Electronic Supplementary Material (ESI) for MedChemComm.

Fluorinated thiazolidinols cause cell death in A549 lung cancer cells *via* PI3K/AKT/mTOR and the MAPK /ERK signalling pathways

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Supporting Information

Table of contents ……Pages

1. Material and Methods:

Cell Cultures and maintenance

The four human tumour cell lines A549 (lung carcinoma), Hela (Cervical cancer), MCF7(breast adenocarcinoma, estrogen-dependent ER (+)) and MDA-MB-231(breast adenocarcinoma, estrogen-independent ER (-)), used in the present study were purchased from the American Type Culture Collection (Manassas, VA, USA) and were grown in the specific growth medium (Dulbecco's Modified Eagles medium supplemented with 10% fetal bovine serum and 100U/ml penicillin, 100 µg/ml streptomycin sulphate antibiotics at 37 °C in a humidified atmosphere containing 5% CO_2). The cells were trypsinized when subconfluent from T25 flasks/60 mm dishes and seeded either to T25 or T75 flasks or 96 well plates depending on the assay to be performed.

Growth Inhibition Assay:

MTT assay (Vybrant® MTT Cell Proliferation Assay Kit (V-13154)) was performed to assess the cytotoxicity (/growth inhibition) of the test compounds on the chosen cancer cell lines. Cells were plated onto 96 well microtitre plates at $10x10^3$cells/well and allowed to adhere overnight. Following the incubation period, test compounds were added to each of the wells at concentrations of 0, 2, 4, 8 and16 µM and incubated for 24 h. After 24 h, the media was replaced with 100µl of fresh media followed by addition of 10µl of 12mM MTT reagent/well and incubated for 2 hrs in dark at 37°C according to the manufacturer's instructions. Finally, the reaction was terminated by addition of 100µl of SDS-HCL. Readings were taken at 570 nm using Multimode Varioskan Flash (Thermo Fisher Scientifics) with media as blank. The IC_{50} values were obtained from the results of triplicate determinations of atleast three independent experiments.

Cell Cycle Analysis:

Cells (~2×10^5 cells) were seeded in 60 mm dish and allowed to grow for 24h. Cells were treated with test compounds at their respective IC50 concentration for 24h. Attached cells were harvested with Trypsin-EDTA and washed twice with Phosphate Buffered Saline (PBS). Cells were fixed by adding 1ml of ice cold 70% ethanol dropwise with vortexing followed by incubation at 4°C overnight. Fixed cells were pelleted by centrifugation at 2,000 rpm for 2 min, washed with PBS and repelleted. The cells were resuspended in PBS containing 40 µg/ml PI, 0.1 mg/ml RNase and 0.1% Triton X-100 in dark for 30 minutes at

37°C. The DNA content of 20,000 events was measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed using Summit 4.3 Software.

Protein Extraction and Western Blot Analysis:

For analysis of protein levels by western blotting, A549 cells were treated with the compounds at the respective concentrations and the total cell lysates were collected at 24h post treatment. Cells were scraped into media and collected by centrifugation, rinsed with PBS and recentrifuged. Cell pellets were resuspended in ice-cold RIPA buffer (1XPBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) containing 100 μg/ml PMSF, 5 μg/ml Aprotinin, 5 μg/ml leupeptin, 5 μg/ml pepstatin and 100 μg/ml NaF. The protein in the supernatant was quantified by Bradford method (Biorad) using multimode varioskan instrument (Thermo-Fischer Scientifics). 50 micrograms of protein per lane was electrophoresed in 10% SDS-polyacrylamide gel. After electrophoresis, the protein in the gel was transferred onto polyvinylidinedifluoride (PVDF) membrane (GE Healthcare). Blocking of the membranes was performed using 5% Blocking Buffer (Skimmed Milk in TBS + 0.1% Tween20 (TBST) at room temperature for 2h, followed by primary antibody treatment at 4°C overnight. The following primary antibodies were used: Phospho-Akt (Ser473)(Cell signaling Technology), Akt (pan) (C67E7) (Cell signaling Technology), Anti-PI3 Kinase (EMD Millipore), mTOR (Cell signalling Technology), Anti-beta Actin (abcam), LC3A/B(Cell signaling Technology), Anti-Atg7. Following primary antibody treatment, the membrane was washed with TBST for 10 min (3x). After washing, the membranes were incubated with corresponding horseradish peroxidase-labeled secondary antibody (1:2000) for 1h at room temp. Membranes were washed with TBST (3x for 10 min) and the blots were visualized using Luminata™ HRP Chemiluminescence Detection Reagents (MERCK MILLIPORE) and developed on BioradChemiDoc™ XRS+ System.

Immunodetection of PTEN protein:

A549 Human lung carcinoma cells were seeded on coverslips and treated with compounds at desired concentration for 24 h. After treatment, cells were fixed with freshly prepared paraformaldehyde solution (4% in 1X PBS) for 20 min at room temperature. Cells were permeabilized by administration of Triton X-100 solution (0.2% in 1X PBS) for 5 min followed by incubation in 100% methanol at 4°C over night. Subsequently, blocking was

achieved with a 1% BSA solution for 60 min. Cells were then incubated with primary antibody PTEN (Cell signaling Technology) at room temperature for 2 h. The slides were washed three times in PBST, for 5min each. Then cells were incubated with a Cy3-conjugated anti-rabbit secondary antibody (Jackson Immuno Research Laboratories Inc., Pennsylvania, USA) for one hour followed by three times wash with PBST solution and mounted with DAPI solution. Then cells were observed under confocal microscope (Olympus FV1000). Images taken were processed with the support of the flow view version 1.7c software program.

2. Chemistry

General

All solvents were distilled prior to use. Dry reactions were conducted under a nitrogen atmosphere. Crude products were purified by column chromatography on silica gel of 60–120 or 100–200 mesh. Thin layer chromatography plates were visualized by exposure to ultraviolet light, exposure to iodine vapors, and/or exposure to methanolic acidic solution of *p*-anisaldehyde (anis) followed by heating (<1 min) on a hot plate (~250 ^{0}C). IR spectra were recorded on FT-IR spectrometer. ^{1}H and ^{13}C NMR (proton decoupled) spectra were recorded in $CDCl_3$ solvent on 300 and 500 MHz NMR spectrometers. Chemical shifts (δ) were reported in parts per million (ppm) with respect to TMS as an internal standard. Coupling constants (*J*) are quoted in Hertz (Hz). High resolution mass specta (HRMS) were obtained by ionizing sample using electron spray ionization (ESI) and an orbitrap mass analyzer.

Procedure for synthesis of 2-imino-4-(trifluoromethyl)thiazolidin-4-ol derivatives (8a-v)

To a stirred solution (for 5 minutes) of primary amine **5** (1 mmol) and aryl isothiocyanate **6** (1 mmol) in *N*,*N*-dimethylformamide (5 mL), was added 3-bromo-1,1,1-trifluoromethyl propanone **7** (1 mmol) dropwise and the reaction mixture was stirred at room temperature for 20-30 minutes. After completion (monitored by TLC), the reaction mixture was quenched with saturated $NaHCO_3$ solution (1 mL), diluted with water and extracted with EtOAc (3x10 mL). The combined organic layer was washed with brine solution, dried (Na_2SO_4), filtered and concentrated. The resulting crude product was purified by column chromatography using EtOAc/*n*-hexane gradients to afford pure product **8**.

(*Z*)-2-(Phenylimino)-3-(thiophen-2-ylmethyl)-4-(trifluoromethyl)thiazolidin-4-ol (8a).

Yield, 347 mg, 96%; Solid, m.p. 126–128 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.09 (broad s, 1H), 3.16 (d, *J* = 12.1 Hz, 1H), 3.53 (d, *J* = 12.1 Hz, 1H), 4.72 (d, *J* = 15.9 Hz, 1H), 5.38 (d, *J* = 15.9 Hz, 1H), 6.93–6.98 (m, 1H), 7.01–7.07 (m, 2H), 7.08–7.17 (m, 2H), 7.27–7.38 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 35.1, 41.7, 91.3 (q, *J* = 32.7 Hz), 121.5, 124.0, 126.2, 127.0, 127.3, 129.0, 140.0, 150.1, 156.4; IR (KBr): ν_{max} 3063, 2363, 1607, 1584, 1437, 1338, 1303, 1176 cm^{-1}; MS (ESI): *m/z* 359 $[M+H]^+$; HRMS (ESI): calcd. for $C_{15}H_{14}ON_2F_3S_2$, 359.0484 $[M+H]^+$, found 359.0494. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-3-(Furan-2-ylmethyl)-2-(phenylimino)-4-(trifluoromethyl)thiazolidin-4-ol (8b).

Yield, 335 mg, 98%; Solid, m.p. 105–107 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.20 (d, *J* = 12.1 Hz, 1H), 3.55 (d, *J* = 12.1 Hz, 1H), 3.98 (broad s, 1H), 4.46 (d, *J* = 16.2 Hz, 1H), 5.22 (d, *J* = 16.2 Hz, 1H), 6.34–6.39 (m, 1H), 6.40–6.46 (m, 1H), 6.85–6.94 (m, 2H), 7.02-7.12 (m, 1H), 7.23–7.34 (m, 2H), 7.34-7.44 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 34.8, 39.6, 90.7 (q, *J* = 32.4 Hz), 109.8, 110.9, 121.4, 123.8, 125.1, 129.0, 142.0, 150.0, 150.2, 156.0; IR (KBr): ν_{max} 3061, 2690, 1605, 1581, 1427, 1331, 1302, 1192, 1153 cm^{-1}; MS (ESI): *m/z* 343 $[M+H]^+$; HRMS (ESI): calcd. for $C_{15}H_{14}O_2N_2F_3S$, 343.0713$[M+H]^+$, found 343.0722. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-3-(2-(1H-Indol-3-yl)ethyl)-2-(phenylimino)-4-(trifluoromethyl)thiazolidin-4-ol (8c).

Yield, 360 mg, 89%; Solid, m.p. 128–130 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.78–2.94 (m, 2H), 2.95–3.07 (m, 1H), 3.40–3.60 (m, 2H), 3.62–3.76 (m, 1H), 4.01–4.15 (m, 1H), 6.98–7.28 (m, 6H), 7.30–7.42 (m, 3H), 7.68–7.78 (m, 1H), 8.15 (broad s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 23.0, 34.8, 45.4, 111.4, 113.5, 118.9, 119.9, 121.8, 122.5, 123.8, 125.3, 127.0,

129.0, 136.0, 150.3; IR (KBr): ν_{max} 3409, 3062, 2683, 1606, 1585, 1420, 1311, 1182, 1147 cm^{-1}; MS (ESI): *m/z* 406 $[M+H]^+$; HRMS (ESI): calcd. for $C_{20}H_{19}ON_3F_3S$, 406.1182 $[M+H]^+$, found 406.1195. (CF_3 (on same carbon of -OH) group signal and coupling of carbon (containing CF_3 group) are not visualise in the spectra)

(*Z*)-3-cyclohexyl-2-(phenylimino)-4-(trifluoromethyl)thiazolidin-4-ol (8d).

Yield, 282 mg, 82%; Solid, m.p. 130–132 °C; ^1H NMR ($CDCl_3$, 500 MHz): δ 1.05–1.38 (m, 6H), 1.58–1.76 (m, 4H), 3.18 (d, *J* = 12.2 Hz, 1H), 3.40–3.51 (m, 1H), 3.65 (d, *J* = 12.2 Hz, 1H), 6.80–6.91 (m, 2H), 7.04–7.10 (m, 1H), 7.30–7.36 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 32.4, 35.3, 38.6, 52.6, 92.3 (q, *J* = 32.1 Hz), 117.1, 121.3, 123.6, 126.5, 130.2, 131.1, 150.1, 155.9; IR (KBr): ν_{max} 3098, 2126, 1613, 1596, 1476, 1298, 1157 cm^{-1}; MS (ESI): *m/z* 345 $[M+H]^+$; HRMS (ESI): calcd. for $C_{16}H_{20}ON_2F_3S$, 345.0864 $[M+H]^+$, found 303.0870. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-3-Cyclopropyl-2-(phenylimino)-4-(trifluoromethyl)thiazolidin-4-ol (8e).

Yield, 286 mg, 95%; Solid, m.p. 159–161 °C; ^1H NMR ($CDCl_3$, 500 MHz): δ 0.79–1.01 (m, 3H), 1.14–1.25 (m, 1H), 2.52–2.61 (m, 1H), 3.16 (d, *J* = 11.7 Hz, 1H), 3.51 (d, *J* = 11.7 Hz, 1H), 3.61 (s, 1H), 6.86–6.95 (m, 2H), 7.06–7.12 (m, 1H), 7.27–7.34 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 5.0, 5.6, 24.8, 32.4, 89.7 (q, *J* = 31.3 Hz), 116.7, 120.1, 121.8, 124.3, 127.2, 127.4, 149.7, 156.0; IR (KBr): ν_{max} 3103, 2115, 1609, 1586, 1486, 1342, 1163 cm^{-1}; MS (ESI): *m/z* 303 $[M+H]^+$; HRMS (ESI): calcd. for $C_{13}H_{14}ON_2F_3S$, 303.0765 $[M+H]^+$, found 303.0773. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-3-(2-Morpholinoethyl)-2-(phenylimino)-4-(trifluoromethyl)thiazolidin-4-ol (8f).

Yield, 348 mg, 93%; Solid, m.p. 124–126 °C; ^1H NMR ($CDCl_3$, 500 MHz): δ 2.29– 2.39 (m, 1H), 2.41–2.95 (m, 4H), 2.98–3.11 (m, 1H), 3.22 (d, *J* = 12.0 Hz, 1H), 3.42–3.55 (m, 2H),

3.62–3.94 (m, 4H), 4.19–4.29 (m, 1H), 6.87–6.96 (m, 2H), 7.03–7.12 (m, 1H), 7.24–7.35 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 34.9, 41.1, 53.7, 55.0, 66.0, 89.9 (q, *J* = 31.2 Hz), 121.5, 123.7, 123.9 (q, *J* = 288.7 Hz), 129.0, 150.6, 157.3; IR (KBr): ν_{max} 3065, 2669, 1619, 1594, 1456, 1356, 1303, 1163 cm^{-1}; MS (ESI): *m/z* 376 $[M+H]^+$; HRMS (ESI): calcd. for $C_{16}H_{21}O_2N_3F_3S$, 376.1292 $[M+H]^+$, found 376.1301.

(*Z*)-2-(Phenylimino)-3-(2-(pyridin-2-yl)ethyl)-4-(trifluoromethyl)thiazolidin-4-ol (8g).

Yield, 330 mg, 90%; Solid, m.p. 103–105 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.18 (d, *J* = 12.1 Hz, 1H), 3.21–3.32 (m, 1H), 3.42–3.48 (m, 1H), 3.52 (d, *J* = 12.1 Hz, 1H), 3.78–3.90 (m, 1H), 4.38–4.51 (m, 1H), 6.52–6.61 (m, 2H), 6.95–7.04 (m, 1H), 7.14–7.36 (m, 4H), 7.68–7.76 (m, 1H), 8.41–8.49 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 33.6, 36.0, 41.3, 91.1 (q, *J* = 31.3 Hz), 121.5, 121.9, 123.4, 125.1, 125.8, 128.7, 138.0, 146.4, 150.3, 156.7, 158.6; IR (KBr): ν_{max} 2989, 2649, 1619, 1588, 1443, 1295, 1248, 1186 cm^{-1}; MS (ESI): *m/z* 368 $[M+H]^+$; HRMS (ESI): calcd. for $C_{17}H_{17}ON_3F_3S$, 368.1029 $[M+H]^+$, found 368.1038. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-2-(4-fluorophenylimino)-3-phenyl-4-(trifluoromethyl)thiazolidin-4-ol (8h).

Yield, 286 mg, 80%; Solid, m.p. 143–145 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.34 (d, *J* = 12.1 Hz, 1H), 3.60 (broad s, 1H), 3.69 (d, *J* = 12.1 Hz, 1H), 6.84–6.98 (m, 2H), 7.02–7.08 (m, 1H), 7.09–7.18 (m, 1H), 7.22–7.52 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 34.7, 91.1 (q, *J* = 31.8 Hz), 116.4 (d, *J* = 22.50 Hz), 120.8, 121.5, 122.8 (d, *J* = 7.6 Hz), 124.0, 128.9, 129.5, 130.3, 132.1, 136.6, 150.4, 162.2 (d, *J* = 249.1 Hz); IR (KBr): ν_{max} 3020, 1632, 1596, 1476, 1333, 1173 cm^{-1}; MS (ESI): *m/z* 357 $[M+H]^+$; HRMS (ESI): calcd. for $C_{16}H_{13}ON_2F_4S$, 357.0753 $[M+H]^+$, found 357.0763. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-2-(3-chloro-4-fluorophenylimino)-3-phenyl-4-(trifluoromethyl)thiazolidin-4-ol (8i).

Yield, 342 mg, 87%; Solid, m.p. 152–154 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.32 (d, *J* = 11.8 Hz, 1H), 3.57 (broad s, 1H), 3.72 (d, *J* = 11.8 Hz, 1H), 6.82–6.96 (m, 2H), 7.01–7.06 (m, 1H), 7.26–7.56 (m, 4H), 7.87 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 34.5, 90.6 (q, *J* = 32.8 Hz), 114.8 (d, *J* = 21.7 Hz), 120.6, 120.9, 123.0, 124.3, 129.1, 129.8, 130.6, 132.9, 137.1, 150.6, 161.8 (d, *J* = 247.3 Hz); IR (KBr): ν_{max} 3010, 1611, 1589, 1446, 1313, 1183 cm^{-1}; MS (ESI): *m/z* 391 $[M+H]^+$; HRMS (ESI): calcd. for $C_{16}H_{12}OClN_2F_4S$, 391.0653 $[M+H]^+$, found 391.0663. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

***(Z)*-2-(Phenylimino)-3-propyl-4-(trifluoromethyl)thiazolidin-4-ol (8j).**

Yield, 279 mg, 92%; Solid, m.p. 130–132 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.94 (t, *J* = 7.6 Hz, 3H), 1.60–1.93 (m, 2H), 3.16 (d, *J* = 12.1 Hz, 1H), 3.23–3.61 (m, 4H), 6.89– 6.95 (m, 2H), 7.04–7.12 (m, 1H), 7.25–7.34 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 11.3, 21.4, 35.1, 45.8, 90.9 (q, *J* = 31.9 Hz), 121.6, 123.7, 129.0, 150.6, 156.1; IR (KBr): ν_{max} 3062, 2658, 1606, 1584, 1494, 1361, 1319, 1183 cm^{-1}; MS (ESI): *m/z* 305 $[M+H]^+$; HRMS (ESI): calcd. for $C_{13}H_{16}ON_2F_3S$, 305.0916 $[M+H]^+$, found 305.0929. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-3-Phenyl-2-(phenylimino)-4-(trifluoromethyl)thiazolidin-4-ol (8k).

Yield, 310 mg, 92%; Solid, m.p. 152–154 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.31 (d, *J* = 12.1 Hz, 1H), 3.62 (broad s, 1H), 3.71 (d, *J* = 12.1 Hz, 1H), 6.84–6.91 (m, 2H), 7.01–7.09 (m, 1H), 7.22–7.30 (m, 2H), 7.32–7.51 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 34.5, 91.0 (q, *J* = 31.9 Hz), 120.8, 121.6, 124.0, 124.6, 128.9, 129.3, 130.4, 136.7, 150.6, 159.0; IR (KBr): ν_{max} 3025, 1619, 1585, 1490, 1324, 1183 cm^{-1}; MS (ESI): *m/z* 339 $[M+H]^+$; HRMS (ESI):

calcd. for $C_{16}H_{14}ON_2F_3S$, 339.0763 $[M+H]^+$, found 339.0773. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-4-(Trifluoromethyl)-3-(3-(trifluoromethyl)phenyl)-2-(3-(trifluoromethyl)phenylimino)thiazolidin-4-ol (8l).

Yield, 369 mg, 78%; Solid, m.p. 106–108 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.40 (d, *J* = 12.1 Hz, 1H), 3.70 (broad s, 1H), 3.81 (d, *J* = 12.1 Hz, 1H), 7.0 –7.20 (m, 2H), 7.29–7.46 (m, 2H), 7.55–7.79 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 35.2, 91.1 (q, *J* = 32.4 Hz), 118.6, 120.7, 121.7, 124.4, 124.8, 125.3, 125.9, 127.3, 129.5, 129.9, 131.3 (q, *J* = 32.4 Hz), 131.4 (q, *J* = 32.4 Hz), 133.9, 137.2, 150.4, 159.0; IR (KBr): ν_{max} 3160, 2660, 1583, 1451, 1334, 1158 cm^{-1}; MS (ESI): *m/z* 475 $[M+H]^+$; HRMS (ESI): calcd. for $C_{18}H_{12}ON_2F_9S$, 475.0498 $[M+H]^+$, found 475.0497. (CF_3 (on same carbon of -OH), CF_3 (of phenyl) groups signal are not visualise in the spectra)

(*Z*)-3-(Furan-2-ylmethyl)-4-(trifluoromethyl)-2-(4-(trifluoromethyl)phenylimino) thiazolidin-4-ol (8m).

Yield, 356 mg, 87%; Solid, m.p. 98–100 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.26 (d, *J* = 11.76 Hz, 1H), 3.60 (d, *J* = 11.76 Hz, 1H), 3.89 (broad s, 1H), 4.47 (d, *J* = 16.3 Hz, 1H), 5.23 (d, *J* = 16.3 Hz, 1H), 6.35–6.45 (m, 2H), 6.95–7.00 (m, 2H), 7.39–7.43 (m, 1H), 7.51–7.57 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 34.9, 39.7, 90.8 (q, *J* = 32.7 Hz), 110.0, 111.0, 121.7, 123.2 (q, *J* = 287.9 Hz), 125.8, 126.2, 126.3, 142.2, 149.7, 153.2, 156.4; IR (KBr): ν_{max} 3431, 2926, 1590, 1427, 1329, 1304, 1166, 1124 cm^{-1}; MS (ESI): *m/z* 411 $[M+H]^+$; HRMS (ESI): calcd. for $C_{16}H_{13}O_2N_2F_6S$, 411.0582 $[M+H]^+$, found 411.0596. (CF_3 (on phenyl ring) group and coupling of fluorine with carbon bearing (CF_3) signal are not visualise in the spectra)

(*Z*)-3-(2-(pyridin-2-yl)ethyl)-4-(trifluoromethyl)-2-(4-(trifluoromethyl)phenylimino)thiazolidin-4-ol (8n).

Yield, 389 mg, 89%; Solid, m.p. 138–140 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.21 (d, *J* = 12.1 Hz, 1H), 3.24–3.32 (m, 1H), 3.41–3.48 (m, 1H), 3.55 (d, *J* = 12.1 Hz, 1H), 3.79–3.88 (m, 1H), 4.38–4.48 (m, 1H), 6.63 (d, *J* = 7.7 Hz, 2H), 7.27–7.34 (m, 2H), 7.44 (d, J = 7.72 Hz, 2H), 7.70–7.77 (m, 1H), 8.41–8.46 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 33.6, 36.1, 41.4, 91.3 (q, *J* = 31.7 Hz), 121.8, 122.0, 125.1, 125.9, 138.0, 146.5, 153.5, 157.2, 158.5; IR (KBr): ν_{max} 2991, 1628, 1590, 1464, 1391, 1278, 1192 cm^{-1}; MS (ESI): *m/z* 436 $[M+H]^+$; HRMS (ESI): calcd. for $C_{18}H_{16}ON_3F_6S$, 436.1023 $[M+H]^+$, found 436.1034. (CF_3 (on same carbon of -OH) and CF_3 (of phenyl) group signal are not visualise in the spectra)

(*Z*)-3-(4-fluorophenyl)-2-(4-fluorophenylimino)-4-(trifluoromethyl)thiazolidin-4-ol (8o).

