SUPPLEMENTARY MATERIAL

Metformin, Cu(II)/Metformin and Cu(I)/Metformin complexes antitµMoral activity.

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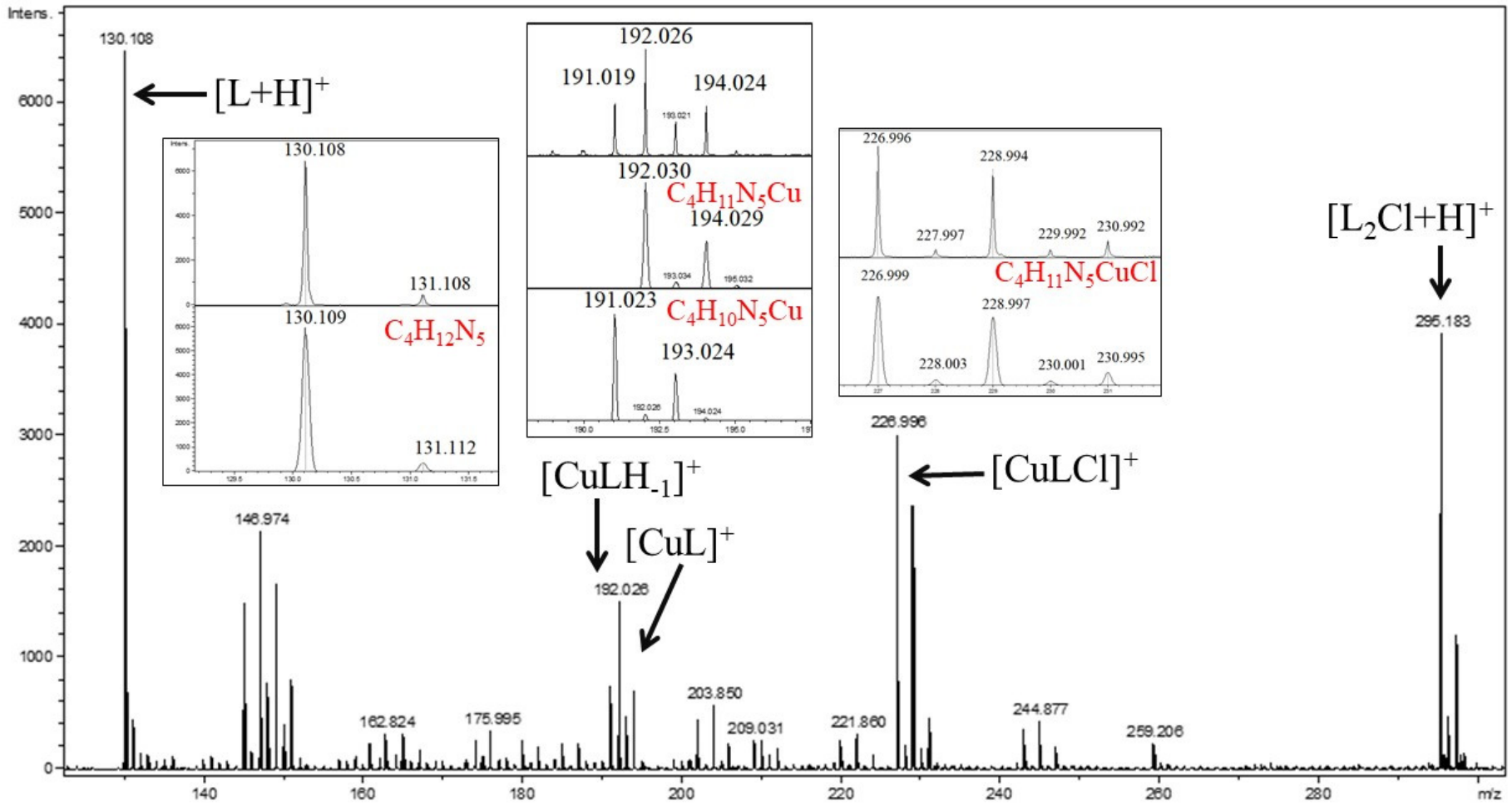
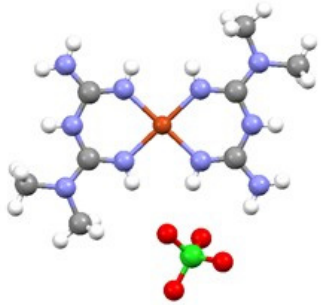


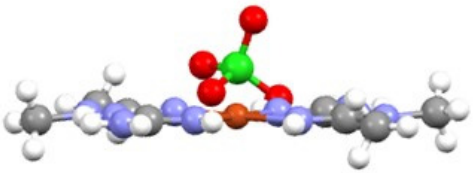
Figure S1. ESI-MS spectrµM of metformin and Cu(II)/Metformin complexes. Enlargement boxes: Comparison of signals of Copper/(metformin (=L) complexes with simulated spectra (Down).

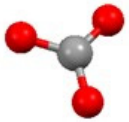
FRONT VIEW

LATERAL VIEW

A







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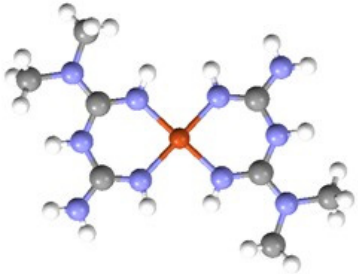
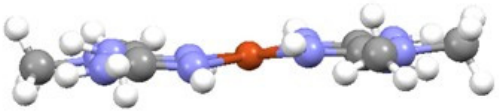
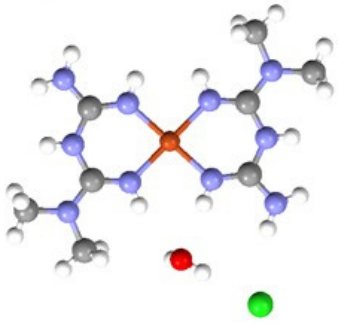
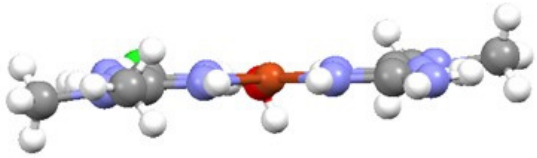




