Supporting Information

Amino- and chloro-8-hydroxyquinolines and their copper complexes as proteasome inhibitors and antiproliferative agents

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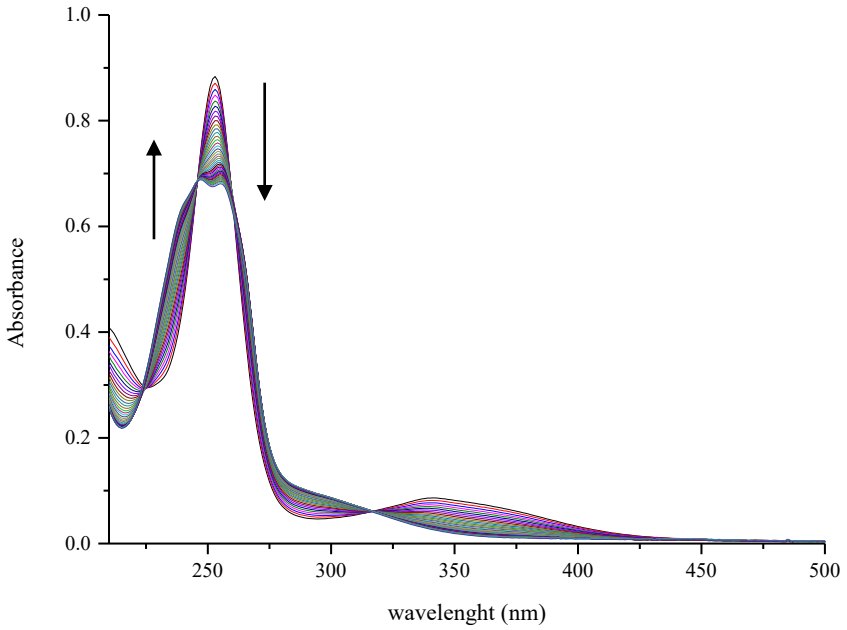

Figure S1. UV-vis spectra of 5-AHQ (4.0×10^{-5} M) in MOPS (pH 7.4, 10 mM). The spectra were collected every 180 s for 2 h.

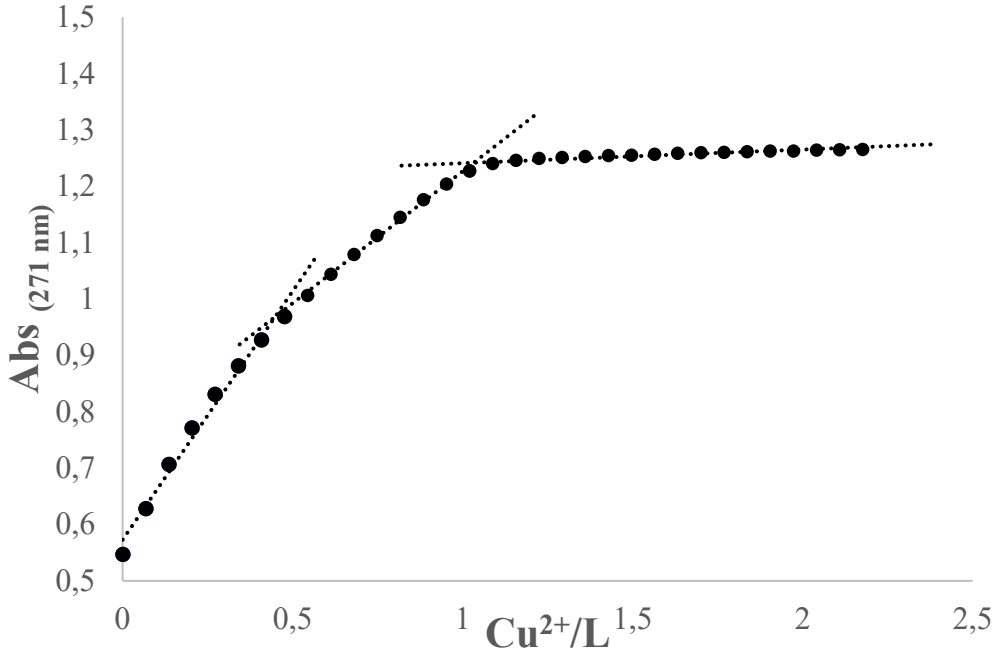

Figure S2. Plot of the absorbance at 271 nm versus the molar ratio Cu^{2+}/L with L=2-AHQ (MOPS/dioxane 50:50 v/v).

