Spring 2015

Synthesis and Anti-Proliferative Activity of N,N'-bis(arylmethyl)benzimidazolium Salts

Travis M. Williams
University of Akron Main Campus, traviswilliams818@yahoo.com

Synthesis and Anti-Proliferative Activity of *N,N'*-bis(arylmethyl)benzimidazolium Salts

by Travis Williams

3150:497:001

4/22/2015

Synthesis and Anti-Proliferative Activity of *N,N'*-bis(arylmethyl)benzimidazolium Salts

Travis M. Williams

Abstract:

A series of *N,N'*-bis(arylmethyl)benzimidazolium salts with hydrophilic and lipophilic substituents have been synthesized, characterized, and tested against select non-small cell cancer cell lines. Substituent variations on the imidazole ring have shown that lipophilicity and hydrophilicity can influence the imidazolium salts' anti-proliferative activity and aqueous solubility.

Introduction

Cancer is a major disease with more than one million people being diagnosed each year in the United States alone. Pharmaceuticals presently used can be toxic to the patient and, over time, can lead to an increased tolerance level of the patient's cancer cells. Cancer is characterized by the abnormal cell growth and rapid cell division due to damaged DNA, and results in hundreds of thousands of deaths every year.[1] According to the American Cancer Society, 585,720 cancer related deaths are predicted in the United States alone during the year of 2014.[2] Presently, research efforts are being devoted to drug formulation and alternative treatments for the growing cancer epidemic. The disadvantage of current cancer medications is the association with numerous side effects. For example, cisplatin, a commonly used chemotheraputic, has been known to affect auditory and digestive functions, cause skin damage, and induce kidney failure.[3] Current research is focused on finding alternatives to cisplatin with equivalent anti-proliferative

activity and fewer side effects. This has led to the exploration of imidazolium salts. Synthetic modifications to these compounds are intended to maximize their anti-proliferative activity and their aqueous solubility, with the intention of minimizing additional health risks.[4,5,6,7] Research in the Youngs group has been concentrated on developing *N,N'*-bis(naphthylmethtyl)imidazolium salts. One *N,N'*-bis(naphthylmethyl)imidazolium salt shown to have anti-proliferative activity is **IC23** (Figure 1).[8] This salt has shown comparable activity to cisplatin.

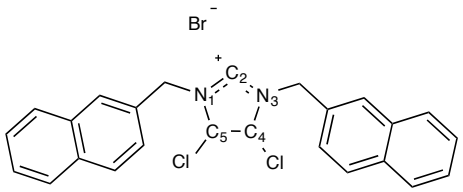


Figure 1. Structure of **IC23** (**1**).

Initial studies began with the C^4 and C^5 positions of the imidazole ring and led to the synthesis and screening of salts **2** and **3** (Figure 2).[8] Salts **1-3** showed anti-proliferative activity comparable to the control, cisplatin, and further proved that the salts' activity was not hindered by the substituent variation in the C^4 and C^5 positions. However, salt **2**, like **IC23**, was limited by its aqueous solubility, which led to the investigation of hydrophilic solubilizing groups on the benzimidazole ring.

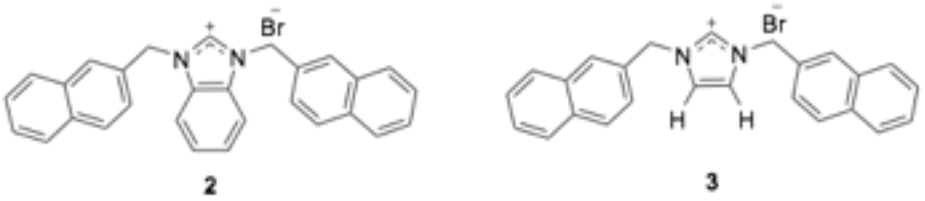


Figure 2. Structures of *N,N'*-bis(naphthylmethyl)imidazolium salt of **2** and **3**.

A series of *N,N'*-bis(naphthylmethyl)benzimidazolium salts with solubilizing groups substituted on the benzimidazole ring have been synthesized and screened. Recently, focus has been concentrated on changing the functionality at the C^2 position of the benzimidazole ring to monitor the substituent's effect on the anti-proliferative activity. Functional groups previously explored at the C^2 position included acids, esters, and alkyl groups. Two additional functionalities of interest are alcohol and ether linkages at the C^2 position. Alcohol and ether linkages warranted investigation because, theoretically, the oxygen atoms can offer sites of hydrogen bonding with water molecules, increasing its aqueous solubility.

The alcohol functional groups provide hydrophilicity through hydrogen bonding. This additional hydrophilicity can help to increase the aqueous solubility of the benzimidazole. However, previously synthesized compounds have shown that substituting a short chained alcohol group directly onto the imidazole ring can adversely affect the anti-proliferative activity. The anti-proliferative activity of these salts, a series of *N,N'*-bis(arylmethyl)benzimidazolium salts with an extended alcohol functionality at the C^2 position, were tested against a panel of non-small cell lung cancers (NSCLCs) and reported. The propanol substituent is useful for determining if increasing the distance of the alcohol group from the benzimidazole ring would

allow these benzimidazolium salts to retain the anti-proliferative activity and provide additional the aqueous solubility (Figure 3).

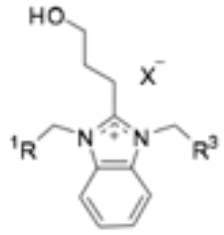


Figure 3. Structures of 2-(3-hydroxypropyl)-*N,N'*-bis(arylmethyl)benzimidazolium salts.

Variation of the substituents at the N^1 and N^3 positions of the 2-(3-hydroxypropyl) series included quinolinylmethyl and (6-methoxynaphthyl)methyl substituents, along with the naphthylmethyl explored in this paper. The modification of the naphthalene portion with heteroatoms and additional substitution was theorized to aid in providing additional water solubility for this novel series of benzimidazolium salts.

In addition to the alcohol moiety, the water solubility of *N,N'*-bis(arylmethyl)benzimidazolium salts can also be modified by incorporating other solubilizing groups onto the benzimidazole, such as a polymeric ethylene glycol (PEG) chain. These PEG groups have previously been explored by researchers to increase the hydrophilic properties of molecules and to increase the overall solubility of molecules. These PEG chains were used in shells for encapsulating metal ions and as a potential delivery system for drugs.[9,10] In an attempt to increase the aqueous solubility and maintain anti-proliferative activity of these benzimidazolium salts, two *N,N'*-bis(arylmethyl)benzimidazolium salts with a triethylene glycol

(TEG) group, a derivative of the PEG family, at the C^2 position were synthesized, characterized, and tested against a panel of NSCLC cell lines (Figure 4). These two salts with an extended ether functionality at the C^2 position were theorized to have increased hydrophilic properties and solubility of benzimidazolium salts through neighboring hydrogen bonding interactions of the solvent. Variation of the substituents at the N^1 and N^3 positions included a quinolinylmethyl substituent, along with the naphthylmethyl explored in this paper. The comparison of bis(quinolinylmethyl) versus bis(naphthylmethyl) salts also provided insight for a novel system of benzimidazolium salts.

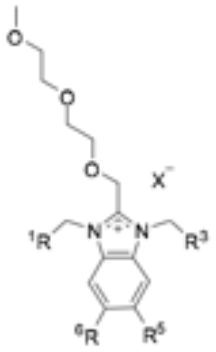
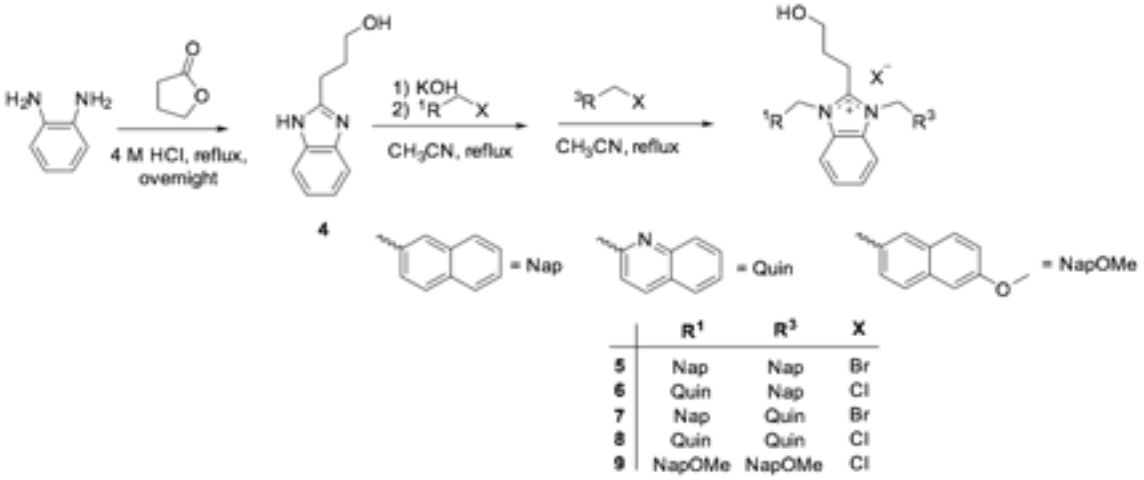


Figure 4. Structures of 2-((2-(2-methoxyethoxy)ethoxy)methyl)-*N,N'*-bis-(arylmethyl)benzimidazolium salts.

Results and Discussion

The 2-(3-hydroxypropyl)-benzimidazole (**4**) and the 2-(3-hydroxypropyl)-*N,N'*-bis(arylmethyl)-benzimidazolium salts (**5-9**) were synthesized according to the reaction in **Scheme 1**. Compound **4** was synthesized by a literature reaction[11] and the addition of the substituents to N^1 and N^3 atoms followed procedures similar to those previously used by the

group on other imidazole based compounds.[8] Compound **4** was stirred with 1.1 equivalent of potassium hydroxide and one equivalent of the appropriate alkyl bromide or chloride in refluxing acetonitrile overnight to generate the mono-substituted intermediate, which was later stirred with a second equivalent of the appropriate alkyl halide to yield the corresponding salts **5-9**.[8]



	R^1	R^3	X
5	Nap	Nap	Br
6	Quin	Nap	Cl
7	Nap	Quin	Br
8	Quin	Quin	Cl
9	NapOMe	NapOMe	Cl

Scheme 1. Synthesis of 2-(3-hydroxypropyl)-*N,N'*-bis(arylmethyl)benzimidazolium salts, **5-9**.

