Supporting Information

Synthesis and Anti-malarial Activity of 4-Methylaminoquinoline Compounds Against Drug Resistant Parasite

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General information:

Chemical Methods:

All the chemicals were purchased from Sigma-Aldrich, Alfa-Aesar, TCI-chemicals, GLR Innovations and Spectrochem Pvt. Ltd. Solvents were procured from Merck, CDH, Finar Ltd., GLR Innovations and QUALIGENS. Unless noted all the chemicals and the solvents were used as received. Reactions were monitored by thin layer chromatography (TLC) on readymade TLC silica gel $60F_{254}$ plates (Merck, Dermstadt, Germany). The TLC plates were either seen directly under UV light or developed under iodine vapors, Dragendorff's stain or by charring with HBr/Ninhydrin solution. Silica gel of 230-400 mesh was used for column chromatography. High resolution mass spectra were taken with a 3000 mass spectrometer, using Waters Agilent 6520-Q-TofMS/MS system and JEOL-AccuTOF JMST100LC. ^{1}H NMR spectra were recorded on 300,400 and 500MHz spectrometers at room temperature in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts (δ) are shown in ppm scales. ^{13}C spectra were recorded at 75, 100 and 125MHz with complete proton decoupling.

Biological Methods:

In-Vitro:

1. Parasites strains

Parasite lines used in the study were Plasmodium falciparum 3D7 (Pf3D7-Chloroquine sensitive) and Plasmodium falciparum K1 (PfK1-Chloroquine resistant) obtained from Malaria Research and Reference Reagent Resource Center (MR4), ATCC Manassas Virginia, USA.

2. Parasite culture

Plasmodium falciparum parasite lines (Pf3D7 & PfK1) were cultured using a modified method described by Trager and Jensen, 1976. Plasmodium strains were maintained in RPMI-1640 medium at 3-5% hematocrit supplemented with 0.5% w/v AlbuMaxII, 0.2% w/v glucose, 0.2% w/v NaHCO3, 30µg/ml gentamycin and additionally 15 µM hypoxanthine was added and incubated at 37˚C with 5% CO2, 5% O2 and 90% N2. Medium was changed preferably after 24 h. On attainment of high parasitaemia (6-8% mature stage parasite) the culture was sub-passaged with fresh human RBCs.

3. Preparation of blood smear

Thin blood films of Plasmodium culture were made to assess parasite maturity and parasitaemia. To prepare a thin blood film, 2-5µl RBCs from culture were taken and smeared on the glass slide, air dried, fixed with 100% methanol and stained for 30 minutes using 30% Giemsa in staining buffer solution. Slides were examined microscopically at 1000 x magnification under an oil immersion lens. Parasitaemia was determined by counting the number of infected and uninfected red blood cells up to a total of 10000 cells.

4. Preparation of Human RBCs for culture

Human blood was collected aseptically in ACD (Acid Citrate Dextrose) solution with the permission of Institutional Human Ethics Committee (CDRI/IEC/2019/A8). Prior to use in parasite culture, the blood was centrifuged at 2000 rpm for 10 minutes, residual plasma and leukocyte aggregates were removed by aspiration; the remaining packed erythrocyte pellet was washed thrice with complete RPMI (CRPMI) at 2000rpm for 10 minutes for complete removal of white blood cells and then resuspended to 50% hematocrit in CRPMI. The cells were stored at 4˚C and can be kept for 15 days.

5. Parasite synchronization (D-sorbitol synchronization)

5% w/v D-sorbitol method was used for the parasite synchronization. Briefly, 5% of D-sorbitol (Sigma Aldirich) were prepared in MilliQ and passed through a 0.22µM syringe filter. Parasite supernatant was removed after centrifugation at 2000rpm for 5 minute, and then 1:5 ratio of D-sorbitol (1 part parasite pellet & 5 part d-sorbitol) was added to parasite palette, and incubated at 37ºC for 10-15 minutes. The tube was then centrifuged at 2000 rpm for 5 minute. Pellet was washed thrice with complete RPMI for removal of late stage parasite by lysis caused by D-sorbitol and then resuspended in CRPMI.

6. Stock solution of compounds preparation

All tested compounds were dissolved in DMSO to get optimum stock solution 10mM. Chloroquine diphosphate was used for reference drug was dissolved in CRPMI. At the time of experiment, working solutions were made from stock solution after diluting in CRPMI medium. The maximum concentration of DMSO used in this study was <1%. It had no parasiticidal effect.

7. Antimalarial assay (IC50 determination)

Synchronous culture of P. falciparum (0.8-1% parasitaemia and 1% hematocrit) was exposed for 72 h (37˚C, 5% CO2, 5%O2 and 90%N2) to the serially diluted drug in 96 well plates. Hundred micro litre RBCs lytic buffer (20 mM Tris pH 7.5, 5 mM EDTA, 0.008% saponin,

and 0.08% Triton X-100) containing SYBR green 1-X final concentration was added to each well, incubated for 1 h at room temp in dark. Plates were read under fluorescence reader at excitation 485±20nm, emission 535±25nm. IC50 values were determined on the basis of DNA content of the parasite relative to controls (Johnson et al. 2007). Inhibitory concentration 50% (IC50) was determined using MS-EXEL template. The signal to noise ratio was found to be 1:5-10.

8. Cytotoxicity assay against Vero cell line

For the cytotoxicity assay, Vero cells were washed with PBS, trypsinized and a suspension of 1x105 cells/ml was prepared. Hundred micro litre of cell suspension was added to the 96 well microtiter plates and allowed to adhere overnight. Next day, serial dilutions of test compounds were prepared in these plates and incubated for 72 h. After 72h, ten microlitre resazurin solution (12.5mg/100ml PBS) was added in each well and after 3 h, plates were read under florescence reader (Biotek) at excitation wavelength of 530 nm and an emission wavelength of 590 nm (Sperandeo and Brun, 2003). Cytotoxic concentration (CC50) was determined using MS-EXEL template.

The selectivity indices of the compounds were determined to select them for in vivo evaluation using the following formula-

Animal studies

9. Experimental animals and parasite

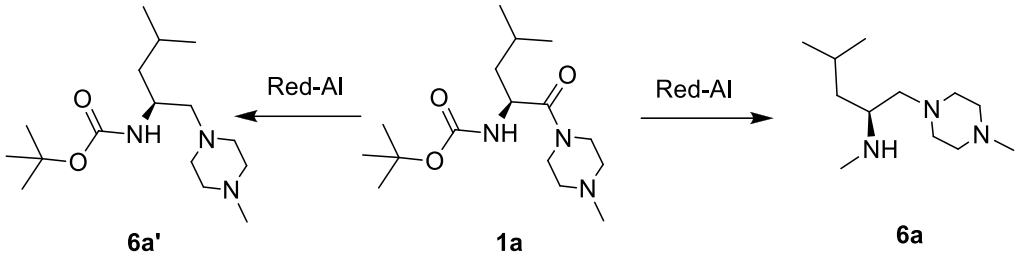
Laboratory bred Swiss albino mice of either sex weighing 20-22 gram obtained from breeding colonies at National Laboratory Animal center at the CSIR-CDRI Lucknow with due permission from animal ethics committee (IAEC/2018/16/Renew-1/dated 05.05.2019). Rodent malaria parasite Plasmodium yoeliinigreiensis was initially obtained from Prof. P.C.C Garnham in 1978 and later it was made multi drug resistant parasite in our laboratory. This parasite is maintained by serial passage in healthy Swiss mice of either sex.

10. In-vivo antimalarial assay:

Swiss mice were inoculated with standard inoculum of 2X105 parasitized erythrocytes intraperitoneally. Each mouse was injected with 0.5ml (infected RBCs) volume using a 22-gauge hypodermic needle. Treatment was started from day 0-3 (total 4 day) once daily. For in-vivo evaluation, in-vitro active compounds were dissolved in MilliQ with a few drops of Tween 80. The maximum Tween 80 concentration was 0.5% v/v. The efficacy of test compounds was

evaluated at 100 mg/kg/day and the required daily dose was administered in 0.2 mL volume via oral route. For parasitemia estimation a drop of blood from tail of mice was collected at regular intervals of throughout the period of experiment. The mean value determined for a group of five mice was used to calculate the percent suppression of parasitemia with respect to the untreated control group.

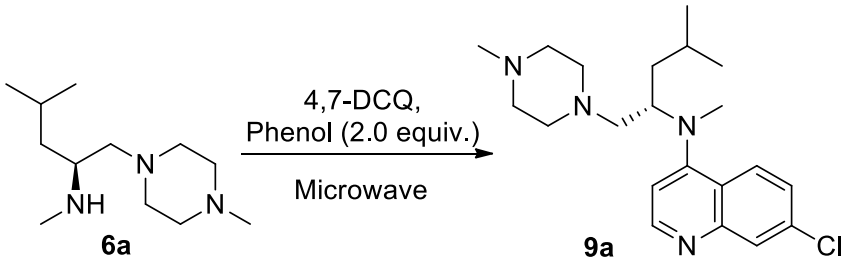
Table S1. Reaction optimization of reduction reaction with Red-Al[a]



Entry	Solvent	Temp. (ºC)	Red-Al (equiv.)	Time (h)	Conversion	Product amine
1	THF	Reflux	10	1.0	100 %	6a
2	THF	RT	10	1.0	100 %	6a’
3	THF	Reflux	3	5.0	Incomplete conversion	6a
4	THF	Reflux	4	5.0	Incomplete conversion	6a
5	**THF**	**Reflux**	**5**	**0.5**	**100 %**	**6a**

[a] Reaction condition was optimized in 1.0 mmol scale of the corresponding amide **1a**.

Table S2. Optimization reaction for microwave assisted fusion reaction[a]



Entry	Eq. of 6a	Temp. °C	Power (W)	Time (min.)	% yield of 9a
1.	1.5	125	25	30	18
2.	1.5	125	50	15	20
3.	1.5	145	25	30	34
4.	**1.5**	**145**	**50**	**30**	**60**
5.	2.0	145	50	30	Not purified
6.	1.5	160	50	15	Charring of reaction

a[a]Reaction condition was optimized in 1.0 mmol scale of the corresponding amide **6a**.

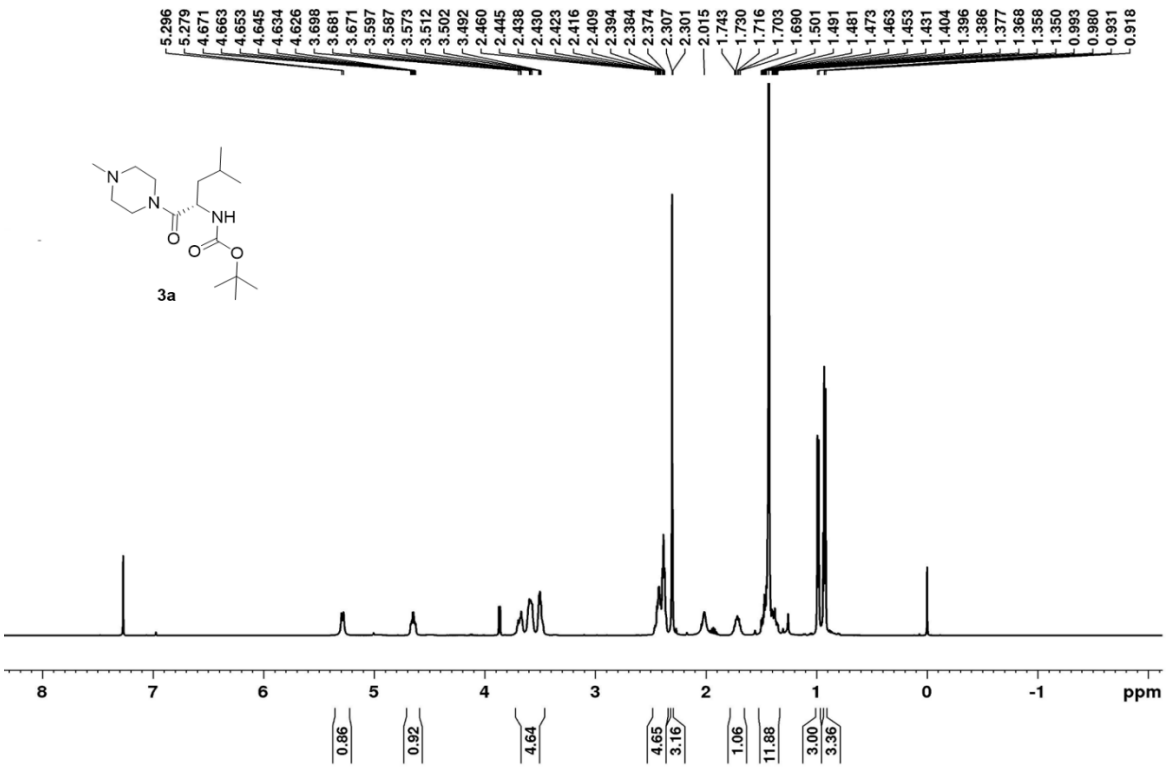


Figure S1: ^{1}H NMR spectrum of compound **3a** (500MHz, $CDCl_3$ 300 K)

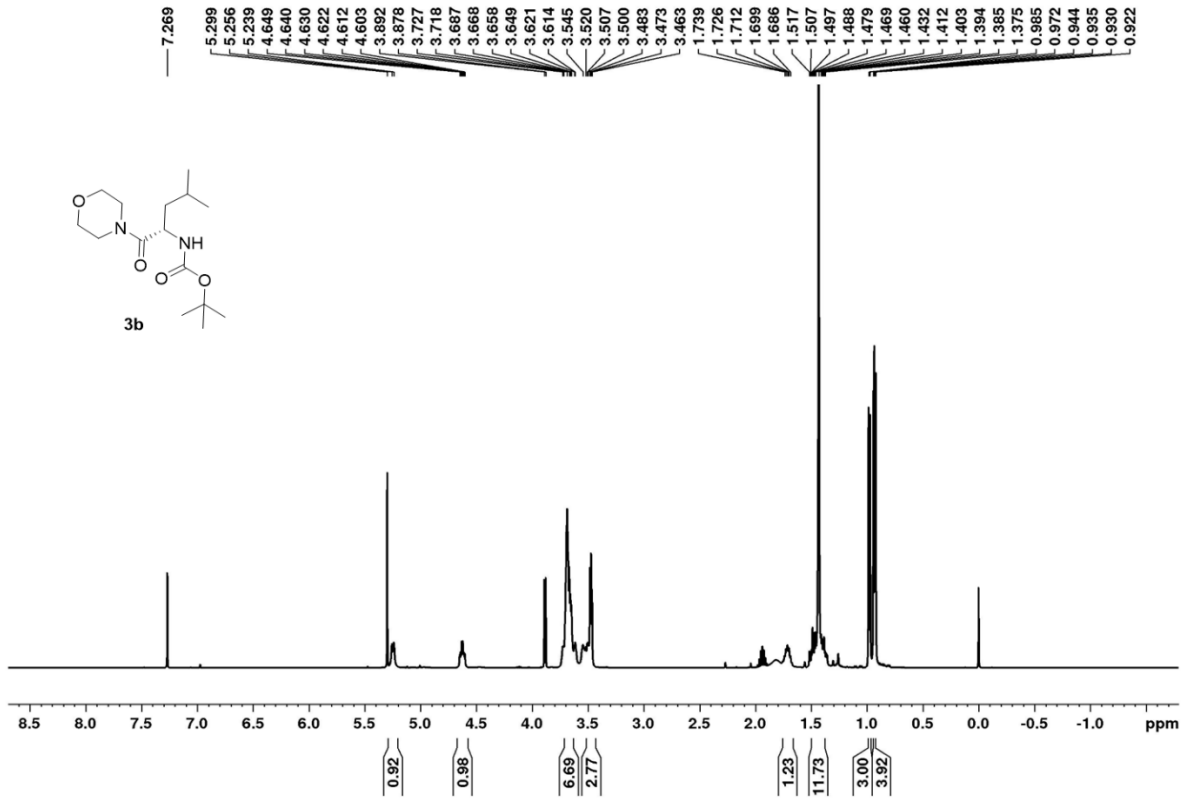


Figure S2: ^{1}H NMR spectrum of compound **3b** (500MHz, $CDCl_3$ 300 K)

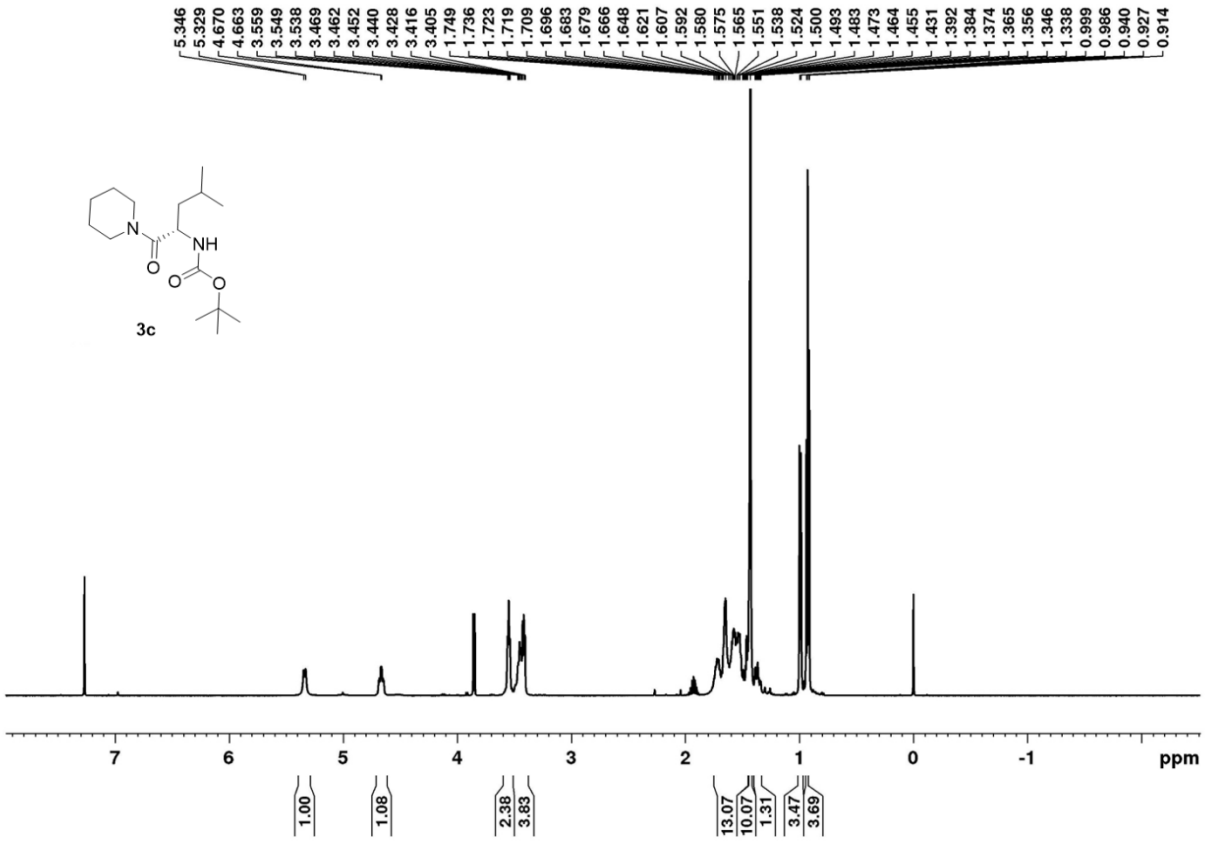


Figure S3: ^{1}H NMR spectrum of compound **3c** (500MHz, $CDCl_3$ 300 K)

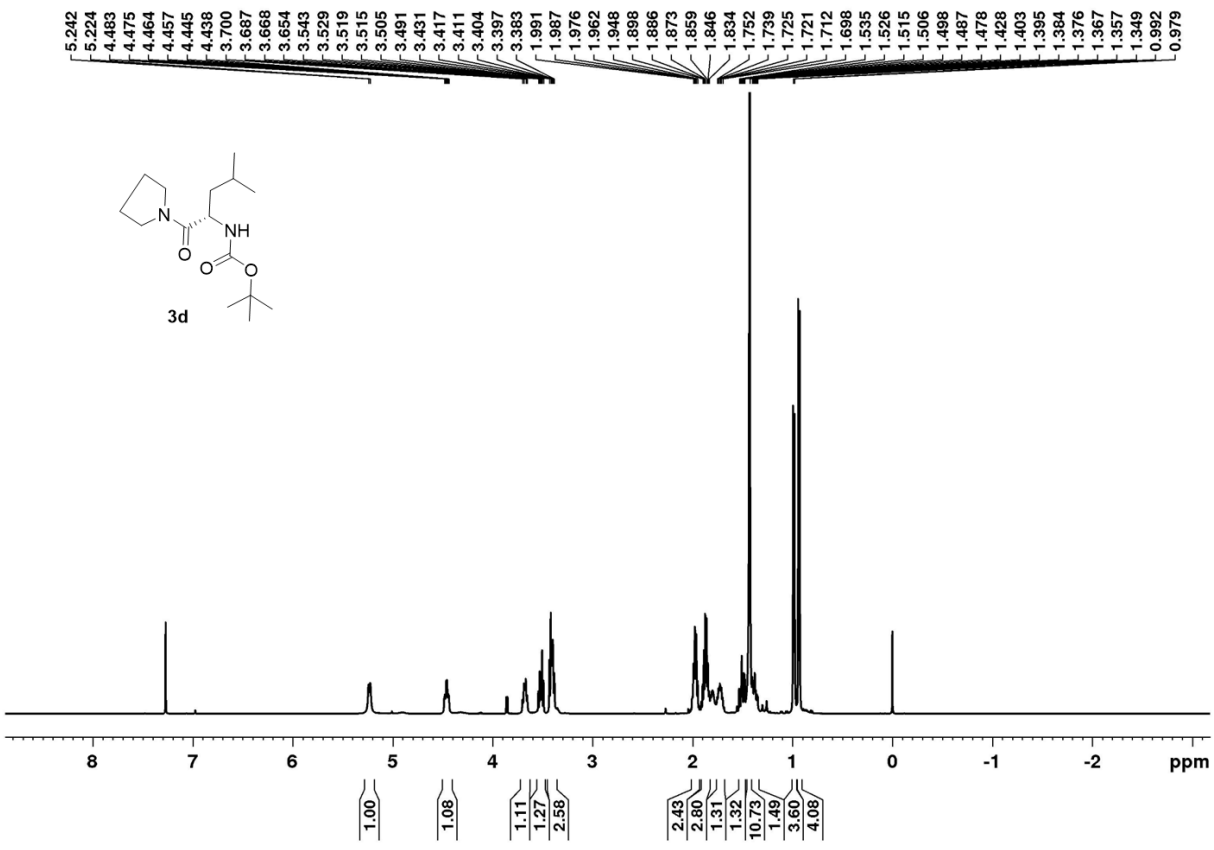


Figure S4: ^{1}H NMR spectrum of compound **3d** (500MHz, $CDCl_3$ 300 K)

