Supporting Information for

Construction of Viscosity-Sensitive RNA Fluorescent Probes and Exploration of Their Applications in Inflammatory Imaging

Meini Tang, Enxiang Ge, Guofang Li, Dingkun Xiong Weiying Lin*

Institute of Optical Materials and Chemical Biology, Guangxi Key Laboratory of Electrochemical Energy Materials, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi 530004, P. R. China

Email: weiyinglin2013@163.com

*Correspondence to: Weiying Lin, Institute of Optical Materials and Chemical Biology, Guangxi Key Laboratory of Electrochemical Energy Materials, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi 530004, P. R. China

Email: weiyinglin2013@163.com.

Table of contents

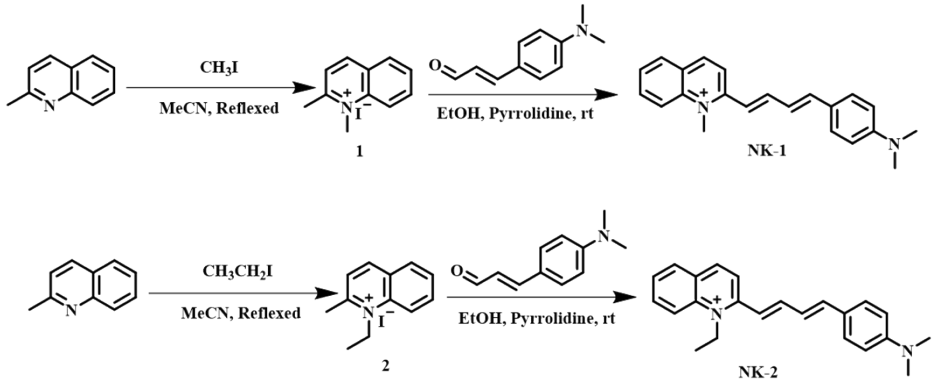
Experimental

1. Equipment and Reagents

All the chemical reagents used in this study were purchased from reagent suppliers with proper qualifications and were directly used in the experiments without pre - treatment. The experimental instruments include: UV - Vis spectrophotometer (Model UV - 2700), fluorescence spectrometer (Model F - 4700): Provided by Beijing Branch of Japanese Scientific Instruments Co., Ltd.; Confocal microscope (Model TCS - SP8 CARS); Small animal in vivo imaging system (Model IVIS Lumina Series III).

2. Steps of synthesis

2.1 Synthesis route of the fluorescent probe NK-1and NK-2.



2.2 Synthesis procedures of compound 1

2-Methylquinoline (800 mg, 5.59 mmol) was added to a 25 mL round-bottom flask and dissolved in 5 mL of acetonitrile. Then methyl iodide (0.42 mL, 5.16 mmol) was added, and the mixture was refluxed at 85 °C for 12 h. After the reaction was completed, it was cooled to room temperature, and the product precipitated. The product was filtered and dried, and further purified by recrystallization to obtain a yellow **compound 1** (1.45 g, 91.2%).^{1}H NMR (600 MHz, DMSO-d_6) δ 9.11 (d, J = 8.5 Hz, 1H), 8.60 (dd, J = 9.1, 1.1 Hz, 1H), 8.41 (dd, J = 8.1, 1.5 Hz, 1H), 8.24 (ddd, J = 8.8, 7.0, 1.5 Hz, 1H), 8.14 (dd, J = 8.5, 1.4 Hz, 1H), 8.00 (ddd, J = 8.0, 6.9, 0.9 Hz, 1H), 4.46 (d, J = 1.1 Hz, 3H), 3.10 (d, J = 1.8 Hz, 3H).2.3 Synthesis procedures of compound 2

2-Methylquinoline (800 mg, 5.59 mmol) was added to a 25 mL round-bottom flask and dissolved in 5 mL of acetonitrile. Then ethyl iodide (0.54 mL, 6.77 mmol) was added, and the mixture was refluxed at 85 °C for 12 h. After the reaction was completed, it was cooled to room temperature, and the product precipitated. The product was filtered and dried, and further purified by recrystallization to obtain a pale yellow **compound 2** (1.48 g, 88.6%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.12 (d, J = 8.5 Hz, 1H), 8.63 (d, J = 9.0 Hz, 1H), 8.43 (dd, J = 8.1, 1.5 Hz, 1H), 8.24 (ddd, J = 8.8, 7.0, 1.6 Hz, 1H), 8.14 (d, J = 8.5 Hz, 1H), 8.00 (ddd, J = 8.0, 7.0, 0.8 Hz, 1H), 5.01 (q, J = 7.3 Hz, 2H), 3.13 (s, 3H), 1.54 (t, J = 7.3 Hz, 3H).

3. DFT calculations

Quantum chemical calculations based on density-functional theory (DFT) and time varying density-functional theory (TD-DFT) were performed using the software Gaussian 09 and the B3LYP/6-31G(d,p) basis set. The initial geometry of the probe **NK-1** and **NK-2** was generated using the software Gaussian View.

4. Molecular docking

Using Autodock technology, perform molecular docking of **NK-1** and **NK-2** with the RNA crystal structure (PDB: 1FJE). Evaluate the binding ability and affinity of the two to RNA by calculating the binding energy, analyzing the binding sites and interaction modes.

