antibiotics

MDPI

Article

Profluorescent Fluoroquinolone-Nitroxides for Investigating Antibiotic–Bacterial Interactions

Anthony D. Verderosa [1,2], Rabeb Dhouib [2], Kathryn E. Fairfull-Smith [1,*] and Makrina Totsika [2,*]

Supplementary Material

Table of Contents

Methods

MIC susceptibility assay (in 96-well plate) for unfunctionalized nitroxides:

Nitroxides 2,2,6,6-tetramethylpiperidin-1-yloxyl (TEMPO), 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO), and 1,1,3,3-tetraethylisoindolin-2-yloxyl (TEIO) were also subjected to MIC susceptibility assays utilising the same methodology detailed above. TEMPO, TMIO, and TEIO were tested between the concentration range of 1200 to 2 μM. MIC values (Table S1) were obtained from at least 3 biological replicates, each with at least 3 technical replicates.

Results

MIC susceptibility assay for unfunctionalized nitroxides:

Table 1. Measured MIC values for TEMPO, TMIO, and TEIO against Gram-negative *P. aeruginosa* and *E. coli*, and Gram-positive *S. aureus*, and *E. faecalis*.[a]

Compound	*P. aeruginosa* ATCC 27853 MIC (μM)	*E. coli* ATCC 25922 MIC (μM)	*S. aureus* ATCC 29213 MIC (μM)	*E. faecalis* ATCC 14933 MIC (μM)
TEMPO	> 1200[b]	> 1200[b]	> 1200[b]	> 1200[b]
TMIO	> 1200[b]	> 1200[b]	> 1200[b]	> 1200[b]
TEIO	> 1200[b]	> 1200[b]	> 1200[b]	> 1200[b]

[a] All MICs were determined via broth microdilution method in accordance with CLSI standard; [b] Highest concentration tested.

Fluorescence microscopy images:

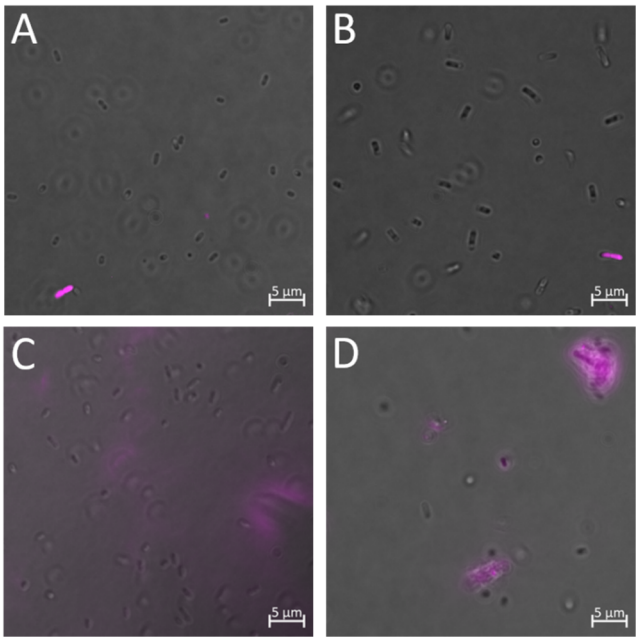

Figure S1. Fluorescent and brightfield overlay micrographs images of bacterial cells treated with FN 14 or FM 17. (A) FN 14 (150 μM) and *P. aeruginosa*; (B) FN 14 (150 μM) and *E. coli*; (C) FN 17 (150 μM) and *P. aeruginosa*; (D) FN 17 (150 μM) and *E. coli*. Scale bars are 5 μM in length.

^{1}H NMR and ^{13}C NMR Spectra

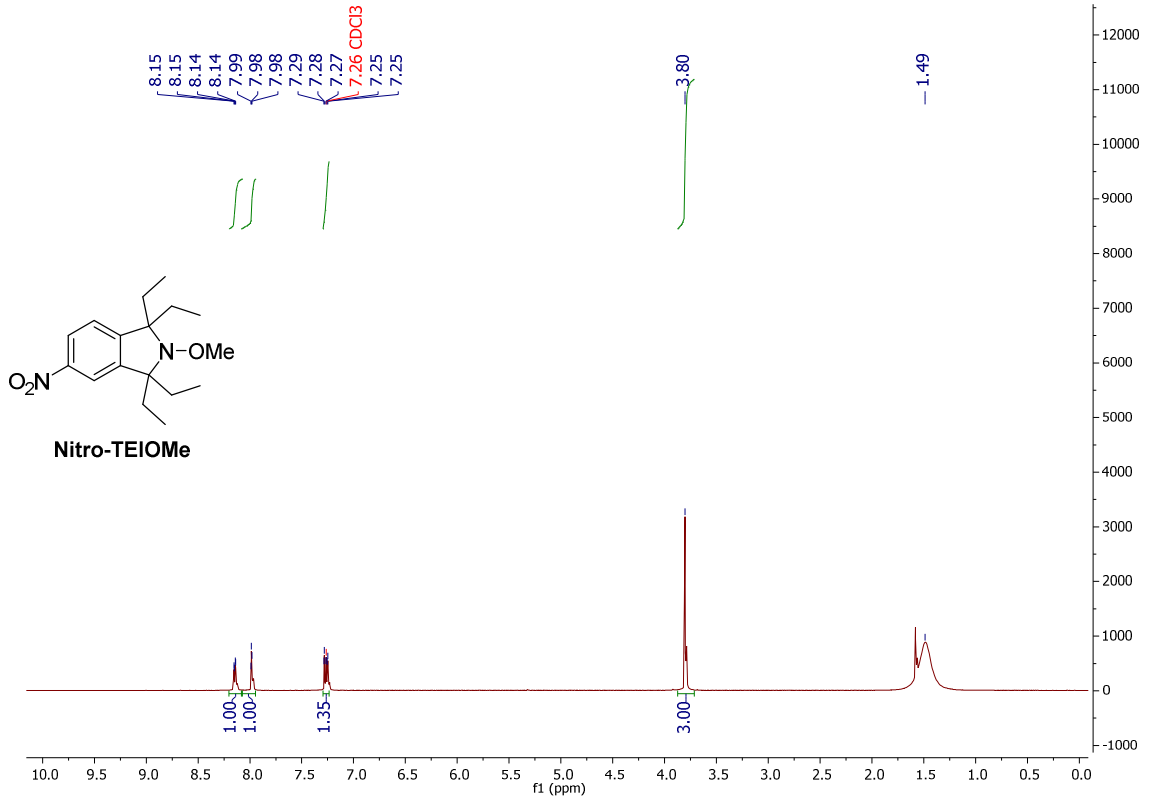

Figure S2. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of Nitro-TEIOMe.

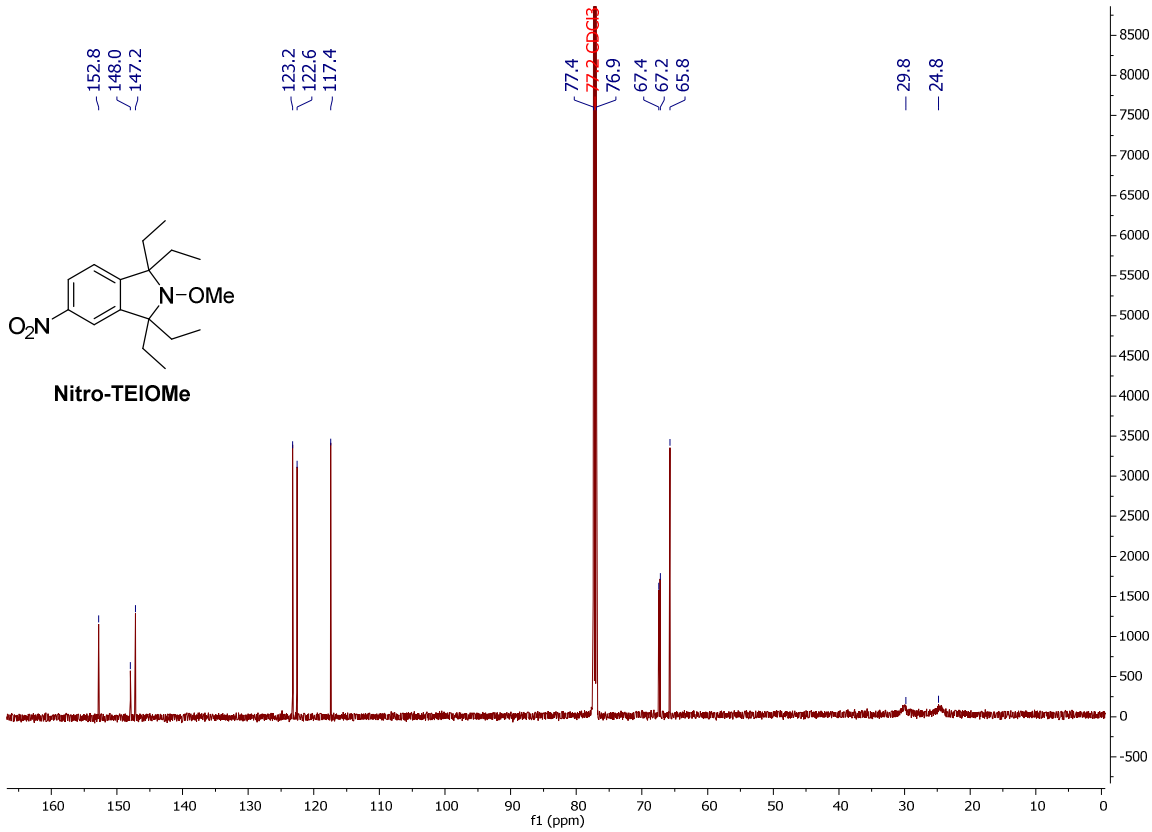

Figure S3. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of Nitro-TEIOMe.

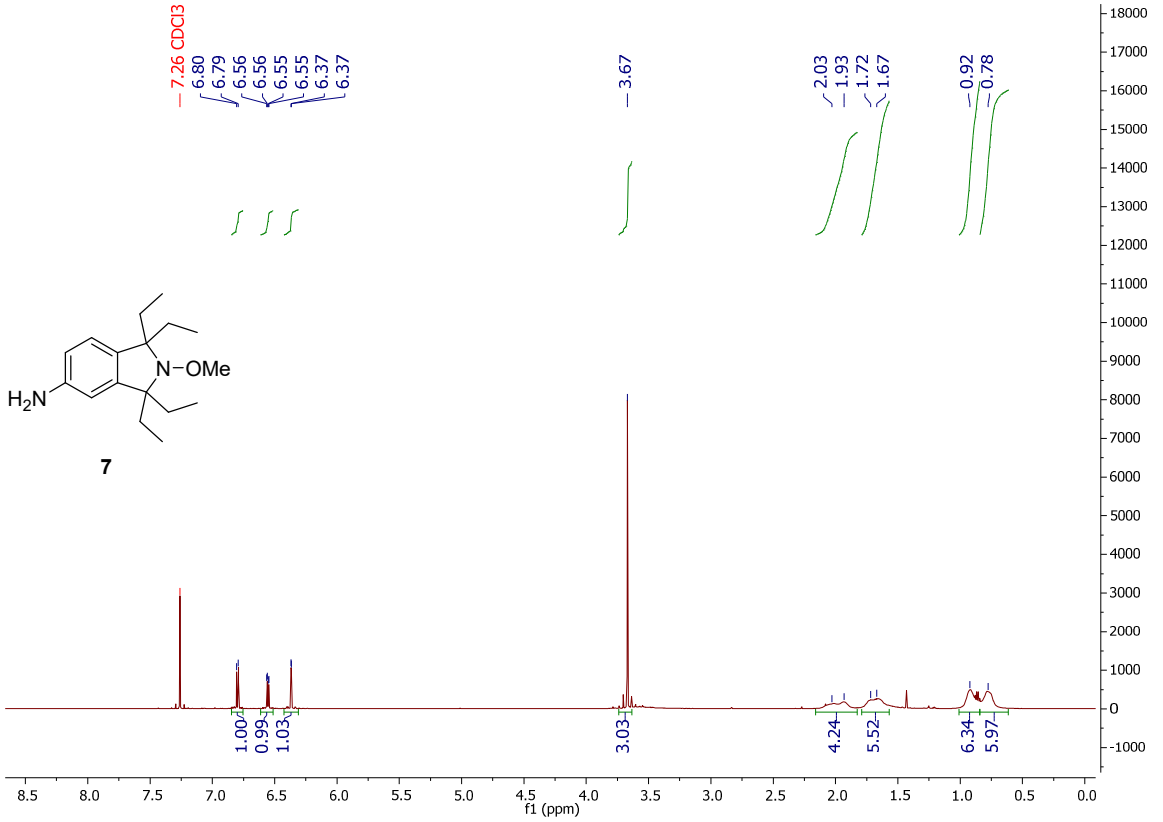

Figure S4. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **7**.

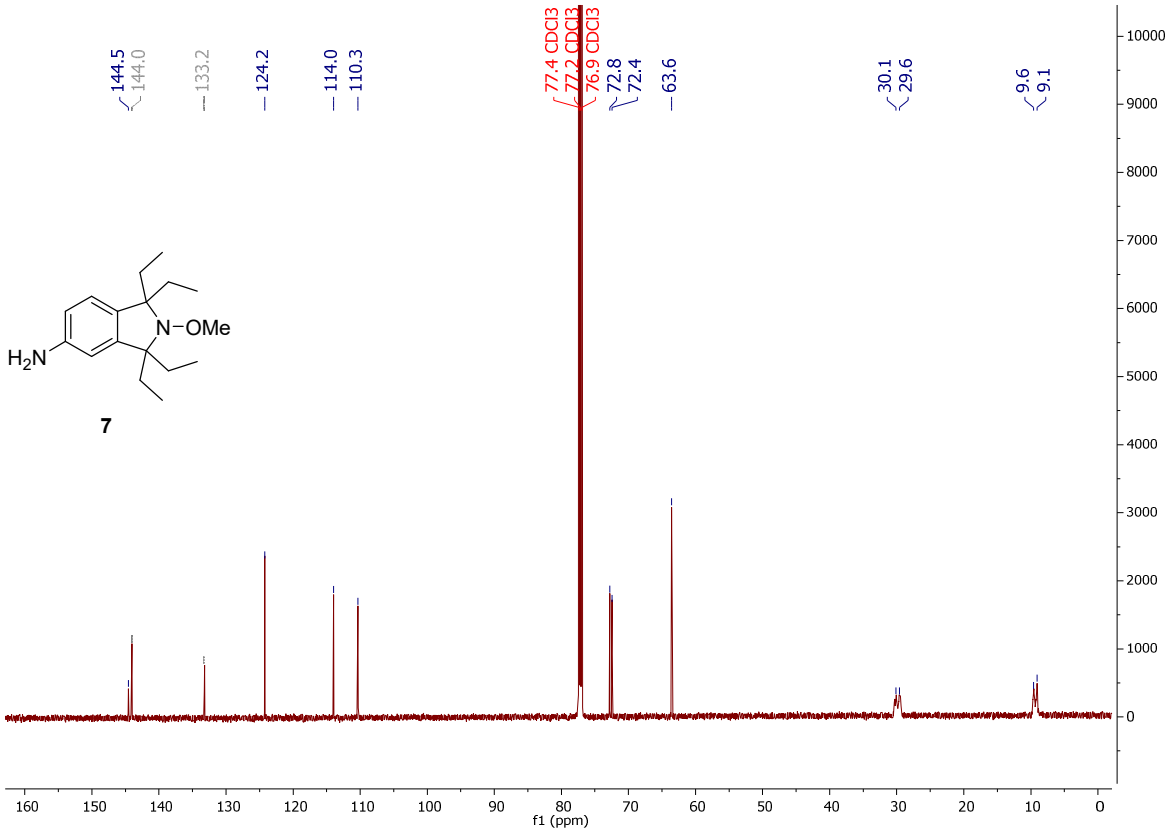

Figure S5. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **7**.

