Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Fluorescent probes for detecting monoamine oxidase activity and cell imaging

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Supporting Information

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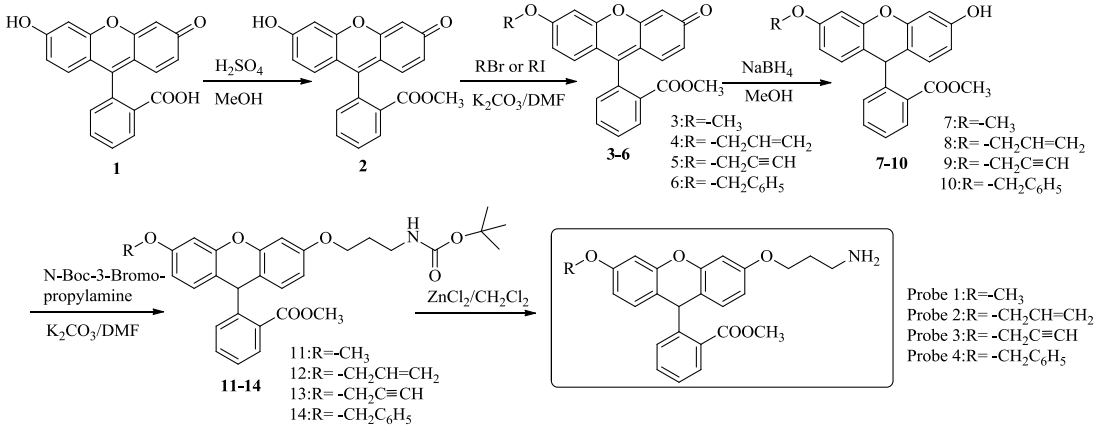
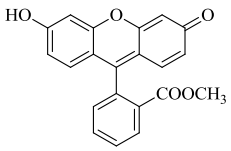
1. General information

Compounds were visualized by UV light (254 and 365 nm) and all reactions were monitored by thin layer chromatography (TLC). ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400M Bruker instrument (400 MHz and 100 MHz, respectively). Data for ^{1}H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity(s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet or unresolved, coupling constant (J)in Hz, integration). Data for ^{13}C NMR are reported in terms of chemical shift (δ, ppm). Mass spectra (MS) were measured with Bruker instrument. Fluorescence spectra were determined on a Multi-Mode Microplate Readers.

2.Preparation and characterization of probes

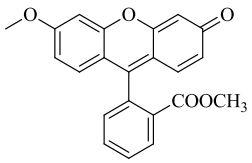


A solution of fluorescein(8 g, 0.24 mmol) in MeOH (45 mL) was added dropwise into concentrated sulfuric acid (6 mL). The reaction was carried out at 85℃ monitored by TLC until completion (~12h). The reaction mixture was poured into 20g of ice-water. Then $NaHCO_3$ (24 g) was added to the solution in portions and stirred vigorously at room temperature. A red precipitate was formed; this was collected by filtration, washed with water and petroleum ether, dried and obtained 6.90g product **2**. Yield: 82.7%.

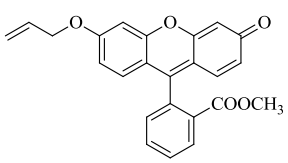


2-(6-Hydroxy-3-oxo-3H-xantene-9-yl)-benzoic acid methyl ester (2):red solid, Yield 82.7%,^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (dd, *J*= 1.2, 0.8 Hz, 1H), 7.74 (m, 2H), 7.64 (m, 1H), 6.41 (d, *J*= 9.2 Hz, 2H), 6.03 (dd, *J*= 2.0, 2.0 Hz, 2H), 5.97 (d, *J*= 2.0 Hz, 2H), 3.55 (s, 3H); ESI-MS m/z 347.1 $(M+1)^{+}$.

