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Electronic Supplementary Information

A quinoline-benzothiazole hybrid as the first near-infrared fluorescent probe for transthyretin

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General Information

All chemicals were purchased from commercial sources unless otherwise specified. All the solvents were of analytical reagent grade and were used without further

purification.

Various amino acids (Ala, Arg, Asp, Cys, Glu, GSH, Lys, Ile, Met, Ser) were purchased from Sigma-Aldrich and its stock solution (10 mM) were obtained by diluting in PBS (pH 7.4). Metal ion (Na^+，K^+，Fe^{2+}, Fe^{3+}, Zn^{2+}, Al^{3+}, Cu^+, Cu^{2+}, Mg^{2+}, Ni^+, Cd^{2+}) stock solutions (10 mM) were obtained by diluting the standard solutions of the corresponding chlorine salt or Sulfate salt. Column-layer chromatographic silica gel was purchased from Branch of Qingdao Haiyang Chemical Co, Ltd. Flash column chromatography was performed with silica gel (100-200 mesh) purchased from Qingdao Haiyang Chemical Co., Ltd. ^1H and ^{13}C NMR spectra were recorded using TMS as the internal standard in $CDCl_3$ with a Bruker BioSpin GmbH spectrometer at 400 MHz and 100 MHz, respectively. Ultra-high resolution mass spectra (UHR-MS) were taken on a Thermo QExactiveplus instrument.

The UV-vis absorption spectra were recorded on a UV-2600 spectrophotometer (Shimadzu, Japan). Fluorescence measurements were performed on an RF-6000 fluorescence spectrophotometer (Shimadzu, Japan) equipped with quartz cell of 10.0 mm path length. And the fluorescent spectra were measured by Tecan Spark Fluorescence Plate Reader by using BeyoGold™ 96-Well Black Opaque plates. The cell Culture and MTT assay can be seen in our previous work[1].

Fluorescence assay with WT-TTR

WT-TTR was expressed and purified from an E. coli expression system as described previously. **QCN-2** (1 μL of a 1.44 mM solution in DMSO, final concentration 14.4

μM) was added to 100 μL of solution of WT-TTR homotetramer (0.4 mg/mL, 14.4 μM) in 10 mM phosphate, 100 mM KCl, and 1 mM EDTA (pH 7.0 phosphate buffer) in a 2 mL EP tube. The mixture was vortexed and preincubated at room temperature for 30 min. The fluorescence changes were monitored using a Tecan Spark Fluorescence Plate Reader.

Fluorometric assay in solvent of variable polarity

Probe **QCN-2** (2 μL of a 10 mM solution in DMSO) was added to 4mL of solvent (DCM, THF, EtOH, EA, MeOH, ACN, DMSO, H_2O) to give a final concentration of 5 μM. The absorption and emission spectra were record at room temperature using a UV-2600 spectrophotometer and RF-6000 fluorescence spectrophotometer.

Viscosity titration assay

Probe **QCN-2** (10 mM solution in DMSO) was dissolved in a solution of glycerol-water system (glycerin ratio was 0 %-90 %) to give a final concentration of 10 μM. The samples were vortexed, and the fluorescence spectrum was detected using RF-6000 fluorescence spectrophotometer. The excitation wavelength was 587 nm.

WT-TTR titration assay

Probe **QCN-2** (1.5 μL, 6 mM solution in DMSO) were added to 150 μL of a solution of WT-TTR (0−57.6 μM) in 1×PBS. The samples were vortexed, and incubated for 1h at room temperature. The fluorescence changes were monitored using a Tecan Spark Fluorescence Plate Reader.

TTR stability assay

TTR-L55P protein and **QCN-2** were mixed together in acidic aggregation buffer (NaOAc 200 μM, KCl 100 μM, acidified by AcOH to Ph=4.4). Final concentration of TTR-L55P protein was 7.2 μM and Final concentration of **QCN-2** was 0μM, 3.6μM, 7.2μM, 14.4μM and 28.8 μM. The mixture was incubated at temperature (37°C). The fluorescence changes were monitored using a Tecan Spark Fluorescence Plate Reader.

