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Synthesis and anticancer activity of silver(I)–N-heterocyclic carbene complexes derived from the natural xanthine products caffeine, theophylline and theobromine†

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A new library of silver(I)–N-heterocyclic carbene complexes prepared from the natural products caffeine, theophylline and theobromine is reported. The complexes have been fully characterised using a combination of NMR spectroscopy, mass spectrometry, elemental analysis and X-ray diffraction analysis. Furthermore, the hydrophobicity of the complexes has been measured. The silver(I)–N-heterocyclic carbenes have been evaluated for their antiproliferative properties against a range of cancer cell lines of different histological types, and compared to cisplatin. The data shows different profiles of response when compared to cisplatin in the same panel of cells, indicating a different mechanism of action. Furthermore, it appears that the steric effect of the ligand and the hydrophobicity of the complex both play a role in the chemosensitivity of these compounds, with greater steric bulk and greater hydrophilicity delivering higher cytotoxicity.

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Introduction

Since their isolation in 1991,[1] N-heterocyclic carbenes (NHCs) have become ubiquitous in organometallic chemistry, particularly in the field of catalysis.[2–5] In more recent years, metal–NHCs have shown promise as antimicrobial (silver–NHCs) and as antitumour (palladium–, copper–, silver– and gold–NHCs) agents.[6–11] The antimicrobial properties of silver have been exploited for centuries, with silver now being incorporated into several materials such as wound dressings, creams, deodorants and even clothing. The efficacy of a silver-based antibacterial compound appears to be linked to its bioavailability and prolonged release of silver over a long period of time to prevent reinfection.[12] The release rate is linked to the ancillary ligand and, as NHCs are strong σ-donors, silver–NHCs can have a slow silver release rate. In addition to antimicrobial activity, several studies have demonstrated that silver–NHCs have a potential future in the field of cancer chemotherapy.[13–17]

Although platinum-based drugs such as cisplatin have been pivotal in the fight against cancer,[18] severe side-effects and the development of drug resistance necessitate the need for new organometallic anticancer drugs. As silver is thought to have relatively low toxicity and is already used widely in biomedicine, it is a judicious choice of metal to study in cancer chemotherapy. However, it is imperative that the material (delivery agent) surrounding the silver also has a low toxicity profile. Xanthine derivatives (Fig. 1) are natural products and are often found in beverages and foods such as coffee and chocolate. As xanthine derivatives contain an imidazole ring, these are potential precursors to NHCs. Due to the low toxicity and high versatility of these compounds, they are ideal to study in combination with silver as potential chemotherapeutic compounds. Furthermore, these compounds have themselves been used medicinally and have potential chemotherapeutic effects.[19] Youngs and co-workers have previously prepared a silver(I)–NHC complex starting from caffeine, which was found

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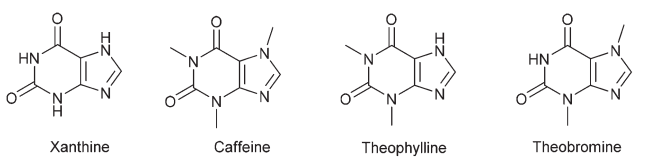


Fig. 1 Xanthine and naturally occurring derivatives of xanthine.

to be active against resistant respiratory pathogens.[20] The same group also added a hydroxyethyl group to the backbone of the NHC, with the silver(I) complex of this ligand being effective against a variety of cystic fibrosis relevant pathogens.[21] Caffeine-based NHC complexes of gold(I) have recently been reported and their biological properties investigated.[22] Herein, we report the synthesis of a family of xanthine-derived imidazolium salts, prepared from caffeine, theophylline and theobromine. Silver(I)–NHC complexes were prepared from the imidazolium salt precursors and evaluated against eight cancer cell lines.

