



Supplementary Materials

Differences in the Formation of Reactive Oxygen Species and Their Cytotoxicity between Thiols Combined with Aqua- and Cyanocobalamins

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Table S1. Dependence of integral chemiluminescence response ($\text{JLCL} \pm \text{SD}$) on the concentration of the compounds.

HOCbl (25 μM) + Reducing agent					
[Reducing agent], μM	AA	NAC	DTT	TNB	GSH
0	5008 \pm 360	4377 \pm 504	3369 \pm 288	4934 \pm 384	4934 \pm 456
15.6	5372 \pm 216	4325 \pm 528	31,657 \pm 4320#	2977\pm312*	4567\pm384*
31.3	11,379\pm2640*#	4380\pm480*	100,763 \pm 5760#	3034\pm408*	6490\pm504*
62.5	59,570\pm4560*#	4950\pm432*	252,490 \pm 8880#	3020\pm360*	12,064\pm768*#
125	125,013\pm5520*#	17,473\pm1536*#	539,531\pm7920*#	3130\pm384*	28,783\pm4320*#
250	136,746\pm5760*#	40,352\pm14,160*#	831,704\pm19,680*#	3465\pm264*	70,924\pm5040*#
500	105,146\pm7925*#	42,465 \pm 7440#	419,433\pm13,920*#	4190\pm264*	44,043\pm11,040*#
1000	43,015\pm3782*#	22,705 \pm 4560#	95,182 \pm 6960#	3027 \pm 432	31,535 \pm 8880#
CNCbl (25 μM) + reducing agent					
[Reducing agent], μM	AA	NAC	DTT	TNB	GSH
0	5219 \pm 240	3349 \pm 240	4150 \pm 528	4934 \pm 408	4934 \pm 384
15.6	4465 \pm 624	4965 \pm 264	48,507 \pm 5040#	23,727\pm4080*#	55,652\pm3120*#
31.3	4559\pm288*	11,628\pm2640*#	126,176 \pm 5040#	60,410\pm3840*#	95,679\pm2880*#
62.5	4567\pm576*	42,791\pm4320*#	257,989 \pm 8640#	119,158\pm3360*#	147,798\pm4080*#
125	4690\pm336*	146,049\pm13,440*#	266,179\pm26,640*#	147,779\pm3840*#	210,120\pm4320*#
250	4594\pm408*	141,838\pm11,629*#	215,574\pm35,520*#	155,870\pm4800*#	212,621\pm8880*#
500	4742\pm384*	52,359 \pm 8561#	130,578\pm29,520*#	70,343\pm2183*#	81,134\pm2232*#
1000	4716\pm288*	18,474 \pm 4806#	71,179 \pm 13,200#	6801 \pm 660	36,722 \pm 2040#

* significant differences between the combinations of a reducing agent with CNCbl and HOCbl (bold); # significant differences between the cobalamin-reducing agent combinations and the cobalamin (baseline), $p < 0.05$.

