Summer 2015

Synthesis and Characterization of Imidazolium Salt Derivatives for Anti-Tumor Activity

Ryan W. Pearce
University of Akron Main Campus, rwp15@zips.uakron.edu

Synthesis and Characterization of Imidazolium Salt Derivatives for Anti-Tumor Activity

Ryan Pearce

The University of Akron Department of Chemistry

3150:499 and 3150:497

04 May 2015

Abstract

Several aldehydes (butanal, pentanal, hexanal, 4-hydroxybenzaldehyde) were reacted with 1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (**1**) to produce novel C^2 substituted imidazolium salts for the potential use against non-small cell lung cancer in humans. Compounds 2-(1-hydroxypentyl)-1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (**3**) and 2-(1-hydroxyhexyl)-1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (**5**) were successfully synthesized with structures supported by NMR and mass spectrometry. Characterization by ^{1}H NMR showed evidence of **1** in both compounds. The tumor cell growth inhibition of **3** against non-small cell lung cancer lines NCI-A549, NCI-H460, HCC827, and NCI-H1975 was tested and found to be comparable to cisplatin as measured by MTT assay. Compounds were compared by their IC_{50} values against a panel of human cancer cell lines. The IC_{50} values for **3** were: 9 μM for A549 cells, 7μM for H460 cells, 5 μM forHCC827 cells, and 3 μM for H1975 cells. Cisplatin had IC_{50} values in the range of 3-8 μM for these cell types, indicating that our compound had similar efficiency to a current chemotherapeutic agent. Similar IC_{50} values for **1** in the literature suggest C^2 substituents may not significantly affect tumor cell growth inhibition.[1] This may allow for different functional groups to be substituted at the C^2 position in order to optimize properties such as water solubility and toxicity while not hindering therapeutic benefits.

Introduction

Developing new chemotherapeutic agents is of utmost importance as cancer continues to pose a great threat to human health. In the United States alone, it is predicted that 158,040 people will die from lung cancer in 2015 amounting to 27 % of total cancer-related deaths.[2] Non-small cell lung cancer (NSCLC) accounts for 83 % of lung cancer diagnoses with early treatment being a combination of surgery and chemotherapy.[2] A very low five-year survival rate of 17 %, even with continued chemotherapy, has prompted the investigation for novel chemotherapeutics.[2] The goal of synthesizing new anti-tumor agents is to produce compounds with equivalent activity and better side effect profiles than currently available drugs. Cisplatin (Fig. 1) has been used in the treatment of many types of cancer including head and neck, testicular, and NSCLC but with numerous side effects.[3] Cisplatin works by forming an intrastrand crosslink adduct with DNA resulting in tumor cell death (Fig. 1).[4] A common problem when using cisplatin alone is that tumor cells can rapidly develop resistance, decreasing its effectiveness.[5] To circumvent this problem, new treatment guidelines and drugs have been developed and used in combination with cisplatin. Combination therapy with inorganic cisplatin and additional drugs such as the natural product derivative, vinorelbine or the biologic, bevacizumab, helps to prevent resistance while maintaining therapeutic efficacy.[6]

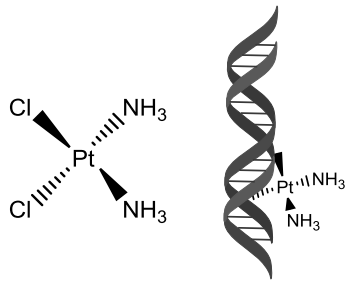


Fig. 1: Structure of cisplatin and representation of intrastrand DNA crosslink formation.

Imidazolium salts (Fig. 2) have recently been investigated for their activity against cancer cells by using MTT assays.[1,7,8] An MTT assay is a procedure that enables the viability of cells to be determined spectrophotometrically when a tetrazolium salt dye (MTT) is added. A measurable absorbance shift from the maximum (540 nm, DMSO) occurs when viable cells reduce the MTT compound to its colored formazan form.[9] The ability for certain compounds to decrease cell viability can be compared using an IC_{50} value; the concentration at which 50 % cell growth occurs relative to control cells. Several Ag(I) N-heterocyclic carbene complexes derived from imidazolium salts were synthesized and found to inhibit tumor cell growth to a comparable level with cisplatin.[10] These results prompted investigation into the anti-tumor activity of the imidazolium salts without the silver complex such as derivatives of 4,5-dichloro-1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (IC23).[1] A derivative of IC23, compound **1** (IC23H, Scheme 1), served as the template for the synthesis of numerous compounds with substitutions made at the C^2 position. It was found that optimal tumor cell growth inhibition occurred when the imidazolium salts maintained both 1,3-bisnaphthylmethyl groups allowing C^2 substitutions to be tuned for improved water solubility and other functions.[1] The focus of this experiment was the substitution of secondary alcohol groups onto the C^2 position of **1**, as the synthesis of these types of compounds had not previously been attempted. In addition to the anti-tumor activity, the synthesis and characterization was of interest because the results may give insight into improving the methodology involved with purifying this class of imidazolium salts.

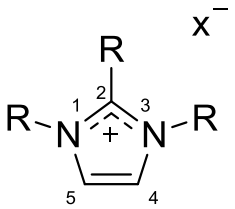


Fig. 2: General structure of an imidazolium salt with carbon atoms labeled numerically.

Methods

Chemicals and Instrumentation: The chemicals used in this experiment were obtained from: Fisher Scientific (sodium bromide, acetonitrile, acetic acid), Sigma-Aldrich (acetone, methanol, ethanol, mixed hexanes, chloroform, dichloromethane), Fluka Analytical (fluorescence indicator green 254 nm), Alfa Aesar (hydrobromic acid, N,N-diisopropylethylamine, hexanal 98 %, 4-hydroxybenzaldehyde 98 %), Cambridge Isotope Laboratories Inc. (DMSO-δ_6), Acros Organics (imidazole, valeraldehyde 97 %), EMD Millipore Chemicals (sodium bicarbonate, diethyl ether, heptane, N,N-dimethylformamide, toluene), and Waterstone Tech LLC (2-(bromomethyl)naphthalene). Butanal was obtained from the organic chemistry lab at the University of Akron and kept in a refrigerator. Instruments used include: Apex Duo XRD (x-ray crystallography), Electrothermal Mel-Temp (melting point), Mettler AE200 (analytical balance), Fisher Scientific Analog Vortex Mixer (sample mixing), Corning Stirring Hot Plate (oil bath heating), Schlenck vacuum glassware (compound purification by vacuum), Büchi Heating Bath B-490 (rotary evaporation bath), Büchi Rotavapor RE111 (rotary evaporation), and Isotemp Fischer Scientific Refrigerator Freezer (recrystallization and storage). NMR spectroscopy was performed on a Varian 300, 400, and 500 MHz NMR with all spectra referenced to 2.50 ppm (^{1}H) and 39.5 ppm (^{13}C) corresponding to the deuterated solvent DMSO-δ_6. A Trevigen MTT Cell Proliferation Assay kit was used by Michael DeBord to conduct MTT assays. The reagent compounds for the synthesis of **2-5** (Scheme 2) was provided by Dr. Wiley Youngs' lab at the University of Akron. Mass spectrometry was performed by Dr. Wesdemiotis' analytical chemistry group at the University of Akron. All reactions were conducted in aerobic conditions and no further purification was used on any reagents or solvents. The synthesis of

compounds **2-5** were modified from procedures for substituting alcohols onto the C^2 position of an imidazolium salt provided in the literature.[1,11]

MTT Assay Protocol: All MTT assays were performed by Michael DeBord. The following standards are provided in the literature.[1] Human non-small cell lung cancer lines NCI-A549, NCI-H460, HCC827, and NCI-H1975 were incubated for 24 h with 5000 – 7000 cells per well (depending on cell line) in a 96-well plate. Stock solutions of compound **3** and cisplatin were each dissolved in a 1 % DMSO/H_2O solution and further diluted to 1, 4, 16, and 32 μM in cell medium (DMEM, maintained at 37 degrees and 5% CO_2). Six replicates at each concentration were added to the plates and incubated for 72 h. A solution of 10 μL MTT reagent in PBS was added to each well and incubated for 3 - 4 h (depending on cell line) followed by removal of the cell medium and the addition of 100 μL DMSO.[1] The plates were then read with a Biotek Epoch plate reader at 540 nm.[1]

Synthesis of 1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (1): The synthesis of compound **1** was modified from a procedure provided in the literature.[1] Imidazole (0.540 g, 7.93 mmol) and potassium hydroxide (0.670 g, 11.9 mmol) were stirred in 10 mL acetonitrile and refluxed for 60 min. This was followed by the slow addition of 2-(bromomethyl)naphthalene (1.78 g, 8.05 mmol) with continued reflux at 82 °C for 60 min. The solution was filtered to remove the yellow/red precipitate (1.06 g). A second equivalent of 2-(bromomethyl)naphthalene (1.78 g, 8.05 mmol) and 10 mL acetonitrile was added to the remaining solution and refluxed overnight. The volatile components were removed by rotary evaporation and the remaining oil precipitated with acetone. The product was then ground to a fine powder with mortar and pestle and stirred in acetone for 24 h. ^{1}H NMR (300 MHz, DMSO-δ_6): δ = 9.50 (1H, s, NCHN), 8.01 – 7.91 (12H, m, Ar), 7.59 – 7.53 (6H, m, Ar), 5.62 (4H, s, CH_2), 2.08 (CH_3CN, impurity).

Synthesis of 2-(1-hydroxybutyl)-1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (2): Compound **1** (0.501 g, 1.17 mmol) was dissolved in 1.5 mL DMF and 3.0 mL (17.4 mmol) N,N-diisopropylethylamine (DIPEA). Butyraldehyde (0.400 mL, 4.44 mmol) was subsequently added and the solution heated and stirred in a pressure vessel at 80 °C overnight. The orange/red liquid was diluted with 60 mL dichloromethane and extracted twice with saturated brine (sodium bromide in water) and a 1:10 (v/v) hydrobromic acid in water solution. Volatiles were then removed with rotary evaporation yielding a red/brown oil which was stirred and precipitated in diethyl ether overnight. The product was then filtered yielding a brown solid. Crude product yield: 0.462 g, 79 % yield. ^{1}H NMR (300 MHz, DMSO-δ_6) did not confirm synthesis.

Synthesis of 2-(1-hydroxypentyl)-1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (3): Compound **1** (0.500 g, 1.16 mmol) was dissolved in 1.5 mL (8.70 mmol) DIPEA and 3.0 mL DMF. Valeraldehyde (0.500 mL, 4.70 mmol) was added and the solution heated and stirred at 80 °C overnight. The orange/red liquid was diluted with 60 mL dichloromethane and extracted twice with a 1:10 (v/v) hydrobromic acid in water and saturated brine solution (sodium bromide in water). The volatile components were then removed with rotary evaporation generating a red/orange oil which was stirred and precipitated with diethyl ether overnight. A silica gel column was prepared using a green fluorescence indicator and 3:1 (v/v) dichloromethane and methanol solution allowing the product to be separated into five fractions using flash column chromatography. The first two fractions were combined and reduced using rotary evaporation and precipitated with diethyl ether. The product was then placed under vacuum using a Schlenk line for 24 h followed by recrystallization in cold acetonitrile and placed under vacuum overnight. The crystals were ground to a fine powder and placed under vacuum overnight. The flask containing the crystals was heated to 80 °C using an oil bath. Obtained 0.110 g (18 %

yield) of a fine white powder. MP: 174 – 176 °C. MS: m/z = 435.6 (theoretical for M^+ = 435.2). ^{1}H NMR (500 MHz, DMSO-δ_6): δ = 9.50 (IC23H, impurity), 7.99 – 7.90 (10H, m, Ar), 7.56 – 7.48 (6H, m, Ar), 6.60 (1H, d, OH, J = 3.90 Hz), 5.82 (4H, q, CH_2, J = 5.81 Hz), 5.65 (IC23H, impurity), 5.34 (1H, quin, CH, J = 9.2 Hz), 2.07 (CH_3CN, impurity), 1.58 (2H, d, CH_2, J = 9.8 Hz), 1.19 – 1.07 (2H, m, CH_2), 0.80 (2H, m, CH_2), 0.41 (3H, m, CH_3). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-δ_6): δ = 147.6 (NCN), 132.6 (Ar), 132.2 (Ar), 128.7 (Ar), 127.7 (Ar), 127.6 (Ar), 126.8 (Ar), 126.6 (Ar), 125.1 (Ar), 123.1 (Ar), 64.0 (COH), 51.4 (CH_2), 34.0 (CH_2), 27.0 (CH_2), 21.3 (CH_2), 13.1 (CH_3).

Synthesis of 2-(hydroxyl(4-hydroxyphenyl)methyl)-1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (4): Compound **1** (0.501 g, 1.17 mmol) was dissolved in 1.5 mL (8.70 mmol) DIPEA and 3.0 mL DMF followed by the addition of 4-hydroxybenzaldehyde (0.152 g, 1.25 mmol). The solution was heated at 80 °C overnight then diluted with 60 mL dichloromethane. The solution was then extracted twice with 1:10 (v/v) hydrobromic acid in water and saturated brine (sodium bromide in water). Volatiles were removed with rotary evaporation giving an oil which was then stirred and precipitated with diethyl ether. The product was filtered and washed with acetone to remove colored impurities. ^{1}H NMR (300 MHz, DMSO-δ_6) did not confirm synthesis.

