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Heterocyclic dithiocarbazate iron chelators: Fe coordination chemistry and biological activity†

Maram T. Basha,‡a Jy D. Chartres,a Namfon Pantarat,b Mohammad Akbar Ali,c Aminul Huq Mirza,c Danuta S. Kalinowski,a Des R. Richardsonbc and Paul V. Bernhardt*a

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The iron coordination and biological chemistry of a series of heterocyclic dithiocarbazate Schiff base ligands is reported with regard to their activity as Fe chelators for the treatment of Fe overload and also cancer. The ligands are analogous to tridentate heterocyclic hydrazone and thiosemicarbazone chelators we have studied previously which bear NNO and NNS donor sets. The dithiocarbazate Schiff base ligands in this work also are NNS chelators and form stable low spin ferric and ferrous complexes and both have been isolated. In addition an unusual hydroxylated ligand derivative has been identified via an Fe-induced oxidation reaction. X-ray crystallographic and spectroscopic characterisation of these complexes has been carried out and also the electrochemical properties have been investigated. All Fe complexes exhibit totally reversible FeIII/II couples in mixed aqueous solvents at potentials higher than found in analogous thiosemicarbazone Fe complexes. The ability of the dithiocarbazate Schiff base ligands to mobilise Fe from cells and also to prevent Fe uptake from transferrin was examined and all ligands were effective in chelating intracellular Fe relative to known controls such as the clinically important Fe chelator desferrioxamine. The Schiff base ligands derived from 2-pyridinecarbaldehyde were non-toxic to SK-N-MC neuroepithelioma (cancer) cells but those derived from the ketones 2-acetylpyridine and di-2-pyridyl ketone exhibited significant antiproliferative activity.

Introduction

In the treatment of acute iron (Fe) overload disorders, the administration of Fe chelating ligands is essential.1–4 In humans, intestinal Fe absorption is tightly regulated5 and when Fe levels are adequate, further Fe uptake is inhibited. In cases where severe Fe overload occurs, commonly through necessary blood transfusions in the treatment of anaemias such as β-thalassemia, Fe overload becomes acute, as humans have no mechanism for actively excreting excess Fe. Failure to remove excess Fe leads to its accumulation in vital organs such as the heart and liver, and if left untreated, leads to irreversible organ damage and death.

Few Fe chelators have been successful in treating Fe overload in the clinic. Desferrioxamine (DFO; Chart 1) was for many years the only drug approved worldwide for the treatment of Fe overload. Its widespread use continues today, despite its major drawback of not being orally active.4 In fact, DFO must be administered by long and frequent periods of subcutaneous infusion (12–24 h per day, 5–6 times a week). In 2005, the triazole deferasirox (Exjade®, Chart 1)5–8 was approved by the US Food and Drug Administration (FDA) for oral administration in the treatment of Fe overload and was the first drug of its type to be approved worldwide. However, possible side effects such as renal and liver damage and gastrointestinal haemorrhage in some patients were added as warnings to the packaging of this drug in 2010.†

The hydroxypyridinones (such as deferiprone also known as L1 and more recently Ferriprox®) emerged in the 1980s9 and also offered the hope of oral activity, but controversy surrounding their efficacy and toxicity10,11 hampered drug development over the years. In 2011, the FDA finally granted approval for the ‘second line’ use of deferiprone as an orally administered drug in the treatment of Fe overload,§ although there are still unresolved questions over its efficacy. Notably, the most promising clinical results with deferiprone have been obtained when it is co-administered with DFO where it appears to be effective against Fe loading in heart tissue.12

The tridentate 2-pyridinecarbaldehyde isonicotinoyl hydrazine (HPCIH) family of Fe chelators (Chart 1) have shown...
promise as alternatives to existing compounds for the treatment of Fe overload.\textsuperscript{13–16} Investigations by our laboratories have shown that they form stable Fe\textsuperscript{II} complexes, in contrast to the Fe\textsuperscript{III} complexes formed by DFO, deferiprone and deferasirox.\textsuperscript{1,2,4} Subsequent studies have shown that substitutions to the C-atom adjacent to the pyridyl ring lead to a marked change in biological activity, for example the HPCIH analogues based on 2-acetylpyridine (HAPIH series)\textsuperscript{17} or di-2-pyridyl ketone (HPKIH analogues)\textsuperscript{18} exhibit significant cytotoxicity in contrast to the benign HPCIH chelators.\textsuperscript{16} Although deleterious for the treatment of chronic Fe overload (where chelators must be administered lifelong), cytotoxic Fe chelators may prove to be a novel and effective method in cancer therapy.\textsuperscript{19–26} Of interest, a significant increase in cytotoxicity and activity against neoplastic cells was found by substitution of the O-donor of the HPKIH and HAPIH hydrazones with an S-atom leading to the thiohydrazone (HPKTBH)\textsuperscript{27} and thiosemicarbazone (HDp44mT)\textsuperscript{28–30} families of chelators (Chart 1).

In the current investigation, we have assessed a series of dithiocarbazate Schiff base analogues (Chart 2) in their reactions with Fe. The ligands share the same NNS, tridentate donor set as the HDp44mT and HPKTBH analogues, but the terminal substituent is a mercaptomethyl or mercaptobenzyl group rather than an amine or aromatic ring. Dithiocarbazate Schiff bases are well described and a number of transition metal complexes are known\textsuperscript{31–41} and these compounds have shown a wide spectrum of biological activity. Little is known about the Fe coordination chemistry of these dithiocarbazate Schiff base ligands\textsuperscript{42,43} and given the resemblance to the highly active thiosemicarbazone Fe chelators we have studied previously,\textsuperscript{21,28–30} this was the focus of the current work. Of particular interest are the relationships between chelator structure, redox properties of the Fe complex and the biological activity (including anti-proliferative efficacy). Modification of the substituents adjacent to the donor group is known to have a marked effect on redox potentials in compounds from the heterocyclic thiosemicarbazone\textsuperscript{28–30} and

\begin{chart}{Fe chelators in clinical use for the treatment of Fe overload disease (i.e. DFO, deferiprone and deferasirox) as well as hydrazone, thiohydrazone, thiosemicarbazone and hydrazine Fe chelators with known biological activity. Donor atoms are in bold type.}

\begin{align*}
\text{desferrioxamine B (desferal, DFO)} \\
\text{deferiprone (L1)} \\
\text{deferasirox}
\end{align*}

\begin{align*}
\text{HPCIH} \\
\text{HAPIH} \\
\text{HPKIH}
\end{align*}

\begin{align*}
\text{HPKTBH} \\
\text{HDp44mT} \\
\text{H}_{2}\text{NIH (311)} \\
\text{H}_{2}\text{IPH}
\end{align*}
hydrazone series due to extended conjugation along the ligand backbone. Replacement of the terminal amino group with a thioether brings about both differences in inductive and resonance effects, which then are communicated to the coordinated metal ion. In this contribution, we investigate the Fe coordination chemistry of these dithiocarbazate ligands, their effectiveness at mobilising intracellular Fe and their anti-proliferative activity.

