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Anti-tumor activity of lipophilic imidazolium salts on select NSCLC cell lines

Brian D. Wright, **Michael C. Deblock**, **Patrick O. Wagers**, **Ernest Duah**, **Nikki K. Robishaw**, **Kerri L. Shelton**, **Marie R. Southerland**, **Michael A. DeBord**, **Kortney M. Kersten**, **Lucas J. McDonald**, **Jason A. Stiel**, **Matthew J. Panzner**, **Claire A. Tessier**, **Sailaja Paruchuri**, and **Wiley J. Youngs**

Department of Chemistry, The University of Akron, Akron, OH 44325-3601, USA

Wiley J. Youngs: youngs@uakron.edu

Abstract

The anti-tumor activity of imidazolium salts is highly dependent upon the substituents on the nitrogen atoms of the imidazolium cation. We have synthesized and characterized a series of naphthalene-substituted imidazolium salts and tested them against a variety of non-smallcell lung cancer cell lines. Several of these complexes displayed anticancer activity comparable to cisplatin. These compounds induced apoptosis in the NCI-H460 cell line as determined by Annexin V staining, caspase-3, and PARP cleavage. These results strongly suggest that this class of compounds can serve as potent chemotherapeutic agents.

Graphical Abstract

Correspondence to: Wiley J. Youngs, youngs@uakron.edu.

Brian D. Wright, Michael C. Deblock, and Patrick O. Wagers have contributed equally to the manuscript.

Present Address: B. D. Wright, Mallinckrodt Institute of Radiology, Washington University, School of Medicine, Campus Box 8225, St. Louis, MO 63110, USA

M. C. Deblock, Department of Biomedical Engineering, Cleveland Clinic Lerner Research Institute, 9500 Euclid Avenue, Cleveland, OH 44195, USA

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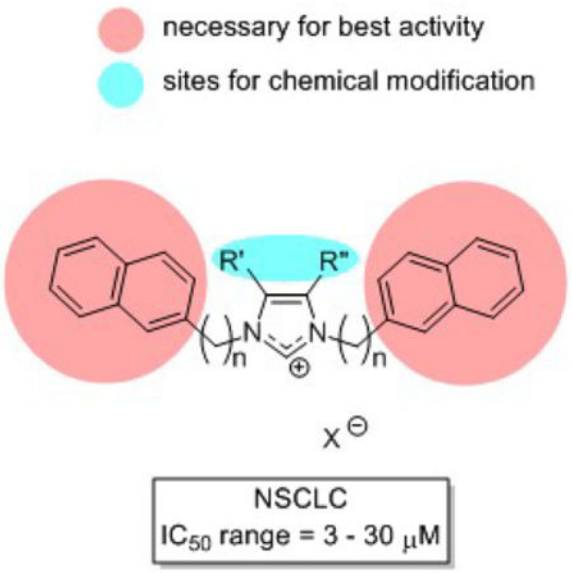
Supporting information: Crystallographic information files (CIF) for compounds **2, 4, 6, 12, 13, 15, 16, 17, 20,** and **21**. This material is available free of charge via the Internet at http://pubs.acs.org.



Keywords

Imidazolium cation; Imidazolium salt; Anti-tumor; Anticancer; Apoptosis

Introduction

Cancer is the second leading cause of death in the United States, and roughly 30 % of all cancer-related deaths are attributed to lung cancer. Lung cancer is divided into two major categories: small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC), with the latter accounting for 80 % of all diagnosed lung cancers (Siegel *et al*., 2012). NSCLC is made up of several different types of lung cancers, including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma, and large-cell carcinoma. Common treatments involve a mixture of organic and heavy metal-based drugs, such as cisplatin. These chemotherapeutic drugs can have devastating side effects on the patient, including kidney, liver, and nerve damage (Yao *et al*., 2007). Additionally, resistance to chemotherapeutic agents ordinarily develops due to the diverse protein expression found in cancerous cells, leading to a relapse in a large number of treated patients (Katano *et al*., 2002).

The necessity of reducing the toxic side effects of anticancer drugs and increasing their longevity by avoiding resistance has spurred the development of several new potential chemotherapies. One strategy that has been investigated to overcome both the issues of toxicity and resistance is the use of silver carbene complexes (SCCs) as chemotherapeutic agents. Silver, specifically the bioactive silver cation, has a long history as an efficacious antimicrobial agent, and *N*-heterocyclic carbenes have shown to be effective, relatively nontoxic ligands with which to deliver the silver cations (Garrison and Youngs, 2005; Garrison *et al*., 2001a, b; Kascatan-Nebioglu *et al*., 2006; Hindi *et al*., 2008; Ornelas-Megiatto *et al*., 2012). Furthermore, bacterial resistance to silver has been infrequent, allowing the medicinal use of silver since the 1800s (Russel *et al*., 1994; Landsdown, 2002,

2004; Lansdown and Williams, 2004). To this point, the use of SCCs as potential anti-tumor agents has focused on lipophilic compounds derived from 4,5-dichloroimidazole (Medvetz *et al*., 2008; Hindi *et al*., 2009a, b; Panzner *et al*., 2009; Lin *et al*., 2009). Several of these complexes have shown in vitro anti-tumor activity at clinically relevant levels, spurring the investigation of additional, analogous compounds (Youngs *et al*., 2012a; Gautier and Cisnetti, 2012; Oehinger *et al*., 2013).

Based on our anticancer studies on lipophilic SCCs, it was determined that several of the corresponding imidazolium salts also possessed potent anti-tumor activity. Imidazolium salts are the immediate precursors to all of the SCCs studied, as well as the primary degradation product of a majority of the complexes. This observation supported several recent reports of imidazolium salts displaying activity against cancer cell lines (Riduan and Zhang, 2013). Tolcher et al. have discussed the suppression of survivin, an inhibitor of apoptosis, by a hydrophilic imidazolium salt. In this phase I human trial, Tolcher observed the inhibition of non-Hodgkin's lymphoma, prostate cancer, and minor inhibition of non-small-cell lung cancer lesions (Tolcher *et al*., 2008). Serebrennikova et al. reported three novel imidazolium-based glycerolipids that induce apoptosis in chronic human promyelocytic leukemia cells (K562). These data suggest that at low concentrations, imidazolium salts disturb the cell cycle, but at higher concentrations, apoptosis is stimulated (Markova *et al*., 2010). Malhotra has tested several long alkyl chain-containing imidazolium salts against a sizable panel of cancer cell lines and found high activity (Malhotra and Kumar, 2010).

With the potential of imidazolium salts as anticancer agents well established, our focus has been the improvement of the lipophilic imidazolium salts. Although these systems have shown impressive anticancer activity, their principal drawback remains their limited solubility in water, which severely impairs the ability to administer these compounds systemically. Through the rational selection of substituents of the *N*-heterocycle, we have strived to synthesize the corresponding salts to have a higher solubility in water, while still maintaining the lipophilicity that is believed to contribute to their efficacy (Wright *et al*., 2012; Li *et al*., 2010; Youngs *et al*., 2012b, c). Additionally, by creating several analogs of an already effective lipophilic imidazolium salt, IC23 (Fig. 1), and observing their anticancer activity, we hope to begin to decipher the mechanism of action of these compounds. In this paper, we report new lipophilic imidazolium and xanthinium salts derived from a variety of compounds containing an imidazole group, including their synthesis, characterization, and in vitro anticancer activity. Furthermore, we identified that the mechanism of cell death induced by IC23 is mediated through apoptosis.

Results and discussion

In Schemes 1, 2, 3, and 4, the synthetic routes of compounds **1–23** are outlined. Compounds **1–9** were prepared by stirring 4,5-dichloroimidazole with 1.1 equivalents of potassium hydroxide and one equivalent of the appropriate alkyl bromide or iodide in refluxing acetonitrile for 12–15 h to yield the corresponding monoalkylated imidazoles, which were stirred with one equivalent of (2-bromomethyl)naphthalene in refluxing acetonitrile for 12–15 h to generate the imidazolium salts in yields ranging from 20 to 67 % (Scheme 1; Figs. 2,

3, and 4). The symmetric compound 1,3-dibenzyl-4,5-dichloroimidazolium bromide (**10**) was synthesized by stirring 4,5-dichloroimidazole with 1.1 equivalents of potassium hydroxide and two equivalents of benzyl bromide, introduced in two separate additions to allow for the removal of the generated potassium bromide, in refluxing acetonitrile for a total of 3.5 h, producing **10** in a 42 % yield. The synthesis of the asymmetric compound 1-benzyl-4,5-dichloro-3-(napthalen-2-ylmethyl)imidazolium bromide (**11**) proceeded in a similar fashion. 4,5-Dichloroimidazole was stirred with 1.1 equivalents of potassium hydroxide and one equivalent of (2-bromomethyl)naphthalene in refluxing acetonitrile for 3 h to yield the monosubstituted imidazole. To this intermediate was added one equivalent of benzyl bromide, and the mixture was heated in refluxing acetonitrile for 8 h to give the imidazolium salt **11** in 33 % yield. With this asymmetric substitution, a “scrambling” of the substituents was observed when the reaction was allowed to proceed longer than 8 h, producing the desired compound **11**, as well as the symmetric compounds 4,5-dichloro-1,3-bis (naphthalen-2-ylmethyl)imidazolium bromide (IC23) and **10**. The symmetric compounds 1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (**12**) and 1,3-bis(naphthalen-2-ylmethyl)benzimidazolium bromide (**13**) were synthesized by stirring either imidazole or benzimidazole with 1.1 equivalents of potassium hydroxide and two equivalents of (2-bromomethyl)naphthalene, introduced in two separate additions to allow for the removal of the generated potassium bromide, in refluxing acetonitrile. Compounds **12** and **13** were produced in 35 and 84 % yield, respectively (Scheme 2; Figs. 5, 6).

The xanthine-based imidazolium salt 1,3,9-trimethyl-7-(naphthalen-2-ylmethyl)xanthinium iodide (**15**) was synthesized by stirring theophylline and an excess of both potassium hydroxide and (2-bromomethyl)naphthalene in acetonitrile at room temperature for 2 days, which generated the monosubstituted product, **14**. This intermediate was stirred with a large excess of iodomethane in DMF heated to 80 °C for 20 h to produce **15** in a 58 % yield (Scheme 3; Fig. 7).

The monosubstituted imidazole 4,5-dichloro-1-(2-(naphthalen-1-yl)ethyl)-imidazole (**16**) was synthesized by stirring 4,5-dichloroimidazole with 1.1 equivalents of potassium hydroxide and one equivalent of 1-(2-bromoethyl)naphthalene in refluxing acetonitrile for 2.5 h. Compound **16** served as the immediate precursor to the imidazolium salts 4,5-dichloro-1,3-bis(2-(naphthalen-1-yl)ethyl)-imidazolium bromide (**17**), 4,5-dichloro-1-(2-(naphthalen-1-yl)ethyl)-3-(naphthalen-2-ylmethyl)-imidazolium bromide (**18**), and 3-benzyl-4,5-dichloro-1-(2-(naphthalen-1-yl)ethyl)-imidazolium bromide (**19**). In a sealed pressure vessel, **16** was stirred with an excess of 1-(2-bromoethyl)naphthalene in acetonitrile and heated to an external temperature of 120 °C overnight to give **17** in a 48 % yield. Compounds **18** and **19** were synthesized by a similar procedure, as **16** was stirred with one equivalent of (2-bromomethyl)naphthalene or benzyl bromide in refluxing acetonitrile to generate the corresponding imidazolium salt in 77 and 44 % yield, respectively (Scheme 4; Figs. 8, 9).

A related series of compounds was created from the monosubstituted imidazole 4,5-dichloro-1-(2-(naphthalen-2-yl)ethyl)-imidazole (**20**). Compound **20** was synthesized by the same procedure as the isomeric imidazole **16**, with 2-(2-bromoethyl)naphthalene being used

to generate **20** in 31 % yield. 4,5-Dichloro-1,3-bis(2-(naphthalen-2-yl)ethyl)-imidazolium bromide (**21**) and 4,5-dichloro-1-(2-(naphthalen-2-yl)ethyl)-3-(naphthalen-2-ylmethyl)-imidazolium bromide (**22**) were synthesized by the same method as their isomeric analogs, **17** and **18**, respectively, with minor deviations. Compound **21** was produced in 41 % yield by the addition of an equimolar amount of 2-(2-bromoethyl)-naphthalene to **20**. Compound **22** was produced in 52 % yield by the addition of (2-bromomethyl)naphthalene to **20** (Scheme 5; Figs. 10, 11).

The asymmetric imidazolium salt 4,5-dichloro-1-(naphthalen-2-ylmethyl)-3-(quinolin-2-ylmethyl)imidazolium bromide (**23**) was synthesized by stirring 4,5-dichloroimidazole and (2-chloromethyl)quinoline hydrochloride with a slight excess of potassium hydroxide in refluxing acetonitrile overnight to yield the monosubstituted imidazole. (2-Bromomethyl)naphthalene was added to the intermediate and stirred in refluxing acetonitrile for 3.5 h to generate **23** in 86 % yield (Scheme 6; Fig. 12).

All symmetric and asymmetric imidazolium salts, as well as select monosubstituted imidazole precursors, were characterized by ^{1}H and ^{13}C NMR, mass spectrometry, elemental analysis, and melting point determination. In addition, the structures of imidazolium salts **2, 4, 6, 12, 13, 15, 17, 21** and the precursors **16** and **20** were determined by single-crystal X-ray diffraction.

The ^{1}H NMR spectra of all imidazolium and xanthinium salts show a characteristic downfield resonance from the C^{2}–H proton in the range of 9.38–10.15 ppm due to the positive charge carried by the NC^{2}HN portion of the imidazolium cation. These chemical shifts are consistent with those observed in the ^{1}H NMR spectra of structurally similar compounds. In the ^{13}C NMR spectra, the corresponding NC^{2}N carbon resonances do not undergo a downfield shift as substantial as that observed in the ^{1}H NMR spectra; however, the number of and chemical shifts of the resonances in each ^{13}C NMR spectrum fully support the formation of the desired products. Additionally, ESI mass spectrometry in the positive mode was performed on the imidazolium salts, and the identity of each product was strongly supported by the presence of the corresponding $[M–Br]^{+}$ or $[M–I]^{+}$ fragment in its spectrum. The melting points of the imidazolium salts range from 155 to 195 °C, with the exception of **13**, which melts at a significantly higher temperature (261–263 °C).

In an attempt to synthesize asymmetric imidazolium salts containing aromatic substituents, an unexpected result was obtained. The addition of an equimolar amount of benzyl bromide to the reaction mixture presumably containing 4,5-dichloro-1-(naphthalen-2-ylmethyl)imidazole yielded the expected asymmetric imidazolium salt **11**, as well as the symmetric salts IC23 and **10**. In the ^{1}H NMR spectrum, three separate downfield resonances in the C^{2}–H region suggested the presence of three unique imidazolium salts, and two of the chemical shift values were identical to those observed in the spectra of pure samples of IC23 and **10**. The identities of the three salts were further supported by positive mode ESI mass spectrometry data, which gave peaks that corresponded to the expected $[M–Br]^{+}$ fragment of **10**, **11**, and IC23. These results suggested that the substituents of these imidazolium salts have some degree of lability.

In order to confirm that the substituents of **10**, **11**, and IC23 can undergo elimination and subsequent "scrambling," an analytically pure sample of IC23 was dissolved in acetonitrile and an equimolar amount of benzyl bromide was added. After refluxing the mixture overnight, the products were characterized by ^{1}H NMR spectroscopy. The spectrum showed the resonances for **10**, **11**, and IC23 previously observed in the synthesis of **11**. The addition of an equimolar amount of (2-bromomethyl)naphthalene to an analytically pure sample of **10** and subsequent heating at reflux resulted in the same mixture of products, as identified by ^{1}H NMR spectroscopy, indicating some degree of reversibility in these reactions (Fig. 13). Compound **11** was eventually isolated by following the progress of the reaction between 4,5-dichloro-1-(napthalen-2-ylmethyl)-imidazole and benzyl bromide by ^{1}H NMR spectroscopy. When the reaction was allowed to proceed for 8 h, only **11** was isolated. At reaction times longer than 8 h, the ^{1}H NMR spectra of aliquots of the reaction mixture indicated the presence of multiple imidazolium salts.