Appropriate bromide and iodide (3.6 mmol) was added to the mixture of **2**(1.0 g, 2.9 mmol) and K_2CO_3(0.598 g, 4.5 mmol) in 30 mL of DMF at room temperature. After stirring for 24 hours, the product was extracted withCH_2Cl_2 (100 mL× 3), and the organic layer was washed with water (100 mL×2) and brine (100mL), dried over Na_2SO_4 and concentrated under reduced pressure. Yield: 91.5 % for **3** (0.94 g, orange solid); 91.5 % for **4** (1.00 g, orange powder); 90 % for **5** (0.99 g, orange powder); 80 % for **6** (1.00 g, orange solid).

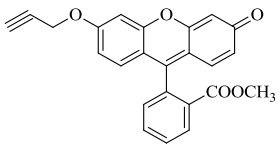


2-(6-Methoxy-3-oxo-3H-xantene-9-yl)-benzoic acid methyl ester (3):orange solid, Yield 91.5%, ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 8.22 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.72 (td, *J* = 7.5, 1.4 Hz, 1H), 7.65 (td, *J* = 7.6, 1.4 Hz, 1H), 7.29 (dd, *J* = 7.5, 1.2 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.85 (dd, *J* = 14.6, 9.3 Hz, 2H), 6.72 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.52 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.44 (d, *J* = 1.9 Hz, 1H), 3.91 (s, 3H), 3.63 (s, 3H); ESI-MS m/z 361.1 $(M+1)^{+}$.

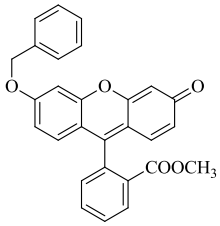


2-(6-allyloxy-3-oxo-3H-xanthen-9-yl) benzoic acid methyl ester (4):orangepowder, 91.5 %, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.68 (td, *J* = 7.4, 1.3 Hz, 1H), 7.61 (td, *J* = 7.6, 1.3 Hz, 1H), 7.25 (dd, *J* = 7.4, 1.0 Hz, 1H), 6.90 (d,

J = 2.4 Hz, 1H), 6.82 (dd, *J* = 15.2, 9.2 Hz, 2H), 6.71 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.47 (dd, *J* = 9.6, 1.8 Hz, 1H), 6.38 (d, *J* = 1.8 Hz, 1H), 5.99 (m, 1H), 5.42 (d, *J* = 1.3 Hz, 1H), 5.38 (d, *J* = 1.3 Hz, 1H), 4.60 (d, *J* = 5.3 Hz, 2H), 3.61 (s, 3H); ESI-MS m/z 387.1 $(M+1)^+$.



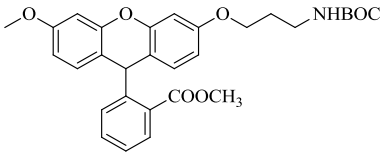
2-(6-(prop-2-ynyloxy)-3-oxo-3H-xanthen-9-yl) benzoic acid methyl ester (5): orange solid, yield 90%,^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, *J* = 7.8 Hz, 1H), 7.73 (t, *J* = 7.4 Hz, 1H), 7.67 (t, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.05 (d, *J* = 2.2 Hz, 1H), 6.90 (d, *J* = 8.9 Hz, 1H), 6.85 (d, *J* = 9.6 Hz, 1H), 6.79 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.56 – 6.48 (m, 1H), 6.44 (d, *J* = 1.1 Hz, 1H), 4.79 (d, *J* = 2.1 Hz, 2H), 3.64 (s, 3H), 3.52 (s, 1H); ESI-MS m/z 385.1 $(M+1)^+$.



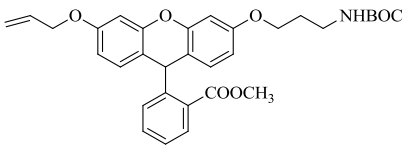
2-(6-benzyloxy-3-oxo-3*H*-xanthen-9-yl) benzoic acid methyl ester (6): orange solid, yield 80%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 7.8 Hz, 1H), 7.72 (t, *J* = 7.4 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.37 (dt, *J* = 13.3, 7.4 Hz, 5H), 7.32 – 7.02 (m, 2H), 7.00 (d, *J* = 2.3 Hz, 1H), 6.89 (d, *J* = 8.9 Hz, 1H), 6.87 – 6.77 (m, 2H), 6.52 (d, *J* = 9.7 Hz, 1H), 5.13 (s, 2H), 3.62 (s, 3H); ESI-MS *m/z* 437.1 $(M+1)^+$.

