Electronic Supplementary Information (ESI)

**Disulfide/thiol switches in thiosemicarbazone ligands for redox-directed iron chelation**

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**General methods and materials.** All reactions were carried out under an inert (N\(_2\) or Ar) atmosphere using dry solvents unless otherwise noted. Tetrahydrofuran (THF) and dichloromethane (CH\(_2\)Cl\(_2\)) were dried by passage through a Vacuum Atmospheres solvent purifier. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (aluminum-backed, 60 W F\(_{254}\), EMD Millipore). 2,2'-Dithiodibenzaldehyde was prepared from 2-mercaptobenzaldehyde as previously reported.\(^1\)\(^2\) All other reagents were obtained commercially and used as received.

**Physical measurements.** \(^1\)H NMR and \(^{13}\)C NMR spectra were recorded at the University of Arizona NMR Facility on Bruker DRX-600, Bruker DRX-500, or Bruker DRX-400 instruments and calibrated using residual undeuterated solvent as an internal reference. High-resolution mass spectra were acquired at the University of Arizona Mass Spectrometry Facility. Elemental analyses were performed by ALS Environmental, Tucson Laboratories. Cyclic voltammograms (CV) were carried out with a Gamry Reference 600 potentiostat employing a glassy carbon working electrode, platinum wire auxiliary electrode and Ag/AgNO\(_3\) reference electrode (0.01 M AgNO\(_3\), 0.1 M (n-Bu\(_4\)N)(PF\(_6\)) triply recrystallized) as an auxiliary electrolyte. Sample concentrations were 1–2 mM. All reported voltammograms were run at a scan rate of 100 mV/s. All electrochemical data were referenced internally to the ferrocene/ferrocenium couple at 0.00 V. UV/Vis spectra were recorded on an Agilent 8453 UV/Vis spectrophotometer. Stock solutions were freshly prepared in DMF containing 0.1 M (n-Bu\(_4\)N)(PF\(_6\)) (triply recrystallized) as an auxiliary electrolyte. Sample concentrations were 1–3 µL were added to 3 mL of buffered aqueous solutions (100 mM KCl, 50 mM PIPES (1,4-piperazinediethanesulfonic acid), pH 7.5). The buffered solutions were degassed prior to use. Solution magnetic moments were measured by the Evans method\(^3\) using reported diamagnetic corrections.\(^4\) A solution of each paramagnetic complex (in the range of 5–15 mg/mL in acetone-d\(_6\) for complexes 3a and 3c and in DMSO-d\(_6\) for complex 3b) was transferred into a 5-mm NMR tube and a Wilmad\(^\text{®}\) coaxial insert filled with the deuterated solvent was placed inside as an internal reference. Solution magnetic susceptibilities were calculated based on the difference in chemical shift for the residual \(^1\)H NMR resonance of the methyl group (of either acetone or DMSO) in neat solvent and in the solution containing the paramagnetic species.

**Synthesis of disulfides 1a-c**

![Chemical structure](image)

Procedures were adapted from previously reported syntheses of thiosemicarbazone ligands.\(^5\) 2, 2'-Dithiodibenzaldehyde (0.50 g, 1.82 mmol) was suspended in ethanol (85 mL) and the appropriate thiosemicarbazide (3.65 mmol, 2 equiv) was added. The reaction mixture was refluxed for 3 h and then allowed to cool to room temperature. Following reduction of the volume to approximately 15 mL by rotary evaporation, a yellowish-green solid was collected by filtration and washed multiple times with CH\(_3\)CN. Drying overnight under vacuum over CaCl\(_2\) gave a free-flowing powder, which could be used without further purification.
• Disulfide 1a (from thiosemicarbazide, 0.332 g, 3.65 mmol)

0.580 g, 76%, mp 232-233 °C

\(^1\)H NMR (500 MHz, DMSO-d\(_6\)) \(\delta\) 11.63 (s, 2H), 8.55 (s, 2H), 8.33–8.29 (m, 2H), 8.09–8.03 (m, 2H), 7.84–7.78 (m, 2H), 7.61–7.55 (m, 2H), 7.41–7.32 (os, 4H)

\(^{13}\)C NMR (125 MHz, DMSO-d\(_6\)) \(\delta\) 177.97, 139.97, 134.67, 133.54, 130.15, 129.98, 128.31, 128.17

HRMS m/z (MH\(^+\)) calculated for C\(_{16}\)H\(_{17}\)N\(_6\)S\(_4\), 421.03885; found, 421.03885

