Supporting Information

Substituted kynurenic acid derivatives as fluorophore-based probes for D- and L-amino acid oxidase assays and their *in vitro* application in eels

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S. 1 Chemicals for compound synthesis

Special-grade hexane, methanol (MeOH), 35% HCl, 99.5% ethanol (EtOH), 85% phosphoric acid (H_3PO_4), glacial acetic acid (AcOH), ethyl acetate (AcOEt), toluene, sodium bicarbonate ($NaHCO_3$), sodium hydroxide (NaOH), potassium bicarbonate (K_2CO_3), potassium iodide (KI), potassium hydroxide (KOH), potassium dihydrogen phosphate (KH_2PO_4), diphenylether (Ph_2O), 2-methylphenylboronic acid, 4-methylphenylboronic acid, 3,5-dimethylphenylboronic acid, diethyl acetylenedicarboxylate, cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), D-aminoacylase "AMANO", diethylamine, HPLC-grade formic acid, and first-grade anhydrous Na_2SO_4 were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Palladium(II) diacetate ($Pd(OAc_2)$), 2-(dicyclohexylphosphino)-2′-(dimethylamino)biphenyl (DavePhos), phenylboronic acid, and 2,6-dimethylphenylboronic acid, chloroacetonitrile, anhydrous aluminum trichloride ($AlCl_3$), boron trichloride in dichloromethane, sodium ethoxide (NaOEt), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and diethyl acetamidomalonate were purchased from Tokyo Chemical Industry (Tokyo, Japan). Dry dimethoxyethane (DME) was purchased from Kanto Kagaku (Tokyo, Japan). Acylase I from *Aspergillus melleus* was purchased from Sigma-Aldrich Co., Ltd. (St. Louis, CA, USA). Water was purified using

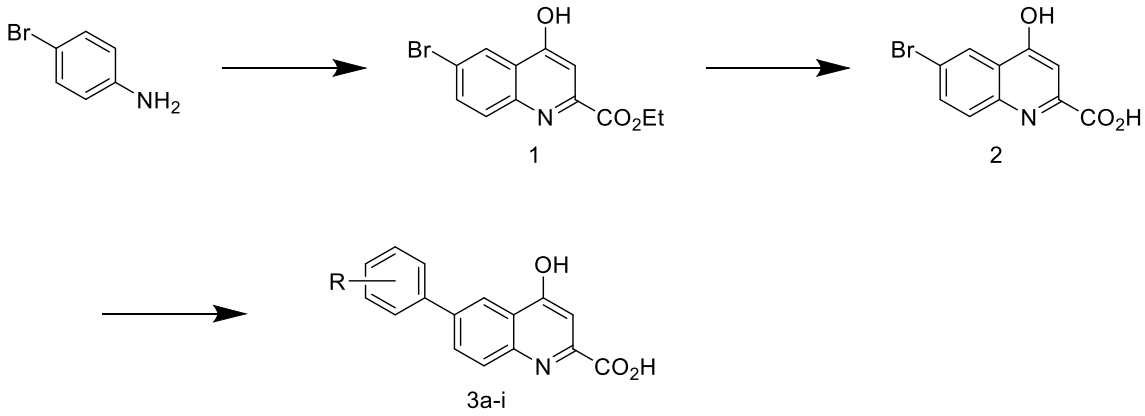
a Milli-Q Labo system (Nihon Millipore Co. Ltd., Tokyo, Japan).

S. 2 Synthesis and identification

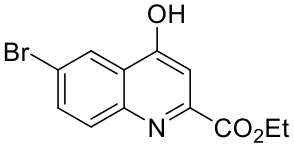
NMR spectra were recorded on a JMS-ECS 400 spectrometer (JEOL Ltd., Tokyo, Japan). The chemical shift (ppm) was referenced using the residual solvent peak.[1] Mass spectra were recorded using a time-of-flight mass spectrometer (JMS-100LP "AccuTOF LC-plus", JEOL Ltd.) equipped with an ESI ion source. Melting points (m.p.) were measured using a MEL-TEMP II® capillary melting point apparatus (Laboratory Devices, Holliston, MA, USA). Overviews of the compound synthesis are shown in **Schemes S1** and **S2**.

S. 2.1 Phenyl-substituted kynurenic acid (KYNA) derivatives

Scheme S1 Synthetic route for the phenyl-substituted KYNA fluorophores.



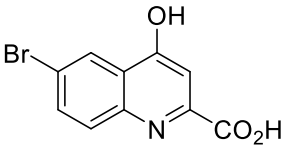
Ethyl 6-bromokynurenate (1)



Diethyl acetylenedicarboxylate (40.1 mmol) was slowly added to a solution of 4-bromoaniline (39.7 mmol) dissolved in MeOH (100 mL). The mixture was stirred at 65 °C for 0.5 h. The solvent was then evaporated under reduced pressure. The residue was redissolved in Ph_2O (100 mL) and heated at 250 °C for 1 h. The solution was cooled to room temperature (r.t., approximately 25 °C) and diluted with hexane (200 mL) to form a precipitate. The precipitate was filtered, washed with hexane, and recrystallised from EtOH to afford **1** as a grey crystalline solid (53% yield). *m/z* 295.99402 (calcd for [M+H] 295.99223); m.p. 246–251 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 12.21 (s, 1H, O*H*), 8.14 (d, J = 2.1 Hz, 1H, Ar*H*), 7.90–7.83 (m,

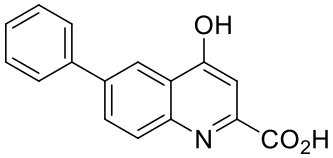
2H, Ar*H*), 6.68 (s, 1H, Ar*H*), 4.41 (q, J = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 1.35 (t, J = 7.1 Hz, 3H, OCH$_2$C*H*$_3$); ^{13}C-NMR (101 MHz, DMSO-D6) δ 176.3, 161.9, 138.9, 138.1, 135.3, 127.2, 126.8, 122.1, 116.9, 110.4, 62.7, 13.9.

6-Bromokynurenic acid (2, Br-KYNA):



Compound **1** (10.0 mmol) and KOH (45.0 mmol) were dissolved in 50% H_2O/EtOH (40 mL), and the mixture was stirred at r.t. for 2 h. The solution was acidified with 2 M HCl to precipitate **2**. The precipitate was filtered, washed with H_2O, and dried under reduced pressure to furnish a pale orange solid (89% yield). *m*/*z* 267.96456 (calcd for [M+H] 267.96093); m.p. 283–286 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 12.16 (s, 1H, O*H*), 8.14 (d, J = 2.3 Hz, 1H, Ar*H*), 7.91 (d, J = 8.9 Hz, 1H, Ar*H*), 7.86–7.83 (m, 1H, Ar*H*), 6.65 (s, 1H, Ar*H*); ^{13}C-NMR (101 MHz, 5% NaOD-D2O) δ 174.9, 172.8, 156.7, 147.4, 132.5, 129.4, 127.4, 125.4, 116.9, 107.1.

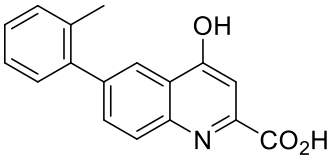
6-Phenylkynurenic acid (3a, Ph-KYNA):



Br-KYNA (1.02 mmol), phenylboronic acid (1.14 mmol), and K_2CO_3 (2.05 mmol) were dissolved in H_2O (50 mL). A catalytic amount (~5 mg) of $Pd(OAc)_2$ was added to the solution followed by stirring for 3 h. The solution was diluted with 1 M HCl. The precipitate was filtered, washed with

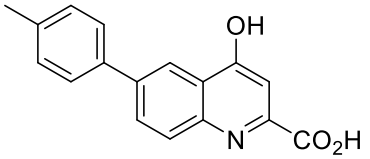
H_2O, and dried under reduced pressure to afford a white solid (96% yield). *m*/*z* 266.07989 (calcd for [M+H] 266.08172); m.p. 271–273 °C; ^{1}H-NMR (400 MHz, 5% NaOD-D2O) δ 7.98 (s, 1H, Ar*H*), 7.48 (s, 2H, Ar*H*), 7.32 (d, J = 7.3 Hz, 2H, Ar*H*), 7.07 (t, J = 7.7 Hz, 2H, Ar*H*), 6.97 (t, J = 7.3 Hz, 1H, Ar*H*), 6.53 (s, 1H, Ar*H*); ^{13}C-NMR (101 MHz, 5% NaOD-D2O) δ 175.0, 174.0, 156.4, 148.2, 140.0, 135.5, 129.1, 128.6, 128.1, 127.5, 126.8, 126.3, 120.7, 106.9.