Calculation of the detection limit

The detection limit was calculated according to the following equation (1)[2]. The fluorescence spectrum of fluorescence probe and the standard deviation of blank measurement were measured. The linear relationship between fluorescence intensity of probe at 648 nm and concentration of QCN-2 was measured to get the slope.

$$\text{Detection limit} = 3\sigma/k \quad (1)$$

σ and k are the standard deviation and the slope of the regression line, respectively.

Determination of quantum yield

The fluorescence quantum yields of **QCN-2** were tested by the following equation (2)[3].

$$\Phi = \Phi s \times \frac{A_s \times OD \times \eta^2}{A \times OD_s \times \eta_s^2} \quad (2)$$

Φ is the quantum yield of **QCN-2**, Φ_s is the quantum yield of R6G (Φ=0.95, ethanol). OD and A represented the fluorescence integral area and the absorbance, respectively.

Table S1: Reported examples of WT-TTR fluorescent probes or sensors

Probe Name	Structure	Excitation wavelength	Emission wavelength	Stock's shift	FI fold Increase	Kd	Ref.
10		357nm	476nm	119 nm	111	–	[4]
5		388nm	513nm	125 nm	13	–	[4]
5		411nm	486nm	75nm	35	–	[5]
A2		328nm	430nm	102 nm		–	[6]
11		395nm	505nm	110 nm	8.6	2.9±0.2 μM	[7]
5		337nm	415nm	78nm	15.8	200± 10	[8]

						nM	
Nile red		530nm	610nm	80nm	11	_	[9]
4		327nm	395nm	68nm	_	_	[10]
5		365nm	520nm	155 nm	_	_	[11]
4		345nm	481nm	136 nm	_	2nM	[12]
QCN-2		487nm	694nm	207 nm	25	_	this work

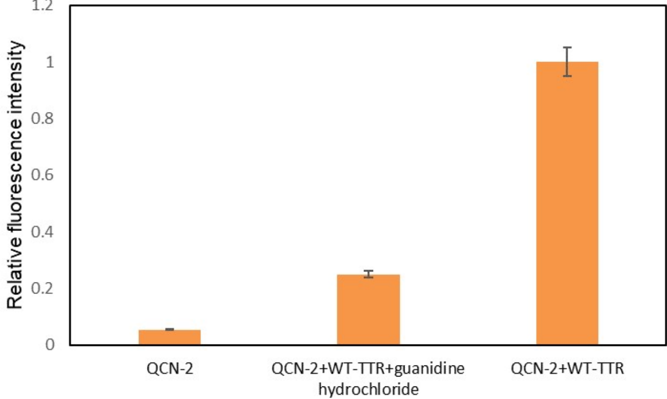

Figure S1: The emission intensity histogram of **QCN-2**(14.4μM) before and after

incubation 1h with WT-TTR (14.4μM) in PBS and incubation with WT-TTR unfolded by guanidine hydrochloride (6M) dissociated. λ_{em}=648nm