Yield, 300 mg, 80%; Solid, m.p. 164–166 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.25 (d, *J* = 12.8 Hz, 1H), 3.68 (d, *J* = 12.8 Hz, 1H), 3.88 (broad s, 1H), 6.77–6.89 (m, 2H), 6.91–7.04 (m, 2H), 7.08-7.21 (m, 2H), 7.25-7.39 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 34.3, 91.0 (q, *J* = 32.9 Hz), 115.5, 115.9 (d, *J* = 21.9 Hz), 116.4, 122.5 (d, *J* = 287.6 Hz), 123.0, 123.1, 131.9 (d, *J* = 8.7 Hz), 132.2, 146.3, 158.1, 160.4, 160.8, 161.3, 164.1; IR (KBr): ν_{max} 3068, 1618, 1522, 1482, 1409, 1334, 1165 cm^{-1}; MS (ESI): *m/z* 375 $[M+H]^+$; HRMS (ESI): calcd. for $C_{16}H_{12}ON_2F_5S$, 375.0572 $[M+H]^+$, found 375.0562. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-3-(4-Methoxyphenyl)-4-(trifluoromethyl)-2-(4-(trifluoromethyl)phenylimino) thiazolidin-4-ol (8p).

Yield, 370 mg, 85%; Solid, m.p. 154–156 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.39 (d, *J* = 12.1 Hz, 1H), 3.43 (broad s, 1H), 3.75 (d, *J* = 12.1 Hz, 1H), 3.81 (s, 3H), 6.91–7.02 (m, 4H),

7.24–7.32 (m, 2H), 7.46–7.55 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 34.4, 55.3, 91.0 (q, *J* = 31.8 Hz), 114.8, 120.8, 121.9, 124.6, 126.1, 128.6, 131.4, 153.7, 159.8; IR (KBr): ν_{max} 3158, 2847, 1589, 1510, 1462, 1410, 1322, 1160 cm^{-1}; MS (ESI): *m/z* 437 $[M+H]^+$; HRMS (ESI): calcd. for $C_{18}H_{15}O_2N_2F_6S$, 437.0728 $[M+H]^+$, found 437.0752. (CF_3 (on same carbon of -OH) and CF_3 (on Phenyl) groups signal are not visualise in the spectra)

(*Z*)-2-(4-fluorophenylimino)-3-(4-methoxyphenyl)-4-(trifluoromethyl)thiazolidin-4-ol (8q).

Yield, 330 mg, 85%; Solid, m.p. 144–146 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.22 (d, *J* = 11.99 Hz, 1H), 3.62 (d, *J* = 11.99 Hz, 1H), 3.77 (s, 3H), 6.79–6.87 (m, 2H), 6.89–6.98 (m, 3H), 7.04–7.10 (m, 1H), 7.18-7.29 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 34.4, 55.3, 91.2 (q, *J* = 31.2 Hz), 114.2, 114.7, 115.6 (d, *J* = 21.9 Hz), 116.2, 116.5, 122.9, 123.1 (d, *J* = 8.2 Hz), 128.6, 131.4, 132.2, 146.1, 154.4, 156.6, 159.7 (d, *J* = 242.0 Hz), 159.7, 164.2; IR (KBr): ν_{max} 3028, 2797, 1590, 1507, 1484, 1411, 1317, 1172 cm^{-1}; MS (ESI): *m/z* 387 $[M+H]^+$; HRMS (ESI): calcd. for $C_{17}H_{15}O_2N_2F_4S$, 387.0826 $[M+H]^+$, found 387.0838. (CF_3 (on same carbon of -OH) group signal is not visualise in the spectra)

(*Z*)-2-(4-chloro-3-(trifluoromethyl)phenylimino)-3-(2-morpholinoethyl)-4-(trifluoromethyl)thiazolidin-4-ol (8r).

Yield, 417 mg, 87%; Solid, m.p. 114–116 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.16– 3.11 (m, 6H), 3.23–3.37 (m, 1H), 3.46–3.53 (m, 1H), 3.56 (d, *J* = 12.0 Hz, 1H), 3.68–3.96 (m, 4H), 4.22 (d, J = 12.0 Hz, 1H), 7.01–7.08 (m, 1H), 7.23–7.29 (m, 1H), 7.36–7.44 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 31.5, 36.8, 42.0, 46.0, 57.8, 108.4, 110.5, 122.1 (q, *J* = 273.9 Hz), 123.4, 128.5, 129.6, 132.6, 135.7, 142.5, 149.6, 162.8; IR (KBr): ν_{max} 3055, 2210, 1622, 1579, 1444, 1348, 1304, 1171 cm^{-1}; MS (ESI): *m/z* 478 $[M+H]^+$; HRMS (ESI): calcd. for

$C_{17}H_{19}O_2ClN_3F_6S$, 478.0296 $[M+H]^+$, found 478.0306. (Fluorine coupling with carbon (carbon having CF_3 and –OH group) and CF_3 group (on Phenyl), signal are not visualise in the spectra)

(*Z*)-2-(4-chloro-3-(trifluoromethyl)phenylimino)-3-(furan-2-ylmethyl)-4-(trifluoromethyl)thiazolidin-4-ol (8s).

Yield, 400 mg, 90%; Solid, m.p. 132–134 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.11 (broad s, 1H), 3.25 (d, *J* = 12.1 Hz, 1H), 3.61 (d, *J* = 12.1 Hz, 1H), 4.68 (d, *J* = 15.6 Hz, 1H), 5.33 (d, *J* = 15.6 Hz, 1H), 6.92–7.00 (m, 1H), 7.12–7.21 (m, 2H), 7.30–7.36 (m, 1H), 7.38–7.42 (m, 1H), 7.44–7.55 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 35.1, 40.0, 90.8 (q, *J* = 32.4 Hz), 110.2, 111.2, 121.1, 126.0, 132.2, 142.5, 149.1, 149.7, 157.6; IR (KBr): ν_{max} 3066, 1601, 1485, 1434, 1323, 1177 cm^{-1}; MS (ESI): *m/z* 445 $[M+H]^+$; HRMS (ESI): calcd. for $C_{16}H_{12}O_2N_2ClF_6S$, 445.0769 $[M+H]^+$, found 445.0879. (CF_3 (on same carbon of -OH) and CF_3 (on Phenyl) groups signal are not visualise in the spectra).

(*Z*)-2-(4-Chloro-3-(trifluoromethyl)phenylimino)-3-(thiophen-2-ylmethyl)-4-(trifluoromethyl)thiazolidin-4-ol (8t).

Yield, 418 mg, 91%; Solid, m.p. 123–125 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.09 (broad s, 1H), 3.23 (d, *J* = 12.3 Hz, 1H), 3.59 (d, *J* = 12.3 Hz, 1H), 4.73 (d, *J* = 15.8 Hz, 1H), 5.36 (d, *J* = 15.8 Hz, 1H), 6.94–7.02 (m, 1H), 7.10–7.20 (m, 2H), 7.28–7.33 (m, 1H), 7.34–7.39 (m, 1H), 7.41–7.51 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 35.3, 41.8, 121.1, 125.9, 126.4, 127.2, 127.5, 132.0, 139.5, 148.8, 157.8; IR (KBr): ν_{max} 3074, 2704, 1585, 1475, 1415, 1313, 1161 cm^{-1}; MS (ESI): *m/z* 461 $[M+H]^+$; HRMS (ESI): calcd. for $C_{16}H_{12}ON_2ClF_6S_2$, 460.9967 $[M+H]^+$, found 460.9978. (Coupling of fluorine with carbon (having CF3 and –OH groups),

CF_3 (on same carbon of -OH) and CF_3 (on Phenyl) groups signal are not visualise in the spectra).

(*Z*)-3-tert-Butyl-2-(4-chloro-3-(trifluoromethyl)phenylimino)-4-(trifluoromethyl) thiazolidin-4-ol (8u).

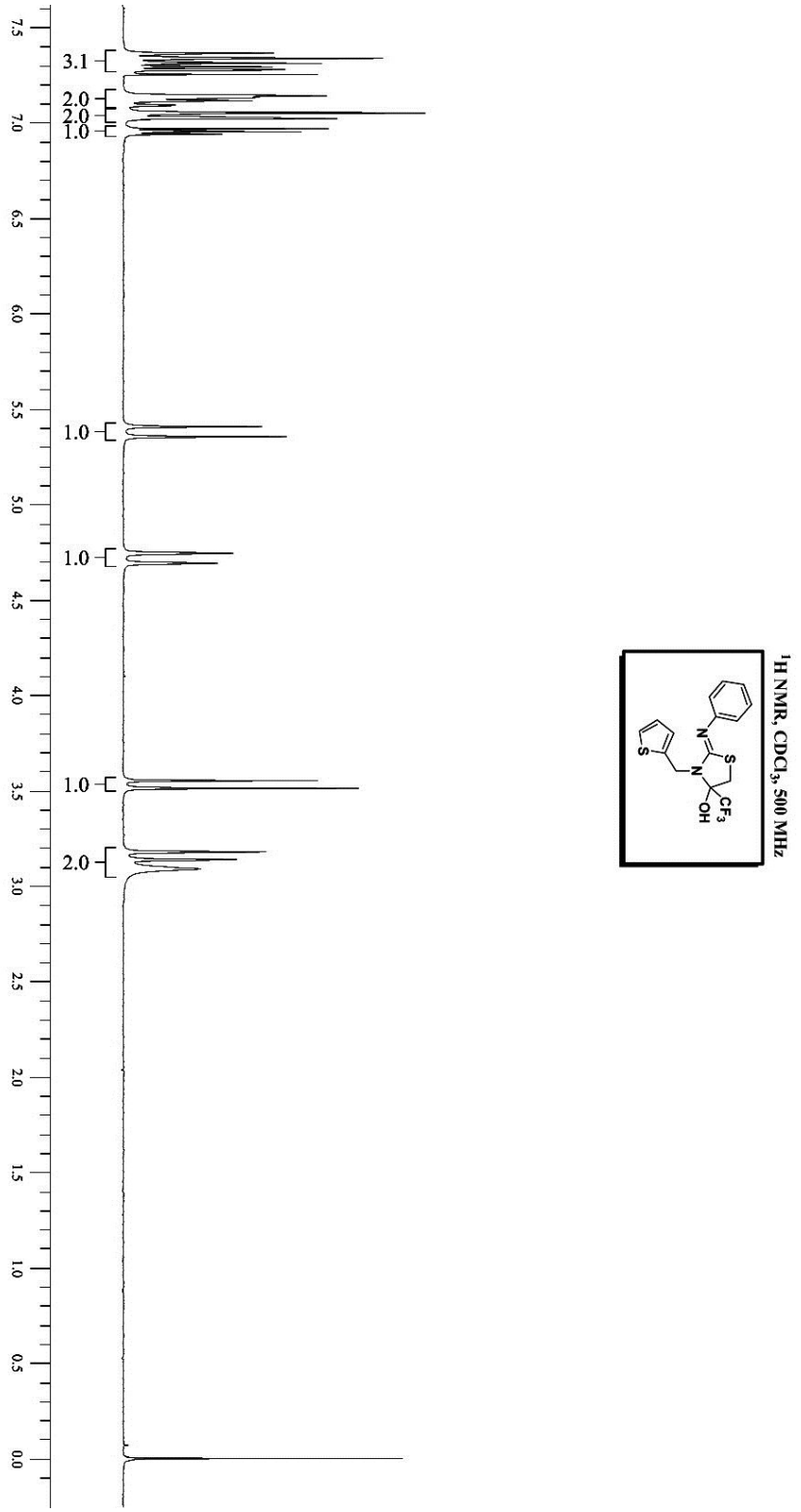
Yield, 361 mg, 86%; Solid, m.p. 80–82 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.95 (t, *J* = 7.0 Hz, 3H), 1.18–1.44 (m, 3H), 1.57–1.84 (m, 2H), 3.23 (d, *J* = 13.0 Hz, 1H), 3.45–3.68 (m, 3H), 7.00–7.06 (m, 1H), 7.22–7.29 (m, 1H), 7.36–7.43 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.7, 20.2, 30.1, 35.1, 44.2, 91.0 (q, *J* = 32.4 Hz), 120.9, 121.2, 121.3, 123.0 (q, *J* = 287.6 Hz), 124.5, 126.0, 126.4, 131.9, 149.3, 157.6; IR (KBr): ν_{max} 3078, 2667, 1607, 1582, 1496, 1350, 1182 cm^{-1}; MS (ESI): *m/z* 421 $[M+H]^+$; HRMS (ESI): calcd. for $C_{15}H_{16}ON_2ClF_6S$, 421.0556 $[M+H]^+$, found 421.0570. (CF_3 (on Phenyl) group signal are not visualise in the spectra).

(*Z*)-2-(4-Chloro-3-(trifluoromethyl)phenylimino)-3-(2-(pyridin-2-yl)ethyl)-4-(trifluoromethyl)thiazolidin-4-ol (8v).

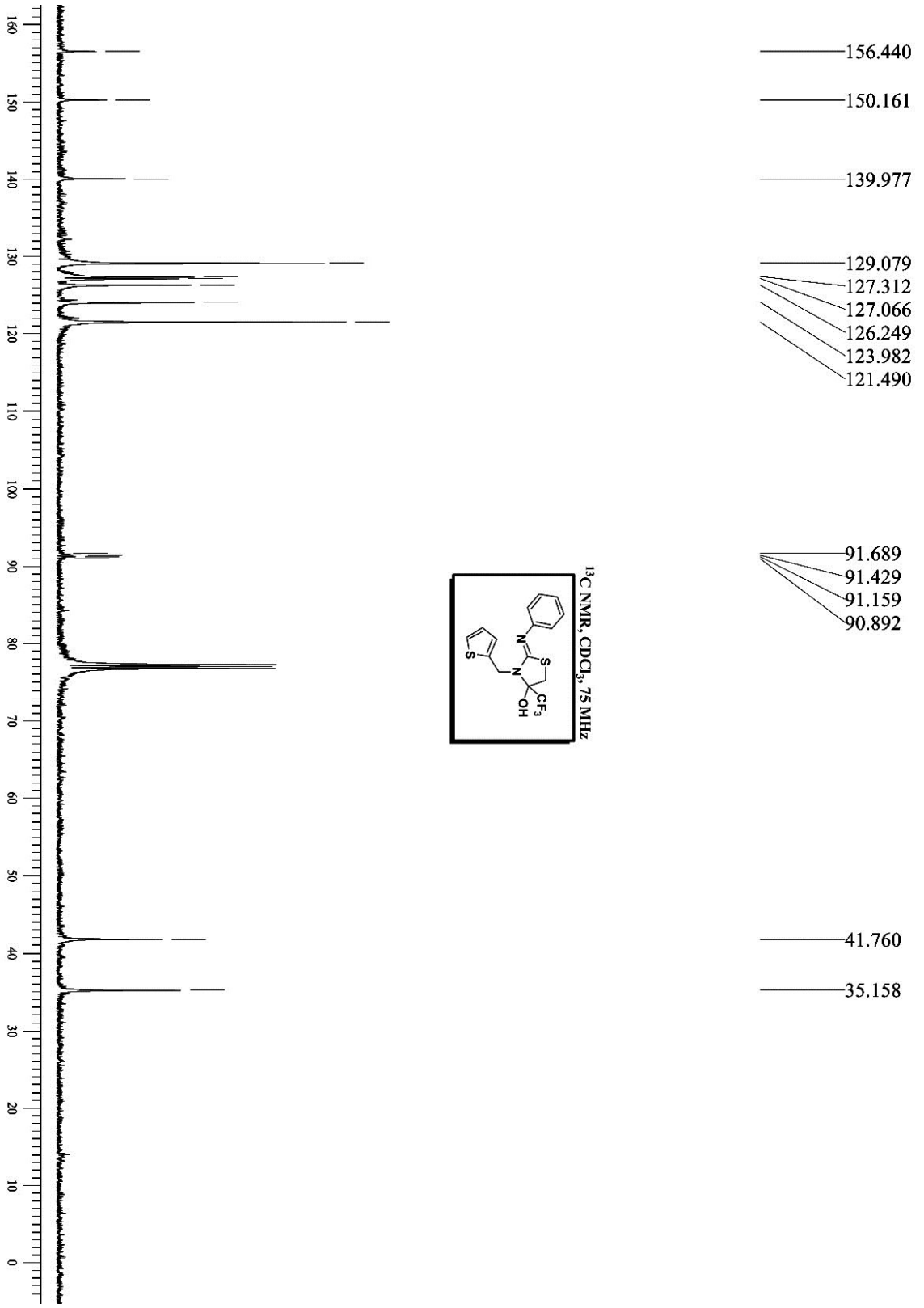
Yield, 408 mg, 87%; Solid, m.p. 118–120 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.22 (d, *J* = 11.5 Hz, 1H), 3.25–3.33 (m, 1H), 3.34–3.41 (m, 1H), 3.56 (d, *J* = 11.5 Hz, 1H), 3.76–3.88 (m, 1H), 4.38–4.50 (m, 1H), 6.66–6.78 (m, 2H), 7.22–7.37 (m, 3H), 7.69–7.78 (m, 1H), 8.40–8.48 (m, 1H), 11.29 (broad s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 33.8, 36.2, 41.6, 91.4 (q, *J* = 31.3 Hz), 121.3, 122.0, 125.2, 125.7, 131.7, 138.0, 146.6, 149.2, 158.2, 158.5; IR (KBr): ν_{max} 3031, 2720, 1611, 1587, 1477, 1420, 1343, 1197, 1142 cm^{-1}; MS (ESI): *m/z* 470 $[M+H]^+$; HRMS (ESI): calcd. for $C_{18}H_{15}ON_3ClF_6S$, 470.0505 $[M+H]^+$, found 470.0523. (CF_3 (on same carbon of -OH) and CF_3 (on Phenyl) groups signal are not visualise in the spectra).