Reactions similar to those previously discussed were performed on the symmetric imidazolium salts **12** and **13** and the asymmetric xanthinium salt **15**. Analytically pure samples of these three compounds, each containing at least one naphthylmethyl substituent, were dissolved in an appropriate solvent and refluxed overnight with an equimolar amount of benzyl bromide. Interestingly, the ^{1}H NMR spectra of the resulting products contained only one downfield resonance that could be attributed to the C^{2}–H (or C^{8}–H for the xanthinium salt) proton, indicating that the naphthylmethyl substituents remained bound to the *N*-heterocycle. This result suggests that electron withdrawing groups off of the C^{4} and C^{5} atoms (Cl in IC23, **10**, and **11**) may be facilitating the observed reversibility of these reactions. The substituents of the C^{4} and C^{5} atoms have a large influence on the electronics of the front portion of the imidazole, as evidenced by the stability studies of metal *N*-heterocyclic carbene complexes, making this a reasonable theory (Hindi *et al*., 2008). The notion that electronics are playing a significant role in this observed reversibility is further supported by the synthesis of **23**, an asymmetric imidazolium salt containing both a naphthylmethyl and a quinolylmethyl substituent. Although **23** is based on 4,5-dichloroimidazole, it can be synthesized in high yield as the only product with no evidence of the formation of IC23 or the bis-quinoline imidazolium salt. The presence of the nitrogen atom provides some degree of stability for the quinolylmethyl substituent, allowing for the formation of the asymmetric product.

It is also of note that the observed reversibility affects only aromatic substituents with a methyl linker. Compounds **1–9**, each containing an alkyl substituent, were the only imidazolium salts detected in their respective syntheses. Furthermore, a series of asymmetric compounds containing ethylnaphthalene substituents, with the linker in both the 1 and 2 positions of naphthalene, was synthesized without any observed reversibility. Compounds **18**, **19**, and **22** were all synthesized in moderate to good yields as the only imidazolium salt detected, despite the presence of C^{l} atoms as the C^{4} and C^{5} substituents.

IC23 has displayed anticancer activity against several cancer cell lines when applied in single-digit micromolar concentrations despite its severe lack of solubility in water (<0.1 mg/mL), which is problematic for the systemic delivery of the complex. We believe the activity may be a result of the hydrophobic nature of the substituents, specifically the two

naphthylmethyl groups. The aromaticity and planarity of these groups are traits shared by several other anticancer agents that are efficacious due to their intercalation into the base pairs of DNA and introduce structural distortions that inhibit transcription and replication.

Due to the low water solubility of these imidazolium salts, it was difficult to determine the amount of compound that would dissolve into water. A saturated solution of the compound was stirred for several hours. The solution was passed through a syringe with a filter attached to remove any of the undissolved solid. A known amount of this solution was added to a pre-weighed round bottom flask. The solvent was evaporated to dryness by rotary evaporation. The mass of the flask was compared to the mass of the preweighed flask to determine the degree of water solubility. Several analogs of IC23 were synthesized in an effort to increase the water solubility of the complexes relative to IC23 while maintaining the anti-tumor efficacy.

Similar to IC23, **1–9** are derived from 4,5-dichloroimidazole and contain one naphthalene group in order to allow for possible interaction with DNA base pairs; however, the second substituent was replaced with alkyl chains of varying length. These alkyl chains allow the molecule to maintain its hydrophobic nature, and the replacement of a naphthalene substituent reduces the stabilizing pi-stacking interactions, which can exclude solvent molecules. As a result, the water solubility of the compounds was markedly improved from that of IC23, and a trend was observed. The highest water solubility was observed for **1**, containing an ethyl substituent, at 13.5 mg/mL. Compound **3**, with an isopropyl substituent, also displayed a large increase in water solubility at 10.8 mg/mL. Compounds **2** and **4**, containing a propyl and butyl chain, respectively, showed moderate water solubility at 5.2 and 5.7 mg/mL. As the alkyl chain length was increased, the water solubility of the compounds decreased, presumably as a result of the increase in intermolecular forces between the longer alkyl chains acting in a similar manner to the $\pi-\pi$ interactions of naphthalene groups.

In a separate attempt to confer water solubility to IC23 analogs, compounds **17** and **18** were synthesized, with each containing at least one ethyl linker between the *N*-heterocycle and the naphthalene ring, which was bound in the C^1 position. The rationale behind the design of these complexes centered on the introduction of additional degrees of freedom via the introduction of the ethyl, rather than methyl, linker. The naphthalene portion of the substituent has additional freedom with the ethyl linker compared to the methyl linker, which can reduce the intermolecular pistacking that likely occurs (pi-stacking has been observed in the solid state for similar molecules). Intermolecular pi-stacking may also be perturbed by the orientation of the naphthalene bound in the C^1 position, rather than in the C^2 position as in IC23. The water solubility of **17** was mildly increased from that of IC23 to 0.4 mg/mL. Compound **18**, containing one naphthylethyl and one naphthylmethyl substituent, did not show a significant increase in water solubility compared to IC23. Compound **21**, the symmetric isomeric analog to **17**, was synthesized to determine the effects of changing the point of attachment of the linker group to the naphthalene ring.

A modest increase in water solubility was obtained by including a heteroatom in the aromatic substituent. The inclusion of a quinoline group bound with a methyl linker in **23**

increased the solubility of the compound relative to IC23 to 0.9 mg/mL. The increase in water solubility is believed to be a consequence of the inclusion of a hydrogen bond acceptor into the fused aromatic substituent, allowing for a greater interaction of solvent molecules with the compound. The addition of the nitrogen atom does not perturb the planarity of the aromatic system, allowing the substituent to maintain its ability to possibly intercalate into other planar environments.

A compound derived from xanthine also demonstrated a significant increase in water solubility. Xanthine and several of its derivatives possess a high degree of water solubility, are relatively nontoxic, and have demonstrated clinical significance as treatments for several pulmonary ailments. A naphthylmethyl substituent was added to the xanthine derivative theophylline to yield the imidazolium salt **15**, which has solubility in water at 2.6 mg/mL.

The previous results demonstrate that one of the major drawbacks of the efficacious imidazolium salt IC23, a lack of water solubility and resulting inability to deliver the compound systemically, can be remedied using several different strategies. The analogous compounds discussed have also been analyzed for their in vitro anticancer activity.

The in vitro anticancer activity of **1–23** against the NSCLC cell lines NCI-H460, NCI-H1975, and HCC827 was evaluated, and the results are summarized in Table 1. The cells were exposed to compounds for 72 h after which a standard MTT assay was used to determine the cell viability. From these data, IC_{50} values were determined, where the IC_{50} value denotes the median concentration of the compound that causes a 50 % inhibition in cell viability, relative to control cells. Several of the imidazolium salts displayed IC_{50} values in the single-digit micromolar range against one or more of the NSCLC lines, which is on par with the activity of cisplatin.

Imidazolium salts **1–9**, each containing a naphthylmethyl substituent and a varying alkyl substituent, varied greatly in their effectiveness. Compounds **1–3** showed little to no efficacy against any of the cell lines, despite having the highest solubility in water, with IC_{50} values ranging from 19 μM to greater than 30 μM against the panel of cancer cell lines. As the alkyl substituent was lengthened, the efficacy of the corresponding compounds began to improve but the solubility decreased with the added lipophilicity. Compound **5** displayed IC_{50} values of 10–12 μM, while the IC_{50} values of **6–9** were less than or equal to 10 μM. These values are in the same range as those observed for cisplatin (H460—3 μM; H1975—10 μM; HCC827—4 μM) and IC23 (5 μM; 6 μM; 8 μM). In this series of compounds, **1–9** have either sufficient solubility or comparable activity to IC23, but none have both of these characteristics. There needs to be a balance between hydrophilicity and lipophilicity, which is not met with **1–9** when exchanging one of the naphthylmethyl groups for an alkyl chain.

The symmetric imidazolium salt **10** containing two benzyl substituents did not demonstrate any relevant anticancer activity, with an IC_{50} value of greater than 30 μM for all three cell lines. The lack of activity demonstrated by **10** suggests that an extended planar, aromatic system as a substituent, is essential to anticancer efficacy in NSCLC cell lines. This notion is supported by **11**, in which one of the benzyl substituents in **10** has been replaced with a naphthylmethyl group. The IC_{50} values of **11** fall between 12 and 15 μM against the three

cell lines, a substantial improvement over the activity of **10**. Anticancer activity near that of IC23 and cisplatin was restored in **12** (H460—4 μM; H1975— 6 μM; HCC827—9 μM) and **13** (3 μM; 4 μM; 5 μM), both of which contain two methylnaphthyl substituents.

The trisubstituted xanthine precursor **14** was tested against the panel of cancer cells and showed moderate efficacy, especially against the H460 (8 μM) and H1975 (12 μM) lines. This result demonstrated that the cationic nature of the imidazole or imidazole-containing molecule was not a requirement for anticancer activity. In fact, the formation of xanthinium salt via the methylation of **14** yielded **15**, which had no observable anti-tumor activity against the three cell lines. Compound **15** had increased water solubility compared to IC23 but severely decreased activity. These results are not surprising considering compound **1**, with one naphthylmethyl and one methyl group bound to the imidazole base, had high solubility but was also rendered inactive.

The imidazolium salts containing naphthalene substituents bound in the 1 or 2 position of the naphthalene ring with either methyl or ethyl linkers demonstrated promising anticancer activity against all three lines tested. The IC_{50} values of **18** were all in the range of cisplatin and IC23 at 5 μM, 6 μM, and 7 μM for the H460, H1975, and HCC827 lines, respectively. Also in this range were **17** (8 μM; 8 μM; 11 μM), **21** (8 μM; 8 μM; 8 μM), and **22** (4 μM; 7 μM; 9 μM). Consistent with earlier results, the benzyl-substituted salt **19** possessed IC_{50} values (12 μM, 14 μM, >30 μM) noticeably higher than those of the naphthalene-containing compounds. Although **17** and **18** had similar IC_{50} values to IC23, the solubilities were not significantly higher than IC23. Therefore, changing the methyl linker to an ethyl linker is not a sufficient modification to increase the solubility.

Compound **23** demonstrated that the inclusion of a heteroatom into the extended planar, aromatic system, did not substantially alter the anticancer activity of the system. The IC_{50} values of **23** were very similar to that of IC23 at 10 μM (H460), 5 μM (H1975), and 9 μM (HCC827). The added hydrogen-bonding acceptor caused a dramatic increase in the aqueous solubility with at least a 10-fold increase from IC23 while maintaining the high activity. Various functional groups had drastic effects on the solubility and activity of these imidazolium salts, but it is clear that there needs to be a balance between hydrophilicity and lipophilicity for the compound to remain active while achieving solubility at clinically relevant levels.

Treatment of H460 cells with the listed imidazolium compounds causes morphological changes that include cell shrinking, rounding, and the eventual detachment of the cells from the culture plate as early as 1 h after the treatment (Fig. 14). The efficacy of these compounds as determined by MTT assay is comparable to cisplatin that we use as a standard chemotherapeutic drug in not only H460 cells (Fig. 15), but also in H1975 and HCC827 NSCLC lines. The IC_{50} data shown in the table above strongly suggest that these compounds are effective against the selected NSCLC lines. In an attempt to analyze the mechanism of cell death induced by the IC23, an Annexin V (conjugated to FITC) apoptosis detection kit was employed. This assay detects phosphatidylserine (PS) expressed on the surface of apoptotic cells and fluoresces green after interacting with the labeled Annexin V. During early apoptosis, membrane asymmetry is lost, and PS translocates from the

cytoplasmic side of the membrane to the external leaflet. Propidium iodide (PI), the counterstain used in this assay, has the ability to cross only compromised membranes to intercalate into the DNA. Therefore, PI is used to detect the different stages of apoptosis by the absence or presence of red fluorescence (Van Engeland *et al*., 1998). Figure 16 shows the progression of apoptosis caused by the introduction of compound IC23 into the growth medium at increasing time frames. As a comparison, negative and positive controls were tested alongside the IC23-treated cells. The negative control consisted of H460 cells grown in medium, and H460 cells grown in the presence of cisplatin constituted the positive control, as cisplatin is a known inducer of apoptosis. Treatment of cells with IC23 for a period of 1 h revealed cells in the early stage of apoptosis. These results were comparable to the cisplatin positive control images after treatment for 6 h. Cells treated with IC23 for a period of 3 h showed latestage apoptotic cells based on the visualization of both red and green fluorescence. Blebbing, a common indicator of apoptosis, was able to be visualized at this time point (Figs. 16d, 17). After treatment of IC23 for 6 h, imaged cells showed both red and green fluorescence. This combined with the absence of blebbing lead to the conclusion that these cells were in the end stage of apoptosis and were no longer viable. These results indicate that treatment of H460 cells with IC23 induces apoptosis at a faster rate than what is observed with cisplatin.

In a similar experiment, H460 cells were treated with the IC23 analog **12**, which employs hydrogen in the C^4 and C^5 positions rather than chlorine. While cells could be visualized in the early stages of apoptosis, this was not seen until the latest time period at 6 h. These cells were relatively comparable to the cisplatin-treated cells visualized at the same time period. Treatment with **12** at 1 and 3 h was similar to the control cells. When comparing these cells to the IC23-treated cells, it is obvious that while **12** can cause H460 cells to begin the early stages of apoptosis, it takes a longer time frame to do so than IC23. This experiment shows that while there may be a difference in time needed for full effect of naphthalene-substituted analogs, they do cause cells to go through the process of apoptosis.

To further confirm that these compounds induce apoptotic cell death, in parallel experiments, we also analyzed the expression and cleavage of apoptotic proteins caspase-3 and PARP cleavage by Western blotting (Fig. 18). We found the presence of full-length PARP and procaspase-3 proteins with no cleaved products in the control cell lysates indicating the absence of apoptosis. However, cell lysates treated with IC23 at 12 h showed the presence of cleaved PARP and substantial reduction in the levels of procaspase-3 confirming apoptotic cell death. A possible reason that we could not detect any cleaved caspase-3 could be explained by its short half-life in the cells. Cell lysates from cells treated with cisplatin served as a control. Further, equal protein loading in the samples was confirmed by stripping the blots and probing with a housekeeping protein, GAPDH, and the levels of GAPDH were equal in all the analyzed samples.

Conclusions

In this paper, we have presented a series of novel imidazolium salts that have anticancer activity comparable to that of cisplatin as tested on the three NSCLC cell lines NCI-H460, NCI-H1975, and HCC827 using MTT assays. The efficacy of these compounds is tied to the

interaction of the imidazolium cation and at least one naphthylmethyl substituent. A combination of naphthylmethyl on one nitrogen atom of an imidazole and an alkyl group on the other nitrogen atom gives significant anticancer activity as demonstrated by compounds **6, 7, 8,** and **9**. Compounds IC23, **12, 13, 17,** and **18** with a naphthylmethyl substituent on each of the imidazole nitrogen atoms have similar IC_{50} values with either hydrogens or chlorides in the C^4 and C^5 positions of the imidazole ring or as the benzimidazole analog.

Further studies based on the morphological changes in tested cells showed that IC23 produced similar staining patterns using an Annexin V assay, but at different time frames as cisplatin, a known apoptosis inducer. Western blot staining of NCI-H460 cells showed IC23-induced PARP-1 cleavage and a reduction in procaspase-3 as compared to cisplatin. The Annexin V, caspase-3, and PARP-1 results indicate that IC23 is inducing the apoptotic pathway in the cancer cells.

In whole, these results show that imidazolium salts with naphthylmethyl substituents should be studied further to understand their mechanism of action and possible uses as chemotherapeutics agents.

Experimental details

General considerations

All reactions were carried out under aerobic conditions unless otherwise specified. Benzimidazole, imidazole, 4,5-dichloroimidazole, and the alkyl halides were purchased from Alfa Aesar. 2-(Bromomethyl)naphthalene was purchased from Waterstone Technologies. 1-(2-Bromoethyl)naphthalene, 2-(naphthalen-1-yl)ethanol and 2-(naphthalen-2-yl)ethanol were purchased from Sigma-Aldrich. 1-(2-Bromoethyl)naphthalene and 2-(2-bromoethyl)naphthalene were synthesized via a literature procedure (Ellames and Herbert, 2010). All solvents were purchased from Fisher Scientific and used without further purification. All reagents were used as received without further purification. Melting points were obtained on a MelTemp apparatus. ^1H and ^{13}C NMR spectra were collected on a Varian 500 MHz instrument with all spectra referenced to residual deuterated solvents (DMSO-d_6: 2.50 ppm). Mass spectrometry was performed by the University of Akron mass spectrometry laboratory. Elemental analysis was performed by the Microanalysis Laboratory at University of Illinois at Urbana-Champaign.

