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Aust. J. Chem. **2011**, *64*, 244–252

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Water-soluble Bis(thiosemicarbazonato)copper(II) Complexes

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The synthesis of four new water-soluble bis(thiosemicarbazone) ligands and their copper(II) complexes is presented and their potential to be new ligands for copper radiopharmaceuticals is discussed. The ligands and complexes have been characterized by a combination of NMR spectroscopy, mass spectrometry, and X-ray crystallography. The electrochemical behaviour of two of the copper(II) complexes was investigated by cyclic voltammetry and revealed that both complexes exhibited a quasi-reversible redox process attributed to a Cu^{II}/Cu^{I} process. Two of the new ligands were radiolabelled with positron-emitting ^{64}Cu with a view to assessing their potential as ligands that bind radioactive copper isotopes for application in diagnostic radiopharmaceuticals. The cellular uptake of the copper complexes was investigated in SH-SY5Y cells.

Manuscript received: 17 December 2010.
Manuscript accepted: 17 January 2011.

Introduction

The biological activity of copper complexes of bis(thiosemicarbazone) ligands has led to them being investigated in models of relevance to the treatment of cancer and Alzheimer's disease.[1–5] There is also interest in the use of this family of ligands to act as delivery vehicles for radioactive isotopes in the development of copper radiopharmaceuticals. The decay profile of copper-64 includes positron emission (β^+; E_{av} 278 keV, 17.9%) and β^- emission (37%) with a half-life of 12.7 h, and consequently offers the potential for both positron emission tomography (PET) imaging and radiotherapy.[6] As a non-invasive imaging technique, PET can provide valuable diagnostic information to assist in clinical diagnosis. The technique relies on a positron-emitting tracer that is detected as it passes through the body. The use of copper radioisotopes in radiopharmaceuticals is dependent on the ability to selectively deliver the radioisotope to target tissue. One approach is to incorporate the radioactive copper isotope into a coordination complex. The biodistribution of the copper complex is dictated by a variety of factors including lipophilicity, size of the complex, and redox properties. A wide range of bis(thiosemicarbazonato) ligands have been investigated as delivery vehicles for copper radioisotopes as they form stable ($K_a = 10^{18}$), neutral, membrane permeable copper complexes.[7–15] The stable, neutral complexes can diffuse into cells where a reducing environment renders the complexes susceptible to intracellular reduction (Cu^{II} to Cu^{I}). The cellular metabolism of bis(thiosemicarbazonato)copper(II) complexes is remarkably sensitive to the substituents on the diimine backbone of the ligand.[16,17] For example, the copper complex of the ligand diacetyl-bis (N^4-methyl-3-thiosemicarbazone) (H_2atsm), with two methyl substituents on the diimine backbone, Cu^{II}(atsm), is harder to reduce than Cu^{II}(ptsm), the derivative that only possesses a single methyl functional group on the backbone of the ligand pyruvaldehyde-bis(N^4-methyl-3-thiosemicarbazone) (H_2ptsm) (Fig. 1). Consequently, $^{64}Cu^{II}$(atsm) is only reduced in hypoxic cells and is being investigated as a hypoxia imaging agent,[17–25] whereas Cu^{II}(ptsm) is under investigation as a perfusion tracer, and more recent developments have focussed on an ethyl derivative.[7–9,26]

In general, bis(thiosemicarbazonato)copper(II) complexes suffer from poor solubility in water or aqueous mixtures. For example, Cu^{II}(atsm) has an octanol/water partition coefficient, log *P*, of 1.48 and is insoluble in water. It is normally dissolved in dimethyl sulfoxide for synthetic preparations.[12]

It was thought that new water-soluble bis(thiosemicarbazonato)copper(II) complexes could have markedly different biological activity, and be of potential interest in the development of copper radiopharmaceuticals with different biodistribution when compared with more traditional derivatives such as Cu^{II}(atsm) and Cu^{II}(ptsm). The new water-soluble ligands were prepared using a selective transamination reaction on asymmetric bis(thiosemicarbazone) precursors. The synthesis of four new ligands is presented as well the synthesis of their Cu^{II} complexes. Representative examples of the new ligands were readily radiolabelled with positron-emitting copper-64 and

10.1071/CH10463 0004-9425/11/030244

preliminary cell uptake data in neuronal-like SH-SY5Y cells are presented. One of the ligands presented in this paper, $[H_2\mathbf{L}^3]^-$, and its use as a zinc sensor have been reported in a preliminary communication.[27]

Results and Discussion

Synthesis

The stability and reduction potential of bis(thiosemicarbazonato) copper(II) complexes is more dependent on the substituents on the diimine backbone than modifications to the terminal N^4-substitutents. Attempts to maintain the subtle control of the Cu^{II}/Cu^{I} redox couple of bis(thiosemicarbazonato)copper complexes have focussed on the addition of functional groups at the terminal N^4-positions of the ligand framework.[28–30] Four new ligands were prepared by the addition of water-solubilizing aromatic sulfonate functional groups to the N^4-position of the ligand framework via a selective transamination reaction of asymmetric bis(thiosemicarbazone) precursors H_2atsm/m_2 (diacetyl-4,4-dimethyl-4-methyl-bis(thiosemicarbazone), $H_2\mathbf{L}^1$) (/m_2 refers to 4,4′-dimethyl functional group) and H_2ptsm/m_2 (pyruvate-4,4-dimethyl-4-methyl-bis(thiosemicarbazone), $H_2\mathbf{L}^2$) with aromatic amine sulfonates. The addition of aromatic sulfonate groups is a standard approach for introducing water solubility to otherwise lipophilic ligands. An analogous transamination reaction was described previously to prepare bifunctional bis(thiosemicarbazone) chelators.[31]

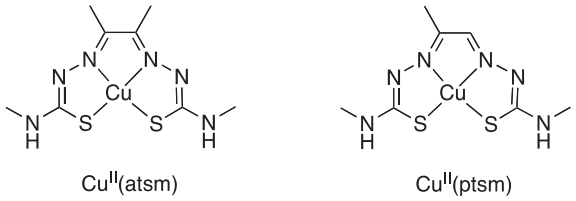
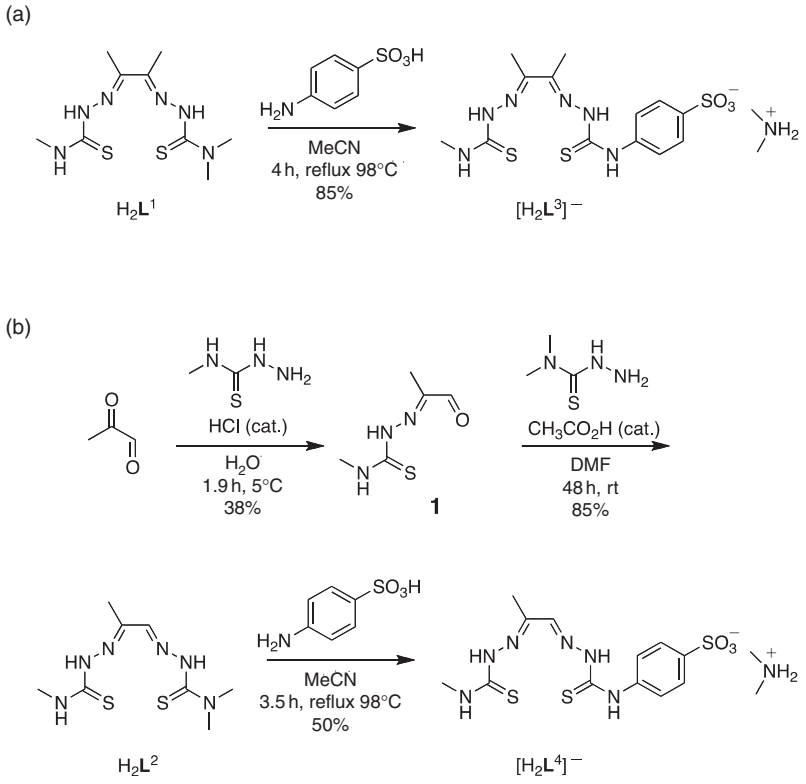


