Supplementary Materials

Construction of a Mito-targeting fluorescent probe for specific identification of Al3+ in environmental and biological systems

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Contents

1. Materials and apparatus

All chemical reagents were procured in their analytical or spectroscopic grade from commercial sources, specifically from Energy Chemical and Aladdin Bio-Chem Technology Co., Ltd., and were utilized directly without further modification. The Bruck AV-600 spectrometer was employed to record the ^{1}H NMR and ^{13}C NMR spectra, utilizing TMS and DMSO-d_6 as solvents. The chemical structural information of the compound was assessed through ESI-MS and FT-IR spectra, which were acquired using the Waters Xevo UPLC/Xevo G2-XS Q-Tof MS spectrometer and the Bruker SEWERE ALPHA-T, respectively. Absorption and fluorescence spectral data were gathered using the Shimadzu UV-2700 UV–vis spectrometer and the Hitachi FL 4700 fluorescence spectrometer at room temperature.

2. Synthetic of HMH-Al

Compounds 1 and 2 were synthesized according to previously reported literature articles [1].

The intermediate **2** (0.351 mmol) and 4-methoxybenzoyl hydrazine (0.351 mmol) were first dissolved together in 15.0 mL of absolute ethanol [2]. After that, two drops of glacial acetic acid were introduced into the resulting reaction mixture, which was then subjected to stirring at room temperature. Throughout the process, the reaction progress was tracked via thin-layer chromatography (TLC), until the TLC spot corresponding to the starting material was no longer visible. Once the reaction was complete, the mixture was filtered under reduced pressure to collect the light yellow solid product, and the solid was rinsed with ethanol three times, using 5.0 mL of ethanol for each rinse. Yield: 85.9%. m.p.: 255.4-256.2 °C. ^{1}H NMR (Fig. S1) (600 MHz, DMSO-d_6) δ 12.10 (s, 1H), 11.46 (s, 1H), 8.78 (s, 1H), 8.13 (d, J = 7.9 Hz, 1H), 8.07 (d, J = 8.1 Hz, 1H), 7.96 (d, J = 8.1 Hz, 3H), 7.68 (d, J = 2.0 Hz, 1H), 7.54 (ddd, J = 8.3, 7.1, 1.3 Hz, 1H), 7.45 (td, J = 7.6, 1.2 Hz, 1H), 7.12 – 7.08 (m, 2H), 3.98 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (Fig. S2) (151 MHz, DMSO-d_6) δ 167.48, 162.89, 162.72, 154.06, 150.25, 149.04, 146.16, 134.78, 130.14, 127.06, 125.67, 125.31, 124.66, 123.00, 122.70, 120.39, 119.98, 114.32, 111.29, 56.58, 55.96. IR (cm^{-1}) (Fig. S1): ν (O–H), 3198; ν (C=O), 1643; ν (C=N), 1611; 1530, 1506, 1465, 1335, 1312, 1286, 1260, 1189, 1116, 1025. HRMS: m/z (TOF MS ES^{+}) (Fig. S3): Calcd. For $C_{23}H_{19}N_3O_4S$: 433.1096 [**HMH-Al**-H^{+}]$^{-}$, found: 432.1023.

3. Spectral measurement preparation

The stock solutions of various metal ions Zn^{2+}, Fe^{3+}, Cu^{2+}, Cd^{2+}, Mn^{2+}, K^{+}, Ca^{2+}, Co^{2+}, Fe^{2+}, Na^{+}, Hg^{2+}, Mg^{2+}, Cr^{3+}, Pb^{2+}, Ag^{+}, Ni^{2+}, Ba^{2+} and Al^{3+} (10 mM) were meticulously prepared by dissolving accurately weighed quantities of the respective components in ultrapure water. The test solution of the probe **HMH-Al**, at a concentration of 1 mM, was diluted in EtOH and further diluted into a mixed solvent consisting of EtOH/H_2O (v/v, 3/2) to achieve a final concentration of 10 μM, intended for the subsequent measurement of fluorescence and UV-vis spectra.