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 a Bruker APEX CCD diffractometer with graphite-monochromated Mo K_α radiation (λ = 0.71073 Å) or a Bruker APEX II Duo CCD system equipped with a Cu ImuS microfocus source (λ = 1.54178 Å). The unit cells were determined by using reflections from three different orientations. The data sets were integrated using SAINT (Bruker, 1997, 2007). An empirical absorption correction and other corrections were applied to the data sets using multi-scan SADABS (Ellames and Herbert, 2010). Structure solution, refinement, and modeling were accomplished by using the Bruker SHELXTL package (Bruker, 1997; Sheldrick, 2008). 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.

Cell culture

All cell lines were grown in RPMI-1640 medium supplemented with 10 % fetal bovine serum and maintained at 37 °C in humidified 5 % CO_2 environment, unless otherwise stated.

MTT assay

The human cancer cell lines NCI-H460, NCI-H1975, and HCC827 were grown to confluency and then plated in 96-well plates at 5,000–7,000 cells per well and incubated for 24 h. Tested compounds and blanks were then suspended in a 1 % DMSO/water solution and diluted into cell culture medium to the desired concentrations. Medium was removed and replaced with fresh medium containing the test compounds (six replicates each in triplicate). The plates were allowed to incubate for 72 h, after which the optional MTT protocol was followed. MTT (10 μL) in PBS was added to each well, and the plates were incubated for 4 h. The medium was carefully removed, and 100 μL of DMSO was added per well. The optical density of each well was read at 540 nm on a SpectraMax microplate reader.

Cell lysis, gel electrophoresis, and immunoblotting

NCI-H460 cells (1×10^5 cells per well) were grown to confluency in a 6-well plate, using RPMI-1640 medium supplemented with 10 % fetal bovine serum and 1 % penicillin-streptomycin, and then serum-starved for 24 h. Cells were stimulated with indicated compounds for the indicated periods of time and lysed with ice-cold lysis buffer (50 mM Tris [pH 7.5], 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 1 % Triton X-100, 50 mM NaF, 5 mM sodium pyrophosphate, 10 mM sodium glycerophosphate, 4 mg/mL Leupeptin, and 30 mg/mL phenylmethanesulfonyl-fluoride, PMSF) supplemented with protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Pierce) as described earlier (Kondeti *et al*., 2013; Duah *et al*., 2013). Briefly, cell lysates were boiled at 100 °C for 5 min in a sample buffer [62 mM Tris (pH 6.8), 1.0 % SDS, 10 % glycerol, 15 mg/mL dithiothreitol, and 0.05 % bromophenol blue]. Equal amount of lysates was subjected to SDS-PAGE on 4–12 %—gels (Bio-Rad).

The separated proteins were electrophoretically transferred to a PVDF membrane. All membranes were blocked for 1 h with 5 % nonfat dried milk in TBS containing 0.05 % Tween 20 (TBS-T) at room temperature and then incubated with a primary antibody overnight at 4 °C. A 1:1,000 dilution of PARP, caspase-3 (cell signaling), and GAPDH (Fitzgerald) primary antibodies was used. Membranes were washed extensively in TBS-T and incubated with a horseradish-peroxidase-linked goat anti-rabbit antibody (1:5,000) for 1 h at room temperature. Thereafter, the membrane was again washed extensively, incubated with ECL Western blot detection reagents (GE Healthcare), and finally, the bands were visualized using an imager (Protein Simple®). Blots were stripped and reprobed with GAPDH antibody for normalization.

Annexin V assay

The Annexin V assay was conducted using the components of a FITC Annexin V Apoptosis Detection Kit I (BD Biosciences). The NCI-H460 cell line was grown in 6-well plates at a density of 1×10^5 cells per well. After allowing cells to adhere overnight, they were treated with cisplatin and IC23 at a concentration of 40 μM. Cisplatin and IC23 were first solubilized in a 1 % DMSO/water solution and were further diluted to the aforementioned concentration in culture medium. The first treatment consisted of aspirating off medium of a 6-well plate and replacing with fresh medium for control cells, cisplatin supplemented medium for cisplatin-treated cells, and IC23 supplemented medium for IC23-treated cells (2 wells per treatment type). The remaining IC23 treatments were conducted in the same manner at their respective times. After treatment, the medium was aspirated, and cells were washed twice with cold PBS. The provided 10× binding buffer was diluted to 1×, and 300 μL was added to each well. Subsequently, 15 μL each of the provided FITC Annexin V and PI was also added to each well. The plates were incubated for 15 min on a platform shaker while covered with aluminum foil at room temperature. After this time, the binding buffer was aspirated from the wells, and 1 mL of the 1× binding buffer was added to each well for imaging. The fluorescence microscope used was an EVOS fl Digital Inverted Microscope with the 10× and 20× objectives.

Synthesis of 4,5-dichloro-1-ethyl-3-(naphthalen-2-ylmethyl)-imidazolium bromide (1)—4,5-Dichloroimidazole (1.23 g, 9.0 mmol) was dissolved in 27 mL of acetonitrile, potassium hydroxide (0.61 g, 9.9 mmol) was added, and the mixture was stirred for 0.5 h. Bromoethane (0.66 mL, 9.0 mmol) was added, and the solution was refluxed overnight. The solution was filtered hot to remove a white precipitate (presumed to be KBr), (2-bromomethyl)naphthalene (1.98 g, 9.0 mmol) was added, and the mixture was refluxed overnight. The mixture was allowed to cool to room temperature, resulting in a white precipitate that was isolated by filtration and air-dried. (0.71 g, 20 % yield). Mp: 192–194 °C. Found: C, 49.6; H, 3.8; N, 7.1 Calc. for $C_{16}H_{15}Cl_2N_2Br_1$: C, 49.8; H, 3.9; N, 7.2 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.62 (1H, s, NCHN), 7.61 (4H, m, Ar), 7.58 (3H, m, Ar), 5.66 (2H, s, NCH_2), 4.27 (2H, q, NCH_2), 1.47 (3H, t, CH_3). ^{13}C{ ^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.2 (NCN), 132.8 (Ar), 132.6 (Ar), 130.1 (Ar), 128.7 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 126.8 (Ar), 126.7 (Ar), 125.5 (Ar), 119.0 (Ar), 118.6 (Ar), 51.6 (CH_2), 44.2 (CH_2), 13.7 (CH_3). MS: *m/z* = 304.8 (theor for M^+ $C_{16}H_{15}Cl_2N_2$ = 306.2).

Synthesis of 4,5-dichloro-3-(naphthalen-2-ylmethyl)-1-propylimidazolium bromide (2)—4,5-Dichloroimidazole (1.23 g, 9.0 mmol) was dissolved in 27 mL of acetonitrile, potassium hydroxide (0.61 g, 9.9 mmol) was added, and the mixture was stirred for 0.5 h. 1-Iodopropane (0.88 mL, 9.0 mmol) was added, and the solution was refluxed overnight. The solution was filtered hot to remove a white precipitate (presumed to be KI), 2-bromomethylnaphthalene (1.98 g, 9.0 mmol) was added, and the mixture was refluxed overnight. The mixture was allowed to cool to room temperature, resulting in a yellow precipitate that was isolated by filtration and air-dried (1.13 g, 31 % yield). Mp: 157–159 °C. Found: C, 50.4; H, 4.2; N, 6.7 Calc. for $C_{17}H_{17}Cl_2N_2Br_1$: C, 51.0; H, 4.3; N, 7.0 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.58 (1H, s, NCHN), 8.02 (4H, m, Ar), 7.57 (3H, m, Ar), 5.66 (2H, s, NCH_2), 4.21 (2H, t, NCH_2), 1.85 (2H, tq, CH_2), 0.94 (3H, t, CH_3). ^{13}C{^{1}H}

NMR (125 MHz, DMSO-d_6) δ = 136.4 (NCN), 132.8 (Ar), 132.6 (Ar), 130.0 (Ar), 128.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 126.9 (Ar), 126.8 (Ar), 125.5 (Ar), 119.1 (Ar), 118.8 (Ar), 51.6 (CH_2), 50.0 (CH_2), 21.7 (CH_2), 10.3 (CH_3). MS: *m/z* = 318.8 (theor for M^+ $C_{17}H_{17}Cl_2N_2$ = 319.2).

Crystal data for 2—$C_{18}H_{19}BrCl_2N_2$, M = 414.16, triclinic, a = 10.5375(3) Å, b = 12.6980(3) Å, c = 14.5472(4) Å, α = 108.360(2)°, β = 100.900(2)°, γ = 90.726(2)°, V = 1,808.62(8) Å ^{3}, T = 100(2) K, space group $P\bar{1}$, Z = 4,13,391 reflections measured, 5,647 independent reflections (R_{int} = 0.0352). The final R_1 values were 0.0531 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1372 ($I > 2\sigma(I)$). The final R_1 values were 0.0648 (all data). The final $wR(F^2)$ values were 0.1499 (all data).

Synthesis of 4,5-dichloro-1-isopropyl-3-(naphthalen-2-ylmethyl)imidazolium bromide (3)—4,5-Dichloroimidazole (1.23 g, 9.0 mmol) was dissolved in 27 mL of acetonitrile, potassium hydroxide (0.61 g, 9.9 mmol) was added, and the mixture was stirred for 0.5 h. 2-Iodopropane (0.88 mL, 9.0 mmol) was added, and the solution was refluxed overnight. The solution was filtered hot to remove a white precipitate (presumed to be KI), (2-bromomethyl)naphthalene (1.98 g, 9 mmol) was added, and the mixture was returned to reflux overnight. The mixture was then allowed to cool to room temperature, resulting in a white crystalline solid that was isolated by filtration and air-dried (1.33 g, 36 % yield). Mp: 182–0184 °C. Found: C, 50.9; H, 4.2; N, 6.7 Calc. for $C_{17}H_{17}Cl_2N_2Br_1$: C, 51.0; H, 4.3; N, 7.0 %. ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 9.82 (1H, s, NCHN), 7.99 (4H, m, Ar), 7.57 (3H, m, Ar), 5.66 (2H, s, NCH_2), 4.71 (1H, 5, NCH), 10.94 (6H, t, CH_3). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 135.2 (NCN), 132.8 (Ar), 132.6 (Ar), 130.0 (Ar), 128.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 126.9 (Ar), 126.8 (Ar), 125.5 (Ar), 119.1 (Ar), 118.8 (Ar), 51.6 (CH_2), 50.0 (CH_2), 21.7 (CH_2), 10.3 (CH_3). MS: *m/z* = 318.8 (theor for M^+ $C_{17}H_{17}Cl_2N_2$ = 319.2).

Synthesis of 1-butyl-4,5-dichloro-3-(naphthalen-2-ylmethyl)-imidazolium bromide (4)—4,5-Dichloroimidazole (1.23 g, 9.0 mmol) was dissolved in 27 mL of acetonitrile, potassium hydroxide (0.61 g, 9.9 mmol) was added, and the mixture was stirred for 0.5 h. 1-Iodobutane (1 mL, 9.0 mmol) was added, and the solution was refluxed overnight. The solution was filtered hot to remove a white precipitate (presumed to be KI), (2-bromomethyl)naphthalene (1.98 g, 9.0 mmol) was added, and the mixture was refluxed overnight. The mixture was then allowed to cool to room temperature, resulting in a yellow precipitate that was isolated by filtration and air-dried (1.96 g, 53 % yield). Mp: 160–163 °C. Found: C, 52.3; H, 4.6; N, 6.6 Calc. for $C_{18}H_{19}Br_1Cl_2N_2$: C, 52.2; H, 4.6; N, 6.8 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.66 (1H, s, NCHN), 8.02 (4H, m, Ar), 7.57 (3H, m, Ar), 5.66 (2H, s, NCH_2), 4.24 (2H, t, NCH_2), 1.80 (2H, tt, CH_2), 1.37 (2H, tq, CH_2), 0.93 (3H, t, CH_3). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.4 (NCN), 132.8 (Ar), 132.6 (Ar), 130.0 (Ar), 128.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 126.8 (Ar), 126.7 (Ar), 125.5 (Ar), 119.1 (Ar), 118.7 (Ar), 51.6 (CH_2), 48.3 (CH_2), 30.1 (CH_2), 18.7 (CH_2), 13.2 (CH_3). MS: *m/z* = 332.9 (theor for M^+ $C_{18}H_{19}Cl_2N_2$ = 334.2).

Crystal data for 4—$C_{17}H_{17}Cl_2N_2 \cdot Br$, M = 400.14, triclinic, a = 10.2051(3) Å, b = 12.9647(4) Å, c = 13.5156(4) Å, α = 105.576(2)°, β = 96.998(2)°, γ = 90.045(2)°, V = 1,708.64(9) Å^3, T = 100(2) K, space group $P\bar{1}$, Z = 4,37,315 reflections measured, 8,647 independent reflections (R_{int} = 0.0509). The final R_1 values were 0.0420 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0954 ($I > 2\sigma(I)$). The final R_1 values were 0.0671 (all data). The final $wR(F^2)$ values were 0.1118 (all data).

Synthesis of 4,5-dichloro-3-(naphthalen-2-ylmethyl)-1-pentylimidazolium bromide (5)—4,5-Dichloroimidazole (1.23 g, 9.0 mmol) was dissolved in 27 mL of acetonitrile, potassium hydroxide (0.61 g, 9.9 mmol) was added, and the mixture was stirred for 0.5 h. 1-Bromopentane (1.1 mL, 9.0 mmol) was added, and the solution was refluxed overnight. The solution was filtered hot to remove a white precipitate (presumed to be KBr), (2-bromomethyl)naphthalene (1.98 g, 9.0 mmol) was added, and the mixture was refluxed overnight. The mixture was then allowed to cool to room temperature, resulting in a yellow precipitate that was isolated by filtration and air-dried (1.79 g, 49 % yield). Mp: 168–169 °C. Found: C, 53.1; H, 4.7; N, 6.4 Calc. for $C_{19}H_{21}Cl_2N_2Br_1$: C, 53.3; H, 4.9; N, 6.5 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.66 (1H, s, NCHN), 8.02 (4H, m, Ar), 7.57 (3H, m, Ar), 5.66 (2H, s, NCH_2), 4.24 (2H, t, NCH_2), 1.80 (2H, tt, CH_2), 1.37 (2H, tq, CH_2), 0.93 (3H, t, CH_3). ^{13}C{ ^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.4 (NCN), 132.8 (Ar), 132.6 (Ar), 130.0 (Ar), 128.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 126.8 (Ar), 126.7 (Ar), 125.5 (Ar), 120.9 (Ar), 114.1 (Ar), 112.9 (Ar), 51.6 (CH_2), 48.5 (CH_2), 27.8 (CH_2), 27.4 (CH_2), 21.4 (CH_2), 13.6 (CH_3). MS: m/z = 346.8 (theor for M^+ $C_{19}H_{21}Cl_2N_2$ = 348.3).

Synthesis of 4,5-dichloro-1-hexyl-3-(naphthalen-2-ylmethyl)-imidazolium bromide (6)—4,5-Dichloroimidazole (1.23 g, 9.0 mmol) was dissolved in 27 mL of acetonitrile, potassium hydroxide (0.61 g, 9.9 mmol) was added, and the mixture was stirred for 0.5 h. 1-Bromohexane (1.26 mL, 9.0 mmol) was added, and the solution was refluxed overnight. The solution was filtered hot to remove a white precipitate (presumed to be KBr), (2-bromomethyl)naphthalene (1.98 g, 9.0 mmol) was added, and the mixture was refluxed overnight. The mixture was then allowed to cool to room temperature, resulting in a yellow precipitate that was isolated by filtration and air-dried (2.57 g, 65 % yield). Mp: 165–166 °C. Found: C, 54.3; H, 5.2; N, 6.2 Calc. for $C_{20}H_{23}Cl_2N_2Br_1$: C, 54.3; H, 5.2; N, 6.3 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.64 (1H, s, NCHN), 8.02 (4H, m, Ar), 7.57 (3H, m, Ar), 5.66 (2H, s, NCH_2), 4.22 (2H, t, NCH_2), 1.80 (2H, tt, CH_2), 1.30 (6H, m, CH_2), 0.93 (3H, t, CH_3). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.4 (NCN), 132.8 (Ar), 132.6 (Ar), 130.0 (Ar), 128.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 126.8 (Ar), 126.7 (Ar), 125.5 (Ar), 119.1 (Ar), 51.6 (CH_2), 48.3 (CH_2), 30.5 (CH_2), 28.0 (CH_2), 24.9 (CH_2), 21.8 (CH_2), 13.2 (CH_3). MS: m/z = 361.1 (theor for M^+ $C_{20}H_{23}Cl_2N_2$ = 362.3).

