Electronic Supplementary Material (ESI) for MedChemComm.


# Quinolone-isoniazid hybrids: Synthesis and preliminary *in vitro* cytotoxicity and anti-tuberculosis evaluation

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## Contents

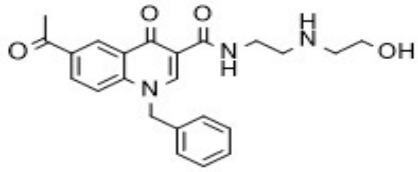
## 1. Experimental procedure

All the chemicals and solvents used were purchased from various chemical suppliers and were used without further purification. Melting points were determined using a Reichert hot stage microscope and are uncorrected. The progress of the reactions was monitored by thin layer chromatography (TLC) using Merck F254 silica gel plates supported on aluminium. The desired intermediates were purified by a silica gel column chromatography using Merck Kieselgel 60 Å: 70 – 230 (0.068 – 0.2 mm) silica gel mesh. $^{1}H$ and $^{13}C$ NMR spectra were recorded on Bruker Biospin 300 MHz, or 400 MHz spectrometers, and the chemical shifts are given in δ values referenced to deuterated DMSO-$d_6$ and are reported in parts per million (ppm). The high-resolution mass spectrometric data (HRS-MS) of final compounds was recorded on Bruker Daltonics Compact QTOF mass spectrometer (Rhodes University, South Africa), or Waters Synapt G2 quadrupole time-of-flight (QTOF) mass spectrometer (Stellenbosch University) using electrospray ionization (ESI) in the positive ionization mode. Purity was determined by HPLC, and all compounds were confirmed to have purity >95%. The chromatographic system consisted of an Agilent HP1100 LC-MSD, which is equipped with a quaternary pump, in-line degasser, DAD detector, 1100 MSD and ChemStation for collection and analysis of data. A ZORBAX Elipse Plus C18 4.6 i.d. x 150 mm x 5 µm column was used for reversed-phase HPLC analysis. A mixture of aqueous solution of monobasic sodium phosphate 0.01M and acetonitrile (90:10) on isocratic elution mode was used as the mobile phase. Five different concentrations (5 - 500 µg/mL) of samples to be analysed were made, filtered using 0.45 µm Millipore filters before their injection.

### 1.1 General synthesis for aroylhydrazone conjugates

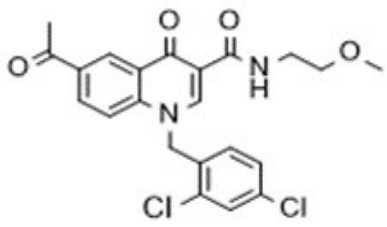
A 100 mL round bottom flask was charged with 20 mL of 95 % ethanol, 400-500 mg of 4 or 5, few drops of glacial acetic acid and 1.5 equivalent of isoniazid. The mixture was stirred under reflux for 12-24 h. The products precipitated out during the course of reaction, and were filtered, washed twice with 10 mL portions of ethanol and dried to obtain 200-400 mg of target compounds in 30-70 % yields.

**6-Acetyl-1-benzyl-N-(2-((2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide**, **8**



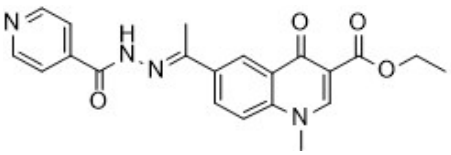
Orange powder, 0.320 g (48%), m.p. 169-171 °C; $^{1}$H NMR (300 MHz, DMSO) δ 9.93 (t, *J* = 4.8 Hz, 1H, -CONH-), 9.10 (s, 1H, Ar-H), 8.85 (d, *J* = 1.7 Hz, 1H, Ar-H), 8.19 (dd, *J* = 8.9, 1.7 Hz, 1H, Ar-H), 7.79 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.54 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.20 (d, *J* = 8.3 Hz, 2H, Ar-H), 5.79 (s, 2H, -$\underline{CH_2}$-Ar ), 5.03 (s, 1H,-OH )4.62 (t, *J* = 5.2 Hz, 2H, -$CH_2$-), 3.60 – 3.49 (m, 6H, -$CH_2$- × 3 ), 2.43 (s, 3H, -$CH_3$). $^{13}$C NMR (75 MHz, DMSO) δ 197.1, 176.2, 164.1, 150.2, 142.2, 135.7, 133.4, 132.2, 132.0, 129.3, 127.4, 121.6, 118.9, 112.7, 111.9, 72.7, 69.7, 60.7, 55.02, 39.7, 27.2. ESI-HRMS *m/z* calcd for $C_{23}H_{26}N_30_4$ 408.1923 $[M+H]^+$, found 408.1923. HPLC Purity: 96 %, $t_R$ = 8.2 min.

**6-Acetyl-1-(2,4-dichlorobenzyl)-N-(2-methoxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide**, **9**



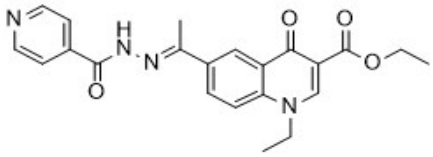
Brown powder, 0.370 g (44%), m.p. 203-205 °C; $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 9.99 (t, *J* = 5.1 Hz, 1H, -CONH-), 9.16 (s, 1H, Ar-H), 8.92 (d, *J* = 2.2 Hz, 1H, Ar-H), 8.26 (dd, *J* = 8.9, 2.2 Hz, 1H, Ar-H), 7.85 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.72 – 7.59 (m, 2H, Ar-H), 7.21 (dd, *J* = 8.3, 2.2 Hz, 1H, Ar-H), 5.87 (s, 2H, -$\underline{CH_2}$-Ar), 3.56 – 3.39 (m, 4H, -$CH_2$- × 2), 3.37 (s, 3H, -$OCH_3$), 2.70 (s, 3H, -$CH_3$). $^{13}$C NMR (75 MHz, DMSO) δ 197.1, 176.3, 164.0, 150.2, 142.1, 137.4, 133.4, 132.1, 131.9, 131.6, 131.1, 129.4, 127.7, 127.4, 127.2, 118.7, 112.8, 71.2, 58.5, 55.3, 39.0, 27.2. ESI-HRMS *m/z* calcd for $C_{22}H_{21}Cl_2N_20_4$ 447.0878 $[M+H]^+$, found 447.0873. HPLC Purity: 96 %, $t_R$ = 7.3 min.

**Ethyl (*E*)-6-(1-(2-isonicotinoylhydrazono)ethyl)-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate, 10**



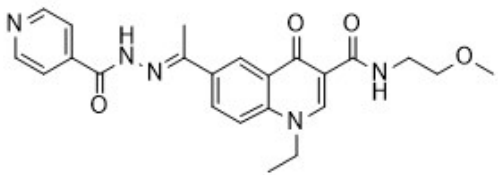
Red powder, 303 mg (56%), m.p. 224-226 °C; $^{1}$H NMR (300 MHz, DMSO) δ 11.14 (s, 1H, -C(=O)NH-N-), 8.78 (s, 1H, Ar-H), 8.65 (d, *J* = 9.0 Hz, 2H, Ar-H), 8.38 (s, 1H, Ar-H), 7.93 – 7.64 (m, 4H, Ar-H), 4.34 (q, *J* = 7.1 Hz, 2H, -$CH_2$), 3.94 (s, 3H, -$CH_3$), 2.42 (s, 3H, -$CH_3$), 1.28 (t, *J* = 7.1 Hz, 3H, -$CH_3$). $^{13}$C NMR (75 MHz, DMSO) δ 164.8, 163.1, 158.6, 153.2, 150.3, 141.8, 140.9, 134.5, 131.0, 128.2, 124.6, 122.5, 118.2, 110.6, 109.3, 59.9, 41.4, 15.12, 14.7. ESI-HRMS *m/z* calcd for $C_{21}H_{21}N_4O_4$ 393.1479 $[M+H]^+$, found 393.1479. HPLC Purity: 96 %, $t_R$ = 8.7 min.

**Ethyl (*E*)-1-ethyl-6-(1-(2-isonicotinoylhydrazono)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate, 11**



Orange powder, 350 mg (57%), m.p. 201-203 °C; $^{1}$H NMR (300 MHz, DMSO) δ 11.12 (s, 1H, -C(=O)NH-N-), 8.76 (s, 2H, Ar-H), 8.66 (d, *J* = 7.9 Hz, 1H, Ar-H), 8.30 (d, *J* = 7.9 Hz, 1H, Ar-H), 8.04 – 7.75 (m, 4H, Ar-H), 4.43 (q, *J* = 6.5 Hz, 2H, -$CH_2$-), 4.23 (q, *J* = 7.0 Hz, 2H, -$CH_2$-), 2.43 (s, 3H, -$CH_3$), 1.32 (dt, *J* = 6.5, 7.0 Hz, 6H, -$CH_3$ × 2). $^{13}$C NMR (75 MHz, DMSO) δ 173.5, 172.6, 164.9, 155.9, 150.6, 150.0, 140.0, 134.4, 131.0, 128.4, 125.1, 126.4, 122.3, 117.9, 111.1, 60.2, 49.0, 15.1, 14.8, 14.7. ESI-HRMS *m/z* calcd for $C_{22}H_{23}N_4O_4$ 407.1718 $[M+H]^+$, found 407.1717. HPLC Purity: 97 %, $t_R$ = 8.2 min.

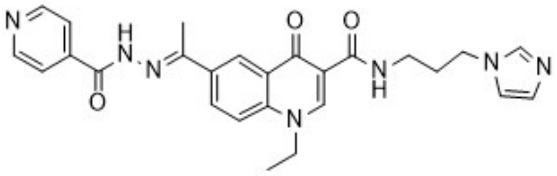
**(*E*)-1-ethyl-6-(1-(2-isonicotinoylhydrazono)ethyl)-N-(2-methoxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 12**



Yellow powder, 324 mg (58%), m.p. 215-217 °C; $^{1}$H NMR (400 MHz, DMSO) δ 11.15 (s, 1H, -C(=O)NH-N-), 10.07 (s, 1H, -CONH-), 8.89 (s, 1H,

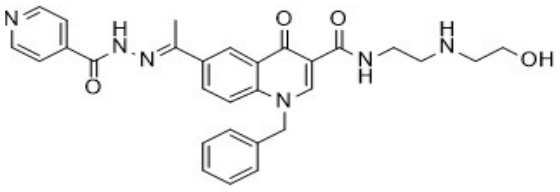
Ar-H), 8.75 (d, $J$ = 8.9 Hz, 1H, Ar-H), 8.34- 8.04 (m, 4H, Ar-H), 7.99 (d, $J$ = 8.9 Hz, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 4.57 (q, $J$ = 6.5 Hz, 2H, -$CH_2$-), 3.58 – 3.50 (m, 4H, -$CH_2$- × 2), 3.31 (s, 3H, -$OCH_3$), 2.43 (s, 3H, -$CH_3$), 1.42 (t, $J$ = 6.5 Hz, 3H, -$CH_3$). $^{13}$C NMR (101 MHz, DMSO) δ 176.0, 175.7, 164.3, 155.9, 150.6, 148.6, 140.3, 134.9, 131.2, 127.5, 124.8, 122.5, 122.2, 118.2, 111.8, 72.7, 58.6, 48.9, 38.2, 15.2, 14.9. ESI-HRMS *m/z* calcd for $C_{23}H_{26}N_5O_4$ 436.1985 $[M+H]^+$, found 436.1983. HPLC Purity: 96 %, $t_R$ = 9.3 min.

**(*E*)-N-(3-(1H-imidazol-1-yl)propyl)-1-ethyl-6-(1-(2-isonicotinoylhydrazono)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 13**



Yellow powder, 400 mg (63%), m.p. 211-213 °C; $^1$H NMR (300 MHz, $D_2O$) δ 8.88 (s, 1H, Ar-H), 8.42 (d, $J$ = 8.4 Hz, 1H, Ar-H), 8.15 – 7.97 (m, 4H, Ar-H), 7.86 – 7.20 (m, 4H, Ar-H), 4.36 (q, $J$ = 6.7 Hz, 2H, -$CH_2$-), 4.26 (d, $J$ = 6.9 Hz, 2H, -$CH_2$-), 3.34 (t, $J$ = 6.9 Hz, 2H, -$\underline{CH_2}$-NHCO-), 2.23 (dd, $J$ = 12.6, 6.9 Hz, 2H, -$CH_2$-), 2.06 (s, 3H, -$CH_3$), 1.49 (t, $J$ = 6.8 Hz, 3H, -$CH_3$). $^{13}$C NMR (75 MHz, $D_2O$) δ 177.4, 175.7, 165.3, 154.5, 148.8, 148.0, 147.9, 146.8, 139.1, 134.5, 133.2, 132.3, 130.6, 123.8, 122.3, 121.6, 119.7, 110.1, 49.6, 47.2, 35.9, 29.2, 14.0, 13.8. ESI-HRMS *m/z* calcd for $C_{26}H_{28}N_7O_3$ 486.2255 $[M+H]^+$, found 486.2257. HPLC Purity: 98 %, $t_R$ = 4.3 min.

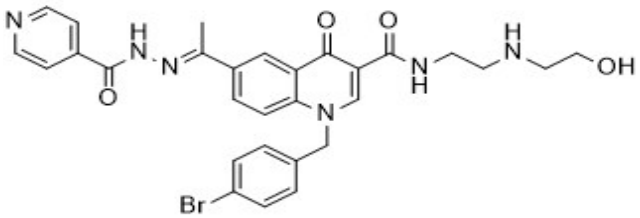
**(*E*)-1-benzyl-N-(2-((2-hydroxyethyl)amino)ethyl)-6-(1-(2-isonicotinoylhydrazono)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 14**



White powder, 270 mg (52%), m.p. 210-212 °C; $^1$H NMR (300 MHz, DMSO) δ 11.19 (s, 1H, -C(=O)NH-N-), 10.08 (s, 1H, -CONH-), 9.08 (s, 1H, Ar-H), 8.63 (d, $J$ = 8.0 Hz, 1H, Ar-H), 8.24 (d, $J$ = 8.0 Hz, 1H, Ar-H), 7.85–7.5 (m, 5H, Ar-H), 7.44 – 7.10 (m, 5H, Ar-H), 5.82 (s, 2H, -$\underline{CH_2}$-Ar), 5.27 (s, 1H, -OH), 3.77 – 3.57 (m, 2H, -$CH_2$), 3.17 (t, $J$ = 6.0 Hz, 2H, -$CH_2$-), 3.12 – 2.95 (m, 4H, -$CH_2$- × 2), 2.35 (s, 3H, -$CH_3$). $^{13}$C NMR (75 MHz, DMSO) δ 176.3, 165.4, 163.1, 155.3, 150.5, 149.5, 141.8, 139.5, 136.2, 135.0, 131.0,

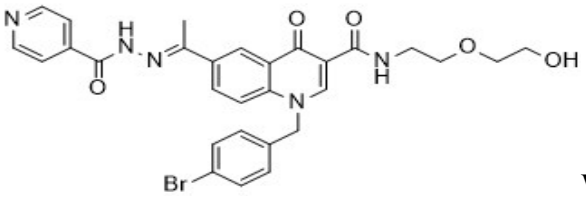
129.4, 129.0, 128.4, 127.5, 127.0, 125.1, 123.8, 122.4, 118.7, 111.8, 57.9, 56.8, 49.6, 47.1, 35.9, 15.5. ESI-HRMS *m/z* calcd for $C_{29}H_{31}N_6O_4$ 527.2308 $[M+H]^+$, found 527.2308. HPLC Purity: 97 %, $t_R$ = 11.4 min.

**(*E*)-1-(4-bromobenzyl)-N-(2-((2-hydroxyethyl)amino)ethyl)-6-(1-(2-isonicotinoylhydrazono)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 15**



Red powder, 250 mg (47%), m.p. 227-229 °C; $^1$H NMR (400 MHz, DMSO) δ 11.20 (s, 1H, -C(=O)NH-N-), 10.08 (s, 1H, -CONH-), 9.12 (s, 1H, Ar-H), 8.81 (d, *J* = 8.6, Hz, 2H, Ar-H), 8.25 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.83 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.57 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.23 (d, *J* = 7.4 Hz, 1H, Ar-H), 5.82 (s, 2H, -$\underline{CH_2}$-Ar), 5.28 (s, 1H, -OH), 3.75 – 3.64 (m, 4H, -$CH_2$- × 2), 3.15 (t, *J* = 5.7 Hz, 2H, -$CH_2$-), 3.04 (t, *J* = 5.1 Hz, 2H, -$CH_2$-), 2.49 – 2.38 (m, 3H, -$CH_3$ overlapping with DMSO-$d_6$). $^{13}$C NMR (101 MHz, DMSO) δ 176.3, 172.0, 163.6, 151.3, 149.2, 141.5, 140.6, 135.9, 134.9, 132.5, 131.2, 129.5, 127.6, 124.5, 123.6, 122.5, 121.5, 119.2, 112.2, 56.7, 55.6, 49.6, 47.0, 36.3, 15.2. ESI-HRMS *m/z* calcd for $C_{29}H_{30}BrN_6O_4$ 605.1515 $[M+H]^+$, found 605.1514. HPLC Purity: 96 %, $t_R$ = 5.0 min.

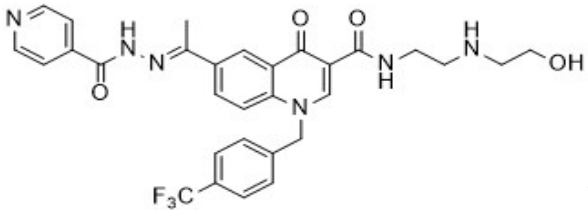
**(*E*)-1-(4-bromobenzyl)-N-(2-(2-hydroxyethoxy)ethyl)-6-(1-(2-isonicotinoylhydrazono)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 16**



White powder, 290 mg (32%), m.p. 203-205 °C; $^1$H NMR (300 MHz, DMSO) δ 11.17 (s, 1H, -C(=O)NH-N-), 10.03 (s, 1H, -CONH-), 9.08 (s, 1H, Ar-H), 8.75 (d, *J* = 6.4 Hz, 2H, Ar-H), 8.24 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.01-7.38 (m, 6H, Ar-H), 7.22 (d, *J* = 6.4 Hz, 2H, Ar-H), 5.79 (s, 2H, -$\underline{CH_2}$-Ar), 4.65 (s, 1H, -OH), 3.69 – 3.23 (m, 8H, -$CH_2$- × 2), 2.36 (s, 3H, -$CH_3$). $^{13}$C NMR (75 MHz, DMSO) δ 176.7, 172.6, 164.3, 150.5, 150.0, 149.2, 141.5, 140.0, 135.8, 134.6, 132.2, 129.1, 127.3, 124.5, 123.6, 122.8, 121.4, 118.3, 112.2,

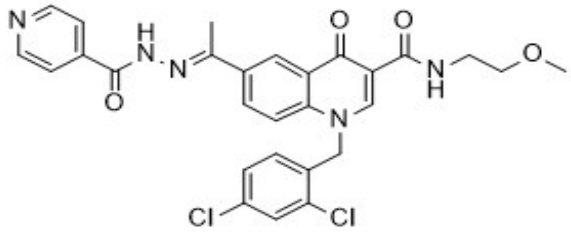
72.6, 69.8, 60.7, 55.9, 39.3, 20.5. ESI-HRMS *m/z* calcd for $C_{29}H_{29}BrN_5O_5$ 606.1352 $[M+H]^+$, found 606.1349. HPLC Purity: 96 %, $t_R$ = 4.7 min.

