Supplementary Materials

A comparative study of ROS production and cytotoxicity induced by combinations of thiols with HOCbl and CNCbl

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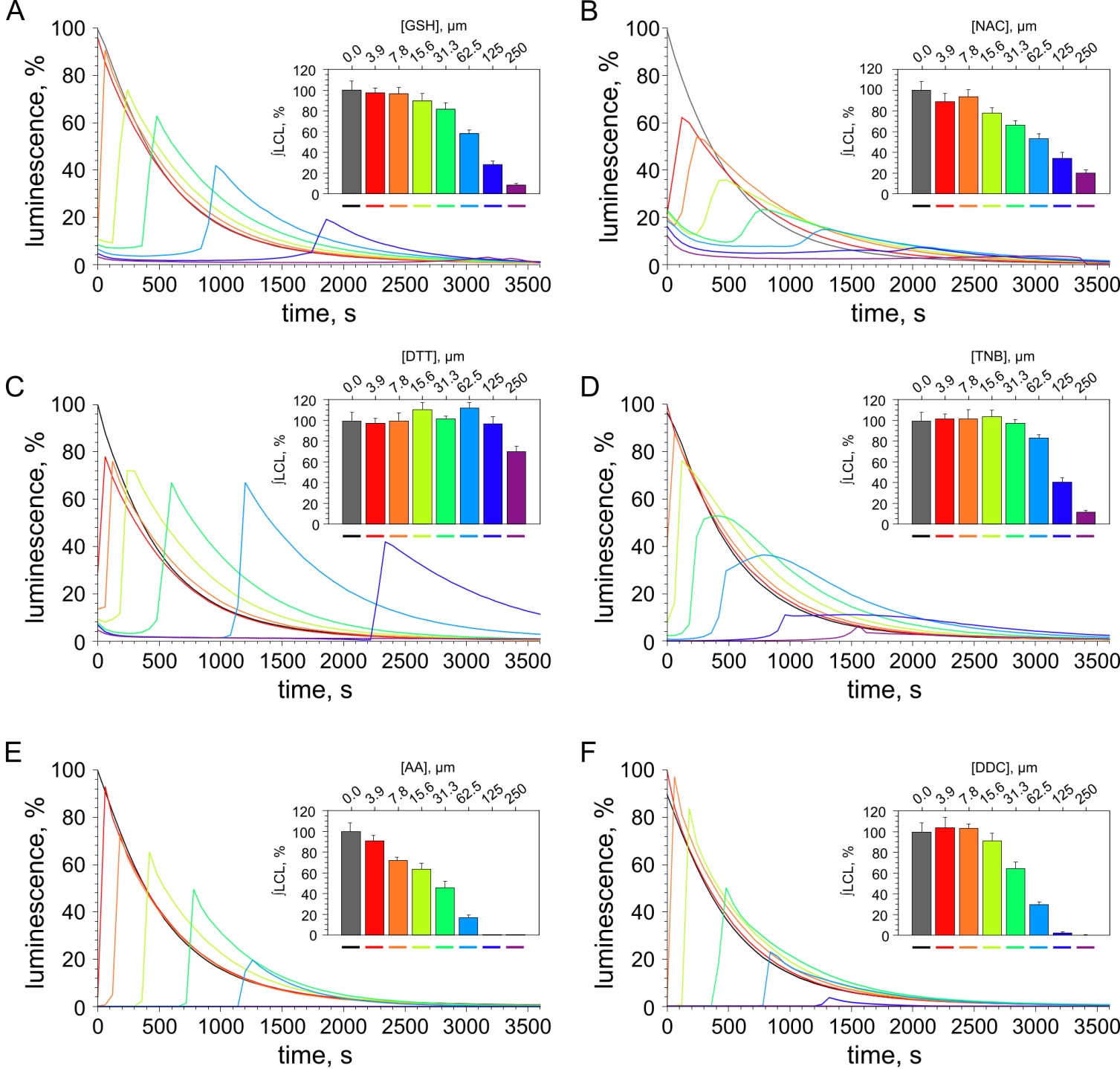
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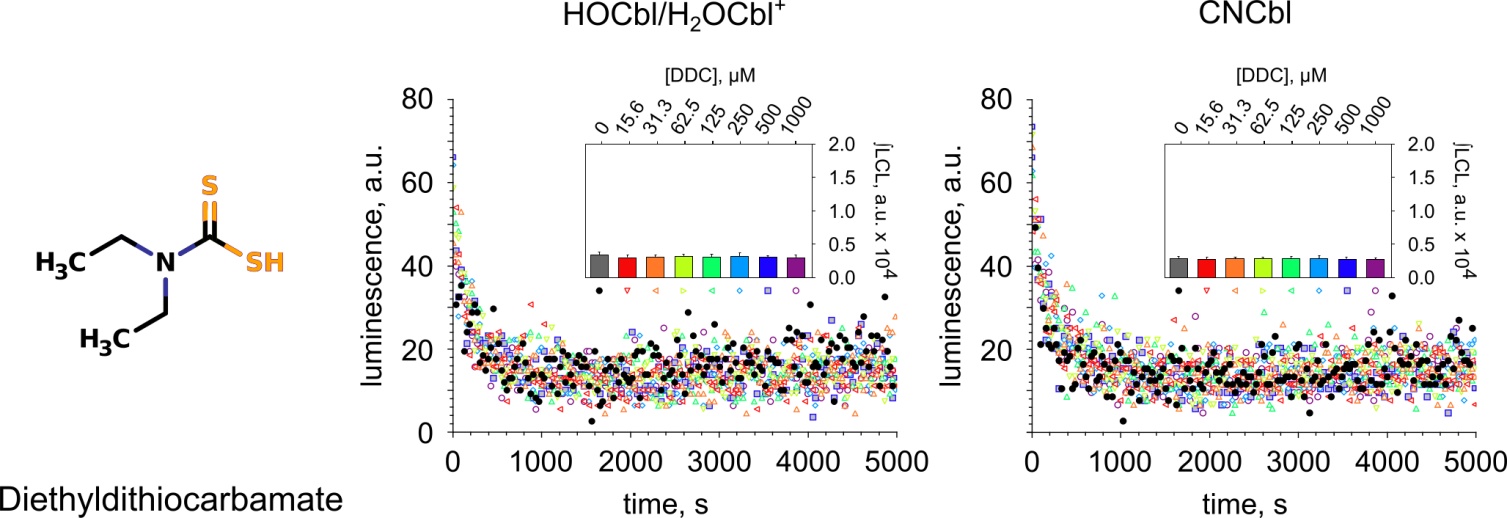
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| --- | --- | --- | --- | --- | --- |
| HOCbl (25 µM) + compound | | | | | |
| [Compound], µM | AA | NAC | DTT | TNB | GSH |
| 0 | 5008±3600 | 4377±5040 | 3369±2880 | 4934±3840 | 4934±4560 |
| 15,6 | 5372±2160 | 4325±5280 | 31657±4320 | 2977±3120 | 4567±3840 |
| 31,3 | 11379±2640 | 4380±4800 | 100763±5760 | 3034±4080 | 6490±5040 |
| 62,5 | 59570±4560 | 4950±4320 | 252490±8880 | 3020±3600 | 12064±7680 |
| 125 | 125013±5520 | 17473±15360 | 539531±7920 | 3130±3840 | 28783±4320 |
| 250 | 136746±5760 | 40352±14160 | 831704±19680 | 3465±2640 | 70924±5040 |
| 500 | 105146±7925 | 42465±7440 | 419433±13920 | 4190±2640 | 44043±11040 |
| 1000 | 43015±3782 | 22705±4560 | 95182±6960 | 3027±4320 | 31535±8880 |