C





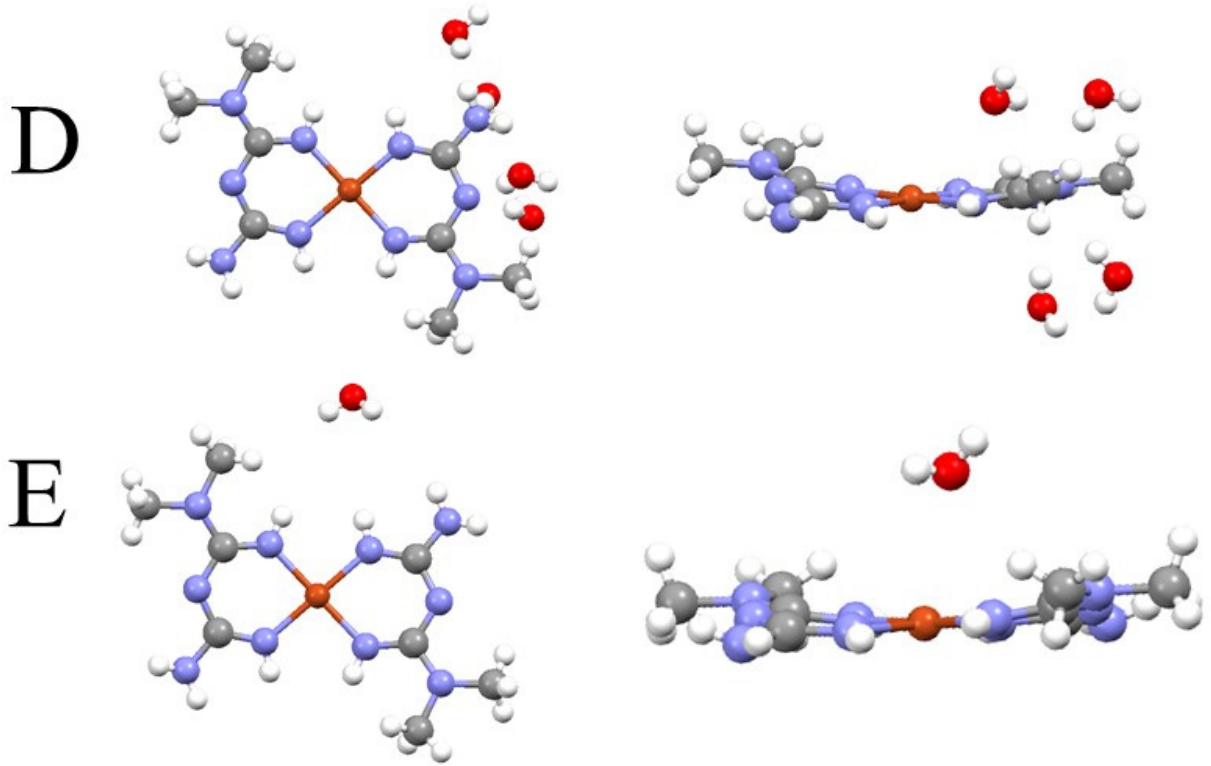


Figure S2. X-ray structures AJUHUJ (A), HIBPOX (B), HIHDUX (C), EFIXμM (D) and ETOFOI (E) of Cu(II)/Metformin complexes in different experimental conditions. Data available in CCDS database (https://www.ccdc.cam.ac.uk/structures/).

