Electronic Supplementary Information (ESI)

for

Iron(III)-binding of the anticancer agents doxorubicin and vosaroxin

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Figure S1. UV-VIS spectra of one titration run of Hvox to determine the pK_as of the test solution.

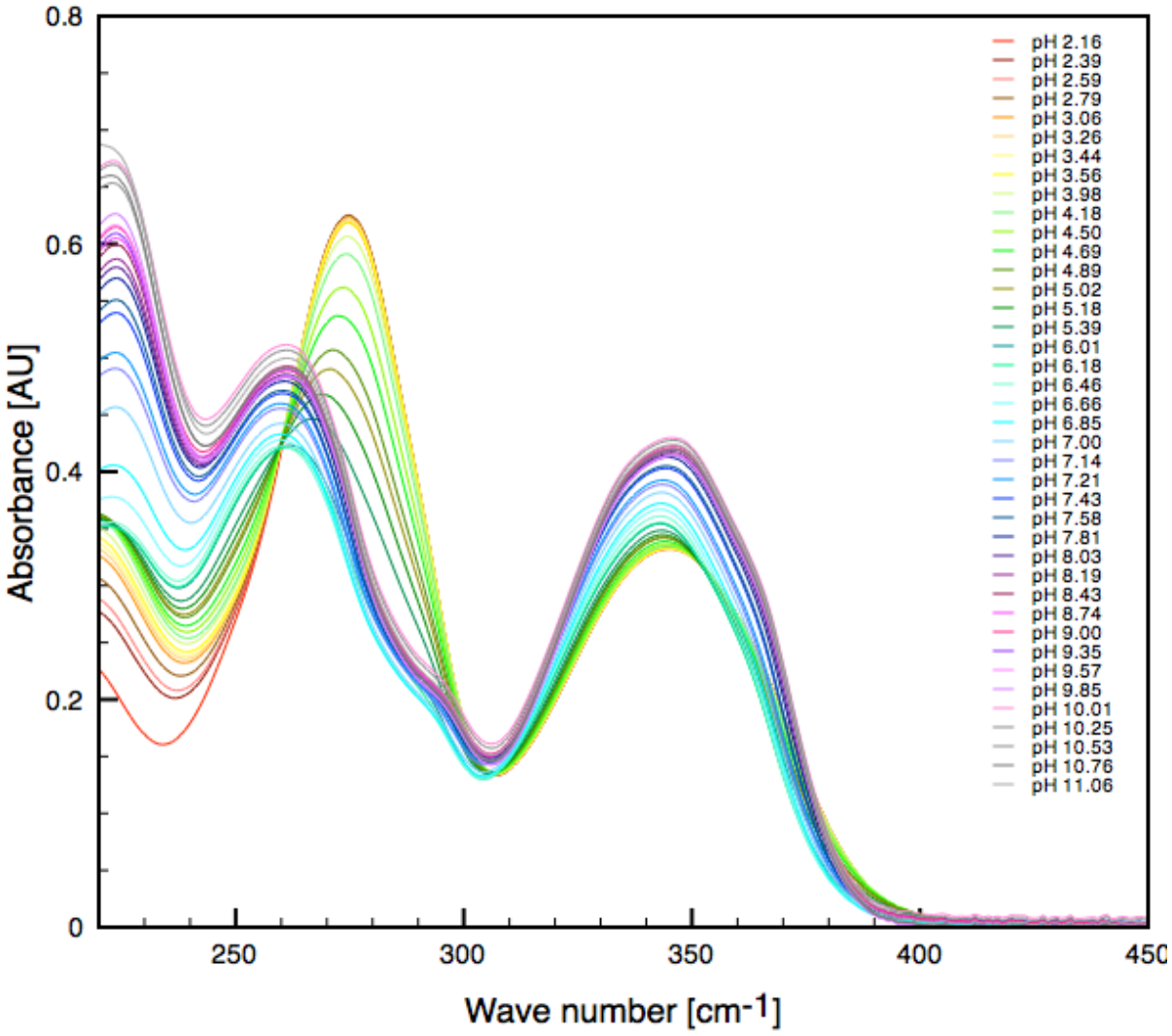


Figure S2. UV-VIS spectra of one titration run of Hvox:Fe in the ratio of 1:1.

