Supporting Information

Antitubercular activity of novel 2-(quinoline-4-yloxy)acetamides with improved drug-like properties

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TABLE OF CONTENTS

1. **Experimental Section and Synthesis**
2. **^{1}H NMR and ^{13}C NMR spectra**
3. **Minimum Inhibitory Concentration**

4. **Cellular Viability Evaluation**
5. **Intracellular activity in a macrophage model of *M. tuberculosis* infection**
6. **Chemical Stability**
7. **Solubility**
8. **Permeability (PAMPA)**
9. **Metabolic stability assay**
10. **Pharmacokinetic experiments**

1. Experimental Section and Synthesis

Commercially available reactants and solvents were obtained from commercial suppliers and were used without additional purification. The progress of the reaction was monitored using thin-layer chromatography (TLC, Kenilworth, NJ, USA) with Merck TLC Silica gel 60 F254. The melting points were measured using a Microquímica MQAPF-302 apparatus. ^{1}H and ^{13}C NMR spectra were acquired on an Avance III HD Bruker spectrometer (Bruker Corporation, Fällanden, Switzerland). Chemical shifts (δ) were expressed in parts per million (ppm) relative to $CDCl_3$ or DMDO-d_6, which were used as the solvent, and to TMS, as an internal standard. High-resolution mass spectra (HRMS) were obtained on an LTQ Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific, Bremer, Germany). This system combines an LTQ XL linear ion-trap mass spectrometer and an Orbitrap mass analyzer. The analyses were performed through the direct infusion of the sample in positive-ion mode using electrospray ionization (ESI). For the elemental composition, the calculations used the specific tool included in the Qual Browser module of Xcalibur (Thermo Fisher Scientific, release 2.0.7) software. Compound purity was measured using an Dionex ultimate 3000 HPLC system (Thermo Fisher Scientific inc., Waltham, MA, USA) equipped with a dual pump, automatic injector, and UV detector. For data acquisition, processing, elementary composition, calculations were performed using the Chromeleon 6.80 SR11 software (Build 3160). The HPLC conditions: RP column, 5 μm Nucleodur C-18 (250 × 4.6 mm); flow rate, 1.5 mL/min; UV detection, 260 nm; 100% water (0.1% acetic acid). was maintained from 0 to 7 min, followed by a linear gradient from 100% water (0.1%

acetic acid) to 90% acetonitrile/methanol (1:1, v/v) from 7 to 15 min (15–30 min) and subsequently returned to 100% water (0.1% acetic acid) in 5 min (30–35 min) and maintained for more 10 min (35–45 min). All the evaluated compounds were ≥ 92% pure. Important to mention that no unexpected or unusually high safety hazards were encountered during the synthetic procedures.

1.1 General procedure for the synthesis of 2-alkyl-4-hydroxyquinolines **4**, **7a–h**.

The synthesis of 2-alkyl-4-hydroxyquinolines was carried out using the reaction between substituted anilines (25 mmol) and *β*-ketoesters (29.4 mmol) in the presence of magnesium sulfate (3.611 g, 30 mmol) and acetic acid (0.429 mL, 7.5 mmol) using ethanol (30 mL) as solvent. The mixture was heated under stirring at 80 °C for 16 h. Afterwards, the magnesium sulfate was filtered off and the ethanol was evaporated under reduced pressure yielding the corresponding *β*-acrilate intermediate. Thermal cyclization of this intermediary was carried out by heating in Dowtherm® A (30 mL) at 230-250 °C for 15 min. Then the residue formed was washed with hexane (100 mL). Finally, the solid formed was washed with chloroform (100 mL) and then dried under reduced pressure. The product was used without any further purification method.

1.2 General procedure for the synthesis of bromoacetamides **5a–o**, **8**.

Bromoacetyl chloride (0.425 mL, 5.1 mmol) in dry dichloromethane (5 mL) was added dropwise to a solution containing the respective amine (4.1 mmol) and a catalytic amount of dimethylaminopyridine (DMAP) (0.150 g, 30 mmol%) in dry dichloromethane (20 mL) maintained in an ice bath under an argon atmosphere. The resulting solution was stirred at 0 °C for 30 min, and the temperature was then increased to 25 °C. After stirring for an additional 4 hours, the reaction mixture was diluted with diethyl ether (50 mL). All the stirring time was accomplished under an argon atmosphere. The organic layers were washed sequentially with a solution of 1 N HCl (2× 50 mL), water (1 × 100 mL), saturated aqueous $NaHCO_3$ (3 × 50 mL), and brine (5% w/v, 1 × 50 mL). Finally,

the organic solution was dried over anhydrous $MgSO_4$ and evaporated under vacuum, and the residue was purified by flash chromatography on silica gel.

1.3 General procedure for the synthesis of 2-(quinoline-4-yloxy)acetamides **6a–o**, **9a–h**.

4-Hydroxyquinoline **4** (1.1 mmol) were added to a stirred solution containing bromoacetamides (1.0 mmol) and K_2CO_3 (3.12 mmol) in 6 mL of *N*,*N*-dimethylformamide (DMF) under an argon atmosphere. After being stirred for 18 h, the reaction mixture was dissolved in 15 mL of distilled water. The precipitated product was filtered off, washed with water, and dried under a vacuum. Purification of the compounds was performed by recrystallization from MeOH.

***N*-Cyclobutyl-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (6a)**: Yield: 45%; m.p.: 179-181 °C; HPLC: 97% (t_R = 12.46 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.65 (dtd, *J*=12.9, 6.9, 2.8 Hz, 2H), 1.93–2.08 (m, 2H), 2.14–2.24 (m, 2H), 2.54 (s, 3H), 3.89 (s, 3H), 4.30 (h, *J*=8.2 Hz, 1H), 4.73 (s, 2H), 6.77 (s, 1H), 7.34 (dd, *J*=9.1, 2.9 Hz, 1H), 7.50 (d, *J*=2.9 Hz, 1H), 7.77 (d, *J*=9.1 Hz, 1H), 8.45 (d, *J*=7.8 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 14.6, 25.0, 30.0 (2C), 43.8, 55.3, 67.0, 100.4, 102.2, 119.7, 121.4, 129.4, 144.1, 156.1, 156.8, 159.3, 165.7; FTMS (ESI) *m/z:* 301.1542 $[M+H]^+$; calcd. for $C_{17}H_{21}N_2O_3$: 301.1547.

***N*-Cyclopentyl-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (6b)**: Yield: 44%; m.p.: 99-101 °C; HPLC: 96% (t_R = 10.23 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.43–1.58 (m, 3H), 1.61–1.73 (m, 2H), 1.77–1.88 (m, 3H), 2.84 (s, 3H), 3.95 (s, 3H), 4.03–4.14 (m, 1H), 5.09 (s, 2H), 7.39 (s, 1H), 7.57 (d, *J*=2.8 Hz, 1H), 7.70 (dd, *J*=9.3, 2.8 Hz, 1H), 8.24 (d, *J*=9.3 Hz, 1H), 8.54 (d, *J*=7.3 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 20.0, 23.4 (2C), 32.1 (2C), 50.4, 55.9, 68.2, 101.3, 104.1, 120.1, 121.5, 125.8, 134.1, 155.6, 158.2, 164.8, 165.2; FTMS (ESI) *m/z:* 315.1690 $[M+H]^+$; calcd. for $C_{18}H_{23}N_2O_3$: 315.1703.

***N*-Cyclohexyl-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (6c)**: Yield: 54%; m.p.: 186-187 °C; HPLC: 99% (t_R = 14.25 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.15 (d, *J*=12.1 Hz, 1H), 1.23–1.35 (m, 4H), 1.60–1.51 (m, 1H), 1.62–1.73 (m, 2H), 1.76 (dd, *J*=9.4, 4.5 Hz, 2H), 2.54 (s, 3H), 3.61–3.73 (m, 1H), 3.89 (s, 3H), 4.73 (s, 2H), 6.76 (s, 1H), 7.33 (dd, *J*=9.1, 2.9 Hz, 1H), 7.47 (d, *J*=2.9 Hz, 1H), 7.77 (d, *J*=9.1 Hz, 1H), 7.91 (d, *J*=7.9 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6)

δ ppm 24.2 (2C), 24.8, 24.9, 31.9 (2C), 47.3, 55.2, 67.1, 100.2, 102.0, 119.6, 121.1, 129.3, 144.0, 156.1, 156.6, 159.3, 165.5; FTMS (ESI) *m/z:* 329.1868 $[M+H]^+$; calcd. for $C_{19}H_{25}N_2O_3$: 329.1860.

***N*-Cycloheptyl-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (6d)**: Yield: 64%; m.p.: 141-143 °C; HPLC: 92% (t_R = 15,50 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.00 (d, *J*=7.8 Hz, 1H), 7.87 (dd, *J*=9.1, 3.3 Hz, 1H), 7.51 (d, *J*=2.9 Hz, 1H), 7.44 (dt, *J*=9.1, 3.0 Hz, 1H), 6.92 (s, 1H), 4.83 (d, *J*=3.1 Hz, 2H), 3.91 (s, 3H), 3.85 (tt, *J*=8.3, 4.1 Hz, 1H), 2.62 (s, 2H), 1.81 (ddd, *J*=13.3, 7.6, 3.9 Hz, 2H), 1.68–1.37 (m, 10H). ^{13}C NMR (400 MHz, DMSO-*d*6) δ ppm 23.4, 23.5 (2C), 27.4 (2C), 33.9 (2C), 49.5, 55.3, 67.4, 100.5, 102.5, 119.7, 122.3, 127.0, 141.2, 156.2, 156.6, 160.9, 164.8; FTMS (ESI) *m/z:* 343.2009 $[M+H]^+$; calcd. for $C_{20}H_{27}N_2O_3$: 343.2016.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(4-methylcyclohexyl)acetamide (6e): isolated as two conformers (approximately 1:1 ratio). Yield: 49%; m.p.: 186-188 °C; HPLC: 99% (t_R = 14.30 min); <u>*Conformer A*</u>: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.86 (dd, *J*=6.6, 3.4 Hz, 3H), 0.97 (dt, *J*=14.5, 10.6 Hz, 1H), 1.18–1.35 (m, 3H), 1.48 (qd, *J*=9.2, 4.3 Hz, 3H), 1.64 (td, *J*=12.7, 10.1, 4.3 Hz, 2H), 1.74–1.82 (m, 1H), 2.53 (d, *J*=1.8 Hz, 3H), 3.58 (dtd, *J*=12.1, 8.0, 4.2 Hz, 1H), 3.88 (s, 3H), 4.73 (s, 2H), 6.76 (dd, *J*=13.1, 1.7 Hz, 1H), 7.33 (dd, *J*=9.1, 2.7 Hz, 1H), 7.45 (dd, *J*=8.6, 2.8 Hz, 1H), 7.76 (dt, *J*=9.1, 1.9 Hz, 1H), 7.94 (d, *J*=7.6 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 22.60, 25.54, 29.04, 30.32, 32.55, 34.03, 45.31, 48.23, 55.82, 67.65, 100.76, 102.68, 120.22, 122.03, 130.01, 144.68, 156.74, 157.26, 159.90, 166.44. <u>*Conformer B*</u>: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.86 (dd, *J*=6.6, 3.4 Hz, 3H), 0.97 (dt, *J*=14.5, 10.6 Hz, 1H), 1.18–1.35 (m, 3H), 1.48 (qd, *J*=9.2, 4.3 Hz, 3H), 1.64 (td, *J*=12.7, 10.1, 4.3 Hz, 2H), 1.74–1.82 (m, 1H), 2.53 (d, *J*=1.8 Hz, 3H), 3.58 (dtd, *J*=12.1, 8.0, 4.2 Hz, 1H), 3.88 (s, 3H), 4.80 (s, 2H), 6.76 (dd, *J*=13.1, 1.7 Hz, 1H), 7.33 (dd, *J*=9.1, 2.7 Hz, 1H), 7.45 (dd, *J*=8.6, 2.8 Hz, 1H), 7.76 (dt, *J*=9.1, 1.9 Hz, 1H), 8.05 (d, *J*=8.1 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 21.38, 25.52, 29.04, 29.83, 31.90, 34.03, 45.31, 48.23, 67.51, 100.40, 102.68, 120.22, 121.92, 129.94, 144.66, 156.67, 157.24, 159.86, 166.27; FTMS (ESI) *m/z:* 343.2001 $[M+H]^+$; calcd. for $C_{20}H_{27}N_2O_3$: 343.2016.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(2-methylcyclohexyl)acetamide (6f): Yield: 52%; m.p.: 215-217 °C; HPLC: 96% (t_R = 13.28 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.85 (d, *J*=6.6 Hz, 3H), 1.01 (qd, *J*=12.4, 3.5 Hz, 1H), 1.21 (ddtd, *J*=21.3, 15.8, 12.6, 9.2 Hz, 3H), 1.33 (s, 0H), 1.42 (tdt, *J*=15.3, 8.6, 4.0 Hz, 1H), 1.60 (dd, *J*=9.4, 5.6 Hz, 1H), 1.65–1.81 (m, 3H), 2.53 (s, 3H), 3.34 (qd, *J*=10.0, 3.8 Hz, 1H), 3.88 (d, *J* = 3.6 Hz, 3H), 4.68–4.82 (m, 2H), 6.75 (s, 1H), 7.33 (dd, *J*=9.1, 2.9 Hz, 1H), 7.48 (d, *J*=3.0 Hz, 1H), 7.76 (d, *J*=9.1 Hz, 1H), 7.89 (d, *J*=8.9 Hz, 1H); ^{13}C

NMR (400 MHz, DMSO-d_6) δ ppm 19.4, 25.5, 25.6, 26.4, 32.7, 34.9, 37.7, 54.0, 56.6, 68.0, 100.9, 102.7, 120.3, 121.9, 130.0, 144.7, 156.8, 157.2, 159.9, 166.5; FTMS (ESI) *m/z:* 343.2000 $[M+H]^+$; calcd. for $C_{20}H_{27}N_2O_3$: 343.2016.

***N*-(Cyclohexylmethyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (6g)**: Yield: 63%; m.p.: 161-163 °C; HPLC: 97% (t_R = 15.85 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.83–0.94 (m, 2H), 1.10–1.19 (m, 4H), 1.45 (dqd, *J*=10.6, 6.9, 3.6 Hz, 1H), 1.63–1.69 (m, 4H), 2.54 (s, 3H), 3.02 (t, *J*=6.4 Hz, 2H), 3.89 (s, 3H), 4.78 (s, 2H), 6.78 (s, 1H), 7.35 (dd, *J*=9.1, 2.9 Hz, 1H), 7.50 (d, *J*=2.9 Hz, 1H), 7.78 (d, *J*=9.1 Hz, 1H), 8.21 (t, *J*=6.0 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 24.9, 25.4 (2C), 26.0, 30.3 (2C), 37.5, 44.5, 55.3, 67.2, 100.3, 102.2, 119.7, 121.5, 129.4, 144.1, 156.2, 156.7, 159.3, 166.6; FTMS (ESI) *m/z:* 343.2009 $[M+H]^+$; calcd. for $C_{20}H_{27}N_2O_3$: 343.2016.

(*R*)-*N*-(1-Cyclohexylethyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (6h): Yield: 50%; m.p.: 222-223 °C; HPLC: 99% (t_R = 16.73 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.84–1.01 (m, 2H), 1.06 (d, *J*=6.8 Hz, 3H), 1.06-1.21 (m, 3H), 1.33 (ddd, *J*=11.1, 7.2, 3.3 Hz, 1H), 1.59 (d, *J*=11.2 Hz, 1H), 1.63–1.76 (m, 4H), 2.53 (s, 3H), 3.18 (s, 3H), 3.73 (dt, *J*=8.4, 6.6 Hz, 1H), 3.89 (s, 3H), 4.75 (q, *J*=14.2 Hz, 2H), 6.76 (s, 1H), 7.33 (dd, *J*=9.1, 2.9 Hz, 1H), 7.47 (d, *J*=2.9 Hz, 1H), 7.77 (dd, *J*=9.2, 4.7 Hz, 2H). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 17.3, 24.7, 25.4 (2C), 25.7, 28.5, 28.7, 42.1, 48.4, 55.1, 67.2, 100.1, 102.0, 119.6, 121.2, 129.3, 144.0, 156.1, 156.5, 165.6; FTMS (ESI) *m/z:* 357.2164 $[M+H]^+$; calcd. for $C_{21}H_{29}N_2O_3$: 357.2173.

(*S*)-*N*-(1-Cyclohexylethyl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (6i): Yield: 67%; m.p.: 222-223 °C; HPLC: 99% (t_R = 15.39 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.78–0.99 (m, 3H), 0.99-1.25 (m, 5H), 1.28-1.43 (s, 1H), 1.53-1.81 (m, 5H), 2.50 (s, 3H), 3.70-3.75 (m, 1H), 3.88 (s, 3H), 4.75 (q, *J*=14.1 Hz, 2H), 6.76 (s, 1H), 7.33 (d, *J*=9.1 Hz, 1H), 7.47 (s, 1H), 7.73-7.86 (m, 2H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 17.4, 24.8, 25.5 (2C), 25.7, 28.5, 28.7, 42.1, 48.4, 55.1, 67.2, 100.1, 102.0, 119.6, 121.2, 129.3, 144.0, 156.1, 156.5, 159.2, 165.7; FTMS (ESI) *m/z:* 357.2158 $[M+H]^+$; calcd. for $C_{21}H_{29}N_2O_3$: 357.2173.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-((1*S*,2*S*,3*S*,5*R*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)acetamide (6j): Yield: 72%; m.p.: 169-170 °C; HPLC: 99% (t_R = 14.80 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.01 (d, *J*=4.5 Hz, 6H), 1.20 (s, 3H), 1.62 (ddd, *J*=13.5, 6.5, 2.3 Hz, 1H), 1,77 (td, *J*=5.8, 1.8 Hz, 1H), 1.84-2.00 (m, 2H), 2.36 (dqd, *J*=20.3, 6.7, 5.9, 3.5 Hz, 2H), 2.53 (s, 3H), 3.89 (s, 3H), 4.15-4.27 (m, 1H), 4.79 (d, *J*=2.9 Hz, 2H), 6.76 (s, 1H), 7.34

(dd, *J*=9.1, 2.9 Hz, 1H), 7.48 (d, *J*=2.9 Hz, 1H), 7.77 (d, *J*=9.1 Hz, 1H), 8.26 (d, *J*=8.6 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 20.4, 23.1, 25.0, 27.8, 33.9, 35.8, 38.2, 41.0, 44.0, 46.8, 47.2, 55.3, 67.3, 100.1, 102.2, 119.7, 121.5, 129.4, 144.1, 156.1, 156.7 159.4, 166.1; FTMS (ESI) *m/z:* 383.2320 $[M+H]^+$; calcd. for $C_{23}H_{31}N_2O_3$: 383.2329.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-((1*R*,2*R*,3*R*,5*S*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)acetamide (6k): Yield: 70%; m.p.: 173-175 °C; HPLC: 99% (t_R = 20.29 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.97–1.04 (m, 6H), 1.20 (s, 3H), 1.61 (ddd, *J*=13.5, 6.4, 2.2 Hz, 1H), 1.74-1.80 (m, 1H), 1.87-1.97 (m, 2H), 2.28–2.42 (m, 2H), 2.53 (s, 3H), 3.88 (s, 3H), 4.15-4.26 (m, 1H), 4.78 (d, *J*=2.9 Hz, 2H), 6.76 (s, 1H), 7.34 (dd, *J*=9.1, 2.9 Hz, 1H), 7.47 (d, *J*=2.9 Hz, 1H), 7.77 (d, *J*=9.1 Hz, 1H), 8.26 (d, *J*=8.6 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 20.4, 23.1, 25.0, 27.9, 33.9, 35.8, 38.2, 41.0, 44.0, 46.8, 47.2, 55.3, 67.3, 100.1, 102.2, 119.7, 121.5, 129.4, 144.1, 156.2, 156.7, 159.4, 166.1; FTMS (ESI) *m/z:* 383.2321 $[M+H]^+$; calcd. For $C_{23}H_{31}N_2O_3$: 383.2329.

