Supporting Information

3-Hydroxypyridin-2-thione as Novel Zinc Binding Group for Selective Histone Deacetylase Inhibition

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[+] These authors contributed equally to the manuscript.

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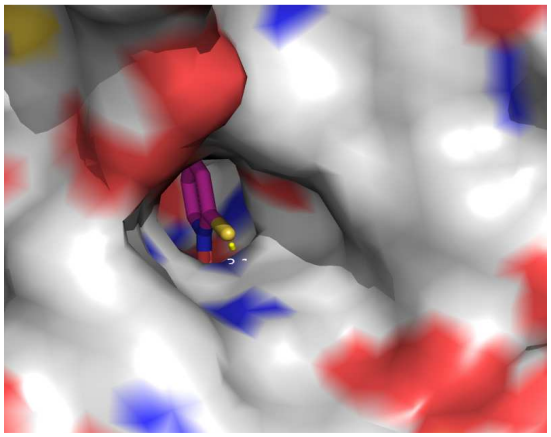

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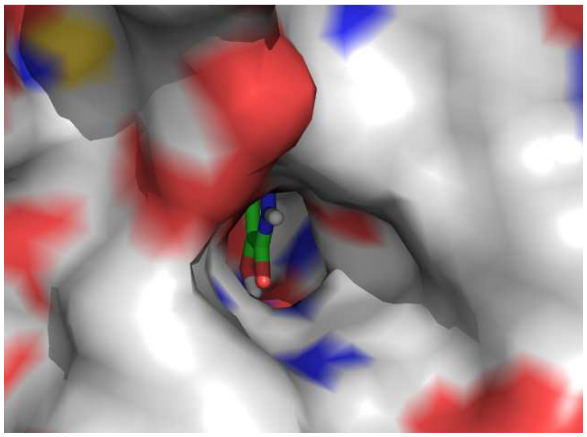

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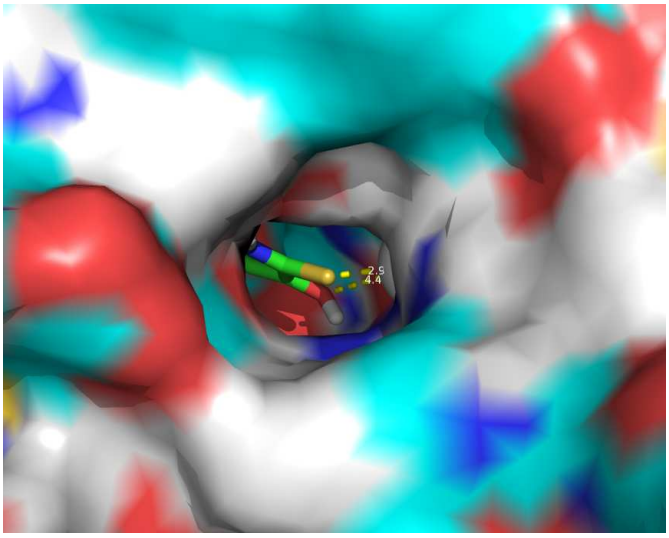

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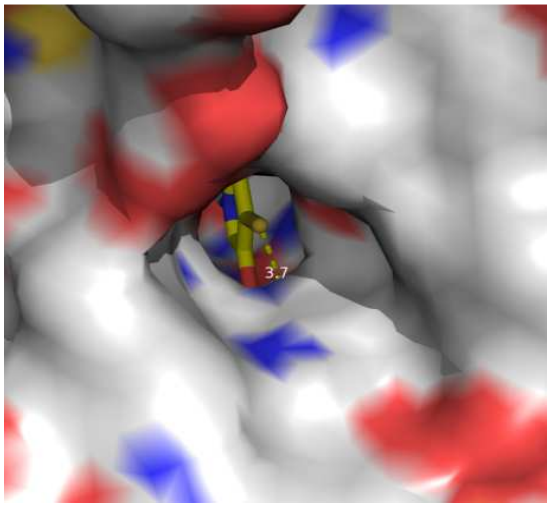

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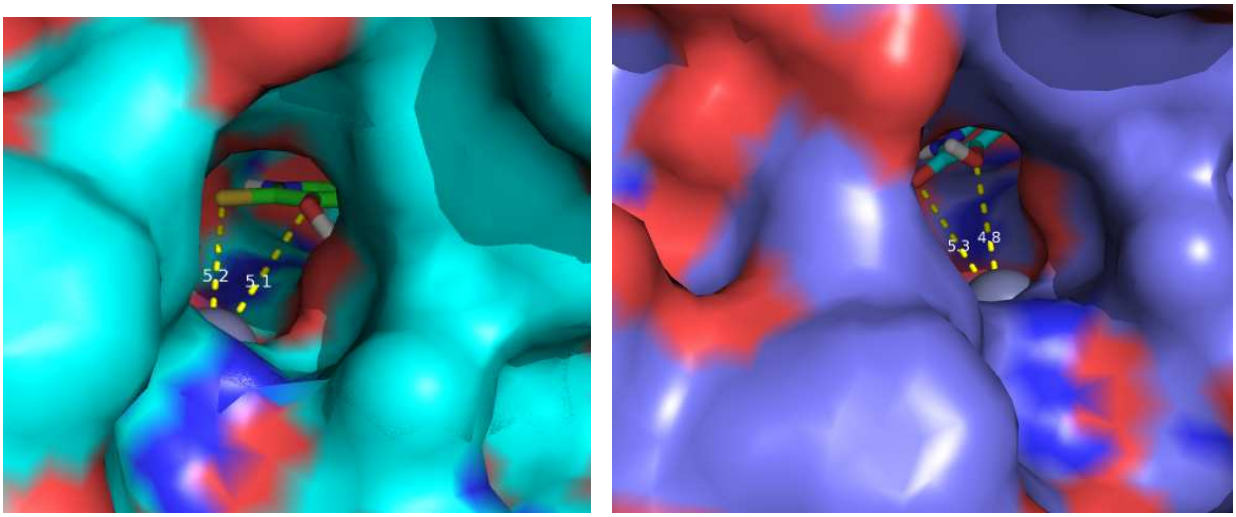

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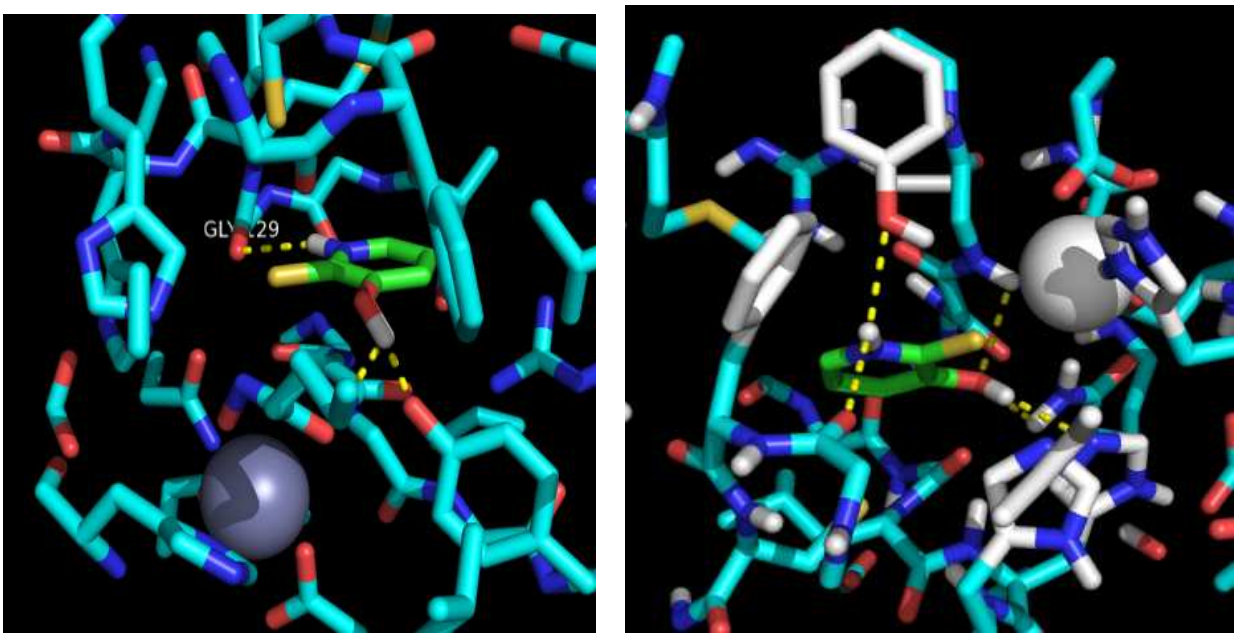


i) ii)

Figure S1. A) HDAC 1 homology model docking. (i) Zn^{2+} ion chelation by 1-hydroxypyridin-2-thione; (ii) N1 of 3-hydroxypyridin-2-one (3HP) pointing towards the outside of the channel; (iii) positioning of the N1 of 3-hydroxypyridin-2-thione (3HPT) towards the outside of the channel; (iv) N1 of 3-hydroxypyridin-4-thione position toward the base of the channel. **B) Docking of 3HPT and 3HP with HDLP.** (i) N1 of 3HPT points towards the base of the channel; ii) N1 of 3HP is orientated towards the base of the channel; **C) Interactions of 3HPT at the active site of HDLP and HDAC 1 homology model.** (i) In HDLP, N1 of 3HPT H-bonding with the C=O of GLY 129 thus orienting N1 towards the

base of the channel; ii) In HDAC1 homology model, N1 of 3HPT possibly form H-bonds with TYR303 phenol group and GLY149 backbone leading to an outwards orientation.

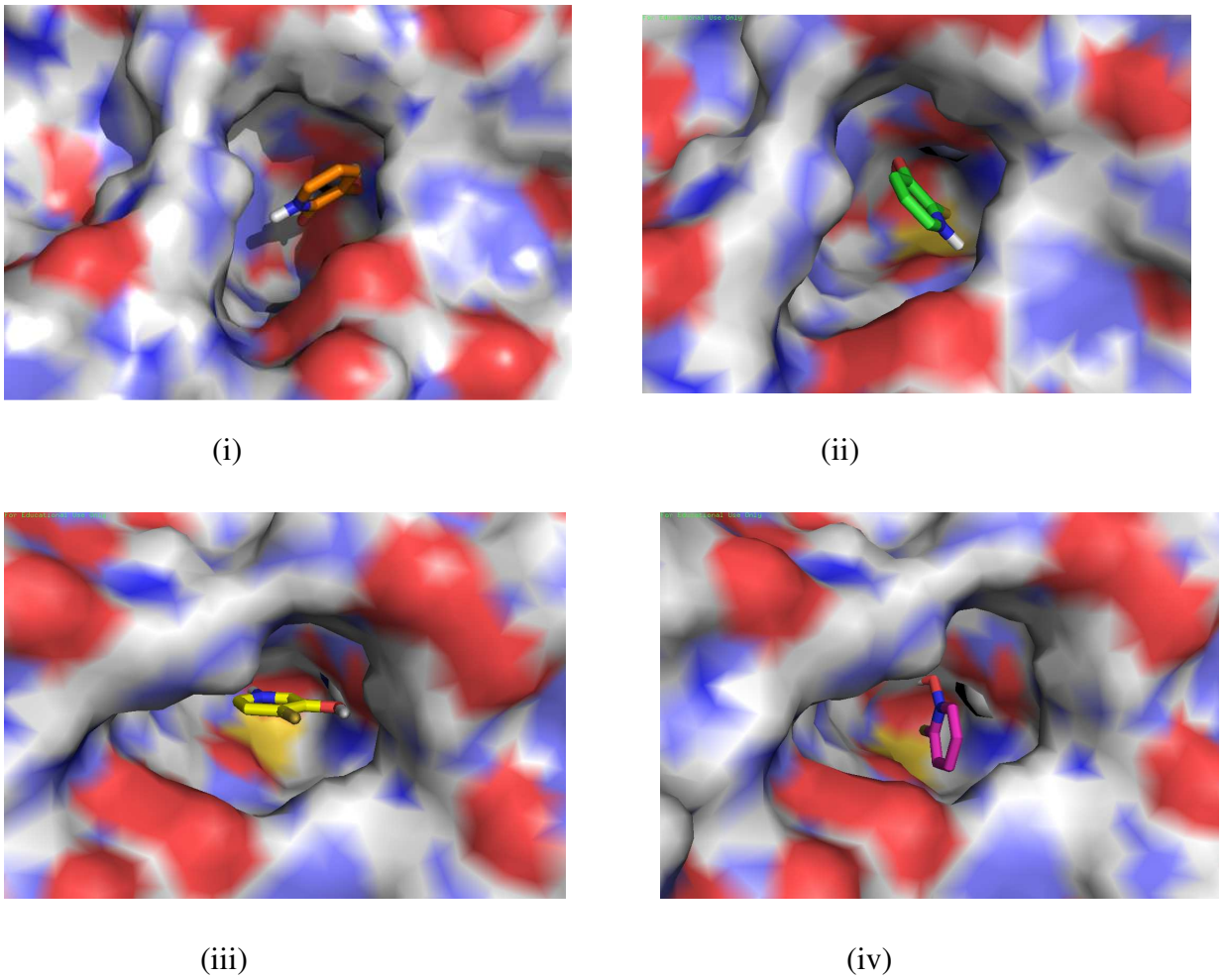
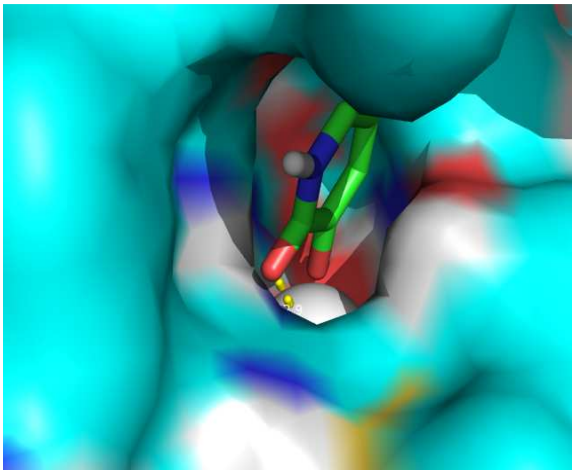


Figure S2. HDAC 6 homology model docking (i) N-1 of 3-hydroxypyridin-2-one oriented towards the surface of the channel; (ii) N-1of 3-hydroxypyridin-2-thione oriented towards the surface of the channel; (iii) N-1 of 3-hydroxypyridin-4-thione oriented towards the base of the channel; (iv) Zn^{2+} chelation by 1-hydroxypyridin-2-thione.



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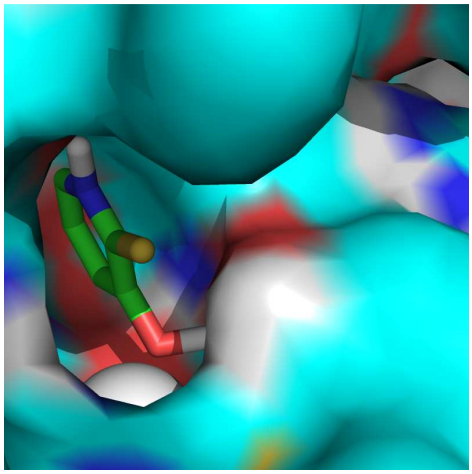

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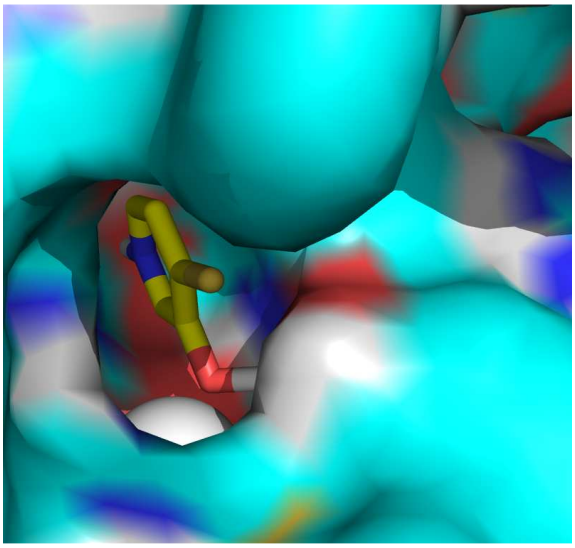

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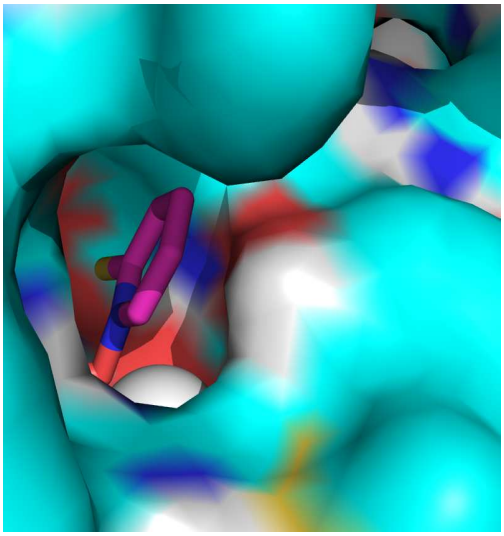
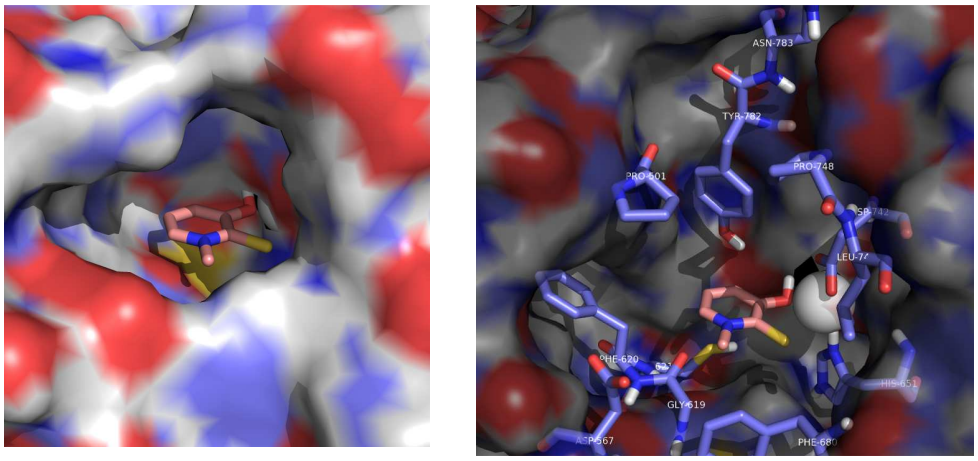


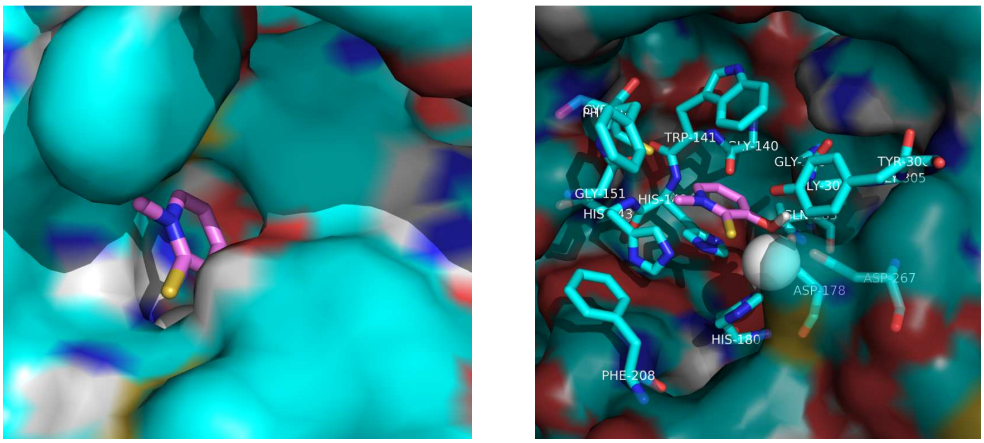
(iv)

Figure S3: HDAC 8 docking (i) N-1 of 3-hydroxypyridin-2-one oriented towards the surface of the channel; (ii) N-1of 3-hydroxypyridin-2-thione oriented towards the surface of the channel; (iii) N-1 of 3-hydroxypyridin-4-thione oriented towards the base of the channel; (iv) Zn^{2+} chelation by 1-hydroxypyridin-2-thione.



(a) (b)

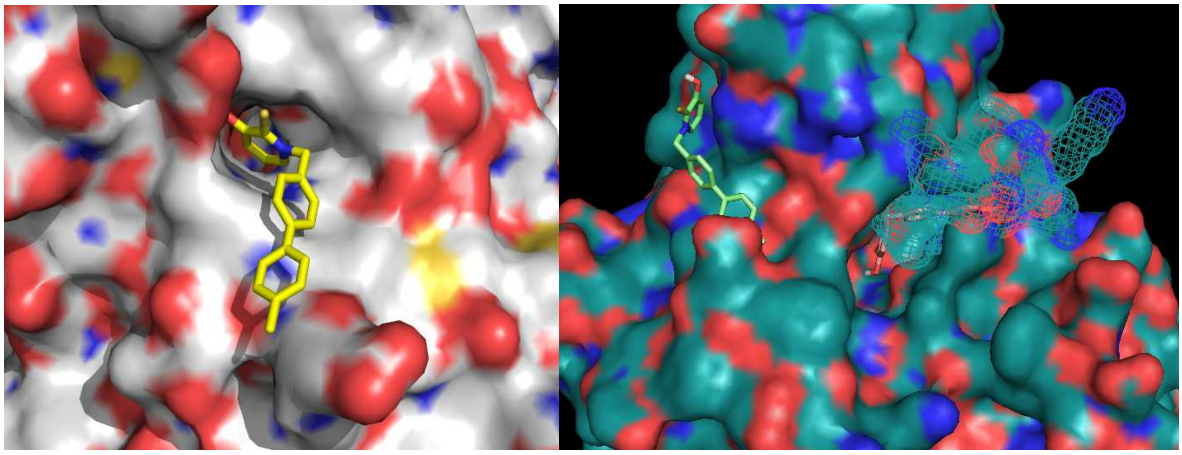
Figure S4: Docking of N-methyl-3-hydroxypyrydin-2-thione against HDAC6 homology model; (a) The N-methyl points towards the surface of the channel; (b) Amino acid residues in a 5 Å diameter surrounding the N-methyl-3-hydroxypyridin-2-thione fragment.



(a) (b)

Figure S5: Docking of N-methyl-3-hydroxypyrydin-2-thione against HDAC8; (a) N-methyl oriented towards the surface of the HDAC channel; (b) Amino acid residues surrounding the docked fragment (within 5 Å).

Methylation of the N-1 position of 3-HPT did not alter its docking at the active sites of both HDAC6 and 8, notably; the N-methyl is still pointing towards the surface of the channel (Figure S4 and S5). We analyzed amino acid residues surrounding the docked structure of N-methyl-3-hydroxypyridin-2-thione to further refine our design of the linker region. The tunnel which connects the active site of HDAC6 to its surface consists of several hydrophobic amino acid (AA) residues including Leu74, Pro501, Gly619, Phe620, Phe680, Pro748, and Tyr782. Moreover, some of the hydrophobic aromatic residues (Phe620, Phe680, and Tyr782) are placed in close proximity of *N*-methyl of N-methyl-3-hydroxypyridin-2-thione (Figure S4b). Similarly, hydrophobic AA lining the channel of HDAC8 such as Phe152, Phe208, Tyr306 are also positioned in proximity of N-methyl group (Figure S5b).



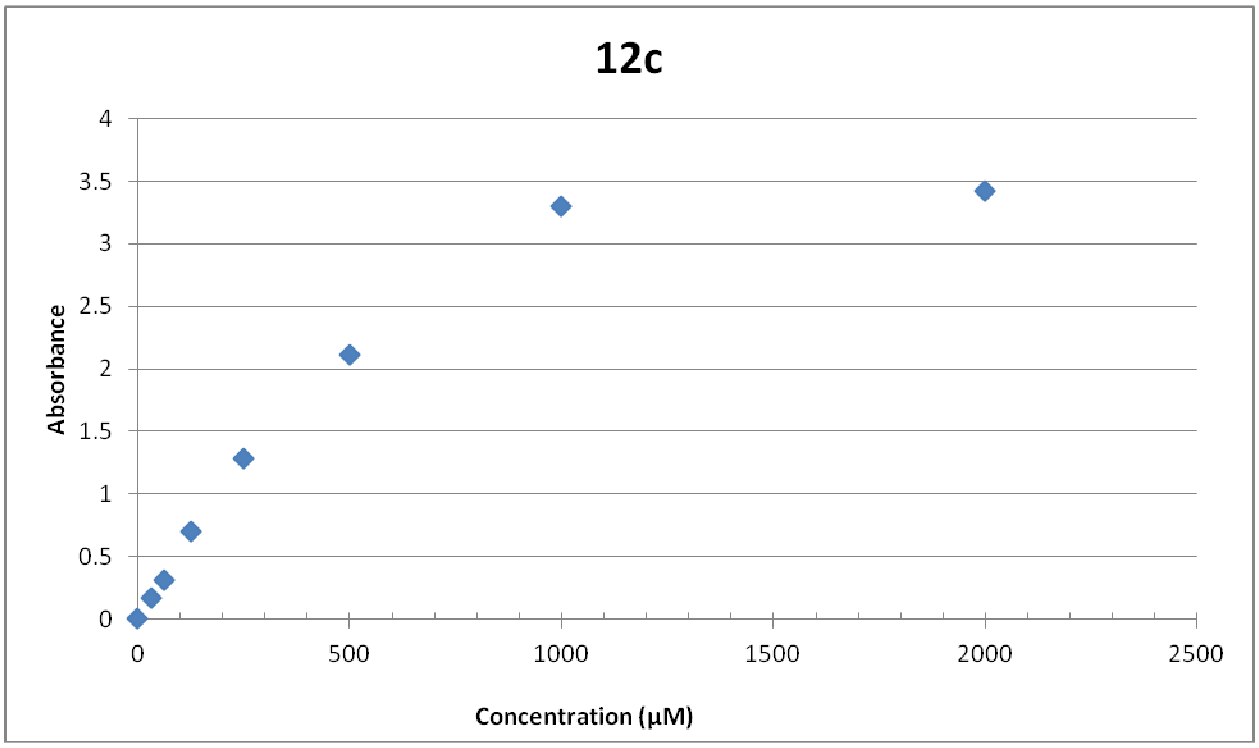
i) ii)

Figure S6:Molecular Docking of 12d. i) Homology model of human HDAC1. ii) HDLP

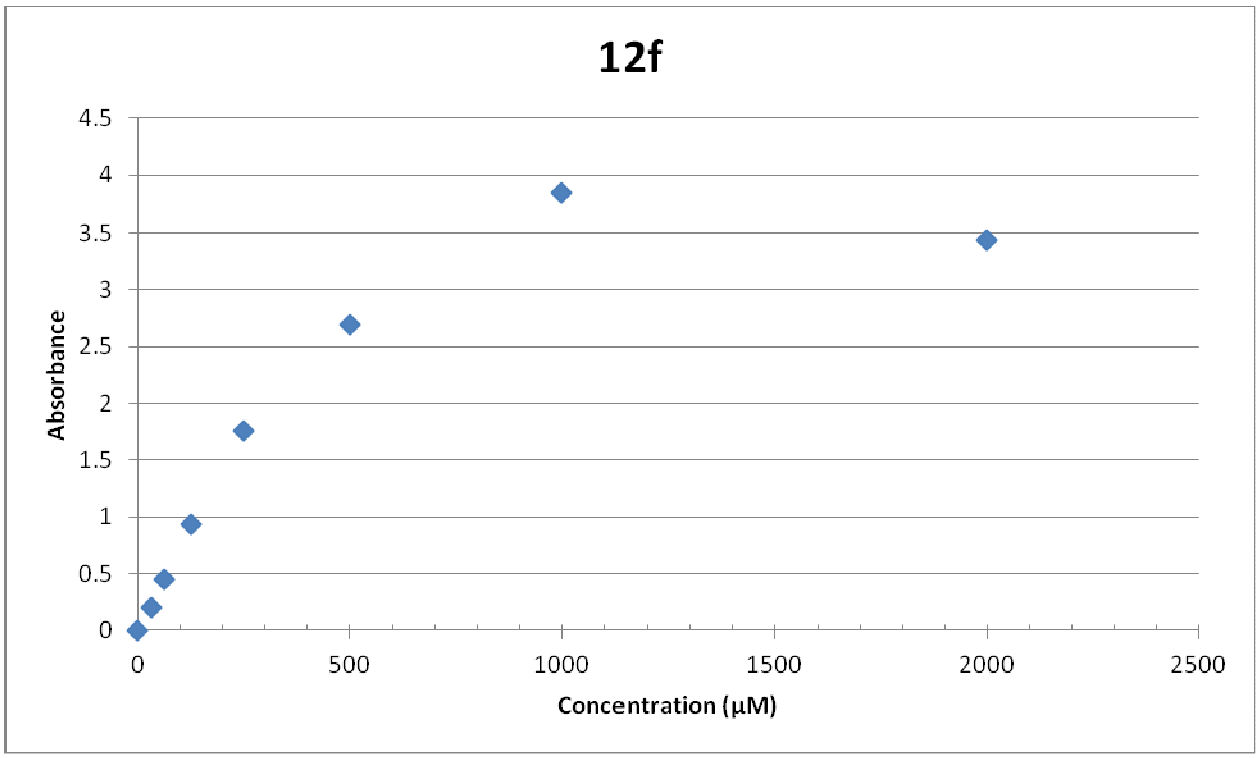
This representative compound is incapable of entering HDAC 1 pocket to chelate the zinc at the active site, instead it is bound to the residues at the surface of the binding pocket. With HDLP, **12d** binds to mutiple spots on HDLP, however none of its conformation is able to present the 3HPT ZBG for chelation to the active site zinc ion. These observations may explain the lack of activity of this class of compounds against HDAC1.

Solubility Measurement.

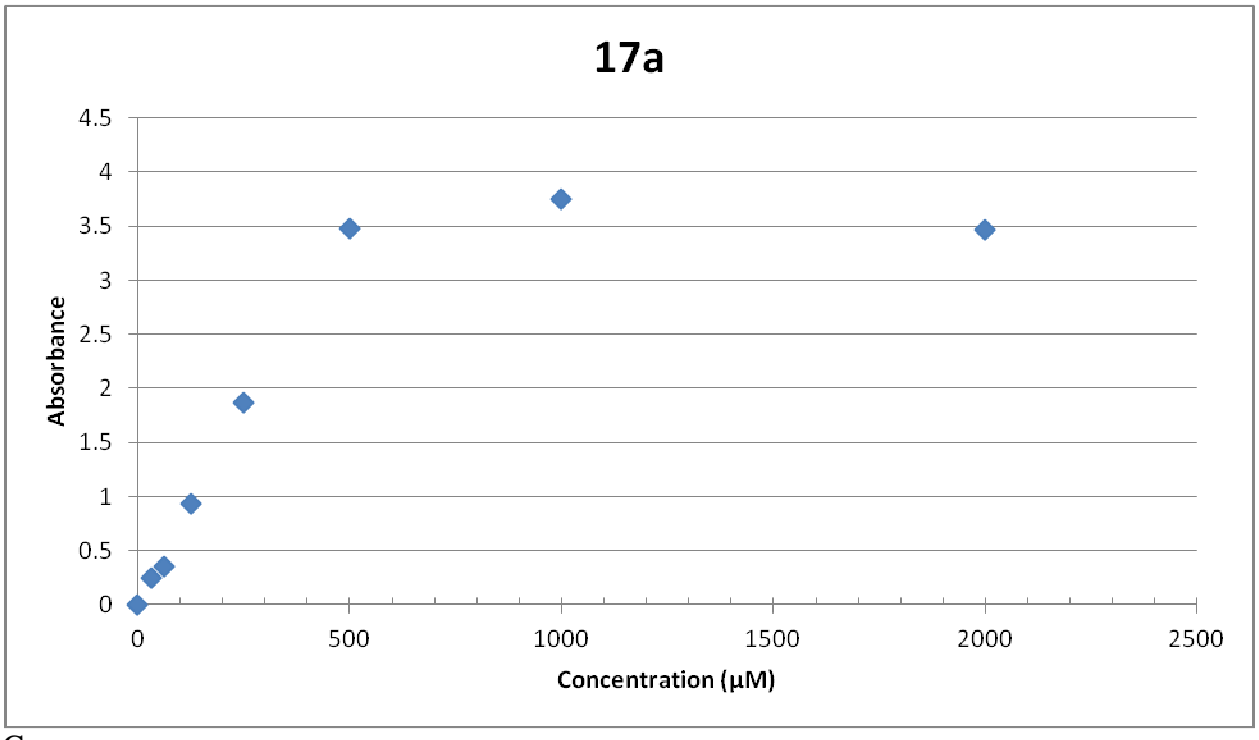
This experiment was performed according to reported protocol using a 10 mM stock solution of the test compound in DMSO.[1] DMSO was kept at 5% of the final volume. Following incubation at room temperature and centrifugation to remove precipitate, absorbance was measured using a UV-vis spectrophotometer (Varian, Palo Alto, CA). Data are presented in Figure S7 and Table S1 below:



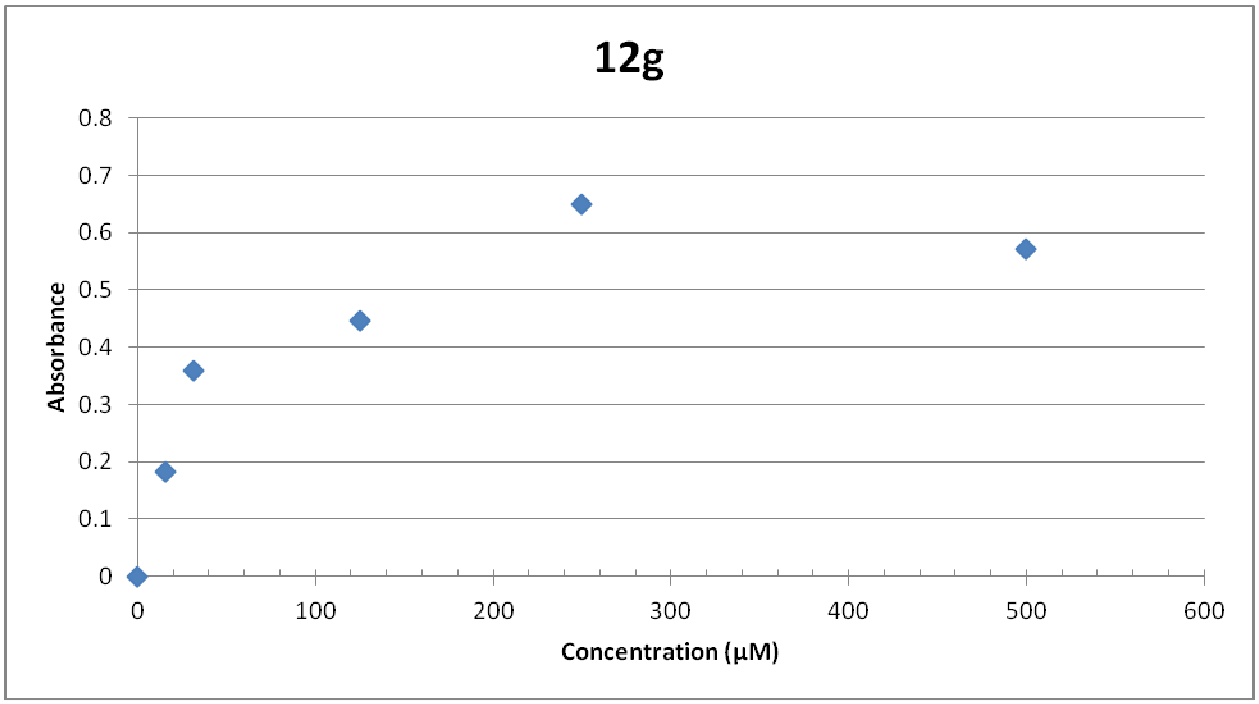
A


12f
Absorbance
Concentration (µM)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
500
1000
1500
2000
2500

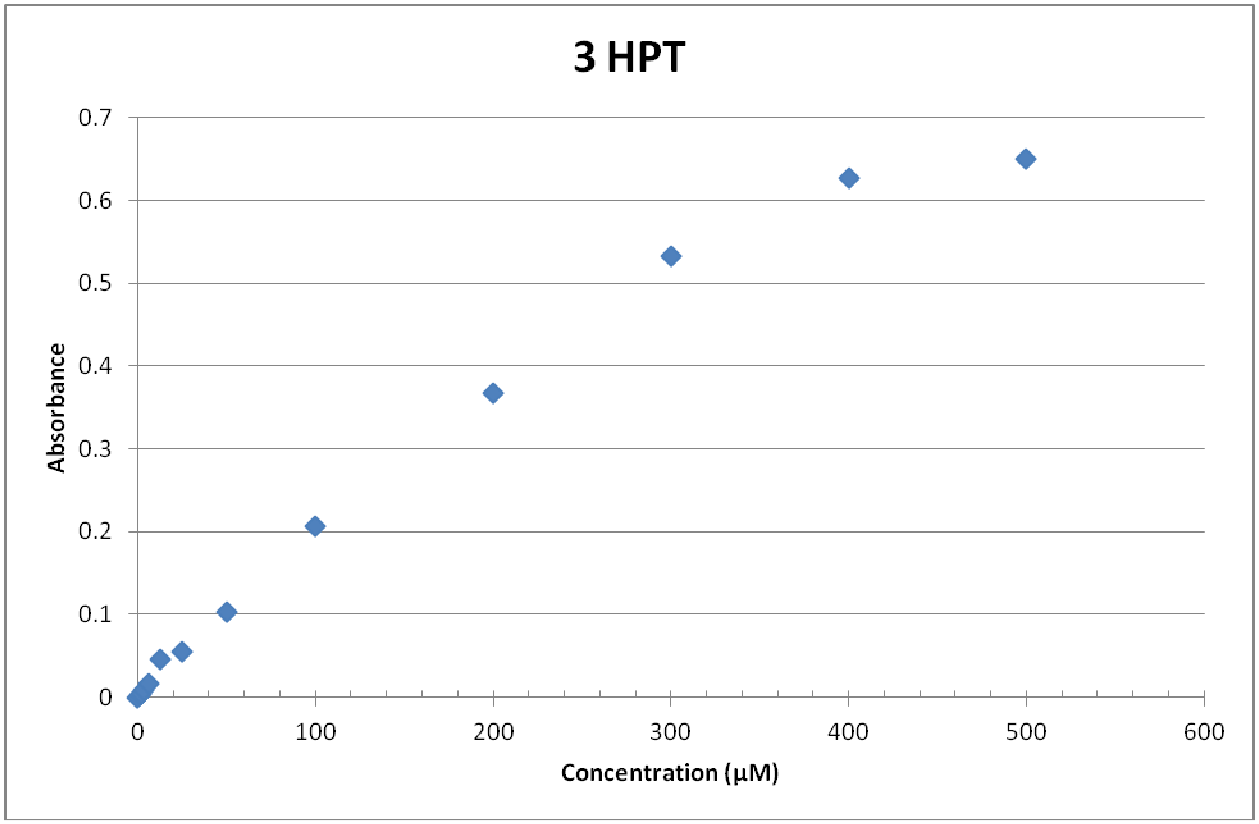
B


17a
Absorbance
Concentration (µM)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
500
1000
1500
2000
2500

C


12g
Absorbance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
Concentration (µM)

D


3 HPT
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
Concentration (µM)

E

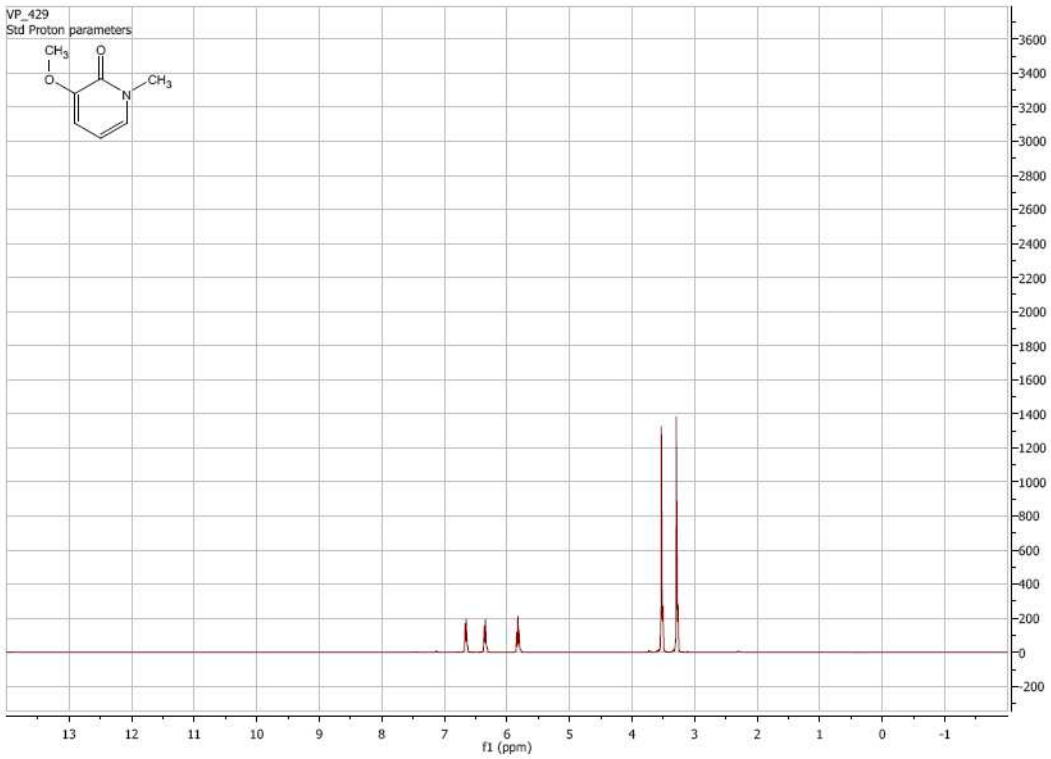
Figure S7: Absorbance vs concentration of compounds. The solubility of each compound is estimated to be the concentration at which saturation was reached (plateau). λ_{max} at which absorbance was measured: A) 365nm, B) 380nm, C) 350nm, D) 375nm, E) 345nm.

Table S1: Aqueous solubility of the tested compounds.