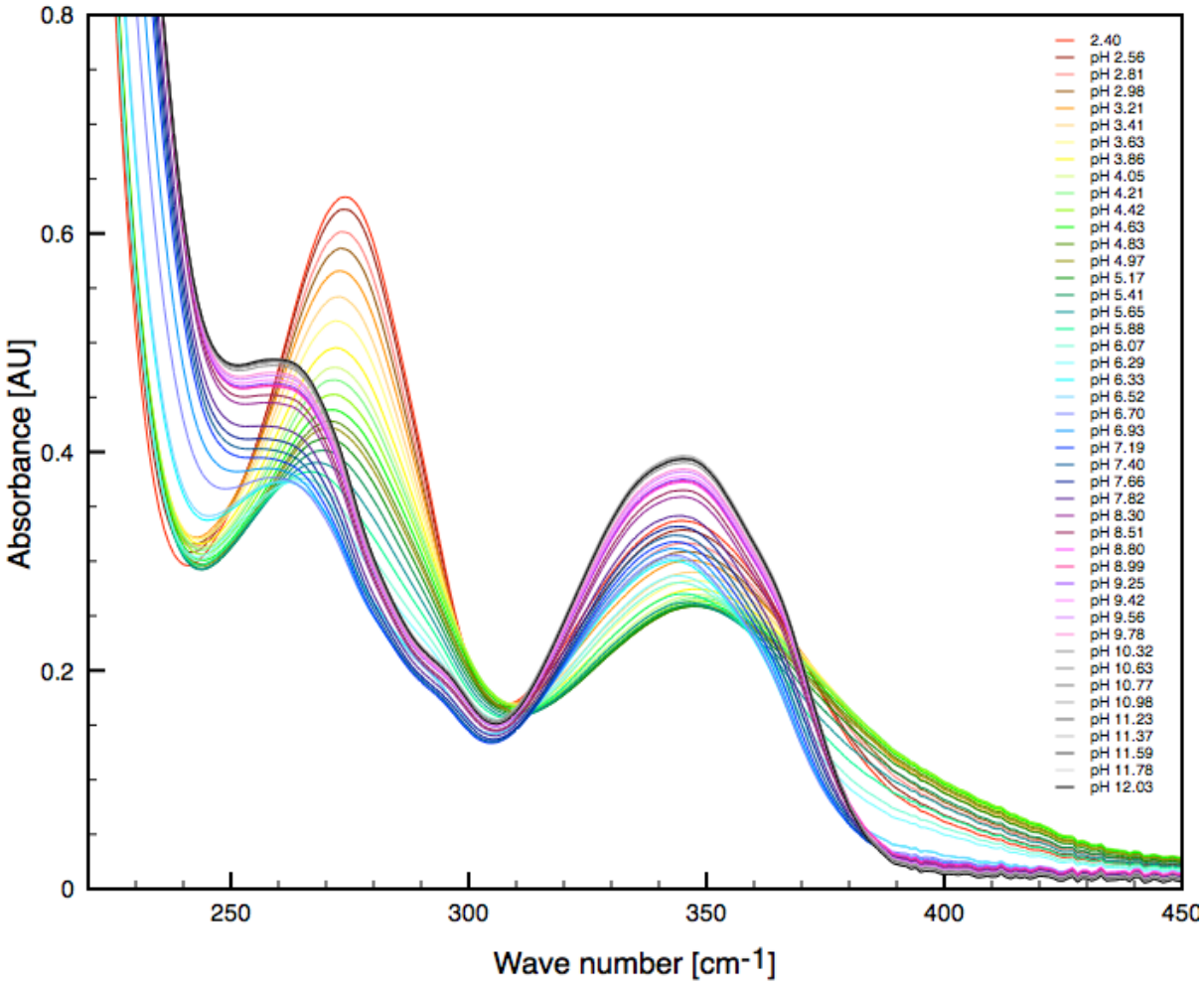


Figure S3. UV-VIS spectra of one titration run of Hvox:Fe in the ratio of 2:1.

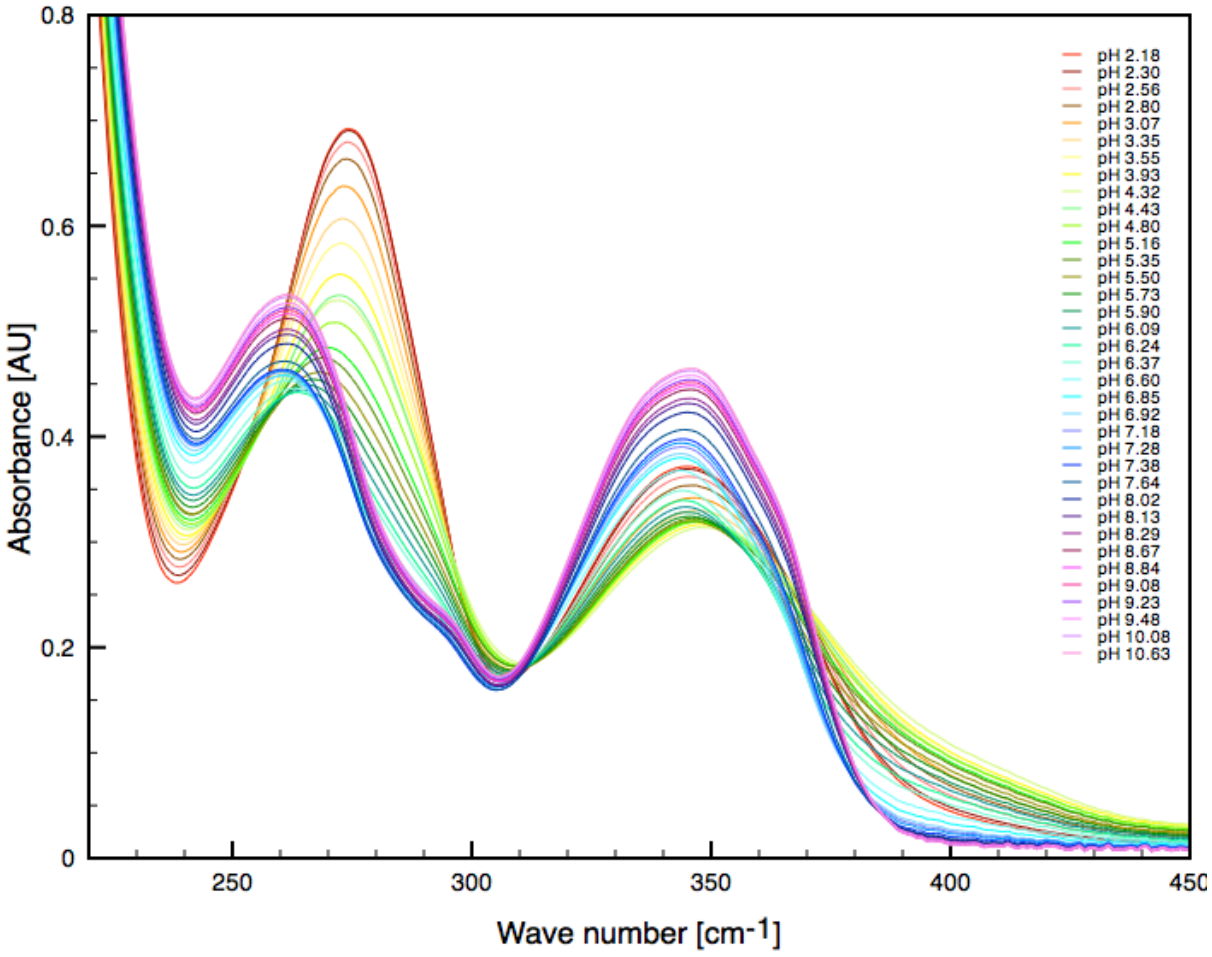


Figure S4. UV-VIS spectra of one titration run of Hvox:Fe in the ratio of 3:1.