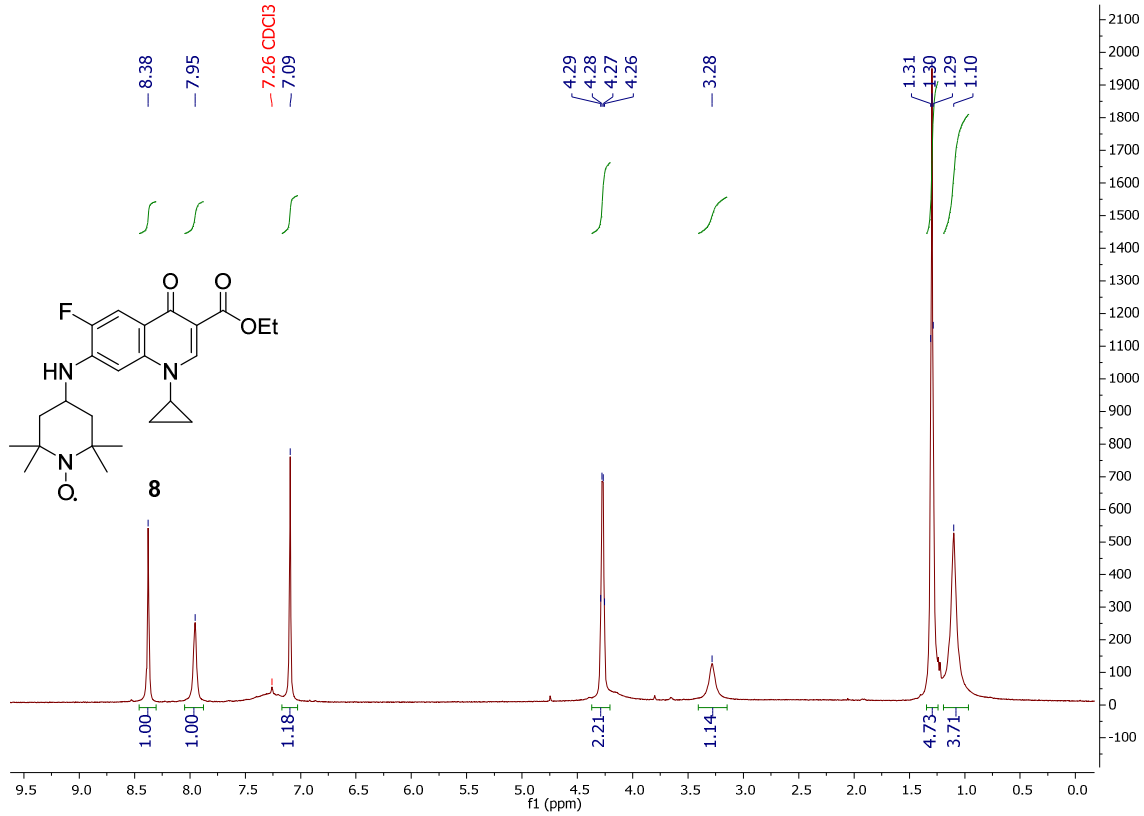

Figure 6. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **8**.

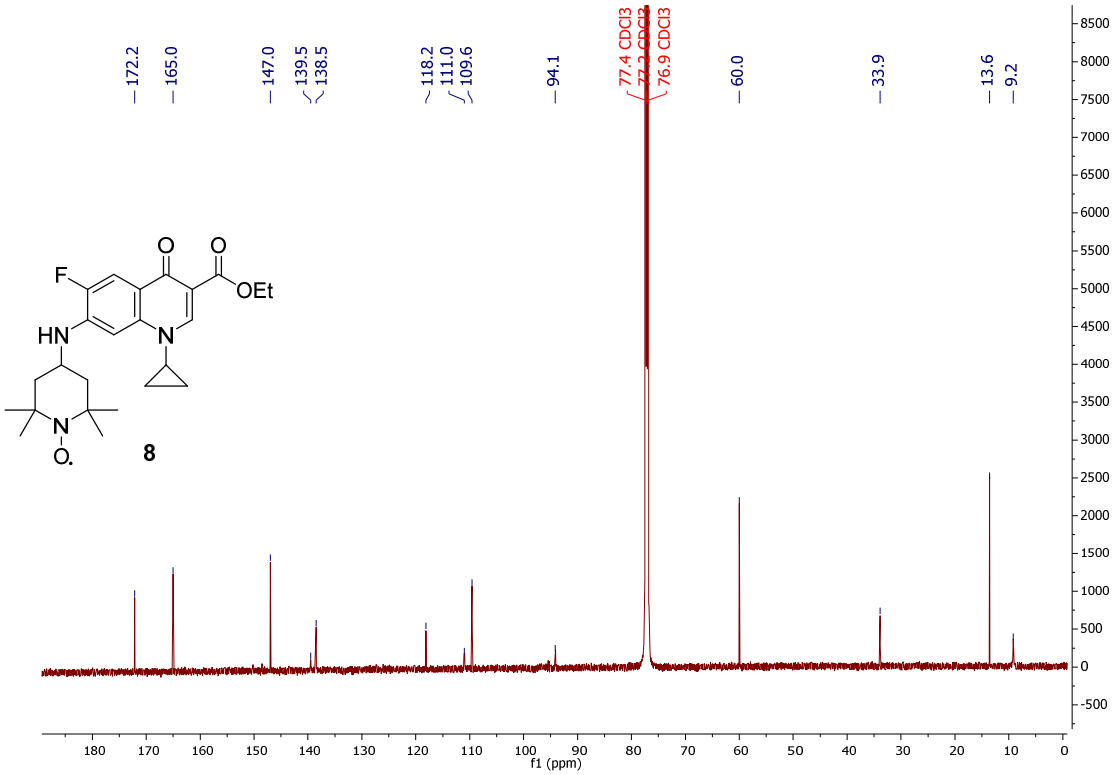

Figure S7. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **8**.

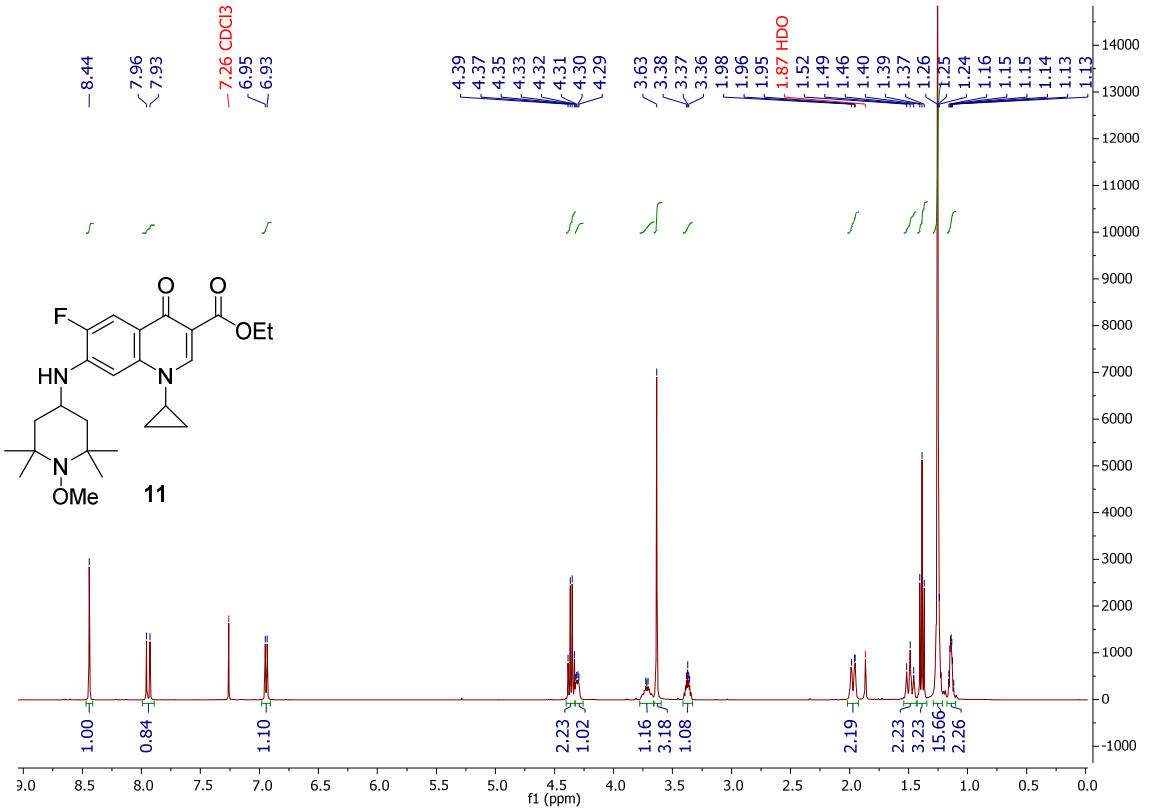

Figure 8. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **11**.

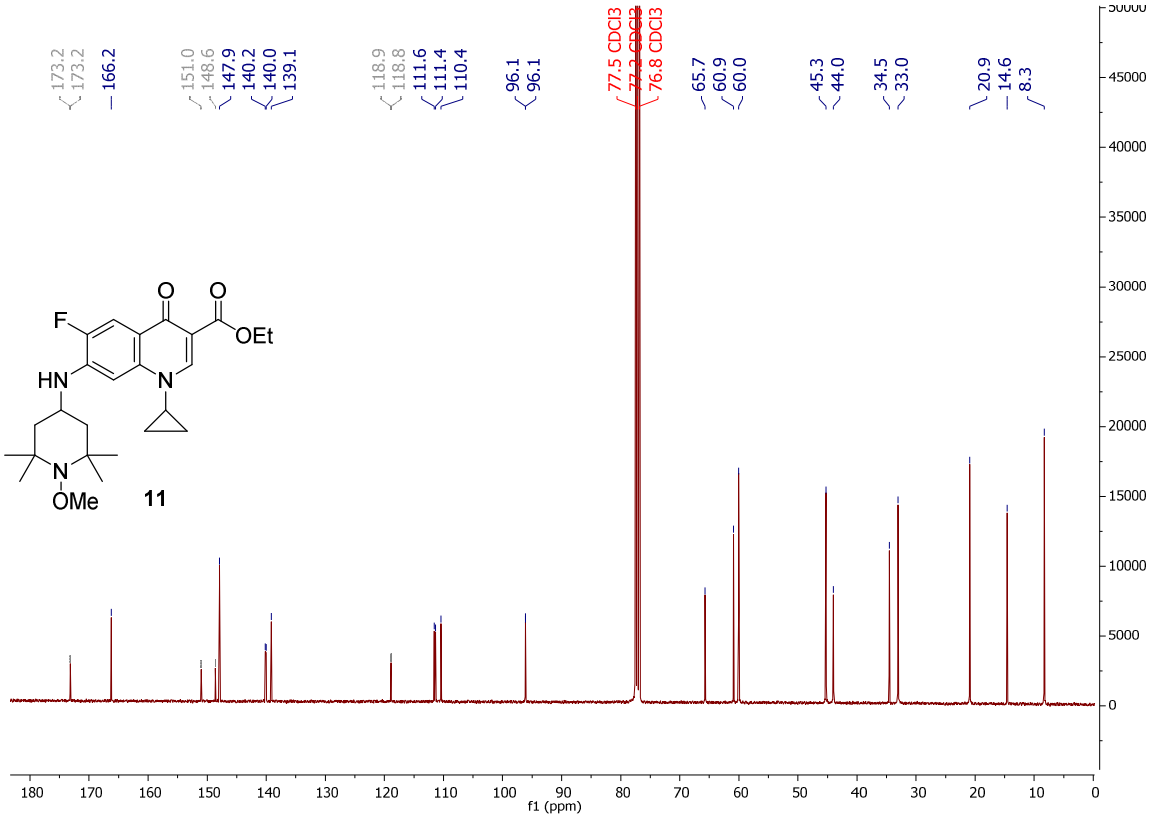

Figure S9. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **11.**

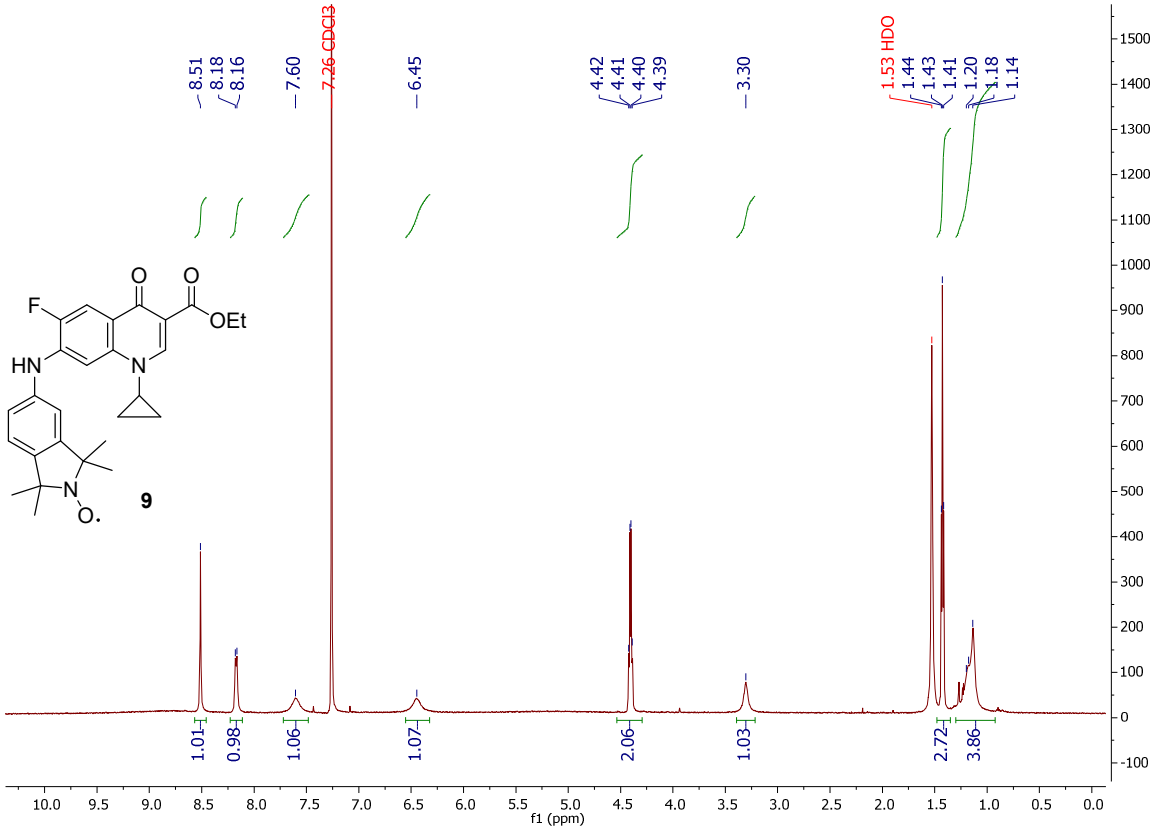

Figure S10. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **9**.