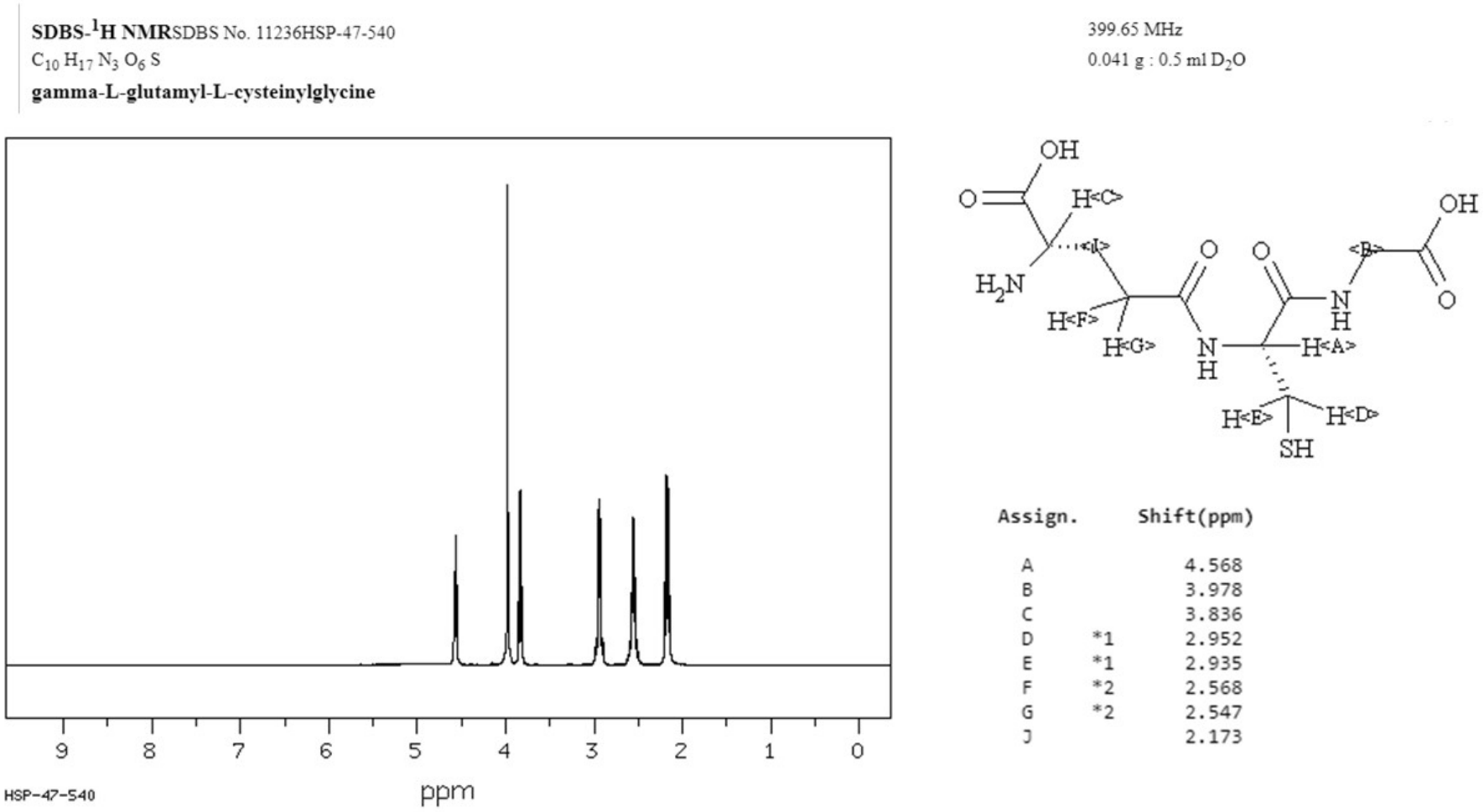
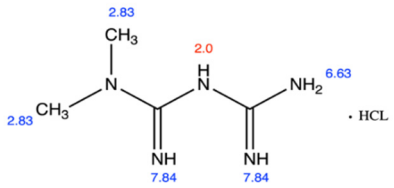
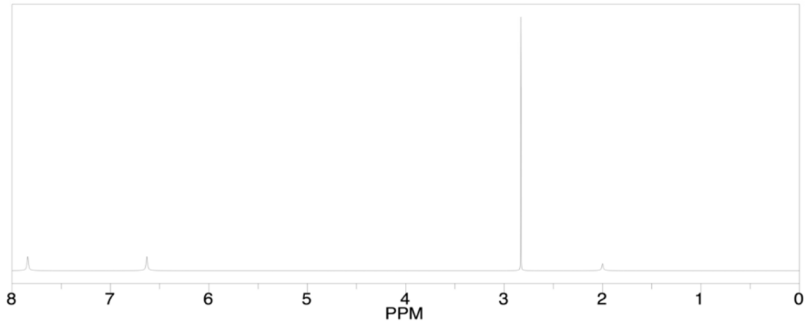


Figure S3. 1HNMR spectrμM and proton assigned of GSH according to SDBS database (https://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi).

ChemNMR ^{1}H Estimation



Estimation quality is indicated by color: good, medium, rough

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Protocol of the H-1 NMR Prediction (Lib=SU Solvent=DMSO 300 MHz):

Node      Shift    Base + Inc.   Comment (ppm rel. to TMS)

NH   7.84              7.50      guanidine
                        0.34        general corrections
NH   7.84              7.50      guanidine
                        0.34        general corrections
NH   2.0               2.00      guanidine
                          ?         1 unknown substituent(s) from sec. amide
                          ?         1 unknown substituent(s) from sec amine
                          ?         1 unknown substituent(s) from amine
                                    -> 2 increment(s) not found
NH2 6.63              2.00      guanidine
                        4.63        general corrections
CH3 2.83              0.86      methyl
                        1.81        1 alpha -N-C=R
                        0.16        general corrections
CH3 2.83              0.86      methyl
                        1.81        1 alpha -N-C=R
                        0.16        general corrections

1H NMR Coupling Constant Prediction

shift   atom index  coupling partner, constant and vector

7.84        5
7.84        6
2.0         2
6.63        4
2.83        8
2.83        9
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Figure S4. ^{1}H NMR prediction of metformin by Chemdraw.