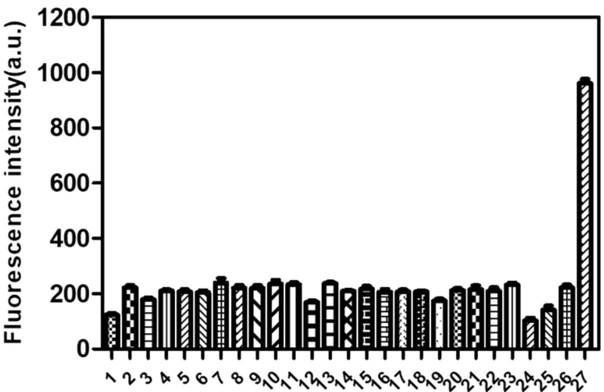

Figure S2: The fluorescence intensity histogram of **QCN-2** (1 μM) with different metal ions, amino acids or proteins (10 μM; 1: control, 2: Al^{3+}, 3: Cu^{+}, 4: Zn^{2+},5: Fe^{3+}, 6: Fe^{2+}, 7: K^{+}, 8: Na^{+}, 9: Mg^{2+}, 10: Pd^{2+}, 11: Cu^{2+}, 12: Cd^{2+}, 13: Ni^{+}, 14: Met, 15: Arg, 16: Glu, 17: Cys, 18: Ala, 19: GSH, 20: Asp, 21:Lys, 22: Ser, 23: Ile, 24: HAS, 25: BSA, 26: Aβaggregates, 27: WT-TTR).

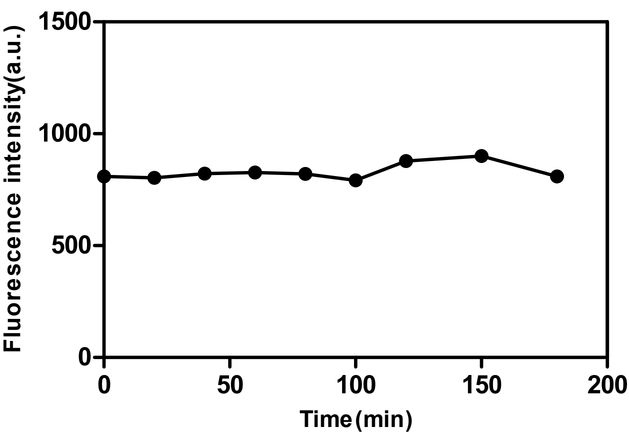

Figure S3：The photostability assay of **QCN-2** (10μM) under UV-light.

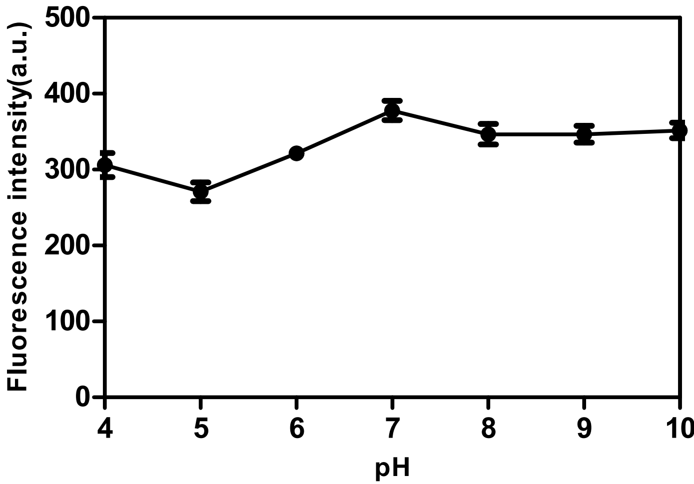

Figure S4：The pH stability assay of **QCN-2** (10 μM) in Na_2HPO_4-NaH_2PO_4 solution.

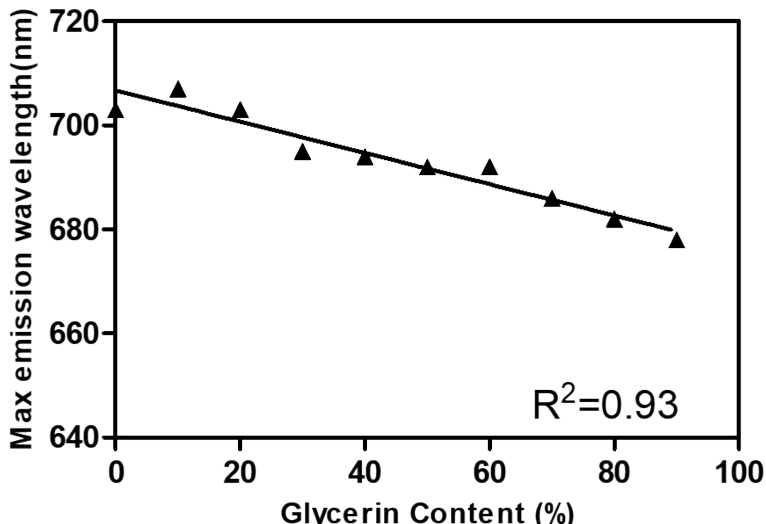

Figure S5：The linear relationship of **QCN-2** (10 μM) between max emission wavelength and glycerol fraction.

