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SUPPLEMENTARY INFORMATION

Salicylaldehyde thiosemicarbazone copper complexes: impact of hybridization with estrone on cytotoxicity, solution stability and redox activity

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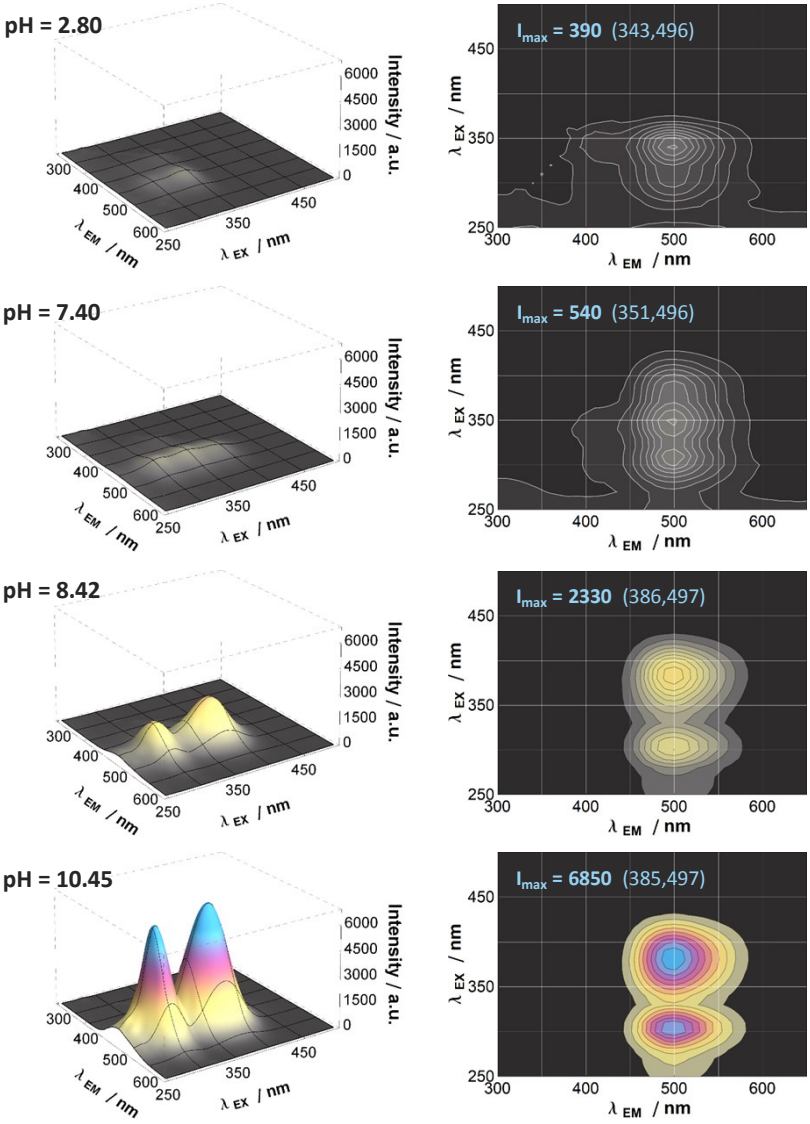

Fig. S1. Three dimensional fluorescence spectra of estrone-TSC and corresponding contour plot representations at the indicated pH values. Maximal peak intensities (I_{max}) and wavelength coordinates (λ_{EX}, λ_{EM}) are indicated in the figure. {c_L = 1 μM; T = 25.0 °C; I = 0.1 M (KCl)}