Three symmetric compounds, **5**, **8**, and **9**, featuring the propanol group at the C^2 position were synthesized, each with different substituents at the N^1 and N^3 positions (Figures 5 and Figure 8). The bis(naphthylmethyl) salt **5** was synthesized and tested in order to draw a comparison to **IC23**, due to the previous salt's high anti-proliferative activity. The quinolinylmethyl and (6-methoxynaphthyl)methyl substituents of **8** and **9**, respectively, provided structural similarity to **5** and with the addition of heteroatom – nitrogen and oxygen – potentially provided sites for more hydrogen bonding interactions. Additionally, two asymmetric compounds **6** and **7** were synthesized using both the naphthylmethyl and quinolinylmethyl substituents,

featuring two variations in the accompanying anion (Figures 6 and Figure 7). The asymmetric nature of the compounds were used to test what effect two differing substituent groups at the N^1 and N^3 positions had on the anti-proliferative activity and aqueous solubility of the salts. The variation in the anion of **6** and **7** was due to the different alkylating agent included at the second step when synthesizing the salts.

Compounds **5-9** were analyzed via proton NMR spectroscopy. The compounds all had resonance indicative of the benzimidazole ring and the aromatic substituents of the nitrogen atoms at 8.50, 8.04-7.87, 7.79, 7.77-7.67, 7.63-7.55, 7.48, the resonance of the hydrogen of the alcohol group at 6.28, the resonance of the methylene groups off of the two substituents at 6.15, and the resonances of the hydrogen atoms bonded to the carbon atoms of the propyl chain at 4.85, 3.53-3.47, and 1.86.

Crystals of the salts, **5-8**, suitable for single X-ray diffraction were obtained by slow evaporation. Data collected through X-ray crystallography helped determine the structures for **5-8**. The data compiled for compound **5**, which was crystallized from methanol, showed that the naphthylmethyl substituents appeared to repel each other and the alcohol group (Figure 5). The structure of compound **8**, crystallized from water and acetone, featured the two quinolinylmethyl substituents and appeared to adopt a different orientation (Figure 8). The substituents are directed straight forward off the benzimidazole ring and the alcohol functionality stretches up from the benzimidazole ring, appearing to form a 90 degree angle with the quinoline groups. This may be due to the salt maximizing hydrogen bonding interactions with surrounding water molecules by stretching the heteroatoms, the nitrogen atoms and the oxygen, away from each other.

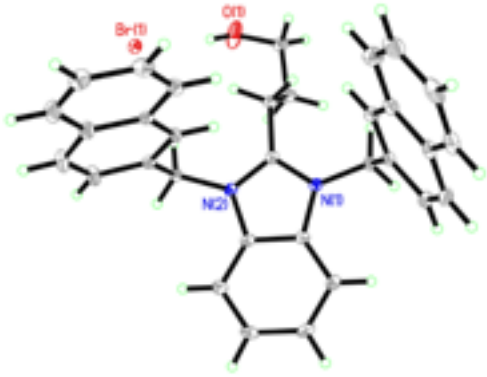


Figure 5. Thermal ellipsoid plot of **5** with thermal ellipsoid plots at 50% probability level.

The two asymmetric 2-(3-hydroxypropyl)-*N,N'*-bis(arylmethyl) benzimidazolium salts, compounds **6** and **7**, showed similar results in their X-ray crystallography data. Compound **6** was crystallized in a combination of water, ethyl acetate, and acetone. Compound **7** was crystallized in a combination of water, methanol, and acetone. Both **6** and **7** had the alcohol orient toward the quinoline-portion of the substituent (Figure 6 and Figure 7). This may imply hydrogen bonding interactions between the nitrogen atom and the alcohol group, which may cause the alcohol and the quinoline substituent to hydrogen bond in the same area. However, reviewing the thermal ellipsoid plots of compound **8**, it was shown that the alcohol seemed to repel the quinoline groups. The configuration of compounds **6** and **7** may be because the alcohol was interacting with the hydrogen of the nitrogen atom of the quinolinylmethyl substituent in a way it was unable to do in compound **8**. This configuration was also an excellent example of potential intramolecular hydrogen bonding, a phenomenon which is further illustrated in the 2-((2-(2-methoxyethoxy)ethoxy)methyl)-*N,N'*-bis-substituted benzimidazolium salts.

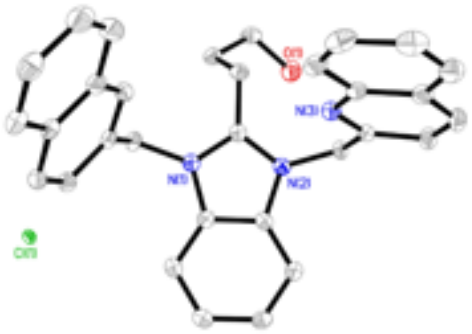


Figure 6. Thermal ellipsoid plot of **6** with thermal ellipsoid plots at 50% probability level.

Hydrogen atoms omitted for clarity.

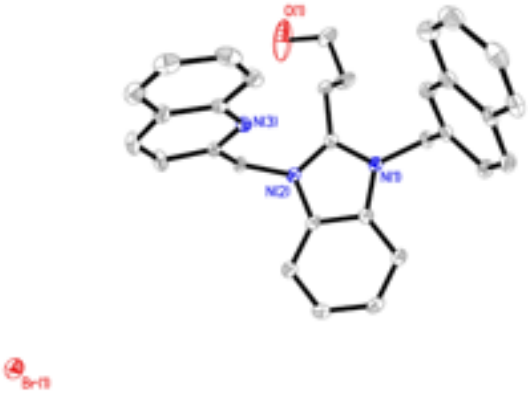


Figure 7. Thermal ellipsoid plot of **7** with thermal ellipsoid plots at 50% probability level.

Hydrogen atoms omitted for clarity.

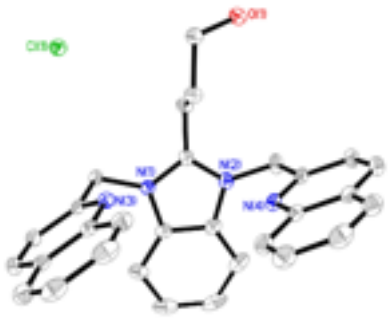
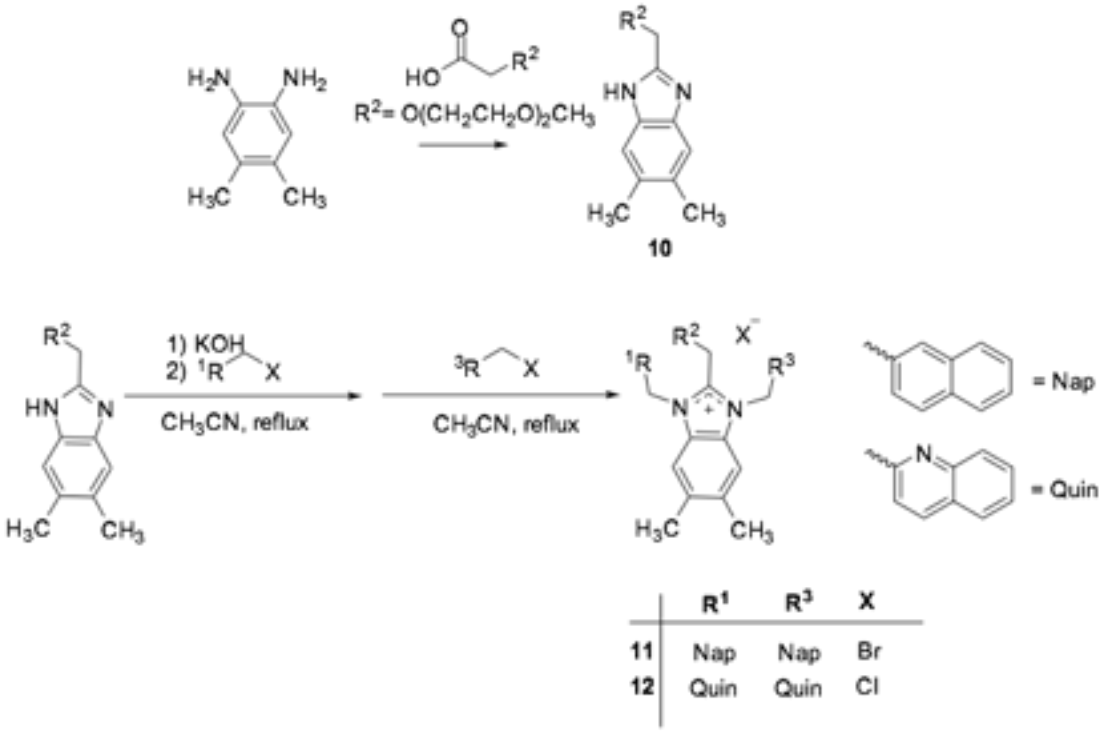


Figure 8. Thermal ellipsoid plot of **8** with thermal ellipsoid plots at 50% probability level. Hydrogen atoms omitted for clarity.

A series of 2-((2-(2-methoxyethoxy)ethoxy)methyl)-*N,N'*-bis-substituted benzimidazolium salts were synthesized using the method found in **Scheme 2**. Equivalent portions of 5,6-Dimethyl-1,2-benzenediamine and 2-(2-[2-methoxy]ethoxy)ethoxy-acetic acid were refluxed in 4M aqueous hydrochloric acid. Following the synthesis and characterization of **10**, the reaction was optimized to minimize possible contamination or side products. The two reagents, 5,6-dimethyl-1,2-benzenediamine and 2-(2-[2-methoxy]ethoxy)ethoxy-acetic acid, were heated at 140 °C in a neat reaction. Furthermore, the purity of the 2-(2-[2-methoxy]ethoxy)ethoxy-acetic acid was increased from a synthesis grade (≥ 90%) to a technical grade (≥ 95%).