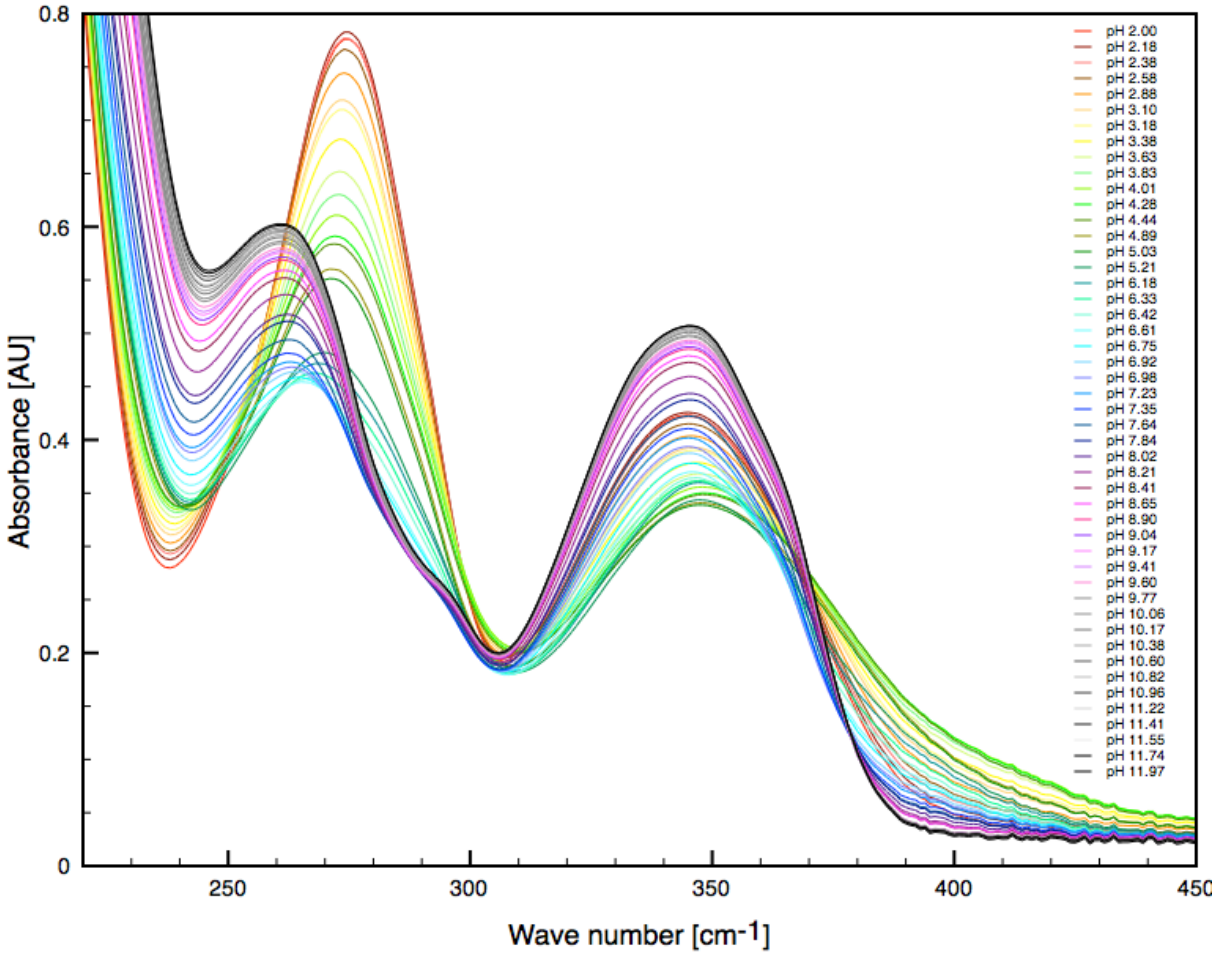


Figure S5. UV-VIS spectra of one titration run of Hdox to determine the pK_as of the test solution.

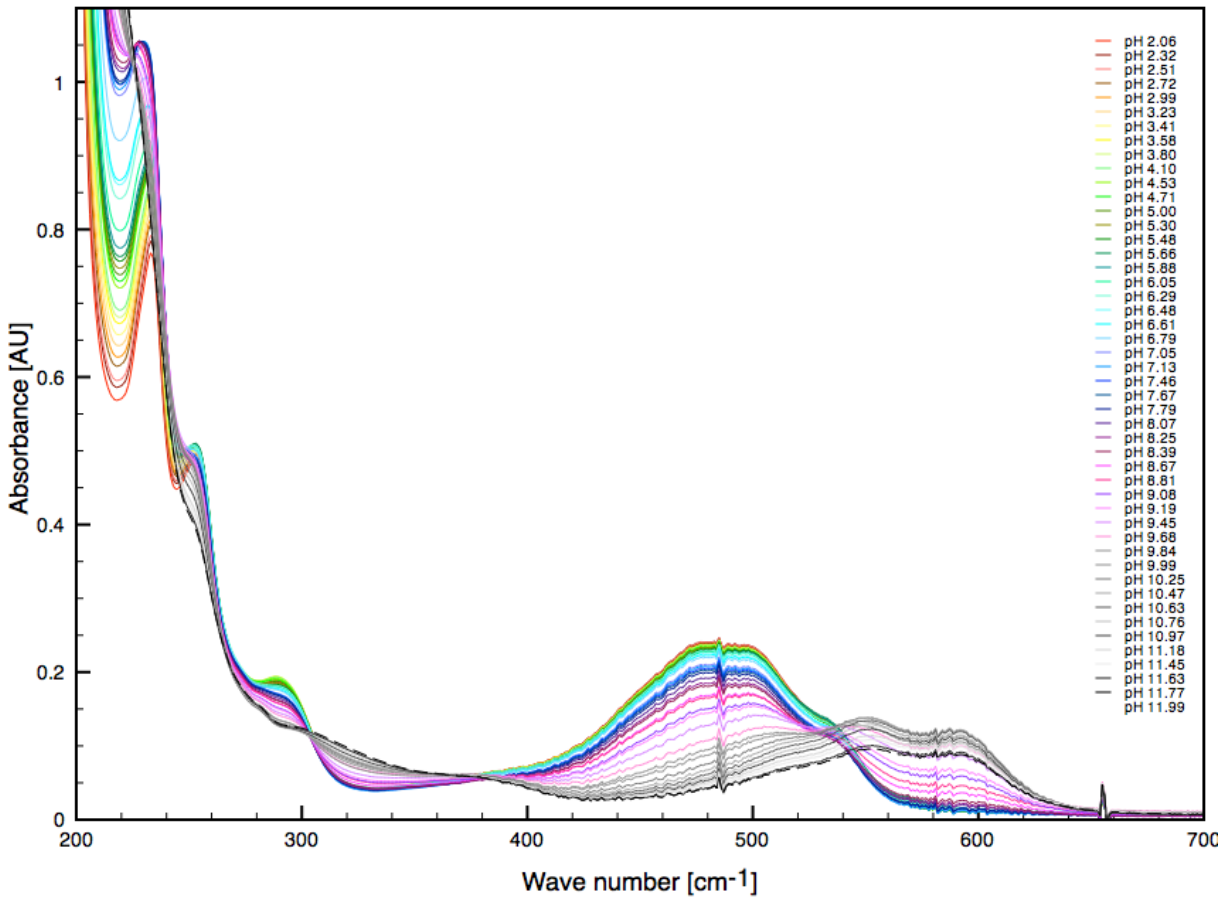


Figure S6. UV-VIS spectra of one titration run of Hdox:Fe in the ratio of 1:1.