3. Copies of ^{1}H and ^{13}C NMR spectra of products 8a-v

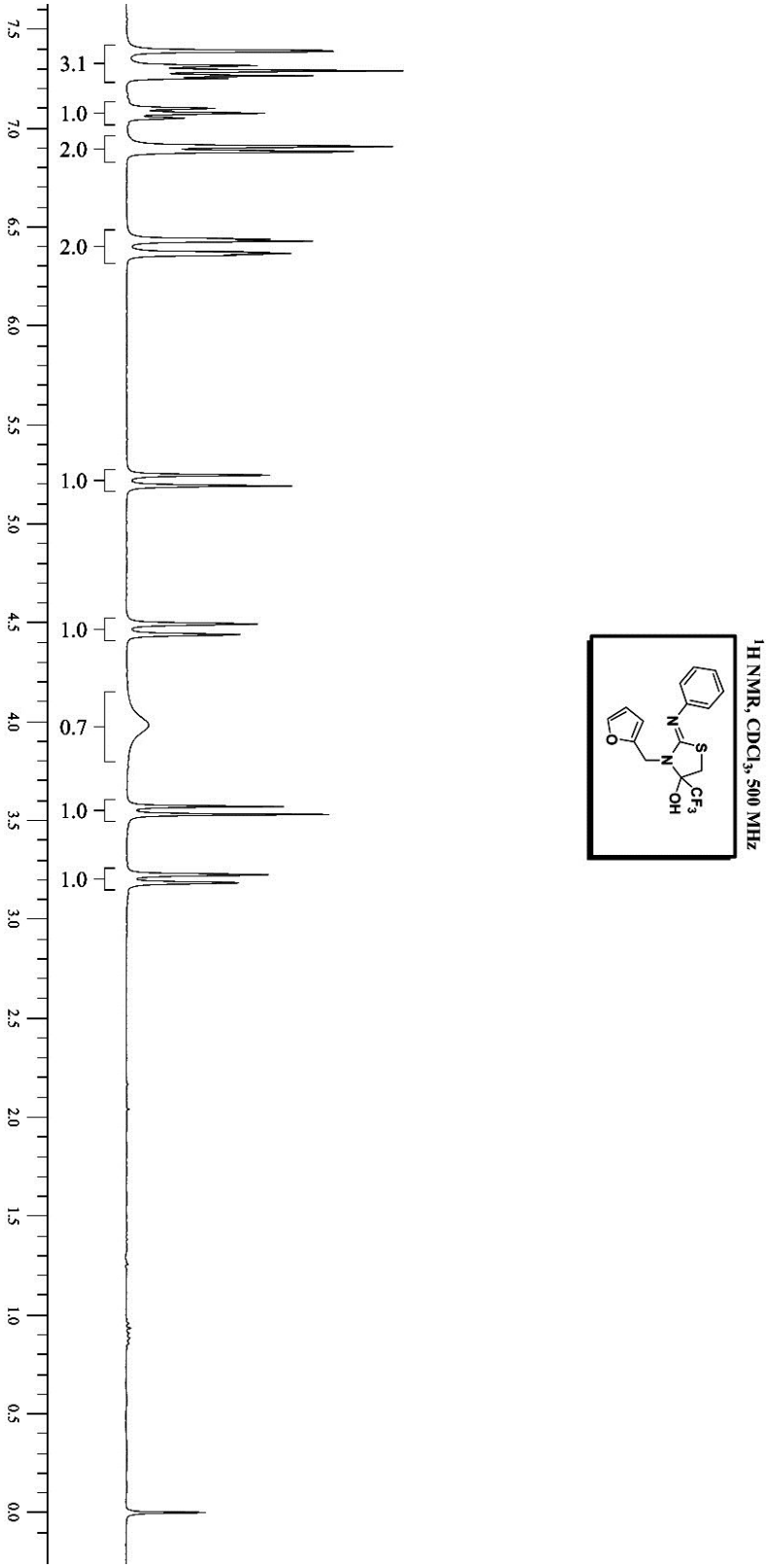
^{1}H NMR spectra of 8a



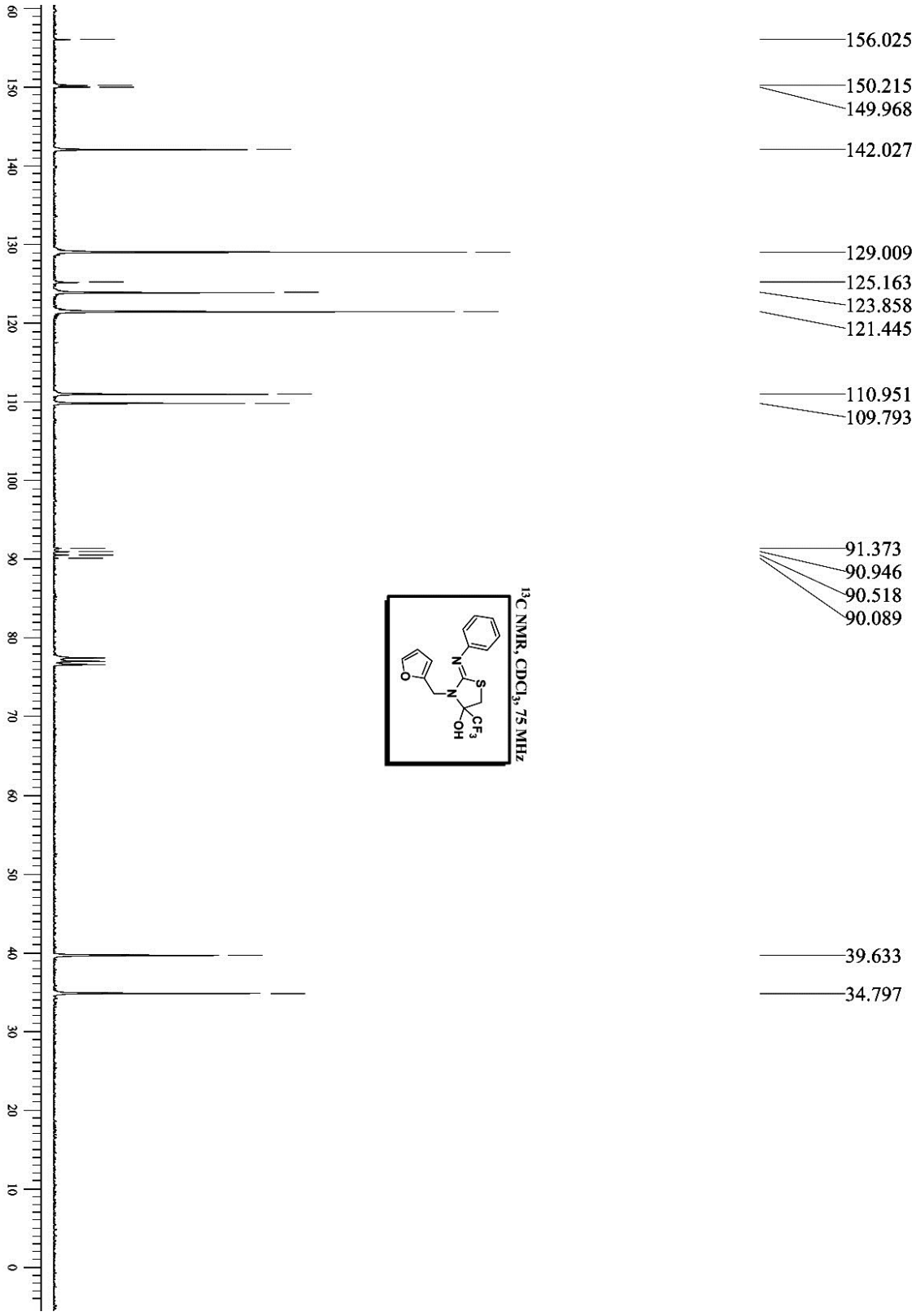
^{13}C NMR spectra of 8a



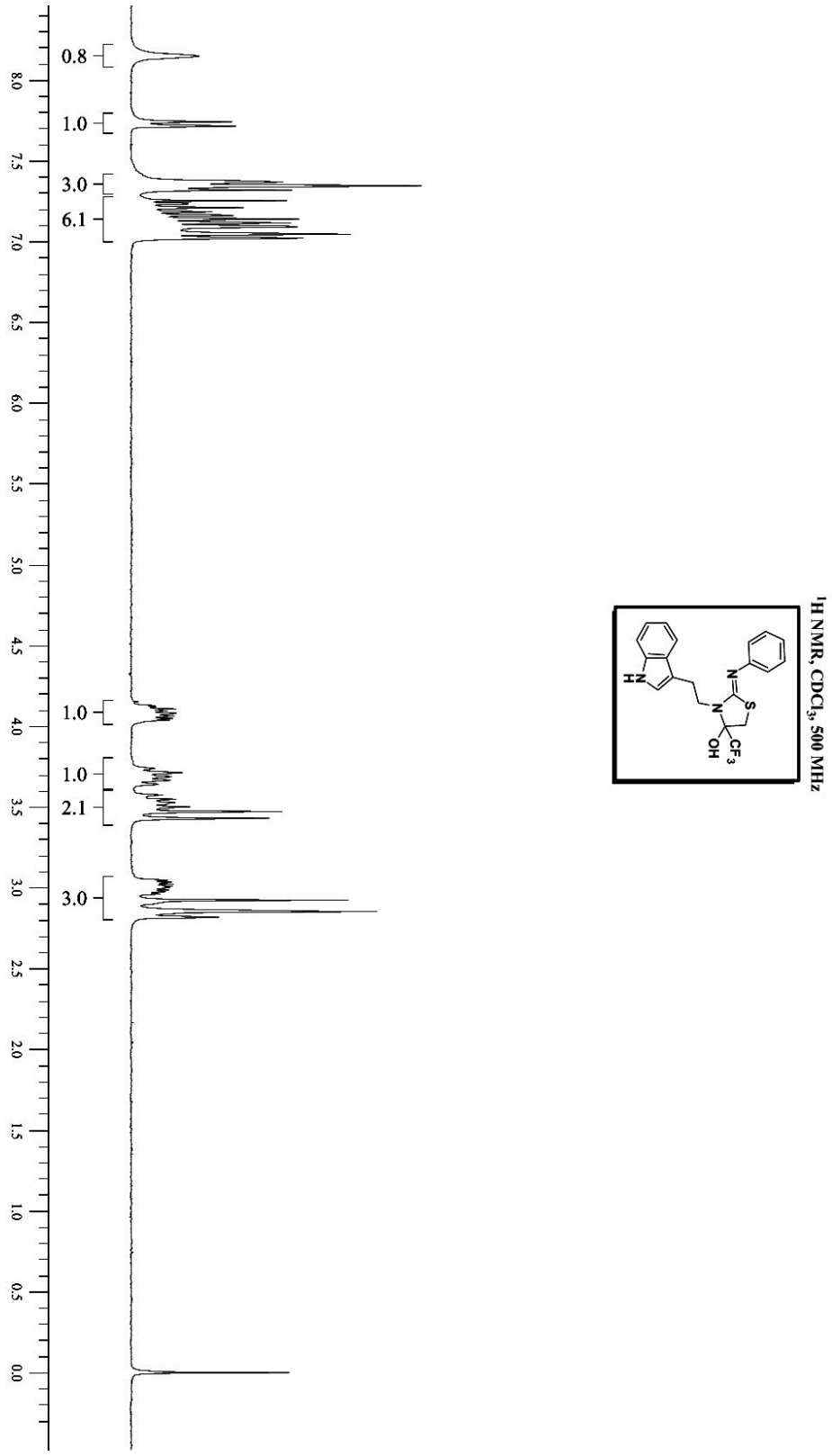
^{1}H NMR spectra of 8b



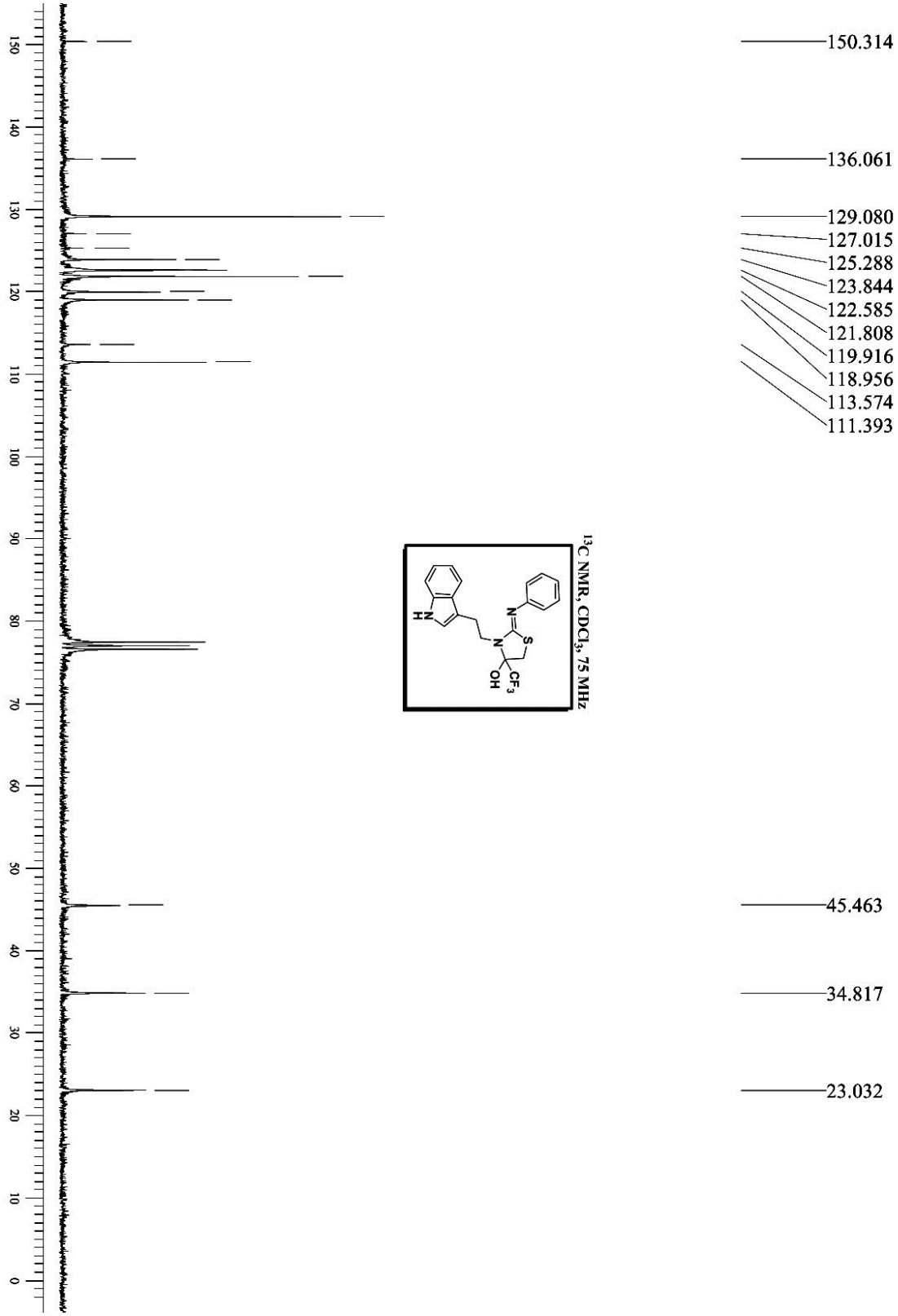
^{13}C NMR spectra of 8b



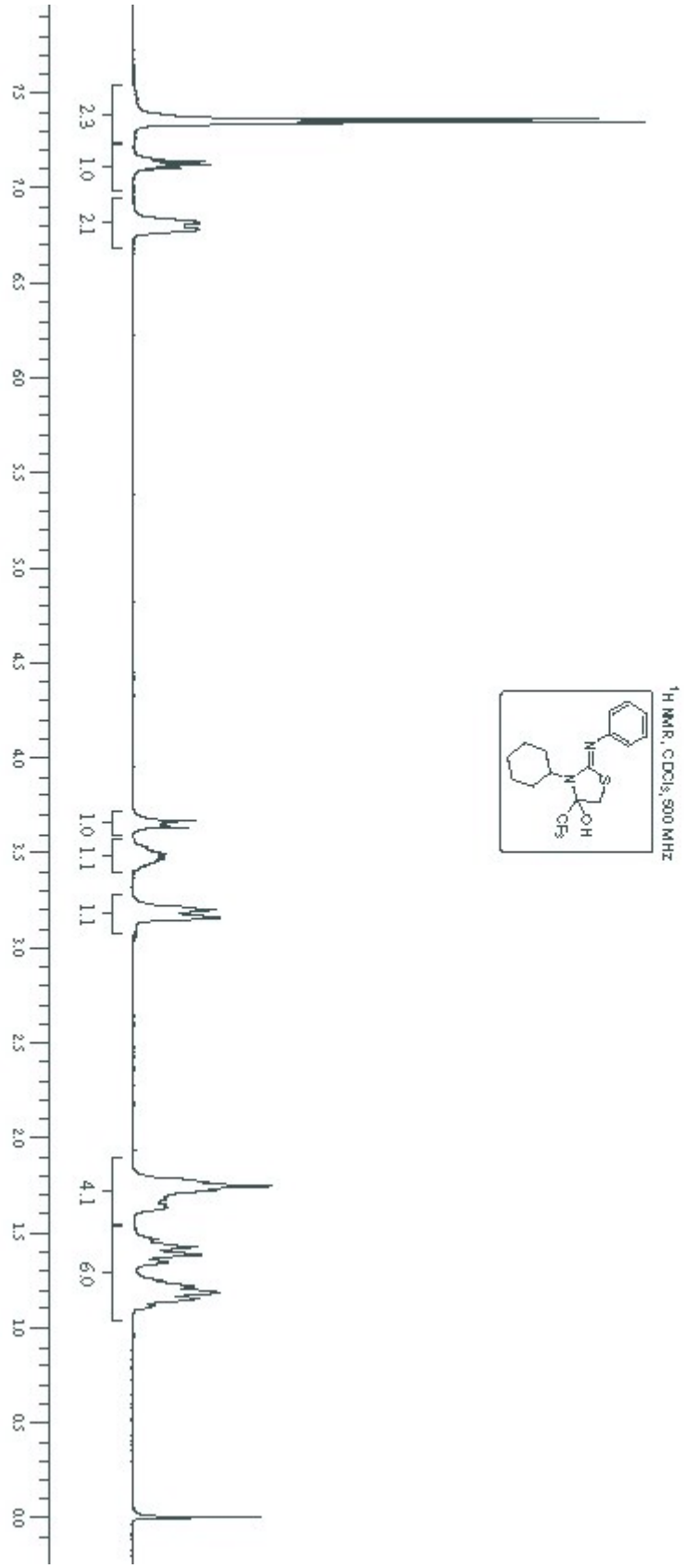
^{1}H NMR spectra of 8c



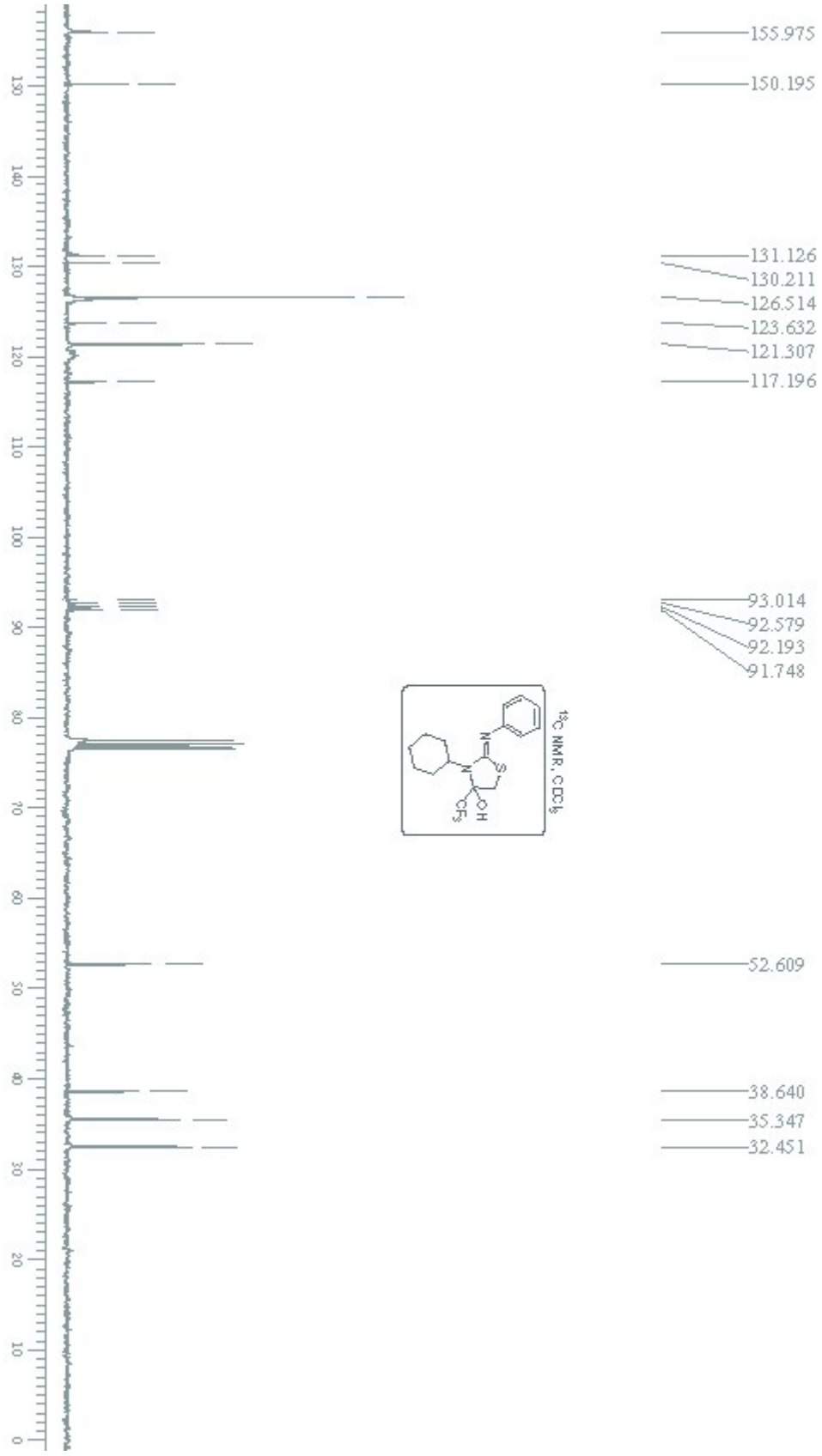
^{13}C NMR spectra of 8c