To a solution ofcorresponding **3-6**(3.0 mmol) in MeOH (10 mL) was added $NaBH_4$ (0.57 g，15.0 mmol) at 0 °C. The reaction was stirred in an ice-water bath for 30 min, and at room temperature for 2 h. After removal of the solvent, the product was extracted with CH_2Cl_2 (50 mL×3), and the organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure to yield the homologous air-sensitive product **7-10** as yellow powder.

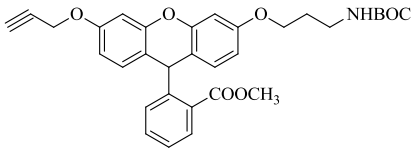
Appropriate compound **7-10** (2.7 mmol) was dissolved in DMF (30 mL). Then, K_2CO_3(0.559 g, 4.05 mmol) and N-Boc-3-bromopropylamine (0.771 g, 3.24 mmol) were added to the solution. The mixture was stirred at r.t for 6 hours. The remaining solid residue was removed by filtration. The filtrate was concentrated and purified by SiO_2 chromatography (CH_2Cl_2: MeOH = 50:1). Yield: 70.4 % for **11**(1.00 g, white solid); 72% for **12**(1.07 g, white solid); 75 % for **13**(1.11 g, white solid); 80 % for **14**(1.30 g, white solid).



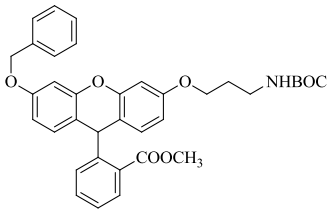
methyl2-(3-(3-((tert-butoxycarbonyl)amino)propoxy)-6-methoxy-9H-xanthen-9-yl)benzoate (11):white solid, yield 70.4%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, *J* = 7.8 Hz, 1H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.06 – 6.94 (m, 2H), 6.82 (dd, *J* = 8.5, 4.2 Hz, 2H), 6.54 – 6.48 (m, 2H), 6.42 – 6.34 (m, 2H), 6.10 (s, 1H), 4.96 (s, 1H), 3.88 – 3.76 (m, 5H), 3.61 (s, 3H), 3.17 (d, *J* = 5.8 Hz, 2H), 1.86 – 1.77 (m, 2H), 1.33 (s, 9H); ESI-MS *m/z* 520.1 $(M+1)^+$.



methyl2-(3-(allyloxy)-6-(3-((tert-butoxycarbonyl)amino)propoxy)-9H-xanthen-9-yl)benzoate (12): white solid, yield 72%,^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, *J* = 8.9 Hz, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 7.10 – 6.96 (m, 2H), 6.82 (dd, *J* = 8.6, 1.9 Hz, 2H), 6.53 (dd, *J* = 9.5, 2.5 Hz, 2H), 6.41 (ddd, *J* = 13.0, 8.5, 2.5 Hz, 2H), 6.09 (s, 1H), 5.87 (m, 1H), 5.29 (d, *J* = 17.2 Hz, 1H), 5.16 (d, *J* = 10.5 Hz, 1H), 4.73 (s, 1H),4.40 (d, *J* = 5.3 Hz, 2H), 3.90 – 3.83 (m, 5H), 3.20 (d, *J* = 5.9 Hz, 2H), 1.89 – 1.82 (m, 2H), 1.34 (s, 9H); ESI-MS *m/z* 546.1 $(M+1)^+$.