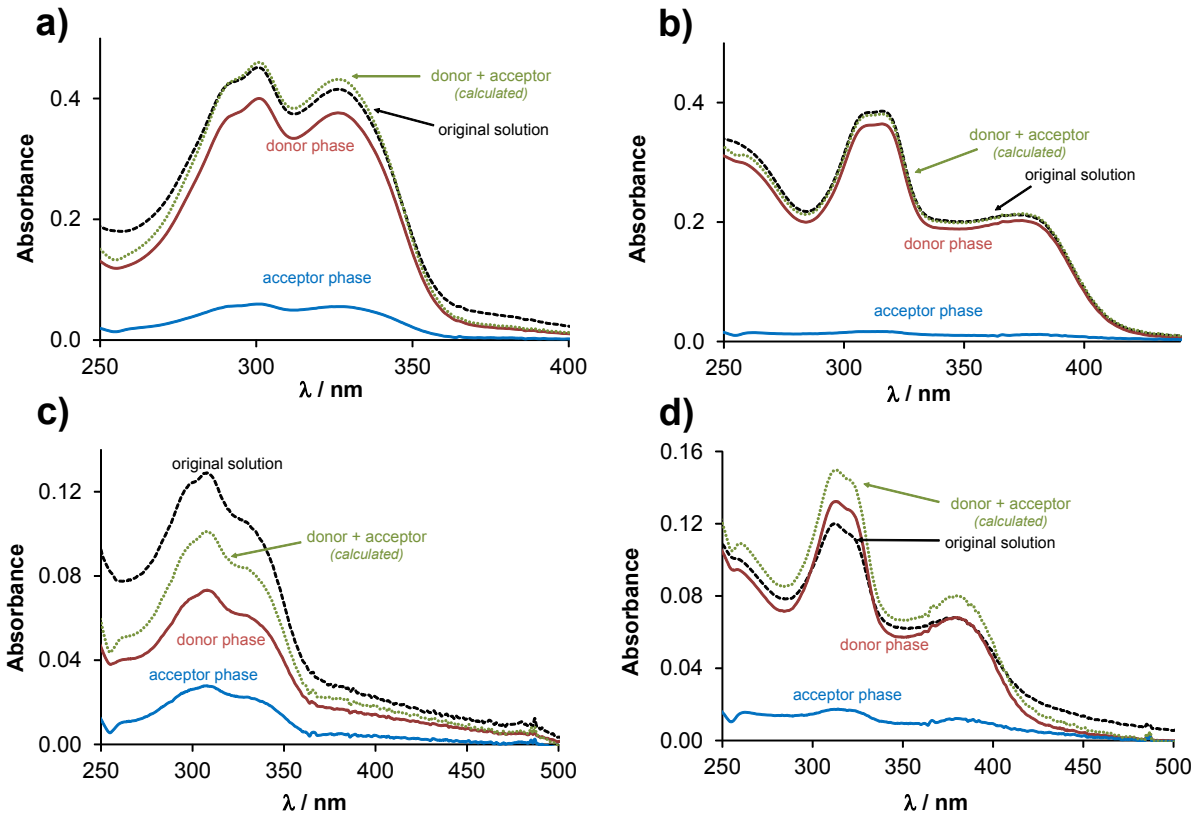

Fig. S2. UV-vis absorption spectra recorded for the acceptor (blue line) and donor (red line) phases using the PAMPA assay for STSC (a), its copper(II) complex (b), thn-TSC (c) and its copper(II) complex (d). The spectrum of the original solution (dashed black line) and the summed spectra of the acceptor and donor phases (green dotted line) are also shown. {$c_{compound}$ = 20 μM; pH = 7.4 (PBS); 4% (v/v) DMSO/water; t = 25 °C; l = 1 cm}

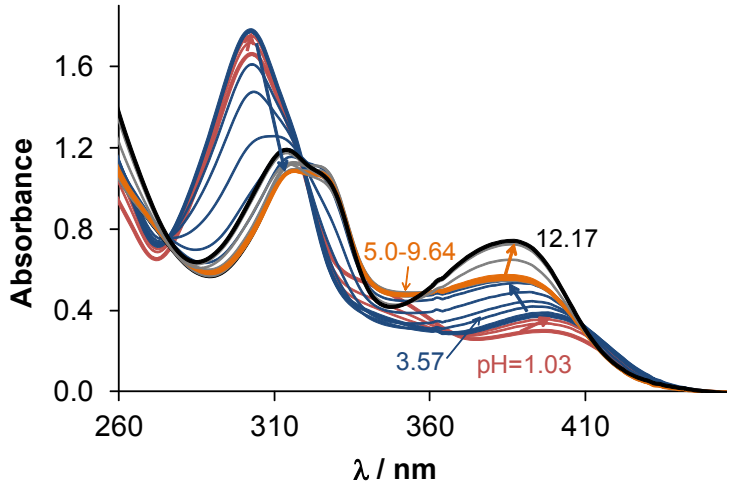

Fig. S3. UV-vis absorption spectra of the copper(II) – estrone-TSC (1:1) system in the pH range 1.0–12.2 in 30% (v/v) DMSO/H_2O solvent mixture. {c_{ligand} = 50 μM; $c_{Cu(II)}$ = 50 μM; T = 25.0 °C; I = 0.1 M (KCl)}