| CNCbl (25 µM) + compound | | | | | |
| [Compound], µM | AA | NAC | DTT | TNB | GSH |
| 0 | 5219±2400 | 3349±2400 | 4150±5280 | 4934±4080 | 4934±3840 |
| 15,6 | 4465±6240 | 4965±2640 | 48507±5040 | 23727±4080 | 55652±3120 |
| 31,3 | 4559±2880 | 11628±2640 | 126176±5040 | 60410±3840 | 95679±2880 |
| 62,5 | 4567±5760 | 42791±4320 | 257989±8640 | 119158±3360 | 147798±4080 |
| 125 | 4690±3360 | 146049±13440 | 266179±26640 | 147779±3840 | 210120±4320 |
| 250 | 4594±4080 | 141838±11629 | 215574±35520 | 155870±4800 | 212621±8880 |
| 500 | 4742±3840 | 52359±8561 | 130578±29520 | 70343±2183 | 81134±22320 |
| 1000 | 4716±2880 | 18474±4806 | 71179±13200 | 6801±6600 | 36722±20400 |

**Table S1.** Dependence of integral chemiluminescence response (ʃLCL±SD) on the concentration of the compounds.

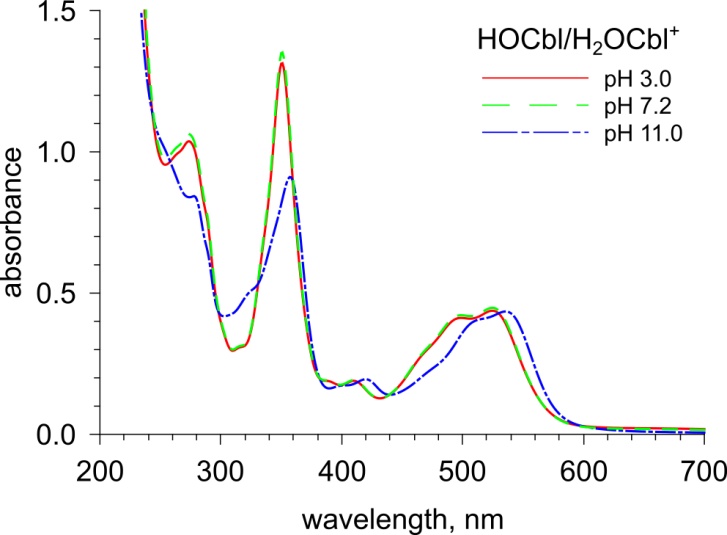
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**Figure S1.** Effect of compounds being tested on chemiluminescence: (A) GSH, (B) NAC, (C) DTT, (D) TNB, (E) AA, (F) DDC. Inserts: Dependence of integral chemiluminescence response (ʃLCL) on the concentration of the compounds.

