Supplementary Information

For

Tyrosinase detection via enzyme-triggered structural transformation of a ratiometric fluorescent probe

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Experimental

All chemicals used in the synthesis were of reagent or analytical grade (≥98% purity) and used without further purification. 3-Hydroxybenzaldehyde, phosphorus tribromide (PBr_3), 2-hydroxy-1-naphthaldehyde, 2-aminothiophenol, iron(II) chloride ($FeCl_2$), iron(III) chloride ($FeCl_3$), magnesium chloride ($MgCl_2$), cysteine, glutathione, pepsin, β-galactosidase, lysozyme, acetylcholinesterase, trypsin, esterase, α-glucosidase, thrombin, and tyrosinase were purchased from Sigma-Aldrich. Normal human serum was also obtained from Sigma-Aldrich, and its supplier specifications are provided in Table S3. Tyrosine and alanine were purchased from Alfa Aesar Company. Zinc chloride and urea were purchased from Daejung Company. Nickel(II) chloride, calcium chloride, silver nitrate ($AgNO_3$), and ethanol were purchased from Duksan Company. Potassium carbonate, glycine, vitamin C, and glucose were purchased from Fluka Company. Potassium chloride was purchased from JUNSEI Company. Alkaline phosphatase was purchased from Roche Company. Acetonitrile, sodium borohydride, dimethyl sulfoxide, chloroform, and sodium chloride were purchased from Samchun Company. Glutamic acid and valine were purchased from Tokyo Chemical Industry (TCI) Company. Silica gel (Merck, 230-400 mesh) was used for chromatographic purification of all intermediate and target molecules. All other chemicals and solvents were purchased from Sigma-Aldrich, Fisher Scientific, or TCI and used without further purification.

Instrumentation

NMR spectra were recorded using a Bruker (AVANCE III 500), operating at 500 MHz for ^{1}H-NMR and at 125 MHz for ^{13}C-NMR. UV/Vis absorption spectra were recorded using a Shimadzu UV-2600i UV spectrophotometer. Steady-state fluorescence spectra were obtained with a

Shimadzu fluorometer RF-6000. HPLC studies were performed with Waters 515 (HPLC pump) and Thermo Scientific Hypersil Gold (5 μm 4.0 × 250 mm) column.

Sample preparation

A 1 cm quartz cuvette was used for all spectral measurements. A 2.0 mM stock solution of compound **1** in MeOH was prepared and kept at room temperature for one hour before use. Alkaline phosphatase (ALP), pepsin, β-galactosidase, lysozyme, acetylcholinesterase, trypsin, esterase, α-glucosidase, thrombin, tyrosinase, potassium chloride (KCl), sodium chloride (NaCl), nickel(II) chloride ($NiCl_2$), iron(II) chloride ($FeCl_2$), zinc chloride ($ZnCl_2$), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), iron(III) chloride ($FeCl_3$), glycine, glutamic acid, tyrosine, cysteine, valine, alanine, glutathione, vitamin C, glucose, and urea (stock solutions: [ALP] = [pepsin] = [β-galactosidase] = [lysozyme] = [acetylcholinesterase] = [trypsin] = [esterase] = [α-glucosidase] = [thrombin] = 2.0 mg/mL in H_2O; [tyrosinase] = 2.0×10^4 U/mL in H_2O; [KCl] = [NaCl] = [$NiCl_2$] = [$FeCl_2$] = [$ZnCl_2$] = [$MgCl_2$] = [$CaCl_2$] = [$FeCl_3$] = [glycine] = [glutamic acid] = [tyrosine] = [cysteine] = [valine] = [alanine] = [glutathione] = [vitamin C] = [glucose] = [urea] = 0.20 M in H_2O) were tested to evaluate the selectivity of compound **1**. All measurements were performed in Tris-HCl buffer (10 mM, pH 7.5). For tyrosinase activity assays and selectivity tests, samples were incubated at 37 °C for 7 hours.

Serum lipid extraction with organic solvent

Normal human serum was used, and lipids were removed by extraction with a chloroform-methanol mixture according to the method of Folch et al.[1] 4 mL of serum was mixed with 10 mL of chloroform-methanol (2/1, *v/v*), manually agitated for 1 minute, and centrifuged at 4000 rpm

for 20 minutes at room temperature (rt). After centrifugation, the aqueous phase was collected and washed once with 40.0 mL of *n*-hexane to remove any remaining lipids.

Limit of Detection

The limit of detection (LOD) was calculated using the equation LOD = $3\delta_{bk}/m$, where δ_{bk} is the standard deviation of the blank and m is the slope of the calibration plot.

Fluorescence Quantum yield

Fluorescence quantum yield was determined relative to known standards (9,10-diphenylanthracene, Φ_{FL} = 95.5% in cyclohexane).