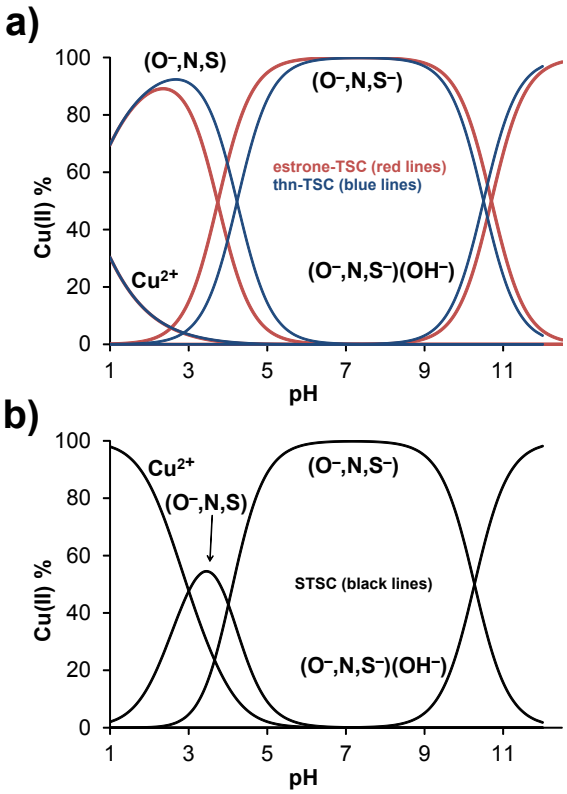

Fig. S4. Concentration distribution curves for the copper(II) – estrone-TSC (red lines) and thn-TSC (blue lines) systems (a) and for the copper(II) – STSC (b) system based on stability constants taken from Ref.[14]. {c_{ligand} = 50 μM; $c_{Cu(II)}$ = 50 μM; T = 25.0 °C; I = 0.1 M (KCl); 30% (v/v) DMSO/water}

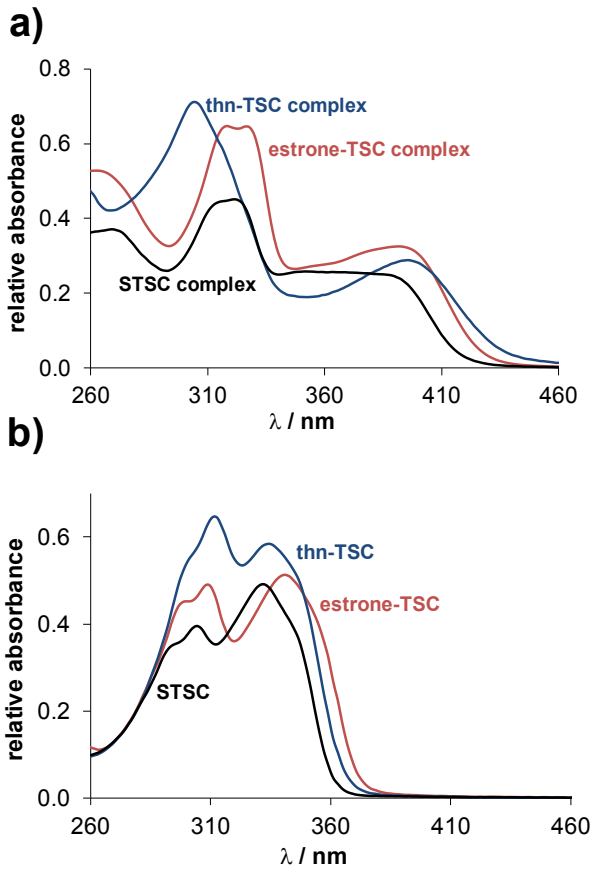

Fig. S5. UV-vis absorption spectra recorded for the copper(II) complexes of estrone-TSC (red line), thn-TSC (blue line) and STSC (black line) in methanol (a) and for the proligands for the sake of comparison (b). {$c_{\text{compound}} \sim 25$ μM; $t = 25$ °C; $l = 1$ cm}

