Supplementary materials

Reasonable design and comparison of three curcumin-based fluorescent probes for viscosity detection in vivo and vitro

Wenhao Du, Yue Gu, Xin Zhou, Zhonglong Wang*, Shifa Wang*

Co-Innovation Center of Efficient Processing and Utilization of Forest Resources, International Innovation Center for Forest Chemicals and Materials, College of Light Industry and Food, College of Chemical Engineering, Nanjing Forestry University, Nanjing, 210037, China

*Corresponding author: Dr. Zhonglong Wang;

Phone: +86-25-85428369; Fax: +86-25-85428369;

Email: wang_zhonglong@njfu.edu.cn

*Corresponding author: Dr. and Prof. Shifa Wang;

Phone: +86-25-85428369; Fax: +86-25-85428369;

Email: wangshifa65@163.com

2.1. Materials and reagents

The chemical reagents used in the experiments were normally available on the market and used directly. Curcumin was purchased from Shanghai Titan Technology Co., Ltd. Boron trifluoride diethyl ether was obtained from Shanghai Lingfeng Chemical Reagent Co., 85% hydrazine hydrate, hydroxylamine hydrochloride and phenylhydrazine hydrochloride were bought from Shanghai Haohong Biomedical Technology Co., Ltd. The ^{1}H and ^{13}C NMR spectra were recorded with Bruker AV-600 NMR spectrometer. High resolution mass spectrometry (HRMS) was analyzed on a JMS-800D mass spectrometer. The UV-vis absorption spectra were recorded with Shimadzu UV-2450 spectrometer. The fluorescence emission spectra was recorded using a Perkin Elmer LS55 fluorescence spectrometer.

2.2. Synthesis of probes CNO, CNN and CNNB

Synthesis of probe CNO

Curcumin (1 mmol, 0.37 g) was dissolved in ethanol (50 mL) and agitated for 10 min at room temperature, and hydroxylamine hydrochloride (4 mmol, 0.28 g) was then added to the solution, and refluxed for 6 h. After completing the reaction, saturated brine (25 mL) was added, and the solution was extracted with ethyl acetate for three times. The combined organic phase was condensed to obtain the crude **CNO**, which was purified by silica column chromatography (PE : EA = 1:1, v/v) to obtain white solid product **CNO**. Yield: 70.3%; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.42 (s, 1H), 9.34 (s, 1H), 7.32 – 7.25 (m, 4H), 7.12 – 7.02 (m, 4H), 6.85 (s, 1H), 6.80 (dd, J = 8.1, 4.4 Hz, 2H), 3.84 (s, 6H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ 168.35, 162.25, 148.16, 147.98, 147.95, 147.83, 136.45, 134.79, 127.33, 126.99, 121.69, 121.33, 115.61, 115.54, 112.64, 110.35, 110.33, 110.09, 97.87, 55.70, 55.66 ppm. HRMS (m/z): $[M + H]^+$ calcd for $C_{21}H_{20}NO_5$ $+H^+$, 366.1337; found, 366.1341.

Synthesis of probe CNN

Curcumin (1 mmol, 0.37 g), $NaHCO_3$ (1 mmol, 0.11 g), and ethanol (10 mL) were charged into reaction flask along with stirring at ambient temperature for 10 min. and hydrazine hydrate solution (85%, 70 μL) was then slowly added to the solution and refluxed for 9 h. Saturated brine was added after ending the reaction, and extracted with ethyl acetate for three times. The combined organic phase underwent washing with distilled water and saturated brine, drying

with anhydrous $MgSO_4$, and evaporating to remove the solvent, and the obtained crude product was furtherly purified with silica column chromatography (PE : EA = 2 : 1) to obtain the yellow solid product **CNN**. Yield: 67.2%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.80 (s, 1H), 9.16 (s, 2H), 7.14 (s, 2H), 7.04 (d, J = 16.5 Hz, 2H), 6.97 – 6.88 (m, 4H), 6.77 (d, J = 8.1 Hz, 2H), 6.62 (s, 1H), 3.83 (s, 6H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ 148.03, 146.93, 129.75, 120.22, 115.75, 109.63, 99.46, 59.91, 55.73 ppm. HRMS (m/z): $[M + H]^+$ calcd for $C_{21}H_{21}N_2O_4$ $+H^+$, 365.1493; found, 365.1501.

