Electronic Supplementary Material (ESI) for Dalton Transactions

Electronic Supplementary Information (ESI)

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

Tsuhen M. Chang and Elisa Tomat*

University of Arizona, Department of Chemistry and Biochemistry, 1306 E. University Blvd., Tucson AZ 85721-0041, USA

Email: *tomat@email.arizona.edu*

Contents

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_2Cl_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}s, 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.

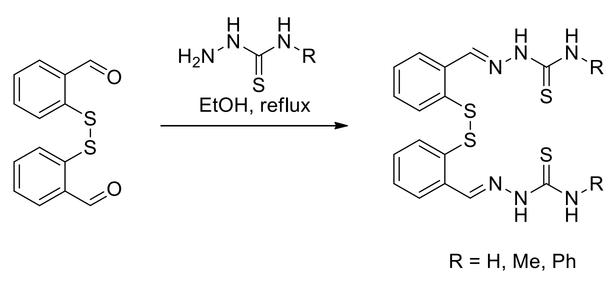
Physical measurements. ^{1}H NMR and ^{13}C NMR spectra were recorded at the University of Arizona NMR Facility on Bruker DRX-600, Bruker DRX-500, or Bruker DRX-400 instruments and calibrated using residual undeuterated solvent as an internal reference. High-resolution mass spectra were acquired at the 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_4N)(PF_6)). Measurements were conducted at ambient temperature under an argon atmosphere in DMF containing 0.1 M (n-Bu_4N)(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 DMSO and 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® 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



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_3CN. 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 ºC

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 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$_6$) δ 177.97, 139.97, 134.67, 133.54, 130.15, 129.98, 128.31, 128.17

HRMS *m/z* (MH$^+$) calculated for $C_{16}H_{17}N_6S_4$, 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 ºC

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 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$_6$) δ 177.67, 139.04, 134.73, 134.22, 131.14, 130.06, 128.54, 127.48, 30.90

HRMS *m/z* (MH$^+$) calculated for $C_{18}H_{21}N_6S_4$, 449.07101; found, 449.07050

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

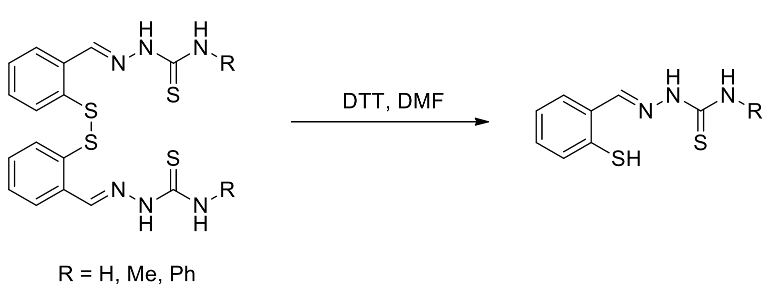
0.862 g, 83%, mp 206-208 ºC

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 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$_6$) δ 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 $C_{28}H_{25}N_6S_4$, 573.10261; found, 573.10180

Synthesis of thiols 2a-c



The disulfide compound was added to a solution of dithiothreitol (DTT) in DMF (25 mL) to the 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 ºC

^{1}H NMR (500 MHz, $CDCl_3$) δ 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) δ 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, $CDCl_3$) δ 178.39, 142.56, 131.67, 131.21, 130.51, 130.02, 129.33, 125.21

HRMS *m/z* (MH^+) calculated for $C_8H_{10}N_3S_2$, 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 ºC

^{1}H NMR (500 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 178.30, 141.06, 131.90, 131.61, 130.68, 129.96, 129.93, 125.88, 31.41

HRMS *m/z* (MH^+) calculated for $C_9H_{12}N_3S_2$, 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 ºC

^{1}H NMR (500 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{14}H_{14}N_3S_2$, 228.06272; found, 228.06237

Synthesis of iron complexes 3a-c

• **3a**, **[Fe^{III}(2a-H)$_2$][BF$_4$]•2THF•H$_2$O**

Thiol **2a** (100 mg, 0.47 mmol) was dissolved in THF (100 mL) under an argon atmosphere. Upon addition of $Fe(BF_4)_2$•$6H_2O$ (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, μ_{eff} = 1.9 μ_B

HRMS *m/z* (M^+) calculated for $C_{16}H_{16}FeN_6S_4$, 475.96572; found, 475.96633

Anal. Calcd. for $C_{24}H_{34}BF_4FeN_6O_3S_4$: C, 39.73; H, 4.72; N, 11.58%; found: C, 39.65; H, 4.45; N, 11.82%

