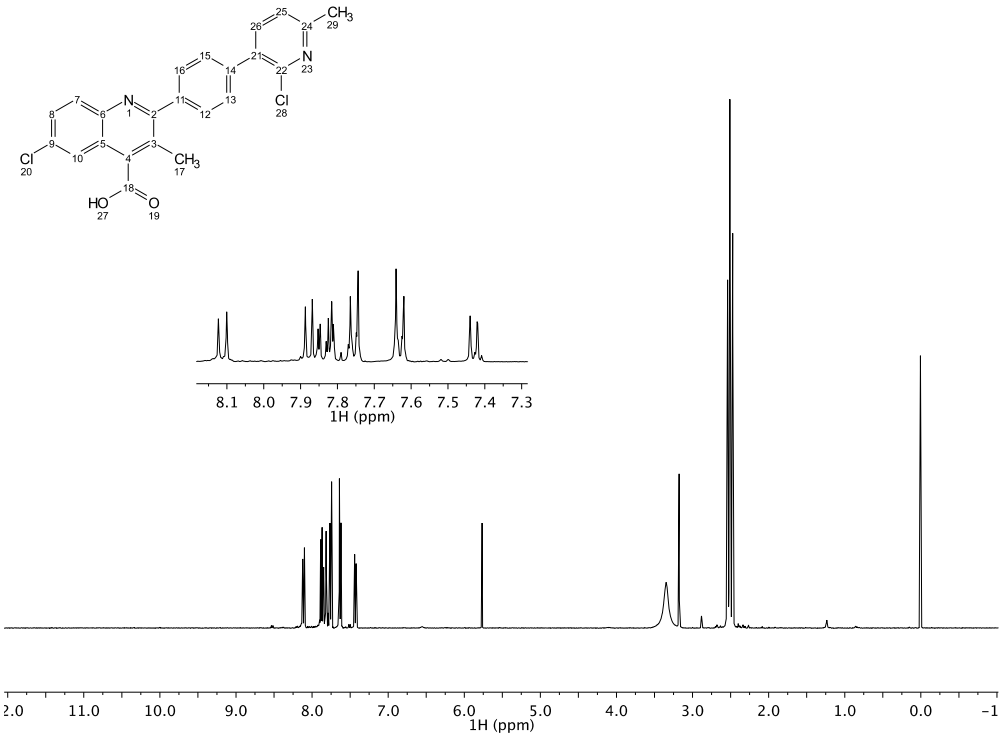

1H (ppm)