Synthesis of probe CNNB

Curcumin (1 mmol, 0.37 g), phenylhydrazine hydrochloride (1.5 mmol, 0.22 g), and ethanol (10 mL) were added to the reaction flask along with agitation for 10 min, and acetic acid solution (10% aqueous acetic acid 0.05 mL) was then added to the solution and refluxed for 15 h. Saturated brine was added to end the reaction, and extracted with ethyl acetate for three times. The combined organic phase underwent washing with distilled water and saturated brine, drying with anhydrous $MgSO_4$, and evaporating to remove the solvent, and the obtained crude product **CNNB** which was furtherly purified with silica column chromatography (PE : EA=2:1) to obtain the white solid product **CNNB**. Yield: 83.2%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.27 (s, 1H), 9.16 (s, 1H), 7.60 – 7.52 (m, 4H), 7.46 (t, J = 7.1 Hz, 1H), 7.23 – 7.12 (m, 3H), 7.08 – 6.92 (m, 5H), 6.81 – 6.72 (m, 3H), 3.81 (d, J = 37.3 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 151.05, 147.91, 147.82, 147.40, 146.85, 142.33, 139.30, 132.83, 130.71, 129.35, 128.37, 127.79, 127.64, 124.74, 120.22, 117.39, 115.75, 115.57, 112.22, 110.57, 109.63, 100.78, 55.65, 55.60. HRMS (m/z): $[M + H]^+$ calcd for $C_{27}H_{25}N_2O_4$ $+H^+$, 441.1818; found, 441.1814.

