Supplementary information

Biological Assessments of Novel Ultrasound-Synthesized 2-Arylbenzimidazole Derivatives: Antiproliferative and Antibacterial Effects

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Contents

Structural characterization of 2-arylbenzimidazole derivatives 12–33 by ^{1}H- and ^{13}C-NMR spectroscopy

The structures of the prepared 2-arylbenzimidazole derivatives **12**–**33** were confirmed by one-dimensional ^{1}H- and ^{13}C-NMR spectroscopy. The assignment of ^{1}H-NMR spectra was performed based on chemical shifts, signal intensity, resonance multiplicity and H-H coupling constants, while the assignment of ^{13}C-NMR spectra was assigned based on chemical shifts, in accordance with the atom numbering shown in the Figure S1.

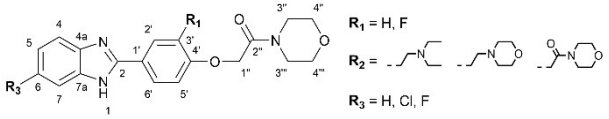


Figure S1. Structure of 2-arylbenzimidazole derivatives with numbered atoms

The ^{1}H-NMR spectra of *O*-alkylated benzimidazole derivatives **12**–**27** (ESI† Fig. S16–S31) exhibit the expected number of signals in both the aromatic and aliphatic regions at characteristic chemical shifts. Additionally, the spectra of 1,2,3-triazole derivatives **28**–**33** (ESI† Fig. S32–S37) include a signal corresponding to the triazole ring proton. In the ^{1}H-NMR spectra of *O*-alkylated derivatives of 2-arylbenzimidazoles **12**–**27**, signals are observed for the protons of the benzimidazole ring, along with additional signals for the protons of the substituted benzene ring at the C-2 position. A characteristic signal for the NH proton of the imidazole ring appears at ~12 ppm, and in the aliphatic region, signals corresponding to the protons of the introduced amine substituents are present. By comparing the selected ^{1}H-NMR spectra (Table 1, Fig. S2) of compound **14** (ESI† Fig. S18) with those of compounds **17** (ESI† Fig. S21) and **20** (ESI† Fig. S24), the absence of a signal for proton H-6 was observed in compounds substituted with chlorine and fluorine at the C-6 position of benzimidazole. It was also observed that protons H-7 and H-5 in the 6-fluorobenzimidazole derivative 20 are shifted upfield, while protons H-4 and H-7 exhibit additional splitting due to coupling with the fluorine atom through two, three, or four bonds: 7.55 ppm (ddd, *J* = 54.14, 8.77, 4.93 Hz, 1H, H-4) and 7.34 ppm (ddd, *J* = 53.2, 9.5, 2.4 Hz, 1H, H-7). Furthermore, the NH proton signal at 12.85 ppm couples with the fluorine atom through five bonds (d, *J* = 4.2 Hz, 1H) (Fig. S2).

Table 1. Chemical shifts (δ/ppm) of compounds **14**, **17** and **20** in ^{1}H-NMR spectra

	δ/ppm								
Compd	H-4	H-5	H-6	H-7	H-2'	H-3'	H-5'	H-6'	NH
14	7.56	7.17	7.17	7.56	8.09	7.10	7.10	8.09	12.73
17	7.54	7.16	/	7.58	8.09	7.10	7.10	8.09	/
20	7.55	7.03	/	7.34	8.07	7.10	7.10	8.07	12.85

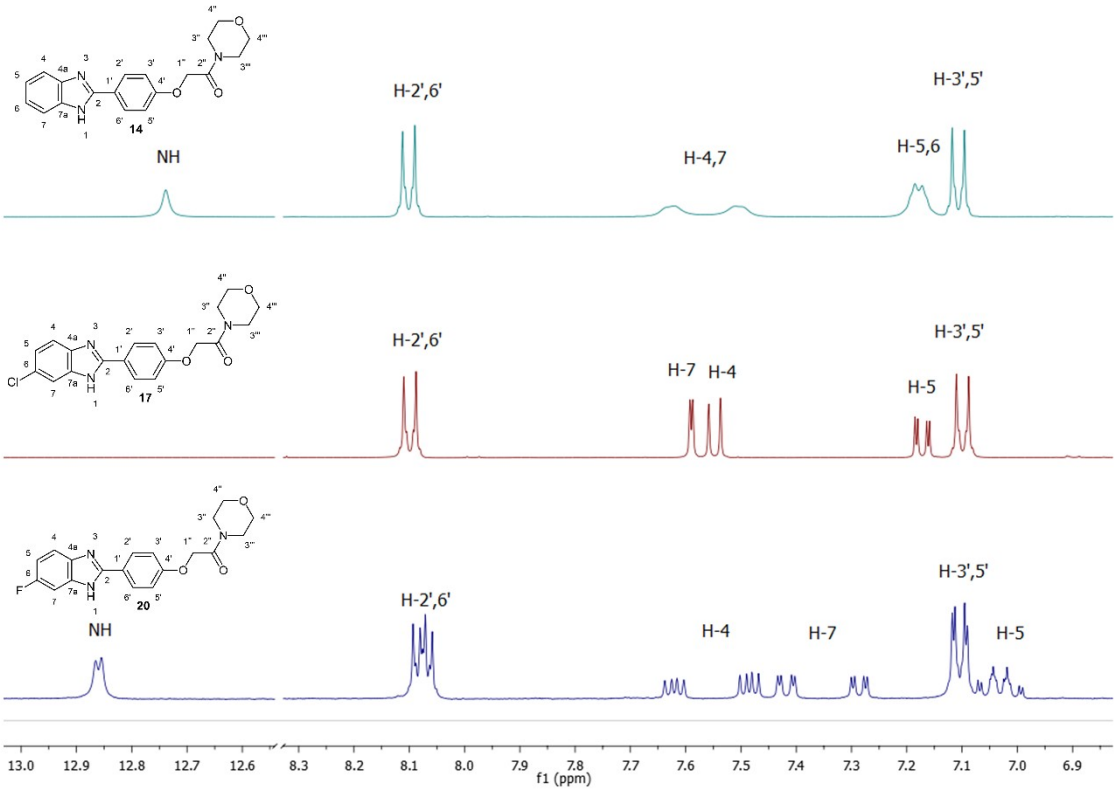


Figure S2. ^{1}H-NMR spectra of compounds **14**, **17** and **20**

The APT spectra of 2-arylbenzimidazole derivatives **12**–**27** (ESI† Fig. S48–S63) exhibit signals for the carbons of the benzimidazole ring in the aromatic region at characteristic chemical shifts (δ 158.61–97.47 ppm), along with the expected number of signals in the aliphatic region. A comparison of the selected APT spectra of s compounds **14** and **20** (Table 2, Fig. S3) reveals differences in chemical shifts in the aromatic region due to substitution with a fluorine atom, as well as additional signals arising

Table 2. Chemical shifts (*δ*/ppm) of compounds **14** and **20** in APT spectra

	δ/ppm						
Compd	C-2/ C-2•	C-4/ C-4•	C-4a/ C-4a•	C-5/ C-5•	C-6/ C-6•	C-7/ C-7•	C-7a/ C-7a•
14	151.76	119.00	144.36	121.94	122.58	111.52	135.45
20	153.04 152.2	119.27 111.5	140.49 131.6	109.94 109.4	158.61 158.3	104.00 97.47	144.27 135.0

from the presence of tautomeric structures in compound **20**. The largest difference in chemical shifts was observed for the tautomeric C-6/C-6• of compound **20**, which are shifted downfield to 158.61 ppm and 158.39 ppm and appear as doublets due to coupling with fluorine through one bond (*J* = 236.3 Hz, 233.9 Hz). Due to splitting with fluorine through one, two, or three bonds, the spectra exhibit two doublets each for the tautomeric C-4/C-4•, C-5/C-5•, and C-7/C-7•, all of which are also shifted downfield compared to the unsubstituted benzimidazole derivative **14**. Thus, the doublets at 119.27

ppm (*J* = 9.8 Hz) and 111.56 ppm (*J* = 10.5 Hz) for C-4/C-4• arise due to splitting with fluorine through three bonds, while the doublets at 109.94 ppm (*J* = 25.9 Hz) and 109.42 ppm (*J* = 24.8 Hz) for C-5/C-5•, as well as at 104.00 ppm (*J* = 23.7 Hz) and 97.47 ppm (*J* = 26.7 Hz) for C-7/C-7•, result from splitting with fluorine through two bonds. Furthermore, the doublets at 144.27 ppm for the quaternary carbon C-7a/C-7a• arise due to splitting with fluorine through three bonds (J = 13.2 Hz, 13.3 Hz). In contrast, the quaternary carbons C-2/C-2• at 153.04 ppm and 152.23 ppm, as well as C-4a/C-4a• at 140.49 ppm and 131.60 ppm, appear as singlets.

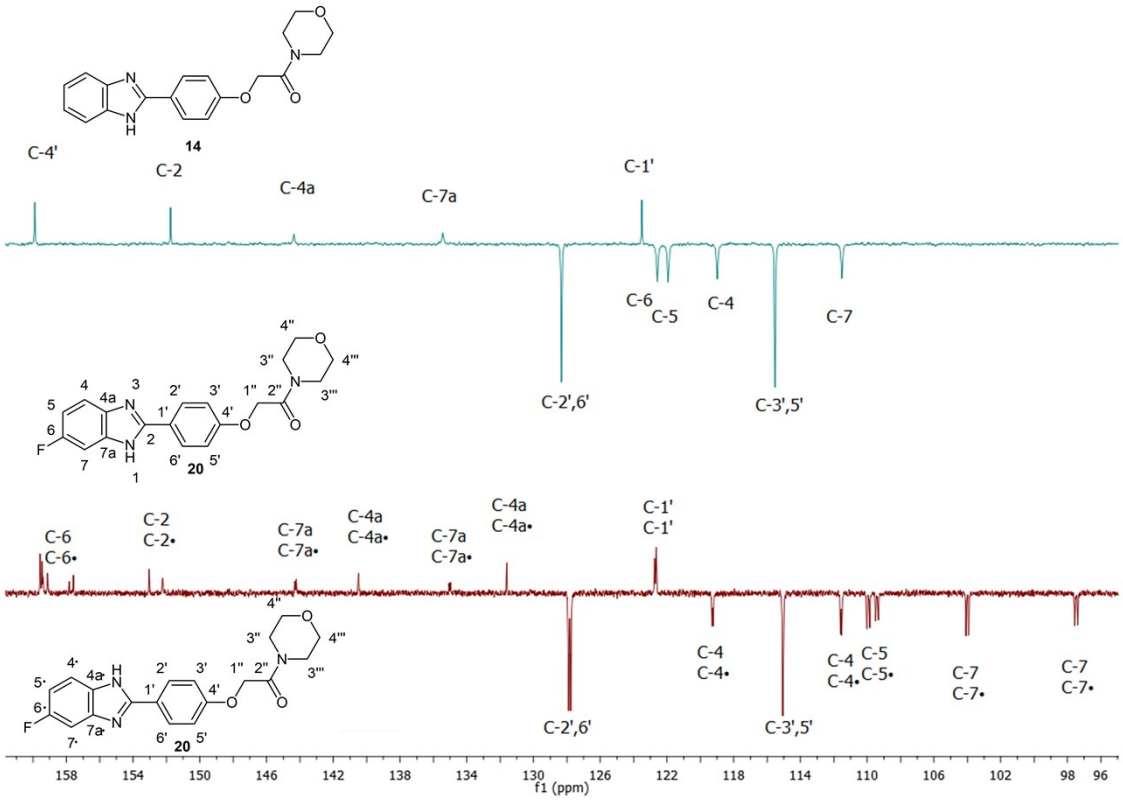


Figure S3. ^{13}C-NMR spectra of compounds **14** and **20**

The structure of the benzimidazole derivative **20** was further confirmed using heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) spectroscopy. In the HSQC spectrum of compound **20** (Fig. S4), heteronuclear coupling is observed through one bond between protons and carbon atoms. In the aliphatic region of the spectrum, a correlation is observed between protons H-3'' and H-3''' at 3.47 ppm and carbons at 41.58 and 44.66 ppm, as well as between H-4'' and H-4''' at 3.67–3.55 ppm and the C-4''/C-4''' carbon at 65.97 and 65.70 ppm, which are superimposed in the same signal. Additionally, the correlation of proton H-1'' at 4.94 ppm with carbon C-1'' at 66.03 ppm is evident. In the aromatic region, a correlation is observed between protons H-2'/H-6' at 8.10–8.04 ppm and a carbon at ~128 ppm, as well as between H-3'/H-5' at 7.10 ppm and a carbon at ~115 ppm. Proton H-4, shifted upfield to ~7.55 ppm, couples with carbons at 119.27 ppm (*J* = 9.8 Hz) and 111.57 ppm (*J* = 10.5 Hz). The signals for C-5 carbons are overlapped due to their similar

chemical shifts (109.94 and 109.42 ppm) and are coupled to proton H-5, which is shifted upfield (7.07–6.98 ppm). Finally, the signal for H-7 correlates with C-7/C-7• at 104.00 and 97.47 ppm.

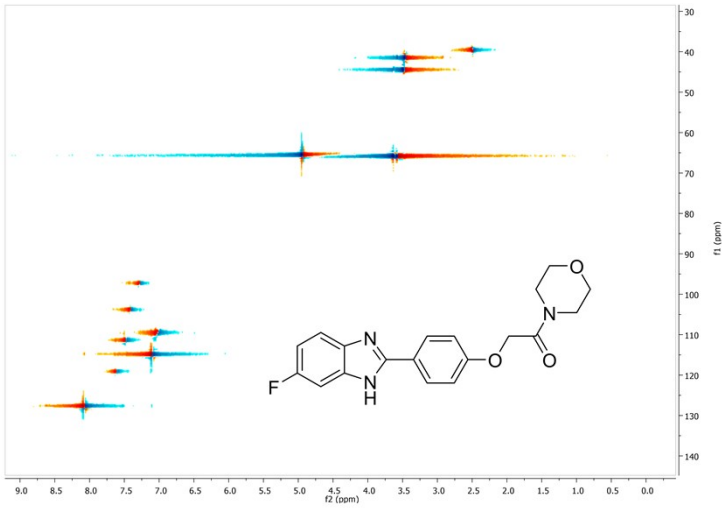


Figure S4. HSQC spectrum of compound **20**

In the HMBC spectrum of compound **20** (Fig. S5), couplings of H-5 at 7.07–6.98 ppm with C-4a/C-4a• at 131.60 ppm and 140.49 ppm through two bonds are observed. Additionally, coupling through three bonds between C-4a/C-4a• at 131.60 ppm and 140.49 ppm and H-7 at 7.34 ppm is present. Furthermore, a weak cross-peak of C-4a at 131.60 ppm with H-5 at ~7 ppm of the benzimidazole ring is detected due to coupling through three bonds, while C-4a• at 140.49 ppm exhibits a stronger cross-peak with the NH proton due to coupling through two bonds. The signal of C-7a at 135.02 ppm correlates with H-4 at 7.55 ppm, while the signal of C-7a• at 144.27 ppm also shows coupling with proton H-4. A weak cross-peak at 104.00 ppm and 97.47 ppm corresponds to the correlation of H-5 and H-7 with C-7/C-7•.

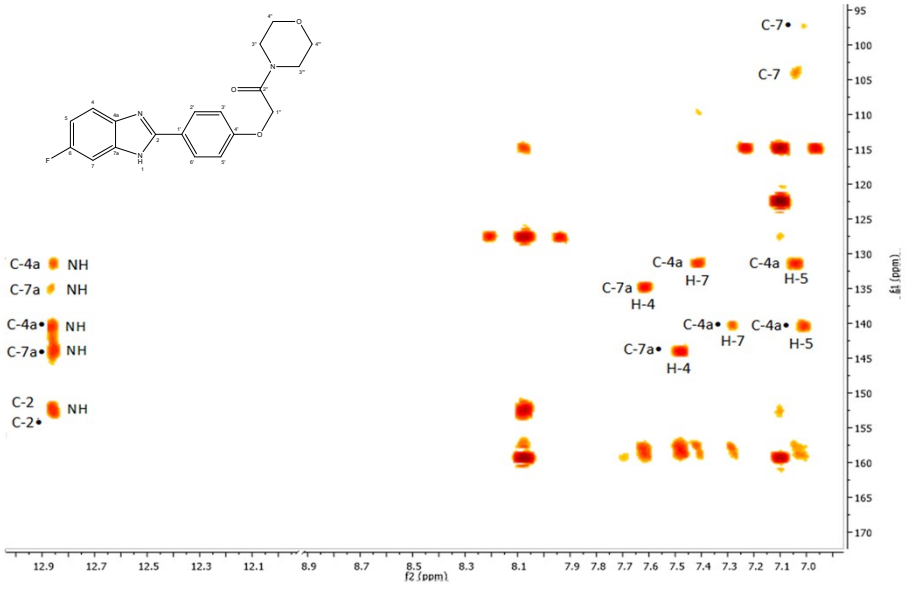


Figure S5. HMBC spectrum of compound **20**

[1]H-NMR spetra for compounds **1**–**8** (Fig. S6–S13) and **10**–**33** (Fig. S14–S37)