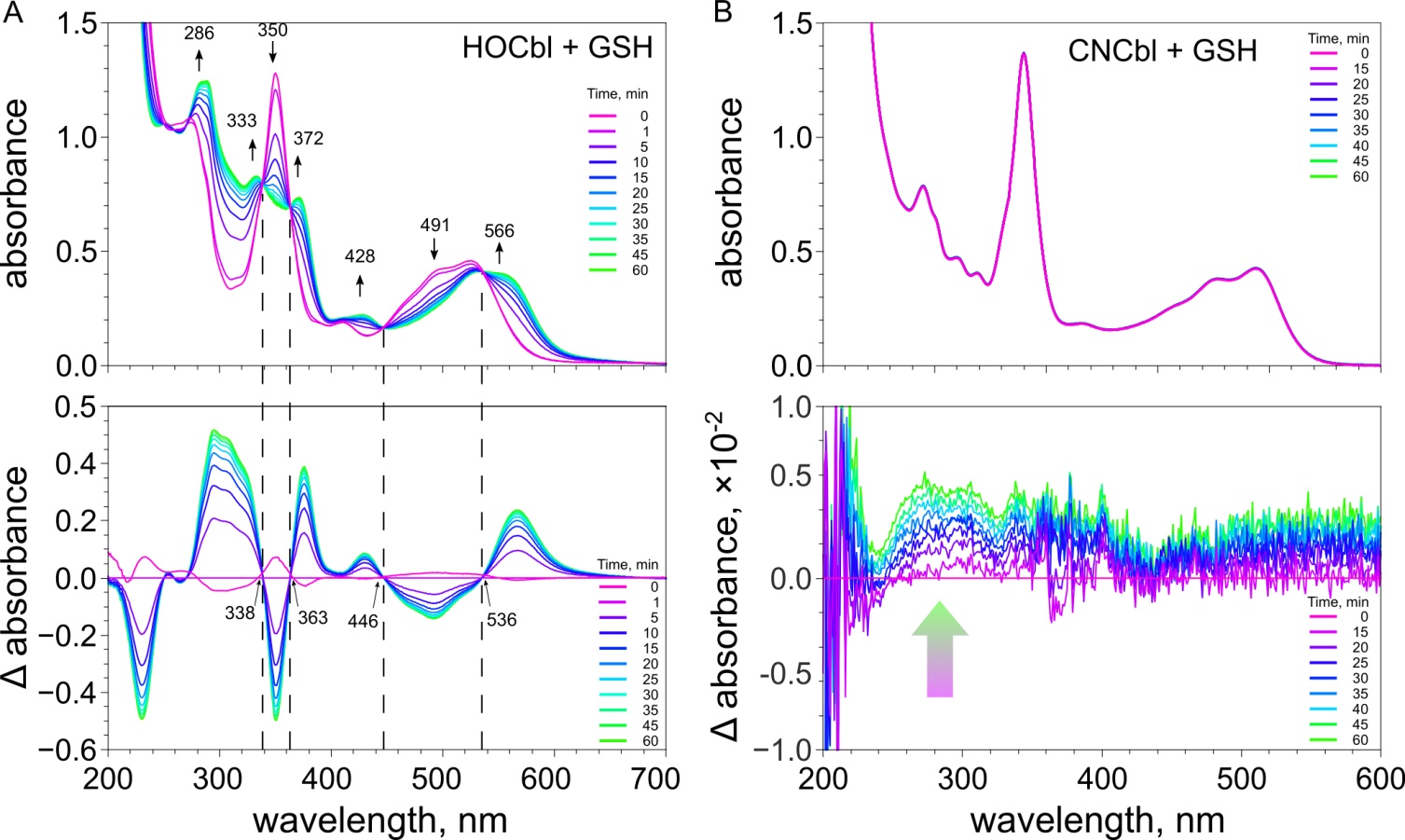
The antioxidant activity of compounds was estimated by luminol-dependent chemiluminescence (LCL) as described previously [doi.org/10.3390/antiox10081262].