4. Detection of Al^{3+} in actual water samples

Detections of $Al^{3+}$was performed on three real samples: ultrapure water, tap water, Youjiang River water, the Youjiang River water was treated with simple decompression filtration [3]. Varying concentrations of Al^{3+} (2, 4, 6, 8, and 10 μM) were prepared using each of the water samples and introduced into the solution of probe **HMH-Al**, with the fluorescence intensity at 485 nm being recorded for each system, and the recovery rates were computed in the end.

5. The test strips of HMH-Al

The discs of the same size test paper were soaked in probe **HMH-Al** (10 μM) solution for 30 min and removed to air dry [4]. Then these paper strips were treated with Al^{3+} prepared in pure aqueous media. The differences were observed under a 365 nm UV lamp.

6. Cytotoxicity of HMH-Al in vitro

HeLa cells were purchased from Wuhan Proceed Life Technology Co., Ltd. (China). The cells were cultured in DMEM (Dulbecco's modified Eagle's medium) containing 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) and 10% FBS (fetal bovine serum) in an atmosphere of 37°C and 5% CO_2. When the cell density reached 90% of confluence, a subculture was done and the medium was changed approximately every day. The cytotoxicity of MBC-V was evaluated by CCK-8 counterstaining assay done by using commercial kits purchased from Beyotime (Nantong, China).

7. Co-localization experiments

HeLa cells to grow and adhere for 24 h in a constant temperature incubator, probe **HMH-Al** (10 μM) was added and incubated for 0.5 h. Subsequently, commercial subcellular fluorescent

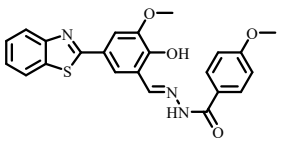
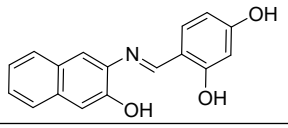
biosensors Mito-Tracker Green (200 nM), Lyso-Tracker Green (100 nM) and LD-Tracker Green (1 μM) were added and co-incubated for 0.5 h. After incubation, the medium was removed, and the cells were washed three times with PBS. The cells were then fixed with 4% paraformaldehyde solution at room temperature for 15-20 min. Finally, the confocal dishes were placed under a confocal microscope for fluorescence imaging. Co-localization experiments were performed on a confocal laser scanning microscope. Channel of probe **HMH-Al**, green channel: λ_{ex} = 405 nm; λ_{em} = 450-500 nm; Mito-Tracker Green: λ_{ex} = 488 nm, λ_{em} = 500-550 nm; Channel of Lyso-Tracker Green: λ_{ex} = 488 nm, λ_{em} = 500-550 nm; Channel of LD-Tracker (BODIPY 493/503) Green: λ_{ex}= 488 nm, λ_{em} = 500-550 nm.

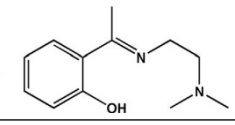
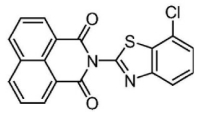
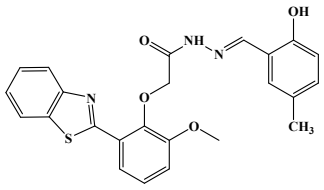
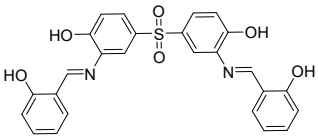
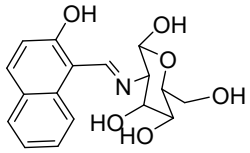
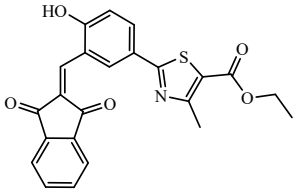
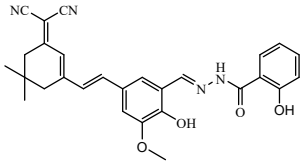
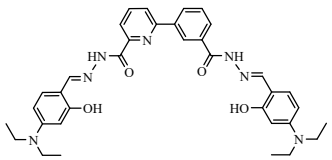
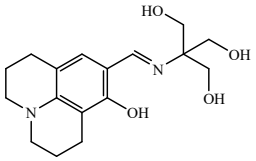
8. Detection of Al^{3+} in living A549 and HeLa cells