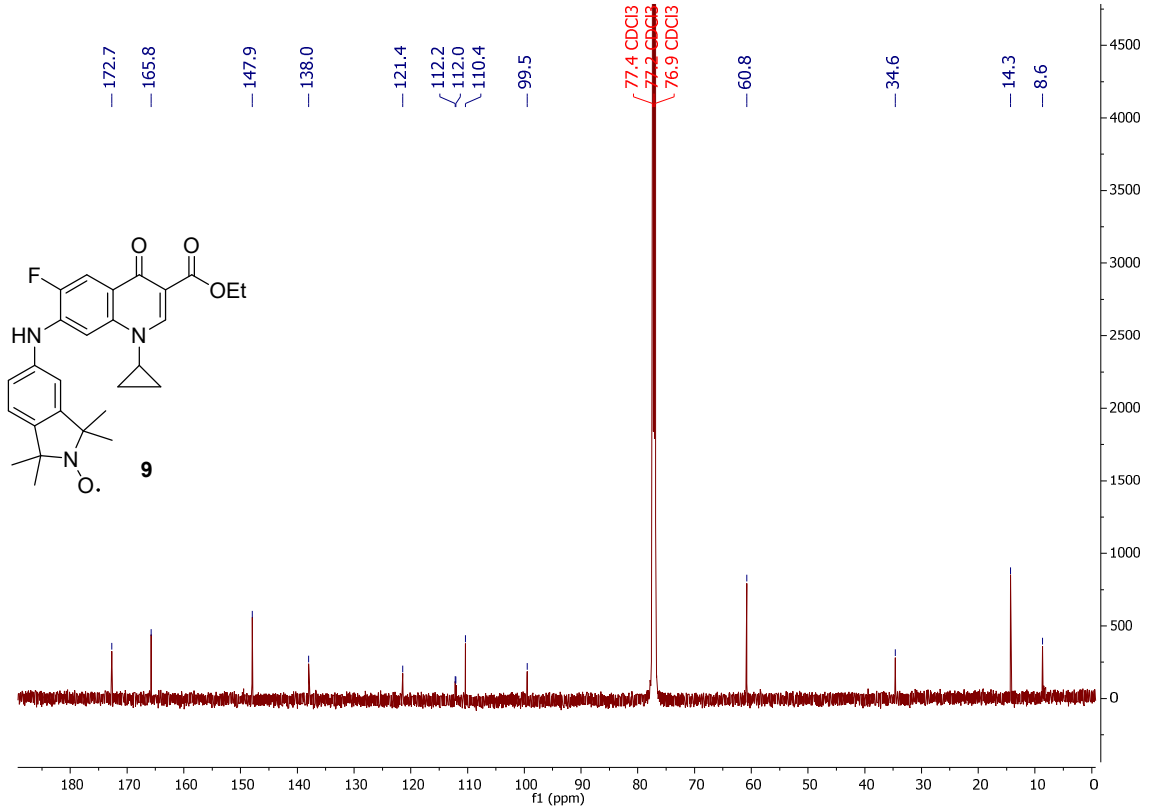

Figure S11. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **9**.

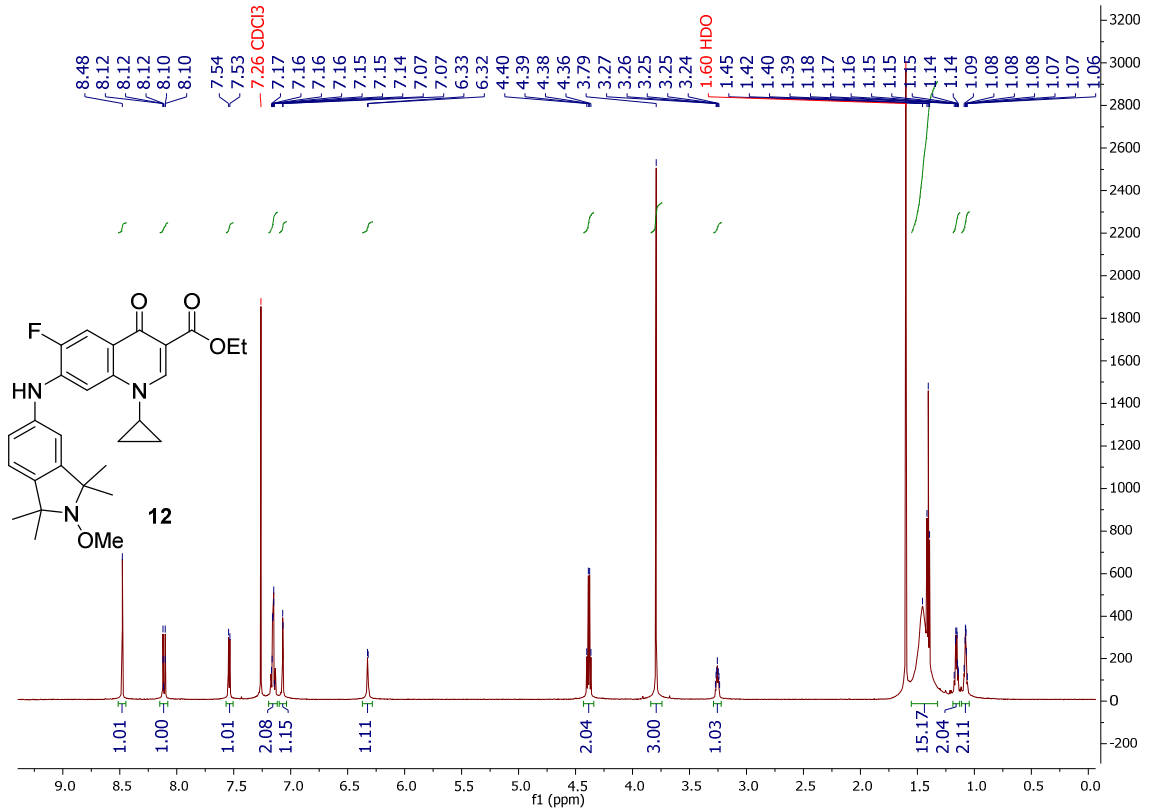

Figure S12. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **12**.

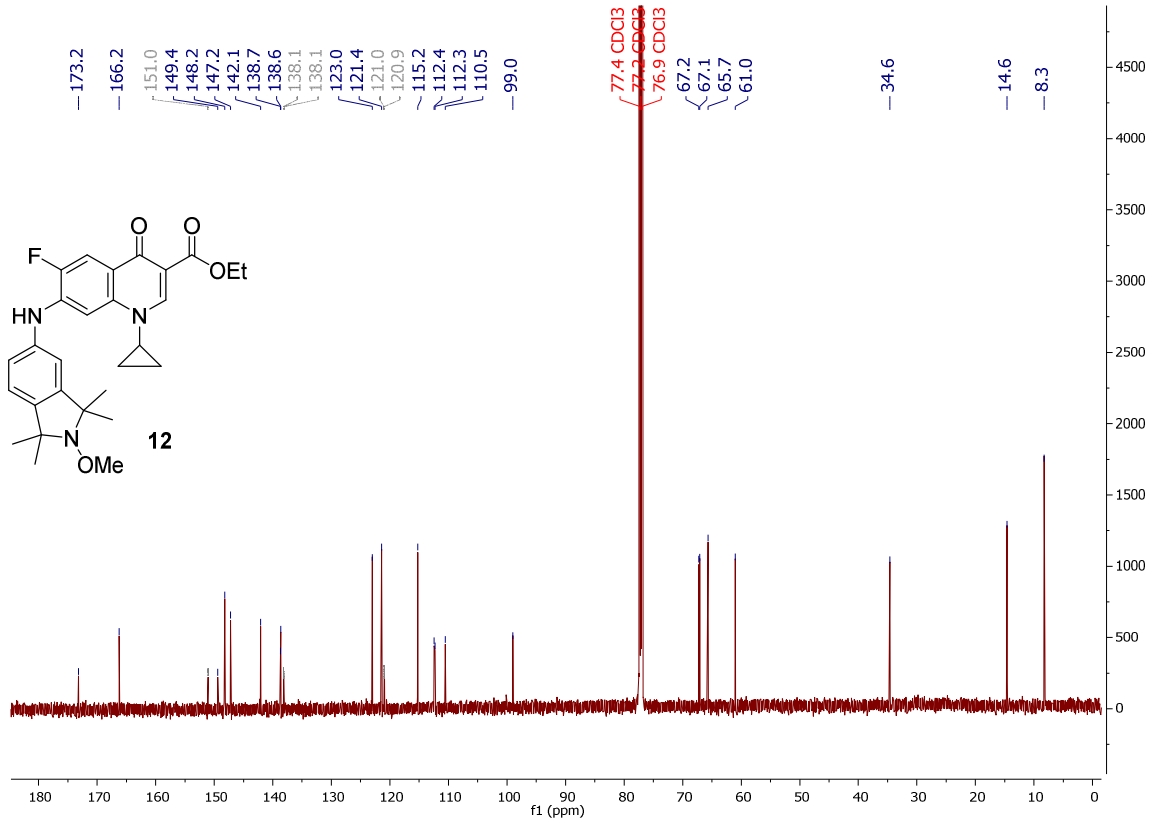

Figure S13. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **12**.

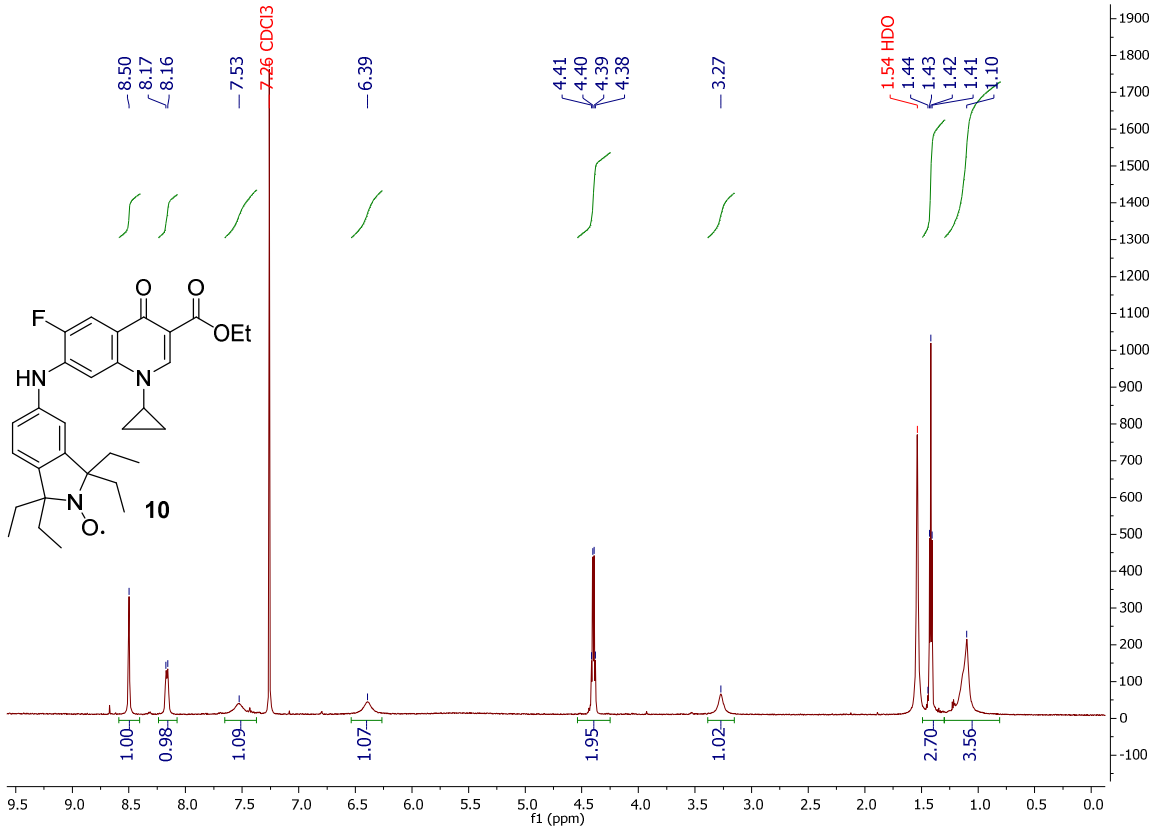

Figure S14. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **10**.

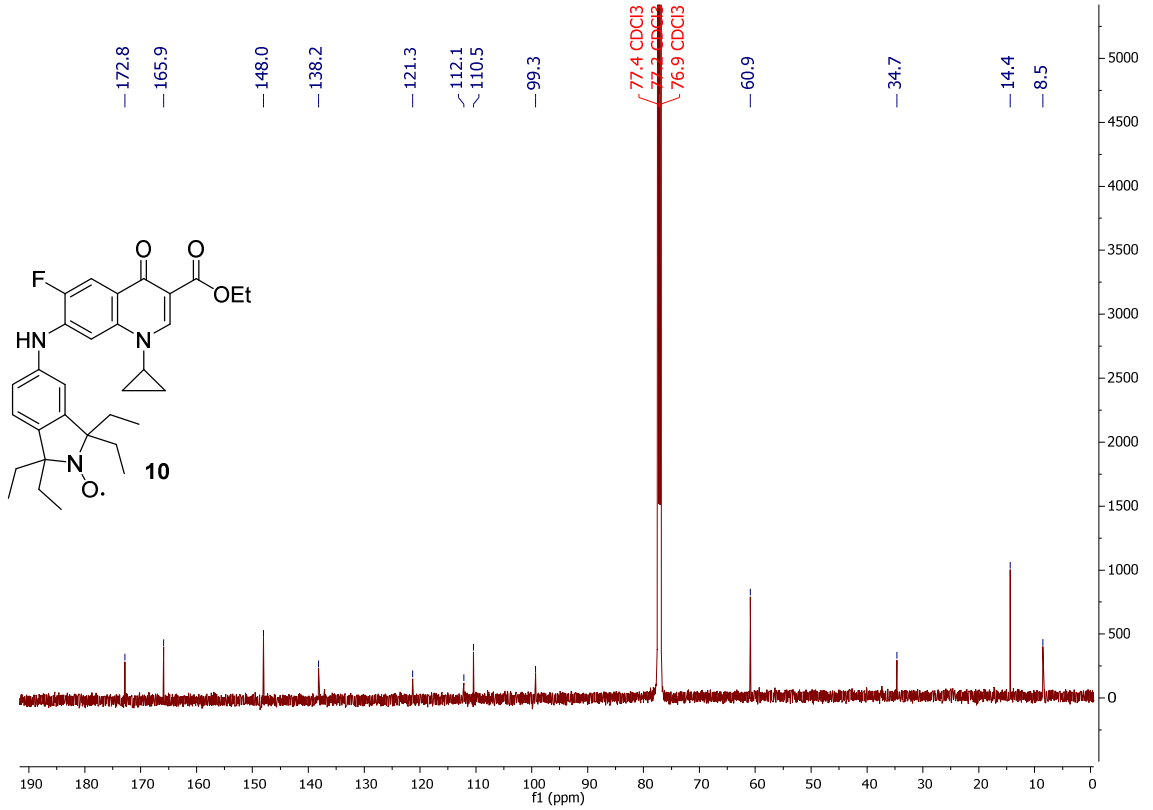

Figure S15. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **10**.