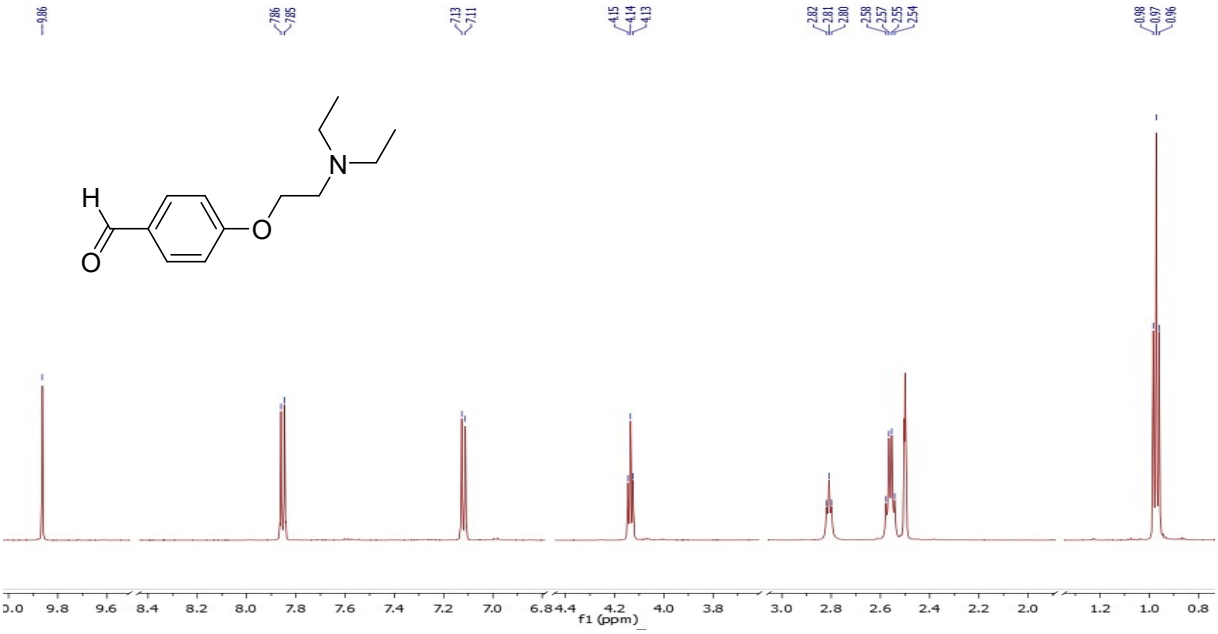


Figure S6. [1]H-NMR spectrum of compound **1**

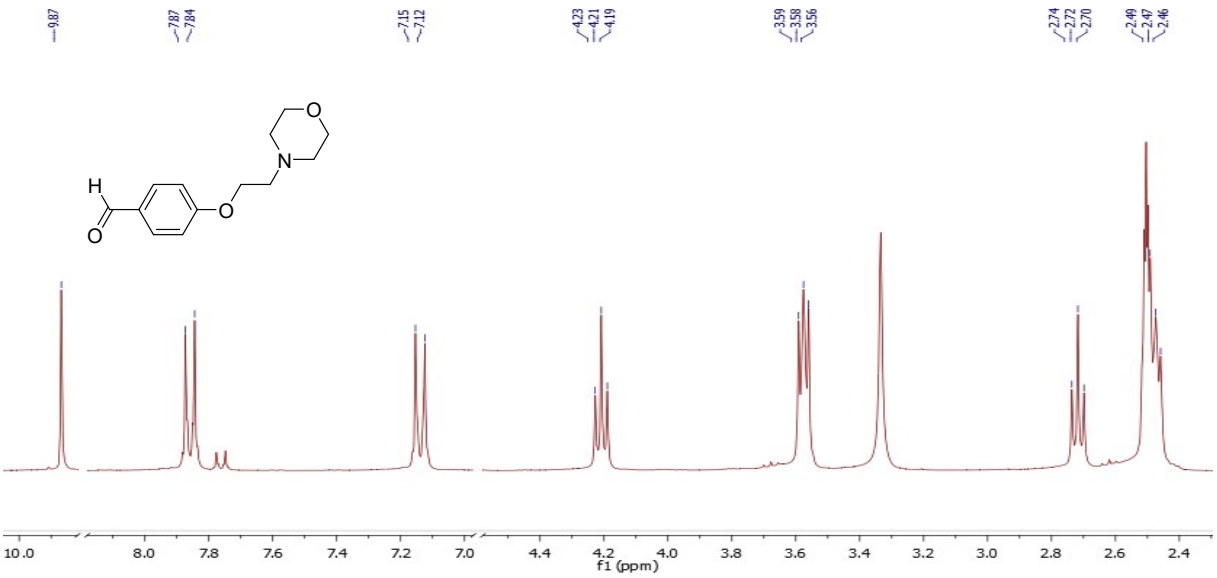


Figure S7. [1]H-NMR spectrum of compound **2**

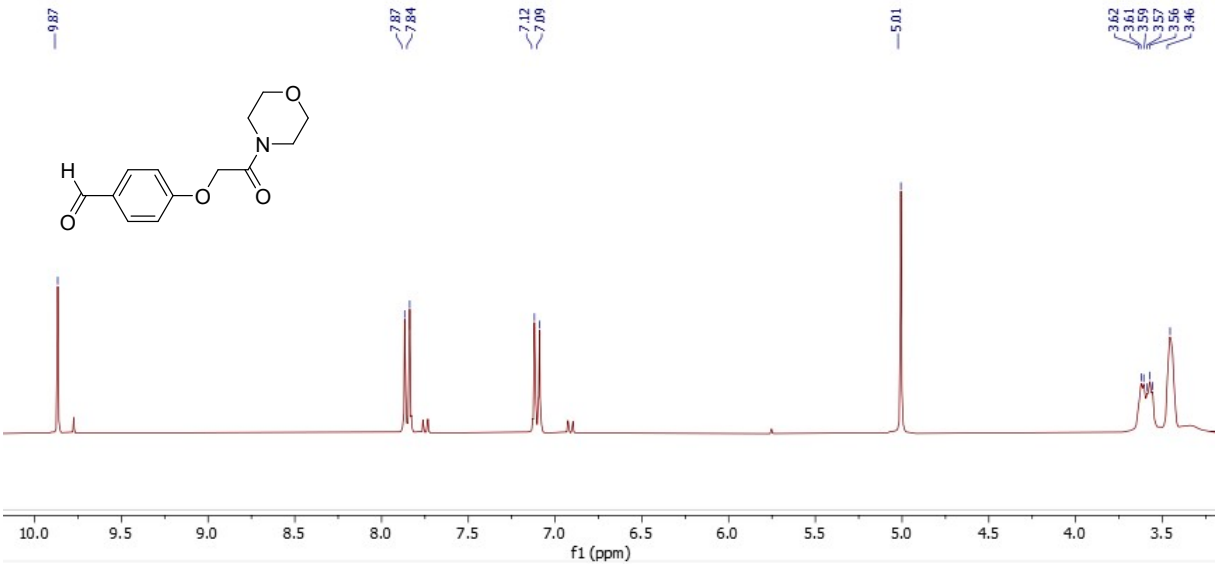


Figure S8. ^{1}H-NMR spectrum of compound **3**

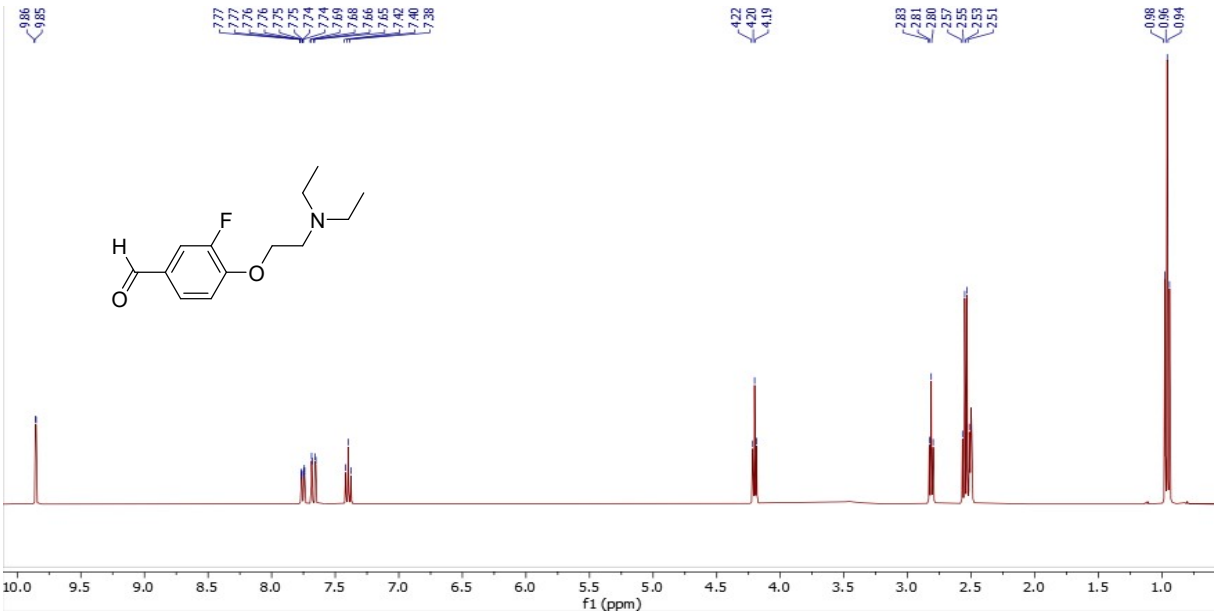


Figure S9. ^{1}H-NMR spectrum of compound **4**

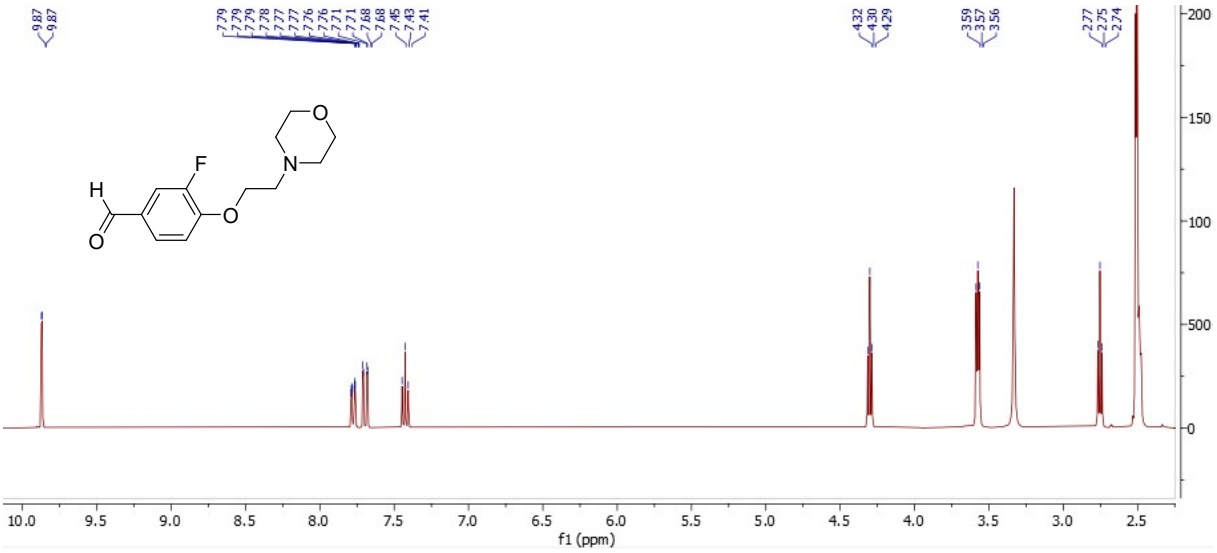


Figure S10. ^{1}H-NMR spectrum of compound **5**

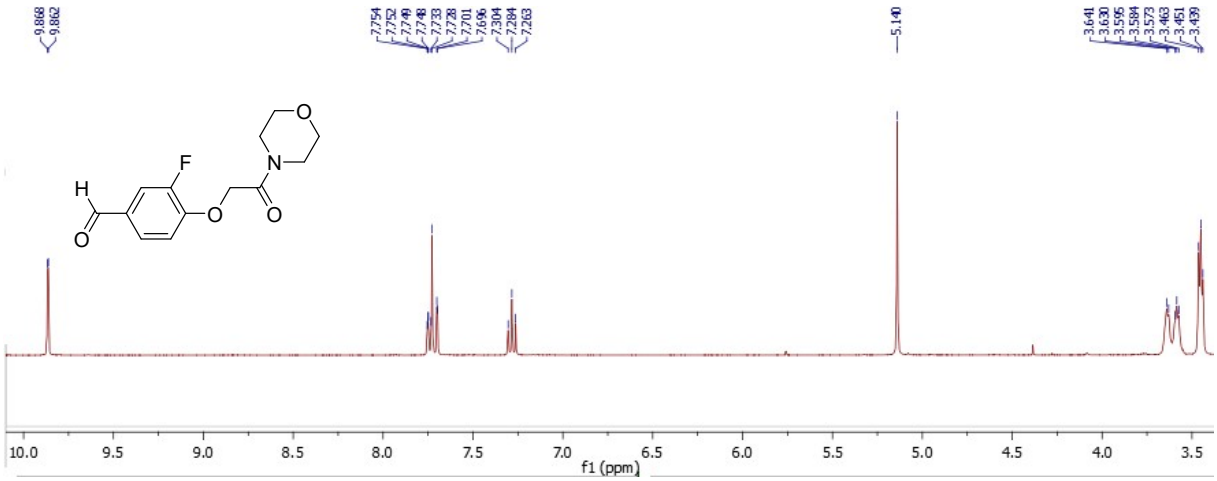


Figure S11. ^{1}H-NMR spectrum of compound **6**

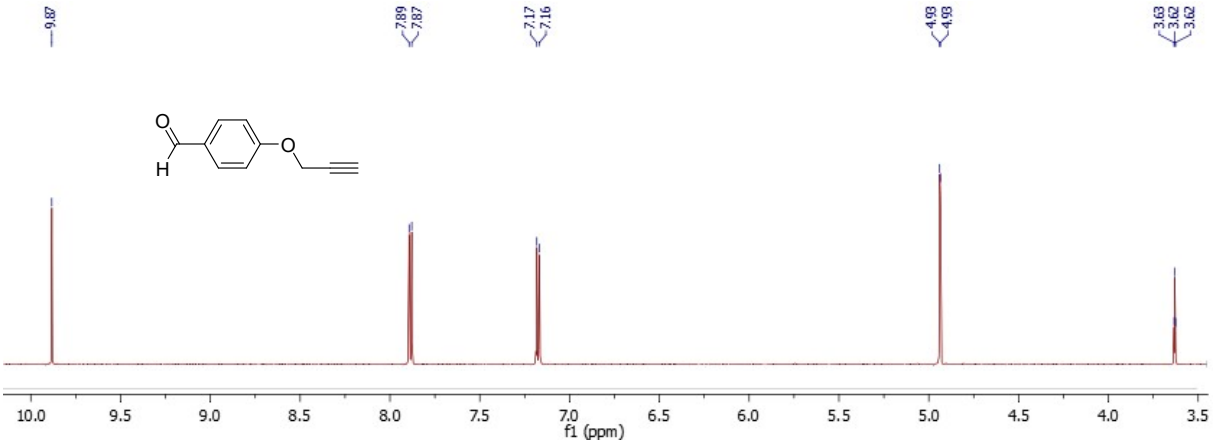


Figure S12. ^{1}H-NMR spectrum of compound **7**

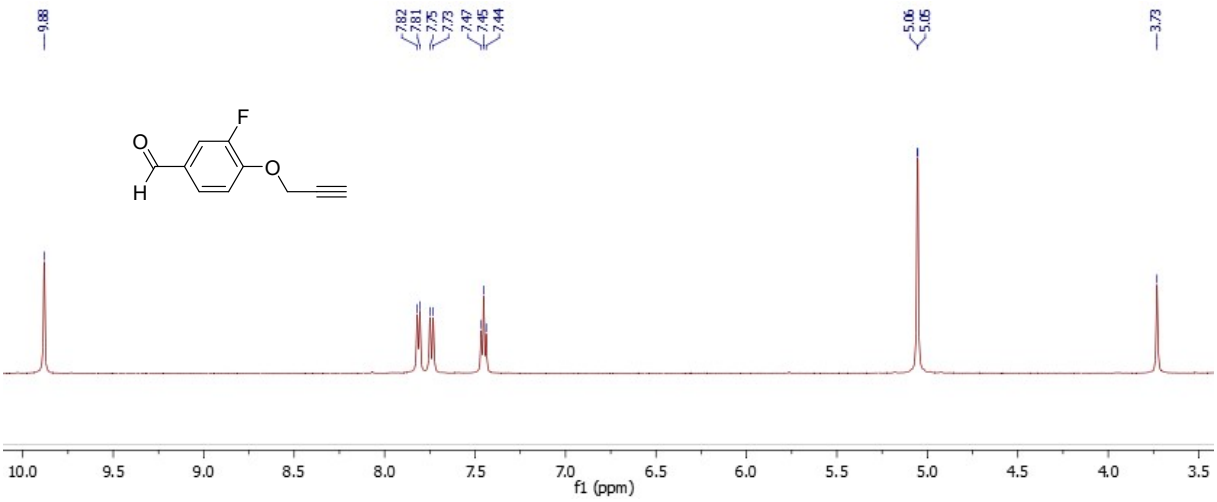


Figure S13. ^{1}H-NMR spectrum of compound **8**

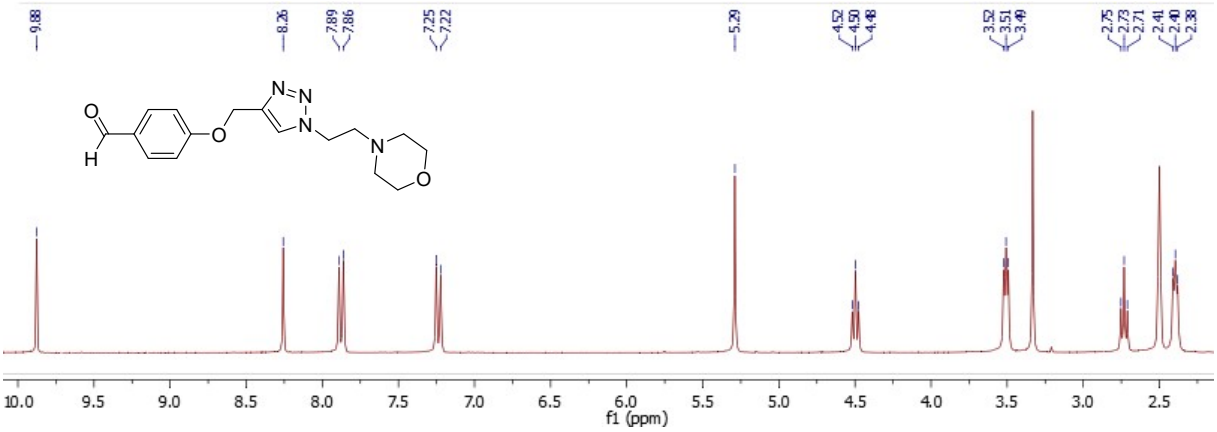


Figure S14. ^{1}H-NMR spectrum of compound **10**

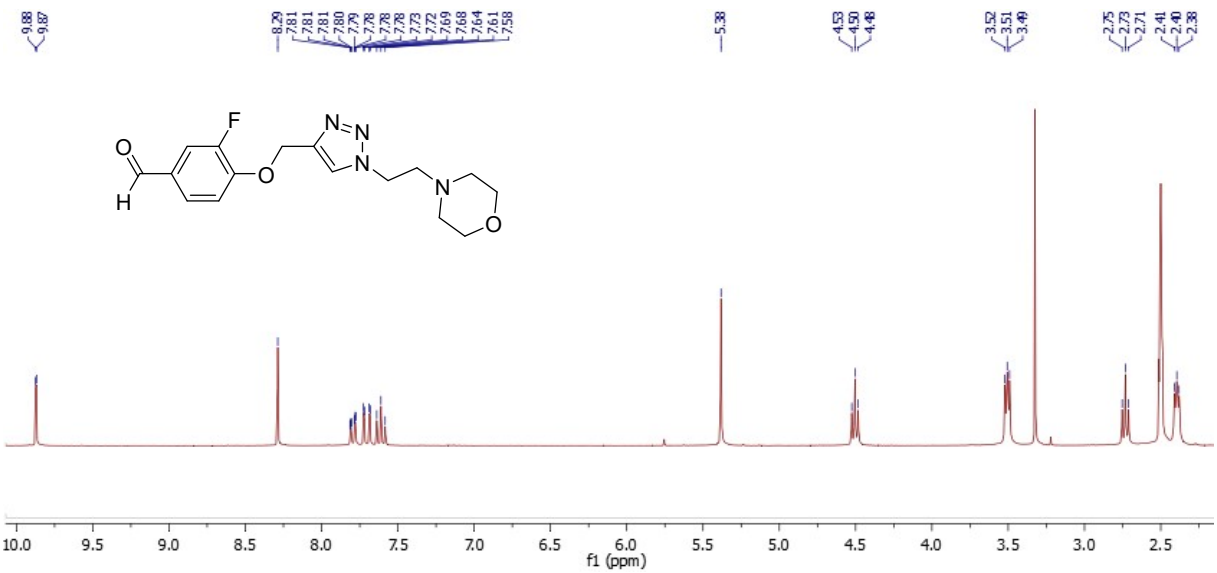


Figure S15. ^{1}H-NMR spectrum of compound **11**

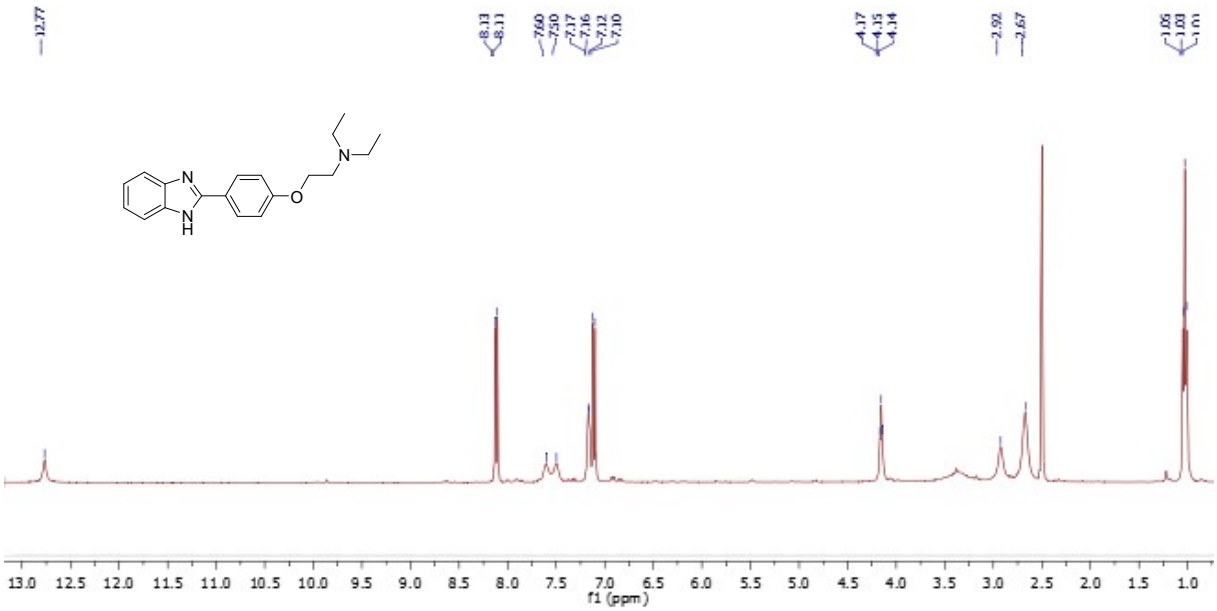


Figure S16. ^{1}H-NMR spectrum of compound **12**

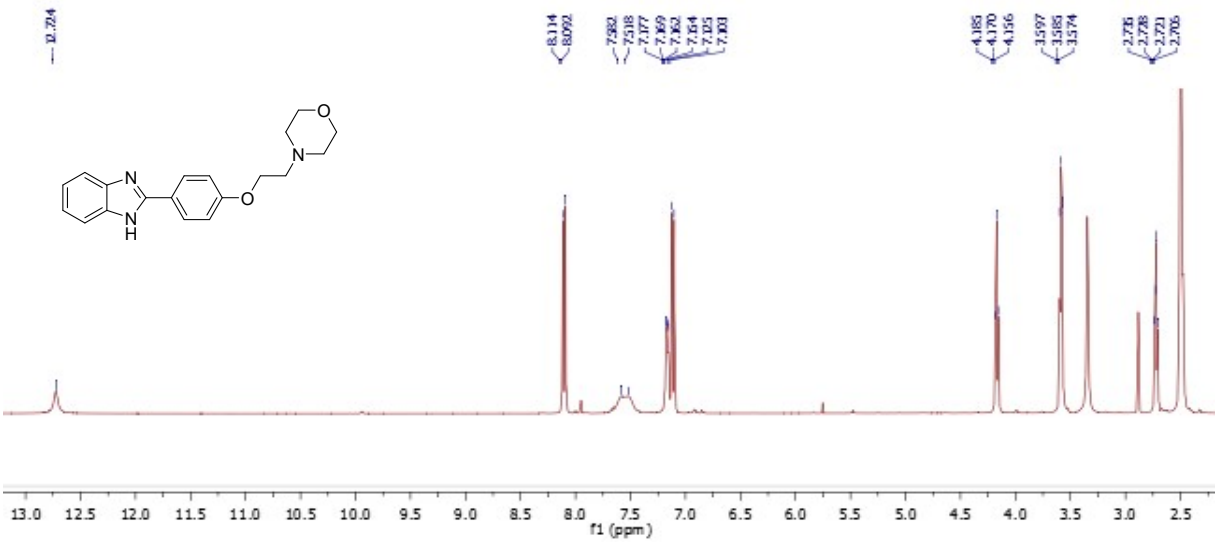


Figure S17. ^{1}H-NMR spectrum of compound **13**

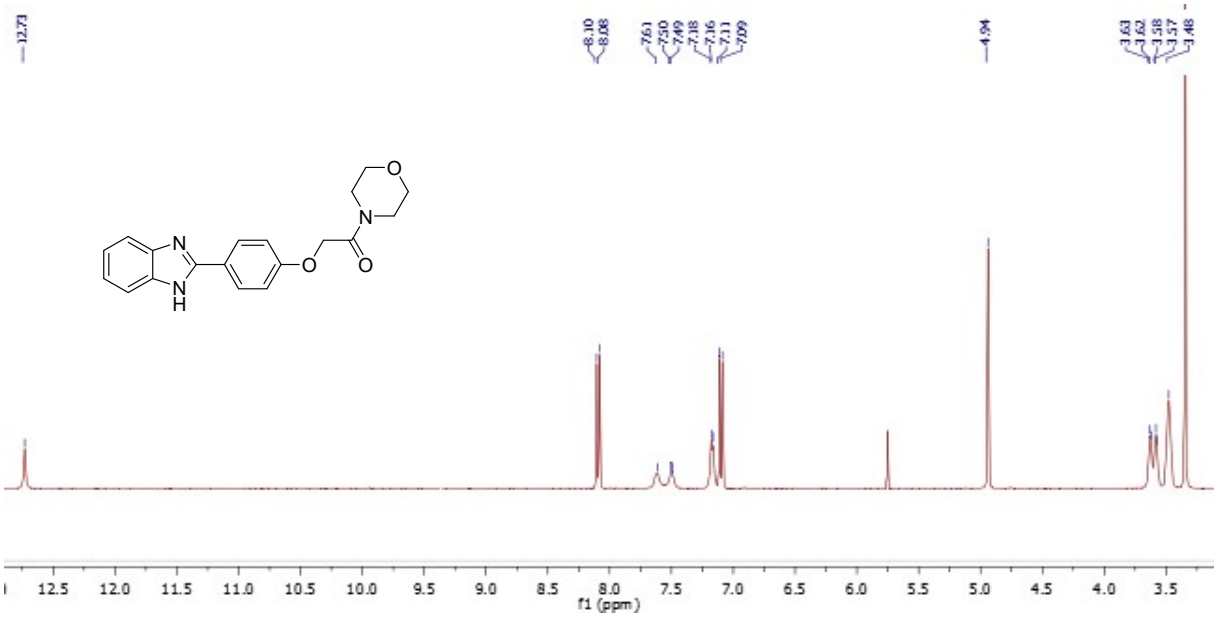


Figure S18. ^{1}H-NMR spectrum of compound **14**

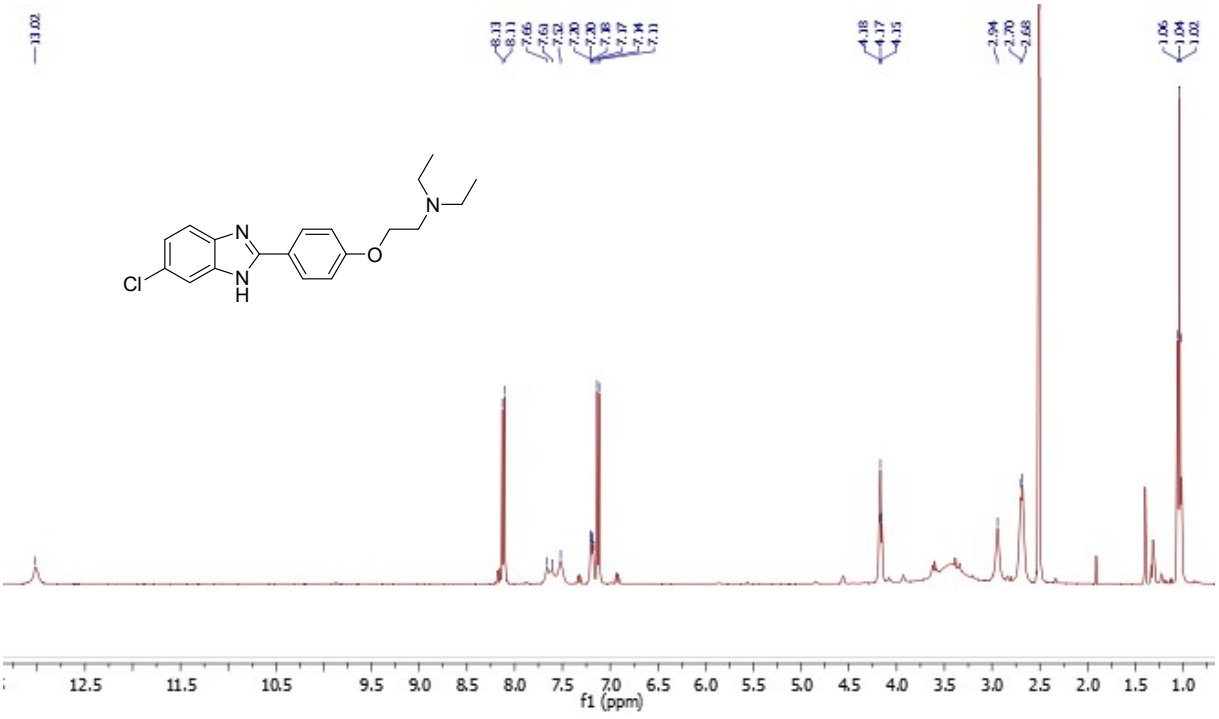


Figure S19. ^{13}C-NMR spectrum of compound **15**

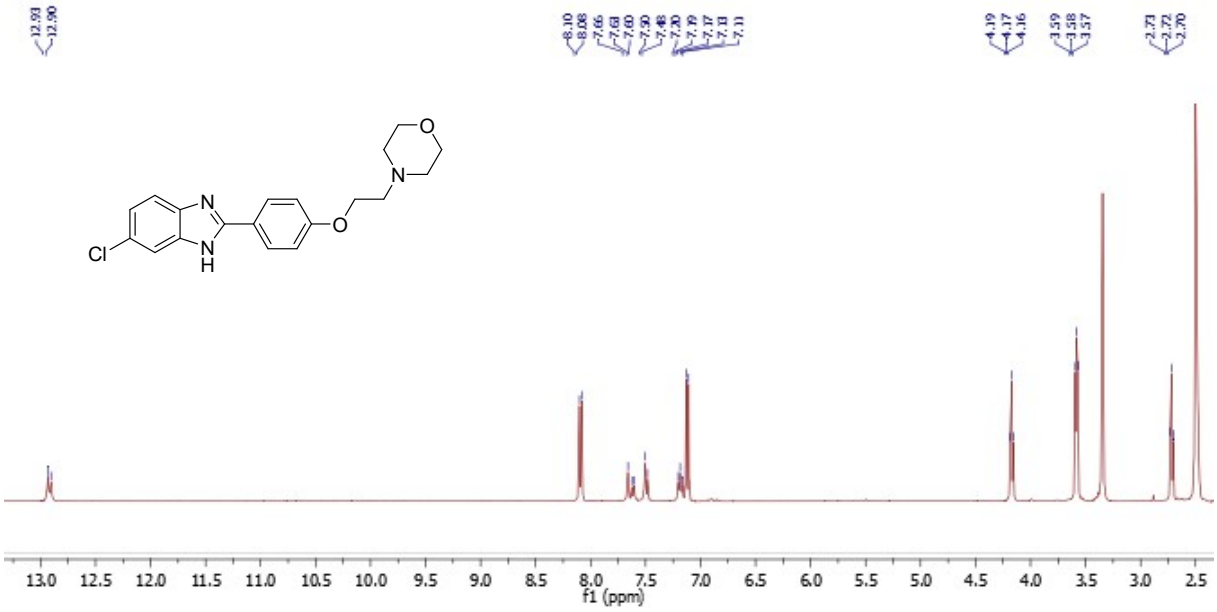


Figure S20. ^{1}H-NMR spectrum of compound **16**

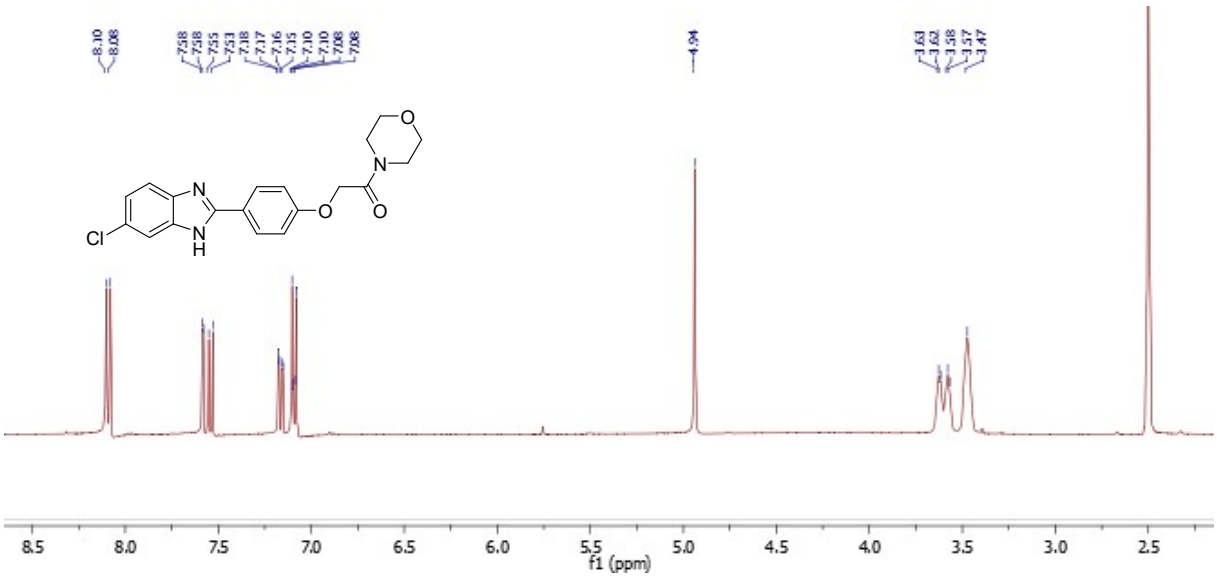


Figure S21. ^{1}H-NMR spectrum of compound **17**

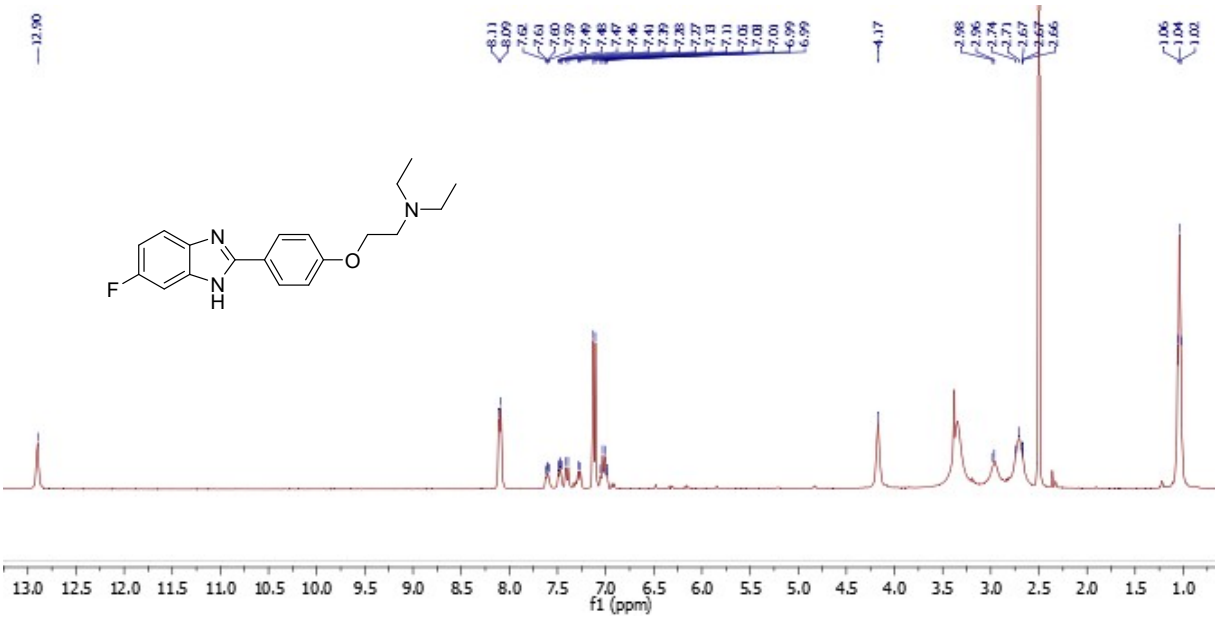