Synthesis of 2-(1-hydroxyhexyl)-1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (5): Compound **1** (0.501 g, 1.17 mmol) was dissolved in 1.5 mL DMF and 3.0 mL (17.4 mmol) DIPEA followed by the addition of hexanal (0.500 mL, 4.07 mmol). The solution was heated at 80 °C overnight then diluted with 60 mL dichloromethane. The solution was then extracted twice with 1:10 (v/v) hydrobromic acid in water and saturated brine (sodium bromide in water). The volatile components were removed by rotary evaporation and the remaining oil precipitated in

diethyl ether and filtered giving an orange/white powder. The product was then ground and placed under vacuum. A fluorescent silica gel column was prepared using a green fluorescence indicator and 9:1 (v/v) dichloromethane and methanol solution with the addition of 20 mL acetic acid. The product was dissolved in ~ 3 mL of the solvent system resulting in 10 fractions after flash column chromatography. Fractions 1 – 4 were combined and the volatile components removed by rotary evaporation giving an oil. The oil was stirred and precipitated with diethyl ether, filtered, and placed under vacuum for ~ 12 h. Obtained 0.271 g (44 % yield) of a fine white powder. MP: 144 – 146 °C. MS: m/z = 449.3 (theoretical for M^+ = 449.3). ^{1}H NMR (500 MHz, DMSO-δ_6): δ = 9.49 (IC23H, impurity), 7.99 – 7.86 (11H, m, Ar), 7.57-7.46 (7H, m, Ar), 6.57 (1H, s, OH), 5.78 (4H, m, CH_2), 5.61 (IC23H), 5.30 (1H, s, CH), 1.55 (2H, d, CH_2, J = 10.3 Hz), 1.20 (2H, comp, CH_2), 1.05 (2H, comp, CH_2), 0.86 – 0.63 (6H, comp, CH_2), 0.53 (3H, t, CH_3, J = 7.1 Hz). 13C{^{1}H} NMR (125 MHz, DMSO-δ_6): δ = 147.5 (NCN), 132.6 (Ar), 132.3 (Ar), 128.7 (Ar), 127.8 (Ar), 127.6 (Ar), 126.7 (Ar), 125.0 (Ar), 123.2 (Ar), 64.1 (COH), 51.4 (CH_2), 34.4 (CH_2), 30.4 (CH_2), 24.5 (CH_2), 21.5 (CH_2), 13.5 (CH_3).

Results and Discussion

Synthesis and Characterization: Compound **1** served as the foundation for the synthesis of novel compounds with secondary alcohol substituents at the C^2 position. Compound **1** was synthesized using imidazole and two equivalents of 2-(bromomethyl)naphthalene in acetonitrile and potassium hydroxide at 82 °C (Scheme 1). The yellow/red precipitate filtered after the first addition of 2-(bromomethyl)naphthalene was most likely potassium bromide which remained colored due to an insufficient washing with acetonitrile. A ^{1}H NMR (300 MHz, DMSO-δ_6) spectrum was then obtained (Fig. 4) to confirm the presence of the intermediate monosubstituted imidazole (Scheme 1). Resonance at 9.47 ppm and in the 7.96 – 7.76 ppm region indicated the

presence of a proton at the C^2 position and aromatic protons, respectively. Resonance at 5.60 ppm indicated presence of the methylene protons corresponding to the "bridge" between the imidazole ring and naphthalene. This chemical shift was also evident on the spectrum for the final product (Fig. 3, $\delta = 5.62$). Obtaining this intermediate spectrum was useful for verifying the first step of the reaction had occurred. Precipitation and stirring in acetone for 24 h gave a nearly pure product confirmed by ^{1}H NMR (Fig. 3). Resonance at 2.08 ppm was most likely an acetonitrile impurity which could be removed if the compound were placed under vacuum. Due to acetonitrile being used in the future synthesis of **2-5**, no additional purification was necessary for this material. Resonance at 9.50 ppm (Fig. 3) corresponded to the proton at the C^2 position of **1**, a key structural feature for identifying the compound, shifted downfield due to the nearby nitrogen atoms in the imidazole ring.

The synthesis of **2-5** (Scheme 2) followed the same general procedure for reaction of various aldehydes with **1** to give secondary alcohol substituents at the C^2 position. This general procedure began by combining the imidazolium salt reagent **1** with the base DIPEA before addition of the aldehyde and heating to 80 °C. A molar ratio of approximately 2:1 DIPEA to aldehyde was used in the synthesis of **3** and **4** and 4:1 in the synthesis of **2** and **5**. The base's function was to deprotonate **1** at the C^2 position enabling the aldehyde substitution. Compound **2** was unable to be synthesized to a substantial degree due to oxidation of the reagent, butanal. The downfield shift of the carboxylic acid proton a_1 (11.98 ppm) from the aldehyde proton a (9.66 ppm) suggests partial oxidation occurred. The reduced amount of available butanal may have prevented the reaction from occurring. Fig. 5 does not display resonance at 6.50 ppm which is expected for the intended hydroxyl proton, nor does it display a sufficient number of chemical shifts in the alkyl region for the 1-hydroxybutyl chain. Due to some aldehyde remaining present

in the oxidized reagent, it was expected that some of the intended product was still formed despite poor yield and lack of substantial NMR evidence in Fig. 5.

The synthesis of **3** and **5** proved challenging due to difficulties in removing the starting material. Ultimately, IC23H impurities remained in small amounts based on ^{1}H NMR (Fig. 7, 9). After extraction and rotary evaporation of **3**, a ^{1}H NMR was obtained (Fig. 13) showing evidence of **1** at chemical shifts 9.46 ppm and 5.61 ppm. Residual diethyl ether from precipitation was apparently due to resonance at 3.63 ppm.[12] Flash column chromatography was selected in an attempt to separate the residual starting material from the product due to the expected differences in polarities between the compounds. Thin-layer chromatography (TLC) was used to determine the optimum solvent combination and ratio of solvents for the column. A 3:1 (v/v) mixture of dichloromethane to methanol provided the greatest amount of separation. For the purposes of TLC, acetic acid (6 drops) was added to decrease the amount of "smearing" of the spots, but was not added to the column. Acetic acid's high polarity and ability to decrease fraction size may have enabled better separation. It was noted that **3** traveled further on the TLC plates and eluted first from the column compared to **1**. These results were interesting, as the hydroxyl group on **3** was expected to have increased polarity compared to **1** thereby decreasing its R_f value. However, the naphthalene substituents and imidazole ring may have created a great electron delocalization effect negating any substantial polarity differences leading to this result. Fractions containing no starting material were combined, reduced by rotary evaporation, and precipitated with diethyl ether. The remaining solids were then placed under vacuum for attempted removal of residual diethyl ether. Based on ^{1}H NMR (Fig. 14), the column was successful in removing the starting material but showed evidence of diethyl ether impurities at 3.38 ppm and 3.16 ppm. Recrystallizing in acetonitrile, followed by vacuum, grinding to a fine powder, and repeated

vacuum with heat gave the final product with an 18 % yield. The yield could possibly be improved by increasing the amount of base (DIPEA) used (such as in the synthesis of **5**). Mass spectrometry data (Appendix 2, Chart 1) supported the synthesis of **3** as the experimental mass (435.6 g/mol) was near the theoretical M^+ of 435.2 g/mol. Significant product loss occurred during column chromatography and purification. A more highly concentrated sample analyzed with a 500 MHz ^{1}H NMR (Fig. 7) showed evidence of residual IC23H resonance at 9.60 ppm and 5.65 ppm, in addition to acetonitrile at 2.07 ppm. The $^{13}C\{^1H\}$ (125 MHz, DMSO-δ_6) spectrum suggested the presence of the pentyl chain due to the 4 unique upfield shifts (Fig. 8). It is clear that further purification techniques were needed to remove the starting material. The nature of these substituted imidazolium salts may be such that there is not a significant difference in polarity or solubility between **1** and **3**, or that better separation conditions are needed. A longer column or different technique such as HPLC may improve the results. Crystal trials were attempted for mixed solvents (acetone/water, methanol/water) and single solvents (ethanol, acetonitrile, methanol) giving visible crystals for ethanol and acetone/water; however, none of these crystals were suitable to obtain high quality X-ray crystallographic data.

Compound **5** followed a similar synthetic and purification methodology as **3** but did not require recrystallization in acetonitrile after elution from the silica gel column and resulted in a nearly 2.5 times greater yield (44 %). Mass spectrometry data (Appendix 2, Chart 3) supported the synthesis of **5** as the experimental mass (449.3 g/mol) matched the theoretical M^+ of 435.2 g/mol. Unlike **3**, this compound was synthesized using 17.4 mmol of the base DIPEA compared to 8.70 mmol and was placed under vacuum before flash column chromatography. A slightly different solvent system for column chromatography was used (9:1 (v/v) dichloromethane and methanol) but with the addition of 20 mL acetic acid. Visibly, the column did not appear to give

significant separation as the effluent elongated across the entire length of the column. Despite this, 10 fractions were obtained with the first 4 showing no evidence of **1** by TLC. These fractions were combined, reduced by rotary evaporation, and precipitated to give a white powder which was then placed under vacuum. A ^{1}H NMR (300 MHz) was obtained (Fig. 15) giving no evidence of IC23H impurities. However, a ^{1}H NMR (500 MHz, DMSO-δ_6) (Fig. 9) showed resonance at 9.49 ppm and 5.61 ppm corresponding to **1**, but did not show evidence of acetonitrile as was apparent in Fig. 7 for **3**. The ^{13}C{^{1}H} (125 MHz, DMSO-δ_6) spectrum suggested the presence of the hexyl chain due to the 5 unique upfield shifts (Fig. 10). Crystal trials were attempted using mixed-solvents (ethanol/water, methanol/water, acetone/water) and single solvents (acetone, ethanol). Crystals grew for methanol/water, acetone, and ethanol but again, none were suitable for obtaining high quality data. The chiral center at the carbon to which the hydroxyl group is bonded in both **5** and **3** (Scheme 2) may have led to a racemic product containing both stereoisomers increasing the complexity of the crystal structure.

The successfully synthesized compounds **3** and **5** demonstrated unique NMR features. Both compounds exhibit chirality (Scheme 2) at the carbon to which the hydroxyl group is attached. The methylene groups bridging the nitrogen of the imidazole ring to the naphthalene substituent on both imidazolium salts resonate as apparent quartets at chemical shifts 5.82 ppm (**3**, Fig. 16) and 5.78 ppm (**5**, Fig. 17). Due to the chirality of both compounds, each methylene group will exist in a different conformation resulting from a lack of symmetry. The apparent quartet could be considered “facing doublets” as each methylene group splits as an independent doublet with one equivalent proton. Furthermore, on the ^{1}H NMR (500 MHz, DMSO-δ_6) for **3** (Fig. 7), the shift corresponding to the hydroxyl proton (δ = 6.60) is a doublet (Fig. 18), which supports the

chirality of the structure due to slightly different splitting patterns for each isomer; however, this doublet is not apparent in **5** (Fig. 9).

Compound **4** was unique in that it was the only attempted synthesis with a non-alkyl aldehyde but was unable to be synthesized most likely due to lack of reaction of **1** with 4-hydroxybenzaldehyde. The ^{1}H NMR spectrum for **4** is similar to that of **1** (Fig. 11) and the spectrum for 4-hydroxybenzaldehyde (Fig. 12) does not show evidence of oxidation to carboxylic acid. If no reaction occurred, the aldehyde would have been removed during extraction giving the original imidazolium salt that was observed by NMR. The 4-hydroxybenzaldehyde reagent (Fig. 12) has a singlet at chemical shift 10.60 ppm corresponding to the hydroxyl proton c. The chemically equivalent protons at position a are split by b, which are split by a. This splitting by neighboring equivalent protons resulted in the doublets resonating at chemical shifts 6.91 – 6.94 ppm and 7.74 – 7.77 ppm, respectively. The singlet resonating at 9.78 ppm was shifted downfield by the nearby carbonyl group. The difference in structure and increased molecular weight for this reagent compared to the alkyl aldehydes in the synthesis of **2**, **3**, and **5** may have prevented reaction. A 1:1 molar ratio of aldehyde to **1** was also used instead of the typical ~ 4:1 ratio which was successful in the synthesis of **3** and **5**. The 4-hydroxybenzaldehyde structure contains a benzene ring which acts as an electron donating group, creating an electron delocalization effect that increases the electrophilicity of the carbonyl carbon. Furthermore, the bulk of the benzene ring may have created significant steric hindrance. Increasing the molar equivalent of the base DIPEA may have also helped increase reactivity by ensuring the starting material was deprotonated for substitution.