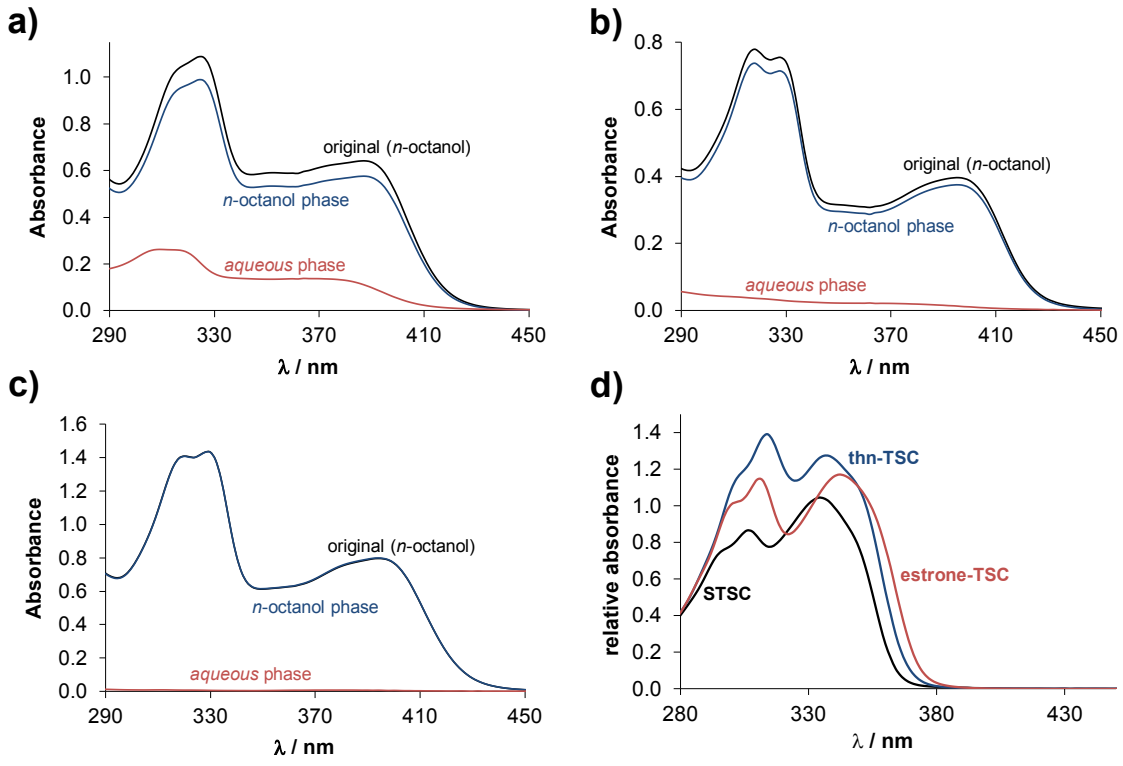

Fig. S6. UV-vis absorption spectra recorded for copper(II) complexes STSC (a), thn-TSC (b) and estrone-TSC (c) in *n*-octanol before (black line) and after (blue lines) partitioning and in the aqueous phase (red lines) after partitioning. UV-vis spectra recorded for the ligands in *n*-octanol for comparison (d). {pH = 7.4 (20 mM HEPES); I = 0.1 M (KCl); t = 25 °C}

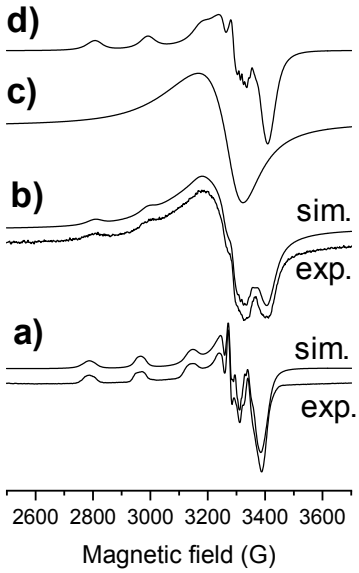

Fig. S7. Experimental (exp.) and simulated (sim.) anisotropic EPR spectra of complexes of [Cu(estrone-TSCH$_{-2}$)] (a) and [Cu(thn-TSCH$_{-2}$)] (b) in 50% (v/v) DMSO/H_2O. Calculated component EPR spectra of a dimeric species (c) and a monomer species (d) taken in 76% and 24%, respectively, in the simulation of spectrum (b). The dimeric species was simulated with an isotropic spectrum with g_o = 2.092.