• Disulfide 1b (from 4-methyl-3-thiosemicarbazide, 0.383 g, 3.65 mmol)

0.650 g, 79%, mp 224-226 °C

\(^1\)H NMR (500 MHz, DMSO-d\(_6\)) \(\delta\) 11.63 (s, 2H), 8.56 (s, 2H), 8.43 (q, J = 4.5 Hz, 2H), 8.15–8.09 (m, 2H), 7.56–7.53 (m, 2H), 7.41–7.33 (os, 4H), 3.03 (d, J = 4.6 Hz, 6H)

\(^{13}\)C NMR (125 MHz, DMSO-d\(_6\)) \(\delta\) 177.67, 139.04, 134.73, 134.22, 131.14, 130.06, 128.54, 127.48, 30.90

HRMS m/z (MH\(^+\)) calculated for C\(_{18}\)H\(_{21}\)N\(_6\)S\(_4\), 449.07101; found, 449.07050

• Disulfide 1c (from 4-phenylthiosemicarbazide, 0.610 g, 3.65 mmol)

0.862 g, 83%, mp 206-208 °C

\(^1\)H NMR (500 MHz, DMSO-d\(_6\)) \(\delta\) 12.01 (s, 2H), 10.05 (s, 2H), 8.68 (s, 2H), 8.26 (dd, J = 6.1, 3.3 Hz, 2H), 7.60 (d, J = 7.2 Hz, 6H), 7.44–7.31 (os, 8H), 7.20 (t, J = 7.4 Hz, 2H)

\(^{13}\)C NMR (125 MHz, DMSO-d\(_6\)) \(\delta\) 175.75, 140.24, 138.83, 134.87, 133.86, 130.82, 130.39, 128.49, 128.29, 128.10, 125.25

HRMS m/z (MH\(^+\)) calculated for C\(_{28}\)H\(_{25}\)N\(_6\)S\(_4\), 573.10261; found, 573.10180

**Synthesis of thiols 2a-c**

![Synthesis of thiols 2a-c](image)

R = H, Me, Ph
The disulfide compound was added to a solution of dithiothreitol (DTT) in DMF (25 mL) to give an amber brown solution. The reaction mixture was allowed to stir overnight and then evaporated in the presence of toluene (multiple additions). The resulting solid was transferred to a fritted funnel and washed with water to remove DTT and residual DMF. Drying overnight under vacuum over CaCl$_2$ gave a free-flowing powder, which was used without further purification.

- **Thiol 2a** (from DTT, 0.293 g, 1.90 mmol and 1a, 0.400 g, 0.95 mmol)

  0.361 g, 90%, mp 166-168 °C

  $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 10.91 (s, 1H), 8.18 (s, 1H), 7.53–7.45 (m, 1H), 7.24 (os, 1H), 7.14 (os, 3H), 6.65 (s, 1H), 3.77 (s, 1H)

  (400 MHz, DMSO-d$_6$) $\delta$ 11.55 (s, 1H), 8.32 (d, J = 22.6 Hz, 2H), 7.92 (d, J = 7.7 Hz, 1H), 7.76 (s, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.20 (dt, J = 27.7, 7.1 Hz, 2H), 5.59 (s, 1H)

  $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 178.39, 142.56, 131.67, 131.21, 130.51, 130.02, 129.33, 125.21

  HRMS $m/z$ (MH$^+$) calculated for C$_8$H$_{10}$N$_3$S$_2$, 212.03084; found, 212.03107

- **Thiol 2b** (from DTT, 0.254 g, 1.64 mmol and 1b, 0.400 g, 0.82 mmol)

  0.358 g, 89%, mp 106-107 °C

  $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.58 (s, 1H), 8.07 (s, 1H), 7.56 (dd, J = 6.0, 3.2 Hz, 1H), 7.52 (s, 1H), 7.33 (dd, J = 6.1, 3.0 Hz, 1H), 7.21 (dd, J = 6.1, 3.0 Hz, 2H), 3.71 (s, 1H), 3.28 (d, J = 4.8 Hz, 3H)

  $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 178.30, 141.06, 131.90, 131.61, 130.68, 129.96, 129.93, 125.88, 31.41

  HRMS $m/z$ (MH$^+$) calculated for C$_9$H$_{12}$N$_3$S$_2$, 226.04680; found, 226.04672

- **Thiol 2c** (from DTT, 0.269 g, 1.75 mmol and 1c, 0.500 g, 0.87 mmol)

