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COMMUNICATION

Synthesis of N-aryl and N-alkyl substituted imidazolium silver complexes. Cytotoxic screening using human cell lines modelling acute myeloid leukemia

Eirin Alme,[a] Karl Wilhelm Törnroos,[a] Bjørn Tore Gjertsen,[b, c] and Hans-René Bjørsvik*[a]

[a] Ms Eirin Alme, Prof. Dr. Karl Wilhem Törnroos, Prof. Dr. Hans-René Bjørsvik
Department of Chemistry
University of Bergen
Allégaten 41, 5007 Bergen (Norway)
E-mail: hans.bjorsvik@kj.uib.no
[b] Prof. Dr. B. T. Gjertsen
Center for Cancer Biomarkers CCBIO,
Department of Clinical Science, University of Bergen,
5020 Bergen (Norway)
[b] Prof. Dr. B. T. Gjertsen
Department of Internal Medicine
Hematology Section, Haukeland University Hospital
P.B. 1400, 5021 Bergen (Norway)

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Abstract: A series of *N*-aryl and *N*-alkyl substituted imidazoles were synthesized and complexed with Ag^+ to obtain silver-NHC complexes of the form $[Ag(NHC)_2]X$. These silver-NHC complexes were tested *in-vitro* versus the human cell lines HL-60 and MOLM-13 that both model acute myeloid leukemia (AML). A substantial difference in cytotoxicity was revealed varying in the range 13–4 μM (for HL-60) and 22–9 μM (for MOLM-13), respectively. Furthermore, this study revealed that when an alkyl group was installed on the imidazole scaffold, its position influenced substantially the cytotoxicity of the corresponding silver NHC complex.

The imidazole ring constitute an embedded moiety of an assorted selection of biologically active compounds,[1] including analgesic,[2] antibacterial,[3] cytotoxic,[4] and anticancer compounds.[5] Imidazole derivatives also constitute an essential moiety in a variety of alkaloids,[6] as a precursor for N-heterocyclic carbene (NHC) ligands[7] used in transition metal catalysis,[8] furthermore NHCs have also been proven to be versatile organocatalysts.[9]

Elemental silver has been known for its bactericidal properties since ancient time.[10] Silver has reemerged as a workable alternative for treatment of infections,[11] exemplified by silver sulfadiazine introduced for clinical use in the early 1960s,[12,13] which has been used as a topical antibacterial for second- and third-degree burn treatment. NHC complexes of late transition metals have materialized as promising compounds with potent activity as antibacterial, antiviral, and anticancer agents.[14]

A long standing project in our laboratory has been dedicated to the design and development of synthetic methods for late stage functionalization of the imidazole ring,[15,16,17,18,19] whose results have been exploited to realize total syntheses of Ag-NHC complexes, see **NHC-I** and **NHC-II**[20] of Figure 1. The **NHC-I** and **NHC-II** complexes were explored by *in vitro* experiments involving the human cell lines HL-60 and MOLM-13 that both model acute myeloid leukemia. These studies revealed **NHC-II**, which contain a heptylated imidazole backbone, to be four- to six-fold more potent than the corresponding Ag-NHC complex with a methyl group installed on the same position (**NHC-I**). Previous investigations in our laboratories suggest that backbone alkylation might contribute to the ligand-to-metal donation (NHC→Ag),[21] which was supported by the examinations of the complexes **NHC-I** and **NHC-II**. Although, the effect of an overall more lipophilic compound may also be the reason for the potent activity observed for **NHC-II** silver complex.

In a previous study,[20] we were not able to conclude whether the variation of cytotoxicity actually was due to the altered electronic properties of the imidazole ring owing to the alkylated backbone[21] or if the observed effect was only due to the overall augmented lipophilicity of the actual Ag-NHC complex, backbone heptylated **NHC-II** and backbone methylated **NHC-I**.

In our attempt to address this issue, in this study we have designed and produced a small library of target molecules (Figure 1) that was synthesized and examined against the two human cell lines HL-60 and MOLM-13. Moreover, we wanted also to further optimize the cytotoxicity towards a potent compound where only a very small dose is required and thus fit into an appropriate therapeutic window.

Chemical Synthesis and Structure Determination

The syntheses of the defined target silver-NHC complexes, whose structures are outlined in Figure 1, were accomplished through two different pathways: (1) via a three-step linear synthetic pathway that was used for the preparation of the complexes **NHC-IX**, **NHC-X**, **NHC-XII**, and **NHC-XIII**, Scheme 1, and (2) a second pathway that comprises a multicomponent reaction (MCR) that produce the ligand with all functionality embedded. The only additional reaction step was the silver complexation to approach complex **NHC-XI**, see Scheme 2.

