Substrate-competitive activity-based profiling of ester prodrug activating enzymes

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SUPPLEMENTARY INFORMATION
SUPPLYMENTARY METHODS

Materials. FP-PEG-biotin was synthesized following previously described methods¹. All other materials and reagents are from commercial source except where noted.

General synthetic methods. ¹H and ¹³C NMR spectra were obtained on Bruker 300 or Bruker 500 MHz spectrometers with CDCl₃ as solvent, and chemical shifts are reported relative to the residual solvent peak in δ (ppm). Mass spectrometry analysis was performed by using a Waters LCT time-of-flight mass spectrometry instrument. Flash column chromatography was performed with silica gel (220–240 mesh). Thin-layer chromatography (TLC) was performed on silica gel GHLF plates (250 microns) purchased from Analtech. Developed TLC plates were visualized with a UV lamp at 254 nm or by iodine staining. Extraction solutions were dried over MgSO₄ prior to concentration. WWL79 and WWL50 were synthesized as previously described².

Supplementary Scheme S1. Synthetic scheme of WWL79 and WWL50.

WWL79. A mixture of phenol (94.11 mg, 1 mmol), N,N’-disuccinimidyl carbonate (256.17 mg, 1 mmol), triethylamine (140 µL, 10 mmol) and acetonitrile (4 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with ethyl acetate and washed sequentially with 0.5% aqueous HCl and brine. Following drying and concentration of the organic phase,
compound 2 was isolated and used directly without further purification. A mixture of compound 2 (235.05 mg, 1 mmol), 3-morpholinopropylamine (146.1 µL, 1 mmol), triethylamine (140 µL, 10 mmol) and dichloromethane (2 mL) was stirred at room temperature for 8 h. The mixture was distributed between dichloromethane and water, and the organic phase dried over MgSO₄. Concentration provided a residue that was purified by flash silica gel chromatography, eluting with dichloromethane/methanol (98:2). Product fractions were pooled and concentrated to leave pure **WWL79** (211 mg) as a white powder: ¹H NMR (400 MHz, CDCl₃) δ 7.33 (m, 2 H), 7.16-7.09 (m, 3 H), 3.71 (t, J = 7 Hz, 4 H), 3.32 (dd, J = 7 Hz, J = 13 Hz, 2 H), 2.46-2.43 (m, 6 H), 1.74-1.68 (m, 2 H).

**WWL50.** A solution of cyclohexyl isocyanate (100 µL, 0.78 mmol), 4-fluoro-3-methylphenol (87.1 µL, 0.78 mmol), triethylamine (100 µL, 7.1 mmol) and toluene (5 mL) was refluxed overnight. Concentration of the reaction mixture gave a pale solid powder, which was purified by flash silica gel chromatography eluting with hexane/ethyl acetate (10:1). Product fractions were pooled and concentrated to leave pure **WWL50** (175.7 mg) as a white powder: ¹H NMR (400 MHz, CDCl₃) δ 6.98-6.6.82 (m, 3 H), 4.87 (b, 1 H), 3.51 (m, 1 H), 2.25 (s, 3 H), 2.00 (m, 2 H), 1.74 (m, 2 H), 1.62 (m, 1 H), 1.39-1.29 (m, 2 H), 1.21 (m, 3 H).
Supplementary Figure 1. Time versus concentration screening of competitive ABPP between FP-PEG-TAMRA and oseltamivir.
Supplementary Figure 2. Time dependent FP-PEG-TAMRA competitive binding to CES1.
REFERENCES