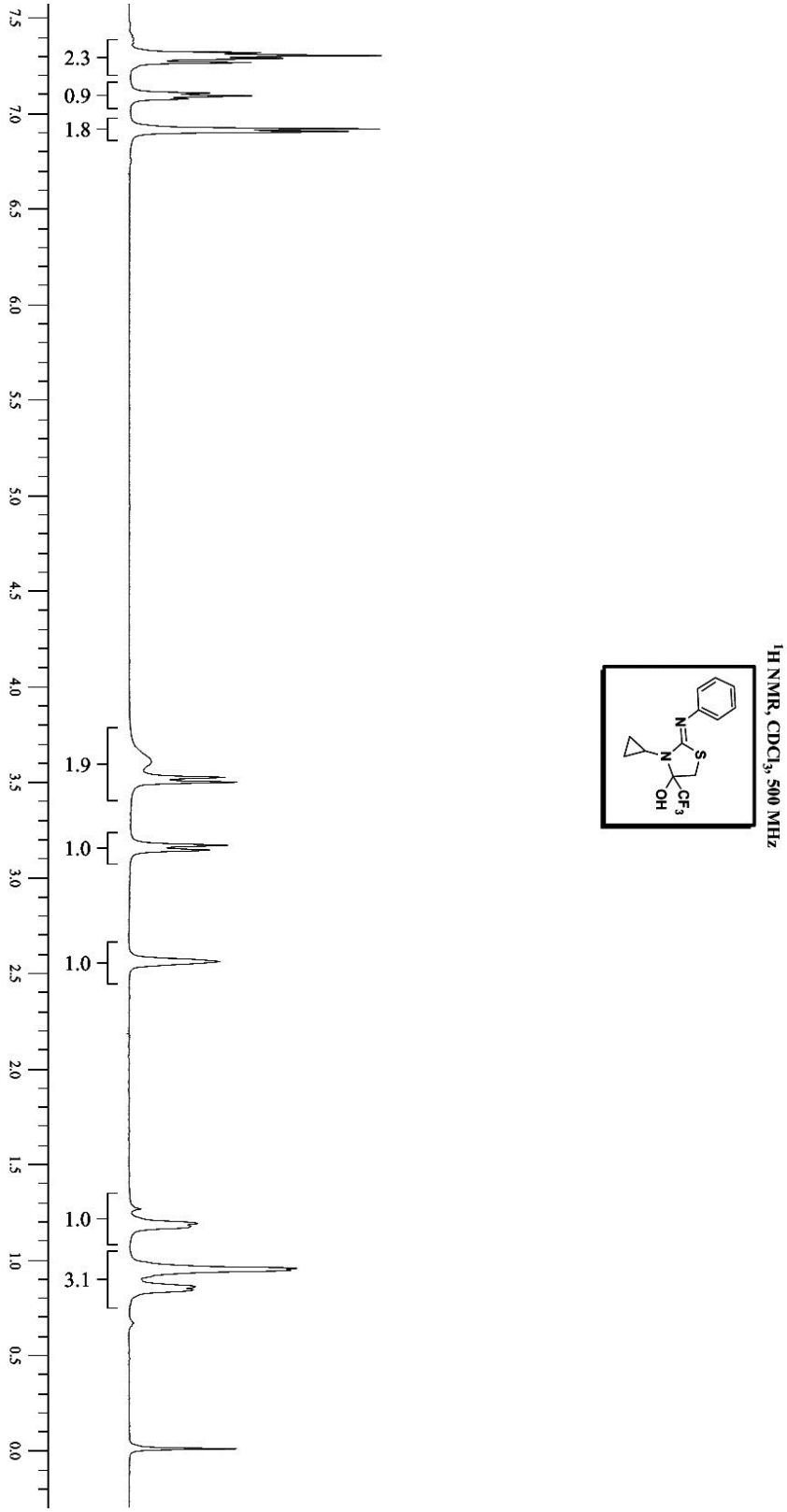
[1]H NMR spectra of 8d



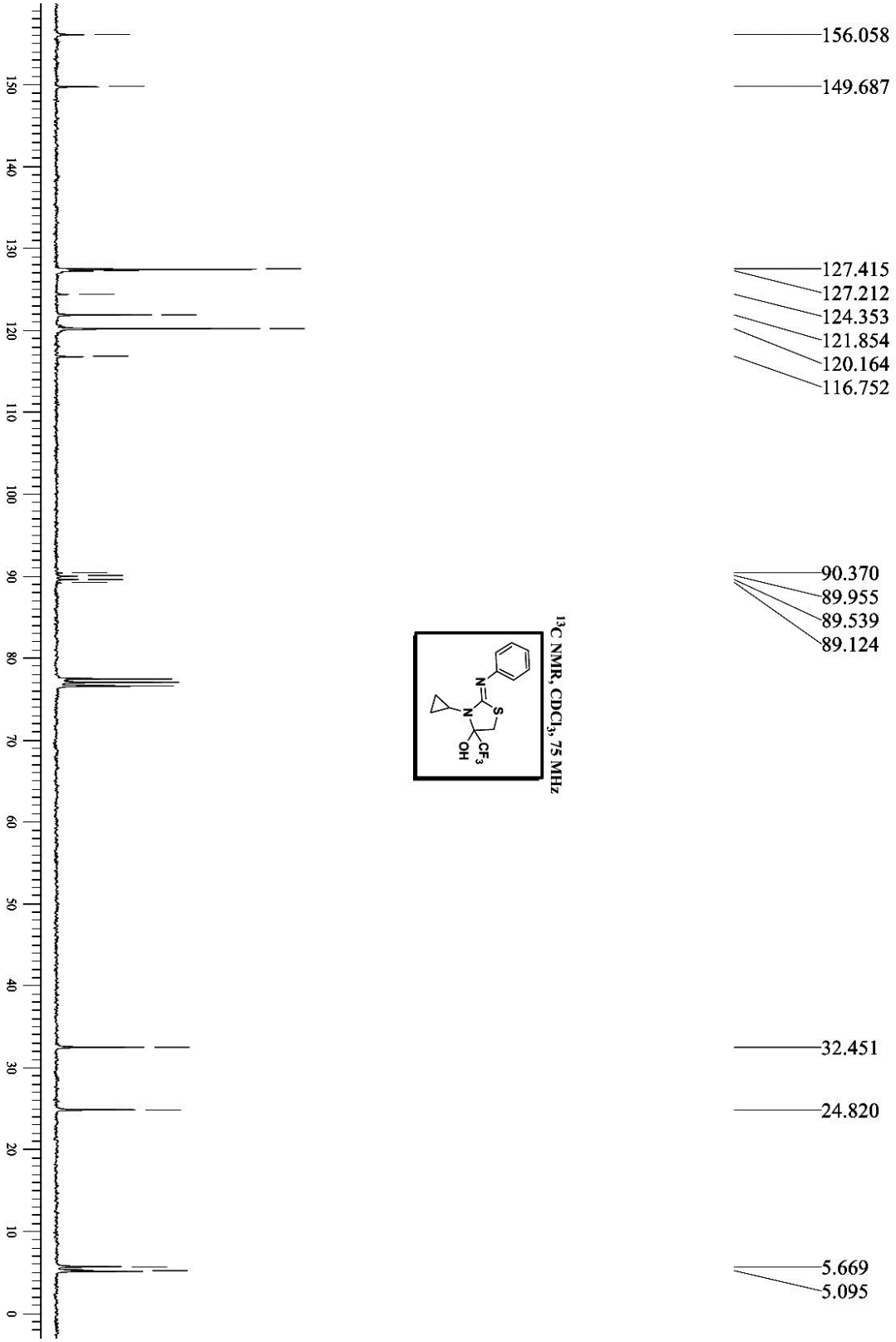
^{13}C NMR spectra of 8d



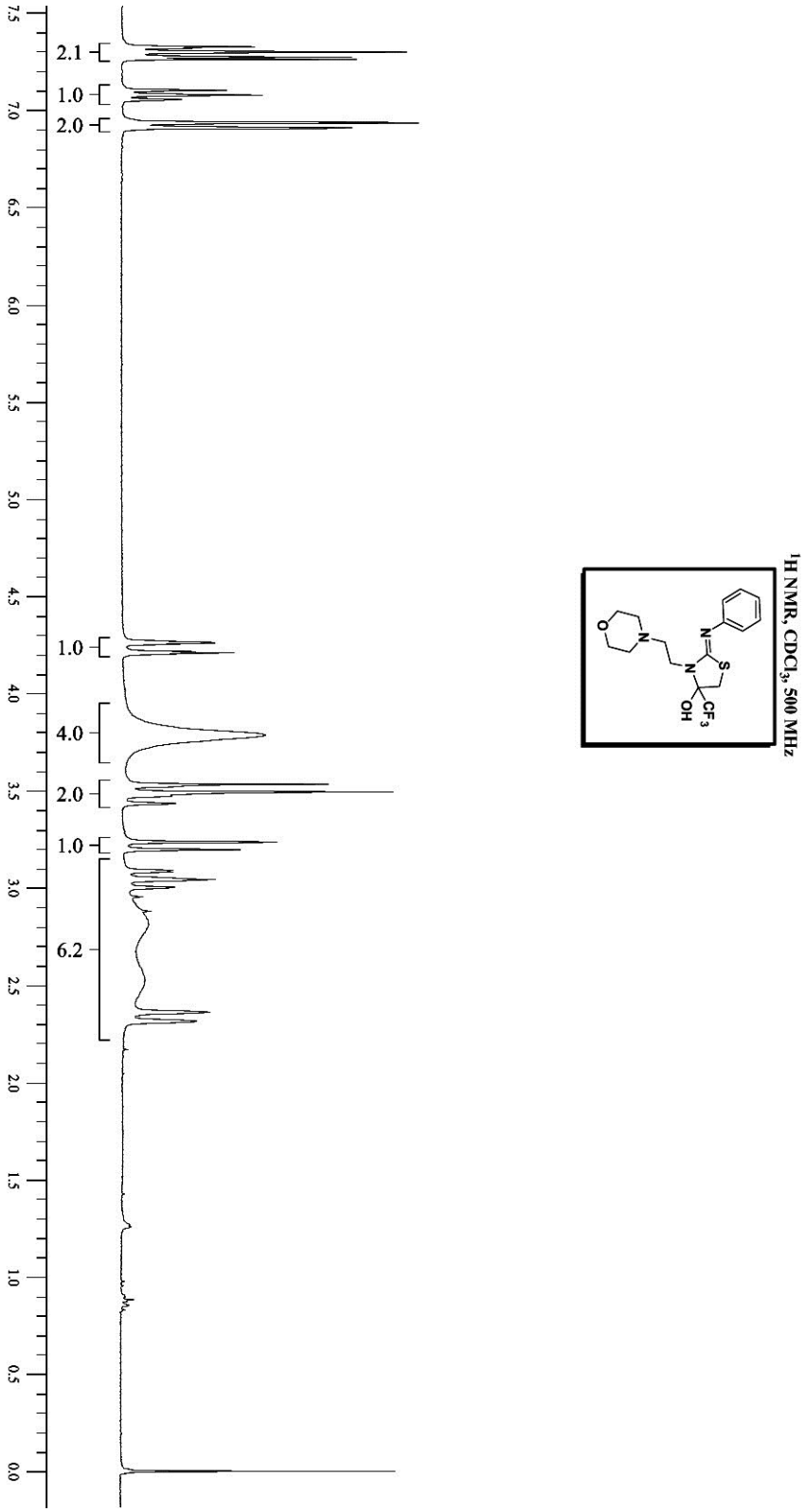
^{1}H NMR spectra of 8e



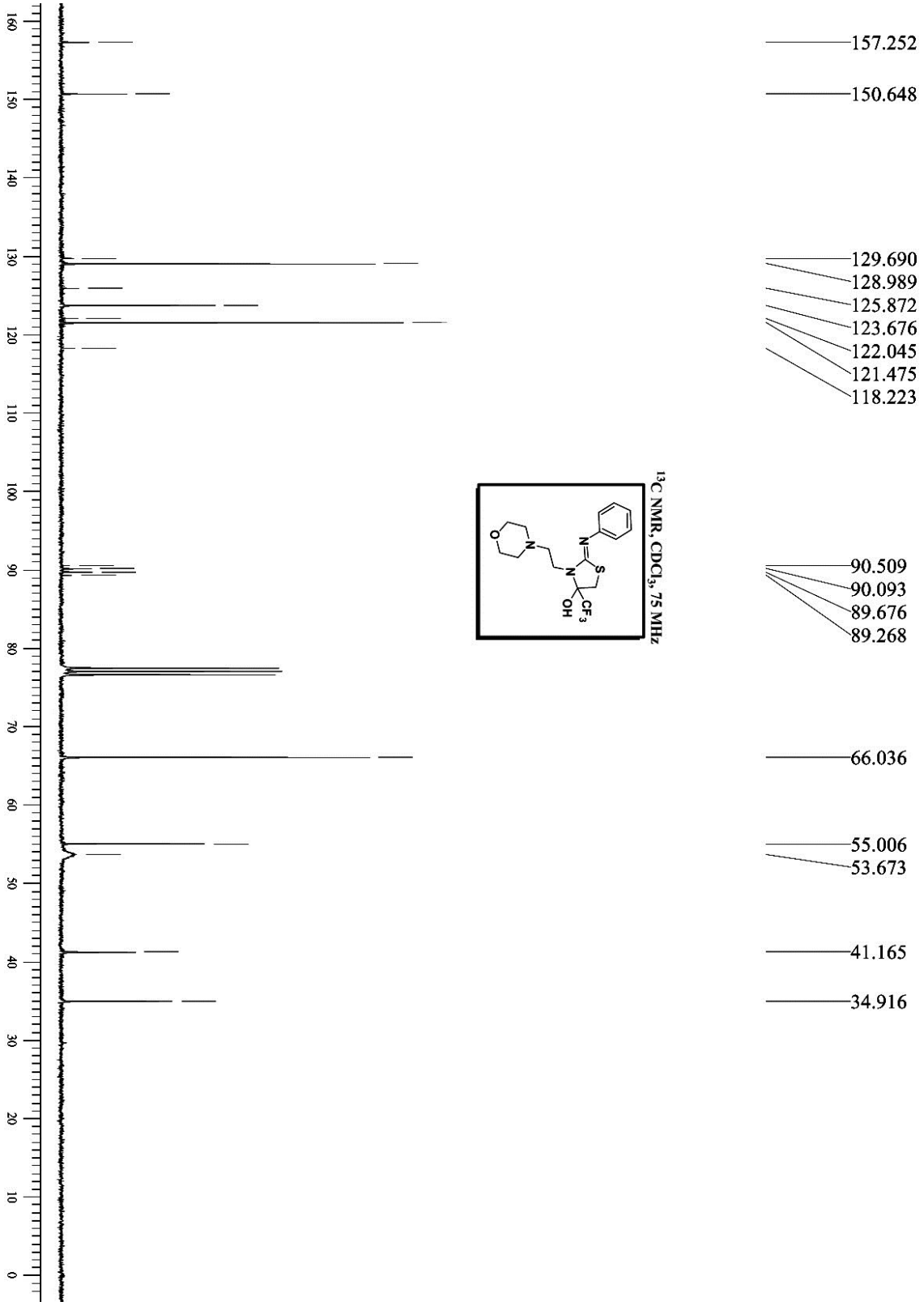
^{13}C NMR spectra of 8e



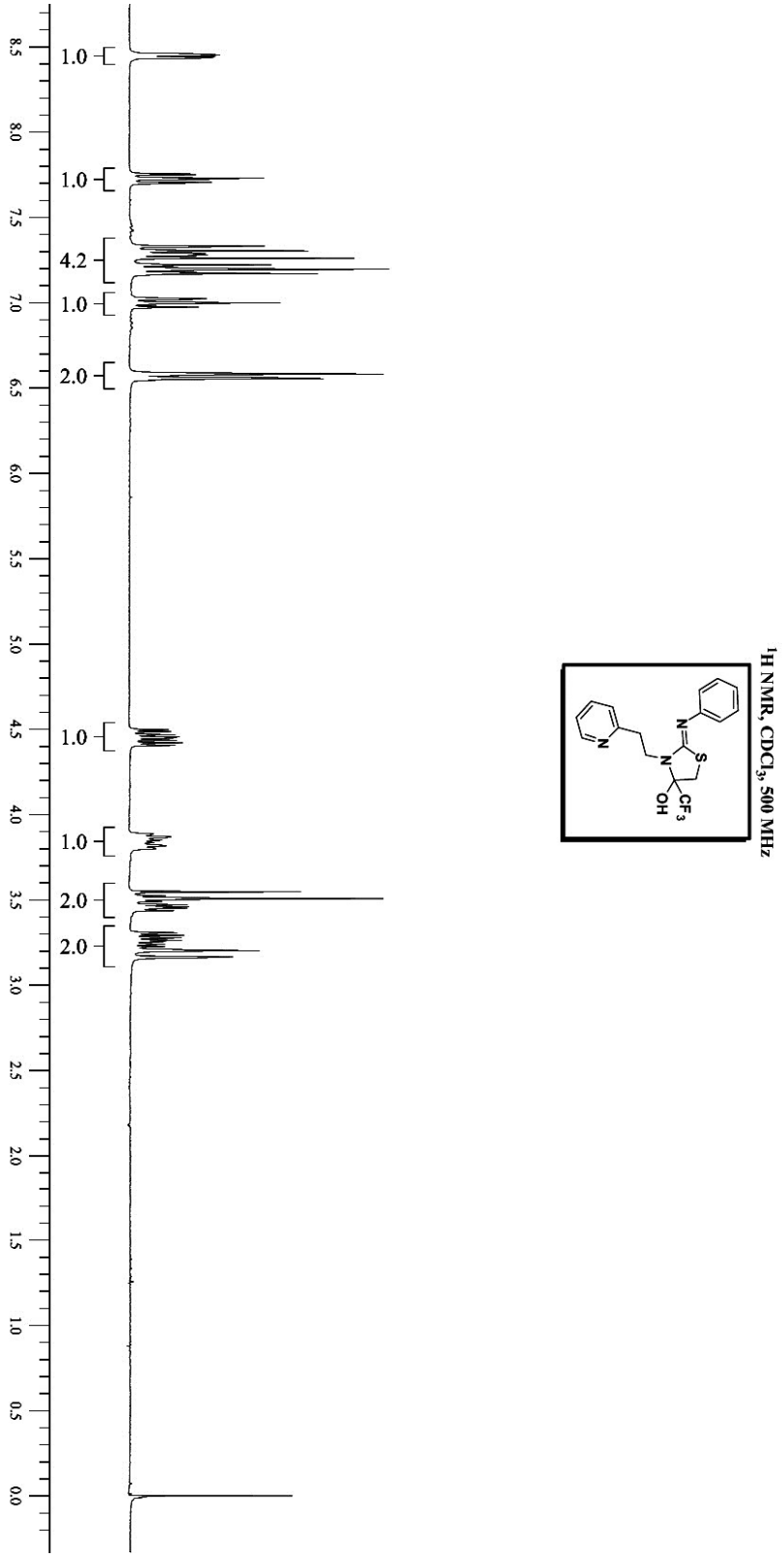
^{1}H NMR spectra of 8f



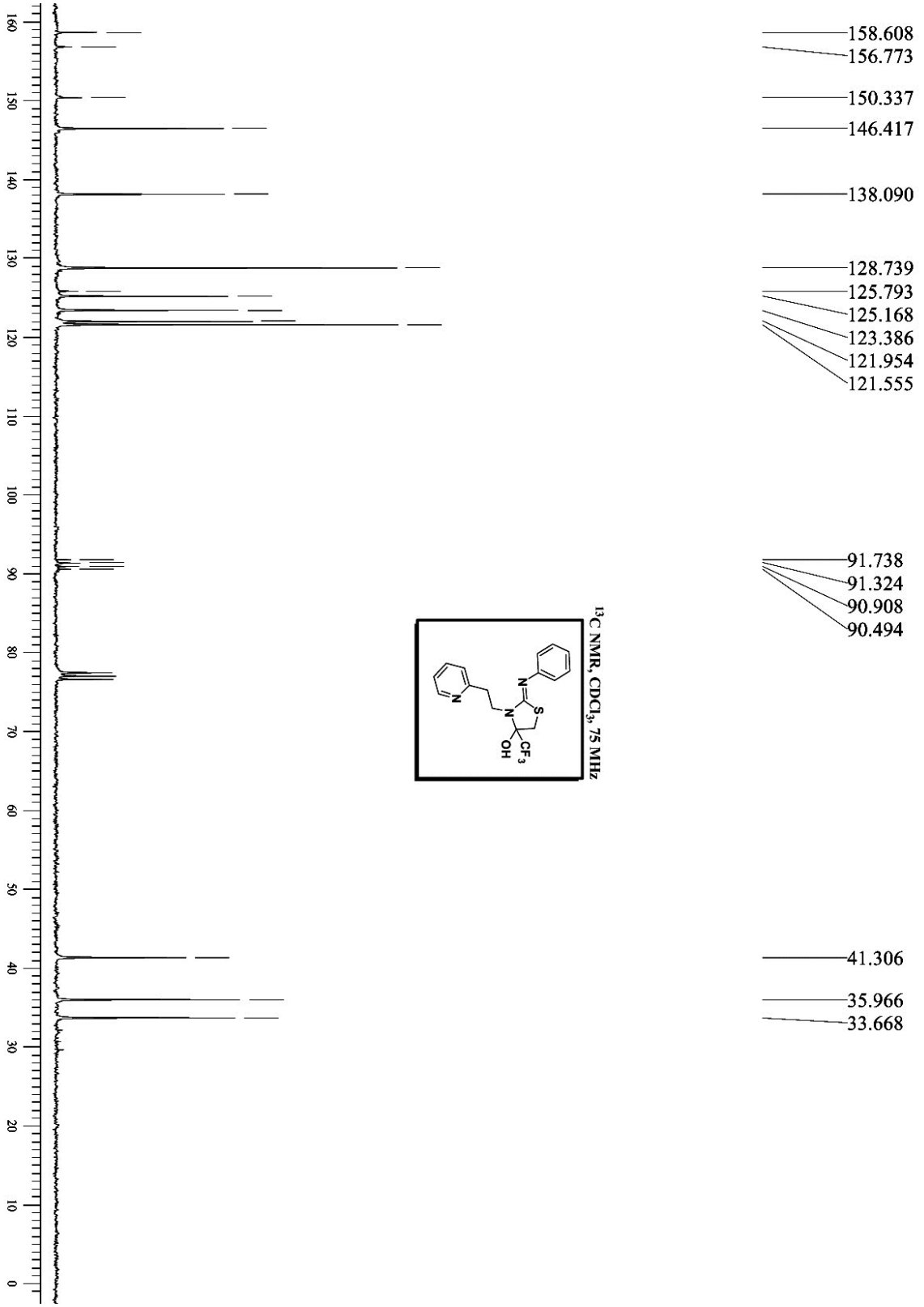
^{13}C NMR spectra of 8f



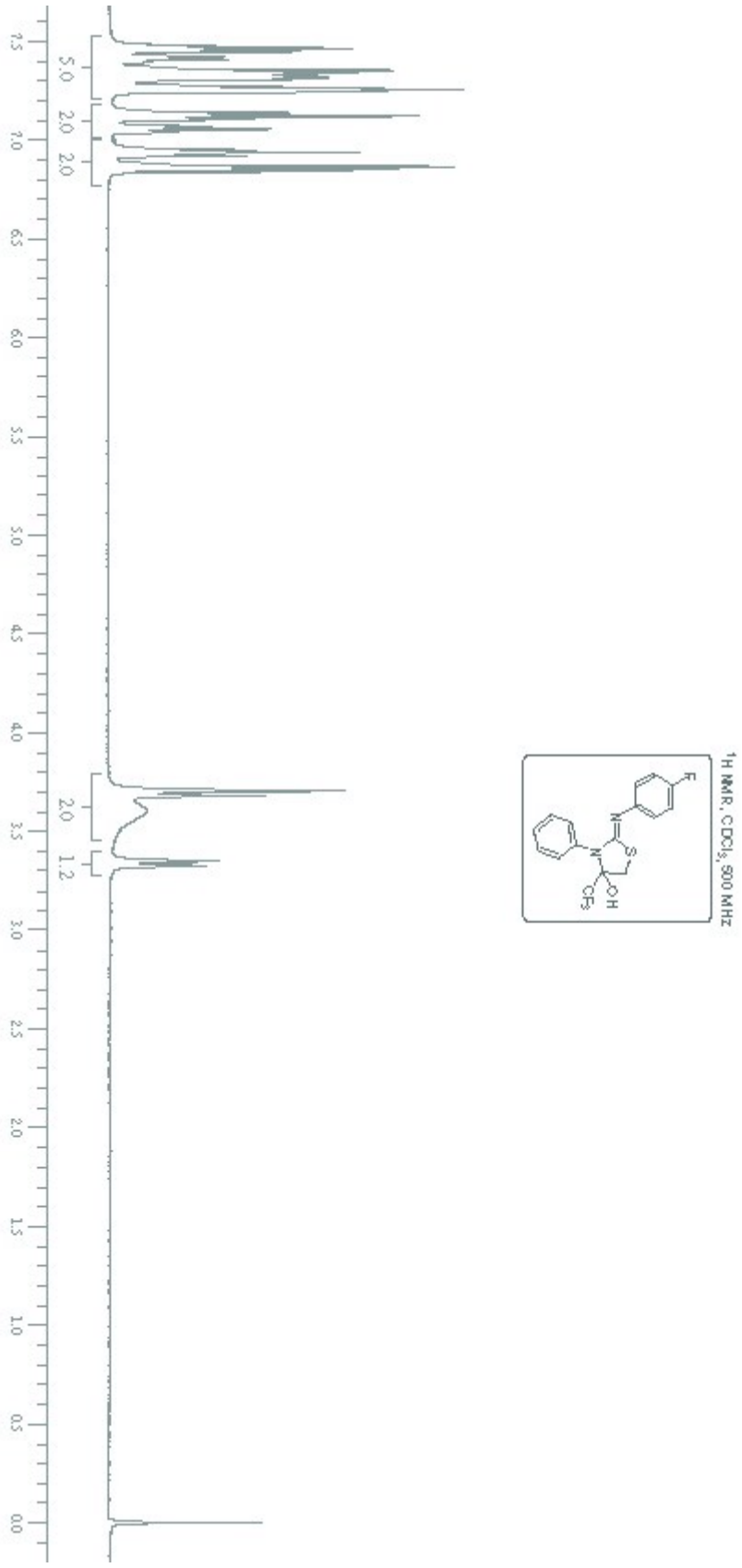
^{1}H NMR spectra of 8g



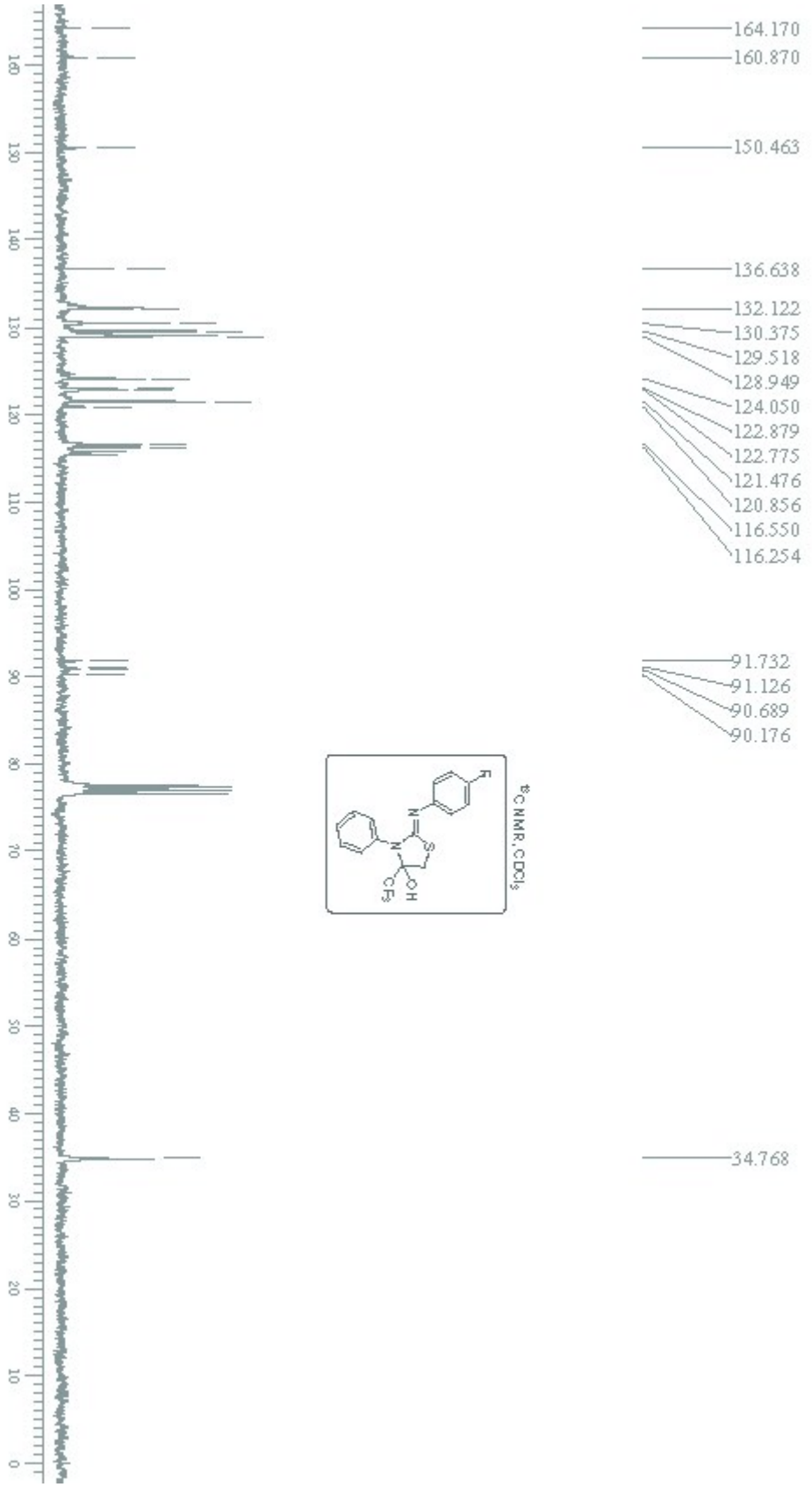