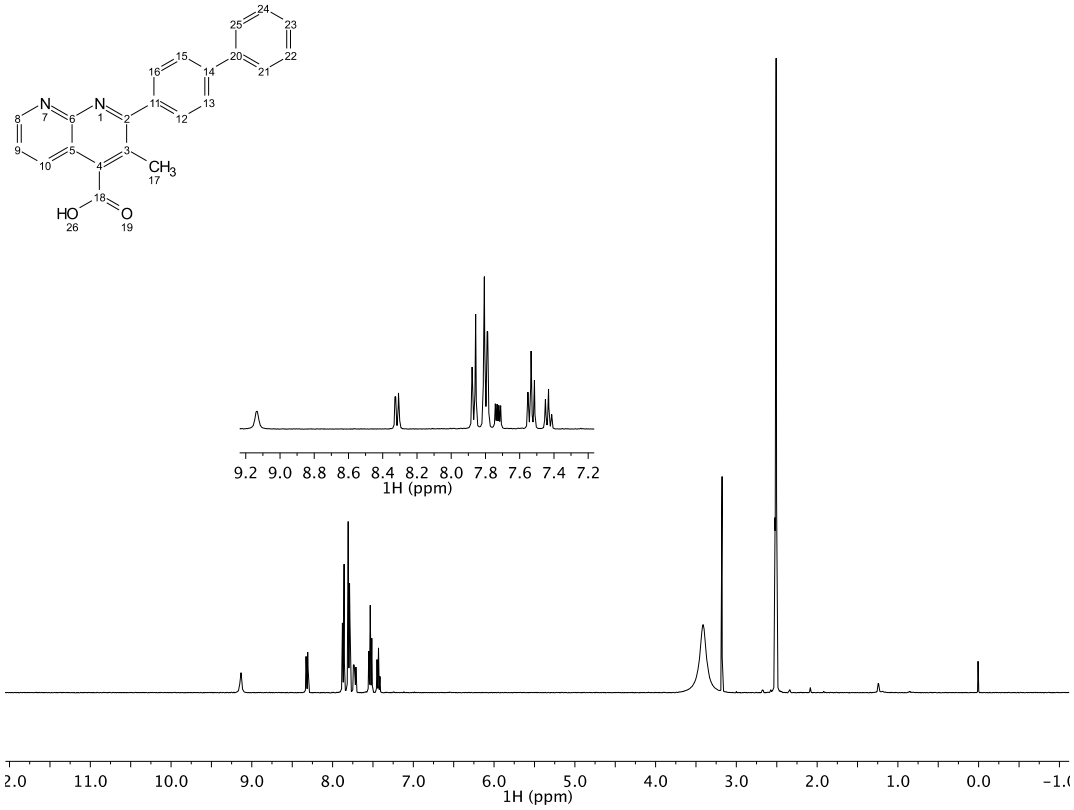

N
Cl
F
CH3
HO
O

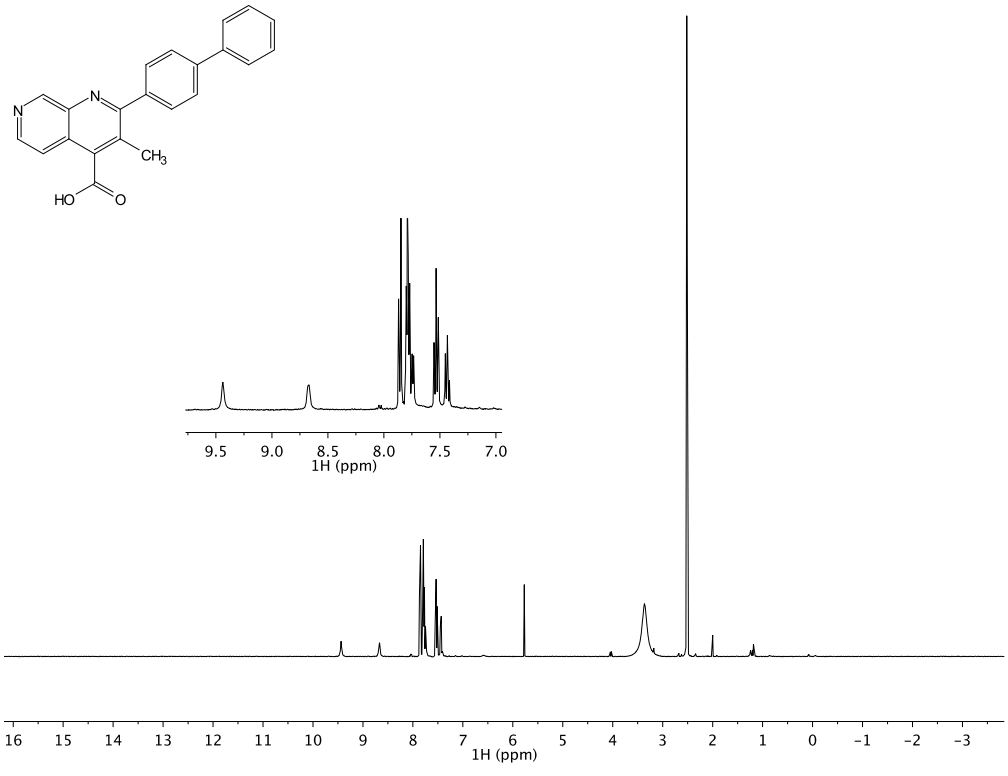

8.5
8.0
7.5
1H (ppm)
13.5
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
1H (ppm)

