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Synthesis, Characterization and Anti-Proliferative Activity of an Imidazolium Salt and its Silver Carbene Complex: Utilization of Picolyl and Quinolylmethyl Substituents

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Honors Research Project

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Table of Contents

Executive Summary

Problem Statement

Due to the lack of a universal cure for cancer, our research group has continued to research and synthesize compounds that exude anti-proliferative activity on cancer cells, but has a more favorable toxicity profile than that of cisplatin. Imidazolium salts (IS) have shown the possibility to provide excellent anti-proliferative activity; however, many times they lack sufficient solubility (*1*). Likewise, silver carbene complexes (SCC) synthesized in our lab have provided a relatively straightforward synthetic process by which silver (in the form of silver acetate) can be used to simulate ligand-metal coordination (*2*), (*3*). Ideally, future experiments would explore the use of platinum (II) in the ligand-metal complex (similar to that of cisplatin).

The IS and SCC that are studied in this report are 3-(pyridin-2-ylmethyl)-1-(quinolin-2-ylmethyl)imidazolium chloride (**1**) and 3-(pyridin-2-ylmethyl)-1-(quinolin-2-ylmethyl)imidazole – silver acetate complex (**2**). The heteroatoms found on the picolyl and quinolylmethyl substituents of **1** and **2** were expected to increase solubility in water while retaining anti-proliferative activity. In a similar manner, the picolyl substituent was anticipated to help increase solubility due to its lesser lipophilic nature in comparison to other substituents used in our lab (eg. naphthylmethyl substituent).

Results

Synthesis and Characterization

^{1}H NMR spectroscopy (300 MHz) was used to confirm the successful synthesis of both **1** and **2**. Because of limited time and the extreme hygroscopic nature of **2**, only **1** was able to be characterized using ^{1}H NMR (500 MHz), ^{13}C NMR (500 MHz) and mass spectrometry.

When viewing the ^{1}H NMR (300 MHz), ^{1}H NMR (500 MHz), and ^{13}C NMR (500 MHz) spectra for **1**, the integration of all peaks correlates to the number of hydrogen and carbon atoms

found in **1**: seventeen hydrogen atoms and nineteen carbon atoms. Likewise, the locations of the peaks found in the spectra are in ranges deemed acceptable based on the characterization of other ISs (*1*). However, **1** is anticipated to have strong interactions with solvent shown by a small peak in the ^{1}H NMR (300 MHz) spectrum, possibly from residual chloroform. Likewise, **1** exhibits hygroscopic behavior.

In performing mass spectrometry the calculated exact mass of **1** was 301.1 m/z. The results obtained, as seen in **Appendix D**, illustrate that the exact mass found was 301.3 m/z. These results further support the synthesis of **1**.

The ^{1}H NMR (300 MHz) spectrum for **2** provides the confirmation of successful synthesis. However, like **1**, compound **2** exhibits strong interactions with solvent which is demonstrated by the peak at 1.80 ppm that has a greater integration than expected; it is anticipated that acetic acid is the culprit for this larger than expected integration. As mentioned previously, the extreme hygroscopic nature of **2** prevented further studies to be conducted. **Appendix A**, **B**, and **C** show all NMR spectra.

Anti-Proliferative Activity

Because it was anticipated that the anti-proliferative activity of both **1** and **2** would be similar, the MTT assay was only completed for **1**; the silver cation in SCCs, such as **2,** typically results in anti-microbial activity rather than anti-cancer activity (*2*), (*4*). The MTT assay results showed that **1** has an IC_{50} value of greater than 30 μM for both NCI-H460 and A549 cell lines (non-small cell lung cancers), while cisplatin exhibited IC_{50} values of 3 μM and 5 μM, respectively.

Conclusions

Based on results obtained (via NMR spectroscopy and mass spectrometry), it is concluded that the synthetic processes for **1** and **2** were effective and reproducible. However, the

hygroscopic nature of both **1** and **2** represents an issue with pursuing these compounds as anti-cancer drugs; they are difficult to work with. Likewise, IC_{50} values obtained for **1**, on both NCI-H460 and A549 cell lines, demonstrate the lack of effectiveness these compounds will have as anti-cancer drugs.

Broader Impacts

Unfortunately, because of the learned inefficiency of picolyl groups as substituents on the imidazolium salts, further research will not be focused on these substituents. However, in any research environment, it is important to determine what does work along with what does not work. This project has further narrowed substituents for future study. Ultimately, we are one step closer to the final goal: determining an effective anti-cancer drug.

Throughout this project, many other skills were learned. Aside from new chemistry knowledge gained, performing NMR spectroscopy and interpreting the results obtained are new technical skills that I will carry into my career. Likewise, the understanding of mass spectrometry as well as the results obtained from MTT assays was developed.

Aside from the tangible skills learned throughout this project, I have developed a more independent stature in the laboratory environment. This improvement alone will prove to be invaluable in my future career.

Recommendations

It is difficult to recommend a "next step" due to the dynamic atmosphere of a research environment. Likewise, because it is difficult to determine the exact mechanism by which some of the IS compounds are effective as anti-proliferative compounds, it is difficult to recommend the study of a specific family of compounds. However, based on what has worked thus far and what has not worked, room for further exploration of different compounds exists.

Introduction

Cancer, a worldwide epidemic, continues to be the target of many research and development groups due to the lack of a universal cure. Approximately 25% of deaths in the United States are due to cancer (*5*). Of the 1,658,370 new cancer diagnoses estimated to occur in 2015, approximately 15% of these cancer diagnoses will be respiratory system related (*5*). Due to the prevalence of respiratory cancer diagnoses, specifically lung cancer, our research lab has targeted non-small cell lung cancer (NSCLC) cell lines when determining anti-tumor activity of newly formed compounds.

The development of cis-diamminedichloroplatinum(II) (cisplatin), shown in **Table 1,** and its efficacy in anti-tumor activity has paved the way for many other researchers' goals in determining suitable anti-tumor compounds (*6*). However, due to the myriad of side effects associated with cisplatin (e.g., renal dysfunction) and the known ability of several cancer types to develop resistance to the drug, there are ongoing efforts in our lab to synthesize new anti-cancer drugs that maintain the efficacy of cisplatin against NSCLC cell lines, but have a more favorable toxicity profile (*7*), (*8*). Cisplatin is used as the control in our anti-proliferative studies due to its prevalent, albeit relatively ineffective, use in the treatment of NSCLCs.

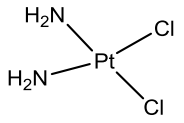
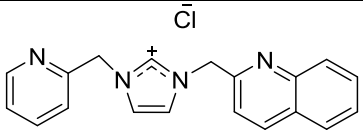
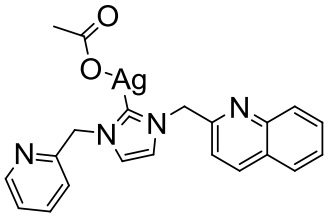
Silver carbene complexes (SCC) and their imidazolium salt (IS) precursors have shown anti-microbial and anti-cancer activity, respectively (*1*), (*9*). Silver has been actively used in our lab because it has been noted that silver is relatively non-toxic to humans (and their cell membranes); likewise, very few cases of silver-resistance have been noted which supports the use of silver as a component of an anti-bacterial agent (*3*), (*4*), (*10*). The benefits of silver are sufficient evidence to rationalize the persistent study of the SCC and IS compounds. This report investigates two compounds (an IS and its corresponding SCC, respectively): 3-(pyridin-2-

ylmethyl)-1-(quinolin-2-ylmethyl)imidazolium chloride (**1**) and 3-(pyridin-2-ylmethyl)-1-(quinolin-2-ylmethyl)imidazole – silver complex (**2**). These compounds are shown in **Table 1**.

Because the known effectiveness of platinum as an anti-tumor agent (in cisplatin and its derivatives) is a highly desirable characteristic, compound **1** was rationally designed to chelate a platinum (II) center. However, due to a lack of time, the platinum coordination chemistry was unable to be explored. Instead, silver was the metal chosen to explore the ligand-metal coordination of **1**, primarily due to the familiarity of the synthetic process toward SCCs and the aforementioned benefits of silver (*3*), (*4*), (*9*), (*10*). Additionally, SCCs are well known carbene transfer agents, and it is possible that the ligand of **2**, or a similar derivative, can eventually be transferred to a separate metal center, such as Pt(II); these possibilities have been previously reviewed (*10*).

Compounds **1** and **2** were characterized by various methods, including ^{1}H NMR, ^{13}C NMR and mass spectrometry, in order to determine their structure and confirm their purity. The anti-proliferative activity of **1** against NSCLC lines was determined by the MTT assay.

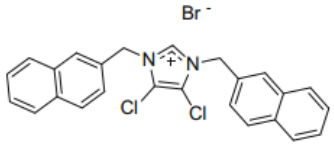
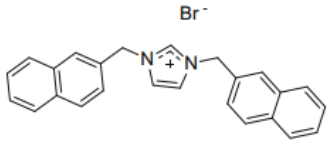
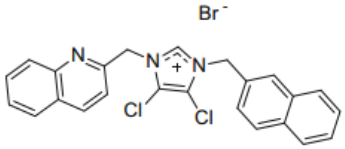
Table 1. Structures of cisplatin, compound **1,** and compound **2**

Compound	**Structure**
Cisplatin	
1	
2	

Background

Because of the anti-proliferative properties of imidazolium salts against NSCLC lines, our lab has found it necessary to continue to synthesize different analogs of IC23-Br, a compound shown to have similar anti-proliferative activity on NSCLC cell lines as cisplatin despite its extremely limited solubility in aqueous solutions (*1*). Derivations to this compound have therefore focused on improving the solubility profile of the analogs in water while maintaining efficacy. Though different analogs of IC23-Br have been synthesized in our lab, IC29-Br and IC30-Br, solubility still remains an issue (*1*). Compounds **1** and **2** were of interest to synthesize in hopes of developing a compound that retained anti-proliferative activity while having sufficient solubility. Note that anti-proliferative activity is represented by IC_{50} values as can be seen in **Table 2**. IC_{50} values (in μM) are the concentration of the anti-tumor drug that reduces cell viability to 50%, relative to untreated control cells (*1*) .