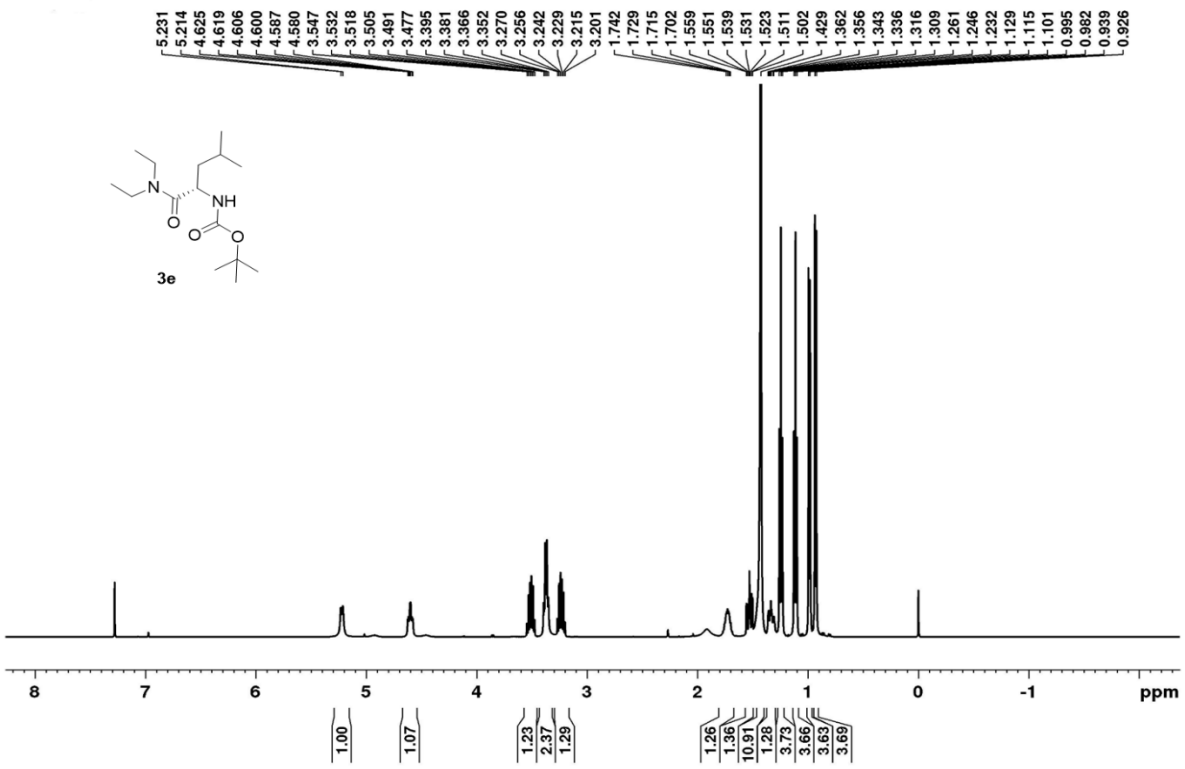


Figure S5: ^{1}H NMR spectrum of compound **3e** (500MHz, $CDCl_3$ 300 K)

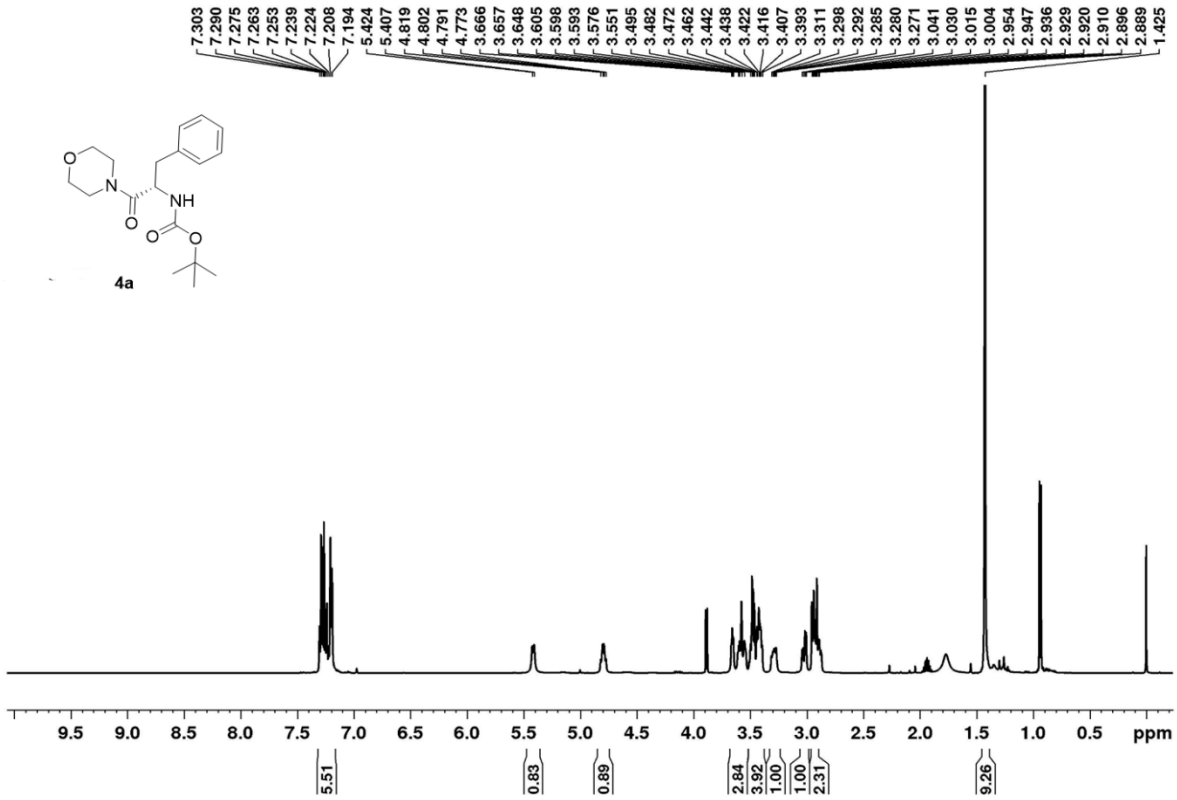


Figure S6: ^{1}H NMR spectrum of compound **4a** (500MHz, $CDCl_3$ 300 K)

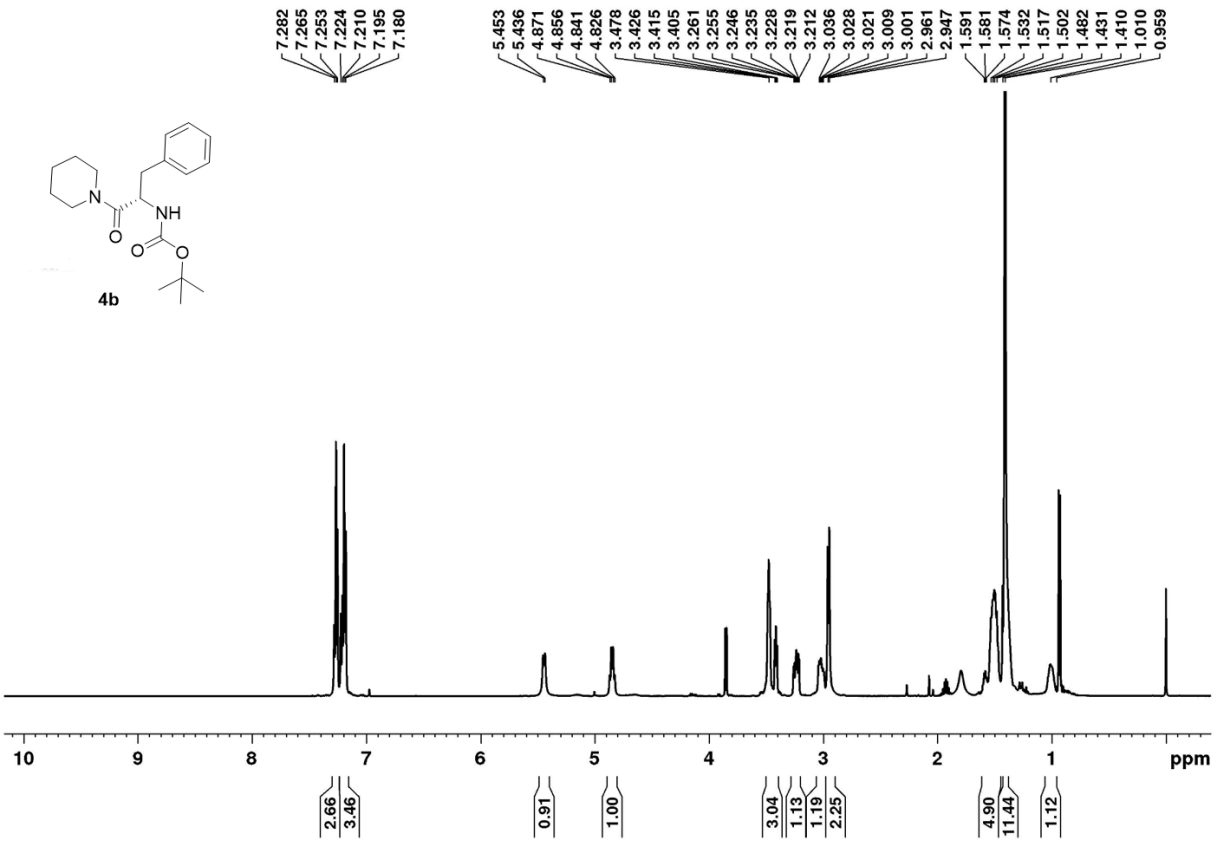


Figure S7: ^{1}H NMR spectrum of compound **4b** (500MHz, $CDCl_3$ 300 K)

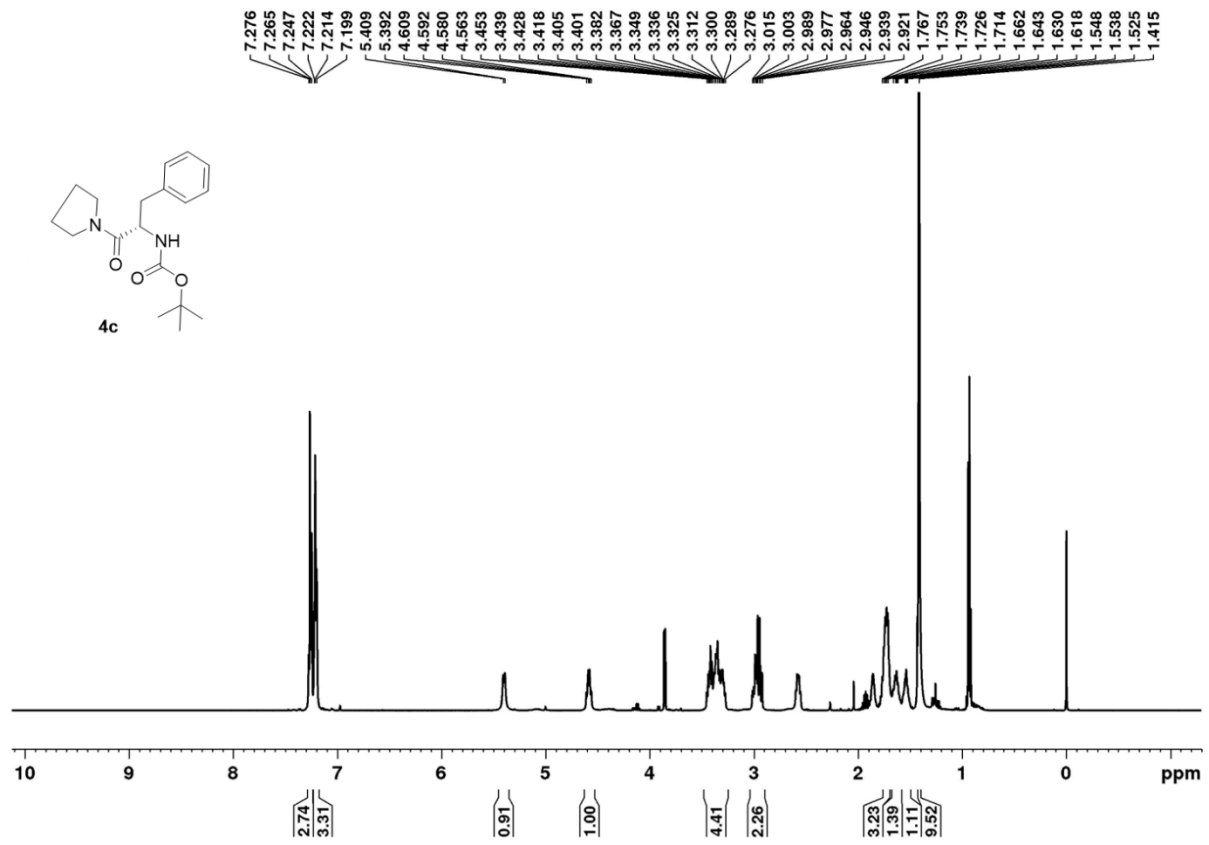


Figure S8: ^{1}H NMR spectrum of compound **4c** (500MHz, $CDCl_3$ 300 K)

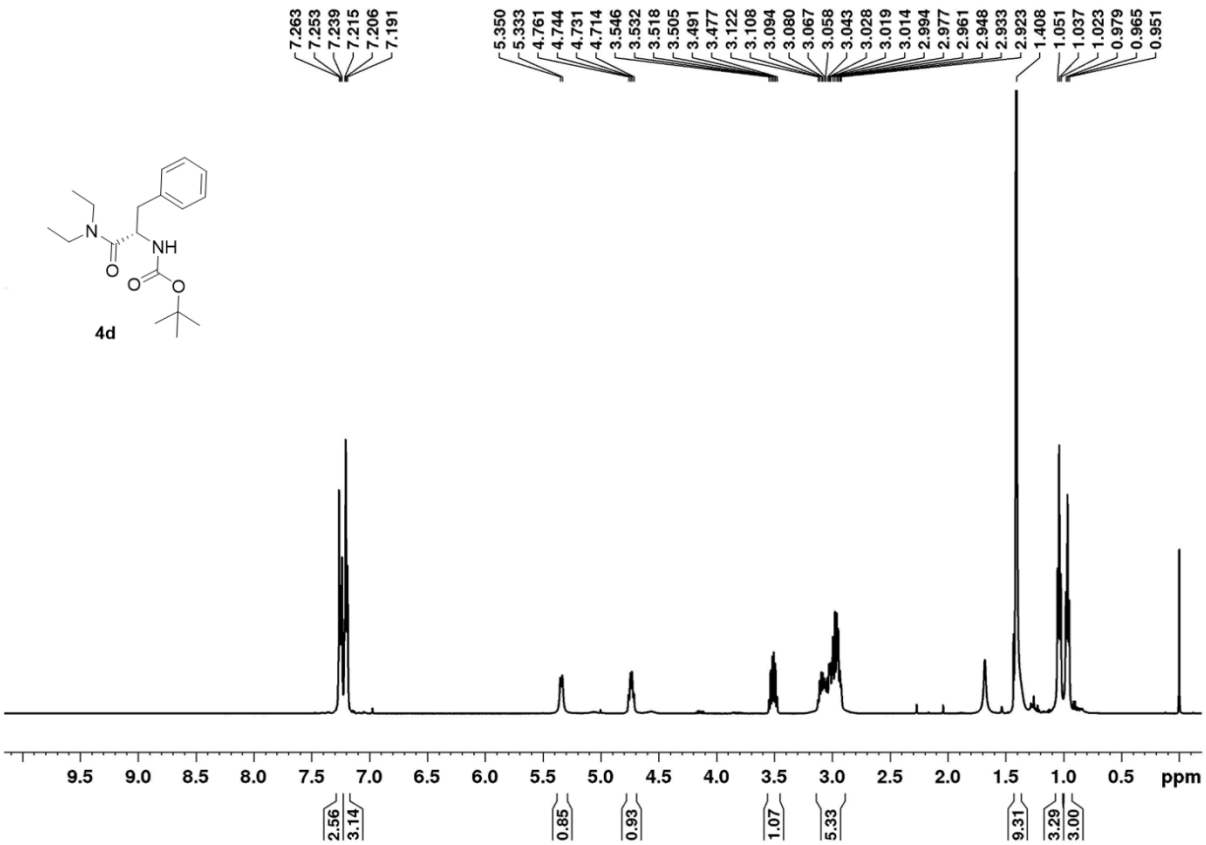


Figure S9: ^{1}H NMR spectrum of compound **4d** (500MHz, $CDCl_3$ 300 K)

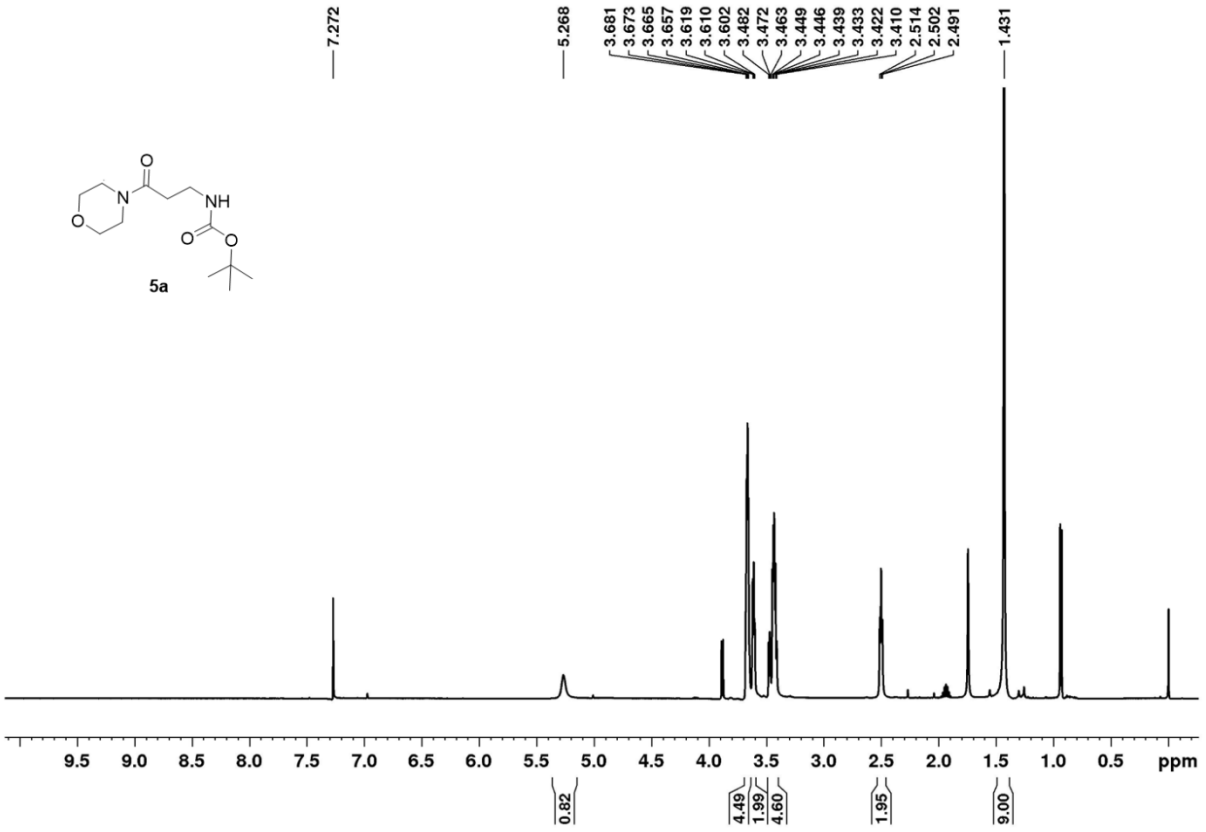


Figure S10: ^{1}H NMR spectrum of compound **5a** (500MHz, $CDCl_3$ 300 K)

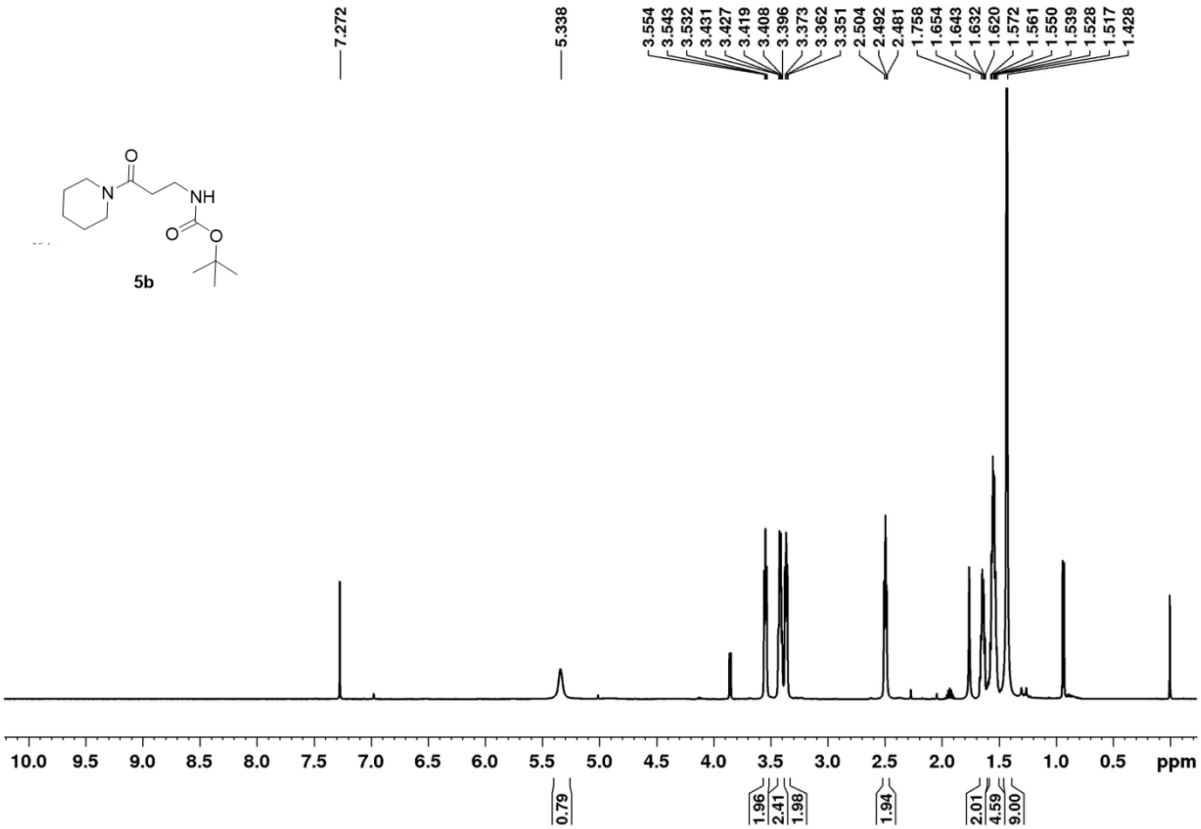


Figure S11: ^{1}H NMR spectrum of compound **5b** (500MHz, $CDCl_3$ 300 K)

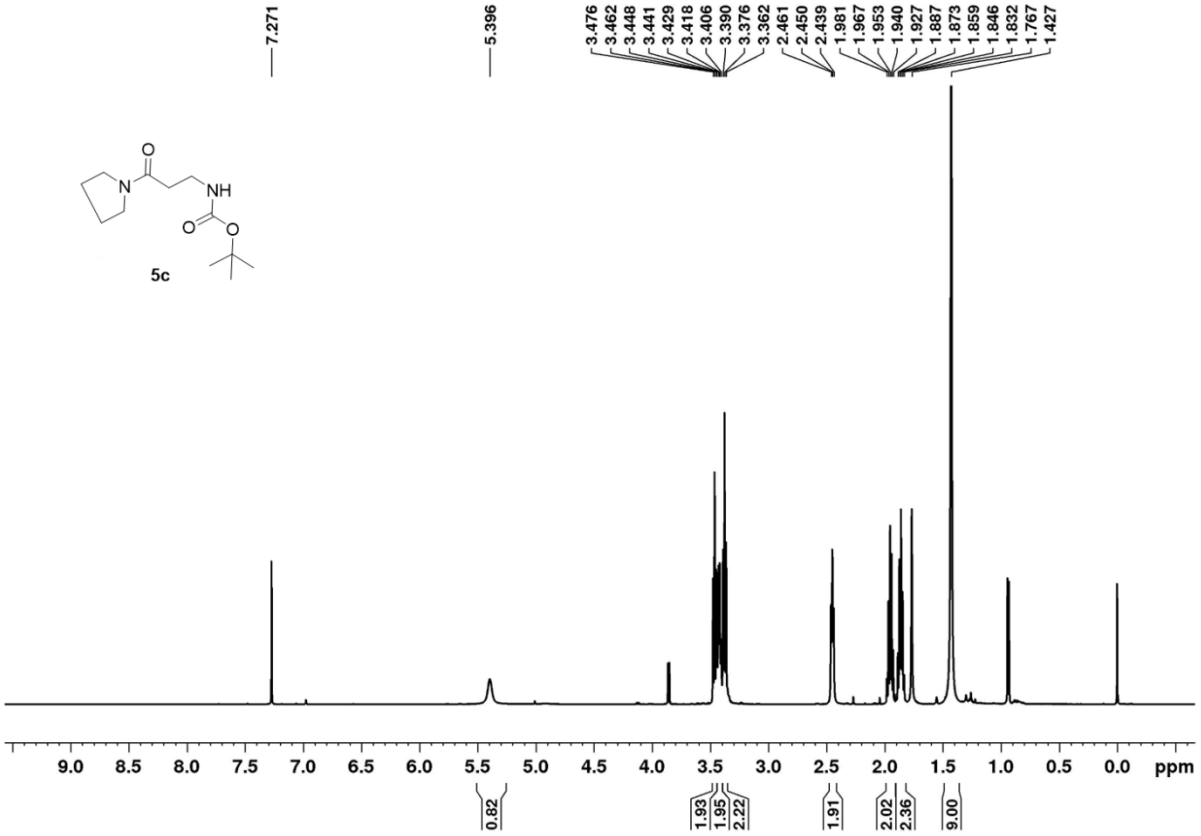


Figure S12: ^{1}H NMR spectrumof compound **5c** (500MHz, $CDCl_3$ 300 K)