6-(2-Methylphenyl)kynurenic acid (3b, 2-MePh-KYNA):



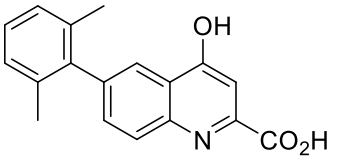
Br-KYNA (2.00 mmol), 2-methylphenylboronic acid (2.19 mmol), and K_2CO_3 (10.6 mmol) were dissolved in H_2O (50 mL). $Pd(OAc)_2$ (10.3 μmol) was added to this solution, which was then refluxed for 1 h. The solution was diluted with 1 M HCl. The precipitate was filtered, washed with H_2O, and dried under reduced pressure to afford a white solid (85% yield). *m*/*z* 280.09846 (calcd for [M+H] 280.09737); m.p. 255–259 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 12.06 (s, 1H, O*H*), 8.01 (d, J = 8.7 Hz, 1H, Ar*H*), 7.96 (d, J = 2.1 Hz, 1H, Ar*H*), 7.71 (dd, J = 8.7, 2.3 Hz, 1H, Ar*H*), 7.32–7.23 (m, 4H, Ar*H*), 6.66 (s, 1H, Ar*H*), 2.23 (s, 3H, C*H*$_3$); ^{13}C-NMR (101 MHz, DMSO-D6) δ 177.6, 163.8, 140.4, 139.8, 139.0, 136.8, 134.8, 133.5, 130.5, 129.7, 127.6, 126.1, 125.6, 124.3, 119.6, 109.8, 20.2.

6-(4-Methylphenyl)kynurenic acid (3c, 4-MePh-KYNA):



Br-KYNA (1.00 mmol), 4-methylphenylboronic acid (1.32 mmol), and K_2CO_3 (2.34 mmol) were dissolved in H_2O (50 mL). A catalytic amount (~5 mg) of $Pd(OAc)_2$ was added to this solution, which was then stirred for 1 h. The solution was diluted with 1 M HCl. The precipitate was filtered, washed with H_2O, and dried under reduced pressure to furnish a white solid (quant.). *m/z* 280.09906 (calcd for [M+H] 280.09737); m.p. 274–278 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 12.03 (s, 1H, O*H*), 8.24 (t, J = 1.1 Hz, 1H, Ar*H*), 7.99 (s, 2H, Ar*H*), 7.61 (d, J = 8.2 Hz, 2H, Ar*H*), 7.27 (d, J = 8.2 Hz, 2H, Ar*H*), 6.62 (s, 1H, Ar*H*), 2.32 (s, 3H, CH_3); ^{13}C-NMR (101 MHz, 10% METHANOL-D4/PYRIDINE-D5) δ 179.8, 165.5, 148.9, 142.1, 139.9, 137.4, 131.4, 130.0, 127.0, 127.0, 124.0, 123.1, 120.0, 110.7, 20.7.

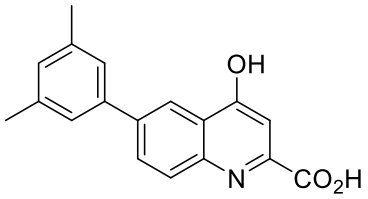
6-(2,6-Dimethylphenyl)kynurenic acid (3d, 2,6-diMePh-KYNA):



Br-KYNA (2.11 mmol), 2,6-dimethylphenylboronic acid (2.25 mmol), and K_2CO_3 (10.4 mmol) were dissolved in 50% H_2O/DME (20 mL). $Pd(OAc)_2$ (0.243 mmol) and DavePhos (0.166 mmol) were added to the solution, which was then refluxed under an argon atmosphere for 40 h. The product was purified by reversed-phase chromatography (ODS, 50% MeOH/0.1 M phosphate buffer, pH 7.5). The target fraction was concentrated and

acidified with HCl to precipitate **3d**. The precipitate was filtered, washed with H_2O, and dried under reduced pressure to afford a white solid (62% yield). *m*/*z* 294.11436 (calcd for [M+H] 294.11302); m.p. 257–260 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 12.06 (s, 1H, O*H*), 8.04 (d, J = 8.5 Hz, 1H, Ar*H*), 7.78 (d, J = 2.1 Hz, 1H, Ar*H*), 7.49 (dd, J = 8.5, 2.1 Hz, 1H, Ar*H*), 7.18–7.10 (m, 3H, Ar*H*), 6.68 (s, 1H, Ar*H*), 1.94 (s, 6H, CH_3); ^{13}C-NMR (101 MHz, DMSO-D6) δ 177.5, 163.8, 140.4, 139.2, 139.0, 136.1, 135.4, 133.7, 127.4, 127.3, 125.8, 124.3, 120.2, 109.9, 20.6.

6-(3,5-Dimethylphenyl)kynurenic acid (3e, 3,5-diMePh-KYNA):

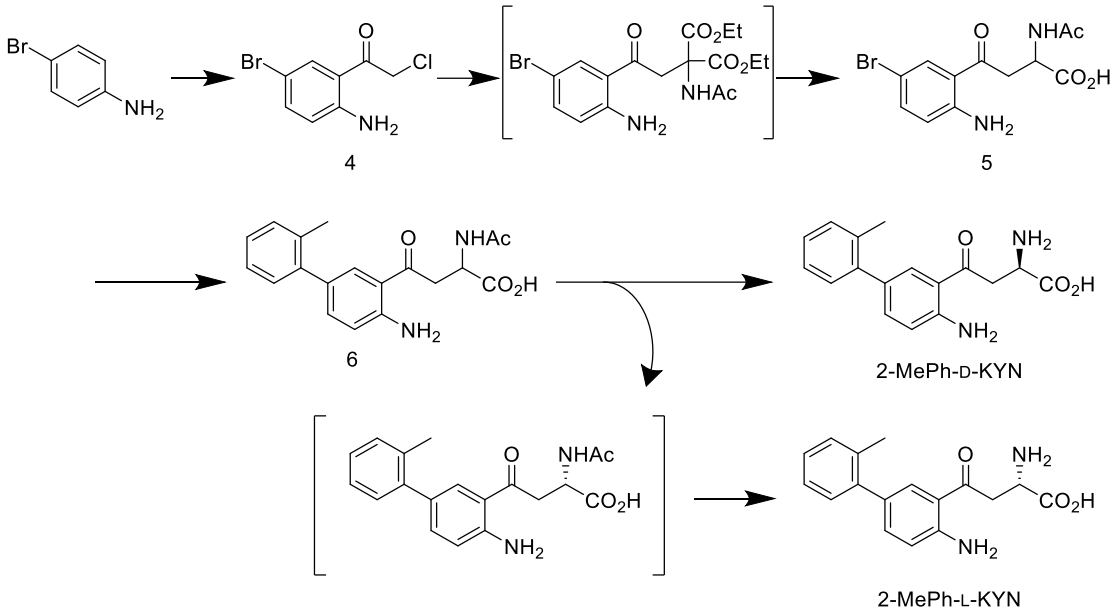


Br-KYNA (0.996 mmol), 3,5-dimethylphenylboronic acid (1.04 mmol), and K_2CO_3 (2.76 mmol) were dissolved in H_2O (50 mL). A catalytic amount (~5 mg) of $Pd(OAc)_2$ was added to the solution, which was refluxed for 1 h. The solution was diluted with 1 M HCl. The precipitate was filtered, washed with H_2O, and dried under reduced pressure to afford a pale-yellow solid (89% yield). *m*/*z* 294.11384 (calcd for [M+H] 294.11302); m.p. 273–275 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 12.06 (s, 1H, O*H*), 8.27 (t, J = 1.4 Hz, 1H, Ar*H*), 8.01 (d, J = 1.4 Hz, 2H, Ar*H*), 7.33 (s, 2H, Ar*H*), 7.01 (s, 1H, Ar*H*), 6.65 (s, 1H, Ar*H*), 2.35 (s, 6H, CH_3); ^{13}C-NMR (101 MHz, 10% METHAOL-D4/PYRIDINE-D5) δ 167.8, 153.1, 130.8, 127.6, 127.4, 126.2, 124.6, 119.3, 117.0, 114.2, 112.6, 110.8, 107.5,

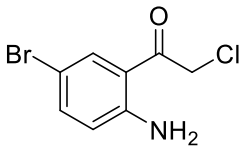
97.8, 8.6.