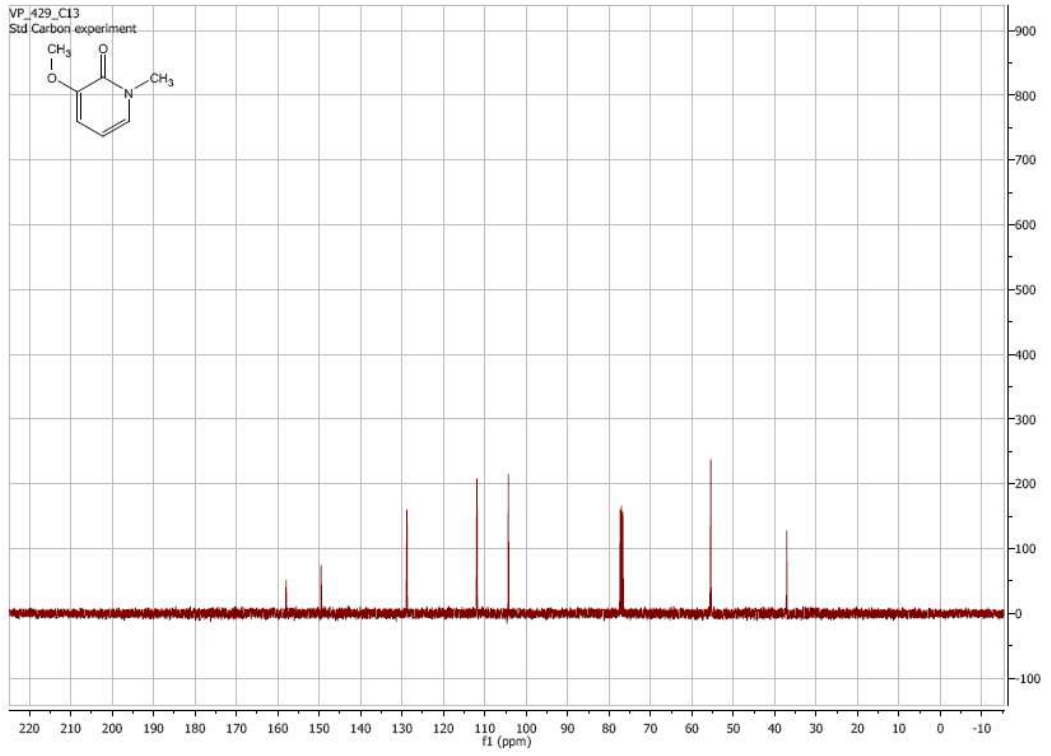
Compound	Estimated solubility (µg/mL)
3HPT	**46**
12g	**34**
17a	**142***
12f	**148***
12c	**172***

* 40 mM DMSO stock solution was made in order to achieve final concentrations above 500 µM (Figure S7- A, B and C).

^{1}H NMR of **4a:**



^{13}C NMR of **4a:**



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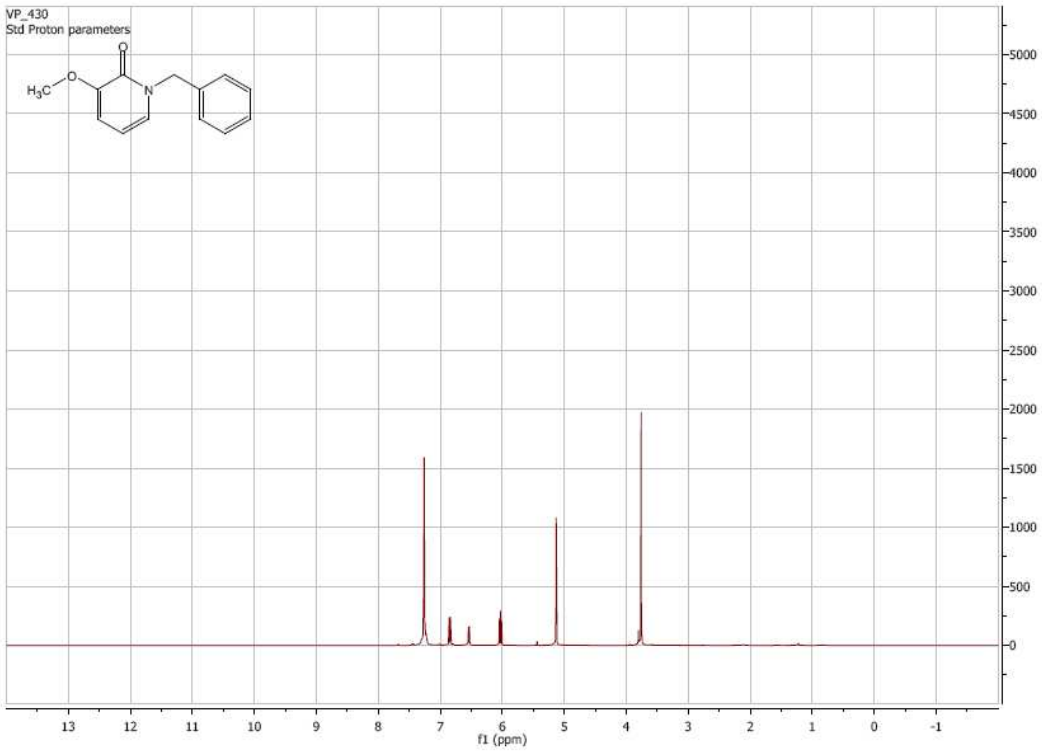

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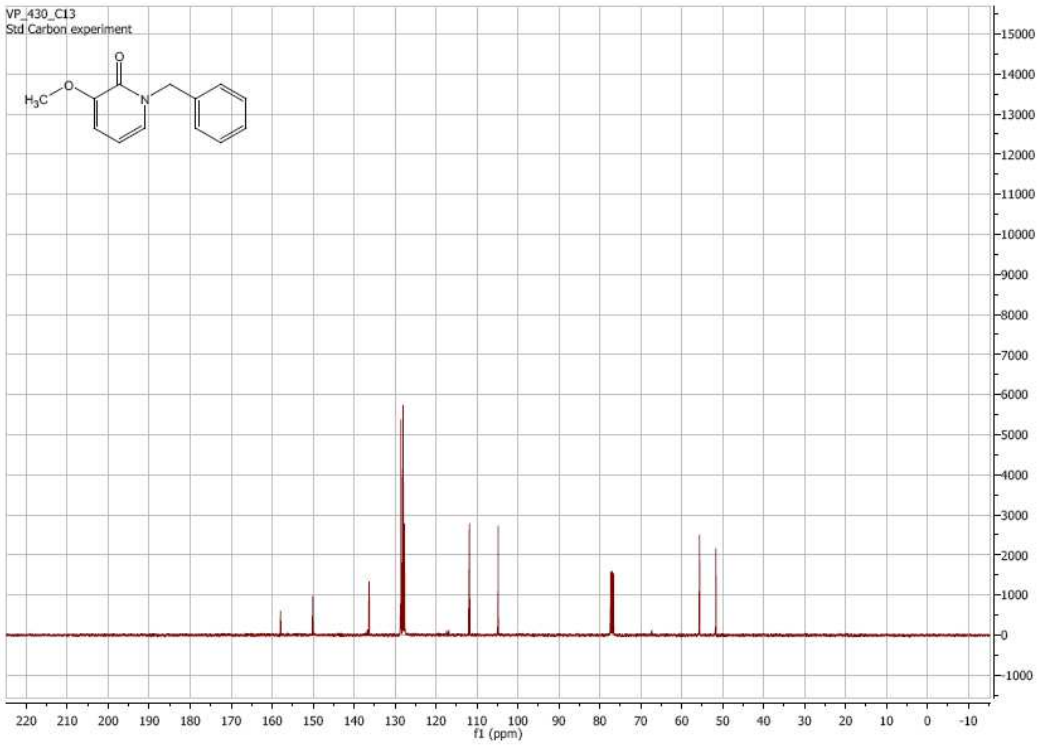
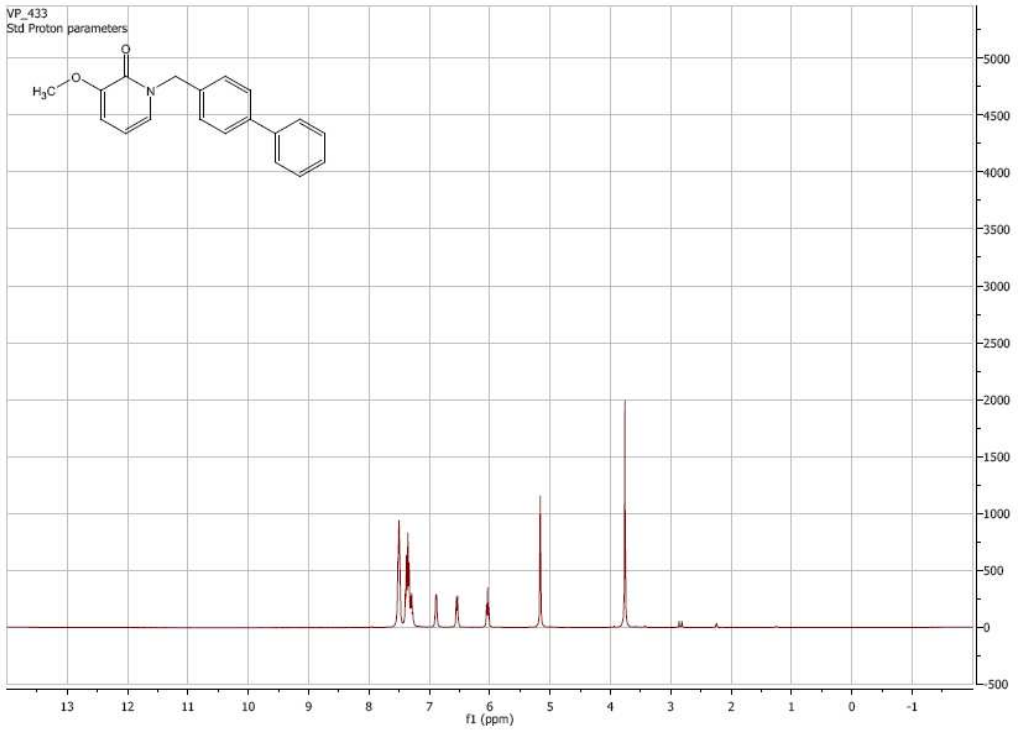


^{1}H NMR of **4c:**



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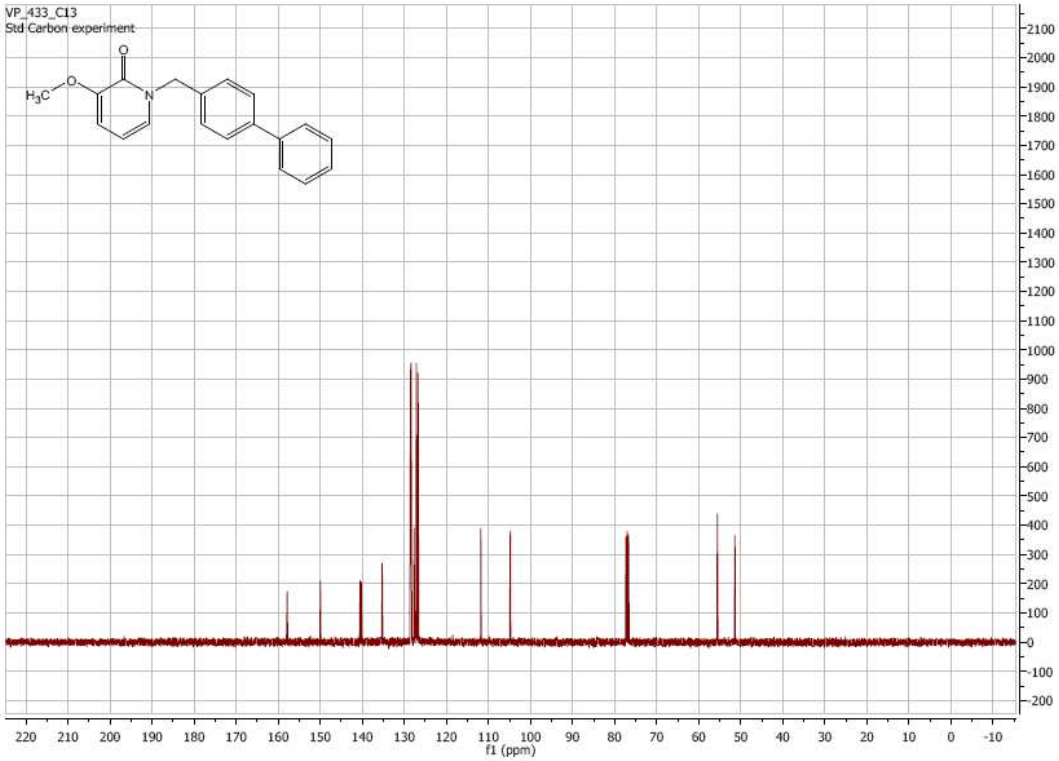

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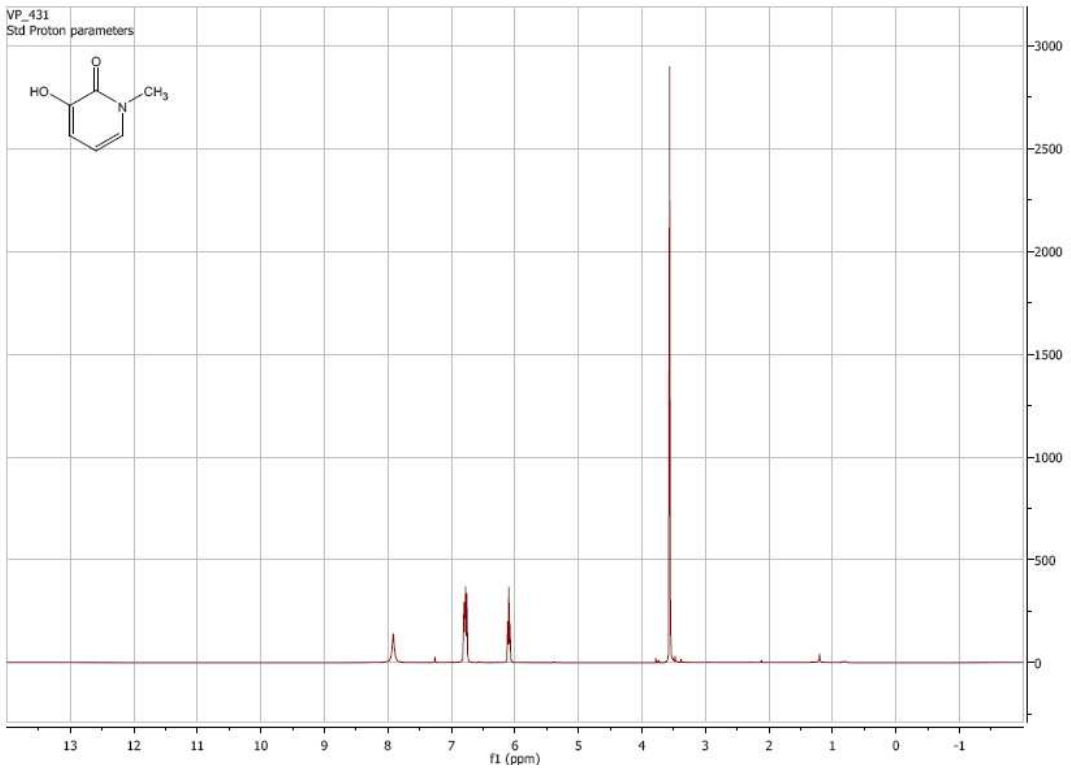

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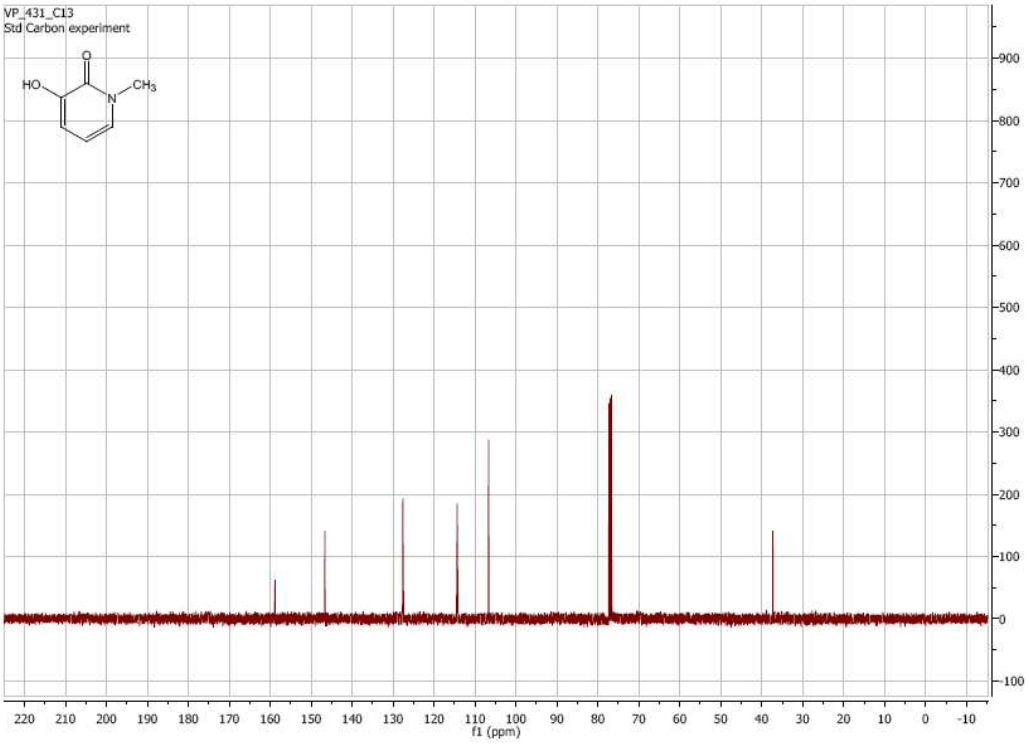

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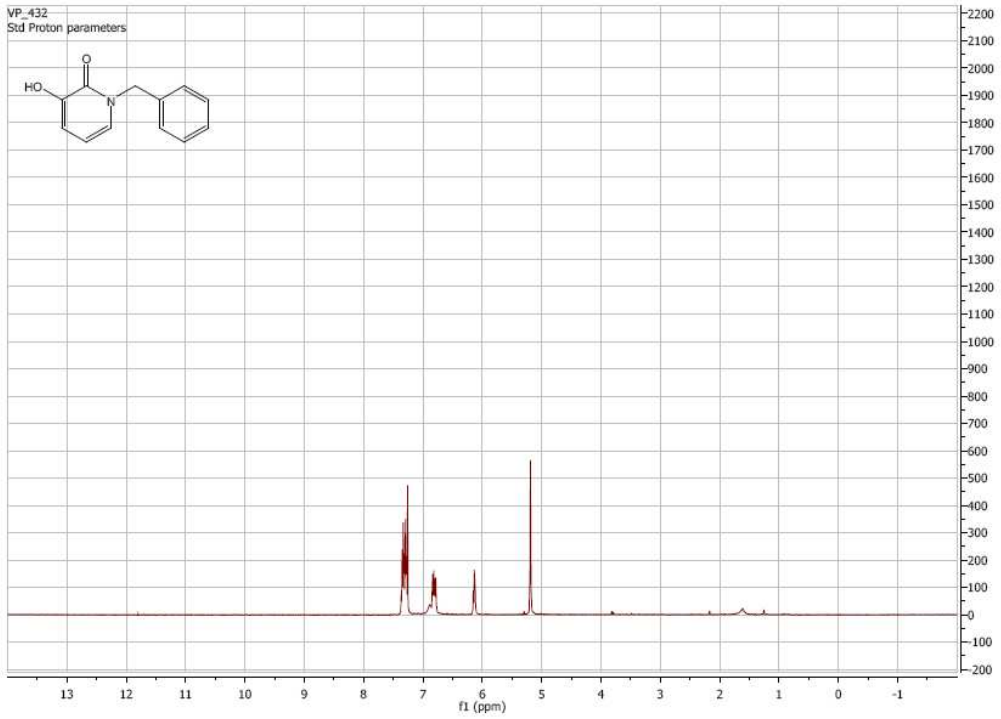

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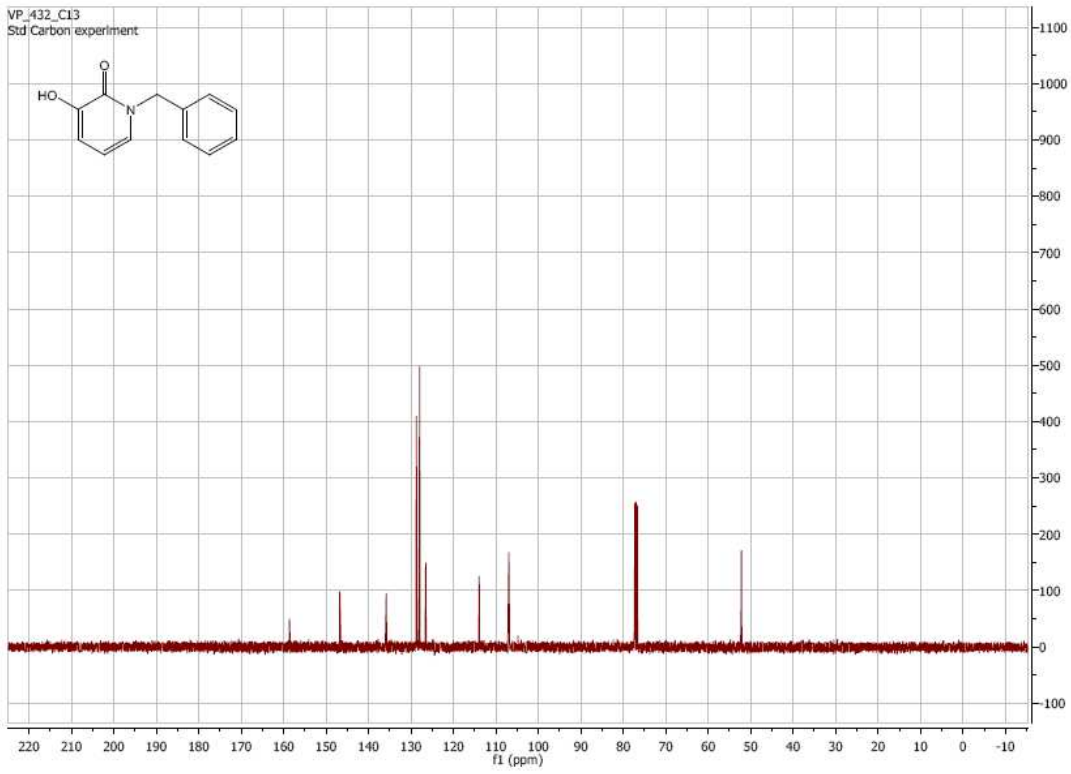

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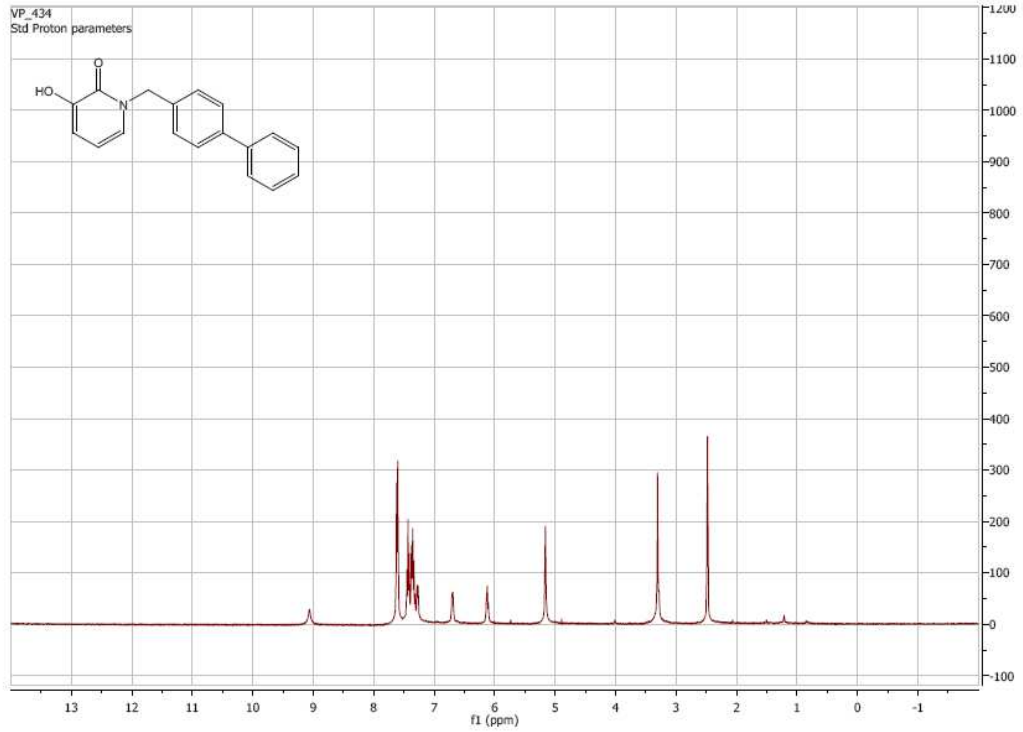

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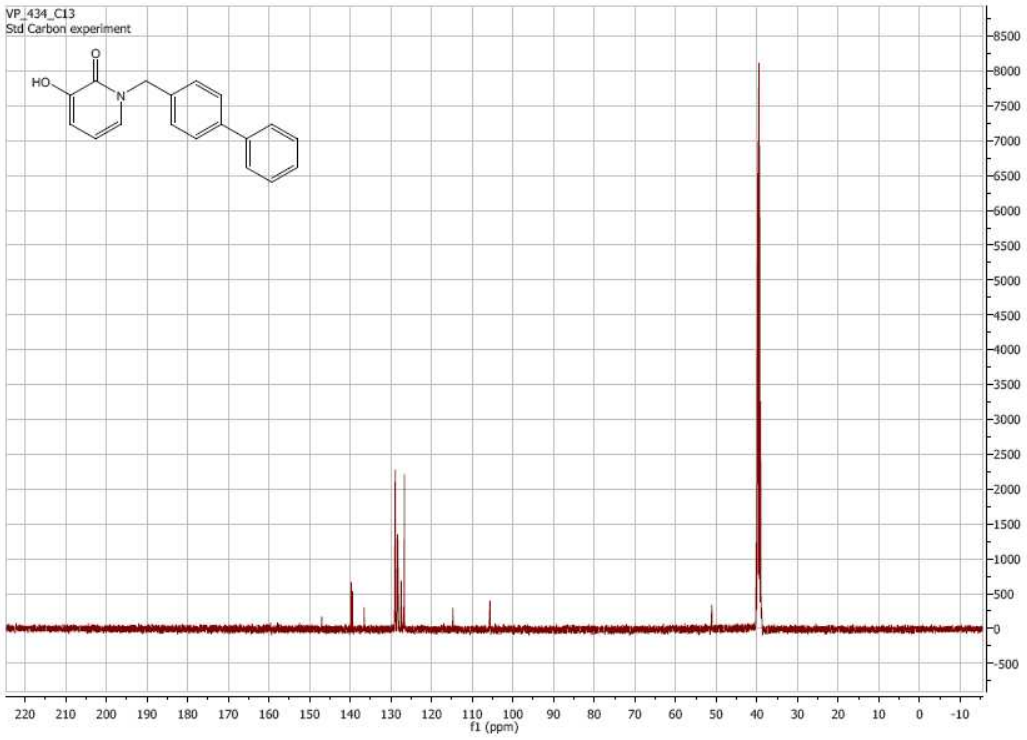

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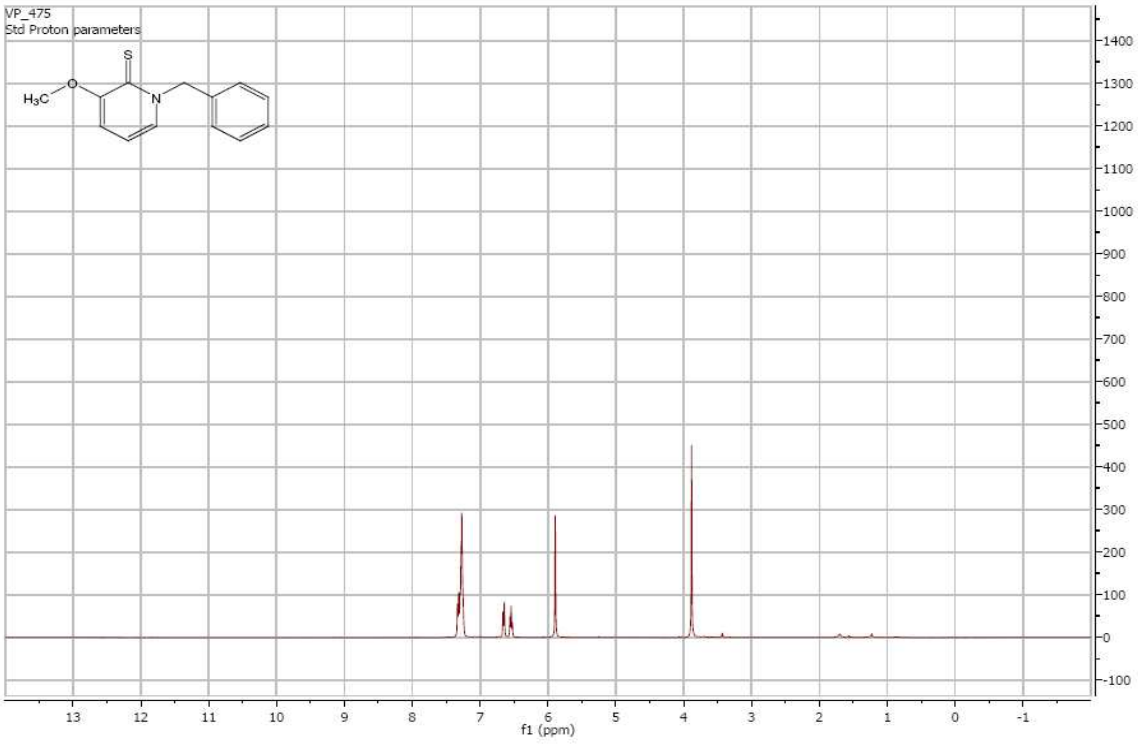

6b:

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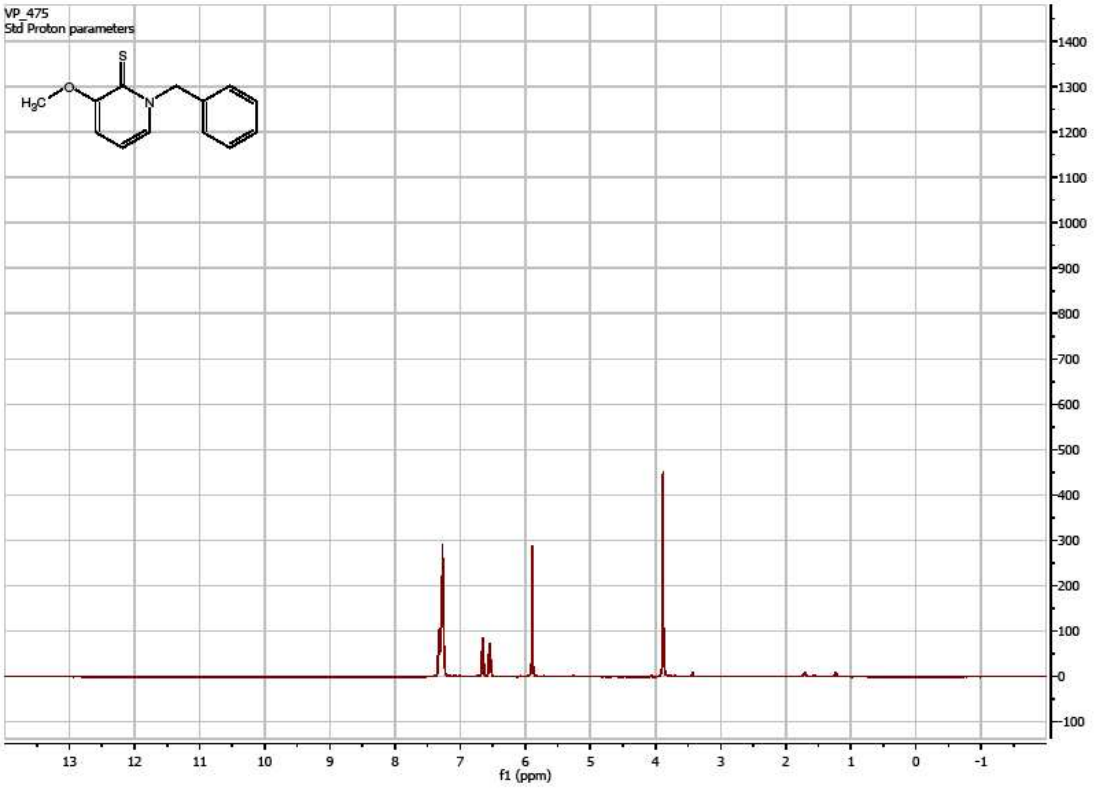

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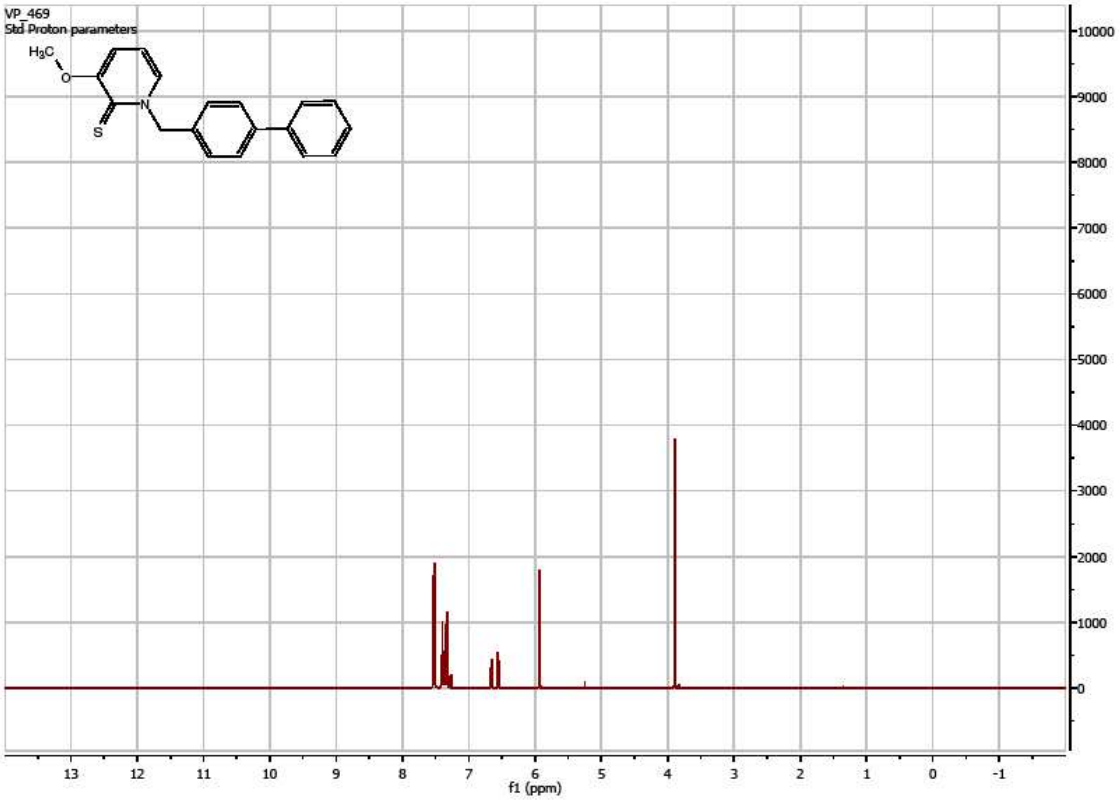

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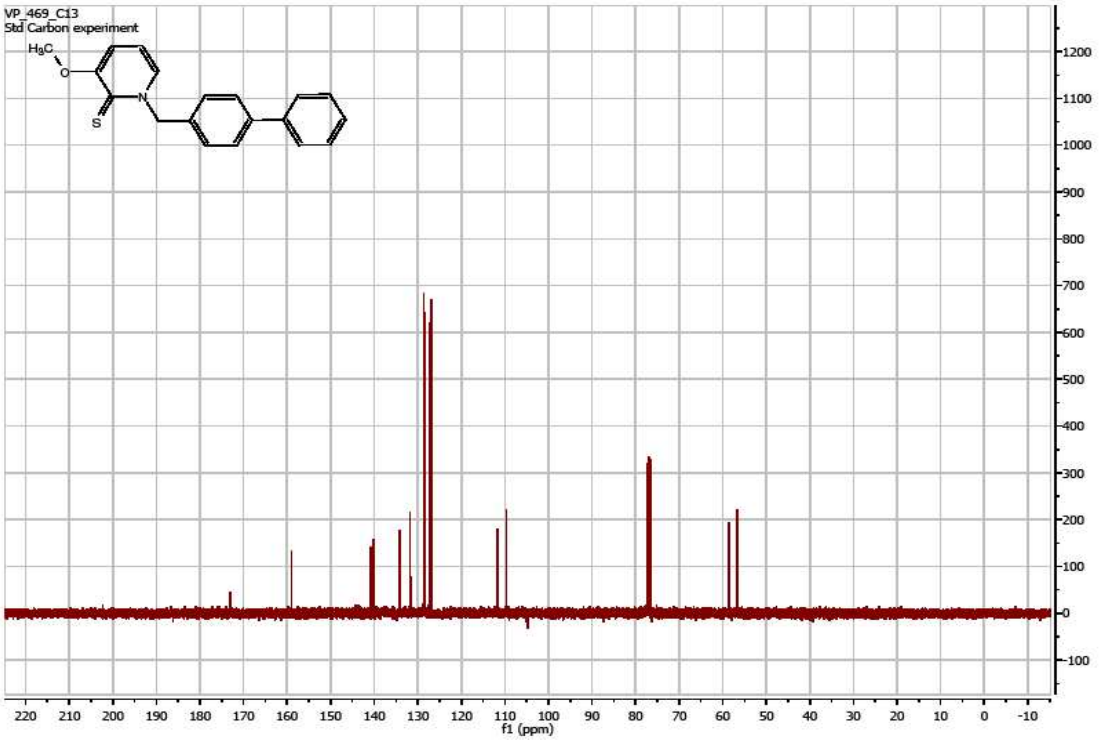

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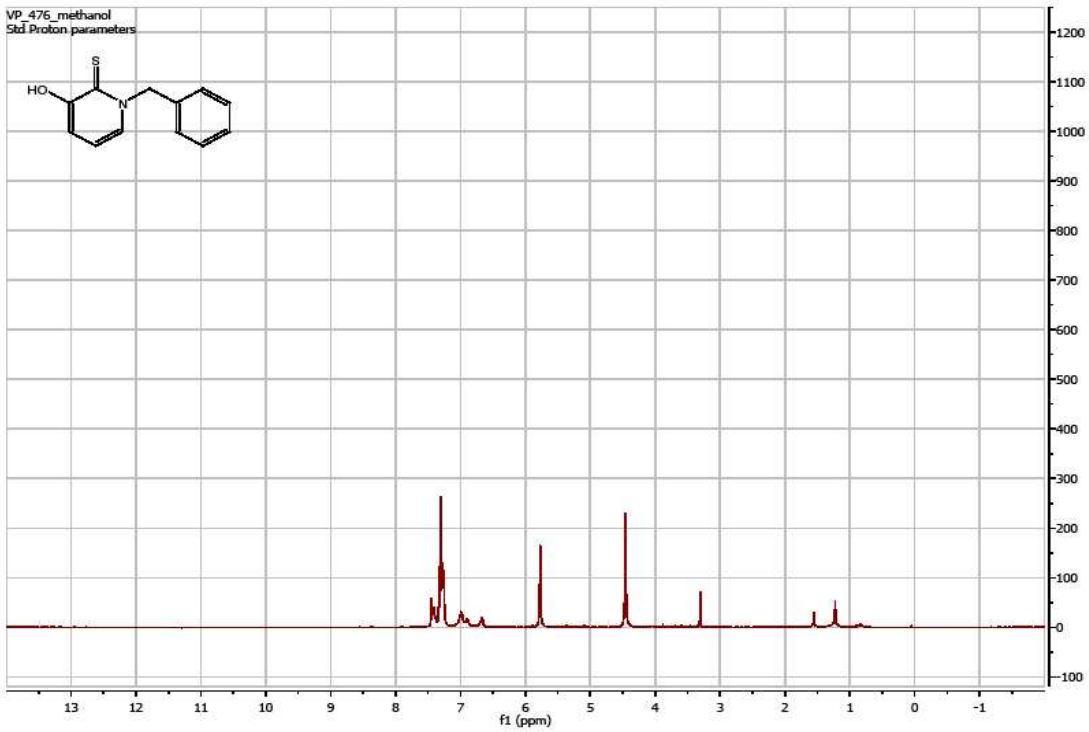

