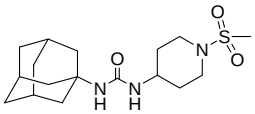
Supplementary Data

Synthesis methods for sEH inhibitors 17, 18, 25, 26, 27, 29, 30, 31, and 32.

General

The synthesis of compounds and the intermediates prepared specifically for this study are described below. Other inhibitors are from published studies. All reagents and anhydrous solvents were purchased from Aldrich Chemical Company (Milwaukee, WI, USA) unless otherwise noted and used as received. All chemical reactions were conducted under nitrogen atmosphere unless otherwise noted. Reaction progress was monitored using thin-layer chromatography (TLC) 0.2 mm glass plates precoated with silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). Chemical detection was based on the quenching of fluorescence from 254 nm ultraviolet light. For those compounds not possessing a suitable chromophore, TLC plates were visualized using either a $KMnO_4$ solution or I_2 vapor. Flash chromatography was performed with 32-63 μm silica gel (Sorbent Technologies, Atlanta, GA). NMR spectra were recorded on a Varian Mercury 300 (Varian Inc., Palo Alto, CA) in $CDCl_3$ or DMSO-d_6 using tetramethylsilane (TMS) as an internal reference unless otherwise noted. NMR peaks are reported in parts per million (ppm, δ) relative to TMS.

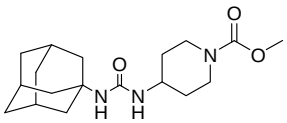
sEHI 17



1-Adamantan-1-yl-3-(1-methanesulfonyl-piperidin-4-yl)-urea, The adamantyl scaffold was synthesized as described previously [1]. The desired scaffold (807 mg, 2.91 mmol) and 1 mL of TEA were all combined in 15 mL dichloromethane at 0°C. The reaction was allowed to stir. At this point, methanesulfonyl chloride (3.4 mmol), was added and the reaction was allowed to warm to room temperature over 2 hours. After reaching room temperature, the reaction was allowed to stir for 18 hrs. The reaction was then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The organic layer was dried and evaporated to give a white powder. Recrystallization from acetone afforded the pure product (917 mg, Yield=89%).

1H NMR (300 MHz, $CDCl_3$.) δ ppm 4.16-4.00 (m, 2H), 3.82-3.60 (m, 3H), 2.78 (s, 3H), 2.76-2.69 (m, 2H), 2.12-1.61 (m, 17H), 1.53-1.36 (m, 2H)

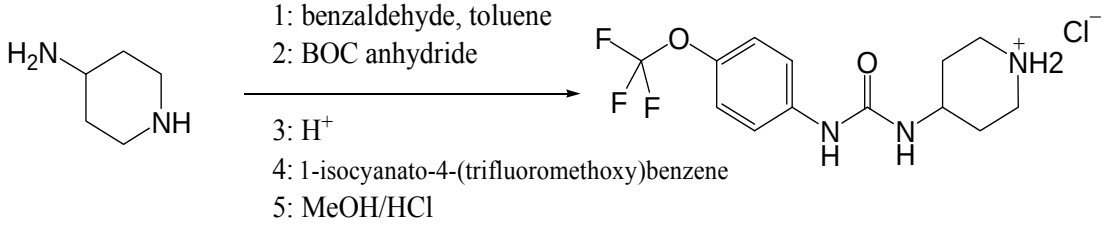
sEHI 18



4-(3-Adamantan-1-yl-ureido)-piperidine-1-carboxylic acid methyl ester. The adamantyl scaffold was synthesized as described previously. The desired scaffold (592 mg, 2.13 mmol) and 1 mL of TEA were all combined in 15 mL dichloromethane at 0°C. The reaction was allowed to stir. At this point, methyl chloroformate (2.7 mmol), was added and the reaction was allowed to warm to room temperature over 2 hours. After reaching room temperature, the reaction was allowed to stir for 18 hrs. The reaction was

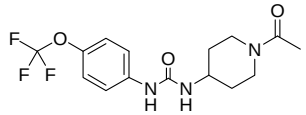
then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The organic layer was dried and evaporated to give a white powder. Recrystallization from acetone afforded the pure product (459 mg, Yield=64%).
1H NMR (300 MHz, $CDCl_3$) δ ppm 5.68 (d, *J* = 7.56 Hz, 1H), 5.42 (s, 1H), 3.82-3.72 (m, 2H), 3.57 (s, 3H), 3.53-3.40 (m, 1H), 3.00-2.85 (m, 2H), 2.03-1.52 (m, 17H), 1.20-1.04 (m, 2H)

Synthesis of the 4- trifluoromethoxyphenyl scaffold:



4-Aminopiperidine (4.29 g, 42.8 mmol) was dissolved in toluene (100 mL). To this was added benzaldehyde (4.54 g, 44.6 mmol). The reaction fitted with a Dean-Stark trap and a condenser and was refluxed for 3 hours under an atmosphere of nitrogen. At this point, when no additional water was seen to form, the reaction was cooled to 0 ^oC and BOC anhydride (9.34g, 42.8 mmol) was added via syringe over 10 minutes. The reaction was allowed to warm to room temperature over 1 hr and was stirred for an additional 17 hrs. The solvent was removed *in vacuo* and the resulting oil was treated with $KHSO_{4(aq)}$ (1 M, 42.8 mL). This was stirred for 2 hours. Water (30 mL) and diethylether (50 mL) was added to and stirred for 1.25 hours. The solution was washed with ether. Because of the resulting emulsion, 50 mL of saturated sodium chloride was added. The solution was then basified with $KOH_{(s)}$ (pH = 10) and was extracted with dichloromethane (3 x 100 mL). The organic layer was dried over $MgSO_4$ and evaporated to give 7.24 g of a yellow/orange oil. To this oil (1.0 g) was added THF (10 mL). This was stirred for 5 minutes until the oil was completely dissolved. The reaction was cooled to 0^oC and 1-isocyanato-4-(trifluoromethoxy)benzene (1.03 mg, 5.0 mmol, 1eq) was added and the reaction stirred overnight under an atmosphere of nitrogen. The solvent was removed and the residue was chromatographed on silica with 5:95 ethylacetate:dichloromethane. The major fraction was collected (1.711g). The resultant residue was treated with a solution of HCl in methanol (20 mL, 1M). This was stirred for 18 hours. Because TLC showed incomplete reaction, another HCl (12M, 1 eq) was added and refluxed for 30 minutes. The solvent was removed and the residue was chromatographed on silica with methanol saturated with ammonia and dichloromethane. The major fraction was collected as white powder (1.03g, 69% yield overall).

