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Graphical Abstract

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Compound	IC_{50} values (μM)		
	NCI-H460	NCI-H1975	HCC827
11	3	3	4
12	3	< 1	2

Synthesis, anti-proliferative activity, and toxicity of $C^4(C^5)$ substituted *N,N'*-bis(arylmethyl)imidazolium salts

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Keywords: Lung cancer, Imidazolium salt, Anti-proliferative, Anti-tumor

Abstract

The syntheses and characterization of C^4 and C^5 substituted *N,N'*-bis(arylmethyl)imidazolium salts with hydrophilic or lipophilic substituents on the imidazole ring are reported. A structure-activity relationship study revealed that the lipophilicity of groups at the C^4 and C^5 positions plays a crucial role in modulating the efficacy against select non-small cell lung cancer cell lines tested. Compounds **11 – 17** were determined to be the most active against the panel of cell lines studied. Compounds **11** and **12** were examined by the National Cancer Institute's Developmental Therapeutic Program where they were tested against the NCI-60 human cancer cell line panel in a one-dose and five-dose assay. Compound **11** had high activity against the nine lung cancer lines tested while **12** had cytotoxic effects against 59 of the 60 cell lines. Compound **11** was also studied in a murine model to determine its in vivo toxicity.

1. Introduction

Cancer is the second leading cause of death in the United States, with lung cancer accounting for 27% of all cancer deaths.[1] Despite significant advances in the treatment of cancer, one of the major drawbacks of many clinically used chemotherapeutics is their substantial toxic

side effects. An ongoing goal of cancer research is to obtain chemotherapeutics with high efficacy and reduced side effects.

Imidazolium salts as potential anti-cancer agents are under active investigation by several research groups.[1-6] Research in our group has focused on *N,N'*-bis(naphthylmethyl)imidazolium salts, many of which have been shown to exhibit high anti-proliferative activity against non-small cell lung cancer (NSCLC) cell lines. One notable example is **IC23** (Figure 1).[6] Despite its substantial activity, the low water solubility of **IC23** – similar to many bis(naphthylmethyl)imidazolium salts – presents a severe challenge to clinical applications. Herein we report the influence of the nature of hydrophilic and lipophilic functional groups in the C^4 and C^5 position of *N,N'*-bis(naphthylmethyl)imidazolium salts, along with the impact of hydrophilic and lipophilic naphthalene-based solubilizing substituents at the nitrogen (N^1 and N^3) atoms of 4,5-diphenyl-*N,N'*-bis(arylmethyl)imidazolium salts as it relates to aqueous solubility and anti-tumor activity against NSCLC cell lines.

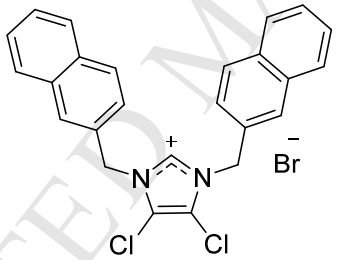


Figure 1. Structure of **IC23**.

2. Results and Discussion

2.1 Synthesis and Characterization

To investigate the influence of substituents at the C^4 and C^5 positions on the in vitro anti-cancer activity of *N,N'*-bis(naphthylmethyl)imidazolium salts, we first chose to explore the naturally occurring imidazole derivatives urocanic acid and histamine (Figure 2). Both azoles consist of an imidazole ring substituted at the C^4 position with a two carbon linker connected to a terminal hydrophilic group.

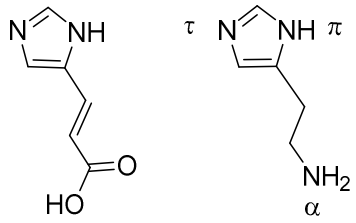
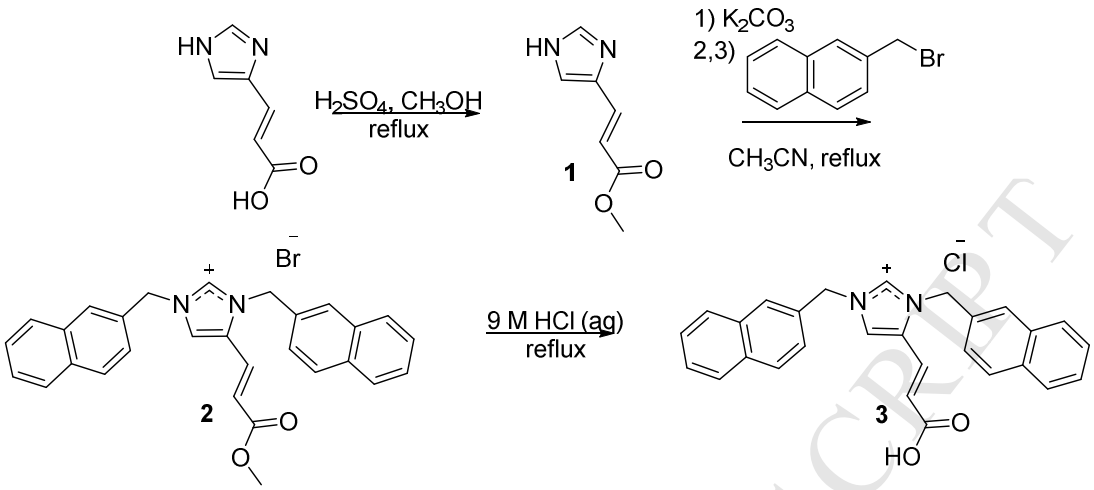


Figure 2. Urocanic acid (left) and histamine (right).

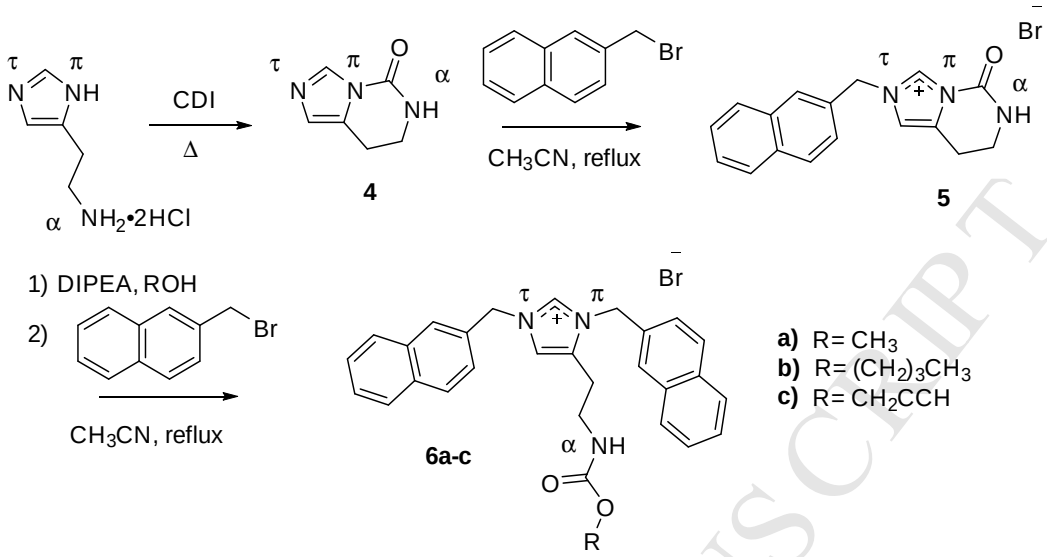
The imidazolium salts 4-(3-methoxy-3-oxoprop-1-en-1-yl)-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**2**) and 4-(2-carboxyvinyl)-1,3-bis(naphthalen-2-ylmethyl)imidazolium chloride (**3**) were synthesized from commercially available urocanic acid as outlined in Scheme 1. Urocanic acid was esterified to methyl-3-(imidazol-4-yl)acrylate (**1**) according to literature procedure.[7,8] Compound **1** was deprotonated with potassium carbonate and reacted with one equivalent of 2-(bromomethyl)naphthalene. Removal of the generated precipitate and the subsequent addition of a second equivalent of 2-(bromomethyl)naphthalene afforded the imidazolium salt **2** in moderate yield. The formation of **2** was confirmed by ^{1}H NMR spectroscopy. The most distinctive resonance in the spectrum was that of the C^{2}-H imidazolium proton which was observed at 9.54 ppm. This was a significant downfield shift from the C^{2} proton resonance of **1** (7.73 ppm) and was indicative of cation formation.

Compound **2** was refluxed for 5 days in 9 M aqueous HCl to form the carboxylic acid derivative, compound **3**. This transformation was confirmed by ^{1}H NMR spectroscopy by the appearance of the carboxylic acid proton resonance at 12.84 ppm and the loss of the proton resonance at 3.66 ppm corresponding to the methyl group of **2**. Additionally, a shift in the C=O stretching frequency from 1716 cm^{-1} (**2**) to 1693 cm^{-1} (**3**) was observed via infrared (IR) spectroscopy.



Scheme 1. Synthesis of the urocanic acid-derived *N*,*N*'-bis(naphthylmethyl)imidazolium salts **2** and **3**.

A series of *N*,*N*'-bis(naphthylmethyl)imidazolium salts that contain carbamate substituents at the C^4 position were synthesized from histamine (**6a-c**, Scheme 2). Because alkylation of histamine is known to occur at all available nitrogen atoms (N^{α}, N^{π} and N^{τ}),[9,10,11] differentiation of these three positions was achieved by converting the histamine to 5,6,7,8-tetrahydro-5-oxoimidazo[1,5-c]pyrimidine (**4**) using N,N'-carbonyldiimidazole (CDI). The combination of **4** with 2-(bromomethyl)naphthalene resulted in the alkylation of the N^{τ} atom and the formation of 2-(naphthalen-2-ylmethyl)-5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidinium bromide (**5**). The formation of **5** was confirmed by ^{1}H NMR spectroscopy. Again the downfield shift of the C^2 proton, which was observed at 9.99 ppm, was indicative of the formation of the cationic species. Crystals of **5** suitable for single crystal X-ray diffraction were obtained by slow evaporation of a concentrated solution of the compound in acetonitrile (see supplementary information). Refluxing a mixture of **5** and *N*,*N*-diisopropylethylamine (DIPEA) with methanol, n-butanol, or propargyl alcohol resulted in the deprotection of the N^{π} atom and produced the respective carbamates. These carbamates were substituted with 2-(bromomethyl)naphthalene to yield the corresponding imidazolium salts **6a-c**.



Scheme 2. Synthesis of the carbamate-substituted *N*,*N*'-bis(naphthylmethyl)imidazolium salts **6a-c**.

The C^2-H imidazolium protons of compounds **6a-c** were observed at 9.40 ppm, 9.39 ppm, and 9.35 ppm, respectively, by ^{1}H NMR spectroscopy. IR spectroscopy indicated a shift in the C=O stretching frequency from 1758 cm^{-1} (**5**) to 1709 cm^{-1} (**6a**), 1701 cm^{-1} (**6b**), and 1709 cm^{-1} (**6c**). The emergence of a C≡C stretching frequency at 2114 cm^{-1} in the spectrum of **6c** indicated the presence of the propargyl group. Crystals of **6b** suitable for single crystal X-ray diffraction were obtained by slow evaporation of a concentrated solution of the compound in a mixture of acetonitrile and chloroform (1:1) (Figure 3).

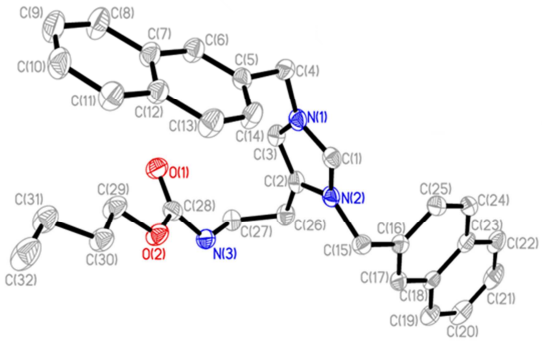
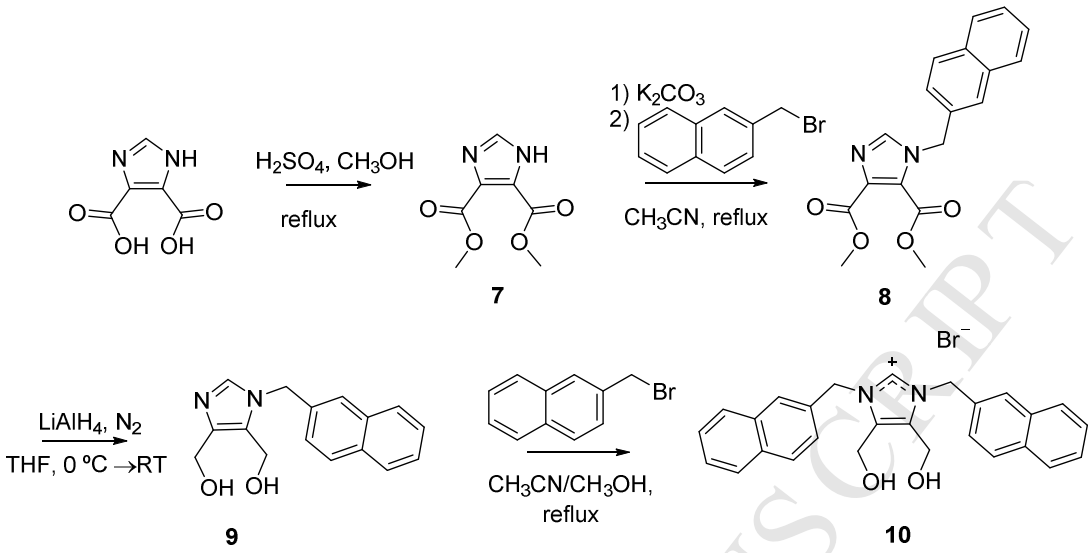


Figure 3. Thermal ellipsoid plot of the cationic portion of **6b**, with thermal ellipsoids drawn at the 50% probability level. Hydrogen atoms and bromide anion omitted for clarity.