  0.477 g, 95%, mp 140-142 °C

  $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.90 (s, 1H), 9.40 (s, 1H), 8.16 (s, 1H), 7.76 (d, J = 7.9 Hz, 2H), 7.58 (dd, J = 6.2, 3.2 Hz, 1H), 7.43 (t, J = 7.7 Hz, 2H), 7.41–7.38 (dd, J = 6.2, 3.2 Hz, 1H), 7.30–7.24 (os, 3H), 3.81 (s, 1H)

  $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 141.57, 137.91, 131.91, 131.84, 130.85, 130.21, 130.07, 128.76, 125.99, 125.81, 123.87

  HRMS $m/z$ (MH$^+$) calculated for C$_{14}$H$_{14}$N$_3$S$_2$, 228.06272; found, 228.06237
Synthesis of iron complexes 3a-c

- **3a, [Fe\text{III}(2a-H)₃][BF₄]•2THF•H₂O**

  Thiol 2a (100 mg, 0.47 mmol) was dissolved in THF (100 mL) under an argon atmosphere. Upon addition of Fe(BF₄)₂•6H₂O (80 mg, 0.24 mmol) to this pale yellow solution, the reaction mixture turned dark brown. The reaction was allowed to stir for 30 min. Slow diffusion of n-pentane into the reaction mixture in THF yielded dark brown crystals within a few days. The light brown THF/pentane supernatant was cannulated out and the crystals were dried overnight under vacuum.

  0.102 g, 60%, mp 162-170 ºC, $\mu_{\text{eff}} = 1.9 \mu_B$

  HRMS $m/z$ (M⁺) calculated for C₁₆H₁₆FeN₆S₄, 475.96572; found, 475.96633

  Anal. Calcd. for C₂₄H₃₄BF₄FeN₆O₃S₄: C, 39.73; H, 4.72; N, 11.58%; found: C, 39.65; H, 4.45; N, 11.82%

- **3b, [Fe\text{III}(2b-H)₂][BF₄]•THF**

  According to the procedure above, 2b (20 mg, 89 µmol) was combined with Fe(BF₄)₂•6H₂O (15 mg, 44 µmol) in THF (20 mL) and the reaction mixture was allowed to stir for 30 min. Slow diffusion of n-pentane into the THF reaction mixture yielded dark brown crystals within a few days. Single crystals for X-ray diffraction analysis were grown in acetone/n-pentane mixtures.

  13 mg, 44%, mp 170-174 ºC, $\mu_{\text{eff}} = 1.7 \mu_B$

  HRMS $m/z$ (M⁺) calculated for C₁₈H₂₀FeN₆S₄, 503.99765; found, 503.99763

  Anal. Calcd. for C₂₂H₂₈BF₄FeN₆O₅S₄: C, 39.83; H, 4.25; N, 12.67%; found: C, 39.75; H, 4.53; N, 12.63%

- **3c, [Fe\text{III}(2c-H)₂][BF₄]•H₂O**

  According to the procedure above, 2c (20 mg, 70 µmol) was combined with Fe(BF₄)₂•6H₂O (12 mg, 35 µmol) in THF (1 mL) and the reaction mixture was allowed to stir for 30 min. After addition of CH₂Cl₂ (3 mL), the reaction mixture was layered with pentane (5 mL) and crystallization occurred overnight.

  18 mg, 70%, mp 186-188 ºC, $\mu_{\text{eff}} = 1.9 \mu_B$

  HRMS $m/z$ (M⁺) calculated for C₂₈H₂₄FeN₆S₄, 628.02844; found, 628.02893

  Anal. Calcd. for C₂₈H₂₈BF₄FeN₆O₄S₄: C, 45.85; H, 3.57; N, 11.46%; found C, 45.91; H, 3.64; N, 11.28%
**X-ray diffraction data**

Data were collected on a Bruker Kappa APEX II DUO diffractometer at the University of Arizona X-ray Diffraction Facility.

For compound 3a, crystals grew as brown/black plates during slow diffusion of n-pentane in a THF solution of the complex. Each asymmetric unit in the crystal structure contains an iron complex with a BF$_4$ counter ion and two disordered THF solvent molecules. Both THF molecules are hydrogen-bound to the thiosemicarbazone ligands. All nitrogen-bound hydrogen atoms were refined with nitrogen-hydrogen bond lengths restrained.

