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Supporting Information

A novel coelenterate luciferin-based luminescent probe for selective and sensitive detection of thiophenols

Mingliang Yuan,[a,†] Xiaojie Ma,[b,†] Tianyu Jiang,[a] Chaochao Zhang,[a] Hui Chen,[a] Yuqi Gao,[a] Xingye Yang,[a]Lupei Du[a] and Minyong Li[a,*]

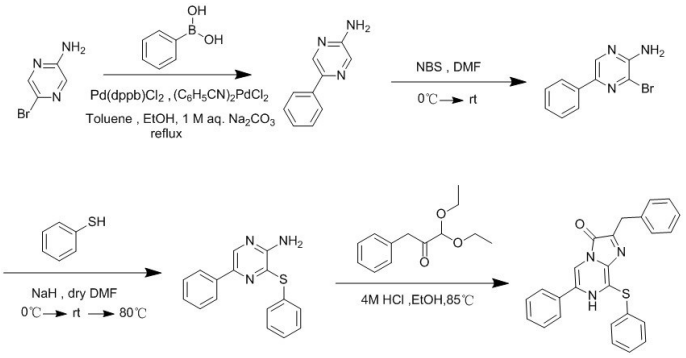
[a]*Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (MOE),School of Pharmacy, Shandong University, Jinan, Shandong 250012, China.*

[b]*Department of Otorhinolaryngology, Qilu Hospital of Shandong University, Jinan, Shandong 250012, China.*

*Corresponding author. Tel./fax: +86-531-8838-2076; e-mail: mli@sdu.edu.cn

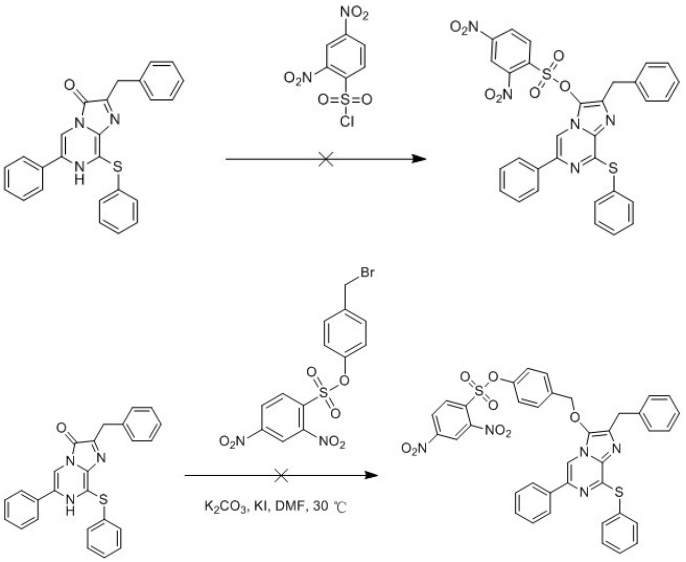
Content

1. Probe design and synthesis

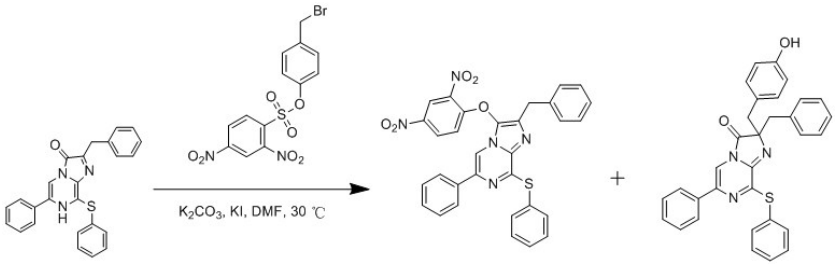


Scheme S1 The synthesis of compound**2**

Note: The detailed procedure was described in the literature. (M.Yuan, T.Jiang, L.Du, et al., *Chin. Chem. Lett.*, 2016, 27, 550-554.)

