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## Electronic Supporting Information

# A fluorescent probe for alkylating agents and its quantification of triflate as a genotoxic impurity

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#### 1. Materials and instruments

All chemical reagents and solvents were analytical grade products and they were obtained from commercial suppliers (Adamas Reagents or Cologne Reagents) without further purification. Silica gel (Qingdao, mesh 100-200) was used for TLC analysis and column chromatography.

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were measured on a Bruker AVANCE NMR instrument (400 MHz) and chemical shifts were given in ppm using the peak of residual proton signals of DMSO as the internal standard. Mass spectra were carried out on a Finnigan LCQ<sup>DECA</sup> spectrometer with ESI mode. UV-vis absorption and fluorescence spectra were recorded on a SHIMADZU UV-2450 spectrophotometer and a VARIAN Cary Eclipse FL1003 M013 spectrometer, respectively.

#### 2. Synthesis and characterizations of BI-Py

#### Synthesis of BI-Py

To the solution of 1,1,2-trimethyl-1*H*-benzo[*e*]indole (1.90 g, 9.07 mmol) in acetic acid (1.50 mL) was added 4-pyridinecarboxaldehyde (1.0 g, 9.33 mmol) followed by two drops of concentrated hydrochloric acid. After stirring for 5 h at room temperature, the reaction mixture was poured into brine (50 mL) and then extracted with dichloromethane (20 mL×3), the combined organic phase was dried over sodium sulfate and evaporated under vacuum. The residue was purified by column chromatograph on silica gel (cyclohexane/ethyl acetate = 5/1) to yield yellow solid (1.97 g, 67.9%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.64 (d, *J* = 6.1 Hz, 2H), 8.20 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 7.98 (d, *J* = 8.5 Hz, 1H), 7.84 (d, *J* = 5.0 Hz, 1H), 7.83 - 7.79 (m, 3H), 7.68 (d, *J* = 16.4 Hz, 1H), 7.62 (d, *J* = 7.0 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 1H), 1.65 (s, 6H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  184.82, 151.25, 150.72, 143.51, 134.91, 132.70, 129.98, 129.56, 127.27, 125.42, 124.08, 123.59, 122.25, 120.69, 54.74, 22.33. HR-MS (ESI<sup>+</sup>, *m/z*) calcd for [C<sub>21</sub>H<sub>18</sub>N<sub>2</sub> + H]<sup>+</sup>: 299.1543, found: 299.1542.



Fig. S1 <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ) spectra of **BI-Py**.





Fig. S3 HR-MS of probe BI-Py.

#### **3.** Experimental procedures and results of spectroscopic analysis

Stock solutions of **BI-Py** (2 mM) and various alkylating agents (10 mM) were prepared in pure organic solvents including dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), acetonitrile (MeCN), dichloromethane (DCM), tetrahydrofuran (THF), methanol (MeOH), ethanol (EtOH) and ethyl acetate (EA), and they were diluted with pure solvents to obtain different samples for spectral measurements. The concentration of the probe was 10  $\mu$ M throughout the spectroscopic experiments. Samples were contained in 10-mm quartz cuvettes and the absorption and fluorescence spectra ( $\lambda_{ex} / \lambda_{em} = 350 / 475$  nm or  $\lambda_{ex} / \lambda_{em} = 435 / 625$  nm, slit width: 10 nm /10 nm) were recorded after the samples were mixed well and incubated at room temperature for 200 s unless otherwise stated.

#### **Determination of quantum yields**

With quinine sulfate as the standard ( $\Phi_R = 0.54$ ), the quantum yields of **BI-Py** in eight solvents were determined according to the formula listed below.

$$\Phi_{\rm s} = \Phi_{\rm R} \cdot A_{\rm R} / A_{\rm S} \cdot I_{\rm S} / I_{\rm R} \cdot \eta_{\rm S}^2 / \eta_{\rm R}^2$$

 $\Phi_R$  is the quantum yield of the standard, A is the absorbance of the solution, I is the integrated fluorescence intensity,  $\eta$  is the refractive index of the solution. The results were demonstrated in Table S1.

#### **Determination of LOD**

The detection limit of **BI-Py** for ET was calculated based on the equation defined by IUPAC.

$$LOD = 3s / k$$

s is the standard deviation of blank measurement (11 times) and k represents the slop of the linear relationship between fluorescence intensity at 475 nm and the concentration of ET.

Thus, LOD =  $3s / k = 3 \times 6179.922 / 17129.226 = 1.08 \mu M$ .

Solvent	Maximal absorption	Maximal emission	Stokes shift	Refractive	Quantum
	wavelength / nm	wavelength / nm	/ nm	index	yield
DMSO	357	478	121	1.477	0.38
DMF	356	476	120	1.3972	0.33
MeCN	354	475	121	1.344	0.19
DCM	355	470	115	1.4244	0.18
THF	358	460	102	1.405	0.22
MeOH	355	495	140	1.329	0.20
EtOH	354	490	136	1.3611	0.11
EA	355	463	108	1.3719	0.15

Table S1 Spectral parameters of probe BI-Py in different organic solvents.



Fig. S4 UV-vis absorption spectra of BI-Py (10  $\mu M)$  in different solvents.



Fig. S5 Fluorescence spectra of BI-Py (10  $\mu$ M) in different solvents,  $\lambda_{ex} = 350$  nm.



Fig. S6 The fluorescence intensities of probe BI-Py (10  $\mu$ M) in different organic solvents ( $\lambda_{ex}$  =

350 nm).



Fig. S7 Fluorescence spectra of BI-Py (10  $\mu$ M) before and after the addition of 1,3-propane sultone (100  $\mu$ M) in DCM,  $\lambda_{ex} = 350$  nm, slit width (ex/em) = 10/10 nm.