The benzimidazolium salts 2-((2-(2-methoxyethoxy)ethoxy)methyl)-5,6-dimethyl-1,3-bis(naphthalen-2-ylmethyl)-benzimidazolium bromide (**11**) and 2-((2-(2-methoxyethoxy)ethoxy)methyl) -5,6-dimethyl-1,3- bis(quinolin-2-ylmethyl)-benzimidazolium chloride (**12**) were synthesized from **10**, according to an established procedure in our laboratory for similar lipophilic systems (**Scheme 2**). Compound **10** was stirred with 1.2 equivalent of potassium hydroxide and one equivalent of the appropriate alkyl bromide or chloride in refluxing

acetonitrile overnight to generate the mono-substituted intermediate, which was later stirred with a second equivalent of the appropriate alkyl halide to yield the corresponding salts **11-12**. These benzimidazolium salts featured the inclusion of methyl groups at the C^5 and C^6 positions of the benzimidazole ring that gave the molecules more lipophilicity. Like the 2-(3-hydroxypropyl)-*N,N'*-bis(arylmethyl)benzimidazolium salts **5-9**, **11** and **12** were fully characterized. Crystals of **12**, suitable for single X-ray diffraction was obtained by slow evaporation of a concentrated solution of isopropanol and minimal chloroform (Figure 9).



Scheme 2. Synthesis of 2-((2-(2-methoxyethoxy)ethoxy)methyl)-*N,N'*-bis(arylmethyl)benzimidazolium salts, **11-12**.

Interestingly, the (2-(2-(2-methoxyethoxy)ethoxy)methyl)-portion was quite complex in the NMR spectra when progressing from **10** to both **11** and **12**. Compound **10** appeared as a yellow oil, and showed slight impurities in the material following analysis in the 300 Hz ^{1}H NMR. These impurities were removed by column chromatography using a (50:50) ethyl acetate and hexanes mixture as the eluent. The fractions were evaluated using TLC plates and NMR spectroscopy, with the fourth fraction clearly containing the desired product (Figure 9). In the final and nearly-purified form, the product was a yellow oil. There was one remaining impurity in the NMR data at 4.04 ppm. Comparison of the NMR spectra with NMR spectra of the two starting materials showed that the resonance of 4.04 ppm was the result of unreacted 2-(2-[2-methoxy]ethoxy)ethoxy-acetic acid. The remaining acid was removed using a sodium bicarbonate wash on the product (Figure 10). The post-wash NMR spectroscopy also shows a slight differentiation between the hydrogens at the C^4 and C^7 positions (7.20 ppm and 7.32 ppm) of the benzimidazole and the hydrogens of the methyl groups at the C^5 and C^6 positions (2.26 ppm and 2.28 ppm). This separation of peaks implies there may be hydrogen bonding interactions between the hydrogen of the nitrogen atom in the benzimidazole and one or more of the oxygen atoms in the triethylene glycol chain, resulting in an asymmetric molecule. This data supports the original hypothesis that the triethylene glycol chain is capable of hydrogen bonding interactions and may increase the overall hydrophilicity of the molecule.

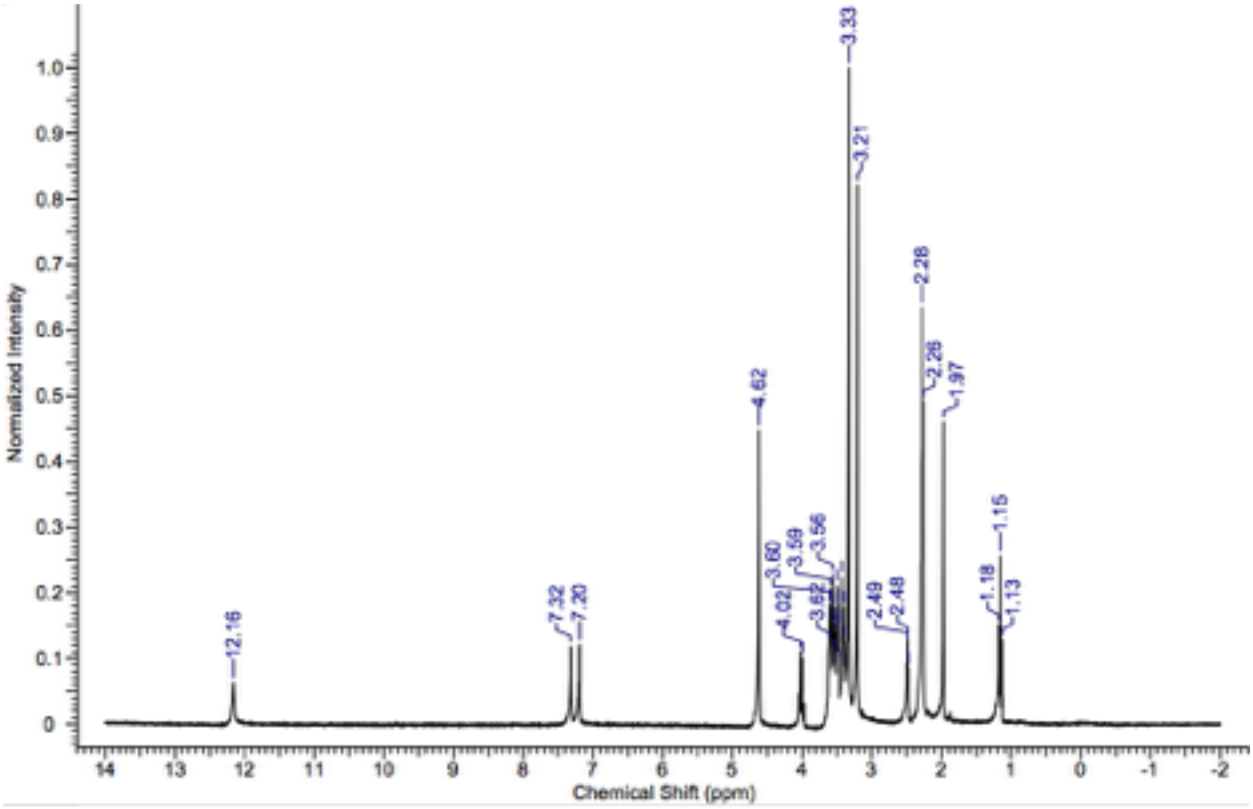


Figure 9. 300 Hz ^{1}H NMR data for fraction 4. Spectrum displayed resonances of 5,6-dimethyl-2-methoxy-triethyleneglycol-benzimidazole and column eluent, (50:50) ethyl acetate and hexanes.

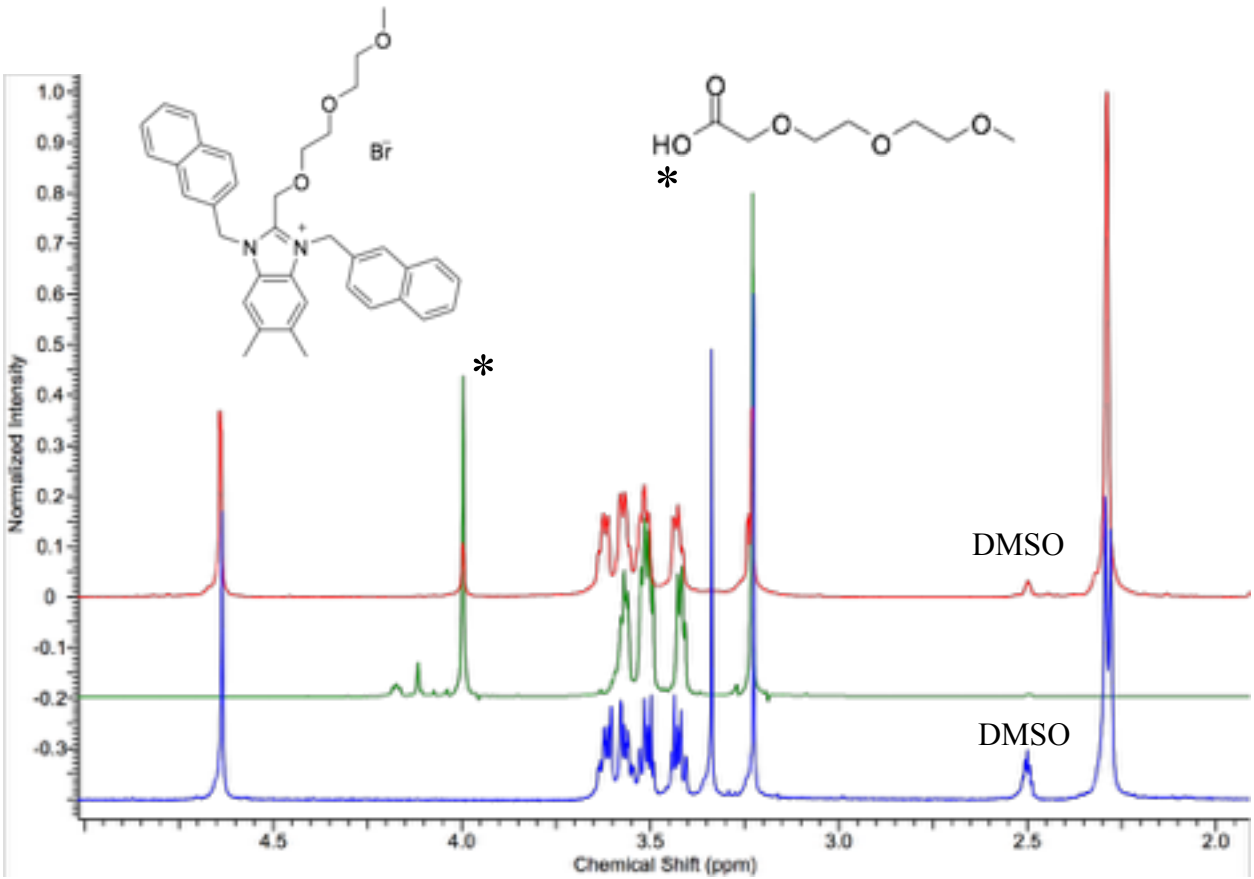


Figure 10. 300 Hz ^{1}H NMR data of 5,6-dimethyl-2-methoxy-triethyleneglycol-benzimidazole pre-wash, 2-(2-[2-methoxy]ethoxy)ethoxy-acetic acid, and 5,6-dimethyl-2-methoxy-triethyleneglycol-benzimidazole post-wash. Resonances per salt **11** depicted in red (pre-wash) and blue (post-wash). Resonances per acid starting material depicted in green.