**(*E*)-N-(2-((2-hydroxyethyl)amino)ethyl)-6-(1-(2-isonicotinoylhydrazono)ethyl)-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxamide, 17**



White powder, 300 mg (48%), m.p. 213-215 °C; $^1$H NMR (400 MHz, DMSO) δ 11.20 (s, 1H, -C(=O)NH-N-), 10.09 (s, 1H, -CONH-), 9.19 (s, 1H, Ar-H), 8.83-8.25 (m, 5H, Ar-H), 8.24 (d, *J* = 9.1 Hz, 1H, Ar-H), 7.82 (d, *J* = 9.1 Hz, 1H, Ar-H), 7.74 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.46 (d, *J* = 7.6 Hz, 2H, Ar-H), 5.92 (s, 2H, -$\underline{CH_2}$-Ar), 5.31 (s, 1H, -OH), 3.70 (t, *J* = 13.9 Hz, 2H, -$CH_2$-), 3.12 (t, *J* = 12.9 Hz, 4H, -$CH_2$- × 2), 3.06 (t, *J* = 4.6 Hz, 2H, -$CH_2$-), 2.45 (s, 3H, -$CH_3$). $^{13}$C NMR (101 MHz, DMSO) δ 173.4, 165.3, 164.3, 155.9, 153.8, 150.5, 145.6, 140.7, 136.4, 132.1, 130.2, 128.1, 127.7, 127.5, 127.3, 126.3, 126.1, 125.5, 123.8, 120.9, 116.0, 112.0, 57.0, 51.0, 49.6, 47.1, 35.8, 15.4. ESI-HRMS *m/z* calcd for $C_{30}H_{30}F_3N_6O_4$ 595.2167 $[M+H]^+$, found 595.2169. HPLC Purity: 96 %, $t_R$ = 4.9 min.

**(*E*)-1-(2,4-dichlorobenzyl)-6-(1-(2-isonicotinoylhydrazono)ethyl)-N-(2-methoxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 18**



Grey powder, 286 mg (56%), m.p. 220-222 °C; $^1$H NMR (400 MHz, DMSO) δ 11.26 (s, 1H, -C(=O)NH-N-), 10.05 (s, 1H, -CONH), 9.10 (s, 1H, Ar-H), 8.79- 8.36 (m, 4H, Ar-H), 8.26 (s, 1H, Ar-H), 7.72-7.21 (m, 4H, Ar-H), 7.17 (s, 1H, Ar-H), 5.82 (s, 2H, -$\underline{CH_2}$-Ar), 3.50 (s, 3H, -$OCH_3$), 3.35-3.01 (m, 4H, -$CH_2$- × 2), 2.34 (s, 3H, -$CH_3$). $^{13}$C NMR (101 MHz, DMSO) δ 176.3, 166.0, 164.3, 155.6, 151.6, 146.9, 138.6, 135.3, 133.9, 131.9, 131.5, 130.8, 129.4, 128.8, 127.5, 126.1, 124.8, 122.0, 118.8, 115.0, 111.7, 71.2, 59.3, 58.5, 38.8, 15.2. ESI-HRMS *m/z* calcd for $C_{28}H_{26}Cl_2N_5O_4$ 566.1253 $[M+H]^+$, found 566.1255. HPLC Purity: 96 %, $t_R$ = 13.8 min.

### 1.2 *In vitro* cytotoxicity assay

HeLa cells seeded in 96-well plates were incubated with 20 μM test compounds for 24 hours and cell viability assessed using a resazurin fluorescence assay as previously described.[41]

### 1.3 *In vitro* antimycobacterial assay

The minimum inhibitory concentration (MIC) was determined using the standard broth micro dilution method, where a 10 mL culture of *Mycobacterium tuberculosis* pMSp12::GFP,[42] was grown to an absorbance (OD600) of 0.6 – 0.7. The medium used was Middlebrook 7H9 supplemented with 0.03% casitone, 0.4% glucose, and 0.05% tyloxapol.[43] Cultures grown in this medium are diluted 1:500, prior to inoculation of the MIC assay. The compounds to be tested were reconstituted to a concentration of 10 mM in DMSO. Two-fold serial dilutions of the test compound were prepared across a 96-well micro titre plate, after which, 50 μL of the diluted *M. tuberculosis* cultures was added to each well in the serial dilution. The plate layout was a modification of the method previously described.[44] Assay controls used were a minimum growth control (Rifampicin at 2 × MIC), and a maximum growth control (5% DMSO). The micro titre plates were sealed in a secondary container and incubated at 37 °C with 5% $CO_2$ and humidification. Relative fluorescence (excitation 485 nM; emission 520 nM) was measured using a plate reader (FLUOstar OPTIMA, BMG LABTECH), at day 7 and day 14. The raw fluorescence data were archived and analysed using the CDD Vault from Collaborative Drug Discovery, in which, data were normalised to the minimum and maximum inhibition controls to generate a dose response curve (% inhibition), using the Levenberg-Marquardt damped least squares method, from which the $MIC_{90}$ was calculated (Burlingame, CA www.collaborativedrug.com). The lowest concentration of drug that inhibited growth of more than 90 % of the mycobacterial population was considered to be the $MIC_{90}$.

### 1.4 Kinetic solubility determination using nephelometry

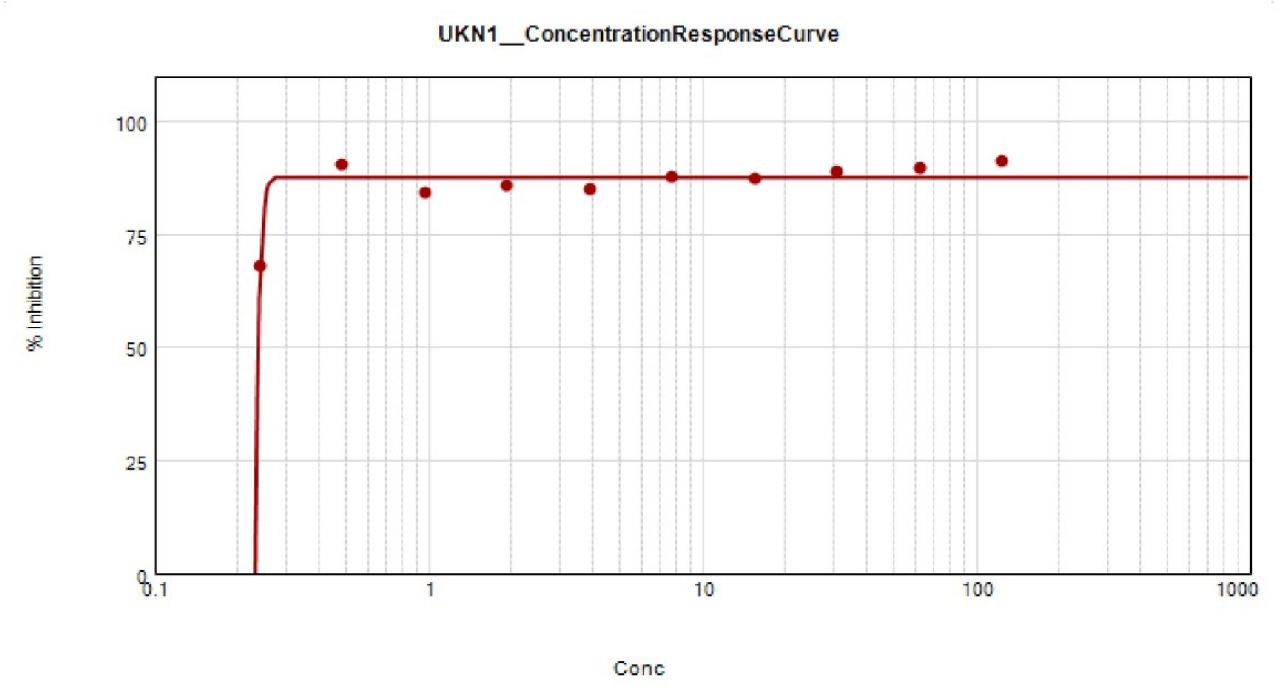
The solubility assay was performed (H3-D, University of Cape Town) using a miniaturised shake flask method.[45] 10 mM stock solutions of each of the test compounds were used to prepare calibration standards (10-220 μM) in DMSO, and to spike (1:50) duplicate aqueous samples of phosphate buffered saline (pH 6.5). The DMSO was dried off using a GeneVac (MiVac, 90 min, 37 °C). After shaking (20 hours, 25 °C), the solutions were filtered and analysed by means of HPLC-DAD (Agilent 1200 Rapid Resolution HPLC with a diode array detector). Best fit

calibration curves were constructed using the calibration standards, which were used to determine the aqueous samples' solubility.

### 1.5 *In vitro* metabolic stability using human, rat and mouse liver microsomes

All protocols for in vitro metabolic studies were done in collaboration with Drug Discovery and Development Centre (H-3D), University of Cape Town. Animal studies were conducted in accordance to guidelines and policies as stipulated in the UCT Research Ethics Code for Use of Animals in Research and Teaching after review and approval of the experimental protocol by the UCT Senate Animal Ethics Committee (Protocol FHS-AEC 013/032). Metabolic stability was performed (H-3D, University of Cape Town) in duplicate in a 96-well micro titre plate. The test compounds (1 μM) were incubated individually in mouse, rat and pooled human liver microsomes (0.4 mg/mL) at 37 °C for predetermined time points, in the presence and absence of the co-factor NADPH (1 mM). Reactions were quenched by adding 300 μL of ice cold acetonitrile containing internal standard (carbamazepine, 0.0236 μg/mL). Test compounds in the supernatant were analysed by means of LC-MS/MS (Agilent Rapid Resolution HPLC, AB SCIEX 4000 QTRAP MS). Metabolite searches were not conducted during the metabolic stability assay.[46] The scaling factors were used to calculate clearance and hepatic extraction.[47]

## 2. *In vitro* anti-TB assay



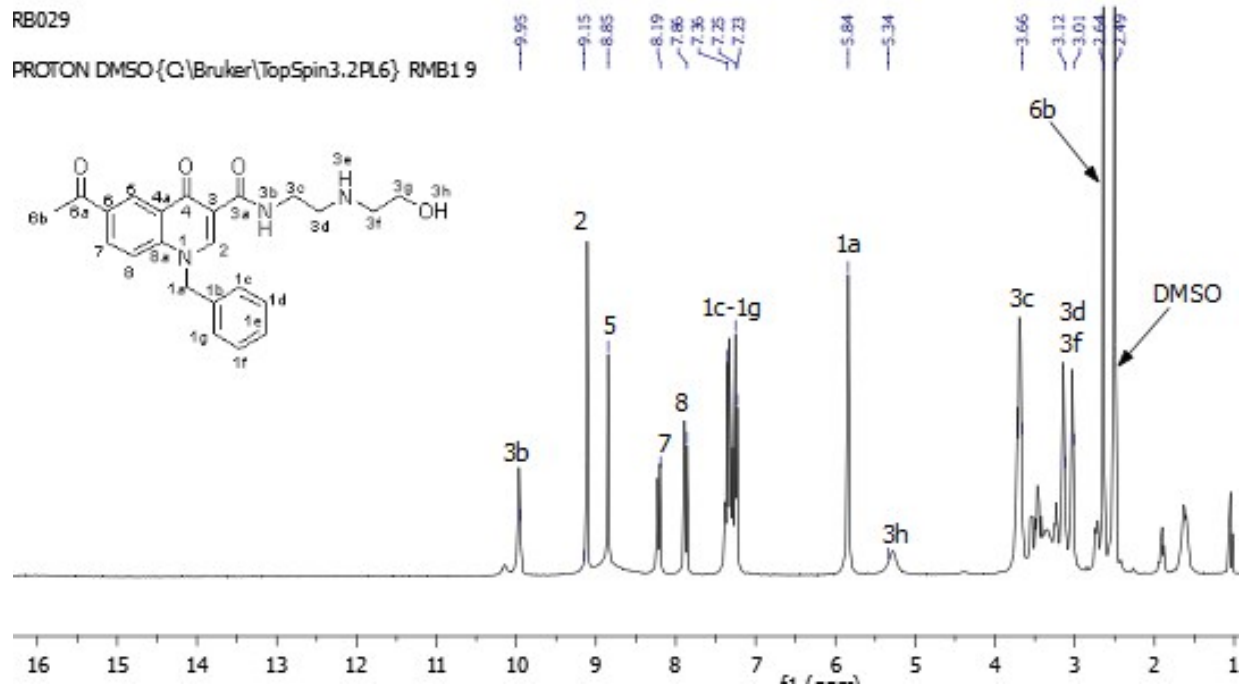

Curve Fit Results ▲

Curve Fit : 4-Parameter $y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$

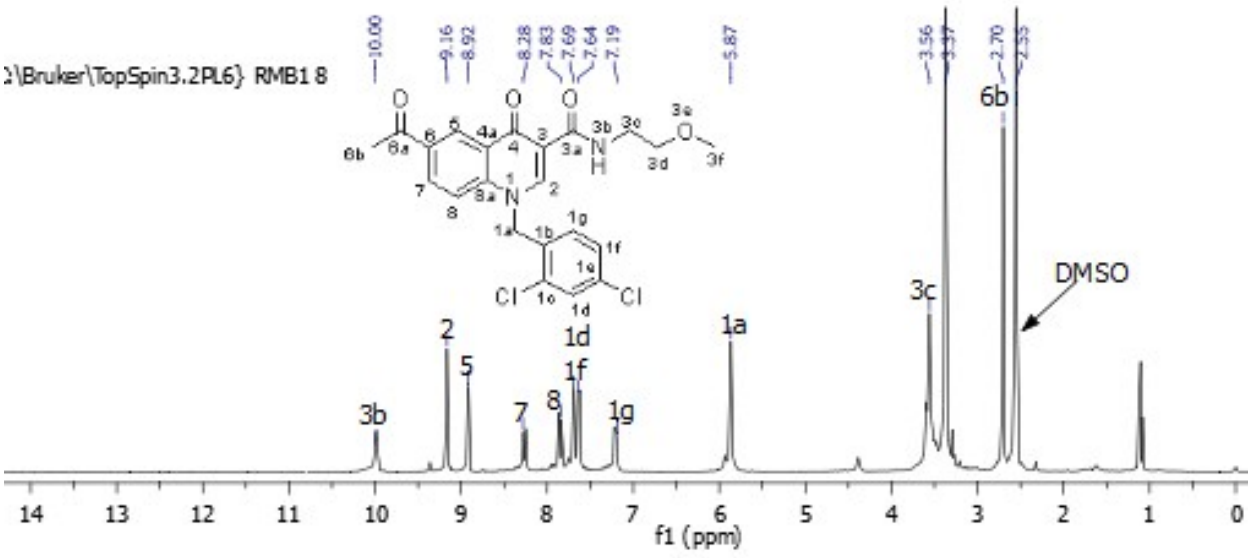
| | Parameter | Estimated Value | Std. Error | Confidence Interval |
|---|---|---|---|---|
| **Unknown1** $R^2 = 0.885$ EC50 = 0.232 | A | -96.61 | | |
| | B | 40.81 | 2.28e+7 | [-5.57e+7, 5.57e+7] |
| | C | 0.232 | 8.39e+8 | [-2.05e+9, 2.05e+9] |
| | D | 87.82 | 1.03e-8 | [87.82, 87.82] |

**Figure 1S:** Data reported for CALCULATED MIC90 7D 7H9 GLU CAS Tx

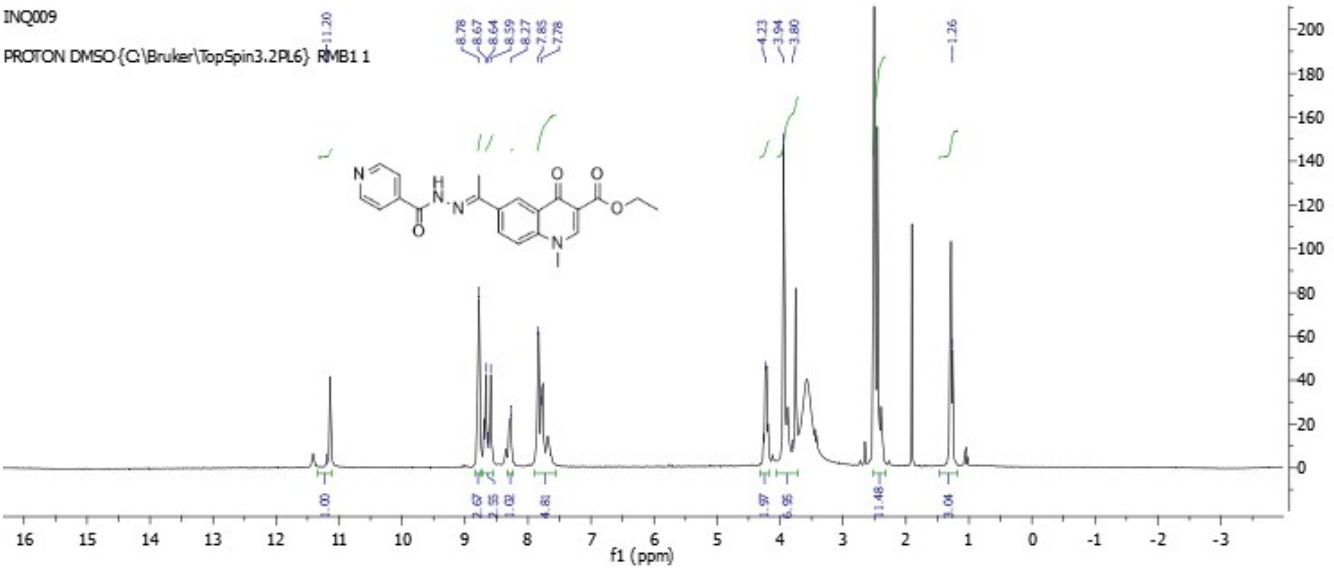
## 3. [1]H NMR spectra of synthesized compound