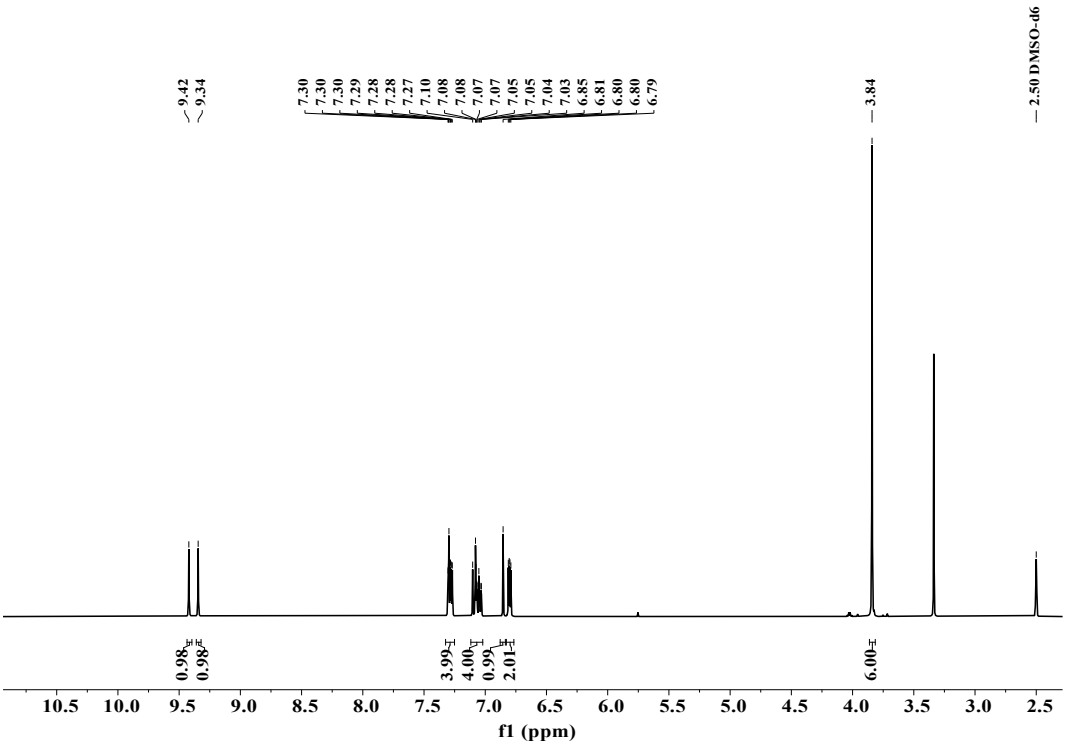

Fig. S1 ^{1}H NMR spectra of **CNO**

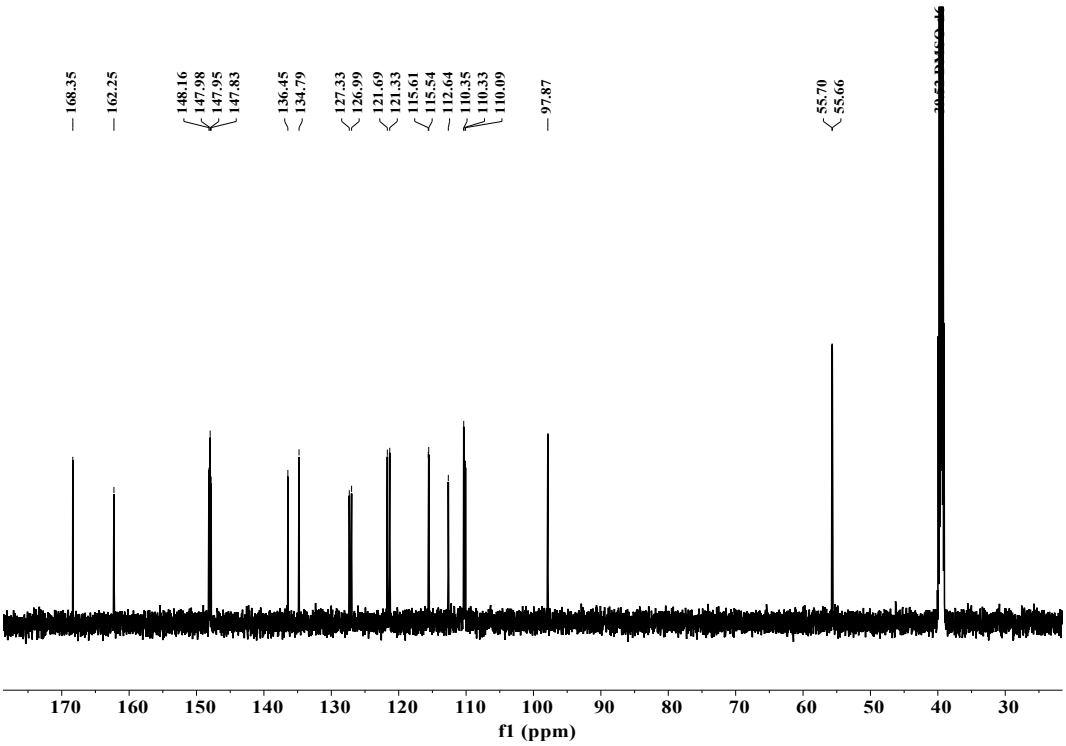