Table 2. Imidazolium salt structures, concentrations and solubilities (*1*)

Compound	**Structure**	**Solubility in H^2O**	**IC_{50} (μM)**
IC23-Br		<0.1 mg/mL	NCI-H460 – 5 μM NCI-H1975 – 6 μM HCC827 – 8 μM
IC29-Br		N.D.	NCI-H460 – 4 μM NCI-H1975 – 6 μM HCC827 – 9 μM
IC30-Br		0.9 mg/mL	NCI-H460 – 10 μM NCI-H1975 – 5 μM HCC827 – 9 μM

Without sufficient solubility, these anti-proliferative drugs would be unable to be administered properly and effectively (*1*). When comparing the different compounds in **Table 2**, it is important to note that the quinolylmethyl substituent on the 1 position (and naphthylmethyl substituent on the 3 position) on IC30-Br increased the solubility of the imidazolium salt when compared to the imidazolium salt with naphthylmethyl groups on both the 1 and 3 positions (IC23-Br) (*1*). For reference, **Figure 1** illustrates the different positions of the IS, **1**; note that the 1 position on the imidazole is the nitrogen where the first substituent is added, and the 3 positions on the imidazole is the nitrogen where the second substituent is added.

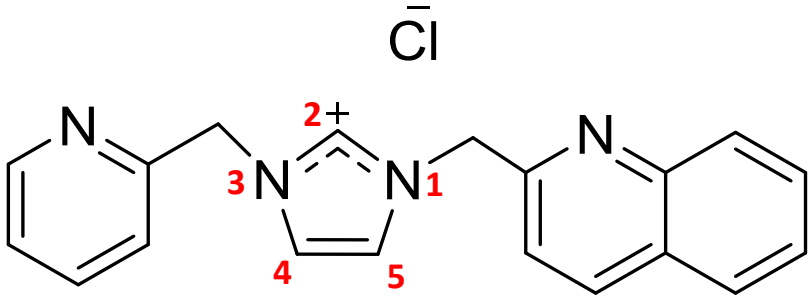
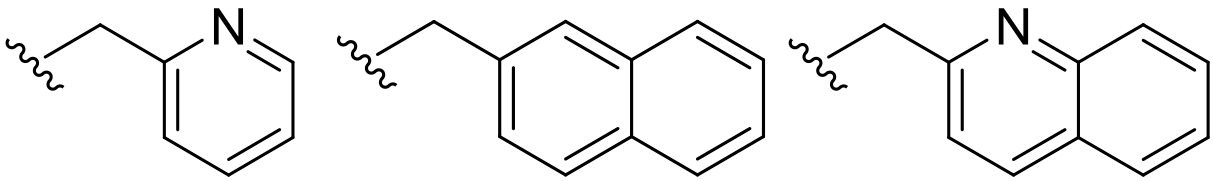


Figure 1. Numbering scheme of imidazole rings, as demonstrated on compound **1**

The heteroatom on the quinolylmethyl ring proved to be beneficial for the imidazolium salt construction, which ultimately led to the decision of incorporating a picolyl group as a substituent. Compounds **1** and **2** were synthesized utilizing a quinolylmethyl and picolyl group in hope of further increasing solubility. **Figure 2** shows the structures of the picolyl group, the naphthylmethyl group and the quinolylmethyl group.



(a) (b) (c)

Figure 2. (a) picolyl group (b) naphthymethyl group (c) quinolylmethyl group

By incorporating quinolylmethyl and picolyl groups, it was also anticipated that there would be an increased possibility to successfully bind metals to this heteroatom due to the less sterically hindered position the picolyl group would pose to the silver. Ideally, when using a different metal (platinum (II)), these heteroatoms would bind to the platinum in a similar manner as shown in cisplatin (**Table 1**). Based on the crystal structure of IC30-Br, the heteroatom of the quinolylmethyl group was not able to interact with the metal center (eg. silver).

Experimental Section

Materials

Reactions were performed in aerobic conditions or in a nitrogen environment using standard anaerobic techniques. All solvents were purchased from commercial sources. Solvents for anaerobic reactions were dried on a system manufactured by Innovative Technology. Imidazole was purchased from Acros Organics, 2-(chloromethyl)pyridine hydrochloride and silver acetate were purchased from Alfa Aesar, and 1-(quinolin-2-ylmethyl)imidazole was synthesized in our laboratory.

^{1}H NMR spectra were obtained on a Varian 300 MHz instrument as well as a Varian 500 MHz instrument. The ^{13}C NMR spectrum was obtained on a Varian 500 MHz instrument. All NMR spectra were collected in d_6-DMSO (Cambridge Isotope Laboratories) and referenced to residual protons in the solvent (^{1}H: 2.50 ppm, ^{13}C: 39.51 ppm). Mass spectrometry was performed by the University of Akron mass spectrometry laboratory.

Synthesis of 2-(chloromethyl) pyridine

2-(Chloromethyl)pyridine hydrochloride (1.00 g, 6.10 mmol) was dissolved in deionized water (6 mL). Sodium bicarbonate was added to neutralize the complex. Ethyl acetate (9 mL) was added to the mixture, and additional sodium bicarbonate was added to ensure neutralization of the acid. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (1 x 9 mL). The organic portions were combined, washed with brine (NaCl), and dried with anhydrous sodium sulfate. The volatiles were removed via rotary evaporation, yielding a red oil (0.68 g, 87%). This synthetic route was based on a previous literature procedure (*11*). The ^{1}H NMR spectrum of the product was compared to that reported in literature to ensure the desired product was formed.

Synthesis of **1**

2-(Chloromethyl)pyridine (1.07 g, 8.42 mmol) and 1-(quinolin-2-ylmethyl)imidazole (1.60 g, 7.65 mmol) were added to a 15 mL flask with acetonitrile (9 mL). The mixture was stirred for 23 h at reflux, slowly becoming red in color. The volatiles were removed by rotary evaporation, yielding the crude product as a red oil. The product was purified by column chromatography (silica gel, 20:80 v/v methanol/chloroform) and isolated as a hygroscopic brown solid after extensive drying under vacuum (1.20 g, 47%). ^{1}H NMR (300 MHz, d_6-DMSO): δ 9.67 (s, 1 H, NCHN), 8.58 (d, ^{3}J = 4.7 Hz, 1 H, ArH), 8.49 (d, ^{3}J = 8.58 Hz, 1 H, ArH), 8.41 (s, 1 H, CHCl3), 8.02 (d, ^{3}J = 8.20 Hz, 1 H, ArH), 7.85-7.94 (m, 4 H, ArH), 7.78 (dd, ^{3}J = 8.75, 6.73 Hz, 1 H, ArH), 7.61-7.65 (m, 2 H, ArH), 7.54 (d, ^{3}J = 7.90 Hz, 1 H, ArH), 7.42 (dd, ^{3}J = 7.30, 5.0 Hz, 1 H, ArH), 5.91 (s, 2 H, CH2), 5.72 (s, 2 H, CH2). ^{13}C NMR (500 MHz, d_6-DMSO): δ 154.3, 153.7, 149.5, 146.8, 138.0, 137.5, 137.4, 130.1, 128.5, 127.9, 127.2, 126.9, 123.6, 123.5, 123.1, 122.4, 119.9, 53.4, 53.1. MS (ESI+) m/z: $C_{19}H_{17}N_4$ $[M\text{-}Cl]^+$, Calcd 301.1, Found 301.3.

Synthesis of **2**

Synthesis of **2** was based on a similar procedure for SCC synthesis (*2*). All manipulations were carried out under anaerobic conditions using standard Schlenk technique. Compound **1** (0.25 g, 0.74 mmol) was dissolved in dry methylene chloride (10 mL). Silver acetate (1.48 mmol, 0.25 g) was added to the mixture. The reaction mixture was stirred at room temperature in the dark for 1 h. The reaction was stored in the freezer overnight. After the mixture returned to room temperature, the solid (presumably silver chloride) was removed by filtration. The remaining volatiles were slowly removed under a dynamic vacuum, yielding a light brown solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 8.50 (bs, 1 H, ArH), 8.42 (d, ^{3}J = 8.2 Hz, 1 H, ArH), 7.99 (d, ^{3}J = 8.5 Hz, 1 H, ArH), 8.08 – 7.33 (m, 9 H, ArH), 5.63 (s, 2 H, CH2), 5.44 (s, 2 H, CH2), 1.80 (s, 3 H, CH3COO$^-$).

Results and Discussion

Compound **1**: 3-(pyridin-2-ylmethyl)-1-(quinolin-2-ylmethyl) imidazolium chloride

The synthesis and purification of **1** was able to be obtained with a 47% yield. ^{1}H NMR and mass spectrometry (MS) have been completed in order to ensure a full characterization of the compound and to ensure the synthesis was successful. The characterization methods can be seen in **Figures A.1, A.2, A.3, B.1, Figure C.1** and **Figures 3** and **4** (^{1}H NMR, ^{13}C NMR and mass spectrometry results).