In a separate attempt to introduce aqueous solubility to *N,N'*-bis(naphthylmethyl)imidazolium salts, the amphiphilic imidazolium salt 4,5-bis(hydroxymethyl)-1,3-bis(naphthalen-2-ylmethyl) imidazolium bromide (**10**) was synthesized (Scheme 3). Commercially available 4,5-imidazoledicarboxylic acid was used to prepare dimethyl imidazole dicarboxylate (**7**).[12] The reaction of **7** with an equimolar amount of 2-(bromomethyl)naphthalene yielded dimethyl 1-(naphthalen-2-ylmethyl)imidazole-4,5-dicarboxylate (**8**). Reduction of **8** with lithium aluminum hydride in tetrahydrofuran produced the vicinal diol, (1-(naphthalen-2-ylmethyl)imidazole-4,5-diyl)dimethanol (**9**). This functional group transformation was indicated by the loss of the C=O frequency from the IR spectra of **8** (1713 cm^{-1}) to that of **9**, as well as the emergence of the expected doublet (CH_2) and triplet (OH) resonances in the ^1H NMR spectrum of **9**. Although **9** theoretically could be synthesized from the reaction of 4,5-imidazoledicarboxylic acid with 2-(bromomethyl)naphthalene and subsequent reduction of the carboxylic acid functional groups, this route was determined to be less desirable due to the difficulties anticipated from the extremely low solubility of 4,5-imidazoledicarboxylic acid in solvents permissible for the reduction reaction. Compound **9** was reacted with a slight molar excess of 2-(bromomethyl)naphthalene to yield **10**. The formation of **10** was indicated by the characteristic downfield shift of the C^2-H imidazolium proton resonance in the ^1H NMR spectroscopy, observed at 9.45 ppm. The structure of **10** was confirmed from characterization by X-ray crystallography (Figure 4). Single crystals were grown from slow evaporation of a concentrated solution of acetonitrile and diethyl ether.



Scheme 3. Synthesis of the dimethanol-substituted *N*,*N*'-bis(naphthylmethyl)imidazolium salt **10**.

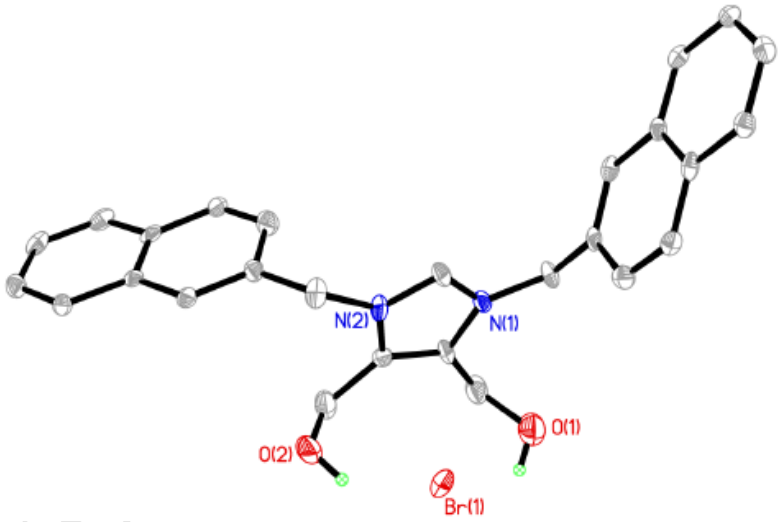
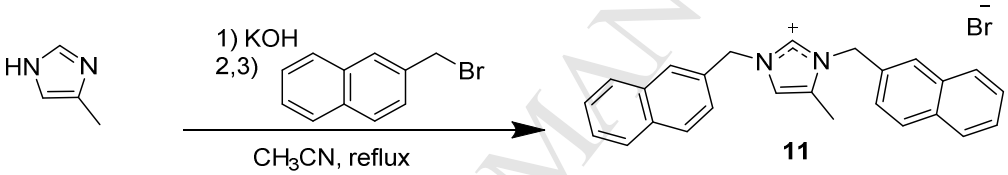


Figure 4. Thermal ellipsoid plot of **10**, with thermal ellipsoids drawn to the 50% probability level. Hydrogen atoms, with the exception of those of the hydroxyl groups, and carbon labels have been removed for clarity.

Whereas the synthesis of **2**, **3**, **6a-c**, and **10** was aimed to increase the aqueous solubility of *N*,*N*'-bis(naphthylmethyl)imidazolium salts, the synthesis of 4-methyl-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**11**) provided a lipophilic imidazolium salt to test against the

selected NSCLC cell lines. The methyl substituent in the C^4 position of **11** increased the lipophilicity of the imidazolium salt without significantly adding steric bulk. Compound **11** was synthesized according to an established procedure in our laboratory for analogous lipophilic imidazolium salts.[6] In refluxing acetonitrile, 4-methylimidazole was deprotonated with potassium hydroxide and alkylated with one equivalent of 2-(bromomethyl)naphthalene. After removal of the precipitate, a second equivalent of 2-(bromomethyl)naphthalene was added to the refluxing mixture to generate the imidazolium salt **11** (Equation 1). ^{1}H NMR spectroscopy indicated the formation of the imidazolium salt, with the downfield shift of the C^2-H imidazolium proton resonance observed at 9.57 ppm for **11**. Additionally, crystals of **11** suitable for single crystal X-ray diffraction were obtained by slow evaporation of a concentrated solution of the compound in acetonitrile (Figure 5).



Equation 1. Synthesis of the lipophilic *N,N'*-bis(naphthylmethyl)imidazolium salt **11**.

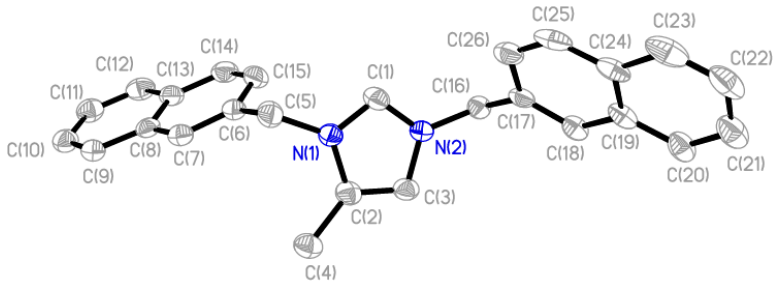
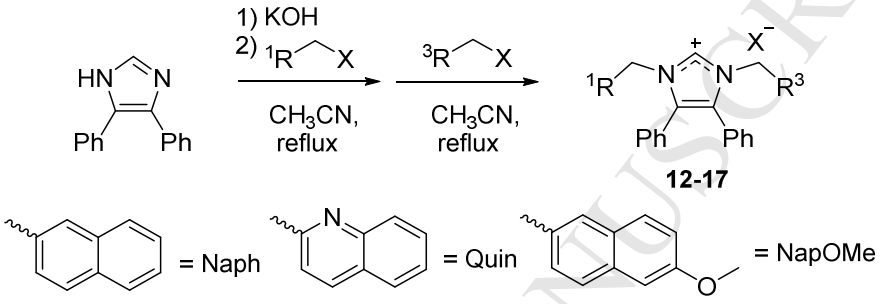


Figure 5. Thermal ellipsoid plot of the cationic portion of **11**, with thermal ellipsoids drawn at the 50% probability level. Hydrogen atoms and bromide anion omitted for clarity.

Substituents were also introduced at the nitrogen atoms (N^1 and N^3) of the imidazole ring, rather than at the C^4 and C^5 positions, to help understand the potential anti-proliferative activity and aqueous solubility of these imidazolium salts. 4,5-Diphenylimidazole was functionalized with quinolinylmethyl and (6-methoxynaphthyl)methyl substituents, in addition to the

naphthylmethyl group to create a series of six 4,5-diphenyl-*N*,*N*'-bis(arylmethyl)imidazolium salts **12 – 17**. The synthesis of compounds **12 – 17** were executed by the same route used to obtain the analogous imidazolium salts.[6] Compounds **12 – 17** were prepared by stirring 4,5-diphenylimidazole with a minimum of 1.2 equivalents of potassium hydroxide and one equivalent of the appropriate alkyl bromide or chloride in refluxing acetonitrile overnight. Removal of the generated precipitate and the addition of one equivalent of the naphthalene-based halide in refluxing acetonitrile produced compounds **12 – 17** (Scheme 4).



	R^1	R^3	X
12	Naph	Naph	Br
13	NapOMe	NapOMe	Cl
14	NapOMe	Naph	Br
15	Quin	NapOMe	Cl
16	Quin	Naph	Br
17	Quin	Quin	Br

Scheme 4. Synthesis of the 4,5-diphenyl-*N*,*N*'-bis(arylmethyl)imidazolium salts **12 – 17**.

All 4,5-diphenyl-*N*,*N*'-bis(arylmethyl)imidazolium salts **12 – 17** were characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry, elemental analysis and melting point determination. The ^{1}H NMR spectra of compounds **12 – 17** show a representative resonance for the C^2-H imidazolium proton within the range of 9.77 – 9.88 ppm and was a significant downfield shift from the C^2 proton resonance of the 4,5-diphenylimidazole starting material (7.78 ppm). The number of chemical resonances in the ^{13}C NMR spectra fully support the series of 4,5-diphenyl-*N*,*N*'-bis(arylmethyl)imidazolium salts **12 – 17**. ESI-mass spectrometry in the positive mode was conducted for the salts **12 – 17** and the corresponding $[M\text{-}Br]^+$ and $[M\text{-}Cl]^+$ of the salts further suggested compound identification of the desired products. The structures of compound **16** and **17** were determined by single crystal X-ray diffraction, following the slow evaporation of a

concentrated solution of the compounds in a water/acetone (1:1) mixture and ethanol, respectively (Figure 6 and Figure 7). Compound **16** co-crystallized with a water molecule and **17** co-crystalized with an ethanol solvent molecule.

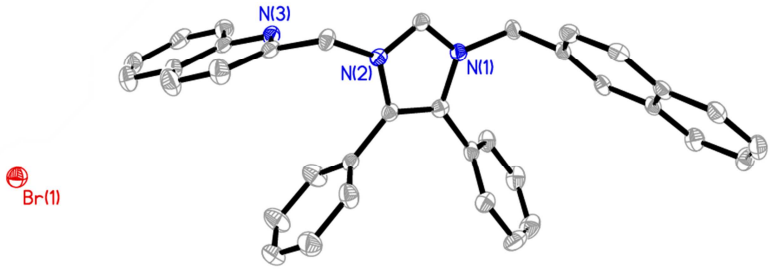


Figure 6. Thermal ellipsoid plot of **16** from solvate **16•(H_2O)**. All thermal ellipsoids have been drawn at the 50% probability level. Hydrogen atoms, carbon labels, and the co-crystalized water molecule have been removed for clarity.

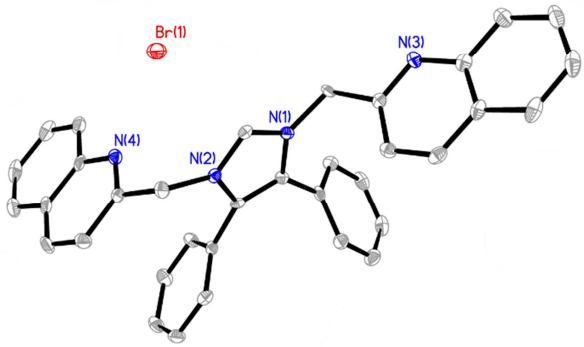


Figure 7.Thermal ellipsoid plot of **17** from solvate **17•(C_2H_6O)**. All thermal ellipsoids have been drawn at the 50% probability level. Hydrogen atoms, carbon labels, and the co-crystalized ethanol molecule have been removed for clarity.

2.2 In vitro Anti-tumor Studies

The in vitro anti-tumor activity of compounds **2**, **3**, **6a-c**, **10** and **11** - **17** was evaluated against the NSCLC cell lines NCI-H460, NCI-H1975, and HCC827 in order to establish two structure-activity relationships (SAR). The first SAR study considers compounds **2**, **3**, **6a-c**, **10**,

11, and **12**. Each of these compounds has different functional groups at the C^4 and/or the C^5 position(s). These various functional groups contain a wide range of hydrophobic to hydrophilic functional groups. The second SAR study involves compounds **12 - 17**. Each compound is functionalized with phenyl substituents at both the C^4 and C^5 positions with varying arylmethyl groups at the N^1 and N^3 positions. Previously we have shown that naphthylmethyl substituents at the N^1 and N^3 positions yield compounds with high anti-cancer activity. However, these highly hydrophobic substituents do not allow for sufficient water solubility for systemic administration. Therefore, the naphthalene substituents were varied by adding a nitrogen heteroatom to the ring and/or an ether substituent off the ring in attempt to increase water solubility without lessening the anti-cancer activity.

