Supplementary Information

A highly selective marker reaction for measuring the activity of human carboxylesterase 1 in complex biological samples

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Synthesis and characterization of NMHN
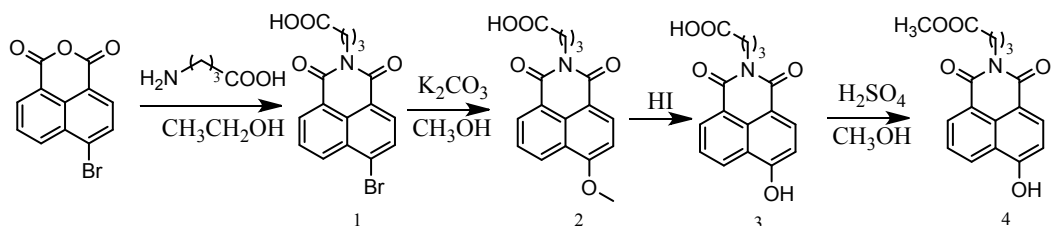
Scheme S1. The synthetic route of **NMHN**

Synthesis of compound 1

4-Bromo-1, 8-naphthalic anhydride (1 g, 3.62 mmol) and 4-aminobutyric acid (0.412 g, 3.98 mmol) were dissolved in ethanol (50 mL). The reaction mixture was stirred and refluxed for 8 h. After cooling to room temperature, the mixture was poured into ice water; the precipitate was isolated, washed with water, and dried to yield 1.21 g of a yellowish-color solid (92.6%). ^{1}H-NMR (400 MHz, DMSO) δ = 12.01 (s, 1H), 8.54 (dd, J = 10.6, 8.0 Hz, 2H), 8.32 (d, J = 7.9 Hz, 1H), 8.21 (d, J = 7.9 Hz, 1H), 8.04-7.94 (m, 1H), 4.08 (t, J = 6.9 Hz, 2H), 2.31 (t, J = 7.3 Hz, 2H), 1.89 (p, J = 7.1 Hz, 2H). ^{13}C-NMR (100 MHz, DMSO) δ 174.49, 163.31, 132.84, 131.85, 131.64, 131.23, 130.05, 129.42, 129.08, 128.60, 123.11, 122.33, 39.97, 31.82, 23.38. HRMS calcd for $C_{16}H_{13}BrNO_4$ $^+$ ([M + H] $^+$) 362.0022, found 362.0021.

Synthesis of compound 2

A mixture of compound 1 (0.8 g, 2.22 mmol) and K_2CO_3 (2.54 g, 18.4 mmol) in 30 mL CH_3OH was refluxed for 10 h. After cooling to room temperature, the pH value was adjusted to about 2 by the addition of concentrated HCl. The precipitate was filtered, washed with water and dried to yield compound 2 as a yellow-color solid (0.562 g, yield: 80.1%). ^{1}H-NMR (400 MHz, DMSO) δ = 12.05 (s, 1H), 8.49 (dd, J = 8.4, 7.8, 1.1, 2H), 8.43 (d, J = 8.3, 1H), 7.80 (dd, J = 8.3, 7.4, 1H), 7.31 (d, J = 8.4, 1H), 4.13 (s, 3H), 4.06 (t, J = 7.0, 2H), 2.30 (t, J = 7.4, 2H), 1.88 (d, J = 7.2, 2H).^{13}C-NMR (100 MHz, DMSO) 23.59, 31.82, 39.33, 56.99, 116.36, 114.39, 122.01, 122.86, 126.45, 131.15, 133.39, 160.48, 163.27, 163.93, 174.45. HRMS calcd for $C_{17}H_{16}NO_5$ $^+$ ([M + H]$^+$) 314.1023, found 314.1025.

Synthesis of compound 3

A mixture of compound 2 (0.3 g, 0.96 mmol) and 10 mL concentrated HI (55-58%) was refluxed for 14 h. After cooling the mixture was poured into water; the precipitate was isolated, washed with water, and dried to yield 0.235 g of the yellow needles solid (81.9%). ^{1}H-NMR (400 MHz, DMSO) δ = 11.87 (s, 1H), 8.54 (dd, J = 8.3, 1.1, 1H), 8.48 (dd, J = 7.3, 1.1, 1H), 8.37 (d, J = 8.2, 1H), 7.84-7.70 (m, 1H), 7.17 (d, J = 8.2, 1H), 4.07 (t, J = 6.9, 2H), 2.29 (t, J = 7.4, 2H), 2.01-1.71 (m, 2H). ^{13}C-NMR (100 MHz, DMSO) 23.59, 31.82, 39.33, 110.32, 113.05, 122.22, 122.75, 125.91, 129.21, 129.61, 131.46, 133.89, 160.61, 162.52, 164.20, 174.42. HRMS calcd for $C_{16}H_{14}NO_5$ $^+$ ([M + H] $^+$) 300.0866, found 300.0864.

