

## **7-*N*-Substituted-3-Oxadiazole Quinolones with Potent Antimalarial Activity Target the Cytochrome bc<sub>1</sub> Complex**

William Nguyen,<sup>a,b,#</sup> Madeline G. Dans,<sup>a,b,#</sup> Iain Currie,<sup>a,b</sup> Jon Kyle Awalt,<sup>a,b</sup> Brodie L. Bailey,<sup>a,b</sup> Chris Lumb,<sup>a</sup> Anna Ngo,<sup>a</sup> Paola Favuzza,<sup>a,b</sup> Josephine Palandri,<sup>a,b</sup> Saishyam Ramesh,<sup>c</sup> Jocelyn Penington,<sup>a,b</sup> Kate E. Jarman,<sup>a,b</sup> Partha Mukherjee,<sup>d</sup> Arnish Chakraborty,<sup>d</sup> Alexander G. Maier,<sup>c</sup> Giel G. van Dooren,<sup>c</sup> Tony Papenfuss,<sup>a,b</sup> Sergio Wittlin,<sup>e,f</sup> Alisje Churchyard,<sup>g</sup> Jake Baum,<sup>g,h</sup> Elizabeth A. Winzeler,<sup>i</sup> Delphine Baud,<sup>j</sup> Stephen Brand,<sup>j</sup> Paul F. Jackson,<sup>k</sup> Alan F. Cowman,<sup>a,b</sup> and Brad E. Sleebs.<sup>a,b,\*</sup>

<sup>a</sup> The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia.

<sup>b</sup> Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia.

<sup>c</sup> Research School of Biology, The Australian National University, Canberra 2600, Australia.

<sup>d</sup> TCG Lifesciences, Kolkata, West Bengal, 700091, India.

<sup>e</sup> Swiss Tropical and Public Health Institute, Kreuzstrasse 2, 4123 Allschwil, Switzerland.

<sup>f</sup> University of Basel, 4003 Basel, Switzerland.

<sup>g</sup> Department of Life Sciences, Imperial College London, South Kensington, SW7 2AZ UK.

<sup>h</sup> School of Biomedical Sciences, University of New South Wales, Sydney, 2031, Australia.

<sup>i</sup> School of Medicine, University of California San Diego, 9500 Gilman Drive 0760, La Jolla, CA, 92093, USA

<sup>j</sup> Medicines for Malaria Venture, Geneva 1215, Switzerland.

<sup>k</sup> Global Public Health, Janssen R&D LLC, La Jolla 92121, USA.

\* Correspondence to:

Brad E. Sleebs

The Walter and Eliza Hall Institute of Medical Research

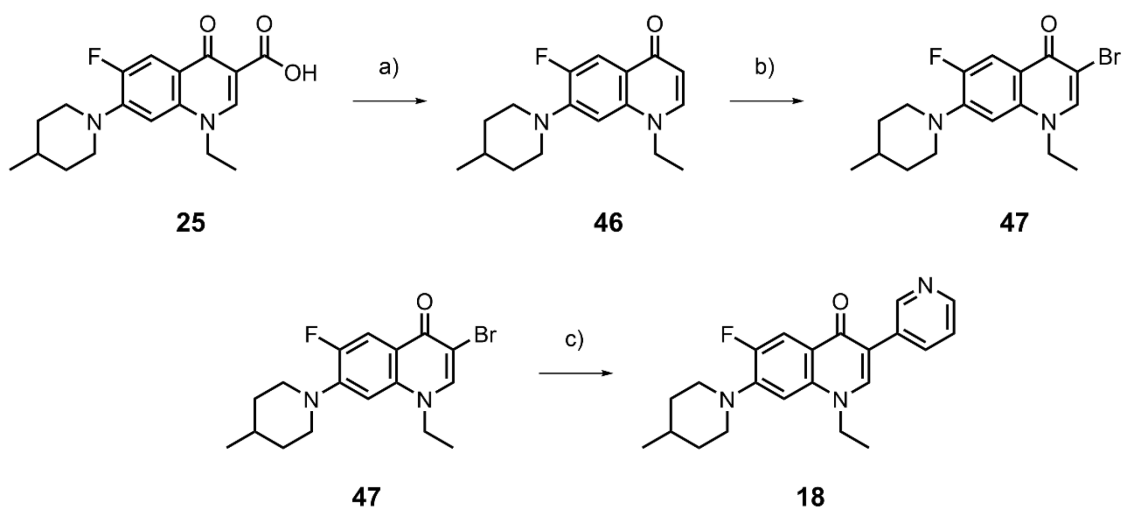
1G Royal Parade, Parkville 3052, Victoria, Australia

Email: [sleebs@wehi.edu.au](mailto:sleebs@wehi.edu.au)

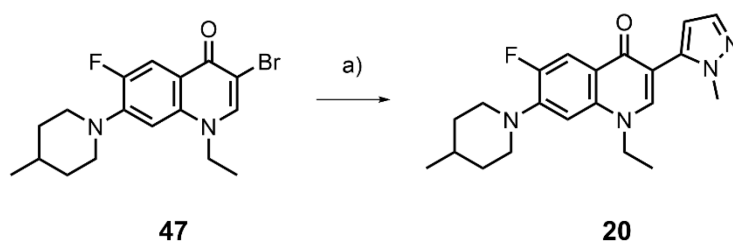
# These authors contributed equally.

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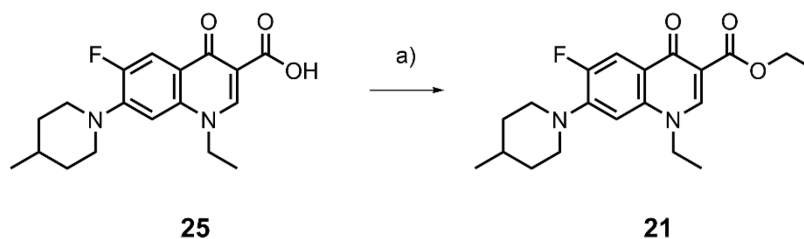
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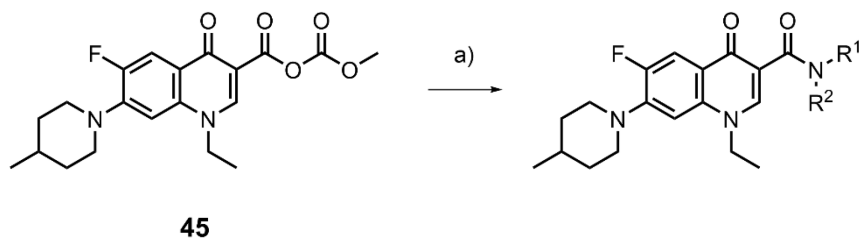
**Scheme S1.** Synthesis of **18**. *Reagents and conditions:* (a) 37% HCl, 20 °C (b) Br<sub>2</sub>, AcOH, 20 °C; (c) 3-pyridylboronic acid, K<sub>2</sub>CO<sub>3</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, 1,4-dioxane/H<sub>2</sub>O (6:1), 100 °C.



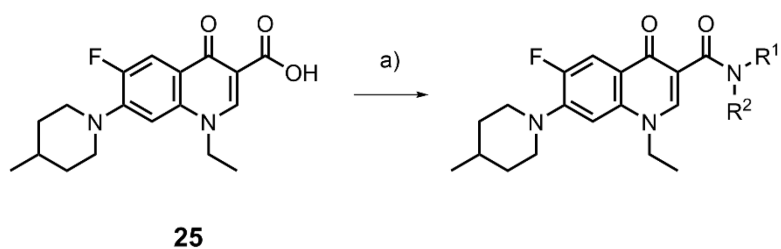
**Scheme S2.** Synthesis of **20**. *Reagents and conditions:* (a) 1-methylpyrazole-5-boronic acid pinacol ester, K<sub>2</sub>CO<sub>3</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, 1,4-dioxane/H<sub>2</sub>O (6:1), 100 °C.



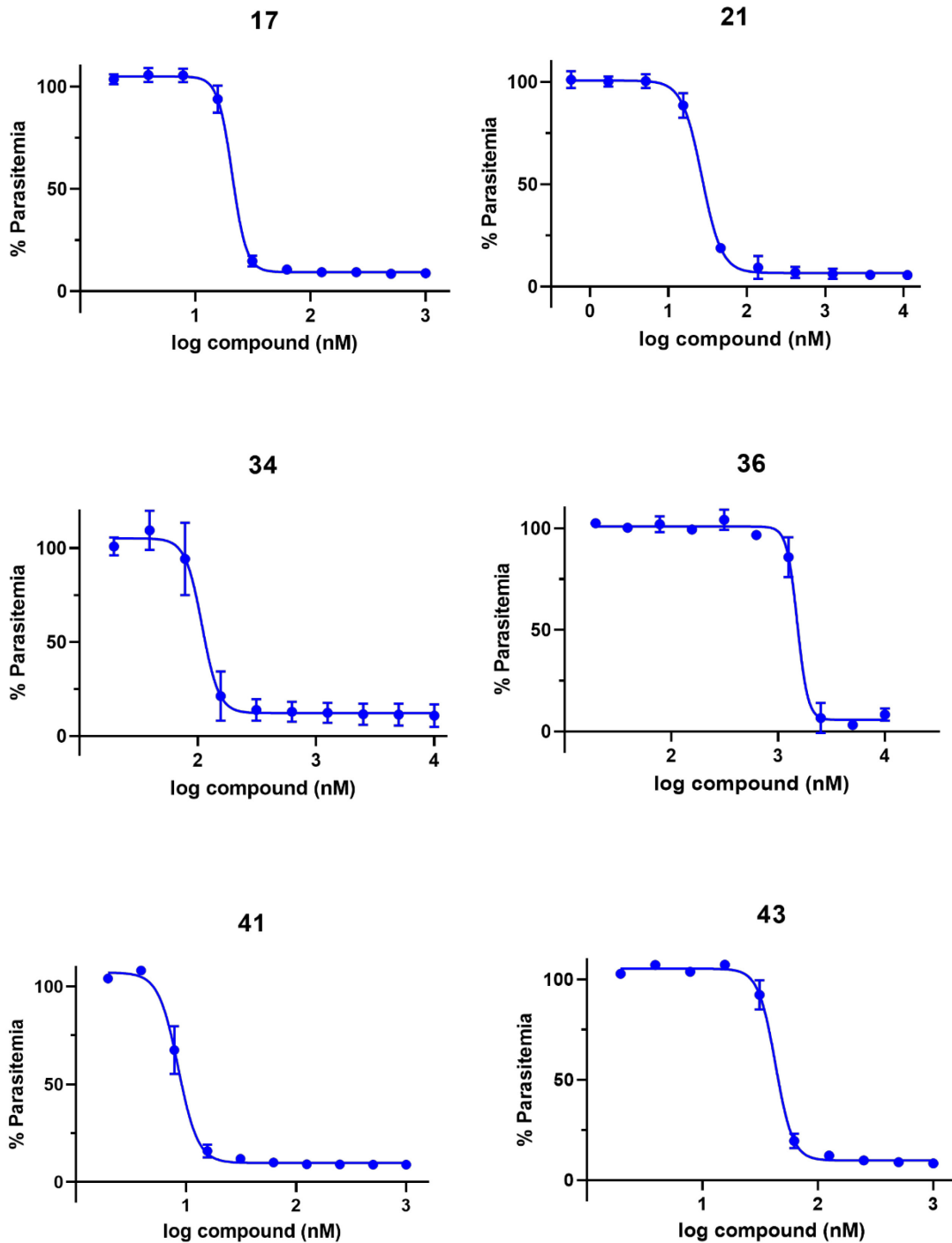
**Scheme S3.** Synthesis of **21**. *Reagents and conditions:* (a) H<sub>2</sub>SO<sub>4</sub>, EtOH, 78 °C.



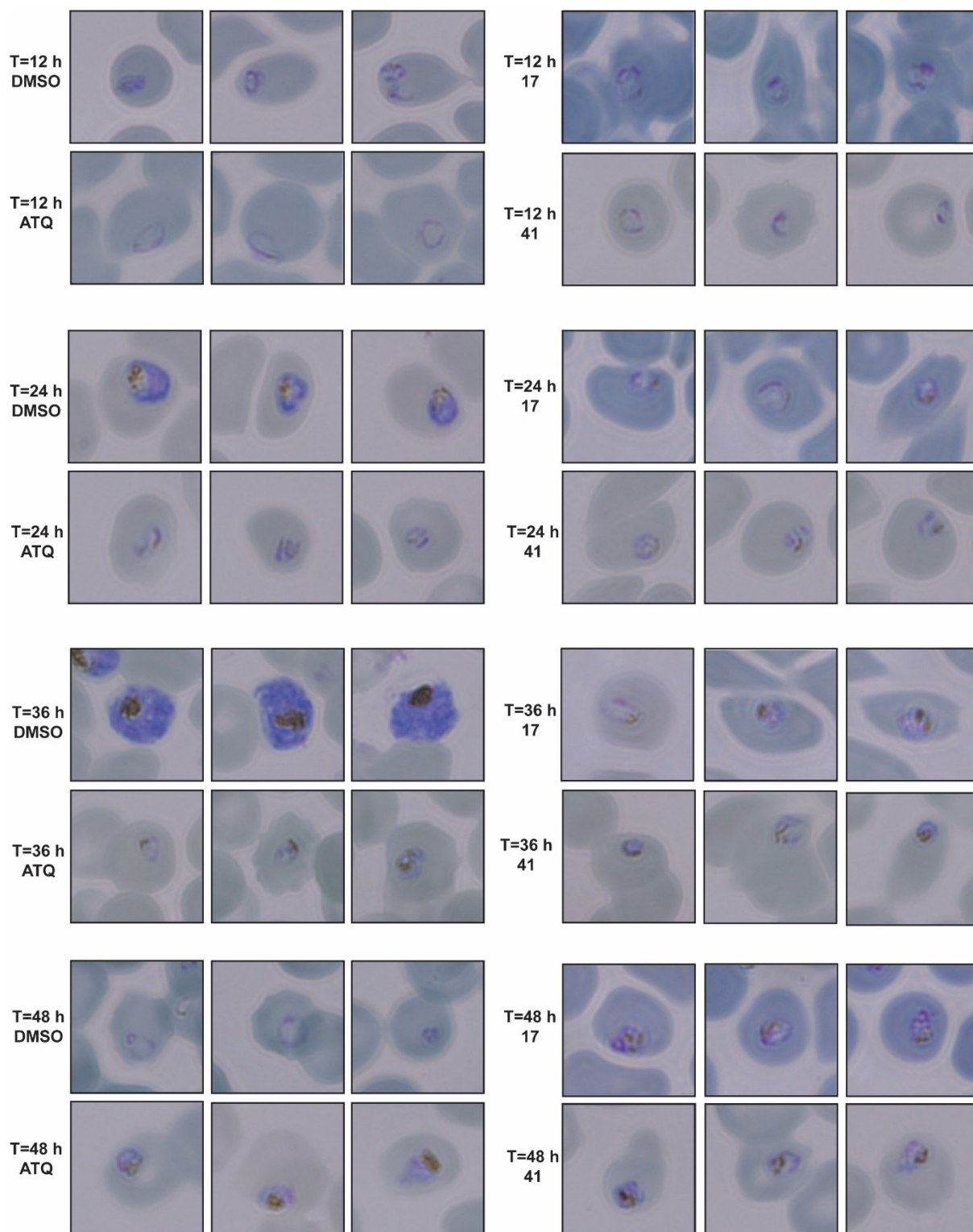
**Scheme S4.** Synthesis of **22**, **23** and **24**. *Reagents and conditions:* (a) substituted amine, 20 °C.



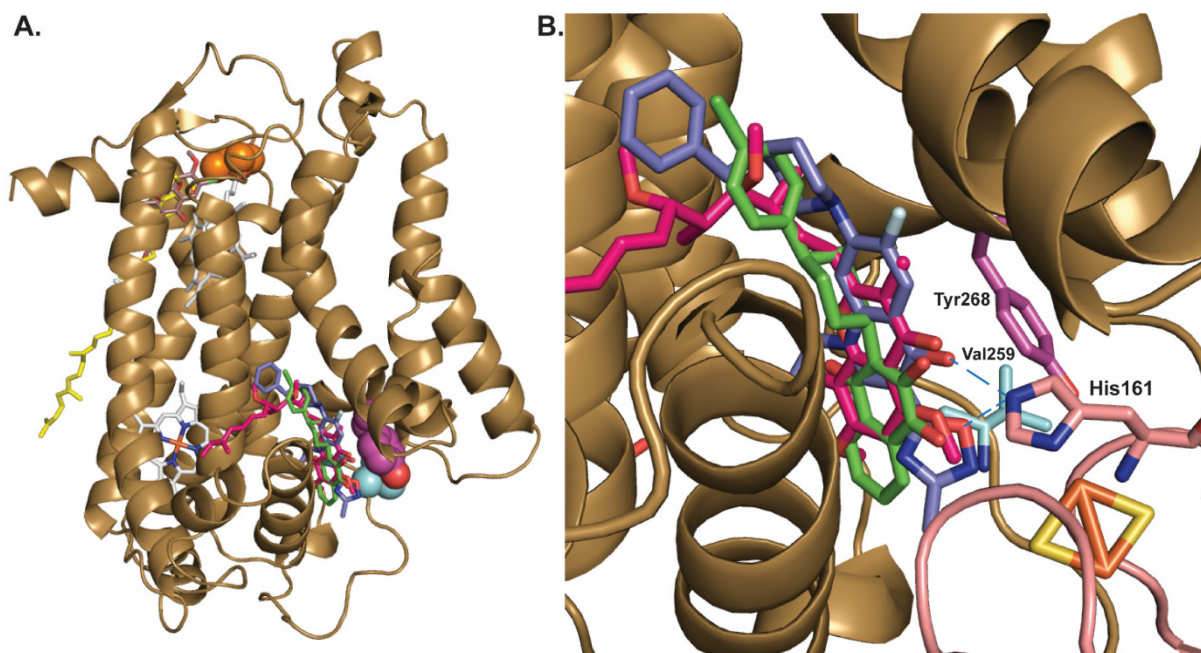
**Scheme S5.** Synthesis of **26** and **27**. *Reagents and conditions:* (a) substituted secondary amine, HATU, DIPEA, DMF, 20 °C.