Figure S22. ^{1}H-NMR spectrum of compound **18**

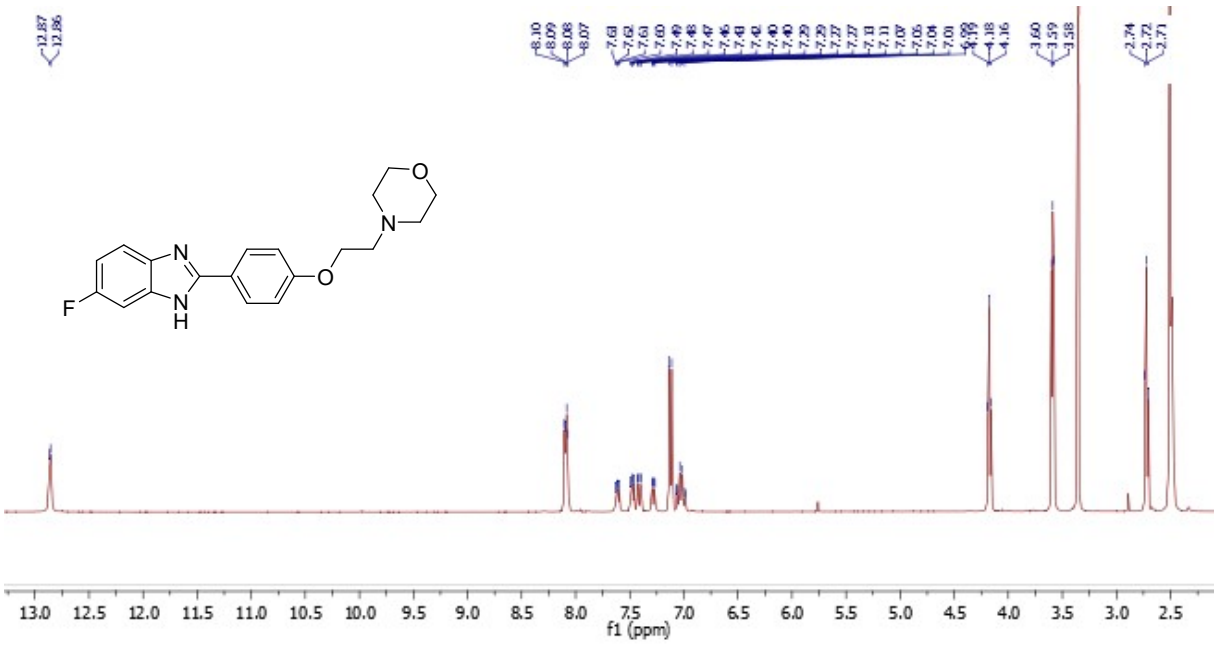


Figure S23. ^{1}H-NMR spectrum of compound **19**

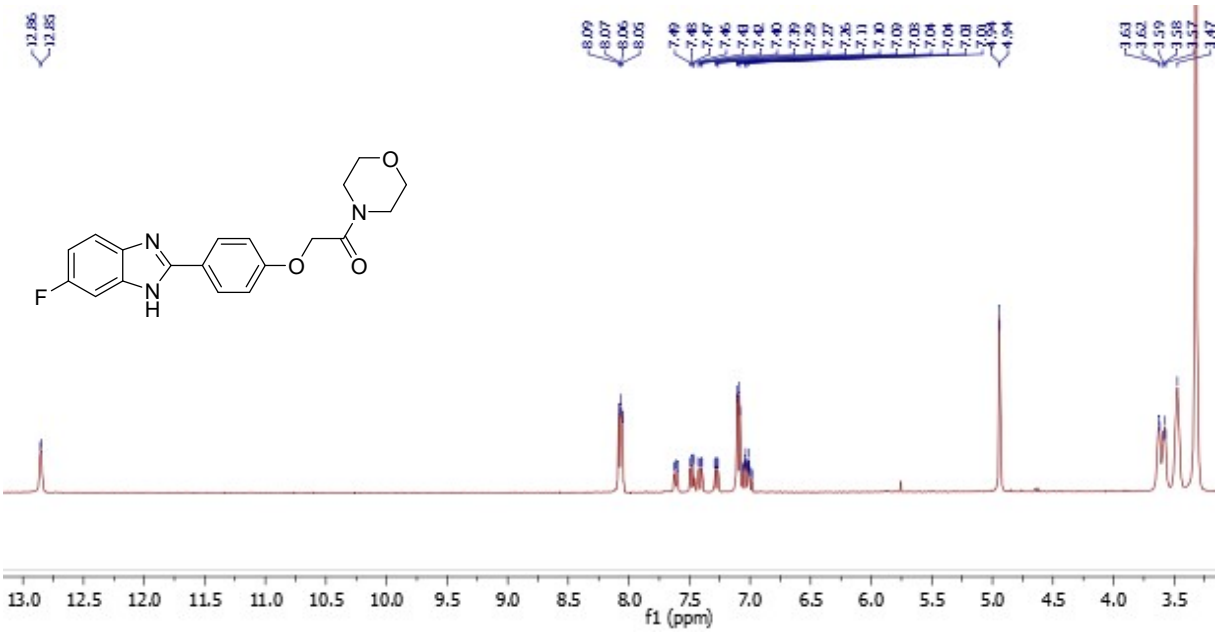


Figure S24. ^{1}H-NMR spectrum of compound **20**

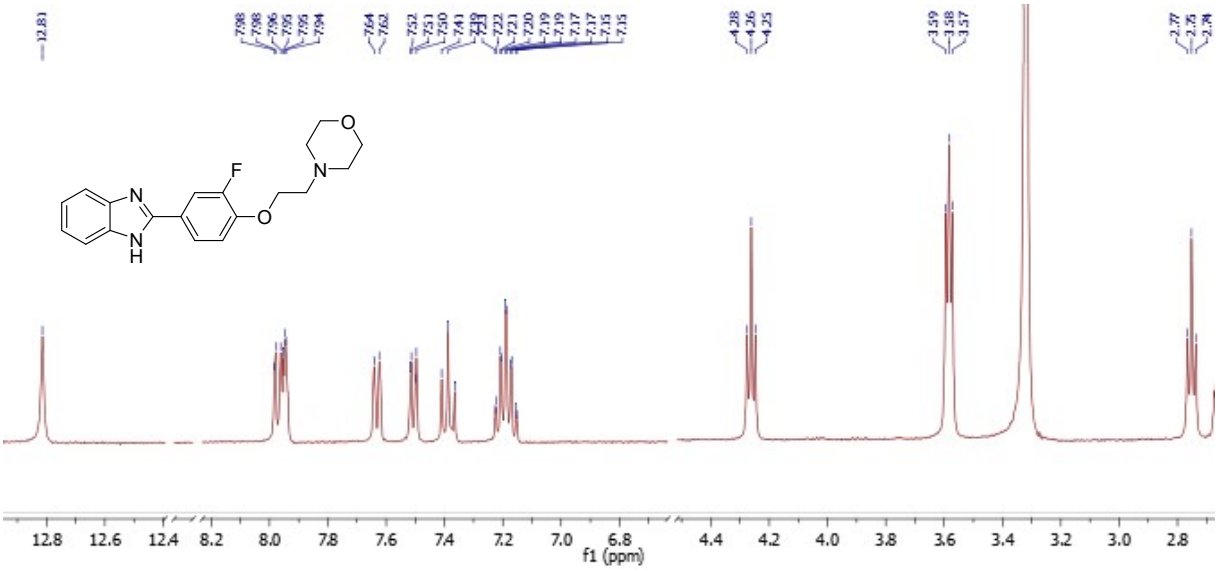


Figure S25. ^{1}H-NMR spectrum of compound **21**

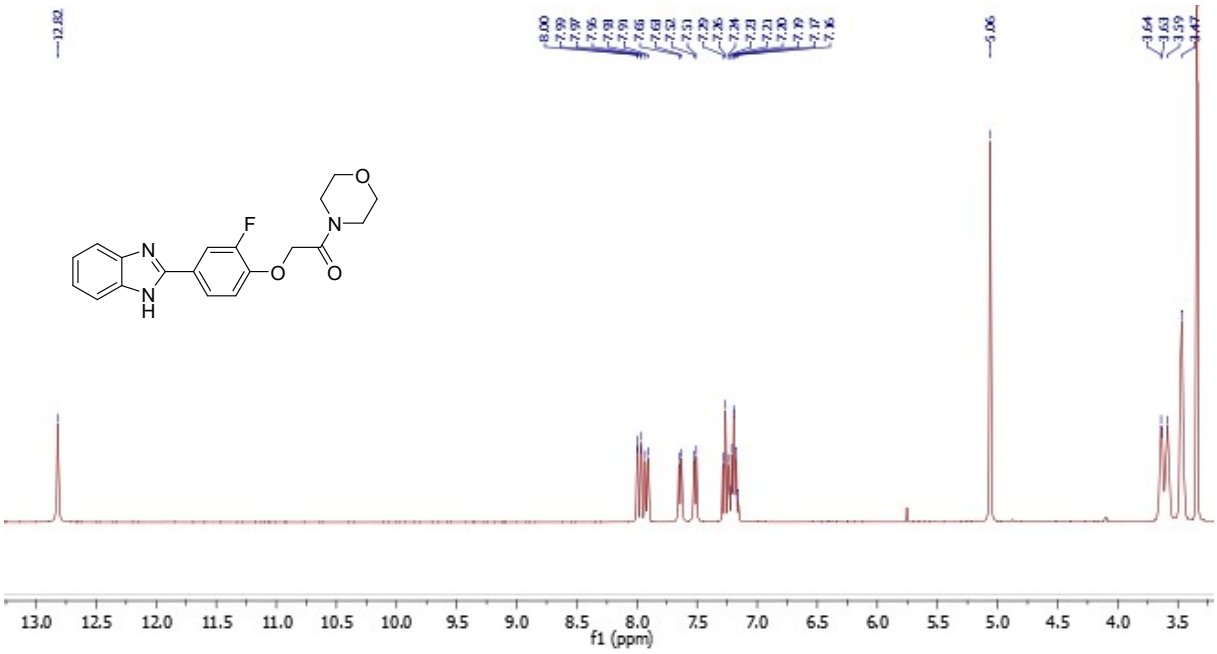


Figure S26. ^{1}H-NMR spectrum of compound **22**

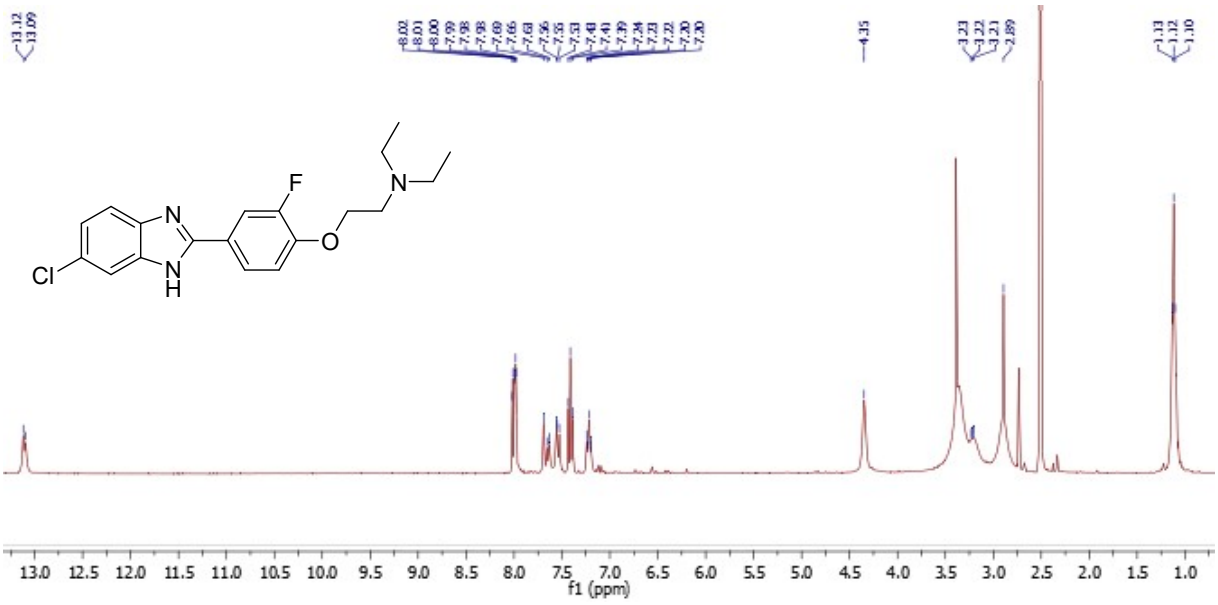


Figure S27. ^{1}H-NMR spectrum of compound **23**

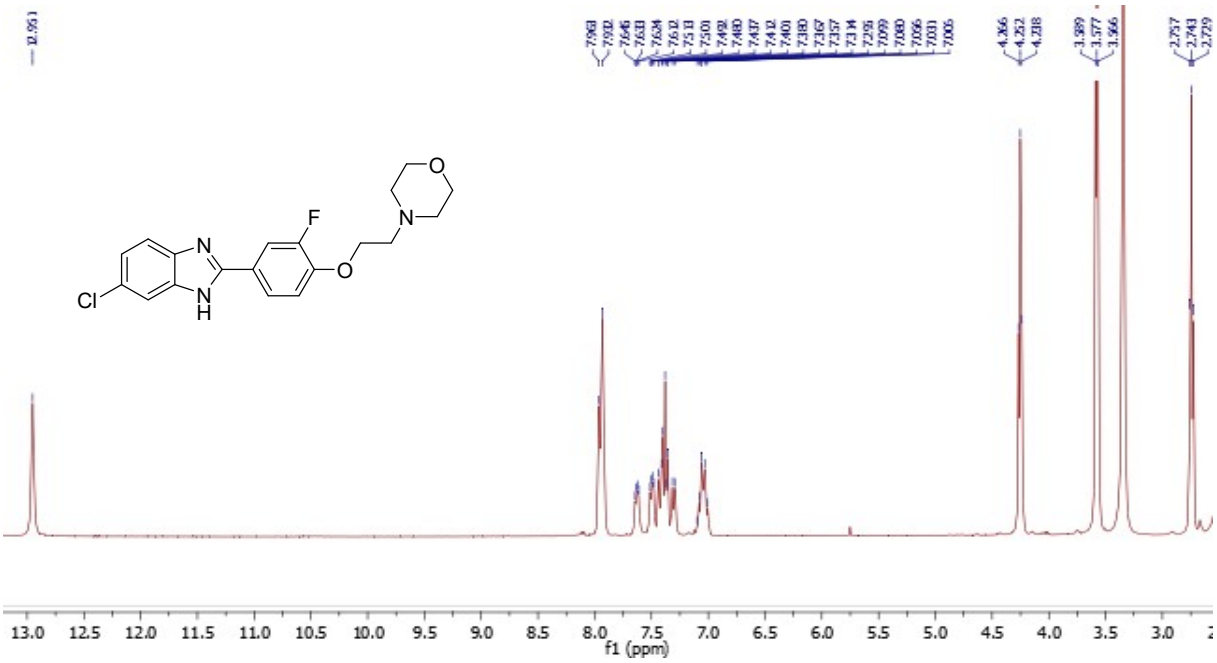


Figure S28. ^{1}H-NMR spectrum of compound **24**

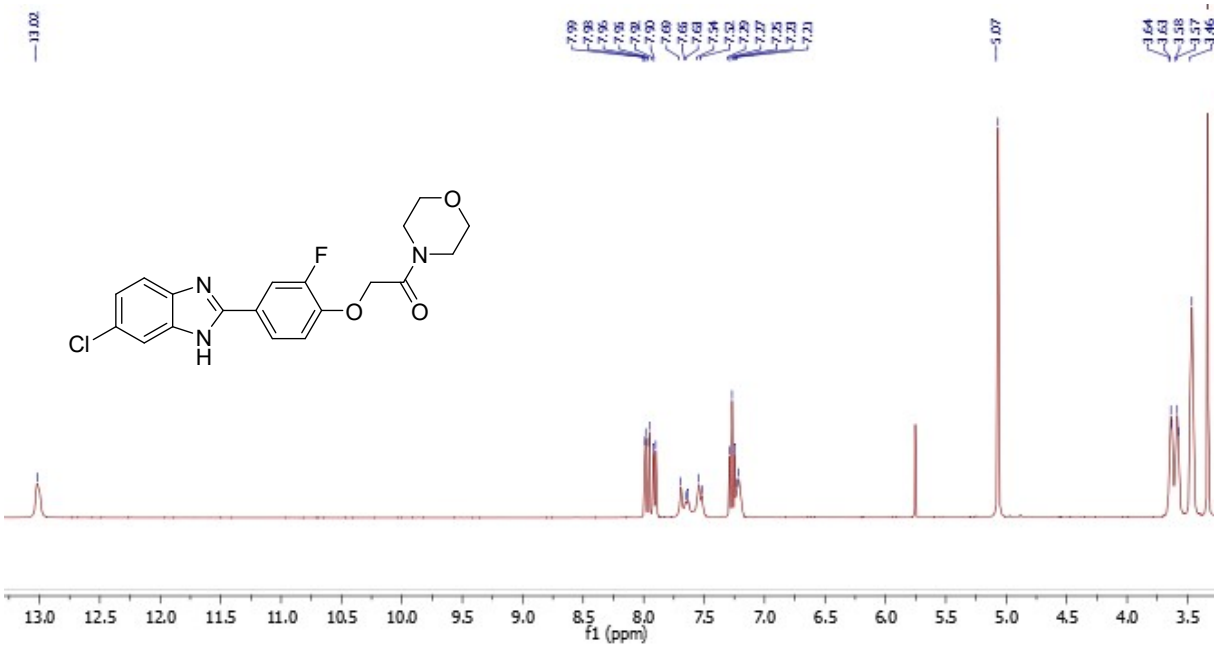


Figure S29. ^{1}H-NMR spectrum of compound **25**

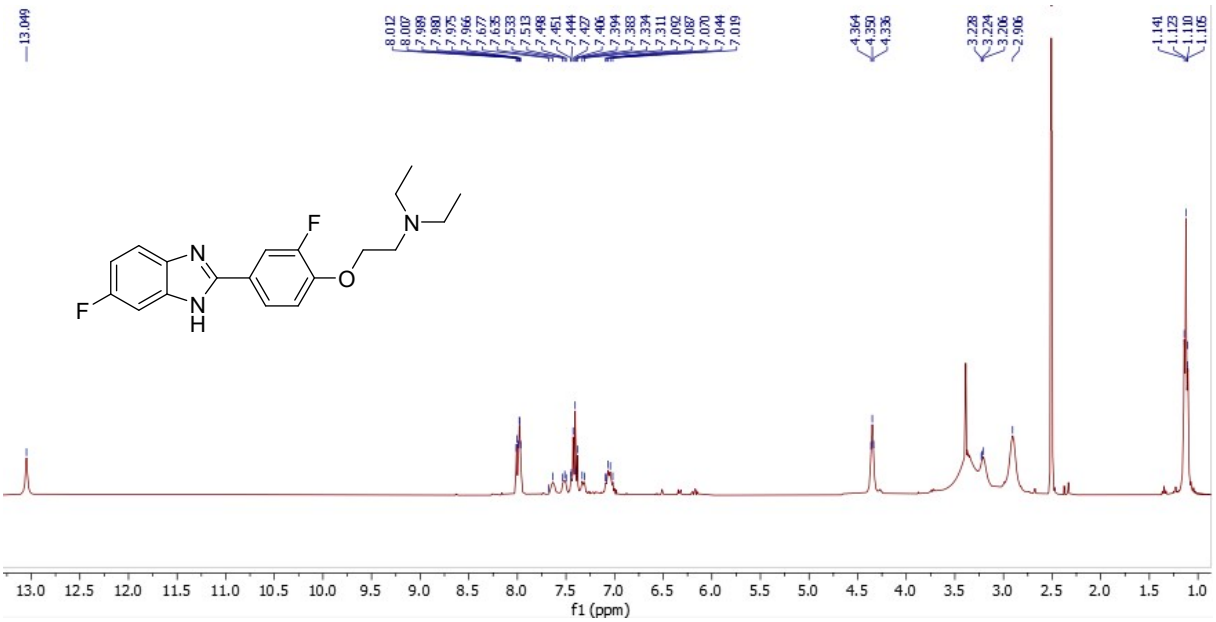


Figure S30. ^{1}H-NMR spectrum of compound **26**

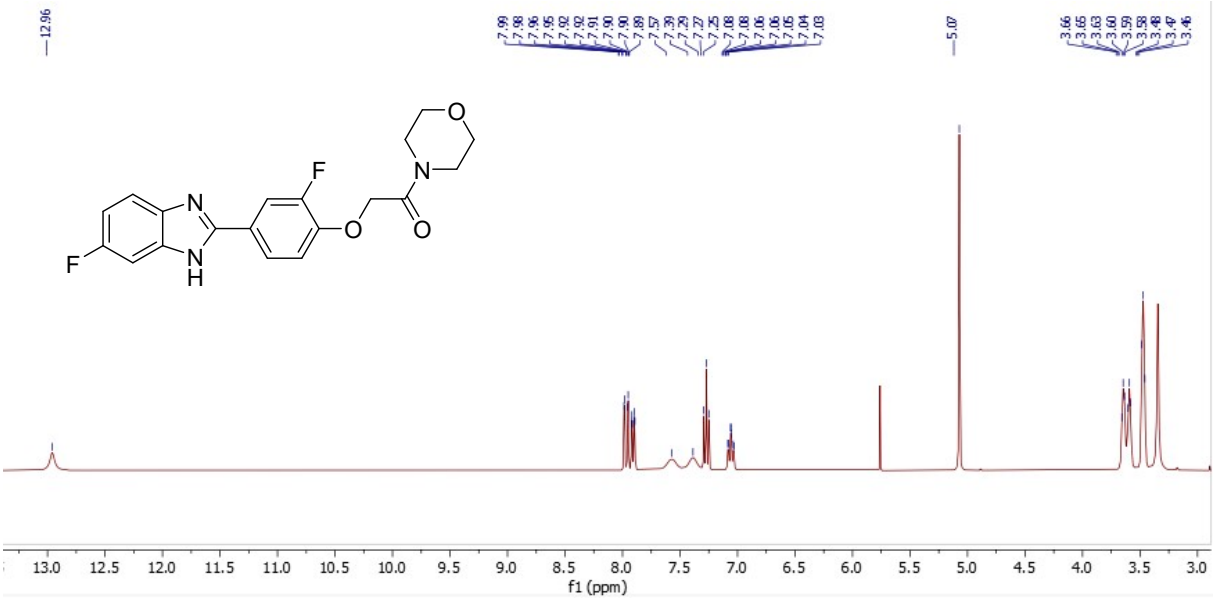


Figure S31. ^{1}H-NMR spectrum of compound **27**

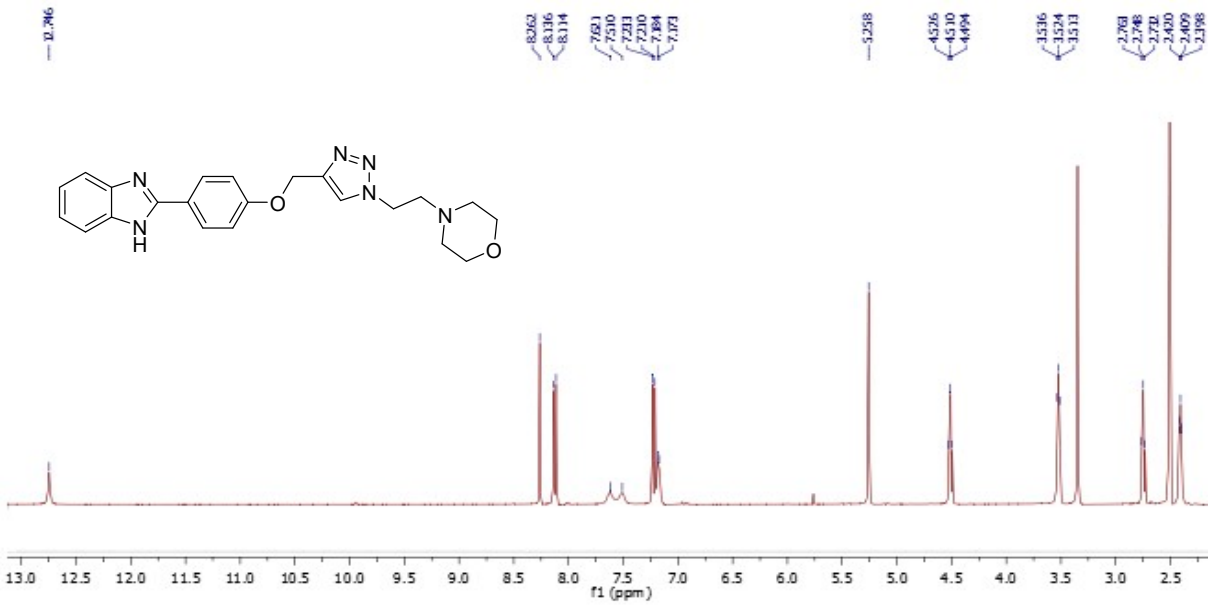


Figure S32. ^{1}H-NMR spectrum of compound **28**

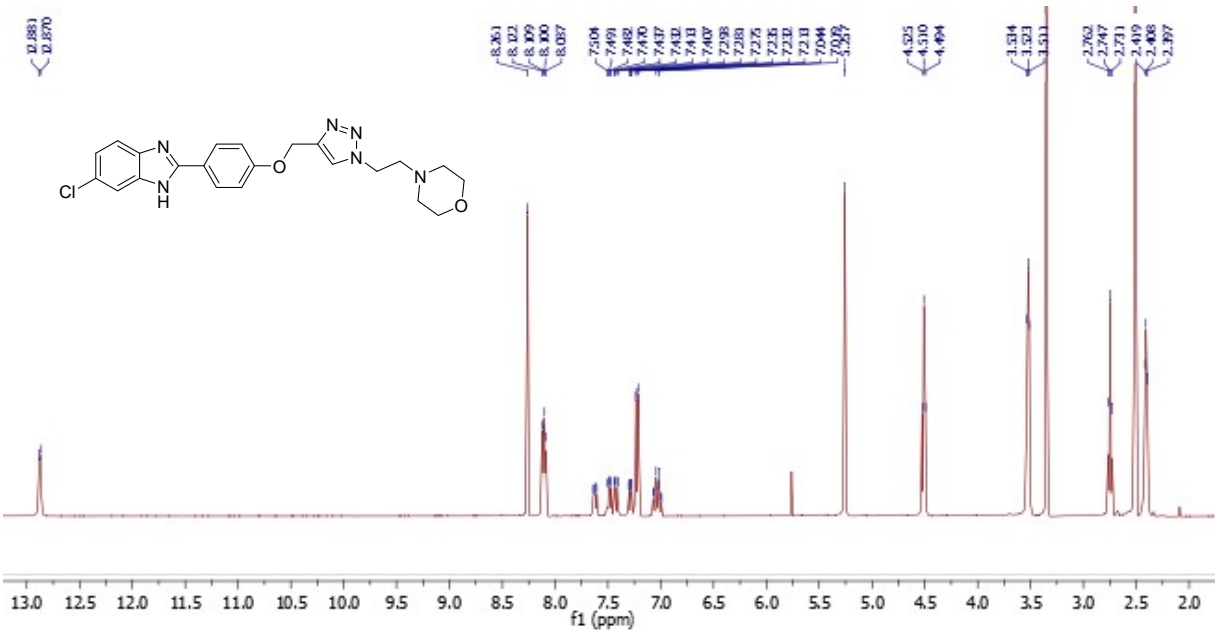


Figure S33. ^{1}H-NMR spectrum of compound **29**

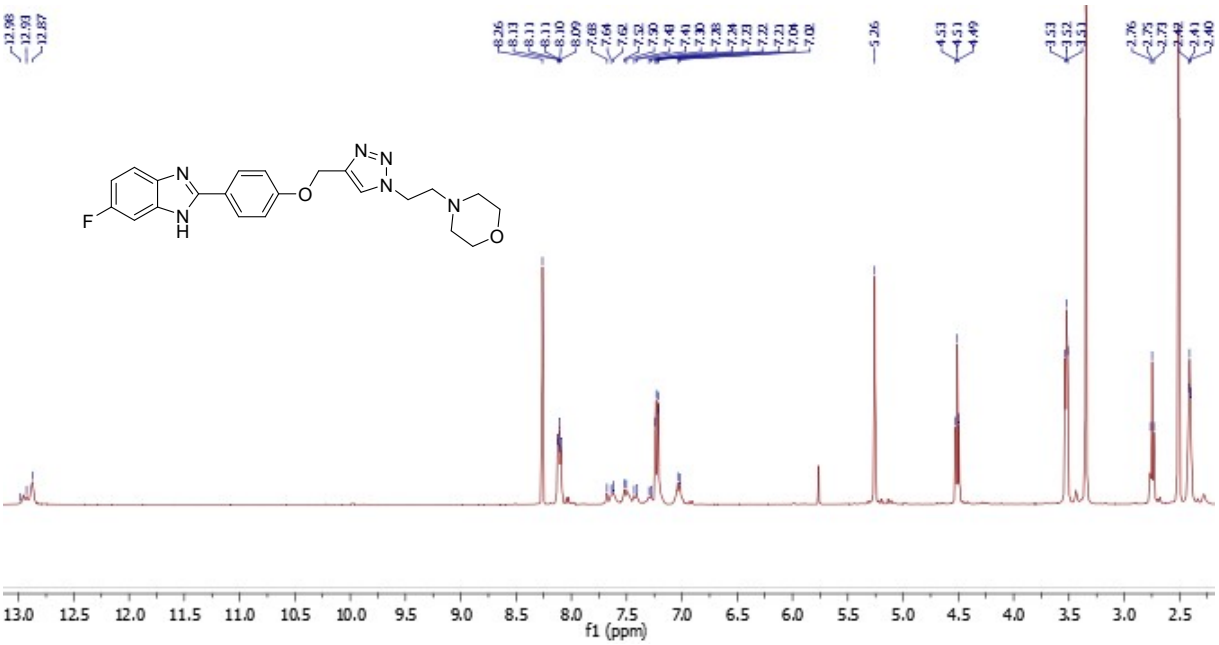


Figure S34. ^{1}H-NMR spectrum of compound **30**

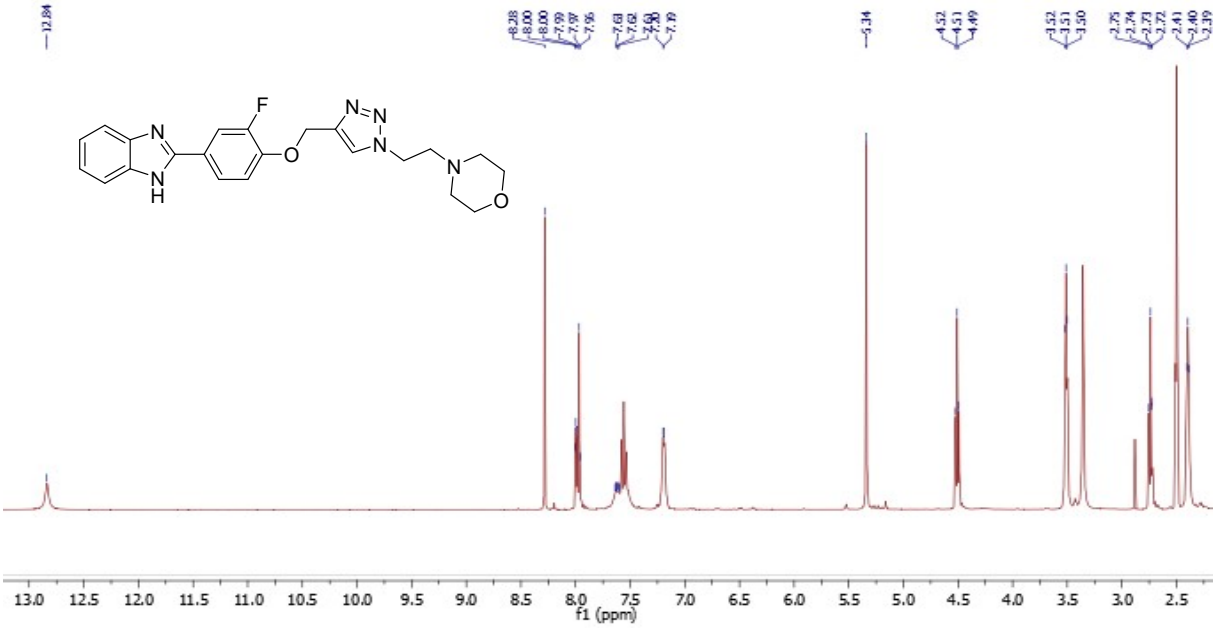


Figure S35. ^{1}H-NMR spectrum of compound **31**

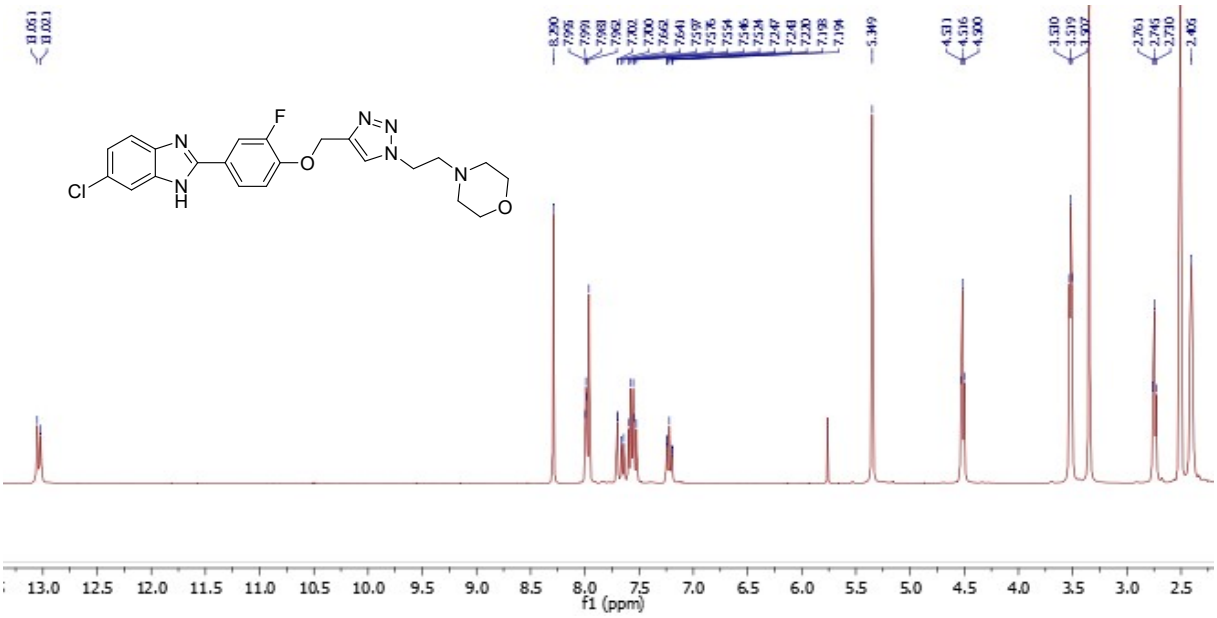


Figure S36. ^{1}H-NMR spectrum of compound **32**

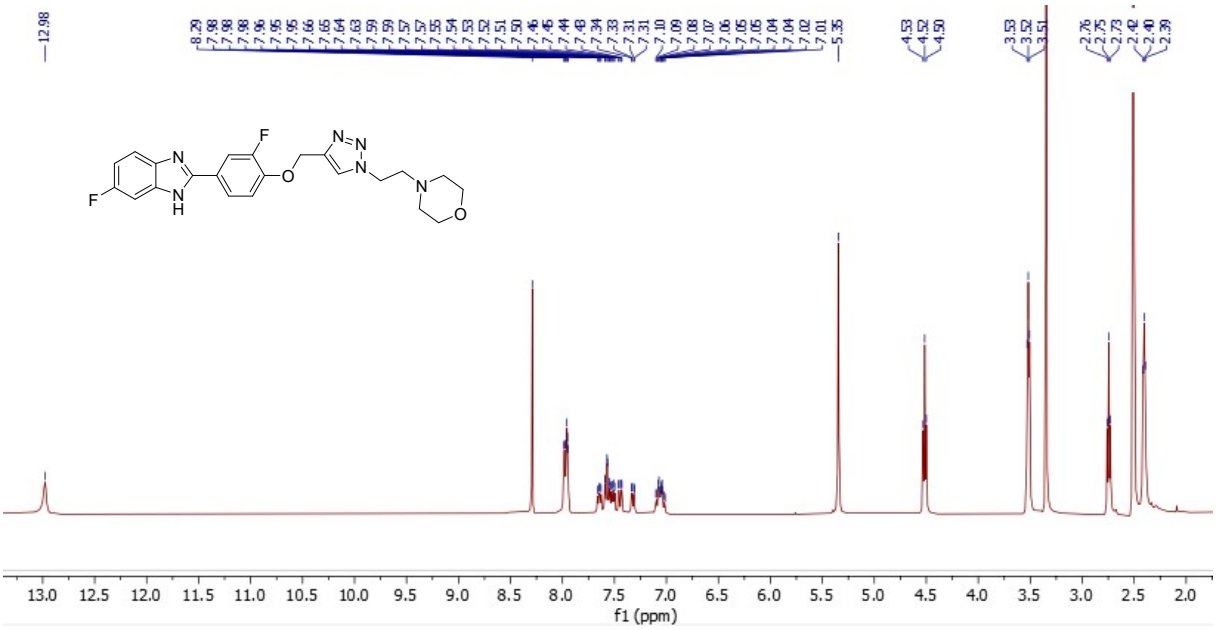


Figure S37. ^{1}H-NMR spectrum of compound **33**

^{13}C-NMR spectra for compounds **1**–**8** (Fig. S38–S45) and **10**–**33** (Fig S46–S69)

