Supporting Information

Synthesis and Antimicrobial Activity of Novel 4-Hydroxy-2-quinolone Analogues

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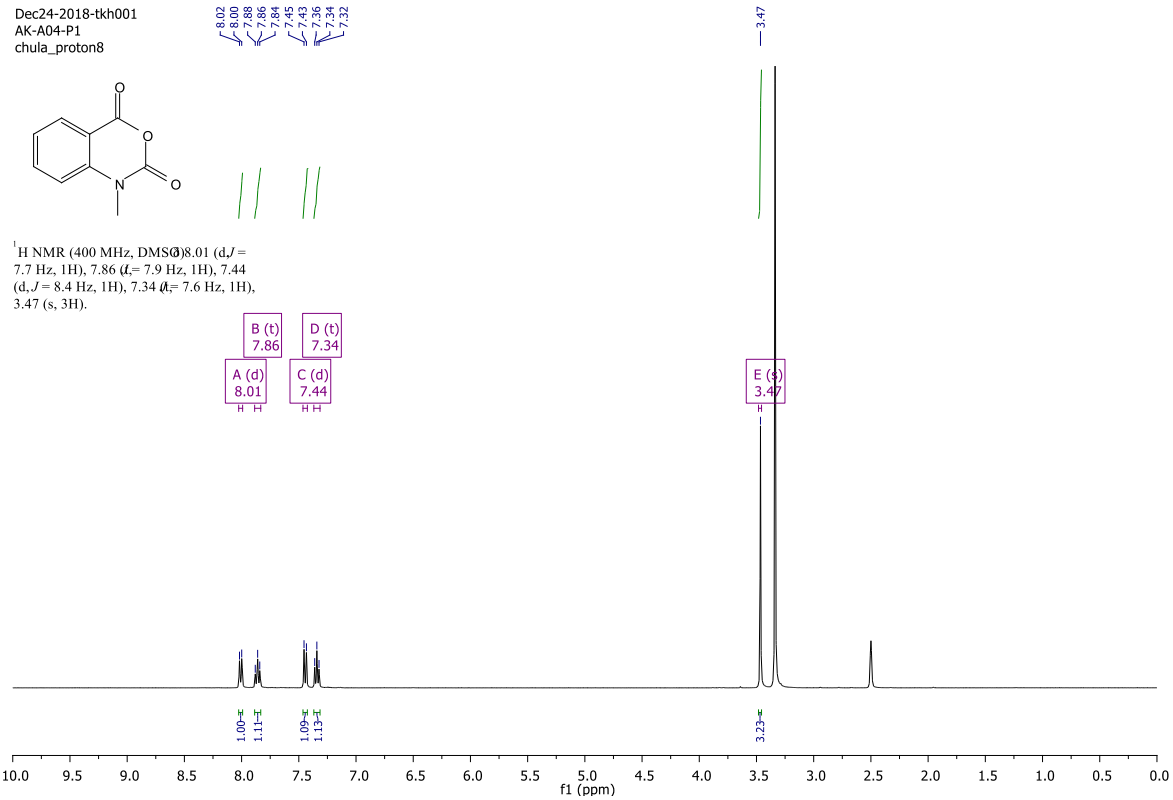
Content

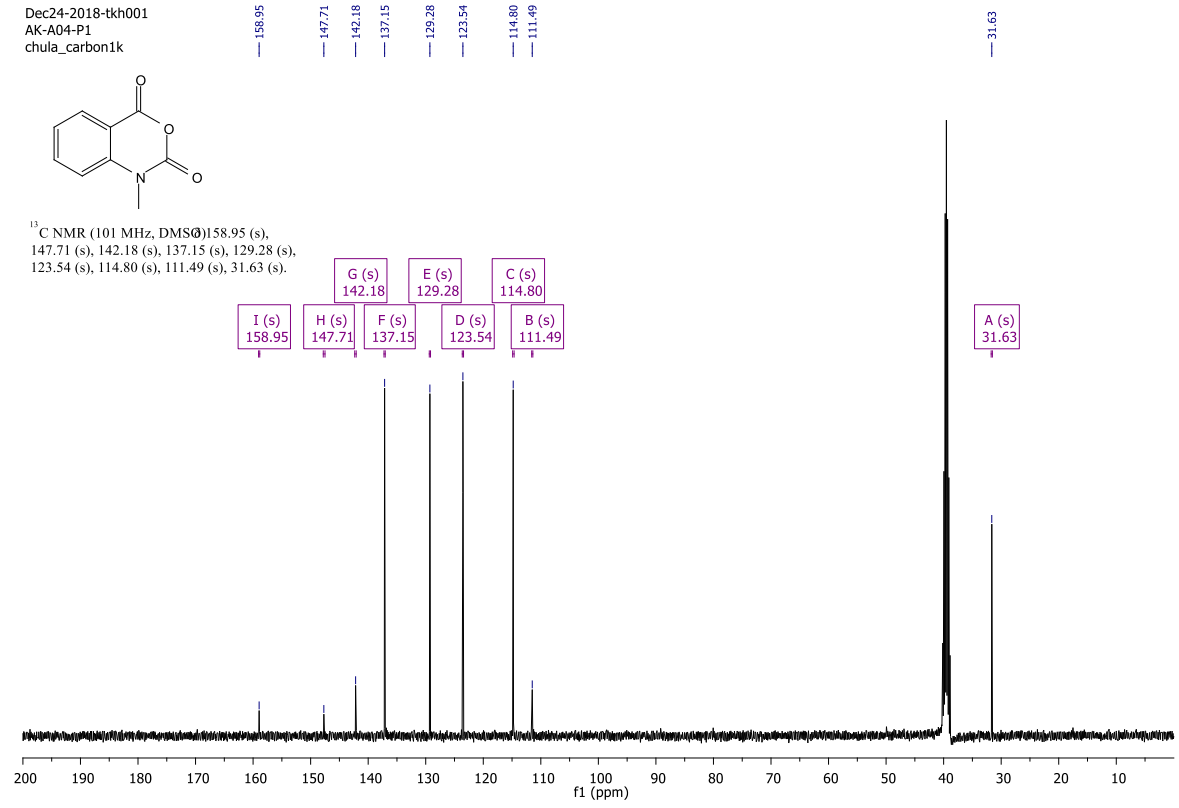
1. NMR and HRMS spectra

1.1. NMR spectra of 1a – 1g

1-Methyl-2H-benzo[d] [1,3] oxazine-2,4(1H)-dione (**1a**)

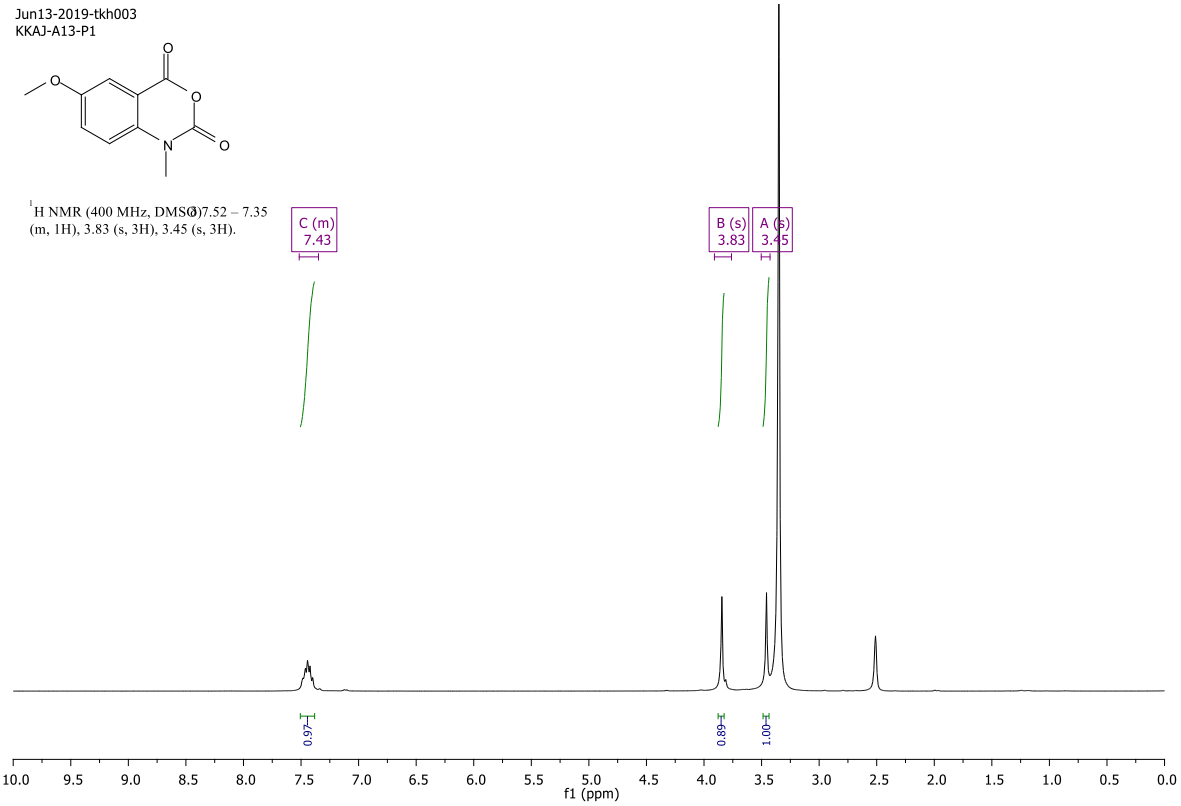


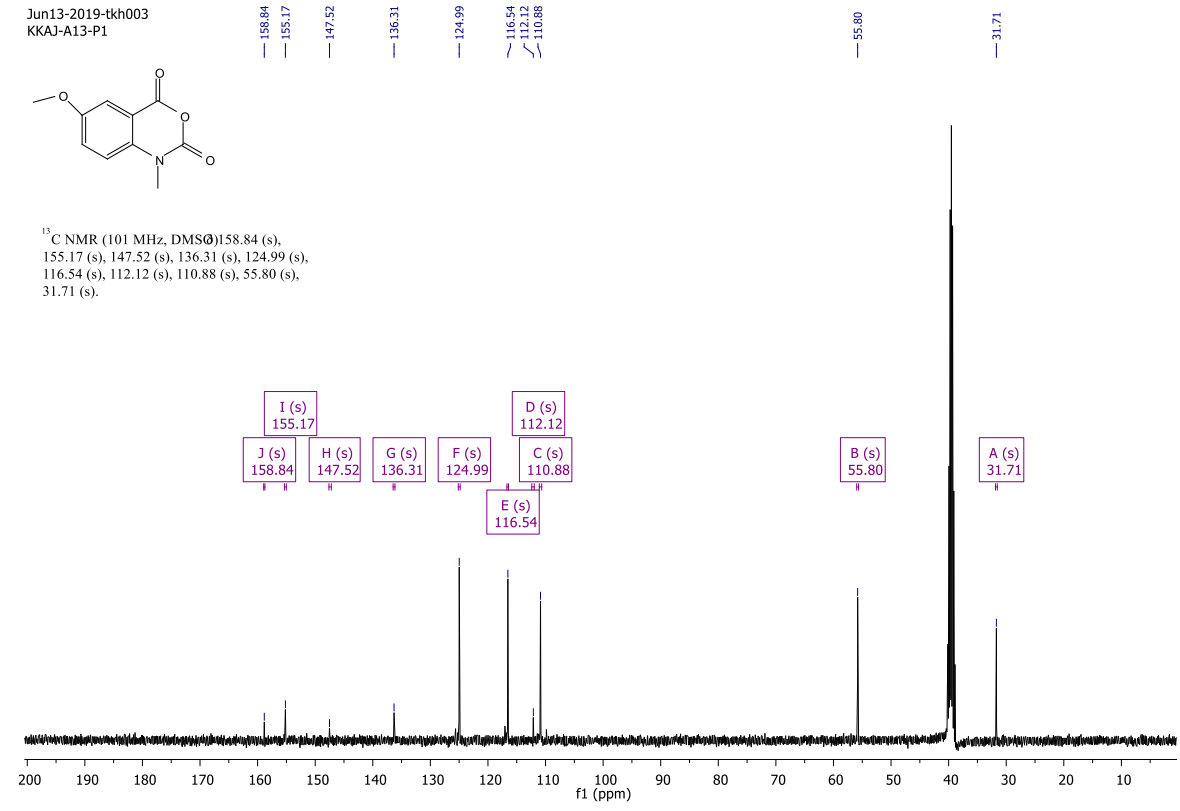
^{1}H NMR (400 MHz, DMSO-d_6) 8.01 (d, J = 7.7 Hz, 1H), 7.86 (t, J = 7.9 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 3.47 (s, 3H).



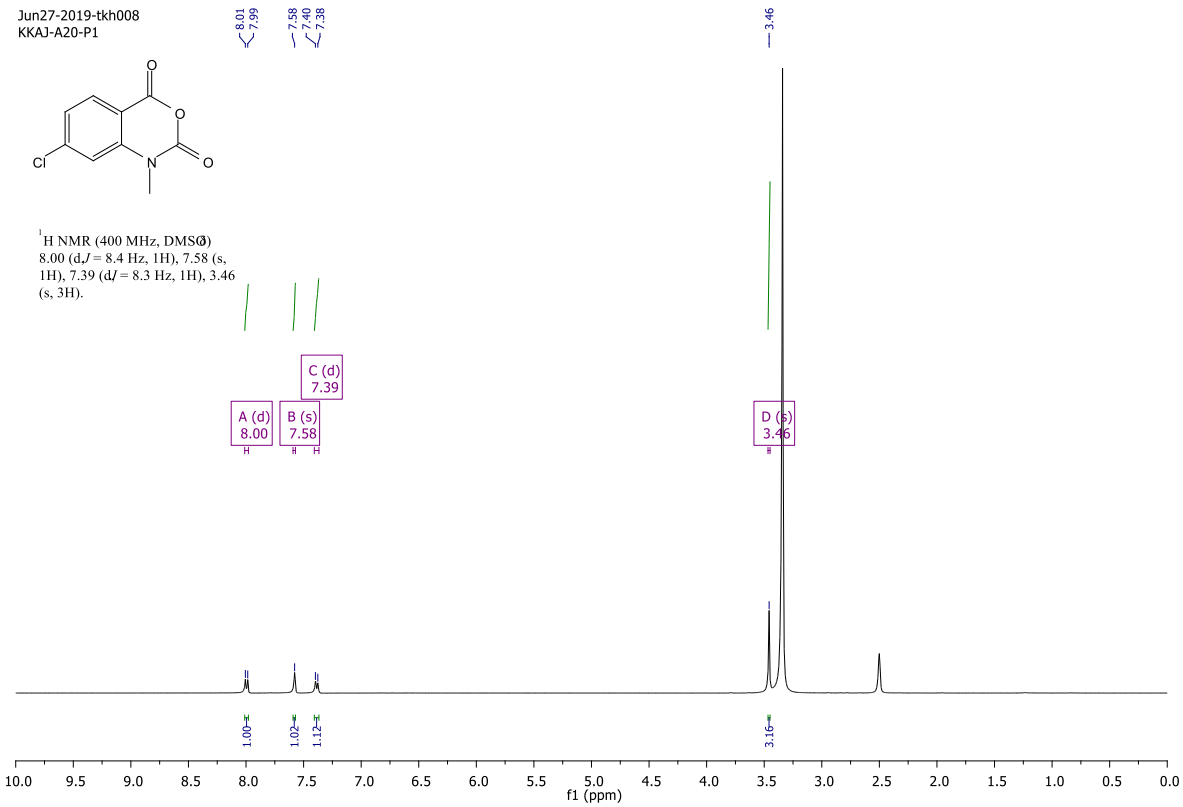
^{13}C NMR (101 MHz, DMSO-d_6) 158.95 (s), 147.71 (s), 142.18 (s), 137.15 (s), 129.28 (s), 123.54 (s), 114.80 (s), 111.49 (s), 31.63 (s).

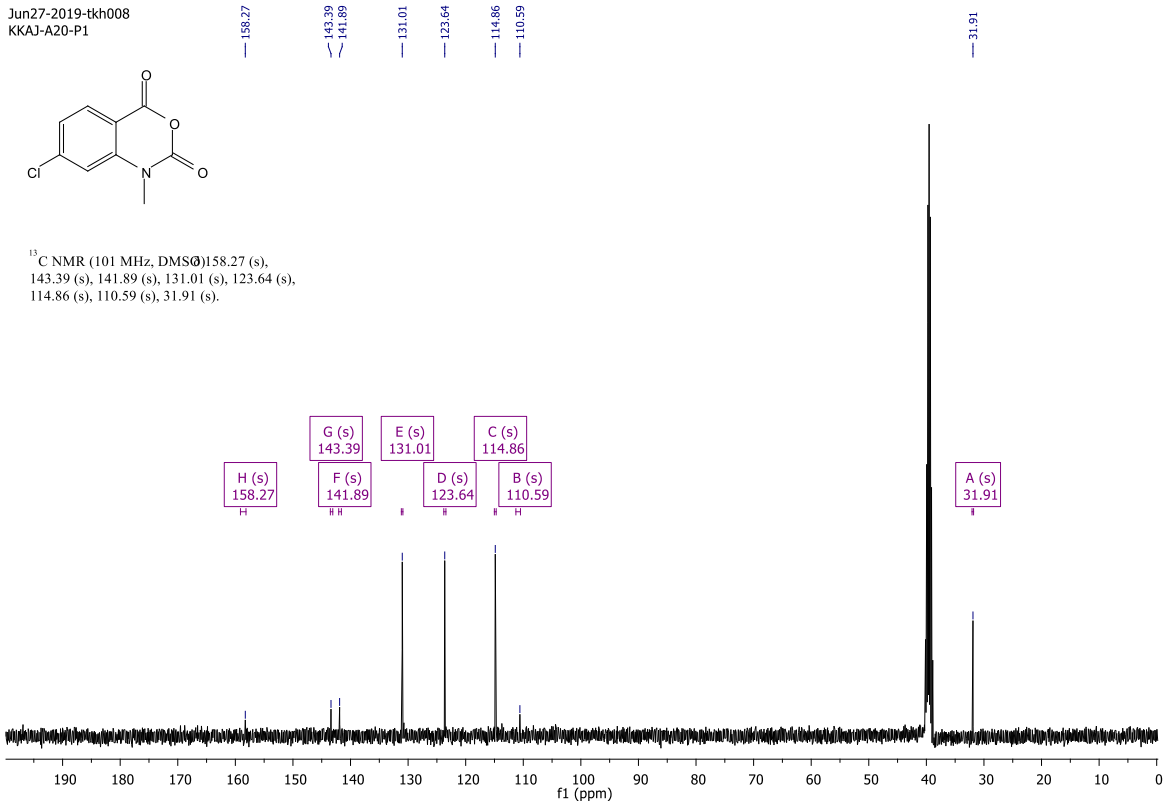
6-Methoxy-1-methyl-2H-benzo[d] [1,3] oxazine-2,4(1H)-dione (**1b**)



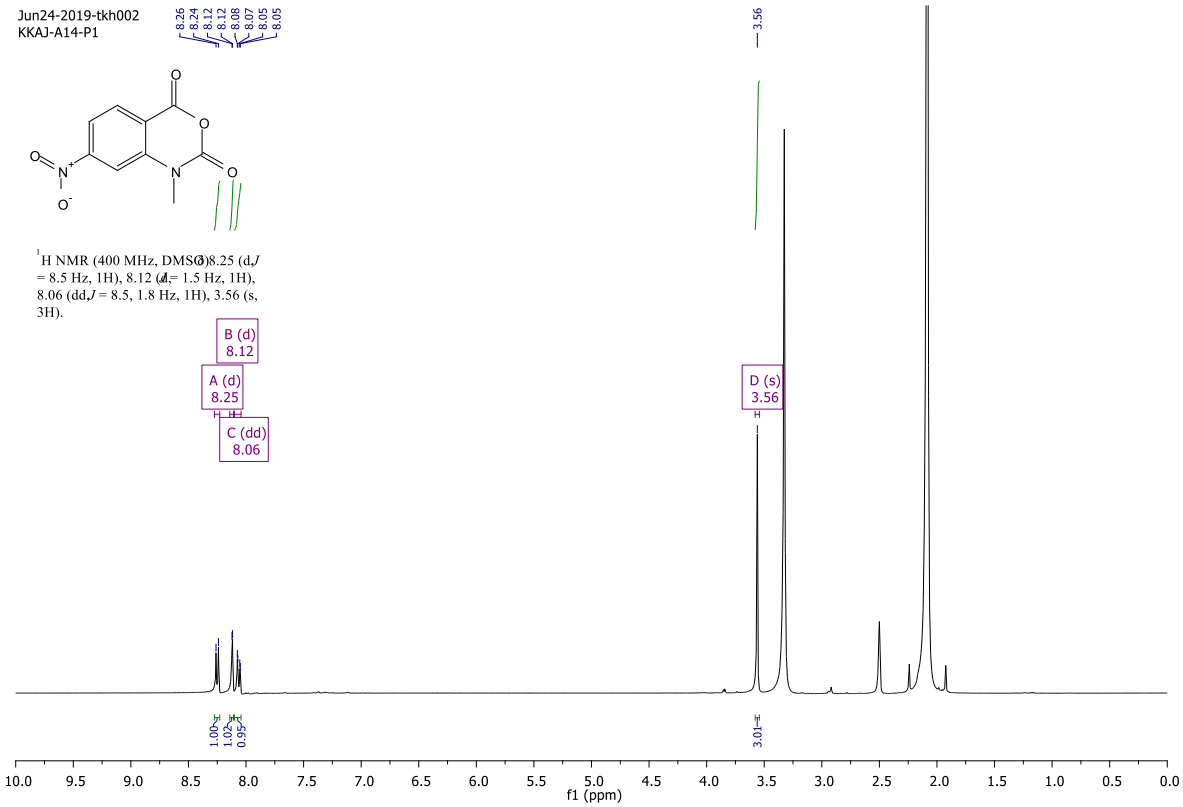


7-Chloro-1-methyl-2H-benzo[d] [1,3] oxazine-2,4 (1H)-dione (**1c**)