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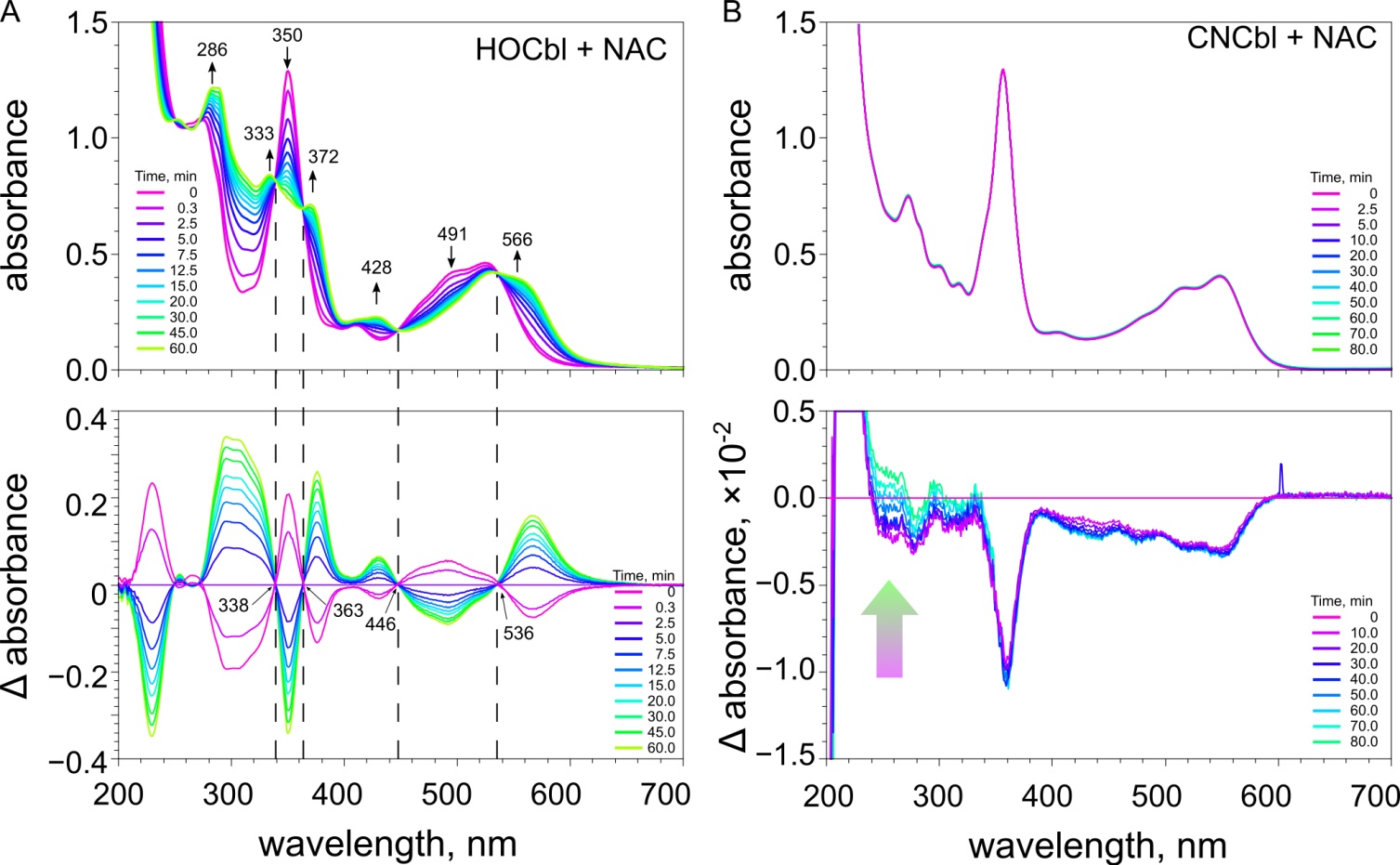
**Figure S2.** Chemiluminescence recorded during the oxidation of DDC in the presence of cobalamins. Inserts: Dependence of integral chemiluminescence response (ʃLCL) on the concentration of DDC.