The optional MTT assay protocol was used to determine the IC_{50} values (the concentration of the compound that inhibits 50% of the growth of cells, relative to controls) of each compound (Table 1). The well-known chemotherapy agent cisplatin was used as a positive control in each assay with IC_{50} values of 3 μM (H460), 10 μM (H1975), and 4 μM (HCC827). Although **IC23** had activity comparable to that of cisplatin, with IC_{50} values of 5 μM (H460), 6 μM (H1975), and 8 μM (HCC827), potential use of the compound as a drug is limited by poor water solubility. Of the urocanic acid-derived imidazolium salts, compound **2** proved to be the most active against the NSCLC cell lines, with IC_{50} values of 13 μM (H460), 4 μM (H1975), and 16 μM (HCC827). The conversion of the ester functionality in **2** to the carboxylic acid group in **3** led to a severe reduction in efficacy, as the IC_{50} values of **3** were > 30 μM for all three cell lines.

The imidazolium salts **6a-c** derived from histamine all showed comparable activity (IC_{50} values between 8 -14 μM) against all three cell lines, with the exception of **6a** against the HCC827 line (IC_{50} > 30 μM). As expected, the least hydrophobic compound of the three, **6a**, was the only compound to fully dissolve in the 1% DMSO aqueous stock solution.

Compound **10**, which was designed to have a relatively high aqueous solubility and the most hydrophilic compound tested, was ineffective against the H460 and HCC827 lines (IC_{50} > 30 μM) and showed only moderate activity against the H1975 line (IC_{50} = 11 μM). Compound **10**, which fully dissolved in the 1% DMSO aqueous stock solution, demonstrated a lack of significant anti-proliferative activity against the majority of the cell lines, similar to compound **3**. The data from **3** and **10** suggests that the inclusion of polar hydrophilic groups at

the C^4 and C^5 positions of imidazole leads to a substantial decrease in anti-proliferative activity of those compounds.

Supporting this observation, **11** and **12**, which did not contain any polar, hydrophilic substituents, demonstrated high anti-proliferative activity against all three cell lines. Compound **11** was fully soluble in the 1% DMSO aqueous stock solution and had IC_{50} values of 3 μM (H460 and H1975) and 4 μM (HCC827). Compound **12** had IC_{50} values of 3 μM (H460), < 1 μM (H1975), and 2 μM (HCC827), displaying activity comparable to that of **11**, despite not being fully soluble in the stock solution. It is also important to note that **11** and **12** had in vitro activity comparable to cisplatin and **IC23**.

Compounds **2**, **3**, **6a-c**, **10**, **11**, and **12** were all substituted with naphthylmethyl groups at both the N^1 and N^3 positions and differences between the IC_{50} values for these compounds establishes our first SAR. The most active compounds were **11** and **12** which have added lipophilicity at the C^4 and/or C^5 positions. Compound **2** with an ester functional group has higher activity than **3** with a carboxylic acid group. Compounds **6a-c** with a carbamate functional group all had moderate activity while **10** with two hydroxyl groups had the lowest activity observed. The conclusion from this series of compounds is that added lipophilicity is beneficial to high anti-cancer activity while highly hydrophilic groups such as hydroxyls severely inhibits activity.

The second SAR study concerns compounds **12-17** which all have phenyl substituents at the C^4 and C^5 positions while the arylmethyl groups at the N^1 and N^3 positions were varied. Imidazolium salts **12 – 17** displayed IC_{50} values in the single-digit micromolar or high nanomolar range against all three of the NSCLC cell lines (IC_{50} < 1 – 3 μM). The anti-proliferative activities of the 4,5-diphenyl-*N,N'*-bis(arylmethyl)imidazolium salts **12 – 17** were an improvement relative to both **IC23** and cisplatin. The variants of the naphthylmethyl-based substituents at the N^1 and N^3 atoms appeared to have no effect on the IC_{50} values of the 4,5-diphenyl compounds. However, the solubility of **17**, which contains two quinolinylmethyl groups, was increased considering this was the only 4,5-diphenyl derivative to be solubilized by the 1% DMSO solution, though its solubility in pure water is still too low to be systemically administered. Summarization of the second SAR study suggests that adding heteroatoms to the naphthylmethyl rings can increase the aqueous solubility and maintain the potent anti-cancer activity of the more lipophilic derivatives. The combined data from salts **2**, **3**, **6a-c**, **10** and **11 – 17** further affirmed the direct relationship between the hydrophobicity of the substituents and the

anti-proliferative activity of the imidazolium salts. Hydrophilic substituents at the C^4 and C^5 positions provided a more pronounced effect to the imidazolium salt's anti-tumor activity and aqueous solubility, as compared to the N^1 and N^3 positions.

Table 1. IC_{50} values of synthesized *N,N'*-bis(arylmethyl)imidazolium salts **2**, **3**, **6a-c**, **10** and **11 – 17**.

Compound	**IC_{50} values (μM)**		
	NCI-H460	NCI-H1975	HCC827
cisplatin	3	10	4
IC23	5	6	8
2	13	4	16
3	> 30	> 30	> 30
6a	9	11	> 30
6b	11	8	12
6c	11	10	14
10	> 30	11	> 30
11	3	3	4
12	3	< 1	2
13	2	2	2
14	2	< 1	< 1
15	3	< 1	2
16	2	< 1	2
17	3	< 1	2

The National Cancer Institute's (NCI) Developmental Therapeutics Program (DTP) agreed to test **11** and **12** in the NCI-60 human cancer cell line screen using a one-dose assay and a five-dose assay, in tandem with our own MTT viability assays. The NCI-60 human cancer cell screen contains nine NSCLC cell lines. Results from the one-dose assay for **11** and **12** are summarized in Table 2. In this assay the compounds were exposed to the 60 cell lines at one concentration (10 μM). Results were given as a growth percentage of cells treated with **11** and **12** compared to growth of control cells. Compound **11** was lethal against two cell lines tested (HOP-

92 and NCI-H522) defined as leaving fewer live cells after treatment with **11** than before treatment began. Combining the results of **11** from all nine cell lines showed an average growth of 27% meaning **11** significantly slowed the growth of the nine NSCLC cell lines at a concentration of 10 µM. Compound **12** proved more potent, as it was lethal against all nine cell lines and had a negative average growth rate of 70.81%.

Table 2. Results from the one-dose assay (10 µM) performed by the DTP. All cell lines in the table are NSCLC cell lines. Values are percent growth relative to control cells. Negative values depict the compound was lethal at the concentration tested.

Compound	Growth percentage								
	A549/ ATCC	EKVX	HOP-62	HOP-92	NCI-H226	NCI-H23	NCI-H322M	MCI-H460	NCI-H522
11	60.54	38.14	33.16	-20.86	31.78	27.66	65.66	21.86	-17.86
12	-81.83	-46.67	-86.12	-83.90	-75.79	-74.86	-20.79	-78.57	-85.76

In the five-dose assay, compounds were exposed to all 60 cell lines at concentrations of 10 nM, 100 nM, 1 µM, 10 µM, and 100 µM. Results were given as concentrations of 50% growth inhibition (GI50), total growth inhibition (TGI), and 50% lethal concentration (LC50) relative to cell growth with no drug added. Results of the five-dose assay for **11** are shown in Table 3 and Figure 8. The GI50 concentration ranges from the mid nanomolar range (448 nm) to the low µM range (5.05 µM), the TGI concentration ranges from 2.02 µM to 18.7 µM, and the LC50 concentration ranges from 6.49 µM to > 100 µM. These results are consistent with the IC_{50} values determined in our lab and give further evidence that **11** has potent anti-proliferative effects against NSCLC and is a quality candidate for further studies.

Table 3. Results of 5-dose study for compound **11**. Values are concentration in molarity for the growth inhibition 50% (GI50), total growth inhibition (TGI), and lethal concentration 50% (LC50).

Cell line	Concentration (μM)		
	GI50	TGI	LC50
A549/ATCC	5.05	18.7	53.5
EKVX	2.15	9.71	43.8
HOP-62	1.61	3.80	8.97
HOP-92	0.448	2.02	6.49
NCI-H226	2.12	5.87	> 100
NCI-H23	1.49	3.97	12.5
NCI-H332M	2.87	10.3	33.7
NCI-H460	1.72	3.65	7.75
NCI-H522	1.41	3.06	6.63

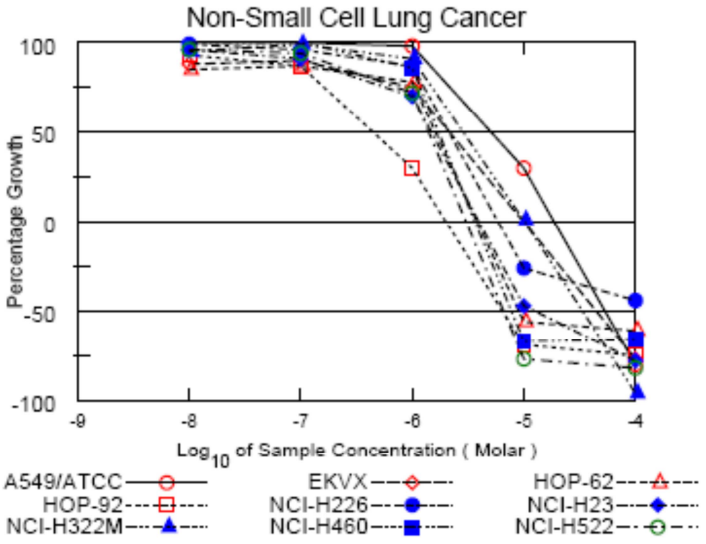


Figure 8. Dose chart for compound **11** against nine NSCLC cell lines.

The five-dose results for **12** are shown in Table 4 and Figure 9. The GI50 concentration ranges from the low nanomolar range (122 nm) to the low μM range (1.41 μM), the TGI concentration ranges from 1.26 μM to 3.16 μM, and the only LC50 concentration observed was

for the HOP-92 cell line at a concentration of 3.98 µM. Strangely, the cell growth percentage was higher at the 100 µM dose than the 10 µM dose for all NSCLC cell lines (Figure 9). No explanation was given to us by the DTP and we cannot explain this trend. Totality of these results give further evidence of high anti-cancer activity previously demonstrated by this compound qualifying it as a prime candidate for further studies. Experimental procedures for the one-dose and five-dose assays can be found on the DTP website and were based on the concentration of protein at the examined time points. Results from all other cell lines tested including figures and the tables provided by the DTP are included in the supplemental information.

Table 4. Results of 5-dose study for compound **12**. Values are concentration in molarity for the growth inhibition 50% (GI50), total growth inhibition (TGI), and lethal concentration 50% (LC50).

Cell line	Concentration (M)		
	GI50	TGI	LC50
A549/ATCC	1.00	2.48	N/A
EKVX	0.837	2.29	N/A
HOP-62	1.41	2.97	N/A
HOP-92	0.122	1.26	3.98
NCI-H226	1.20	3.16	N/A
NCI-H23	0.445	1.88	N/A
NCI-H332M	1.20	2.50	N/A
NCI-H460	0.384	1.65	N/A
NCI-H522	0.325	1.78	N/A

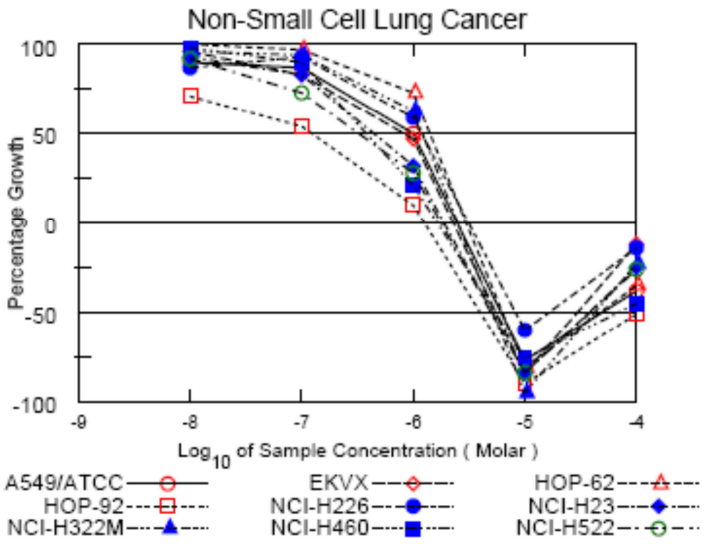


Figure 9. Dose chart for compound **12** against nine NSCLC cell lines.

2.3 Annexin V Assay

Compounds **11** and **12** were also studied using the Annexin V assay to determine the mode of cell death that NCI-H460 cells undergo when exposed to these compounds. In cells that are undergoing apoptosis, phosphatidylserine is translocated to the outer leaflet of the cell membrane resulting in a loss of both cell integrity and cell asymmetry. At the outer leaflet, the phosphatidylserine can interact with Annexin V. The Annexin V is conjugated to FITC which can be visualized by fluorescence microscopy. A secondary stain, propidium iodide, is also used which fluoresces red when interacting with DNA. This interaction is only seen in cells whose membrane integrity is lost, as propidium iodide cannot cross intact cell membranes.[13]

The Annexin V assay employed in these in vitro studies has the ability to distinguish between apoptosis and necrosis by use of the propidium iodide. As is well known, apoptosis is programmed cell death whereas necrosis is a disordered form of cell death. The progression over time of observed fluorescence in conjunction with changes in cell membrane morphology indicates the specific mode of cell death. In the case of apoptosis, cell membrane integrity is not immediately compromised as the phosphatidylserine is translocated to the outer leaflet. This corresponds to an observation of green fluorescence with the absence of any red fluorescence from the propidium iodide. At longer treatment time points, apoptosis is observed as both green and red fluorescence with the presence of a blebbing membrane. In contrast to this is necrosis,

which exhibits both green and red fluorescence independent of the treatment time. This is due to the swelling and eventual breakdown of the cell membrane, which is permeable to propidium iodide even at early time points. The progression in time as well as cell morphology is what can assist in distinguishing these two modes of cell death in this assay. However, due to ambiguity between identification of cells in the late stage of apoptosis or those that are necrotic, further studies can be completed in order to validate the Annexin V assay.