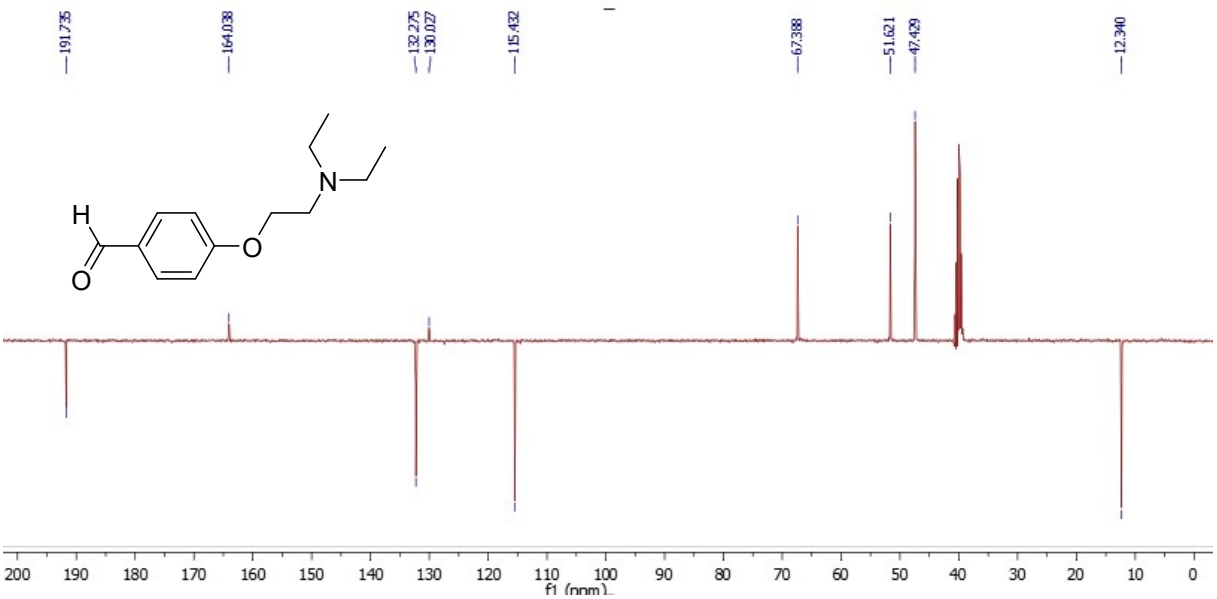


Figure S38. ^{13}C-NMR spectrum of compound **1**

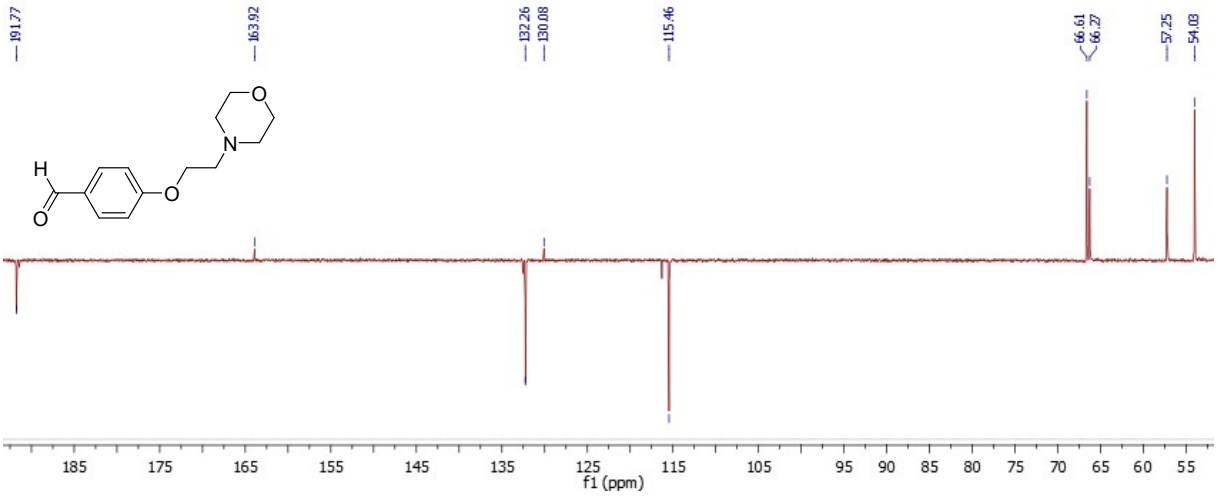


Figure S39. ^{13}C-NMR spectrum of compound **2**

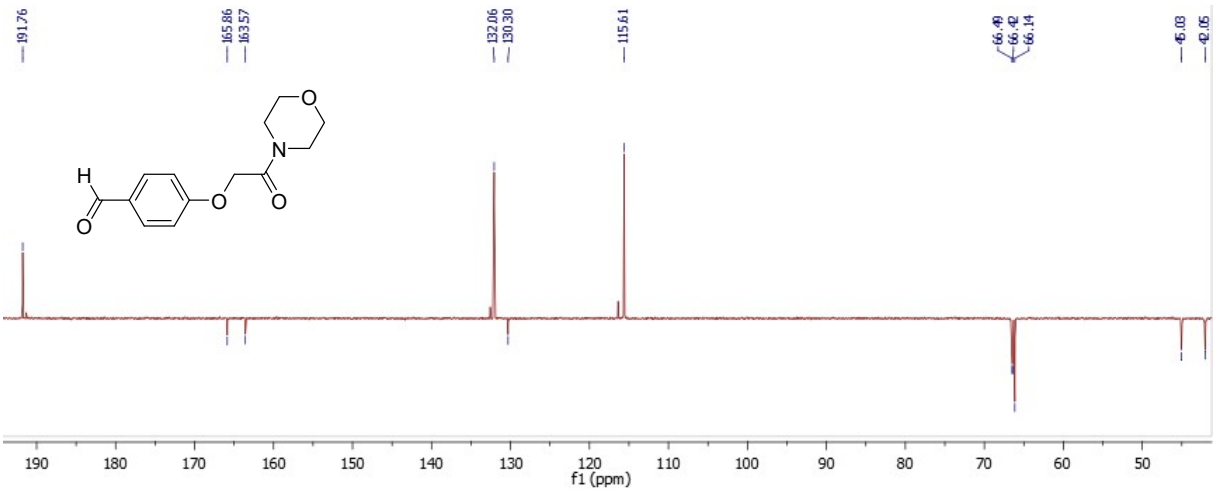


Figure S40. ^{13}C-NMR spectrum of compound **3**

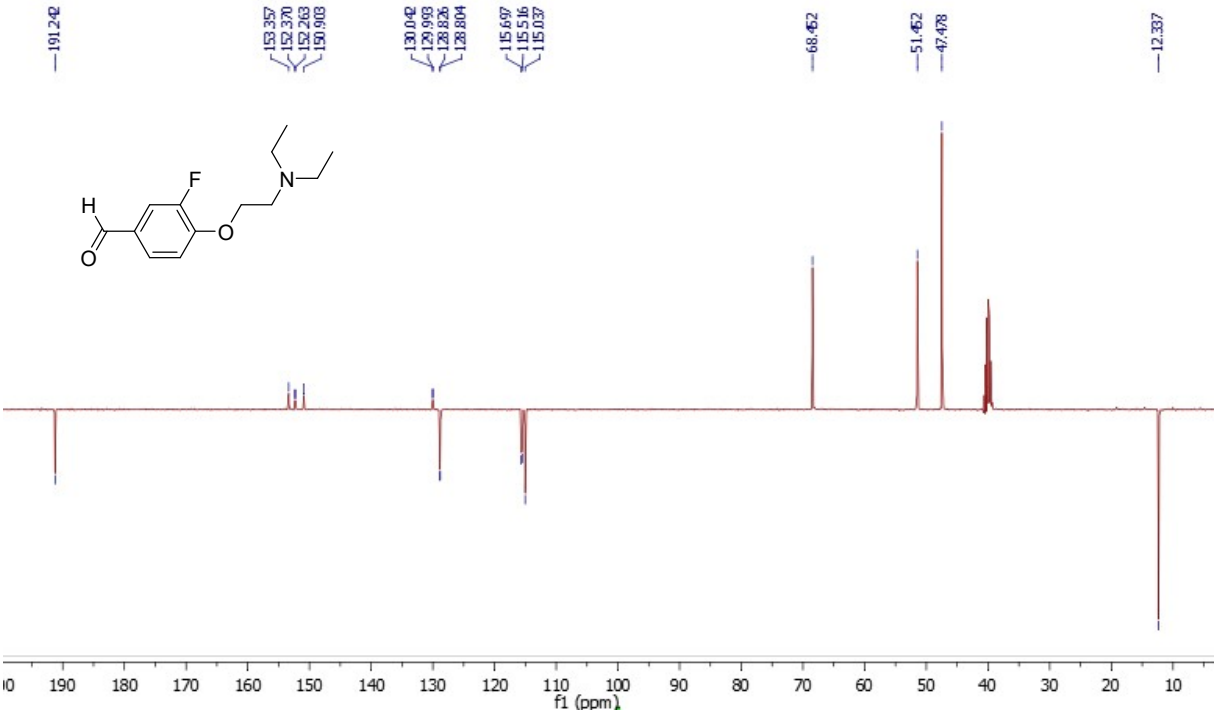


Figure S41. ^{13}C-NMR spectrum of compound **4**

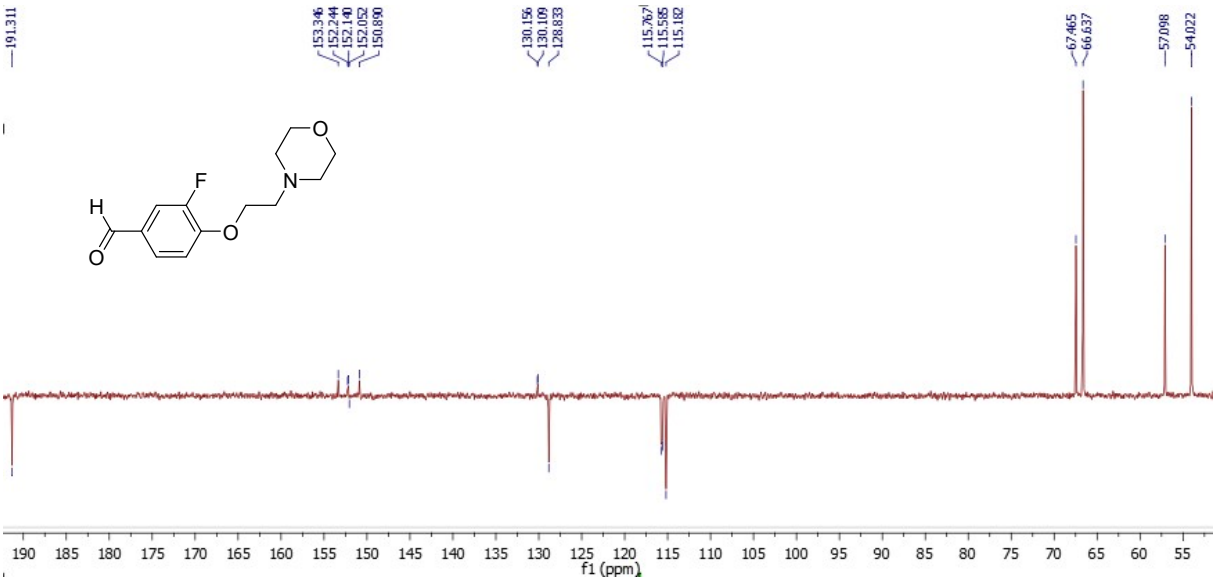


Figure S42. ^{13}C-NMR spectrum of compound **5**

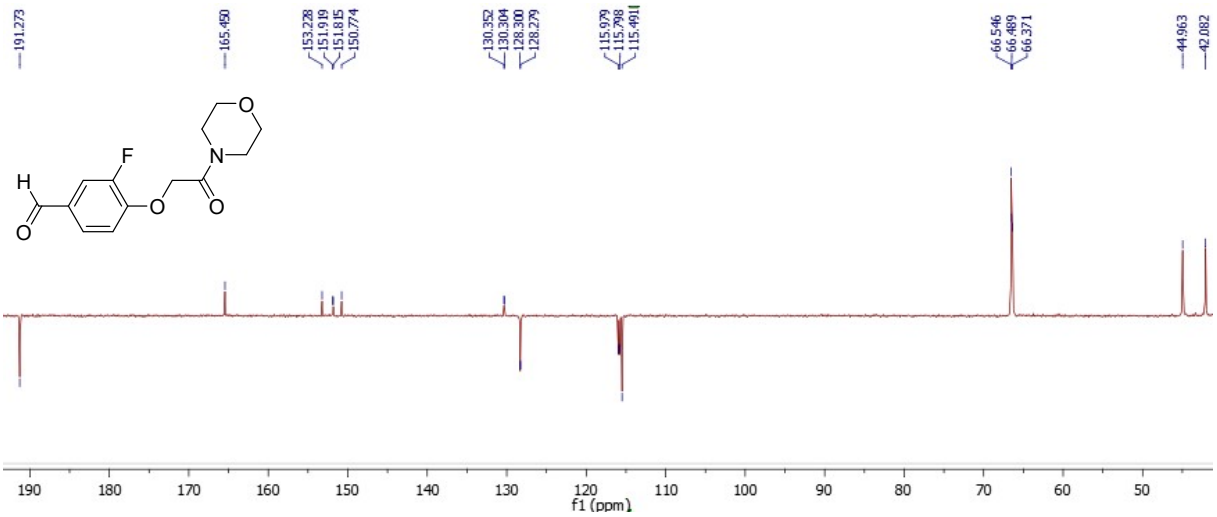


Figure S43. ^{13}C-NMR spectrum of compound **6**

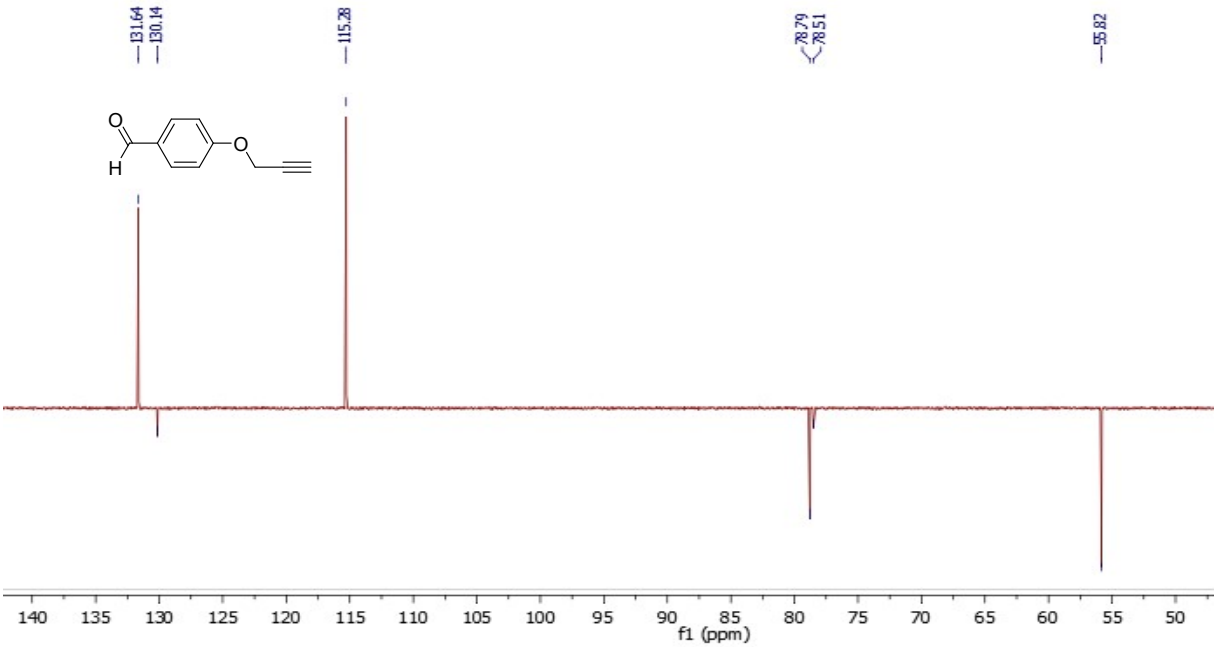


Figure S44. ^{13}C-NMR spectrum of compound **7**

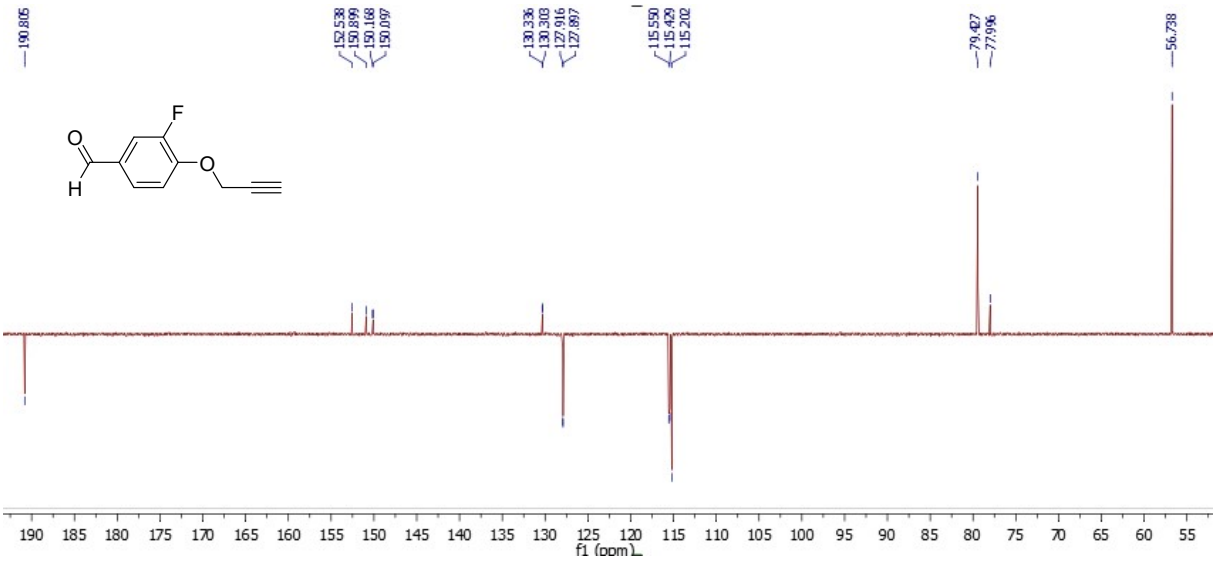


Figure S45. ^{13}C-NMR spectrum of compound **8**

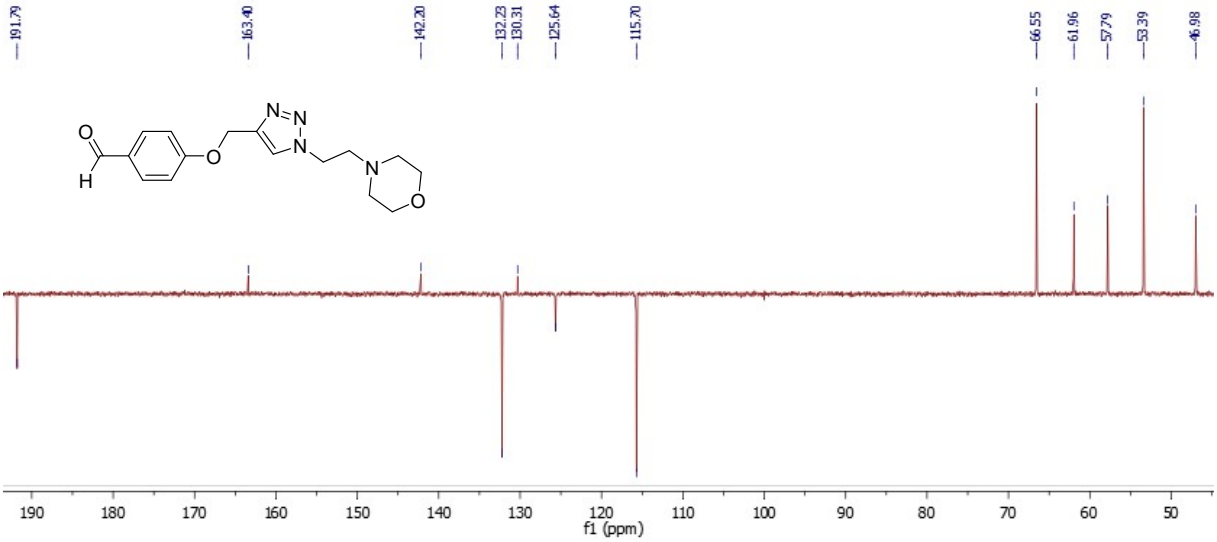


Figure S46. ^{13}C-NMR spectrum of compound **10**

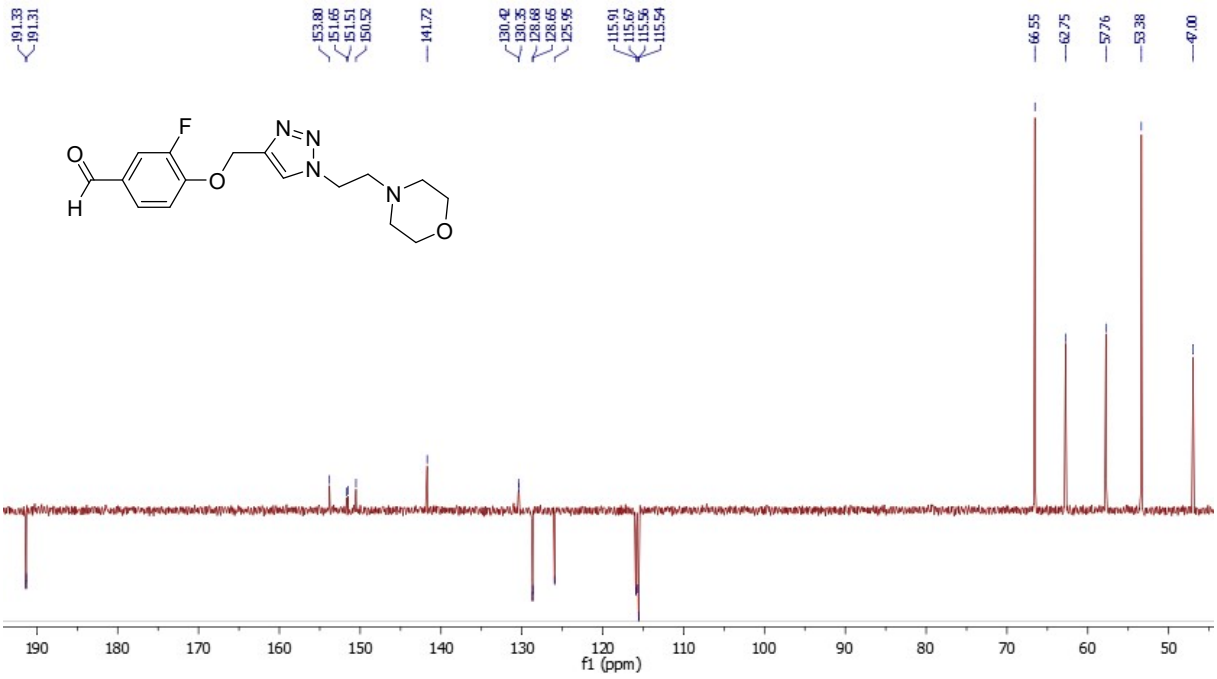


Figure S47. ^{13}C-NMR spectrum of compound **11**

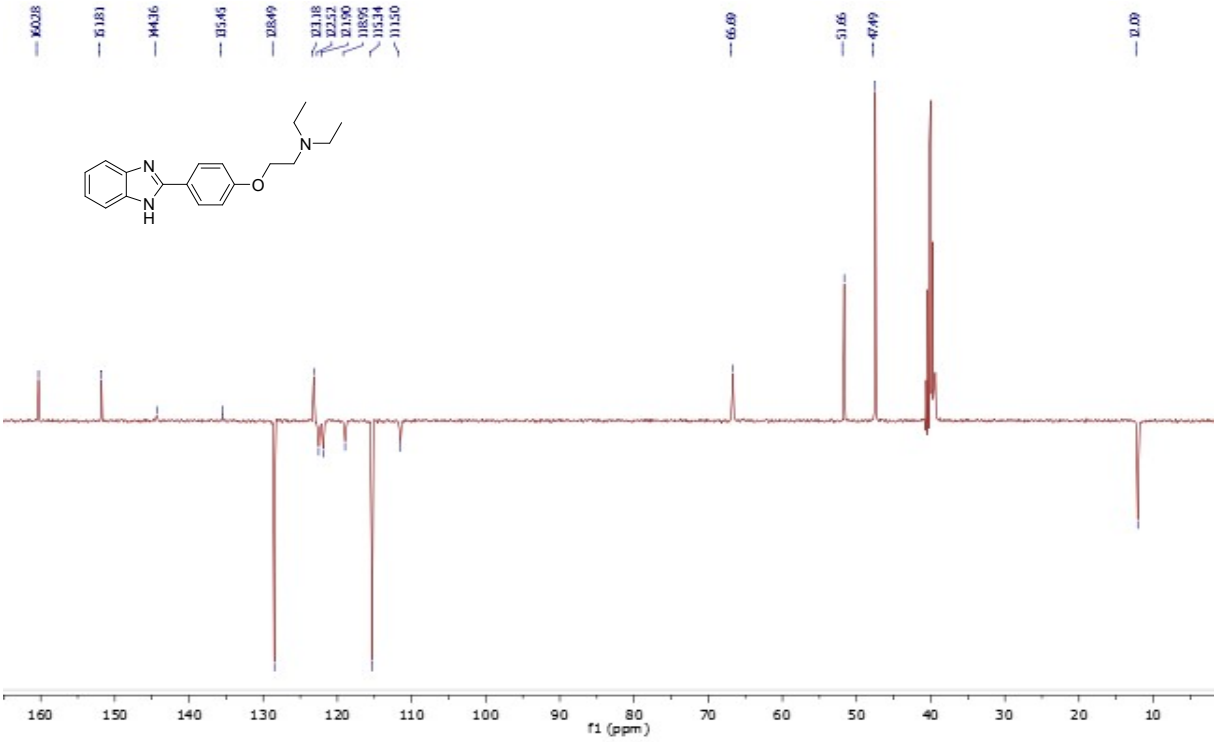


Figure S48. ^{13}C-NMR spectrum of compound **12**