Fig. S2 ^{13}C NMR spectra of **CNO**

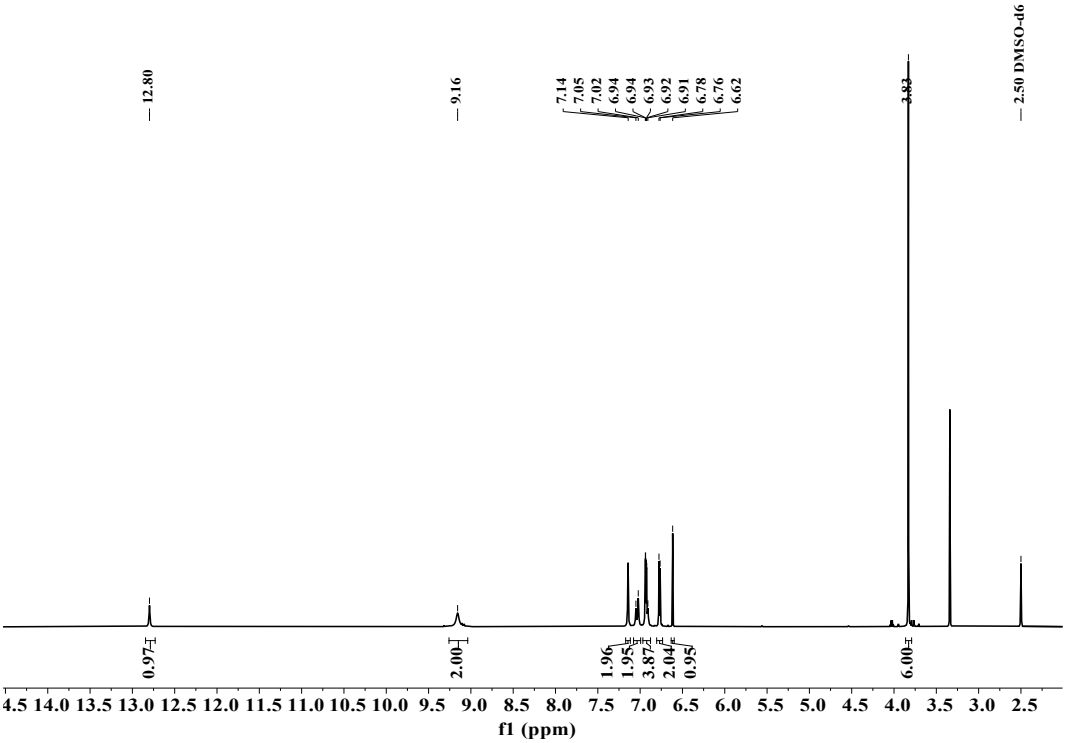

Fig. S3 ^{1}H NMR spectra of **CNN**

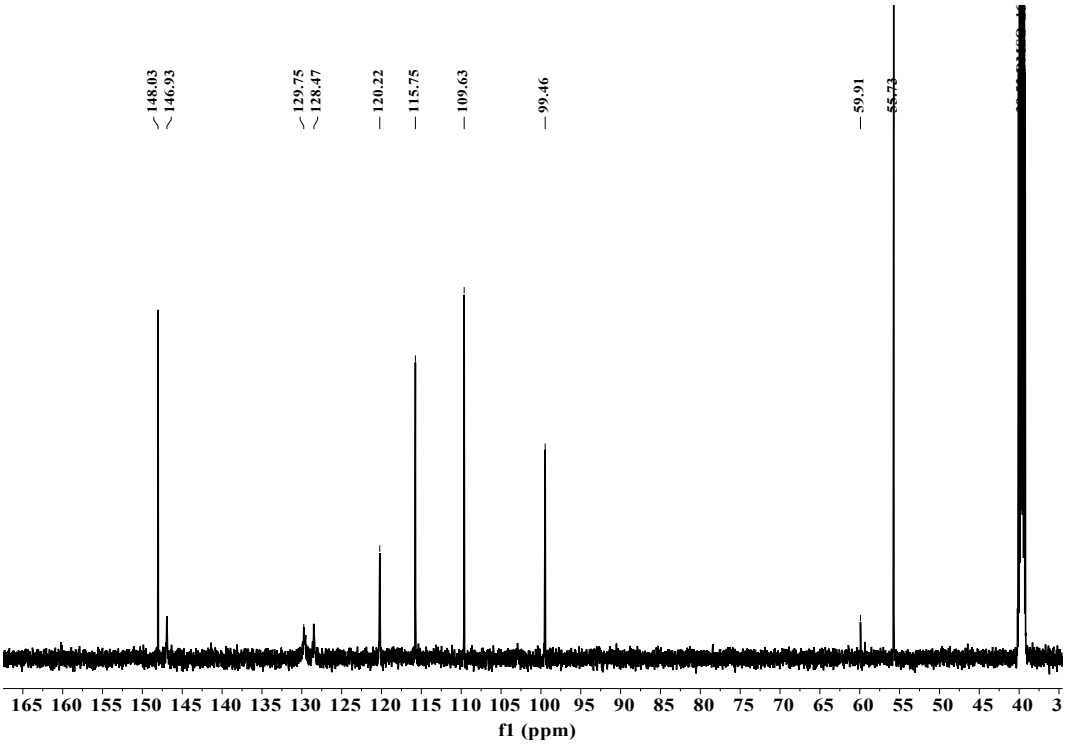