Synthesis of compound 4

Compound 3 (0.1 g, 0.33 mmol) dissolved in methanol (15 mL), and then a few drops of concentrated sulfuric acid were added. The reaction mixture was stirred and refluxed for 8 h. After cooling to room temperature, removal of solvent under reduced pressure and purification by silica gel column chromatography with dichloromethane/methanol (10:1) as the eluent generated compound 4 as a yellow-color solid: yield 86 mg, 82.1%. ^{1}H-NMR (400 MHz, DMSO) δ 11.85 (s, 1H), 8.54 (dd, J = 8.3, 0.9 Hz, 1H), 8.51 - 8.45 (m, 1H), 8.36 (d, J = 8.2 Hz, 1H), 7.83 - 7.72 (m, 1H), 7.16 (d, J = 8.2 Hz, 1H), 4.07 (t, J = 6.9 Hz, 2H), 3.51 (s, 3H), 2.38 (t, J = 7.3 Hz, 2H), 1.91 (p, J = 7.1 Hz, 2H). ^{13}C-

NMR (100 MHz, DMSO) 173.32, 164.21, 163.53, 160.64, 133.88, 131.45, 129.61, 129.23, 125.90, 122.75, 122.20, 113.03, 110.32, 51.63, 39.12, 3145, 23.48. HRMS calcd for $C_{17}H_{16}NO_5^+$ ([M + H]$^+$) 314.1023, found 314.1022.
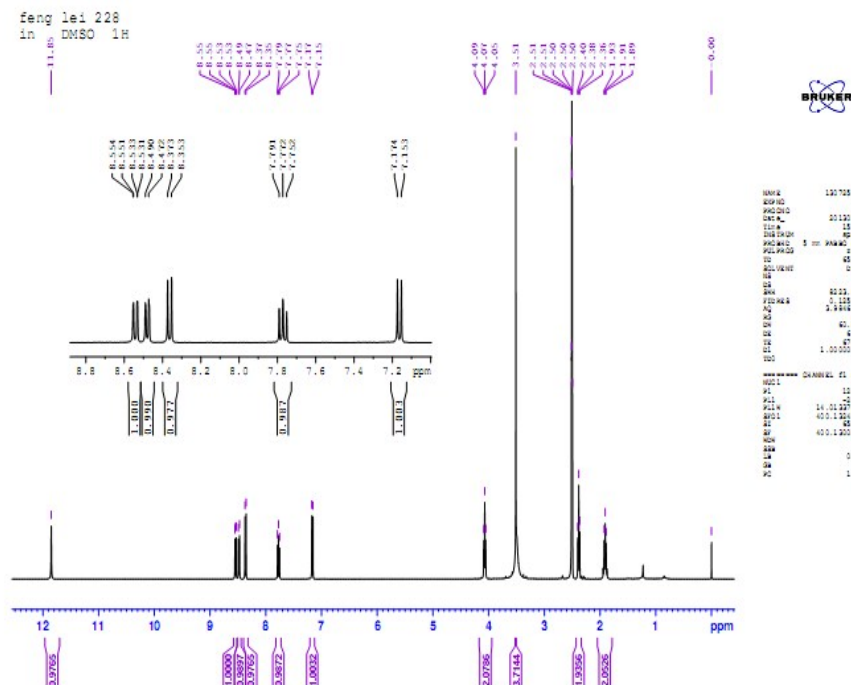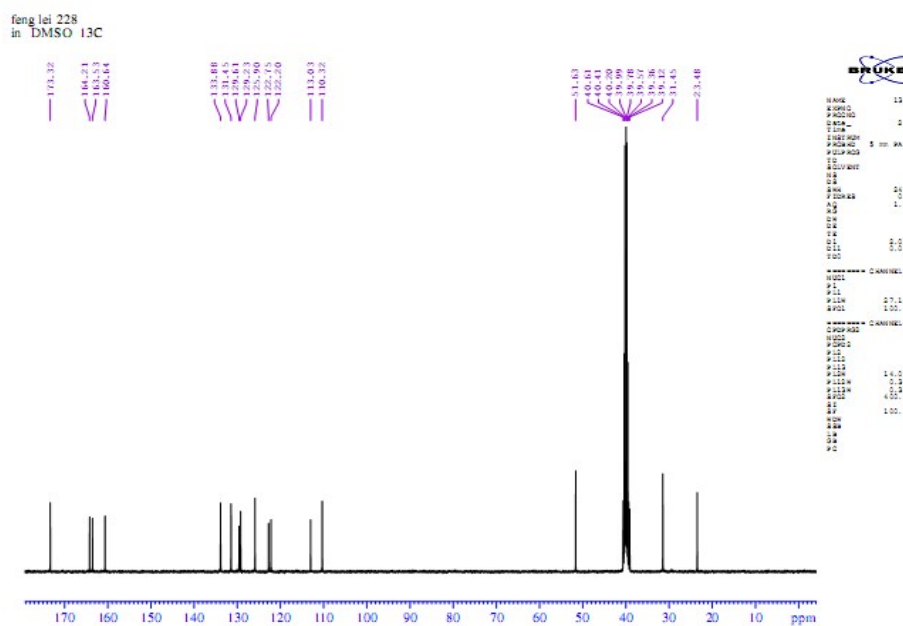