The *N,N'*-bisnapthyl-5,6-dimethyl-2-methoxy-TEG-benzimidazolium salt, compound **11**, was the first to be synthesized. The initial stirring of the 5,6-dimethyl-2-methoxy-triethyleneglycol-benzimidazole with the base for 30 minutes is meant to deprotonate the nitrogen atom, allowing the naphthalene substituent to bind to the nitrogen. 2-(Bromomethyl)-naphthalene was added one equivalent at a time so that the potassium bromide salt formed in the first step of the reaction may be removed at the halfway point via a hot vacuum filtration.

However, the *N,N'*-bisnapthyl-5,6-dimethyl-2-methoxy-TEG-benzimidazolium salt still appeared as a yellow oil, and ^{1}H NMR showed impurities in the spectrum, so it was purified through column chromatography.

Following the TLC analysis, which showed salt **11** was more polar than the starting benzimidazole 5,6-dimethyl-2-methoxy-TEG-benzimidazole **10**, the eluent used to purify **11** was a (5:95) methanol and chloroform mixture. The solvent from the final fraction of the column was removed under reduced pressure to produce a white powder, and the powder was analyzed on the 400 Hz NMR (Figure 11). The NMR spectroscopy data clearly shows resonance at 6.06, which is indicative of the methylene hydrogens on the naphthalene substituents, another resonance at 5.35, which is indicative of the methylene at the 2 position of the benzimidazole, and splitting ranging from 3.72 to 3.09, which is representative of the hydrogens in the triethylene glycol chain.

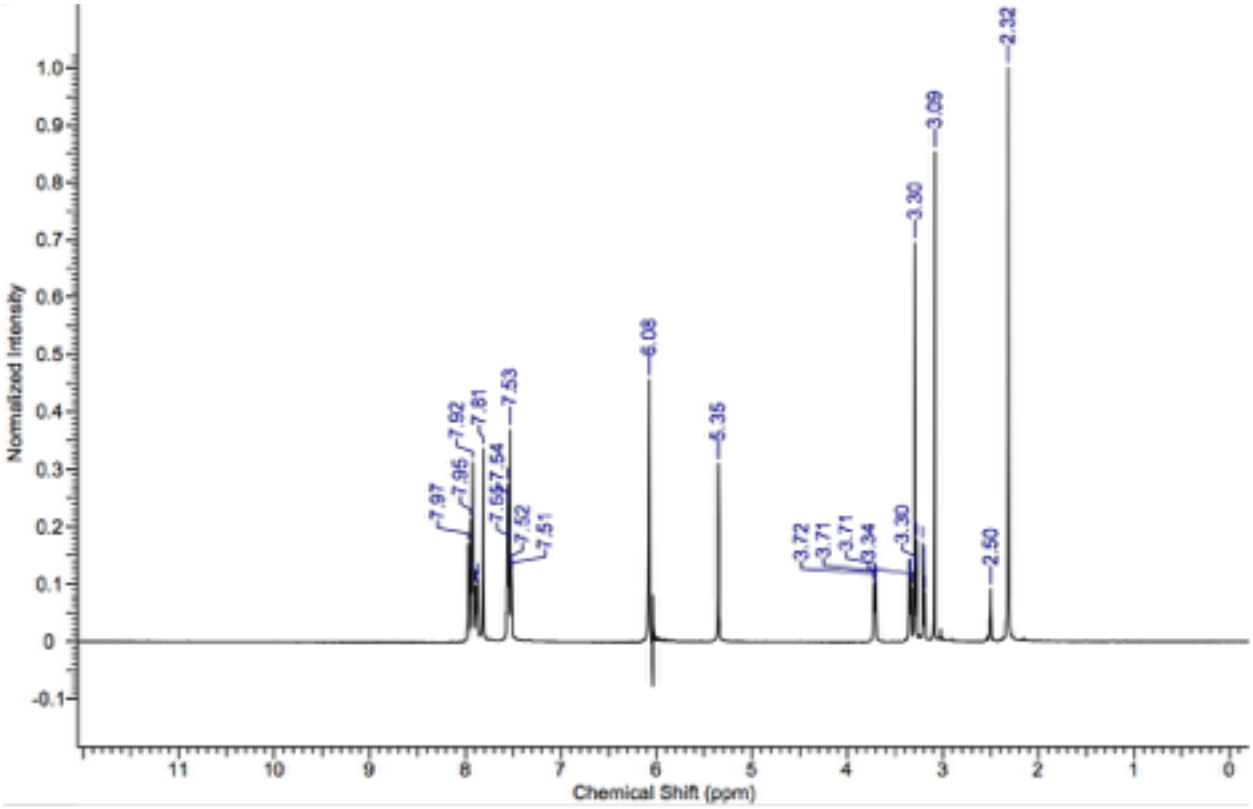


Figure 11. 400 Hz ^{1}H NMR data; post-column *N,N'*-bisnapthyl-5,6-dimethyl-2-methoxy-TEG-benzimidazolium salt, **11**. (Artifact at approximately 6.00 ppm)

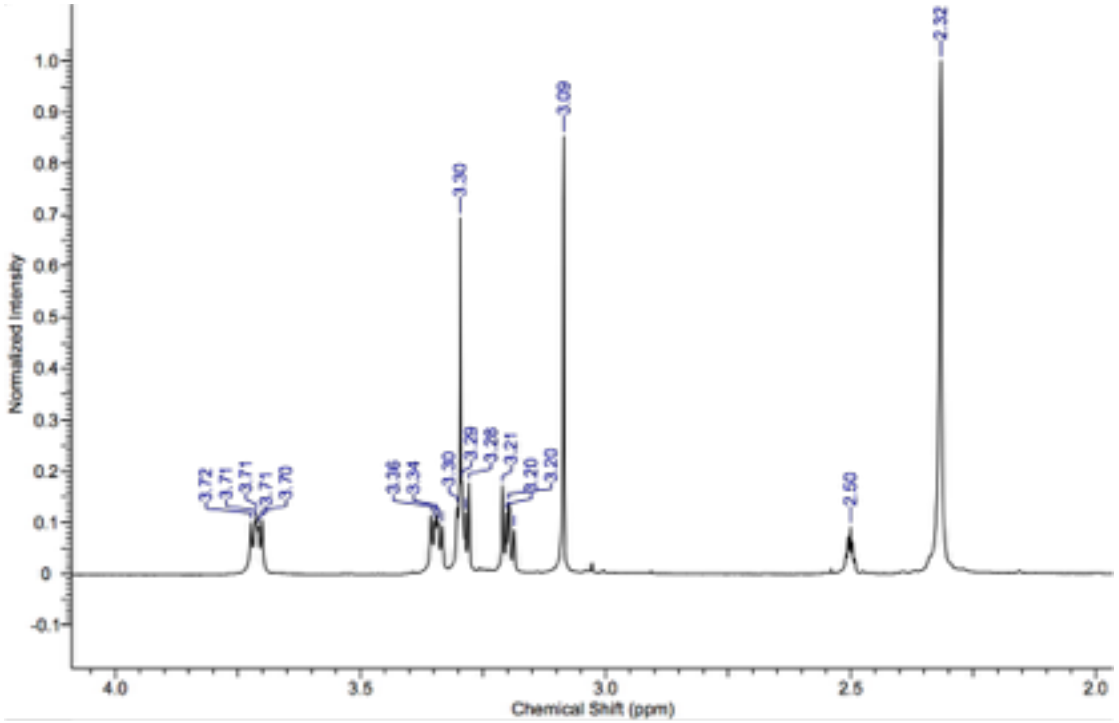


Figure 12. Aliphatic region of the 400 Hz ^{1}H NMR data shown in Figure 11; post-column *N,N'*-bisnapthyl-5,6-dimethyl-2-methoxy-TEG-benzimidazolium salt, **12**.

Compound **12** was synthesized incorporating quinolinylmethyl substituents at the N^{1} and N^{3} positions, using the same procedure of compound **11**. Similar to **11**, compound **12** appeared as a yellow oil following the final hot acetonitrile wash. ^{1}H NMR data showed that the desired product was present in the oil, and the product was purified and isolated by column chromatography using a (5:95) methanol and chloroform mixture as the eluent. The product still appeared as a yellow oil following the column filtration, however it was determined that an isopropanol wash could precipitate a white powder out of the solution.

In Vitro Anti-Proliferative Activity

Following synthesis and characterization, the activity of these compounds were tested against NSCLC lines, including NCI-H460, NCI-H1975, HCC827 and NCI-A549 to determine their anti-proliferative activity via MTT assay analysis (Table 1). The activity was analyzed by the concentration required to inhibit function of half the cells in a given cell colony, known as IC_{50} values. Cisplatin, a commonly used chemotherapeutic, was used as a control for the assays. The target of the assays was to screen for synthesized compounds that had IC_{50} values comparable to or lower than those of cisplatin against the four NSCLC lines.

The *N,N'*-bis(arylmethyl)-2-(3-hydroxypropyl) benzimidazolium salts, **5-9**, had a wide range of anti-proliferative activity. Compound **5**, the least hydrophilic of the salts, had IC_{50} values comparable to both the cisplatin control and the compound **IC23** (**1**), ranging from 6-10 μM, which is indicative of moderate activity. Furthermore, **5** had IC_{50} values lower than cisplatin against the cell line NCI-H1975, with a value of 4 μM (which indicates high activity) compared to cisplatin's value of 11 μM for NCI-H1975 (which indicates moderate to low activity). Compound **8** had IC_{50} values ranging from 11-25 μM, indicative of moderate to poor activity, on three of the four cell lines, and fell far short of the high activity values of cisplatin. The poor activity values of **8** may be due to the nitrogen atoms found in the quinolinylmethyl substituents, which were used to increase the molecules hydrophilicity. This theory is further supported by the fact that compound **9**, which featured the two (6-methoxynaphthyl)methyl substituents, had IC_{50} values of 7 μM,4 μM, and 8 μM for NCI-H460, NCI-H1975, and HCC827, respectively. Compound **9** has heteroatoms in the (6-methoxynaphthyl)methyl substituents to increase hydrophilicity, but uses oxygen atoms rather than nitrogen atoms. Since the IC_{50} values of **9** are

lower than those of **8**, it could be hypothesized that the nitrogen atoms are the cause of the decrease in activity. The asymmetric salt, compound **7**, had IC_{50} values for cell lines NCI-H460 and HCI-H1975 comparable to cisplatin and **5**, however the IC_{50} values for lines HCC827 and NCI-A549 were still higher than those for compound **5**.