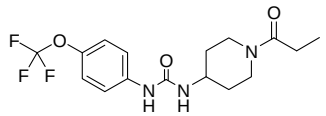
sEHI 24



1-(1-Acetyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea. The desired scaffold (258 mg, 0.85 mmol) and an appropriate carboxylic acid (or ester-acid) (0.96 mmol), and DMAP (104 mg, 0.85 mmol) were all combined in dichloromethane at 0^oC. The reaction was allowed to stir for 10 minutes. At this point, EDCI (162 mg, 0.85 mmol) was added

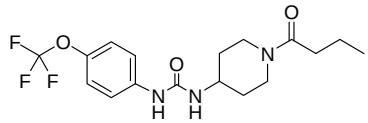
and the reaction was allowed to warm to room temperature over 2 hours. After reaching room temperature, the reaction was allowed to stir for 18 hrs. The reaction was then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The organic layer was dried and evaporated to give a white powder. Recrystallization from acetone afforded the pure product (167 mg, Yield=57%).
1H NMR (300 MHz, D6 DMSO) δ ppm 8.07 (s, 1H), 7.48-7.28 (m, 2H), 7.09 (d, *J* = 8.93 Hz, 2H), 5.87 (d, *J* = 7.57 Hz, 1H), 4.53-4.26 (m, 1H), 4.05-3.82 (m, 1H), 3.82-3.70 (m, 1H), 3.29-3.06 (m, 1H), 3.01-2.69 (m, 1H), 2.11 (s, 3H), 2.14-1.87 (m, 2H), 1.40-1.25 (m, 2H), Mp 204-206°C

sEHI 25



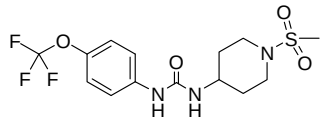
1-(1-Propionyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea. The desired scaffold (242 mg, 0.84 mmol) and 1 mL of TEA were all combined in 15 mL dichloromethane at 0°C. The reaction was allowed to stir. At this point, propionyl chloride (1.1 mmol), was added and stirred for 5 hours and the reaction was allowed to warm to room temperature over 4 hours The reaction was then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The solvent was removed and the residue was chromatographed on silica with 1:1 ethyl acetate:dichloromethane. Recrystallization from acetone afforded the pure product (178 mg, Yield=63%).
1H NMR (300 MHz, $CDCl_3$) δ ppm 7.95 (s, 1H), 7.44-7.35 (m, 2H), 7.10 (d, *J* = 8.99 Hz, 2H), 5.89-5.64 (m, 1H), 4.54-4.36 (m, 1H), 3.96-3.68 (m, 2H), 3.26-3.06 (m, 1H), 2.95-2.78 (m, 1H), 2.36 (q, *J* = 7.48, 7.47, 7.47 Hz, 2H), 2.14-2.00 (m, 1H), 2.00-1.86 (m, 1H), 1.42-1.21 (m, 2H), 1.15 (t, *J* = 7.45, 7.45 Hz, 3H)

sEHI 26



1-(1-Butyryl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea,The desired scaffold (242 mg, 0.84 mmol) and 1 mL of TEA were all combined in 15 mL dichloromethane at 0°C. The reaction was allowed to stir. At this point, butyryl chloride (1.1 mmol), was added and stirred for 5 hours and the reaction was allowed to warm to room temperature over 4 hours. The reaction was then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The solvent was removed and the residue was chromatographed on silica with 1:1 ethyl acetate:dichloromethane. Recrystallization from acetone afforded the pure product (197 mg, Yield=66%).
1H NMR (300 MHz, $CDCl_3$) δ ppm 7.75-7.58 (m, 1H), 7.39 (d, *J* = 8.97 Hz, 2H), 7.12 (d, *J* = 8.86 Hz, 2H), 5.55-5.40 (m, 1H), 4.49 (d, *J* = 13.96 Hz, 1H), 4.02-3.87 (m, 1H), 3.82 (d, *J* = 13.63 Hz, 1H), 3.15 (d, *J* = 12.19 Hz, 1H), 2.78 (d, *J* = 11.44 Hz, 1H), 2.44-2.23 (m, 2H), 2.22-2.09 (m, 1H), 1.97-1.87 (m, 1H), 1.72-1.54 (m, 2H), 1.27-1.09 (m, 2H), 0.96 (t, *J* = 7.37 Hz, 3H)

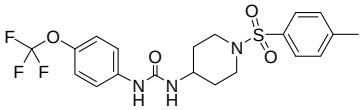
sEHI 27



1-(1-Methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea, The desired scaffold (250 mg, 1.3 mmol) and 1 mL of TEA were all combined in 15 mL dichloromethane at 0^{o}C. The reaction was allowed to stir. At this point, methanesulfonyl chloride (1.4 mmol), was added and stirred for 13 hours. The reaction was then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The solvent was removed and the residue was chromatographed on silica with 1:1 ethyl acetate:dichloromethane. Recrystallization from acetone afforded the pure product (250 mg, Yield=53%).

1H NMR (300 MHz, $CDCl_3$.) δ ppm 8.04 (s, 1H), 7.44-7.38 (m, 2H), 7.09 (d, *J* = 8.36 Hz, 2H), 5.93 (d, *J* = 7.70 Hz, 1H), 4.00-3.55 (m, 3H), 2.90-2.78 (m, 2H), 2.81 (s, 3H), 2.61-2.55 (m, 2H), 2.15-1.99 (m, 2H), 1.61-1.45 (m, 2H)

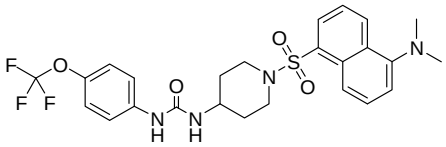
sEHI 29



1-[1-(Toluene-4-sulfonyl)-piperidin-4-yl]-3-(4-trifluoromethoxy-phenyl)-urea, The desired scaffold (250 mg, 1.3 mmol) and 1 mL of TEA were all combined in 15 mL dichloromethane at 0^{o}C. The reaction was allowed to stir. At this point, 4-methylbenzene-1-sulfonyl chloride (266 mg, 1.4 mmol), was added and stirred for 13 hours. The reaction was then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The solvent was removed and the residue was chromatographed on silica with 1:1 ethyl acetate:dichloromethane. Recrystallization from acetone afforded the pure product (297 mg, Yield=52%).