^{13}C NMR spectra of 8g



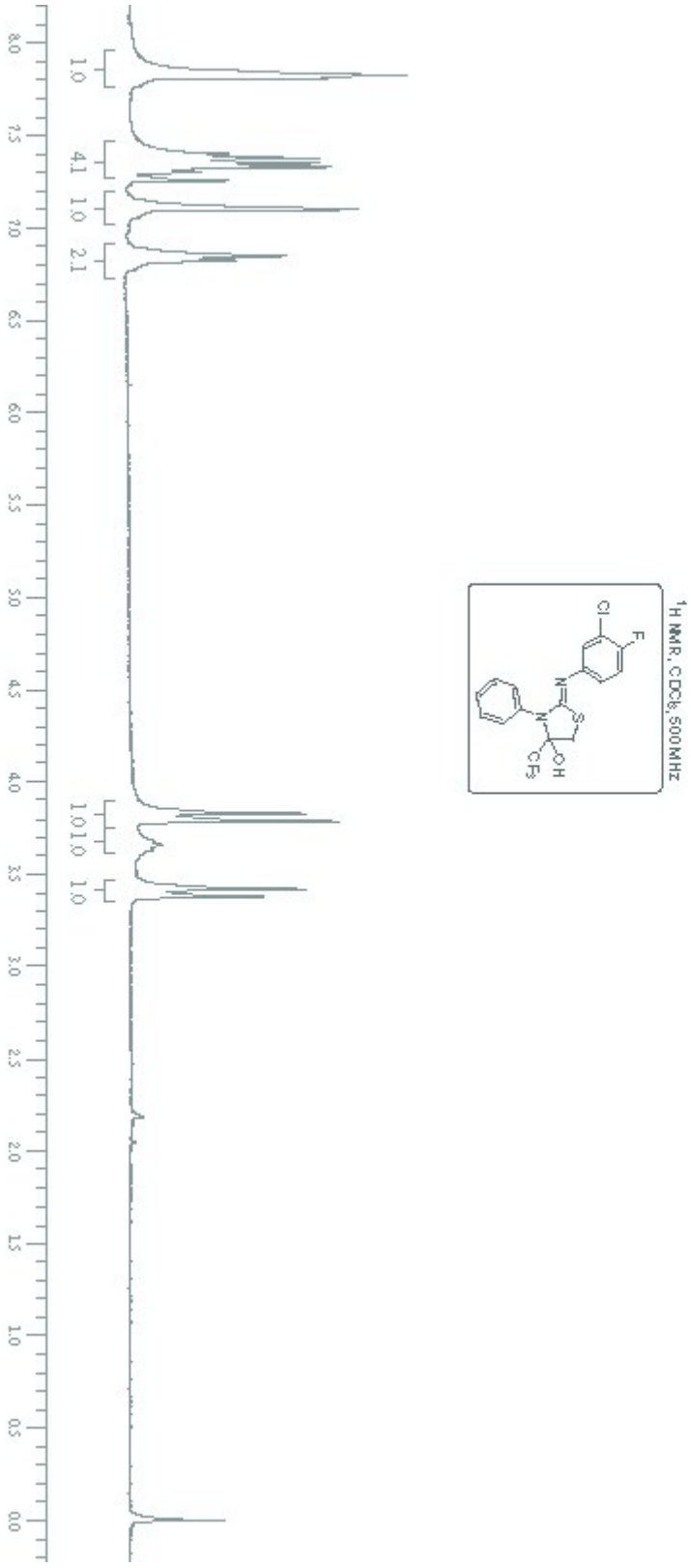
^{1}H NMR spectra of 8h



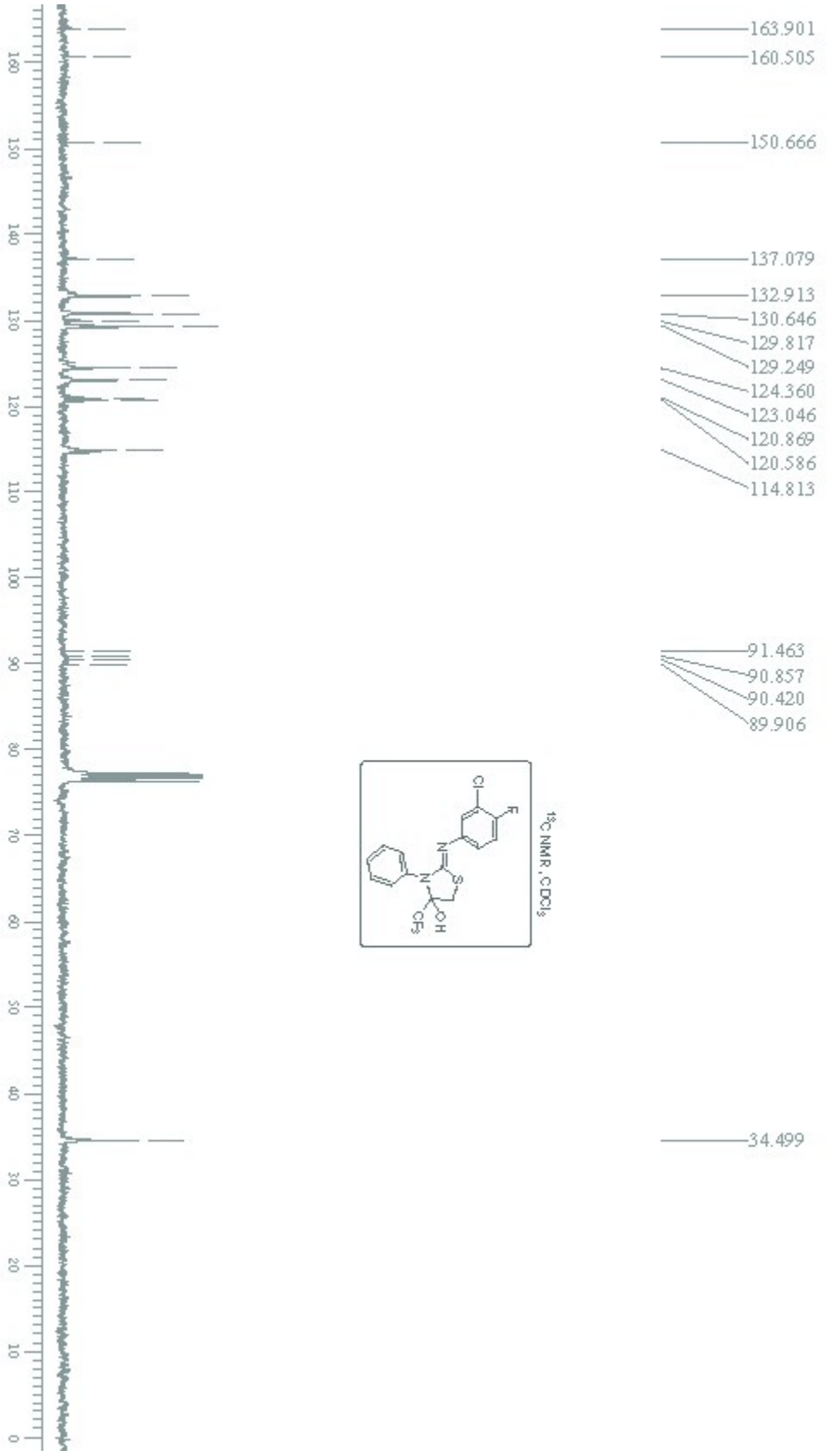
^{13}C NMR spectra of 8h



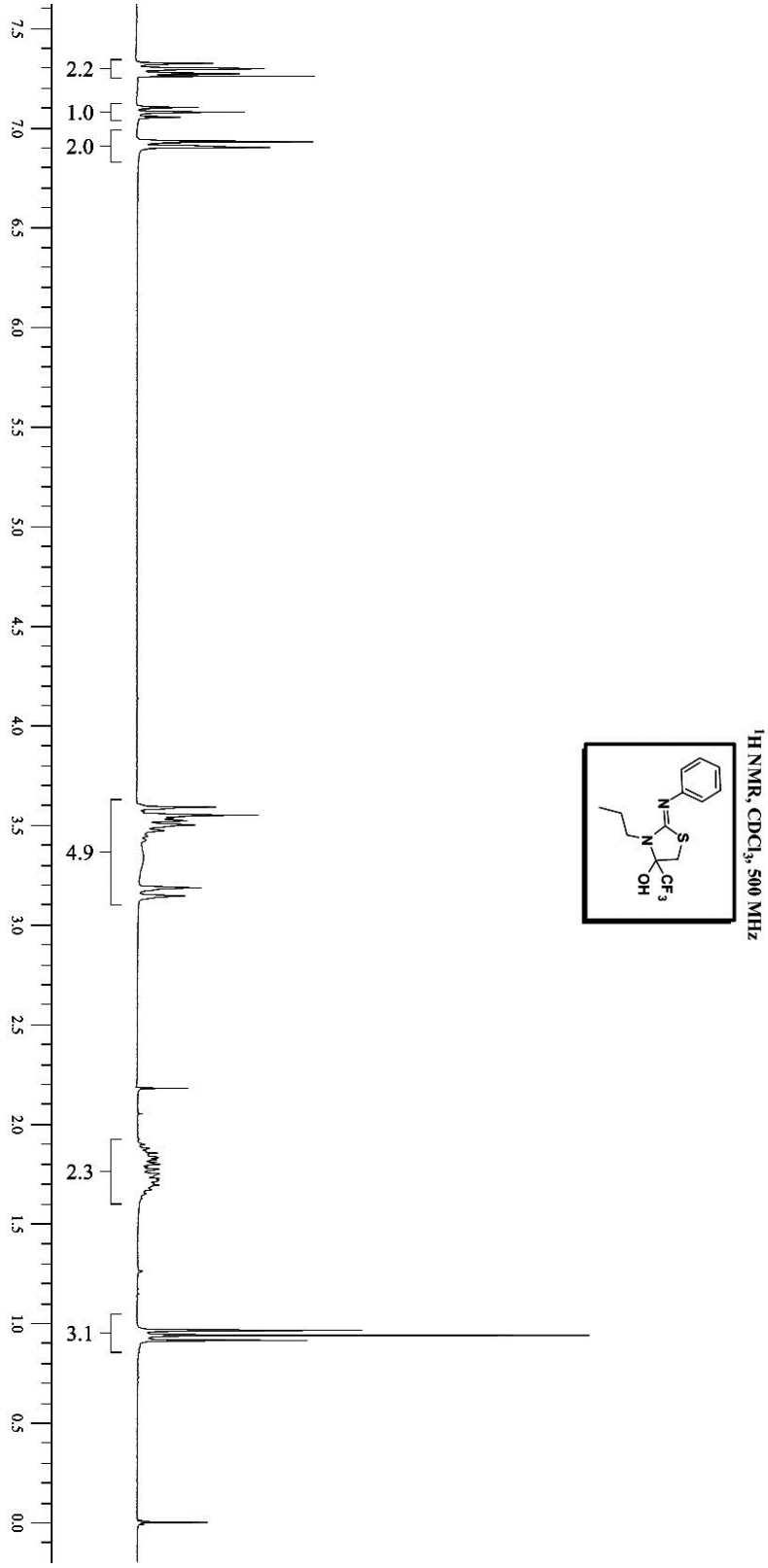
^{1}H NMR spectra of 8i



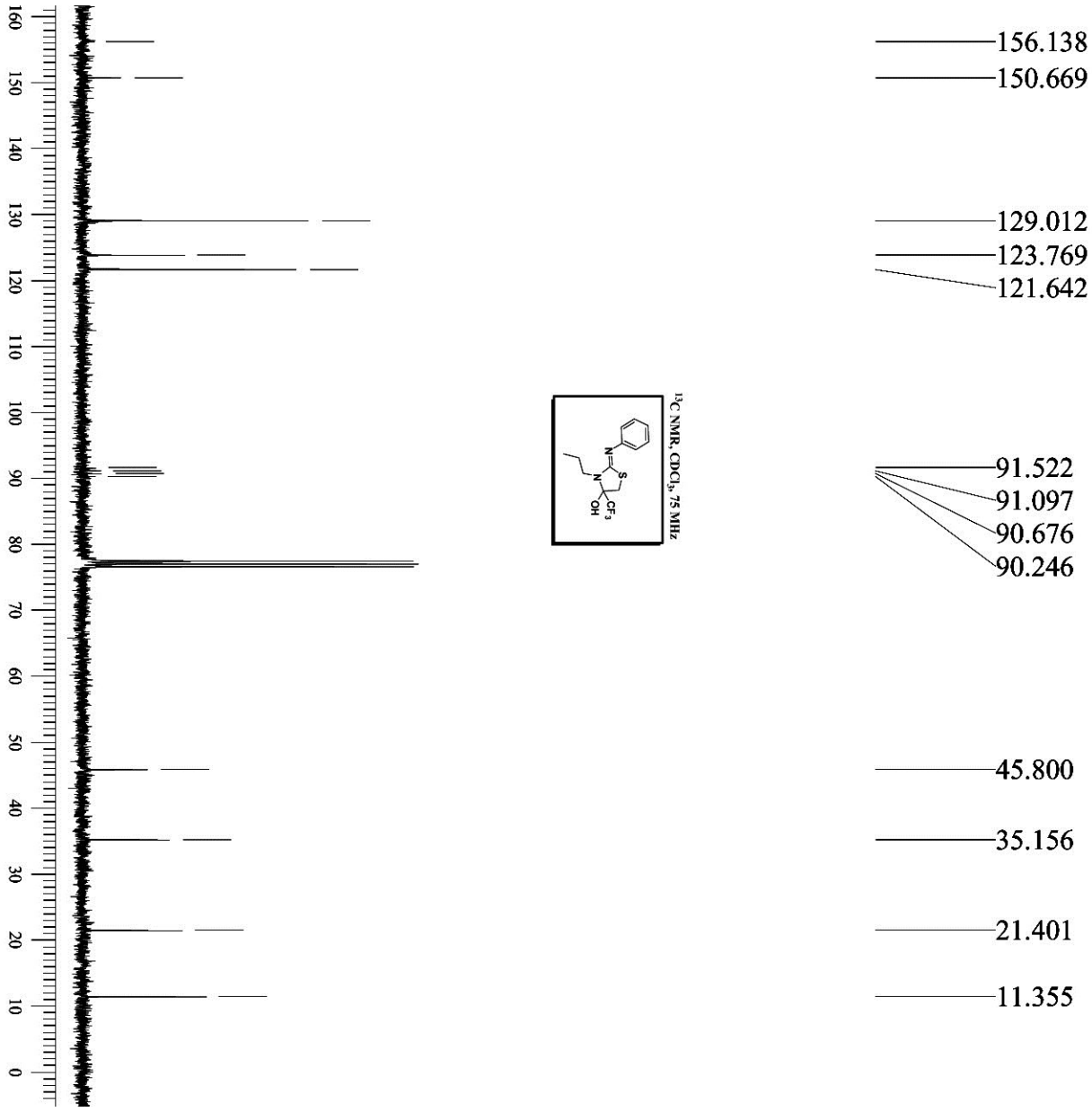
^{13}C NMR spectra of 8i



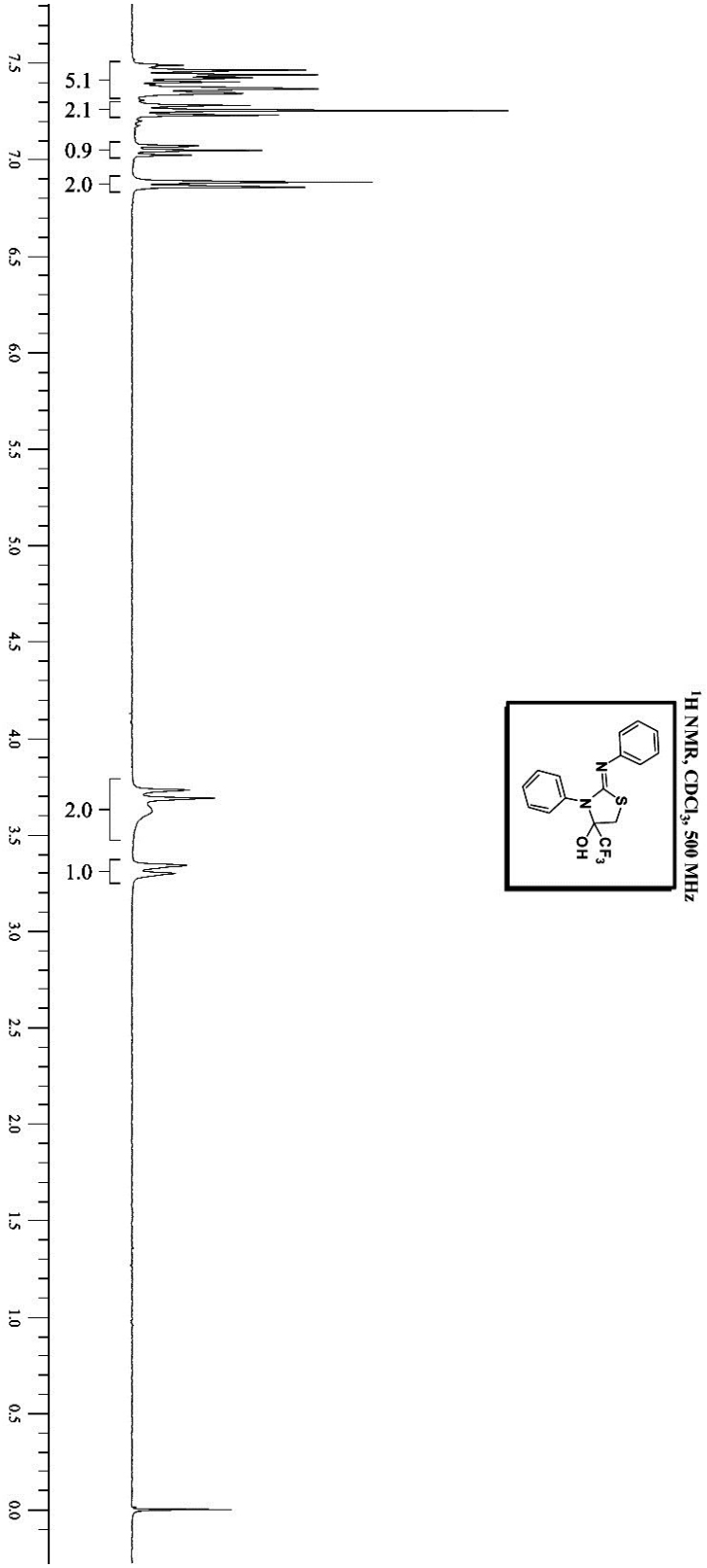
^{1}H NMR spectra of 8j



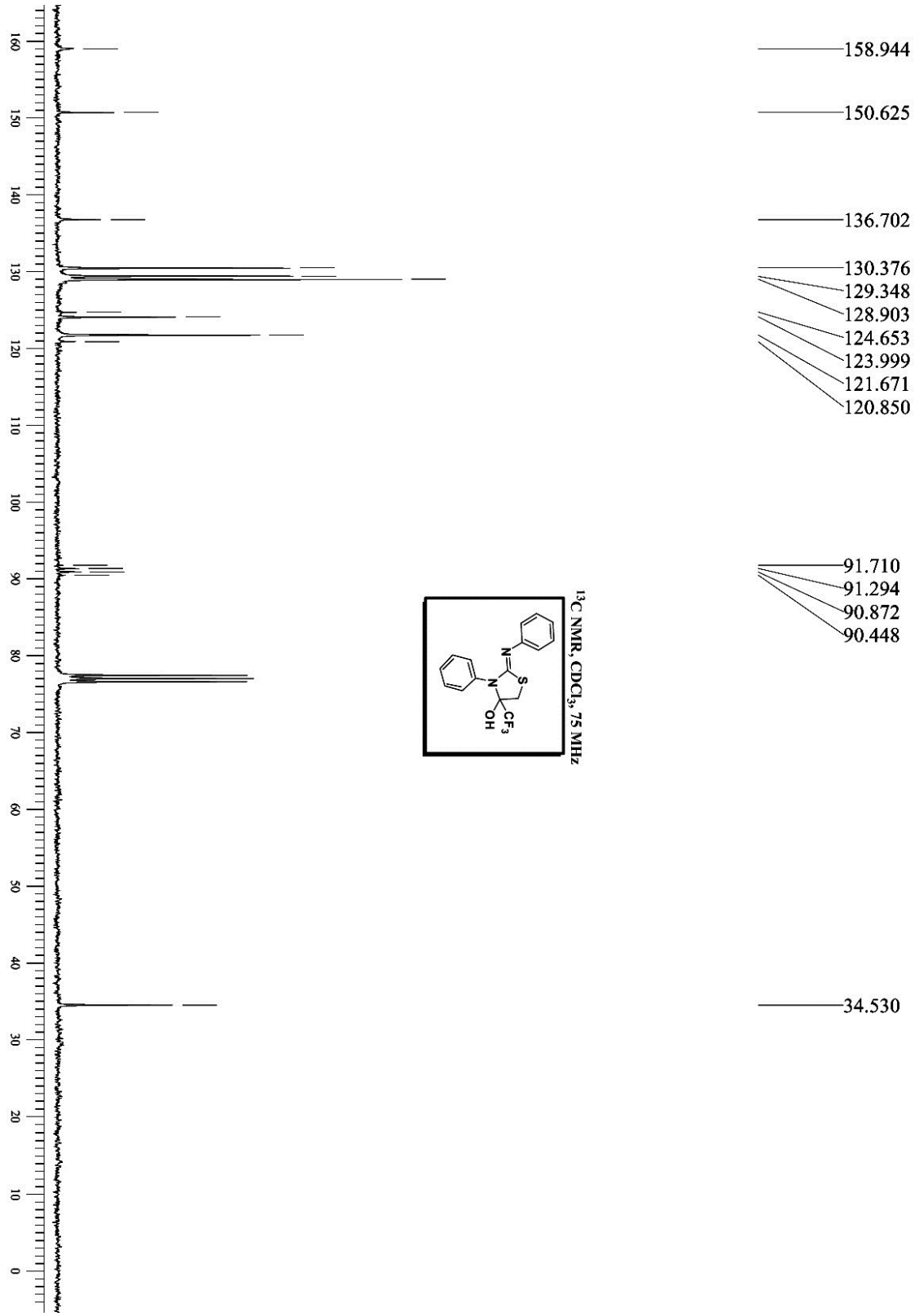
^{13}C NMR spectra of 8j



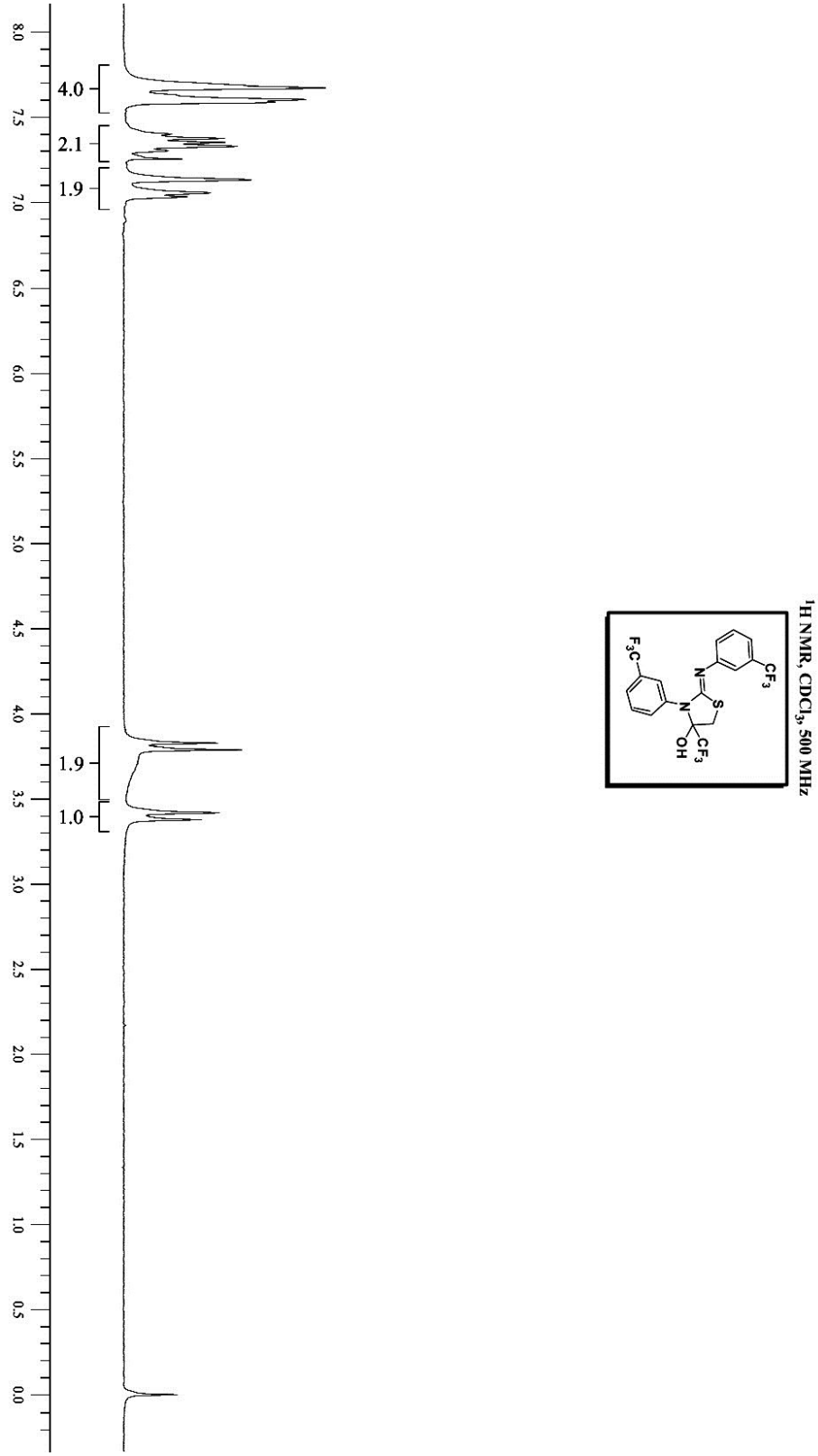