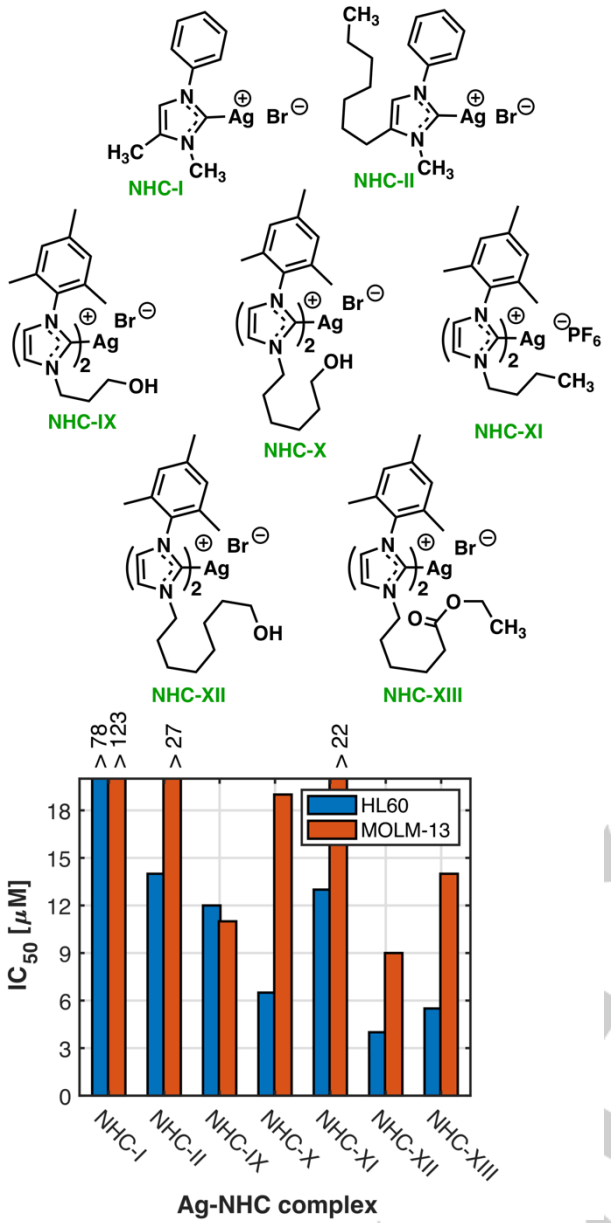


Figure 1. Designed and synthesized Ag-NHC complexes (**NHC-IX** – **NHC-XIII**) and measurements of IC_{50} versus the two different human cell lines HL-60 and MOLM-13. The silver-NHC complexes **NHC-I** and **NHC-II** were previously published in a work from our laboratory.[20] The numerical values provided on top of bar diagram indicate highest tested concentration (>78 μM, >123 μM, etc.).

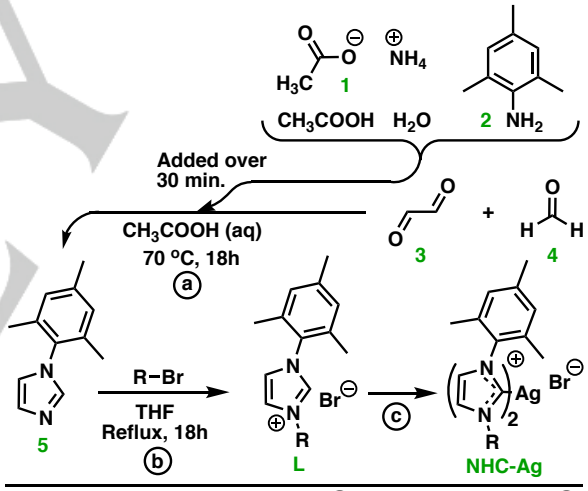
The three-step NHC-ligand synthesis of Scheme 1 incorporated the following procedures. Step (a): 2,4,6-Trimethylaniline **2**, oxalaldehyde **3**, formaldehyde **4**, and ammonium acetate **1** were mixed and reacted by means of a MCR protocol[22] to obtain a the intermediate 1-mesityl-1*H*-imidazole **5**. In step (b) involved the installation of the functionalized alkyl chains using the Ω-bromoalkyl derivatives **6**-**9**. The Ω-bromoalkyl derivatives were installed on *N3* of the imidazole ring to obtain the various NHC ligand precursors **10-13**. Ultimately, each of these NHC precursors were complexed with silver[23] following one of two distinct methods that involve $AgNO_3$ or Ag_2O as silver sources, respectively.

The silver NHC complex **NHC-XI** was obtained via a different three-step synthesis, whereof the two first steps (a) and (b) were telescoped. The pre-cursor NHC ligand **14**, was obtained by reacting 2,4,6-trimethylaniline **2** and oxalaldehyde **3**, formaldehyde **4**, and butan-1-amine **15** as described elsewhere.[24]

From our previous work with complexes **NHC-I** and **NHC-II**, any clear evidences existed on whether these complexes comprised one or more NHC ligands. In this work, however, we were able to produce crystals complex **NHC-IX**. X-ray crystallographic analysis of the obtained crystals revealed the complex to hold two ligands, see Figure 2.