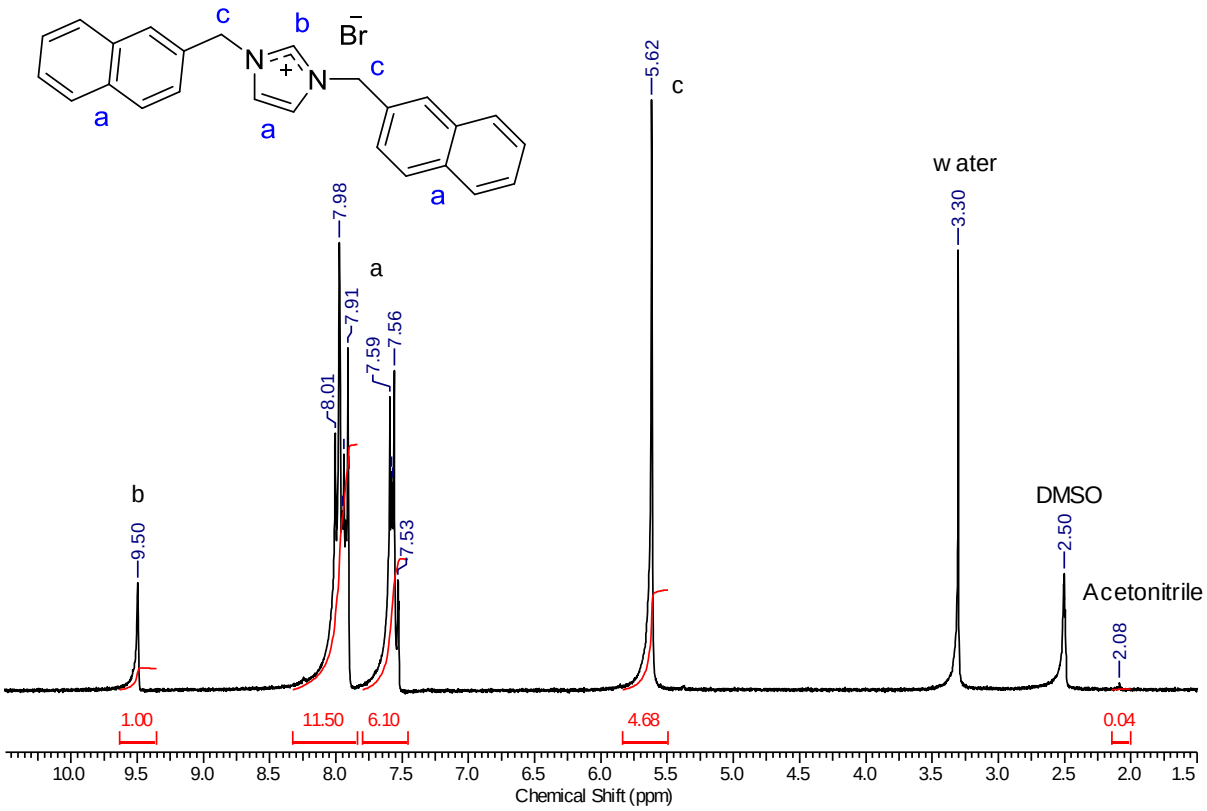


Fig. 3: ^{1}H NMR (300 MHz, DMSO-δ_6) for compound **1**.

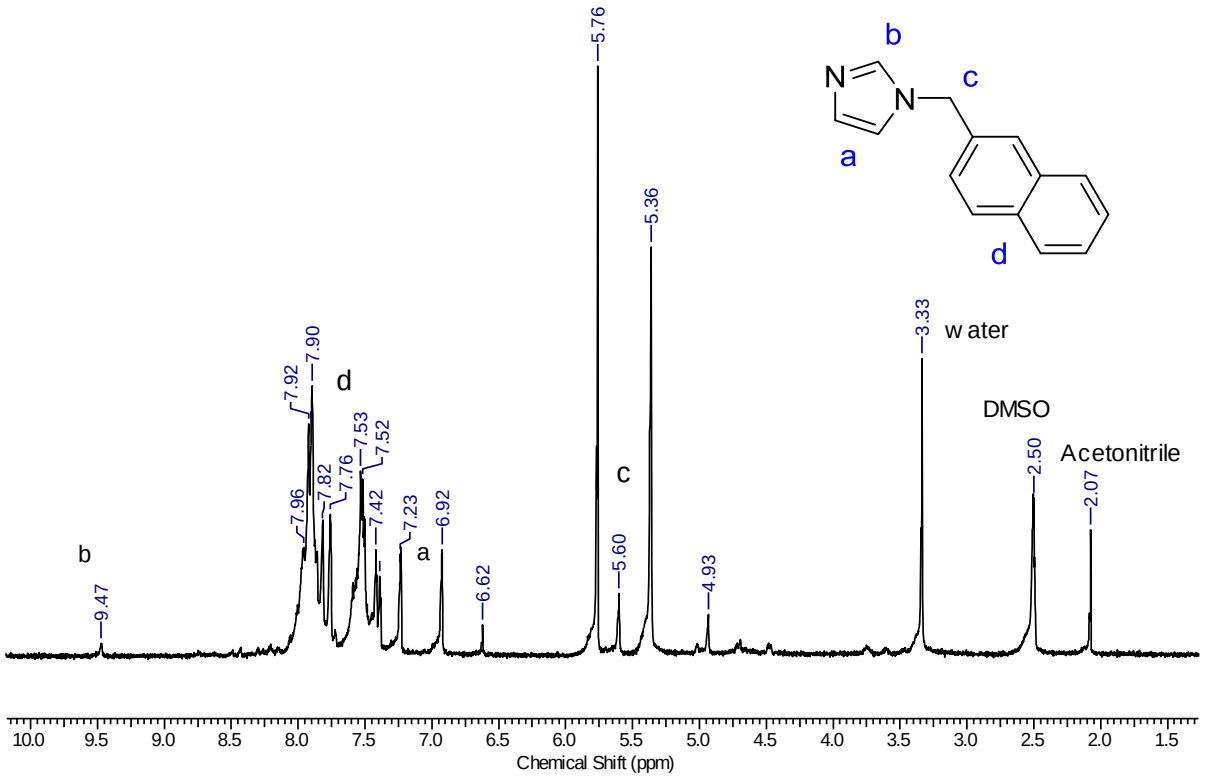


Fig. 4: ^{1}H NMR (300 MHz, DMSO-δ_6) of intermediate monosubstituted imidazolium salt, 1-(naphthalen-2-ylmethyl)-imidazole in the synthesis of **1**.

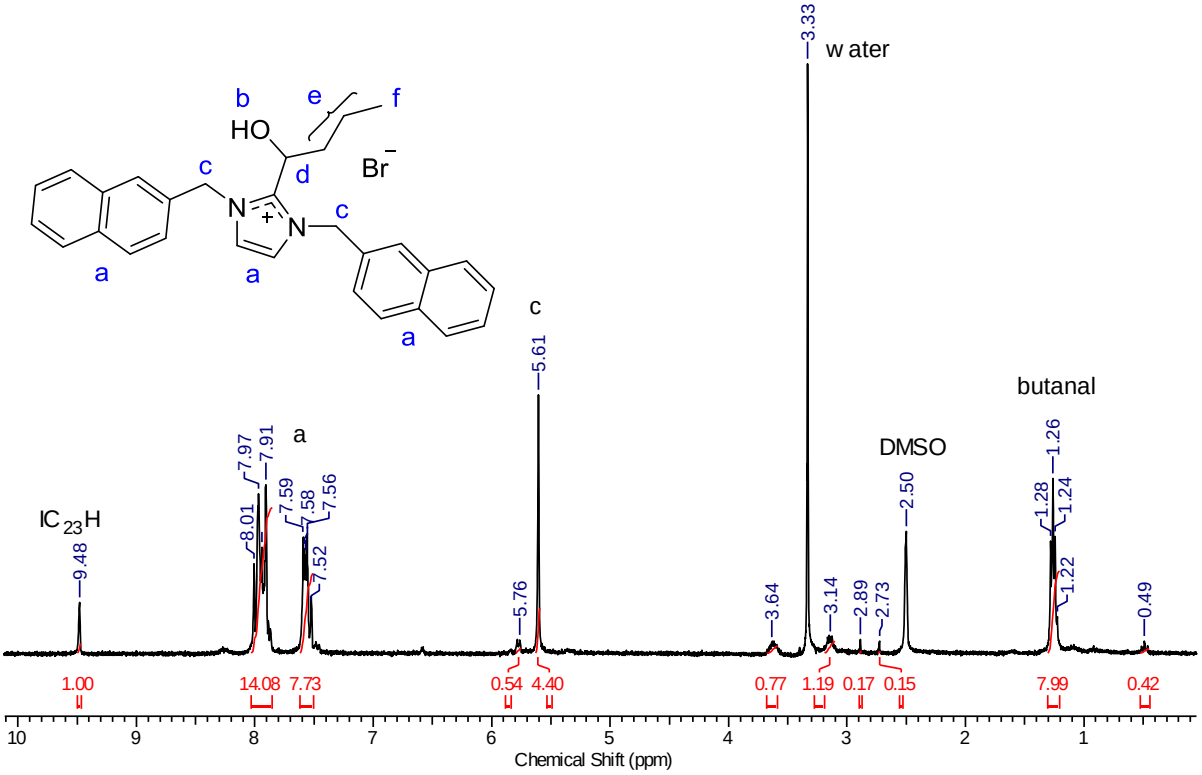


Fig. 5: ^{1}H NMR (300 MHz, DMSO-δ_6) for the synthesis of **2**.

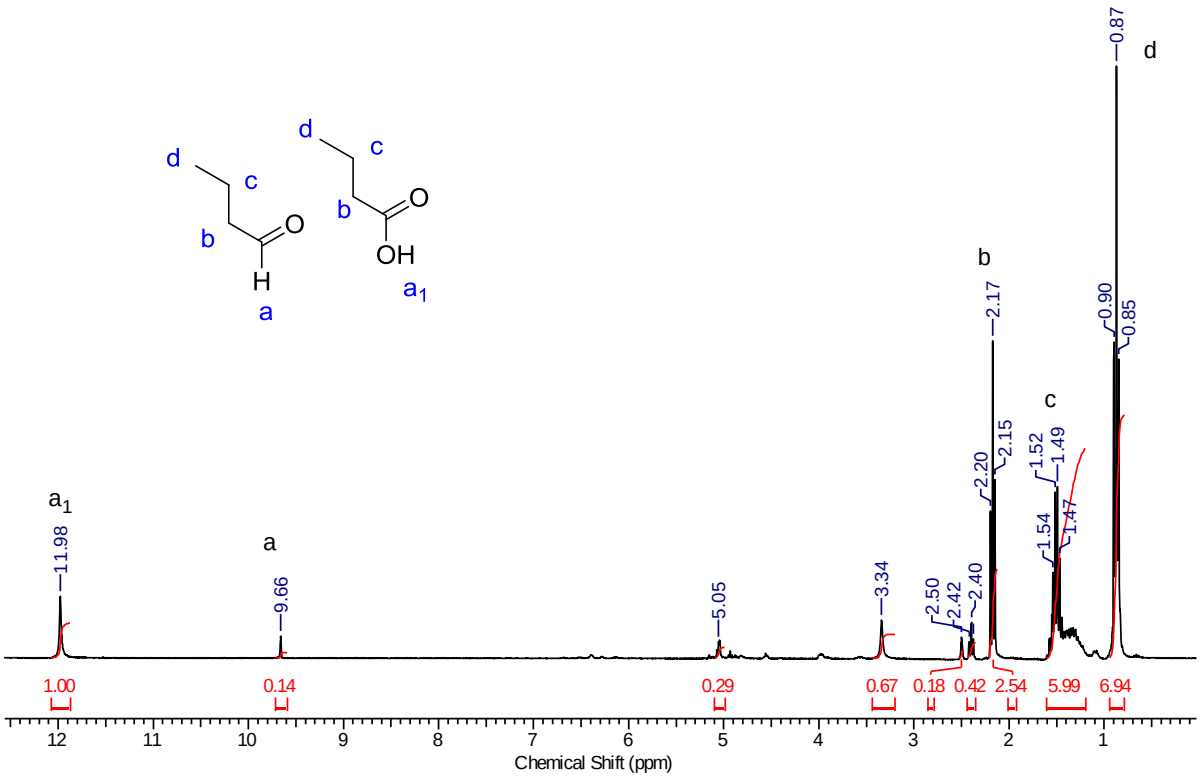


Fig. 6: ^{1}H NMR (300 MHz, DMSO-δ_6) for butanal in the synthesis of **2** displaying evidence of oxidation at δ = 11.98.

