

Straightforward conversion of decoquinatate into inexpensive tractable new derivatives with significant antimalarial activities

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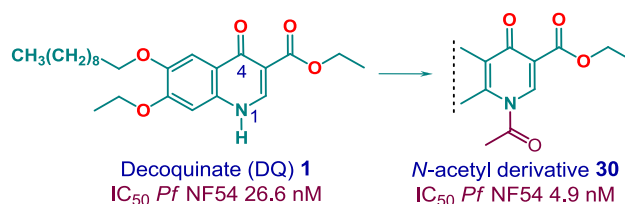
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Abstract: As part of a programme aimed at identifying rational new triple drug combinations for treatment of malaria, tuberculosis and toxoplasmosis, we have selected quinolones as one component, given that selected examples exhibit exceptionally good activities against the causative pathogens of the foregoing diseases. The quinolone decoquinatate (DQ), an old and inexpensive coccidiostat, displays anti-malarial activity *in vitro* against *Plasmodium falciparum* (*Pf*). However, because of its exceedingly poor solubility in water or organic solvents, development of DQ as a drug is problematical. We have therefore converted DQ in straightforward fashion into tractable new derivatives that display good activities *in vitro* against chloroquine-sensitive NF54 and multidrug-resistant K1 and W2 *Pf*; and relatively low toxicities against human fibroblast cells. The most active compound, the *N*-acetyl derivative **30**, is 5-fold more active than DQ against NF54 and K1 and equipotent with DQ against W2. It possesses an activity profile against all strains comparable with that of the artemisinin derivative artesunate. Overall, this compound and the other accessible and active derivatives serve as an attractive template for development of new and economic lead quinolones.

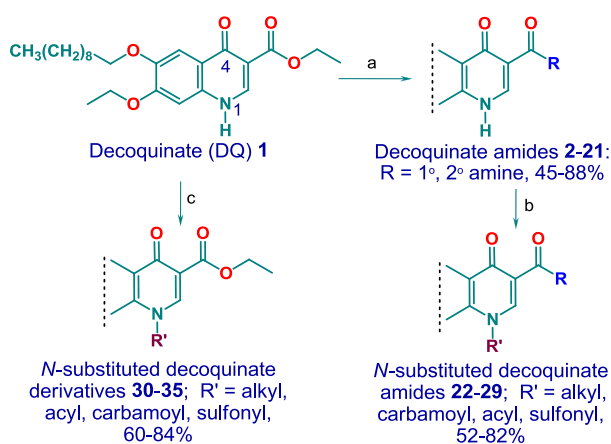
Graphical Abstract



Under a programme designed to develop new triple drug combinations for the treatment of malaria, tuberculosis, and toxoplasmosis,¹ we are preparing and evaluating efficacies of compound sets based on combinations of oxidant and redox drugs² coupled with a third partner with a different mode of action. In the case of malaria, the need to develop new drug combinations is particularly pressing.³ Chemotherapy coupled with vector control and inculcation of public awareness has reduced mortality due to malaria by over 66% since 2000.⁴ However, the emergence of malaria parasites resistant to the current clinically-used artemisinin derivatives⁵ mandates the urgent development of newer artemisinin derivatives. Such derivatives must be incapable of providing the active metabolite dihydroartemisinin (DHA) common to the current clinical artemisinins, and should be rationally combined with the redox drug and a third combination partner to counter resistance and inhibit spread of resistant phenotypes by blocking transmission.⁶ The third partner is logically constructed about the 4(1*H*)-quinolone scaffold. In addition to being used clinically against a variety of infectious diseases including tuberculosis,⁷ certain quinolones have acquired lead status for development of drugs for treatment of toxoplasmosis^{8,9} and malaria^{10,11,12} respectively.

Our attention is drawn to decoquinate (DQ, **1**) that has been used for many years in veterinary medicine largely co-administered with poultry feed for treatment of coccidiosis wherein it displays negligible toxicity.^{13,14} It is also used against other apicomplexan infections in animals¹⁵ and is highly active in a murine model against *Toxoplasma gondii*.¹⁶ Activity of DQ against malaria including *P. berghei*¹⁷ in mice and *P. cynomolgi*¹⁸ in monkeys has been known for some time. DQ is active *in vitro* against the liver, blood and gametocyte parasite stages of *P. falciparum* (*Pf*).^{19,20} Although such multistage activity matches the profile required for blocking transmission of the malaria parasite,⁶ DQ like other 4(1*H*)-quinolones including the advanced lead compound ELQ 300,¹⁰⁻¹² is highly lipophilic with a Log *P* of 8.19,²¹ has exceedingly low solubility in water (0.06 mg L⁻¹)¹⁴ and other solvents,¹² and has low bioavailability.^{19,22} The decisive benefit of DQ is its low cost, ≤\$10 per kg,²³ which renders it substantially cheaper than the other 4(1*H*)-quinolones proposed for further development as antimalarial drugs. However, the poor solubility markedly complicates screening both *in vitro* and *in vivo* and hampers evaluation of pharmacokinetic parameters, thereby restricting its further development as a drug. Because of this, resort has been made to preparation of nanoparticle formulations that appear to enhance efficacy against malaria in an animal model.²² Our approach consistent with the overall aims of our work is to convert DQ into accessible and more tractable derivatives that in the first instance are amenable to screening *in vitro*. We report here the preparation and preliminary *in vitro* antimalarial activity and cytotoxicity of such derivatives.

Chemistry. The proposed steps involve conversion of the ethyl ester into the less readily metabolized amide²⁴ and replacement of H-1 largely by acyl, carbamoyl or sulfonyl groups, operations that should improve physicochemical properties associated with enhanced polarity (Scheme 1). *N*-alkyl derivatives are also prepared based on structure-activity considerations vis-à-vis the *N*-acyl and *N*-sulfonyl derivatives.



a. 1° or 2° amine (5 equiv.), DBU (0.9 equiv.), CHCl₃, reflux 24-72 h; b. DQ amide, alkyl, acyl or sulfonyl halide (5 equiv.), DBU (0.6 equiv.), CHCl₃, reflux, 15 h; c. DQ, as for b.

Scheme 1. Conversion of decoquinate (DQ) into DQ amide derivatives.

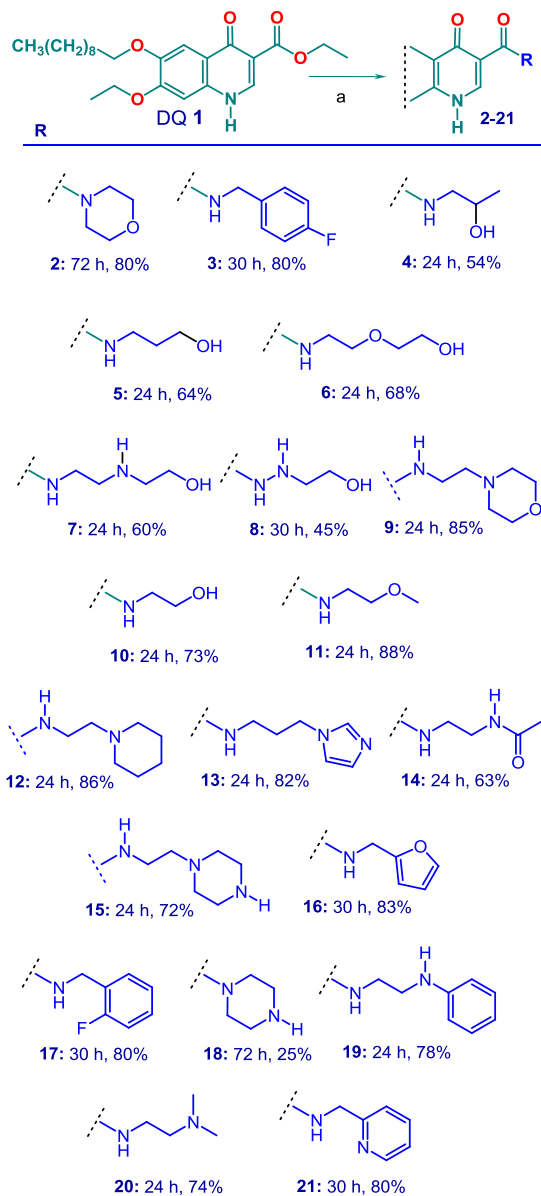


Figure 1. Amides obtained from direct aminolysis of decoquinolate **1** with primary and secondary amines in chloroform according to method a, Scheme 1; reaction time (h), overall yields given.

For the ester aminolysis (step a, Scheme 1), DQ is acidic with a pKa of 9.81,21 and thus a basic amine nucleophile under standard aminolysis conditions²⁵ is likely to undergo competitive equilibrating proton transfer with H-1 of the 4(1*H*)-quinolone (*cf.* Figure 2 below). Whilst this problem may be countered by the use of Lewis acidic reagents,²⁶ base-catalyzed aminolysis with an amine and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)²⁷ attracts because of its economy. Although the reactions may be conducted with the neat reactants and reagents, the most expeditious involved the heating of DQ and excess of primary or secondary amine with DBU in a solvent. We use chloroform here because of lack of solubility of DQ in other solvents, although it is emphasized that our initial concern is to obtain the new derivatives in the first place and then to optimize preparation for hit compounds using industrially acceptable solvents. Amides **2-21** (Fig 1) were obtained in good yields. Characterization data for the compounds are given in the Supplementary Material.

For functionalization at N-1 according to route b, Scheme 1, the decoquinolate morpholino amide **2** was selected. Although the pKa of amide **2** with respect to deprotonation at N-1 is unknown, it is likely this will be efficiently deprotonated by DBU in aprotic solvents to give the conjugate base **2⁻** that as an ambident anion may react through N-1 or O-4. In the event, the reactions with various acyl and sulfonyl chlorides, and alkyl halide proceeded smoothly through N-1 to give the products

22-29 in good yields (Figure 2). Whereas the IR spectrum of compound **2** shows an absorption at 3162 cm^{-1} due to the N-H stretching vibration in the quinolone nucleus, the peak is absent in derivatives **22-29**, and a signal due to C-4 bearing an oxo group is evident at *ca.* 170 ppm in the ^{13}C NMR spectra.

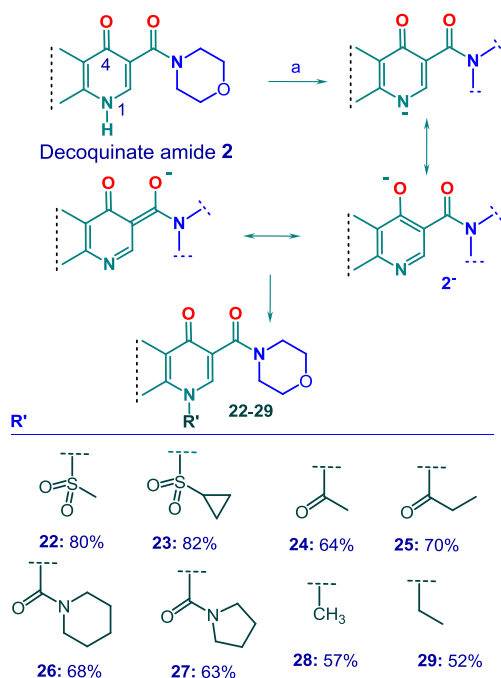


Figure 2. *N*-Substituted amide derivatives obtained from decoquinate amide **2** by treatment with DBU in the presence of sulfonyl, acyl and alkyl halides in chloroform with a reaction time of 15 h according to method b, Scheme 1; overall yields given.

Decoquinate **1** was also functionalized at N-1 according to Scheme 1 route c to give the *N*-substituted decoquinate derivatives **30-35** in good yields (Figure 3). Data for derivatives **30**, **31** and **33** appear in the Supplementary Material. As above, reaction through N-1 occurs in all cases. This is evident from the ^{13}C NMR spectra, which contain signals due to C-4 bearing an oxo group at *ca.* 170 ppm and the ester carbonyl at 160-165 ppm.