1H NMR (300 MHz, $CDCl_3$.) δ ppm 7.91 (s, 1H), 7.63 (d, *J* = 8.22 Hz, 2H), 7.38-7.30 (m, 4H), 7.06 (d, *J* = 8.84 Hz, 1H), 5.78 (s, 1H), 3.77-3.47 (m, 3H), 2.48-2.35 (m, 2H), 2.44 (s, 3H), 2.05-1.93 (m, 2H), 1.57-1.41 (m, 2H)

sEHI 30

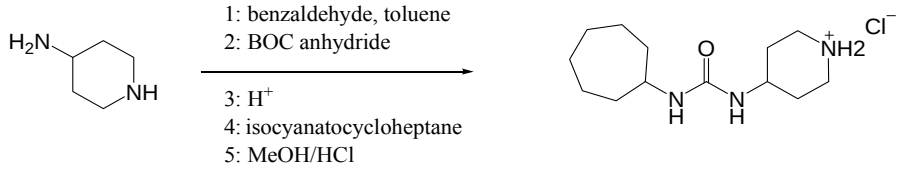


1-[1-(5-Dimethylamino-naphthalene-1-sulfonyl)-piperidin-4-yl]-3-(4-trifluoromethoxy-phenyl)-urea, The desired scaffold (200 mg, 0.7 mmol) and 1 mL of TEA were all combined in 15 mL dichloromethane at 0^{o}C. The reaction was allowed to stir. At this point, 5-(dimethylamino)naphthalene-1-sulfonyl chloride (207 mg, 0.77 mmol), was added and stirred for 13 hours. The reaction was then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The solvent was removed and the residue was chromatographed on silica with 1:1 ethyl acetate:dichloromethane. Recrystallization from acetone afforded the pure product (338 mg, Yield=93%).

1H NMR (300 MHz, $CDCl_3$.) δ ppm 8.59 (d, *J* = 8.50 Hz, 1H), 8.32 (d, *J* = 8.71 Hz, 1H),

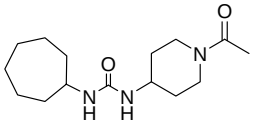
8.18 (dd, *J* = 7.35, 1.19 Hz, 1H), 7.54 (ddd, *J* = 8.50, 7.51, 3.20 Hz, 2H), 7.29-7.22 (m, 2H), 7.19 (d, *J* = 7.15 Hz, 1H), 7.02 (d, *J* = 8.45 Hz, 2H), 6.93 (s, 1H), 5.19 (d, *J* = 7.73 Hz, 1H), 3.91-3.64 (m, 3H), 2.89 (s, 6H), 2.86-2.72 (m, 2H), 2.06-1.92 (m, 2H), 1.64-1.46 (m, 2H)

Synthesis of the cycloheptyl scaffold:



4-Aminopiperidine (4.29 g, 42.8 mmol) was dissolved in toluene (100 mL). To this was added benzaldehyde (4.54 g, 44.6 mmol). The reaction fitted with a Dean-Stark trap and a condenser and was refluxed for 3 hours under an atmosphere of nitrogen. At this point, when no additional water was seen to form, the reaction was cooled to 0 ^{o}C and BOC anhydride (9.34g, 42.8 mmol) was added via syringe over 10 minutes. The reaction was allowed to warm to room temperature over 1 hr and was stirred for an additional 17 hrs. The solvent was removed *in vacuo* and the resulting oil was treated with $KHSO_{4(aq)}$ (1 M, 42.8 mL). This was stirred for 2 hours. Water (30 mL) and diethylether (50 mL) was added to and stirred for 1.25 hours. The solution was washed with ether. Because of bad emulsion, 50 mL of saturated sodium chloride was added. The solution was then basified with $KOH_{(s)}$ (pH = 10) and was extracted with dichloromethane (3 x 100 mL). The organic layer was dried over $MgSO_4$ and evaporated to give 7.24 g of a yellow/orange oil. To this oil (1.51 g) was added THF (10 mL). This was stirred for 5 minutes until the oil was completely dissolved. The reaction was cooled to 0^{o}C and isocyanatocycloheptane (1.0 ml, 7.55 mmol, 1eq) was added and the reaction stirred overnight under an atmosphere of nitrogen. The solvent was removed and the residue was chromatographed on silica with 1:1 ethylacetate:dichloromethane. The major fraction was collected (2.18 g, 85%). The resultant residue (1.19 g) was treated with a solution of HCl in methanol (15 mL, 1M). This was refluxed for 4 hours. The solvent was removed and the residue was washed with acetone. The major fraction was isolated as white powder (0.833 g).

sEHI 31

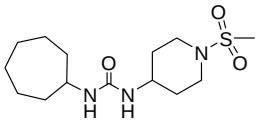


1-(1-Acetyl-piperidin-4-yl)-3-cycloheptyl-urea. The desired scaffold (133 mg, 0.48 mmol) and an appropriate carboxylic acid (or ester-acid) (1.1 mmol), and DMAP (58 mg, 0.48 mmol) were all combined in dichloromethane at 0^{o}C. The reaction was allowed to stir for 10 minutes. At this point, EDCI (95 mg, 1.1 mmol) was added and the reaction was allowed to warm to room temperature over 2 hours. After reaching room temperature, the reaction was allowed to stir for 18 hrs. The reaction was then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The organic layer was dried and evaporated to give a white powder. Recrystallization from acetone afforded the pure product (48 mg, Yield=36%).

1H NMR (300 MHz, $CDCl_3$.) δ ppm 4.68-4.60 (m, 2H) , 4.49 (d, *J* = 11.54 Hz, 1H),

3.92-3.66 (m, 3H), 3.24-3.10 (m, 1H), 2.83-2.71 (m, 1H), 2.11 (s, 3H), 2.08-1.11 (m, 16H)

sEHI 32



1-Cycloheptyl-3-(1-methanesulfonyl-piperidin-4-yl)-urea,The desired scaffold (257 mg, 0.94 mmol) and 1 mL of TEA were all combined in 3 mL dichloromethane at 0^{o}C. The reaction was allowed to stir. At this point, methanesulfonyl chloride (1.2 mmol), was added and stirred for 6 hours. The reaction was then washed with $K_2CO_{3(aq)}$ (1M, 2 x 10 mL) followed by $HCl_{(aq)}$ (1M, 2 x 10 mL). The solvent was removed and the residue was chromatographed on silica with 1:1 ethyl acetate:dichloromethane. Recrystallization from acetone afforded the pure product (176mg, Yield=56%).
1H NMR (300 MHz, $CDCl_3$.) δ ppm 4.24-4.13 (m, 1H), 4.12-4.01 (m, 1H), 3.85-3.61 (m, 2H), 2.86-2.74 (m, 2H), 2.80 (s, 3H), 2.14-2.02 (m, 2H), 2.00-1.88 (m, 2H), 1.68-1.35 (m, 14H)