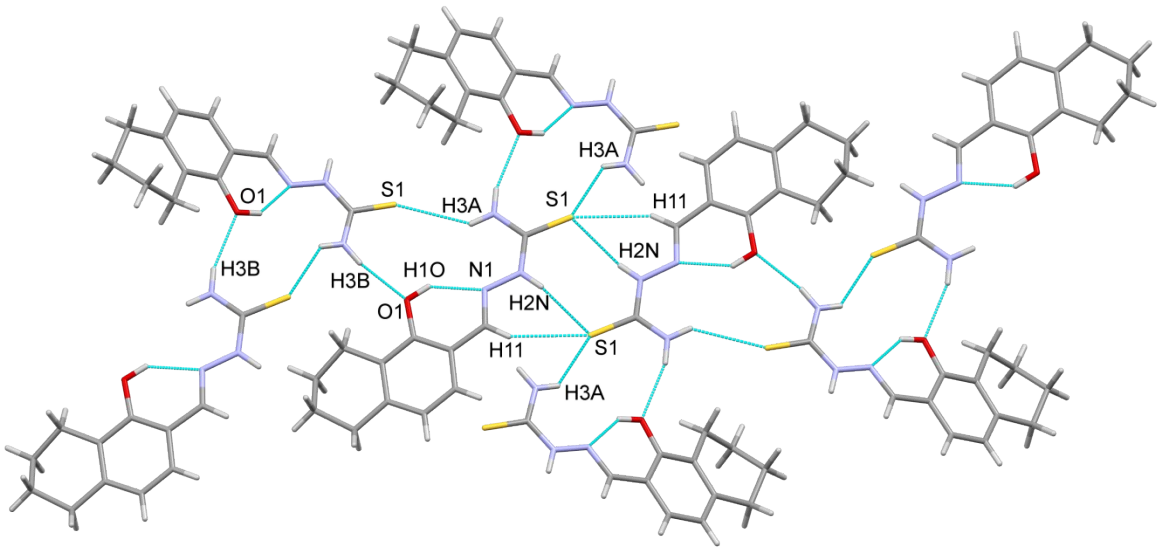

Fig. S8. Packing arrangements in crystal thn-TSC (**I**) showing the N-H…S, C-H…S and N-H…O hydrogen bond interactions (only the major conformer is shown)

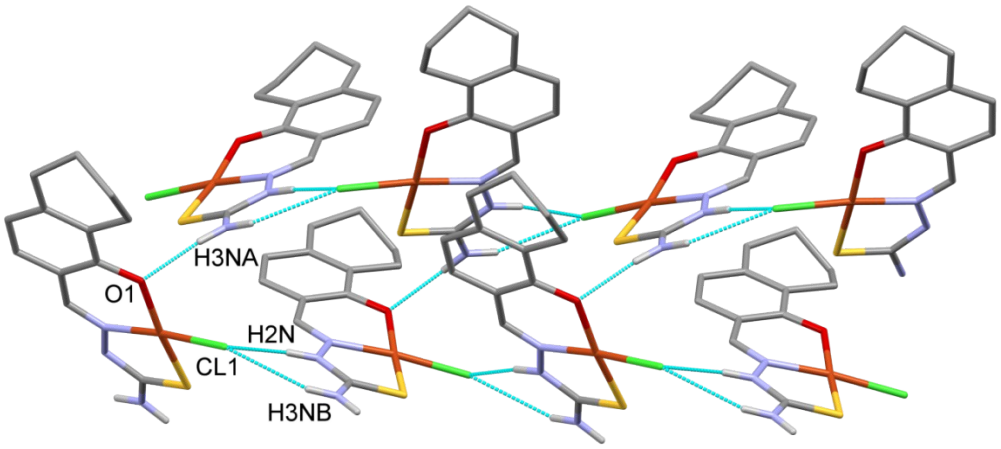

Fig. S9. Crystal packing in crystal [Cu(thn-TSCH$_{-1}$)Cl] (**II**) arranged by N-H…Cl and N-H…O hydrogen bonds (hydrogen atoms involved in hydrogen bonds are only showed for clarity)

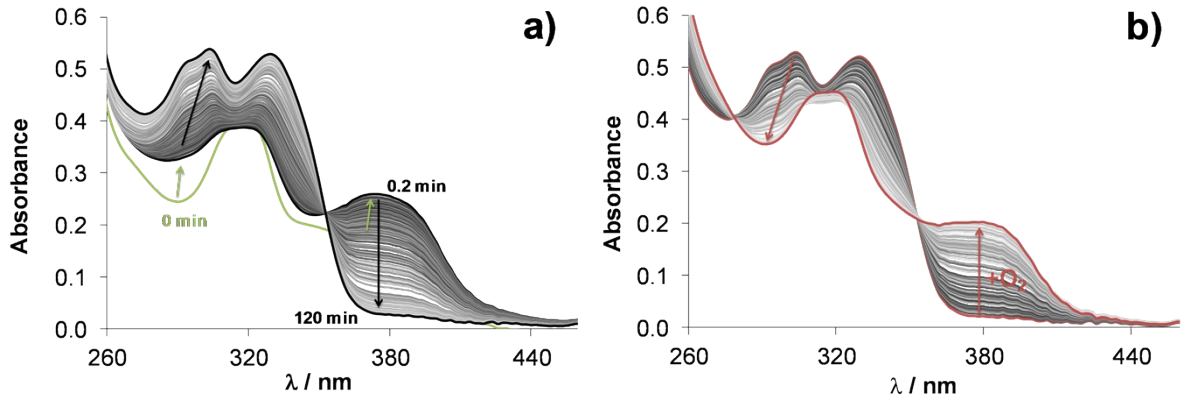

Fig. S10. Time dependence of the UV-vis spectra of the [Cu(STSCH$_{-2}$)] complex (25 μM) in the presence of 50 equivalents GSH (1.25 mM) at pH 7.4 in 30% (v/v) DMSO/H_2O under anaerobic conditions (a), and effect of bubbling O_2 through the sample following the reaction with GSH (b). {T = 25 °C; I = 0.1 M (KCl)}