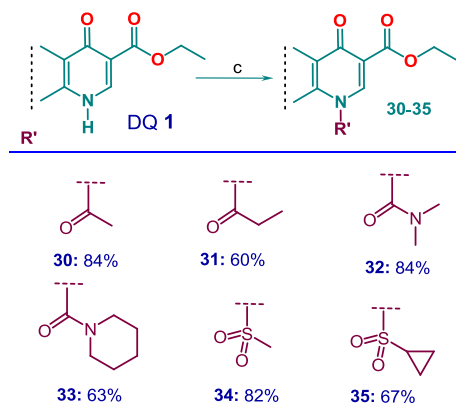


Figure 3. *N*-Substituted decoquinate derivatives obtained from decoquinate **1** and DBU in the presence of acyl, carbamoyl, and sulfonyl chlorides in chloroform, with a reaction time of 15 h according to method c, Scheme 1; overall yields given.

In summary, decoquinate **1** is converted readily via aminolysis of the ethyl ester with primary and secondary amines in the presence of DBU in chloroform under reflux into the corresponding secondary and tertiary amides. Decoquinate **1** and the morpholino amide **2** under the foregoing conditions react with acyl and sulfonyl halides and alkyl halides through N-1 to provide new *N*-substituted derivatives in good yields.

Antimalarial activities: Decoquinatone and the new derivatives were screened *in vitro* for antimalarial activity with the SYBR Green I-based fluorescence assay²⁸ against *Pf* NF54 asexual blood stage parasites to determine the % inhibition of proliferation at initial compound concentrations of 1 and 5 μ M (Figure S1, Supplementary Material). Compounds showing the highest activity (>50% inhibition at 5 μ M and >70% inhibition at 1 μ M) were submitted to a second dual point assay at 100 nM and 500 nM (Figure S2, Supplementary Material). Compounds showing near complete inhibition of parasite proliferation were carried forward for IC₅₀ determination against chloroquine (CQ)-sensitive *Pf* NF54 and multidrug resistant K1 and W2. CQ, the artemisinins artemether and artesunate, and decoquinatone **1** were used as controls. Cytotoxicity of the compounds was also evaluated with the sulforhodamine B (SRB) assay on the WI-38 cell line²⁹ using emetine as reference standard. IC₅₀ values are summarized in Table 1. Of all compounds, only five, namely the decoquinatone amides **3** and **6** (Fig. 1) and the N-substituted decoquinatone derivatives **30**, **31** and **33** (Fig. 3) showed near complete inhibition at 1 and 5 μ M regardless of the parasite strain (Fig. S2, Supplementary Material). Decoquinatone **1** and the derivatives showed no appreciable toxicity to mammalian WI-38 cells; their IC₅₀ values are all substantially greater than the IC₅₀ of 0.05 μ M of emetine. The derivatives are thus selective in their antimalarial action as evidenced by the SI values \geq 400 (Table 1). The amides **3** and **6** (Fig. 1) are as active as DQ, but less active than both artemether and artesunate against all three parasite strains. The N-acyl derivative **30** (Fig. 3) on the other hand is equipotent with the two clinically used artemisinins against all strains, and is 5-fold more potent than DQ against *Pf* NF54 and K1, whilst being equipotent with DQ against W2. In general according to the resistance indices (Table 1), the other compounds are less active against K1 with respect to drug sensitive NF54, and essentially equipotent with DQ against W2. Although derivative **30** shows slight differences in activities between the three parasite strains, the selectivity indices indicate it is more selective than DQ in its antimalarial activity. Thus the balance between strain susceptibility, efficacy and safety favors derivative **30** over the parent decoquinatone. It is to be noted that with the exception of compound **3**, cLogP values indicate that all compounds are less lipophilic than decoquinatone, and were able to be screened by preparing solutions in DMSO.

Table 1. Calculated LogP and *in vitro* biological data of selected derivatives.

Compound	cLog P ^a	Antimalarial activity IC ₅₀ ±SEM, nM ^b			Resistance Index		Cytotoxicity	Selectivity
		NF54	K1	W2	RI ^c	RI ^d	IC ₅₀ , μ M	Index
							WI-38 ^e	SI ^f
CQ	nd	10±3	154.73±14.96	233.18±49.49	15.4	23.3	nd	nd
Artemether	nd	5.77±1.33	7.26±2.34	4.5±0.78	1.3	0.8	nd	nd
Artesunate	nd	7.88±1.35	8.97±2.26	6.77±1.13	1.4	0.8	>100	>12690
DQ 1	8.2	26.6±1.4	64.9±8.8	15.4±3.4	2.4	0.6	>100	>3759
3	8.0	33.1±2.5	63.1±9.8	8.6±4.3	1.9	0.2	>100	>3021
6	6.0	40.4±1.3	64.8±7.2	18.5±4.5	1.6	0.5	43.9	1087
30	5.9	4.9±0.9	12.0±3.5	8.5±6.3	2.5	1.7	>100	>20408
31	6.5	15.4±0.5	48.9±9.9	11.5±1.2	3.2	0.8	>100	>6494
33	6.5	18.3±4.8	89.1±6.0	28.2±11.7	4.9	1.5	8.5	465
Emetine	nd	nd	nd	nd	nd	nd	0.05	nd

^a cLogP values calculated with ACD/ChemSketch Version 14.02; ^b data from at least three independent biological repeats, each performed in triplicate; CQ = chloroquine; nd = not determined; ^c resistance index (RI) = IC₅₀ K1/IC₅₀ NF54; ^d resistance index (RI) = IC₅₀ W2/IC₅₀ NF54; ^e WI-38 cell line of normal human fetal lung fibroblast (HFLF); ^f selectivity index (SI) = IC₅₀ WI-38-HFLF/IC₅₀ NF54.

To conclude, the derivative **30** that has an *in vitro* activity profile comparable to that of artesunate coupled with a favorable incipient toxicity profile, and others of Table 1 represent easily accessible and economic hit compounds to guide the preparation of new and potent derivatives. As foreshadowed in the introduction, activities of these and related compounds against *Mycobacterium tuberculosis* and *Toxoplasma gondii* have also been recorded, and will be reported elsewhere.

Acknowledgments

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Supplementary Material

Preparation of decoquinone derivatives, spectroscopic data for all derivatives; illustration of ^1H , ^{13}C -NMR and mass spectra for derivatives **2**, **3**, **6**, **24**, **30**, **31**, **33**; details of *in vitro* malaria and cytotoxicity screening assays; Figs.S1, S2.

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Supporting Information

Experimental Section

General procedures

The amines, acyl, sulfonyl, carbamoyl and alkyl halides in this study were purchased from either Sigma Aldrich or AKSci and were used as supplied. NMR spectra were obtained on a 600 MHz Bruker Avance™ III spectrometer as solutions in deuterated solvents (CDCl₃, or DMSO-*d*₆). All chemical shifts (δ) are reported in parts per million (ppm) values. ¹H chemical shifts are reported downfield of tetramethylsilane (TMS), and were internally referenced to the residual proton in CDCl₃ (7.26 ppm), or DMSO-*d*₆ (2.50 ppm). ¹³C chemical shifts were internally referenced to resonances in CDCl₃ (δ 77.16 ppm), or DMSO-*d*₆ (δ 39.51 ppm). Peak multiplicities are abbreviated as follows: s (singlet), d (doublet), dd(doublet of doublet), t (triplet), q (quartet), p (pentet), and m (multiplet). Coupling constant (*J*) are reported in Hz. NMR data were analyzed using MestReNova Software, version 5.3.2-4936. Exact mass measurements were recorded on Bruker MicroTOF Q II mass spectrometer with its APCI source set at 300 °C coupled with Bruker Compass Data analysis 4.0 software. A full scan, ranging between 50–1500 *m/z*, was generated at a capillary voltage of 4500 V, end plate offset voltage of -500 V, and a collision cell RF voltage of 100 V. Infrared (IR) spectra were recorded on a Bruker Alpha-P FTIR instrument. Melting points (mp) were determined with a Büchi melting point B-545 instrument and were uncorrected. Thin layer chromatography (TLC) was performed using silica gel plates (60F₂₅₄) (Merck) and components were visualized by ultraviolet light (254 nm). Silica gel 230-400 (particle size 40-63 μ m) mesh was used for column chromatography. Calculated LogP (cLogP) values were generated using ACD/ChemSketch version 14.02.

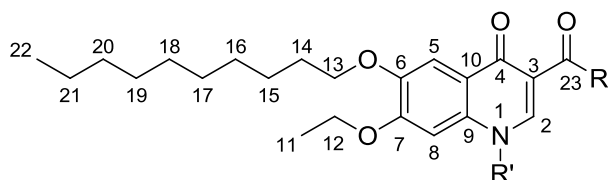
Syntheses

(a) To a 100 mL two neck flask containing decoquinatone **1** (1 g, 2.34 mmol) and DBU (320 μ L, 0.33 g, 2.1 mmol, 0.9 eq.) in chloroform (25 mL) was added the primary or secondary amine (5 eq.). The resulting mixture was stirred under reflux for 24-72 h. Progress of the reactions was monitored by tlc. Upon completion, the reaction mixture was concentrated *in vacuo*. The concentrated residue was taken into chloroform (20 mL) and washed with distilled water (4 x 20 mL). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product, which was subjected to flash column chromatography on silica gel. The product was eluted with a mixture of dichloromethane/methanol (10:1, v/v). The product was recrystallized from ethyl acetate and air dried. The amide derivatives **2-21** were thereby obtained in good yields using this procedure. Data for derivatives **2**, **3** and **6** are given below.

(b) To a 100 mL two neck flask containing DQ (0.5 g 1.22 mmol, 1 eq), DBU (100 μ L 0.7 mmol, 0.6 eq.) in chloroform (15 mL) was added the appropriate acyl or sulfonyl halide, or alkyl halide (5 eq). The mixture was stirred under reflux for 15 h, and then washed with aqueous saturated NH_4Cl solution (3 x 20 mL). The organic layer was dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness to leave the crude product, which was submitted flash column chromatography on silica gel. Elution with dichloromethane/methanol (20:1, v/v) gave the product mixture, that was evaporated under reduced pressure to leave the crude product that was recrystallized from acetone and air dried overnight. The *N*-substituted derivatives **30-35** were obtained in 50-80% yields. Data for derivatives **30**, **31** and **33** are given below.

The foregoing procedure was applied to the morpholino amide **1** (0.35 g, 0.8 mmol, 1 eq.) to give the *N*-substituted derivatives **22-29**.

DQ and key derivatives in this study have the general structure below.



Compd	R	R'	Compd	R	R'
DQ 1		H	24		
2		H	30		
3		H	31		
6		H	33		

6-Decoxy-7-ethoxy-3-(morpholin-4-ylcarbonyl)quinolin-4(1*H*)-one **2**

White powder; yield: 80%, 0.87 g; m.p.: 182-184 °C; HPLC: 30% ACN/ 70% 0.1% H₃PO₄ gradient, >93% pure, rt = 5 min; APCI-HRMS (*m/z*): 459.2793 [M+H]⁺ (calculated for C₂₆H₃₉N₂O₅: 459.2853); IR (ATR) ν_{\max} cm⁻¹: 3167, 3135, 2988, 2916, 1633, 1604, 1566, 1539, 1000-574; ¹H NMR (600 MHz, CDCl₃) δ (ppm): 11.96 (s, 1H, H-1), 7.67 (s, 1H, H-2), 7.52 (s, 1H, H-5), 6.70 (s, 1H, H-8), 4.01 – 3.92 (m, 4H, H-12, H-13), 3.76 – 3.68 (m, 8H, H-2',-H-5'), 1.81 (m, *J* = 7.0 Hz, 2H, H-14), 1.45 –

1.35 (m, 4H, H-15, H-16), 1.37 – 1.18 (m, 13H, H-11, H-17,-H-21), 0.85 (t, $J = 6.9$ Hz, 3H, H-22); ^{13}C NMR (151 MHz, CDCl_3) δ (ppm): 172.75 (C-4), 167.79 (C-23), 153.61 (C-6), 147.80 (C-7), 138.63 (C-2), 135.00 (C-9), 120.22 (C-10), 115.47 (C-3), 105.57 (C-5), 99.84 (C-8), 69.17 (C-13) , 67.23 (C-3', C-4') , 64.61 (C-12), 48.08 (C-2', C-5'), 31.88 (C-14), 29.54 (C-15, C-16), 29.35 (C-17, C-18), 28.97 (C-19), 25.93 (C-20), 22.66 (C-21), 14.45 (C-11), 14.10 (C-22).