By means of crystallography, we revealed that the silver-NHC complex **NHC-IX** contained two ligands coordinated to a silver atom, see Figure 2. From the NMR data alone, it was difficult to determine whether one or two ligands were present in the Ag-NHC complex.

For the silver-NHC complexes **NHC-X**, **NHC-XI**, **NHC-XII**, and **NHC-XIII**, we were not able, even after several attempts to produce crystals suitable for crystallography analysis. The NMR spectra of these compounds revealed however a similar pattern as for complex **NHC-IX**, but this did not deliver conclusive evidence for whether the complexes consist of one silver atom coordinated to two NHC-ligands. HRMS analyses of the complexes revealed expected molecular formula for the bis-ligand-Ag complexes.



R−Br	L	(b)	Method	NHC-Ag	(c)
6	10	39%	1	NHC-IX	45%
7	11	39%	2	NHC-X	93%
8	12	35%	1	NHC-XII	67%
9	13	32%	1	NHC-XIII	61%

Scheme 1. Synthesis of the Ag-NHC complexes **NHC-IX**, **NHC-X**, **NHC-XII**, and **NHC-XIII.** Reagents and conditions: (a) ammonium acetate **1**, 2,4,6-trimethylaniline **2**, oxalaldehyde **3**, formaldehyde **4** in CH_3COOH and H_2O, 70 °C, 18 h. b) THF, reflux, 18 h, R—Br. (c) Method 1: $AgNO_3$, K_2CO_3, DCM, 20 °C, 24 h. Method 2: Ag_2O, DCM, 20 °C, 24h.

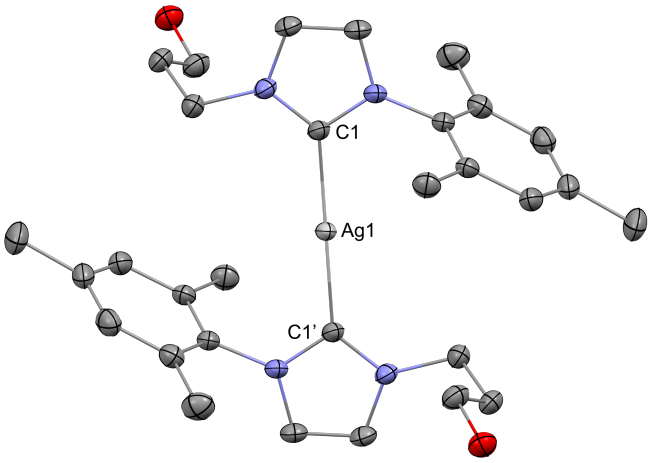
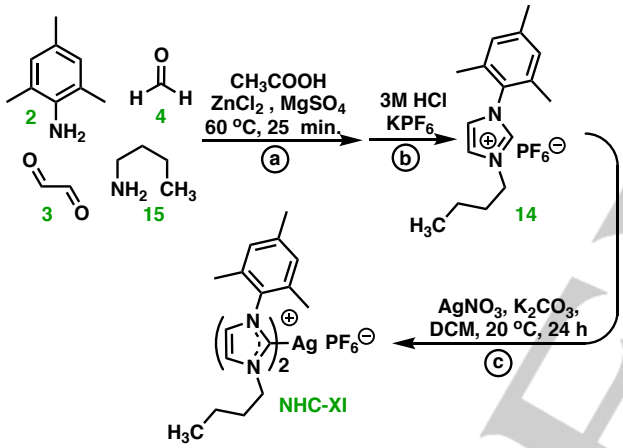


Figure 2. X-ray crystal structure of the cationic moiety of silver-NHC complex **NHC-IX**. The distance C1-Ag is 2.068(3) Å, the angle C1-Ag-C1' is 180.00(0)° and the angle between NHC-planes is 0.0(2) °. The symmetry operator for C1 to C1' is (-x+1, -y+1, -z+1). The atomic displacement factors are given at a 50 % probability. Hydrogens are omitted for clarity. Further details are provided in the supporting information file.



Scheme 2. Synthesis of (1-butyl-3-mesityl-2,3-dihydro-1*H*-imidazol-2-yl) silver(I) hexafluorophosphate **NHC-XI**. Reagents and conditions: (a) 2,4,6-trimethylaniline **2**, oxalaldehyde **3**, butan-1-amine **15**, Reflux, 25 min. (b) 3M HCl, KPF_6. (c) $AgNO_3$, K_2CO_3, DCM, 20 °C, 24 h.