^{1}H NMR spectra of 8k



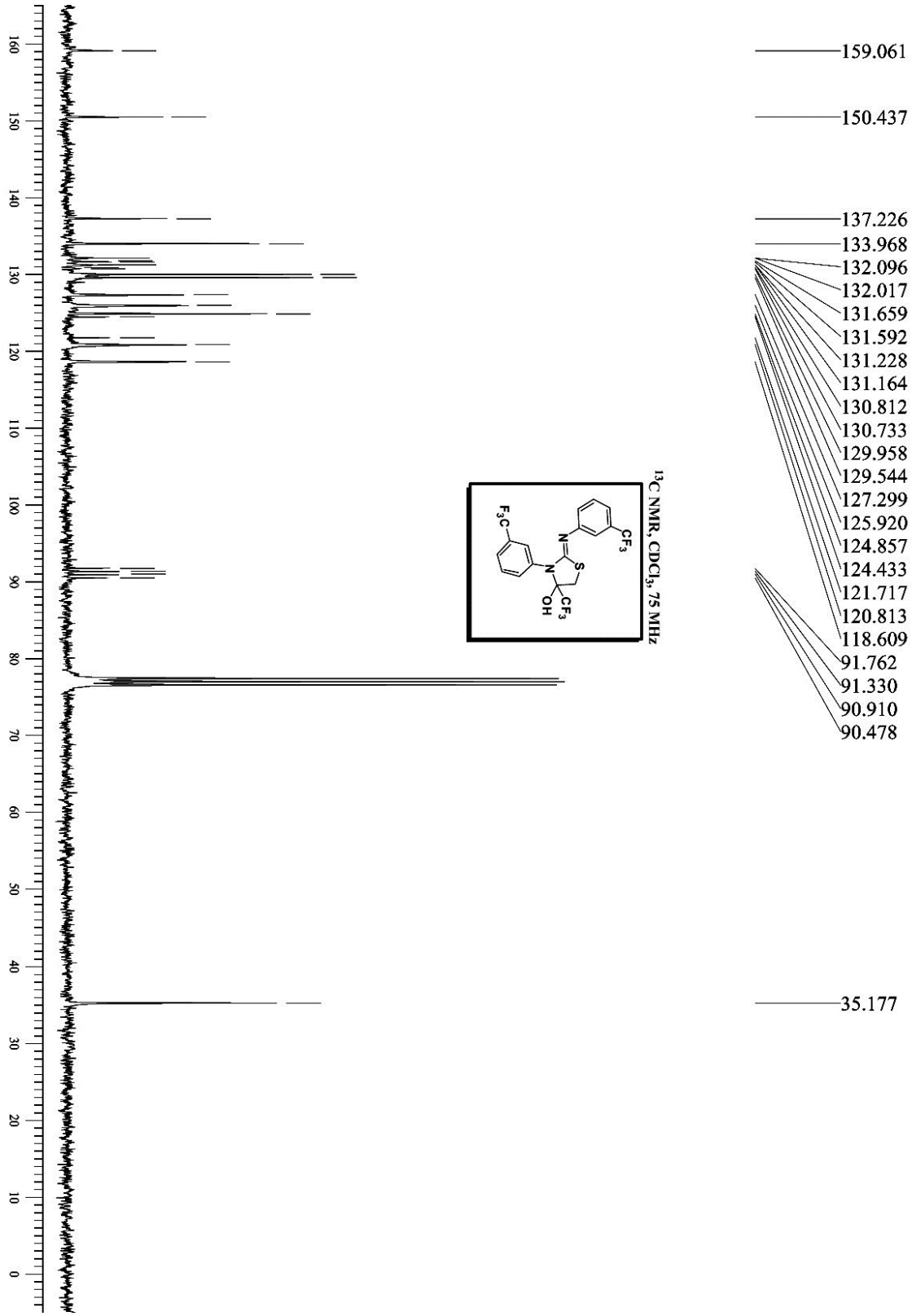
^{13}C NMR spectra of 8k



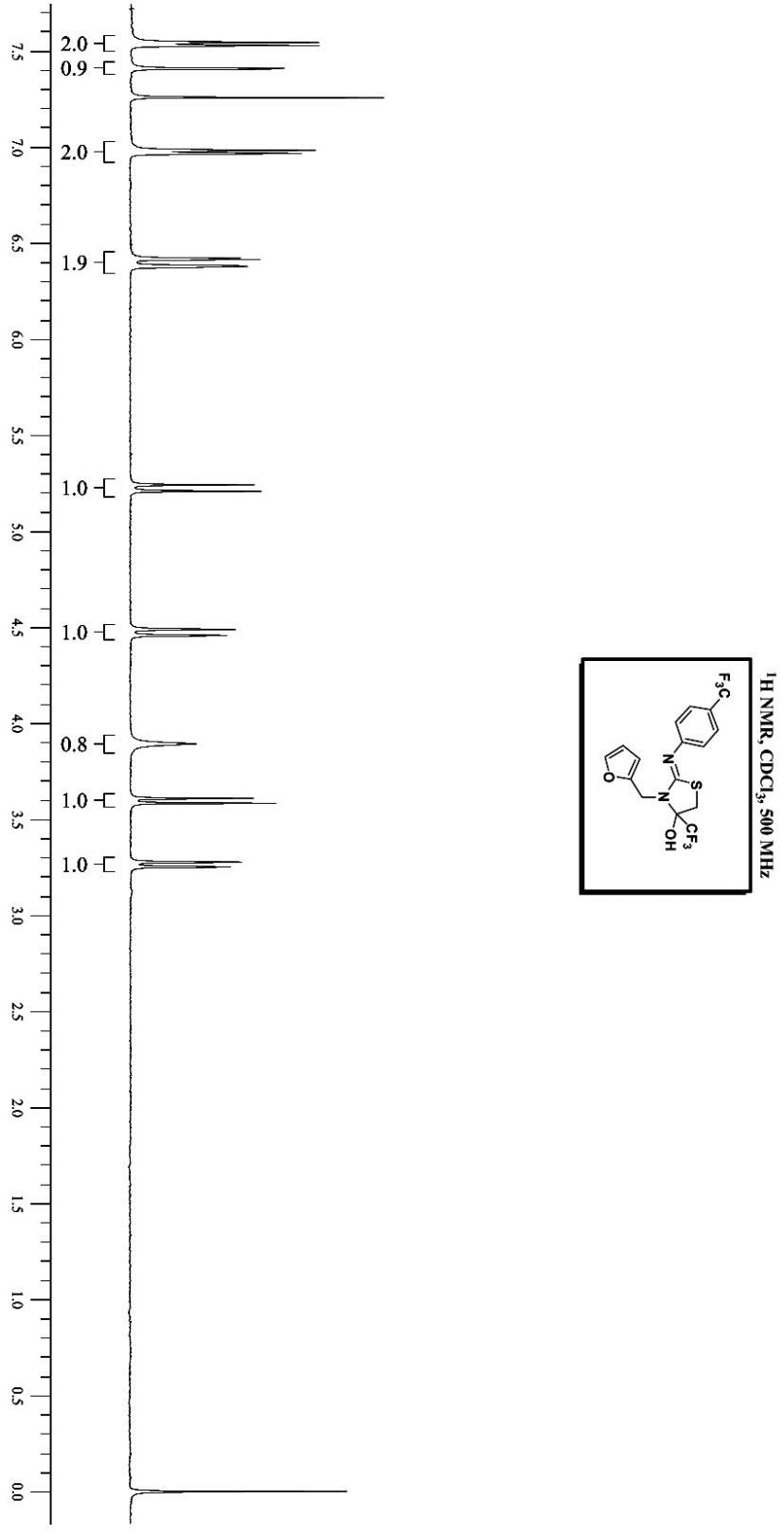
^{1}H NMR spectra of 8l



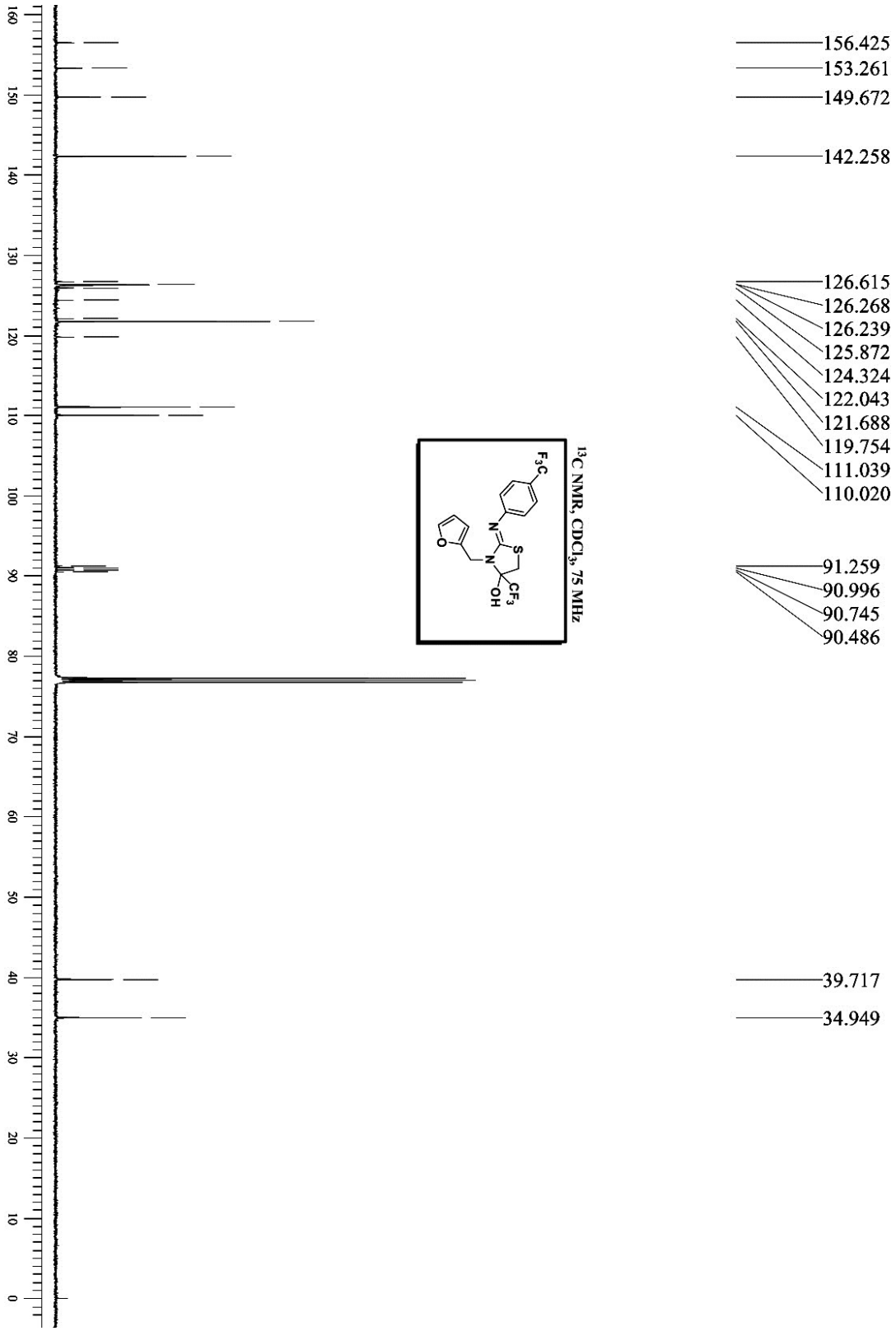
^{13}C NMR spectra of 8l



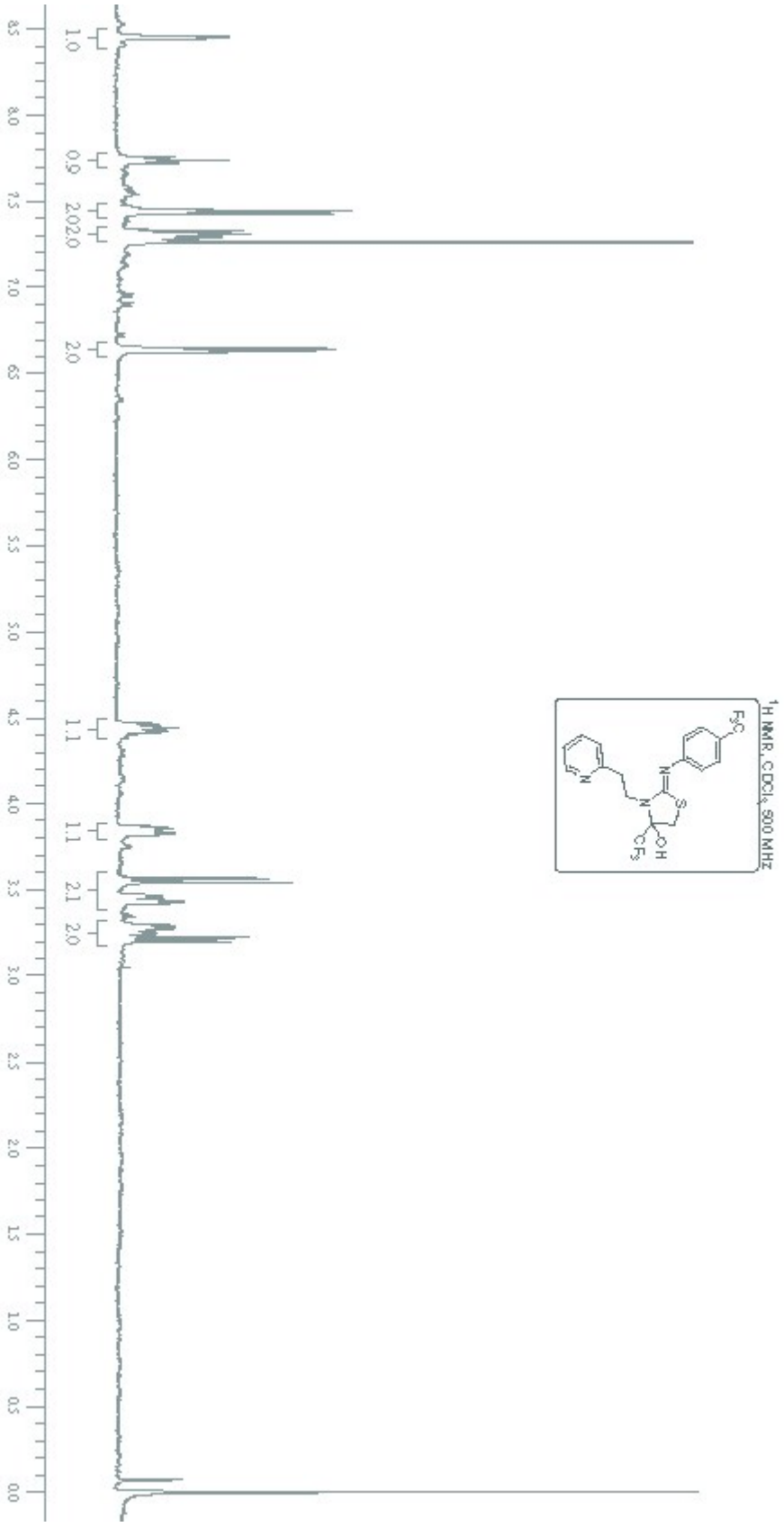
^{1}H NMR spectra of 8m



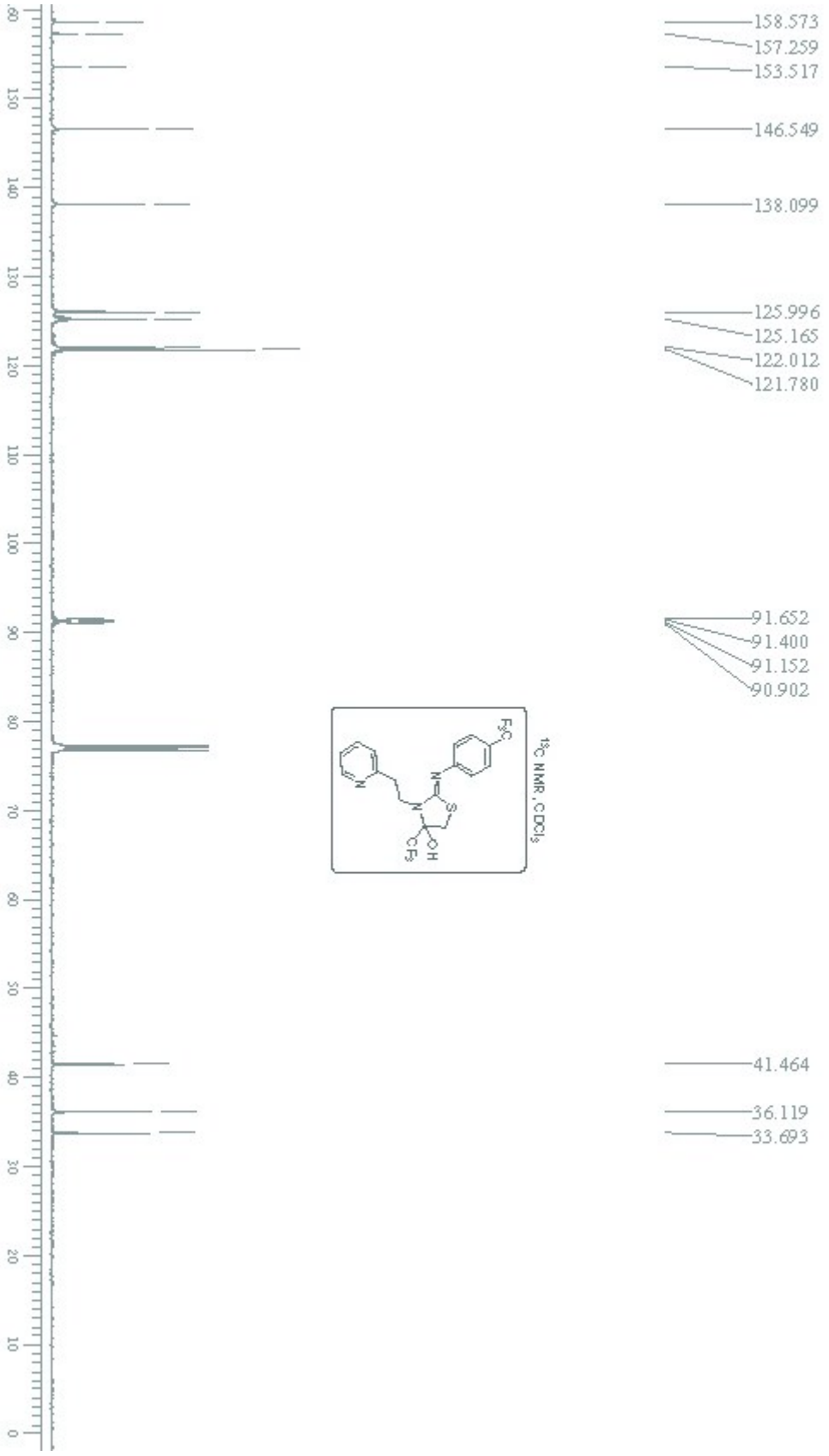
^{13}C NMR spectra of 8m



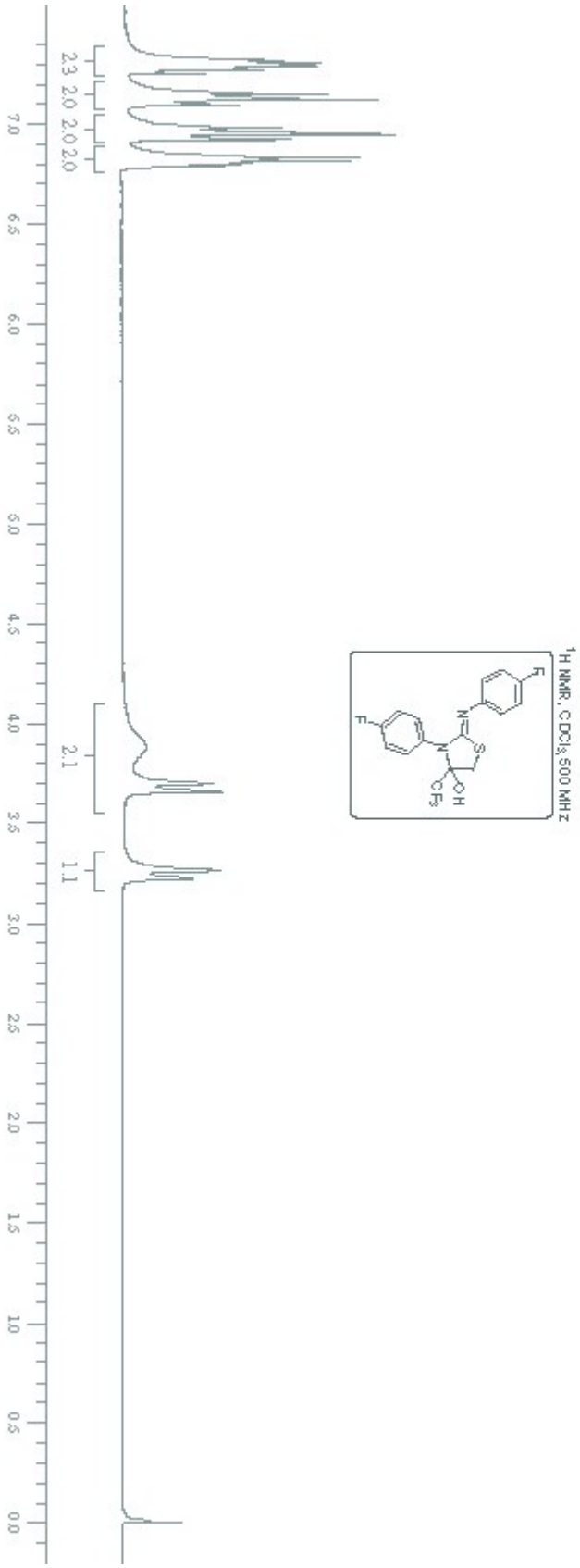
^{1}H NMR spectra of 8n



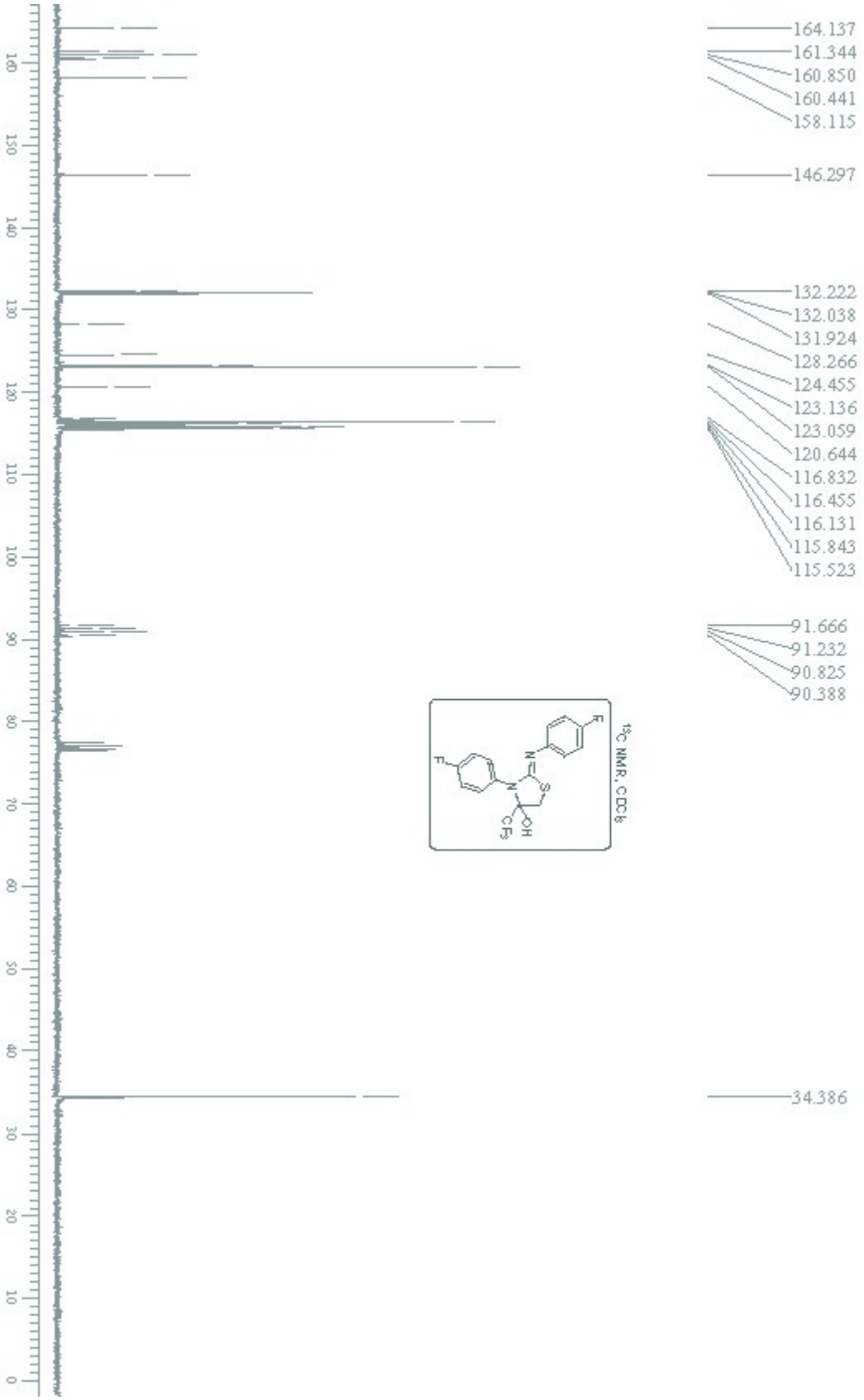
^{13}C NMR spectra of 8n



