Interaction of 2,6,7-trihydroxy-xanthene-3-ones with iron and copper, and biological effect of the most active derivative on breast cancer cells and erythrocytes

SUPPLEMENTARY DATA

9 pages

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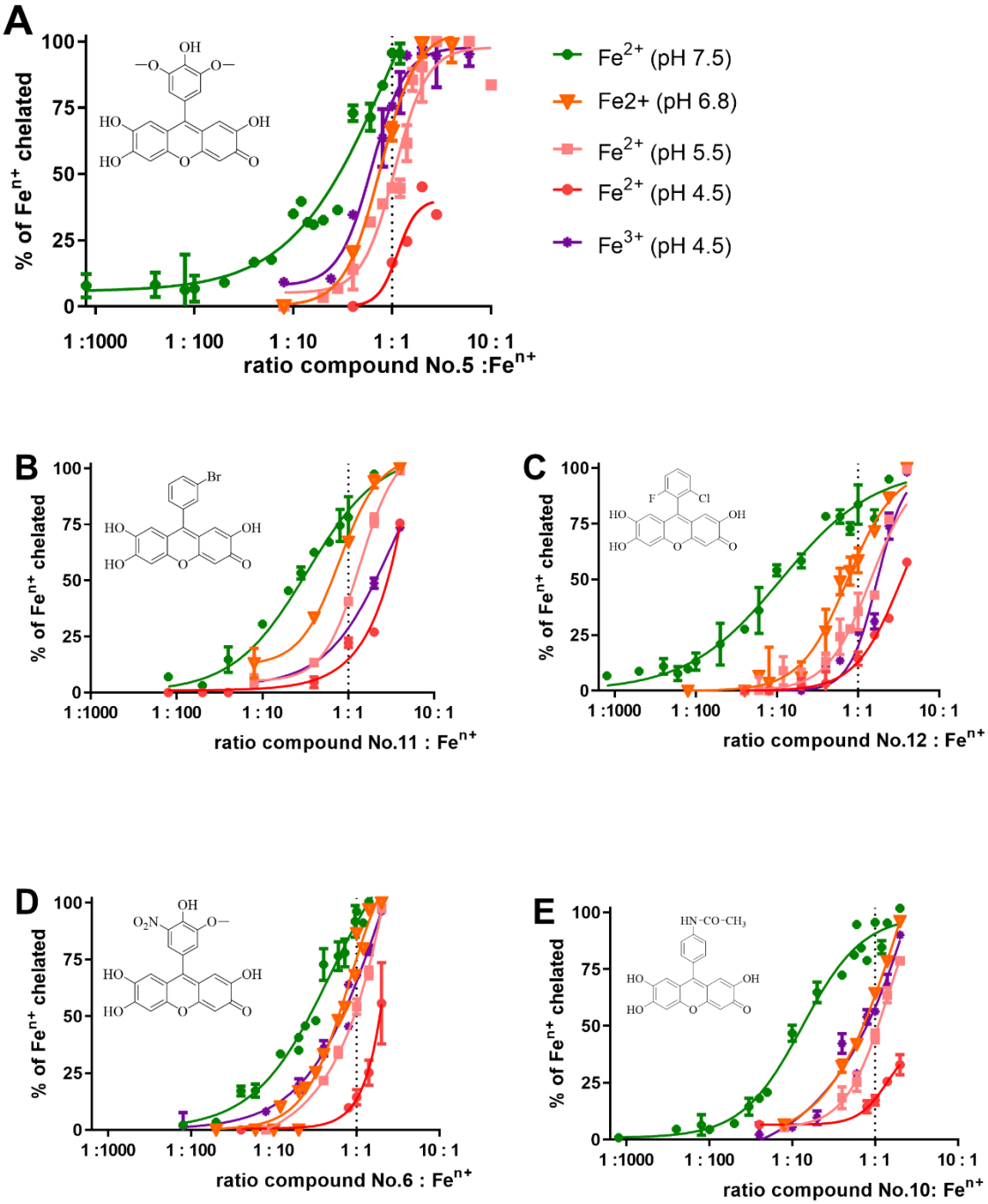

Figure S1. Iron chelation by other compounds not shown in Figure 1. A: 4´-hydroxy-3´,5´-dimethoxy (**5**), B: 3´-bromo (**11**), C: 2´-chloro-6´-fluoro (**12**), D: 4´-hydroxy-3´-methoxy-5´-nitro (**6**) and E: 4´-acetamido (**10**) derivatives.