For compound 3b, crystals grew as brown needles during slow diffusion of hexanes in an acetone solution of the complex. Each asymmetric unit in the crystal structure contains an iron complex with a BF$_4$ counter ion and a disordered solvent pocket. The site occupancy factors for acetone and hexane in this pocket refined to a 75:25 ratio. The terminal carbon of the hexane solvent molecule lies on a special position. Hydrogen atoms have not been added to this carbon atom.

<table>
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<th>Compound reference</th>
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<th>3b</th>
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<tr>
<td>Chemical formula</td>
<td>C$<em>{16}$H$</em>{16}$FeN$_6$S$_4$•BF$_4$•2(C$_4$H$_8$O)</td>
<td>C$<em>{18}$H$</em>{20}$FeN$_6$S$_4$•BF$_4$•0.75(C$_3$H$_6$O)</td>
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<td>Final $R_I$ values ($I &gt; 2\sigma(I)$)</td>
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<td>Final $R_I$ values (all data)</td>
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APEX2 (data collection)

SAINT (integration and reduction)

SADABS (absorption correction)

SHELXTL (structure solution and refinement)

**MERCURY** (molecular graphics – hydrogen bonding and packing)


**OLEX2** (structure refinement)


**Biological studies.** Human SK-N-MC (ATCC® HTB-10™) neuroepithelioma cells, MDA-MB-231 (ATCC® HTB-26™) breast adenocarcinoma cells, and MRC-5 (ATCC® CCL-171™) normal lung fibroblasts were cultured at 37 °C under a 5% CO₂ humidified atmosphere in Eagle’s Minimal Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS), glutamine (2 mM), sodium pyruvate (1 mM), sodium bicarbonate (1.5 mg/L), penicillin (100 units/mL), and streptomycin (100 µg/mL).

For MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) viability assays, growth media were additionally supplemented with 1.25 µM human holo-transferrin (Aldrich) prior to use. Cells were seeded in 96-well plates (7 x 10⁴ cells/well) and allowed to incubate for 24 h. Stock solutions of the test compounds were freshly made in DMSO prior to use and added up to a maximum of 0.1% v/v DMSO in growth media. Stock solutions of DFO were prepared in PBS buffer. Cells were incubated in the presence of the test compounds for 48 h or 72 h and then viability was assessed by MTT addition using standard methods. Absorbance (570 nm) was determined using a BioTek Synergy™ 2 microplate reader. All experiments were performed in triplicate. Data analysis was performed using the Dose Response Analysis method of the MasterPlex ReaderFit software (Hitachi Software Engineering America Ltd., USA). Fitting procedures employed either a four- or five-parameter logistic equation and results are expressed as the mean value +/- Root Mean Square Error (RMSE) of experiments run in triplicate.

**References**

Figures

Fig. S1 $^1$H NMR spectra (500 MHz, DMSO-$d_6$) of disulfides 1a, 1b, and 1c.

Fig. S2 $^1$H NMR spectra (500 MHz, CDCl$_3$) of thiols 2a, 2b, and 2c.
**a, R = H**

![Absorbance spectrum for R = H](image)

**b, R = Me**

![Absorbance spectrum for R = Me](image)

**c, R = Ph**

![Absorbance spectrum for R = Ph](image)

**Fig. S3** UV-visible spectra of disulfides 1a-c before (black) or after (blue) addition of 1 equiv of Fe(BF$_4$)$_2$, and of thiols 2a-c before (green) or after (orange) addition of 0.5 equiv of Fe(BF$_4$)$_2$ in aqueous solutions (100 mM KCl, 50 mM PIPES (1,4-piperazinediethanesulfonic acid), pH 7.5).
**Fig. S4** Crystal structure of the cation in compound 3b showing a partial labeling scheme. The BF$_4^-$ counterion, as well as disordered acetone and hexane molecules, are not shown. Thermal ellipsoids are scaled to the 50% probability level. CCDC 915454

**Fig. S5** Cyclic voltammograms of iron complexes 3a-c at a glassy carbon electrode in DMF with (n-Bu$_4$N)(PF$_6$) as a supporting electrolyte. Data collected at a 100 mV/s sweep rate using a Ag/AgNO$_3$ reference electrode and a platinum wire auxiliary electrode.
Table S1  Antiproliferative activity of pro-chelator \textit{1c} and chelator \textit{2c} in MRC-5 (normal lung fibroblast) cell cultures (IC$_{50}$ values from MTT assays after exposure to tested compounds for 48 h or 72 h).

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<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
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<td>MRC-5, 48 h</td>
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<td>1c</td>
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<td>2c</td>
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