Ghdhayed, Haque, and collaborators[25] claimed that it is possible to produce either mono-ligand-silver or bis-ligand-silver complexes from the same NHC ligand. The key factor that induced the formation of the mono- or bis-NHC Ag complex was the reaction temperature used during the silver complexation. The bis-NHC-Ag complex was produced when the complexation was conducted at 20 °C, while the mono-NHC-Ag complex was produced when the reaction mixture was refluxed (that is at 82 °C) in CH_3CN as the reaction medium. We conducted a similar experiment to see if this observation was of general validity using our ligand (that was used for **NHC-IX**). Our trials resulted in the formation of the bis-NHC-Ag complex both at low and high reaction temperature.

Biology

The screening method used in this study was based on WST-1 and reflects cellular metabolism and mitochondrial activity, and has been described in detail previously.[20] We used the cell line HL-60 in this screen to test the new silver-NHC complexes **NHC-IX**, **NHC-X**, **NHC-XII**, and **NHC-XIII** against a model for acute myeloid leukemia with complex karyotype and deletions of both alleles of tumor suppressor TP53 (see atcc.org for details about cell lines). HL-60 reflects thereby the most therapy refractory leukemia. The monocytic derived MOLM-13 model a more therapy responsive leukemia, but likely dependent on its mutated receptor tyrosine kinase FLT3 gene, and with severe chromatin remodulations due to the MLL gene fusion. MOLM-13 is in general highly sensitive for ATP antagonists that inhibits FLT3. Surprisingly, we find that six of the synthesized Ag-NHC complexes were more effective in HL-60 than in MOLM-13 (Figure 1). We may speculate that the mechanism of action of the Ag-NHC is reflected in the biology of the two cell lines. Particularly **NHC-X** and **NHC-XII** would be interesting to test in normal bone marrow cells and other tumor cells to determine if cytotoxicity is beneficial for cancer cell eradication. **NHC-XII** may be a complex that has lost its discriminative power between HL-60 and MOLM-13, but represents a cytotoxic compound with a wider application. Overall, the structure–activity optimization accomplished in present study afforded a substantial augmentation of the cytotoxic activity. Comparison of the most active silver complex **NHC-XII** synthesized in this study with the Ag-NHC complexes prepared in our previous works revealed a high potency improvements: **NHC-I** : **NHC-XII** = 20 for the HL-60 cell line and **NHC-I** : **NHC-XII** = 18 for the MOLM-13 cell line, and **NHC-II** : **NHC-XII** = 3.5 for the HL-60 cell line and **NHC-II** : **NHC-XII** = 3.3 for the MOLM-13 cell line.

Conclusions

Five different silver-3-alkyl-1-mesityl-1*H*-imidazol-3-ium complexes (**NHC-IX**–**NHC-XIII**) were synthesized, characterized, and biologically evaluated for cytotoxicity. The complexes were found to be cytotoxic in the micromolar concentration range towards the two human cell lines HL-60 and MOLM-13. Space demanding substituents installed on the N1 of the imidazole ring concurrently with an alkyl chain on N3 augmented the cytotoxicity of the silver NHC complex, revealing that the ligand molecular structure is of paramount importance for the cytotoxicity of the final silver-NHC complex. X-ray crystallography shown that a bis-ligand silver complex (**NHC-IX**) was formed, which apparently act in opposition to a mechanism of action that comprises "slow release" of Ag^+ ions. For now, we suggest that the mechanism of action involves the entire Ag-NHC complex participating in an outer sphere SET process[26] intercepting a redox process such as the thioredoxin (Trx) system[27] that recently was observed as a modulator of tumor expansion.[28] The Trx system plays an important role for DNA synthesis and as a means to cope with oxidative stress.[29] Moreover, the Trx system serves as a large disulfide reductase, which catalyzes proteindisulfid-ditiol transformation with a preserving CGPC-active site motif. Reductase catalyzes TrxR selenium-sulfide-selenium-thiol transformation and supplies electrons to a wide range of enzymes.[29] Such a biological interaction involving Au-NHC complexes towards TrxR have been revealed,[30] which make it reasonable to believe that an analogous mechanism of action might occurs with Ag-NHCs.

The brief structural-activity studies carried through in this study, afforded an Ag-NHC complex (**NHC-XII**) comprising substantial enhanced cytotoxicity, **NHC-I** versus **NHC-XII** ≈ 20 ×. Further structure-activity optimization studies involving novel silver-NHC complexes are underway and will be reported in near future. Mechanism of action studies are currently in course of planning.

Experimental part

Chemistry

General Methods

^{1}H and ^{13}C NMR spectra were recorded at ambient temperature at frequencies of 500 MHz and 125 MHz, respectively. The chemical shifts were reported in ppm relative to residual $CHCl_3$ for proton (δ = 7.26 ppm) and $CDCl_3$ for carbon (δ = 77.16 ppm) and with DMSO-d_6 for proton (δ = 2.50 ppm) and for carbon (δ = 39.0 ppm) with an external reference (TMS). Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), multiplet (m), broad (br) and overlapped (o).