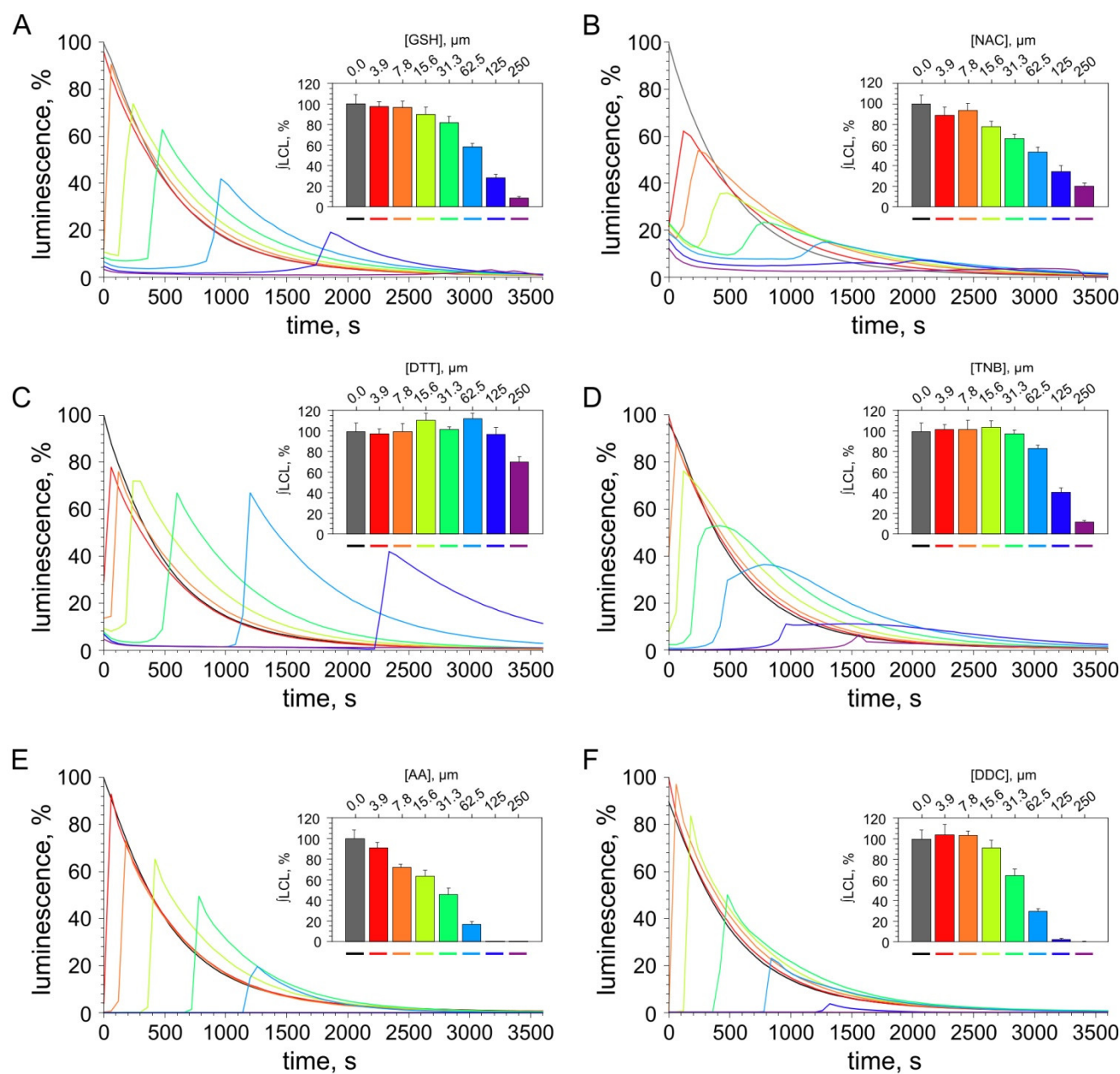


Figure S1. Effect of compounds being tested on chemiluminescence: (A) GSH, (B) NAC, (C) DTT, (D) TNB, (E) AA, (F) DDC. Inserts: Dependence of integral chemiluminescence response (I_{LCL}) on the concentration of the compounds.

The antioxidant activity of compounds was estimated by luminol-dependent chemiluminescence (LCL) as described previously [doi.org/10.3390/antiox10081262].

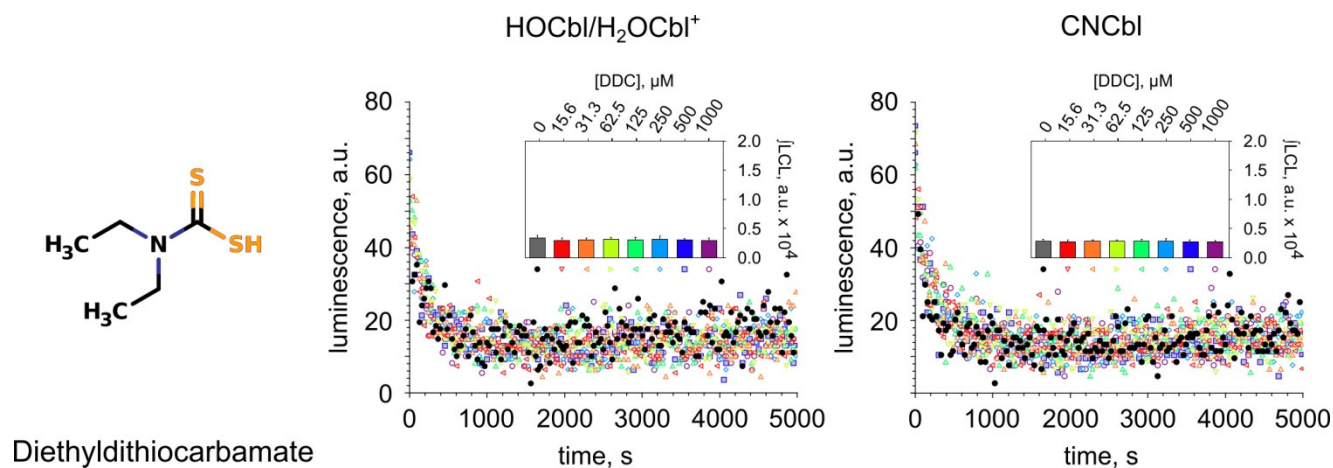


Figure S2. Chemiluminescence recorded during the oxidation of DDC in the presence of cobalamins. Inserts: Dependence of integral chemiluminescence response (JLCL) on the concentration of DDC.