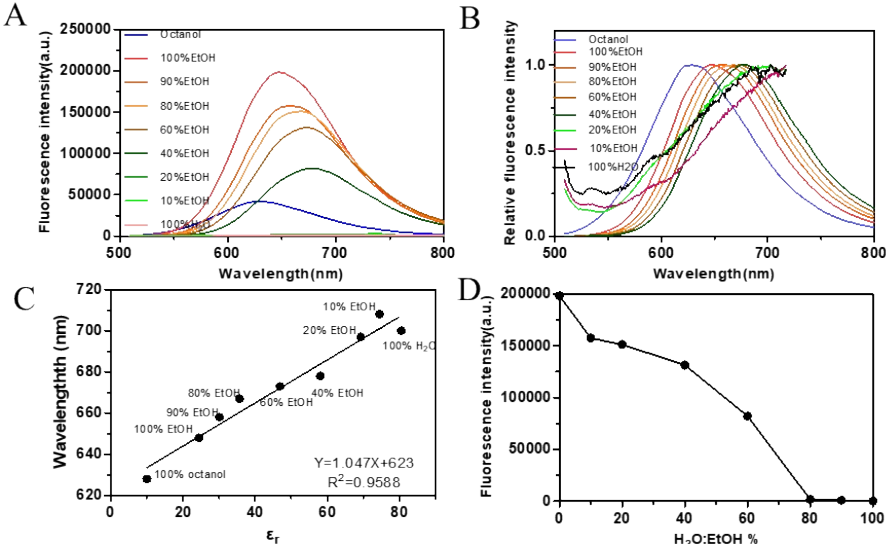

Figure S6: (A) The fluorescence intensity (B) The relative fluorescence intensity of **QCN-2** (5 μM) in octanol and EtOH-H_2O system; (C) The max emission wavelength of **QCN-2** in octanol and EtOH-H_2O system; (D) The fluorescence intensity of **QCN-2** in different percentages of H_2O: EtOH system.

Table S2: The spectroscopy properties of **QCN-2**.

$\lambda_{abs}{}^{a}$ (nm)	$\lambda_{em1}{}^{b}$ (nm)	Stock's shift (nm)	$\lambda_{em2}{}^{c}$ (nm)	fold[d]	$\Phi^{e}{}_{DCM}$	Φ_{THF}	Φ_{EtOH}	Φ_{ACN}	Φ_{MeOH}	Φ_{H2O}
487	694	207	648	25	0.317	0.213	0.242	0.245	0.220	0.007

[a]Absorption wavelength measured in PBS; [b]emission wavelength measured in PBS; [c]emission wavelength after binding with WT-TTR; [d]fold increase in fluorescence intensity upon binding with WT-TTR; [e]fluorescence quantum yield in different solvents.

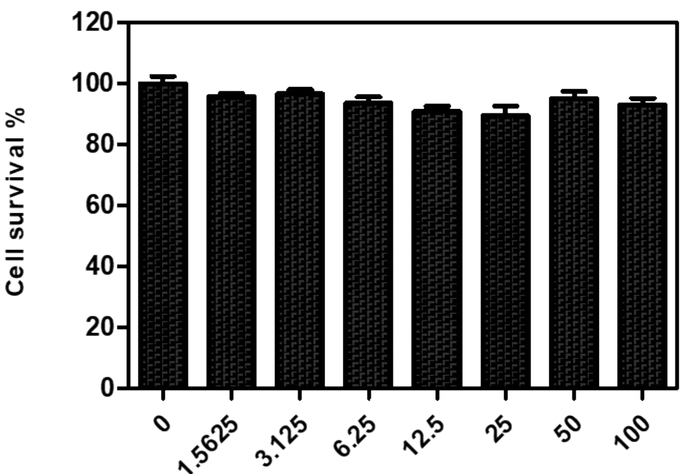

Figure S7：The SH-SY5Y cells survival assay of **QCN-2** from 0 μM to 100 μM.