5. Spectral test method

5.1 Optical studies and analysis

Sample selectivity test: When investigating the responses of **NK-1** and **NK-2** to DNA and RNA, ions such as GSH, Hcy, Al^{3+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, Fe^{2+}, HSO_3^-/SO_3^{2-}, ClO^-, $O_2^{\cdot-}$, $ONOO^-$ were dissolved in PBS for testing. In addition, DNA and RNA were dissolved in PBS buffer solution, and their concentrations were roughly calculated. Then the specific concentrations were calculated by the Lambert-Beer formula and absorption spectrum tests. Spectral test parameters: The detection used 520 nm as the excitation wavelength, and the widths of both the excitation slit and the emission slit were set to 10 nm.

5.2 Preparation of PBS buffer solution

The procured analytical-grade phosphate buffer formulation powder was dissolved in ultrapure water, made up to the mark in a 2 L volumetric flask, the pH of the solution was recorded as 7.55, and autoclaved for use.

5.3 pH buffer system solution configuration

A pH (3.0-8.0) gradient system was constructed by microtitration of 0.1 M HCl/NaOH using PBS as the base buffer, which was confirmed by a pH meter calibration before the experiment.

6. Calculation method of RNA and DNA concentration

RNA and DNA concentration were calculated by follow equation. The ultraviolet absorption intensity stranded for size of the electron energy level transition probability and abides by the lambert-beer's law (1).

$$A = -\log\frac{I}{I_0} = \varepsilon_{cl}$$

A stand for absorbance; *ε* stand for extinction coefficient, extinction coefficient of RNA is 7700, extinction coefficient of DNA is 6600; *c* stand for molar concentration; *l* stand for length of sample pool; I_0 and I stand for intensity of the incident light and transmission light, respectively.

7. Calculation of Fluorescence Quantum Yield

Using Rhodamine B as the reference standard, the fluorescence quantum yields (Φ) of **NK-1** and **NK-2** in various solvents were measured. This operation was carried out in accordance with the Φ measurement standard specified for Rhodamine B. The calculation formula for Φ is as follows:

$$\Phi_{Sample} = \Phi_{Standard} * \left(\frac{A_{Standard}F_{Sample}}{A_{Sample}F_{Standard}}\right)$$

where Φ represents the fluorescence quantum yield, $F_{Standard}$ represents the integrated area of the fluorescence spectrum of the standard sample, F_{Sample} represents the integrated area of the fluorescence spectrum of the sample

to be tested, $A_{Standard}$ represents the absorbance of the standard sample at the excitation wavelength, and A_{sample} represents the absorbance of the sample to be tested at the excitation wavelength.

8. Culture and preparation of HepG2 cells

HepG2 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Before the experiments, the HepG2 cells in 35-mm glass-bottomed dishes were cultured to a density of 2×10^5 cells per dish. Incubate the cells for 24 h. Cells will attach to the glass surface during this time.

9 Cytotoxicity assay

The cytotoxicity of **NK-1**and **NK-2** were evaluated on HepG2. Cells were seeded in 96-well plates (5×10^3 cells/well) and incubated for 24 h, and then **NK-1** and **NK-2** (final concentration 0, 1, 2, 5, 10, 20, 30 and 40 μM) was added to the cell culture medium. The corresponding cells were incubated with or without (control) **NK-1** and **NK-2** for 12 h, followed by the addition of MTT assay (10 μL, 5 mg/mL) for 4 h. The absorption value was measured at 490 nm wavelength by an enzyme labeler. The assays were performed in six sets for each concentration. The cellular viability of each group was determined by defining the absorbance of the controls group to be 100 % viability. The cell viability of different concentrations of probes was calculated according to the formula:

$$\text{Cell viability (\%)} = (A_s\text{-}A_b) / (A_c\text{-}A_b) \times 100\ \%$$

Where A_s is the absorbance value of the experimental group;

A_c is the absorbance value of the control group;

A_b is the absorbance value of the blank group.

10. RNase digestion experiment

Studies have shown that ribonuclease (RNase) digestion experiments are usually used to test the presence or absence of RNA in cells. Composition of the cell culture medium: DMEM: bovine serum albumin: antibiotics (penicillin, streptomycin) = 100:10:1. The cells were cultured in a CO_2 incubator. 24 h before imaging, the cells were transferred to an imaging dish for culture. When the cell culture coverage reaches 70%, place the cell imaging dish on the operating table, added 1 mL of 4% formalin fixative for cell fixation for 3 h. Then, use 5% Triton X-100 for cell permeabilization for 30 min, and then wash with PBS. Added 1 mL of PBS buffer solution and 4 μL of RNase (5 mg/mL) to the experimental group and culture for 2 h. Added 1004 μL of PBS to the control group and culture for 2 h. Added **NK-1** and **NK-2** respectively and incubate for 30 min, then wash with PBS solution. After washing, removed the PBS and added new PBS for imaging.