Preparation of sEH-depleted canine liver cytosol
Cytosol from canine liver that was depleted in sEH was prepared by binding the sEH selectively to a hydrophobic affinity gel optimized to bind the sEH. Five affinity gels were prepared by similar methods and the one optimized for use with canine cytosol was synthesized following the method. Sepharose CL-6B was washed extensively and successively with water, water/methanol (1:1), and 0.1 M NaOH. To 100 g of moist gel in 200 ml of 0.3 M NaOH, 300 mg of $NaBH_4$, and 20 ml of 1,4-butanediol diglycidyl ether were added. The mixture was swirled at room temperature overnight. The epoxy-activated gel was then sequentially and extensively washed with water, methanol/water (1:1), methanol, methanol/water (1:1), and water. To insure that the water used for washing the gel had not become acidic, NaOH was used to insure that the solutions were slightly basic as evaluated by pH test paper. The concentration of free epoxide groups was then evaluated [2]. The gel was synthesized with a ligand density of 10 μmol/g of wet gel. A fivefold excess of 4-chlorophenyl thiol in 20 ml of methanol was added to the activated gel in 10 ml of 0.1 M $NaHCO_3$. The gel was then gently swirled on a rotating table overnight. After mixing, the derivatized Sepharose was washed extensively and successively with methanol/water (1:1), methanol, methanol/water (1:1), water, 0.5 M NaCl, water, 1 mM HCl, water, and ethanol/water (1:1). The resulting gel was stored at 4°C in absolute ethanol containing 0.1% butylatedhydroxyanisole to reduce oxidation of the thiol ether. To bind canine sEH, 0.5 ml of the gel was used and washed extensively with phosphate buffer. 1 ml of dog liver cytosol was mixed with gel overnight at 4°C. After binding, the unbound cytosol was collected and the gel was washed twice with phosphate buffer. The protein remaining bound on the gel was eluted with 1% SDS solution. The protein concentrations were determined by BCA assay. The sEH activity of unbound cytosol determined with the *t*-DPPO radiolabeled assay was depleted to 13% of the original value. The collected unbound cytosol and eluted binding protein were evaluated by SDS-PAGE.

Table S1 - Optimized conditions for monitoring parent sEH inhibitors by tandem mass spectrometry

Compound		Transition	Cone	Collision	LOD[c]	Ref[d]
Text #[a]	Laboratory #[b]		Voltage (V)	Voltage (V)	(ng/mL)	
1	700	393 → 135	28	30	1.0	[3]
2	800	449 → 135	33	29	0.5	[4]
3	950	397 → 220	30	25	0.7	[5]
4	972	451 → 274	35	22	0.3	[5]
5	1029	408 → 135	45	35	0.3	[5]
6	1084	642 → 465	30	20	0.7	[6]
7	1438	580 → 299	12	10	1.6	[3]
8	1663	419 → 178	25	15	5.9	
9	1664	475 → 224	25	20	0.3	
10	1661	403 → 384	20	18	4.7	
11	1662	459 → 224	22	18	0.1	
12	1659	355 → 216	25	18	2.6	
13	1660	411 → 272	28	20	0.7	
14	1153	320 → 143	18	15	0.5	[1]
15	1163	334 → 157	19	12	<0.1	[1]
16	1606	374 → 197	22	20	<0.1	[1]
17	1701	356 →179	18	17	<0.1	
18	1702	336 → 159	15	13	<0.1	
19	1157	348 → 171	20	15	<0.1	[1]
20	1206	406 → 229	28	20	0.1	[1]
21	1159	382 → 205	18	10	0.1	[1]
22	1201	383 → 206	20	20	0.1	[1]
23	1204	440 → 263	20	15	<0.1	[1]
24	1555	346 → 169	22	15	0.2	
25	1770	360 →183	22	18	<0.1	
26	1771	374 → 197	22	20	<0.1	
27	1709	382 → 178	22	18	0.6	
28	1753	418 → 386	20	15	<0.1	
29	1711	458 → 281	20	15	2.0	
30	1710	537 → 360	25	20	0.2	
31	1645	282 → 143	18	13	0.4	
32	1748	318 → 179	18	15	<0.1	
33	981	409 → 275	28	18	0.7	[5]
34	1061	420 → 135	45	50	0.8	[5]
35	1565	438 → 135	22	15	0.1	
36	1135	387 → 210	28	15	<0.1	[7]
37	1037	419 → 135	40	30	0.3	[7]
38	1513	438 → 275	18	14	0.2	
39	1471	413 → 135	40	30	0.2	[7]

Table S1 *continued*

Compound		Transition	Cone Voltage (V)	Collision Voltage (V)	LOD (ng/mL)	Ref[c]
Text #[a]	Laboratory #[b]					
40	1675	413 → 236	25	15	<0.1	[7]
41	1761	413 → 236	25	23	1.9	[7]
42	1470	441 → 135	42	28	0.3	[7]
43	1168	473 → 322	32	30	<0.1	
44	1519	413 → 301	24	15	0.2	[8]
45	1686	439 → 301	25	18	0.9	
46	1615	511 → 178	22	15	0.5	
47	1517	397 → 285	25	15	0.4	[8]
48	1515	349 → 210	23	15	0.2	[8]
49	1193	329 → 135	35	25	<0.1	
50	1443	409 → 135	38	25	0.4	
51	1444	381 → 135	35	25	<0.1	
52	1026	403 → 135	35	25	1.2	[5]
53	1622	457 → 135	42	33	0.3	[5]
54	1425	662 → 485	30	22	1.5	[6]
55	1183	287 → 135	35	25	<0.1	[5]
56	1647	301 → 135	35	25	0.4	[9]
57	1648	355 → 135	35	20	2.7	
58	1650	387 → 135	35	20	6.2	
59	1195	363 → 135	32	25	0.3	[9]
60	1222	391 → 135	35	25	0.7	[9]
61	1849	449 → 287	30	20	<0.1	[9]
62	1671	345 → 135	30	25	0.7	[9]
63	1672	331 → 135	30	22	4.1	[9]
64	1197	400 → 135	45	32	<0.1	[5]
65	1167	356 → 205	42	28	<0.1	[9]
66	1179	384 → 135	30	15	<0.1	[9]
67	1180	467 → 135	45	30	<0.1	[9]
68	1618	503 → 135	45	35	0.6	
69	1774	371 → 178	22	20	6.0	
70	1775	357 → 136	25	22	4.2	
71	1064	292 → 135	40	35	0.3	[10]
72	1642	319 → 143	25	20	<0.1	[1]
73	1644	373 →135	25	20	<0.1	[1]

[a] text numbers of sEH inhibitors.
[b] laboratory numbers of sEH inhibitors.
[c] limit of detection.
[d] published reference for previously published compounds.