Compound **11** is a highly lipophilic compound and has poor solubility in aqueous solution. Previously, we have described lipophilic imidazolium salts that could be solubilized by a chemical excipient, 2-hydroxypropyl-β-cyclodextrin (2-HPβCD).[14] In vitro MTT assays suggested there was no difference in the anti-cancer activity of compounds solubilized by the 1% DMSO solution described above or solubilization by the vehicle, 2-HPβCD. Therefore, compound **11** was dissolved in a 20% (w/v) solution of 2-HPβCD and exposed to cells at a concentration of 40 μM for 12, 14, 17, and 20 hour time points. At the end of the treatment periods, the Annexin V apoptosis detection kit was utilized and cells were visualized by fluorescence microscopy (Figure 10). Images were compared to cells exposed to only the 2-HPβCD vehicle solution and cells treated with cisplatin. There was minimal fluorescence from cells treated with vehicle which is expected. A significant amount of fluorescence and blebbing was observed from cells treated with cisplatin as this compound is known to induce apoptosis. Signs of apoptosis including green fluorescence of the cell membrane, blebbing, and staining of the nucleus from propidium iodide were observed at all the time points for cells treated with **11** (Figure 11). However, not all cells were in the same stage of cell death as indicated by the progression of fluorescence in individual wells. At the 12 hour time point, a fraction of the cells were visualized with green fluorescence only while some had green and red fluorescence together and the rest had no fluorescence. At the 17 and 20 hour time point, a significant percentage of the cells are both green and red fluorescent, show blebbing and thus suggesting apoptosis. We predicted **11** to induce an apoptotic mode of cell death considering the structure is similar to previously reported imidazolium salts that also induce apoptosis in NSCLC cells.[6]

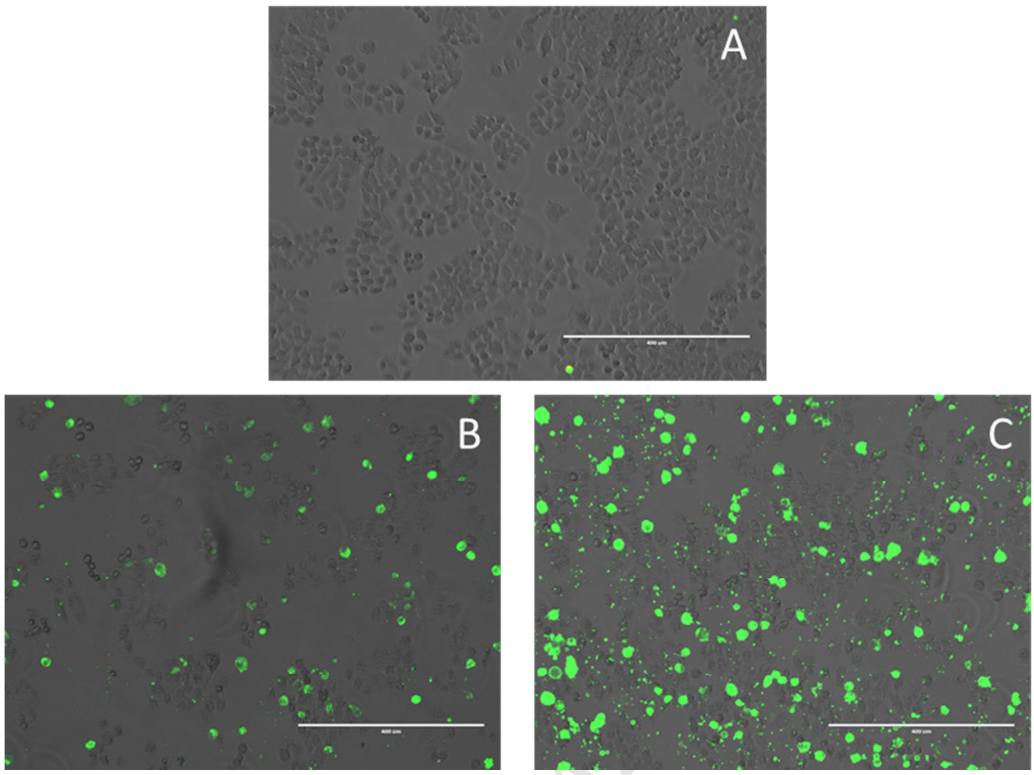


Figure 10. Images of the Annexin V assay on H460 cells grown in 6-well plates using compound **11** in 20% (w/v) 2-HPβCD aqueous solution as the compound treatment. All images were taken using a 10x objective. Images are presented as a merged image of the normal transmitted light, green fluorescence and red fluorescence figures. (A) 2-HPβCD control, 20 hours. (B) Compound **11**, 14 hours. (C) Compound **11**, 20 hours. Scale bars equal 400 μm.

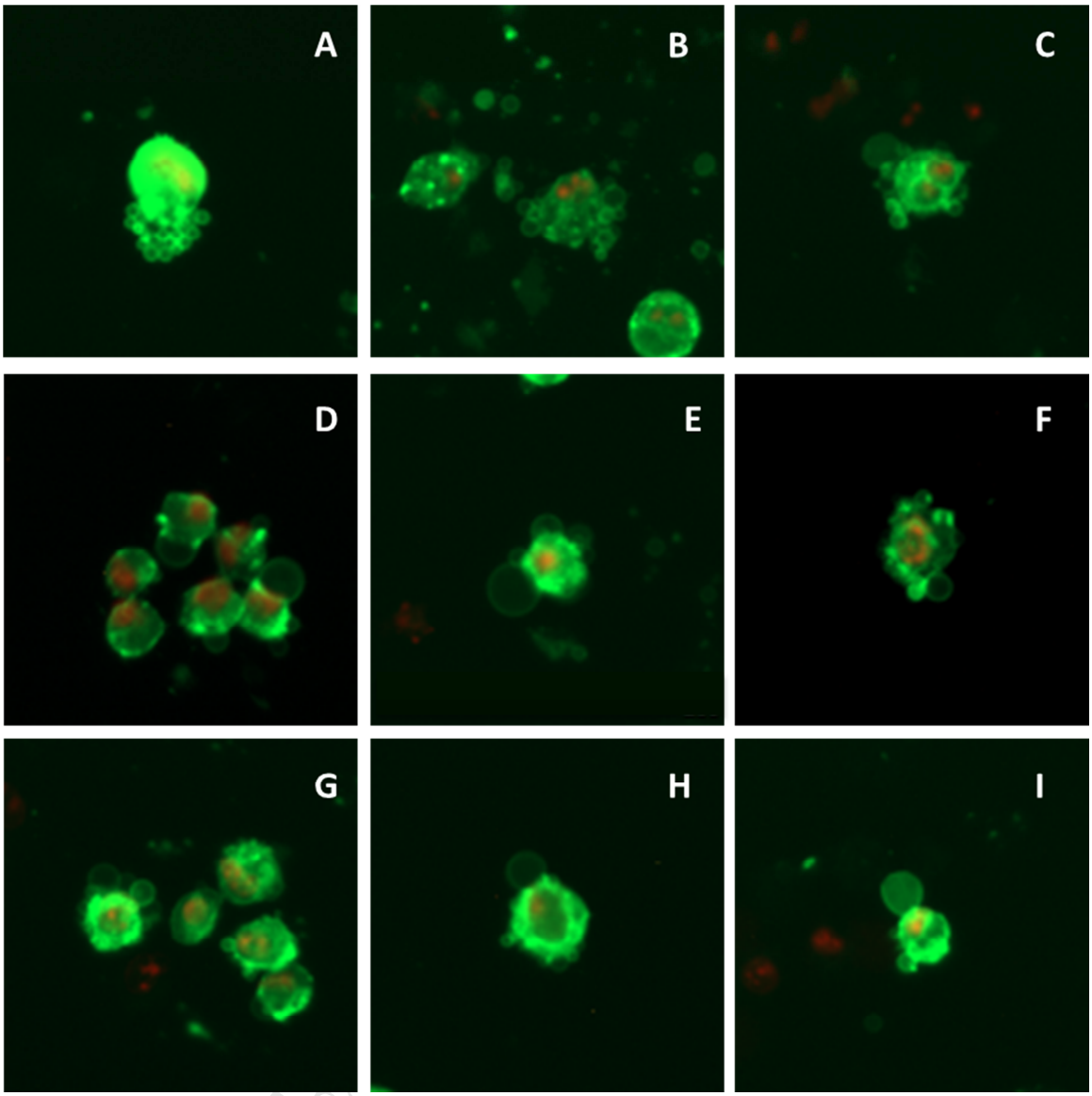


Figure 11. Images of the Annexin V assay on H460 cells grown in 6-well plates using compound **11** in 20% (w/v) 2-HPβCD aqueous solution as the compound treatment. All images were taken using a 20x objective. Images are presented as a merged image of the green fluorescence and red fluorescence figures, omitting the normal transmitted light image for blebbing clarity. Images A-C were taken at the 20 hour time point, D-E were taken at the 17 hour time point, F-G were taken at the 14 hour time point, and images H-I were taken at the 12 hour time point.

Compound **12** was dissolved in a 1% DMSO solution, because it could not be solubilized by the 2-HPβCD vehicle solution, and H460 cells were exposed at a concentration of 40 µM for

1, 3, 6, and 12 hour time points. The vehicle control and cisplatin positive control also contained the same percentage of DMSO as the cells treated with **12**. Again, the Annexin V assay detection kit was utilized. Minor green fluorescence was observed with cells treated with vehicle, while early signs of apoptosis are observed in cells treated with cisplatin as would be expected at the **12** hour time point (Figure 12). Strong green fluorescence is observed as early as the 1 hour time point and continues to be observed at all of the later time points in cells treated with **12**. However, cells treated with **12** were abnormal when compared to the controls, especially in that the morphology of the membrane is vastly different. No blebbing was observed at any of the time points and green fluorescence as well as red fluorescence were observed at all time points. This suggests that compound **12** induces a necrotic cell death pathway versus an apoptotic cell death pathway as observed with **11** (Figure 13). While observation of membrane morphology and the progression of fluorescence can give great insight into the mode of cell death, future work will also incorporate other assays that can confirm the findings of the Annexin V assay.

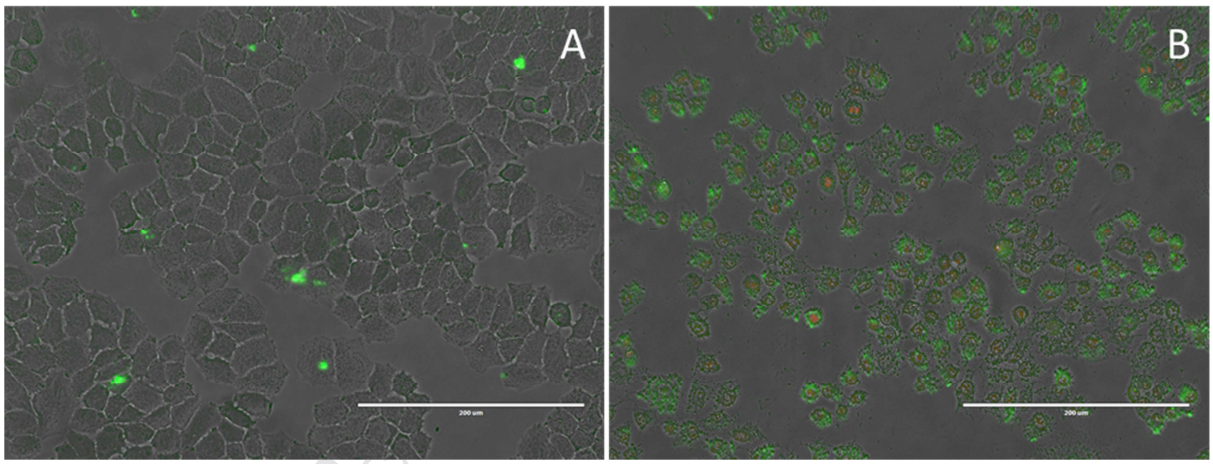


Figure 12. Images of the Annexin V assay on H460 cells grown in 6-well plates using **12** dissolved in a 1% DMSO aqueous solution as the compound treatment. All images were taken using a 20x objective. Images are presented as a merged image of the normal transmitted light, green fluorescence and red fluorescence figures. (A) DMSO control, 12 hours. (B) Compound **12**, 12 hours. Scale bars equal 200 μm

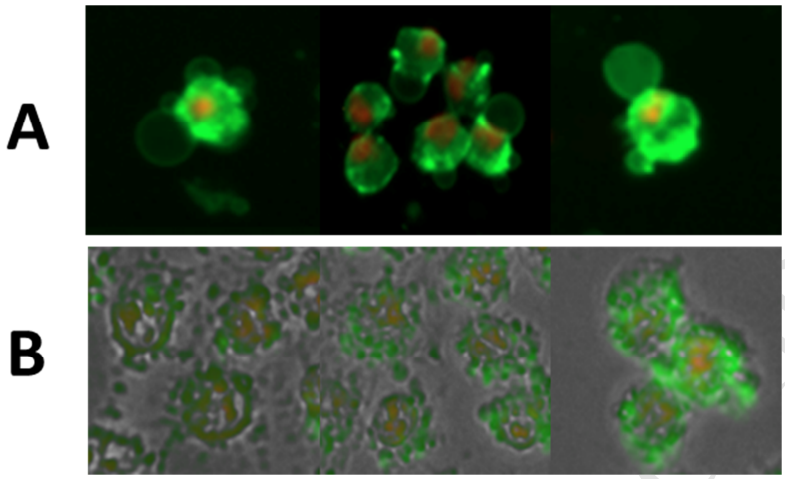


Figure 13. Image depicting the comparison of compound **11** versus compound **12** treated cells. (Row A) H460 blebbing cells after treatment with compound **11** (normal transmitted light image omitted for blebbing clarity). (Row B) H460 cells after treatment with compound **12**.