Crystal data for 6—$C_{20}H_{23}Cl_2N_2 \cdot Br$, M = 442.21, monoclinic, a = 16.787(7) Å, b = 10.075(4) Å, c = 12.824(5) Å, β = 110.121(7)°, V = 2,036.7(14) Å^3, T = 100(2) K, space group $P2(1)/c$, Z = 4, 15,705 reflections measured, 4,126 independent reflections (R_{int} = 0.0879). The final R_1 values were 0.0542 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0956 ($I > 2\sigma(I)$). The final R_1 values were 0.0883 (all data). The final $wR(F^2)$ values were 0.1058 (all data).

Synthesis of 4,5-dichloro-1-heptyl-3-(naphthalen-2-ylmethyl)-imidazolium bromide (7)—4,5-Dichloroimidazole (1.23 g, 9.0 mmol) was dissolved in 27 mL of acetonitrile, potassium hydroxide (0.61 g, 9.9 mmol) was added, and the mixture was stirred for 0.5 h. 1-Bromoheptane (1.41 mL, 9.0 mmol) was added, and the solution was refluxed overnight. The solution was filtered hot to remove a white precipitate (presumed to be KBr), (2-bromomethyl)naphthalene (1.98 g, 9.0 mmol) was added, and the mixture was refluxed overnight. The mixture was then allowed to cool to room temperature, resulting in a light yellow precipitate that was isolated by filtration and air-dried (2.73 g, 67 % yield). Mp: 165–166 °C. Found: C, 55.9; H, 5.4; N, 5.9 Calc. for $C_{21}H_25Cl_2N_2Br_1$: C, 55.3; H, 5.5; N, 6.1 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.67 (1H, s, NCHN), 7.97 (4H, m, Ar), 7.57 (3H, m, Ar), 5.67 (2H, s, NCH_2), 4.23 (2H, t, NCH_2), 1.82 (2H, m, CH_2), 1.29 (8H, m, CH_2), 0.86 (3H, t, CH_3). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.4 (NCN), 132.8 (Ar), 132.6 (Ar), 130.1 (Ar), 128.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 126.9 (Ar), 126.7 (Ar), 125.5 (Ar), 119.0 (Ar), 118.7 (Ar), 51.6 (CH_2), 48.6 (CH_2), 31.0 (CH_2), 28.1 (CH_2), 28.0 (CH_2), 25.3 (CH_2), 21.9 (CH_2), 13.9 (CH_3). MS: *m/z* = 374.8 (theor for M^+ $C_{21}H_{25}Cl_2N_2$ = 376.3).

Synthesis of 4,5-dichloro-3-(naphthalen-2-ylmethyl)-1-nonylimidazolium bromide (8)—4,5-Dichloroimidazole (1.23 g, 9.0 mmol) was dissolved in 27 mL of acetonitrile, potassium hydroxide (0.61 g, 9.9 mmol) was added, and the mixture was stirred for 0.5 h. 1-Bromononane (1.72 mL, 9.0 mmol) was added, and the solution was allowed to reflux overnight. The solution was filtered hot to remove a white precipitate (presumed to be KBr), (2-bromomethyl)naphthalene (1.98 g, 9.0 mmol) was added, and the mixture was refluxed overnight. The mixture was then allowed to cool to room temperature, resulting in a yellow/white precipitate that was isolated by filtration and air-dried (2.73 g, 56 % yield). Mp: 160–161 °C. Found: C, 57.0; H, 5.9; N, 5.7 Calc. for $C_{23}H_{29}Cl_2N_2Br_1$: C, 57.0; H, 6.0; N, 5.8 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.67 (1H, s, NCHN), 7.97 (4H, m, Ar), 7.57 (3H, m, Ar), 5.67 (2H, s, NCH_2), 4.23 (2H, t, NCH_2), 1.81 (2H, m, CH_2), 1.28 (12H, m, CH2), 0.85 (3H, t, CH3). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.4 (NCN), 132.8 (Ar), 132.6 (Ar), 130.1 (Ar), 128.7 (Ar), 127.8 (Ar), 127.6 (Ar), 127.6 (Ar), 126.8 (Ar), 126.7 (Ar), 125.5 (Ar), 119.0 (Ar), 118.7 (Ar), 51.6 (CH_2), 48.6 (CH_2), 31.2 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 28.3 (CH_2), 28.1 (CH_2), 25.3 (CH_2), 22.0 (CH_2), 13.9 (CH_3). MS: *m/z* = 402.8 (theor for M^+ $C_{23}H_{29}Cl_2N_2$ = 402.4).

Synthesis of 4,5-dichloro-1-dodecyl-3-(naphthalen-2-ylmethyl)-imidazolium bromide (9)—4,5-Dichloroimidazole (1.23 g, 9.0 mmol) was dissolved in 27 mL of acetonitrile, potassium hydroxide (0.61 g, 9.9 mmol) was added, and the mixture was stirred for 0.5 h. 1-Bromododecane (2.16 mL, 9.0 mmol) was added, and the solution was refluxed overnight. The solution was filtered hot to remove a white precipitate (presumed to be KBr), (2-bromomethyl)naphthalene (1.98 g, 9.0 mmol) was added, and the mixture was refluxed overnight. The mixture was then allowed to cool to room temperature, resulting in a yellow/white precipitate that was isolated by filtration and air-dried (2.22 g, 47 % yield). Mp: 155–156 °C. Found: C, 59.5; H, 6.7; N, 5.2 Calc. for $C_{26}H_{35}Cl_2N_2Br_1$: C, 59.3; H, 6.7; N, 5.3 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.58 (1H, s, NCHN), 7.99 (4H, m, Ar), 7.58 (3H, m, Ar), 5.65 (2H, s, NCH2), 4.22 (2H, t, NCH2), 1.80 (2H, tt, CH2), 1.24 (12H, m, CH2), 0.87

(3H, t, CH3). $^{13}C\{^{1}H\}$ NMR (125 MHz, DMSO-d_6) δ = 136.4 (NCN), 132.8 (Ar), 132.6 (Ar), 130.0 (Ar), 128.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 126.8 (Ar), 126.7 (Ar), 125.5 (Ar), 119.1 (Ar), 118.7 (Ar), 51.6 (CH_2), 48.6 (CH_2), 31.2 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 25.3 (CH_2), 22.0 (CH_2), 13.9 (CH_3). MS: *m/z* = 445.0 (theor for M^+ $C_{26}H_{35}Cl_2N_2$ = 446.4).

Synthesis of 1,3-dibenzyl-4,5-dichloroimidazolium bromide (10)—4,5-Dichloroimidazole (1.37 g, 10 mmol) was dissolved in 10 mL of acetonitrile, potassium hydroxide (0.617 g, 11 mmol) was added, and the mixture was refluxed for 0.5 h. Benzyl bromide (1.71 g, 10 mmol) was added to the solution and refluxed for 2.5 h. The solution was filtered hot to remove a white precipitate (presumed to be KBr), a second equivalent of benzyl bromide (1.71 g, 10 mmol) was added, and the mixture was refluxed for 1 h. The resulting white precipitate was filtered and air-dried (1.61 g, 42 % yield). Mp: 177–178 °C. Found: C, 46.87; H, 3.44; N, 5.61 Calcd for $C_{17}H_{15}Cl_2N_2Br_1$: C, 47.14; H, 3.54; N, 5.79. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.86 (1H, s, NCHN) 7.42, 7.46 (10H, m, Ar), 5.55 (4H, s, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 136.68 (NCN), 132.61 (Ar), 128.93 (Ar), 128.84 (Ar), 128.27 (Ar), 119.11 (Ar), 51.50 (CH_2). MS: *m/z* = 317.1, (theor for M^+ $C_{17}H_{15}Cl_2N_2$ = 317.1).

Synthesis of 1-benzyl-4,5-dichloro-3-(naphthalen-2-ylmethyl)-imidazolium bromide (11)—4,5-Dichloroimidazole (0.68 g, 5.0 mmol) was dissolved in 10 mL of acetonitrile, potassium hydroxide (0.31 g, 5.5 mmol) was added, and the mixture was stirred for 1 h. (2-Bromomethyl)naphthalene (1.11 g, 5.0 mmol) was added, and the solution was refluxed for 3 h. The solution was filtered hot to remove a white precipitate (presumed to be KBr), benzyl bromide (0.58 g, 5.0 mmol) was added, and the mixture was refluxed for an 8 h. The mixture was cooled to room temperature, and the resulting white precipitate was filtered and air-dried (0.74 g, 33 %). Mp: 156–157 °C. Found: C, 56.3; H, 3.63; N, 6.15. Calcd for $C_{21}H_{16}Cl_2N_2Br_1$: C, 56.4; H, 3.61; N, 6.26. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.65 (1H, s, NCHN), 7.42–7.43, 7.45–7.46 (10H, m, Ar), 5.53 (s, 2H, CH_2). $^{13}C\{^{1}H\}$ NMR (125 MHz, DMSO-d_6) δ = 136.7 (NCN), 132.8 (Ar), 132.7 (Ar), 132.6 (Ar), 129.9 (Ar), 129.0 (Ar), 128.9 (Ar), 128.8 (Ar), 128.2 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 126.9 (Ar), 126.8 (Ar), 125.5 (Ar), 119.4 (C–Cl), 119.2 (C–Cl), 51.8 (CH_2), 51.5 (CH_2). MS: *m/z* = 317.1, (theor for M^+ $C_{17}H_{15}Cl_2N_2$ = 317.1).

Synthesis of 1,3-bis(naphthalen-2-ylmethyl)imidazolium bromide (12)—Imidazole (0.68 g, 10 mmol) was dissolved in 10 mL of acetonitrile, potassium hydroxide (0.62 g, 11 mmol) was added, and the mixture was heated at reflux for 0.5 h. (2-Bromomethyl)naphthalene (2.21 g, 10 mmol) was added, and the mixture was refluxed for 2.5 h, during that time a white precipitate was formed. The solid was filtered off, and to the filtrate was added a second equivalent of (2-bromomethyl)naphthalene (2.21 g, 10 mmol). The mixture was returned to reflux for 1 h. Volatiles were removed by rotary evaporation under reduced pressure, yielding a viscous brown oil. Hot water (∼90 °C) was added to the oil, stirred, and slowly decanted. This was repeated several times, and the water layers were combined. A white solid, **12**, was precipitated from the cooled water layers and was isolated by filtration and air-dried (1.47 g, 35 % yield). Mp: 165–168 °C. Found: C, 67.5; H, 5.0; N,

6.3 Calcd for $C_{25}H_{21}N_2Br_1$: C, 69.9; H, 4.9; N, 6.5 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.50 (s, 1H, NCHN), 7.95 (m, 10H, Ar), 7.56 (m, 6H, Ar), 5.62 (s, 4H, CH_2). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ = 136.5 (NCN), 132.7 (Ar), 132.7 (Ar), 132.1 (Ar), 128.8 (Ar), 127.8 (Ar), 127.6 (Ar), 127.6 (Ar), 126.7 (Ar), 126.7 (Ar), 125.7 (Ar), 123.0 (Ar), 52.3 (CH_2). MS: *m/z* = 348.9, (theor for M^+ $C_{25}H_{21}N_2$ = 349.2).

Crystal data for 12—$C_{25}H_{21}N_2$·Br, M = 429.35, monoclinic, a = 19.810(6) Å, b = 8.060(2) Å, c = 12.591(4) Å, β = 95.941(5)°, V = 1,999.8(10) Å^3, T = 100(2) K, space group $P2(1)/c$, Z = 4, 15,271 reflections measured, 4,046 independent reflections (R_{int} = 0.0560). The final R_1 values were 0.0467 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1225 ($I > 2\sigma(I)$). The final R_1 values were 0.0594 (all data). The final $wR(F^2)$ values were 0.1313 (all data).

Synthesis of 1,3-bis(naphthalen-2-ylmethyl)benzimidazolium bromide (13)— Benzimidazole (0.40 g, 3.4 mmol) was dissolved in 5 mL of acetonitrile, potassium hydroxide (0.29 g, 5.1 mmol) was added, and the mixture was stirred for 0.5 h. (2-Bromomethyl)naphthalene (0.82 g, 3.7 mmol) was added, and the solution was refluxed overnight. The solution was filtered hot to remove a white precipitate (presumed to be KBr), the resulting solution was washed into a flask with 2 mL of acetonitrile, a second equivalent of (2-bromomethyl)naphthalene (0.82 g, 3.72 mmol) was added, and the solution was refluxed overnight. The resulting off-white precipitate was collected and washed with ethyl ether and air-dried (1.35 g, 84 % yield). Mp: 261–263 °C. Found: C, 61.2; H, 3.9; N, 9.5 Calc. for $C_{15}H_{12}Cl_2N_2Br_1$: C, 61.9; H, 4.1; N, 9.6 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 10.21 (1H, s, NCHN), 8.14 (2H, d, Ar), 8.03 (2H, m, Ar), 7.99 (2H, m, Ar), 7.94 (3H, m, Ar), 7.63 (4H, m, Ar). 7.57 (4H, m, Ar), 6.00 (4H, t, CH_2). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ = 142.9 (NCN), 132.7 (Ar), 132.7 (Ar), 131.3 (Ar), 131.2 (Ar), 128.8 (Ar), 127.8 (Ar), 127.6 (Ar), 127.6 (Ar), 126.8 (Ar), 126.7 (Ar), 126.7 (Ar), 125.6 (Ar), 114.0 (C–Cl), 50.25 (CH_2). MS: *m/z* = 398.9 (theor for M^+ $C_{29}H_{22}N_2$ = 398.5).

Crystal data for 13—$C_{29}H_{23}N_2$·Br·$C_1H_1Cl_3$, M = 598.77, orthorhombic, a = 17.135(5) Å, b = 11.182(4) Å, c = 27.555(9) Å, V = 5,280(3) Å^3, T = 100(2) K, space group $Pbca$, Z = 8, 25,105 reflections measured, 2,894 independent reflections (R_{int} = 0.1045). The final R_1 values were 0.0481 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1094 ($I > 2\sigma(I)$). The final R_1 values were 0.0731 (all data). The final $wR(F^2)$ values were 0.1253 (all data).

Synthesis of 1,3-dimethyl-7-(naphthalen-2-ylmethyl)xanthine (14)—Theophylline (10 g, 55 mmol) was dissolved in 700 mL of acetonitrile, potassium hydroxide (6.23 g, 111 mmol) was added, and the reaction was stirred for 1 h. (2-Bromomethyl)naphthalene (22.1 g, 100 mmol) was added, and the mixture was stirred at room temperature for 48 h. A light brown solid was filtered off (presumed to be KBr), the remaining filtrate was collected, and the volatiles were removed via rotary evaporation to yield a light tan solid. The crude product was washed with methanol for purification, resulting in a light tan product (5.567 g, 31 % yield). Mp: 173–175 °C. Found C, 67.8; H, 5.0; N, 17.3. Calcd for $C_{18}H_{16}N_4O_2$: C, 67.5; H, 5.0; N, 17.5. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.33 (1H, s, NCHN), 7.79 (7H, m, Ar), 5.65 (2H, s, CH_2), 3.42 (3H, s, CH_3), 3.18 (3H, s, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ = 154.4 (s, NCOC), 151.0 (s, NCON), 148.5 (s, CH_3NCN), 142.7 (s,

CH_2NCN), 134.5 (s, Ar), 132.7 (s, Ar), 132.4 (s, Ar), 128.3 (s, Ar), 127.8 (s, Ar), 127.6 (s, Ar), 126.4 (s, Ar), 126.3 (s, Ar), 126.2 (s, Ar), 125.4 (s, Ar), 105.9 (s, NCCO), 49.2 (s, NCH_2), 29.5 (s, CH_3), 27.5 (s, CH_3). MS: *m/z* = 429.0 (theor for $C_{18}H_{16}N_4O_2Ag^+$ = 429.3).

