Supplementary Data

Biologically Active Ester Derivatives as Potent Inhibitors of the Soluble Epoxide Hydrolase

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Syntheses

All melting points were determined with a Thomas-Hoover apparatus (A.H. Thomas Co.) and are uncorrected. Mass spectra were measured by LC-MS/MS (Waters 2790) using positive mode electrospray ionization. The purity of novel key compounds was determined by elemental analyses (C, H, N) that were performed by Midwest Microlab, IN, and was ≥95% as shown in Table **1**. ^{1}H-NMR spectra were recorded on QE-300 spectrometer, using tetramethylsilane as an internal standard.

4-(3-Adamantan-1-ylureido)-butyric acid 1-methylpentyl ester (**5**)

To a suspension of 4-aminobutyric acid (1.03 g, 10.1 mmol) in DMF (40 mL) was added 1-adamantyl isocyanate (1.20 g, 6.77 mmol) at room temperature. The reaction mixture was stirred overnight. Then an aqueous solution of 1N HCl (40 mL) was added into the reaction at 0℃, and the mixture was stirred for 30 min. The solid product crystalized was filtered and washed with water (40 mL) and ethyl acetate (30 mL). The resulting solid was dried in the vacuum oven at 50℃ to give 4-(3-adamantan-1-ylureido)-butyric acid (1.90 g; **3**) as a white solid in 100% yield. To a solution of **3** (0.30 g, 1.07 mmol) and DMAP (0.14 g, 1.18 mmol) in dichloromethane (10 mL) was added 2-hexanol (0.16 g, 1.61 mmol) at room temperature. After stirring for 10 min, EDCI (0.23 g, 1.18 mmol) was added portionwise to this mixture. The reaction was stirred for 12 hrs. An aqueous solution of 1N HCl (20 mL) was poured into the reaction mixture and the product was extracted with ether (30 mL). The ether solution was washed with water (50 mL), dried over $MgSO_4$, and concentrated. The residue was

purified by column chromatography on silica gel eluting with hexane and ethyl acetate (3:1) to afford **5** (0.25 g, 64%) as a solid. ^{1}H NMR δ ($CDCl_3$) 0.90 (3H, t, *J* = 6.9 Hz), 1.20 (3H, d, *J* = 6.9 Hz), 1.25-1.38 (4H, m), 1.57-1.69 (8H, m), 1.80 (2H, quint, *J* = 6.9 Hz), 1.94-1.98 (6H, m), 2.05-2.10 (3H, m), 2.33 (2H, t, *J* = 6.9 Hz), 3.16 (2H, q, *J* = 6.9 Hz), 4.14 (1H, s), 4.32 (1H, s), 4.87 (1H, m), LC-MS (ESI) *m/z* calcd for $C_{21}H_{36}N_2O_3$ $[M + H]^+$ 365.27, found $[M + H]^+$ 365.42, mp 100°C.

4-(3-Adamantan-1-yl-ureido)-butyric acid 1-ethylpentylester (**6**)

Compound **6** was prepared with the same procedure as that used for the preparation of compound **5** by using 3-heptanol instead of 2-hexanol. ^{1}H NMR δ ($CDCl_3$) 0.89 (6H, m), 1.21-1.387(6H, m), 1.57-1.69 (8H, m), 1.80 (2H, quint, *J* = 6.9 Hz), 1.96(6H, brs), 2.07 (3H, brs), 2.33 (2H, t, *J* = 6.9 Hz), 3.16 (2H, q, *J* = 6.9 Hz), 3.98 (1H, s), 4.29 (1H, s), 4.81 (1H, quint, *J* = 6.9 Hz), LC-MS (ESI) *m/z* calcd for $C_{22}H_{38}N_2O_3$ $[M + H]^+$ 378.29, found $[M + H]^+$ 378.31, mp 69-72°C.

