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# Rational construction of the novel probe for rapid detection of stress changes in butyrylcholinesterase in apoptotic cells

Zhenqing Yu[a], Xiang Li[a, b], Xiaofeng Lu[a]*, and Yong Guo[a]*

[a]Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, 730000, P. R. China；[b]University of Chinese Academy of Sciences, Beijing, 100049, P. R. China.

## Contents

# 1. $^{1}$H NMR, $^{13}$C NMR and Mass Spectra for Synthesis

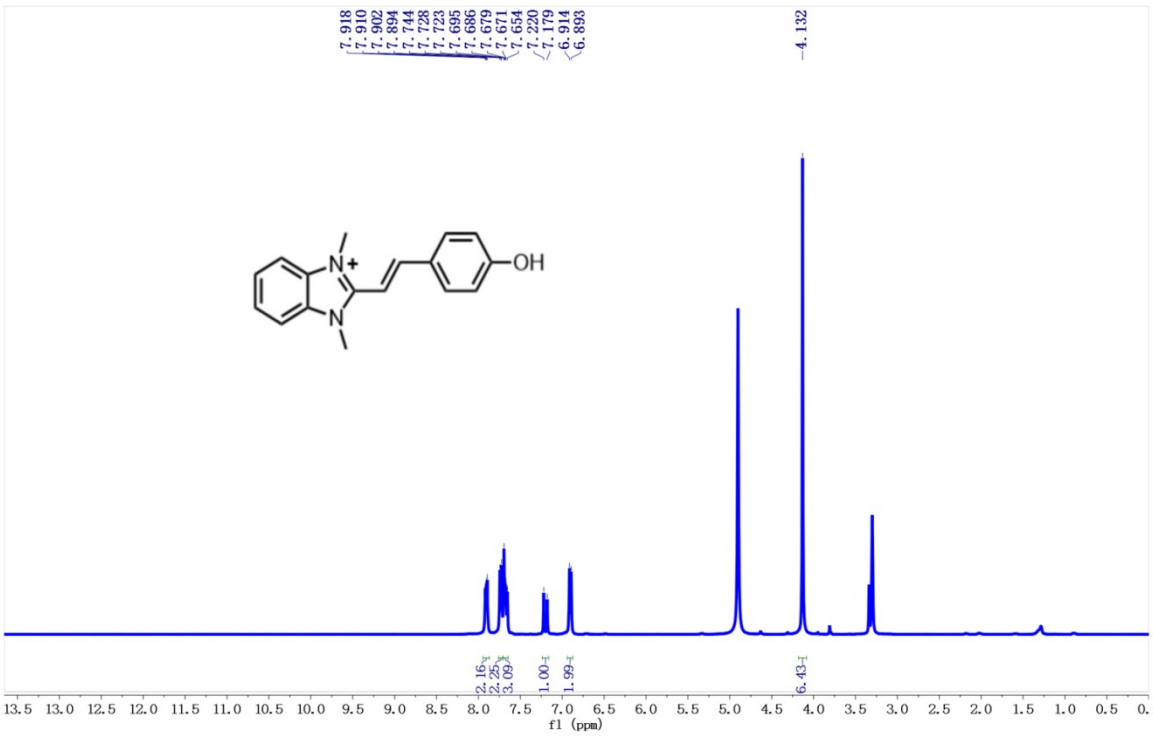



**Fig. S1.** $^{1}$H NMR (400 MHz) spectrum of **CnOH** in MeOD.

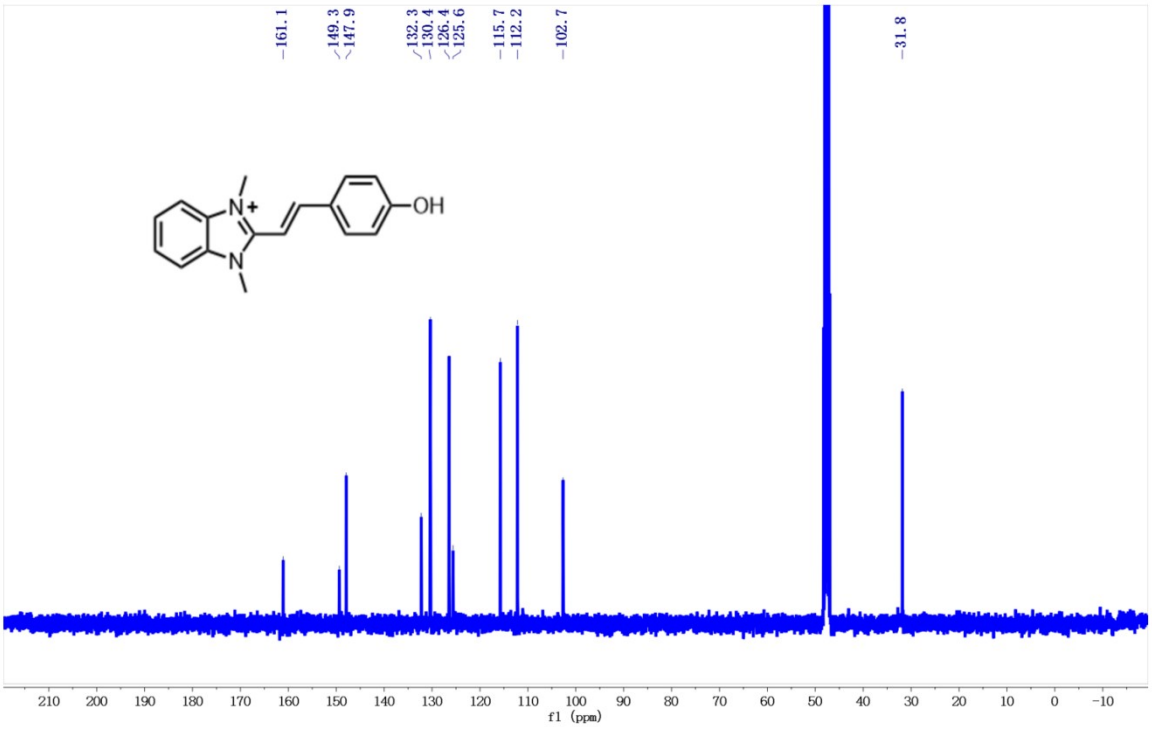


**Fig. S2.** $^{13}$C NMR (100 MHz) spectrum of **CnOH** in MeOD.

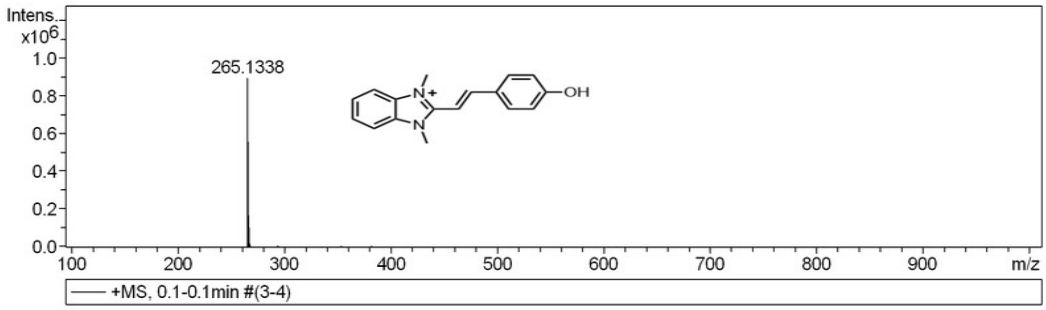