Fig. S1 ^{1}H-NMR spectrum of **NMHN** in scheme 1

Fig. S2 ^{13}C-NMR spectrum of **NMHN** in scheme 1
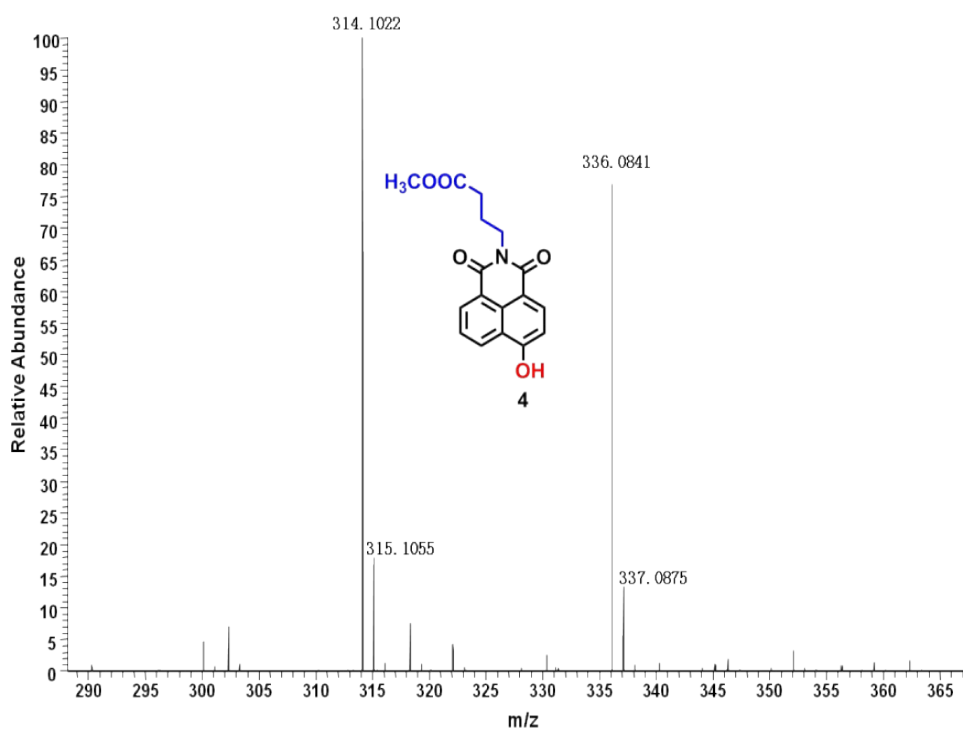
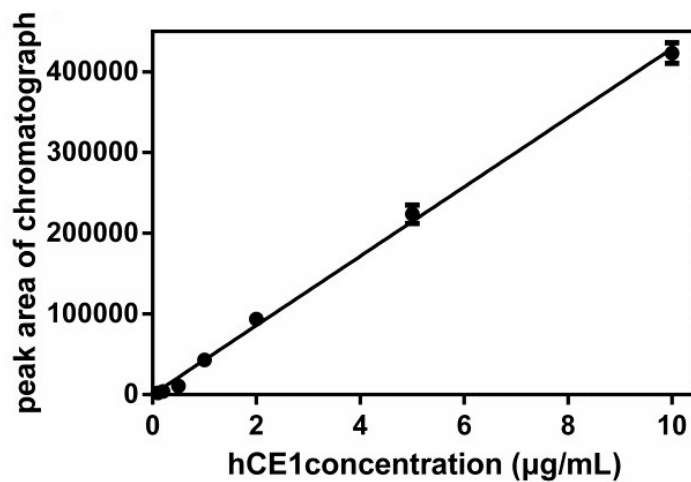**Fig. S3** HR-MS spectrum of **NMHN** in scheme 1