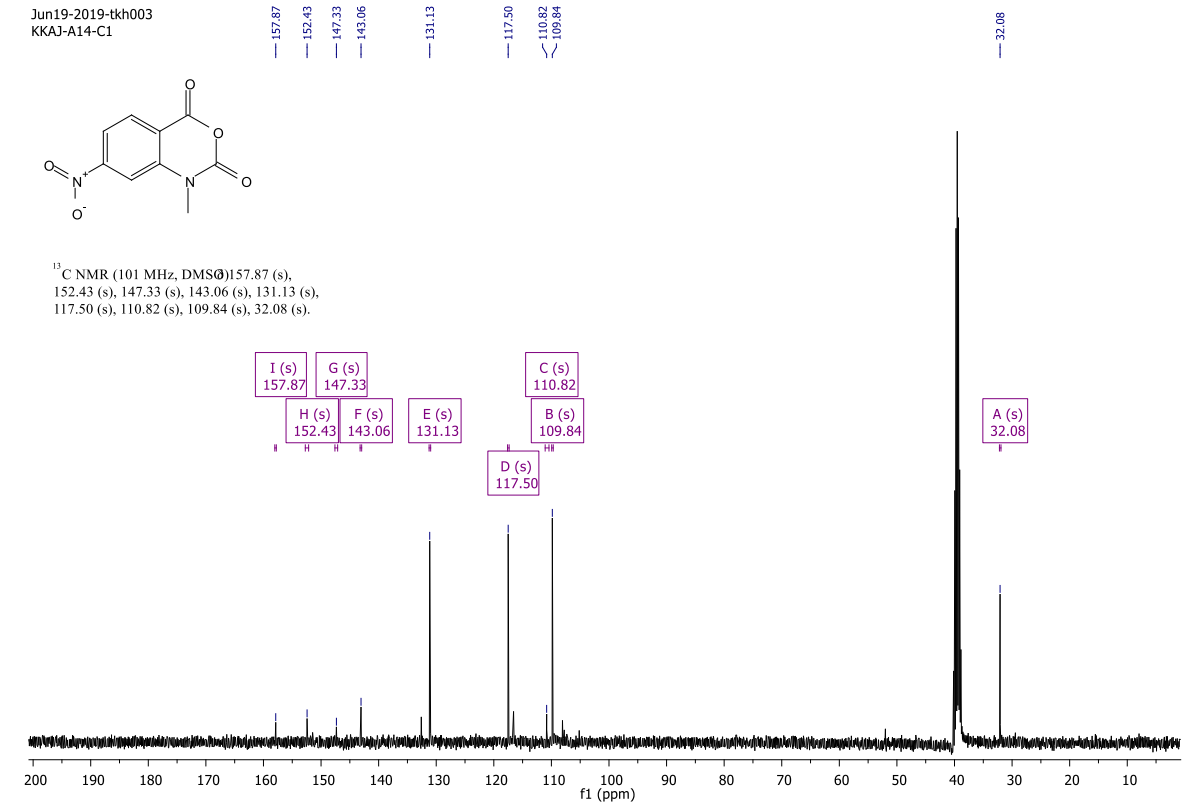




1-Methyl-7-nitro-2H-benzo[d][1,3] oxazine-2,4(1H)-dione (**1d**)

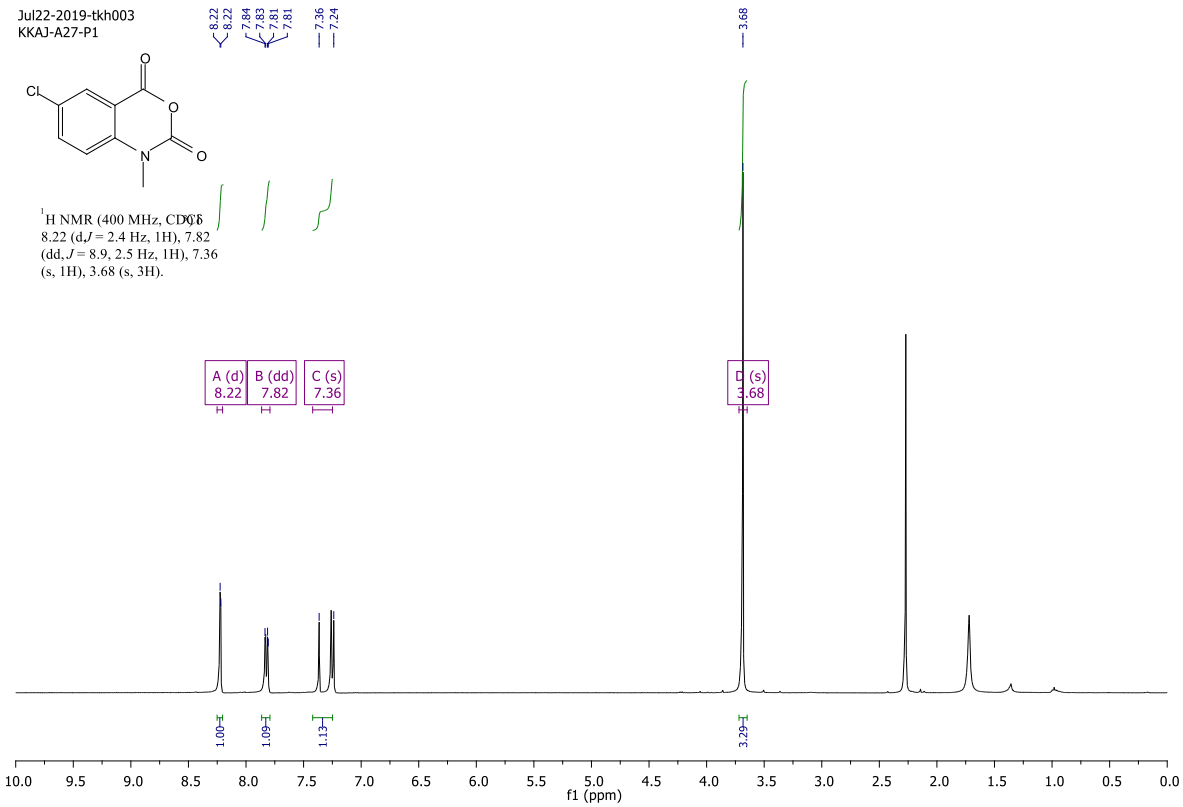


^{1}H NMR (400 MHz, DMSO) δ 8.25 (d, J = 8.5 Hz, 1H), 8.12 (d, J = 1.5 Hz, 1H), 8.06 (dd, J = 8.5, 1.8 Hz, 1H), 3.56 (s, 3H).

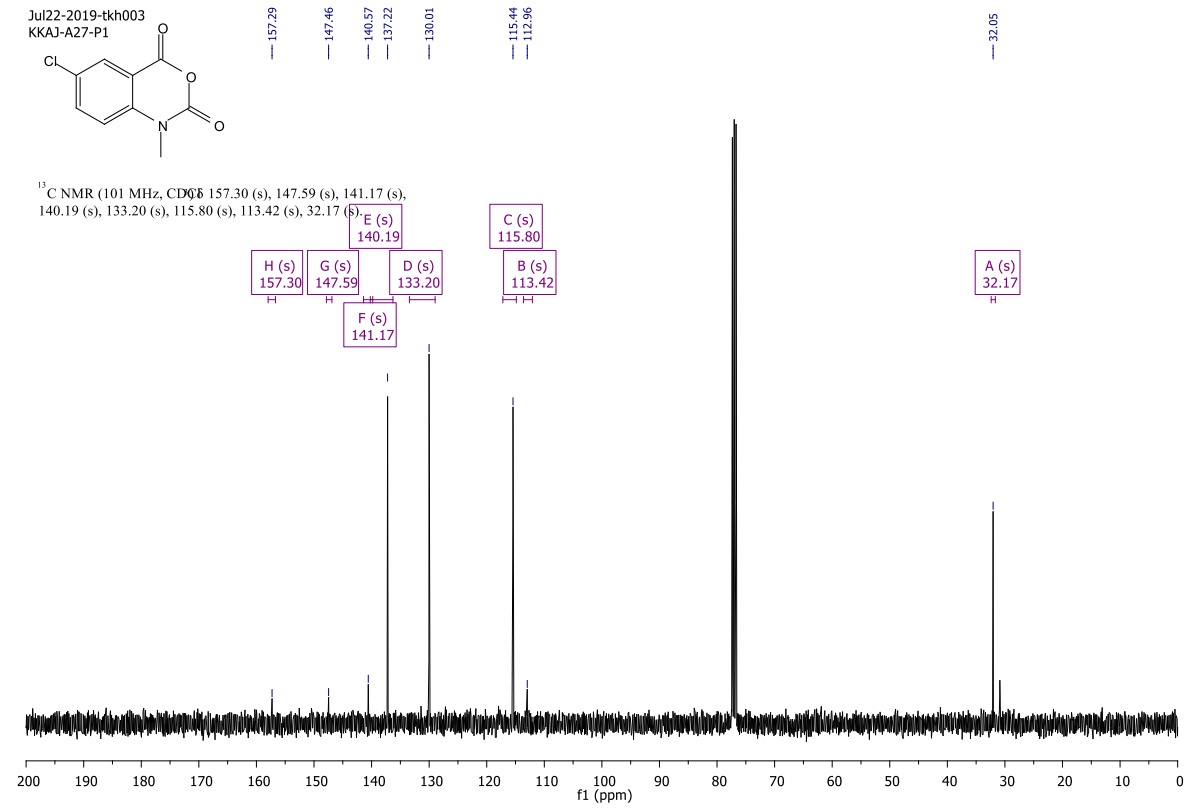


^{13}C NMR (101 MHz, DMSO) δ 157.87 (s), 152.43 (s), 147.33 (s), 143.06 (s), 131.13 (s), 117.50 (s), 110.82 (s), 109.84 (s), 32.08 (s).

6-Chloro-1-methyl-2H-benzo[d] [1,3] oxazine-2,4(1H)-dione (**1e**)

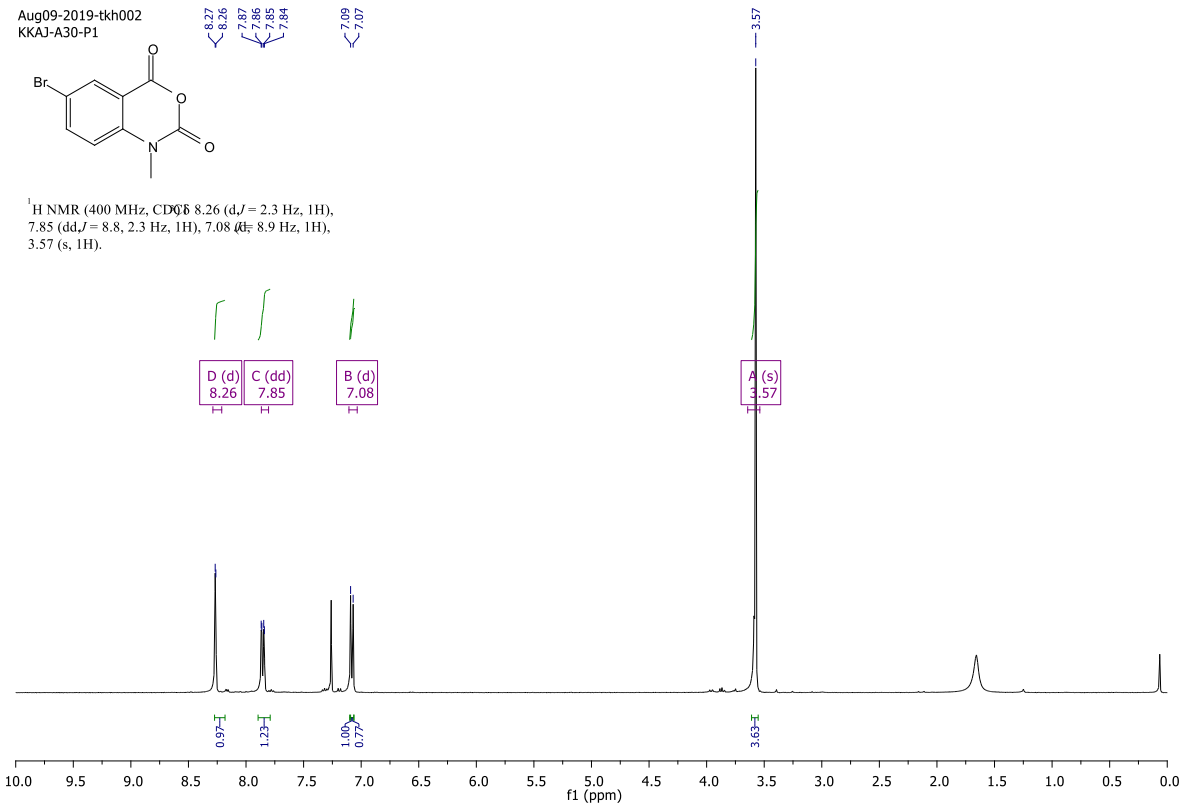


^{1}H NMR (400 MHz, CDCl$_3$) δ 8.22 (d, *J* = 2.4 Hz, 1H), 7.82 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.36 (s, 1H), 3.68 (s, 3H).

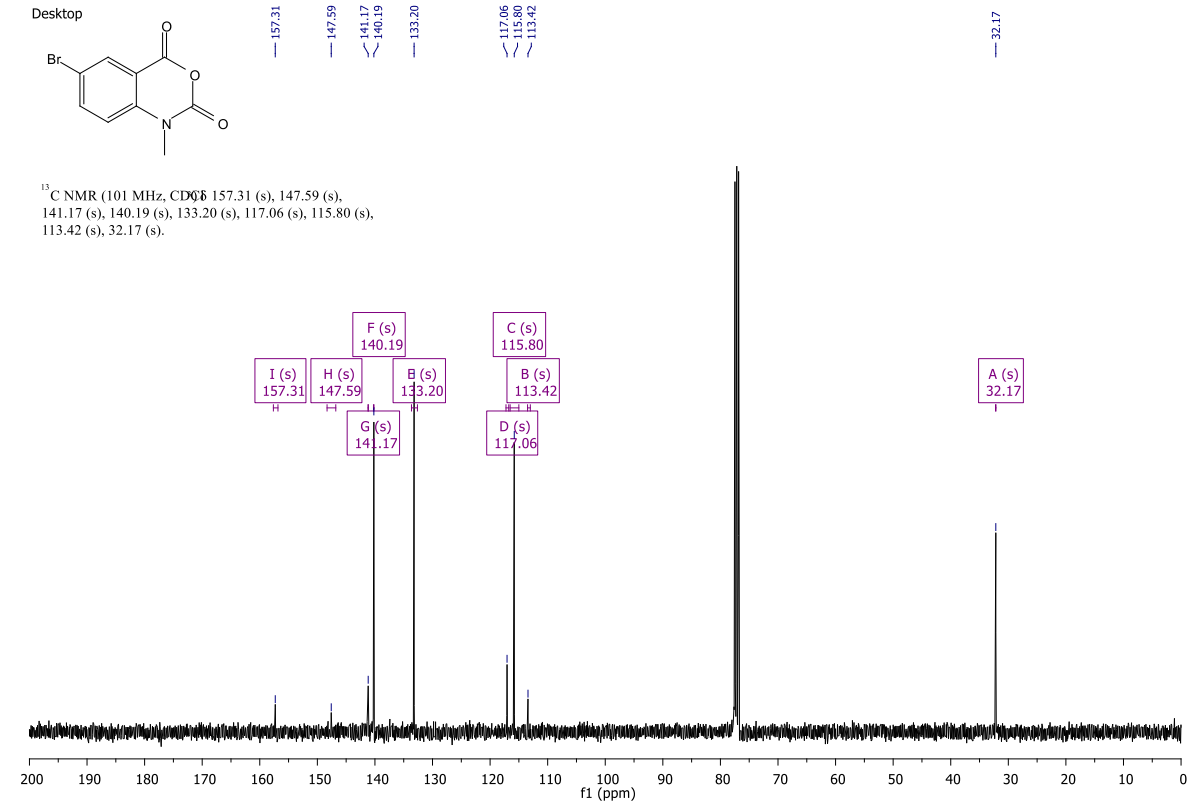


^{13}C NMR (101 MHz, CDCl$_3$) δ 157.30 (s), 147.59 (s), 141.17 (s), 140.19 (s), 133.20 (s), 115.80 (s), 113.42 (s), 32.17 (s).

6-Bromo-1-methyl-2H-benzo[d] [1,3] oxazine-2,4(1H)-dione (**1f**)

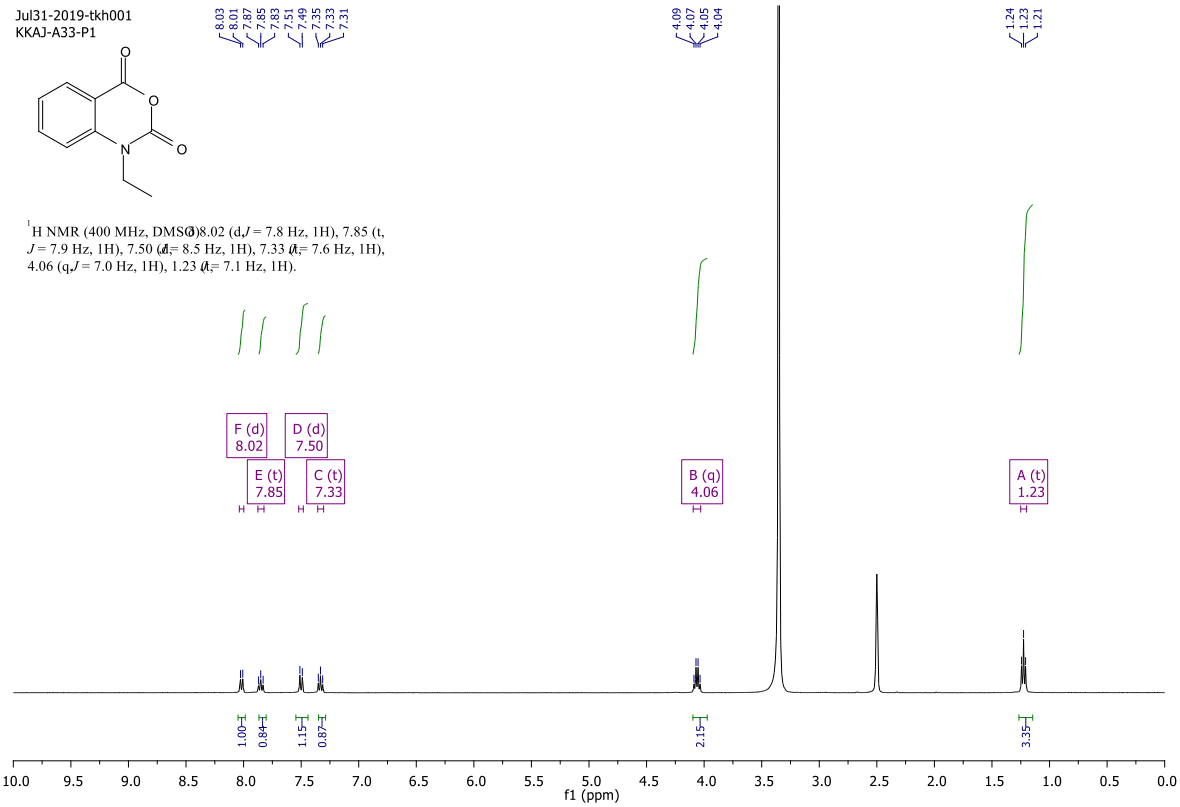


^{1}H NMR (400 MHz, CDCl$_3$) δ 8.26 (d, *J* = 2.3 Hz, 1H), 7.85 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.08 (d, *J* = 8.9 Hz, 1H), 3.57 (s, 1H).

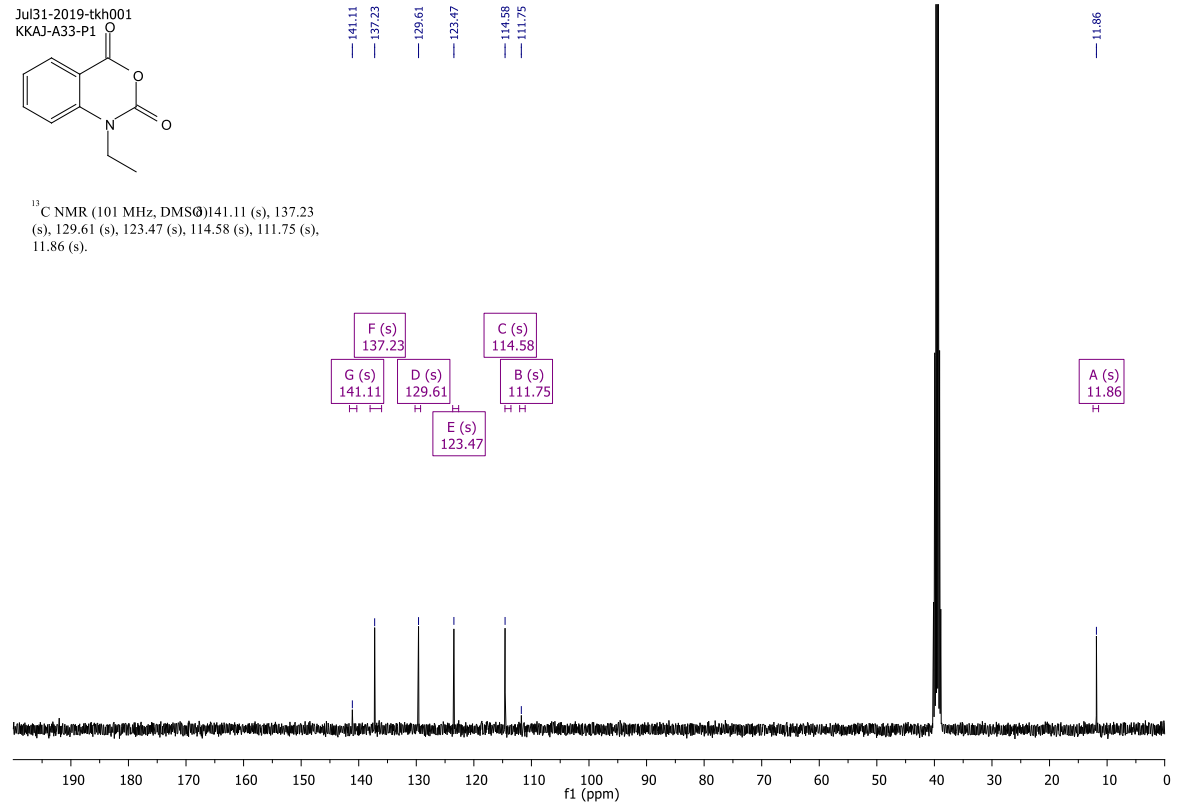


^{13}C NMR (101 MHz, CDCl$_3$) δ 157.31 (s), 147.59 (s), 141.17 (s), 140.19 (s), 133.20 (s), 117.06 (s), 115.80 (s), 113.42 (s), 32.17 (s).