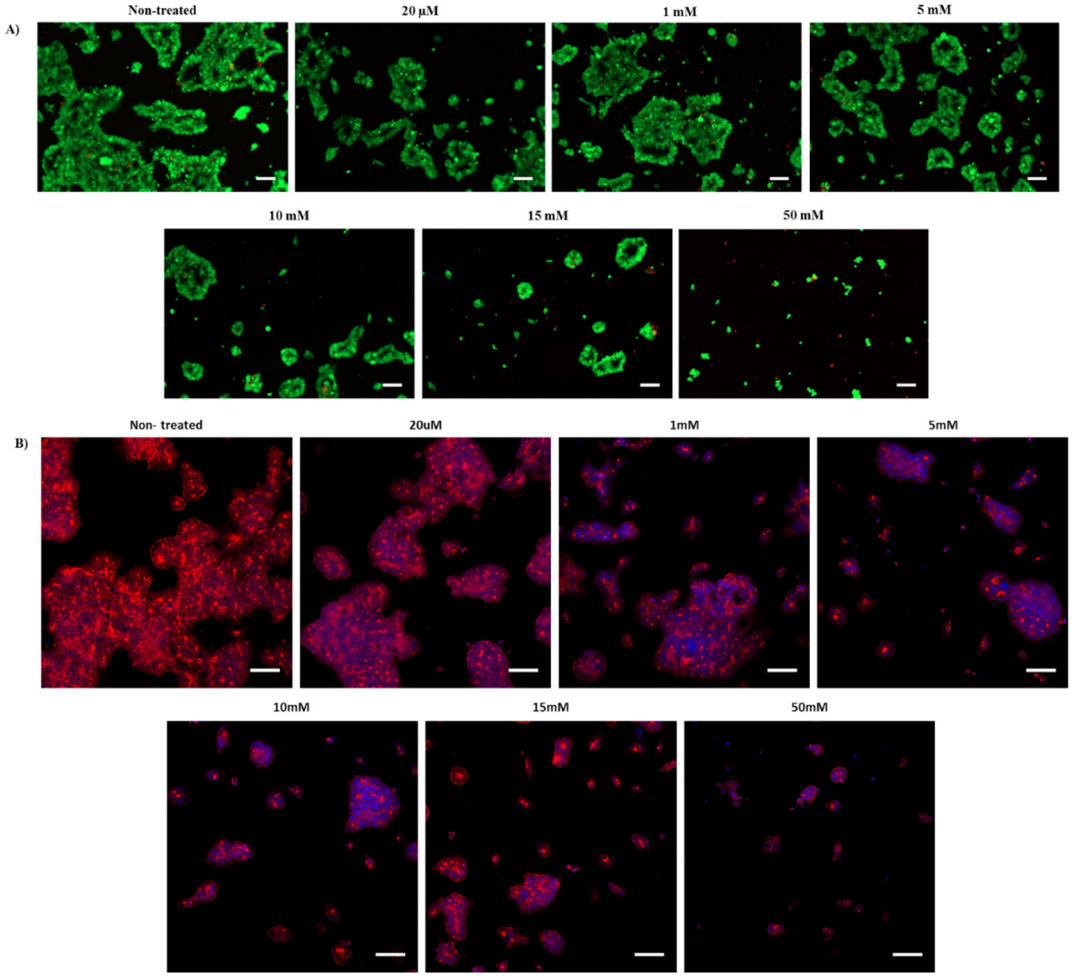

Figure S5. Anticancer effect of metformin on colorectal cancer (SW1222) cells. (A) Live/dead staining of SW1222 cells treated with different concentrations of metformin. The fluorescent images show live cells (green) and dead cells (red). (B) High resolution confocal images of SW1222 showing actin fibers organization (red) and nucleus (blue). Scale bar (100 µm).

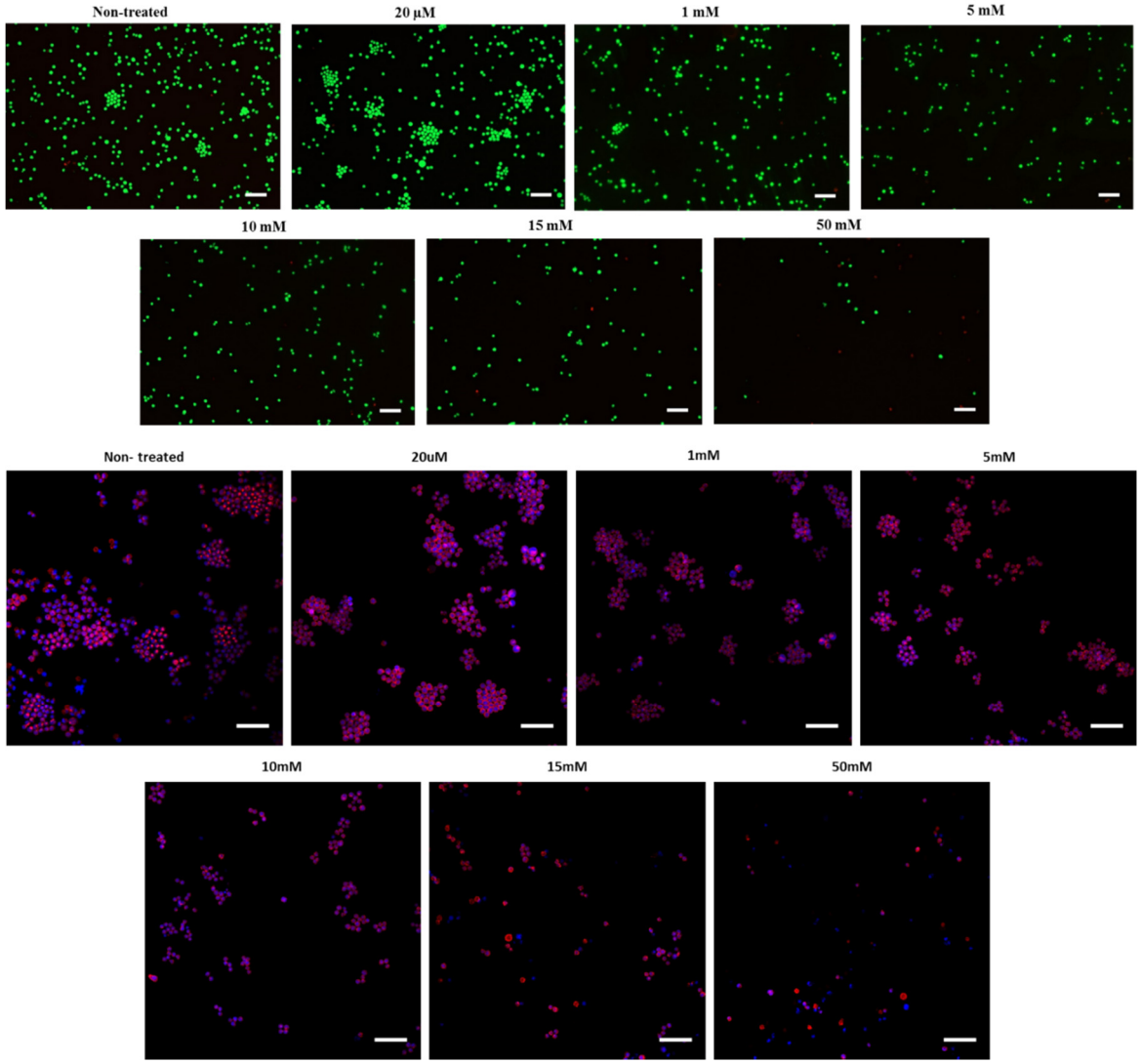

Figure S6. Anticancer effect of metformin on leukemic cell line (K562). (A) Live/dead staining of K562 cells treated with different concentrations of metformin. The fluorescent images show live cells (green) and dead cells (red). (B) High resolution confocal images of K652 showing actin fibers organization (red) and nucleus (blue). Scale bar (100 µm).