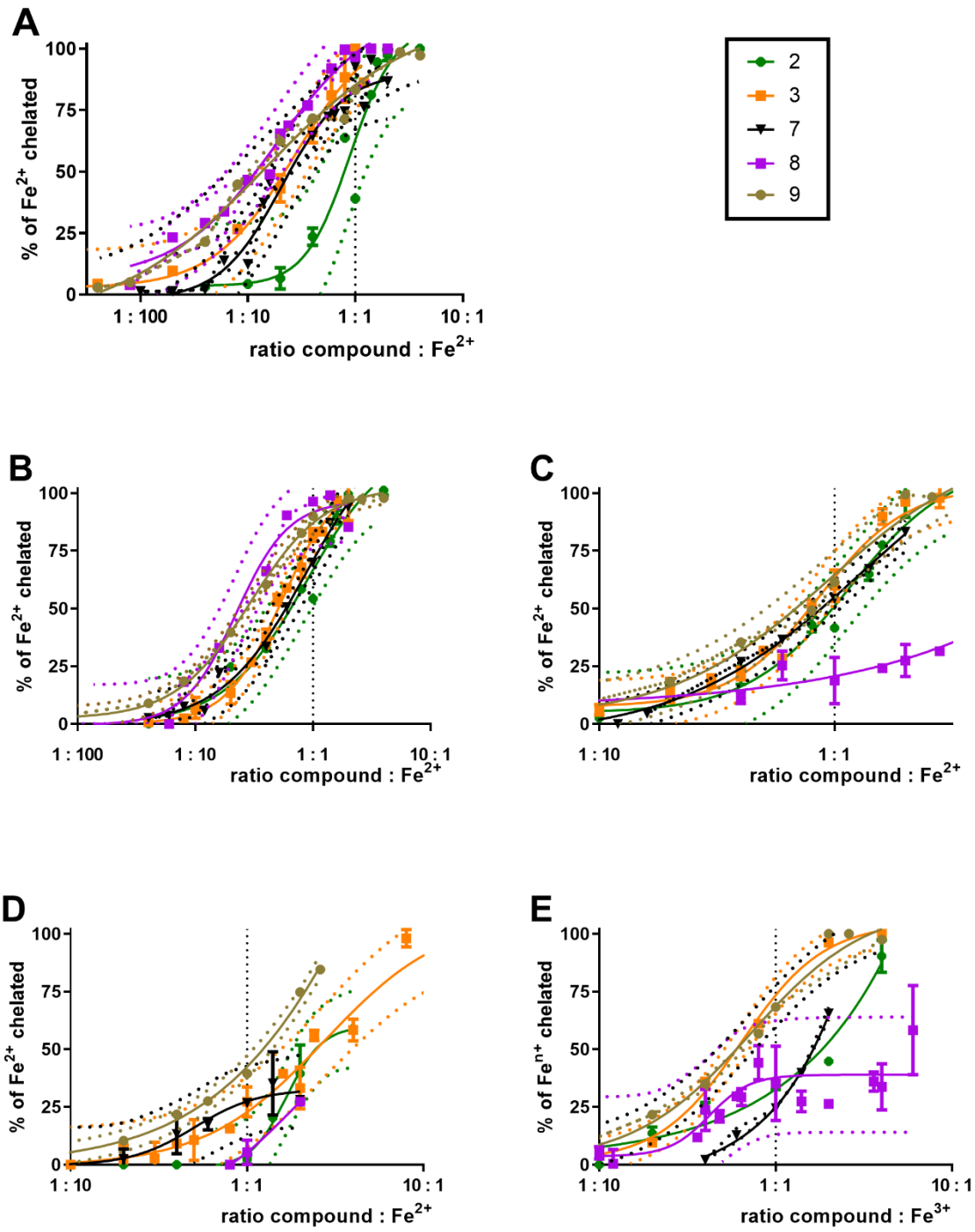

Figure S2. Comparison of iron chelation of representative compounds. A: ferrous chelation at pH 7.5, B: ferrous chelation at pH 6.8, C: ferrous chelation at pH 5.5, D: ferrous chelation at pH 4.5 and E: ferric (total) chelation at pH 4.5.

4´-trifluoromethyl (**9**), 4´-dimethylamino (**8**), 4´-ethoxy (**7**), 3´,4´-dihydroxy (**3**), 2´-hydroxy-3-methoxy (**2**) derivatives.