**Experimental**

**Safety note**

Perchlorate salts are potentially explosive. Although we experienced no problems with the compounds described below they should never be heated in the solid state or scraped from sintered glass frits.

**Reagents**

*S*-Methylthiocarbazate, and *S*-benzylthiocarbazate were prepared according to literature syntheses. All other reagents were obtained commercially and used without further purification.

**Free ligand syntheses**

**2-Acetylpyridine *S*-methylthiocarbazate (HAPSMC).** A suspension of *S*-methyl dithiocarbazate (6.11 g, 0.05 mol) in 75 mL of isopropanol was heated until it dissolved. This mixture was cooled to room temperature then filtered and the residue was washed with isopropyl alcohol then discarded. The filtrate was treated with 2-acetylpyridine (6.05 g, 0.05 mol) added drop-wise. The mixture was stirred for 24 h at room temperature. The resulting yellow precipitate was filtered off, washed with isopropanol and recrystallised from ethanol to give a yellow crystalline product. Yield (7.3 g, 65%). Microanalysis found C, 48.1; H, 5.0; N, 18.8%; Calculated for C9H11N3S2: C, 48.0; H, 4.9; N, 18.7%. IR: \( \ddot{\nu} \text{ (cm}^{-1}\text{)} \): 3144m, 2913w, 2114w, 1665w, 1615w, 1568m, 1562m, 1484m, 1460s, 1428s, 1366m, 1336m, 1254vs, 1144w, 115w, 1060vs, 1045m, 990w, 949vs, 886w, 774vs, 734vs, 678m, 642s, 620w, and 562m. \( ^1\text{H NMR (DMSO-d}_6\text{)}: \delta \text{ 12.57 (s, 1H, NH), 8.62 (m, 1H, py), 8.07 (m, 1H, py), 7.88 (m, 1H, py), 7.45 (m, 1H, py), 2.52 (s, 3H, S–CH}_3\text{), 2.45 (s, 3H, C–CH}_3\text{).} \)

**2-Acetylpyridine *S*-benzylthiocarbazate (HAPSCBC).** *S*-Benzyl dithiocarbazate (1.98 g, 0.01 mol) was dissolved in hot absolute ethanol (50 mL). To this solution was added drop-wise...
an equimolar amount of 2-acetylpyridine (1.21 g, 0.01 mol). The mixture was heated with stirring for 30 min and allowed to stand at room temperature until the product precipitated. The product was filtered off and washed with ethanol to afford dark yellow crystals. Yield (2.29 g, 76%). Microanalysis found C, 59.5; H, 5.1; N, 14.0%. Calculated for C14H12N4S2: C, 59.8; H, 5.0; N, 13.9%. IR: ν (cm⁻¹): 3171, 2969, 2120, 1668, 1600, 1580, 1563, 1489s, 1461vs, 1416s, 1367m, 1328m, 1268s, 1230m, 1146w, 1062vs, 990w, 961m, 919w, 889w, 776s, 720s, 681m, 622s, 589w, and 566m. ¹H NMR (DMSO-d₆): δ 12.6 (s, 1H, NH), 8.59 (m, 1H, py), 8.01 (s, 1H, py), 7.83 (m, 1H, py), 7.25 (m, 1H, py), 7.43–7.27 (m, 5H, Ph), 4.48 (s, 2H, CH₂), 2.45 (s, 3H, CH₃). ¹³C NMR (DMSO-d₆): 12.9, 120.4, 124.6, 127.2, 128.5, 129.3, 136.7, 136.8, 142.1, 148.2, 152.2 ppm.

**Di-2-pyridyl ketone S-methyldithiocarbazate (HPKSMC).** S-Methyl dithiocarbazate (1.22 g, 0.01 mol) was dissolved in ethanol (25 mL) and added to a solution of di-2-pyridylketone in ethanol (15 mL). The mixture was refluxed for 1 h and then left to stand overnight at room temperature. The product was filtered off and recrystallised from ethanol to give bright yellow crystals. Yield (2.19 g, 76%). Microanalysis found C, 54.2; H, 4.2; N, 19.8%. Calculated for C₁₄H₁₂N₄S₂: C, 54.1; H, 4.2; N, 19.4%. IR: ν (cm⁻¹): 3042, 2981, 2916, 1698w, 1564w, 1584m, 1481m, 1452s, 1416vs, 1327s, 1281m, 1242s, 1130s, 1060s, 998m, 962vs, 890m, 802s, 733vs, 692s, 693m, 614m, and 589s. ¹H NMR (CDCl₃): δ 15.28 (s, 1H, NH), 8.78 (m, 1H, py), 8.61 (m, 1H, py), 8.03 (m, 1H, py), 7.82 (m, 2H, py), 7.70 (m, 1H, py), 7.37 (m, 2H, py), 2.65 (s, 3H, SCH₃). ¹³C NMR (CDCl₃): 174.1, 123.9, 124.3, 124.5, 127.2, 137.1, 137.3, 142.1, 148.1, 151.1, 155.4 and 202.3 ppm.

**Di-2-pyridyl ketone S-benzyldithiocarbazate (HPKSB).** Di-2-pyridyl ketone (1.84 g, 0.01 mol) was dissolved in EtOH (15 mL) and mixed with a hot solution of S-benzyl dithiocarbazate (1.98 g, 0.01 mol) in EtOH (30 mL). The mixture was refluxed for 1 h and then left to stand overnight at room temperature. The product was filtered off and recrystallised from ethanol to obtain yellow crystals. Yield (2.94 g, 79.9%). Microanalysis found C, 58.3; H, 4.6; N, 14.6%. Calculated for C₁₃H₁₁N₃S₂: C, 58.5; H, 4.6; N, 14.6%. IR: ν (cm⁻¹): 2923w, 2794m, 1582w, 1566w, 1533s, 1464s, 1438w, 1366w, 1317s, 1278vs, 1108m, 1044s, 1000m, 928s, 830w, 773vs, 714m, 680s, 633, and 600. ¹H NMR (DMSO-d₆): δ 13.44 (s, 1H, NH), 8.60 (m, 1H, py), 8.26 (s, 1H, CH), 7.86 (m, 2H, py), 7.45–7.25 (m, 5H, Ph, 1H, py), 4.49 (s, 2H, CH₂). ¹³C NMR (DMSO-d₆): 137.7, 120.2, 124.9, 127.3, 128.5, 129.3, 163.5, 137, 146.7, 149.8 and 152.2 ppm.