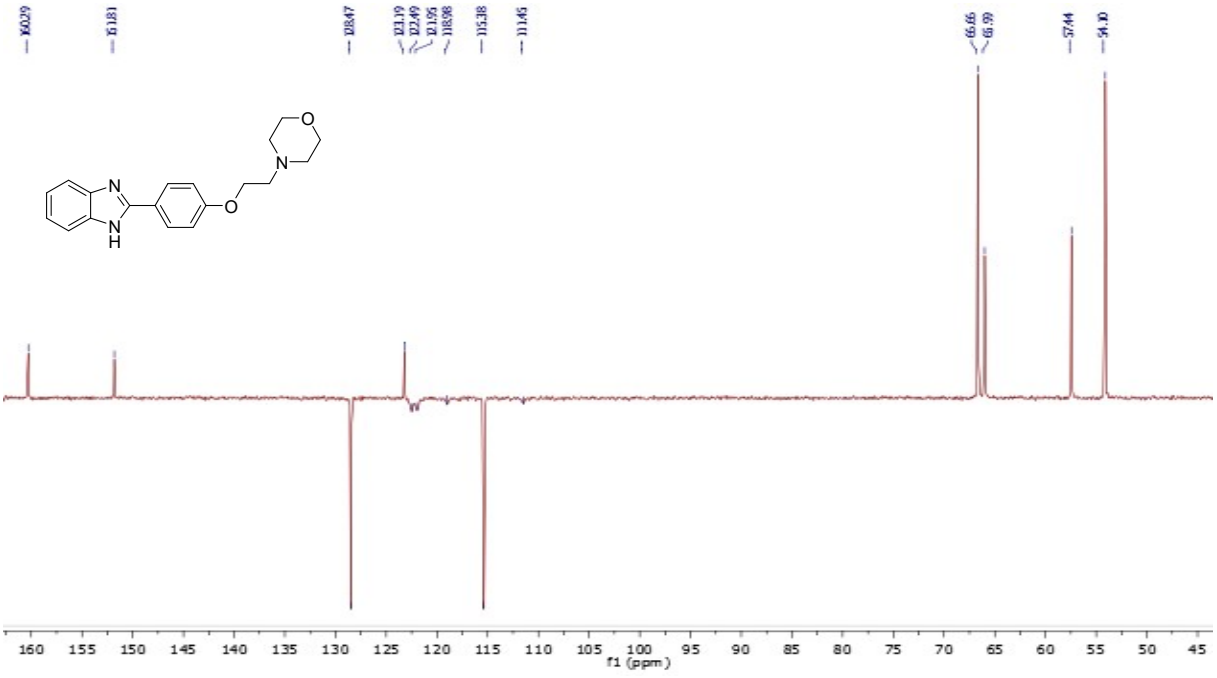


Figure S49. ^{13}C-NMR spectrum of compound **13**

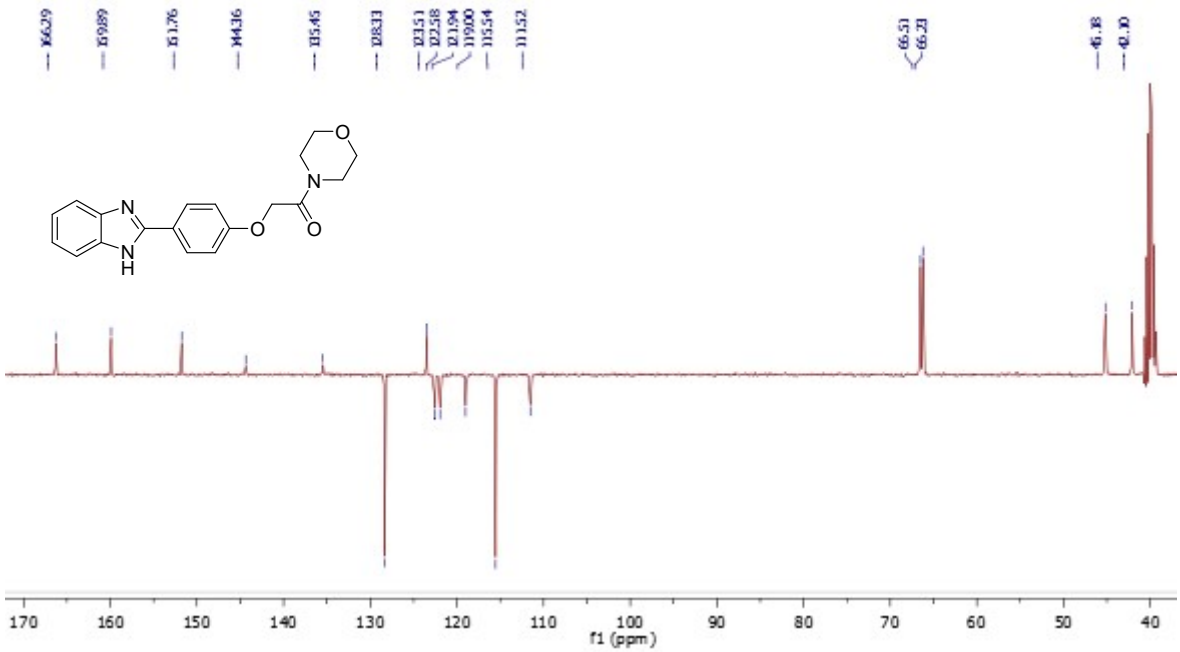


Figure S50. ^{13}C-NMR spectrum of compound **14**

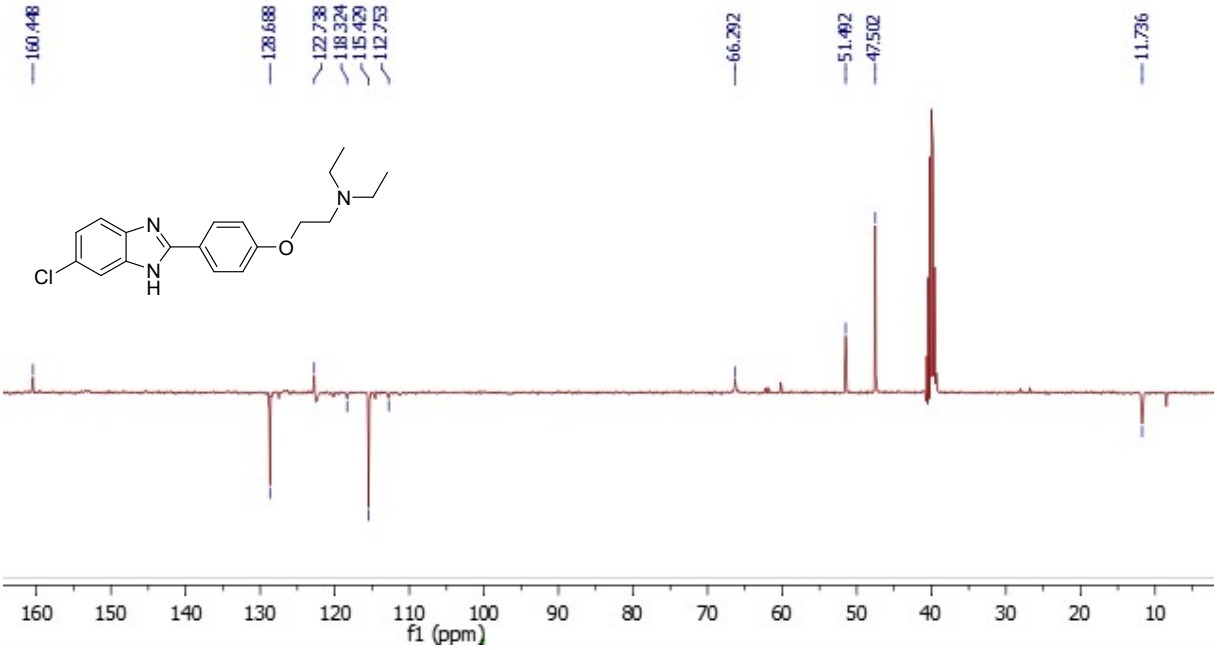


Figure S51. ^{13}C spectrum of compound **15**

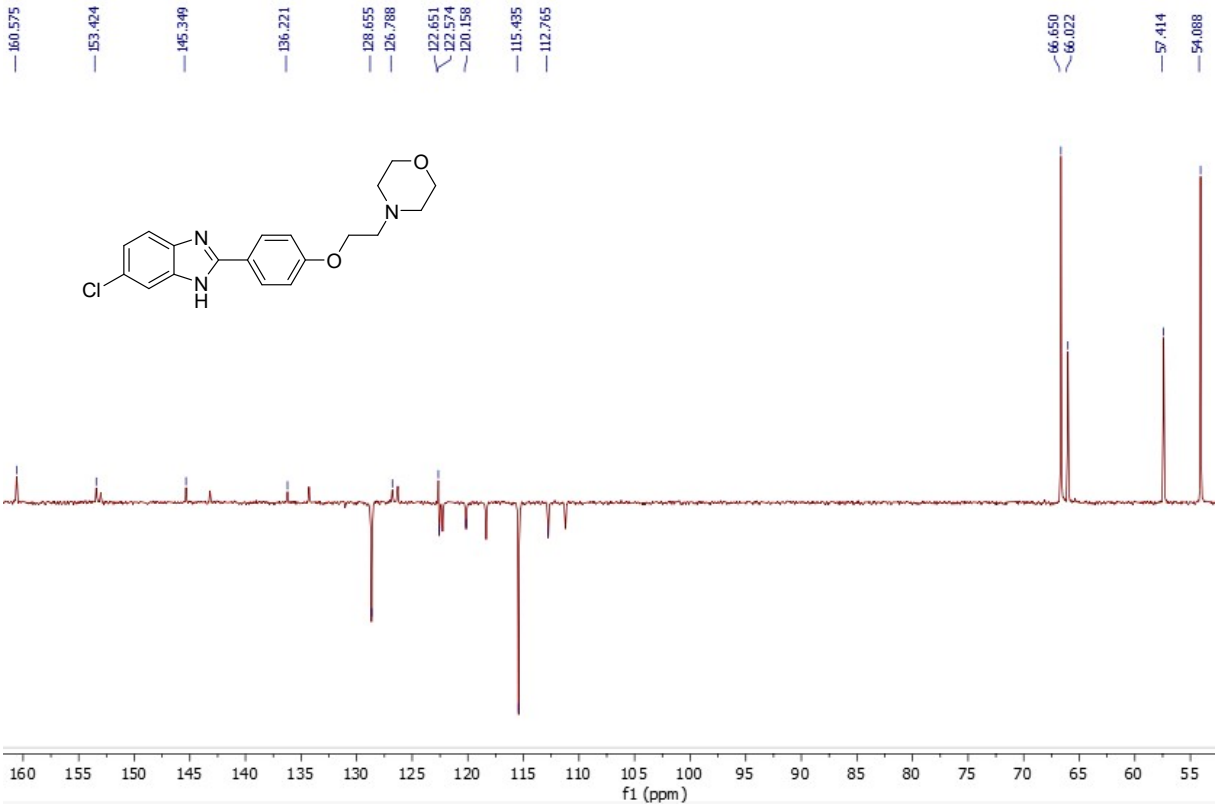


Figure S52. ^{13}C-NMR spectrum of compound **16**

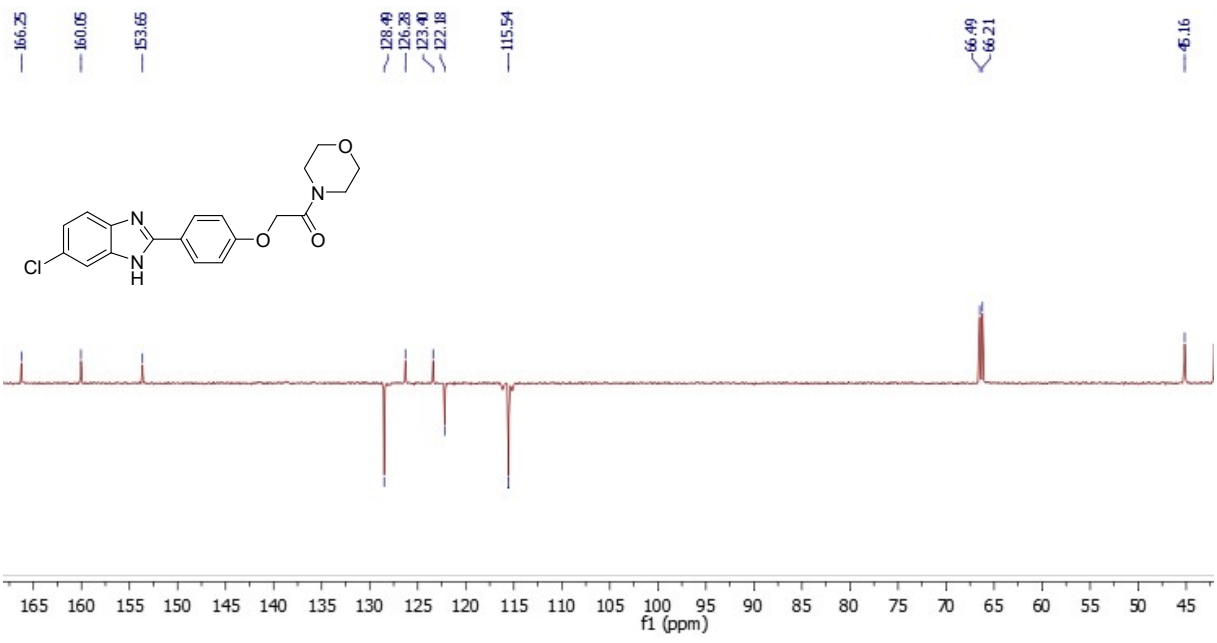


Figure S53. ^{13}C-NMR spectrum of compound **17**

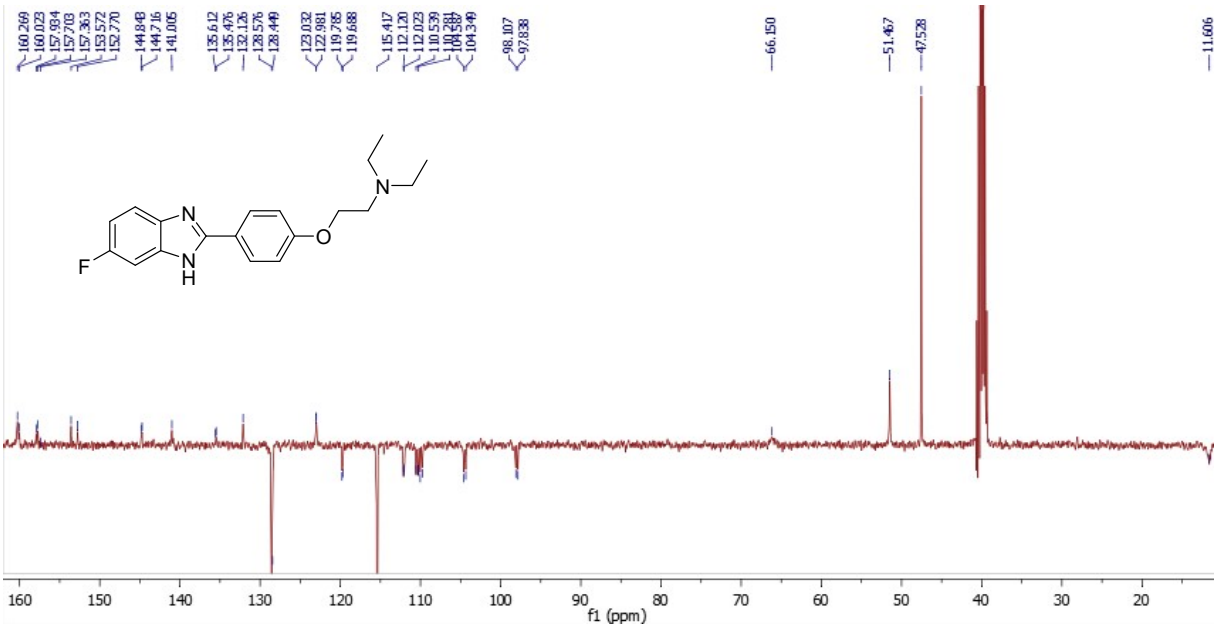


Figure S54. ^{13}C-NMR spectrum of compound **18**

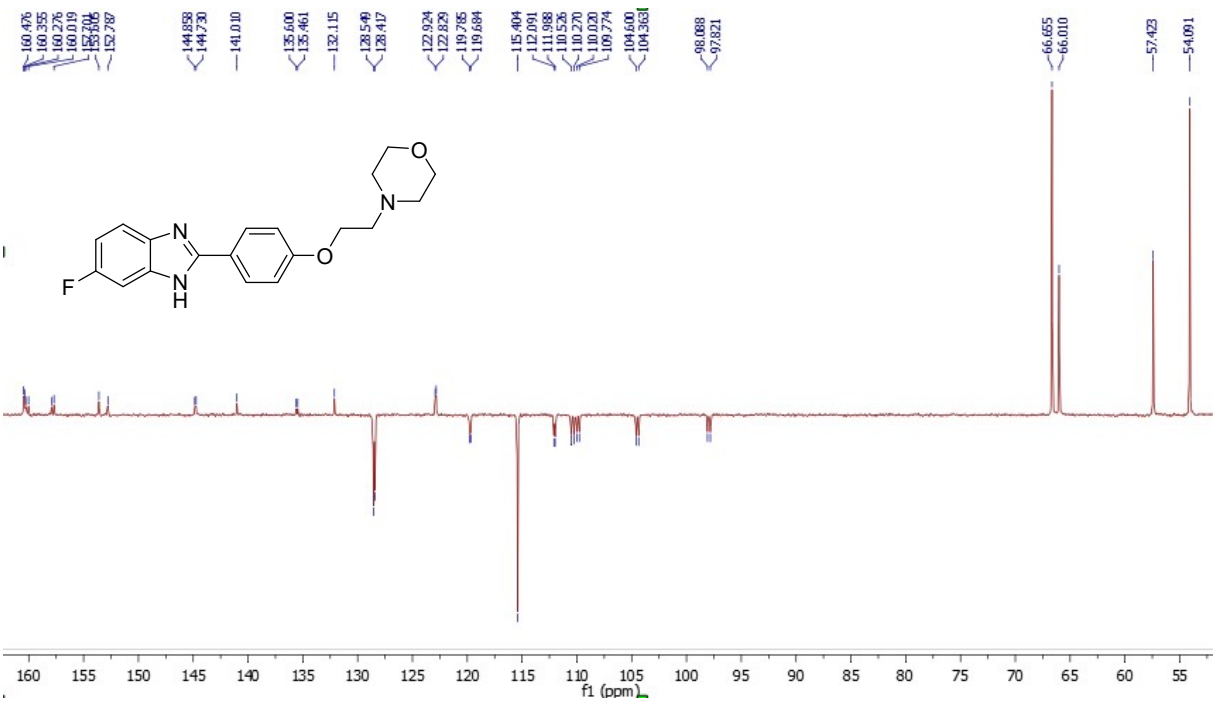


Figure S55. ^{13}C-NMR spectrum of compound **19**

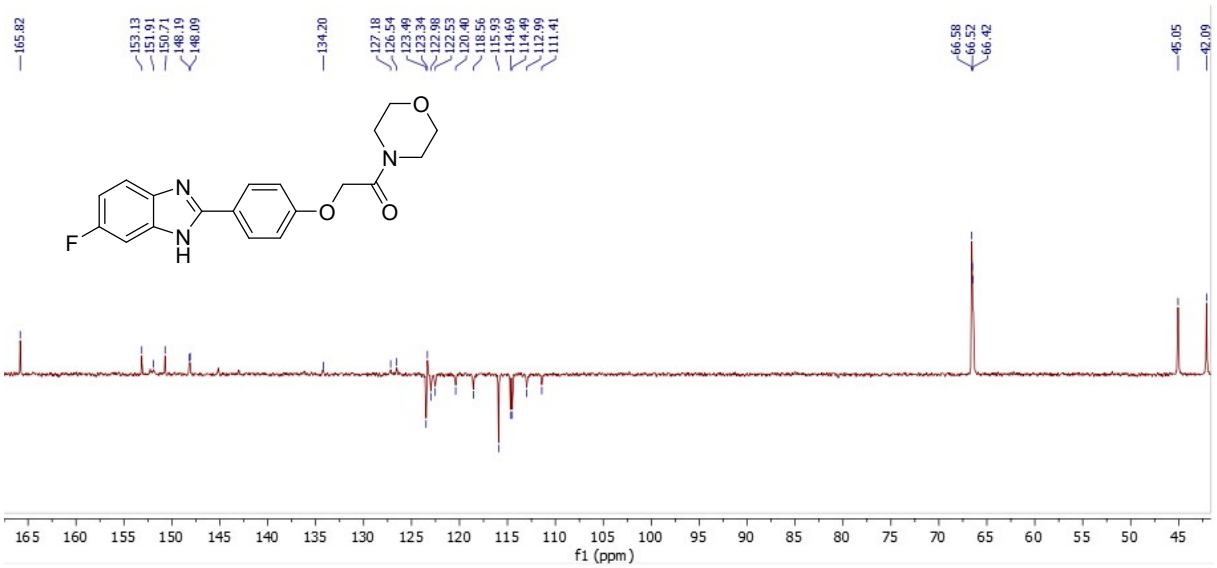


Figure S56. ^{13}C-NMR spectrum of compound **20**

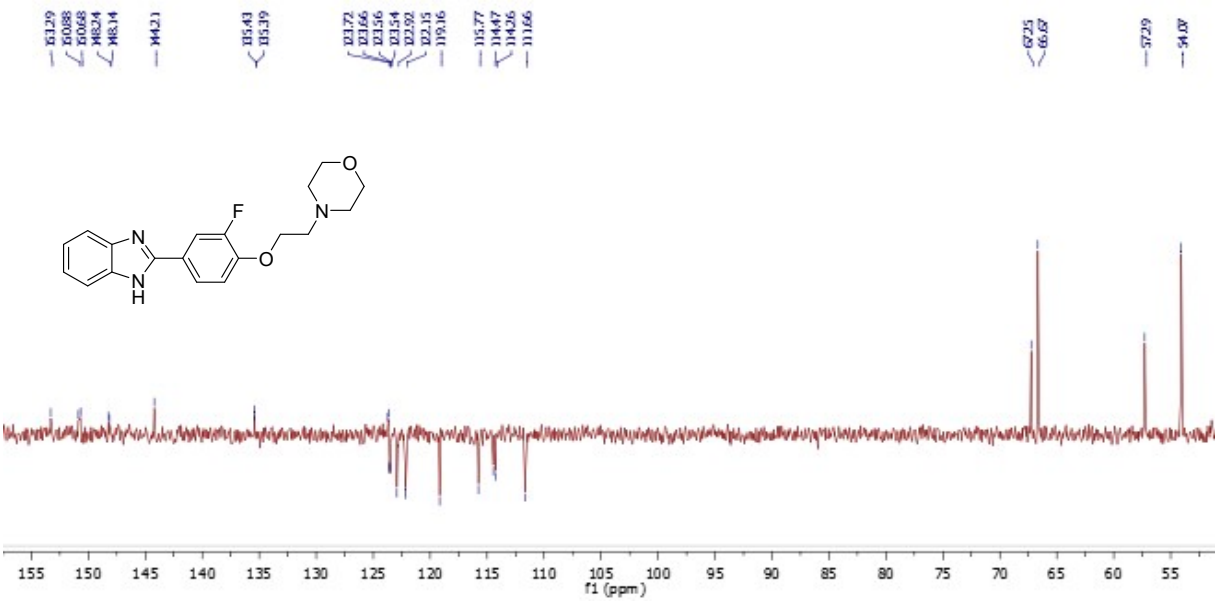


Figure S57. ^{13}C-NMR spectrum of compound **21**

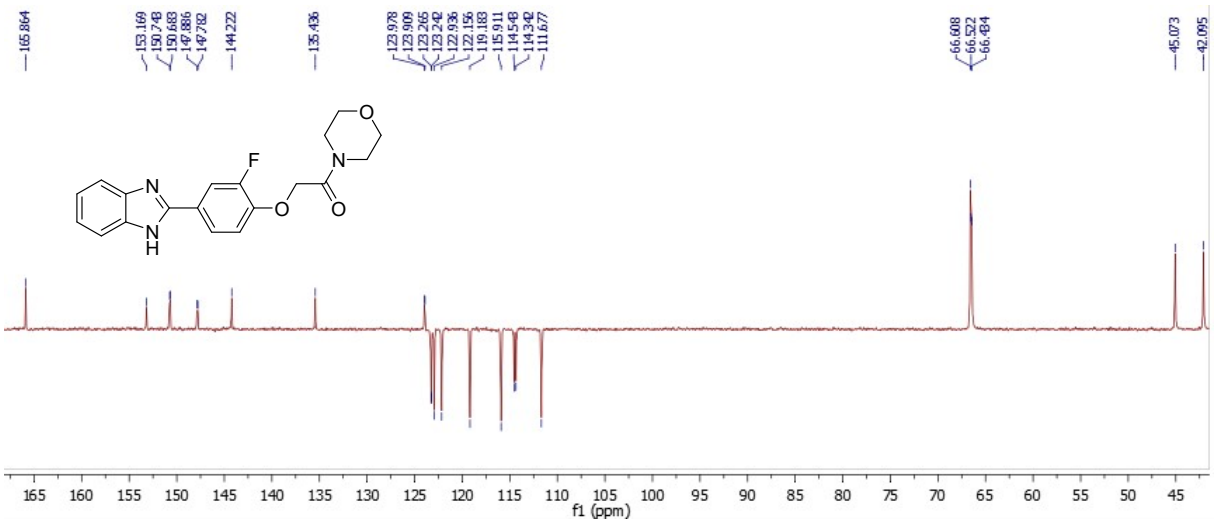


Figure S58. ^{13}C-NMR spectrum of compound **22**

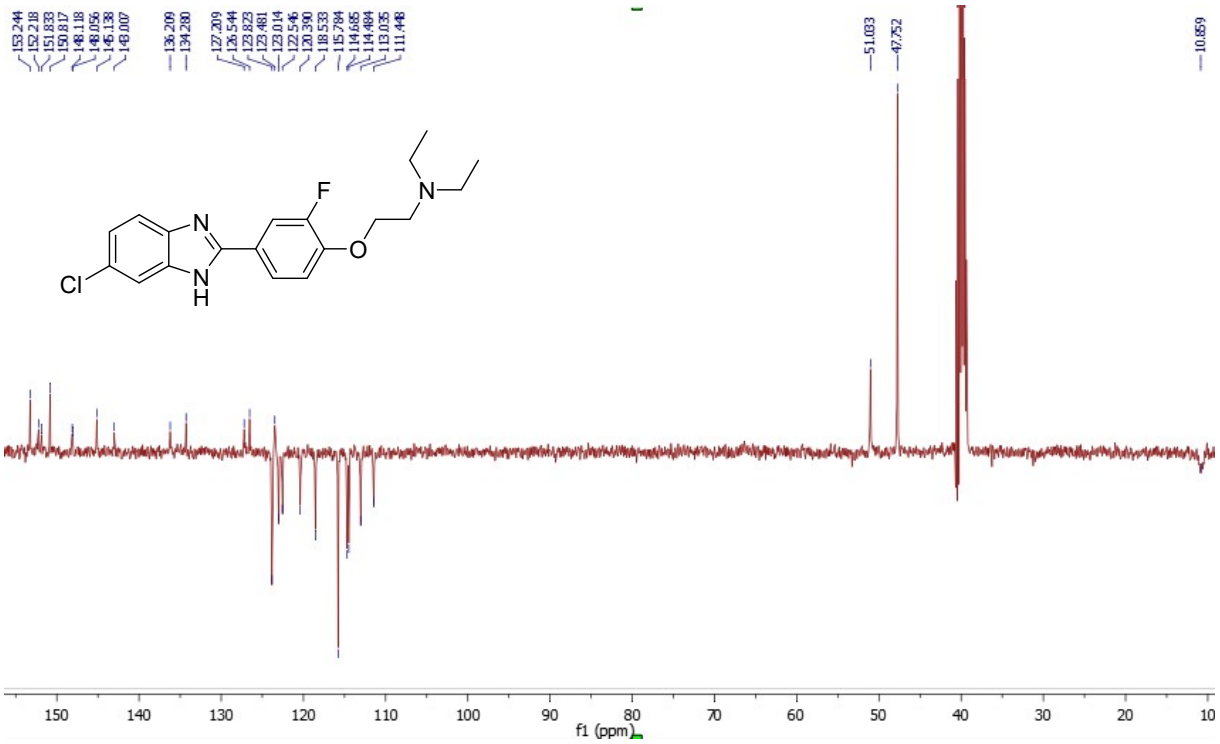


Figure S59. ^{13}C-NMR spectrum of compound **23**