4-(3-Adamantan-1-ylureido)-butyric acid 1-cyanopentyl ester (**7**)

To a solution of 1-pentanal (0.6 g, 6.97 mmol) in dry chloroform (10 mL) was added trimethylsilyl cyanide (0.76 g, 7.66 mmol; TMSCN) and zinc iodide (0.11 g, 0.35 mmol) at 0°C under nitrogen. After stirring for 1 hr at room temperature, the product was extracted with ether (40 mL), and the ether solution was washed with water (40 mL), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate (10:1)

to give trimethylsilylated intermediate (0.63 g, 80%). To this intermediate (0.21 g, 1.86 mmol) was added an aqueous solution of 3N HCl (7 mL) at room temperature. After stirring for 30 min, the cyanohydrin was extracted with ether (20 mL). The ether solution was washed with water (20 mL), dried over Na_2SO_4, and concentrated. The residue dissolved in dichloromethane (10 mL) was added to a solution of 4-(3-adamantan-1-ylureido)butyric acid **3** (0.40 g, 1.43 mmol) and DMAP (0.035 g, 0.29 mmol) in dichloromethane (40 mL) at room temperature. After 10 min, EDCI (0.30 g, 1.57 mmol) was added portionwise to the mixture. The reaction was stirred for 12 hrs, and then an aqueous solution of 1N HCl (20 mL) was poured into the reaction mixture. The product was extracted with ether (60 mL). The ether solution was washed with water (60 mL), dried over Na_2SO_4, and concentrated. The residue was purified using silica gel column chromatography (hexane:ethyl acetate = 2:1) to afford **7** (0.36 g, 68%) as a solid. ^{1}H NMR δ ($CDCl_3$) 0.93 (3H, t, *J* = 6.9 Hz), 1.32-1.52 (4H, m), 1.63-1.70 (6H, m), 1.78-1.95 (10H, m), 2.03-2.08 (3H, m), 2.43 (2H, t, *J* = 6.9 Hz), 3.17 (2H, q, *J* = 6.9 Hz), 4.21 (1H, s), 4.31 (1H, s), 5.27 (1H, t, *J* = 6.9 Hz), LC-MS (ESI) *m/z* calcd for $C_{21}H_{33}N_3O_3$ $[M + H]^+$ 376.25, found $[M + H]^+$ 376.18, mp 101°C.

4-(3-Adamantan-1-ylureido)-butyric acid 2-(2-ethoxyethoxy)-ethyl ester (**8**)

To a solution of **3** (0.5 g, 1.78 mmol), DMAP (0.22 g, 1.78 mmol), and diethylene glycol monoethyl ether (0.24 g, 1.78 mmol) in dichloromethane (15 mL) was added portionwise EDCI (0.34 g, 1.78 mmol) at room temperature. After stirring for 2 hrs, the product was extracted with ether (45

mL). The ether solution was washed with water (50 mL), a solution of 1N HCl (10 mL), and water (40 mL), dried over $MgSO_4$, and concentrated. The residue was purified with silica gel column chromatography eluting with ethyl acetate to give compound **8** (0.18 g) as an oil in 25% yield. ^{1}H NMR δ ($CDCl_3$) 1.21 (3H, t, *J* = 6.9 Hz), 1.75 (6H, brs) 1.84 (2H, quint, *J* = 6.9 Hz), 1.95 (6H, brs), 2.05 (3H, brs), 2.38 (2H, t, *J* = 6.9 Hz), 3.18 (2H, q, *J* = 6.9 Hz), 3.53 (2H, q, , *J* = 6.9 Hz), 3.61 (2H, t, *J* = 6.9 Hz), 3.67 (2H, t, *J* = 6.9 Hz), 3.74 (2H, t, *J* = 6.9 Hz), 4.25 (2H, t, *J* = 6.9 Hz), 4.47 (1H, s), 4.62 (1H, s). LC-MS (ESI) *m/z* calcd for $C_{21}H_{36}N_2O_5$ $[M + H]^+$ 397.26, found $[M + H]^+$ 397.32.