**Fe³⁺ complexes**

[Fe³⁺(APSMC)₂(ClO₄)]Cl₄. HAPSMC (1 g, 4.4 mmol) was dissolved in 15 mL of hot ethanol and then triethylamine (0.617 g, 4.4 mmol) was added. Solid Fe(ClO₄)₂·6H₂O (1.025 g, 2.2 mmol) was added directly to the basic ligand solution and stirred for 30 min. After cooling to room temperature, the resulting solid was filtered off and washed with ethanol (10 mL), followed by diethyl ether (10 mL). It was then dried in a vacuum desiccator and recrystallised from ethanol to give dark brown powder. Yield (1.09 g, 82%). Microanalysis found C, 36.3; H, 3.6; N, 14.2%. Calculated for C₁₃H₂₀ClFeO₄N₆S₄: C, 35.8; H, 3.3; N, 13.9%. IR: ν (cm⁻¹): 3078w, 2926w, 1700w, 1597m, 1481w, 1423s, 1310m, 1148w, 1084vs, 1032vs, 945s, 818m, 774s, 742s, 621s, and 557w. Electronic spectrum (MeCN): 425 nm (11 300 M⁻¹ cm⁻¹).
1330m, 1242w, 1077vs, 942s, 812w, 785s, and 620s. Electronic spectrum (MeCN): 428 nm (8530 M$^{-1}$ cm$^{-1}$), 350 (25 600).

$\text{[Fe}^{\text{III}}(\text{PCSBC})_2(\text{ClO}_4)]\text{1.5H}_2\text{O}$. HPCSBC (0.7 g, 2.4 mmol) was dissolved in 300 mL of hot ethanol and then triethylamine (0.34 mL, 2.4 mmol) was added. Solid Fe(ClO$_4$)$_2$·6H$_2$O (0.54 g, 1.2 mmol) was added to the basic ligand solution with stirring and the mixture was gently refluxed for 1 h. The reaction mixture was cooled to room temperature and the resulting crude dark brown solid containing a mixture of compounds was filtered off, washed with 10 mL of ethanol followed by 10 mL of diethyl ether. A column of dry silica (100 × 32 mm diameter, Scharlau or Merck Silica gel 60, 230–400 mesh) was prepared. The crude product (0.3 g) was dissolved in CH$_2$CN (∼50 mL) and adsorbed onto Celite (∼1.3 g) by evaporation to dryness on a rotary evaporator at room temperature (see Safety Note). The resulting solids were packed dry onto the column which was tapped gently to remove air pockets. The column was eluted with a step-wise gradient of CH$_3$CN resulting solids were packed dry onto the column which was gently refluxed for 1 h. The reaction mixture was cooled to room temperature and the resulting crude dark brown solid was recrystallised with acetone–diethyl ether. Column chromatography on silica (see previous synthesis) was carried out using a solvent gradient of CH$_2$OH (in increasing quantities) in DCM. After elution of a green band of $\text{[Fe}^{\text{II}}(\text{PCSBC})_2]$, the desired mixed-ligand complex $\text{[Fe}^{\text{III}}(\text{PCSBC})(\text{PSMC})]$ eluted. This was evaporated to dryness. Yield 50%. The complex was recrystallised with acetone–diethyl ether. Microanalysis found C, 38.6; H, 4.1; N, 12.1%; calculated for C$_{38}$H$_{35}$ClFe·N$_8$O$_6$·S$_4$: C, 38.9; H, 5.2; N, 12.4%. IR: $\nu$ (cm$^{-1}$): 3401m, 2921m, 1583vs, 1371s, 1283m, 1192w, 1073vs, 995m, 916m, 761, 686w, and 620m. Crystals suitable for crystallography were obtained by slow diethyl ether vapour diffusion into a concentrated acetone solution. Electronic spectrum (MeCN): 618 nm (1440 M$^{-1}$ cm$^{-1}$), 441 (3850), 300 (11 300).

$\text{[Fe}^{\text{III}}(\text{PKSMC})_2(\text{ClO}_4)].$ HPKSMC (0.8 g, 2.8 mmol) was dissolved in 30 mL of hot ethanol and (0.64 1.4 mmol) of solid Fe(ClO$_4$)$_2$·6H$_2$O was added directly to the solution. Et$_3$N (0.39 mL, 2.8 mmol) was added to the dark brown mixture which was gently refluxed for 30 min. The reaction mixture was filtered while still hot to obtain an initial crop. The filtrate was allowed to cool at room temperature and refrigerated overnight. The dark brown product was collected by vacuum filtration and washed with ethanol and diethyl ether to give a second batch of brown solid. Yield (0.83 g, 83%). Microanalysis found C, 49.3; H, 3.6; N, 12.3%; calculated for C$_{38}$H$_{35}$ClFe·N$_8$O$_6$·S$_4$: C, 49.2; H, 3.8; N, 12.1%. IR: $\nu$ (cm$^{-1}$): 3090w, 1620m, 1584m, 1529w, 1413s, 1340s, 1313m, 1159m, 1073v, 1000s, 821w, 792m, 743s, and 615vs. Crystals of $\text{[Fe}^{\text{III}}(\text{PKSMC})_2]\cdot\text{MPPT}(\text{ClO}_4)_2$ (MPPT being a triazinum product of HPKSMC desulphurisation, see Scheme 1) suitable for X-ray work were obtained from diethyl ether vapour diffusion into a concentrated acetone solution of the crude first crop. Electronic spectrum (MeCN): 445 nm (12 100 M$^{-1}$ cm$^{-1}$), 362 (27 000).