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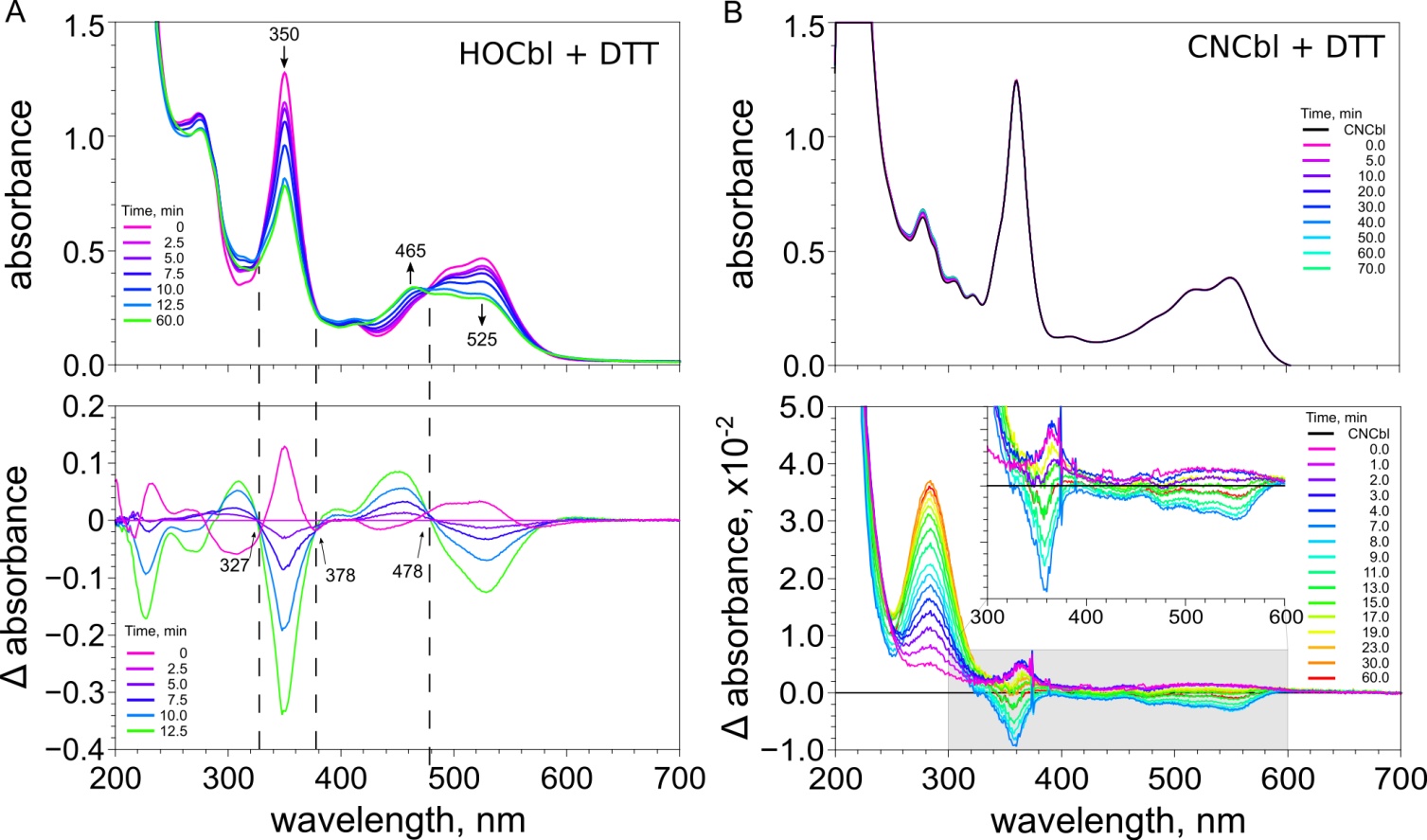
**Figure S3.** UV/Vis absorption spectra H2OCbl+/HOCbl at different pH values. The concentration of HOCbl is 50 μM.

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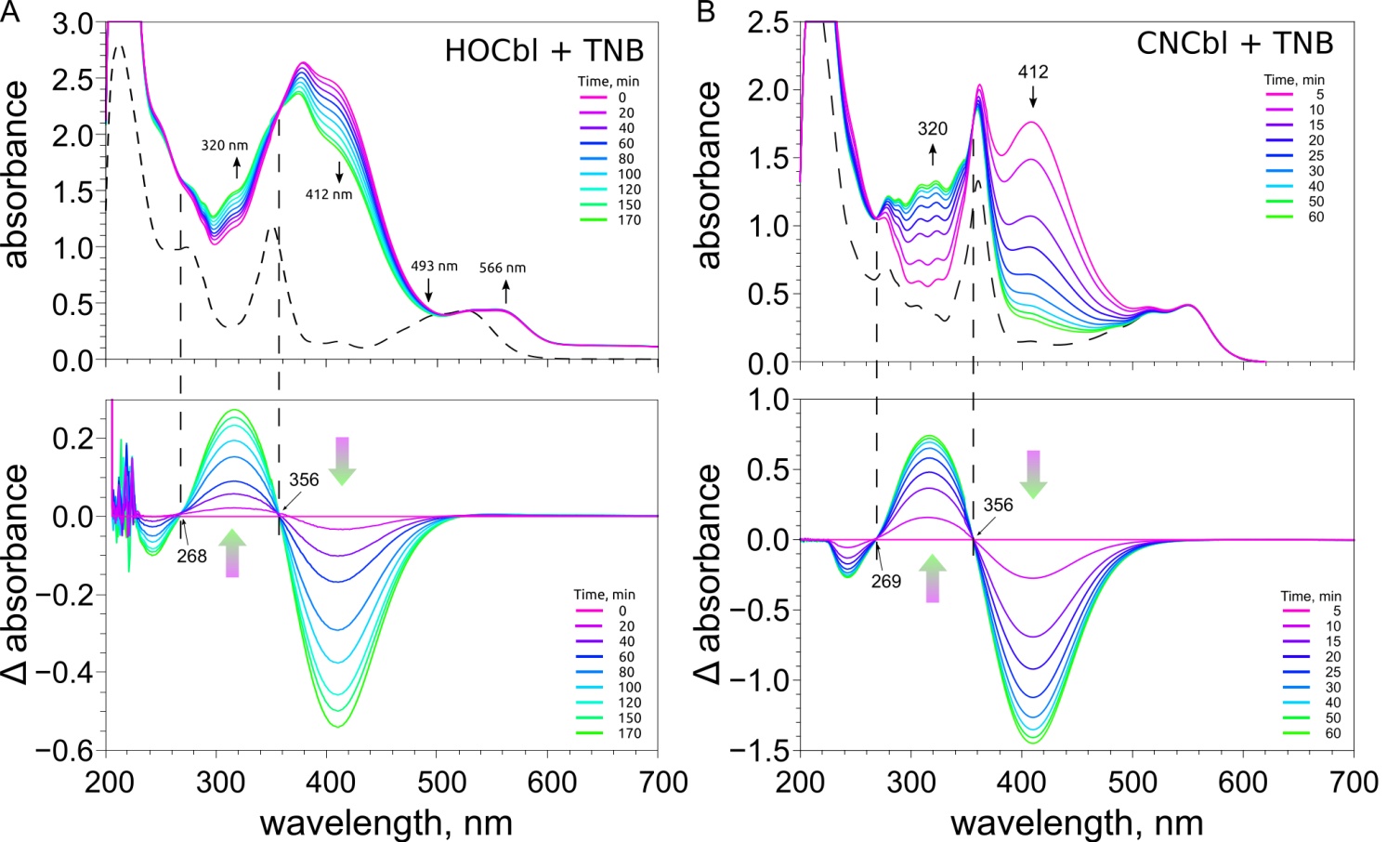
**Figure S4.** UV-Vis spectra recorded during the reactions between