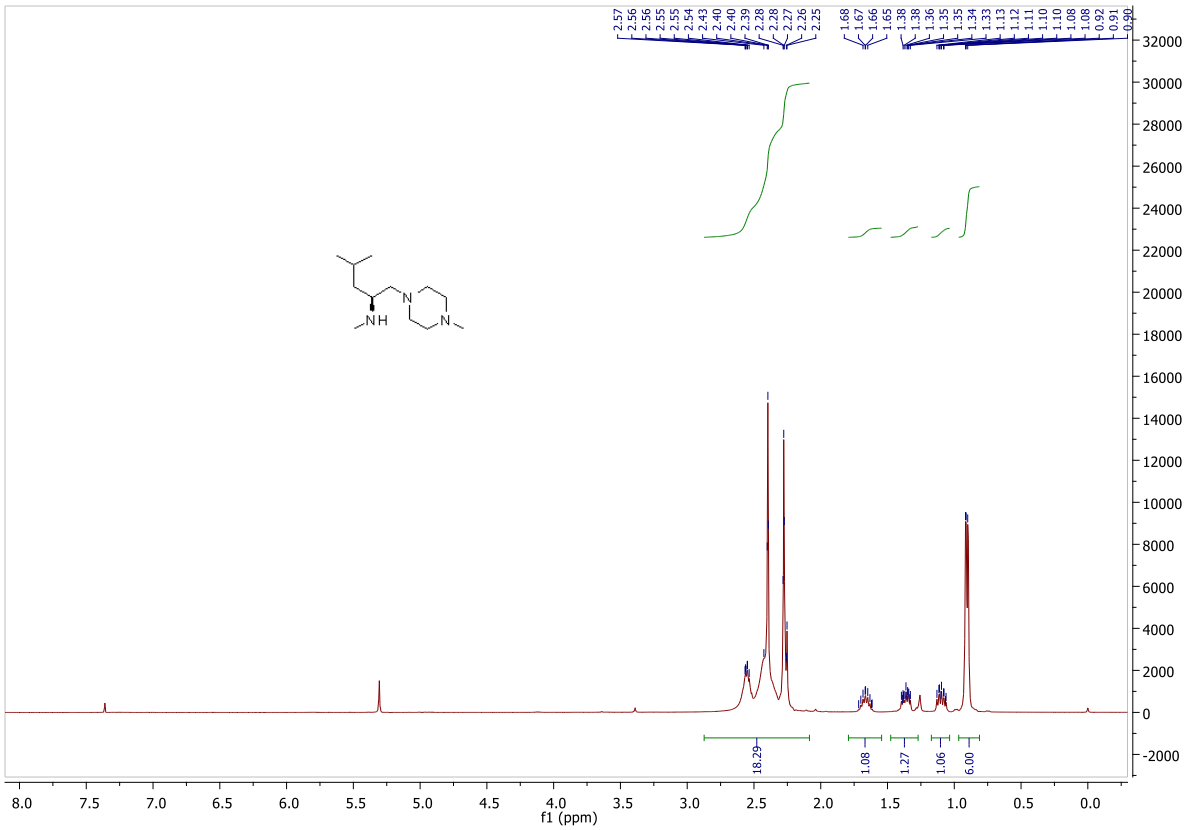


Figure S13: ^{1}H NMR spectrum of compound **6a** (400MHz, $CDCl_3$ 300 K)

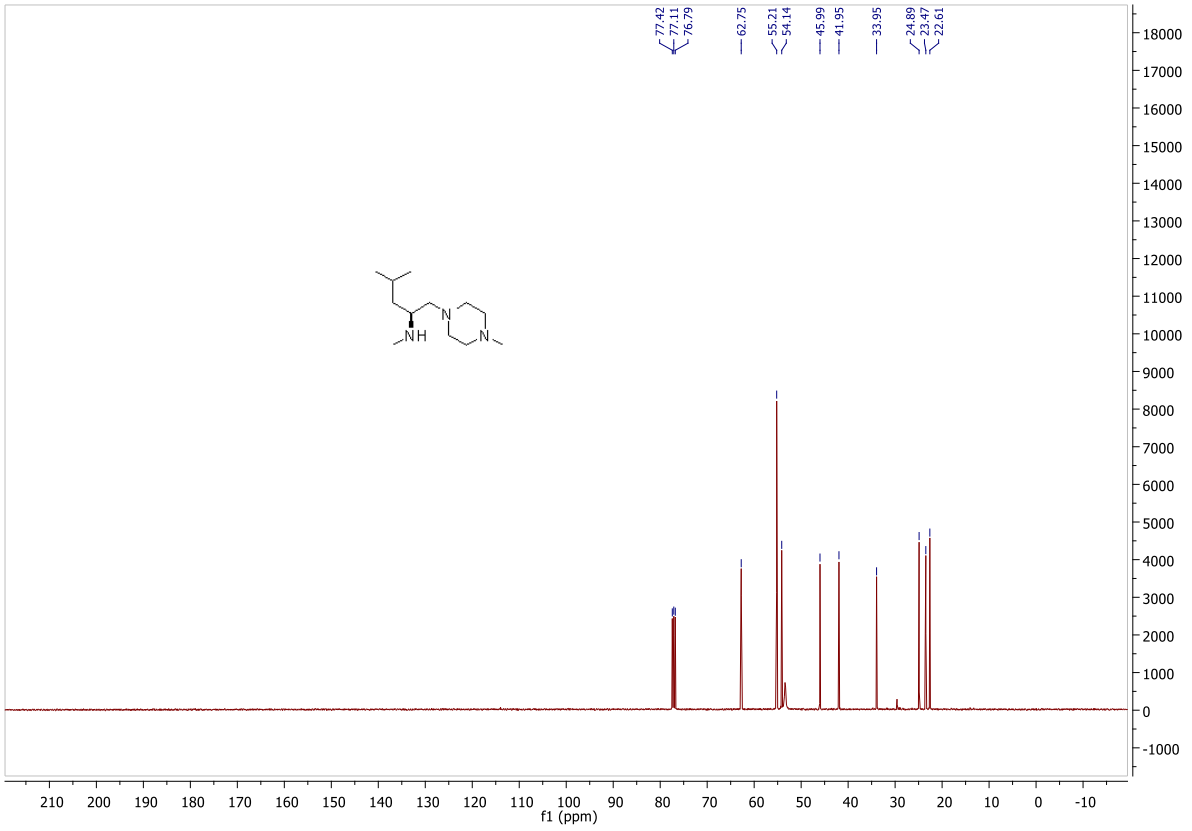


Figure S14: ^{13}C NMR spectrum of compound **6a** (100MHz, $CDCl_3$ 300 K)

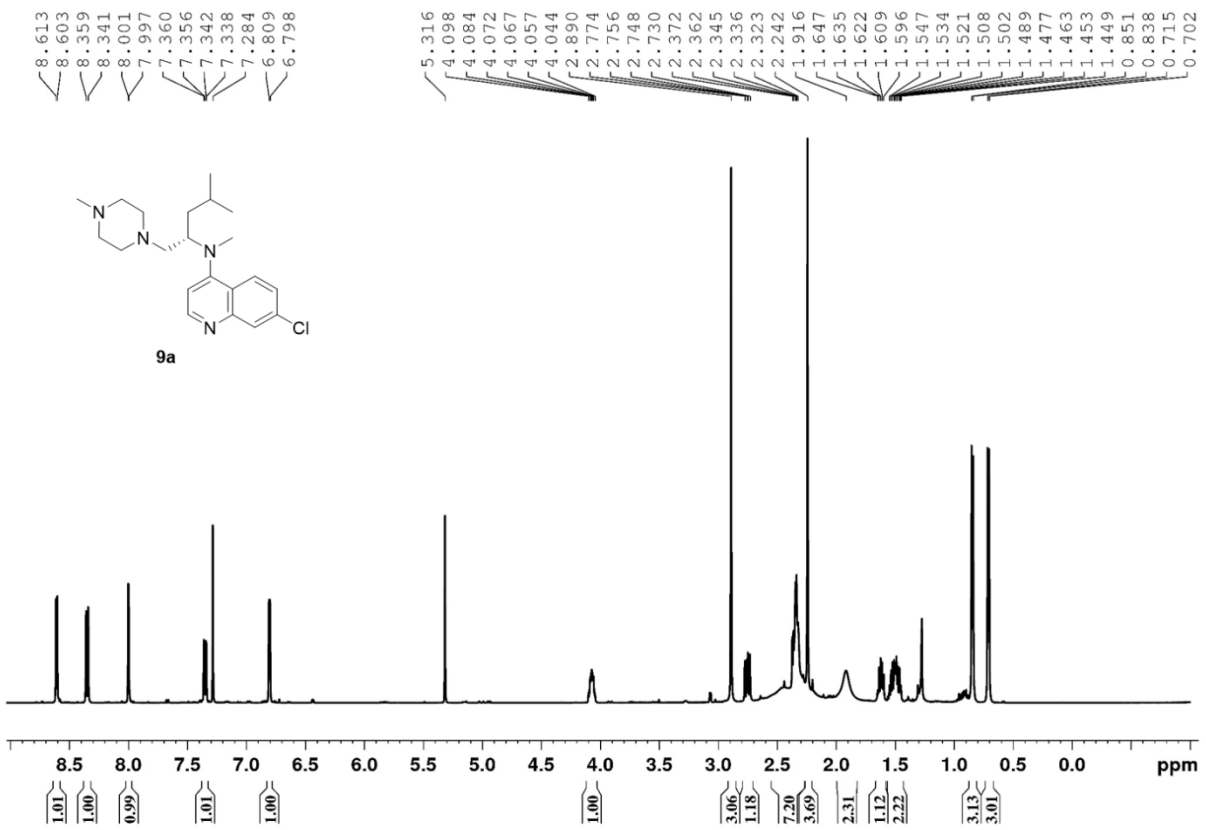


Figure S15: ^{1}H NMR spectrum of compound **9a** (500MHz, $CDCl_3$ 300 K)

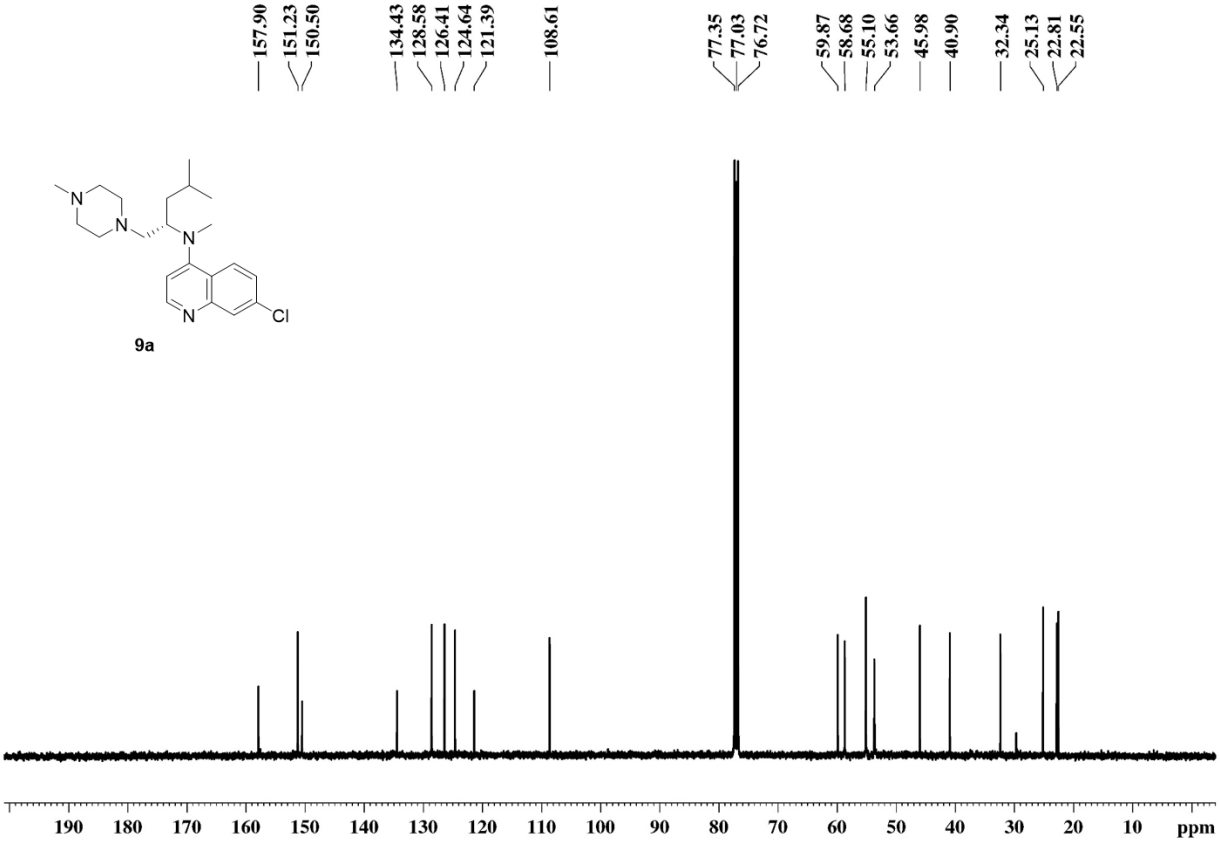


Figure S16: ^{13}C NMR spectrum of compound **9a** (100MHz, $CDCl_3$ 300 K)

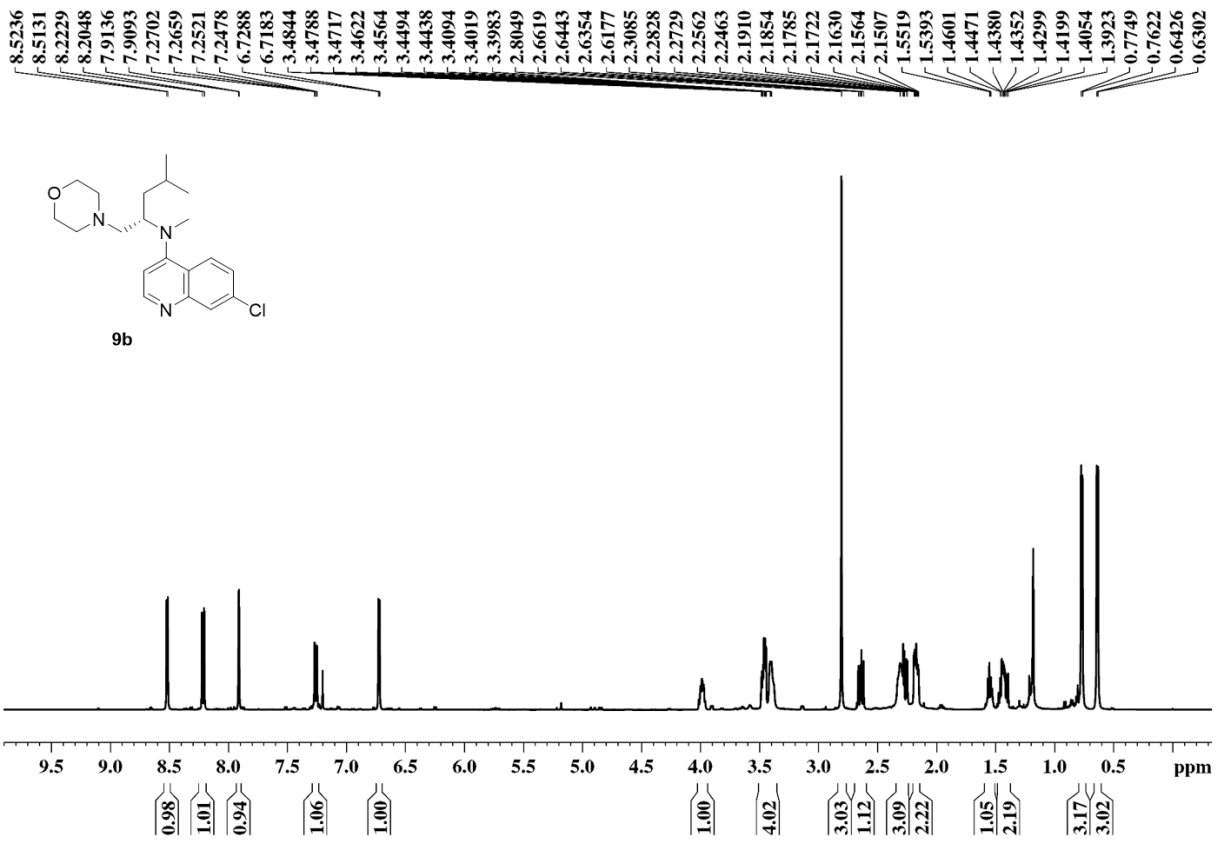


Figure S17: ^{1}H NMR spectrum of compound **9b** (500MHz, $CDCl_3$ 300 K)

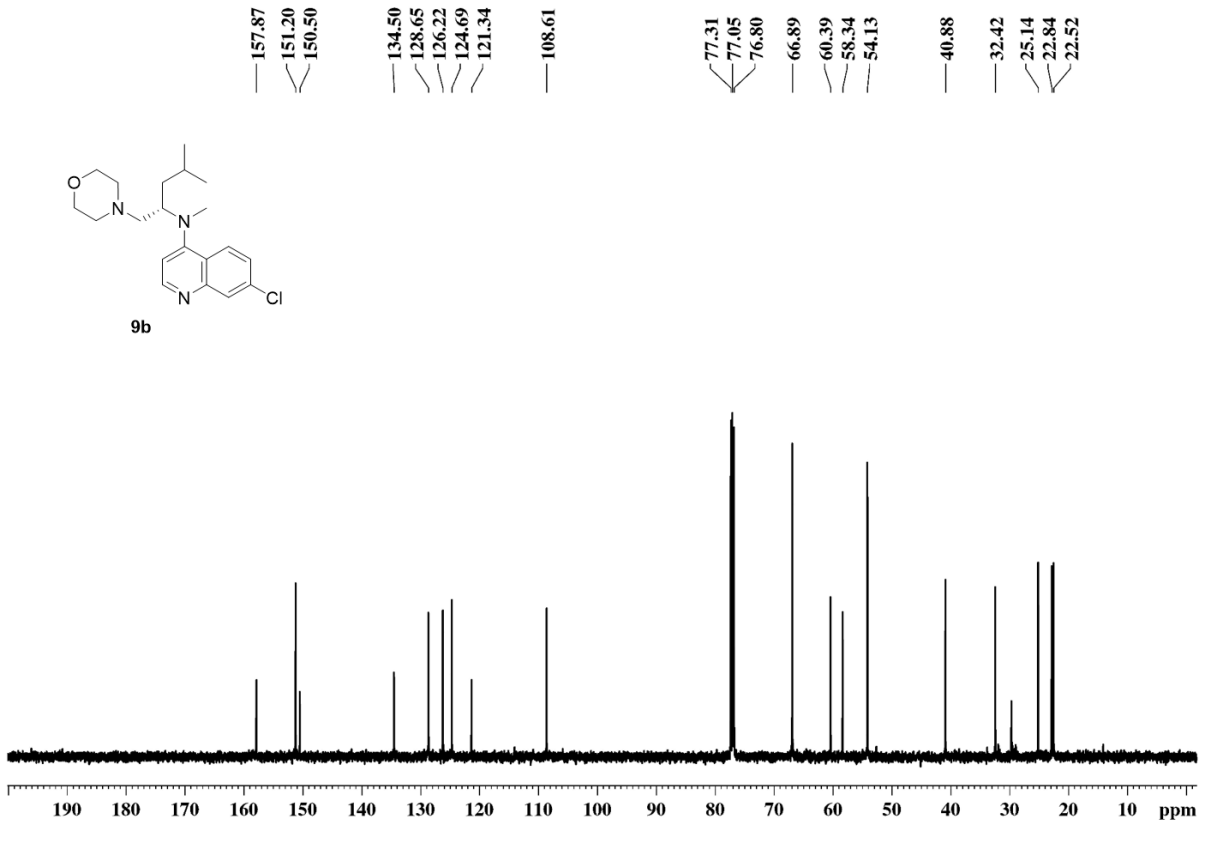


Figure S18: ^{13}C NMR spectrum of compound **9b** (125MHz, $CDCl_3$ 300 K)

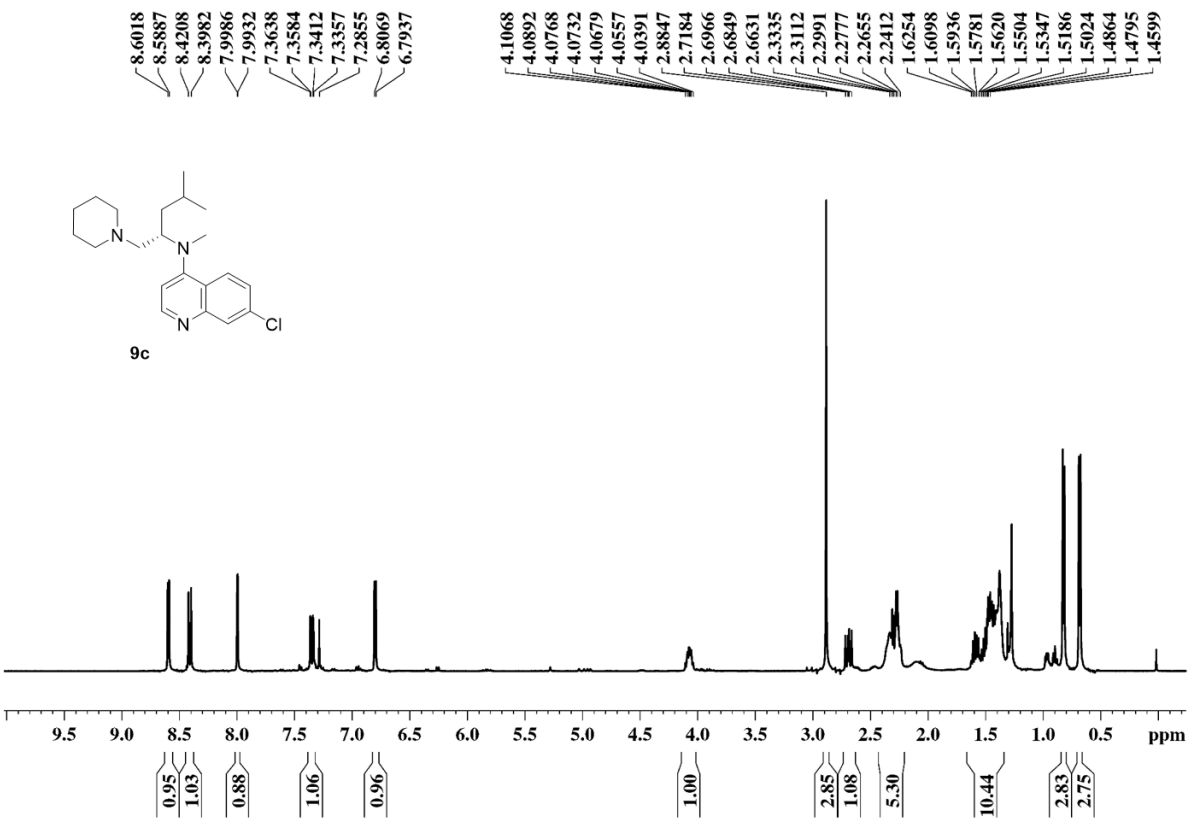


Figure S19: ^{1}H NMR spectrum of compound **9c** (400MHz, $CDCl_3$ 300 K)

