A direct continuous fluorometric turn-on assay for monoamine oxidase B and inhibitor screening based on the abnormal fluorescent behavior of silole

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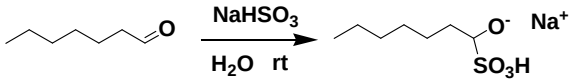
1. Synthesis and characterization of compound 2



Scheme S1 The synthetic route for compound **2**

Synthesis of compound 2[S1]*:* Sodium bisulfite (5.2 g, 50 mmol) were added to the aqueous solution (10 mL) of heptylaldehyde (1.4 mL, 10 mmol) at room temperature. Immediately, white precipitates appeared. The precipitates were filtered and crystallized with water, and then dried under vacuum. A water-soluble white solid was obtained (1.9 g, 87.2%). ^{1}H-NMR (400 MHz, D_2O, ppm): δ 0.87-0.90 (t, J = 6.6 Hz, 3H), 1.32-1.46 (m, 8H), 1.54-1.56 (m, 1H), 1.67-1.71 (m, 1H), 1.94-1.97 (m, 1H), 4.37-4.41 (q, 1H); ^{13}C NMR (100 MHz, D_2O, ppm): δ 84.2, 31.0, 30.9, 28.1, 24.9, 22.1, 13.5.

Reference：

S1. Y. Itoh, J. P. Horiuchi and K. Takahashi, *Colloids and Surfaces A: Physicochem. Eng. Aspects.,* 2007, **308**, 118-122.

2. Fluorescence spectra of the ensemble of silole 1 and heptylaldehyde containing $NaHSO_3$ after incubation for different times

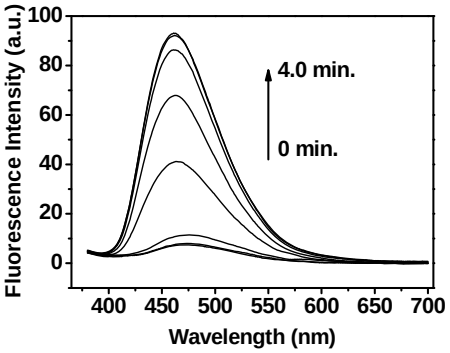


Fig. S1 Fluorescence spectra ($\lambda_{ex.}$ = 370 nm) of the ensemble of silole **1**(7.5×10^{-5} M) and heptylaldehyde (1.0×10^{-4} M) and $NaHSO_3$ (1.0×10^{-4} M) in the mixture of HEPES buffer (10 mM, pH = 7.4) and THF (200:1, v/v) after incubation for different times.

3. Isothermal titration microcalorimetry (ITC) study of compound 2

The calorimetric measurements were conducted using a TAM 2277-201 microcalorimetric system (Thermometric AB, Järfälla, Sweden) with a stainless steel sample cell of 1.0 mL. The cell was initially loaded with H_2O. The concentrated solution of compound **2** was injected into the sample cell via a 500 μL Hamilton syringe controlled by a 612 Thermometric Lund pump. A series of injections were made until the desired range of concentration had been covered. The system was stirred at 50 rpm with a gold propeller. The observed enthalpy (ΔH_{obs}) was obtained by integration over the peak for each injection in the plot of heat flow P against time t. All of the measurements were conducted at 25 ^{o}C.

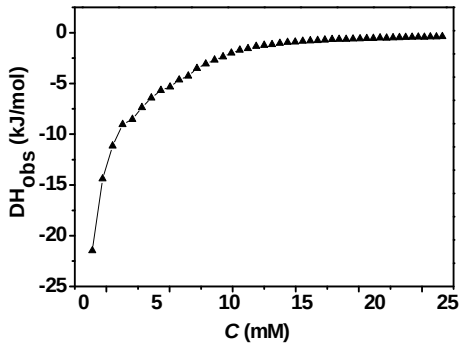


Fig. S2 Variation of the observed enthalpy (ΔH_{obs}) with the concentration (C) of compound **2** dissolved in the mixture of HEPES buffer (10 mM, pH = 7.4) and THF (200:1, v/v).