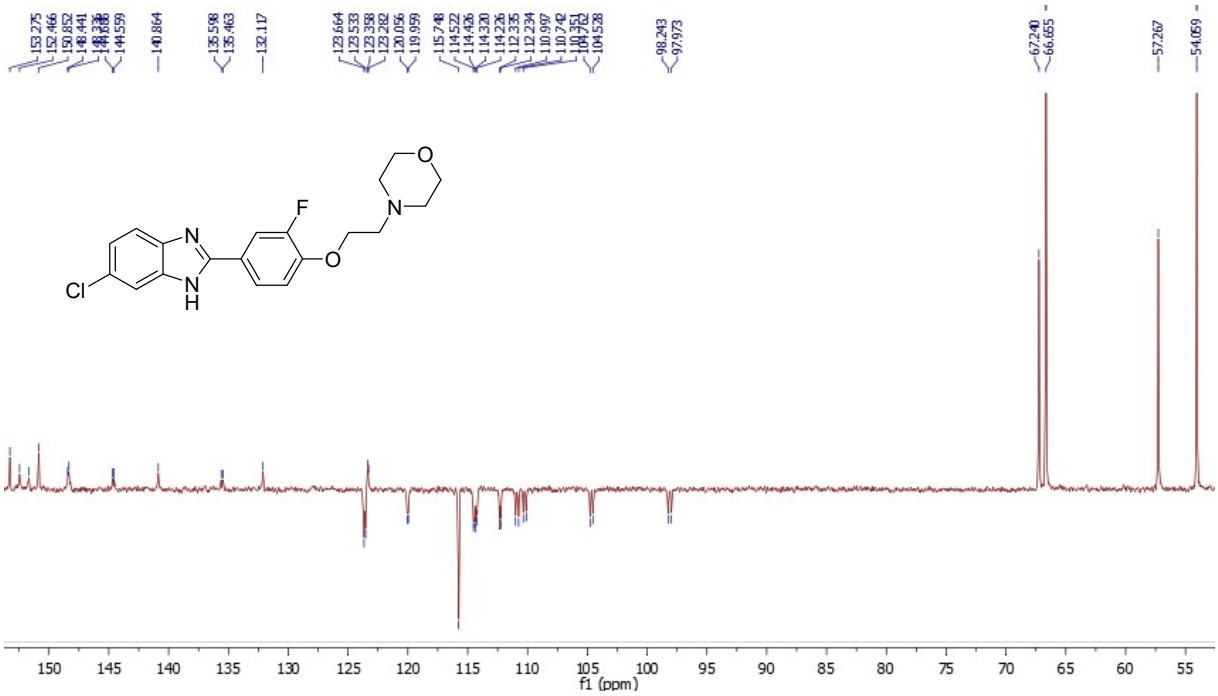


Figure S60. ^{13}C-NMR spectrum of compound **24**

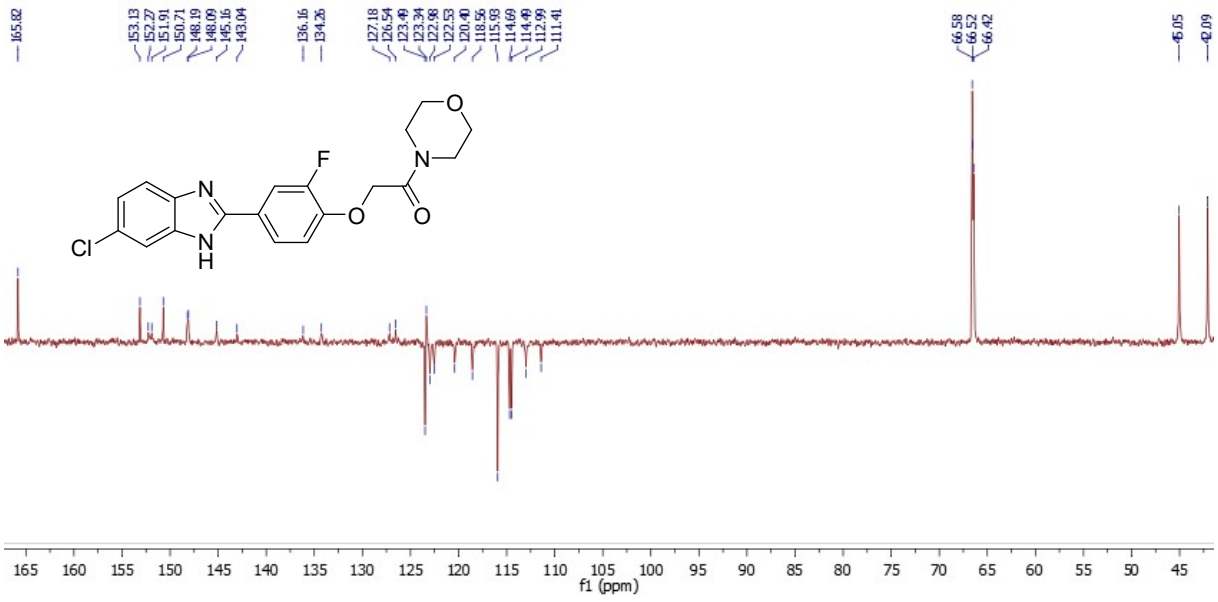


Figure S61. ^{13}C-NMR spectrum of compound **25**

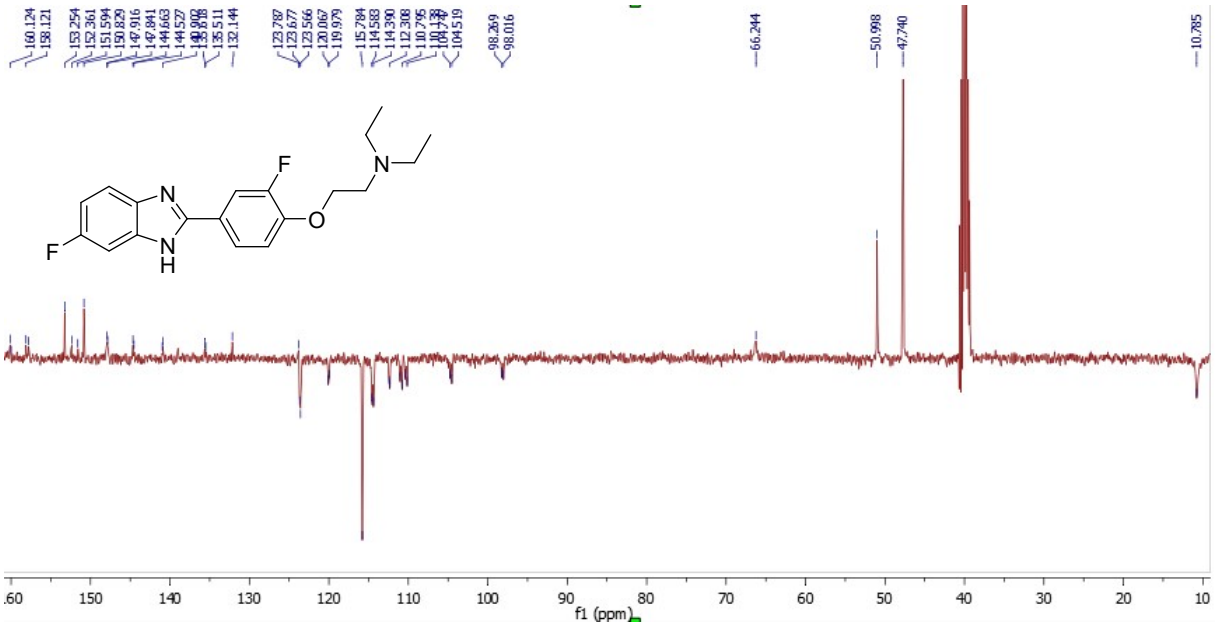


Figure S62. ^{13}C-NMR spectrum of compound **26**

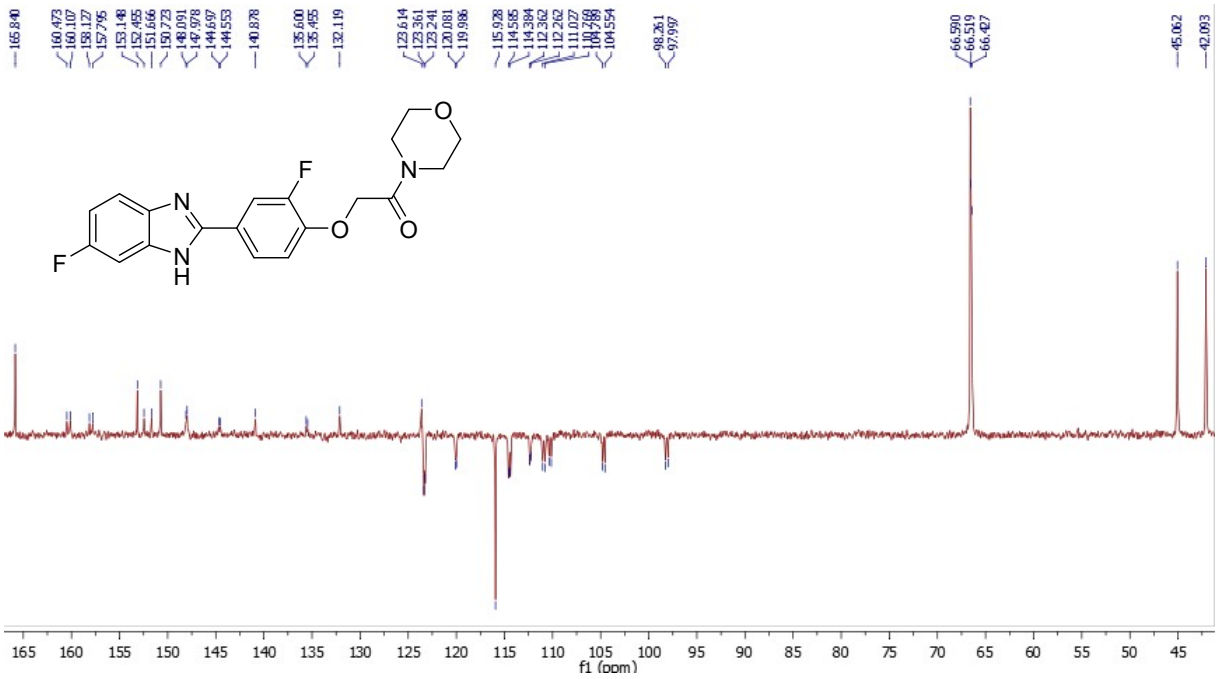


Figure S63. ^{13}C-NMR spectrum of compound **27**

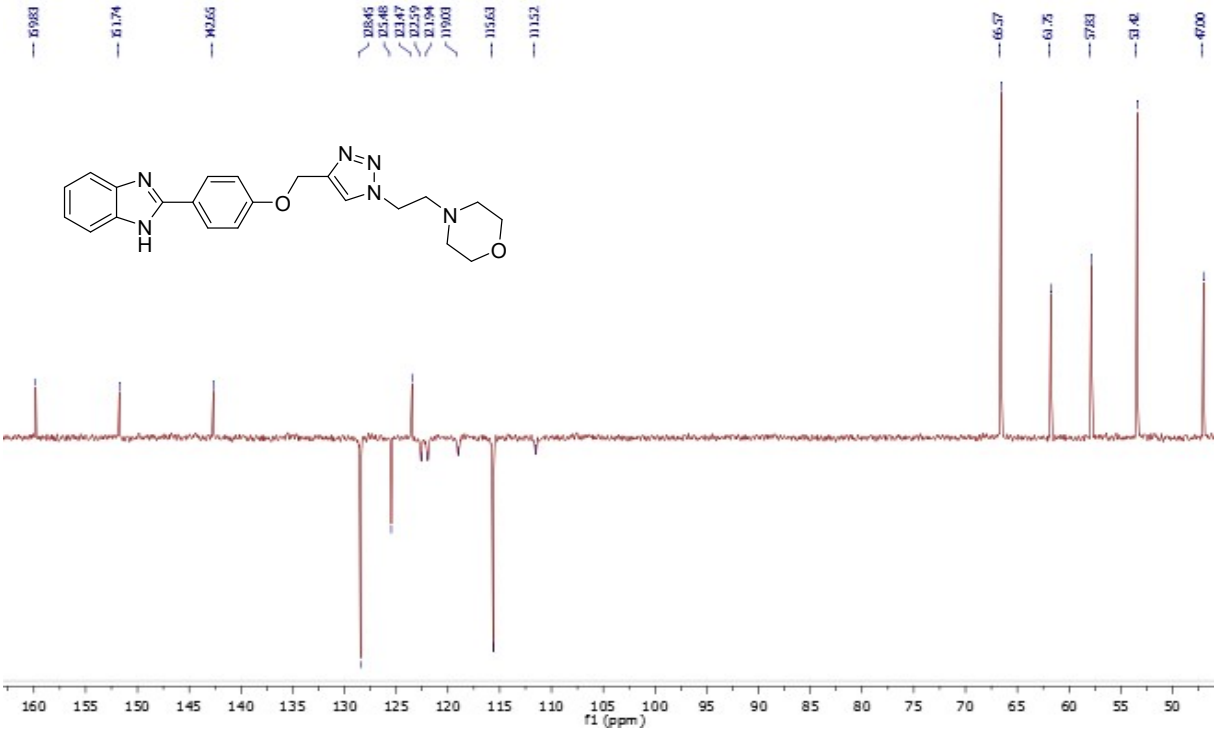


Figure S64. ^{13}C-NMR spectrum of compound **28**

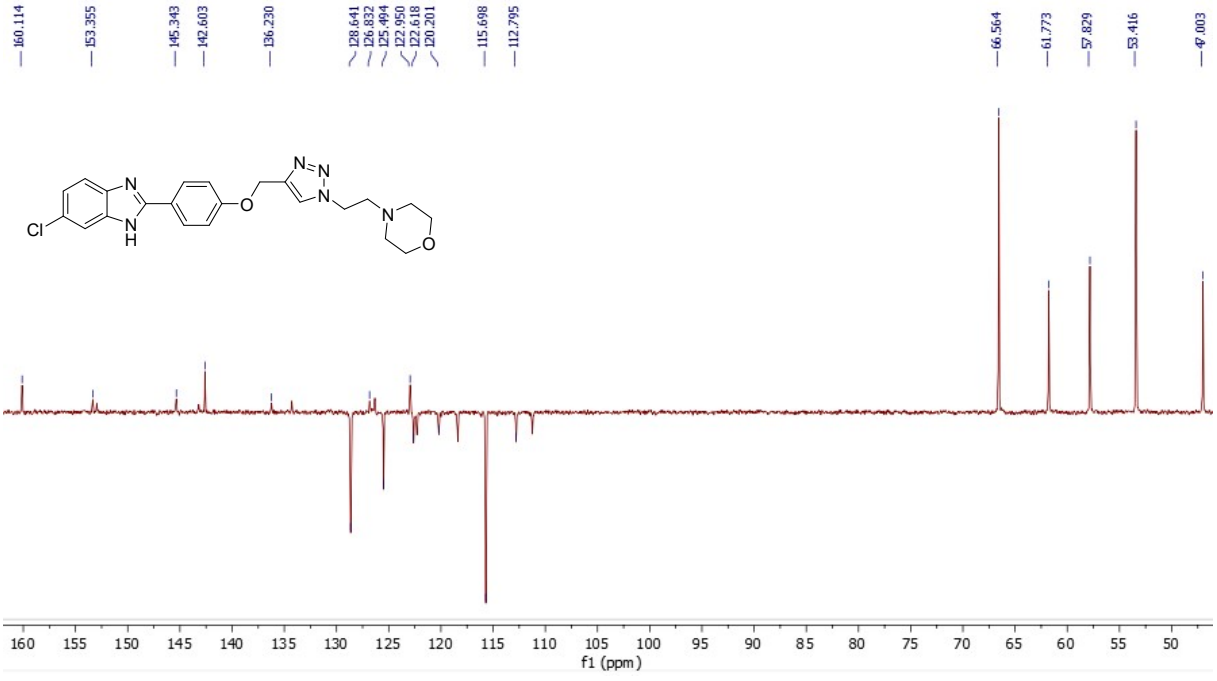


Figure S65. ^{13}C-NMR spectrum of compound **29**

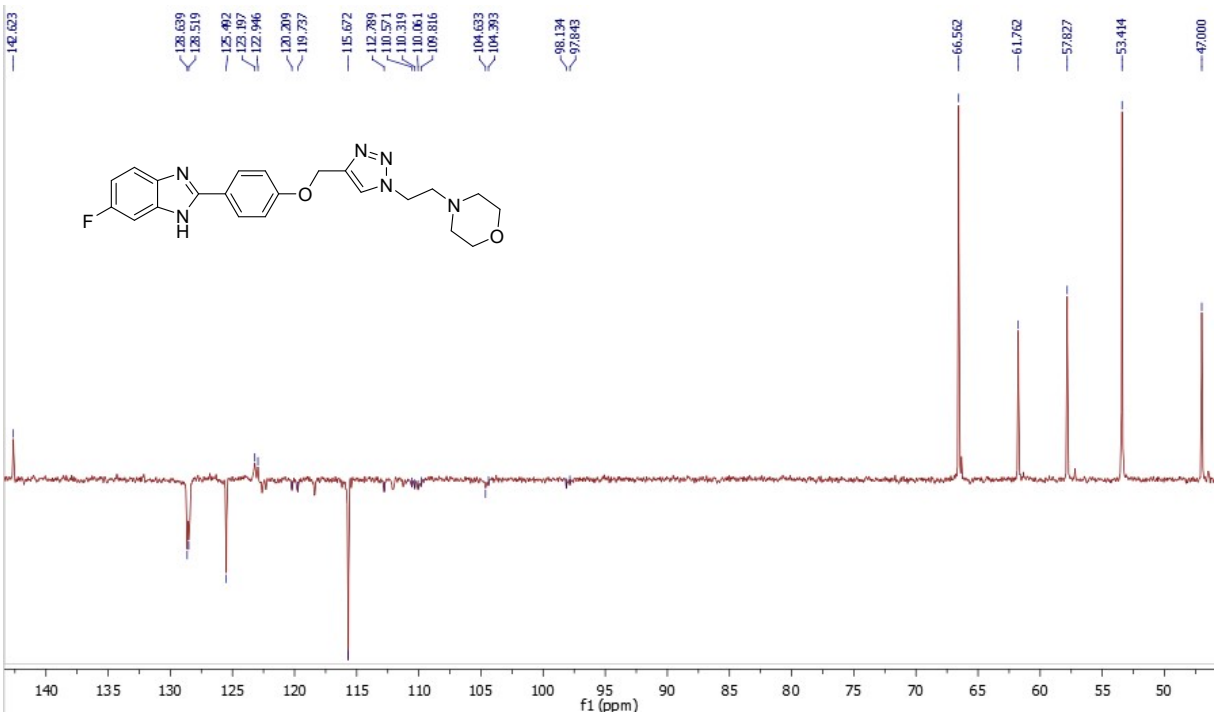


Figure S66. ^{13}C-NMR spectrum of compound **30**

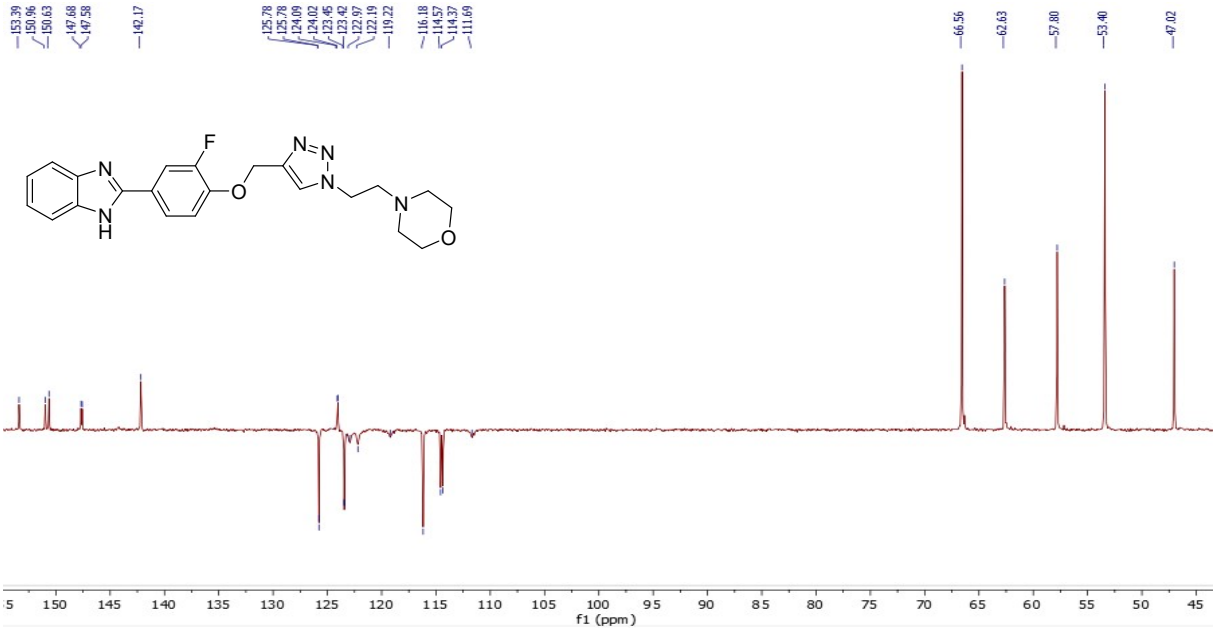


Figure S67. ^{13}C-NMR spectrum of compound **31**

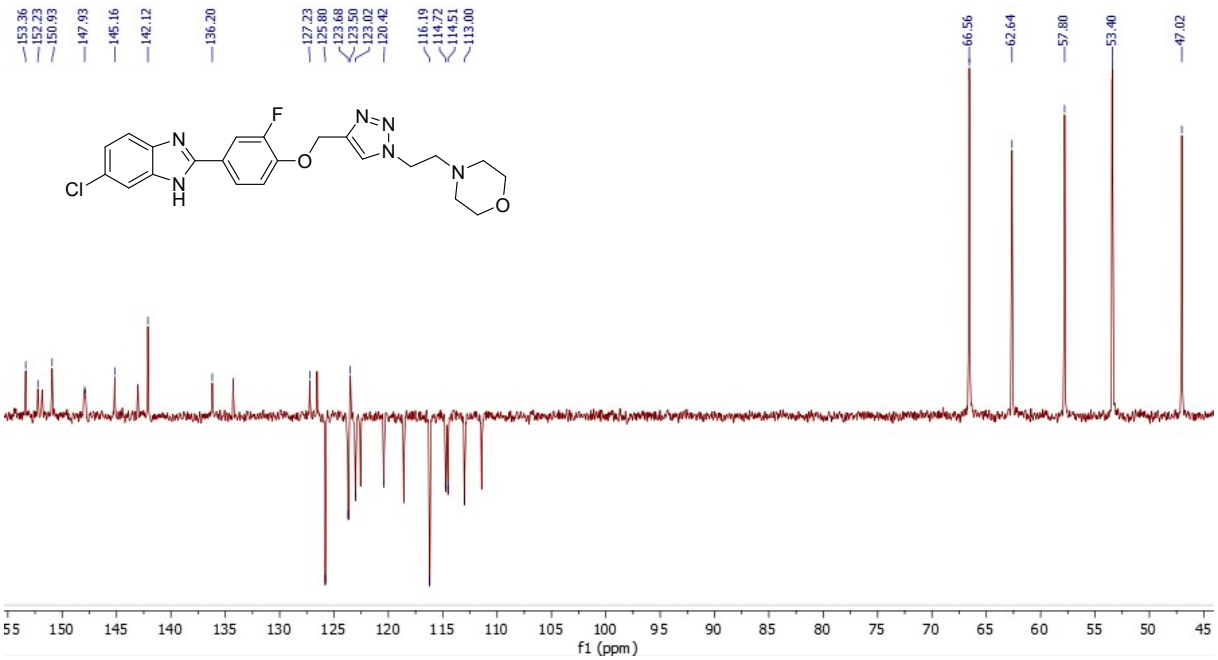


Figure S68. ^{13}C-NMR spectrum of compound **32**

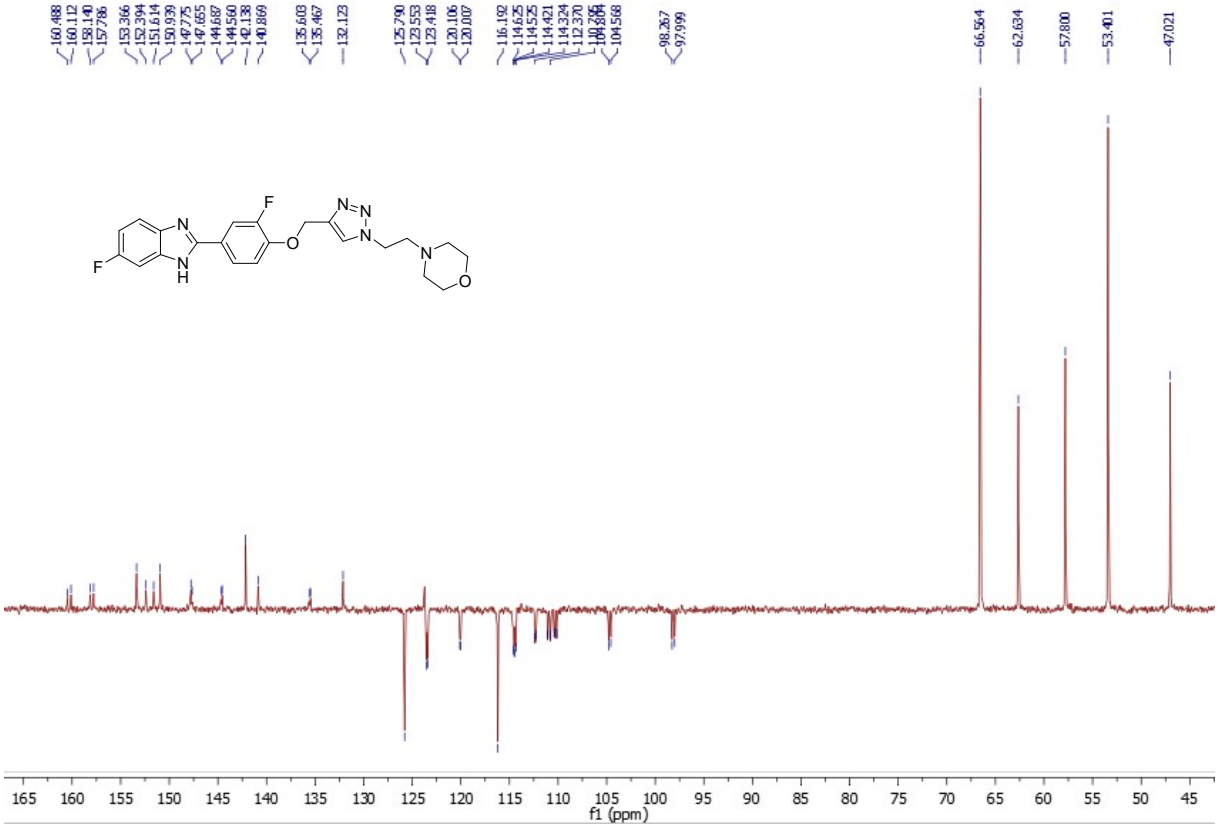


Figure S69. ^{13}C-NMR spectrum of compound **33**

IR spectra for compounds **12**–**33** (Fig. S70–S87)