Fig. S8 Fluorescence spectra of BI-Py (10  $\mu$ M) before and after the addition of ET (100  $\mu$ M) in MeOH,  $\lambda_{ex} = 350$  nm, slit width (ex/em) = 10/10 nm.



Fig. S9 Fluorescence spectra of BI-Py (10  $\mu$ M) before and after the addition of ET (100  $\mu$ M) in THF,  $\lambda_{ex} = 350$  nm, slit width (ex/em) = 10/10 nm.



Fig. S10 Fluorescence spectra of BI-Py (10  $\mu$ M) before and after the addition of ET (100  $\mu$ M) in EA,  $\lambda_{ex} = 350$  nm, slit width (ex/em) = 10/10 nm.



Fig. S11 Fluorescence spectra of BI-Py (10  $\mu$ M) before and after the addition of ET (100  $\mu$ M) in MeCN,  $\lambda_{ex} = 350$  nm, slit width (ex/em) = 10/10 nm.



**Fig. S12** Fluorescence changes of **BI-Py** (10  $\mu$ M) before and after the treatment of different alkylating agents (1, ET, 100  $\mu$ M; 2, 1, 3-propane sultone, 2000  $\mu$ M; 3, methyl iodide, 2000  $\mu$ M; 4, benzyl chloride, 2000  $\mu$ M; 5, epichlorhydrin, 2000  $\mu$ M; 6, methyl sulfate, 2000  $\mu$ M) in acetonitrile.  $\lambda_{ex} / \lambda_{em} = 350 / 475$  nm, slit width (ex/em) = 10/10 nm.



Fig. S13 Time-dependent fluorescence intensities of BI-Py (10  $\mu$ M) after the addition of ET (100  $\mu$ M) in MeCN,  $\lambda_{ex} = 350$  nm, slit width (ex/em) = 10/10 nm.



**Fig. S14** Fluorescence spectral changes of **BI-Py** (10  $\mu$ M) upon the addition of ET (0-90  $\mu$ M) in MeCN. (b) Plot of the linear relationship between fluorescence intensity at 625 nm and the concentration of ET (0-90  $\mu$ M). Reaction time = 200 s,  $\lambda_{ex} / \lambda_{em} = 435 / 625$  nm, slit width (ex/em) = 10/10 nm.



**Fig. S15** (a) Fluorescence spectra of **BI-Py** (0, 10  $\mu$ M) upon the addition of interfering substances (1, 1,2-dibromoethane; 2, 1-chlorobutane; 3, 3-bromopropane; 4, methyl iodide; 5, benzyl chloride; 6, 2-(2-bromoethyl)-1,3-dioxane; 7, 2-bromomethyl tetrahydrofuran; 8, 3-allyl bromide; 9, propargyl bromide; 10, methyl bromoacetate; 11, epichlorhydrin; 12, dimethyl carbonate; 13, dimethyl sulfate; 14, 1,3-propane sultone; 15, ethyl methanesulfonate, 200  $\mu$ M) and ET (16, 100  $\mu$ M) in MeCN. (b) Fluorescence intensities at 625 nm of **BI-Py** (10  $\mu$ M) upon the addition of interfering substances.  $\lambda_{ex} / \lambda_{em} = 435 / 625$  nm, slit width (ex/em) = 10/10 nm.

#### 4. Verification of sensing mechanism



Fig. S16 HR-MS of BI-Py after the addition of an excess of ET in MeCN.

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Synthesis of BI-Py-Et
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Scheme S1 Synthetic route of BI-Py-Et.

4-pyridinecarboxaldehyde (214.2 mg, 2.0 mmol) and ethyl triflate (356.3 mg, 2.1 mmol) were dissolved in acetonitrile (10 mL) and the solution was stirred for 4 h at room temperature. Then, the reaction mixture was concentrated under vacuum and the residue was purified by column chromatograph on silica gel to give crude 1-ethyl-4formylpyridin-1-ium as an oil (1.30 g). The crude intermediate (285 mg, 1.0 mmol) and 1,1,2-trimethyl-1*H*-benzo[*e*]indole (230 mg, 1.1 mmol) were dissolved in dichloromethane (5 mL) and stirred for 2 h at room temperature. On completion of the reaction, the orange precipitate was collected by filtration and washed with dichloromethane. After drying under vacuum, **BI-Py-Et** (237.0 mg) was obtained. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  9.09 (d, J = 6.4 Hz, 2H), 8.55 (d, J = 6.4 Hz, 2H), 8.24 (d, *J* = 8.2 Hz, 1H), 8.11 - 8.06 (m, 2H), 8.05 - 8.00 (m, 2H), 7.87 (d, *J* = 8.5 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.57 (t, J = 7.5 Hz, 1H), 4.59 (q, J = 7.3 Hz, 2H), 1.68 (s, 6H), 1.57 (t, J = 7.3 Hz, 3H). <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  184.11, 151.83, 151.07, 144.90, 133.07, 131.50, 130.53, 130.02, 129.85, 128.45, 127.45, 125.94, 125.70, 123.88, 120.86, 56.15, 54.98, 21.96, 16.70. HR-MS (ESI<sup>+</sup>, m/z) calcd for  $[C_{23}H_{23}N_2]^+$ : 327.1856, found: 327.1839.

a) BI-Py



**Fig. S17** <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ) spectra of (a) **BI-Py** and (b) **BI-Py** (10 mM) with the addition of an excess of ET.



Fig. S18 <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) spectra of BI-Py-Et.



**Fig. S19** <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) spectra of (a) **BI-Py** upon the addition of an excess of ET and (b) the synthesized sensing product **BI-Py-Et**.



Fig. S20 HR-MS of BI-Py-Et.



Fig. S21 <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) spectra of **BI-Py-Et**.