cobalamins and GSH. (A) - H2OCbl+/HOCbl, (B) - CNCbl. Above: absorption spectra, below: difference spectra.

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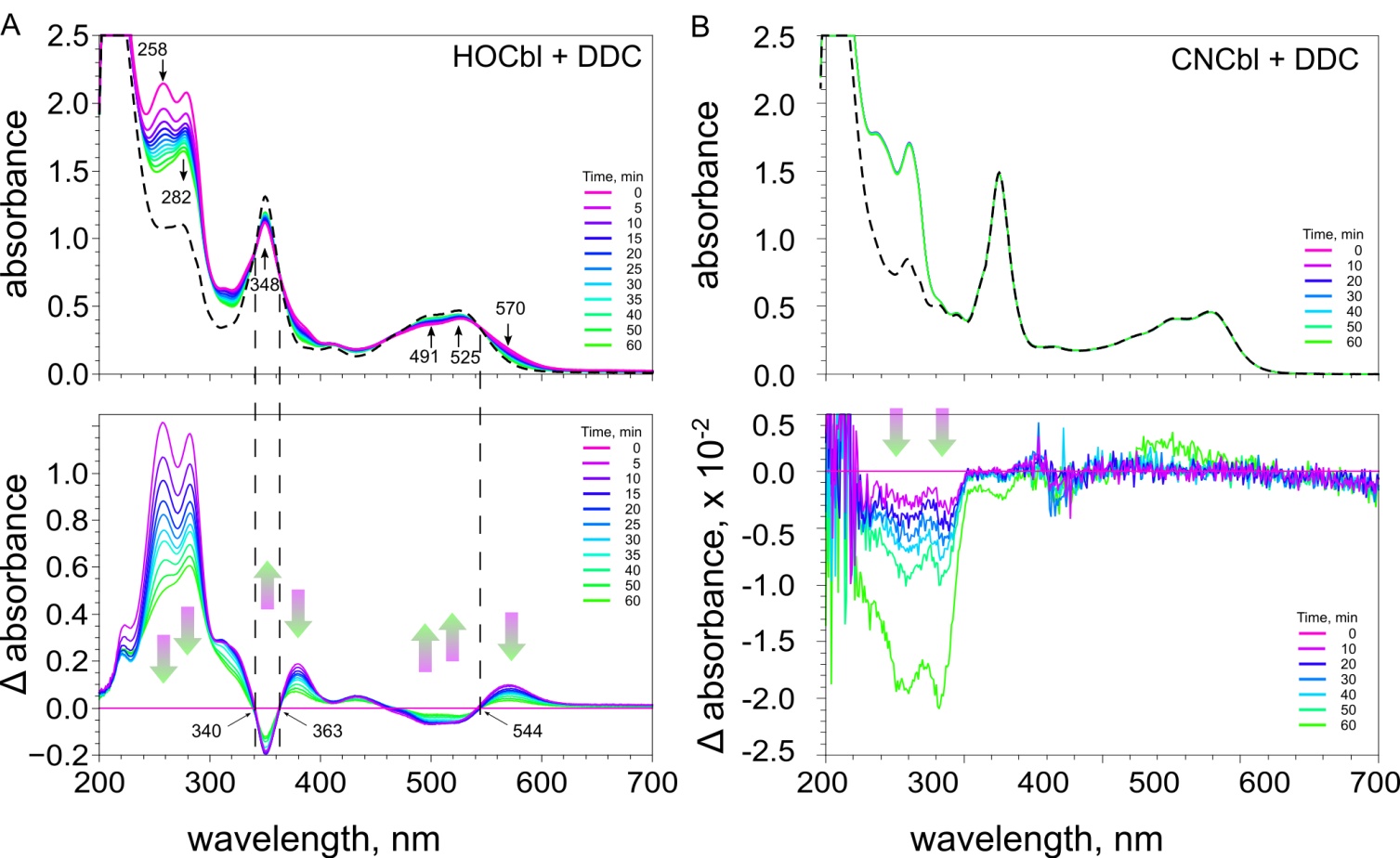
**Figure S5.** UV-Vis spectra recorded during the reactions between cobalamins and NAC. (A) - H2OCbl+/HOCbl. (B) - CNCbl. Above: absorption spectra, below: difference spectra.