**Fig. S3.** High resolution mass spectrum of **CnOH**.

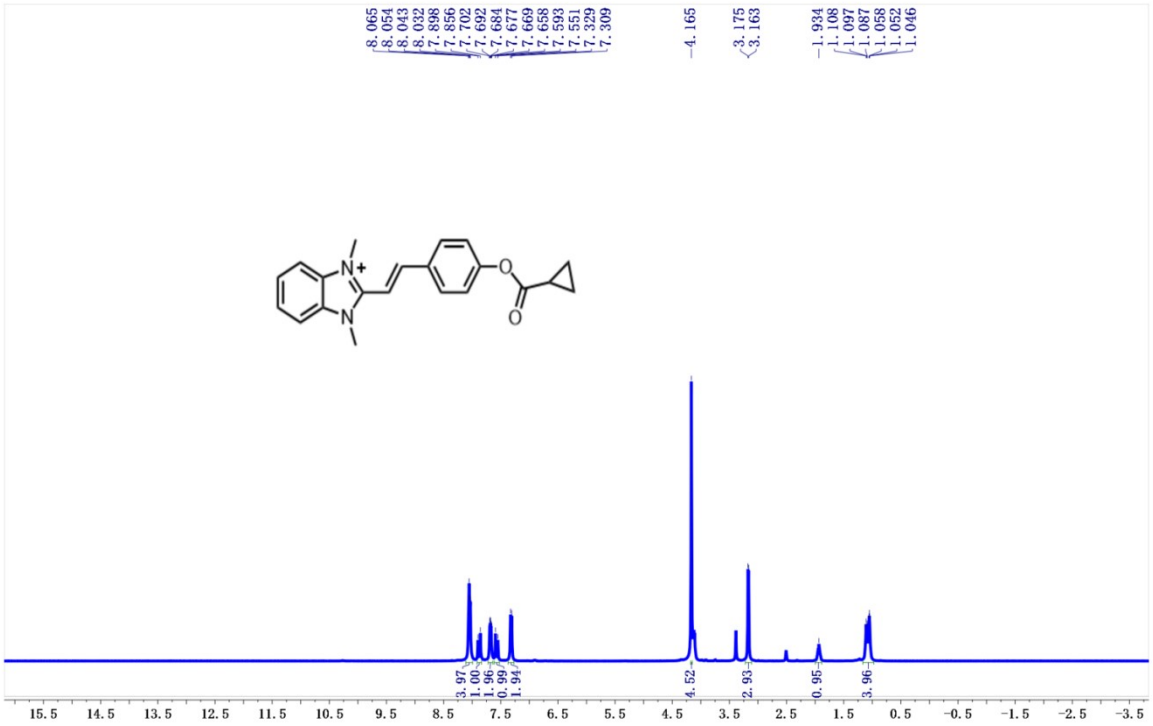



**Fig. S4.** $^{1}$H NMR (400 MHz) spectrum of **CnN** in DMSO−d6.

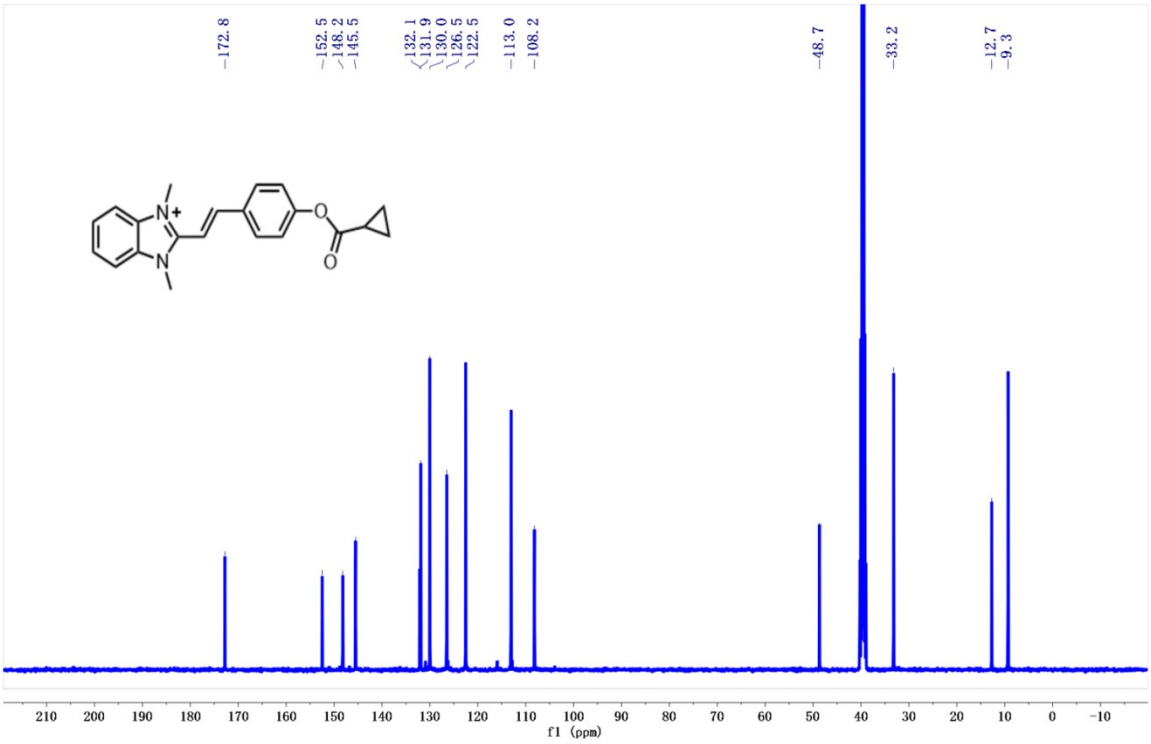



**Fig. S5.** $^{13}$C NMR (100 MHz) spectrum of **CnN** in DMSO−d6.

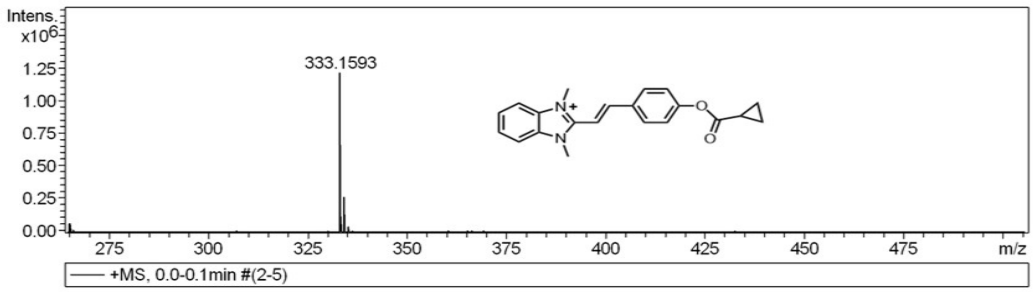