In order to obtain a pure product, it was necessary to perform column chromatography. Using Thin Layer Chromatography (TLC), it was shown that **1** and 1-(quinolin-2-ylmethyl)imidazole could be separated effectively by using a methanol/chloroform solution (20:80 v/v). 95 g of silica gel was used to provide a sufficient stationary phase for column chromatography. The silica gel was embedded with a fluorescent indicator. Compounds with aromatic substituents quench the fluorescence allowing one to visualize these compounds as dark bands on the column under UV light. Using the methanol/chloroform solution (20:80 v/v) as the mobile phase, the first band visible in the column was assumed to contain the 1-(quinolin-2-ylmethyl)imidazole and any remainder 2-(chloromethyl) pyridine due to their lack of polarity shown by TLC; these two compound move faster through the stationary phase. The second visible band contained **1**. Once the first band was eluted, a methanol/chloroform solution (50:50 v/v) was used to quickly elute **1**. ^{1}H NMR of the second band (assumed **1**) revealed **1** along with unknown solvents. The product separated using column chromatography was placed under vacuum for five days, and the ^{1}H NMR showed that only **1** remained. Compound **1** becomes "sticky" as it is exposed to air for lengths of time and appears to be highly hygroscopic.

Because of the success in synthesizing **1**, a MTT assay was done incorporating the following NSCLC cell lines: NCI-H460 and A549. This test was done in order to compare the

anti-proliferative activity of **1** in comparison to the anti-proliferative activity of cisplatin. In the following figures, **Figures 5** and **6**, data collected from the MTT assay are represented. The IC_{50} values of **1** and cisplatin can be extracted from the data in **Figures 5** and **6**.

^{1}H NMR Results (300 MHz)

Based on the ^{1}H NMR spectrum obtained shown in **Figure A.1**, all peaks associated with **1** can be accounted for based on the integration of the peaks; seventeen hydrogen atoms are found within **1**. **Figure B.1** illustrates the ^{1}H NMR spectrum obtained from the 500 MHz spectrometer.

The singlet found at 8.41 ppm, not associated with **1**, is anticipated to be that of chloroform. This rationale was formed primarily on the observation that **1** has strong interactions with solvents and that resonance of chloroform in d_6-DMSO is usually observed at 8.32 ppm (*12*). Because of the compound-solvent interaction, this peak has most likely shifted to 8.41 ppm. Integration of all other peaks correlates to all hydrogen atoms found in **1**; the integration of the 8.41 ppm peak is extremely small, which would suggest a small amount of chloroform present.

The most notable feature of the spectra of imidazolium salts, and often indicative of their formation, is the resonance found downfield of (most) other peaks between 9-10 ppm; it is a result of the positive charge on the imidazole ring (*1*). The hydrogen atom representing this peak is found on the 2 position of the imidazole ring. In the spectrum of **1**, this peak occurs at 9.67 ppm, while the next closest peak occurs at 8.58 ppm. The peaks found between 6 and 9 ppm correlate to the hydrogens found in the aromatic rings of **1**; there are 12 hydrogen atoms in these aromatic rings, and all can be accounted for based on the integration of the singlet, doublet, doublet of doublet or muliplet splitting patterns shown in **Figures A.1, A.2,** and **A.3**. The peaks found at 5.91 and 5.72 ppm are the results of the methyl linkers on both the 1 and 3 positions of

the imidazole (*1*). Based on integration and their expected coupling patterns (singlet), all four of these hydrogen atoms are accounted for.

^{13}C NMR Results (500 MHz)

The ^{13}C NMR results show nineteen singlet peaks, which directly correlate to the nineteen carbons found in **1**. This further proves that **1** was able to be successfully synthesized. The peaks observed at 53.4 and 53.1 ppm are representative of the carbons found on the methyl group linkers on the 1 and 3 positions of the imidazole ring (*1*). The peaks found between 155 ppm and 119 ppm are all peaks that correlate to the carbons found in the aromatic rings.

Mass Spectrometry Results

In order to prove that **1** was able to be successfully synthesized, mass spectrometry was done in order to show that the only peak on the spectrum (when the solvent peaks are negated) is that of **1**. As shown in **Figure 3,** the only peak shown is at 301.3 m/z. The charge for the compound observed, the imidazolium salt minus the chloride ion ($[M\text{-}Cl]^+$), is 1. The mass of the peak shown is the actual mass of the compound (301.3). The calculated mass of $[M\text{-}Cl]^+$ is 301.1. This mass spectrum shows that the synthesis of **1** was successful.

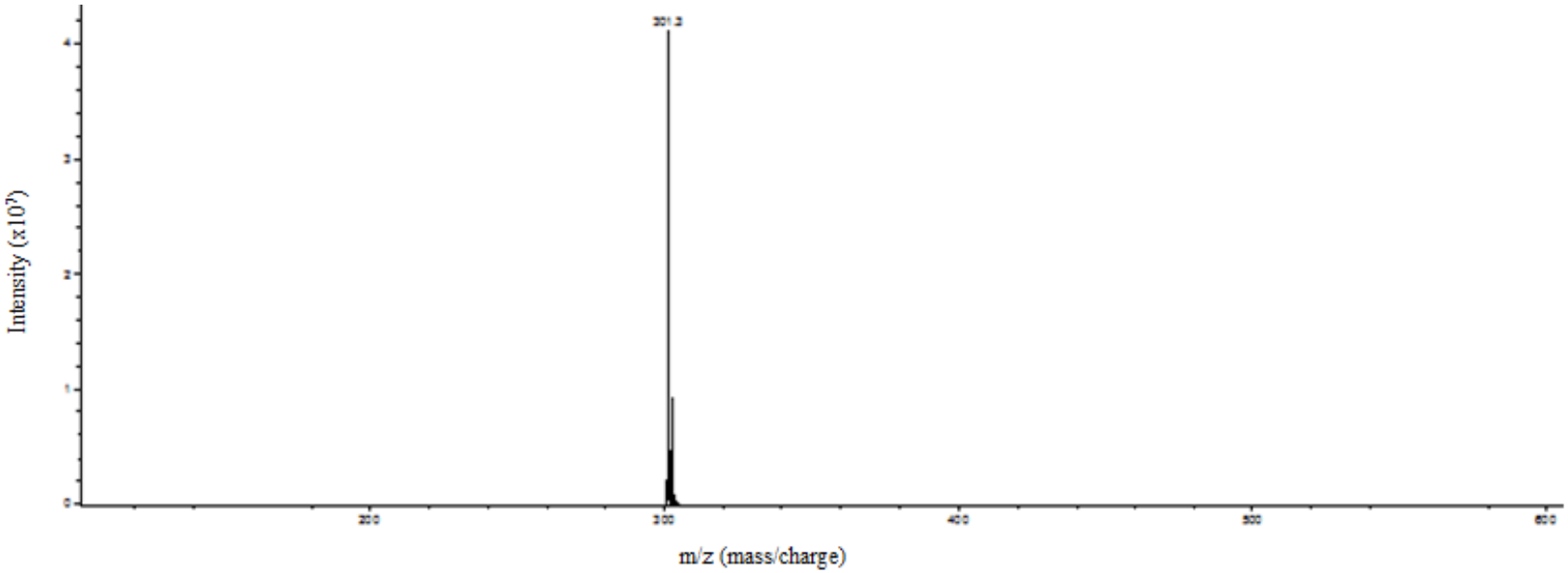


Figure 3. Mass spectrum of **1**

Because the base solvent (methanol) used had an impurity that showed a peak at 301.4, it was of interest to pursue MS-MS; it was done in order to see the fragmented pieces of the parent compound, **1** minus the chloride anion, and the 301.4 peak found from the mass spectrum of methanol. The mass spectrum of both **1** and methanol and the MS-MS spectrum of **1** and the 301.4 impurity peak found in methanol can be found in **Appendix D**. **Figure 4** shows the spectrum of all the fragments found from 1. This spectrum is different than that of the methanol impurity 301.4 peak, suggesting that the 301.3 peak seen in mass spectrum is the desired compound.

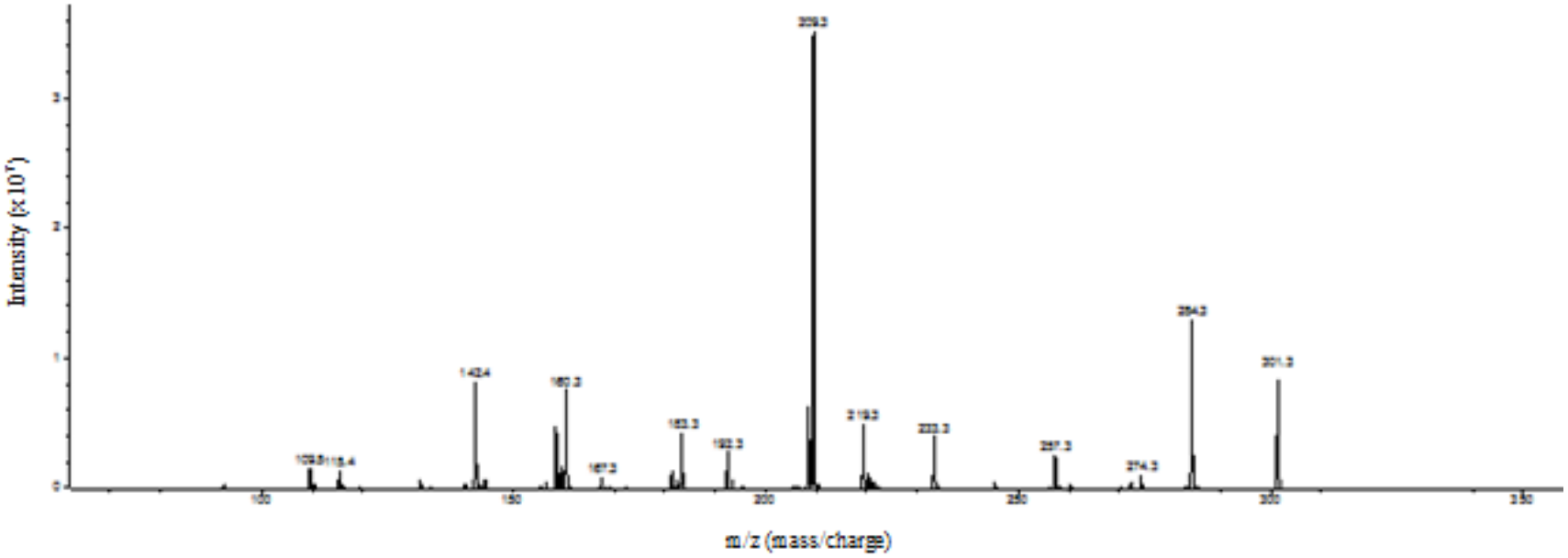


Figure 4. MS-MS spectrum of **1**

MTT Assay Results

The IC_{50} values of **1** as shown in **Figures 5** and **6** are greater than 30 μM for both the NCI-H460 and A549 cell lines, while the IC_{50} values of cisplatin are 3 μM and 5 μM, respectively. Note, the IC_{50} value is the concentration of drug that reduces cell viability to 50% (*1*). In the following figures, 1-Quin-3-Pyridine Cl represents **1**.