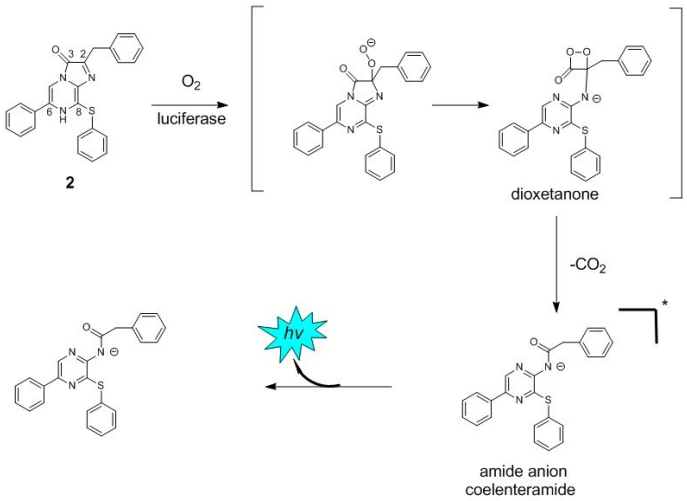


Scheme S2 Two failed reactions.



Scheme S3 An unexpected reaction

2. The mechanism of compound 2 luminescence



Scheme S4 A possible mechanism of the CTZ derivative **2** luminescence reaction.

3. HPLC analysis

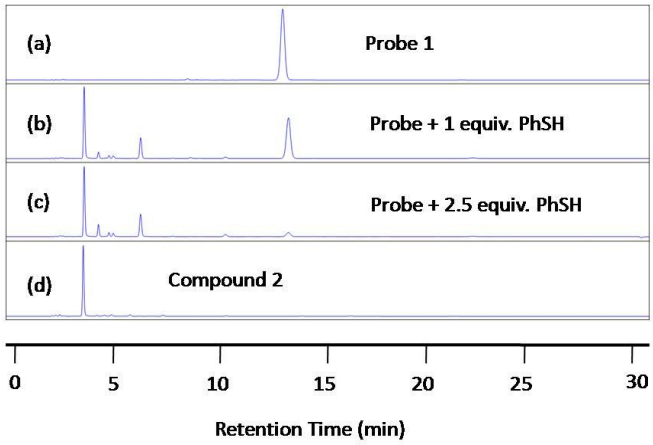


Fig. S1 The HPLC chromatograms: (a) Probe **1** (60.0 μM); (b,c) Probe **1** (60.0 μM) with thiophenol (1.0 and 2.5 equiv., respectively) incubated in pH = 7.4 Tris-HCl buffer/CH_3CN (v/v, 2/3) for 30 min at 37 °C; (d) reference compound **2**(60.0 μM).

