

Supporting Information

Title: Unexpected enhancement of HDACs inhibition by MeS substitution at C-2 position of fluoro largazole

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Experimental Section

General Methods. The chemicals and reagents were purchased from Acros, Alfa Aesar, and National Chemical Reagent Group Co. Ltd., P. R. China, and used without further purification. Anhydrous solvents (THF, MeOH, DMF, CH₂Cl₂, and CH₃CN) used in the reactions were dried and freshly distilled before use. All the reactions were carried out under Ar atmosphere, otherwise stated else. The progress of the reactions was monitored by TLC (silica-coated glass plates) and visualized under UV light, and by using iodine or phosphomolybdic acid. Melting points were measured on a SGW X-4 microscopy melting point apparatus without correction. ¹H NMR and ¹³C NMR spectra were recorded either on a 400 MHz Varian Instrument at 25 °C or 600 MHz Bruke Instrument at 25 °C, using TMS as an internal standard, respectively. Multiplicity is tabulated as s for singlet, d for doublet, dd for doublet of doublet, t for triplet, and m for multiplet. HRMS spectra were recorded on Finnigan-Mat-95 mass spectrometer, equipped with ESI source.

(S,Z)-1-((R)-4-benzyl-2-thioxothiazolidin-3-yl)-4-fluoro-3-hydroxy-7-(tritylthio)hept-4-en-1-one
(8).

S-9-55-2_H1-CDCL3-50303
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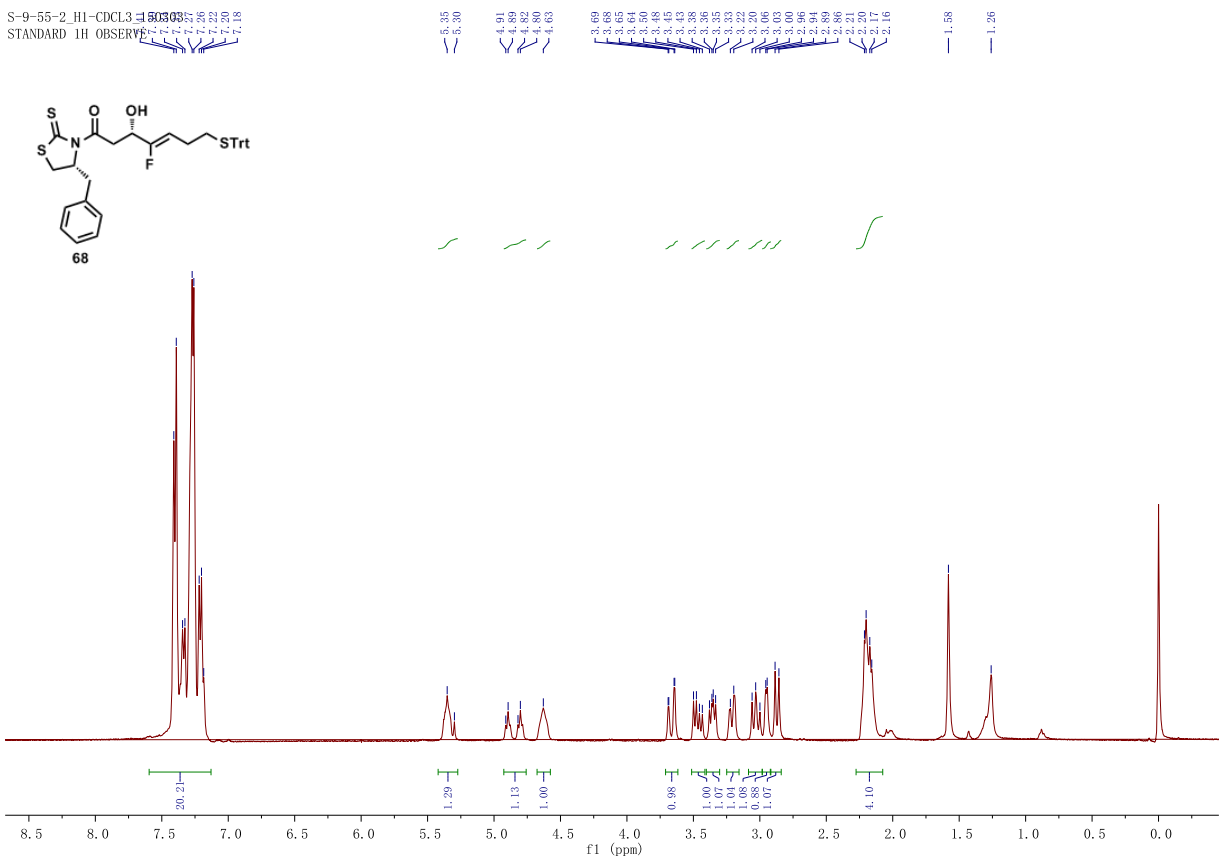


Figure S1. ¹H spectra of compound **8**

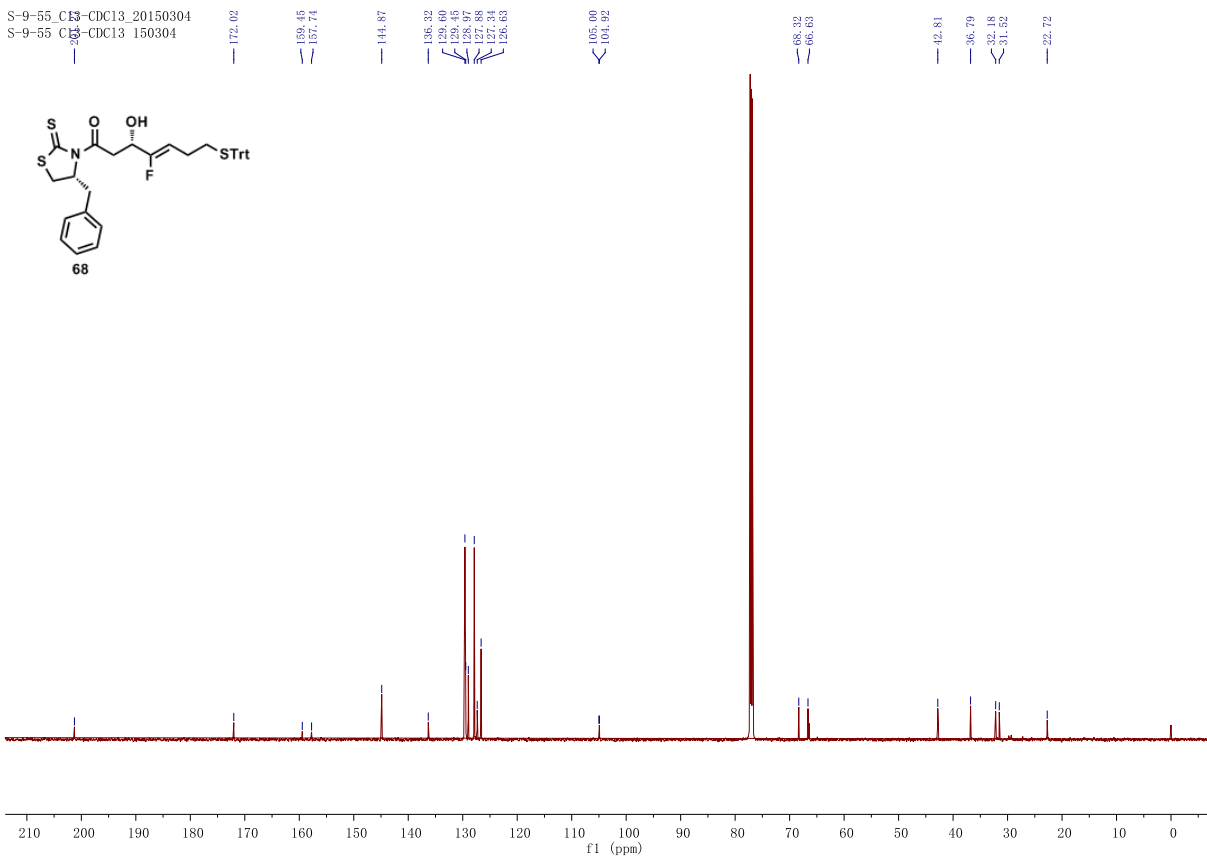


Figure S2. ^{13}C spectra of compound **8**

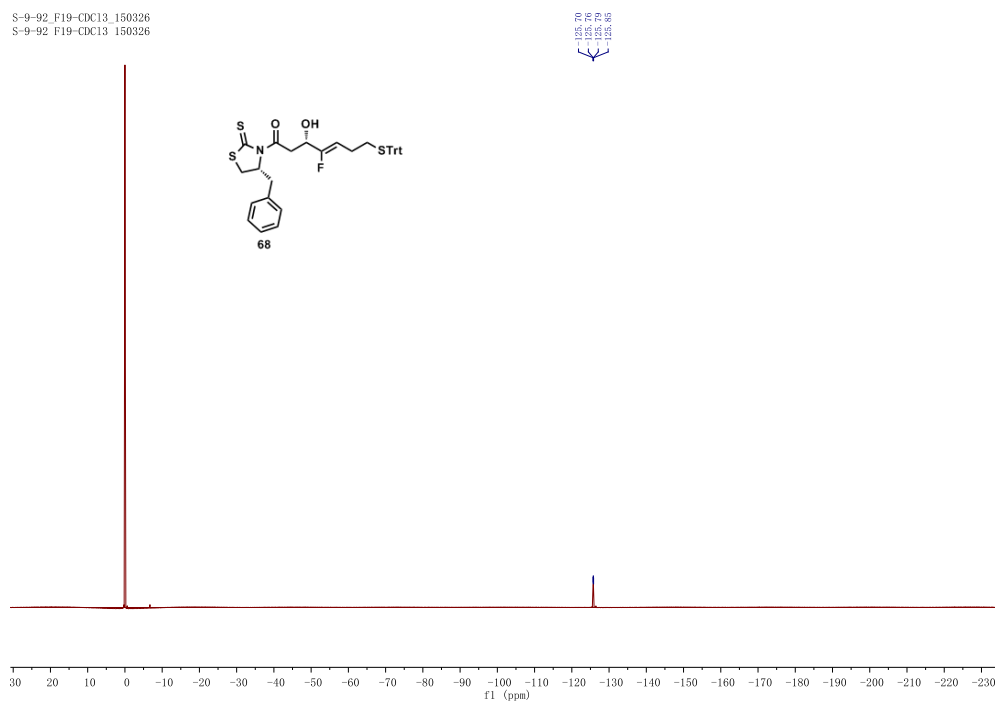


Figure S3. ^{19}F spectra of compound **8**

(R)-2'-(((tert-butoxycarbonyl)amino)methyl)-4-methyl-4,5-dihydro-[2,4'-bithiazole]-4-carboxylic acid (11)

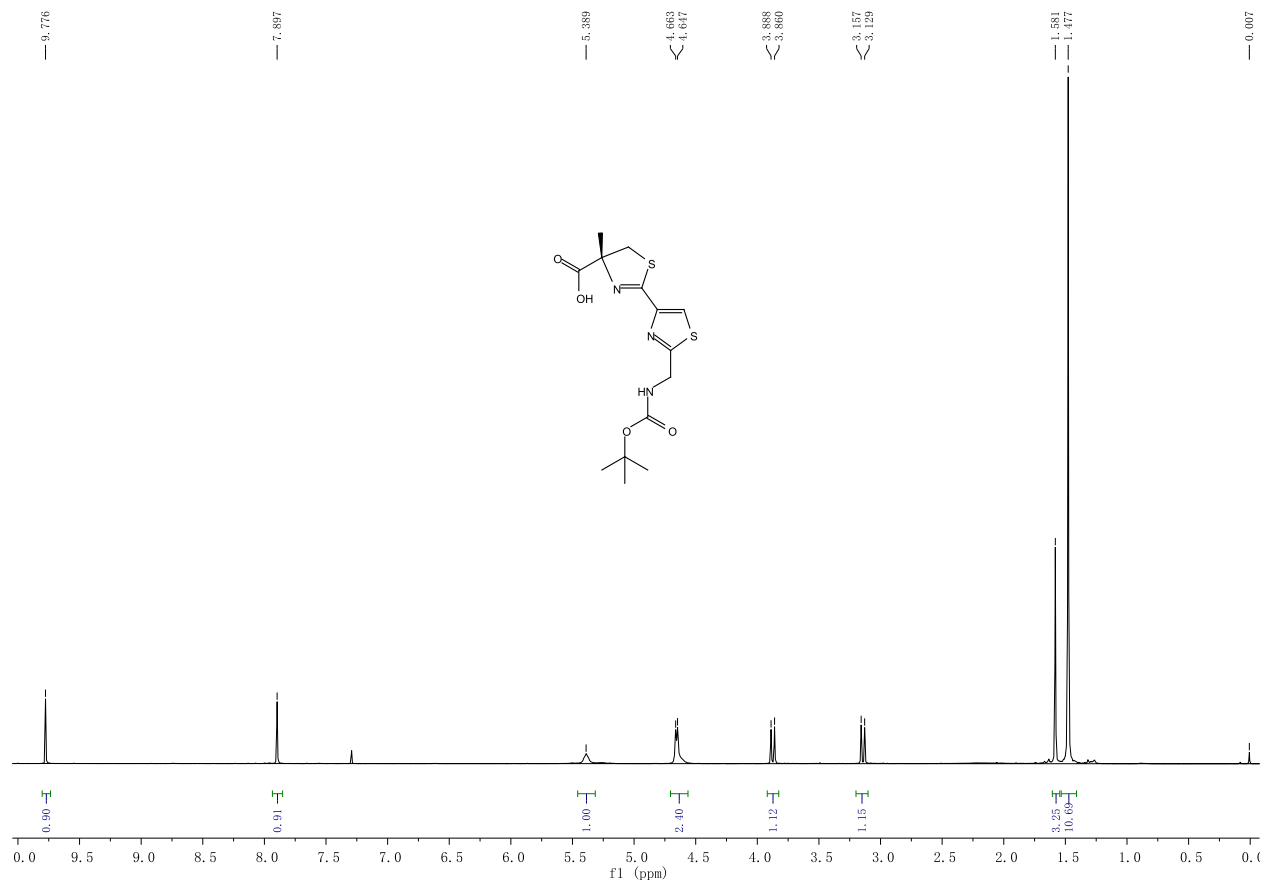


Figure S4. ¹H spectra of compound 11

2-(trimethylsilyl)ethyl (S,Z)-4-fluoro-3-hydroxy-7-(tritylthio)hept-4-enoate (9)