The time frame differences of the Annexin V studies on **11** and **12** arise from the use of different solubilizing agents. We have noted previously that the use of 2-HPβCD does not change activity by MTT assay analysis. However, the treatment period and compound concentrations used for the MTT assay are greatly different from those used for the Annexin V assay. Previous studies have shown that at the same concentration, solubilization in 2-HPβCD delays the effects of imidazolium salts which we believe comes from the equilibration time needed for the compound to be released from the lipophilic core of 2-HPβCD before it can exert its effect on the cancer cells.[14] This equilibration time leads to each cancer cell being at a different stage of cell death when imaging occurs. This can be seen where some cells have no fluorescence while others have either green fluorescence or green paired with red fluorescence (see Suppmental Information).

2.4 In vivo toxicity study

Considering **11** had high anti-cancer activity and it could be completely solubilized in aqueous solution by 2-HPβCD at high concentrations (whereas 12 could not be solubilized by 2-HPβCD), **11** was chosen for a preliminary analysis of the in vivo toxicity using C57BL/6 mice. Six week old C57BL/6 male mice were allowed to acclimate in their cages (n = 3 mice per group, each group housed in separate cages) for 5 days. Following acclimation, the control group

received 100 µL of vehicle (20% (w/v) 2-HPβCD) solution by intraperitoneal (IP) injection while the experimental group received 100 µL of a 20 mg/kg dose of **11** (assuming an average weight of 20 g) dissolved in the vehicle solution by IP injection on day 0. The weights and health of all animals were closely monitored for the next 6 days (Figure 14). There was a steady increase in weight for all control animals. All animals survived the initial injection of **11**, but a sharp decrease in weight occurred for animals treated with **11**. However, this was followed by complete weight recovery and weight gain by 66% of animals by day six. Animals in both groups were again injected on days 0, 7, 14, 21, 25, and 29 with similar patterns concerning weight loss and gain. All mice survived for the duration of the study and the last injection, on day 29, was given one hour before perfusion.

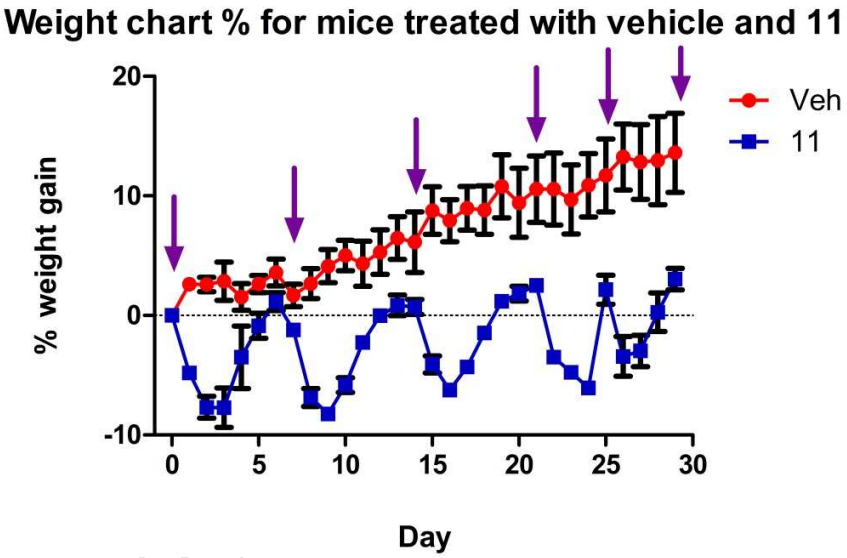


Figure 14. Weight chart for C57BL/6 mice injected with **11** dissolved in vehicle solution. Red arrows signify days the animals were injected.

Selected reaction monitoring mass spectrometry (SRM-MS) studies were performed on extraction samples from the brain, heart, lung, liver, kidney, and spleen of all mice. The highest concentration of **11** was found in the liver, followed closely by the kidney and spleen (Figure 15). Accumulation was also observed in the heart, lung, and brain but at lower concentrations.

Minimizing side effects is a major goal of potential chemotherapeutics considering current treatments such as cisplatin have severe side effects, especially renal toxicity. Although **11** did show accumulation in the kidney, collectively, **11** was less cytotoxic against the panel of renal cancer cells than the panel of lung cancer cells tested in the NCI-60 cell line assays (see supplemental information). Results from the in vivo toxicity study of compound **11** were very promising. All animals survived and although animals lost weight after each injection, they were able to fully recover during rest periods in-between injections. Compound **11** is a promising candidate to move on to further in vivo studies with lung cancer models.

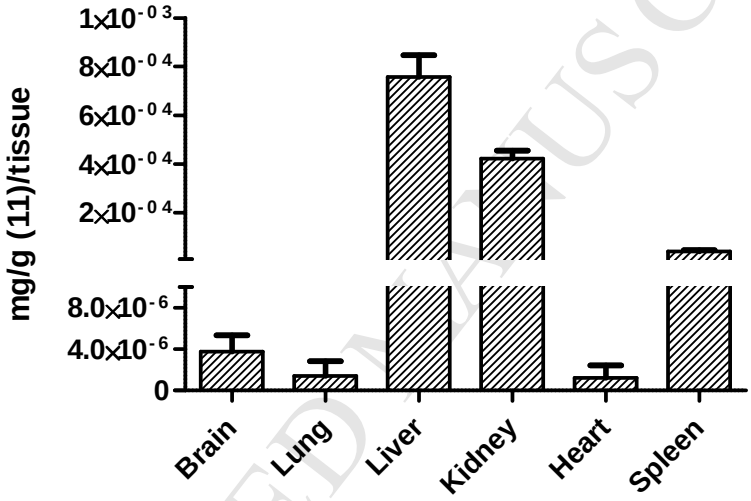


Figure 15. Bar graph depicting the concentration of **11** deposited in the brain, lung, liver, kidney, heart, and spleen as determined by selective reaction monitoring.

3. Conclusion

In this work, two in vitro SAR studies were performed to establish a route to increase the solubility of highly active imidazolium salts without reducing the activity. The first SAR study included a series of *N,N'*-bis(naphthylmethyl)imidazolium salts with varied hydrophilic and hydrophobic substituents at the C^4 and C^5 positions. Compounds **2** and **6a-c**, all containing moderately hydrophobic substituents in the C^4(C^5) position(s), displayed IC_{50} values between 8 – 16 μM, with the exception of **6a** against the HCC827 line. Although less efficacious than cisplatin, these values are in a clinically achievable range and compounds **2** and **6a** were fully soluble in the 1% DMSO aqueous stock solution. In addition, the propargyl group of **6c** provides

a site for the potential coupling of targeting and/or imaging moieties bearing an azide group through 'click chemistry'.[15] The modification would presumably enhance the effectiveness of the compound in vivo.

The compounds with highly polar, hydrophilic groups, **3** and **10,** showed almost no significant anti-proliferative effects. With the exception of **10** against the H1975 line, all IC_{50} values for these complexes were > 30 μM against the three cell lines. Conversely, compounds **11** and **12** with highly hydrophobic substituents demonstrated activity comparable to that of cisplatin, with IC_{50} values of 4 μM or less against all cell lines tested. Results from the in vitro MTT assays on compounds **2**, **3**, **6a-c**, **10**, **11**, and **12** strongly suggests that the hydrophobic substituents are necessary for high anti-proliferative activity against these NSCLC cell lines. Compound **11** and **12** were also studied by the NCI's DTP with the NCI-60 human cancer cell line panel. Both compounds were active against the nine NSCLC lines tested while **12** was highly cytotoxic against almost all 60 cell lines. Compound **11** was shown to induce cell death by an apoptotic pathway while **12** induced a necrotic cell death pathway. Considering **11** had high solubility when dissolved in an aqueous 2-HPβCD solution and high-anticancer activity, it was chosen for an in vivo toxicity study where it showed a favorable side effect profile. Although compound **11** was solubilized by 2-HPβCD, the aqueous solubility of potential therapeutics is still of high importance, and future work should focus on increasing the water solubility of compounds by means other than inclusion of highly polar substituents, while still maintaining anti-proliferative activity.

The second SAR study involved the 4,5-diphenyl-*N,N'*-bis(arylmethyl)imidazolium salts **12 – 17**. The heteroatoms added to the aryl groups in **13**-**17** proved to add little solubility to the highly lipophilic derivative **12**. However, **17,** with two quinolylmethyl moieties, was solubilized in the 1% DMSO aqueous solution. Unfortunately, its solubility was limited without the use of the DMSO vehicle. This second SAR study suggests that heteroatoms can be added to the aryl groups at the N^1 and N^3 positions without reducing activity. Therefore, future derivatives will focus on utilizing the quinolylmethyl and methoxynaphthylmethyl groups to increase solubility. It may also be possible to increase solubility by protonating the nitrogen of quinoline substituents.[16] However, it is unknown if that will alter the anti-cancer activity of the compound and resulting pharmacokinetics considering potential differences in pH. Imidazolium salts with a combination of satisfactory solubility and high anti-proliferative activity are still actively

investigated and future work should concentrate on substituents with multiple heteroatoms or other hydrophilic groups.

4. Experimental

4.1 General Considerations

All reactions were carried out under aerobic conditions unless otherwise specified. All acids, bases and solvents were purchased from Fisher Scientific and used without further purification. All reagents were used as received without further purification. Histamine dihydrochloride, urocanic acid, N,N'-carbonyldiimidazole (CDI), *N*,*N*–diisopropylethylamine (DIPEA), 4,5-diphenylimidazole, 4,5-imidazoledicarboxylic acid, 4-methylimidazole and primary alcohols were purchased from Alfa Aesar. 2-(Bromomethyl)naphthalene was purchased from Waterstone Technologies. 2-Hydroxypropyl-β-cyclodextrin (average molecular weight: 1400) was purchased from Tocris (a Biotechne brand). The TACS MTT cell proliferation assay kit was purchased from Trevigen. All C57BL/6 mice were purchased from Charles River Laboratories and housed in the biology department at the University of Akron. The FITC annexin V apoptosis detection kit was purchased from BD Pharmingen. HPLC grade (≥99.9%) acetonitrile, methanol, and water used for extractions and LC-MS analysis were purchased from Fisher Scientific (Fair Lawn, NJ, USA). 2-(Chloromethyl)-6-methoxynaphthalene was synthesized according to literature procedure.[17] Melting points were obtained on a MelTemp apparatus. Methyl 3-(imidazol-4-yl)acrylate (**1**) was synthesized according to literature procedures and used without further purification.[7,8] Dimethyl imidazole-4,5-dicarboxylate (**7**) was synthesized according to literature procedure and used without further purification.[12] Column chromatography utilized silica gel (60 Å, ICN Medicals) embedded with a green 254 nm fluorescent indicator (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 300 or 500 MHz instrument referenced to DMSO-d_6 (2.50 ppm, 39.51 ppm) or CD_3OD-d_4 (3.31 ppm, 49.00 ppm, respectively). Electrospray ionization mass spectrometry (ESI-MS) or high resolution mass spectrometry (ESI-HRMS) was performed by the University of Akron mass spectrometry laboratory. Elemental analysis was performed by the microanalysis laboratory at Atlantic Microlabs (Atlanta, GA) and the University of Akron Department of Geology.

The human non-small cell lung cancer cell lines NCI-H1975 and HCC827 were donated by Dr. Daniel 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.

4.2 X-ray Structure Determination Details

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 SMART APEX I 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 sets were collected using SMART or APEX II software packages. All data sets were processed using the APEX II software suite.[18,19] The data sets were integrated using SAINT.[20] An empirical absorption correction and other corrections were applied to the data sets using multi-scan SADABS.[21] Structure solution, refinement, and modelling were accomplished by using the Bruker SHELXTL package.[22] The structures were determined by full-matrix least-squares refinement of F^2 and the selection of the appropriate atoms from the generated difference map. Hydrogen atom positions were calculated and $U_{iso}(H)$ values were fixed according to a riding model.

4.3 MTT Assay Protocol

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 μM. 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 μL) was added to each well and cells were incubated for 3-4 h depending on the cell line. Medium was removed by vacuum and DMSO (100 μL) 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.