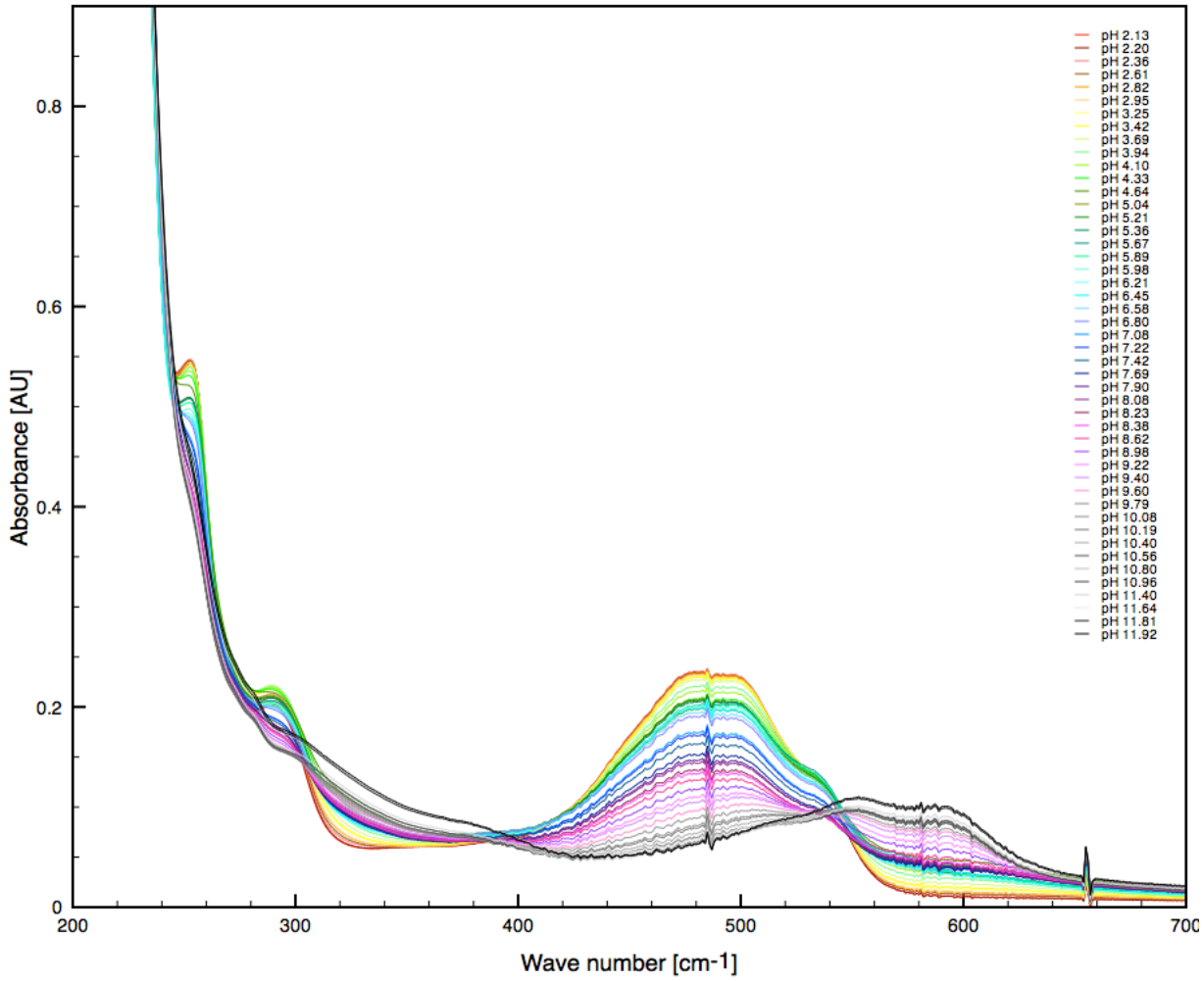


Figure S7. UV-VIS spectra of one titration run of Hdox:Fe in the ratio of 2:1.

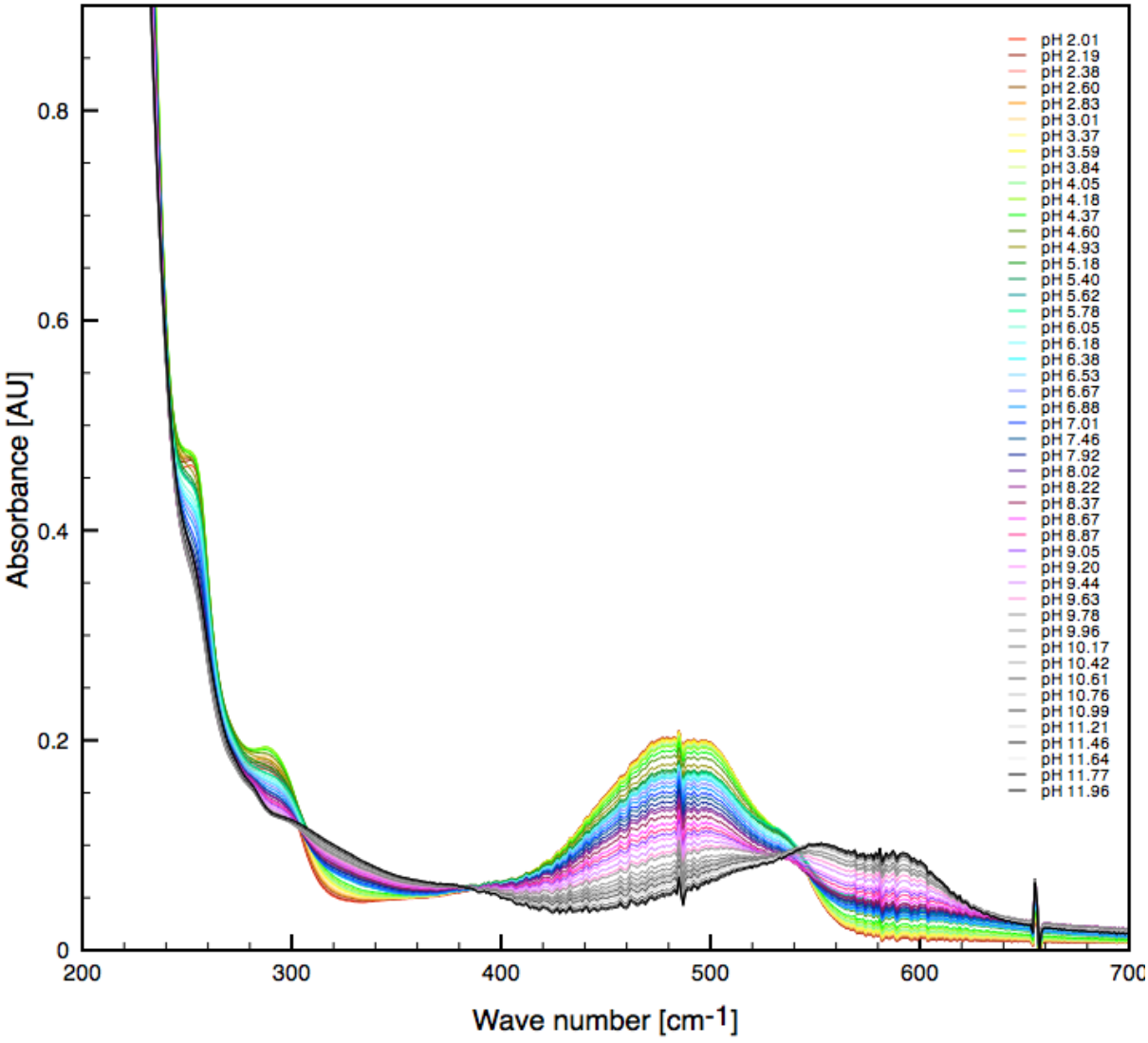


Figure S8. UV-VIS spectra of one titration run of Hdox:Fe in the ratio of 3:1.