***N*-(Adamantan-2-yl)-2-((6-methoxy-2-methylquinolin-4-yl)oxy)acetamide (6l)**: Yield: 40%; m.p.: 169-171 °C; HPLC: 99% (t_R = 14.80 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.55 (d, *J*=12.7 Hz, 2H). 1.70 (s, 2H), 1.74–1.83 (m, 7H), 1.87 (s, 2H), 1.98 (d, *J*=12.9 Hz, 2H), 2.53 (s, 3H), 3.87 (s, 3H), 3.93 (d, *J*=7.4 Hz, 1H), 4.85 (s, 2H), 6.79 (s, 1H), 7.30–7.36 (m, 1H), 7.41 (d, *J*=2.8 Hz, 1H), 7.77 (d, *J*=9.1 Hz, 1H), 7.99 (d, *J*=7.5 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 25.0, 26.6, 26.7, 31.0 (2C), 31.3 (2C), 36.6 (2C), 37.0, 55.2, 52.9, 66.9, 99.6, 102.1, 119.7, 121.6, 129.5, 144.1, 156.2, 156.7, 159.3, 166.0; FTMS (ESI) *m/z:* 381.2174 $[M+H]^+$; calcd. for $C_{23}H_{29}N_2O_3$: 381.2173.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(4-(piperidin-1-yl)phenyl)acetamide (6m): Yield: 80%; m.p.: 198-199 °C; HPLC: 99% (t_R = 12.89 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.47-1.54 (m, 2H), 1.60 (t, *J*=5.7 Hz, 4H), 2.55 (s, 3H), 3.04–3.09 (m, 4H), 3.89 (s, 3H), 4.95 (s, 2H), 6.85 (s, 1H), 6.87–6.91 (m, 2H), 7.35 (dd, *J*=9.1, 2.9 Hz, 1H), 7.43–7.49 (m, 2H), 7.52 (d, *J*=2.9 Hz, 1H), 7.78 (d, *J*=9.1 Hz, 1H), 10.02 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 23.8, 25.0 (2C), 25.2 (2C), 49.9, 55.3, 67.2, 100.2, 102.1, 116.1 (2C), 119.7, 120.9 (2C), 121.5, 129.4, 129.8, 144.2, 148.3, 156.2, 156.8, 159.4, 164.9; FTMS (ESI) *m/z:* 406.2127 $[M+H]^+$; calcd. for $C_{24}H_{28}N_3O_3$: 406.2125.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(4-morpholinophenyl)acetamide (6n): Yield: 68%; m.p.: 234-236 °C; HPLC: 98% (t_R = 12.90 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.55

(s, 3H), 3.01–3.08 (m, 4H), 3.69–3.76 (m, 4H), 3.89 (s, 3H), 4.96 (s, 2H), 6.85 (s, 1H), 6.89–6.95 (m, 2H), 7.35 (dd, *J*=9.1, 2.9 Hz, 1H), 7.46–7.54 (m, 3H), 7.78 (d, *J*=9.1 Hz, 1H), 10.06 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 25.1, 48.8 (2C), 55.4, 66.1 (2C), 67.2, 100.1, 102.2, 115.4 (2C), 119.7, 120.8 (2C), 121.5, 129.5, 130.5, 144.2, 147.6, 156.2, 156.8, 159.4, 165.0; FTMS (ESI) *m/z:* 408.1932 $[M+H]^+$; calcd. for $C_{23}H_{26}N_3O_4$: 408.1918.

2-((6-Methoxy-2-methylquinolin-4-yl)oxy)-*N*-(4-thiomorpholinophenyl)acetamide (6o): Yield: 63%; m.p.: 212-214 °C; HPLC: 99% (t_R = 14.80 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.55 (s, 3H), 2.64–2.68 (m, 4H), 3.41–3.47 (m, 4H), 3.89 (s, 3H), 4.96 (s, 2H), 6.85 (s, 1H), 6.87–6. 93 (m, 2H), 7.35 (dd, *J*=9.1, 2.9 Hz, 1H), 7.46–7.53 (m, 3H), 7.78 (d, *J*=9.1 Hz, 1H), 10.04 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 25.0, 25.6 (2C), 51.4 (2C), 55.4, 67.2, 100.2, 102.2, 116.7 (2C), 119.7, 121.0 (2C), 121.5, 129.4, 130.2, 144.1, 147.3, 156.2, 156.8, 159.5, 165.0; FTMS (ESI) *m/z:* 424.1671 $[M+H]^+$; calcd. for $C_{23}H_{26}N_3O_3S$: 424.1689.

2-((6-Chloro-2-methylquinolin-4-yl)oxy)-*N*-(4-(piperidin-1-yl)phenyl)acetamide (9a): Yield: 83%; m.p.: 235-237 °C; HPLC: 94% (t_R = 16.43 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.44–1.70 (m, 6H), 2.59 (d, *J*=5.7 Hz, 3H), 3.02–3.13 (m, 4H), 4.96 (s, 2H), 6.89 (d, *J*=9.1 Hz, 2H), 6.97 (s, 1H), 7.44 (d, *J*=8.6 Hz, 2H), 7.71 (dd, *J*=9.2, 2.5 Hz, 1H), 7.88 (d, *J*=8.9 Hz, 1H), 8.20–8.29 (m, 1H), 9.91 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 23.6, 25.05 (2C), 25.09, 49.8 (2C), 67.4, 102.8, 115.9 (2C), 119.9, 120.7, 121.1 (2C), 129.2, 129.5, 129.8, 130.0, 146.6, 148.3, 159.5, 160.3, 164.5; FTMS (ESI) *m/z:* 410.1625 $[M+H]^+$; calcd. for $C_{23}H_{25}ClN_3O_2$: 410.1630.

2-((6-Bromo-2-methylquinolin-4-yl)oxy)-*N*-(4-(piperidin-1-yl)phenyl)acetamide (9b): Yield: 86%; m.p.: 230-232 °C; HPLC: 92% (t_R = 17.35 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.49–1.57 (m, 2H), 1.58–1.67 (m, 4H), 2.61 (s, 3H), 3.09 (t, *J*=5.4 Hz, 4H), 4.98 (s, 2H), 6.88–6.95 (m, 2H), 7.00 (s, 1H), 7.42–7.49 (m, 2H), 7.79–7.89 (m, 2H), 8.41 (d, *J*=2.0 Hz, 1H), 9.99 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 23.8, 25.2 (3C), 50.0 (2C), 67.4, 103.0, 116.1 (2C), 117.8, 120.5, 121.2 (2C), 124.1, 129.8, 129.9, 132.9, 146.6, 148.3, 159.6, 160.6, 164.7; FTMS (ESI) *m/z:* 454.1117 $[M+H]^+$; calcd. for $C_{23}H_{25}BrN_3O_2$: 454.1125.

2-((2-Ethyl-6-methoxyquinolin-4-yl)oxy)-*N*-(4-(piperidin-1-yl)phenyl)acetamide (9c): Yield: 58%; m.p.: 166-168 °C; HPLC: 99% (t_R = 13.99 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.27 (t, *J*=7.6 Hz, 3H), 1.46–1.54 (m, 2H), 1.56–1.64 (m, 4H), 2.83 (q, *J*=7.6 Hz, 2H), 3.02–3.10 (m, 4H), 3.90 (s, 3H), 4.98 (s, 2H), 6.86–6.92 (m, 3H), 7.36 (dd, *J*=9.1, 2.9 Hz, 1H), 7.42–7.48 (m, 2H), 7.53

(d, *J*=2.9 Hz, 1H), 7.81 (d, *J*=9.1 Hz, 1H), 10.05 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 13.5, 23.8, 25.2 (2C), 31.3, 50.0 (2C), 55.4, 67.3, 100.2, 101.3, 116.1 (2C), 120.0, 120.9 (2C), 121.7, 129.3, 129.8, 143.7, 148.3, 156.3, 159.9, 161.7, 164.9; FTMS (ESI) *m/z:* 420.2281 $[M+H]^+$; calcd. for $C_{25}H_{30}N_3O_3$: 420.2282.

2-((6-Chloro-2-ethylquinolin-4-yl)oxy)-*N*-(4-(piperidin-1-yl)phenyl)acetamide (9d): Yield: 86%; m.p.: 208-209 °C; HPLC: 94% (t_R = 13.50 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.29 (t, *J*=7.6 Hz, 3H), 1.48–1.55 (m, 2H), 1.57–1.66 (m, 4H), 2.51 (p, *J*=1.9 Hz, 3H), 2.87 (q, *J*=7.6 Hz, 2H), 3.07 (t, *J*=5.4 Hz, 4H), 4.99 (s, 2H), 6.86–6.95 (m, 2H), 7.00 (s, 1H), 7.41–7.51 (m, 2H), 7.73 (dd, *J*=8.9, 2.5 Hz, 1H), 7.91 (d, *J*=8.9 Hz, 1H), 8.27 (d, *J*=2.5 Hz, 1H), 10.03 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 13.3, 23.8, 25.2 (2C), 31.8, 49.9 (2C), 67.4, 102.0, 116.0 (2C), 120.2, 120.8, 121.2 (2C), 129.4, 129.6, 130.1, 130.2, 146.8, 148.4, 159.6, 164.7, 165.3; FTMS (ESI) *m/z:* 424.1798 $[M+H]^+$; calcd. for $C_{24}H_{27}ClN_3O_2$: 424.1786.

2-((6-Bromo-2-ethylquinolin-4-yl)oxy)-*N*-(4-(piperidin-1-yl)phenyl)acetamide (9e): Yield: 88%; m.p.: 210-212 °C; HPLC: 97% (t_R = 14.52 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.28 (t, *J*=7.6 Hz, 3H), 1.45–1.55 (m, 2H), 1.55–1.65 (m, 4H), 2.86 (q, *J*=7.6 Hz, 2H), 3.03–3.10 (m, 4H), 4.98 (s, 2H), 6.86–6.92 (m, 2H), 6.98 (s, 1H), 7.40–7.47 (m, 2H), 7.83 (d, *J*=1.4 Hz, 2H), 8.40 (t, *J*=1.4 Hz, 1H), 10.02 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 13.3, 23.8, 25.2 (2C), 31.8, 49.9 (2C), 67.4, 102.0, 116.0 (2C), 117.8, 120.7, 121.2 (2C), 124.0, 129.6, 130.4, 132.7, 146.9, 148.4, 159.5, 164.7, 165.4. FTMS (ESI) *m/z:* 468.1275 $[M+H]^+$; calcd. for $C_{24}H_{27}BrN_3O_2$: 468.1281.

2-((6-Methoxy-2-(trifluoromethyl)quinolin-4-yl)oxy)-*N*-(4-(piperidin-1-yl)phenyl)acetamide (9f): Yield: 76%; m.p.: 197-198 °C; HPLC: 99% (t_R = 16.30 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.44–1.55 (m, 2H), 1.55–1.65 (m, 4H), 3.02–3.09 (m, 4H), 3.96 (s, 3H), 5.16 (s, 2H), 6.86–6.92 (m, 2H), 7.35 (s, 1H), 7.41–7.47 (m, 2H), 7.55 (dd, *J*=9.2, 2.9 Hz, 1H), 7.61 (d, *J*=2.8 Hz, 1H), 8.03 (d, *J*=9.2 Hz, 1H), 10.09 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 23.8, 25.2 (2C), 49.9 (2C), 55.7, 67.7, 98.1 (d, *J*=2.6 Hz), 100.0, 116.1 (2C), 120.8 (2C), 121.6 (q, *J*=275.2 Hz), 122.3, 123.7, 129.7, 130.8, 143.3, 145.0 (q, *J*=33.8 Hz), 148.4, 158.5, 161.2, 164.5; FTMS (ESI) *m/z:* 460.1846 $[M+H]^+$; calcd. for $C_{24}H_{25}F_3N_3O_3$: 460.1843.

2-((6-Chloro-2-(trifluoromethyl)quinolin-4-yl)oxy)-*N*-(4-(piperidin-1-yl)phenyl)acetamide (9g): Yield: 80%; m.p.: 210-211 °C; HPLC: 99% (t_R = 17.10 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.51 (td, *J*=7.3, 6.7, 3.5 Hz, 2H), 1.60 (p, *J*=5.6 Hz, 4H), 3.06 (t, *J*=5.4 Hz, 4H), 5.17 (s, 2H),

6.89 (dq, *J*=10.2, 3.6 Hz, 2H), 7.40–7.45 (m, 2H), 7.48 (s, 1H), 7.93 (dd, *J*=9.0, 2.5 Hz, 1H), 8.14 (d, *J*=9.0 Hz, 1H), 8.40 (d, *J*=2.5 Hz, 1H), 10.06 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 23.8, 25.2 (2C), 49.9 (2C), 68.0, 99.0, 116.0 (2C), 121.1 (2C), 121.2, 121.3 (q, *J*=275.7 Hz), 121.9, 129.5, 131.2, 132.0, 132.8, 146.0, 148.1 (q, *J*=33.9 Hz), 148.4, 161.9, 164.2; FTMS (ESI) *m/z:* 464.1335 $[M+H]^+$; calcd. for $C_{23}H_{22}ClF_3N_3O_2$: 464.1347.

2-((6-Bromo-2-(trifluoromethyl)quinolin-4-yl)oxy)-*N*-(4-(piperidin-1-yl)phenyl)acetamide (9h): Yield: 76%; m.p.: 210-211 °C; HPLC: 99% (t_R = 17.70 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.48-1.54 (m, 2H), 1.59-1.64 (m, 4H), 3.06-3.09 (m, 4H), 5.18 (s, 2H), 6.91 (d, *J*=8.4 Hz, 2H), 7.44 (d, *J*=8.5 Hz, 2H), 7.49 (s,1H), 8.06 (s, 2H), 8.56 (s, 1H), 10.09 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 23.7, 25.2 (2C), 49.9, 67.9 (2C), 99.0, 116.0, 119.9, 121.05 (2C), 121.4, 122.1 (q, *J*=275.8 Hz), 124.4, 131.2, 134.6, 146.1, 148.1 (q, *J*=33.8 Hz), 161.7, 169.2; FTMS (ESI) *m/z:* 508.0827 $[M+H]^+$; calcd. for $C_{23}H_{22}BrF_3N_3O_2$: 508.0842.

2. ^{1}H and ^{13}C NMR spectra of 2-(quinoline-4-yloxy)acetamides **6a–o**, **9a–h**.

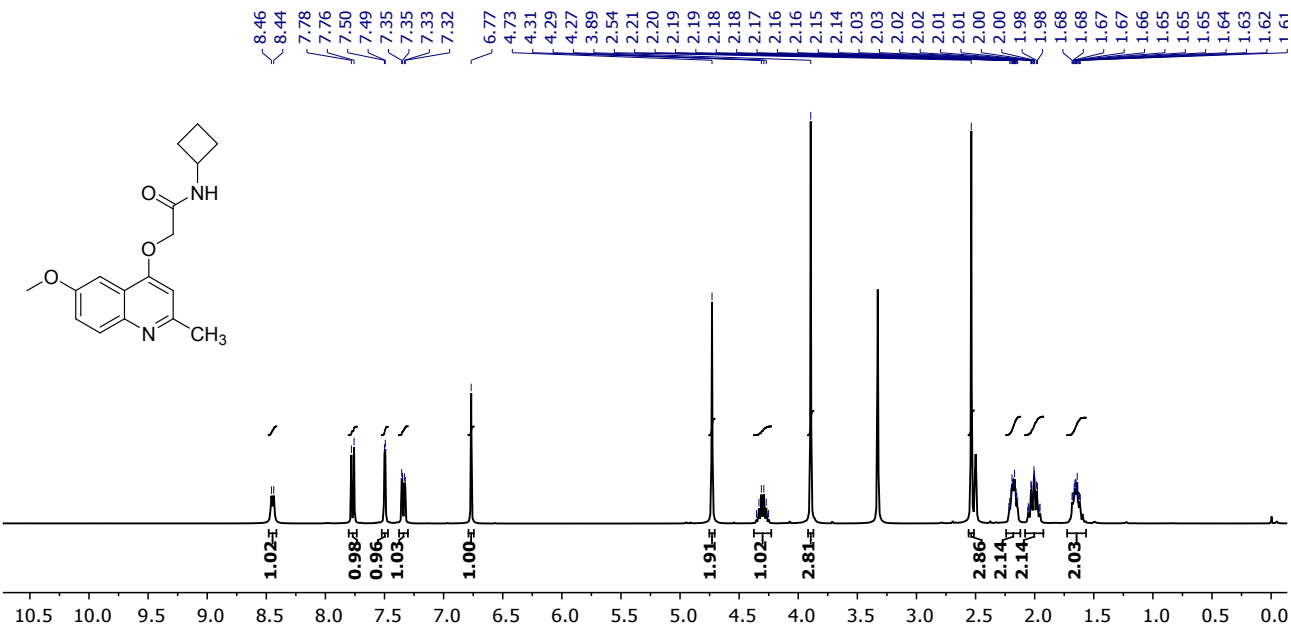

Figure S1 – ^{1}H NMR Spectrum of compound **6a** in DMSO-d_6.

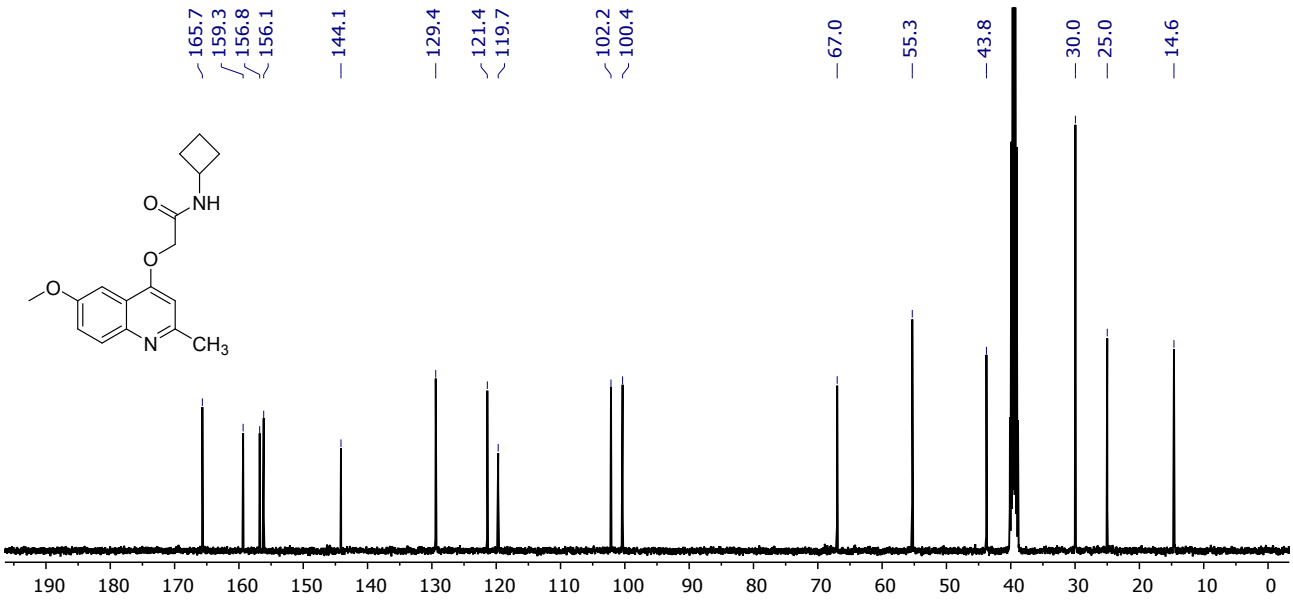

Figure S2 – ^{13}C NMR Spectrum of compound **6a** in DMSO-d_6.

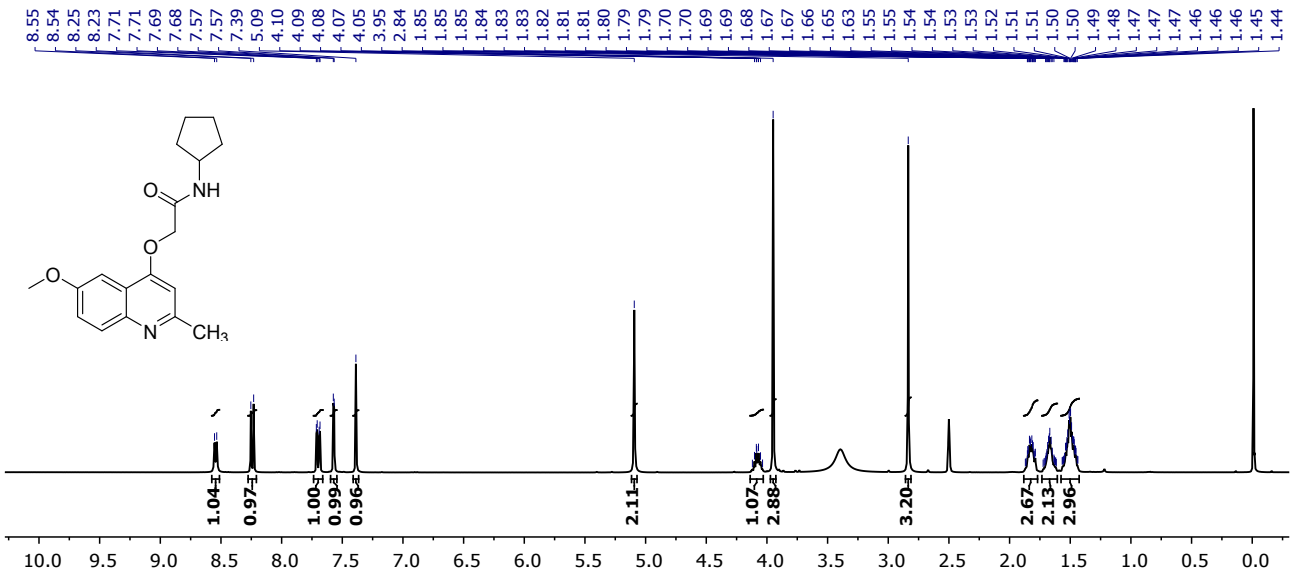

Figure S3 – ^{1}H NMR Spectrum of compound **6b** in DMSO-d_6.