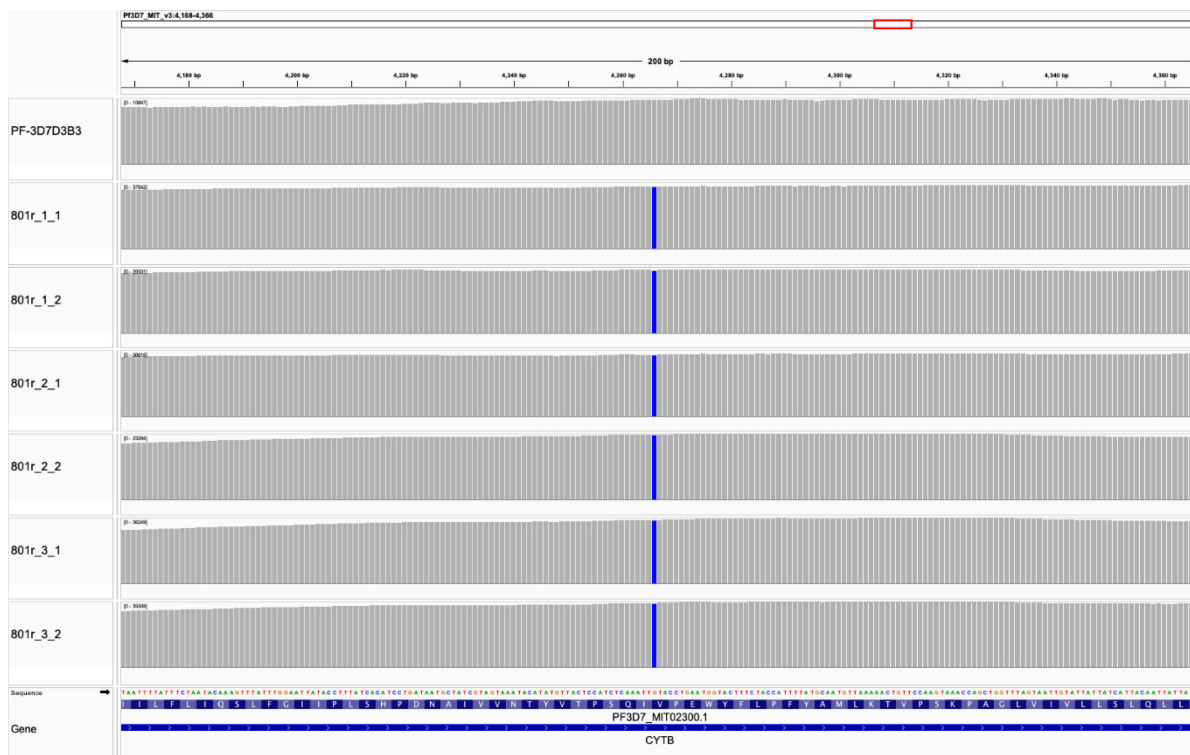
**Figure S1.** *P. falciparum* 3D7 asexual LDH dose response curves of representative compounds. EC<sub>50</sub> data represents means and SDs for four or more technical replicates measuring LDH activity of *P. falciparum* 3D7 parasites following exposure to compounds in 10-point dilution series for 72 h.



**Figure S2.** Giemsa stained microscopy images showing the asexual stage of arrest treated with ATQ (**3**), **17** and **41** at different time points to support representative images shown in Figure 2B.

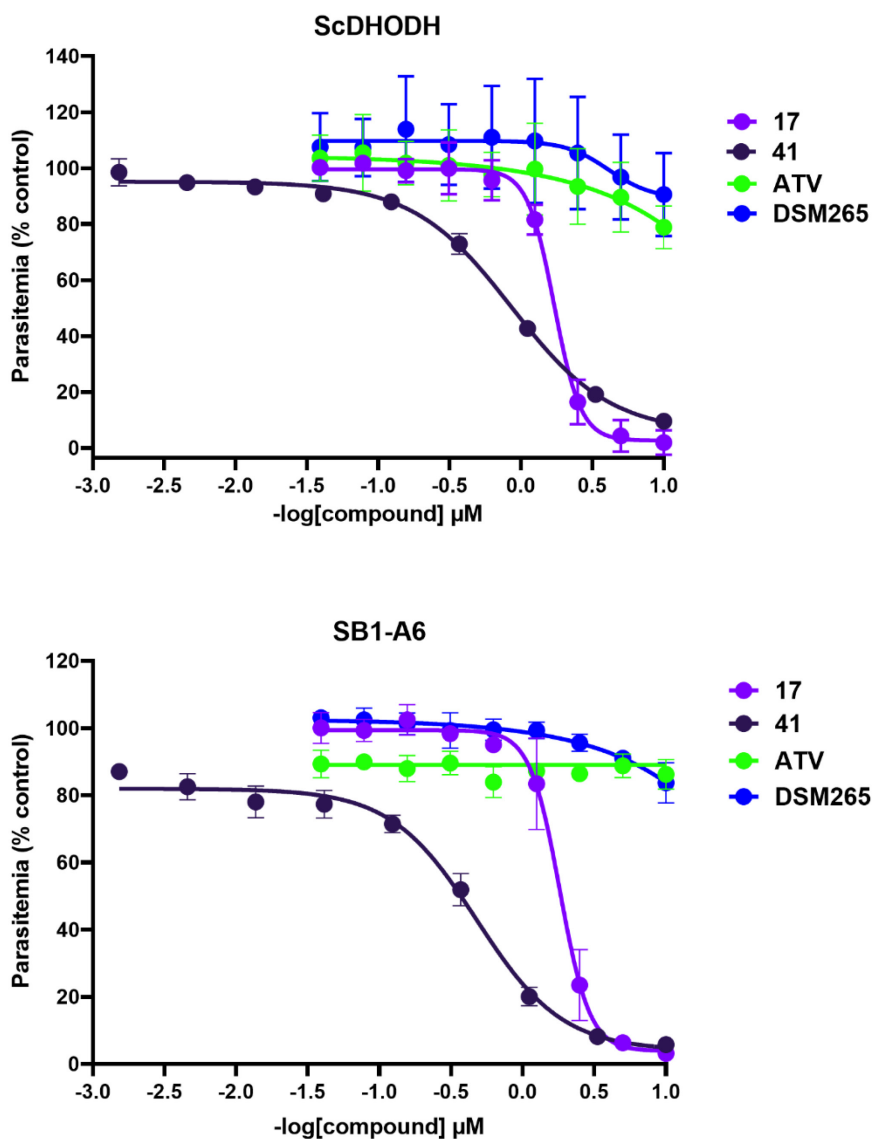


**Figure S3.** A. A homology model of *P. falciparum* cyt *b* showing mutations found in **41**, ELQ300 and ATQ *P. falciparum* resistant strains is shown in Figure 3 and Table 5 respectively. The homology model of *P. falciparum* cyt *b* was created from *Gallus gallus* cyt *bc*<sub>1</sub> (PDB: 3H11).<sup>1</sup> TM90-C2B strain cyt *b* Y268S Q<sub>o</sub> site mutation is shown in magenta; Dd2 strain cyt *b* I222L Q<sub>i</sub> site mutant is shown in orange; 3D7 **41** resistant strain cyt *b* V259L Q<sub>o</sub> site mutation is shown in cyan. Heme molecules are shown in grey. The relative position of **41** (blue) is predicted by docking to the Q<sub>o</sub> site, while ATQ (green) and ubiquinol (pink) in the Q<sub>o</sub> site, and ELQ300 (brown), ubiquinone (yellow) in the Q<sub>i</sub> site were overlaid using previous structural data.<sup>2,3</sup> B. Q<sub>o</sub> site of cyt *b* showing the interaction of **41** with Rieske protein (salmon) and the proximity to Fe-S cluster (orange/yellow).

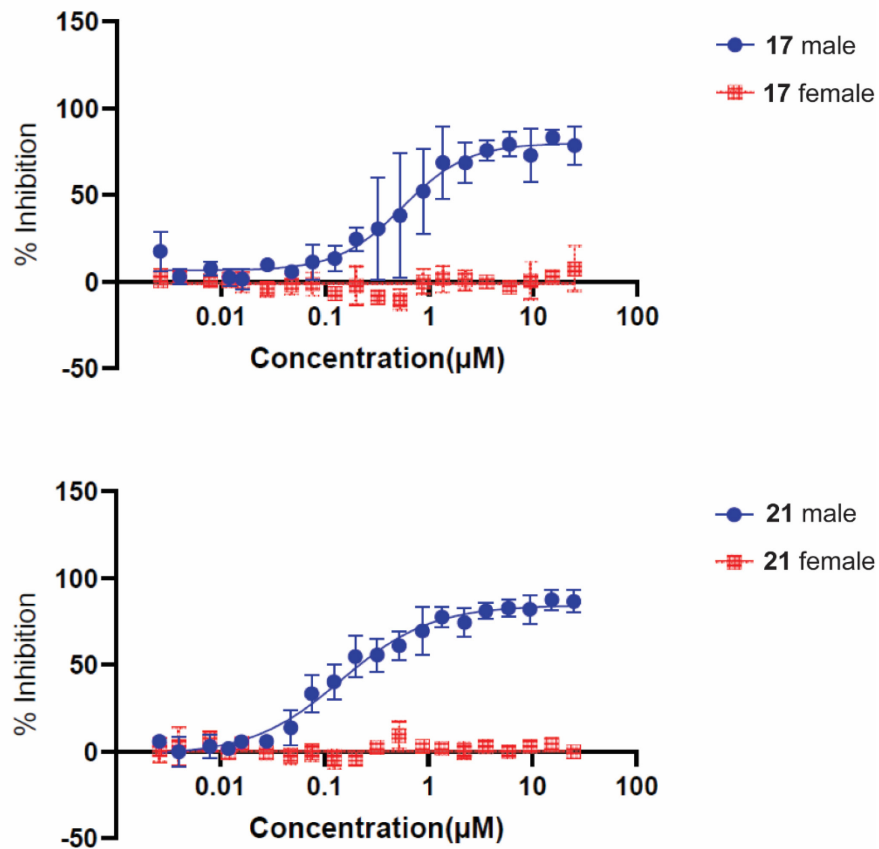


**Figure S4.** Non-synonymous single nucleotide polymorphism identified in *cyt b* (PF3D7\_MIT02300) from compound **41** resistant parasite populations r1, r2 and r3 (samples in duplicate).



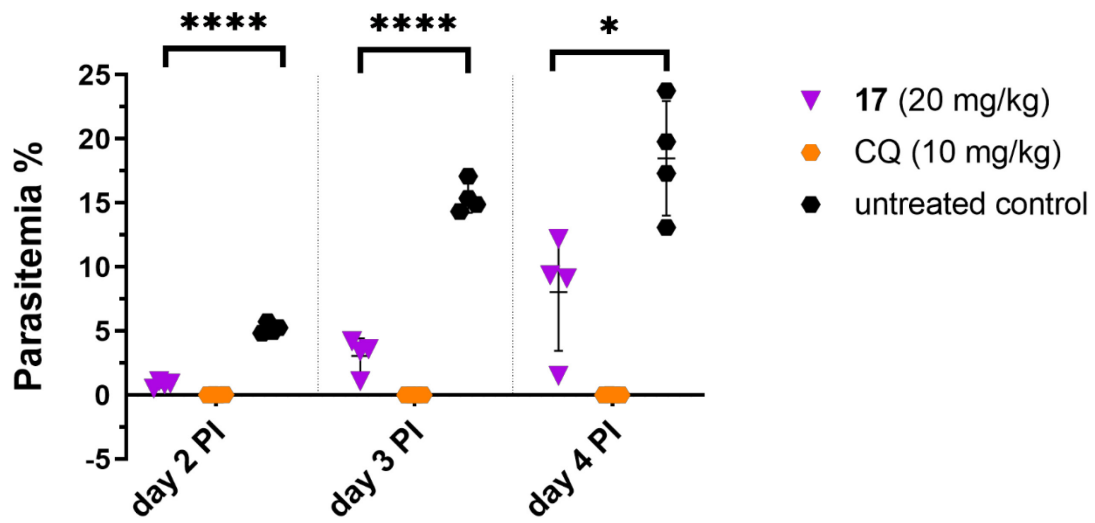


**Figure S5.** Dose response curves represent averages and SDs of 3 independent experiments against the Pf SB1-A6 strain with a CNV (~2-fold) and a C276F mutation in DHODH or Pf Dd2 expressing ScDHODH over 72 h measuring SYBR green by FACS.

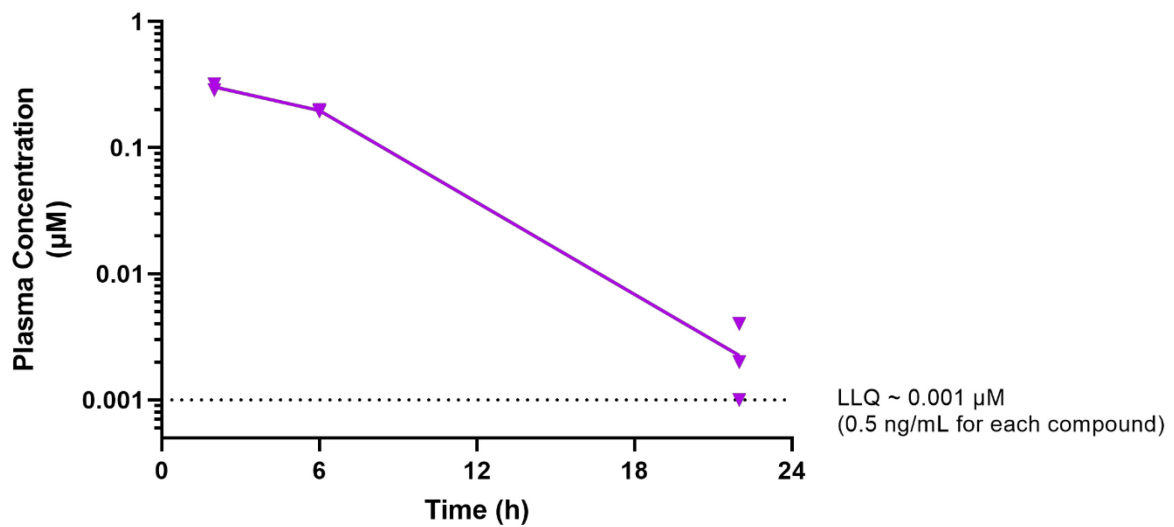


**Figure S6.** *P. falciparum* NF54 DFGA dose response curves from a 20-pt dilution series of compounds **17** and **21**. Data represents means and SD of 4 technical replicates.

A.



B.



**Figure S7. A.** Evaluation of **17** in a *P. berghei* 4 day mouse model. *P. berghei ANKA* parasites expressing GFP were injected into the tail vein to infect mice on day 0. Compound **17** was administered q.d. at 20 mg/kg by p.o. 2 h after infection (day 0) and then on days 1, 2, and 3. Parasitemia was measured by flow cytometry on days 2, 3 and 4. unpaired t test (vs vehicle), P values. **B.** Plasma concentration of **17** after dose 1 in the *P. berghei* mouse model.

**Table S1.** Non-synonymous and synonymous single nucleotide polymorphisms identified from compound **41** resistant genomes.

Chromosome	Pos	Base Change	AA change	AA pos in transcript	transcript ID
Pf3D7_02_v3 <sup>a</sup>	110010	A->C	F->V	155	PF3D7_0202100.1
Pf3D7_10_v3 <sup>b</sup>	1436924	T->G	D->E	203	PF3D7_1036400.1
Pf3D7_10_v3	1436954	A->G	Synonymous	213	PF3D7_1036400.1
Pf3D7_10_v3	1437149	G->A	Synonymous	278	PF3D7_1036400.1
Pf3D7_10_v3	1437158	A->G	Synonymous	281	PF3D7_1036400.1
Pf3D7_10_v3	1437287	T->A	Synonymous	324	PF3D7_1036400.1
Pf3D7_10_v3	1437302	G->A	Synonymous	329	PF3D7_1036400.1
Pf3D7_10_v3	1437305	A->G	Synonymous	330	PF3D7_1036400.1
Pf3D7_10_v3	1437307	A->G	E->G	331	PF3D7_1036400.1
Pf3D7_10_v3	1437321	T->C	Synonymous	336	PF3D7_1036400.1
Pf3D7_10_v3	1437456	C->A	Q->K	381	PF3D7_1036400.1
Pf3D7_10_v3	1437695	T->A	Synonymous	460	PF3D7_1036400.1
Pf3D7_10_v3	1437701	A->G	Synonymous	462	PF3D7_1036400.1
Pf3D7_14_v3	3000703	T->C	Synonymous	551	PF3D7_1473700.1
Pf3D7_14_v3	3000763	C->T	Synonymous	531	PF3D7_1473700.1
Pf3D7_MIT_v3	4266	G->C	V->L	259	PF3D7_MIT02300.1

<sup>a</sup> Pf3D7\_02\_v3:110010 is a miscall due to a deletion.