Synthetic procedures

1-(2,4,6-trimethylphenyl)-1*H*-imidazole[22] **5 [25364-44-7]**. Acetic acid (10 mL), aqueous formaldehyde **4** (40 mmol, 3.00 mL) and aqueous glyoxal **3** (40 mmol, 4.60 mL) was transferred to a three necked round bottom flask (50 mL) equipped with a condenser and a magnetic stirring bar. The mixture was heated at 70 °C, whereupon an mixture composed of acetic acid (10 mL), ammonium acetate **1** (40 mmol, 3.08 g) in water (2 mL) and mesitylamine **2** (39 mmol, 5.60 mL) were added drop-wise to the solution over a period of 20 min. by means of a dropping-funnel. The mixture was stirred at 70°C for another 18 h. The mixture was then cooled at room temperature and drop-wise added to a stirred aqueous solution of sodium bicarbonate (29.40 g / 300 mL). A brown solid was formed, which was filtrated off by using a Büchner funnel with filter paper and washed with water (3 × 20 mL). The product was left to dry, and finally recrystallized using ethyl acetate to obtain target product in an isolated yield of 31% (2.27 g). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.62 (s, 1H), 7.17 (s, 1H), 7.11 (s, 1H), 7.03 (s, 2H), 2.29 (s, 3H), 1.92 (s, 6H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ = 137.97, 137.55, 134.66, 133.40, 128.74, 128.66, 120.34, 20.48, 16.91 ppm.

3-(3-hydroxypropyl)-1-(2,4,6-trimethylphenyl) imidazolium bromide[31] **10 [656832-57-4]**. 1-(2,4,6-trimethylphenyl)-1*H*-imidazole **5** (10.0 mmol, 1.86 g), 3-bromo-1-propanol **6** (10 mmol, 0.90 mL) and THF (18 mL) was added to a round bottle flask equipped with a condenser and a stirring bar. The solution was heated and stirred at reflux for 18 h. The mixture was then diluted with methyl *tert*-butyl ether (MTBE) (30 mL) whereupon the product precipitated. The mixture was extracted with distilled water (2 × 30 mL) (give the product some time to dissolve). The aqueous phases were combined and washed with MTBE (3 × 20 mL). The water was then removed under reduced pressure using a rotary evaporator. Resulting in a light brown waxy compound that was isolated in a yield of 39% (1.27 g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.49 (s, 1H), 8.13 (s, 1H), 7.94 (s, 1H), 7.15 (s, 2H), 4.38 (t, *J* = 6.9 Hz, 1H), 3.47 (t, *J* = 5.8 Hz, 1H), 2.34 (s, 3H), 2.05 (m, 2H), 2.03 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.14, 137.42, 134.28, 131.13, 129.17, 123.74, 123.25, 57.21, 46.94, 31.96, 20.53, 16.84 ppm.

3-(6-hydroxyhexyl)-1-(2,4,6-trimethylphenyl) imidazolium bromide[31] **11 [656832-48-3]**. 1-(2,4,6-trimethylphenyl)-1*H*-imidazole **5** (5.0 mmol, 0.93 g), 6-bromo-1-hexanol **7** (5.0 mmol, 0.90 mL), and THF (9.20 mL) were transferred to a round bottle flask (25 mL) equipped with a condenser and a magnetic stirrer bar. The mixture was heated at reflux and stirred for 18 h. The mixture was diluted with MTBE (15 mL) whereupon the product precipitated. The mixture was extracted with distilled water (2 × 15 mL) (give the product some time to dissolve). The aqueous phases were combined and washed with MTBE (3 × 10 mL). The water was then removed under reduced pressure using a rotary evaporator. The resulting target product was isolated as a brown waxy compound in an isolated yield of 39% (0.71 g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.12 (s, 1H), 7.95 (s, 1H), 7.16 (s, 2H), 4.29 (t, *J* = 6.9 Hz, 2H), 2.34 (s, 3H), 2.03 (s, 6H), 1.90 (p, *J* = 7.4 Hz, 2H), 1.44 – 1.40 (m, 2H), 1.36 – 1.32 (m, 2H), 1.28 – 1.24 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.19, 137.30, 134.26, 131.14, 129.23, 123.88, 123.17, 60.42, 49.22, 32.22, 29.10, 25.30, 24.78, 20.57, 16.88.