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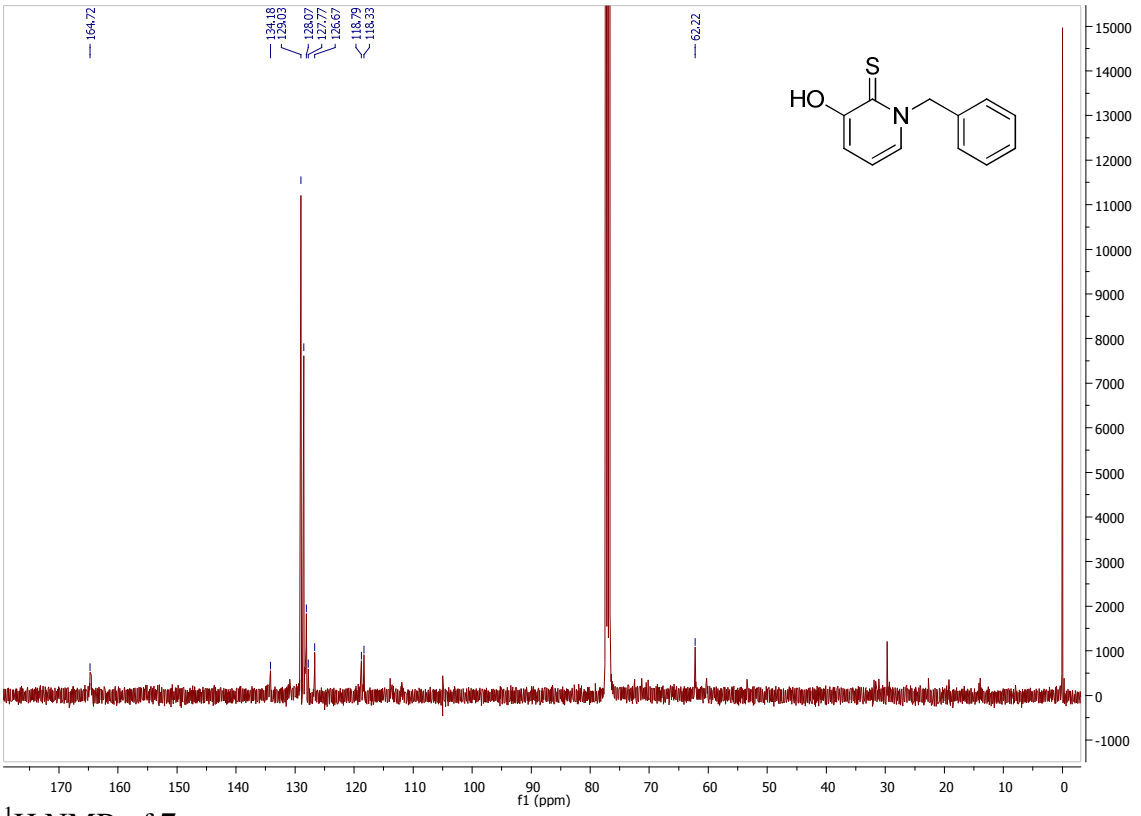
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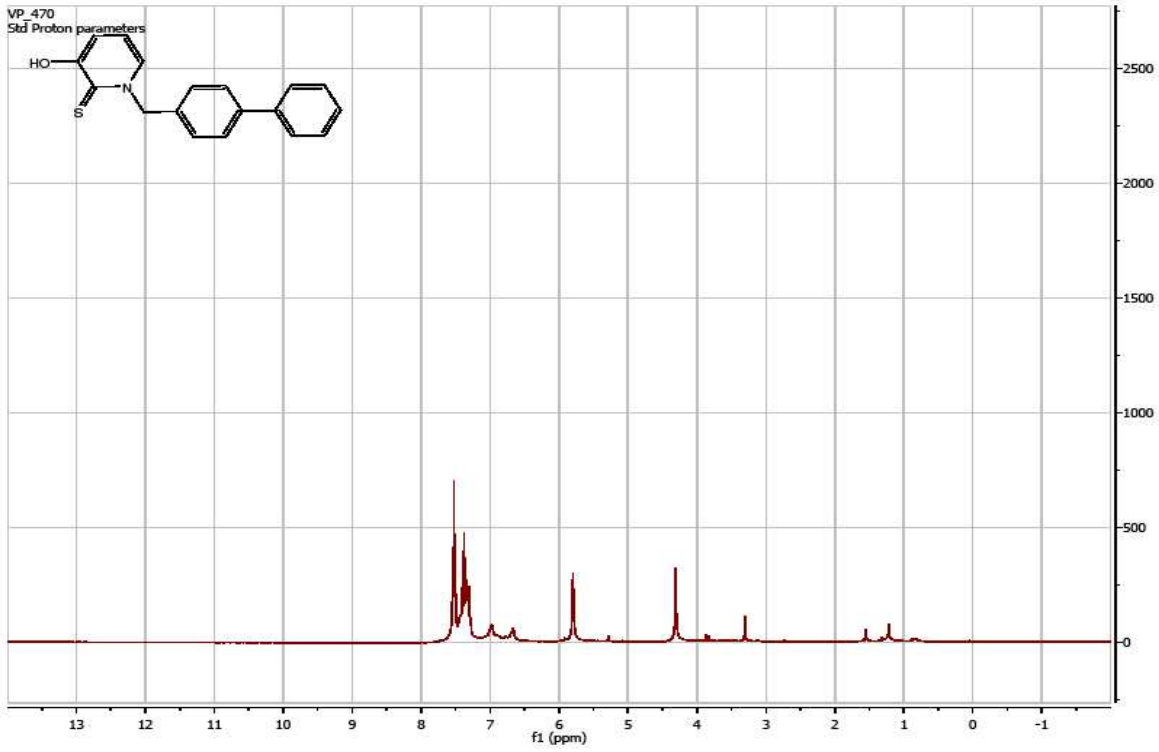
134.18
129.03
128.07
127.77
126.67
118.79
118.33
62.22
HO
S
N
f1 (ppm)

^{1}H NMR of **7c:**


VP_470
Std Proton parameters
HO
N
S
f1 (ppm)

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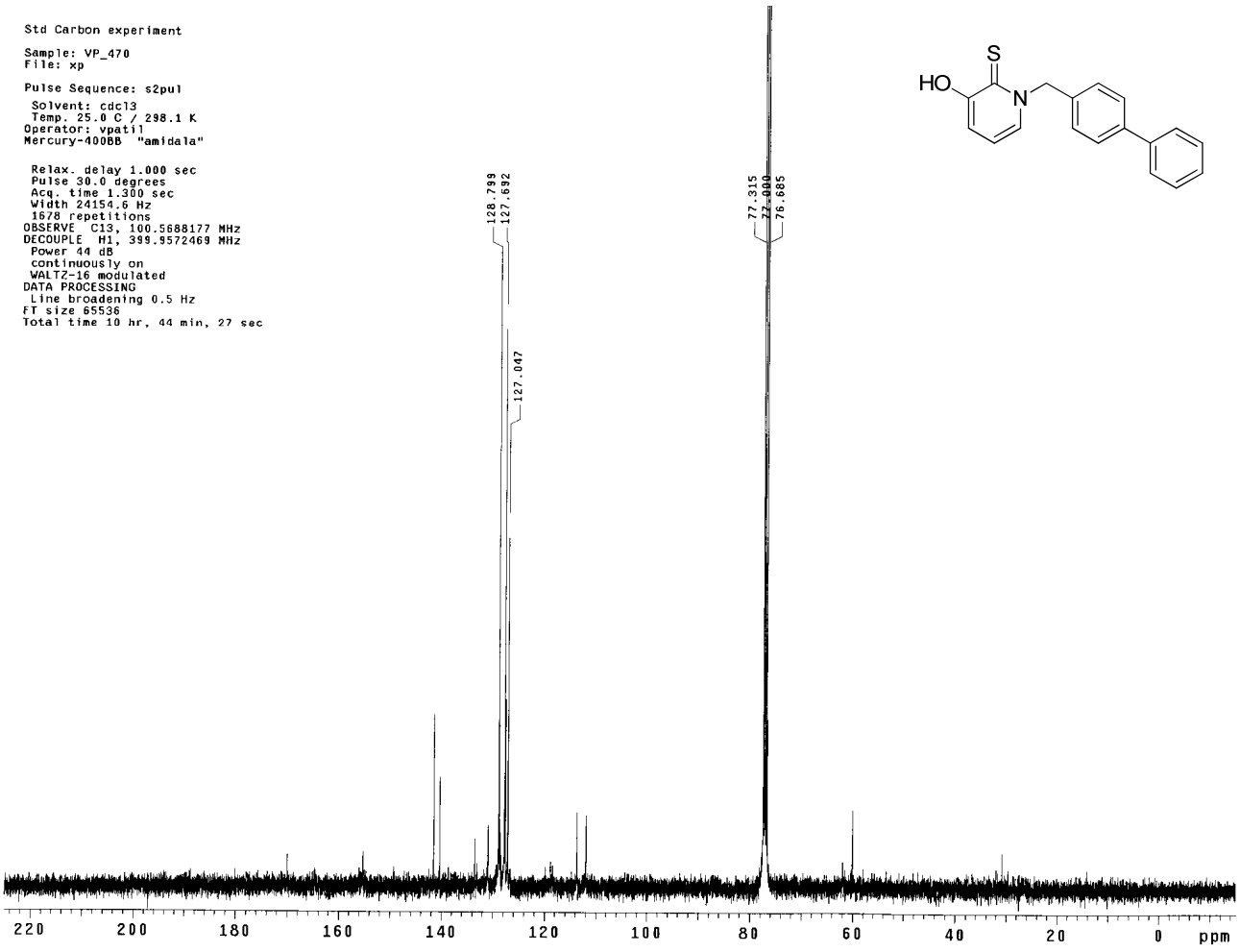
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Solvent: cdcl3
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Operator: vpatil
Mercury-400BB "amidala"
Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 1.300 sec
Width 24154.6 Hz
1678 repetitions
OBSERVE C13, 100.5688177 MHz
DECOUPLE H1, 399.9572469 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 10 hr, 44 min, 27 sec
128.799
127.692
127.047
77.315
77.000
76.685
HO
S
N
220
200
180
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140
120
100
80
60
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20
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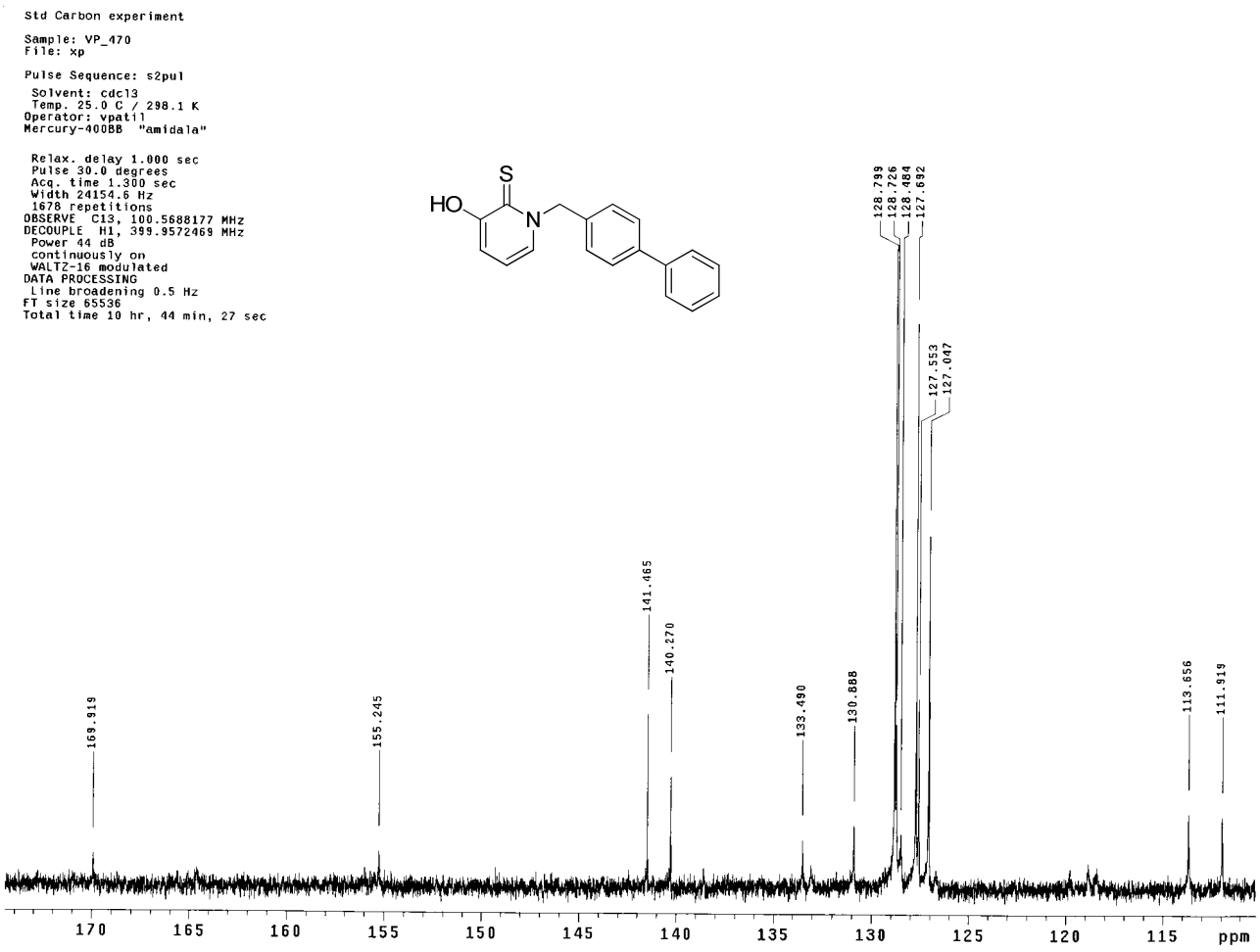
ppm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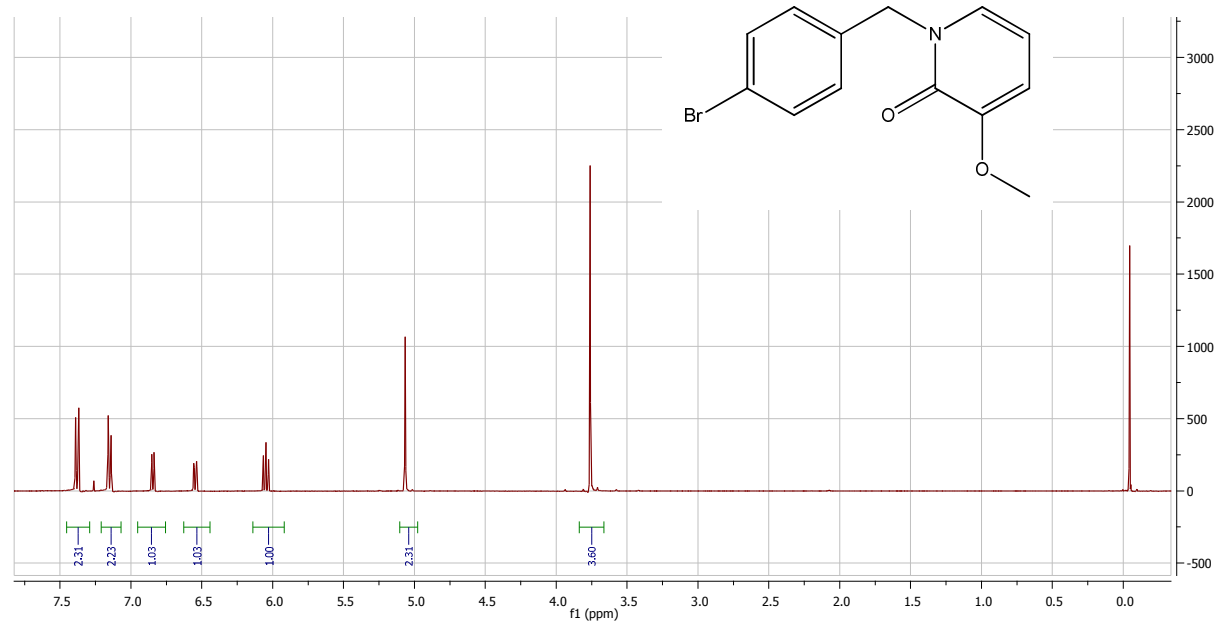
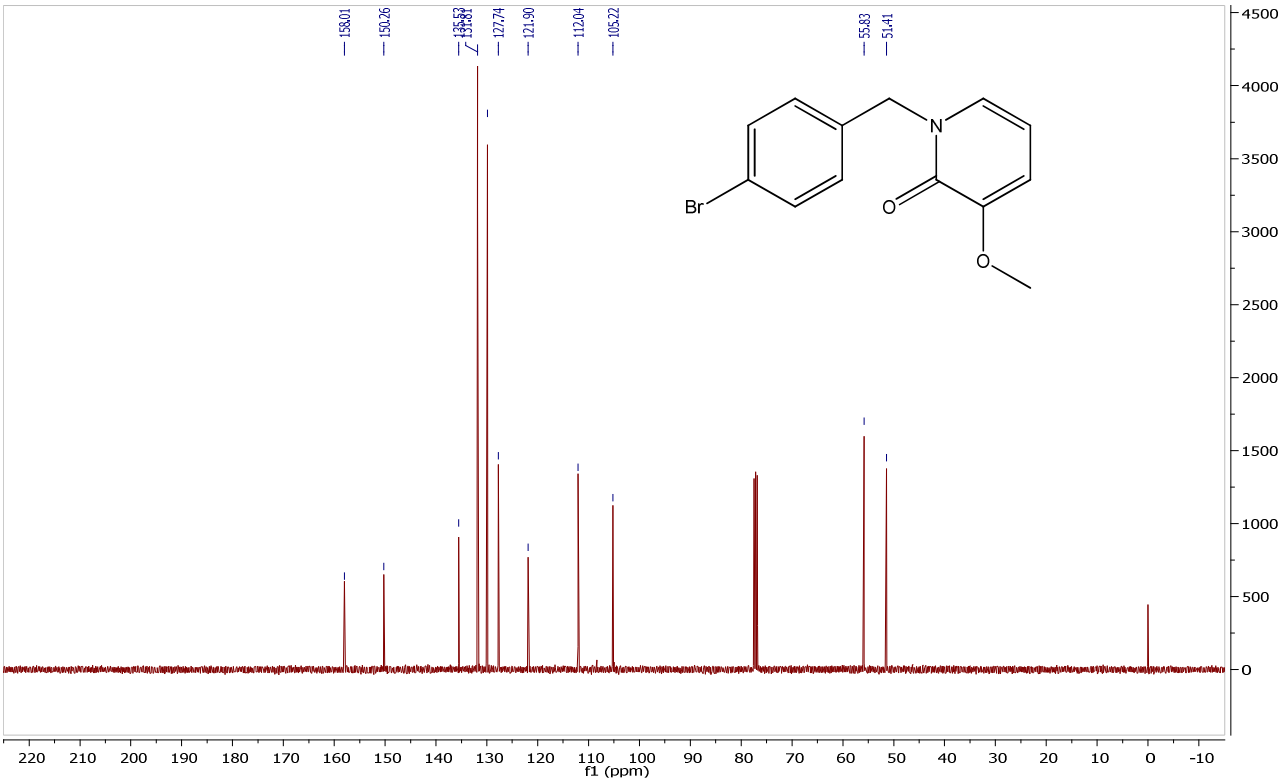
File: xp
Pulse Sequence: s2pul
Solvent: cdcl3
Temp. 25.0 C / 298.1 K
Operator: vpatil
Mercury-400BB "amidala"
Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 1.300 sec
Width 24154.6 Hz
1678 repetitions
OBSERVE C13, 100.5688177 MHz
DECOUPLE H1, 399.9572469 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 10 hr, 44 min, 27 sec
HO
S
N
169.919
155.245
141.465
140.270
133.490
130.888
128.799
128.726
128.484
127.692
127.553
127.047
113.656
111.919
170
165
160
155
150
145
140
135
130
125
120
115
ppm