Synthesis of 1,3,9-trimethyl-7-(naphthalen-2-ylmethyl)xanthinium iodide (15)— Compound **14** (2.90 g, 9.1 mmol) was dissolved in 15 mL of DMF, iodomethane (10 mL, 160 mmol) was added, and the mixture was heated at 80 °C for 20 h. Volatiles were removed via rotary evaporation to produce a dark brown oil. The oil was washed with cold acetone to precipitate a light yellow powder. The precipitate was collected and washed with 50 mL of chloroform and 50 mL of water to yield **15** (2.44 g, 58 % yield). Mp: 184–186 °C. Found C, 48.8; H, 3.9; N, 11.8. Anal. Calcd for $C_{19}H_{19}N_4O_2I$: C, 49.4; H, 4.2; N, 12.1. ^1H NMR (500 MHz, DMSO-d_6) δ = 9.54 (1H, s, NCHN), 7.95 (4H, m, Ar), 7.56 (3H, m, Ar), 5.89 (2H, s, CH_2), 4.18 (3H, s, CH_3), 3.74 (3H, s, CH_3), 3.24 (3H, s, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 153.0 (s, NCO), 150.0 (s, NCON), 139.7 (s, NCN), 139.5 (s, NC^+N), 132.5 (s, Ar), 132.7 (s, Ar), 132.6 (s, Ar), 131.4 (s, Ar), 128.6 (s, Ar), 127.9 (s, Ar), 127.6 (s, Ar), 127.2 (s, Ar), 126.7 (s, Ar), 126.6 (s, Ar), 125.6 (s, Ar), 107.1 (s, NCCO), 51.3 (s, CH_2), 37.4 (s, CH_3), 31.5 (s, CH_3), 28.5 (s, CH_3). MS: *m/z* = 335.1 (Theor for $C_{19}H_{19}N_4O_2^+$ = 335.3).

Crystal data for 15—$C_{19}H_{19}N_4O_2{\cdot}I$, M = 462.28, orthorhombic, a = 8.382(3) Å, b = 12.722(5) Å, c = 17.040(6) Å, V = 1,817.0(11) $Å^3$, T = 100(2) K, space group $P2(1)2(1)2(1)$, Z = 4, 14,350 reflections measured, 3,691 independent reflections (R_{int} = 0.0706). The final R_1 values were 0.0414 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0900 ($I > 2\sigma(I)$). The final R_1 values were 0.0644 (all data). The final $wR(F^2)$ values were 0.0938 (all data).

Synthesis of 1-(2-ethyl-1-naphthyl)-4,5-dichloroimidazole (16)—4,5-Dichloroimidazole (0.14 g, 1.0 mmol) was dissolved into 1 mL of acetonitrile, potassium hydroxide (61 mg, 1.1 mmol) was added, and the mixture was stirred for 0.5 h. 1-(2-Bromoethyl)naphthalene (0.14 mL, 0.24 g, 1.0 mmol) was added, and the solution was refluxed for 2.5 h. The solution was filtered hot to remove a white precipitate (presumed to be KBr), and the solution was allowed to cool to room temperature to yield a tan crystalline material that was isolated by filtration and air-dried (0.09 g, 29 % yield). Mp: 145–147 °C. Found: C, 61.3; H, 3.9; N, 9.5 Calc. for $C_{15}H_{12}Cl_2N_2$: C, 61.9; H, 4.1; N, 9.6 %. ^1H NMR (500 MHz, DMSO-d_6) δ = 8.13 (1H, d, Ar), 7.93 (1H, d, Ar), 7.84 (1H, d, Ar), 7.69 (1H, s, NCHN), 7.58 (2H, m, Ar), 7.42 (1H, t, Ar), 7.27 (1H, d, Ar), 4.31 (2H, t, NCH_2), 3.49 (2H, t, CH_2). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ = 135.9 (NCN), 133.4 (Ar), 133.3 (Ar), 131.3 (Ar), 128.6 (Ar), 127.4 (Ar), 126.9 (Ar), 126.3 (Ar), 125.7 (Ar), 125.5 (Ar), 124.0 (Ar–Cl), 123.3 (Ar), 111.9 (Ar–Cl), 46.2 (CH_2), 32.8 (CH_3). MS: *m/z* = 290.04 (theor for M^+ $C_{15}H_{12}Cl_2N_2$ = 291.0).

Crystal data for 16—$C_{15}H_{12}Cl_2N_2$, M = 291.17, orthorhombic, a = 24.323(3) Å, b = 12.5855(15) Å, c = 8.5446(10) Å, V = 2,615.6(5) $Å^3$, T = 100(2) K, space group *Aba*2, Z = 8, 10,902 reflections measured, 2,970 independent reflections (R_{int} = 0.0437). The final R_1 values were 0.0352 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0822 ($I > 2\sigma(I)$). The final R_1 values were 0.0393 (all data). The final $wR(F^2)$ values were 0.0845 (all data).

Synthesis of 4,5-dichloro-1,3-bis(2-(naphthalen-1-yl) ethyl)-imidazolium bromide (17)—Compound **16** (0.52 g, 1.7 mmol) was dissolved in 2 mL of acetonitrile and combined with 1-(2-Bromoethyl)-naphthalene (1.28 mL, 2.1 g, 9.0 mmol) in a pressure tube. The mixture was heated to 120 °C overnight and was then allowed to cool to room temperature. The volatiles were removed via rotary evaporation, and the resulting tan solid was washed with acetonitrile. The resulting solid was then collected, washed with ethyl ether, and dried to yield **17**. (0.43 g, 48 % yield). Mp: 192–194 °C. Found: C, 61.8; H, 4.1; N, 9.6 Calc. for $C_{15}H_{12}Cl_2N_2$: C, 61.9; H, 4.1; N, 9.6 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.49 (1H, s, NCHN), 8.13 (2H, d, Ar), 7.98 (2H, d, Ar), 7.89 (2H, d, Ar), 7.64 (2H, m, Ar), 7.47 (2H, t, Ar), 7.32 (2H, d, Ar), 7.08 (2H, d, Ar), 4.54 (4H, t, NCH_2), 3.54 (4H, t, CH_2). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.5 (NCN), 133.5 (Ar), 132.1 (Ar), 131.2 (Ar), 128.8 (Ar), 127.9 (Ar), 127.2 (Ar), 126.5 (Ar), 125.9 (Ar), 125.6 (Ar), 123.1 (Ar), 118.6 (Ar), 48.8 (CH_2), 31.7 (CH_2). MS: *m/z* = 444.9 (theor for M^+ $C_{27}H_{23}Cl_2N_2$ = 446.4).

Crystal data for 17—$C_{27}H_{23}Cl_2N_2$·Br, M = 526.28, triclinic, a = 10.5812(3) Å, b = 13.8204(4) Å, c = 17.5946(5) Å, α = 68.1350(10)°, β = 83.1580(10)°, γ = 83.1330(10)°, V = 2,362.97(12) Å^3, T = 100(2) K, space group P, Z = 4, 27,520 reflections measured, 7,773 independent reflections (R_{int} = 0.0242). The final R_1 values were 0.0228 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0589 ($I > 2\sigma(I)$). The final R_1 values were 0.0237 (all data). The final $wR(F^2)$ values were 0.0619 (all data).

4,5-Dichloro-1-(2-(naphthalen-1-yl) ethyl)-3-(naphthalen-2-ylmethyl)-imidazolium bromide (18)—Compound **16** (0.52 g, 1.7 mmol) was dissolved in 2 mL of acetonitrile, (2-bromomethyl)naphthalene (0.38 g, 1.7 mmol) was added, and the mixture was refluxed at 80 °C for 3.5 h, during which a white precipitate was formed. The precipitate was collected and washed with ethyl ether (0.67 g, 77 % yield). Mp: 193–195 °C. Found: C, 61.0; H, 4.0; N, 5.5 Calc. for $C_{26}H_{21}Cl_2N_2Br_1$: C, 60.1; H, 4.1; N, 5.5 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.46 (1H, s, NCHN), 8.13 (1H, d, Ar), 7.96 (1H, d, Ar), 7.92 (1H, d, Ar), 7.61 (2H, m, Ar), 7.57 (4H, m, Ar), 7.39 (1H, d, Ar), 7.34 (2H, m, Ar), 5.62 (2H, s, CH_2), 4.60 (2H, t, NCH_2), 3.63 (2H, t, CH_2). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.5 (NCN), 133.5 (Ar), 132.8 (Ar), 132.6 (Ar), 132.2 (Ar), 131.2 (Ar), 129.8 (Ar), 128.9 (Ar), 128.8 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.2 (Ar), 126.9 (Ar), 126.8 (Ar), 126.5 (Ar), 125.9 (Ar), 125.5 (Ar), 125.3 (Ar), 123.1 (Ar), 51.6 (CH_2), 49.0 (CH_2), 31.4 (CH_2). MS: *m/z* = 430.9 (theor for M^+ $C_{26}H_{21}Cl_2N_2$ = 431.1).

Synthesis of 3-benzyl-4,5-dichloro-1-(2-(naphthalen-1-yl)-ethyl)-imidazolium bromide (19)—Compound **16** (0.09 g, 0.28 mmol) was dissolved in 1 mL of acetonitrile, benzyl bromide (33 μL, 0.48 g, 0.28 mmol) was added, and the mixture was refluxed at 80 °C for 9 h, during which a white precipitate was formed. The precipitate was collected, washed with ethyl ether, and dried to give **19** (0.06 g, 44 % yield). Mp: 183–185 °C. Found: C, 56.7; H, 3.9; N, 5.9 Calc. for $C_{22}H_{18}Cl_2N_2Br_1$: C, 57.3; H, 3.9; N, 6.1 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.52(1H, s, NCHN), 8.15 (1H, d, Ar), 7.90 (1H, d, Ar), 7.92 (1H, d, Ar), 7.61 (1H, m, Ar), 7.57 (1H, t, Ar), 7.39 (1H, d, Ar), 7.34 (1H, d, Ar), 5.62 (2H, s, CH_2), 4.60 (2H, t, NCH_2), 3.63 (2H, t, CH_2). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.1

(NCN), 133.5 (Ar), 132.6 (Ar), 132.2 (Ar), 131.2 (Ar), 129.9 (Ar), 128.9 (Ar), 128.8 (Ar), 128.0 (Ar), 127.9 (Ar), 127.2 (Ar), 126.5 (Ar), 125.9 (Ar), 125.5 (Ar), 123.1 (Ar), 119.2 (Ar), 118.5 (Ar), 51.3 (CH_2), 49.0 (CH_2), 31.3 (CH_2). MS: *m/z* = 380.9 (theor for M^+ $C_{22}H_{19}Cl_2N_2$ = 382.3).

Synthesis of 4,5-dichloro-1-(2-(naphthalen-2-yl) ethyl)-imidazole (20)—4,5-Dichloroimidazole (0.27 g, 2.0 mmol) was dissolved into 2 mL of acetonitrile, potassium hydroxide (0.12 g, 2.2 mmol) was added, and the mixture was stirred for 1 h. 2-(2-Bromoethyl)naphthalene (0.47 g, 2.0 mmol) was added, and the solution was refluxed for 3 h. The solution was filtered hot to remove a white precipitate (presumed to be KBr), and the solution was allowed to cool to room temperature to yield a tan crystalline material that was isolated by filtration and air-dried (0.18 g, 31 % yield). Mp: 84–86 °C. Found: C, 61.8; H, 4.05; N, 9.63 Calc. for $C_{15}H_{12}Cl_2N_2$: C, 61.9; H, 4.1; N, 9.6 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.87 (3H, m, Ar), 7.65 (1H, S, NCHN), 7.48 (2H, d, Ar), 7.38 (1H, d, Ar), 4.32 (2H, t, NCH_2), 3.19 (2H, t, CH_2). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 135.9 (NCN), 133.4 (Ar), 133.3 (Ar), 131.3 (Ar), 128.6 (Ar), 127.4 (Ar), 126.9 (Ar), 126.3 (Ar), 125.7 (Ar), 125.5 (Ar), 124.0 (Ar-), 123.3 (Ar), 111.9 (Ar), 46.6 (CH_2), 35.5 (CH_2). MS: *m/z* = 290.7 (theor for M^+ $C_{15}H_{12}Cl_2N_2$ = 290.0).

Crystal data for 20—$C_{15}H_{12}Cl_2N_2$, M = 291.17, orthorhombic, a = 5.9421(7) Å, b = 8.5102(10) Å, c = 26.505(3) Å, V = 1,340.3(3) Å^3, T = 100(2) K, space group $P2(1)2(1)2(1)$, Z = 4, 11,696 reflections measured, 3,074 independent reflections (R_{int} = 0.0382). The final R_1 values were 0.0358 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0874 ($I > 2\sigma(I)$). The final R_1 values were 0.0403 (all data). The final $wR(F^2)$ values were 0.0903 (all data).

Synthesis of 4,5-dichloro-1,3-bis(2-(naphthalen-2-yl) ethyl)-imidazolium bromide (21)—Compound **20** (0.25 g, 0.87 mmol) was dissolved in 3 mL of acetonitrile and combined with 1-(2-Bromoethyl)naphthalene (0.20 g, 0.87 mmol) in a pressure tube. The mixture was heated to 120 °C overnight and was then allowed to cool to room temperature. The volatiles were removed via rotary evaporation, and the resulting oil was washed with ethyl ether. The resulting brown solid was then collected and dried to yield **21**. (0.19 g, 41 % yield). Mp: 164–166 °C. Found: C, 61.2; H, 3.9; N, 9.5 Calc. for $C_{15}H_{12}Cl_2N_2Br_1$: C, 61.9; H, 4.1; N, 9.6 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.38 (1H, s, NCHN), 7.85 (2H, m, Ar), 7.98 (2H, d, Ar), 7.89 (2H, d, Ar), 7.64 (2H, m, Ar), 7.47 (2H, t, Ar), 7.32 (2H, d, Ar), 7.08 (2H, d, Ar), 4.54 (4H, t, NCH_2), 3.54 (4H, t, CH_2). ^{13}C {^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.5 (NCN), 133.5 (Ar), 132.1 (Ar), 131.2 (Ar), 128.8 (Ar), 127.9 (Ar), 127.2 (Ar), 126.5 (Ar), 125.9 (Ar), 125.6 (Ar), 123.1 (Ar), 118.6 (Ar), 48.8 (CH_2), 31.7 (CH_2). MS: *m/z* = 444.9 (theor for M^+ $C_{27}H_{23}Cl_2N_2$ = 446.4).

Crystal data for 21—$C_{27}H_{23}Cl_2N_2$,Br,H_2O, M = 542.28, triclinic, a = 7.4894(4) Å, b = 12.5503(6) Å, c = 14.7480(7) Å, α = 112.815(2)°, β = 93.953(2)°, γ = 104.639(2)°, V = 1,214.44(10) Å^3, T = 100(2) K, space group $P\bar{1}$, Z = 2, 30,871 reflections measured, 11,762 independent reflections (R_{int} = 0.0244). The final R_1 values were 0.0452 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1202 ($I > 2\sigma(I)$). The final R_1 values were 0.0598 (all data). The final $wR(F)$ values were 0.1284 (all data).

Synthesis of 4,5-dichloro-1-(2-(naphthalen-2-yl) ethyl)-3-(naphthalen-2-ylmethyl)-imidazolium bromide (22)—Compound **20** (0.23 g, 0.79 mmol) and (2-bromomethyl)naphthalene (0.19 g, 0.86 mmol) were dissolved in 1 mL of acetonitrile and heated at reflux for 3 **h**, during that time a precipitate was formed. The precipitate was collected via filtration and washed several times with acetonitrile to yield **22** as a white powder (0.21 g, 52 % yield). Mp: 192–193 °C. Found: C, 60.9; H, 3.9; N, 5.4 % Calc. for $C_{26}H_{21}Cl_2N_2Br_1$: C, 61.0; H, 4.1; N, 5.5 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.58 (1H, s, NCHN), 7.87 (7H, m, Ar), 7.76 (1H, s, Ar), 7.58 (2H, m, Ar), 7.51 (2H, m, Ar), 7.44 (1H, dd, Ar), 7.25 (1H, dd, Ar), 5.63 (2H, s, CH_2), 4.65 (2H, t, CH_2-), 3.34 (2H, t, CH_2). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 136.4 (NCN), 133.6 (Ar), 133.0 (Ar), 137.2 (Ar), 132.5 (Ar), 132.0 (Ar), 129.8 (Ar), 128.7 (Ar), 128.3 (Ar), 127.8 (Ar), 127.6 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 127.4 (Ar), 126.9 (Ar), 126.8 (Ar), 126.7 (Ar), 126.2 (Ar), 125.8 (Ar), 125.1 (Ar), 119.2 (Ar), 118.8 (Ar), 51.6 (CH_2), 49.4(CH_2), 34.2 (CH_2). MS: *m/z* = 430.9 (theor for M^+ $C_{26}H_{21}Cl_2N_2$ = 431.1).