11. viscosity response experiment

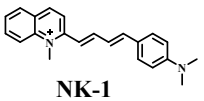
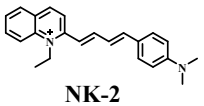
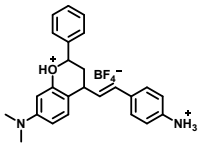
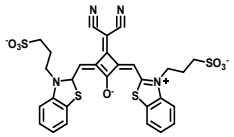
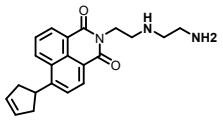
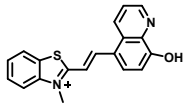
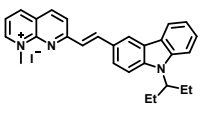
HepG2 cells were incubated with nystatin (10 μM) and Cu^{2+} (10 μM) for 30 min and then incubated with the probe **NK-1** and **NK-2** for 30 min. Before performing upright and inverted imaging, the cultured cells probed with **NK-1** and **NK-2** were rinsed twice with PBS buffer. λ_{ex} = 640 nm, λ_{em} = 690 nm.

12. Visualization experiment of cellular inflammation

For cellular viscosity change, the first group of HepG2 cells was cultured with 0.5 mg/mL, 1 mg/mL, 1 mg/mL LPS and 2 μM Dex, and the second group of HepG2 cells was cultured with 1 μM Rap, 2 μM Rap, 2 μM Rap and 2 μM Dex. Before upright and inverted imaging, the cultured cells probed with **NK-1** and **NK-2** were washed twice with PBS buffer. λ_{ex} = 640 nm, λ_{em} = 690 nm.

13. Supplementary Figures

Table S1 Comparison of the probe in this work with the reported probes for Viscosity/RNA (From 2024 to 2026).

Probe structure	$\lambda_{ex}/\lambda_{em}$ (nm)	Targeted location	Fluorescence enhancement multiplier	Application	Ref.
 **NK-1**  **NK-2**	**NK-1**: 520/650 **NK-2**: 520/650	Nucleolus	Viscosity of **NK-1:** 228-fold/ **NK-2**: 190-fold RNA of **NK-1**: 30-fold/ **NK-2**: 26-fold	Living cells	This work
 **YY-2**	610/706	Nuclear	Viscosity: 289-fold	Living cells	[1]
 **CSTS**	680/705	Cell membrane	/	Living cells/ zebrafish inflammation model	[2]
 **probe 1**	457/530	Nucleolus	RNA: 28.9-fold	Living cells	[3]
 **HQBT**	370/570	Lysosome/ Nuclear	DNA: 6-fold/ RNA: 33-fold	Living cells	[4]
 **1b**	521/709	Mitochondrion	DNA: 143-fold/ RNA: 127-fold	Living cells	[5]

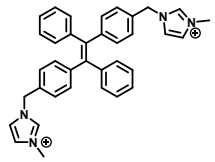
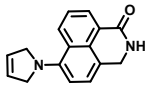
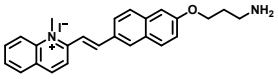
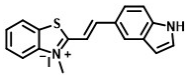
 **TPE1**	305/468	/	DNA: 5-fold/ RNA: 5-fold	Living cells	6
 **Nu-AN**	460/529	Nucleolar	Viscosity: 4-fold/ dsDNA: 4.7-fold/ ssDNA: 2.4-fold/ RNA: 22-fold	Living cells	7
 **NPn**	405/580	Plasma membrane/ Nuclear	DNA: 7.5-fold/ RNA: 4.5-fold	Living cells	8
 **SYN**	440/500	Mitochondrion /Nucleolar	Viscosity: 15-fold/ DNA: 99-fold/ RNA: 113-fold	Living cells	9

Table S2. The fluorescence quantum yield (Φ) of NK-1 in various solvents.

Solvents	λ_{ex}/nm	F_{sample}	$F_{standard}$	Φ/%
EtOH	536 nm	16182.247	505575.857	1.00
DMSO	525 nm	6988.466	486929.118	0.44
MeCN	520 nm	14913.491	491719.014	0.95
THF	540 nm	13901.092	488133.90	0.88
PBS	502 nm	6649.492	485843.22	0.43
1,4-Dioxane	530 nm	57856.332	484295.833	3.77
MeOH	527 nm	8815.441	493088.608	0.55
DMF	528 nm	16628.764	485835.532	1.08
Glycerol	540 nm	113303.541	486843.22	7.79

Table S3. The fluorescence quantum yield (Φ) of NK-2 in various solvents.

Solvents	λ_{ex}/nm	F_{sample}	$F_{standard}$	Φ/%

EtOH	542 nm	18858.236	484581.22	1.23
DMSO	540 nm	14210.639	482683088	0.93
MeCN	524 nm	4981.417	481968.715	0.32
THF	545 nm	9320.512	479603.953	1.24
PBS	500 nm	22283.703	480225.812	1.43
1,4-Dioxane	537 nm	24052.831	480054.357	1.58
MeOH	537 nm	13360.906	483494.698	0.87
DMF	537 nm	8174.966	484028.095	0.53
Glycerol	540 nm	147162.182	480658.40	9.68