The A549/HeLa cells were obtained from iCell Bioscience Inc, Shanghai. The cells were divided into three groups as control, probe **HMH-Al**, **HMH-Al**+Al^{3+}. All groups were added 1 mL of PBS to wash twice, then added 1 mL of serum-free medium, incubated for 1 h [5]. After that, the medium was sucked off, and 1 mL serum-free medium containing (20, 40 μM) Al^{3+} ions was added according to the experimental group, respectively. After incubation for 1 h, the culture solution was removed, the cells were washed with PBS for three times, then added and cultured for 1 h with 4% paraformaldehyde (1 mL/dish), which was freshly prepared and fixed at room temperature. After 3 times washing with PBS, 1 mL probe solution (20 μM) was added and the cells were incubated for 30 min, then washed with PBS for 3 times.

Table S1

Comparison between the performance of **HMH-Al** with recent benzothiazole or Schiff-base probes

Ref.	Probes	Analyte	Test Medium	LOD	Application
This Work		Al^{3+}	EtOH/H_2O (3/2)	0.28 μM	Water samples Cell imaging Test strips
6		Al^{3+}	DMF/H_2O (9:1)	0.49 μM	Test strips

7		Al^{3+}	EtOH/H_2O (9/1)	4.32 μM	DFT
8		Al^{3+}	DMSO/H_2O (1:9, v/v)	2.85 μM	logic gate Cell imaging
9		Al^{3+}	DMF/H_2O (2：3)	1.51 μM	Cell imaging Test strips
10		Al^{3+}	DMSO/H_2O (9:1)	0.22 μM	Water sample
11		Al^{3+}	EtOH	4.08 nM	Cell imaging
12		Al^{3+}	DMF/H_2O (1:9)	-	live cells AD rat model
13		Al^{3+}	DMF/H_2O (6:4)	58.5 nM	Zebrafish imaging Living mice
14		Al^{3+}	DMSO/H_2O (7/3)	10.98 nM	Cell imaging Zebrafish imaging
15		Al^{3+}	DMSO	25 nM	Zebrafish imaging water and drug samples

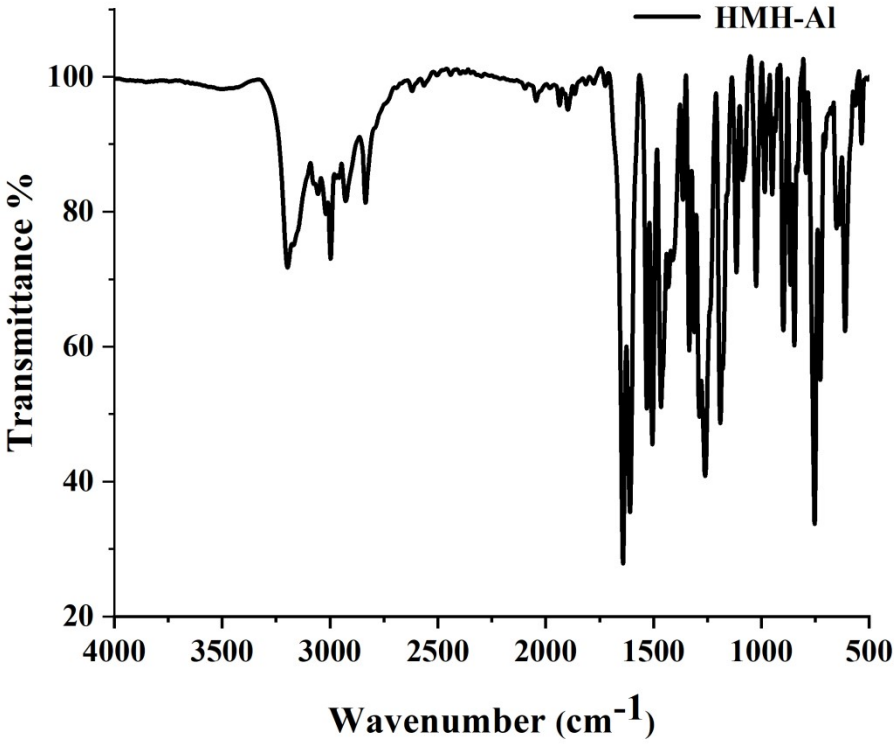


Fig. S1 IR of target product probe **HMH-Al**.