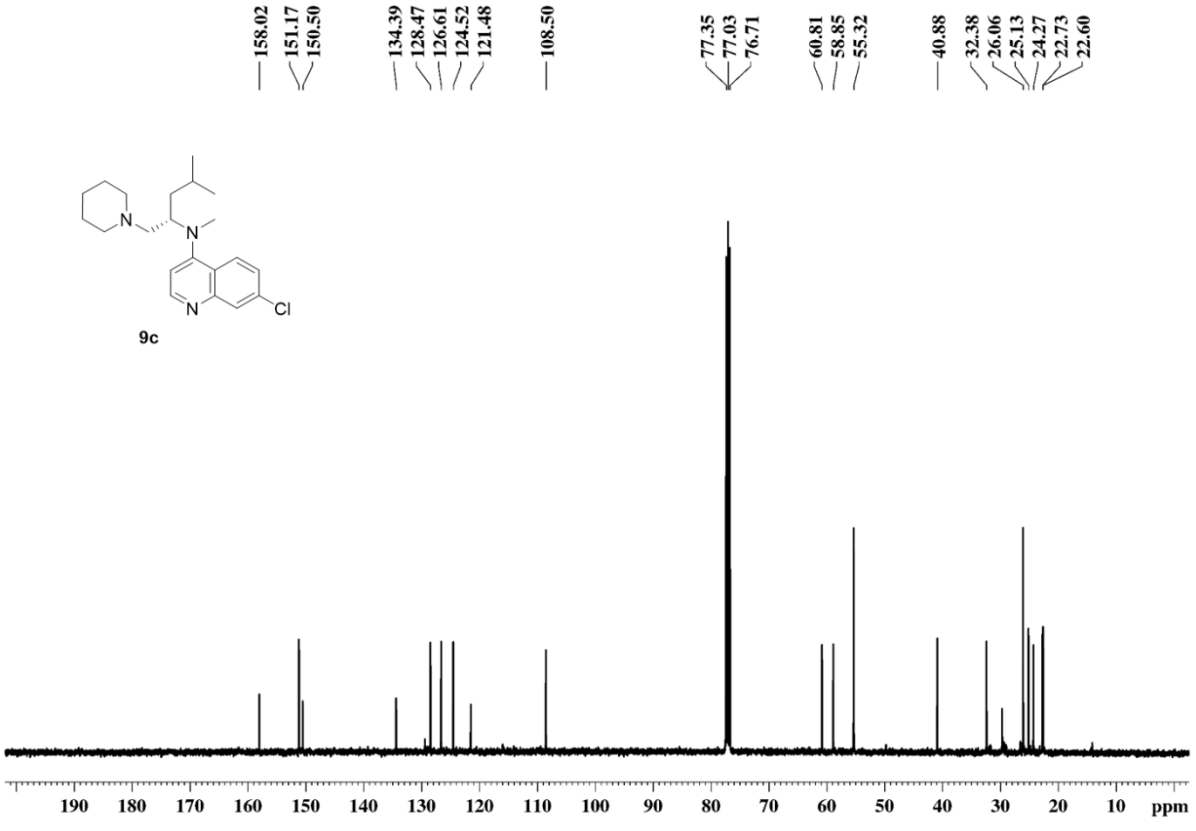


Figure S20: ^{13}C NMR spectrum of compound **9c** (100MHz, $CDCl_3$ 300 K)

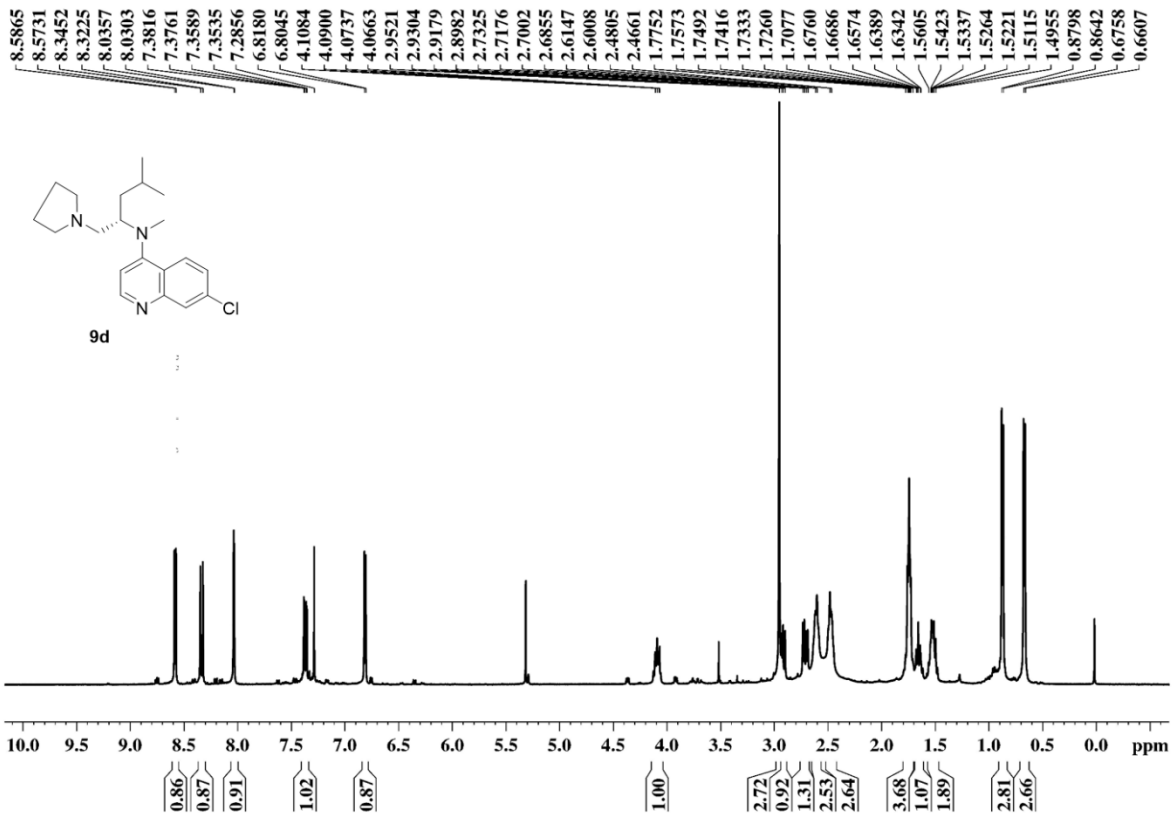


Figure S21: ^{1}H NMR spectrum of compound **9d** (400MHz, $CDCl_3$ 300 K)

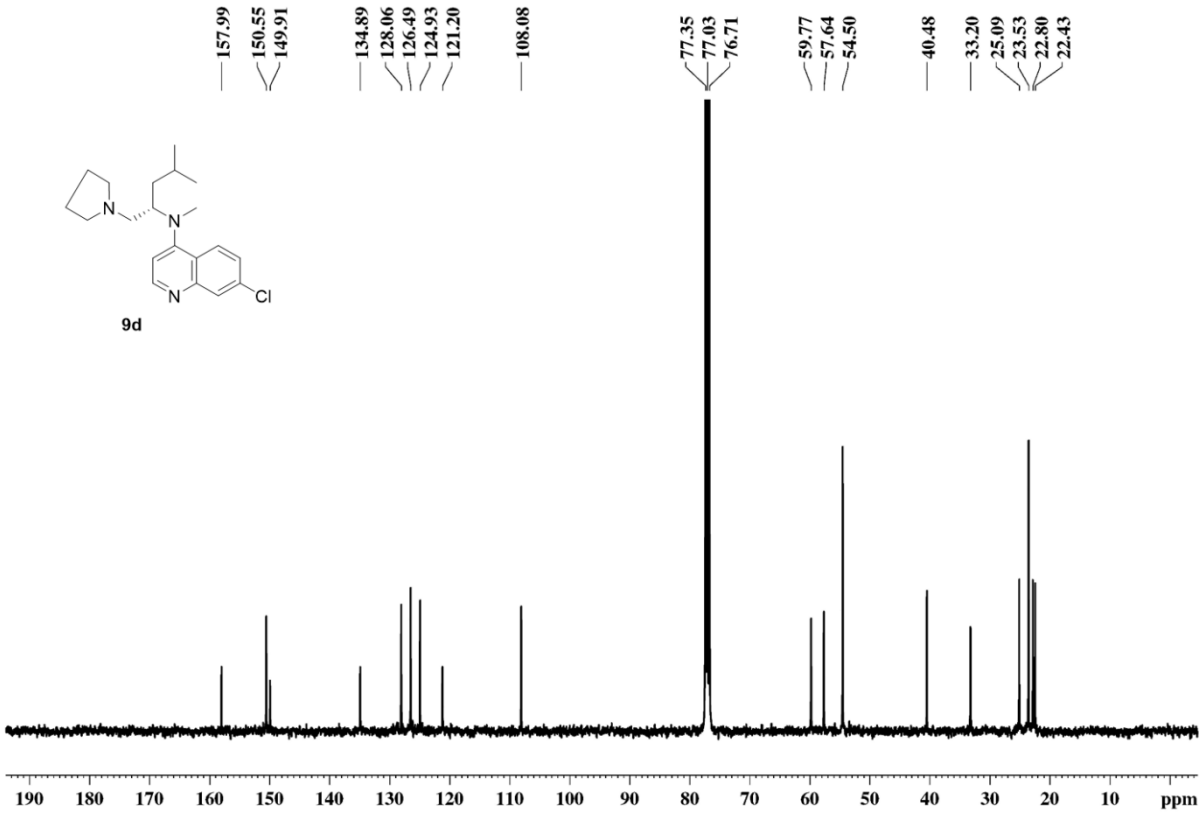


Figure S22: ^{13}C NMR spectrum of compound **9d** (100MHz, $CDCl_3$ 300 K)

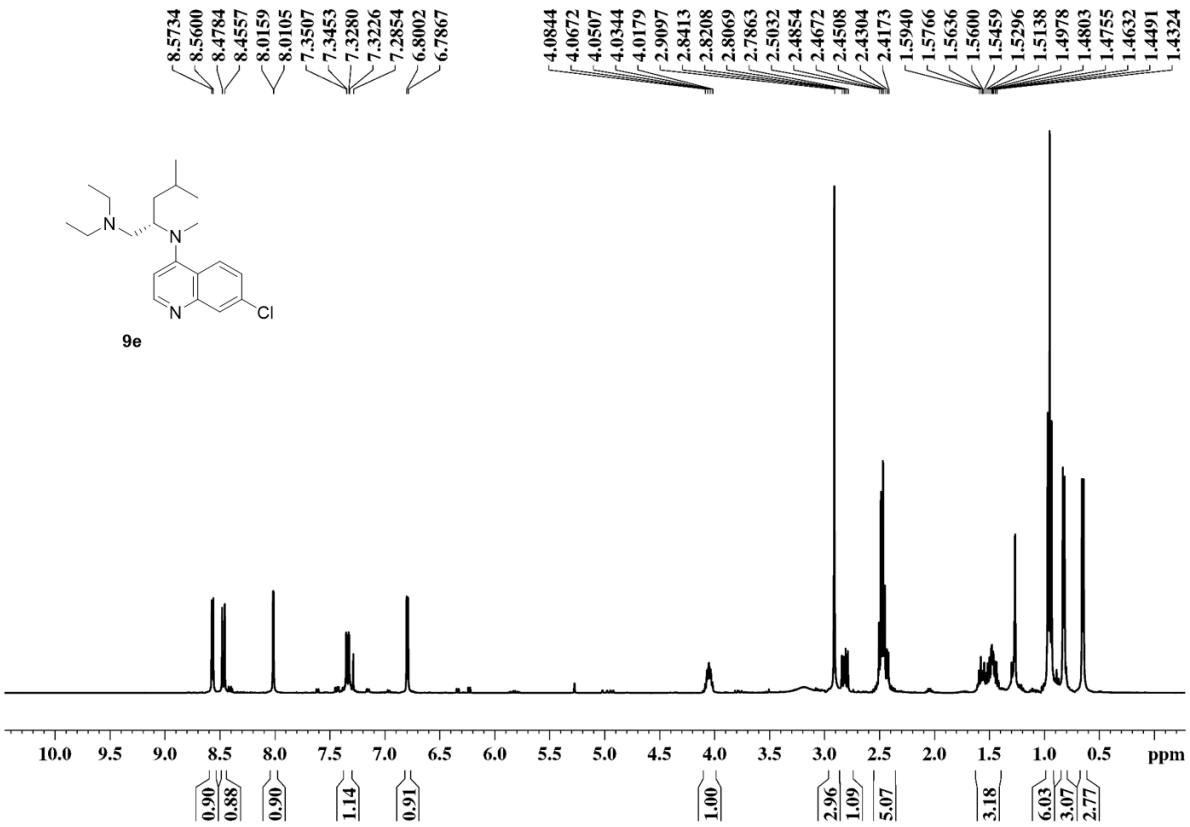


Figure S23: ^{1}H NMR spectrum of compound **9e** (400MHz, $CDCl_3$ 300 K)

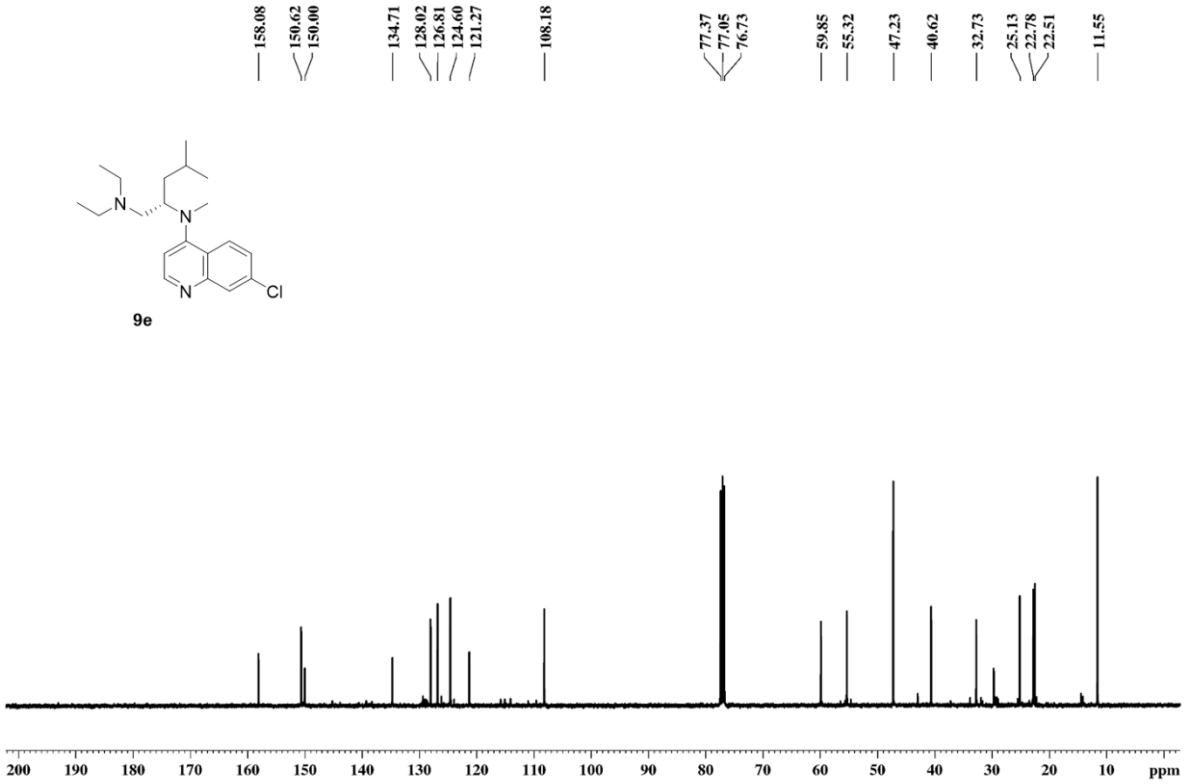


Figure S24: ^{13}C NMR spectrum of compound **9e** (100MHz, $CDCl_3$ 300 K)

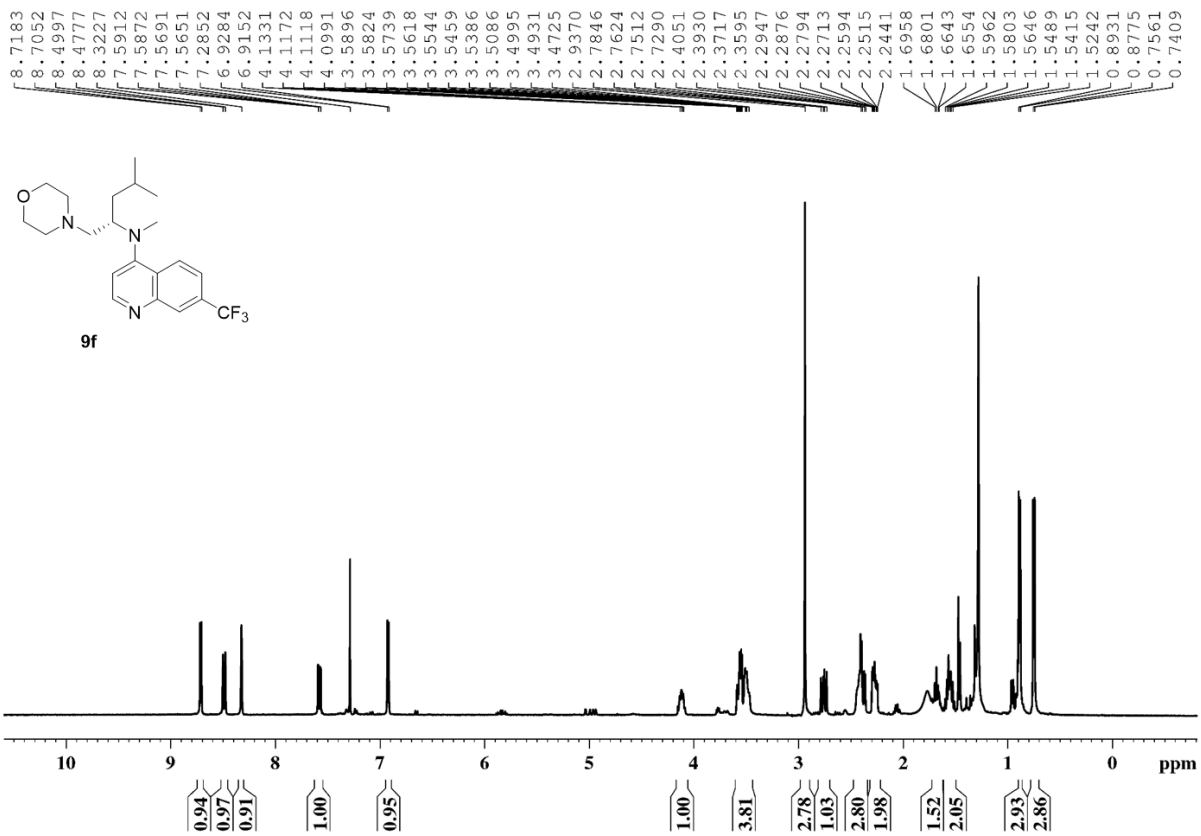


Figure S25: ^{1}H NMR spectrum of compound **9f** (400MHz, $CDCl_3$ 300 K)

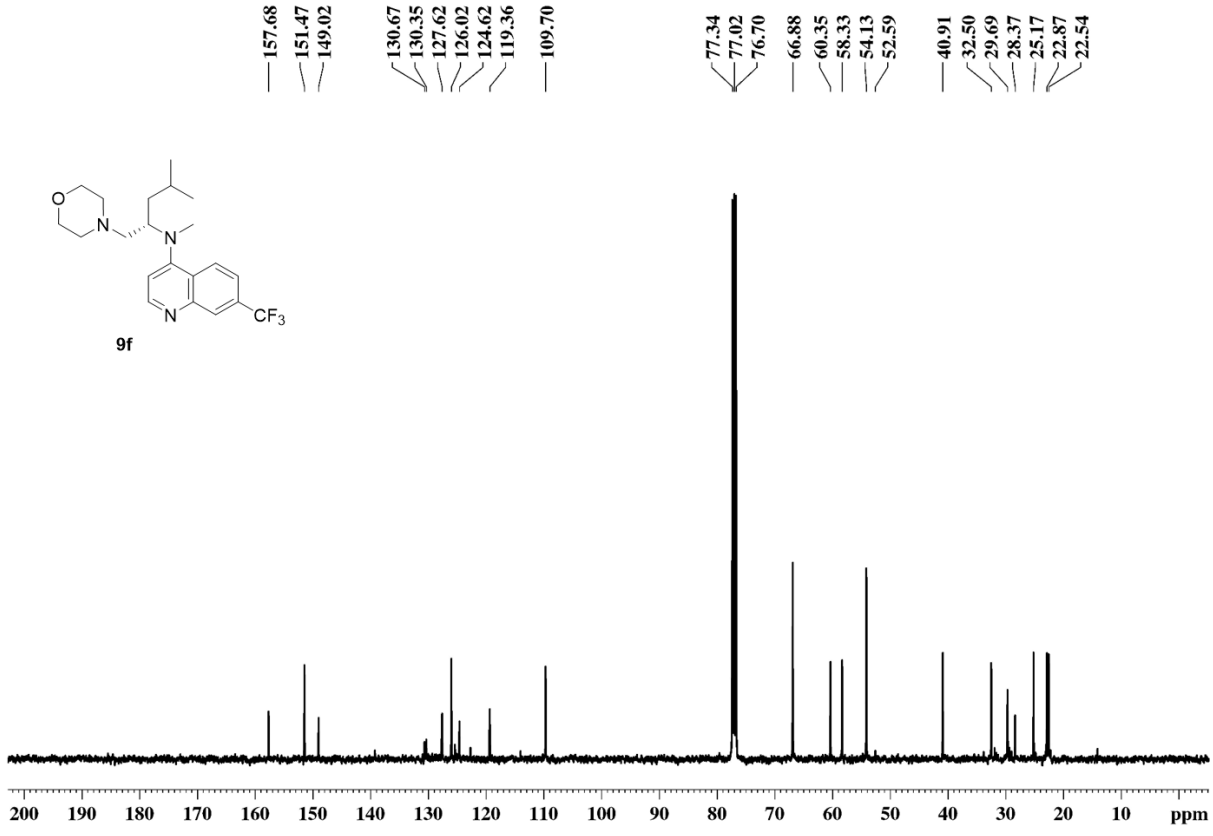


Figure S26: ^{13}C NMR spectrum of compound **9f** (100MHz, $CDCl_3$ 300 K)

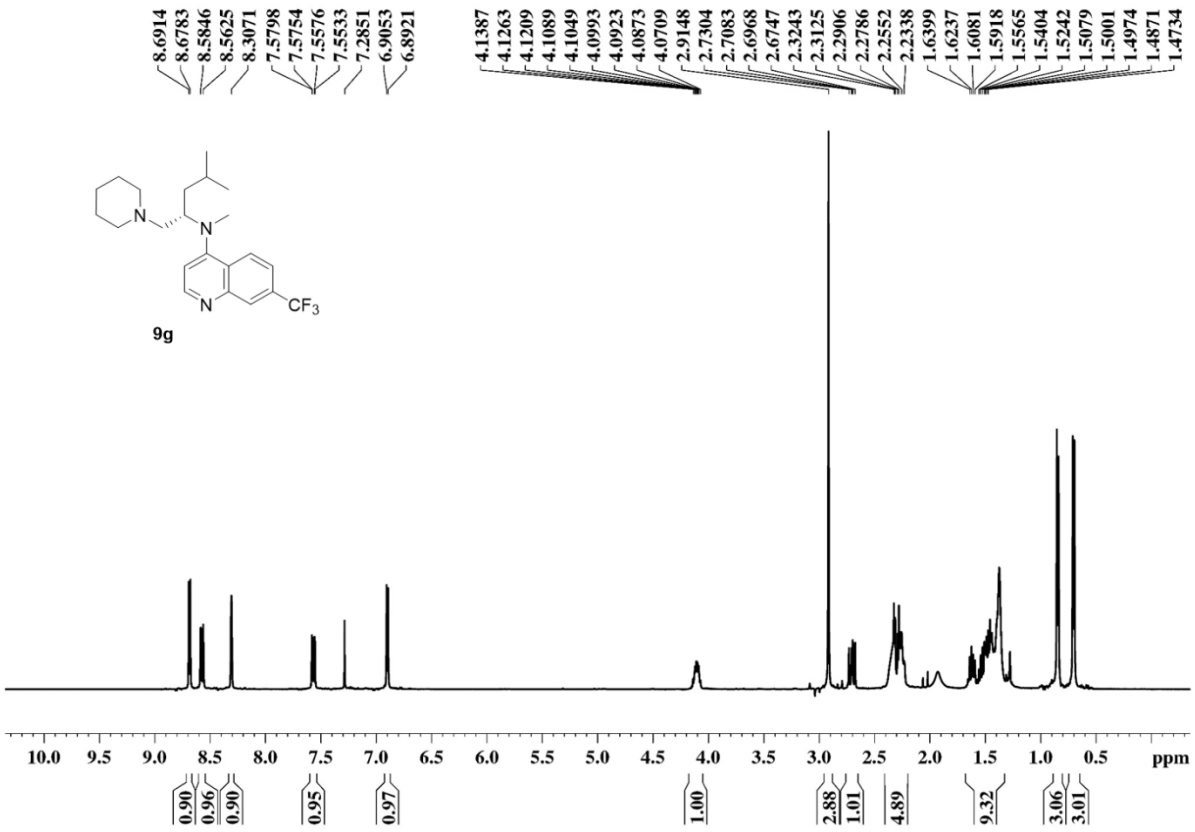


Figure S27: ^{1}H NMR spectrum of compound **9g** (400MHz, $CDCl_3$ 300 K)