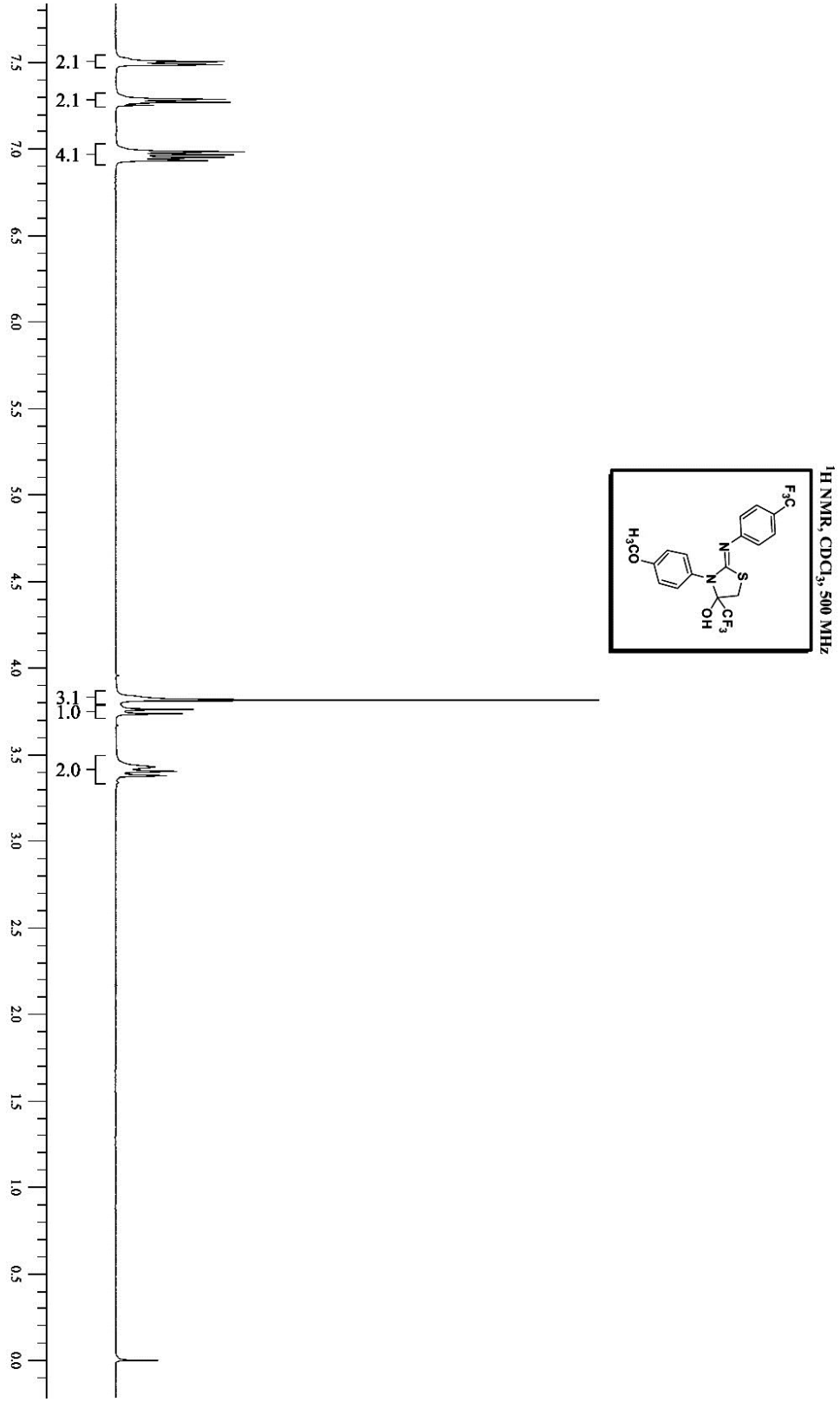
^{1}H NMR spectra of 8o



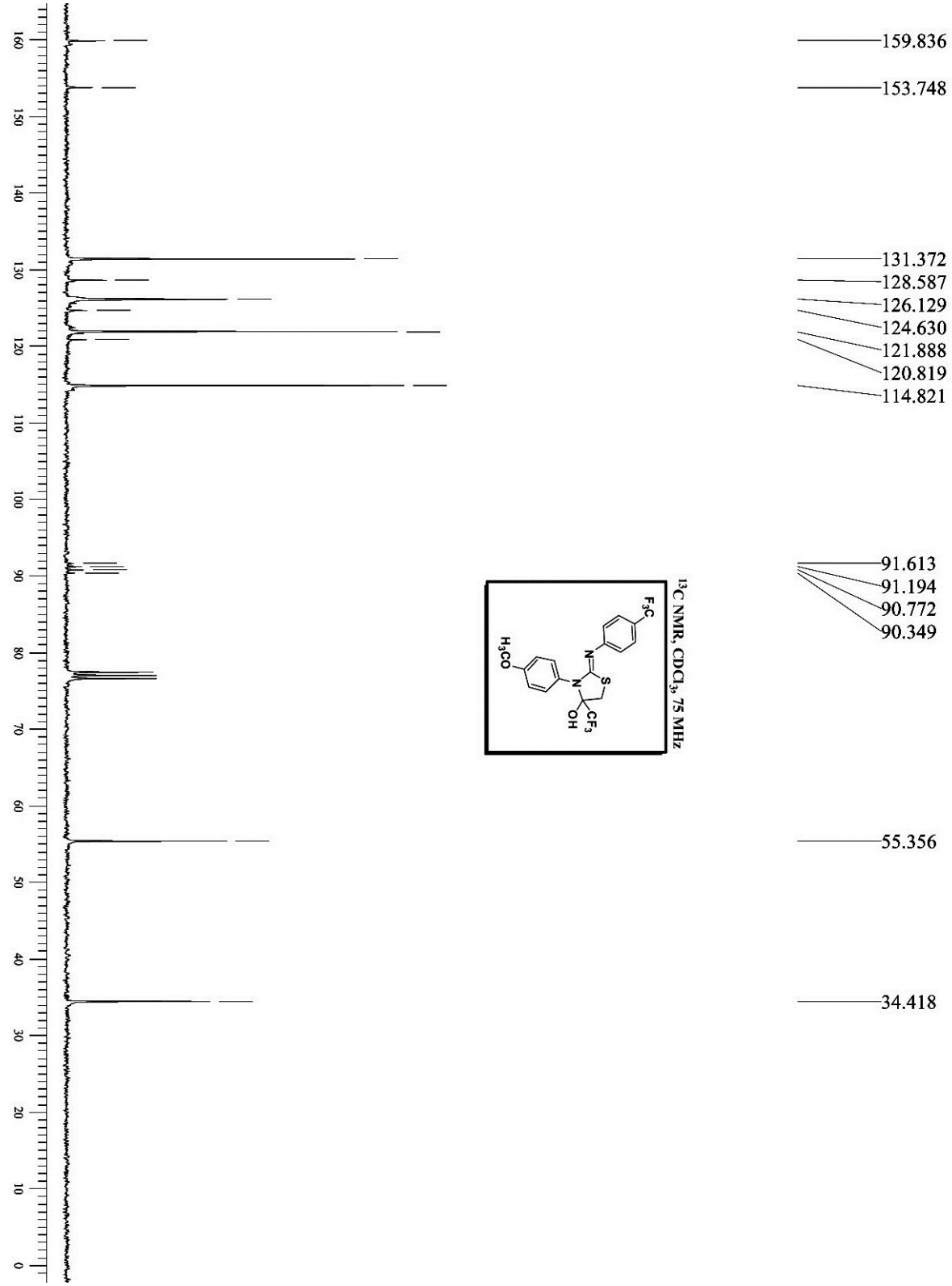
^{13}C NMR spectra of 8o



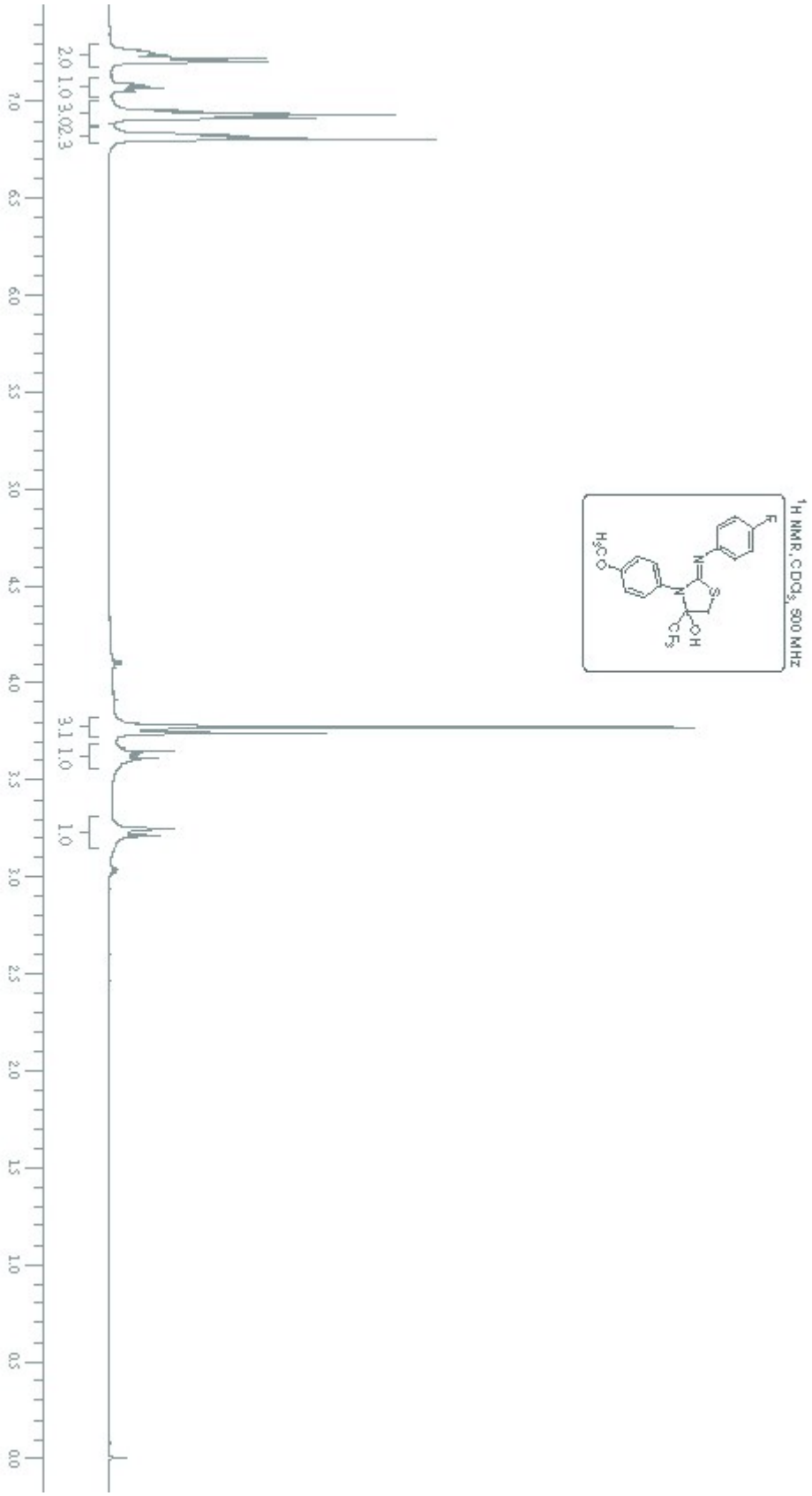
^{1}H NMR spectra of 8p



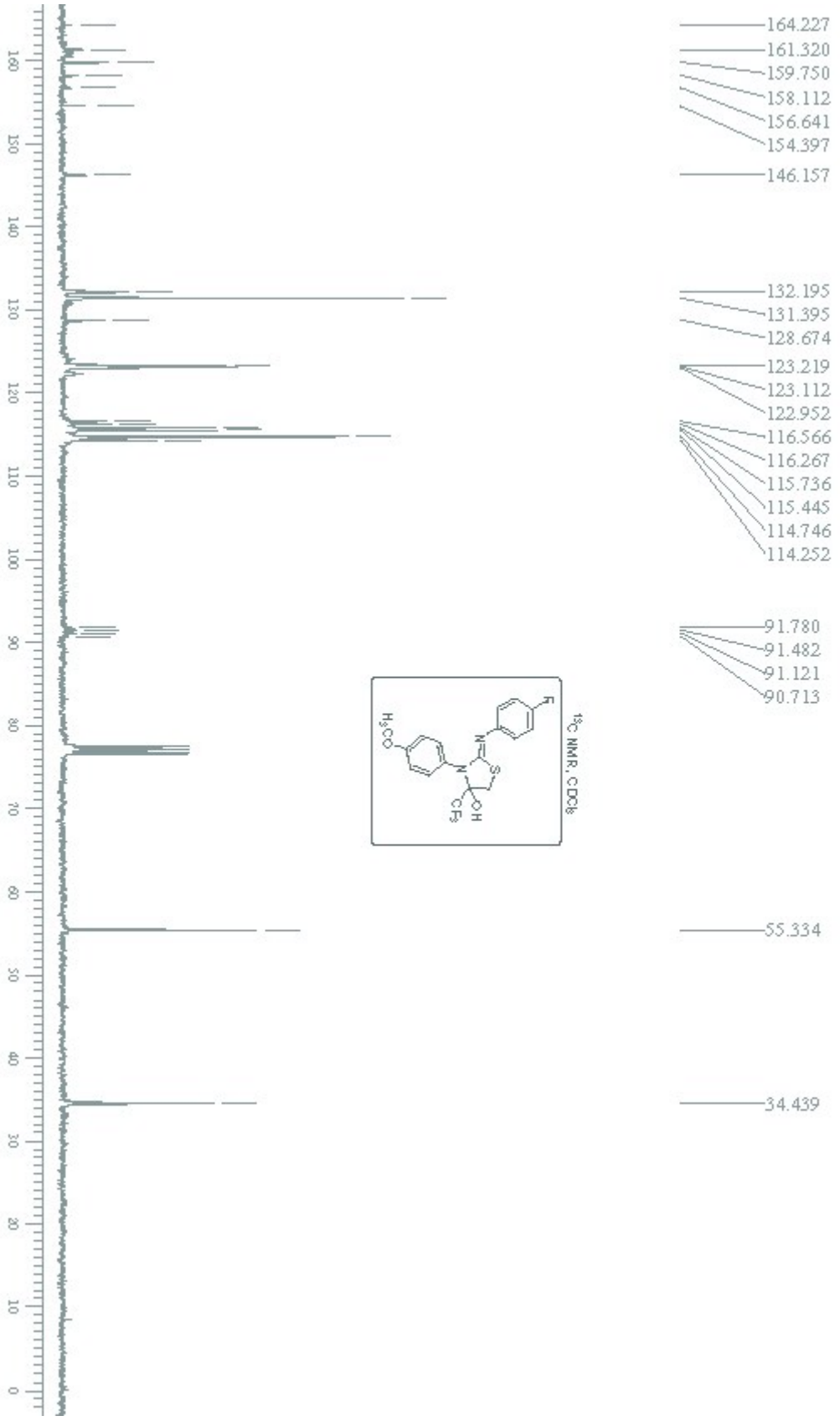
^{13}C NMR spectra of 8p



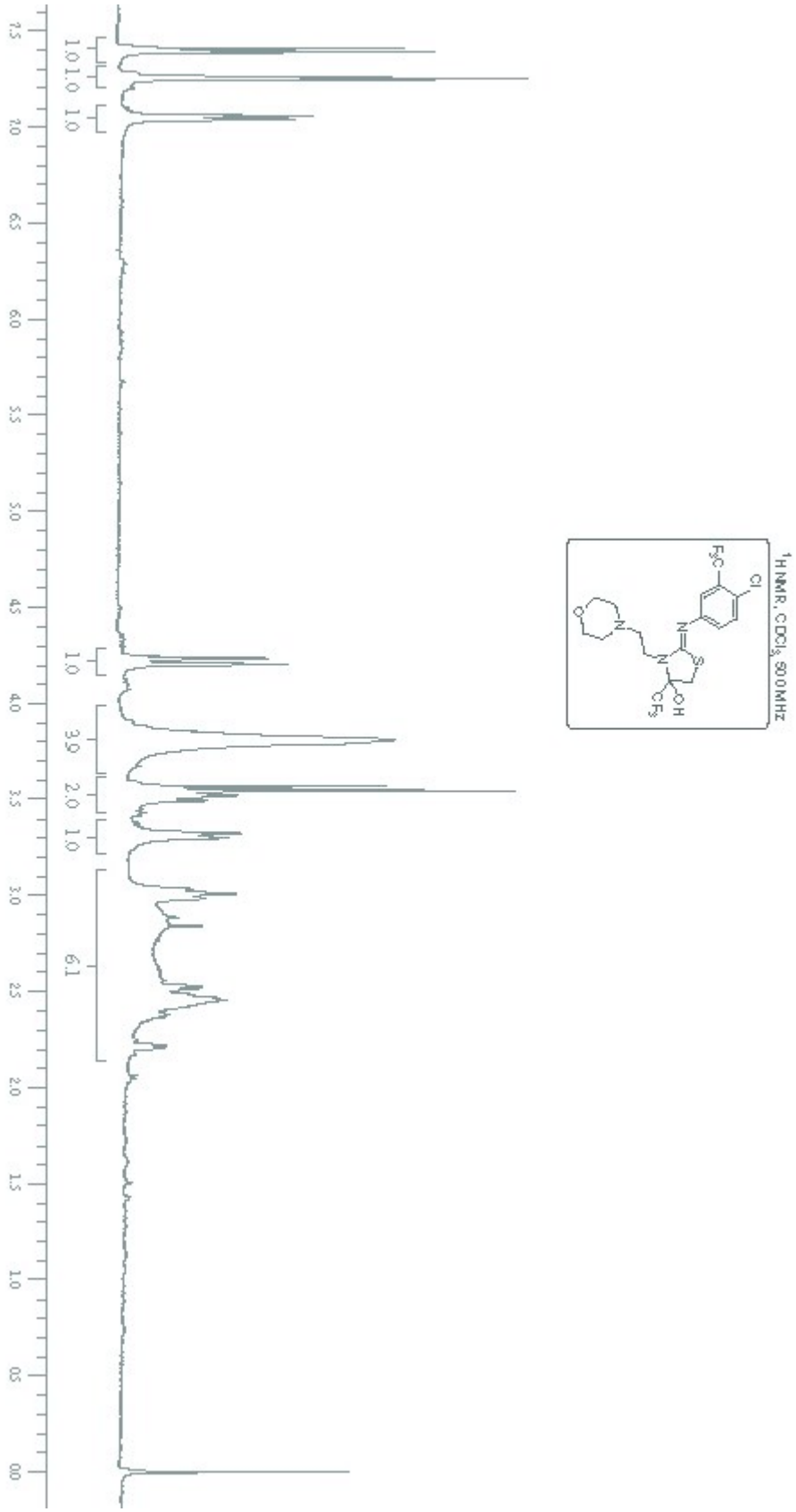
^{1}H NMR spectra of 8q



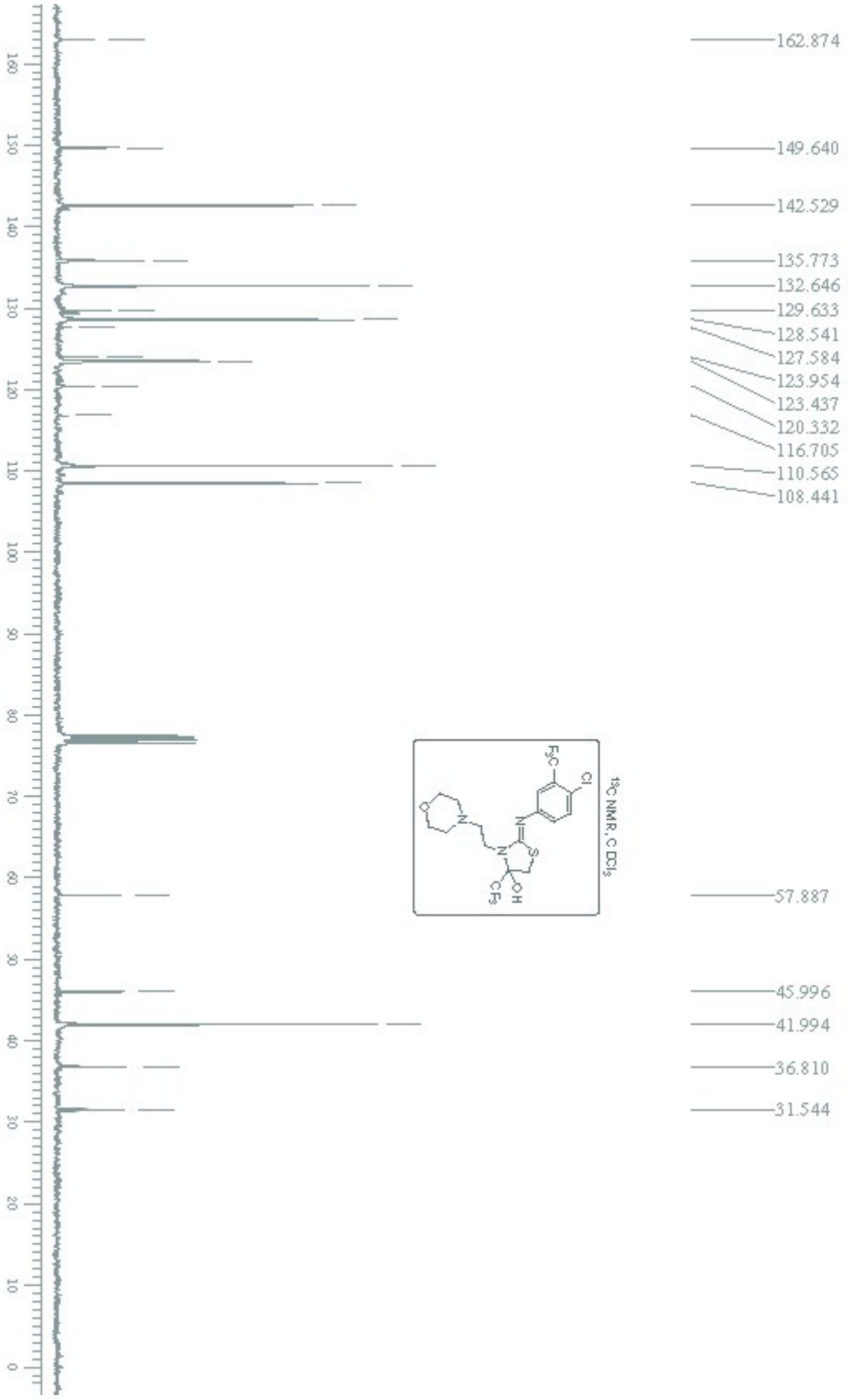
^{13}C NMR spectra of 8q



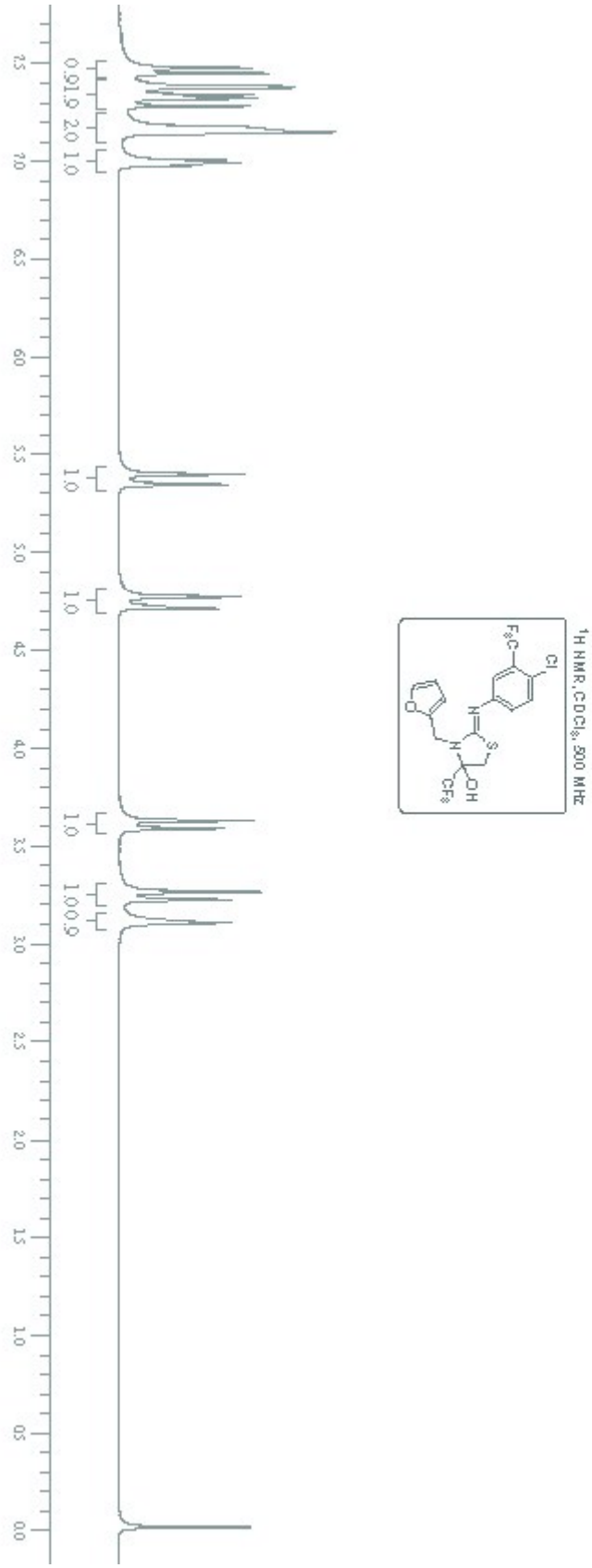
^{1}H NMR spectra of 8r