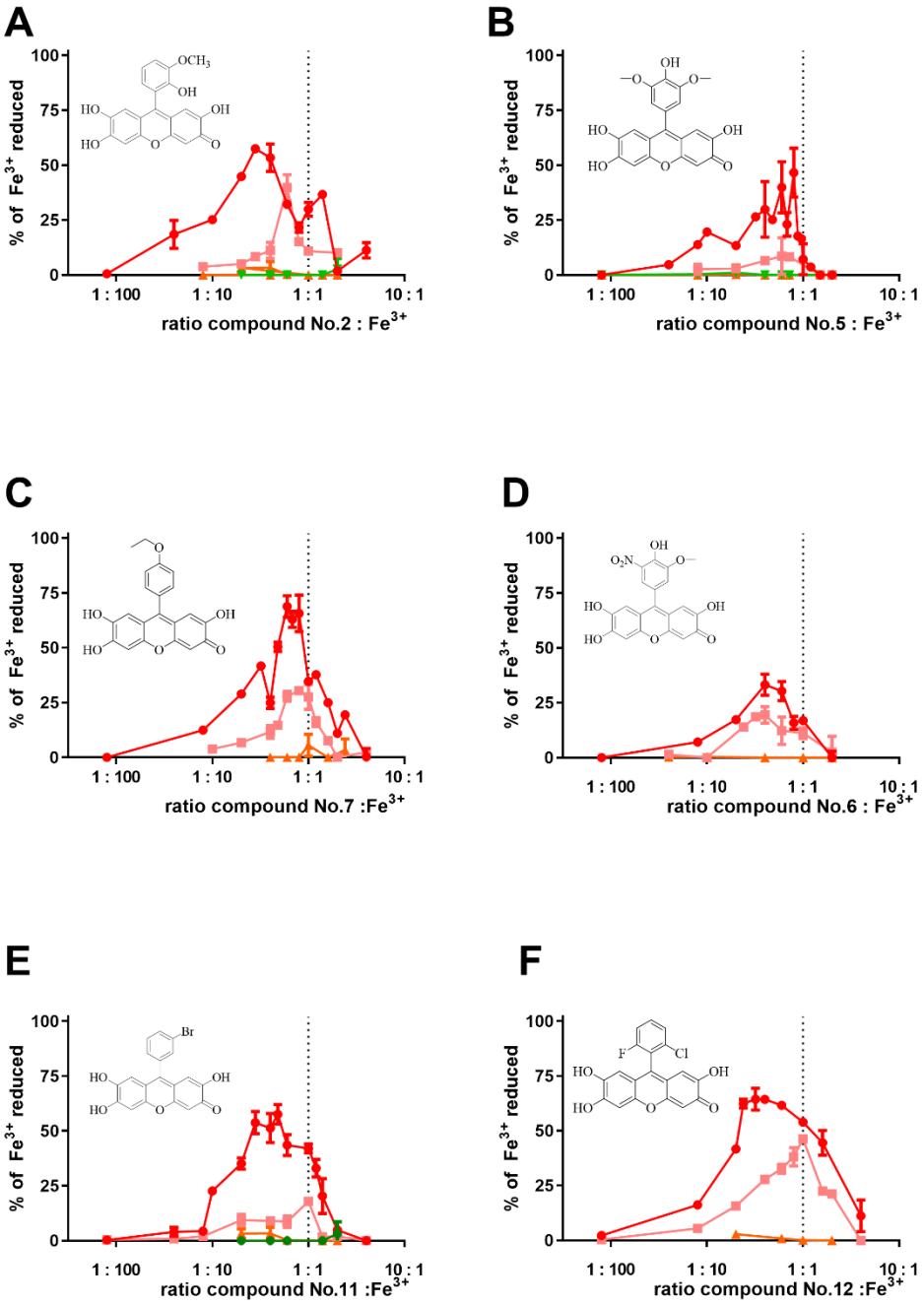

Figure S3. Iron reduction – other compounds not shown in Figure 3. Red color means pH 4.5, pink pH 5.5, orange 6.8 and green pH 7.5. A: 2´-hydroxy-3-methoxy (**2**), B: 4´-hydroxy-3´,5´-dimethoxy (**5**), C: 4´-ethoxy (**7**), D: 4´-hydroxy-3´methoxy-5´-nitro (**6**), E: 3´-bromo (**11**) and F: 2´-chloro-6´-fluoro derivatives (**12**) derivatives.