4. Sensitivity measurement

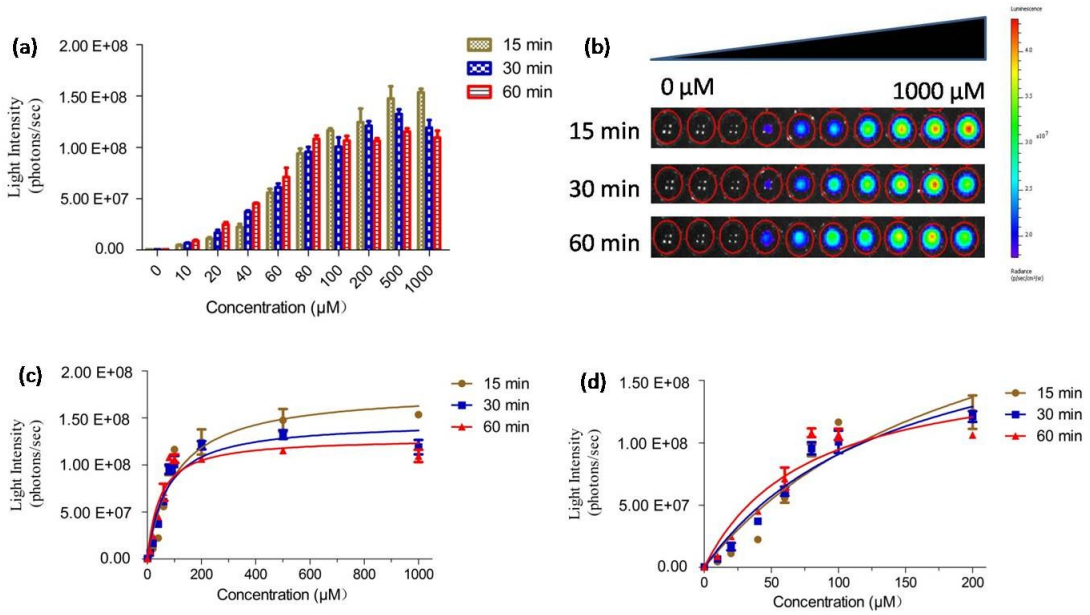


Fig. S2 Bioluminescence assay:(a) Bioluminescent response of probe **1** (20 μM) to different concentrations of thiophenol in Tris-HCl buffer (50 mM, pH 7.4) with an incubation time for 15, 30, and 60 min,respectively,at37 °C; (b)Representative bioluminescence images after the addition of luciferase (1 μg/mL) in 96-well plates;(c) Concentration-dependent luminescence intensity changes of probe **1** (20 μM) to different concentrations of thiophenol (0-1000 μM) for an incubation time for 15, 30, and 60 min, respectively; (d) Concentration-dependent luminescence intensity changes of probe **1** (20 μM) to different concentrations of thiophenol (0-200 μM) for an incubation time for 15, 30, and 60 min, respectively.

5. Selectivity assay

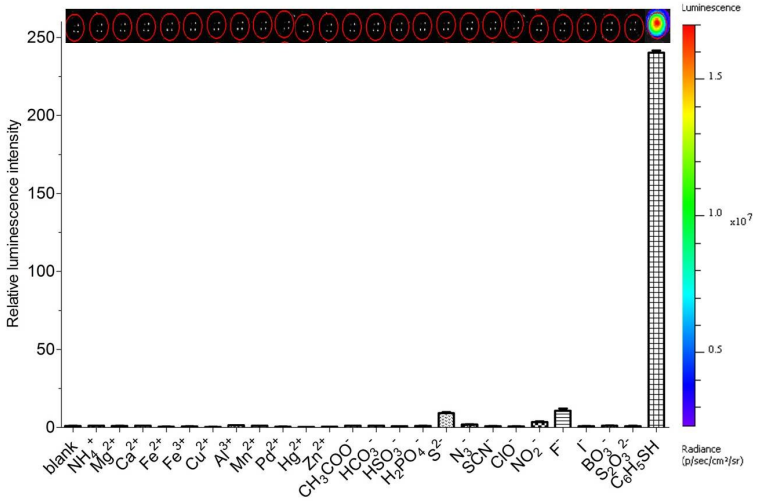


Fig. S3 Selectivity of probe **1** (20 μM) toward thiophenol and various anions and metal ions (500 μM) in Tris-HCl buffer (50 mM, pH 7.4, 37 °C) for 30 min.

6. Cytotoxicity assay

In vitro cytotoxicity of probe **1** was studied according to the standard procedure using the sulphorhodamine B (SRB) dye. ES-2-Rluc cells were cultured in Dulbecco's modified Eagle's medium (DMEM; high glucose with L-glutamine; Gibco,) containing 10% fetal bovine serum (FBS) and 0.5 µg/ mL puromycin at 37 °C in a humidified atmosphere in a 5% CO_2 incubator. 10^4 cells per well were added in 96-well culture plates. After overnight incubation, the culture media was removed and serial dilutions of probe **1** (22.5 µM, 45 µM, 90 µM, 180 µM, 360 µM, 720 µM) in complete growth medium (100 µL) were added. Untreated cells served as control. After 48 h incubation, cells were treated with chilled trichloroacetic acid (50% w/v, 50 µL per well) and then were incubated at 4°C for one hour to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently decanted and discarded. The plates were washed five times with distilled water and air-dried. Wells were stained for 20 min at room temperature with sulforhodamine B dye (0.4% w/v in 1% acetic acid). Then the plates were washed and air-dried. The adsorbed dye was dissolved in Tris-Buffer (100 µL, 10 mM, pH 10.4) and plates were gently stirred for 5 min on a shaker. The optical density (OD) was recorded at 540 nm with a microplate reader (BioTek, MQX200, USA). All the assays were performed in triplicate. IC_{50} value of probe **1** (IC_{50} = 701.1±16.39 µM) was calculated using non-liner regression analysis in the Prism 5.0 GraphPad software.