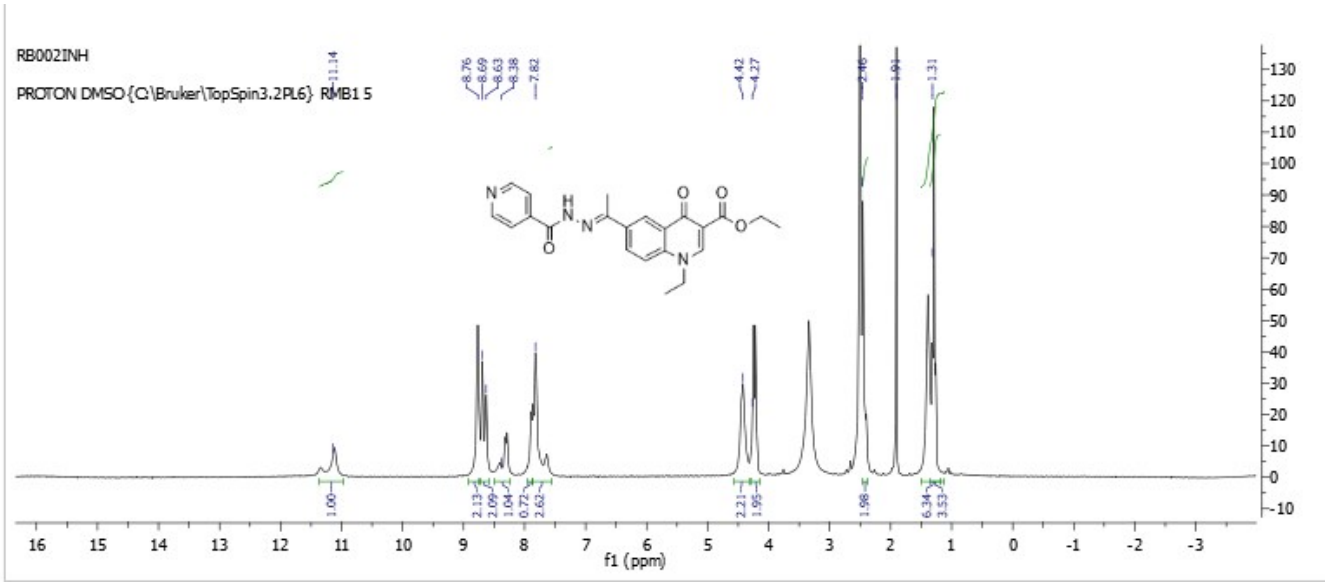

**Compound 8**



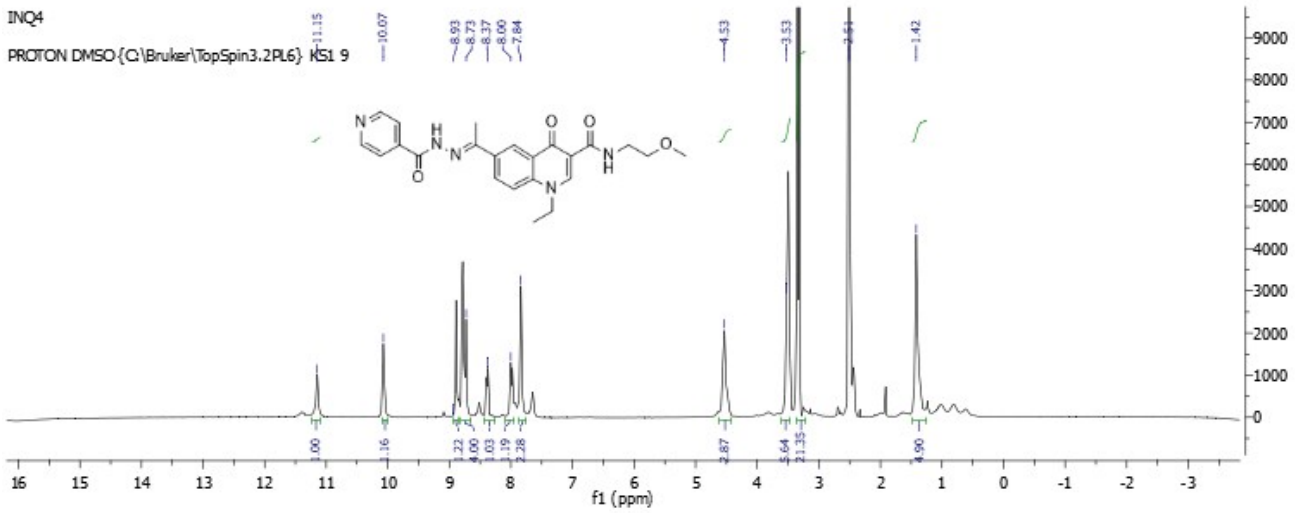

**Compound 9**



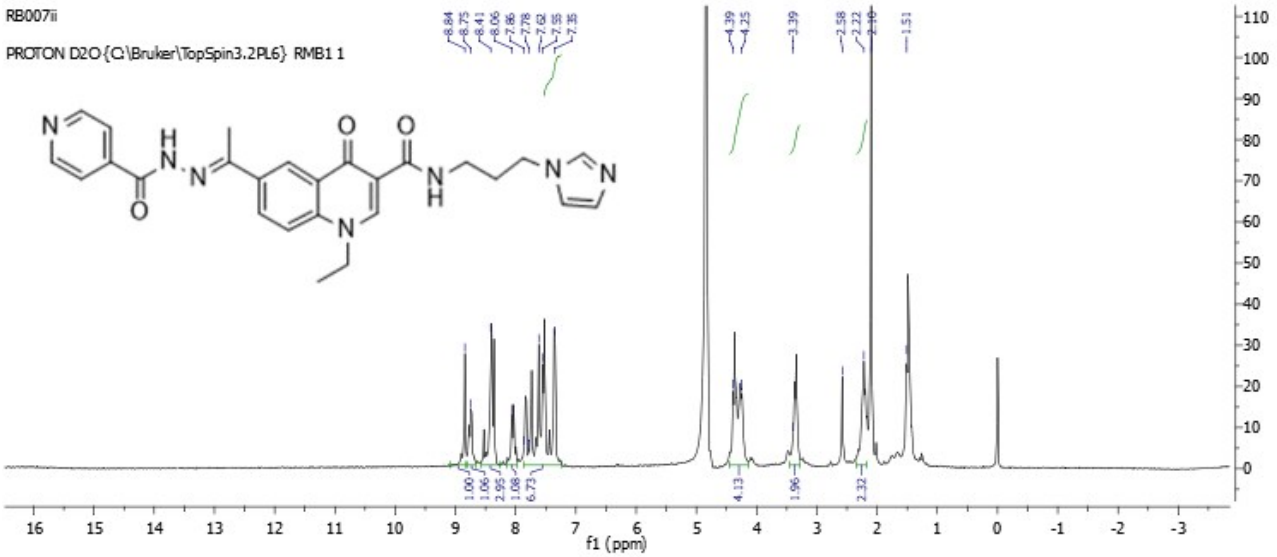

**Compound 10**



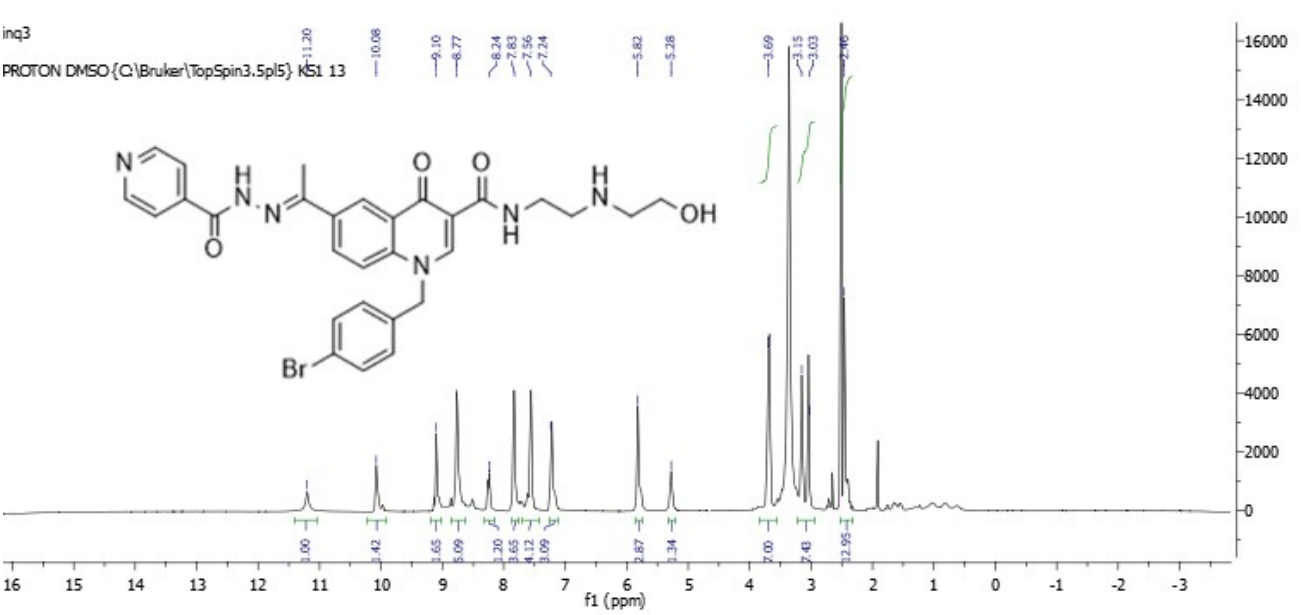

**Compound 11**



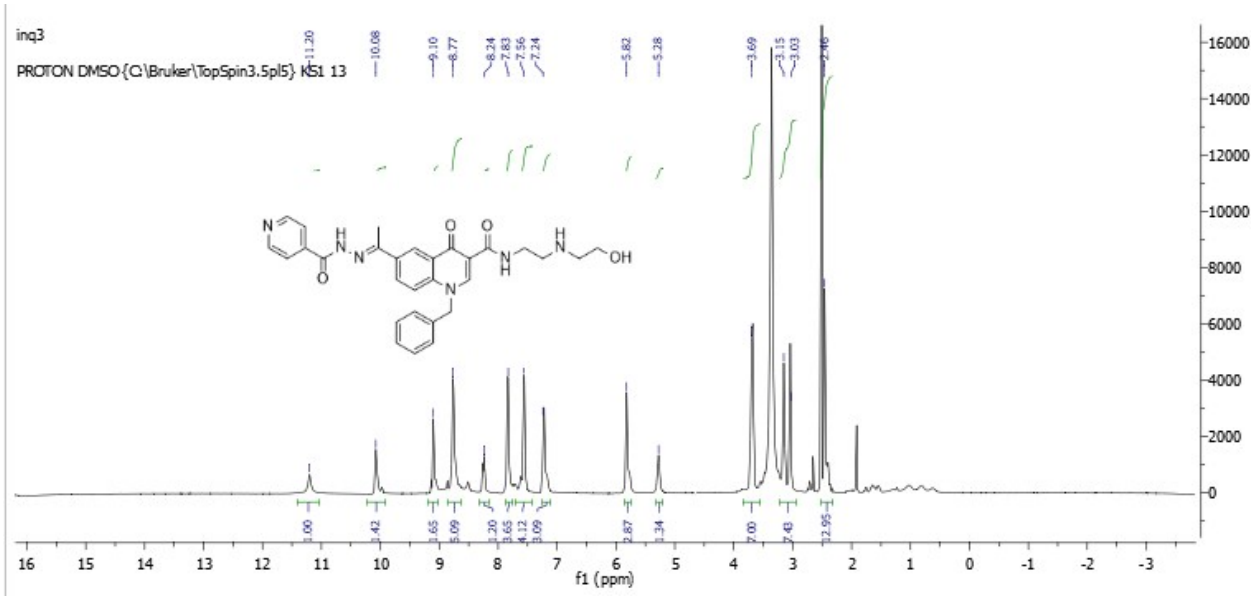

**Compound 12**



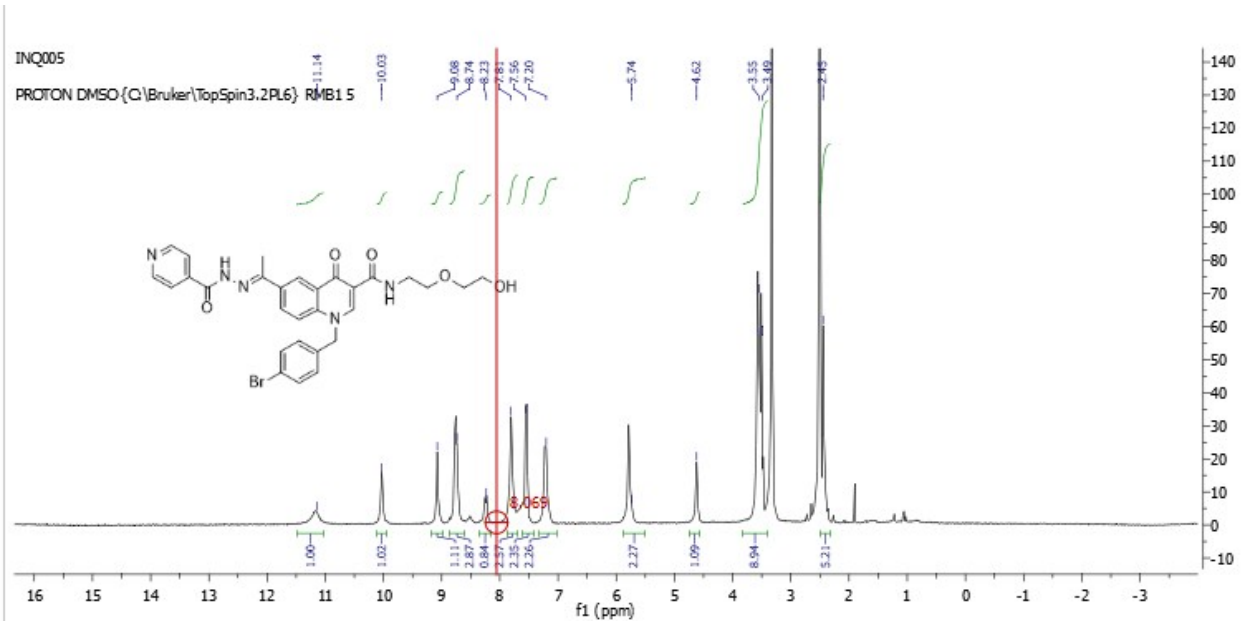

**Compound 13**



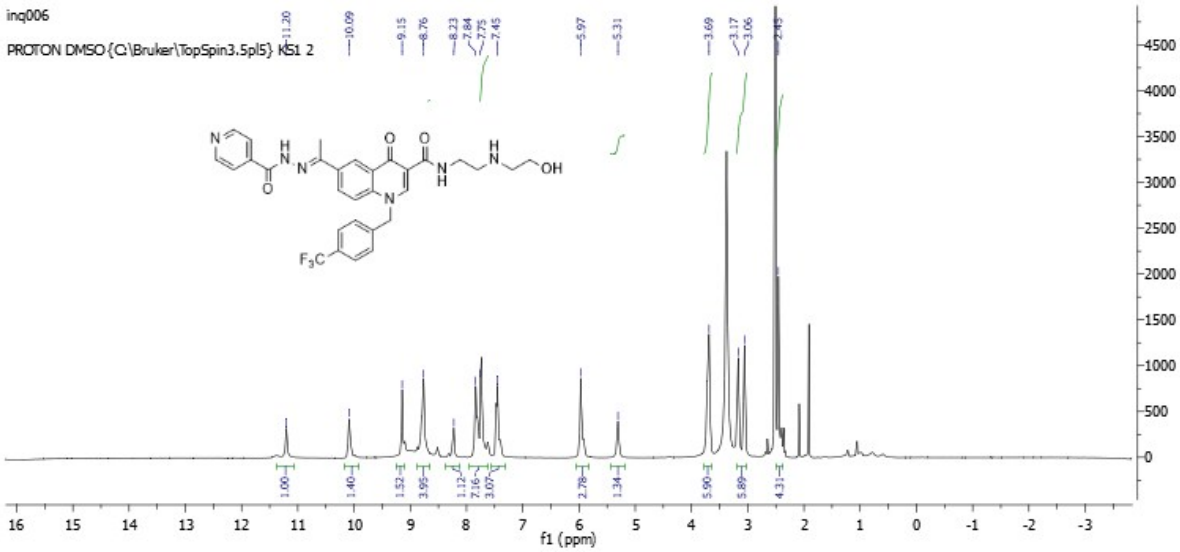

**Compound 14**



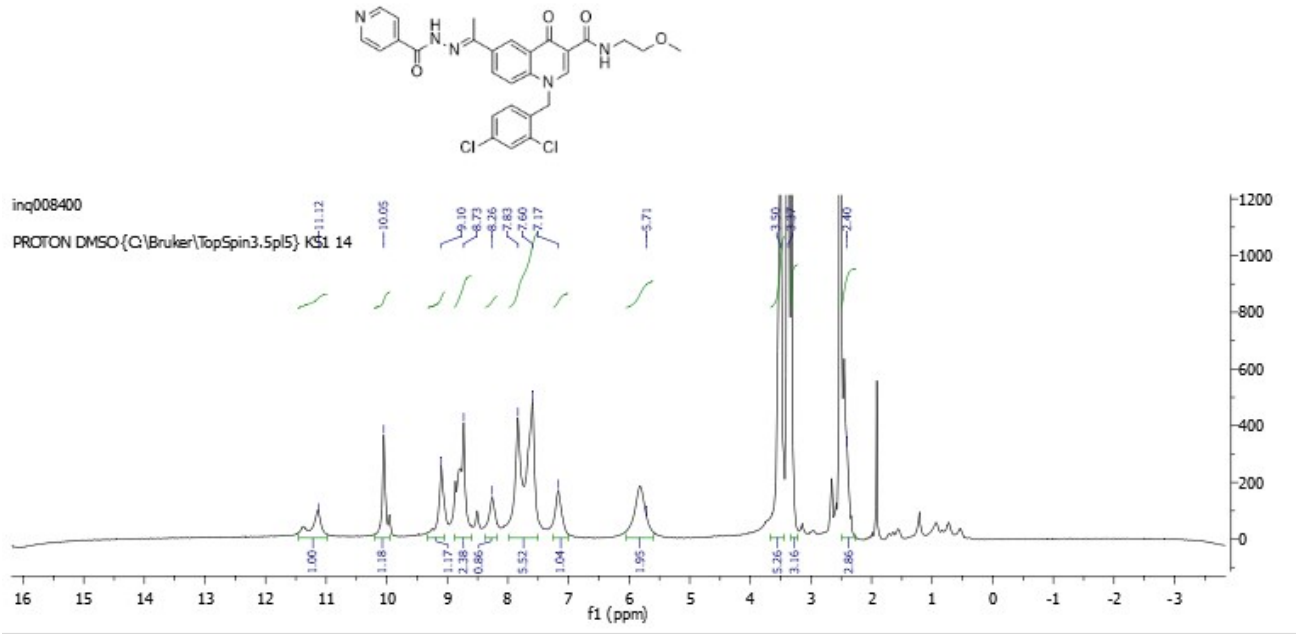

**Compound 15**




**Compound 16**

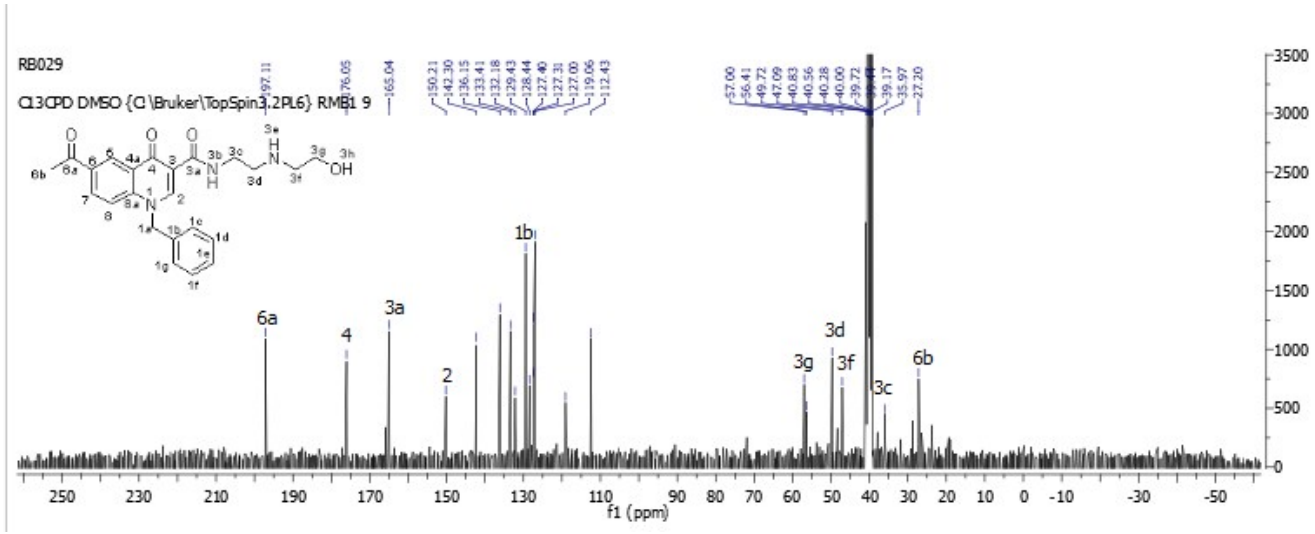


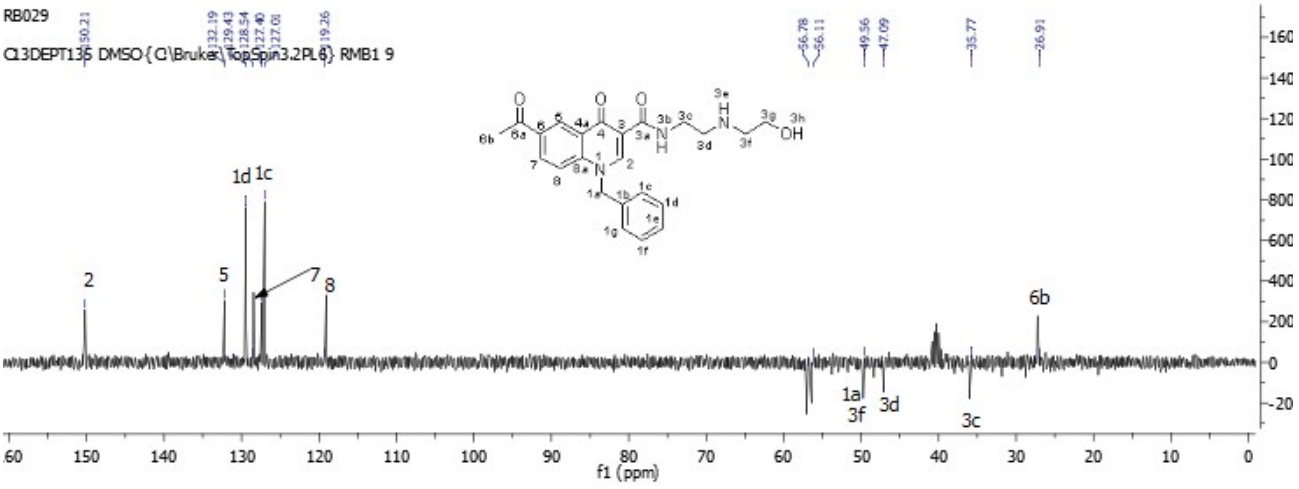

**Compound 17**


inq008400
PROTON DMSO{C:\Bruker\TopSpin3.5pl5} KS1 14
11.12
10.05
9.10
8.73
8.26
7.83
7.60
7.17
5.71
3.50
2.40
1.00
1.18
1.17
2.38
0.86
5.52
1.04
1.95
5.26
3.16
2.86
f1 (ppm)

