

Supporting Information

Novel mycophenolic acid precursor–based fluorescent probe for intracellular H₂O₂ detection in living cells and *Daphnia magna* and Zebrafish model systems.

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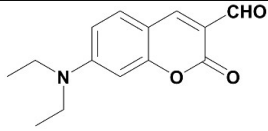
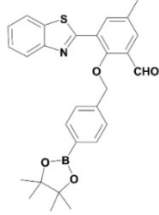
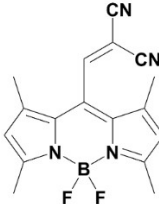
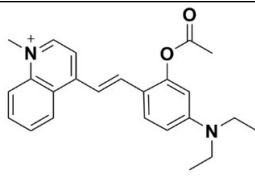
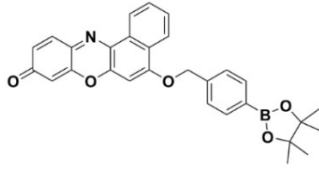
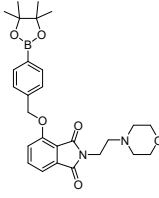
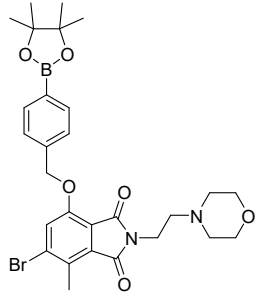
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Table S1. Comparison of related and previously reported fluorescent probes for the detection of H₂O₂.

No. and Ref.	Probe structure	Photo mechanism	Reaction time	Detection limit
1. ¹		TICT	20 min	31 nM
2. ²		ESIPT	45 min	109 nM
3. ³		PET	20 min	31 nM
4. ⁴		ICT	30 min	0.85 μM
5. ⁵		ICT	40 min	91 nM
6. ⁶		ESIPT	30 min	84 nM
This work		ESIPT	45 min	13 nM

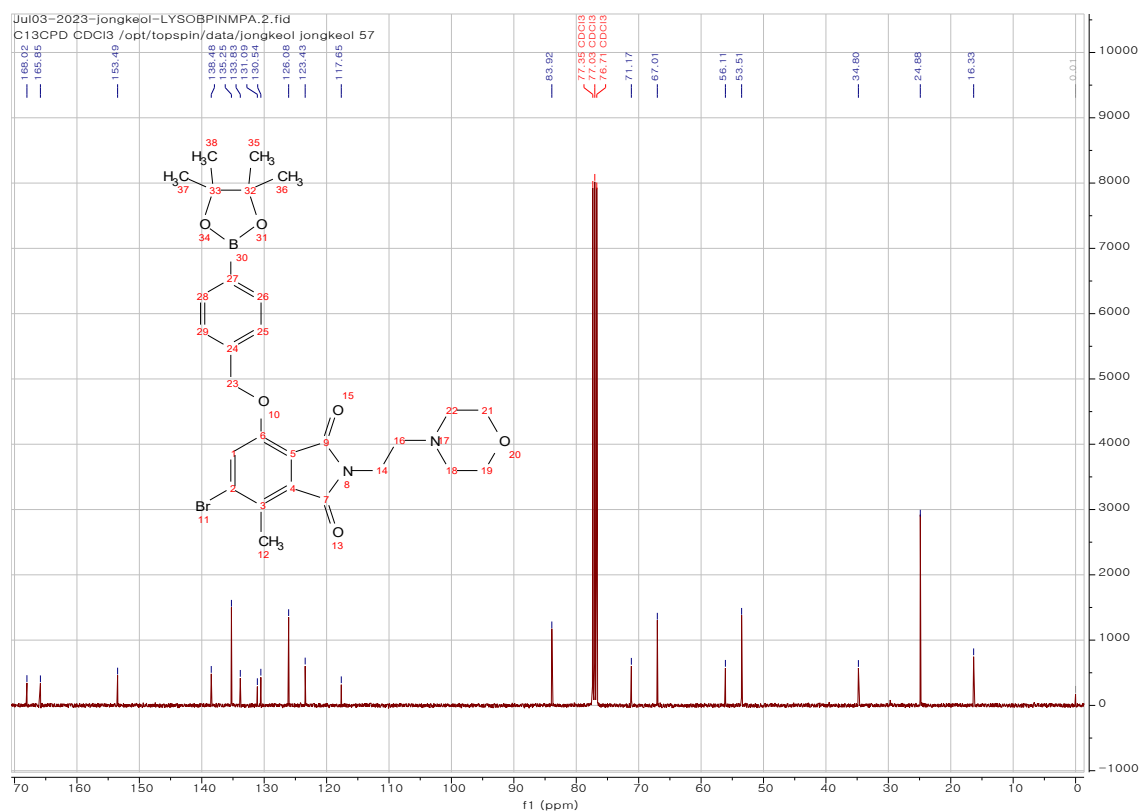
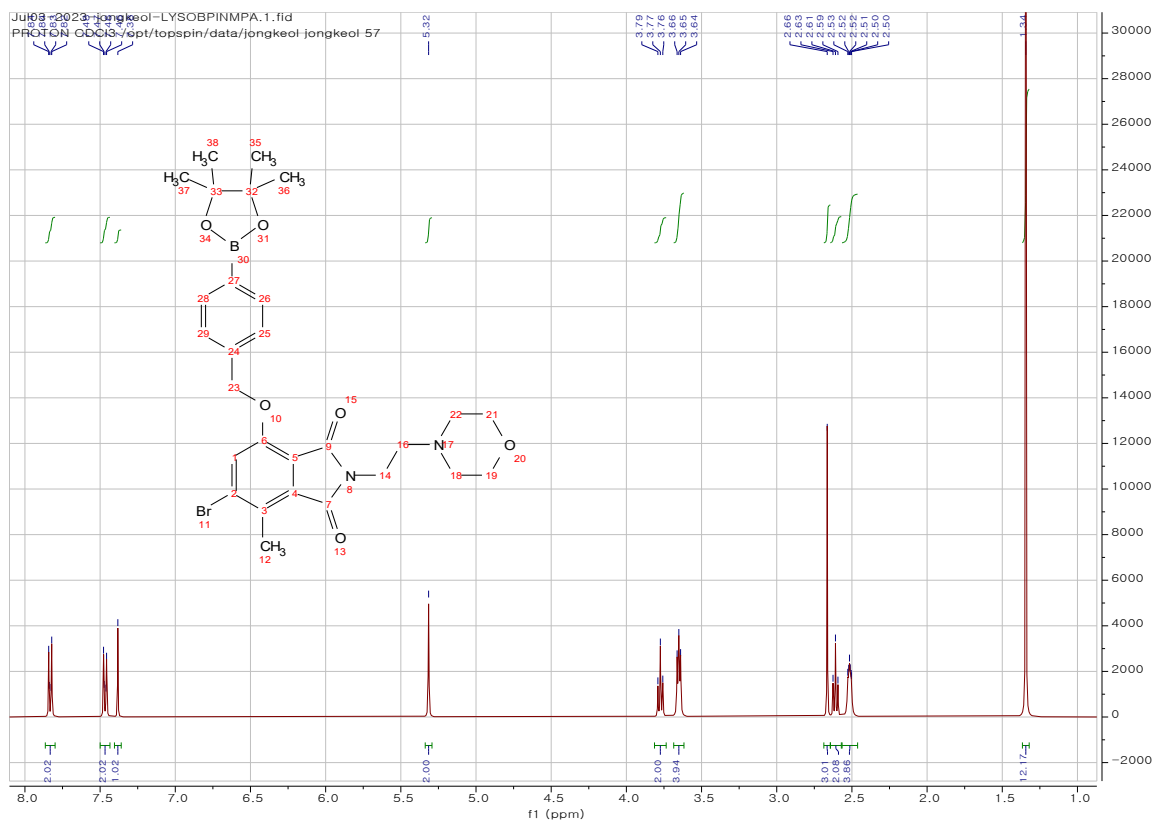


Fig. S1. ¹H NMR (top) and ¹³C NMR (bottom) spectra of LBM.