6-Decoxy-7-ethoxy-N-(4-fluorobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide 3

White powder; yield: 80%, 0.82 g; m.p.: 202-203 °C; HPLC: 30% ACN/ 70% 0.1% H_3PO_4 gradient, >92% pure, rt = 16.5 min; APCI-HRMS (m/z): 497.2734 $[\text{M}+\text{H}]^+$ (calculated for $\text{C}_{29}\text{H}_{38}\text{FN}_2\text{O}_4$: 497.2810); IR (ATR) ν_{max} cm^{-1} : 3167, 3135, 2988, 2916, 1648, 1627, 1614, 1580, 1531,1498; ^1H NMR (600 MHz, CDCl_3) δ (ppm): 11.85 (s, 1H, H-1), 11.11 (t, $J = 5.8$ Hz, 1H, H-1'), 8.67 (s, 1H, H-2), 7.67 (s, 1H, H-5), 7.28 (dd, $J = 8.4, 5.3$ Hz, 2H, H-5', H-7'), 6.94 (t, $J = 8.5$ Hz, 2H, H-4', H-8'), 6.81 (s, 1H, H-8), 4.66 (d, $J = 5.8$ Hz, 2H, H-2'), 4.05 (t, $J = 6.9$ Hz, 2H, H-13), 3.92 (t, $J = 6.9$ Hz, 2H, H-12), 1.84 (q, $J = 7.4, 6.7$ Hz, 4H, H-14,-15), 1.47 – 1.18 (m, 15H, H-11, H-16,-H-21,), 0.92 – 0.82 (m, 3H, H-22); ^{13}C NMR (151 MHz, CDCl_3) δ (ppm): 176.11 (C-4), 166.87 (C-23), 162.83 (C-6'), 153.82 (C-6,) , 148.18 (C-7), 141.18 (C-2), 134.77 (C-9), 133.94 (C-3'), 128.93 (C-4', C-8'), 120.70 (C-10), 115.51 (C-5', C-7'), 110.05 (C-3), 105.92 (C-5), 99.85 (C-8), 69.26 (C-13), 64.70 (C-12) , 42.72 (C-2'), 31.87 (C-14), 29.53 (C-15, C-16), 29.31 (C-17, C-18), 28.85 (C-19), 25.90 (C-20) , 22.66 (C-21), 14.38 (C-11) , 14.10 (C-22).

6-Decoxy-7-ethoxy-N-[2-(2-hydroxyethoxy)ethyl]-4-oxo-1,4-dihydroquinoline-3-carboxamide 6

White powder; yield: 68%, 0.68 g; m.p.: 133-136 °C; HPLC: 30% ACN/ 70% 0.1% H₃PO₄ gradient, > 96% pure, rt= 8.7 min; APCI-HRMS (*m/z*): 477.2911 [M+H]⁺ (calculated for C₂₆H₄₁N₂O₆: 477.2959); IR (ATR) ν_{\max} cm⁻¹: 3336, 3178, 3135, 3085, 2922, 2849, 1639, 1581, 1561, 1523, 1499; ¹H NMR (600 MHz, CDCl₃) δ (ppm): 11.99 (s, 1H, H-1), 10.73 (t, *J* = 5.3 Hz, 1H, H-1'), 8.64 (s, 1H, H-2), 7.39 (s, 1H, H-5), 6.80 (s, 1H, H-8), 4.43 (s, 1H, H-7'), 4.01 (t, *J* = 7.0 Hz, 2H, H-13), 3.86 (t, *J* = 6.8 Hz, 2H, H-12), 3.83 – 3.78 (m, 2H, H-2'), 3.71 – 3.60 (m, 6H, H-3', H-5', H-6'), 1.76 (t, *J* = 6.9 Hz, 2H, H-14), 1.46 – 1.19 (m, 17H, H-11, H-15,-H-21), 0.87 (dt, *J* = 21.8, 7.3 Hz, 3H, H-22); ¹³C NMR (151 MHz, CDCl₃) δ (ppm): 175.76 (C-4), 166.62 (C-23), 153.66 (C-6), 147.86 (C-7), 141.49 (C-2), 134.73 (C-9), 120.40 (C-10), 110.13 (C-3), 105.39 (C-5), 99.88 (C-8), 72.38 (C-2'), 69.80 (C-6'), 68.54 (C-3', C-5'), 64.63 (C-13), 61.73 (C-12), 31.90 (C-14), 29.65 (C-15, C-16), 29.27 (C-17, C-18), 28.92 (C-19, C-20), 22.67 (C-21), 14.44 (C-11), 14.10 (C-22).

1-Acetyl-6-decoxy-7-ethoxy-3-(morpholin-4-ylcarbonyl) quinolin-4(1H)-one 24

White powder; yield: 64%, 0.24 g; m.p. 142-144 °C; HPLC: 30% ACN/ 70% 0.1% H₃PO₄ gradient, > 83% pure, rt = 12.6 min; APCI-HRMS (*m/z*): 501.2895 [M+H]⁺ (calculated for C₂₈H₄₁N₂O₆: 501.2959); IR (ATR) ν_{\max} cm⁻¹: 2988, 2916, 1633, 1604, 1566, 1539, 1000-574; ¹H NMR (600 MHz, CDCl₃) δ (ppm): 8.57 (s, 1H, H-2), 7.3 (s, 1H, H-5), 6.92 (s, 1H, H-8), 4.18 – 4.11 (m, 2H, H-13), 4.09 – 4.03 (m, 2H, H-12), 3.74 – 3.30 (m, *J* = 16.1 Hz, 8H, H-2',-H-5'), 2.43 (s, 3H, H-b), 1.88 – 1.73 (m, 2H, H-14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m,

11H, H-11, H-18,-H-21), 0.87 (dt, $J = 14.0, 7.3$ Hz, 3H, H-22); ^{13}C NMR (151 MHz, CDCl_3) δ (ppm) :172.83 (C-4), 167.24 (C-a), 165.61 (C-23), 153.25 (C-6), 147.71 (C-7), 144.17 (C-2), 140.83 (C-9), 134.49 (C-10), 120.37 (C-3), 107.01 (C-5), 99.63 (C-8), 69.24 (C-13), 67.32 (C-12), 66.79 (C-3'), 64.83 (C-4'), 47.94 (C-2'), 42.85 (C-5'), 31.86 (C-14), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 27.03 (C-b), 25.89 (C-20), 22.64 (C-21), 14.41 (C-11), 14.08 (C-22).

Ethyl-1-acetyl-6-decoxy-7-ethoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate 30

White powder; yield: 84%, 0.42 g; m.p.: 159-162 °C; HPLC: 30% ACN/ 70% 0.1% H_3PO_4 gradient, > 97% pure, $t_r = 8.2$ min; APCI-HRMS (m/z): 460.2656 $[\text{M}+\text{H}]^+$ (calculated for $\text{C}_{26}\text{H}_{38}\text{N}_2\text{O}_6$: 460.2694); IR (ATR) ν_{max} cm^{-1} : 2988, 2916, 1743, 1604, 1566, 1539, 1000-574; ^1H NMR (600 MHz, CDCl_3) δ (ppm): 9.15 (s, 1H, H-2), 7.41 (s, 1H, H-5), 7.12 (s, 1H, H-8), 4.47 – 4.30 (m, 2H, H-24), 4.31 – 4.21 (m, 2H, H-13), 4.13 – 4.03 (m, 2H, H-12), 2.51 (s, 3H, H-b), 1.88 – 1.73 (m, 2H, H-14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m, 14H, H-11, H-18, -21, -25), 0.87 (dt, $J = 14.0, 7.3$ Hz, 3H, H-22); ^{13}C NMR (151 MHz, CDCl_3) δ (ppm):172.83 (C-4), 165.61 (C-23), 154.46 (C-a), 154.06 (C-6), 150.44 (C-7), 149.37 (C-2), 130.86 (C-9), 117.55 (C-10), 113.16 (C-3), 108.72 (C-5), 100.60 (C-8), 69.15 (C-13), 68.11 (C-12), 64.19 (C-24), 31.87 (C-14), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 25.89 (C-20), 22.64 (C-21), 21.13 (C-b), 14.39 (C-25), 14.27 (C-11), 14.09 (C-22).

Ethyl-6-decoxy-7-ethoxy-4-oxo-1-propionyl-1,4-dihydroquinoline-3-carboxylate

31

White powder; yield: 60%, 0.30 g; m.p.: 160-162 °C; HPLC: 30% ACN, 70% 0.1% H₃PO₄ gradient, > 96% pure, rt= 9.3 min; APCI-HRMS (*m/z*): 474.2806 [M+H]⁺ (calculated for C₂₇H₄₀N₆: 474.2850); IR (ATR) ν_{\max} cm⁻¹: 2988, 2916, 1741, 1604, 1566, 1539, 1000-574; ¹H NMR (600 MHz, CDCl₃) δ (ppm): 9.15 (s, 1H, H-2), 7.41 (s, 1H, H-5), 7.12 (s, 1H, H-8), 4.47 – 4.30 (m, 2H, H-24), 4.31 – 4.21 (m, 2H, H-13), 4.13 – 4.03 (m, 2H, H-12), 2.91 – 2.81 (m, 2H, H-b), 1.88 – 1.73 (m, 2H, H-14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m, 17H, H-11, H-18, H-21, H-25, H-c), 0.87 (dt, *J* = 14.0, 7.3 Hz, 3H, H-22); ¹³C NMR (151 MHz, CDCl₃) δ (ppm): 172.83 (C-4), 165.61 (C-23), 154.40 (C-a), 154.06 (C-6), 150.44 (C-7), 149.37 (C-2), 130.86 (C-9), 117.55 (C-10), 113.16 (C-3), 108.72 (C-5), 100.60 (C-8), 69.15 (C-13), 68.11 (C-12), 64.19 (C-24), 38.68 (C-b), 31.87 (C-14), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 25.89 (C-20), 22.64 (C-21), 14.39 (C-c), 14.25 (C-25), 14.11 (C-11), 14.09 (C-22).