$\text{[Fe}^{\text{II}}(\text{PKSBC})_2(\text{ClO}_4)]_2\cdot2\text{H}_2\text{O}$. HPKSBC (0.8 g, 2.2 mmol) was dissolved in 65 mL of hot ethanol and solid Fe(ClO$_4$)$_2$·6H$_2$O was added directly to the solution. Et$_3$N (0.39 mL, 2.2 mmol) was added to the dark brown mixture which was gently refluxed for 30 min. The reaction mixture was filtered while still hot and the filtrate was allowed to cool at room temperature and refrigerated overnight. The dark brown product was collected by vacuum filtration and washed with ethanol and diethyl ether to give a second batch of brown solid. Yield (0.46 g, 48%). Microanalysis found C, 49.1; H, 3.6; N, 12.3% calculated for C$_{38}$H$_{35}$ClFe·N$_8$O$_6$·S$_4$: C, 49.2; H, 3.8; N, 12.1%. IR: $\nu$ (cm$^{-1}$): 3059w, 1585m, 1494w, 1408w, 1355s, 1243m, 1074v, 989s, 820w, 745m, 700m, and 617vs. Electronic spectrum (MeCN): 431 nm (9370 M$^{-1}$ cm$^{-1}$), 356 (20 100).

$\text{Fe}^{\text{II}}$ complexes

The $\text{Fe}^{\text{II}}$ complexes were synthesised by the following general method. 2.4 mmol of the appropriate dithiocarbazate ligand was dissolved in ethanol (30–150 mL) and the mixture was purged
Upon cooling, the green product was filtered off and washed with 10 mL of ethanol followed by 10 mL diethyl ether.

\[ \text{Fe}^{II}(\text{APSMC})_2\cdot0.5\text{H}_2\text{O}. \] Yield (0.93 g, 80%). Microanalysis found C, 41.0; H, 3.9; N, 15.9%; Calculated for C\(_{18}\)H\(_{22}\)FeN\(_6\)S\(_4\): C, 41.4; H, 4.2; N, 16.1%. IR: \(\nu\) (cm\(^{-1}\)) - 3374b, 2927w, 1629w, 1590s, 1496s, 1459m, 1379vs, 1323s, 1239w, 1112w, 1049s, 953s, 882m, 844m, 754s, 666w. Electronic spectrum (MeCN): 679 nm (7120 M\(^{-1}\) cm\(^{-1}\)).

\[ \text{Fe}^{II}(\text{APSCB})_2\cdot2\text{H}_2\text{O}. \] Yield (0.58 g, 72%). Microanalysis found C, 37.9; H, 3.5; N, 16.0%; Calculated for C\(_{28}\)H\(_{25}\)FeN\(_8\)O\(_0.5\)S\(_4\): C, 57.6; H, 4.0; N, 14.2%. IR: \(\nu\) (cm\(^{-1}\)) - 3414b, 2927w, 1629w, 1590s, 1496s, 1459m, 1379vs, 1323s, 1239w, 1112w, 1049s, 953s, 882m, 844m, 754s, 666w, and 566m. Electronic spectrum (MeCN): 679 nm (5920 M\(^{-1}\) cm\(^{-1}\)), 429 (sh, 12 500), 331 (17 800).

\[ \text{Fe}^{II}(\text{PKSMC})_2\cdot2\text{H}_2\text{O}. \] Yield (0.4 g, 52%). Microanalysis found C, 52.7; H, 4.0; N, 12.9%; Calculated for C\(_{38}\)H\(_{31}\)FeN\(_8\)O\(_0.5\)S\(_4\): C, 57.6; H, 4.0; N, 14.2%. IR: \(\nu\) (cm\(^{-1}\)) - 3395b, 2927w, 1629w, 1590s, 1496s, 1459m, 1379vs, 1323s, 1239w, 1112w, 1049s, 953s, 882m, 844m, 754s, 666w, and 566m. Electronic spectrum (MeCN): 684 nm (7120 M\(^{-1}\) cm\(^{-1}\)), 441 (sh, 7210), 346 (17 800).

\[ \text{Fe}^{II}(\text{PKSCB})_2\cdot0.5\text{H}_2\text{O}. \] Yield (0.8 g, 70%). Microanalysis found C, 47.4; H, 3.8; N, 16.7%; Calculated for C\(_{26}\)H\(_{23}\)FeN\(_6\)O\(_{1.5}\)S\(_2\): C, 47.5; H, 3.8; N, 17.0%. IR: \(\nu\) (cm\(^{-1}\)) - 3059w, 2083w, 1595s, 1496s, 1394vs, 1333m, 1287m, 1207.61m, 1153w, 1108s, 1040s, 968w, 852m, 759s, 699s, and 561w. Electronic spectrum (MeCN): 682 nm (6320 M\(^{-1}\) cm\(^{-1}\)), 445 (sh, 10 500), 344 (25 300).

\[ \text{Fe}^{II}(\text{PKSMC})_2\cdot1.5\text{H}_2\text{O}. \] Yield (0.8 g, 70%). Microanalysis found C, 57.5; H, 3.9; N, 14.4%; Calculated for C\(_{36}\)H\(_{31}\)FeN\(_6\)O\(_{0.5}\)S\(_4\): C, 57.6; H, 4.0; N, 14.2%. IR: \(\nu\) (cm\(^{-1}\)) - 3063w, 2119w, 1581m, 1481m, 1379vs, 1322s, 1239w, 1112w, 1061vs, 1015w, 980vs, 858w, 785s, 766m, 740s, 699m, 601s, and 556w. Electronic spectrum (MeCN): 684 nm (7120 M\(^{-1}\) cm\(^{-1}\)), 441 (sh, 8000), 331 (17 800).

**Physical methods**

UV-vis spectra were recorded on a Perkin Elmer Lambda 40 spectrophotometer. Solutions were analysed immediately before any redox reactions occurred. This was particularly important for the Fe\(^{II}\) complexes which slowly oxidised in aerated solutions. IR spectra were recorded as undiluted solids on a Perkin Elmer 1600 FTIR spectrometer in attenuated total reflectance mode. NMR spectra were acquired on a Bruker Avance 300 MHz spectrometer and all resonances were given relative to tetramethylsilane (\(\delta = 0\) ppm). Cyclic voltammetry was recorded with a BAS100B/W potentiostat employing a glassy carbon working electrode, Pt wire counter and Ag–AgCl reference electrode. The solvent was MeCN–H\(_2\)O (7:3) and the supporting electrolyte was 0.1 M Et\(_4\)NClO\(_4\). All solutions were purged with N\(_2\) before measurement. Approximately 1 mM solutions of analyte were used.

**Biological assays**

**Cell culture.** The chelators were dissolved to make 10 mM stock solutions in DMSO and diluted in medium containing 10% foetal calf serum (Commonwealth Serum Laboratories, Melbourne, Australia). A final [DMSO] < 0.5% (v/v) was used to ensure that DMSO had no effect on proliferation.\(^{46}\) Fe uptake, or Fe\(^{59}\) mobilisation from cells.\(^{46}\) The human SK-N-MC neuroepithelioma cell line (American Type Culture Collection, Manassas, VA) was grown using standard procedures\(^{27}\) in a humidified atmosphere of 5% CO\(_2\)–95% air at 37 °C in an incubator (Forma Scientific, Marietta, OH).