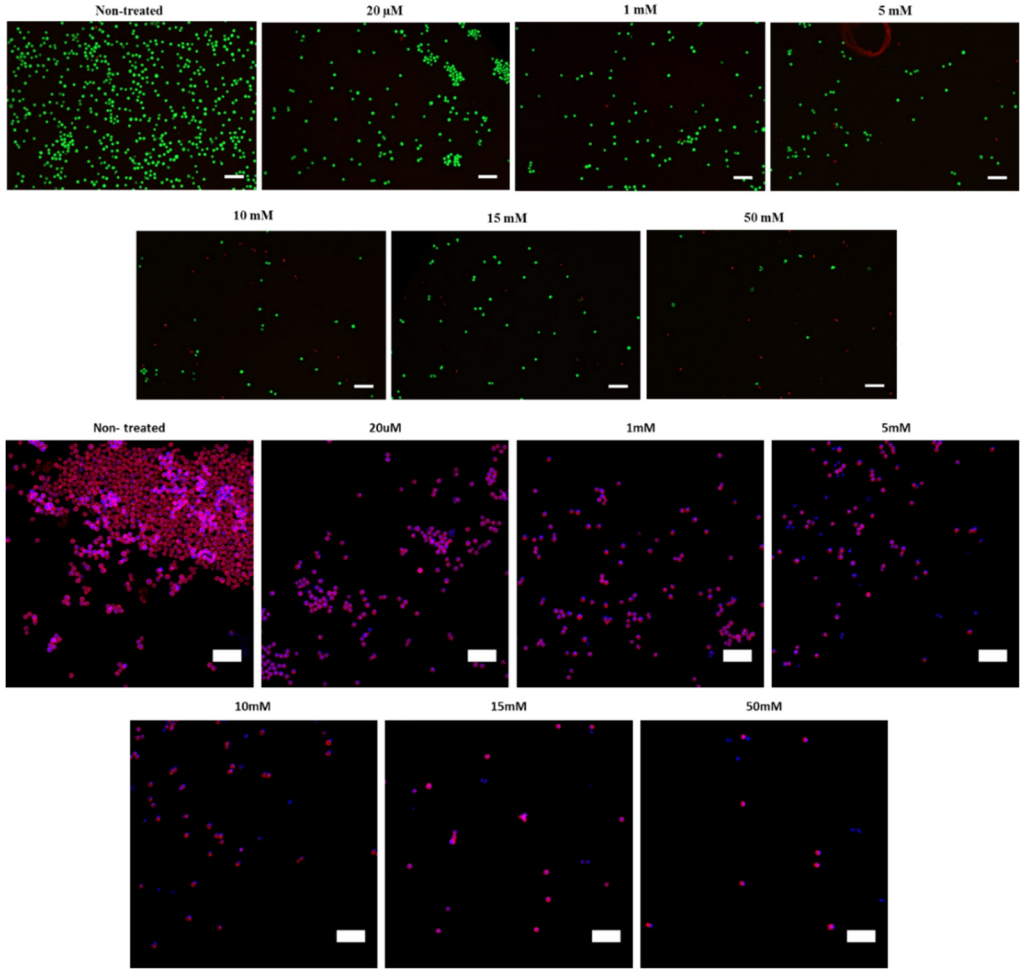

Figure S7. Anticancer effect of metformin on MDR leukemic cell line (K562-200). (A) Live/dead staining of K562-200 cells treated with different concentrations of metformin. The fluorescent images show live cells (green) and dead cells (red). (B) High resolution confocal images of K652-200 showing actin fibers organization (red) and nucleus (blue). Scale bar (100 µm).