Ethyl-6-decoxy-7-ethoxy-4-oxo-1-(piperidin-1-ylcarbonyl)-1,4-dihydroquinoline-3-carboxylate 33

White powder; yield: 63.7%, 0.32 g; m.p.: 170-172 °C; HPLC: 30% ACN/ 70% 0.1% H₃PO₄ gradient, > 96% pure, rt= 7.3 min; APCI-MS (*m/z*): 529.3236 [M+H]⁺ (calculated for C₃₀H₄₅N₂O₆: 529.3272); IR (ATR) ν_{\max} cm⁻¹: 2988, 2916, 1733, 1604, 1566, 1539, 1000-574; ¹H NMR (600 MHz, CDCl₃) δ (ppm): 9.15 (s, 1H, H-2), 7.42 (s, 1H, H-5), 7.12 (s, 1H, H-8), 4.47 – 4.30 (m, 2H, H-24), 4.31 – 4.21 (m, 2H, H-13), 4.13 – 4.03 (m, 2H, H-12), 2.71 – 2.01 (m, 10H, H-b, -H-f), 1.88 – 1.73 (m, 2H, H-

14), 1.57 – 1.45 (m, 4H, H-15, H-16), 1.43 – 1.37 (m, 2H, H-17), 1.33 – 1.17 (m, 14H, H-11, H-18, H-21, H-25), 0.87 (dt, $J = 14.0, 7.3$ Hz, 3H, H-22).; ^{13}C NMR (151 MHz, CDCl_3) δ (ppm): 172.83 (C-4), 165.61 (C-23), 154.48 (C-a), 154.06 (C-6), 150.44 (C-7), 149.37 (C-2), 130.86 (C-9), 117.55 (C-10), 113.16 (C-3), 108.72 (C-5), 100.60 (C-8), 69.15 (C-13), 68.11 (C-12), 64.19 (C-24), 54.58 (C-b, C-f), 31.89 (C-c, C-e), 31.87 (C-14), 29.57 (C-d'), 29.52 (C-15), 29.49 (C-16), 29.31 (C-17), 29.28 (C-18), 28.88 (C-19), 25.89 (C-20), 22.64 (C-21), 14.25 (C-25), 14.11 (C-11), 14.09 (C-22).

***In vitro* antimalarial assays**

P. falciparum parasites were maintained at 37 °C in human erythrocytes (O^+) suspended in complete culture medium [RPMI 1640 medium (Sigma-Aldrich) supplemented with 25 mM HEPES (Sigma-Aldrich), 20 mM D-glucose (Sigma-Aldrich), 200 μM hypoxanthine (Sigma-Aldrich), 0.2% sodium bicarbonate, 24 $\mu\text{g}/\text{mL}$ Gentamycin (Sigma-Aldrich) and 0.5% AlbuMAX II] in a gaseous environment of 90% N_2 , 5% O_2 , and 5% CO_2 .¹ *In vitro* ring-stage intra-erythrocytic *P. falciparum* parasite cultures (Genotyped drug sensitive strains; W2 (Chloroquine, quinine, pyrimethamine and cycloguanil resistant), K1 (chloroquine, pyrimethamine, mefloquine and cycloguanil resistant) and NF54 (drug sensitive) (200 μL at 1% haematocrit, 1% parasitaemia) were treated with the DQ derivatives series.

The controls for this assay included chloroquine disulphate (1 μM , as positive control) and complete RPMI media (as negative control) and grown for 96 h at 37 °C under the 90% N_2 , 5% O_2 , and 5% CO_2 gas mixture in 96-well plates.

At the end of the 96 h growth period, equal volumes (100 μL each) of the *P. falciparum* parasite cultures were combined with SYBR Green I lysis buffer (0.2 $\mu\text{L}/\text{mL}$ 10 000 x SYBR Green I, Invitrogen; 20 mM Tris, pH 7.5; 5 mM EDTA; 0.008% (w/v) saponin; 0.08% (v/v) Triton X-100).

The samples were incubated at 37 °C for 1h after which the fluorescence was measured using a Fluoroskan Ascent FL microplate fluorometer (Thermo Scientific, excitation at 485 nm and emission at 538 nm). The 'background' fluorescence (i.e. that measured in the samples derived from chloroquine-treated iRBC samples in

which parasite proliferation was completely inhibited) was subtracted from the total fluorescence measured for each sample to provide a measure of parasite proliferation.

Data obtained were analysed in Excel 2010 and sigmoidal dose-response curves were plotted using GraphPad 6.0.

Decoquinatone **1** and the derivatives were screened at 5 μ M and 1 μ M against the NF54 strain of asexual *P. falciparum* parasites to determine their % inhibition. Compounds showing near complete inhibition at 5 μ M and 1 μ M (Figure S1), were further screened at 500 and 100 nM against NF54 parasites (Figure S2). Compounds were weighed (1 mg) and dissolved in 100% DMSO and made up to a 10 mM stock and stored at -20°C. Some compounds, however, did not completely dissolve (**2**, **8**, **12**, **14**, **22**, **31**, **32**, **33**, **34** and **35**) despite being incubated at 37 °C for >1h. These were dissolved at lower stock concentrations (<5 mM) and treated with 1xPBS. Therefore, prior to assay these compounds were vortexed thoroughly and the suspensions were immediately used to make up the working solutions in complete RPMI-1640 media.

Solutions/suspensions of compounds were thawed at room temperature (~1 h) prior to an assay to prepare working solutions. The final starting concentrations (5, 1 μ M and 500, 100 nM) were prepared in complete RPMI-1640 media and aliquoted in triplicate into a 96-well plate. The %DMSO in the starting concentration was 0.05% for the 5 μ M, 0.000125% for the 1 μ M, 0.05% for the 500 nM and 0.00000125% for the 100 nM drug concentrations; these concentrations were below the parasite inhibition threshold as previously determined in our laboratory. Following dual point assays, compounds were subjected to *in vitro* IC₅₀ determinations against asexual *P. falciparum* NF54, K1 and W2 parasites. The respective IC₅₀ values for compounds in the series showing activity <100 nM were determined (DQ **1** and compounds **3**, **6**, **30**, **31**, **33**). The same methods and conditions were applied for dissolving and storing the compounds as described for the dual point assays. Fresh stock solutions of the compound used for the IC₅₀ determinations were prepared prior to assays and stored at -20 °C. All assays were performed in triplicate for at least three independent biological repeats.

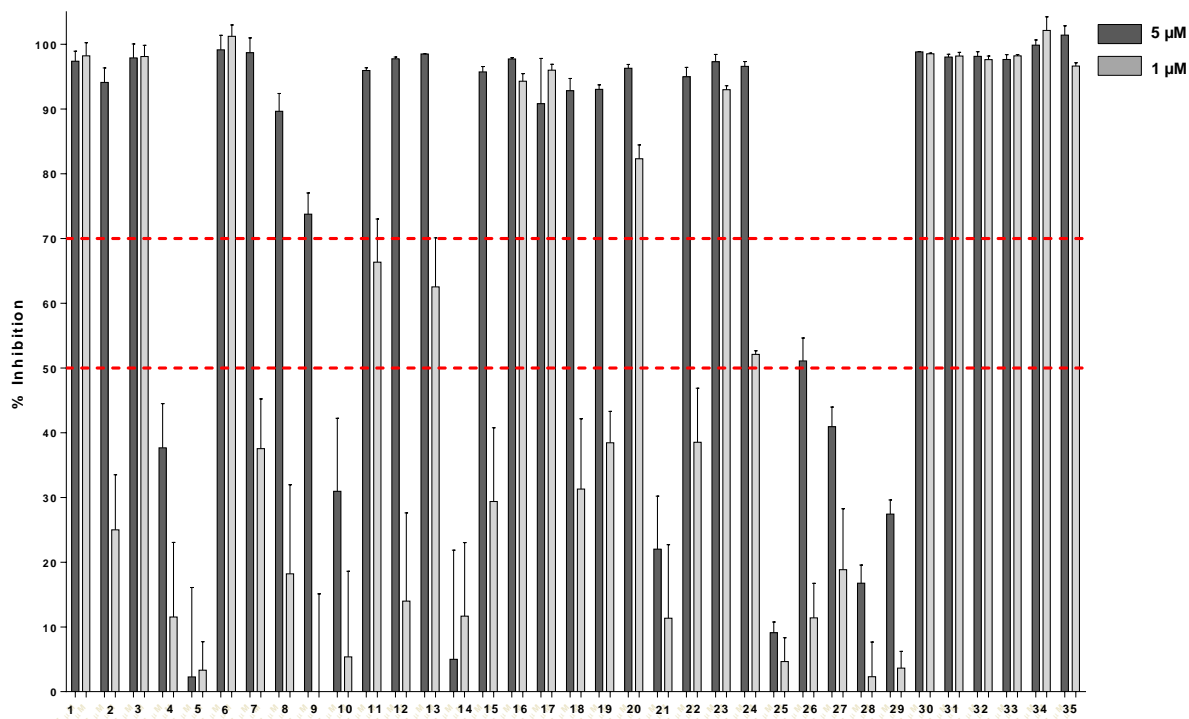


Figure S1: Inhibitory potential against asexual NF54 *P. falciparum* parasites. Compounds were incubated at 5 µM and 1 µM against asexual NF54 *P. falciparum* parasites. Several compounds showed near complete inhibition of parasite proliferation as determined with the SYBR Green I fluorescence based assay following incubation at 37 °C for 96 h with decoquinatone **1** as control. Data analysis were performed in triplicate for n = 3 independent biological repeats with GraphPad 6.0; the average % inhibition is shown and error bars indicate SEM.

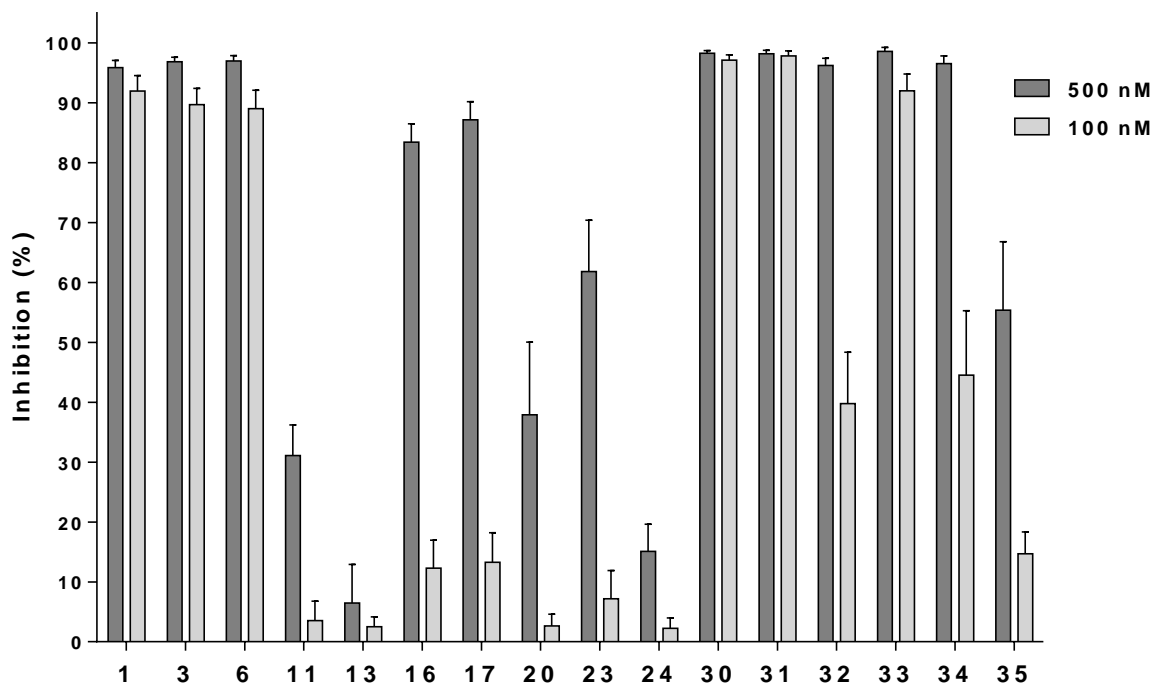


Figure S2: Inhibitory potential against asexual NF54 *P. falciparum* parasites.

The most active compounds at 5 μ M and 1 μ M were incubated at 500 nM and 100 nM against asexual NF54 *P. falciparum* parasites. Eight compounds showed near complete inhibition of parasite proliferation as determined with the SYBR Green I fluorescence based assay following incubation at 37 °C for 96 h. Data analysis were performed in triplicate for n=3 independent biological repeats with GraphPad 6.0; the average % inhibition is shown and error bars indicate SEM.