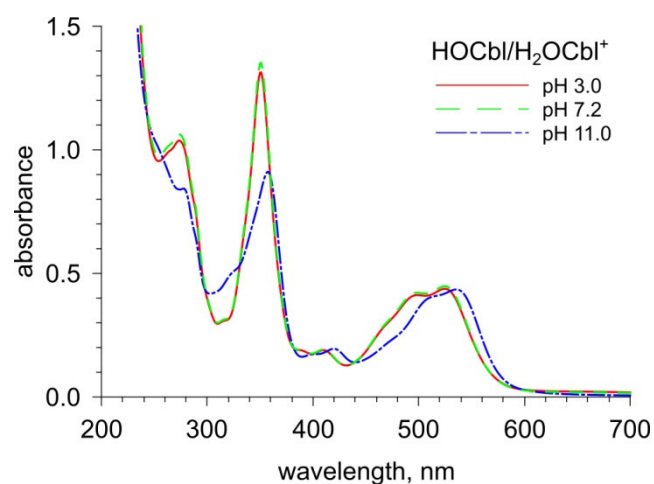


Figure S3. UV/Vis absorption spectra H₂OCbl⁺/HOCbl at different pH values. The concentration of HOCbl is 50 μM.

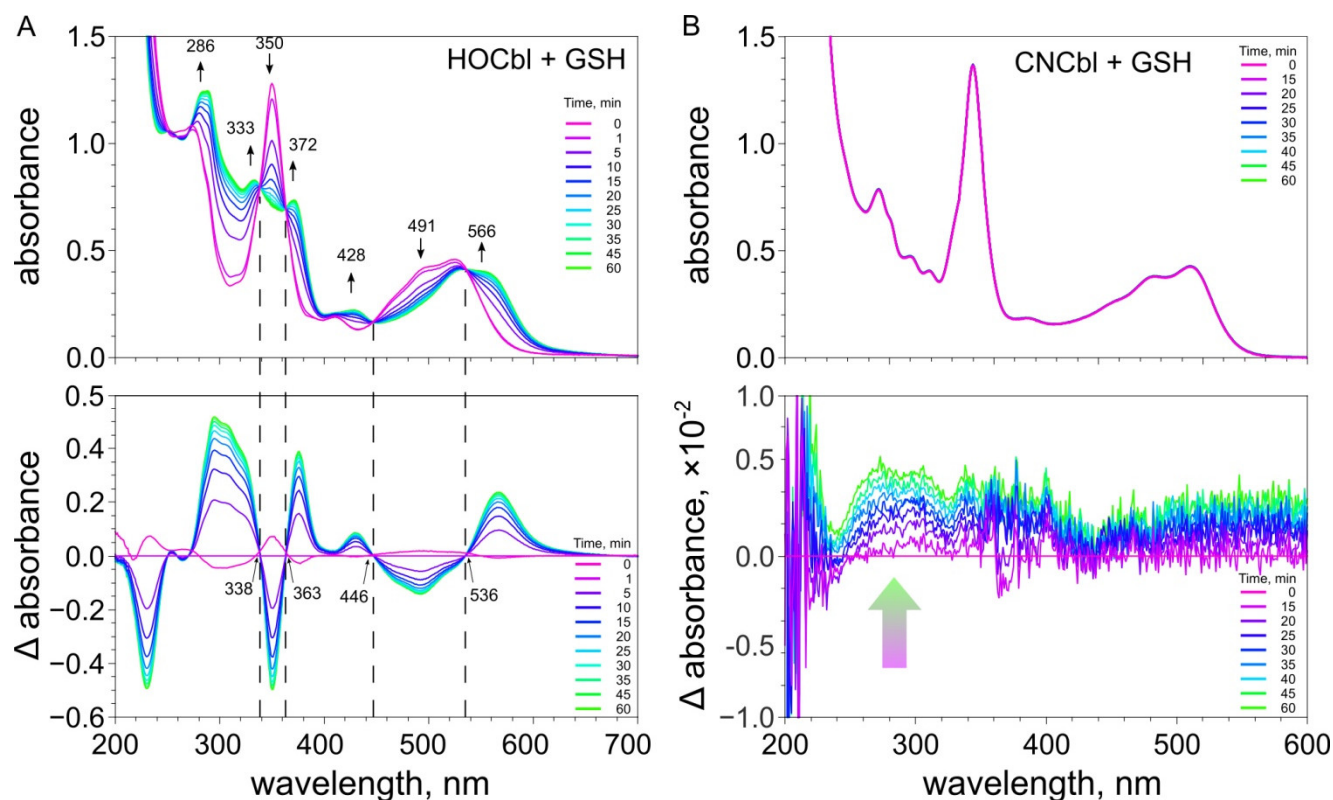


Figure S4. UV-Vis spectra recorded during the reactions between cobalamins and GSH. (A) - $\text{H}_2\text{OCbl}^+/\text{HOCbl}$, (B) - CNCbl . Above: absorption spectra, below: difference spectra.