**Effect of the chelators on cellular proliferation**

The effect of the chelators on cellular proliferation was determined using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] (MTT) assay via standard methods.\(^{27,46}\) The SK-N-MC cell line was seeded at 1.5 × 10\(^4\) cells per well in 96-well microtiter plates in medium containing the Fe uptake protein, human Fe\(^{59}\)-transferrin (Fe\(^{59}\)-Tf; 1.25 μM) and chelators at a range of concentrations (0–25 μM). The control samples contained medium with Fe\(^{59}\)-Tf (1.25 μM) in the absence of the ligands.
The cells were then incubated in a humidified atmosphere containing 5% CO₂ and 95% air at 37 °C for 72 h. Subsequently, 10 µL of MTT (5 mg mL⁻¹) was added to each well and incubated at 37 °C for 2 h. The cells were solubilised with 100 µL of 10% SDS–50% isobutanol in 10 mM HCl and the plates were then read at 570 nm using a scanning multi-well spectrophotometer. The inhibitory concentration (IC₅₀) was defined as the chelator concentration needed to decrease the absorbance to 50% of the untreated control. Absorbance was shown to be directly proportional to cell counts, as shown previously.⁴⁶

**Preparation of ⁵⁶Fe- and ⁵⁹Fe-Tf**

Human Tf (Sigma) was labelled with either ⁵⁶Fe or ⁵⁹Fe (Dupont NEN, MA) to produce ⁵⁶Fe₂-Tf and ⁵⁹Fe₂-Tf, respectively, as described previously.⁴⁶ Any unbound ⁵⁹Fe was removed by passage through a Sephadex G25 column and exhaustive vacuum dialysis against an excess of 0.15 mM NaCl buffered to pH 7.4 with 1.4% NaHCO₃ via standard methods.⁴⁷

**Effect of chelators on ⁵⁹Fe efflux from SK-N-MC cells**

The ability of the chelators to mobilise ⁵⁹Fe from SK-N-MC cells was performed by established techniques.⁴⁶ After prelabeling cells with ⁵⁹Fe₂-Tf (0.75 µM) for 3 h at 37 °C, the cell monolayer was washed four times on ice with ice-cold PBS and subsequently incubated with the chelators (25 µM) for 3 h at 37 °C. The overlying media containing released ⁵⁹Fe was separated from the cells using a Pasteur pipette. Radioactivity in the cell pellet and the supernatant was measured using a γ-scintillation counter (Wallac Wizard 3, Turku, Finland). In these studies, the novel ligands were compared to the well-characterised chelators, DFO, H₂N(NH₃)₃ (311) and HDp44mT.¹⁹,⁴⁶,⁴⁷

**Effect of chelators at preventing ⁵⁹Fe uptake from ⁵⁹Fe₂-Tf by SK-N-MC cells**

The ability of the novel ligands to prevent cellular ⁵⁹Fe uptake from the Fe transport protein, ⁵⁹Fe₂-Tf, was examined via established methods.⁴⁶ Cells were incubated with ⁵⁹Fe₂-Tf (0.75 µM) for 3 h at 37 °C in the presence of the chelators (25 µM). The cells were washed on ice four times with ice-cold PBS and the internalised ⁵⁹Fe was determined by standard procedures by incubating the monolayer for 30 min at 4 °C with the protease, Pronase (1 mg mL⁻¹; Sigma).⁴⁶,⁴⁷ The cells were removed using a plastic spatula and centrifuged at 14 000 rpm for 1 min. The pronase-insensitive fraction represents internalised ⁵⁹Fe, while the supernatant represents the membrane-bound, pronase-sensitive ⁵⁹Fe that was released by the protease.⁴⁶,⁴⁷ The ligands were compared to the previously characterised chelators, DFO, 311 and HDp44mT.¹⁹,⁴⁶

**Crystallography**

Crystallographic data were acquired at 23 °C on an Oxford Diffraction Gemini CCD diffractometer employing either Mo-Kα or Cu-Kα radiation (see Table 1) and operating in the ω-scan mode. Data reduction and empirical absorption corrections (multi-scan) or analytical absorption corrections were performed with Oxford Diffraction CrysAlisPro software. Structures were solved by direct methods with SHELXS and refined by full-matrix least-squares analysis with SHELXL-97.⁴⁹ All non-H atoms were refined with anisotropic thermal parameters. Molecular structure diagrams were produced with ORTEP.⁵⁰ All calculations were carried out within the WinGX graphical user interface.⁵¹ In the structure of [Fe(PCSME)(PSME)]·Me₂CO the acetone molecule was modelled as a diffuse contribution to the overall scattering without specific atom positions using SQUEEZE/PLATON.⁵² Crystal and refinement data are summarised in Table 1.

**Results and discussion**

**Free ligand characterisation**

The dithiocarbazate Schiff base ligands in Chart 2 were prepared by variations on published procedures³¹,³³ involving condensation between S-methyl (or S-benzyl) thio carbamate and the corresponding aldehyde or ketone. These Schiff base ligands

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**Table 1 Crystal data**

<table>
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<tr>
<th></th>
<th>HAPS PMC</th>
<th><a href="ClO%E2%82%84">Fe(PCSME)₂</a>₂</th>
<th><a href="MPPT">Fe(PKSMC)₂</a>(ClO₄)₂</th>
<th>[Fe(PSMC)(PCSME)]·Me₂CO</th>
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<tr>
<td>Formula</td>
<td>C₉H₁₃N₃S₂</td>
<td>C₁₆H₁₆ClFeN₁₀O₄S₄</td>
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<tr>
<td>Mol. Wt</td>
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<td>7.9883(3)</td>
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<tr>
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<td>C2/c</td>
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<td>R3</td>
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<td>3881 (0.0425)</td>
<td>7365 (0.0398)</td>
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<td>wR₂ (all data)</td>
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</table>

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6542 | *Dalton Trans.*, 2012, 41, 6536–6548

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present a homologous series bearing a common NNS donor set. As a representative example, the single crystal structure of HAPSMC was determined. A view of the ligand appears in Fig. 1. The compound adopts an approximately planar conformation. Of note is that the donor atoms (N1, N2 and S1) are not in the required conformation for tridentate coordination and 180° rotation of the C8–N3 and C5–C6 bonds are required. The C8–S1 bond has double bond character in contrast to the S-methyl single bond (S2–C9). The C8–S2 bond is slightly shorter than S2–C9 indicating some electronic delocalisation. Selected bond lengths appear in Table 2.