• **3b**, **[Fe^{III}(2b-H)$_2$][BF$_4$]•THF**

According to the procedure above, **2b** (20 mg, 89 µmol) was combined with $Fe(BF_4)_2$•$6H_2O$ (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, μ_{eff} = 1.7 μ_B

HRMS *m/z* (M^+) calculated for $C_{18}H_{20}FeN_6S_4$, 503.99765; found, 503.99763

Anal. Calcd. for $C_{22}H_{28}BF_4FeN_6OS_4$: C, 39.83; H, 4.25; N, 12.67%; found: C, 39.75; H, 4.53; N, 12.63%

• **3c**, **[Fe^{III}(2c-H)$_2$][BF$_4$]•H$_2$O**

According to the procedure above, **2c** (20 mg, 70 µmol) was combined with $Fe(BF_4)_2$•$6H_2O$ (12 mg, 35 µmol) in THF (1 mL) and the reaction mixture was allowed to stir for 30 min. After addition of CH_2Cl_2 (3 mL), the reaction mixture was layered with pentane (5 mL) and crystallization occurred overnight.

18 mg, 70%, mp 186-188 ºC, μ_{eff} = 1.9 μ_B

HRMS *m/z* (M^+) calculated for $C_{28}H_{24}FeN_6S_4$, 628.02844; found, 628.02893

Anal. Calcd. for $C_{28}H_{26}BF_4FeN_6OS_4$: 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.

Compound reference	**3a**	**3b**
Chemical formula	$C_{16}H_{16}FeN_6S_4{\bullet}BF_4{\bullet}2(C_4H_8O)$	$C_{18}H_{20}FeN_6S_4{\bullet}BF_4{\bullet}0.75(C_3H_6O)$
Formula Mass	707.46	650.39
Crystal system	monoclinic	orthorhombic
a/Å	10.8891(8)	18.6075(8)
b/Å	10.8996(7)	15.6202(7)
c/Å	25.8263(19)	19.2840(8)
α/°	90.00	90
β/°	94.284(3)	90
γ/°	90.00	90
Unit cell volume/Å^3	3056.7(4)	5604.9(4)
Temperature/K	100(2)	100(2)
Space group	$P2_1/n$	Pbca
No. of formula units per unit cell,	4	8
No. of reflections measured	25909	49096
No. of independent reflections	5387	5132
R_{int}	0.0594	0.0514
Final R_1 values ($I > 2\sigma(I)$)	0.0382	0.0299
Final $wR(F^2)$ values ($I > 2\sigma(I)$)	0.1040	0.0689
Final R_1 values (all data)	0.0669	0.0480
Final $wR(F^2)$ values (all data)	0.1266	0.0799
CCDC No.	915453	915454

APEX2 (data collection)
Bruker (2007). *APEX2*. Bruker AXS Inc., Madison, Wisconsin, USA.
SAINT (integration and reduction)
Bruker (2007). *SAINT*. Bruker AXS Inc., Madison, Wisconsin, USA.
SADABS (absorption correction)
Sheldrick, G. M. (1996). SADABS. University of Göttingen, Germany.
SHELXTL (structure solution and refinement)

Sheldrick, G. M. (2008). *Acta Cryst.* **A64**, 112–122.
MERCURY (molecular graphics – hydrogen bonding and packing)
Macrae, C. F., Bruno, I. J., Chisholm, J. A., Edgington, P. R., McCabe, P. Pidcock, E., Rodriguez-Monge, L. Taylor, R., van de Streek, J. & Wood, P. A. (2008). *J. Appl. Cryst.* **41**, 466-470.
OLEX2 (structure refinement)
Dolomanov, O.V., Bourhis, L.J., Gildea, R.J., Howard, J.A.K., Puschmann H. (2008), *J. Appl. Cryst.* **42**, 339-341.

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% CO_2 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 10^4 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.[6] 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

1. Kasmai, H.; Mischke, S. *Synthesis* **1989**, 763-765.
2. See Supporting Information in: Roberts, C.; Hartley, R.C. *J. Org. Chem*. **2004**, *69*, 6145-6148.
3. (a) Evans, D. F. *J. Chem. Soc.* **1959,** 2003-2005; (b) Schubert, E.M. *J. Chem. Ed.* **1992**, *69*, 62.
4. Bain, G.A.; Berry, J.F. *J. Chem. Ed.* **2008**, *85*, 532-536.
5. Stelzig, L.; Kötte, S.; Krebs, B., *J. Chem. Soc., Dalton Trans*. **1998**, 2921-2926.
6. Richardson, D.R.; Tran, E.H.; Ponka, P. *Blood* **1995**, *86*, 4295-4306.