7. ^{1}H NMR, ^{13}C NMR and HR-MS spectra

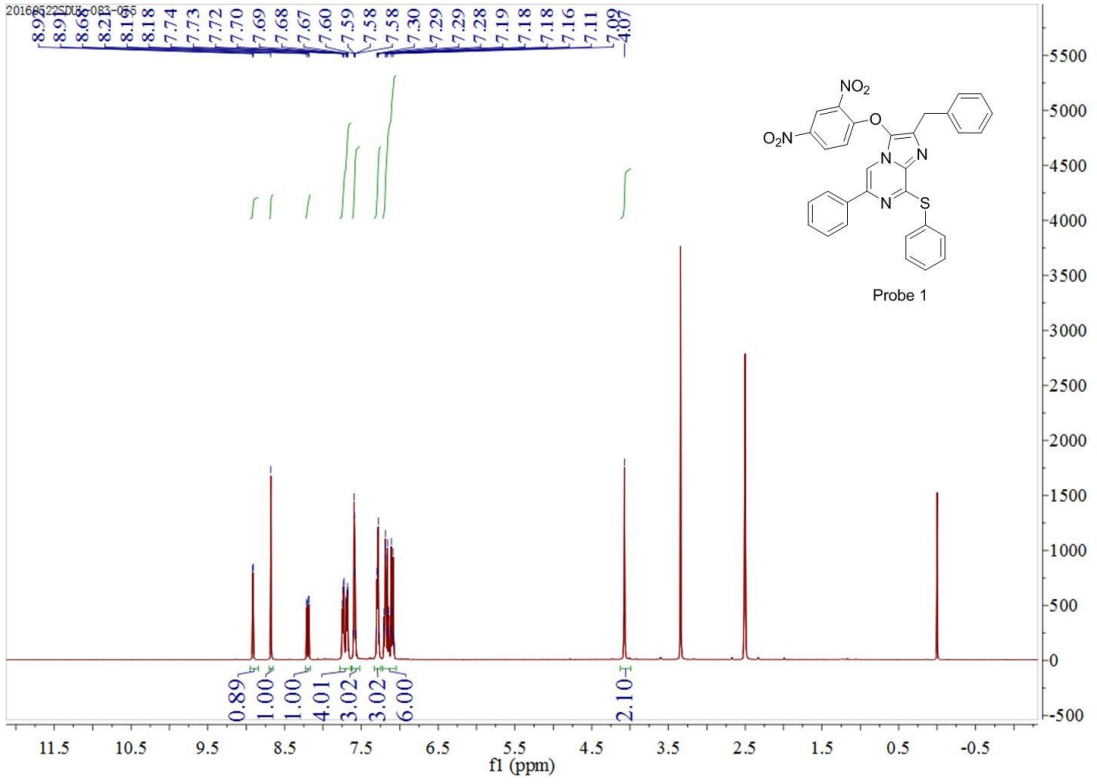


Fig. S3 ^{1}H NMR spectrum of Probe **1**.