Compound **11** was shown to have excellent activity, with IC_{50} values for all cell lines ranging from less than 1 μM to 2 μM, but was also found to have very little aqueous solubility. Even with the addition of multiple heteroatoms at the C^2 position, there was not enough hydrophilicity for **11** to easily solubilize in water. However, the low IC_{50} values are encouraging in that they are both lower than the IC_{50} values of cisplatin and show that other *N,N'*-bis(arylmethyl)-5,6-dimethyl-2-methoxy-TEG-benzimidazolium salts with more heteroatoms substituted into the molecules may have the combination of aqueous solubility and anti-proliferative activity that has been the goal of this research.

Table 1. Synthesized *N,N'*-bis(arylmethyl)benzimidazolium salts with their relevant IC_{50} values.

Compound	IC_{-50} (μM)			
	NCI-H460	NCI-H1975	HCC827	NCI-A549
Cisplatin	3	11	5	7
1	5	6	8	9
2	4	4	5	5
3	5	6	9	10
5	4	4	6	10
6	13	3	7	17
7	4	3	11	12
8	11	7	17	25
9	7	4	8	13
11	< 1	< 1	2	2

Conclusion

The investigation by the Youngs research group into imidazole based compounds with anti-proliferative activity has yielded results that may impact the development of new treatments for NSCLC and possibly other cancer lines. The key to these compounds was finding a balance between the anti-proliferative activity and the aqueous solubility. Work with the 2-(3-hydroxypropyl)-*N,N'*-bis(arylmethyl) benzimidazolium salts, **5-9**, showed the relationship between activity and aqueous solubility clearly in the MTT assays.

Compound **5** had IC_{50} values comparable to those of cisplatin, whereas compound **8**, which featured quinolinylmethyl substituents containing nitrogen atoms, proved to have much

higher values, though better aqueous solubility. The exception was compound **9**, which featured (6-methoxynapthyl)methyl substituents. Compound **9** proved to have lower IC_{50} values than compound **8**, but also maintained higher levels of aqueous solubility. This result may imply some relationship between the addition specifically of nitrogen atoms and a loss of anti proliferative activity.

The *N,N'*-bis(arylmethyl)-5,6-dimethyl-2-methoxy-TEG benzimidazolium salts, **11** and **12**, proved to be complex to synthesize, however tracking the synthesis through NMR spectroscopy was able to show not only the purification of the products, but some weak hydrogen bonding interactions. Compound **11** showed high anti proliferative activity in the MTT assay screenings, though it had poor aqueous solubility. However, due to the low IC_{50} values of **11**, it may be inferred that similar salts with more hydrophilic groups substituted at the N^1 and N^3 positions, such as those found in quinolinylmethyl and (6-methoxynapthyl)methyl, may provide the balance of aqueous solubility and anti-proliferative activity needed to advance to the next stage of medical testing.

Experimental

General Considerations

All reactions were carried out under aerobic conditions unless otherwise specified. All reagents and solvents were purchased from commercial sources and used without further purification. 2-((2-(2-Methoxyethoxy)ethoxy)-acetic acid (≥95%) was purchased from Merck. 4,5-Dimethyl-o-phenylene diamine mono-hydrate (98%) was purchased from Alfa Aesar. 2-(Bromomethyl)naphthalene and 2-(chloromethyl)quinolone were purchased from Waterstone

Technologies. 2-(Chloromethyl)-6-methoxynaphthalene, which was synthesized according to the literature.[12] 3-(Benzimidazol-2-yl)propan-1-ol (**4**) was synthesized according to literature procedure.[12] Column chromatography utilized silica gel (60 Å, ICN Medicals) embedded with fluorescent indicator green 254 nm (Fluka Analytical). Melting points were obtained on a MelTemp apparatus. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were collected on a Varian 500 MHz instrument referenced to DMSO-d_6 (Cambridge Isotope Laboratories, (δ = 2.50 ppm, 39.51 ppm). Infrared spectroscopy (IR) was performed with a Thermo Scientific ATR -IR Nicolet iS5 FT-IR spectrometer equipped with an iD5 ATR attachment. Electrospray ionization mass spectrometry (ESI-MS) and (ESI-HRMS) were performed by the University of Akron mass spectrometry laboratory (Akron, OH). Elemental analysis was performed by the microanalysis laboratory at Atlantic Microlabs, Inc. (Norcross, GA) or by the University of Akron Department of Geology (Akron, OH).

Crystal structures were obtained by Patrick O. Wagers. Crystals of the compounds were coated in paratone oil, mounted on a CryoLoop and placed on a goniometer under a stream of nitrogen. Crystal structure data sets were collected on either a Bruker APEX CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) or a Bruker Kappa APEX II Duo CCD system equipped with a Mo ImuS source and a Cu ImuS micro-focus source equipped with QUAZAR optics (λ = 1.54178 Å). The unit cells were determined by using reflections from three different orientations. Data integration and reduction were performed in the Apex2 suite using SAINT.[13] An empirical absorption correction and other corrections were applied to the data using multi-scan SADABS.[14] Structure refinement and solution was accomplished using the Bruker SHELXTL software package.[15] The structures were determined by full-matrix least-

squares refinement of F2 and the selection of the appropriate atoms from the generated difference map. Hydrogen atom positions were calculated and Uiso(H) values were fixed according to a riding model.

The human non-small cell lung cancer cell lines NCI-H1975, NCI-H460, NCI-A549 and HCC827 were donated by Dr. Lindner from the Cleveland Clinic. The human non-small cell lung cancer cell line NCI-H460 was purchased from ATCC (Manassas, VA, USA). All cell lines were grown in RPMI 1640 media supplemented with 10% fetal bovine serum. All cell lines were grown under physiological conditions, specifically at 37 °C with 5% CO_2 and passed every 2-3 days.

Cells were grown to confluency and plated in 96-well plates at 5,000-7,000 cells per well. Cells were incubated for 24 h prior to adding the compounds. All compounds were dissolved in a 1% DMSO solution and diluted in fresh media to the desired concentrations of 1, 4, 16, and 32 mM. Compounds were added in sextuplet and cells were incubated for 72 h at which time the optional MTT assay protocol was followed. MTT reagent (10 mL) was added to each well and cells were incubated for 3-4 h. Media was removed by vacuum and DMSO (100 mL) was added to each well. Plates were incubated for 15 min at 37 °C. The optical density was read at 540 nm on a BioTek Epoch plate reader.

3-(1-(naphthalen-2-ylmethyl)-benzimidazol-2-yl)propan-1-ol (**4a**). 3-(Benzimidazol-2-yl)propan-1-ol (**4**) (1.99 g, 11.3 mmol) was dissolved in acetonitrile (57 mL) potassium hydroxide (0.718 g, 12.8 mmol) was added, and the mixture was refluxed for 30 min. 2-(Bromomethyl)naphthalene (2.50 g, 11.3 mmol) was added and the mixture was refluxed for 30

min. The reaction mixture was filtered hot to remove a white solid, presumed to be potassium bromide. The resulting filtrate was slowly evaporated to yield crystalline material. The crystals were washed in ether (20 mL), collected via vacuum filtration and air dried to yield a white powder **4a** (1.85 g, 51%). Mp: 126 – 127 °C. Anal. Calcd for $C_{21}H_{20}N_2O$: C, 79.72; H, 6.37; N, 5.06%. Found: C, 79.79; H, 6.39; N, 8.85%. ^{1}H NMR (500 MHz, DMSO- d_6) 7.89-7.87 (m, 2H), 7.83-7.80 (dd, 1H, J = 6.0, 3.4 Hz), 7.61-7.57 (m, 2H), 7.51-7.45 (m, 3H), 7.27 (dd, 1H, J = 8.5, 1.8 Hz), 7.18-7.10 (m, 2H), 5.65 (s, 2H), 4.57 (s, 1H), 3.50 (q, 2H, J = 6.1 Hz), 2.94 (t, 2H, J = 7.6 Hz), 1.94 (dt, 2H, J = 6.8 Hz). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) 155.3, 142.3, 135.3, 134.6, 132.8, 132.2, 128.4, 127.6, 126.4, 124.8, 124.6, 121.6, 121.3, 118.4, 110.1, 60.0, 46.2, 30.2, 23.4.

2-(3-hydroxypropyl)-1,3-bis(naphthalen-2-ylmethyl)-benzimidazolium bromide (**5**). 3-(Benzimidazol-2-yl)propan-1-ol (**4**) (1.50 g, 8.51 mmol) was dissolved in acetonitrile (40 mL) potassium hydroxide (0.90 g, 16.0 mmol) was added, and the mixture was refluxed for 30 min. 2-(Bromomethyl)naphthalene (1.90 g, 8.59 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white solid, presumed to be potassium bromide. 2-(bromomethyl)naphthalene (1.90 g, 8.59 mmol) was added to the filtrate, which was refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot mixture. The solid in the funnel as washed with diethyl ether (10 mL) and air dried to yield a white powder **5** (2.20 g, 48 %). Mp: 229 – 231 °C. Anal. Calcd for $C_{32}H_{29}BrN_2O$: C, 71.02; H, 5.61; N, 5.08%. Found: C, 71.51; H, 5.44; N, 5.21%. ^{1}H NMR (500 MHz, DMSO- d_6) 7.98 (d, 2H, J = 8.6 Hz), 7.94 (, 2H), 7.91-7.86 (m, 6H), 7.59-7.53 (m, 6H), 7.49 (dd, 2H, J = 8.4,

1.6 Hz), 6.09 (s, 4H), 4.79 (bs, 1H), 3.54 (t, 2H), 3.49 (t, 2H, J = 5.6 Hz), 1.76 (dt 2H,). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) 155.3, 132.7, 132.5, 131.8, 131.4, 128.7, 127.7, 127.6, 126.7, 126.51, 126.45, 125.7, 124.7, 113.6, 59.2, 48.7, 29.7, 21.0. MS (ESI^+) calcd for $C_{32}H_{29}N_2O^+$ [M-Br]$^+$: m/z = 457.2, found m/z = 457.1.

