Electronic Supplementary Information (ESI)

Direct assay of butyrylcholinesterase activity using a fluorescent substrate

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Stability test of 1 in buffer condition

Fluorescence spectra of 1 (50 μM) was recorded under different pH conditions (pH = 7.0, 7.4, 8.0) using Tris buffer (20 mM) for 1 h to find the appropriate conditions for enzymatic assay.

In all experiments, the final sample volume was 1 mL and all measurements were performed in 1 cm quartz cells at 25 °C with excitation at 355 nm.

Figure S1. Stability test of 1 (50 μM, Tris buffer 20 mM) in different pH condition (A) pH 7.0, (B) pH 7.4, (C) pH 8.0. λ<sub>ex</sub> = 355 nm.
Mechanism studies of BChE assay based on 1

BChE (2 U/mL) was then added to the sample containing 1 (100 μM) and Tris buffer (20 mM, pH 7.0) and the mixture was incubated for 90 min. By mass spectroscopy, the fragment formed by the enzymatic hydrolysis of 1 was confirmed.

A sample containing 1 (50 μM) and Tris buffer (20 mM, pH 7.0) in distilled H₂O and another sample containing 3 (50 μM) and Tris buffer (20 mM, pH 7.0) in 1% DMSO were prepared. Then, the fluorescence spectrum of each sample was recorded.

Figure S2. Mass spectrum of 1 (100 μM, Tris buffer pH 7.0 20 mM) containing BChE (2 U/mL) after incubation for 2 hours.

Figure S3. Comparison of fluorescence property of 1 (50 μM, Tris buffer pH 7.0 20 mM) and 3 (50 μM, Tris buffer pH 7.0 20 mM). λ_ex = 355 nm.
Selectivity test of 1 for BChE

BChE and other esterase were used; carboxylesterase from porcine liver (CES, EC 3.1.1.1), lipase from porcine pancreas (EC 3.1.1.3), and phospholipase C from *Clostridium perfringens* (PLC, EC 3.1.4.3). Each of esterase (2 U/mL) was added to the sample containing 1 (50 μM) and Tris buffer (20 mM, pH 7.0), and fluorescence spectra of each sample were recorded for 30 min.

![Fluorescence spectra of 1 (50 μM, Tris buffer 20 mM) with different esterase (2 U/mL), (A) without esterase, (B) BChE, (C) CES, (D) PLC, (E) lipase, (F) Normalized fluorescence intensities at 520 nm of 1 versus different esterase (2 U/mL) after 30 min incubation. λ<sub>ex</sub> = 355 nm.](image)

*S4*
Model study of 3,6-dihydroxy phthalimide derivatives

In order to confirm the substitution effect from dimethoxy phthalimide part to dihydroxy phthalimide in compound 1, we synthesized 3,6-dihydroxy phthalimide derivative (S1, S2) for comparison study (Scheme S1).

Scheme S1. Synthesis of 3,6-dihydroxy phthalimide derivatives (S1, S2) for model study.

1) Synthesis of 3,6-dihydroxyphthalic acid (S4) and 4,7-dihydroxyisobenzofuran-1,3-dione (S3).

S4 and S3 was synthesized by following literature procedures. (Phys. Chem. Chem. Phys., 2015, 17, 30659-30669.)

2) Synthesis of benzyl 2-(4,7-dihydroxy-1,3-dioxoisoinolin-2-yl)acetate (S2).

Et$_3$N (0.74 mL, 5.25 mmol) and S3 (0.90 g, 5.00 mmol) were added to a solution of Glycine benzylester hydrochloride (1.06 g, 5.25 mmol) in DMF (6 mL) at 0 °C. After stirring for 30 min, reaction mixture was heated at 70 °C for 14 h. The brown solution was cooled to r.t. and diluted with TDW (50 mL), and extracted with EtOAc (50 mL × 3). The organic phase was washed with TDW and brine and dried with anhydrous Na$_2$SO$_4$. The filtrate was then concentrated and residue as purified by column chromatography (silica gel, CHCl$_3$: MeOH = 10: 1, v/v) to obtain S2 (0.43 g, 26 %) as a pale red powder. $^1$H NMR (400 MHz, DMSO-d$_6$): δ 9.91 (br.s, 2H), 7.40-7.32 (m, 5H), 7.10 (s, 2H), 5.18 (s, 2H), 4.35 (s, 2H) ppm. $^{13}$C NMR
(100 MHz, DMSO-d₆): δ 167.90, 165.59, 148.43, 135.58, 128.51, 128.25, 128.00, 126.32, 113.82, 66.57, 38.34 ppm.