***In vitro* cytotoxicity**

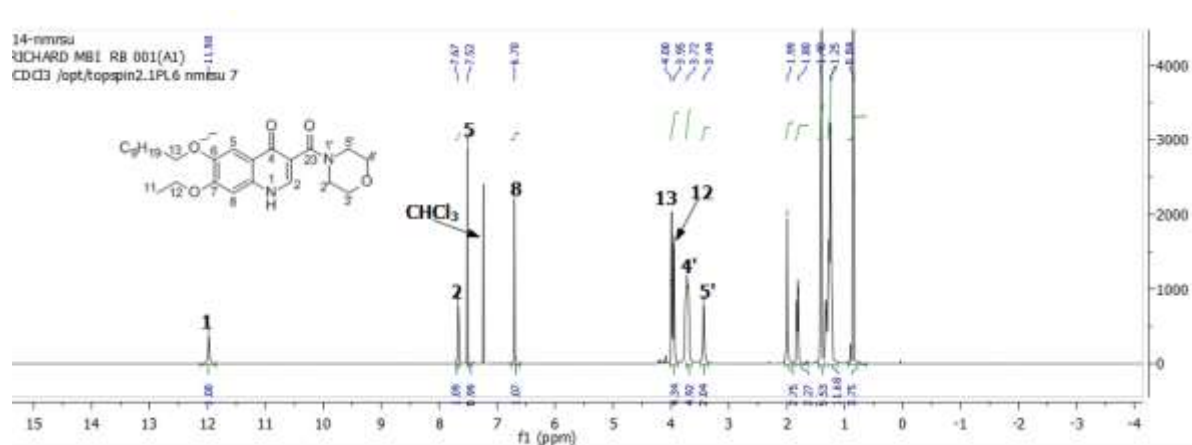
The WI-38 cell line (normal human fetal lung fibroblast) from ECACC was maintained as a monolayer cell culture at 37 °C, 5% CO₂, 95% air and 100% relative humidity in EMEM containing 10% fetal bovine serum, 2 mM L-glutamine and 50 μ g/mL gentamicin. For this screening experiment, the cells (21 - 50 passages) were inoculated in 96-well microtiter plates at plating densities of 10 000 cells/well and incubated for 24 hours. After 24 hours, the cells were treated with the experimental drugs; which had previously been dissolved in DMSO and diluted in medium to produce five concentrations. Neat cells served as the control. The blank contained complete medium without cells. Parthenolide was used as a standard. The plates were incubated for 48 hours after addition of the compounds. Viable cells were fixed

to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein bound dye was extracted with 10 mM Tris base for optical density determination at a wavelength of 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression.

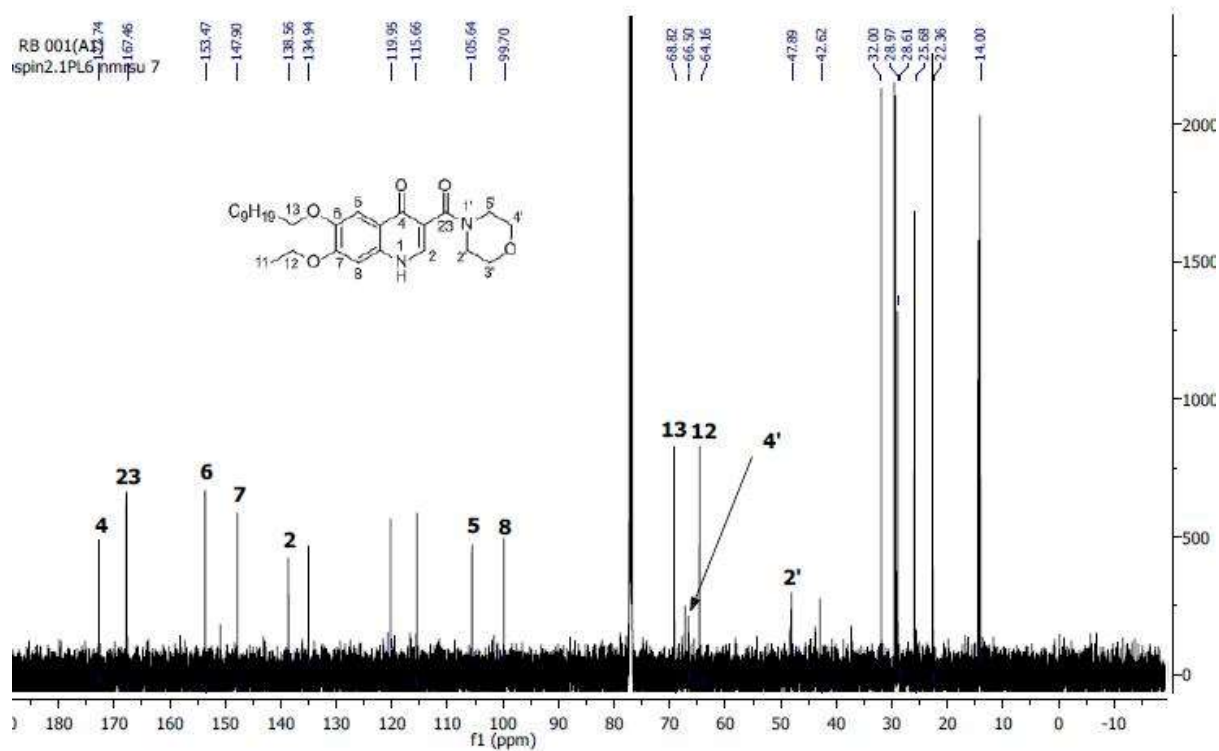
NMR and HRMS spectra of key derivatives

Compound 2

¹H NMR spectrum



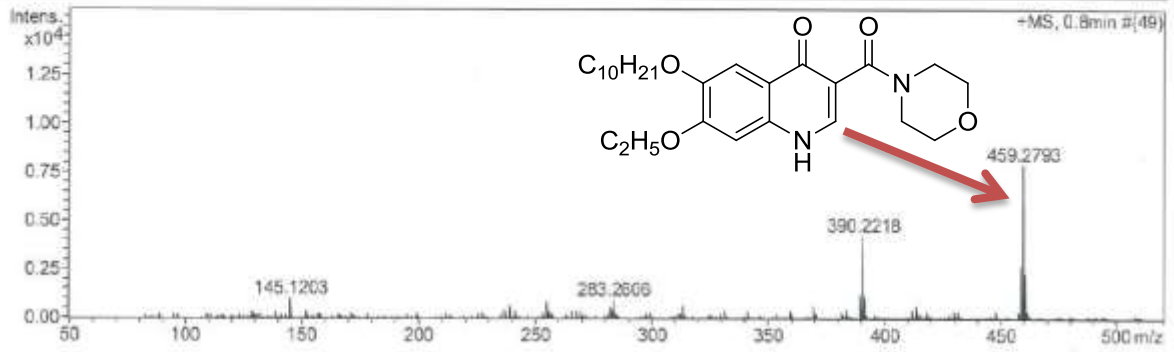
¹³C NMR spectrum



HRMS spectrum

Acquisition Parameter

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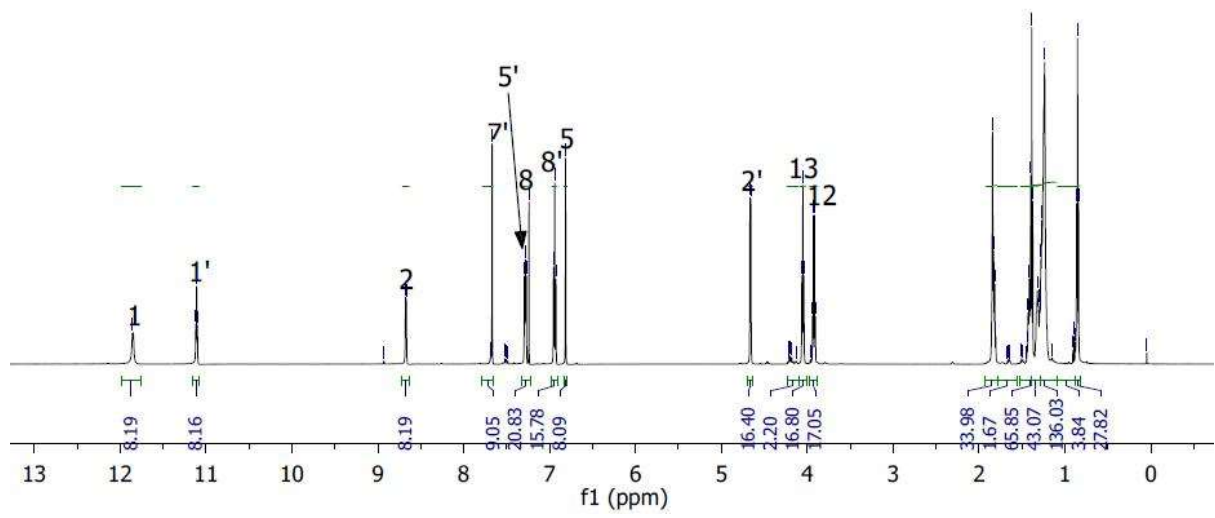
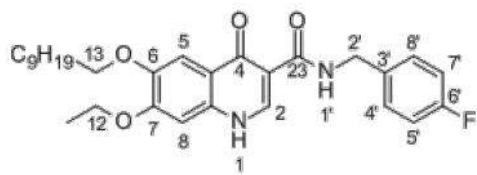


Compound 3

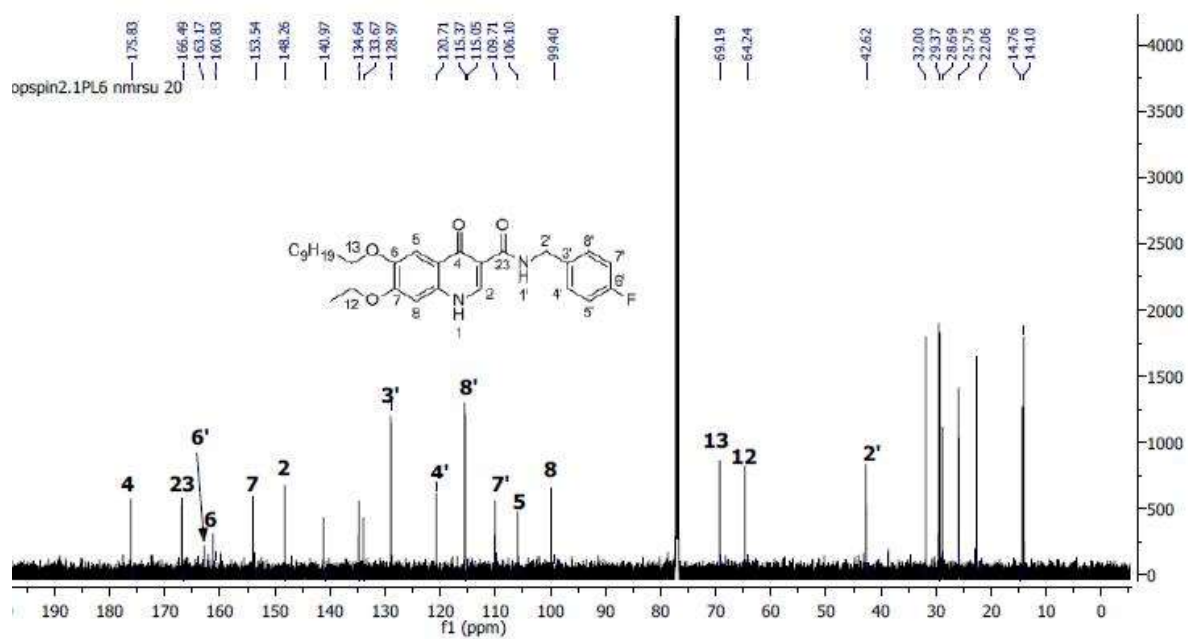
¹H NMR spectrum

11.85
11.12
11.11
11.10
n2.1PL6 nmrsu 20

8.93 8.68 8.67 7.67 7.29 7.28 7.28 7.27 7.24 6.95 6.94 6.93 6.81 4.67 4.66 4.21 4.20 4.06 4.05 4.04 3.94 3.93 3.92 3.90 1.84 1.83 1.40 1.39 1.37 1.27 1.24 0.86 0.85 0.84