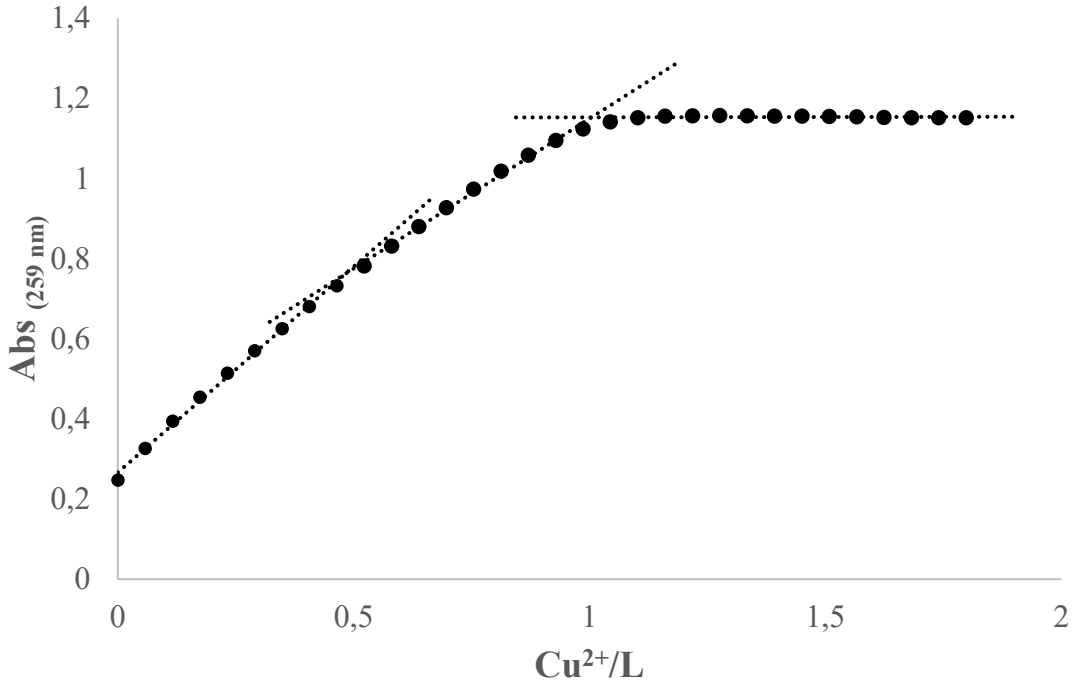

Figure S3. Plot of the absorbance at 259 nm versus the molar ratio Cu^{2+}/L with L=AMHQ (MOPS/dioxane 50:50 v/v).

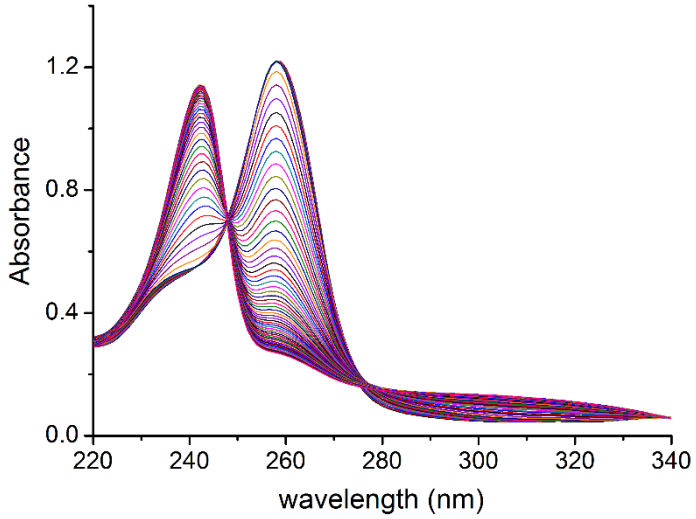

Figure S4.UV-vis competition titration of EDTA (5.49×10^{-4} M) into a Cu^{2+}/AMHQ (1:1) solution in water/dioxane 50/50 v:v at pH 7.4 (MOPS 0.01 M). $C_L = 4.07 \times 10^{-5}$ M.