S. 2.2 2-MePh-D, L-kynurenine (KYN)

Scheme S2 Synthetic route for 2-MePh-D-KYN and 2-MePh-L-KYN.



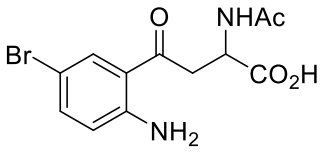
1-(2-Amino-5-bromophenyl)-2-chloroethan-1-one (4):



Boron trichloride (*ca.* 1 M, in dichloromethane, 72 mL), $AlCl_3$ (75.1 mmol), and chloroacetonitrile (5 mL) were added to a solution of 4-bromoaniline (66.3 mmol) in toluene (72 mL) at r.t. The mixture was refluxed under an argon atmosphere for 16 h. Then, 0.5 M HCl (100 mL) was added to quench the reaction, and the solution was cooled to r.t. The target compound was extracted in $CHCl_3$ (200 mL ×3). The organic layer was then dried (Na_2SO_4), filtered, and

evaporated to dryness. Toluene (50 mL) and hexane (200 mL) were added to solidify the residue. The supernatant was discarded and the residue was dried under reduced pressure to afford **4** as a brown solid (14% yield). *m*/*z* 247.95136 (calcd for [M+H] 247.94778); m.p. 129–132 °C; ^{1}H-NMR (400 MHz, CHLOROFORM-D) δ 7.72 (d, J = 2.3 Hz, 1H, Ar*H*), 7.36 (dd, J = 8.8, 2.2 Hz, 1H, Ar*H*), 6.60 (d, J = 8.9 Hz, 1H, Ar*H*), 6.35 (s, 2H, NH_2), 4.64 (s, 2H, CH_2); ^{13}C-NMR (101 MHz, CHLOROFORM-D) δ 191.6, 149.9, 137.9, 132.7, 119.3, 116.2, 106.8, 46.3.

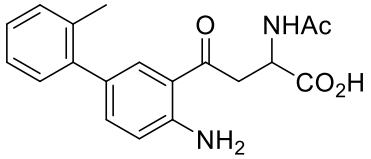
2-Acetamido-4-(2-amino-5-bromophenyl)-4-oxobutanoic acid (5):



Compound **4** (37.1 mmol), diethyl acetamidomalonate (38.0 mmol), NaOEt (77.6 mmol), NaI (3.96 mmol), and DBU (3 mL) were dissolved in EtOH (100 mL). The solution was refluxed for 3 h and transferred to a separating funnel. Saturated $NaHCO_3$ (100 mL) was then added and the product was extracted with $CHCl_3$ (100 mL×3). The organic layer was dried with Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was partially purified by chromatography (silica gel, 50% AcOEt/hexanes) to afford a crude α-substituted diethyl acetamidomalonate intermediate. The intermediate and NaOH (103 mmol) were dissolved in H_2O (100 mL). The mixture was refluxed for 30 min, AcOH (10 mL) was added to it, and the resultant mixture was refluxed for 1.5 h. The mixture was transferred

to a separating funnel and extracted with AcOEt (100 mL×4). The organic layer was dried with Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was partially purified using chromatography (ODS, AcOH/H_2O/MeOH [0.1:50:50]) and recrystallised in H_2O to furnish compound **5** (9.5% yield). *m/z* 329.01680 (calcd for [M+H] 329.01369); m.p. 177–180 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 12.59 (s, 1H, CO_2H), 8.11 (d, J = 8.0 Hz, 1H, Ar*H*), 7.84 (d, J = 2.3 Hz, 1H, Ar*H*), 7.36 (dd, J = 8.9, 2.3 Hz, 1H, Ar*H*), 7.32 (s, 2H, NH_2), 6.75 (d, J = 8.9 Hz, 1H, N*H*Ac), 4.70–4.65 (m, 1H, α-CH_2), 3.35-3.33 (m, 2H, β-CH_2), 1.81 (s, 3H, Ac); ^{13}C-NMR (101 MHz, DMSO-D6) δ 197.5, 173.1, 169.1, 150.2, 136.8, 132.9, 119.4, 117.5, 104.6, 48.0, 40.3, 22.4.

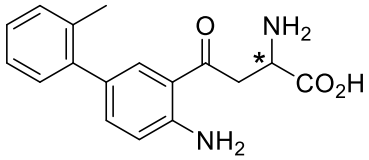
2-Acetamido-4-(4-amino-2′-methyl-[1,1′-biphenyl]-3-yl)-4-oxobutanoic acid (6): Compound **5** (2.09 mmol), 2-methylphenylboronic acid (2.24 mmol), and K_2CO_3 (4.20 mmol) were dissolved in



50% H_2O/MeOH (20 mL). A catalytic amount (~5 mg) of $Pd(OAc)_2$ was added to the solution. The mixture was stirred at r.t. for 1 h and refluxed for another 2 h. The remaining solution was acidified with HCl and passed through an ODS chromatography column (50% H_2O/MeOH as mobile phase). The target fraction was concentrated to afford **6** as a white solid (59% yield). *m/z* 341.14693 (calcd for [M+H] 341.15013); m.p. 177–180 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 12.55 (s, 1H, CO_2H), 8.10 (d, J = 7.8 Hz, 1H, AcN*H*),

7.64 (d, J = 2.1 Hz, 1H, Ar*H*), 7.29–7.24 (m, 4H, Ar*H*)), 7.23–7.21 (m, 2H, NH_2), 7.21–7.18 (m, 1H, Ar*H*), 6.84 (d, J = 8.7 Hz, 1H, N*H*Ac), 4.74–4.69 (m, 1H, α-C*H*), 3.43–3.33 (m, 2H, β-CH_2), 2.26 (s, 3H, PhCH_3), 1.81 (s, 3H, Ac); ^{13}C-NMR (101 MHz, DMSO-D6) δ 198.3, 173.2, 169.1, 150.1, 140.8, 135.2, 134.8, 131.2, 130.3, 129.5, 127.3, 126.8, 126.0, 116.9, 115.9, 48.0, 40.3, 22.4, 20.4.