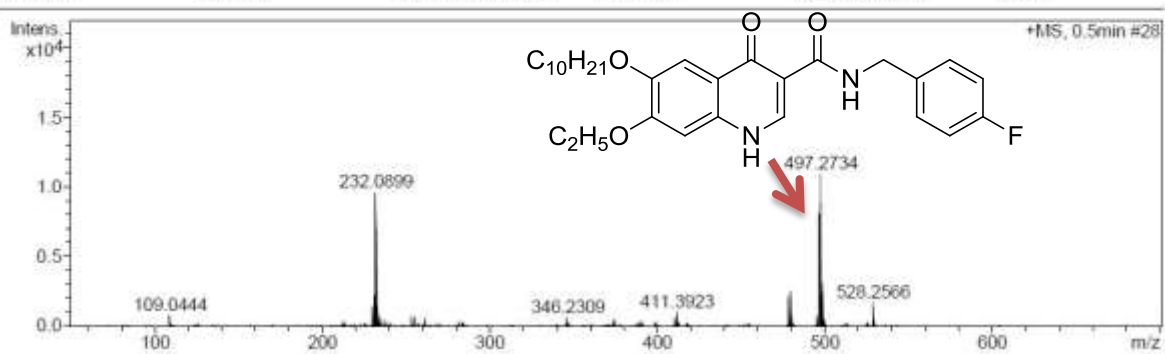
¹³C NMR spectrum



HRMS Spectrum

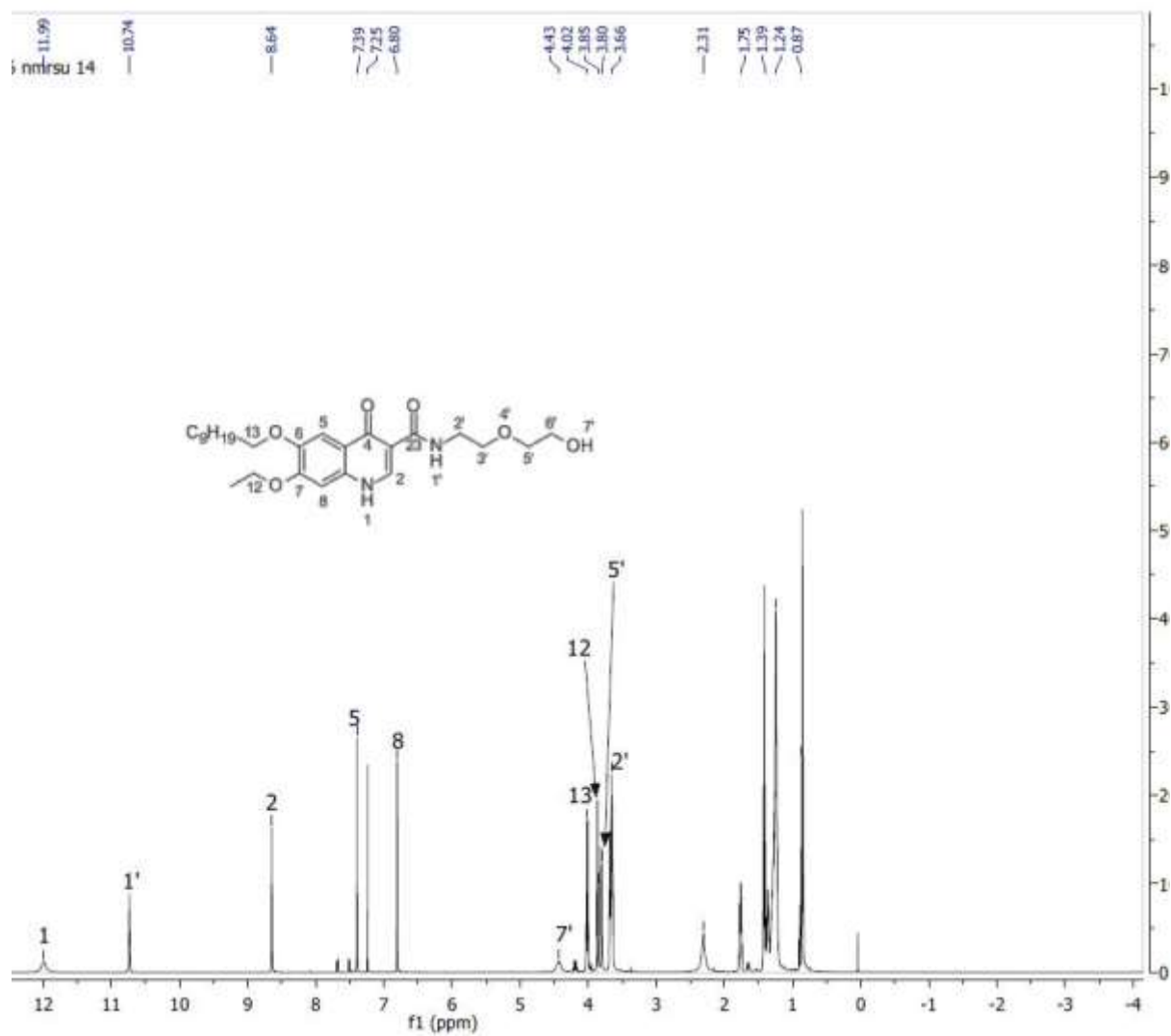
Acquisition Parameter

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Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste

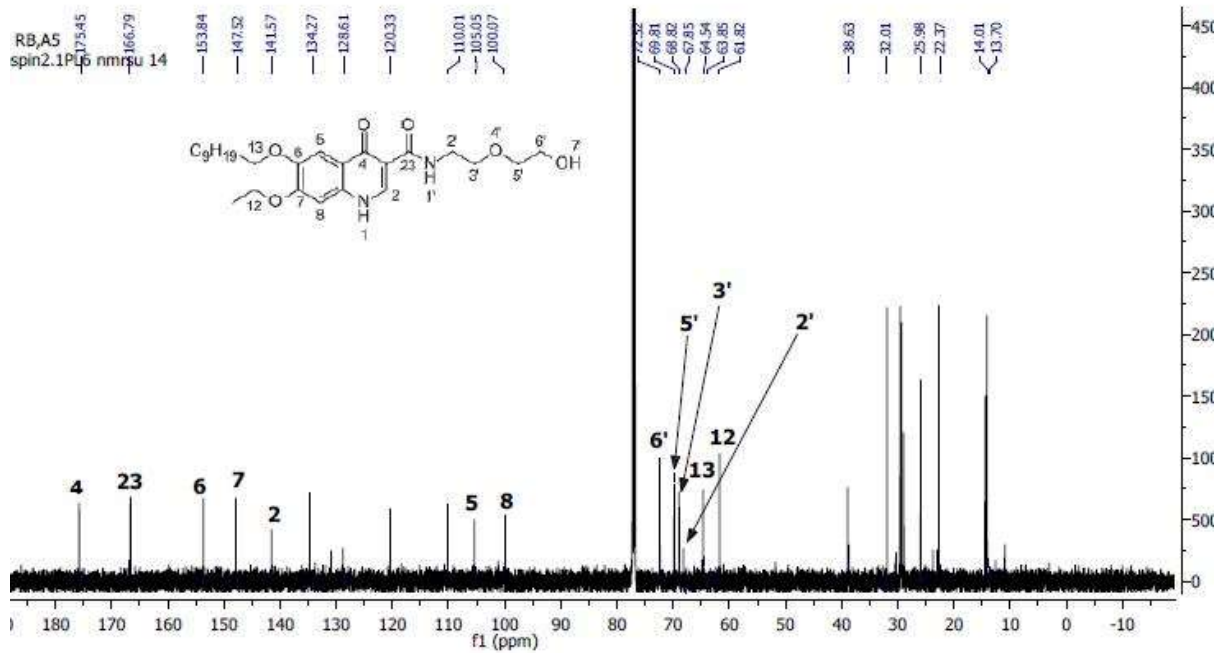


Compound 6

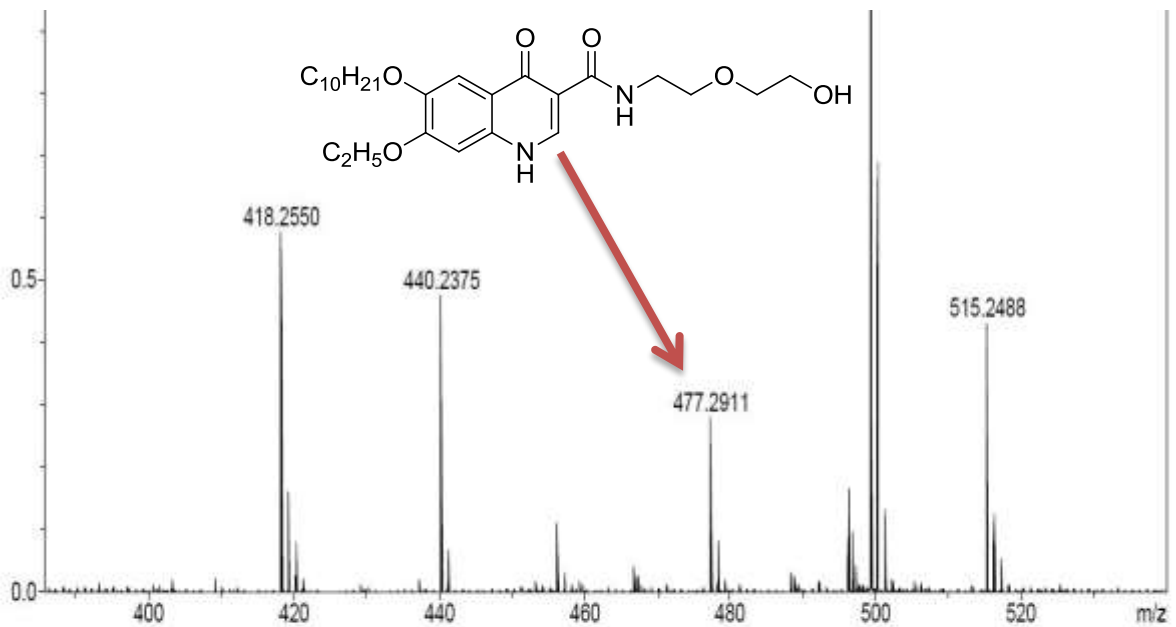
¹H NMR spectrum



¹³C NMR spectrum

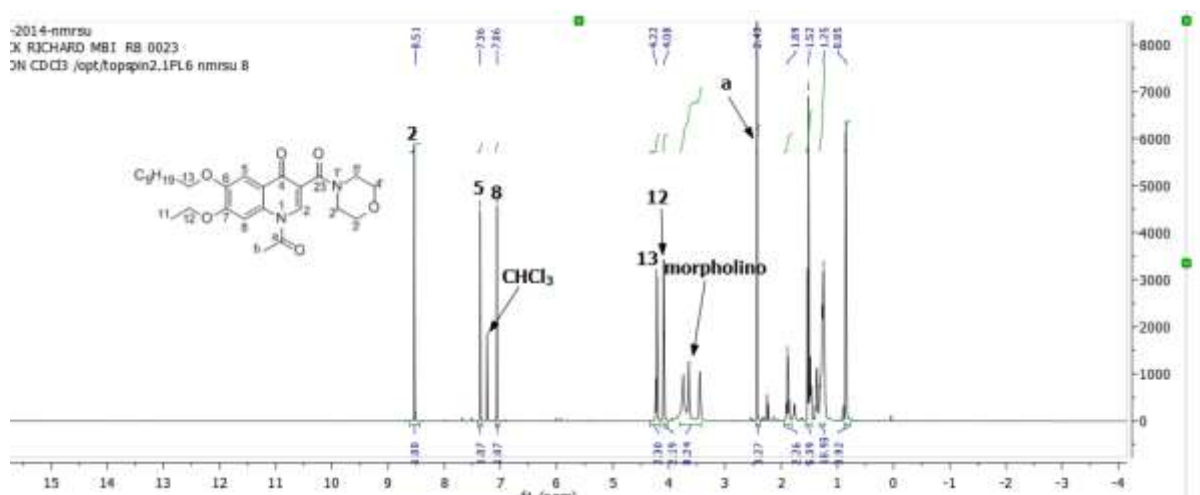


HRMS spectrum

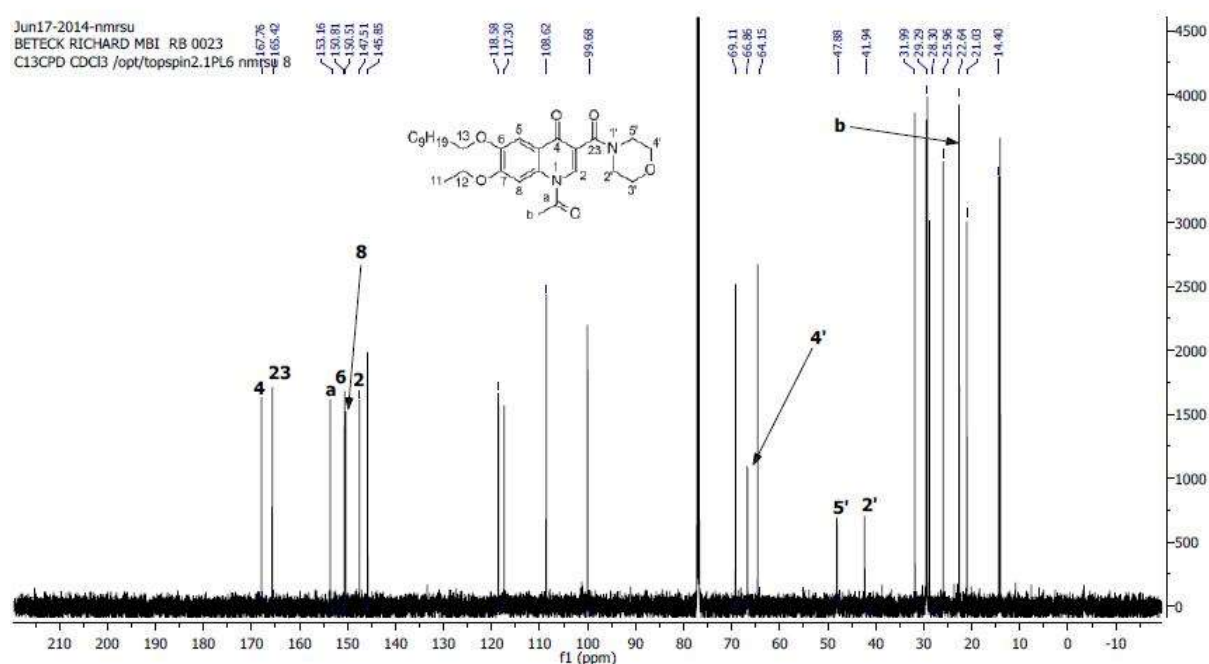


Compound 24

¹H NMR spectrum



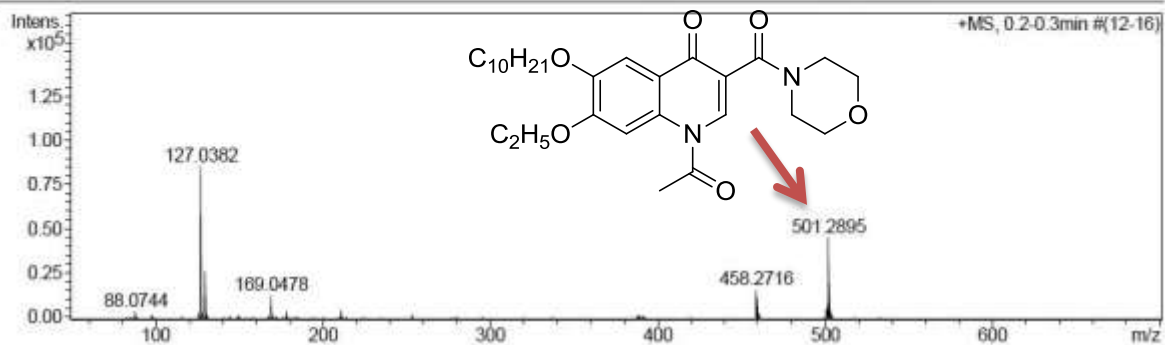
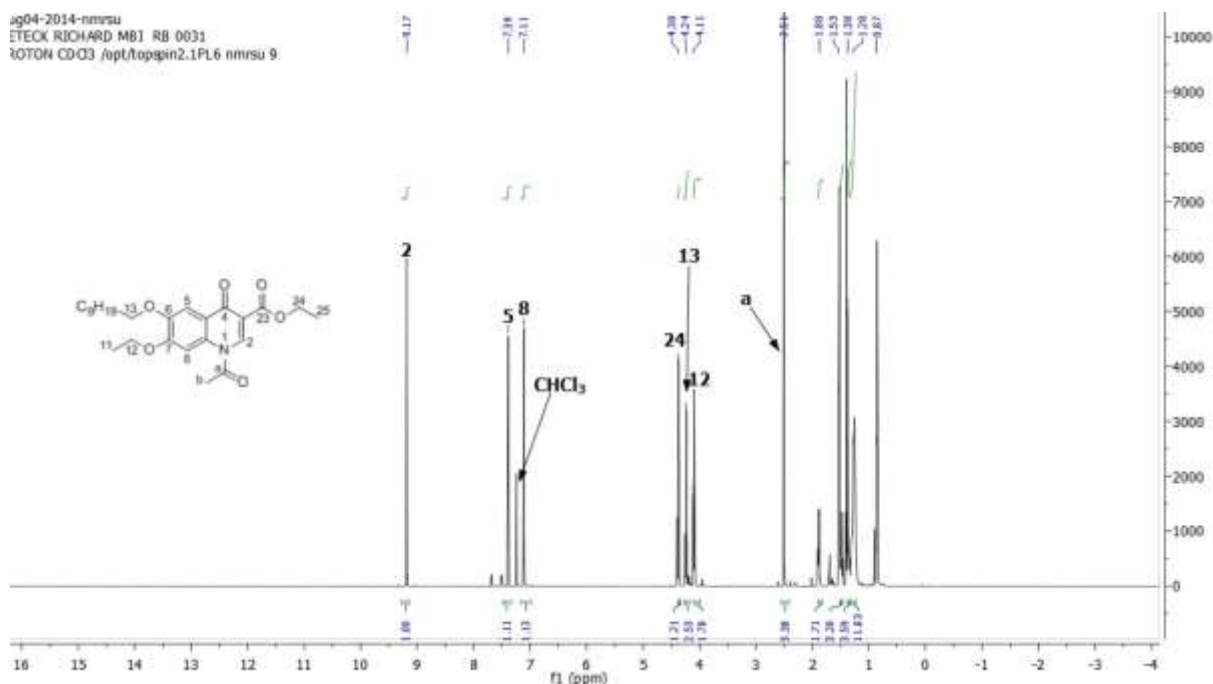
¹³C NMR spectrum



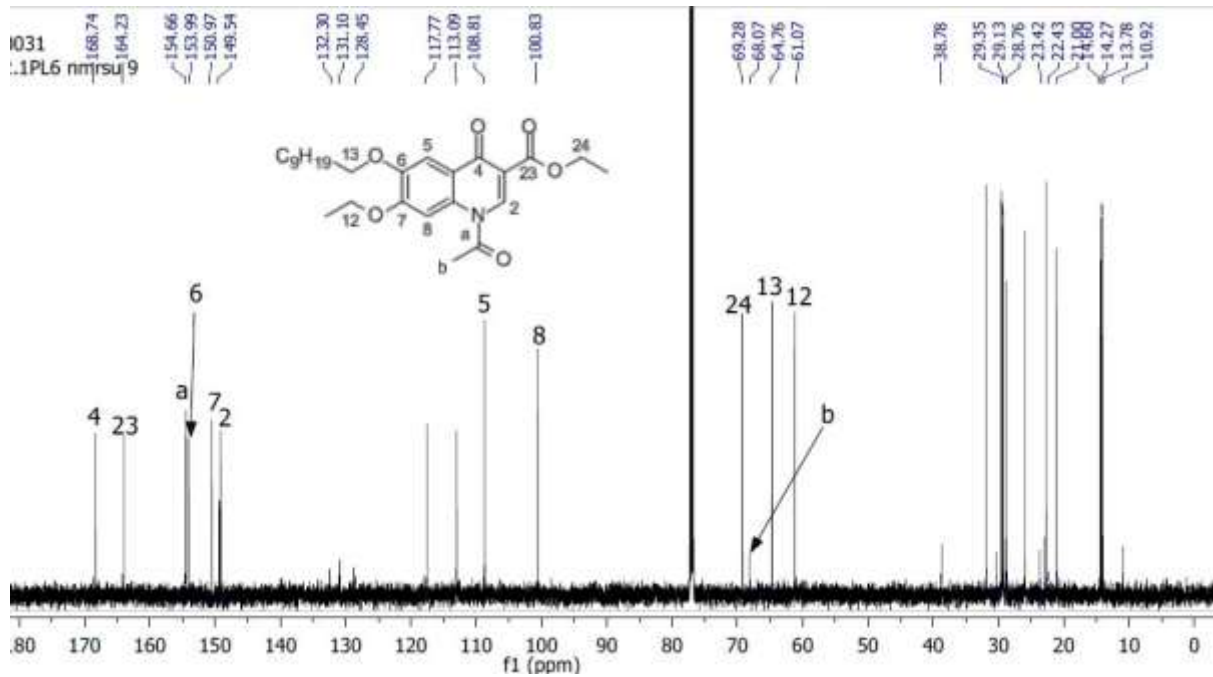
HRMS spectrum

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
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Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