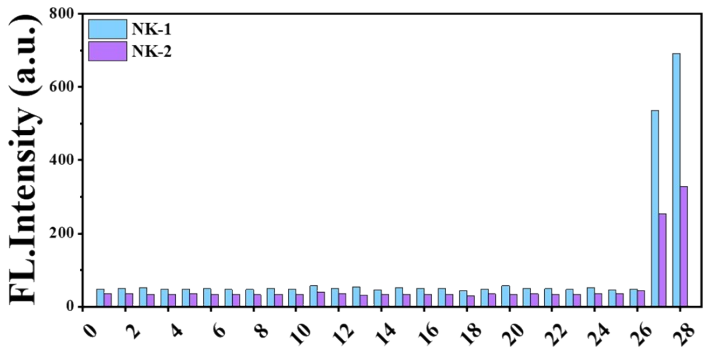


Fig. S1 Fluorescence properties of probe **NK-1** (10 μM) and probe **NK-2** (10 μM) in different analyses. From left to right: (1) Blank, (2) Ala, (3) Glu, (4) Arg, (5) Ser, (6) His, (7) GSH, (8) Hcy, (9) Al^{3+},(10) Ca^{2+},(11) Cu^{2+},(12) Mg^{2+}, (13) Fe^{2+}, (14) Fe^{3+}, (15) K^{+},(16) Mn^{2+},(17) Na^{+},(18) Zn^{2+}, (19) HCO_3^-/CO_3^{2-}, (20) HSO_3^-/SO_3^{2-}, (21) ClO^-, (22) H_2O_2, (23) ·OH, (24) HS^-, (25) $O_2^{\cdot}$, (26) $ONOO^-$,(27) RNA, (28) DNA . All analyses: 100 μM. λ_{ex} = 520 nm, λ_{em} = 540 nm.

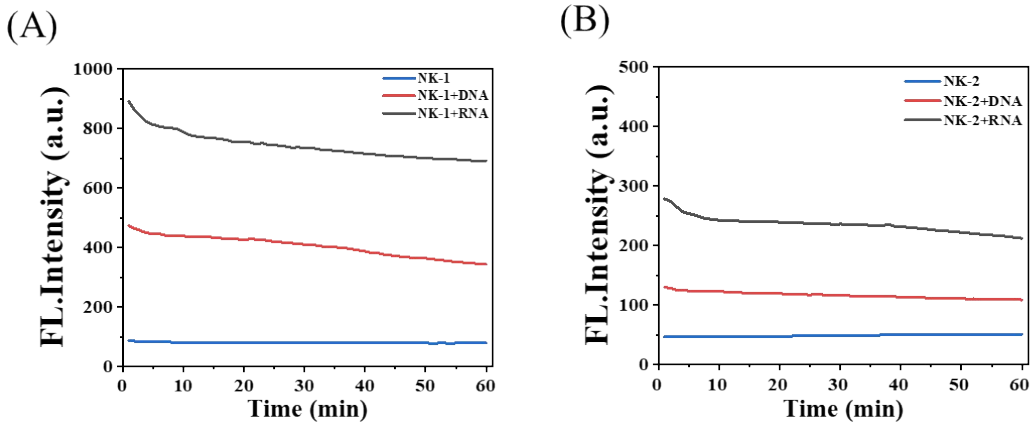


Fig. S2 (A) Photostability of **NK-1** (10 μM) under constant laser irradiation (60 min). (B) Photostability of **NK-2** (10 μM) under constant laser irradiation (60 min).

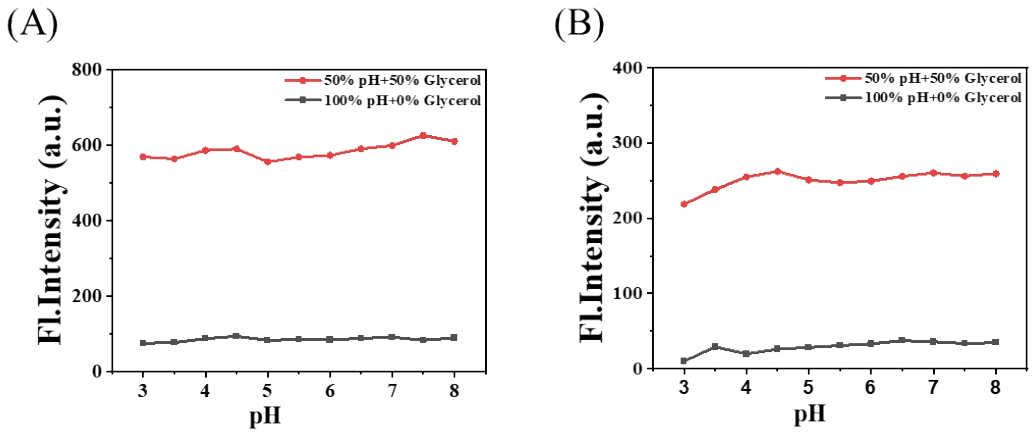


Fig. S3 (A) Fluorescence intensity of **NK-1** (10 μM) in different viscosity and pH systems. (B) Fluorescence intensity of **NK-2** (10 μM) in different viscosity and pH systems.