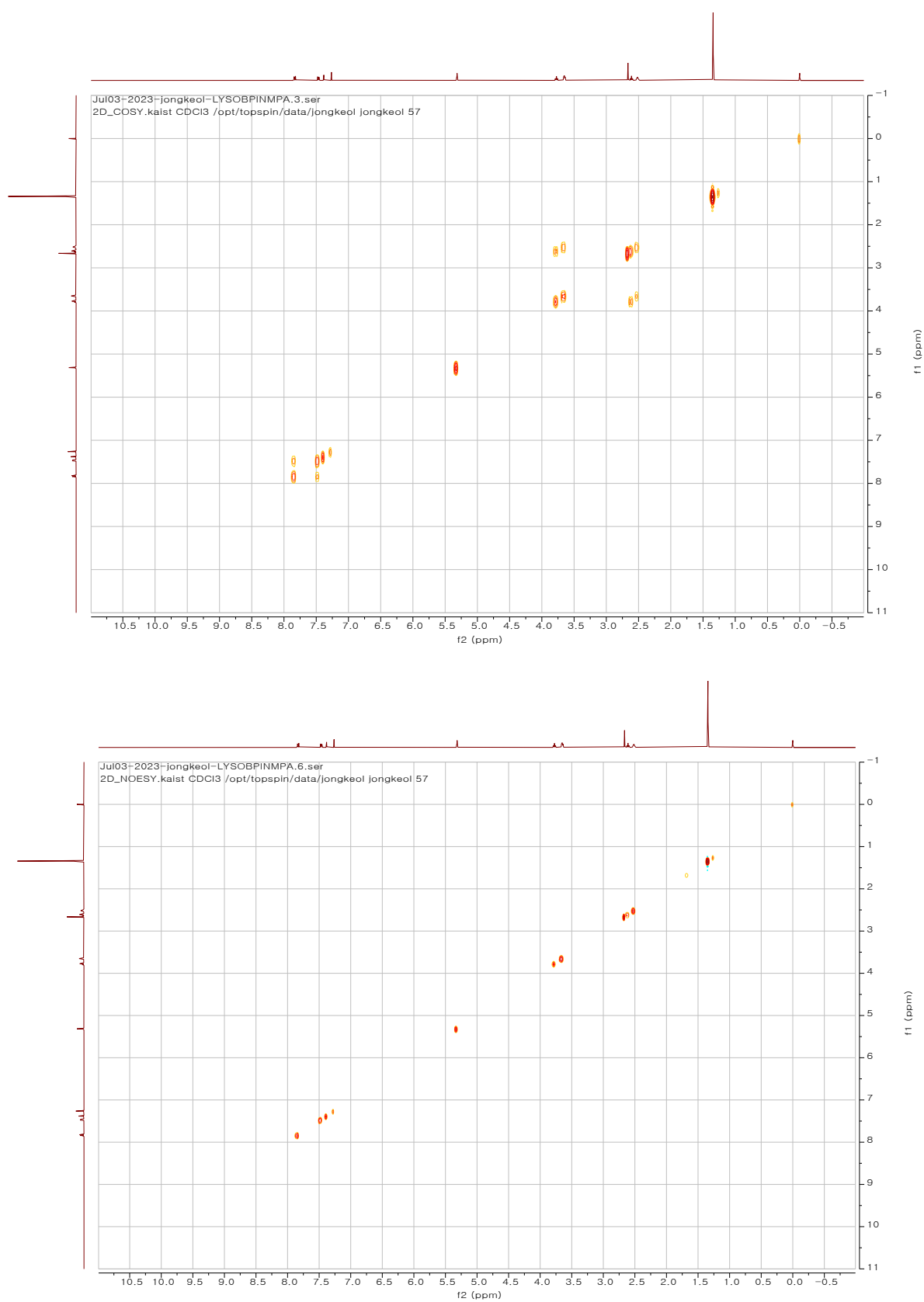


Fig S2. ^1H - ^1H COSY (top) and ^1H - ^1H NOESY (bottom) NMR spectra of **LBM**.

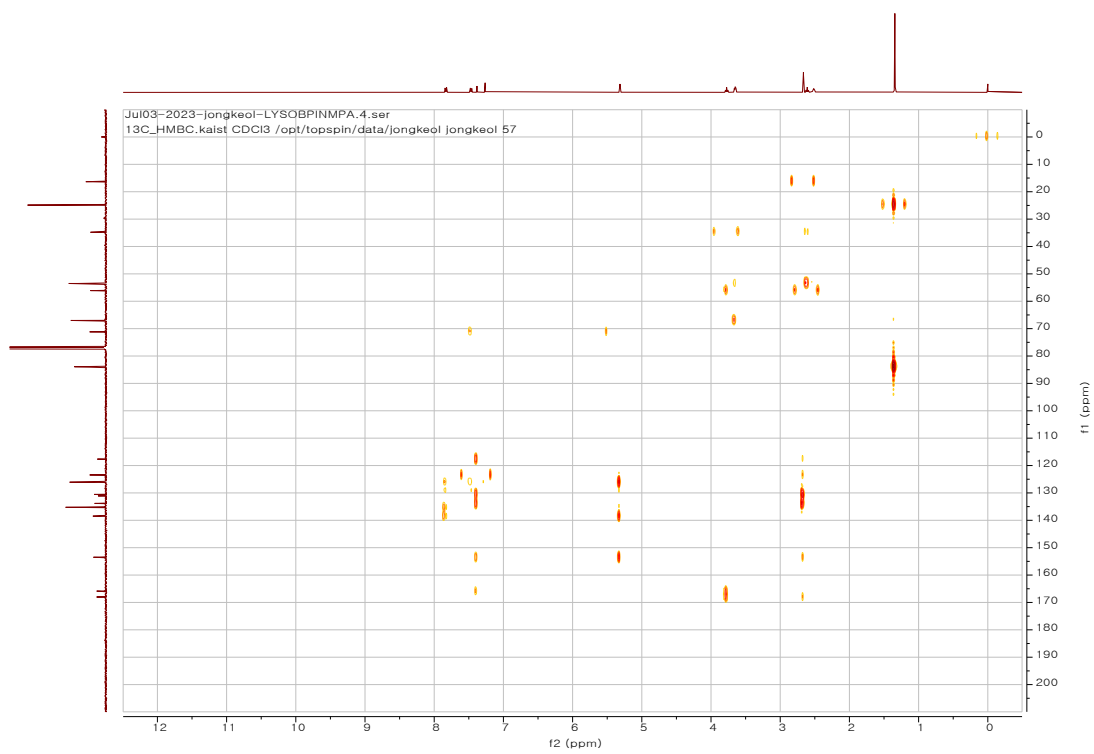
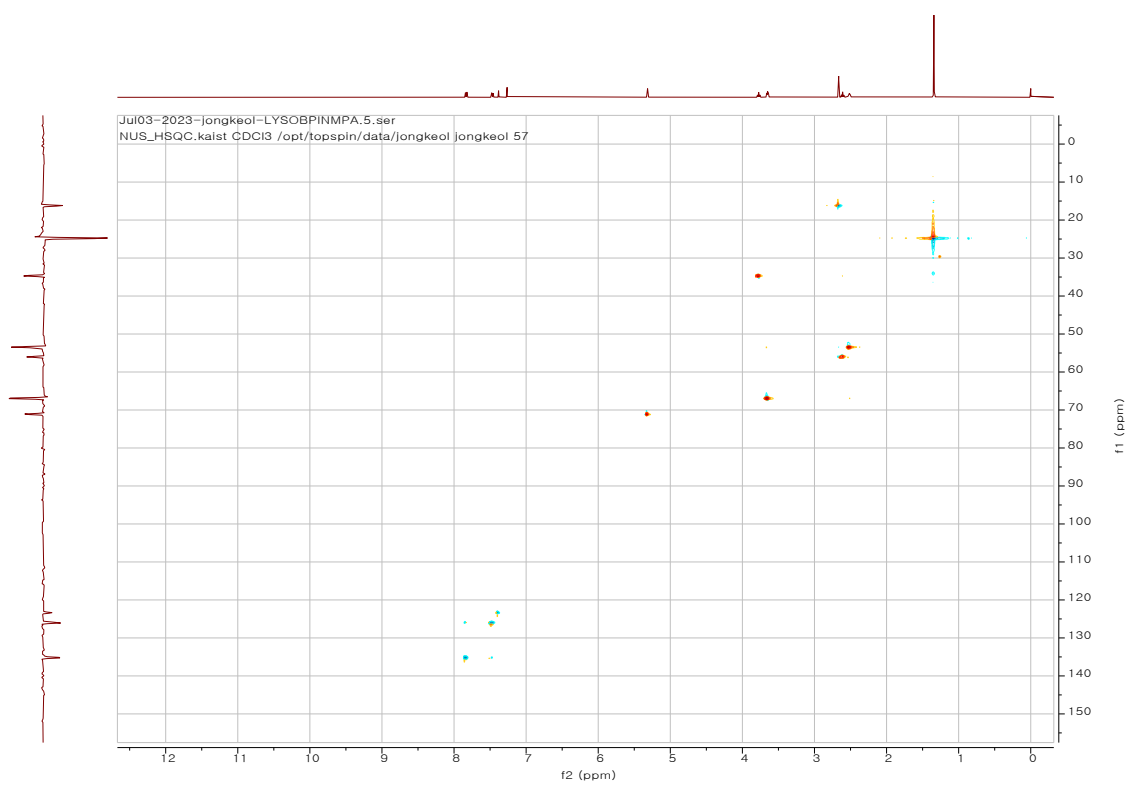