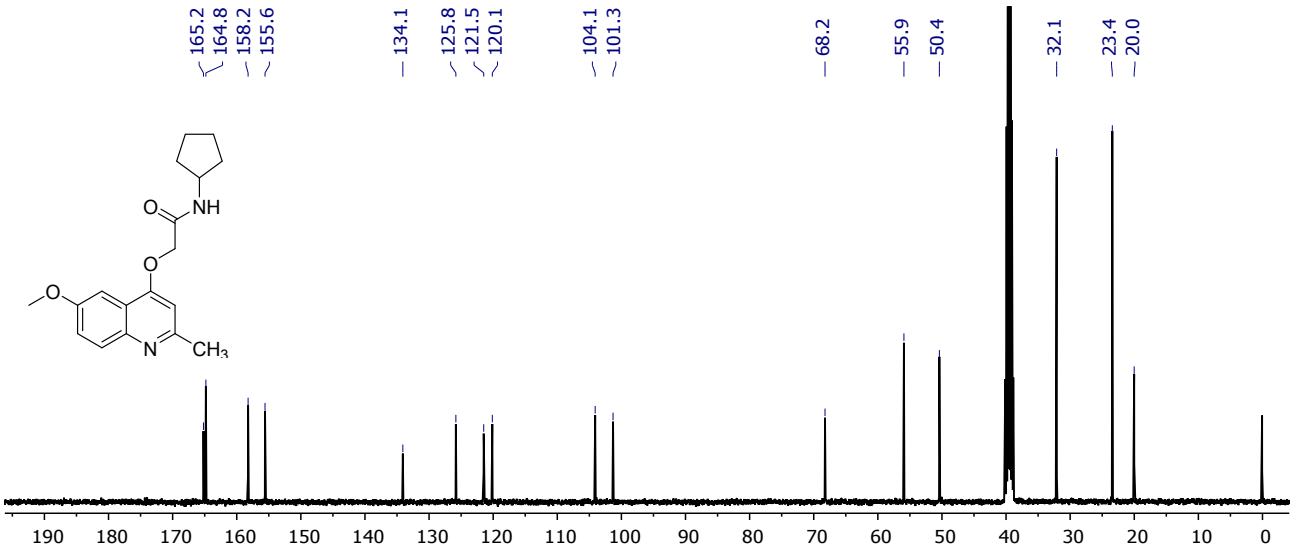

Figure S4 – ^{13}C NMR Spectrum of compound **6b** in DMSO-d_6.

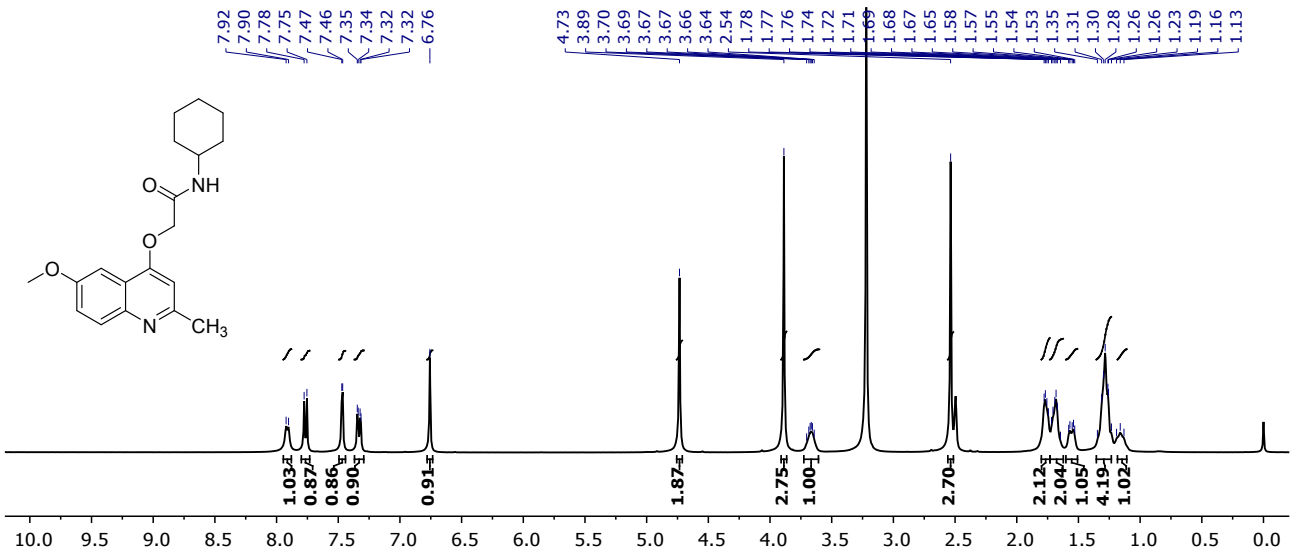

Figure S5 – ^{1}H NMR Spectrum of compound **6c** in DMSO-d_6.

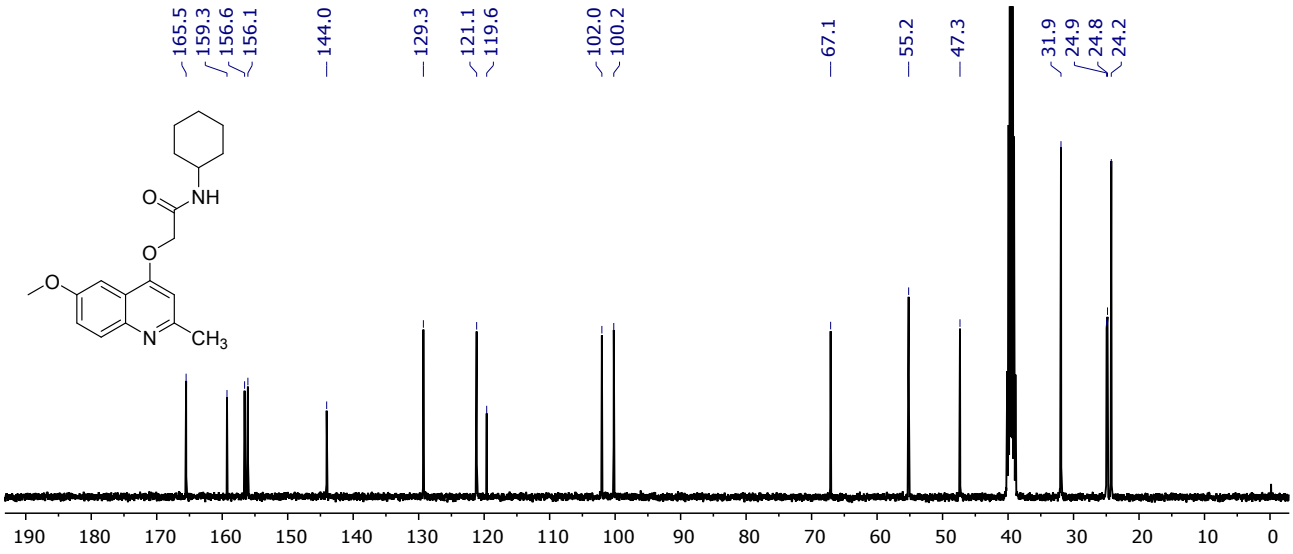

Figure S6 – ^{13}C NMR Spectrum of compound **6c** in DMSO-d_6.

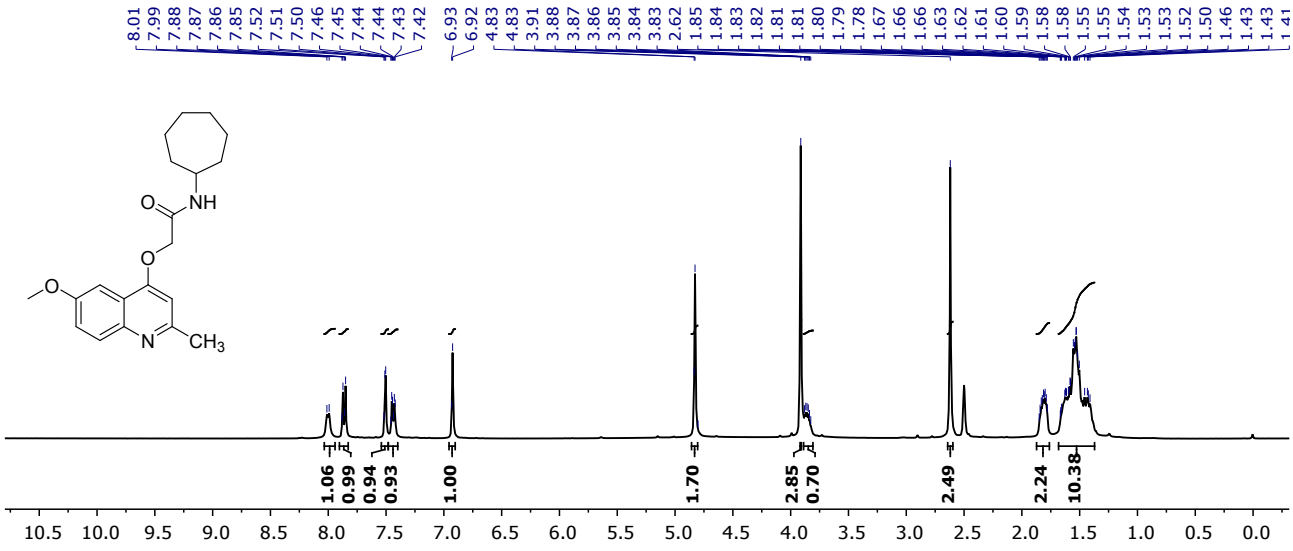

Figure S7 – ^{1}H NMR Spectrum of compound **6d** in DMSO-d_6.

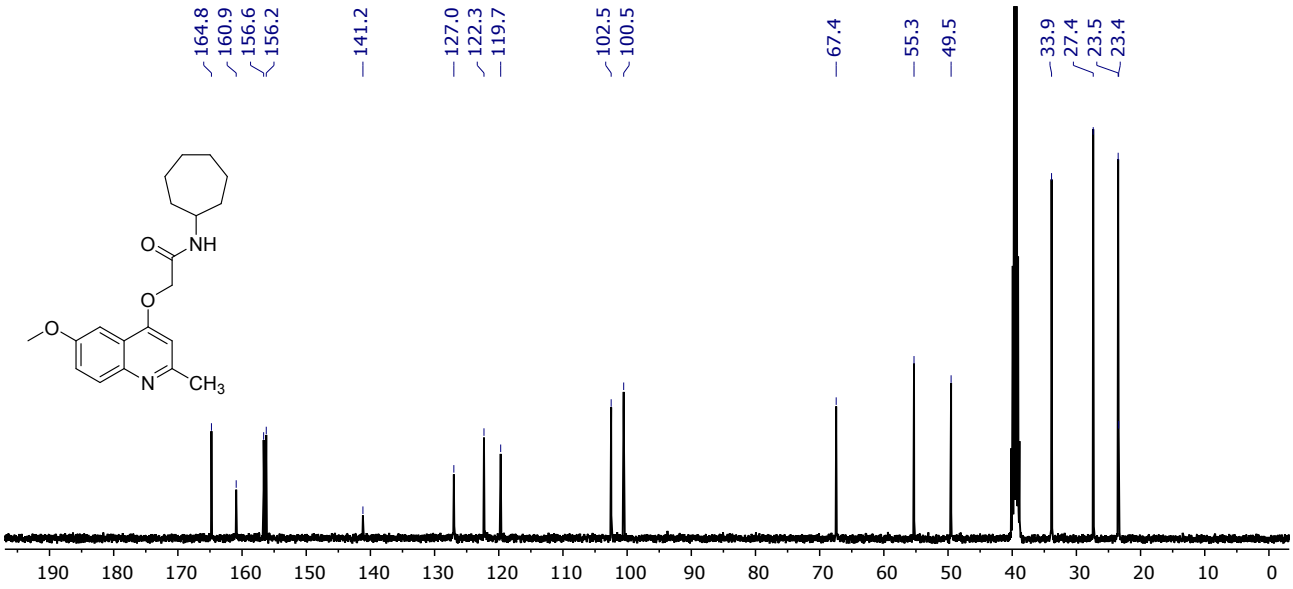

Figure S8 – ^{13}C NMR Spectrum of compound **6d** in DMSO-d_6.

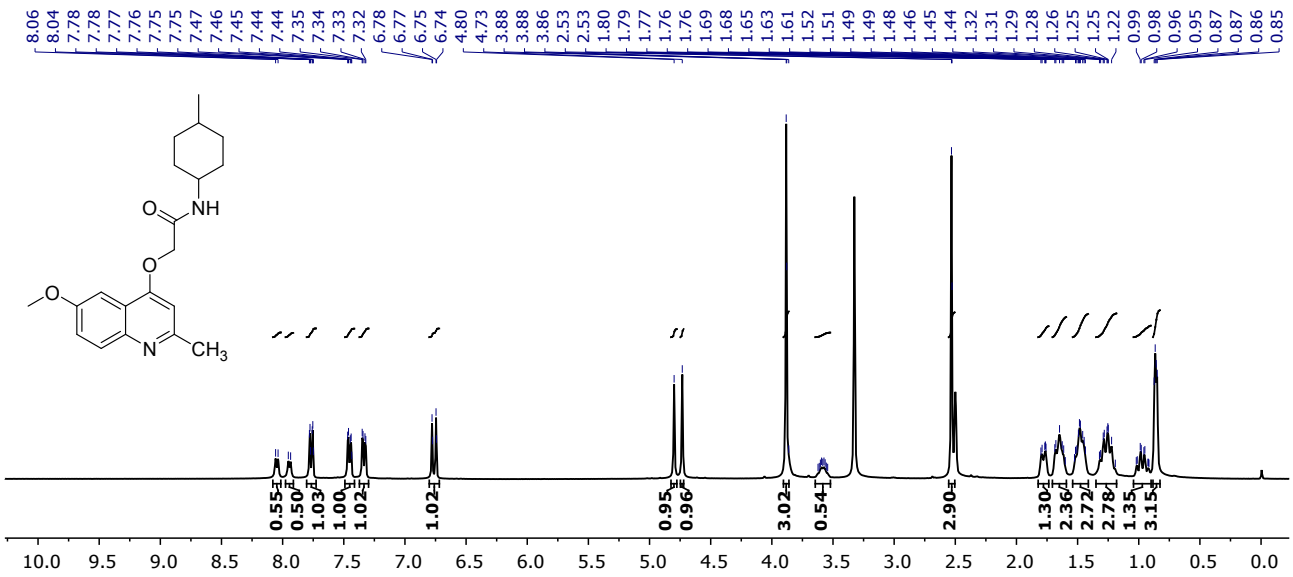

Figure S9 – ^{1}H NMR Spectrum of compound **6e** in DMSO-d_6.

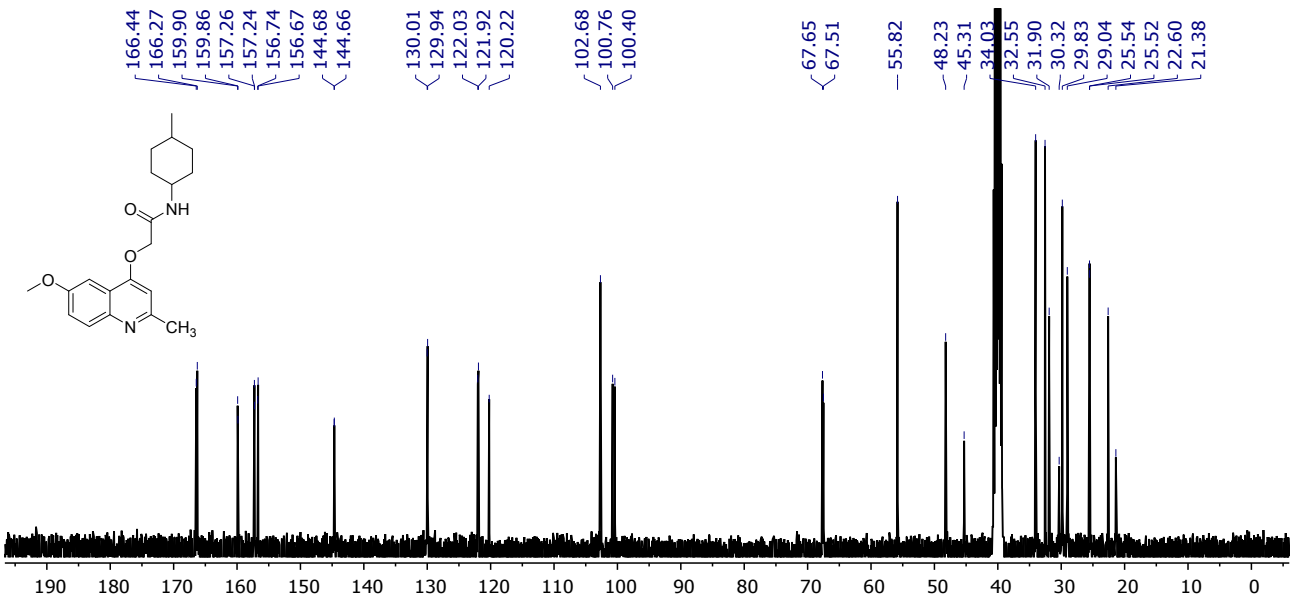

Figure S10 – ^{13}C NMR Spectrum of compound **6e** in DMSO-d_6.

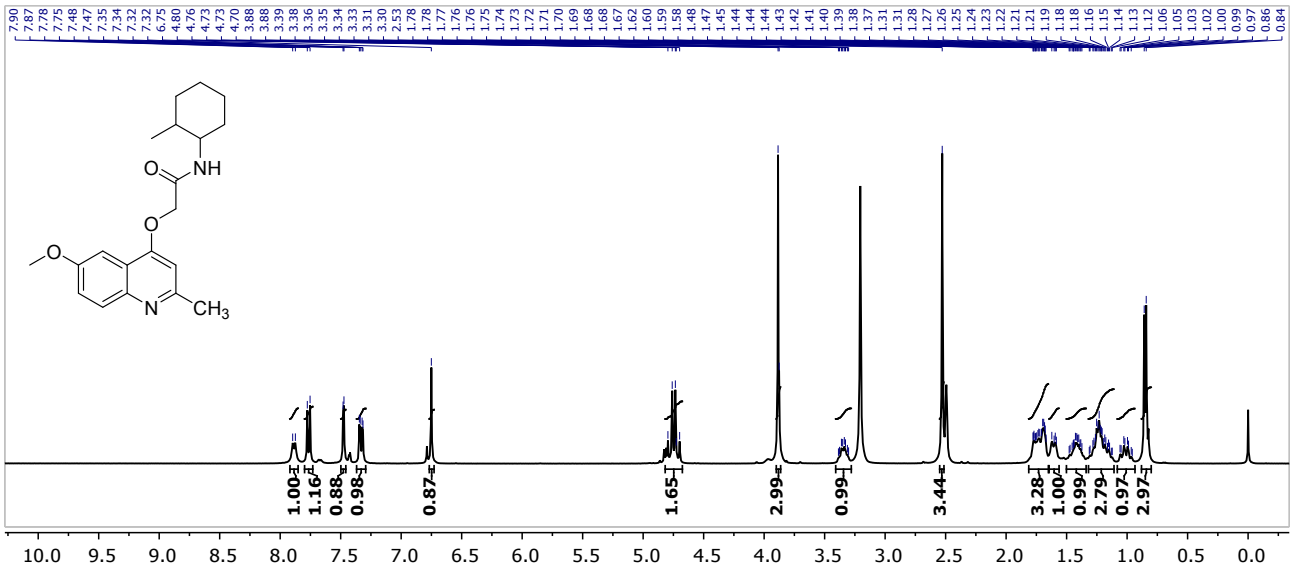

Figure S11 – ^{1}H NMR Spectrum of compound **6f** in DMSO-d_6.