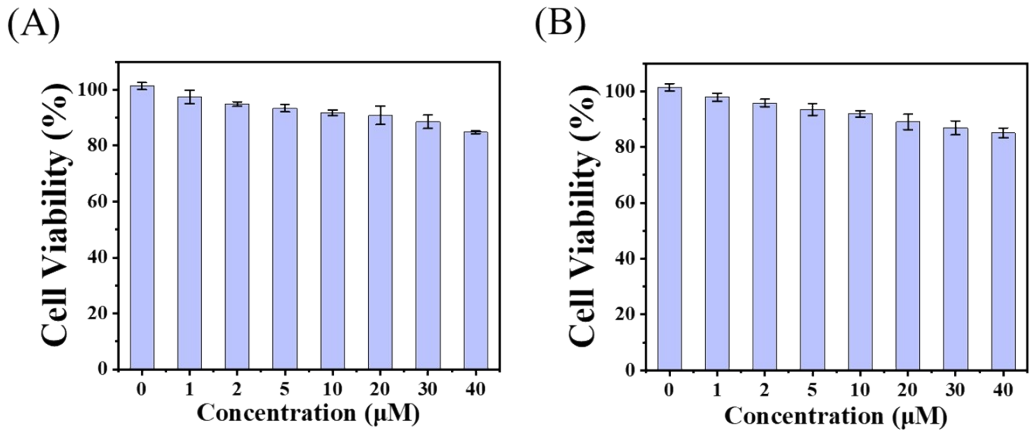


Fig. S4 (A) 12 h Cytotoxicity tests of **NK-1** in HepG2 cells. (B) 12 h Cytotoxicity tests of **NK-2** in HepG2 cells.

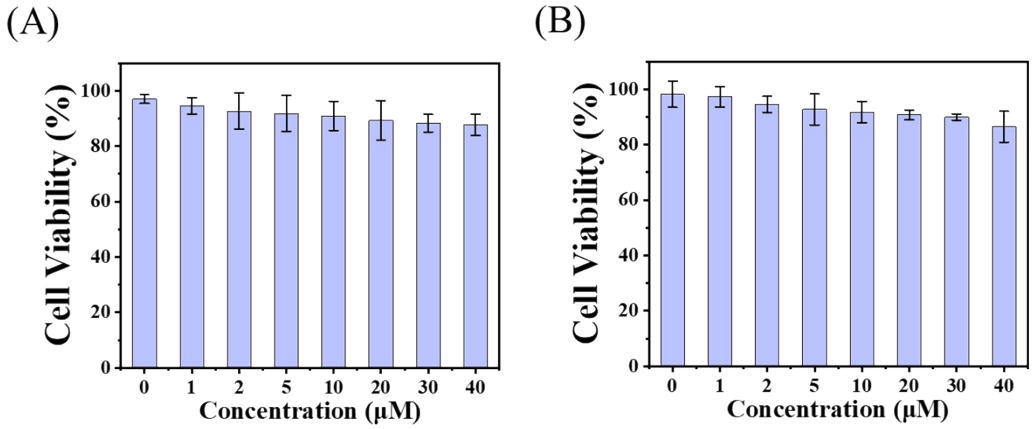


Fig. S5 (A) 24 h Cytotoxicity tests of **NK-1** in HepG2 cells. (B) 24 h Cytotoxicity tests of **NK-2** in HepG2 cells.

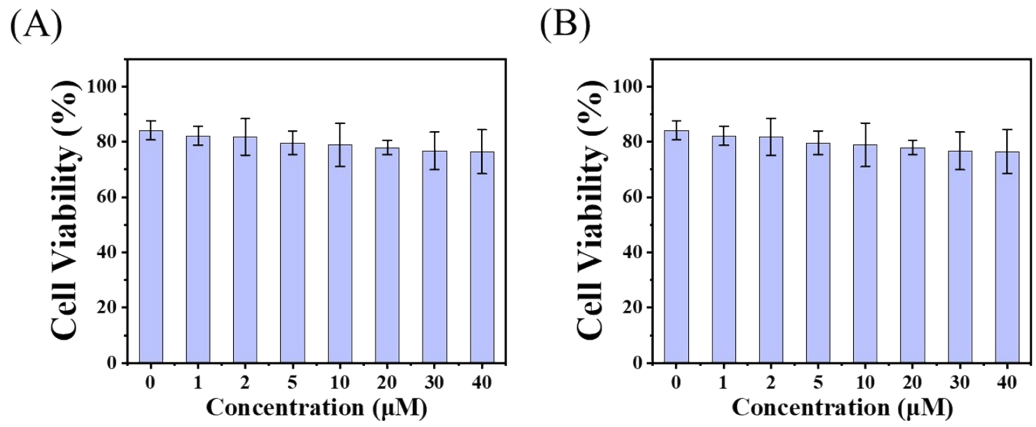


Fig. S6 (A) 48 h Cytotoxicity tests of **NK-1** in HepG2 cells. (B) 48 h Cytotoxicity tests of **NK-2** in HepG2 cells.