Fig S3. ^1H - ^{13}C HSQC (top) and ^1H - ^{13}C HMBC (bottom) NMR spectra of **LBM**.

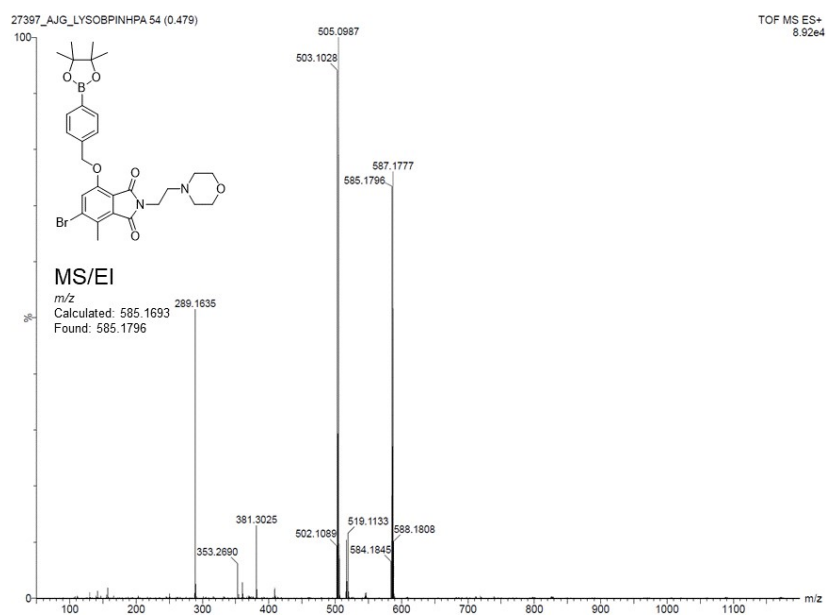
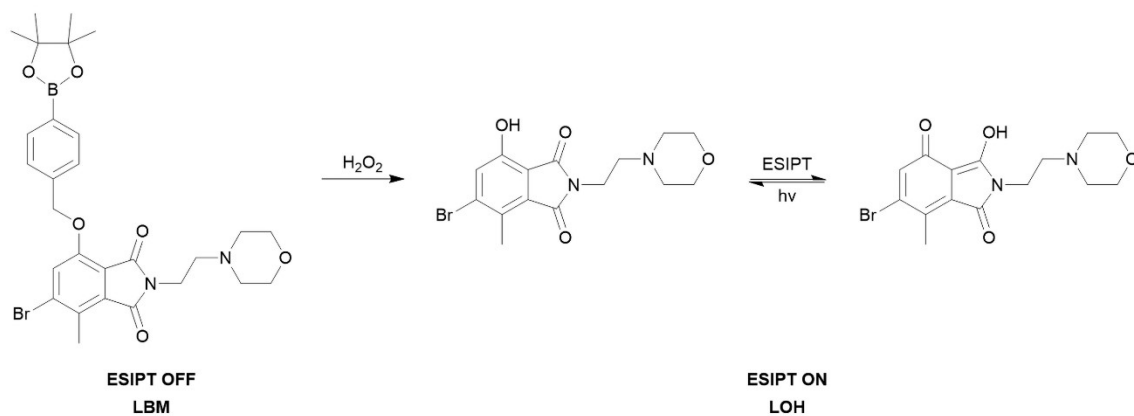


Fig. S4. HR-MS spectra of **LBM**



Scheme S1. A simplified illustration of the cleavage reaction occurring between **LBM** (Probe) and H_2O_2 followed by the ensuing tautomerization phenomenon.

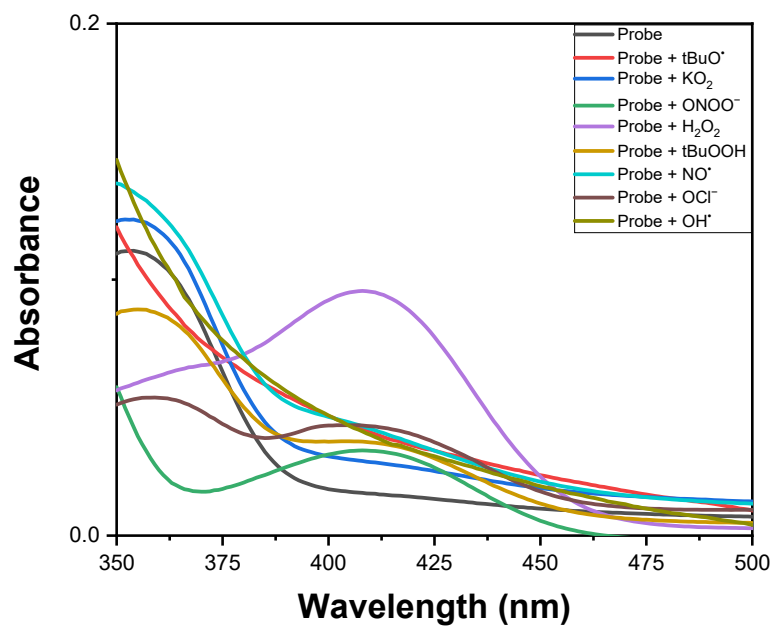


Fig. S5a. Absorption spectra of the **LBM** (15 μM) when treated with 10 equiv. of H₂O₂ in PBS buffer solution (10 mM, pH = 7.4) and incubated for 45 minutes at room temperature.

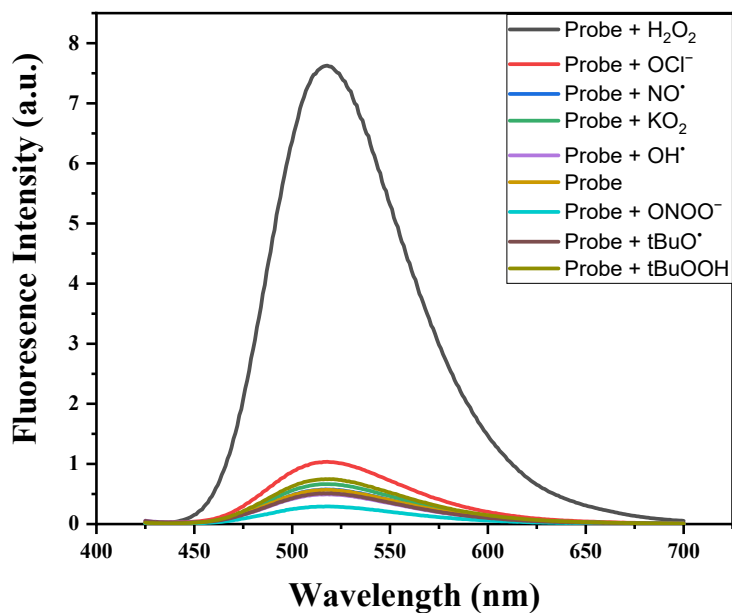


Fig. S5b. Fluorescence emission spectra of the **LBM** (15 μM) when treated with 10 equiv. of H₂O₂ and other ROS in PBS buffer solution (10 mM, pH = 7.4) and incubated for 45 minutes at room temperature.