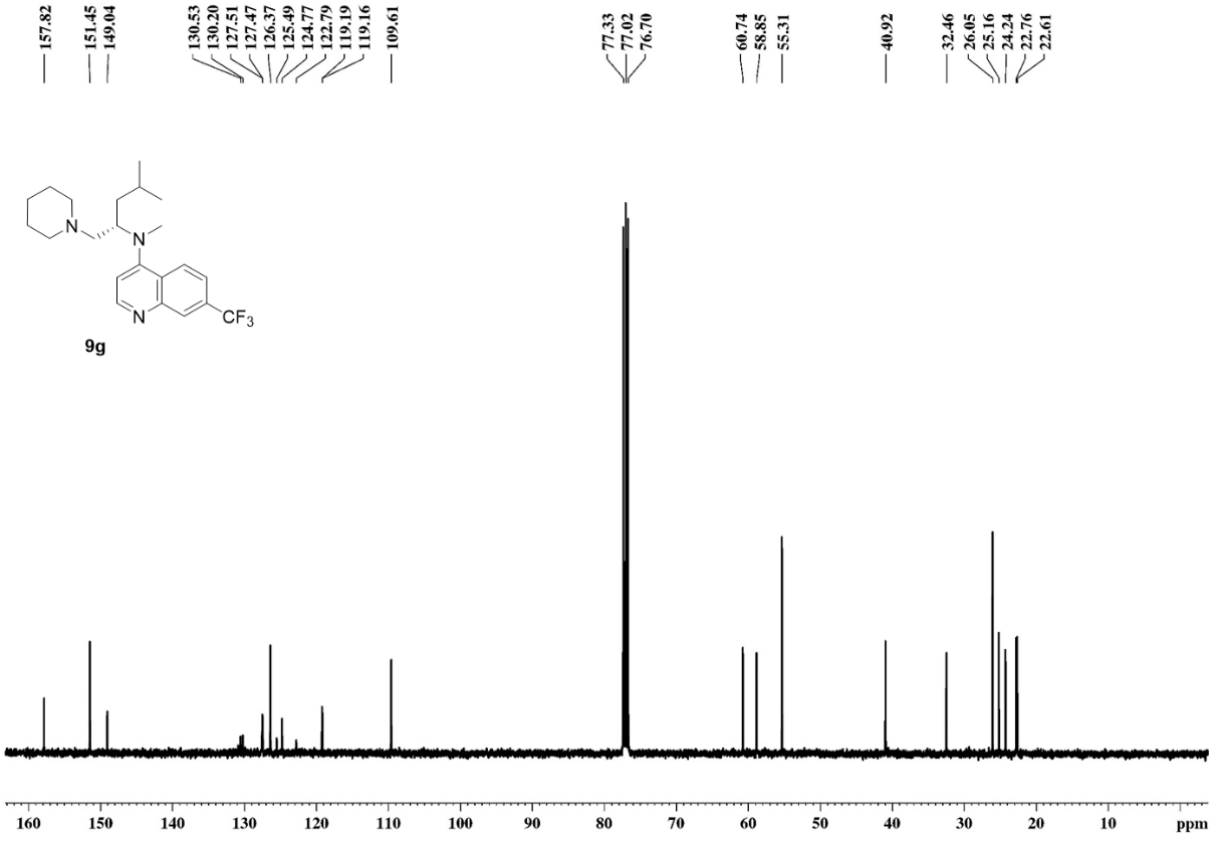


Figure S28: ^{13}C NMR spectrum of compound **9g** (100MHz, $CDCl_3$ 300 K)

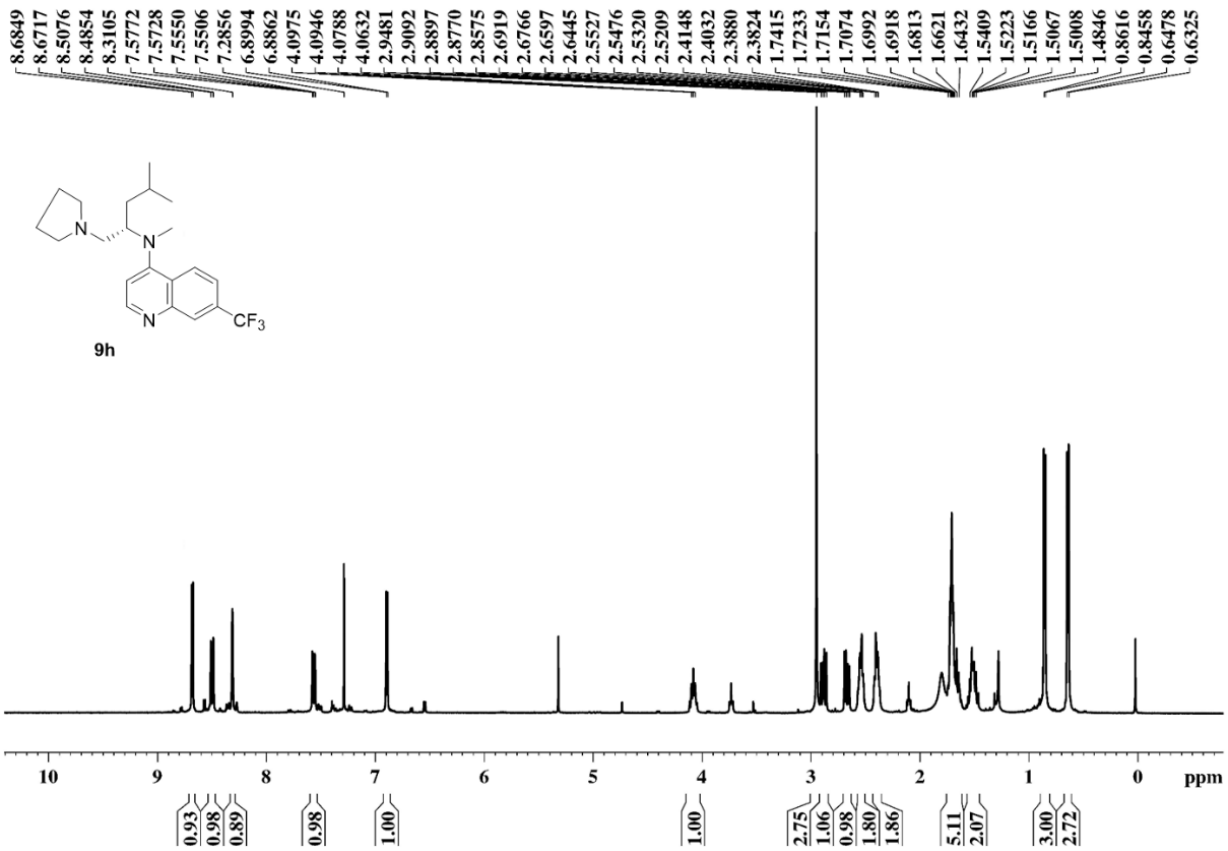


Figure S29: ^{1}H NMR spectrum of compound **9h** (400MHz, $CDCl_3$ 300 K)

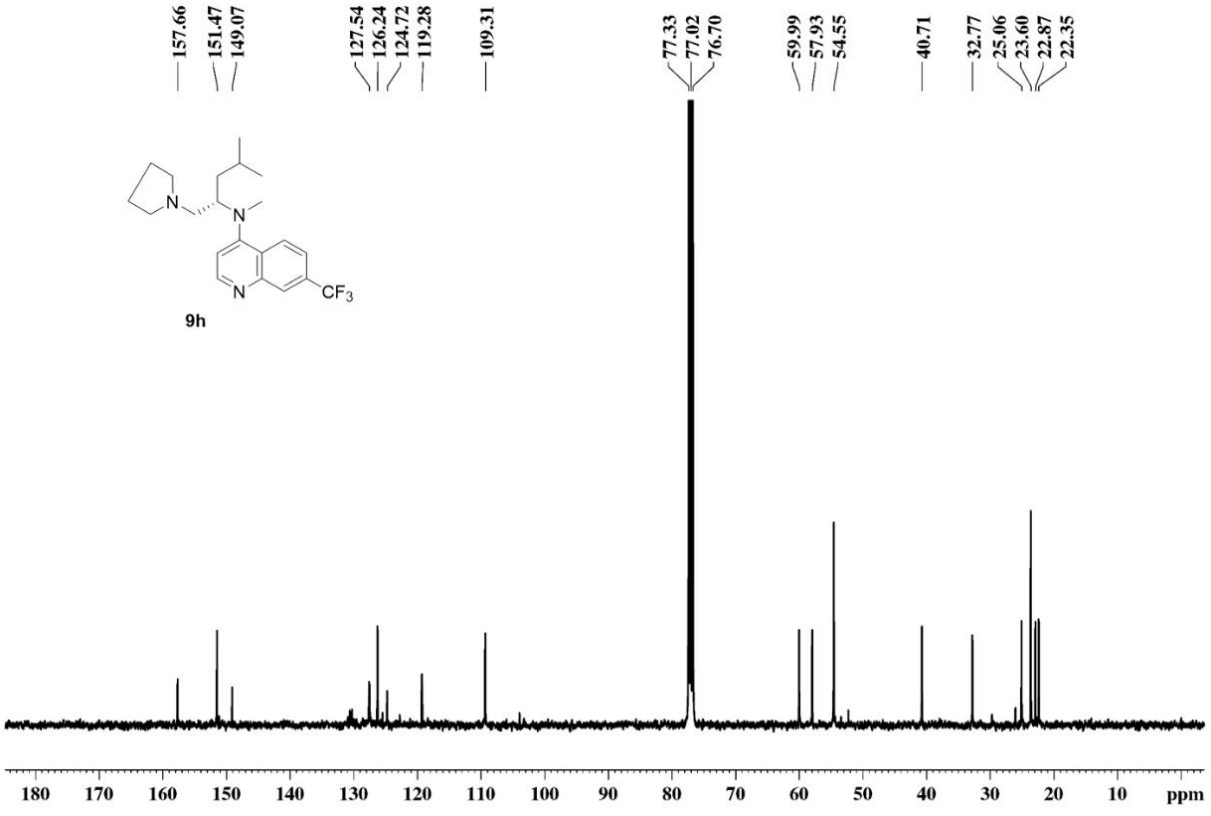


Figure S30: ^{13}C NMR spectrum of compound **9h** (100MHz, $CDCl_3$ 300 K)

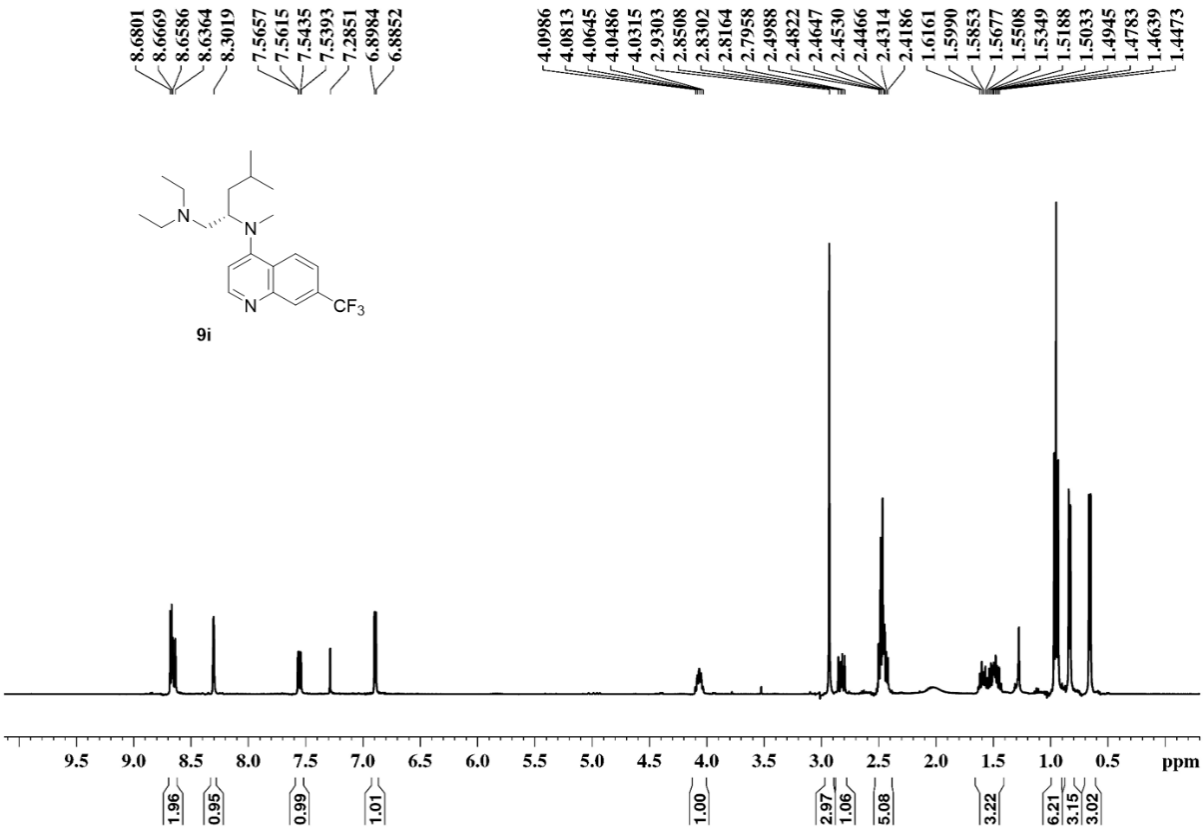


Figure S31: ^{1}H NMR spectrum of compound **9i** (400MHz, $CDCl_3$ 300 K)

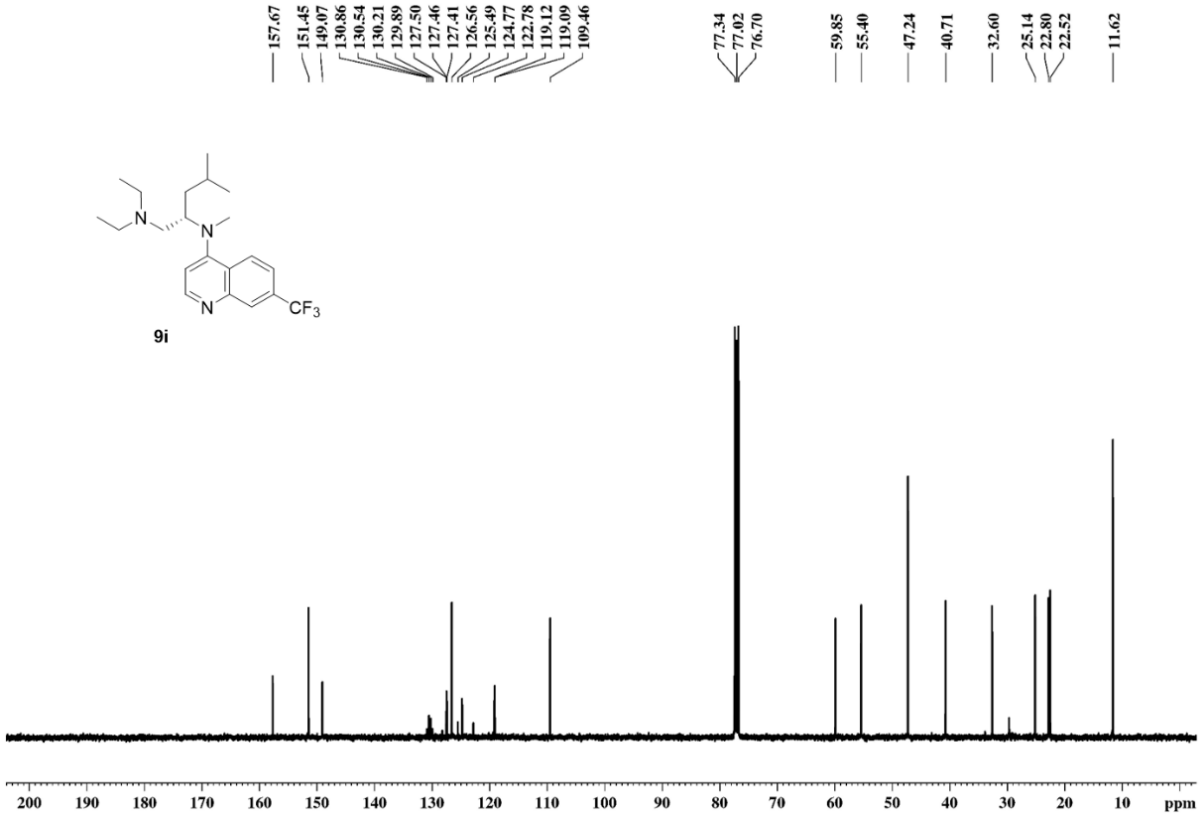


Figure S32: ^{13}C NMR spectrum of compound **9i** (100MHz, $CDCl_3$ 300 K)

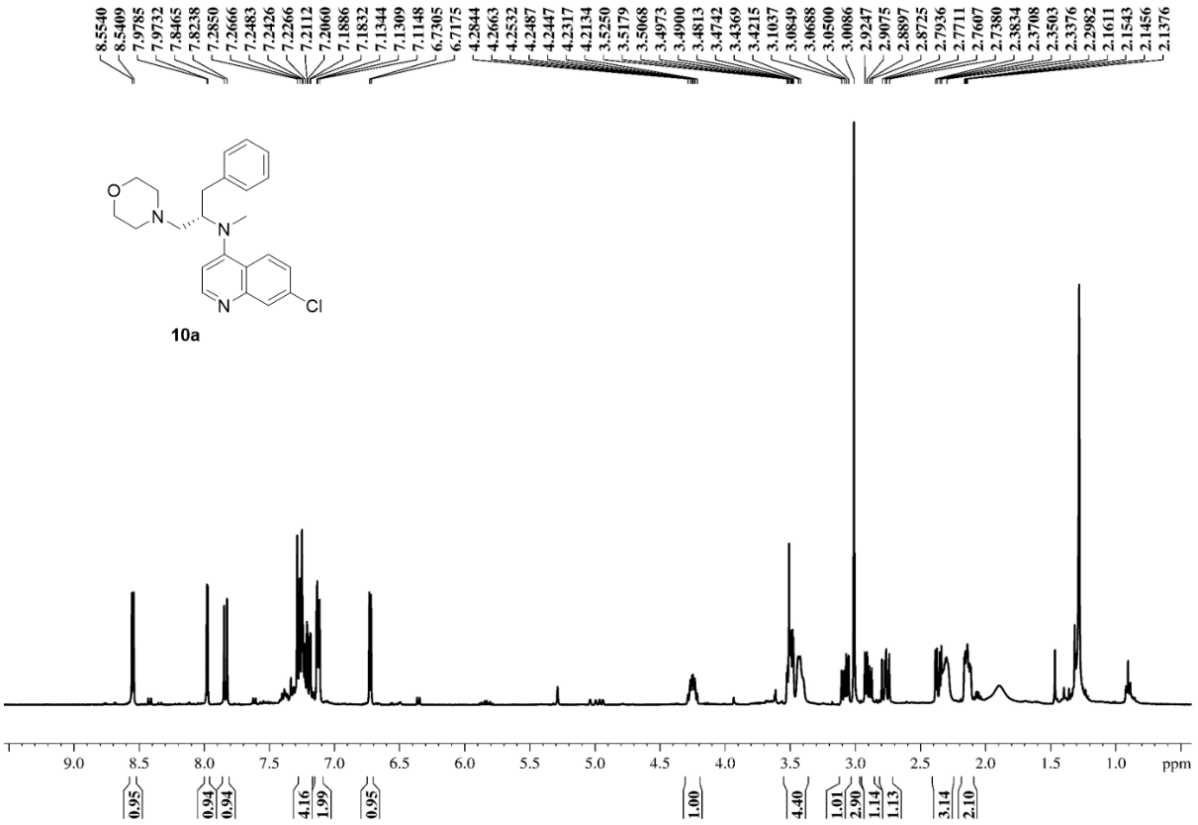


Figure S33: ^{1}H NMR spectrum of compound **10a** (400MHz, $CDCl_3$ 300 K)

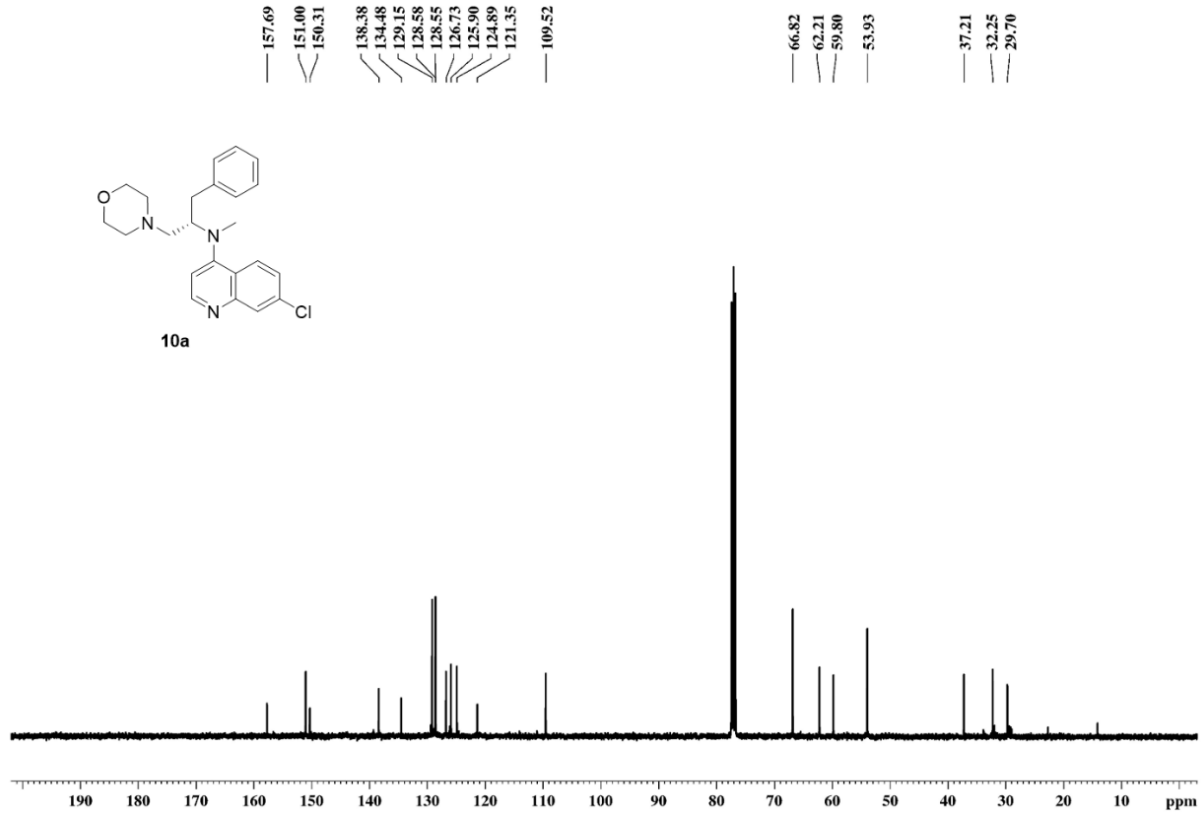


Figure S34: ^{13}C NMR spectrum of compound **10a** (100MHz, $CDCl_3$ 300 K)