1-Ethyl-2H-benzo[d] [1,3] oxazine-2,4(1H)-dione (**1g**)



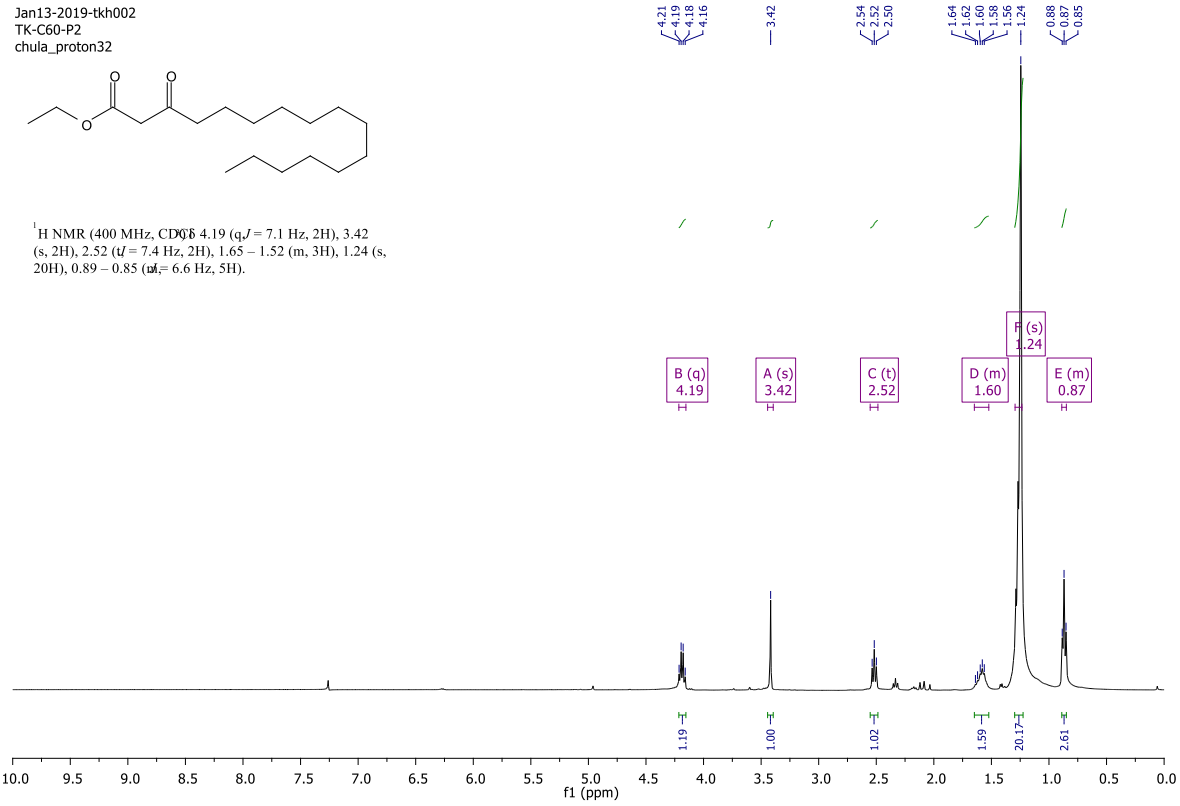
^{1}H NMR (400 MHz, DMSO) δ 8.02 (d, J = 7.8 Hz, 1H), 7.85 (t, J = 7.9 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 4.06 (q, J = 7.0 Hz, 1H), 1.23 (t, J = 7.1 Hz, 1H).



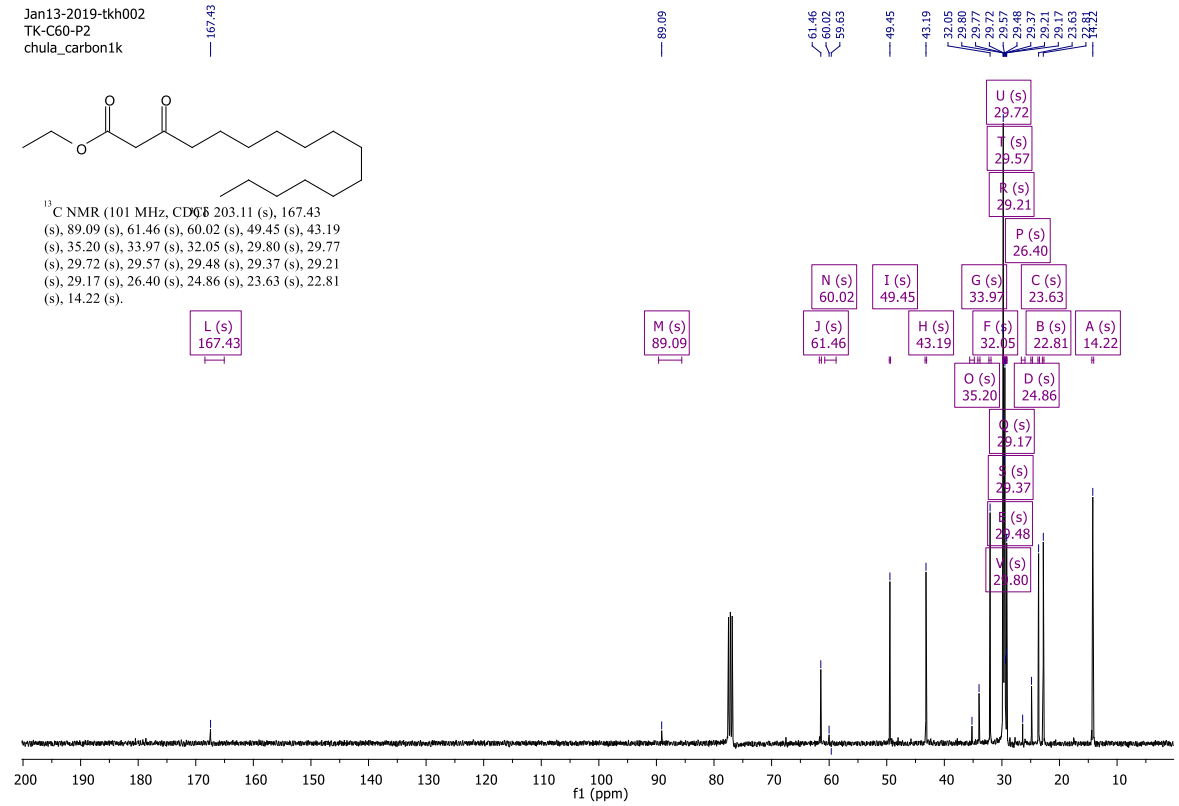
^{13}C NMR (101 MHz, DMSO) δ 141.11 (s), 137.23 (s), 129.61 (s), 123.47 (s), 114.58 (s), 111.75 (s), 11.86 (s).

1.2 NMR spectra of 2a and 2b

Ethyl 3-oxohexadecanoate (**2a**)

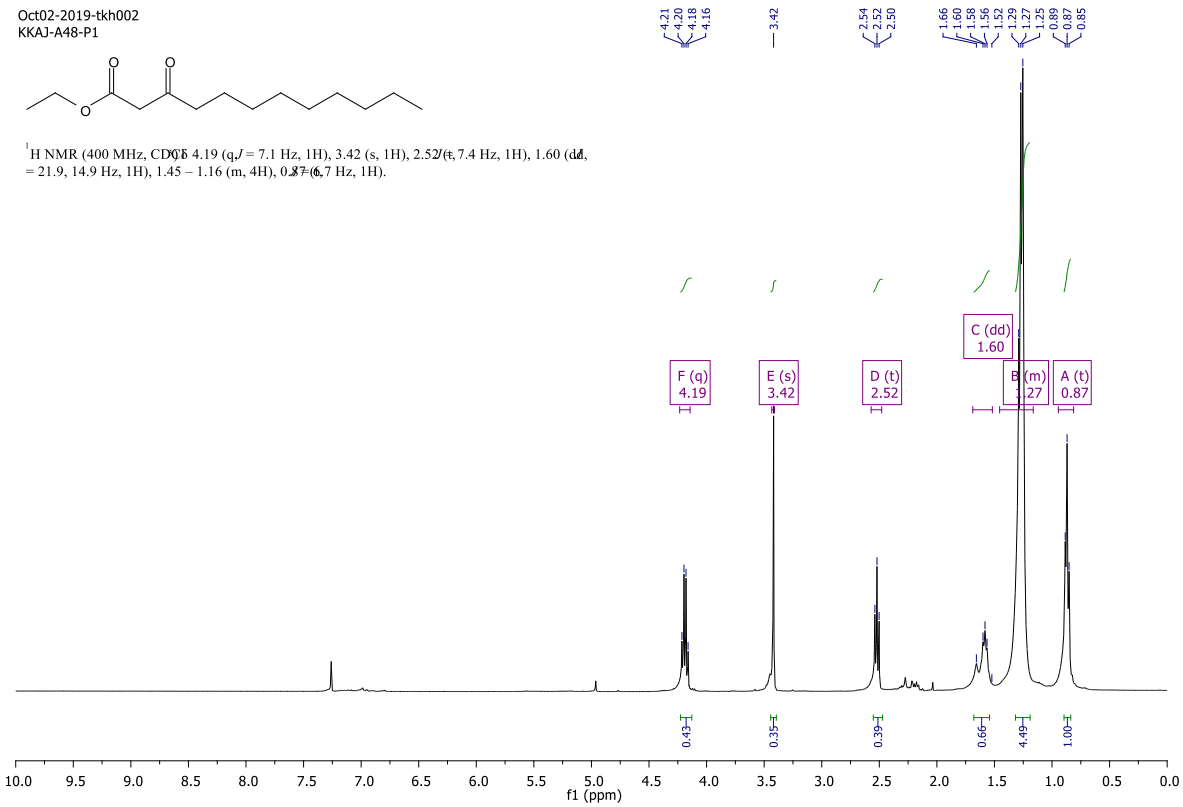


^{1}H NMR (400 MHz, CDCl$_3$) δ 4.19 (q, *J* = 7.1 Hz, 2H), 3.42 (s, 2H), 2.52 (t, *J* = 7.4 Hz, 2H), 1.65 – 1.52 (m, 3H), 1.24 (s, 20H), 0.89 – 0.85 (m, *J* = 6.6 Hz, 5H).

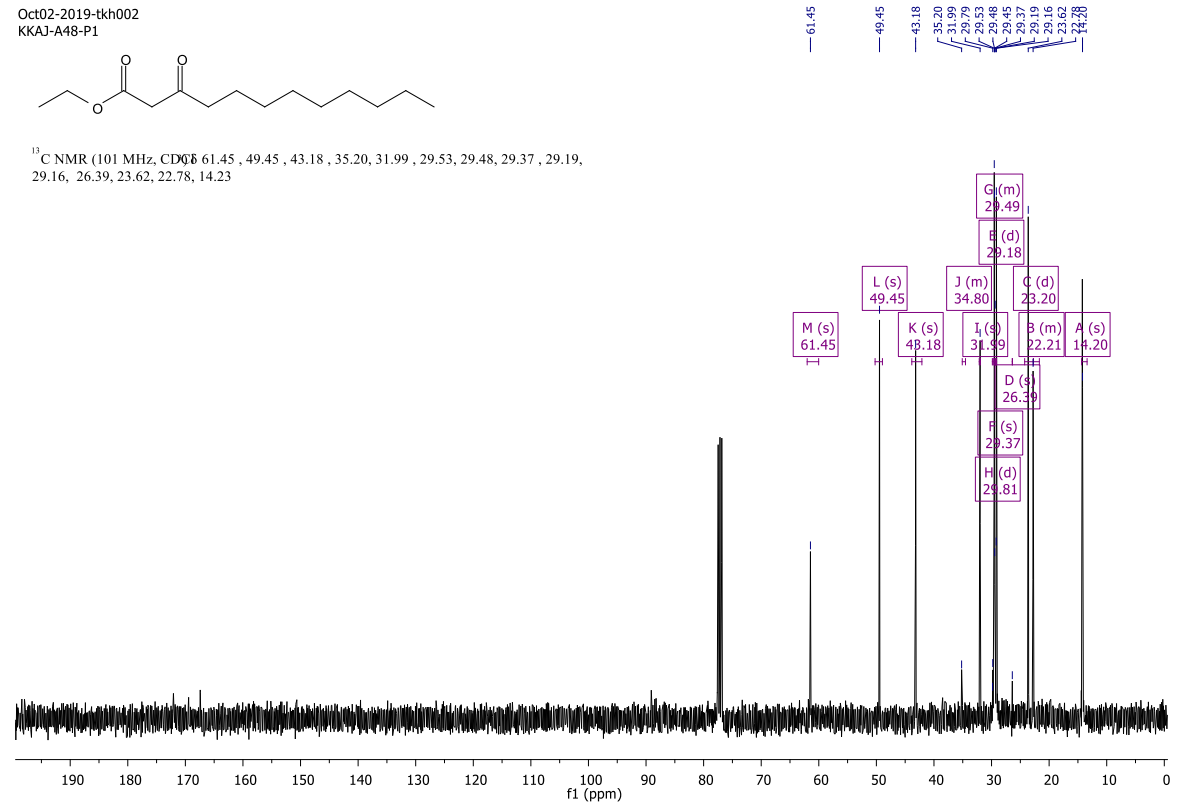


^{13}C NMR (101 MHz, CDCl$_3$) δ 203.11 (s), 167.43 (s), 89.09 (s), 61.46 (s), 60.02 (s), 49.45 (s), 43.19 (s), 35.20 (s), 33.97 (s), 32.05 (s), 29.80 (s), 29.77 (s), 29.72 (s), 29.57 (s), 29.48 (s), 29.37 (s), 29.21 (s), 29.17 (s), 26.40 (s), 24.86 (s), 23.63 (s), 22.81 (s), 14.22 (s).

Ethyl 3-oxododecanoate (**2b**)



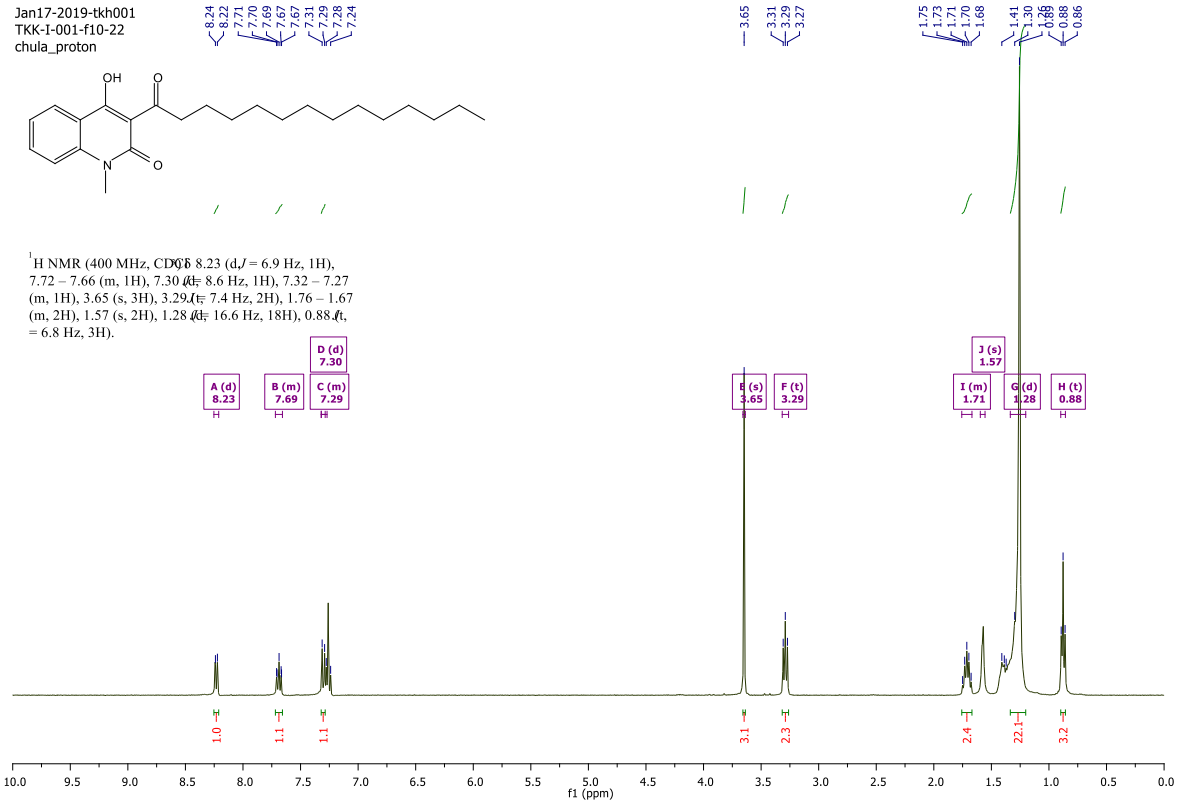
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (q, *J* = 7.1 Hz, 1H), 3.42 (s, 1H), 2.52 (t, *J* = 7.4 Hz, 1H), 1.60 (dd, *J* = 21.9, 14.9 Hz, 1H), 1.45 – 1.16 (m, 4H), 0.87 (t, *J* = 6.7 Hz, 1H).



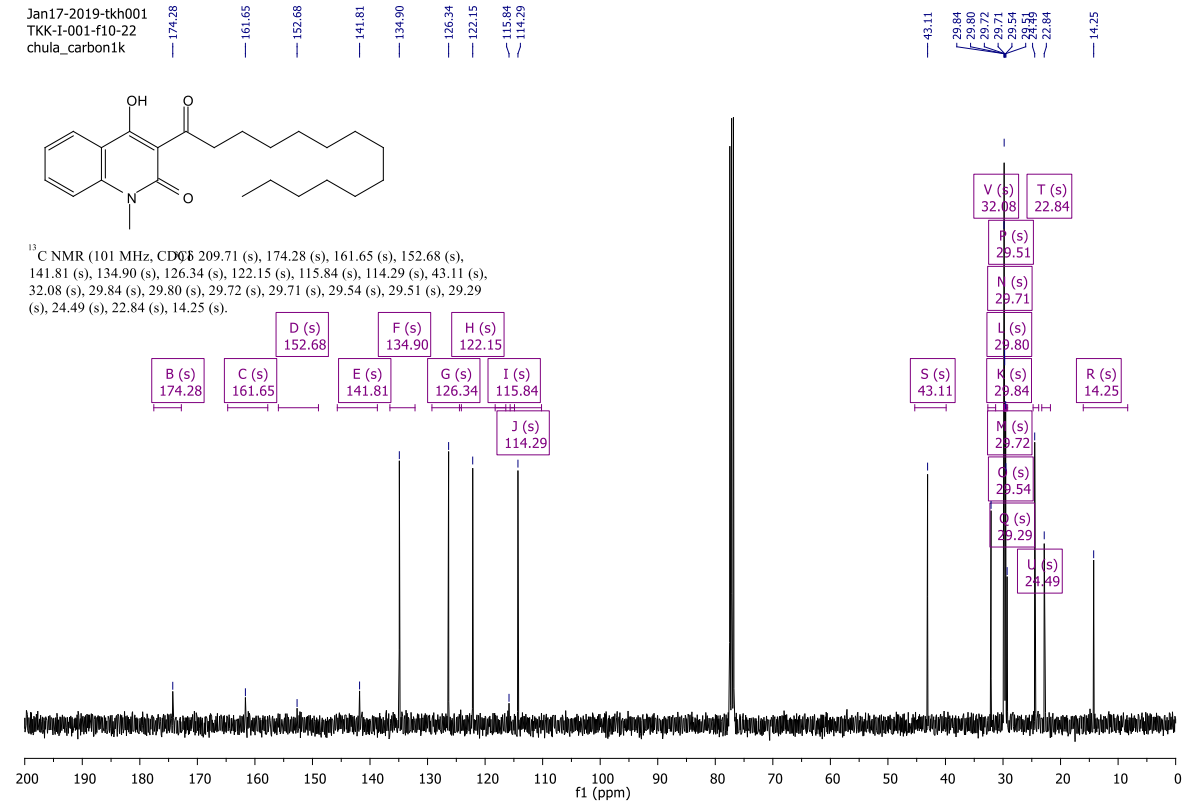
^{13}C NMR (101 MHz, $CDCl_3$) δ 61.45 , 49.45 , 43.18 , 35.20, 31.99 , 29.53, 29.48, 29.37 , 29.19, 29.16, 26.39, 23.62, 22.78, 14.23

1.3 NMR and HM spectra of 3a-3j, 4d and 5d

4-Hydroxy-1-methyl-3-tetradecanoylquinolin-2(1H)-one (**3a**)



^{1}H NMR (400 MHz, CDCl$_3$) δ 8.23 (d, *J* = 6.9 Hz, 1H), 7.72 – 7.66 (m, 1H), 7.30 (d, *J* = 8.6 Hz, 1H), 7.32 – 7.27 (m, 1H), 3.65 (s, 3H), 3.29 (t, *J* = 7.4 Hz, 2H), 1.76 – 1.67 (m, 2H), 1.57 (s, 2H), 1.28 (d, *J* = 16.6 Hz, 18H), 0.88 (t, *J* = 6.8 Hz, 3H).



^{13}C NMR (101 MHz, CDCl$_3$) δ 209.71 (s), 174.28 (s), 161.65 (s), 152.68 (s), 141.81 (s), 134.90 (s), 126.34 (s), 122.15 (s), 115.84 (s), 114.29 (s), 43.11 (s), 32.08 (s), 29.84 (s), 29.80 (s), 29.72 (s), 29.71 (s), 29.54 (s), 29.51 (s), 29.29 (s), 24.49 (s), 22.84 (s), 14.25 (s).