**Fig. S6.** High resolution mass spectrum of **CnN**.

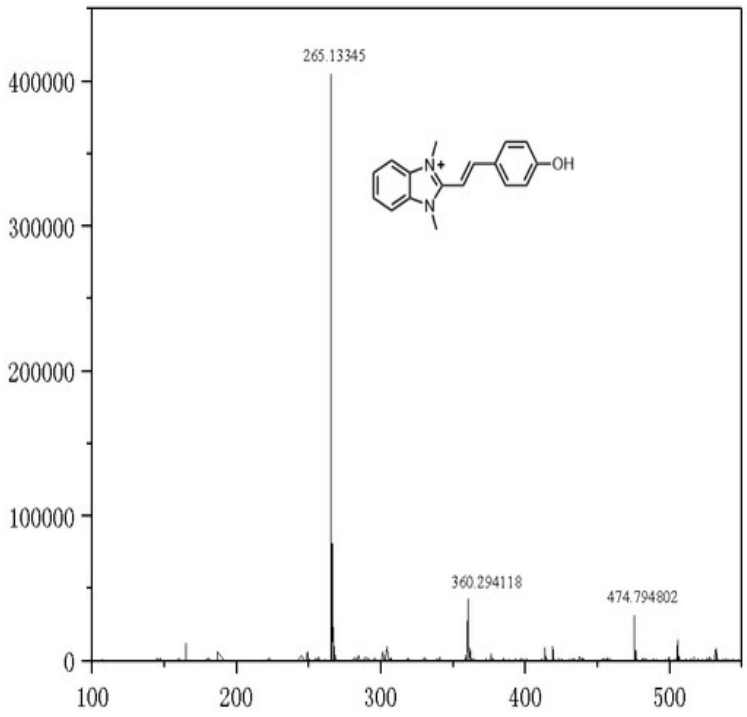



**Fig. S7.** Mass spectrometry of **CnN** reacted with BChE after 2h.

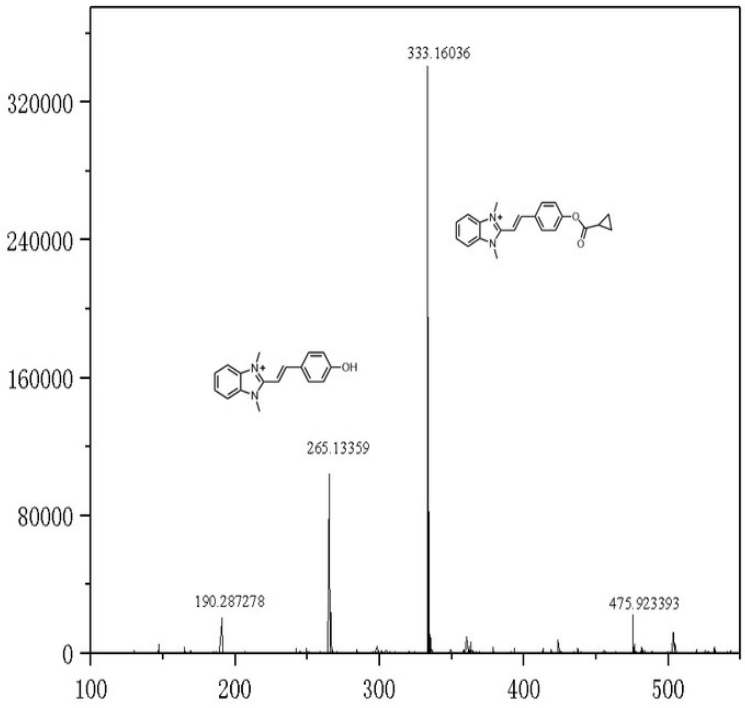



**Fig. S8.** Mass spectrometry of the reaction of **CnN** with BChE for 10 min.