Synthesis of 4,5-dichloro-1-(naphthalen-2-ylmethyl)-3-(quinolin-2-ylmethyl)imidazolium bromide (23)—4,5-Dichloroimidazole (2.00 g, 14.6 mmol) was dissolved in 15 mL of acetonitrile, potassium hydroxide (0.90 g, 16.0 mmol) was added, and the mixture was refluxed for 15 min. 2-Chloromethylquinoline hydrochloride (3.13 g, 14.6 mmol) and potassium hydroxide (0.82 g, 14.6 mmol) were added to a second flask and stirred in 60 mL acetonitrile at reflux for 10 min. The contents of the two flasks were combined, and the mixture was refluxed overnight. The reaction mixture was filtered hot to remove the resulting white precipitate (presumed KCl). Slow evaporation and cooling of the filtrate yielded tan crystals of 4,5-dichloro-1-(quinolin-2-ylmethyl)imidazole, which were collected by a second filtration. The crystals (0.66 g, 2.4 mmol) and (2-bromomethyl)naphthalene (0.58 g, 2.6 mmol) were dissolved in 2 mL acetonitrile and refluxed for 3.5 h, during that time a white precipitate was formed. The precipitate was collected via filtration of the reaction mixture and washed with acetonitrile to yield a white powder (1.02 g, 86 % yield). The analytical sample was recrystallized from the slow cooling of a concentrated solution of **23** in hot acetonitrile. Mp: 182–184 °C. Found: C, 57.7; H, 3.4; N, 8.3 Calc. for $C_{24}H_{18}Cl_2N_3Br_1$: C, 57.7; H, 3.6; N, 8.4 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.82 (1H, s, NCHN), 8.52 (1H, d, Ar), 8.06 (3H, m, Ar), 8.00 (1H, m, Ar), 7.96 (1H, m, Ar), 7.81 (2H, m, Ar), 7.71 (1H, d, Ar), 7.66 (1H, t, Ar), 7.61 (3H, m, Ar), 5.95 (2H, s, CH_2), 5.82 (2H, s, CH_2). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6) δ = 152.6 (Ar), 146.7 (Ar), 137.9 (NCHN), 137.6 (Ar), 132.8 (Ar), 132.7 (Ar), 130.2 (Ar), 130.2 (Ar), 128.9 (Ar), 128.4 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.3 (Ar), 127.1 (Ar), 126.9 (Ar), 126.8 (Ar), 125.3 (Ar), 119.9 (CCl), 119.7 (Ar), 118.8 (CCl), 52.5 (CH_2), 51.7 (CH_2). MS: *m/z* = 417.9 (theor. for M^+ $C_{24}H_{18}Cl_2N_3^+$ = 418.1).

Crystal data for 23—$C_{24}H_{18}BrCl_2N_3$, *M* = 499.23, monoclinic, *a* = 14.850(2) Å, *b* = 12.7441(16) Å, *c* = 11.5771(17) Å, β = 110.063(5)°, *V* = 2,058.0(5) Å^3, *T* = 100(2) K, space group *P*2(1)/*c*, *Z* = 4, 15,852 reflections measured, 3,968 independent reflections (R_{int} = 0.0739). The final R_1 values were 0.0520 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1118 ($I > 2\sigma(I)$). The final R_1 values were 0.0965 (all data). The final $wR(F^2)$ values were 0.1310 (all data).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CCR2	CC chemokine receptor 2
CCL2	CC chemokine ligand 2
CCR5	CC chemokine receptor 5
TLC	Thin-layer chromatography

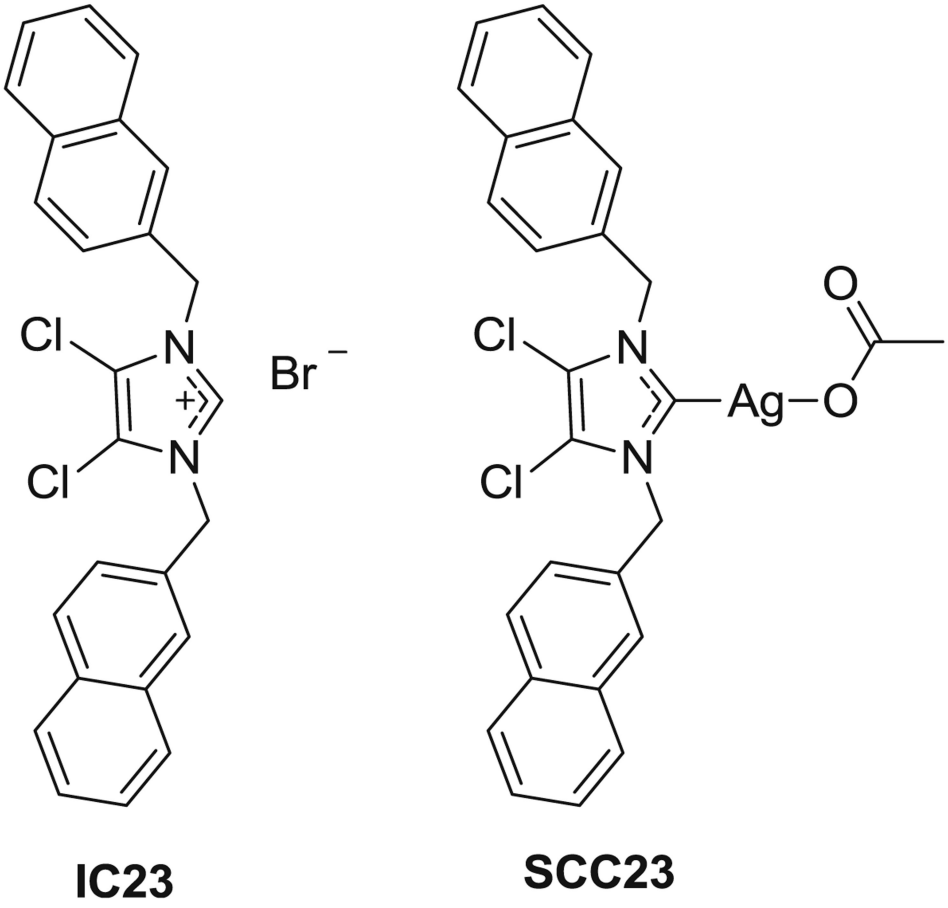


Fig. 1. Schematic representation of SCC23 and its direct precursor and primary degradation product IC23

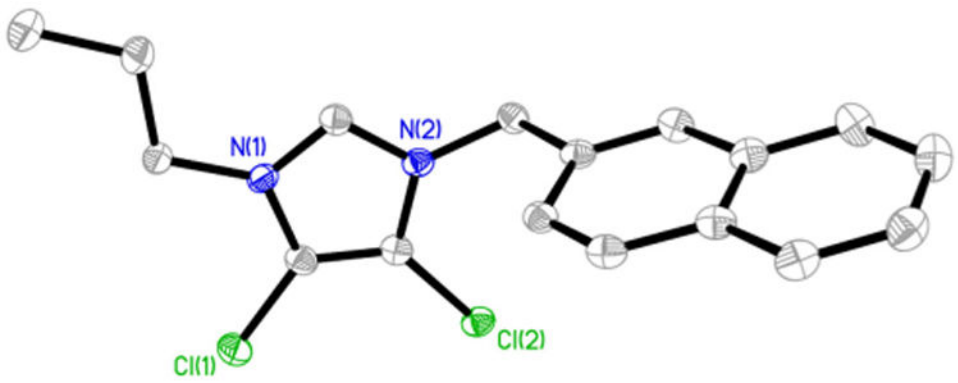


Fig. 2.

Thermal ellipsoid plot of the cationic portion of **2** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and the bromide anion have been removed for clarity

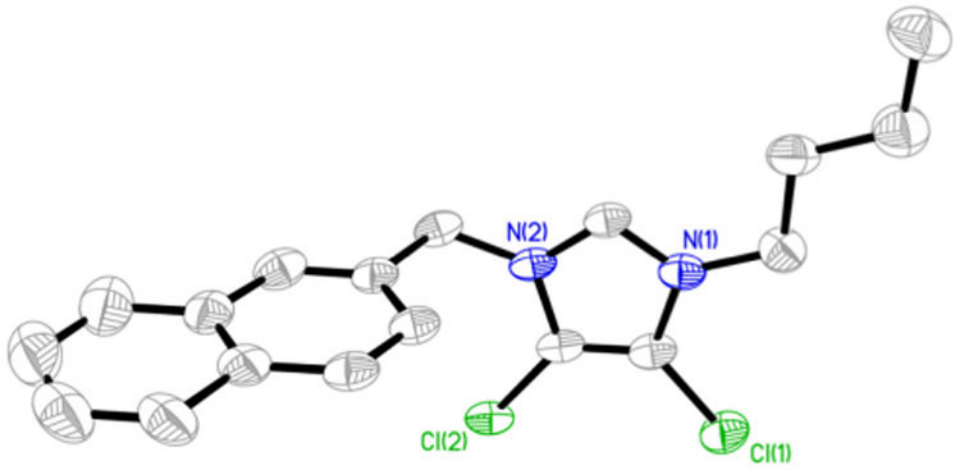


Fig. 3.

Thermal ellipsoid plot of the cationic portion of **4** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and the bromide anion have been removed for clarity

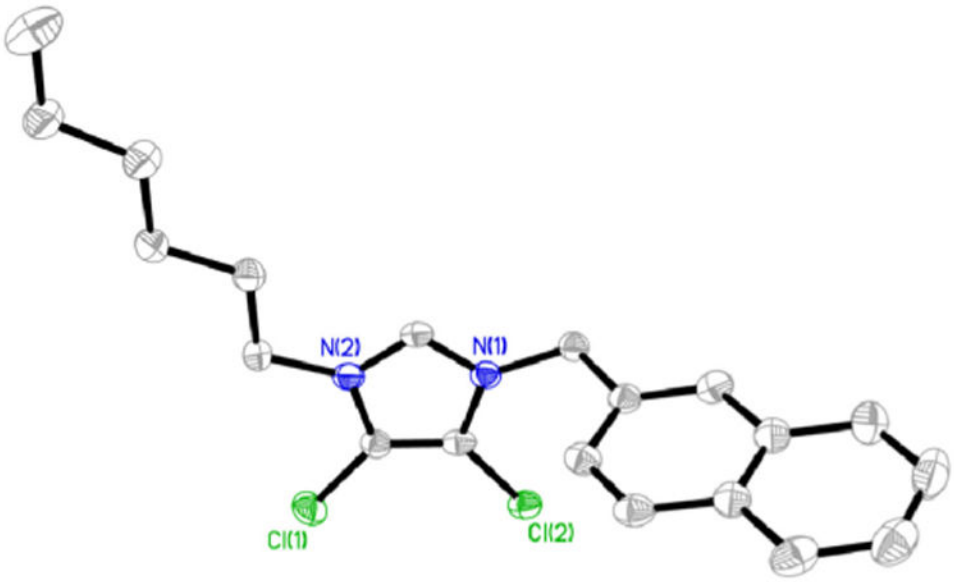


Fig. 4.

Thermal ellipsoid plot of the cationic portion of **6** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and the bromide anion have been removed for clarity

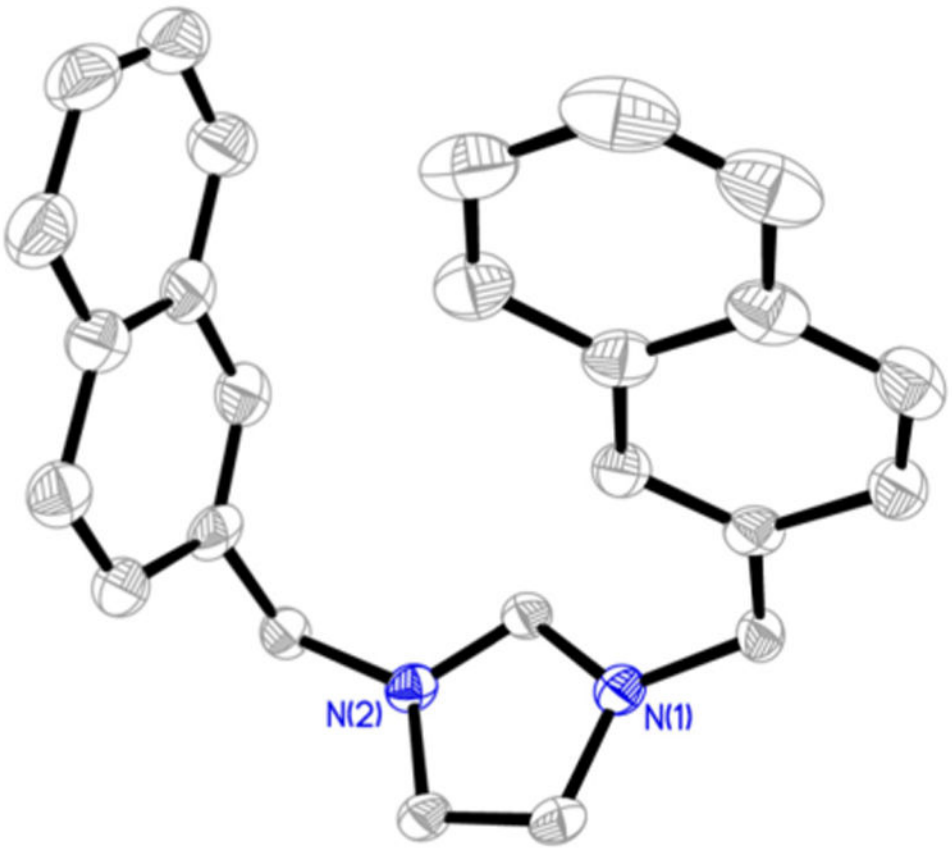


Fig. 5.

Thermal ellipsoid plot of the cationic portion of **12** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and the bromide anion have been removed for clarity

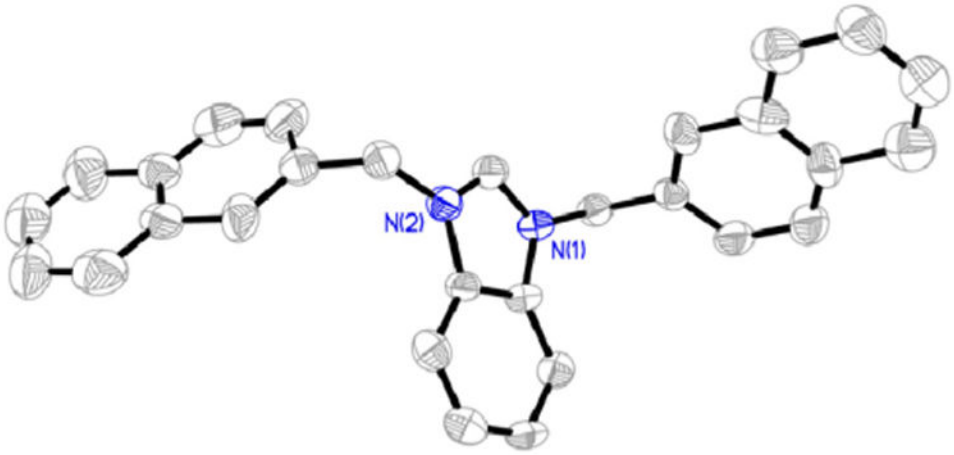


Fig. 6.

Thermal ellipsoid plot of the cationic portion of **13** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms, the bromide anion, and a disordered chloroform molecule have been removed for clarity

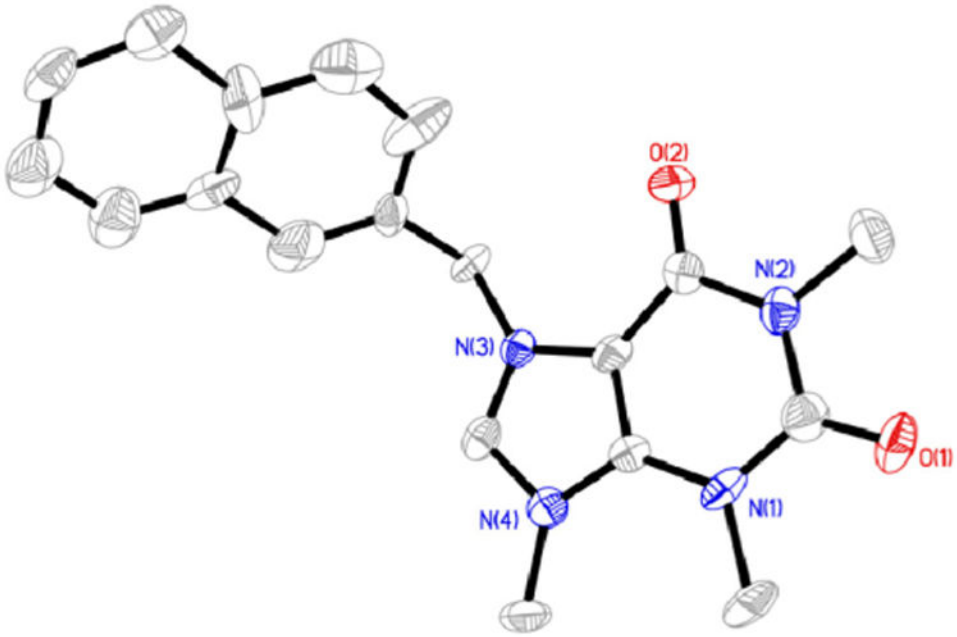


Fig. 7.