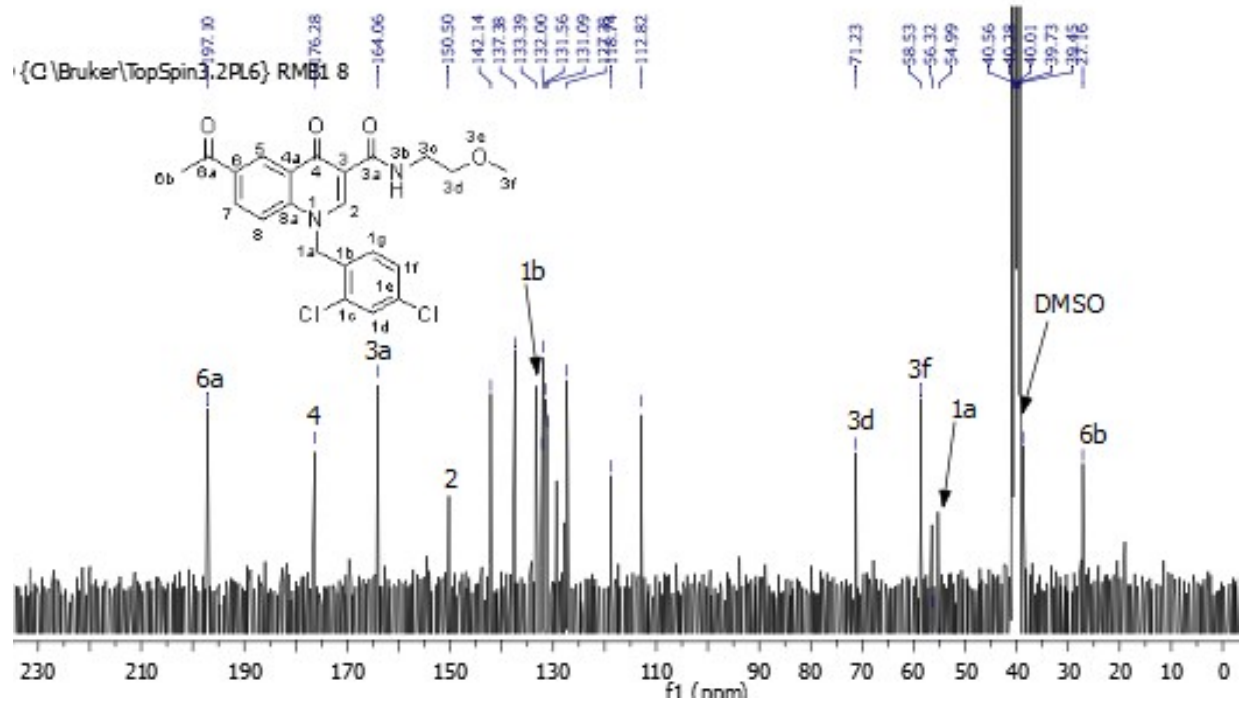
**Compound 18**

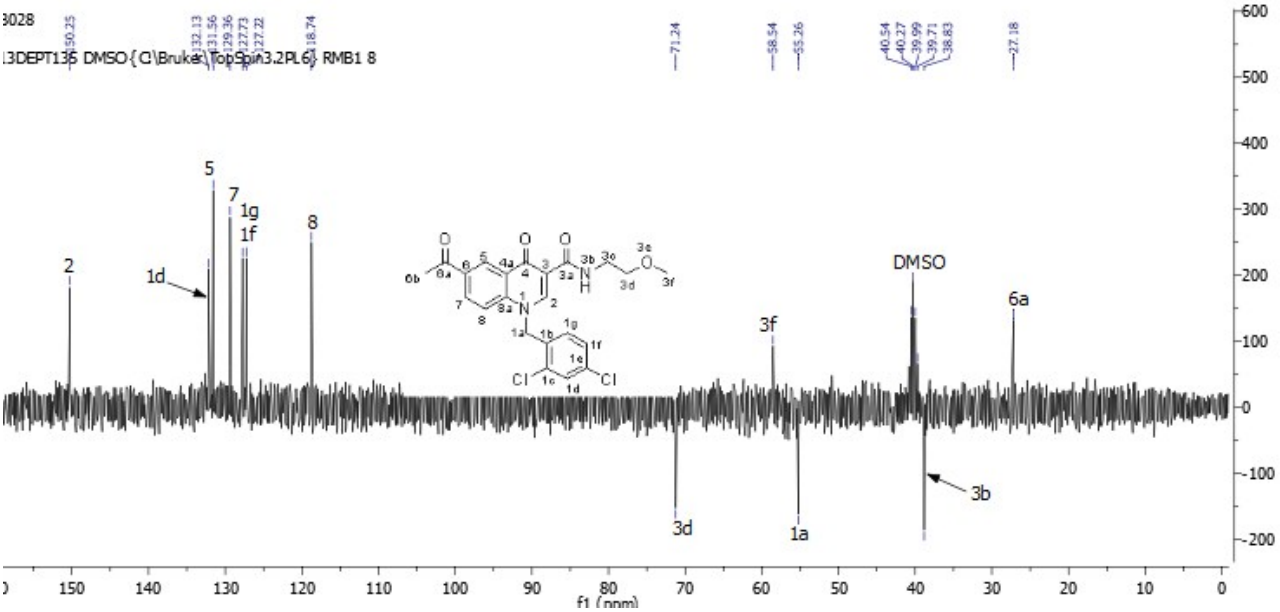
# 4. $^{13}C$ NMR and $^{13}C$ DEPT135 spectra of synthesized compounds



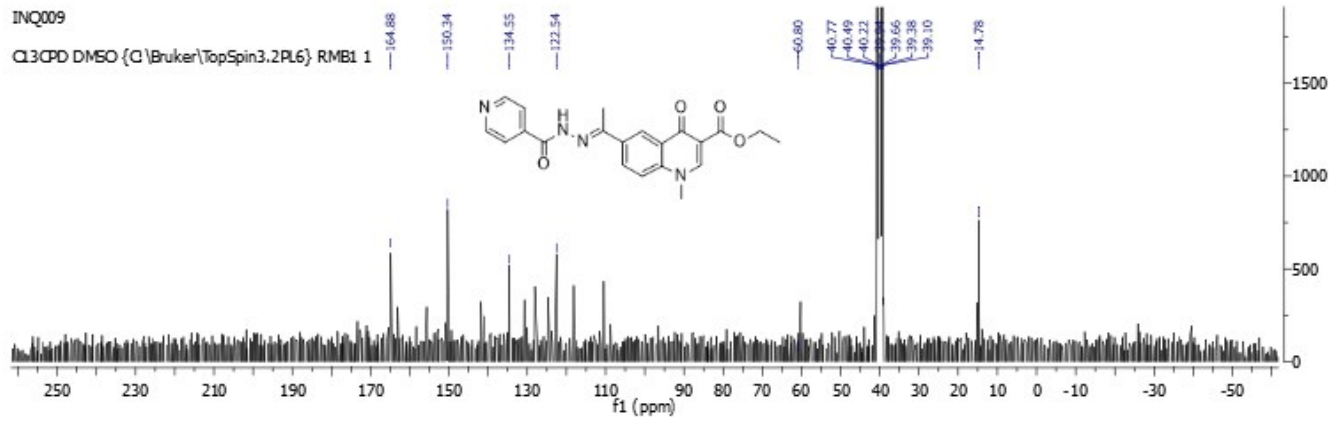



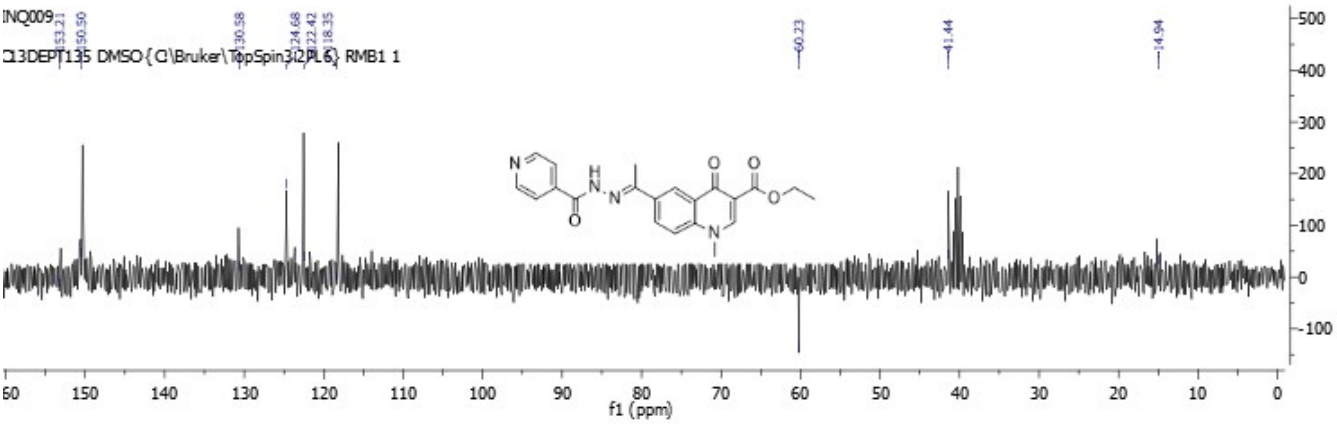

**Compound 8**


{C:\Bruker\TopSpin3.2PL6} RMB1 8
6a
4
3a
2
1b
3d
3f
1a
DMSO
6b
f1 (ppm)

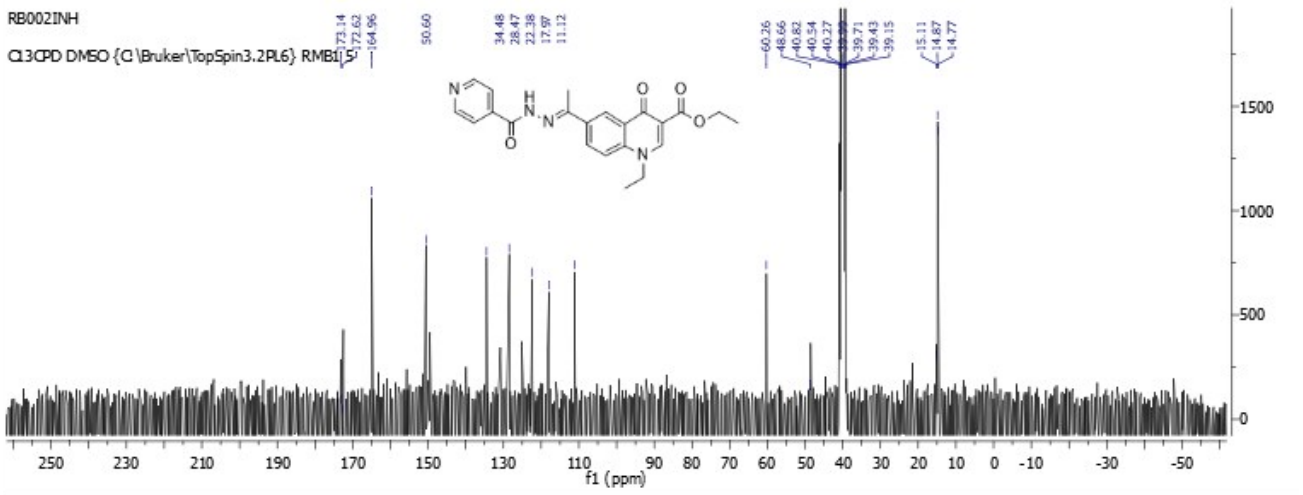

3028
13DEPT135 DMSO{C:\Bruker\TopSpin3.2PL6} RMB1 8
2
1d
5
7
1g
1f
8
3d
1a
3f
DMSO
3b
6a
f1 (ppm)

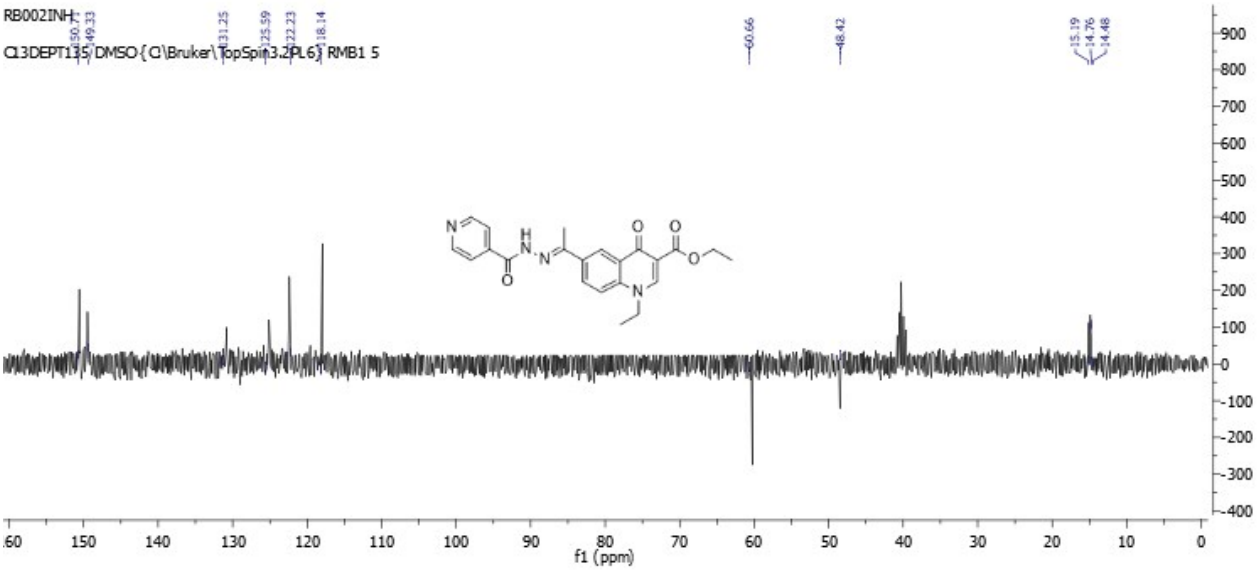
**Compound 9**


INQ009
C13CPD DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 1
164.88
150.34
134.55
122.54
60.80
40.77
40.49
40.22
39.94
39.66
39.38
39.10
14.78
f1 (ppm)

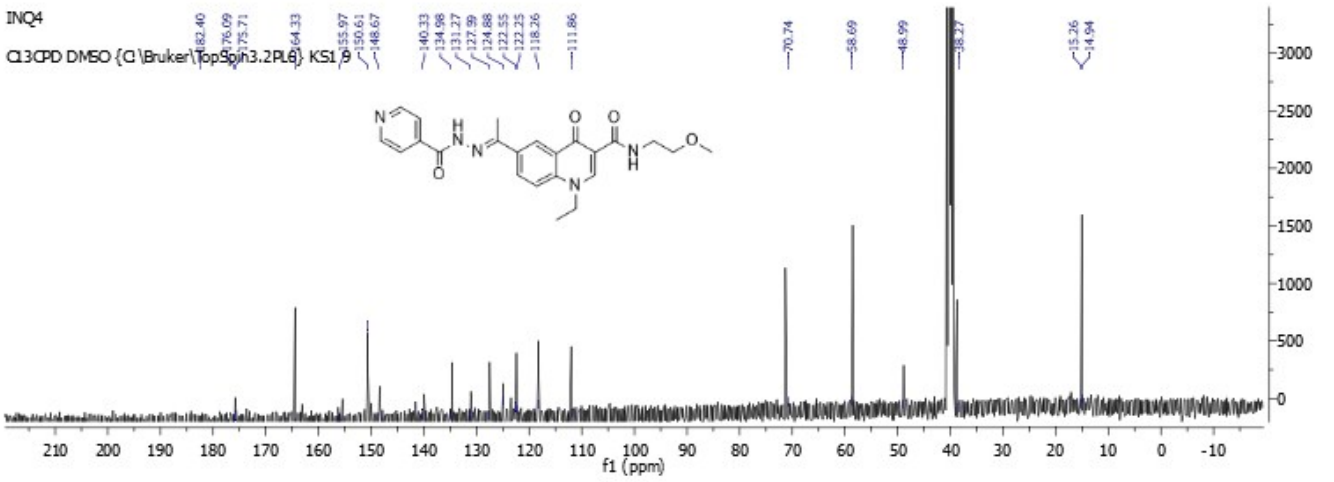

INQ009
C13DEPT135 DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 1
153.21
150.50
130.58
124.68
122.42
118.35
60.23
41.44
14.94
f1 (ppm)

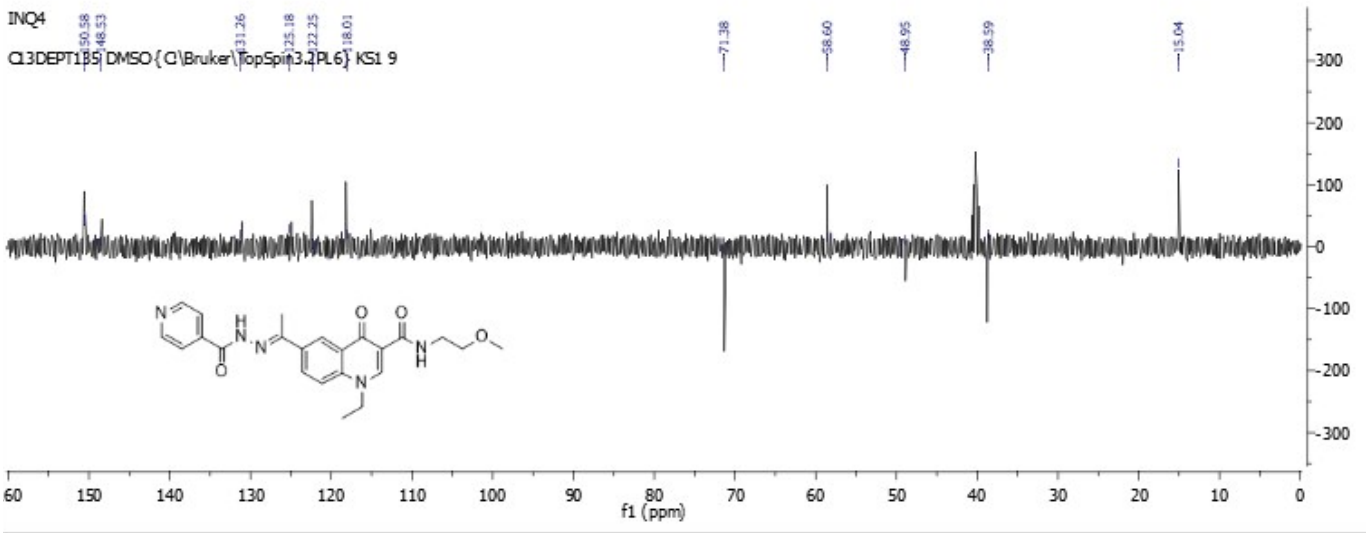
**Compound 10**


RB002INH
C13CPD DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 5
f1 (ppm)