<sup>b</sup> Pf3D7\_10\_v3:1436924 is present in the WT parent at 29%.

**Table S2.** EC<sub>50</sub> values (nM) of **17**, **41**, ATQ and ELQ300 against **41** resistant populations (with a V259L *cyt b* Q<sub>o</sub> site mutation). EC<sub>50</sub> values represent an average of 3 experiments using the LDH assay (Figure 3).

compound	<b>3D7</b>	<b>41 r#1</b>	<b>41 r#2</b>	<b>41 r#3</b>
<b>41</b>	32.1 (21.5-47.1)	809 (593-1234)	733 (533-1171)	896 (701-1131)
<b>17</b>	7.622 (4.0-10.7)	369 (282-491)	248 (213-289)	235 (204-272)
ATQ	1.6 (1.1-2.1)	13.0 (10.9-15.5)	11.9 (9.6-14.6)	13.7 (11.2-16.6)
ELQ-300	17.8 (14.8-21.1)	13.0 (10.9-15.5)	12.3 (10.3-14.6)	12.4 (10.3-14.8)

<sup>a</sup> EC<sub>50</sub> values (95% confidence intervals) from 3 biological replicates (nM).

**Table S3.** Evaluation of selected compounds against *P. falciparum* asexual parasites resistant to mitochondria targeted drugs.

Cmpd	Pf Dd2 EC <sub>50</sub> μM <sup>a</sup>	Pf cytBC1 mutant strains EC <sub>50</sub> μM	
		TM90-C2B <sup>a</sup>	Dd2 <sup>cyt b</sup> (I22L) <sup>a</sup>
<b>17</b>	0.010	1.91	0.006
	0.008	1.29	0.007
<b>21</b>	0.062	0.051	0.051
	0.048	0.030	0.030
<b>41</b>	0.005	0.177	0.003
	0.003	0.206	0.002
<b>43</b>	0.017	0.069	0.014
	0.013	0.042	0.009
<b>ATQ</b>	0.001	6.46	0.005
		4.35	
<b>ELQ300</b>	0.027	-	0.194
	0.014		0.157
<b>DSM265</b>	0.028	-	0.008
	0.020		

<sup>a</sup> Activity values against the PfDd2 parental line, Pf TM90-C2B strain with a Y268S mutation in the Q<sub>o</sub> site of *cyt b* or PfDd2 strain with an I22L mutation in the Q<sub>i</sub> site *cyt b* using a <sup>3</sup>H-hypoxanthine 72 h assay. EC<sub>50</sub> values are an average of two experiments. Average data is shown in Table 4.

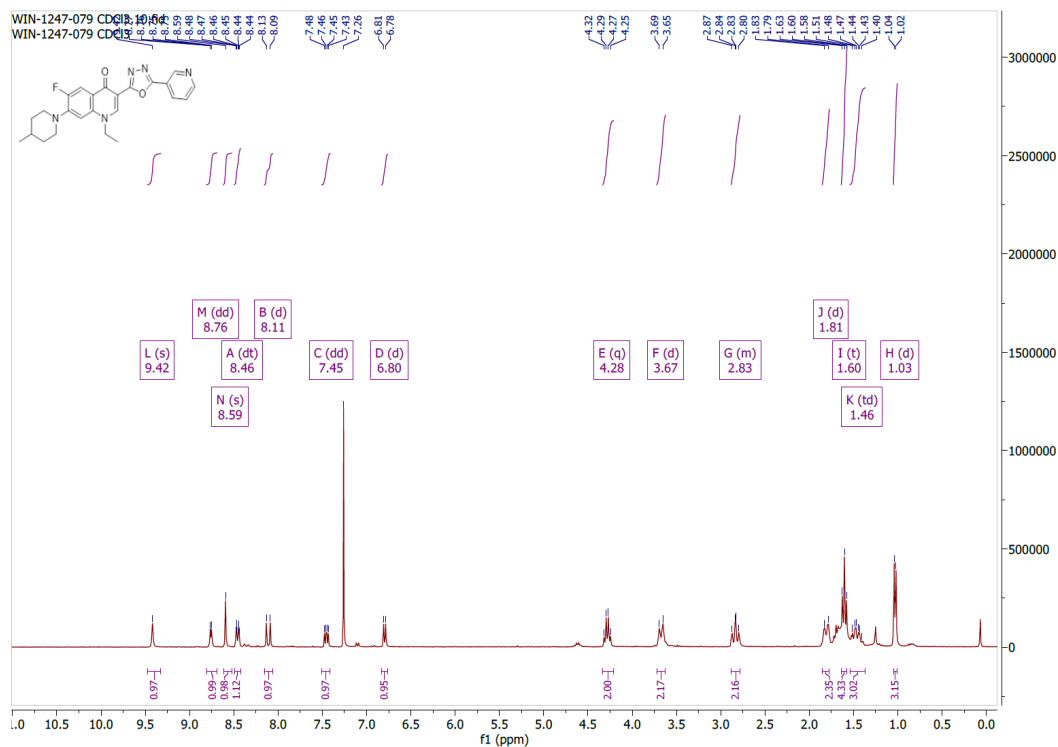
**Table S4.** Activity of selected compounds against drug resistant *P. falciparum* strains.

Cmpd	Dd2 EC <sub>50</sub> μM <sup>a</sup>	Pf multidrug resistant strains. EC <sub>50</sub> μM <sup>a</sup>							
		Dd2 CARL (I1139K)	Dd2 DHODH (C276F)	Dd2 eEF2 (Y86N)	Dd2 PI4K (S743T)	K1	NF54	RF12	7G8
<b>17</b>	0.011	0.009	0.018	0.008	0.011	0.013	0.019	0.007	0.008
	0.009	0.010	0.013	0.008	0.011	0.007	0.006	0.005	0.010
<b>21</b>	0.062	0.014	-	0.016	0.023	0.016	0.041	0.014	0.036
	0.048	0.029	-	0.020	0.031	0.033	0.047	0.023	0.040
<b>41</b>	0.004	0.004	0.006	0.002	0.005	0.006	0.006	0.002	0.003
	0.004	0.004	0.003	0.003	0.005	0.004	0.003	0.001	0.002
<b>43</b>	0.017	0.005	-	0.004	0.009	0.009	0.008	0.004	0.015
	0.014	0.009	-	0.008	0.010	0.021	0.028	0.006	0.010
<b>KAF156</b>	0.008	1.36	-	-	-	-	-	-	-
	0.008	1.69	-	-	-	-	-	-	-
<b>DSM265</b>	0.027	-	0.576	-	-	-	-	-	-
	0.020	-	0.362	-	-	-	-	-	-
<b>DDD 107498</b>	0.001	-	-	0.859	-	-	-	-	-
	0.022	-	-	0.842	-	-	-	-	-
<b>MMV 048</b>	0.009	-	-	-	0.117	-	-	-	-
	0.022	-	-	-	0.116	-	-	-	-

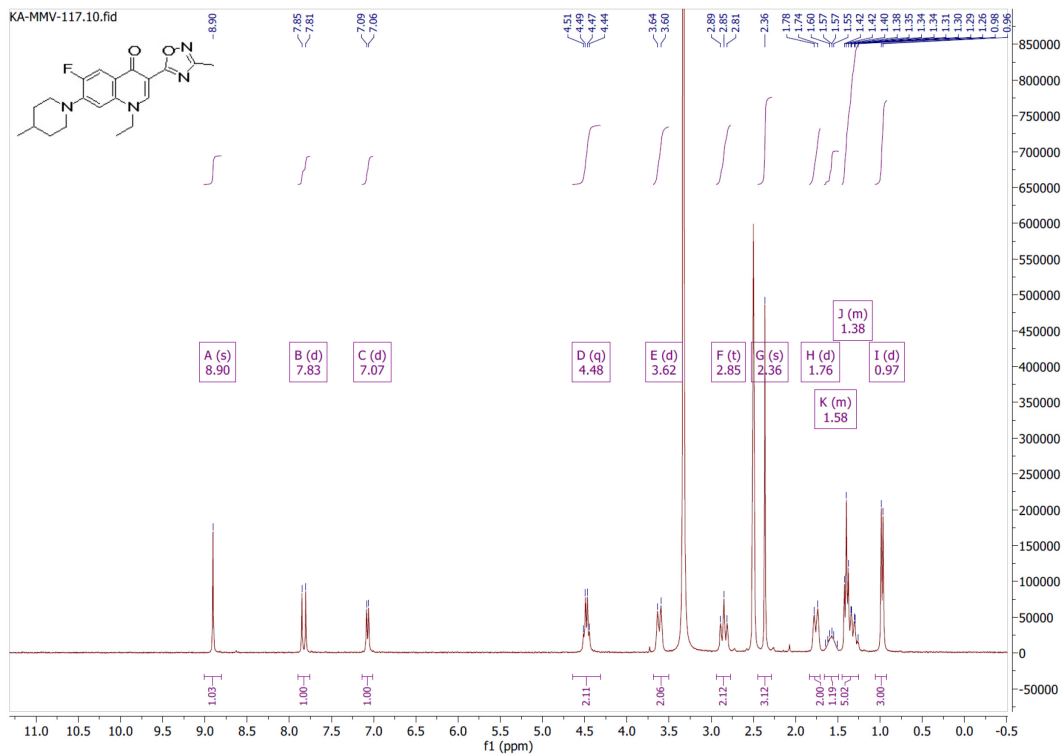
<sup>a</sup> EC<sub>50</sub> data represents means for each <sup>3</sup>H-hypoxanthine experiment using a selection of drug resistant *P. falciparum* strains following exposure to compounds in 10-point dilution series for 72 h. Average data is shown in Table 5.

# <sup>1</sup>H NMR spectra for final compounds

## Compound 16

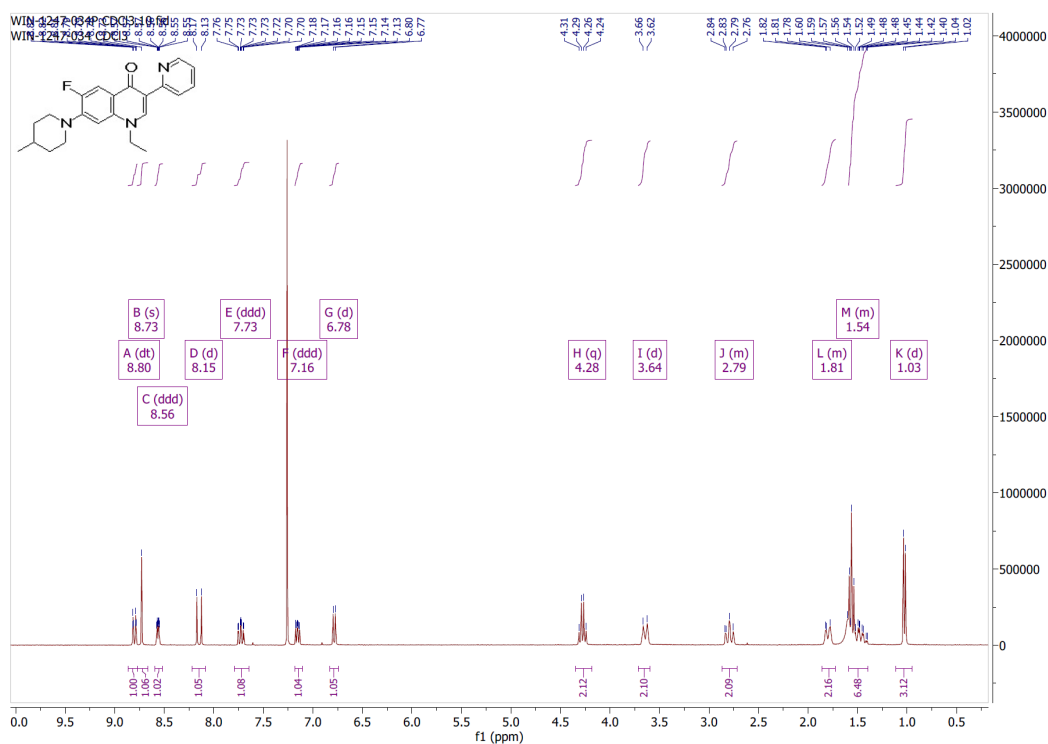


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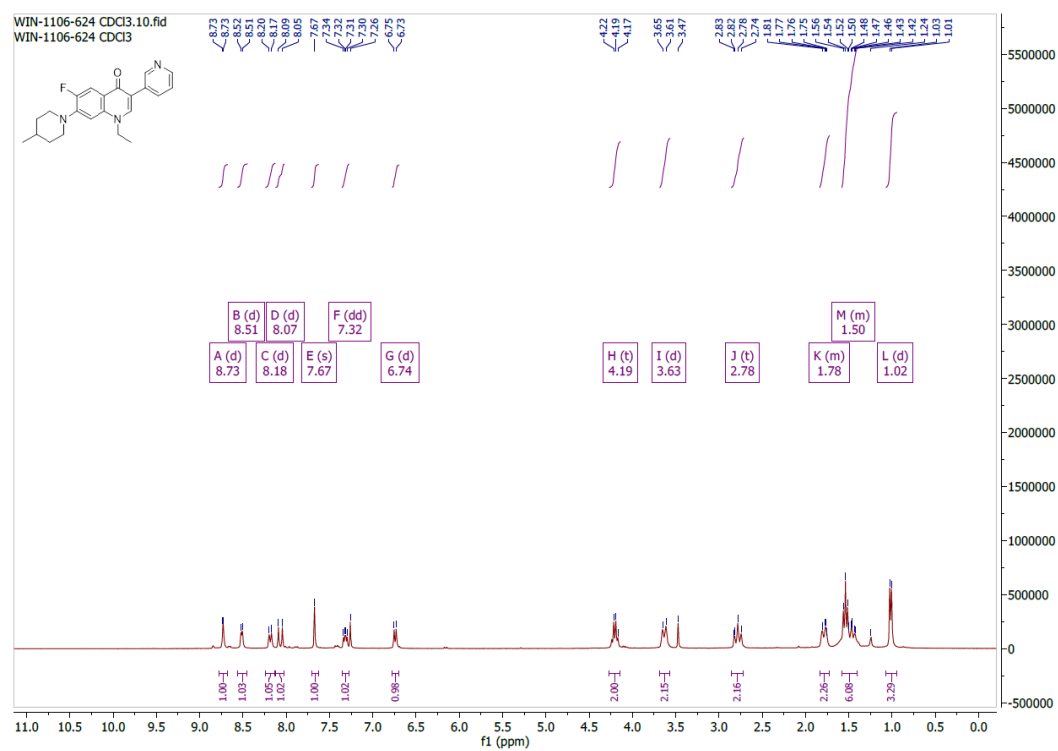




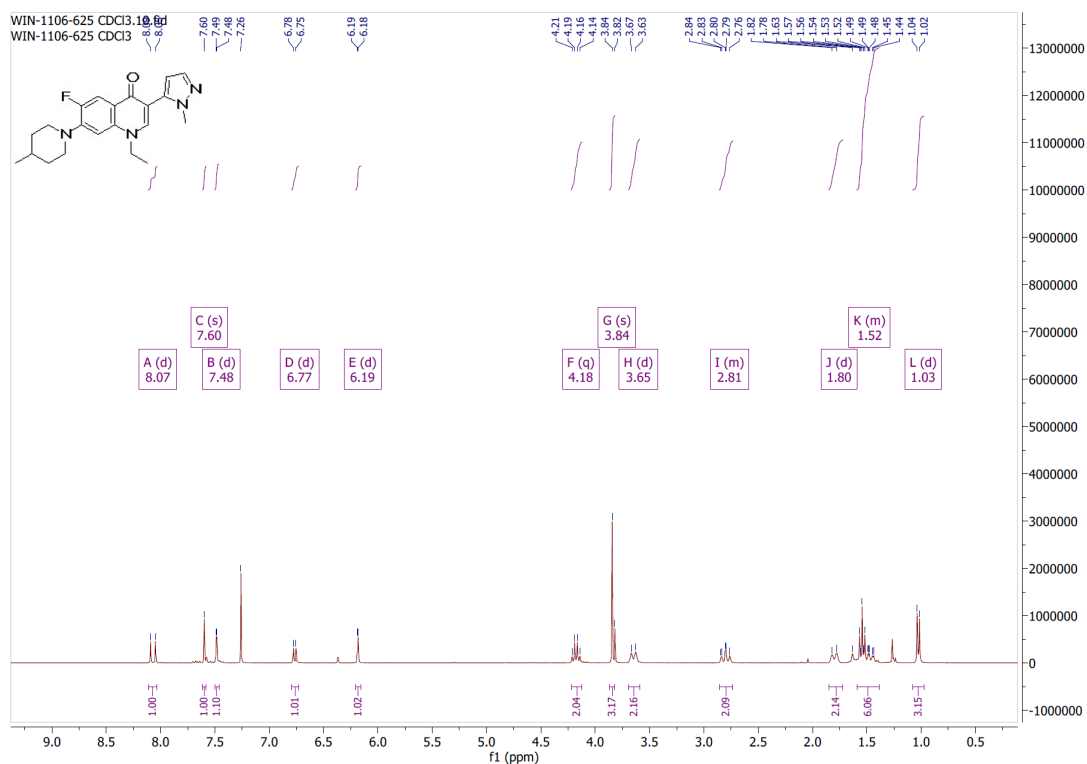
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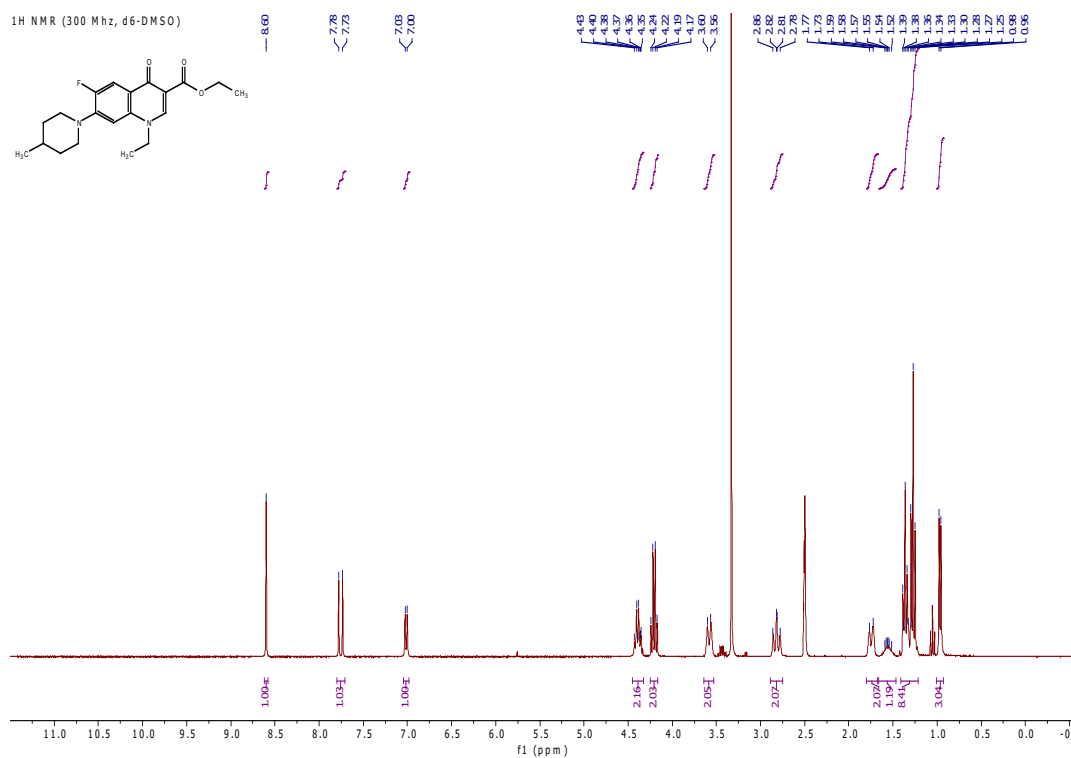
# Compound 19