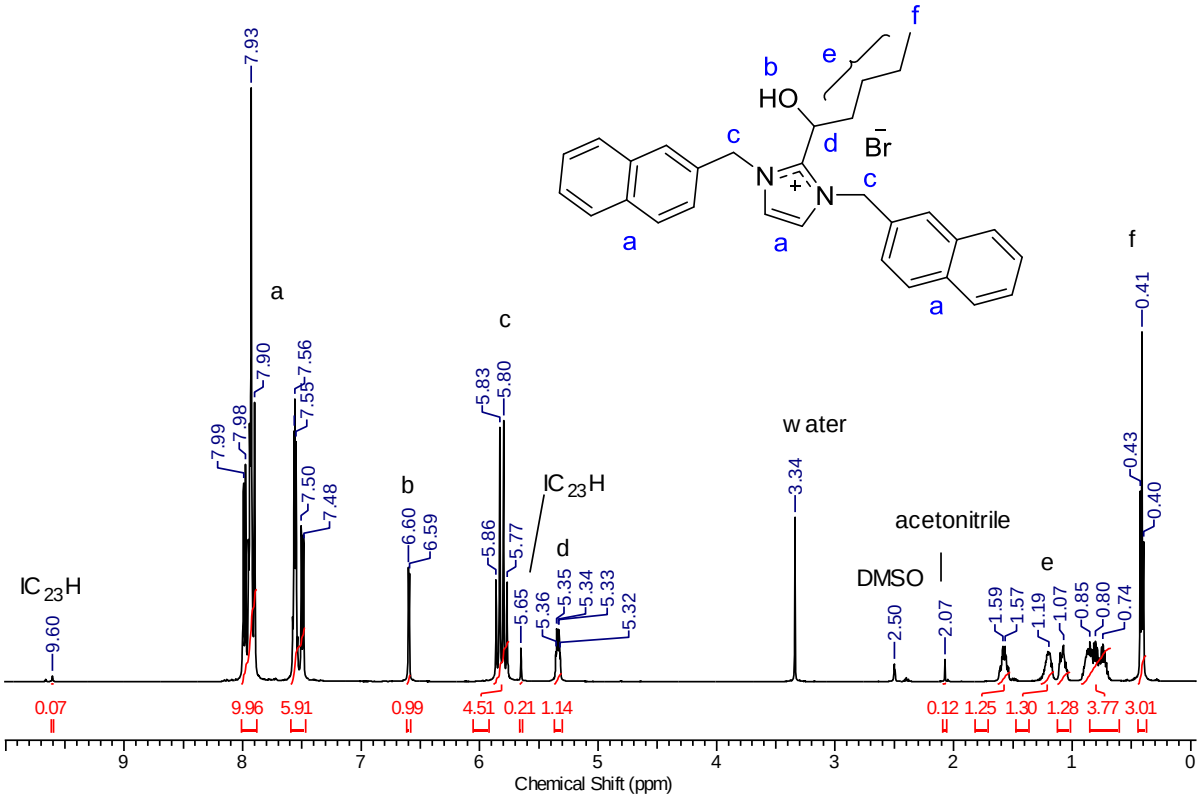


Fig. 7: ^{1}H NMR (500 MHz, DMSO-δ_6) for compound **3**.

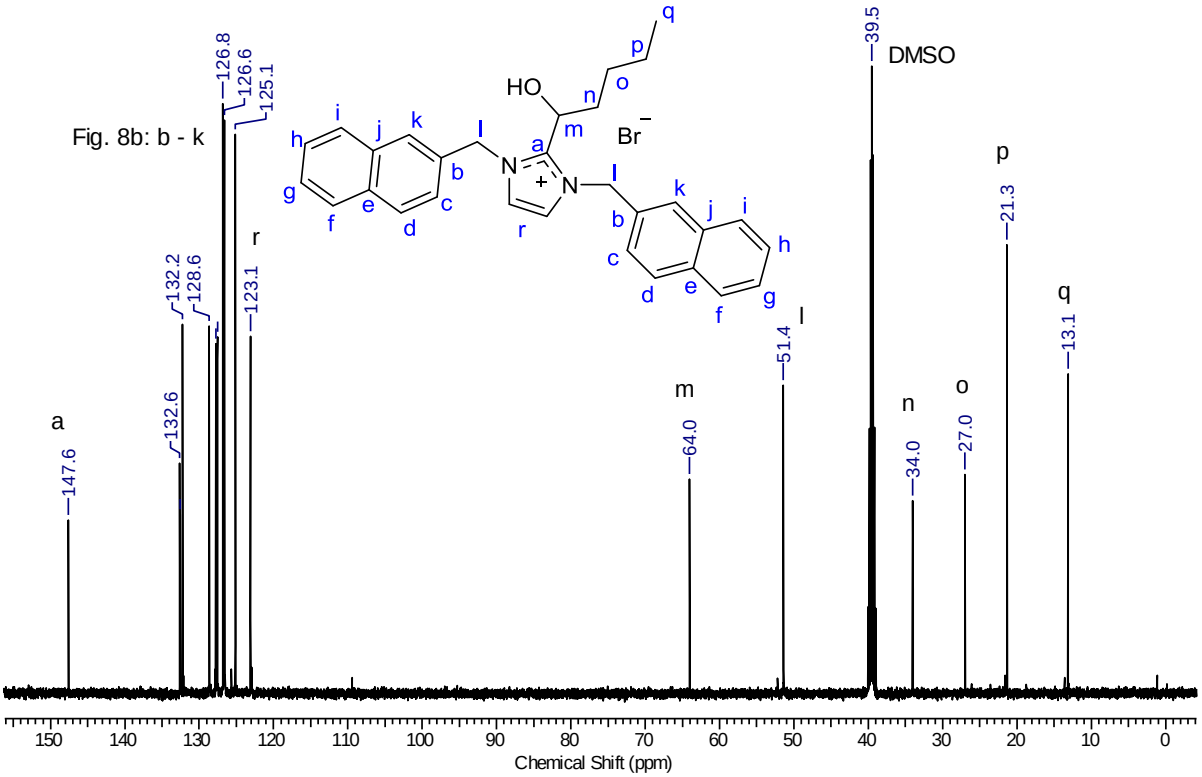


Fig. 8: ^{13}C{^{1}H} NMR (125 MHz, DMSO-δ_6) for compound **3**.

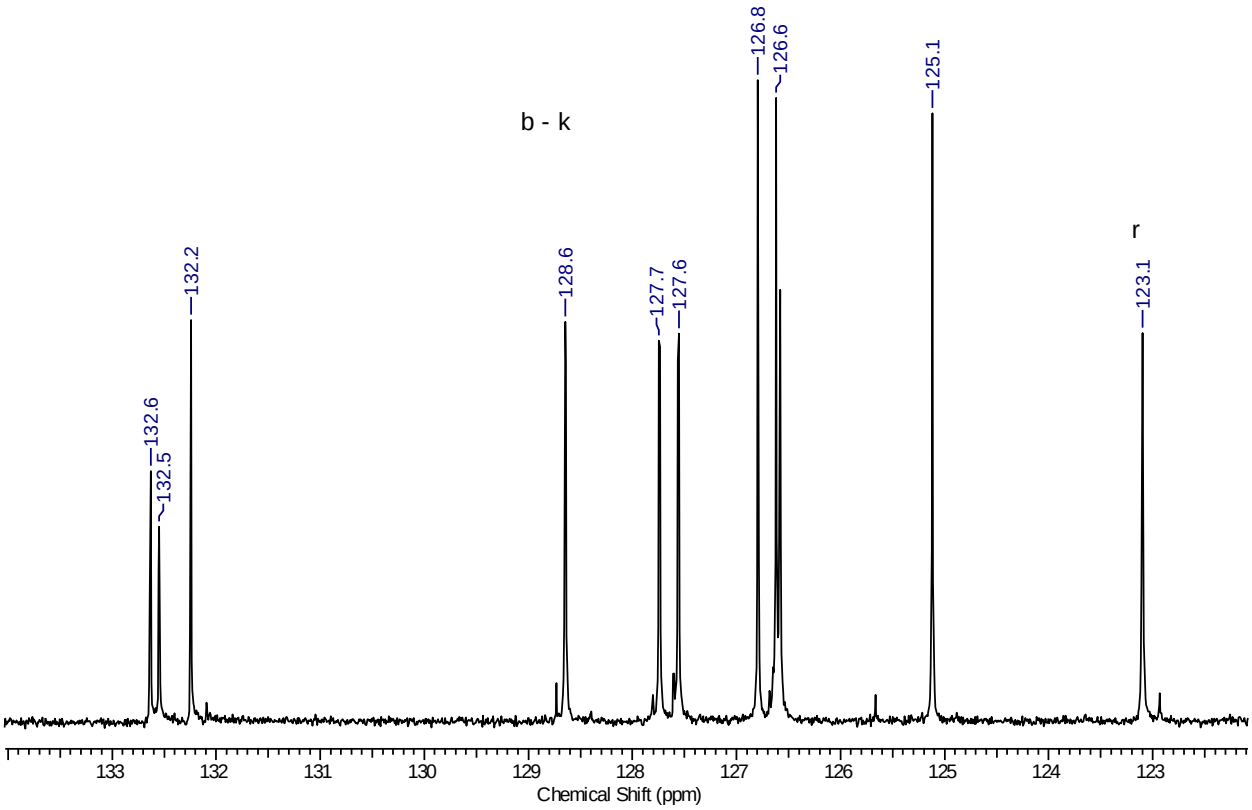


Fig. 8b: Zoomed aromatic region of $^{13}C\{^{1}H\}$ NMR (125 MHz, DMSO-δ_6) for **3** (Ref. Fig. 8).

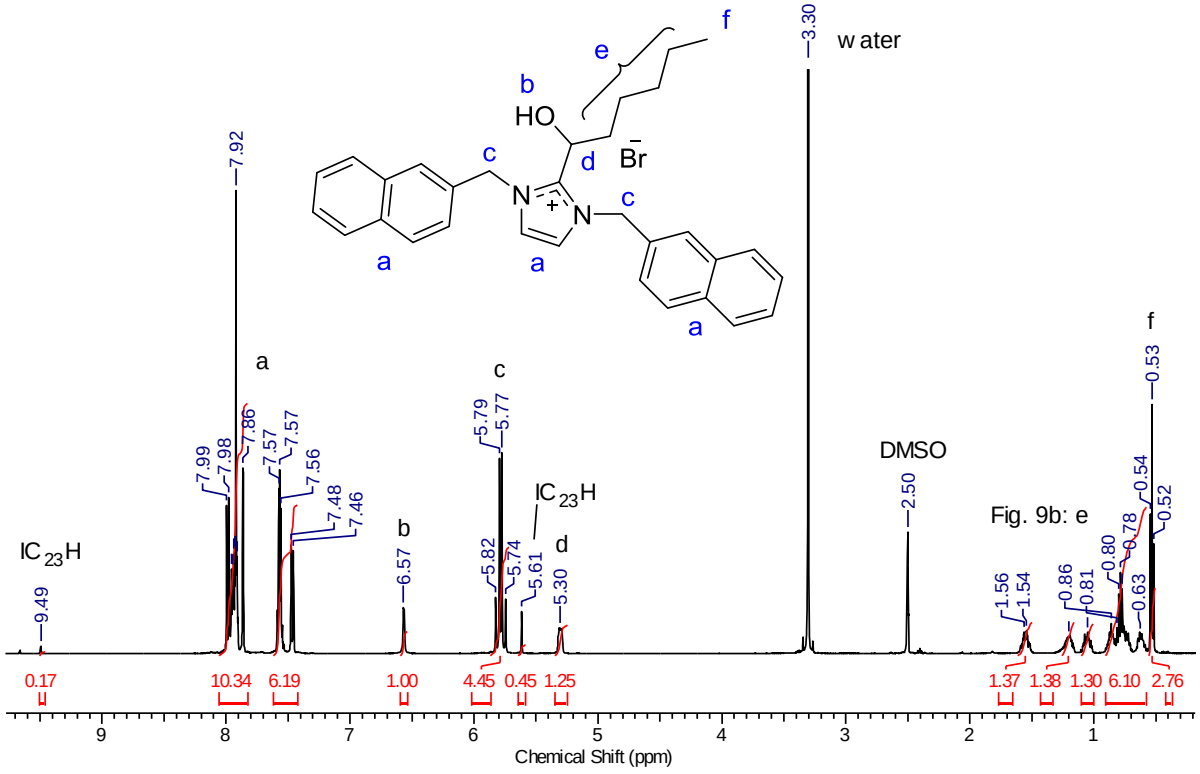


Fig. 9: ^{1}H NMR (500 MHz, DMSO-δ_6) for compound **5**.

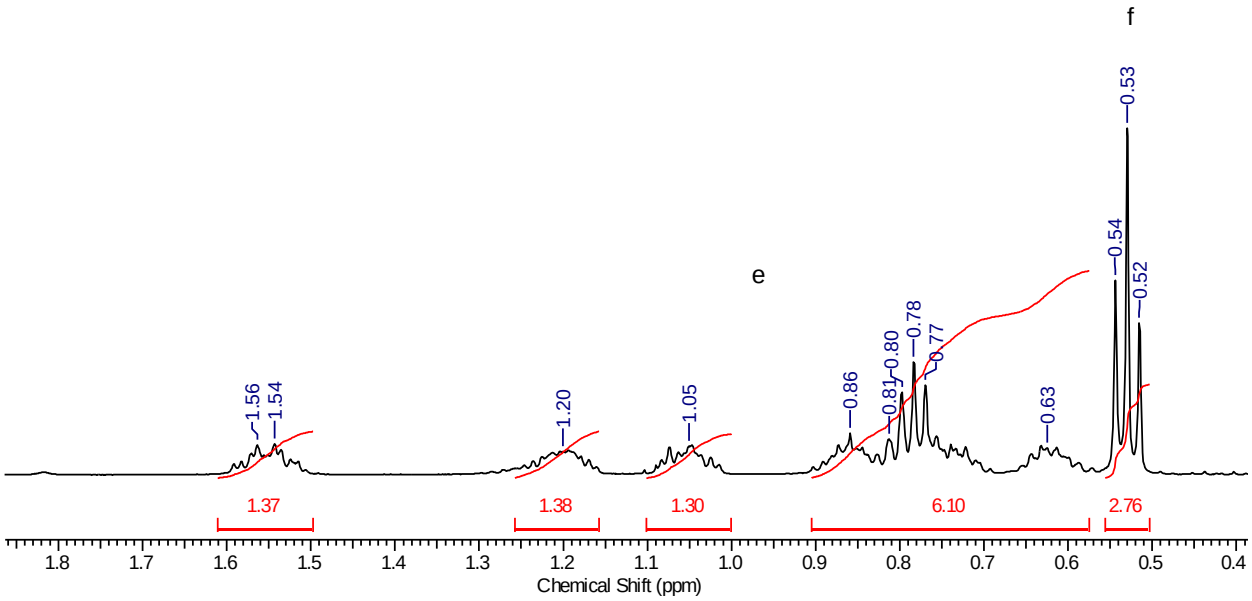


Fig. 9b: Zoomed alkyl region of ^{1}H NMR (500 MHz, DMSO-δ_6) for **5** (Ref. Fig. 9).