Fe complexation
Deprotonation of the thioamide N atom accompanies tridentate coordination of each dithiocarbazate Schiff base ligand. The Fe complexes of these ligands were the focus of our investigation here. Given the parallels with our previous work on the HPCIH (hydrazone)16 and HDp44mT (thiosemicarbazone) series,28 the present set of dithiocarbazate compounds exhibit chemical and biological properties reminiscent of both. The FeII complexes were prepared by reaction of the ligands with ferrous perchlorate in EtOH under an inert atmosphere to avoid oxidation to the trivalent state during synthesis. Each ferrous complex exhibited a characteristically green colour due to a broad and intense MLCT (FeII → pyridine) transition around 670 nm (Fig. 2). Similar maxima have been identified in the FeII complexes of the HPCIH16–18 and HDp44mT28–30 analogues. In the solid state, the FeII complexes are stable and do not undergo oxidation. The FeIII complexes were prepared by direct reaction with ferric perchlorate in EtOH. The ferric complexes are dark brown and exhibit a series of broad, overlapping and intense electronic transitions that span most of the visible region (Fig. 2). The free ligands exhibit no electronic maxima in the visible region and are pale yellow in colour.

Puriﬁcation of the Fe complexes was generally possible by recrystallisation, but column chromatography (silica) was sometimes necessary to separate mixtures of FeII and FeIII complexes that co-precipitated. This is a new method for puriﬁcation of these complexes and facilitated isolation of compounds for single crystal X-ray work.

| Table 2 Selected bond lengths and angles (ligands ‘a’ and ‘b’ defined by labels in Fig. 3 and 4). For the heteroligand complex [Fe(PSMC)(PCSMC)] bonds to the oxidised PSMC− ligand are in italics |
|-----------------|-----------------|-----------------|-----------------|
|                | HAPSMC          | [Fe(PSMC)Cl]+, | [Fe(PSMC)Cl]+, | [Fe(PSMC)Cl]+, |
|                |                 | Ligand a, b     | Ligand a, b     | Ligand a, b     |
| C8–S1          | 1.645(3)        | 1.720(3), 1.713(3) | 1.735(3), 1.735(3) | 1.73(1), 1.751(9) |
| C8–S2          | 1.762(3)        | 1.710(3), 1.713(3) | 1.738(3), 1.733(3) | 1.765(9), 1.740(9) |
| C8–N3          | 1.333(3)        | 1.282(4), 1.288(4) | 1.291(4), 1.301(4) | 1.29(1), 1.26(1) |
| C9–S2          | 1.791(3)        | 1.764(3), 1.767(4) | 1.794(4), 1.796(4) | 1.75(1), 1.78(1) |
| N2–N3          | 1.374(3)        | 1.368(3), 1.371(3) | 1.384(3), 1.385(3) | 1.365(9), 1.369(9) |
| N2–C6          | 1.282(3)        | 1.277(3), 1.277(3) | 1.308(4), 1.309(4) | 1.38(1), 1.27(1) |
| Fe–N1a/b       | —               | 1.968(2), 1.956(3) | 1.984(3), 1.984(2) | 1.992(6), 1.990(7) |
| Fe–N2a/b       | —               | 1.880(2), 1.879(2) | 1.910(2), 1.912(2) | 1.911(7), 1.942(7) |
| Fe–S1a/b       | —               | 2.189(9), 2.201(1) | 2.2255(9), 2.2273(9) | 2.223(2), 2.265(3) |
| N1a/b–Fe–N2a/b | —               | 80.9(1), 80.5(1)  | 80.4(1), 80.4(1)  | 81.6(3), 79.7(3)  |
| N2a/b–Fe–S1a/b | —               | 84.97(7), 85.03(8) | 85.37(8), 85.56(8) | 84.8(2), 82.8(2)  |
| N2a–Fe–N2b     | —               | 176.5(1)         | 177.8(1)         | 176.5(3)         |
The crystal structure of [Fe(PCSMC)\(_2\)](ClO\(_4\)) was determined. A view of the complex cation appears in Fig. 3. The meridional conformation of each ligand is apparent to produce a complex with approximate C\(_2\) symmetry. The Fe–N and Fe–S bond lengths (Table 2) are characteristic of low spin Fe\(^{III}\) complexes seen in related thiosemicarbazone and thiohydrazone ferric complexes from the HDP44mT\(^{19}\) and HPKTBH\(^{27}\) series. There is no conformational flexibility in the complex cation except for the S-methyl groups which each adopt an anti-conformation with respect to the adjacent coordinated S-donor. The most significant variations in intraligand bond lengths (going from free ligand to complex) are in the region of the coordinated S-donors. The C8–S1 and C8–S2 bond lengths in [Fe(PCSMC)\(_2\)]\(^+\) are identical in the complex due to electron delocalisation across the N3–C8 (S1)–S2 moiety in contrast to what is seen in the analogue HAPSMC where the C8=S1 bond has definite double bond character. The C3–N3 bonds in each ligand shorten appreciably upon complexation, defining an effective imine-thiolate (\(\text{N} = \text{C} - \text{S}^\text{−}\)) resonance form of the coordinated ligand anion. No significant variations in the N2–N3 or N2–C6 bonds are apparent on deprotonation and complexation.

The crystal structure of the homologous complex [Fe(PKSMC)\(_2\)]\(^+\) was also determined (Fig. 4). The geometry of the complex cation mirrors that of [Fe(PCSMC)\(_2\)]\(^+\). Again the coordinate bonds and angles are consistent with low spin Fe\(^{III}\) complexes of the dithiocarbazate Schiff base ligands (Table 2) but the Fe–N and Fe–S bonds are slightly longer than seen in [Fe(PCSMC)\(_2\)]\(^+\). This may be attributed to a combination of interligand repulsion brought about by introduction of the non-coordinating pyridyl rings and also electron-withdrawing effects of these groups that may slightly weaken the donor strength of the PKSMC\(^−\) ligand.