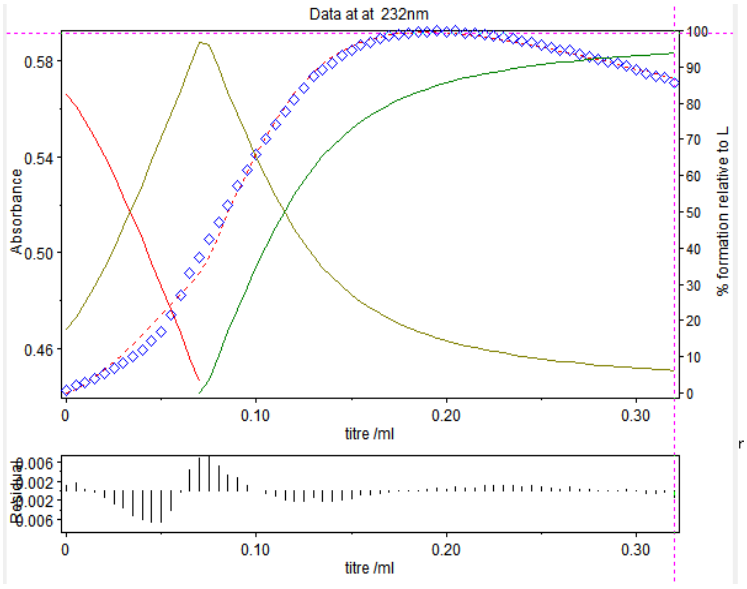

Figure S5. Hyperquad output for the Cu^{2+} – AMHQ system (blue squares: experimental points; red dotted lines: theoretical fit). The fit is reported at one wavelength only (232 nm); however, the entire 220-340 nm range was analyzed through a multi-wavelength treatment of the data. The species distribution diagram is also shown in the same window; residuals (observed/calculated values) are shown below the curves.

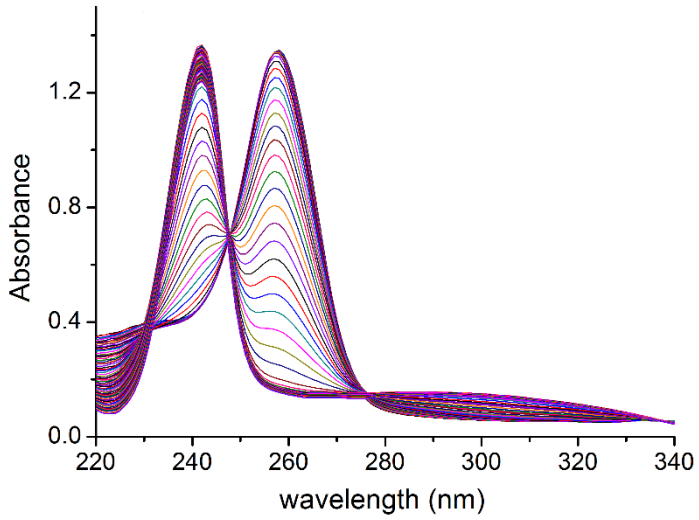

Figure S6. UV-vis competition titration of EDTA (5.41×10^{-4} M) into a Cu^{2+}/HQ (1:1) solution in water/dioxane 50/50 v:v at pH 7.4 (MOPS 0.01 M). $C_L = 4.19 \times 10^{-5}$ M.

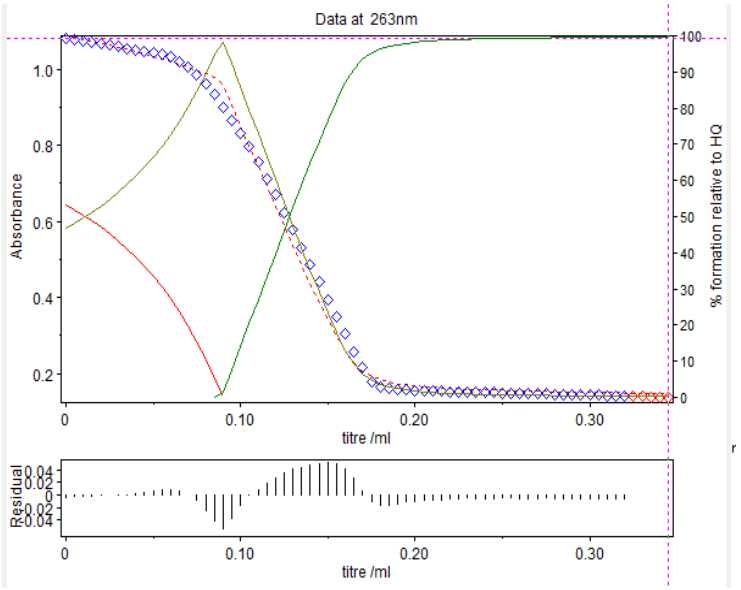

Figure S7. Hyperquad output for the Cu^{2+} – HQ system (blue squares: experimental points; red dotted lines: theoretical fit). The fit is reported at one wavelength only (263 nm); however, the entire 220-340 nm range was analyzed through a multi-wavelength treatment of the data. The species distribution diagram is also shown in the same window; residuals (observed/calculated values) are shown below the curves.