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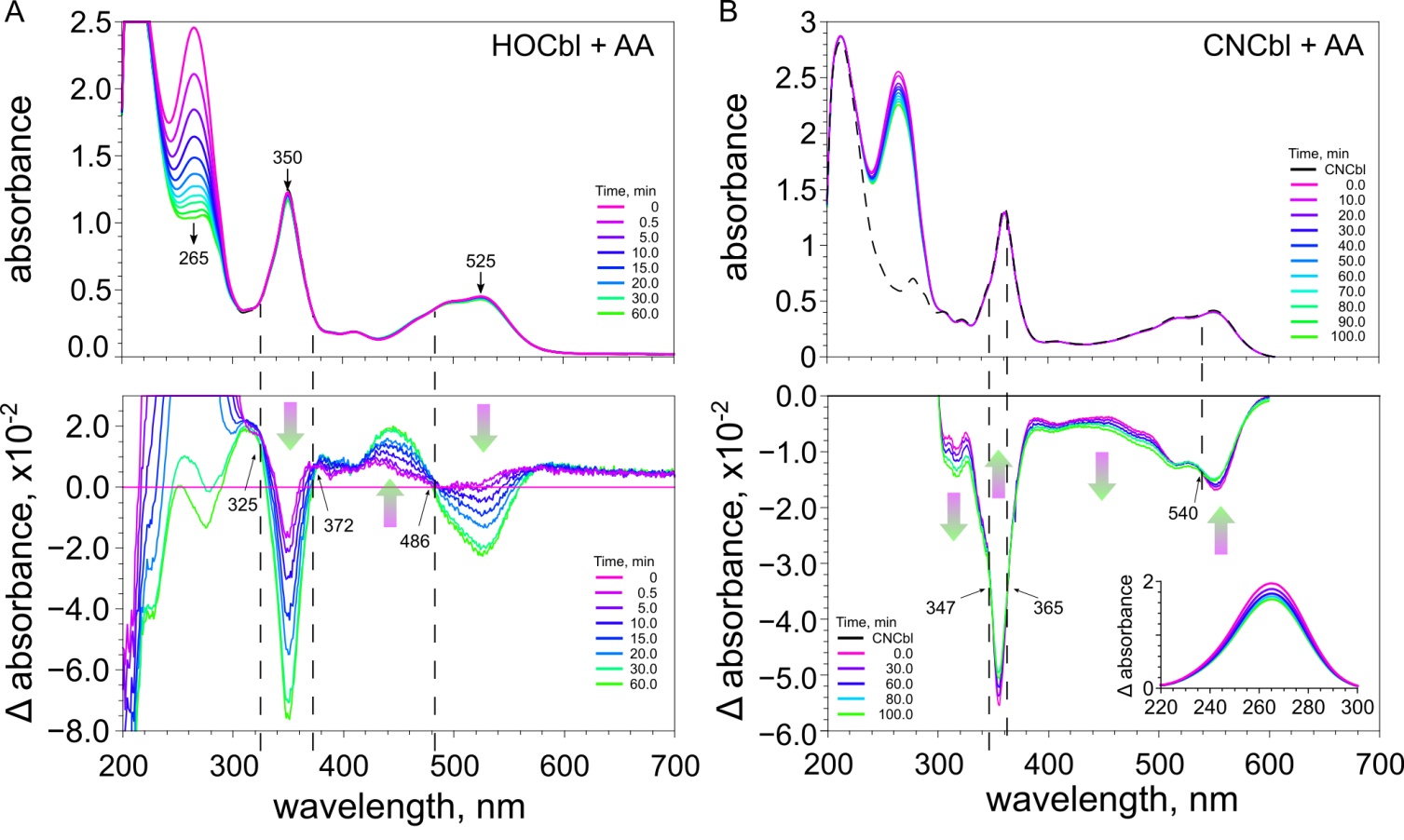
**Figure S6.** UV-Vis spectra recorded during the reactions between cobalamins and DTT. (A) - H2OCbl+/HOCbl, (B) - CNCbl. Above: absorption spectra, below: difference spectra. Inset: The difference spectra (300-600 nm) are shown in an enlarged y-axis.