Fig. 1. The structures of Cu^{II}(atsm) and Cu^{II}(ptsm).

The asymmetric bis(thiosemicarbazone) central to the selective transamination reactions ($H_2\mathbf{L}^1$) was prepared by condensation of diacetyl-*mono*-4-methyl-3-thiosemicarbazone with 4,4-dimethyl-3-thiosemicarbazide and an acetic acid catalyst in DMF at room temperature.[31] The reaction of $H_2\mathbf{L}^1$ with nucleophilic amines results in the selective displacement of dimethylamine from the dimethyl-substituted moiety of the bis (thiosemicarbazone). This reaction occurs under mild conditions exclusively on the N^4,N^4-dimethyl thiosemicarbazone functional group ($N(CH_3)_2$) as it contains a more electrophilic thiocarbonyl carbon atom than the N^4-monosubstituted functional group (N–CH_3). The leaving group is the secondary amine dimethylamine. In this case, the nucleophilic amine is an aromatic amine sulfonate, either sulfanilic acid to give $[H_2\mathbf{L}^3]^-$, 4-amino-3-hydroxy-1-napthalene sulfonic acid to give $[H_2\mathbf{L}^5]^-$, or 5-amino-2-napthalene sulfonic acid to give $[H_2\mathbf{L}^6]^-$. In each case, the leaving amine is protonated to give the dimethylammonium salt of the new proligands, $H_2NMe_2[H_2\mathbf{L}^{3–6}]$.

The synthesis of $H_2\mathbf{L}^2$ is presented here for the first time (Scheme 1). A Schiff base condensation between 4-methyl-3-ethylthiosemicarbazide and pyruvaldehyde allowed isolation of a single product. Condensation at the ketone functional group of pyruvaldehyde occurred selectively on the keto aldehyde 1,2-dione precursor to give 2-(4-*N*-methyl-3-thiosemicarbazone)pyruvaldehyde (**1**). The ^{1}H NMR of **1** clearly identified the regiochemistry of the condensation product, as the aldehyde proton (δ 11.18 ppm) and methyl group protons (δ 1.94 ppm) are readily identified. This product (**1**) may not represent the major product of the reaction as the aldehyde functional group would be expected to be more reactive to condensation with



Scheme 1. The synthesis of new sulfonated proligands (a) $H_2NMe_2[H_2\mathbf{L}^3]$ and (b) $H_2NMe_2[H_2\mathbf{L}^4]$.

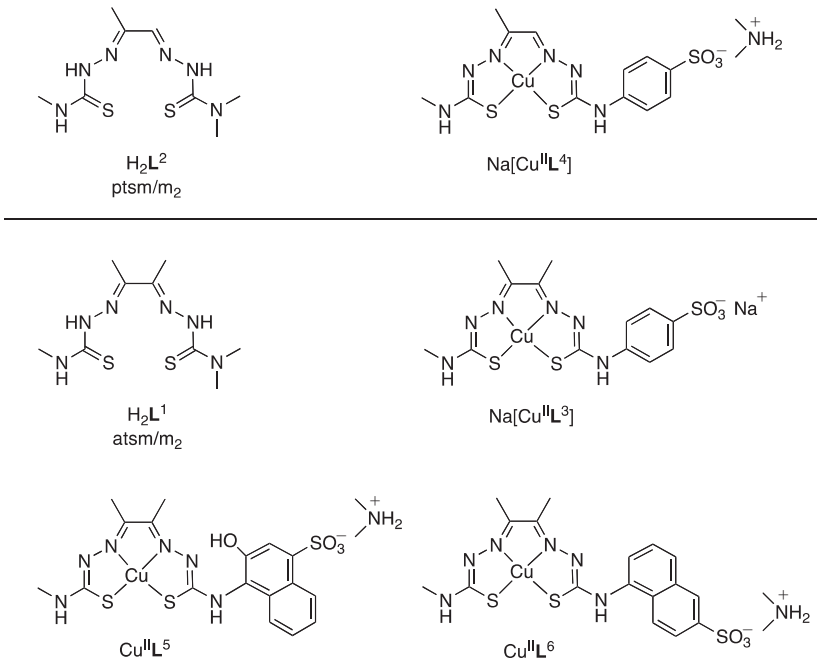


Fig. 2. The water soluble sulfonated $Cu^{II}\mathbf{L}^{3-6}$ complexes prepared from asymmetric bis(thiosemicarbazone) precursors $H_2\mathbf{L}^1$ and $H_2\mathbf{L}^2$.

4-methyl-3-thiosemicarbazide, but **1** is readily isolated in high purity by crystallization, albeit in relatively low yields (~30%). Attempts to recover the product due to condensation at the aldehyde functional group were unsuccessful. A second condensation reaction of 2-(4-*N*-methyl-3-thiosemicarbazone)-pyruvaldehyde (**1**) with 4,4-dimethyl-3-thiosemicarbazide allowed isolation of H_2ptsm/m_2, $H_2\mathbf{L}^2$. This method for the synthesis of asymmetric bis(thiosemicarbazones) derived from pyruvaldehyde is an alternative to previously reported methods that rely on oxidative cleavage of pyruvaldehyde dimethylacetal 2-thiosemicarbazone.[9,10] The transamination reaction of $H_2\mathbf{L}^2$ with sulfanilic acid led to the successful preparation of $Me_2NH_2[H_2\mathbf{L}^4]$, which could be converted to the sodium salt by passage through a cation-exchange column.

Each ligand was characterized by NMR spectroscopy and mass spectrometry. For each ligand, the terminal N–CH_3 protons were split into doublets (δ ~3.0 ppm; $^3J_{HH}$ ~4.5 Hz) with the corresponding secondary amine protons seen as quartets (δ ~8.5 ppm). The dimethyl ammonium counterion could be identified by a triplet (δ ~2.5–2.6 ppm; 6H). Introduction of aromatic sulfonate groups was reflected by downfield shifts of the backbone methyl protons (from 2.1–2.2 to 2.2–2.4 ppm) and all other signals were as expected.