Figures

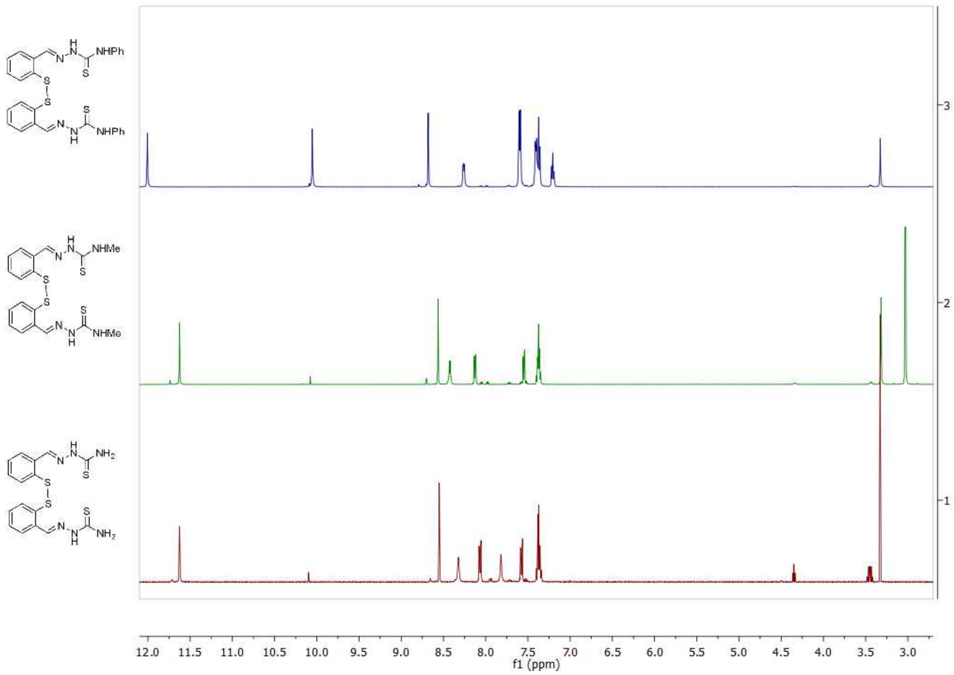


Fig. S1 ^{1}H NMR spectra (500 MHz, DMSO-d$_6$) of disulfides **1a**, **1b**, and **1c**.