Thermal ellipsoid plot of the cationic portion of **15** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and the iodide anion have been removed for clarity

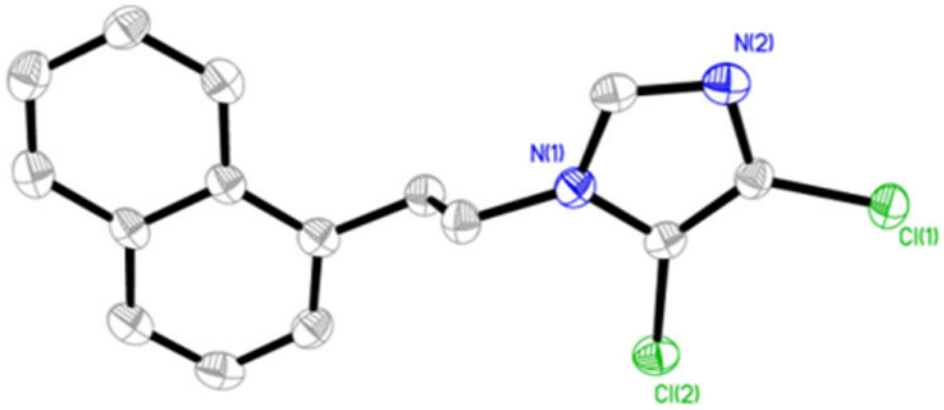


Fig. 8.

Thermal ellipsoid plot of **16** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms have been removed for clarity

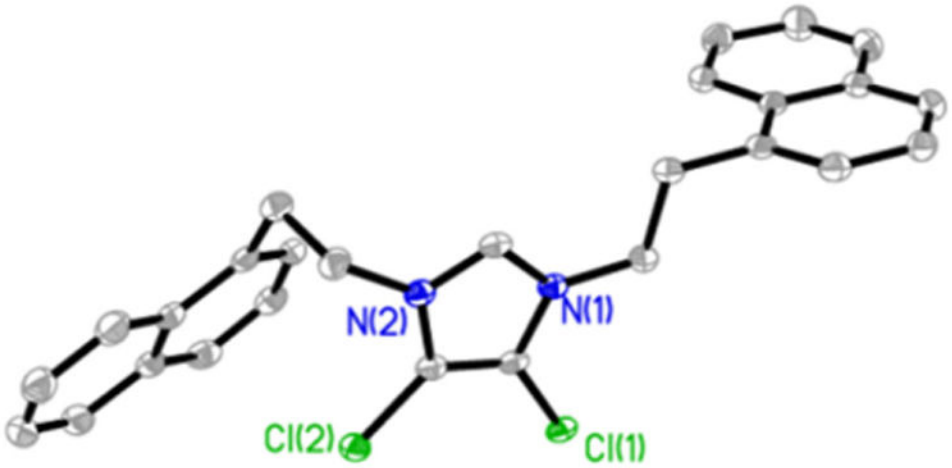


Fig. 9.

Thermal ellipsoid plot of the cationic portion of **17** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and the bromide anion have been removed for clarity

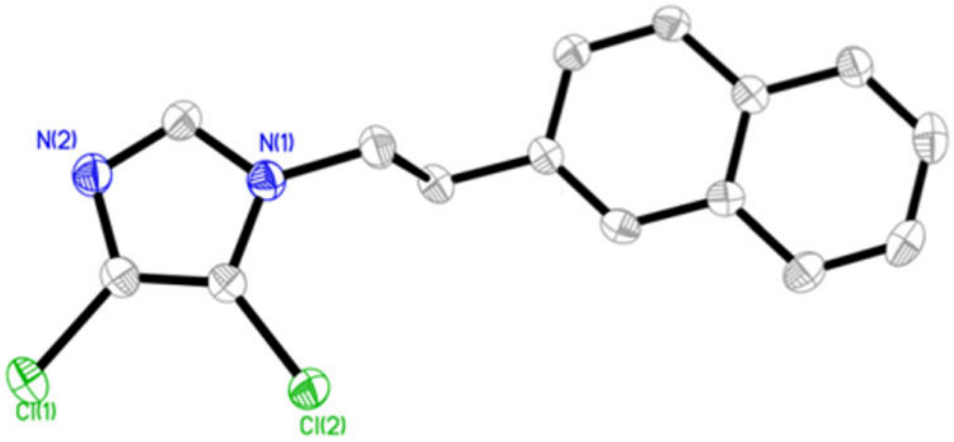


Fig. 10.

Thermal ellipsoid plot of **20** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms have been removed for clarity

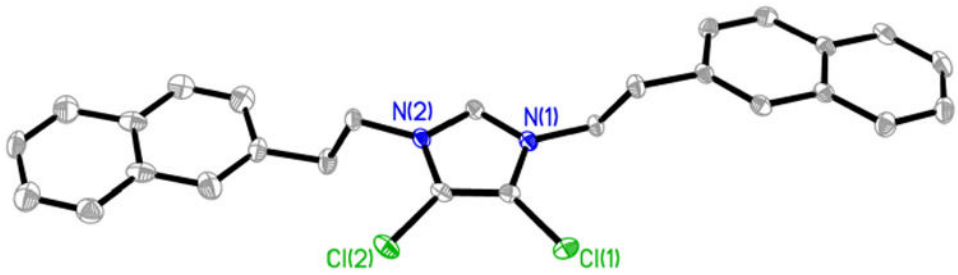


Fig. 11.

Thermal ellipsoid plot of the cationic portion of **21** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms, the bromide anion, and one molecule of water have been removed for clarity

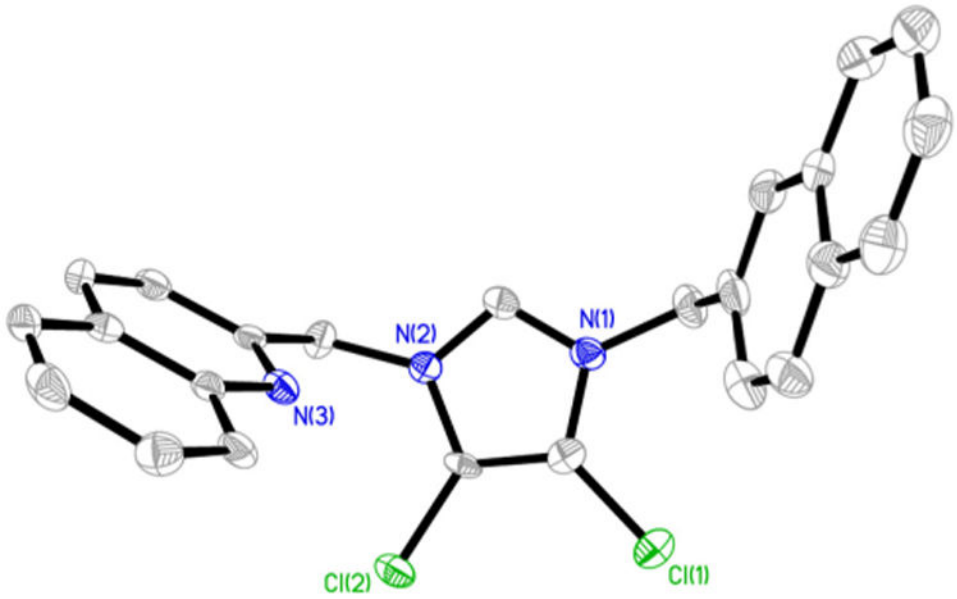


Fig. 12.

Thermal ellipsoid plot of the cationic portion of **23** with thermal ellipsoids drawn at 50 % probability. Hydrogen atoms and the bromide anion have been removed for clarity

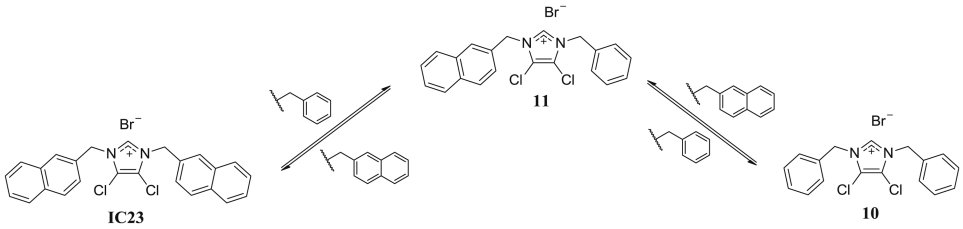


Fig. 13. Reversibility of compounds IC23, 10, and 11

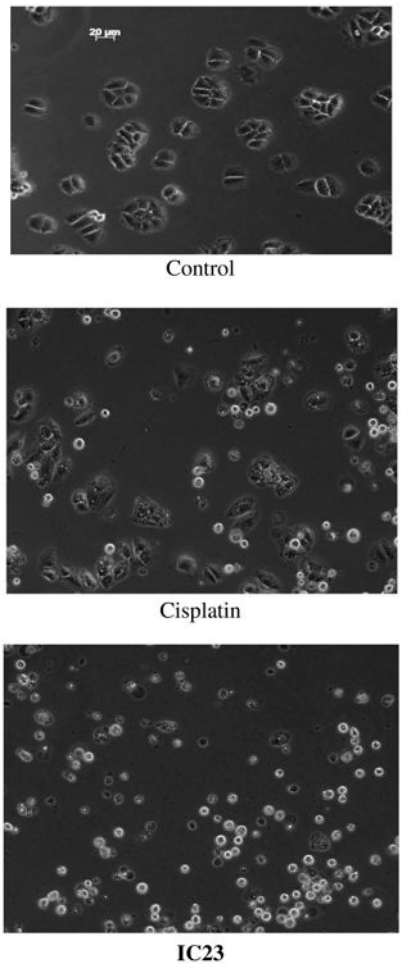


Fig. 14.

These phase-contrast images at 10× are used for morphological comparisons among the control, cisplatin, and IC23 (5 μM) after being treated for 24 h. The control cells are untreated, and cisplatin is a known apoptosis inducer

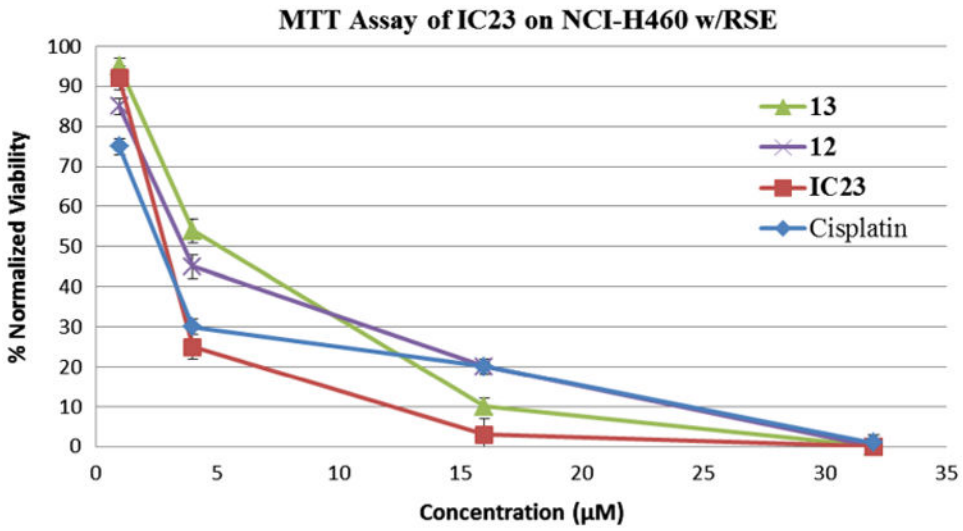


Fig. 15. NCI-H460 MTT assay after 72 h of treatment with cisplatin and compounds IC23, 12, and 13

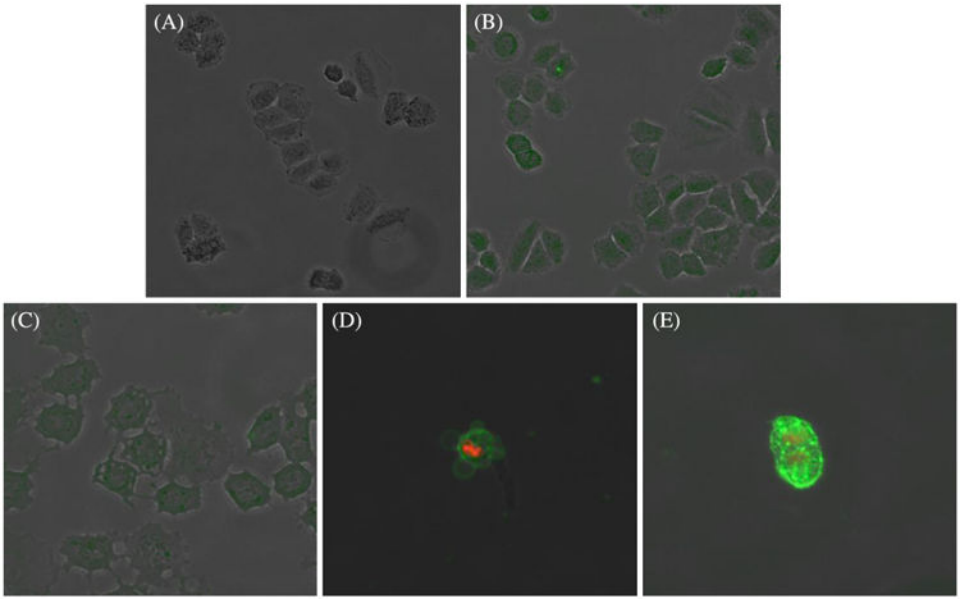


Fig. 16.

H460 cells grown in 6-well plates. Merged images of Annexin V (*green*), propidium iodide (*red*), and normal transmitted light image. Images captured using an EVOS fl Digital Inverted Microscope with the 10× objective (**a, b**), and the 20× objective (**c**– **e**). **a** Control cells grown in the presence of 10 % FBS supplemented RPMI-1640 media. **b** Cells grown in the presence of 40 μM cisplatin. Note that the presence of green *fluorescence* indicates early-stage apoptosis. H460 cells grown in the presence of 40 μM IC23 for a period of **c** 1 h, **d** 3 h, and **e** 6 h. Note the presence of early-stage apoptotic cells comparable to the 6-h cisplatin treatment at 1 h, late-stage apoptotic cells showing blebbing at 3 h, and dead, end-stage apoptotic cells at 6 h (Color figure online)

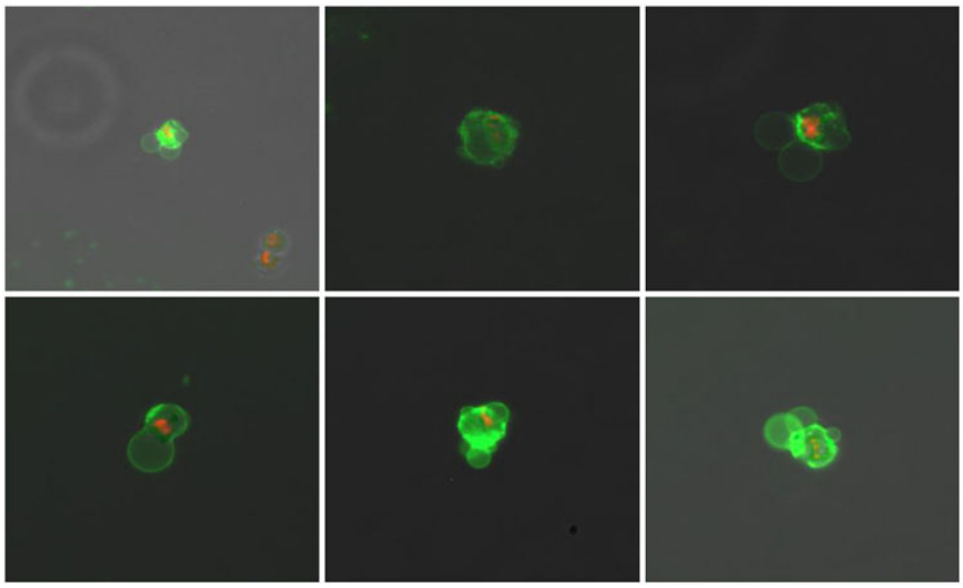


Fig. 17.