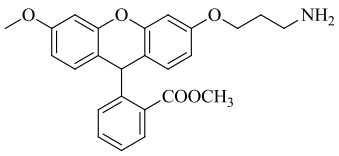


methyl2-(3-(3-((tert-butoxycarbonyl)amino)propoxy)-6-(prop-2-yn-1-yloxy)-9H-xanthen-9-yl)benzoate(13):white solid, yield 75%,^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, *J* = 8.8 Hz, 1H), 7.17 (dd, *J* = 13.9, 6.5 Hz, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 6.97 (d,*J* = 7.8 Hz, 1H), 6.82 (dd, *J* = 16.4, 8.6 Hz, 2H), 6.61 (d, *J* = 2.5 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 6.46 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.39 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.09 (s, 1H), 4.80 (s, 1H), 4.53 (s, 2H), 3.91 – 3.78 (m, 5H), 3.18 (d, *J* = 6.0 Hz, 2H), 2.43 (s, 1H)1.89 – 1.79 (m, 2H), 1.33 (s, 9H); ESI-MS *m/z* 544.3 $(M+1)^+$.

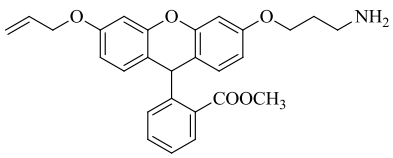


methyl2-(3-(benzyloxy)-6-(3-((tert-butoxycarbonyl)amino)propoxy)-9H-xanthen-9-yl)benzoate(14):white solid, yield80%,^{1}H NMR (400 MHz, $CDCl_3$): δ 7.6 –7.61 (m, 1H), 7.18 (ddd, *J* = 33.4, 17.8, 7.8 Hz, 6H), 6.98 (dd, *J* = 16.7, 7.5 Hz, 2H), 6.82 – 6.76 (m, 2H), 6.58 (d, *J* = 2.5 Hz, 1H), 6.49 – 6.41 (m, 2H), 6.37 – 6.32 (m, 1H), 6.07 (s, 1H), 4.85 (s, 3H), 3.84 – 3.75 (m, 5H), 3.14 (t, 2m), 1.85 – 1.72 (m, 2H), 1.31 (s, 9H);ESI-MS m/z 596.4 $(M+1)^+$.

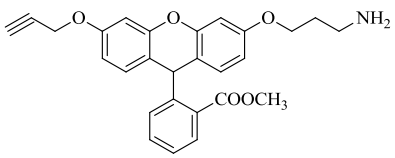
To a solution of compound **11**−14(1 mmol) in CH_2Cl_2 (5mL) was added $ZnCl_2$ (0.27 g，2 mmol) at room temperature and the resulting mixture was stirred for 3 hours. The remaining solid residue was removed by filtration. The filtrate was extracted with CH_2Cl_2 (50 mL×3), and the organic layer was washed with $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated under reduced pressure to yield the air-sensitive product. Yield: 47.7 % for **probe1**(0.2 g, white solid); 50% for **probe2** (0.22 g, white solid); 40 % for **probe3**(0.18 g, white solid); 42 % for **probe4**(0.21 g, white solid).



methyl 2-(3-(3-aminopropoxy)-6-methoxy-9H-xanthen-9-yl)benzoate (probe 1): white solid, yield 47.7%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.22–7.13 (m, 1H), 7.08–7.03 (m, 1H), 7.00–6.95 (m, 1H), 6.81 (dd, *J* = 8.6, 4.4 Hz, 2H), 6.52 (d, *J* = 2.5 Hz, 2H), 6.40 (dt, *J* = 8.5, 2.4 Hz, 2H), 6.08 (s, 1H), 3.90 (t, J = 6.0 Hz, 2H), 3.83 (s, 3H), 3.66 (s, 3H), 3.02 (s, 2H), 2.84 (s, 2H), 1.91–1.81 (m, 2H);^{13}C NMR (101 MHz, $CDCl_3$) δ 168.50, 159.03, 158.24, 151.22, 151.22, 148.05, 132.19, 131.55, 130.38, 130.38, 130.38, 129.32, 129.32, 125.87, 116.82, 110.51, 110.04, 101.70, 101.06, 65.94, 55.39, 52.29, 38.96, 37.92, 31.82. ESI-MS m/z 420.1 $(M+1)^{+}$.