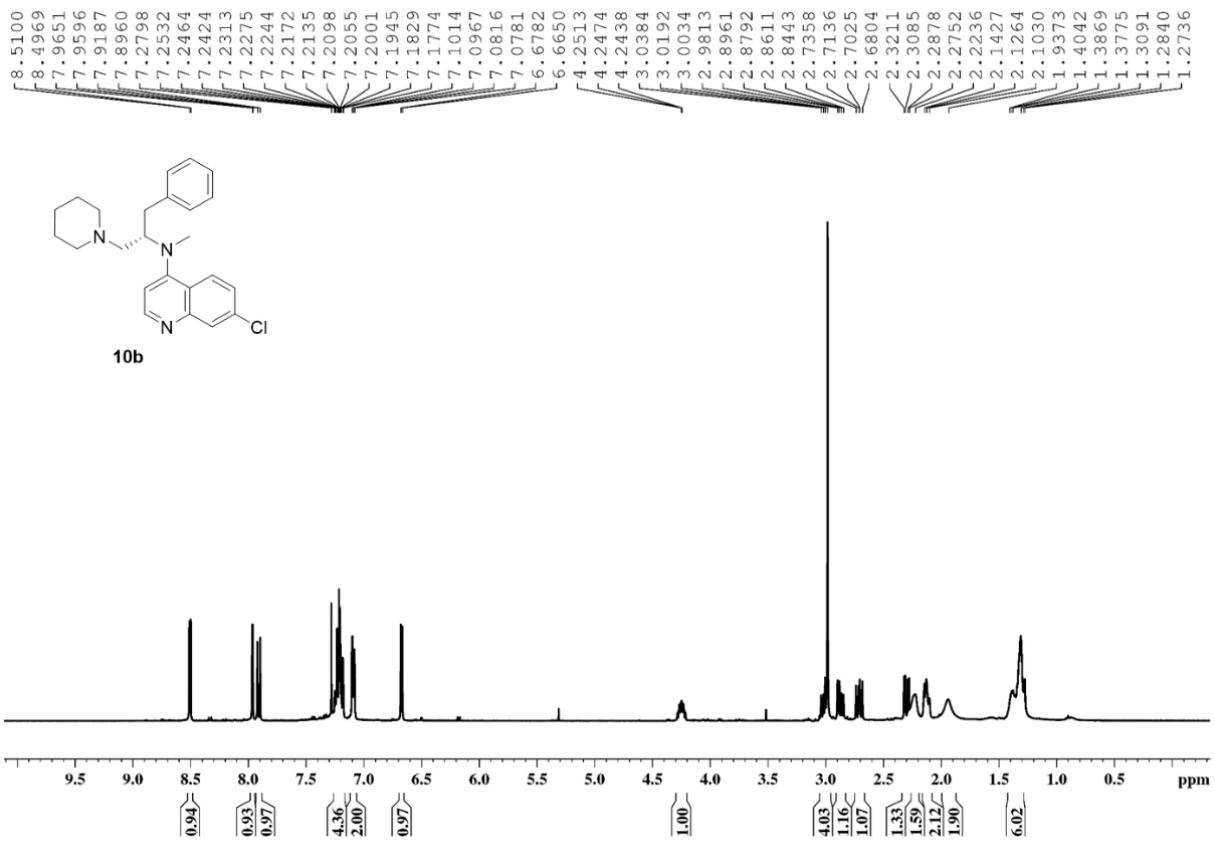


Figure S35: ^{1}H NMR spectrum of compound **10b** (400MHz, $CDCl_3$ 300 K)

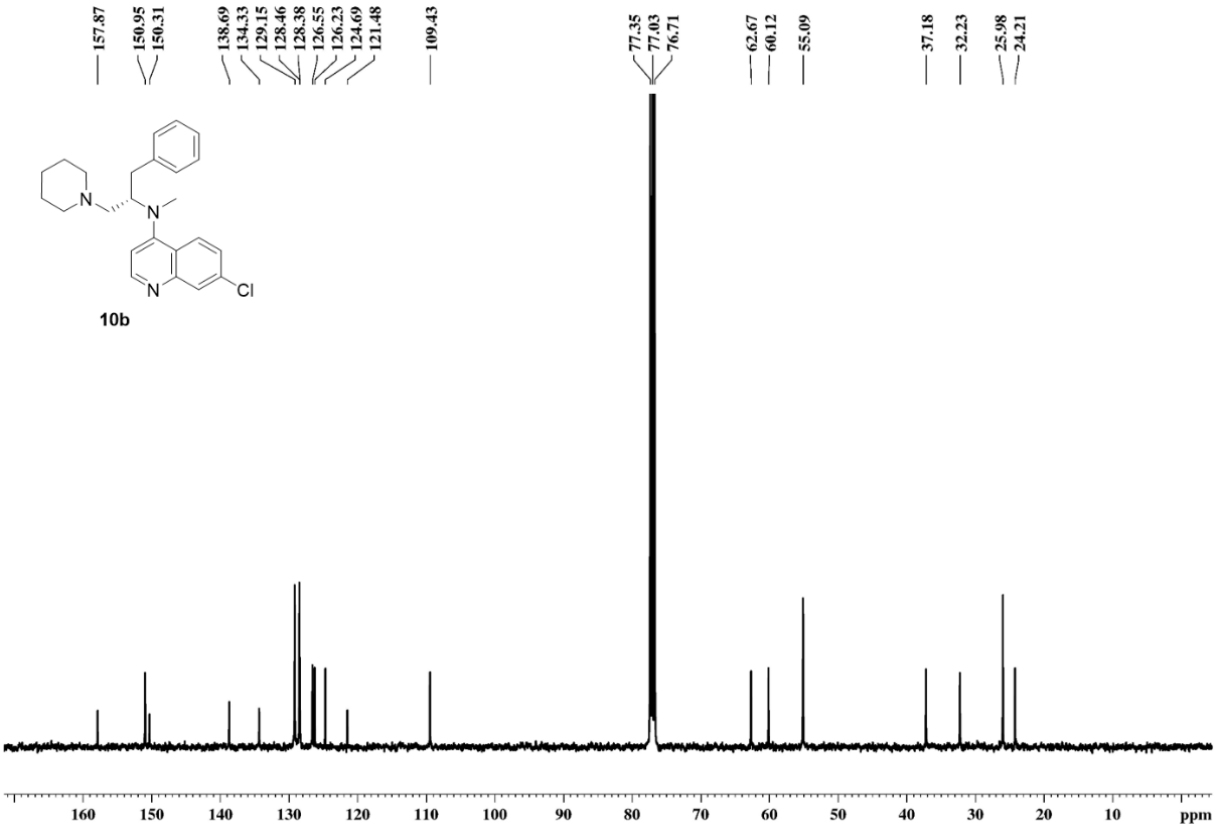


Figure S36: ^{13}C NMR spectrum of compound **10b** (100MHz, $CDCl_3$ 300 K)

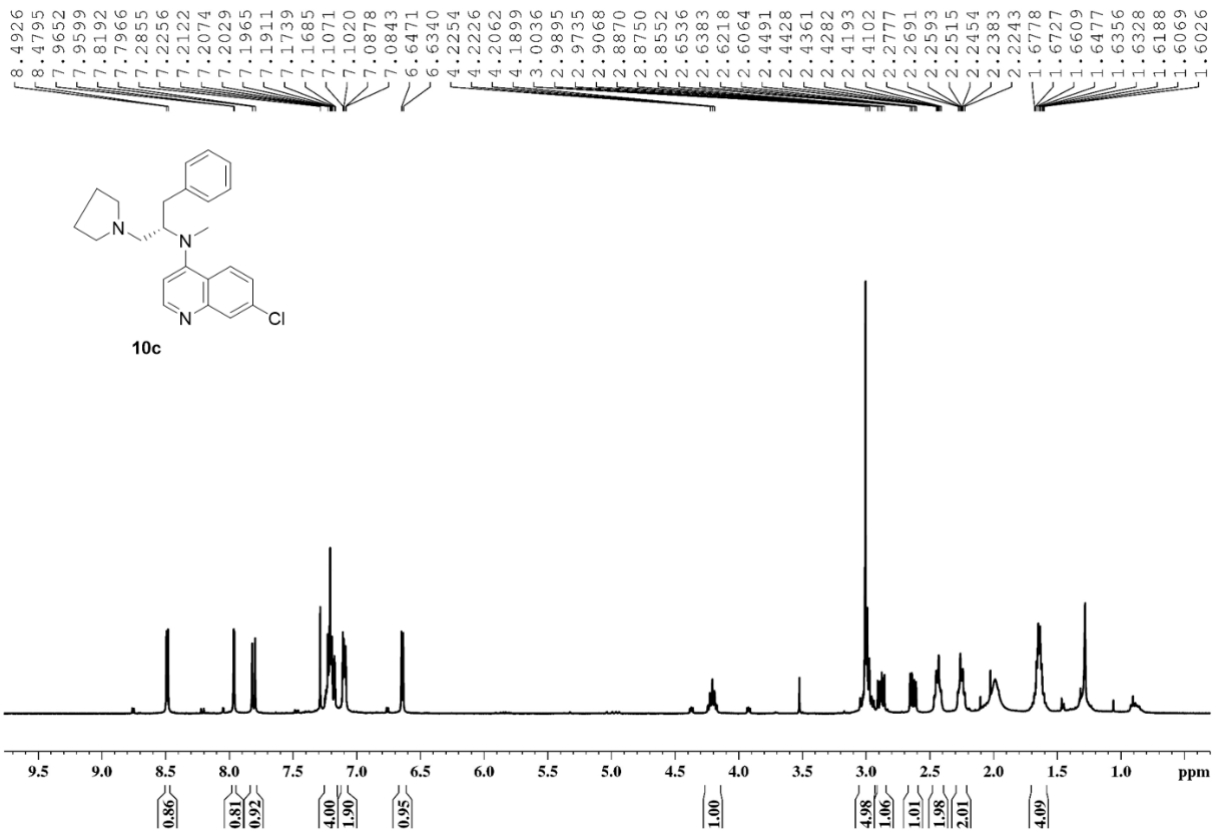


Figure S37: ^{1}H NMR spectrum of compound **10c** (400MHz, $CDCl_3$ 300 K)

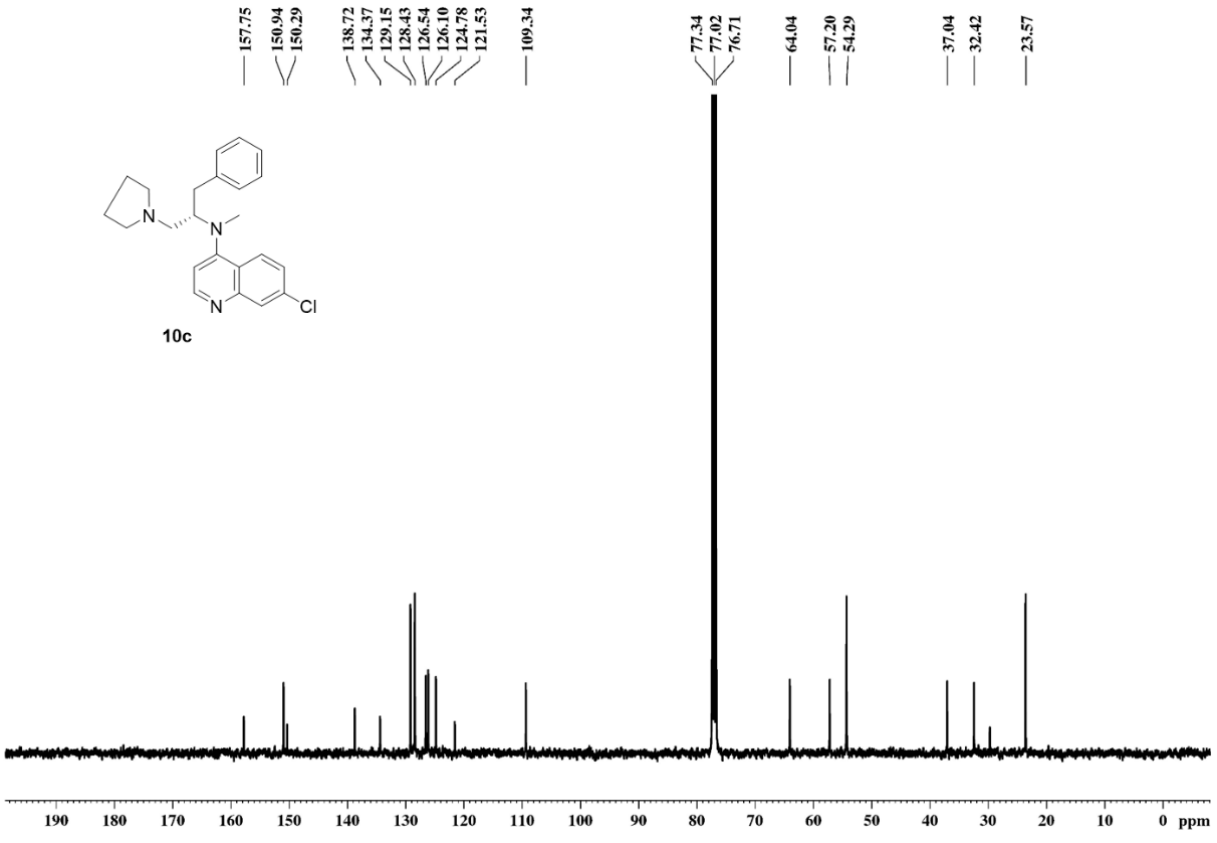


Figure S38: ^{13}C NMR spectrum of compound **10c** (100MHz, $CDCl_3$ 300 K)

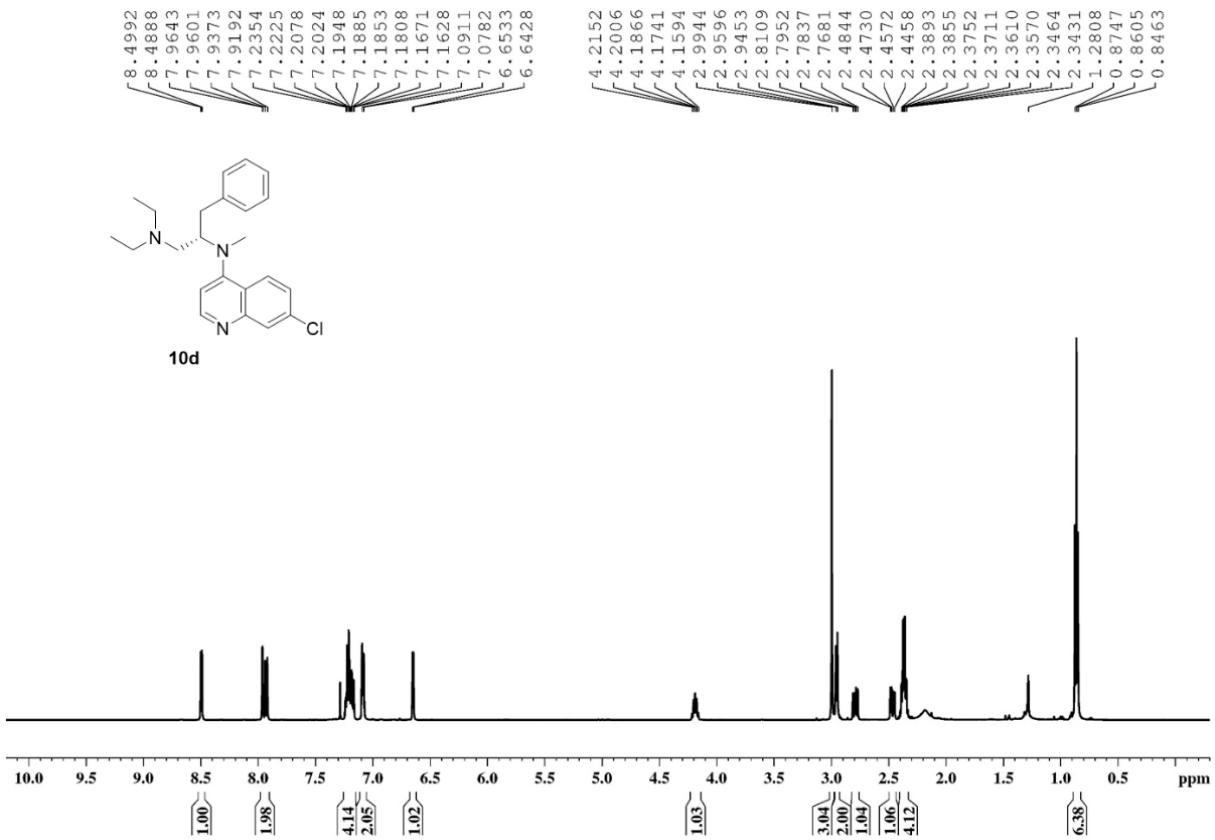


Figure S39: ^{1}H NMR spectrum of compound **10d** (500MHz, $CDCl_3$ 300 K)

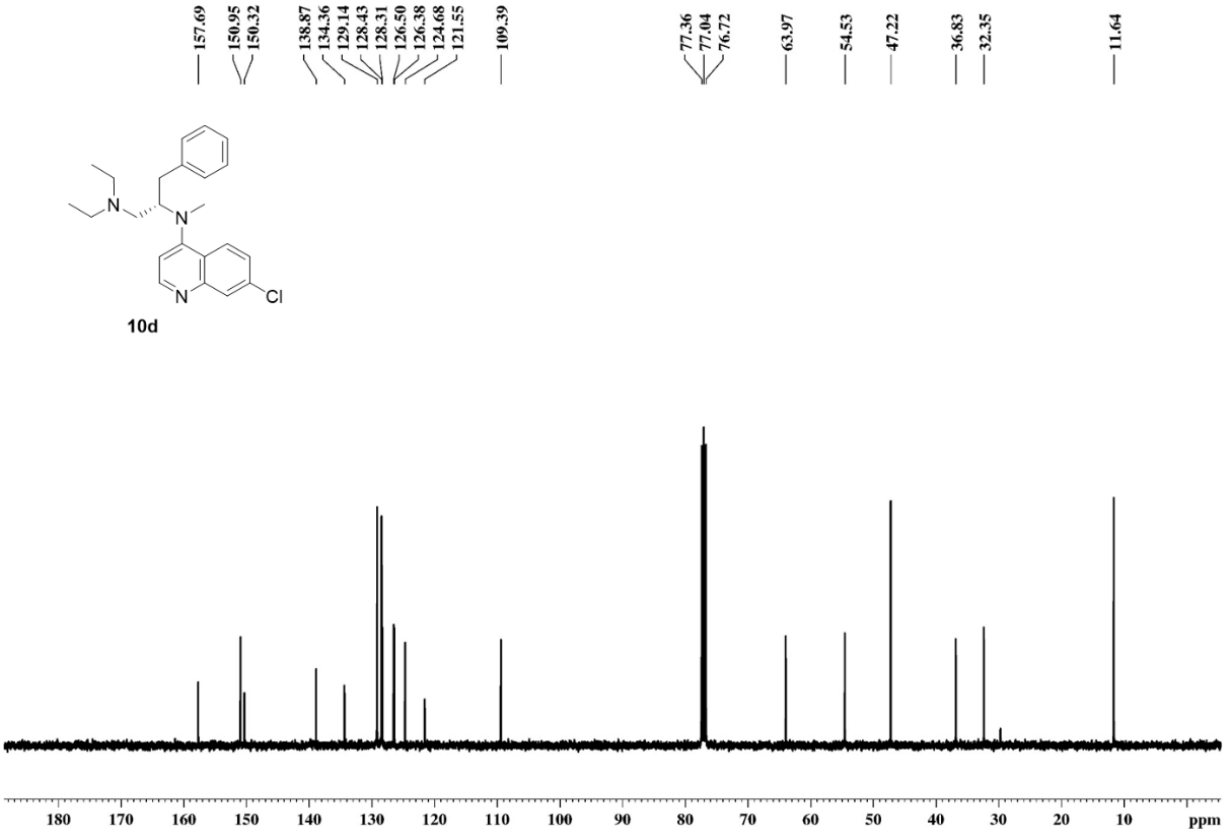


Figure S40: ^{13}C NMR spectrum of compound **10d** (100MHz, $CDCl_3$ 300 K)

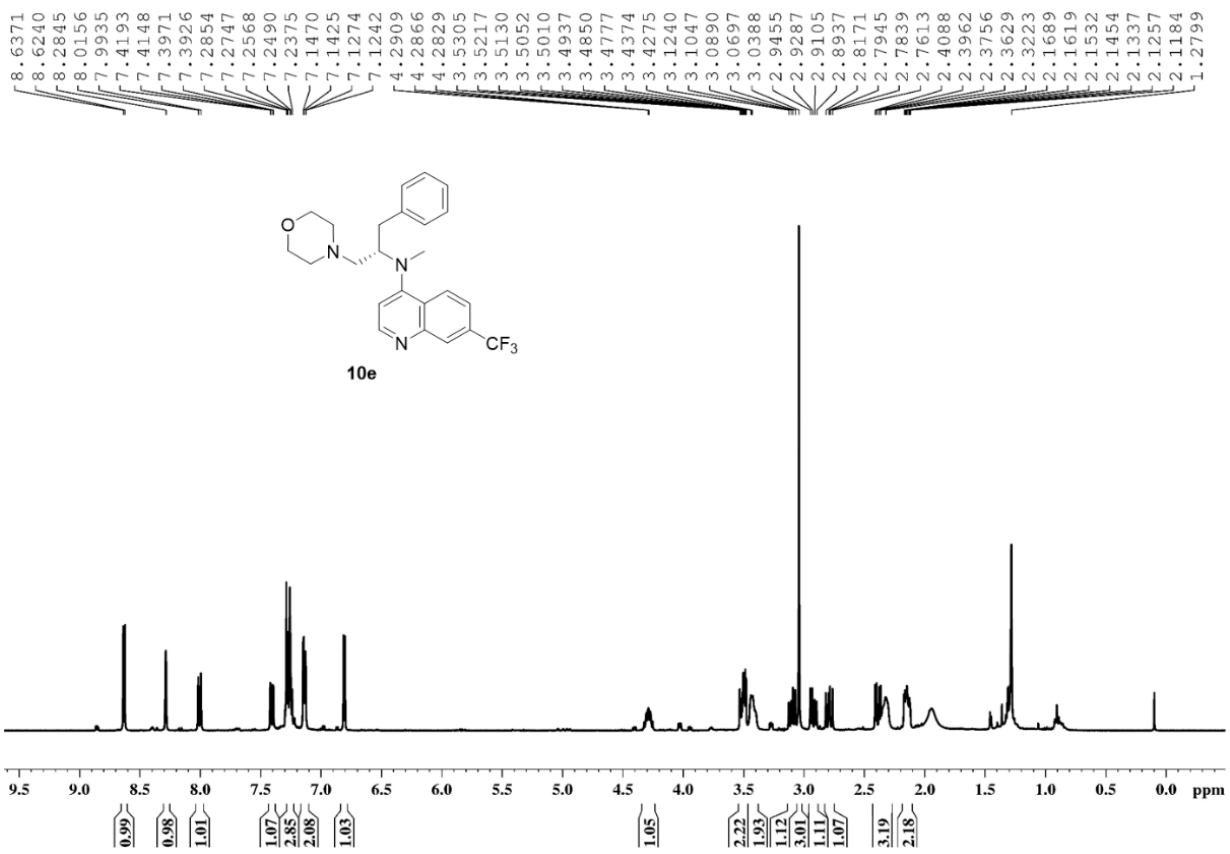


Figure S41: ^{1}H NMR spectrum of compound **10e** (400MHz, $CDCl_3$ 300 K)