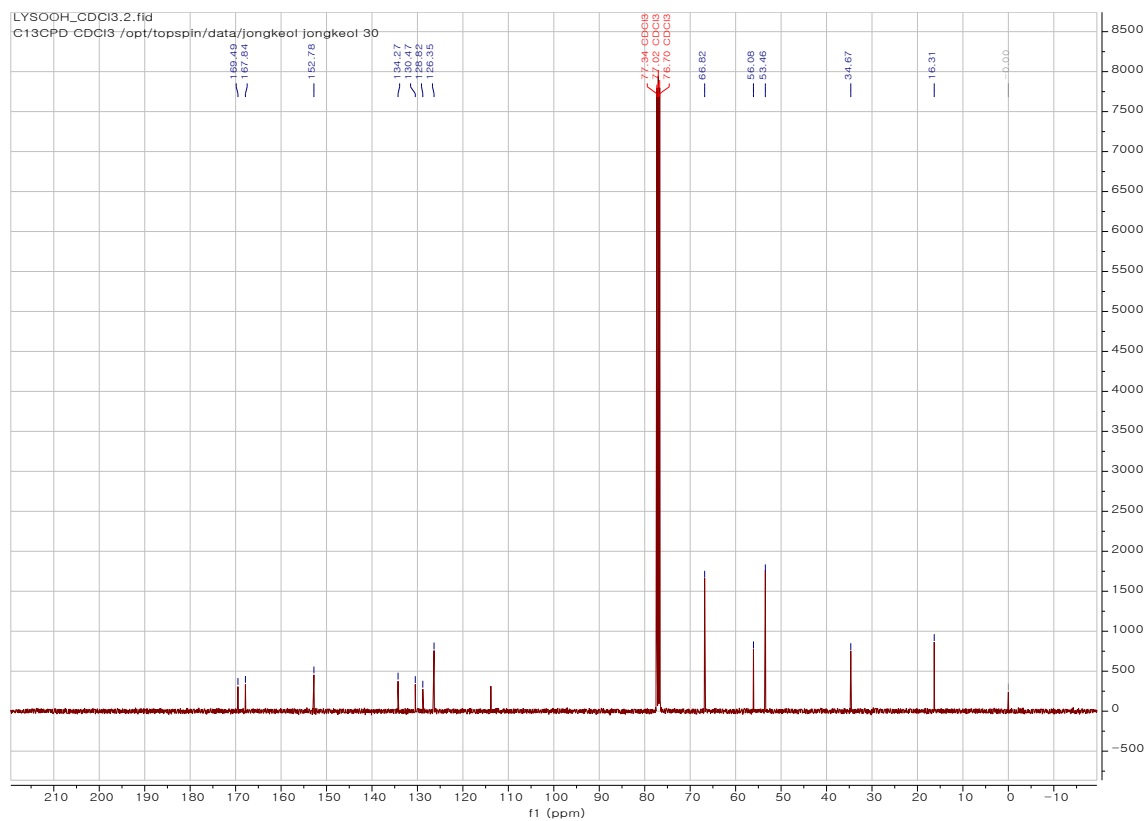
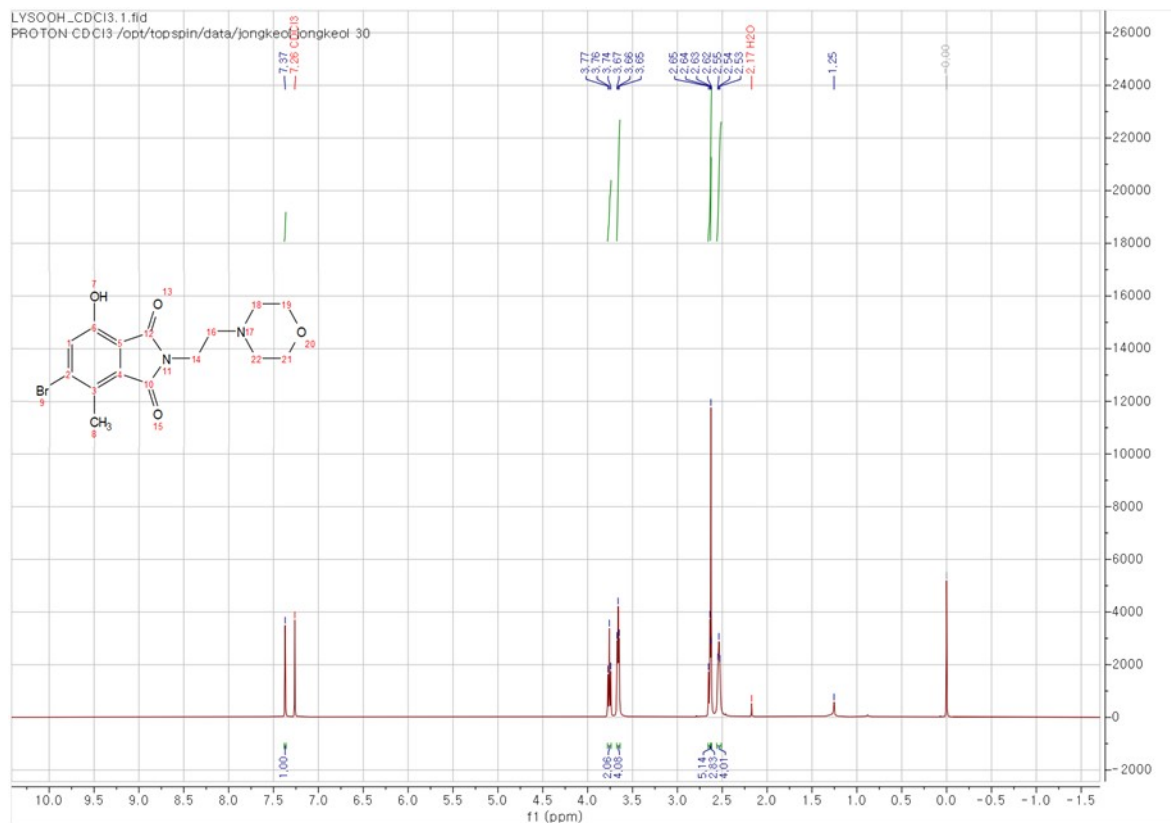