Curiously, the complex [Fe(PKSMC)\(_2\)]\(^+\) co-crystallised with a cationic pyridotriazinium derivative (MPPT\(^+\), Fig. 4); a by-product of ligand desulfuration. It is not known whether Fe catalyses this reaction, although the loss of sulfide (or hydrogen sulfide) is probably assisted by the presence of free metal ions. A tentative mechanism is proposed in Scheme 1. It is known that elimination of methanethiol followed by ring closure is an alternative possibility in dithiocarbazates of this type, leading to an exocyclic thioamide (rather than thioether) group.\(^53\)

Depending on the order of addition of reagents (ligand, ferric perchlorate and base), different products were observed in the synthesis of the Fe\(^{III}\) complex of HPCSRTC. When the ferric salt was added last, an unusual mixed-ligand Fe\(^{III}\) complex was obtained following column chromatography, namely [Fe(PCSMC)(PSMC)]. The ligand dianion PSMC\(^{2−}\) (Chart 2) is
effectively a hydroxylated derivative of PCSMC\(^{-}\). The Fe\(^{III}\) complex, [Fe(PCSMC)(PSMC)] exhibited some characteristic spectroscopic features, including an intense C==O vibrational band in the IR spectrum (1583 cm\(^{-1}\)), which was absent in all other IR spectra reported here.

The structure of the complex was confirmed by X-ray crystallography. A view of the neutral complex [Fe(PCSMC)(PSMC)] is shown in Fig. 5. The presence of O1a on the PSMC\(^{2–}\) ligand is apparent. This has some important structural influences on the complex and particularly on the PSCSMC\(^{−}\) co-ligand. The Fe–N2b and Fe–S1b bonds lengthen considerably in comparison with the symmetrical [Fe(PCSMC)(PSMC)]\(^{−}\) and [Fe(PCSMC)(PSMC)]\(^{2−}\) complexes. This may be partially attributed to a trans influence of the dianionic PSMC\(^{2−}\) ligand. The Fe–N2b bond (from PSCSMC\(^{−}\)) is trans to the Fe–N2a bond (from PSCSMC\(^{2−}\)) where N2a formally carries a negative charge due to amide deprotonation. The extension of the Fe–S1b bond may be a consequence of Fe–N1b elongation as both donor atoms are part of the same rigid chelate ring. Another structural feature is the change in bond order of C6–N2 from a formal (imine) double bond in the dithiocarbazates (Table 2), to an intermediate bond order closer to one (C6a–N2a 1.38(1) Å) characteristic of a deprotonated amide in PSCSMC\(^{2−}\).

Ligand hydroxylation of this kind is unusual but not without precedent. Our previous work has uncovered other Schiff base ligands derived from 2-pyridinecarbaldehyde which undergo Fe\(^{III}\) catalysed oxidation reactions\(^{34,54}\) leading to hydrazine (as opposed to hydrazone) analogues (the H\(_2\)IPH analogues, Chart 1). The mechanism of this reaction is very complicated\(^{39}\) but nucleophilic attack by water on the C6–N2 double bond followed by ligand dehydrogenation are involved. The oxidant is Fe\(^{II}\) and not dioxygen. The properties of Fe complexes from the H\(_2\)IPH series are very different, and in fact, only Fe\(^{II}\) complexes have been isolated in contrast to the Fe\(^{III}\) complexes from the corresponding HPC\(_4\)IH series.

**Redox properties**

The Fe\(^{III/II}\) redox potentials are an important determinant of biological activity and affinity for Fe in each of its oxidation states. Complexes with exceptionally high redox potentials will be most stable in the Fe\(^{II}\) form under biological conditions, while those with low (large negative) redox potentials will be spontaneously oxidised to Fe\(^{III}\). Between these extremes, both Fe\(^{II}\) and Fe\(^{III}\) may co-exist and interconvert. This is germane in a cellular environment due to the ability of redox-active Fe complexes to reduce dioxygen in the presence of hydrogen peroxide. For eqn (1) to be catalytic, the Fe\(^{III}\) complex must be able to be reduced by intracellular reductants (eqn (2)) so the rate of both eqn (1) and (2) will be important in determining the overall yield of \(\cdot\)OH. The higher potential Fe\(^{III}\) complexes will be less reactive toward \(\cdot\)OH but more easily reduced from their reactive Fe\(^{III}\) form.

Cyclic voltammetry of the Fe complexes of the dithio carbazate ligands was undertaken and the results are summarised in Fig. 6 and Table 3. In all cases the Fe\(^{II}\) and Fe\(^{III}\) dithiocarbamate complexes gave identical and totally reversible cyclic voltammograms confirming their common structural features (identical first coordination spheres). Only the voltammograms of the Fe\(^{III}\) complexes are shown in Fig. 6. Any differences in redox potentials are attributed to changes in inductive effects of the substituents. It is immediately apparent that altering the terminal S-substituent (from methyl to benzyl) has little effect (ca.

![Fig. 6](image-url) Cyclic voltammetry of the Fe dithiocarbamate complexes in this work: sweep rate 100 mV s\(^{-1}\), MeCN–H\(_2\)O 7:3 solvent and 0.1 M Et\(_4\)NClO\(_4\) supporting electrolyte.

<table>
<thead>
<tr>
<th>Fe(^{III/II}) redox potentials and biological data for the chelators in this work</th>
<th>(E^o(\text{Fe}^{III/II})) (mV vs. NHE)</th>
<th>IC(_{50}) (μM)</th>
<th>(^{59}\text{Fe efflux} (% \text{ control}))</th>
<th>(^{59}\text{Fe uptake} (% \text{ control}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>4 ± 0.5</td>
<td>100 ± 4</td>
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<tr>
<td>DFO</td>
<td>—</td>
<td>11.4 ± 0.8</td>
<td>10 ± 2</td>
<td>86 ± 7</td>
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<tr>
<td>H(_2)NH</td>
<td>—</td>
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<td>40 ± 3</td>
<td>7 ± 1</td>
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<tr>
<td>Hdp44mT</td>
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<td>0.009 ± 0.004</td>
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</table>

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10 mV) on the FeIII/II redox potential. This is to be expected given the fact that substitution of a phenyl ring with a H-atom occurs at a position remote from the metal. The substituent on atom C6 (as defined in Fig. 1) has a stronger influence on the redox potential, as found in our investigations of the HPCIH17 and HDp44mT30 families and their Fe complexes. The electron-withdrawing non-coordinating pyridyl ring (in PKSMC− and PKSBC−) yields Fe complexes with the highest redox potentials of the series. The electron-donating methyl group gave lower potential Fe complexes of APSMC− and APSBC−. These potentials were born out in the properties of the FeIII and FeII complexes; [FeII(APSMC)2] and [Fe(APSBC)2] being air-sensitive in solution upon standing.