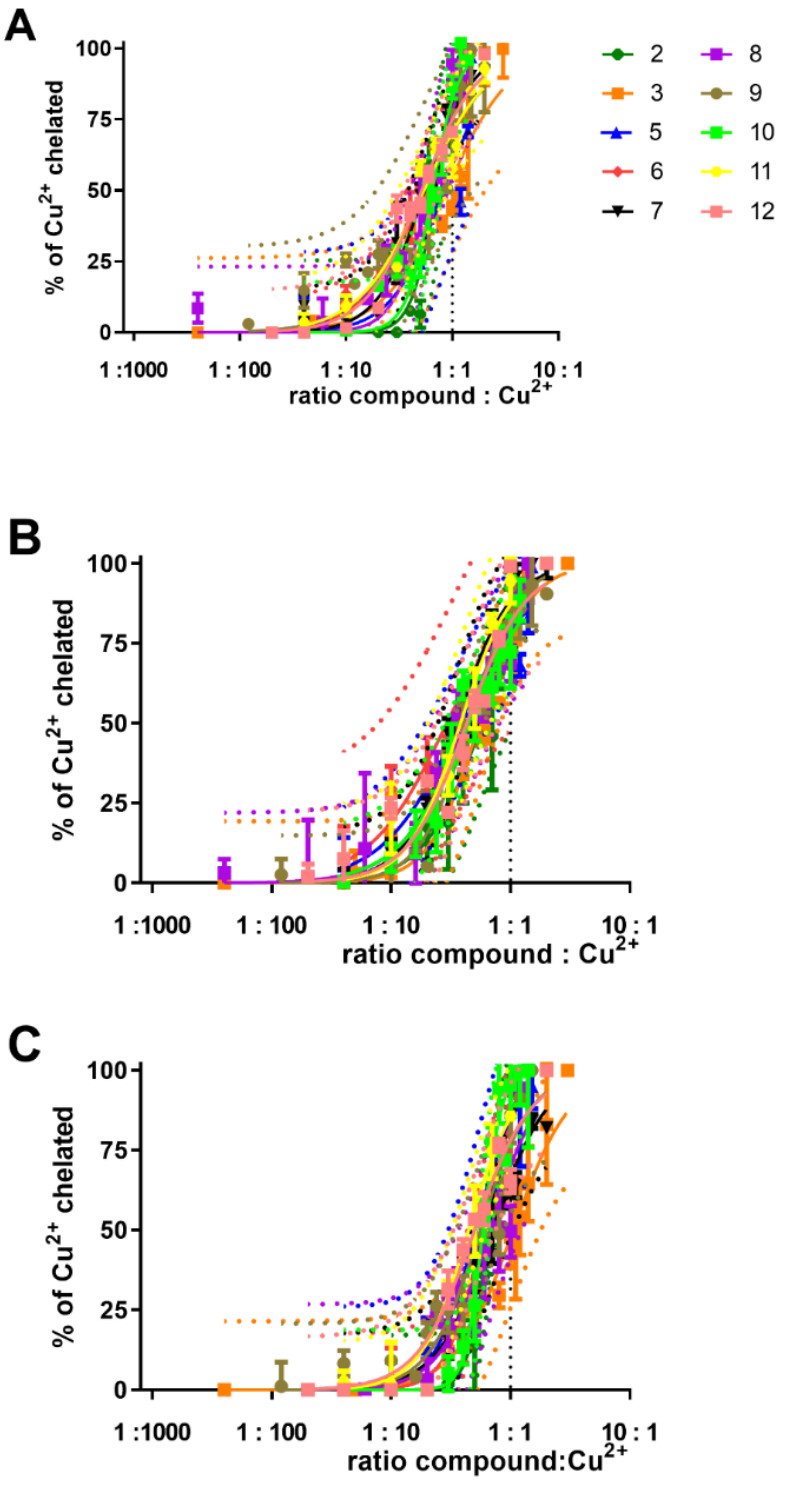

Figure S4. Comparison of cupric chelation (the hematoxylin assay). The graphs show cupric chelation at different pH conditions (A: pH 7.5, B: pH 6.8 and C: pH 5.5).

