Identification of the First Selective Bioluminescent Probe for the Real-Time Monitoring of Carboxylesterase 2 In Vitro and In Vivo

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Synthetic routes

Scheme S1. The syntheses of the designed probes. a) NEt₃, CH₂Cl₂, 98% for 1c, and 61% for 2c; b) K₂CO₃, D-cysteine·HCl, H₂O/MeOH, 36% for CBP 1, 26% for CBP 2 and 85% for CBP 3; c) EDCI, DMAP, CH₂Cl₂, 43%.
Figure S1. The HPLC analyses of CBP 1 with CES2: (A) CBP 1 (10 mM); (B) CBP 1 (10 mM, 0.5 mL) with CES2 (5 µg/mL, 0.5 mL); (C) Luciferin (10 mM); (D) Thiophene-2-carboxylic acid (10 mM).
Figure S2. The HPLC analyses of CBP 2 with CES2: (A) CBP 2 (10 mM); (B) CBP 2 (10 mM, 0.5 mL) with CES2 (5 µg/mL, 0.5 mL); (C) Luciferin (10 mM); (D) Butyric acid (10 mM).
Figure S3. The HPLC analyses of CBP 3 with CES2: (A) CBP 3 (10 mM); (B) CBP 3 (10 mM, 0.5 mL) with CES2 (5 µg/mL, 0.5 mL); (C) Luciferin (10 mM).

Figure S4. The ESI-MS spectrum of CBP 1 (10 mM) upon incubation with CES2 (5 µg/mL).
Figure S5. The influence of pH value on the CES2 detection by CBP 1 (n = 3). Each value was represented as mean ± SEM.

Figure S6. The investigation of the CES2 specificity of CBP 1 by using the cell lysate (n = 3). *** P < 0.001, according to unpaired t test. Each value was represented as mean ± SEM.
Figure S7. The viability of ES-2-Fluc cells after incubating with different concentrations of LPA (n = 3). Each value was represented as mean ± SEM, and the IC$_{50}$ value was fitted by GraphPad Prism 8.
**Table S1.** Representative studies for CES2 detection

<table>
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<th>Chemical structure (Strategy)</th>
<th>λex/em (nm)</th>
<th>Km (μM)</th>
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<td>Value</td>
<td>Concentration (μg/mL)</td>
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| Capillary Electrophoresis | / | / | 0.827 mg/mL | 13 |

*Chemiluminescence*
NMR and HRMS spectra

$^1$H-NMR spectra of compound 1c

$^1$H-NMR spectra of CBP 1
$^{13}$C-NMR spectra of CBP 1

HRMS spectra of CBP 1
$^1$H-NMR spectra of compound 2c

$^1$H-NMR spectra of CBP 2
$^{13}$C-NMR spectra of CBP 2

HRMS spectra of CBP 2
$^1$H-NMR spectra of compound 3c

$^1$H-NMR spectra of CBP 3
$^{13}$C-NMR spectra of CBP 3

$^{19}$F-NMR spectra of CBP 3
HRMS spectra of CBP 3
References