The results indicate that **1**, in comparison to cisplatin, has relatively poor anti-proliferative activity and will not be sufficient as an anti-cancer drug. In reviewing **Table 2**, the IC_{50} values of three other compounds synthesized in our lab are shown; all of these values have IC_{50} values of 10 μM or below, representing comparable anti-proliferative activity to cisplatin (*1*). The main difference between **1** and the compounds found in **Table 2**, is the less bulky substituent, a picolyl group, which may have aided in **1** having inefficient anti-proliferative activity.

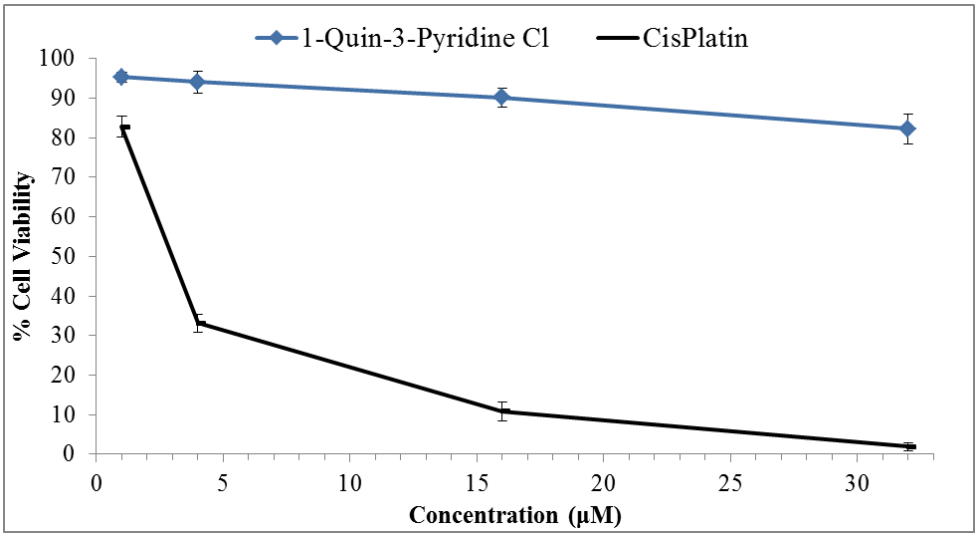


Figure 5. MTT assay results for NCI-H460 cell lines

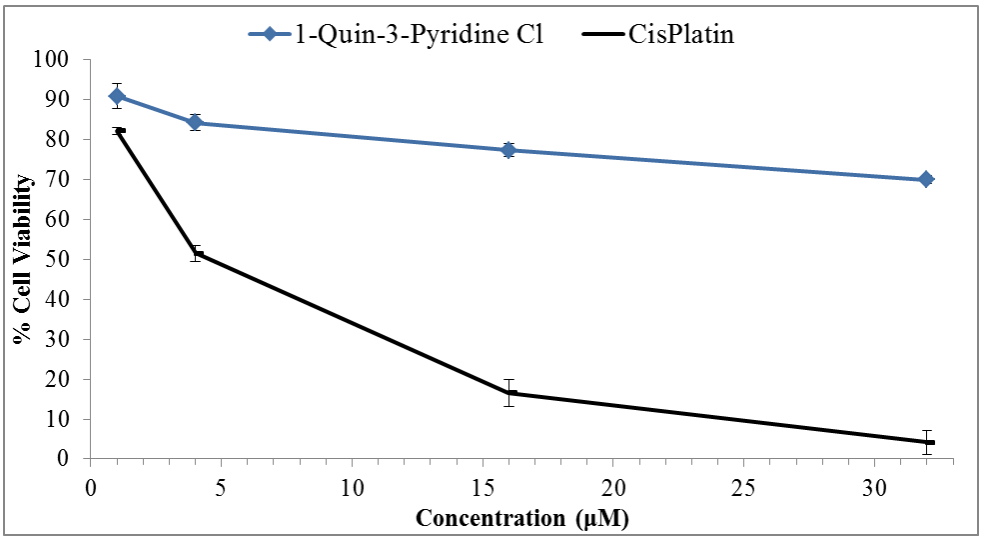


Figure 6. MTT assay results for A549 cell lines

Compound **2**: 3-(pyridin-2-ylmethyl)-1-(quinolin-2-ylmethyl)imidazole – silver complex

The synthesis of **2** was successful; however, the exact mass obtained was unable to be determined due to the inability to efficiently remove the product from the glassware. ^{1}H NMR was completed in order to ensure characterization of the compound. Due to time constraints, mass spectrometry was unable to be completed for **2**. The ^{1}H NMR spectra can be seen in **Figure A.4, A.5 and A.6.**

Though **2** was able to be synthesized, the purity of the component remains an area of concern. This reaction, after being carried out aerobically, was done under anaerobic conditions to determine whether a solid product was able to be obtained; under anaerobic conditions, a solid product remained, whereas performing the reaction under aerobic conditions produced a product that was hygroscopic, resulting in a sticky product could be described as a product in between an oil and solid.

^{1}HNMR (300 MHz) Results

Based on the ^{1}H NMR results obtained from the 300 MHz spectrometer shown in **Figure A.4**, all peaks associated with **2** can be accounted for based on the integration of the peaks;

nineteen hydrogen atoms are found within **1**. The singlet found at 1.80 ppm, which correspond to the hydrogen atoms associated with the acetate, have an integration of 5.50, when the actual integration of the acetate should be 3. This integration of 5.50 illustrates the possibility that acetic acid is present because it is also found near 1.80 ppm (at 1.91 ppm) (*12*). Integration of all other peaks correlates to all hydrogen atoms found in **2**.

The disappearance of the resonance corresponding to the 2-position proton in the imidazolium salt **1** (9.67 ppm) indicates successful formation of the silver carbene complex **2**. Additionally, the appearance of a resonance at 1.80 ppm corresponds with the addition of an acetate methyl group, further supporting the formation of **2**.

Conclusion

It has been recorded that **1** and **2** were able to be successfully synthesized. Compound **1** was characterized by ^{1}H NMR spectroscopy on both the 300 MHz and 500 MHz instruments, ^{13}C NMR on the 500 MHz instrument, and mass spectrometry. The anti-proliferative activity of **1** was studied via MTT assay; the anti-proliferative activity was quantified by its IC_{50} value and compared to cisplatin. Based on the results obtained, **1** will not be efficient as an anti-cancer drug. The IC_{50} values obtained for **1** (for the NCI-H460 and A549 cell lines) is greater than 30 μM while the IC50 values for cisplatin were less than or equal to 5 μM.

Because of the hygroscopic nature of **1**, it needs to be stored under nitrogen. The hygroscopic characteristic of **1** may make it a difficult compound to work with. However, the synthesis process of **1** can be done in aerobic conditions and has proven to be reproducible and consistent.

Compound **2** was characterized by ^{1}H NMR spectroscopy on the 300 MHz instrument. Like **1**, Compound **2** exhibits hygroscopic behavior. Because of the extreme hygroscopic behavior, **2** must be synthesized and stored under anaerobic conditions. This hygroscopic behavior of **2** prevented the determination of the interaction between the picolyl group and silver center by x-ray crystallography because a crystal was unable to be grown.

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Appendix A: 300 MHz ^{1}H NMR Spectra

The following figures represent the ^{1}H NMR spectra obtained for **1** and **2**. Integration of the peaks are shown to ensure synthesis of each of these compounds. A further description of the peaks shown can be found in **Results and Discussion**.