## Compound 20

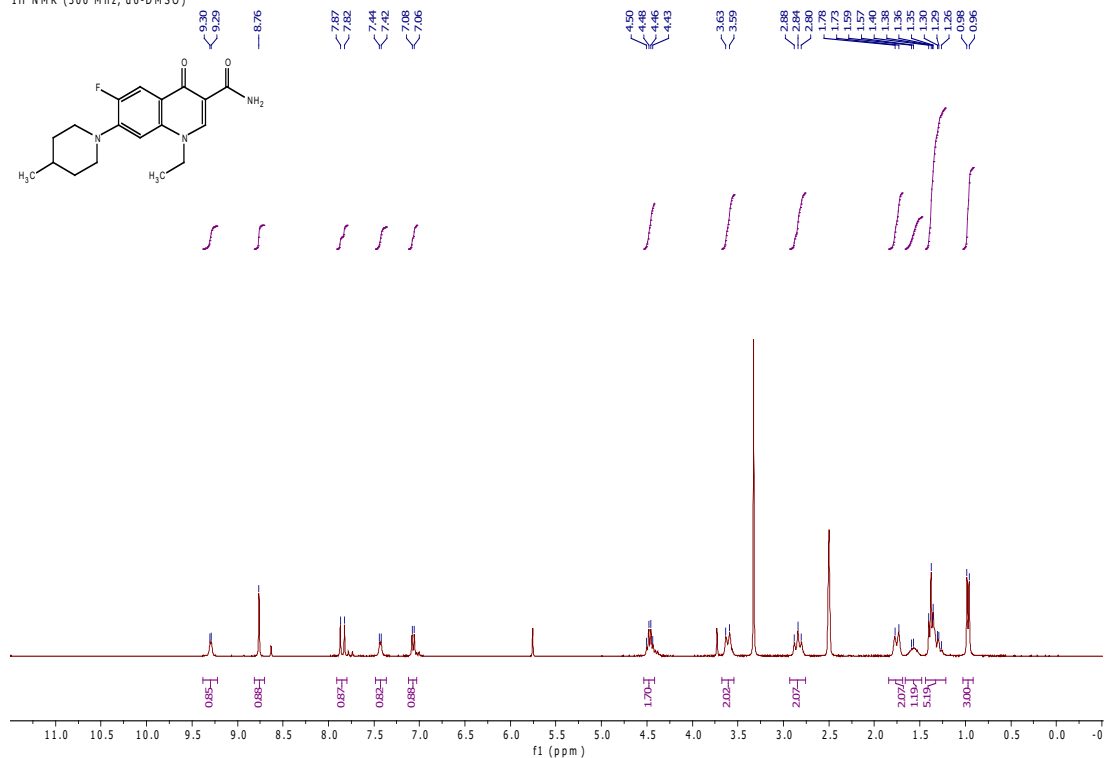


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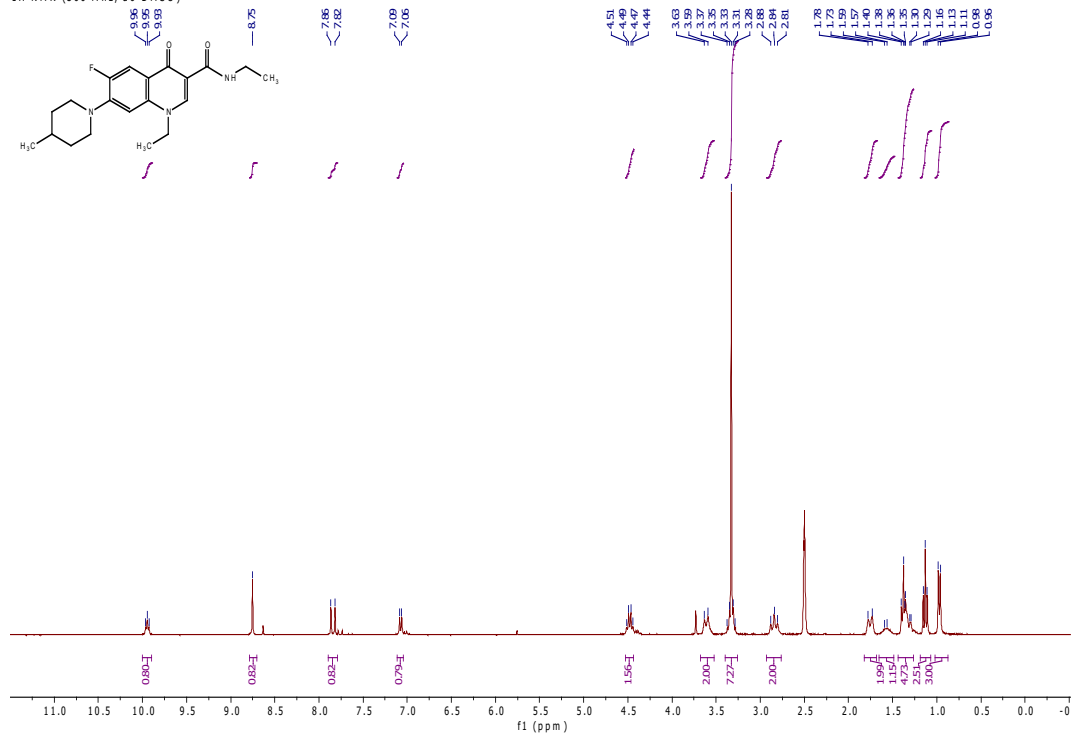
## Compound 22

<sup>1</sup>H NMR (300 Mhz, d6-DMSO)



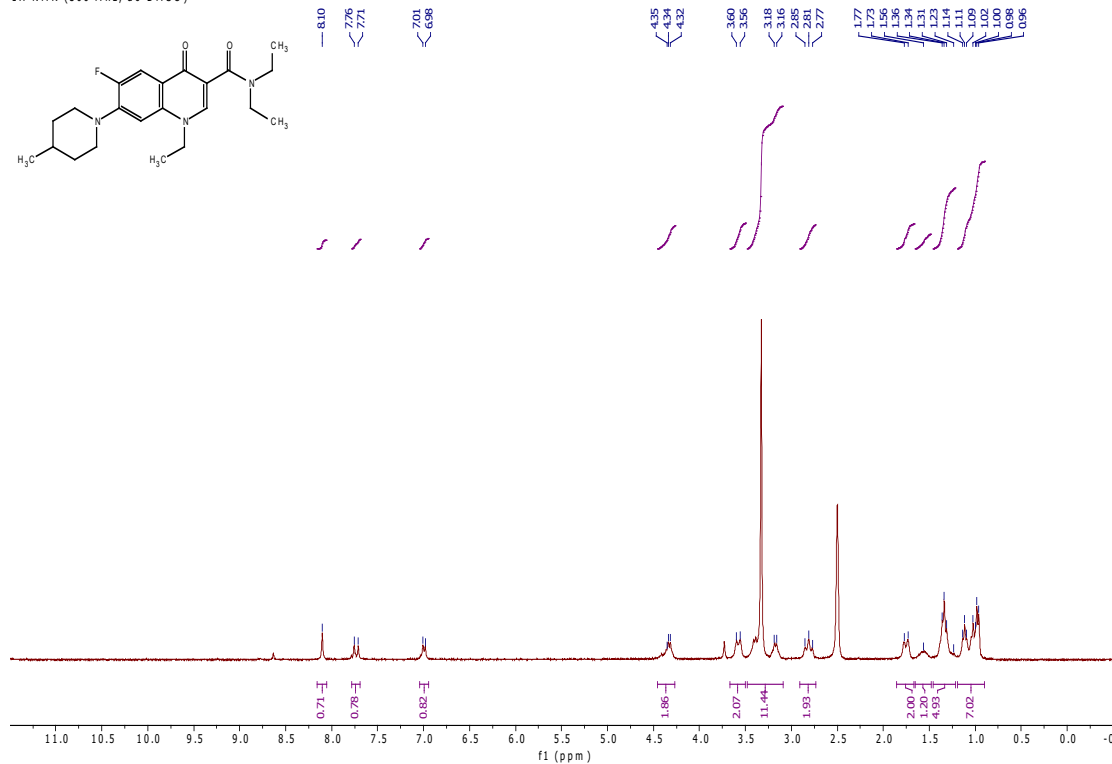
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<sup>1</sup>H NMR (300 Mhz, d6-DMSO)



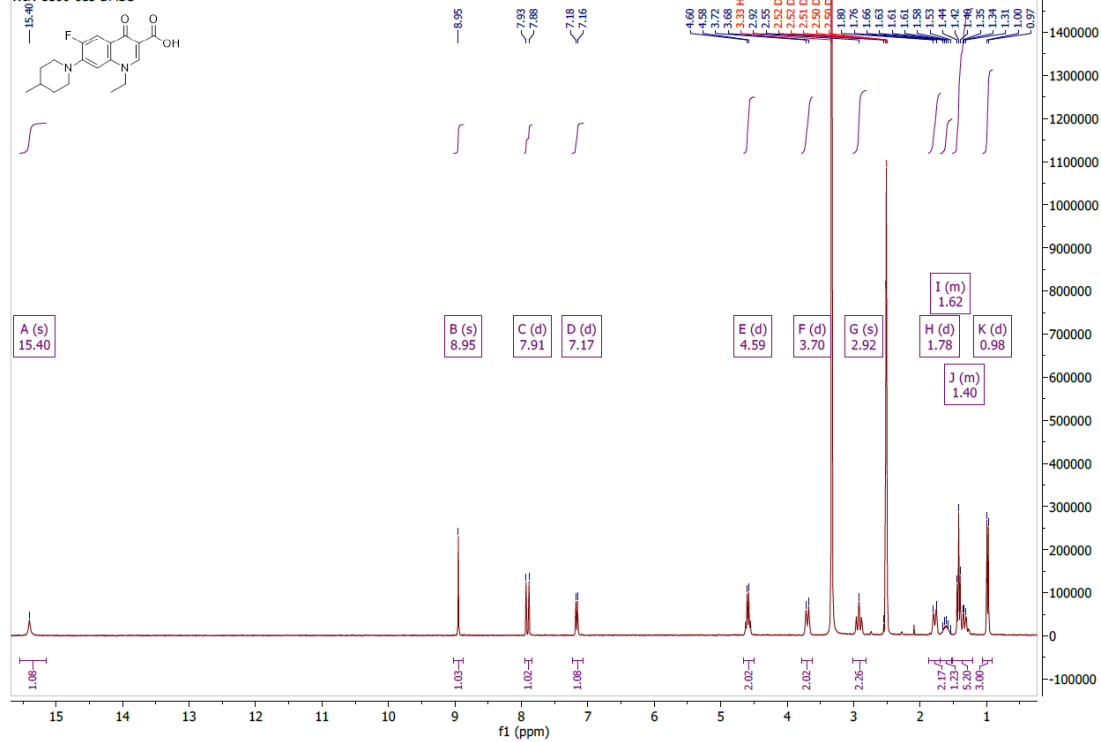
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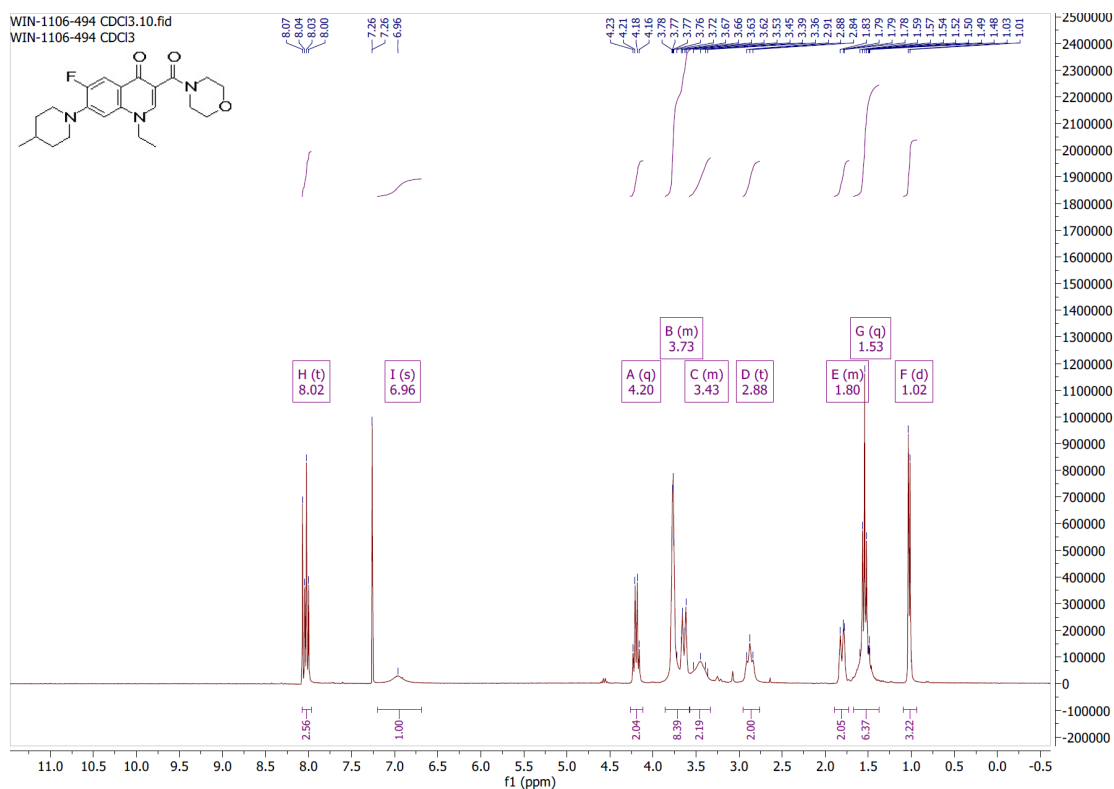