## 2. General Spectral Study of CnN and CnOH

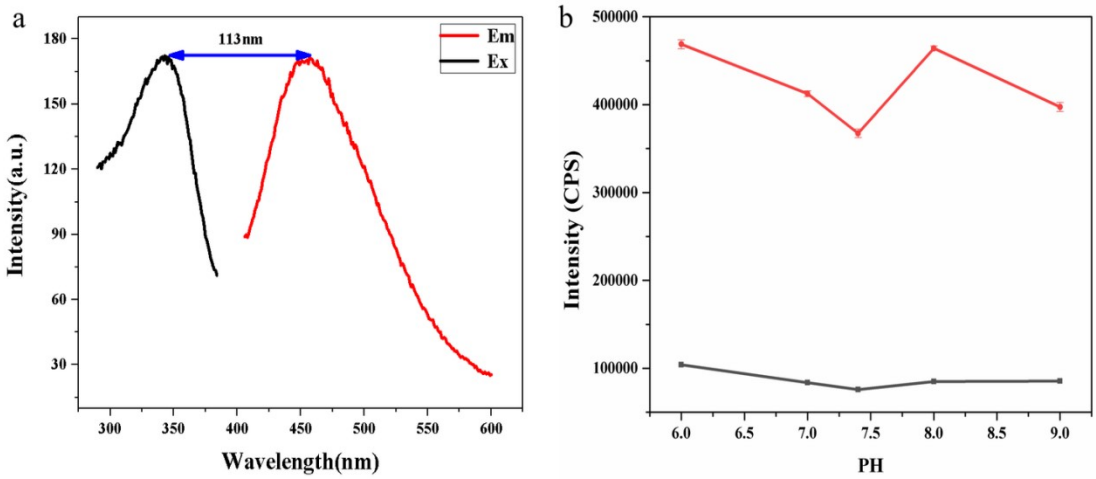



**Fig. S9.** (a) Excitation and emission spectra of **CnOH** (10.0 μM) in 10 mM PBS buffer, pH 7.4. (b) Fluorescence intensity of 10.0 μM **CnN** in the absence (black) and presence of 5.0 U/ml BChE (red) changed with excitation wavelength at 343 nm in the range of pH 6 to 9.