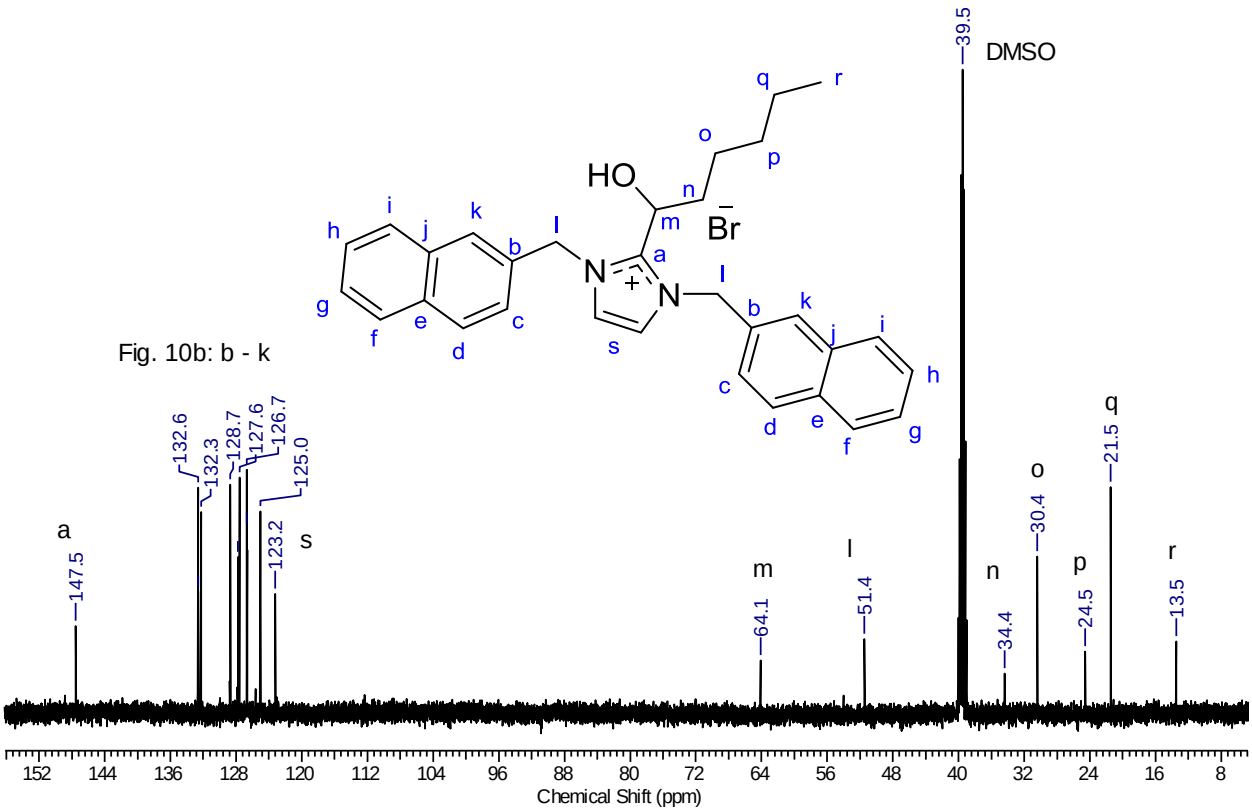


Fig. 10: ^{13}C{^{1}H} NMR (125 MHz, DMSO-δ_6) for compound **5**.

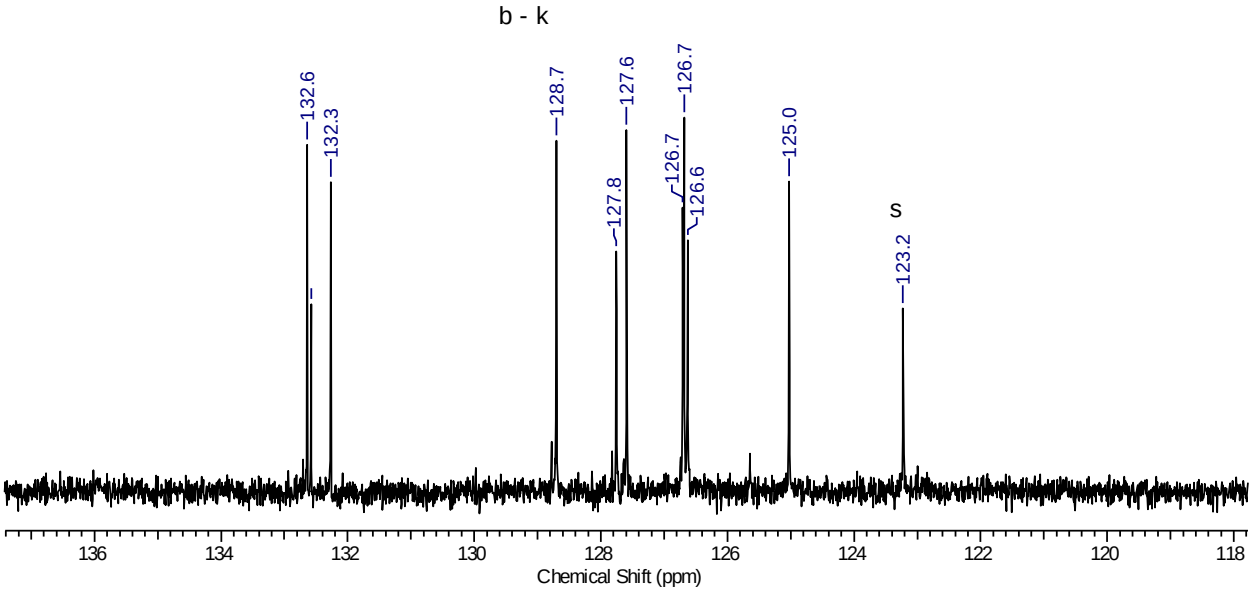


Fig. 10b: Zoomed aromatic region of $^{13}C\{^{1}H\}$ NMR (125 MHz, DMSO-δ_6) for **5** (Ref. Fig. 10).

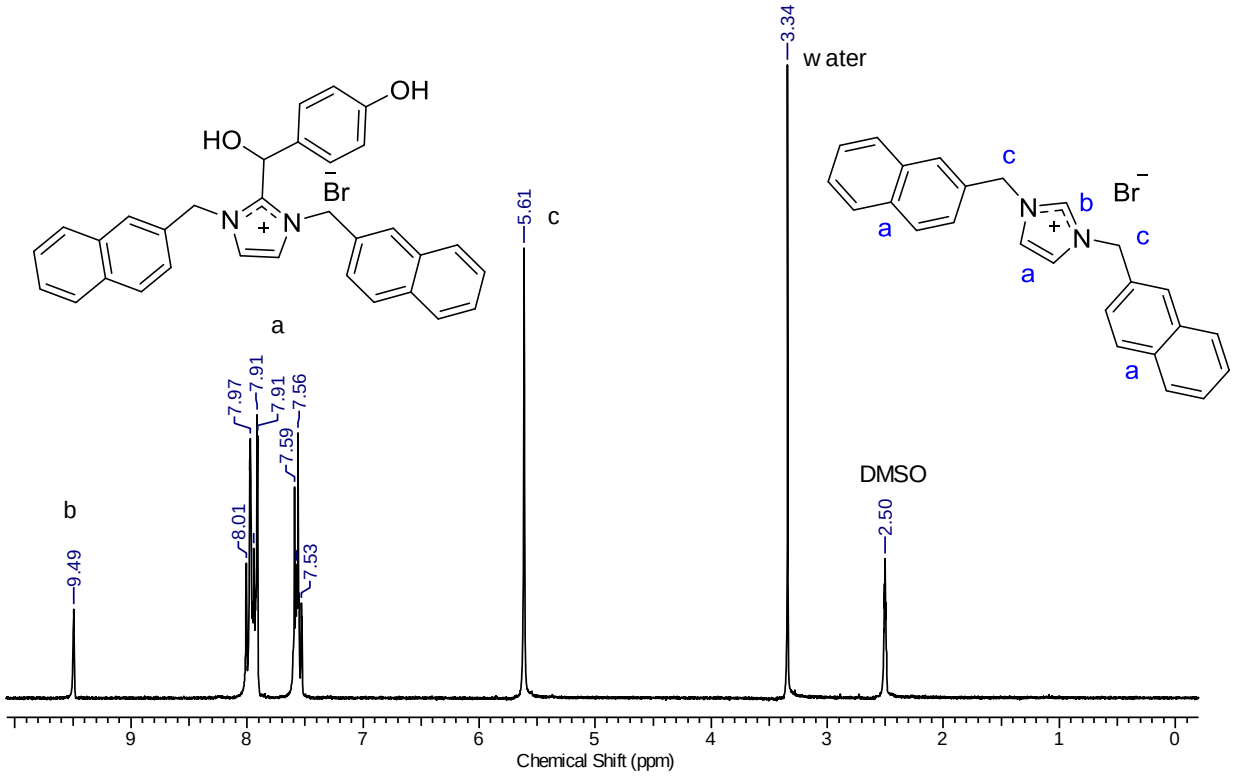


Fig. 11: ^{1}H NMR (300 MHz, DMSO-δ_6) for the synthesis of **4**. Resonance suggests only **1** present.

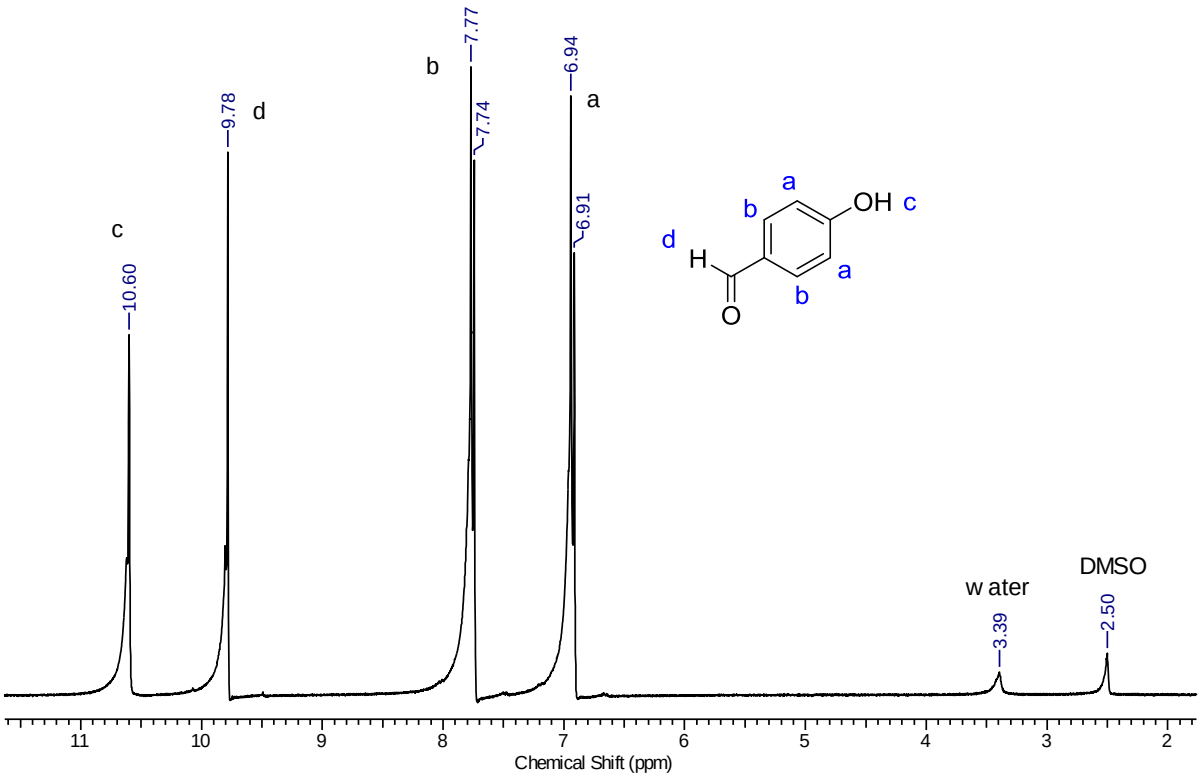


Fig. 12: ^{1}H NMR (300 MHz, DMSO-δ_6) for 4-hydroxybenzaldehyde in the synthesis of **4**.