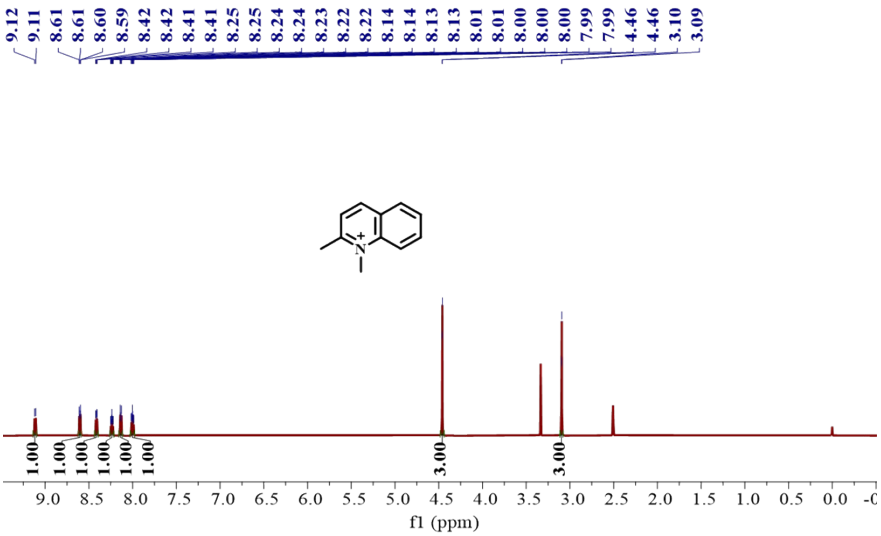


Fig. S7 ^{1}H NMR spectra of **compound 1** in DMSO-d_6.

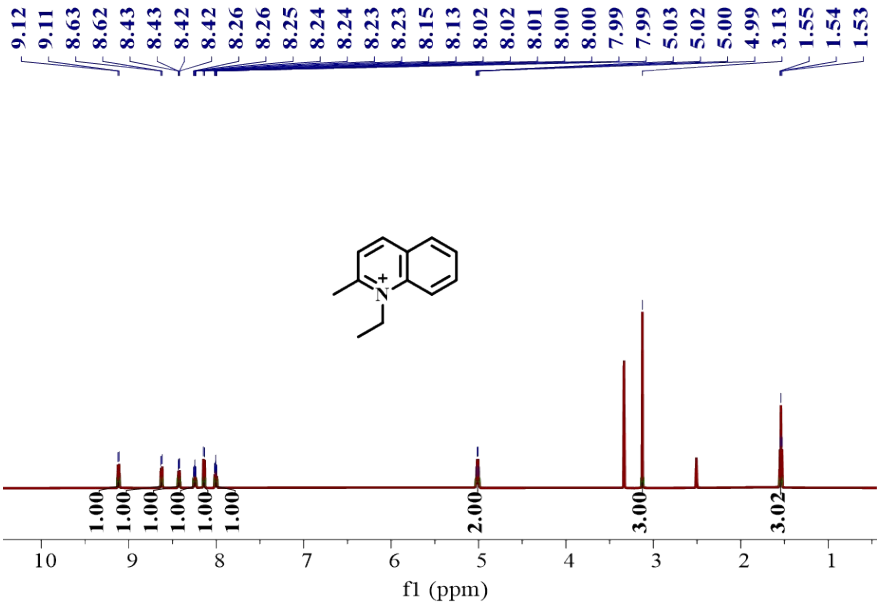


Fig. S8 ^{1}H NMR spectra of **compound 2** in DMSO-d_6.

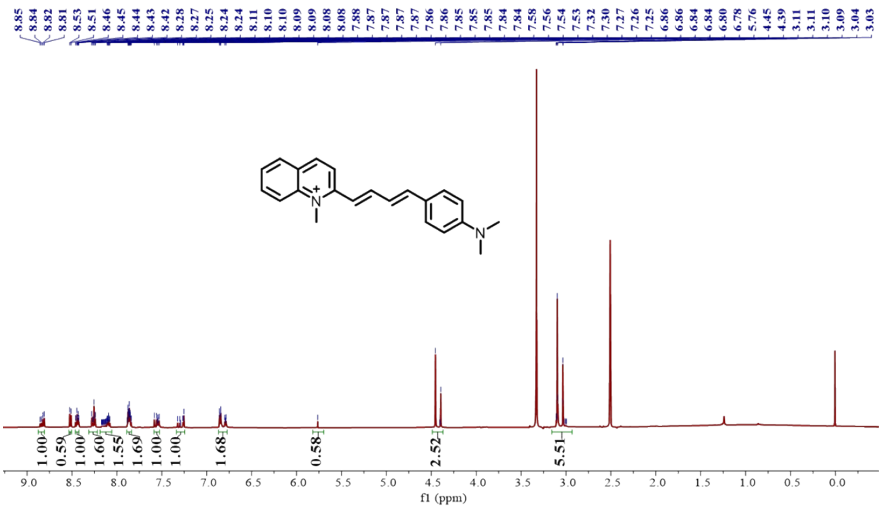


Fig. S9 ^{1}H NMR spectra of **NK-1** in DMSO-d_6.