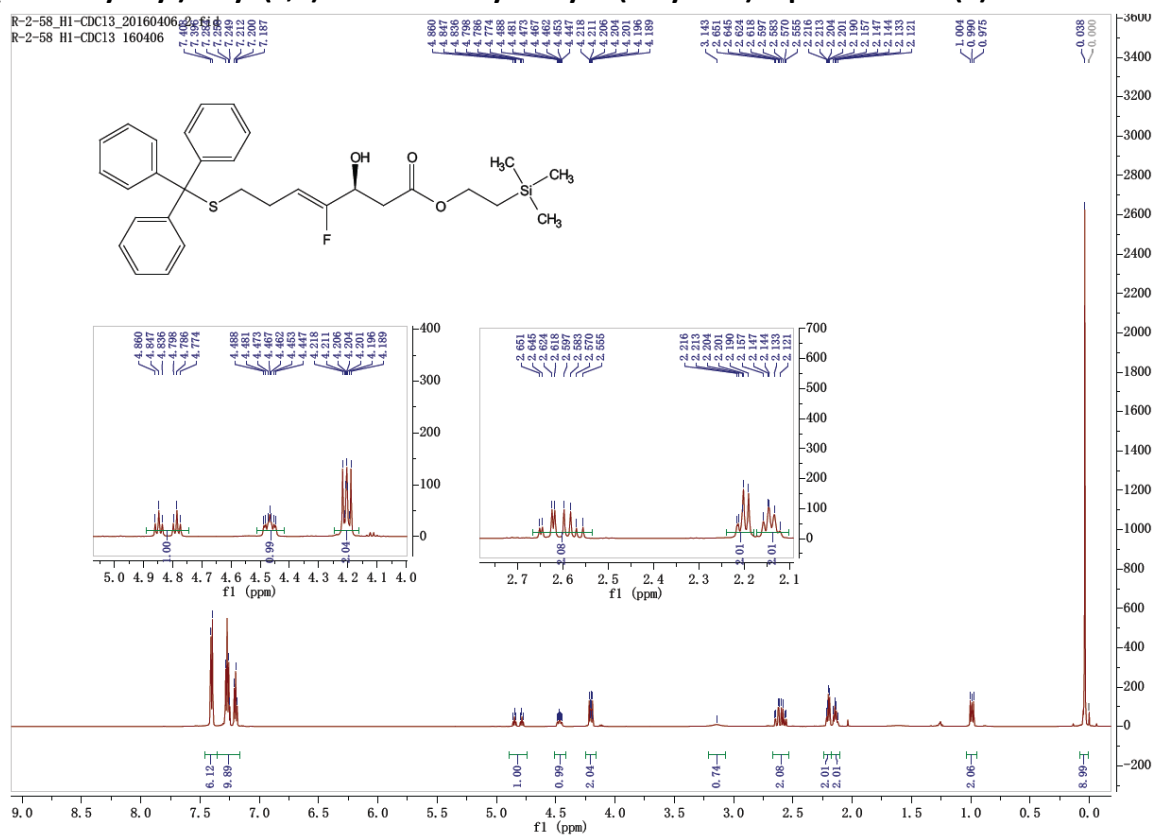


Figure S5. ¹H spectra of compound 9

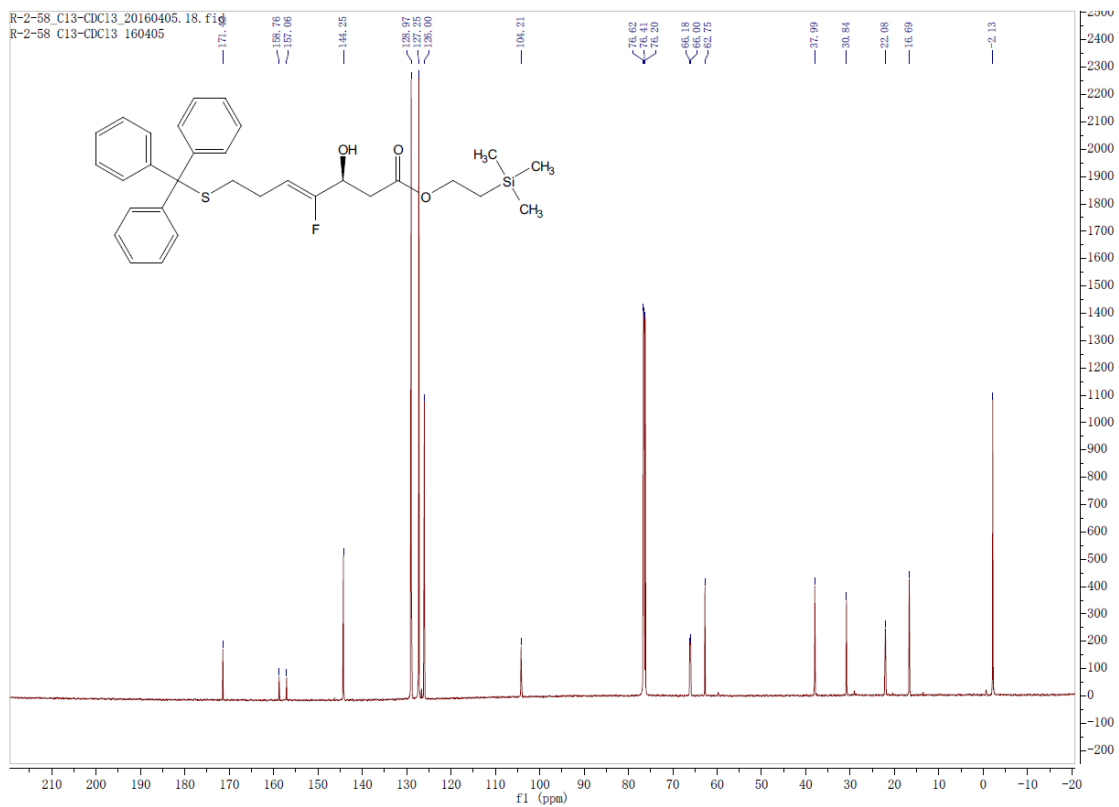


Figure S6. ^{13}C spectra of compound 9

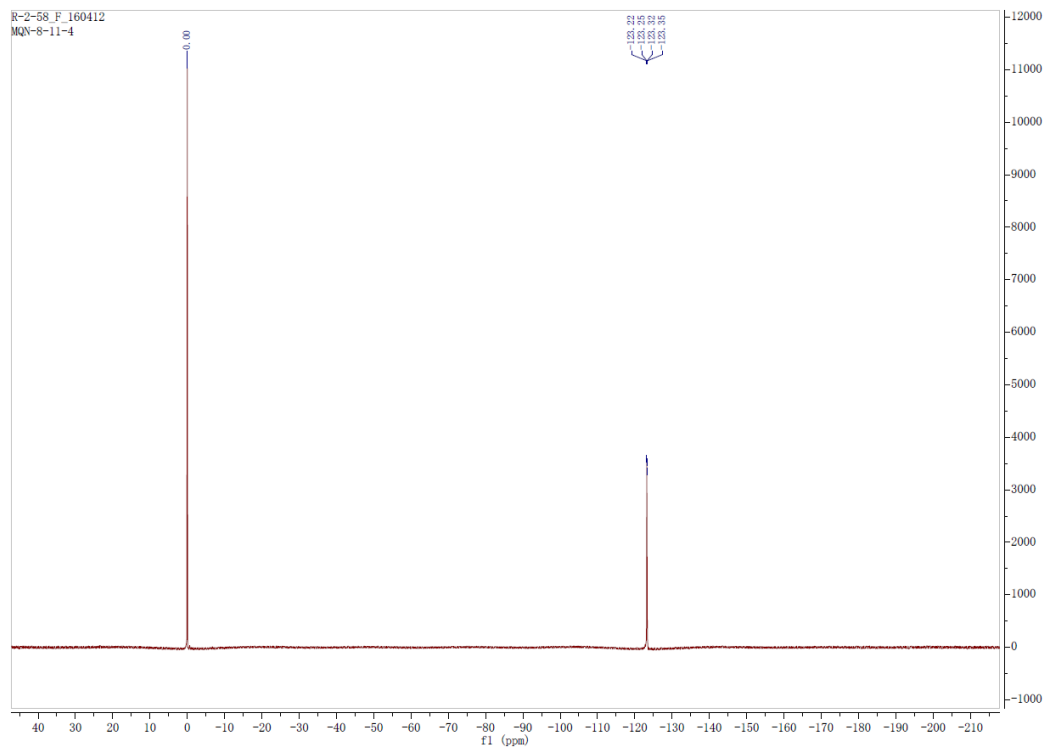
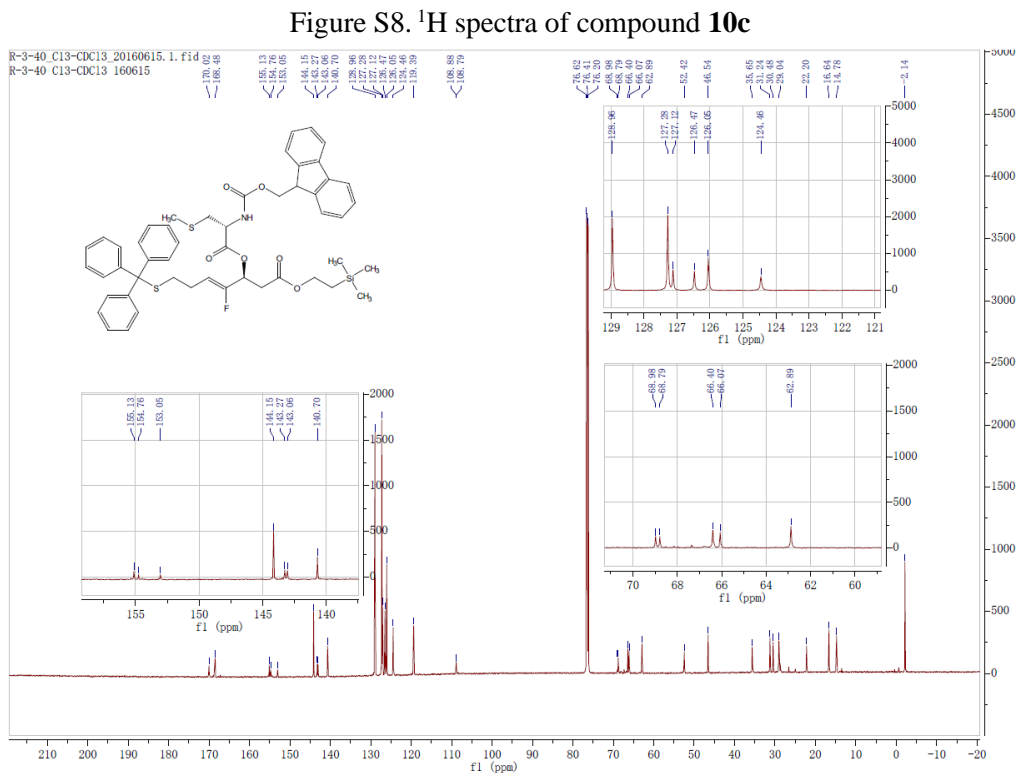
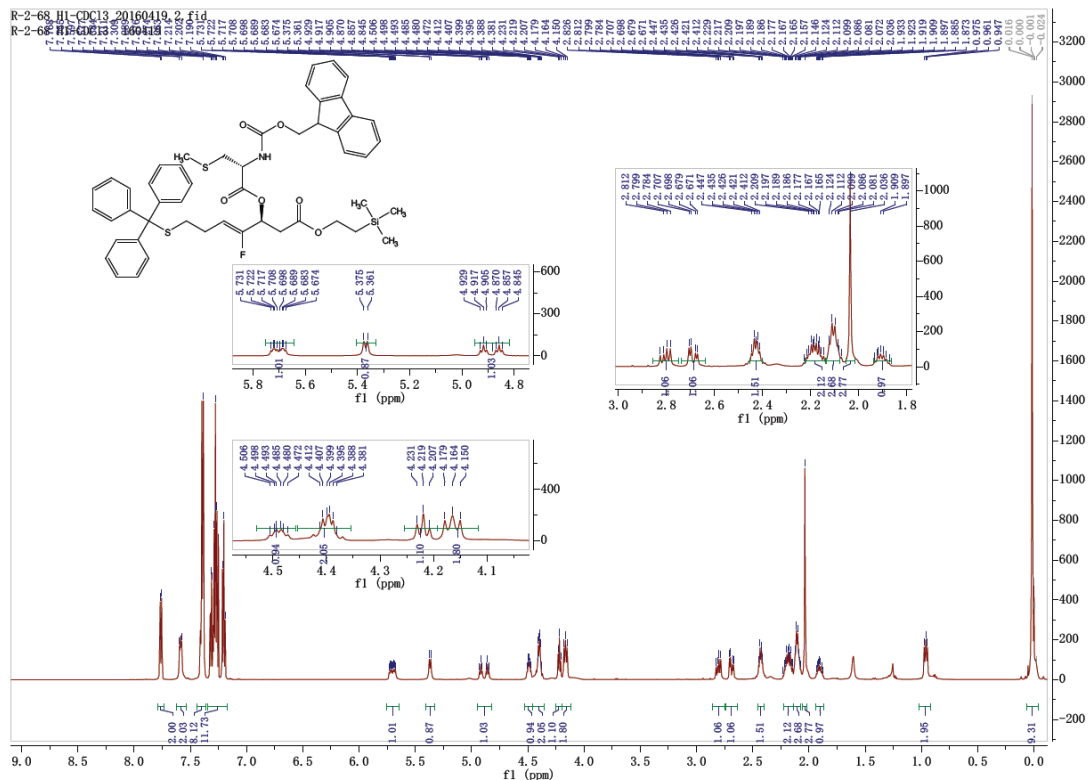