Fig S6. ¹H NMR (top) and ¹³C NMR (bottom) spectra of LOH

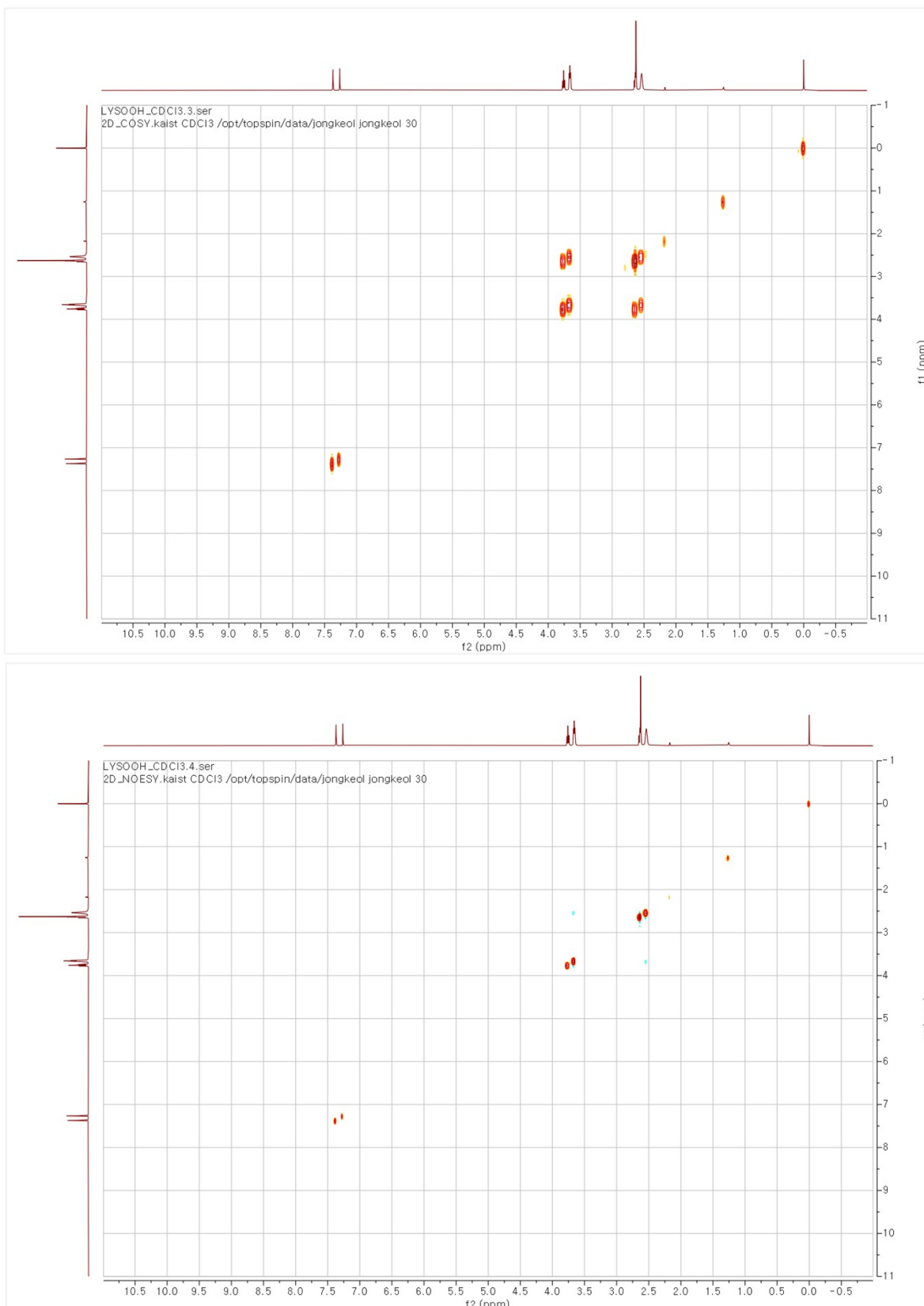


Fig S7. ^1H - ^1H COSY (top) and ^1H - ^1H NOESY (bottom) NMR spectra of **LOH**.

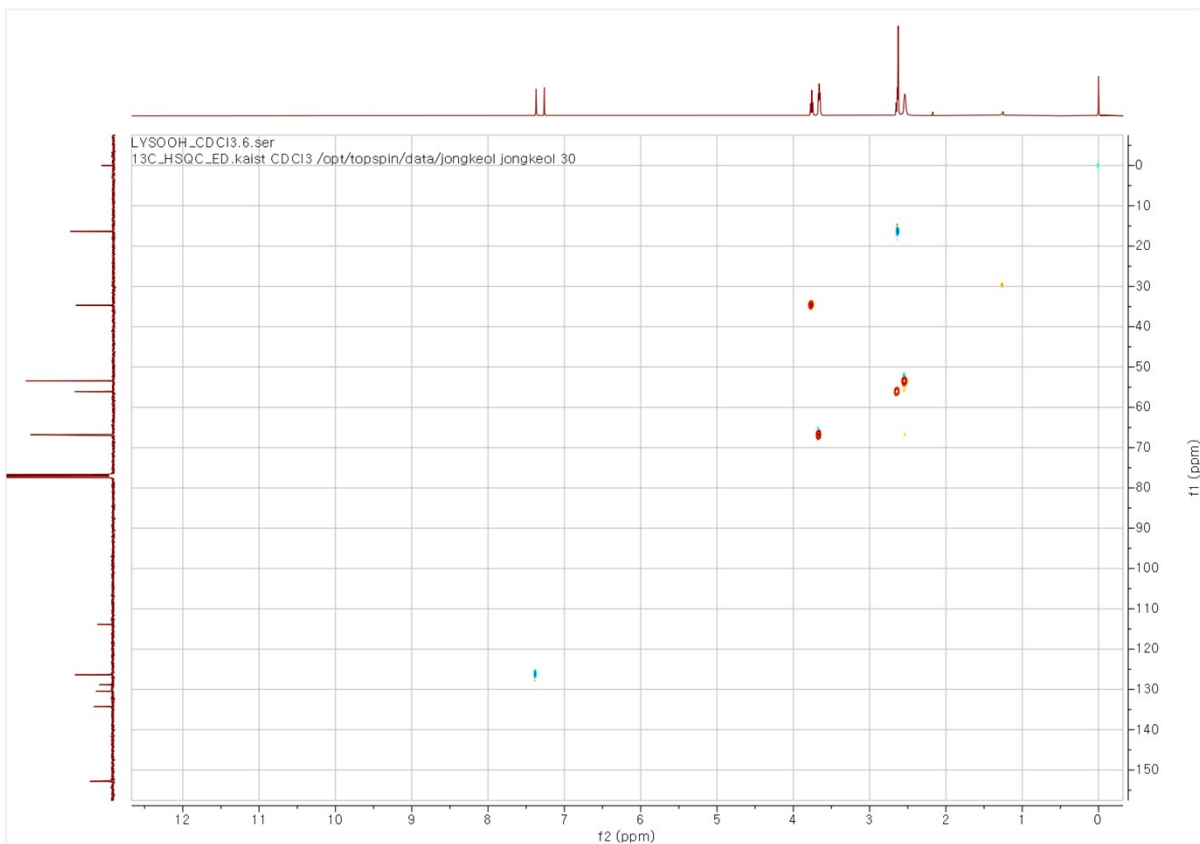
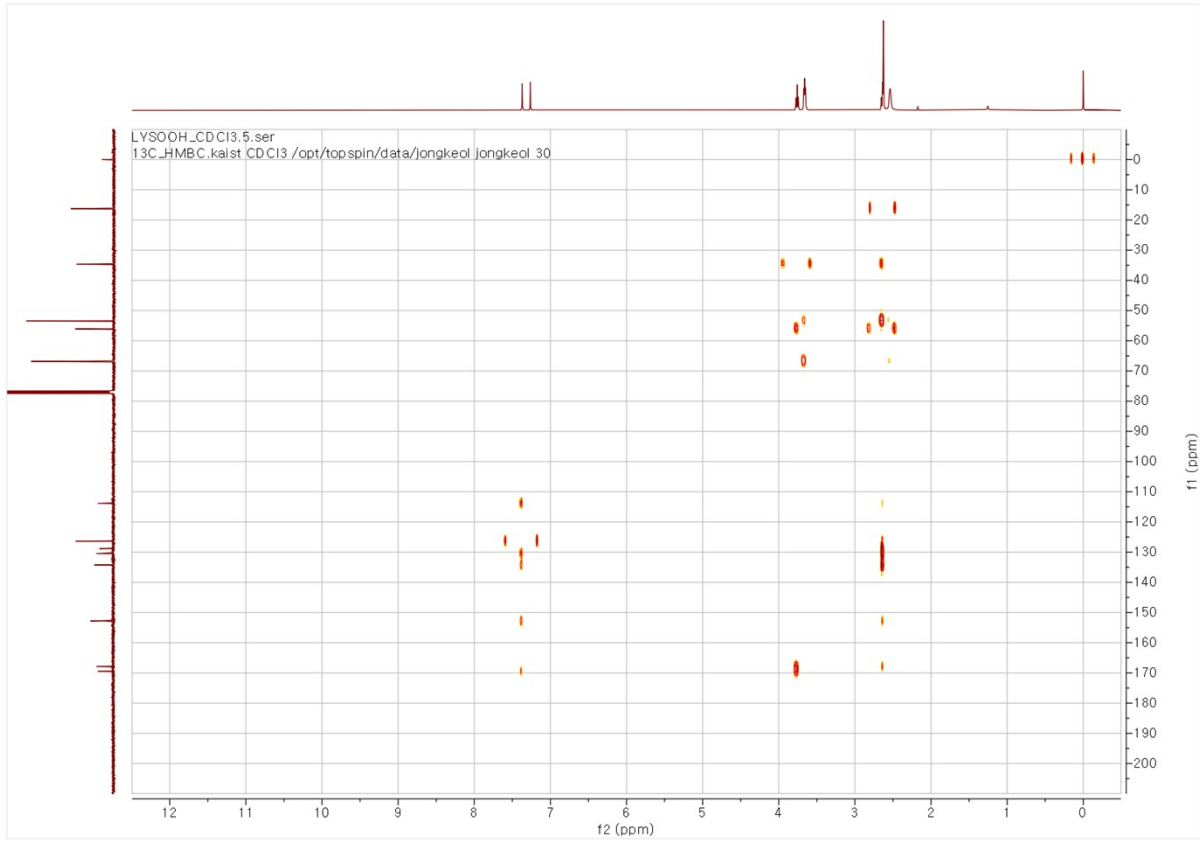