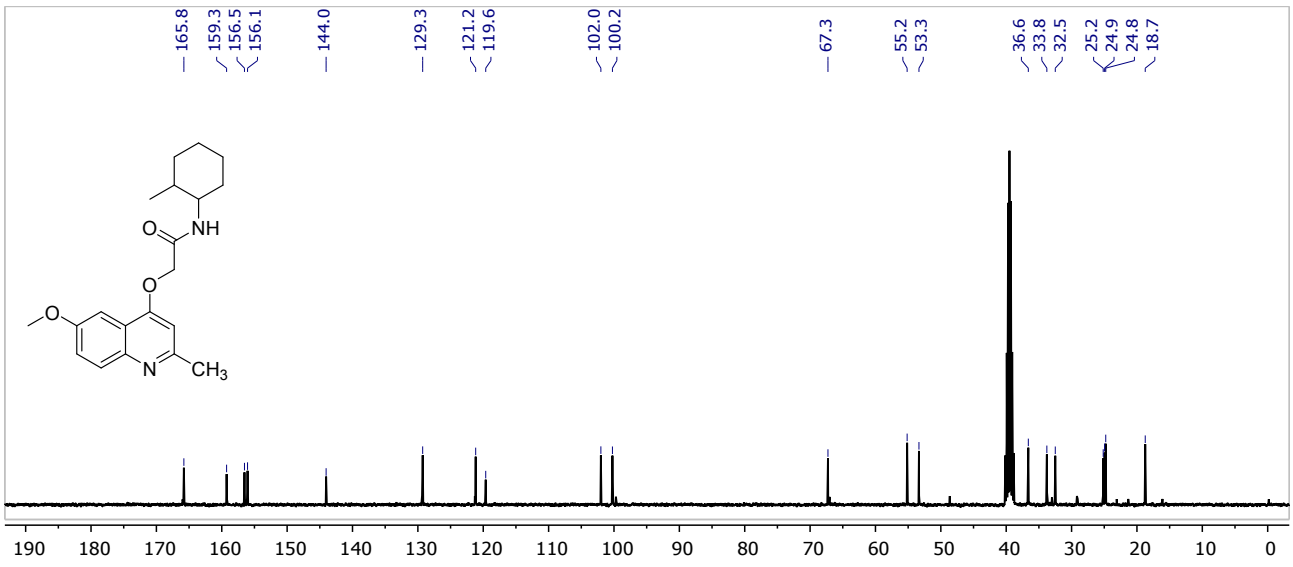

Figure S12 – ^{13}C NMR Spectrum of compound **6f** in DMSO-d_6.

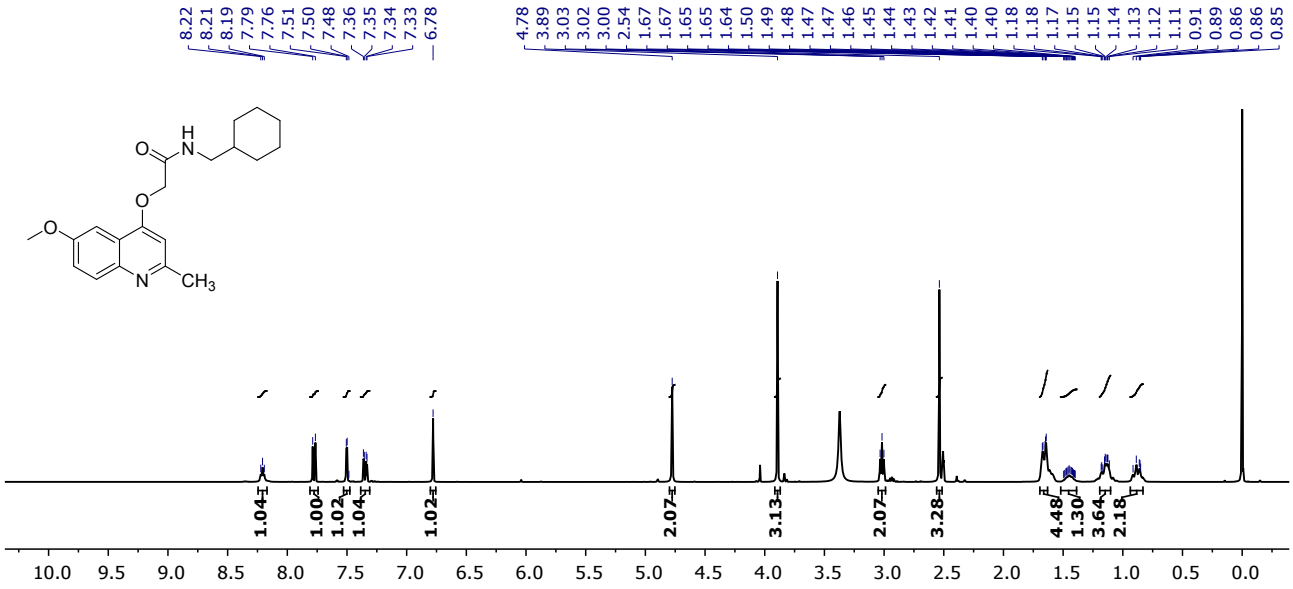

Figure S13 – ^{1}H NMR Spectrum of compound **6g** in DMSO-d_6.

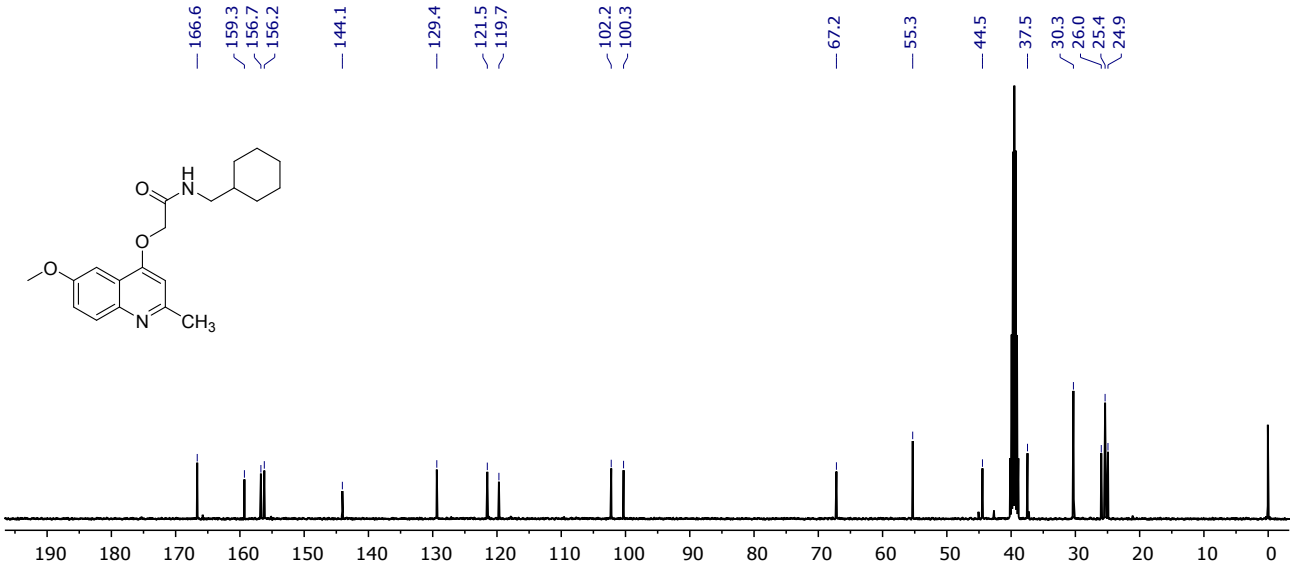

Figure S14 – ^{13}C NMR Spectrum of compound **6g** in DMSO-d_6.

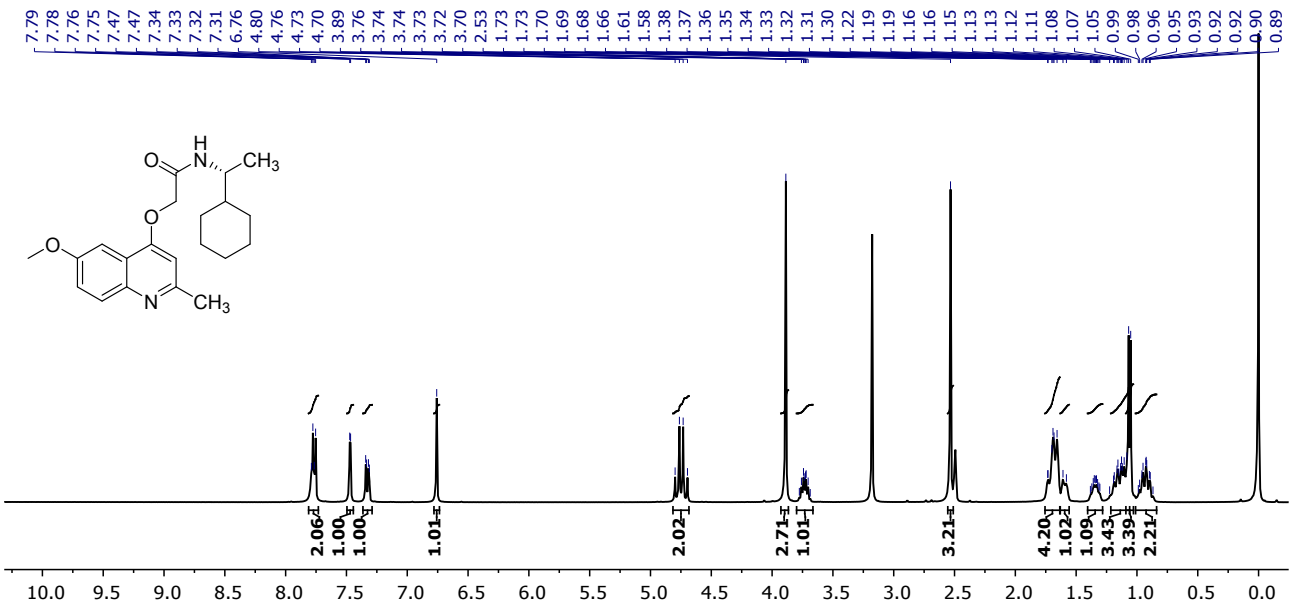

Figure S15 – ^{1}H NMR Spectrum of compound **6h** in DMSO-d_6.

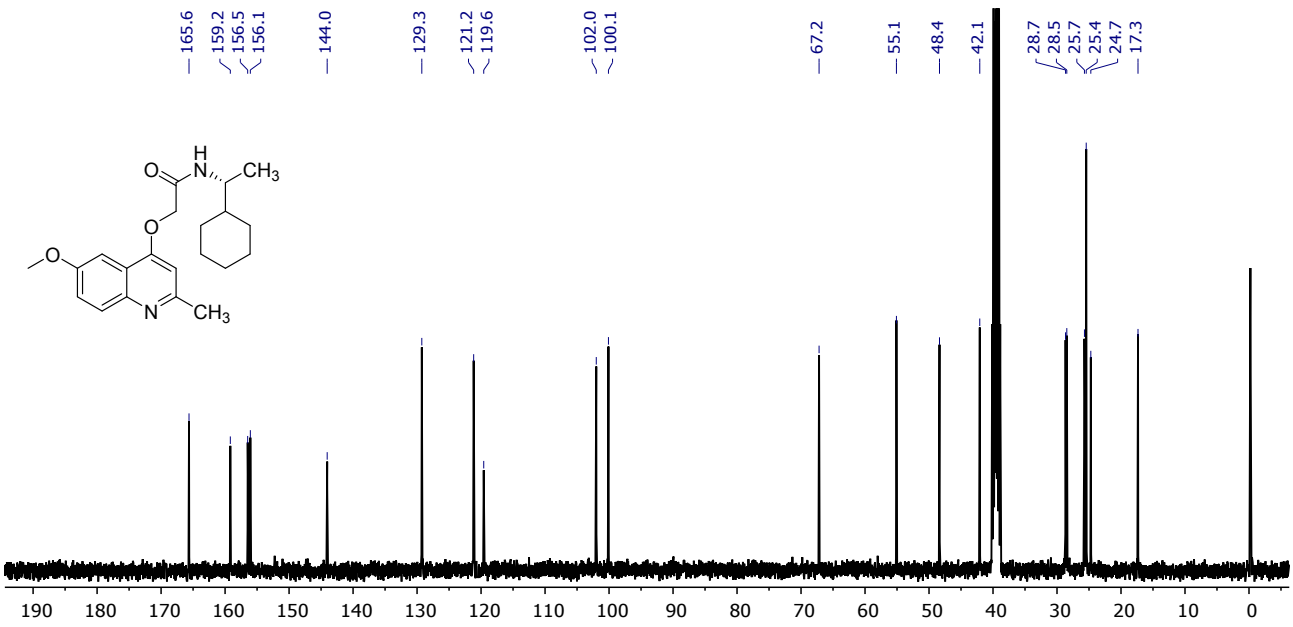

Figure S16 – ^{13}C NMR Spectrum of compound **6h** in DMSO-d_6.

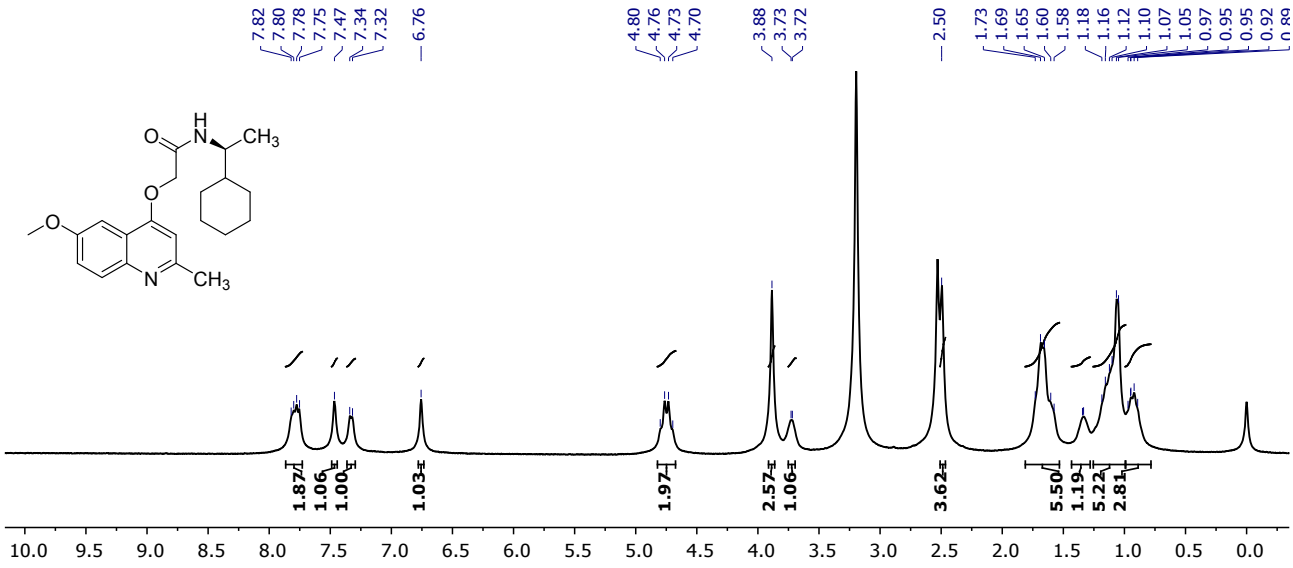

Figure S17 – ^{1}H NMR Spectrum of compound **6i** in DMSO-d_6.

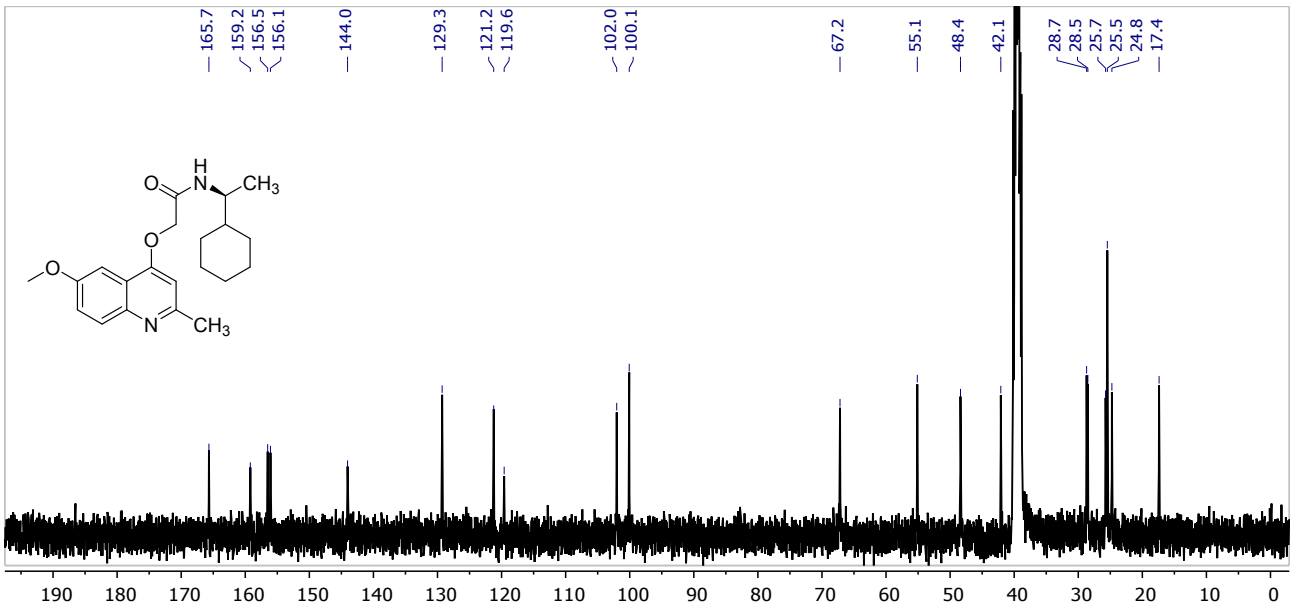

Figure S18 – ^{13}C NMR Spectrum of compound **6i** in DMSO-d_6.

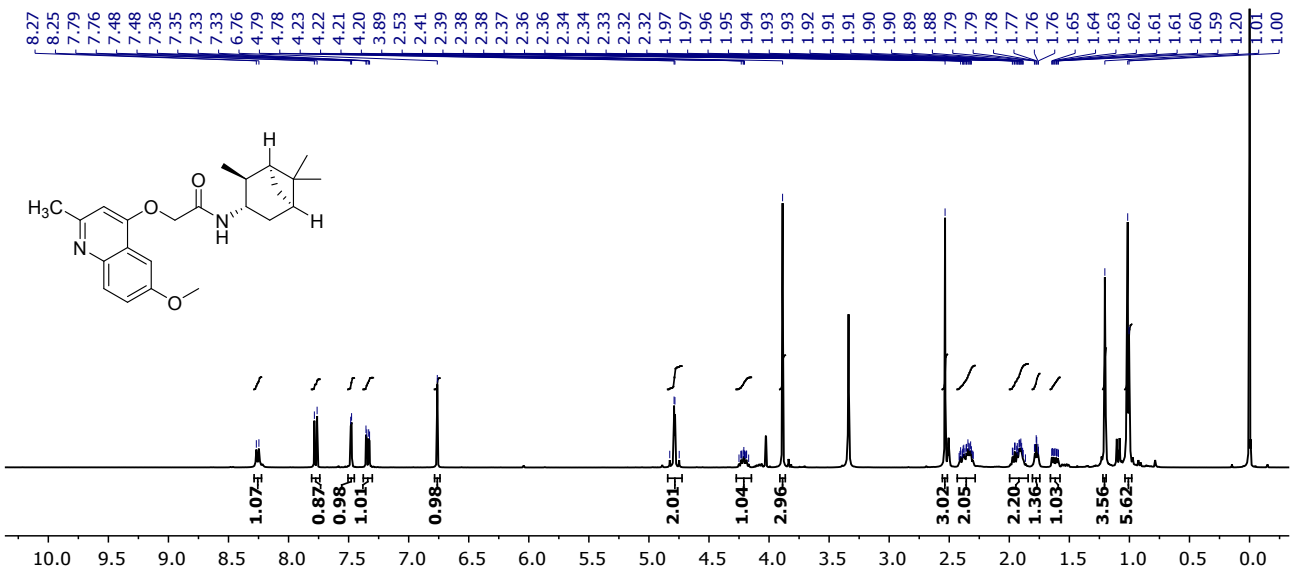

Figure S19 – ^{1}H NMR Spectrum of compound **6j** in DMSO-d_6.