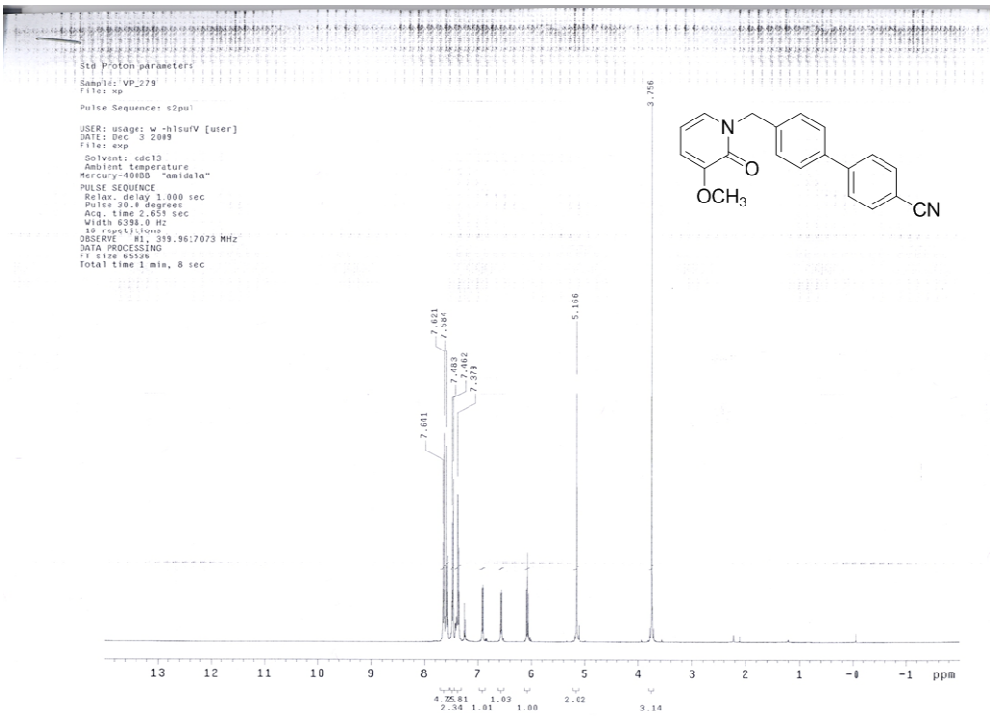
^{1}H NMR of **8:**



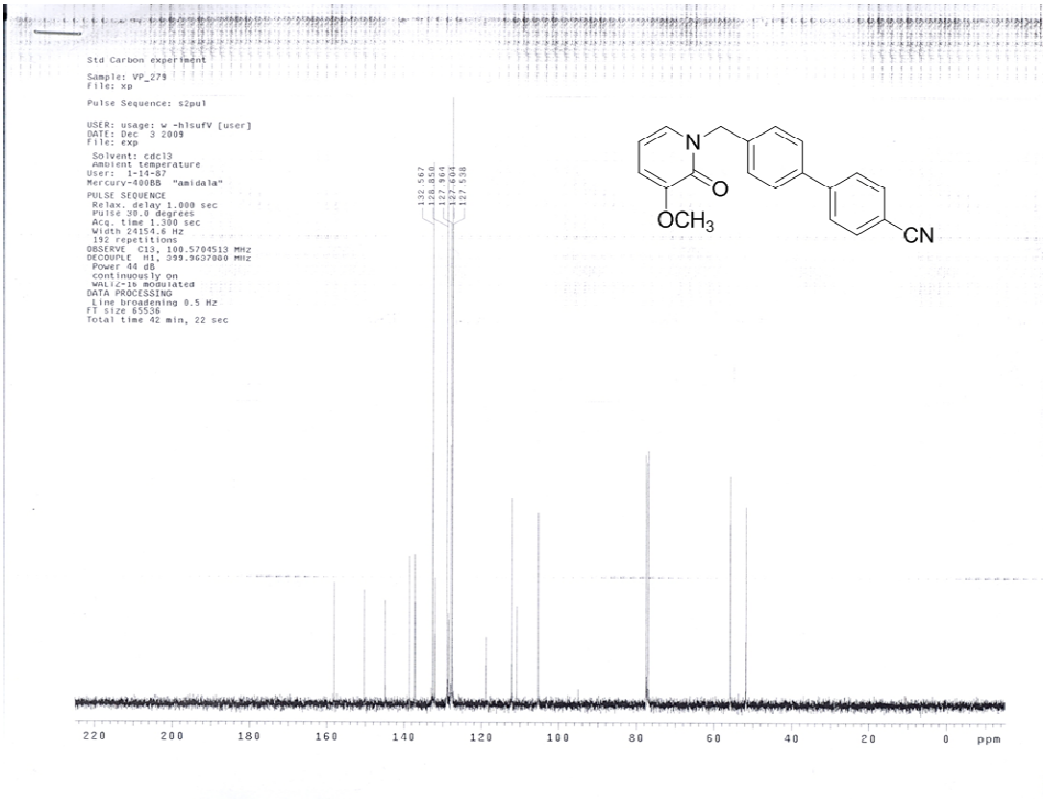
^{13}C NMR of **8:**



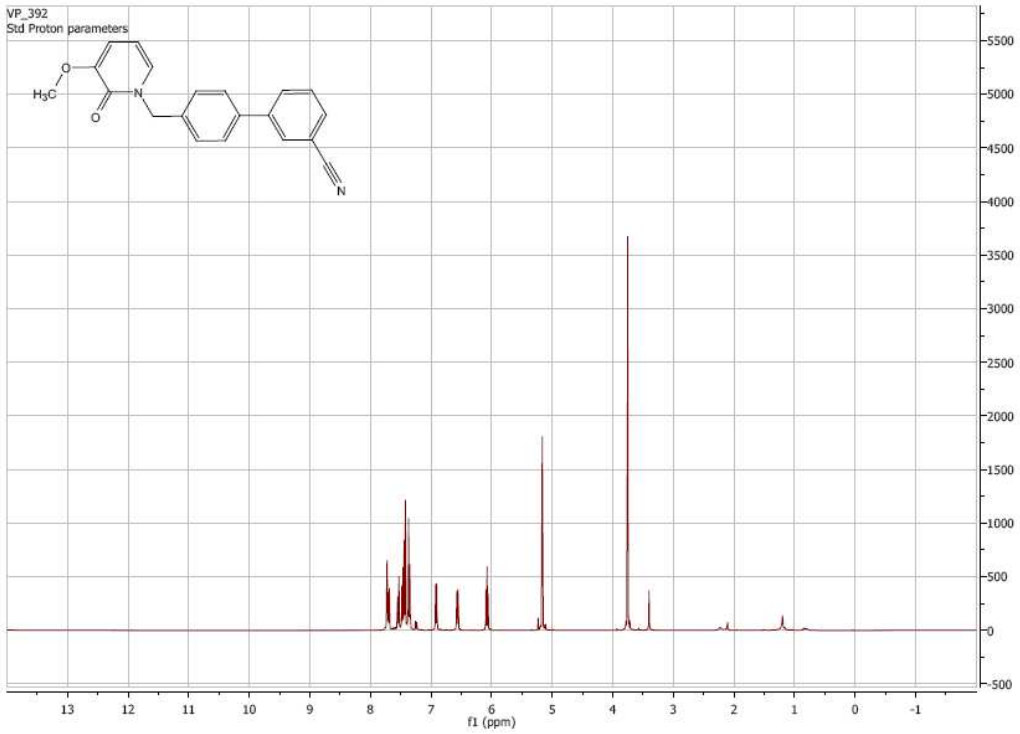
^{1}H NMR of **9a:**



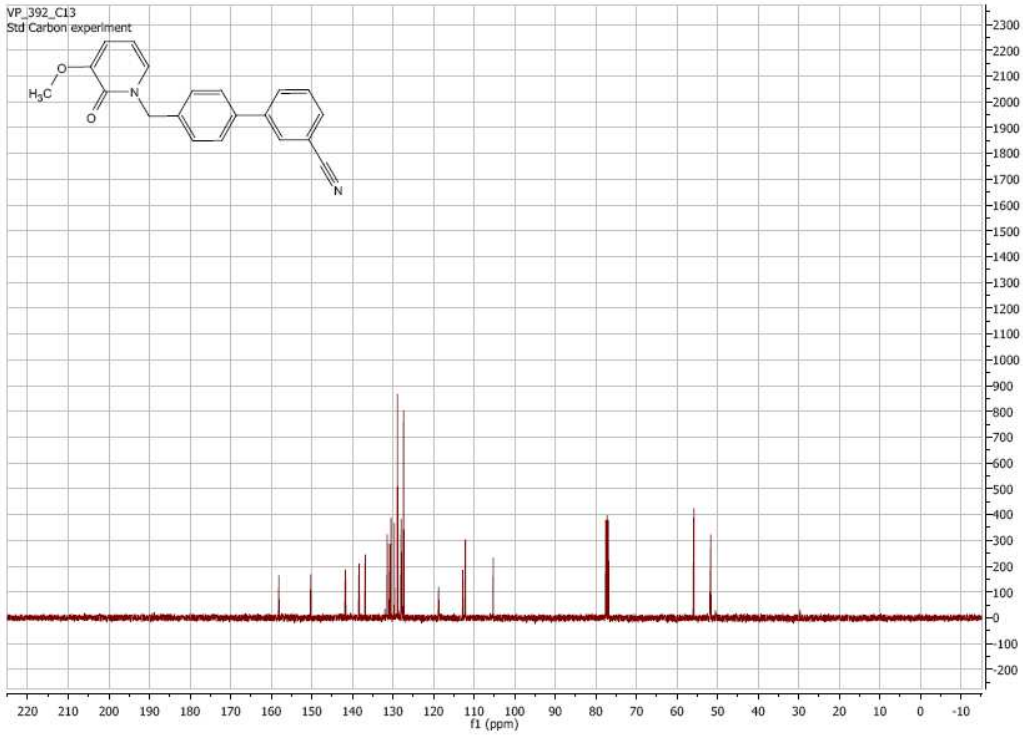
^{13}C NMR of **9a:**



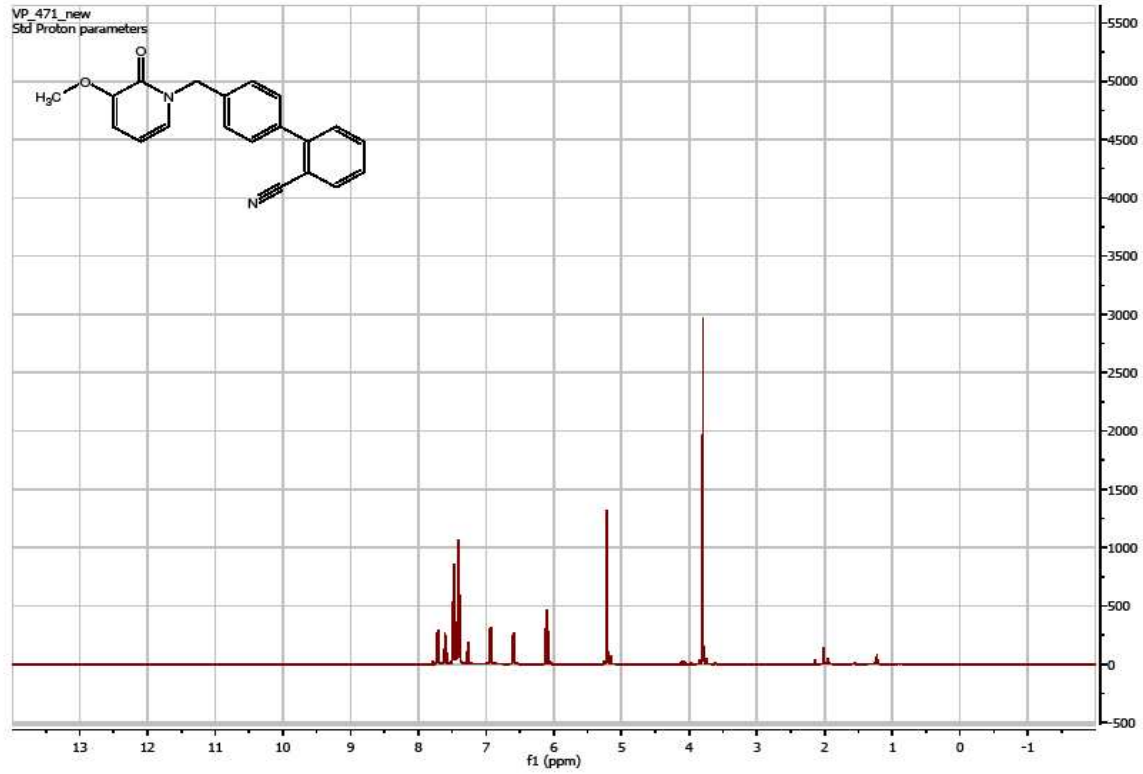
^{1}H NMR of **9b:**



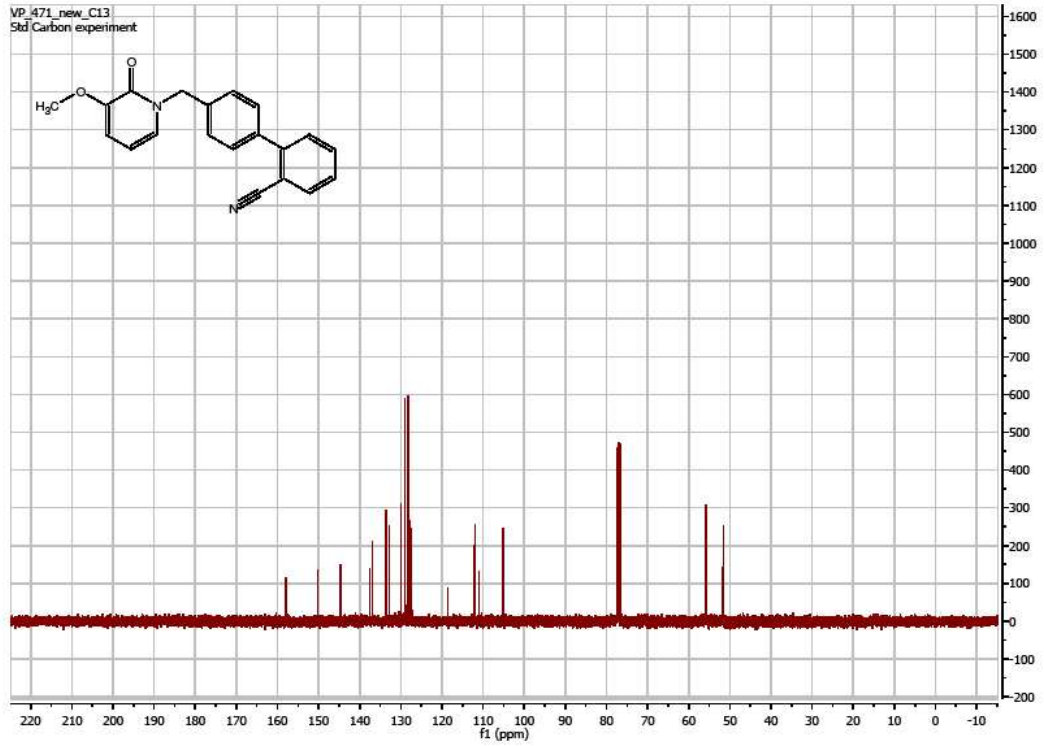
^{13}C NMR of **9b**



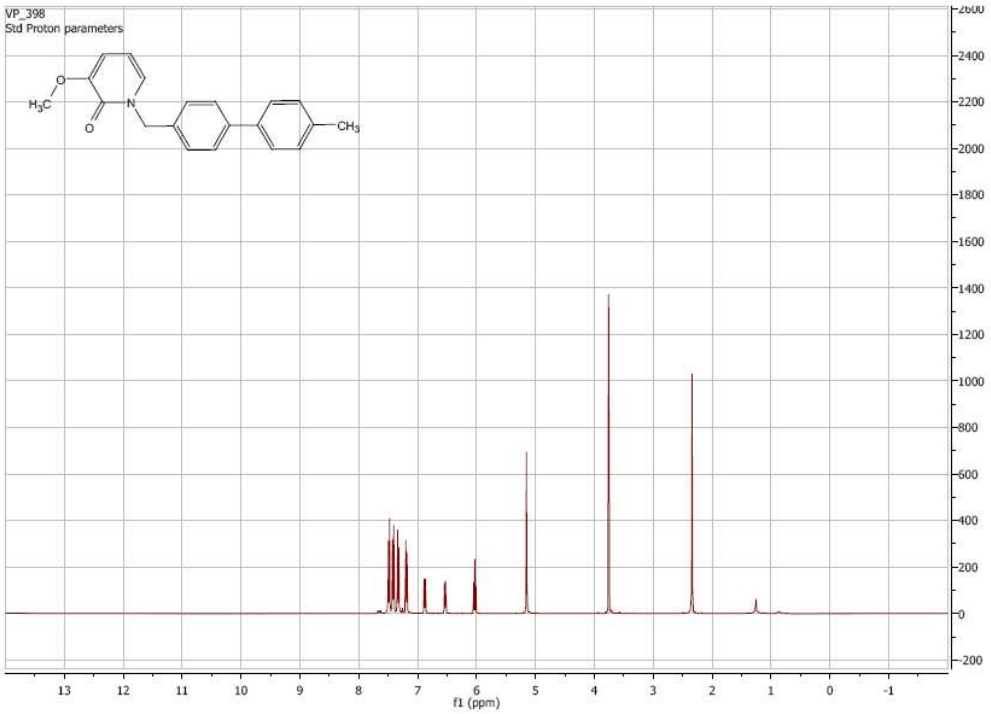
^{1}H NMR of **9c:**



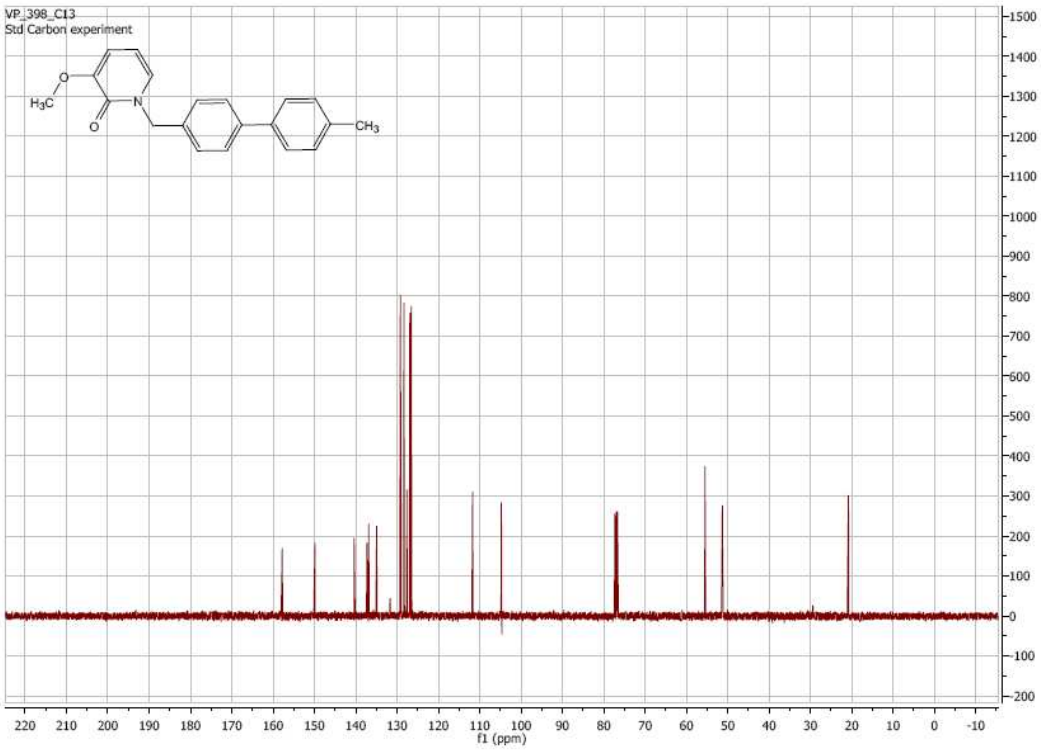
^{13}C NMR of **9c:**



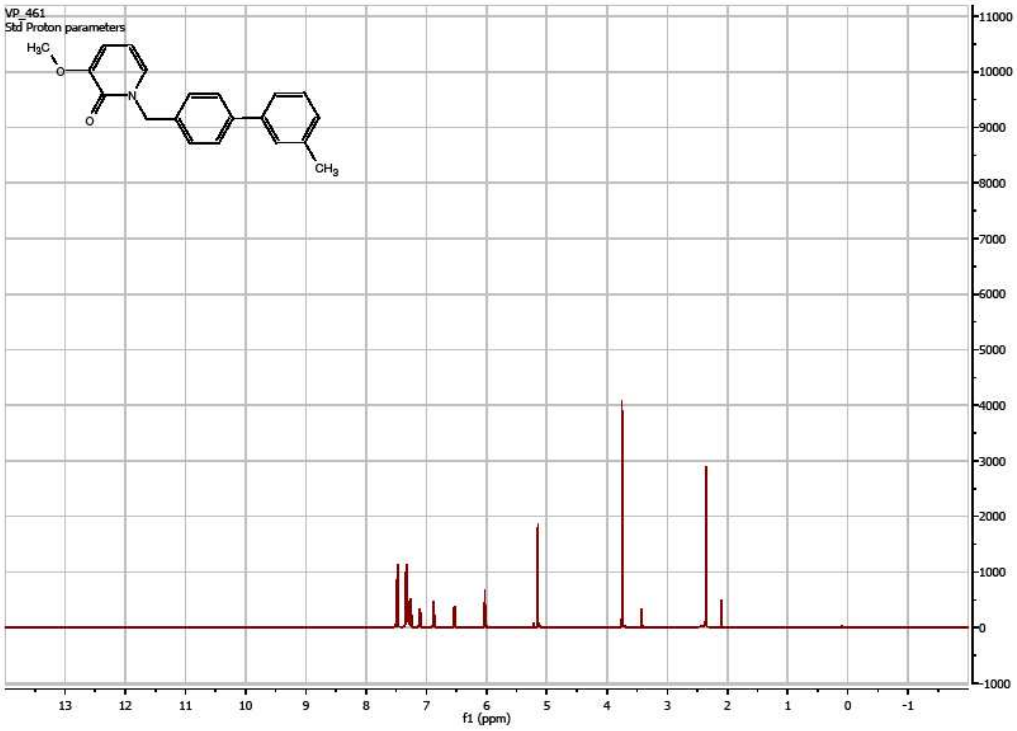
^{1}H NMR of **9d:**



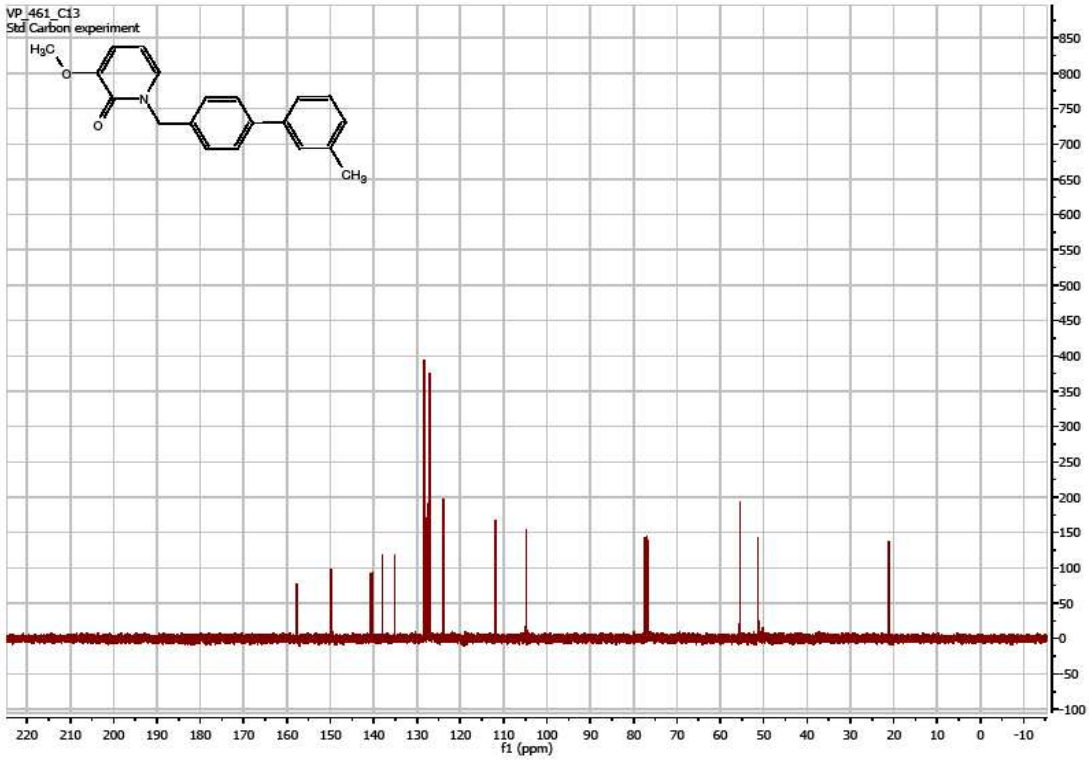
^{1}H NMR of **9d:**



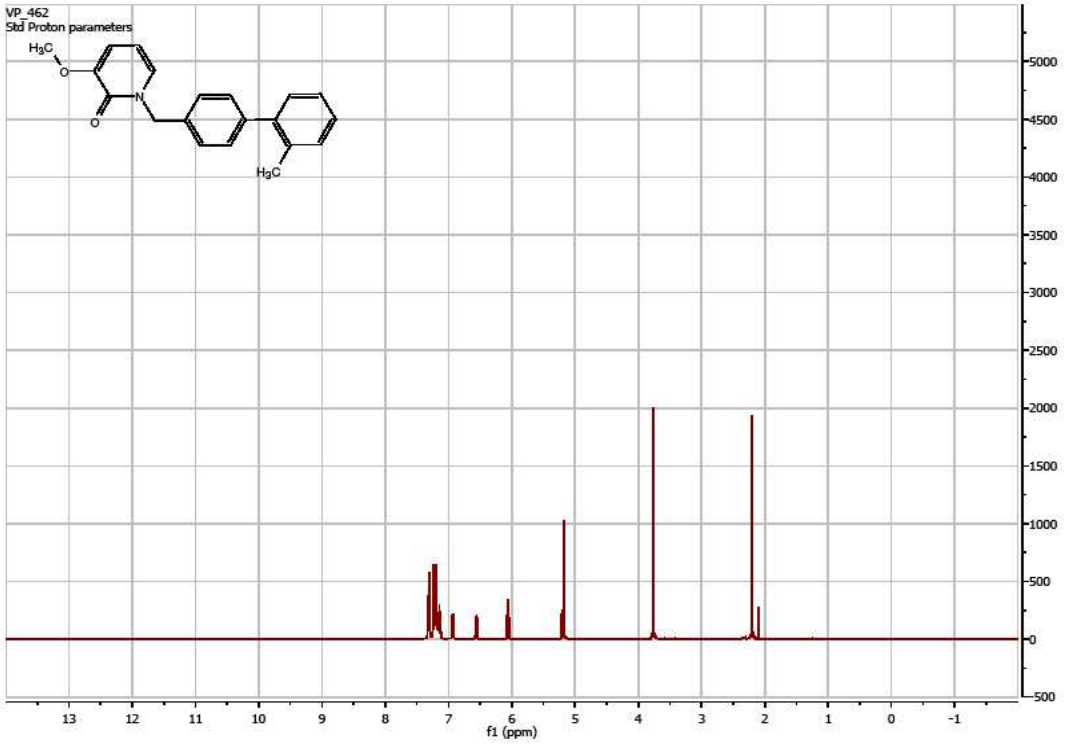
^{1}H NMR of **9e:**



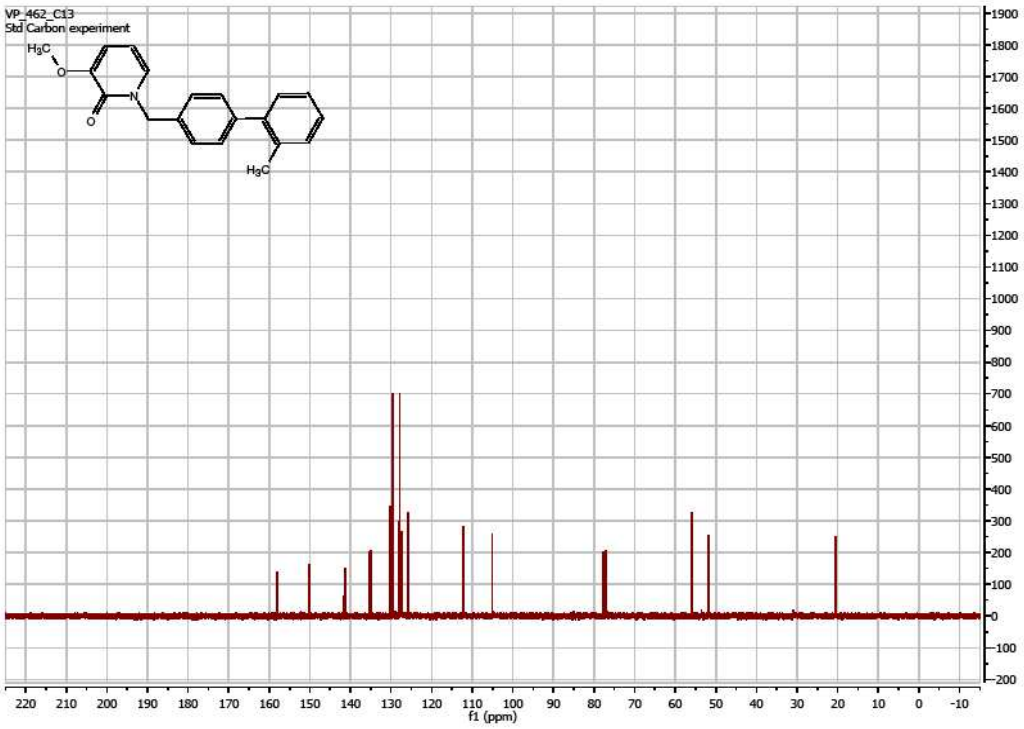
^{13}C NMR of **9e:**



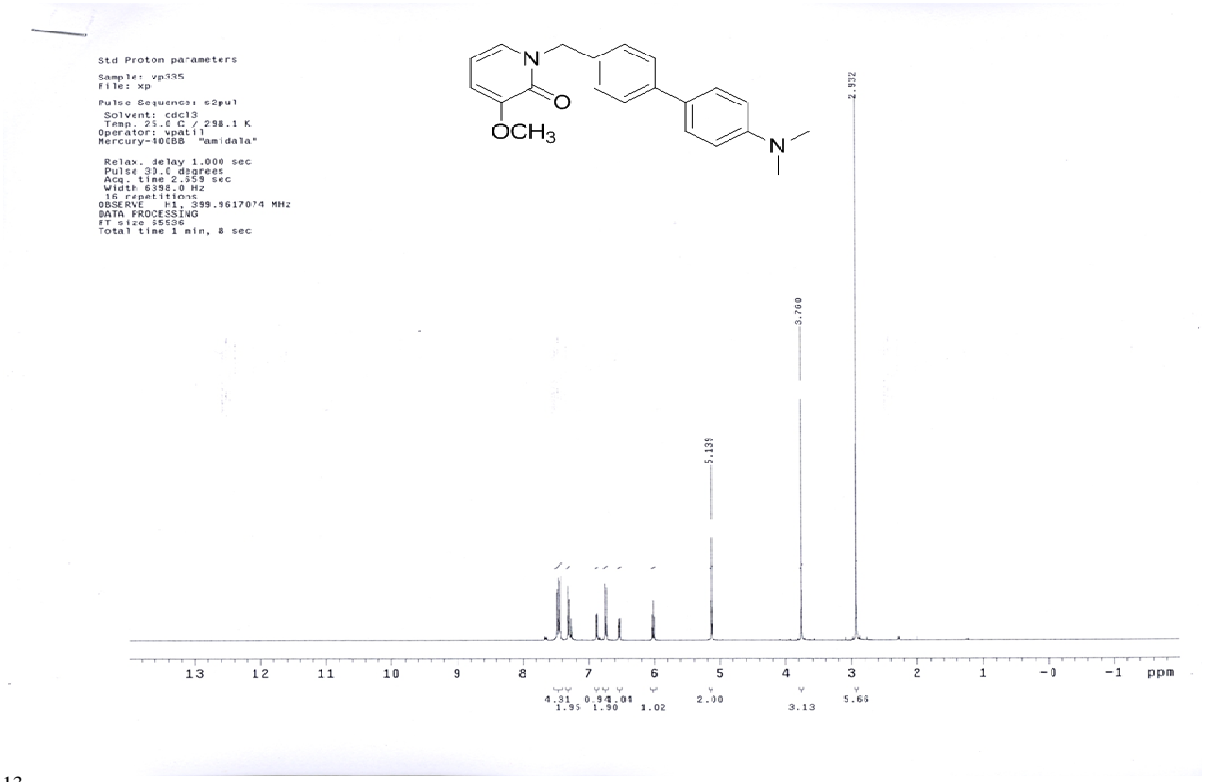
^{1}H NMR of **9f:**



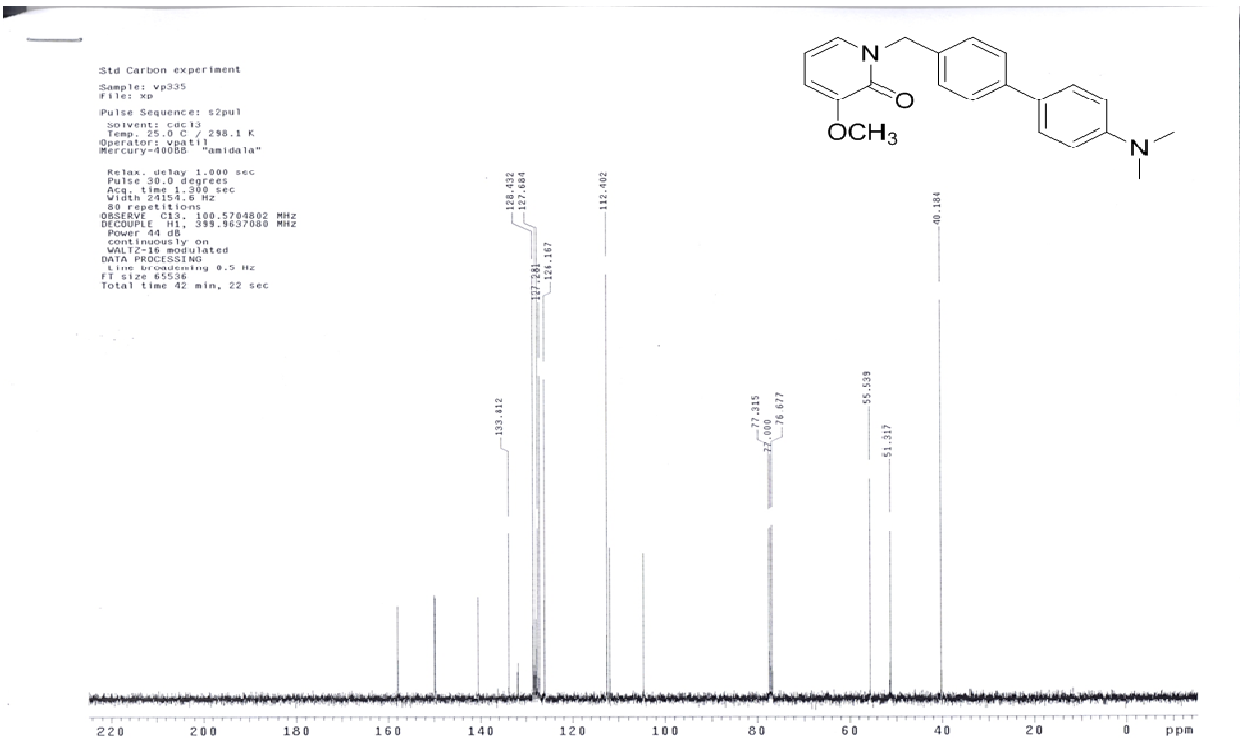
^{13}C NMR of **9f:**



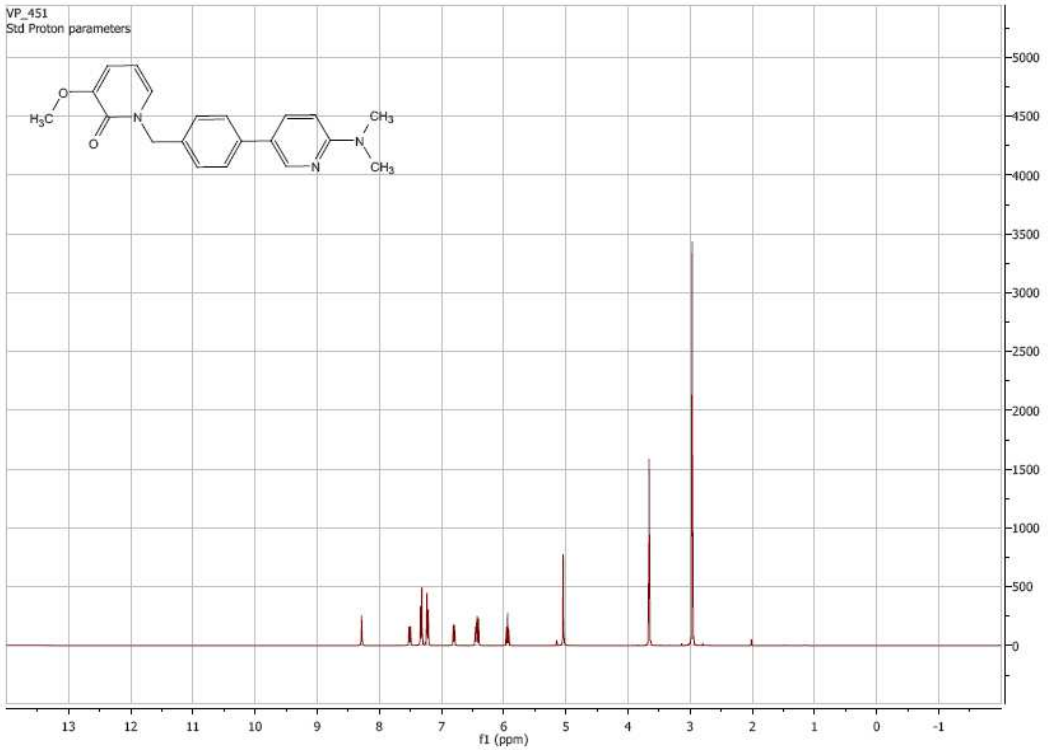
^{1}H NMR of **9g:**



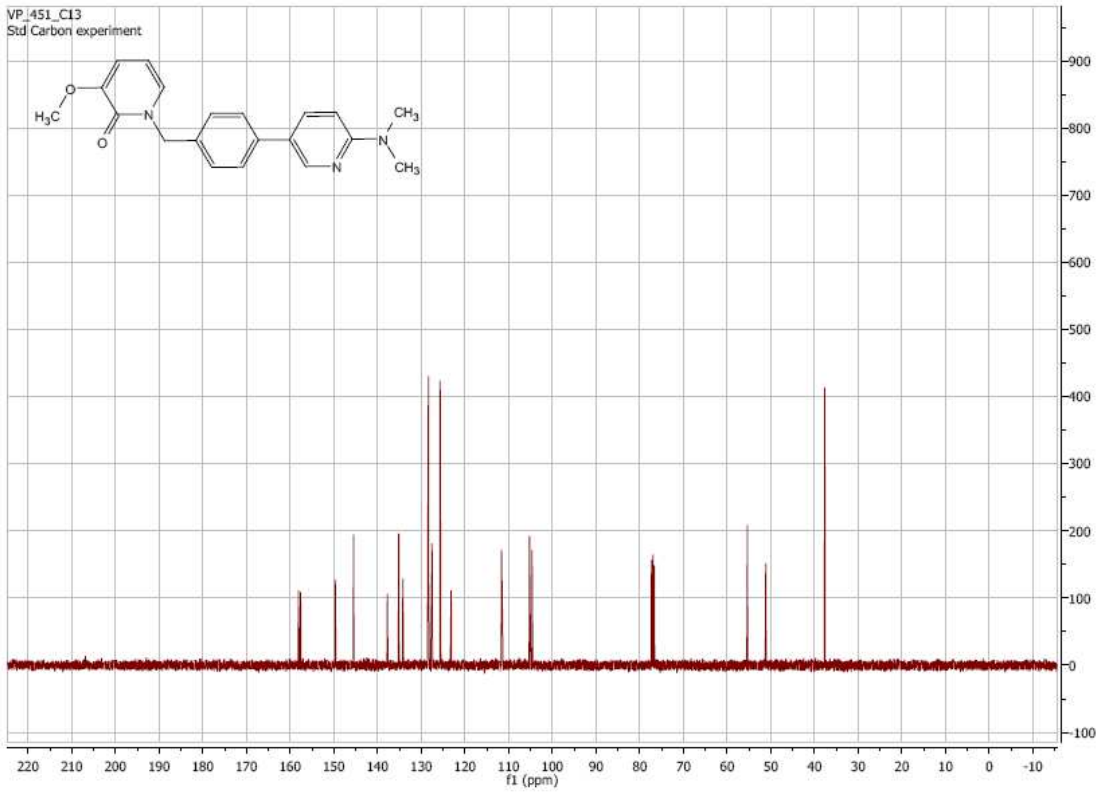
^{13}C NMR of **9g:**



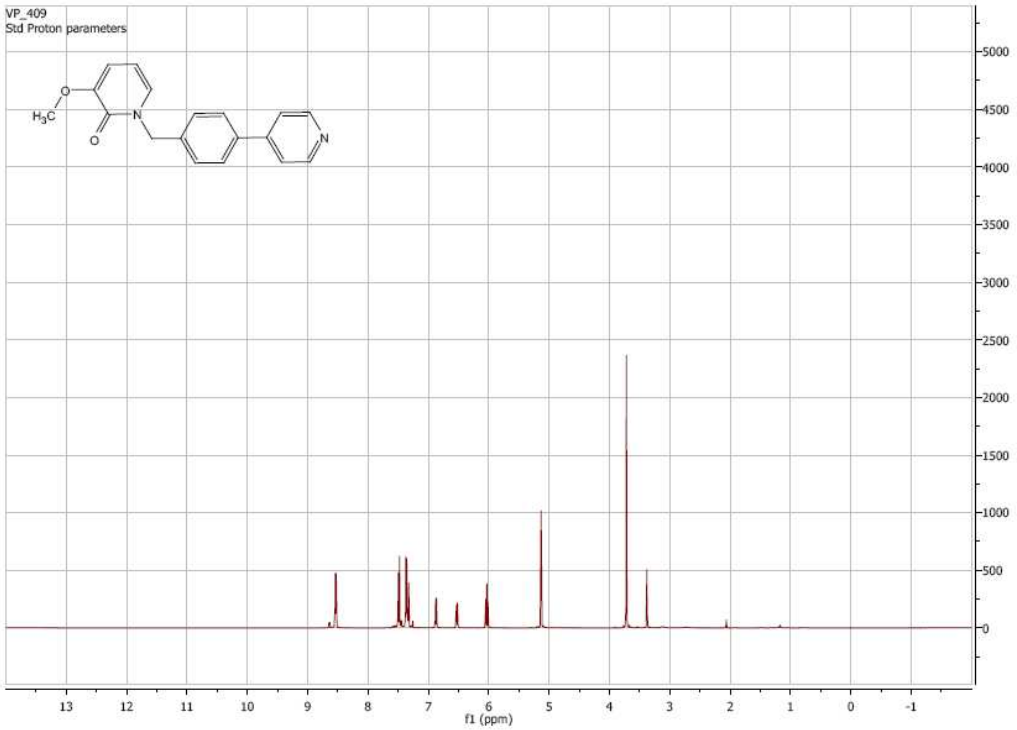
^{1}H NMR of **9h:**



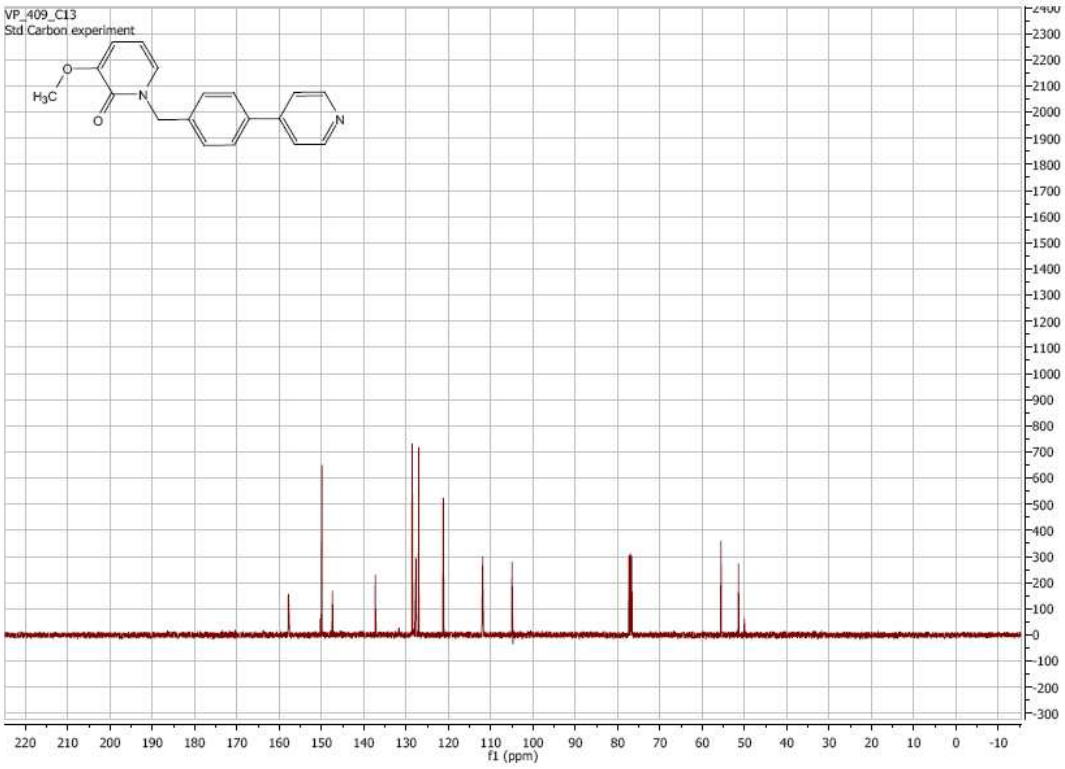
^{13}C NMR of **9h:**



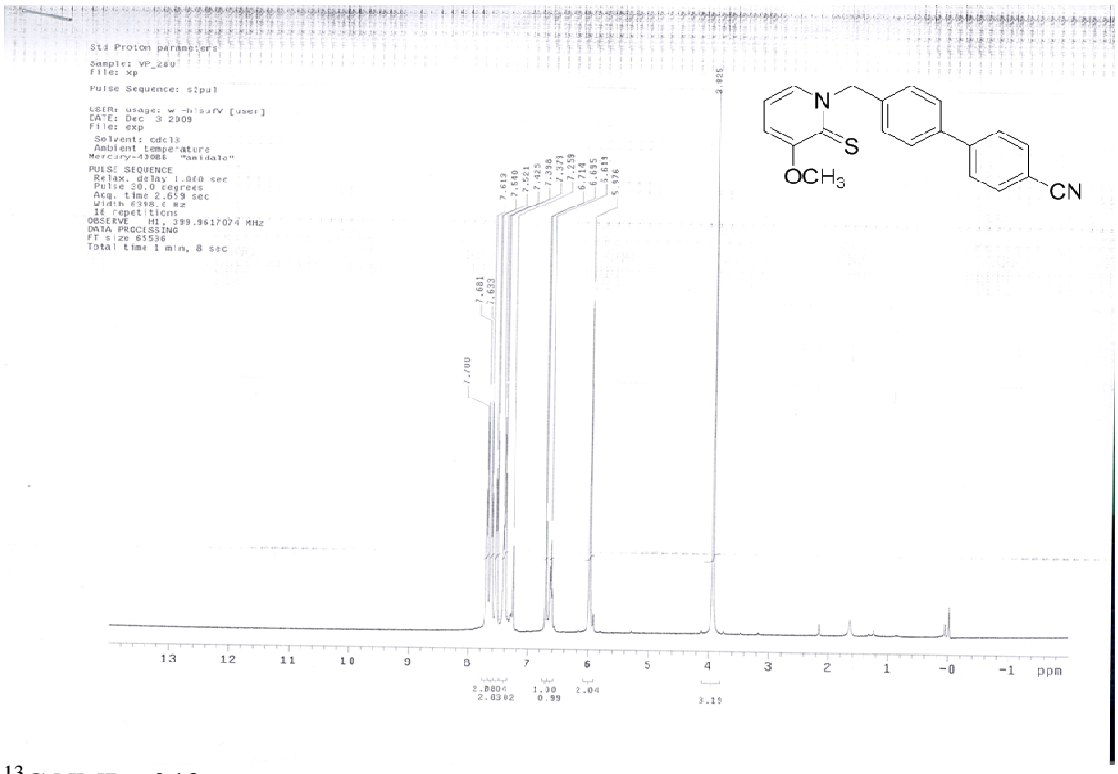
^{1}H NMR of **9i:**



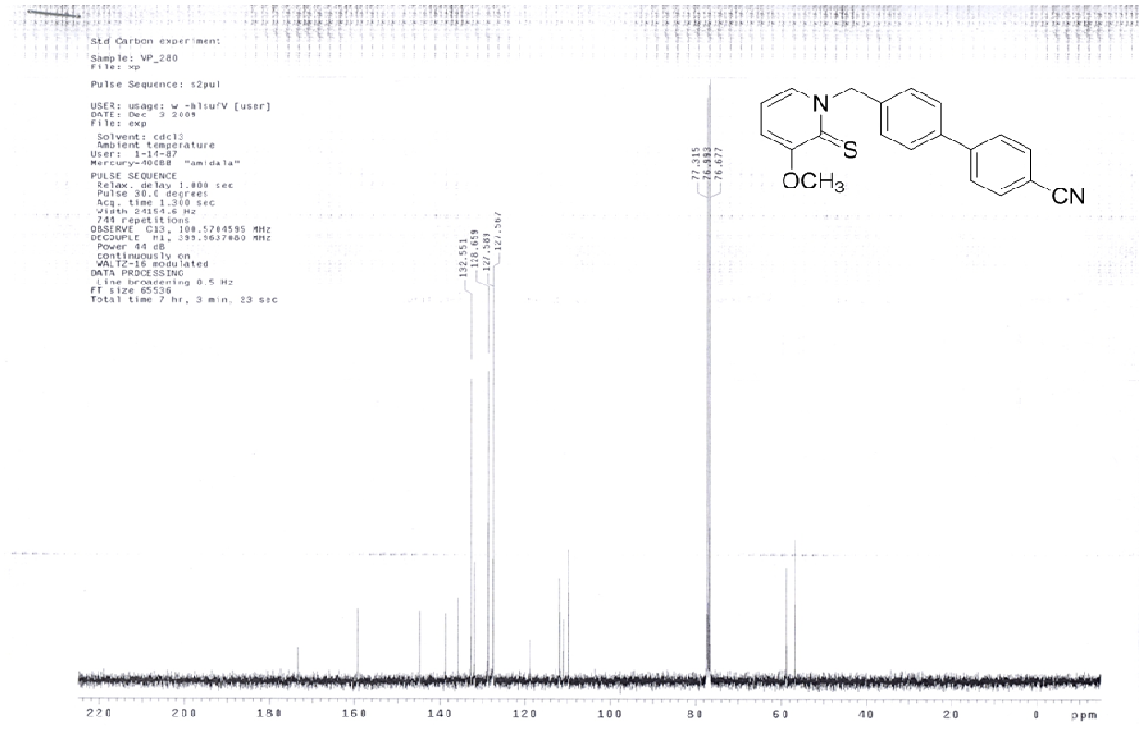
^{13}C NMR of **9i:**



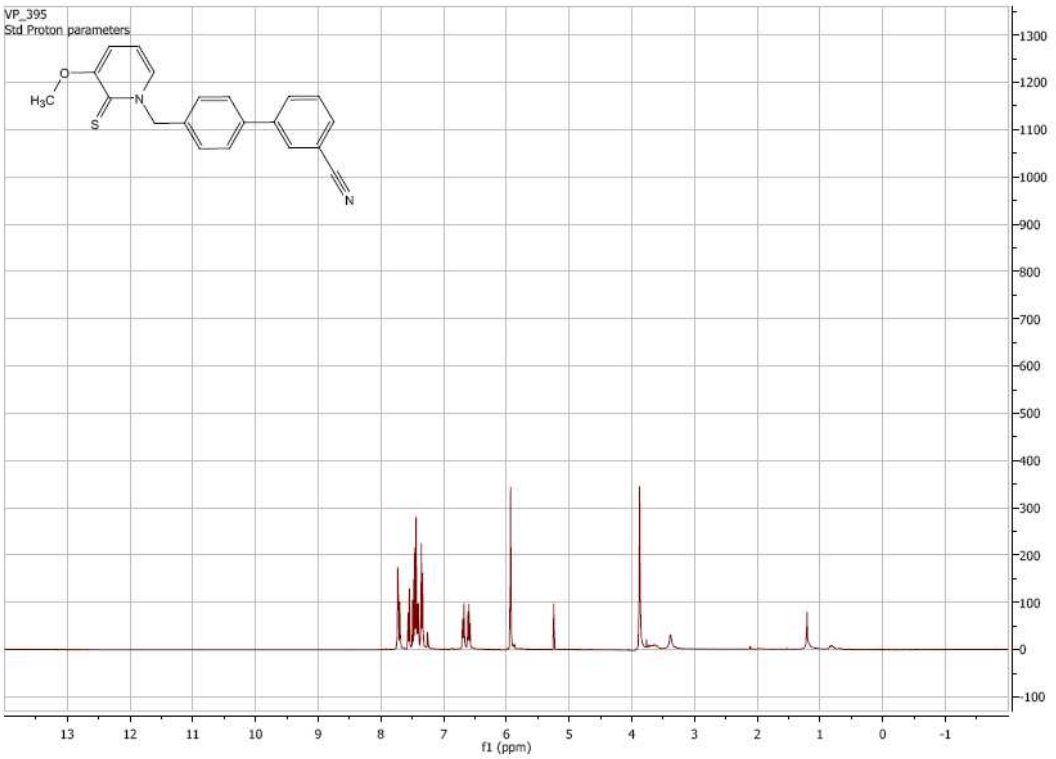