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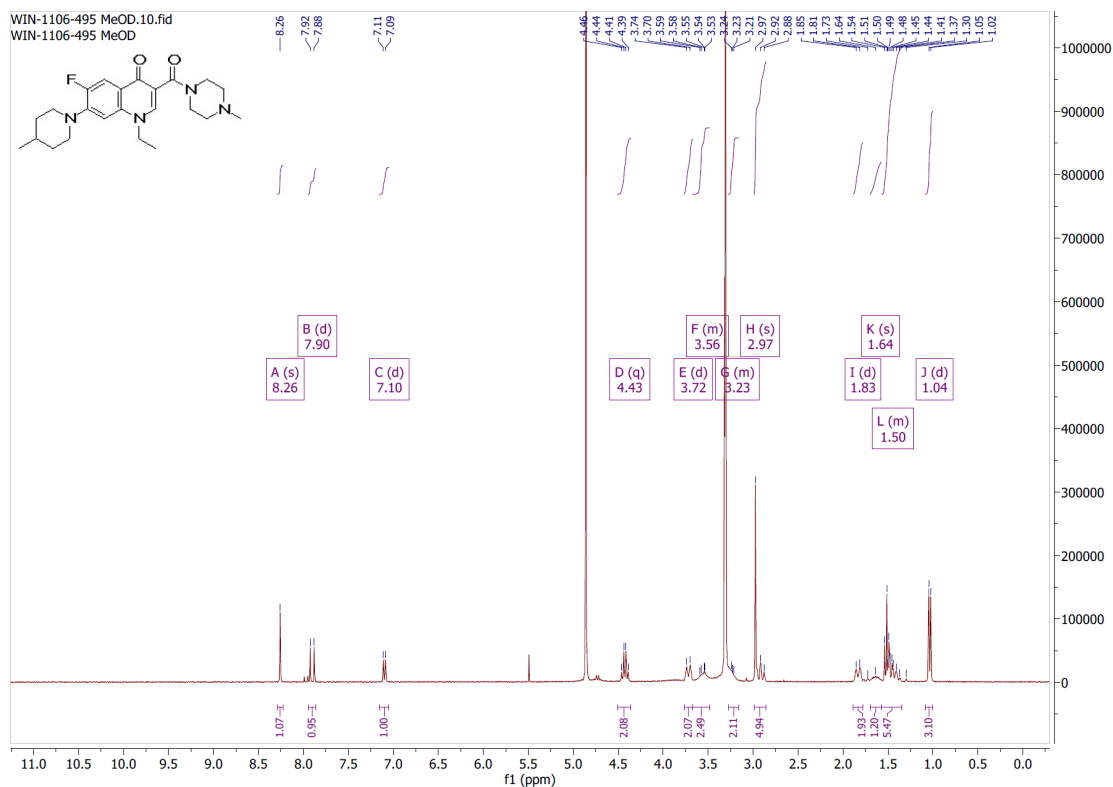
WIN-1106-613 DMSO.10.fid  
WIN-1106-613 DMSO



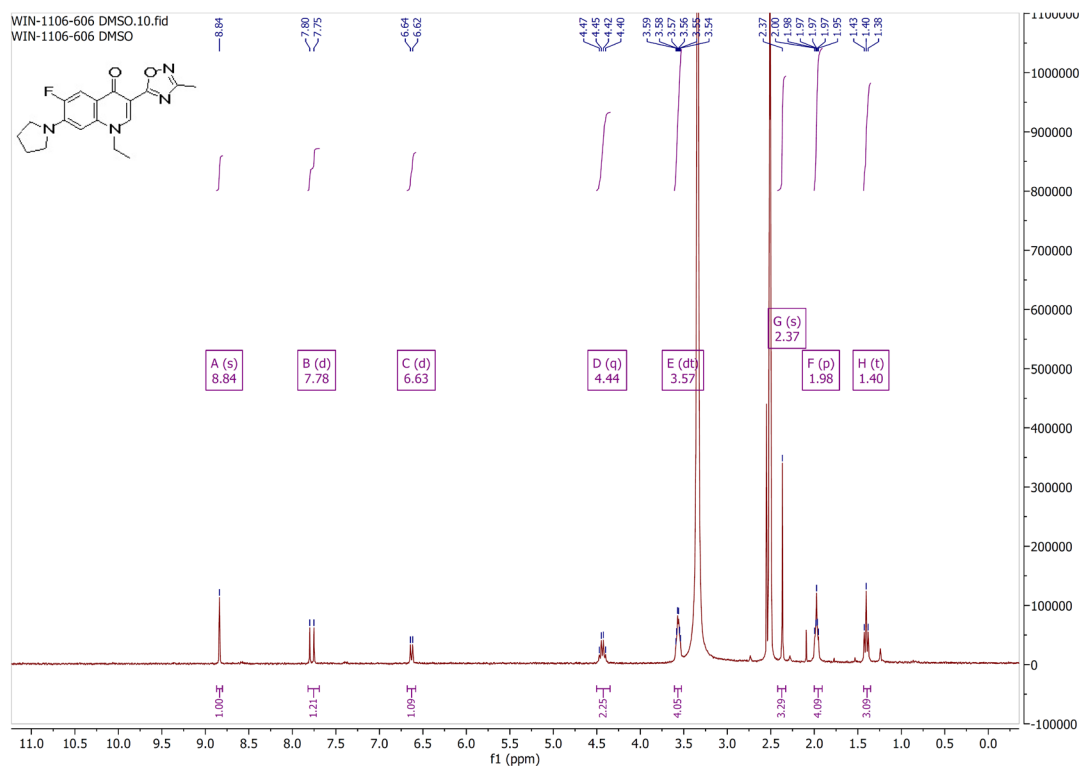
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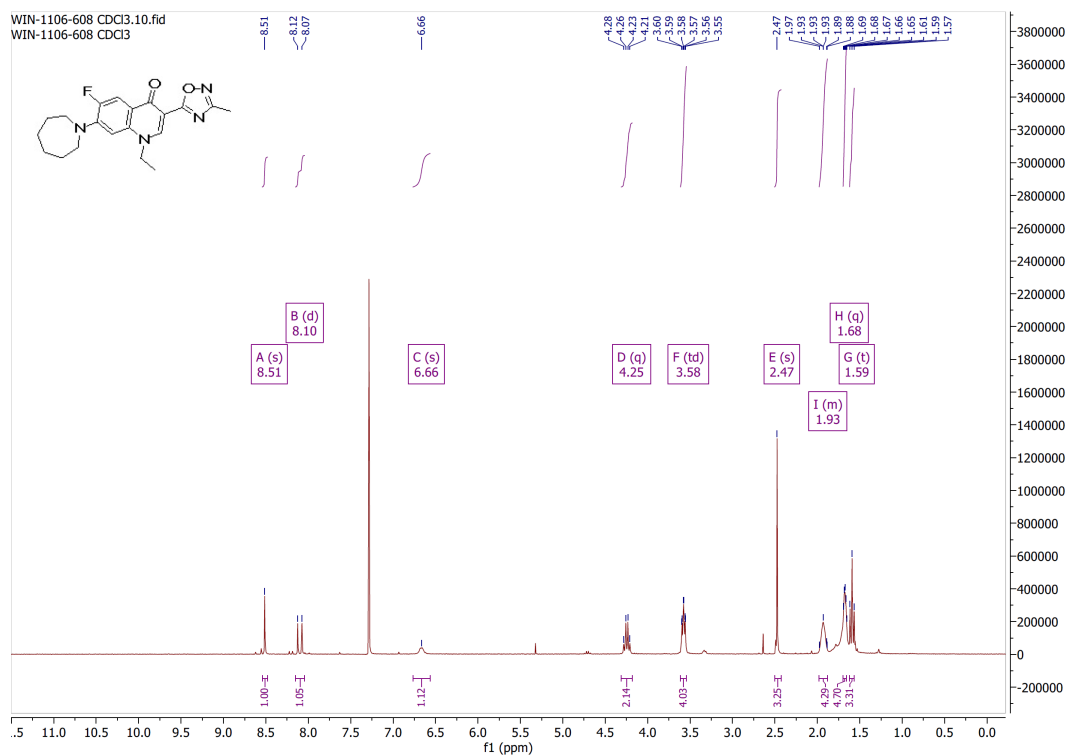
## Compound 27



## Compound 28

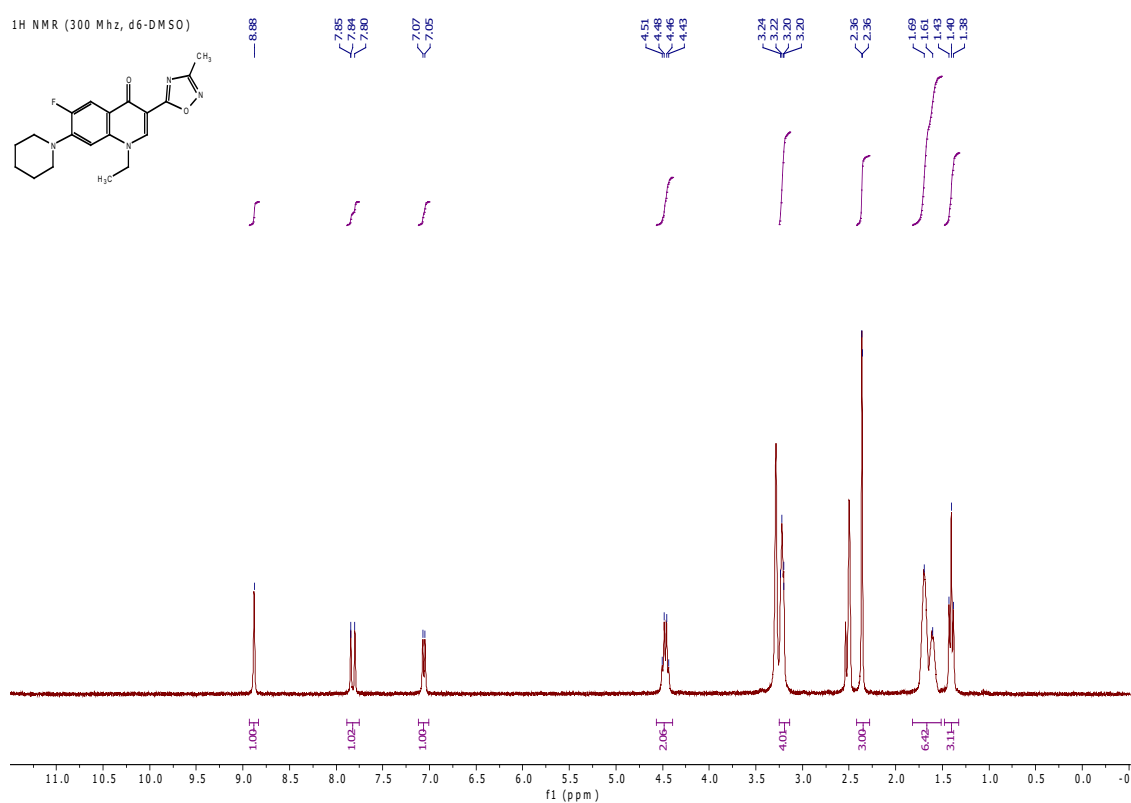
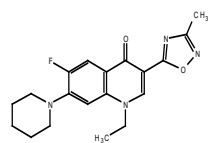


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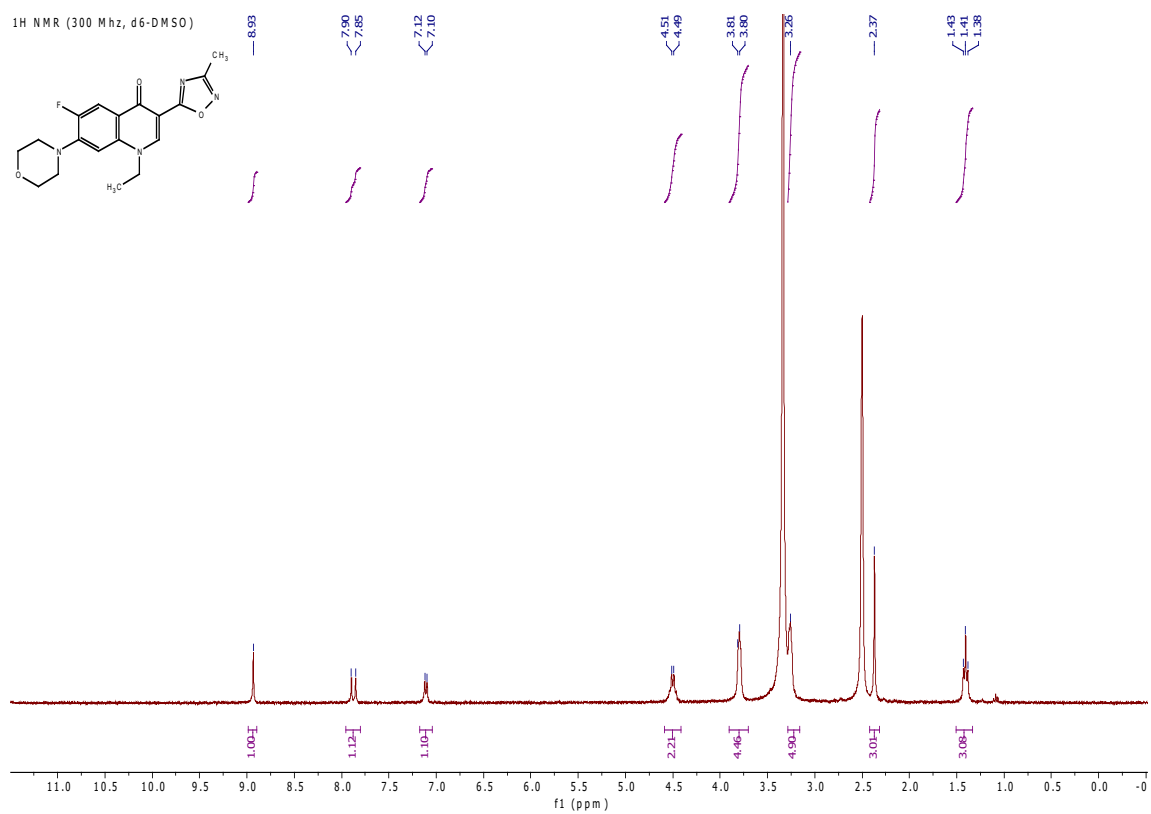
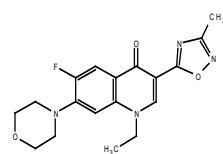
## Compound 30

<sup>1</sup>H NMR (300 Mhz, d6-DMSO)



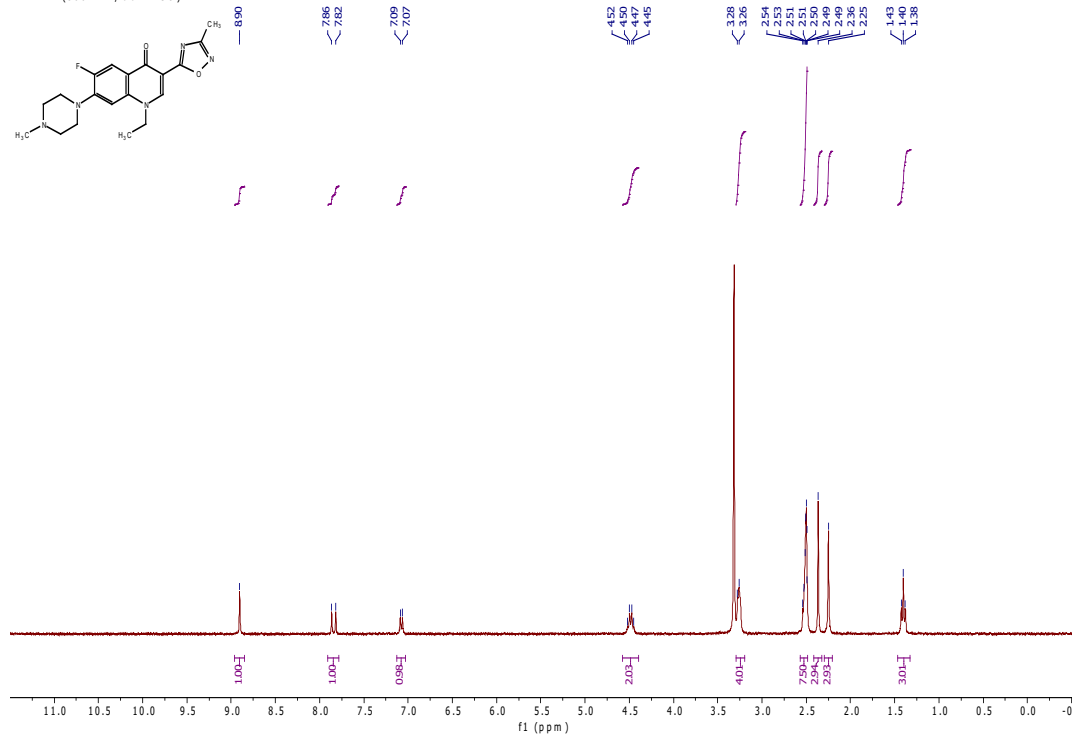
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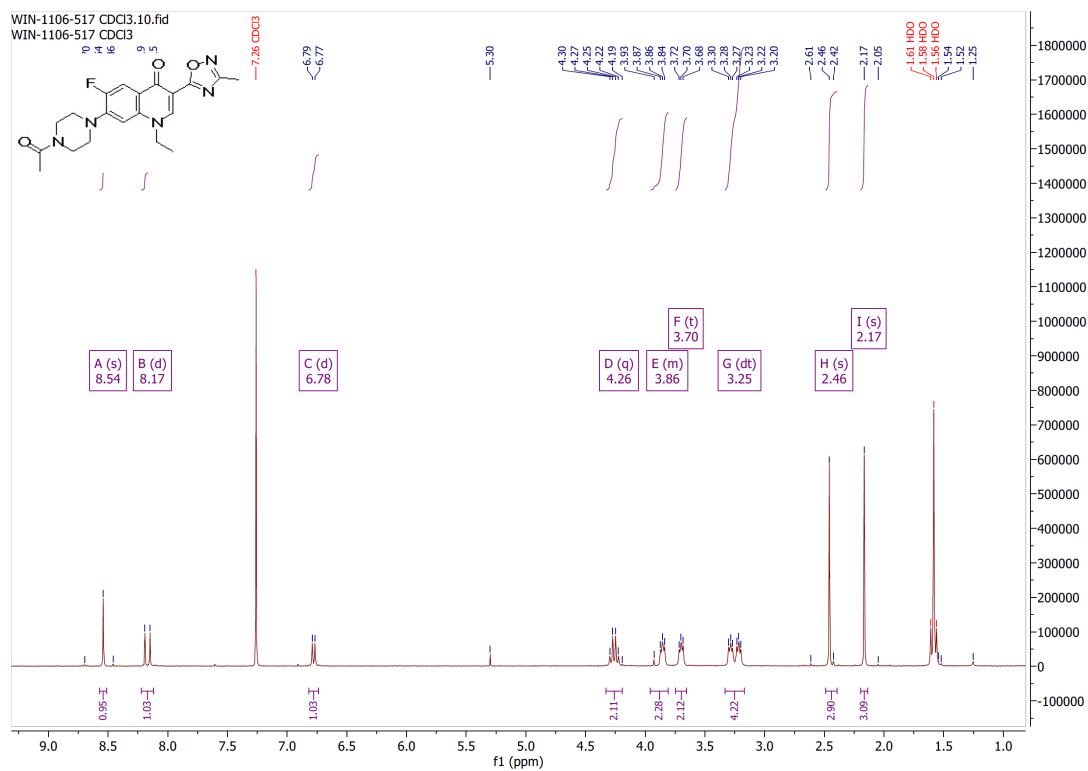
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<sup>1</sup>H NMR (300 MHz, d6-DMSO)