4-(3-Adamantan-1-ylureido)-butyric acid 1-phenyl-2-(2-ethoxyethoxy)-ethyl ester (**9**)

To a suspension of 2-ethoxyethanol (1.13 g, 12.4 mmol) and 60% sodium hydride (0.60 g, 14.9 mmol) in DMF (10 mL) was added a solution of 2-phenyloxirane (1.5 g, 12.4 mmol) in DMF (3 mL) at 0℃. After stirring for 2 hrs at room temperature, the product was extracted with ether (40 mL). The organic solution was washed with water (40 mL x 2), dried over $MgSO_4$, and concentrated. The residue was purified with silica gel column chromatography eluting with hexane and ethyl acetate (2:1) to provide alcohol intermediate (1.83 g, 76%). Compound **9** was synthesized with the same reaction as that used for the preparation of compound **8** using this intermediate and compound **3** in the presence of EDCI and DMAP in dichloromethane in 48% yield. ^{1}H NMR δ ($CDCl_3$) 1.21 (3H, t, *J* = 6.9 Hz), 1.66 (6H, brs) 1.85 (2H, quint, *J* = 6.9 Hz), 1.95 (6H, brs), 2.05 (3H, brs), 2.38 (2H, t, *J* = 6.9 Hz), 3.06-3.17 (1H, m), 3.26-3.29 (1H, m), 3.48-3.71 (8H, m), 4.61 (1H, s), 4.72 (1H, s), 5.97-6.00

(1H, m), 7.33 (5H, s). LC-MS (ESI) *m/z* calcd for $C_{27}H_{40}N_2O_5$ $[M + H]^+$ 473.29, found $[M + H]^+$ 473.11.

3-(3-Adamantan-1-ylureido)-cyclohexanecarboxylic acid pentyl ester (**11**)

A mixture of adamantyl isocyanate (0.30 g, 1.69 mmol) and 3-aminocyclohexanecarboxylic acid (0.61 g, 4.23 mmol) in DMF (30 mL) was stirred at 80°C for 12 hrs, and the reaction mixture was allowed to cool down to room temperature. An aqueous solution of 1N HCl (100 mL) was poured into the reaction mixture, and then the mixture was stirred at 0°C for 1 hr. The solid product crystalized was filtered and washed with water (60 mL) and ethyl acetate (30 mL). The resulting solid was dried in the vacuum oven at 50°C to give **10** (0.54 g, 100%) as a white solid.

To a suspension of **10** (0.30 g, 0.936 mmol) and K_2CO_3 (0.16 g, 1.12 mmol) in DMF (5 mL) was added 1-bromopentane (0.17 g, 1.12 mmol) at room temperature. The mixture was stirred overnight, and the product was extracted with ether (25 mL). The organic solution was washed with water (50 mL X 2), dried over $MgSO_4$, and concentrated. The residue was purified using column chromatography eluting with hexane and ethyl acetate (3:1) to give compound **11** as a while solid in 84% yield. ^{1}H NMR δ ($CDCl_3$) 0.90 (3H, t, *J* = 6.9 Hz), 1.28-1.36 (4H, m), 1.37-1.42 (2H, m), 1.53-1.69 (12H, m), 1.94-1.98 (6H, m), 2.05-2.10 (5H, m), 2.57-2.62 (1H, m), 3.72-3.76 (1H, m), 4.06 (2H, t, *J* = 6.9 Hz), 4.15 (1H, s), 4.24 (1H, s), LC-MS (ESI) *m/z* calcd for $C_{23}H_{38}N_2O_3$ $[M + H]^+$ 391.29,

found $[M + H]^+$ 391.54, mp 133°C.

3-(3-Adamantan-1-ylureido)-cyclohexanecarboxylic acid 1-ethylpentyl ester (**12**)

Compound **12** was prepared with the same manner as that used for the synthesis of compound **6** using compound **10** and 3-heptanol in 74% yield. ^{1}H NMR δ ($CDCl_3$) 0.90-0.92 (6H, m), 1.30-1.36 (4H, m), 1.50-1.67 (14H, m), 1.94-1.98 (10H, m), 2.05-2.10 (3H, m), 2.53-2.57 (1H, m), 3.76-3.80 (1H, m), 4.23 (1H, s), 4.29 (1H, s), 4.82 (1H, quint, J = 6.9 Hz), LC-MS (ESI) *m/z* calcd for $C_{25}H_{42}N_2O_3$ $[M + H]^+$ 419.32, found $[M + H]^+$ 419.23, mp 124°C.