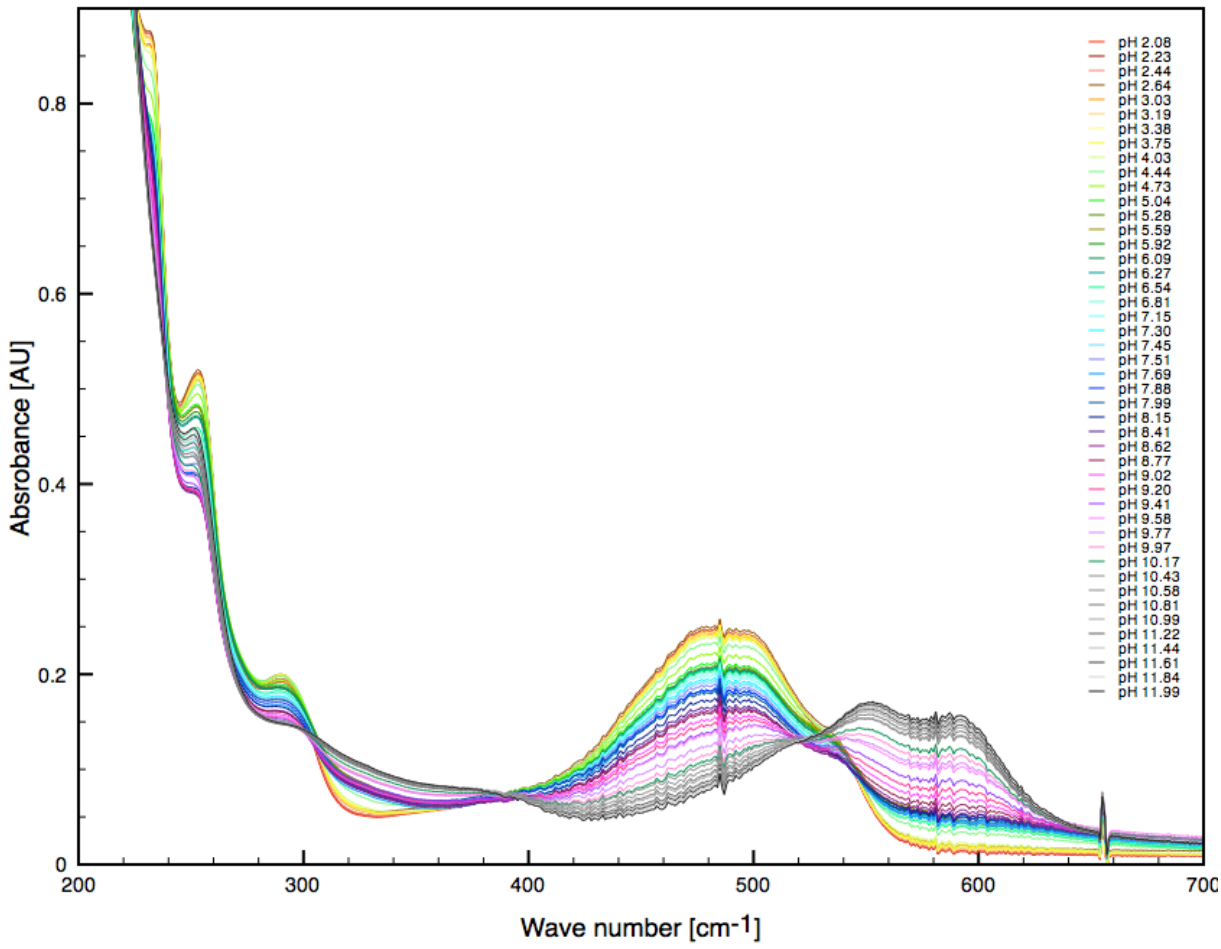


Figure S9. Cyclic voltammogram of vosaroxin (0.001 M, solid line) in DMSO solution; also shown is the blank voltammogram containing tetra(*n*-butyl)ammonium perchlorate 0.1 M (dotted line). Scan rate was 100 mV/s. Potential values are given with reference electrode Ag/AgCl(sat) and against the ferrocene couple Fc^{+}/Fc = +0.64 V vs. SHE (CRC Handbook, 2014).