5-(2-Methylphenyl)-D, L-kynurenine (2-MePh-D, L-KYN):



Compound **6** (1.11 mmol), D-aminoacylase (105 mg), and $CoCl_2 \cdot 6H_2O$ (12.7 mg) were dissolved in 50 mL phosphate buffer (0.1 M, pH 8.4). The solution was stirred at 37 °C for 185 h. The product was separated via chromatography (ODS, AcOH/H_2O/MeOH, 0.1:50:50), where fractions containing 2-MePh-D-KYN and the L-isomer of **6** were obtained. 2-MePh-D-KYN was concentrated and recrystallised with water to furnish pure 2-MePh-D-KYN as a pale-yellow solid (45% yield). The L-isomer of **6** was concentrated to dryness and dissolved in 50 mL phosphate buffer (0.1 M, pH 8.4). Acylase I (101 mg) and $CoCl_2 \cdot 6H_2O$ (12.1 mg) were added to it, and it was stirred at 45 °C for 18 h. The resultant 2-MePh-L-KYN was purified in the same manner as the D-form (51% yield). [**2-MePh-D-KYN**] *m/z* 299.13497 (calcd for [M+H] 299.13957); m.p. 214–216 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 7.60 (d, J = 2.1 Hz, 1H, Ar*H*), 7.28–7.19 (m, 7H, Ar*H* and NH_2),

6.84 (d, J = 8.7 Hz, 1H, Ar*H*), 3.63 (dd, J = 8.6, 3.5 Hz, 1H, α-C*H*), 3.52 (dd, J = 17.9, 3.7 Hz, 1H, β-CH_2), 3.29 (dd, J = 17.9, 8.5 Hz, 1H, β-CH_2), 2.25 (s, 3H, CH_3); ^{13}C-NMR (101 MHz, DMSO-D6) δ 199.3, 169.2, 150.0, 140.7, 135.1, 134.8, 131.0, 130.3, 129.4, 127.3, 126.7, 126.0, 116.8, 116.1, 49.9, 20.3; [**2-MePh-L-KYN**] *m/z* 299.14318 (calcd for [M+H] 299.13957); m.p. 214–215 °C; ^{1}H-NMR (400 MHz, DMSO-D6) δ 7.60 (d, J = 2.1 Hz, 1H, Ar*H*), 7.29–7.17 (m, 7H, Ar*H* and NH_2), 6.84 (d, J = 8.5 Hz, 1H, Ar*H*), 3.61 (dd, J = 8.6, 3.5 Hz, 1H, α-C*H*), 3.52 (dd, J = 17.7, 3.5 Hz, 1H, β-CH_2), 3.27 (q, J = 8.8 Hz, 1H, β-CH_2), 2.25 (s, 3H, CH_3); ^{13}C-NMR (101 MHz, DMSO-D6) δ 199.4, 169.1, 150.0, 140.7, 135.1, 134.8, 131.0, 130.3, 129.4, 127.3, 126.7, 126.0, 116.8, 116.1, 49.9, 20.3.

S. 3 Supplementary experiments and results

S. 3.1 Excitation, fluorescence, and UV-Vis absorption spectra

The samples were prepared by dissolving KYNA derivatives in PBS solution at 10 μM. Excitation and fluorescence spectra were plotted with dashed and solid lines, respectively.

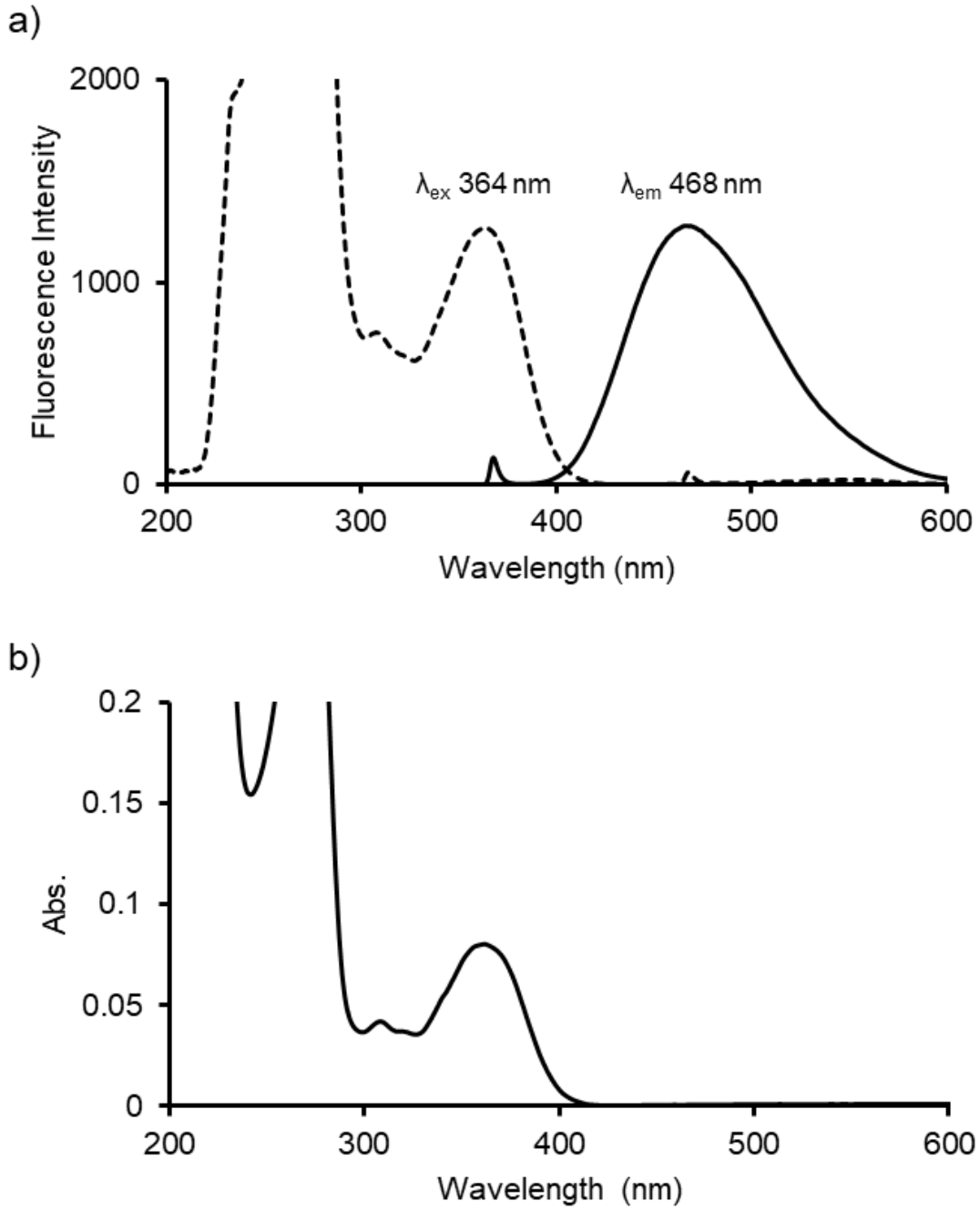


Fig. S1 a) Excitation (dashed line) and fluorescence (solid line) spectra and b) UV-Vis absorption spectrum of MeS-KYNA.