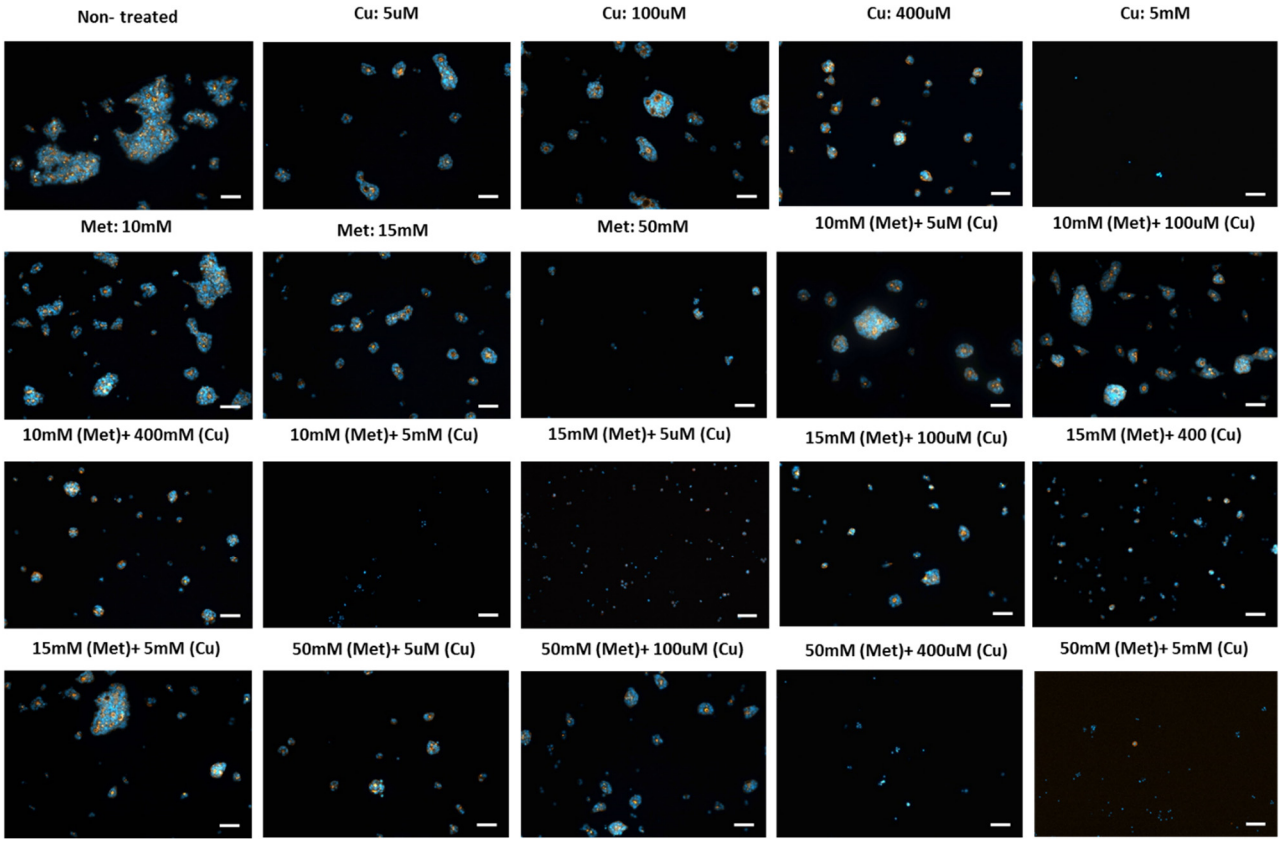

Figure S8. Anticancer effect of metformin on SW1222 cells in the presence and absence of copper sulfate. Scale bar (100 μM). The fluorescent images show cells shrinking and rounding with increasing concentrations of metformin with complete disappearance of actin fibers at the highest concentration used.

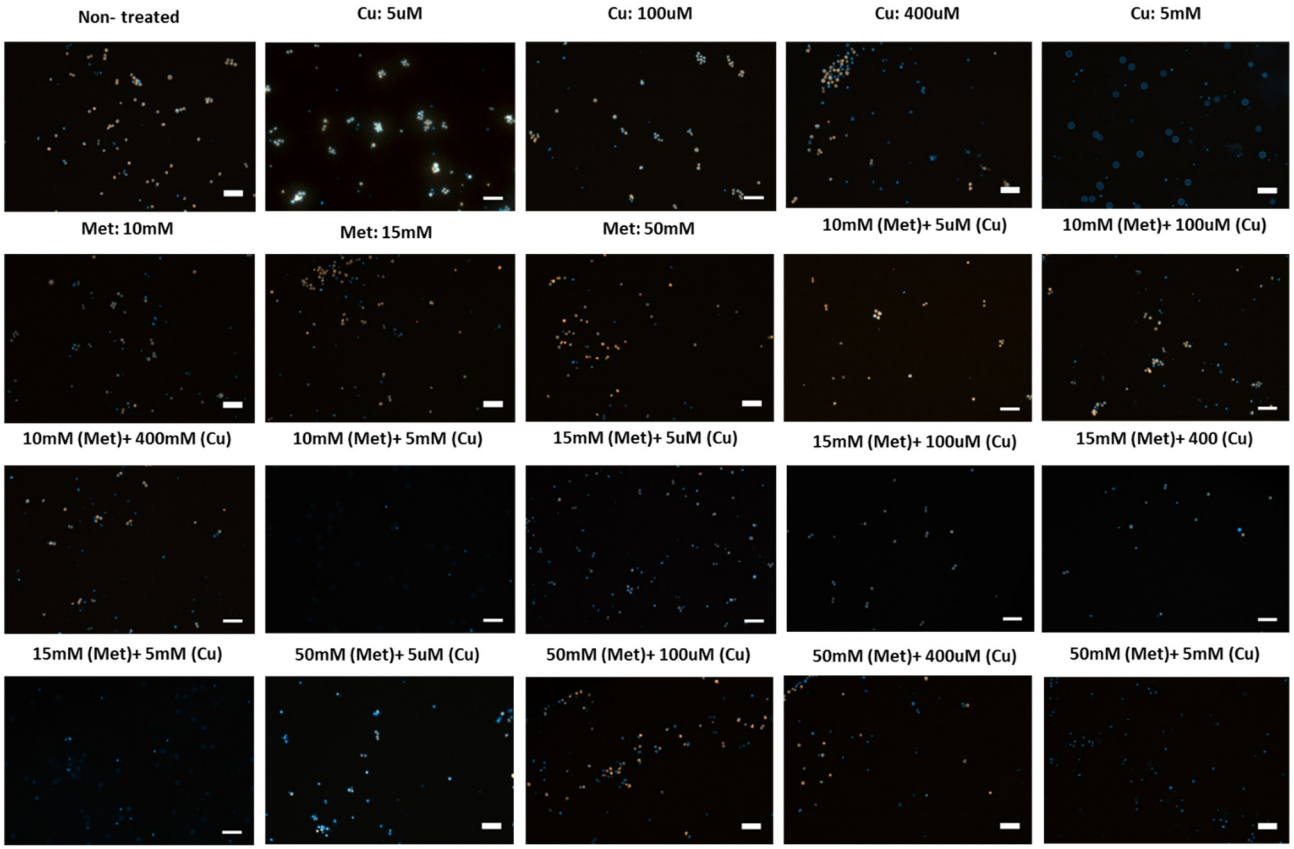

Figure S9. Anticancer effect of metformin on K562 cells in the presence and absence of copper sulfate. Scale bar (100 μM). The fluorescent images show cells shrinking and rounding with increasing concentrations of metformin with complete disappearance of actin fibers at the highest concentration used.