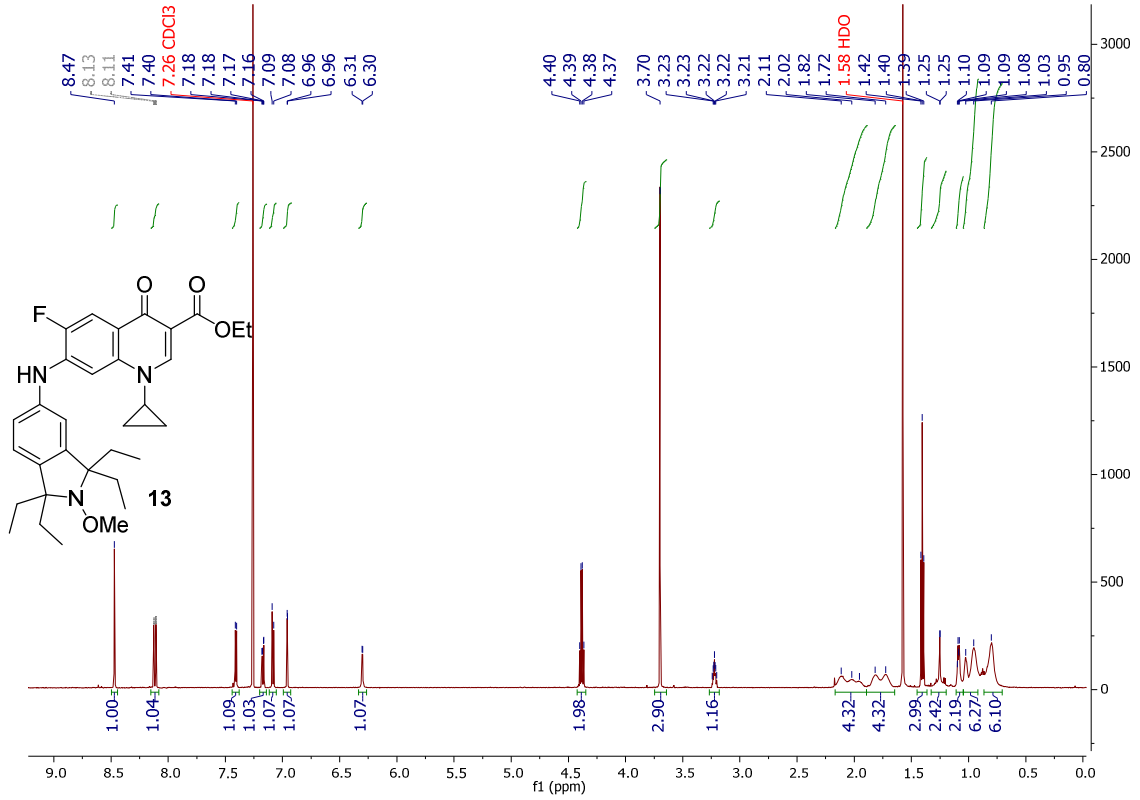

Figure S16. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **13**.

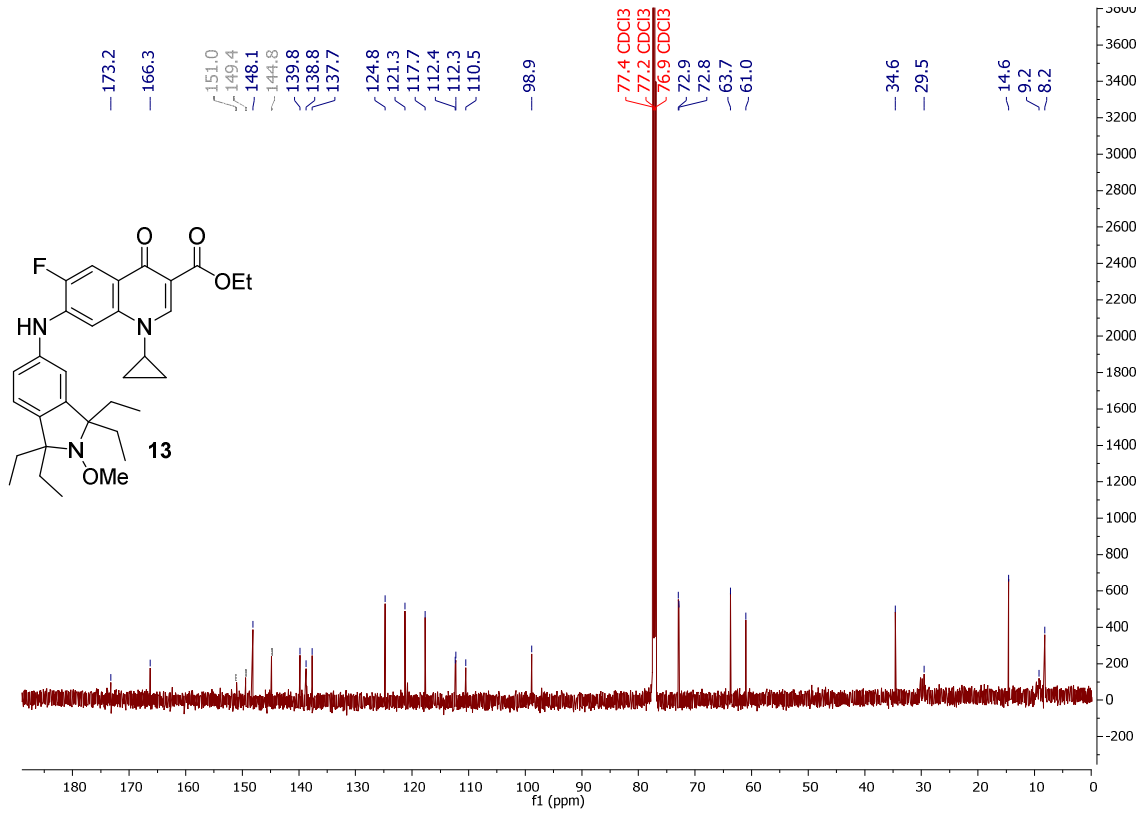

Figure S17. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **13**.

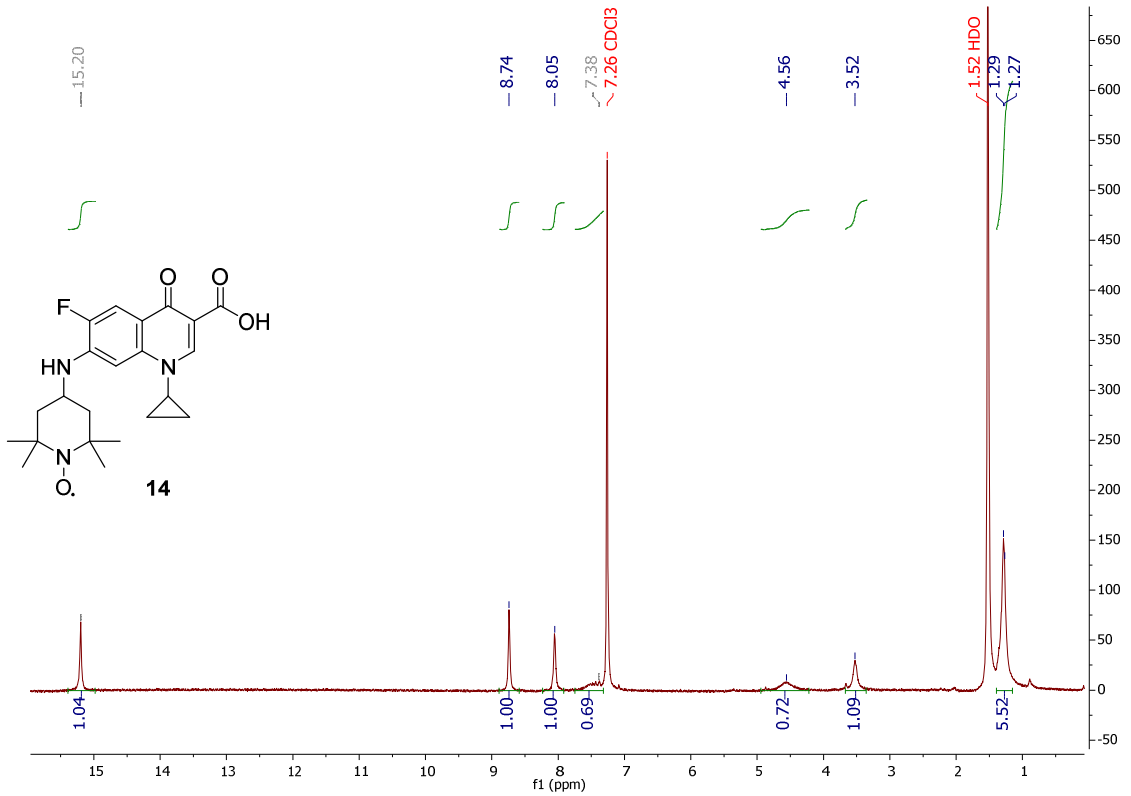

Figure S18. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **14**.

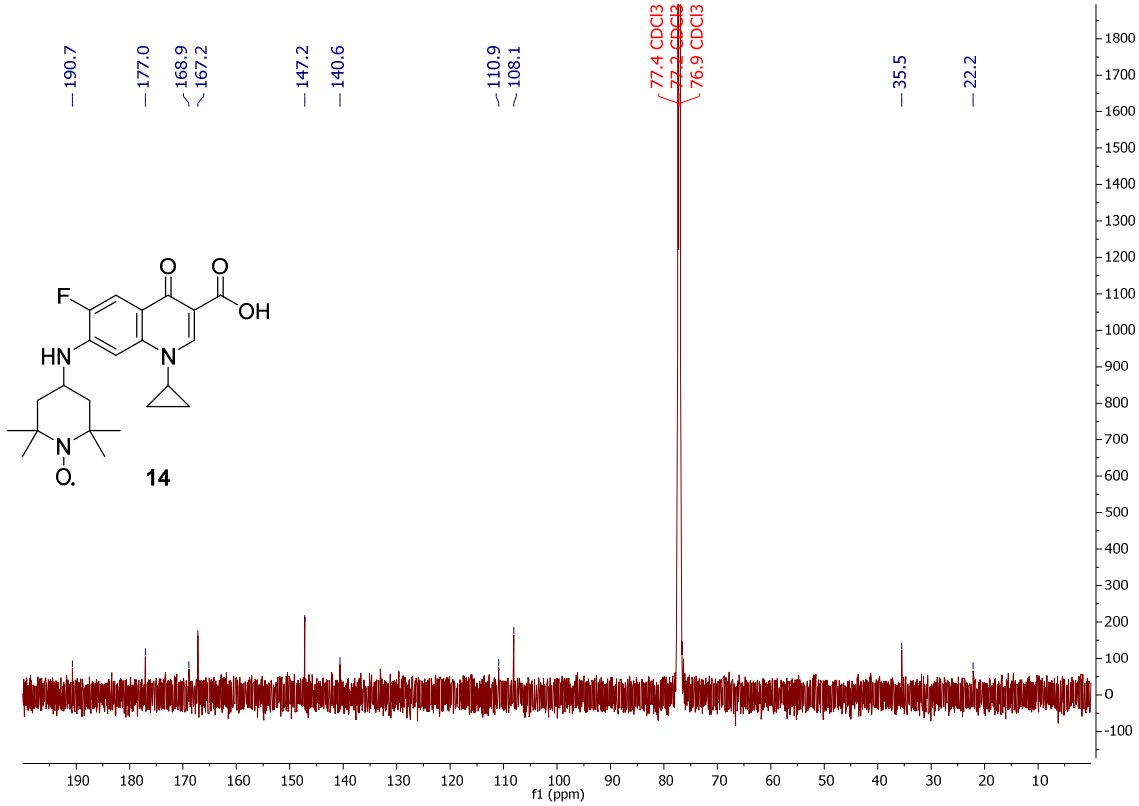

Figure S19. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **14**.

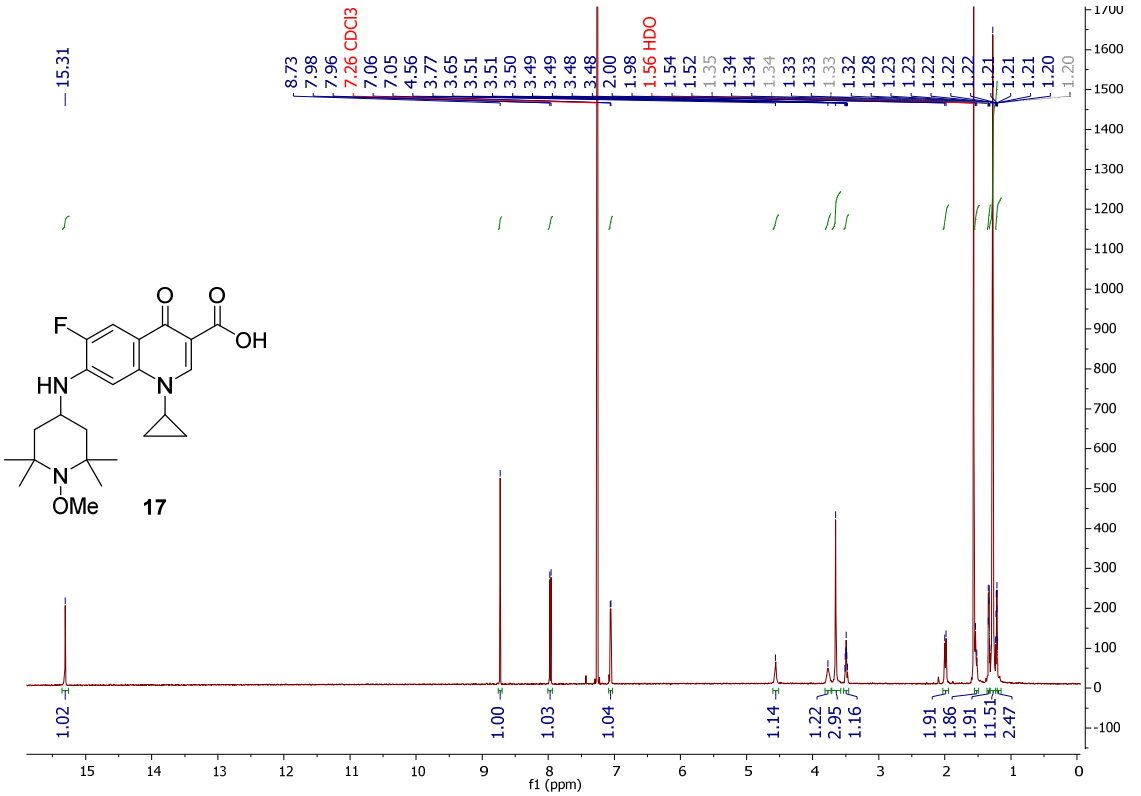

Figure S20. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **17**.