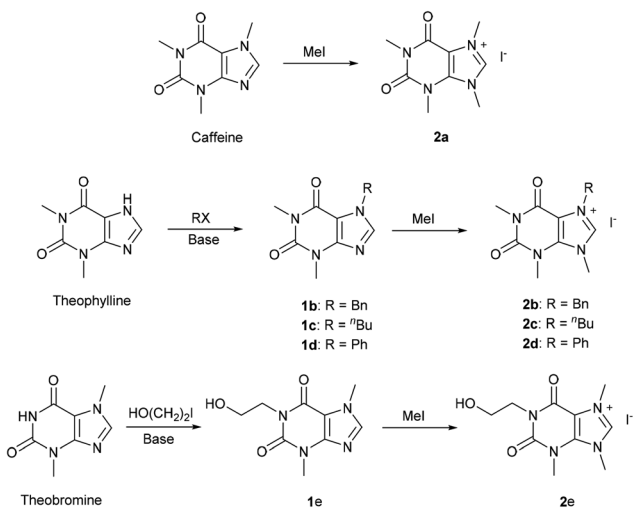
Results and discussion

Imidazolium salts **2a–2e** were prepared from either caffeine, theophylline or theobromine (Scheme 1). Methylation of caffeine to yield the corresponding imidazolium salt **2a** is achieved through reaction with methyl iodide in DMF at reflux.[20] Due to the volatility of methyl iodide, we found it necessary to use a large excess (20 equivalents) and heat in a closed system. We found that dimethyl sulfate and methyl tosylate could also be used as methylating agents to give similar product yields of the corresponding imidazolium methyl sulfate or tosylate salts (>70%). However, reaction of caffeine with other alkylating agents (benzyl bromide, 1-iodobutane) under the same conditions did not result in formation of an imidazolium salt, presumably due to the relatively low basicity of the nitrogen atom of the caffeine. Therefore, theophylline was employed as the precursor to initially add an alternative alkyl group, followed by methylation using methyl iodide. Imidazole compounds **1b** and **1c** were synthesised through reaction of benzyl bromide or 1-iodobutane with theophylline in acetonitrile at reflux, in the presence of a base (K_2CO_3). Imidazole compound **1d** required an Ullmann-type coupling reaction, in which theophylline was reacted with aryl iodide using a copper catalyst (CuI, isobutyrylcyclohexanone, Cs_2CO_3). Imidazolium salts **2b–2d** were then prepared by reaction of the appropriate imidazole with methyl iodide in DMF at reflux, again in a closed system. A hydroxyethyl functionality was added to the ligand architecture by reaction of theobromine with 2-iodoethanol in DMF at reflux, in the presence of a base (Cs_2CO_3), to give imidazole compound **1e**.[21] This was subsequently reacted with methyl iodide under the same reaction conditions as previously described, to give imidazolium salt **2e**.

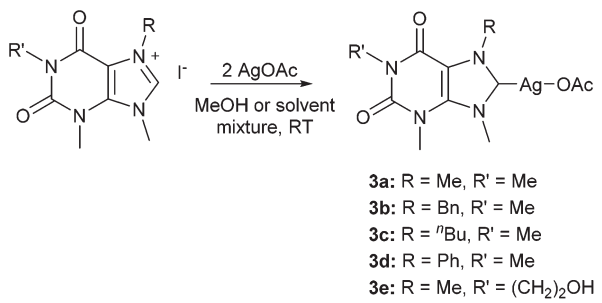
Imidazolium salts **2a–2e** were fully characterised, and solid-state structures were obtained for imidazolium salts **2b–2d** (see ESI†). In each case, the backbone pyrimidinone of the imidazolium salt twists slightly out of the plane as defined by the N(1)–C(2)–N(4) bond. This twisting is most pronounced in imidazolium salt **2d**, with a C(9)–N(4)–C(7)–O(2) torsion angle of 9.19° (analogous torsion angles in **2b** and **2c** are <1°). The greater degree of twisting in **2d** presumably arises due to the steric constraints imposed by the phenyl *N*-substituent.

Reaction of imidazolium salts **2a–2e** with AgOAc at room temperature resulted in formation of complexes of the type Ag(NHC)OAc **3a–3e** (Scheme 2). Complex **3a** was synthesised in methanol as previously reported.[20] We found it necessary to use 1 : 1 mixtures of either methanol–dichloromethane (with ligand precursors **2b**, **2c** and **2e**) or methanol–acetonitrile (with ligand precursor **2d**) to aid solubility of the imidazolium salts for the syntheses of complexes **3b–3e**.

Silver(I)–NHC complexes **3a–3e** were fully characterised, and solid-state structures were obtained for complexes **3b–3d** (Fig. 2). In all three cases the geometry around the silver atom deviates from linearity, with C(2)–Ag(1)–O(3) bond angles of 168.6–171.6° (Table 1). This deviation is consistent with the structure of complex **3a**, which has previously been reported in the literature.[20] The silver–carbene bond lengths for complexes **3c** (2.078(3) Å) and **3d** (2.068(4) Å) are similar to those for complexes **3a** (2.067(3) Å) and **3e** (2.072(4) Å) which have previously been reported. The silver–carbene bond length in complex **3b** (2.052(7) Å), however, is shorter than those observed in other similar complexes. This is likely due to the flexibility of the benzyl substituent, hence lower steric effect, allowing closer



Scheme 1 Synthesis of imidazolium salts **2a–2e** starting from caffeine, theophylline or theobromine.



Scheme 2 Synthesis of silver–NHC complexes **3a–3e**.