Figure S7. ^{19}F spectra of compound 9

2-(trimethylsilyl)ethyl (S,Z)-3-((N-(((9H-fluoren-9-yl)methoxy)carbonyl)-S-methyl-L-cysteinyl)oxy)-4-fluoro-7-(tritylthio)hept-4-enoate (10c).



2-(trimethylsilyl)ethyl (S,Z)-3-((N-((R)-2'-(((tert-butoxycarbonyl)amino)methyl)-4-methyl-4,5-dihydro-[2,4'-bithiazole]-4-carbonyl)-S-methyl-L-cysteinyl)oxy)-4-fluoro-7-(tritylthio)hept-4-enoate (**12c**)

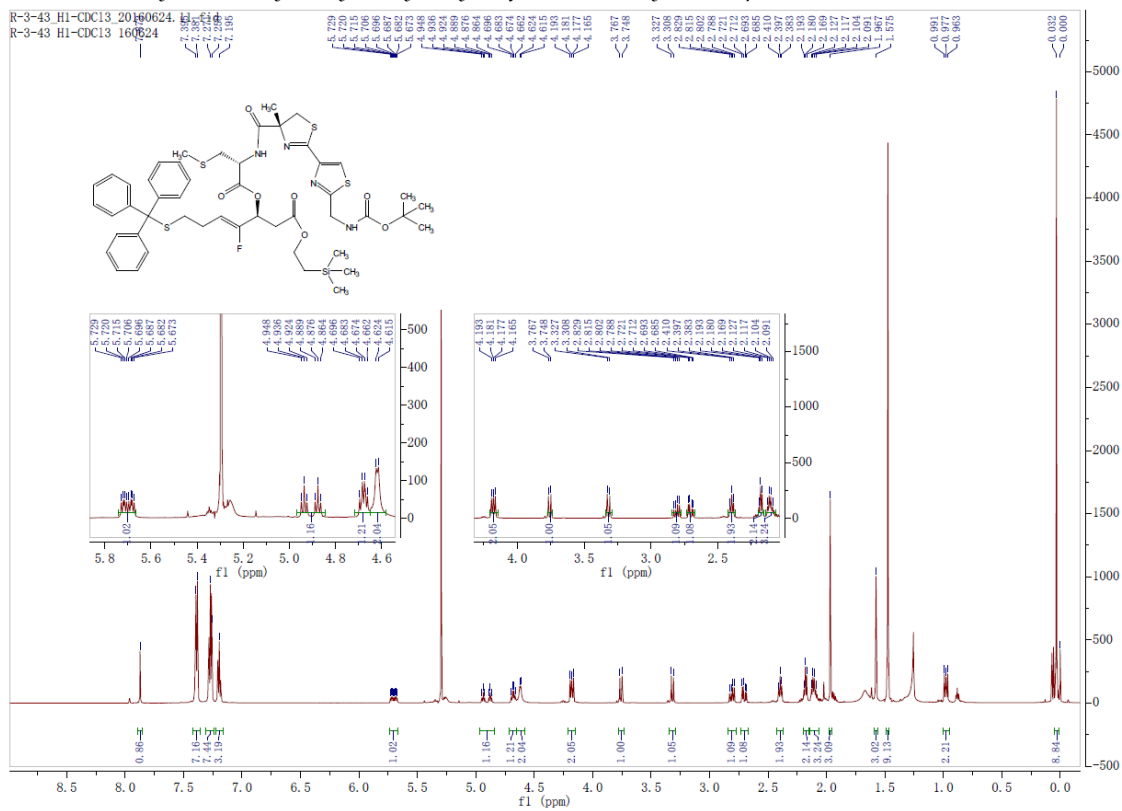


Figure S10. ¹H spectra of compound **12c**

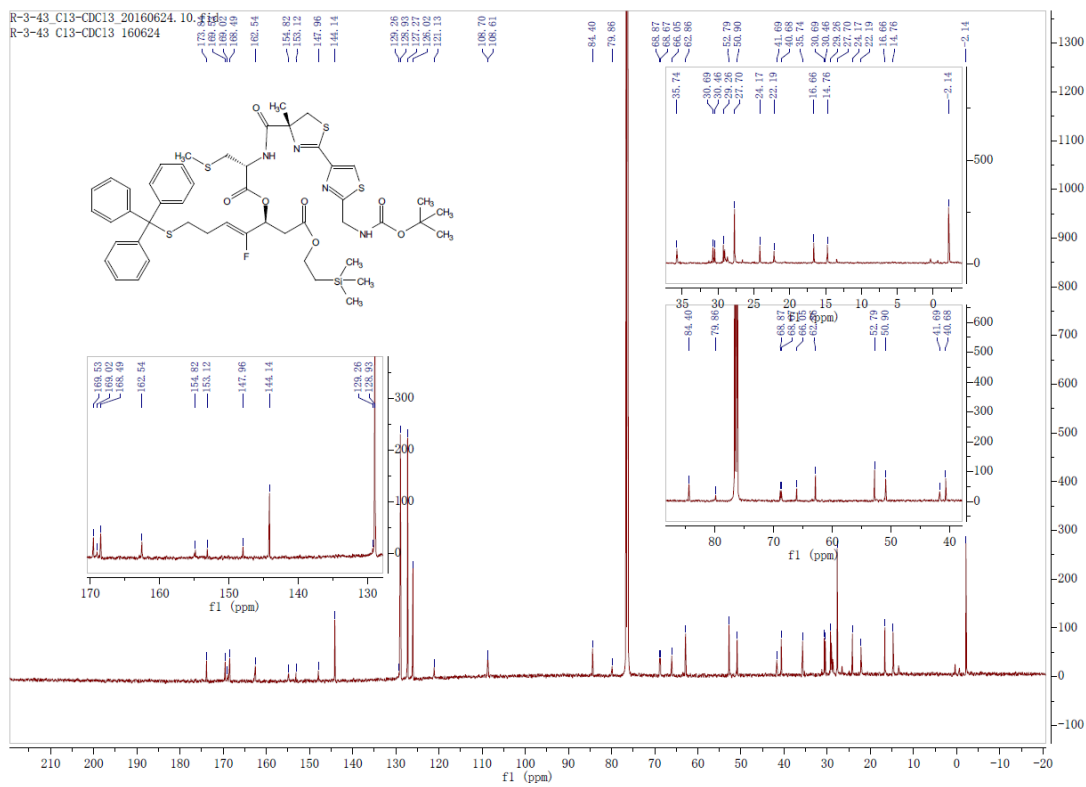


Figure S11. ^{13}C spectra of compound **12c**

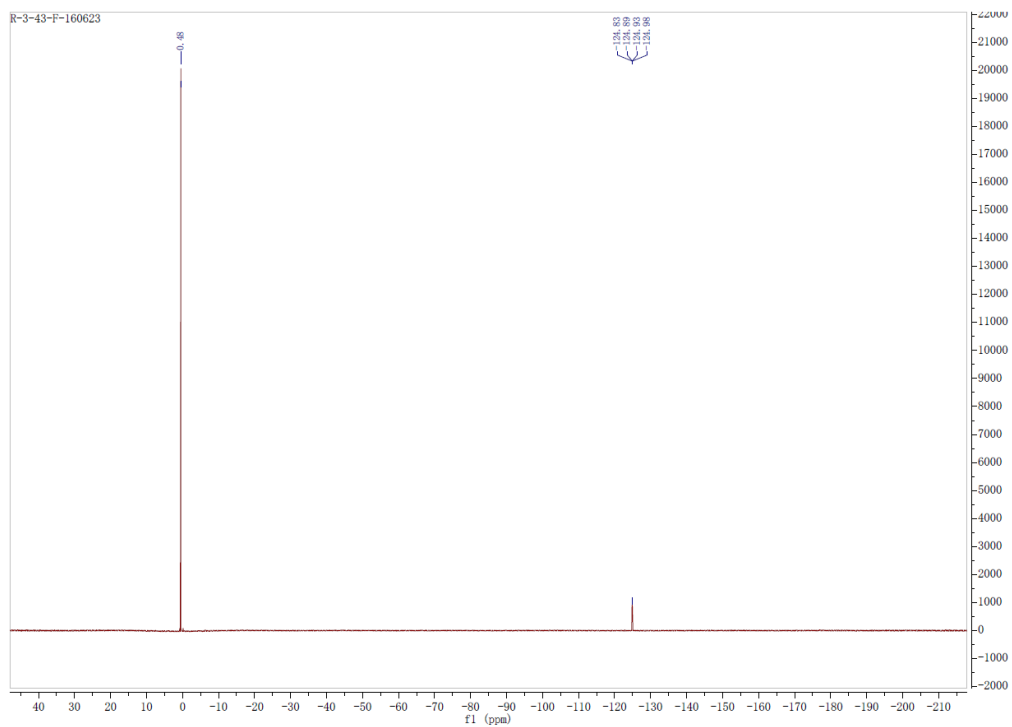


Figure S12. ^{19}F spectra of compound **12c**

2-(trimethylsilyl)ethyl (S,Z)-3-(((R)-2'-(((tert-butoxycarbonyl)amino)methyl)-4-methyl-4,5-dihydro-[2,4'-bithiazole]-4-carbonyl)glycyl)oxy)-4-fluoro-7-(tritylthio)hept-4-enoate (**12d**)