Generic Display Report

Analysis Info

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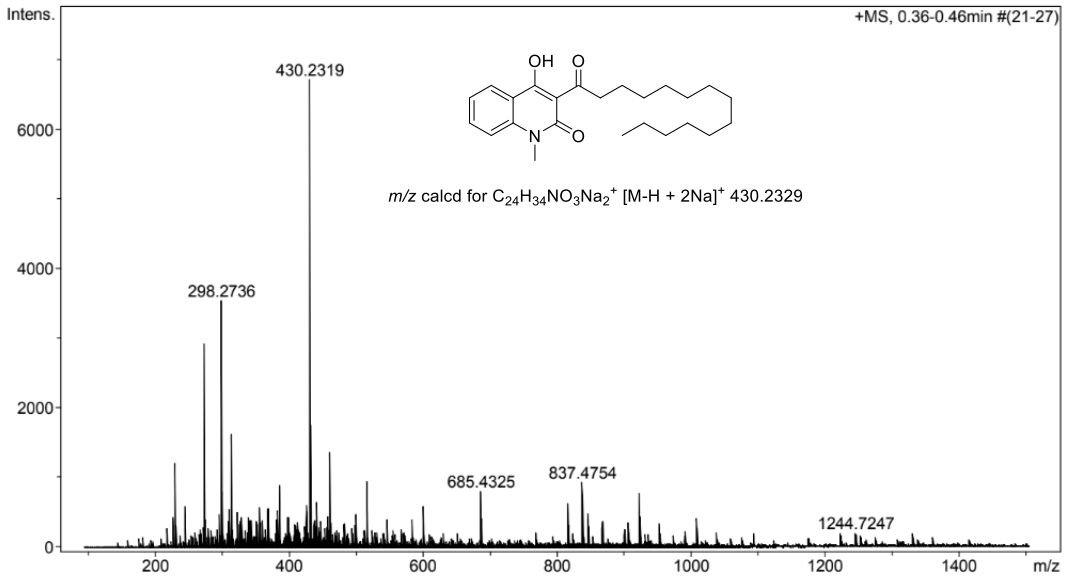
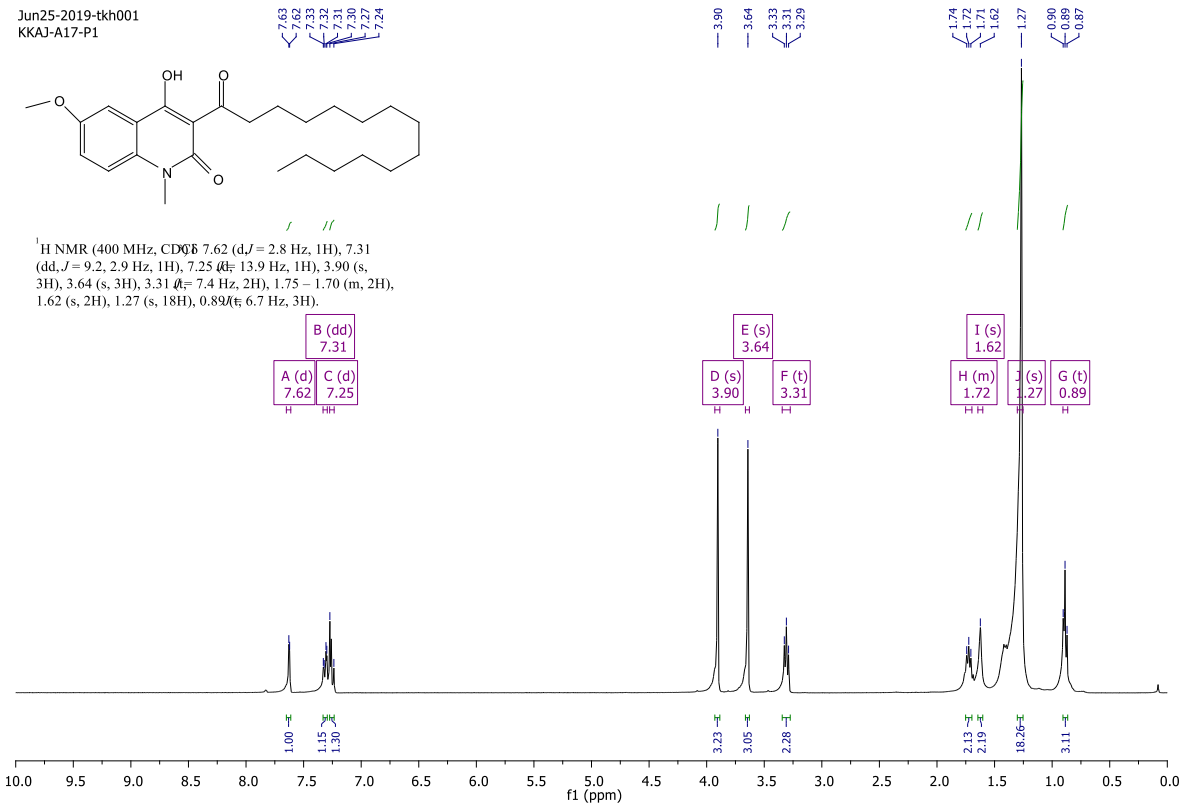
Sample Name	A16	Instrument	micrOTOF-Q II
Comment			

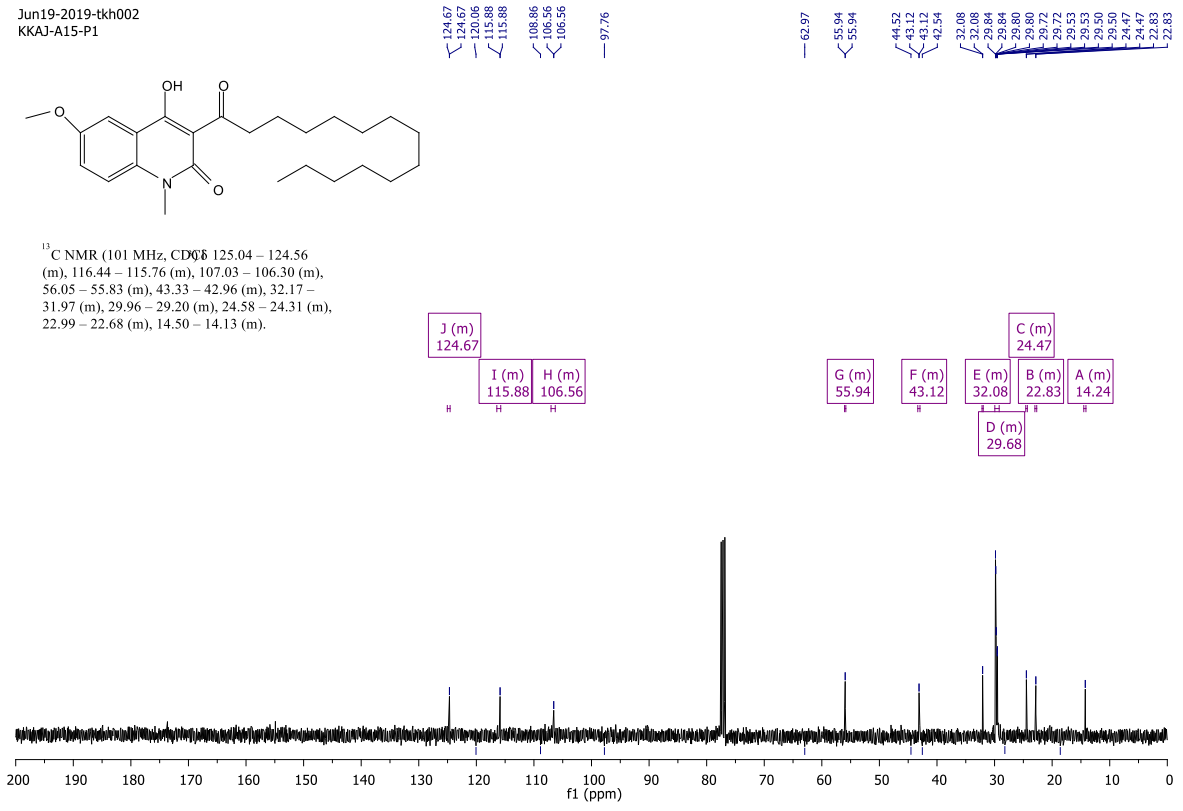


m/z calcd for $C_{24}H_{34}NO_3Na_2^+$ [M-H + 2Na]$^+$ 430.2329

4-Hydroxy-6-methoxy-1-methyl-3-tetradecanoylquinolin-2(1H)-one (**3b**)



^{1}H NMR (400 MHz, CDCl$_3$) δ 7.62 (d, J = 2.8 Hz, 1H), 7.31 (dd, J = 9.2, 2.9 Hz, 1H), 7.25 (d, J = 13.9 Hz, 1H), 3.90 (s, 3H), 3.64 (s, 3H), 3.31 (t, J = 7.4 Hz, 2H), 1.75 – 1.70 (m, 2H), 1.62 (s, 2H), 1.27 (s, 18H), 0.89 (t, J = 6.7 Hz, 3H).



^{13}C NMR (101 MHz, CDCl$_3$) δ 125.04 – 124.56 (m), 116.44 – 115.76 (m), 107.03 – 106.30 (m), 56.05 – 55.83 (m), 43.33 – 42.96 (m), 32.17 – 31.97 (m), 29.96 – 29.20 (m), 24.58 – 24.31 (m), 22.99 – 22.68 (m), 14.50 – 14.13 (m).

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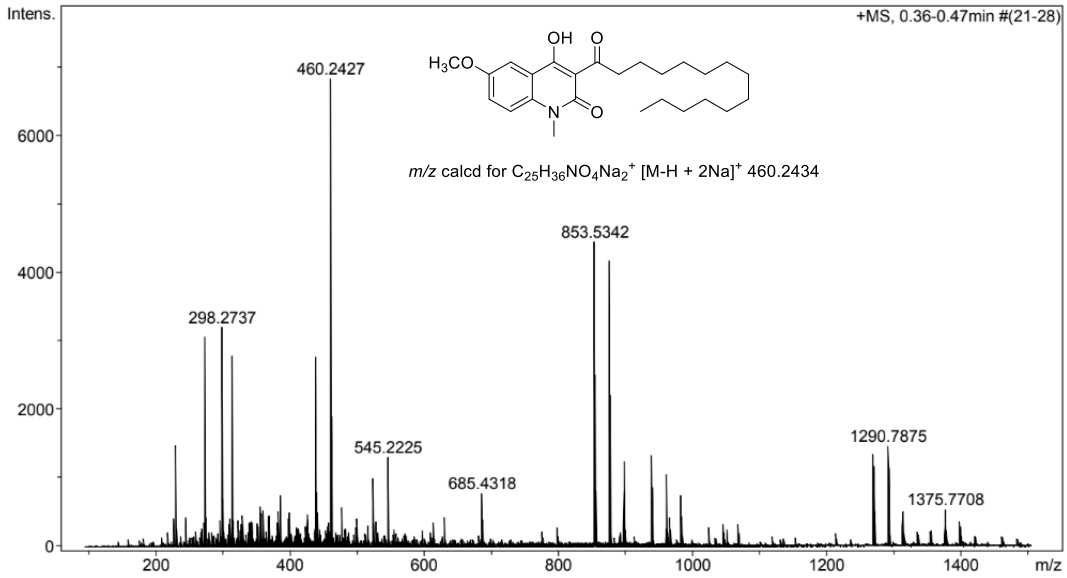
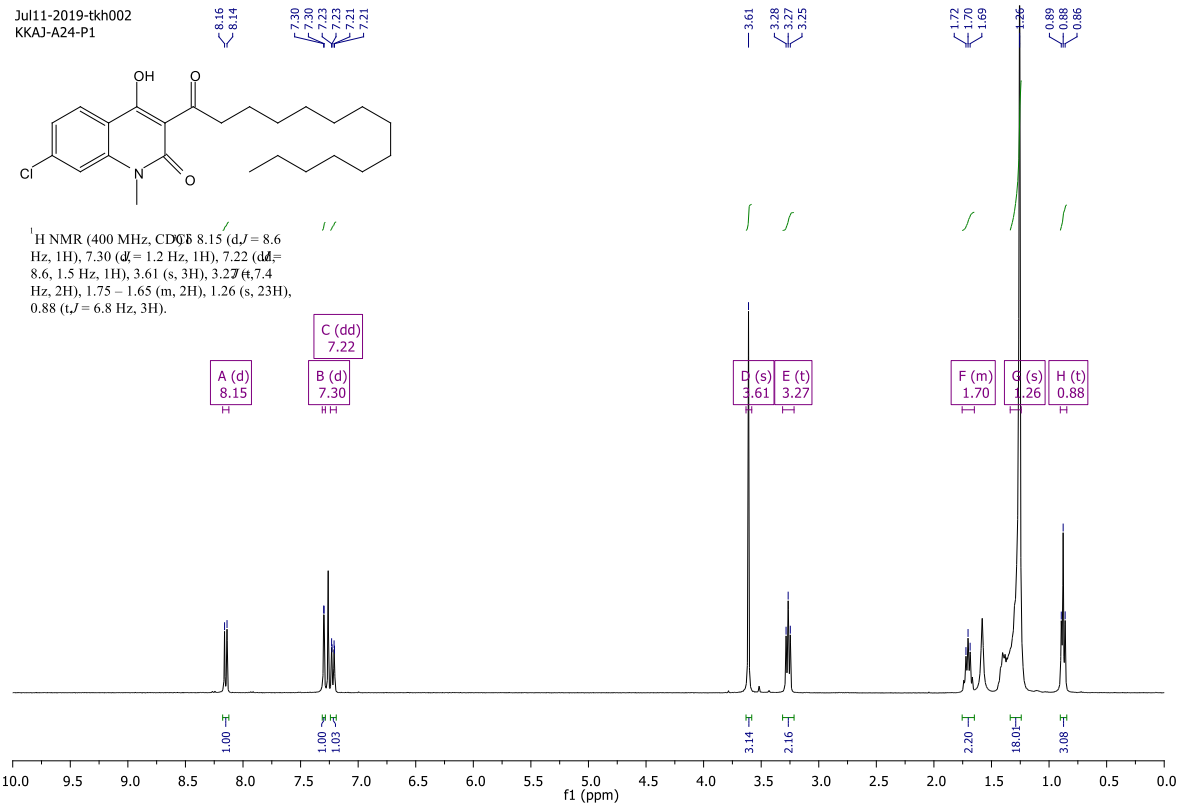
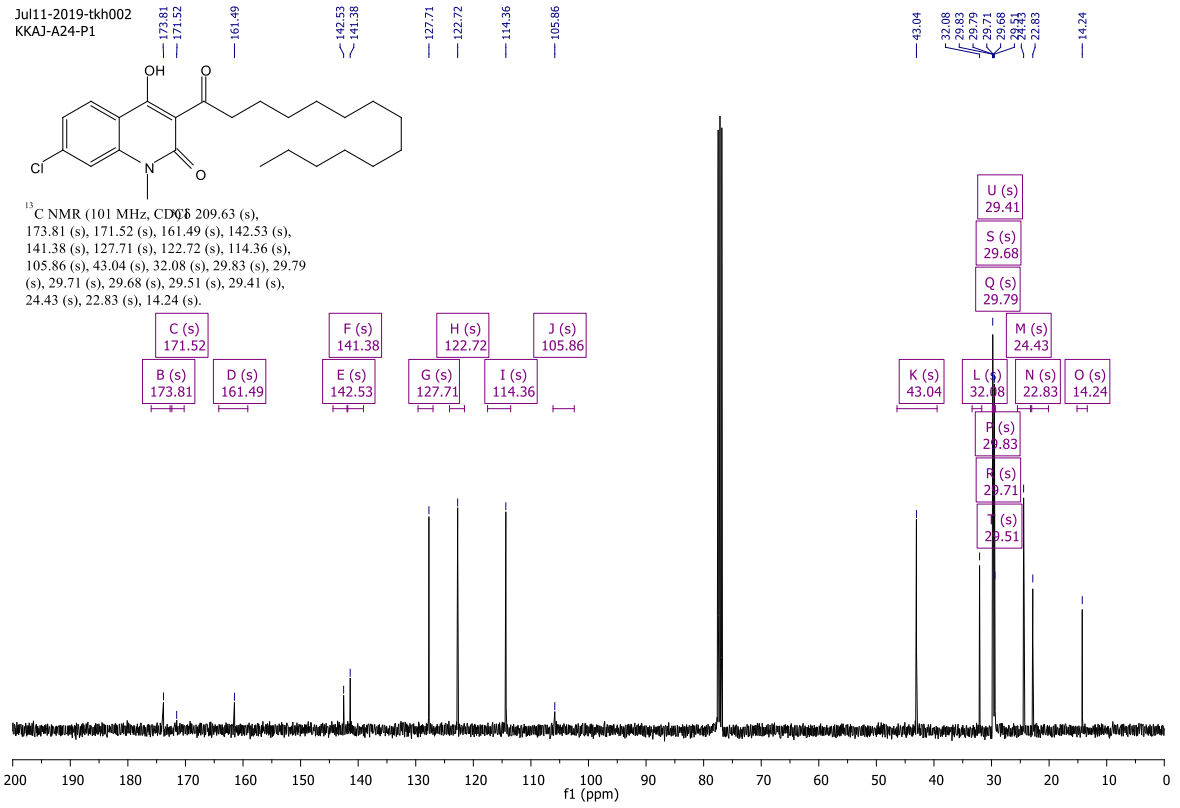


m/z calcd for $C_{25}H_{36}NO_4Na_2^+$ [M-H + 2Na]$^+$ 460.2434

7-Chloro-4-hydroxy-1-methyl-3-tetradecanoylquinolin-2(1H)-one (**3c**)





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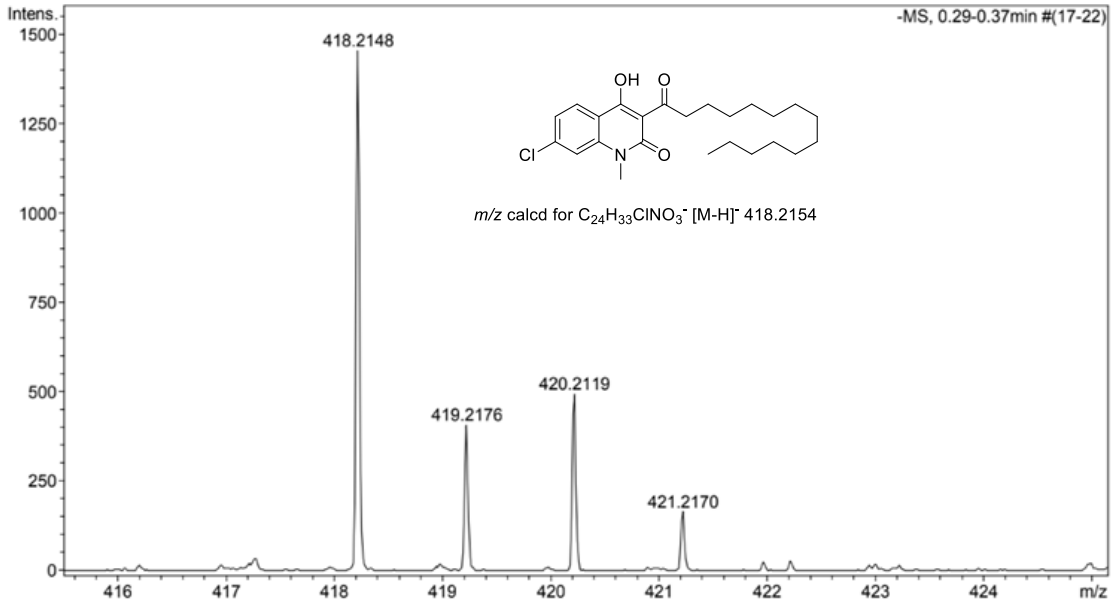
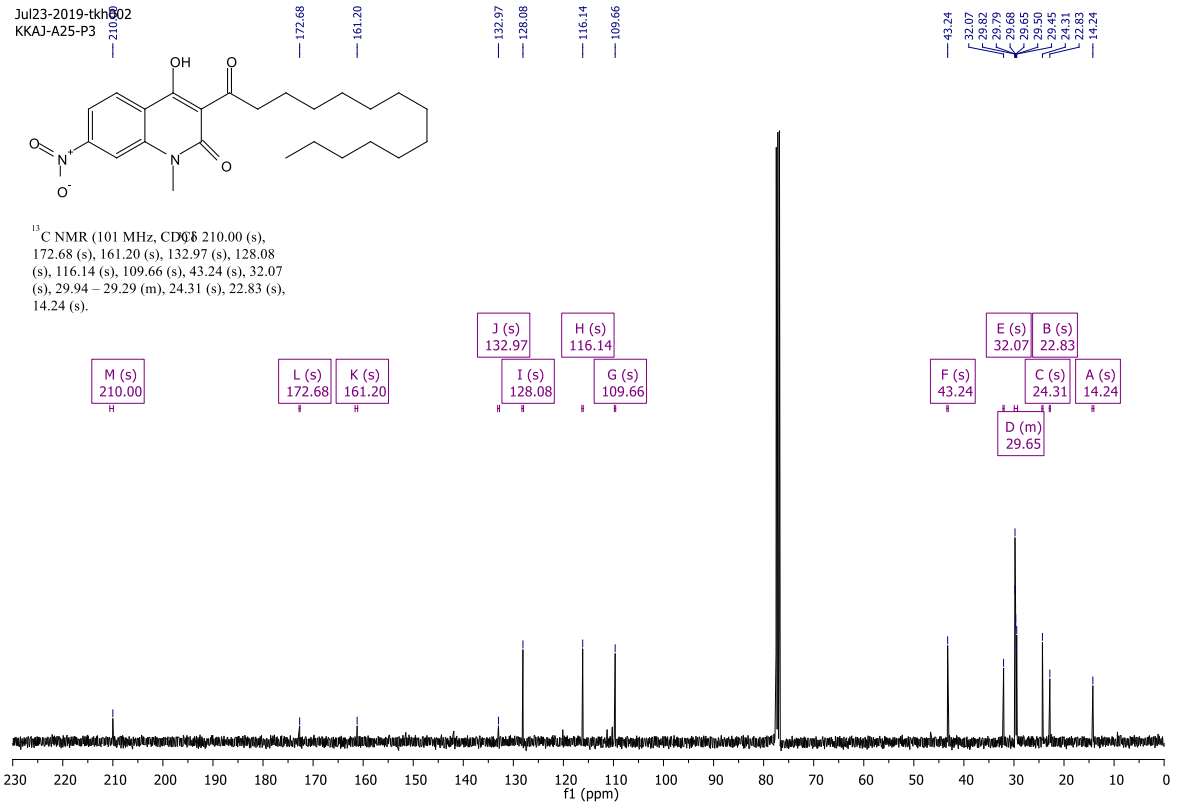


m/z calcd for $C_{24}H_{33}ClNO_3^-$ $[M-H]^-$ 418.2154

4-Hydroxy-1-methyl-7-nitro-3-tetradecanoylquinolin-2(1H)-one (**3d**)

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.40 (d, *J* = 8.8 Hz, 1H), 8.17 (d, *J* = 1.8 Hz, 1H), 8.05 (dd, *J* = 8.7, 1.8 Hz, 1H), 3.72 (s, 3H), 3.30 (t, *J* = 7.4 Hz, 2H), 1.71 (d, *J* = 7.3 Hz, 2H), 1.43 – 1.37 (m, 2H), 1.26 (s, 18H), 0.88 (t, *J* = 6.7 Hz, 3H).



^{13}C NMR (101 MHz, CDCl$_3$) δ 210.00 (s), 172.68 (s), 161.20 (s), 132.97 (s), 128.08 (s), 116.14 (s), 109.66 (s), 43.24 (s), 32.07 (s), 29.94 – 29.29 (m), 24.31 (s), 22.83 (s), 14.24 (s).