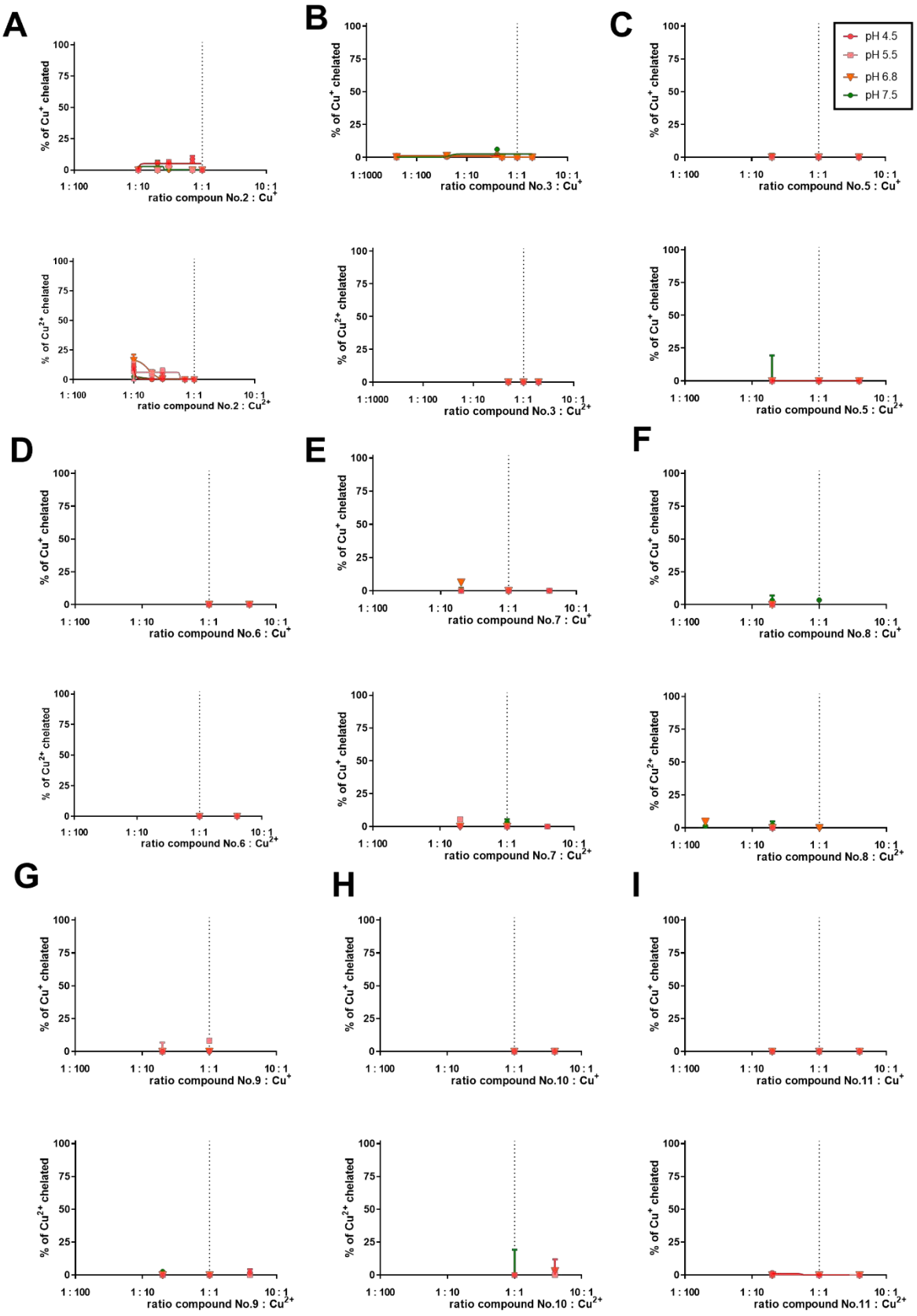

Figure S5. Chelation of copper ions assessed by the BCS method. A-I: compounds **2-11**

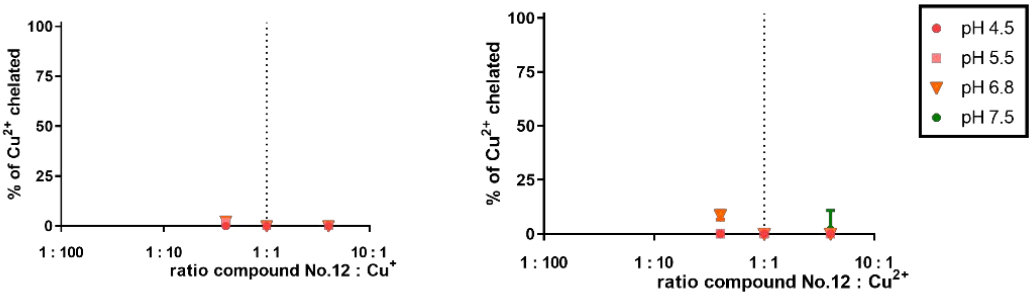

Figure S6. Chelation of cuprous and cupric ions by 2´-chloro-6´-fluoro derivative (12) assessed by the BCS method.