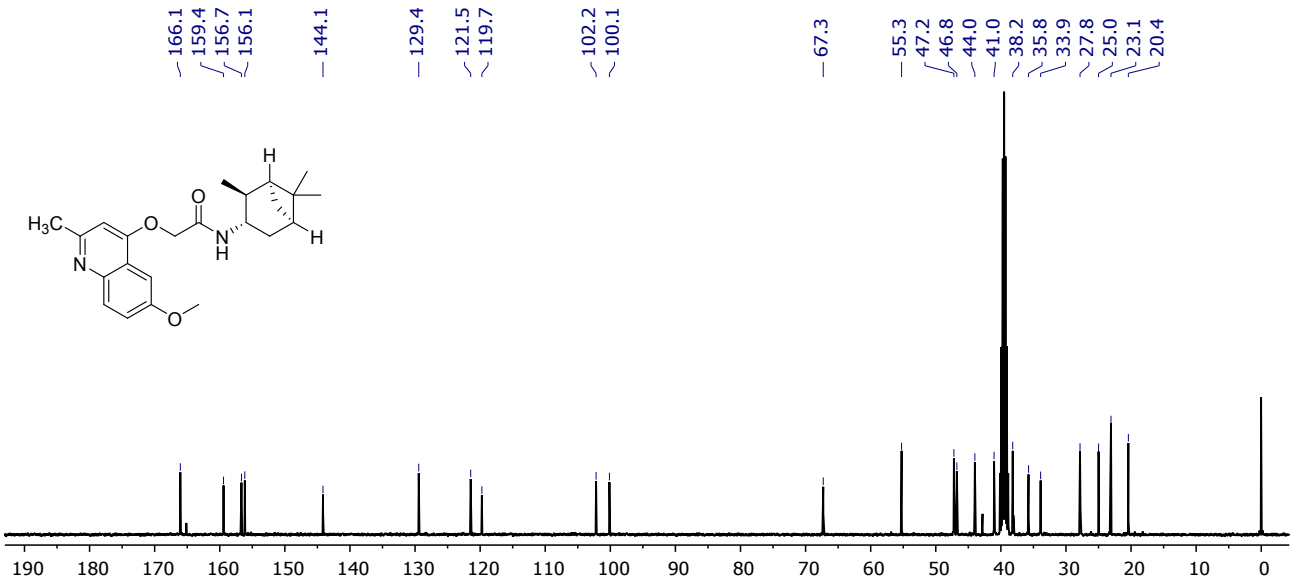

Figure S20 – ^{13}C NMR Spectrum of compound **6j** in DMSO-d_6.

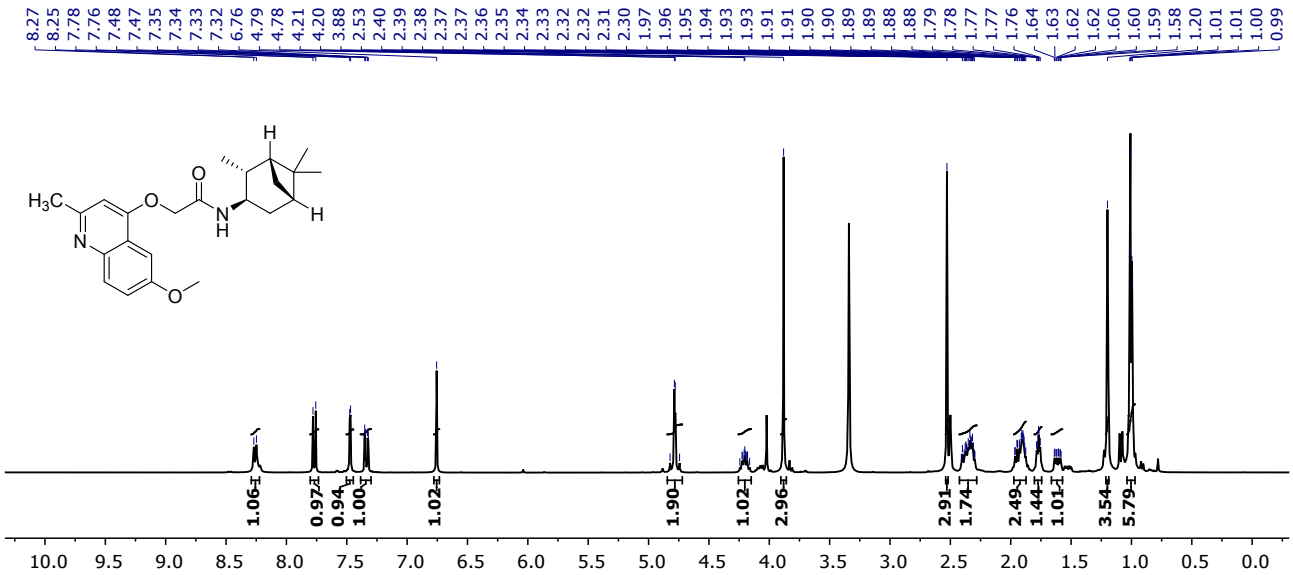

Figure S21 – ^{1}H NMR Spectrum of compound **6k** in DMSO-d_6.

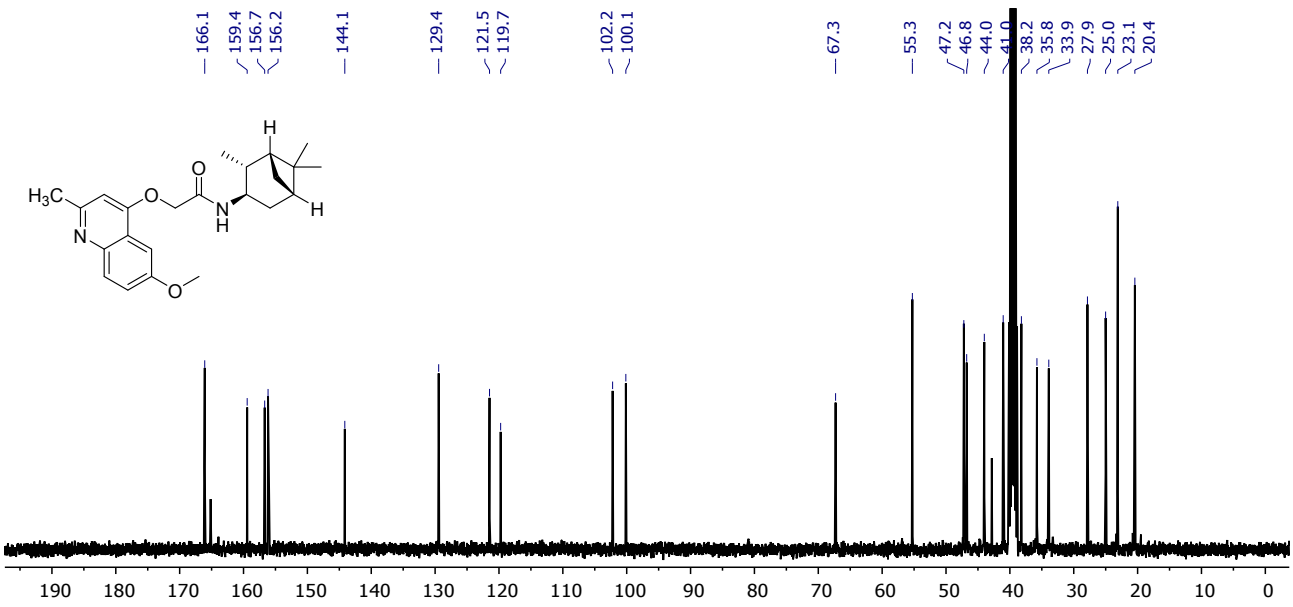

Figure S22 – ^{13}C NMR Spectrum of compound **6k** in DMSO-d_6.

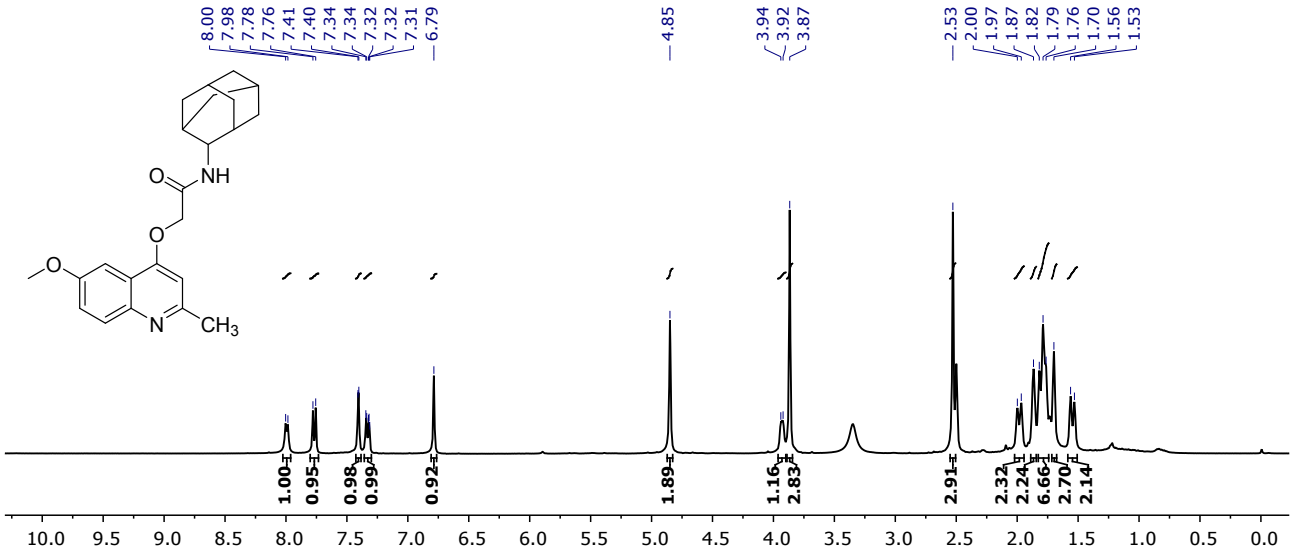

Figure S19 – ^{1}H NMR Spectrum of compound **6l** in DMSO-d_6.

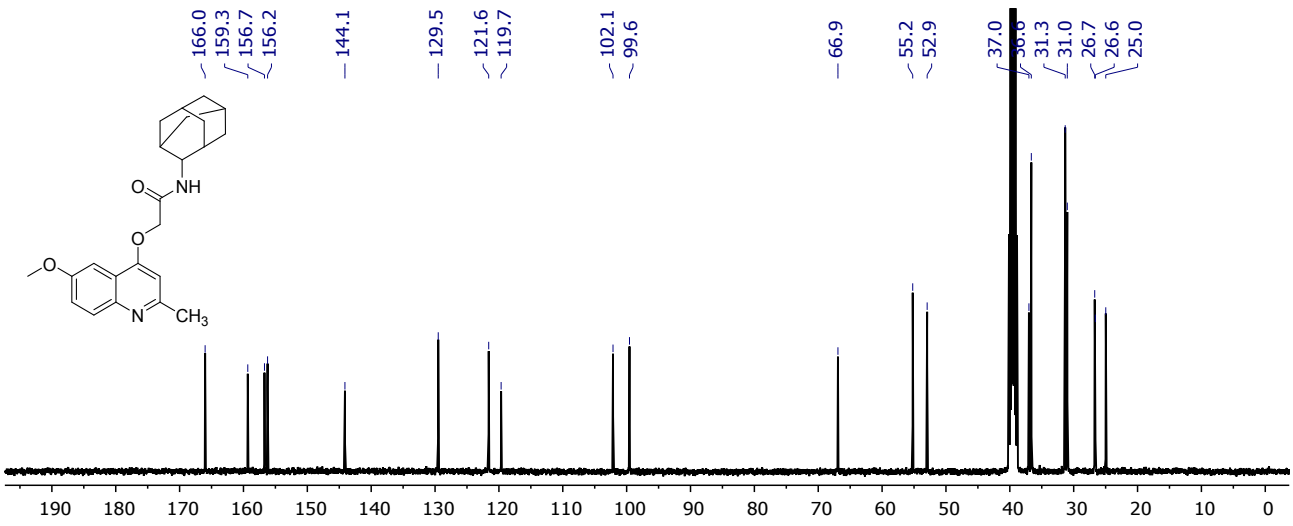

Figure S20 – ^{13}C NMR Spectrum of compound **6l** in DMSO-d_6.

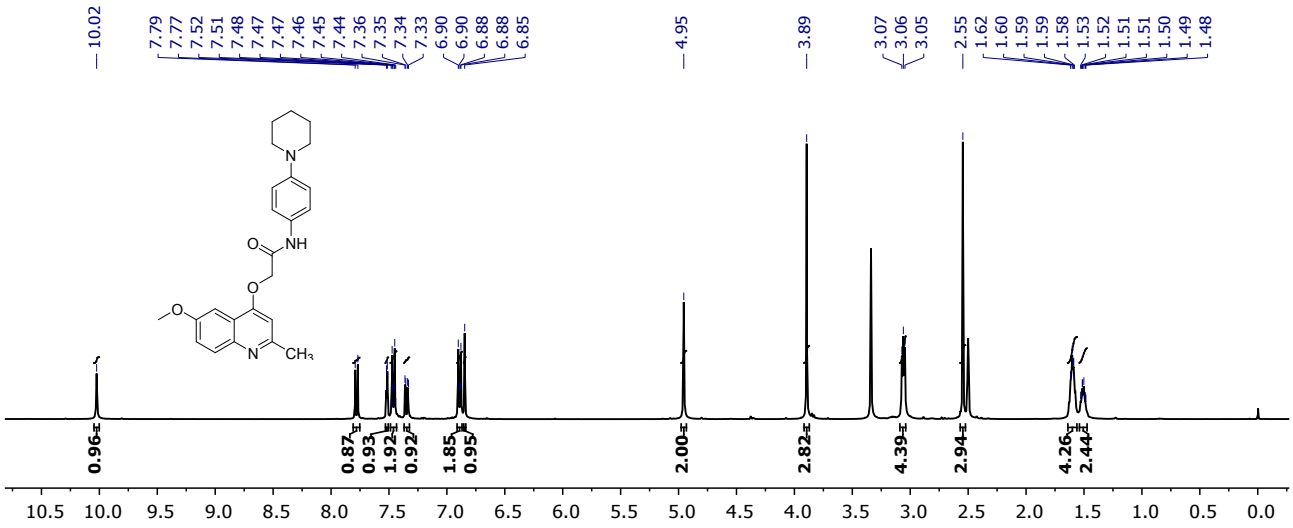

Figure S21 – ^{1}H NMR Spectrum of compound **6m** in DMSO-d_6.

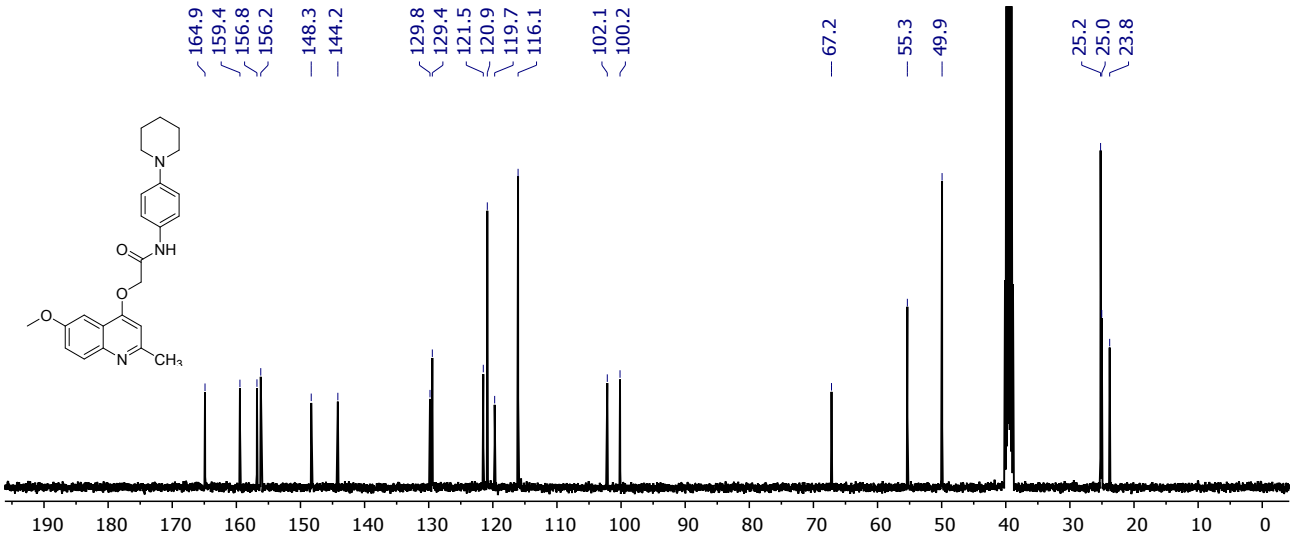

Figure S22 – ^{13}C NMR Spectrum of compound **6m** in DMSO-d_6.

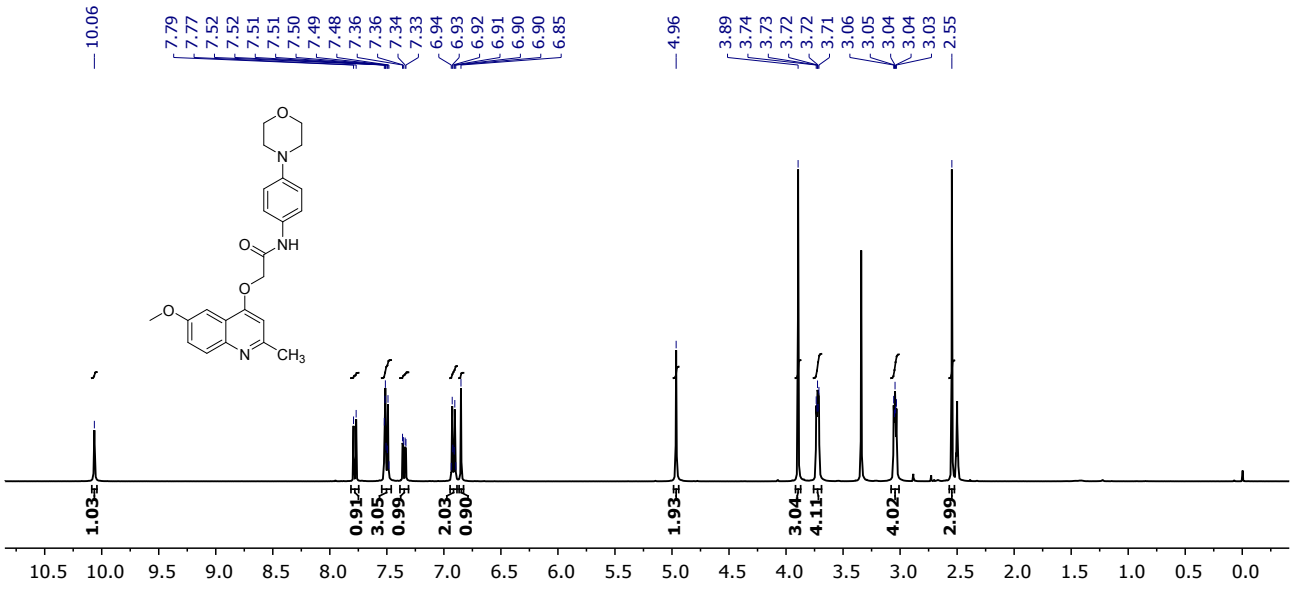

Figure S23 - ^{1}H NMR Spectrum of compound **6n** in DMSO-d_6.

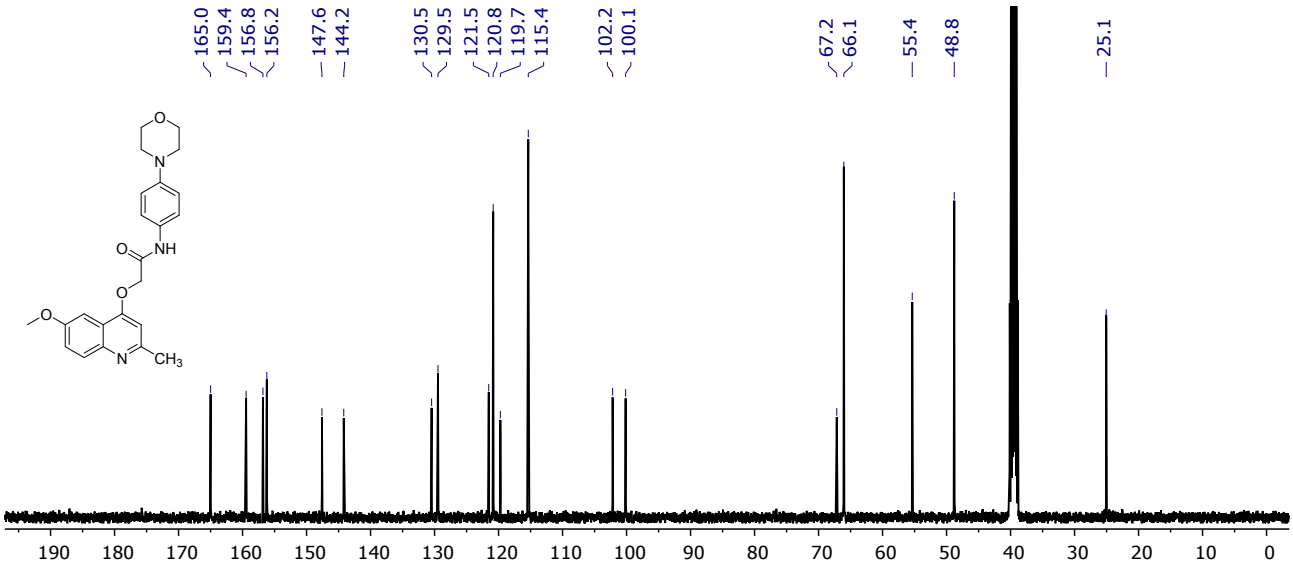

Figure S24 – ^{13}C NMR Spectrum of compound **6n** in DMSO-d_6.