The copper complexes of the new proligands are readily prepared by the addition of a Cu^{II} source to the ligands (Fig. 2). As is common with other bis(thiosemicarbazone) systems, coordination of Cu^{II} is coupled with a double deprotonation, but for the present cases, the ligand now becomes trianionic to give an overall monoanionic complex. The Cu^{II} complexes retain the brown-orange colours common to the better known variants Cu^{II}(atsm) and Cu^{II}(ptsm).[12] For example, electronic spectroscopy in aqueous buffer solution reveals that $[Cu^{II}\mathbf{L}^3]^-$ has an absorption maximum at $\lambda = 460$ nm ($\varepsilon = 1.1(0) \times 10^4\,M^{-1}\,cm^{-1}$), whereas $[Cu^{II}\mathbf{L}^4]^-$ has an absorption maximum at $\lambda = 473$ nm ($\varepsilon = 1.2(0) \times 10^4\,M^{-1}\,cm^{-1}$). All the Cu^{II} complexes could be identified by electrospray ionization (ESI)-mass spectrometry operating in negative-ion mode by molecular ions corresponding to $[Cu^{II}\mathbf{L}^x]^-$ with the correct isotope splitting pattern.

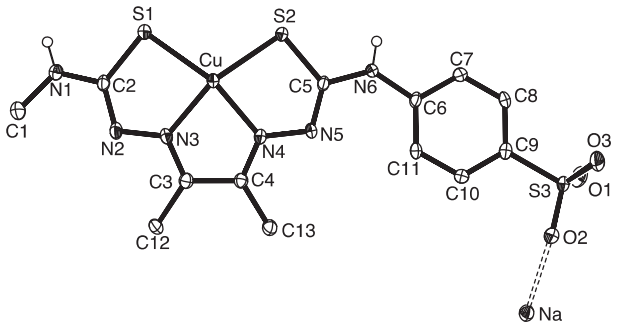


Fig. 3. An *ORTEP* representation (40% probability) of the molecular structure of $Na[Cu^{II}\mathbf{L}^3]\cdot[(CH_3)_2SO]_3$. Solvent omitted for clarity.

The copper complex $[Cu^{II}\mathbf{L}^3]^-$ was characterized as a sodium salt by single-crystal X-ray crystallography (Fig. 3, Table 1). As expected, the Cu^{II} is essentially four-coordinate distorted square planar, although a weak axial interaction with the sulfur atom of an adjacent molecule (Cu1–S1′ 2.872(1) Å) results in a tendency towards square pyramidal geometry. The trianionic ligand coordinates through the two azamethinic nitrogen atoms and two sulfur atoms to give a distorted square planar geometry with a 5–5–5 chelate ring system. The Cu–S bond lengths, Cu–S1 2.274(1) Å and Cu–S2 2.263(1) Å, are similar to the analogous bond lengths in Cu^{II}(atsm), as are the Cu–N bond distances (Table 2).[13,24] The ligand cavity appears a little too small for Cu^{II} and the deviations from ideal square planar geometry are evident in the S1–Cu–S1 bond angle of 109.4(4)° and the N3–Cu–N4 bond angle of 80.3(1)°. The deprotonation of both thiosemicarbazone limbs is reflected in the C2–S1 distance of 1.770(4) Å and the C5–S2 distance of 1.757(4) Å, suggesting more thiolate-like than thione-like character.[32,33] Each complex anion associates with an adjacent molecule through weak Cu1–S1′ interactions and the sulfonate

Table 1. Crystallographic data

Crystal	$Na[Cu^{II}L^3]\cdot[(CH_3)_2SO]_3$	$Me_2NH_2[Cu^{II}L^4]\cdot(CH_3)_2SO$
Chemical formula	$C_{19}H_{33}CuN_6O_6S_6Na$	$C_{16}H_{27}CuN_7O_4S_4$
M_w	720.40	573.23
Crystal system	Triclinic	Monoclinic
Temperature [K]	130.0(2)	130.0(2)
Space group	$P\bar{1}$	$P21/c$
a [Å]	9.4755(5)	7.8258(12)
b [Å]	10.1109(7)	8.8541(13)
c [Å]	17.3555(13)	35.655(5)
α [°]	79.512(6)	90
β [°]	79.796(5)	93.564(3)
γ [°]	69.574(5)	90
V [Å^3]	1520.48(17)	2465.8(6)
Z	2	4
Independent reflections	5776	4337
R_{int}	0.0450	0.0838
R ($I > 2\sigma(I)$)	0.0455	0.0514
wR (all data)	0.1299	0.1119
Residual electron density (min, max) [e Å^{-3}]	0.79, 0.97	−0.43, 0.62
Goodness-of-fit on F^2	1.042	1.038

Table 2. Selected bond lengths [Å] and angles [°] for $Na[Cu^{II}L^3]\cdot[(CH_3)_2SO]_3$ and $Me_2NH_2[Cu^{II}L^4]\cdot(CH_3)_2SO$

	$Na[Cu^{II}L^3]\cdot[(CH_3)_2SO]_3$	$Me_2NH_2[Cu^{II}L^4]\cdot(CH_3)_2SO$
Cu–S1, Cu–S2	2.2742(11), 2.2631(10)	2.2361(11), 2.2509(11)
Cu–N3, Cu–N4	1.959(3), 1.966(3)	1.965(3), 1.957(3)
C3–C4	1.476(5)	1.462(5)
O2–Na	2.258(3)	–
S1–Cu–S2	109.04(4)	109.95(4)
S1–Cu–N3	84.77(10)	85.48(10)
S2–Cu–N4	83.79(10)	83.79(10)
N3–Cu–S2	159.51(11)	164.51(10)
N4–Cu–S1	163.63(10)	166.20(10)
N3–Cu–N4	80.30(13)	80.76(13)

functional group is bound to the sodium counterion (O2–Na 2.257(3) Å). The sodium atom is five-coordinate with two monodentate dimethylsulfoxide molecules coordinating through oxygen and two μ^2-O bridging dimethyl sulfoxide molecules. These interactions result in an elegant infinite chain or coordination polymer (Fig. 4).

An *ORTEP* representation of the anion found in $Me_2NH_2[Cu^{II}L^4]\cdot(CH_3)_2SO$ is shown in Fig. 5. The Cu^{II} is four-coordinate CuN_2S_2 square planar. The Cu–S bonds are slightly shorter when compared with $[Cu^{II}L^3]^-$ Cu–S1 2.251(1) Å and Cu–S2 2.236(1) Å, perhaps reflecting the lack of axial interactions in $[Cu^{II}L^4]^-$. As in the previous structure, the deprotonation of each thiosemicarbazone limb is reflected in the C2–S1 and C5–S2 bond lengths of 1.763(4) and 1.768(4) Å respectively. When focussing on the ligand framework, the most significant difference between the two structures presented is the length of the C–C backbone, C3–C4 1477(6) Å in $Na[Cu^{II}L^3]$ and 1.462(6) Å in $Me_2NH_2[Cu^{II}L^4]$. The dimethylammonium cation that originates from the leaving group in the transamination reaction is involved in hydrogen-bonding interactions to the sulfonate functional group.