Fig S8. ^1H - ^{13}C HMBC (top) and ^1H - ^{13}C HSQC (bottom) NMR spectra **LOH**.

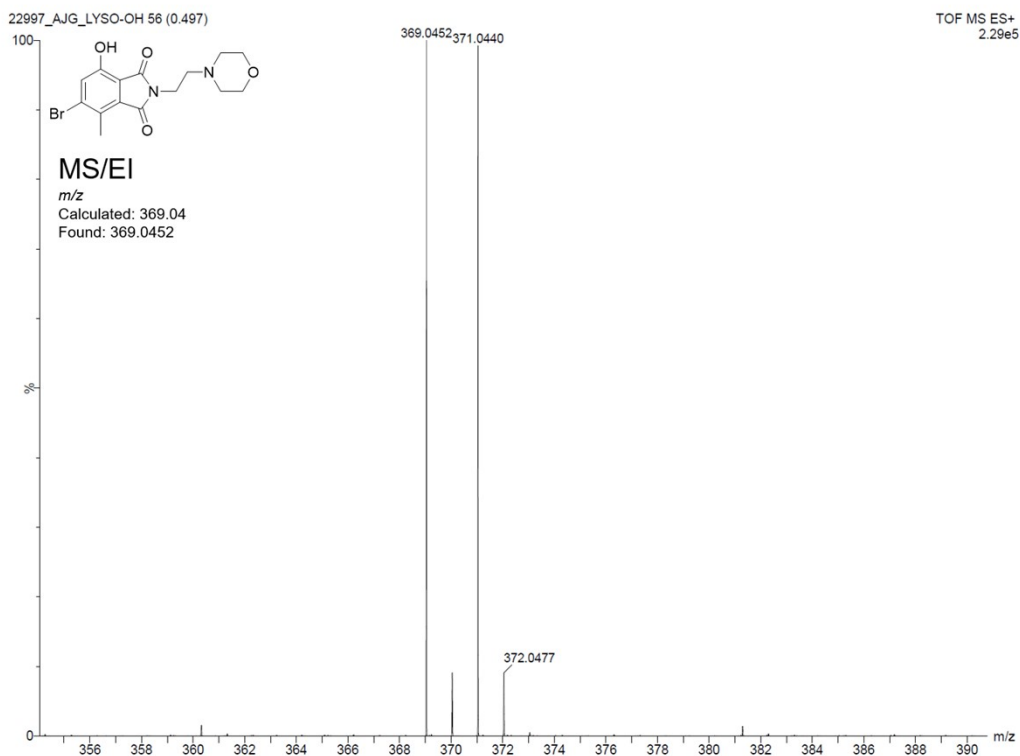


Fig. S9. HR-MS spectra of **LOH**.

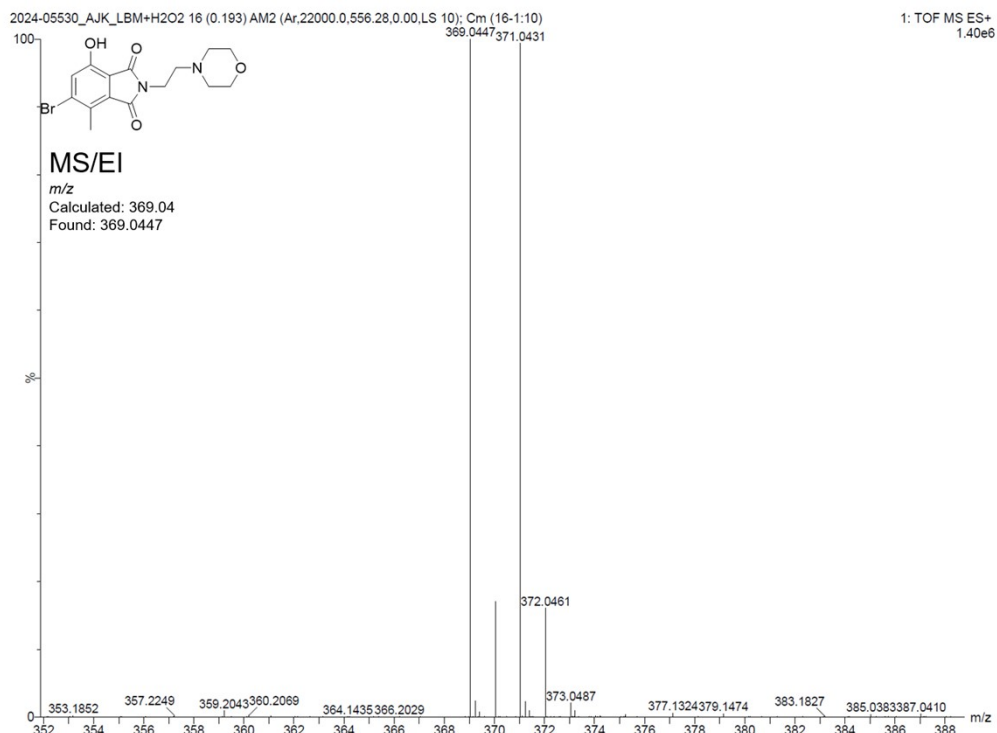


Fig. S10. HR-MS spectra of LBM + H₂O₂.