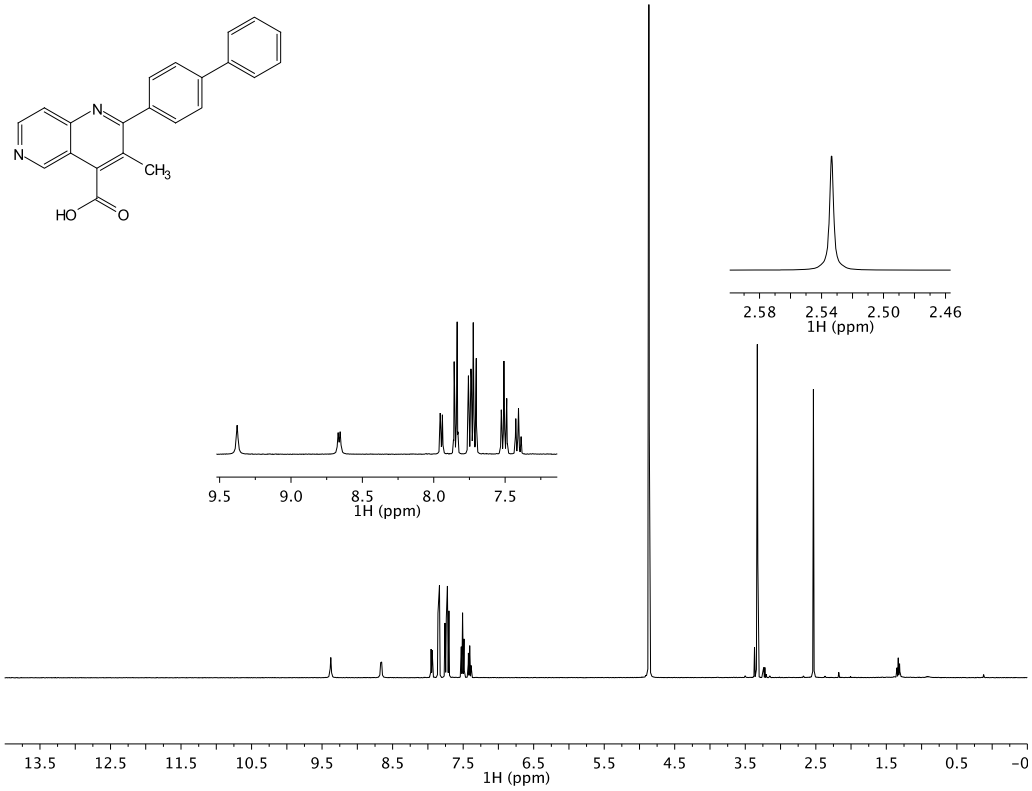

N
Cl
Cl
CH3
HO
O
8.4
8.2
8.0
7.8
7.6
7.4
1H (ppm)
2.6
2.5
2.4
2.3
1H (ppm)
4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
1H (ppm)