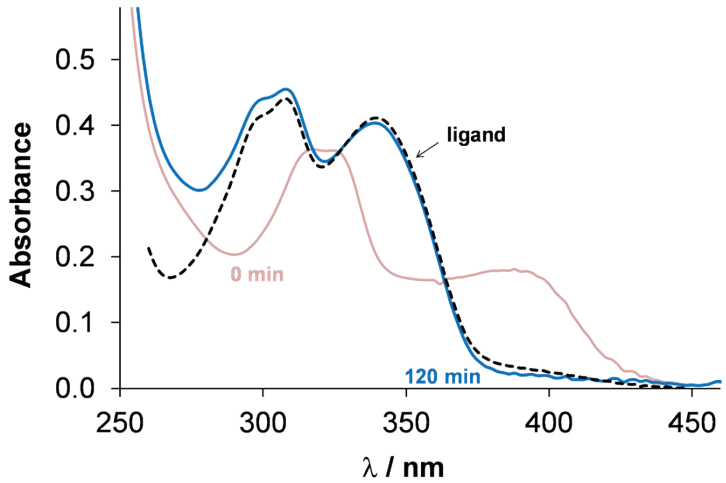

Fig S11. UV-vis spectra of estrone-TSC (25 μM) (black dashed line) and the 'product' formed in the reaction of [Cu(estroneTSCH$_{-2}$)] complex (25 μM) with 50 equiv. GSH (blue solid line) after 120 min at pH 7.4 in 30% (v/v) DMSO/H_2O) under anaerobic conditions with the initial spectrum (light red solid line). {T = 25 °C; I = 0.1 M (KCl)}

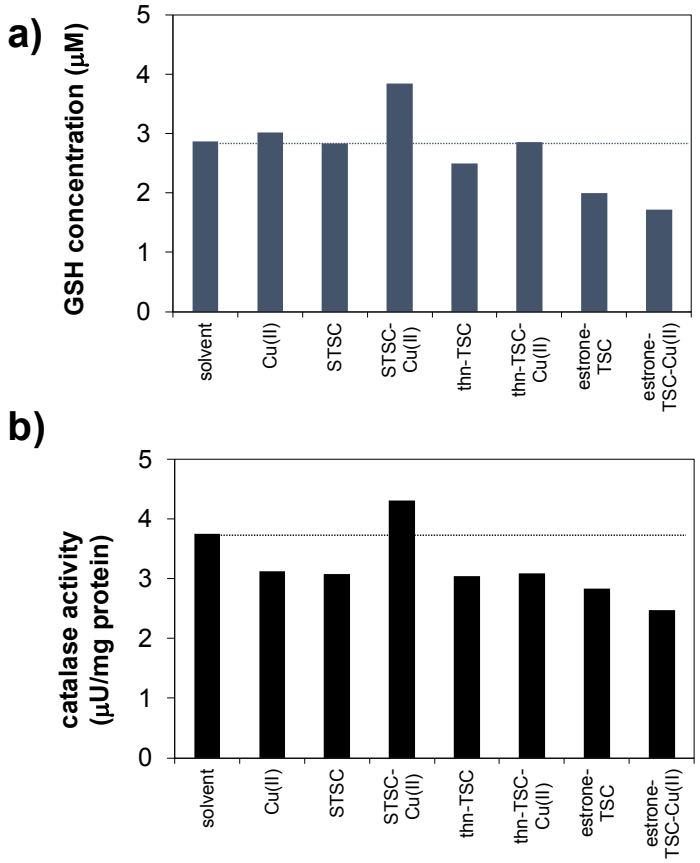

Fig. S12. GSH level (a) and catalase activity (b) in SUM159 cells measured for the solvent control (background DMSO/buffer mixture as in the samples tested), $CuCl_2$ (1 μM) and for the tested compounds (1 μM). Values show the mean of two experiments (relative standard deviation was varied between 1 – 8%).