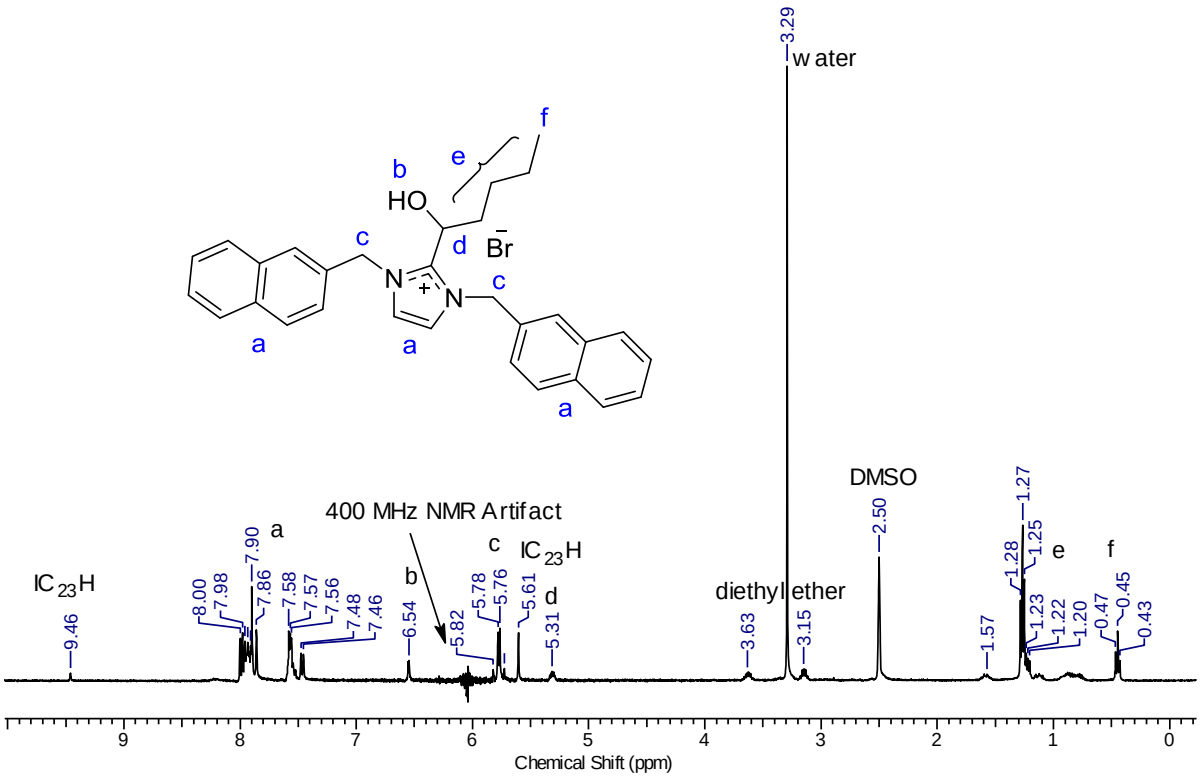


Fig. 13: ^{1}H NMR (400 MHz, DMSO-δ_6) for **3** after initial precipitation of product.

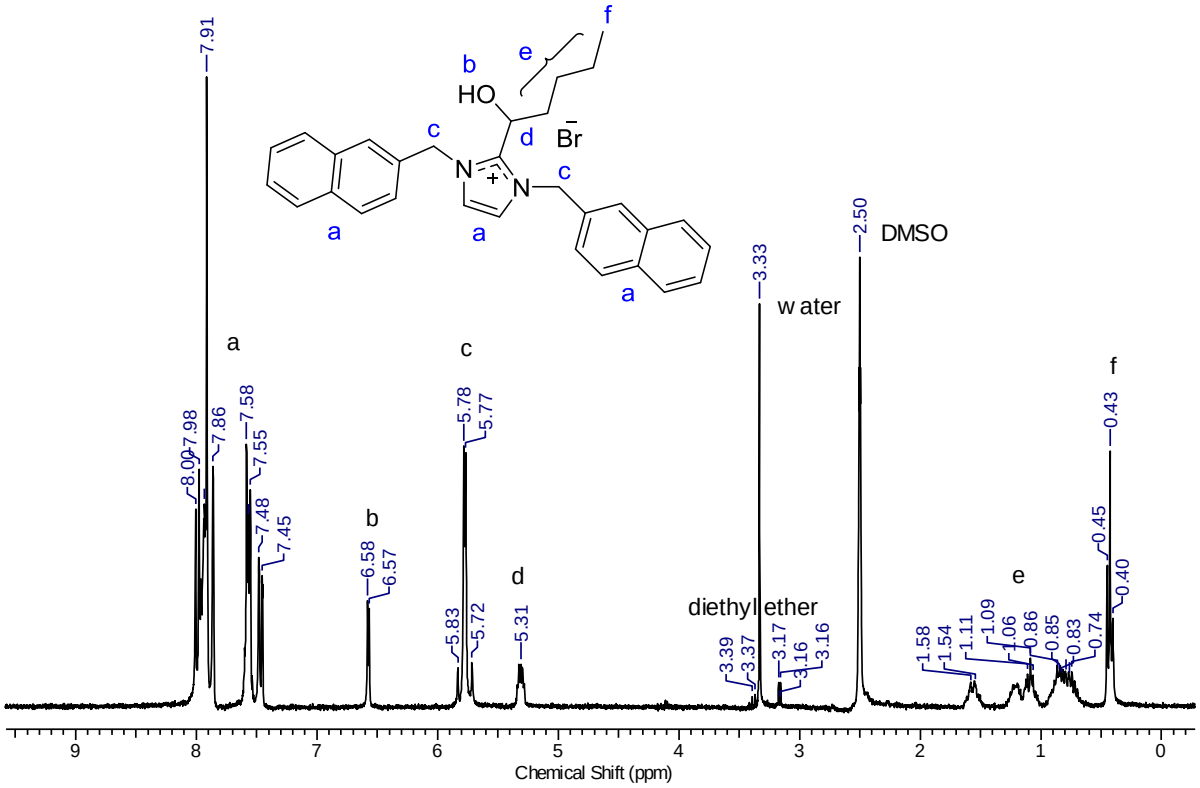


Fig. 14: ^{1}H NMR (300 MHz, DMSO-δ_6) for **3** after column chromatography and vacuum.

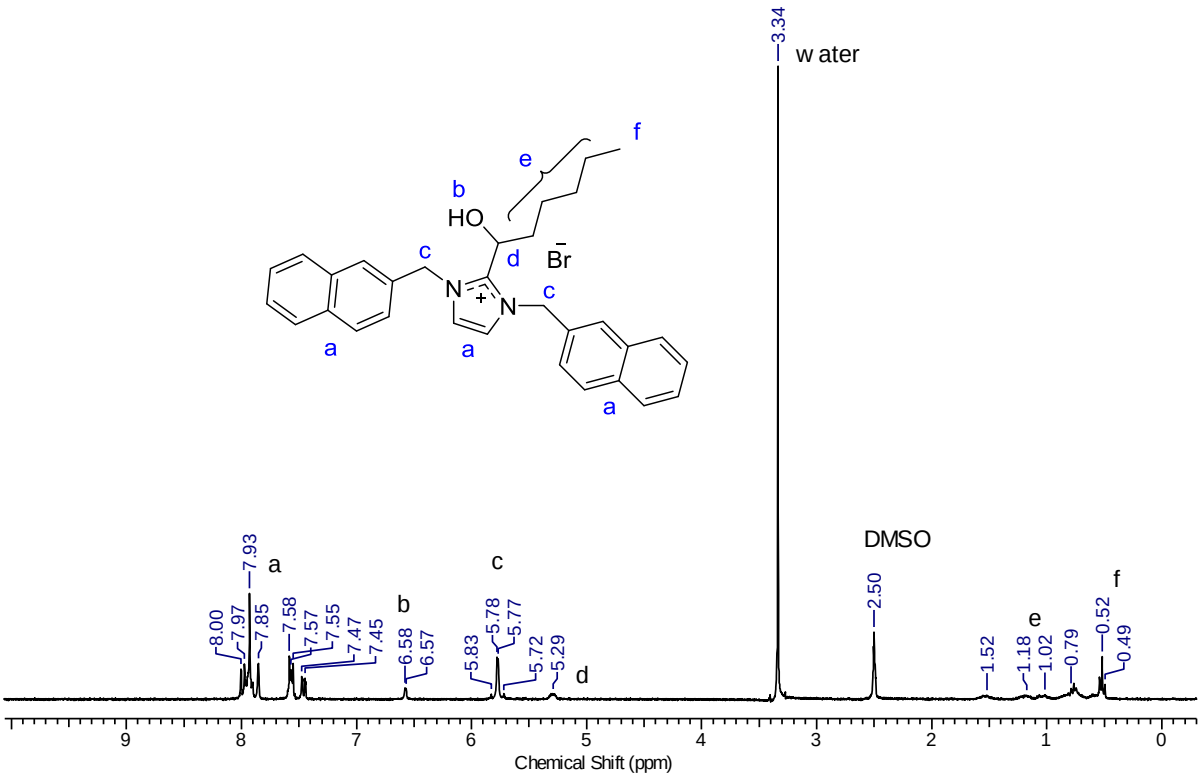


Fig. 15: ^{1}H NMR (300 MHz, DMSO-δ_6) for **5** after column chromatography and vacuum to remove residual diethyl ether.

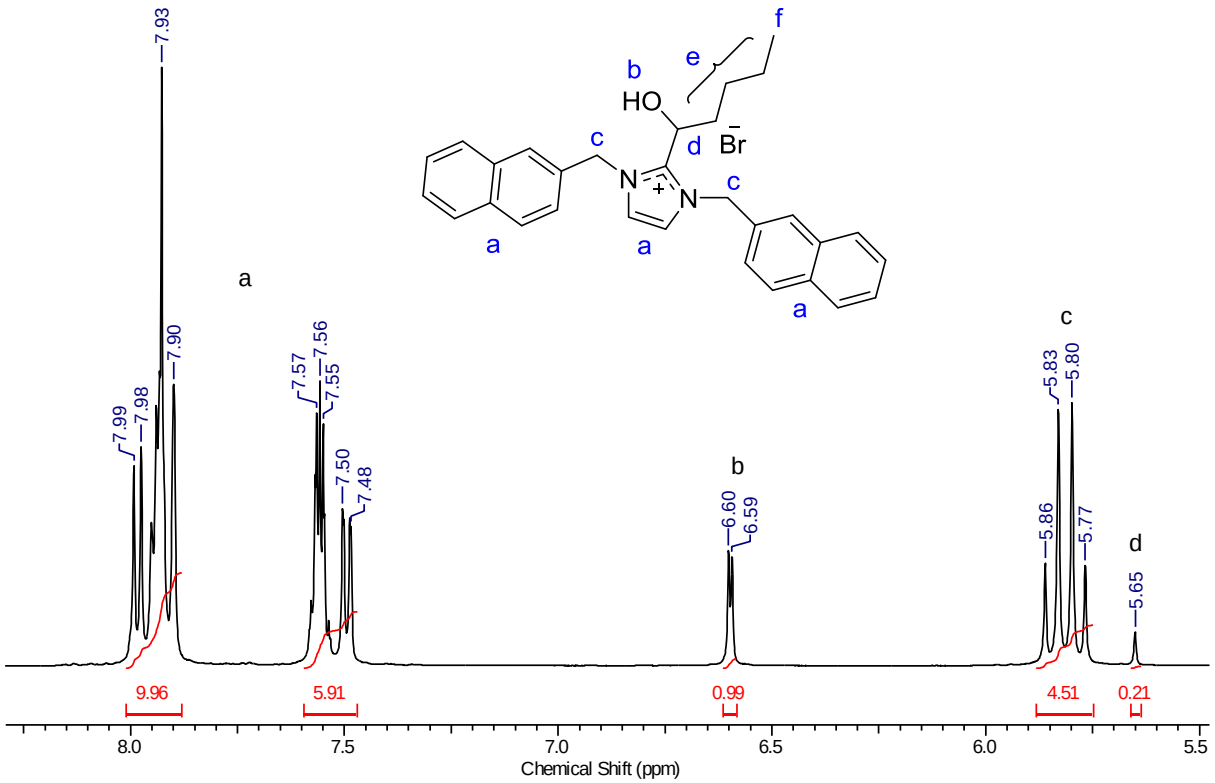


Fig. 16: ^{1}H NMR (500 MHz, DMSO-δ_6) for downfield region of compound **3** (Ref. Fig. 7).