Fig. S4 NCHN formation rate in the presence of increasing concentration of hCE1. NMHN (50 μM) was incubated with increasing concentration of hCE1 (0.05-10 μg/mL).
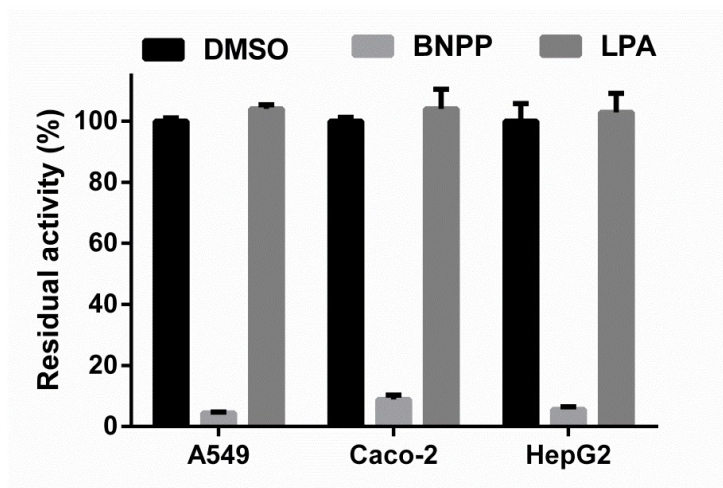
Fig. S5 Inhibitory effects of two selective esterase inhibitors on NMHN hydrolysis in cell preparations.

Table S1 The features of currently used probes for hCE1.

Substrate	Structure	Kinetic behavior	K_m (μM)/V_{max} (nmol/min/mg)	CL_{int} mL·min^{-1}·mg^{-1}
Oseltamivir[1]		Michaelis-Menten	177/102	0.58
Clopidogrel[2]		Michaelis-Menten	62.7/3.6	0.06
BMBT[3]		Substrate Inhibition	NA	NA
Dabigatran[4] etexilate		Michaelis-Menten	24.9/0.7	0.03
NMHN		Michaelis-Menten	53.1/684.3	12.9

NA, not available, the kinetic parameters for BMBT hydrolysis in hCE1 is unavailable because of the kinetic parameters can hardly be exactly calculated due to strong substrate

inhibition effect.

Table S2 Regression coefficient, limit of detection (LOD) and limit of quantification (LOQ) for **NCHN** and **NMHN** by using two detectors.

Detector	Analyte	Conc. Range (µM)	R^2	LOD (µM)	LOQ (µM)
LC-UV	NCHN	0.5-50	0.9997	0.2	0.5
	NMHN	0.5-50	0.9994	0.2	0.5
LC-FD	NCHN	0.5-50	0.9996	0.5	1.0
	NMHN	0.5-50	0.9995	0.1	0.2

R=correlation coefficient; LOD=limit of detection; LOQ= limit of quantification

Table S3 Intra- and inter-day precision and accuracy of the LC-FD method for quantitative determination of **NMHN** and **NCHN**.

Analyte	Added	Intra-day			Inter-day
		Found	RSD (%)	(%)	RSD (%)
NCHN	1.0	1.04±0.04	3.6	103.7	
		1.02±0.01	1.2	102.4	1.8
		1.04±0.02	2.0	103.8	
	5.0	5.03±0.04	0.8	100.6	
		5.07±0.04	0.8	101.3	0.9
		5.03±0.03	0.6	100.6	
	20.0	20.21±0.07	0.3	101.0	
		20.28±0.14	0.7	101.4	1.0
		20.12±0.08	0.4	100.6	
NMHN	1.0	1.05±0.02	2.2	104.7	
		1.03±0.01	0.6	102.5	2.6
		1.03±0.02	1.7	102.8	
	5.0	5.01±0.06	0.8	100.5	
		4.99±0.10	0.7	100.6	0.2
		4.93±0.34	0.6	100.5	
	20.0	19.54±0.54	0.7	100.8	
		19.45±0.41	0.3	100.4	0.5
		19.82±0.50	0.8	100.6	

Table S4 Recovery of the LC-UV method for quantitative determination of NMHN and NCHN in HLM.

Analyte	Added concentration (μM)	Recovery % （Mean ± S.D.)	RSD (%)
NCHN	1	99.5±6.8	6.8
	5	108.5±5.8	5.3
	20	106.3±3.6	3.3
NMHN	1	108.7±8.1	7.5
	5	107.9±2.3	2.1
	20	107.1±1.7	1.6

Table S5 Recovery of the LC-FD method for quantitative determination of **NMHN** and **NCHN** in HLM.

Analyte	Added concentration (μM)	Recovery % （Mean ± S.D.)	RSD (%)
NCHN	1	100.9±3.2	3.1
	5	103.3±2.4	3.1
	20	104.1±4.9	2.3
NMHN	1	101.4±3.1	2.3
	5	102.4±2.3	4.7
	20	105.3±1.5	1.5

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