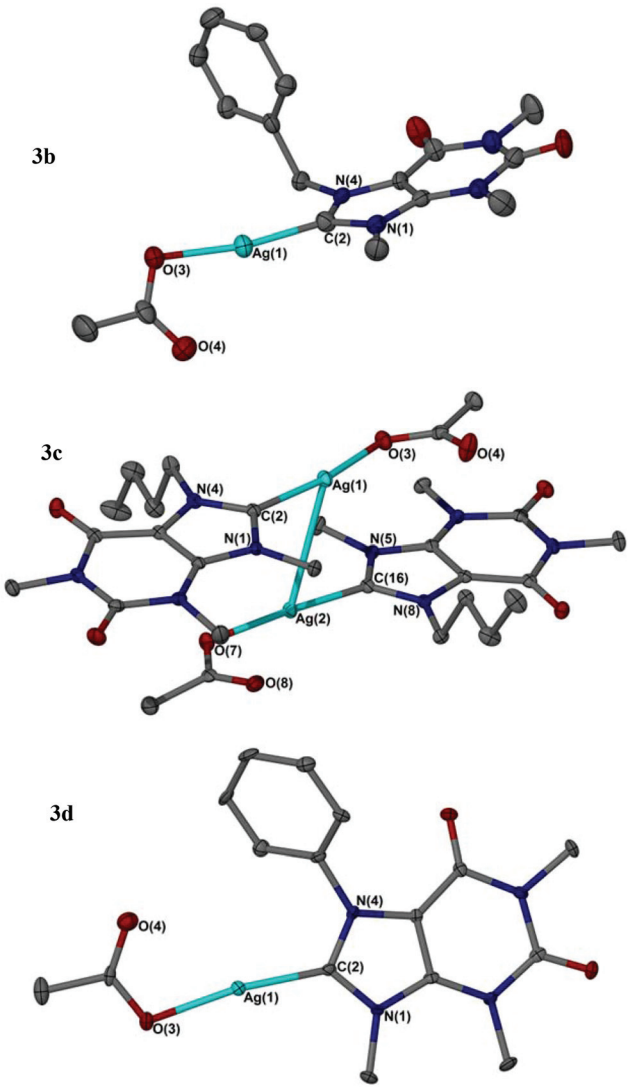


Fig. 2 Molecular structures of silver(I)–NHC complexes **3b–3d**. Hydrogen atoms have been omitted for clarity and ellipsoids are shown at 50% probability.

interaction between the carbenic centre and the silver ion. The ^{n}butyl substituent in complex **3c** is not expected to impart any significant steric constraints on the carbenic carbon. However, a possible argentophilic interaction of approximately 3.2 Å may cause the lengthening of the silver–carbene bond in the solid-state structure of this complex.

Table 1 Selected bond lengths (Å) and angles (°) for silver(I)–NHC complexes **3b–3d**

Bond lengths (Å) and angles (°)	3b	3c	3d
Ag(1)–C(2)	2.052(7)	2.078(3)	2.068(4)
Ag(1)–O(3)	2.120(5)	2.1042(19)	2.111(3)
C(2)–Ag(1)–O(3)	171.4(2)	168.72(9)	168.94(11)
N(1)–C(2)–N(4)	105.2(5)	105.46(18)	105.6(3)
Ag(1)–Ag(2)	—	3.1931(3)	—

The *in vitro* cytotoxicity of silver(I)–NHC complexes **3a–3e** was determined using MTT-based assays involving a 96 hour drug-exposure period. Compounds were tested for their activity against A375 (malignant melanoma), HCT116 (colorectal carcinoma), HT-29 (colorectal adenocarcinoma), LN229 (glioblastoma), Panc-1 (pancreatic carcinoma), SiHa (grade II, squamous cell carcinoma cervix), U-87 MG (glioblastoma) and U-251 (glioblastoma). The results are summarised in Table 2 and Fig. 3. Complexes **3a–3e** exhibit moderate cytotoxicity against the cell lines tested, with IC_{50} values being in the micromolar range.

The profiles of response in this panel of cells is different to that for cisplatin, indicating a different mechanism of action for silver(I)–NHC complexes. The most cytotoxic silver complex in general across these cell-lines is **3d** (see ESI†), in which the NHC ligand bears an *N*-phenyl substituent. The sterically encumbering phenyl group is likely to exert a stabilising effect on the silver–NHC bond, which may lead to a slower silver release rate and enhanced cytotoxicity profile. It is apparent that addition of a hydroxyethyl group to the backbone of the ligand improves activity, with complex **3e** being on average 2-fold more cytotoxic than complex **3a**. This is presumably due to the solubility of the complex, and its ability to cross the cell membrane.