Compound 1

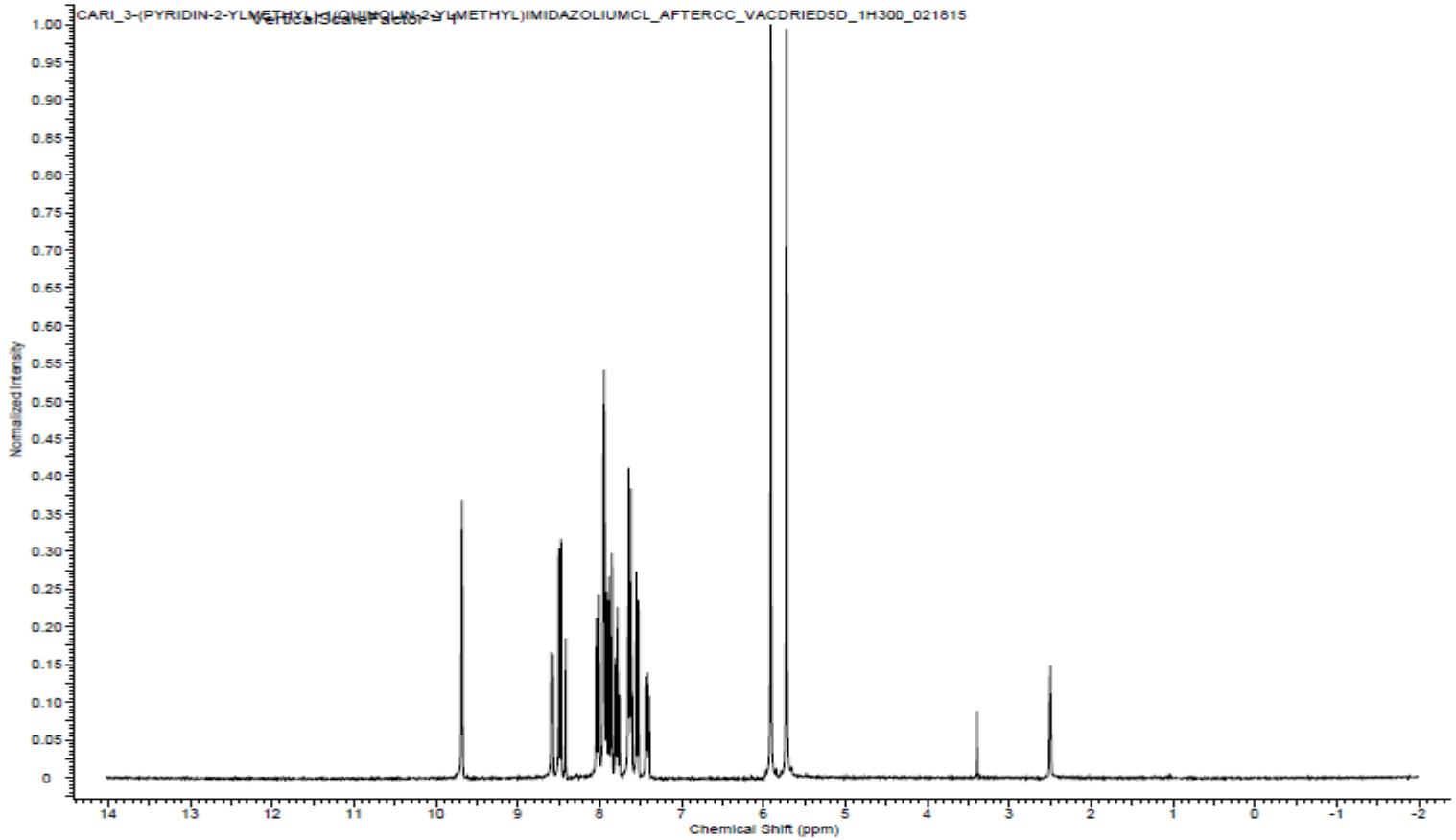

Figure A.1. Full ^{1}H NMR spectrum of **1**

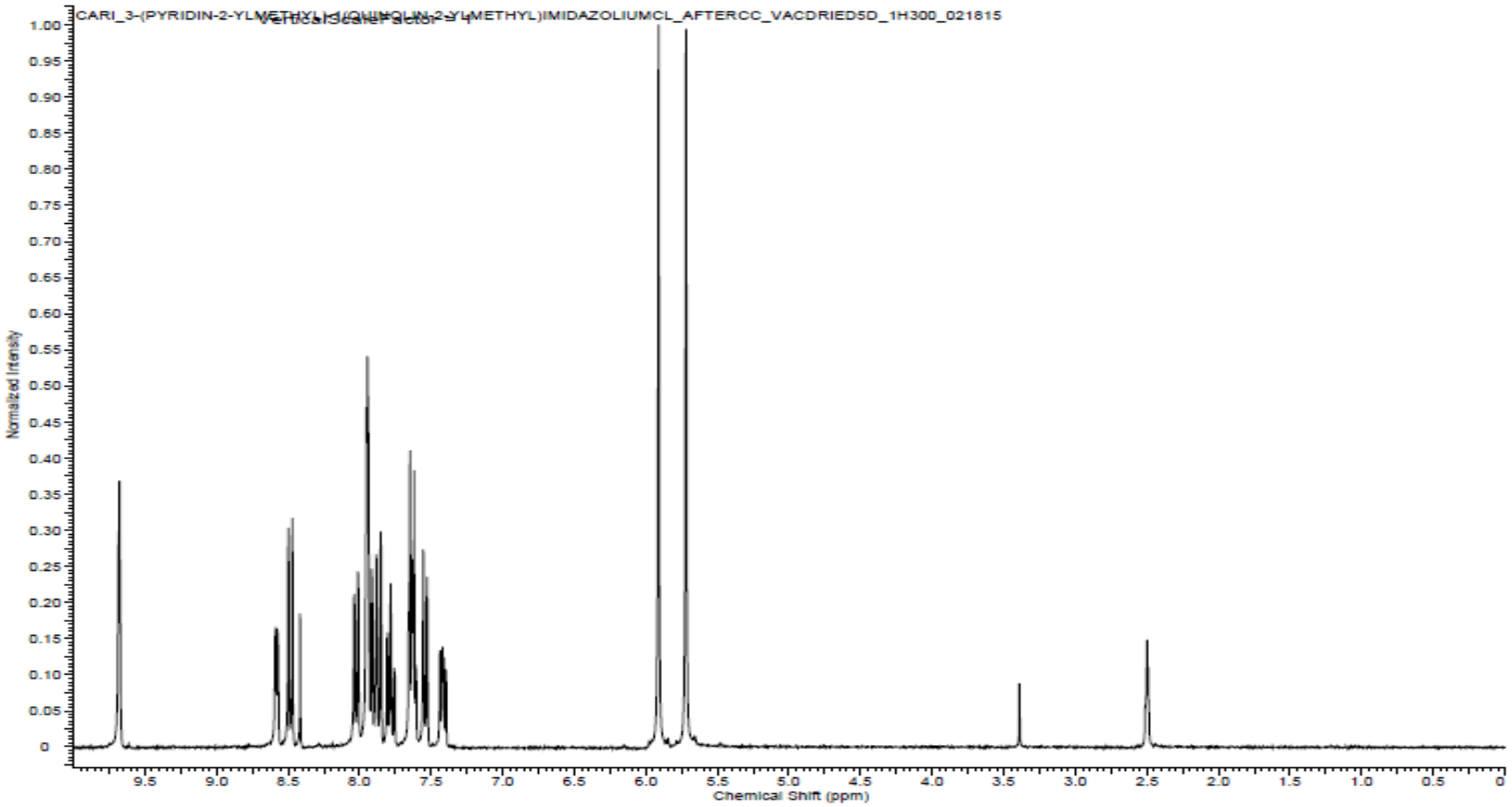

Figure A.2. Zoomed version ^{1}H NMR spectrum of **1**

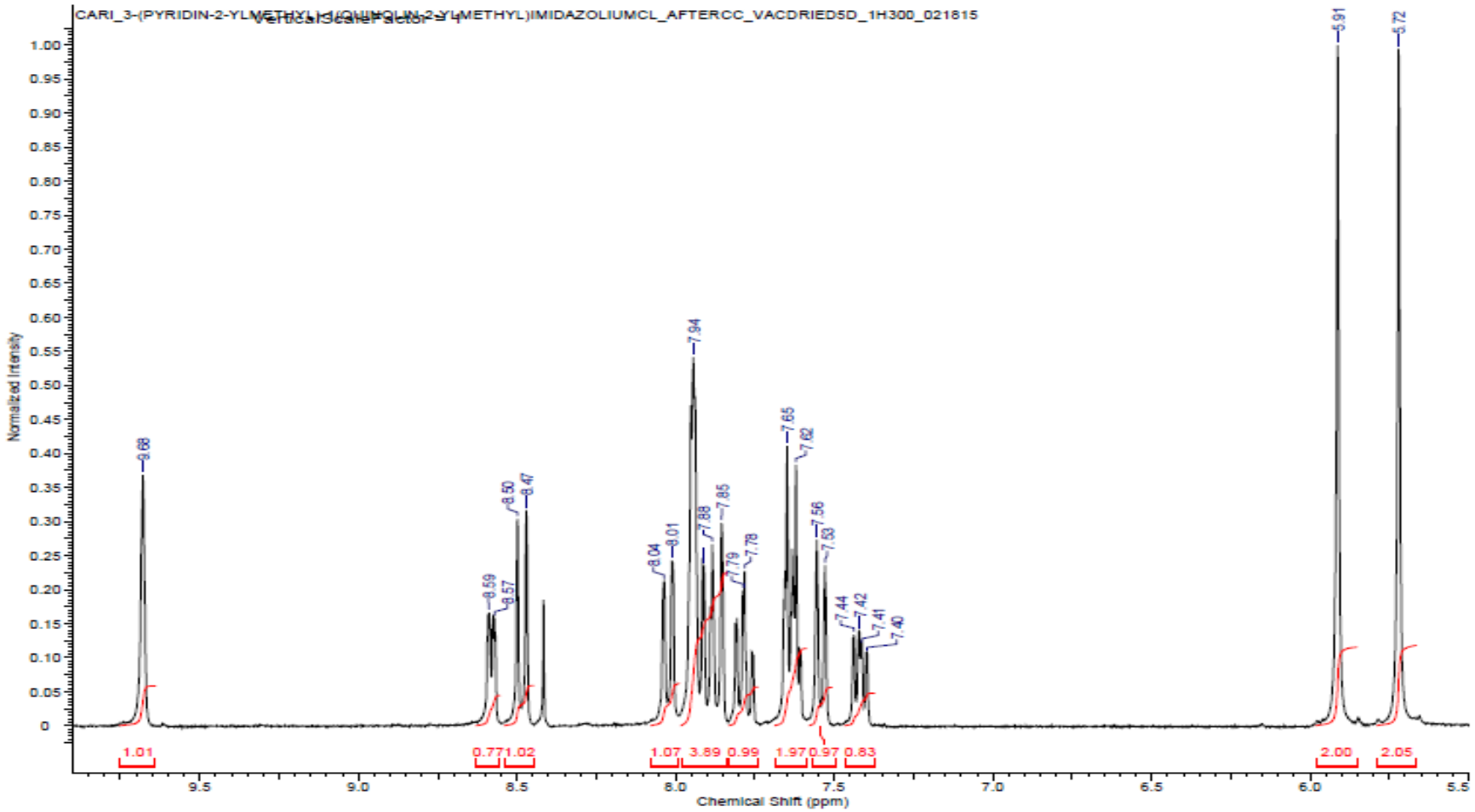

Figure A.3. Integration of peaks shown on ^{1}H NMR spectrum of **1**