Synthesis

3-(Bromomethyl) phenol was prepared in a good yield as described in the literature.[2-5]

Compound 4. To a solution of 2-hydroxy-1-naphthaldehyde (**2**) (1.0 g, 5.8 mmol) in DMSO (10 mL), $AgNO_3$ (0.010 g, 0.058 mmol) was added. After stirring for 30 min, 2-aminobenzenethiol (0.73 mL, 5.8 mmol) was added to the reaction mixture. The resulting mixture was vigorously stirred at room temperature for 24 hours under argon gas. The solvent was removed in vacuo after the reaction was complete at room temperature. The reaction mixture was rinsed with water and extracted with CH_2Cl_2 (200 mL). The organic layer was separated, washed with water (100 mL), dried over anhydrous $MgSO_4$, and the solvent was evaporated to yield a pale-yellow solid. The pure product was then isolated by column chromatography on silica gel using ethyl acetate/petroleum ether (1:7, *v/v*) as the eluent. Yield: 74 %; ^{1}H-NMR (500 MHz, DMSO-d_6): 11.03 (s, 1H), 8.27 (d, 1H), 8.18 (d, 1H), 8.15 (d, 1H), 8.00 (d, 1H), 7.91 (d, 1H), 7.59 (t, 1H), 7.52 (t, 1H), 7.51 (t, 1H), 7.40 (t, 1H), 7.35 (d, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6): 164.24, 155.36,

152.69, 135.84, 132.89, 132.84, 128.83, 128.34, 128.11, 126.54, 125.65, 124.38, 123.85, 123.11, 122.27, 118.78, 112.55.

Compound 1. To a solution of compound **4** (0.35 g, 1.25 mmol) in acetonitrile (100 mL), K_2CO_3 (0.21 g, 1.5 mmol) was added. After stirring for 30 min, 3-(bromomethyl)phenol (0.70 g, 3.74 mmol) in acetonitrile (30 mL) was added to the reaction mixture. The reaction mixture was vigorously stirred at 80 ^oC for 24 hours. The reaction mixture was allowed to cool to room temperature, and the solvent was then removed in vacuo. The mixture was rinsed with water and extracted with CH_2Cl_2 (200 mL). The organic layer was separated, washed with water (100 mL), dried over anhydrous $MgSO_4$, and the solvent was evaporated to yield a white solid. The pure product was then isolated by column chromatography on silica gel using ethyl acetate/*n*-hexane (1:3, *v/v*) as the eluent. Yield: 82 %; ^{1}H-NMR (500 MHz; DMSO-d_6): 9.43 (s, 1H), 8.20 (d, 1H), 8.17 (d, 1H), 8.13 (d, 1H), 7.97 (d, 1H), 7.89 (d, 1H), 7.64 (d, 1H), 7.61 (t, 1H), 7.54 (t, 1H), 7.49 (d, 1H), 7.45 (t, 1H), 7.12 (t, 1H), 6.82 (s, 1H), 6.82 (t, 1H), 6.67 (d, 1H), 5.31 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 163.49, 157.90, 154.91, 153.19, 138.59, 136.36, 132.77, 129.91, 128.90, 128.67, 128.26, 126.60, 125.88, 124.71, 124.56, 123.48, 122.46, 118.26, 116.41, 115.39, 115.25, 114.61, 70.74; ESI(+) MS (*m/z*): [M^+] calcd. for $C_{24}H_{17}NO_2S$, 383.0980; found, 383.0980.

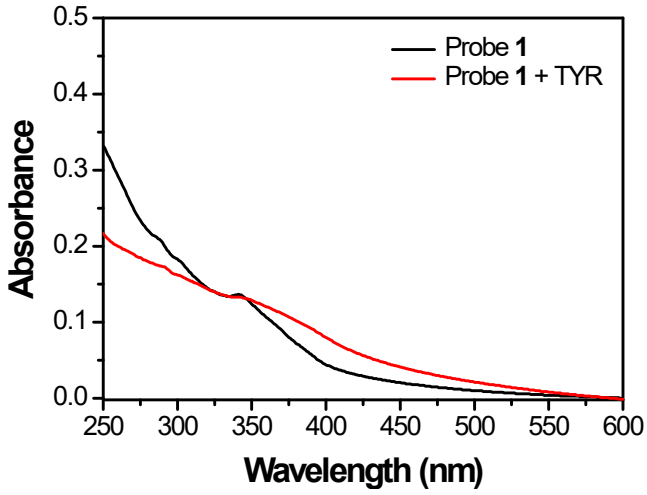


Figure S1. UV/Vis absorbance spectra of **1** after the addition of tyrosinase. Conditions: Tris-HCl buffer (10 mM, pH 7.5); [**1**] = 1.0×10^{-5} M, [tyrosinase] = 200 U/mL.

Table S1. Photophysical parameters of compound **1** and compound **4** in Tris-HCl buffer (10 mM, pH = 7.5).