Both Cu^{II}(ptsm) and Cu^{II}(atsm) are insoluble in water but, as intended, these new complexes with sulfonate functional groups are very soluble in water. The solubility of the $Me_2NH_2[Cu^{II}L^5]$ salts is $> 5\ g\ L^{-1}$ (approximately 10 mM) whereas the solubility of the $Na[Cu^{II}L^3]$ salts is $> 10\ g\ L^{-1}$.

Electrochemistry

Correlations between the reduction potential and likely intracellular reduction of copper complexes in dimethylsulfoxide (DMSO) have proved useful in predicting the retention of copper inside the cell. The hypoxia selectivity of Cu^{II}(atsm) has been explained, in part, by the more negative reduction potential of Cu^{II}(atsm) ($E_m = -0.60$ V versus saturated calomel electrode (SCE) SCE, where $E_m = [E_{pc} + E_{pa}]/2$ and for ferrocene (Fc/Fc^+) $= E_m = 0.54$ V) when measured in anhydrous DMF at a glassy carbon working electrode compared with $E_m = -0.51$ V for Cu^{II}(ptsm).[12,34] Cyclic voltammetry experiments in DMSO using tetrabutylammonium tetrafluoroborate as the supporting electrolyte showed a quasi-reversible processes for the $[CuL^3]^-$ complex with the Cu^{II}/Cu^{I} couple ($E_m = -0.49$ V) and another quasi-reversible process at $E_m = 0.76$ V (versus SCE). For $[CuL^4]^-$, a quasi-reversible process attributed to the Cu^{II}/Cu^{I} couple is at $E_m = -0.39$ V (Fig. 6). The water solubility of $[Cu^{II}L^3]^-$ and $[Cu^{II}L^4]^-$ permitted electrochemical measurements in a 100% aqueous buffer (20 mM PO_4^{2-}, 100 mM NaCl). The Cu^{II}/Cu^{I} process was less reversible in aqueous buffer when compared with measurements in DMSO. The use of faster scan rates (2 V s^{-1}) was required to achieve quasi-reversible Cu^{II}/Cu^{I} couples. For $[Cu^{II}L^3]^-$, the Cu^{II}/Cu^{I} process occurred at $E_m = -0.51$ V (versus Ag/AgCl) whereas for $[Cu^{II}L^4]^-$, it occurred at $E_m = -0.42$ V. Under the same conditions, potassium ferricyanide had $E_m = 0.20$ V.

Radiolabelling with Copper-64 and Cell Uptake Studies

The ligands H_2L^5 and H_2L^6 were radiolabelled with $^{64}Cu^{II}$ at room temperature in aqueous sodium acetate buffer (pH = 4). Analysis by reverse-phase HPLC with radioactivity detection

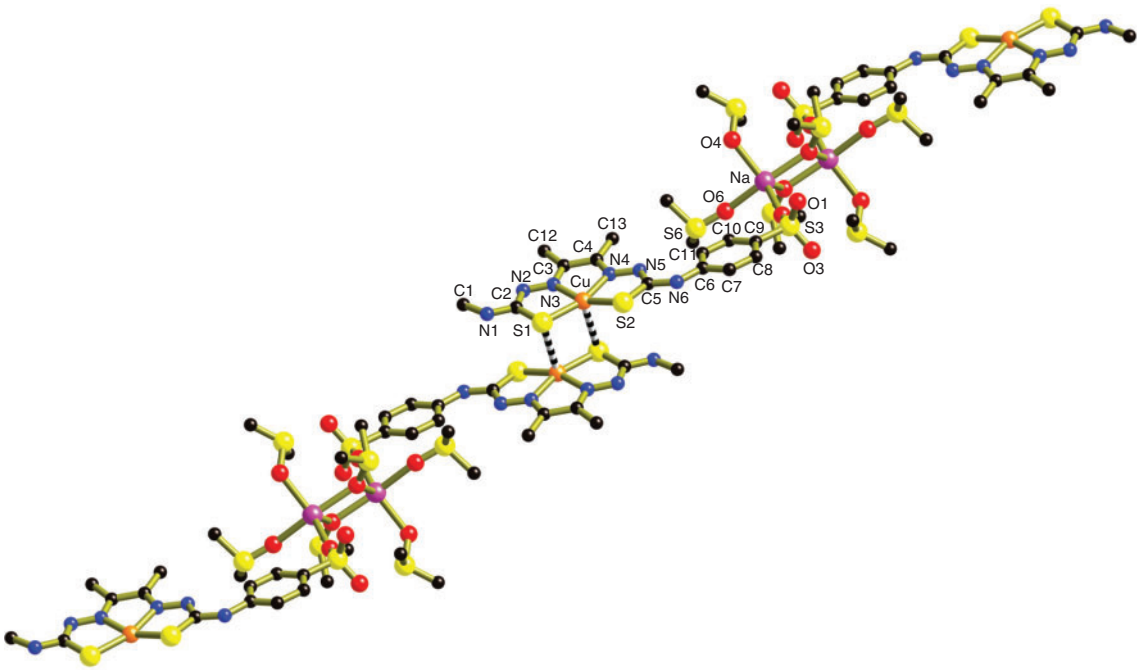


Fig. 4. A representation of an infinite chain from the X-ray structure of $Na[Cu^{II}\mathbf{L}^3]\cdot[(CH_3)_2SO]_3$ generated by axial interactions between Cu^{II} and a sulfur atom of an adjacent complex as well sodium–sulfonate interactions.

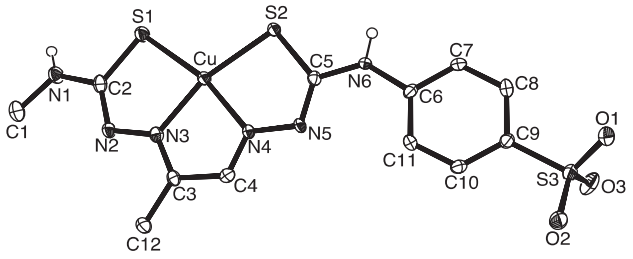


Fig. 5. An *ORTEP* representation (40% probability) of the anion from $Me_2NH_2[Cu^{II}\mathbf{L}^4]\cdot(CH_3)_2SO$. Solvent and counterion omitted for clarity.

(Fig. 7) showed the compounds were prepared with high radiochemical purity (>95%), with no uncoordinated or 'free' $^{64}Cu^{II}$ (under these conditions 'free' $^{64}Cu^{II}$ elutes at ~2 min). The similar retention times (9.08 and 9.32 min respectively) of the two complexes highlights the subtle difference in lipophilicity attributed to the hydroxyl on $H_2\mathbf{L}$.[5]

Neutral copper complexes of bis(thiosemicarbazonato) ligands, such as Cu^{II}(atsm) and Cu^{II}(ptsm), have been shown to be membrane-permeable despite their poor solubility in water or aqueous mixtures. To investigate the cell uptake of the water-soluble bis(thiosemicarbazonato) copper(II) analogues $[Cu\mathbf{L}^3]^-$, $[Cu\mathbf{L}^4]^-$, and $[Cu\mathbf{L}^6]^-$, SH-SY5Y cells were incubated with the three complexes at 10-μM concentration for 1 h and intracellular copper levels were measured by inductively coupled plasma mass spectrometry (ICP-MS) (Fig. 8).