4.4 Annexin V assay protocol

NCI-H460 cells were grown to confluency and plated in 6-well plates at a concentration of 100,000 cells per well. Cells were incubated for 24 hours. At this time, medium was removed by vacuum and fresh medium with vehicle, cisplatin, **11**, or **12** was added. Cisplatin, **11,** and **12** were dissolved at a concentration of 40 µM. Compound **11** was exposed to cells for 12, 14, 17, and 20 hours, while the cisplatin and vehicle control were exposed to cells for 20 hours. Compound **12** was exposed to cells for 1, 3, 6, and 12 hours, while the cisplatin and vehicle control were exposed to cells for 12 hours. At the end of the treatment period, medium was removed and cells were washed twice with cold PBS (1.5 mL). 1X binding buffer (400 µL), FITC annexin V (20 µL), and propidium iodide (20 µL) was added to each well and plates were incubated in the dark for 20 minutes at room temperature. The binding buffer was removed and fresh 1X binding buffer (1 mL) was added for imaging. Cells were imaged on an EVOS fl Digital Inverted Microscope with 10X and 20X objectives.

4.5 In vivo toxicity study protocol

All animal procedures were reviewed and approved by the Institutional Care and Use Committee at the University of Akron. Eight week old male C57BL/6 mice were obtained from Charles River laboratories. Animals were housed in a 12h light/dark cycle and food and water were provided ad libitum. Prior to the toxicity testing, animals were allowed to acclimate for 5 days. Mice received injections on days 0, 7, 14, 21, and 25 that consisted of a 100 µL solution of a 20% 2HPβCD sterile PBS solution (control) or 100 µL of a 4 mg/mL 20% 2HPβCD sterile PBS solution (0.4 mg/ 100 µL or ~ 20 mg/kg assuming 20 g mice) of **11** by IP injection. Animals were closely monitored and weighed on a daily basis. On day 29 animals were sacrificed and the lung, heart, liver, kidney, spleen, and brain were harvested and frozen for later use.

4.6 Tissue extraction and selective reaction monitoring method

To extract and analyze compound deposited in the collected organs, cold methanol (0.2 mL) was added to a centrifuge tube containing the tissue sample of interest. The sample was vortexed, frozen in liquid nitrogen, thawed at room temperature, and sonicated. This series of steps was repeated twice more. The samples were frozen at -20 °C for one hour and centrifuged at 10,000 rpm for 5 minutes at 4 °C. The supernatant was separated from the protein pellet. The protein pellet was extracted by the same procedure outlined above and the resulting supernatants

were combined (the protein pellet was kept for BCA analysis). The samples were concentrated on a speed vac and the resulting residue was resuspended in methanol (50 µL). Samples were processed using an Eksigent micro200 LC equipped with a hydrophilic interaction liquid chromatography column (Luna 3µ NH2 100Å, 150mm×1.0mm, Phenomenex, Torrance, CA, USA) coupled to a 5600+ TripleTOF (Sciex). The mobile phases for separation consisted of water (A) and acetonitrile (B) and the gradient proceeded at a flow rate of 30 uL/min as follows: 0 min 95% B, 2 min 95% B, 20 min 5 % B, 22 min 5 % B. For the mass spectrum the ionspray voltage was set to +5000 V with a declustering potential set at +100. The ion source nebulizer gas and heater gas were both set at 18 psi, and the curtain gas was set to 25 psi. A TOF scan was performed over the mass range of 100-500 Da and a subsequent product ion scan of 363.18 was collected over a range of 50-500 Da using a collision energy of +25 V. The extracted ion chromatogram (XIC) of transition *m/z 363.18→ m/z 141.06* and the MS/MS fragmentation pattern were both use to verify the identify **11**. A standard curve based on peak height versus concentration of standards was constructed to interpolate the tissue extract concentrations.

4.7 Synthetic Procedures

*4.7.1 Synthesis of 4-(3-methoxy-3-oxoprop-1-en-1-yl)-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**2**)*

Compound **1** (0.50 g, 3.29 mmol) was stirred with potassium carbonate (0.64 g, 4.63 mmol) in acetonitrile (4 mL) and the mixture was refluxed for 30 min. 2-(Bromomethyl)naphthalene (0.73 g, 3.30 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white precipitate, presumed to be potassium bromide. 2-(Bromomethyl)naphthalene (0.73 g, 3.30 mmol) was added to the filtrate, and the reaction mixture was refluxed overnight. A white precipitate resulted, which was filtered via vacuum filtration and the white solid was stirred with diethyl ether. The solid product was isolated via vacuum filtration, washed in the funnel with diethyl ether and air-dried to yield a crème powder. The solid was purified by column chromatography using a mobile phase of methanol/dichloromethane (5:95) to yield a white powder **2** (1.52 g, 89%). Mp: 145–147 °C. HRMS (ESI^+) calcd for $C_{29}H_{25}N_2O_2^+$ $[M\text{-}Br]^+$ of m/z = 433.1916, found m/z = 433.1953. ^1H NMR (500 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.55 (m, 1H), 8.03-7.86 (m, 8H), 7.59-7.46 (m, 7H), 6.70 (d, 1H, J = 14.4 Hz), 5.84 (s, 2H), 5.65 (s, 2H), 3.66 (s, 3H). $^{13}C\{^1H\}$ NMR (125 MHz,

DMSO-d_6) δ 165.9, 139.0, 133.3, 133.20, 133.15, 132.21, 131.99, 131.9, 131.3, 130.7, 130.0, 129.41, 129.36, 128.6, 128.4, 128.3, 128.2, 127.5, 127.4, 127.31, 127.28, 127.25, 126.4, 125.6, 123.42, 123.35, 53.34, 52.39, 50.7. ATR-IR (cm^{-1}): 1716 (C=O).

*4.7.2 Synthesis of 4-(2-carboxyvinyl)-1,3-bis(naphthalen-2-ylmethyl)-imidazolium chloride (**3**)*

Compound **2** (0.60 g, 1.17 mmol) in 9 M aqueous HCl (6 mL) was heated at reflux for 5 d. The hydrochloride salt was obtained directly by vacuum filtration of the acidic hydrolysis solution and washed with a small amount of chloroform (5 mL). The hydrochloride salt in the funnel was transferred to a round bottom flask and washed in a mixture of acetone and dichloromethane (1:1 v/v, 3x10 mL). The solid was collected via vacuum filtration and air-dried to yield a cream powder **3** (0.33 g, 62%). Mp: 221–224 °C. HRMS (ESI^+) calcd for $C_{28}H_{23}N_2O_2^+$ $[M\text{-}Cl]^+$ of m/z = 419.1760, found m/z = 419.1887. ^1H NMR (500 MHz, DMSO-d_6) δ 12.84 (bs, 1H), 9.71 (s, 1H), 8.59 (s, 1H), 8.06 (s, 1H), 8.02-7.94 (m, 5H), 7.90 (m, 1H), 7.86 (m, 1H), 7.62-7.55 (m, 5H), 7.48 (dd, 1H, J = 8.6, 1.7 Hz), 7.44 (d, 1H, J = 15.9 Hz), 6.60 (d, 1H), 5.85 (s, 2H), 5.67 (s, 2H). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ 166.2, 138.6, 132.8, 132.73, 132.72, 132.6, 131.72, 131.69, 130.2, 128.9, 128.8, 128.2, 128.0, 127.8, 127.72, 127.70, 126.82, 126.78, 126.73, 126.72, 126.69, 126.3, 126.0, 125.1, 124.5, 122.7, 52.7, 50.1. ATR-IR (cm^{-1}): 1693 (C=O).

*4.7.3 Synthesis of 5,6,7,8-tetrahydro-5-oxoimidazo[1,5-c]pyrimidine (**4**)*

Synthesized according to literature procedure and used without further purification.[10] ^1H NMR and other characterization matched the data in the previously reported literature.[11]

*4.7.4 Synthesis of 2-(naphthalen-2-ylmethyl)-5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidinium bromide (**5**)*

Compound **4** (0.60 g, 4.38 mmol) and 2-(bromomethyl)naphthalene (0.97 g, 4.39 mmol) were dissolved into acetonitrile (20 mL) and refluxed overnight. The solution was concentrated by rotary evaporation to a resulting cream solid. The solid was stirred in a round bottom flask with diethyl ether (30 mL) at room temperature. The product was filtered via vacuum filtration, washed in the funnel with diethyl ether and air dried to yield a crème solid **5** (1.49 g, 95%). Mp: 119-122 °C. Anal. Calcd for $C_{17}H_{16}BrN_3O$: C, 57.00; H, 4.50; N, 11.73%. Found: C, 56.48; H,

4.33; N 11.63%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.02 (s, 1H), 9.06 (s, 1H), 8.05-7.92 (m, 4H), 7.72 (s, 1H), 7.63-7.57 (m, 3H), 5.64 (s, 2H), 3.46 (m, 2H), 2.99 (t, 2H, J = 5.6 Hz). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ 156.7, 136.7, 132.9, 132.71, 132.70, 132.67, 132.6, 132.1, 131.5, 128.9, 128.7, 127.83, 127.82, 127.6, 127.5, 127.0, 126.71, 126.69, 126.67, 125.6, 125.3, 120.1, 52.2, 51.2, 49.8, 38.0, 23.9. MS (ESI^{+}) calcd for $C_{17}H_{16}N_3O^{+}$ [M-Br]+ of m/z = 278.1, found m/z= 277.8. ATR -IR (cm^{-1}): 3395 (N-H), 1758 (C=O).

Crystal data for 2-(naphthalen-2-ylmethyl)-5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidinium bromide (**5**): $C_{17}H_{16}Br_1N_3O$ •C_2H_3N, M = 399.29, monoclinic, *a* = 17.527 (4) Å, *b* = 12.642 (2) Å, *c* = 9.5932 (16) Å, β = 120.684°, *V* = 1827.9 (6) Å^3, *T* = 100(2) K, space group *Cc*, *Z* = 4, 7082 reflections measured, 3562 independent reflections (R_{int} = 0.0398). The final R_1 values were 0.0382 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0658 ($I > 2\sigma(I)$). The final R_1 values were 0.0476 (all data). The final $wR(F^2)$ values were 0.0705 (all data). CCDC #: 1048513.

*4.7.5 Synthesis of 4-(2-((methoxycarbonyl)amino)ethyl)-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**6a**)*

N,*N*-diisopropylethylamine (DIPEA) (0.96 mL, 5.51 mmol) was added to a solution of compound **5** (0.92 g, 2.57 mmol) in methanol (33 mL). The reaction mixture was stirred and refluxed for 2 d under a nitrogen atmosphere. The volatile components were removed in vacuo and the resulting residue was dissolved in dichloromethane (195 mL). The dichloromethane solution was washed with water (195 mL) and dried over magnesium sulfate. The volatile components were removed in vacuo, yielding the presumed intermediate as a white solid. The white solid and 2-(bromomethyl)naphthalene (0.58 g, 2.62 mmol) were dissolved into acetonitrile (45 mL) and refluxed overnight. The volatile components were removed under reduced pressure resulting in a crème solid that was stirred in the round bottom flask with diethyl ether (10 mL). The solution was filtered via vacuum filtration, and the solid was washed in the funnel with diethyl ether and air dried to yield a solid. The solid was purified by column chromatography using a mobile phase of methanol/dichloromethane (10:90) to yield a white solid **6a** (0.66 g, 48%). Mp: 65-67 °C. HRMS (ESI^{+}) calcd for $C_{29}H_{28}N_3O_2^{+}$ [M-Br]$^{+}$ of m/z = 450.2181, found m/z = 450.2149. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.40 (s, 1H), 8.02-7.87 (m, 8H), 7.75 (s, 1H), 7.59-7.46 (m, 6H), 7.29 (t, 1H, J = 5.3 Hz), 5.61 (s, 2H), 5.59 (s, 2H), 3.41 (s, 3H), 3.22 (td, 2H, J = 6.1, 6.0 Hz), 2.75 (t, 2H, J = 6.7 Hz). ^{13}C{^{1}H} NMR (125 MHz, DMSO-

d_6) δ 156.6, 136.7, 132.9, 132.71, 132.70, 132.67, 132.64, 132.1, 131.4, 128.9, 128.7, 127.82, 127.81, 127.64, 127.62, 127.5, 127.0, 126.70, 126.69, 126.67, 125.6, 125.2, 120.1, 52.2, 51.2, 49.8, 38.0, 23.8. ATR-IR (cm^{-1}): 3413 (N-H), 1709 (C=O).