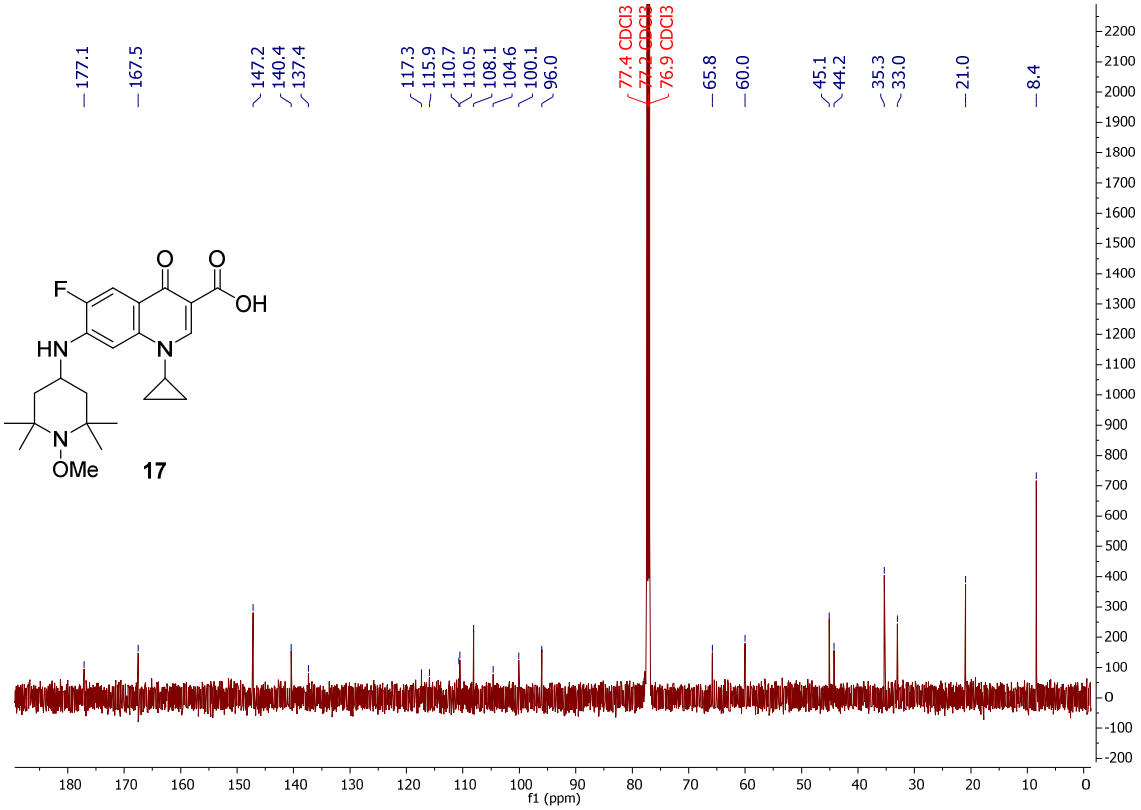

Figure S21. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **17**.

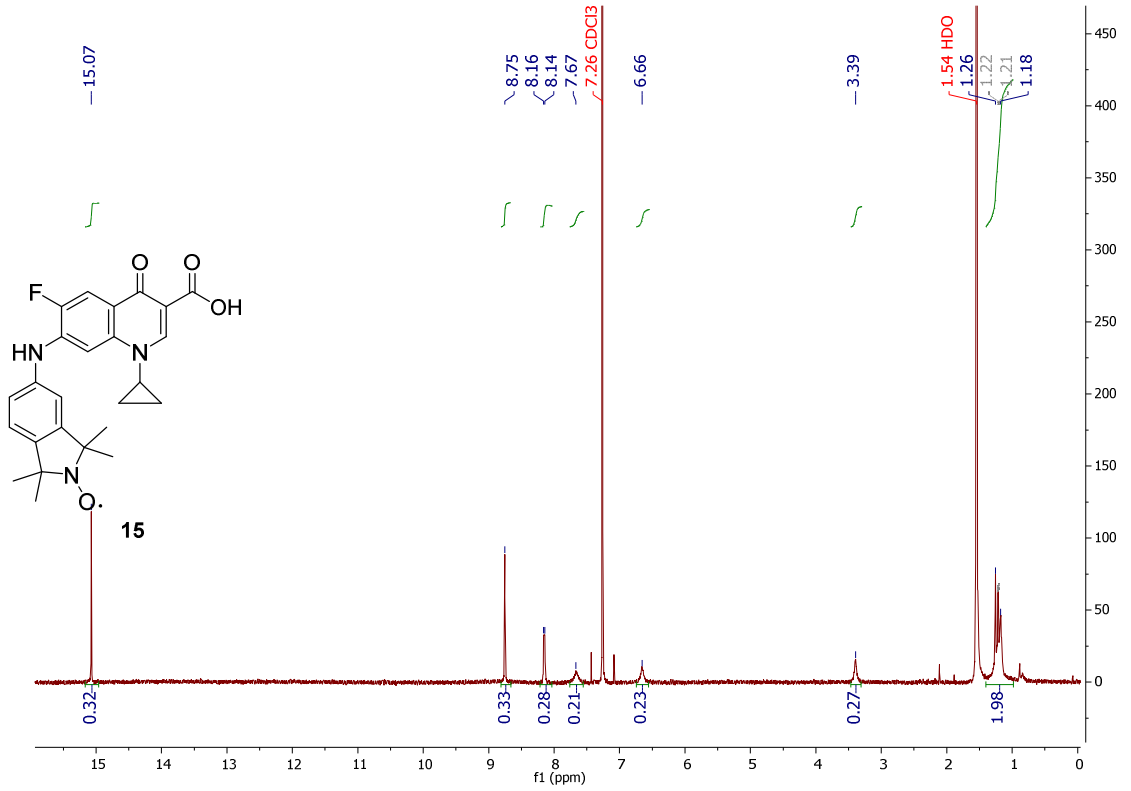

Figure S22. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **15**.

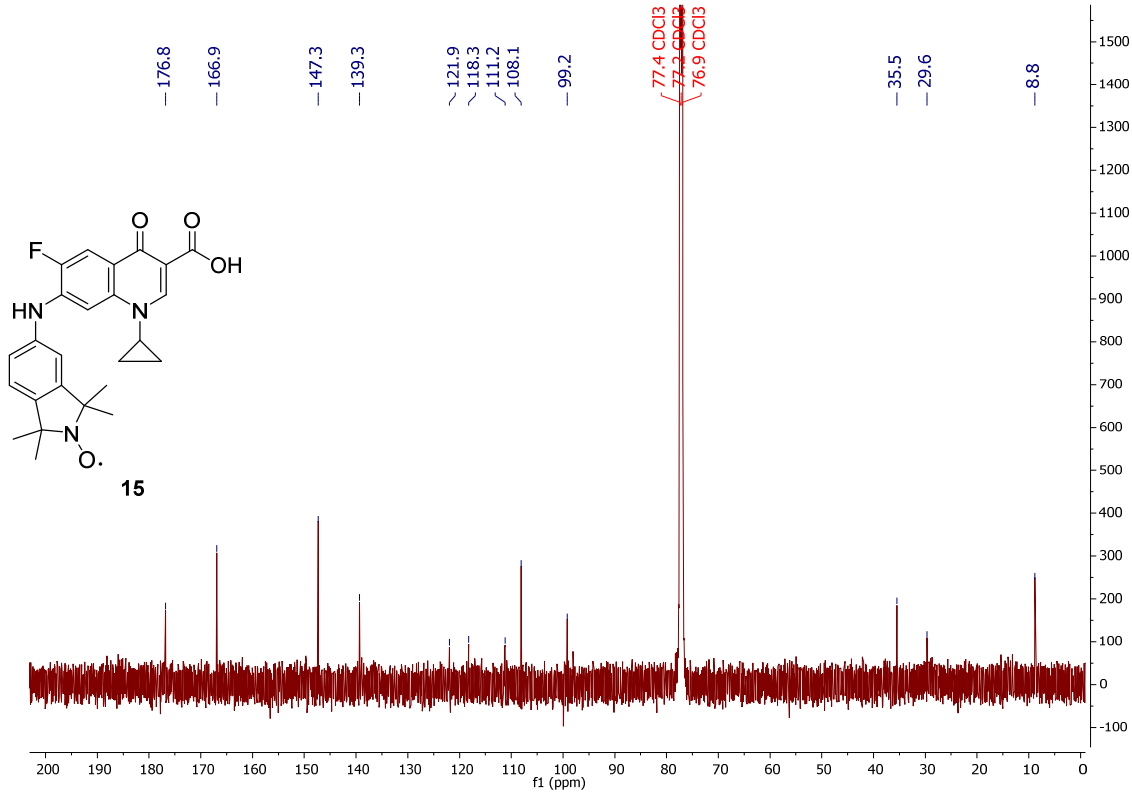

Figure S23. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **15**.

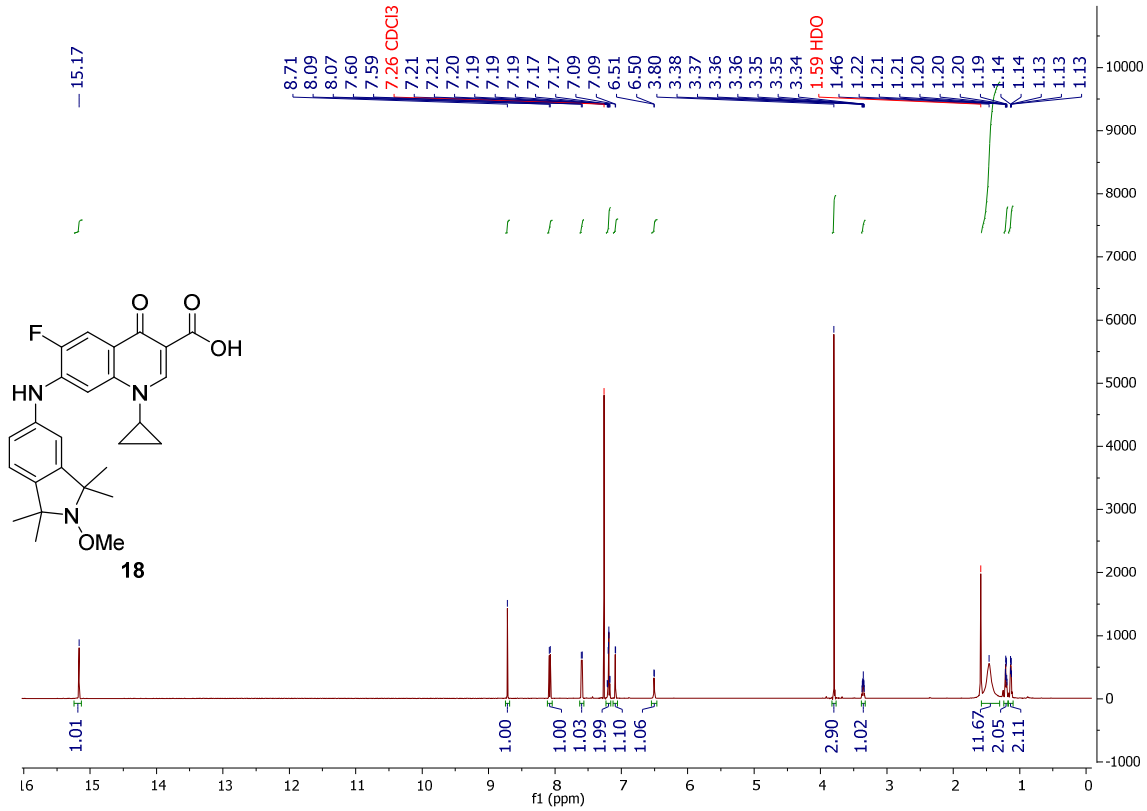

Figure S24. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **18**.

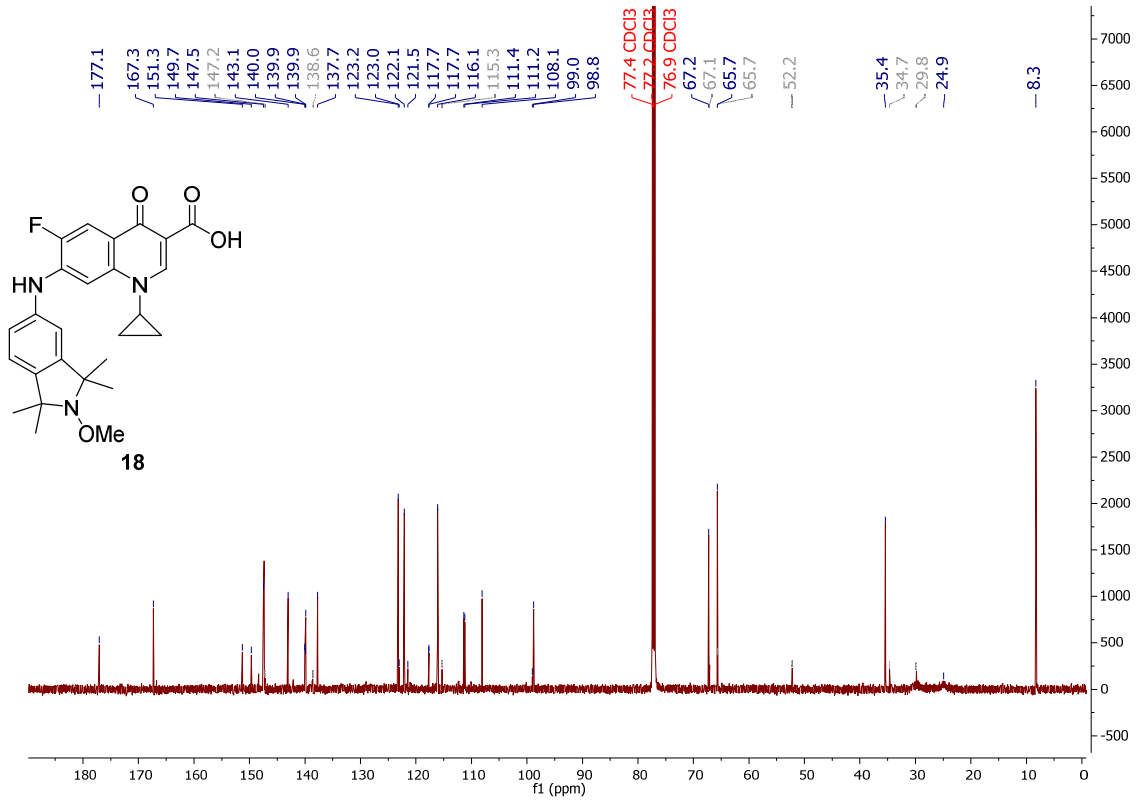

Figure S25. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **18**.