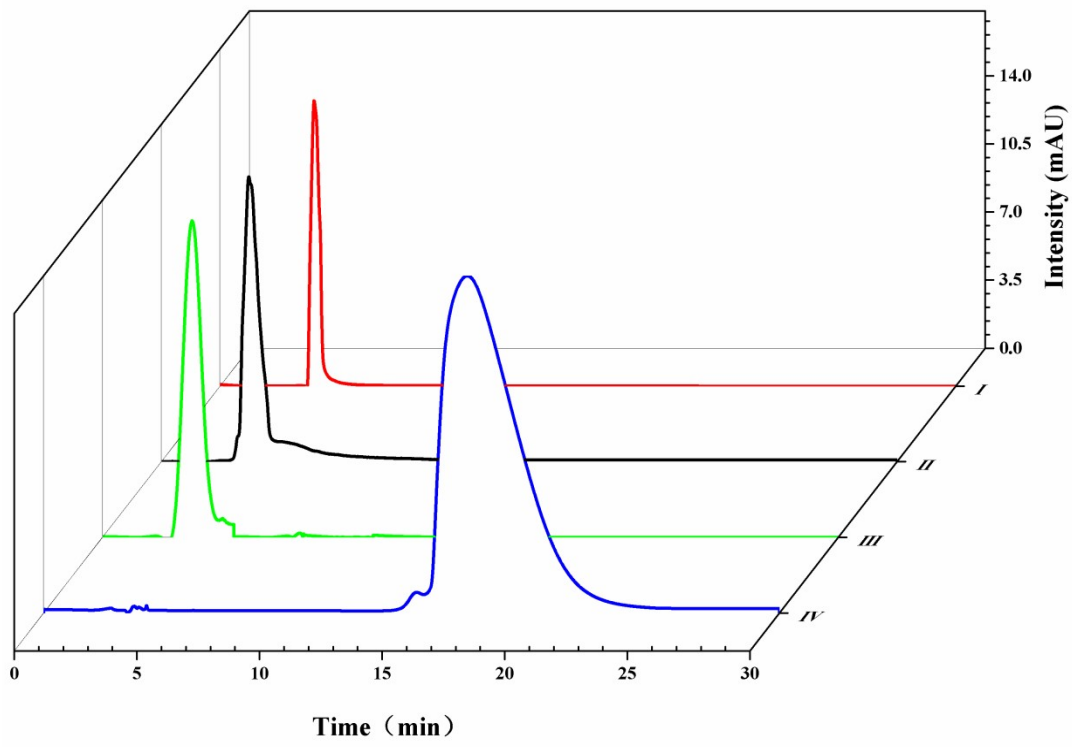



**Fig. S10.** (Ⅰ) **CnOH**; (Ⅱ) **CnN** reacted with AChE; (Ⅲ) **CnN** reacted with BChE and (Ⅳ) **CnN** chromatograms. Mobile phase: solution A (25.0 mM ammonium acetate, pH 7.40), solution B (methanol);

A/B = 1.5/8.5 (v/v), flow rate 0.5 ml/min, detection wavelength 320 nm.