As a means to assess the potential of complexes **3a–3e** to enter a cell membrane,[23] the hydrophobicity ($\log P$) of the five complexes was measured. Accurate amounts of each compound were dissolved in octanol-saturated water, with an

Table 2 Response of eight cell lines to silver(I)–NHC complexes **3a–3e** and cisplatin. Values presented are IC_{50} μM ± SD (in parentheses) for three independent experiments

Cell line	3a	3b	3c	3d	3e	Cisplatin
A357	34.5 ± 3.8	21.4 ± 7.5	27.4 ± 3.9	11.5 ± 5.3	12.4 ± 1.3	1.2 ± 0.3
HCT116	26.7 ± 9.9	29.6 ± 3.5	22.4 ± 4.8	19.5 ± 2.3	19.0 ± 5.1	2.4 ± 0.3
HT-29	41.8 ± 9.8	29.9 ± 16.5	28.5 ± 2.0	21.4 ± 6.5	20.7 ± 1.5	0.6 ± 0.1
LN229	29.2 ± 12.8	18.4 ± 12.0	46.5 ± 9.8	11.2 ± 1.5	7.4 ± 1.8	0.7 ± 0.5
Panc-1	31.7 ± 2.8	29.7 ± 8.2	16.9 ± 1.1	7.6 ± 3.2	23.5 ± 5.9	2.6 ± 0.9
SiHa	21.9 ± 3.8	15.3 ± 2.6	16.4 ± 0.5	13.1 ± 3.7	14.0 ± 2.2	0.9 ± 0.3
U87MG	33.7 ± 1.4	22.8 ± 5.1	14.0 ± 4.3	17.6 ± 4.5	22.1 ± 8.1	0.9 ± 0.4
U-251	54.8 ± 15.7	26.7 ± 8.8	51.4 ± 17.8	14.2 ± 2.5	29.6 ± 5.1	1.0 ± 0.6

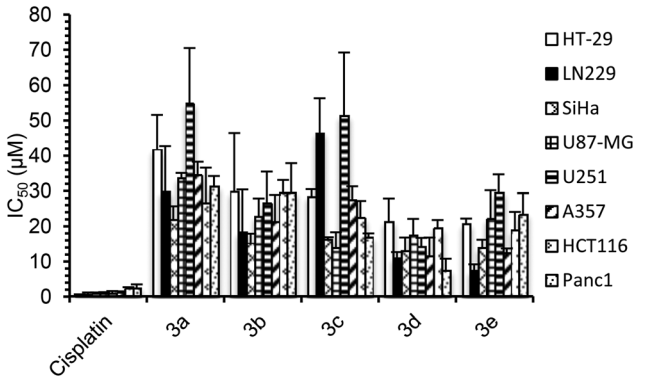


Fig. 3 Response of eight cell lines to silver(I)–NHC complexes **3a–3e** and cisplatin. Values presented are IC_{50} (μM) ± SD for three independent experiments. The presentation of results is based on the sensitivity of cells to cisplatin with the most sensitive line on the far left whereas the most resistant line is on the far right. If the test compounds have a similar mechanism of action to cisplatin, similar patterns of chemosensitivity would be expected.

equal amount of water-saturated octanol being layered on top. This was placed in the vibrax machine for 4 hours at 500 g min^{-1}. The layers were separated and the water-saturated octanol layer was analysed using UV-vis spectroscopy, with an average of at least six runs being taken. The results are summarised in Table 3 and S9 in the ESI,† with the complexes ranging from hydrophilic (−1.96) to hydrophobic (0.56). Cisplatin, with a log *P* value of −2.36, is more hydrophilic than any of the silver(I)–NHC complexes tested. Complex **3e**, like cisplatin, is hydrophilic, with the other complexes being more hydrophobic. Hydrophilicity is likely to lead to improved penetration of the cell membrane by complex **3e**, hence its increased cytotoxicity when compared to complex **3a**. It is clear, however, that hydrophobicity/hydrophilicity is not the single most important factor when considering the effect of these types of complexes against cancer cells. Though complex **3d** is more hydrophobic than complex **3a**, **3d** is on average over 2-fold more cytotoxic than **3a**. This indicates that a combination of both steric and solubility factors should be taken into account when designing new NHC ligands for study in chemotherapeutic applications.

Table 3 Log *P* values for complexes **3a–3e**

Complex	Log *P*	±SD
Cisplatin	−2.36	0.01
3a	−0.01	0.03
3b	0.53	0.02
3c	0.56	0.07
3d	0.1512	0.05
3e	−1.96	0.06

Conclusion

Synthetic procedures have been developed for the preparation of imidazolium salts from caffeine, theophylline and theobromine. Due to the relatively low basicity of the nitrogen donor in caffeine, it was found necessary to initially add varying substituents to theophylline. Following this, methylation was achieved through heating the imidazole compounds with a large excess of methyl iodide in a closed system. The imidazolium salts were reacted with silver acetate to prepare silver(I)–NHC complexes of the type Ag(NHC)OAc. These were fully characterised and the solid-state structures determined through the use of X-ray diffraction techniques. The antiproliferative activities of the silver complexes were examined against eight cancer cell lines, and compared to cisplatin. Whilst these complexes were less active than cisplatin against the cell lines tested, their IC_{50} values were in the micromolar range. The hydrophobicity of each complex was also measured to assess their potential to cross the cell membrane. Upon comparing the chemosensitivity data for each complex and considering their hydrophobicity/hydrophilicity profiles, it is evident that a combination of both steric effects of the ligand and solubility of the silver complex play a role in their activity against cancer cells. Sterically bulky *N*-substituents on the ligand may result in a slower release rate of silver from the complex, leading to increased cytotoxicity, whilst a more hydrophilic complex is likely to have increased penetration through the cell membrane.