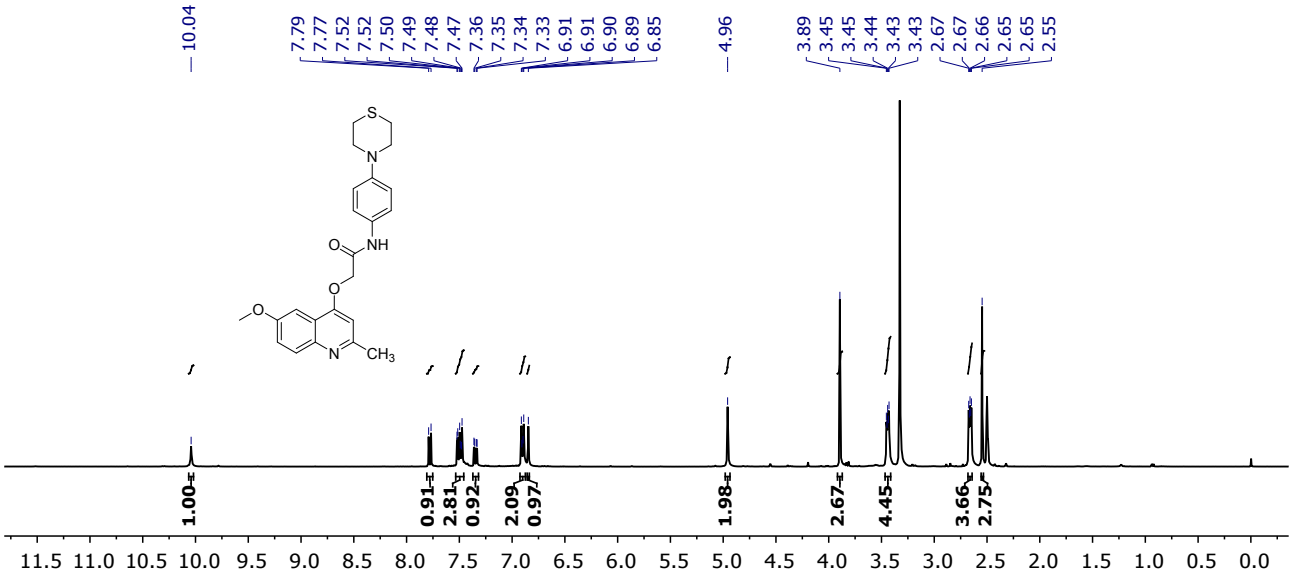

Figure S25 – ^{1}H NMR Spectrum of compound **6o** in DMSO-d_6.

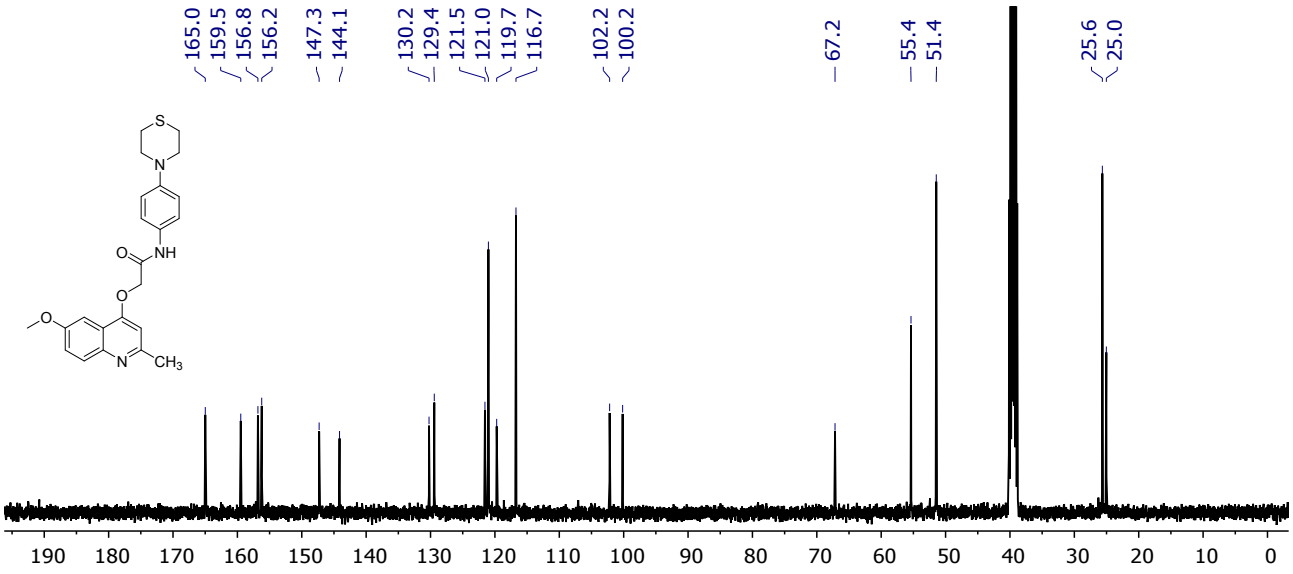

Figure S26 – ^{13}C NMR Spectrum of compound **6o** in DMSO-d_6.

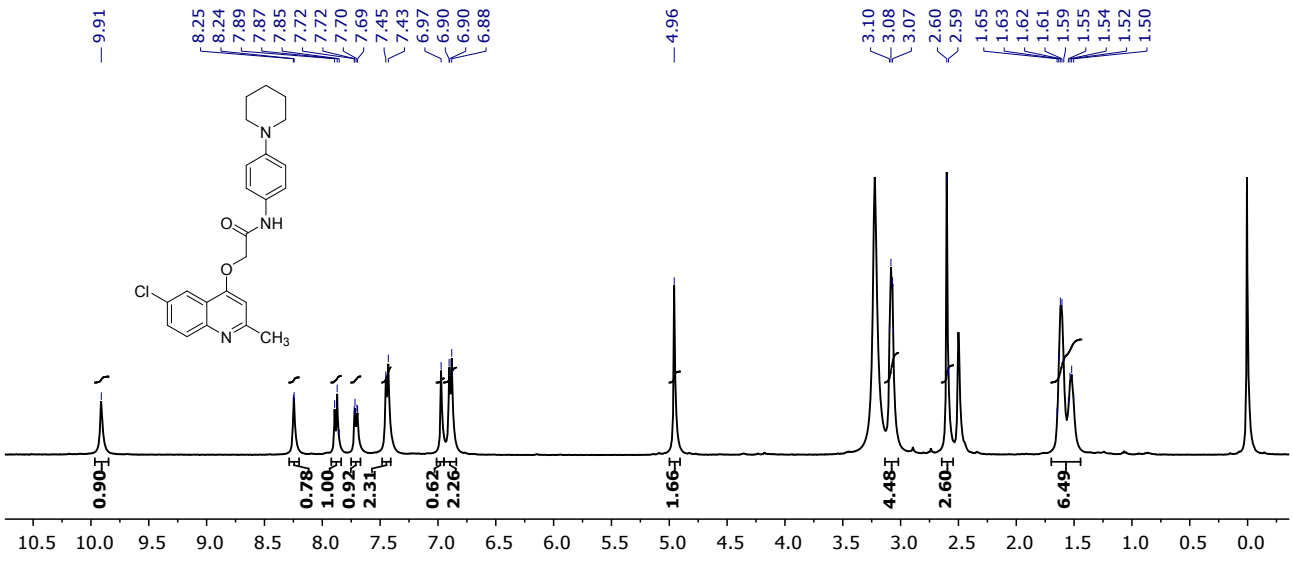

Figure S27 – ^{1}H NMR Spectrum of compound **9a** in DMSO-d_6.

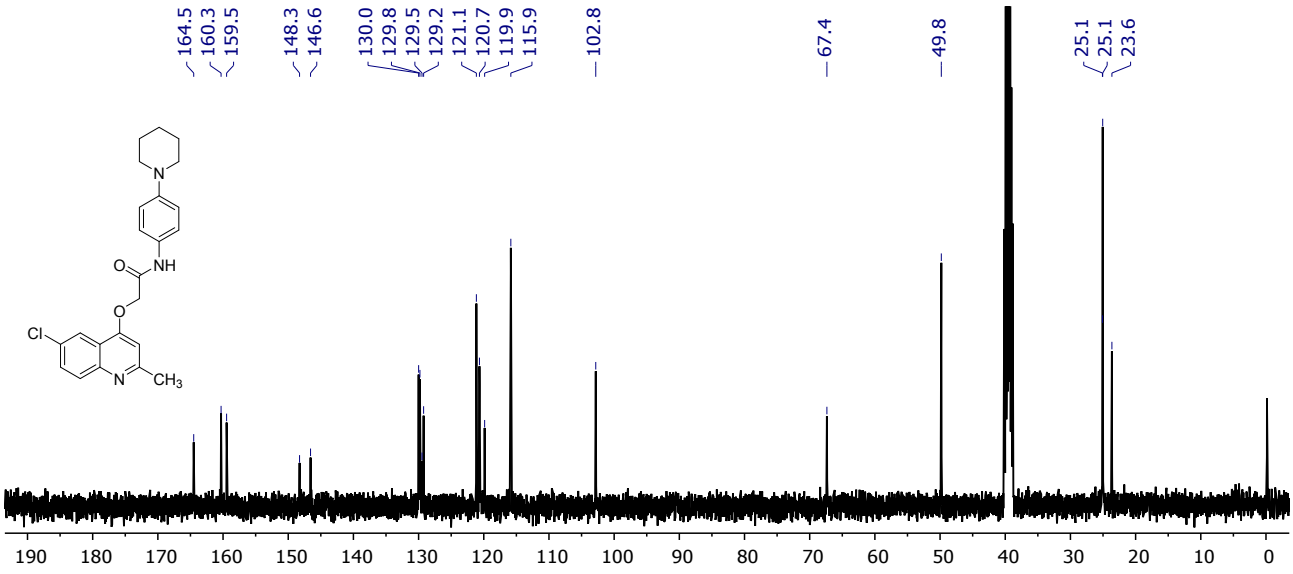

Figure S28 – ^{13}C NMR Spectrum of compound **9a** in DMSO-d_6.

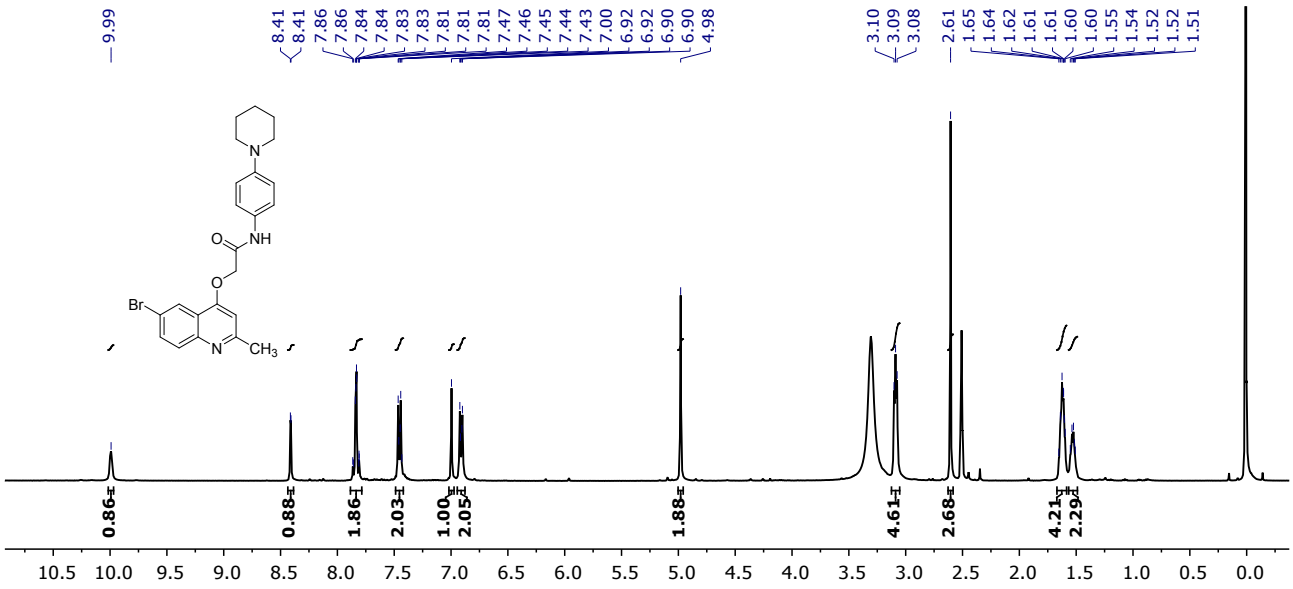

Figure S29 – ^{1}H NMR Spectrum of compound **9b** in DMSO-d_6.

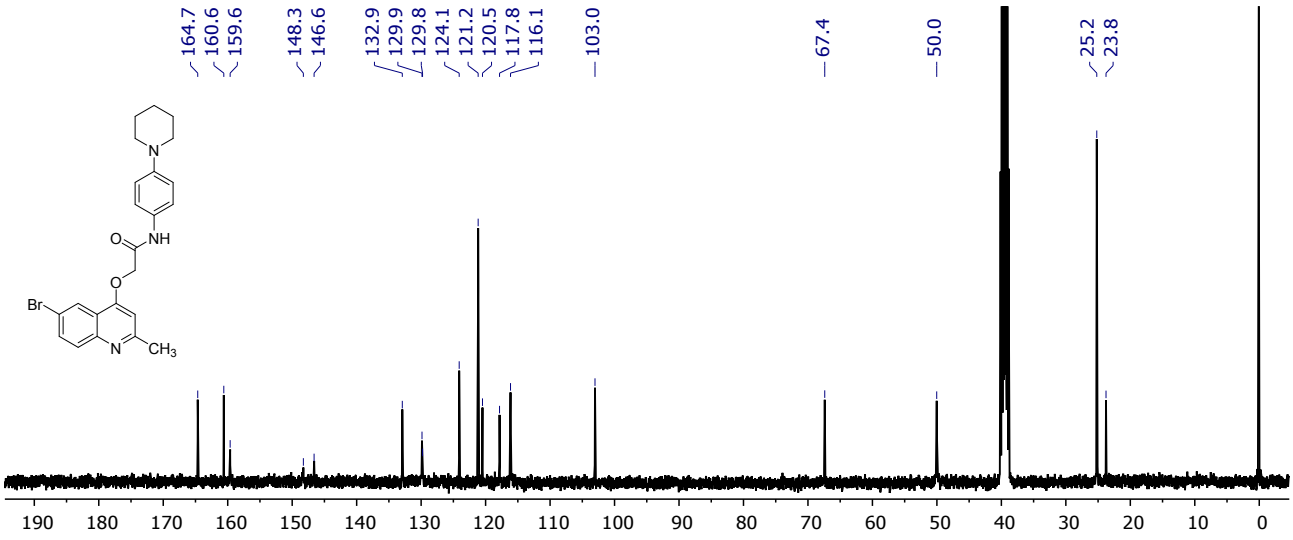

Figure S30 – ^{13}C NMR Spectrum of compound **9b** in DMSO-d_6.

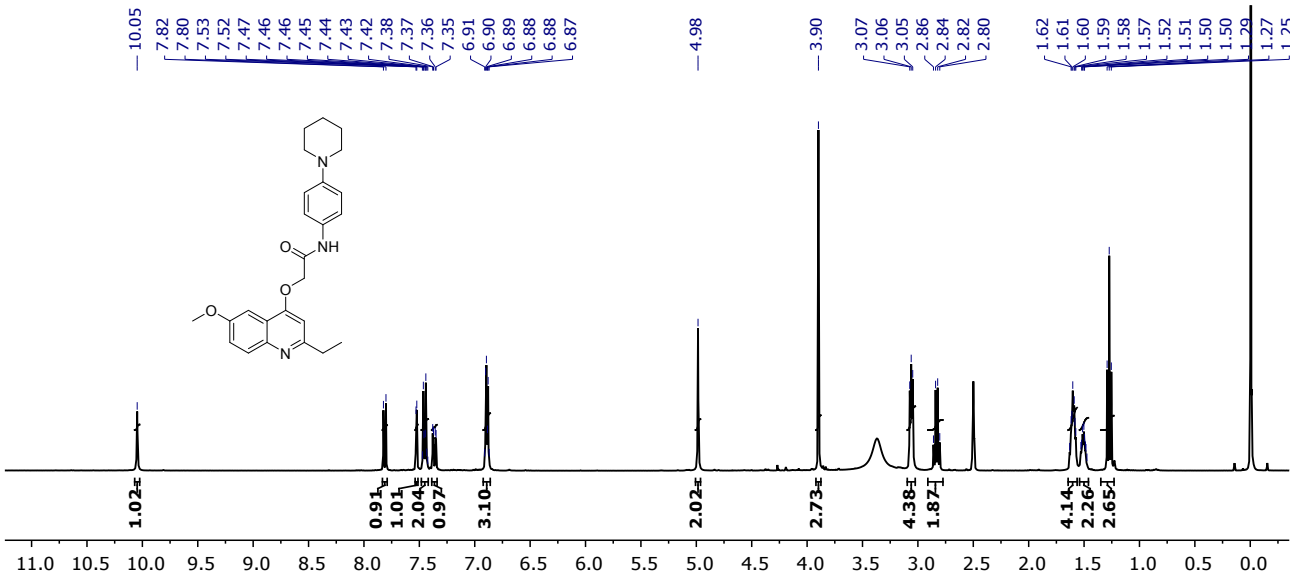

Figure S31 – ^{1}H NMR Spectrum of compound **9c** in DMSO-d_6.

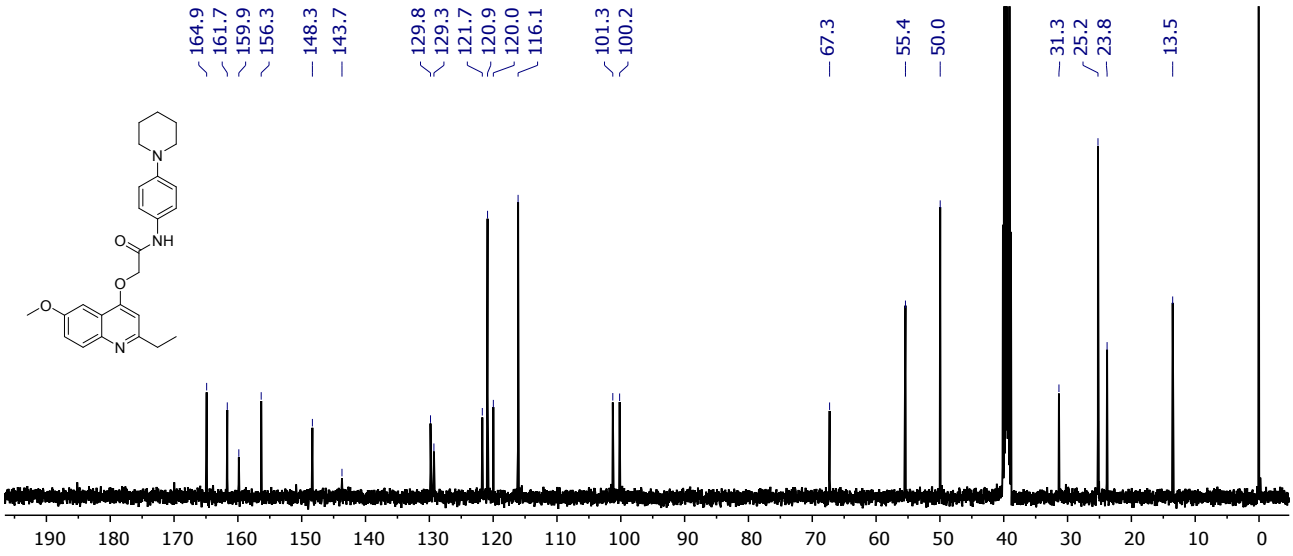

Figure S32 – ^{13}C NMR Spectrum of compound **9c** in DMSO-d_6.