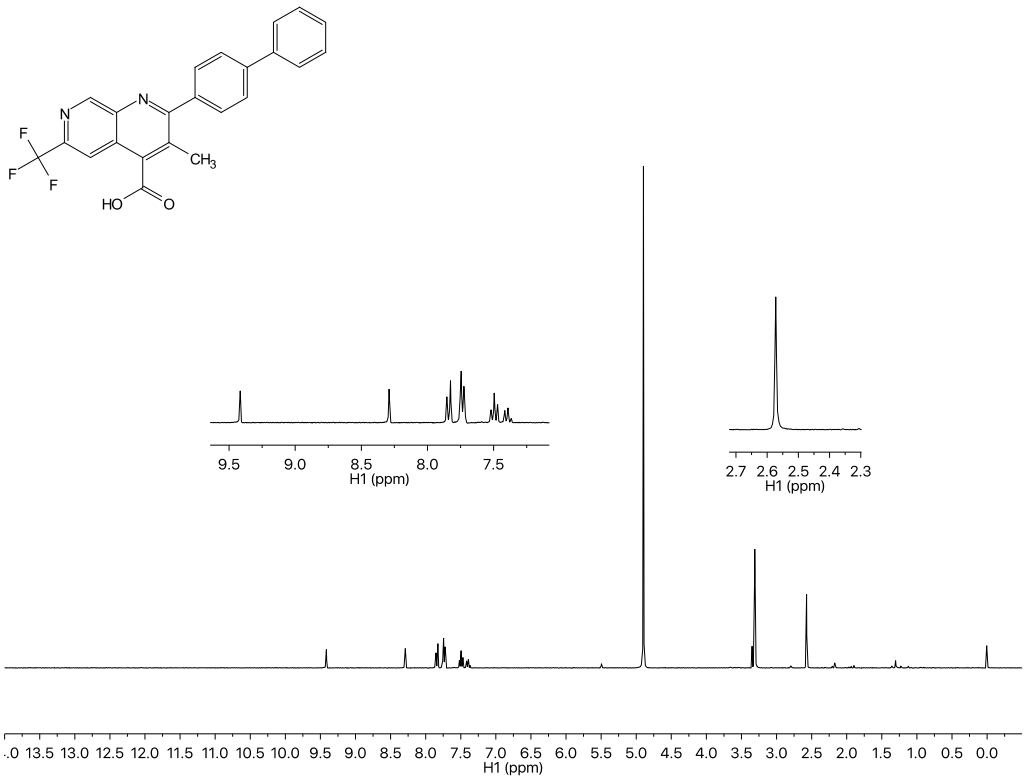

CH3
N
Cl
F
CH3
HO
O
8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2
f1 (ppm)
2.6 2.5 2.4
f1 (ppm)
.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