As the positive control, cells were also treated with Cu^{II}(atsm) (10 μM) and a significant increase of more than 100-fold intracellular copper levels was observed compared with cells treated with solvent alone (DMSO). The negatively charged complexes $[Cu\mathbf{L}^3]^-$, $[Cu\mathbf{L}^4]^-$, and $[Cu\mathbf{L}^6]^-$ induced only a two-fold increase in intracellular copper levels, when compared with untreated cells (DMSO), similarly to the treatment with $CuSO_4$ (10 μM) (Fig. 8). The addition of water-

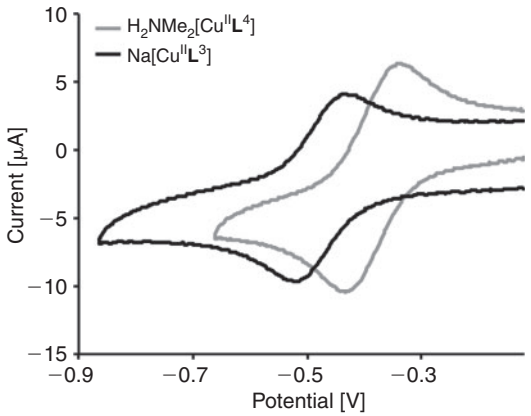


Fig. 6. Cyclic voltammograms of $Na[Cu^{II}\mathbf{L}^3]$ (black) and $H_2NMe_2[Cu^{II}\mathbf{L}^4]$ (grey) (1.0 mM) at a scan rate of 100 mV s^{-1}. Measurements were performed in DMSO (10 mM Bu_4NBF_4) relative to Fc/Fc^+, $E_m = 0.54$ V versus SCE.

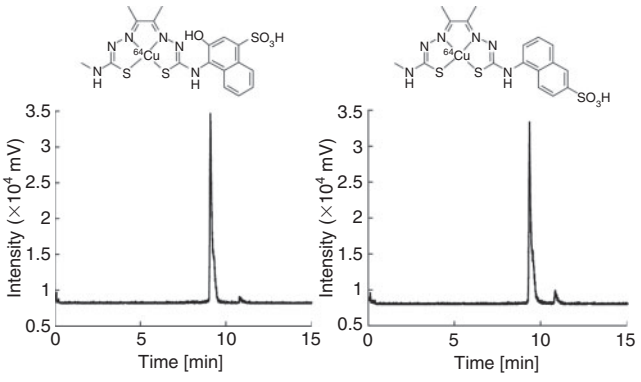


Fig. 7. (Left) Radio-HPLC of $^{64}Cu^{II}\mathbf{L}^5$ (retention time R_t: 9.08 min) and (right) $^{64}Cu^{II}\mathbf{L}^6$ (R_t: 9.32 min).

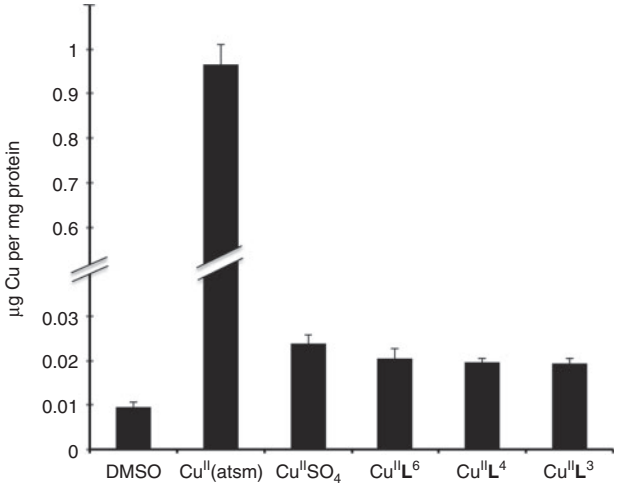


Fig. 8. Cell uptake studies. SH-SY5Y cells were treated with copper complexes (10 µM) or DMSO as the control for 1 h. The copper levels were measured in washed cell pellets by inductively coupled plasma mass spectrometry and calculated as µg Cu per mg protein.

solubilizing functional groups has drastically altered the membrane permeability. Similar effects were seen on a related ligand system.[35]

This corresponds to cellular copper levels of 0.2 and 0.1 µg copper per mg protein for the copper complexes and $CuSO_4$ respectively. The biodistribution of the copper complex is dictated by a variety of factors including lipophilicity, size of the complex, and redox properties. Lipid membranes cannot be regarded as a homogeneous barrier but rather a heterogeneous arrangement of amphiphilic molecules in which a structured hydrophobic core is sandwiched between two polar surfaces.[36] It is likely that the anionic charged copper complexes cannot penetrate the negatively charged top surface of the membrane bilayer. As might be anticipated, uptake into cells is facilitated by neutral complexes.

Conclusions

Four new bis(thiosemicarbazone) ligands were prepared utilizing selective transamination reactions. This synthetic methodology is extremely versatile and allows the preparation of mixed bis(thiosemicarbazones) containing dissimilar thiosemicarbazone functional groups. The introduction of aromatic sulfonate functional groups to bis(thiosemicarbazone) ligands induces significant water solubility in both the ligands and their copper complexes. The solubility of the copper complexes in water as either their Me_2NH_2 or sodium salts is at least 5 g L^{-1} (approximately 10 mM). The copper complexes $[Cu^{II}\mathbf{L}^3]^-$ and $[Cu^{II}\mathbf{L}^4]^-$ retain the quasi-reversible Cu^{II}/Cu^{I} redox couples displayed by the 'parent' molecules Cu^{II}(atsm) and Cu^{II}(ptsm), which are currently under investigation as copper radiopharmaceuticals. Two representative examples of this family of ligands have been readily radiolabelled with $^{64}Cu^{II}$ in high radiochemical yield. Cell uptake studies of three of the four copper complexes, $[Cu\mathbf{L}^3]^-$, $[Cu\mathbf{L}^4]^-$, and $[Cu\mathbf{L}^6]^-$, revealed that the complexes were dramatically less cell-permeable than Cu^{II}(atsm). The addition of water-solubilizing aromatic sulfonate groups has completely eliminated the high membrane permeability often associated with this family of molecules. The presented copper complexes have markedly different cell-uptake properties when compared with the Cu^{II}(atsm) and Cu^{II}(ptsm) and as such, it is likely they will show different in vivo biodistribution and could provide an interesting variant on the use of copper complexes of bis(thiosemicarbazones) as radiopharmaceuticals.