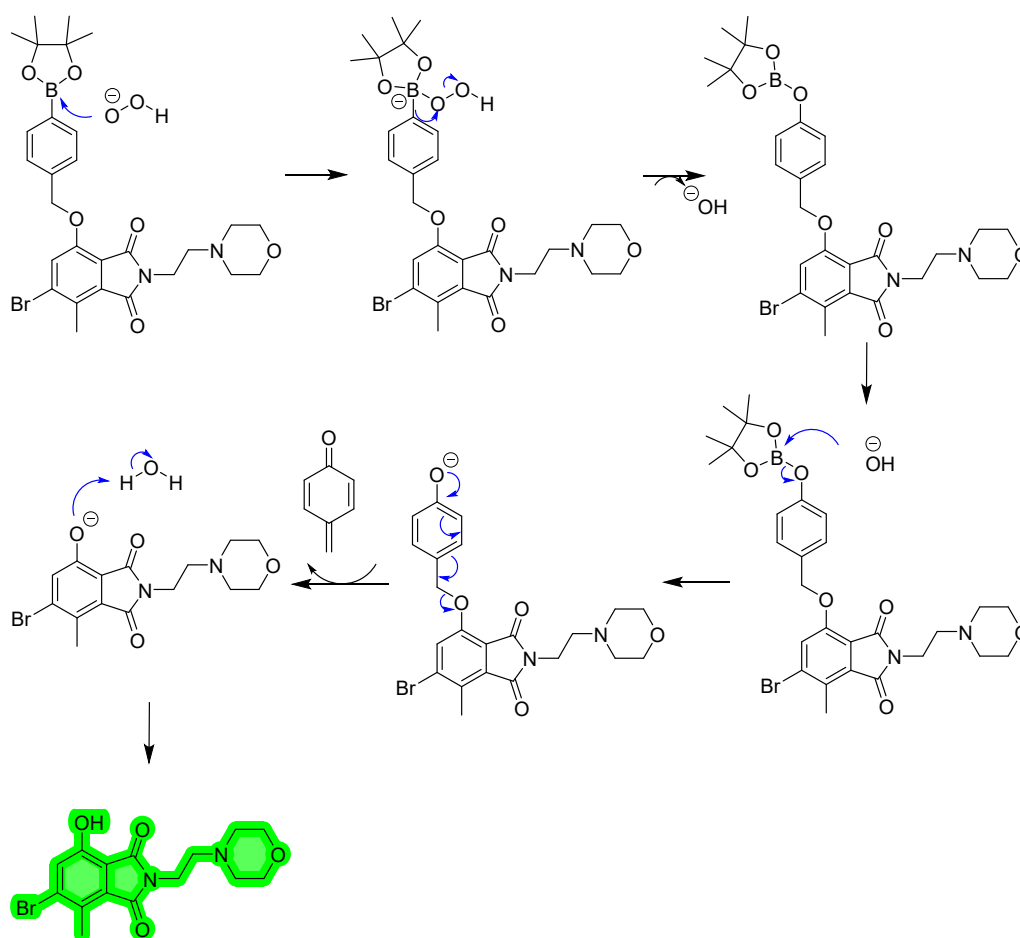


Fig. S11. Proposed sensing mechanism for the detection of H₂O₂ by LBM.

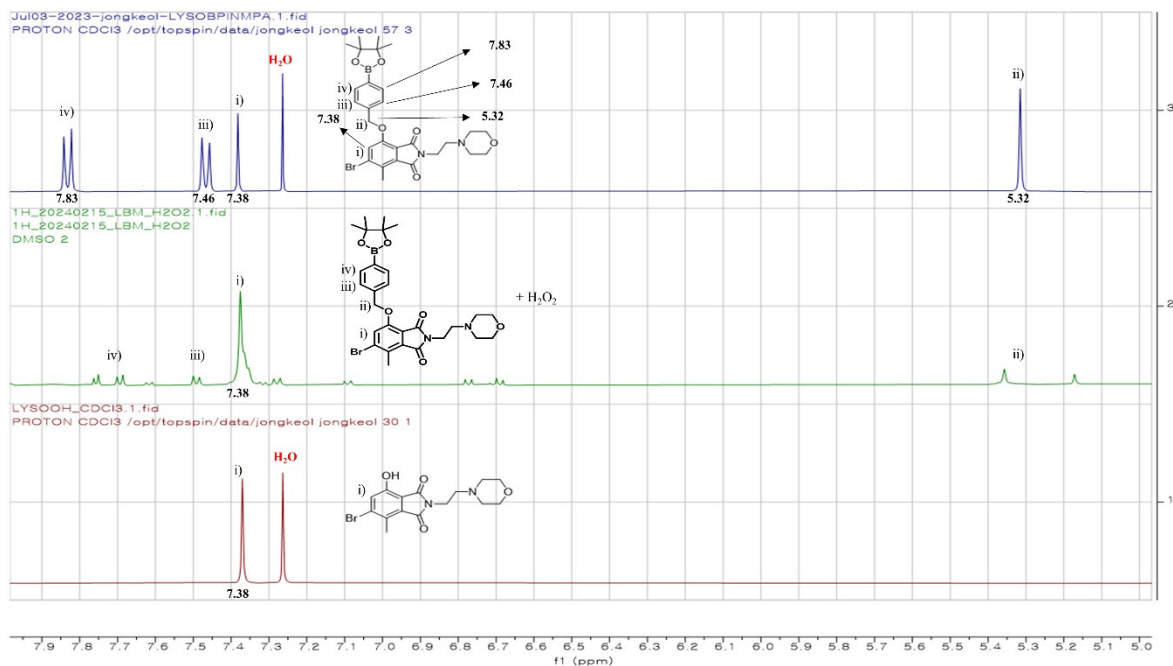


Fig. S12. Comparison of the ^1H NMR spectra of a) **LBM**, b) reaction mixture containing **LBM** + H_2O_2 , c) **LOH**

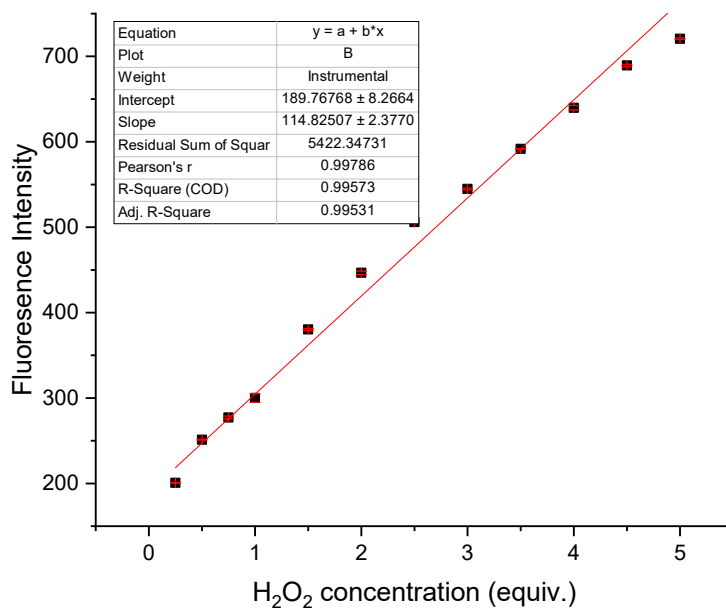


Fig. S13. Plot for the calculation of the limit of detection from the emission of **LBM** ($15 \mu\text{M}$) in the solution of PBS (pH 7.4); λ_{ex} : 417 nm, λ_{em} : 517 nm; slit width 5.0 nm/5.0 nm. Error bars represent mean values \pm SD ($n = 3$).

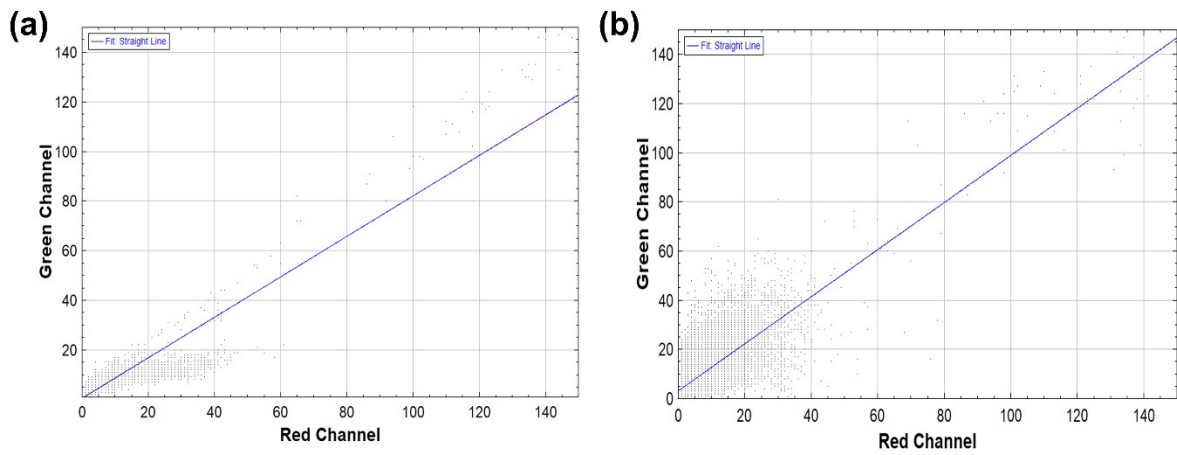


Fig. S14. Co-localization of Lyso-tracker Dye (Red Channel) and LBM (Green Channel) upon treatment of cells with **a)** PMA and **b)** H₂O₂.