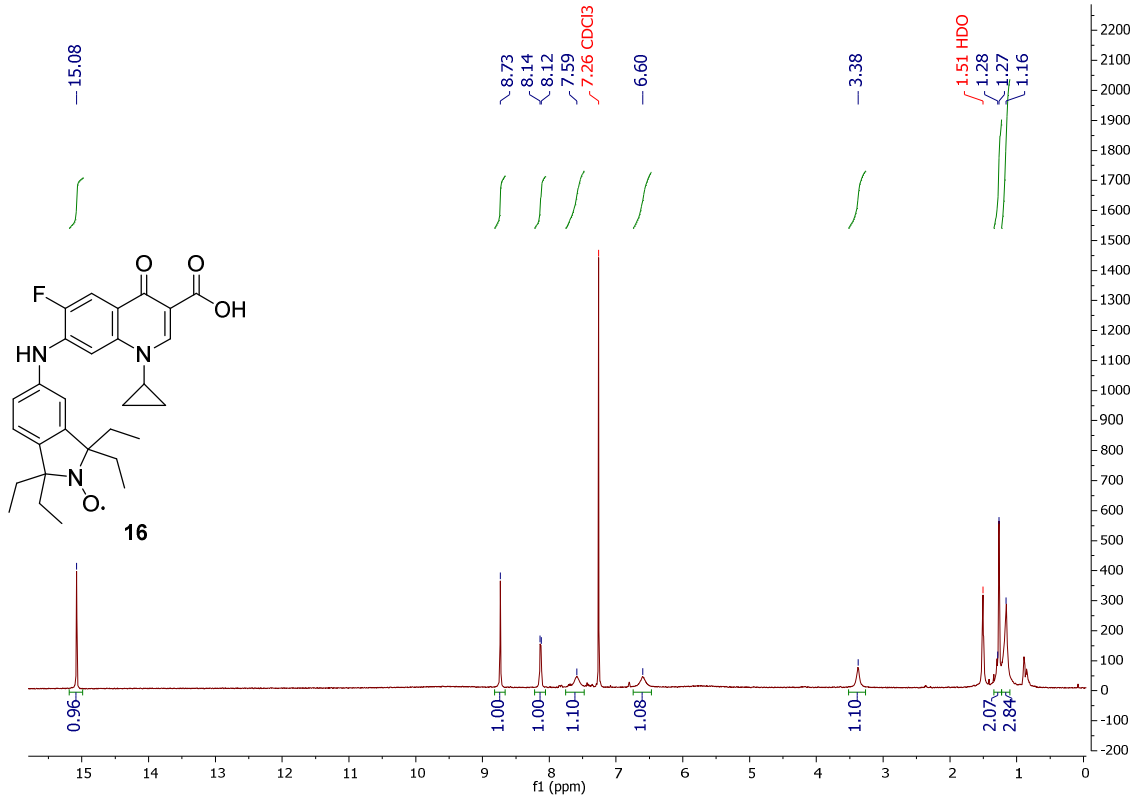

Figure S26. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **16**.

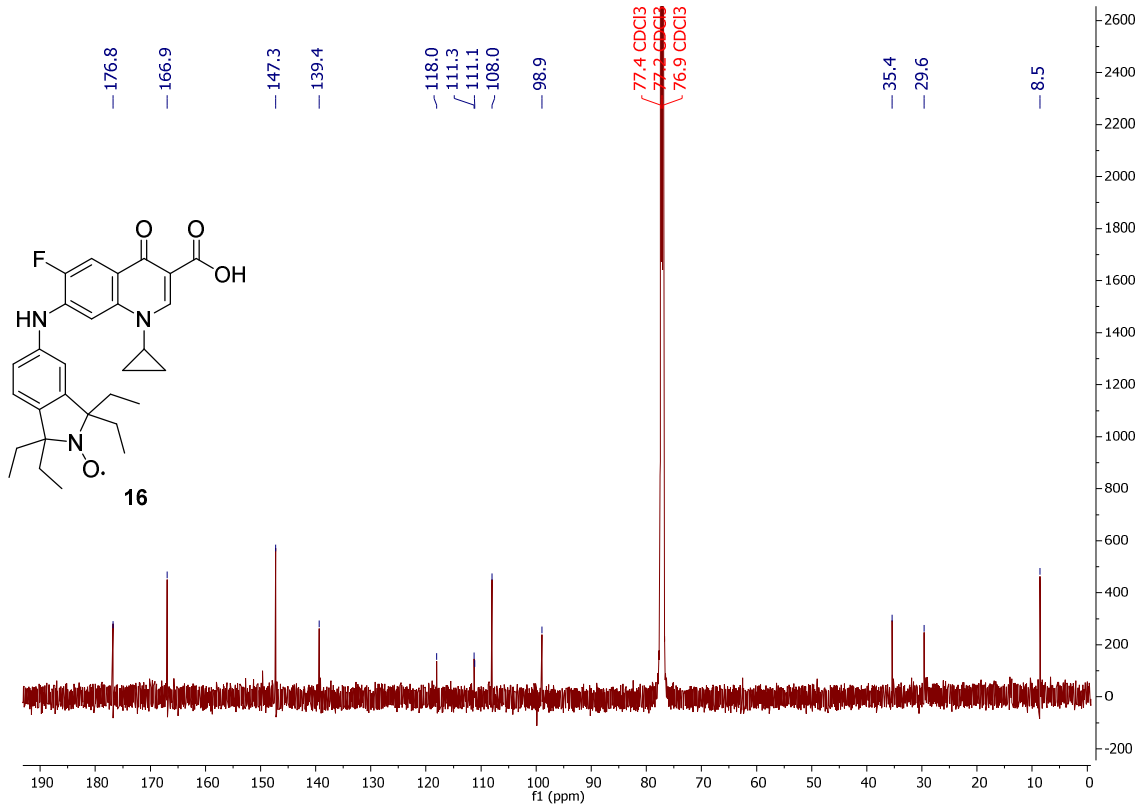

Figure S27. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **16**.

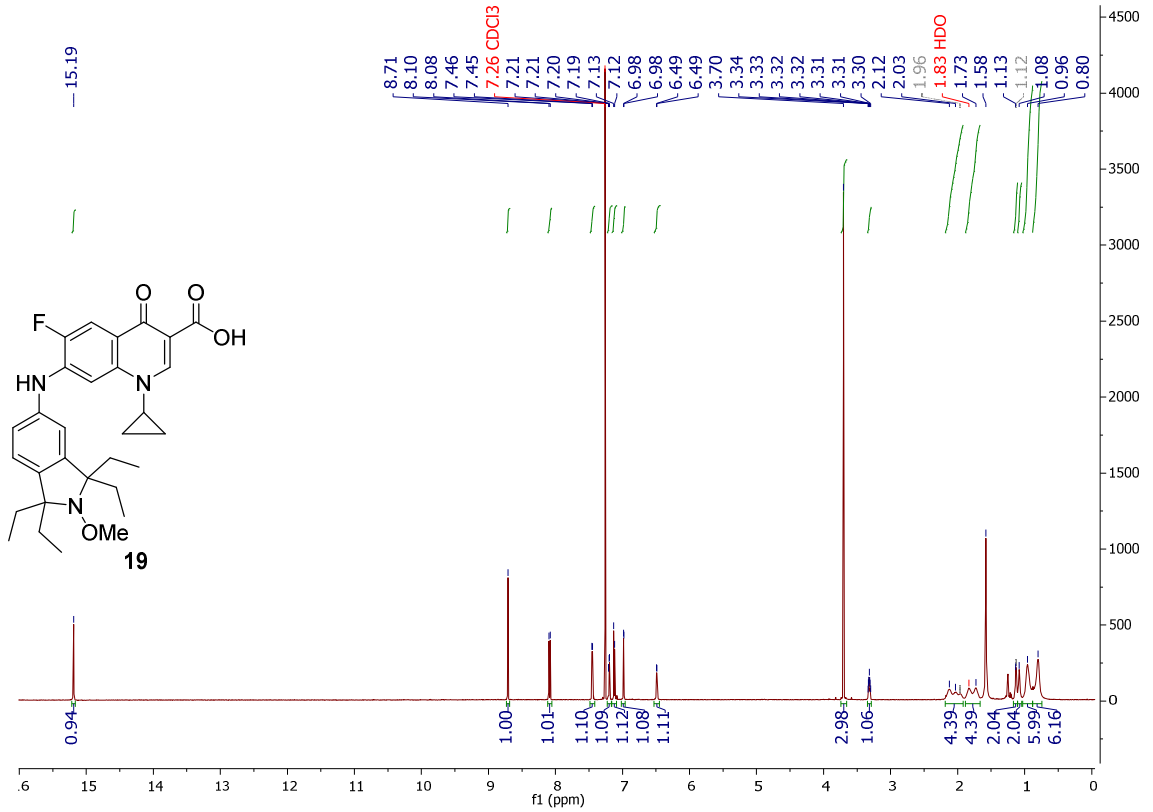

Figure S28. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **19**.

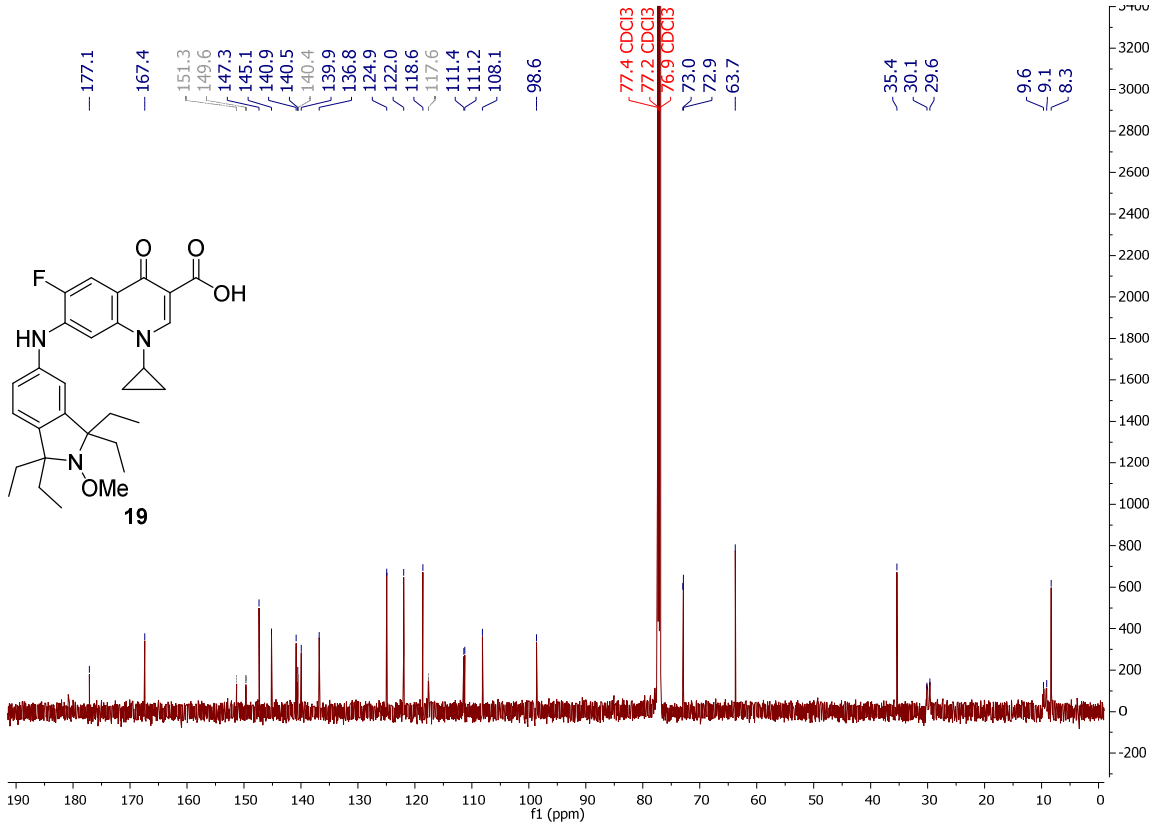

Figure S29. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **19**.

LCMS Chromatograms and HRMS Spectra

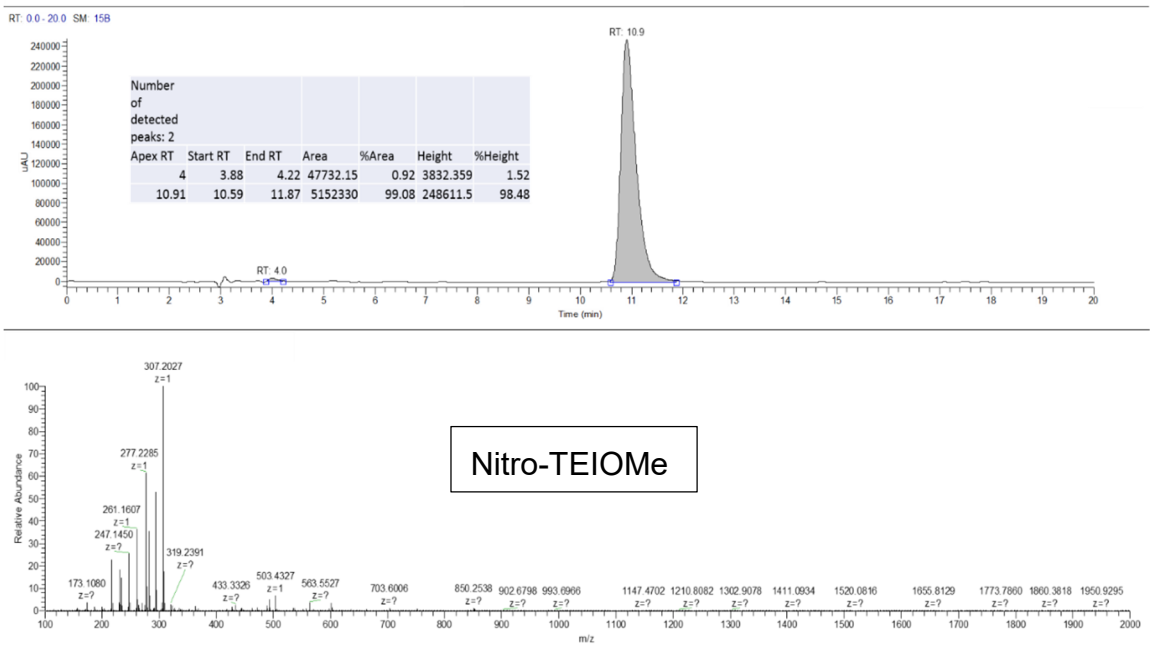

Figure S30. LCMS chromatogram and HRMS spectrum of **Nitro-TEIOMe**.