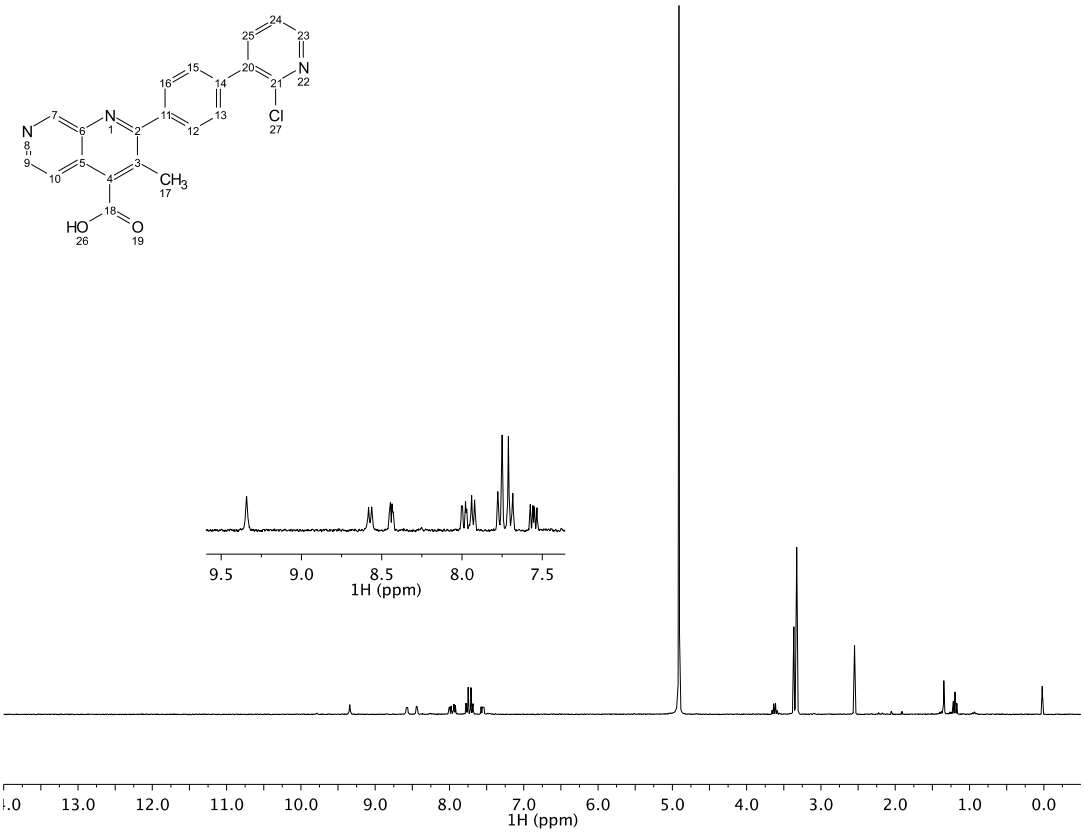

CH3
29
N
23
Cl
28
Cl
20
CH3
17
HO
27
O
19
8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3
1H (ppm)
2.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 −1
1H (ppm)

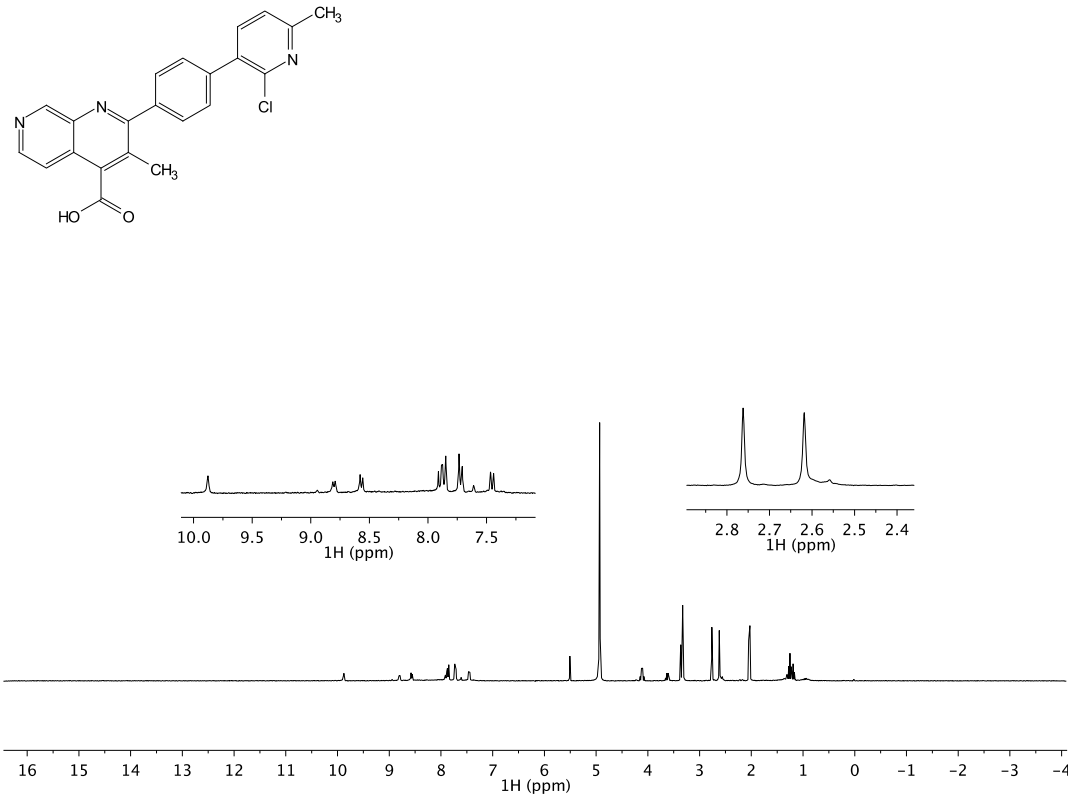

24
25
23
15
20
22
16
14
21
N
N
11
13
8
7
6
1
2
12
9
5
3
CH3
10
4
17
18
HO
O
26
19
9.2 9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2
1H (ppm)
2.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 −1.(
1H (ppm)