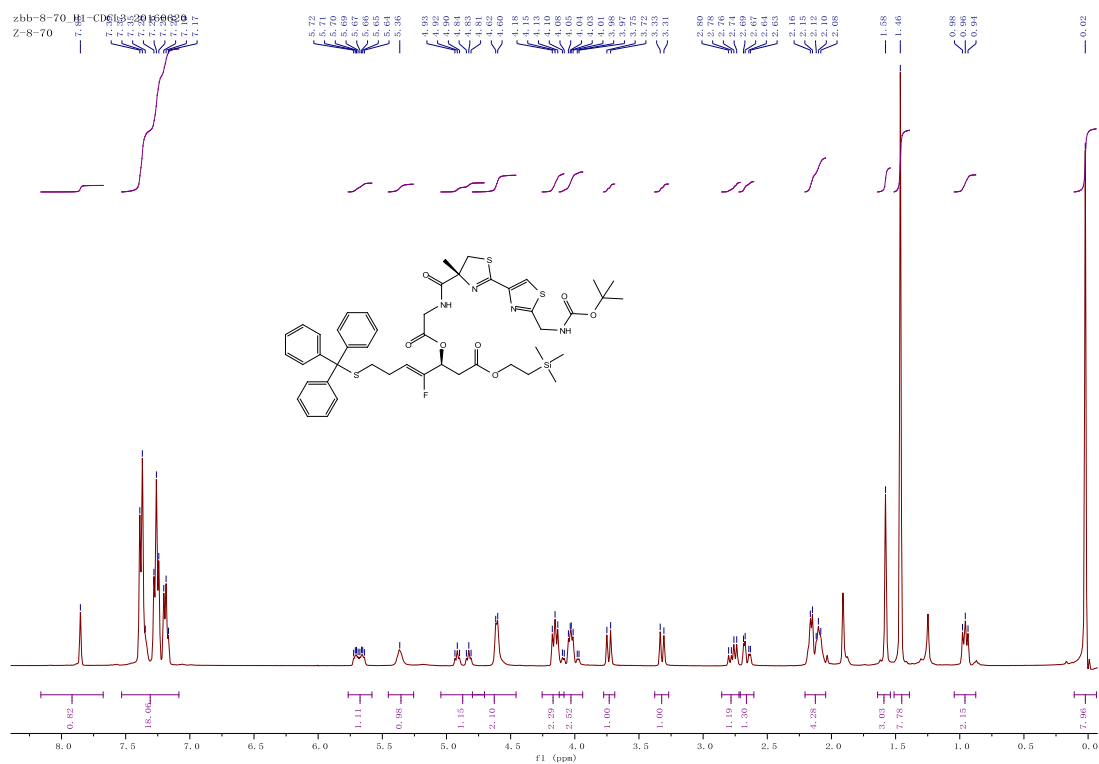
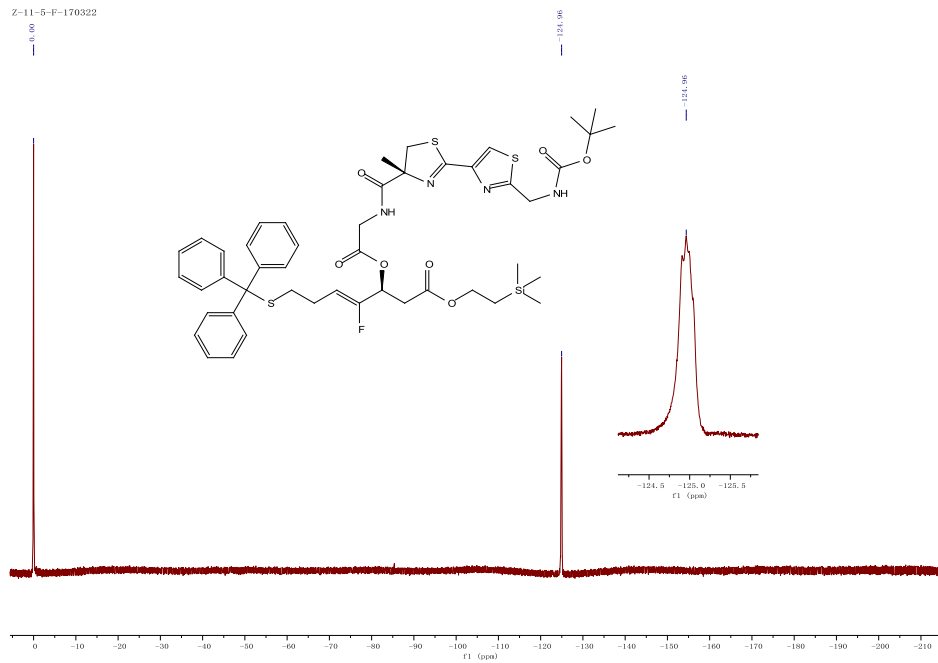
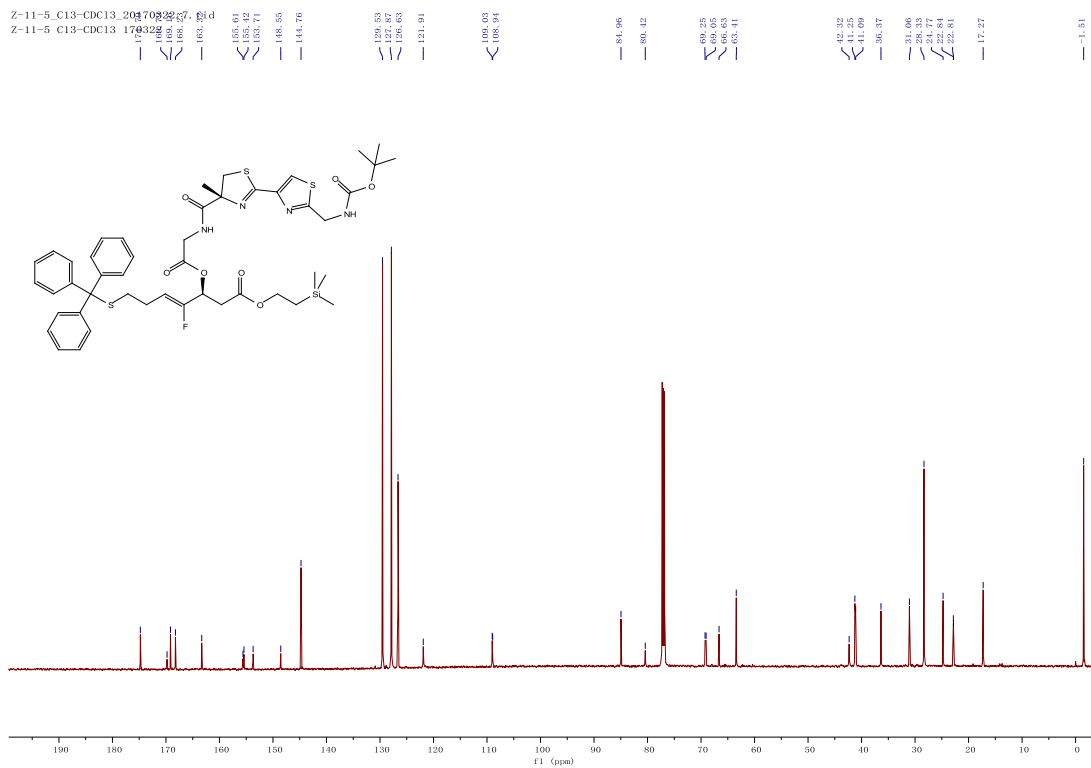


Figure S13. ¹H spectra of compound **12d**



(1²Z,2²Z,2⁴R,5R,8S)-8-((Z)-1-fluoro-4-(tritylthio)but-1-en-1-yl)-2⁴-methyl-5-((methylthio)methyl)-2⁴,2⁵-dihydro-7-oxa-4,11-diaza-1(4,2),2(2,4)-dithiazolacyclododecaphane-3,6,10-trione (**14c**).

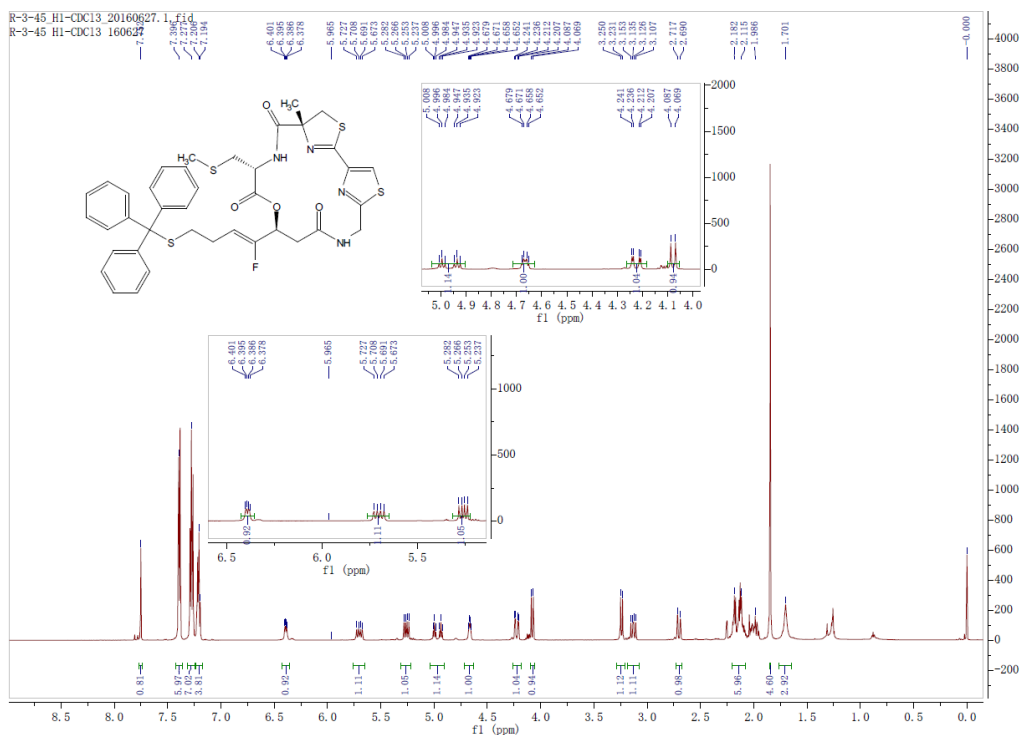


Figure S16. ¹H spectra of compound **14c**

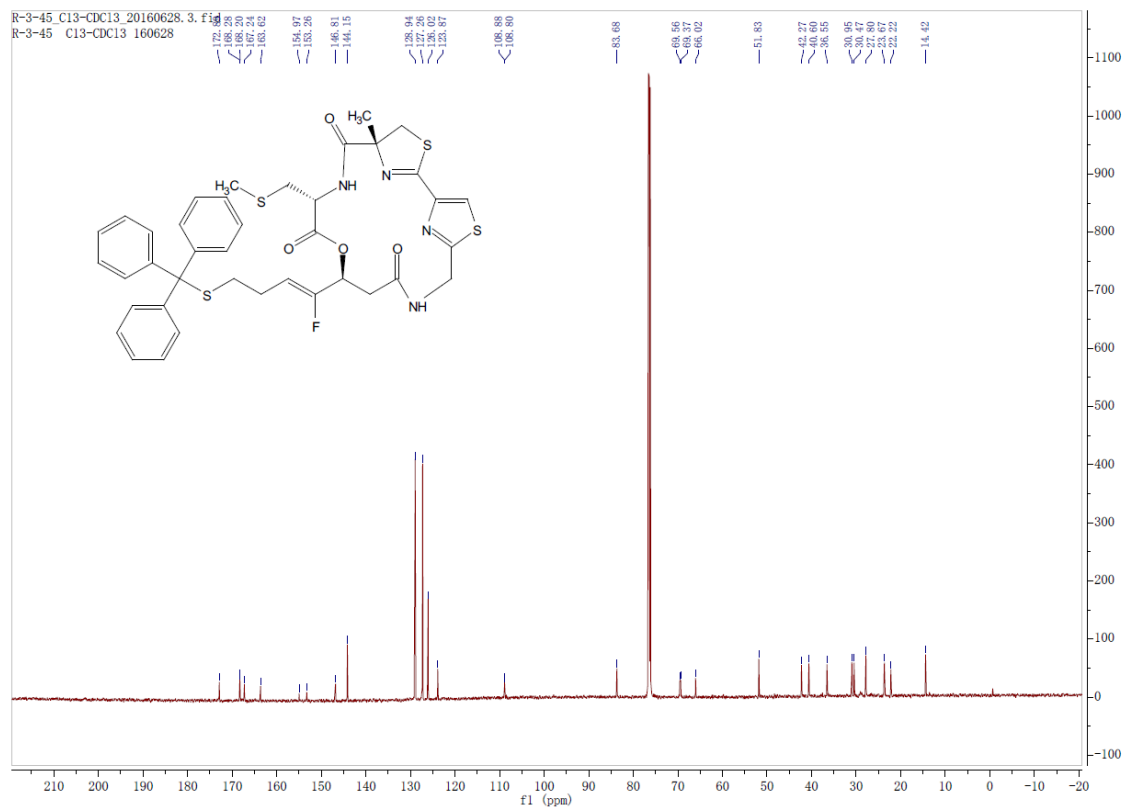


Figure S17. ¹³C spectra of compound **14c**

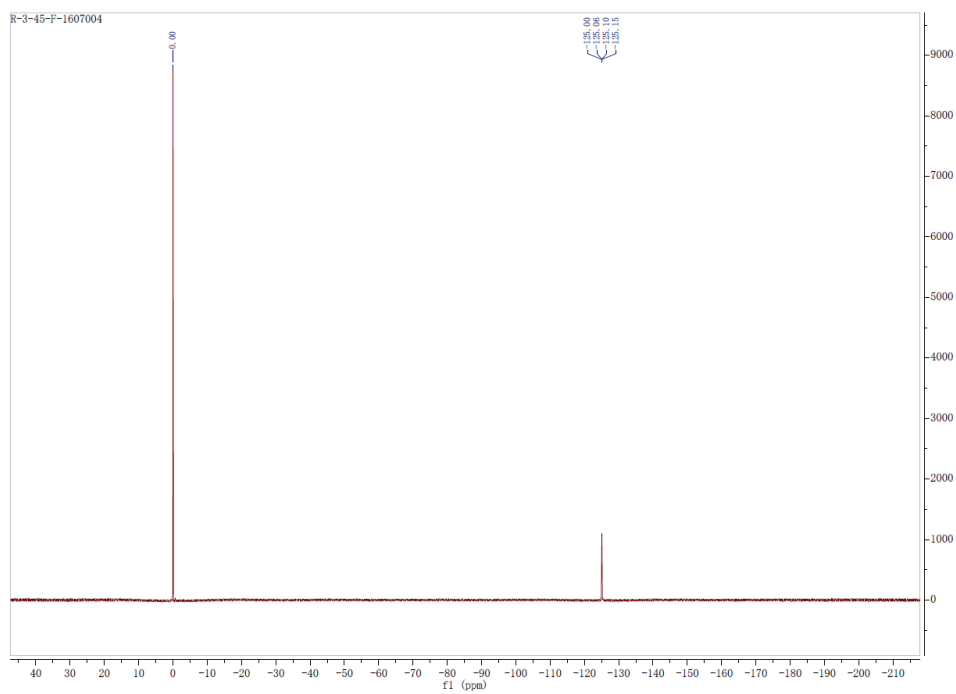


Figure S18. ¹⁹F spectra of compound **14c**

(1²Z,2²Z,2⁴R,8S)-8-((Z)-1-fluoro-4-(tritylthio)but-1-en-1-yl)-2⁴-methyl-2⁴,2⁵-dihydro-7-oxa-4,11-diaza-1(4,2),2(2,4)-dithiazolacyclododecaphane-3,6,10-trione (**14d**).