Fig. S4 ^{13}C NMR spectra of **CNN**

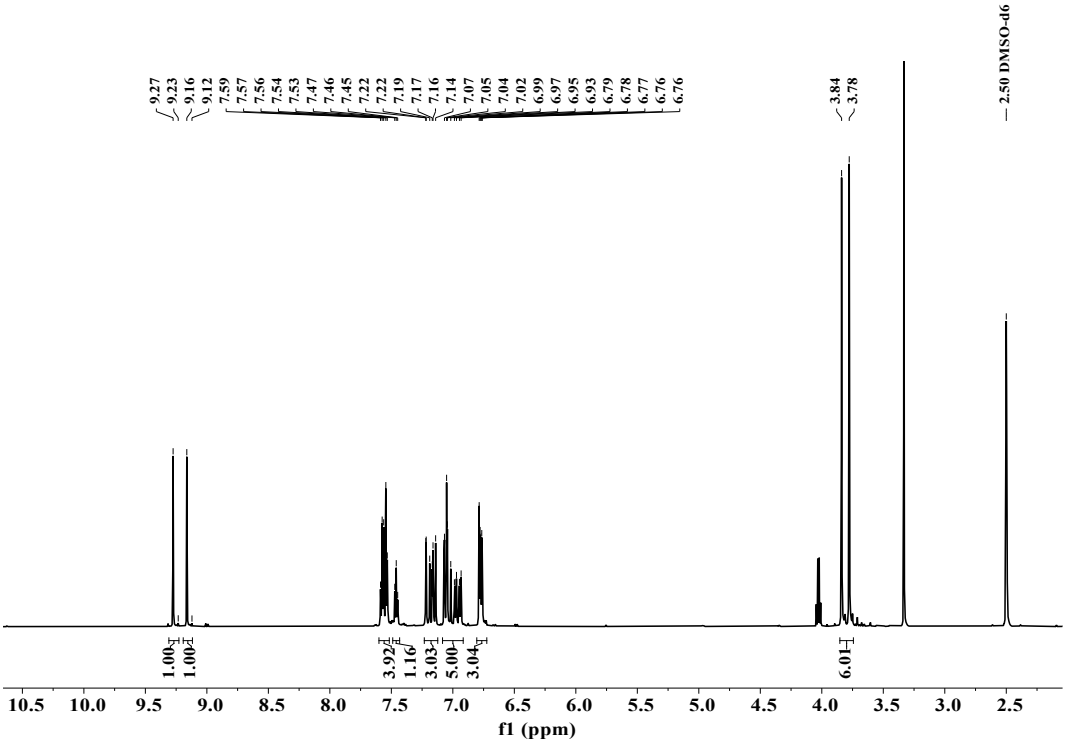

Fig. S5 ^{1}H NMR spectra of **CNNB**