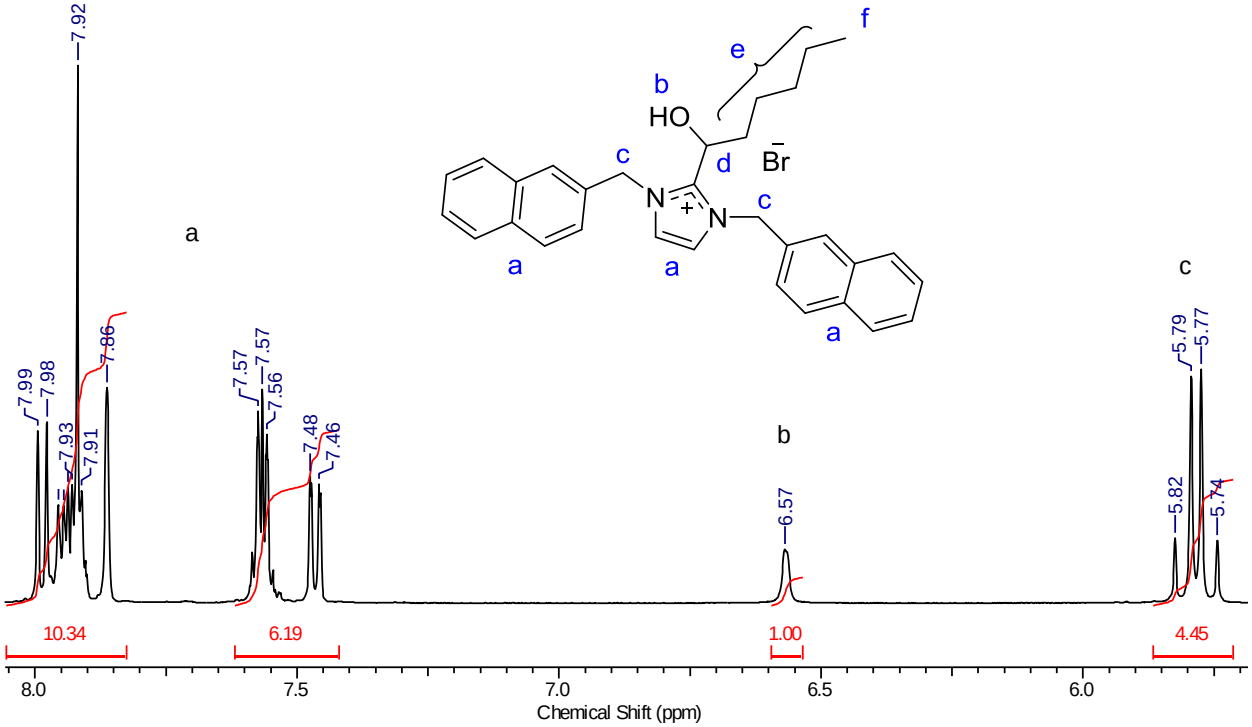


Fig. 17: ^{1}H NMR (500 MHz, DMSO-δ_6) for downfield region of compound **5** (Ref. Fig. 9).

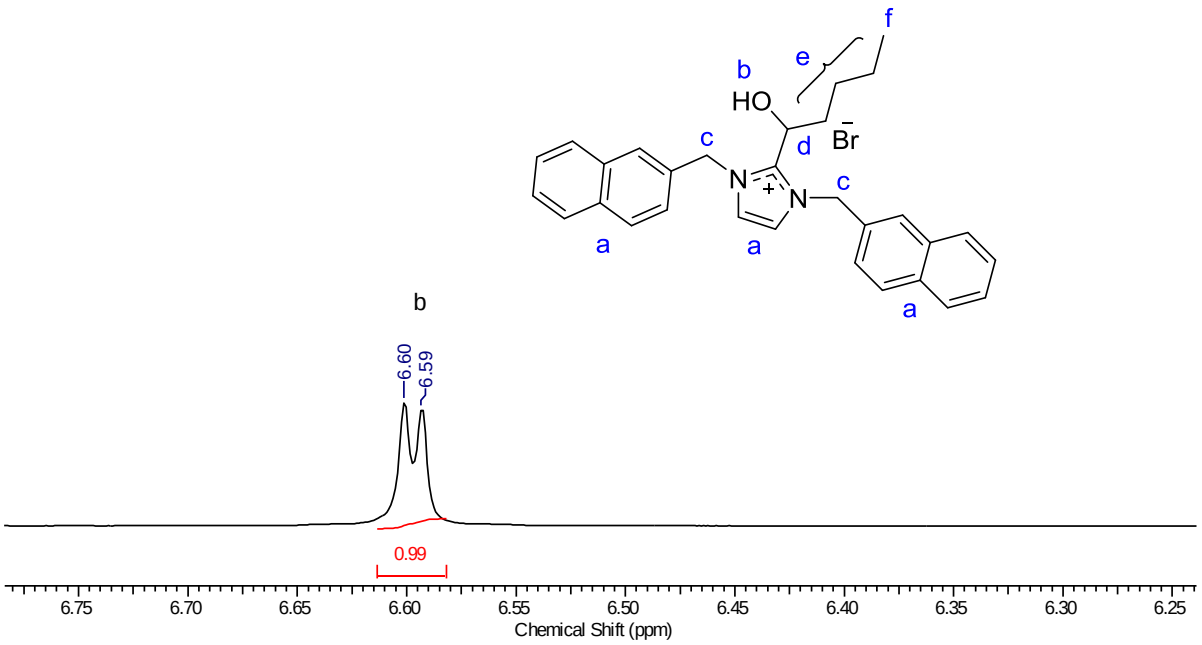
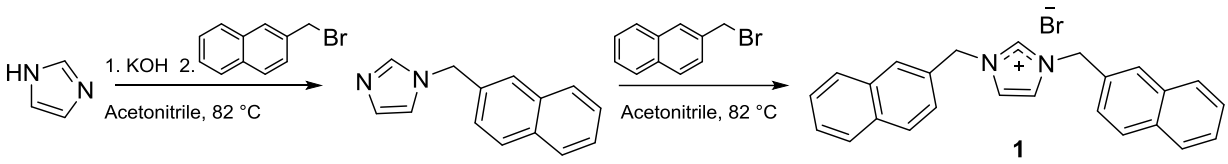
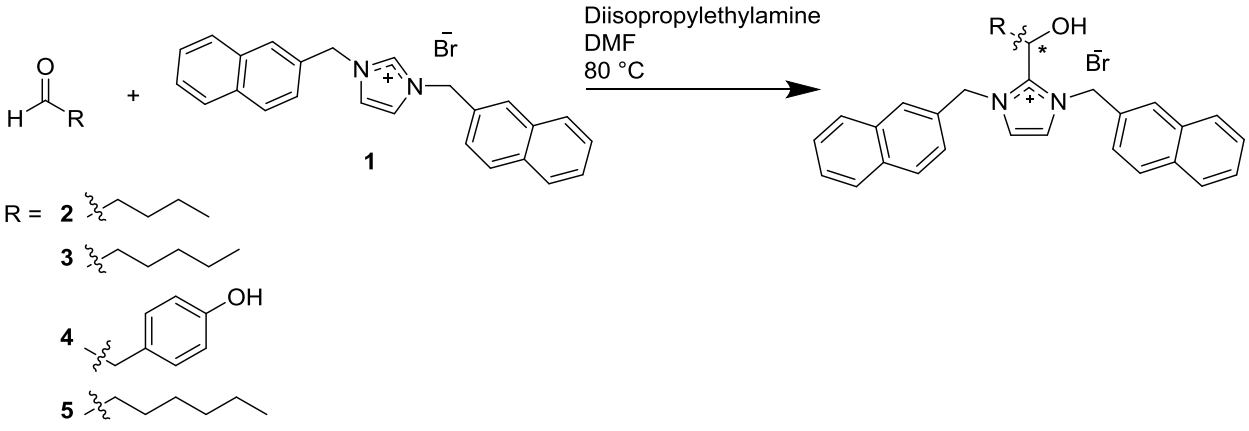


Fig. 18: Zoomed region of ^{1}H NMR (500 MHz, DMSO-δ_6) for **3** (Ref. Fig. 7).



Scheme 1: Synthesis of compound **1**; 1,3-bis(naphthalen-2-ylmethyl)-imidazolium bromide (IC23H).



Scheme 2: Synthesis and general structures of compounds **2-5** showing chirality (*).

Anti-Tumor Activity: An MTT assay was used to determine the in vitro activity of **3** against NSCLC lines NCI-A549, NCI-H460, HCC827, and NCI-H1975. For each cell line, **3** and cisplatin were dissolved in 1 % DMSO/H_2O solutions and diluted to six replicates of 1, 4, 16, and 32 μM. A plot of cell growth (in percent) against concentration was generated for each cell line (Charts 1-4). The IC_{50} value was established to standardize comparisons (Table 1) between each compound. For a more thorough comparison, the results from a similarly conducted MTT assay of **1** obtained in the literature were included in Table 1.[1] Lower IC_{50} values were considered optimal as they indicated greater activity at lower concentrations, therefore increasing effectiveness. Cisplatin had a lower IC_{50} value than **1** and **3** for each cell line except NCI-H1975, and compound **3** demonstrated greater activity against HCC827 and NCI-H1975 than **1**. The overall results suggested compounds **1**, **3**, and cisplatin have similar anti-tumor activity against select NSCLC cell lines. This evidence may allow for the future synthesis of novel imidazolium salts with functional groups at the C^2 position that improve other physical properties, as the secondary alcohol substituent did not substantially affect activity compared to **1**.

IC_{50} (μM)	NCI-A549	NCI-H460	HCC827	NCI-H1975
Compound **3**	9	7	5	3
Cisplatin	5	3	3	8
Compound **1**	n/a	4	9	6

Table 1: Concentration of compound **3**, **1**, and cisplatin at 50 % cell growth for select NSCLC cell lines. Values for **1** were obtained from the literature and were not tested in this experiment.[1]

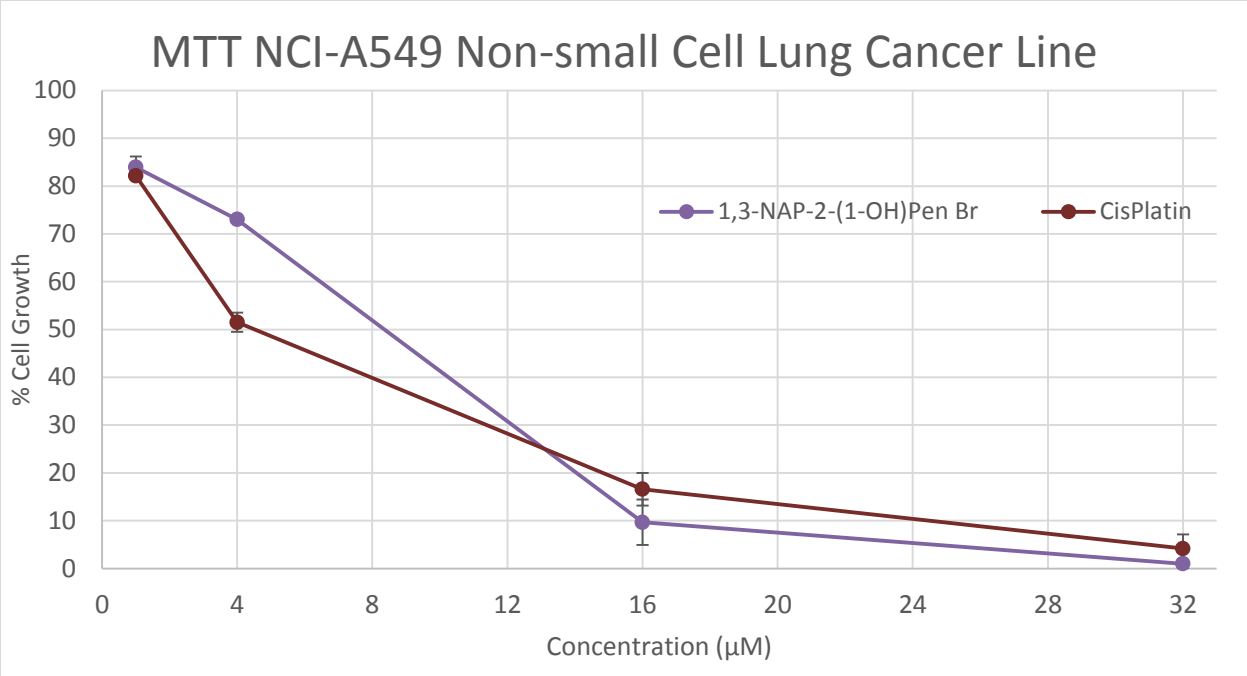


Chart 1: MTT assay results for compound **3** and cisplatin against NSCLC cell line NCI-A549 in-vitro. This chart was modified from MTT data obtained by Michael DeBord.