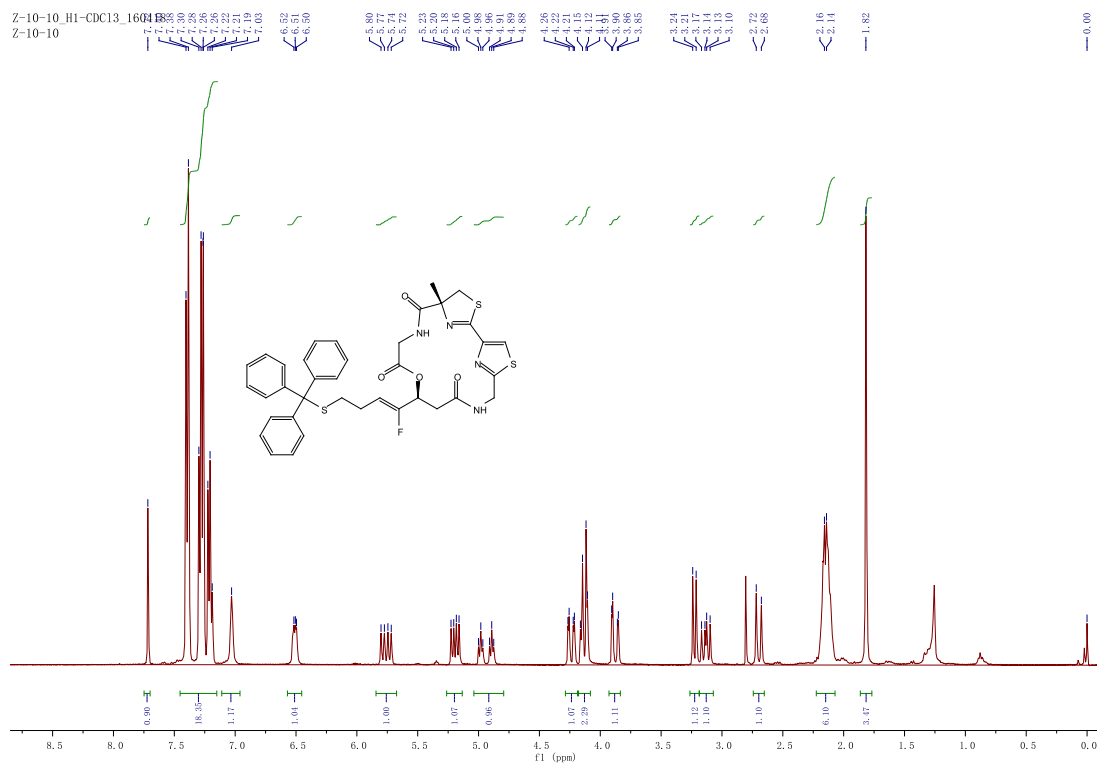


Figure S19. ¹H spectra of compound **14d**

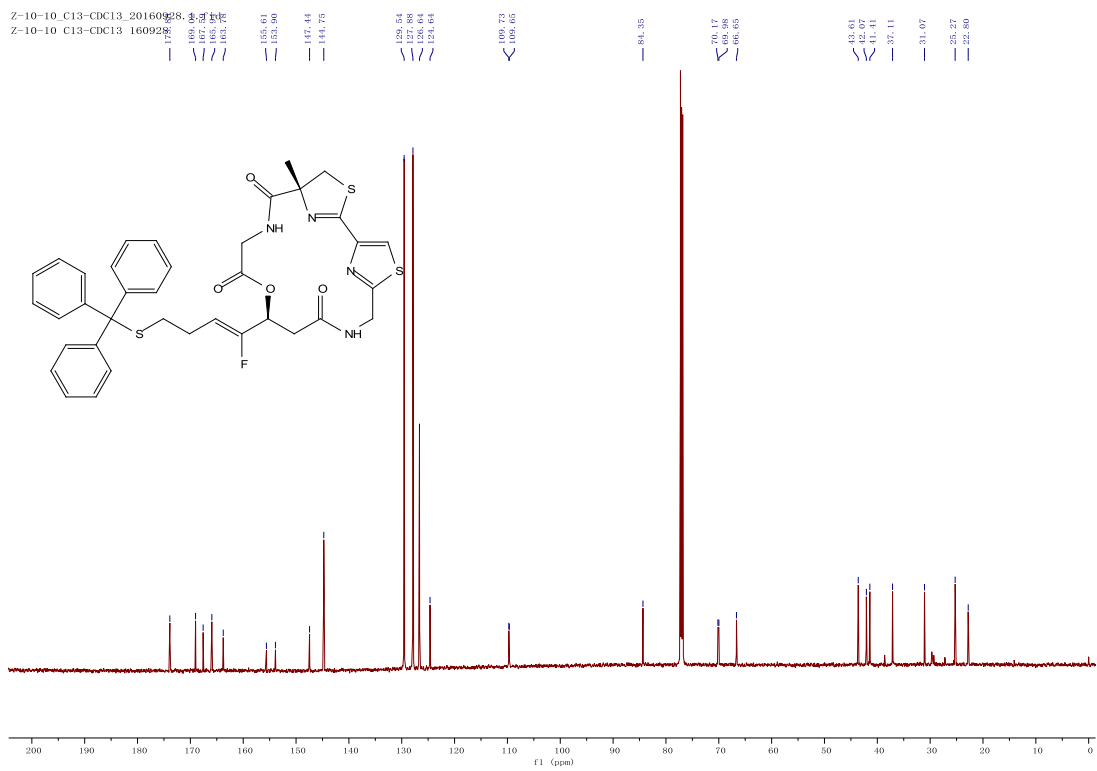


Figure S20. ¹³C spectra of compound 14d

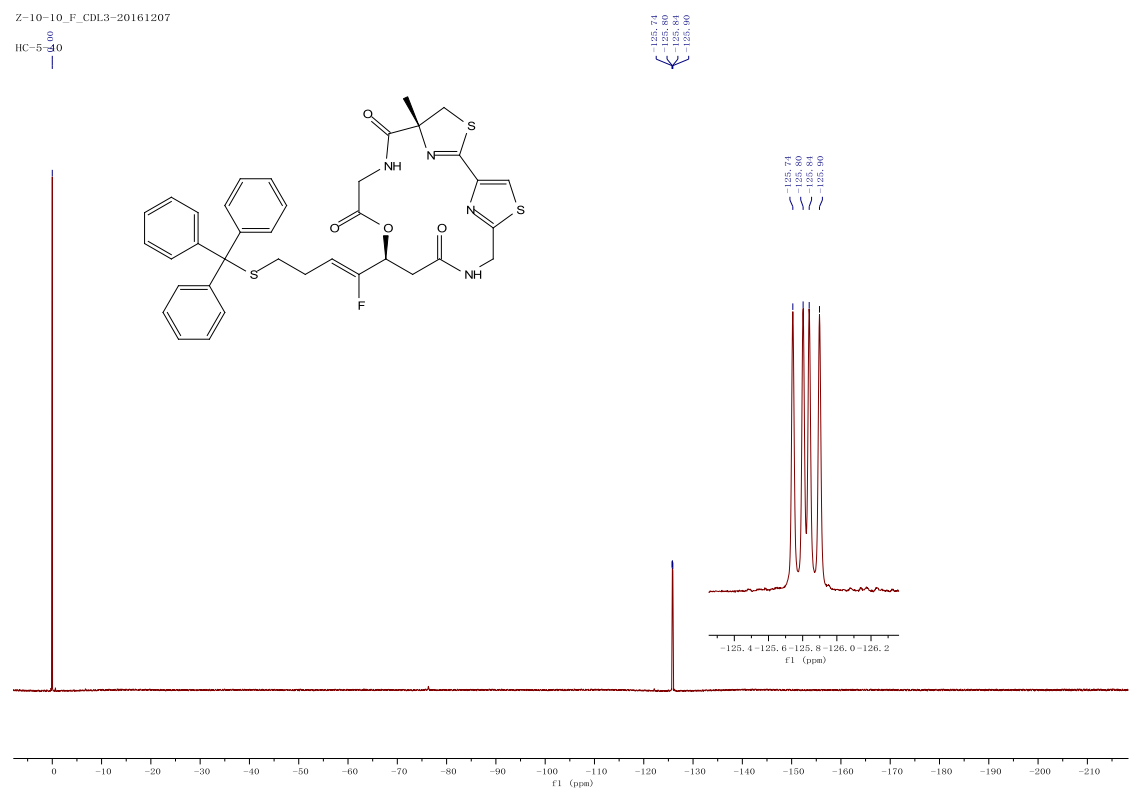


Figure S21. ¹⁹F spectra of compound 14d

(1²Z,2²Z,2⁴R,8S)-8-((Z)-1-fluoro-4-mercaptobut-1-en-1-yl)-2⁴-methyl-5-((methylthio)methyl)-2⁴,2⁵-dihydro-7-oxa-4,11-diaza-1(4,2),2(2,4)-dithiazolacyclododecaphane-3,6,10-trione (**15c**).