^{1}H NMR of **10a:**



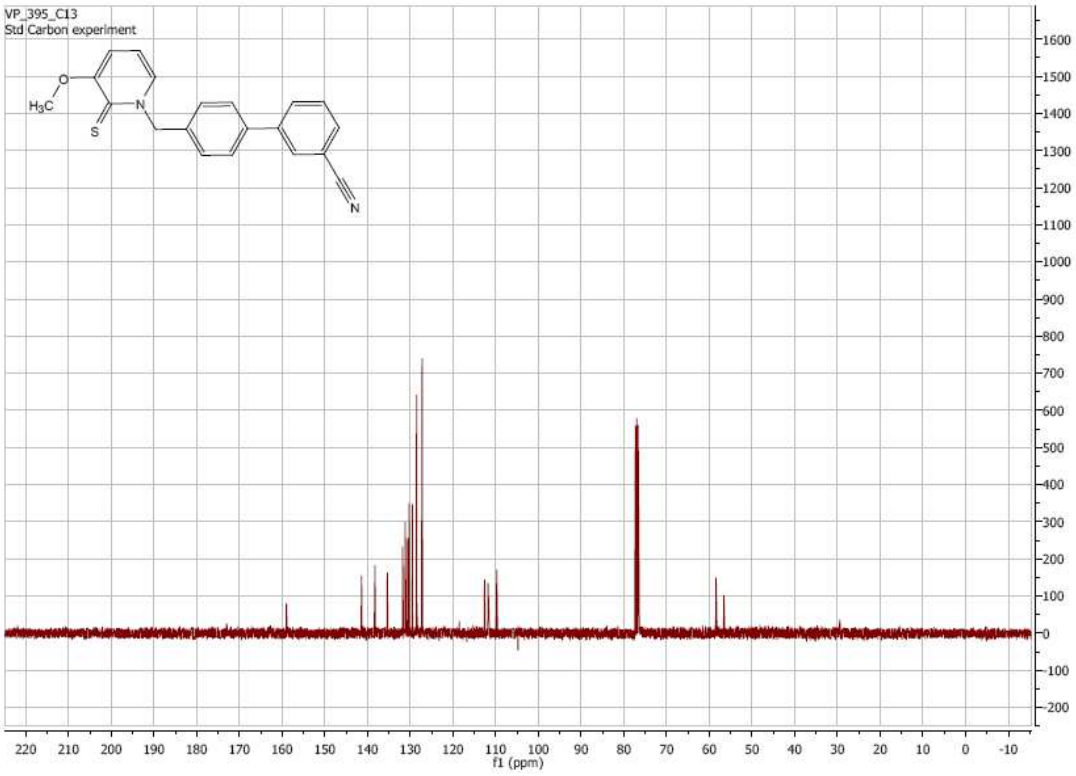
^{13}C NMR of **10a:**



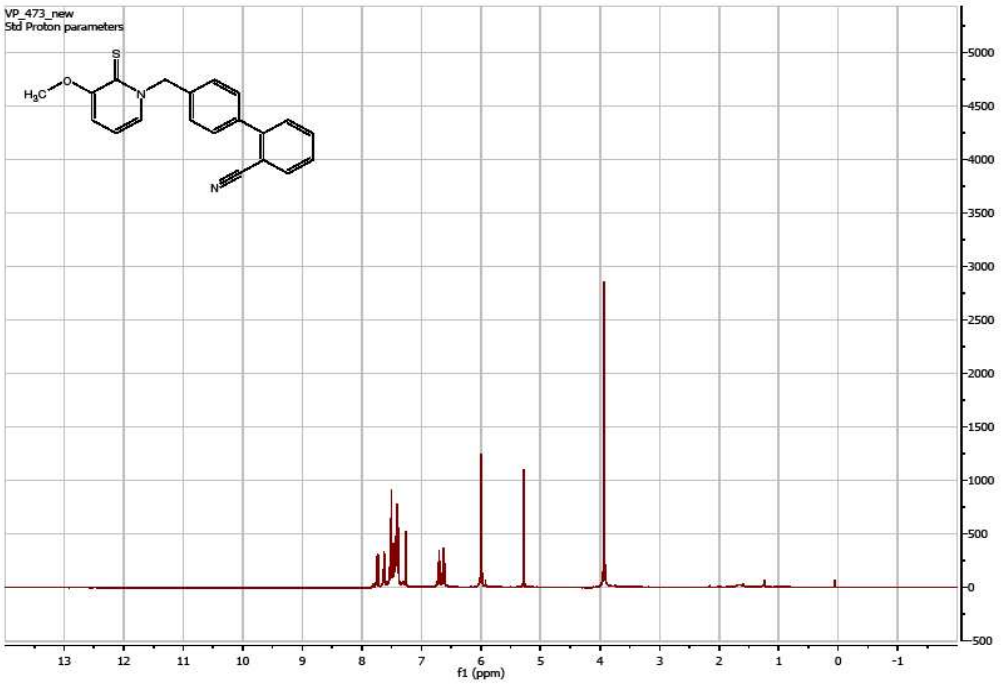
^{1}H NMR of **10b:**



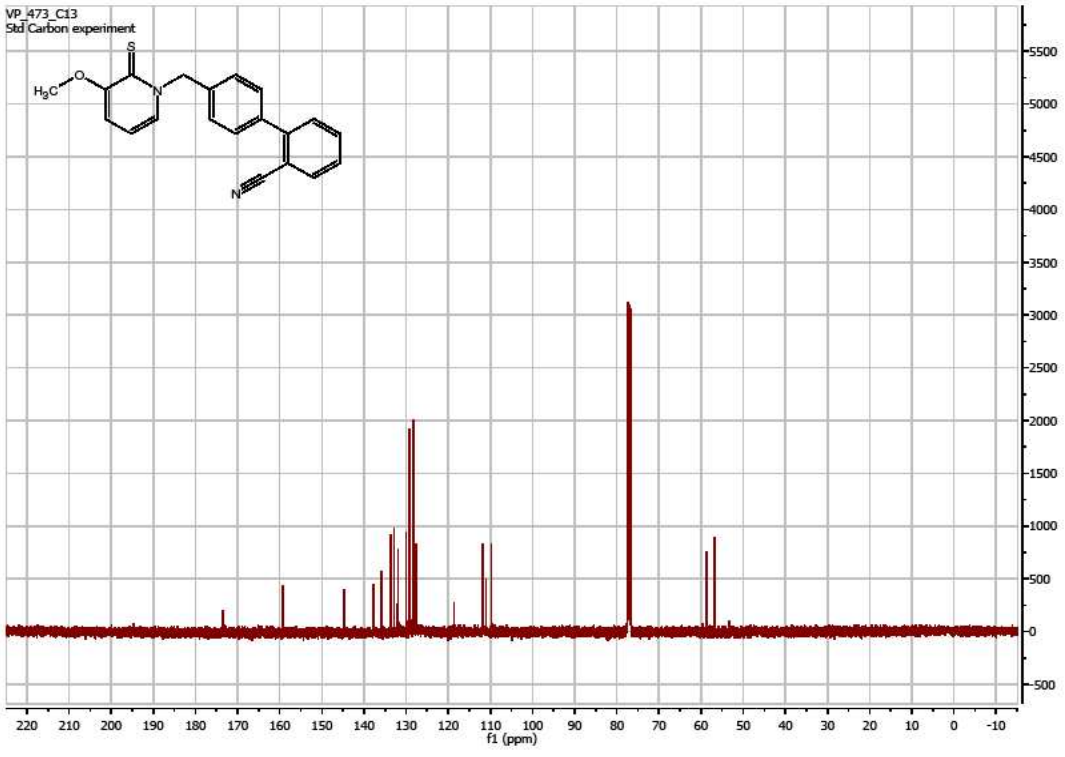
^{1}H NMR of **10b:**



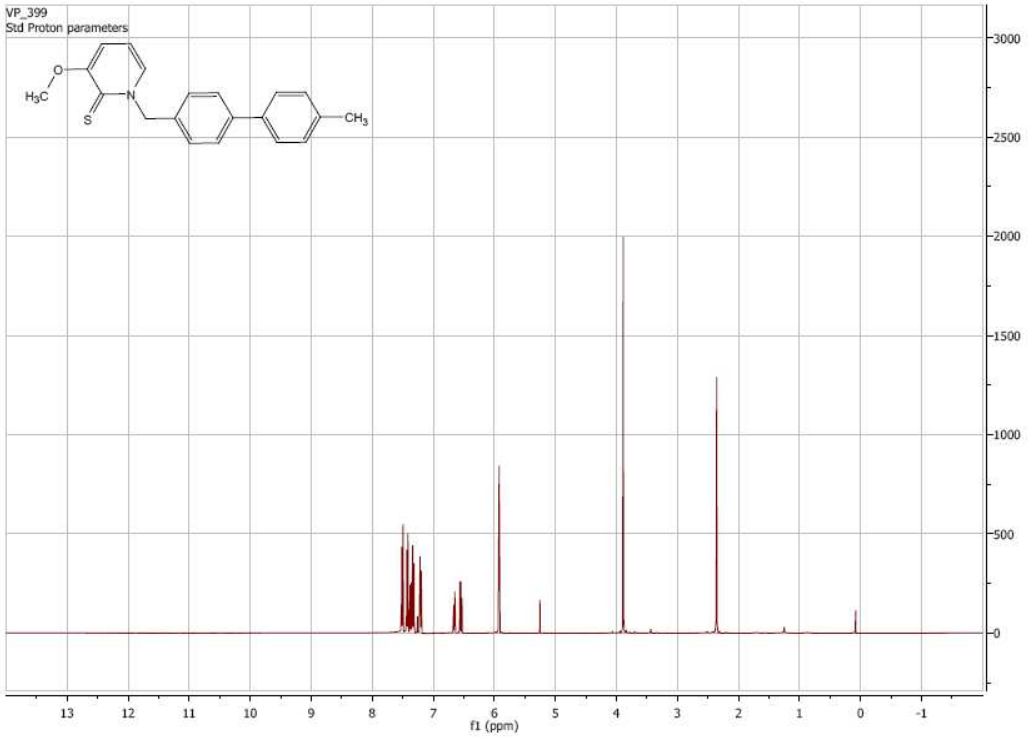
^{1}H NMR of **10c:**



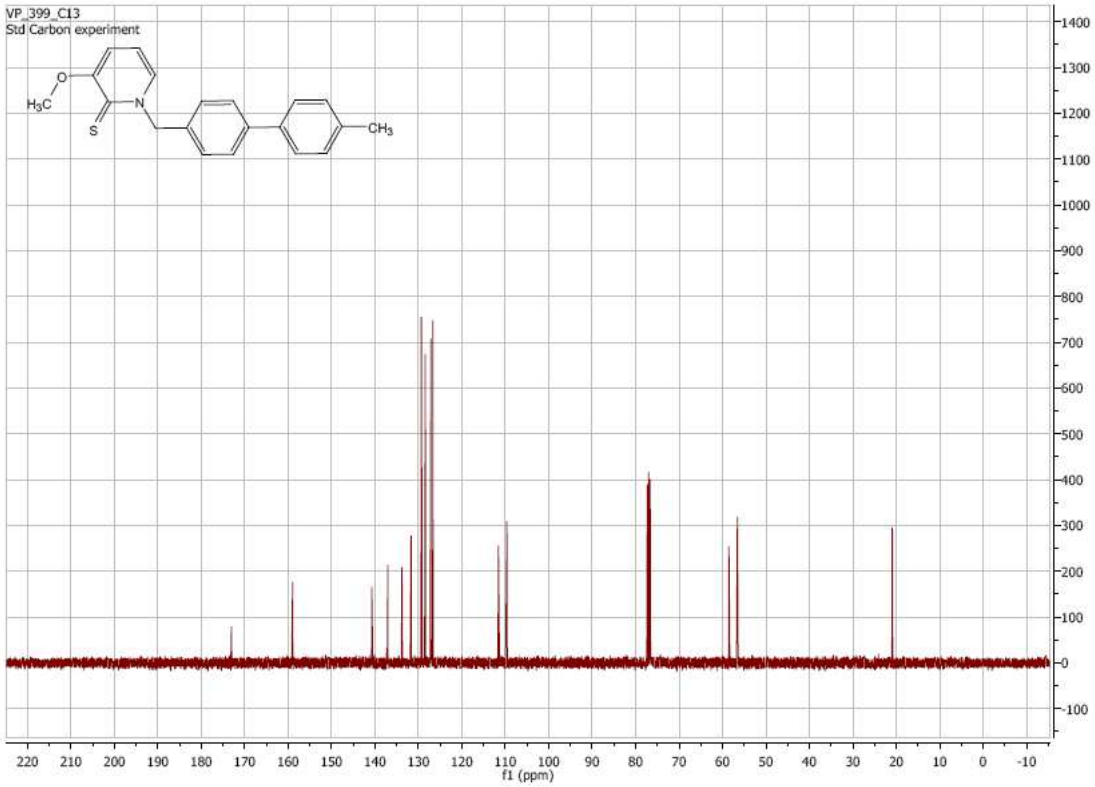
^{13}C NMR **10c:**



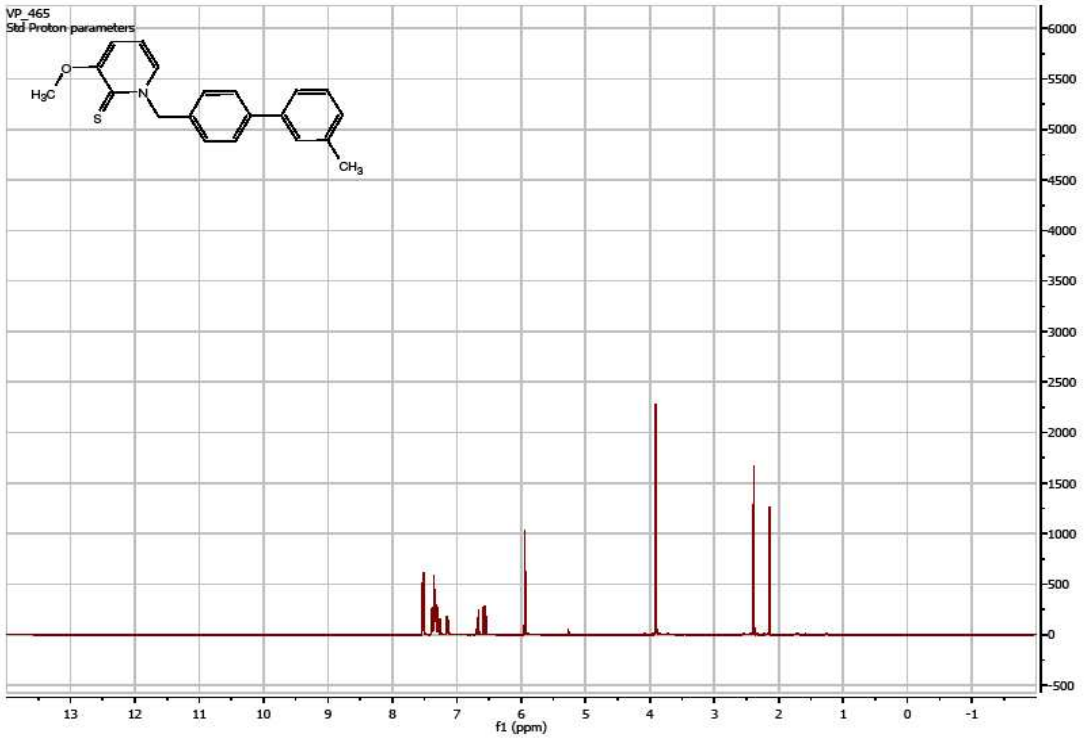
^{1}H NMR of **10d:**



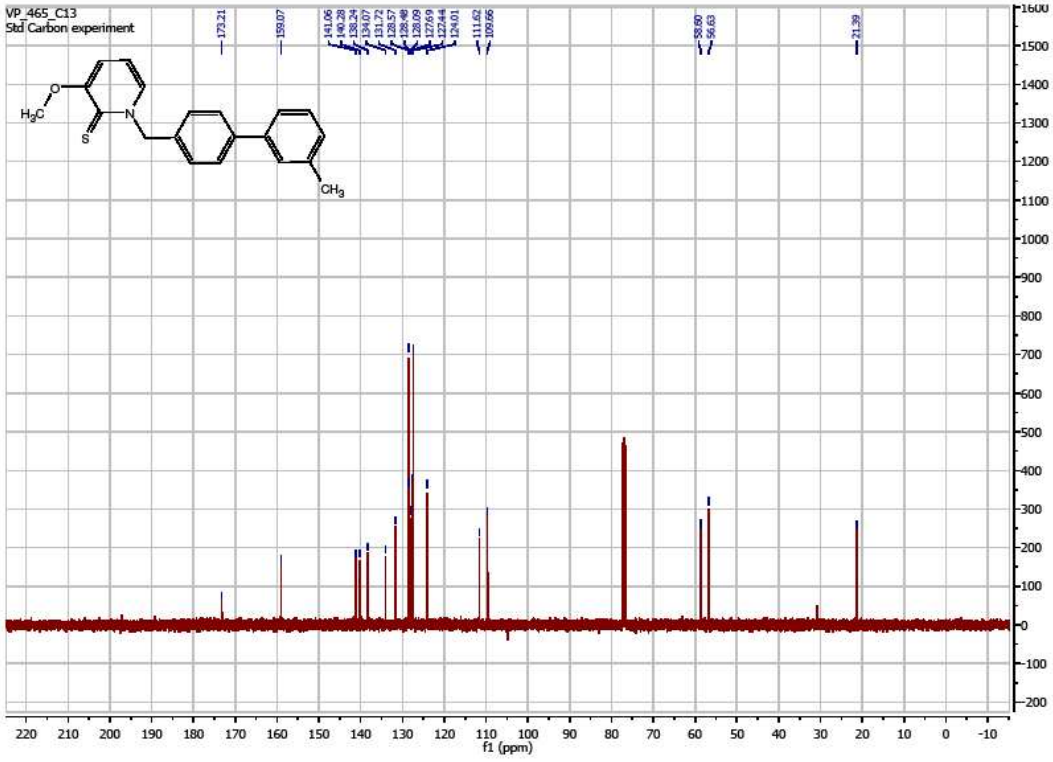
^{13}C NMR of **10d:**



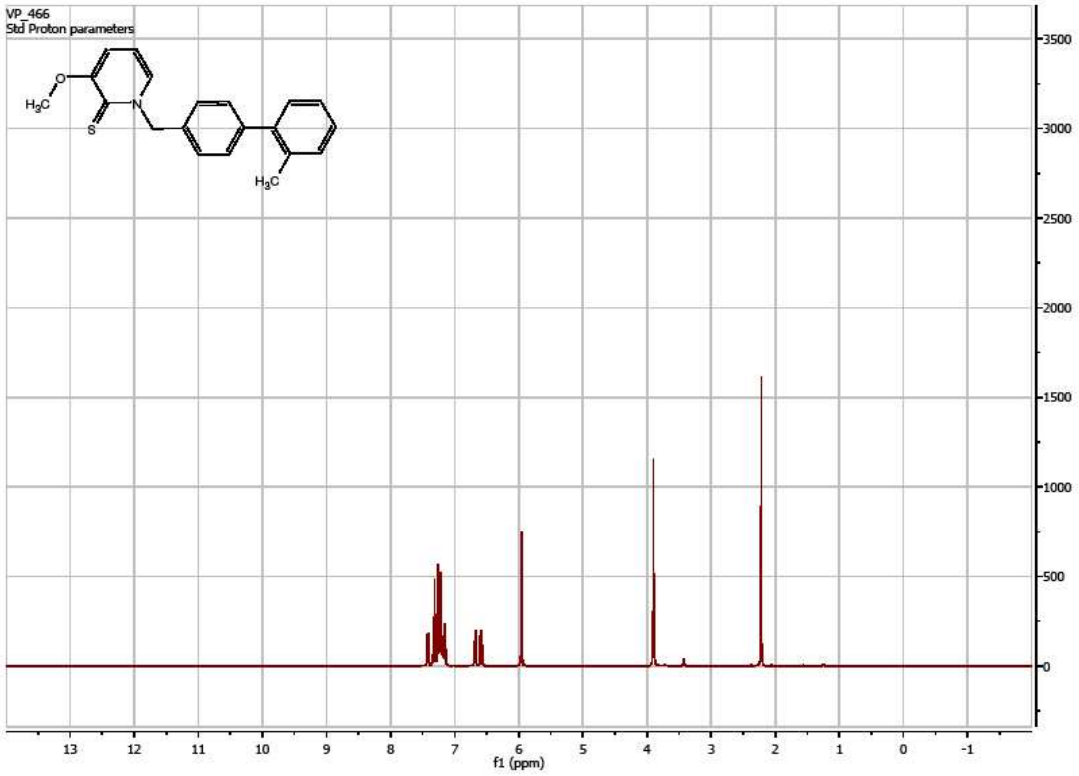
^{1}H NMR of **10e:**



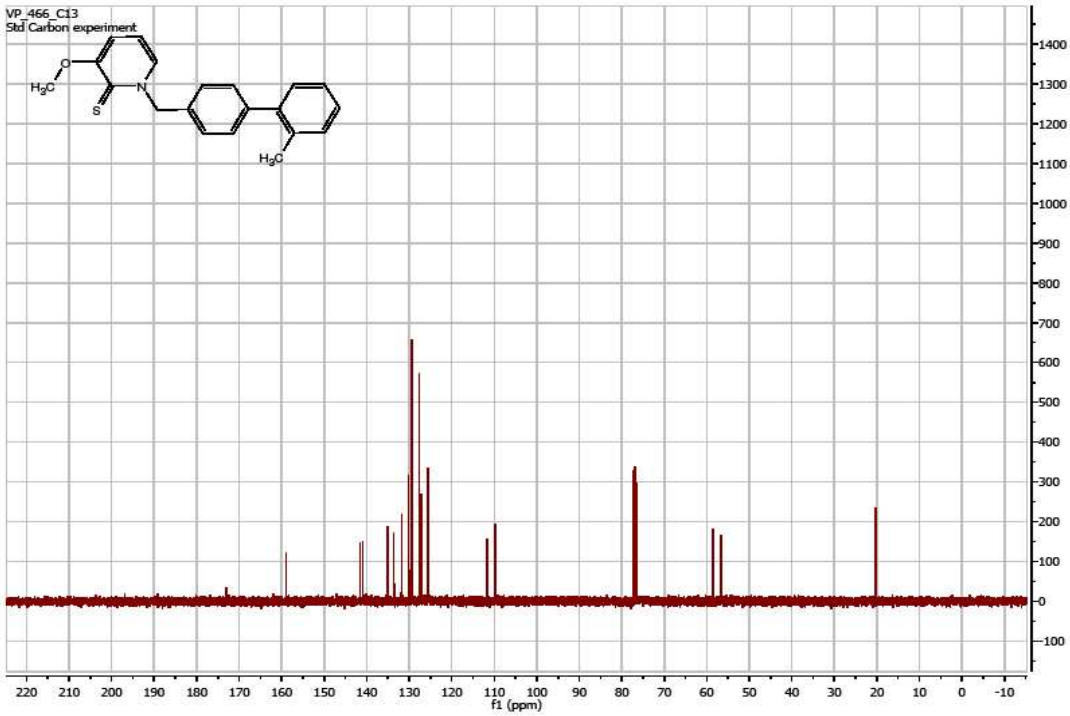
^{13}C NMR of **10e:**



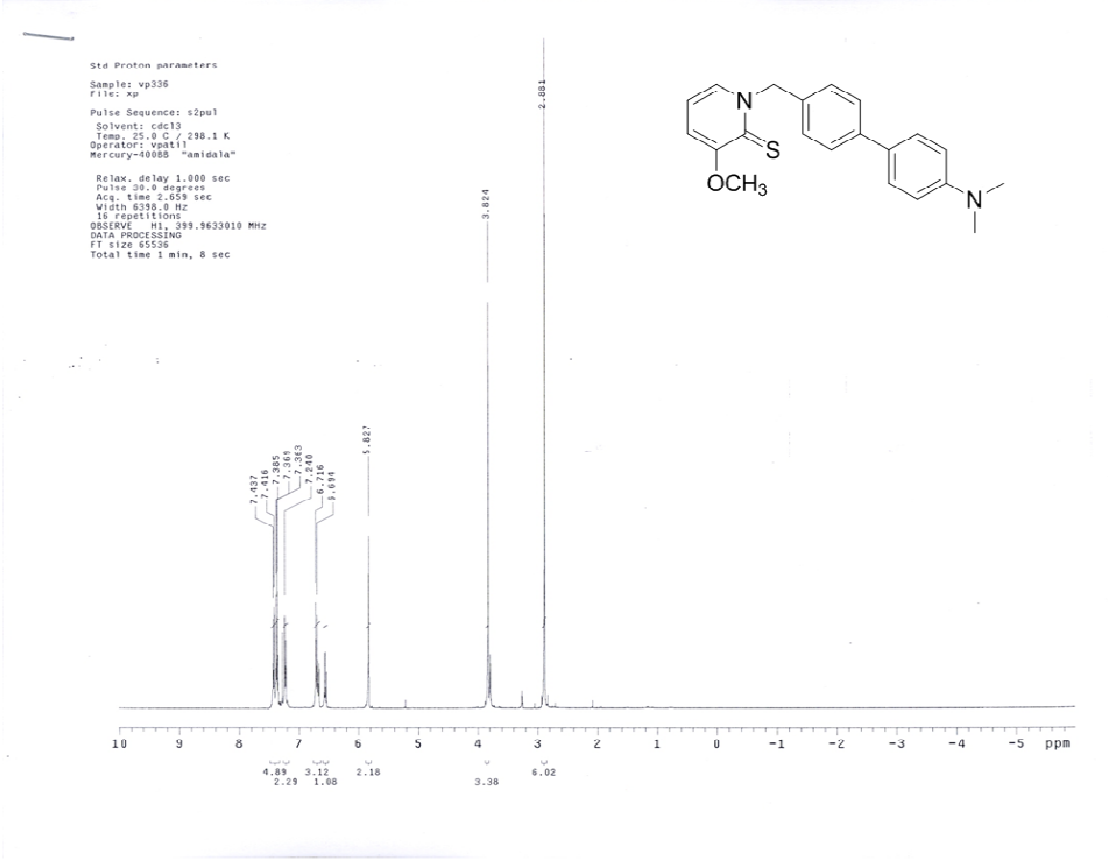
^{1}H NMR of **10f:**



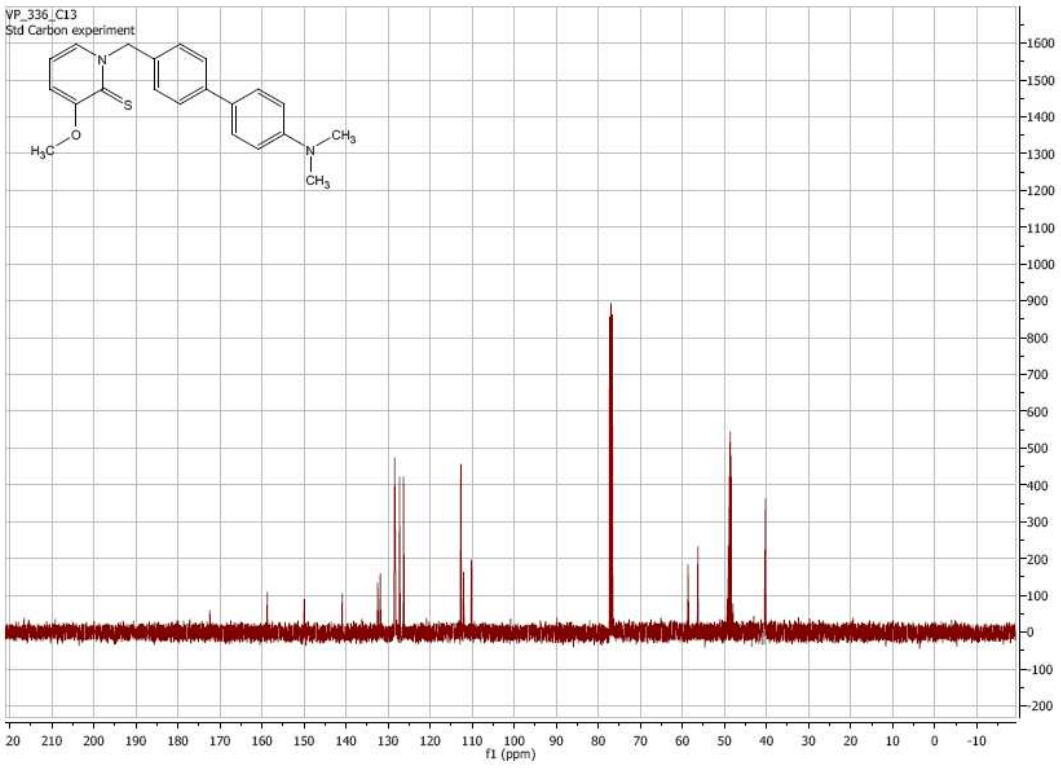
^{13}C NMR of **10f:**



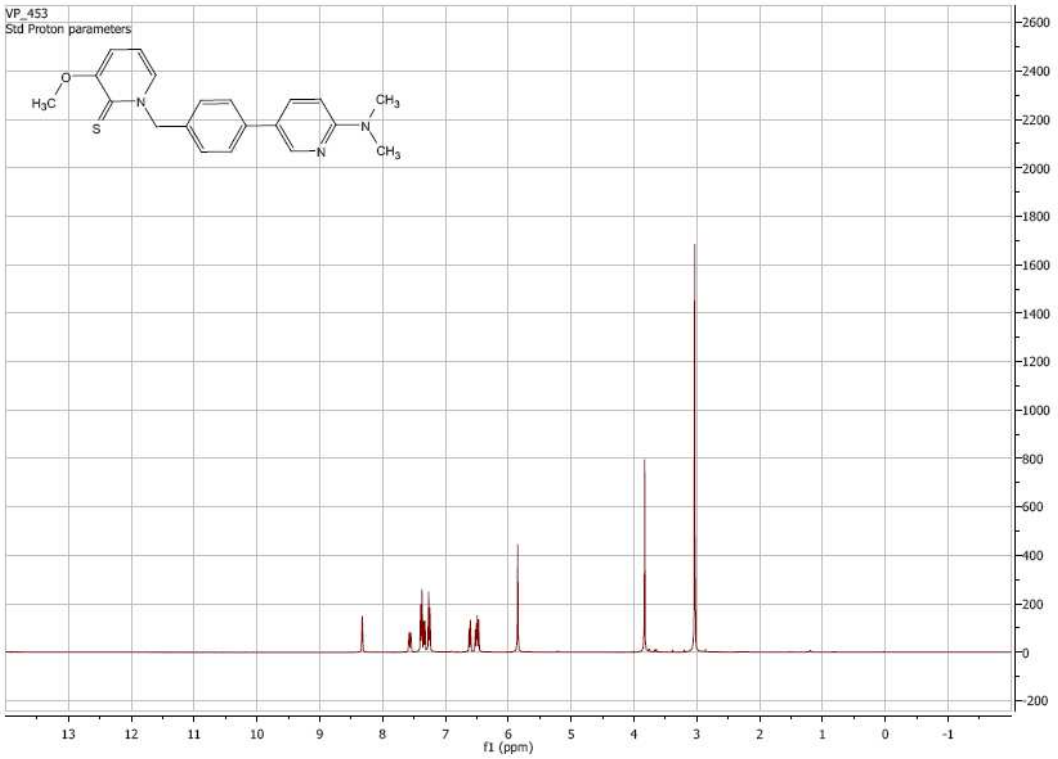
^{1}H NMR of **10g:**



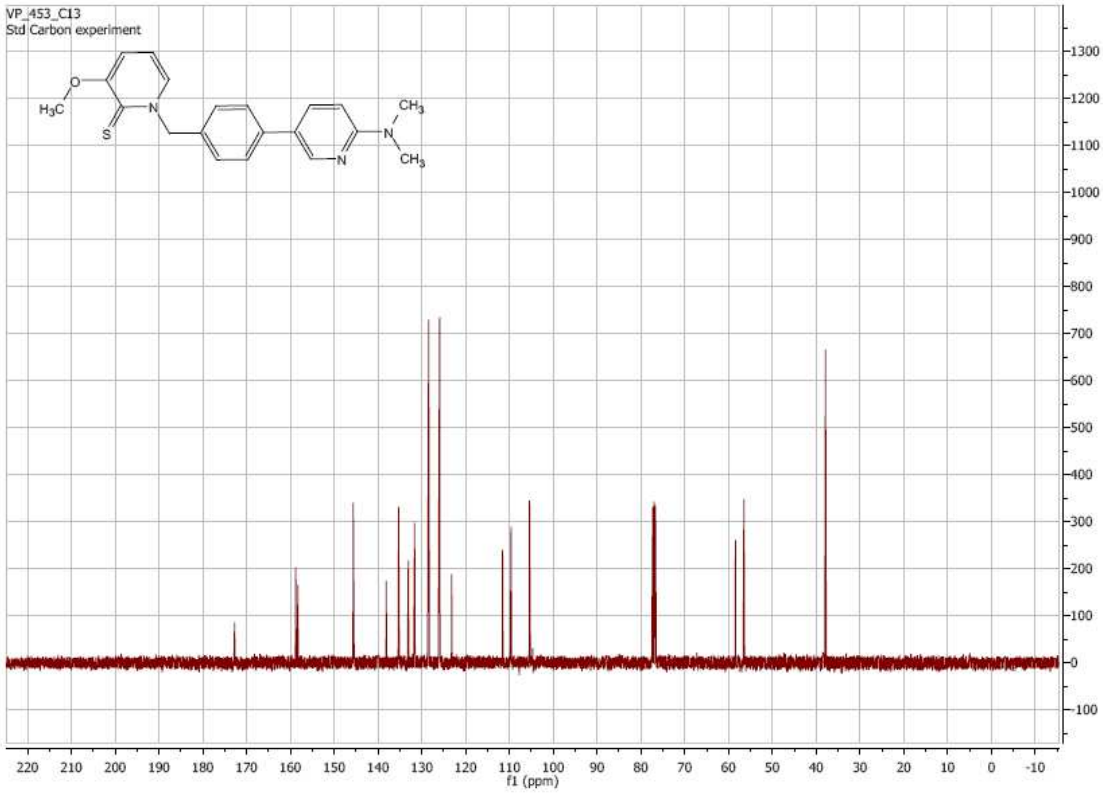
^{13}C NMR of **10g**



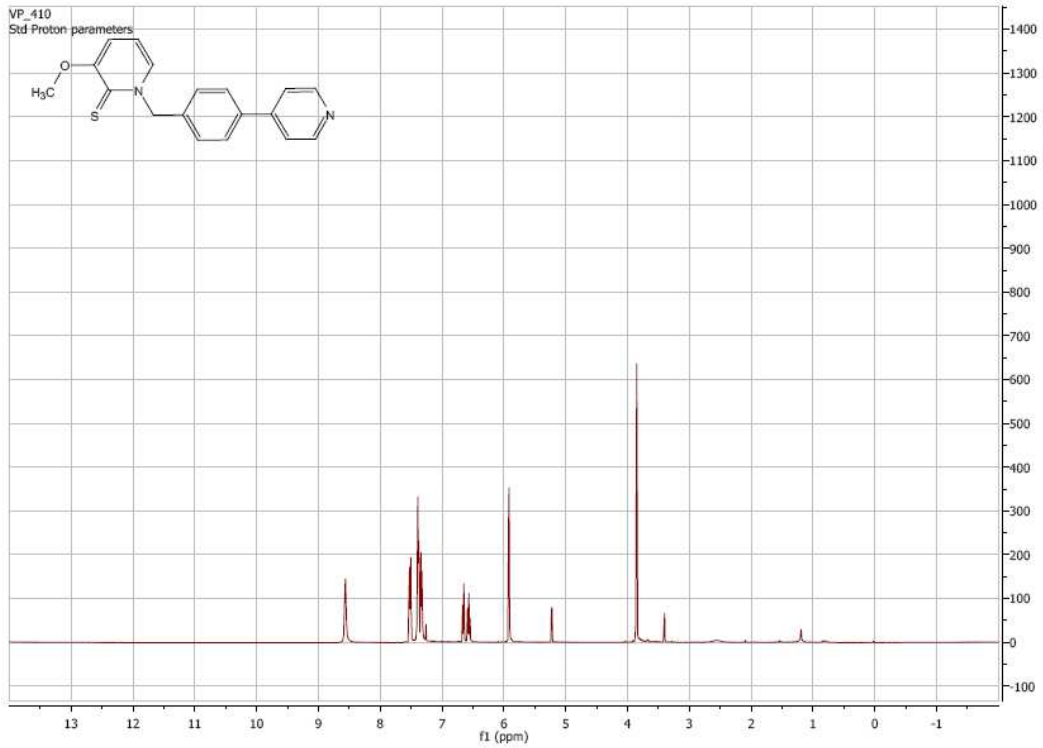
^{1}H NMR of **10h:**



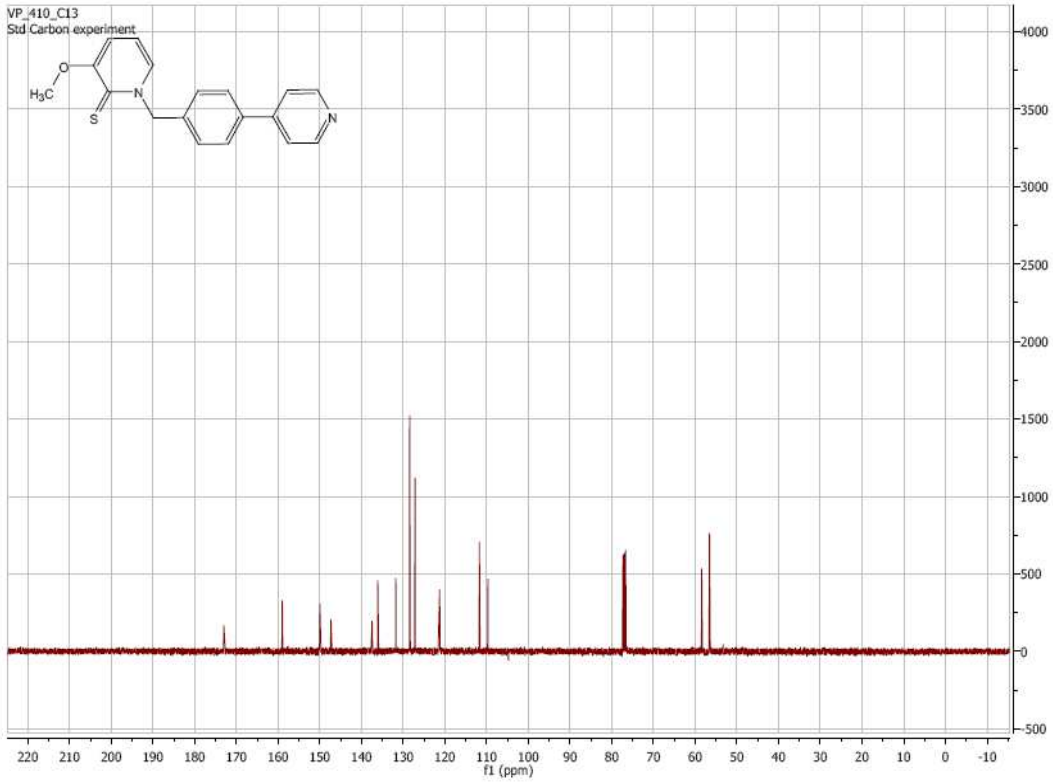
^{13}C NMR of **10h:**



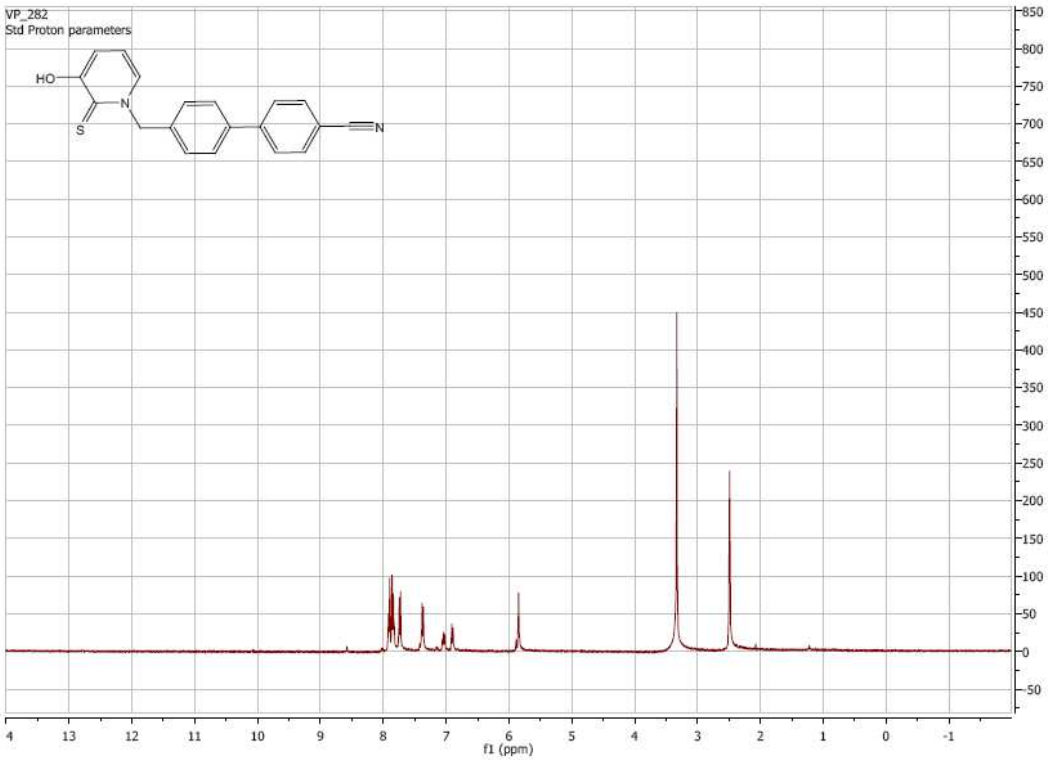
^{1}H NMR of **10i:**



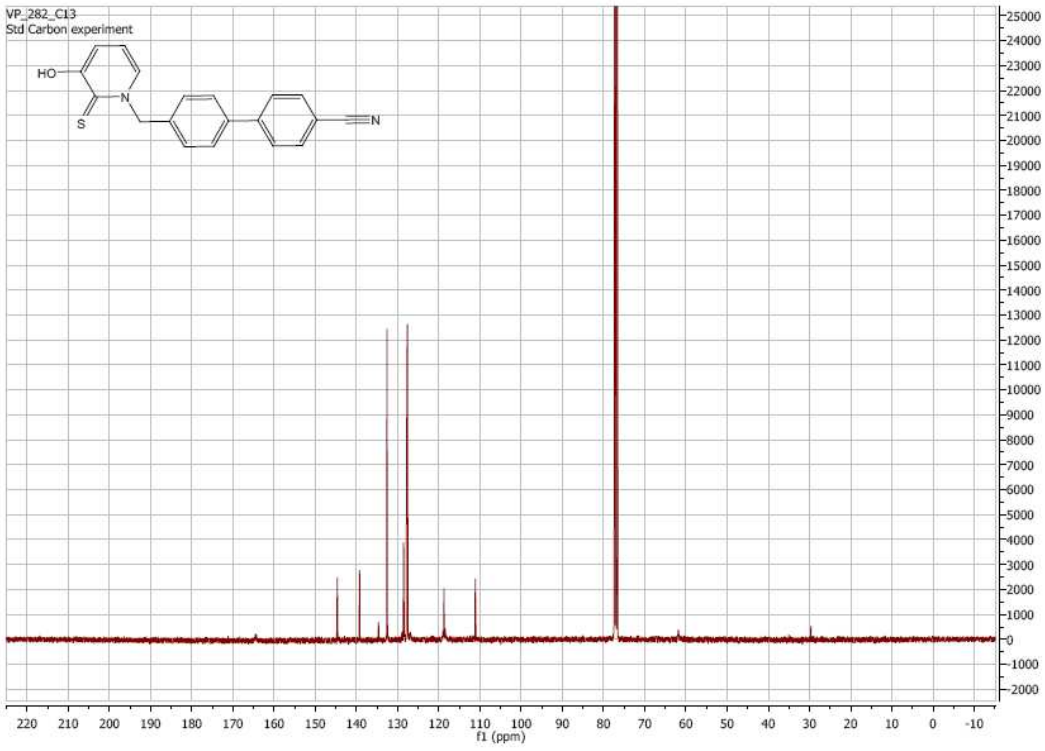
^{13}C NMR of **10i:**



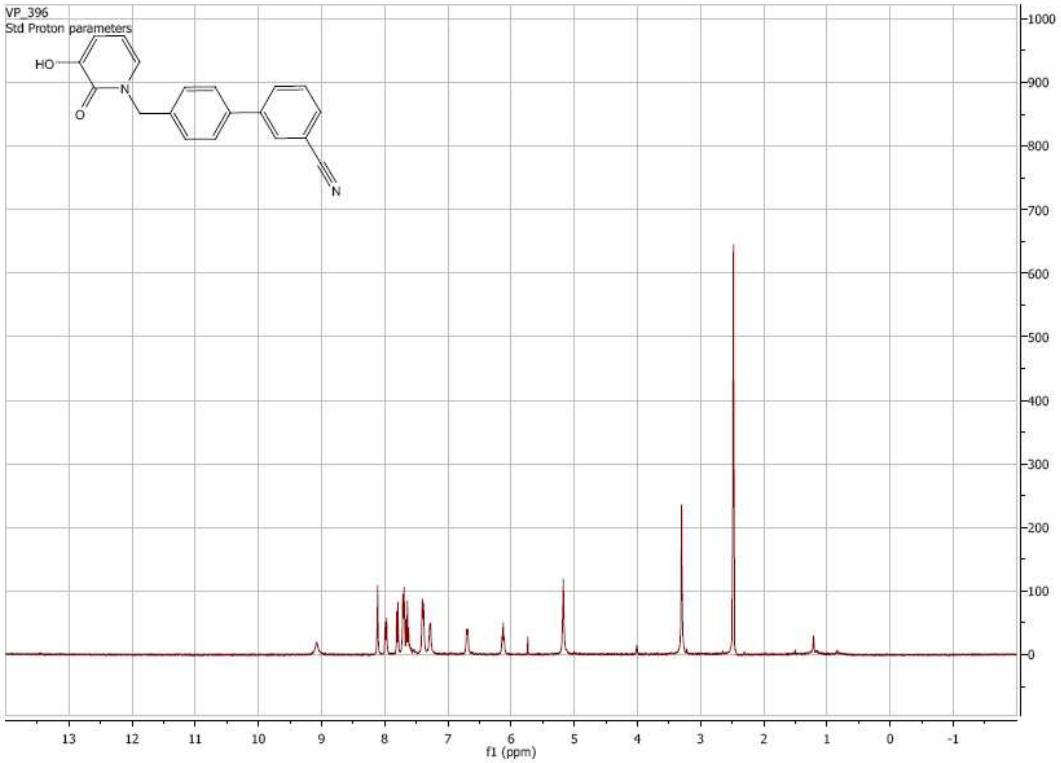
^{1}H NMR of **11a:**



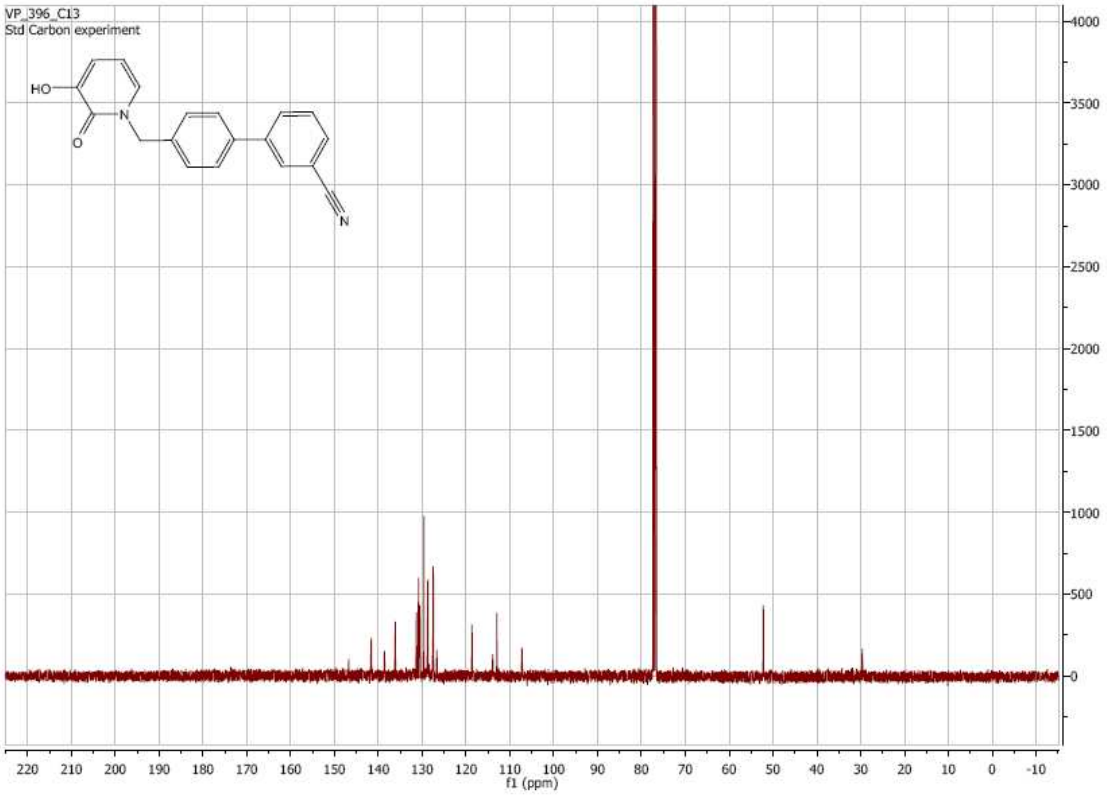
^{13}C NMR of **11a:**



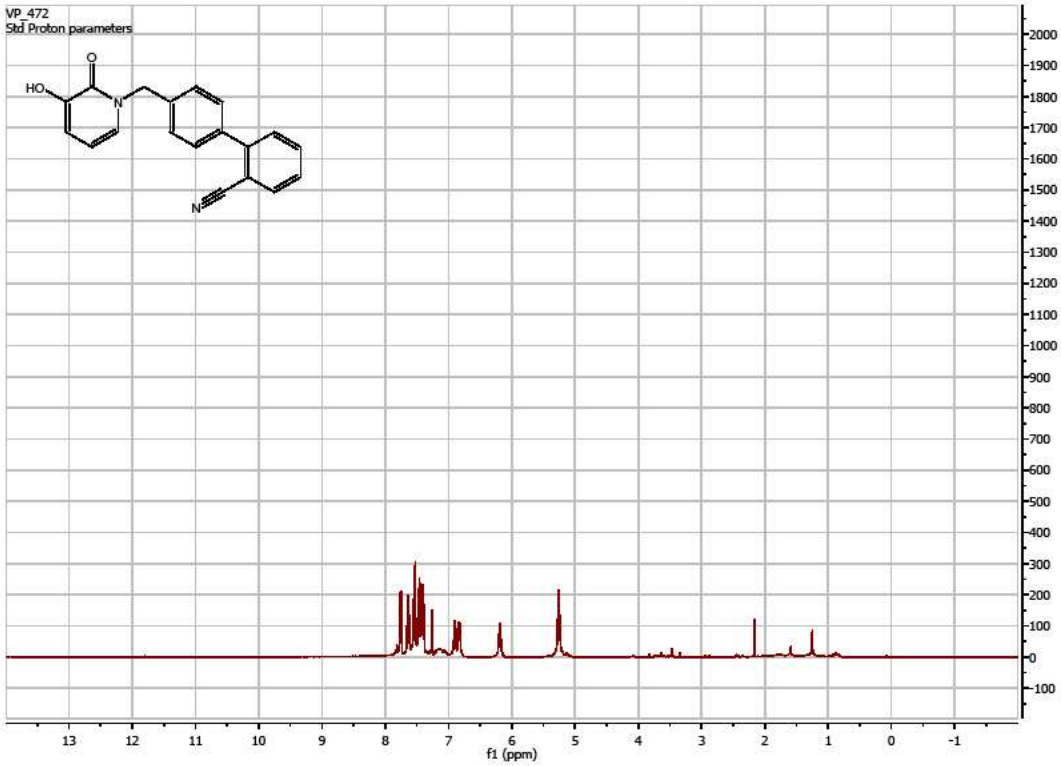