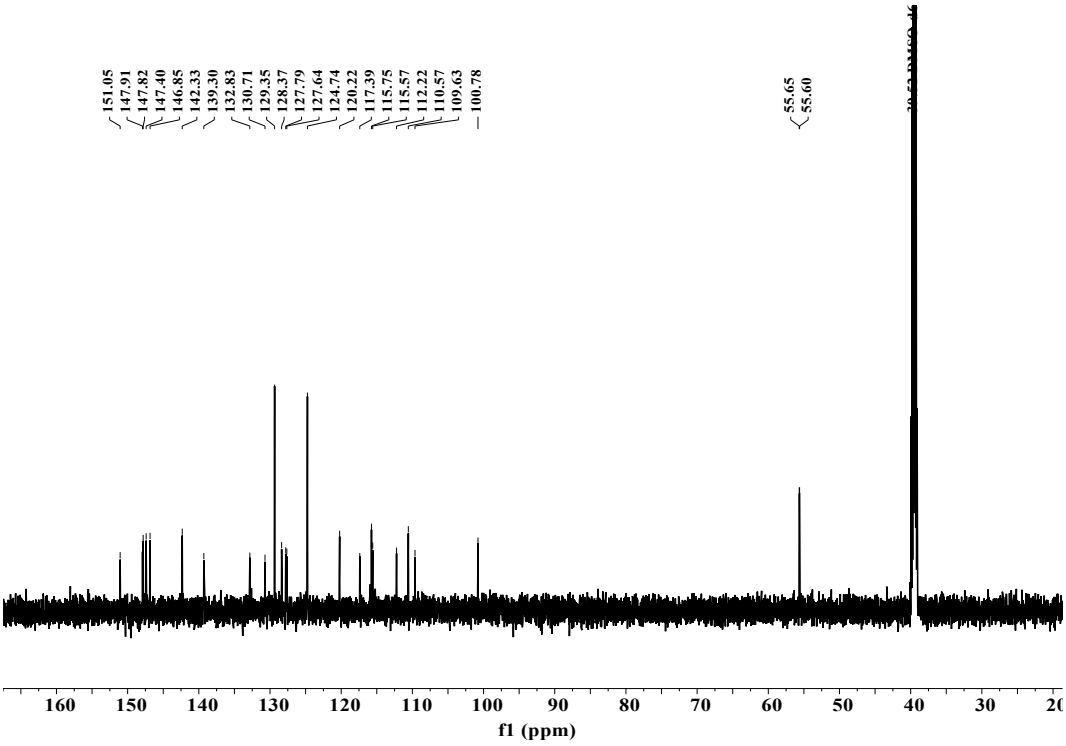

Fig. S6 ^{13}C NMR spectra of **CNNB**

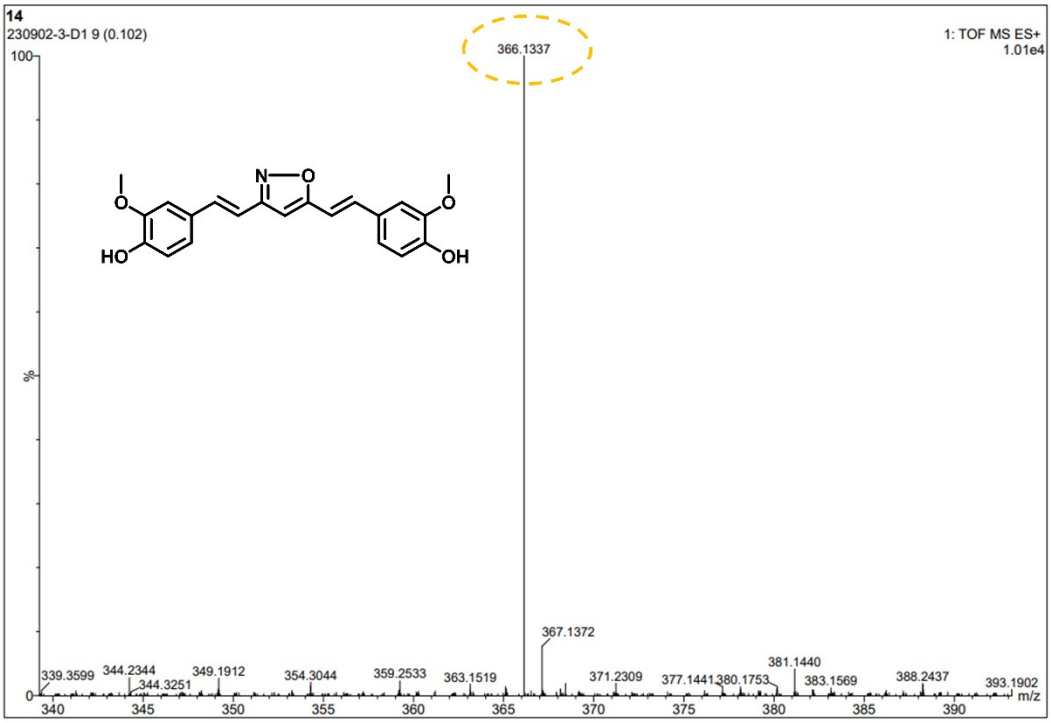

Fig. S7 HRMS of **CNO**