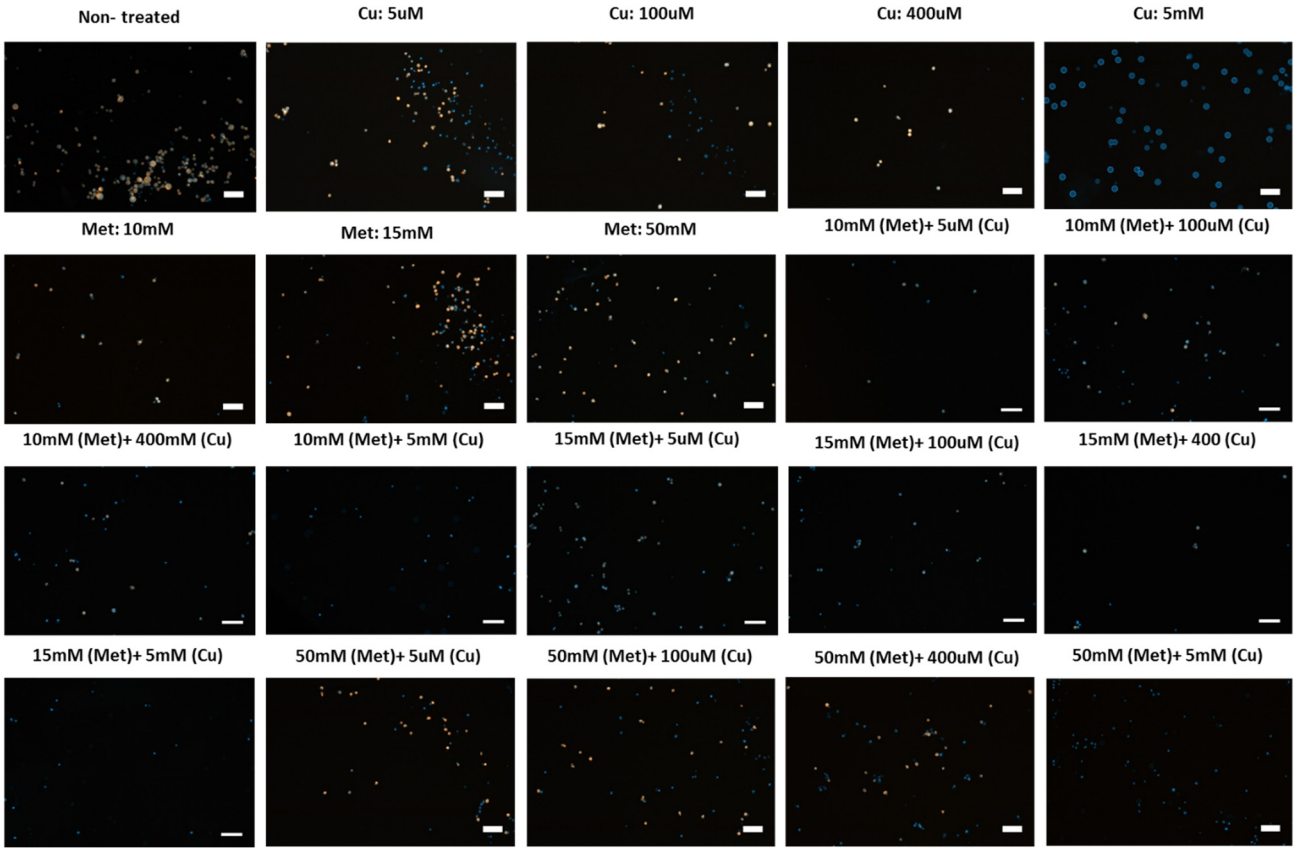

Figure S10. Anticancer effect of metformin on K562-200 cells in the presence and absence of copper sulfate. Scale bar (100 µM). The fluorescent images show cells shrinking and rounding with increasing concentrations of metformin with complete disappearance of actin fibers at the highest concentration used.

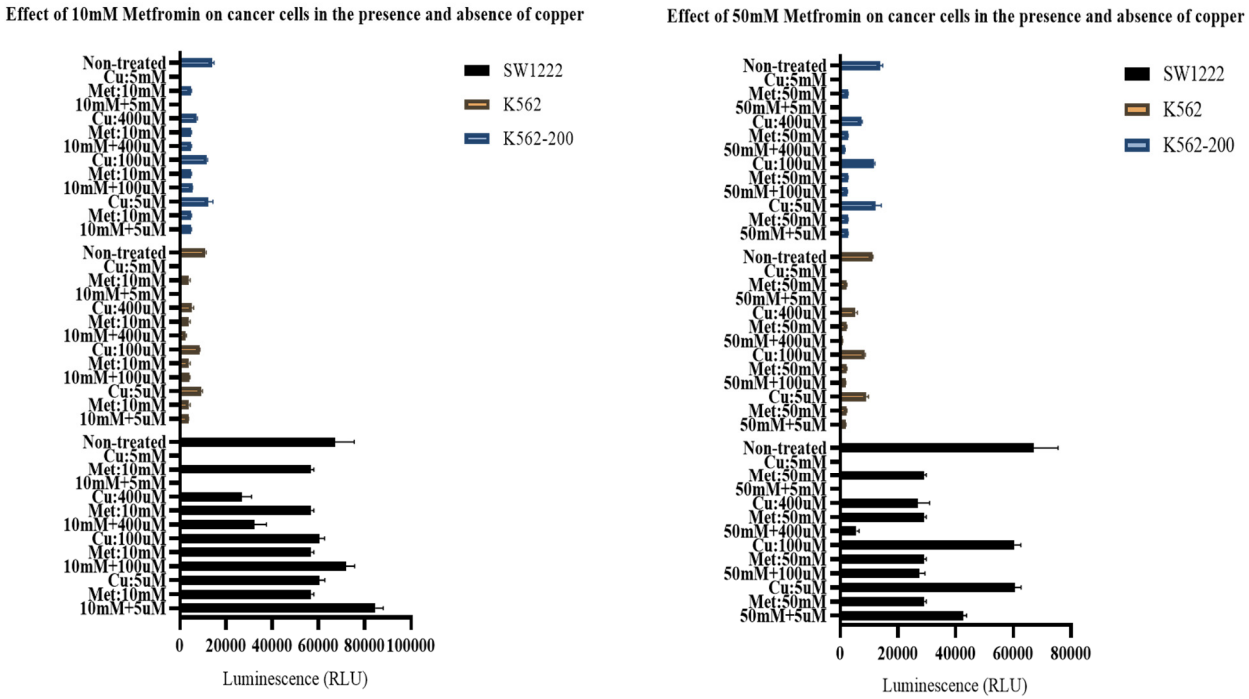

Figure S11. Effect of copper on the anticancer properties of metformin. Comparison between the effect on cancer cells of 10 mM (left) and 50 mM (right) metformin in the absence and presence of different concentrations of copper sulfate and the effect of copper sulfate on the same cells in the absence of metformin. (error bars different color)

Table S1: Effect of Cu(II) on the anticancer properties of metformin.