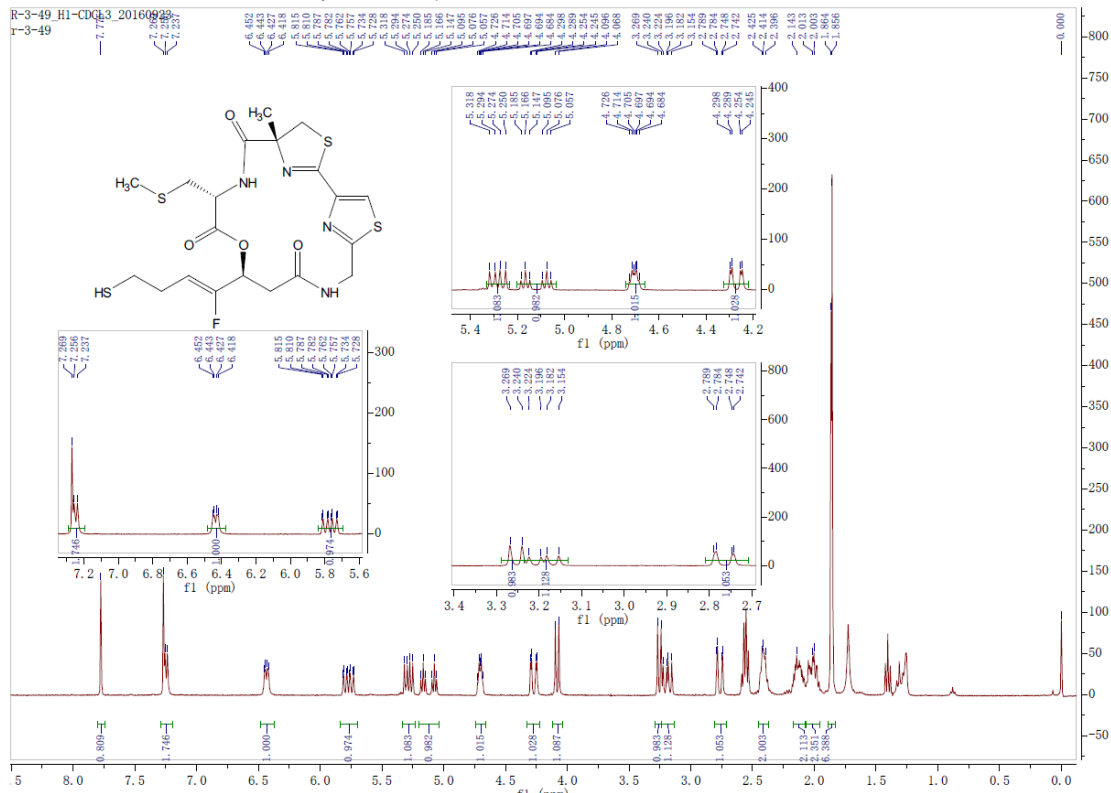


Figure S22. ¹H spectra of compound **15c**

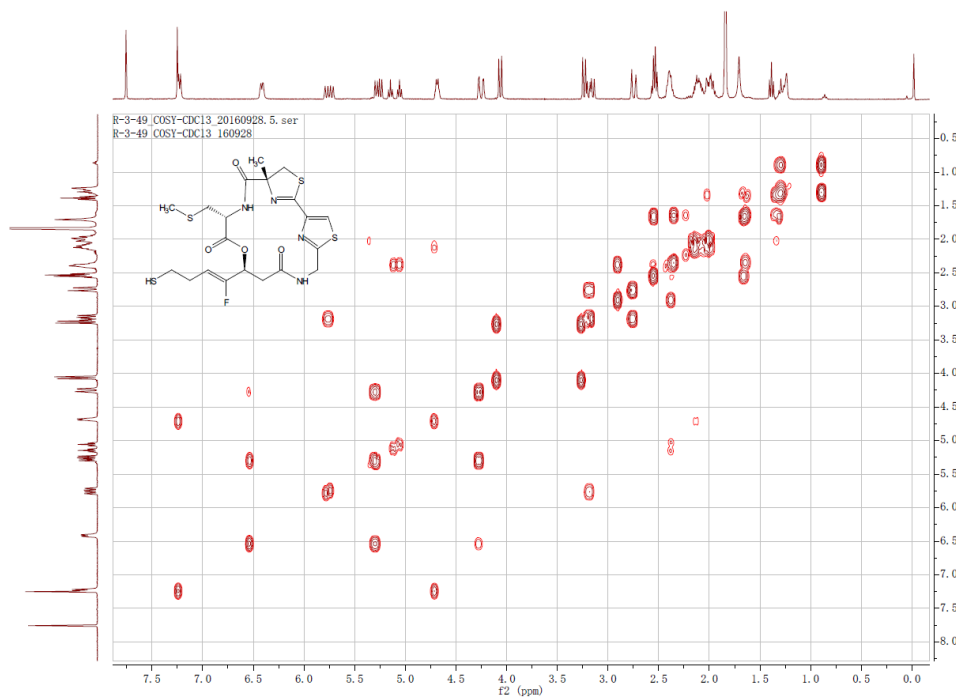


Figure S23. COSY spectra of compound **15c**

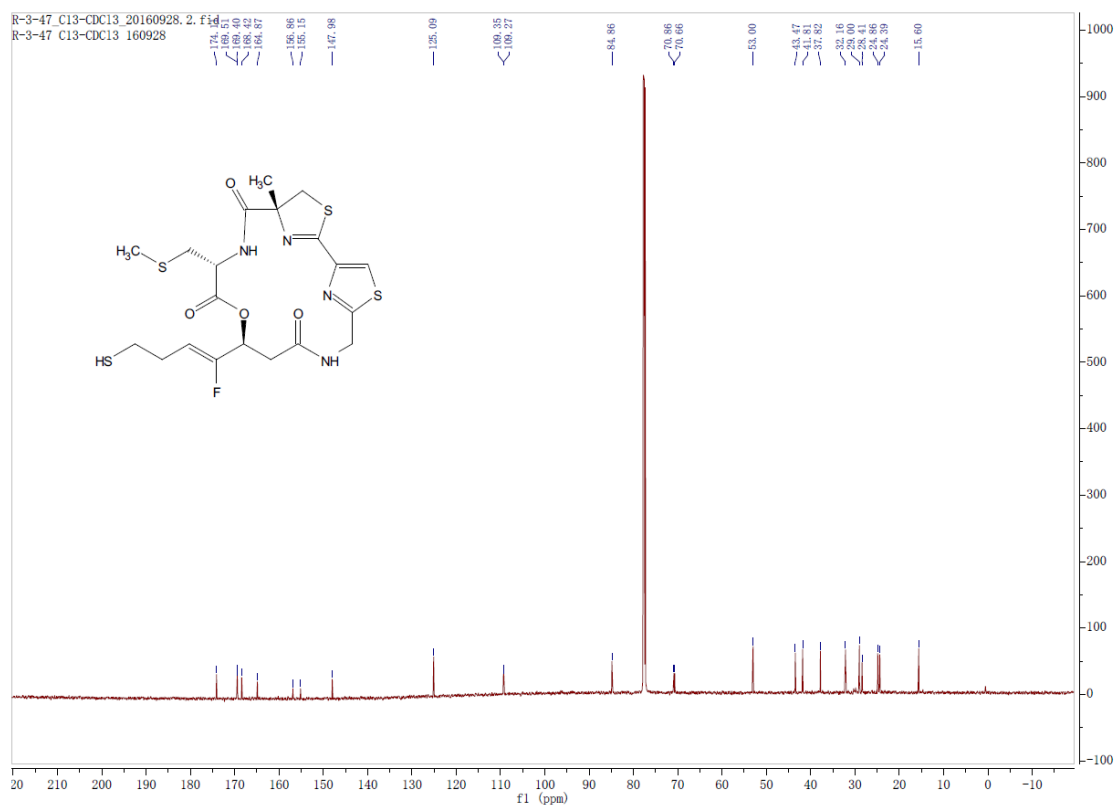


Figure S24. ^{13}C spectra of compound **15c**

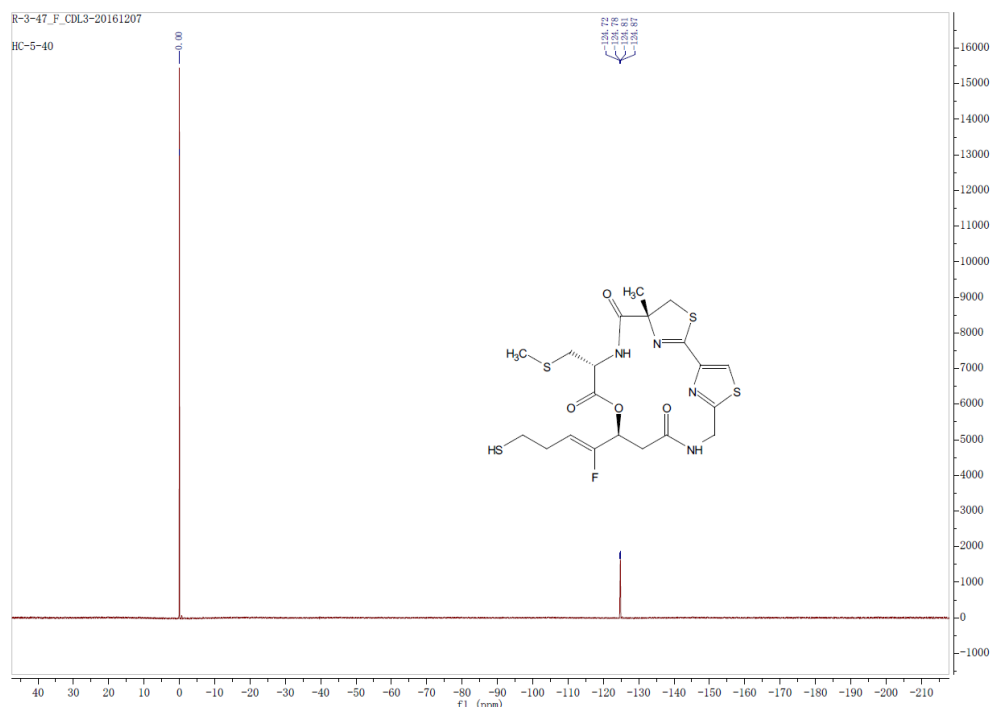


Figure S25. ^{19}F spectra of compound **15c**

S-((*Z*)-4-fluoro-4-((1²*Z*,2²*Z*,2⁴*R*,5*R*,8*S*)-2⁴-methyl-5-((methylthio)methyl)-3,6,10-trioxo-2⁴,2⁵-dihydro-7-oxa-4,11-diaza-1(4,2),2(2,4)-dithiazolacyclododecaphane-8-yl)but-3-en-1-yl) octanethioate (**16c**).