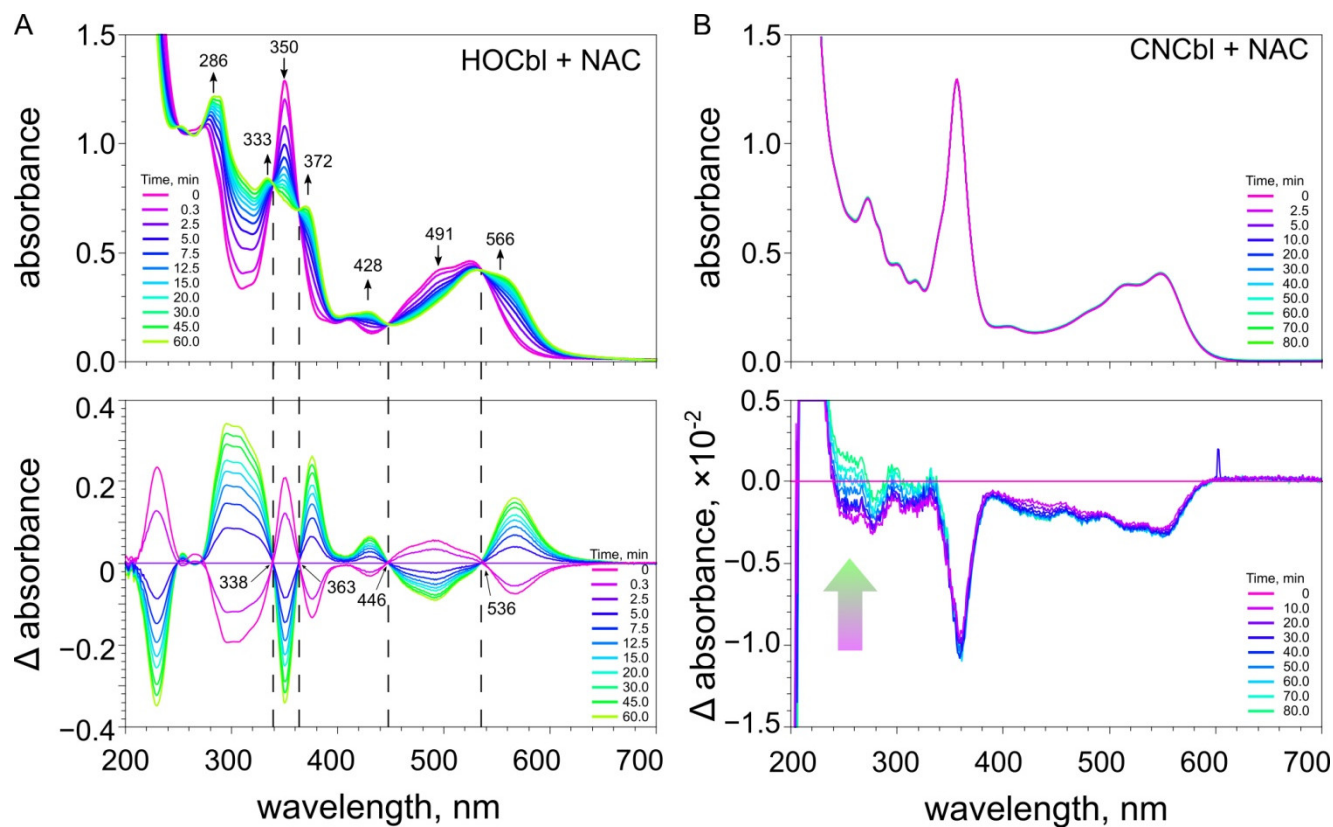


Figure S5. UV-Vis spectra recorded during the reactions between cobalamins and NAC. (A) - $\text{H}_2\text{OCbl}^+/\text{HOCbl}$, (B) - CNCbl . Above: absorption spectra, below: difference spectra.