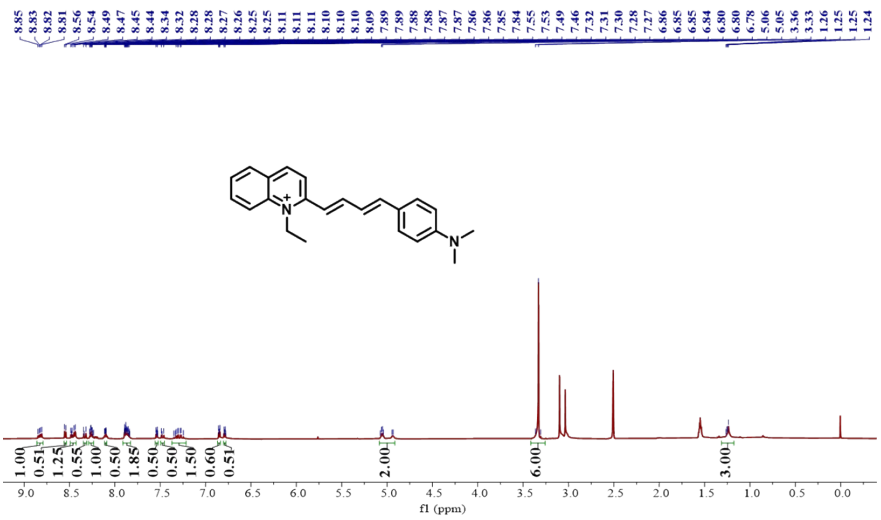


Fig. S10 ^{1}H NMR spectra of **NK-2** in DMSO-d_6.

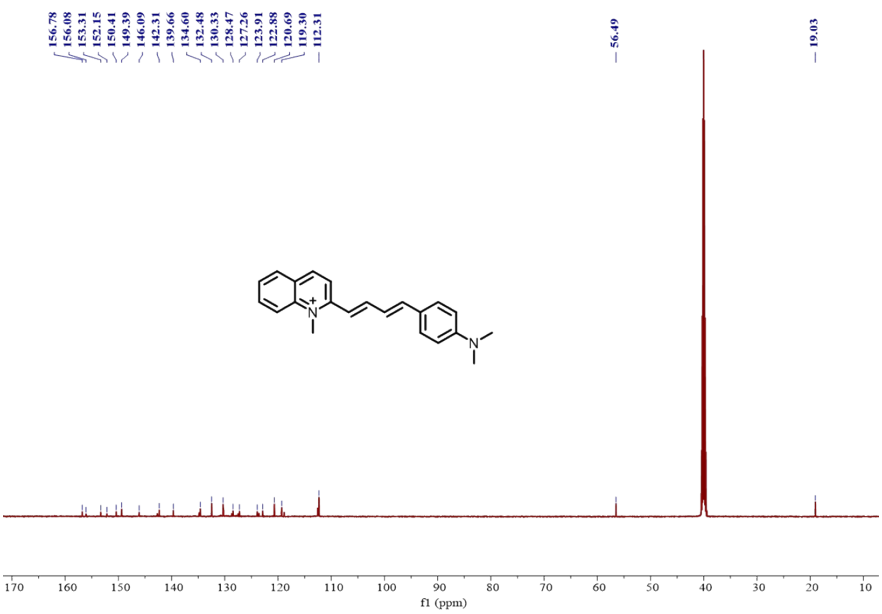


Fig. S11 ^{13}C NMR spectra of **NK-1** in DMSO-d_6.

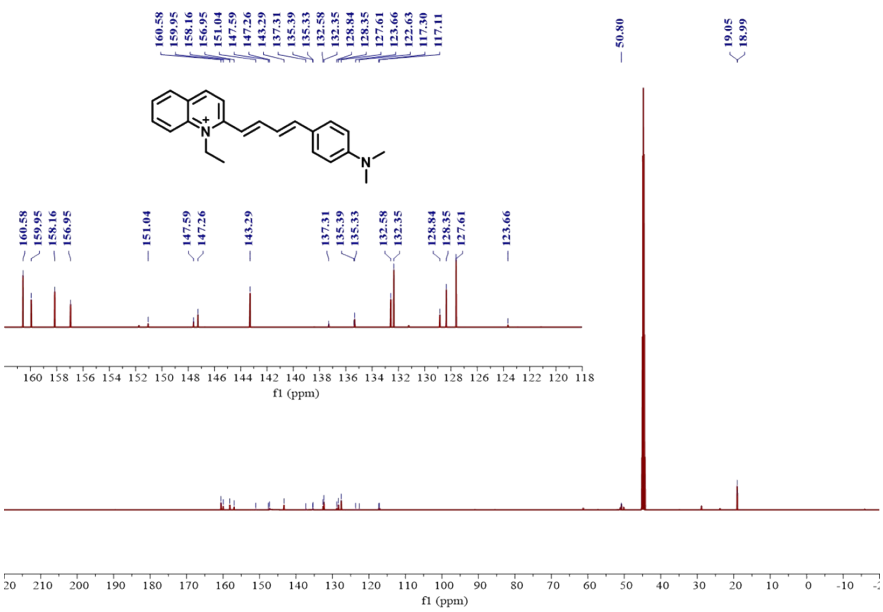


Fig. S12 ^{13}C NMR spectra of **NK-2** in DMSO-d_6.