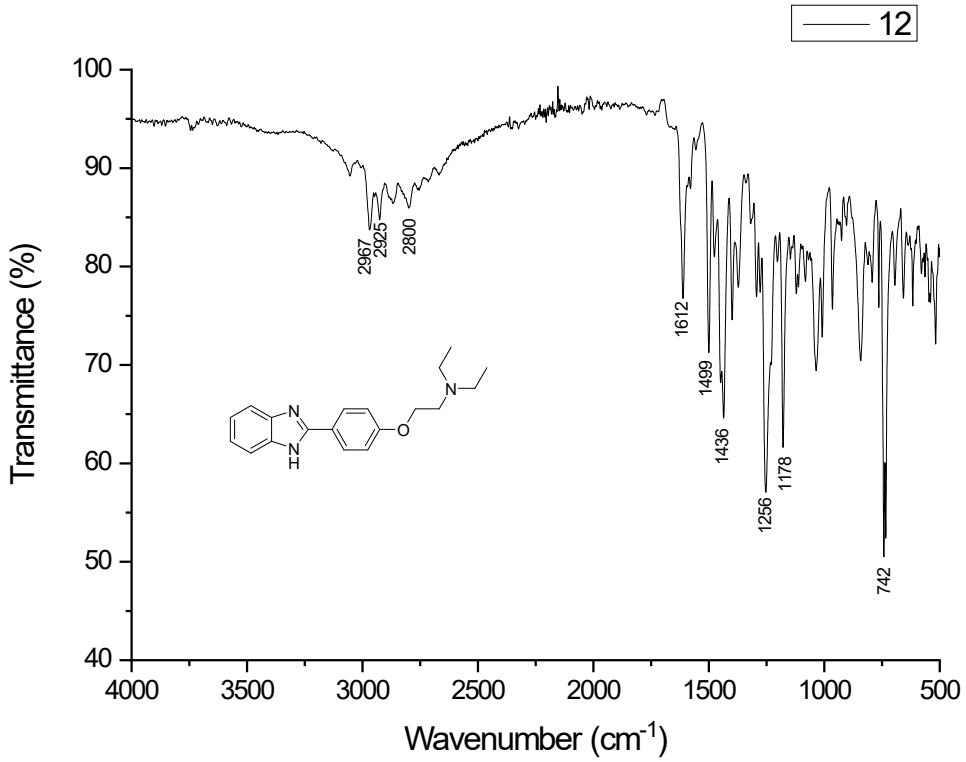


Figure S70. IR spectrum of compound **12**

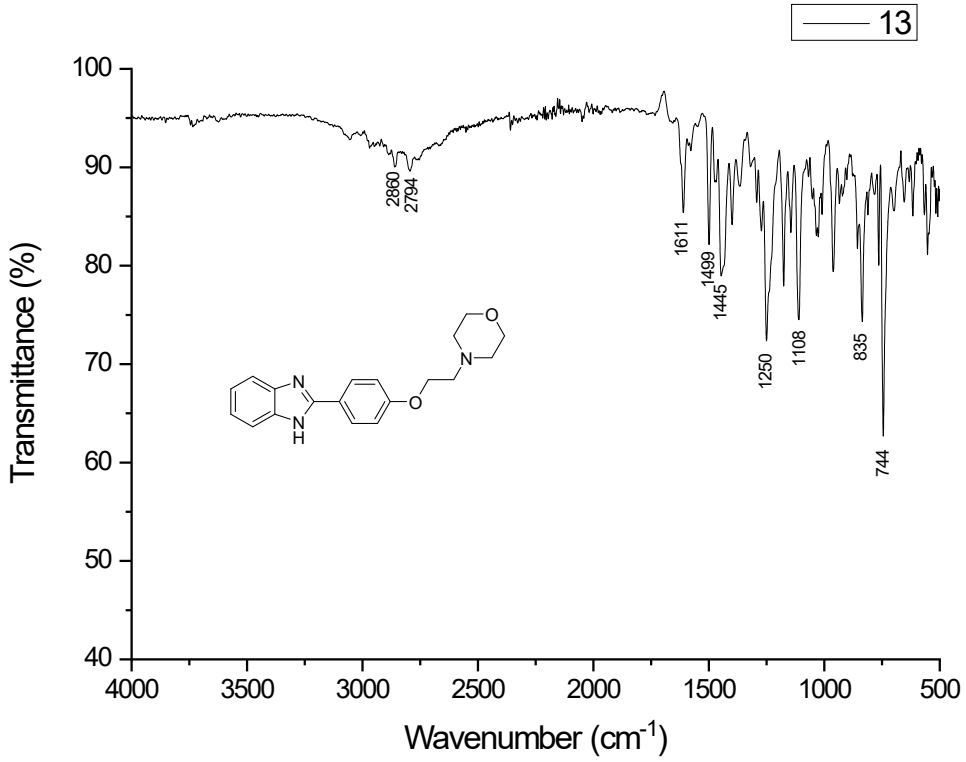


Figure S71. IR spectrum of compound **13**

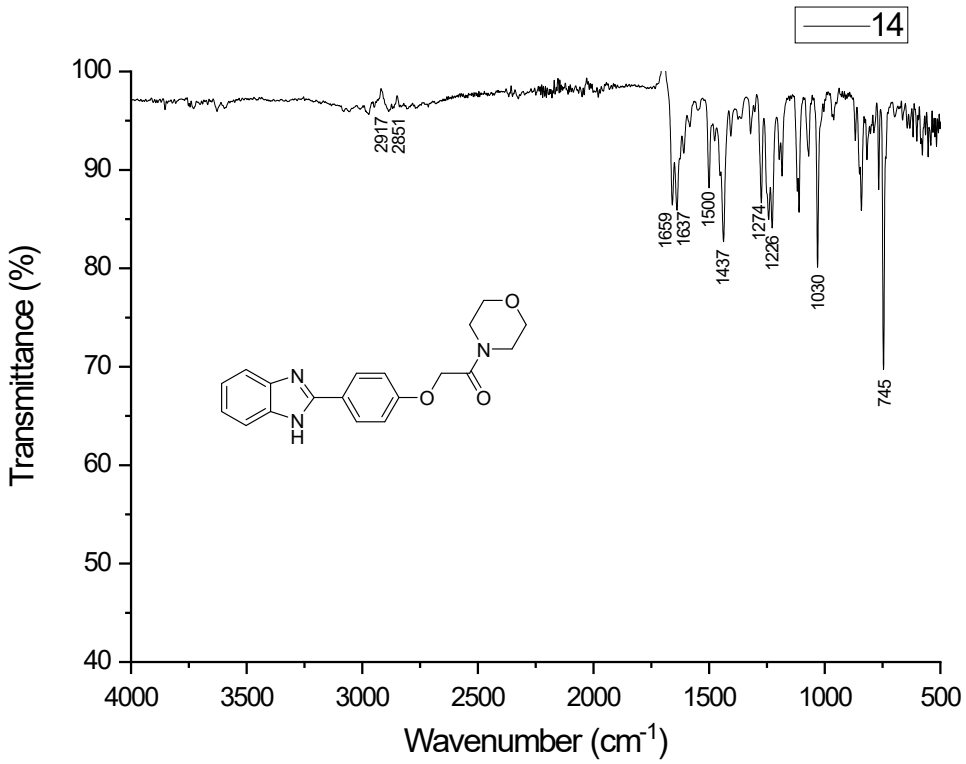


Figure S72. IR spectrum of compound **14**

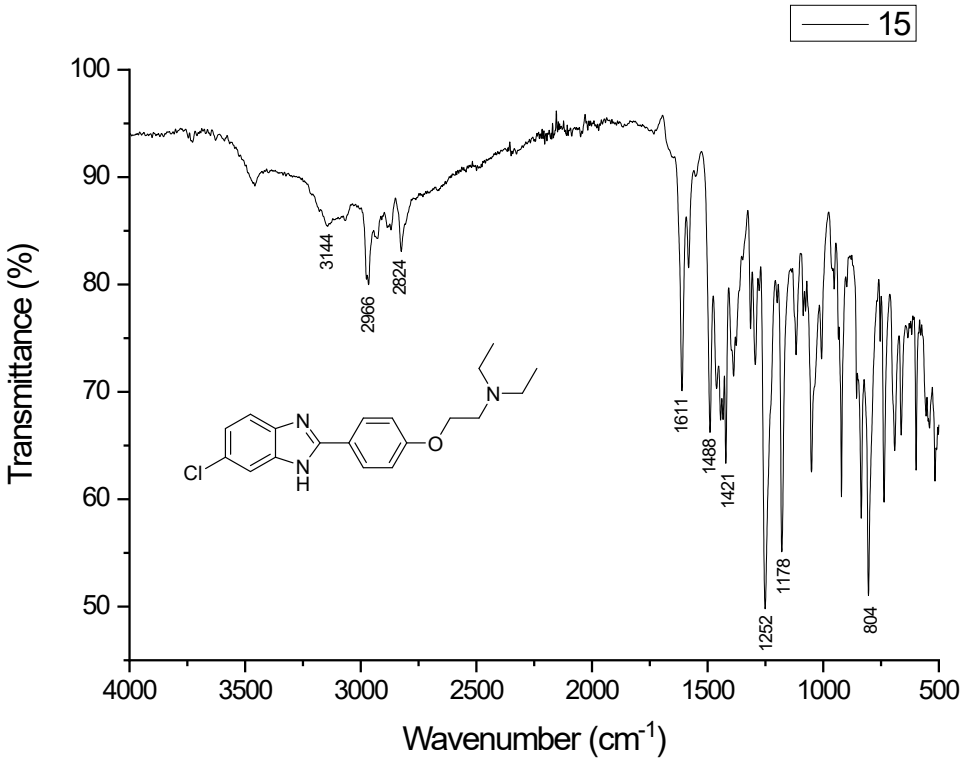


Figure S73. IR spectrum of compound **15**

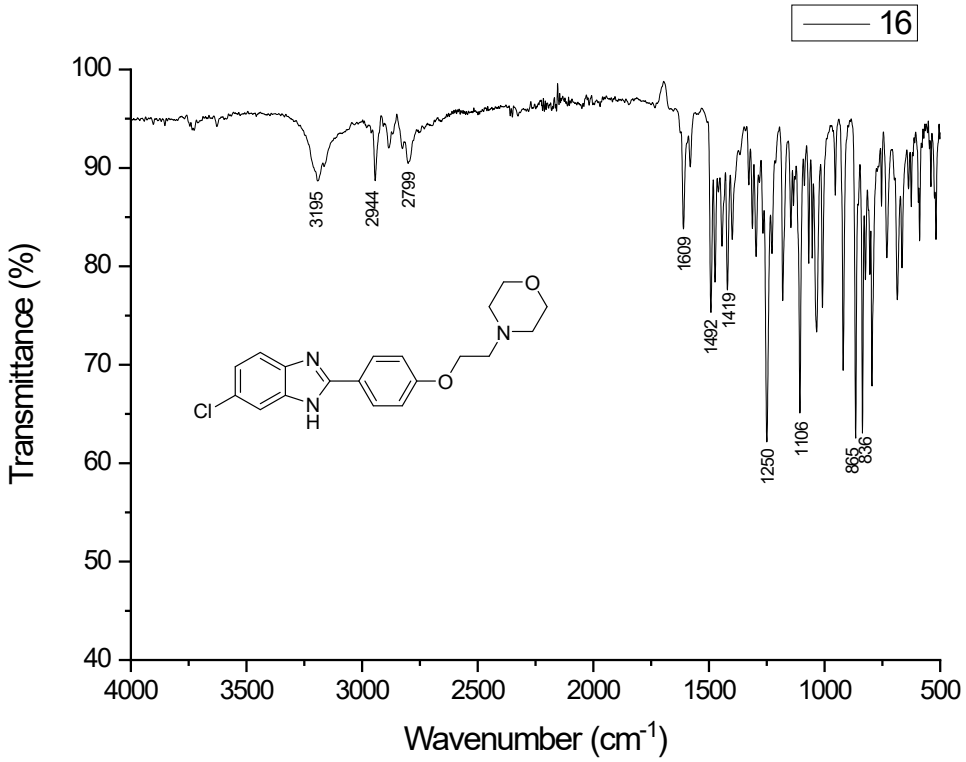


Figure S74. IR spectrum of compound **16**

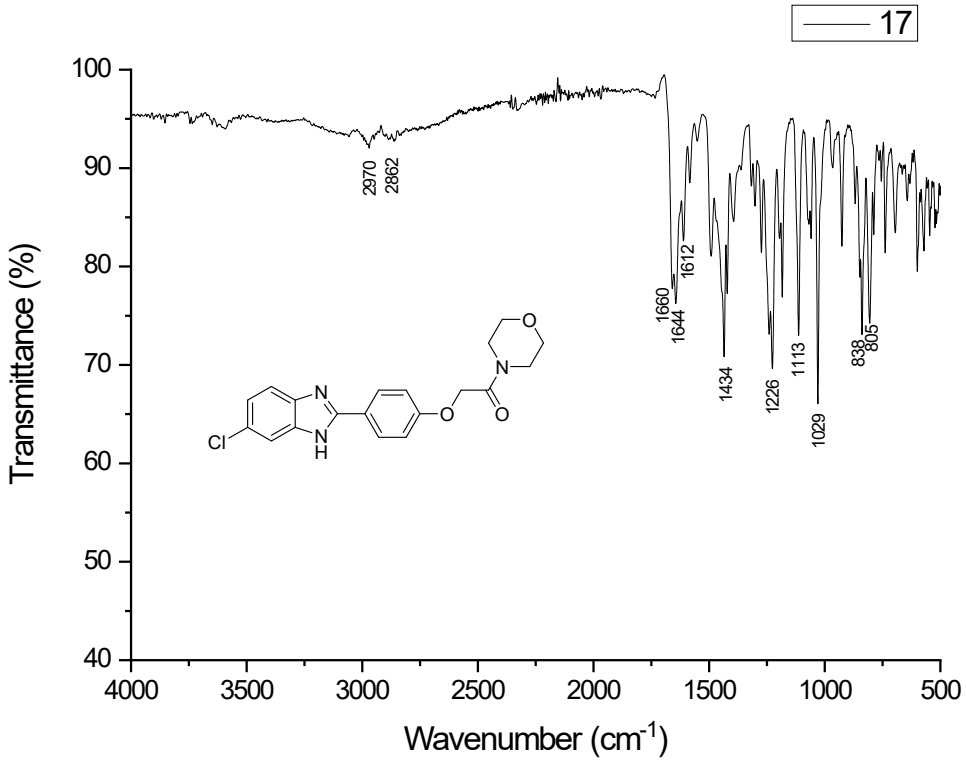


Figure S75. IR spectrum of compound **17**

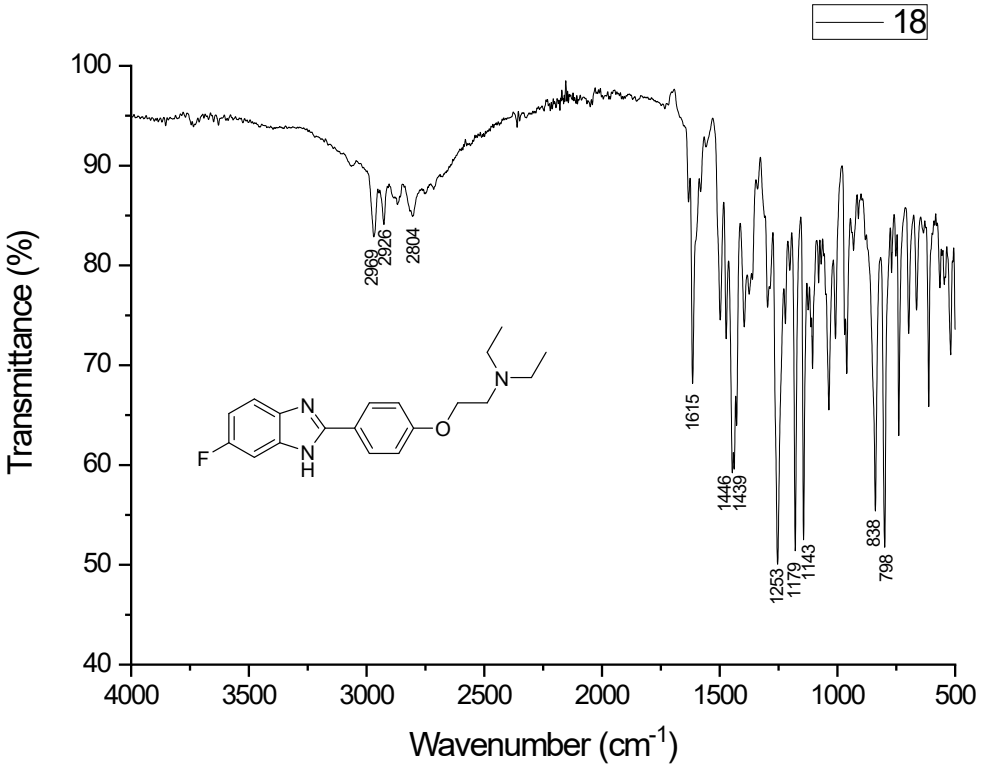


Figure S76. IR spectrum of compound **18**

3063

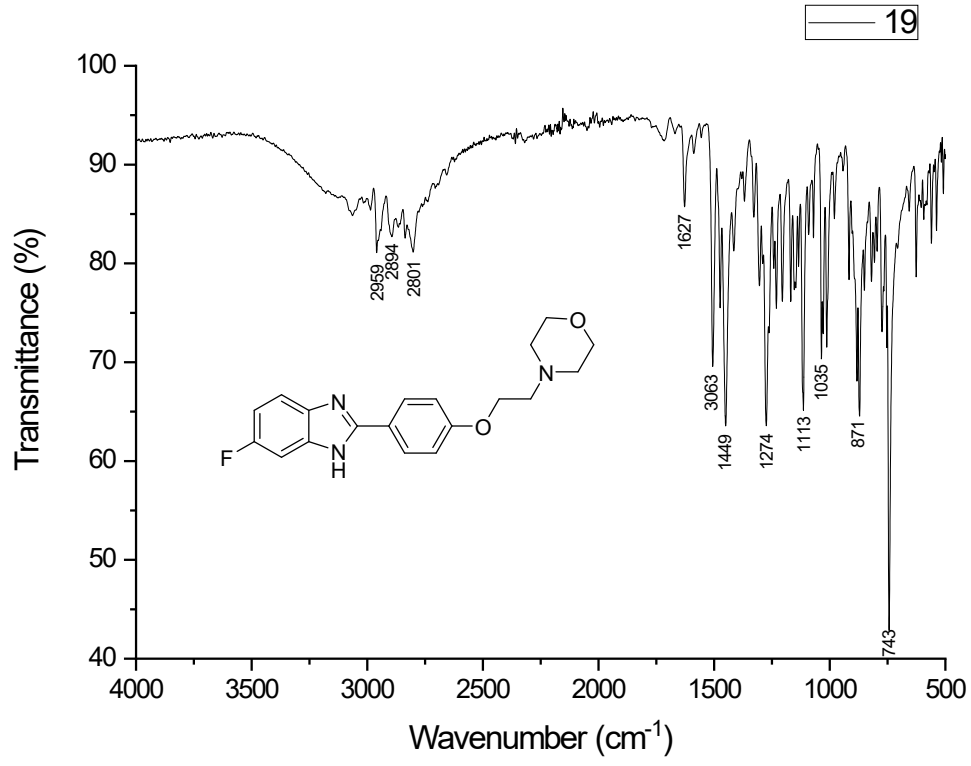


Figure S77. IR spectrum of compound **19**

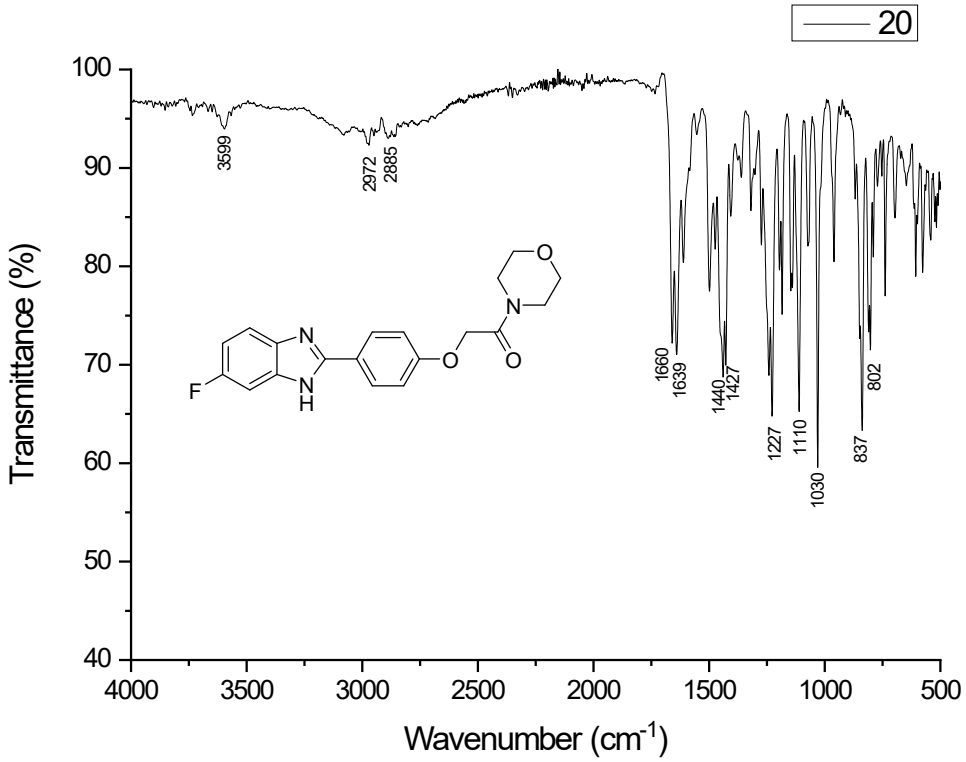


Figure S78. IR spectrum of compound **20**

2862

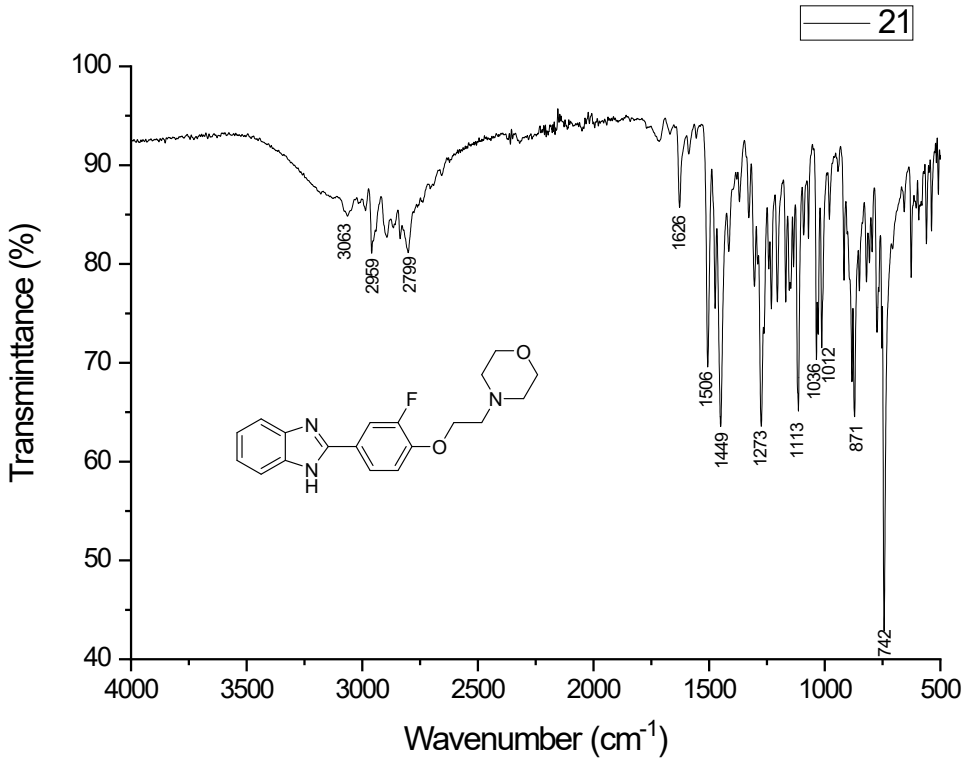
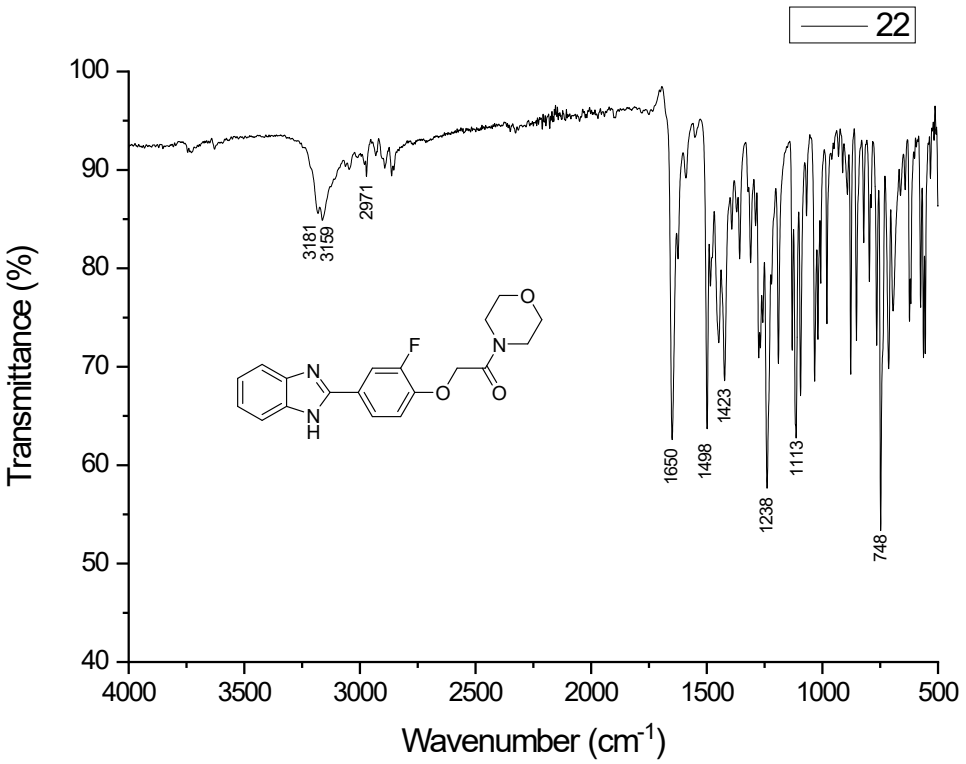


Figure S79. IR spectrum of compound **21**



F

igure S80. IR spectrum of compound **22**

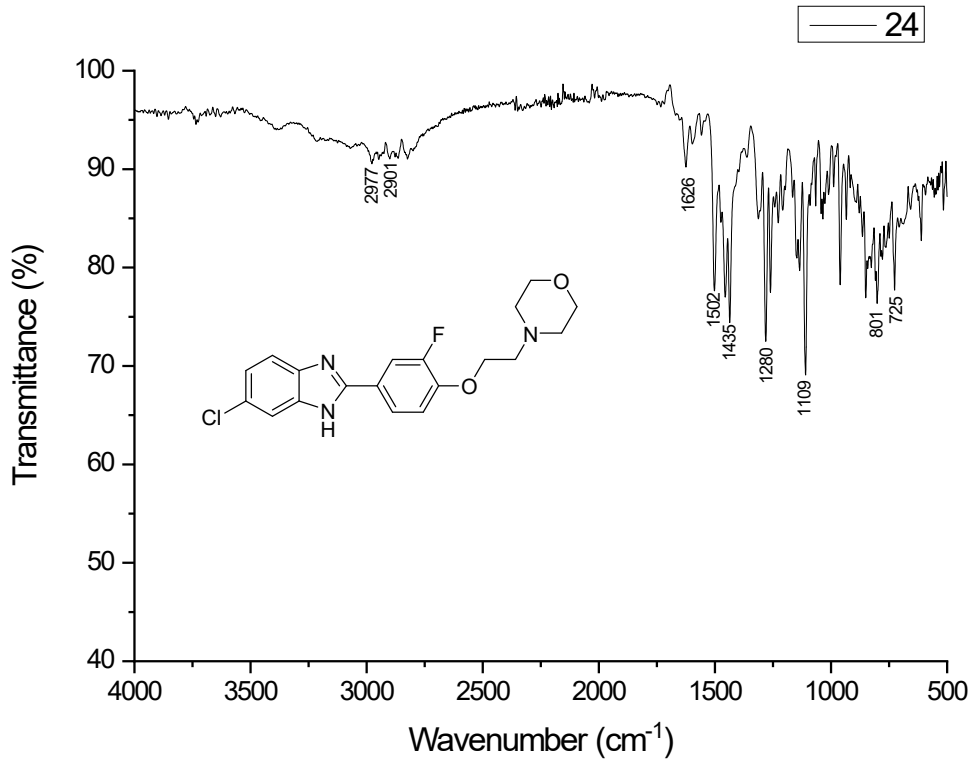


Figure S81. IR spectrum of compound **24**

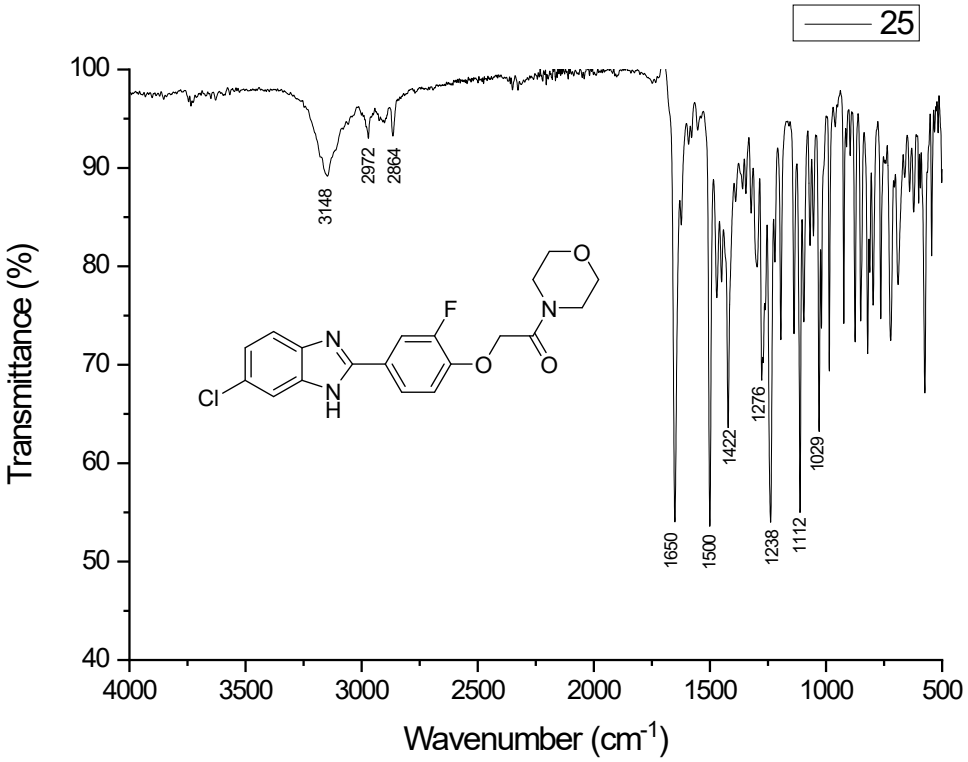


Figure S82. IR spectrum of compound **25**

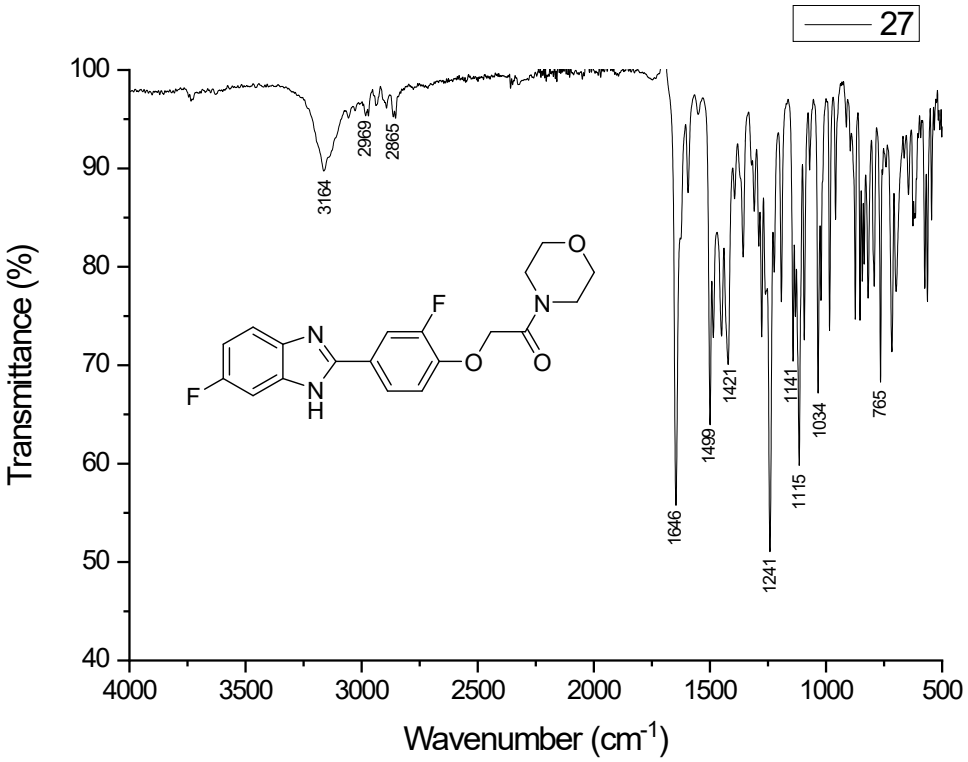


Figure S83. IR spectrum of compound **27**

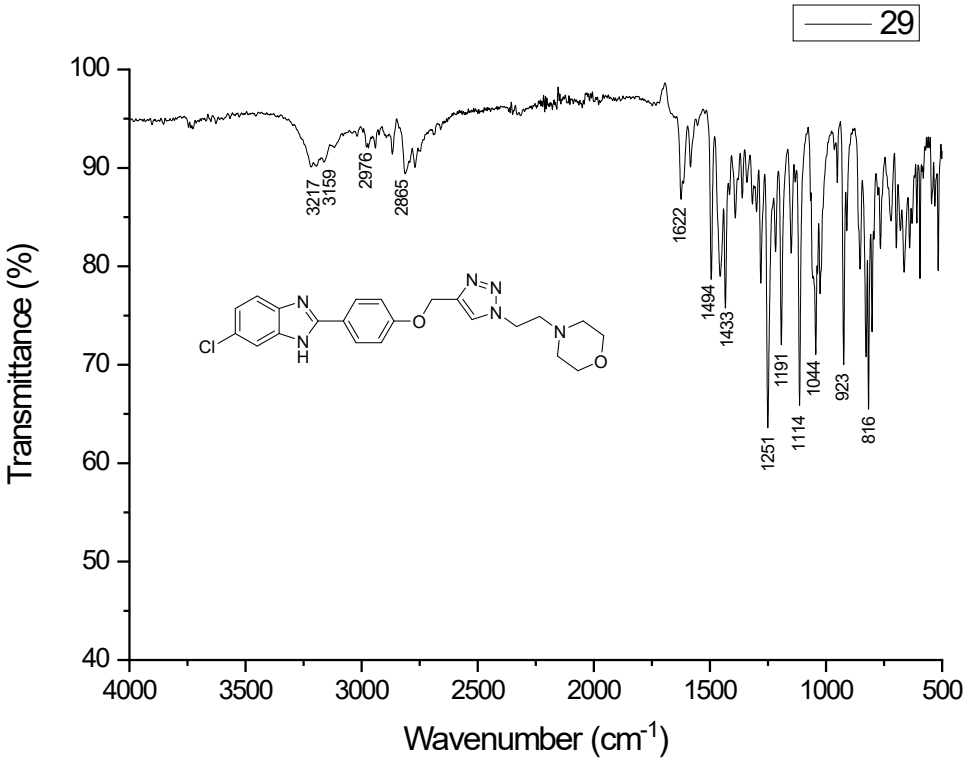


Figure S84. IR spectrum of compound **29**

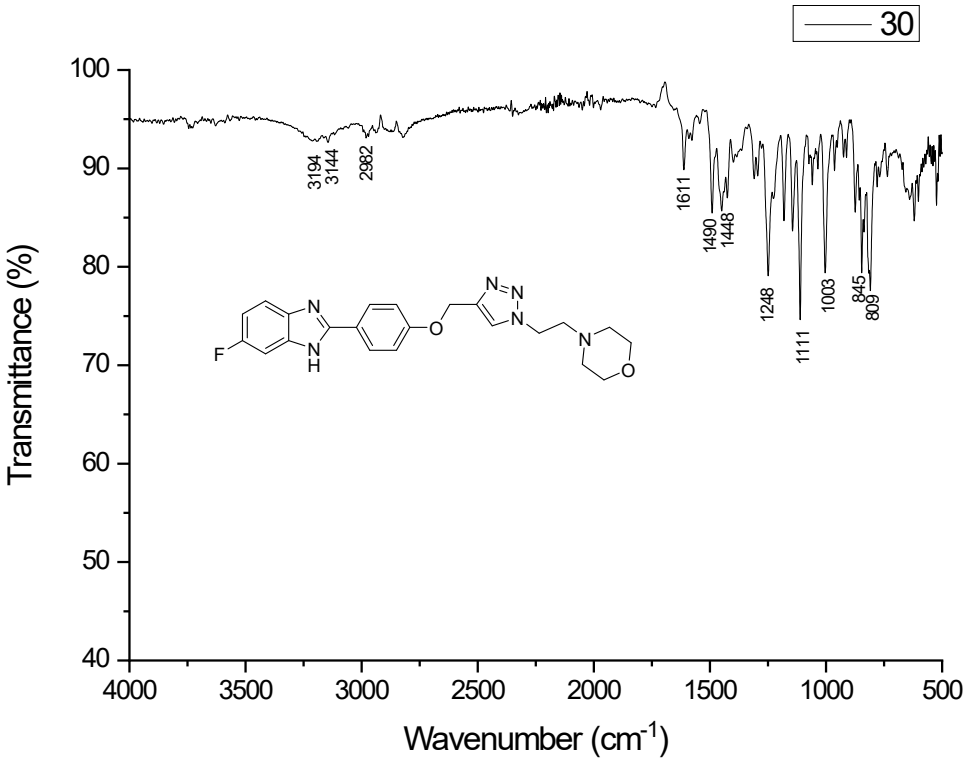


Figure S85. IR spectrum of compound **30**

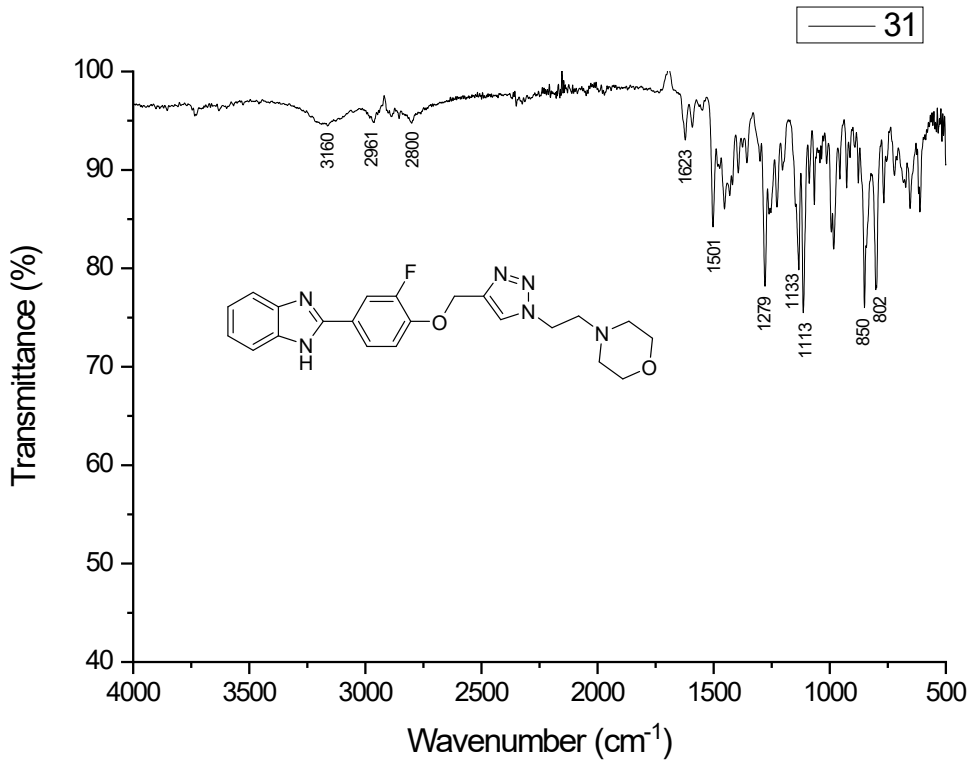


Figure S86. IR spectrum of compound **31**

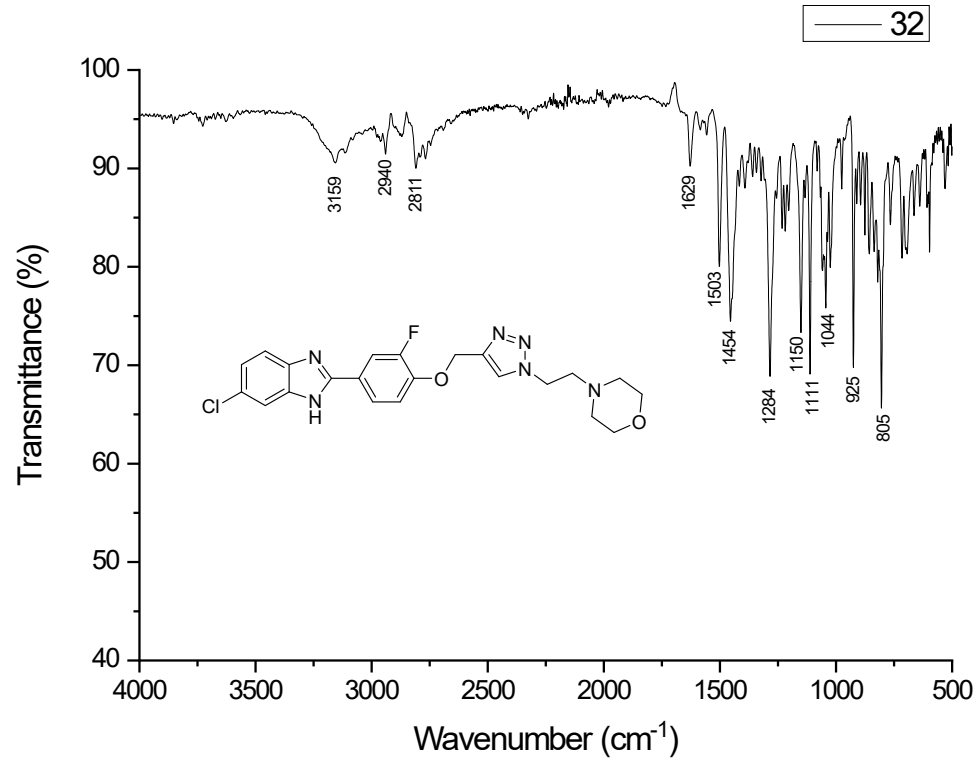


Figure S87. IR spectrum of compound **32**

MS spectra for compounds **12**–**33** (Fig. S88–S106)