Crystal data for 2-(3-hydroxypropyl)-1,3-bis(naphthalen-2-ylmethyl)-benzimidazolium bromide (5):$C_{32}H_{29}Br_1N_2O_1$, M = 537.48, triclinic, a = 10.3959(6) Å, b = 10.4438(6) Å, c = 12.8557(7) Å, α = 67.842(2)°, β = 76.321(2)°, γ = 79.887(2)°,V = 1250.23(12) Å^3, T = 100(2) K, space group P-1, Z = 2, 27063 reflections measured, 5022 independent reflections (R_{int} = 0.0277).The final R_1 values were 0.0221 (I > 2σ(I)). The final wR(F^2) values were 0.0552 (I > 2σ(I)). The final R_1 values were 0.0237 (all data). The final wR(F^2) values were 0.0561 (all data).

2-(3-hydroxypropyl)-1-(naphthalen-2-ylmethyl)-3-(quinolin-2-ylmethyl)-benzimidazolium chloride salt (**6**). 3-(1-(naphthalen-2-ylmethyl)-benzimidazol-2-yl)propan-1-ol (**4a**) (0.20 g, 0.63 mmol) and 2-(Chloromethyl)quinoline (0.29 g, 1.63 mmol) were dissolved in a refluxing mixture of acetonitrile and methanol (4:1, v/v, 5 mL), and the mixture was refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot mixture. The solid in the funnel as washed with diethyl ether (10 mL) and air dried to yield a crème powder **5** (0.18 g, 58%). Mp: 250 – 252 °C. Anal. Calcd for $C_{31}H_{28}ClN_3O$: C, 75.37; H, 5.71; N, 8.51%. Found: C, 74.55; H, 5.81; N, 8.34%. ^{1}H NMR (300 MHz, DMSO- d_6) 8.50 (d, 1H, J = 8.5 Hz), 8.04-7.87 (m, 7H), 7.79 (d, 1H, J = 8.5 Hz), 7.77-7.67 (m, 1H), 7.63-7.55 (m, 6H), 7.48 (d, 1H, J = 8.5 Hz), 6.28 (s, 2H), 6.15 (s, 2H), 4.85 (bt, 1H, J = 4.8 Hz), 3.53-3.47 (m, 4H), 1.86 (dt, 2H). ^{13}C

{^{1}H} NMR (125 MHz, DMSO-d_6) 156.0, 153.6, 146.6, 137.5, 132.7, 132.5, 132.0, 131.6, 130.1, 128.8, 128.2, 128.0, 127.7, 127.2, 126.9, 126.8, 126.59, 126.42, 126.39, 125.7, 124.5, 120.0, 113.4, 113.4, 59.2, 49.5, 48.4, 29.8, 21.0. MS (ESI$^+$) calcd for $C_{31}H_{28}N_3O^+$ [M-Cl]$^+$: m/z = 458.2, found m/z = 458.0.

Crystal data for 2-(3-hydroxypropyl)-1-(naphthalen-2-ylmethyl)-3-(quinolin-2-ylmethyl)-benzimidazolium chloride (6): $C_{31}H_{28}Cl_1N_3O_1$, M = 494.04, triclinic, a = 10.2508(5) Å, b = 10.5009(6) Å, c = 12.5681(7) Å, α = 110.590(3)°, β = 96.855(2)°, γ = 102.225(2)°,V = 1209.63(11) Å^3, T = 100(2) K, space group P-1, Z = 2, 30199 reflections measured, 4908 independent reflections (R_{int} = 0.0319).The final R_1 values were 0.0376 (I > 2σ(I)). The final wR(F^2) values were 0.0955 (I > 2σ(I)). The final R_1 values were 0.0430 (all data). The final wR(F^2) values were 0.0994 (all data).

2-(3-hydroxypropyl)-1-(naphthalen-2-ylmethyl)-3-(quinolin-2-ylmethyl)-benzimidazolium bromide salt (**7**). 3-(Benzimidazol-2-yl)propan-1-ol (**4**) (0.40 g, 2.27 mmol) was dissolved in acetonitrile (11 mL) potassium hydroxide (0.15 g, 2.67 mmol) was added, and the mixture was refluxed for 30 min. 2-(Chloromethyl)quinoline (0.40 g, 2.26 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white solid, presumed to be potassium bromide. 2-(Bromomethyl)naphthalene (0.50 g, 2.26 mmol) was added to the filtrate, which was refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot mixture. The solid in the funnel as washed with diethyl ether (5 mL) and air dried to remove any remaining 2-(bromomethyl)naphthalene. The solid was triturated in a hot

mixture of acetone and dichloromethane (1:1 v/v, 6 mL) and gravity filtered to yield a white powder **7** (0.57 g, 46%). Mp: 246 – 250 °C. Anal. Calcd for $C_{31}H_{28}BrN_3O$: C, 69.15; H, 5.24; N, 7.80%. Found: C, 69.15; H, 5.15; N, 7.72%. ^{1}H NMR (500 MHz, DMSO- *d*$_6$) 8.51 (d, 1H, J = 8.3 Hz), 8.04-7.81 (m, 7H), 7.81-7.48 (d, 1H, J = 8.3 Hz), 6.31 (s, 2H), 6.18 (s, 2H), 4.75 (bt, 1H), 3.55-3.47 (m, 4H), 1.89 (dt, 2H). ^{13}C {^{1}H} NMR (125 MHz, DMSO-*d*$_6$) 156.0, 153.6, 146.6, 137.5, 132.7, 132.5, 132.0, 131.6, 131.1, 130.1, 128.8, 128.0, 127.7, 127.2, 126.9, 126.8, 126.6, 126.42, 126.39, 125.7, 124.5, 120.0, 113.5, 113.4, 59.2, 49.5, 48.4, 29.8.

Crystal data for 2-(3-hydroxypropyl)-1-(naphthalen-2-ylmethyl)-3-(quinolin-2-ylmethyl)-benzimidazolium bromide (7): $C_{31}H_{28}Br_1N_3O_1$, M = 538.47, triclinic, a = 10.4596(3) Å, b = 10.4601(3) Å, c = 12.7946(4) Å, α = 113.0640(10)°, β = 97.215(2)°, γ = 99.6430(10)°,V = 1241.21(6) Å^3, T = 100(2) K, space group P-1, Z = 2, 29636 reflections measured, 5003 independent reflections (R_{int} = 0.0271).The final R_1 values were 0.0295 (I > 2σ(I)). The final wR(F^2) values were 0.0741 (I > 2σ(I)). The final R_1 values were 0.0310 (all data). The final wR(F^2) values were 0.0750 (all data).

2-(3-hydroxypropyl)-1,3-bis(quinolin-2-ylmethyl)-benzimidazolium chloride salt (8). 3-(benzimidazol-2-yl)propan-1-ol (**4**) (0.39 g, 2.21 mmol) was dissolved in acetonitrile (5 mL) sodium carbonate (0.24 g, 2.26 mmol) was added, and the mixture was refluxed for 30 min. 2-(Chloromethyl)quinoline (0.60 g, 3.38 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white solid, presumed to be potassium bromide. 2-(Chloromethyl)quinoline (0.60 g, 3.38 mmol) was added to the filtrate, which was

refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot mixture. The solid in the funnel as washed with diethyl ether (5 mL) and air dried yield a white powder **8** (0.81 g, 72%). Mp: 256 – 260 °C. HRMS (ESI^+) calcd for $C_{30}H_{27}N_4O^+$ $[M\text{-}Cl]^+$: m/z = 459.2185, found m/z = 459.2193 ^1H NMR (500 MHz, DMSO- *d*$_6$) 8.51 (d, 2H, J = 8.3 Hz), 8.02 (d, 2H, J = 8.1 Hz), 7.98 (m, 2H), 7.79 (d, 2H, J = 8.6 Hz), 7.72-7.67 (m, 3H), 7.62-7.59 (ddd, 2H, J = 8.1, 6.1, 2.0 Hz), 7.54 (m, 2H), 6.35 (s, 4H), 4.95 (t, 1H, J = 5.1 Hz), 3.58 (t, 2H, J = 7.7 Hz), 3.46 (q, 2H, J = 5.4 Hz), 1.91 (tt, 2H). ^{13}C {^1H} NMR (125 MHz, DMSO-*d*$_6$) 156.5, 153.9, 146.7, 137.5, 131.4, 130.1, 128.4, 128.0, 127.2, 126.9, 126.3, 119.9, 113.4, 59.2, 49.7, 29.8, 21.0.

Crystal data for 2-(3-hydroxypropyl)-1,3-bis(quinolin-2-ylmethyl)-benzimidazolium chloride (8):$C_{30}H_{27}Cl_1N_4O_1 \bullet H_2O_1$, M = 513.02, monoclinic, a = 13.0575(11) Å, b = 19.4079(13) Å, c = 10.2667(8) Å, α = 90°, β = 90.609(3)°, γ = 90°,V = 2601.6(3) Å^3, T = 100(2) K, space group P2(1)/c, Z = 4, 41453 reflections measured, 5281 independent reflections (R_{int} = 0.0376).The final R_1 values were 0.0336 (I > 2σ(I)). The final wR(F^2) values were 0.0814 (I > 2σ(I)). The final R_1 values were 0.0421 (all data). The final wR(F^2) values were 0.0866 (all data).