3-(8-hydroxyoctyl)-1-mesityl-1*H*-imidazolium bromide[31] **12 [NEW]**. 1-(2,4,6-trimethylphenyl)-1*H*-imidazole **5** (5.00 mmol, 0.92 g), 8-bromo-1-octanol **8** (5 mmol, 0.65 mL) and THF (9.20 mL) were transferred to a round bottle flask (25 mL) equipped with a condenser and a magnetic stirring bar. The mixture was heated at reflux and stirred for 18 h. Then, the mixture was diluted with MTBE (15 mL) whereupon the product precipitated. The mixture was extracted with distilled water (2 × 15 mL) (give the product some time to dissolve). The aqueous phases were combined and washed with MTBE (3 × 10 mL). The water was then removed under reduced pressure using a rotary evaporator. Target product was obtained as a brown waxy compound in an isolated yield of 35% (0.69 g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.49 (s, 1H), 8.12 (s, 1H), 7.95 (s, 1H), 7.15 (s, 2H), 4.29 (t, *J* = 7.0 Hz, 2H), 2.33 (s, 3H), 2.02 (s, 6H), 1.90 (p, *J* = 14.3 2H), 1.40 (p, *J* = 6.7 Hz, 2H), 1.26 (m, 10H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.24, 137.24, 134.26, 131.14, 129.23, 123.93, 123.16, 60.63, 49.29, 32.43, 28.99, 28.71, 28.26, 25.36, 25.30, 20.56, 16.82.

3-(6-ethoxy-6-oxohexyl)-1-mesityl-1*H*-imidazolium bromide[31] **13 [NEW]**. 1-(2,4,6-trimethyl-phenyl)-1*H*-imidazole (10.0 mmol, 1.87 g) **5**, ethyl-6-bromohexanoate **9** (10.0 mmol, 1.79 mL), and THF (19 mL) were transferred to a round bottle flask (100 mL) equipped with a condenser and a magnetic stirrer bar. The solution was heated at reflux and stirred for 18 h. The mixture was diluted with MTBE (30 mL) and the product precipitated. The mixture was extracted with distilled water (2 × 15 mL) (give the product some time to dissolve). The aqueous phases were combined and washed with MTBE (3 × 10 mL). The water was then removed under reduced pressure using a rotary evaporator. Target product was obtained as a brown waxy compound in an isolated yield of 32% (1.30 g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.14 (s, 1H), 7.96 (s, 1H), 7.16 (s, 2H), 4.31 (t, *J* = 7.1 Hz, 2H), 4.04 (q, *J* = 7.1 Hz, 2H), 2.33 (s, 3H), 2.31 (t, *J* = 7.3 Hz, 2H) 2.02 (s, 6H), 1.92 (p, *J* = 7.2 Hz, 2H), 1.58 (p, *J* = 7.4 Hz, 2H), 1.27 (p, *J* = 7.7 Hz, 2H), 1.17 (t, *J* = 7.1 Hz, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 172.67, 140.22, 137.28, 134.27, 131.14, 129.23, 123.90, 123.15, 59.68, 49.07, 33.16, 28.70, 24.82, 23.63, 20.56, 16.85, 14.11.

3-butyl-1-mesityl-1*H*-imidazol-3-ium hexafluorophosphate[32] **14 [1176199-40-8]**. 2,4,6-trimethylaniline **2** (0.14 mL, 1.0 mmol), butylamine **15** (0.1 mL, 1.0 mmol), and acetic acid (0.26 mL, 4.5 mmol) were transferred to a round bottom flask (250 mL) equipped with a magnetic stirrer bar. The mixture was heated at 60 °C for 5 min. Then, $MgSO_4$ (0.241 g, 2.0 mmol) was added to the mixture (denoted as mixture A). Another mixture B was prepared by transferring to a round bottle flask equipped with a magnetic stirrer bar: glyoxal **3** (0.12 mL, 1.0 mmol, 40 % weight in aqueous solution), formaldehyde **4** (0.1 mL, 1.0 mmol, 37% in

aqueous solution), and acetic acid (0.26 mL, 4.5 mmol) that was heated at 60 °C for 5 min. whereupon $ZnCl_2$ (0.164, 1.2 mmol) and Et_2O (1.2 mL) were added. Mixture B was then added to mixture A and the combined mixtures was stirred at 60 °C for 25 min. before cooling at 20 °C. DCM (25 mL) and HCl(aq) (3M, 50 mL) was added and the resulting mixture was stirred at 20 °C for 1 h. The post-reaction mixture was then transferred to a separatory funnel where the organic phase was separated, and water (50 mL) and potassium hexafluorphosphate (0.184 g, 1.0 mmol) was added. The mixture was stirred at 20 °C 1 h, before the organic phase was separated and dried over $MgSO_4$. The organic phase was filtered, and the solvent removed under reduced pressure using a rotary evaporator. The resulting product was a yellow waxy compound in an isolated yield of 73% (0.28 g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.11 (s, 1H), 7.95 (s, 1H), 7.16 (s, 2H), 4.29 (t, *J* = 7.1 Hz, 2H), 2.34 (s, 3H), 2.02 (s, 6H), 1.89 (p, *J* = 7.3 Hz, 2H), 1.29 (h, *J* = 7.4 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.26, 137.20, 134.27, 131.14, 129.23, 123.96, 123.14, 49.06, 31.00, 20.54, 18.73, 16.79, 13.22.