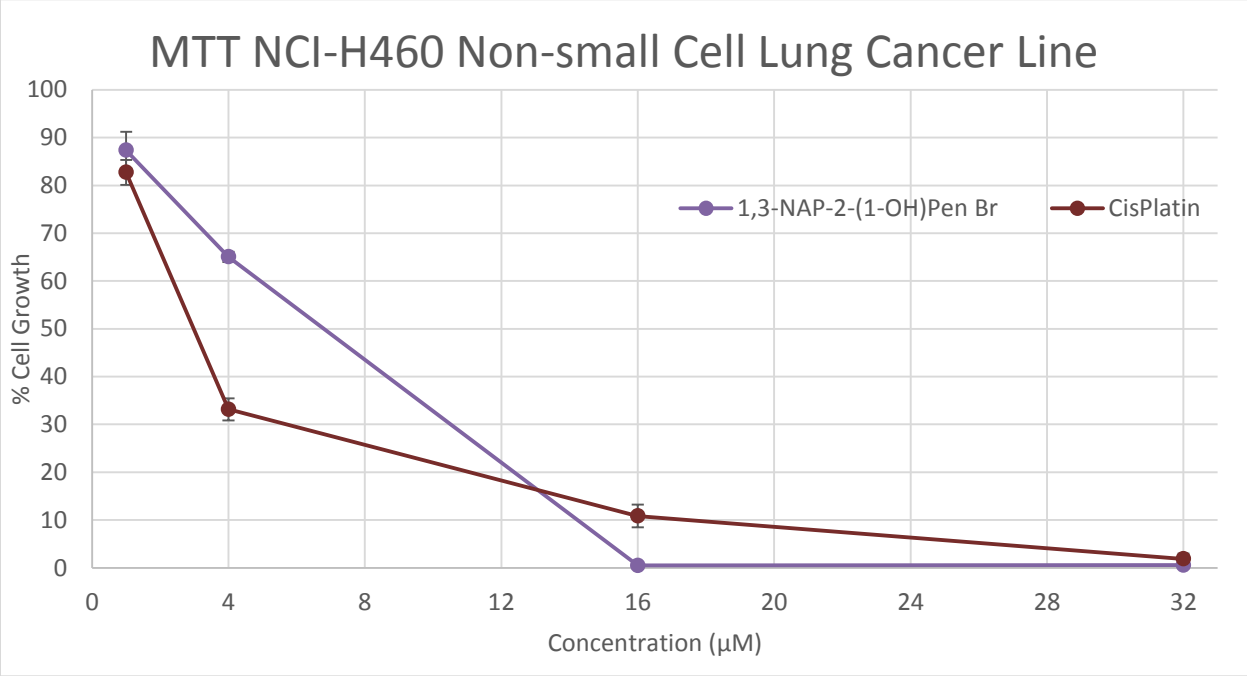


Chart 2: MTT assay results for compound **3** and cisplatin against NSCLC cell line NCI-H460 in-vitro. This chart was modified from MTT data obtained by Michael DeBord.

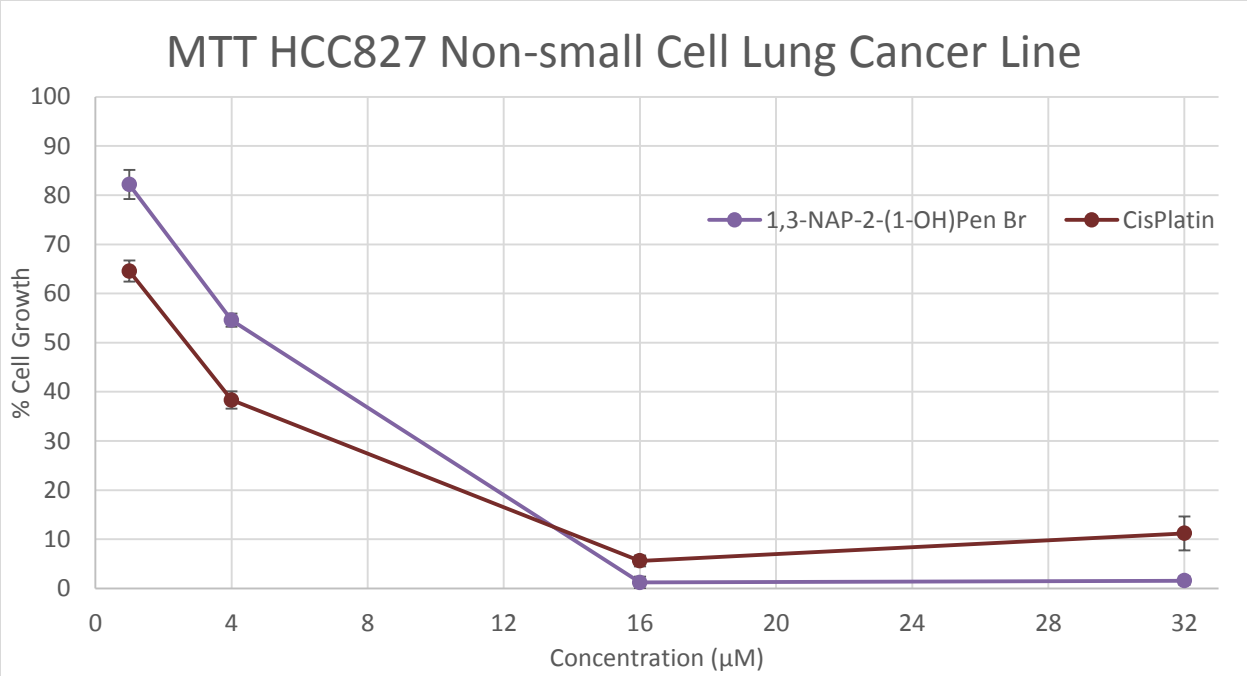


Chart 3: MTT assay results for compound **3** and cisplatin against NSCLC cell line HCC827 in-vitro. This chart was modified from MTT data obtained by Michael DeBord.

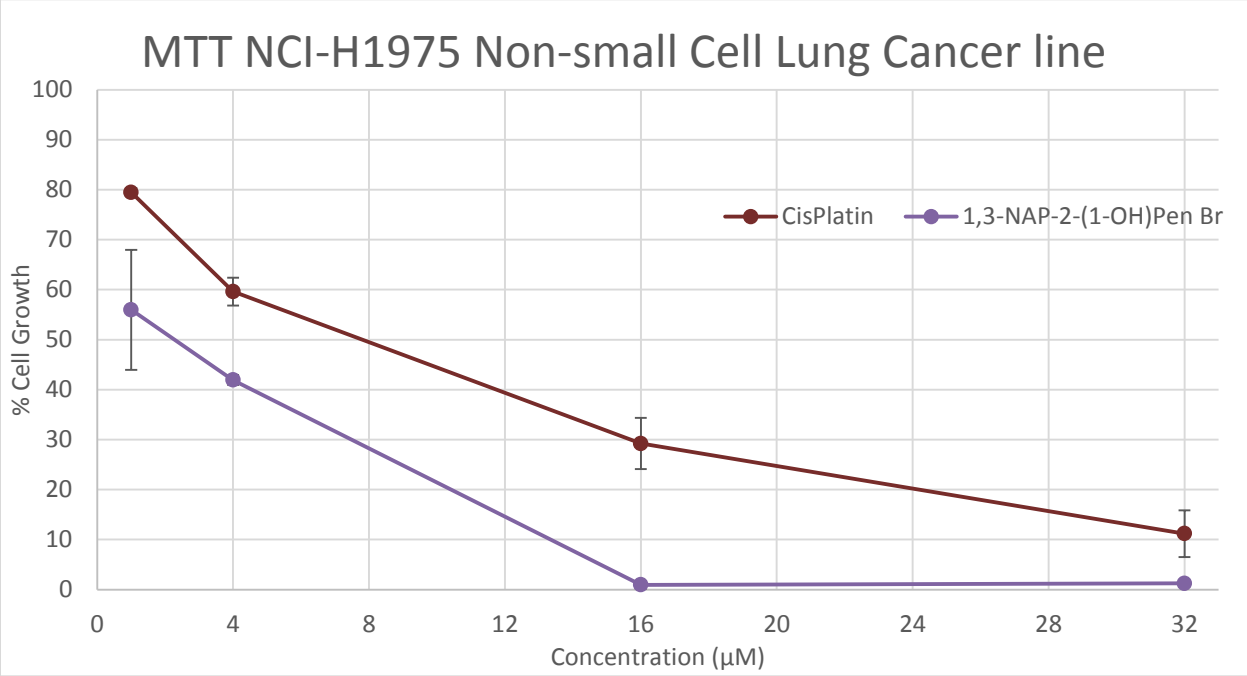


Chart 4: MTT assay results for compound **3** and cisplatin against NSCLC cell line NCI-H1975 in-vitro. This chart was modified from MTT data obtained by Michael DeBord.

Conclusion

Several novel imidazolium salts were synthesized for the study of in vitro activity against NSCLC lines NCI-A549, NCI-H460, HCC827, and NCI-H1975 compared to cisplatin. Various aldehydes were combined with **1** to synthesize imidazolium salts with secondary alcohol groups at the C^2 position. Compounds **3** and **5** were successfully synthesized and characterized in yields of 18 % and 44 %, respectively. Compounds **2** and **5** showed no evidence of synthesis as the aldehyde starting materials were oxidized or did not react. Further work should be done to purify compounds **3** and **5** due to the presence of residual solvent and compound **1** evident in the ^1H NMR spectra. An MTT assay was used to test the anti-tumor activity of **3** and cisplatin for each cell line in concentrations of 1, 4, 16, and 32 μM giving comparable IC_{50} values (Table 1). The IC_{50} values for **1** were obtained from the literature and showed similar results. Compound **3** has the lowest IC_{50} value against the H1975 cell line compared to either **1** or cisplatin. Although cisplatin had a lower IC_{50} value for the other cell lines, the similarity in results suggests that **3** may be effective in treating non-small cell lung cancer, with the addition of a secondary alcohol group not significantly affecting the activity compared to **1**. If compounds **3** and **5** can be completely purified and **2** synthesized, insight into the effects of increasing carbon chain length may be possible with an additional MTT assay. X-ray crystallography of **3** and **5** gave poor results which were not included. Further purification of these compounds may lead to more accurate anti-tumor activity data and future characterization by x-ray crystallography if optimum crystal growth conditions are attained. These results are important because they suggest that C^2 substituents on **1** do not significantly change efficacy against NSCLC. With this in mind, continuing to increase the library of novel compounds may be helpful in understanding the

possible role of imidazolium salts as chemotherapeutic agents. Different C^2 functional groups can be selected to change other physical properties that may be clinically relevant.

References

1. Wright, B.; Deblock, M.; Wagers, P.; Duah, E.; Robishaw, N.; Shelton, K.; Southerland, M.; DeBord, M.; Kersten, K.; McDonald, L.; Stiel, J.; Panzner, M.; Tessier, C.; Paruchuri, S.; Youngs, W. Anti-tumor activity of lipophilic imidazolium salts on select NSCLC cell lines. *Medicinal Chemistry Research* **2015**, 1-24.

2. American Cancer Society *Cancer Facts and Figures 2015;* American Cancer Society: Atlanta, **2015**.

3. Lovejoy, K. S.; Lippard, S. J. Non-Traditional Platinum Compounds for Improved Accumulation, Oral Bioavailability, and Tumor Targeting. *Dalton transactions (Cambridge, England : 2003)* **2009**, 10651-10659.

4. Johnstone, T. C.; Alexander, S. M.; Lin, W.; Lippard, S. J. Effects of Monofunctional Platinum Agents on Bacterial Growth: A Retrospective Study. *J. Am. Chem. Soc.* **2014**, *136*, 116-118.

5. Galluzzi, L.; Senovilla, L.; Vitale, I.; Michels, J.; Martins, I.; Kepp, O.; Castedo, M.; Kroemer, G. Molecular mechanisms of cisplatin resistance. *Oncogene* **2012**, *31*, 1869-1883.

6. Kotsakis, A.; Kentepozidis, N.; Emmanouilidis, C.; Polyzos, A.; Agelidou, A.; Vaslamatzis, M.; Chandrinos, V.; Agelaki, S.; Vamvakas, L.; Kalbakis, K.; Katsaounis, P.; Stoltidis, D.; Nintos, G.; Hatzidaki, D.; Vetsika, E. K.; Mavroudis, D.; Georgoulias, V. Sequential administration of vinorelbine plus cisplatin and bevacizumab followed by docetaxel plus gemcitabine and bevacizumab compared to docetaxel plus cisplatin and bevacizumab regimen as first-line therapy for advanced or metastatic non-squamous non-small cell lung cancer: A multicenter randomized phase II trial of the Hellenic Oncology Research Group (HORG). *Lung Cancer* **2015**, *88*, 57-62.

7. Riduan, S. N.; Zhang, Y. Imidazolium salts and their polymeric materials for biological applications. *Chem. Soc. Rev.* **2013**, 9055.

8. Sun, C.; Chen, W.; Li, Y.; Liu, L.; Wang, X.; Li, L.; Zhang, H.; Yang, X. Design, synthesis and antitumor activity of novel 8-substituted 2,3,5,6-tetrahydrobenzo[1,2-b:4,5-b[prime or minute]]difuran imidazolium salt derivatives. *RSC Adv.* **2014**, 16312.

9. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55-63.

10. Medvetz, D. A.; Hindi, K. M.; Panzner, M. J.; Ditto, A. J.; Yun, Y. H.; Youngs, W. J. Anticancer Activity of Ag(I) N-Heterocyclic Carbene Complexes Derived from 4,5-Dichloro-1H-Imidazole. *Metal-Based Drugs* **2008**, *2008*, 384010.

11. Agelis, G.; Resvani, A.; Koukoulitsa, C.; Tůmová, T.; Slaninová, J.; Kalavrizioti, D.; Spyridaki, K.; Afantitis, A.; Melagraki, G.; Siafaka, A.; Gkini, E.; Megariotis, G.;

Grdadolnik, S. G.; Papadopoulos, M. G.; Vlahakos, D.; Maragoudakis, M.; Liapakis, G.; Mavromoustakos, T.; Matsoukas, J. Rational design, efficient syntheses and biological evaluation of N,N′-symmetrically bis-substituted butylimidazole analogs as a new class of potent Angiotensin II receptor blockers. *Eur. J. Med. Chem.* **2013**, *62*, 352-370.

12. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. *J. Org. Chem.* **1997**, *62*, 7512-7515.