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

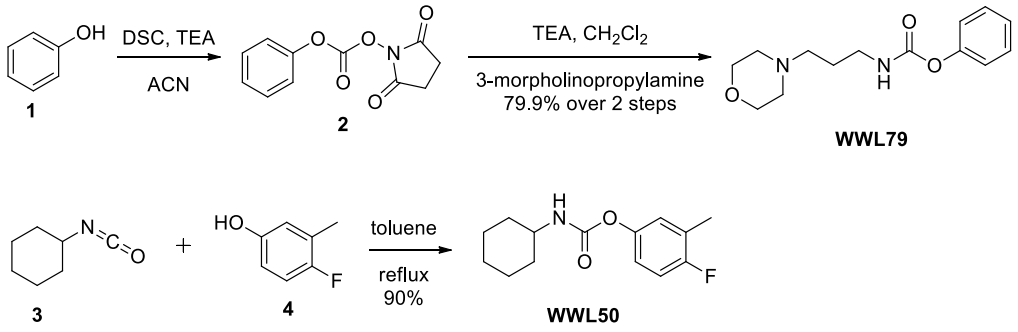
Hao Xu, Jaimeen D. Majmudar, Dahvid Davda, Phani Ghanakota, Ki H. Kim, Heather A. Carlson, Hollis D. Showalter, Brent R. Martin* and Gordon L. Amidon*

SUPPLEMENTARY INFORMATION

SUPPLYMENTARY METHODS

Materials. FP-PEG-biotin was synthesized following previously described methods[1]. All other materials and reagents are from commercial source except where noted.

General synthetic methods. ^{1}H and ^{13}C NMR spectra were obtained on Bruker 300 or Bruker 500 MHz spectrometers with $CDCl_3$ 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 $MgSO_4$ prior to concentration.WWL79 and WWL50 were synthesized as previously described[2].



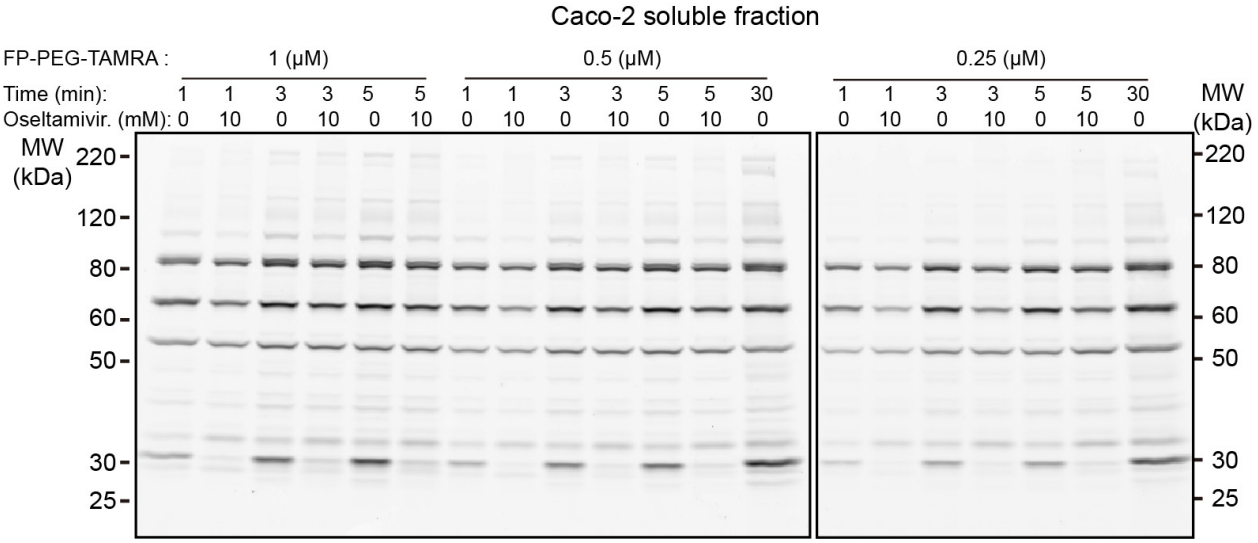
Supplementary Scheme S1. Synthetic scheme of **WWL79** and **WWL50**.