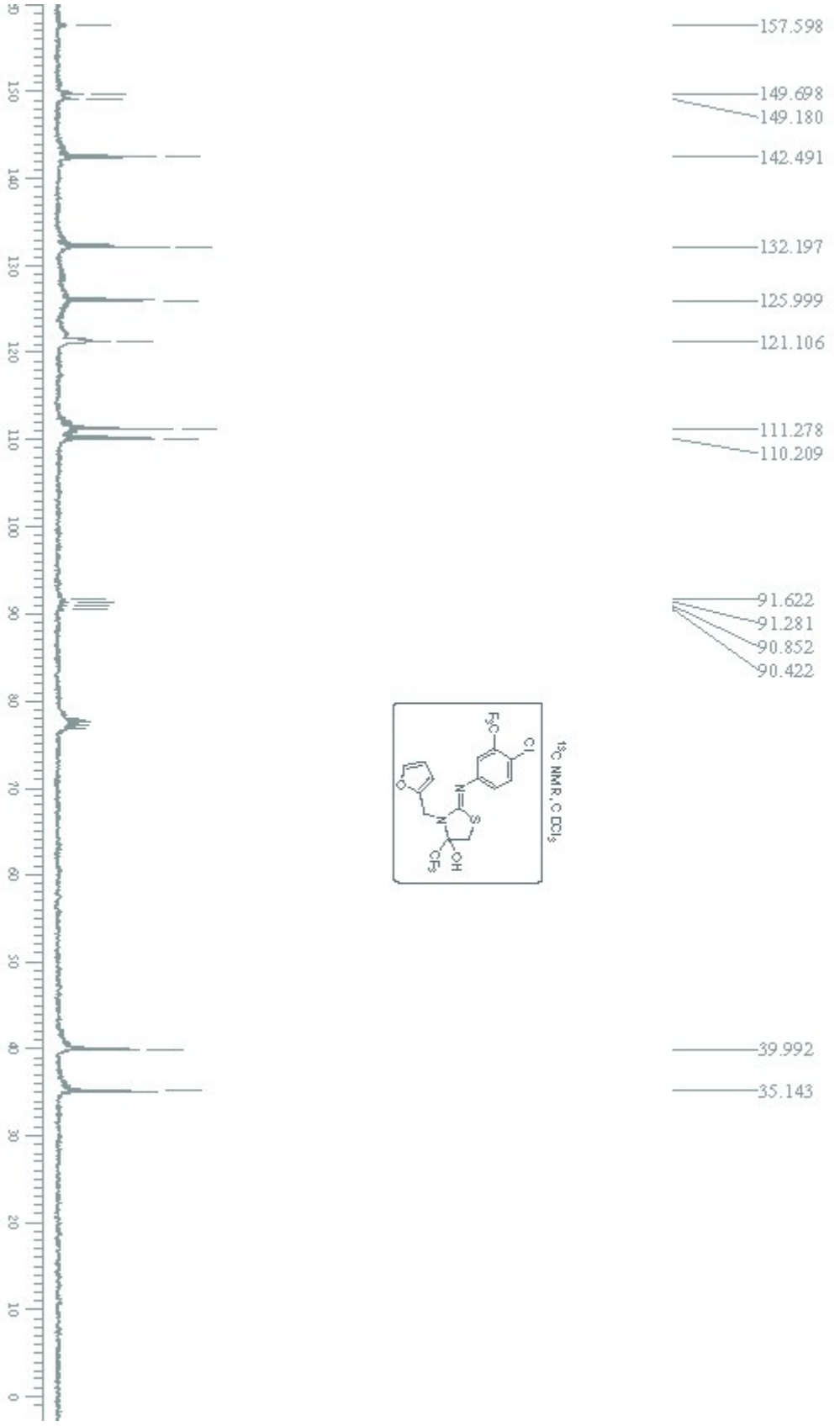
^{13}C NMR spectra of 8r



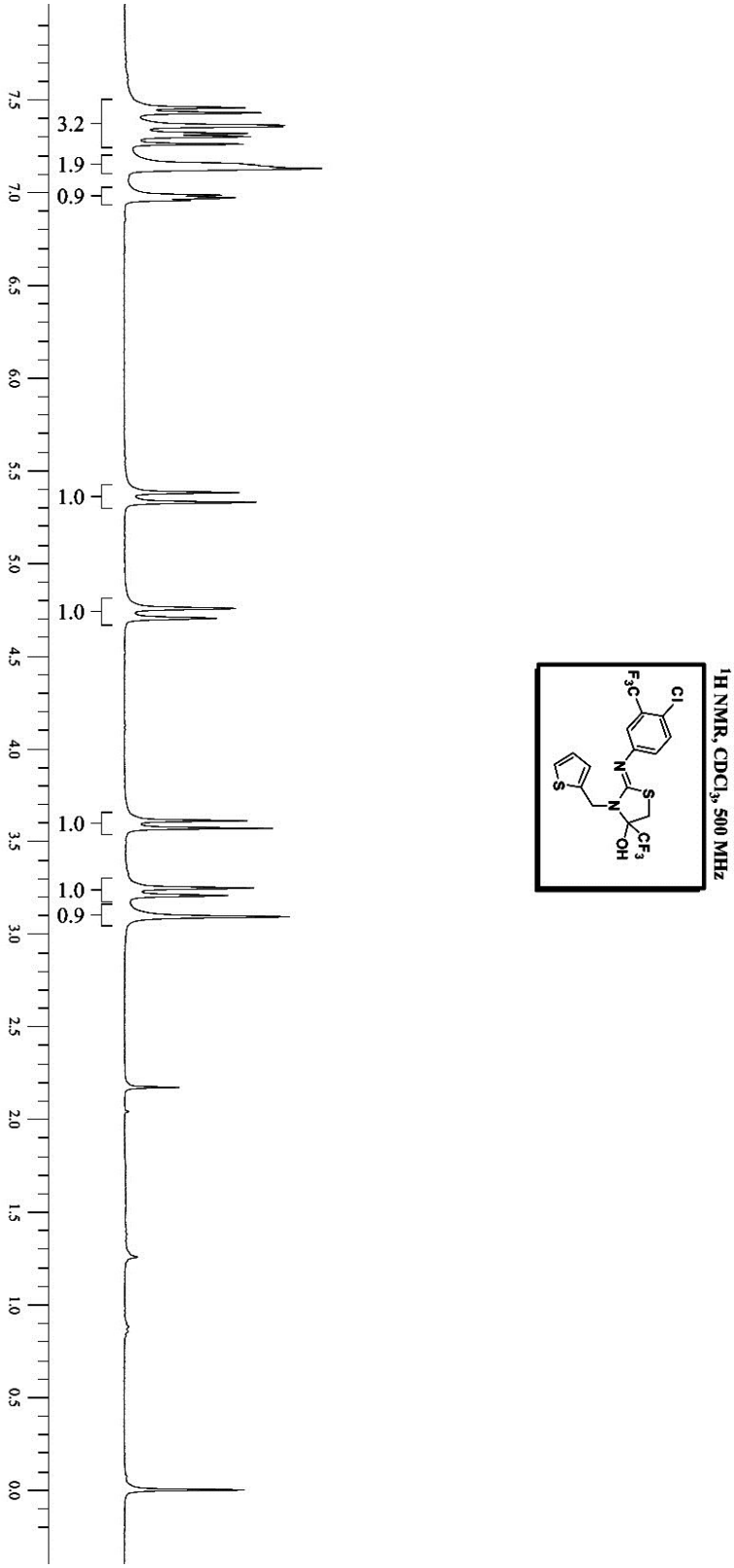
^{1}H NMR spectra of 8s



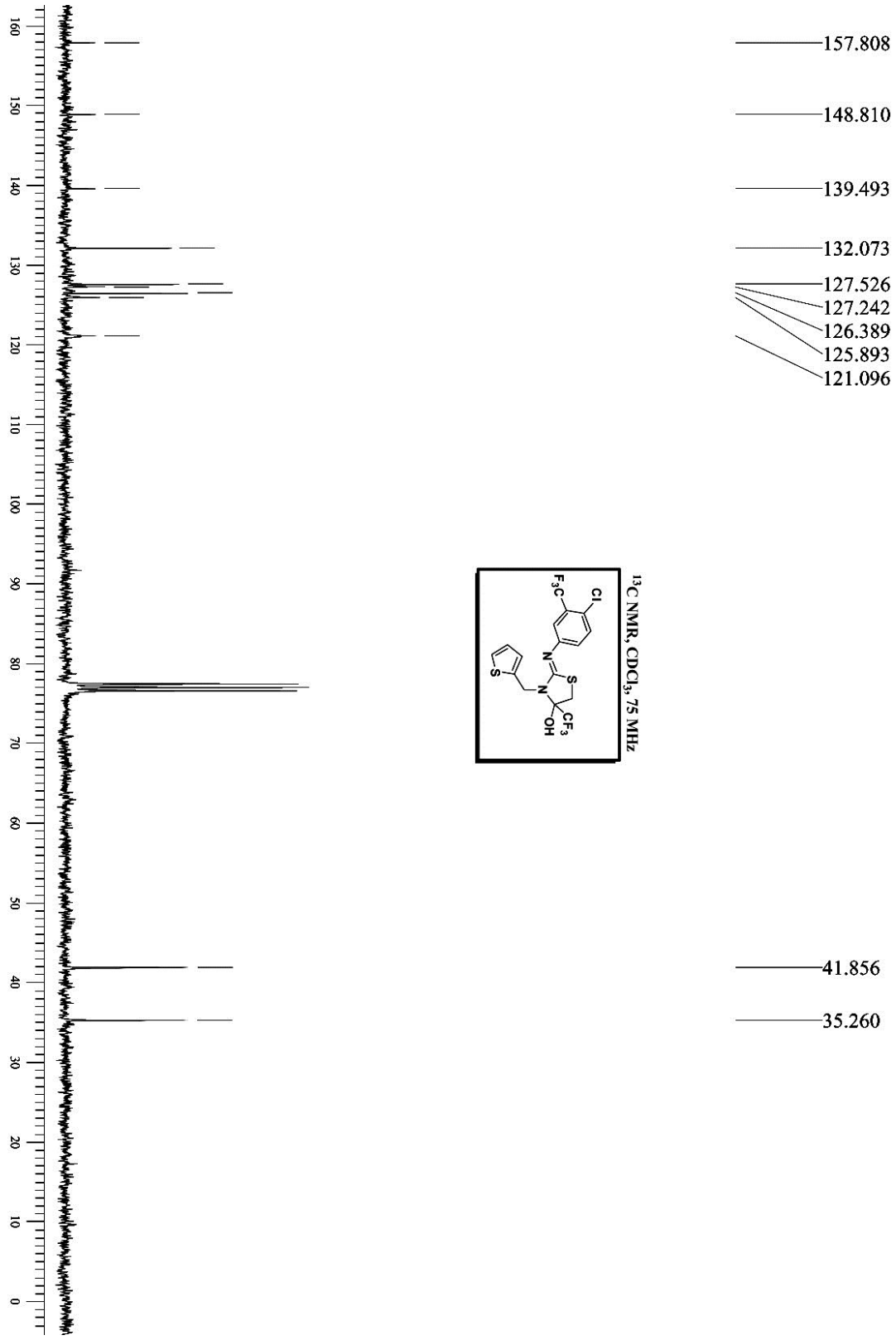
^{13}C NMR spectra of 8s



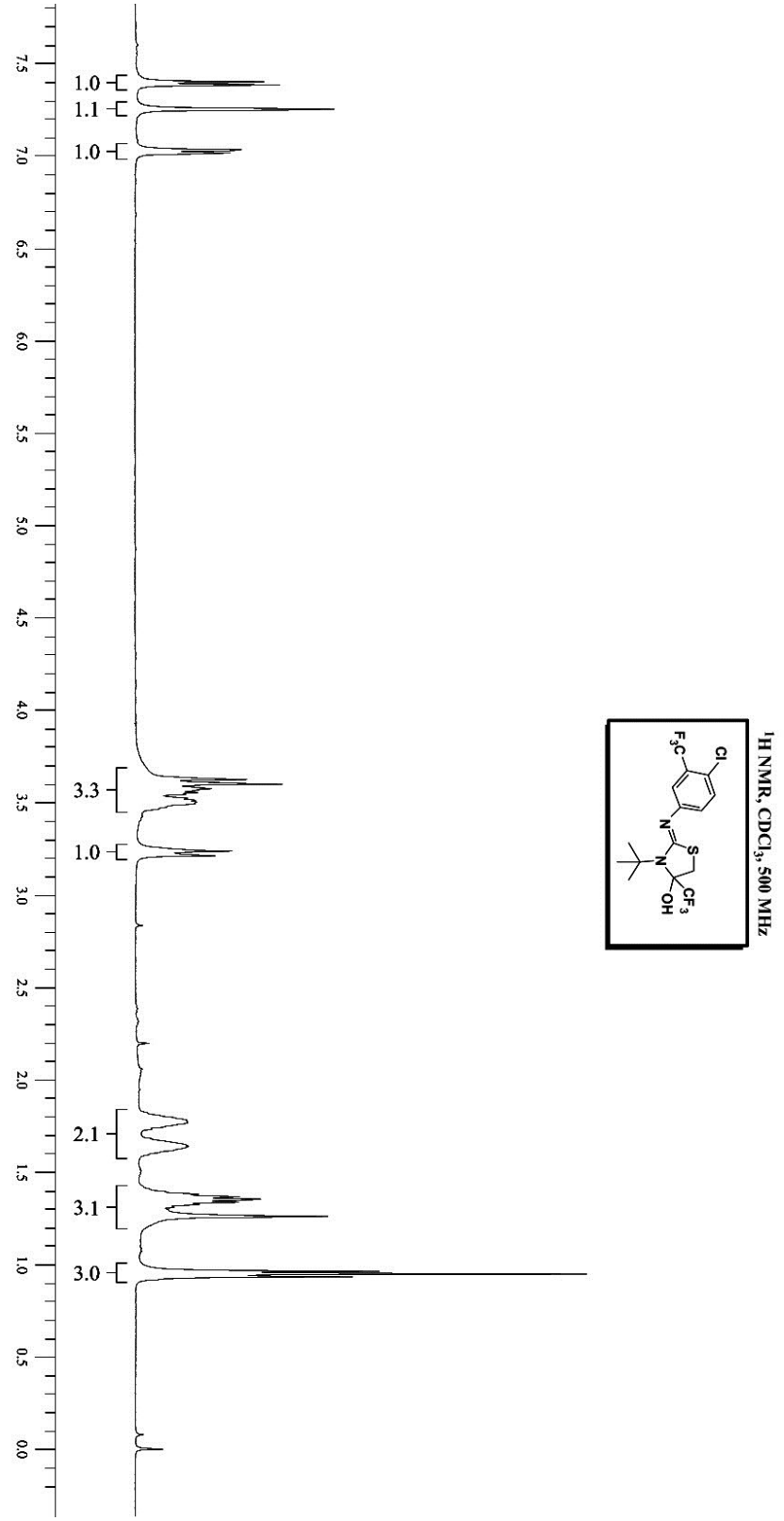
^{1}H NMR spectra of 8t



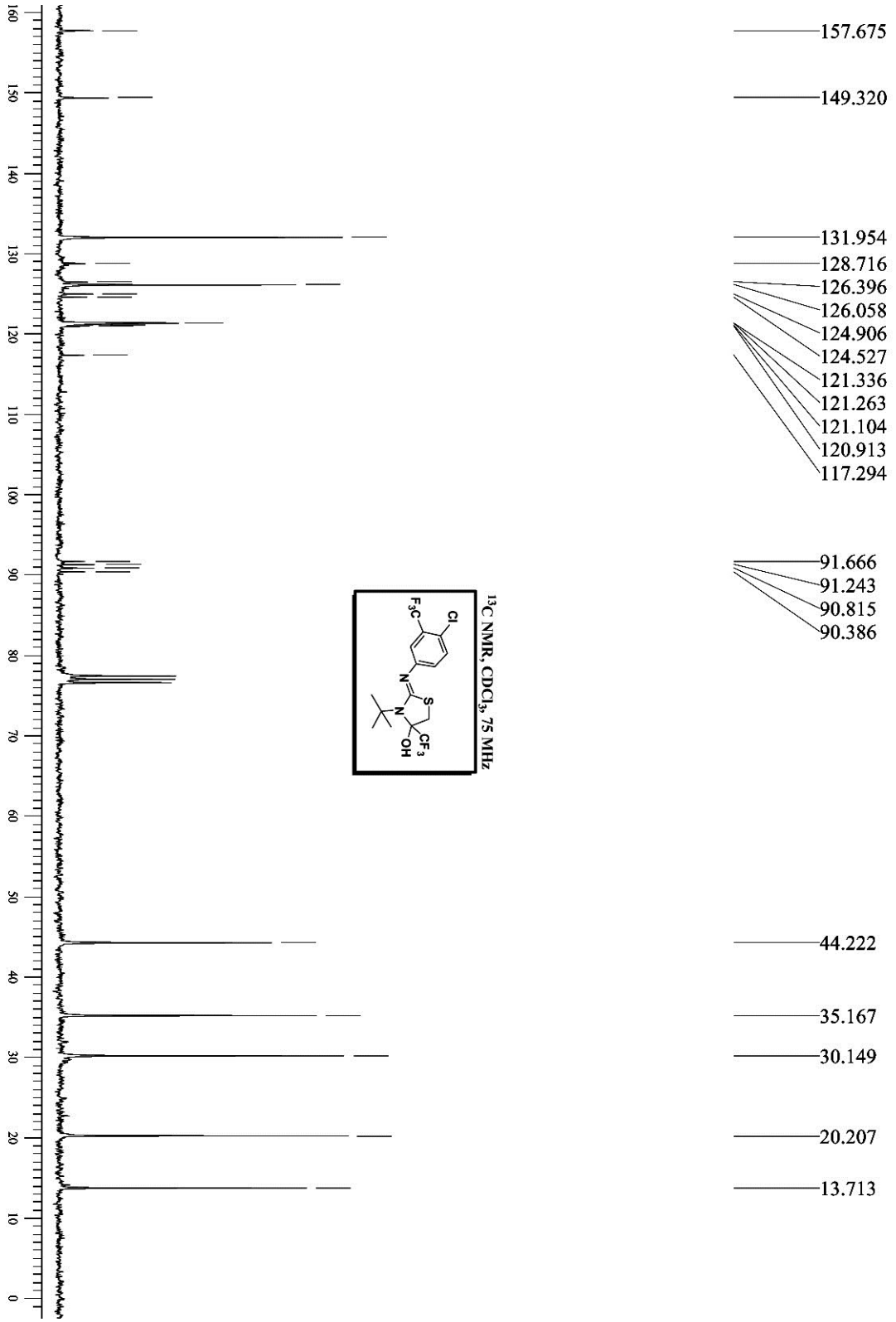
^{13}C NMR spectra of 8t



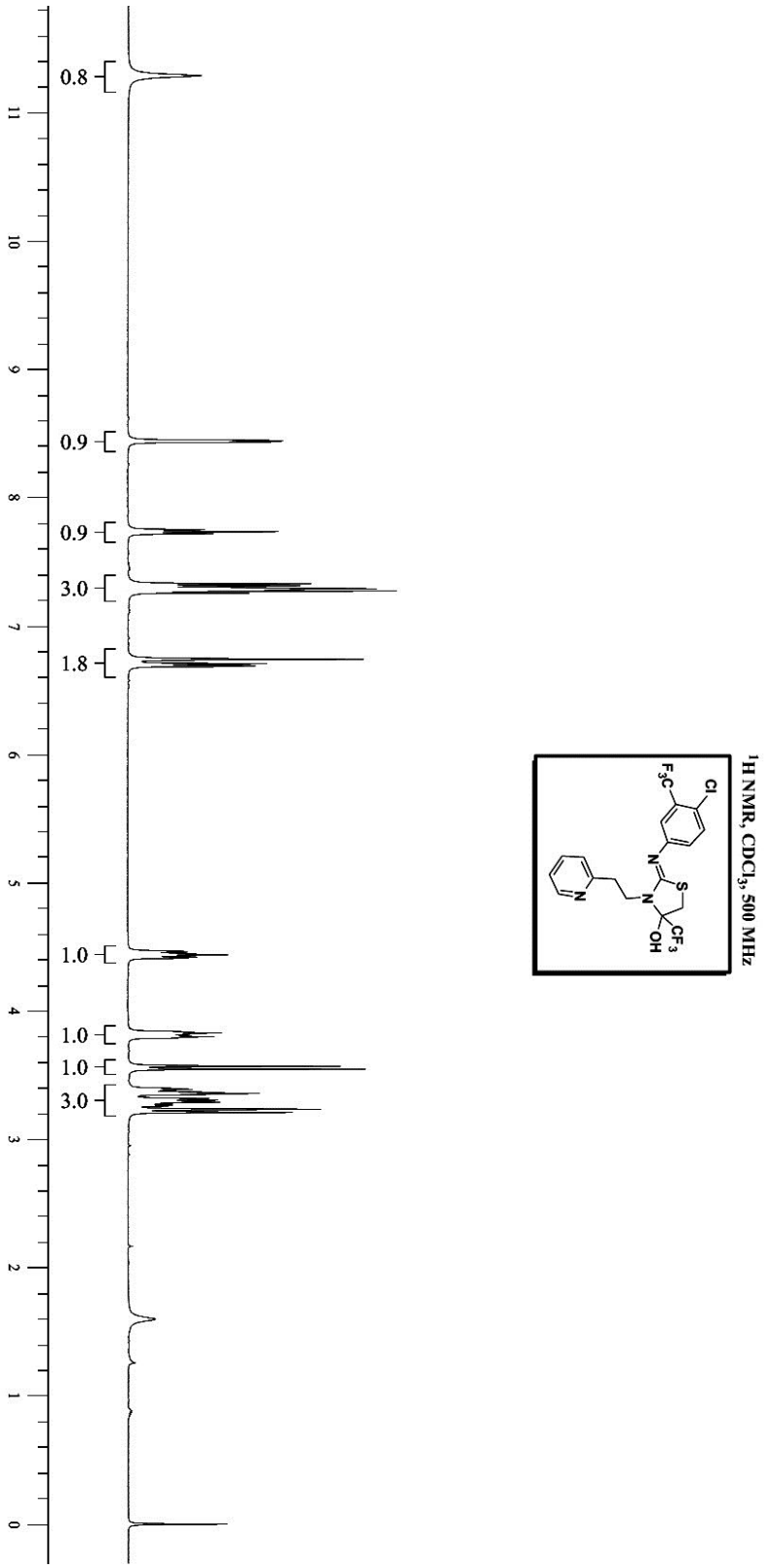
^{1}H NMR spectra of 8u



^{13}C NMR spectra of 8u



^{1}H NMR spectra of 8v



^{13}C NMR spectra of 8v