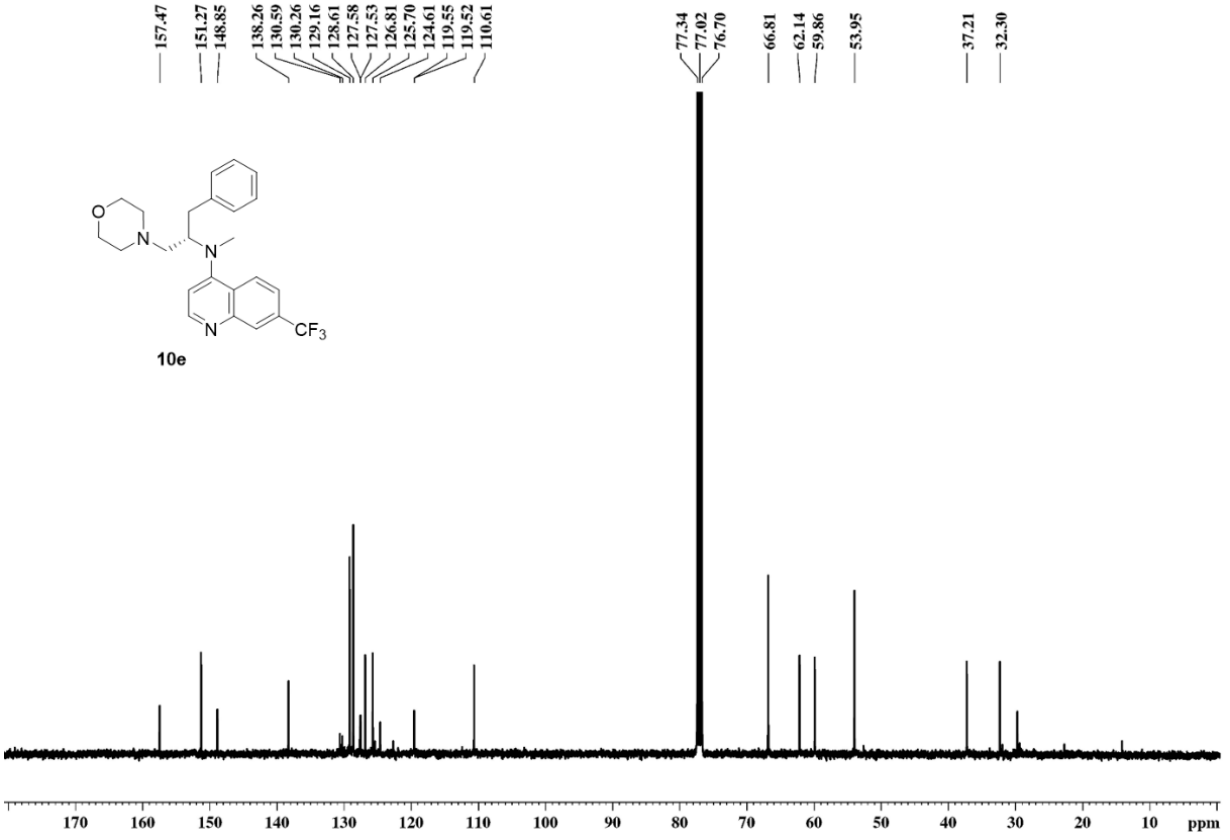


Figure S42: ^{13}C NMR spectrum of compound **10e** (100MHz, $CDCl_3$ 300 K)

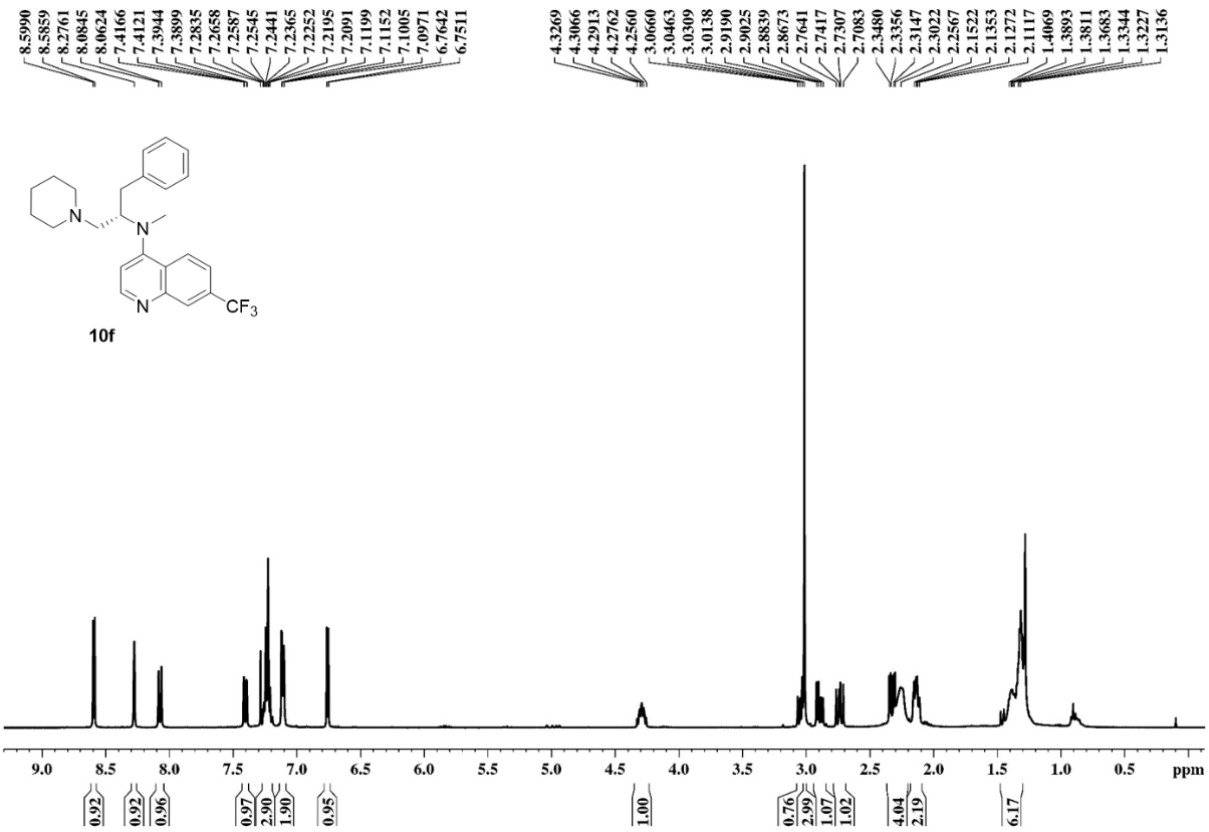


Figure S43: ^{1}H NMR spectrum of compound **10f** (400MHz, $CDCl_3$ 300 K)

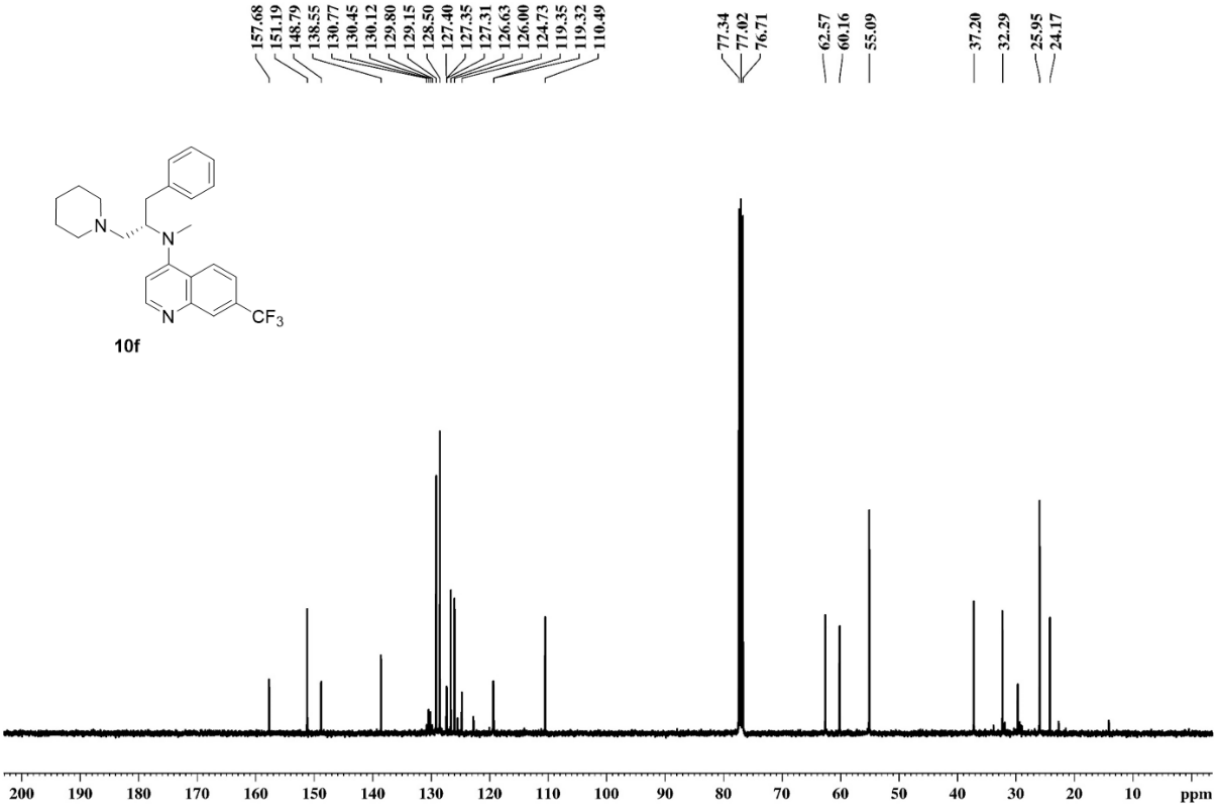


Figure S44: ^{13}C NMR spectrum of compound **10f** (100MHz, $CDCl_3$ 300 K)

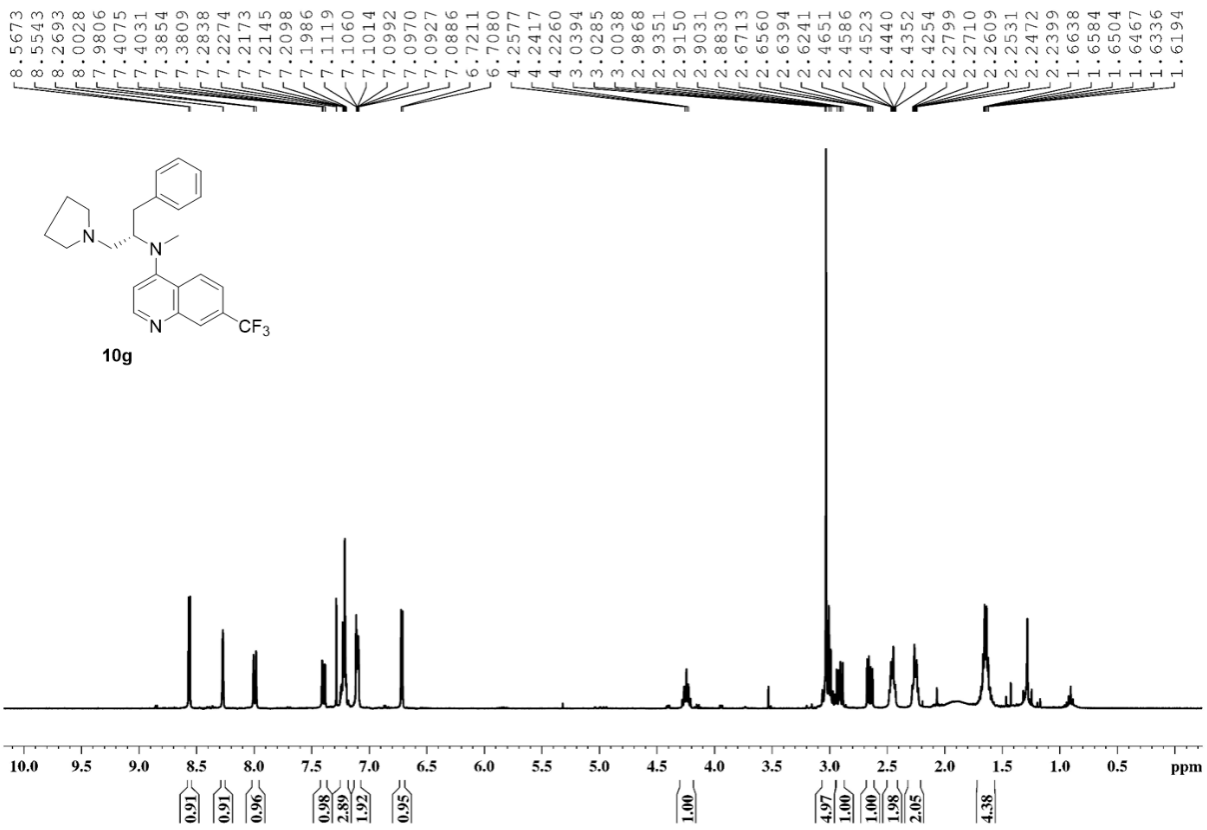


Figure S45: ^{1}H NMR spectrum of compound **10g** (400MHz, $CDCl_3$ 300 K)

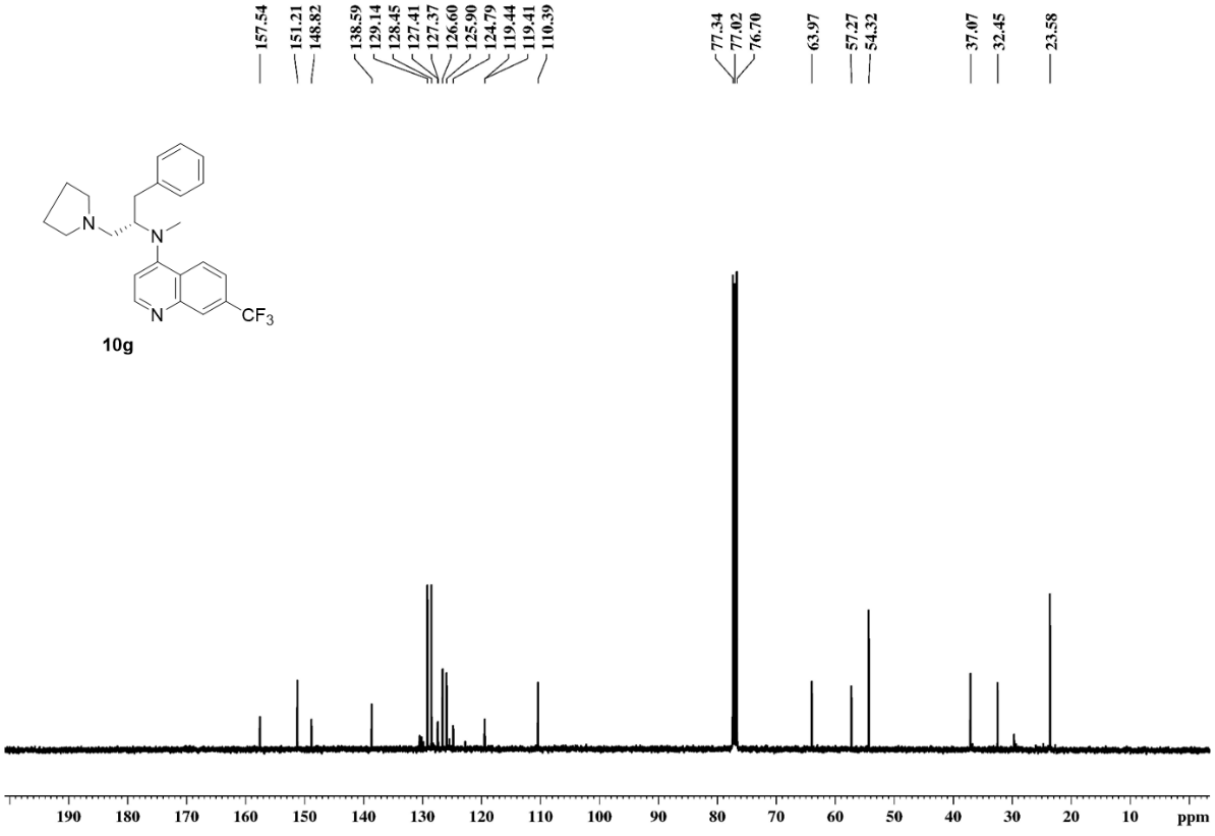


Figure S46: ^{13}C NMR spectrum of compound **10g** (100MHz, $CDCl_3$ 300 K)

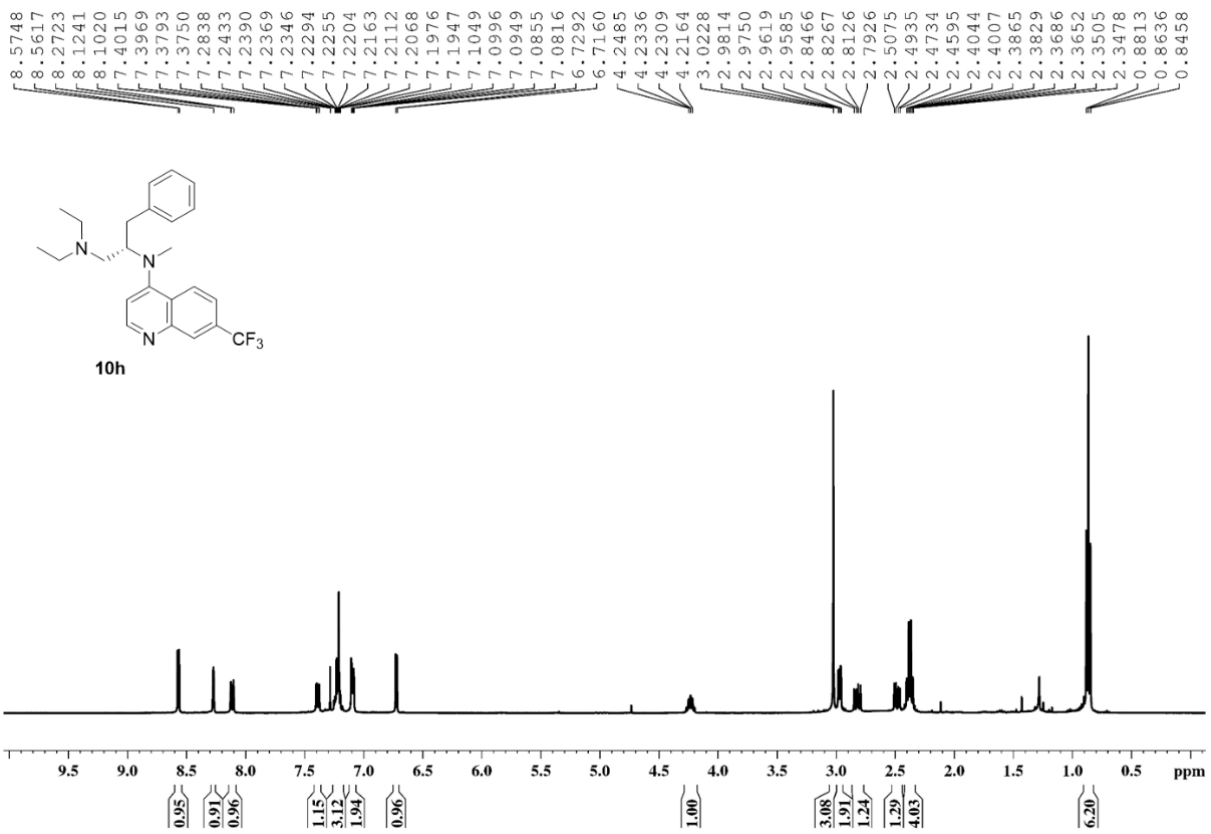


Figure S47: ^{1}H NMR spectrum of compound **10h** (400MHz, $CDCl_3$ 300 K)

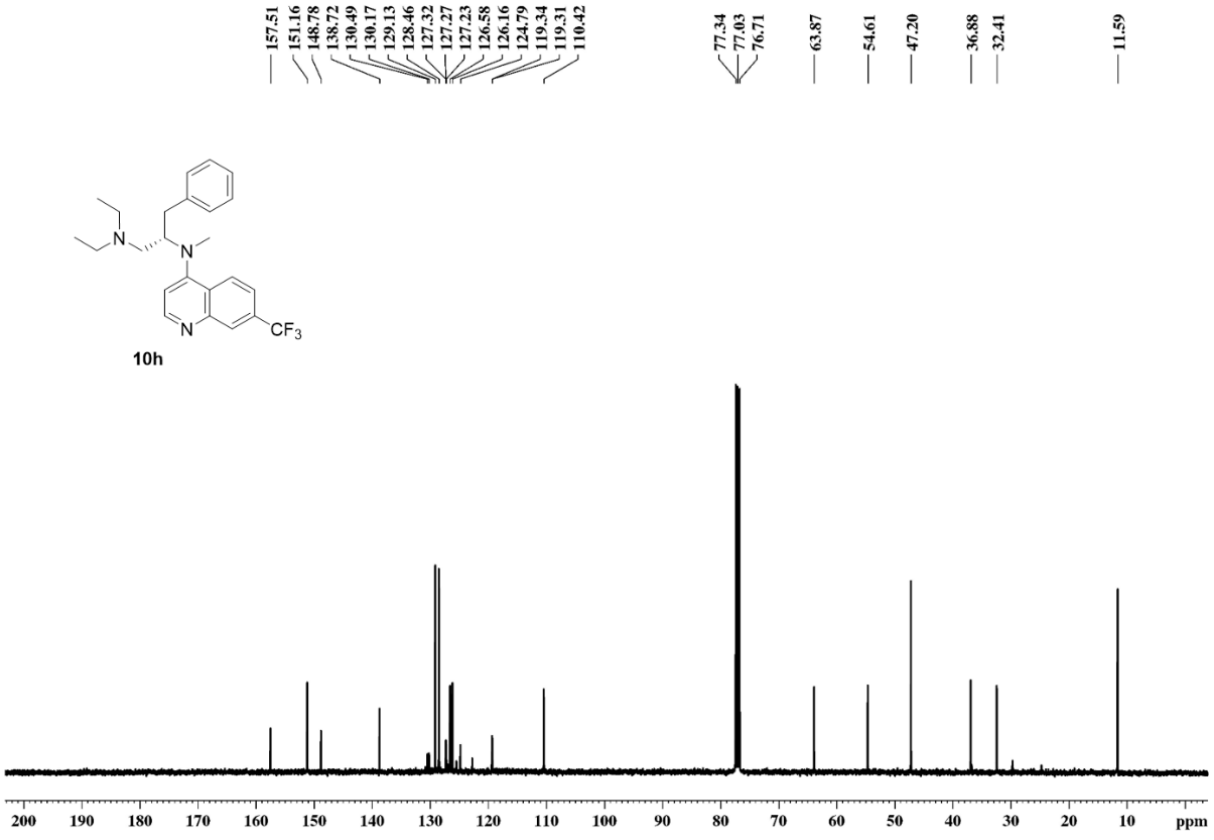


Figure S48: ^{13}C NMR spectrum of compound **10h** (100MHz, $CDCl_3$ 300 K)

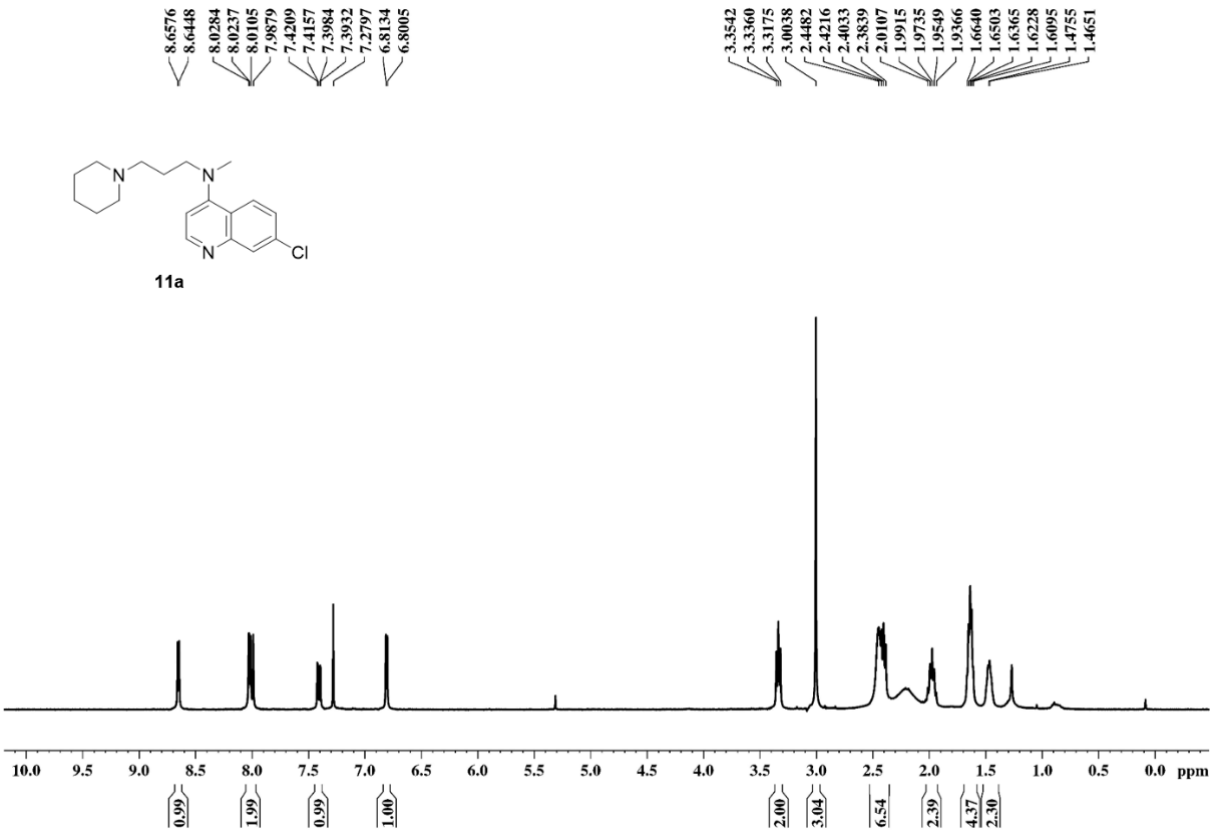


Figure S49: ^{1}H NMR spectrum of compound **11a** (400MHz, $CDCl_3$ 300 K)