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,

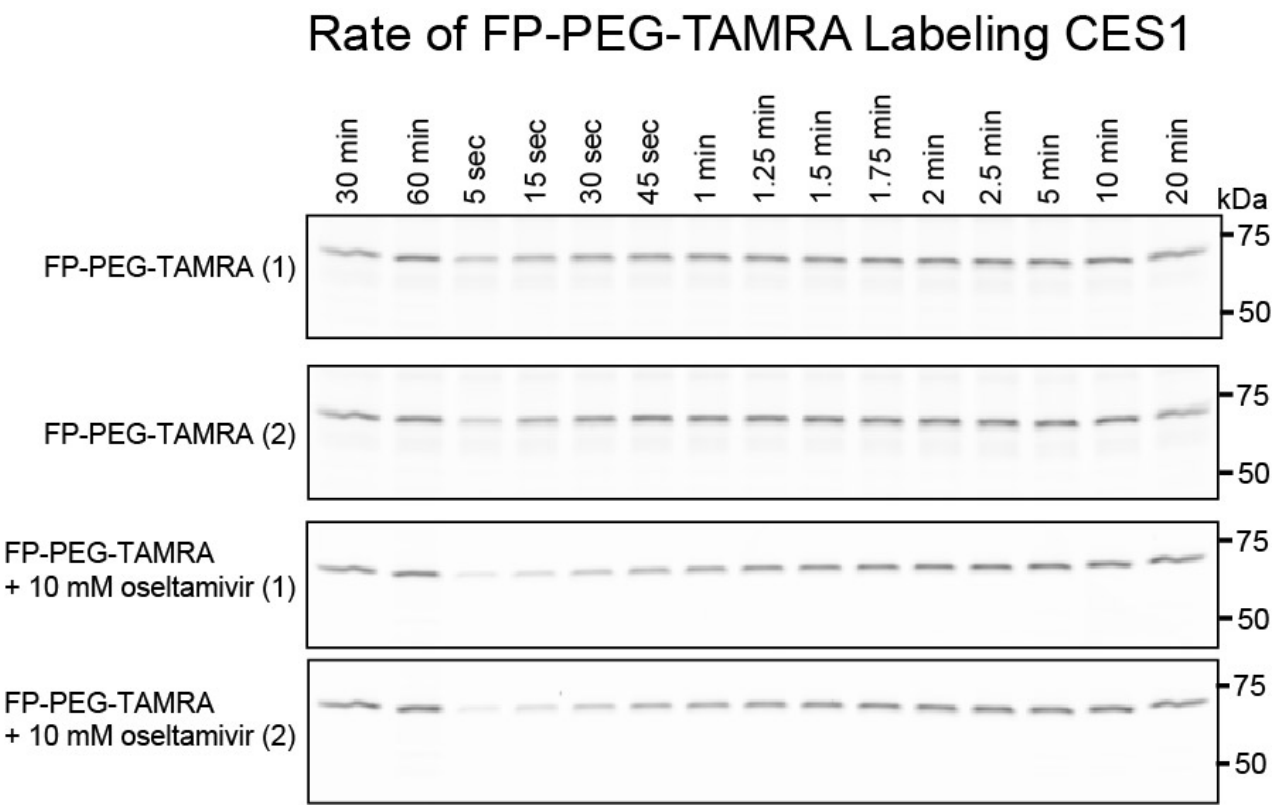
compound **2** was isolated and used directly without further purification. A mixture of compound **2** (235.05 mg, 1 mmol), 3-morpholinopropylamine (146.1 μL, 1 mmol), triethylamine (140 μL, 10 mmol) and dichloromethane (2 mL) was stirred at room temperature for 8 h. The mixture was distributed between dichloromethane and water, and the organic phase dried over $MgSO_4$.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: ^{1}H NMR (400 MHz, $CDCl_3$) 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: ^{1}H NMR (400 MHz, $CDCl_3$) 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 FIGURES



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

1. Xu, H.; Sabit, H.; Amidon, G. L.; Showalter, H. D., An improved synthesis of a fluorophosphonate-polyethylene glycol-biotin probe and its use against competitive substrates. *Beilstein J Org Chem* **2013,** *9*, 89-96.
2. Bachovchin, D. A.; Ji, T.; Li, W.; Simon, G. M.; Blankman, J. L.; Adibekian, A.; Hoover, H.; Niessen, S.; Cravatt, B. F., Superfamily-wide portrait of serine hydrolase inhibition achieved by library-versus-library screening. *Proceedings of the National Academy of Sciences of the United States of America* **2010,** *107* (49), 20941-6.