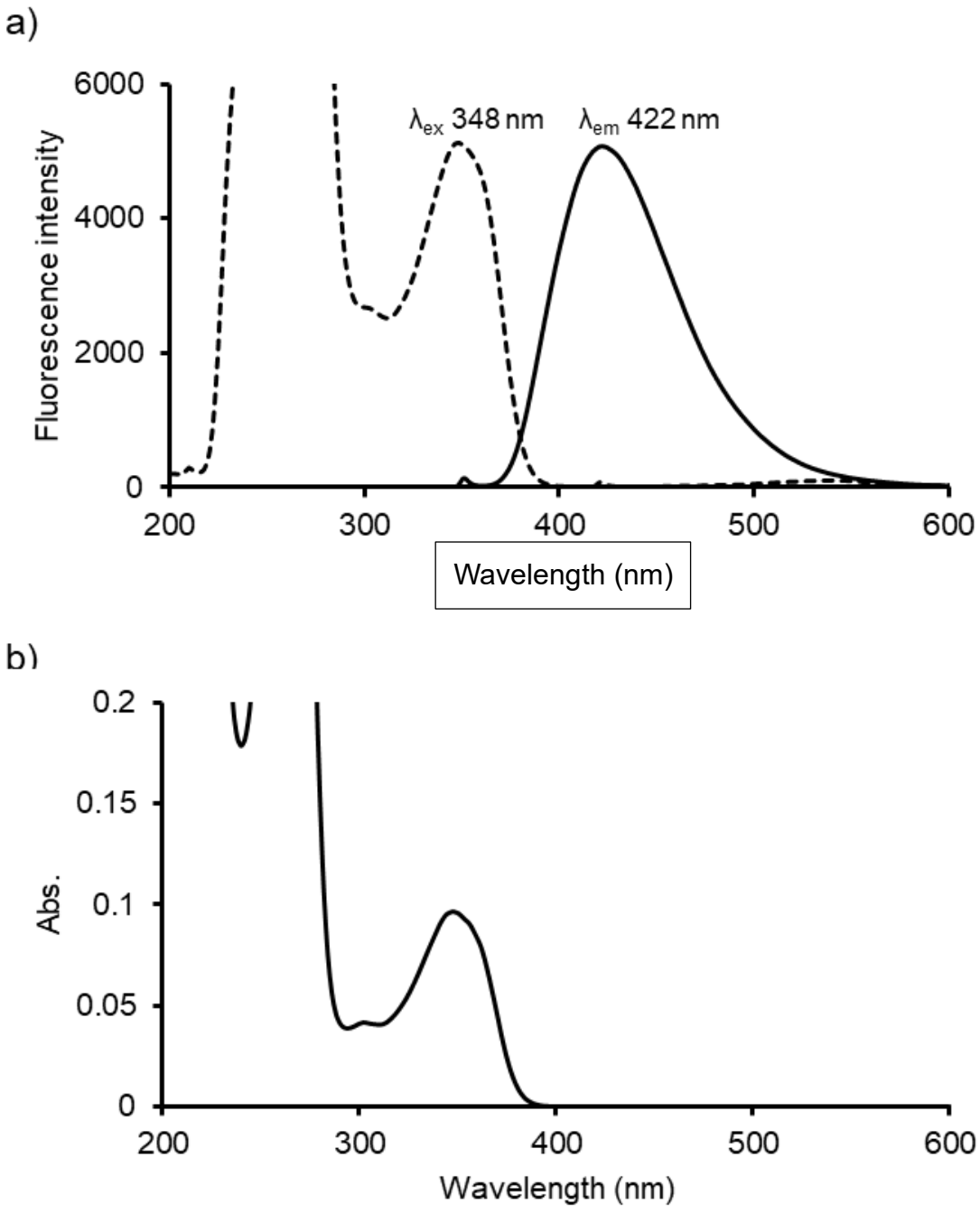


Fig. S2 a) Excitation (dashed line) and fluorescence (solid line) spectra and b) UV-Vis absorption spectrum of Ph-KYNA.

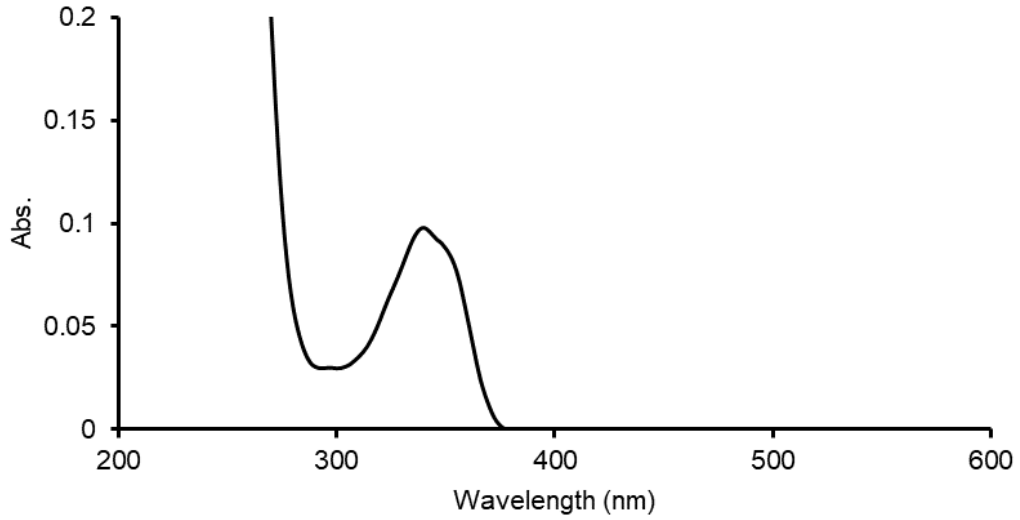


Fig. S3 UV-Vis absorption spectrum of 2-MePh-KYNA. The excitation and fluorescence spectra are shown in Fig. 3 in the main text.

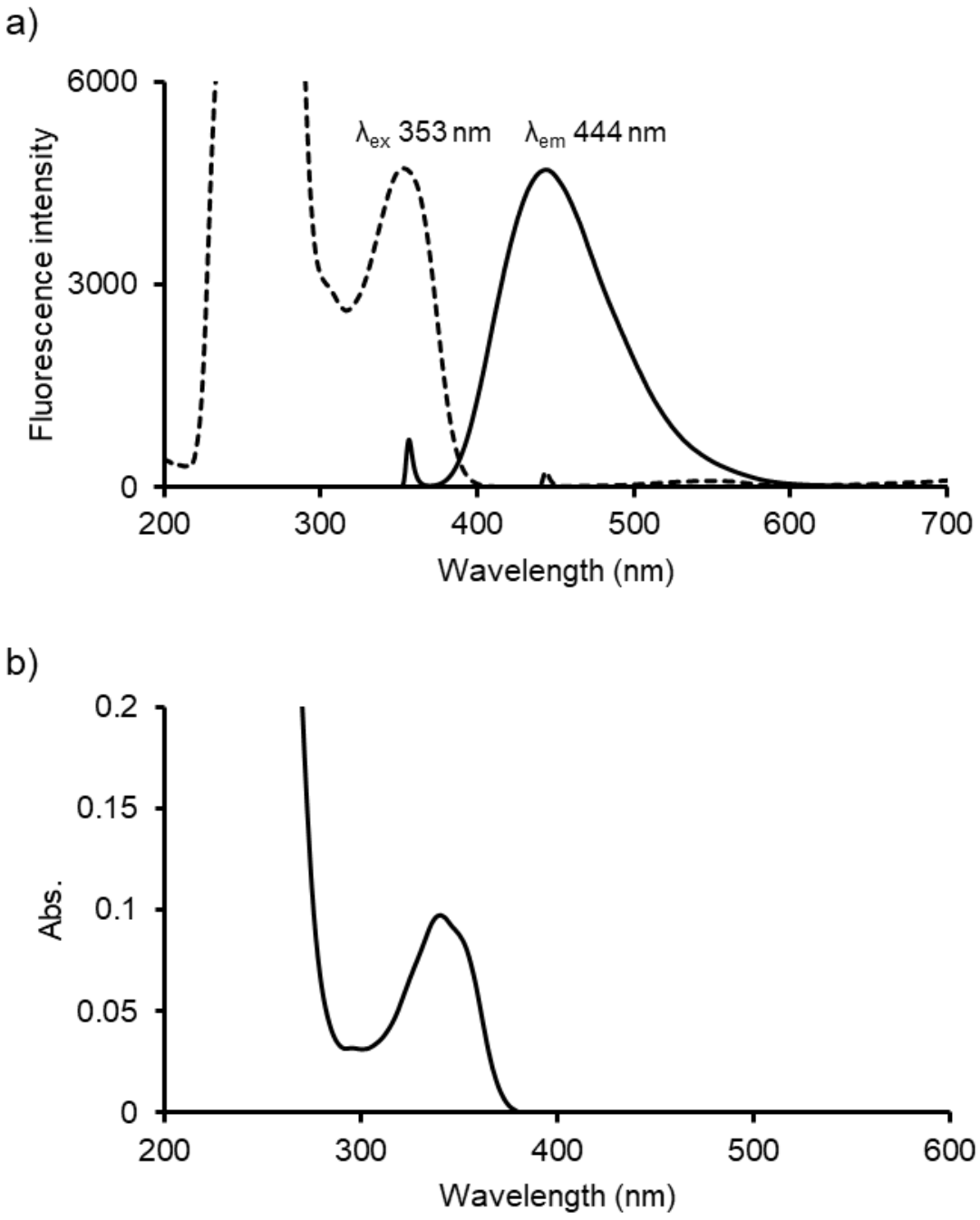


Fig. S4 a) Excitation (dashed line) and fluorescence (solid line) spectra and b) UV-Vis absorption spectrum of 4-MePh-KYNA.