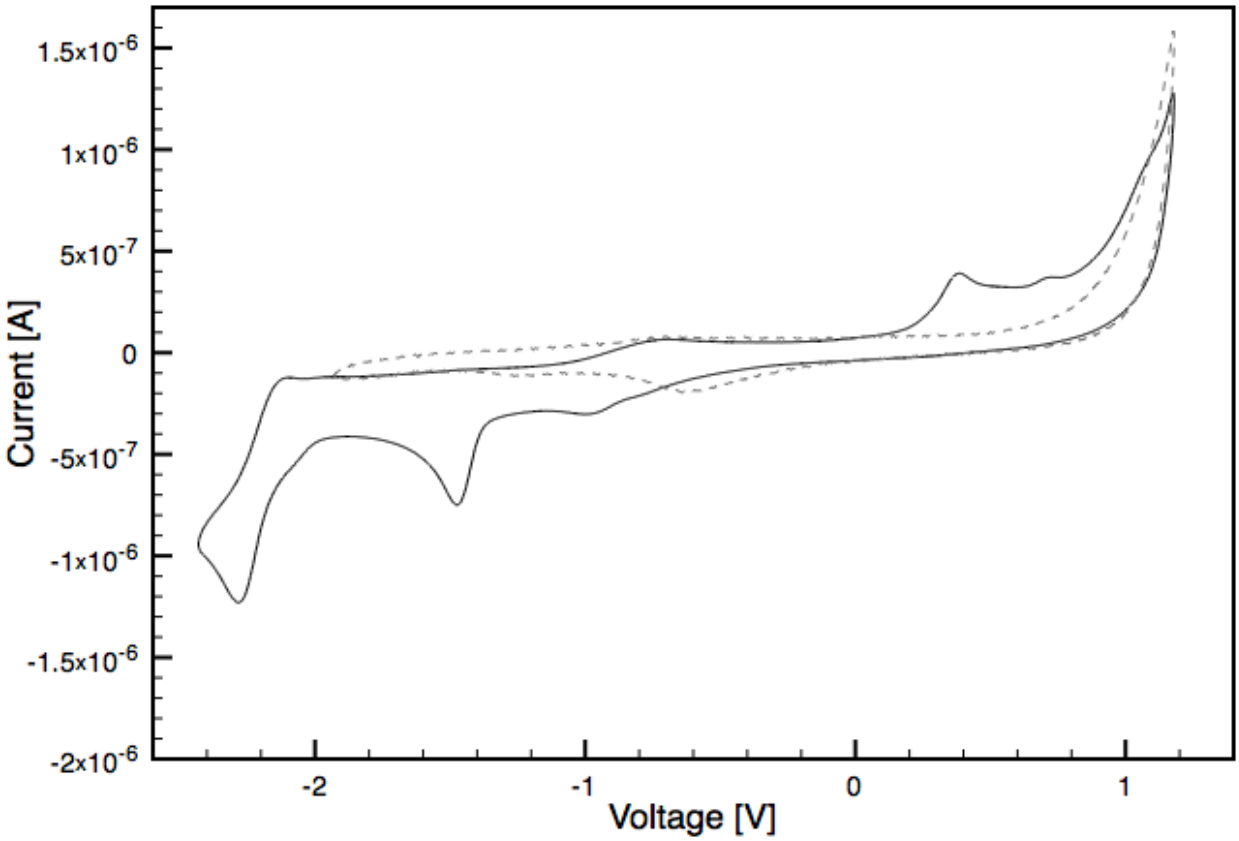


Figure S10. Cyclic voltammogram of [Ga(vox)$_3$] (0.001 M, green) in DMSO solution; also shown is the blank voltammogram containing tetra(*n*-butyl)ammonium perchlorate 0.1 M (dotted line). Scan rate was 100 mV/s. Potential values are given with reference electrode Ag/AgCl(sat) and against the ferrocene couple Fc^+/Fc = +0.64 V vs. SHE (CRC Handbook, 2014).

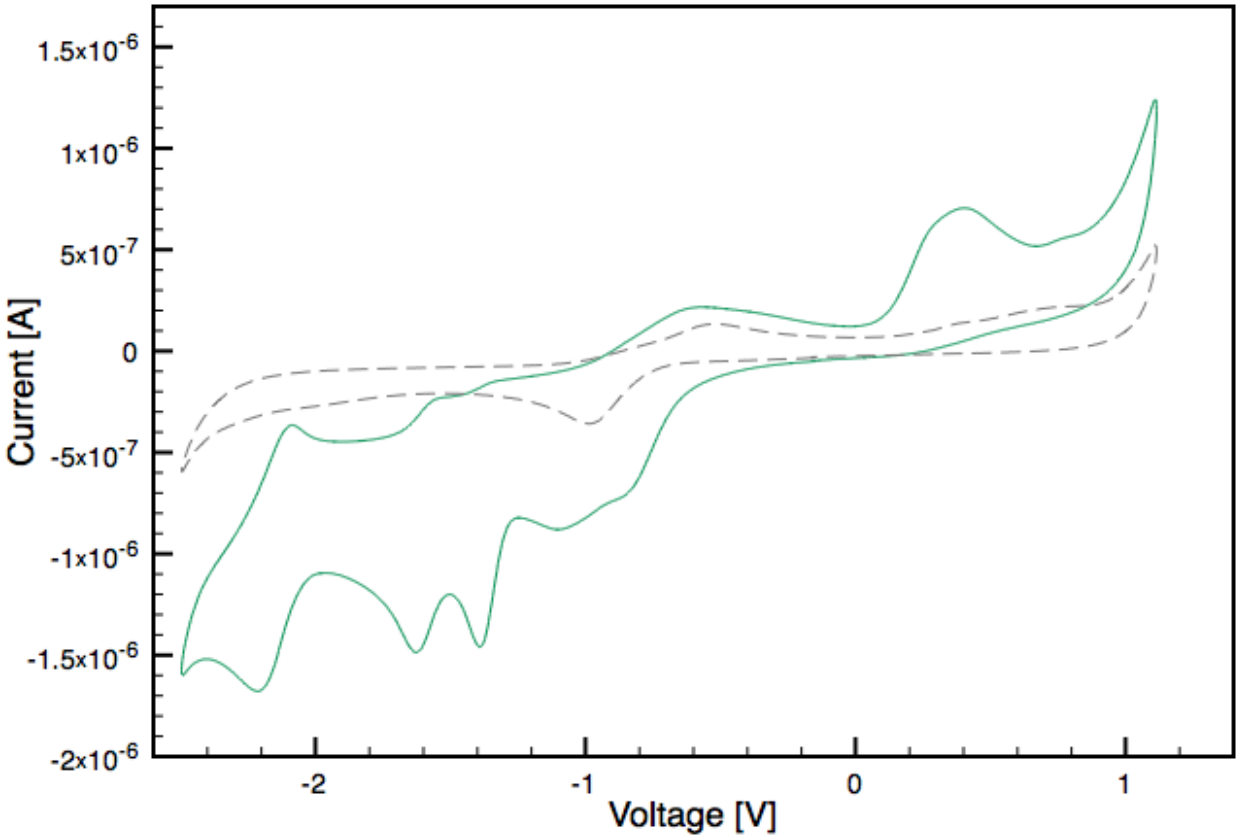


Figure S11. Titration of vosaroxin ($5 \cdot 10^{-4}$ M) in deuterated phosphate buffer ($5 \cdot 10^{-2}$ M) at pD 7.0 with increasing amounts of iron(III) nitrate in D_2O monitored via ^{1}H-NMR (600 MHz, D_2O, 298 K) with a total increase in volume throughout the titration of 0.6 %. The ^{1}H-NMR spectra with different ratios of Fe^{3+}:Hvox are shown with the same intensities for better comparison. From bottom to top: Hvox (dark blue), Fe^{3+}:Hvox = 1:50 (light blue), Fe^{3+}:Hvox = 1:25 (intense blue), Fe^{3+}:Hvox = 1:12.5 (teal), Fe^{3+}:Hvox = 1:6.25 (purple), Fe^{3+}:Hvox = 1:6.25 after 60 min wait time (grey), Fe^{3+}:Hvox = 1:3.33 (black). The NMR signals broaden with increasing amounts of Fe^{3+}. At a ratio of Fe^{3+}:Hvox = 1:6.25, one NMR spectrum was recorded after the standard time of 3 min (purple), and a second spectrum was recorded after the sample had been stirred for 60 min at ambient temperature upon which a precipitate had formed (grey). As this second spectra showed a clear narrowing of signals again, this indicated that the amount of Fe^{3+} ions in solution was reduced, which supports the assumption that the Fe[$(vox)_3$] complex, a complex not soluble in aqueous media, formed over the course of the titration. This assumption is further supported by the fact that the precipitate did not have the characteristic orange color of insoluble $Fe(OH)_{3(s)}$.