2-(3-hydroxypropyl)-1,3-bis((6-methoxynaphthalen-2-yl)methyl)-benzimidazolium chloride salt (**9**). 3-(Benzimidazol-2-yl)propan-1-ol (**4**) (0.30 g, 1.70 mmol) was dissolved in acetonitrile (2 mL), potassium hydroxide (0.13 g, 2.32 mmol) was added, and the mixture was refluxed for 30 min. 2-(Chloromethyl)-6-methoxynaphthalene (0.35 g, 1.69 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white solid, presumed to be potassium bromide. 2-(Chloromethyl)-6-methoxynaphthalene (0.35 g, 1.69

mmol) was added to the filtrate, which was refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot mixture. The solid in the funnel as washed with diethyl ether (5 mL) and air dried to yield a white powder **9** (0.40 g, 48 %). Mp: 217 – 219 °C. HRMS (ESI^+) calcd for $C_{34}H_{33}N_2O_3^+$ $[M\text{-}Cl]^+$: m/z = 517.2491, found m/z = 517.2474. ^1H NMR (125 MHz, DMSO- d_6) 7.91 (m, 2H), 7.87-7.83 (m, 4H), 7.79 (d, 2H, J = 9 Hz), 7.57 (dd, 2H, J = 3.2, 1.2 Hz), 7.43 (d, 2H, J = 8.6 Hz), 7.34 (m, 2H), 7.19 (d, 2H, J = 8.8 Hz), 6.02 (s, 4H), 4.93 (t, 1H), 3.86 (s, 6H), 3.53 (t, 2H, J = 7.3), 3.47 (dt, 2H), 1.73 (dt, 2H). ^{13}C $\{^1H\}$ NMR (125 MHz, DMSO-d_6) 157.7, 155.1, 133.9, 131.4, 129.34, 129.30, 128.1, 127.6, 126.2, 125.9, 125.3, 119.3, 113.7, 105.9, 59.2, 55.2, 48.6, 29.7, 21.0.

2-((2-(2-methoxyethoxy)ethoxy)methyl) -5,6-dimethyl-benzimidazole (10). 4,5-Dimethylbenzene-1,2-diamine (0.50 g, 3.67 mmol) was dissolved in 2-[2-(2-Methoxyethoxy)ethoxy]acetic acid (0.68 mL, 4.41 mmol, d = 1.161 g/mL) and was refluxed overnight. The solution was extracted in dichloromethane (2x32 mL). The organic layers were combined, dried with magnesium sulfate and the volatiles were removed under reduced pressure to a brown, viscous oil. The oil was purified using a mobile phase of ethyl acetate/hexanes (1:1) to yield a clear orange liquid. The orange oil was dissolved in dichloromethane (5 mL) and stirred for 1 hr with sodium bicarbonate. Deionized water (10 mL) was added to the residue and the organic layer was extracted with dichloromethane (3x10 mL). The organic layers were combined, dried with magnesium sulfate, and the volatiles were removed under reduced pressure to yield a viscous, orange oil 10 (0.4605 g, 45%). HRMS (ESI+) calcd for $C_{15}H_{23}N_2O_3^+$ $[M+H]^+$ of m/z = 279.1709, found $[M+H]^+$ of m/z = 279.1713. ^1H NMR (300 MHz, DMSO-d_6) δ 12.17

(s, 1H), 7.33 (s, 1H), 7.21 (s, 1H), 4.63 (s, 2H), 3.64-3.61 (m, 2H), 3.58-3.54 (m, 2H), 3.53-3.49 (m, 2H), 3.44-3.40 (m, 2H), 3.23 (s, 3H), 2.29 (s, 3H), 2.28 (s, 3H). $^{13}C\{^{1}H\}$ NMR (125 MHz, DMSO-d_6) δ 150.4, 129.9, 113.8, 71.27, 69.6, 66.2, 58.0, 30.5, 19.8.

2-((2-(2-methoxyethoxy)ethoxy)methyl) -5,6-dimethyl-1,3-bis(naphthalen-2-ylmethyl)-benzimidazolium bromide (11). Compound 10 (1.00 g, 3.59 mmol) was dissolved in acetonitrile (10 mL) with potassium hydroxide (0.24 g, 4.31 mmol) and was refluxed for 30 min. 2-(Bromomethyl)naphthalene (0.79 g, 3.59 mmol) was added and the mixture was refluxed overnight, during which a precipitate was formed. The reaction mixture was filtered hot to remove a white precipitate, presumed to be potassium bromide. The filtrate was stirred with 2-(bromomethyl)naphthalene (0.79 g, 3.59 mmol) and refluxed overnight. The volatiles were removed under reduced pressure and the residue was washed in a mixture of diethyl ether and acetone (4:1 v/v, 265 mL). The initial precipitate was isolated via a room temperature vacuum filtration. The volatiles of the filtrate were removed under reduced pressure and purified by column chromatography using a mobile phase of methanol/chloroform (5:95) to yield a clear residue. The residue was washed diethyl ether (20 mL) at room temperature to afford a cream solid **11**. (0.48 g, 21%). M_p: 163-166 °C. Anal. Calcd for $C_{37}H_{29}BrN_2O_3$: C, 69.48; H, 6.15; Br, 12.49; N, 4.38. Found: C, 69.28; H, 6.12; N, 4.34%. 1H NMR (400 MHz, DMSO-d_6) δ 7.97 (s, 2H), 7.95-7.87 (m, 6H), 7.81 (s, 2H), 7.57-7.51 (m, 6H), 6.08 (s, 4H), 5.85 (s, 2H), 3.71 (m, 2H), 3.34 (m, 2H), 3.29 (m, 2H), 3.20 (m, 2H), 3.09 (s, 3H), 2.32 (s, 6H). $^{13}C\{^{1}H\}$ NMR (125 MHz, DMSO-d_6) δ 148.1, 137.1, 132.6, 132.5, 131.8, 129.7, 128.6, 127.7, 127.6, 126.6, 126.1, 124.9,

113.4, 70.9, 69.4, 59.8, 57.9, 48.9, 20.0. MS (ESI+) calcd for $C_{37}H_{29}N_2O_3^+$ $[M\text{-}Br]^+$ of m/z = 559.3, found $[M\text{-}Br]^+$ of m/z = 559.2.

2-((2-(2-methoxyethoxy)ethoxy)methyl)-5,6-dimethyl-1,3-bis(quinolin-2-ylmethyl)-benzimidazolium chloride salt (**12**). 2-((2-(2-methoxyethoxy)ethoxy)methyl)-5,6-dimethylbenzimidazole (**10**) (0.200 g, 0.718 mmol) was dissolved in acetonitrile (2 mL), sodium carbonate (0.0774 g, 0.718 mmol) was added, and the mixture was refluxed for 30 min. 2-(Chloromethyl)quinoline (0.143 g, 0.790 mmol) was added to the flask and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white solid, presumed to be potassium bromide. 2-(Chloromethyl)quinoline (0.143 g, 0.790 mmol) was added to the filtrate, which was refluxed overnight. The resulting precipitate was filtered hot and the solid was transferred to a round bottom flask. The brown residue was washed at room temperature in acetonitrile, the solvent was decanted away and the solid was washed with diethyl ether at room temperature to yield an off-white solid. The white solid was purified by column chromatography using a mobile phase of methanol/chloroform (5:95) to yield an off-white solid **12** (0.02 g, XX %). Mp: 200–203 °C. Anal. Calculated for $C_{35}H_{37}ClN_4O_3$: HR-MS (ESI^+) calculated for $C_{35}H_{37}N_4O_3^+$ $[M\text{-}Cl]^+$ of m/z = 561.2866, found $[M\text{-}Cl]^+$ of m/z = 561.2791. ^1H NMR (500 MHz, DMSO- d_6) 8.50 (d, 2H), 8.12 (dd, 2H), 8.02 (d, 2H), 7.76 (d, 2H), 7.68 (m, 4H), 7.66-7.60 (m, 4H), 6.39 (s, 4H), 5.47 (s, 2H), 3.65 (tt, 2H), 3.22 (tt, 4H), 3.19 (tt, 2H), 3.09 (s, 3H), 2.33 (s, 6H). ^{13}C $\{^1H\}$ NMR (100 MHz, DMSO-d_6) 153.8, 149.9, 146.7, 137.5, 136.8, 130.0, 129.9, 128.4, 127.9, 127.0, 126.9, 119.7, 113.1, 70.9, 70.2, 69.3, 69.2, 61.1, 57.9, 49.9, 19.9.

Acknowledgments

This project may be the culmination of my research, but I would not have accomplished so much without a team of experienced researchers helping me along. I would like to give acknowledgement to the following people for their help on this project. Kerri L. Shelton, who acted as my graduate student supervisor. Patrick O. Wagers, for collecting the crystallography data on these compounds. Michael A. DeBord, for performing the MTT Assay studies on these compounds. Marie R. Southerland, for the synthesis and characterization of 4a, as well as biological studies for the group. Dr. Matthew J. Panzner and Dr. Michael Konopka, who agreed to be readers on this project and gave valuable feedback on this paper. Dr. Claire A. Tessier, who was both the honors advisor and final editor for this project. Dr. Wiley J. Youngs, who allowed me to research with his group and acted as my research advisor.

This project has been funded by The University of Akron, the Akron Research Commercialization Corporation, and the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health (R01-DK082546). We thank the National Science Foundation (NSF) for providing funds for the purchase of the NMR instruments (Nos. CHE-0341701 and DMR-0414599), mass spectrometers (CHE-0821313 and CHE-1012636) and X-ray diffractometers (CHE-0116041 and CHE-0840446) used in this work.

References

[1] Siegel, R.L.; Miller, K.D.; Jemal, A. CA-Cancer J. Clin. 2015, 65, 5-29

[2] American Cancer Society. Cancer Facts and Figures 2014. http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151.pdf (accessed Sept 8, 2014)

[3] American Cancer Society. http://www.cancer.org/treatment/treatmentsandsideeffects/guidetocancerdrugs/cisplatin (accessed Sept 8, 2014)

[4] Haque, R.A.; Nasri, S.F.; Iqbal, M.A.; Al-Rawi, S.S.; Jafari, S.F.; Ahamed, M.B.K.; Majid, A.M.S.A. Synthesis, Characterization, and Crystal Structures of Bis-Imidazolium Salts and Respective Dinuclear Ag(I) against "Human Colon Cancer" and "Breast Cancer". *J. of Chem.* **2013**

[5] Markova, A.A.; Plyavnik, N.V.; Tatarskii Jr., V.V.; Shtil, A.A.; Serebrennikova, G.A. Novel Alkyl Cationic Glycerolipids with a Heterocyclic Polar Domain Induce Disturbances of the Cell Cycle and Death of Human Leukemia Cells. *Russ. J. Bioorg. Chem.* **2010**, 36, 532-534.

[6] Malhotra, S.V.; Kumar, V.; Velez, C.; Zayas, B. Imidazolium-derived ionic salts induce inhibition of cancerous cell growth through apoptosis. *Med. Chem. Comm.* **2014**, 5, 1404-1409.