Compound	ε ($M^{-1}cm^{-1}$)	λ_{ex}	λ_{em}	Stokes shift	Φ_F (%)	Φ_F (%)/ 1% serum
1	13500	332	418	86	2.26	1.11
4	28600	332	484	152	3.17	0.42

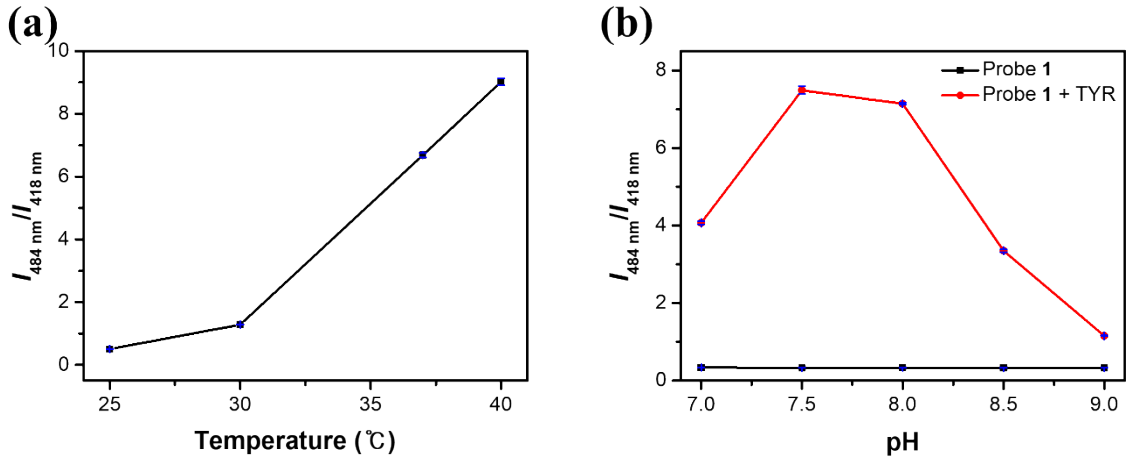


Figure S2. (a) Fluorescence intensity change ($I_{484\ nm}/I_{418\ nm}$) of **1** after 7 hours incubation with tyrosinase at different temperatures (25, 30, 37, and 40 °C); (b) Fluorescence intensity change ($I_{484\ nm}/I_{418\ nm}$) of **1** after 7 hours incubation with tyrosinase at different pH values (7.0, 7.5, 8.0, 8.5, and 9.0). Conditions: Tris-HCl buffer (10 mM, pH 7.5); [**1**] = 1.0×10^{-5} M, [tyrosinase] = 200 U/mL. Excitation was performed at 332 nm, and emission was monitored at the maximum intensities of $I_{418\ nm}$ and $I_{484\ nm}$.

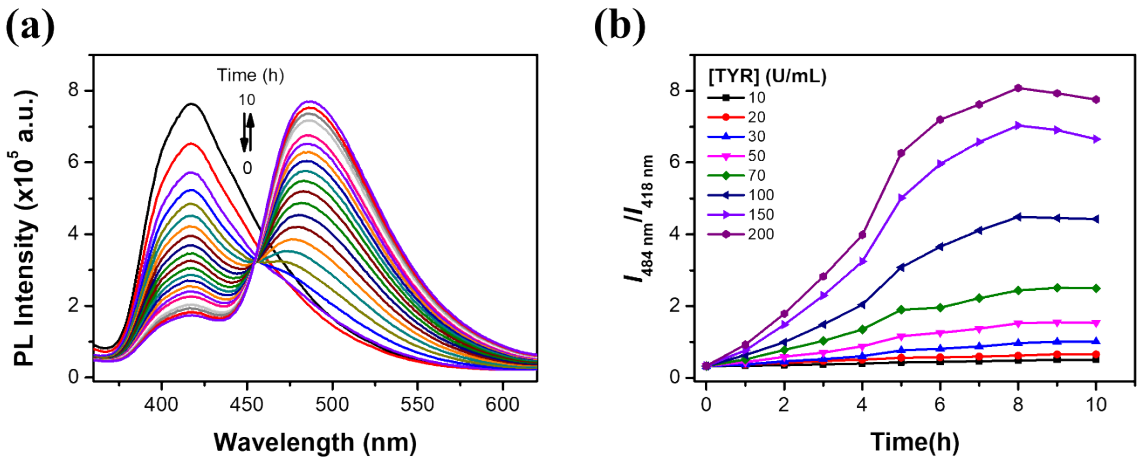


Figure S3. (a) Fluorescence intensity change of **1** after incubation with tyrosinase for 1~10 hours; (b) Corresponding fluorescence profile as a function of time with tyrosinase. Conditions: Tris-HCl buffer (10 mM, pH 7.5); [**1**] = 1.0×10^{-5} M, [tyrosinase] = 200 U/mL. Excitation was performed at 332 nm, and emission was monitored at the maximum intensity of $I_{418\ nm}$ and $I_{484\ nm}$, respectively.