4. Variation of the fluorescence intensity of silole 1 at 467 nm in the different concentration of HEPES buffer

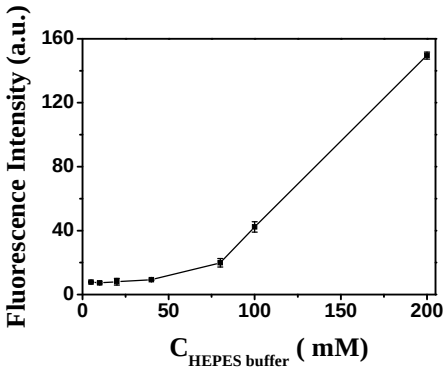


Fig. S3 Variation of the fluorescence intensity of silole **1** (7.5×10^{-5} M) at 467 nm vs. concentration of HEPES buffer in the mixture of HEPES buffer (pH = 7.4) and THF (200:1, v/v).

5. Variation of the fluorescence intensity of silole 1 at 467 nm under different control experimental conditions

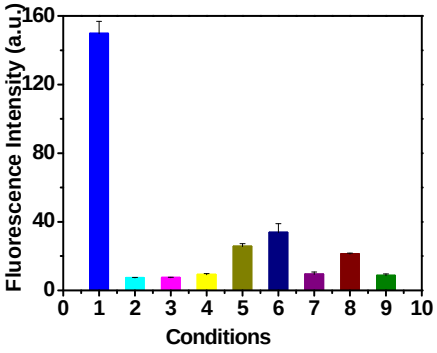


Fig. S4 Variation of the fluorescence intensity at 467 nm under different conditions: 1) silole **1**/heptylamine/$NaHSO_3$/MAO-B; 2) silole **1**/ $NaHSO_3$; 3) silole **1**/heptylamine; 4) silole **1**/heptylaldehyde; 5) silole **1**/MAO-B; 6) silole **1**/ $NaHSO_3$/MAO-B; 7) silole **1**/heptylamine/$NaHSO_3$; 8) silole **1**/heptylamine /MAO-B; 9) silole **1**; the concentrations of silole **1**, heptylamine, heptylaldehyde, $NaHSO_3$ and MAO-B employed for the experiments were 7.5×10^{-5} M**,** 2.0×10^{-4} M**,** 1.0×10^{-4} M, 1.0×10^{-4} M and 2.2 μg/mL, respectively, in the mixture of HEPES buffer (10 mM, pH = 7.4) and THF (200:1, v/v); in each case the solution was incubated for 10.0 min. before the recording of the fluorescence spectrum ($\lambda_{ex.}$ = 370 nm).

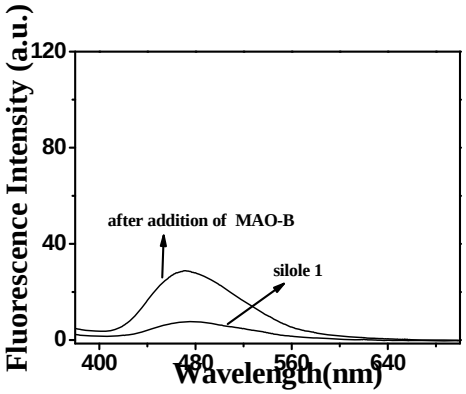


Fig. S5 Fluorescence spectra of silole **1** (7.5×10^{-5} M) in the absence and presence of MAO-B (2.2 μg/mL) in the mixture of HEPES buffer (10 mM, pH = 7.4) and THF (200:1, v/v); in each case the solution was incubated for 10 min. at room temperature before the recording of the fluorescence spectrum ($\lambda_{ex.}$ = 370 nm).