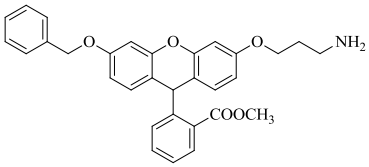


methyl 2-(3-(allyloxy)-6-(3-aminopropoxy)-9H-xanthen-9-yl)benzoate (probe 2): white solid, yield 50%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 6.6 Hz, 1H), 7.15 (t, *J* = 8.0 Hz, 1H), 7.07 – 6.99 (m, 1H), 6.97 (d, *J* = 7.8 Hz, 1H), 6.87 – 6.77 (m, 2H), 6.54 – 6.46 (m, 2H), 6.40 (td, *J* = 8.6, 2.2 Hz, 2H), 6.07 (d, *J* = 6.5 Hz, 1H), 5.89 (ddd, *J* = 22.1, 10.5, 5.3 Hz, 1H), 5.26 (d, *J* = 17.2 Hz, 1H), 5.17–5.10 (m, 1H), 4.36 (d, *J* = 4.8 Hz, 2H), 3.94–3.76 (m, 5H), 2.80 (dt, *J* = 20.4, 6.8 Hz, 2H), 1.93–1.75 (m, 2H); 13C NMR (101 MHz, CDCl3) δ 168.24, 158.09, 157.83, 151.00, 147.89, 132.85, 132.85, 131.99, 131.38, 130.19, 130.19, 130.19, 129.04, 129.04, 125.69, 117.24, 116.81, 110.49, 110.32, 101.80, 101.53, 68.71, 65.67, 52.09, 38.58, 37.69, 31.46. ESI-MS m/z 446.5 $(M+1)^{+}$.



methyl 2-(3-(3-aminopropoxy)-6-(prop-2-yn-1-yloxy)-9H-xanthen-9-yl)benzoate

(probe 3): white solid, yield 40%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.61 (m, 1H), 7.24 – 7.15 (m, 1H), 7.06 (t, *J* = 7.1 Hz, 1H), 6.97 (d, *J* = 7.7 Hz, 1H), 6.88 – 6.77 (m, 2H), 6.60 (s, 1H), 6.48 (dd, *J* = 8.3, 6.5 Hz, 2H), 6.38 (dd, *J* = 13.6, 6.2 Hz, 1H), 6.11 – 6.05 (m, 1H), 4.55 (s, 2H), 4.14 (s, 2H), 4.00 – 3.76 (m, 5H), 2.94 (s, 2H), 2.44 (s, 1H), 2.08 – 1.82 (m, 2H); ESI-MS m/z 444.5 $(M+1)^+$.



methyl 2-(3-(3-aminopropoxy)-6-(benzyloxy)-9H-xanthen-9-yl)benzoate (probe 4): white solid, yield 42%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 7.1 Hz, 1H), 7.32 – 7.15 (m, 6H), 7.03 (t, *J* = 7.5 Hz, 1H), 6.95 (d, *J* = 7.7 Hz, 1H), 6.78 (t, *J* = 8.3 Hz, 2H), 6.57 (d, *J* = 2.4 Hz, 1H), 6.49 – 6.44 (m, 2H), 6.38 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.04 (s, 1H), 5.54 (s, 2H), 4.87 (s, 2H), 3.88 – 3.78 (m, 5H), 3.04 (s, 2H), 2.11 – 1.98 (m, 2H); ESI-MS m/z 496.4 $(M+1)^+$.

3. Limit of detection

The limit of detection, expressed as the concentration, c_L,

$c_L = 3\sigma / m$,

$$\sigma = \sqrt{\frac{\sum(\bar{x}-xi)^2}{n-1}}$$

$\bar{x}$ is the mean of the blank measures(just probe only), xi is the values of blank measures, n is the tested number of blank measure. m is the slope of the linear regression equation.

4. Enzymatic activity assays

MAO-A and MAO-B were prepared from human placenta and beef liver respectively and the crude enzymes were used directly to approach the in vivo environment. The stock solutions of Probe 1-4 were prepared in DMSO (10mM) and diluted in 100 mM aqueous borate buffer (pH = 8.4) to a final concentration 200 μM. All fluorescence spectra were performed in 96-well plates. MAO A, MAO B, diamineoxidase and heat-inactivated enzyme were added to a final protein concentration 16 μg/mL and

incubation for 40 min at 37 ºC, respectively. Fluorescence intensity of Probe 1-4 were collected by microplate reader and the excitation wavelength was 470 nm. The resultsare shown in Figure S1 and Figure S2.