^{1}H NMR of **11b:**



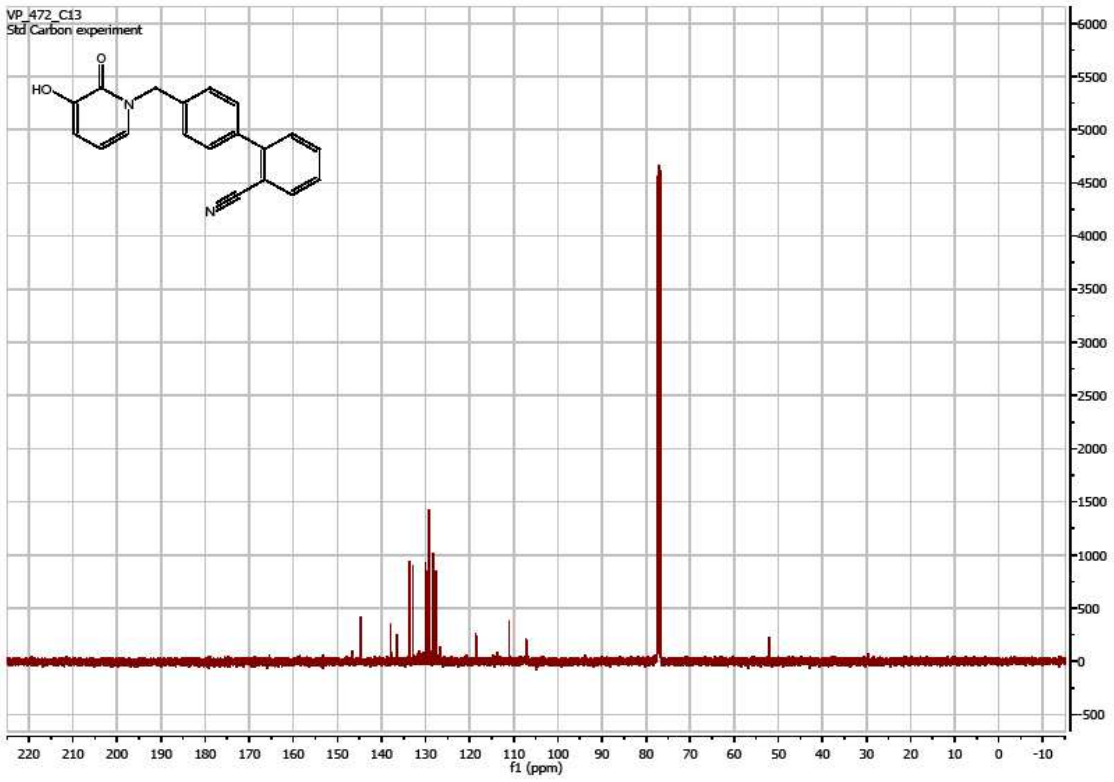
^{13}C NMR of **11b:**



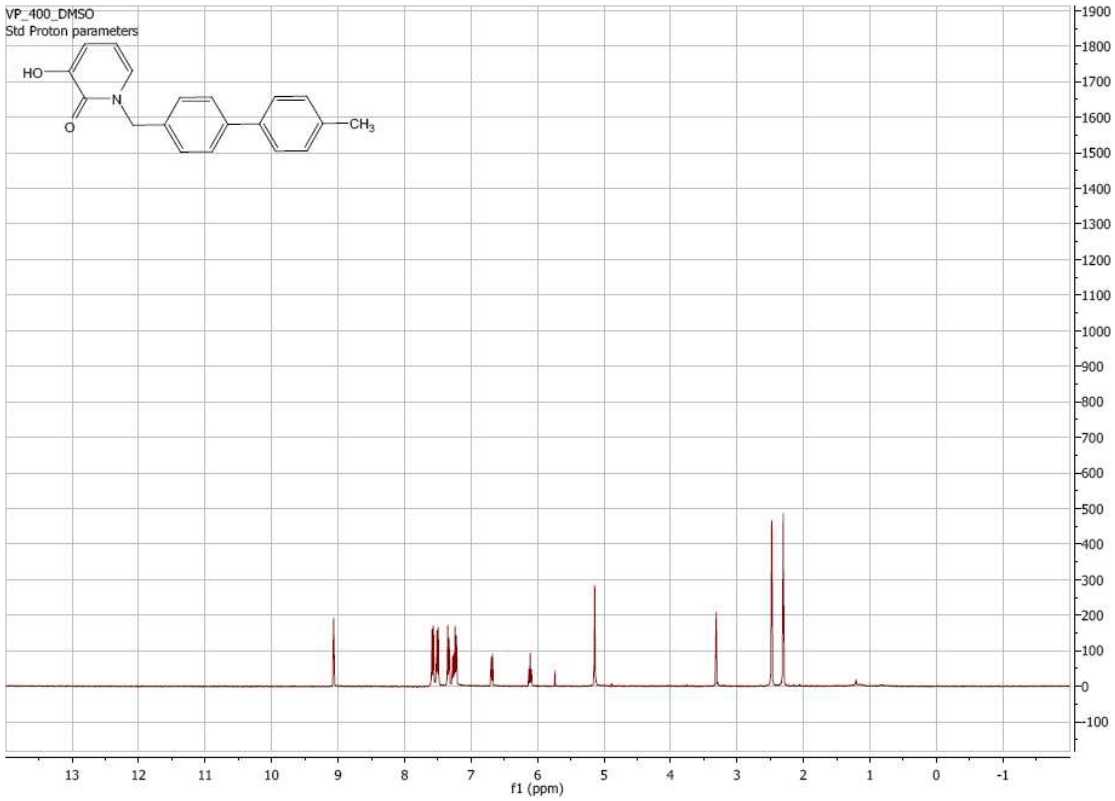
^{1}H NMR of **11c:**



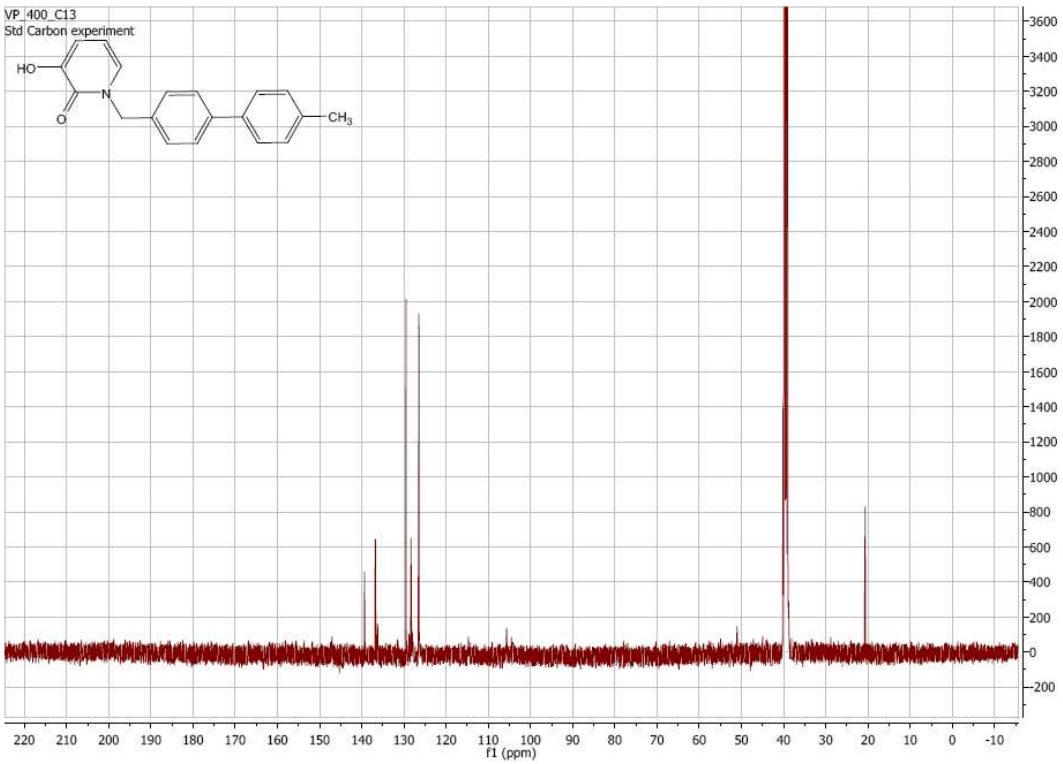
^{13}C NMR of **11c:**



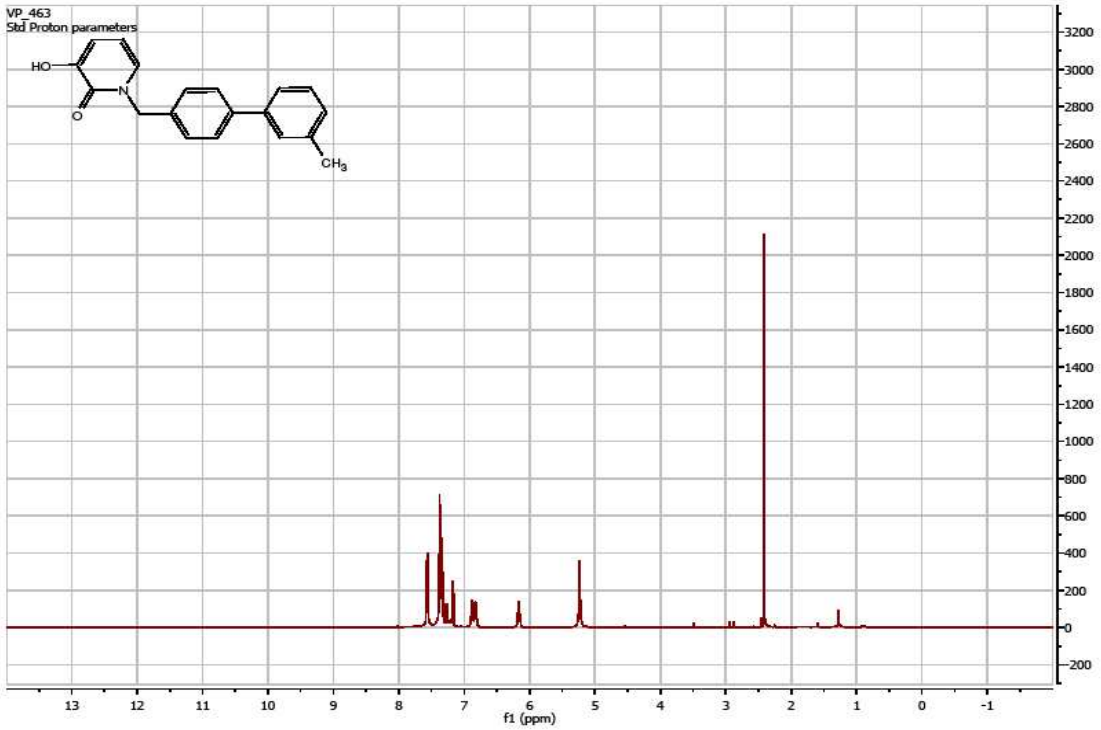
^{1}H NMR of **11d:**



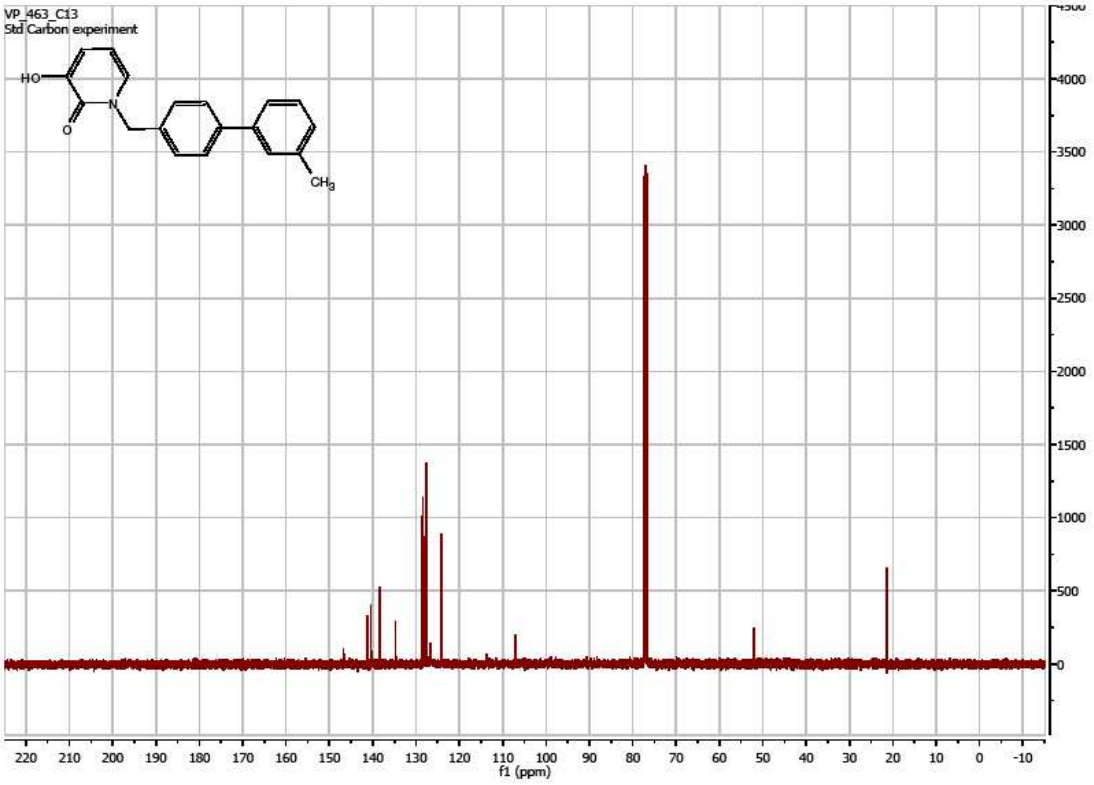
^{13}C NMR of **11d:**



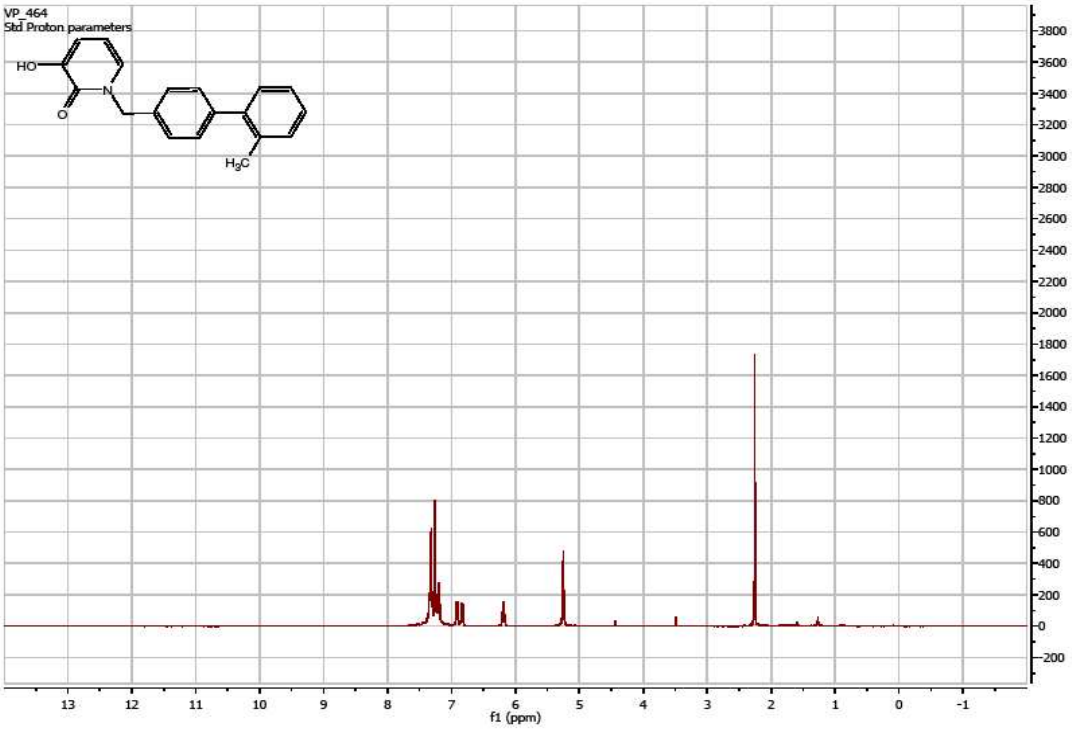
^{1}H NMR of **11e:**



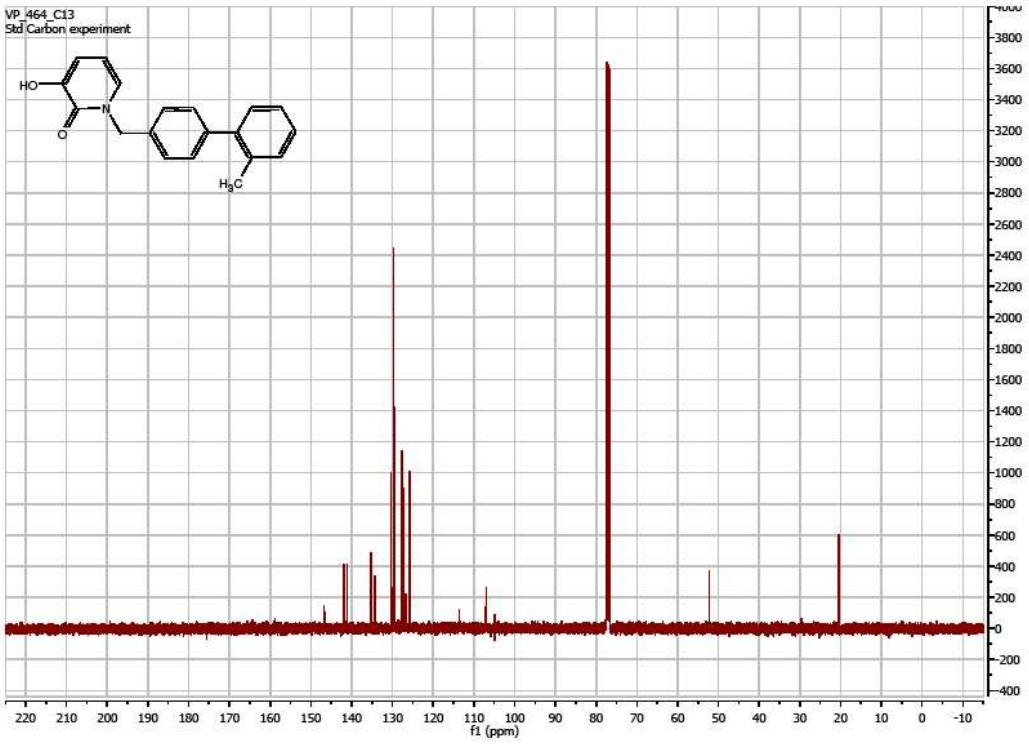
^{13}C NMR of **11e:**



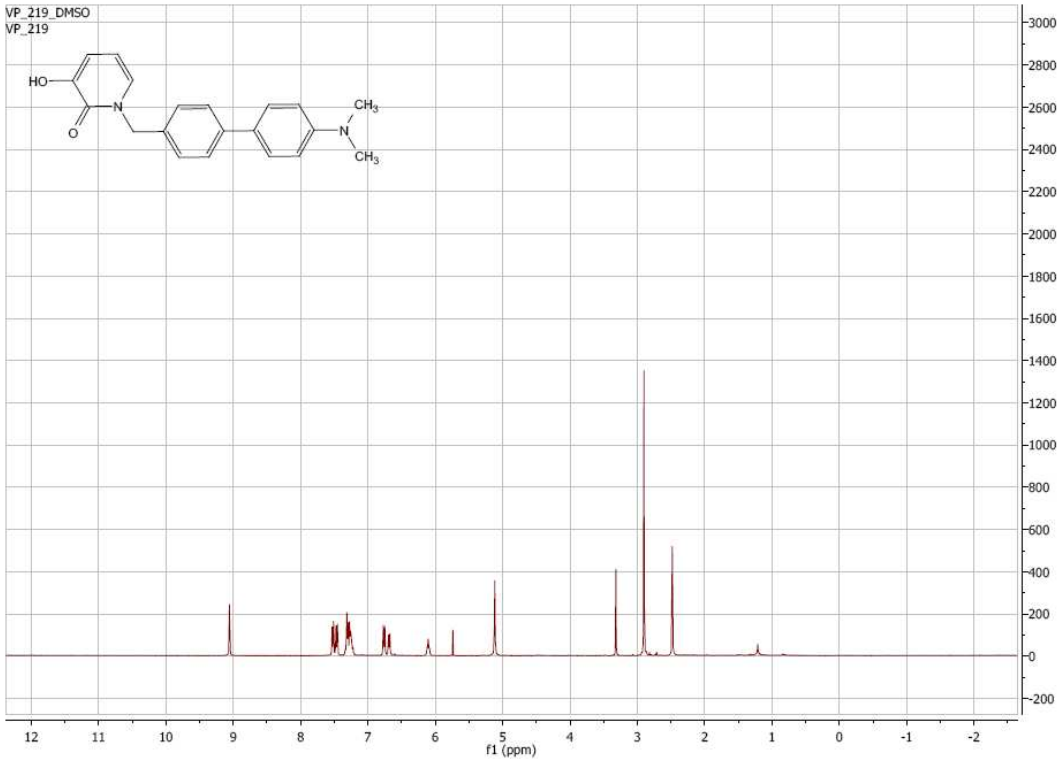
^{1}H NMR of **11f:**



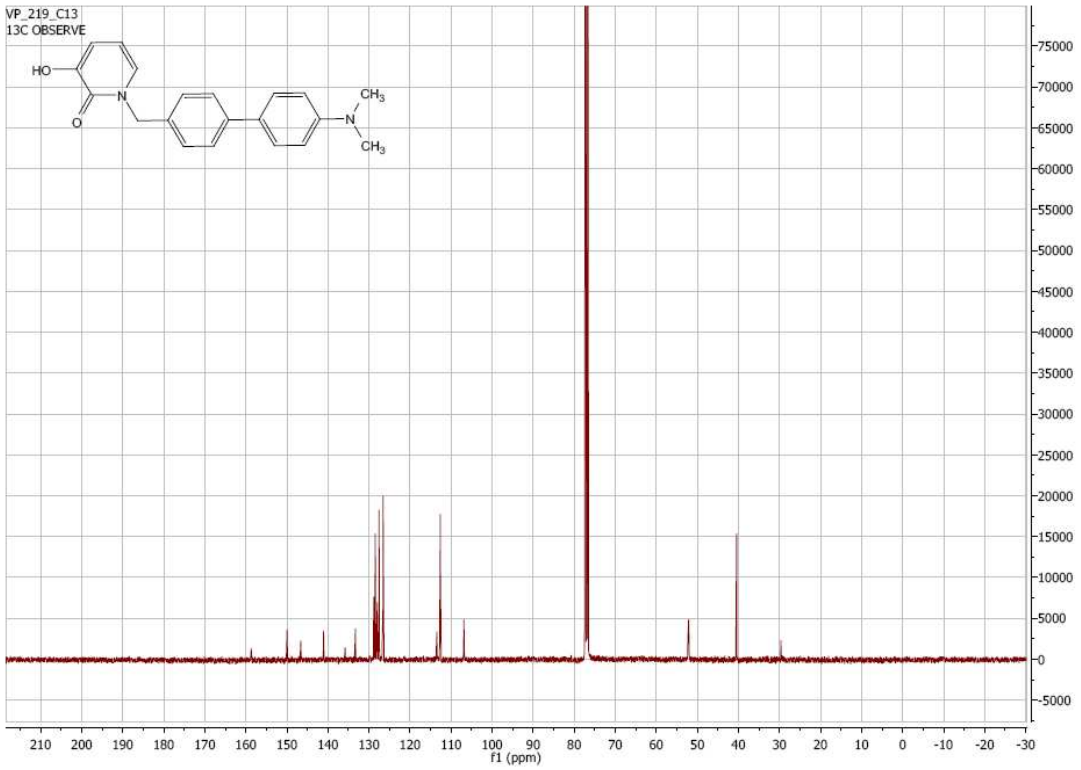
^{13}C NMR of **11f:**


VP_464_C13
Std Carbon experiment
HO
O
N
H3C
f1 (ppm)

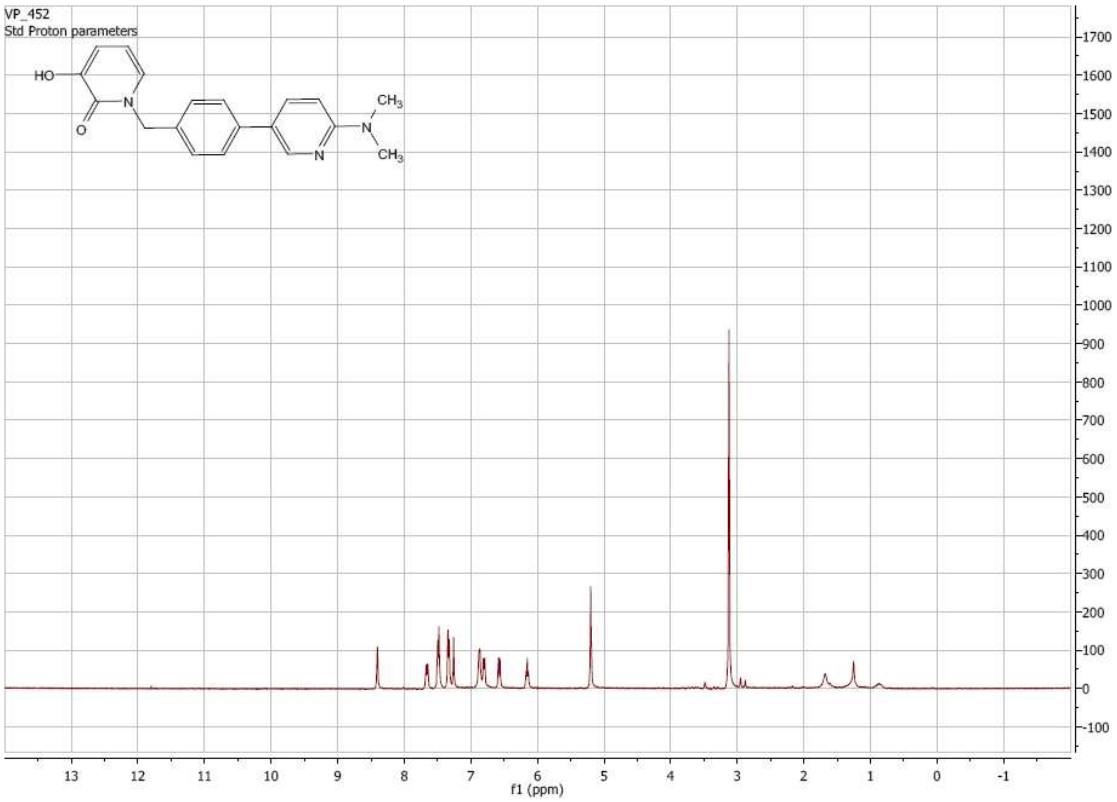
^{1}H NMR of **11g:**


VP_219_DMSO
VP_219
HO
O
N
CH3
N
CH3
f1 (ppm)

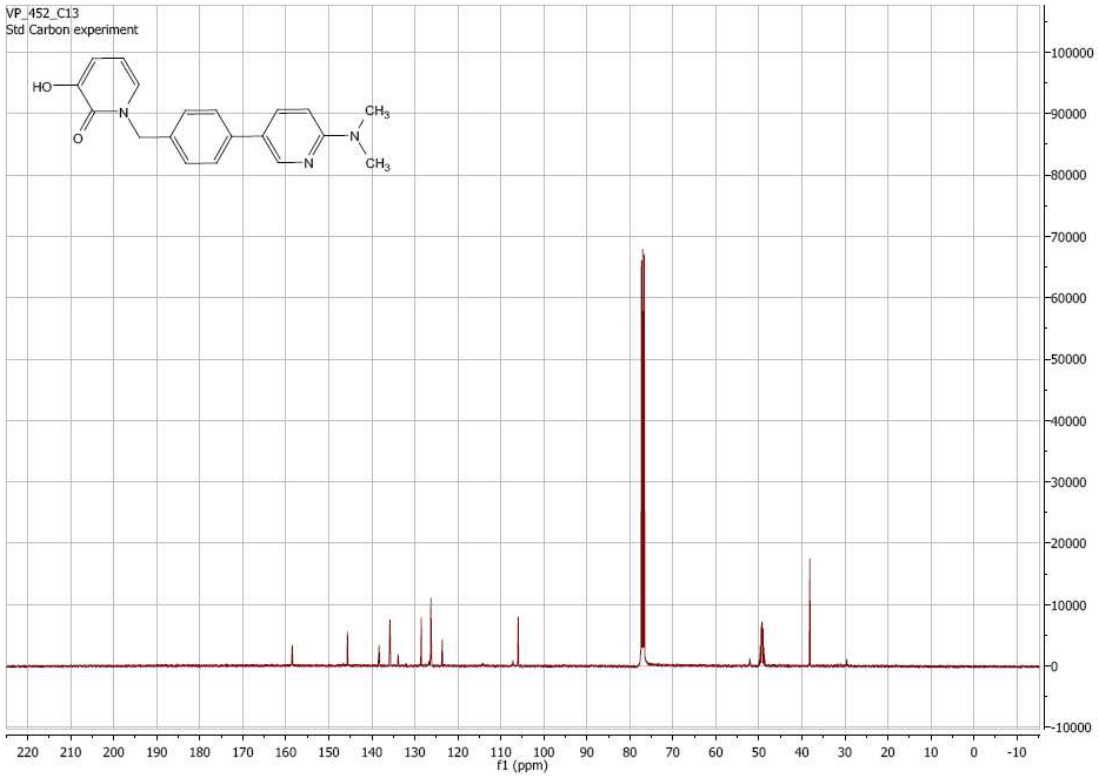
^{13}C NMR of **11g:**



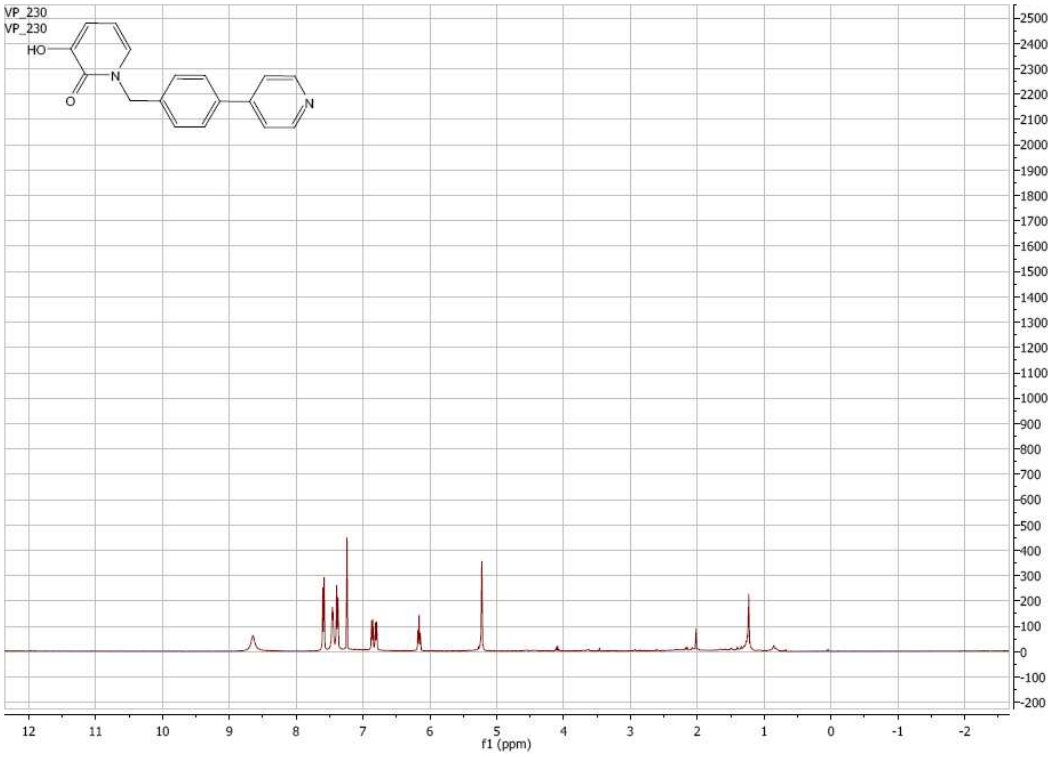
^{1}H NMR of **11h:**



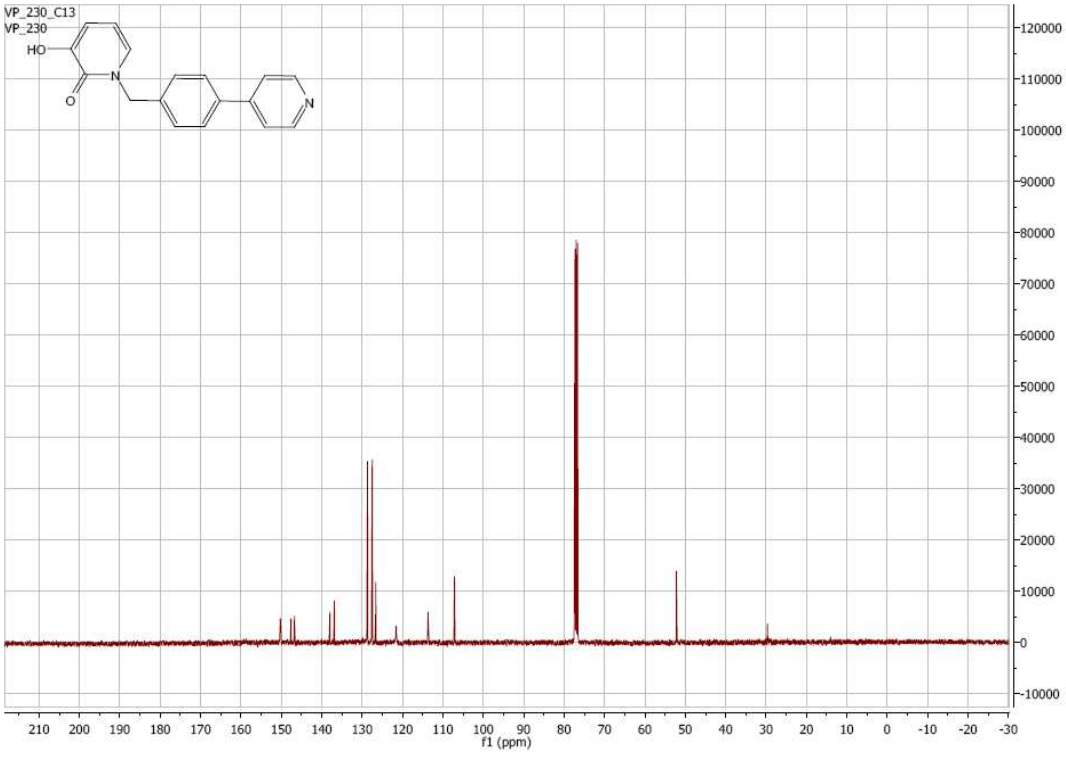
^{13C} NMR of **11h:**



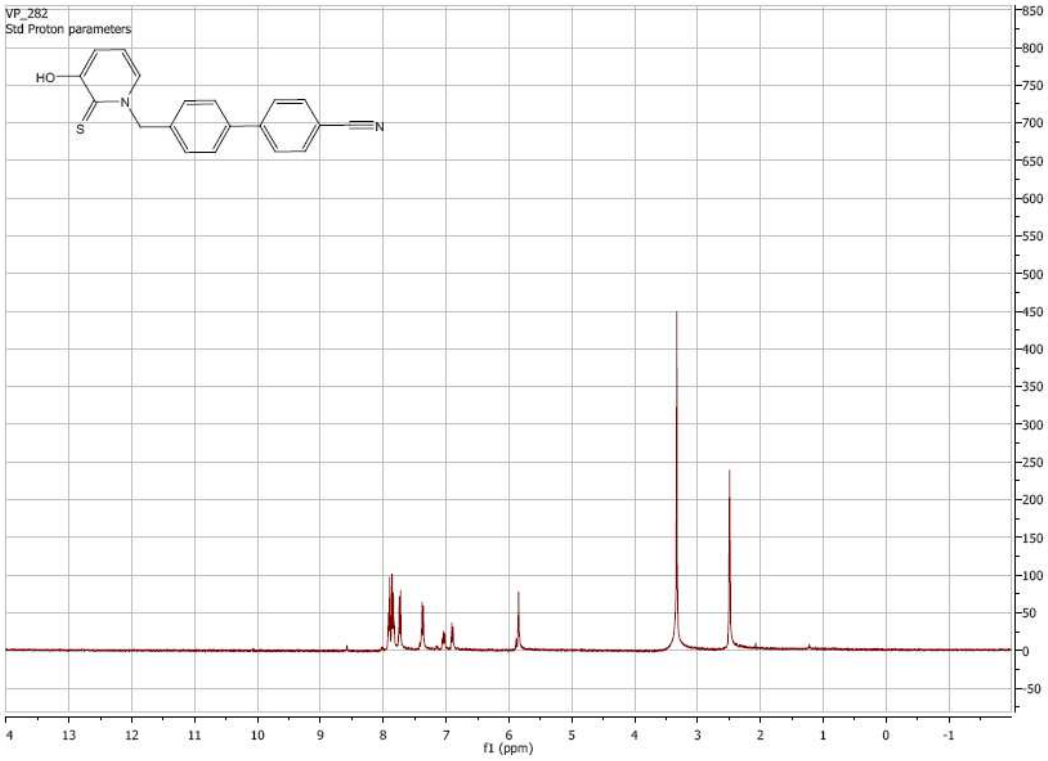
^{1}H NMR of **11i:**



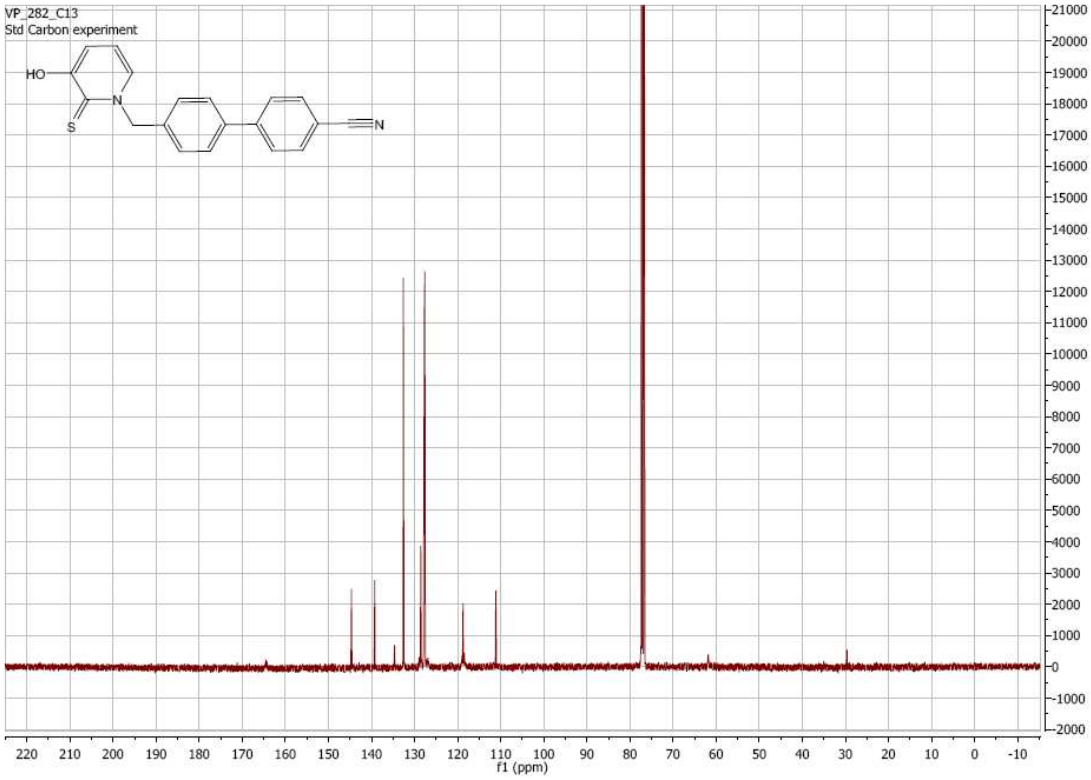
^{13}C NMR of **11i:**



^{1}H NMR of **12a:**



^{13}C NMR of **12a:**



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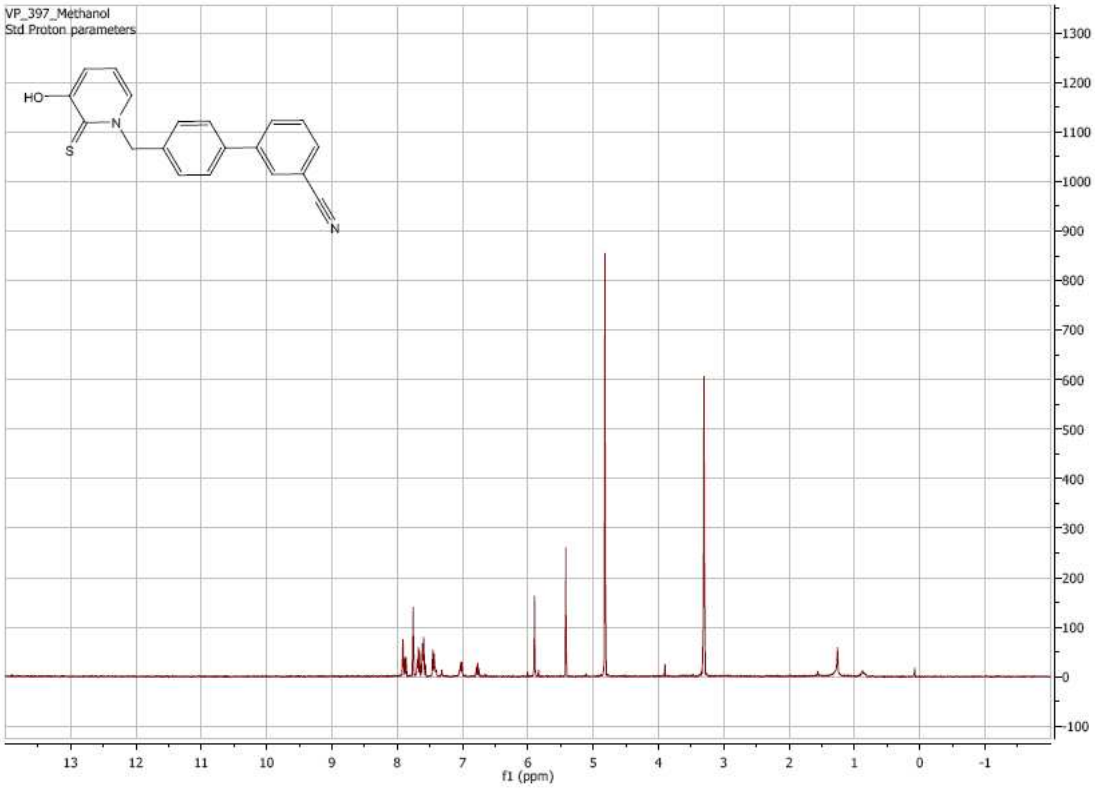
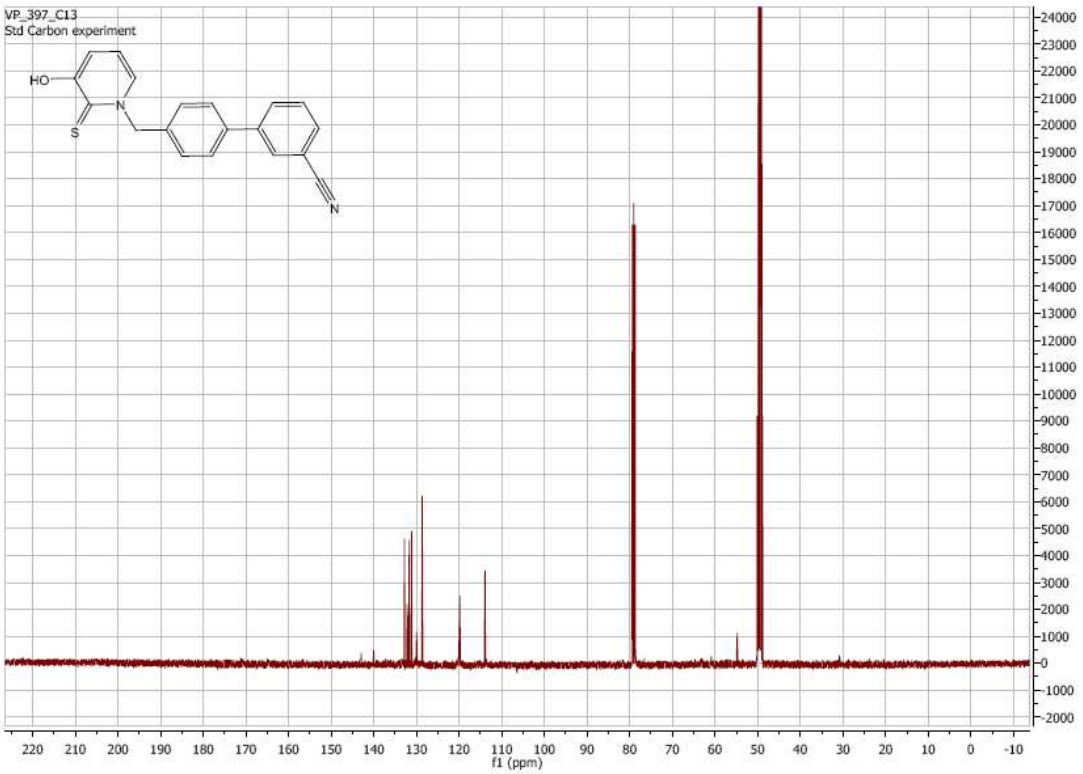
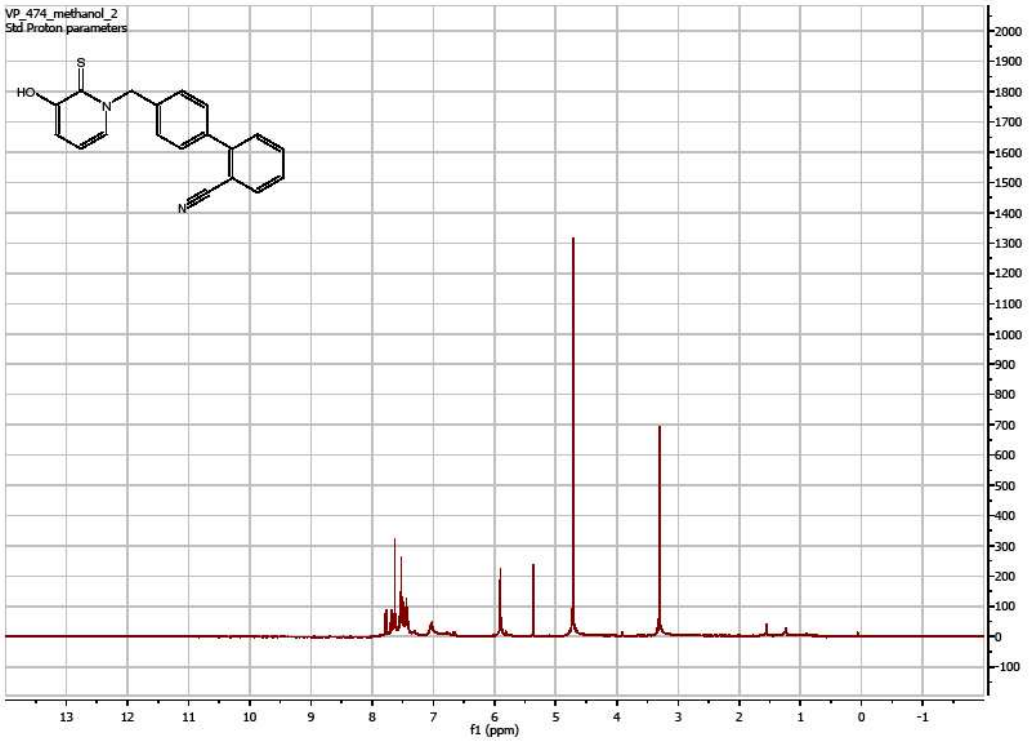