*4.7.6 Synthesis of 4-(2-((butoxycarbonyl)amino)ethyl)-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**6b**)*

Compound **5** (1.02 g, 2.85 mmol) and N,N-diisopropylethylamine (DIPEA) (1.00 mL, 5.74 mmol) were dissolved in n-butanol (2.50 mL) under a nitrogen atmosphere. The reaction mixture was stirred at reflux for 3 d. The volatile components were removed in vacuo and the residue was dissolved in dichloromethane (300 mL). The dichloromethane solution was washed with water (300 mL) and dried over magnesium sulfate. The volatile components of the intermediate were removed in vacuo to yield a crème solid as the presumed intermediate, which was dissolved in acetonitrile (4 mL) with 2-(bromomethyl)naphthalene (0.63 g, 2.85 mmol) and refluxed overnight. The volatile components were removed under reduced pressure to a resulting cream solid, and the solid was stirred in a round bottom flask with diethyl ether (10 mL). The solid was collected via vacuum filtration, washed in the funnel with diethyl ether and air dried to a solid. The solid was purified by column chromatography using a mobile phase of methanol/chloroform (5:95) to yield a white solid **6b** (0.94 g, 57%). Mp: 116-118 °C. Anal. Calculated for $C_{32}H_{34}BrN_3O_2$: C, 67.13; H, 5.99, N, 7.34%. Found: C, 67.10; H, 6.11; N, 7.27%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.39 (s, 1H), 8.01-7.88 (m, 8H), 7.72 (m, 1H), 7.60-7.53 (m, 5H), 7.48 (m, 1H), 7.21 (t, 1H), 5.61 (s, 2H), 5.59 (s, 2H), 3.79 (t, 2H, J = 6.6 Hz), 3.22 (td, 2H, J = 6.4, 6.1 Hz), 2.75 (t, 2H, J = 6.6 Hz), 1.40 (m, 2H), 1.22 (m, 2H), 0.82 (t, 3H, J = 7.2 Hz). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ 156.5, 136.7, 133.6, 133.1, 132.74, 132.73, 132.70, 132.68, 132.1, 131.5, 128.9, 128.8, 127.9, 127.69, 127.68, 127.67, 127.5, 127.1, 126.75, 126.72, 125.7, 125.3, 120.12, 120.11, 63.5, 52.2, 49.8, 37.9, 30.6, 23.9 , 18.5, 13.5. MS (ESI+) calcd for $C_{32}H_{34}N_3O_2^+$ [M-Br]+ of m/z = 492.3, found m/z = 492.2. ATR-IR (cm^{-1}): 3413 (N-H), 1701 (C=O).

Crystal data for 4-(2-((butoxycarbonyl)amino)ethyl)-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**6b**): $C_{32}H_{34}Br_1N_3O_2$, M = 572.52, triclinic, a = 8.370 (3) Å, b = 10.646 (4) Å, c = 17.666 (17) Å, α= 100.436(5)°.β = 97.651(5)°, γ = 108.773(5)°, V = 1434.5 (10) $Å^3$, T = 100(2) K, space group P-1, Z = 2, 11072 reflections measured, 5763 independent

reflections (R_{int} = 0.0270). The final R_1 values were 0.0354 (I > 2σ(I)). The final wR(F^2) values were 0.0863 (I > 2σ(I)). The final R_1 values were 0.0444 (all data). The final wR(F^2) values were 0.0904 (all data). CCDC #: 1048514.

*4.7.7 Synthesis of 1,3-bis(naphthalen-2-ylmethyl)-4-(2-(((prop-2-yn-1-yloxy)carbonyl)amino)ethyl)imidazolium bromide (**6c**)*

N,N-diisopropylethylamine (DIPEA) (1.05 mL, 6.03 mmol) was added to compound **5** (1.00 g, 2.79 mmol) in propargyl alcohol (57 mL). The reaction mixture was refluxed for 3 d under a nitrogen atmosphere. The volatile components were removed in vacuo and the residue was dissolved in dichloromethane (300 mL). The dichloromethane solution was washed with water (300 mL) and dried over magnesium sulfate. The volatile components were removed in vacuo to yield a crème solid as the presumed intermediate, which was dissolved into acetonitrile (2 mL) with 2-(bromomethyl)naphthalene (0.62 g, 2.80 mmol) and refluxed overnight. The volatile components were removed under reduced pressure to a resulting crème residue that was stirred in a round bottom flask with diethyl ether (10 mL). The solid was collected via vacuum filtration, washed in the funnel with diethyl ether, and air dried to yield a solid. The solid was purified by column chromatography using a mobile phase of methanol/dichloromethane (10:90) to yield a white solid **6c** (0.36 g, 23%). Mp: 122-125 °C. HRMS (ESI^+) calcd for $C_{31}H_{28}N_3O_2^+$ of $[M\text{-}Br]^+$ = 474.2181, found m/z = 474.2104. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.35 (s, 1H), 8.01-7.91 (m, 7H), 7.87 (s, 1H), 7.72 (s, 1H), 7.59-7.46 (m, 7H), 5.60 (s, 2H), 5.58 (s, 2H), 4.51 (d, 2H, J = 2.0 Hz), 3.46 (m, 1H), 3.25 (td, 2H, J = 6.1, 6.6 Hz), 2.77 (t, 2H, J = 6.5 Hz). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ 155.4, 136.7, 133.0, 132.82, 132.78, 132.75, 132.1, 131.5, 129.0, 128.9, 128.0, 127.76, 127.75, 127.7, 127.1, 126.86, 126.84, 126.83, 125.7, 125.3, 120.3, 79.2, 77.2, 52.4, 51.7, 50.0, 38.2, 23.9. ATR-IR (cm^{-1}): 3411 (N-H), 2114 (C≡CH), 1709 (C=O).

4.7.9 *Dimethyl 1-(naphthalen-2-ylmethyl)imidazole-4,5-dicarboxylate (**8**)*

Compound **7** (3.45 g, 18.7 mmol) and potassium carbonate (2.84 g, 20.6 mmol) were combined in acetonitrile (10 mL) and stirred at reflux for 30 min. 2-(Bromomethyl)naphthalene (4.14 g, 18.7 mmol) was added and the mixture was heated at reflux overnight (18 h). The volatile components were removed under reduced pressure. To the residue was added ethyl acetate (175 mL) which was washed with a saturated brine solution (4 x 75 mL). The organic

layer was dried over magnesium sulfate and the volatile components were removed under reduced pressure, yielding a viscous yellow liquid smelling strongly of ethyl acetate. The residue was dissolved in ethyl acetate (5 mL) and petroleum ether (35 mL, b.p. 35–60 °C) was added. Vigorous stirring resulted in the formation of a precipitate, which was triturated while in the reaction mixture. The solid was collected via vacuum filtration and air-dried to give the title compound as a light tan powder **8** (5.08 g, 84%). Mp: 84-85 °C. Anal. Calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64%. Found: C, 66.37; H, 4.96; N, 8.46%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.90 (m, 3H), 7.67 (s, 1H), 7.51 (m, 2H), 7.34 (d, 1H, J = 8.6 Hz), 5.60 (s, 2H), 3.77 (s, 3H), 3.71 (s, 3H). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) δ 162.7, 160.0, 141.1, 136.2, 133.9, 132.7, 132.4, 128.4, 127.7, 127.5, 126.5, 126.3, 126.0, 125.0, 124.5, 52.4, 51.8, 49.6. MS (ESI$^+$) calcd for $C_{18}H_{16}N_2NaO_4^+$ $[M+Na]^+$ of m/z = 347.1, found m/z = 346.8. ATR-IR (cm^{-1}): 1713 (C=O).

*4.7.10 (1-(Naphthalen-2-ylmethyl)imidazole-4,5-diyl)dimethanol (**9**)*

Over a period of 30 min, compound **8** (2.77 g, 8.54 mmol) was added in small portions to a stirred mixture of lithium aluminum hydride (0.69 g, 18 mmol) in dry tetrahydrofuran (75 mL) at 0 °C under a nitrogen atmosphere. The stirring was continued at room temperature for 3 h. The reaction mixture was cooled to 0 °C and treated successively with water (3 mL), 15% aqueous sodium hydroxide (3 mL), and water (3 mL). The volatile components were removed under reduced pressure. To the residue was added water (15 mL), which was stirred and the remaining solid collected by vacuum filtration. The solid was stirred in a refluxing mixture of acetonitrile and methanol (1:1 v/v, 100 mL) and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure to a volume of approximately 15 mL and cooled to 15 °C to induce further precipitation. The solid was collected by vacuum filtration to yield a pale yellow microcrystalline solid **9** (1.28 g, 56%). Mp: 172-175 °C. Anal. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44%. Found: C, 69.31; H, 6.24; N, 10.24%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89 (d, 2H, J = 8.3 Hz), 7.86 (m, 1H), 7.70 (s, 1H), 7.69 (s, 1H), 7.51 (m, 2H), 7.38 (dd, 1H, J = 8.3, 1.3 Hz), 5.39 (s, 2H), 5.02 (bs, 1H), 4.67 (t, 1H, J = 5.4 Hz), 4.43 (d, 2H, J = 4.3 Hz), 4.38 (d, 2H, J = 5.4 Hz). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) δ 139.6, 136.9, 135.2, 132.8, 132.3,

128.3, 128.1, 127.64, 127.55, 126.4, 126.1, 125.7, 125.3, 56.4, 51.6, 47.8. MS (ESI+) calcd for $C_{16}H_{17}N_2NaO_2^+$ $[M+Na]^+$ of m/z = 291.1, found m/z = 290.7.

*4.7.11 4,5-Bis(hydroxymethyl)-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**10**)*

2-(Bromomethyl)naphthalene (385 mg, 1.74 mmol) and compound **9** (423 mg, 1.57 mmol) were dissolved in a mixture of acetonitrile and methanol (1:1 v/v, 7 mL). The mixture was heated at reflux for 2 d, with additional solvent (3 mL) added after 1 day. The initial precipitate was collected by vacuum filtration of the hot reaction mixture and rinsed with a small volume of the acetonitrile/methanol mixture. As the filtrate cooled, additional precipitate formed and it was collected via vacuum filtration and rinsed as before. The collected solids were combined and dried in air to yield a white powder **10** (270 mg, 35%). No attempt was made to recover additional product from the remaining filtrate. Mp: 202-203 °C. Anal. Calcd for $C_{27}H_{25}BrN_2O_2$: C, 66.26; H, 5.15; N, 5.72%. Found: C, 65.96; H, 5.31; N, 5.77%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.45 (s, 1H), 8.00 (d, 2H, J = 8.6 Hz), 7.94 (m, 6H), 7.57 (m, 4H), 7.53 (dd, 2H, J =8.6, 1.7 Hz), 5.69 (s, 4H), 5.65 (t, 2H, J = 4.9 Hz), 4.59 (d, 4H, J = 4.9 Hz). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) δ 137.6, 132.7, 132.6, 131.8, 131.5, 128.7, 127.8, 127.6, 127.2, 126.7, 125.4, 50.8, 50.2. MS (ESI^+) calcd for $C_{27}H_{25}N_2O_2^+$ $[M–Br]^+$ of m/z = 409.2, found m/z = 408.9.

Crystal data for 4,5-bis(hydroxymethyl)-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**10)**: $C_{27}H_{25}BrN_2O_2$, M = 489.40, orthorhombic, a = 26.4778(9) Å, b = 15.2081(2) Å, c = 5.6553 (9) Å, V = 2277.3(4) Å3 , T = 100(2) K, space 84 group Pna21, Z = 4, μ(Mo Kα) = 1.832 mm-1 , 11286 reflections collected, 4503 independent reflections (Rint = 0.0530). The final R1 values were 0.0481 (I > 2σ(I)). The final wR(F 2) values were 0.1041 (I > 2σ(I)). The final R1 values were 0.0825 (all data). The final wR(F 2) values were 0.1202 (all data). The goodness of fit on F 2 was 1.023. CCDC #: 1471723.

*4.7.8 Synthesis of 4-methyl-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**11**)*

4-Methylimidazole (1.00 g, 12.2 mmol) was dissolved in acetonitrile (15 mL), potassium hydroxide (0.78 g, 14 mmol) was added, and the mixture was refluxed for 1 h. 2-(Bromomethyl)naphthalene (2.68 g, 12.1 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 (3.20 g, 14.5 mmol) was added to the filtrate which was refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot reaction mixture. The solid in the funnel was washed with diethyl ether, and air dried to provide a white powder **11** (2.66 g, 49%). Mp: 198-200 °C. Anal. Calcd for $C_{26}H_{23}BrN_2$: C, 70.43; H, 5.23; N, 6.32%. Found C, 69.71; H, 5.37; N, 6.25%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.04 (s, 1H), 8.01 (s, 1H), 7.99 (s, 1H), 7.94 (m, 5H), 7.71 (s, 1H), 7.57 (m, 5H), 7.51 (m, 1H), 5.65 (s, 4H), 2.22 (s, 3H). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) δ 136.4, 132.68, 132.67, 132.65, 132.57, 132.1, 131.5, 131.4, 128.8, 128.7, 127.80, 127.77, 127.65, 127.6, 127.0, 126.7, 126.63, 126.60, 125.7, 125.3, 119.8, 52.0, 49.7, 8.9. MS (ESI$^+$) calcd for $C_{26}H_{23}N_2^+$ [M-Br]$^+$ of m/z = 363.2, found m/z = 363.1.

Crystal data for 4-methyl-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**11**): $C_{26}H_{23}Br_1N_2$, M = 443.37, monoclinic, *a* = 10.779(4) Å, *b* = 17.353(7) Å, *c* = 11.919(5) Å, α = 90°, β = 111.99(2)°, γ = 90°,V = 2067.2(13) Å^3, *T* = 100(2) K, space group P2(1)/n, *Z* = 4, 17385 reflections measured, 4200 independent reflections (R_{int} = 0.0747). The final R_1 values were 0.0492 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0986 ($I > 2\sigma(I)$). The final R_1 values were 0.0949 (all data). The final $wR(F^2)$ values were 0.1147 (all data). CCDC #: 1048512.