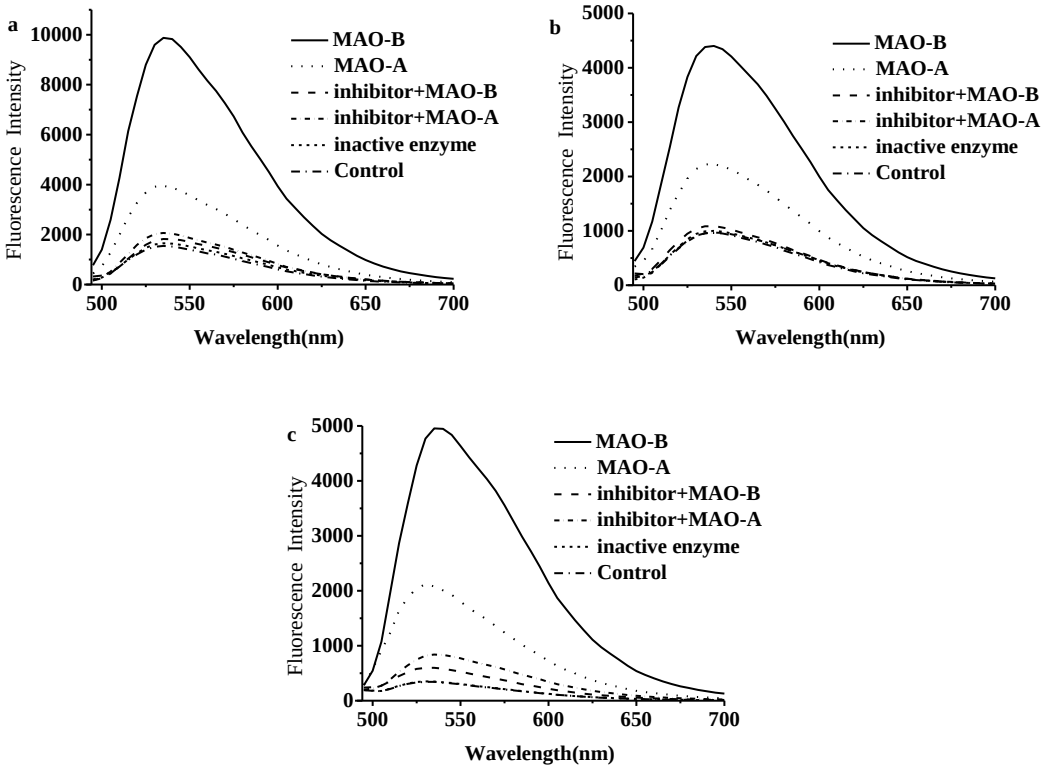


Fig.S1(a-c) Fluorescence of probe 2-4 before and after reaction with MAO-B (dot line), MAO-A (short dash line) and corresponding inhibitor, inactive enzyme. The spectra were recorded in 100 mM borate buffer (pH = 8.4) at λ_{ex}= 470 nm.

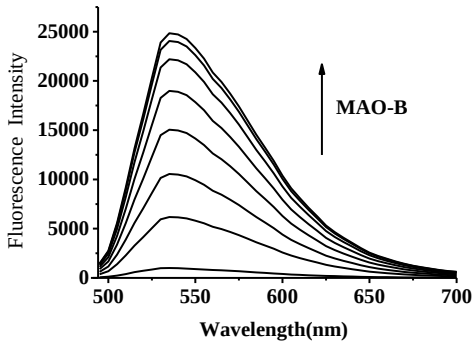


Fig. S2 Fluorescence of probe 1 (200 μM) treated with different concentrations of MAO-B, [Enzyme] =0-300μg/mL, pH=8.4, borate buffer.