Experimental

Crystallography

Crystals of $Na[Cu^{II}\mathbf{L}^3]\cdot[(CH_3)_2SO]_3$ and $Me_2NH_2[Cu^{II}\mathbf{L}^4]\cdot(CH_3)_2SO$ were mounted in low-temperature oil, then flash-cooled to 130 K using an Oxford low-temperature device. Intensity data were collected at 130 K with an Oxford XCalibur X-ray diffractometer with Sapphire CCD detector using Cu-Kα radiation (graphite crystal monochromator $\lambda = 1.54184$ Å). Data were reduced and corrected for absorption.[37] The structures were solved by direct methods and difference Fourier synthesis using the *SHELX* suite of programs[38] as implemented within the *WINGX*[39] software. Thermal ellipsoid plots were generated using the program *ORTEP-3* integrated within the *WINGX* suite of programs. CCDC reference numbers: $Na[Cu^{II}\mathbf{L}^3]\cdot[(CH_3)_2SO]_3$, 807829; $Me_2NH_2[Cu^{II}\mathbf{L}^4]\cdot(CH_3)_2SO$, 807828.

General

All reagents and solvents were obtained from commercial sources (Sigma–Aldrich) and used as received unless otherwise stated. H_2atsm/m_2 ($H_2\mathbf{L}^1$) and sodium diacetyl-4-methyl-4′-*p*-sulfonato-bis(thiosemicarbazone) ($H_2\mathbf{L}^3$) were prepared previously. Cation-exchange chromatography was performed on DOWEX® 50WX2–200 ion-exchange resin (Na^+ form, 1.5 × 10 cm). Elemental analyses for C, H, and N were carried out by Chemical & MicroAnalytical Services Pty Ltd, Vic. NMR spectra were recorded on a Varian FT-NMR 500 spectrometer (^{1}H NMR at 499.9 MHz and $^{13}C\{^1H\}$ NMR at 125.7 MHz) at 298 K and referenced to the internal solvent residual peak. Mass spectra were recorded on an Agilent 6510-Q-TOF LC-MS mass spectrometer and calibrated to internal references.

UV-Visible Spectroscopy

UV-vis spectra were recorded on a Cary 300 Bio UV-vis spectrophotometer, from 800 to 200 nm at 0.5-nm data intervals with a 600-nm min^{-1} scan rate.

High-Performance Liquid Chromatography

Analytical RP-HPLC traces were acquired using two chromatographic systems. System A: Agilent 1200 series HPLC system equipped with an Agilent Zorbax Eclipse XDB-C18 column (4.6 × 150 mm) with a 1 mL min^{-1} flow rate and UV spectroscopic detection at 214, 220, and 270 nm. Retention times (R_t, min) were recorded using a gradient elution method of 0–100% B over 25 min; solution A consisted of water (buffered with 0.1% trifluoroacetic acid) and solution B consisted of acetonitrile (buffered with 0.1% trifluoroacetic acid). System B: Shimadzu LC-20AT HPLC system equipped with a Waters Cosmosil C18 column (4.6 × 150 mm) with a 1-mL min^{-1} flow rate and a sodium iodide scintillation detector and UV-vis detector in series (detection at 220 and 275 nm). Retention times (R_t, min) were recorded using a gradient elution method of 0% B for 1 min, 0–100% B over 10 min, 100–0% B over 2 min, and 0% B for 2 min, where solution A consisted of water (buffered with 0.1% trifluoroacetic acid) and solution B consisted of acetonitrile (buffered with 0.1% trifluoroacetic acid).

Electrochemistry

Cyclic voltammograms were recorded using an Autolab PGSTAT100 equipped with *GPES V4.9* software. Measurements on the complexes were carried out at $\sim 1 \times 10^{-4}$ M in DMSO with tetrabutylammonium tetrafluoroborate (0.1 M) as electrolyte, using a glassy carbon disk (diameter, 3 mm) working electrode, a Pt wire counter/electrode, and an Ag/Ag^+ pseudo reference electrode (silver wire in CH_3CN (NBu_4BF_4 (0.1 M)), $AgNO_3$ (0.01 M)). Ferrocene was used as an internal reference ($E_m(Fc/Fc^+) = 0.54$ V versus SCE), where E_m refers to the midpoint between a reversible reductive (E_{pc}) and oxidative (E_{pa}) couple, given by $E_m = (E_{pc} + E_{pa})/2$.

^{64}Cu Radiolabelling

$^{64}Cu^{II}Cl_2$ (2.15 GBq mL^{-1}, pH 1) was purchased from ANSTO Radiopharmaceuticals and Industrials (ARI, Lucas Heights, NSW, Australia). The radionuclidic purity at calibration {(^{64}Cu)/(^{67}Cu)} was 100% and the radiochemical purity as Cu^{II} was 100%. The chemical purities of copper, zinc, and iron were 3.5, 0.06, and 1 μg mL^{-1} respectively.

General Procedure

An aliquot of $^{64}CuCl_2$ (20 μL, ~43 MBq, pH 1.0) was added to a solution containing the ligand (10 μL, 1 mg mL^{-1} DMSO), sodium acetate (90 μL, 0.1 M), and Milli-Q water (390 μL). The reaction was left for 30 min at room temperature before 100 μL of the reaction solution was injected onto a reverse-phase C18 analytical HPLC column. RP-HPLC (System B) $^{64}Cu^{II}\mathbf{L}^5$, R_t: 9.08 min and $^{64}Cu^{II}\mathbf{L}^6$ R_t: 9.32 min.

Synthetic Procedures

2-(4-N-Methyl-3-thiosemicarbazone)pyruvaldehyde

To a stirred solution of pyruvaldehyde (20 mL, 40% w/w, 133 mmol) in water (30 mL) acidified with hydrochloric acid (12 M, two drops) and cooled to 0°C was added 4-methyl-3-thiosemicarbazide (2.65 g, 25.2 mmol) portionwise over 75 min. After maintaining the same temperature for a further 40 min, chloroform (150 mL) and water (100 mL) were added to the reaction and the organic phase was separated. Subsequent extracts of the aqueous phase (chloroform, 2 × 50 mL) were combined with the organic phase, dried over magnesium sulfate and concentrated under vacuum. Pentane was added to a point of turbidity and the resulting mixture was stored in the freezer for 1 h. The precipitate that formed was collected by filtration, washed with pentane and air-dried on the filter to give 1.10 g (27%) of a light yellow solid. A second crop (0.48 g, 11%) was also recovered. δ_H (500 MHz, [D6]DMSO) 11.18 (s, 1H, O=CH), 9.36 (s, 1H, N–NH–C=S), 9.05–8.95 (bm, 1H, CH_3–NH–C=S), 3.03 (d, $^3J_{HH}$ 4.6, 3H, NH–CH_3), 1.94 (s, 3H, N=C–CH_3). $^{13}C\{^1H\}$ NMR (125.7 MHz, [D6]DMSO) 191.6 (C=O), 179.0 (C=S), 145.4 (C=N), 31.2 (NH–CH_3), 9.1 (C–CH_3). *m/z* (high resolution (HR)MS ESI$^-$) (Calc.) 158.0394 (158.0466) [M – H]$^-$.