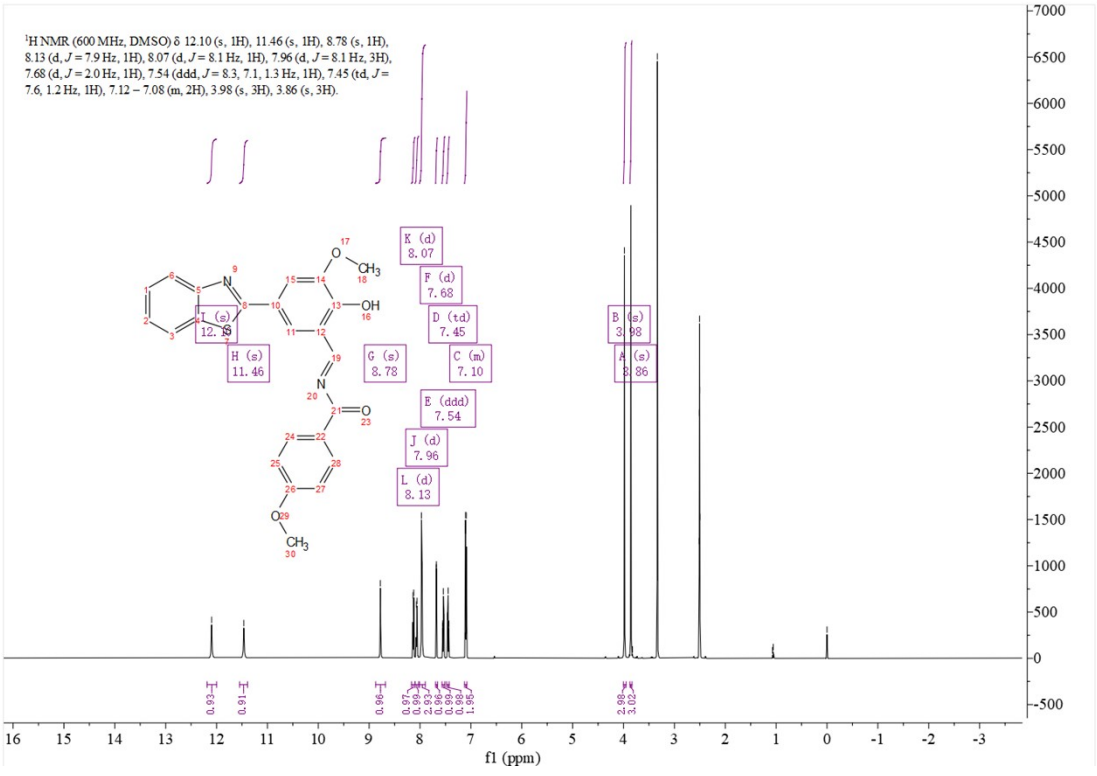


Fig. S2 [1]H NMR of target product probe **HMH-Al**.