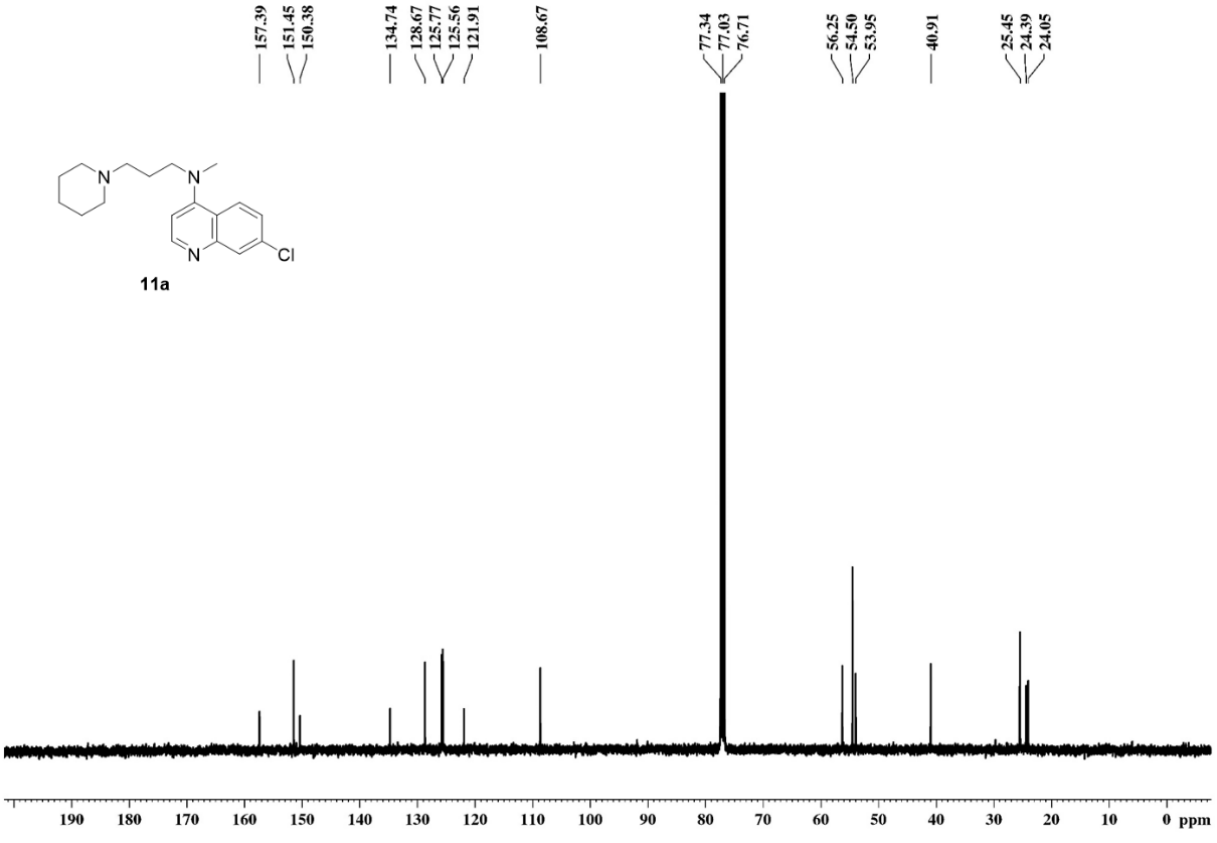


Figure S50: ^{13}C NMR spectrum of compound **11a** (100MHz, $CDCl_3$ 300 K)

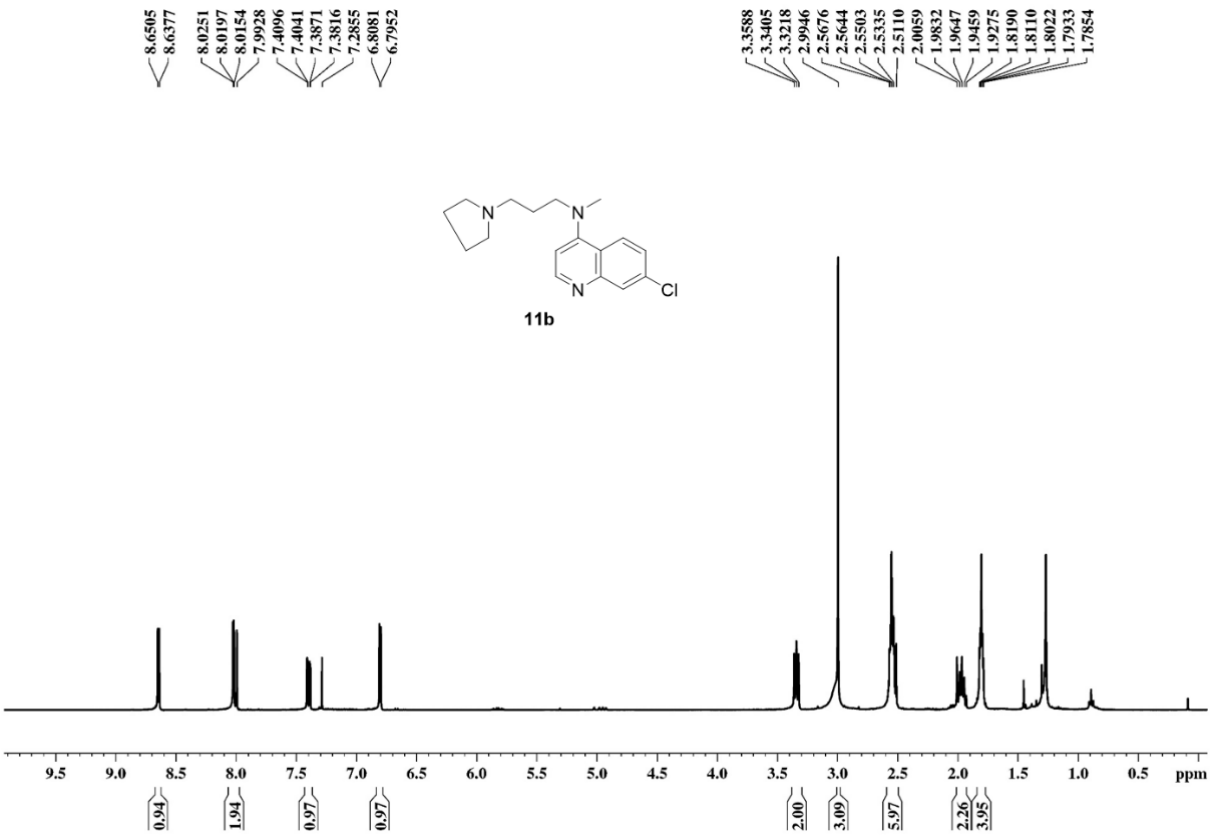


Figure S51: ^{1}H NMR spectrum of compound **11b** (400MHz, $CDCl_3$ 300 K)

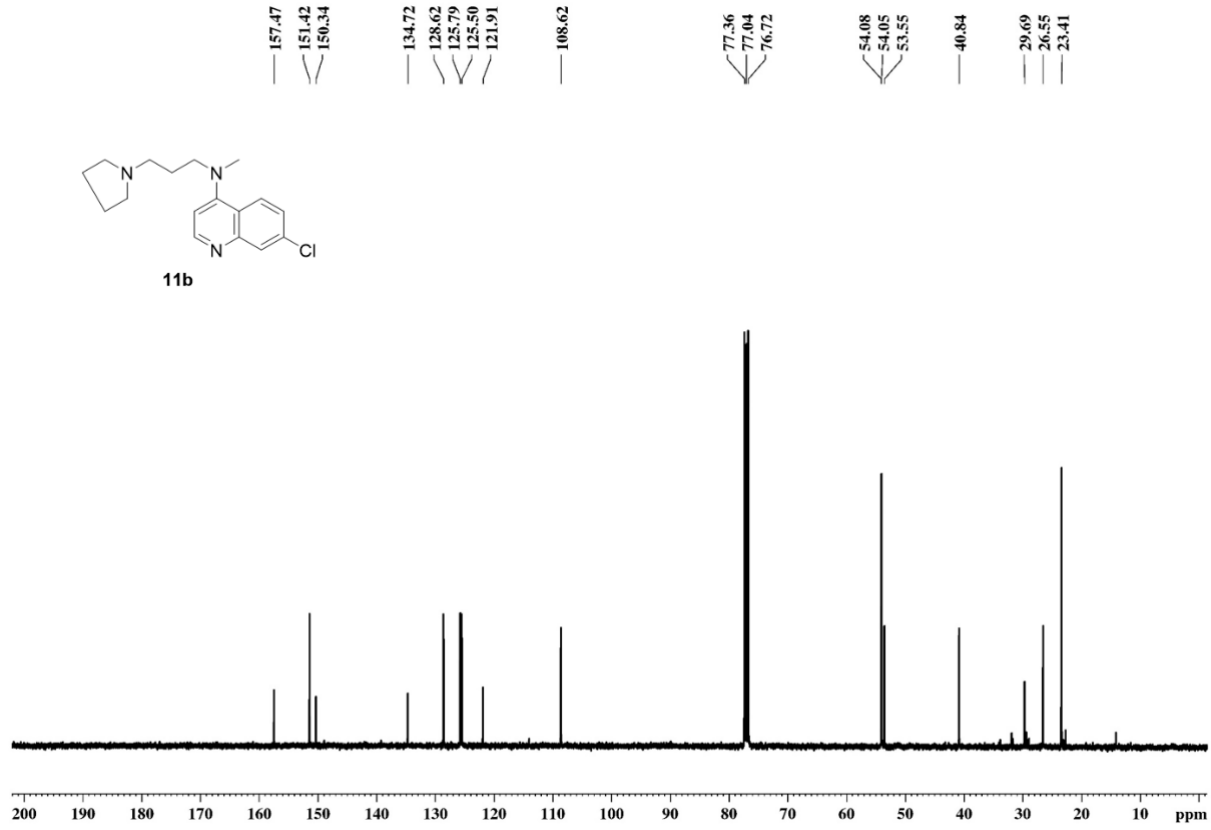


Figure S52: ^{13}C NMR spectrum of compound **11b** (100MHz, $CDCl_3$ 300 K)

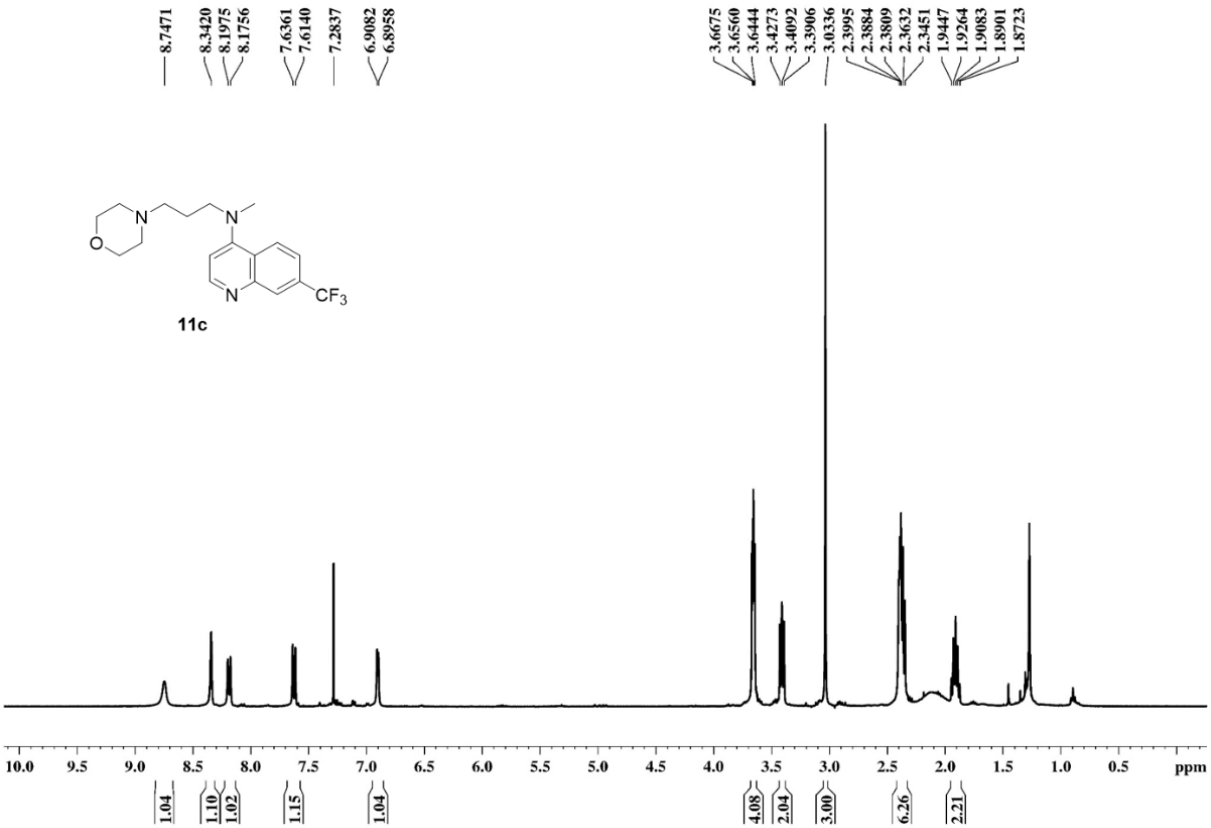


Figure S53: ^{13}C NMR spectrum of compound **11c** (400MHz, $CDCl_3$ 300 K)

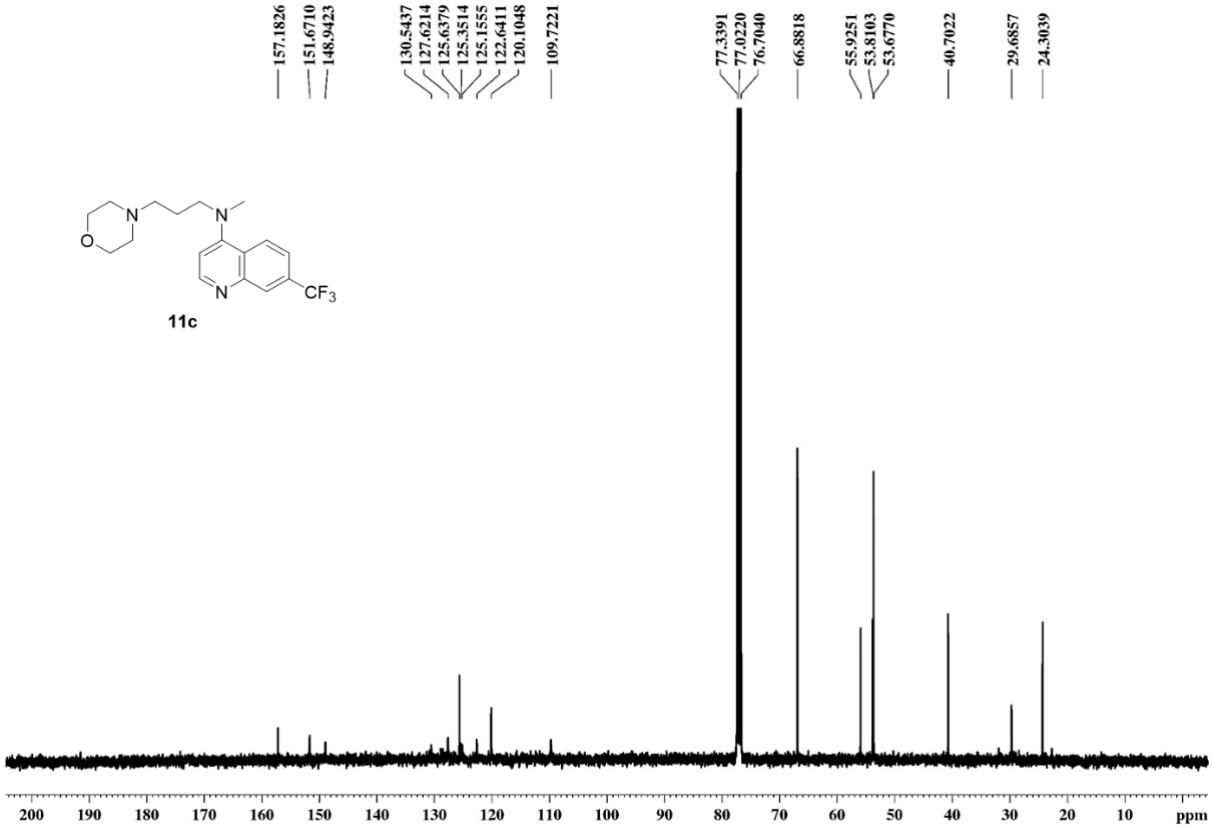


Figure S54: ^{13}C NMR spectrum of compound **11c** (100MHz, $CDCl_3$ 300 K)

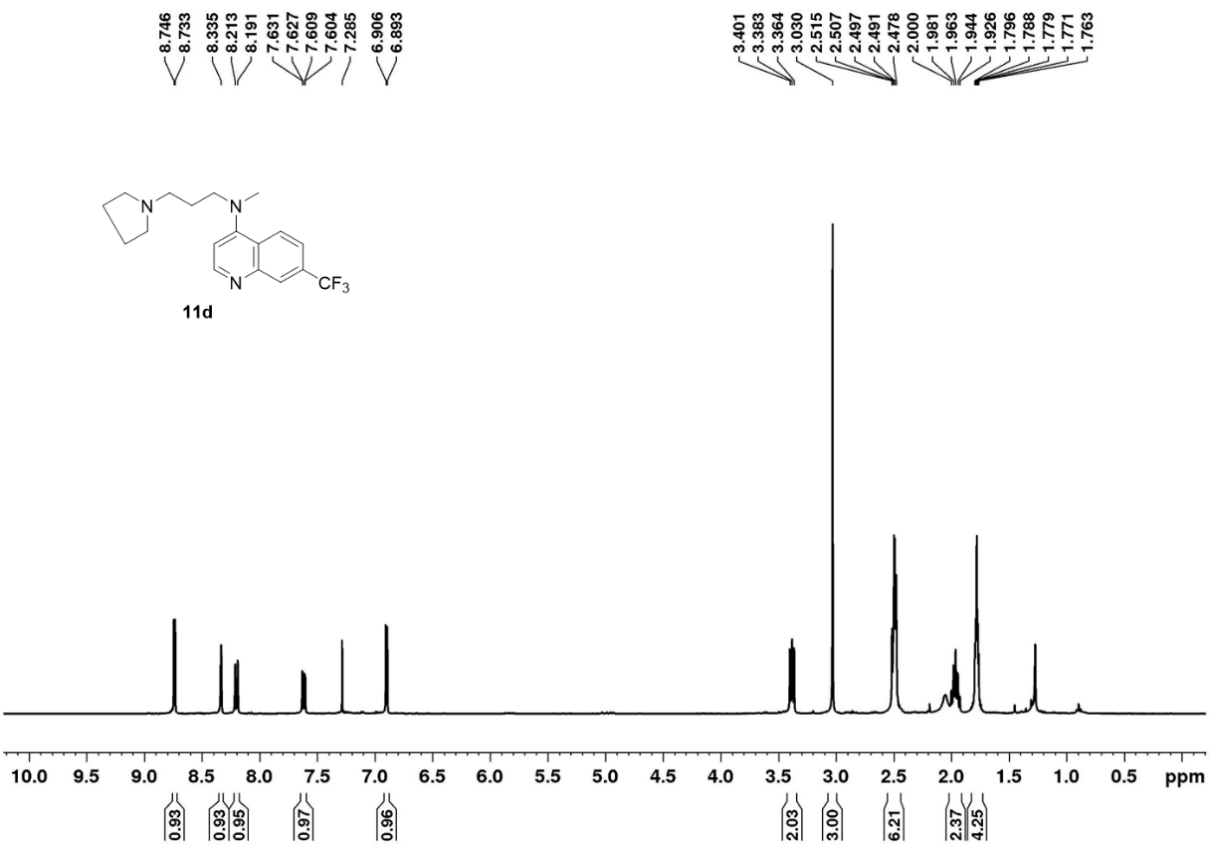


Figure S55: ^{1}H NMR spectrum of compound **11d** (400MHz, $CDCl_3$ 300 K)

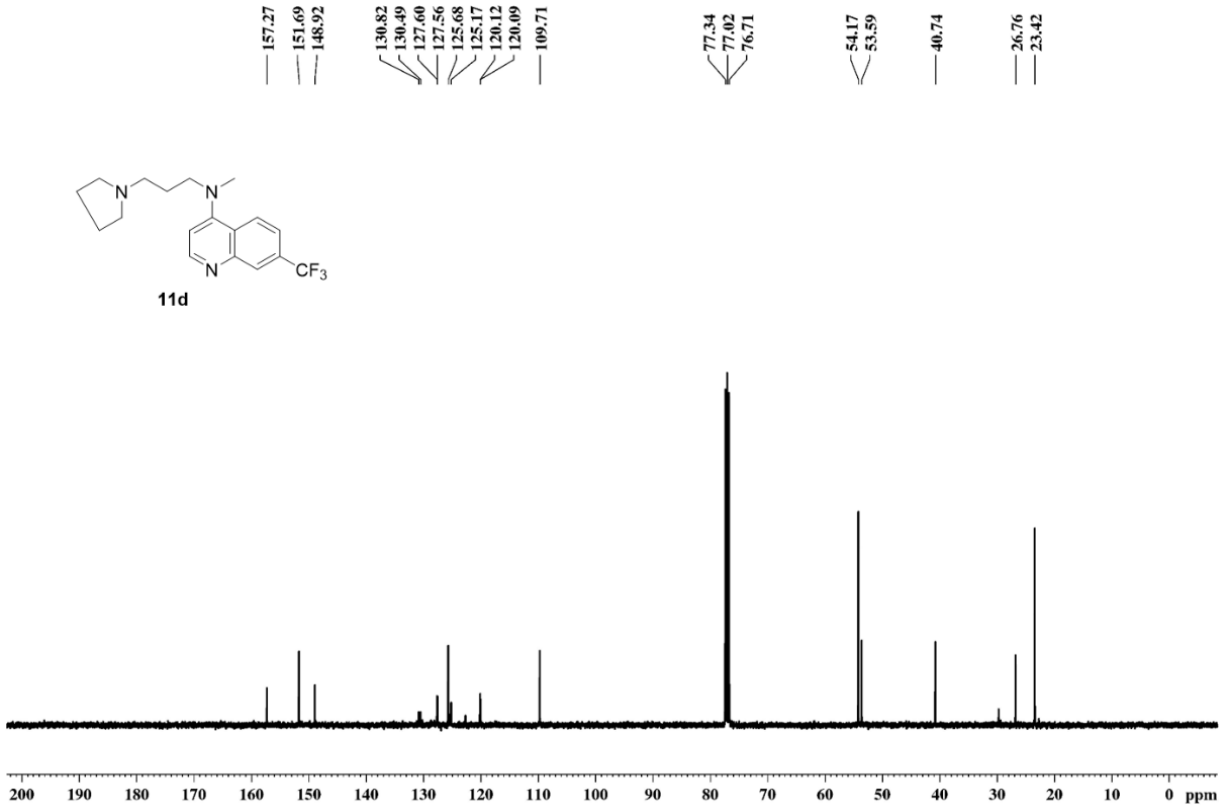


Figure S56: ^{13}C NMR spectrum of compound **11d** (100MHz, $CDCl_3$ 300 K)