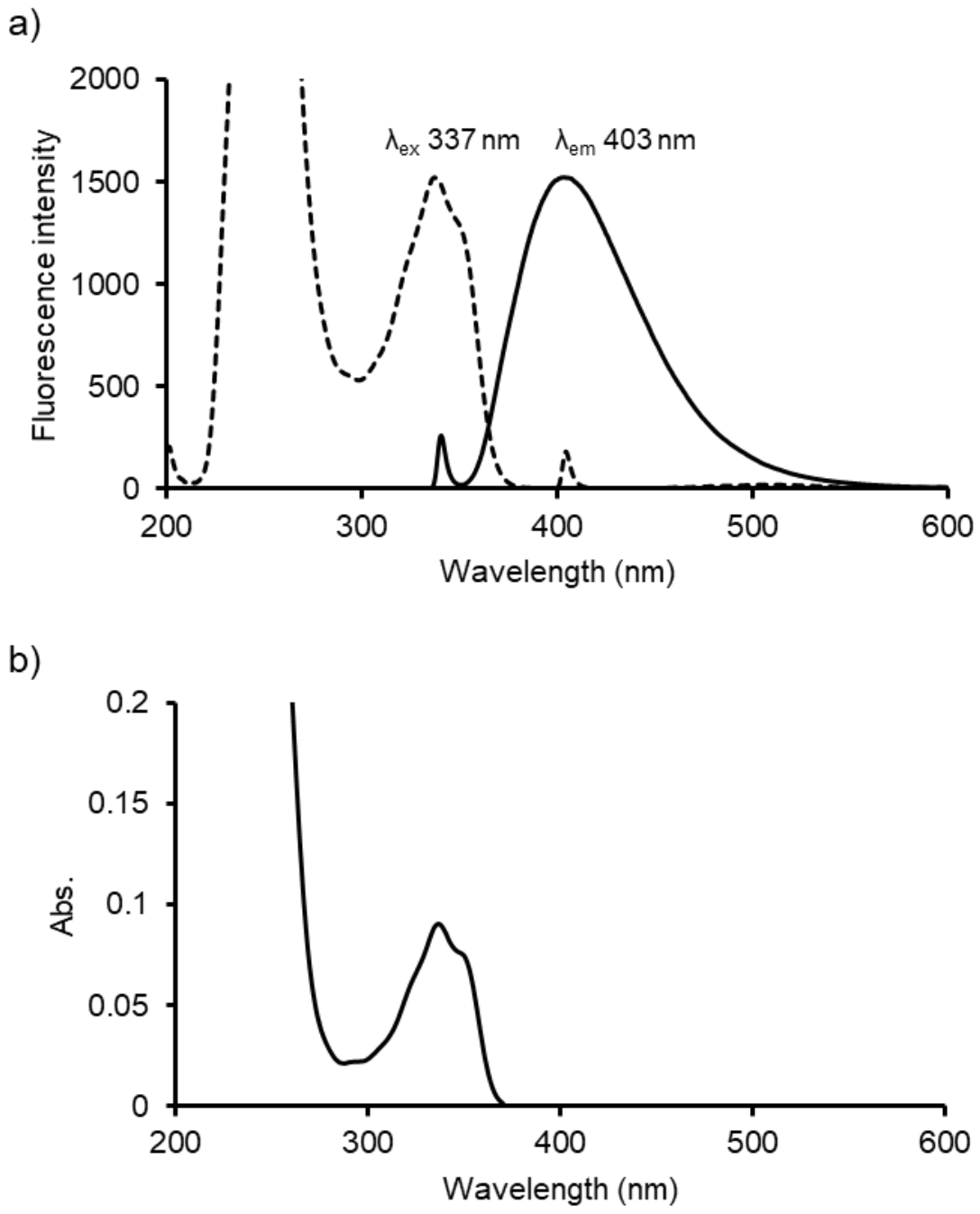


Fig. S5 a) Excitation (dashed line) and fluorescence (solid line) spectra and b) UV-Vis absorption spectrum of 2,6-diMePh-KYNA.

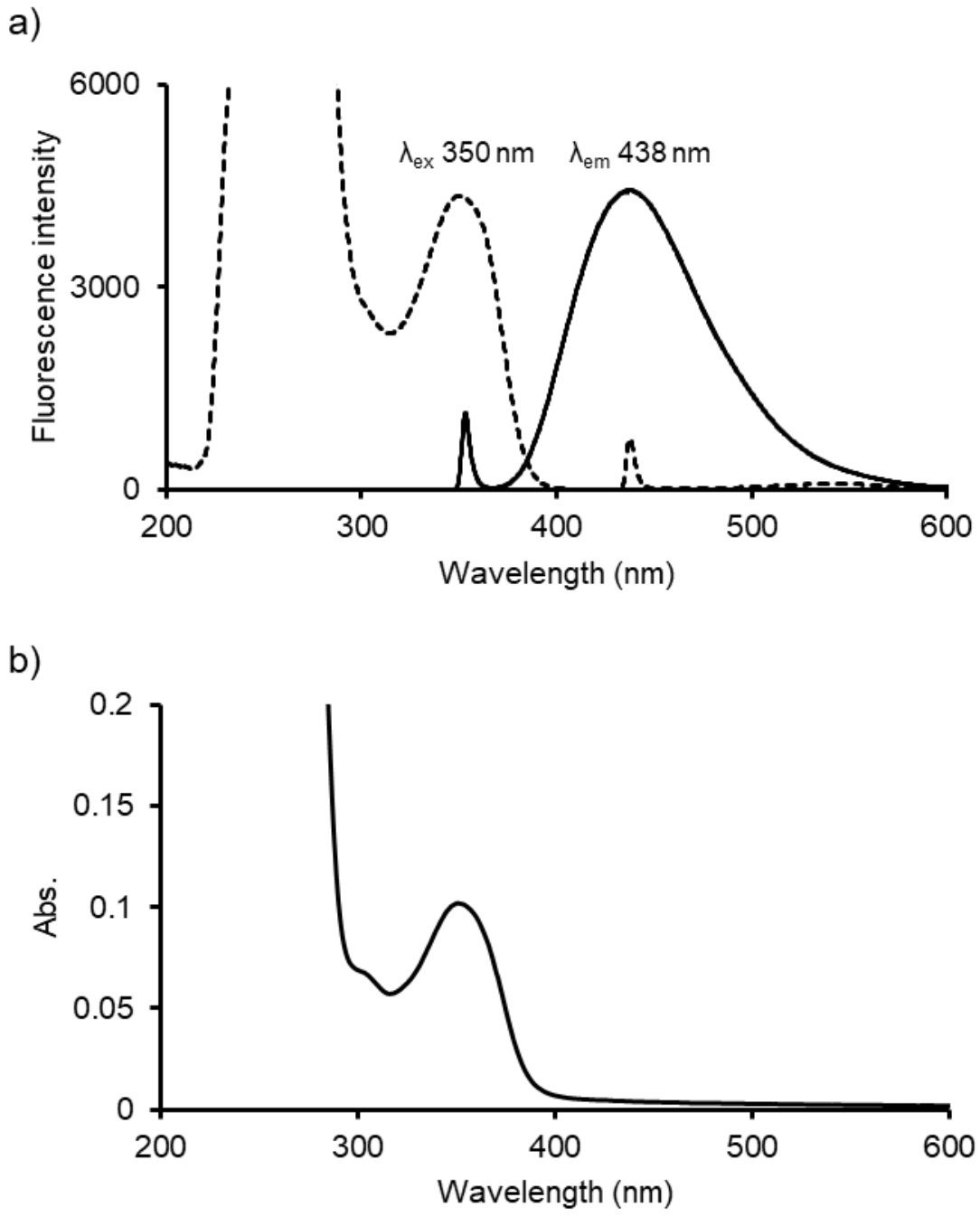


Fig. S6 a) Excitation (dashed line) and fluorescence (solid line) spectra and b) UV-Vis absorption spectrum of 3,5-diMePh-KYNA.