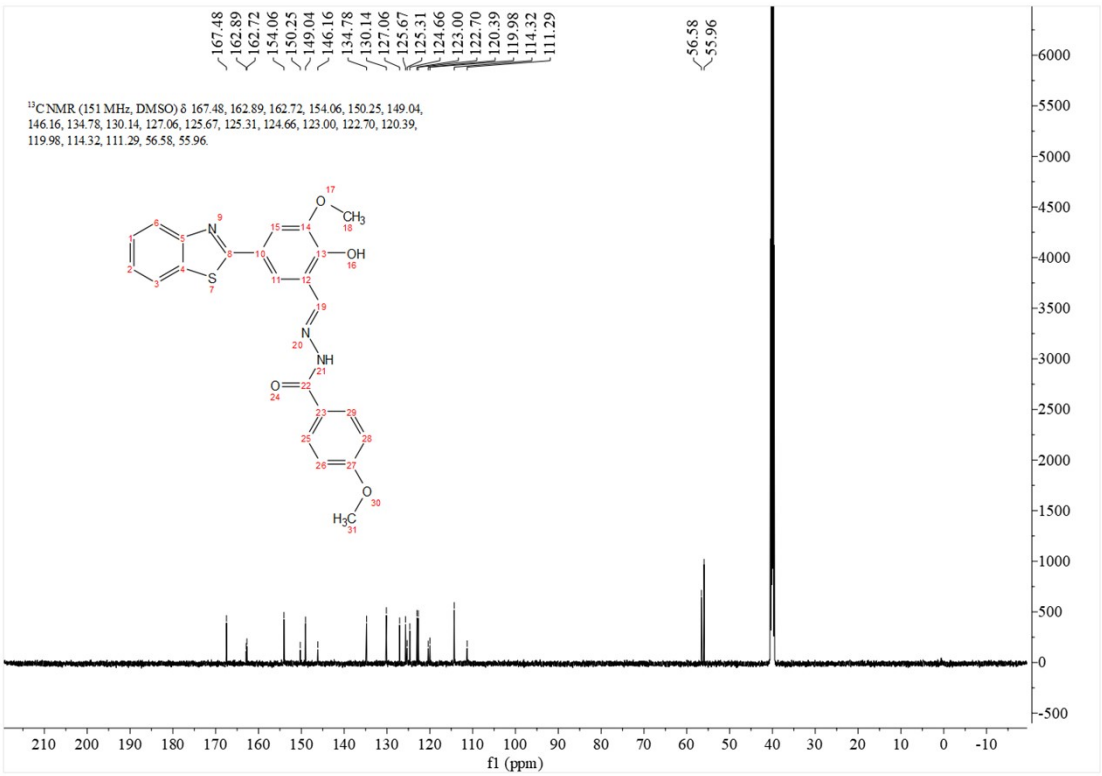


Fig. S3 ^{13}C NMR of target product probe **HMH-Al**.

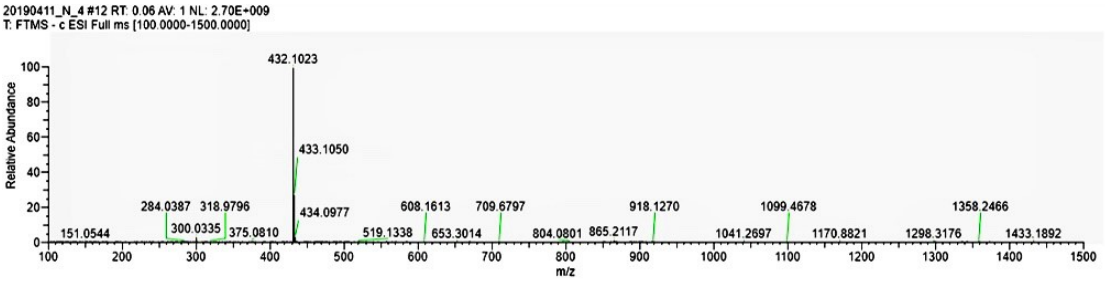


Fig. S4 HRMS of probe **HMH-Al**.

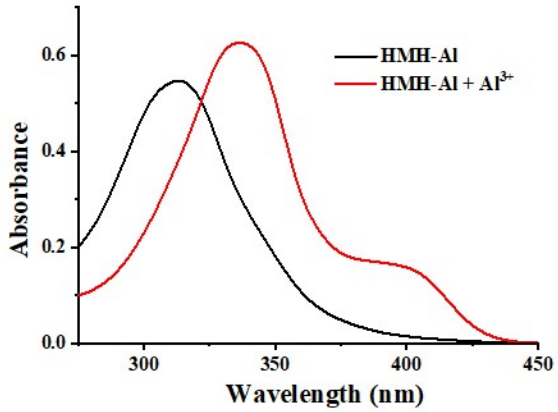


Fig. S5 UV-vis absorbance spectral change of **HMH-Al** (10 μM) and **HMH-Al**/Al^{3+}.