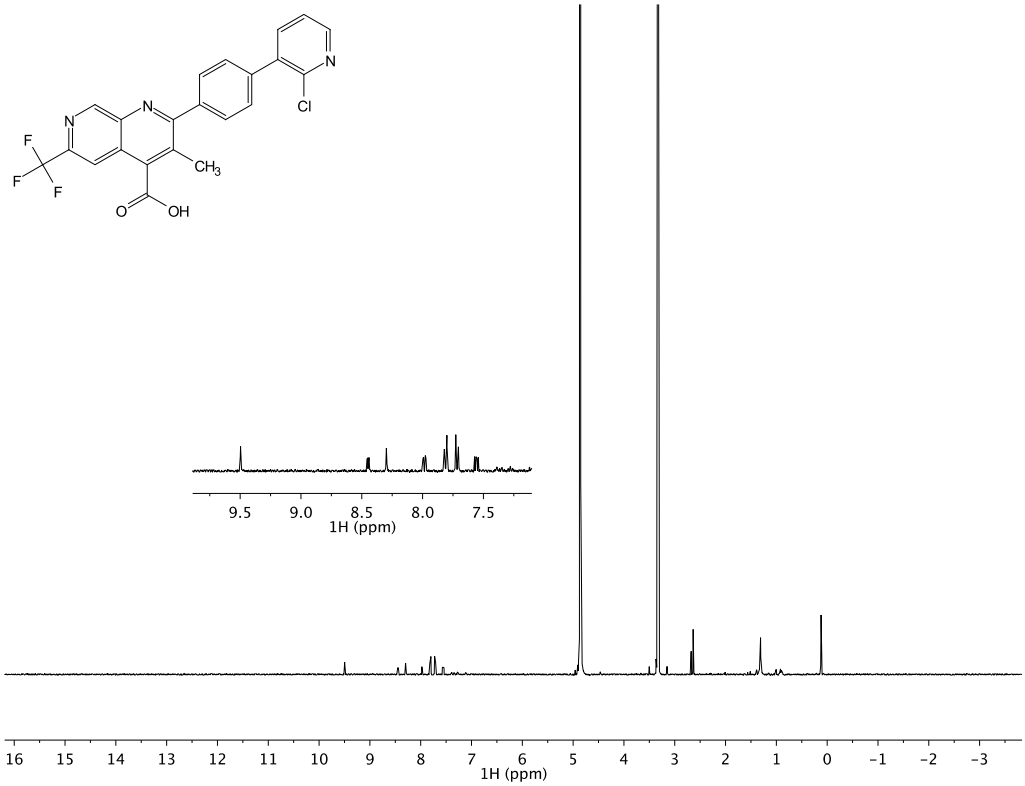

N
N
CH3
HO
O
9.5 9.0 8.5 8.0 7.5 7.0
1H (ppm)
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3
1H (ppm)