3-(3-Adamantan-1-ylureido)-cyclohexanecarboxylic acid 1-phenyl-2-(2-ethoxyethoxy)-ethyl ester (**13**)

Compound **13** was prepared with the same manner as that used for the synthesis of compound **9** using compound **10** instead of compound **3** in 50% yield. ^{1}H NMR δ ($CDCl_3$) 1.21 (3H, t, J = 6.9 Hz), 1.53-1.69 (2H, m), 1.74-1.73 (10H, m), 1.83-1.85 (2H, m), 1.93 (6H, s), 2.06 (3H, s), 2.62-2.65 (1H, m), 3.59-3.77 (8H, m), 4.16-4.18 (1H, m), 4.29 (1H, s), 4.35 (1H, s), 5.98-6.00 (1H, m), 7.33 (5H, s). LC-MS (ESI) *m/z* calcd for $C_{30}H_{44}N_2O_5$ $[M + H]^+$ 513.33, found $[M + H]^+$ 513.44.

Table **1**. Elemental Analyses

(only novel key compounds)

Compound	Calculated (%) Found (%)		
	C	H	N
6	69.80	10.12	7.40
	69.83	10.13	7.38
8	63.61	9.15	7.06
	63.60	9.12	7.01
9	68.62	8.53	5.93
	68.66	8.58	5.93
12	67.21	9.54	6.03
	67.30	9.57	6.00
13	70.28	8.65	5.46
	70.32	8.64	5.46

IC_{50} assay conditions

IC_{50} values were determined as described using a sensitive fluorescent based assay, and a brief description of the procedure is as follows: cyano(2-methoxynaphthalen-6-yl)methyl *trans*-(3-phenyl-oxyran-2-yl)methyl carbonate (CMNPC) was used as a fluorescent substrate. Human sEH (0.96 nM) was incubated with inhibitors for 5 min in pH 7.0 Bis-Tris/HCl buffer (25 mM) containing 0.1 mg/mL of BSA at 30^{o}C prior to substrate introduction ([S] = 5 μM). Activity was measured by determining the appearance of the 6-methoxy-2-naphthaldehyde with an excitation wavelength of 330 nm and an emission wavelength of 465 nm for 10 minutes. IC_{50} results are averages of three replicates.

***In vitro* metabolic stability in human liver microsomes**

Human liver microsomal protein (0.125 mg) was brought to sodium phosphate buffer (0.222 mL; 0.1 M, pH 7.4). The proteins were preincubated for 5 min in open glass tubes immersed in a shaking bath at 37℃. After this preincubation, a solution of test compound (2.5 µL of 100 µM) was added, and the reaction was initiated by the addition of NADPH generating system (25 µL; NADP (2 mM), glucose 6-phosphate (57 mM), glucose 6-phosphate dehydrogenase (3.5 units), and magnesium chloride (50 mM) in 1 mL of sodium phosphate buffer (0.1 M, pH 7.4)). Incubation mixture (0.25 mL total volume) was shaken in a water bath at 37℃ for 60 min. A control was prepared by the addition of acetonitrile (1 mL) immediately after adding the NADPH generating system. The reaction was terminated by the addition of cold acetonitrile (0.75 mL), and a 50 µL aliquot of 500 ng/mL 1-adamantyl-3-decylurea (ADU) was added to the samples. The samples were then vortexed and centrifuged at 6000 rpm (4000 g) for 5 min. The extracts were transferred to a new glass tube and dried under nitrogen. The residue was reconstituted in methanol (0.5 mL). Aliquots (5 µL) were analyzed by LC-MS/MS. The absolute amount of parent compounds remaining after the incubation was converted to a percentage. The results given are averages of triplicate independent analyses.

Water solubility (µg/mL):

Water solubility was determined experimentally using the following procedure at 25 ± 1.5°C. An excess of the test compound was added to a vial containing sodium phosphate buffer, 0.1 M pH

7.4 (1 mL), and a suspension of the mixture was equilibrated during 1 hr of sonication and 24 hrs of shaking, followed by centrifugation (5 min, 200g). The water supernatant (2.5μL) was dissolved in 0.5 mL of methanol, and to the methanol solution was added 0.5 mL of internal standard solution (1-cyclohexyl-3-tetradecylurea **(**CTU); 1000 ng/mL in methanol). A regression curve for each compound was obtained from five standard stock solutions (r^2 = 0.99) by using LC-MS/MS. Then, the absolute amount of each compound was calculated.