Silver(I) NHC complexes[33] **NHC-IX, NHC-X**, **NHC-XI**, **NHC-XII**, **NHC-XIII**. The Imidazolium salt (**10**, **11, 12, 13, 14**) (0.60 mmol), $AgNO_3$ (0.102-0.154 g, 0.60-0.90 mmol), and DCM (30 mL) were transferred to a round bottle flask (50 mL) equipped with a magnetic stirrer bar. The reaction mixture was stirred for 2 min. whereupon K_2CO_3 (10 mmol) was added, and then stirred for another 3.5-24 h. The reaction mixture was then filtered through a pad of Celite and the solvent volume was reduced until a volume of ≈2 mL using a rotary evaporator. Target silver complex precipitated from the concentrate when hexane (15 mL) was added.

Bis(1-(3-hydroxypropyl)-3-mesityl-2,3-dihydro-1H-imidazol-2-yl) silver(I) bromide NHC-IX was isolated in a yield of 45% (using 0.6 mmol of substrate **10** and 0.9 mmol of $AgNO_3$ with a reaction time of 3.5 h), or a yield of 52% when using 0.6 mmol of substrate **10** and 0.6 mmol of $AgNO_3$ and a reaction time of 24 h). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (s, 2H), 6.85 (s, 6H), 4.42 (t, *J* = 6.9 Hz, 4H), 3.63 (t, *J* = 5.4 Hz, 4H), 2.40 (s, 6H), 2.06 (m, 4H), 1.74 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.73, 135.49, 134.91, 129.05, 122.59, 120.99, 57.25, 48.59, 33.91, 21.19, 17.41. HRMS (ESI) m/z: calcd for $C_{30}H_{40}N_4O_2{}^{107}Ag$ 595.22022; found 595.22061 and calcd for $C_{30}H_{40}N_4O_2{}^{109}Ag$ 597.21988; found 597.21736.

Bis(1-(6-hydroxyhexyl)-3-mesityl-2,3-dihydro-1H-imidazol-2-yl) silver(I) bromide NHC-X was isolated in a yield of 93% (using 0.6 mmol of substrate **11** and 0.6 mmol of $AgNO_3$ with a reaction time of 24 h).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 2H), 6.94 (s, 6H), 4.13 (s, 4H), 3.61 (t, *J* = 6.4 Hz, 4H), 2.36 (s, 6H), 1.88 (s, 12H), 1.83 (s, 4H), 1.62 – 1.52 (m, 5H), 1.49 – 1.39 (m, 4H), 1.29 (s, 4H).^{13}C NMR (126 MHz, $CDCl_3$) δ 139.29, 135.43, 134.74, 129.26, 122.67, 121.69, 61.91, 51.72, 32.32, 31.29, 30.95, 26.00, 25.21, 21.11, 17.58. HRMS (ESI) m/z: calcd for $C_{36}H_{52}N_4O_2{}^{107}Ag$ 679.31412; found 679.31443 and calcd for $C_{36}H_{52}N_4O_2{}^{109}Ag$ 681.31378; found 681.30640.

Bis(1-butyl-3-mesityl-2,3-dihydro-1H-imidazol-2-yl) silver PF_6 NHC-XI [33] was isolated in a yield of 83% (using 0.6 mmol of substrate **11** and 0.6 mmol of $AgNO_3$ with a reaction time of 24 h).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 (t, *J* = 1.7 Hz, 2H), 6.95 (s, 4H), 6.93 (t, *J* = 1.6 Hz, 2H), 3.95 (t, *J* = 6.9 Hz, 4H), 2.37 (s, 6H), 1.84 (s, 12H), 1.68 – 1.73 (m, 4H), 1.20 – 1.11 (m, 4H), 0.89 (t, *J* = 7.3 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 139.32, 135.49, 134.83, 129.16, 122.68, 121.99, 51.40, 33.23, 30.90, 21.07, 19.45, 17.42, 13.59. HRMS (ESI) m/z: calcd for $C_{32}H_{44}N_4{}^{107}Ag$ 591.26169; found 591.26193 and calcd for $C_{32}H_{44}N_4{}^{109}Ag$ 593.26135 found 593.26604.

Bis(1-(8-hydroxyoctyl)-3-mesityl-2,3-dihydro-1H-imidazol-2-yl) silver(I) bromide NHC-XII was isolated in a yield of 67% (using 0.6 mmol of substrate **12** and 0.6 mmol of $AgNO_3$ with a reaction time of 24 h).^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87 (s, 8H), 4.10 (s, 4H), 3.54 (t, *J* = 6.6 Hz, 4H), 2.27 (s, 6H), 1.85 (s, 12H), 1.78 (s, 4H), 1.49 (m, 4H), 1.25 (s, 16H).^{13}C NMR (126 MHz, $CDCl_3$) δ 135.41, 134.71, 129.36, 128.98, 122.76, 99.99, 62.53, 51.91, 32.61, 31.30, 29.16, 28.95, 26.17, 25.59, 21.10, 17.62. HRMS (ESI) m/z: calcd for $C_{40}H_{60}N_4O_2{}^{107}Ag$ 735.37672; found 735.37700 and calcd for $C_{40}H_{60}N_4O_2{}^{109}Ag$ 737.37638 found 737.38167.