Table S1. Data fitting relative to the evaluation of the I_{50} values of HQ compounds and their copper complexes for ChT-L peptidase activity of the h-CP. Curve fitting was performed by using equation 1 reported in the main text.

	5-AHQ	2-AHQ	AMHQ	ClHQ	ClMHQ
I_{50} (μM)	0.51	0.44	0.30	n.d	n.d
95% Confidence Intervals	0.25 to 1.3	0.20 to 0.99	0.12 to 0.77	n.d	n.d
R^2	0.91	0.90	0.90	n.d	n.d

	5-AHQ Cu(II)	2-AHQ Cu(II)	AMHQ Cu(II)	ClHQ Cu(II)	ClMHQ Cu(II)
I_{50} (μM)	0.34	1.22	0.85	4.5	1.3
95% Confidence Intervals	0.26 to 0.44	0.77 to 1.9	0.77 to 0.95	1.6 to 12.7	1.06 to 1.60
R^2	0.99	0.96	0.99	0.82	0.99

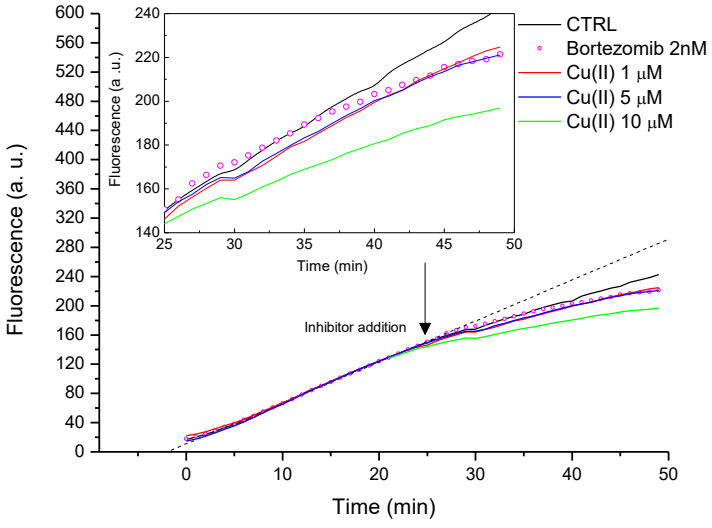

Figure S8. Fluorescence vs time plots of 20S activity in the presence of Bortezomib (2 nM) and Cu(II), (1, 5 and 10 μM).