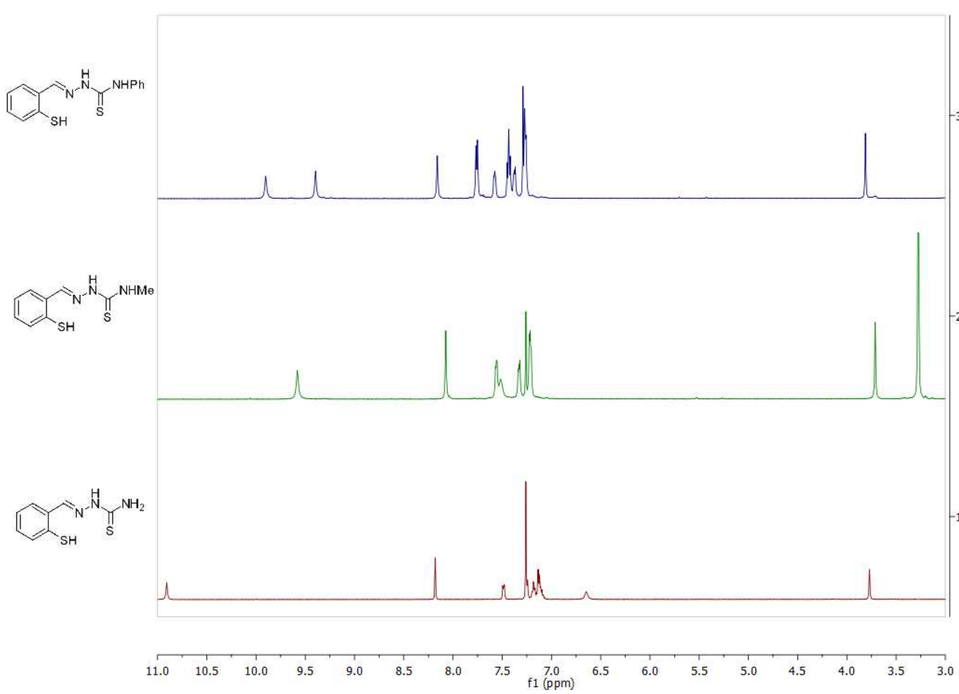
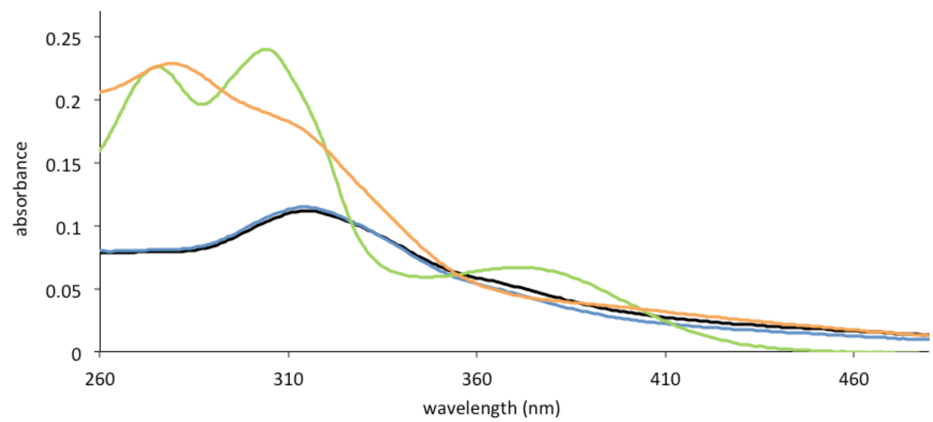
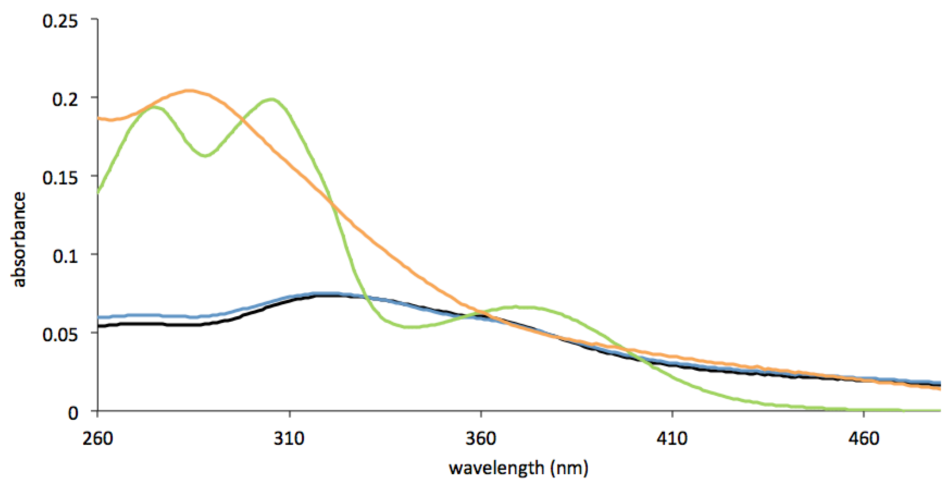


Fig. S2 ^{1}H NMR spectra (500 MHz, $CDCl_3$) of thiols **2a**, **2b**, and **2c**.

a, R = H



b, R = Me



c, R = Ph

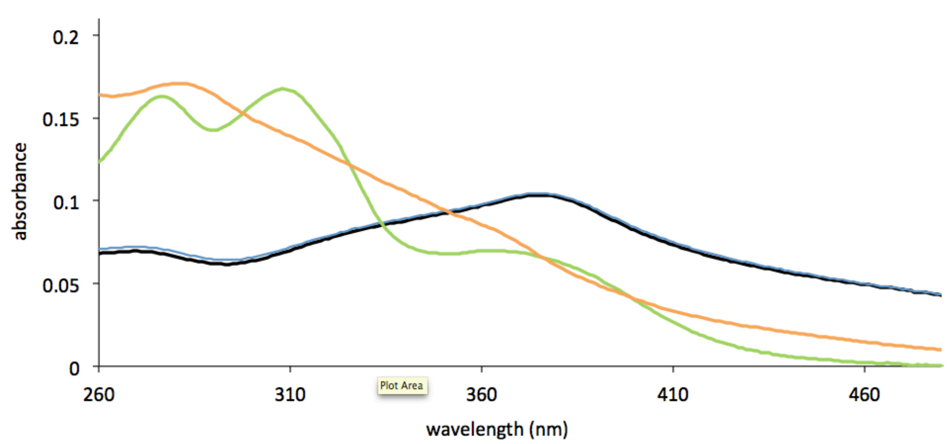


Fig. S3 UV-visible spectra of disulfides **1a-c** before (black) or after (blue) addition of 1 equiv of $Fe(BF_4)_2$, and of thiols **2a-c** before (green) or after (orange) addition of 0.5 equiv of $Fe(BF_4)_2$ in aqueous solutions (100 mM KCl, 50 mM PIPES (1,4-piperazinediethanesulfonic acid), pH 7.5).