*4.7.12 Synthesis of 1,3-bis(naphthalen-2-ylmethyl)-4,5-diphenylimidazolium bromide (**12**)*

A mixture of 4,5-diphenylimidazole (1.00 g, 4.54 mmol) and potassium hydroxide (0.38 g, 6.77 mmol) in acetonitrile (10 mL) was refluxed for 30 min. 2-(Bromomethyl)naphthalene (1.00 g, 4.52 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white precipitate, presumed to be potassium bromide. The filtrate was stirred with 2-(bromomethyl)naphthalene (1.00 g, 4.52 mmol) and refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot reaction mixture. The white solid was stirred in a round bottom flask with diethyl ether (20 mL). The product was collected via vacuum filtration, washed with diethyl ether in the funnel and air-dried to yield a white powder **12** (1.39 g, 52%). Mp: 199–202 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.79 (s, 1H), 7.92 (m, 4H), 7.82 (m, 2H), 7.59 (s, 2H), 7.54 (m, 4H), 7.41 (m, 2H), 7.36 (m, 8H), 7.32 (m, 2H), 5.61 (s, 4H). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) δ 136.8, 132.54, 132.52, 131.9, 131.4, 130.8,

130.1, 128.7, 128.5, 127.8, 127.6, 127.2, 126.63, 126.59, 125.3, 125.0, 50.7. HR-MS (ESI^+) calcd for $C_{37}H_{29}N_2^+$ $[M\text{-}Br]^+$ of m/z = 501.2331, found m/z = 501.2242.

*4.7.13 Synthesis of 1,3-bis((6-methoxynaphthalen-2-yl)methyl)-4,5-diphenylimidazolium chloride salt (**13**).*

A mixture of 4,5-diphenylimidazole (0.30 g, 1.36 mmol) and potassium hydroxide (0.11 g, 1.96 mmol) in acetonitrile (2 mL) was refluxed for 30 min. 2-(Chloromethyl)-6-methoxynaphthalene (0.29 g, 1.40 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white precipitate, presumed to be potassium chloride. 2-(Chloromethyl)-6-methoxynaphthalene (0.29 g, 1.40 mmol) was added to the filtrate and refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot reaction mixture. The white solid was stirred in a round bottom flask with diethyl ether (5 mL). The product was collected via vacuum filtration, washed with diethyl ether in the funnel and air-dried to yield a white powder **13** (0.24 g, 29%). Mp: 240–243 °C. Anal. Calcd for $C_{39}H_{33}ClN_2O_2$: C, 78.44; H, 5.57; N, 4.69%. Found: C, 78.05; H, 5.47; N, 4.64%. ^1H NMR (300 MHz, DMSO-d_6) δ 9.74 (s, 1H), 7.79 (d, 2H, J = 8.5 Hz), 7.70 (d, 2H, J =9.1 Hz), 7.44-7.32 (m, 14H), 7.24 - 7.19 (m, 2H), 5.53 (s, 2H), 3.86 (s, 6H). ^{13}C $\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ 157.7, 136.7, 133.9, 131.8, 130.8, 130.1, 129.3, 128.8, 128.7, 127.9, 127.4, 127.2, 125.8, 125.0, 119.1, 105.9, 55.2, 50.7. HRMS (ESI^+) calcd for $C_{39}H_{33}N_2O_2^+$ $[M\text{-}Cl]^+$ of m/z = 561.2542, found m/z = 561.2500.

*4.7.14 Synthesis of 3-((6-methoxynaphthalen-2-yl)methyl)-1-(naphthalen-2-ylmethyl)-4,5-diphenylimidazolium bromide salt (**14**).*

A mixture of 4,5-diphenylimidazole (0.30 g, 1.36 mmol) and potassium hydroxide (0.11 g, 1.96 mmol) in acetonitrile (2 mL) was refluxed for 30 min. 2-(Chloromethyl)-6-methoxynaphthalene (0.29 g, 1.40 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white precipitate, presumed to be potassium chloride. 2-(Bromomethyl)naphthalene (0.30 g, 1.36 mmol) was added to the filtrate and refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot

reaction mixture. The white solid was stirred in a round bottom flask with diethyl ether (5 mL). The product was collected via vacuum filtration, washed with diethyl ether in the funnel and air-dried to yield a white powder **14** (0.58 g, 69%). Mp: 240–243 °C. Anal. Calcd for $C_{38}H_{31}BrN_2O$: C, 74.63; H, 5.11; N, 4.58%. Found: C, 73.83; H, 4.91; N, 4.52%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.71 (s, 1H), 7.93-7.89 (m, 2H), 7.82-7.79 (m, 2H), 7.71 (d, 1H, J = 8.8 Hz), 7.56-7.50 (m, 4H), 7.45-7.31 (m, 11H), 7.27 (dd, 1H, J = 8.8, 1.8 Hz), 7.20 (m, 2H), 5.59 (s, 2H), 5.54 (s, 2H), 3.87 (s, 3H). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) δ 157.7, 136.7, 134.0, 132.53, 132.50, 131.84, 131.83, 131.4, 130.82, 130.80, 130.1, 129.3, 128.8, 128.7, 128.5, 127.9, 127.8, 127.6, 127.4, 127.2, 126.62, 126.58, 125.8, 125.3, 125.00, 124.97, 119.1, 105.9, 55.2, 50.73, 50.68. HRMS (ESI$^+$) calcd for $C_{38}H_{31}N_2O^+$ [M-Br]$^+$ of m/z = 531.2437, found m/z = 531.2424.

*4.7.15 Synthesis of 3-((6-methoxynaphthalen-2-yl)methyl)-4,5-diphenyl-1-(quinolin-2-ylmethyl)-imidazolium chloride salt (**15**)*

A mixture of 4,5-diphenylimidazole (0.39 g, 1.77 mmol) and potassium hydroxide (0.15 g, 2.67 mmol) in acetonitrile (2 mL) was refluxed for 30 min. 2-(Chloromethyl)quinoline (0.32 g, 1.80 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white precipitate, presumed to be potassium chloride. 2-(Chloromethyl)-6-methoxynaphthalene (0.38 g, 1.84 mmol) was added to the filtrate and refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot reaction mixture. The white solid was stirred in a round bottom flask with diethyl ether (5 mL). The product was collected via vacuum filtration, washed with ethyl acetate in the funnel and air-dried to yield a white powder **15** (0.45 g, 44%). Mp: 233–236 °C. Anal. Calcd for $C_{37}H_{30}ClN_3O$: C, 78.22; H, 5.32; N, 7.40%. Found: C, 74.31; H, 5.57; N, 6.85%. ^{1}H NMR (500 MHz, CD_3OD-d_4) δ 9.54 (s, 1H), 8.37 (d, 2H, J = 8.5 Hz), 7.93- 7.74 (m, 5H), 7.66-7.59 (m, 5H), 7.52-7.15 (m, 18H), 5.71 (s, 2H), 5.60 (s, 2H), 3.92 (s, 3H). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) δ 157.8, 153.8, 146.7, 137.8, 137.3, 134.0, 132.4, 131.4, 130.8, 130.6, 130.14, 130.09, 130.0, 129.0, 128.8, 128.6, 128.5, 127.9, 127.5, 127.01, 126.98, 126.96, 125.7, 125.0, 124.9, 119.5, 119.2, 105.9, 55.2, 51.7, 50.7. HRMS (ESI$^+$) calcd for $C_{37}H_{30}N_3O^+$ [M-Cl]$^+$ of m/z = 532.2389, found m/z = 532.2347.

*4.7.16 Synthesis of 1-(naphthalen-2-ylmethyl)-4,5-diphenyl-3-(quinolin-2-ylmethyl)-imidazolium bromide salt (**16**)*

A mixture of 4,5-diphenylimidazole (0.40 g, 1.82 mmol) and potassium hydroxide (0.12 g, 2.14 mmol) in acetonitrile (7 mL) were refluxed for 30 min. 2-(Chloromethyl)quinoline (0.33 g, 1.86 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white precipitate, presumed to be potassium chloride. 2-(Bromomethyl)naphthalene (0.40 g, 1.81 mmol) was added to the filtrate and refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot reaction mixture. The white solid was stirred in a round bottom flask with diethyl ether (10 mL). The product was collected via vacuum filtration, washed with diethyl ether in the funnel and air-dried to yield a white powder **16** (0.85 g, 80%). Mp: 212–215 °C. Anal. Calcd for $C_{36}H_{28}BrN_3$: C, 74.23; H, 4.84; N, 7.21%. Found: C, 73.66; H, 4.95; N, 7.10%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.77 (s, 1H), 8.37 (d, 2H), 7.99 (d, 1H), 7.95 (m, 2H), 7.90 (d, 1H), 7.82-7.79 (m, 2H), 7.66-7.62 (m, 2H), 7.56 (m, 2H), 7.44-7.41 (m, 2H), 7.37-7.33 (m, 5H), 7.29-7.24 (m, 4H), 5.77 (s, 2H), 5.68 (s, 2H). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ 153.7, 146.7, 137.8, 137.3, 132.5, 132.4, 131.6, 131.5, 130.8, 130.6, 130.14, 130.11, 130.0, 128.8, 128.7, 128.6, 128.5, 127.9, 127.7, 127.6, 127.1, 126.99, 126.96, 126.7, 125.1, 125.0, 124.8, 119.5, 51.7, 50.7. HRMS (ESI$^+$) calcd for $C_{36}H_{28}N_3^+$ $[M\text{-}Br]^+$ of m/z = 502.2283, found m/z = 502.2241.

Crystal data for 1-(naphthalen-2-ylmethyl)-4,5-diphenyl-3-(quinolin-2-ylmethyl)-imidazolium bromide salt (**16**): $C_{36}H_{30}Br_1N_3O{\bullet}H_2O$, M = 600.54, monoclinic, a = 12.0212(5) Å, b = 23.5567(9) Å, c = 10.5859(5) Å, α = 90°, β = 101.101(2)°, γ = 90°,V = 2941.6(2) Å^3, T = 100(2) K, space group P2(1)/c, Z = 4, 24653 reflections measured, 5962 independent reflections (R_{int} = 0.0522).The final R_1 values were 0.0347 (I > 2σ(I)). The final wR(F^2) values were 0.0696 (I > 2σ(I)). The final R_1 values were 0.0572 (all data). The final wR(F^2) values were 0.0768 (all data). CCDC #: 1419705.

*4.7.17 Synthesis of 4,5-diphenyl-1,3-bis(quinolin-2-ylmethyl)-imidazolium bromide salt (**17**)*

A mixture of 4,5-diphenylimidazole (0.40 g, 1.82 mmol) and potassium hydroxide (0.12 g, 2.14 mmol) in acetonitrile (2 mL) was refluxed for 30 min. 2-(Chloromethyl)quinoline (0.33 g, 1.86 mmol) was added and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove a white precipitate, presumed to be potassium chloride. 2-(Bromomethyl)quinoline (0.48 g, 2.16 mmol) was added to the filtrate and refluxed overnight. The resulting precipitate was collected via vacuum filtration of the hot reaction mixture. The

white solid was stirred in a round bottom flask with diethyl ether (10 mL). The product was collected via vacuum filtration, washed with diethyl ether in the funnel and air-dried to yield a cream powder **17** (0.55 g, 51%). Mp: 234–236 °C. Anal. Calcd for $C_{35}H_{27}BrN_4 \bullet C_2H_6O$: C, 70.59; H, 5.28; N, 8.90%. Found: C, 70.55; H, 5.25; N, 8.90%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.40 (m, 2H), 8.01 (m, 2H), 7.91 (m, 2H), 7.81-7.78 (m, 2H), 7.66-7.63 (m, 2H), 7.43 (m, 2H), 7.35-7.26 (m, 10H), 5.85 (s, 4H). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ 153.9, 146.7, 138.4, 137.3, 132.1, 130.7, 130.1, 130.0, 128.7, 128.5, 127.9, 127.06, 126.96, 125.0, 119.4, 51.9. HRMS (ESI$^+$) calcd for $C_{35}H_{27}N_4^+$ $[M\text{-}Br]^+$ of m/z = 503.2236, found m/z = 503.2219.

Crystal data for 4,5-diphenyl-1,3-bis(quinolin-2-ylmethyl)-imidazolium bromide (**17**):$C_{35}H_{27}BrN_2 \bullet C_2H_6O$, M = 629.58, monoclinic, a = 14.3836(5) Å, b = 10.9731(4) Å, c = 19.6587(7) Å, α = 90°, β = 101.2972(13)°, γ = 90°,V = 3042.67(19) Å^3, T = 100(2) K, space group P2(1)/n, Z = 4, 25081 reflections measured, 6175 independent reflections (R_{int} = 0.0623).The final R_1 values were 0.0390 (I > 2σ(I)). The final wR(F^2) values were 0.0869 (I > 2σ(I)). The final R_1 values were 0.0633 (all data). The final wR(F^2) values were 0.0989 (all data). CCDC #: 1419702.

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5.2 Abbreviations: NCI - National Cancer Institute; IC_{50} - inhibitory concentration 50 %; NSCLC – non-small cell lung cancer; IC23 - 4,5-dichloro-1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide; MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 2-HPβCD – 2-hydroxypropyl-β-cyclodextrin; PS – phosphatidylserine; PI – propidium iodide; SRM-MS- selected reaction monitoring mass spectrometry

5.3 Supporting Information. The ^{13}C NMR for compounds **2**, **3**, **6a-c**, **10**, **11**, and **12-17,** the thermal ellipsoid plot of **5,** and 2D NMR HSQC of **13** is included in the supporting information. Crystallographic information files (CIF) for compounds **5**, **6b**, **10**, **11**, **16**, and **17** (CCDC #s 1048512-1048514, 1471723, 1419705, and 1419702) can be found on the Cambridge Crystallographic Data Center website. This material is available free of charge via the Internet at http://www.ccdc.cam.ac.uk/pages/Home.aspx.

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5.5 Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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