Concentration (Metformin)	Concentration Cu(II)	SW1222		K562		K562-200	
		% of Normalized ATP Values vs. Non Treated Cells	*P* Value	% of Normalized ATP Values vs. Non Treated Cells	*P* Value	% of Normalized ATP Values vs. Non Treated Cells	*P* Value
10 mM	5 µM	126	0.05	33.5	<0.001	34.8	0.002
10 mM	100 µM	107.2	0.4	37.7	<0.001	38.7	0.003
10 mM	400 µM	48	0.006	22.5	<0.001	34	0.001
10 mM	5 mM	0.27	0.005	0.36	<0.001	0.34	0.001
15 mM	5 µM	112.5	0.2	41.2	<0.001	34.1	0.003
15 mM	100 µM	83.4	0.2	33.5	<0.001	35	0.003
15 mM	400 µM	37.9	0.01	19.6	<0.001	15.3	<0.001
15 mM	5 mM	0.12	0.005	0.32	<0.001	0.27	0.001
50 mM	5 µM	63.5	0.03	17.5	<0.001	19.7	0.002
50 mM	100 µM	40.9	0.01	16.9	<0.001	17.9	0.002
50 mM	400 µM	8.3	0.006	6.8	<0.001	11.8	0.002
50 mM	5 mM	0.16	0.005	0.32	<0.001	0.27	0.001
10 mM	0	84.6	0.2	35.2	<0.001	34	0.001
15 mM	0	71.2	0.04	34.9	<0.001	27.3	0.003
50 mM	0	43.4	0.02	20.2	<0.001	19.5	0.002
0	5 µM	90.1	0.3	81.8	0.03	88.5	0.3
0	100 µM	89.9	0.3	76.5	<0.001	84.9	0.05
0	400 µM	39.9	0.005	47.2	0.002	52.7	0.003
0	5 mM	0.12	0.005	0.31	<0.001	0.31	0.001

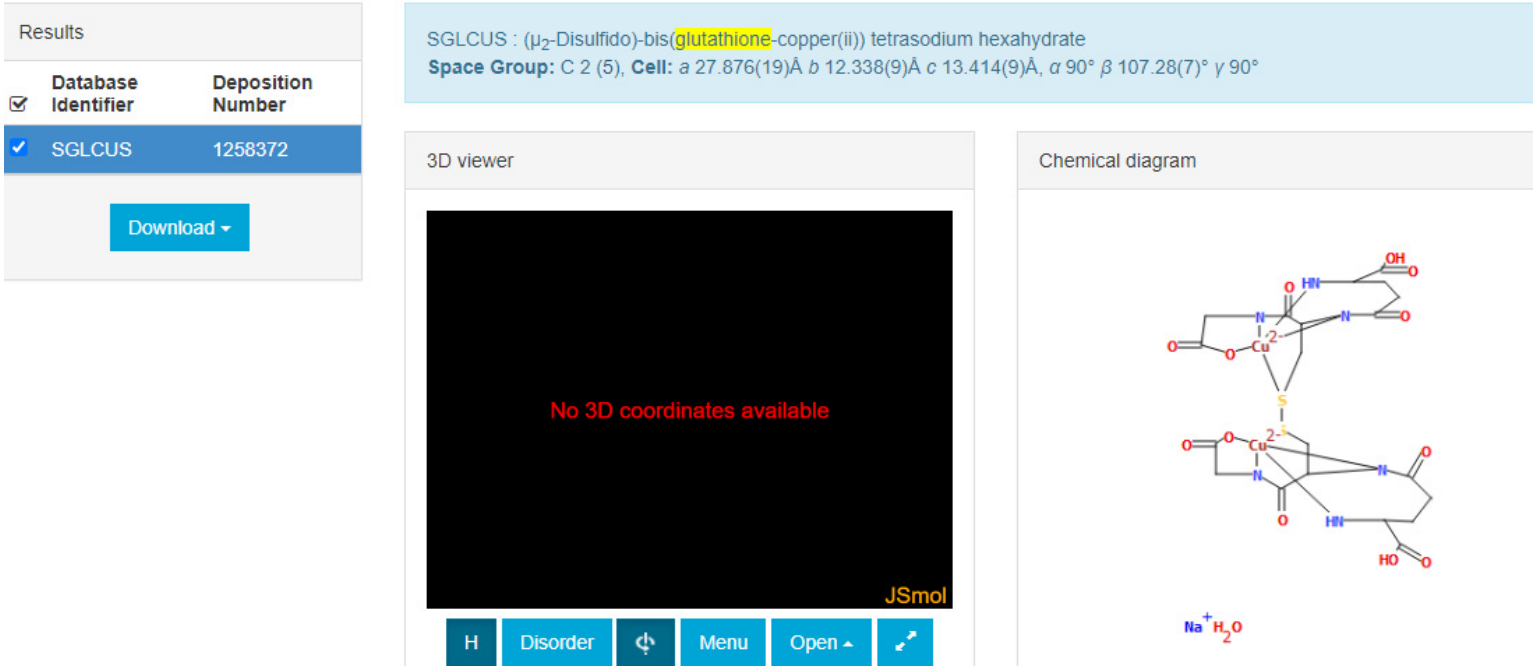

Figure S12. Chemical diagram of the X-ray structures of Cu(II)/GSSG complex [1]. Data available in CCDS database (https://www.ccdc.cam.ac.uk/structures/).

1 Miyoshi, K., Sugiura, Y., Ishizu, K., Iitaka, Y. & Nakamura, H. Crystal structure and spectroscopic properties of violet glutathione-copper (II) complex with axial sulfur coordination and two copper sites via a disulfide bridge. *Journal of the American Chemical Society* 102, 6130-6136 (1980).