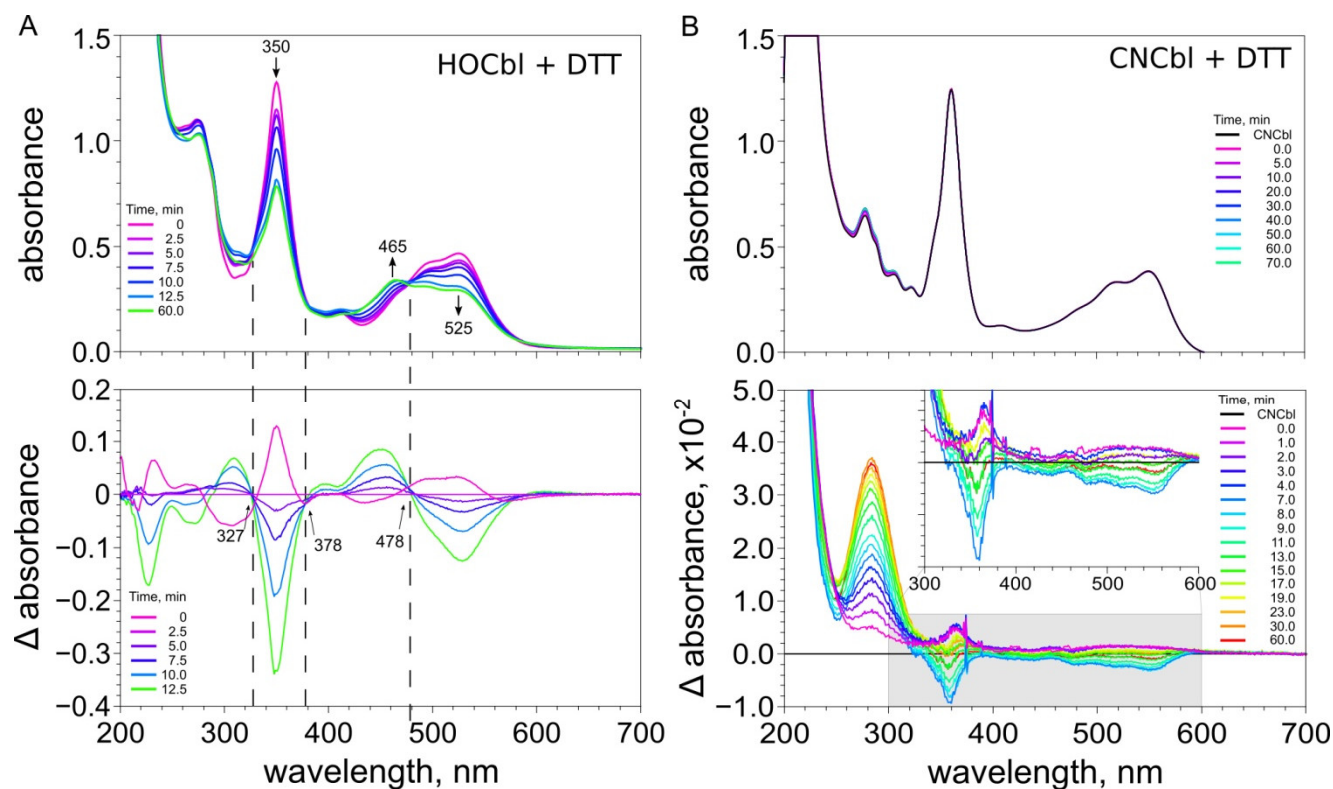


Figure S6. UV-Vis spectra recorded during the reactions between cobalamins and DTT. (A) - $\text{H}_2\text{OCbl}^+/\text{HOCbl}$, (B) - CNCbl . Above: absorption spectra, below: difference spectra. Inset: The difference spectra (300–600 nm) are shown in an enlarged y-axis.