Bis(1-(6-ethoxy-6-oxohexyl)-3-mesityl-2,3-dihydro-1H-imidazol-2-yl) silver(I) bromide NHC-XIII was isolated in a yield of 61% (using 0.6 mmol of substrate **13** and 0.6 mmol of $AgNO_3$ with a reaction time of 24 h). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (d, *J* = 1.7 Hz, 2H), 6.98 (d, *J* = 1.7 Hz, 2H), 6.95 (s, 4H), 4.11 (q, *J* = 7.1 Hz, 8H), 2.35 (s, 6H), 2.29 (t, *J* = 7.4 Hz, 4H), 1.90 (s, 12H), 1.87 – 1.80 (m, 4H), 1.64 (p, *J* = 7.6 Hz, 4H), 1.25 (t, *J* = 7.1 Hz, 10H).^{13}C NMR (126 MHz, $CDCl_3$) δ 173.09, 139.16, 135.37, 134.62, 129.13, 122.68, 121.81, 60.15, 51.50, 33.81, 31.00, 30.85, 25.72, 24.23, 20.98, 17.47, 14.16. HRMS (ESI) m/z: calcd for $C_{40}H_{56}N_4O_4{}^{107}Ag$ 763.33525; found 763.33556 and calcd for $C_{40}H_{56}N_4O_4{}^{109}Ag$ 765.33491; found 765.33260.

Crystallography

A colourless crystal specimen with needle morphology was mounted inside a MiTiGen MicroLoop containing Parabar 10312 oil (Hampton Research) and diffraction data were collected at 100 K using an Oxford Cryosystems, Cryostream 700 Plus N_2 open flow blower, on the PILATUS@SNBL diffractometer at the BM01A station of the Swiss–Norwegian Beamlines at the ESRF (Grenoble, France). The energy (wavelength) of the synchrotron radiation was set to 17.1774 keV (0.72179 Å) using a 16-bunch electron storage ring mode. The crystals scatter weakly and the Bragg profiles are split in two components. The data were collected by way of one φ scans of 1.456 images for a total of 24 min., with an angular step of 0.5° in a shutter-free mode with the PILATUS2M detector.

The structure suffers from disorder of the anionic (Br^-) site, situated on an inversion centre. The positional disorder can in addition possibly be chemical as a fraction of the site might be occupied by a NO_3^- ion. As this disorder cannot be resolved and because there is no independent proof of the chemical composition, the structure is only described with respect to the cationic metal complex moiety. The Bragg diffraction pattern also shows twinning. Only the major part of the two components has been integrated. Further details are provided in the supporting information file.

Biological activity screening.

The human cell lines HL-60 and MOLM-13 (American Type Culture Collection) were cultured in RPMI 1640, 2 mM *L*-

Glutamine, 50 UmL^{-1} penicillin/streptomycin (Sigma Aldrich) and 10% fetal bovine serum (Biowest) and incubated in humidified atmosphere at a temperature of 37°C under 5% CO_2. The cells (2×10^5 cells $\times$ mL^{-1}) were treated with various concentrations (0.001 µM $\rightarrow$ 100 µM) of the five different silver N-heterocyclic carbene complexes (**NHC-IX**, **NHC-X**, **NHC-XI**, **NHC-XII**, and **NHC-XIII**) for a period of 24 h. The metabolic cell activity, that is the cell viability, was measured by using the WST-1 cell proliferation agent (Roche) as described by the supplier protocol and read on a luminescence plate reader (Infinite 200 Pro, Tecan).

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Conflict of Interest

The authors declare no conflict of interest.

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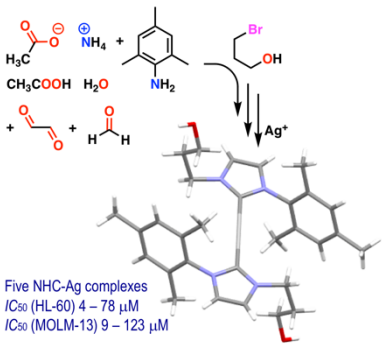

Ag-NHC complexes were designed, synthesised, and biological evaluated versus human cell lines modelling acute myeloid leukemia to reveal high cytotoxicity.

Institute and/or researcher Twitter usernames:

ORCID

- Hans-René Bjørsvik: 0000-0001-9593-6079
- Karl Wilhelm Törnroos: 0000-0001-6140-5915
- Bjørn Tore Gjertsen: 0000-0001-9358-9704
- Eirin Alme: 0000-0003-0690-3465