Generic Display Report

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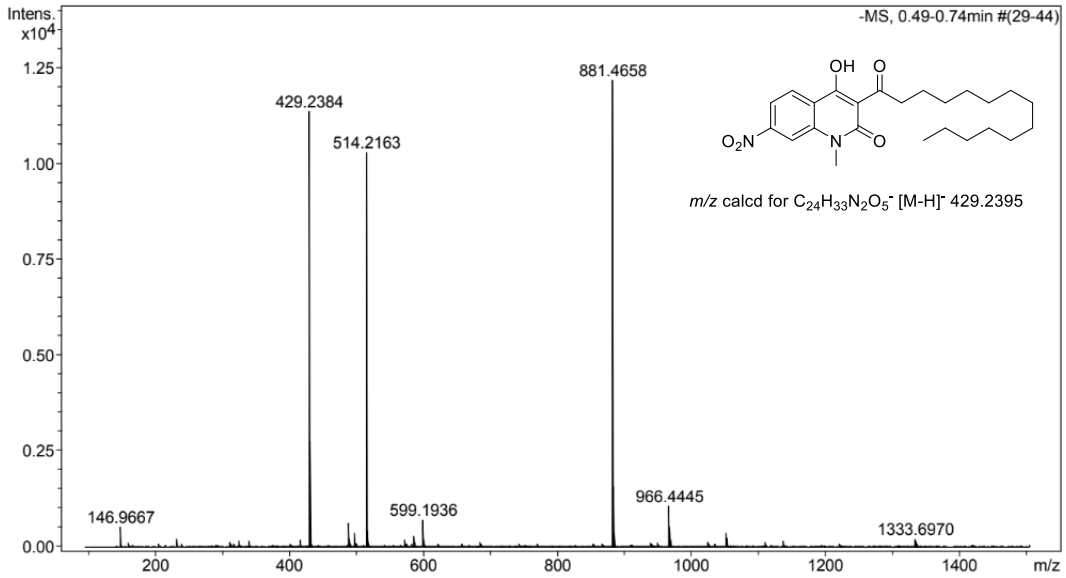
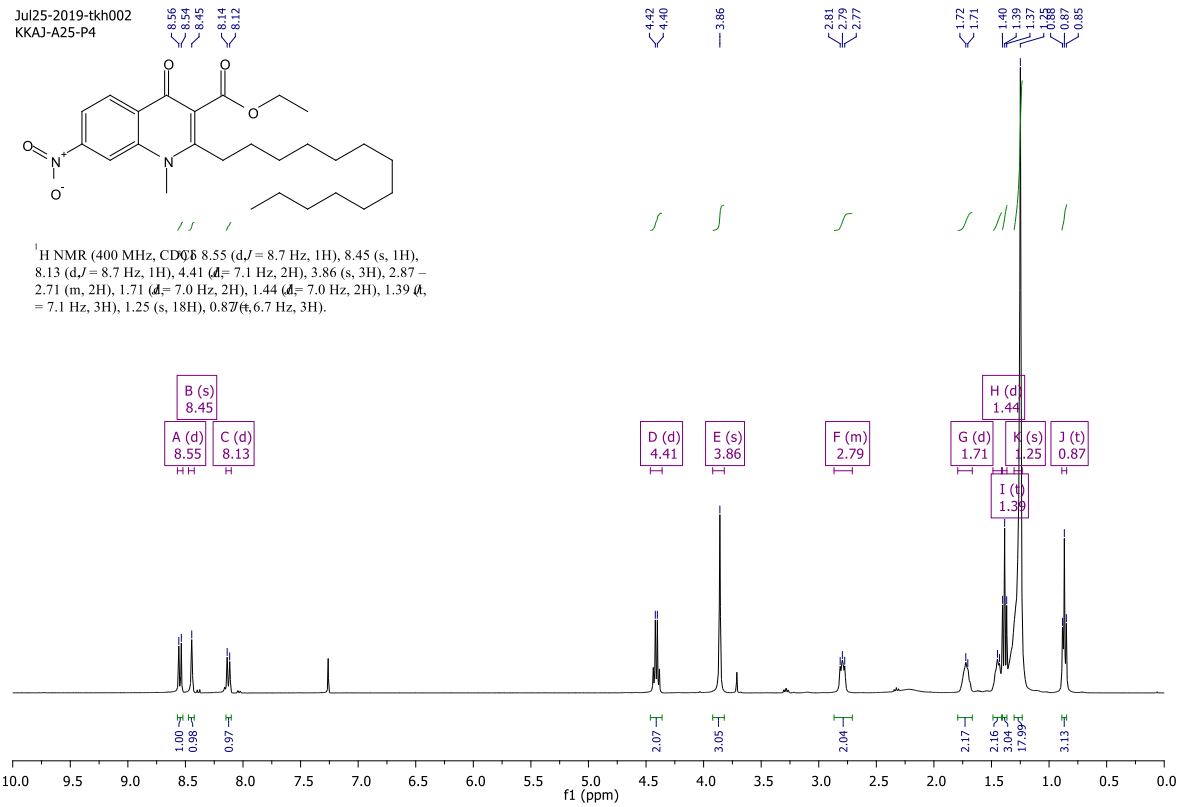
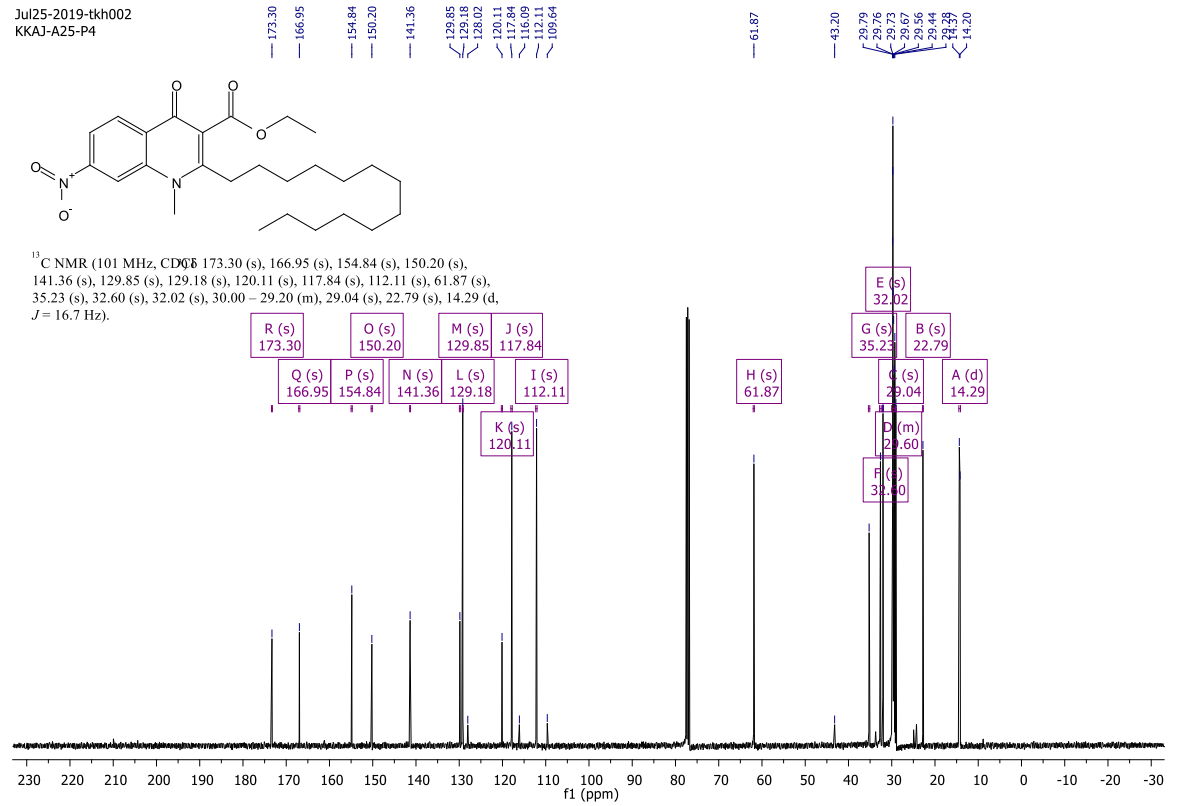


Ethyl 1-methyl-7-nitro-4-oxo-2-tridecyl-1,4-dihydroquinoline-3-carboxylate (**4d**)



^{1}H NMR (400 MHz, CDCl$_3$) δ 8.55 (d, J = 8.7 Hz, 1H), 8.45 (s, 1H), 8.13 (d, J = 8.7 Hz, 1H), 4.41 (d, J = 7.1 Hz, 2H), 3.86 (s, 3H), 2.87 – 2.71 (m, 2H), 1.71 (d, J = 7.0 Hz, 2H), 1.44 (d, J = 7.0 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H), 1.25 (s, 18H), 0.87 (t, J = 6.7 Hz, 3H).



^{13}C NMR (101 MHz, CDCl$_3$) δ 173.30 (s), 166.95 (s), 154.84 (s), 150.20 (s), 141.36 (s), 129.85 (s), 129.18 (s), 120.11 (s), 117.84 (s), 112.11 (s), 61.87 (s), 35.23 (s), 32.60 (s), 32.02 (s), 30.00 – 29.20 (m), 29.04 (s), 22.79 (s), 14.29 (d, J = 16.7 Hz).

Generic Display Report

Analysis Info

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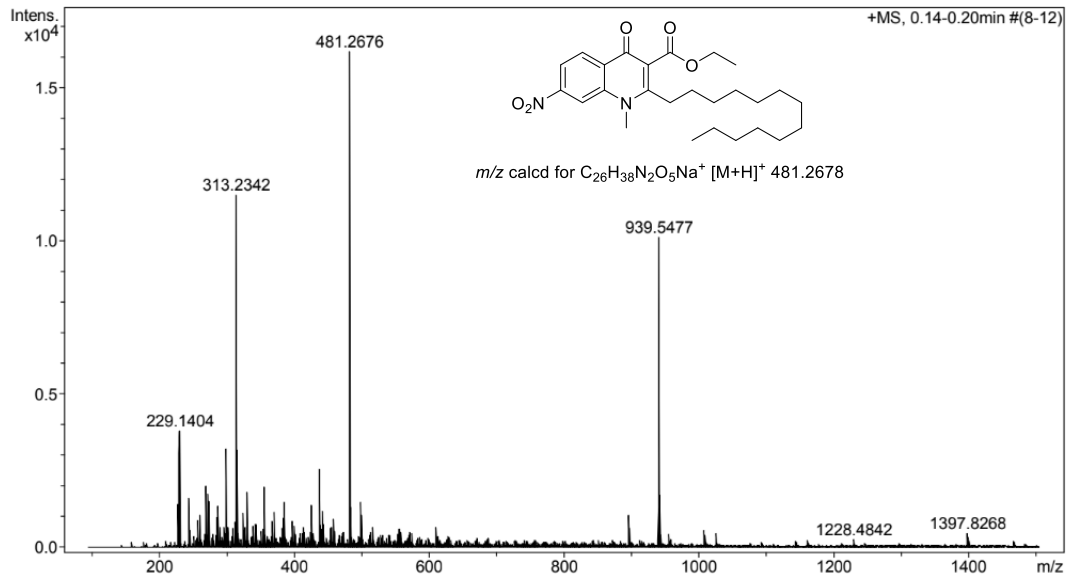
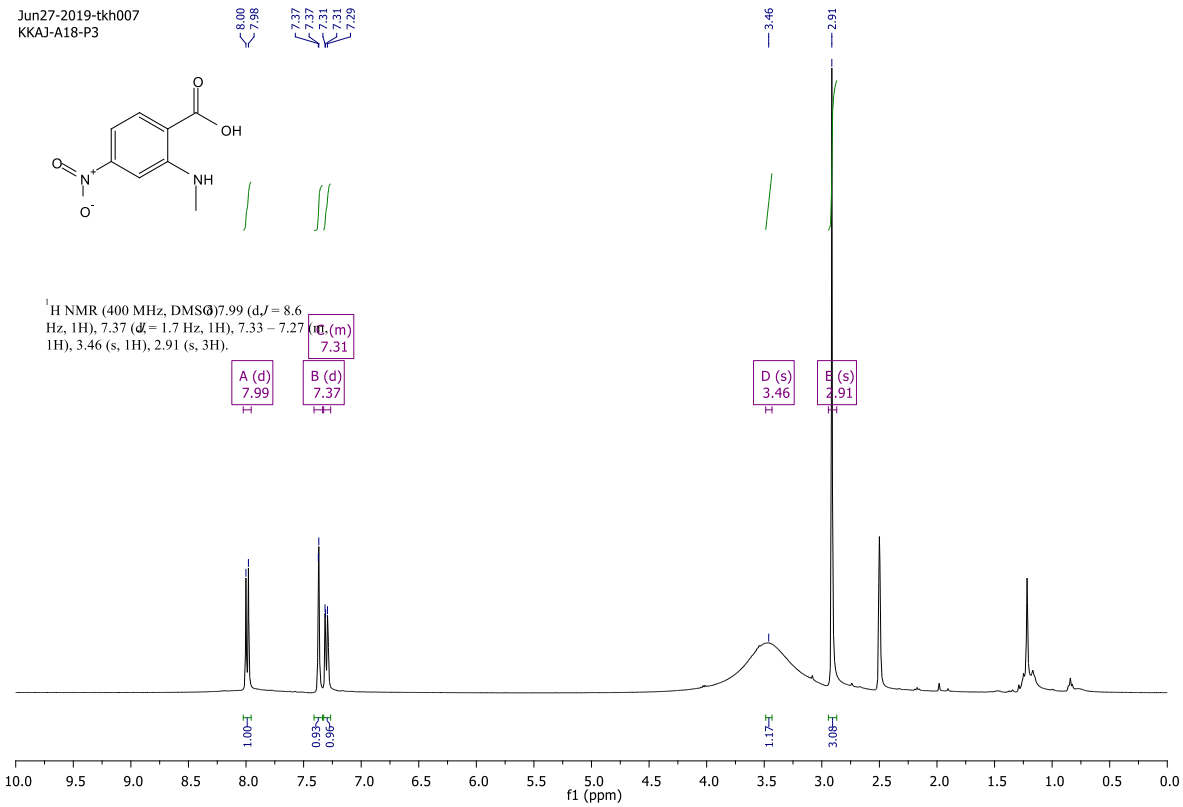
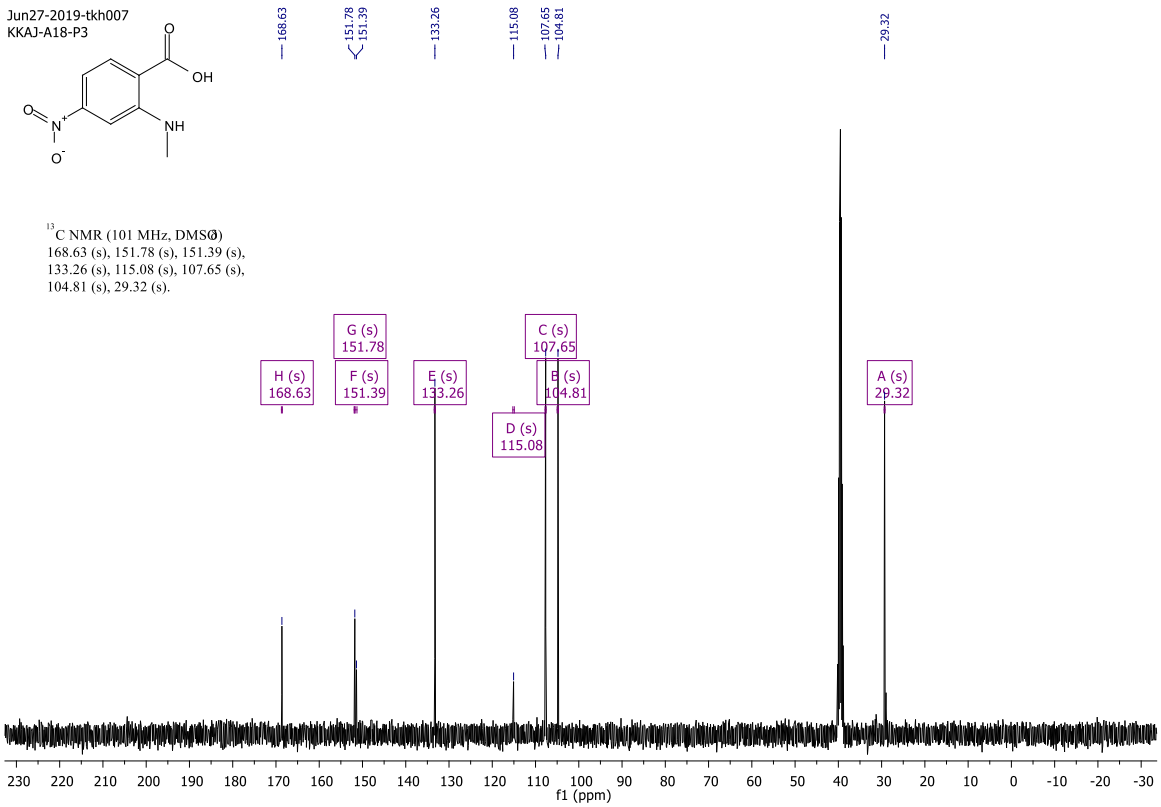
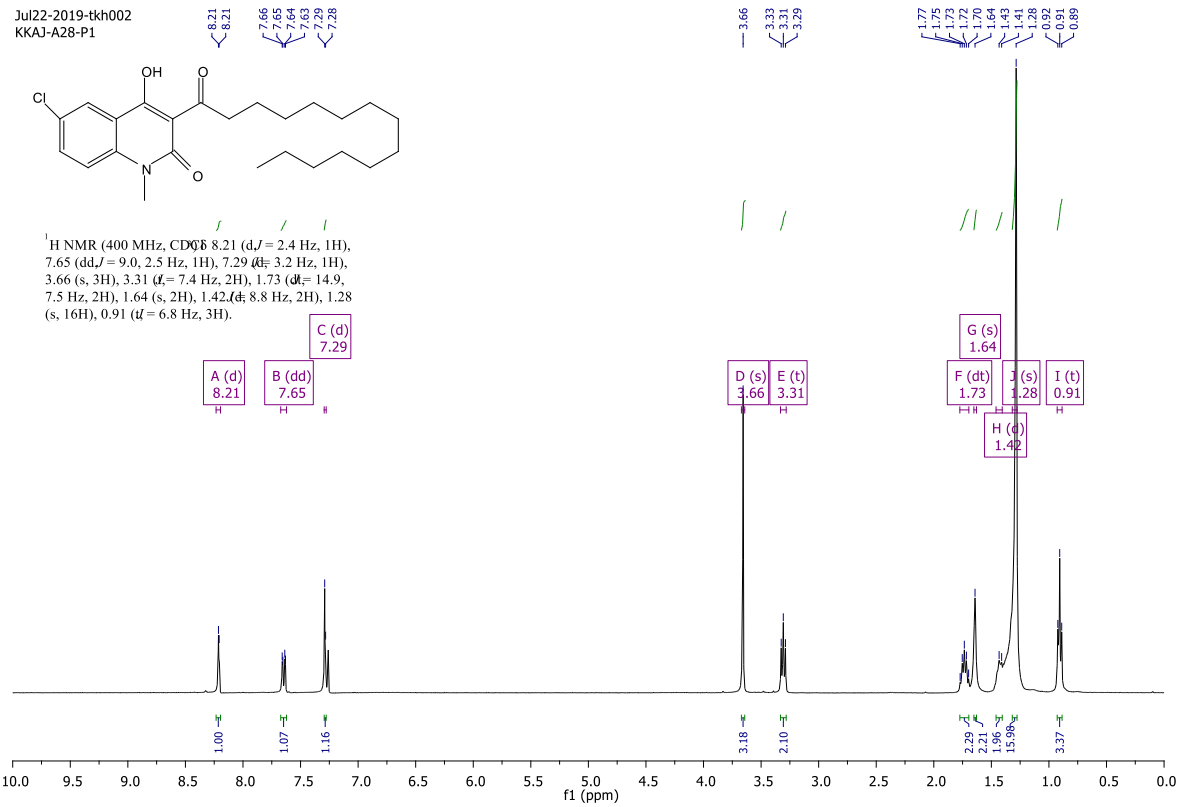


2-(methylamino)-4-nitrobenzoic acid (**5d**)

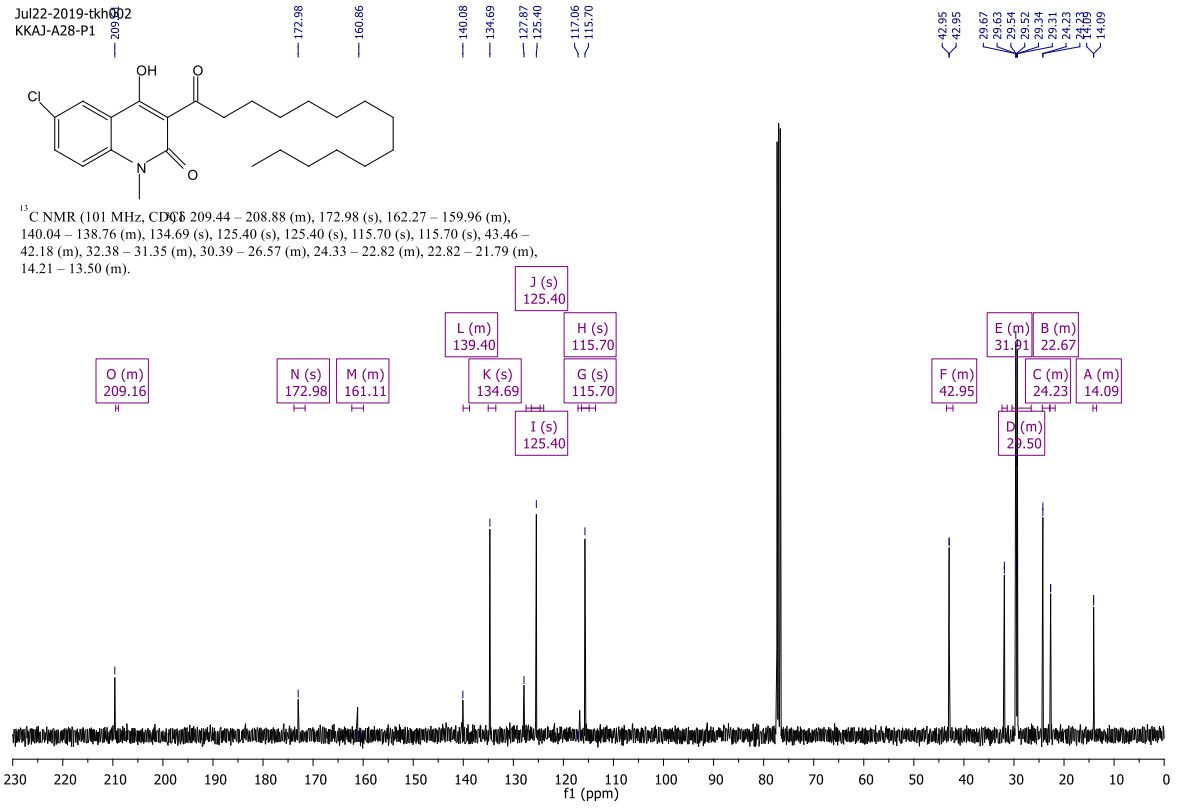




6-Chloro-4-hydroxy-1-methyl-3-tetradecanoylquinolin-2(1H)-one (**3e**)



^{1}H NMR (400 MHz, CDCl$_3$) δ 8.21 (d, J = 2.4 Hz, 1H), 7.65 (dd, J = 9.0, 2.5 Hz, 1H), 7.29 (d, J = 3.2 Hz, 1H), 3.66 (s, 3H), 3.31 (t, J = 7.4 Hz, 2H), 1.73 (dt, J = 14.9, 7.5 Hz, 2H), 1.64 (s, 2H), 1.42 (d, J = 8.8 Hz, 2H), 1.28 (s, 16H), 0.91 (t, J = 6.8 Hz, 3H).