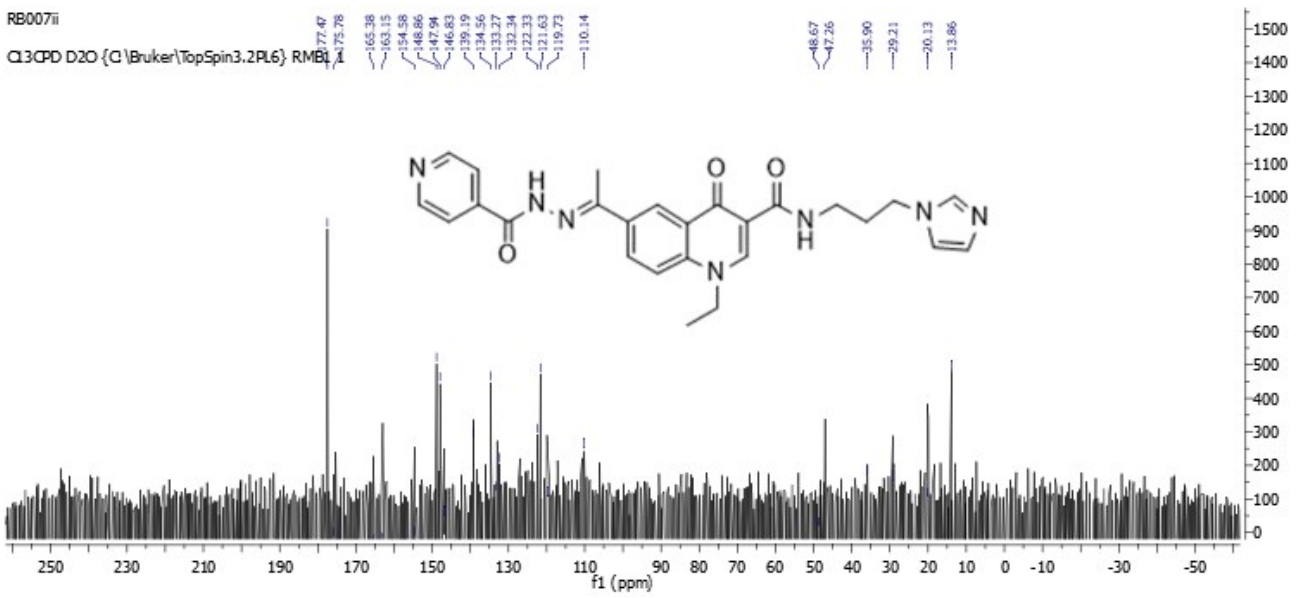

RB002INH
C13DEPT135 DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 5
f1 (ppm)

**Compound 11**


INQ4
C13CPD DMSO {C:\Bruker\TopSpin3.2PL6} KS1 9
f1 (ppm)


INQ4
C13DEPT135 DMSO {C:\Bruker\TopSpin3.2PL6} KS1 9
f1 (ppm)

**Compound 12**


RB007ii
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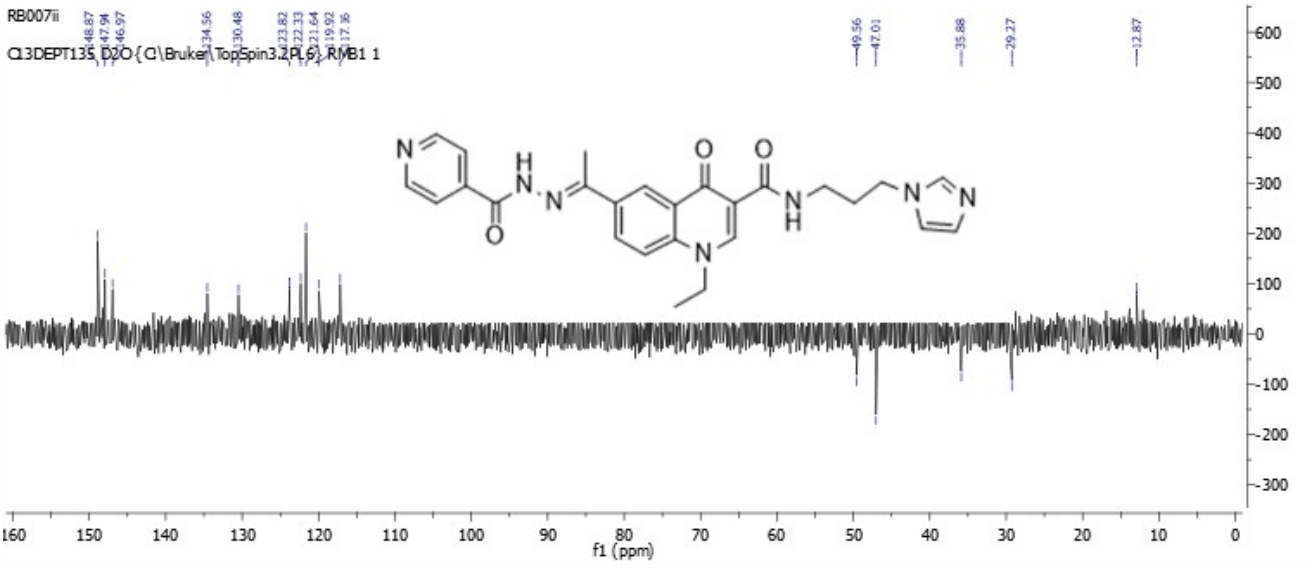
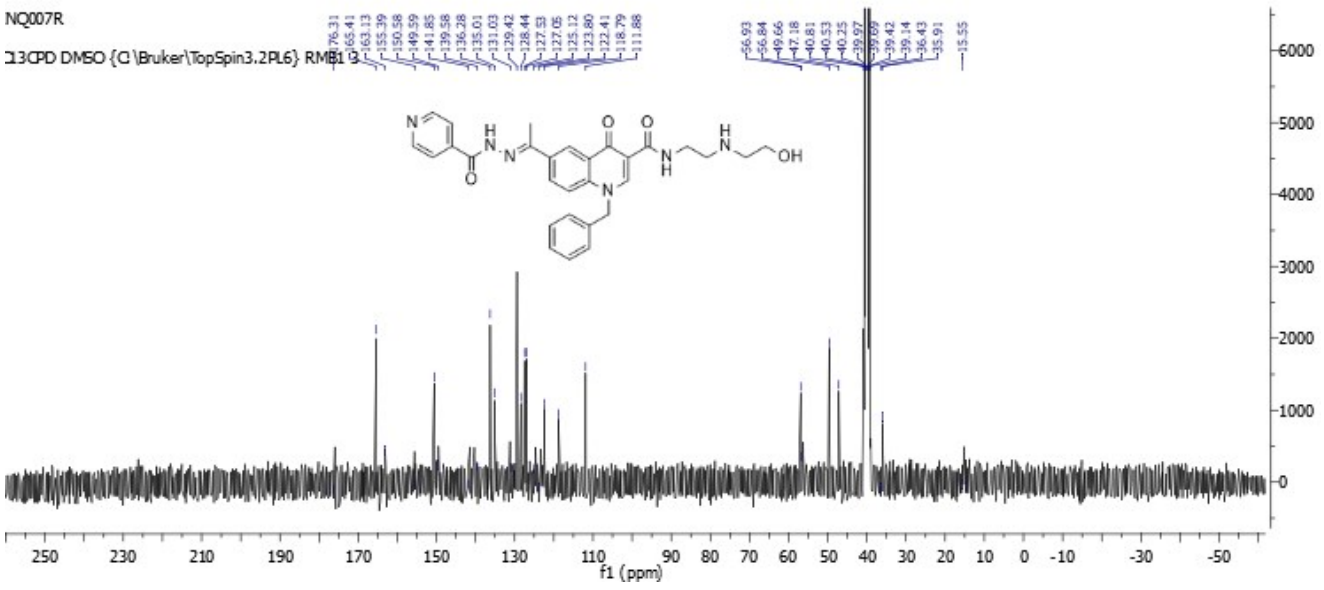
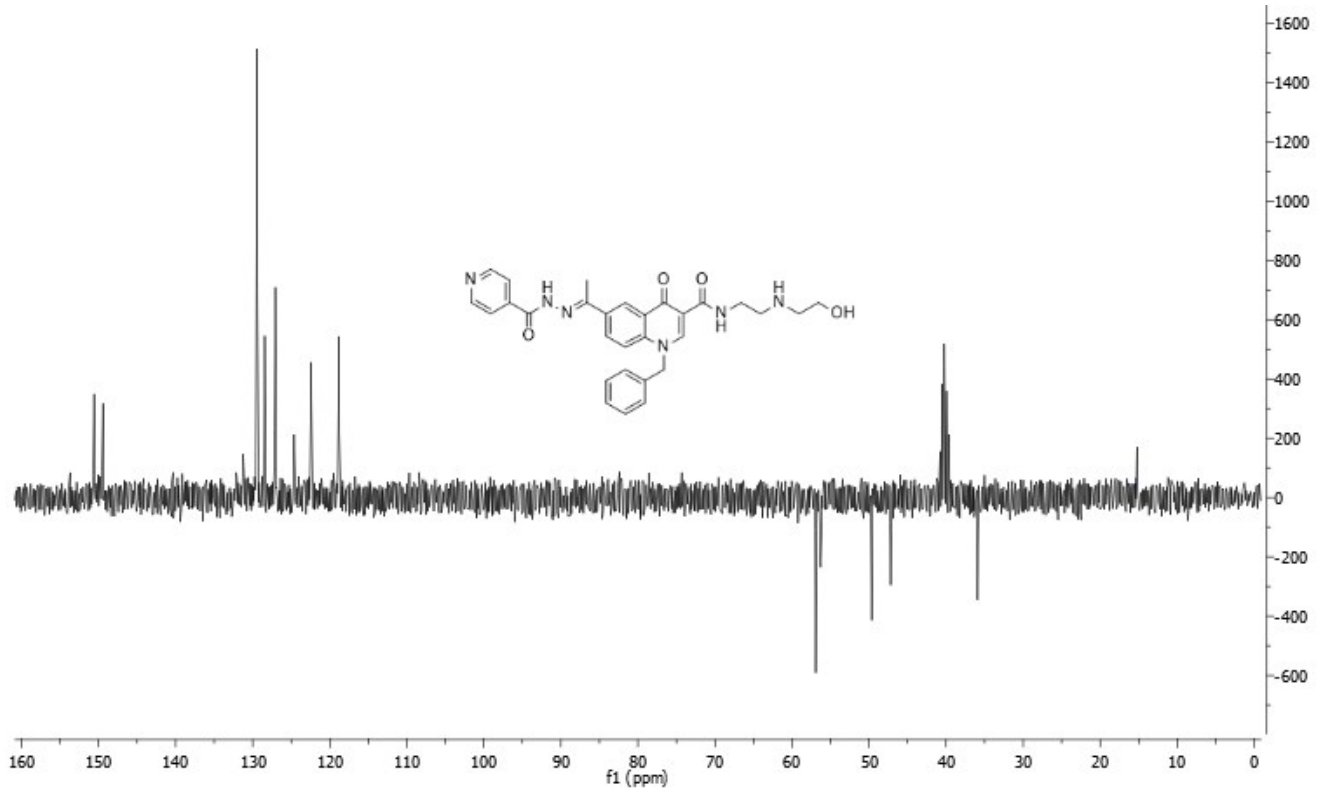
f1 (ppm)

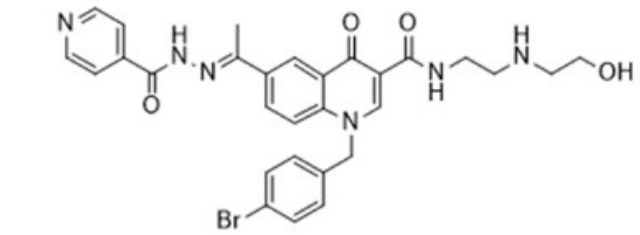

RB007ii
C13DEPT135 D2O {C:\Bruker\TopSpin3.2PL6} RMB1 1
f1 (ppm)

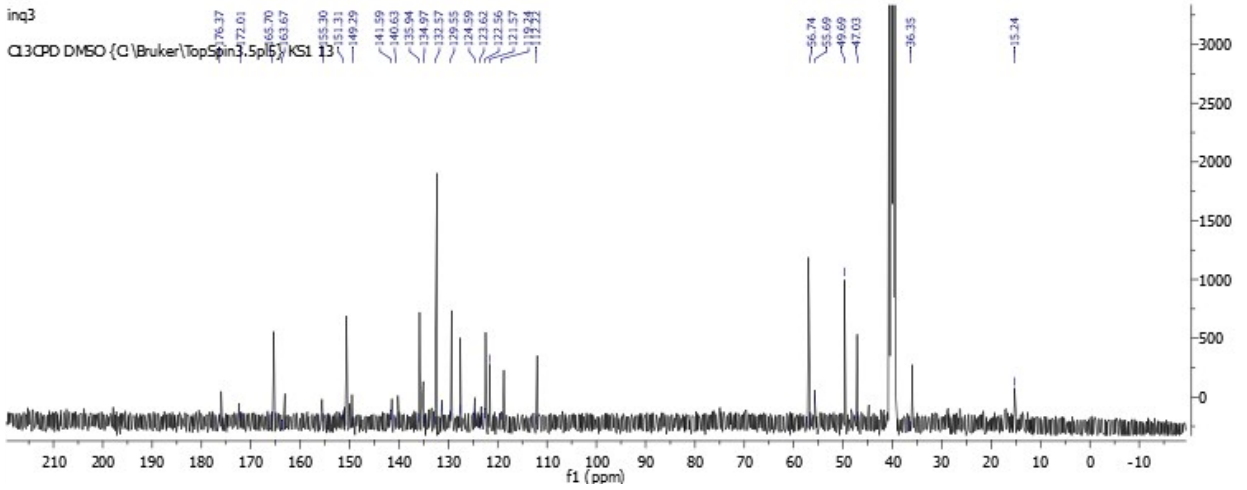
**Compound 13**




**Compound 14**

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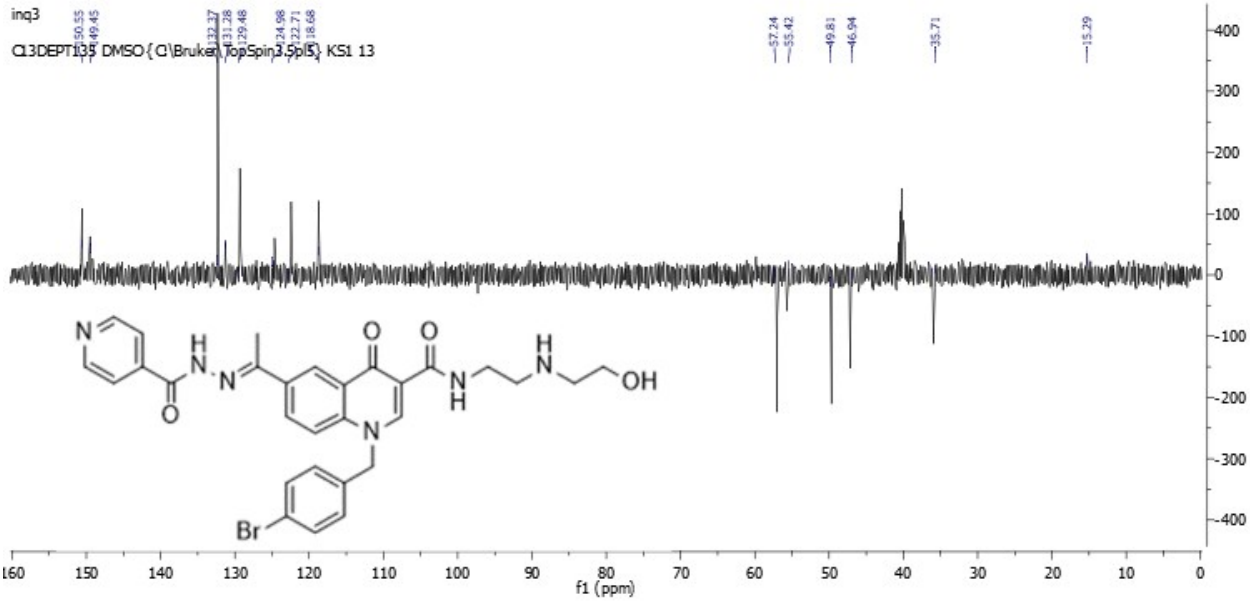
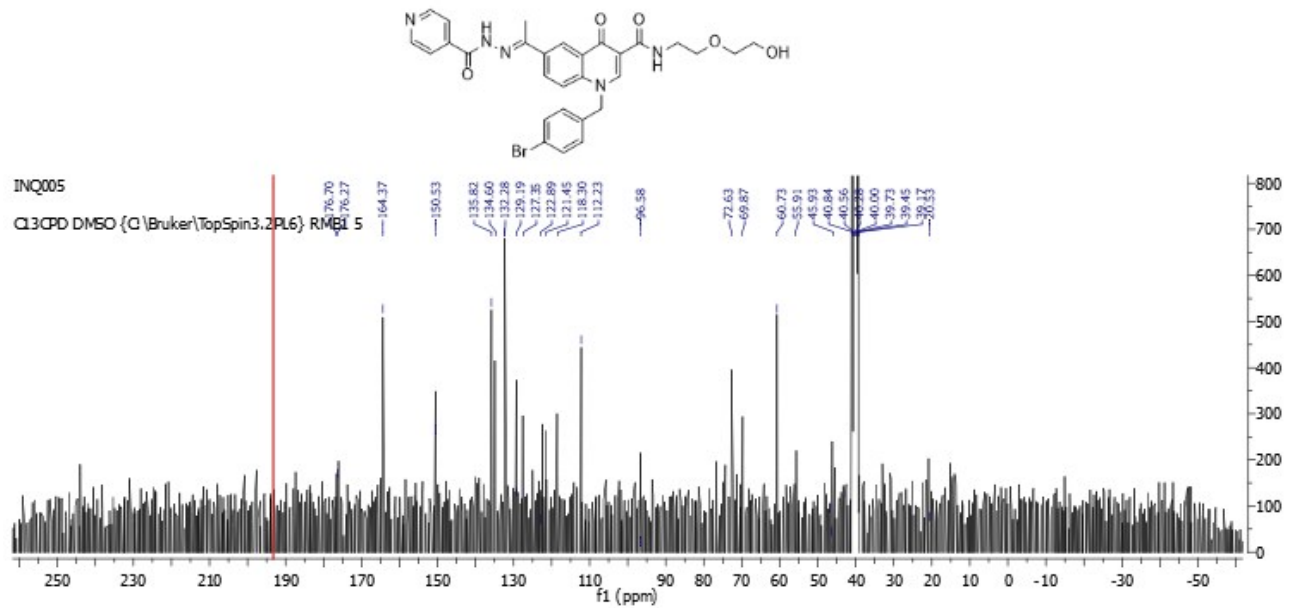
C13CPD DMSO {C:\Bruker\TopSpin3.5pl5} KS1 13
f1 (ppm)


inq3
C13DEPT135 DMSO {C:\Bruker\TopSpin3.5pl5} KS1 13
f1 (ppm)