The thiosemicarbazone complex [Fe(Dp44mT)2]+ (see Chart 1 for ligand structure) exhibits a redox potential (+166 mV) 28 more than 200 mV below the analogous thiocarbazate complexes [Fe(PKSMC)2]+ and [Fe(PKSBC)2]+; all three compounds being of Schiff bases derived from di-2-pyridyl ketone. The thiohydrazone analogue [Fe(PKTBH)2]+ (see Chart 1) has a FeIII/II redox potential of +383 mV vs. NHE27 which is essentially the same as that of the thiocarbazate complex analogues reported here. Replacing the thiohydrazone S-donor (e.g. [Fe(PKTBH)2]1+) with an O-donor ([Fe(PKBH)2]1+) a hydrazone shifts the FeIII/II couple to a very high potential (ca. +500 mV) and in fact the highly oxidising FeIII complexes of these and related hydrazone ligands are not stable in aqueous solution.16–18

The redox potential of the unusual mixed-ligand complex [Fe(PSMC)(PCSMC)] was much lower than the symmetric bis-dithiocarbazate complexes. The voltammetry at three different sweep rates is shown in Fig. 7. Again a totally reversible FeIII/II couple is seen, but this time at a potential of −100 mV vs. NHE; approximately 450 mV lower than the related [Fe(PCSMC)2]+ complex. This large stabilisation of the trivalent oxidation state is a consequence of the PSMC2− ligand which bears two formally anionic donor atoms; the deprotonated amide N2a (Fig. 5) and the thiolate S-donor. This cathodic shift mirrors the behaviour seen in the related hydrazone (HPCIH)16 to hydrazine (H2IPH) series, 57 where the FeIII/II redox potential of [FeIII(IPH)2]− is ca. 750 mV lower than [FeII(PCHI)2]. In the case of [FeIII(PSMC)(PCSMC)], only one of the dithiocarbazate Schiff base ligands has been altered, so the effect on the redox potential is not as pronounced.

Fe mobilisation studies and cytotoxicity

The ability of the dithiocarbazates in Chart 2 to bind intracellular 59Fe was examined in two separate, but complementary assays using 59Fe2-Tf which donates 59Fe to cells.46,47 The ability of the chelators to mobilise cellular 59Fe (Fig. 8A) from human SK-N-MC neuroepithelioma cells pre-labelled with 59Fe2-Tf was expressed as the percentage of 59Fe released in the presence of each ligand. During this assay, the chelators act as “shuttles” to mobilise 59Fe out of the cell. As expected, the control (medium alone with no chelator) resulted in only a small amount (∼4%) of 59Fe being released from the cell over the time course of the experiment. Positive controls were the known Fe chelators, DFO, HDp44mT and H2NIH (311; Chart 1).19,46,47 All dithiocarbazate ligands were more effective than DFO at releasing intracellular Fe and HPCSMC was the most active of all compounds being almost as effective as HDp44mT (Fig. 8A). Furthermore, the three S-methyl dithiocarbazates (HAPSMC, HPCSMC and

Fig. 7 Cyclic voltammetry of [Fe(PSMC)(PCSMC)] at 20, 50 and 100 mV s−1 sweep rate: MeCN–H2O (7 : 3) with 0.1 M Et4NClO4 supporting electrolyte.

Fig. 8 The effect of the dithiocarbazate chelators on (A) cellular 59Fe release from pre-labelled SK-N-MC neuroepithelioma cells and (B) 59Fe uptake from 59Fe2-Tf by SK-N-MC neuroepithelioma cells. The control is treated with medium only. Results are mean ± SD (3 experiments). See Charts 1 and 2 for ligand abbreviations.
HPKSMC) were more effective at mobilising intracellular $^{59}$Fe than their corresponding S-benzyl analogues (HAPSBC, HPCSBc and HPKSBc, respectively).

During the second assay (Fig. 8B), the ability of the chelators to inhibit $^{59}$Fe uptake from $^{59}$Fe$_2$-Tf was assessed. All results are relative to the untreated control (i.e. $^{59}$Fe in the presence of incubation medium alone). The results mirror those obtained with the $^{59}$Fe efflux assay. The three S-methyl dithiocarbazates are consistently more effective at preventing $^{59}$Fe uptake from $^{59}$Fe$_2$-Tf than the three S-benzyl chelators. Again, the novel chelators prepared in this investigation were all more active than DFO, but less effective than H$_2$NH (311) or HDp44mT (Fig. 8B).

Potential chelators for the treatment of Fe overload disorders, must have low toxicity as the condition will in many cases require life-long administration of the drug. Thus, the toxicity of the chelators was examined against the same SK-N-MC neuroepithelioma cell line using DFO as a negative control and the highly potent ligand, HDp44mT, as a positive control. The results are summarised in Table 3, where it can be seen that the two dithiocarbazates derived from 2-pyridinecarbaldehyde exhibit no apparent cytotoxicity (~10 μM). This property, in combination with their high activity in sequestering intracellular Fe, present ideal properties for a chelator in the treatment of Fe overload. The four dithiocarbazates derived from 2-acetylpyridine and di-2-pyridyl ketone show moderate to potent anti-proliferative activity, and as such, may be problematic as useful drugs for the treatment of Fe overload. However, anti-proliferative activity can be advantageous in the treatment of cancer if a large therapeutic index (anti-proliferative activity against cancer cells relative to normal cells) can be defined, and the thiosemicarbazone relatives are an example of this. This aspect is the subject of a separate investigation.

Conclusions

This study has focused on the Fe coordination chemistry of a series of dithiocarbazate Schiff base ligands. Both Fe$^{11}$ and Fe$^{11}$ complexes were isolated and characterised. Cyclic voltammetry showed that all undergo totally reversible redox reactions at potentials dependent on substrate effects inherent to their parent carbonyl compound (2-acetylpyridine, di-2-pyridyl ketone or 2-pyridinecarbaldehyde). All ligands were more effective in chelating intracellular Fe relative to the clinically proven chelator DFO, and the compounds derived from 2-pyridinecarbaldehyde exhibited no marked anti-proliferative activity. The results reported here mirror the trends observed with the HPCIH, HPKIH and HAPIH hydrazones. In those cases, Schiff bases derived from aldehydes (bearing a H-atom at C6) exhibited no pronounced cytotoxicity in contrast to ketone-derived Schiff bases bearing a methyl, phenyl or pyridyl group at C6 which all exhibited significant anti-proliferative efficacy against cancer cells. Exactly the same trend is seen here, suggesting that the 2-pyridinecarbaldehyde group is an ideal pharmacophore for an Fe chelator with low toxicity as seen in the HPCIH analogues.

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