Experimental

General considerations

Manipulations were performed under an atmosphere of dry nitrogen by means of standard Schlenk line techniques. The gas was dried by passing through a twin-column drying apparatus containing molecular sieves (4 Å) and P_2O_5. Anhydrous solvents were prepared by passing the solvent over activated alumina to remove water, copper catalyst to remove oxygen and molecular sieves to remove any remaining water, *via* the Dow-Grubbs solvent system. ^1H and $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker DPX300 spectrometer. The values of chemical shifts are given in ppm and values for coupling constants (*J*) in Hz. Mass spectra were collected on a Bruker Daltonics (micro TOF) instrument operating in the electrospray mode. Microanalyses were performed using a Carlo Erba Elemental Analyser MOD 1106 spectrometer. X-ray diffraction data were collected on an Agilent SuperNova diffractometer fitted with an Atlas CCD detector with Mo-Kα radiation (λ = 0.71073 Å) or Cu Kα radiation (λ = 1.5418 Å). Crystals were mounted under oil on glass or nylon fibres. Data sets were corrected for absorption using a multiscan method, and the structures were solved by direct methods using SHELXS-97 and refined by full-matrix least squares on F^2 using ShelXL-97, interfaced through the program X-Seed. Molecular graphics for all structures were generated using POV-RAY in the X-Seed

program. Imidazolium salts **2a** and **2e** and silver(I)–NHC complexes **3a** and **3e** were prepared using slightly modified literature procedures.[20,21]

Synthesis of 9-benzyl-1,3,5-trimethylxanthine (1b). Theophylline (5.0 g, 27.8 mmol) was dissolved in acetonitrile (80 mL) and benzyl bromide (16.5 mL, 138.7 mmol) and potassium carbonate (4.25 g, 30.8 mmol) were added. The mixture was heated to reflux for 24 hours. The mixture was filtered and washed with acetonitrile. The filtrate was dried *in vacuo* to yield the product as a white solid. Yield: 6.1 g (81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.58 (s, 1H, NCHN), 7.43–7.32 (m, 5H, aromatic), 5.52 (s, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.13 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ: 153.4 (C═O), 150.1 (C═O), 139.4 (C), 136.1 (NCHN), 133.6 (C), 127.2, 126.9, 126.6 (CH), 105.1 (C), 48.3 (CH_3), 31.8 (CH_3), 27.9 (CH_2). HRMS (ESI^+): calcd for $C_{14}H_{15}N_4O_2$ $[M + H]^+$: 271.1195. Found: 271.1188. Anal. Calcd for $C_{14}H_{14}N_4O_2{\cdot}1/3Et_2O$: C, 62.43; H, 5.92; N, 18.99. Found: C, 63.00; H, 5.45; N, 19.40.

Synthesis of 9-butyl-1,3,5-trimethylxanthine (1c). Theophylline (5.0 g, 27.8 mmol) was dissolved in acetonitrile (70 mL), and butyl iodide (12.0 mL, 105.5 mmol) and potassium carbonate (4.25 g, 30.8 mmol) were added. The mixture was heated to reflux for 48 hours, filtered, and washed with acetonitrile. The filtrate was dried *in vacuo* to yield the product as a white solid. Yield: 4.7 g (74%) ^{1}H NMR (300 MHz, d_6-DMSO): δ 8.09 (s, 1H, NCHN), 4.26 (t, J = 7.22 Hz, 2H, CH_2), 3.42 (s, 3H, CH_3), 3.19 (s, 3H, CH_3), 1.74 (quin, J = 7.22 Hz, 2H, CH_2), 1.24 (sext, J = 7.22 Hz, 2H, CH_2), 0.85 (t, J = 7.22 Hz, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 155.1 (C═O), 151.7 (C═O), 148.9 (C), 140.8 (NCHN), 106.9 (C), 47.0 (CH_2), 32.8 (CH_2), 29.7 (CH_2), 27.9 (CH_3), 19.5(CH_3), 13.4 (CH_3). HRMS (ESI^+): calcd for $C_{11}H_{17}N_4O_2$ $[M + H]^+$: 237.1351. Found: 237.1345. Anal. Calcd for $C_{11}H_{16}N_4O_2$: C, 55.92; H, 6.83; N, 23.71. Found: C, 55.40; H, 6.85; N, 23.30.