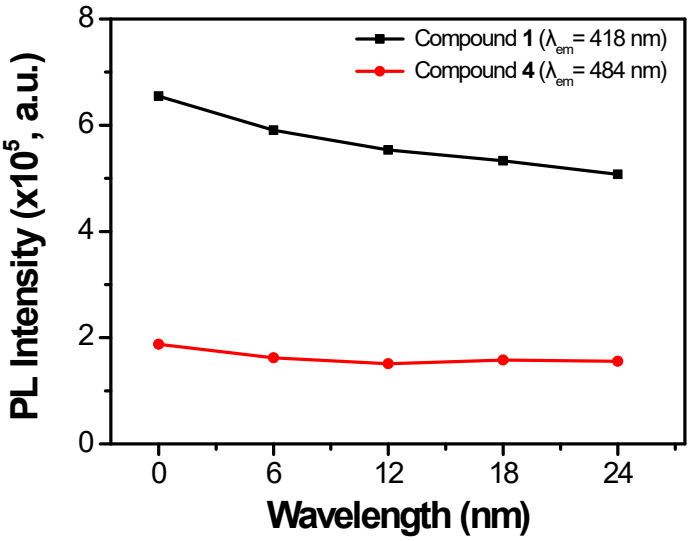


Figure S4. Fluorescence intensity variation of compounds **1** and **4** over 24 h under ambient light. Conditions: Tris-HCl buffer (10 mM, pH 7.5); [**1**] = [**4**] = 1.0×10^{-5} M. Excitation was performed at 332 nm, and emission was monitored at the maximum intensity of 418 nm (compound **1**) and 484 nm (compound **4**), respectively.

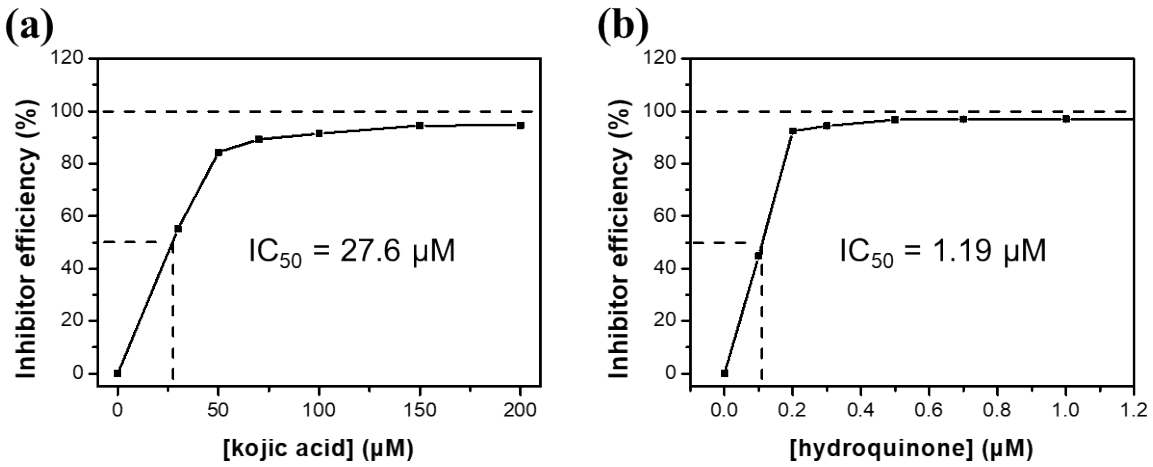


Figure S5. Inhibition efficiency profile of compound **1** incubated with tyrosinase in the presence of varying concentrations of tyrosinase inhibitor (kojic acid and hydroquinone) in Tris-HCl buffer (10 mM, pH 7.5). Conditions: [**1**] = 1.0×10^{-5} M, [tyrosinase] = 200 U/mL.

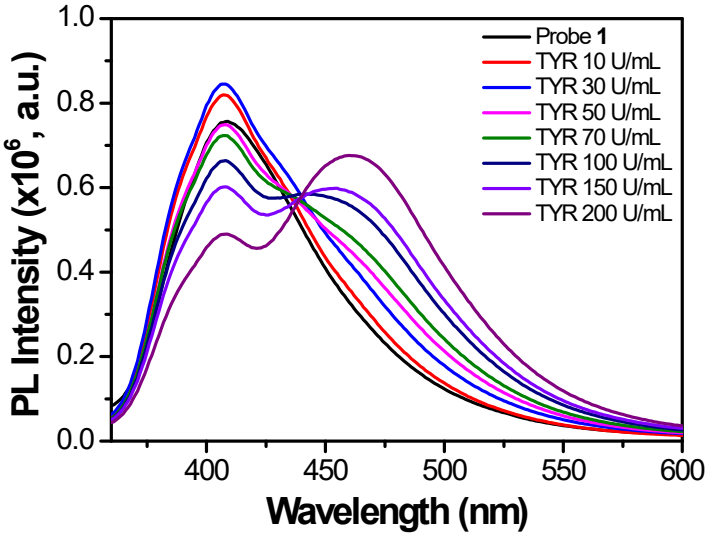


Figure S6. Fluorescence intensity change of **1** incubated with various concentrations of tyrosinase (10.0 to 200.0 U/mL) at 24 hours in human serum (1.0%) at 37 °C, pH 7.5. Conditions: [**1**] = 1.0 × 10^{-5} M. Excitation was performed at 332 nm.