**Compound 15**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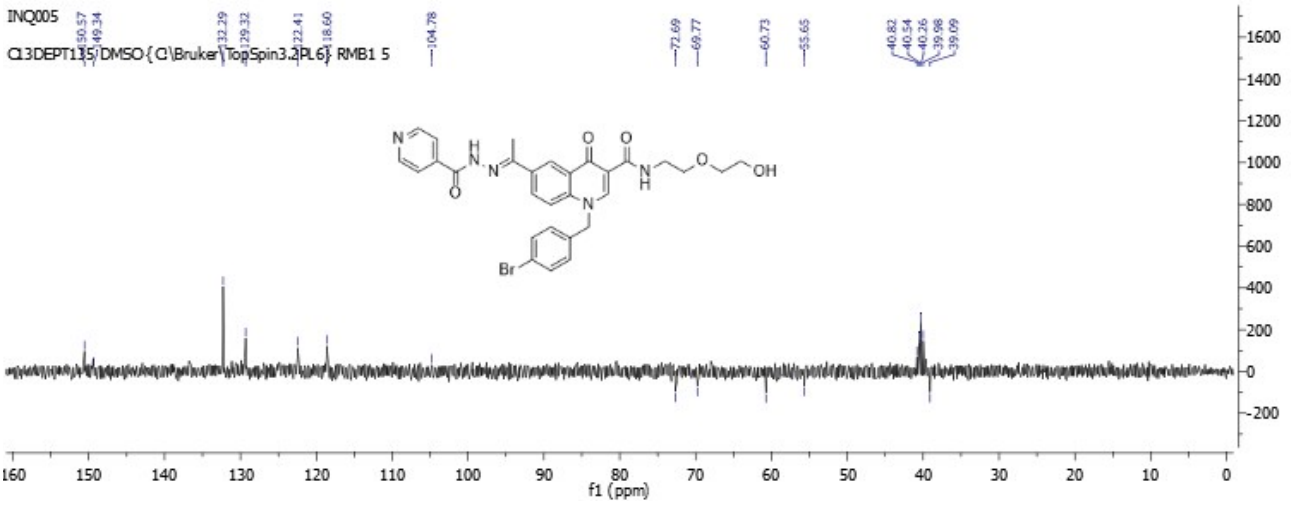
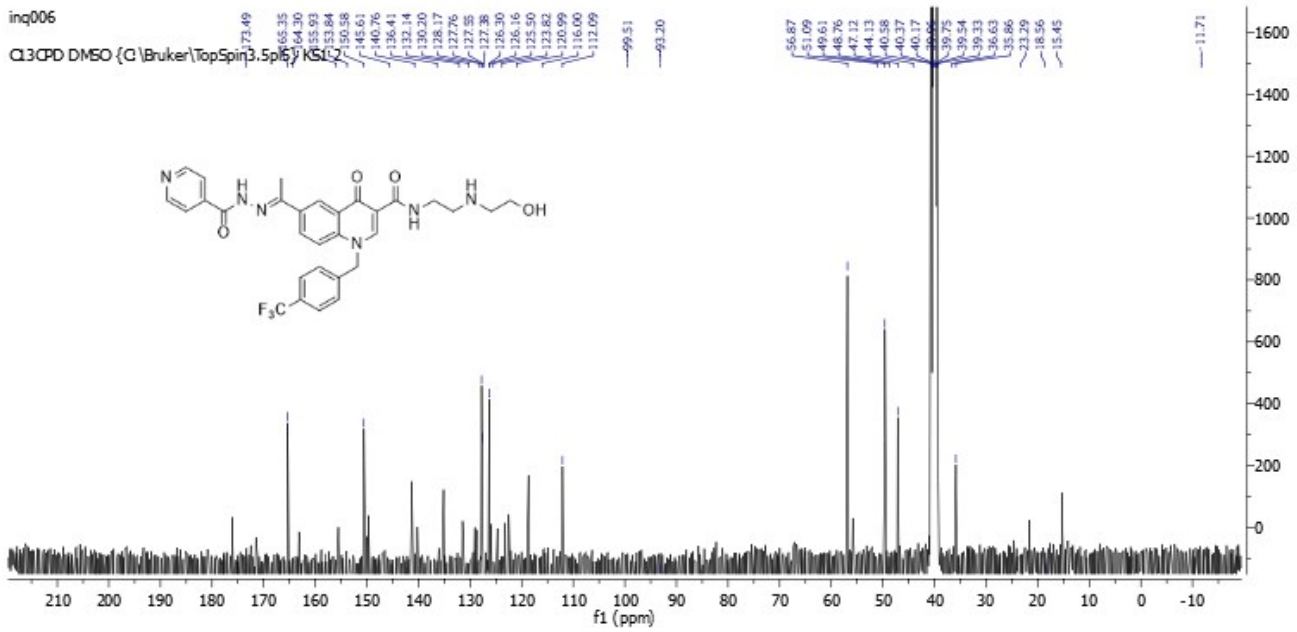
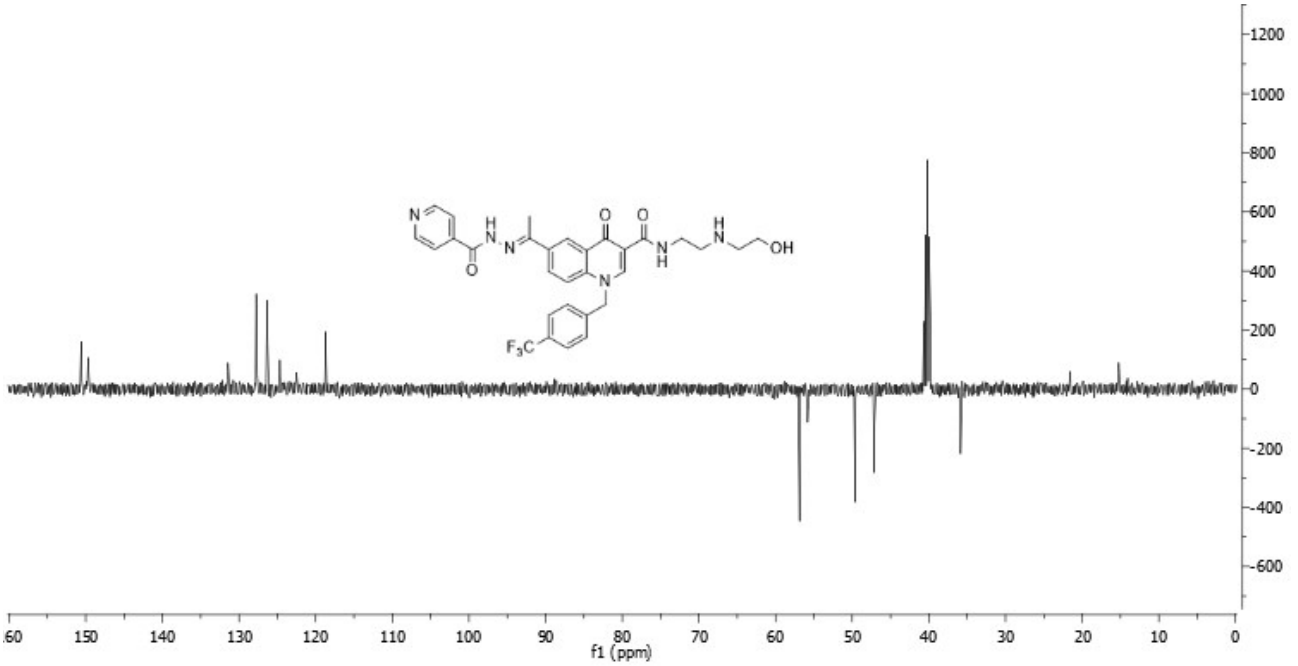
f1 (ppm)

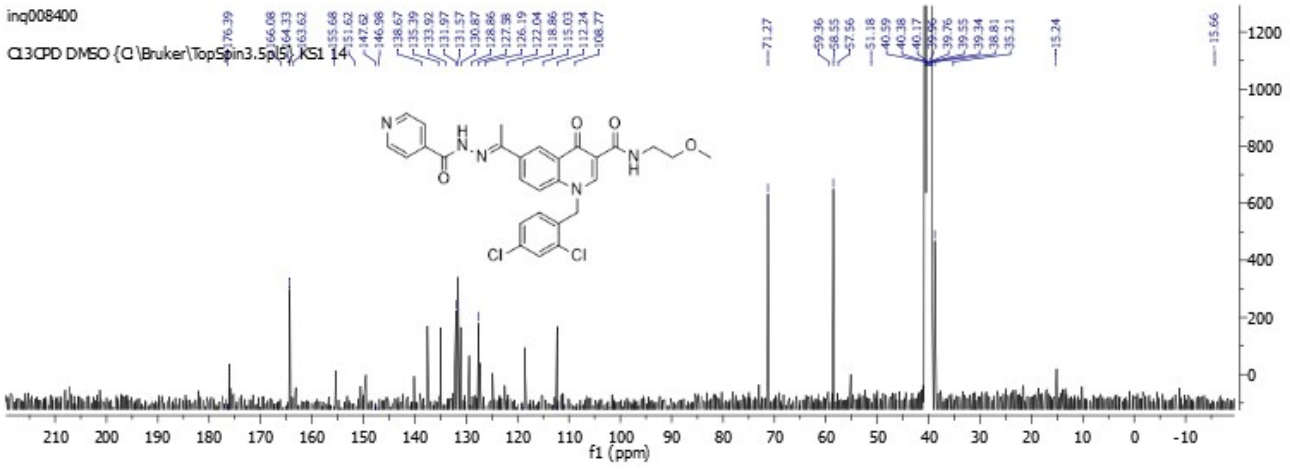

INQ005
C13DEPT135/DMSO {C:\Bruker\TopSpin3.2PL6} RMB1 5
f1 (ppm)

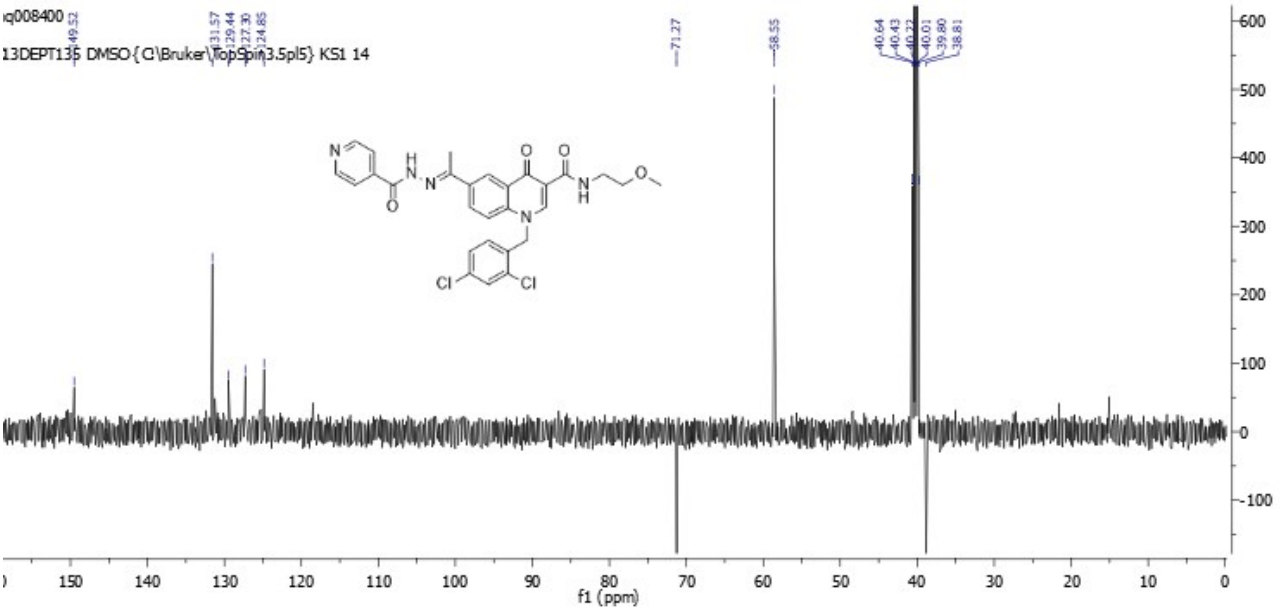
**Compound 16**



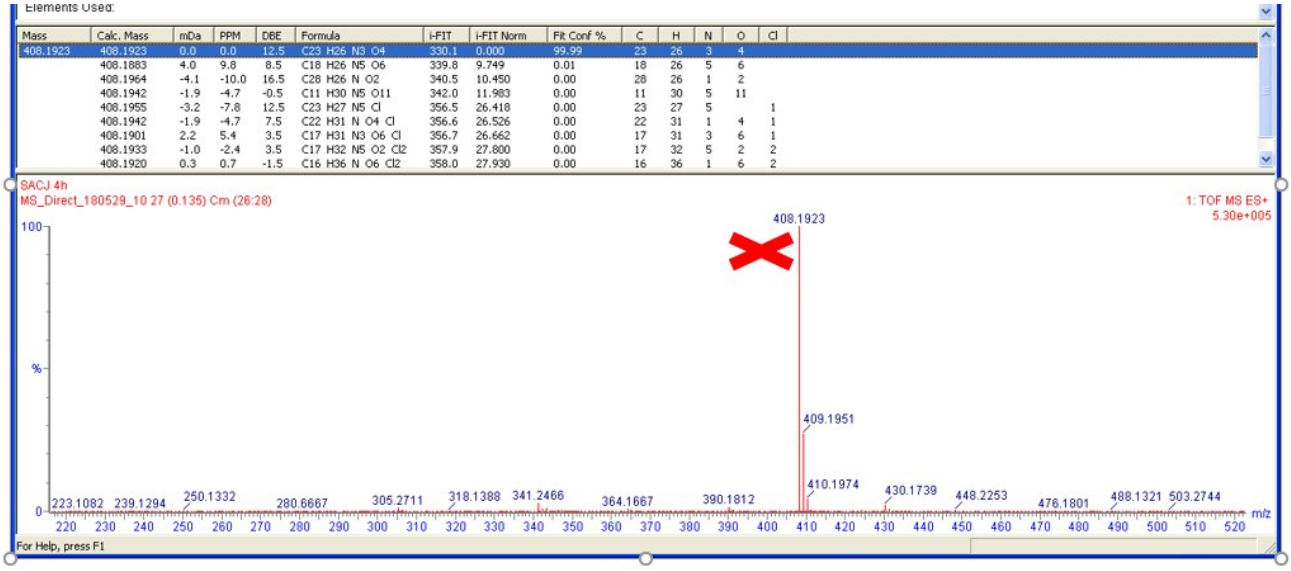

**Compound 17**


inq008400
C13CPD DMSO {C:\Bruker\TopSpin3.5pl5} KS1 14
f1 (ppm)

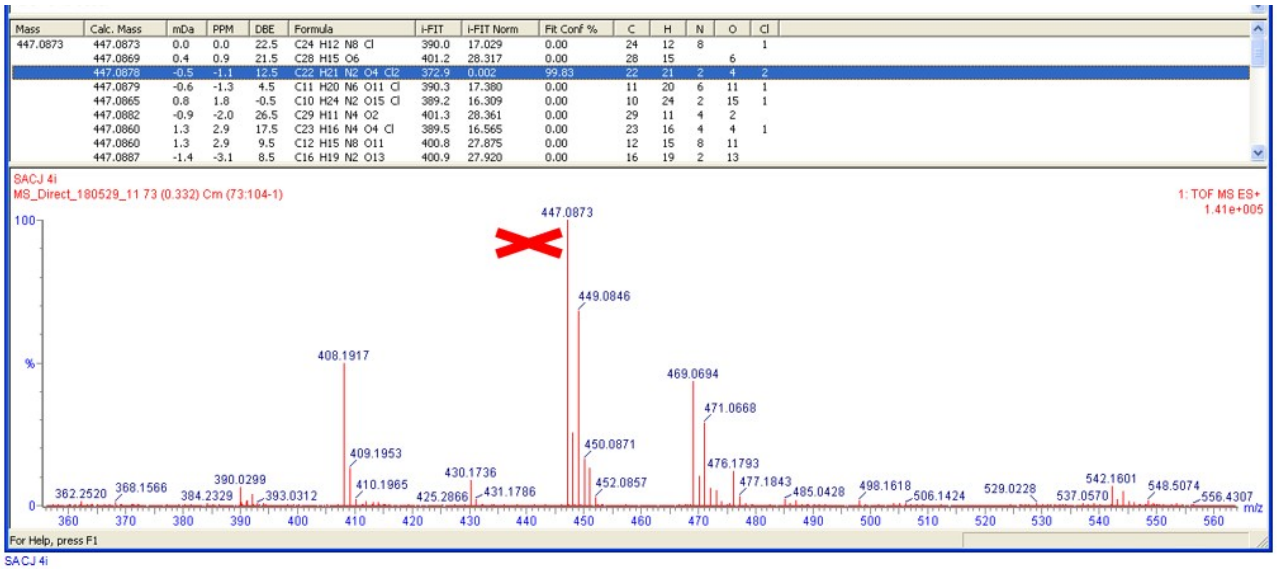

inq008400
13DEPT135 DMSO {C:\Bruker\TopSpin3.5pl5} KS1 14
f1 (ppm)

**Compound 18**

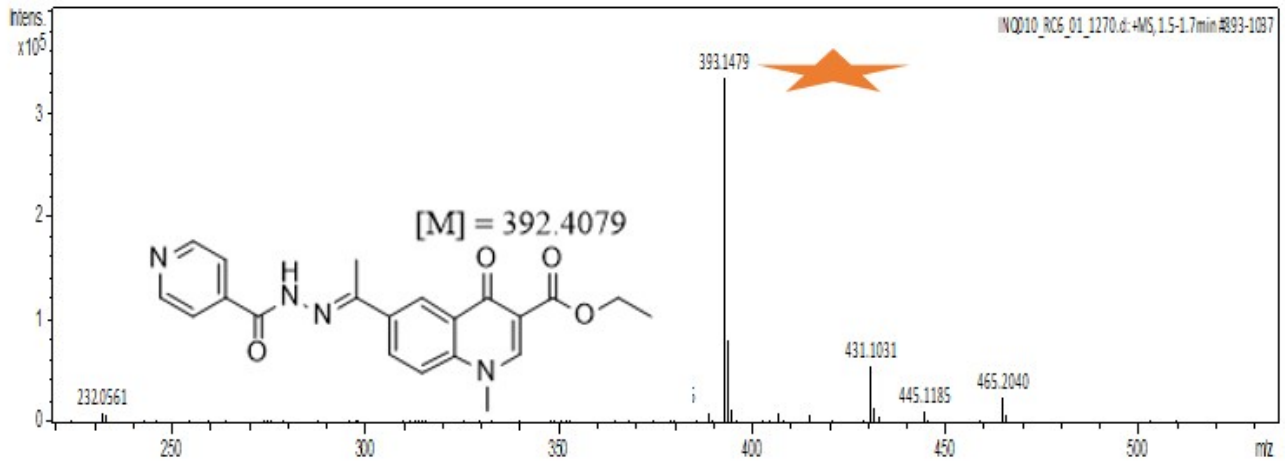
# 5. Mass spectrometry spectra for synthesized compounds