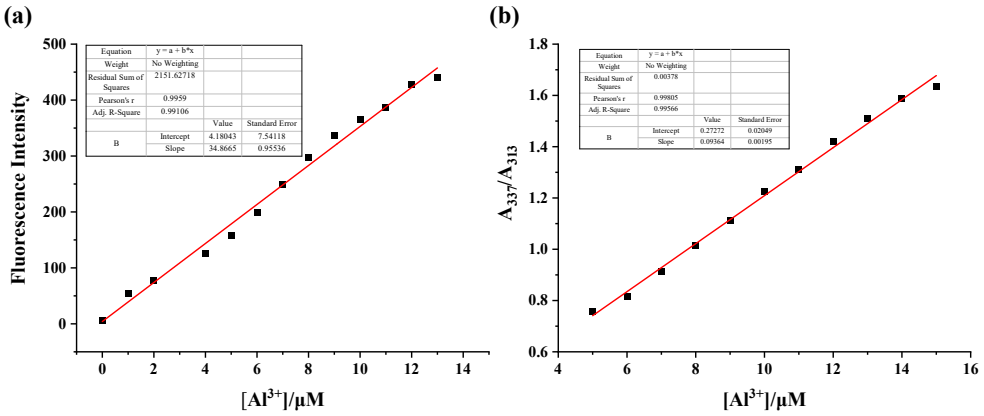


Fig. S6 (a) Fluorescence intensity at 485 nm as a function of Al^{3+} concentrations (0-13 μM) (b) Absorbance intensity at 337 nm/313 nm as a function of Al^{3+} concentrations (0.5-15 μM)

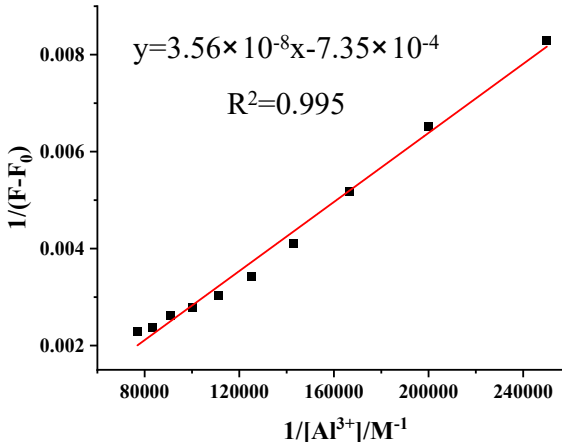


Fig. S7 Benesi-Hildebrand plot of probe **HMH-Al** with Al^{3+}.

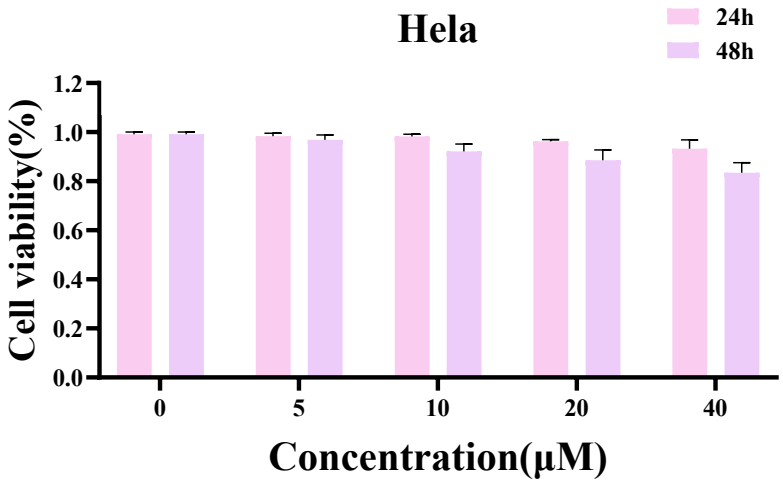


Fig. S8 CCK-8 assay for detecting the effect of different concentrations of **HMH-Al** on cell survival rate.

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