Table S2. Comparison of probe **1** with a recently reported tyrosinase sensors in Tris-HCl buffer (10 mM, pH = 7.5) and human serum.

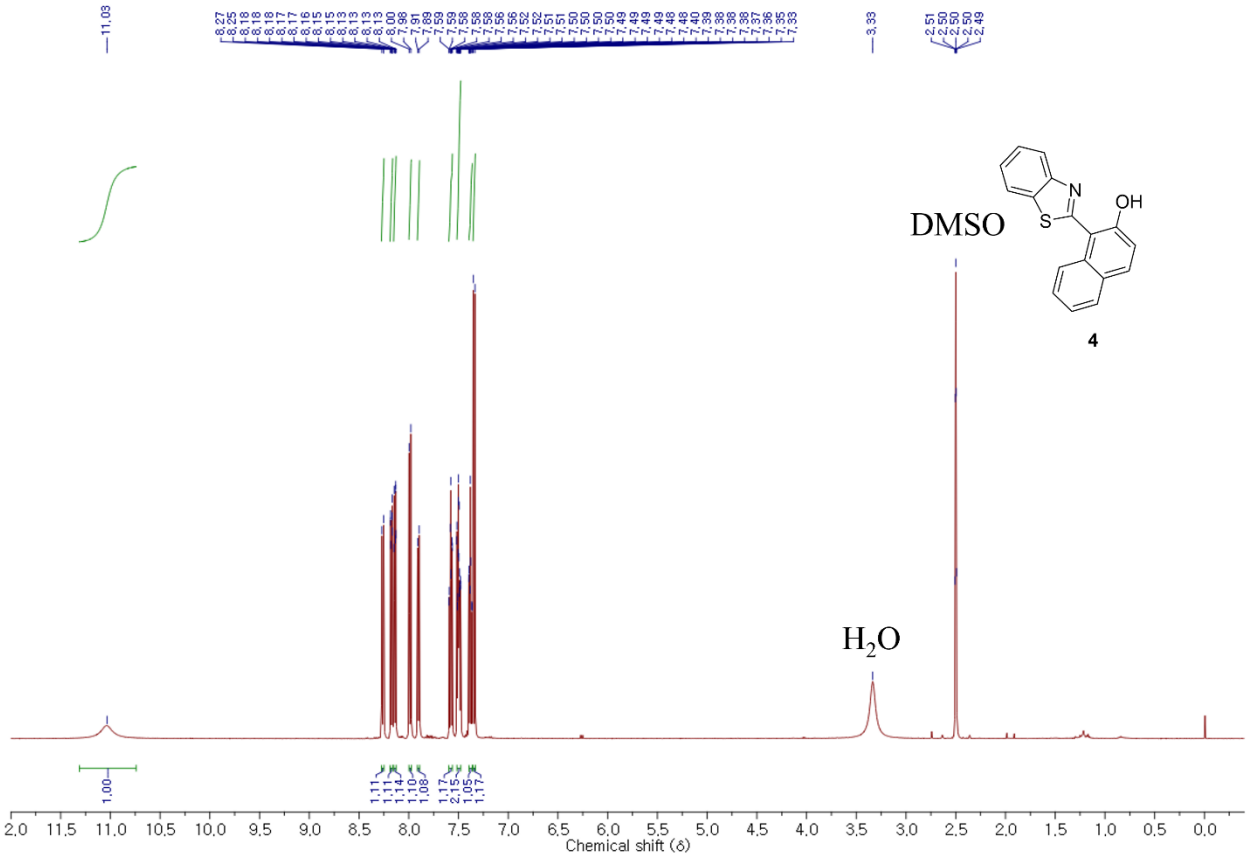
S. No.	LOD (Linear range)	Fluorescence sensing method	Interference of ROS/RNS in detecting TYR	Detection time	Biological application	Ref.
1	0.34 U/mL (0~200 U/mL)	Two-photon, turn-on	x	6 h	B16 and HeLa cells	(S6)
2	0.028 U/mL (0~8 U/mL)	Turn-on	O	30 min	Diluted serum	(S7)
3	2.26 U/mL (0~200 U/mL)	ICT, turn-on	x	1 h 30 min	B16, HeLa cells and zebrafish	(S8)
4	0.08 U/mL (0~190 U/mL)	ICT, turn-on	x	3 h	B16 cells	(S9)
5	0.36 U/mL (0~10 U/mL)	ICT, turn-on	x	2 h 30 min	B16 cells	(S10)
This work	2.33 U/mL (0~200 U/mL)	Ratiometric	x	7 h	Diluted human serum	

Table S3. Specifications for normal human serum[a]. Data was collected from Sigma-Aldrich chemical company sample information.