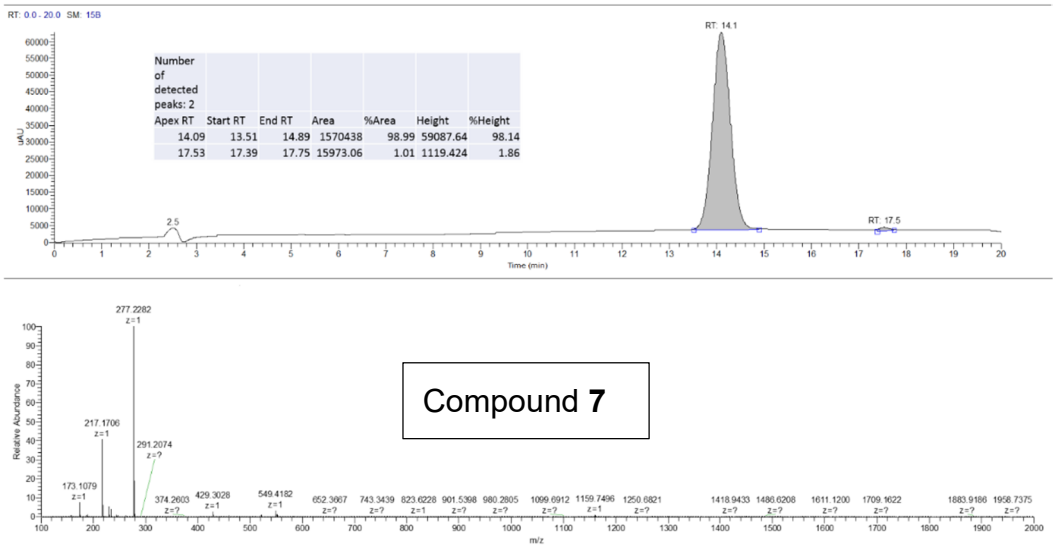

Figure S31. LCMS chromatogram and HRMS spectrum of **7**.

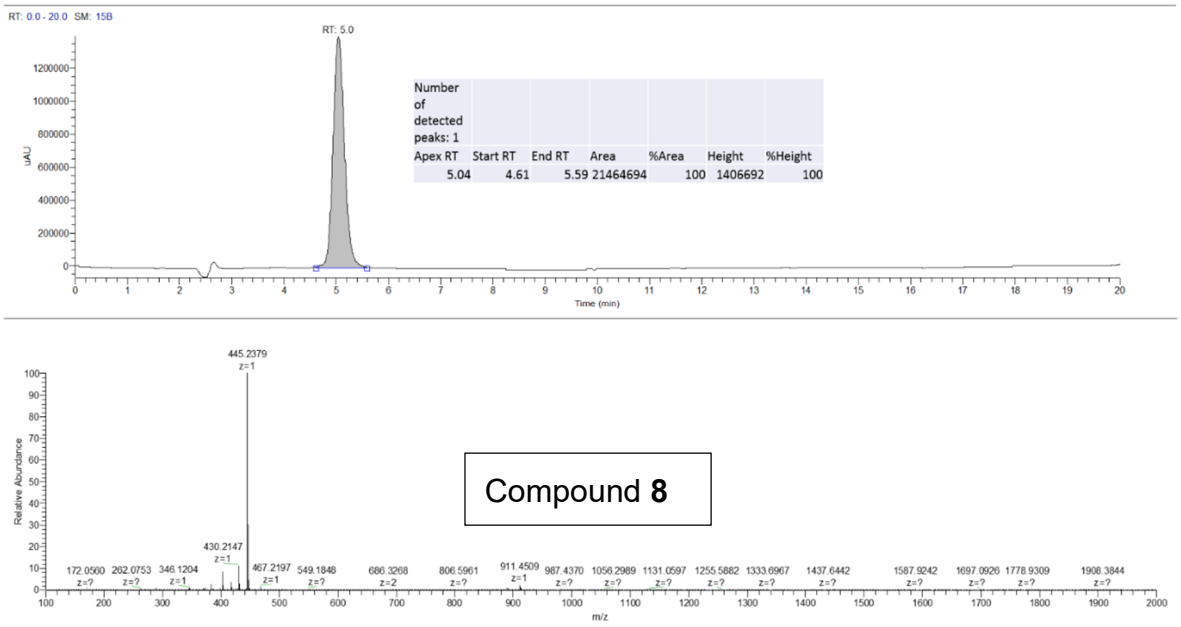

Figure S32. LCMS chromatogram and HRMS spectrum of **8**.

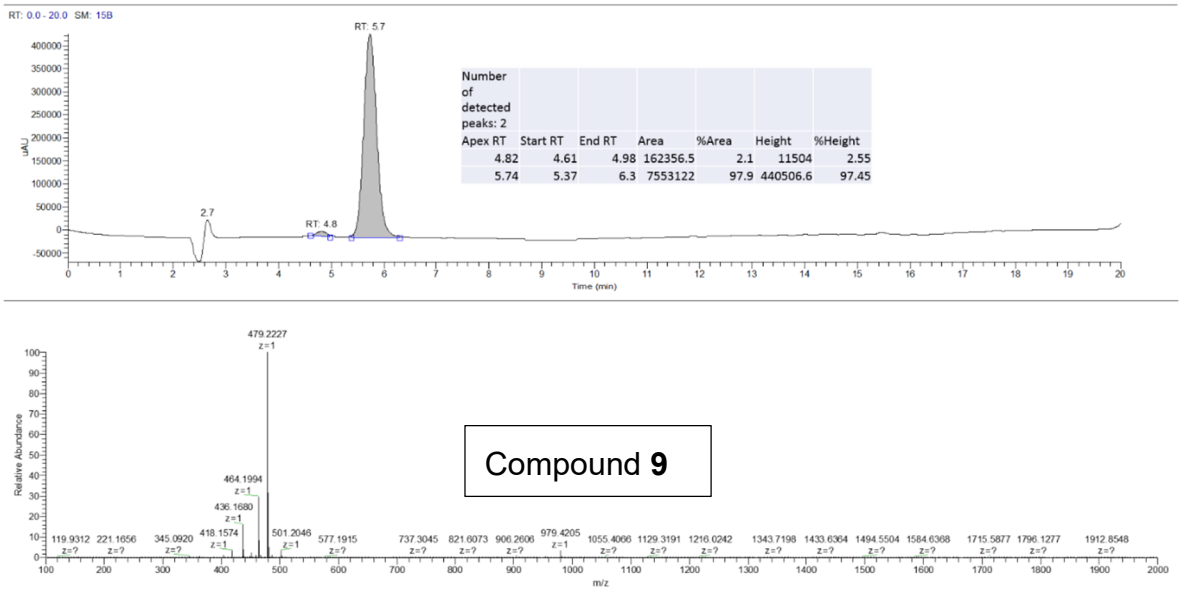

Figure S33. LCMS chromatogram and HRMS spectrum of **9**.

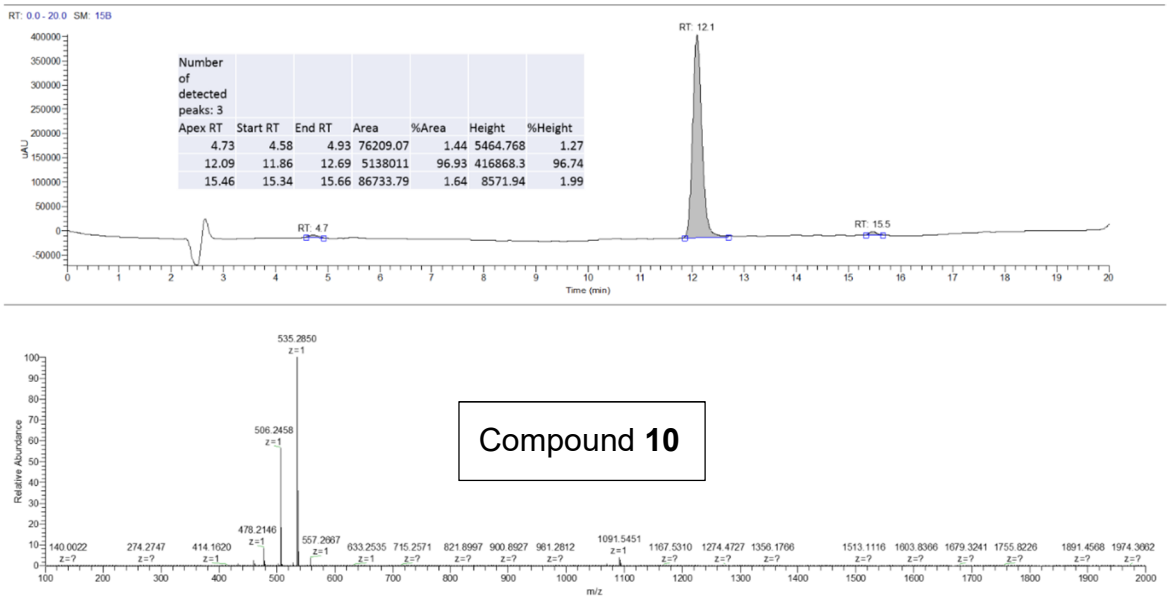

Figure S34. LCMS chromatogram and HRMS spectrum of **10**.

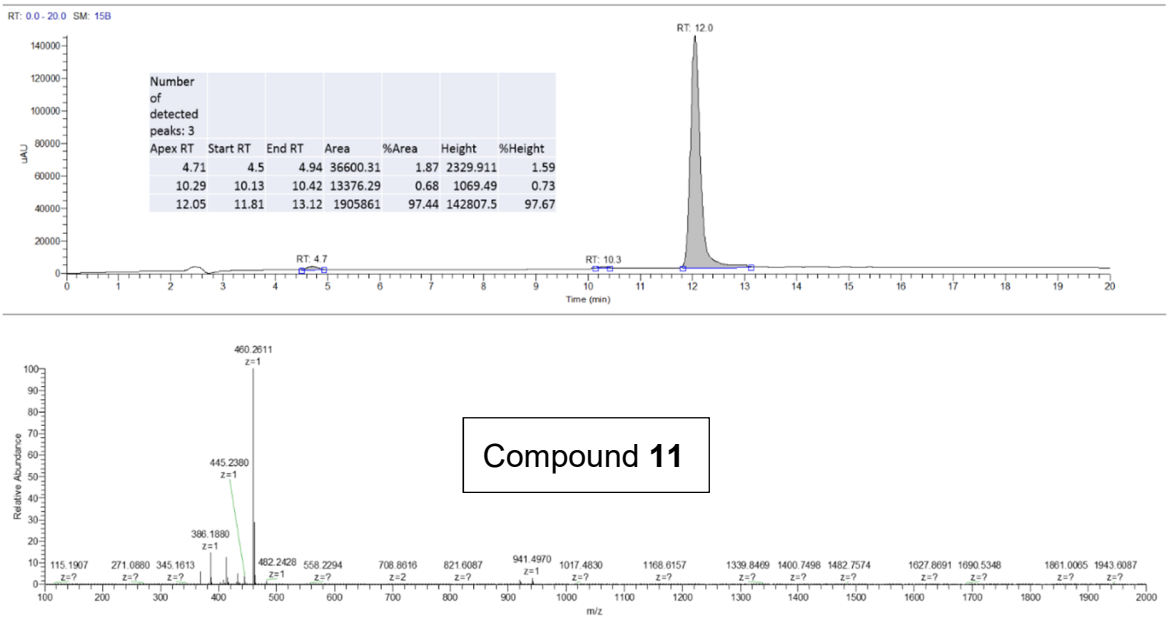

Figure S35. LCMS chromatogram and HRMS spectrum of **11**.

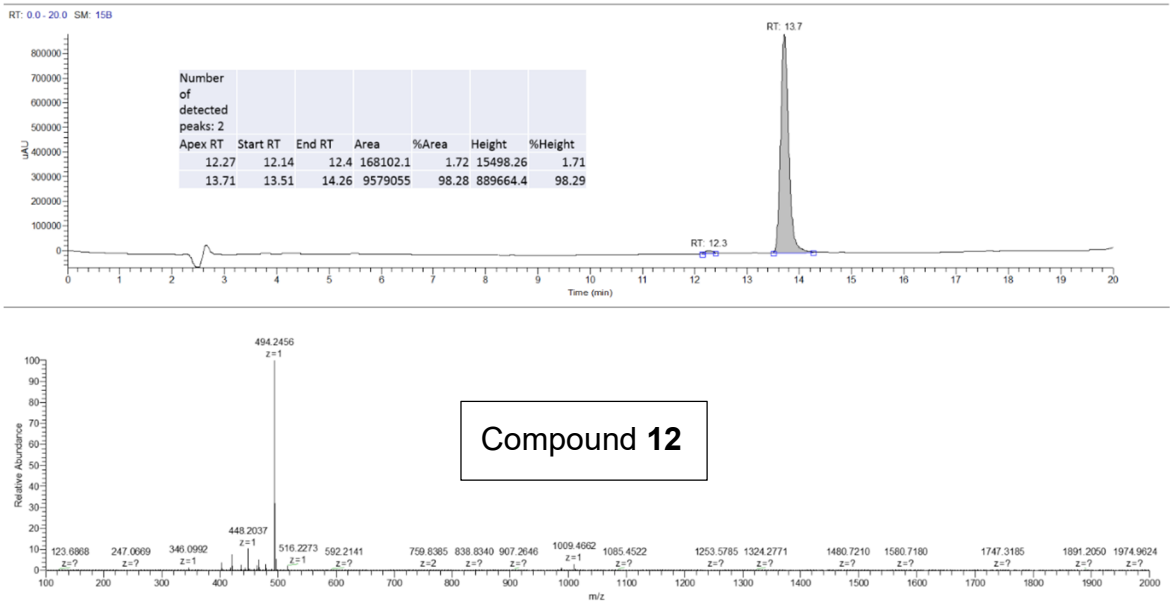

Figure S36. LCMS chromatogram and HRMS spectrum of **12**.

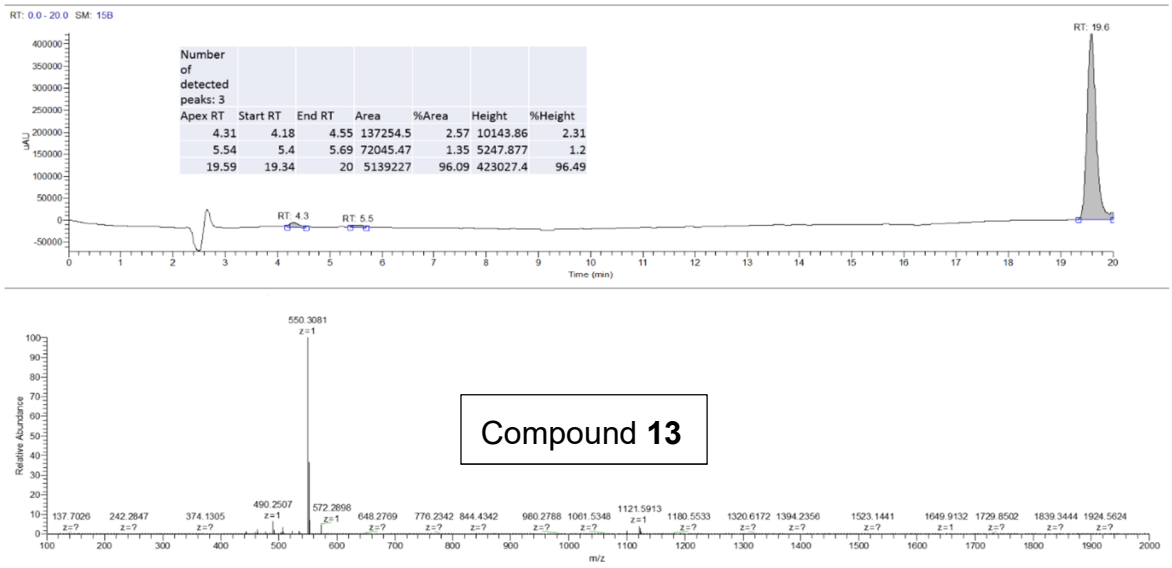

Figure 37. LCMS chromatogram and HRMS spectrum of **13**.