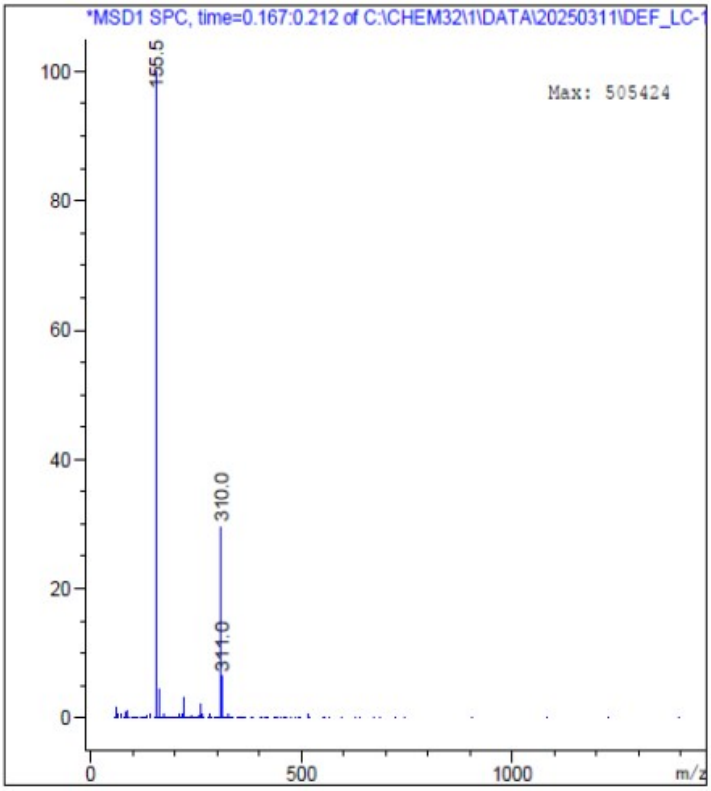


Figure S88. MS spectrum of compound **12**

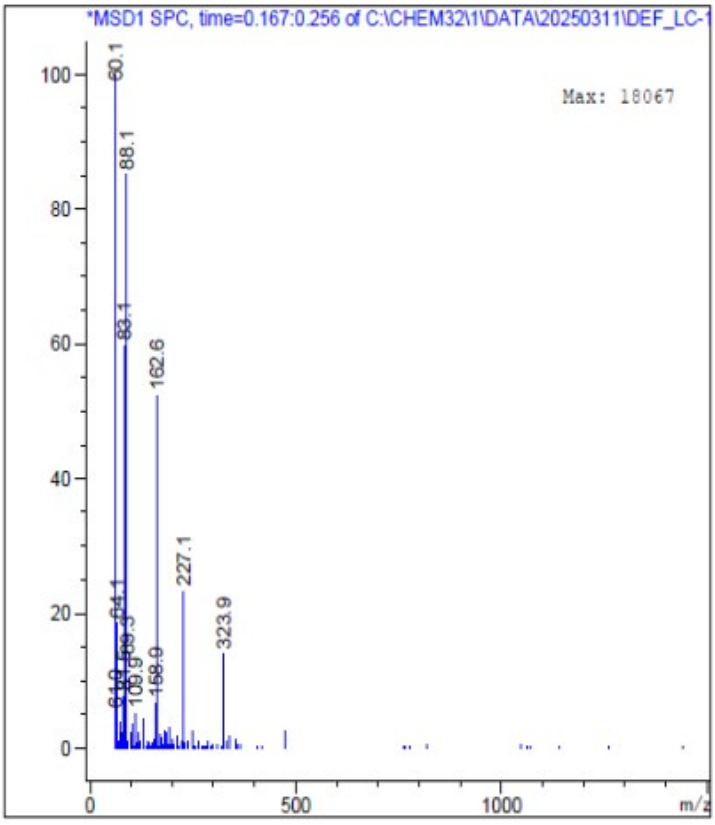


Figure S89. MS spectrum of compound **13**

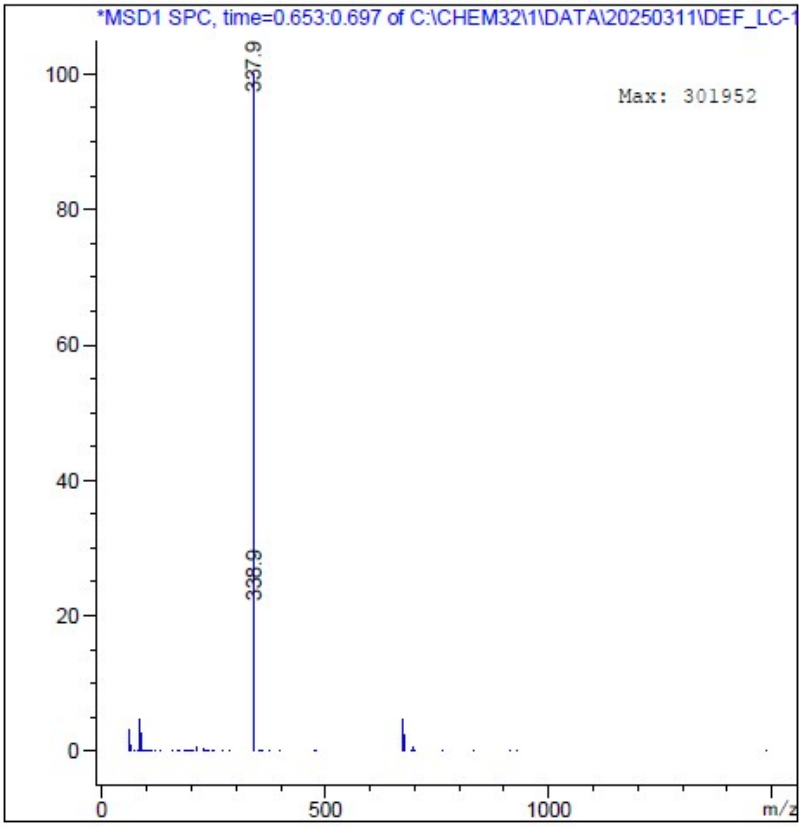


Figure S90. MS spectrum of compound **14**

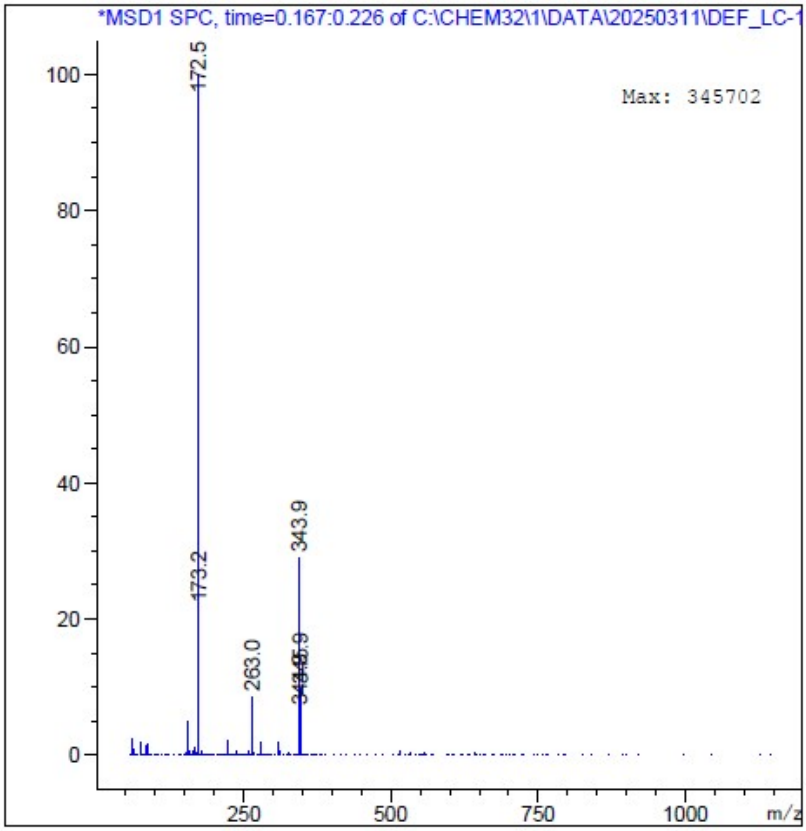


Figure S91. MS spectrum of compound **15**

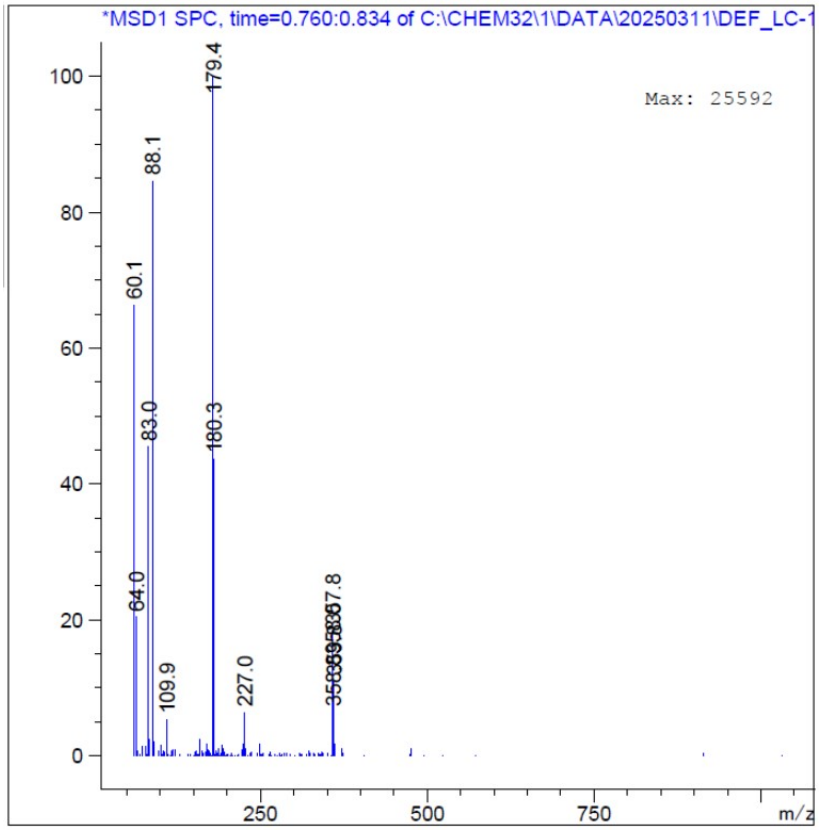


Figure S92. MS spectrum of compound **16**

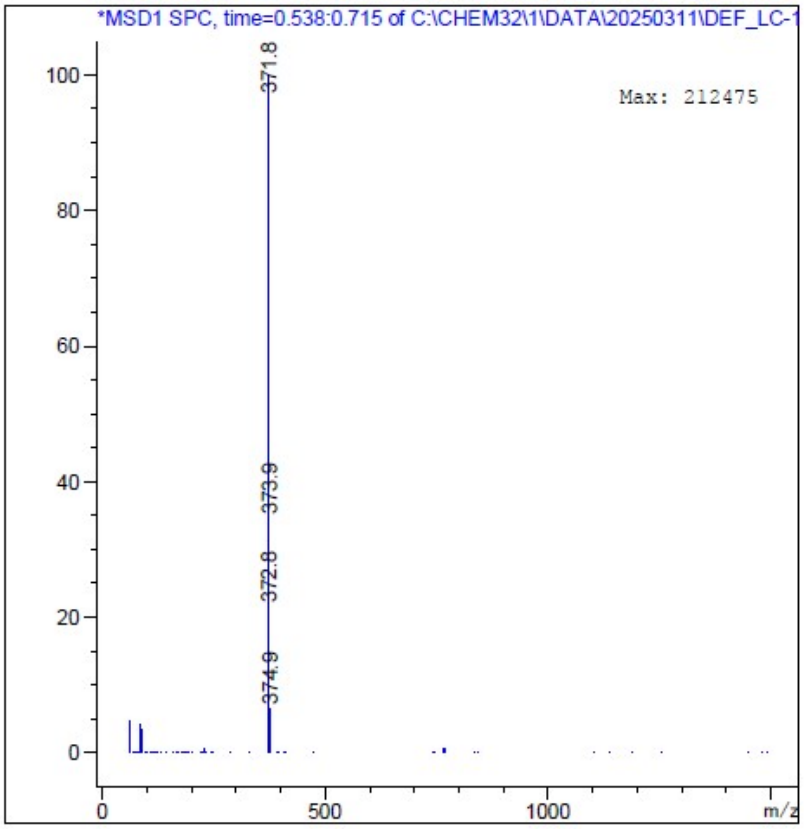


Figure S93. MS spectrum of compound **17**

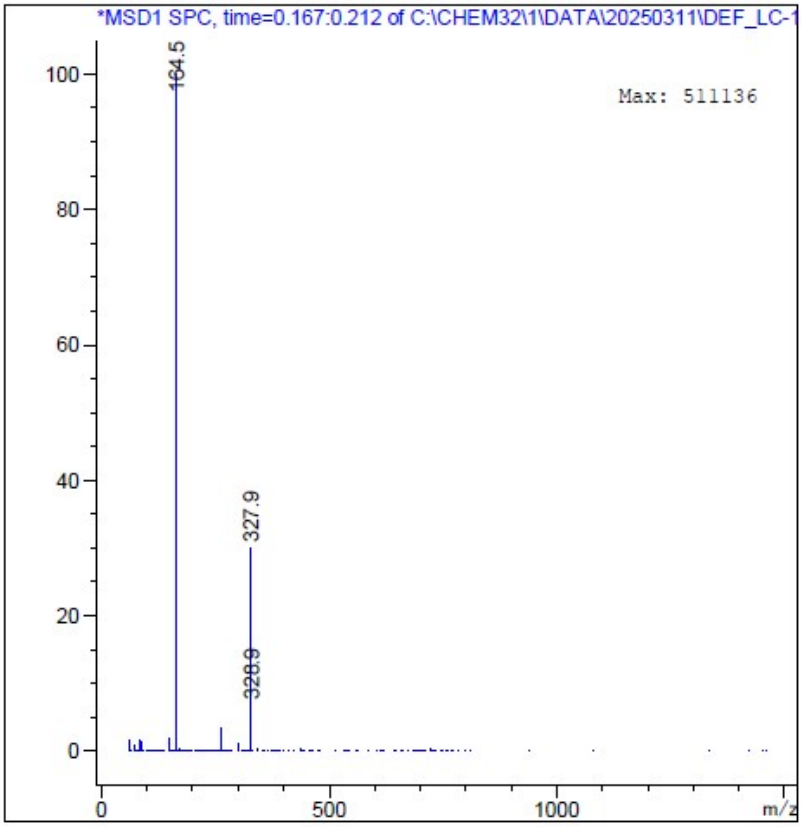


Figure S94. MS spectrum of compound **18**

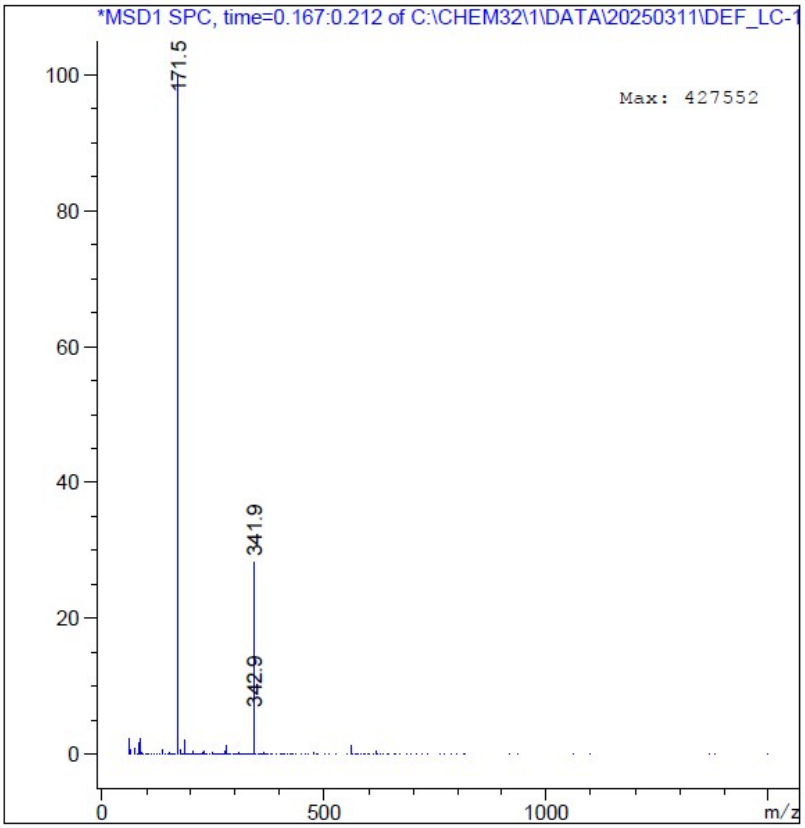


Figure S95. MS spectrum of compound **19**

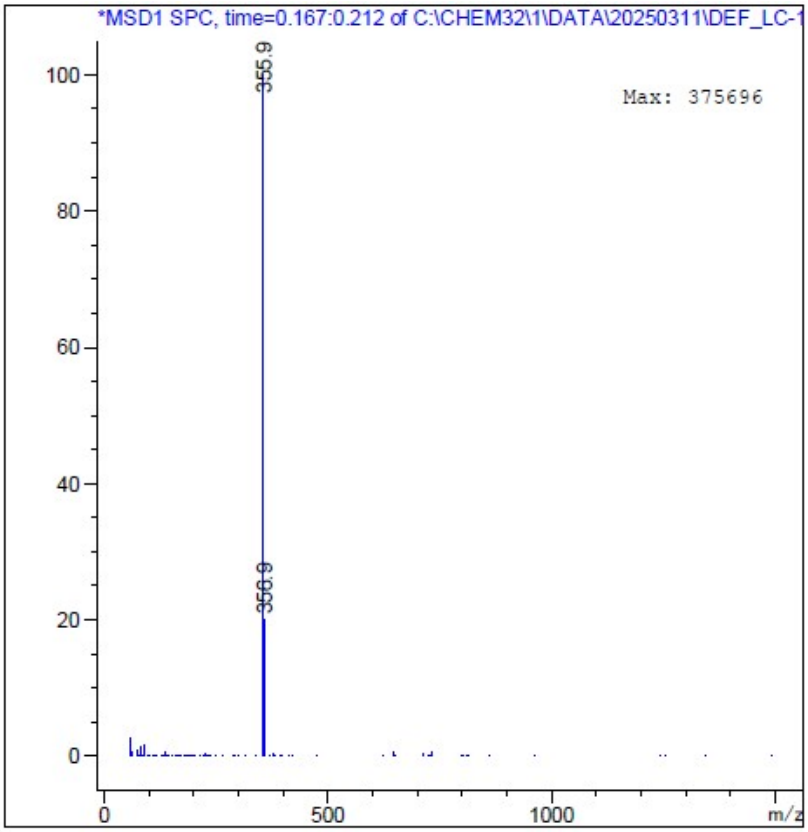


Figure S96. MS spectrum of compound **20**

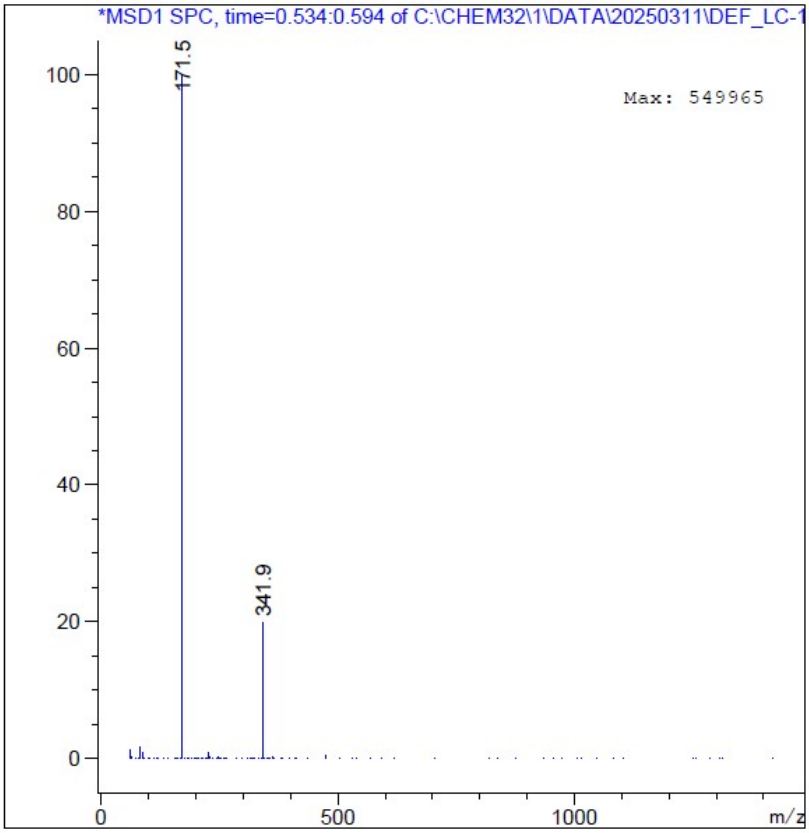


Figure S97. MS spectrum of compound **21**

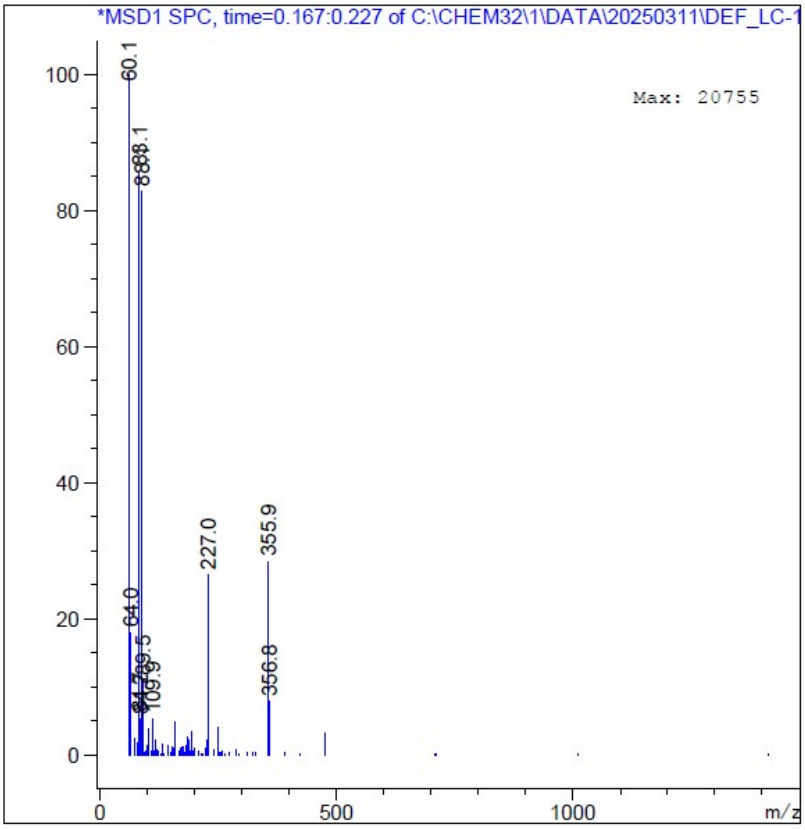


Figure S98. MS spectrum of compound **22**

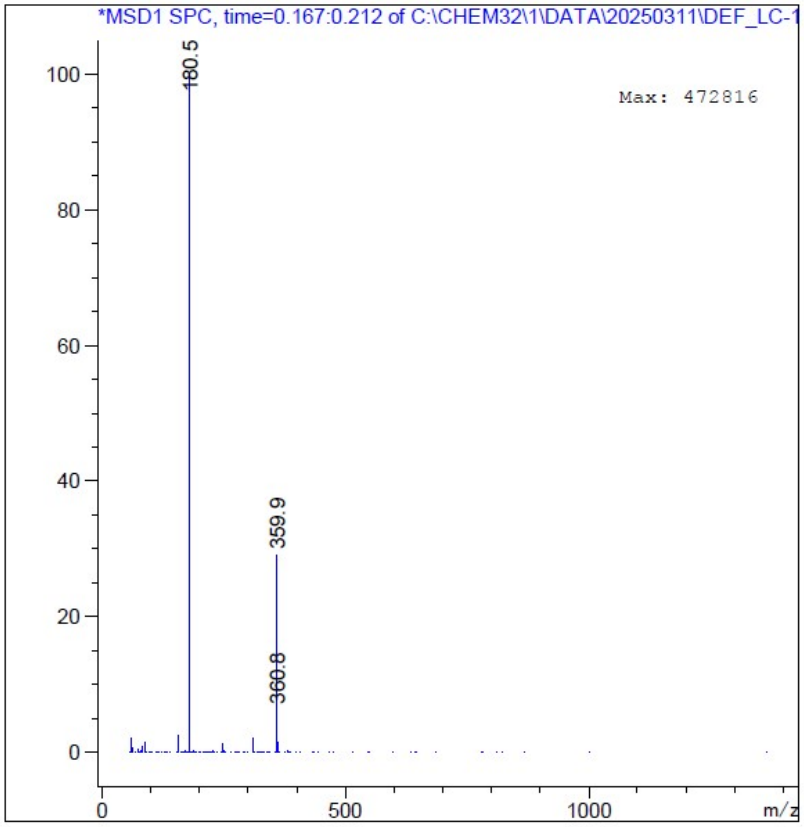


Figure S99. MS spectrum of compound **24**

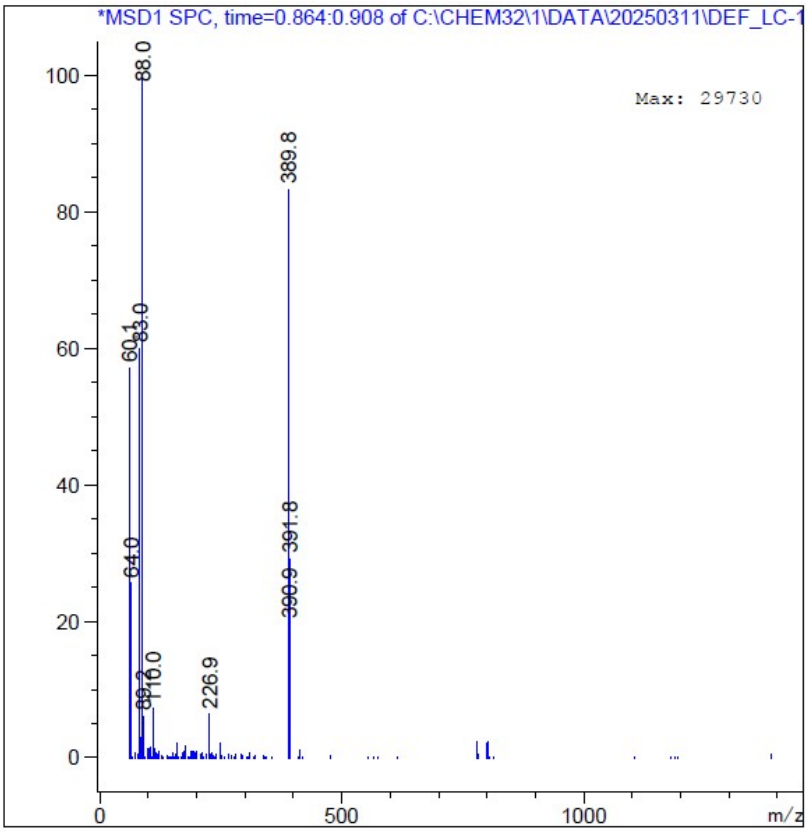


Figure S100. MS spectrum of compound **25**

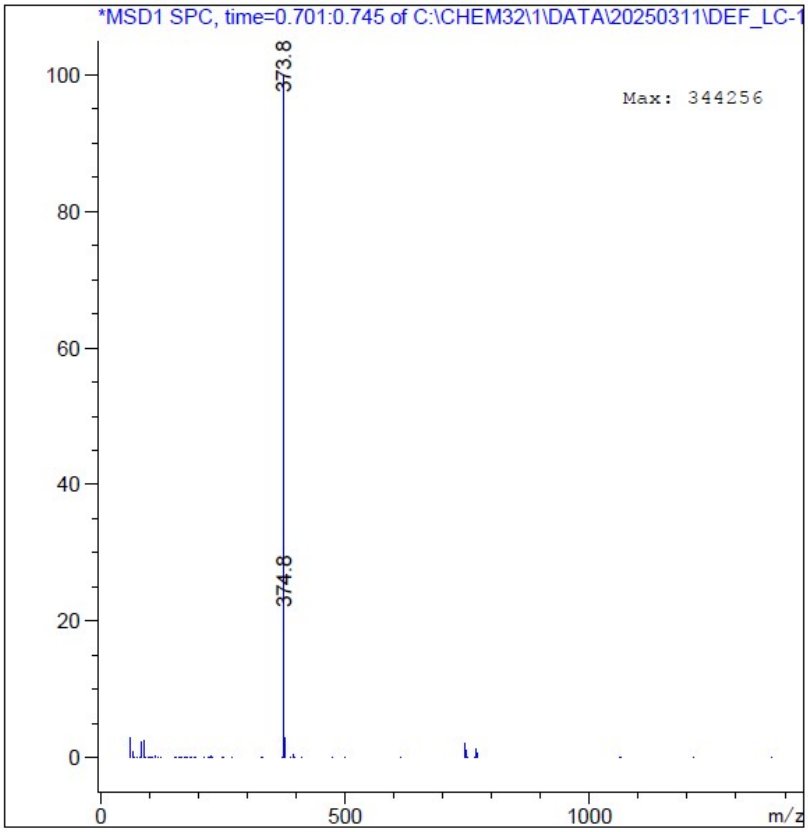


Figure S101. MS spectrum of compound **27**

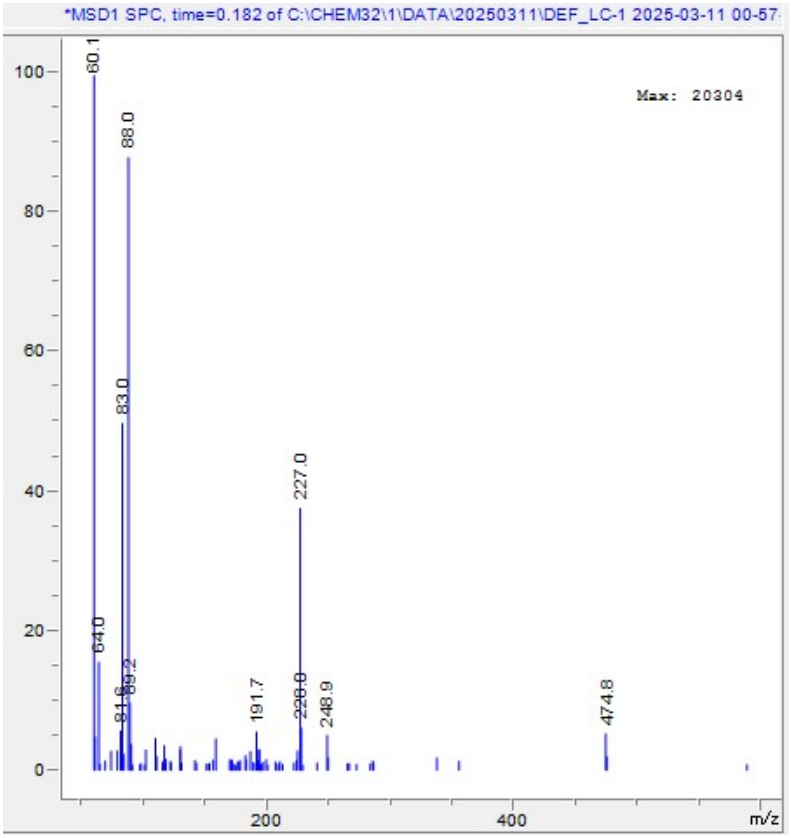


Figure S102. MS spectrum of compound **29**

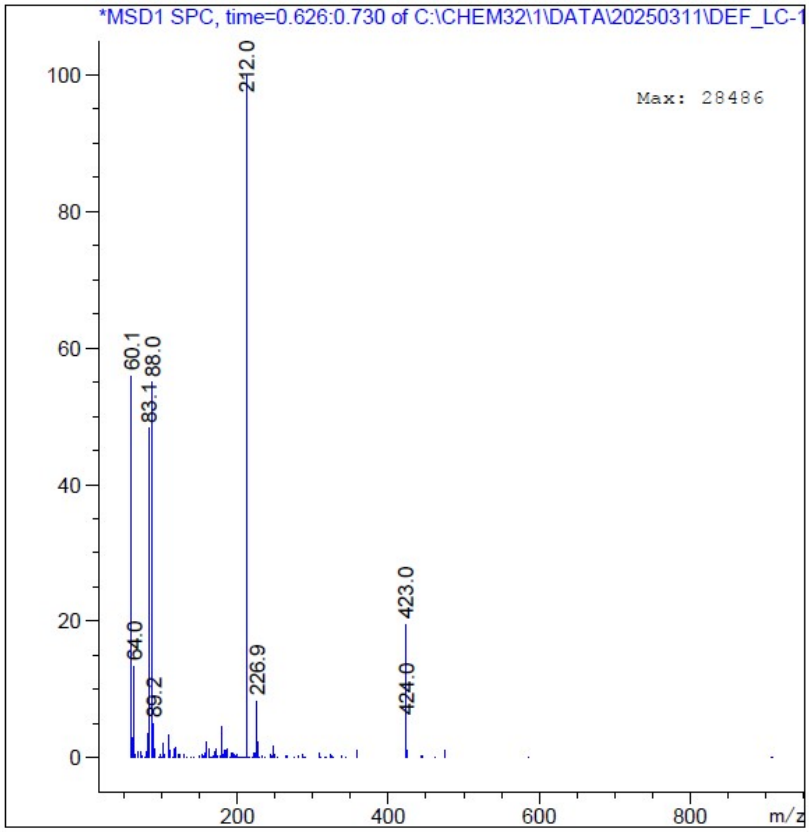


Figure S103. MS spectrum of compound **30**

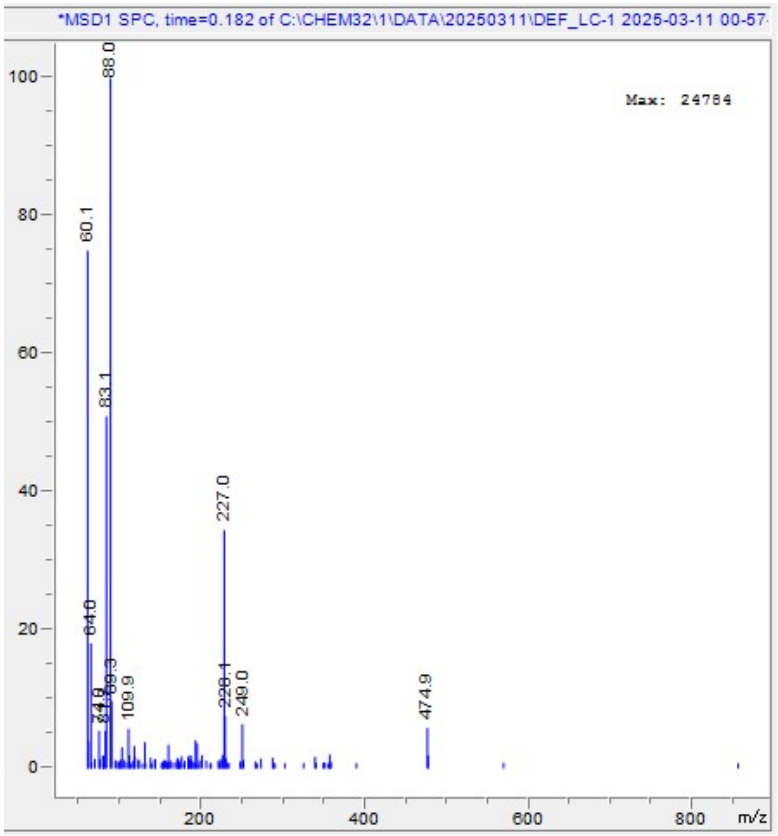


Figure S104. MS spectrum of compound **31**

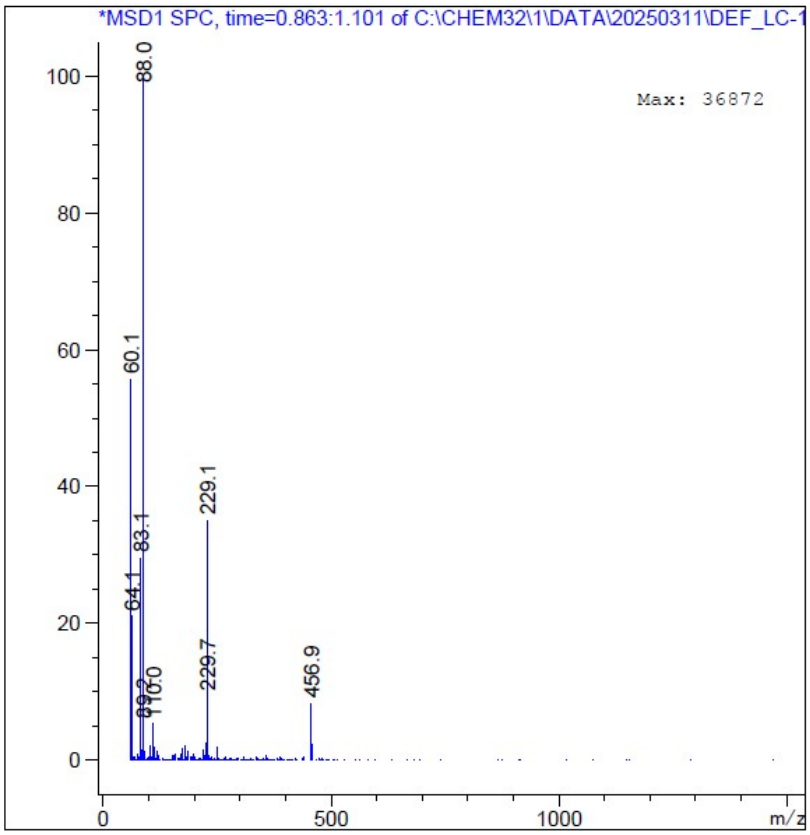


Figure S105. MS spectrum of compound **32**

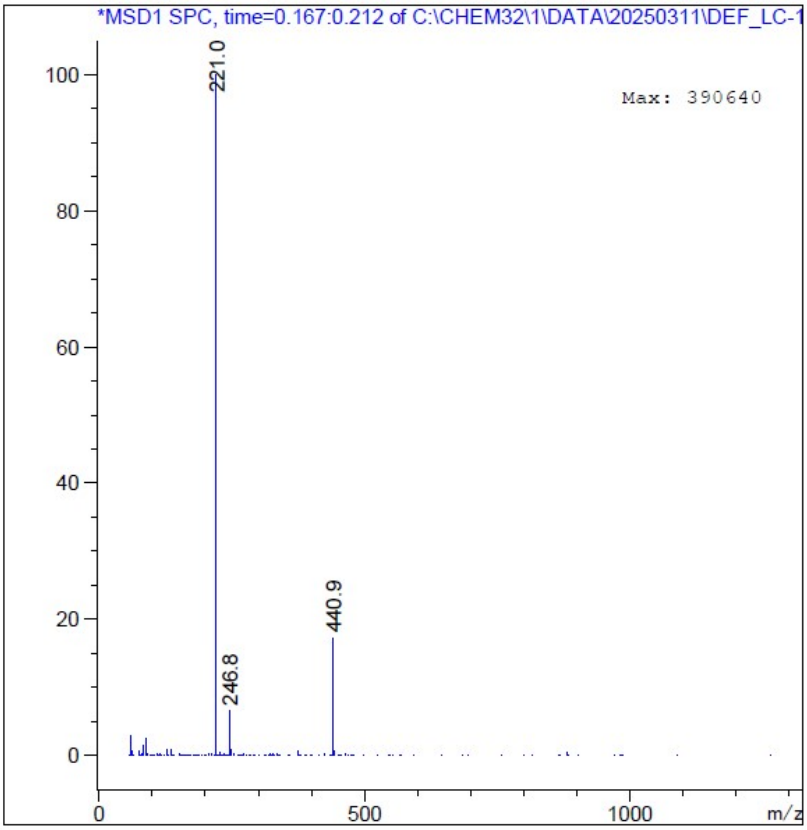


Figure S106. MS spectrum of compound **33**