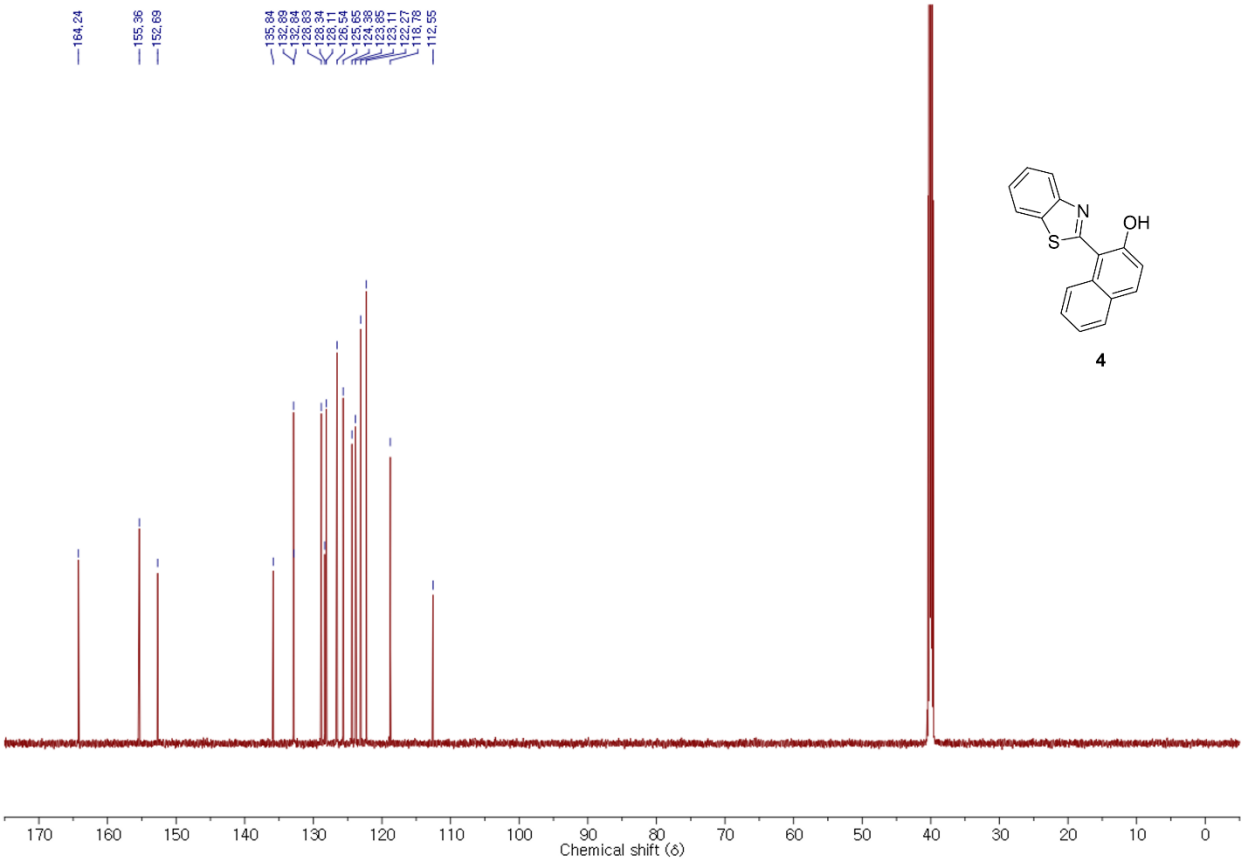
TEST	Specification
Appearance (Color)	Colorless to Brown-Yellow to Brown
Appearance (Form)	Liquid
pH	7.0 ~ 9.0
Iron (UG%)	40 ~ 100
Source: Male donors Within the United States	Conforms
Processing Country of Origin United States	Conforms
Osmolality Expressed in MOSM/KG H_2O	260 ~ 340
Sterility by USP Guidelines	Pass
Hemoglobin	≤ 25 mg/dl
Mycoplasma Test	None Detected
Endotoxin Level	≤ 10 EU/ml
Cholesterol	80 ~ 200 mg/dl
Triglyceride	30 ~ 175 mg/dl
Glucose	50 ~ 180 mg/dl
Sodium (Na)	100 ~ 160 MEQ/L
Protein Content	4.0 ~ 9.0 %
Tested For Infectious Agents	Tested

[a]All donor units are collected in donor centers located in the United States, which are licensed by the FDA.