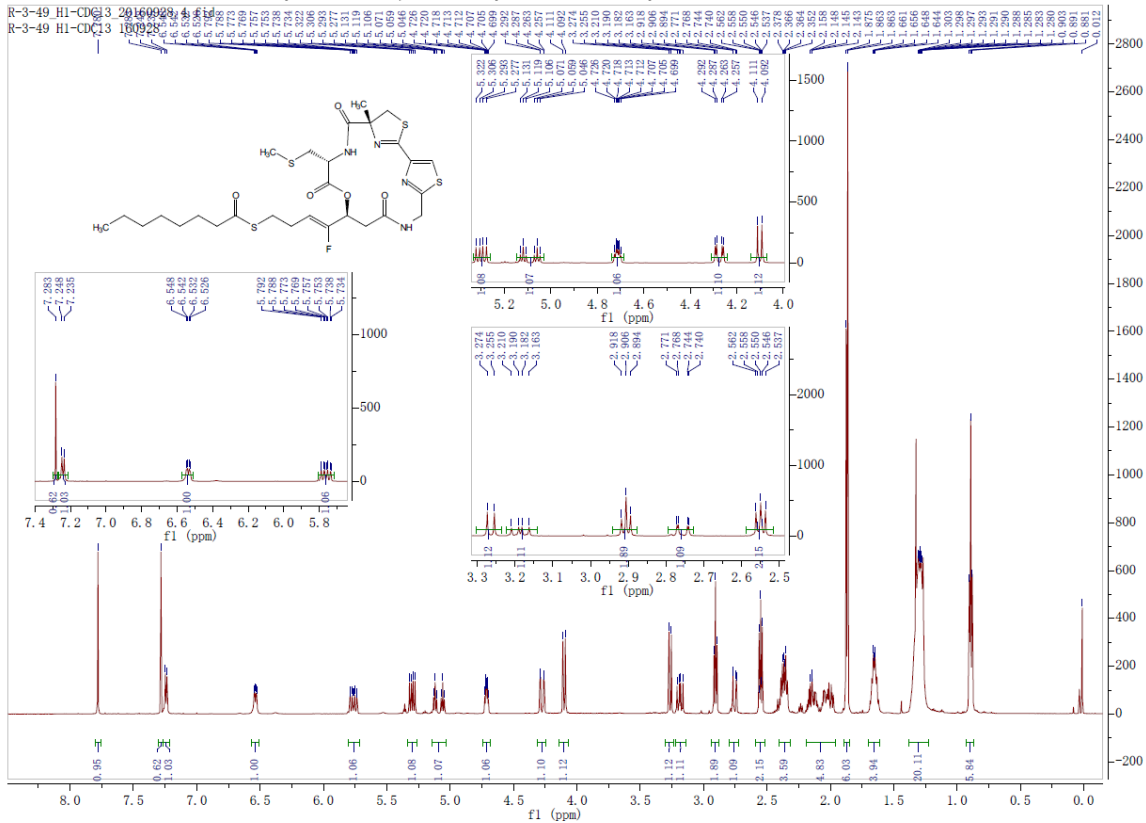


Figure S26. ¹H spectra of compound **16c**

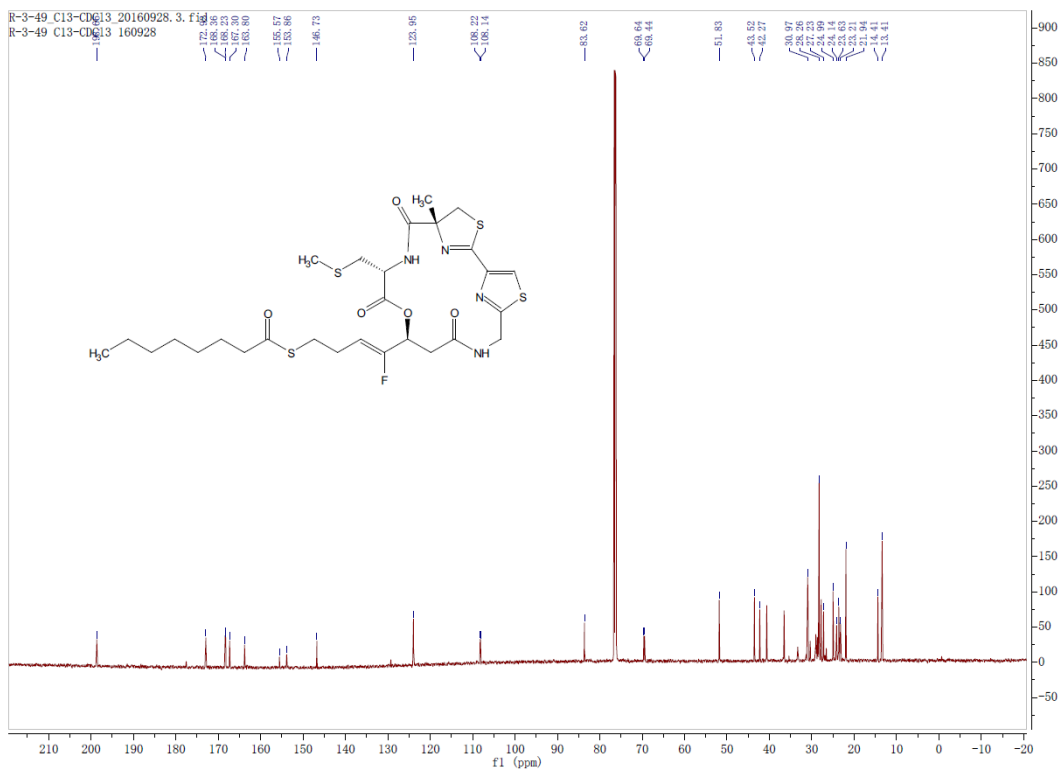


Figure S27. ^{13}C spectra of compound **16c**

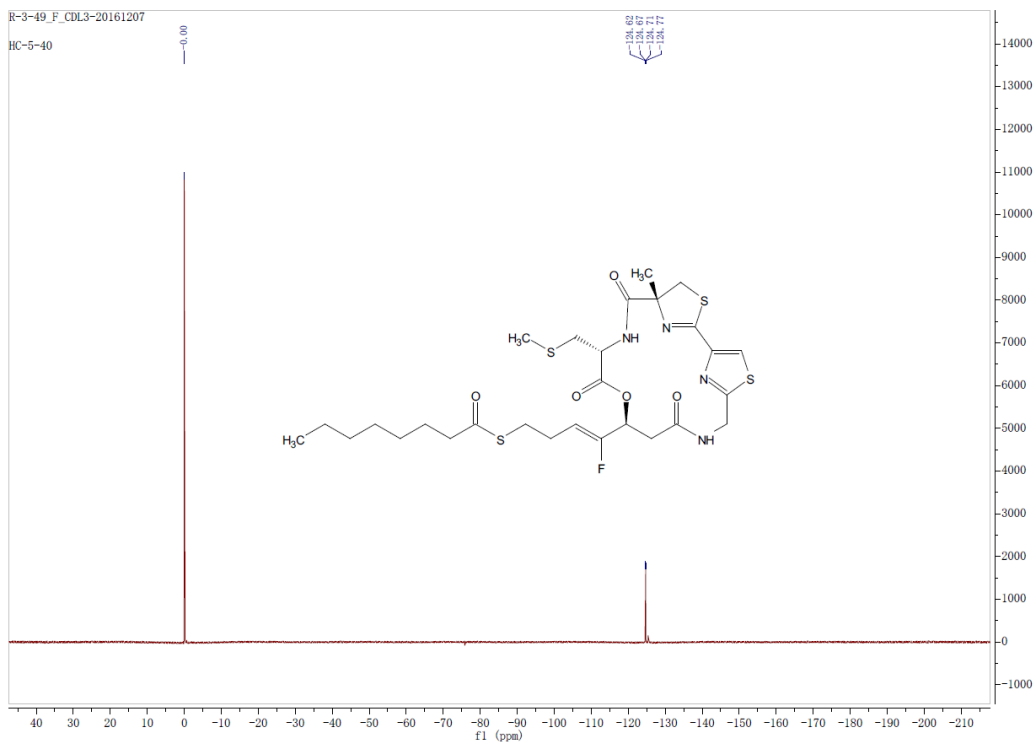


Figure S28. ^{19}F spectra of compound **16c**

S-((*Z*)-4-fluoro-4-((1²*Z*,2²*Z*,2⁴*R*,8*S*)-2⁴-methyl-3,6,10-trioxo-2⁴,2⁵-dihydro-7-oxa-4,11-diaza-1(4,2),2(2,4)-dithiazolacyclododecaphane-8-yl)but-3-en-1-yl) octanethioate (**16d**).

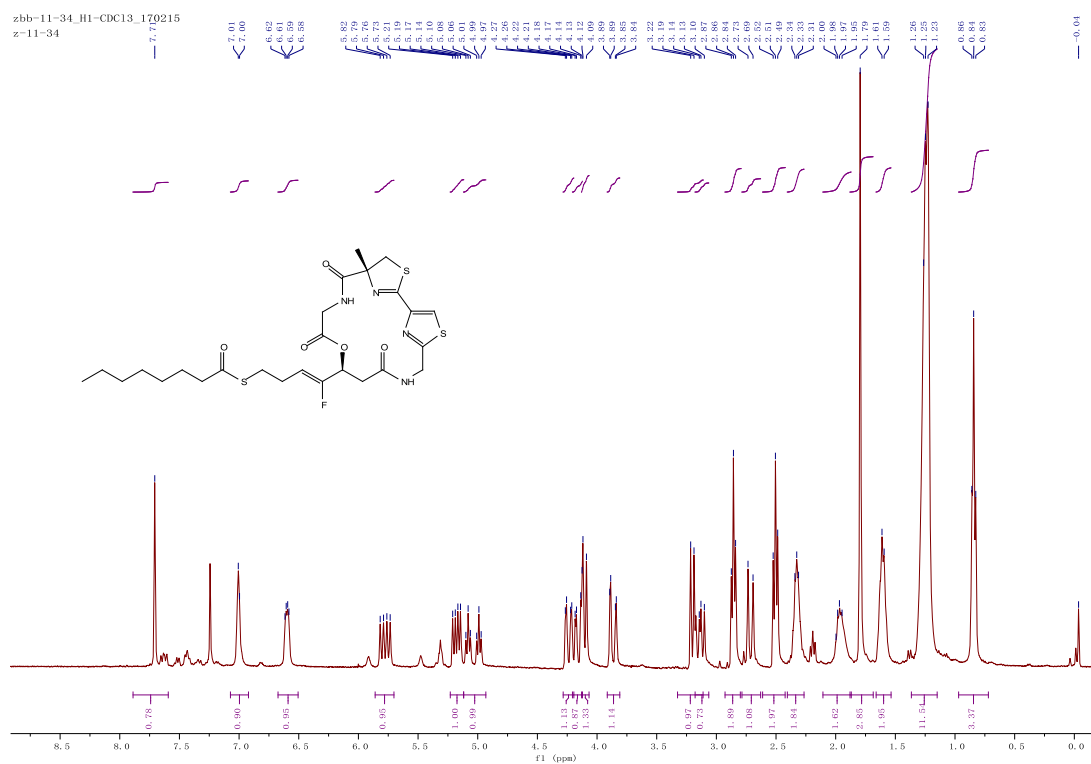


Figure S29. ¹H spectra of compound **16d**

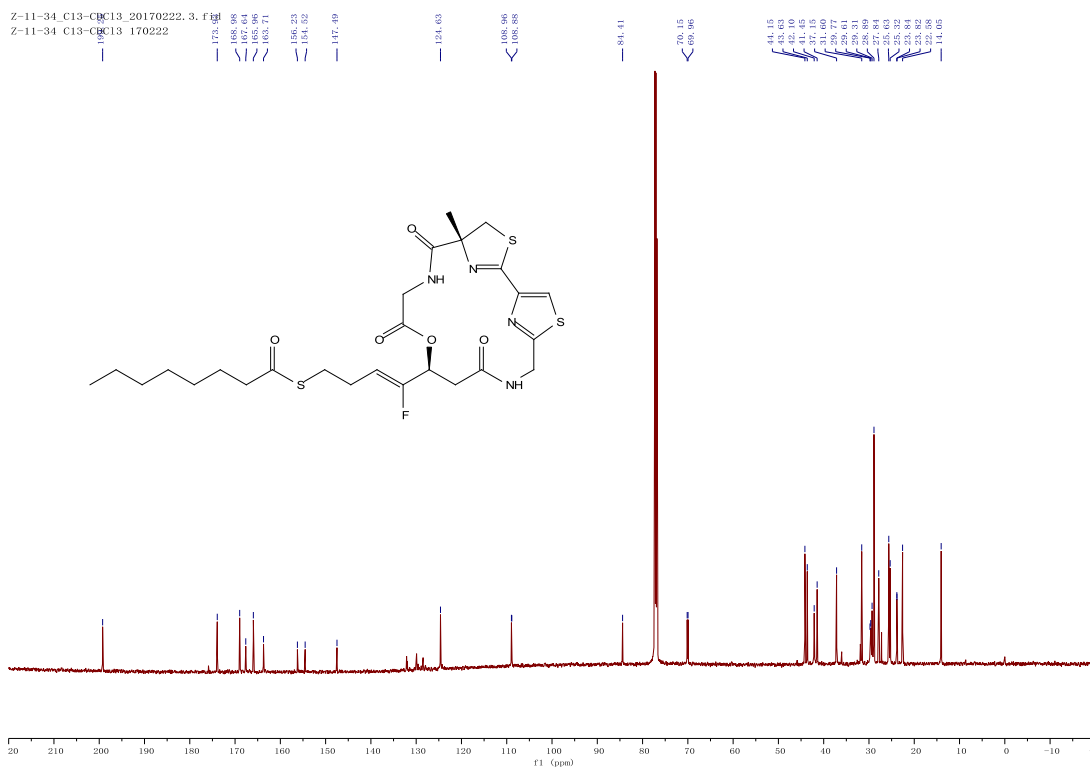


Figure S30. ^{13}C spectra of compound 16d

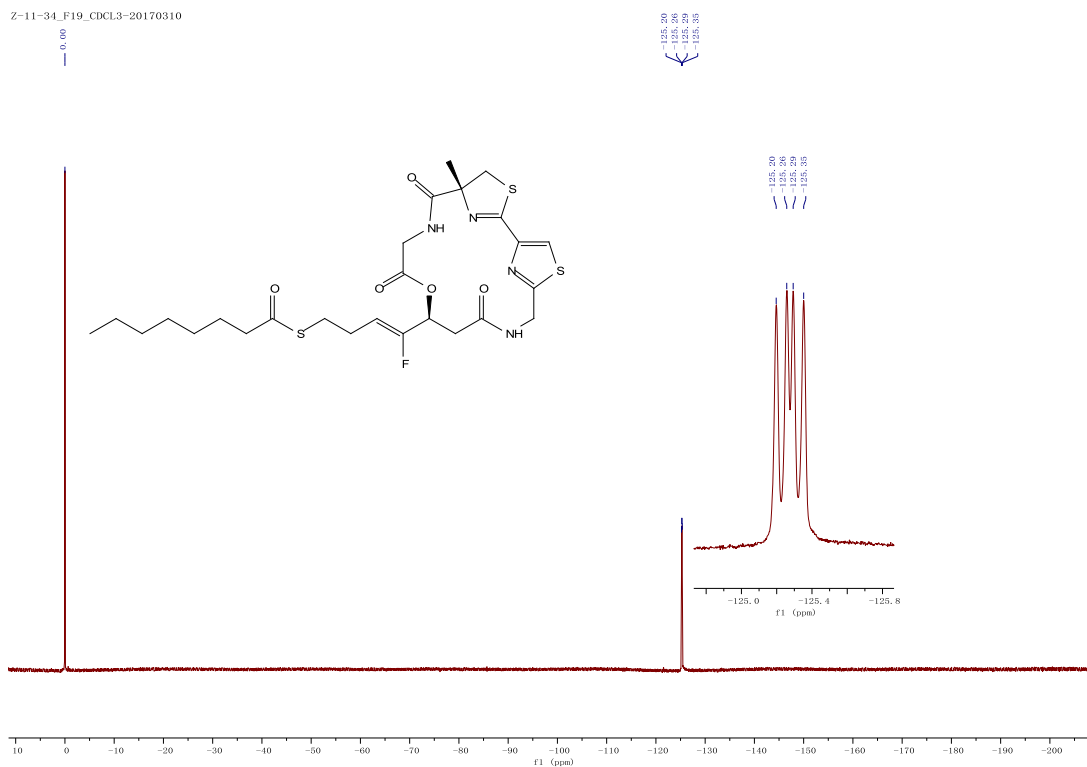


Figure S31. ^{19}F spectra of compound 16d

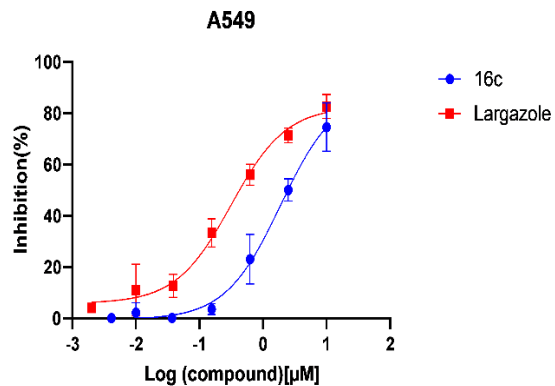


Figure S32. The concentration-response curves of Largazole and 16c in the growth inhibition of A549 cells.

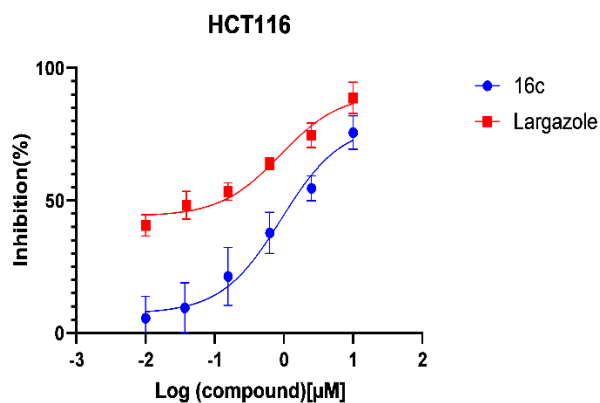


Figure S33. The concentration-response curves of Largazole and 16c in the growth inhibition of HCT116 cells.