## 3. Density functional theory (DFT) calculations for CnN and CnOH

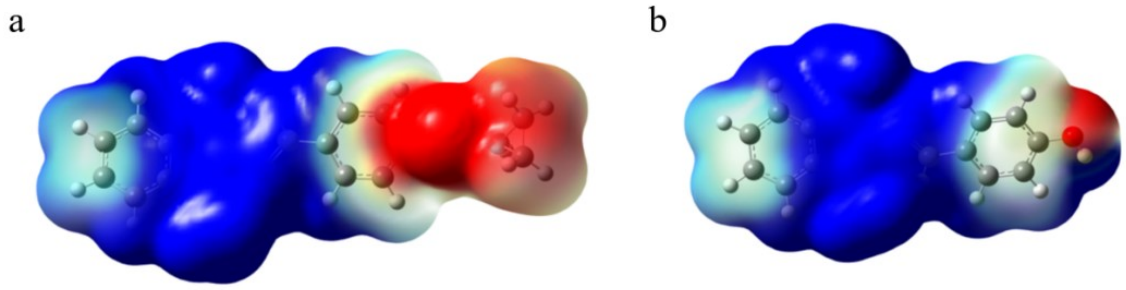



**Fig. S11.** Calculated the electrostatic potential of **CnN** (a) and **CnOH** (b).

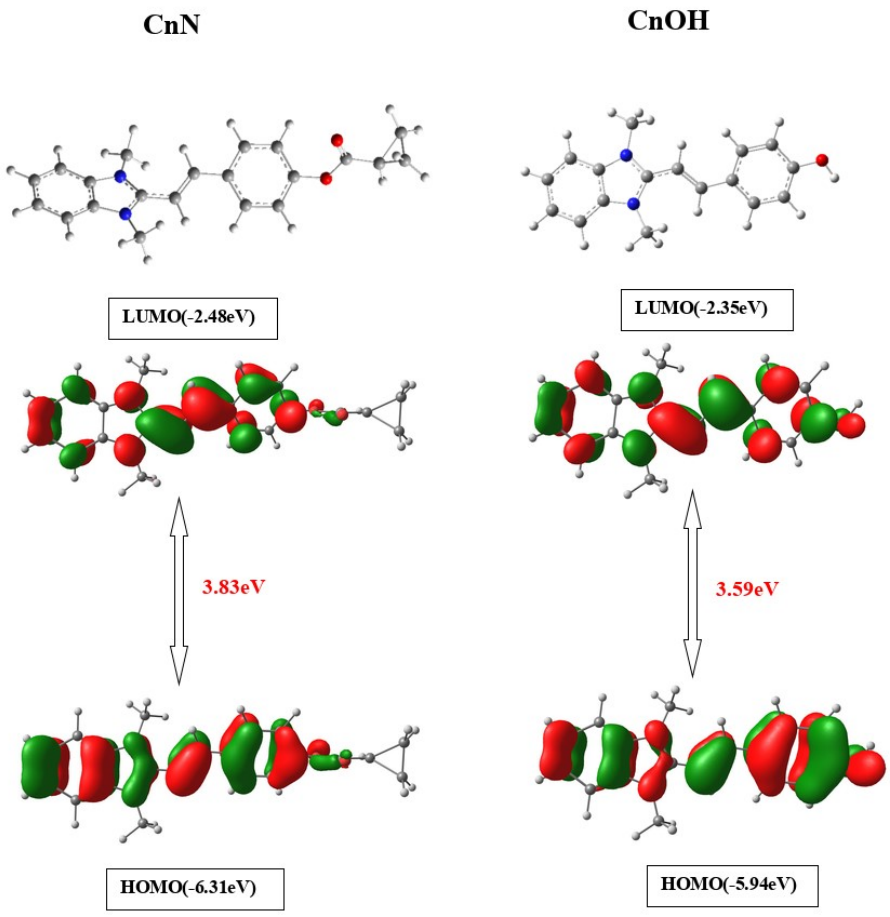
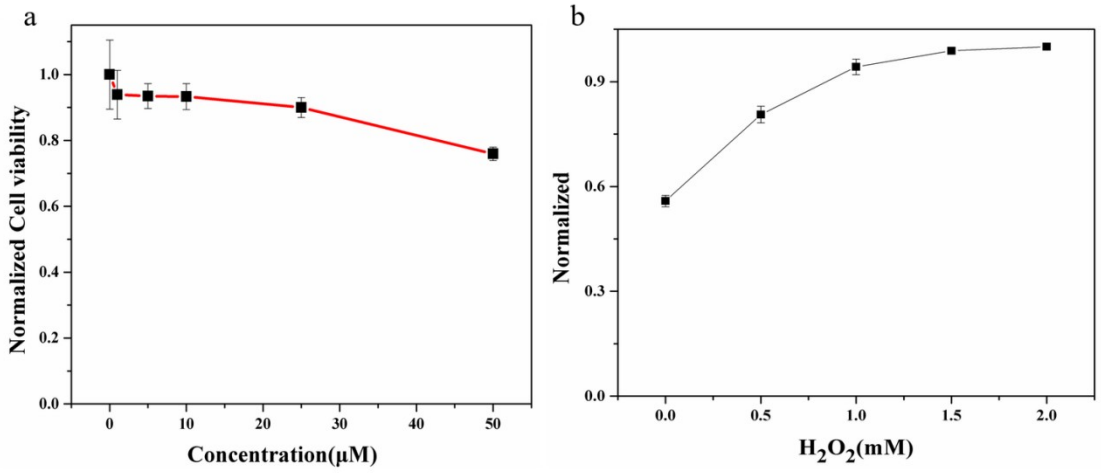