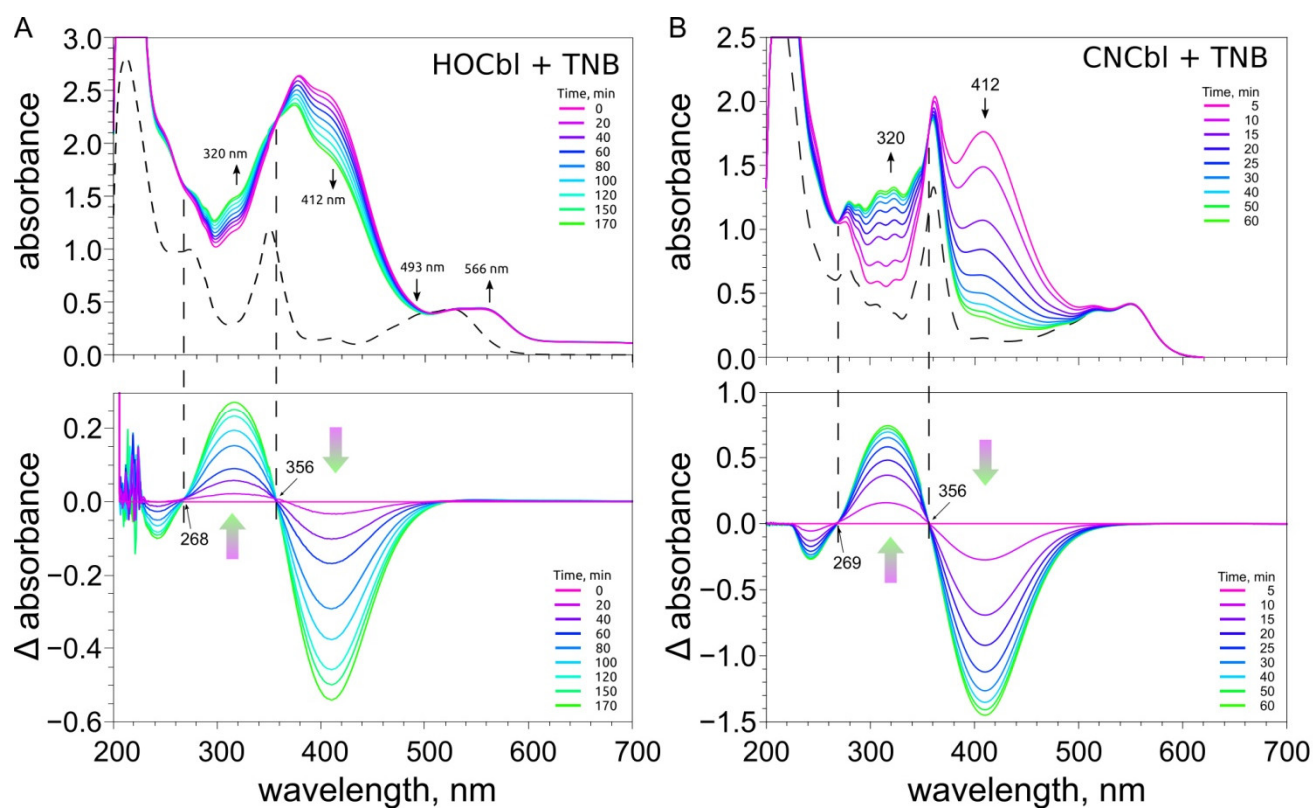


Figure S7. UV-Vis spectra recorded during the reactions between cobalamins and TNB. (A) - $\text{H}_2\text{OCbl}^+/\text{HOCbl}$. Dashed line indicates the spectrum of $\text{H}_2\text{OCbl}^+/\text{HOCbl}$. (B) - CNCbl . Dashed line indicates the spectrum of CNCbl . Above: absorption spectra, below: difference spectra.