Table S2 – HPLC gradient of EETs/diols analysis

Time (min)	B[a] (%)
0	15
0.5	15
0.6	55
4	80
4.1	98
5	98
5.1	15
6	15

[a] organic solvent (methanol: acetonitrile: acetic acid=15:85:0.1) concentration of the gradient.

Table S3 - Chemical structures and IC_{50} and AUC of the sEH inhibitors

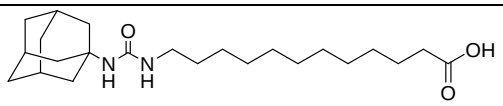
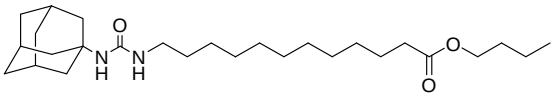
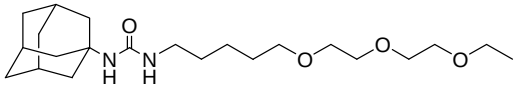
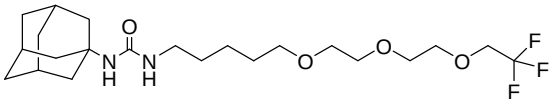
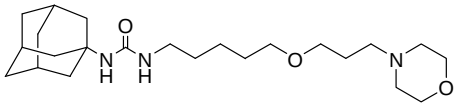
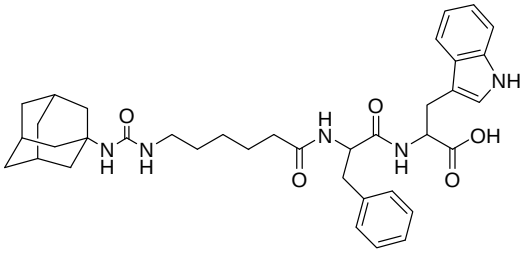
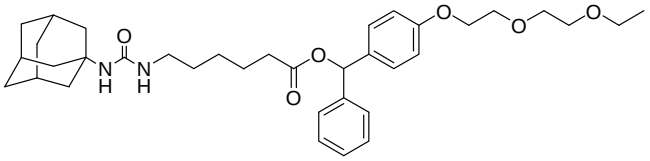
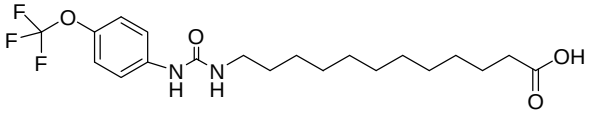
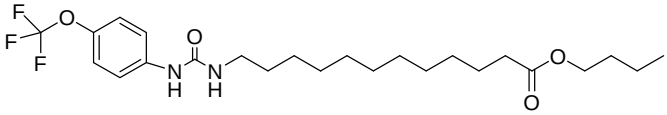
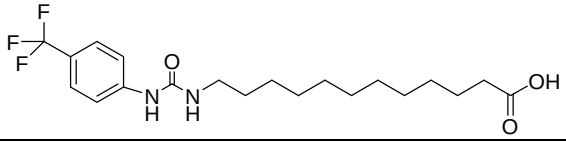
Text #	Structure	Human sEH IC_{50}[a] (nM)	Canine AUC[b] (μM*min)
1		3.2	3.1
2		0.8	<LOD[c] (14.3)[d]
3		14.1	2.4
4		1.4	0.6
5		9.0	<LOD
6		2.5	<LOD
7		5.1	< LOD
8		7.3	8.9
9		2.4	<LOD (<LOD)
10		5.9	<LOD

Table S3 *continued*

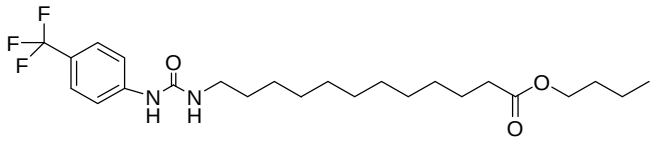
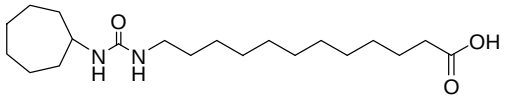
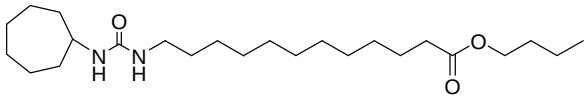
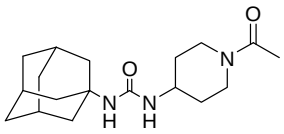
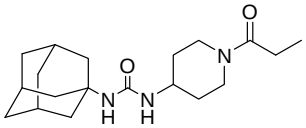
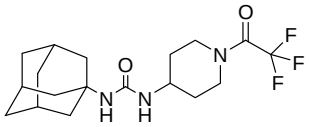
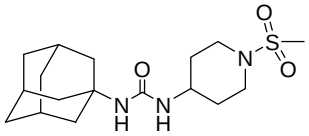
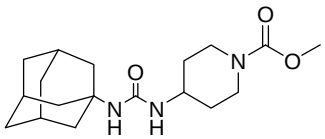
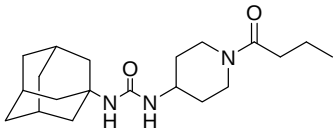
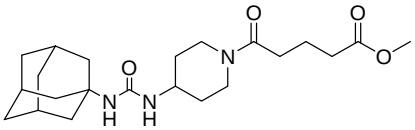
Text #	Structure	Human sEH IC_{50}[a] (nM)	Canine AUC[b] (μM*min)
11		2.1	<LOD (<LOD)
12		3.9	1.7
13		0.8	<LOD (10.5)
14		14.5	36
15		3.2	5.6
16		1.7	2.6
17		1.4	4.9
18		0.9	5.4
19		2.6	2.6
20		2.7	<LOD (<LOD)