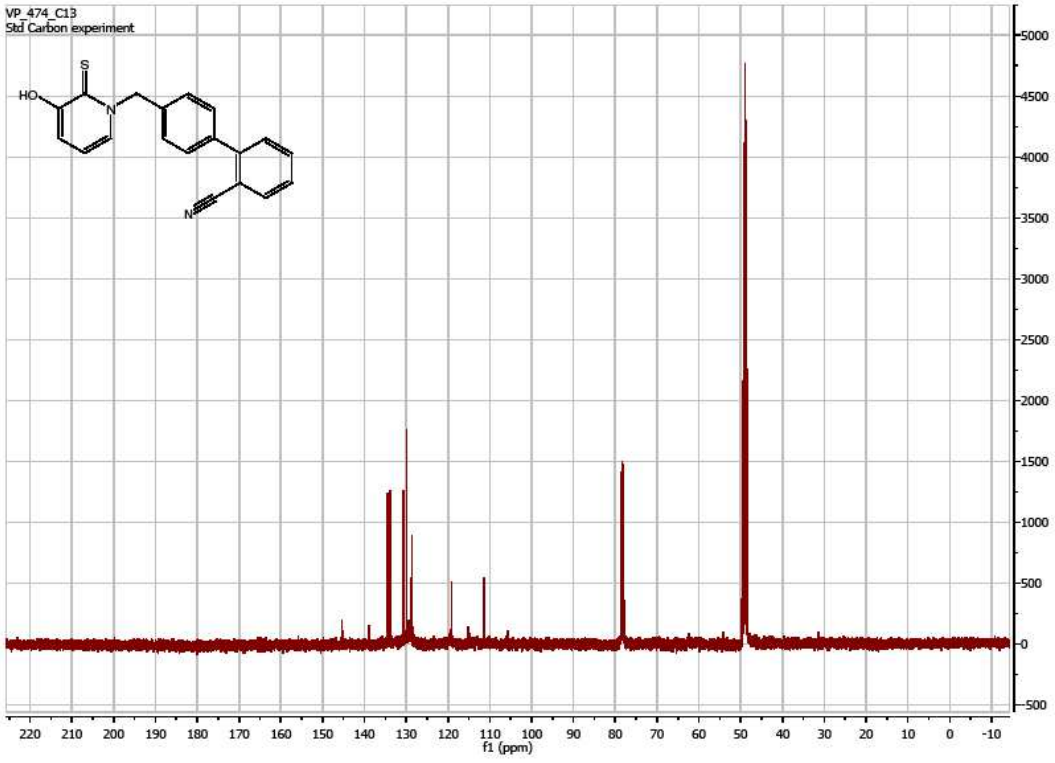
^{13}C NMR of **12b:**



^{1}H NMR of **12c:**



^{13}C NMR of **12c:**



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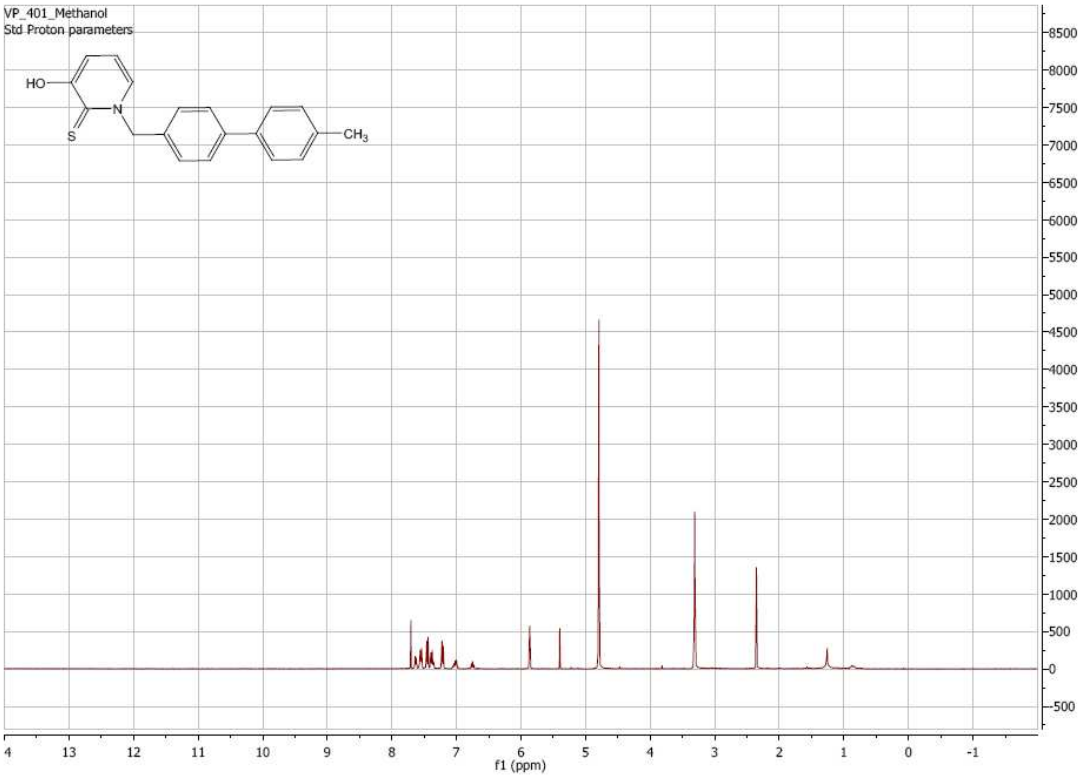
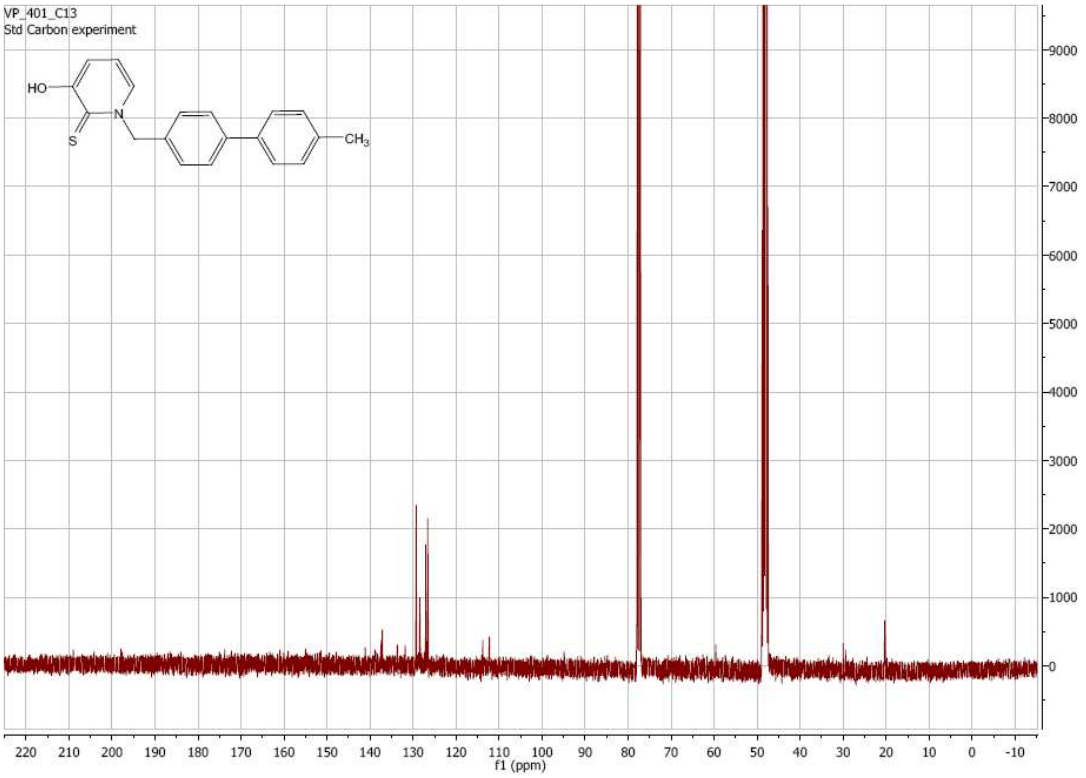


^{13}C NMR of **12d:**



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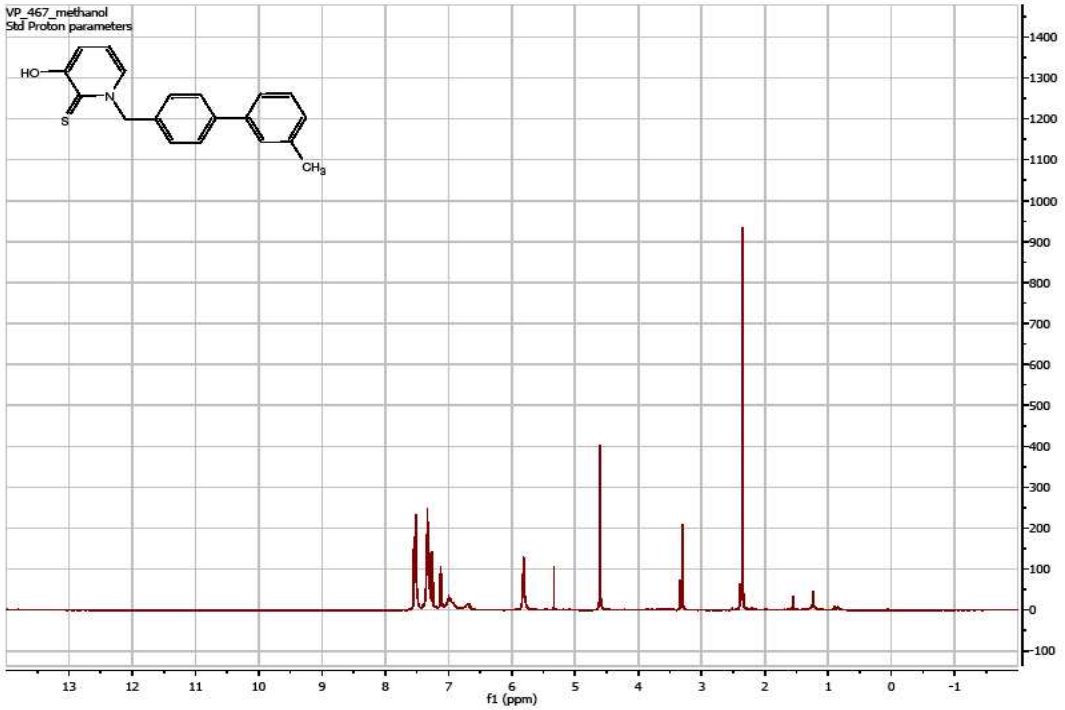
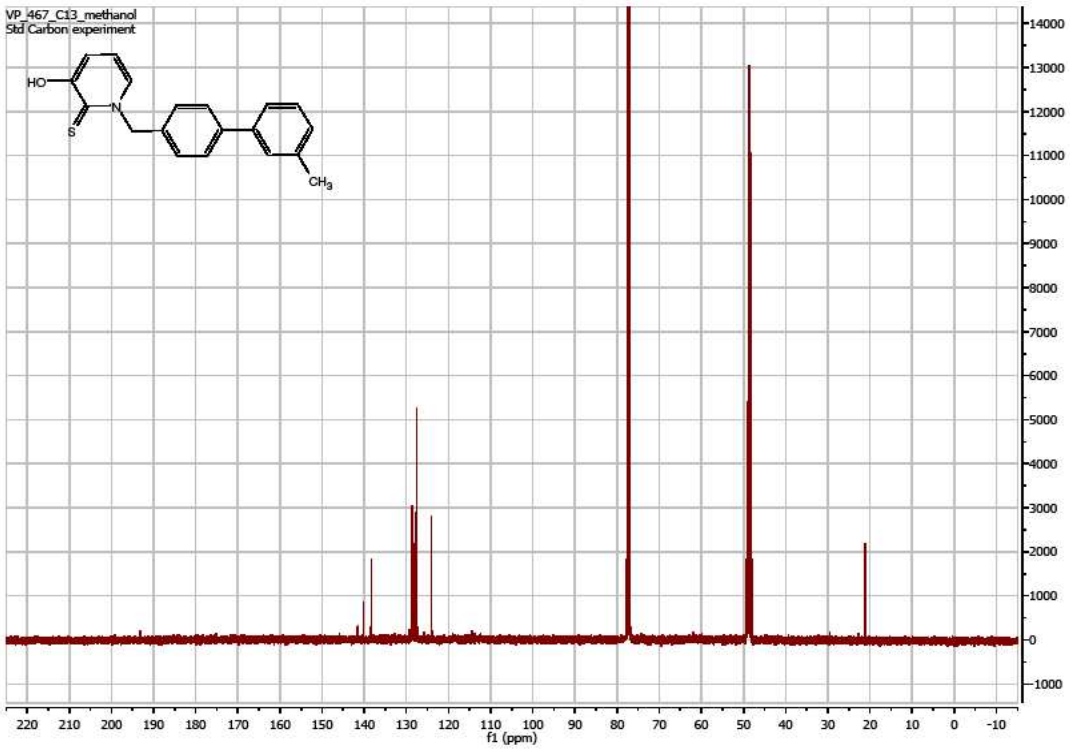


^{13}C NMR of **12e:**



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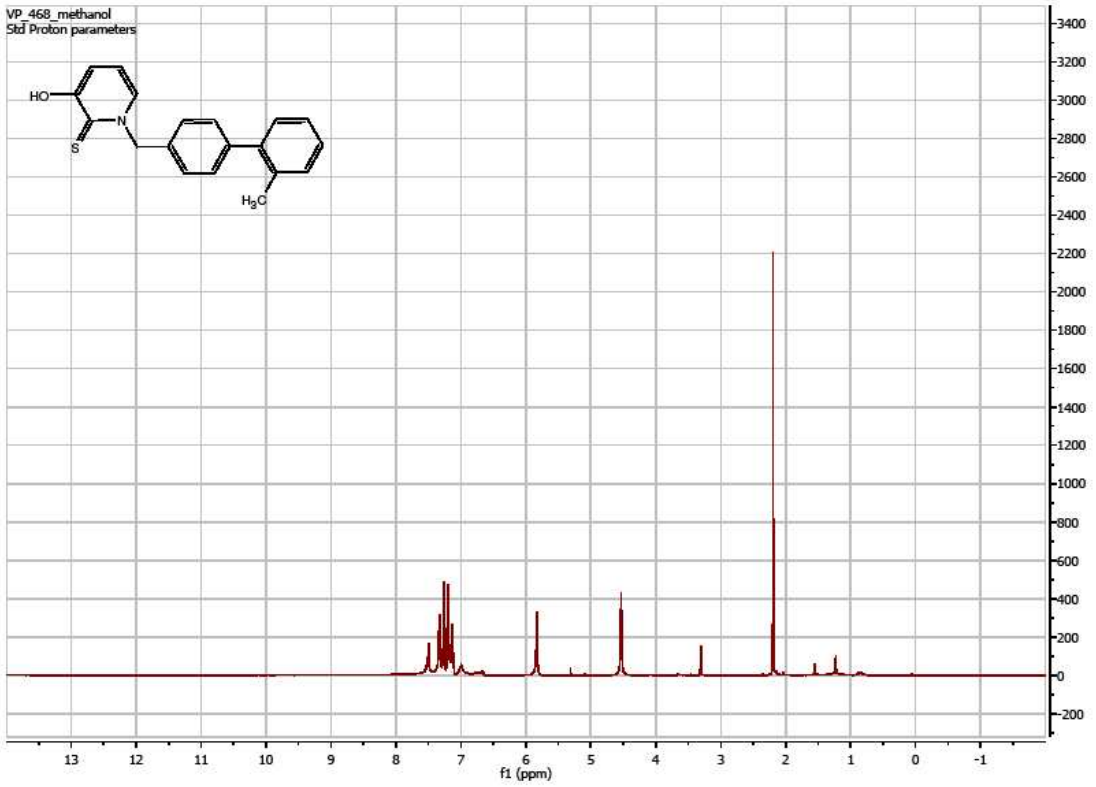
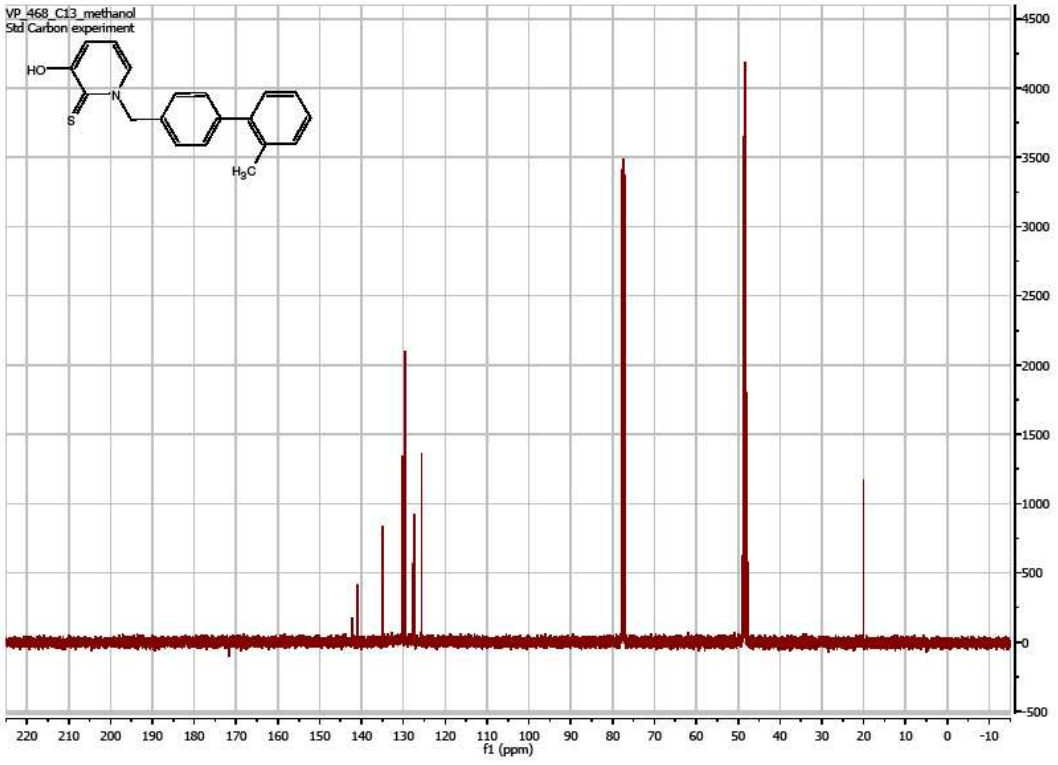
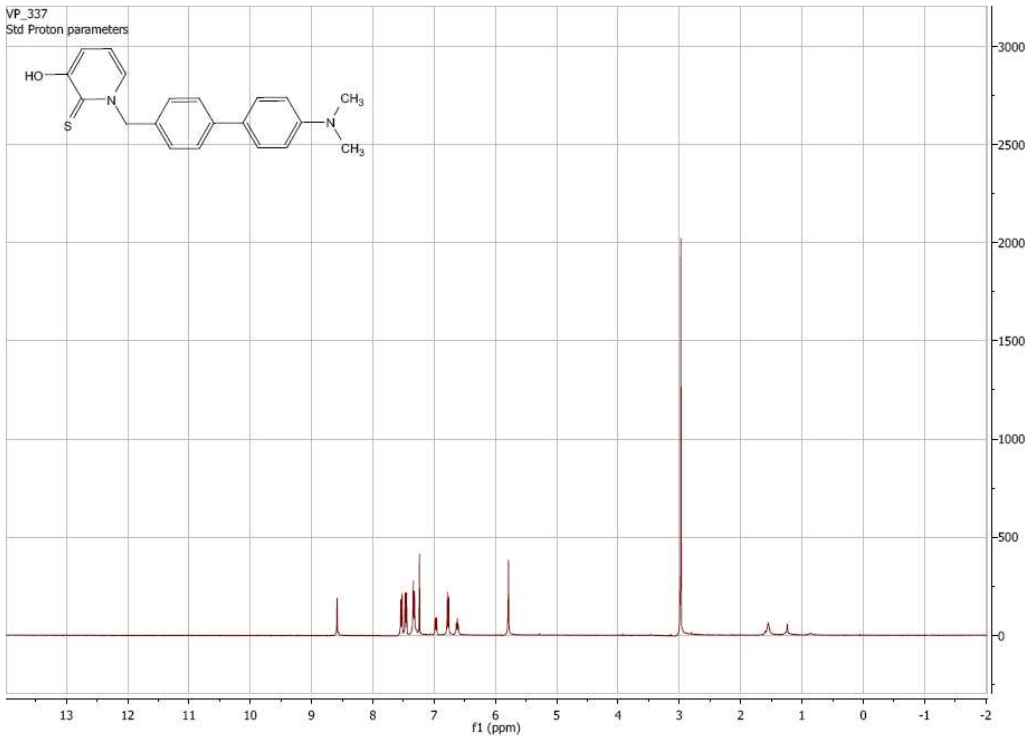


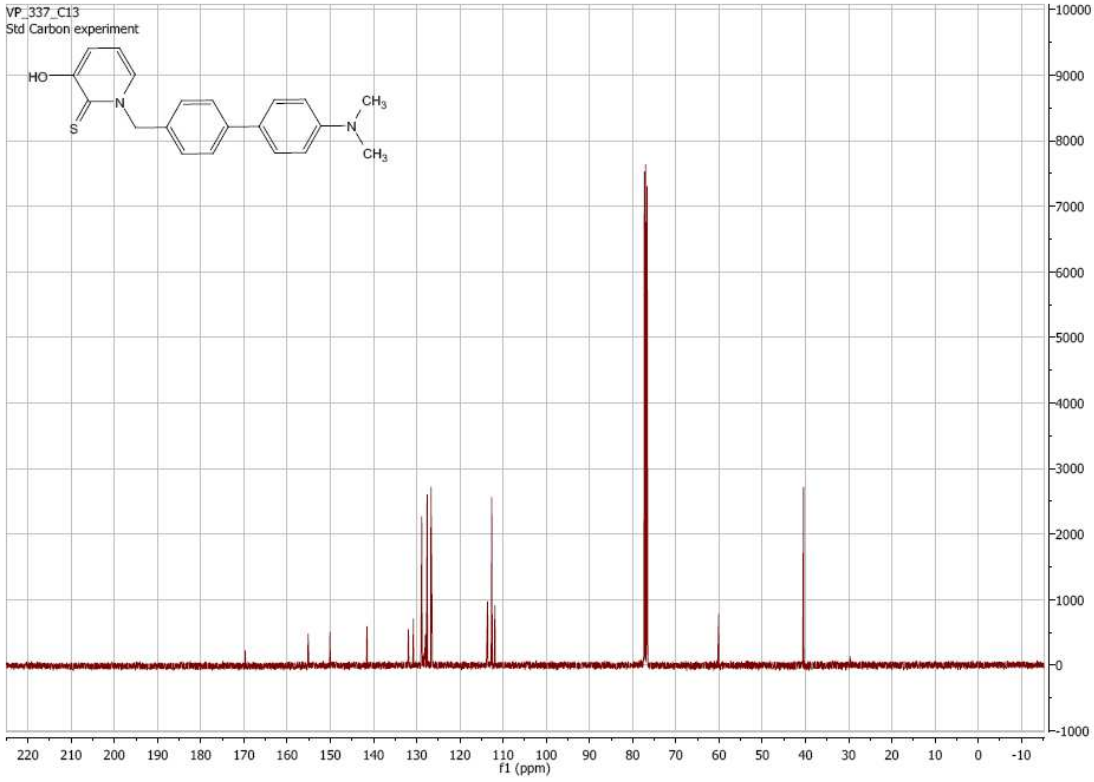
^{13}C NMR of **12f:**



^{1}H NMR of **12g:**



^{13}C NMR of **12g:**



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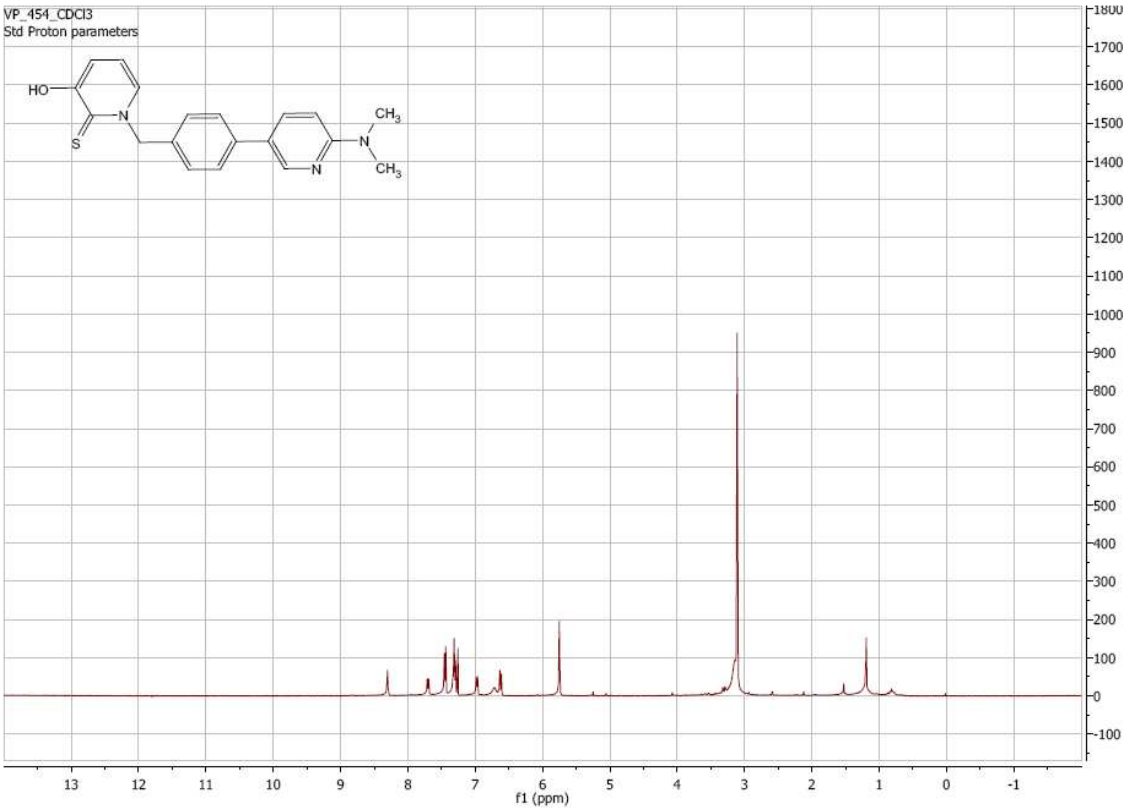
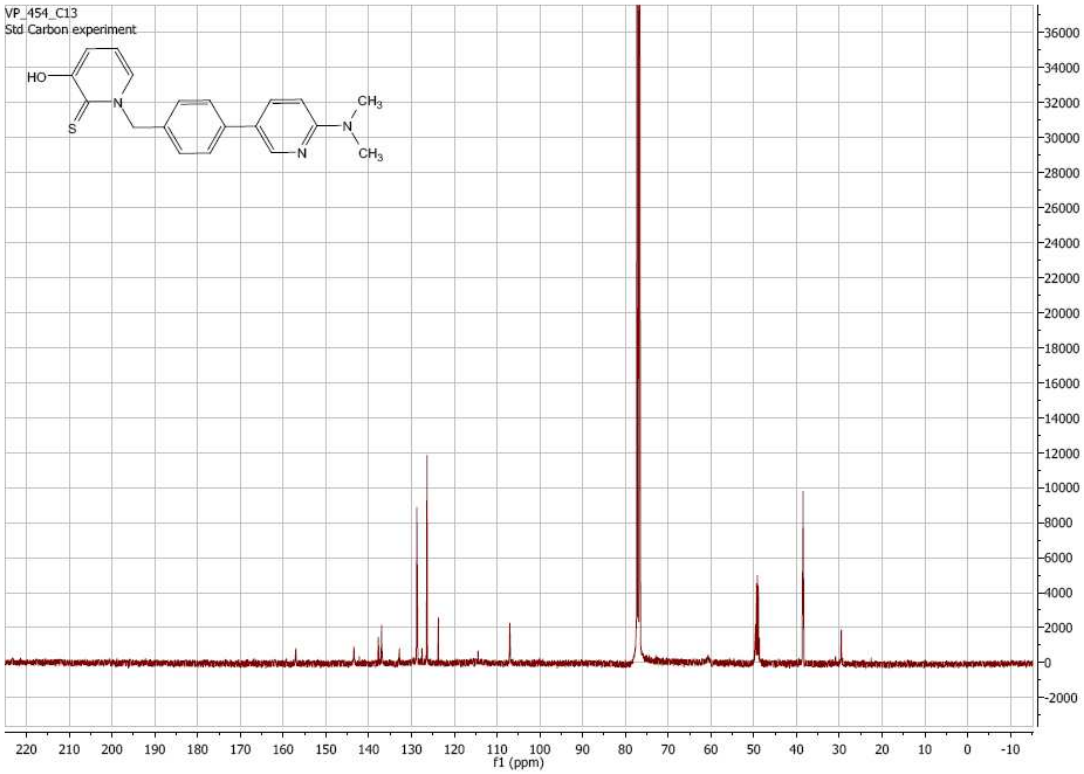
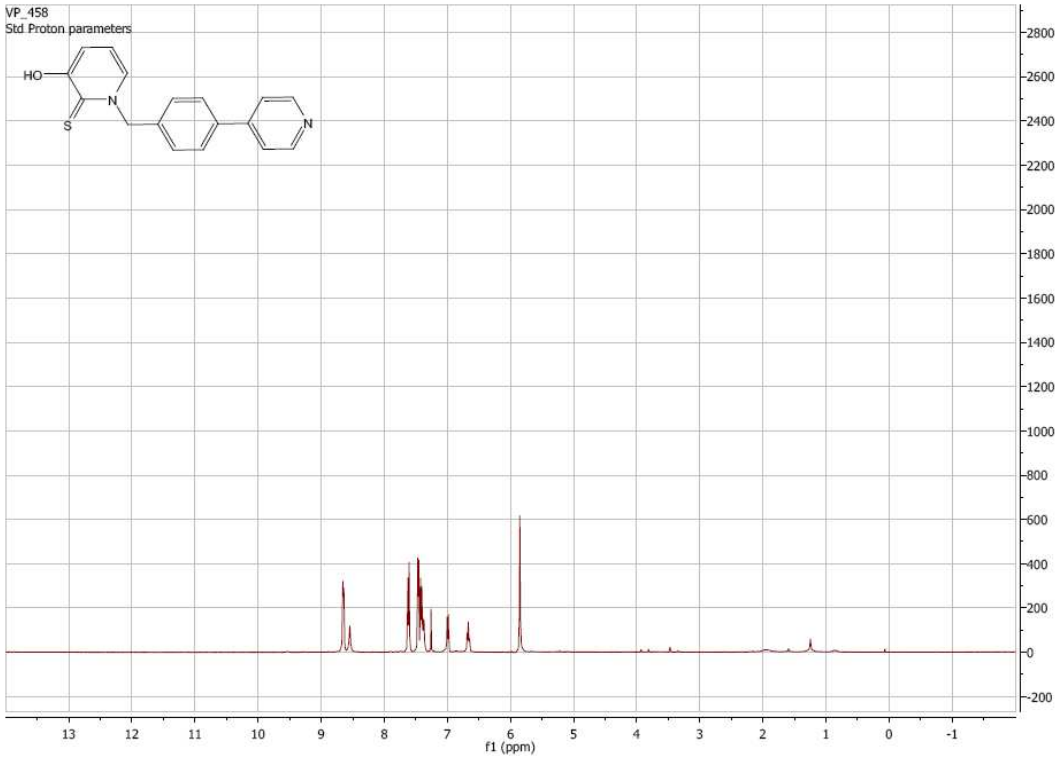