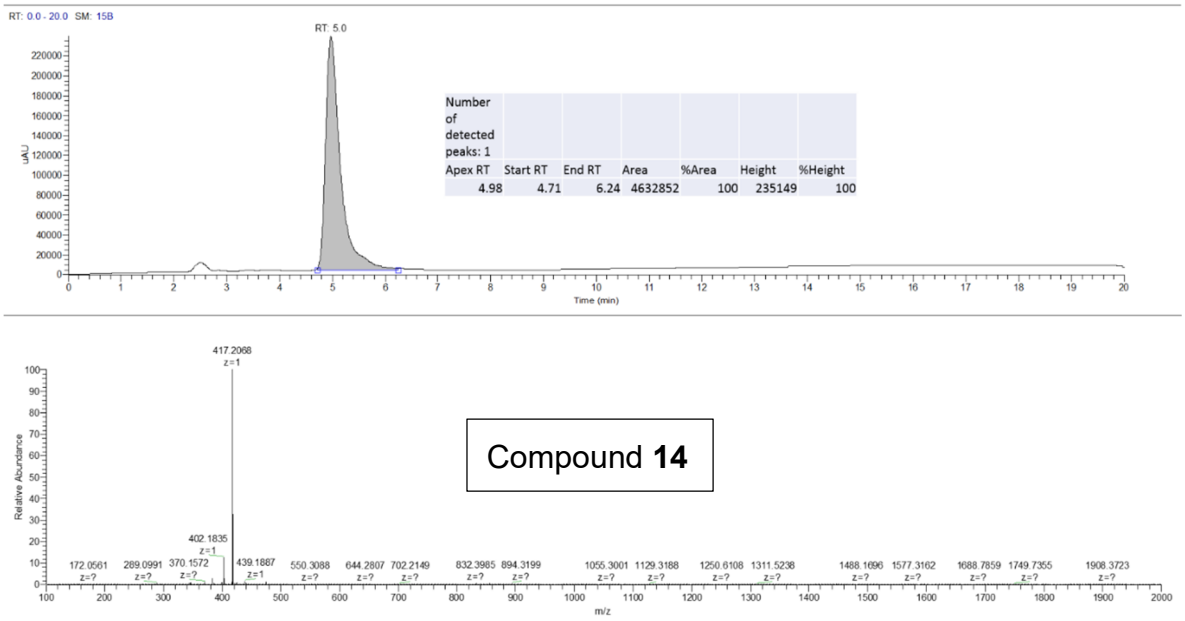

Figure S38. LCMS chromatogram and HRMS spectrum of **14**.

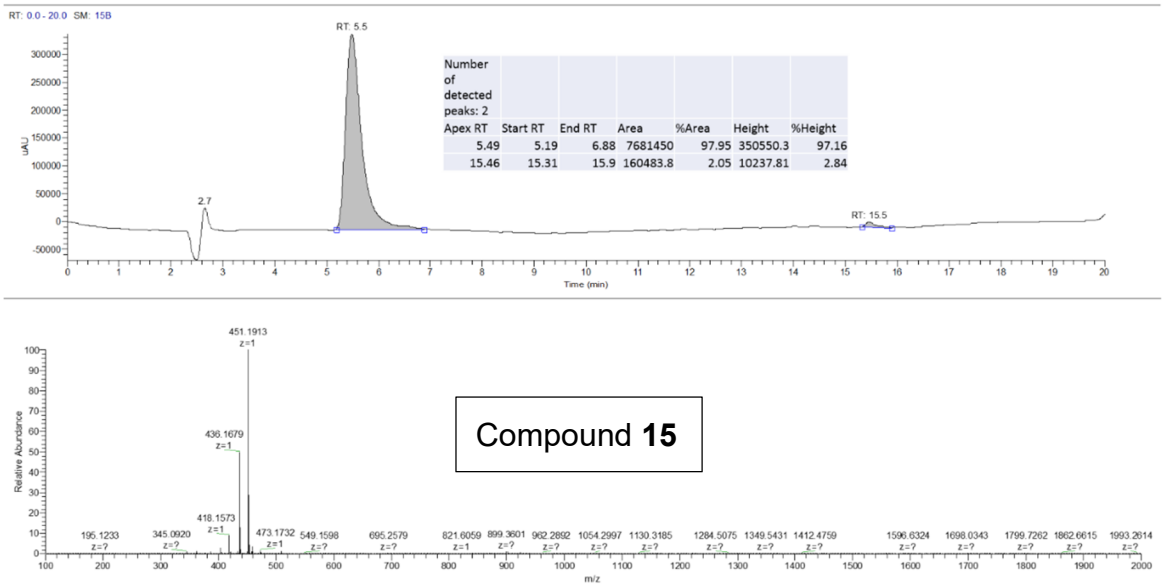

Figure S39. LCMS chromatogram and HRMS spectrum of **15**.

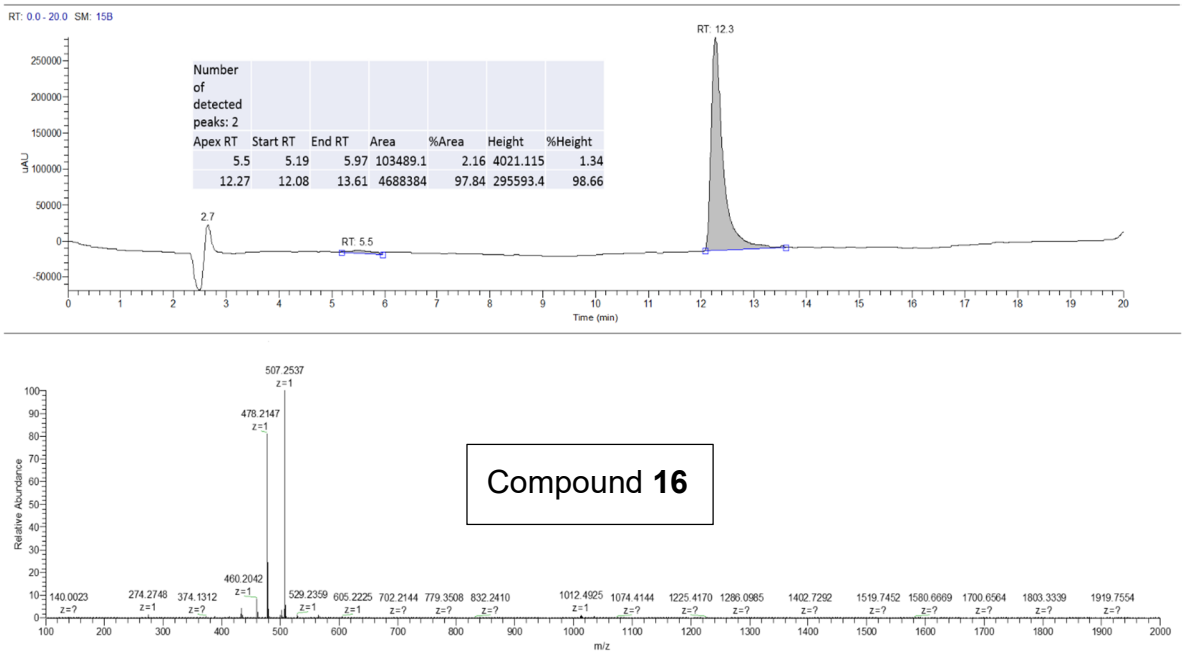

Figure S40. LCMS chromatogram and HRMS spectrum of **16**.

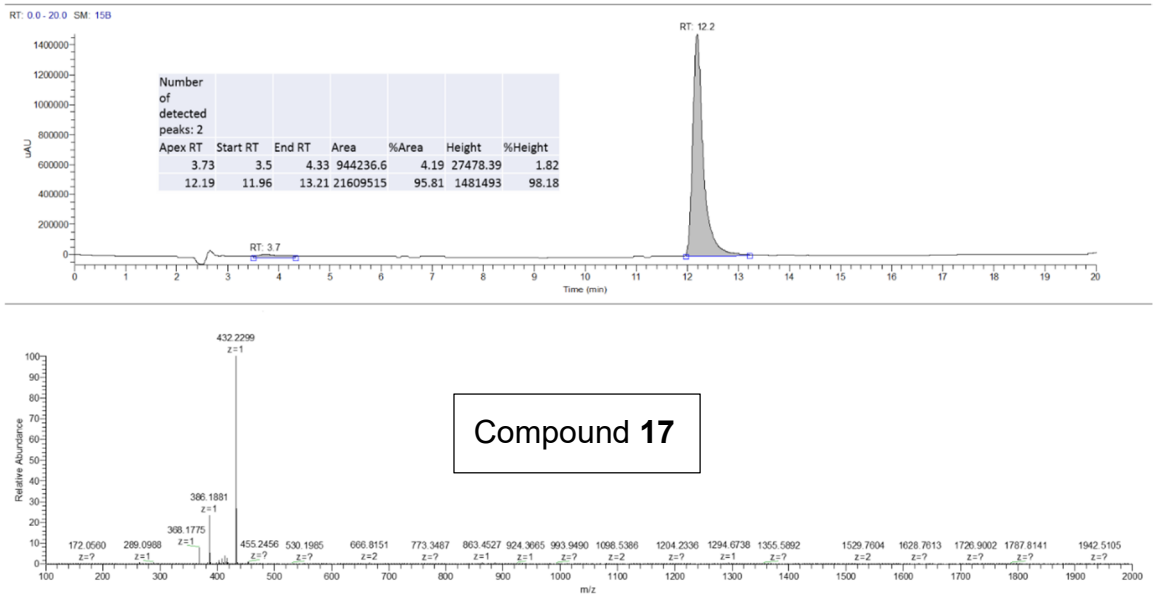

Figure S41. LCMS chromatogram and HRMS spectrum of **17**.

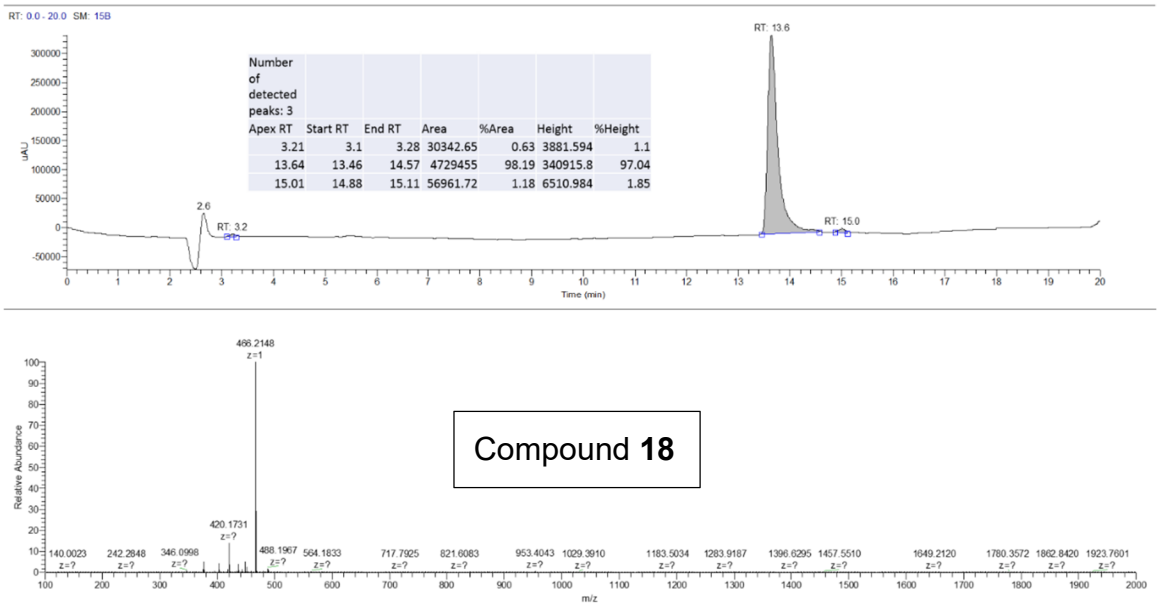

Figure S42. LCMS chromatogram and HRMS spectrum of **18**.

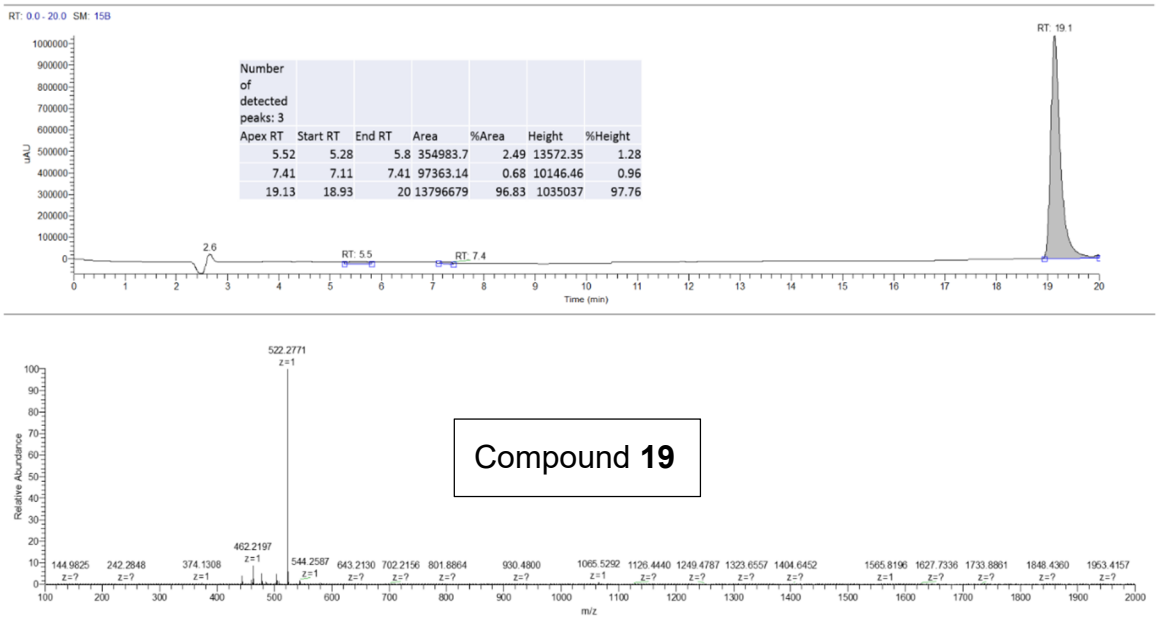

Figure S43. LCMS chromatogram and HRMS spectrum of **19**.