5. Enzymatic kinetics assays

Enzyme kinetics experiments were performed in 96-well fluorescence assay plates. A series of different concentrations of probe 1-4 was diluted in enzyme assay buffer (100 mM Borate buffer, pH = 8.4) to a final concentration containing (0-200 μM). The fluorescence intensity was collected at 535 nm (λex = 470 nm) by using Molecular Devices Spectramax M2 Microplate Fluorometer at 5 min intervals from 0 to 1 h at 37 ºC. Enzyme kinetics experiments with Probe 1-4 and MAO-A or MAO-B were performed on three independent experiments.

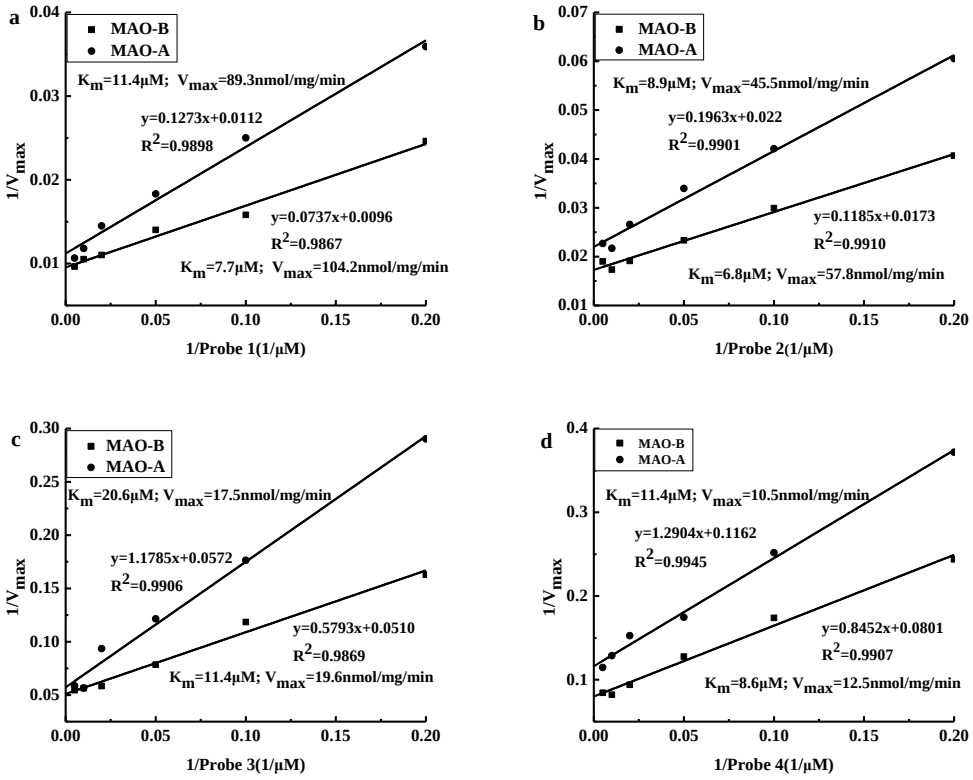


Fig.S3(a-d) Km values of Probe 1-4 with MAO-A and MAO-B. The Km and V_{max} value of MAO-A or MAO-B was assessed by a series concentrations of Probe 1-4(0-200μM) reaction with MAO-A or MAO-B (16μg/mL) at 37 °C in enzyme assay buffer(borate buffer, pH=8.4). The fluorescence intensity was collected at 535nm (λ ex=470nm).

6. Live cell imaging

Experiments were performed as following steps. First, MCF-7 cells were cultured in chamber at 37 °C, and after80% confluence, the medium was removed followed by

washing with PBS buffer twice. Next, the probe1 (50 μM) were then added to the chamber in the growth mediumseparately. After incubation in a 5% CO_2 incubatorfor 3 h at 37 °C, cells werewashed twice with PBS buffer to remove the extracellular probes, and further fixed by 75% ethanol for 30min. Cell imaging experimentswere conducted with a fluorescence microscope. Moreover, the cells were treated with MAO inhibitor selegiline for 90 minutes at 37 ºC in a 5% CO_2 incubator. Probe 1 were then added to final concentrations of 50 μM and incubated for 2 hours, cells were then washed twice with PBS buffer to remove the extracellular inhibitors and probes, and further fixed by 75% ethanol for 30 min. Following that, the cells were imaged with a fluorescence microscope.