**Compound 30****¹H NMR spectrum**

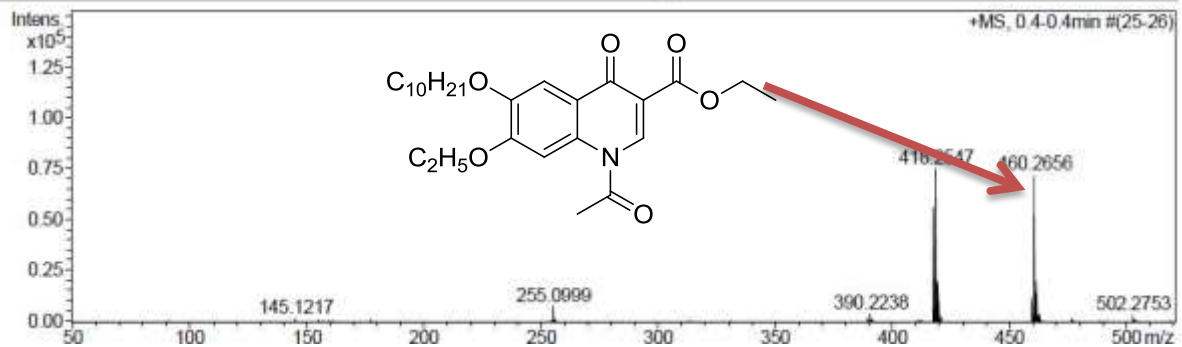
¹³C NMR spectrum



HRMS spectrum

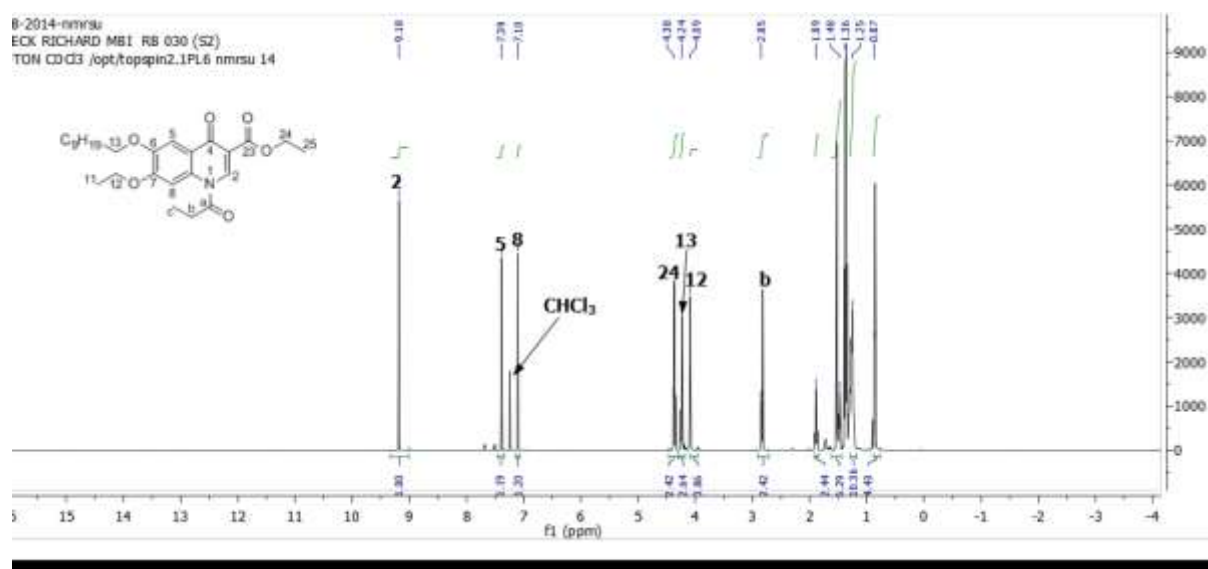
Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

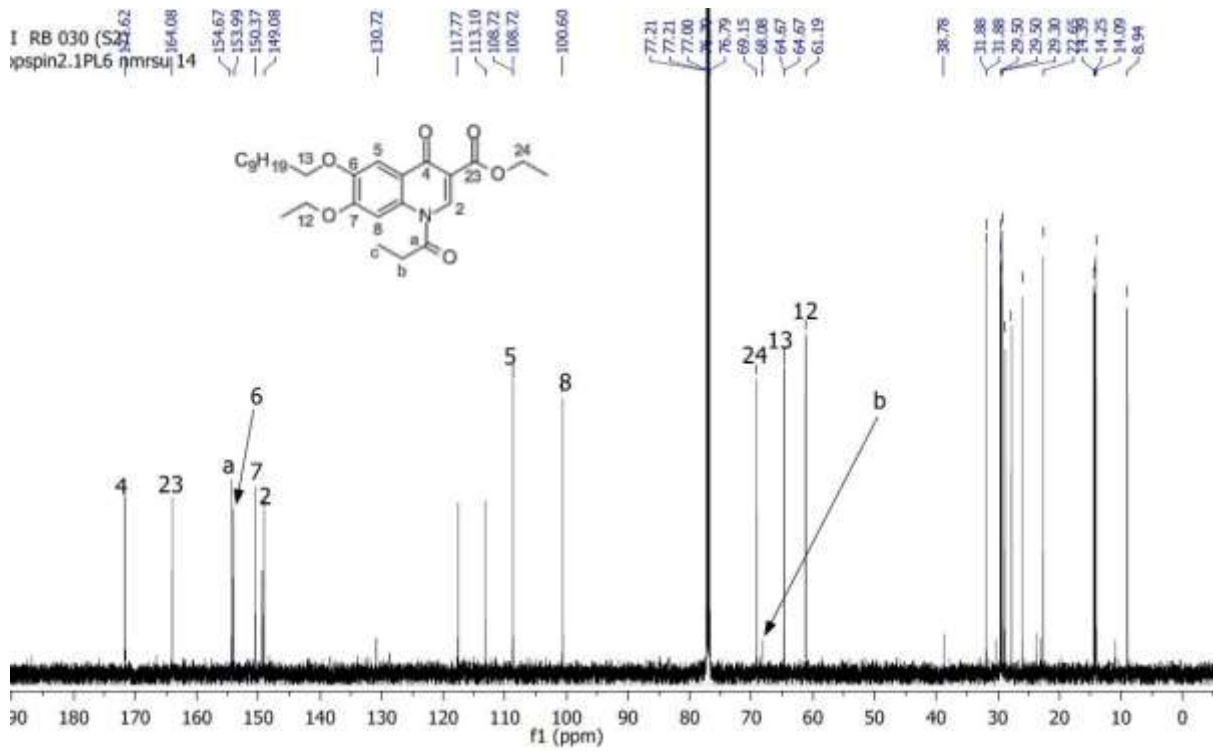


Compound 31

¹H NMR spectrum



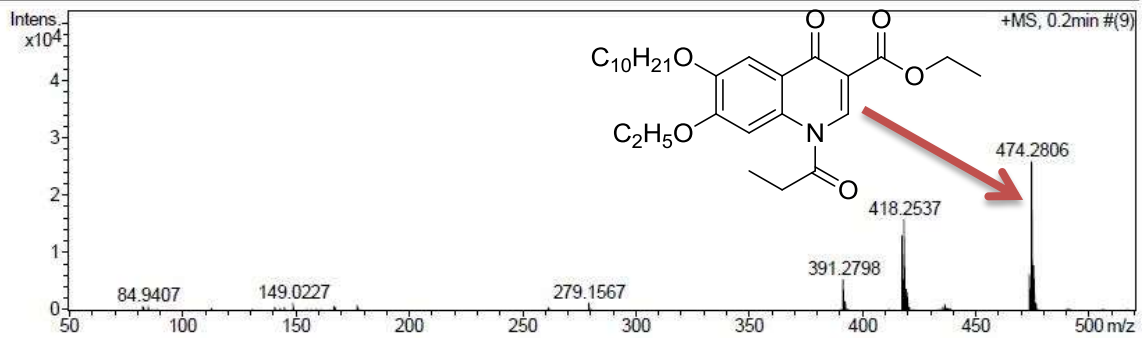
¹³C NMR spectrum



HRMS spectrum

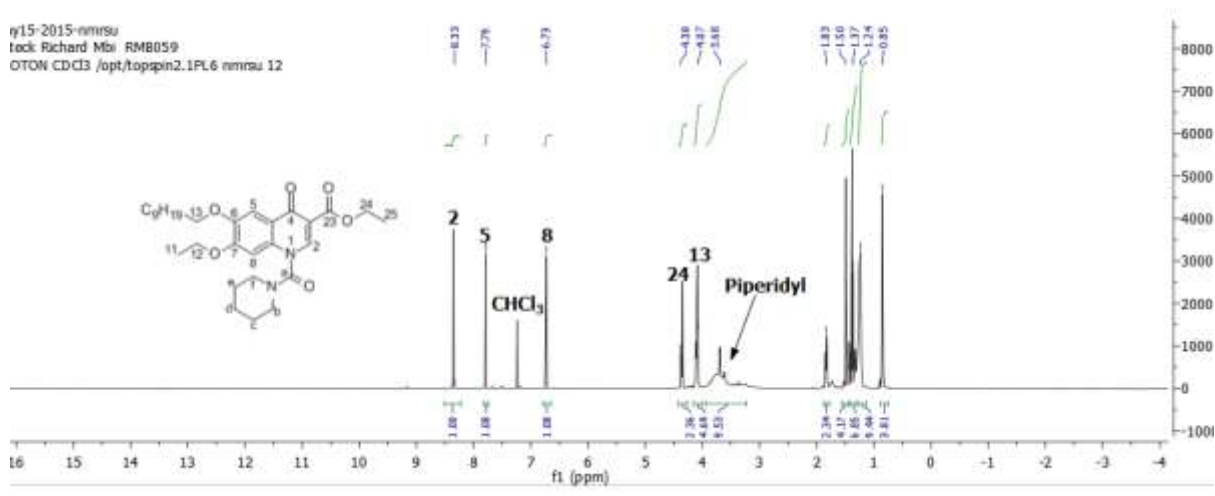
Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

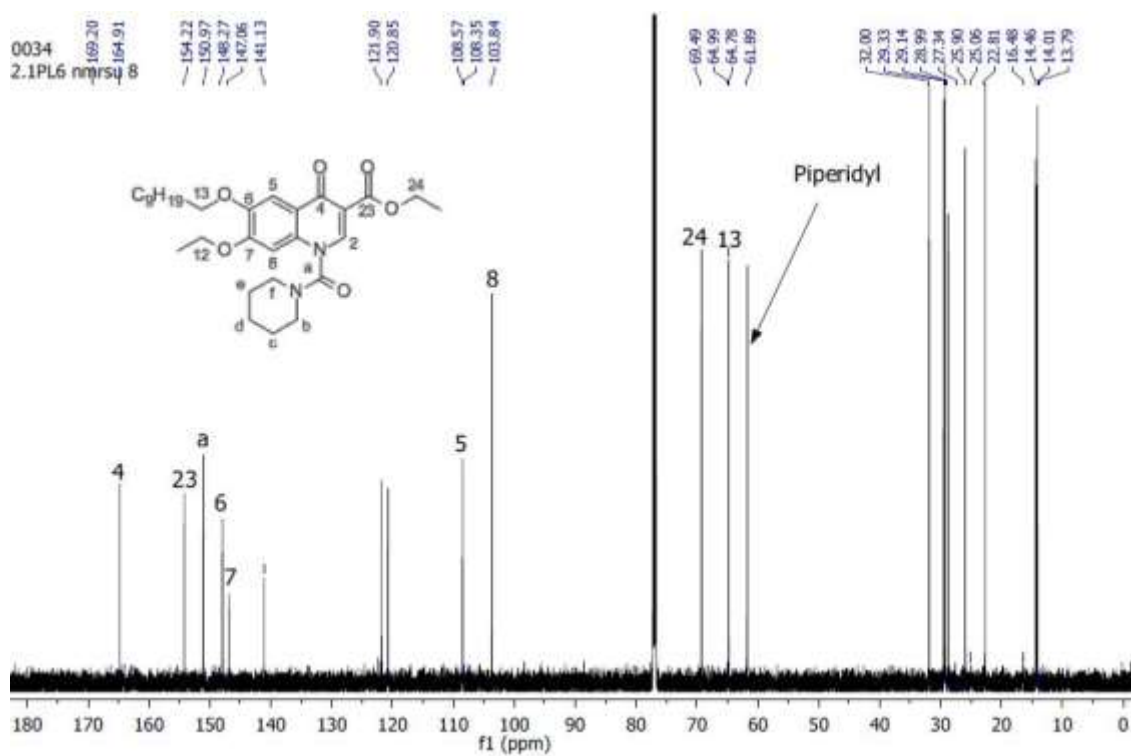


Compound 33

¹H NMR spectrum



¹³C NMR spectrum



HRMS spectrum

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