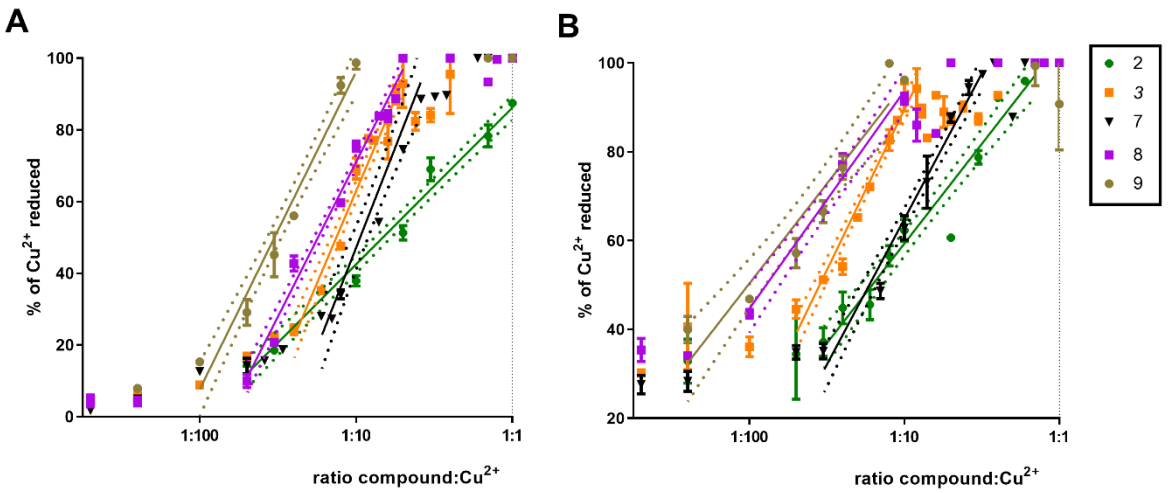

Figure S7. Example of comparisons of copper reduction lines with 95% confidence intervals. A: pH 7.5 and B: pH 4.5.

2 (2´-hydroxy-3-methoxy), **3** (3´,4´-dihydroxy), **7** (4´-ethoxy), **8** (4´-dimethylamino), **9** (4´-trifluoromethyl) derivatives