Compound 2

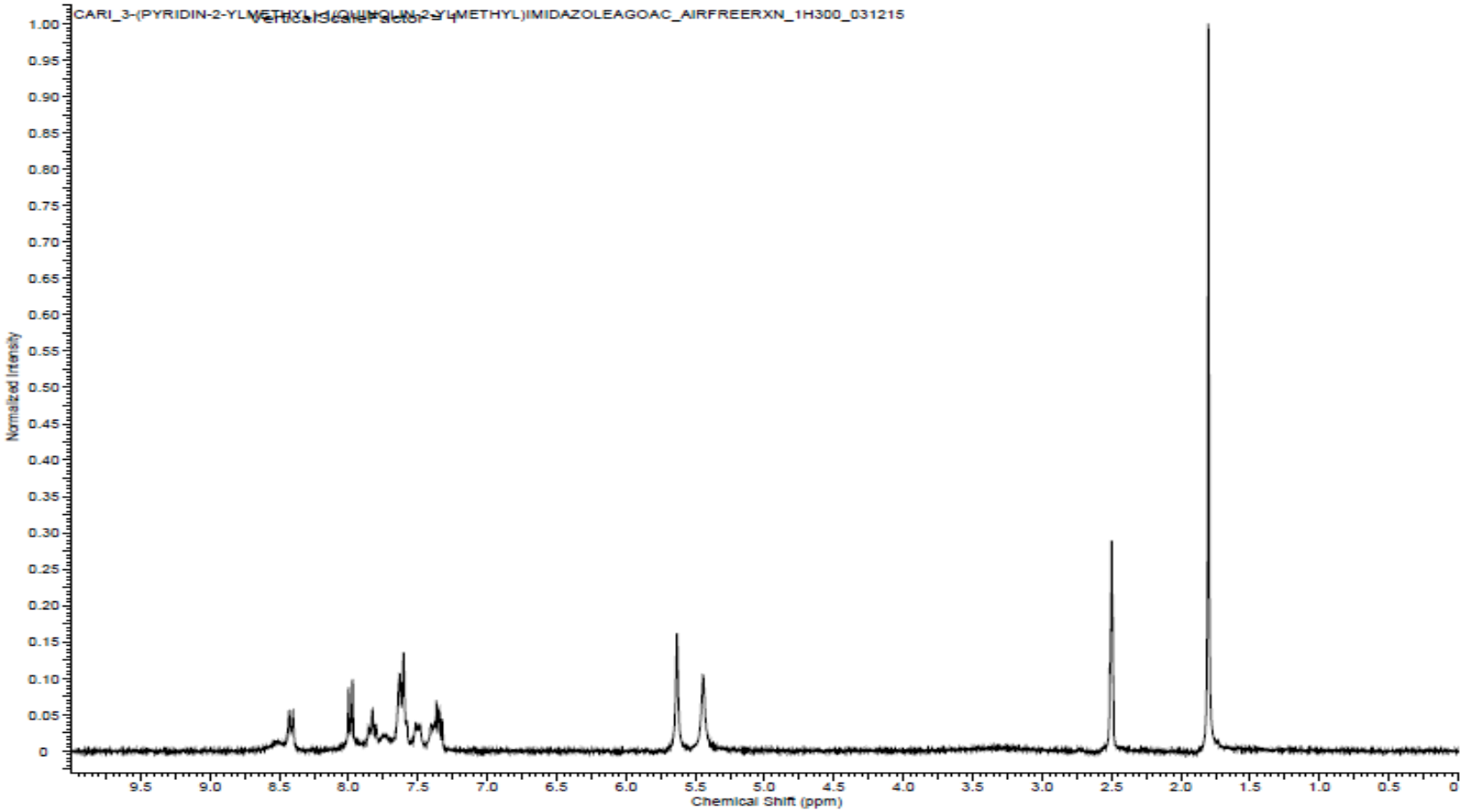

Figure A.4. Full ^{1}H NMR spectrum of **2**

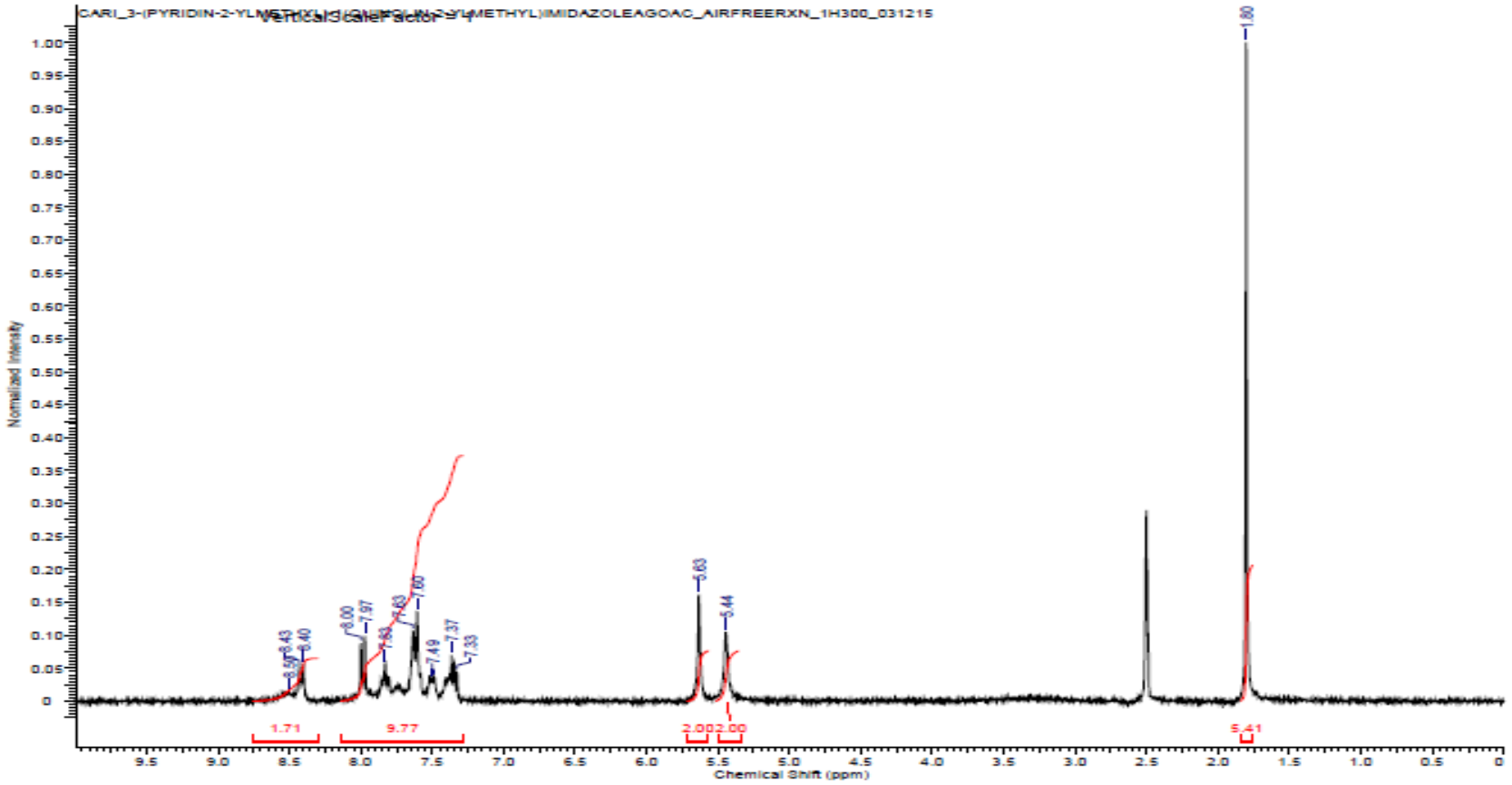

Figure A.5. Integration of peaks shown on ^{1}H NMR spectrum of **2**

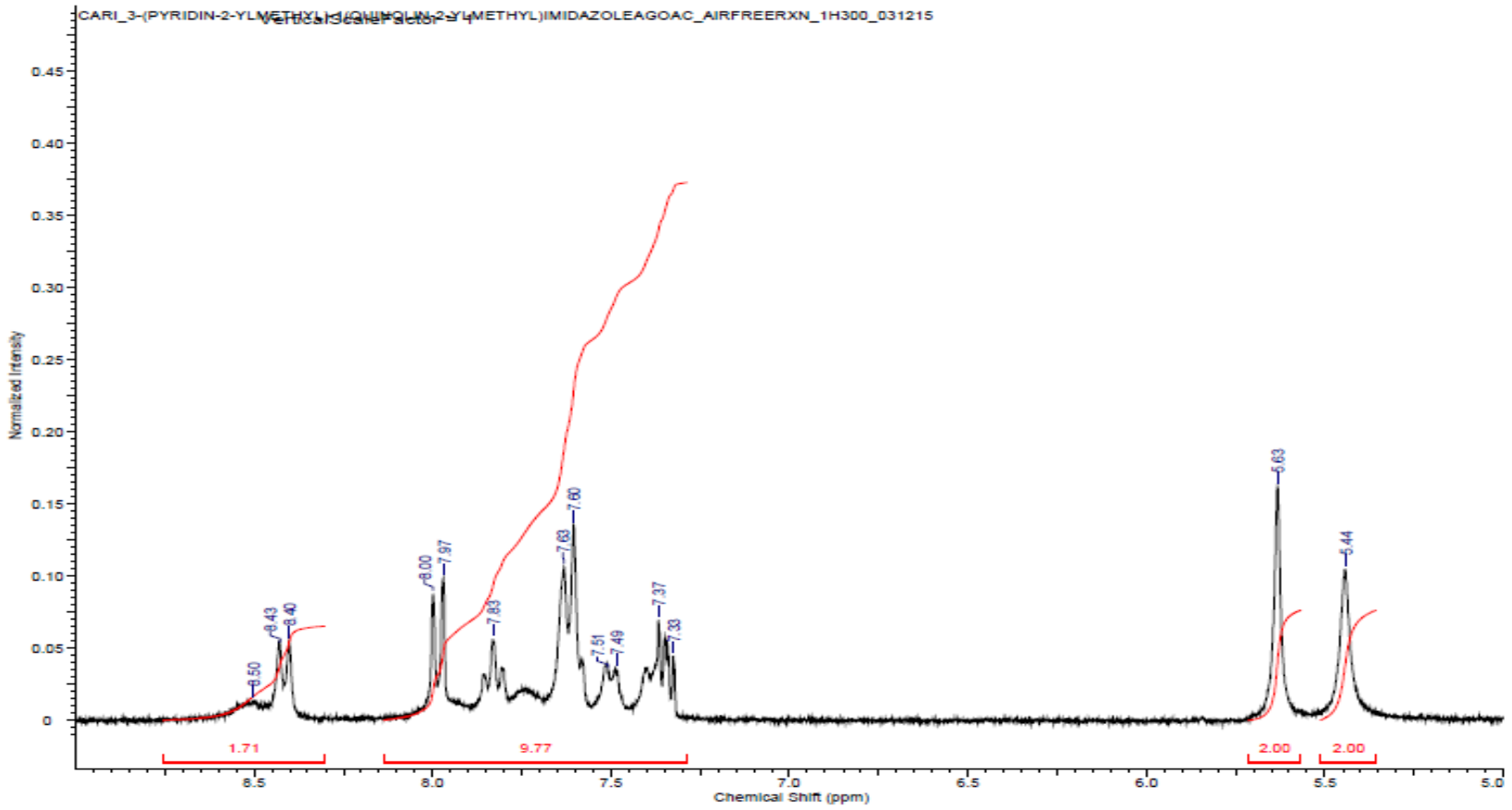