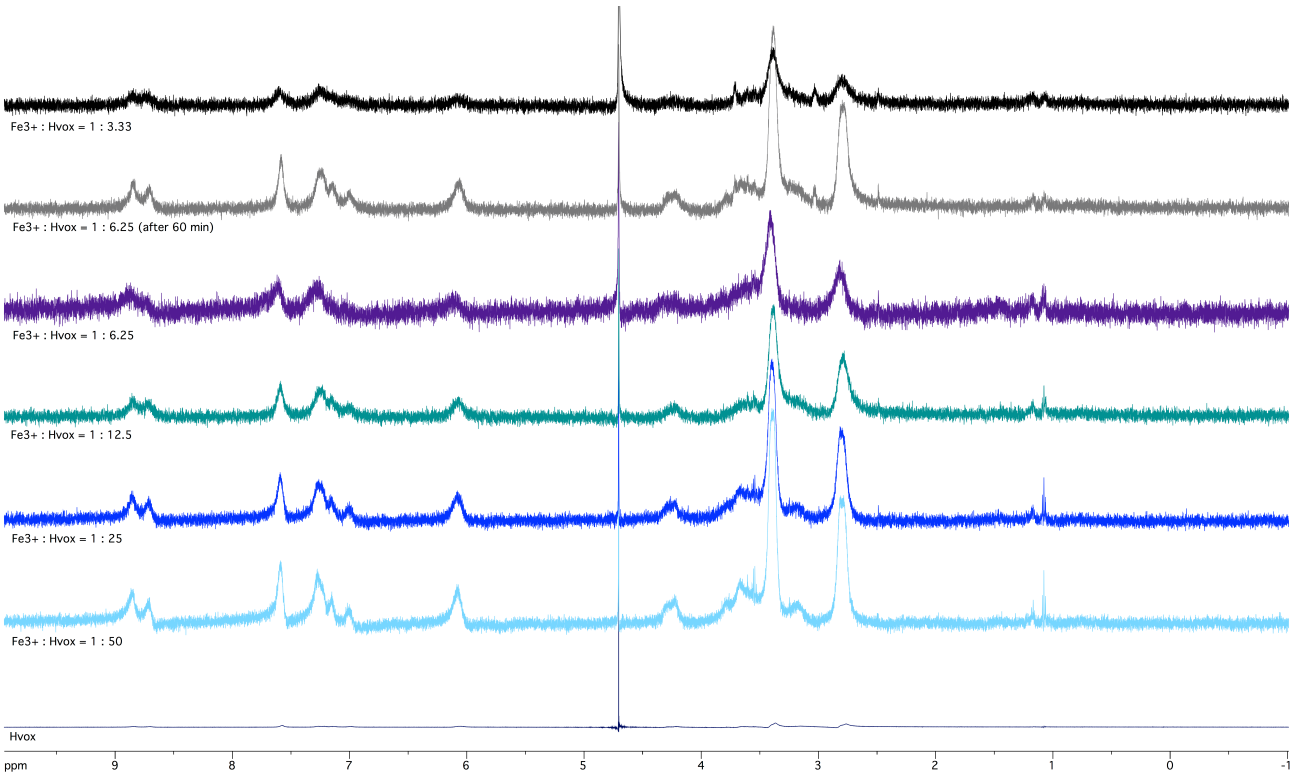


Figure S12. Temperature dependent NMR study (400 MHz, d_6-DMSO) of [Ga(vox)$_3$]. The sample was heated inside the spectrometer from ambient temperature (298 K, bottom, dark blue) to 363 K (red). To conclude the experiment, the sample was cooled down again to ambient temperature (top, black). As it could be expected, the heat accelerated the interchanging of the various stereoisomers in solution, which was reflected in more defined signals in the NMR spectra recorded at higher temperature. This phenomenon was solely temperature dependent and fully reversible, as a comparison of the first spectrum (bottom, dark blue) and the final spectrum (top, black) show.