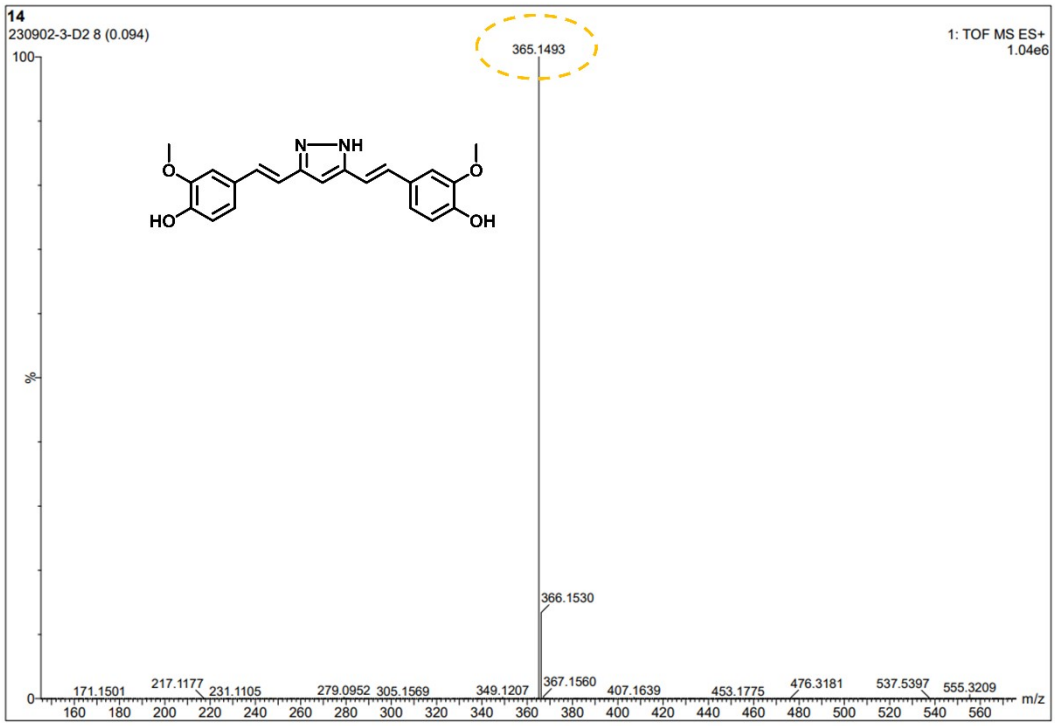

Fig. S8 HRMS of **CNN**

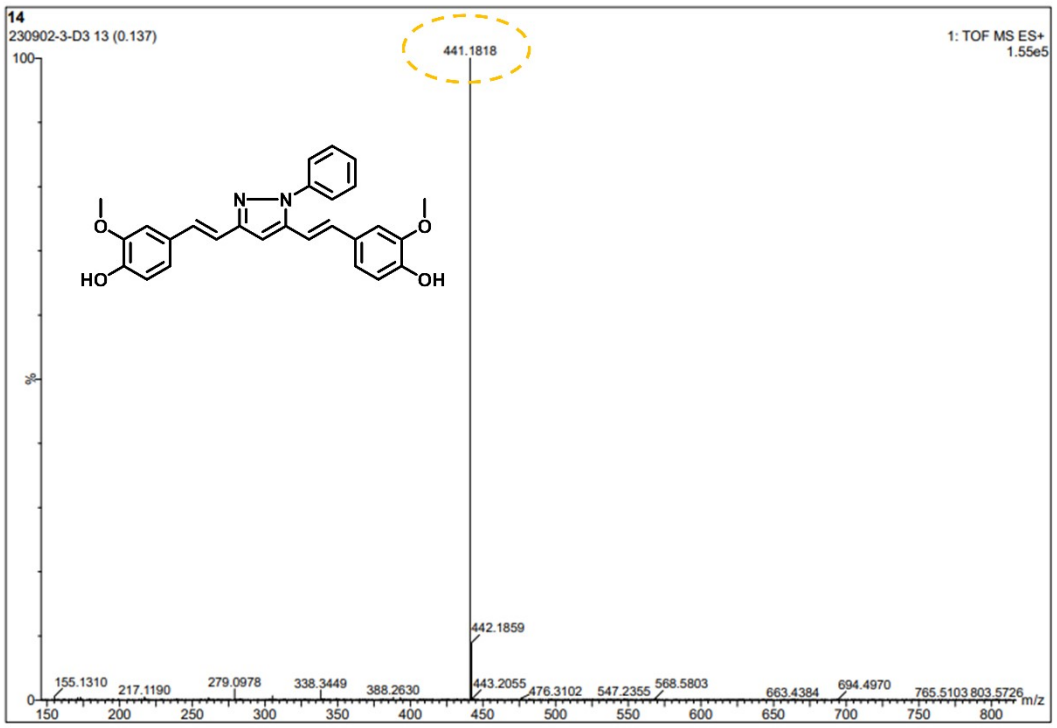

Fig. S9 HRMS of **CNNB**