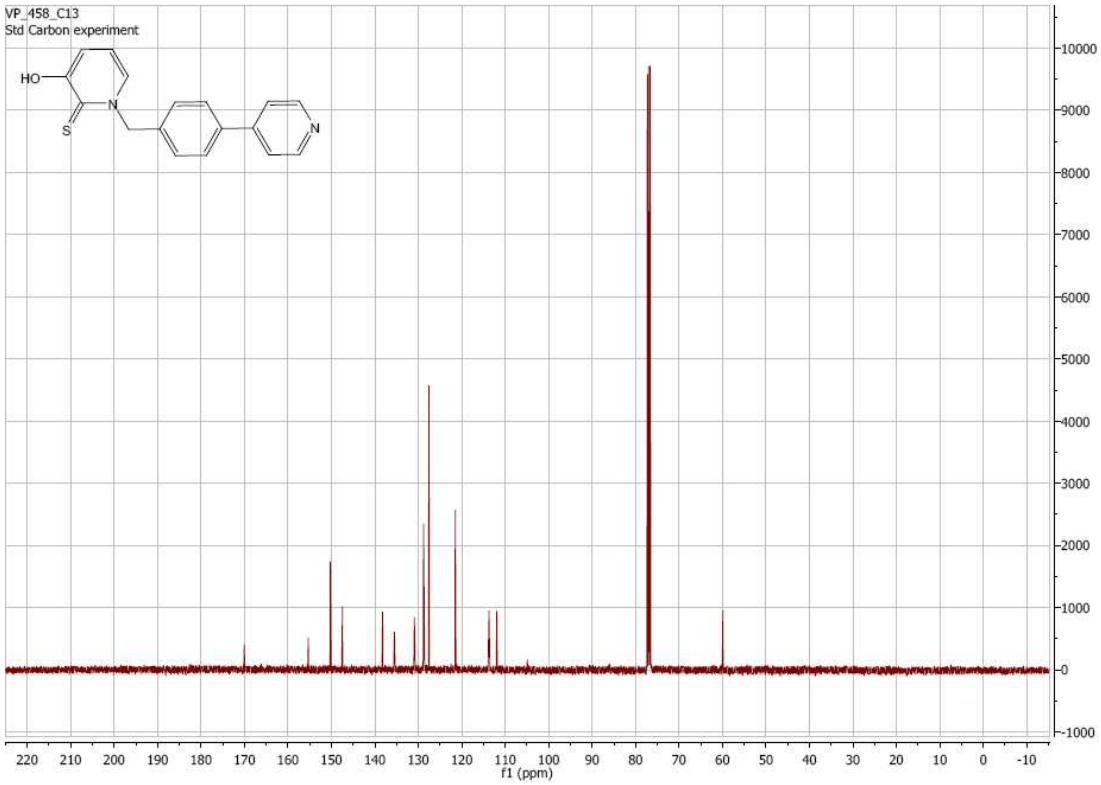
^{13}C NMR of **12h:**



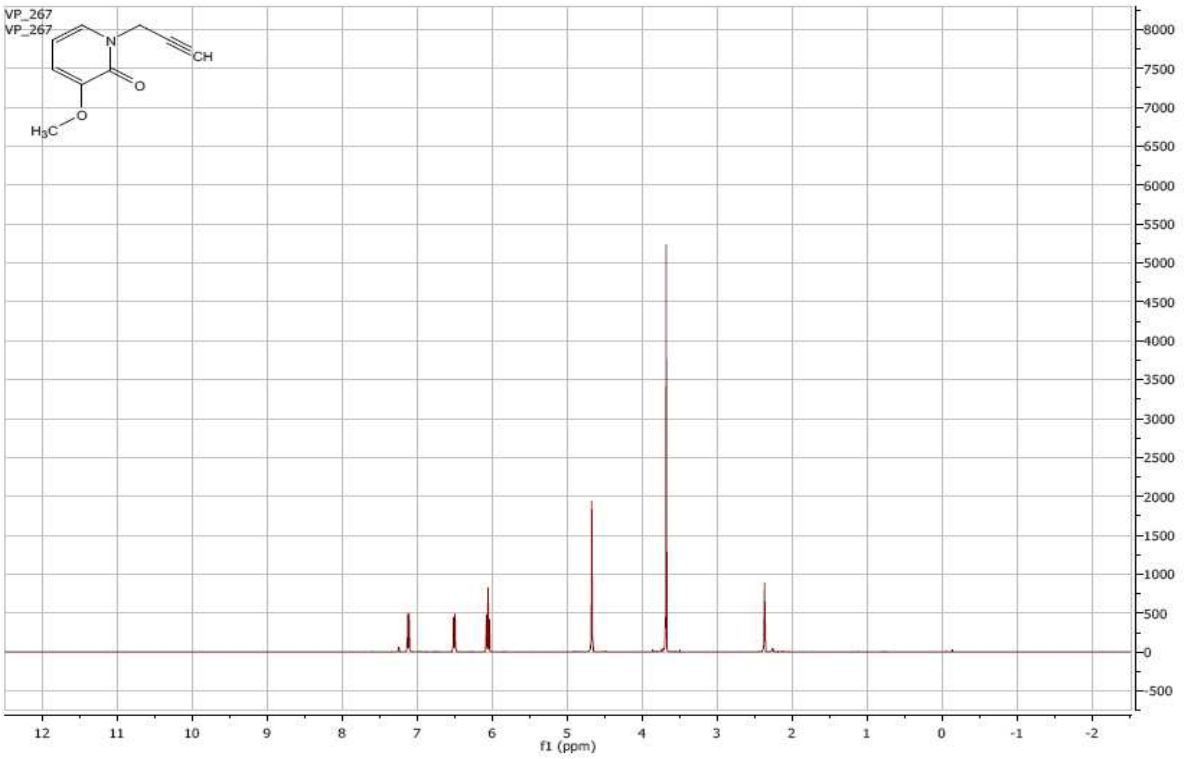
^{1}H NMR of **12i:**



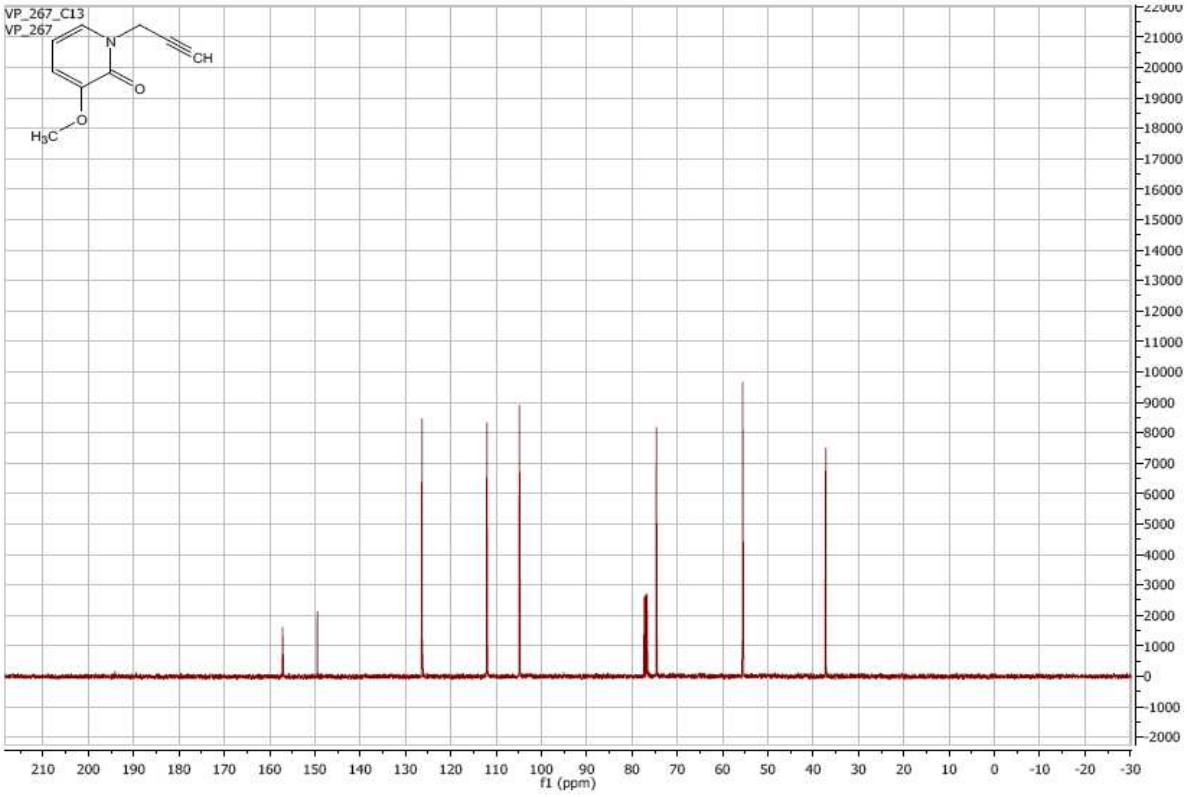
^{13}C NMR of **12i:**



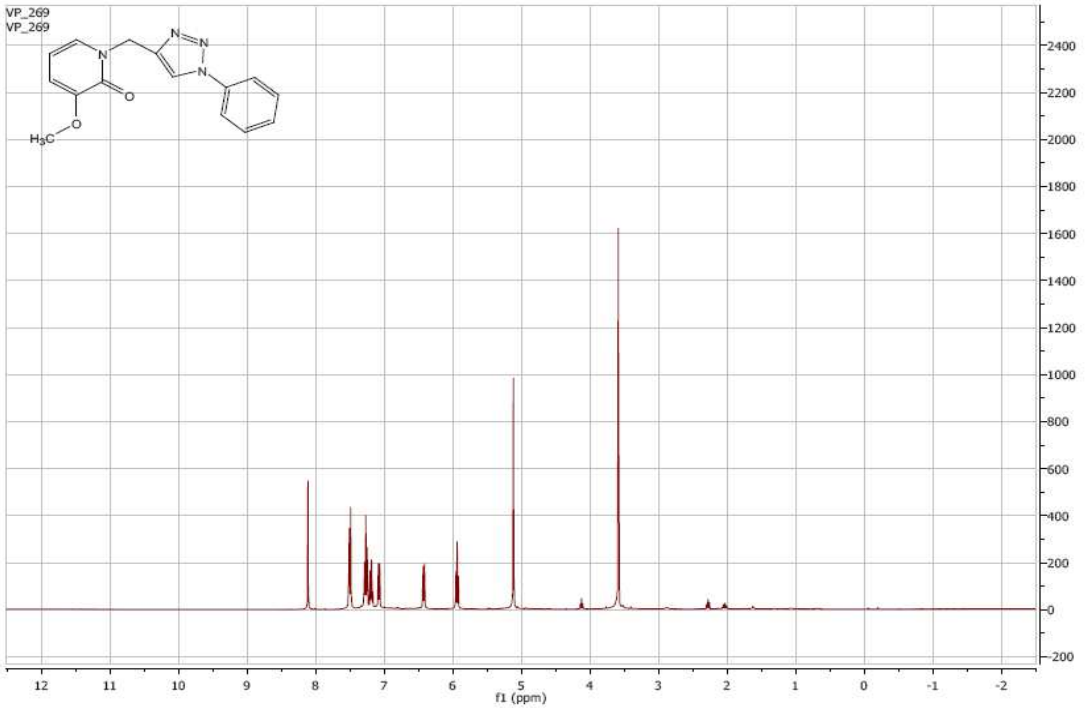
^{1}H NMR of **13:**



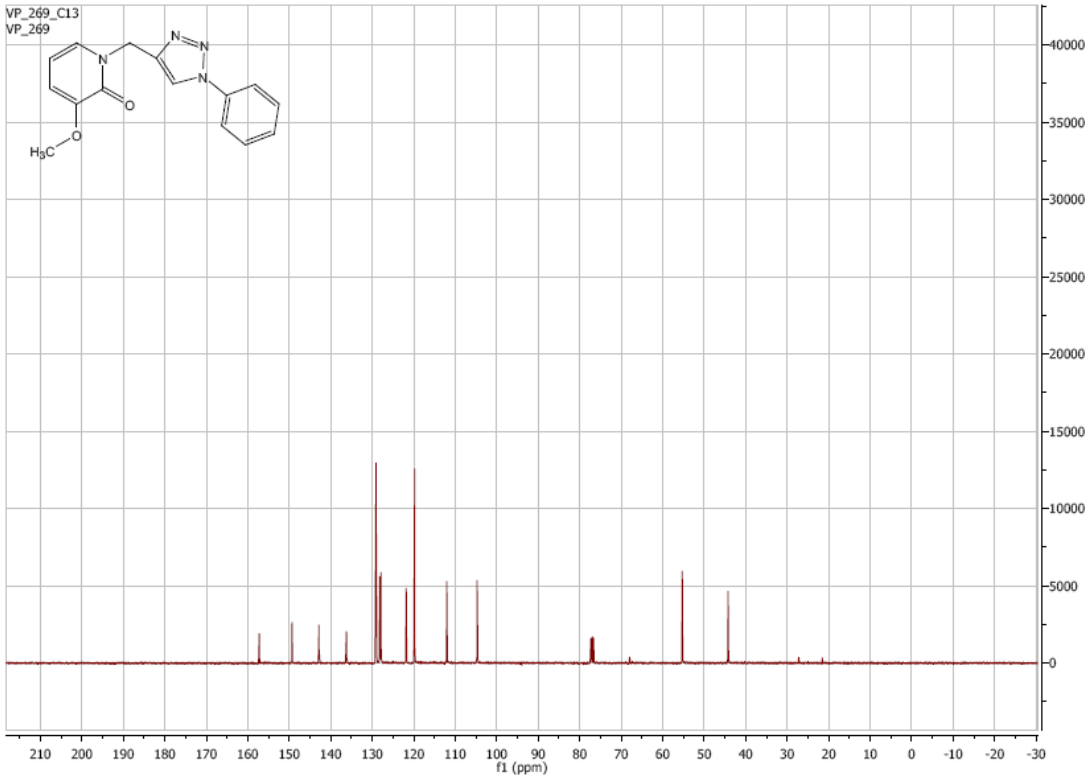
^{13}C NMR of **13:**



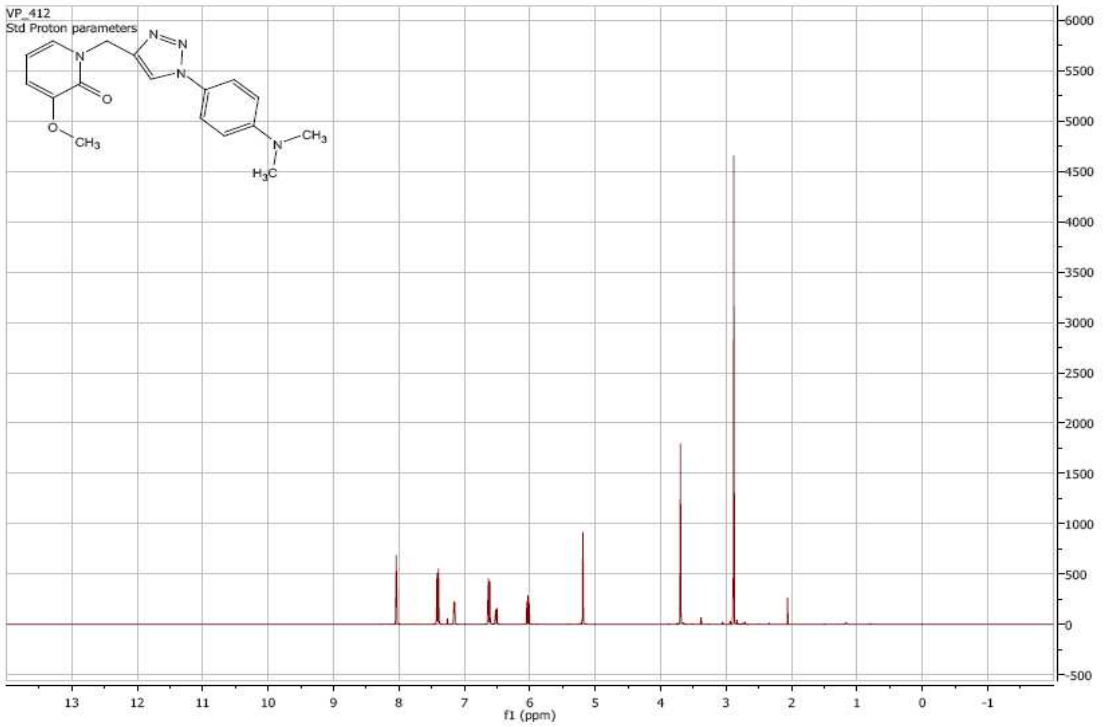
^{1}H NMR of **14a:**



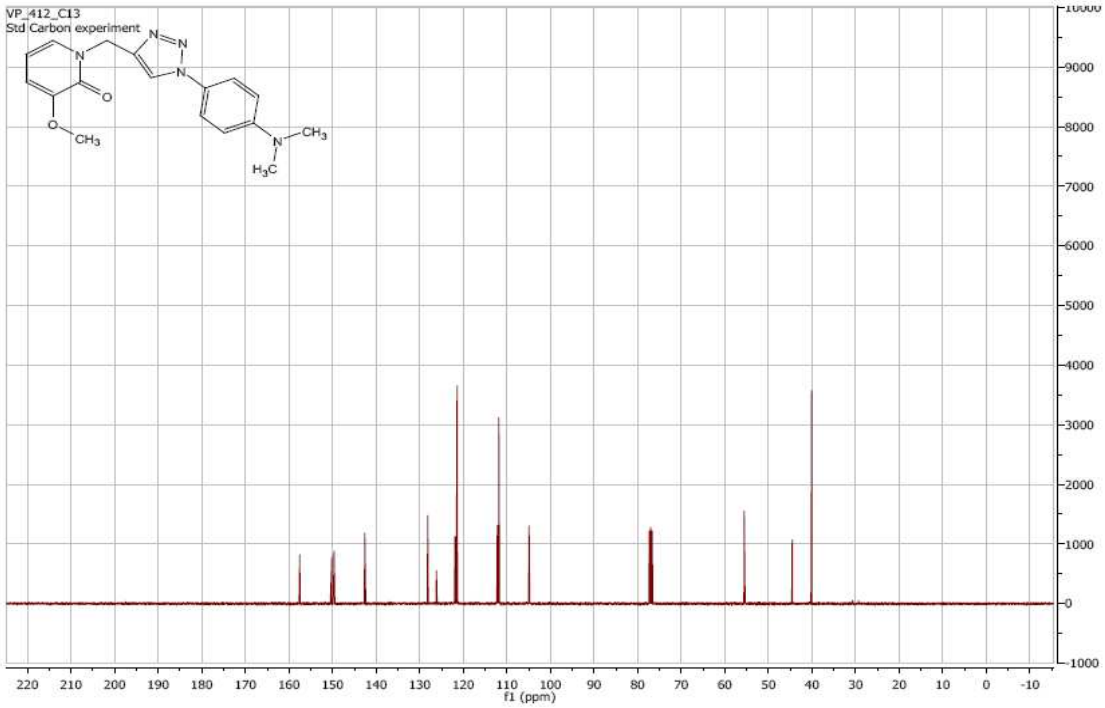
^{13}C NMR of **14a:**



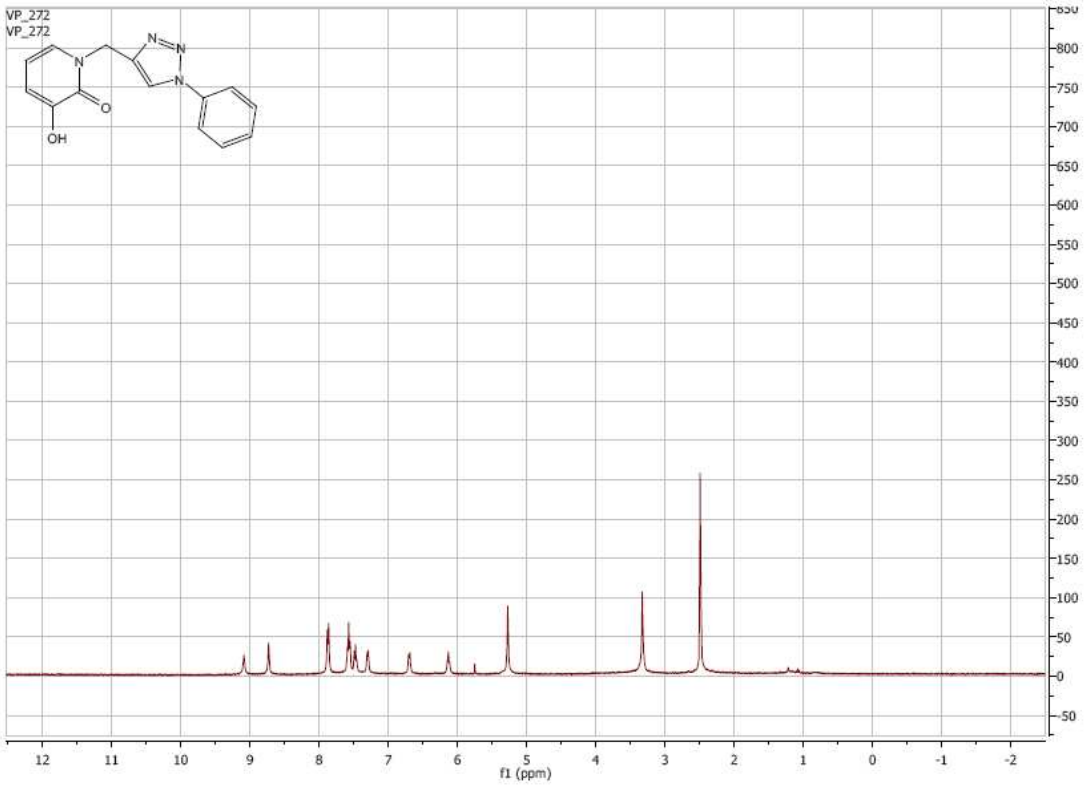
^{1}H NMR of **14b:**



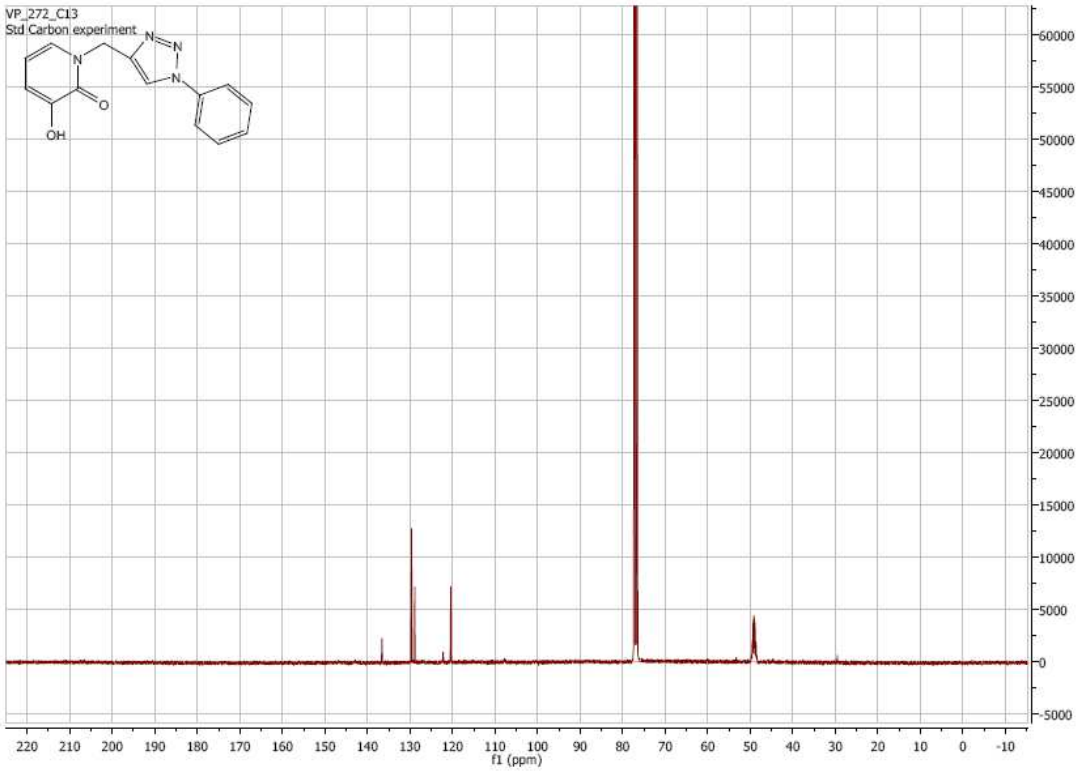
^{13}C NMR of **14b:**



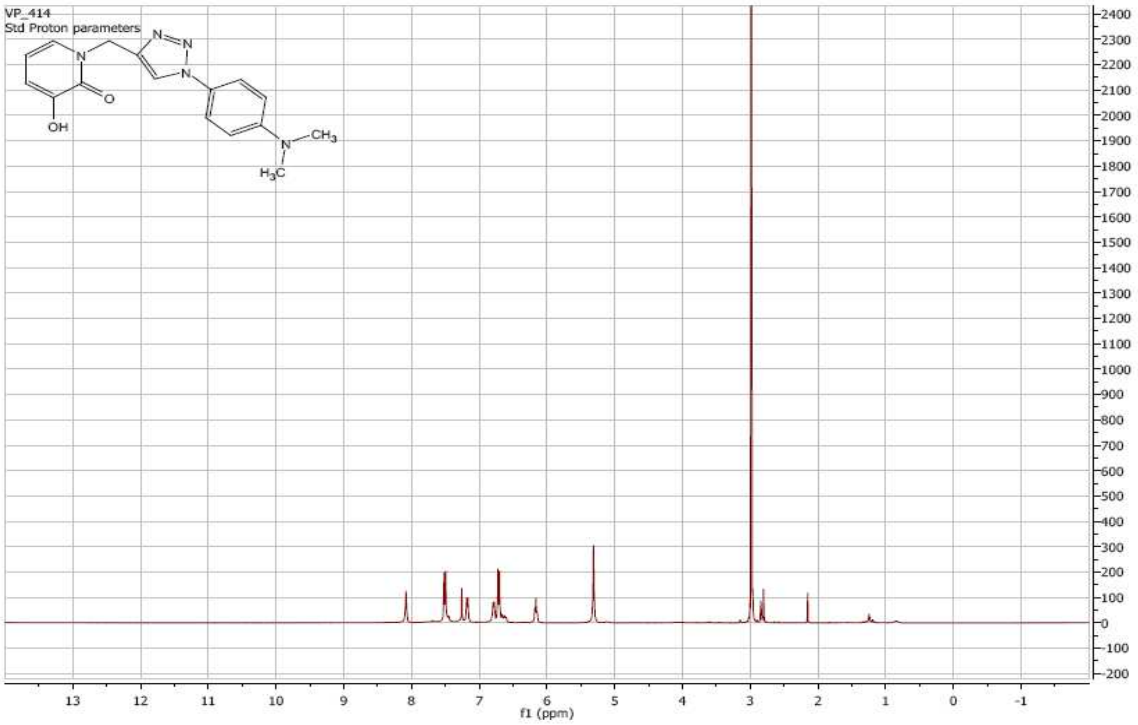
^{1}H NMR of **15a:**



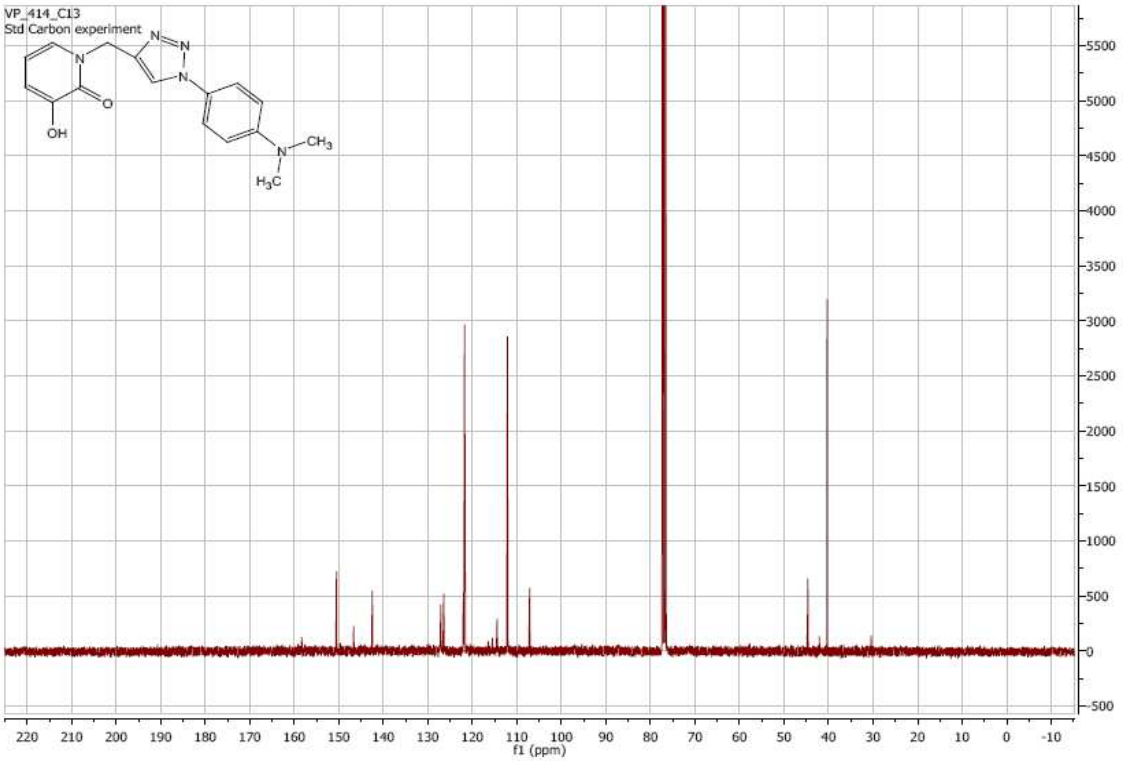
^{13}C NMR of **15a:**



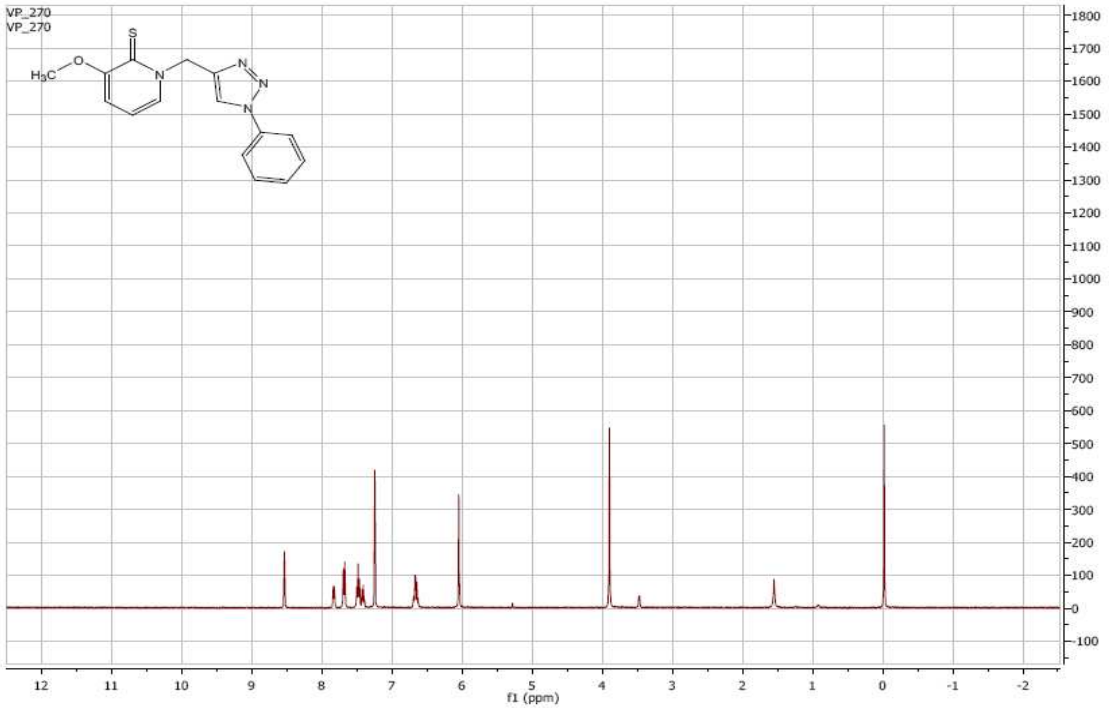
^{1}H NMR of **15b:**



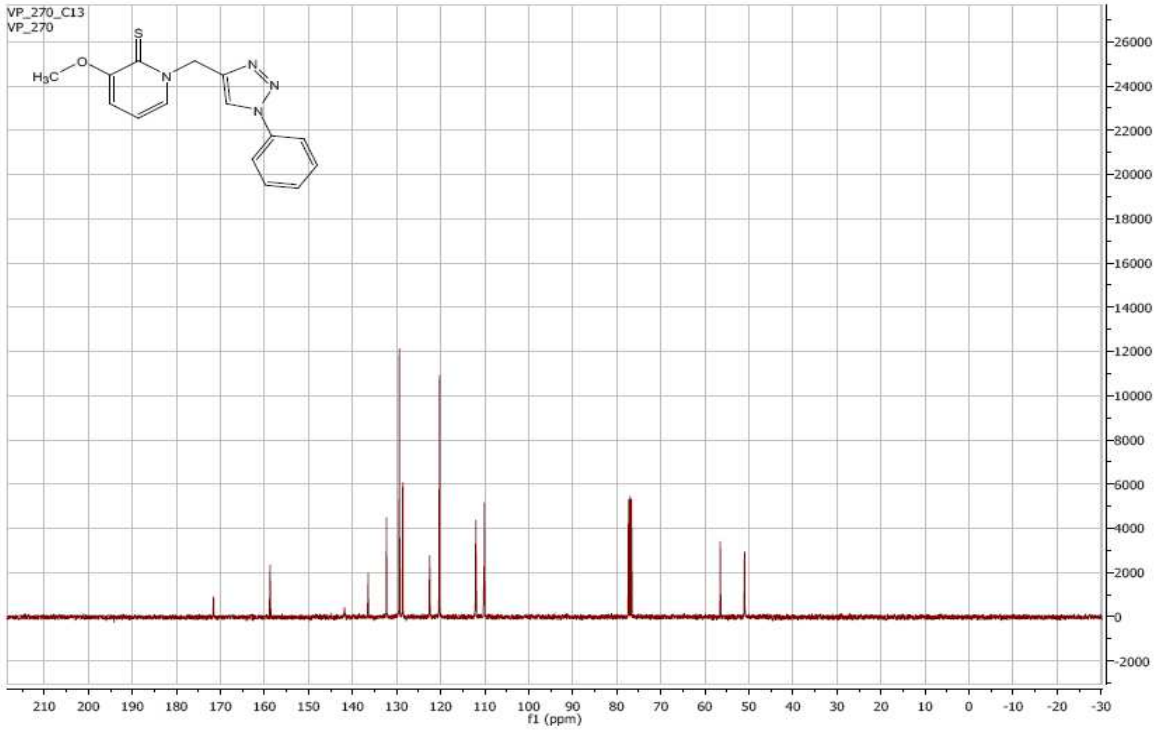
^{13}C NMR of **15b:**



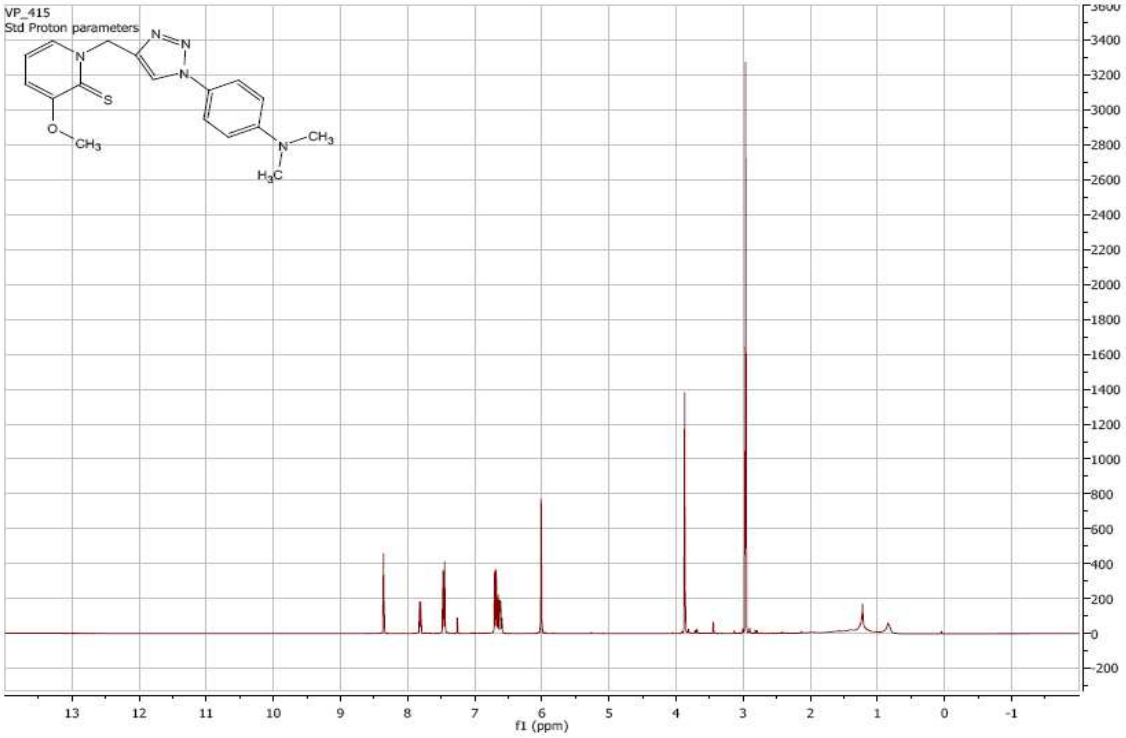
^{1}H NMR of **16a:**



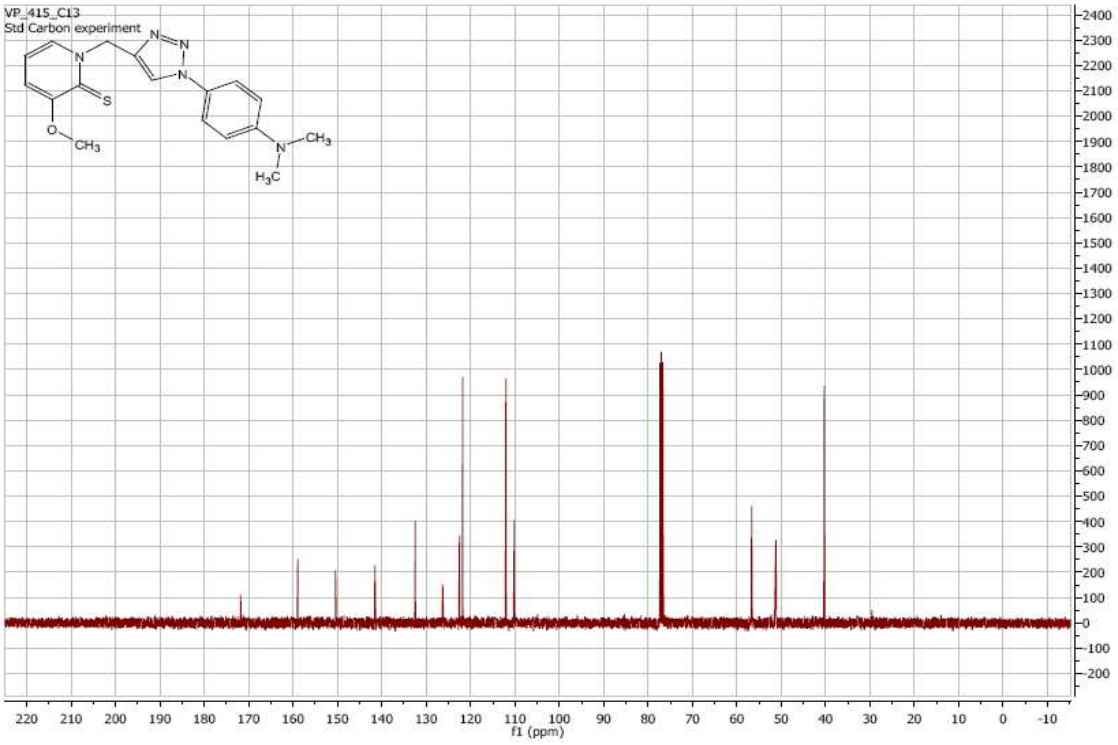
^{13}C NMR of **16a:**



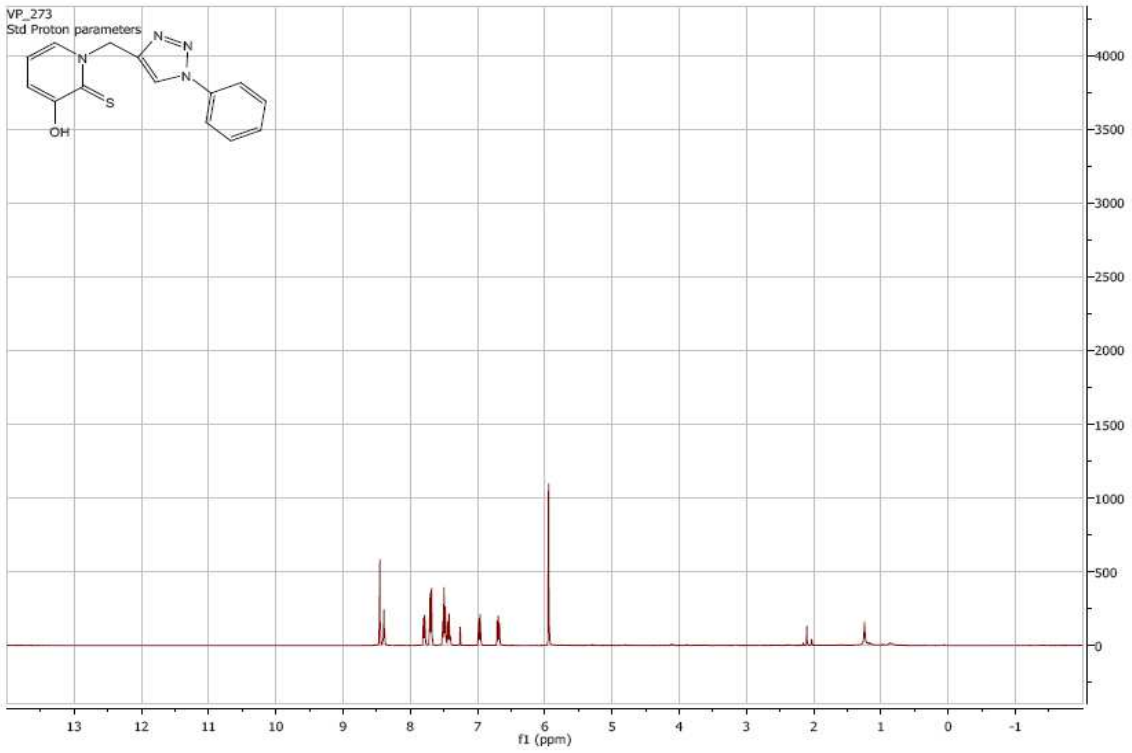
^{1}H NMR of **16b:**



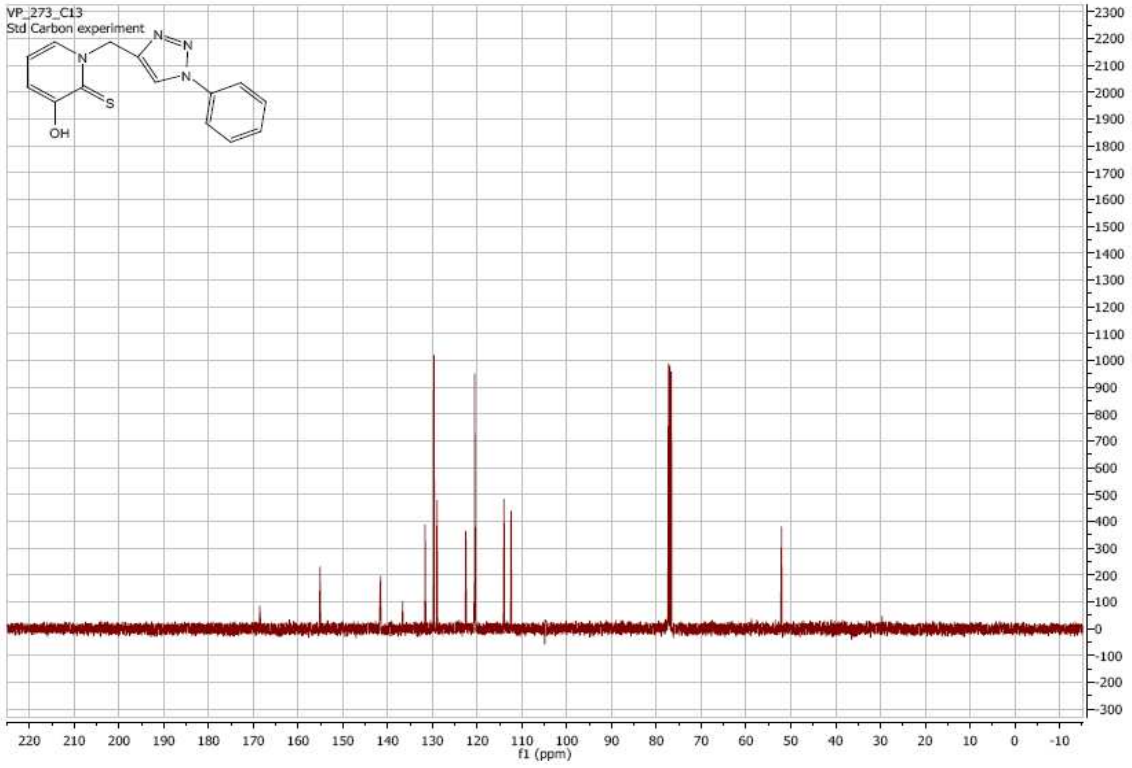
^{13}C NMR of **16b:**



^{1}H NMR of **17a:**



^{13}C NMR of **17a:**



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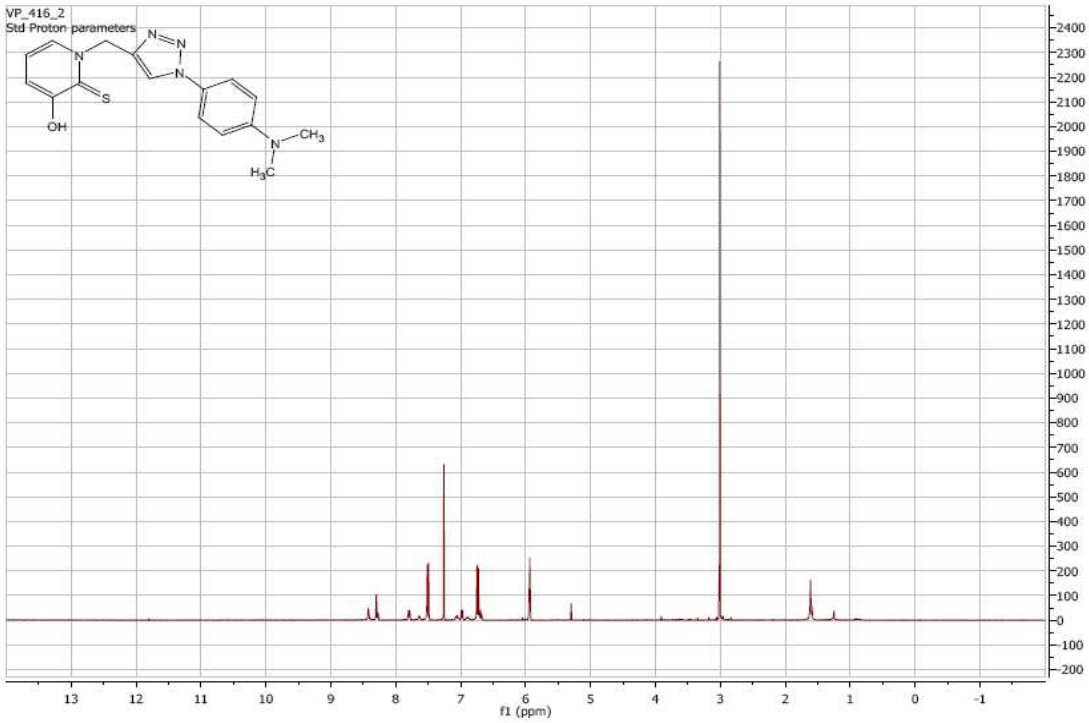
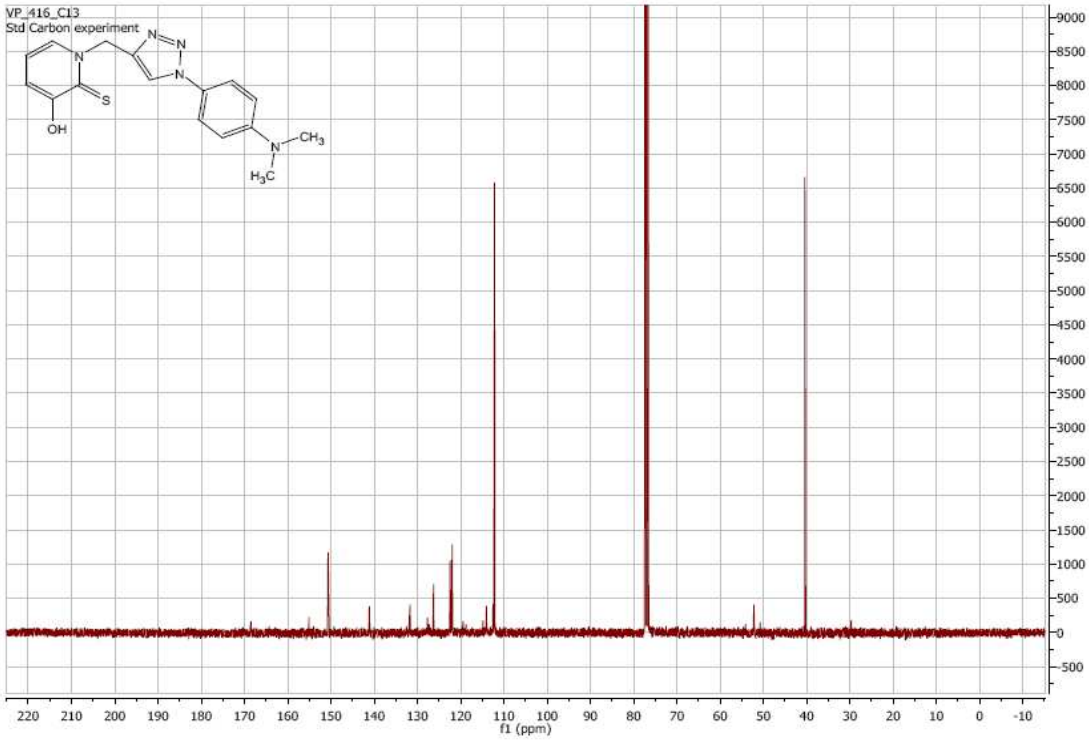


^{13}C NMR of **17b:**



Reference

1. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* **1997,** *23* (1–3), 3-25.