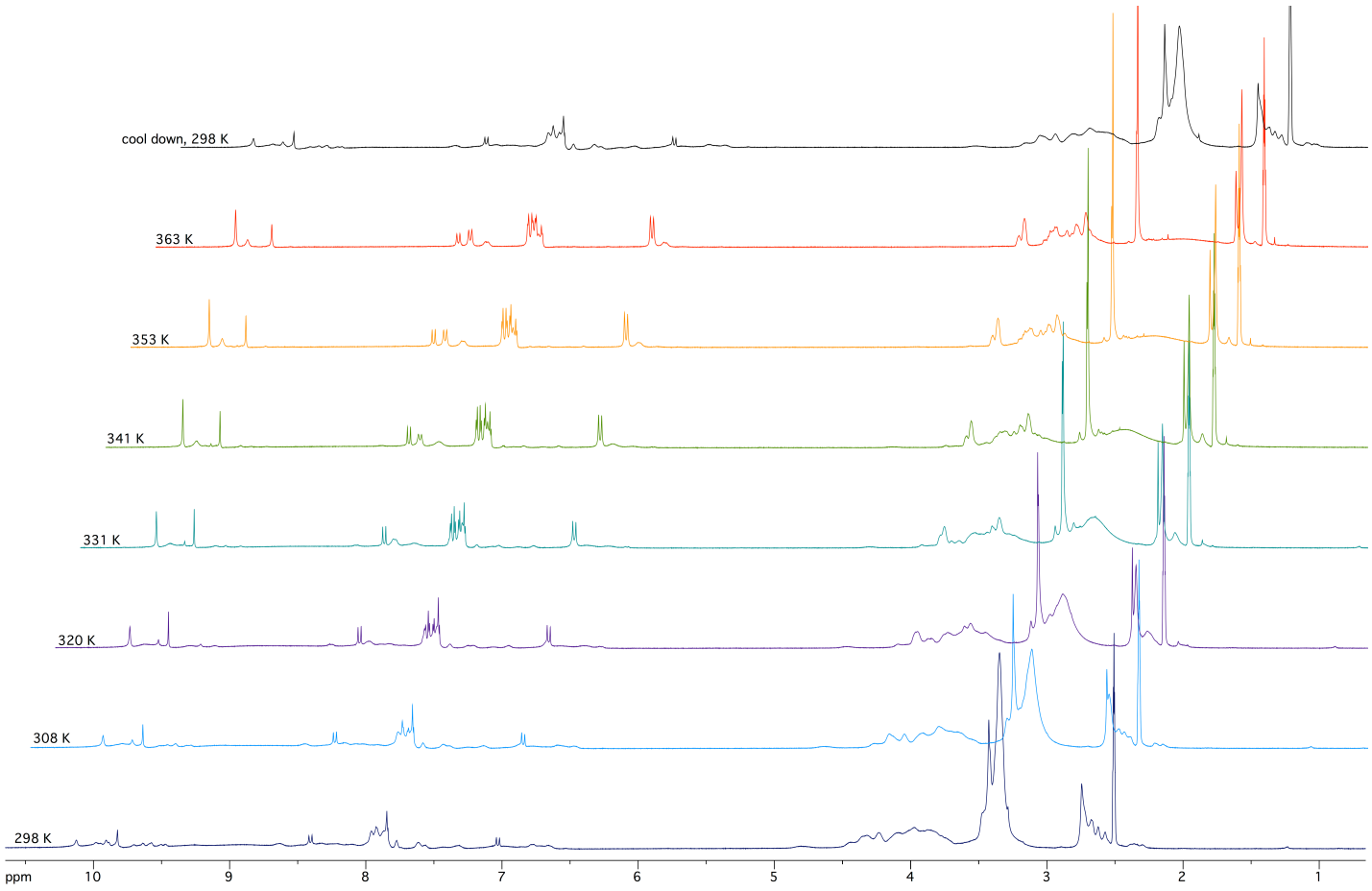
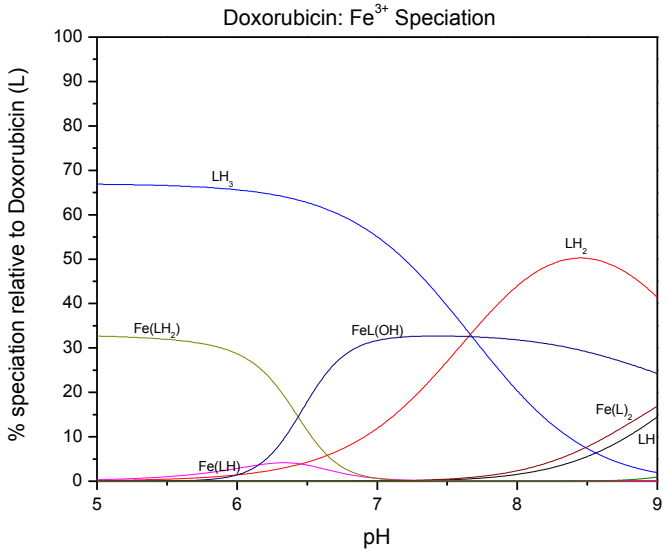
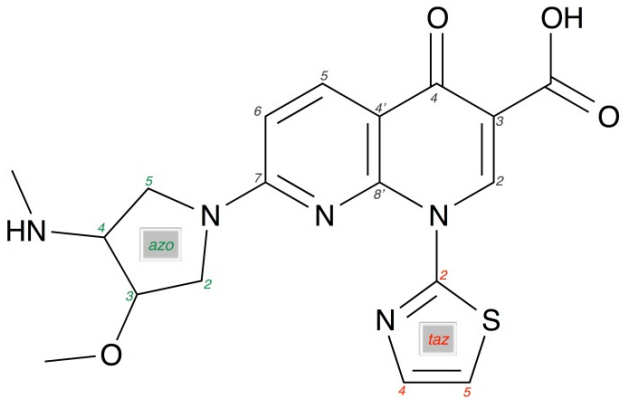


Figure S13. Species distribution curves for the iron(III)-doxorubicin system ($[Fe^{3+}]_T = 3.3 \times 10^{-4}$ M, $[L]_T = 1 \times 10^{-3}$ M).



Characterization of vosaroxin (Hvox)



Amorphous, off-white solid. **Mp** ≥ 240 °C, decomposition to dark yellow solid. **IR**: $\tilde{\nu}$ [cm^{-1}] = 3316 (w), 3088 (m), 3047 (sh), 2988 (w), 2940 (m), 2885 (m), 2819 (w), 1728 (s), 1619 (s), 1548 (s), 1510 (sh), 1491 (s), 1439 (m), 1417 (m); 1386 (m), 1327 (m), 1299 (m), 1261 (sh), 1251 (m), 1220 (w), 1185 (w), 1161 (w), 1113 (s), 1092 (sh), 1016 (w), 958 (s), 871 (w), 854 (w), 830 (m), 799 (s), 764 (m), 736 (m), 698 (w), 681 (m), 657 (m). **NMR**: $\boldsymbol{\delta_H}$ (600 MHz, 298 K, d_6-DMSO) [ppm] = (COO*H* not observed); 9.72 (s, 1 H, $C_{ar_2}H$); 8.23 (d, $^3J_{HH}$ = 9.1 Hz, 1 H, $C_{ar_5}H$); 7.81-7.80 (m, 1 H, $C_{taz_4}H$); 7.78-7.76 (m, 1 H, $C_{ar_6}H$); 6.85-6.83 (m, 1 H, $C_{taz_5}H$); 3.96 (s, 1 H, $C_{azo_3}H$); 3.88-3.80 (m, 1 H, N*H*); 3.78-3.71 (m, 2 H, $C_{azo_5}H_2$); 3.66-3.63 (m, 1 H, $C_{azo_4}H$); 3.52-3.43 (m, 2 H, $C_{azo_2}H_2$); 3.31-3.28 (m, 3 H, OCH_3); 2.37 (d, $^3J_{HH}$ = 2.6 Hz, 3 H, NH(CH_3)). $\boldsymbol{\delta_C}$ (150 MHz, 298 K, d_6-DMSO) [ppm] = 176.8 (C_{ar_4}); 165.3 (*C*OOH); 157.3 (br, C_{ar_7}); 155.2 (br, C_{taz_2}); 147.7, 147.6 ($C_{ar_{8'}}$); 141.9 (br, C_{ar_2}); 137.8, 137.7 (C_{taz_4}); 135.4, 135.3 (C_{ar_5}); 121.6, 121.5 (C_{ar_6}); 110.0 (br, $C_{ar_{4'}}$); 109.3 (br, C_{taz_5}); 109.0, 108.9 (C_{ar_3}); 82.0, 81.6 (C_{azo_3}); 62.4, 62.1 (O*C*H$_3$); 56.3, 56.3 (C_{azo_2}); 53.4, 53.1 (C_{azo_5}); 51.2, 50.8 (C_{azo_4}); 34.2, 34.1 (N*C*H$_3$). **LR-MS** (ES+, methanol): m/z (%) = 402 (100) [LH + H^+]. **HR-ESI-MS** m/z for $C_{18}H_{19}N_5O_4S$ + H^+ calcd. (found): 402.1236 (402.1242). **EA**: Anal. Calcd. (found) [%] for $C_{18}H_{19}N_5O_4S$: C, 53.86 (53.97); H, 4.77 (4.74); N, 17.45 (17.24); S, 7.99 (7.64).