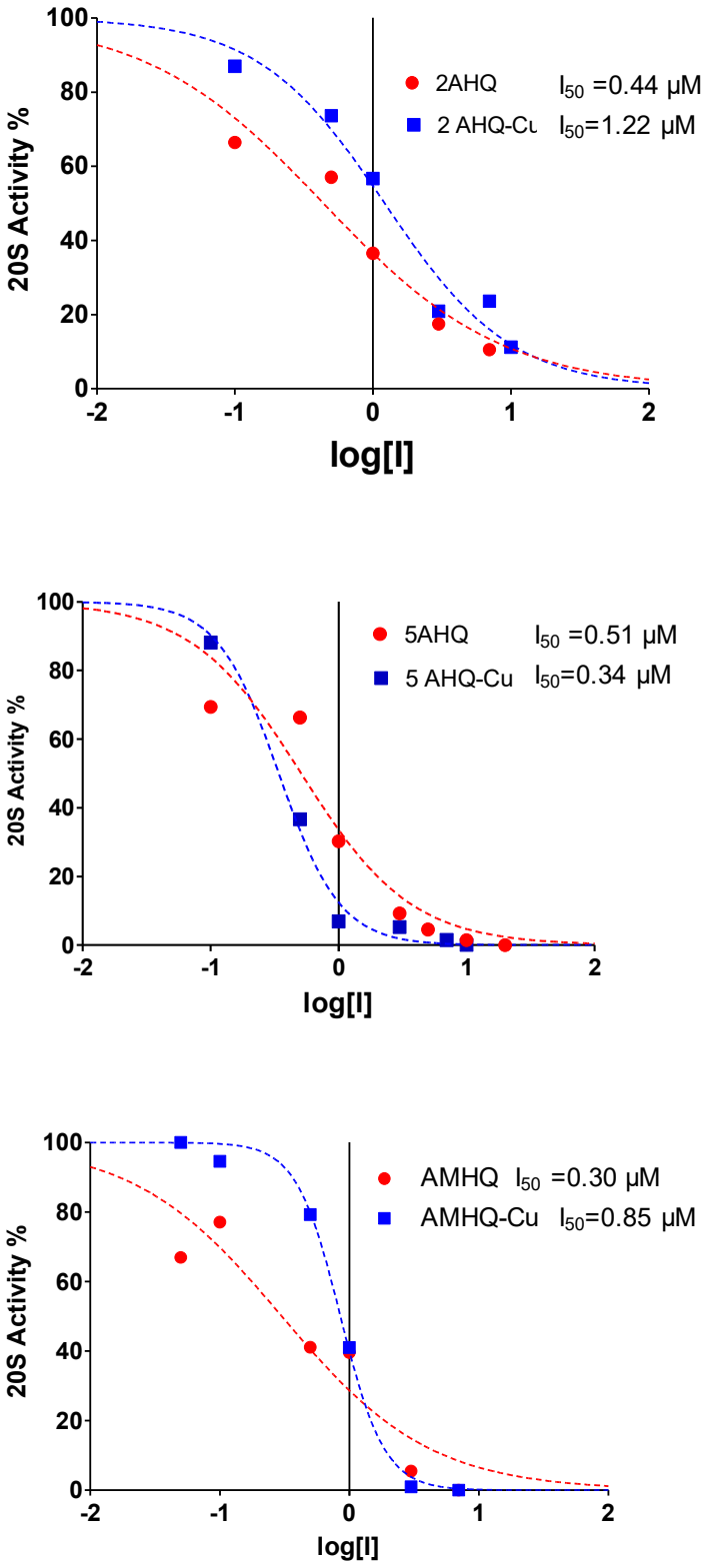

Figure S9. Normalized concentration−response plot for inhibition of ChT-L residual activities of CP reported as a semilog plot fitted by equation 1 in presence of 5-AHQ, 2-AHQ and 5-AMHQ (red points and curves) and their copper complexes (blue points and curves). I_{50} values for the distinct peptidase activities and the related fitting parameters are reported in Table S1.

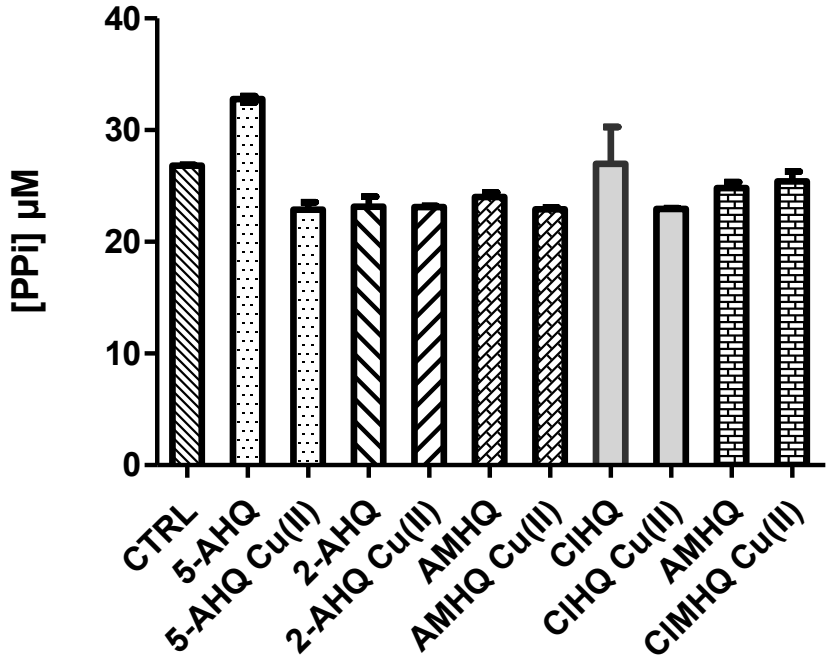

Figure S9. Spectrophotometric determination of $P_2O_7^{2-}$ anion in Lys48-linked polyubiquitination reactions in the presence of HQ compounds and their copper complexes (10 µM).