Table S3 *continued*

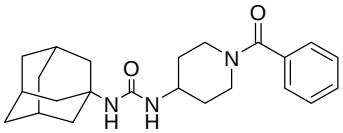
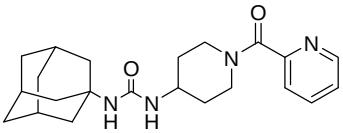
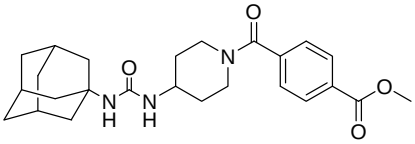
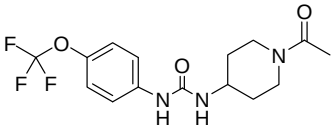
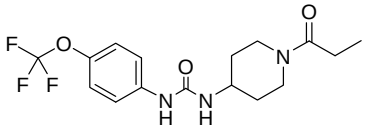
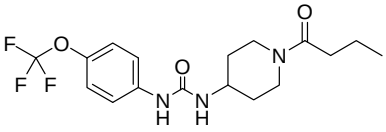
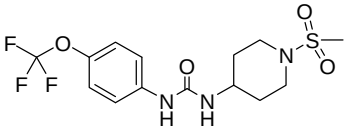
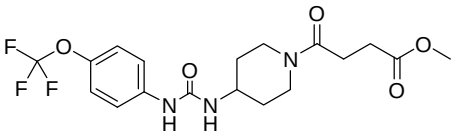
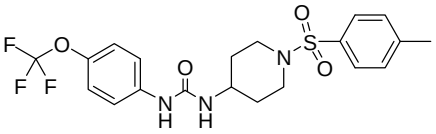
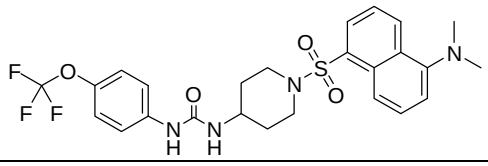
Text #	Structure	Human sEH IC_{50}[a] (nM)	Canine AUC[b] (μM*min)
21		1.3	<LOD
22		1.2	4.8
23		1.1	<LOD
24		11.5	390
25		3.7	600
26		2.1	260
27		2.9	1700
28		10.2	<LOD (<LOD)
29		0.4	<LOD
30		0.8	<LOD

Table S3 *continued*

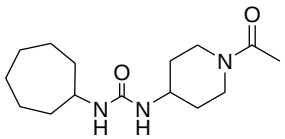
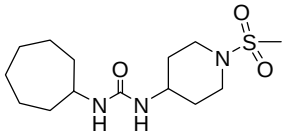
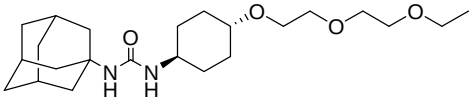
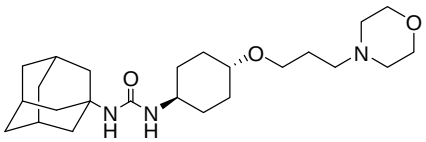
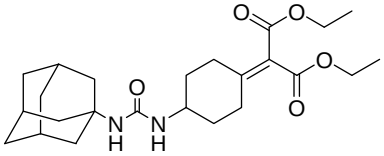
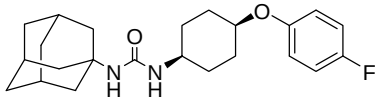
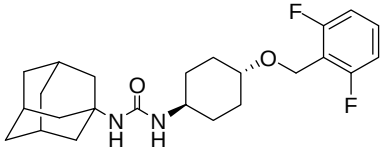
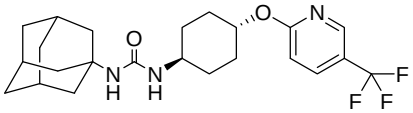
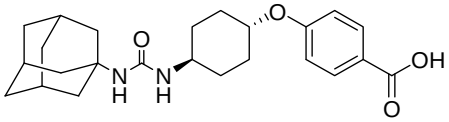
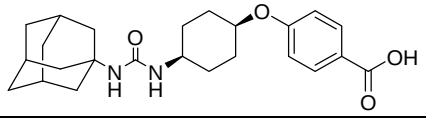
Text #	Structure	Human sEH IC_{50}[a] (nM)	Canine AUC[b] (μM*min)
31		27.6	220
32		2.3	160
33		1.6	2.0
34		1.8	8.0
35		6.6	<LOD
36		0.4	1.2
37		0.4	<LOD
38		4.8	13.7
39		1.5	240
40		1.3	290

Table S3 *continued*

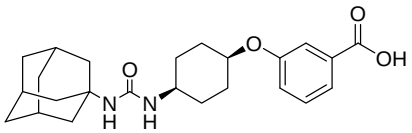
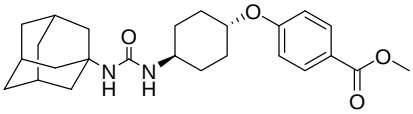
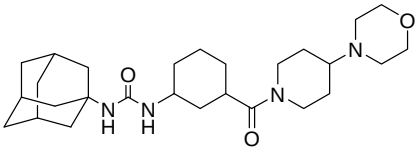
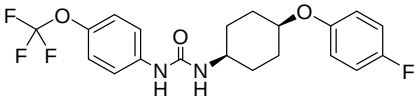
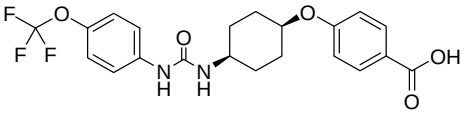
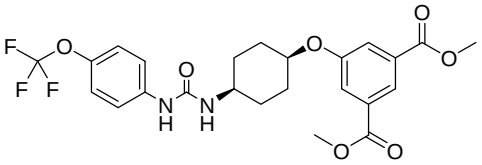
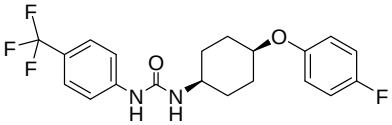
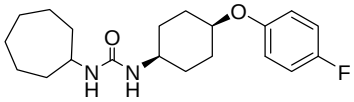
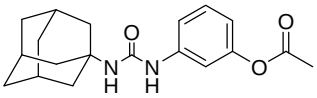
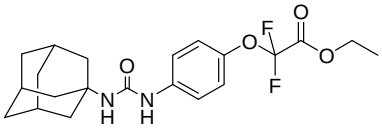
Text #	Structure	Human sEH IC_{50}[a] (nM)	Canine AUC[b] (μM*min)
41		8.1	17.4
42		3.8	4.0 (60.0)
43		6.1	<LOD
44		1.4	29.8
45		0.6	240
46		2.8	0.7
47		1.2	31.2
48		0.4	19.2
49		5.8	<LOD (<LOD)
50		0.8	<LOD (0.2)