[7] Keppler, B.K.; Wehe, D; Endres, H.; Rupp, W. Synthesis, antitumor activity, and x-ray structure of bis(imidazolium) (imidazole)pentachlororuthenate(III), (ImH)2(RuImCl5). *Inorg. Chem*. **1987**, 26, 844-846.

[8] Wright, B.D.; Deblock, M.C.; Wagers, P.O.; Duah, E.; Robishaw, N.K.; Shelton, K.L.; Southerland, M.R.; DeBord, M.A.; Kersten, K.M.; McDonald, L.J.; Stiel, J.; Panzner, M.P.; Tessier, C.A.; Paruchuri, S.; Youngs, W.J. Med. Chem. Res. Anti-tumor Activity of Lipophilic Imidazolium Salts on Select NSCLC Cell Lines. (Manuscript Accepted).

[9] Hahn, U.; Luelf, H.; Winkler, H.D.F.; Schalley, C.A.; Vögtle, F.; De Cola, L. Encapsulation of Luminescent Homoleptic $[Ru(dpp)_3]^{2+}$ -Type Chromophores within an Amphiphilic Dendritic Environment. *Chem. Euro. J.* [Online] **2012**, 18, 15424-15432. (accessed Sept 20, 2014)

[10] Kim, Y.; Klutz, A.M.; Jacobson, K.A. Systematic Investigation of Polyamidoamine Dendrimers Surface-Modified with Poly(ethylene glycol) for Drug Delivery Applications: Synthesis Characterization, and Evaluation of Cytotoxicity. *Bioconjugate Chem.* [Online] **2008**, 19, 1660-1672. http://pubs.acs.org/doi/pdf/10.1021/bc700483s (accessed Sept 20, 2014)

[11] Bai, R.; Wei,Z.; Liu, J.; Xie,W.; Yao, H.; Wu, X.; Jiang,J.; Wang, Q.; Xiu, J. *Bioorg. Med. Chem.* **2012**, *20*, 4661-4667.

[12] Indusegaram, S., Katsifis, A., Ridley, D., Vonwiller, S. Aust. J. Chem., 2003, 56, 819-827.

[13] Bruker (2007). *Apex2, SAINT*. Bruker AXS Inc., Madison, Wisconsin, USA.

[14] Bruker (2001). *SADABS*. Bruker AXS Inc., Madison, Wisconsin, USA.

[15] Sheldrick, G. M. (2008). Acta Cryst. A64, 112-122.

Appendix 1:

Safety Precautions

Basic Precautions

When working in a chemistry lab, safety is a key issue. Every chemist should be aware of basic lab safety, as well as any special safety considerations for any specific chemicals being used in an experiment. Basic safety measures to be aware of include, but are not limited to: wearing gloves, wearing protective eye gear, not wearing any loose clothing or accessories in the lab, no food or drink near the chemicals or in the lab, and always working reactions inside a ventilated hood. All of these safety measures were observed during the completion of this project.

Another safety consideration when working in the lab is being aware of any and all wash stations, including eye wash stations, safety showers, and sinks. Also, it is important to know how to use these wash stations properly. If any chemical splashes or touches the skin when working in the lab, one should immediately wash the affected area for at least 15 minutes, regardless of the toxicity of the chemical spilled.

Broken glassware or glass pipettes must be disposed of in the designated waste containers. This prevents possible injury to anyone unaware of broken glass or pipettes disposed of in the regular trash. Also, other disposable lab equipment, such as syringe and needle units, must be disposed of properly in a designated sharps container. In this project, 5 mL syringes were used to transfer the 2-(2-[2-methoxy]ethoxy)ethoxy acetic acid into the reaction flask. Following use, the soiled syringes and needle caps were disposed of in a designated sharps container.

This experiment also involved work with column chromatography, which has its own specific safety precautions. The stationary phase of the columns consisted of silica gel, which

may induce silicosis if inhaled. To prevent silicosis, a respirator or breathing mask was worn in addition to standard protective equipment.

Chemical-Specific Precautions

One of the main reactants for compounds 4-**9** was o-phenylenediamine (Figure S1). The compound was stored under room temperature conditions on a high shelf. The compound was labelled as a suspected carcinogen, or cancer-inducing substance, so, in addition to basic safety precautions, a long-sleeved lab coat was worn to minimize the chance of the o-phenylenediamine coming into contact with skin.

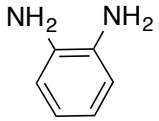


Figure S1. Structure of o-phenylenediamine

The other main reactant for compounds **5-9** was γ-butyroactone (Figure S2). The compound was stored under room temperature conditions on a high shelf. It is a viscous liquid at room temperature and was added to the solution via needle syringe. The only additional safety precaution taken for this compound was the proper disposal of the needle syringe in the designated sharps container.

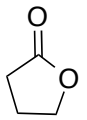


Figure S2. Structure of γ-butyroactone

The two main reactants for compounds **10-12** were 4,5-dimethyl-o-phenylenediamine and 2-(2-[2-methoxy]ethoxy)ethoxy acetic acid (Figure S3). The 4,5-dimethyl-o-phenylenediamine was stored under room temperature conditions on a high shelf. Similar to the o-phenylenediamine, 4,5-dimethyl-o-phenylenediamine is labelled as a suspected carcinogen and a long-sleeved lab coat was worn as an additional precaution to reduce chance of skin contact.

The 2-(2-[2-methoxy]ethoxy)ethoxy acetic acid was stored between 1°C-8°C in an Isotemp Fisher Scientific explosion-proof refrigerator to prevent degradation. The compound is a viscous liquid and was added to the reaction via a needle syringe, which was disposed of properly in the designated sharps container.

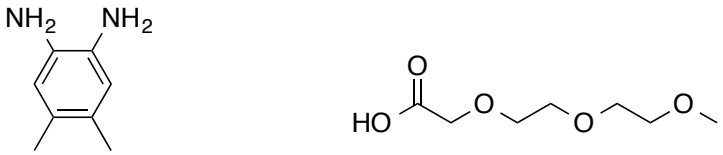


Figure S3. Structures of 4,5-dimethyl-o-phenylenediamine (left) and 2-(2-[2-methoxy]ethoxy)ethoxy acetic acid (right)

Other commonly used compounds in these syntheses were 2-(bromomethyl)naphthalene, 2-(chloromethyl) hydrochloride, and potassium hydroxide, none of which required special safety

measures. The 2-(bromomethyl)napthalene (Figure S4) was stored between 1°C-8°C in an Isotemp Fisher Scientific explosion-proof refrigerator to prevent degradation. Standard safety measures were observed when handling the compound.

The 2-(chloromethyl) hydrochloride (Figure S5) was stored under room temperature conditions on a high shelf. Standard safety measures were observed when handling the compound.

The potassium hydroxide was stored under room temperature conditions on a high shelf. Standard safety measures were observed when handling the compound.

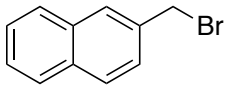


Figure S4. Structure of 2-(bromomethyl)naphthalene

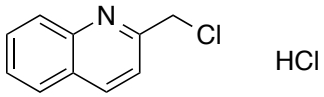


Figure S5. Structure of 2-(chloromethyl)quinoline hydrochloride

Thionyl chloride was one other chemical used in this experiment (Figure S6). This compound was used in the synthesis of the 6-(methoxy)-2-(bromomethyl)naphthalene substituent used in compound **9**. The thionyl chloride was stored under room temperature conditions on a high shelf. This chemical was only used in a hood while wearing a respirator or mask, in addition to basic safety precautions, and under the supervision of a graduate student. Thionyl chloride is

an extremely toxic substance and can react violently with water. This chemical was and should be used with extreme caution.

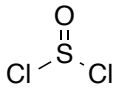
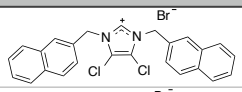
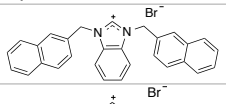
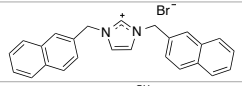
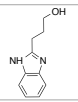
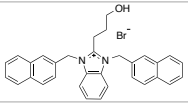
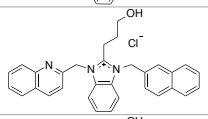
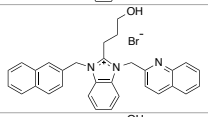
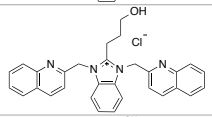
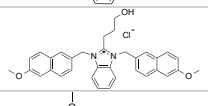
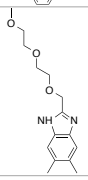
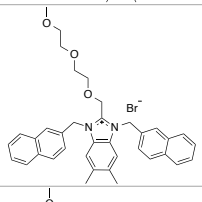


Figure S6. Structure of thionyl chloride

Appendix 2:

Table of Compounds

Table S1. Compound numbers, compound names, and compound structures

Compound #	Compound name	Compound Structure
1	4,5-dichloro-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide; IC23	
2	1,3-bis(naphthalen-2-ylmethyl)benzimidazolium bromide; IC23Benz	
3	1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide; IC23H	
4	2-(3-hydroxypropyl)benzamidizole	
5	2-(3-hydroxypropyl)-*N,N'*-bis(naphthalen-2-ylmethyl)benzimidazolium bromide	
6	2-(3-hydroxypropyl)-N-(naphthalen-2-ylmethyl)-N'-(quinolin-2-ylmethyl)benzimidazolium chloride	
7	2-(3-hydroxypropyl)-N-(naphthalen-2-ylmethyl)-N'-(quinolin-2-ylmethyl)benzimidazolium bromide	
8	2-(3-hydroxypropyl)-N,N'-bis(quinolin-2-ylmethyl)benzimidazolium chloride	
9	2-(3-hydroxypropyl)-N,N'-bis(6-methoxy-naphthalen-2-ylmethyl)benzimidazolium chloride	
10	2-((2-(2-methoxyethoxy)ethoxy)methyl)-5,6-dimethyl-benzimidazole	
11	2-((2-(2-methoxyethoxy)ethoxy)methyl)-5,6-dimethyl-*N,N'*-bis(naphthalen-2-ylmethyl)benzimidazolium bromide	
12	2-((2-(2-methoxyethoxy)ethoxy)methyl)-5,6-dimethyl-*N,N'*-bis(quinolin-2-ylmethyl)benzimidazolium chloride