1-(4,4-N-Dimethyl-3-thiosemicarbazone)-2-(4-N-methyl-3-thiosemicarbazone)pyruvaldehyde ($H_2\mathbf{L}^2$)

To 2-(4-*N*-methyl-3-thiosemicarbazone)pyruvaldehyde (0.50 g, 3.10 mmol) and 4,4-dimethyl-3-thiosemicarbazide (0.38 g, 3.20 mmol) in dimethylformamide (30 mL) was added acetic acid (glacial, three drops). The mixture was stirred at room temperature under nitrogen for 48 h followed by addition of water (50 mL) and chilling on ice for 15 min. A yellow precipitate was collected by filtration, washed with water, ethanol and diethyl ether, and dried under high vacuum to give 0.69 g (85%) of a yellow solid. δ_H (500 MHz, [D6]DMSO) 11.21 (s, 1H, N–NH–C=S), 10.41 (s, 1H, N–NH–C=S), 8.49–8.44 (q, 1H, CH_3–N*H*–C=S), 7.77 (s, 1H, N=C–H), 3.26 (s, 6H, $N(CH_3)_2$), 3.01 (d, $^3J_{HH}$ 4.6, 3H, NH–CH_3), 2.09 (s, 3H, N=C–CH_3). $^{13}C\{^1H\}$ NMR (125.7 MHz, [D6]DMSO) 180.6 (C=S), 178.3 (C=S), 147.0 (C=N), 143.4 (C=N), 42.4 ($N(CH_3)_2$), 31.2 (NH–CH_3), 11.1 (C–CH_3). *m/z* (HRMS ESI$^-$) (Calc.) 259.0823 (259.0878) [M – H]$^-$.

Dimethylammonium 1-(4-N-p-Sufonato-3-thiosemicarbazone)-2-(4-N-methyl-3-thiosemicarbazone)pyruvaldehyde ($H_2NMe_2[H_2\mathbf{L}^4]$)

$H_2\mathbf{L}^2$ (0.34 g, 1.30 mmol) and sulfanilic acid (0.20 g, 1.16 mmol) were suspended in acetonitrile (50 mL) and the mixture was refluxed under nitrogen for 4 h. After cooling to room temperature, a light-yellow precipitate was collected by filtration, washed with acetonitrile and diethyl ether, and dried under high vacuum (0.25 g, 50%). δ_H (500 MHz, [D6]DMSO) 12.11 (s, 1H, Ar–NH–C=S), 10.42 (s, 1H, N–NH–C=S), 10.02 (s, 1H, N–NH–C=S), 8.57 (q, $^3J_{HH}$ 4.5, 1H, CH_3–N*H*–C=S), 8.40–7.99 (bs, 2H, [$H_2N(CH_3)_2$]$^+$), 7.77 (s, 1H, N=C–H), 7.60–7.55 (m, AA′BB′, 2H, ArH), 7.52–7.46 (m, AA′BB′, 2H, ArH), 3.00 (d, $^3J_{HH}$ 4.6, 3H, NH–CH_3), 2.55 (t, $^3J_{HH}$ 5.6, 6H, $N(CH_3)_2$), 2.23 (s, 3H, N=C–CH_3).

Sodium 1-(4-N-p-Sufonato-3-thiosemicarbazone)-2-(4-N-methyl-3-thiosemicarbazone)pyruvaldehyde ($Na[H_2\mathbf{L}^4]$)

Compound $H_2NMe_2[H_2\mathbf{L}^4]$ (0.22 g, 0.51 mmol) in water (75 mL) was passed through a Dowex column in Na^+ form eluting with water. Fractions (5 × 30 mL) were collected and tested for presence of ligand by mixing a few drops of the eluate with copper(II) acetate monohydrate (~2 mg) showing intense brown colour. Second, third, and fourth fractions were combined and concentrated (~20 mL final volume) to give a highly viscous liquid. Acetonitrile (100 mL) was added, and the resulting mixture was concentrated and triturated with acetonitrile repeatedly (3 × 40 mL) to remove traces of water. A precipitate formed that was collected by filtration washed with acetonitrile and dried under high vacuum (80 mg, 38%). δ_H (500 MHz, [D6]DMSO) 12.10 (s, 1H, Ar–NH–C=S), 10.42 (s, 1H, N–NH–C=S), 10.01 (s, 1H, N–NH–C=S), 8.57 (q, $^3J_{HH}$ 4.5, 1H, CH_3–N*H*–C=S), 7.77 (s, 1H, N=C–H), 7.60–7.55 (m, AA′BB′, 2H, ArH), 7.50–7.46 (m, AA′BB′, 2H, ArH), 3.00 (d, $^3J_{HH}$ 4.6, 3H, NH–CH_3), 2.23 (s, 3H, N=C–CH_3). $^{13}C\{^1H\}$ NMR (125.7 MHz, [D6]DMSO) 178.1 (C=S), 176.1 (C=S), 147.0 (C=N), 145.5 (ArC), 143.4 (C=N), 138.8 (ArC), 125.4 (ArCH), 124.8 (ArCH), 31.0 (NH–CH_3), 11.2 (C–CH_3). *m/z* (HRMS ESI$^-$) (Calc.) 387.0405 (387.0368) [M – H]$^-$.

Dimethylammonium Diacetyl-4-(4-amino-3-hydroxynaptholene-p-sufonato)-4′-methyl-bis (3-thiosemicarbazone) ($H_2NMe_2[H_2\mathbf{L}^5]$)

$H_2\mathbf{L}^1$ (0.10 g, 0.36 mmol) and 4-amino-3-hydroxy-1-napthalene sulfonic acid (0.08 g, 0.36 mmol) were suspended in acetonitrile (20 mL) and the mixture was refluxed under nitrogen overnight. After cooling to room temperature, a colourless precipitate was collected by filtration, washed with acetonitrile and diethyl ether, and dried under high vacuum (0.14 g, 82%). δ_H (500 MHz, [D6]DMSO) 10.59 (s, 1H), 10.25 (s, 1H), 9.73–9.69 (m, 2H), 8.77 (d, $^3J_{HH}$ 8.6, 1H, ArH), 8.46 (d, $^3J_{HH}$ 4.5, 1H, CH_3–N*H*–C=S), 8.26–8.18 (br s, 2H, [$H_2N(CH_3)_2$]$^+$), 7.81 (s,

1H, ArH), 7.63 (d, $^3J_{HH}$ 8.4, 1H, ArH), 7.42 (dd, $^3J_{HH}$ 8.1, 7.0, 1H, ArH), 7.33–7.30 (m, 1H, ArH), 3.09 (d, $^3J_{HH}$ 4.5, 3H, NH–CH_3), 2.58 (t, $^3J_{HH}$ 5.5, 6H, N(CH_3)$_2$), 2.34 (s, 3H, N=C–CH_3). $^{13}C\{^1H\}$ NMR (125.7 MHz, [D6]DMSO) 180.0 (C=S), 179.4 (C=S), 150.5, 149.3, 149.1, 145.1, 133.7, 128.5, 126.5, 124.9, 123.1, 120.1, 119.0, 118.5, 35.3 (N(CH_3)$_2$), 32.1 (NH–CH_3), 12.8 (C–*C*H_3), 12.6 (C–*C*H_3). *m*/*z* (HRMS ESI$^-$) (Calc.) 467.0635 (466.92581) [M – H]$^-$.