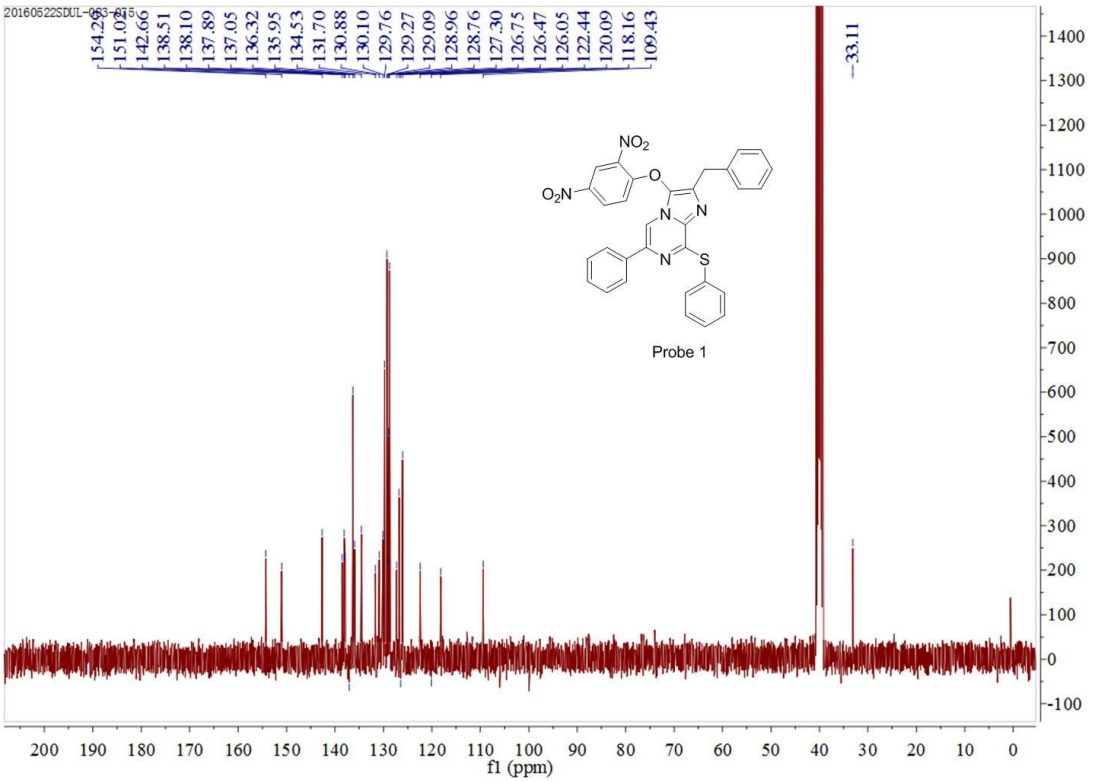


Fig. S4 ^{13}C NMR spectrum of Probe **1**.

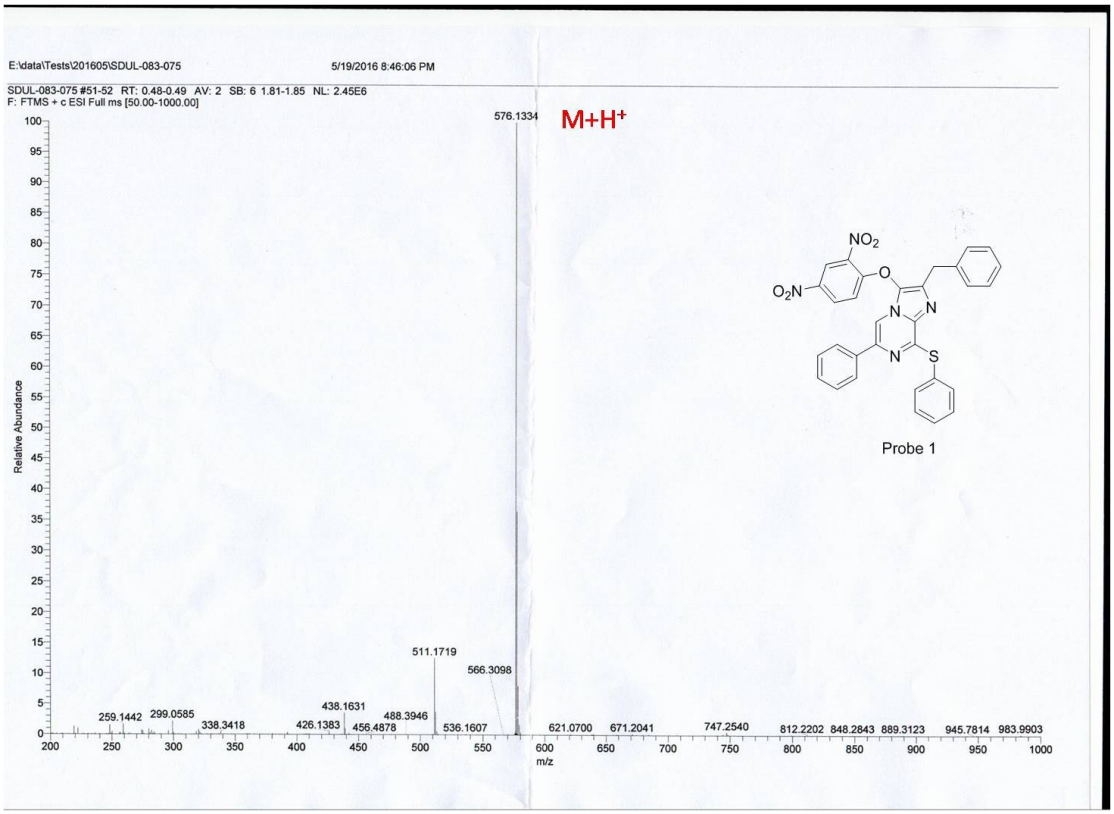


Fig. S5 High-resolution mass spectrum of Probe **1**.