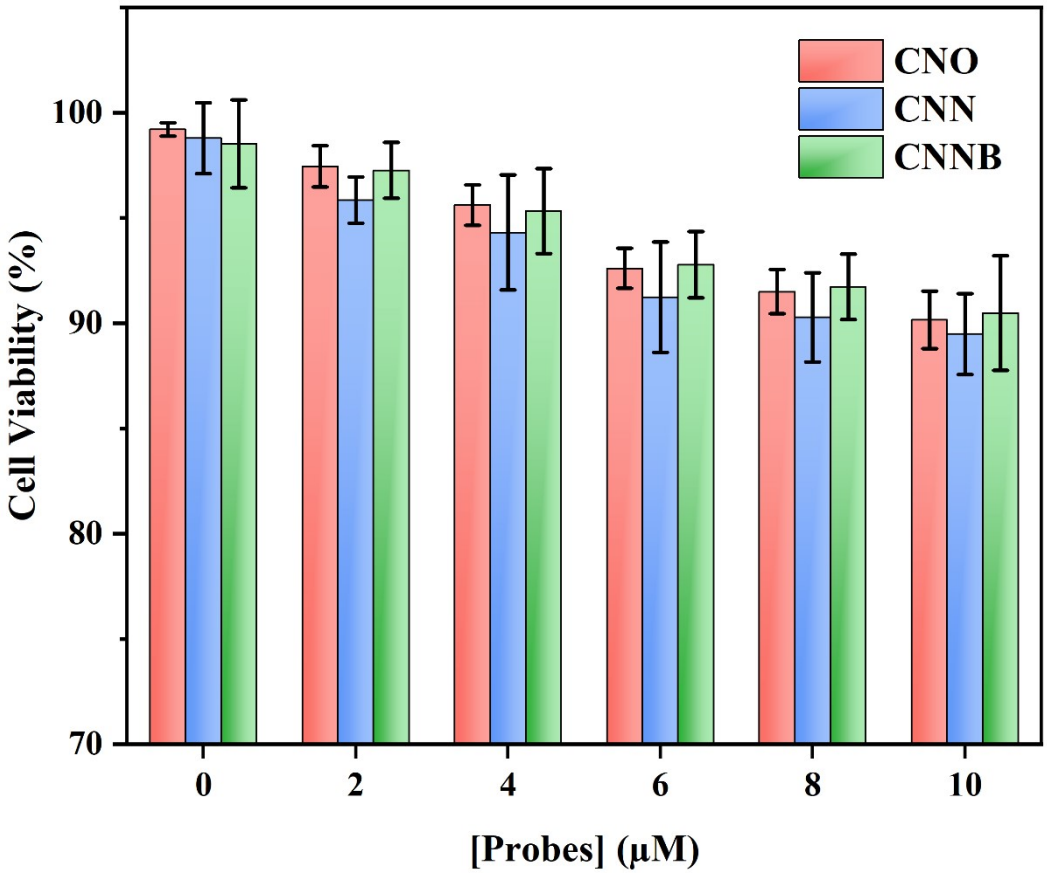

Fig. S10 Survival rate of HeLa cells treated with different concentrations of **CNO, CNN** and **CNNB**