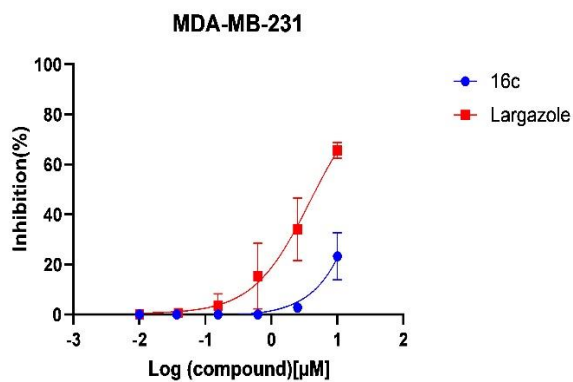


Figure S34. The concentration-response curves of Largazole and 16c in the growth inhibition of MDA-MB-231 cells

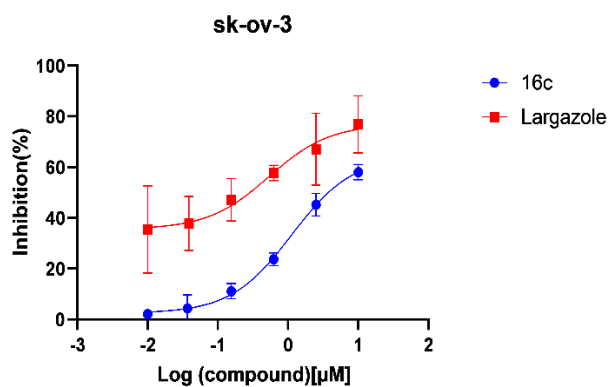


Figure S35. The concentration-response curves of LARGAZOLE and 16c in the growth inhibition of SK-OV-3 cells

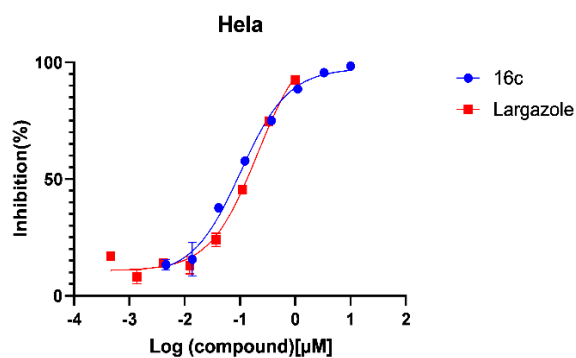


Figure S36. The concentration-response curves of LARGAZOLE and 16c in the growth inhibition of Hala cells

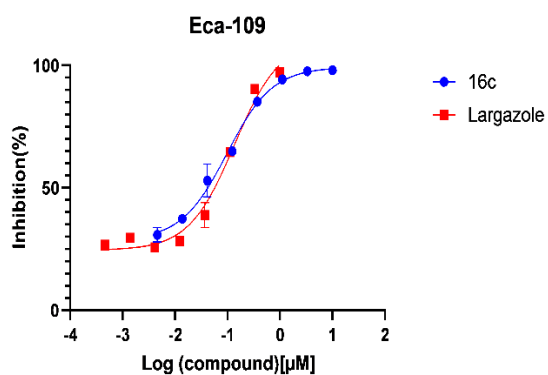


Figure S37. The concentration-response curves of LARGAZOLE and 16c in the growth inhibition of Eca-109 cells

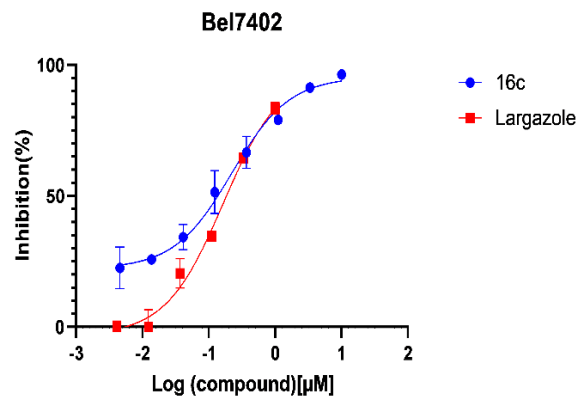


Figure S38. The concentration-response curves of Largazole and 16c in the growth inhibition of Bel7402 cells

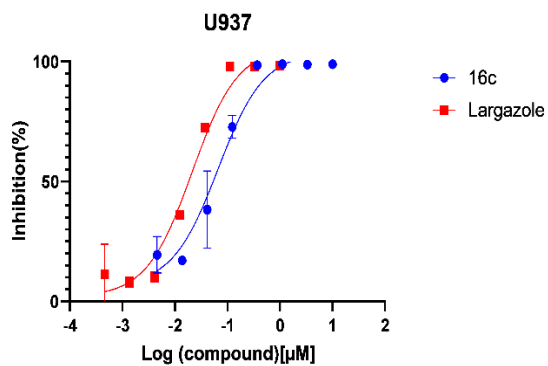


Figure S39. The concentration-response curves of Largazole and 16c in the growth inhibition of U937 cells

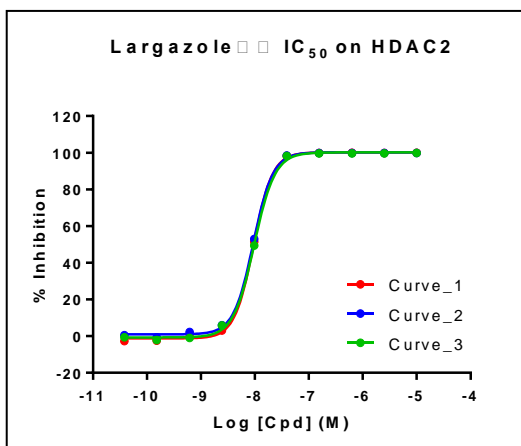
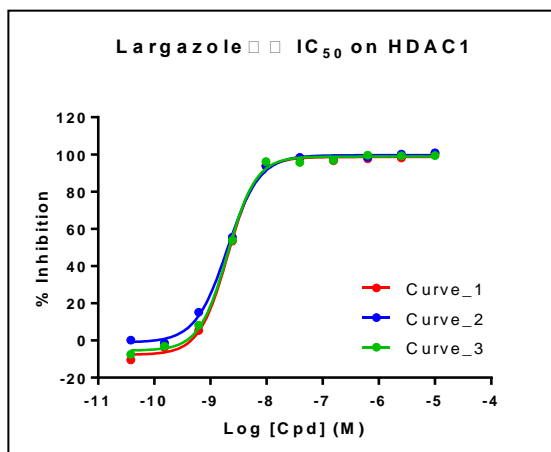


Figure S40. **The concentration-response curves of largazole thiol in HDAC1-2 assays** (Note: Largazole thiol has been named as LARGAZOLE in the figures.)

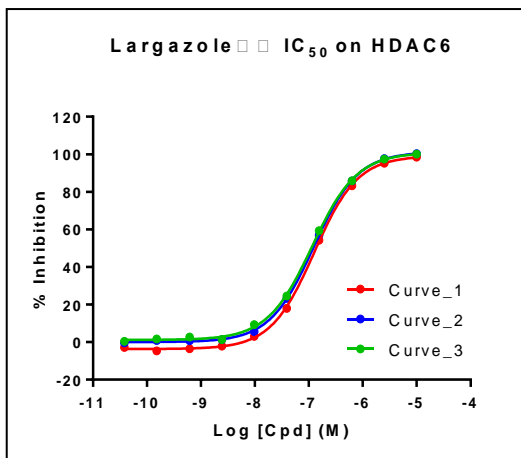
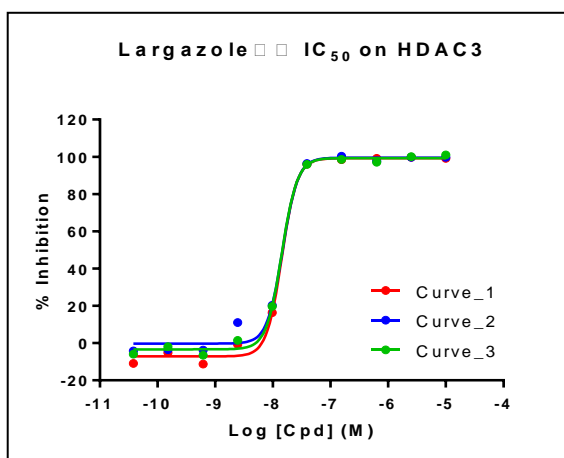


Figure S41. **The concentration-response curves of largazole thiol in HDAC3 and HDAC6 assays** (Note: Largazole thiol has been named as LARGAZOLE in the figures.)

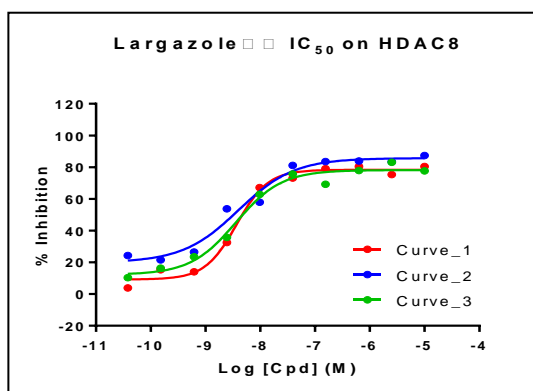


Figure S42. **The concentration-response curves of largazole thiol in HDAC8 assays** (Note: Largazole thiol has been named as LARGAZOLE in the figure.)

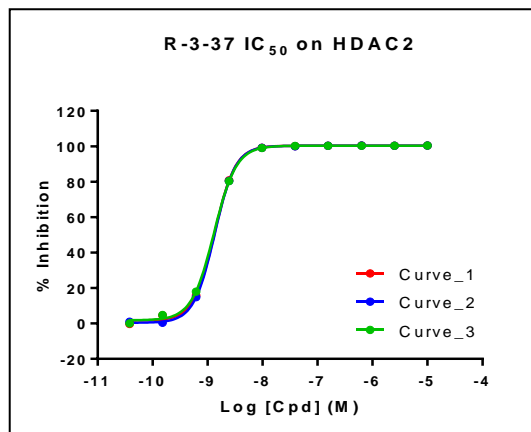
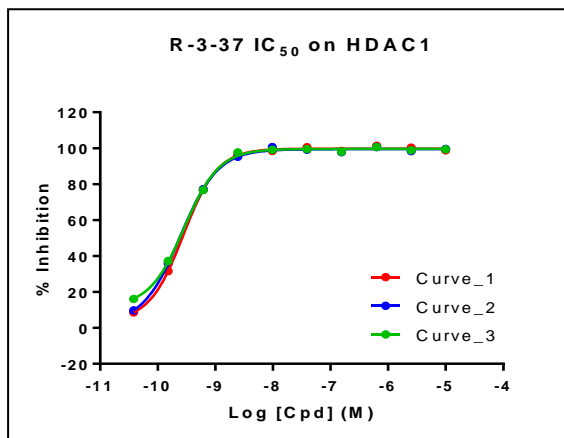


Figure S43. The concentration-response curves of 15c in HDAC1-2 assays (Note: 15c has been named as R-3-37 in the figures.)

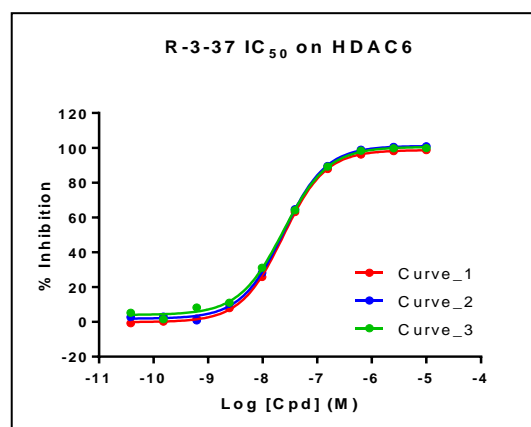
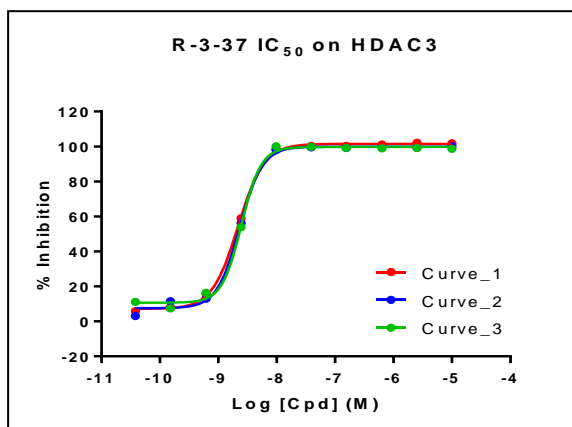


Figure S44. The concentration-response curves of 15c in HDAC3 and HDAC6 assays (Note: 15c has been named as R-3-37 in the figures.)

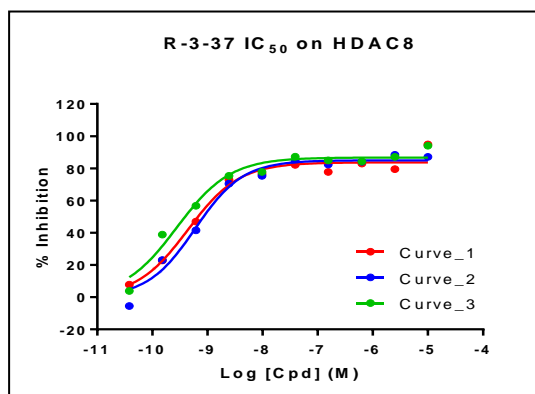


Figure S45. The concentration-response curves of 15c in HDAC8 assays (Note: 15c has been named as R-3-37 in the figure.)