Dimethylammonium Diacetyl-4-(5-amino-2-napthalenesufonato)-4′-methyl-bis(3-thiosemicarbazone) ($H_2NMe_2[H_2\mathbf{L}^6]$)

$H_2\mathbf{L}^1$ (0.2 g, 0.7 mmol) and 5-amino-2-naphtalene sulfonic acid (0.16 g, 0.7 mmol) were suspended in acetonitrile (25 mL) and the mixture was refluxed under nitrogen for overnight. After cooling to room temperature, the precipitate was collected by filtration, washed with acetonitrile and diethyl ether, and dried under high vacuum (0.2 g, 61%). δ_H (500 MHz, [D6]DMSO) 10.68 (s, 1H, NH–C=S), 10.27 (br s, 2H, NH–C=S), 8.44–8.42 (m, 1H, CH_3–N*H*–C=S), 8.25–8.12 (m, 3H, Ar*H*, [H_2N(CH_3)$_2$]$^+$), 8.00–7.95 (m, 1H), 7.80–7.72 (m, 2H, ArH), 7.58–7.51 (m, 2H, ArH), 3.05 (d, $^3J_{HH}$ 4.6, 3H, NH–CH_3), 2.55 (t, $^3J_{HH}$ 5.6, 6H, N(CH_3)$_2$), 2.31 (s, 3H, N=C–CH_3). $^{13}C\{^1H\}$ NMR (125.7 MHz, [D6]DMSO) 179.6 (C=S), 179.4 (C=S), 150.1, 149.0, 146.5, 136.5, 133.8, 131.0, 128.5, 127.5, 126.7, 125.2, 125.0, 123.8, 35.3 (N(CH_3)$_2$), 32.2 (NH–CH_3), 12.9 (C–*C*H_3), 12.6 (C–*C*H_3). *m*/*z* (HRMS ESI$^-$) (Calc.) 451.0686 (450.9350) [M – H]$^-$.

*Sodium Diacetyl-4-*p*-sulfonato-4′-methyl-bis(3-thiosemicarbazonato)copper(II) ($Na[Cu^{II}\mathbf{L}^3]$)*

To Na[$H_2\mathbf{L}^3$] (46 mg, 0.11 mmol) in dimethylformamide (2 mL) was added copper(II) acetate monohydrate (21 mg, 0.12 mmol) in dimethylformamide (1 mL) and the resulting mixture was left to stir under nitrogen for 12 h at room temperature. Diethyl ether (20 mL) was added, resulting in precipitation of a dark-red solid, which was collected by filtration, washed with diethyl ether, and dried under high vacuum (34 mg, 64%). Anal. Calc. for [$C_{13}H_{15}N_6O_3S_3Cu$]Na: C 32.13, H 3.11, N 17.29, Na 4.73. Found C 31.79, H 3.71, N 16.65, Na 5.11%. *m*/*z* (HRMS ESI$^-$) (Calc.) 461.9668 (461.9664) [M – H]$^-$. R_t 8.26 min.

*Dimethylammonium Pyruval-1-(4-*N*-*p*-sufonato)-2-(4-*N*-methyl)-bis(3-thiosemicarbazonato)copper(II) ($H_2NMe_2[Cu^{II}\mathbf{L}^4]$)*

To H_2NMe_2[$H_2\mathbf{L}^4$] (0.10 g, 0.23 mmol) dissolved in dimethylformamide (2 mL) was added copper(II) acetate monohydrate (50 mg, 0.23 mmol) in dimethylformamide (1 mL) and the resulting mixture was left to stir under nitrogen at room temperature for 4 h. Excess diethyl ether was added and the resulting dark-red precipitate was collected by filtration, washed with diethyl ether and dried in air (0.11 g, 97%). Anal. Calc. for [$C_{14}H_{21}N_7O_3S_3Cu$]H_2NMe_2·DMF: C 35.75 (35.94), H 4.93 (4.97), N 19.54 (19.72). *m*/*z* (HRMS ESI$^-$) (Calc.) 447.9551 (447.9507) [M – H]$^-$. R_t 8.19 min.

*Dimethylammonium Diacetyl-4-(4-amino-3-hydroxynapthalene-*p*-sufonato)-4′-methyl-bis(3-thiosemicarbazonato)copper(II) ($H_2NMe_2[Cu^{II}\mathbf{L}^5]$)*

To H_2NMe_2[$H_2\mathbf{L}^5$] (50 mg, 0.10 mmol) dissolved in dimethylformamide (2 mL) was added copper(II) acetate monohydrate (20 mg, 0.10 mmol) in dimethylformamide (1 mL) and the resulting mixture was left to stir under nitrogen at room temperature for 4 h. Excess diethyl ether was added and the resulting dark-red precipitate was collected by filtration, washed with diethyl ether, and dried in air (30 mg, 52%). *m*/*z* (HRMS ESI$^-$) (Calc.) 527.8610 (527.9769) [M – H]$^-$. R_t 9.24 min.

Dimethylammonium Diacetyl-4-(5-amino-2-napthalenesufonato)-4′-methyl-bis(3-thiosemicarbazonato)copper(II) ($H_2NMe_2[Cu^{II}\mathbf{L}^6]$)

To H_2NMe_2[$H_2\mathbf{L}^6$] (54 mg, 0.11 mmol) dissolved in dimethylformamide (2 mL) was added copper(II) acetate monohydrate (22 mg, 0.11 mmol) in dimethylformamide (1 mL) and the resulting mixture was left to stir under nitrogen at room temperature for 12 h. Excess diethyl ether was added and the resulting dark-red precipitate was collected by filtration washed with diethyl ether and dried in air (45 mg, 74%). *m*/*z* (HRMS ESI$^-$) (Calc.) 511.9725 (511.9820) [M – H]$^-$. R_t 9.60 min.

Cell Culture and Inductively Coupled Plasma Mass Spectrometry

SH-SY5Y cells were grown in DMEM:F12 media supplemented with 10% (v/v) FBS, 50 mM Hepes, non-essential amino acids (Invitrogen), and penicillin–streptomycin sulfate (Invitrogen). Cultures were maintained at 37°C in a humidified incubator with 5% (v/v) CO_2 and passaged every 5–6 days at a dilution of 1:10. For cell uptake studies, cells were seeded into 6-cm plates. Once the cells had reached 80% confluency, the media were removed by aspiration and replaced with fresh, FBS-free media supplemented with 10 μM copper complexes. The compounds were prepared freshly as 10 mM stock solutions in DMSO, and control treatments therefore received an equivalent volume of DMSO. After treating for 1 h, cells were harvested by scraping into the treatment media and pelleting by centrifugation (1000*g*, 5 min). Pelleted cells were rinsed three times with PBS. A small aliquot from each sample was used to determine total cellular protein, and the remaining sample analysed for cellular Cu using ICP-MS as described previously.[40] Cellular metal levels were normalized for total cellular protein and are expressed as μg Cu per mg protein.

Acknowledgements

The Australian Research Council and National Health and Medical Research Council are acknowledged for financial support. We thank Irene Volitakis for her expertise in ICP-MS of cell lysates and Brett Paterson for assistance with radiolabelling.

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