Synthesis of 9-phenyl-1,3,5-trimethylxanthine (1d). Theophylline (4.0 g, 22.2 mmol), copper(I) iodide (1.9 g, 10.0 mmol), 2-isobutyrylcyclohexanone (2.2 mL, 13.1 mmol) and caesium carbonate (6.52 g, 20.0 mmol) were placed in a Schlenk flask and degassed. Anhydrous dimethylsulfoxide (17 mL) and iodobenzene (3.5 mL, 31.3 mmol) were added to the flask. The mixture was heated at 130 °C for 24 hours. The dark brown solution obtained was dissolved in dichloromethane and extracted with water three times. The aqueous layers were combined and washed with dichloromethane three times. All of the organic layers were combined and dried *in vacuo* to give a dark brown solid. Recrystallisation from dichloromethane–diethyl ether yielded the product as a white solid. To ensure complete removal of the copper, the solid was dissolved in dichloromethane and a saturated solution of EDTA was added. The organic layer was extracted and dried *in vacuo* to give a white solid. Yield: 1.78 g (34%). ^{1}H NMR (300 MHz, d_6-DMSO): δ 8.31 (s, 1H, NCHN), 7.71–7.14 (m, J = 7.54 Hz, 5H, aromatic), 3.46 (s, 3H, CH_3), 3.18 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, d_6-DMSO): δ 153.7 (C═O), 150.9 (C═O), 149.2 (C), 142.7 (NCHN), 134.8 (C), 128.9 (CH), 128.5 (CH), 125.1 (CH), 106.1 (C), 29.6 (CH_3), 27.8 (CH_3). HRMS (ESI^+): calcd for $C_{13}H_{13}N_4O_2$ $[M + H]^+$: 257.1039. Found: 257.1031. Anal. Calcd for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.86. Found: C, 60.60; H, 4.85; N, 21.80.

Synthesis of 9-benzyl-1,3,5-trimethylxanthinium iodide (2b). Imidazole **1b** (2.6 g, 9.6 mmol) was dissolved in dimethylformamide (3 mL) in an ampoule, and methyl iodide (11.9 mL, 191 mmol) was added. The mixture was heated at 70 °C for 24 hours in a closed system resulting in a clear red solution. Excess diethyl ether was added to the flask to precipitate an orange powder which was filtered. Further recrystallisation using dichloromethane–diethyl ether and acetonitrile–diethyl ether resulted in the product as a yellow solid. Yield: 0.8 g (20%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.49 (s, 1H, NCHN), 7.46–7.37 (m, 5H, aromatic), 5.75 (s, 2H, CH_2), 4.16 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 3.26 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, DMSO-d_6): δ 153.1 (C═O), 150.1 (C═O), 139.8 (C), 139.4 (NCHN), 134.4 (C), 128.8 (CH), 128.7 (CH), 128.2 (CH), 106.9 (C), 51.1 (CH_3), 37.2 (CH_3), 31.3 (CH_3), 28.4 (CH_2). HRMS (ESI^+): calcd for $C_{30}H_{33}N_8O_4$ $[2M - H]^+$: 569.2614. Found: 569.2619. Calcd for $C_{15}H_{16}N_4O_2Na$ $[M - H + Na]^+$: 307.117096. Found: 307.116546. Anal. Calcd for $C_{15}H_{17}IN_4O_2{\cdot}1/4Et_2O$: C, 44.61; H, 4.56; N, 13.01. Found: C, 44.80; H, 4.30; N, 12.70. MP: 200.2–203.4 °C.

Synthesis of 9-butyl-1,3,5-trimethylxanthinium iodide (2c). Imidazole **1c** (4.5 g, 19.1 mmol), methyl iodide (23.7 mL, 381 mmol) and dimethylformamide (10 mL) were added to an ampoule and heated at 70 °C for 24 hours in a closed system. Excess diethyl ether was added to the solution to precipitate a yellow solid. The solid was filtered and recrystallised from dichloromethane–diethyl ether, and dried *in vacuo* to yield the product as a bright yellow solid. Yield: 6.3 g (87%). ^{1}H NMR (300 MHz, d_6-DMSO): δ 9.41 (s, 1H, NCHN), 4.45 (t, J = 7.25 Hz, 2H, CH_2), 4.16 (s, 3H, CH_3), 3.76 (s, 3H, CH_3), 3.26 (s, 3H, CH_3), 1.82 (quin, J = 7.25 Hz, 2H, CH_2), 1.31 (sext, J = 7.25 Hz, 2H, CH_2), 0.91 (t, J = 7.25 Hz, 3H, CH_3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.50 (s, 1H, NCHN), 4.58 (t, J = 7.20 Hz, 2H, CH_2), 4.48 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 1.99 (quin, J = 7.20 Hz, 2H, CH_2), 1.45 (sext, J = 7.20 Hz, 2H, CH_2), 0.98 (t, J = 7.20 Hz, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 153.1 (C═O), 149.9 (C═O), 139.5 (C), 138.6 (NCHN), 107.8 (C), 49.7 (CH_3), 38.9 (CH_2), 32.3 (CH_2), 32.1 (CH_3), 28.7 (CH_2), 19.4 (CH_3), 13.3 (CH_3). HRMS (ESI^+): calcd for $C_{12}H_{19}N_4O_2$ $[M - I]^+$: 251.1508. Found: 251.1589. Anal. Calcd for $C_{12}H_{19}IN_4O_2$: C, 38.11; H, 5.06; N, 14.81. Found: C, 37.80; H, 5.00; N, 14.60. M.P: 142.4–144.7 °C.