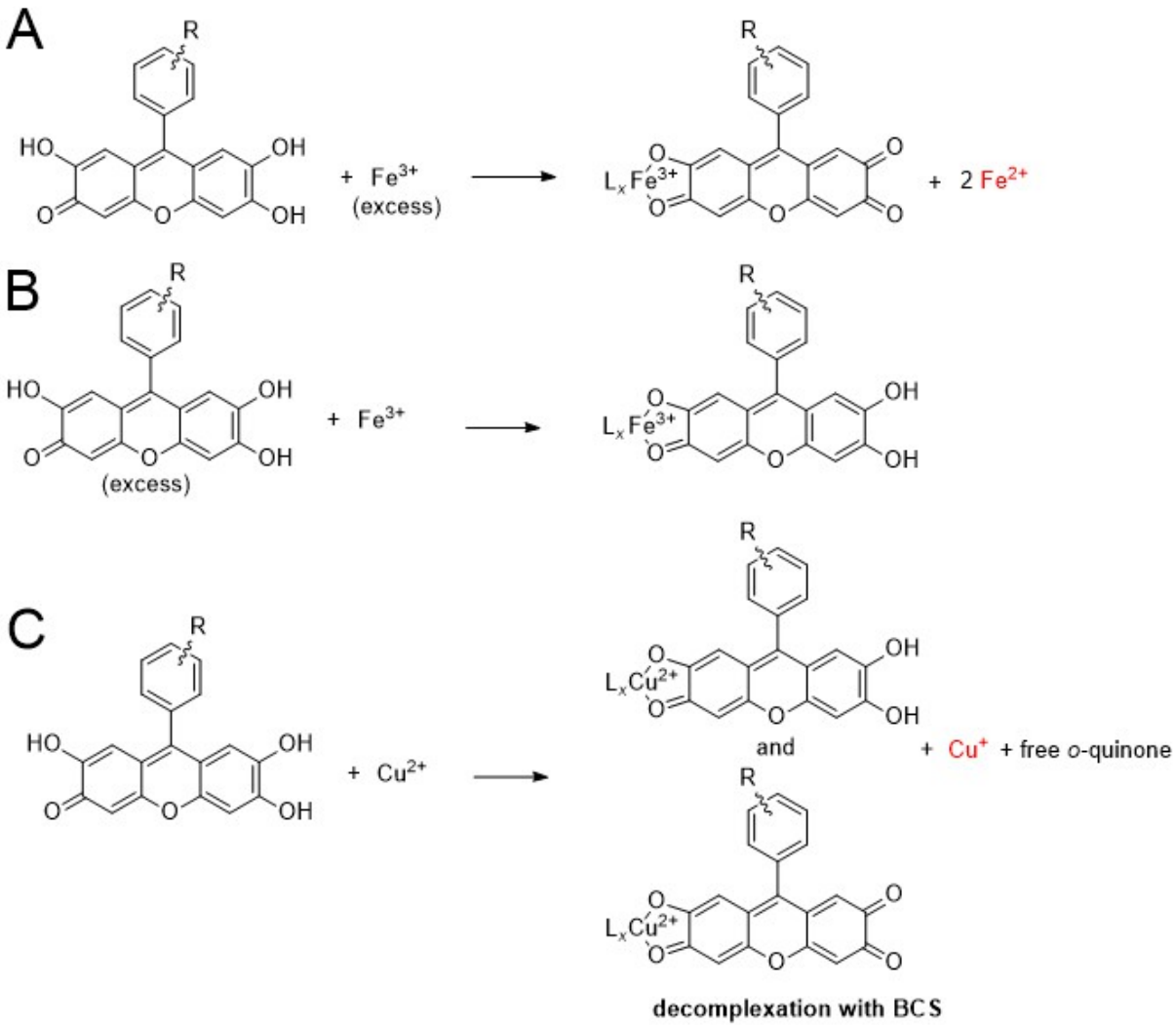

Figure S8. Likely scenarios of the interaction of Fe^{3+} and Cu^{2+} with tested xanthones. For iron, two scenarios, A and B, depending on the xanthone:metal ratio, can be envisaged. In case of excess metal, complexation followed by consecutive reduction, occur (A). With excess xanthone, all Fe^{3+} ions are just chelated, with no reduction taking place (B, see Figure 4). As regards copper (C), it is evident that the metal is reduced more easily, while the complex(es) are less stable. Thus, it is likely that the complexation and reduction events occur simultaneously and, eventually, all copper atoms end up in the form of Cu^{+} (decomplexation is facilitated by the addition of BCS; see Figure 5).

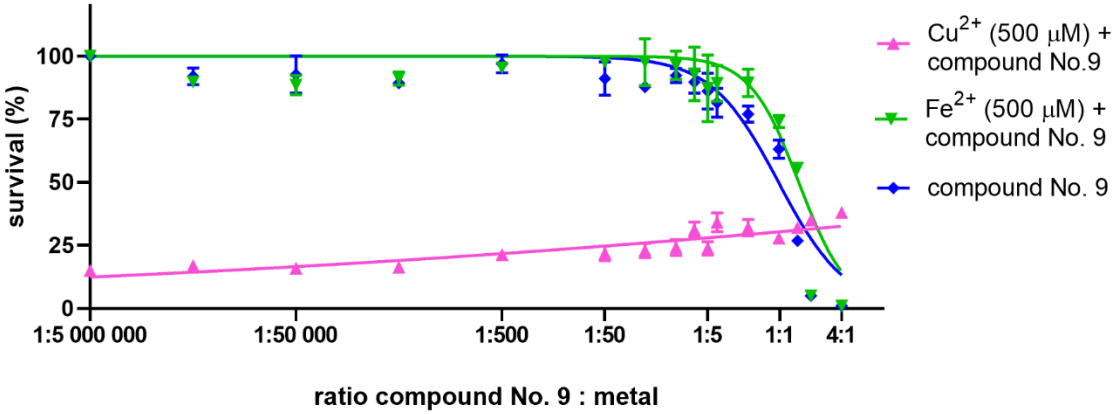

Figure S9. Cytotoxicity of 4'-trifluoromethyl derivate (9) alone and in combination with Cu^{2+} or Fe^{2+} at concentration 500 µM. Viability was determined by cell viability assay after 48 h treatment in MCF7/182R-6 cells. Vehicle-treated cells were set as 100% viability.

Table S1. Cytotoxicity of Cu^{2+}, Fe^{2+} and 4'-trifluoromethyl derivate (**9**) alone and in combination.

	MCF7/182R-6	
	IC_{50}	Viability at 10 µM
Cu^{2+}	406.41 ± 1.04	99.57 ± 6.18
Fe^{2+}	>750	96.57 ± 6.04
compound No.9	447.88 ± 1.13	91.11 ± 6.63
Cu^{2+} (500 µM) + compound No.9	n.a.	21.83 ± 2.96
Fe^{2+} (500 µM) + compound No.9	>750	97.82 ± 1.03

n.a.: not applicable

Viability was determined by cell viability assay 48 hours after treatment in MCF7/182R-6 cells. Vehicle-treated cells were set as 100% viability.