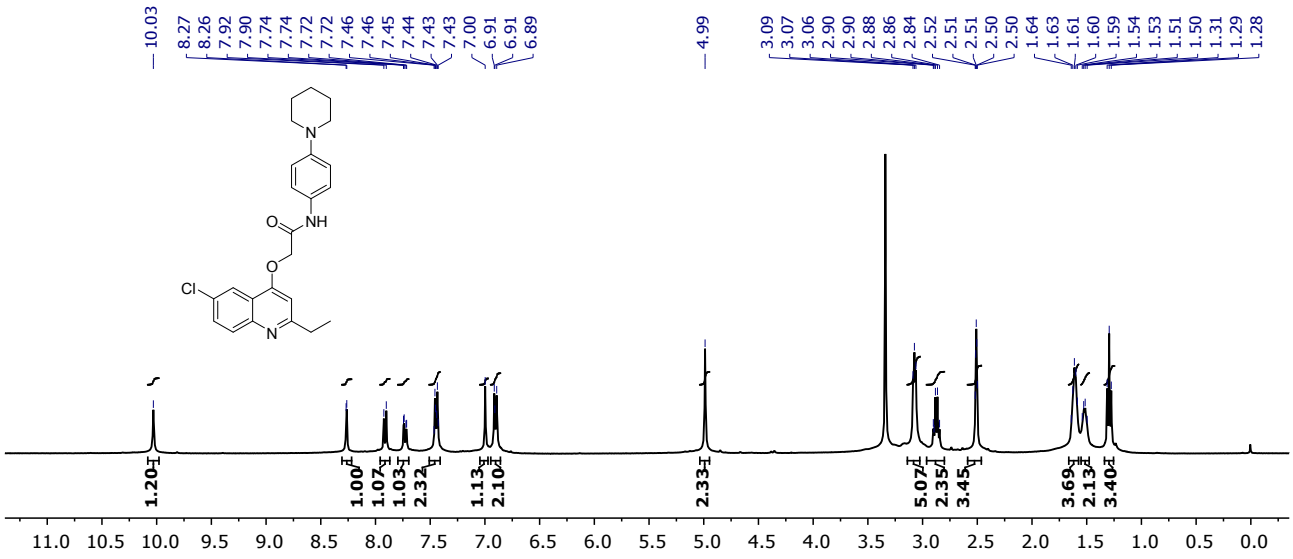

Figure S33 – ^{1}H NMR Spectrum of compound **9d** in DMSO-d_6.

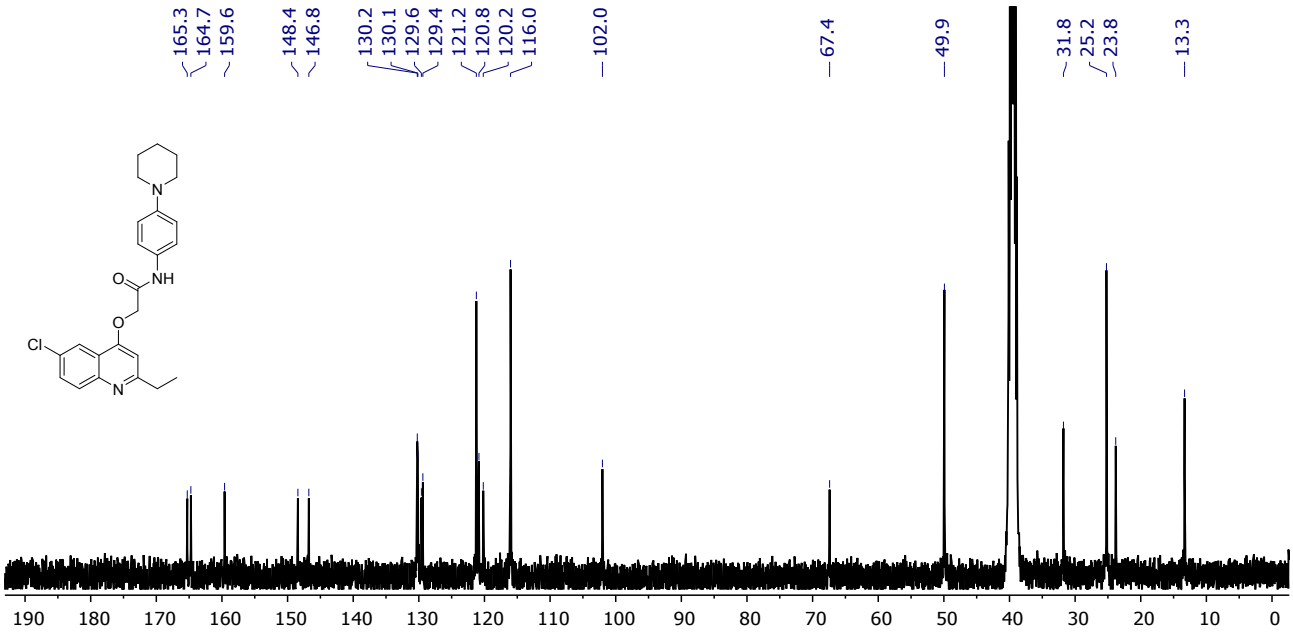

Figure S34 – ^{13}C NMR Spectrum of compound **9d** in DMSO-d_6.

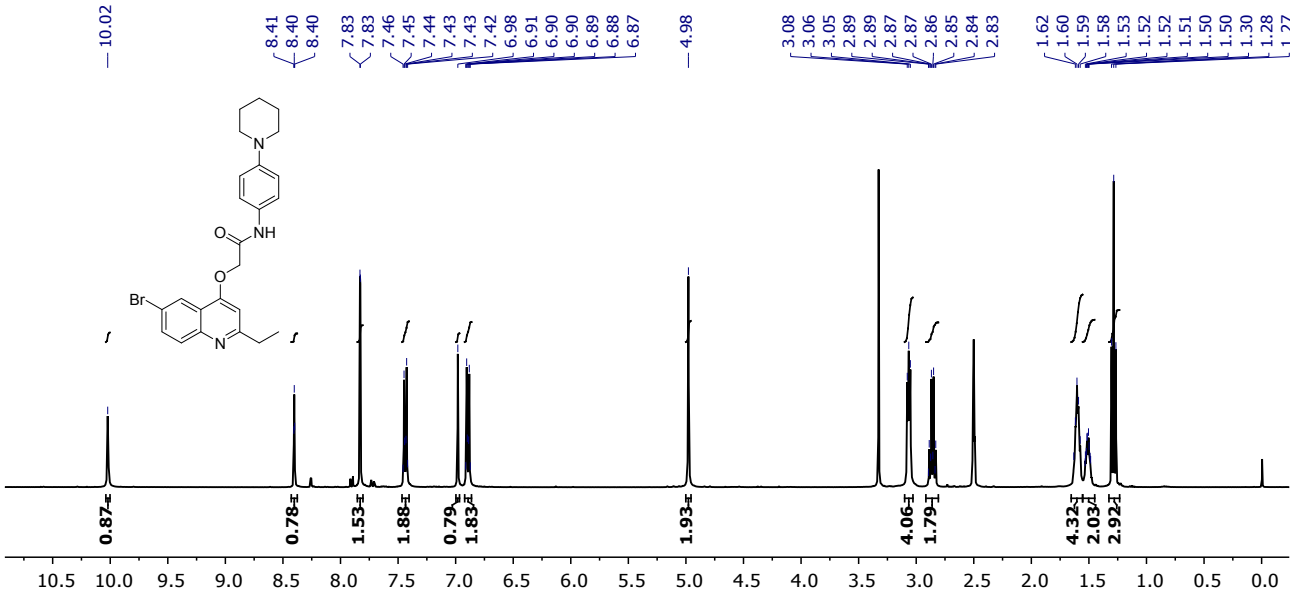

Figure S35 – ^{1}H NMR Spectrum of compound **9e** in DMSO-d_6.

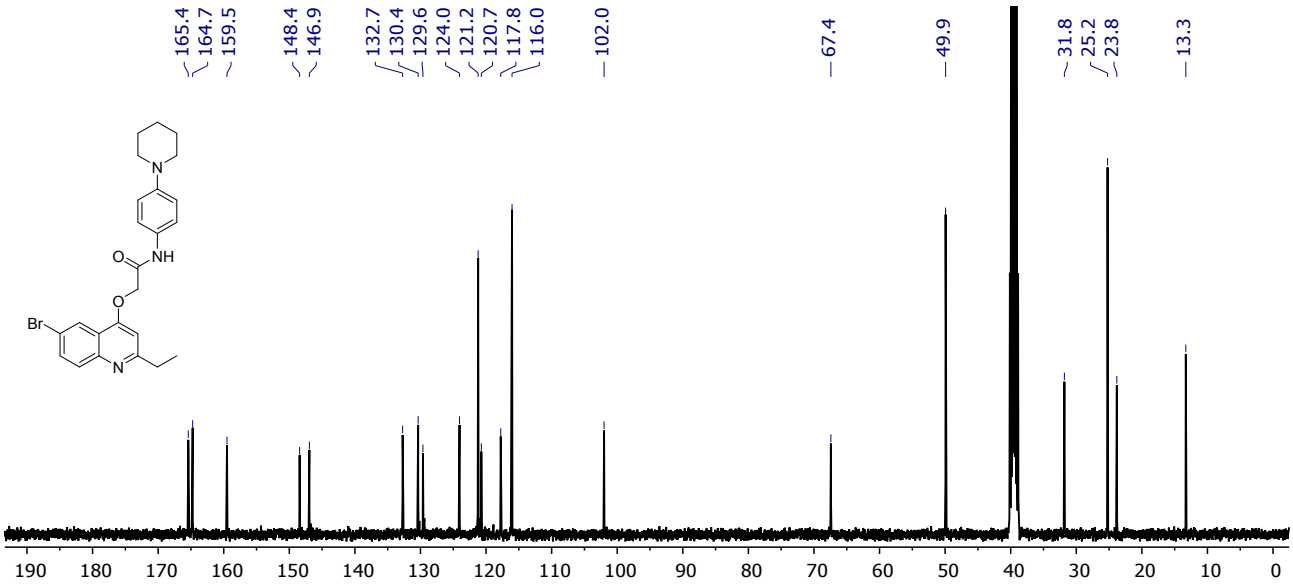

Figure S36 – ^{13}C NMR Spectrum of compound **9e** in DMSO-d_6.

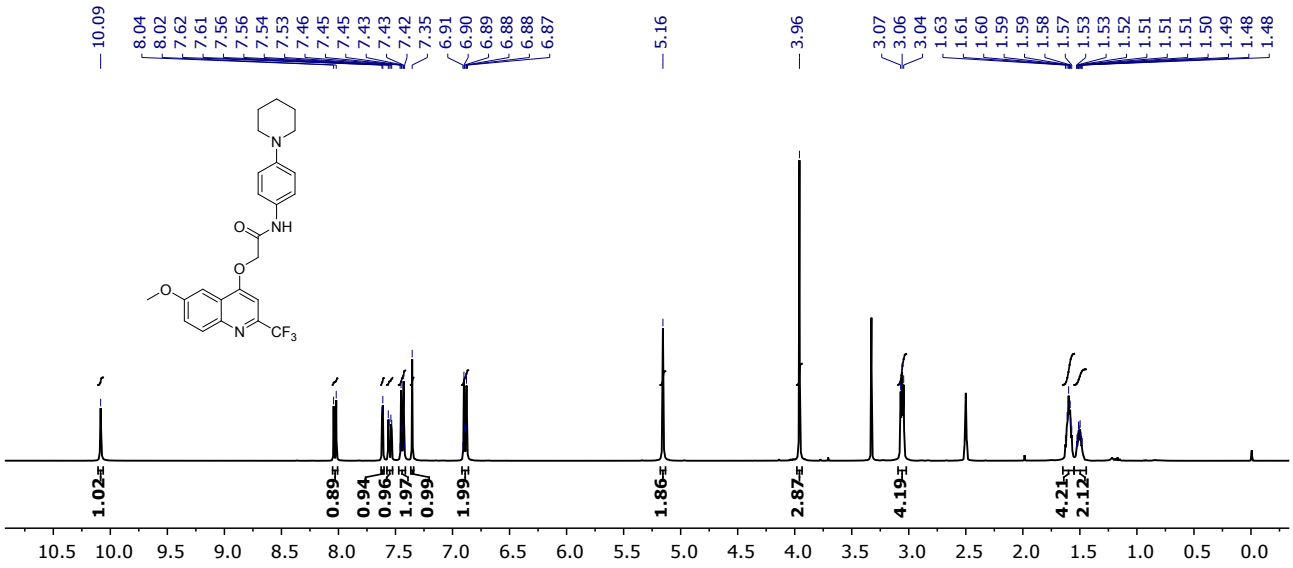

Figure S37 – ^{1}H NMR Spectrum of compound **9f** in DMSO-d_6.

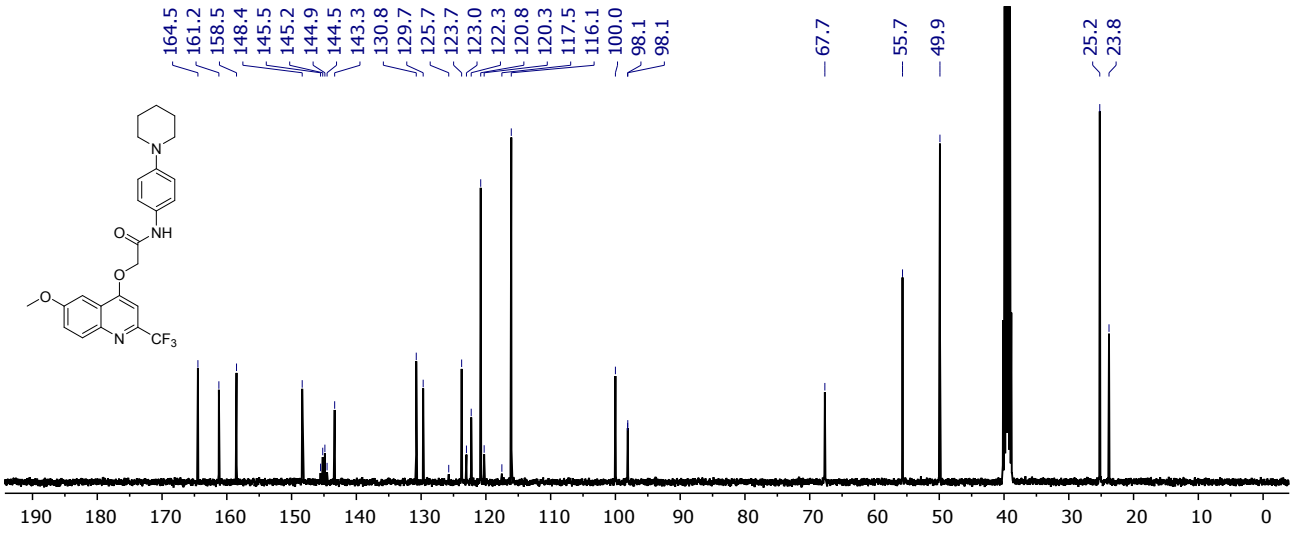

Figure S38 – ^{13}C NMR Spectrum of compound **9f** in DMSO-d_6.

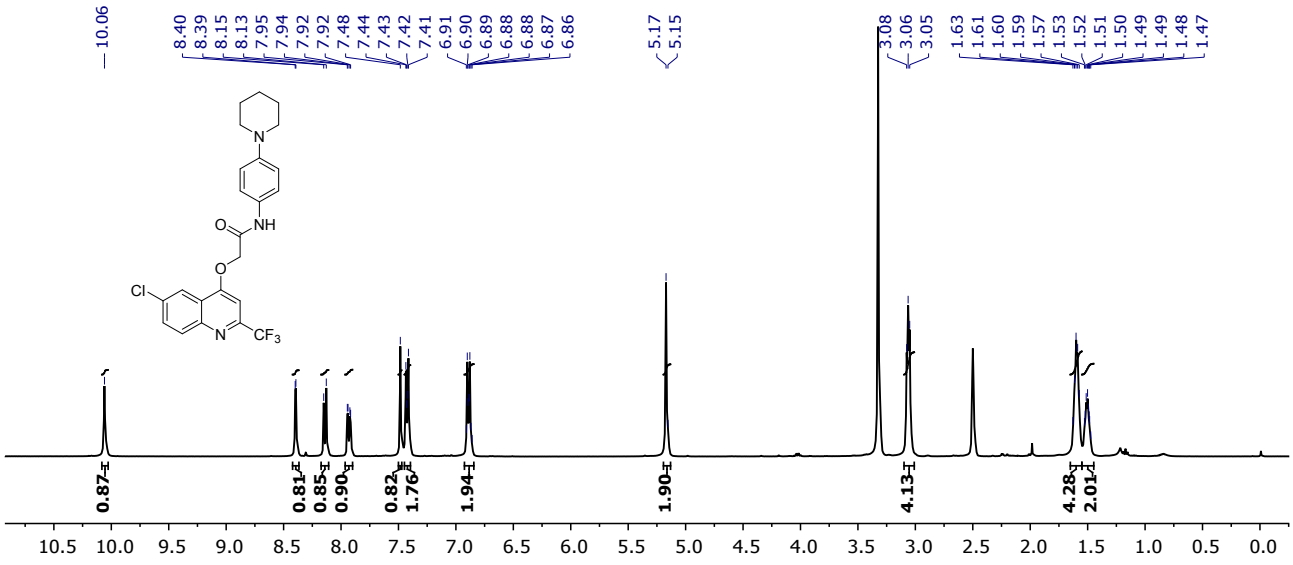

Figure S39 – ^{1}H NMR Spectrum of compound **9g** in DMSO-d_6.

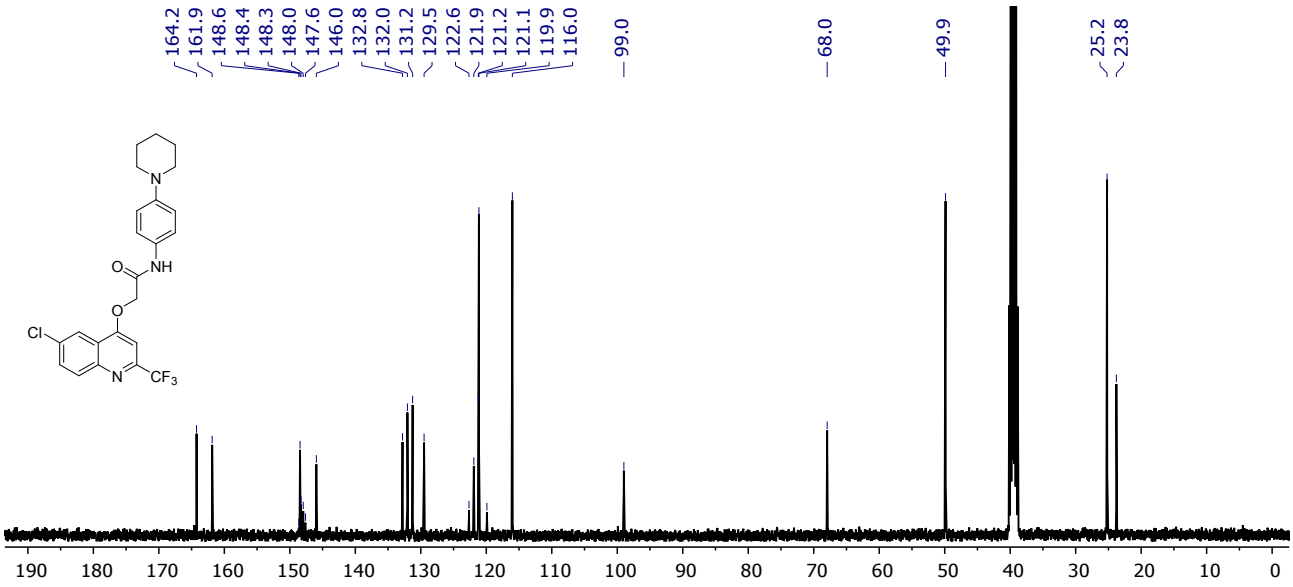

Figure S40 – ^{13}C NMR Spectrum of compound **9g** in DMSO-d_6.