Synthesis of 9-phenyl-1,3,5-trimethylxanthinium iodide (2d). Imidazole **1d** (0.2 g, 0.78 mmol), methyl iodide (0.91 mL, 14.6 mmol) and dimethylformamide (4 mL) were added in an ampoule. The mixture was heated at 70 °C for 24 hours in a closed system. Excess diethyl ether was added to the resulting orange solution to precipitate the product as a yellow solid that was filtered and dried *in vacuo*. Yield: 0.15 g (97%). ^{1}H NMR (300 MHz, d_6-DMSO): δ 9.75 (s, 1H, NCHN), 8.17 (broad s, 5H, aromatic), 4.25 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 3.40 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, d_6-DMSO): δ 154.1 (C═O), 151.9 (C═O), 140.3 (C), 139.8 (NCHN), 132.7 (C), 130.7 (CH),

129.3 (CH), 125.8 (CH), 109.5(C), 35.7 (CH_3), 28.5 (CH_3), 26.3 (CH_3). HRMS (ESI^+): calcd for $C_{14}H_{15}N_4O_2$ $[M - I]^+$: 271.1190. Found: 271.1188. Anal. Calcd for $C_{14}H_{15}IN_4O_2{\cdot}3H_2O$: C, 37.18; H, 4.68; N, 12.39. Found: C, 36.80; H, 4.20; N, 12.20. M.P: 168.1–169.4 °C.

Synthesis of 9-benzyl-1,3,5-trimethylxanthine-8-ylidene silver acetate (3b). Imidazolium salt **2b** (0.25 g, 0.61 mmol) was added to silver acetate (0.2 g, 1.2 mmol) in a mixture of anhydrous dichloromethane (5 mL) and methanol (5 mL). The mixture was stirred at room temperature for two hours in the dark. The mixture was filtered, with the solid being washed with dichloromethane, and the filtrate was dried *in vacuo* to give the product as a white solid which was recrystallized from dichloromethane–pentane. Yield: 0.3 g (40%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49 (m, 2H, aromatic), 7.31 (m, 3H, aromatic), 5.66 (s, 2H, CH_2), 4.21 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 3.38 (s, 3H, CH_3), 2.05 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ Ag–C not observed, 178.2 (C═O), 153.2 (C═O), 150.7 (C═O), 140.4 (C), 135.4 (C), 129.1 (CH), 128.8 (CH), 128.5 (CH), 109.3 (C), 54.4 (CH_3), 40.1 (CH_3), 32.1 (CH_3), 28.9 (CH_2), 22.4 (CH_3). HRMS (ESI^+): calcd for $C_{30}H_{32}N_8O_4Ag$ $[Ag(NHC)_2]^+$: 675.1597. Found: 675.1616. Anal. Calcd for $C_{17}H_{19}AgN_4O_4$: C, 45.25; H, 4.24; N, 12.42. Found: C, 45.30; H, 4.20, N, 12.20.

Synthesis of 9-butyl-1,3,5-trimethylxanthine-8-ylidene silver acetate (3c). Imidazolium salt **2c** (0.10 g, 0.26 mmol) was dissolved in anhydrous methanol (5 mL) and dichloromethane (5 mL) and silver acetate (0.09 g, 0.54 mmol) were added. The mixture was stirred for two hours in the dark. The mixture was filtered, with the solid being washed with dichloromethane, and the filtrate was dried *in vacuo* to yield a white sticky solid. Recrystallisation from dichloromethane–diethyl ether gave the product as a white solid which was further dried *in vacuo*. Yield: 0.06 g (55%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.44 (t, J = 7.35 Hz, 2H, CH_2), 4.22 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 3.38 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.79 (quin, J = 7.35 Hz, 2H, CH_2), 1.37 (sext, J = 7.35 Hz, 2H, CH_2), 0.92 (t, J = 7.35 Hz, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, d_6-DMSO): δ Ag–C and C═O (acetate) not observed, 153.0 (C═O), 150.6 (C═O), 140.8 (C), 108.3 (C), 50.3 (CH_3), 39.4 (CH_2), 33.0 (CH_3), 31.6 (CH_3), 28.3 (CH_2), 19.10 (CH_2), 13.6 (CH_3). HRMS (ESI^+): *m*/*z* Calcd for $C_{24}H_{36}AgN_8O_4$ $[Ag(NHC)_2]^+$: 608.1910. Found: 608.1939. Anal. Calcd for $C_{14}H_{21}AgN_4O_4$: C, 40.30; H, 5.07; N, 13.43. Found: C, 39.90; H, 5.30, N, 13.10.