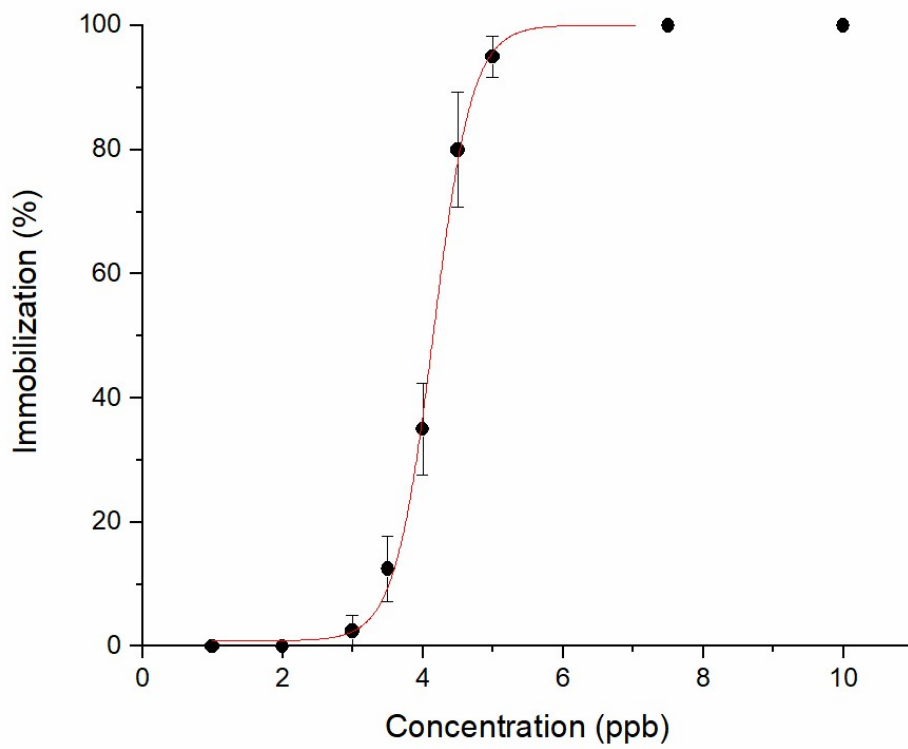


Fig. S15. Immobilization of daphnids after 48 h exposure to various concentrations of silver ion (1.0 to 10.0 $\mu\text{g L}^{-1}$). The graph is expressed as a mean value with SEM with 40 daphnids in a group.

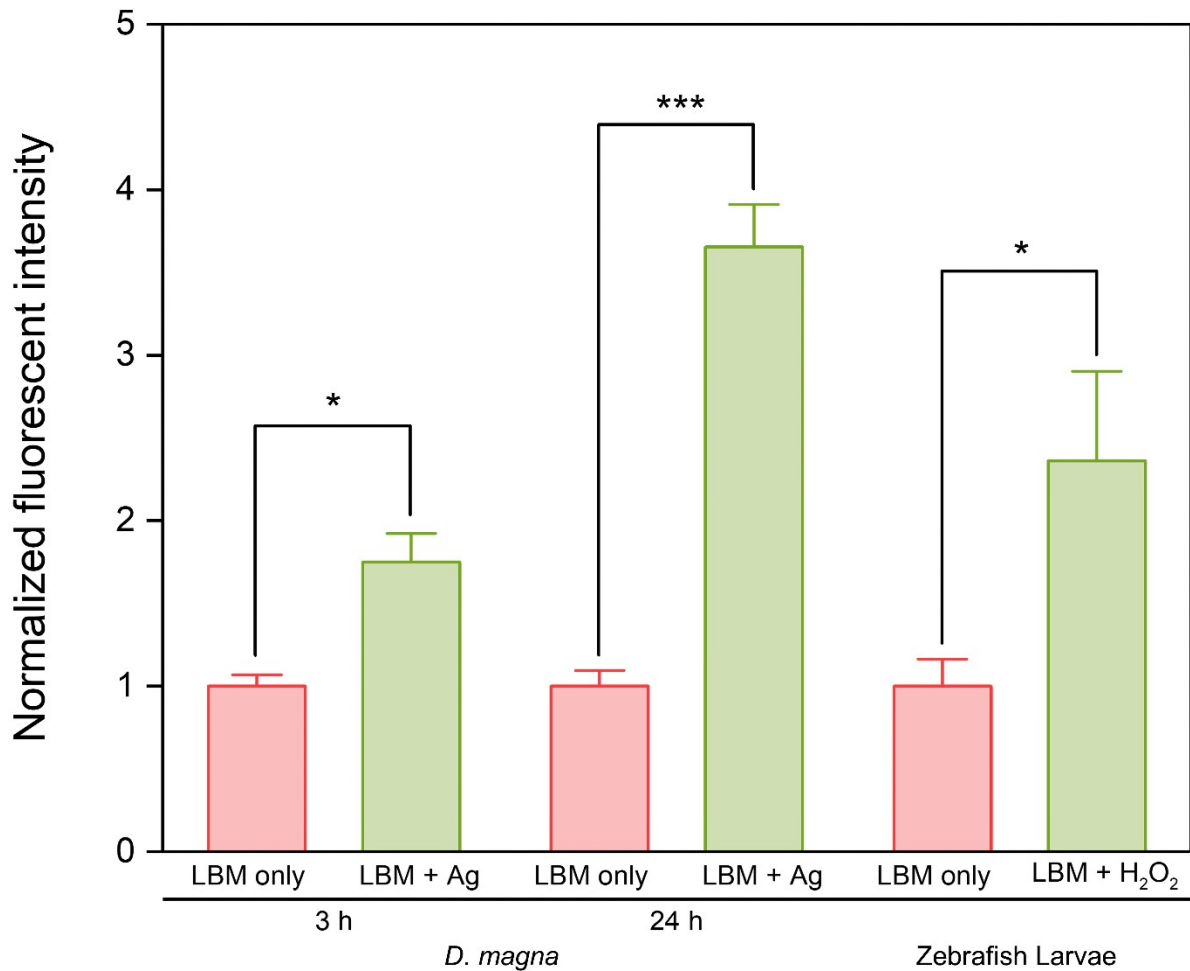


Fig. S16. Relative fluorescent intensities of *D. magna* between the group incubated with only 15.0 μM LBM for 30 min at designated times and the group exposed to 3 ppb Ag for designated times, followed by incubation with 15.0 μM LBM for 30 min. Relative fluorescent intensities of 96 hpf zebrafish larvae between the group incubated with only 15.0 μM LBM for 30 min and the group exposed to 5 mM H₂O₂ for 1 h, followed by incubation with 15.0 μM LBM for 30 min. Statistical differences were analyzed by one-way analysis of variance (ANOVA) and Bonferroni multiple comparison tests. Error bars indicate standard error of the mean; * and *** indicate $p < 0.05$ and < 0.001 , respectively.

Properites (Note: abbreviation same as website)	Value (LOH)	Value (LBM)
miLogP	1.64	4.59
TPSA	71.78	79.25
natom	22	38
MW	369.21	585.30
nON	6	8
nOHNH	1	0
nviolations	0	1
nrotb	3	7
volume	278.15	511.75

Table. S2. Information of the **LOH** and **LBM** calculated through ‘molinspiration’ property engine v2022.08’ at the website, <http://www.molinspiration.com>.

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