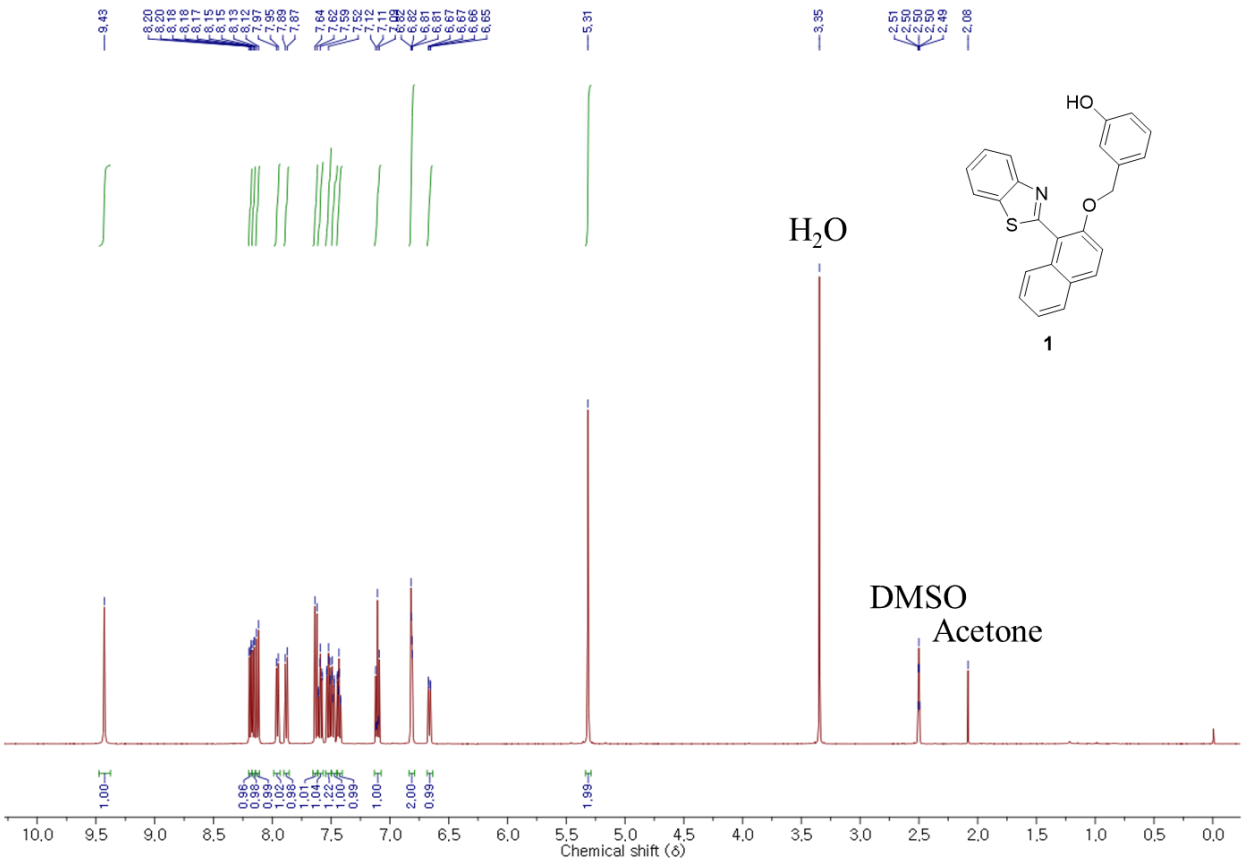
Appendix



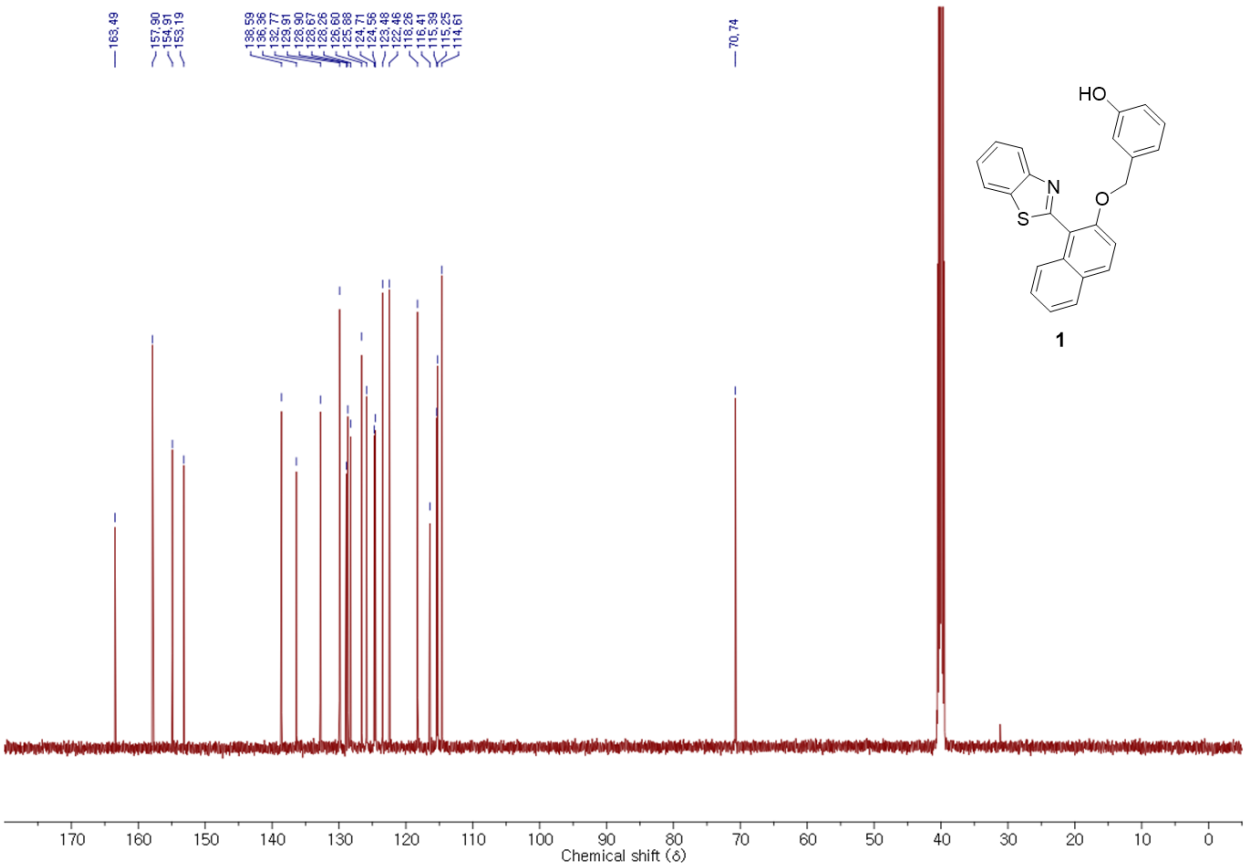
^{1}H-NMR spectrum of **4** in DMSO-d_6



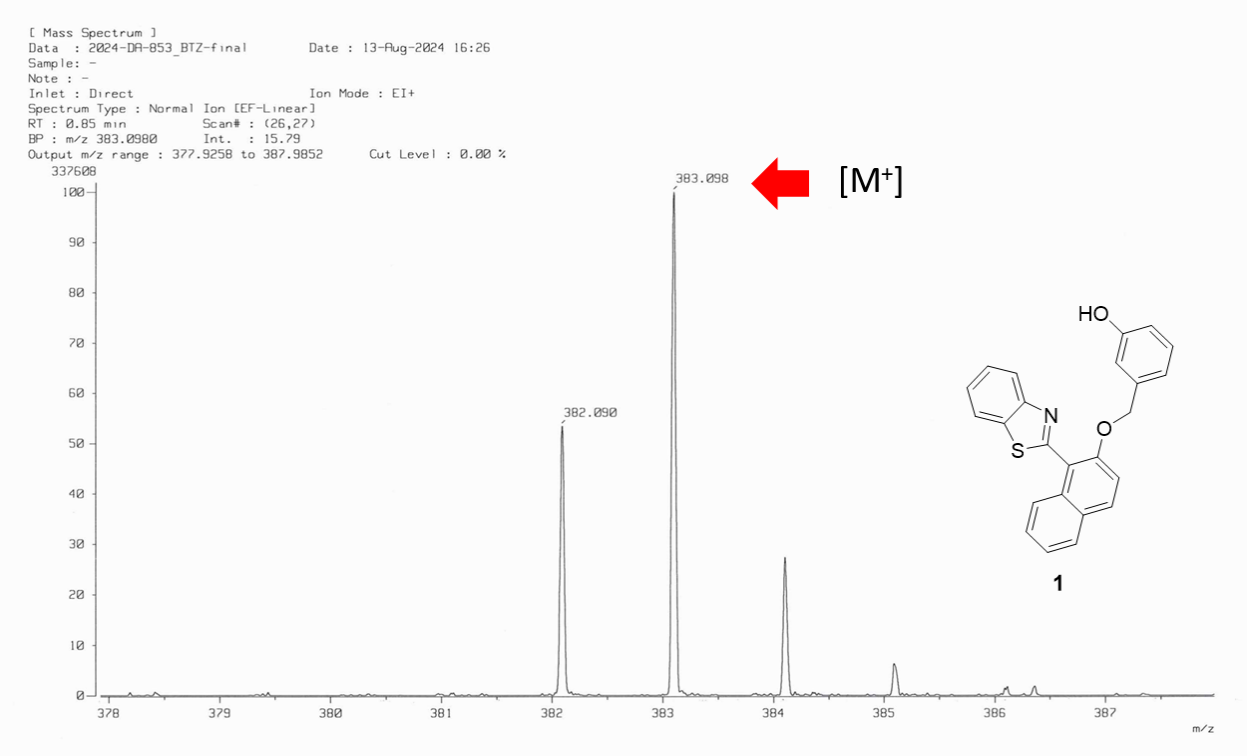
^{13}C-NMR spectrum of **4** in DMSO-d_6



[1]H-NMR spectrum of **1** in DMSO-d_6



^{13}C-NMR spectrum of **1** in DMSO-d_6



ESI Mass spectrum of **1**

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