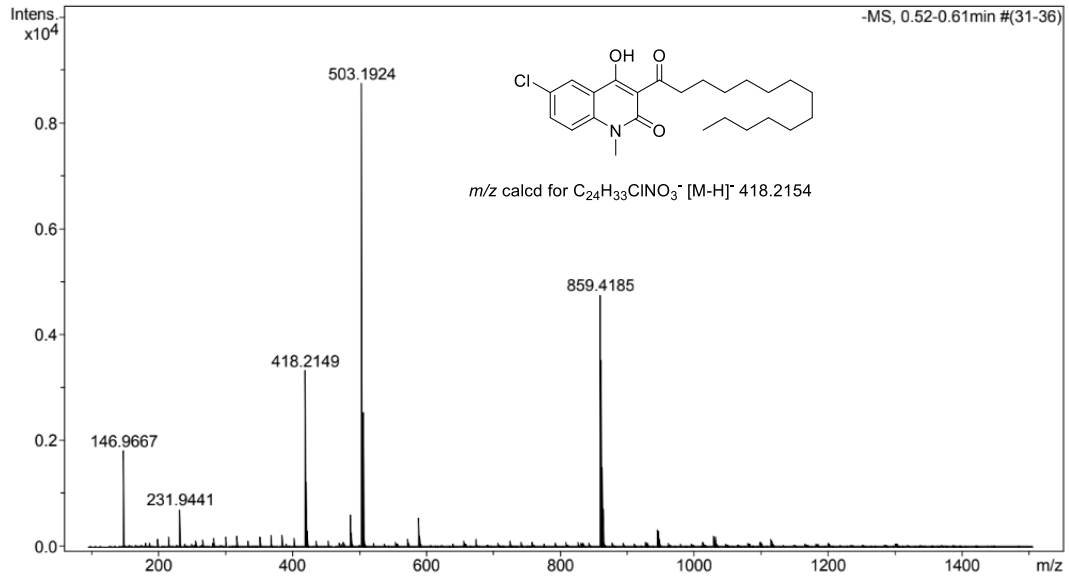


^{13}C NMR (101 MHz, CDCl$_3$) δ 209.44 – 208.88 (m), 172.98 (s), 162.27 – 159.96 (m), 140.04 – 138.76 (m), 134.69 (s), 125.40 (s), 125.40 (s), 115.70 (s), 115.70 (s), 43.46 – 42.18 (m), 32.38 – 31.35 (m), 30.39 – 26.57 (m), 24.33 – 22.82 (m), 22.82 – 21.79 (m), 14.21 – 13.50 (m).

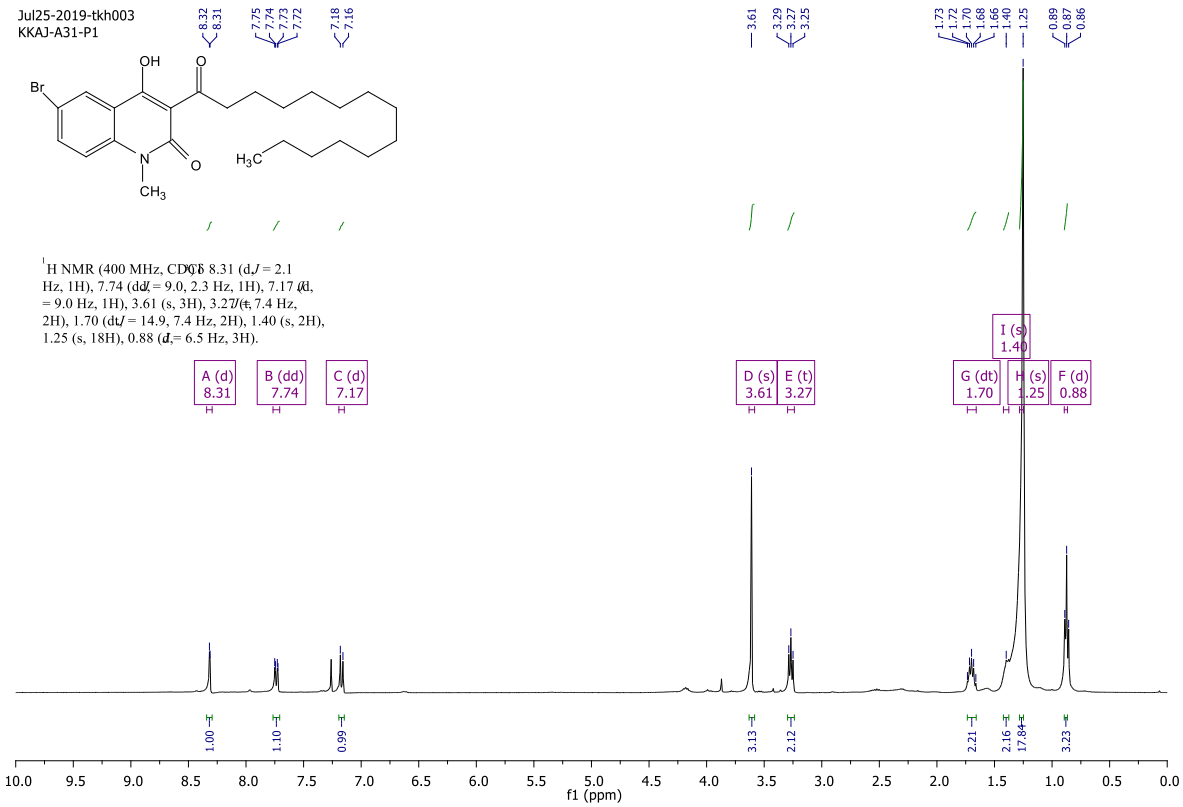
Generic Display Report

Analysis Info

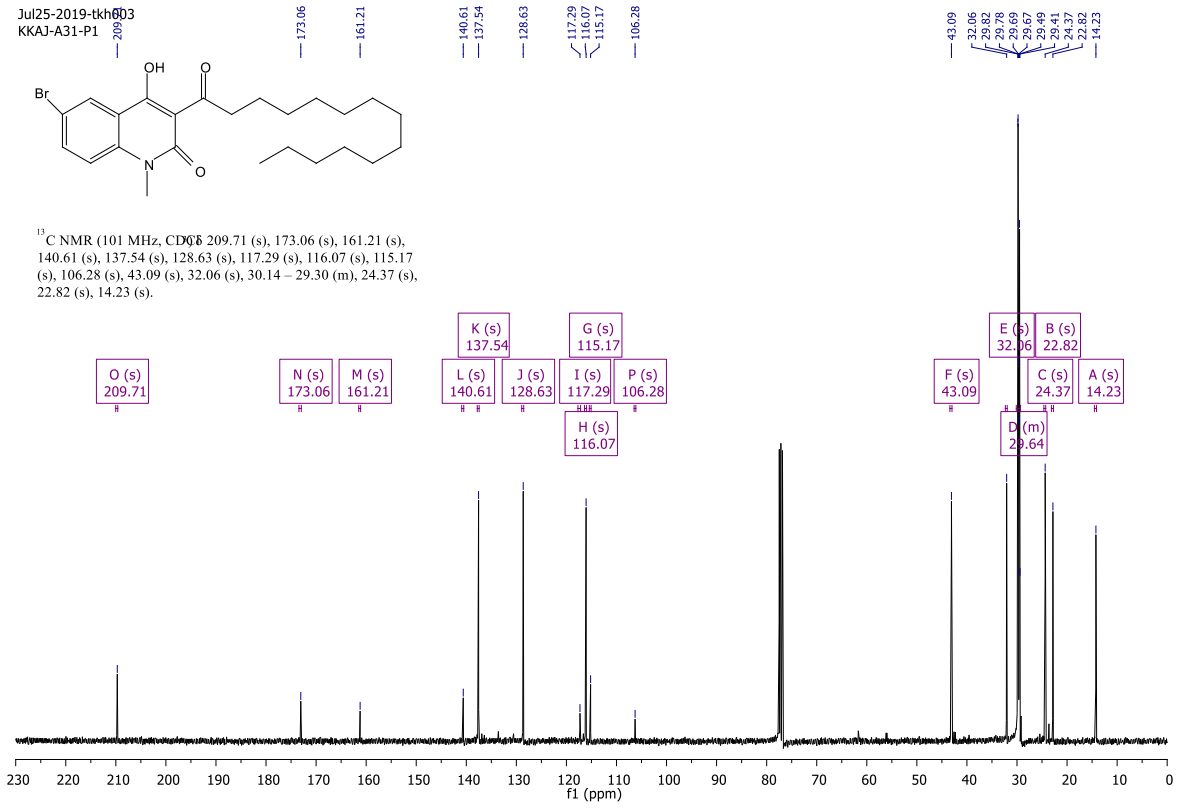
Analysis Name	D:\Data\Data Service\190819\A28_RB8_01_2967.d	Acquisition Date	8/19/2019 8:18:49 PM
Method	nv_neg_6min_profile_wguardcol_190624.m	Operator	CU.
Sample Name	A28	Instrument	micrOTOF-Q II
Comment			



6-Bromo-4-hydroxy-1-methyl-3-tetradecanoylquinolin-2(1H)-one (**3f**)



^{1}H NMR (400 MHz, CDCl$_3$) δ 8.31 (d, J = 2.1 Hz, 1H), 7.74 (dd, J = 9.0, 2.3 Hz, 1H), 7.17 (d, J = 9.0 Hz, 1H), 3.61 (s, 3H), 3.27 (t, J = 7.4 Hz, 2H), 1.70 (dt, J = 14.9, 7.4 Hz, 2H), 1.40 (s, 2H), 1.25 (s, 18H), 0.88 (d, J = 6.5 Hz, 3H).

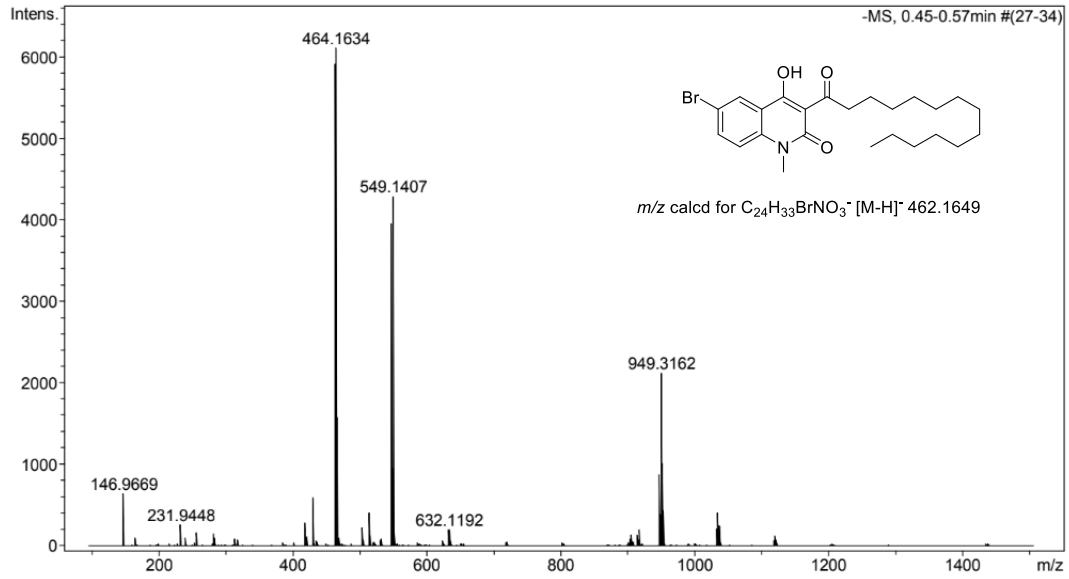


^{13}C NMR (101 MHz, CDCl$_3$) δ 209.71 (s), 173.06 (s), 161.21 (s), 140.61 (s), 137.54 (s), 128.63 (s), 117.29 (s), 116.07 (s), 115.17 (s), 106.28 (s), 43.09 (s), 32.06 (s), 30.14 – 29.30 (m), 24.37 (s), 22.82 (s), 14.23 (s).

Generic Display Report

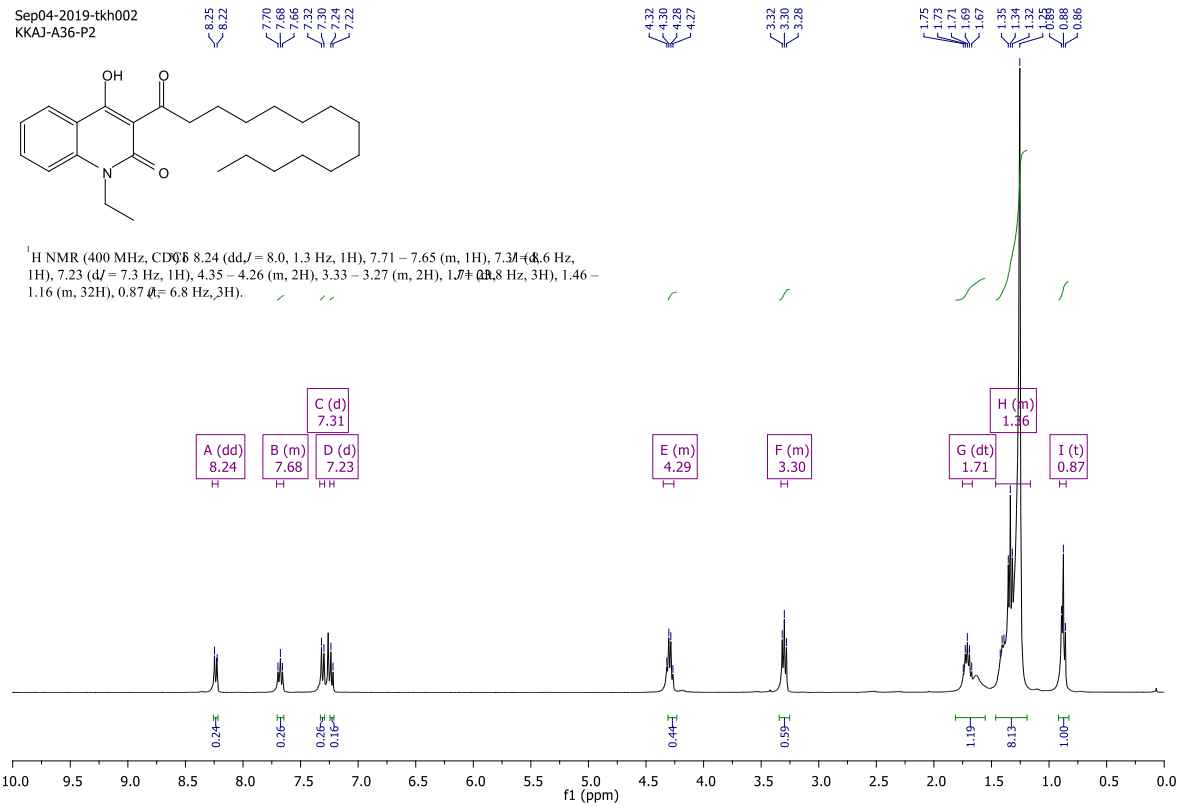
Analysis Info

Analysis Name	D:\Data\Data Service\190819\A31_RC2_01_2969.d	Acquisition Date	8/19/2019 8:31:48 PM
Method	nv_neg_6min_profile_wguardcol_190624.m	Operator	CU.
Sample Name	A31	Instrument	micrOTOF-Q II
Comment			

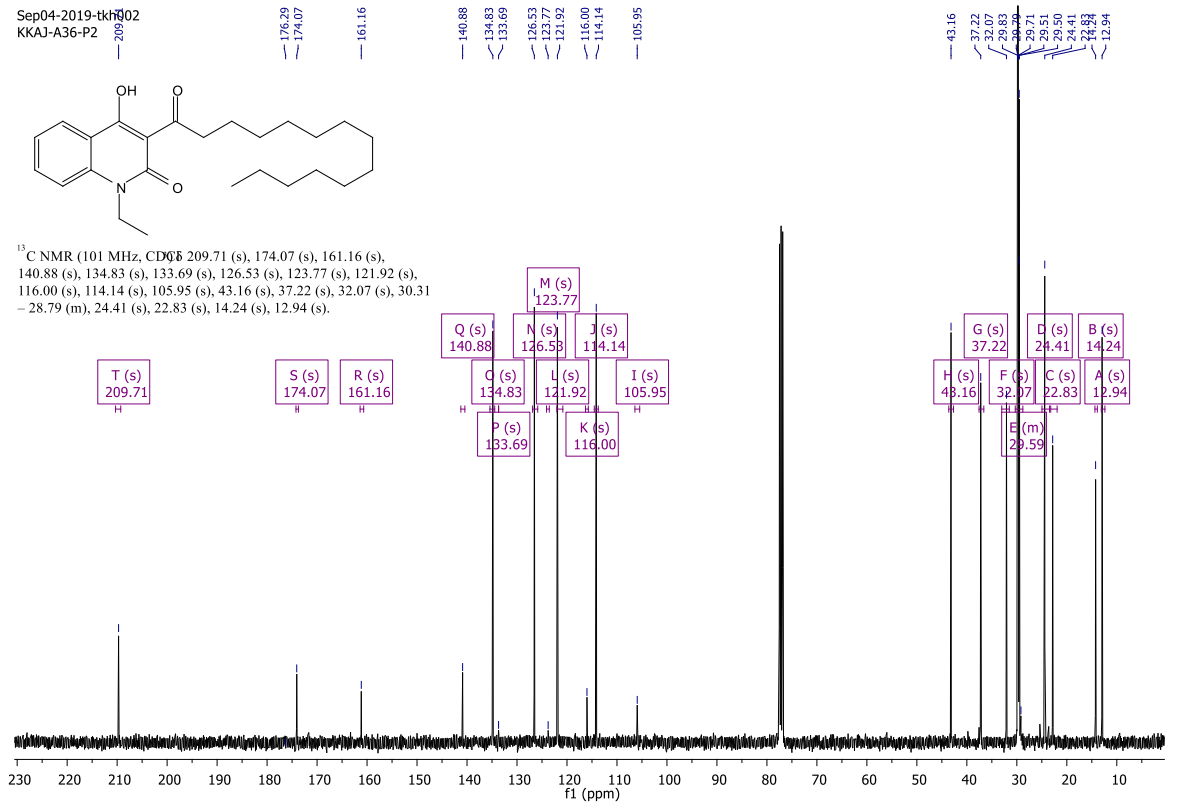


m/z calcd for $C_{24}H_{33}BrNO_3^-$ $[M-H]^-$ 462.1649

1-Ethyl-4-hydroxy-3-tetradecanoylquinolin-2(1H)-one (**3g**)



^{1}H NMR (400 MHz, CDCl$_3$) δ 8.24 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.71 – 7.65 (m, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 7.23 (d, *J* = 7.3 Hz, 1H), 4.35 – 4.26 (m, 2H), 3.33 – 3.27 (m, 2H), 1.71 (dt, *J* = 15.8, 7.8 Hz, 3H), 1.46 – 1.16 (m, 32H), 0.87 (t, *J* = 6.8 Hz, 3H).



^{13}C NMR (101 MHz, CDCl$_3$) δ 209.71 (s), 174.07 (s), 161.16 (s), 140.88 (s), 134.83 (s), 133.69 (s), 126.53 (s), 123.77 (s), 121.92 (s), 116.00 (s), 114.14 (s), 105.95 (s), 43.16 (s), 37.22 (s), 32.07 (s), 30.31 – 28.79 (m), 24.41 (s), 22.83 (s), 14.24 (s), 12.94 (s).

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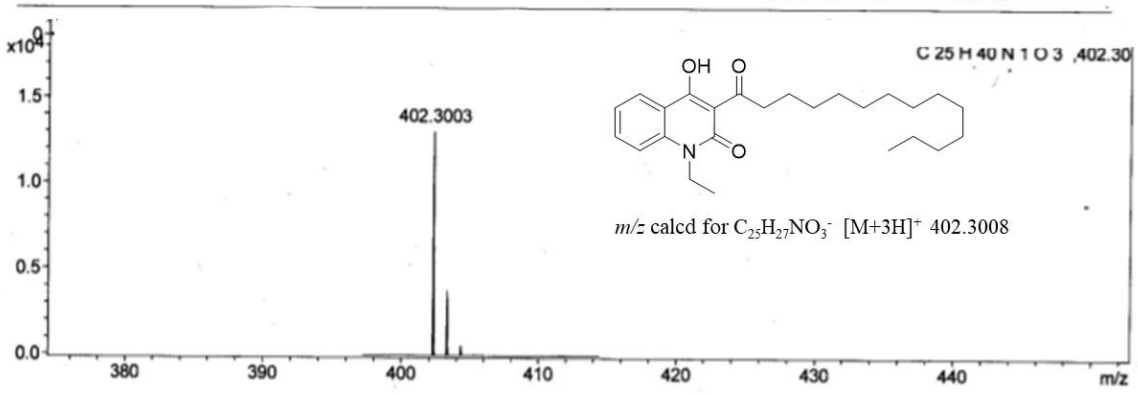
High resolution report

Analysis Name	D:\Data\customer\TK3g.d	Acquisition Date	6/1/2020 10:30:54 AM
Method	NaFormate_pos.m		
Sample Name	TK3g	Operator	Sutichai Ext: 3560
		Instrument	micrOTOF Bruker
		Calibrate by	Sodium Formate