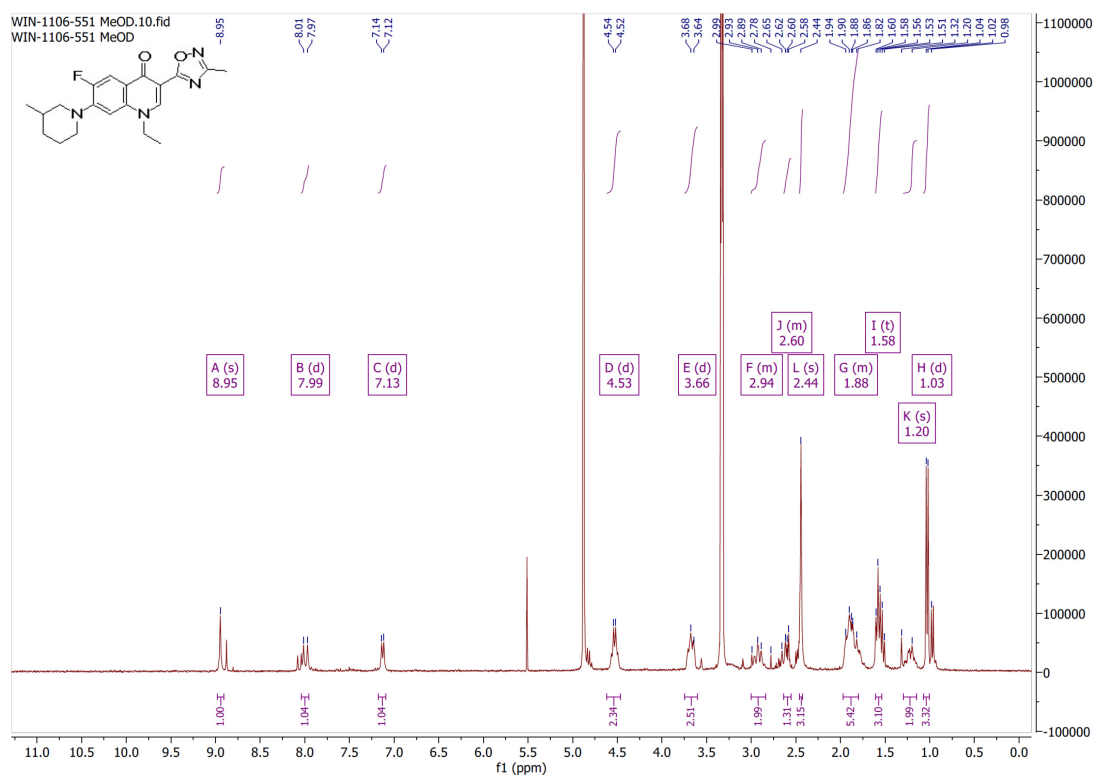
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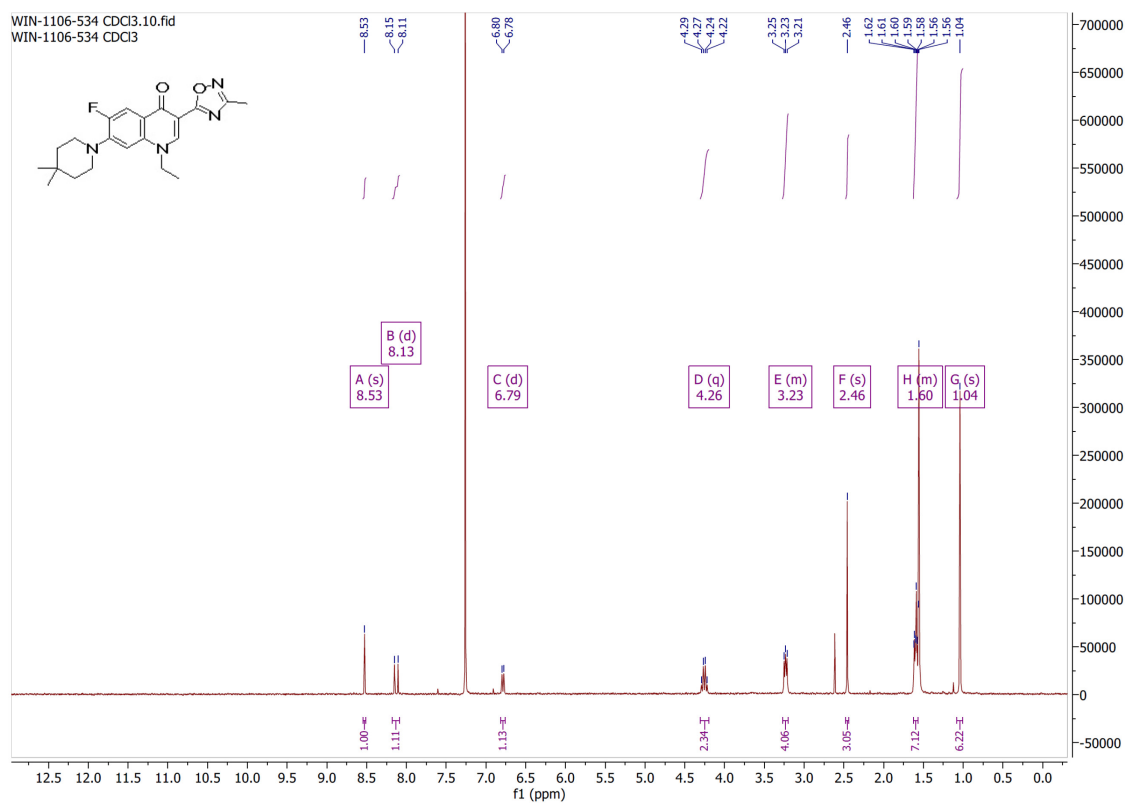




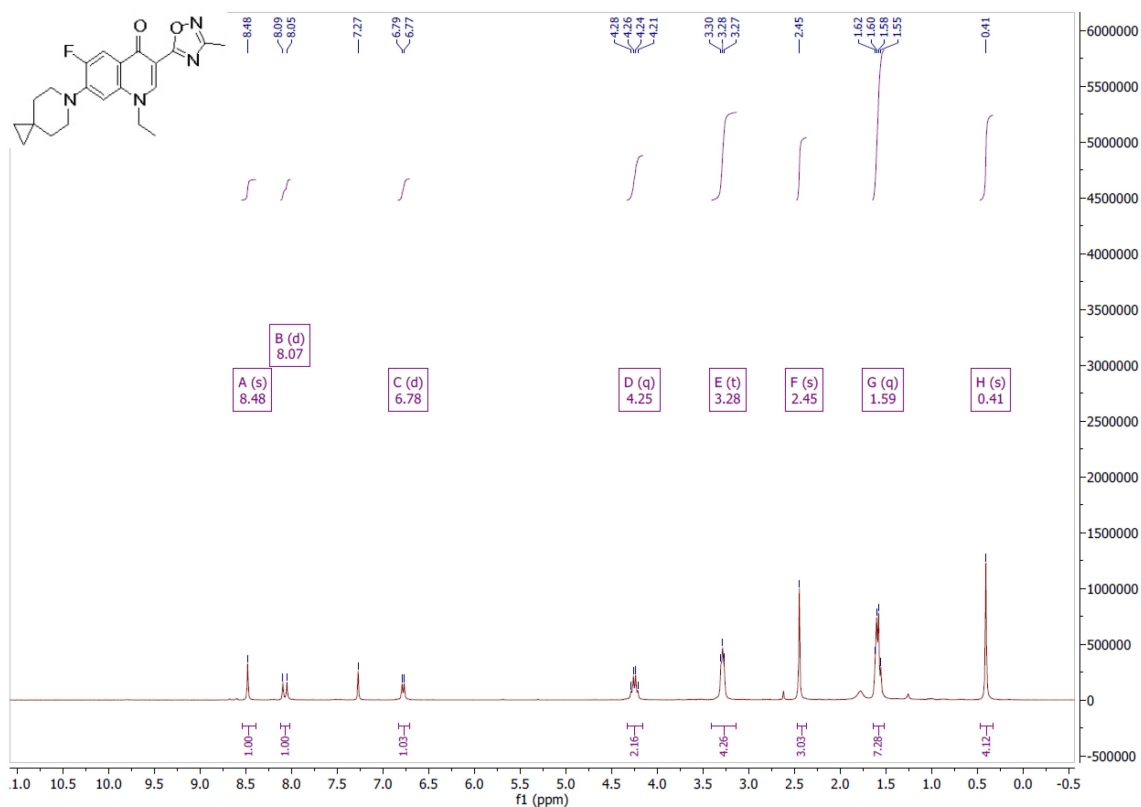
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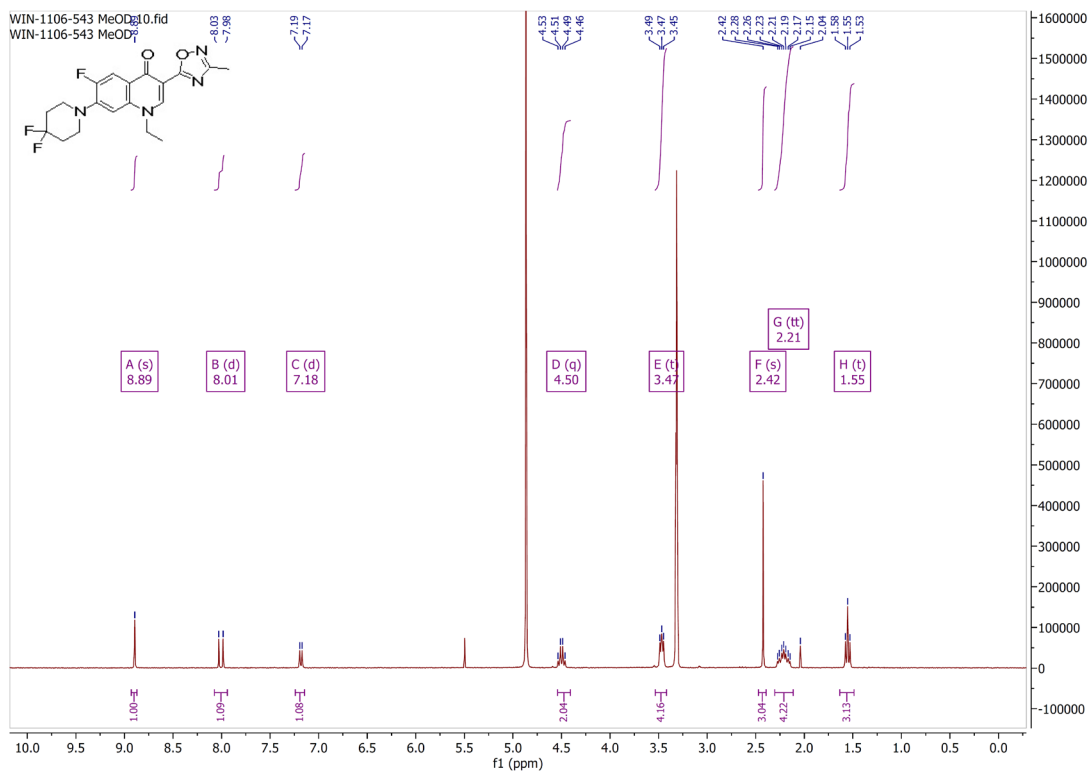
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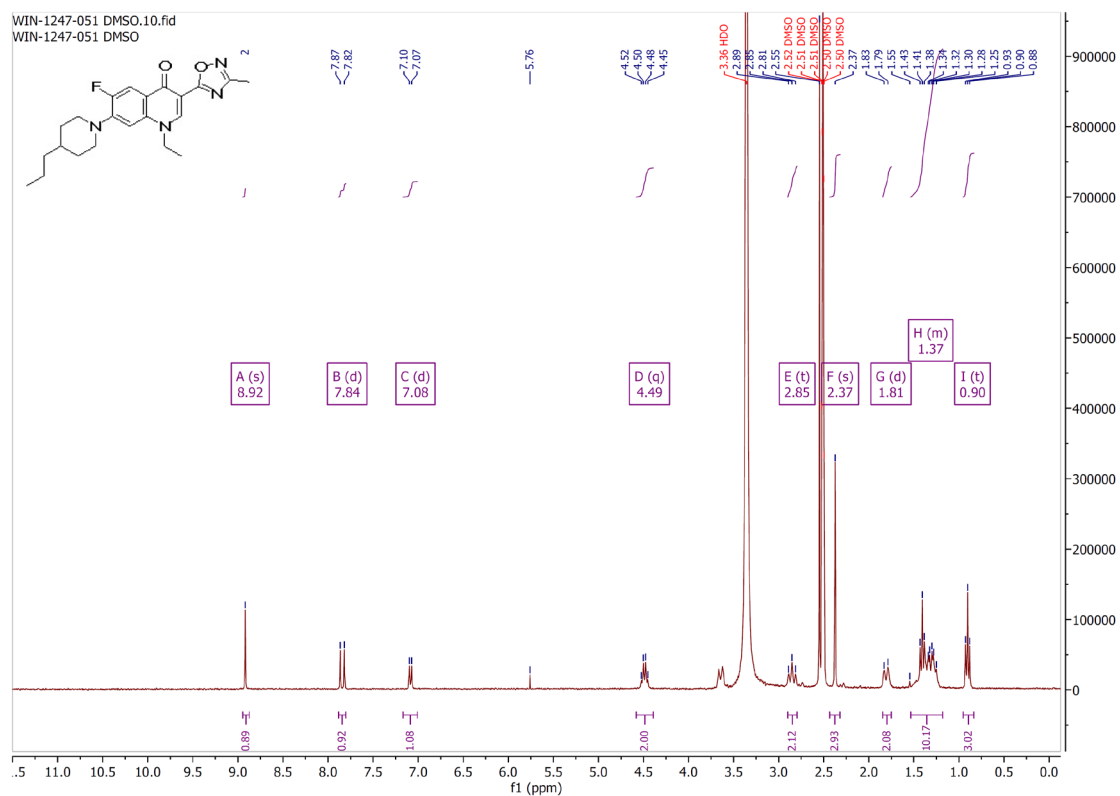
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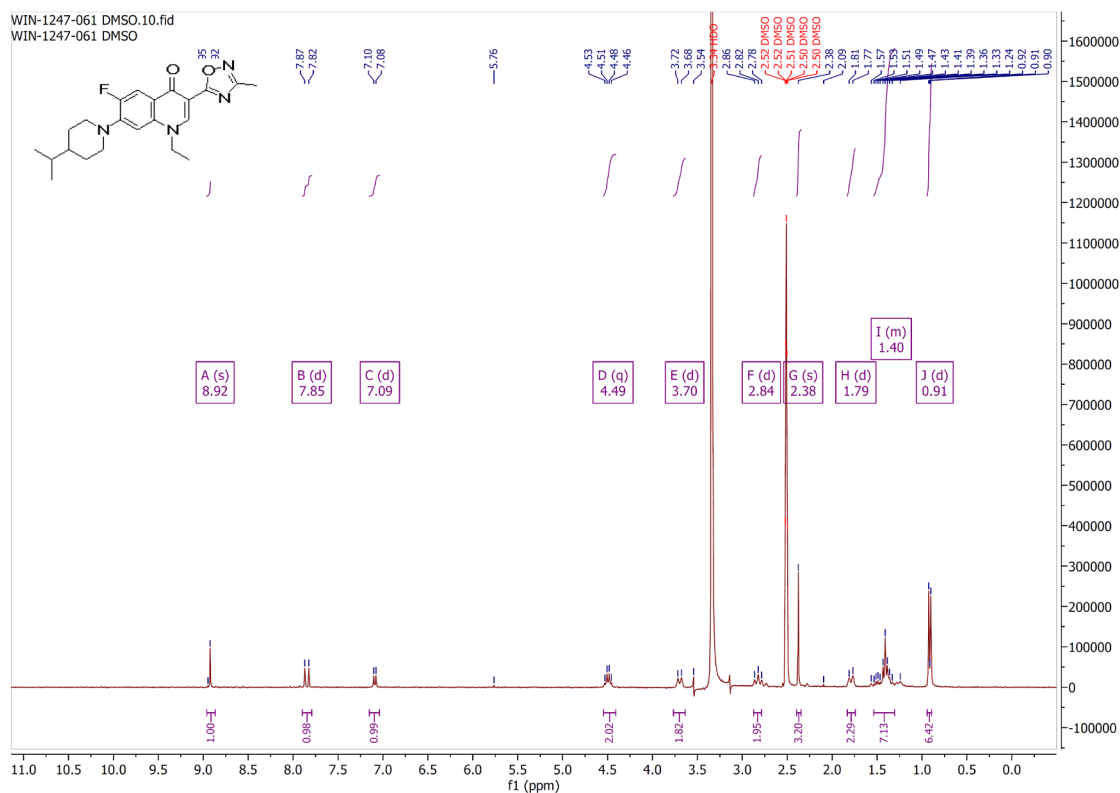
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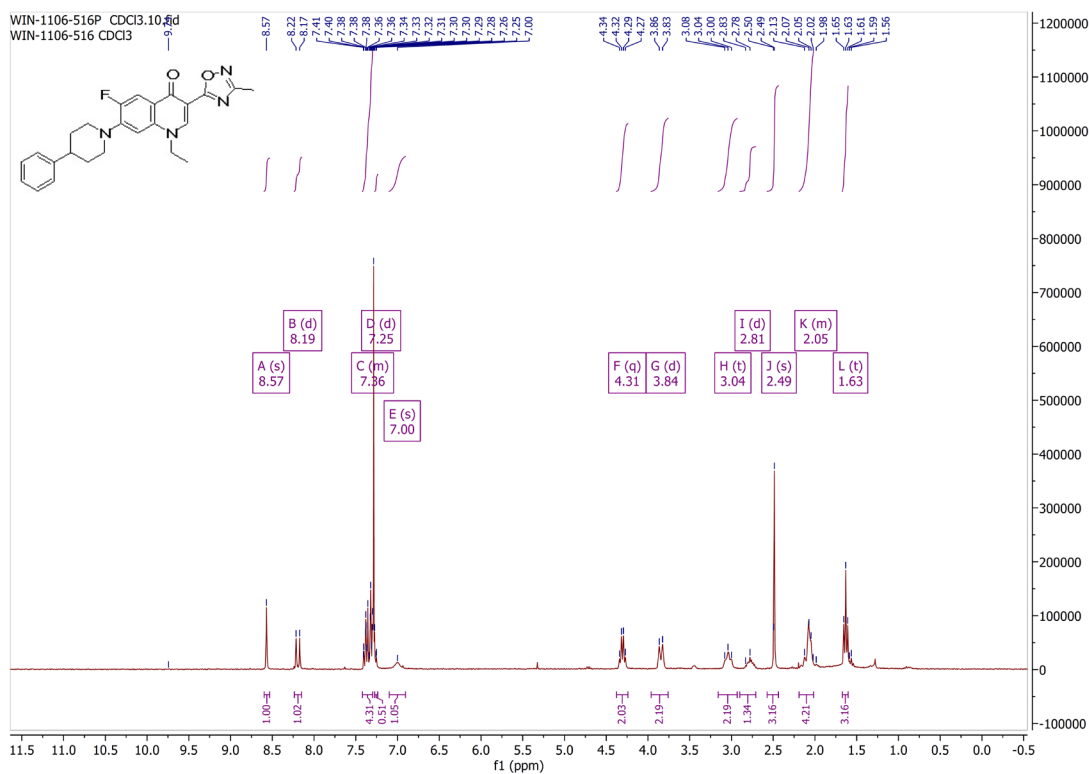
# Compound 38