Table S3 *continued*

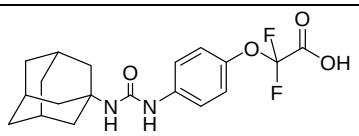
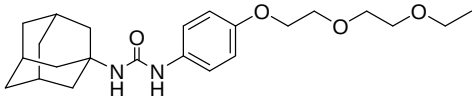
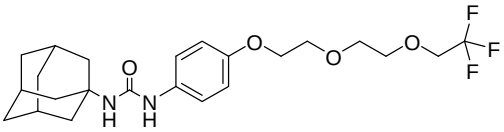
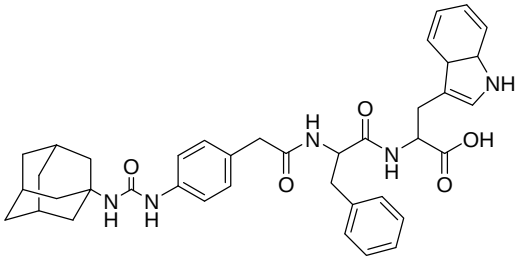
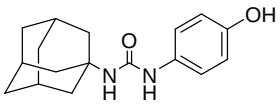
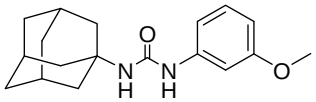
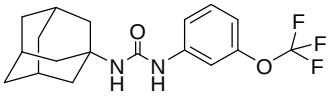
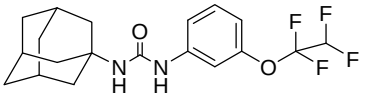
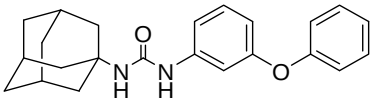
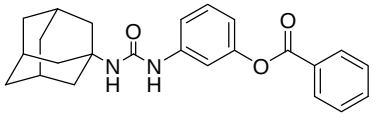
Text #	Structure	Human sEH IC_{50}[a] (nM)	Canine AUC[b] (μM*min)
51		11.6	<LOD
52		1.8	10.2
53		3.3	6.7
54		11.3	<LOD
55		23.6	<LOD
56		4.7	5.9
57		3.1	5.1
58		1.5	1.4
59		0.9	<LOD
60		5.1	<LOD (<LOD)

Table S3 *continued*

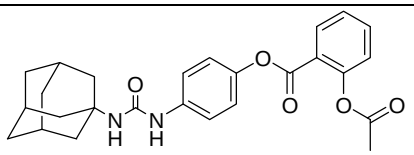
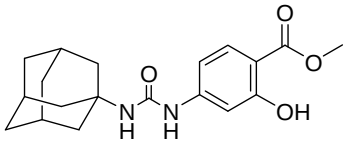
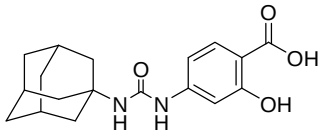
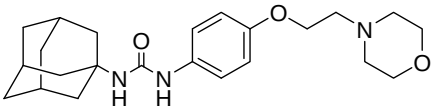
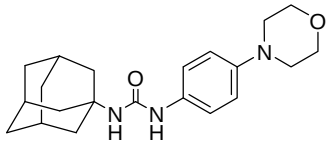
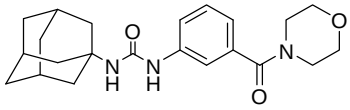
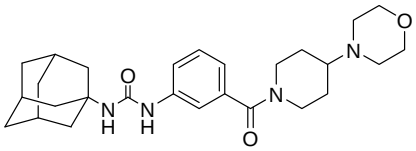
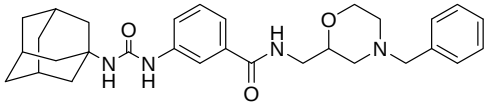
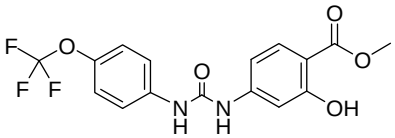
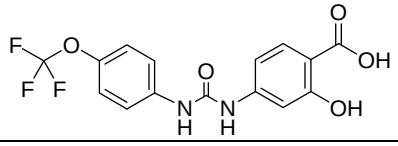
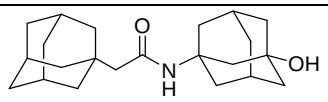
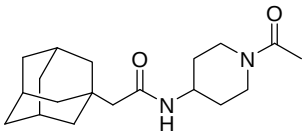
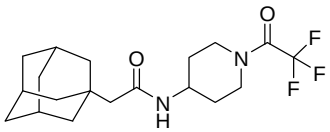
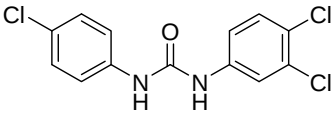
Text #	Structure	Human sEH IC_{50}[a] (nM)	Canine AUC[b] (μM*min)
61		0.8	<LOD (<LOD)
62		2.8	12.6 (<LOD)
63		71.4	<LOD
64		0.9	11.3
65		5.2	<LOD
66		24.6	<LOD
67		28.7	<LOD
68		13.6	<LOD
69		12.2	8.5 (<LOD)
70		94.6	<LOD

Table S3 *continued*

Text #	Structure	Human sEH IC_{50} [a] (nM)	Canine AUC [b] (μM*min)
71		39.9	<LOD
72		275	5.8
73		28.3	<LOD
74		13	1.8

[a] IC_{50} determined by fluorescent assay with recombinant human enzyme. Caution, this assay over estimates the potency of piperidines on the sEH by 10-20x relative to other series of sEHI.
[b] area under the curve, AUC ($Time_{0\text{-}infinite}$).
[c] below the limit of detection.
[d] area under the blood concentration vs time AUC of the corresponding carboxylic acids ($Time_{0\text{-}infinite}$).

Table S4 – Balance sheet for the preparation of sEH-depleted dog liver cytosol

	Protein conc. (mg/ml)	Total protein (mg)[a]	Specific activity (nmol/min/mg)	Total activity (nmol/min)	Activity remaining (%)
Crude	25	25	3.3	83.3	100
sEH-depleted	19	19	0.6	11.2	13

[a] in interpreting this experiment, it is possible that a factor reducing the potency of piperidine compounds remained bound to the column.

[b] the depletion of sEH coupled with the low specific activity of canine cytosol allowed the high amounts of canine cytosol to be added with the human sEH without a major contribution from the canine sEH to the total enzyme activity.

Table S5 – IC_{50} of inhibitors with human recombinant sEH in buffer, dog liver cytosol, or sEH-depleted dog liver cytosol using a *t*-DPPO based radiotracer assay

sEHI	#	Buffer[a]	Dog liver cytosol[b]	Dog liver cytosol sEH depleted[c]
			IC_{50} (nM)	
TUPS	**27**	120	120	150
t-AUCB	**39**	2	3	2

[a] measured with purified recombinant enzyme with a sEH protein concentration of 0.12 μg/ml.