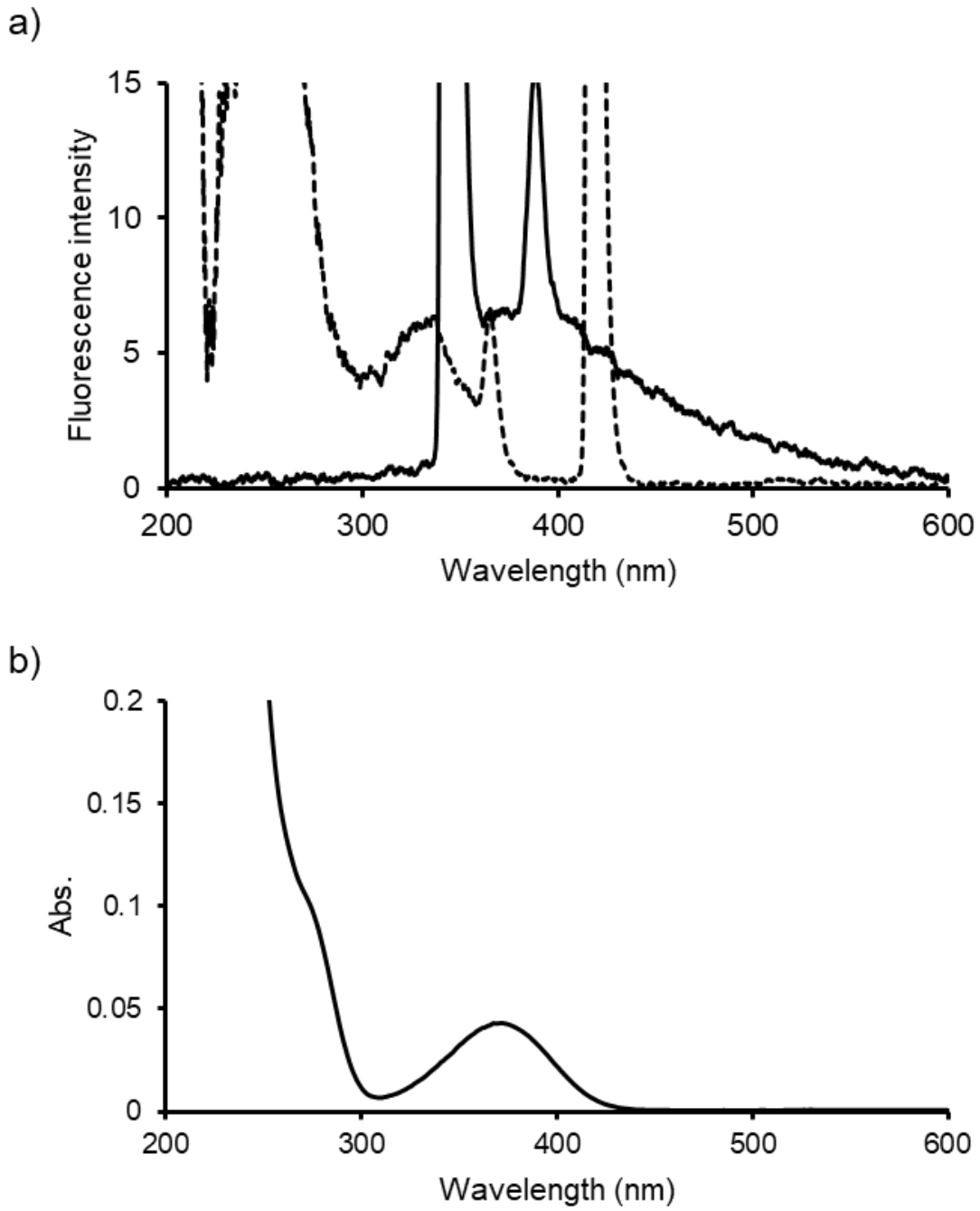


Fig. S7 a) Excitation (dashed line) and fluorescence (solid line) spectra and b) UV-Vis absorption spectrum of 2-MePh-D-KYN.

S. 3.2 pH-dependence of 2-MePh-KYNA fluorescence

To test the pH-dependence of fluorescence, 10 µL of each 2-MePh-KYNA solution (in DMSO, 10 mM) was diluted to 10 µM using a 40 mM Britton-Robinson buffer (pH was adjusted at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12). The fluorescence intensities (ex. 340 and em. 418 nm) were recorded at different pH values using an F-7000 spectrofluorometer.

As shown in Fig. S8, a decrease in fluorescence was observed at pH 4–5 and 10–11. The fluorescence intensity was maintained at its maximum at a pH range of 5–10, including the physiological pH (around 7).

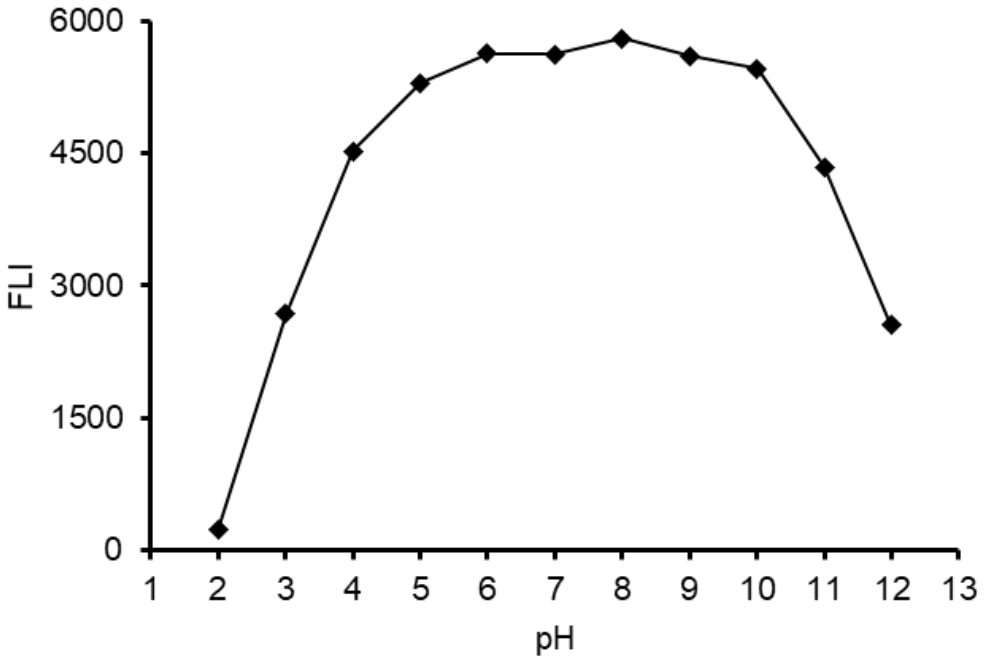


Fig. S8 Fluorescence intensity of 2-MePh-KYNA (mean values in duplicate) at different pH values.

S. 3.3 Determination of enzyme kinetic parameters using HPLC-UV

A HPLC-UV system was used equipped with two LC-10ADVP pumps, a CTO-10ASVP column oven, an SPD-20A UV-vis detector (Shimadzu Corp., Kyoto, Japan), a GL-7420 autosampler (GL Sciences Inc., Tokyo, Japan), and a Cadenza CD-C18 (4.6×150 mm, 3 μm) separation column (Imtakt Corp., Kyoto, Japan).