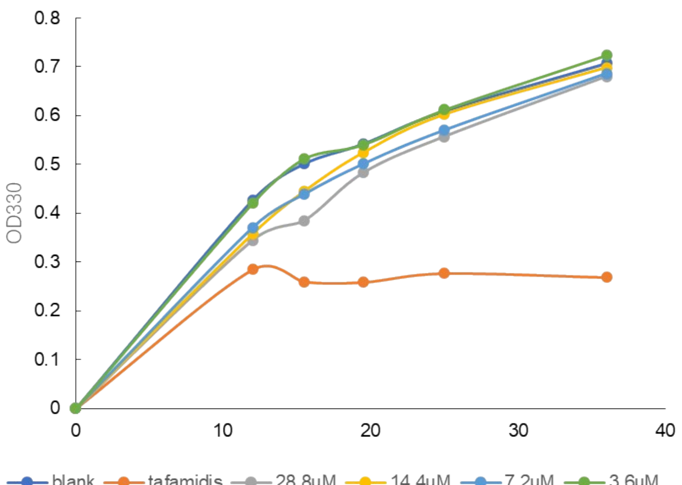

Figure S8：The OD_{330} detecting spectra of **QCN-2** (0 μM, 3.6 μM, 7.2 μM, 14.4 μM and 28.8 μM) and tafamidis (3.6 μM) inhibits TTR-L55P-His protein (7.2 μM) aggregation in PBS (pH 7.4).

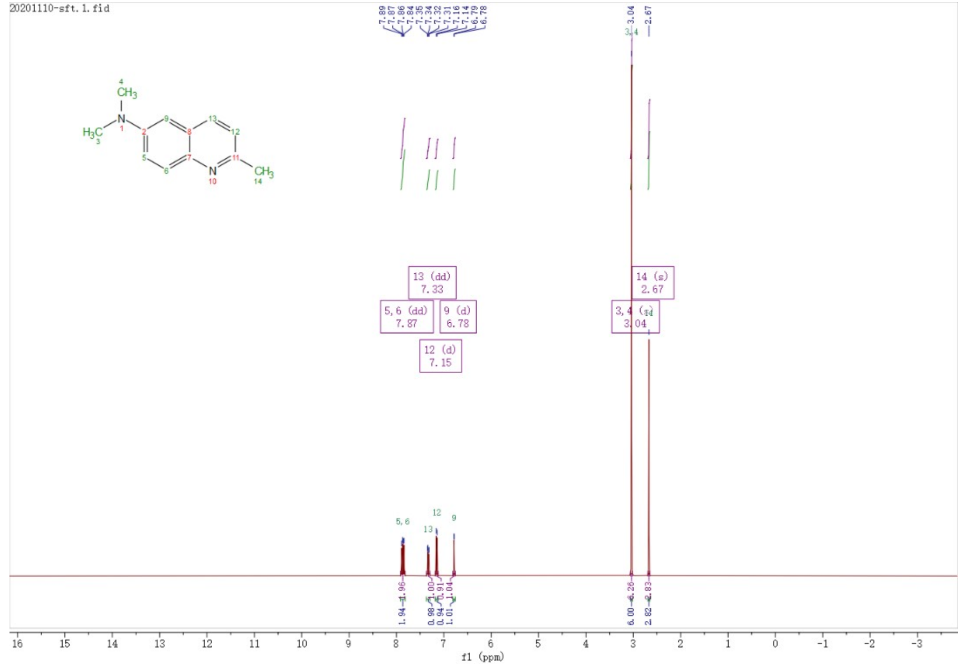

Figure S9：^{1}H NMR (Chloroform-d) spectrum of compound **2** (400 MHZ).