6. The dynamic light scattering results

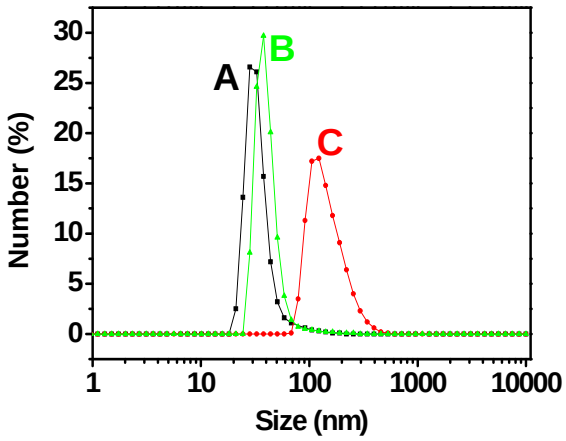


Fig. S6 The dynamic light scattering results for the solution of silole **1** (7.5×10^{-5} M), heptylamine (2.0×10^{-4} M) (A), that after addition of MAO-B (2.2 μg/mL) (B) and that after further addition of $NaHSO_3$ (1.0×10^{-4} M) (C); the mixture of HEPES buffer (10 mM, pH = 7.4) and THF (200:1, v/v) was used as the solvent for all cases.

7. The effect of ionic strength on the fluorescence variation

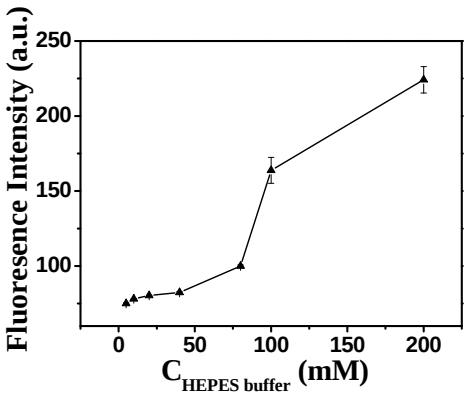


Fig. S7 Variation of the fluorescence intensity at 467 nm for the ensemble of silole **1** (7.5×10^{-5} M), heptylamine (2.0×10^{-4} M), $NaHSO_3$ (1.0×10^{-4} M) and MAO-B (2.2 μg/mL) vs. concentration of HEPES buffer in the mixture of HEPES buffer (pH = 7.4) and THF (200:1, v/v), in each case the solution was incubated for 10.0 min. at room temperature before the recording of the fluorescence spectrum ($\lambda_{ex.}$ = 370 nm).

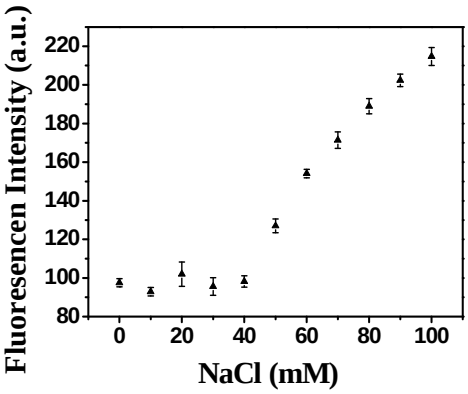


Fig. S8 Variation of the fluorescence intensity at 467 nm for the ensemble of silole **1** (7.5×10^{-5} M), heptylamine (2.0×10^{-4} M), $NaHSO_3$ (1.0×10^{-4} M) and MAO-B (2.2 μg/mL) vs. the concentration of NaCl in the mixture of HEPES buffer (10 mM, pH = 7.4) and THF (200:1, v/v); in each case the solution was incubated for 10.0 min. at room temperature before the recording of the fluorescence spectrum ($\lambda_{ex.}$ = 370 nm).

8. Fluorescence spectra of the ensemble of silole 1, heptylamine and MAO-B in the presence of HSO_3^- and those in the presence of cysteine and glutathione reduced form