The mobile phases A and B were 0.05% H_3PO_4 and CH_3CN, respectively. The mobile phase gradient was as follows: B: 3% (0–2 min), 3–100% (2–15 min), 100% (15–20 min), and initialization for the next analysis (20–30 min).

The flow rate, column temperature, injection volume, and detection wavelength were 1.0 mL/min, 40 °C, 1.0 μL, and 254 nm, respectively.

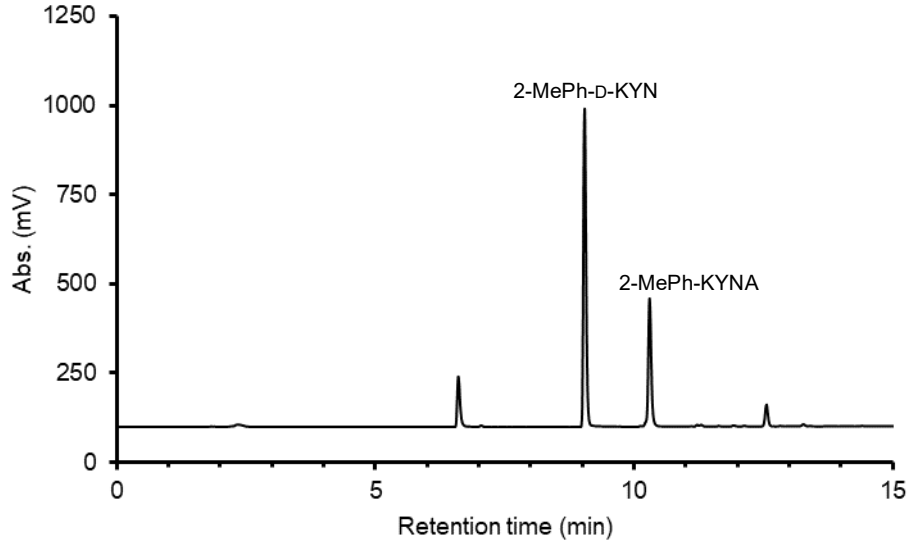


Fig. S9 Chromatogram after the enzymatic reaction with DAO (50 μU DAO + 2000 μM 2-MePh-D-KYN, 37 °C, 1 h).

S. 3.4 Certification of the enantiopurity of 2-MePh-D, L-KYN

An HPLC equipped with a chiral column was used to certify the enantiopurity of 2-MePh-D-KYN and 2-MePh-L-KYN. The following equipment and settings were used: Pump: PU-4180 (JASCO Corp.); column oven: CO-2065PLUS (JASCO Corp.); detector: L-400H (Hitachi High-Tech Corp., Tokyo, Japan); autosampler: AS-4050i (JASCO Corp.); column: CHIRALPAK® ZWIX (+) (4 × 250 mm, 3 μm); column temperature: Room temperature; injection volume: 10 μL; detection: UV 254 nm; flow rate: 0.5 mL/min; mobile phase: 40 mM formic acid and 20 mM diethylamine in [MeOH/H_2O (4/1)].

S. 3.5 Amino acid determination

The amino acid levels in the eel plasma were determined using LC-MS/MS after diastereomeric derivatisation using (*R*)-CIMA-OSu according to a previously reported method.[2,3] The samples for analysis were prepared based on a previously reported method.[3]

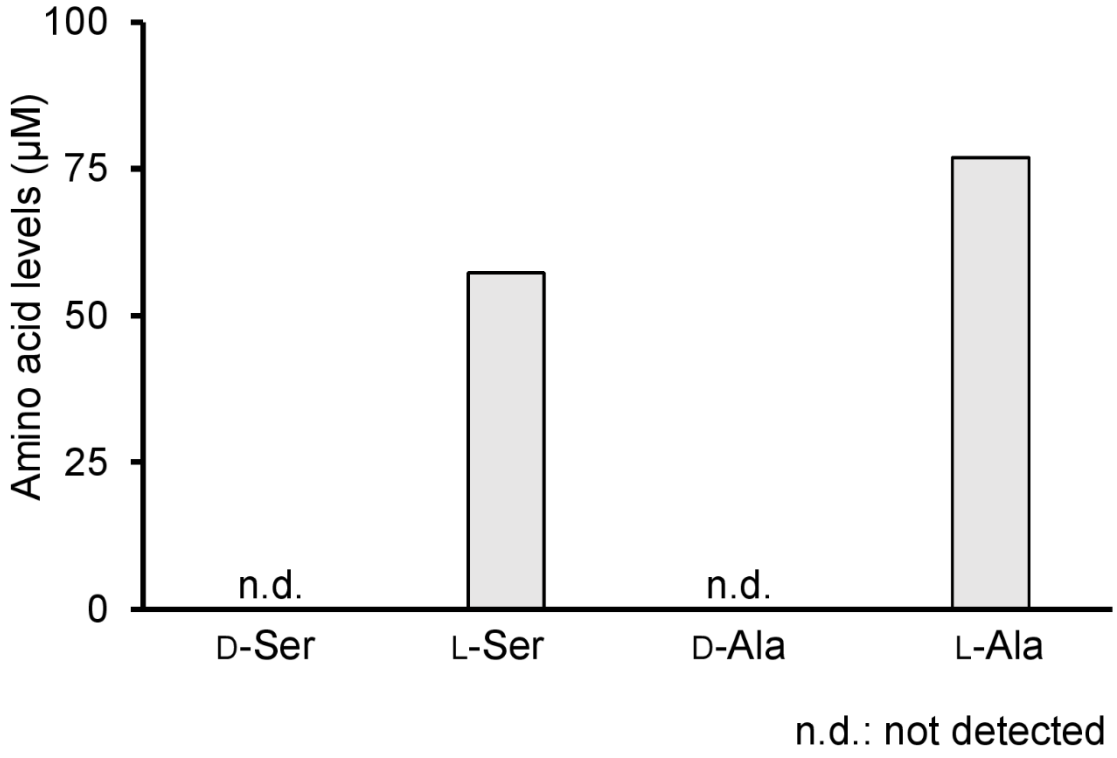


Fig. S10 Eel plasma levels of D, L-Ser and D, L-Ala (mean values in duplicate).

S. 4 Supplementary Tables

Table S1 Tested solvents and their physical properties

Solvents	Abbreviation	Viscosity (mPa·s)	Dielectric constant	pK_a	Protic/Aprotic
methanol	MeOH	0.54[4]	33[5]	15.54[6]	protic
ethanol	EtOH	1.08[4]	24[5]	16[6]	protic
ethylene glycol	EG	16.1[6]	37.7[5]	14.77[6]	protic
glycerine	Glyc	934[7]	42.5[5]	14.4[8]	protic
ethyl acetate	AcOEt	0.4[9]	6[5]	-	aprotic
tetrahydrofuran	THF	0.44[9]	7.5[5]	-	aprotic
2-propanol	IPA	2.04[4]	19[5]	16.5[10]	protic
dimethyl sulfoxide	DMSO	1.99[7]	46.7[5]	-	aprotic
water	H_2O	0.89[7]	80.1[5]	14[10]	protic
acetonitrile	CH_3CN	0.34[5]	37.5[5]	-	aprotic
2,2,2-trifluoroethanol	TFE	1.73[11]	8.55[12]	12.43[13]	protic

Table S2 Maximum excitation and emission wavelengths (λ_{ex} and λ_{em}) and FLI of 2-MePh-KYNA in each solvent

	Solvents	λ_{ex} (nm)	λ_{em} (nm)	FLI
1	MeOH	348	410	301
2	EtOH	350	391	376
3	EG	350	403	1161
4	Glyc	341	410	2024
5	IPA	350	417	275.1
6	H_2O	341	416	4943
7	TFE	340	419	5503
8	AcOEt	302	372	75
9	THF	303	373	36.3
10	DMSO	300	365	22.5
11	CH_3CN	307	386	88

S. 5 References

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