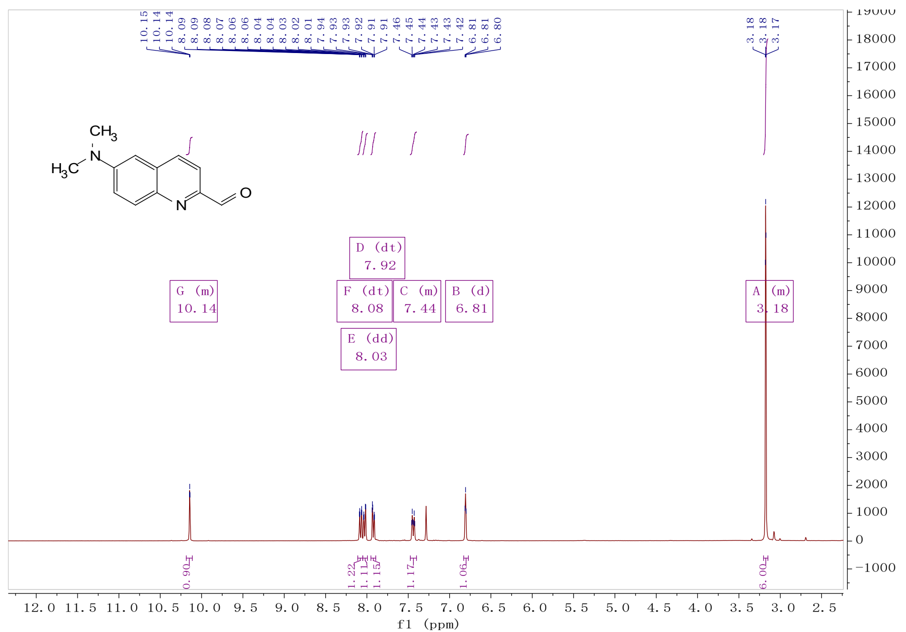

Figure S10：^{1}H NMR (Chloroform-d) spectrum of compound **3** (400 MHZ).

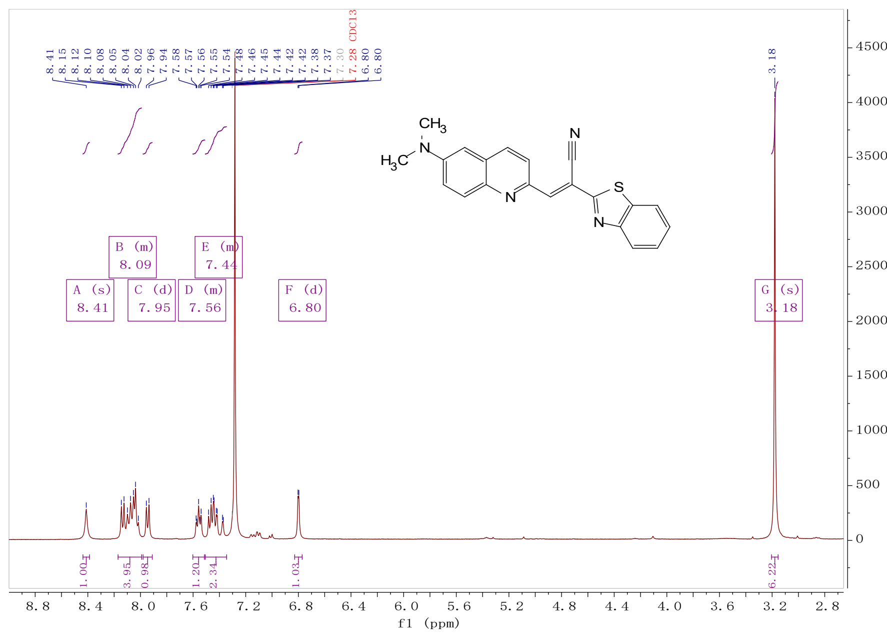

Figure S11 ：^{1}H NMR (Chloroform-d) spectrum of **QCN-2** (400MHZ)