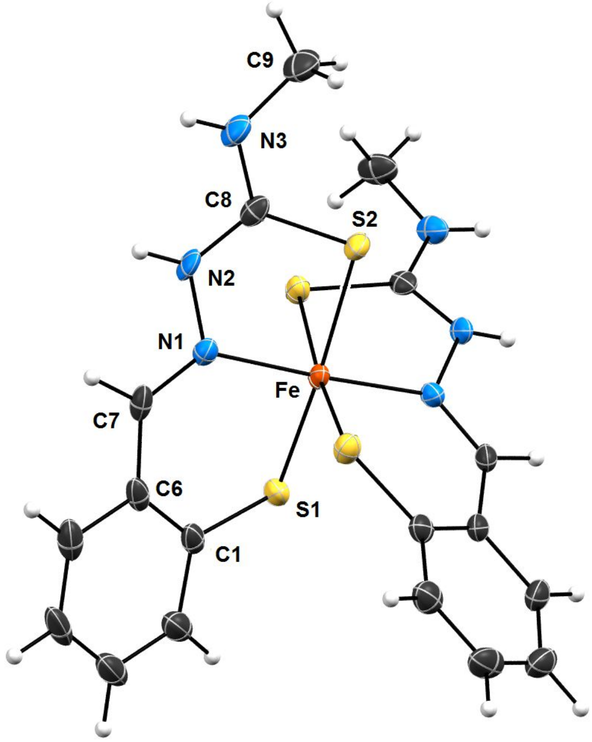


Fig. S4 Crystal structure of the cation in compound **3b** showing a partial labeling scheme. The BF_4^- counterion, as well as disordered acetone and hexane molecules, are not shown. Thermal ellipsoids are scaled to the 50% probability level. CCDC 915454

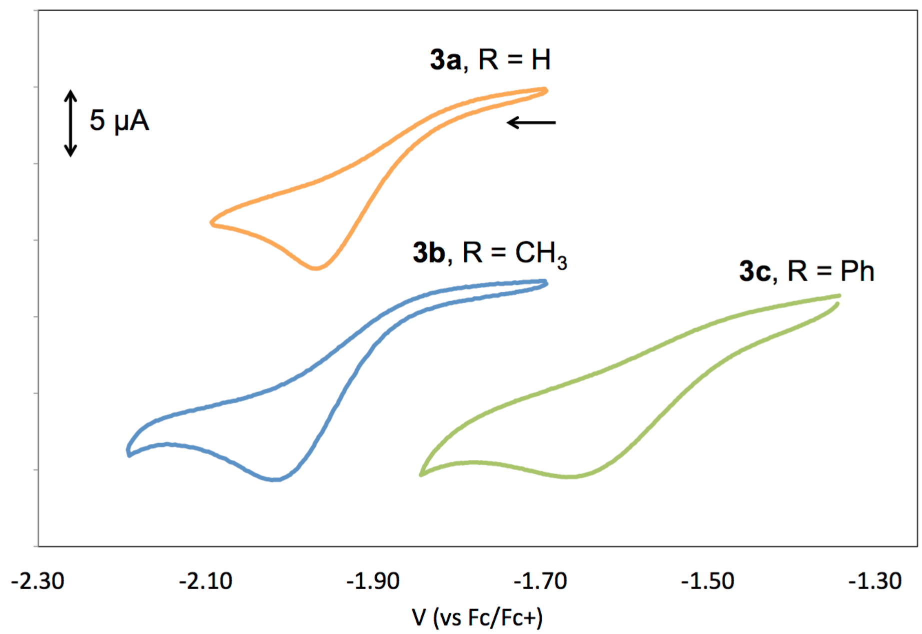


Fig. S5 Cyclic voltammograms of iron complexes **3a-c** at a glassy carbon electrode in DMF with (*n*-Bu_4N)(PF_6) as a supporting electrolyte. Data collected at a 100 mV/s sweep rate using a Ag/$AgNO_3$ reference electrode and a platinum wire auxiliary electrode.

Table S1 Antiproliferative activity of pro-chelator **1c** and chelator **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).

Compound	**IC_{50} (μM)**	
	MRC-5, 48 h	MRC-5, 72 h
DFO	25.68±0.08	8.04±0.03
1c	84.08±0.11	30.91±0.02
2c	27.86±0.02	15.08±0.02