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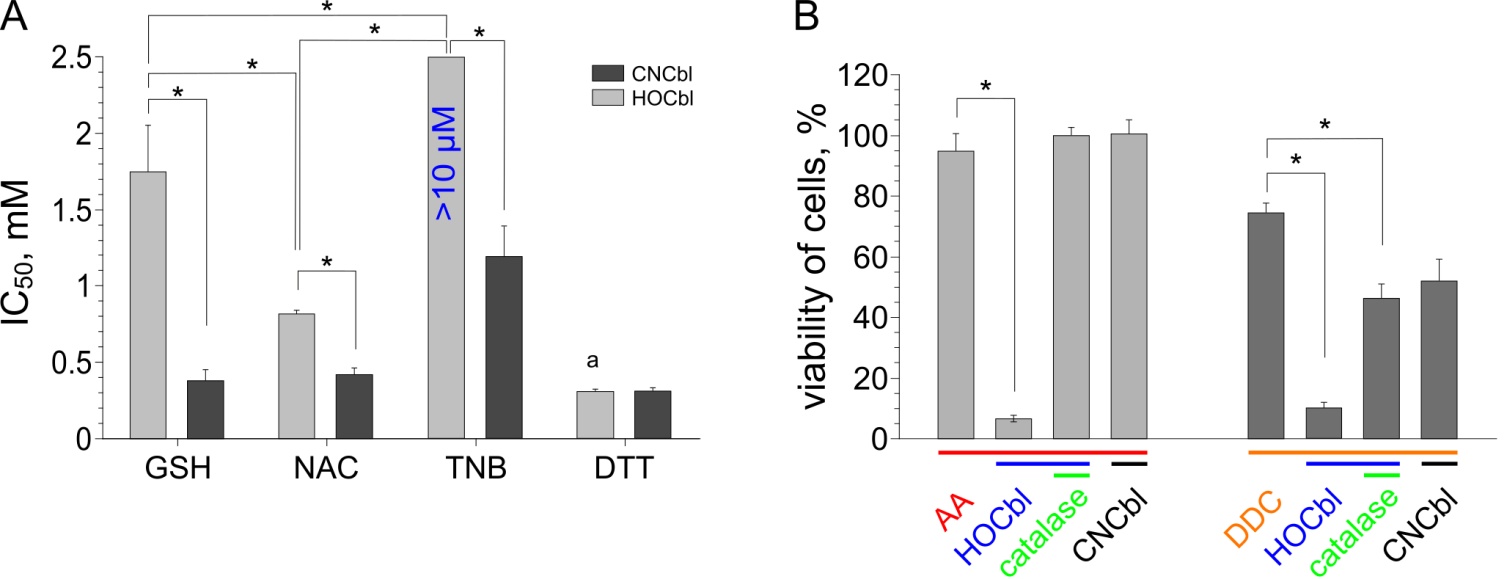
**Figure S7.** UV-Vis spectra recorded during the reactions between cobalamins and TNB. (A) - H2OCbl+/HOCbl. Dashed line indicates the spectrum of H2OCbl+/HOCbl. (B) - CNCbl. Dashed line indicates the spectrum of CNCbl. Above: absorption spectra, below: difference spectra.

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**Figure S8.** UV-Vis spectra recorded during the reactions between cobalamins and DDC. (A) - H2OCbl+/HOCbl. Dashed line indicates the spectrum of H2OCbl+/HOCbl. (B) - CNCbl. Dashed line indicates the spectrum of CNCbl. Above: absorption spectra, below: difference spectra.

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**Figure S9.** UV-Vis spectra recorded during the reactions between cobalamins and AA. A - H2OCbl+/HOCbl. Dashed line indicates the spectrum of H2OCbl+/HOCbl. B - CNCbl. Dashed line indicates the spectrum of CNCbl. Above: absorption spectra, below: difference spectra. Inset: The difference spectra at 220-300 nm.

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**Figure S10.** Cytotoxic effect of combinations of cobalamins with compounds being tested. A - IC50 values for combinations of cobalamins with thiols. B - Cytotoxicity of combinations of cobalamins with AA and DDC. \* signiﬁcant differences between the combinations; a differences are signiﬁcant compared with combinations HOCbl + monothiols, p <0.05.