3) Synthesis of 2-(4,7-dihydroxy-1,3-dioxoisindolin-2-yl)acetic acid (S1).

A solution of S2 (0.23 g, 0.7 mmol) in MeOH (15 mL) was hydrogenated at atmospheric pressure for 30 min at room temperature using 10% palladium-carbon (0.14 g) as a catalyst. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to obtain S1 (0.14 g, 84%) as a yellow powder. ¹H NMR (400 MHz, DMSO-d₆): δ 10.31 (br.s, 2H), 7.10 (s, 2H), 4.16 (s, 2H) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ 169.29, 165.78, 148.30, 126.18, 114.01, 38.38 ppm.

4) UV-Vis and Fluorescence spectra of S1 and S2 for various pH.

Figure S5. (a) UV-vis spectra of S2 (50 μM) and (b) S1 (50 μM) for various pH condition (20 mM, DMSO 5%). pH 5–6: citrate buffer, pH 7–9: Tris buffer.
**Figure S6.** Fluorescence spectra of S2 (50 μM) for various pH condition (20 mM, DMSO 5%). pH 5~6: citrate buffer, pH 7~9: Tris buffer. (a) $\lambda_{ex} = 378$ nm, (b) $\lambda_{ex} = 436$ nm

**Figure S7.** Fluorescence spectra of S1 (50 μM) for various pH condition (20 mM, DMSO 5%). pH 5~6: citrate buffer, pH 7~9: Tris buffer. (a) $\lambda_{ex} = 378$ nm, (b) $\lambda_{ex} = 436$ nm
**Figure S8.** Plot of fluorescence intensity of S2 (50 μM) and S1 (50 μM) at 513 nm. pH 5–6: citrate buffer, pH 7–9: Tris buffer. (a) $\lambda_{ex} = 378$ nm, (b) $\lambda_{ex} = 436$ nm

Based on the UV-Vis spectra of S1 and S2, we recorded fluorescence emission spectra of S1 and S2 for two difference excitation wavelength (378 nm, 436 nm). As shown Figure 4(a), in case of $\lambda_{ex} = 378$ nm, fluorescence intensity of S1 and S2 at 513 nm are almost same in various pH condition. And in case of $\lambda_{ex} = 436$ nm, S2 (benzyl ester moiety) was showed more strong fluorescence intensity than S1 (acid moiety). This experimental result is similar with that of dimethoxy moiety that fluorescence on-off phenomena.
Characterization of compounds

Figure S9. $^1$H-NMR spectrum of 6 in CDCl$_3$.
Figure S10. $^1$H-NMR spectrum of 5 in DMSO-d$_6$. 

![NMR spectrum](image)
Figure S11. $^1$H-NMR spectrum of 4 in CDCl$_3$. 

![Chemical structure of 4](image)
Figure S12. $^1$H-NMR spectrum of 3 in DMSO-d$_6$. 

![Chemical Structure](image)
Figure S13. $^1$H-NMR spectrum of 2 in CDCl$_3$.

Figure S14. $^{13}$C-NMR spectrum of 2 in CDCl$_3$. 
Figure S15. ESI-Mass spectrum of 2.

Figure S16. HPLC chromatogram of 2.
Figure S17. $^1$H-NMR spectrum of 1 in DMSO-d$_6$.

Figure S18. $^{13}$C-NMR spectrum of 1 in DMSO-d$_6$. 
Figure S19. ESI-Mass spectrum of 1.

Figure S20. HPLC chromatogram of 1.
Figure S21. $^1$H-NMR spectrum of S2 in DMSO-d$_6$.

Figure S22. $^{13}$C-NMR spectrum of S2 in DMSO-d$_6$. 
Figure S23. $^1$H-NMR spectrum of S1 in DMSO-$d_6$.

Figure S24. $^{13}$C-NMR spectrum of S1 in DMSO-$d_6$. 