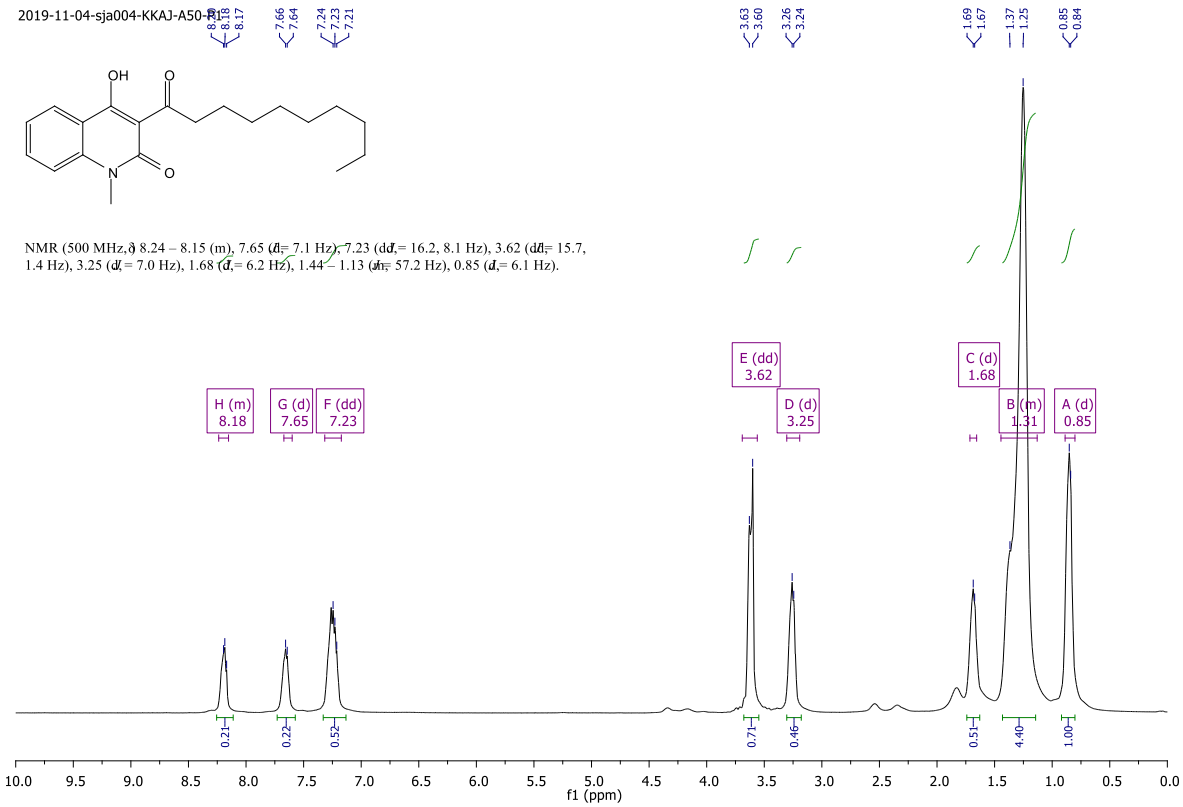
Acquisition Parameter

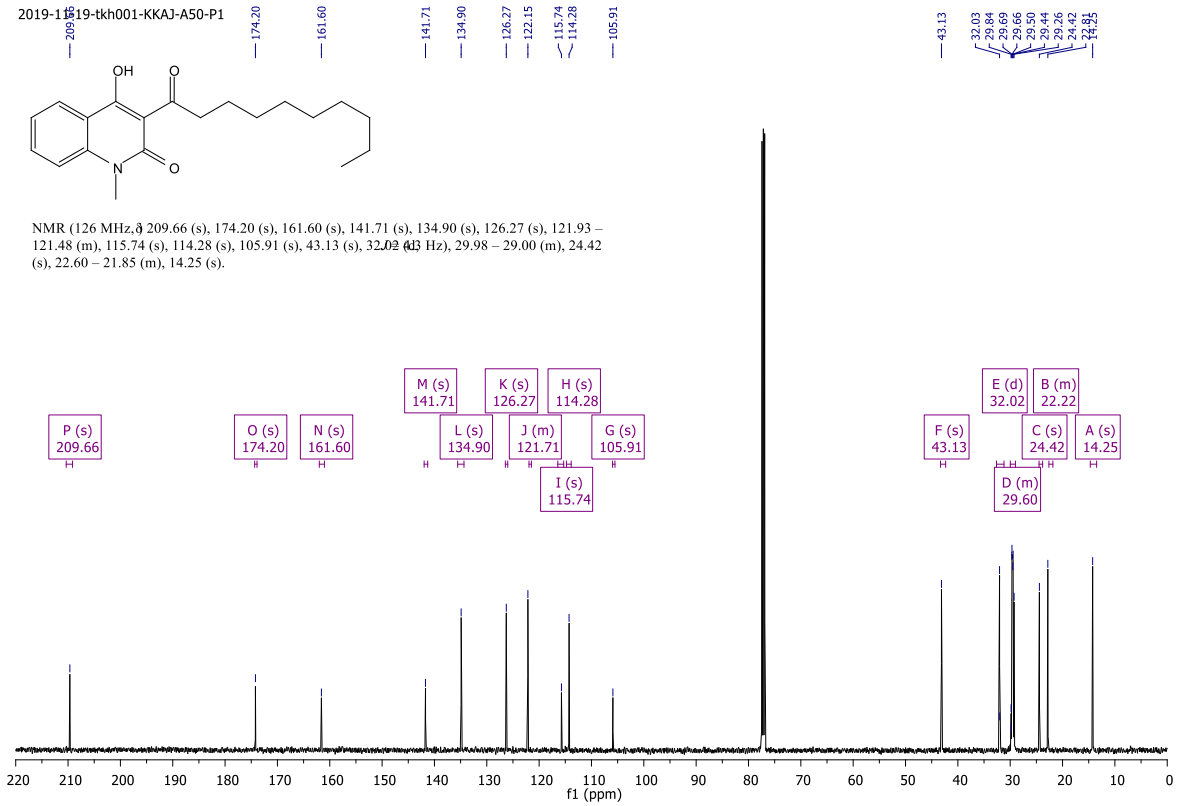
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active			Set Dry Heater	180 ?C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



m/z calcd for $C_{25}H_{27}NO_3^-$ $[M+3H]^+$ 402.3008

3-Decanoyl-4-hydroxy-1-methylquinolin-2(1H)-one (**3h**)



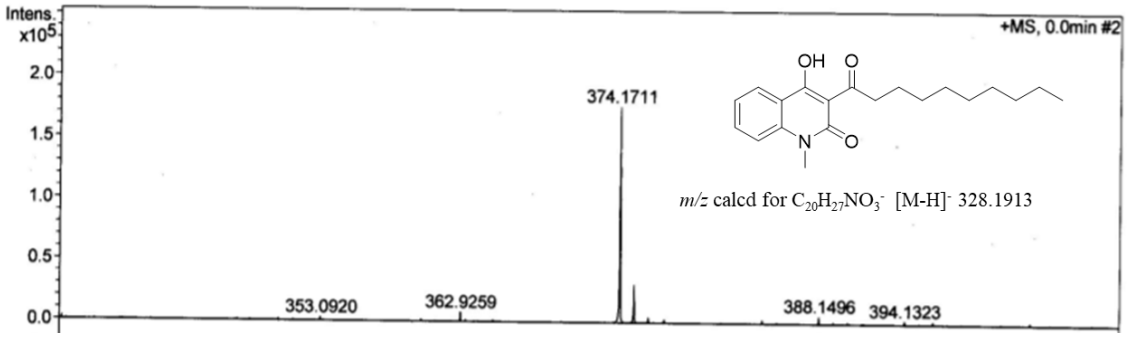


High resolution report

Analysis Name	D:\Data\customer\TK 3h.d	Acquisition Date	6/1/2020 3:03:32 PM
Method	NaFormate_pos.m		
Sample Name	TK 3h	Operator	Sutichai Ext: 3560
		Instrument	micrOTOF Bruker
		Calibrate by	Sodium Formate

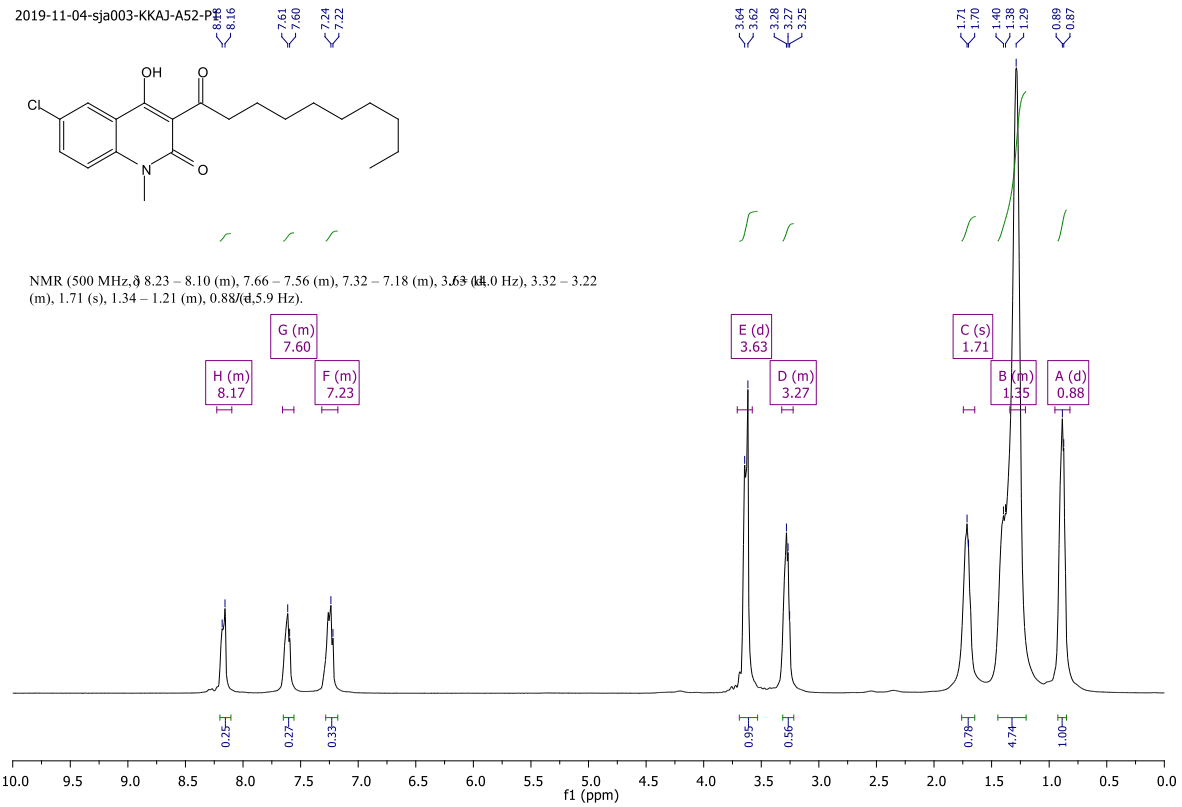
Acquisition Parameter

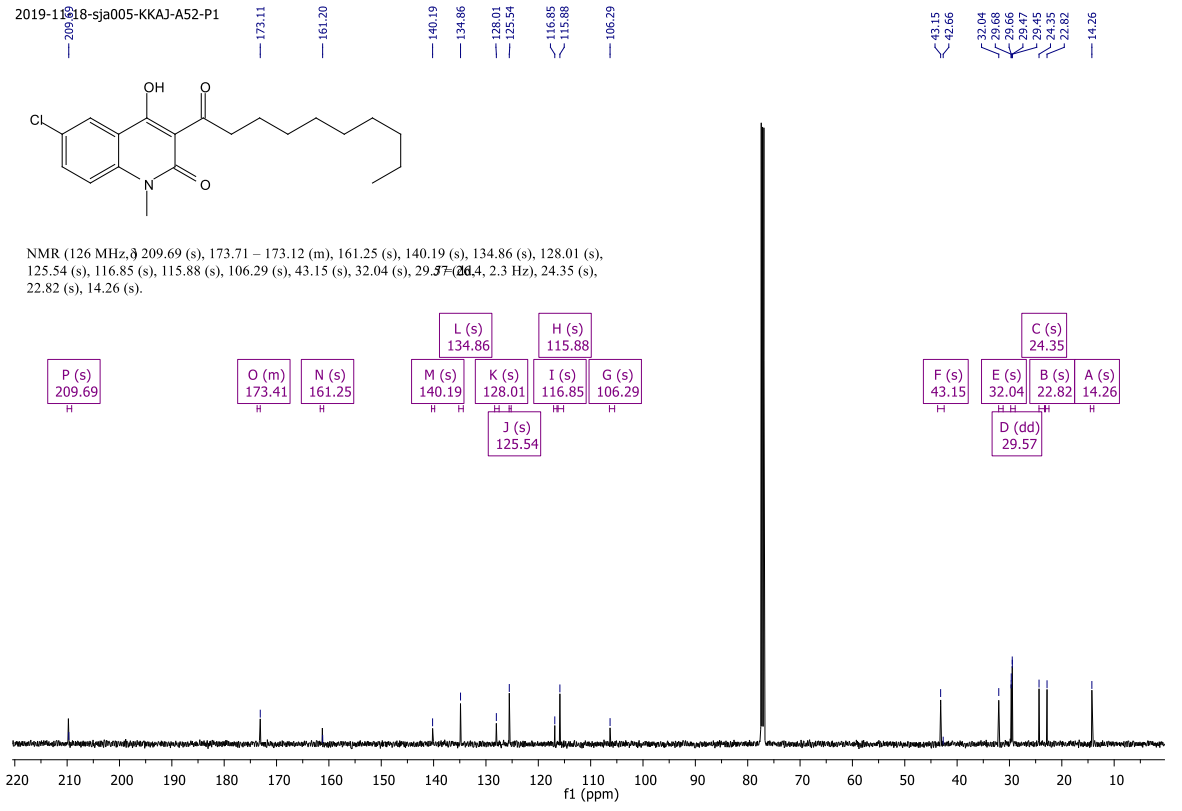
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active			Set Dry Heater	180 ?C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



m/z calcd for $C_{20}H_{27}NO_3^-$ $[M\text{-}H]^-$ 328.1913

6-Chloro-3-decanoyl-4-hydroxy-1-methylquinolin-2(1H)-one (**3i**)





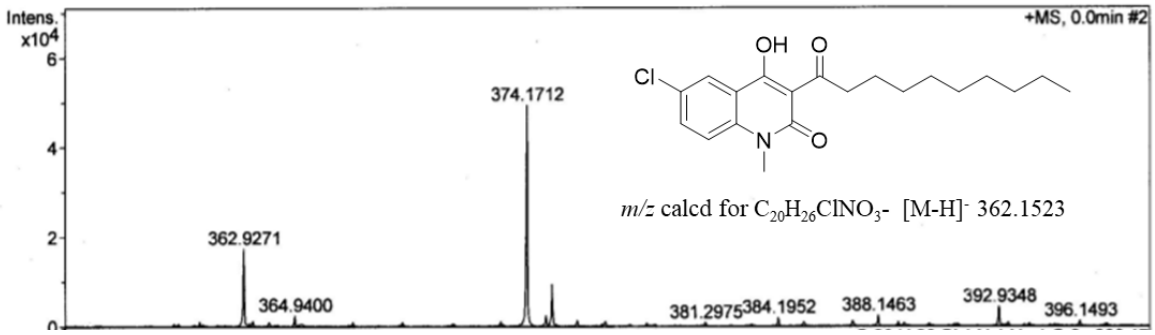
High resolution report

Acquisition Date 6/1/2020 3:14:55 PM

Analysis Name	D:\Data\customer\TK 3i.d		
Method	NaFormate_pos.m	Operator	Sutichai Ext: 3560
Sample Name	TK 3i	Instrument	micrOTOF Bruker
		Calibrate by	Sodium Formate

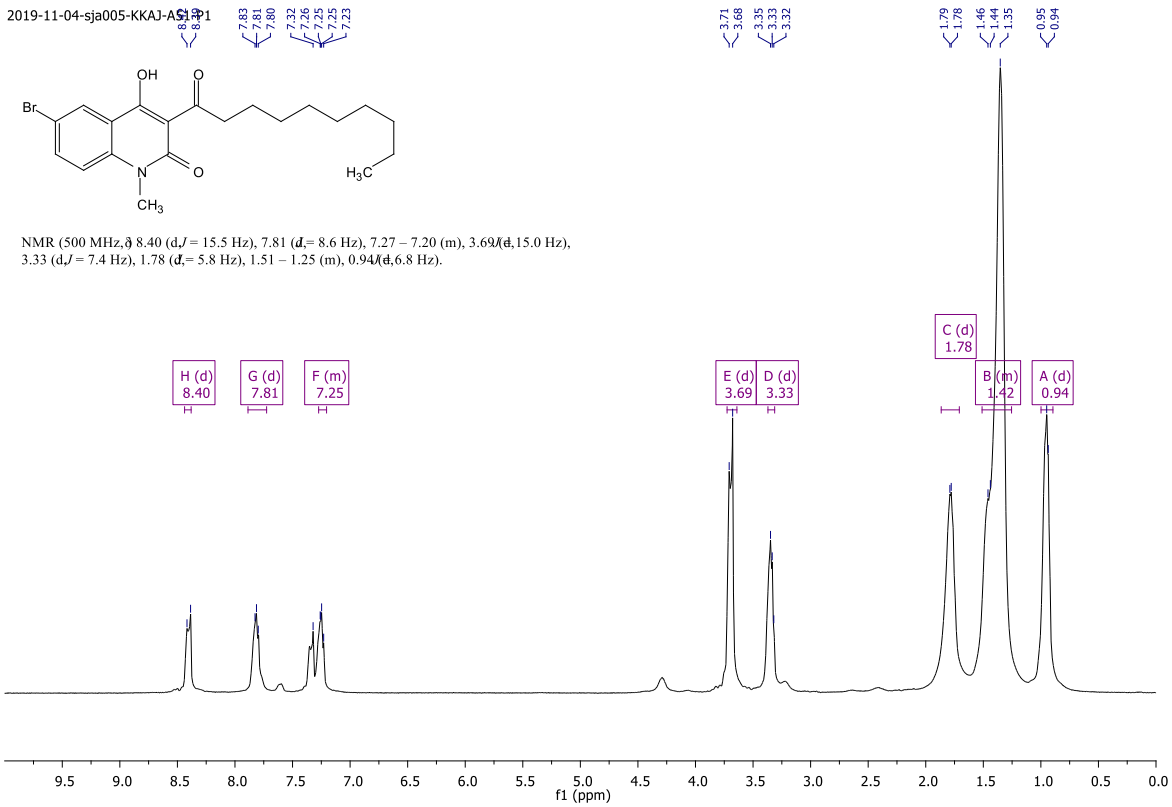
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active			Set Dry Heater	180 ?C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

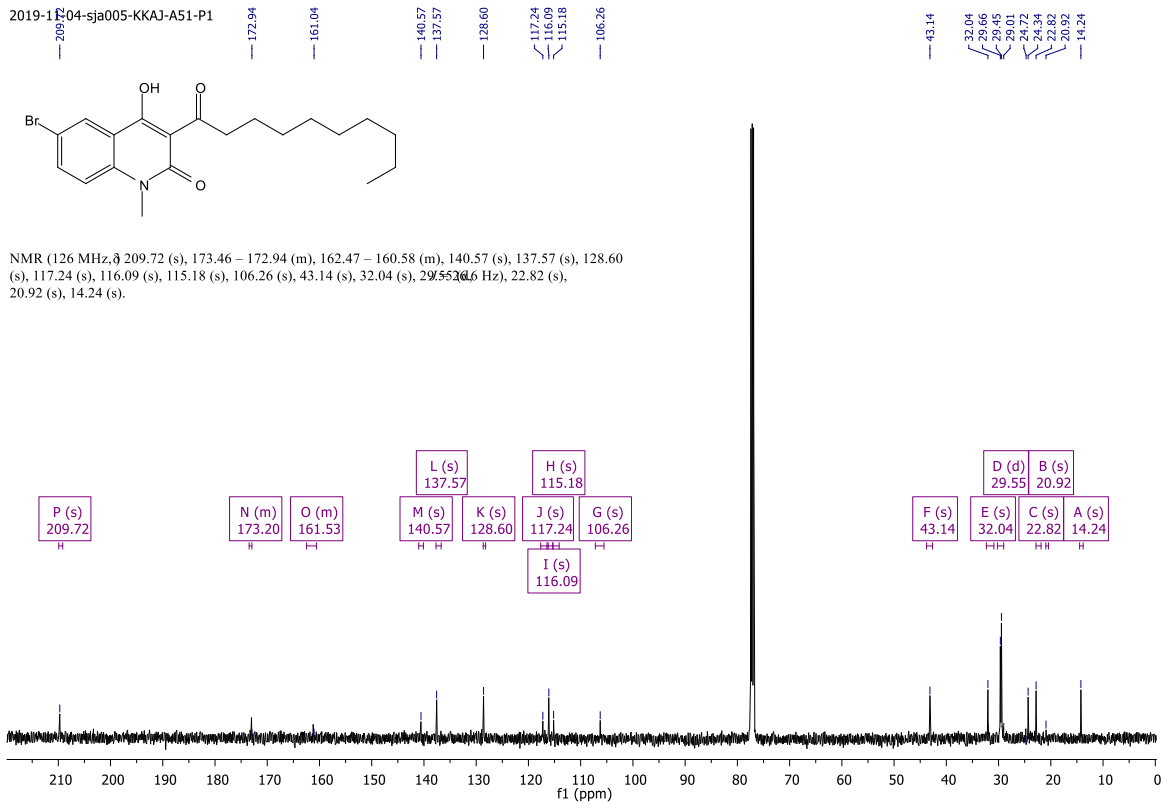


m/z calcd for $C_{20}H_{26}ClNO_3$- [M-H]$^-$ 362.1523

6-Bromo-3-decanoyl-4-hydroxy-1-methylquinolin-2(1H)-one (**3j**)



NMR (500 MHz, ∂ 8.40 (d, *J* = 15.5 Hz), 7.81 (d, *J* = 8.6 Hz), 7.27 – 7.20 (m), 3.69 (d, *J* = 15.0 Hz), 3.33 (d, *J* = 7.4 Hz), 1.78 (d, *J* = 5.8 Hz), 1.51 – 1.25 (m), 0.94 (d, *J* = 6.8 Hz).



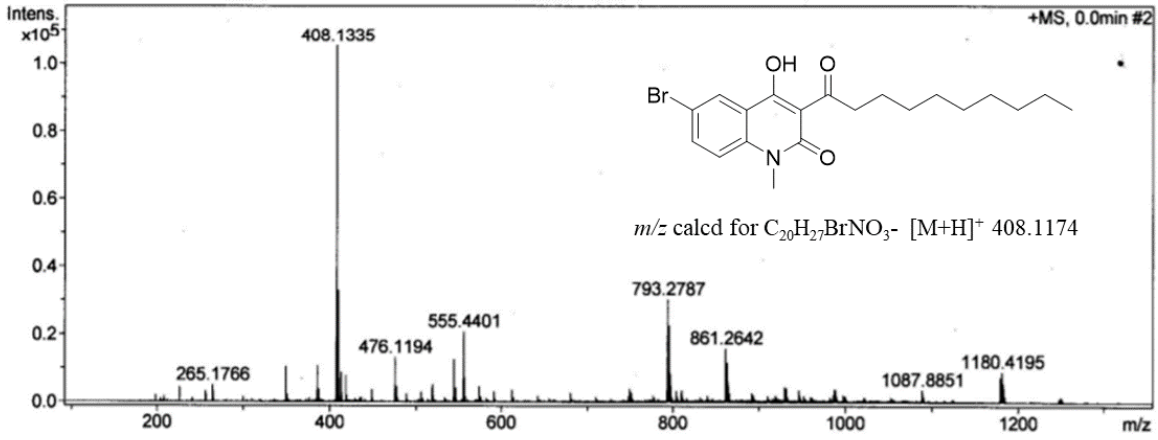
NMR (126 MHz, ∂ 209.72 (s), 173.46 – 172.94 (m), 162.47 – 160.58 (m), 140.57 (s), 137.57 (s), 128.60 (s), 117.24 (s), 116.09 (s), 115.18 (s), 106.26 (s), 43.14 (s), 32.04 (s), 29.55 (d, *J* = 16.6 Hz), 22.82 (s), 20.92 (s), 14.24 (s).