**Fig. S12.** Calculated frontier molecular orbitals and orbital energy levels for **CnN** and **CnOH**. In the ball

and stick model, the carbon, nitrogen, and oxygen atoms are marked in gray, blue, and red, respectively.

# 4. Cytotoxicity Assys and Cell Experiment



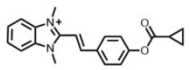
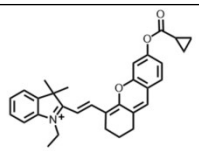
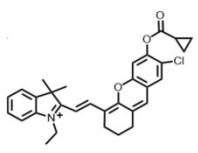
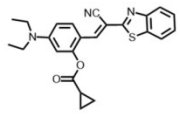
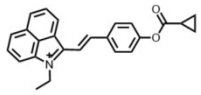
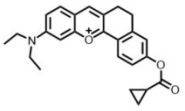
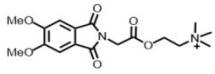
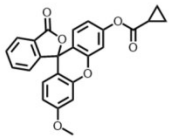
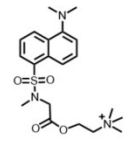

| NO | Structure | LOD | sensing mode | Response time | Stokes shift | Cytotoxicity (Viability/ Concentration) | Re. |
|---|---|---|---|---|---|---|---|
| CnN |  | 0.07969 U/mL | turn-on | 5 min | 113 nm | 90%(25 μM) | This work |
| 1 |  | - | turn-on | 30 min | 45 nm | 80%(20 μM) | 1 |
| 2 |  | 3.75 U/L | turn-on | 35 min | 38 nm | 80%(10 μM) | 2 |
| 3 |  | 0.8 U/L | turn-on | 90 min | 48 nm | 90%(20 μM) | 3 |

**Fig. S13.** (a) MTT assay for the survival rate of LO2 cells. (b) Analysis of fluorescence intensity under different hydrogen peroxide concentrations.

**Table S1.** Properties of respresentive fluorescent BChE probes.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **4** |  | **0.077 μg/mL** | **ratiometric** | **20 min** | **141 nm** | **More than 90%(20 μM)** | **4** |
| **5** |  | **29 ng/mL** | **turn-on** | **10 min** | **48 nm** | **More than 90%(20 μM)** | **5** |
| **6** |  | **-** | **turn-off** | **30 min** | **165 nm** | **-** | **6** |
| **7** |  | **-** | **turn-on** | **-** | **65 nm** | **90%(20 μM)** | **7** |
| **8** | **-** | **6.8 U/L** | **colorimetric** | **-** | **-** | **-** | **8** |
| **9** |  | **1.2 U/L** | **turn-on** | **2 min** | **145 nm** | **-** | **9** |

**References:**


1 S. Liu, H. Xiong, J. Yang, S. Yang, Y. Li, W. Yang and G. Yang, *ACS. Sens.*, 2018, **3**, 2118−2128.

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4 C. Wan, J. Li, J. Gao, H. Liu, Q. Zhang, P. Zhang and C. Ding, *Dyes. Pigments.*, 2022, **197**, 109874.

5 T. Cao, L. Zheng, L. Zhang, Z. Teng, J. Qian, H. Ma, J. Wang, Y. Cao, W. Qin and Y. Liu, *Sen. Actuators. B: Chem.*, 2021, **330**, 129348.

6 S. Kang, S. Lee, W. Yang, J. Seo and M. Han, *Org. Biomol. Chem.*, 2016, **14**, 8815−8820.

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8 X. Wang, K. Koh and H. Chen, *Sen. Actuators. B: Chem.*, 2017, **251**, 869−876.

9 S. Yoo and M. Han, *Chem. Commun.,* 2019, **55**, 14574−14577.