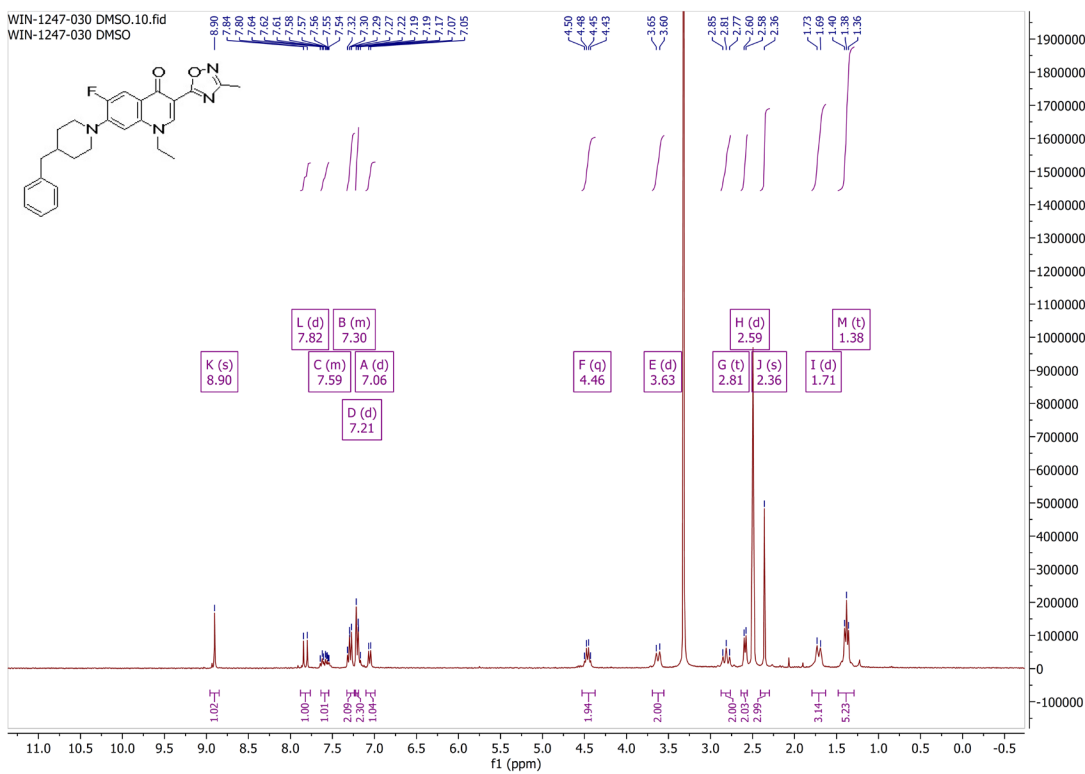
# Compound 39



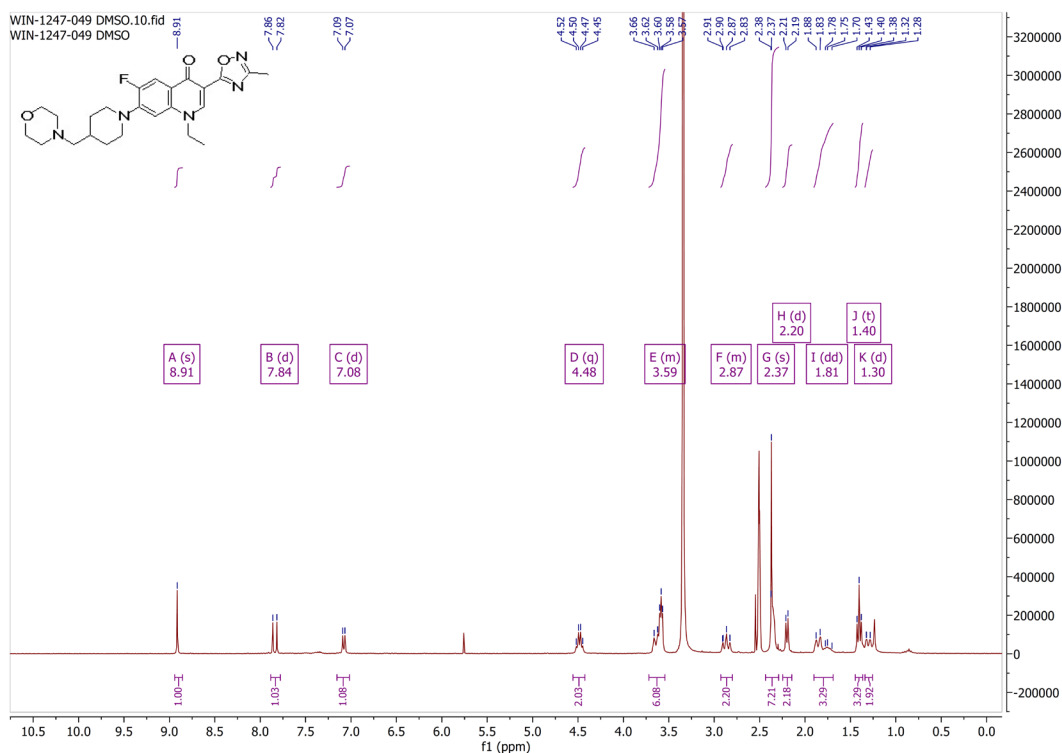
## Compound 40



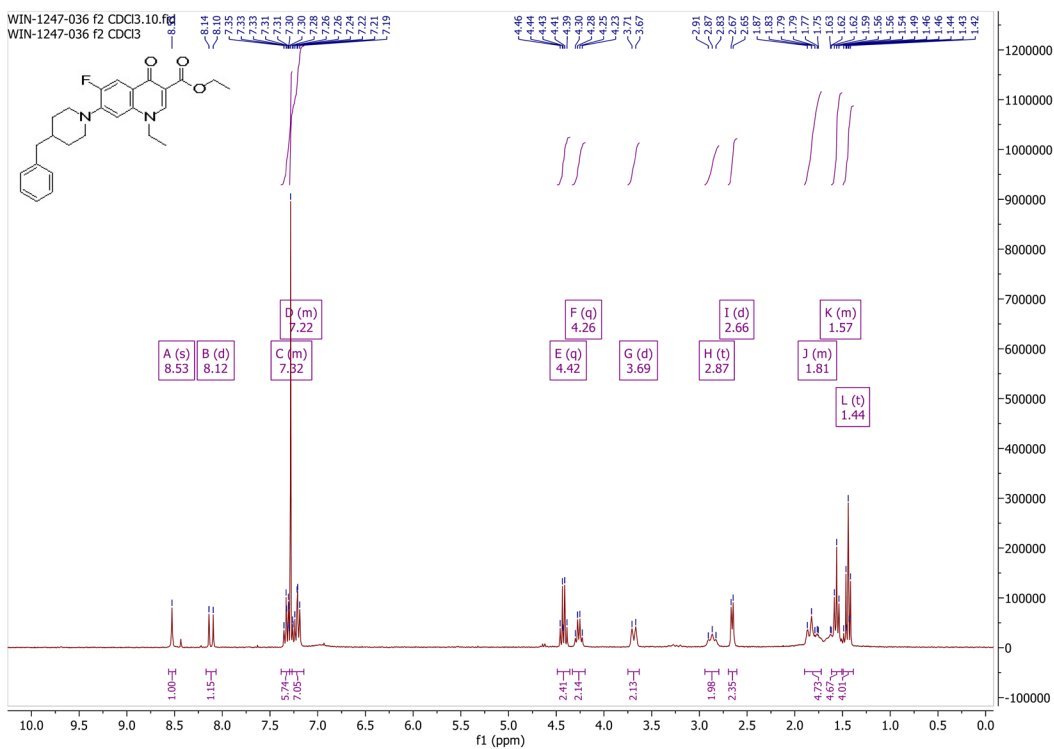
## Compound 41



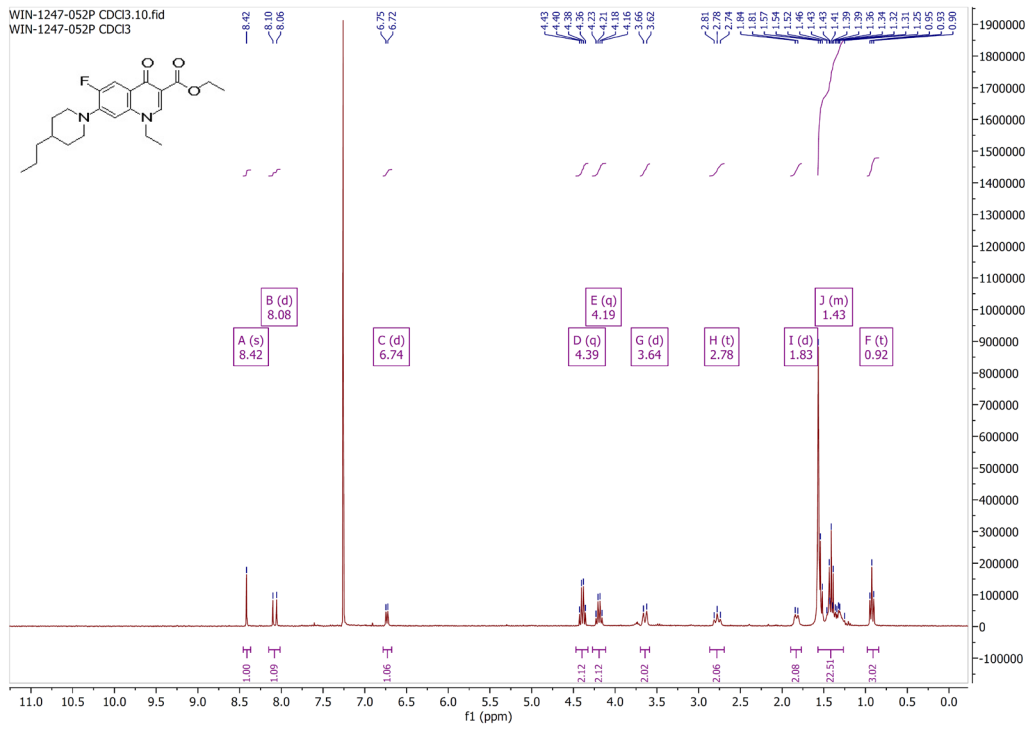
## Compound 42



## Compound 43

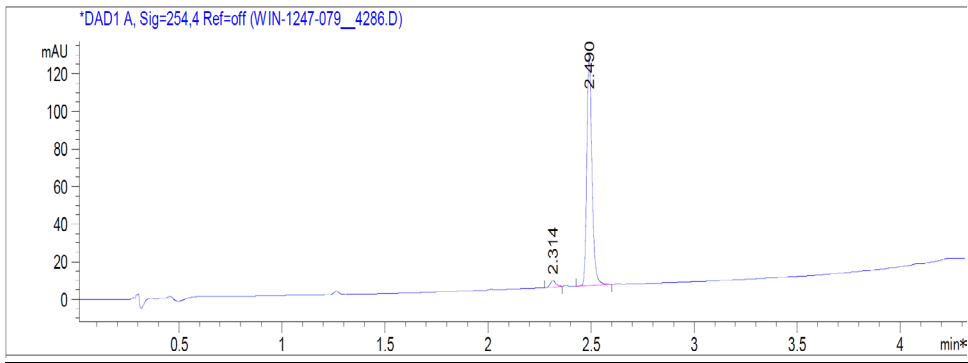


# Compound 44

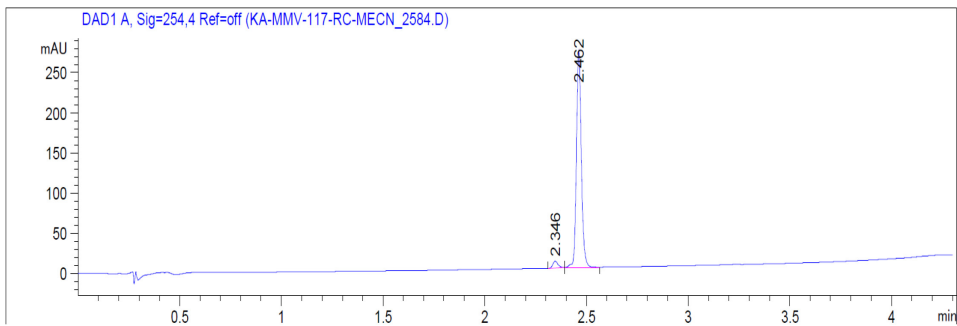


## HPLC traces for final compounds

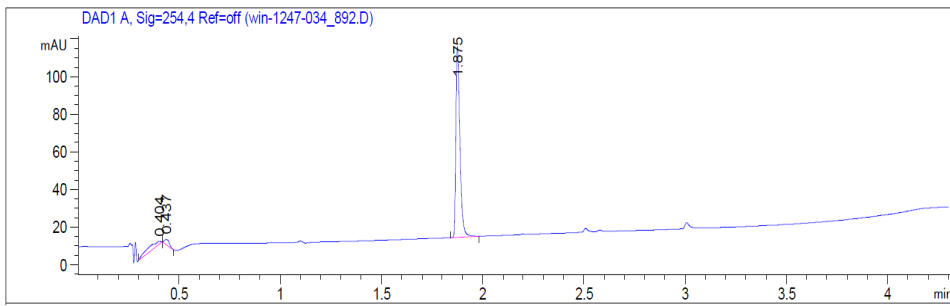
### Compound 16



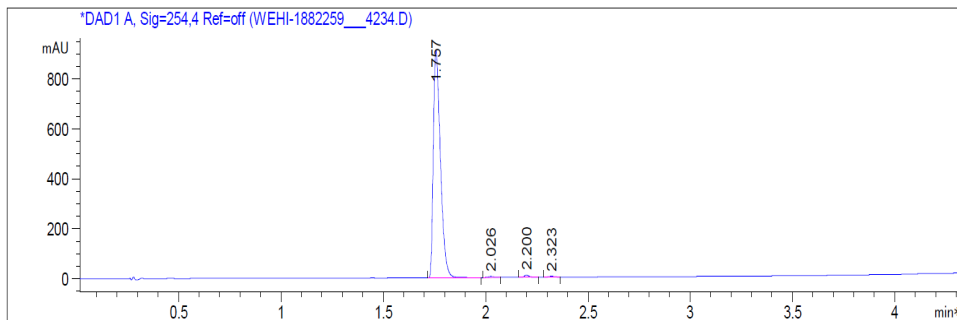
### Compound 17



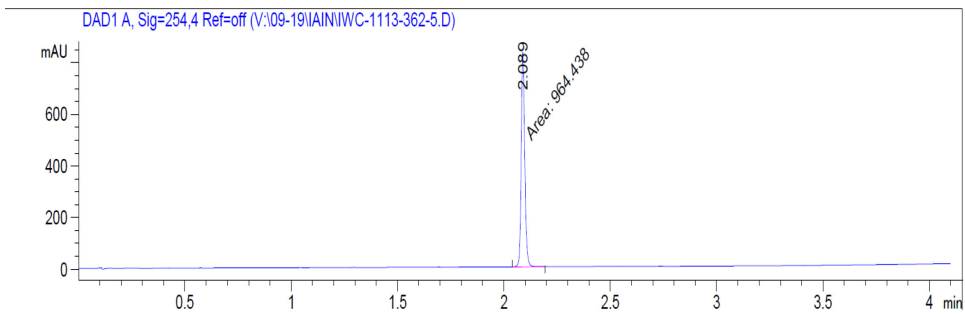
### Compound 18



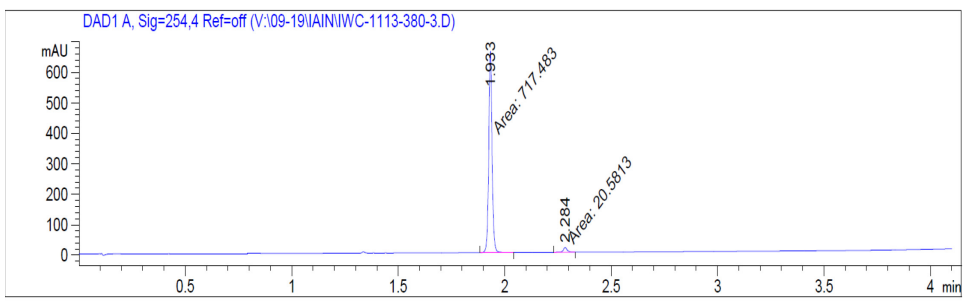
### Compound 19



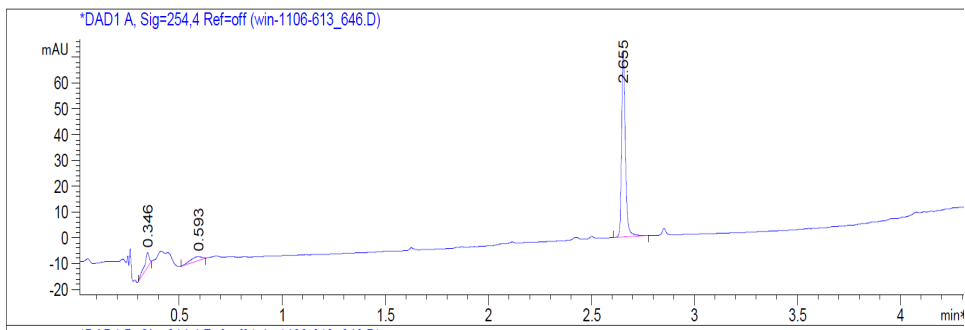
### Compound 21



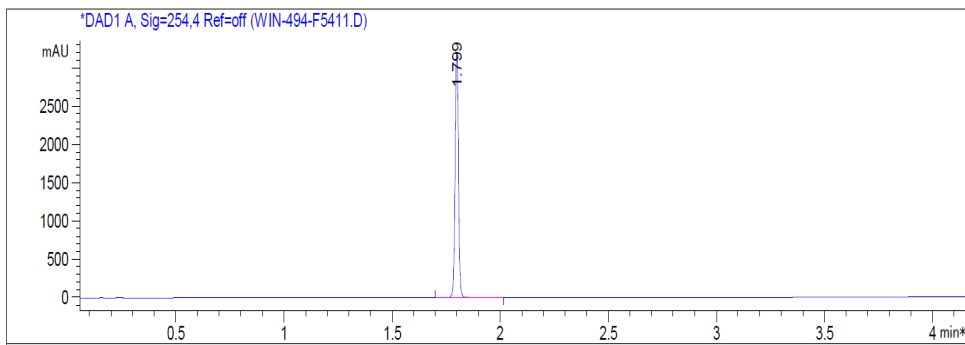
### Compound 22



### Compound 25

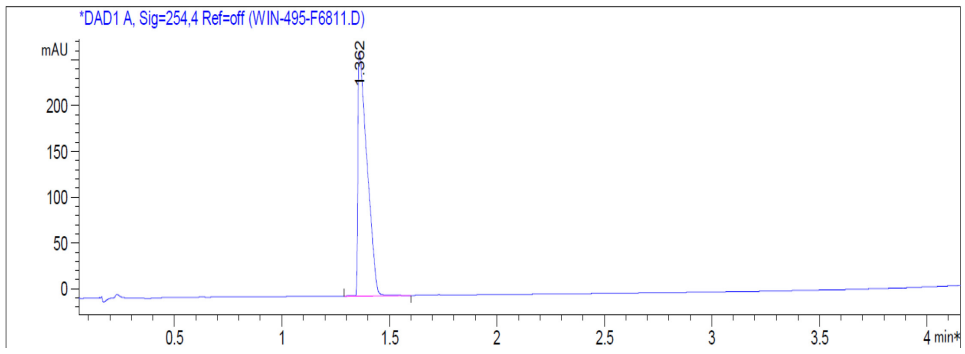


### Compound 26

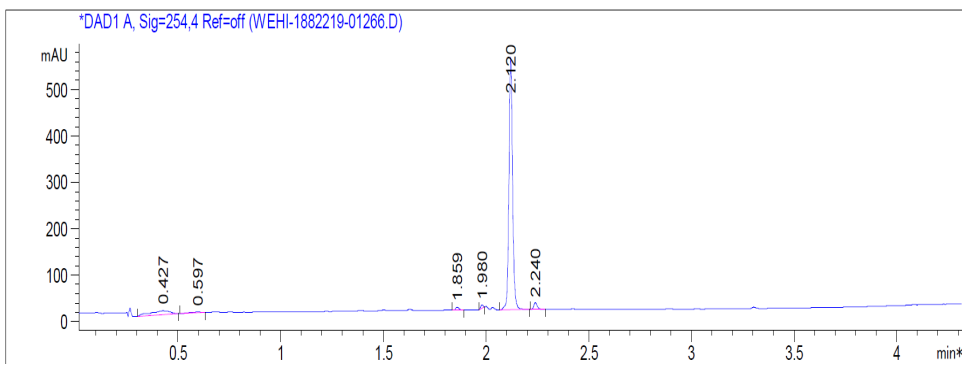




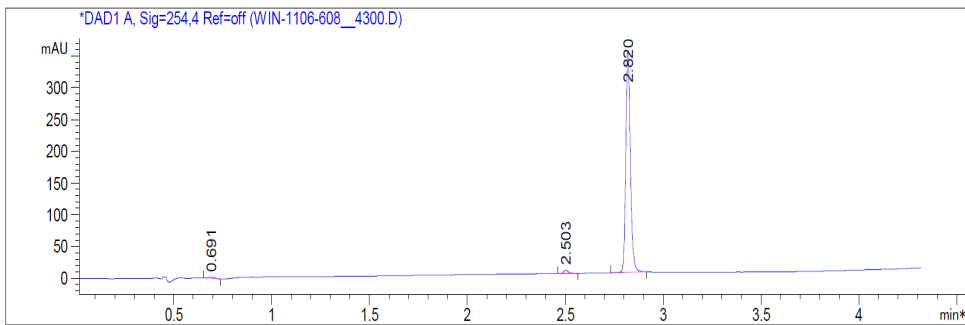
### Compound 27



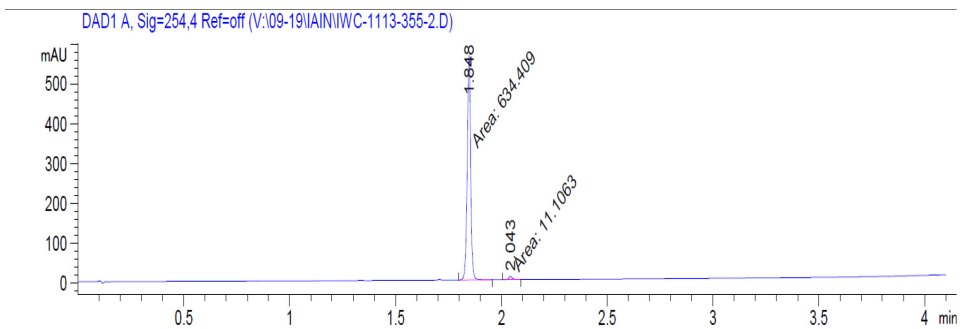
### Compound 28



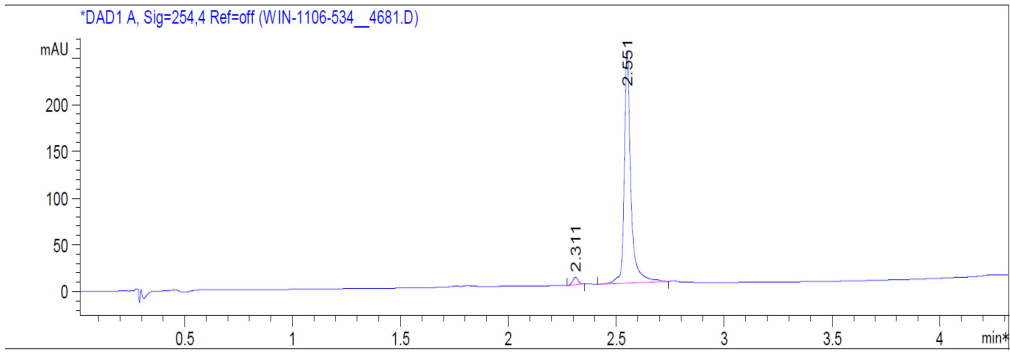