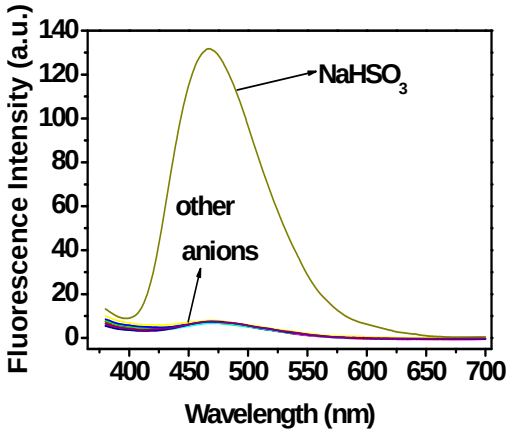


Fig. S9 Fluorescence spectrum ($\lambda_{ex.}$ = 370 nm) of the ensemble of silole **1** (7.5×10^{-5} M), heptylamine (2.0×10^{-4} M) and MAO-B (2.2 μg/mL) in the mixture of HEPES buffer (10 mM, pH = 7.4) and THF (200:1, v/v) in the presence of $NaHSO_3$ and those in the presence of NaOAc, NaF, NaBr, NaCl, NaN_3, $NaNO_3$, $NaHSO_4$, NaH_2PO_4, and $NaClO_4$, respectively; the concentration of $NaHSO_3$ was 2.0×10^{-4} M and those of other salts were 2.0×10^{-3} M; each solution was incubated for 10.0 min. before the recording of the fluorescence spectrum.

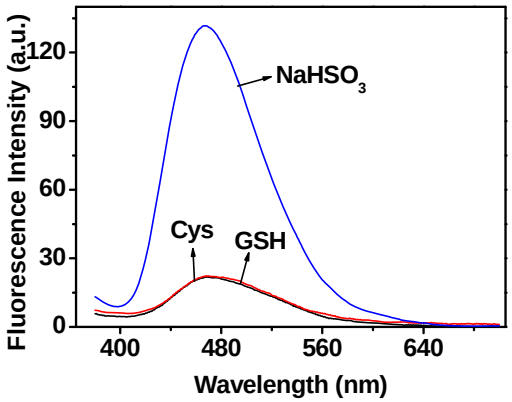


Fig. S10 Fluorescence spectrum ($\lambda_{ex.}$ = 370 nm) of the ensemble of silole **1** (7.5×10^{-5} M), heptylamine (2.0×10^{-4} M) and MAO-B (2.2 μg/mL) in the mixture of HEPES buffer (10 mM, pH = 7.4) and THF (200:1, v/v) in the presence of $NaHSO_3$ and those in the presence of *L*-cysteine and glutathione reduced form (GSH), respectively; the concentration of $NaHSO_3$ was 2.0×10^{-4} M and those of *L*-cysteine and glutathione reduced form were 2.0×10^{-3} M; each solution was incubated for 10.0 min. before the recording of the fluorescence spectrum.

9. The fluorescence spectra of silole 1 in the presence of different amounts of compound 2

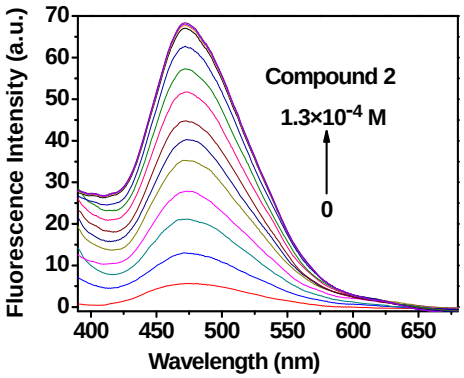


Fig. S11 Fluorescence spectra of the ensemble of silole **1** (7.5×10^{-5} M) in the mixture of HEPES buffer (10 mM, pH = 7.4) and THF (200:1, v/v) in the presence of different concentrations of compound **2** (from 0 to 1.3×10^{-4} M).

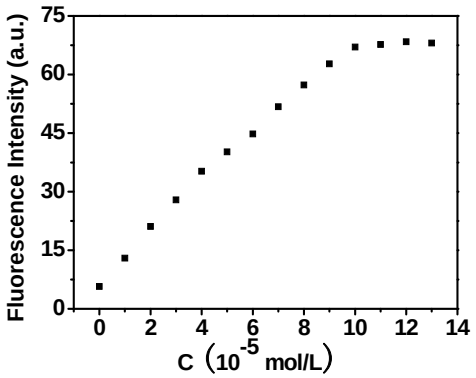


Fig. S12 The plot of fluorescence intensity of silole **1** at 467 nm *vs.* the concentrations (**C**) of compound **2**.

10. ^{1}HNMR and ^{13}CNMR of compound 2