H460 cells grown in 6-well plates. Merged images of Annexin V (*green*), propidium iodide (*red*), and normal transmitted light image. Images captured using an EVOS fl Digital Inverted Microscope with the 20× objective. Blebbing was observed after 1-h treatment with IC23 at 40 μM (Color figure online)

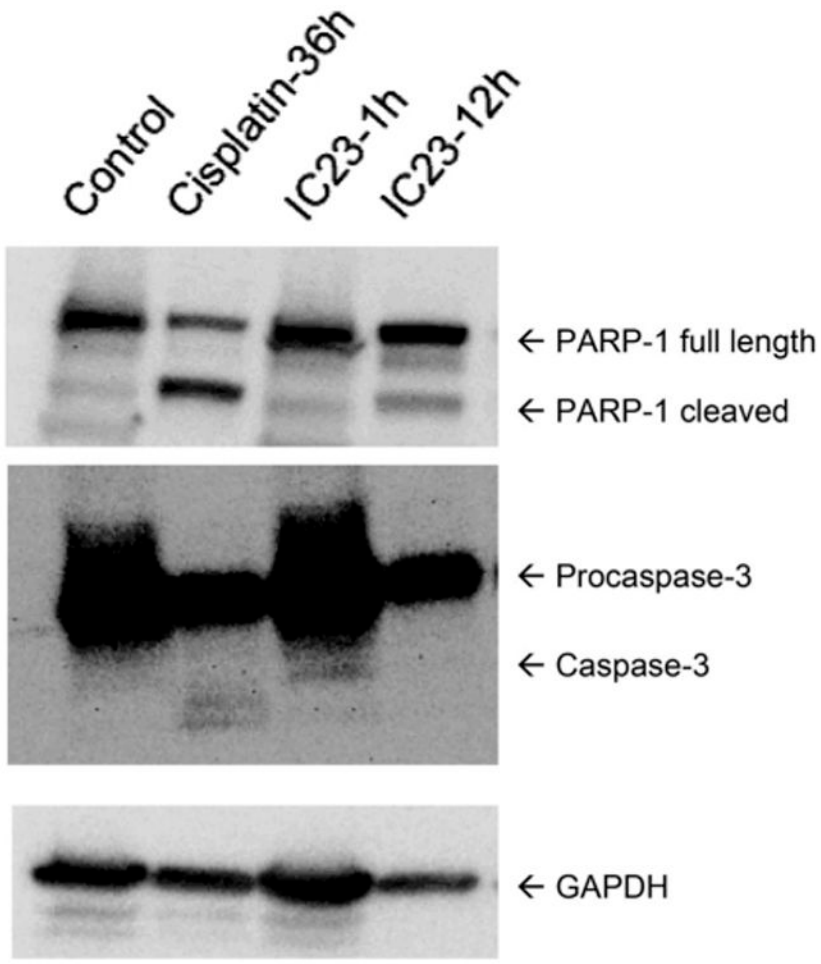
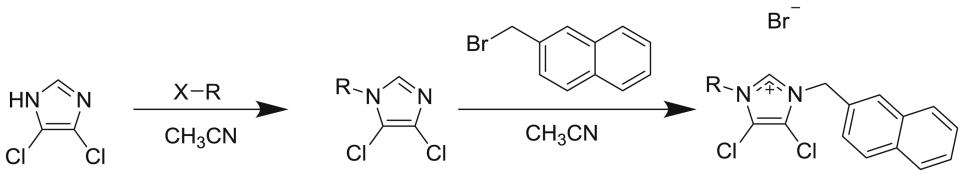


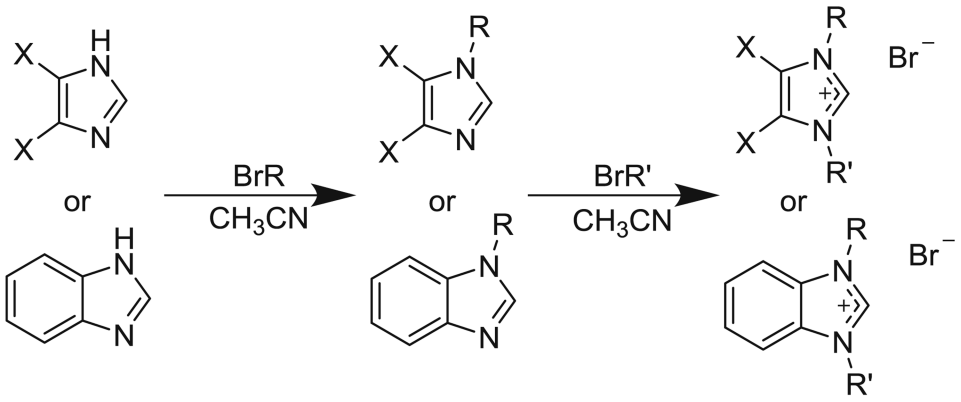
Fig. 18.

Western blots of cells treated with cisplatin and IC23 at the concentration of 5 μM shows PARP-1 and procaspase-3 cleavage in H460 cells. The control cells show no sign of PARP-1 or caspase-3 cleavages. At 36 h, cisplatin treatment shows both PARP-1 cleavage and a reduced amount of procaspase-3. IC23 shows little PARP-1 and procaspase-3 cleavage at 1 h, and shows increased PARP-1 cleavage at 12 h with a substantial reduction in the amount of procaspase-3. Note that visualization of caspase-3 could be limited by its short half-life



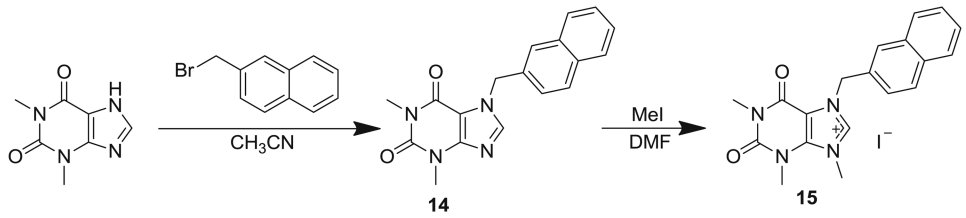
1: R=$(CH_2)CH_3$, X=Br
2: R=$(CH_2)_2CH_3$, X=I
3: R=$CH(CH_3)_2$, X=I
4: R=$(CH_2)_3CH_3$, X=I
5: R=$(CH_2)_4CH_3$, X=Br
6: R=$(CH_2)_5CH_3$, X=Br
7: R=$(CH_2)_6CH_3$, X=Br
8: R=$(CH_2)_8CH_3$, X=Br
9: R=$(CH_2)_{11}CH_3$, X=Br

Scheme 1. Synthesis of 1–9

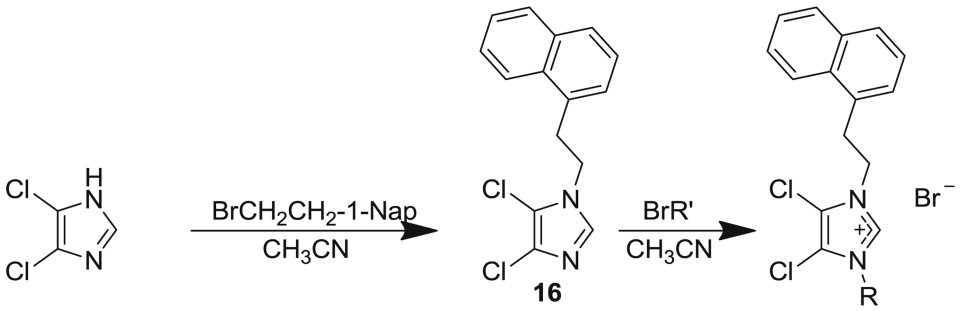


10: X=Cl R,R'=Bz
11: X=Cl R=Bz R'=CH_2Nap
12: X=H R,R'=CH_2Nap
13: R,R'=CH_2Nap

Scheme 2. Synthesis of 10–13

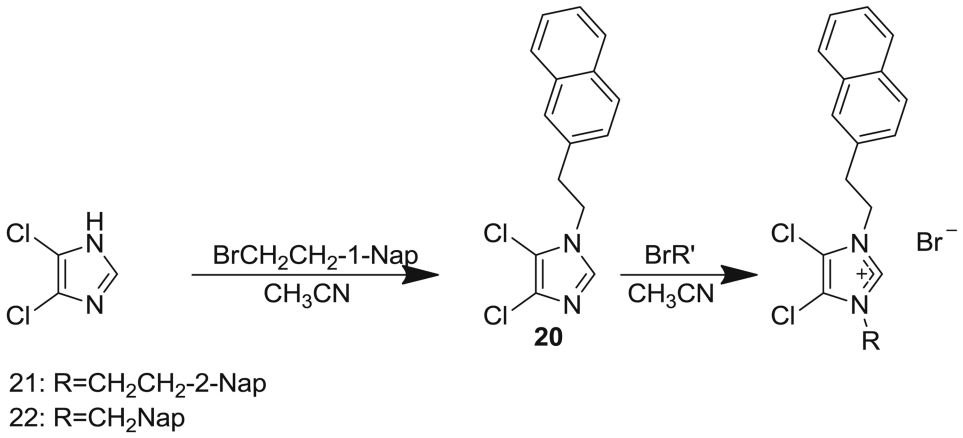


Scheme 3. Synthesis of 14 and 15



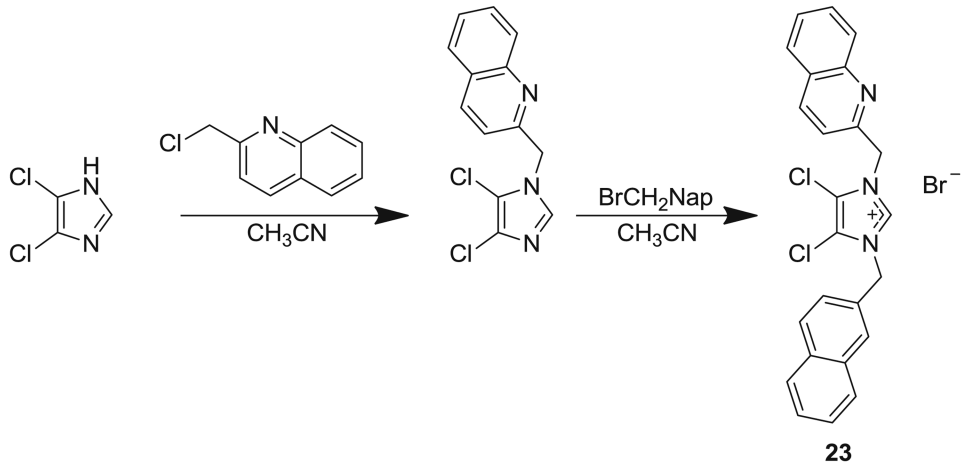
17: R=CH_2CH_2-1-Nap
18: R=CH_2Nap
19: R=Bz

Scheme 4. Synthesis of 16–19



21: R=CH_2CH_2-2-Nap
22: R=CH_2Nap

Scheme 5. Synthesis of 20–22

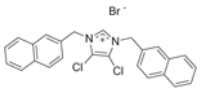
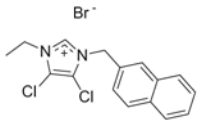
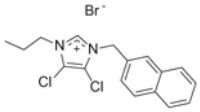
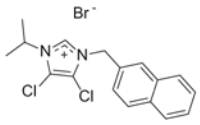
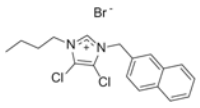
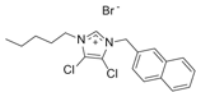
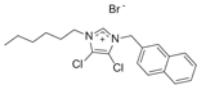
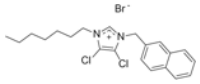
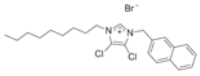
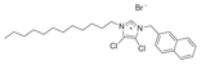
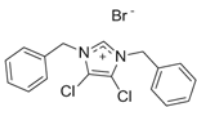


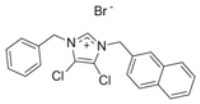
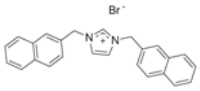
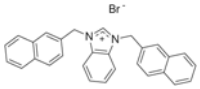
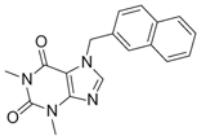
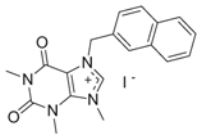
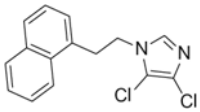
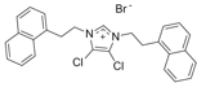
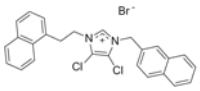
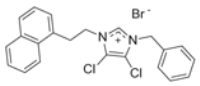
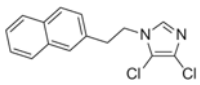
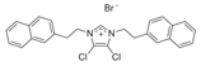
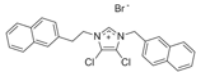
23

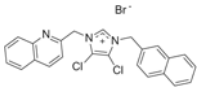
Scheme 6. Synthesis of 23

Table 1

Synthesized imidazolium salts and their relevant IC_{50} and solubility values

Compound	Chemical structure	IC_{50} (at day 3)	H_2O solubility
IC23		5 μM—NCI-H460 6 μM—NCI-H1975 8 μM—HCC827	<0.1 mg/mL
1		26 μM—NCI-H460 >30 μM—NCI-H1975 >30 μM—HCC827	13.5 mg/mL
2		19 μM—NCI-H460 22 μM—NCI-H1975 >30 μM—HCC827	5.2 mg/mL
3		>30 μM—NCI-H460 20 μM—NCI-H1975 >30 μM—HCC827	10.8 mg/mL
4		13 μM—NCI-H460 10 μM—NCI-H1975 12 μM—HCC827	5.7 mg/mL
5		10 μM—NCI-H460 12 μM—NCI-H1975 12 μM—HCC827	3.1 mg/mL
6		5 μM—NCI-H460 5 μM—NCI-H1975 8 μM—HCC827	1.4 mg/mL
7		6 μM—NCI-H460 4 μM—NCI-H1975 6 μM—HCC827	N.D.
8		10 μM—NCI-H460 3 μM—NCI-H1975 3 μM—HCC827	0.3 mg/mL
9		5 μM—NCI-H460 4 μM—NCI-H1975 N.D.—HCC827	N.D.
10		>30 μM—NCI-H460 >30 μM—NCI-H1975 >30 μM—HCC827	N.D.

Compound	Chemical structure	IC_{50} (at day 3)	H_2O solubility
11		15 μM—NCI-H460 12 μM—NCI-H1975 15 μM—HCC827	N.D.
12		4 μM—NCI-H460 6 μM—NCI-H1975 9 μM—HCC827	N.D.
13		3 μM—NCI-H460 4 μM—NCI-H1975 5 μM—HCC827	N.D.
14		8 μM—NCI-H460 12 μM—NCI-H1975 28 μM—HCC827	0.8 mg/mL
15		>30 μM—NCI-H460 >30 μM—NCI-1975 >30 μM—HCC827	2.6 mg/mL
16		>30 μM—NCI-H460 >30 μM—NCI-H1975 >30 μM—HCC827	N.D.
17		8 μM—NCI-H460 8 μM—NCI-H1975 11 μM—HCC827	0.4 mg/mL
18		5 μM—NCI-H460 6 μM—NCI-H1975 7 μM—HCC827	0.1 mg/mL
19		12 μM—NCI-H460 14 μM—NCI-H1975 >30 μM—HCC827	N.D.
20		>30 μM—NCI-H460 >30 μM—NCI-H1975 >30 μM—HCC827	N.D.
21		8 μM—NCI-H460 8 μM—NCI-H1975 8 μM—HCC827	N.D.
22		4 μM—NCI-H460 7 μM—NCI-H1975 9 μM—HCC827	N.D.

Compound	Chemical structure	IC_{50} (at day 3)	H_2O solubility
23		10 μM—NCI-H460 5 μM—NCI-H1975 9 μM—HCC827	0.9 mg/mL