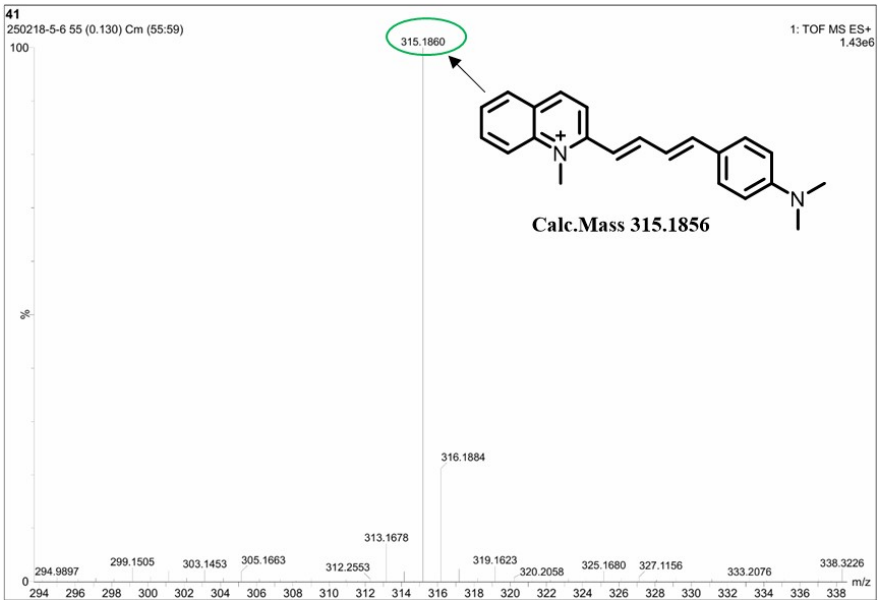


Fig. S13 HR-MS spectrum of **NK-1.**

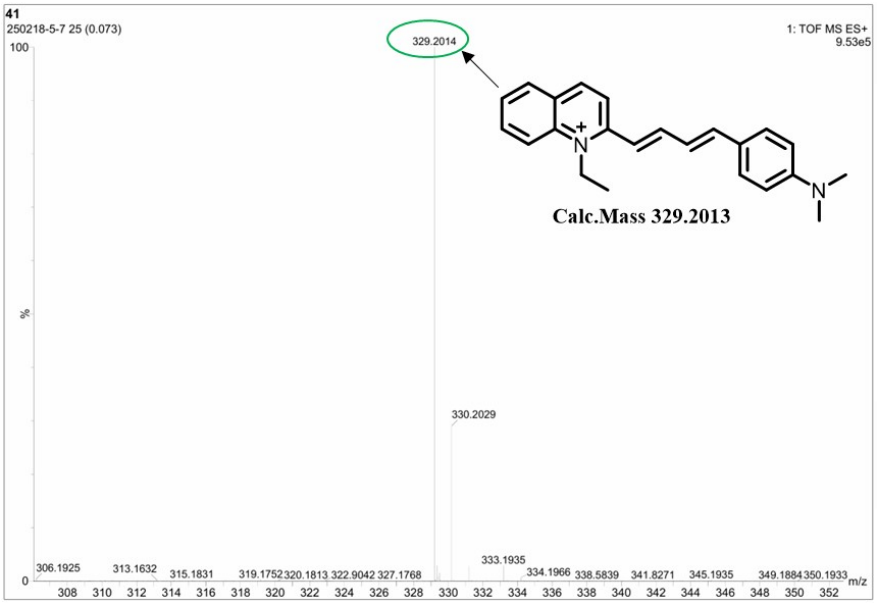


Fig. S14 HR-MS spectrum of **NK-2.**

Reference

1. J. Yu, H. Zhang, S. Yuan, C. Sun, L. Zhang, C. Luo and Q. Wang, *Analytical Chemistry*, 2026, **98**, 2060-2069.
2. Z. Gong, P. Yuan, Y. Tang, K. Li, Z. Liu, Y. Wang, S. Zhong and Y. Yang, *Sensors and Actuators B: Chemical*, 2026, **457**, 139738.
3. W. Jiang, J. Chen, Y. Ruan, X. Wang, Q. Qiao and Z. Xu, *ACS Sensors*, 2025, **10**, 5266-5275.
4. D. Wang, W. Huang, Y. Zhu, Z. Yu, Y. Zhou, Y. Chen, Z. Feng, X. Tian, G. Han and Z. Zhang, *Analytical Chemistry*, 2025, **97**, 2359-2366.
5. H. Ma, W. P. Ni, Q. Lin, R. Sun and J. F. Ge, *Analyst*, 2025, **150**, 642-649.
6. S. Adak, S. A. Rahaman, S. Karmakar and S. Bandyopadhyay, *Chemistry – An Asian Journal*, 2024, **19**, e202400400.
7. W. Jiang, Q. Qiao, J. Chen, P. Bao, Y. Tao, Y. Zhang and Z. Xu, *Advanced Science*, 2024, **11**, 2309743.
8. W. Ge, H. Wang, X. Wu, B. Dong, Q. Lu and M. Tian, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2024, **313**, 124095.
9. Y. Wang, Q. Lin, Y. Liu, C. Li, Z. Liu, X. Yu and K. N. Wang, *Analytical Chemistry*, 2024, **96**, 9808-9816.
10. Z. Wang, Y. Liu, W. Wang, C. Zhao and W. Lin, *New Journal of Chemistry*, 2021, **45**, 2614-2619.
11. Y. Guo, H. Huang, Q. Zhang, H. Wang, M. Liu and W. Lin, *Analytica Chimica Acta*, 2025, **1350**, 343863.
12. M. Tian, J. Sun, B. Dong and W. Lin, *Angewandte Chemie International Edition*, 2018, **57**, 16506-16510.