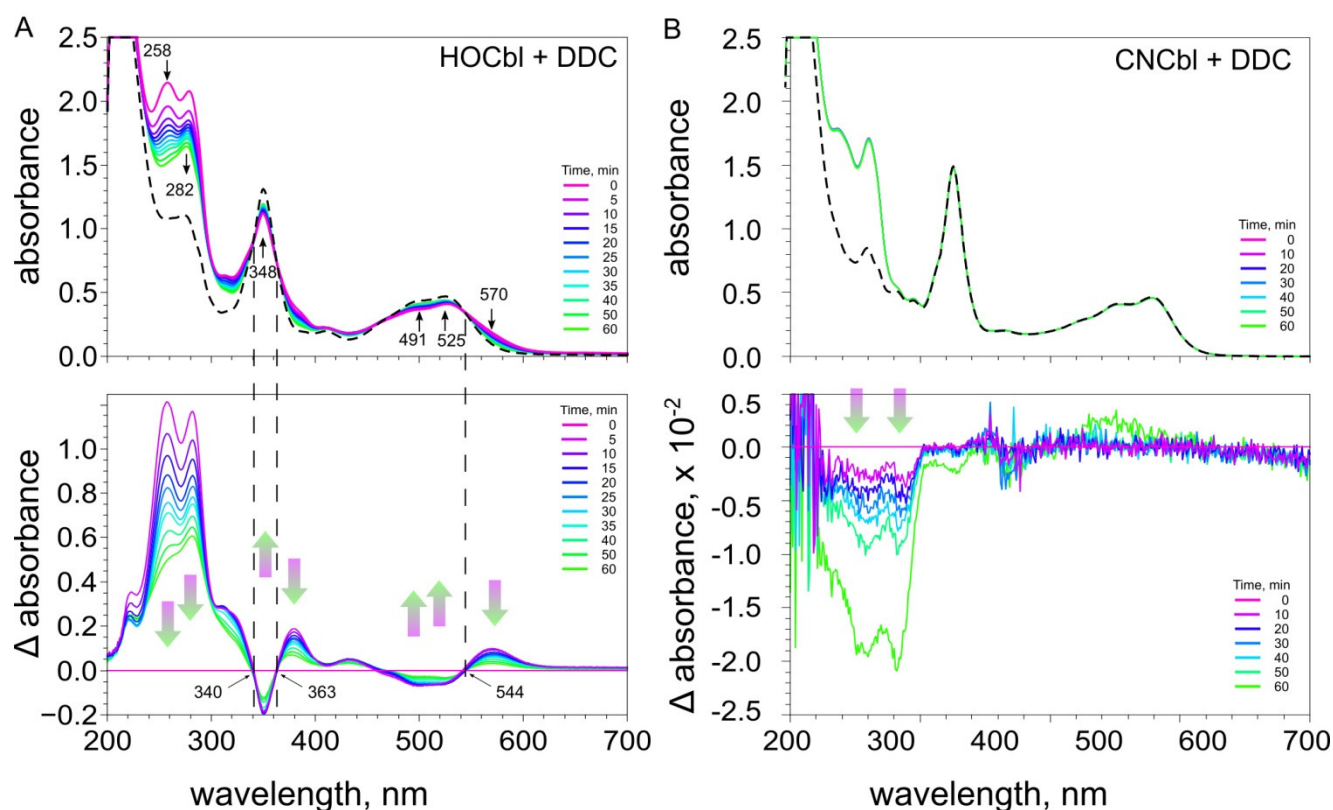


Figure S8. UV-Vis spectra recorded during the reactions between cobalamins and DDC. (A) - $\text{H}_2\text{OCbl}^+/\text{HOCbl}$. Dashed line indicates the spectrum of $\text{H}_2\text{OCbl}^+/\text{HOCbl}$. (B) - CNCbl. Dashed line indicates the spectrum of CNCbl. Above: absorption spectra, below: difference spectra.

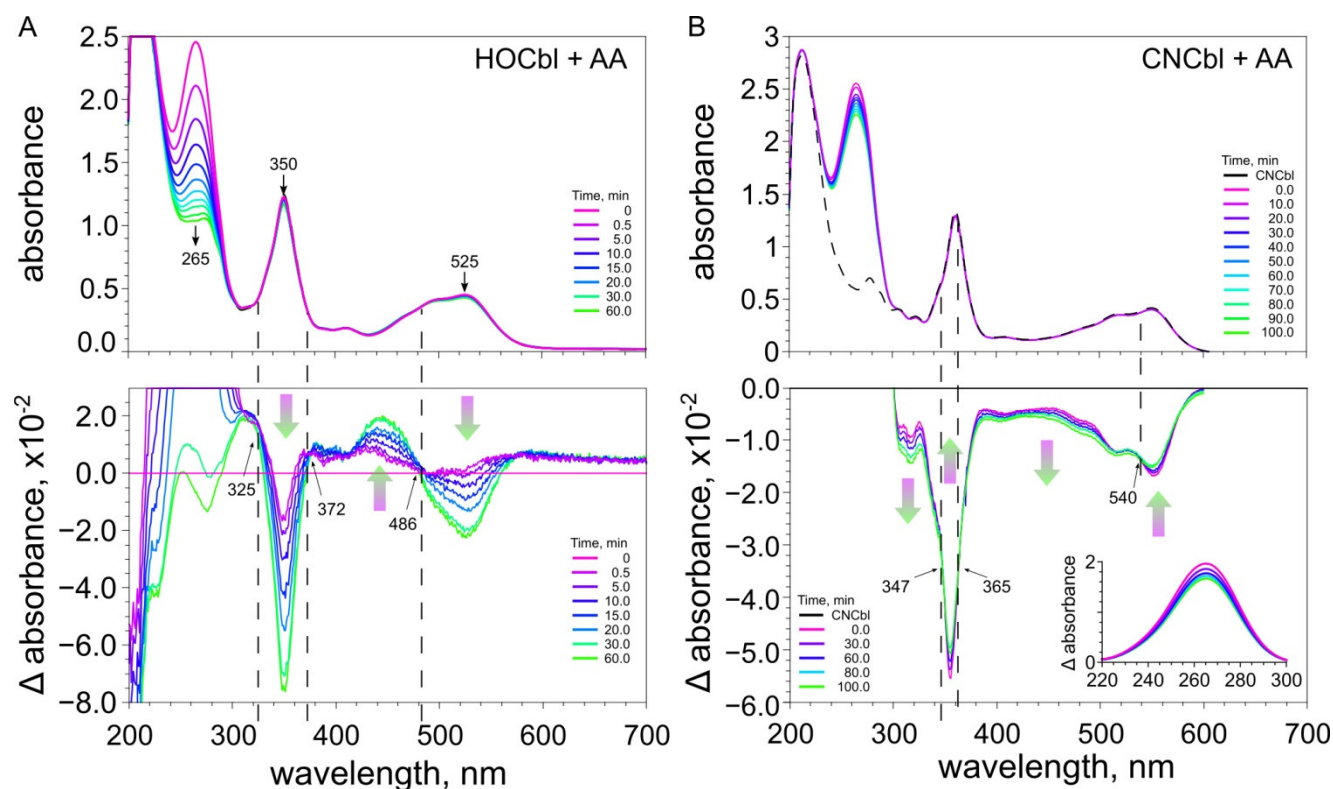


Figure S9. UV-Vis spectra recorded during the reactions between cobalamins and AA. (A) - $\text{H}_2\text{OCbl}^+/\text{HOCbl}$. Dashed line indicates the spectrum of $\text{H}_2\text{OCbl}^+/\text{HOCbl}$. (B) - CNCbl. Dashed line indicates the spectrum of CNCbl. Above: absorption spectra, below: difference spectra. Inset: The difference spectra at 220–300 nm.