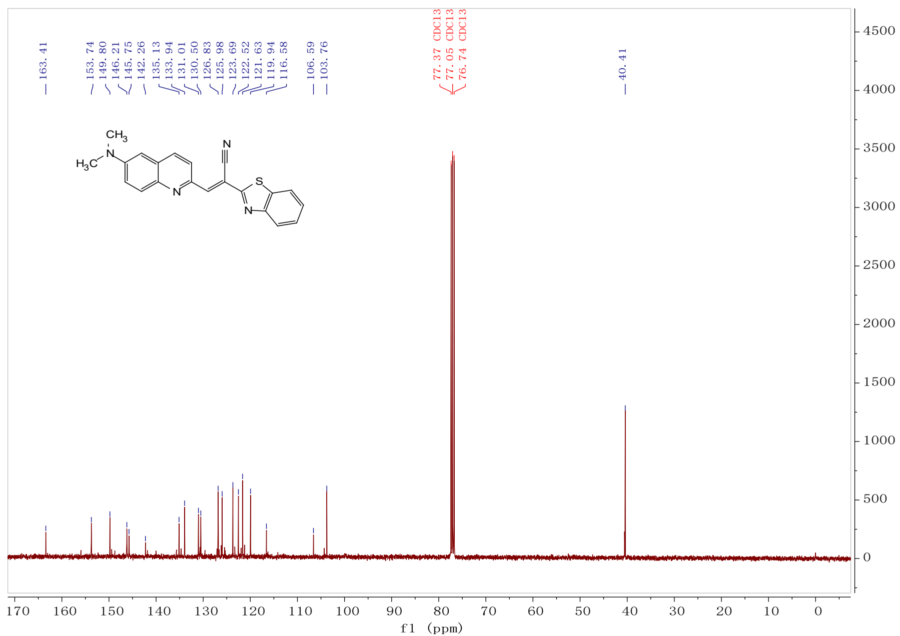

Figure S12 :^{13}C NMR (Chloroform-d) spectrum of **QCN-2**.

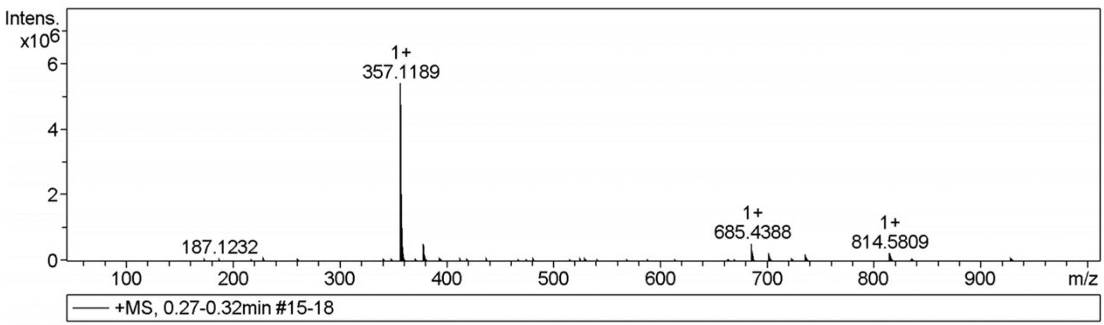

Figure S13: The HRMS spectrum of **QCN-2**.

analytic functional testing
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serial number: 0400.191023

Samples

	Weight [mg]	Name	Method	N [%]	C [%]	H [%]	S [%]	Date	Time
15	1.096000	liu	2mg	15.61	69.57	4.489	9.8811	2021/4/30	15:56

Figure S14: The elemental analysis data of **QCN-2**.

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