Synthesis of 9-phenyl-1,3,5-trimethylxanthine-8-ylidene silver acetate (3d). Imidazolium salt **2d** (0.15 g, 0.38 mmol) was dissolved in anhydrous acetonitrile (5 mL), and methanol (5 mL) and silver acetate (0.125 g, 0.75 mmol) were added. The mixture was stirred at room temperature for two hours in the dark. The mixture was filtered and the solvent removed from the filtrate *in vacuo* to give the product as a white solid. Yield: 0.115 g (69.1%). ^{1}H NMR (300 MHz, d_6-DMSO): δ 7.53 (broad s, 5H, aromatic), 4.27 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 3.19 (s, 3H, CH_3), 1.78 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (75 MHz, d_6-DMSO): δ 186.1 (Ag–C), 175.5 (C═O), 152.1 (C═O), 150.5 (C═O), 141.1 (C), 137.9 (C), 129.3 (CH), 128.9 (CH), 126.6 (CH), 109.3 (C), 31.7 (CH_3), 28.3 (CH_3), 23.2 (CH_3). HRMS (ESI^+): *m*/*z* Calcd for $C_{28}H_{28}AgN_8O_4$ $[Ag(NHC)_2]^+$: 647.1284. Found: 647.1297. Anal. Calcd for $C_{16}H_{17}AgN_4O_4$: C, 43.96; H, 3.92; N, 12.82. Found: C, 44.70; H, 4.40, N, 12.60.

Cytotoxicity studies

In vitro cell tests were performed at the Institute of Cancer Therapeutics, Bradford. Cells were incubated in 96-well plates, at 2×10^3 cells per well in 200 μL of growth media (RPMI 1640 supplemented with 10% foetal calf serum, sodium pyruvate (1 mM) and L-glutamine (2 mM)). Cells were incubated for 24 hours at 37 °C in an atmosphere of 5% CO_2 prior to drug exposure. Silver compounds and cisplatin were dissolved in dimethylsulfoxide at a concentration of 25 mM and diluted with medium to obtain drug solutions ranging from 25 μM to 0.049 μM. The final dimethylsulfoxide concentration was 0.1% (v/v) which is non-toxic to cells. Drug solutions were applied to cells and incubated for 96 hours at 37 °C in an atmosphere of 5% CO_2. The solutions were removed from the wells and fresh medium added to each well along with 20 μL MTT (5 mg mL^{-1}), and incubated for 4 hours at 37 °C in an atmosphere of 5% CO_2. The solutions were removed and 150 μL dimethylsulfoxide was added to each well to dissolve the purple formazan crystals. A plate reader was used to measure the absorbance at 540 nm. Lanes containing medium only, and cells in medium only (no drug), were used as blanks for the spectrophotometer and 100% cell survival respectively. Cell survival was determined as the true absorbance of treated cells divided by the true absorbance of controls and expressed as a percentage. The concentration required to kill 50% of cells (IC_{50}) was determined from plots of % survival against drug concentration. Each experiment was repeated 3 times and a mean value obtained.

Hydrophobicity

Equal volumes of octanol and NaCl-saturated water were stirred at room temperature for 24 hours, and separated to give octanol-saturated water and water-saturated octanol. Five standard concentrations (5, 10, 20, 40 and 60 μM) of the complexes were prepared from the octanol-saturated water. Analysis using UV/vis spectroscopy was used to obtain a calibration curve of absorbance *vs.* concentration for each complex at its maximum absorbance. Accurate amounts of the complexes were dissolved in the octanol-saturated water (25 mL) to make up a concentration of 50 μM. 3 mL of octanol-saturated water containing the complex was placed in a centrifuge tube and 3 mL of water-saturated octanol was layered on top. Six samples prepared in this manner were shaken for 4 hours using a vibrax machine at 500 g min^{-1}. The layers were separated and the octanol-saturated water layer was retained for analysis using UV/vis spectroscopy. The average concentration of the six runs was calculated using the calibration graph and maximum absorbance for each complex. Subtraction of the average concentration obtained from the concentration of an unshaken sample in octanol-saturated water gave the final $[C]_{org}$. The $[C]_{org}$ and $[C]_{aq.}$ were used to determine the partition coefficient log *P*.

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