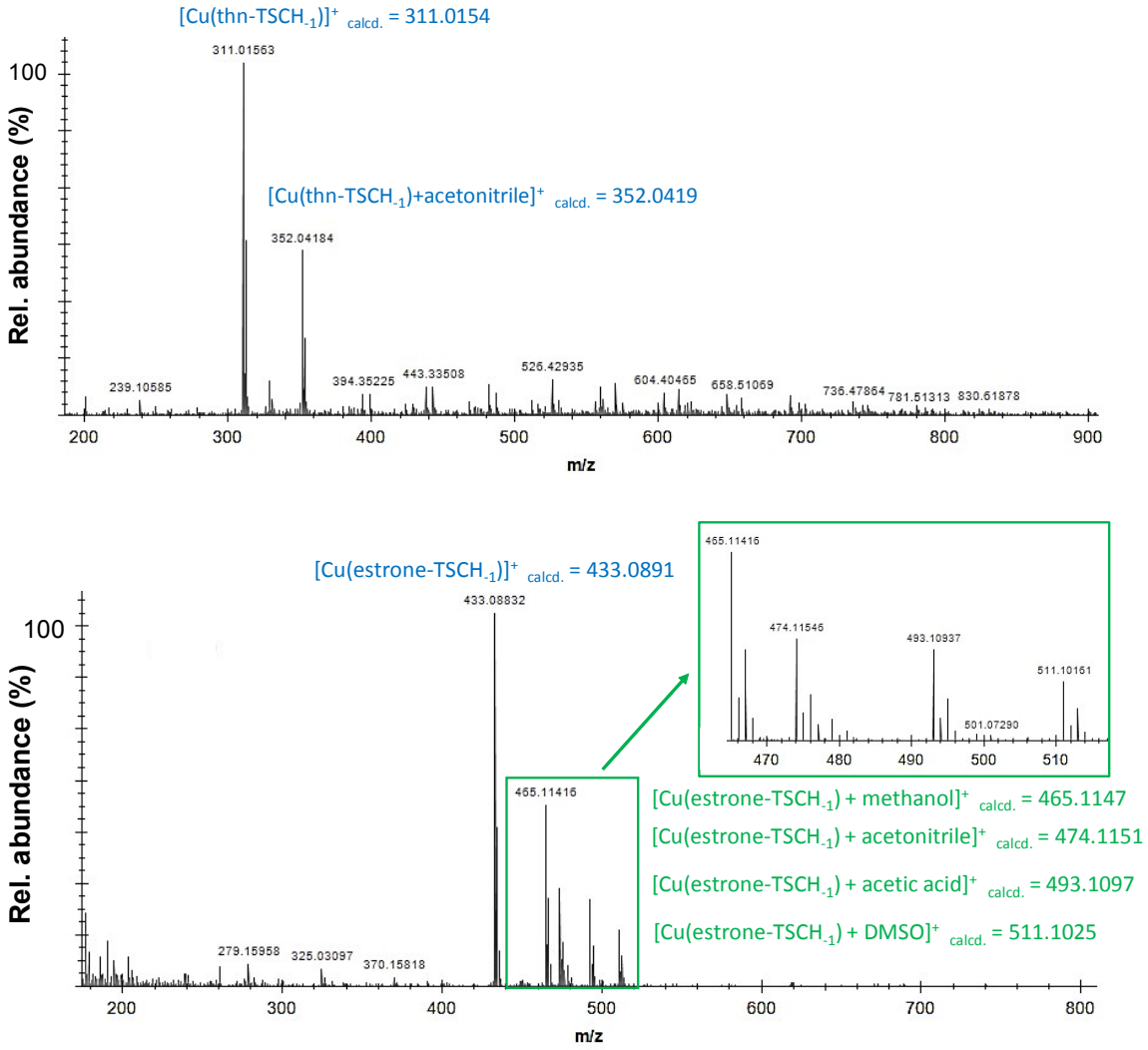

Figure S13. ESI-MS spectra of the indicated novel Cu(II)-TSC complexes. Experimental and calculated m/z values are listed as well. Samples were prepared in methanol containing ca. 0.5 % acetic acid in the case of $[Cu(estrone\text{-}TSCH_{-1}]^+$ complex; the eluent was methanol/acetonitrile.

Table S1. Crystal data and structure refinement for thn-TSC (**I**) and [Cu(thn-TSCH$_{-1}$)Cl] (**II**)