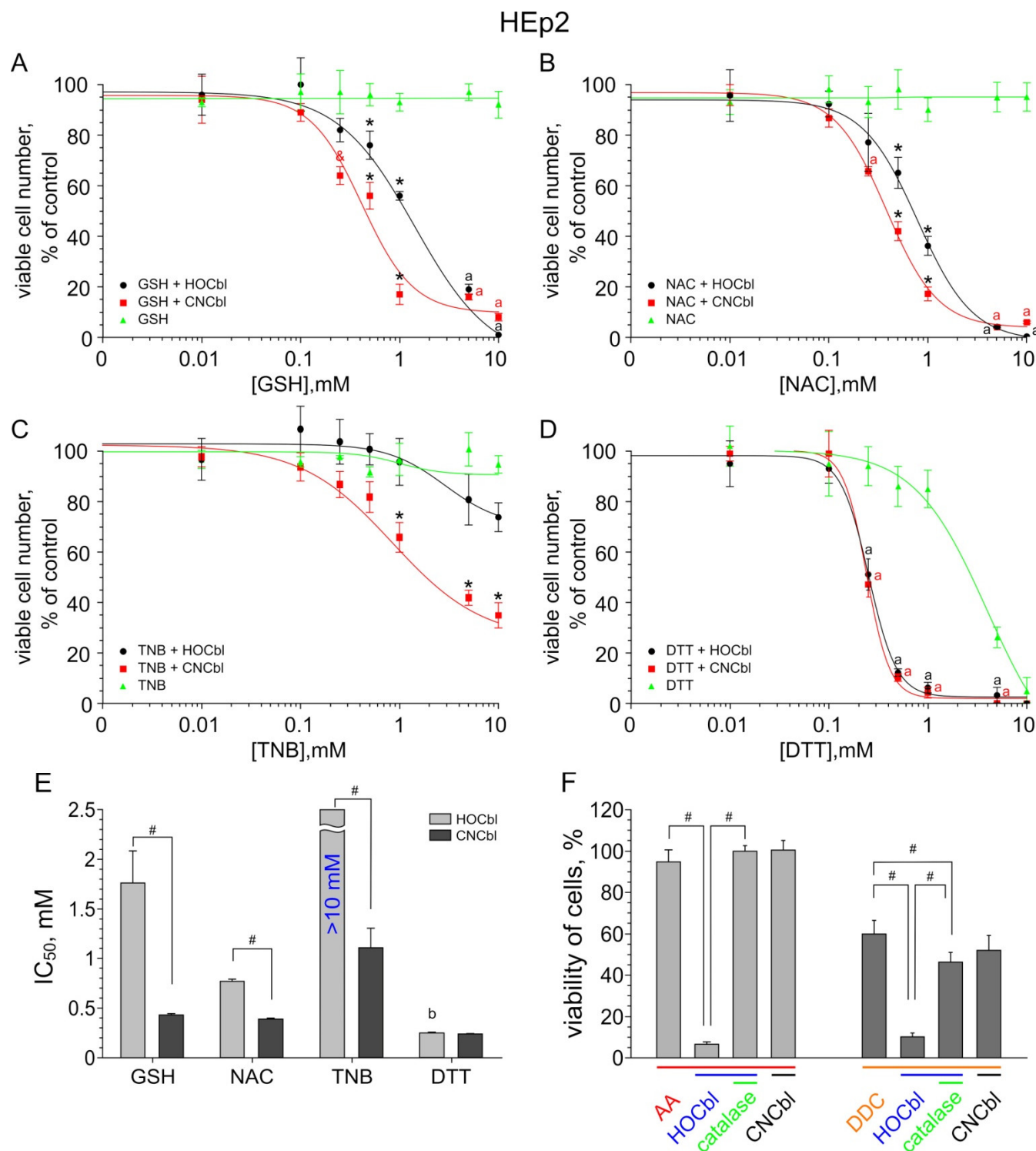


Figure S10. Cytotoxic effect of combinations of cobalamins with thiols on HEp-2 cells (A–D), IC₅₀ values for combinations of cobalamins with thiols (E), and cytotoxicity of combinations of cobalamins (25 μM) with AA (0.5 mM) and DDC (1 mM) (F). The results were expressed as the mean ± standard deviation. Data were analyzed using one-way ANOVA followed by Tukey's test. (A–D) *Differences are significant compared with other two groups, $p < 0.05$; ^a significant differences between the cobalamin+thiol and thiol groups $p < 0.05$. & significant differences between the combinations of a thiol with CNCbl and HOCbl, $p < 0.066$ (E–F) # significant differences between the groups; ^b differences are significant compared with combinations HOCbl + monothiols, $p < 0.05$.