Figure A.6. Integration of peaks shown on ^{1}H NMR spectrum of **2** for the peaks correlating to the hydrogens in the aromatic regions

Appendix B: 500 MHz ^{1}H NMR Spectrum (for Compound 1)

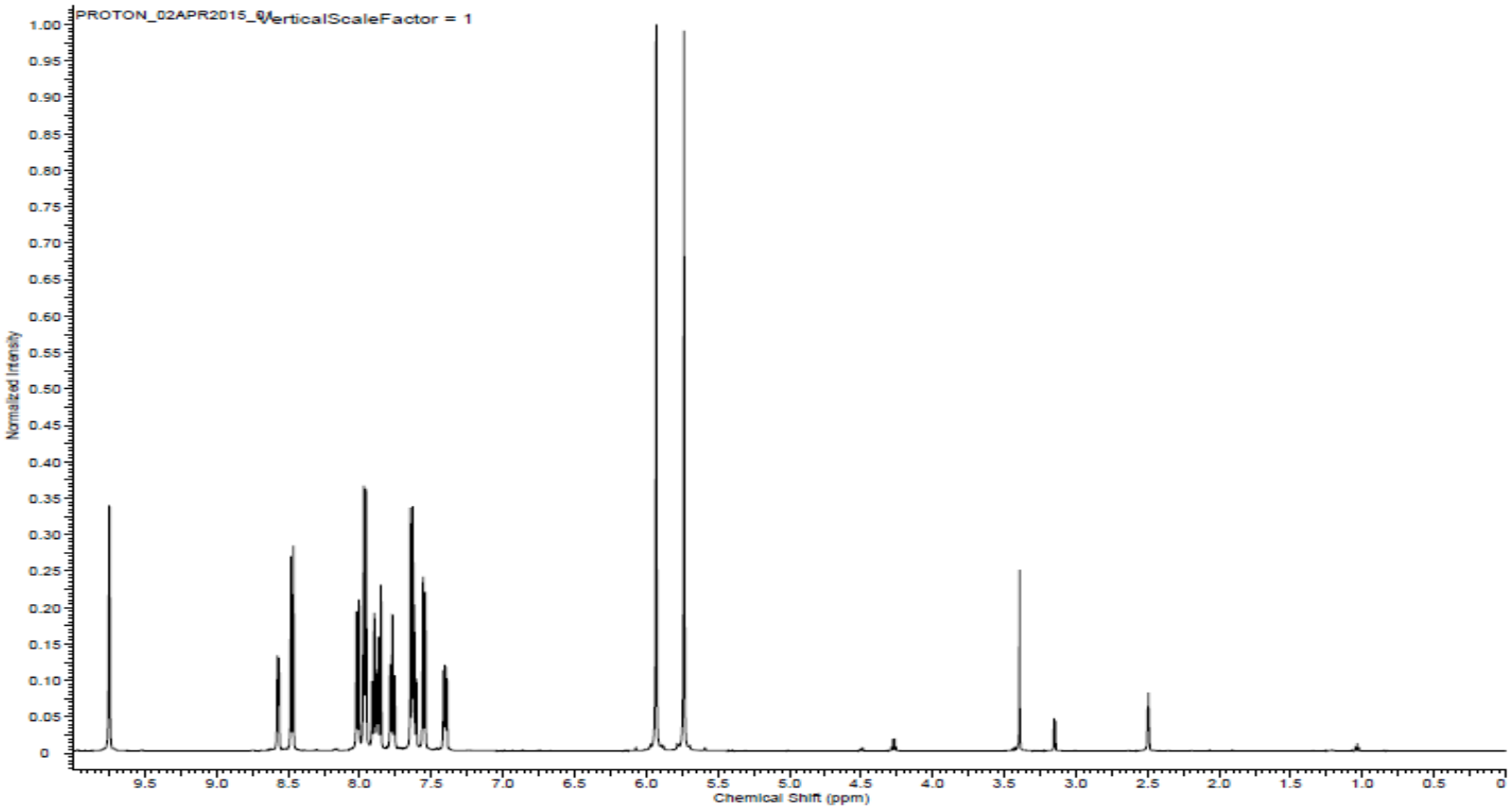

Figure B.1. Full ^{1}H NMR spectrum of **1**

Appendix C: 500 MHz ^{13}C NMR Spectrum (for Compound 1)