	thn-TSCH (I)	**[Cu(thn-TSCH$_{-1}$) Cl] (II)**
Color/Shape	Colorless/Prism	Blue/Platelet
Empirical formula	$C_{12}H_{15}N_3OS$	$C_{12}H_{14}ClCuN_3OS$
Moiety formula	$C_{12}H_{15}N_3OS$	$[Cu(C_{12}H_{14}N_3OS)(Cl)]$
Formula weight	249.33	347.31
Temperature	162(2)	168(2)
Radiation and wavelength λ (Å)	Cu-Kα, 1.54178	Cu-Kα, 1.54187
Crystal system	monoclinic	orthorhombic
Space group	$P2_1/n$	*Pbca*
Unit cell dimensions		
a (Å)	8.8483(7)	12.3620(7)
b (Å)	10.5831(7)	9.1718(5)
c (Å)	13.2516(10)	24.3156(13)
β (°)	102.518(7)	90
Volume (Å^3)	1211.41(16)	2756.9(3)
Z/Z'	4/1	8/1
Density (calculated) (Mg/m^3)	1.367	1.674
Absorption coefficient, μ (mm^{-1})	2.272	5.395
F(000)	528	1416
Crystal size (mm)	0.30 x 0.15 x 0.15	0.30 x 0.15 x 0.05
Absorption correction	numerical	numerical
Max. and min. transmission	0.912 and 0.960	0.881749 and 0.966832
θ–range for data collection (°)	5.400 ≤ θ ≤ 68.206	3.636 ≤ θ ≤ 68.244
Index ranges	$-10 \le h \le 10; -12 \le k \le 12; -15 \le l \le 15$	$-14 \le h \le 13; -11 \le k \le 10; -29 \le l \le 29$
Reflections collected	10216	37412
Completeness to 2θ	0.990	0.996
Independent reflections, (R_{int})	2196, 0.0420	2513, 0.2023
Reflections $I>2\sigma(I)$	1878	1876
Refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2
Data / restraints / parameters	2196 /0 /173	2513 /0 /172
Goodness-of-fit on F^2	1.060	1.262
Final *R* indices [$I>2\sigma(I)$], R_1, wR_2	0.0452, 0.1034	0.1326, 0.2597
R indices (all data), R_1, wR_2	0.0557, 0.1079	0.1704, 0.2774
Max. and mean shift/esd	0.000;0.000	0.000;0.000
Largest diff. peak and hole (e.Å^{-3})	0.320; -0.202	0.982; -0.487

Table S2. Selected bond angles (°) and distances (Å) for crystal I and II

Crystal I	S1-C12	1.689(2)
	N1-C11	1.291(3)
	N2-C12	1.340(3)
	O1-C1	1.366(3)
	N1-N2	1.376(2)
	N3-C12	1.326(3)
Crystal II	Cu1-O1	1.898(8)
	Cu1-S1	2.265(3)
	Cu1-N1	1.93(1)
	Cu1-Cl1	2.268(3)
	S1-C12	1.69(1)
	N1-C11	1.27(2)
	N2-C12	1.35(2)
	O1-C1	1.34(1)
	N1-N2	1.38(1)
	N3-C12	1.33(1)
	O1-Cu1-N1	92.1(4)
	N1-Cu1-S1	86.4(3)
	N1-Cu1-Cl1	175.1(3)
	C12-S1-Cu1	96.7(5)
	O1-Cu1-S1	171.1(3)
	O1-Cu1-Cl1	90.9(2)
	S1-Cu1-Cl1	91.2(1)
	C1-O1-Cu1	126.2(7)

Table S3. Hydrogen-bond geometry of crystal **I** and **II**

D-H…A	**D-H** (Å)	**H…A**(Å)	**D…A**(Å)	**D-H…A** (°)	**symmetry codes**
crystal I					
O1-H1O…N1	0.87	1.98	2.716(2)	142	intra
N2-H2N…S1	0.86	2.53	3.379(2)	166	1-x,-y,-z
N3-H3A…S1	0.86	2.81	3.490(2)	137	3/2-x,1/2+y,1/2-z
N3-H3B…O1	0.86	2.14	2.965(2)	161	3/2-x,-1/2+y,1/2-z
C11-H11…S1	0.93	2.83	3.687(2)	153	1-x,-y,-z
crystal II					
N3-H3NB…Cl1	0.88	2.68	3.456213)	146	1/2+x,y,1/2-z
N3-H3NA…O1	0.88	1.99	2.835(15)	162	1-x,-1/2+y,1/2-z
N2-H2N…Cl1	0.88	2.29	3.144(11)	163	1/2+x,y,1/2-z

Table S4. Fluorescence emission intensity (Int.) measured by the DCFDA assay in MCF-7 and SUM159 cells for the tested compounds (1 μM) without and with NAC (1 mM). {λ_{EX} = 500 nm; λ_{EM} = 529 nm}

Int. (a.u.)	**MCF-7**		**SUM159**	
	without NAC	**with NAC**	**without NAC**	**with NAC**
solvent control	45390 ± 573	34951 ± 6086	28895 ± 6045	18962 ± 709
$CuCl_2$	36599 ± 6332	27045 ± 5577	23521 ± 3843	18187 ± 1792
STSC	42791 ± 543	31772 ± 8123	27243 ± 1009	19664 ± 2265
thn-STSC	40390 ± 1273	28530 ± 2918	23521 ± 3843	18187 ± 1792
estrone-TSC	47692 ± 567	27940 ± 1751	27987 ± 1494	19204 ± 3127
STSC-Cu(II)	45129 ± 1560	25102 ± 5058	35887 ± 3192	24888 ± 2566
thn-STSC-Cu(II)	55631 ± 7100	31208 ± 1777	36501 ± 4974	19391 ± 2922
estrone-TSC-Cu(II)	50708 ± 4325	25419 ± 9046	48022 ± 4625	27064 ± 9899