High resolution report

		Acquisition Date	6/1/2020 3:26:20 PM
Analysis Name	D:\Data\customer\TK 3j.d		
Method	NaFormate_pos.m	Operator	Sutichai Ext: 3560
Sample Name	TK 3j	Instrument	micrOTOF Bruker
		Calibrate by	Sodium Formate

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active			Set Dry Heater	180 ?C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



m/z calcd for $C_{20}H_{27}BrNO_3$- $[M+H]^+$ 408.1174

2. Antimicrobial Activity and Structure-Activity Relationship

2.1 The resazurin microdilution assay to determine MICs of the synthesized quinolones against *S. aureus*.

The synthesized compounds (**3a**, **3b**, **3c**, **3d**, **3e**, **3f**, **3g**, **3h**, **3i**, **3j**, and **4d**) were incubated with *S. aureus* (ATCC 6538P) at the final compound concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. The 4% DMSO in MHB, 4% DMSO in MHB plus *S. aureus*, and ciprofloxacin were used as experimental controls. MIC was defined as a minimum concentration of agents that inhibited the growth of bacteria observed by remaining blue color of resazurin.

The MICs of compound **3i** (*) and **3j** (**) were unclear. However, the **3i** and **3j** seemed to display partial inhibitory effect against *S. aureus* at the concentration between 125-1000 µg/mL and 125-500 µg/mL, respectively.

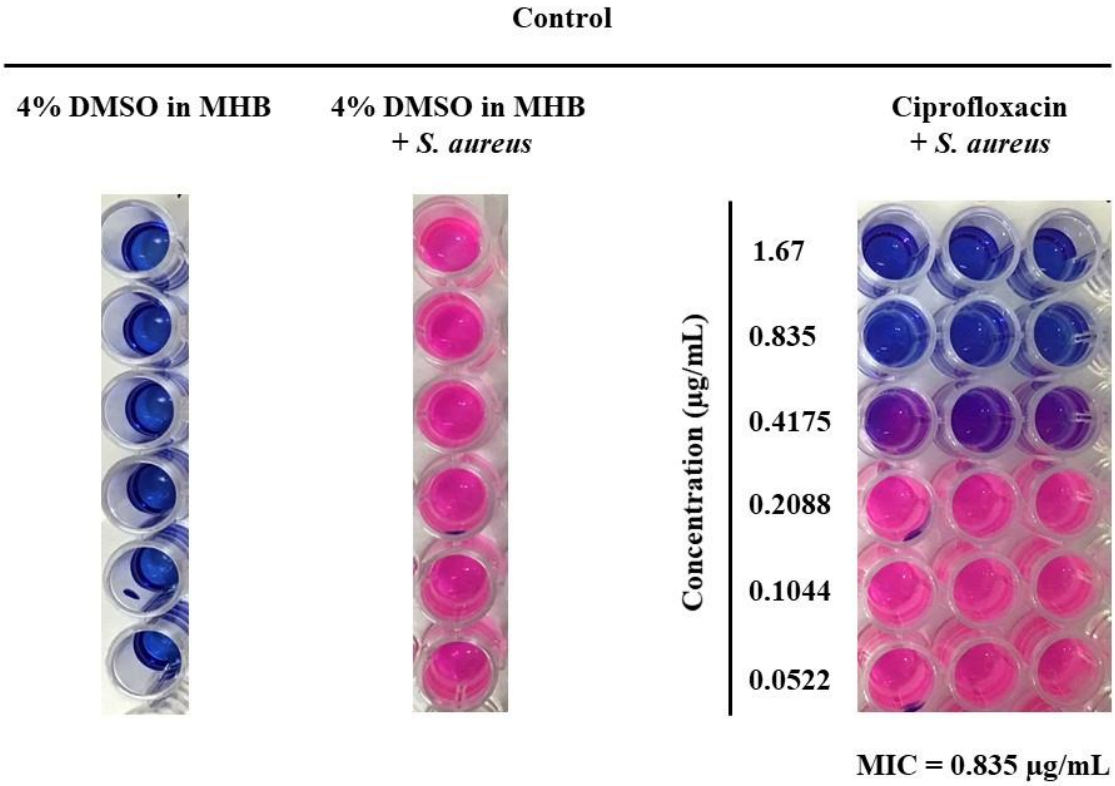


Figure S1. MIC determination of Ciprofloxacin against *S. aureus.*

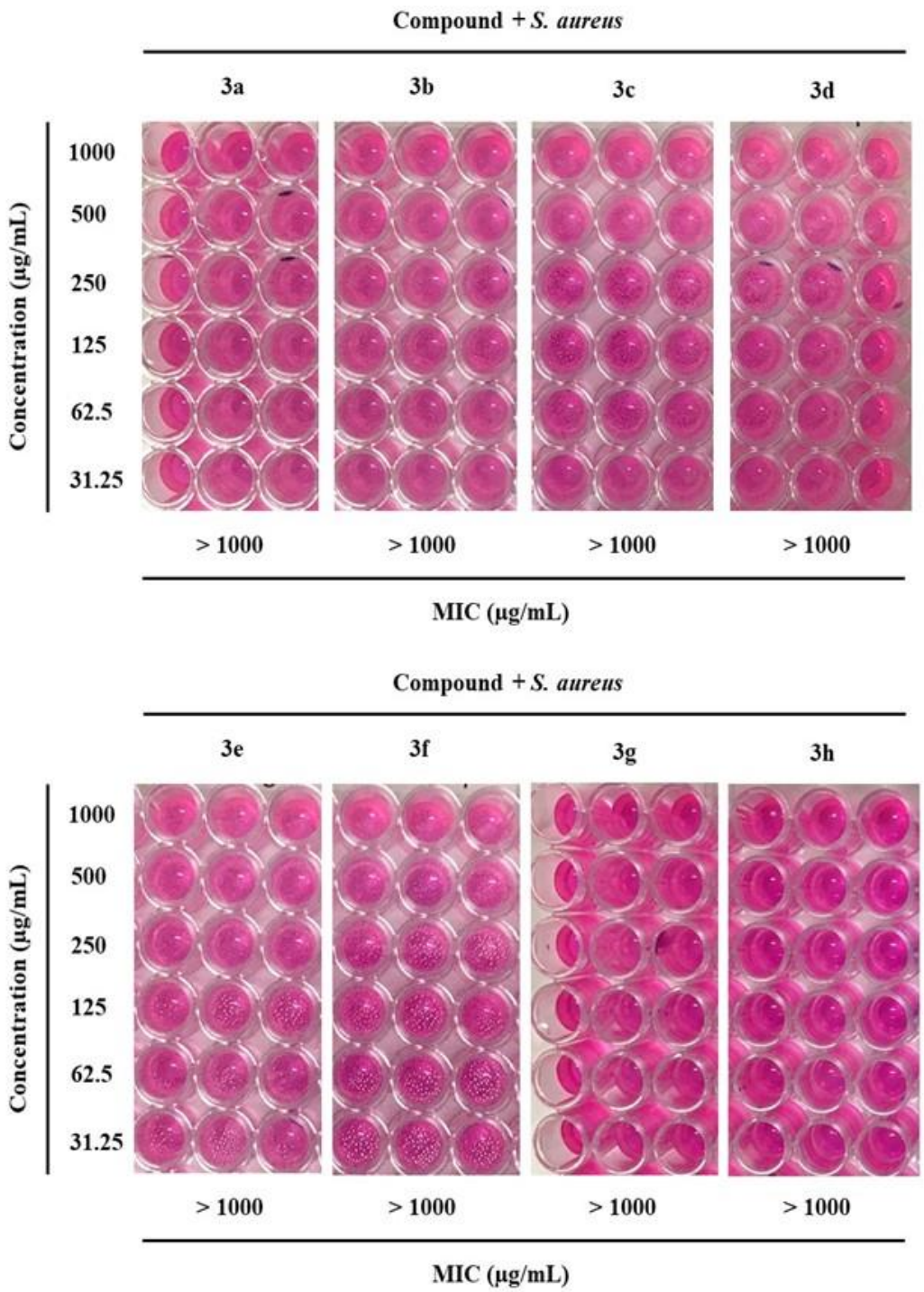


Figure S2. MIC determination of **3a**–**3h** against *S. aureus.*

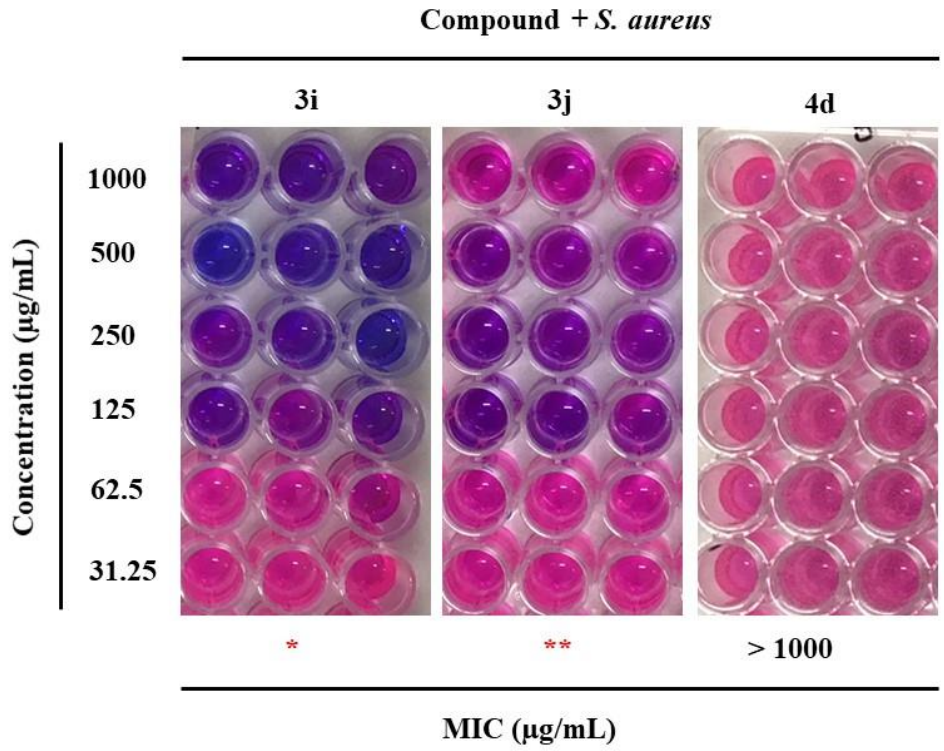


Figure S3. MIC determination of **3i**, **3j** and **4d** against *S. aureus.*

2.2 The MBC results of 3i and 3j against *S. aureus*.

Although 3i and 3j showed inhibitory activity against *S. aureus*, both compounds failed to kill the bacteria confirmed by the presence of bacterial colonies on nutrient agar plates.

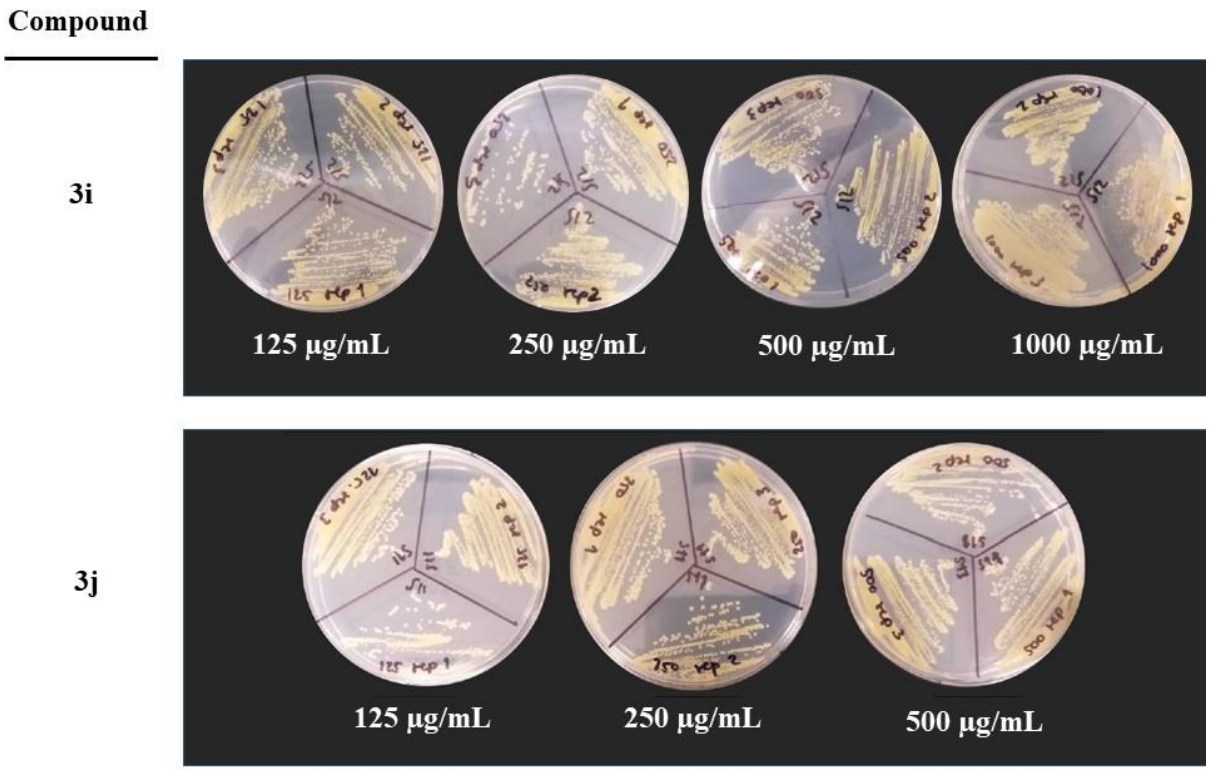


Figure S4. MBC determination of **3i** and **3j** against *S. aureus.*

2.3 The resazurin microdilution assay to determine MICs of the synthesized quinolones against *E. coli*.

The synthesized compounds (**3a**, **3b**, **3c**, **3d**, **3e**, **3f**, **3g**, **3h**, **3i**, **3j**, and **4d**) were incubated with *E. coli* (ATCC 25922) at the final compound concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. The 4% DMSO in MHB, 4% DMSO in MHB plus *E. coli*, and ciprofloxacin were used as experimental controls. MIC was defined as a minimum concentration of agents that inhibited the growth of bacteria observed by remaining blue color of resazurin.

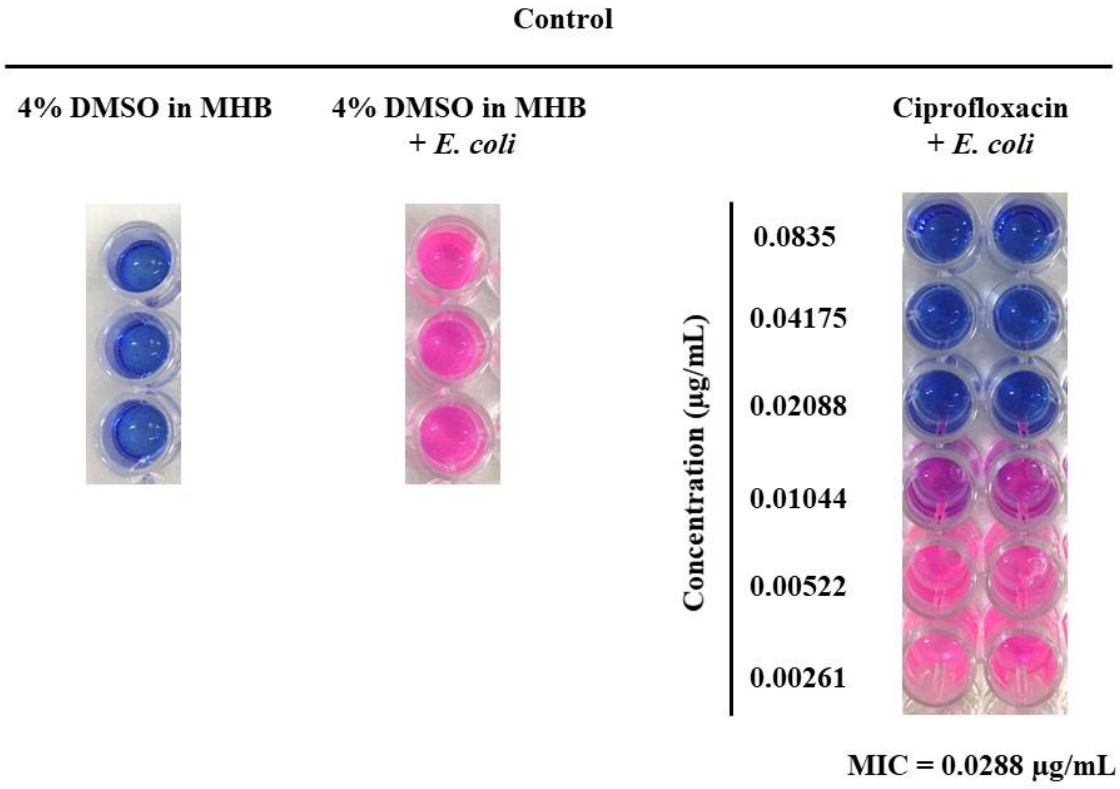


Figure S5. MIC determination of Ciprofloxacin against *E. coli*.

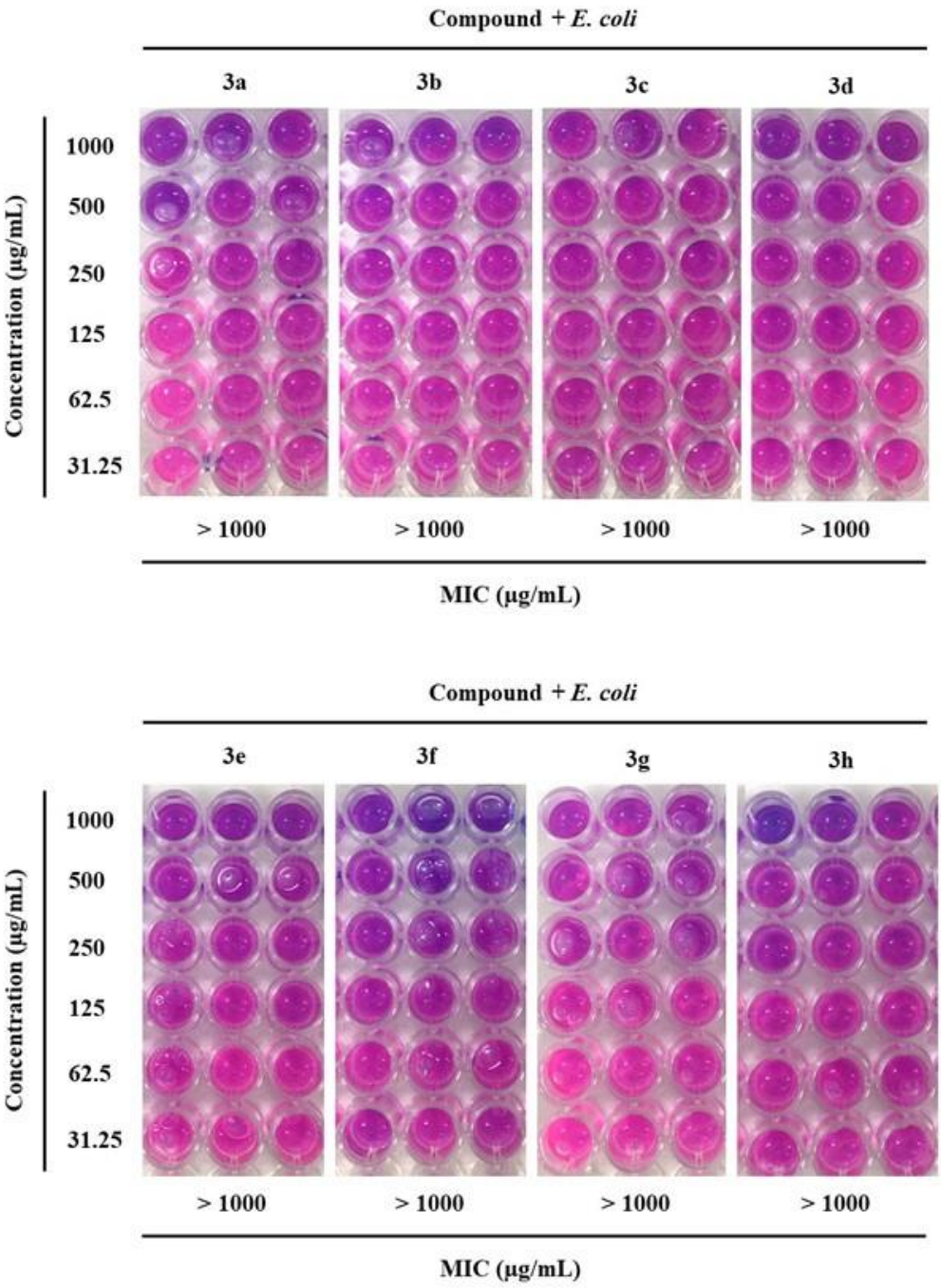


Figure S6. MIC determination of **3a**–**3h** Ciprofloxacin against *E. coli.*

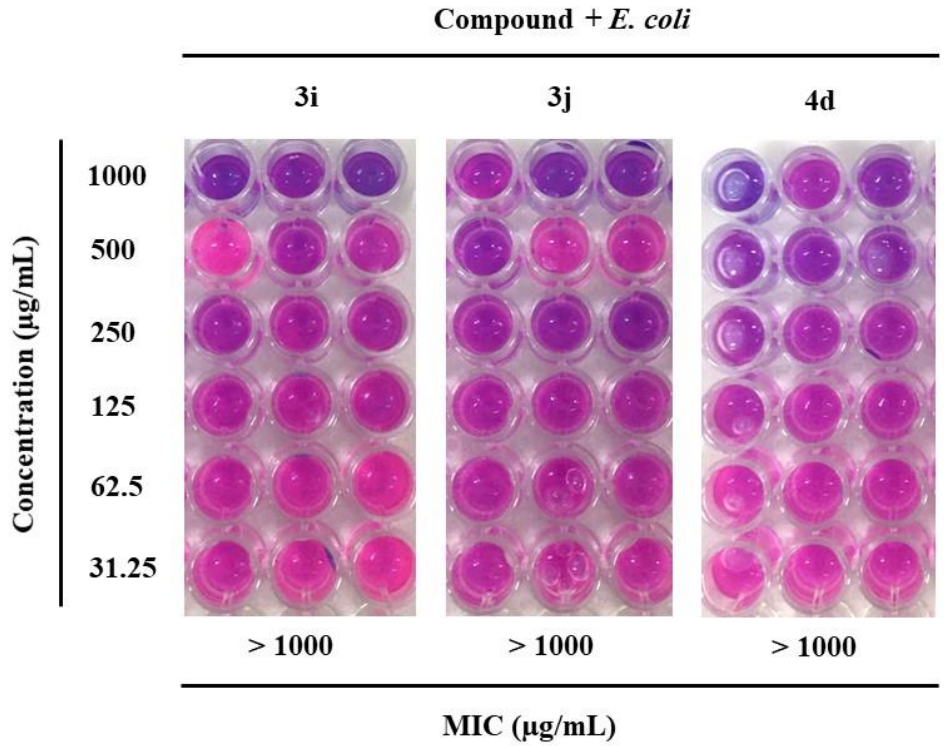


Figure S7. MIC determination of **3i**, **3j** and **4d** against *E. coli.*