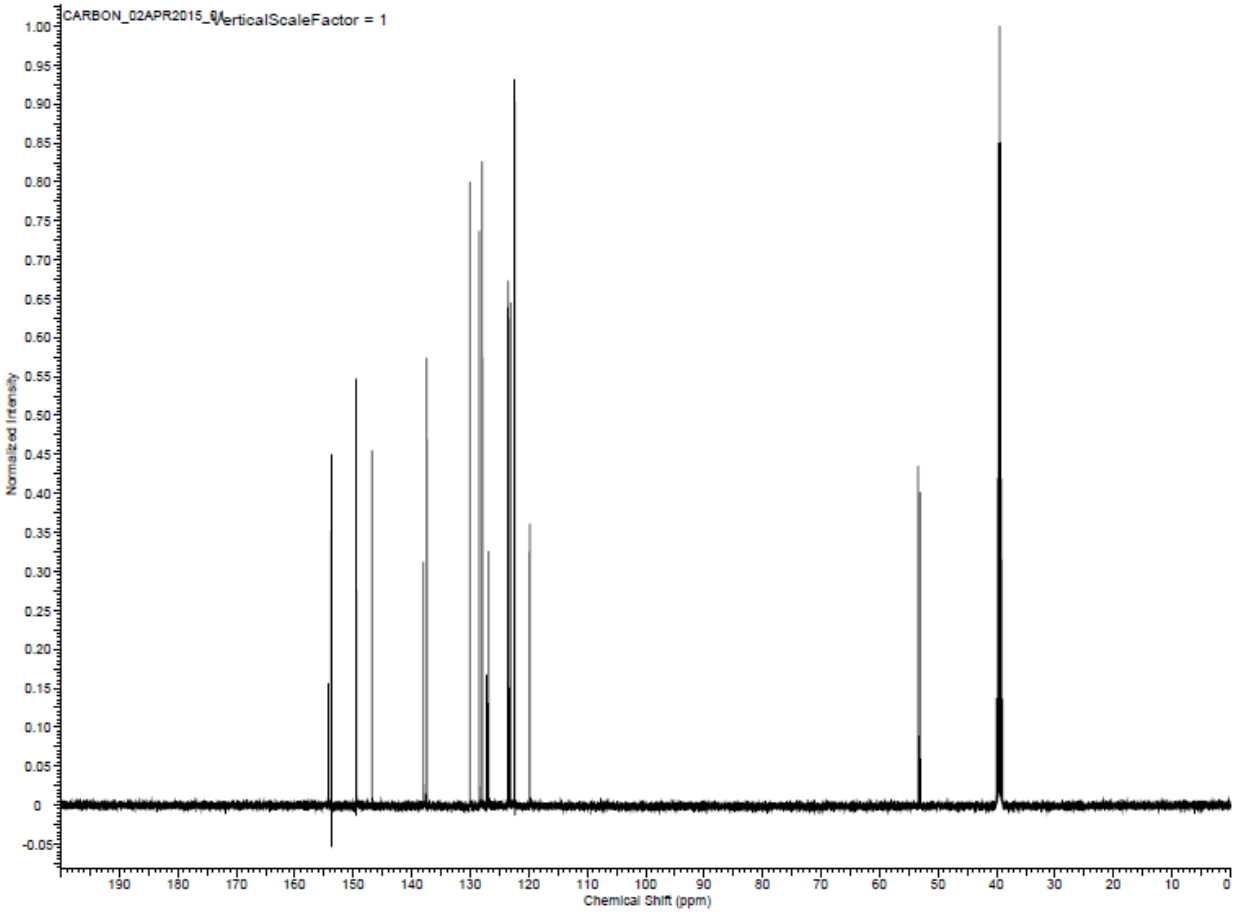

Figure C.1. Full ^{13}C NMR spectrum of **1**

Appendix D: Mass Spectrometry Results for Compound 1

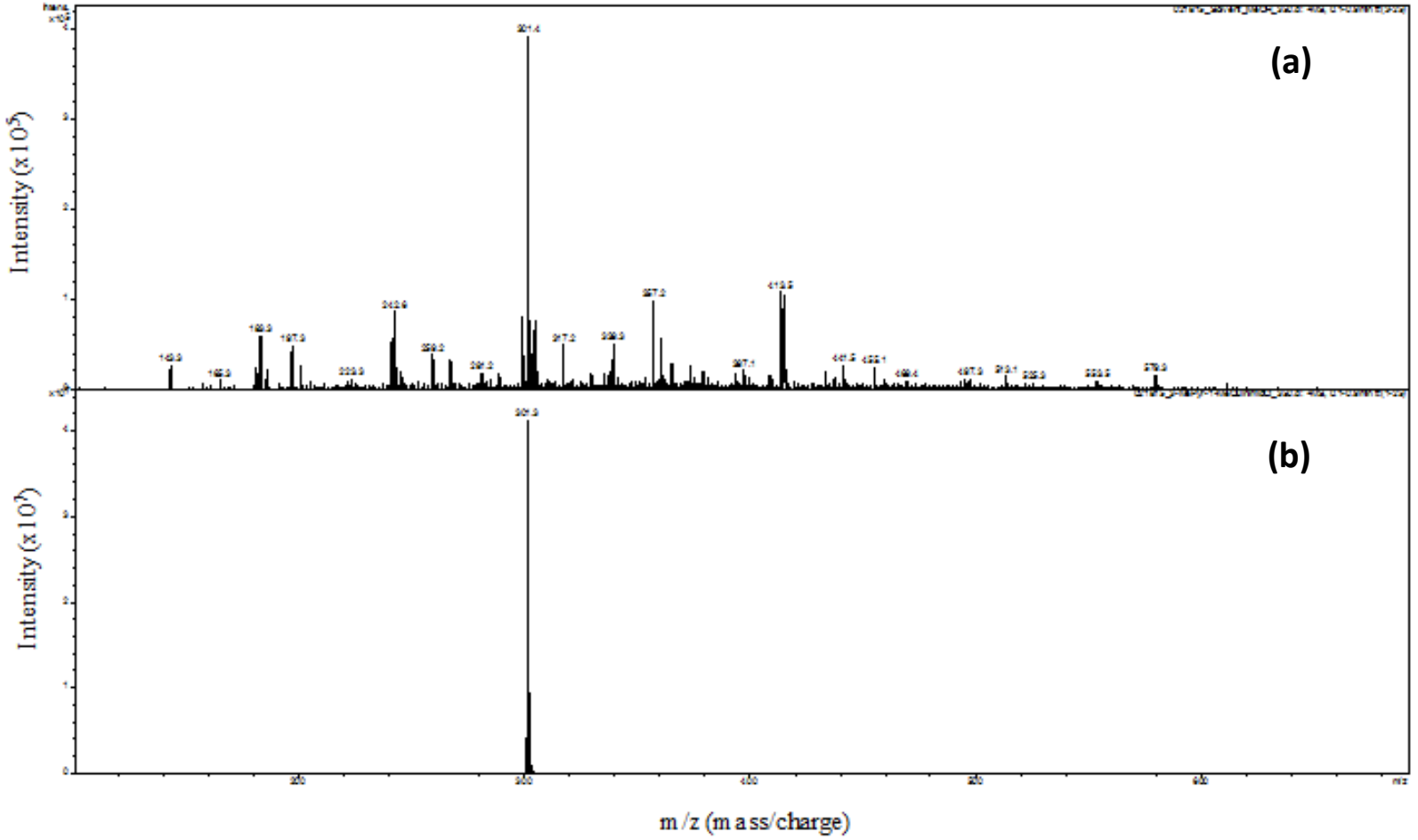

Figure D.1. (a) Mass spectrometry of methanol (b) mass spectrometry of **1**

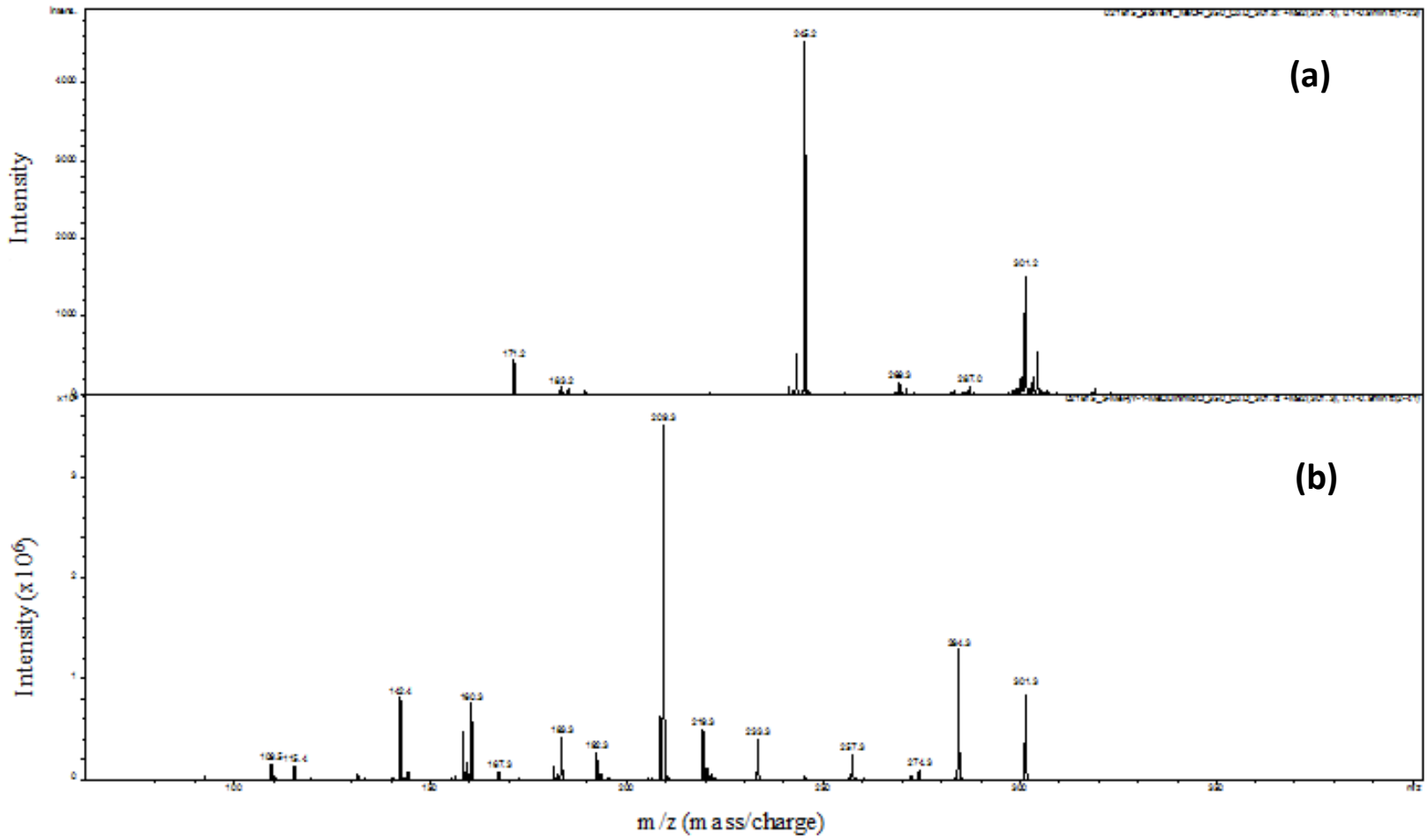

Figure D.2. (a) MS-MS of methanol impurity peak, 301.4 (b) MS-MS of **1**