### Compound 29



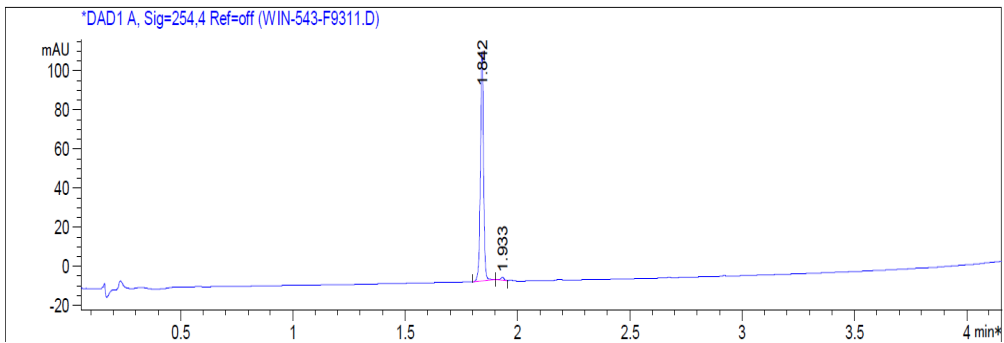
### Compound 30



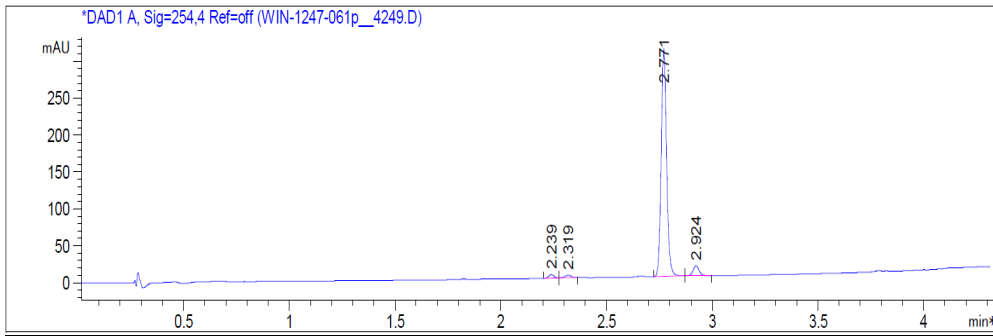




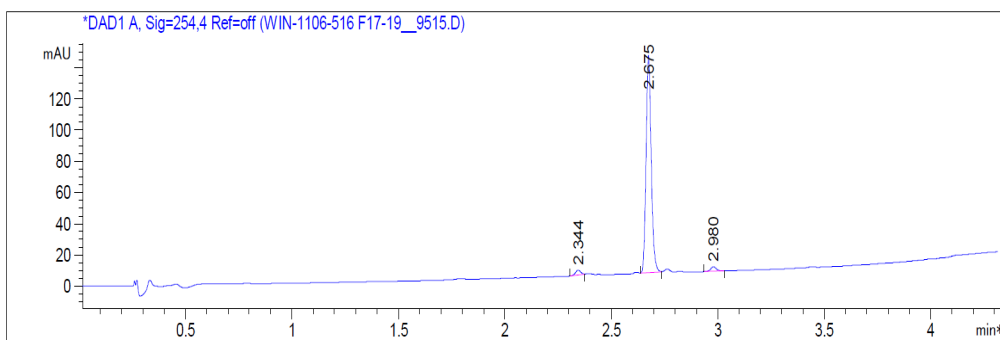
### Compound 37



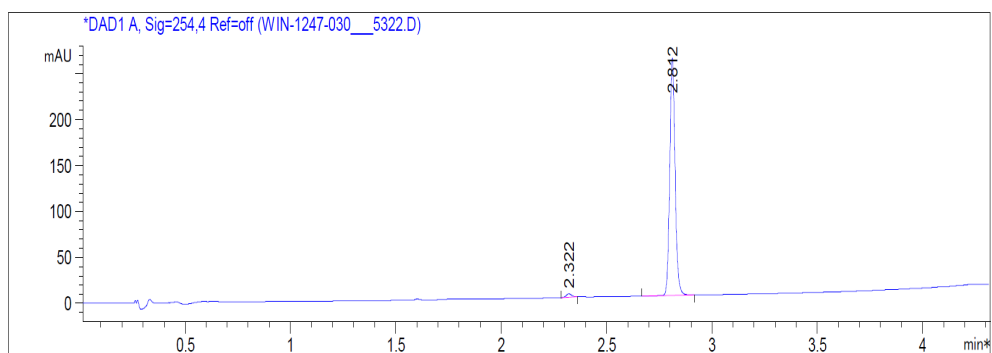
### Compound 39



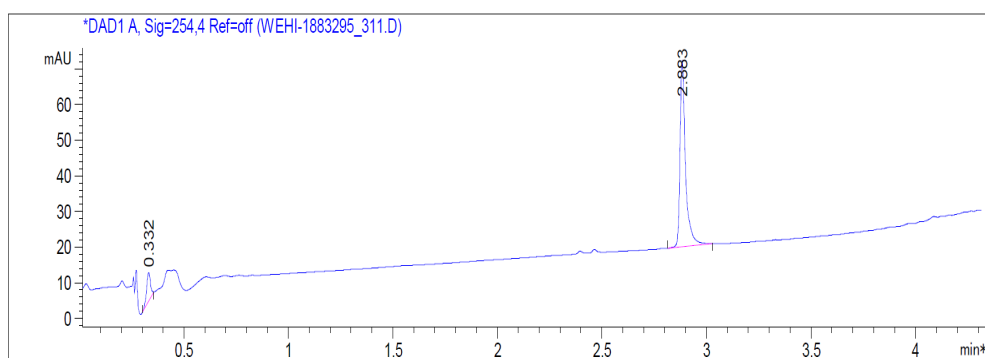
### Compound 40



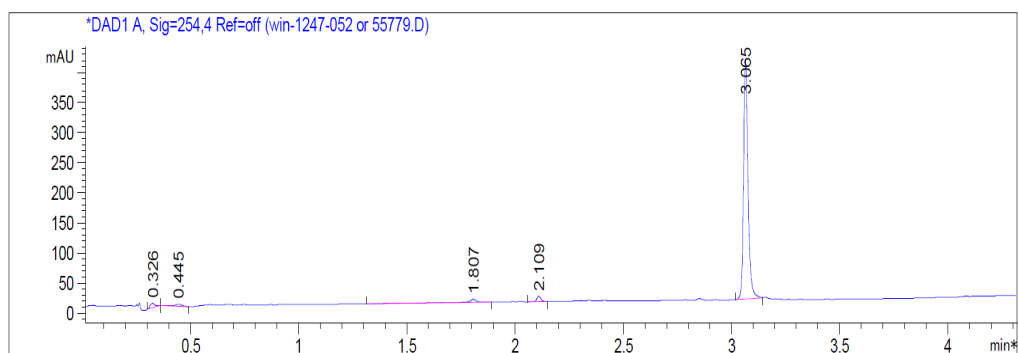
### Compound 41



### Compound 43



### Compound 44



## References

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2. Birth, D.; Kao, W.-C.; Hunte, C., Structural analysis of atovaquone-inhibited cytochrome bc1 complex reveals the molecular basis of antimalarial drug action. *Nat. Commun.* **2014**, *5* (1), 4029.
3. Capper, M. J.; O'Neill, P. M.; Fisher, N.; Strange, R. W.; Moss, D.; Ward, S. A.; Berry, N. G.; Lawrenson, A. S.; Hasnain, S. S.; Biagini, G. A.; Antonyuk, S. V., Antimalarial 4(1H)-pyridones bind to the Qi site of cytochrome bc1. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112* (3), 755-760.