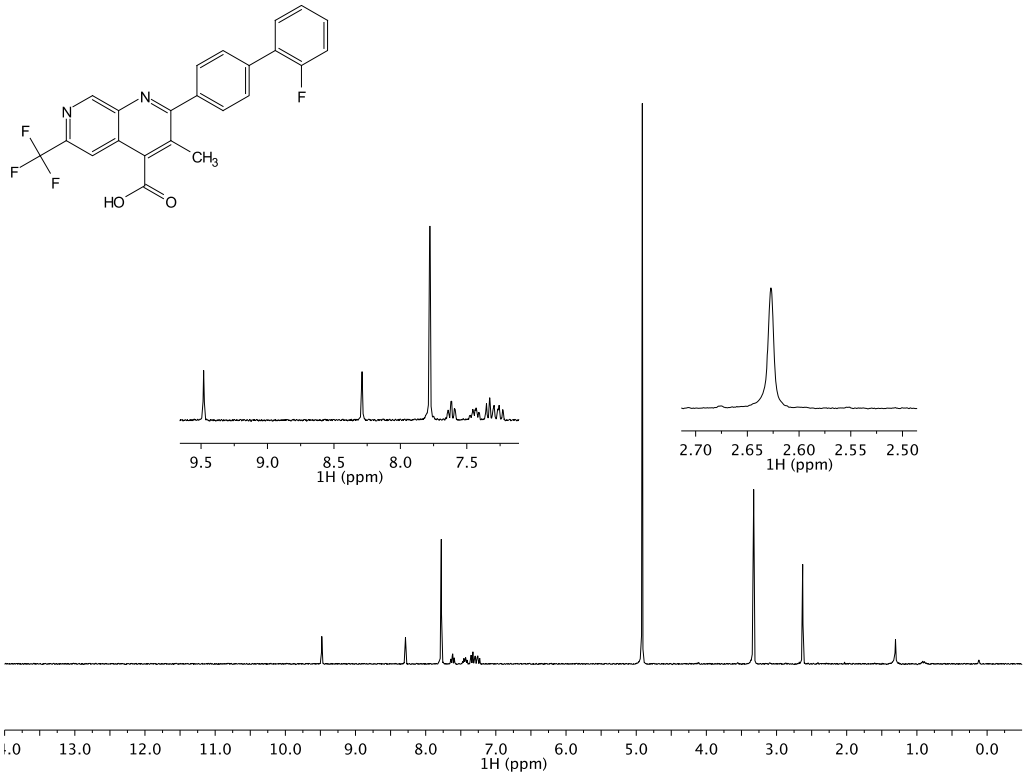

N
N
CH3
HO
O
2.58
2.54
2.50
2.46
1H (ppm)
9.5
9.0
8.5
8.0
7.5
1H (ppm)
13.5
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
1H (ppm)


N
N
F
F
F
CH3
HO
O
9.5
9.0
8.5
8.0
7.5
H1 (ppm)
2.7
2.6
2.5
2.4
2.3
H1 (ppm)
H1 (ppm)


1H (ppm)


1H (ppm)
1H (ppm)


N
N
Cl
F
F
F
CH3
O
OH
9.5
9.0
8.5
8.0
7.5
1H (ppm)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
−3
1H (ppm)


N
N
F
F
F
F
CH3
HO
O
9.5
9.0
8.5
8.0
7.5
1H (ppm)
2.70
2.65
2.60
2.55
2.50
1H (ppm)
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1H (ppm)

Supplemental References:

1. The Oncomine Platform (Thermo Fisher, Ann Arbor, MI) https://www.oncomine.com/resource/main.html - a:1780;cso:sizeSmallLarge;cv:detail;d:1197;dso:geneOverex;dt:predefinedClass;ec:[2];et:over;f:2507079;g:1723;gt:boxplot;p:200001263;pg:1;pvf:10222,35244,35245,35246,35247,35248,35249,35250;scr:summary;ss:analysis;v:18

2. Rhodes, D. R.; Yu, J.; Shanker, K.; Deshpande, N.; Varambally, R.; Ghosh, D.; Barrette, T.; Pandey, A.; Chinnaiyan, A. M. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* **2004**, 6, 1-6.

3. Rhodes, D. R.; Kalyana-Sundaram, S.; Mahavisno, V.; Varambally, R.; Yu, J.; Briggs, B. B.; Barrette, T. R.; Anstet, M. J.; Kincead-Beal, C.; Kulkarni, P.; Varambally, S.; Ghosh, D.; Chinnaiyan, A. M. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* **2007**, 9, 166-80.