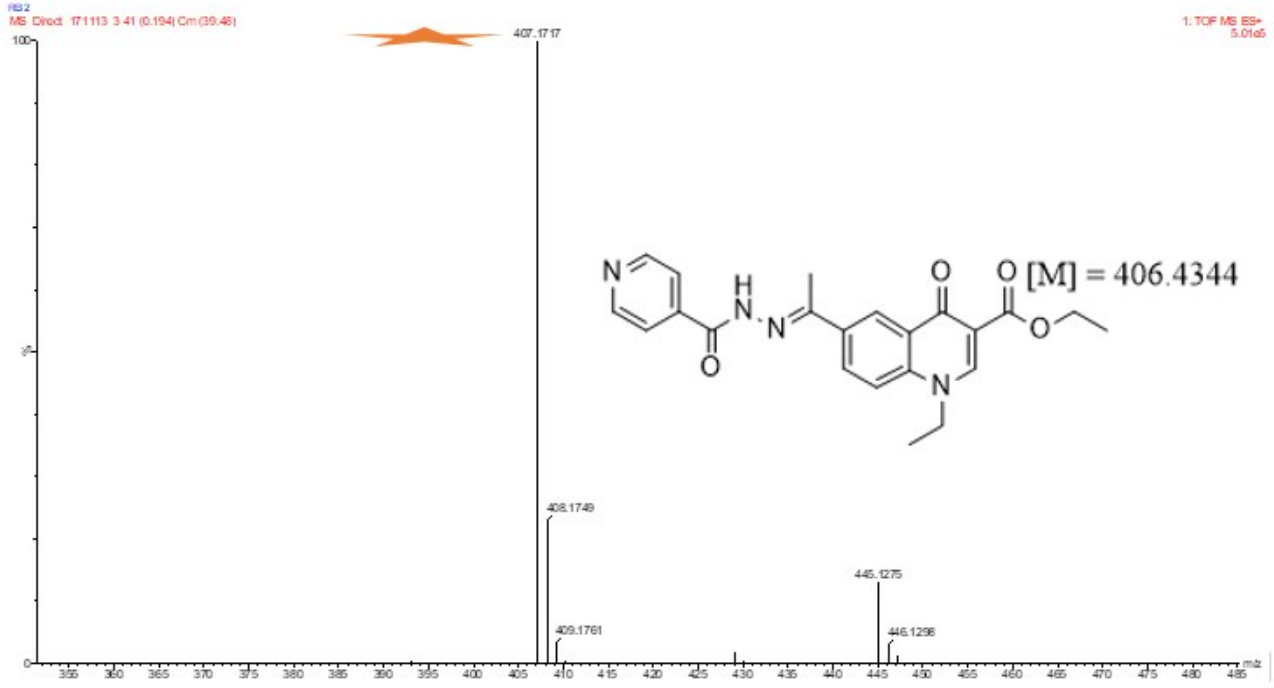

**Compound 8**



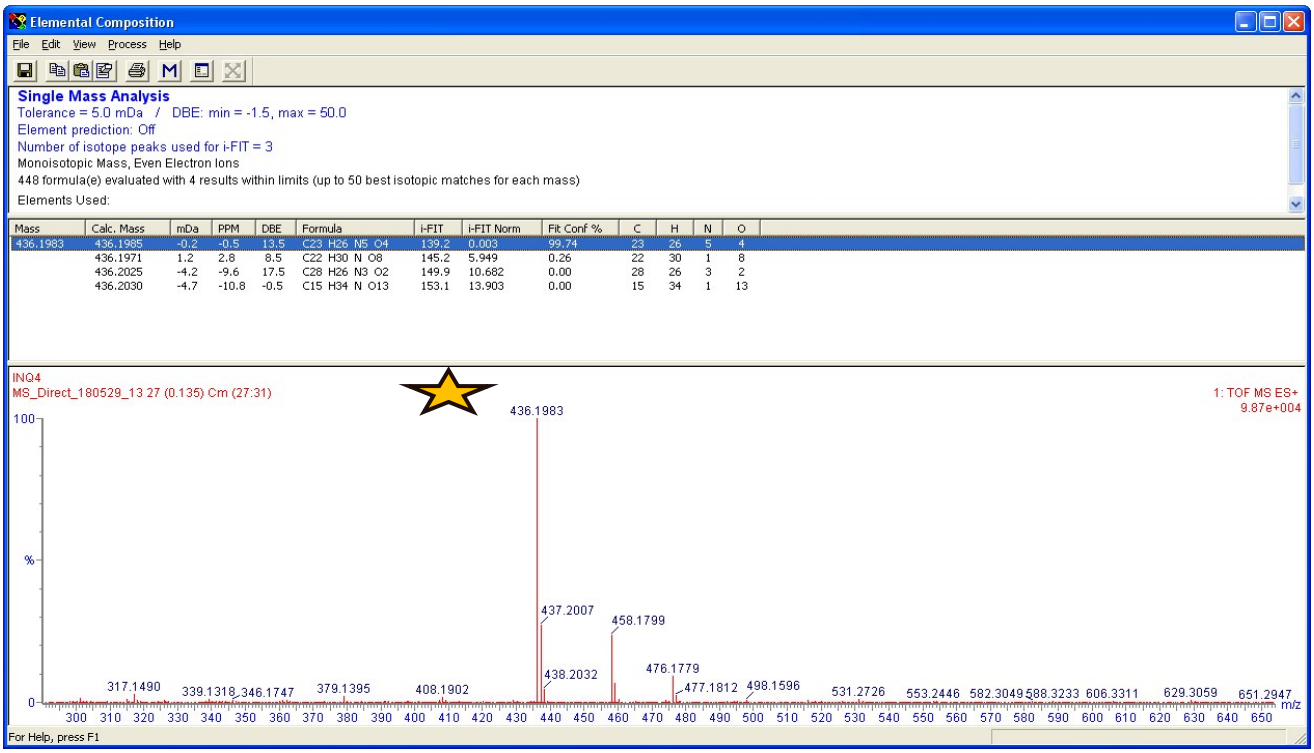

**Compound 9**



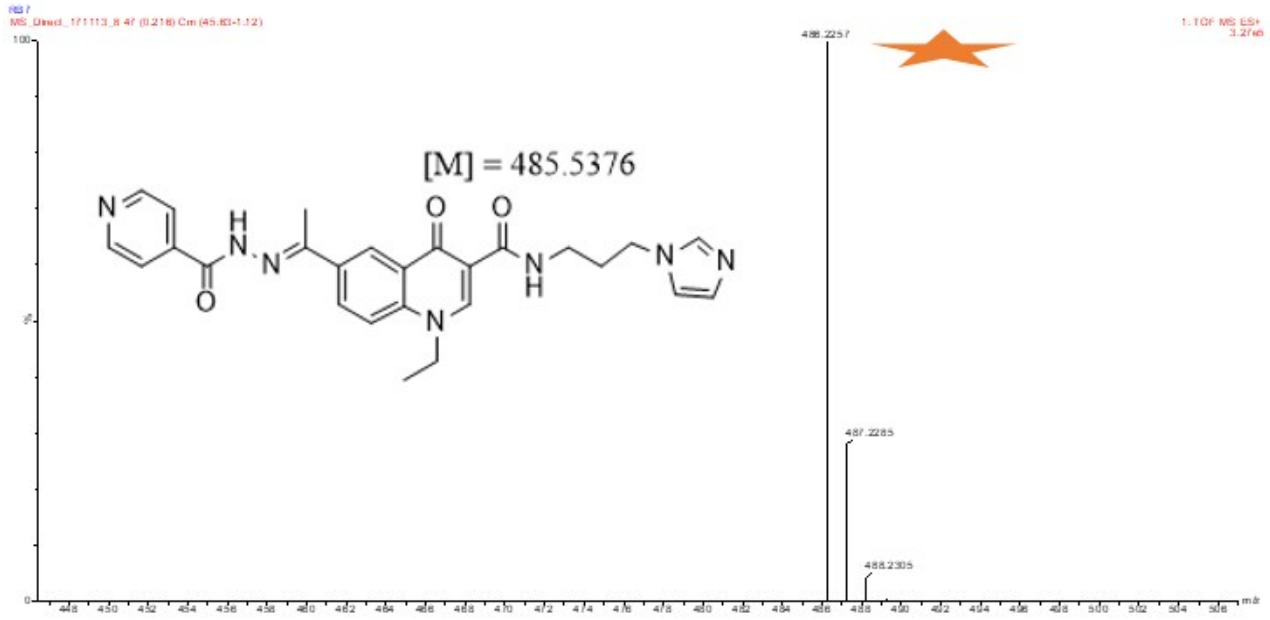

**Compound 10**



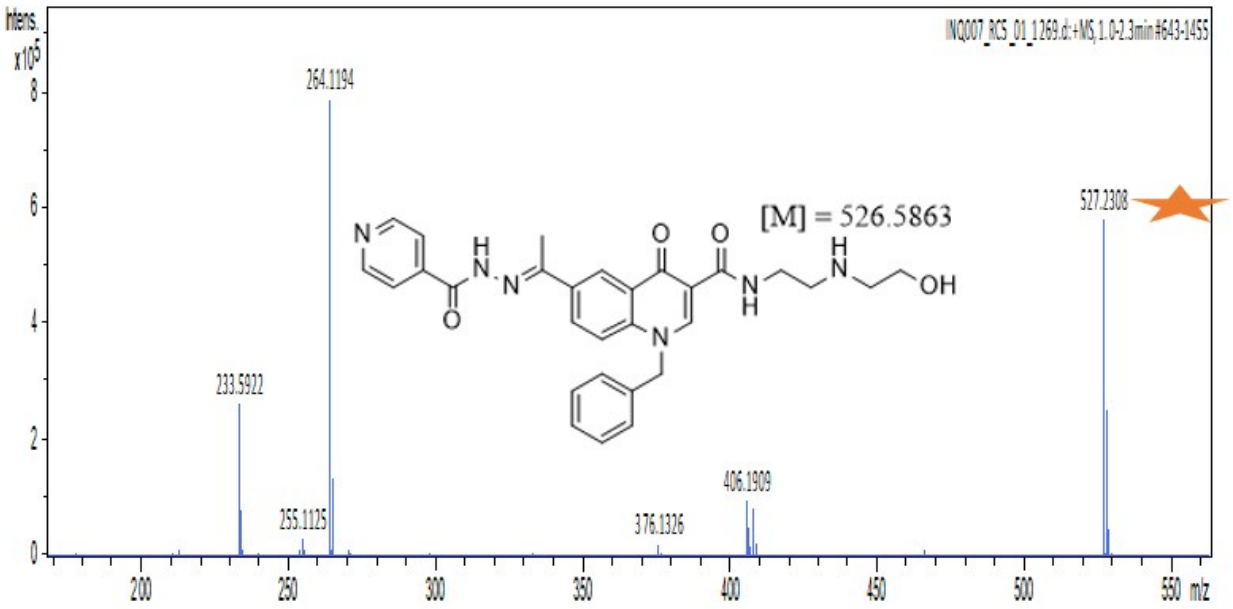

**Compound 11**



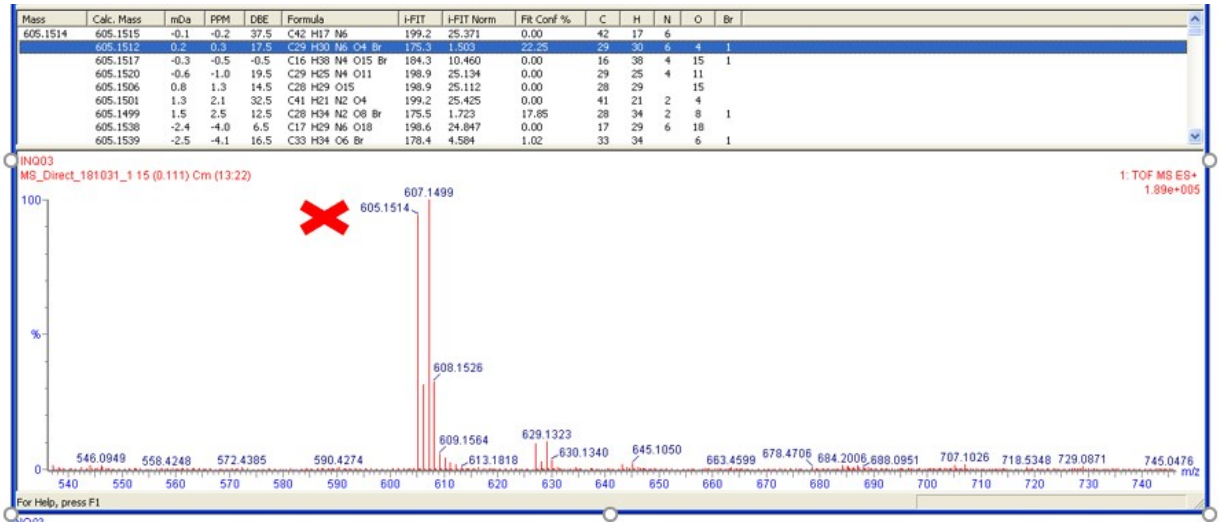

## Compound 12



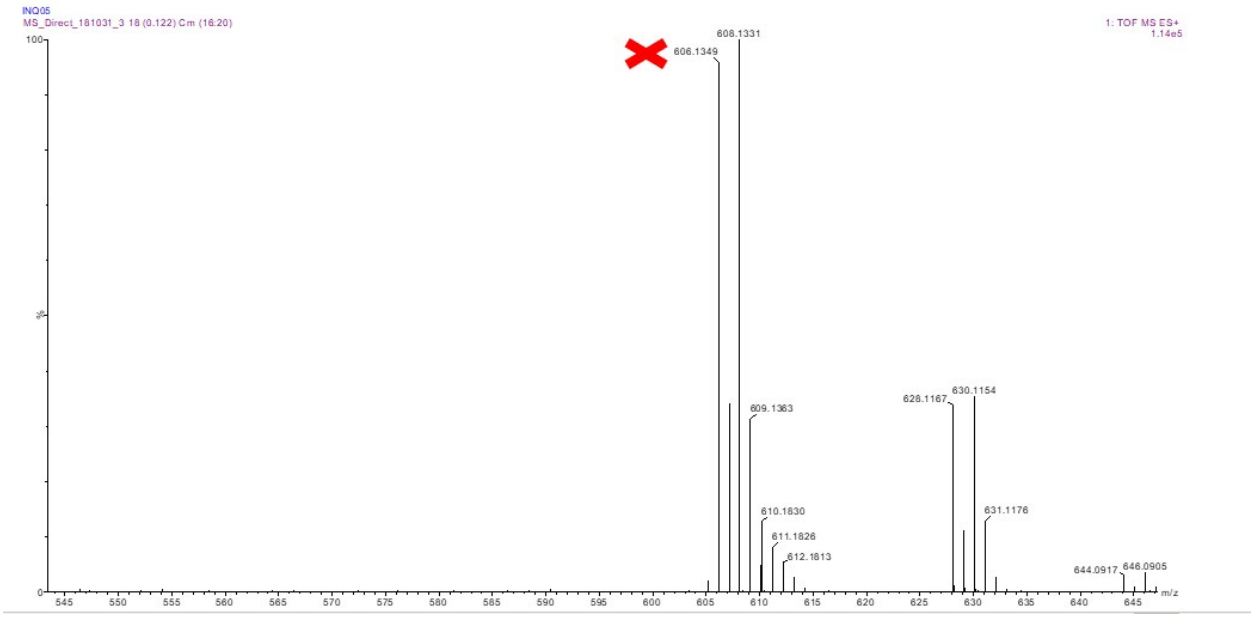

## Compound 13



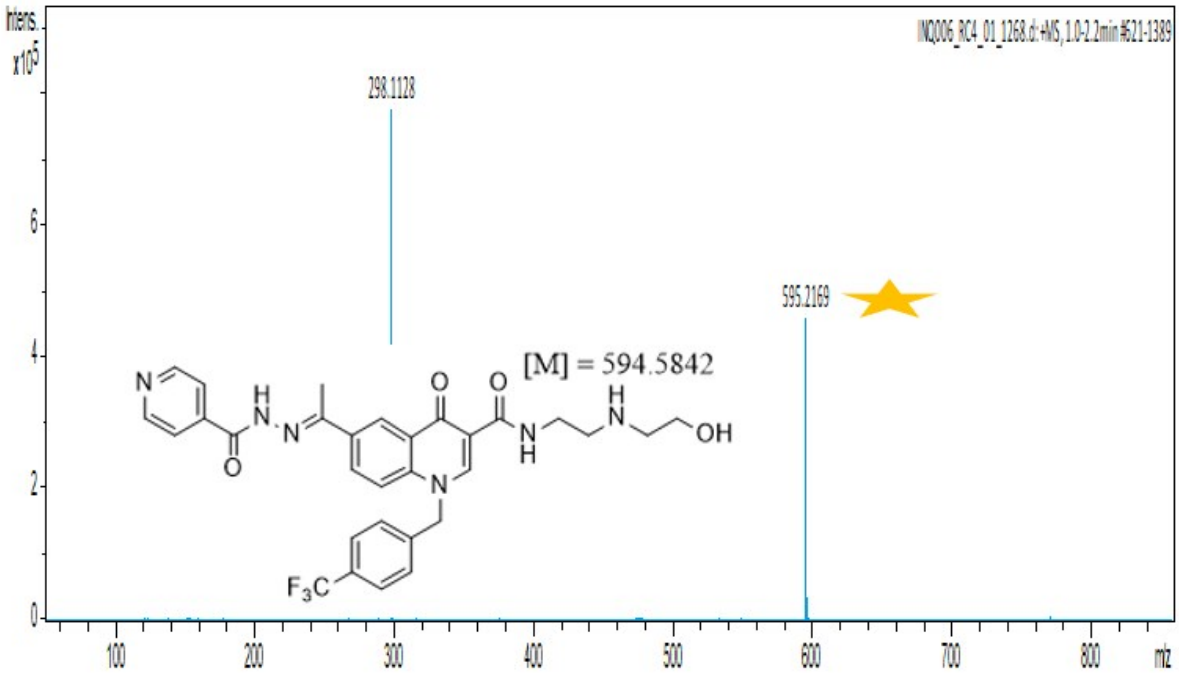

**Compound 14**



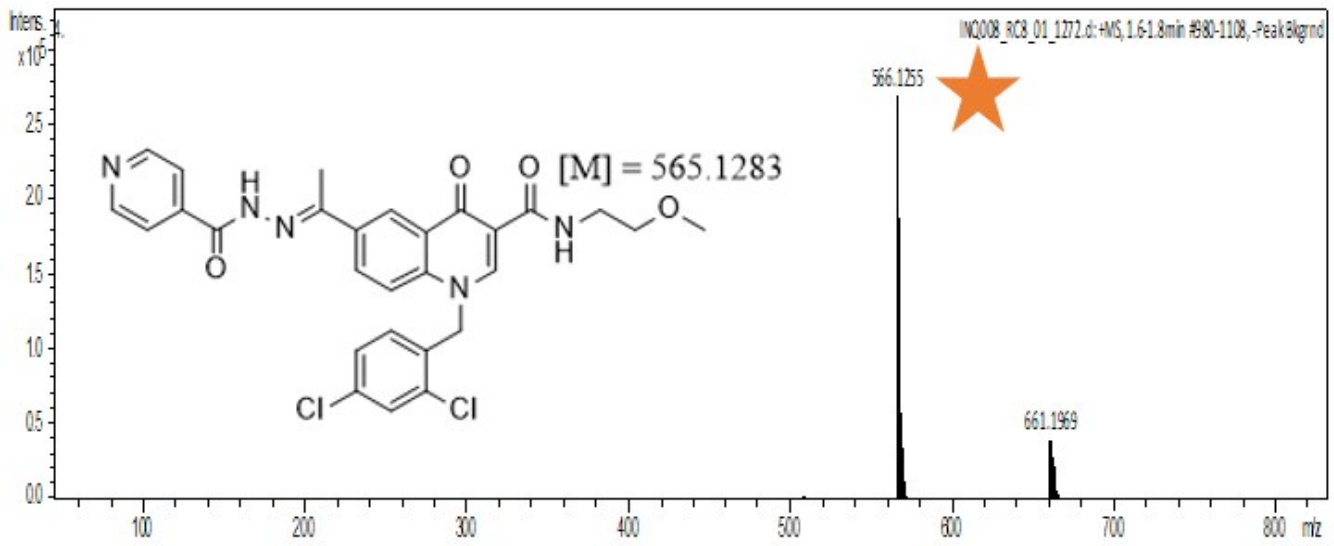

**Compound 15**




**Compound 16**

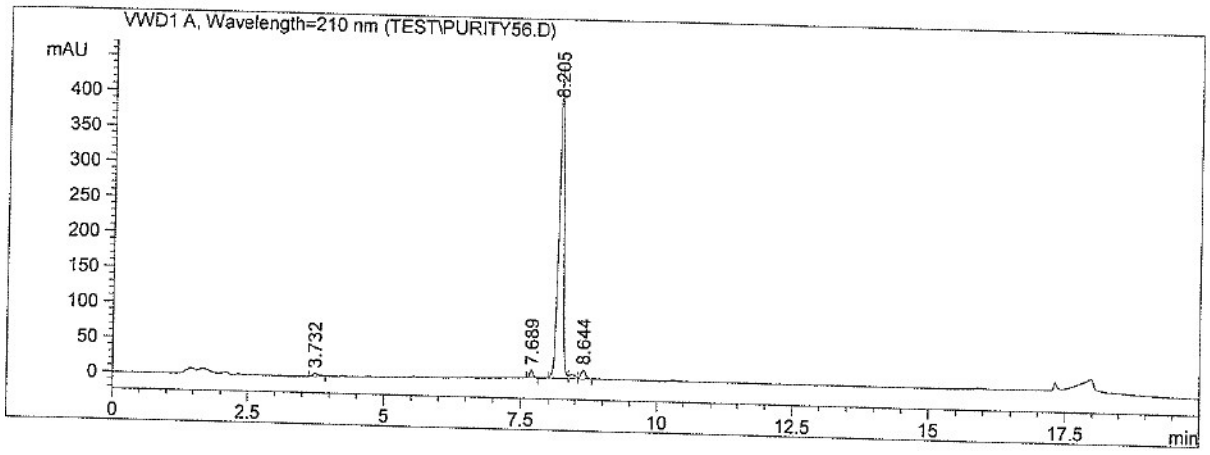



**Compound 17**

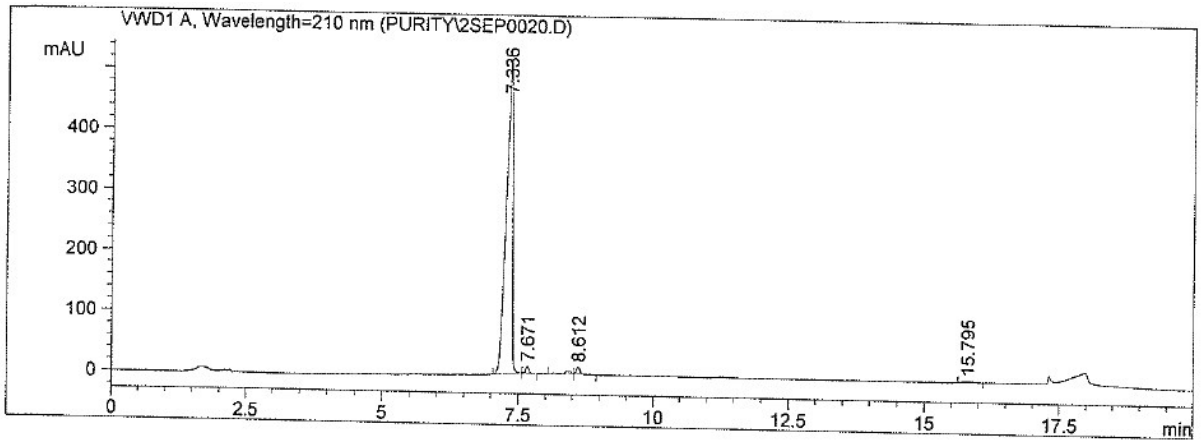

Intens.
x10^5
2.5
2.0
1.5
1.0
0.5
0.0
100
200
300
400
500
600
700
800
m/z
566.1255
661.1959