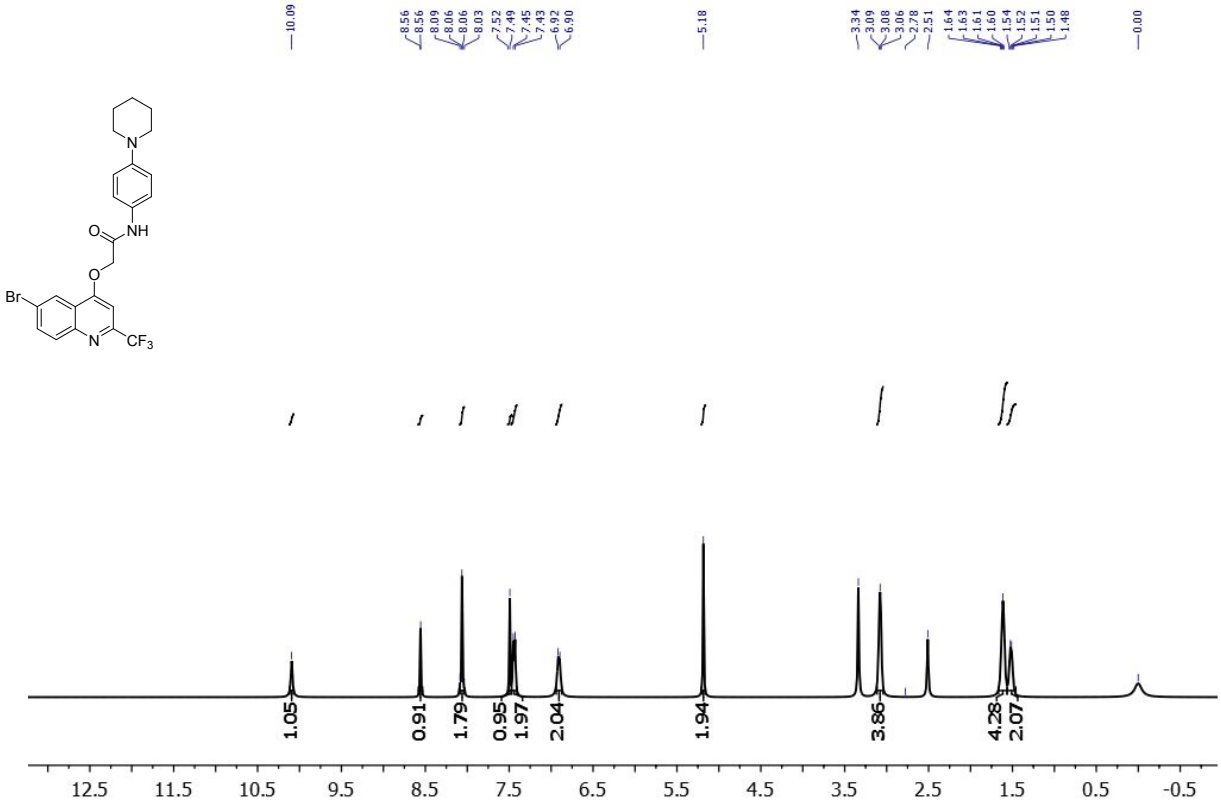

Figure S45 – ^{1}H NMR Spectrum of compound **9h** in DMSO-d_6.

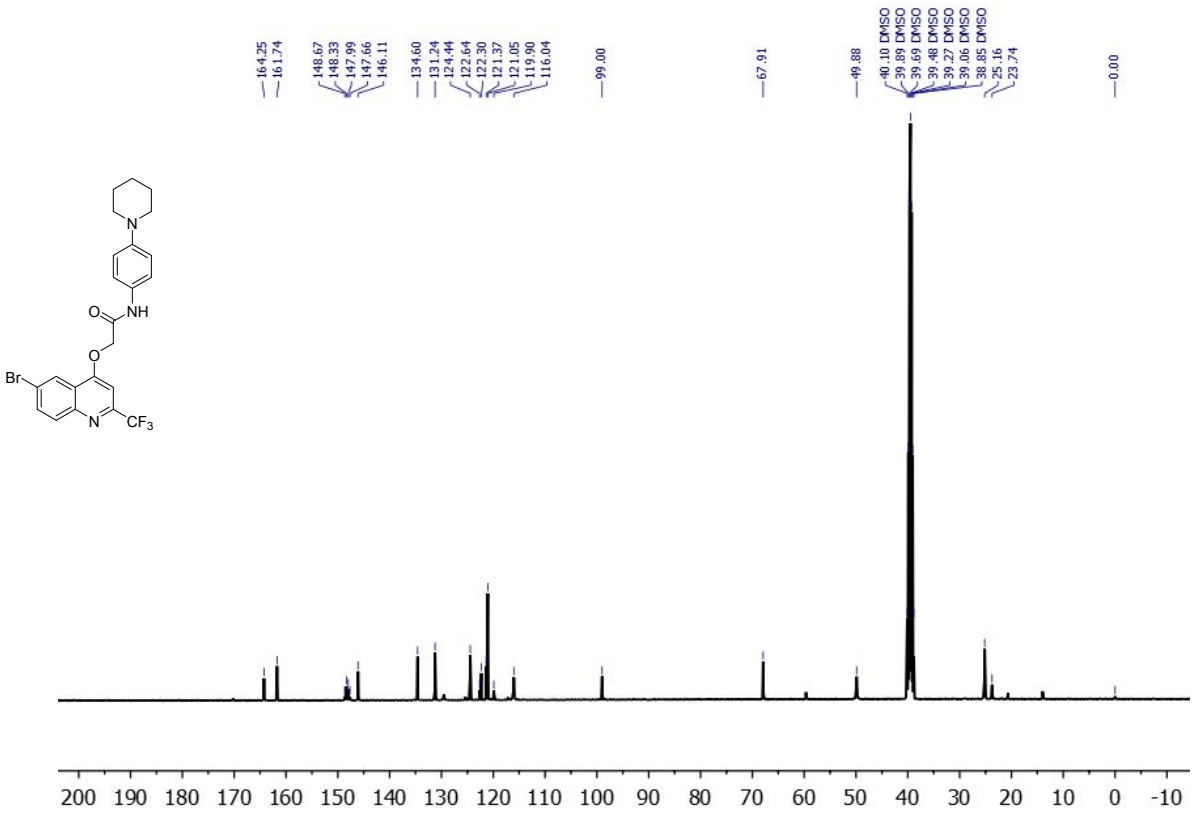

Figure S46 – ^{13}C NMR Spectrum of compound **9h** in DMSO-d_6.

3. Minimum Inhibitory Concentration

The determination of the minimum inhibitory concentrations (MICs) for each synthesized compound was performed in 96-well microplates. Isoniazid and rifampin were used as positive control and compound solutions were prepared at concentrations of 2 mg/mL in DMSO. They were diluted in Middlebrook 7H9 medium containing 10% ADC (albumin, dextrose, and catalase) to a concentration of 20 μg/mL of each compound containing 2% DMSO (Sigma Aldrich), and after were evaluated the possible presence of crystals, if it had crystals, it was diluted once more to half of the previous concentration. It is noteworthy that only molecules capable of forming a real solution were evaluated. Serial two-fold dilutions of each drug in 100 μL of Middlebrook 7H9 medium containing 10% ADC (BD co.) were prepared directly in 96-well plates at concentration ranges starting with the maximum concentration allowed by the solubility of each compound. Growth controls without antibiotic and sterility controls without inoculation were included. Mycobacterial strains were grown

in Middlebrook 7H9 containing 10% OADC (oleic acid, albumin, dextrose, and catalase) and 0.05% tween 80, and cells were vortexed with sterile glass beads (4 mm) for 5 min to disrupt clamps and then allowed to settle for 20 min. The supernatants were measured spectrophotometer at an absorbance of 600 nm. The Mtb suspensions were aliquoted and stored at -20 °C. Each suspension was appropriately diluted in Middlebrook 7H9 broth containing 10% ADC to achieve an optical density of 0.006 at 600 nm, and 100 µl was added to each well of the plate except to the sterility controls. The plates were covered, sealed, and incubated at 37 °C. After 7 days of incubation, 60 µL of 0.01% resazurin solution was added to each well, and the plate was incubated for an additional 48 hours at 37 °C. The MIC assay is based in an alteration in the color which indicate bacterial growth when blue change to pink, and the MIC was stablished as the lowest compound concentration previously the color change. Three tests were performed independently for each chemical structure and the MIC values were reported the highest value among the three assays. It is noteworthy that clinical isolates (named PT2, PT12 and PT20) were obtained from patients in the Lisbon Health Region, Lisbon, Portugal. INH and RIF were used as control drugs to demonstrate the MDR phenotype of these isolates.

4. Cellular Viability Evaluation

Cellular viability determination of was performed by two distinct methods using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) and neutral red uptake (NRU). The cell lines employed in the evaluation were Vero and HepG2 cells. The both cell lines were grown with Dulbecco's Modified Eagle Medium (DMEM-Gibco, Grand Island, NY, USA) and supplemented with 10% disable fetal bovine serum by Invitrogen, 1% antibiotics (penicillin and streptomycin) by Gibco, and 0.1% fungizone by Gibco. For the MTT and NRU assay, Vero (2 x 10^3 cells/well) and HepG2 (4 x 10^3 cells/well) cells were seeded in 96-well culture plates and incubated overnight. The evaluated compounds were diluted at concentrations of 20 µM using DMSO 1% and

were incubated with the cell lines for 72 h at 37 °C. In the MTT investigation, after incubation for 72 h at 37 °C under 5% CO_2, the cells were incubated with MTT solution (5 mg/mL, Sigma) for 4 h. The formazan crystals were solubilized in 100 µL of DMSO. EZ Read 400 microplate reader (Biochrom, Cambridge, UK) was used to measure the absorbance at 570 nm. The mean absorbance of negative control wells was stablished as maximum viability and the values of treated cells were calculated as the percentage of vehicle control (1% DMSO). The precipitated purple formazan crystals were directly equivalent to the number of live cells with active mitochondrial metabolism. In the NRU investigation, after 72 h of cell incubation PBS was used to wash the cells and 200 µL of neutral red dye solution (25 µg/mL, Sigma) prepared in serum-free medium was added to the plate and incubated for 3 h at 37 °C under 5% CO_2. Cells were washed with PBS followed by the addition of 100 µL of desorb solution (ethanol/acetic acid/water, 50:1:49) for 30 min with smoothly homogenizing to extract neutral red dye from the viable cells. EZ Read 400 microplate reader was operated to measure the absorbance at 562 nm, and the cell viability was attributed as a percentage, considering the vehicle control cell (1% DMSO) as maximum cell viability.

5. Intracellular activity in a macrophage model of *M. tuberculosis* infection

Murine macrophage RAW 264.7 cells were cultured in RPMI 1640 medium (Gibco), supplemented with 10% heat-inactivated fetal bovine serum (FBS), without penicillinestreptomycin, and about 5×10^4 cells were seeded in each well of a sterile flat-bottom 24-well plate. After an incubation period of 24 h in a bacteriological chamber (at 37 °C with 5% CO_2 and a humid atmosphere), the adhered cells were washed once with pre-heated sterile PBS (pH 7.4) to remove non-adherent cells, and the infection occurred as follows. One isolated colony of the *M. tuberculosis* H37Rv strain was cultured in 5 mL of 7H9-ADC broth, supplemented with 0.05% (v/v) Tween 80 (Sigma-Aldrich) and 0.2% (v/v) glycerol (MERCK) until the mid-log phase ($OD_{600} \approx 0.5$). The culture was diluted in pre-heated RPMI medium, and approximately 2.5×10^4 CFU was added to each well. The infection was allowed to continue for 3 h at 37 °C with 5% CO_2. Afterwards, the

infected cells were washed twice with sterile PBS to remove non-internalized mycobacteria. Thereafter, the infected cells were treated with 2.5 μM of each test compound in triplicate. Compounds were first solubilized (2 mM) in neat DMSO, and then diluted in 2 mL of RPMI medium to a final concentration of 2.5 μM. The final DMSO concentration was maintained at 0.5% in each well. After 5 days of treatment, the RPMI medium was removed, and each well was gently washed with PBS. The treated macrophages were lysed with 0.025% SDS, serially diluted in 0.9% saline, and plated on 7H10 agar. After an incubation period of 2-3 weeks at 37 °C, the CFU were counted, setting a limit of detection (LOD) between 20 and 200 CFU per plate. The calculated CFU values were converted into logarithms of CFU before statistical analysis, and the result was expressed as the mean of the $\log_{10}$ CFU values per well ± the standard deviation (mean $\log_{10}$ CFU/well ± SD). Groups were compared by one-way analysis of variance (ANOVA), followed by the Tukey post-test, using GraphPad Prism 5.0 (GraphPad, San Diego, CA, USA). Significance between groups was determined using $P < 0.05$.

6. Chemical Stability

The experiment was carried out by Center for Applied Mass Spectrometry (CEMSA), São Paulo, Brazil. In brief, the compound **6m** (10 μM) was incubated at 37 °C for 24 h in the presence of pH-controlled buffer solution pH 1.2 (simulating the pH of the stomach – 0.1 M HCl), pH 7.4 (simulating plasma pH – phosphate buffer) and pH 9.1 – simulating intestinal pH – 0.1M NH_4HCO_3). Afterward, compounds were quantified by HPLC-MS/MS. Alprenolol drug was used as an analytical control. The results were presented as percentage, comparing the signal at time zero of the assay (100%) with the signal produced by concentrations after the incubation period.

7. Solubility

The solubility of **6m** was performed according to protocol implemented in our labs. Accordingly, 1 mL of a prepared solution (1× PBS, pH 7.4 or 0.1 M HCl 0, pH 1.0 solution) was

added to 1 mg of **6m** (in triplicate). The final solutions were vortexed (1 min) and the resulting suspensions were shaken for 4 h at 25 °C. Then, the solutions were centrifuged (13000 rpm for 15 min at 25 °C) obtaining a pellet and the remaining solutions were quantified by liquid chromatography (as per the conditions described in the experimental section) using single-point calibration of a known concentration of the compounds in DMSO.

8. Permeability (PAMPA)

The experiment was carried out by Center for Applied Mass Spectrometry (CEMSA), São Paulo, Brazil. In brief, PAMPA (Parallel Artificial Membrane Permeability Assay) assay consists of quantifying the test compound (via HPLC-MS/MS) after an incubation period, in two solutions separated by an artificial lipid membrane. The result of this test was expressed in units of diffusion rate (permeation). The procedure comprised the following steps: (1) previously preparing the membrane, which contains lipids, with specific activating solutions, creating a hydrophobic surface that simulated the intestinal epithelial cell; (2) add the test compound (**6m**) at a concentration of 10 μM in the donor aqueous phase (buffered pH 7.4); (3) after 5 hours at room temperature an aliquot was removed of the solution receptor (buffered at pH 7.4), in which the compound was transported by passive diffusion, for quantification by HPLC-MS/MS.

9. Metabolic stability assay

The experiment was carried out by Center for Applied Mass Spectrometry (CEMSA), São Paulo, Brazil. In brief, the metabolic stability assay was performed in the presence of rat liver microsomes. Compound **6m** was incubated at 37 °C in a buffered solution containing 1 mg/mL of microsomal protein and nicotinamide adenine dinucleotide (NADH). The reaction was stopped at different times using acetonitrile. The compound concentration was determined by HPLC-MS/MS at each incubation time (0, 5, 15, and 30 min) and the percentage remaining *versus* time curve was determined. Verapamil was used as an analytical control.

10. Pharmacokinetic experiments

10.1. HPLC analysis and sample preparation

The analysis of 2-(quinoline-4-yloxy)acetamide **6m** was performed using a Dionex Ultimate 3000 HPLC with UV/Visible detector. A RP column, 5 mm Nucleodur C-18ec (250 × 4.6 mm) (Macherey-Nagel, Germany) was used. 90% Water (1% acetic acid) and 10% acetonitrile was maintained from 0 to 0.5 min, followed by a linear gradient from 70 % water (1% acetic acid) to 30% acetonitrile from 0.5 to 10.5 min, and subsequently returned to 90% water (1% acetic acid) and 10% acetonitrile (10.5-11 min) and maintained for up to 14.3 min. The flow rate was 1.5 mL/min at 20 °C (column temperature). The autosampler temperature was also maintained at 20 °C and the injection volume was 90 μL. The detection was at 254 nm. Sample processing was accomplished according to previously reported methods used for lipophilic compounds with some slight modifications.[1,2] To each 100 μL of spiked plasma or test samples, 100 μL of acetonitrile was added and vortexed (1 min). After, the samples were centrifuged at 13,000 rpm for 15 min at 4 °C. The supernatants were removed and transferred to Eppendorf tubes with the subsequent addition of 300 μL of dichloroethane. The cloud solutions formed were vortexed for 5 s, and the extraction phases were again transferred to other Eppendorf tubes. The organic solvent was evaporated, and the residues obtained were reconstituted in 150 μL of water (1% acetic acid). The samples were then injected into the HPLC system. The retention time of the compound **6m** was approximately 4.9 min and the calibration curves (triplicate, constructed by spiking commercial blank plasma with **6m**) were linear in the concentration range investigated (0.1–50 μg/mL, r = 0.9951).

10.2. Naïve pooling compartmental analysis

The concentration *versus* time data was analyzed applying the naïve pooled approach (NPA) using NONMEM (Nonlinear mixed effects model, version 7.4.3, Certara, USA) with support of PsN (Perl-Speaks-NONMEM, version 4.9.0) and Pirana (version 2.9.9, Certara) for controlling runs and R (version 4.0, R Foundation for Statistical Computing, Vienna, Austria) for data visualization. The

estimation method was a first-order conditional estimation with interaction (FOCE+I). Since one mice was euthanized at each time point, it was assumed that each data set was obtained from a single subject following different iv or oral dosing. Accordingly, there was no differentiation on between-subject variability and all sort of variability was lumped into residual error model. Plasma concentration data following intravenous dose was modeled as a two-compartment model (ADVAN3, TRANS4), parameterized in terms of CL, V1, Q, and V2. A one-compartment model (ADVAN2, TRANS2), parameterized in terms of CL, V1, and ka, best described the oral data. The assumption of different compartment models for the routes of administration improved the precision estimates and stability of the model. The bioavailiabity was estimated by the area under the curve of the modeled oral dose over the area under the curve of the modeled intravenous dosing taking the doses into consideration.

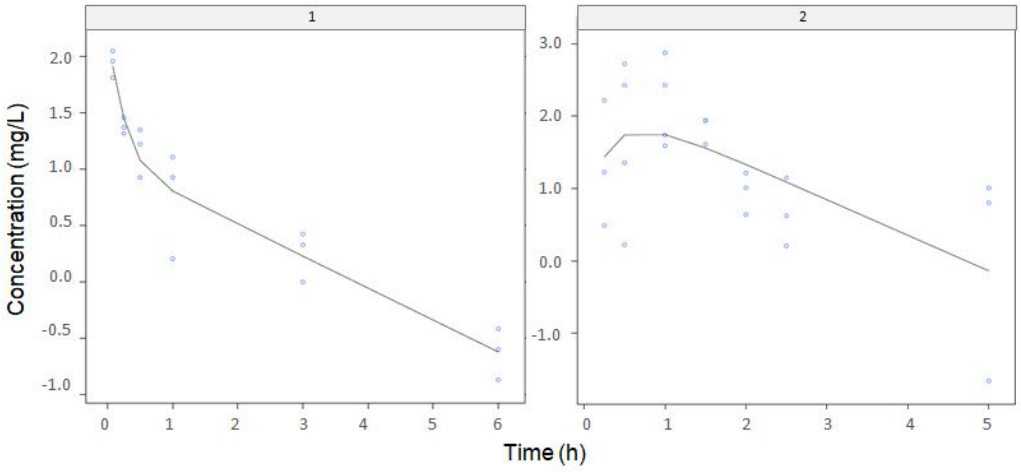

Figure S47. Concentration versus time profiles of compound 1164 following intravenous (25 mg/kg; 61.6 µmol/Kg) (1) and oral (121.6 mg/kg; 300 µmol/Kg) (2) administration to mice. Points indicate observed data (n= 3-4 observations/time point) and the solid lines the fitted model to the data set using naïve-pooled approach.

References:

1. Fardous A. Mohamed, Pakinaz Y. Khashaba, Reem Y. Shahin, Mohamed M. El-Wekil. Determination of donepezil in spiked rabbit plasma by high-performance liquid chromatography with fluorescence detection. R Soc Open Sci 6, 1-12 (2019). doi: http://dx.doi.org/10.1098/rsos.181476.

2. Harshal Patil, Sandeep Sonawane, Paraag Gide. Determination of guaifenesin from spiked human plasma using RP–HPLC with UV Detection. J Anal Chem 69, 390–394 (2014). doi: https://doi.org/10.1134/S1061934814040030