[M] = 565.1283
N
H
N
O
O
O
N
H
O
N
Cl
Cl

**Compound 18**

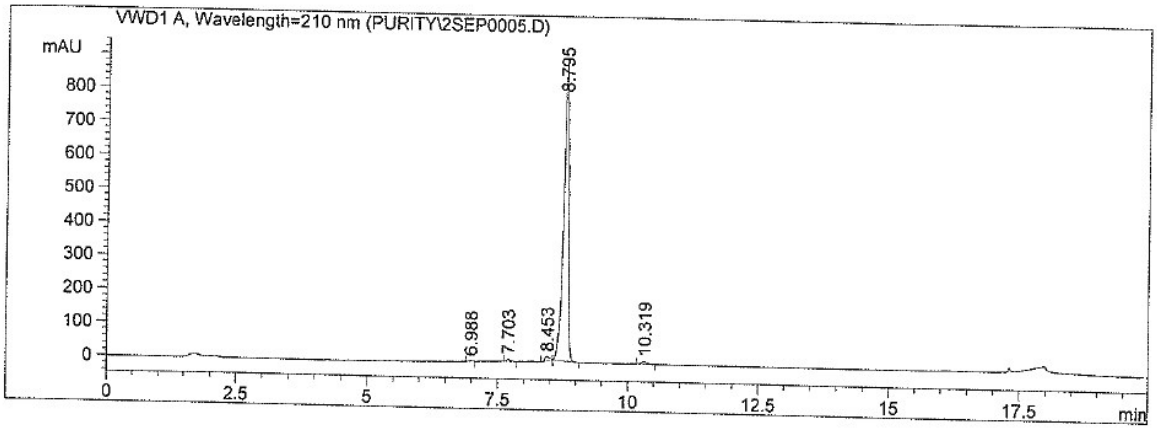
# 6. HPLC chromatograms of synthesized compounds



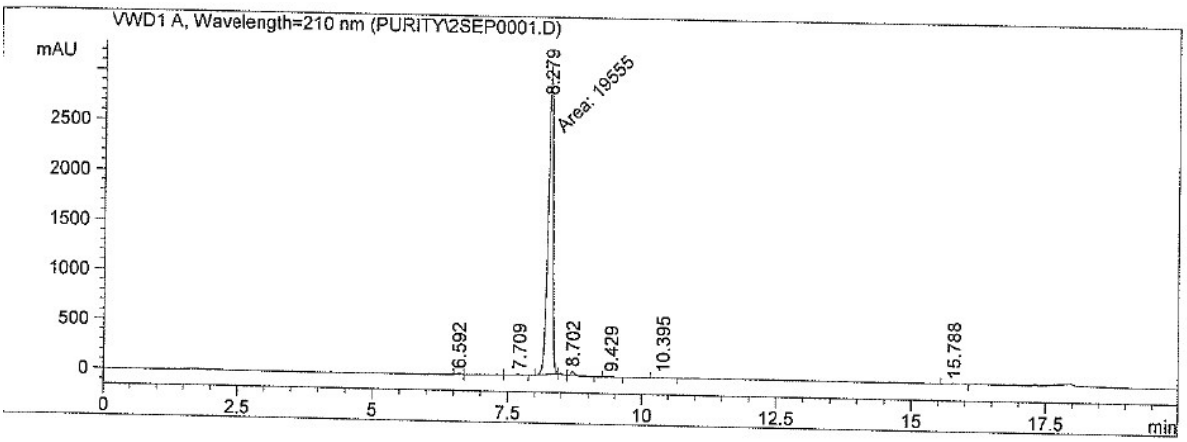

**Compound 8**



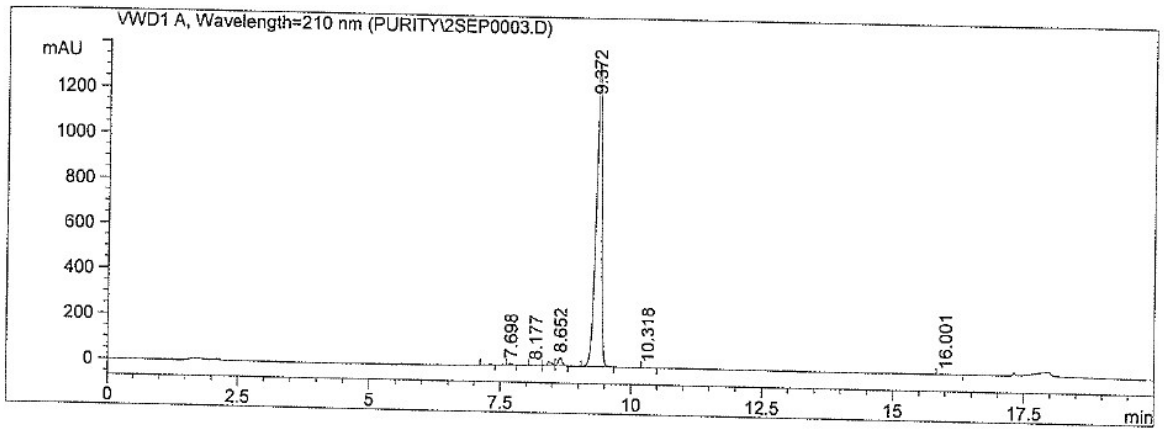

**Compound 9**



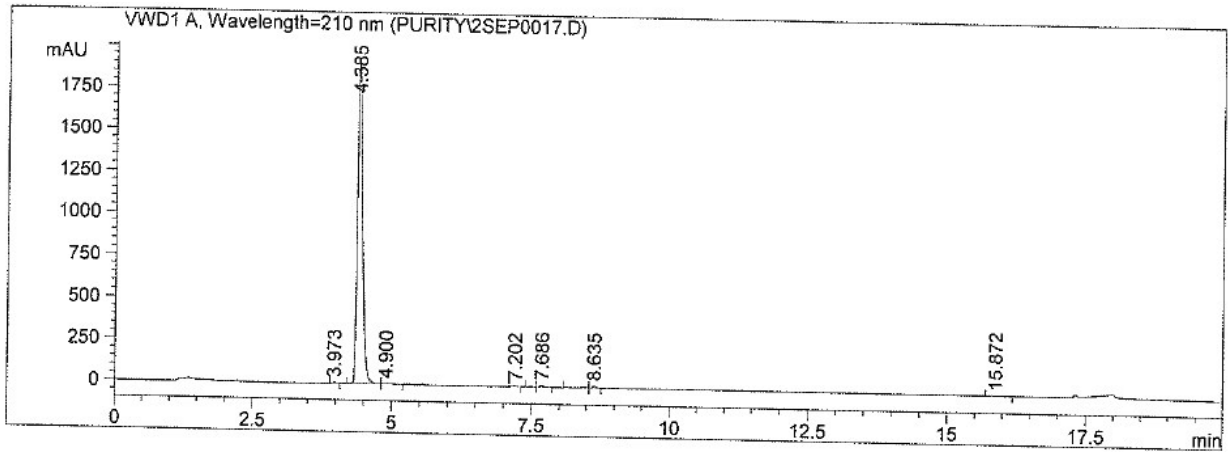

**Compound 10**



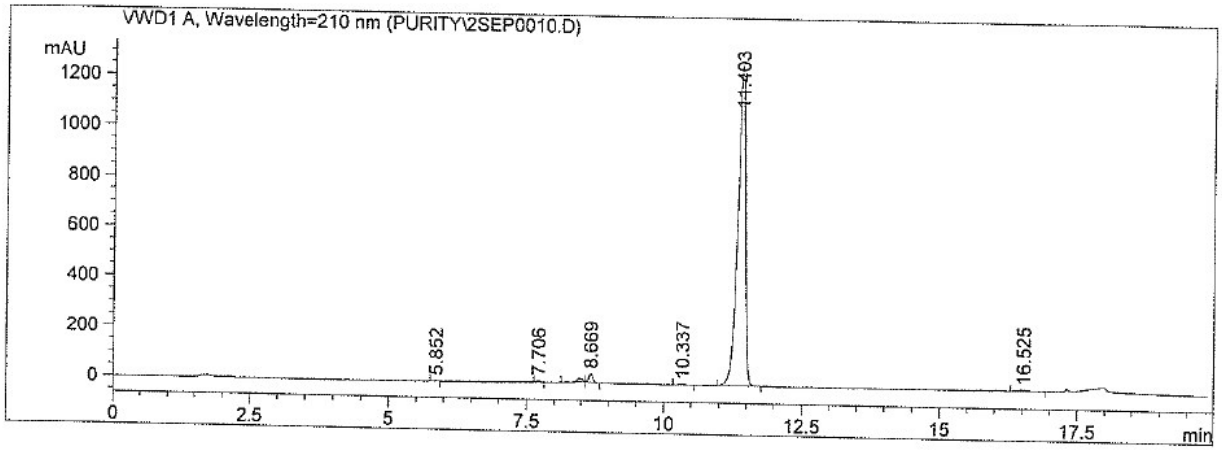

**Compound 11**



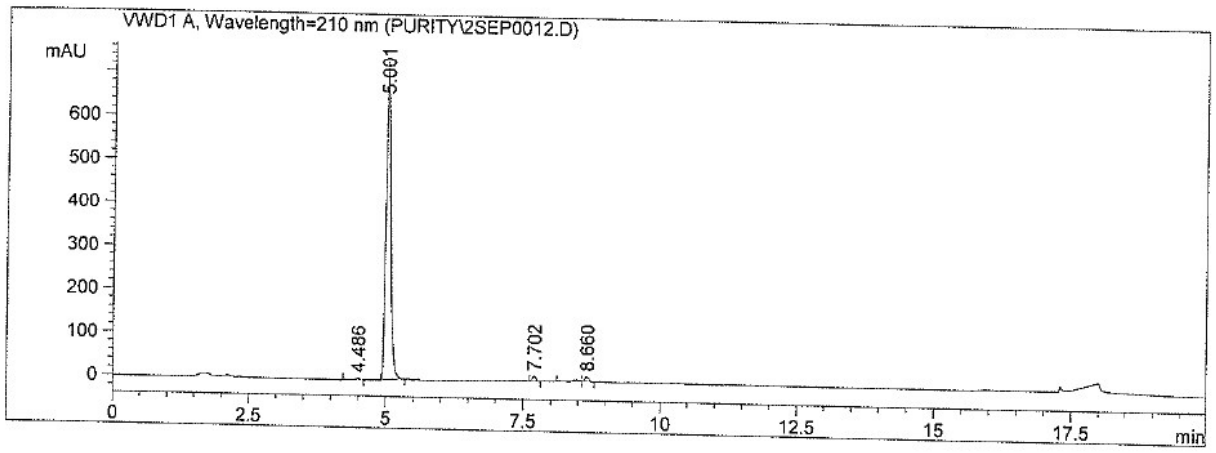

**Compound 12**



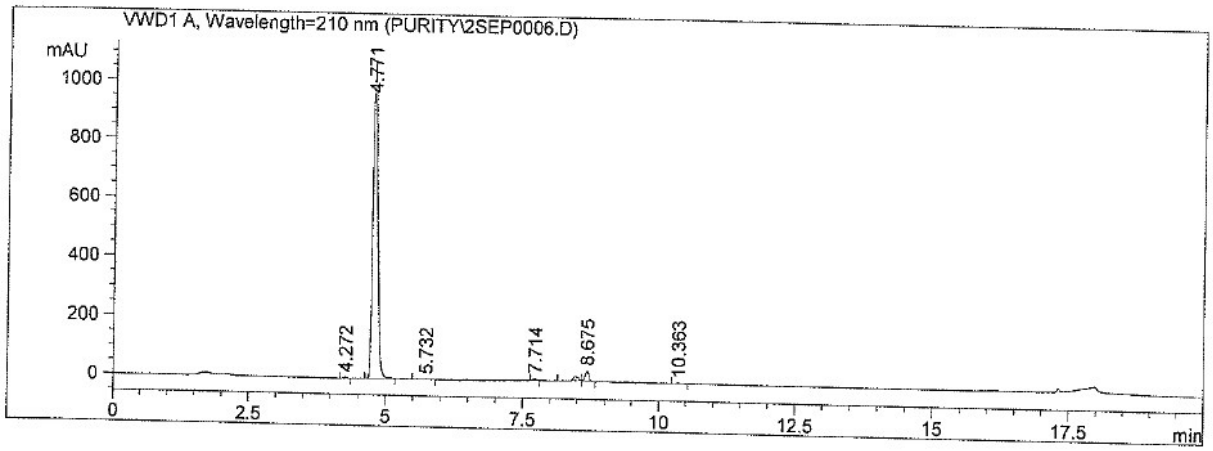

**Compound 13**



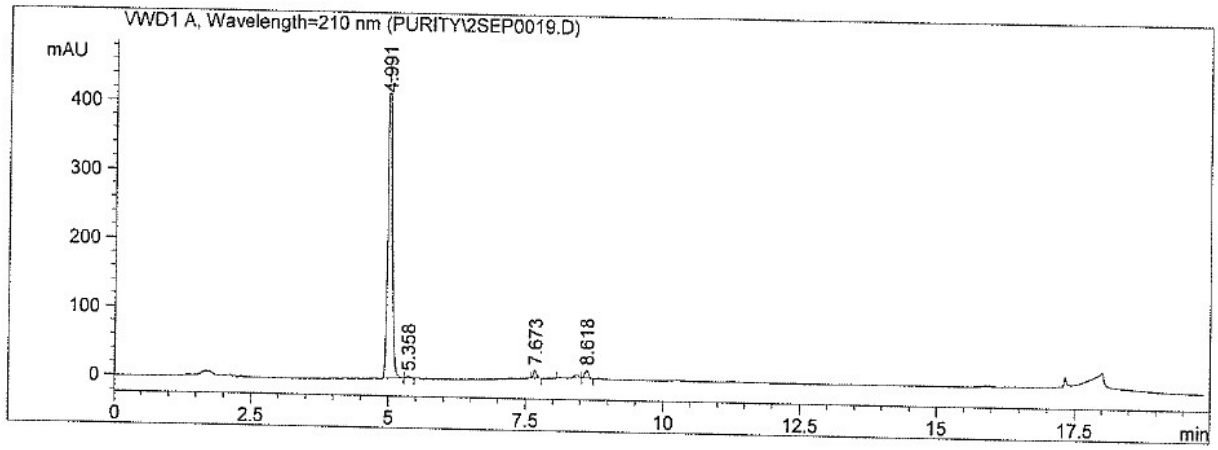

**Compound 14**



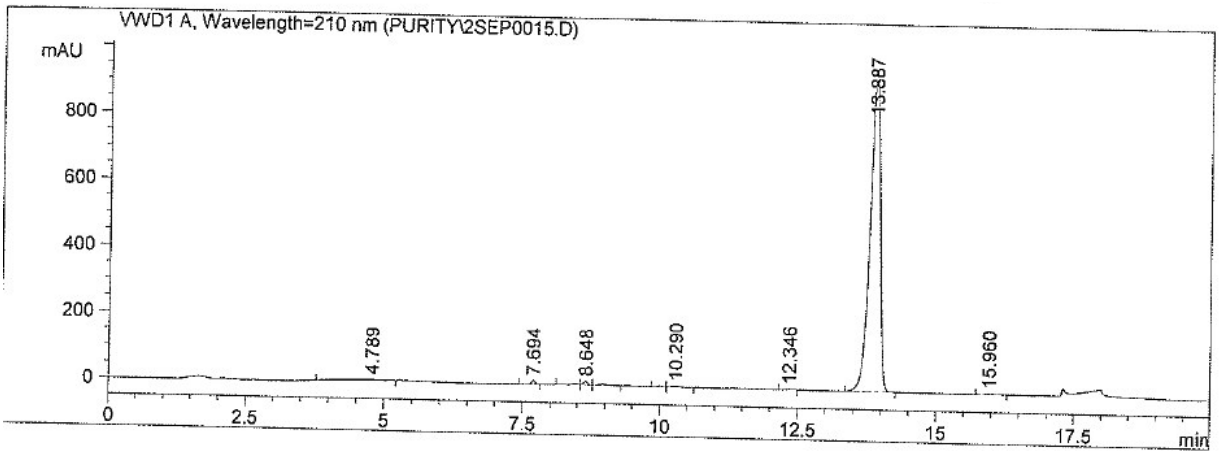

**Compound 15**




**Compound 16**




**Compound 17**




**Compound 18**