[b] measured with dog liver cytosolic preparation at a protein concentration of 0.025 supplemented with purified recombinant human enzyme (0.12 μg/ml). The sEH activity remaining in the dog liver cytosol accounted for under 17% of the total activity in the assay.

[c] measured with dog liver cytosolic preparation at a protein concentration of 0.19 mg/ml supplemented with purified recombinant human enzyme (0.12 μg/ml). The sEH remaining in the depleted dog liver cytosol accounted for under 13% of the total activity in the assay.

Table S6 – IC_{50} of inhibitors with piperidyl and non-piperidyl linker with 14, 15-EET by LC/MS-MS

#	Human recombinant sEH[a]		Human liver cytosol[b]		Dog liver cytosol[c]	
	IC_{50} (nM)					
[S] µM	5	50	5	50	5	50
27	33	40	18	26	1200	1500
39	4	5	2	3	7	7

[a] measured with purified recombinant enzyme at protein concentration of 0.12 µg/ml.
[b] measured with liver cytosolic preparation at protein concentration of 0.05 mg/ml.
[c] measured with liver cytosolic preparation at protein concentration of 0.2 mg/ml.

Table S7 - Compartmental pharmacokinetic parameters of *t*-AUCB after oral gavage at doses of 0.1, 0.3 and 1 mg/kg in dogs (n = 3)

Dose	Compartment	Correlation	C_{max}[a] (nM)	$T_{1/2}$[b] (h)	AUC[c] (μM*min)
0.03	1	0.77± 0.11	0.011 ± 0.004	6 ± 3	8.3 ± 0.2
0.1	1	0.72 ± 0.03	0.05 ± 0.04	6 ± 3	34 ± 25
0.3	1	0.96 ± 0.04	0.15 ± 0.03	12 ± 10	270 ± 100
1	1	0.94 ± 0.01	0.55 ± 0.22	9 ± 4	1300 ± 750

[a] maximum concentration.
[b] terminal half life.
[c] area under the concentration ($Time_{0\text{-}24\ h}$).

Table S8 – Plasma levels of epoxides and diols derived from arachidonic acids at 30 minutes following oral administration of *t*-AUCB **39** at an oral dose of 0.1, 0.3, and 1 mg/kg body weight.

Dose (mg/kg)	5, 6-EET	8, 9-EET	11, 12-EET	14, 15-EET	5, 6-DHET (nM)	8, 9-DHET	11, 12,-DHET	14, 15-DHET
0	27 ± 11	7.6 ± 3.7	7.9 ± 6.4	16.5 ± 7.9	31 ± 15	13.4 ± 6.1	13.1 ± 3.2	11.1 ± 2.1
0.1	9 ± 4	3.0 ± 1.2	3.1 ± 1.5	5.4 ± 2.5	9 ± 6	3.4 ± 1.8	5.9 ± 3.8	4.4 ± 3.0
0.3	15 ± 3	6.8 ± 1.9	9.0 ± 1.5	11.2 ± 2.6	20 ± 7	4.5 ± 2.0	6.5 ± 1.9	3.7 ± 1.4
1	59 ± 31	10.8 ± 5.5	22.7 ± 3.9	37.2 ± 17.6	58 ± 16	22.2 ± 10.3	16.5 ± 3.9	10.3 ± 3.5

Table S9 - Non-compartmental pharmacokinetic parameters of *t*-AUCB **39** after oral administration at a 0.3 mg/kg dose in different formulations (n = 3)

	C_{max}[a] (nM)	AUC_t[b] (μM*hr)
Triglyceride Solution	99	2.5
Saline Solution	170±55	2.7±0.6
Dry Powder	41±45	0.5±0.8
Dry Powder/HPMC/Lactose	67±32	0.6±0.3

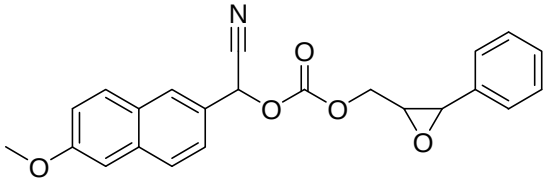
[a] maximum concentration.
[b] area under the curve ($Time_{0-24}$).
[c] the variation represents variation among data from individual dogs.

Table S10 - Compartmental pharmacokinetic parameters of sEHIs between cassette and individual dosing after oral gavage at a 0.3 mg/kg dose in dogs (n = 3)

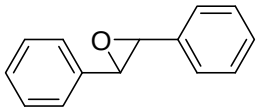
#	Acronym	Dosing	T_{max}[a] (h)	C_{max}[b] (μM)	$T_{1/2}$[c] (h)	AUC[d] (μM*min)
3	AEPU					
		Cassette	0.4	0.03	1.2	2.2
		Individual	0.2 ± 0.1	0.02 ± 0.01	1.7 ± 1.0	1.8 ± 0.6
14	APAU					
		Cassette	0.4	0.3	1.1	35
		Individual	1.0 ± 0.7	0.08 ± 0.03	1.7 ± 0.4	14 ± 1
39	*t*-AUCB					
		Cassette	2.4	0.1	18	220
		Individual	7.3 ± 5.4	0.15 ± 0.03	12 ± 10	270 ± 100

[a] time of maximum concentration.
[b] maximum concentration.
[c] terminal half life.
[d] area under the concentration ($Time_{0\text{-}24\ h}$).

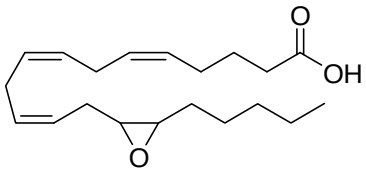
Fig. S1 - Structures of the substrate for the fluorescent assay, CMNPC; substrate for the radiochemical assay, *t*-DPPO; and substrate for the LC/MS based assay, 14, 15-EET.
The fluorescent, radiolabeled and LC/MS based assays are all valuable as sensitive screens for sEH inhibitors *in vitro* [11]. Due to the high-throughput capability of fluorescent assay and comparability to other assays, CMNPC was preferred for screening sEH inhibitors [12]. However, it is difficult to compare IC_{50}s between assays due to different substrate affinities and concentrations, and enzyme concentrations among assays. In the previous study [13], IC_{50} values of previous inhibitors were 1-10 fold higher in the radiolabeled assay than in the fluorescent assay but the values were highly predictable (r^2=0.86). In this study, it is observed that the IC_{50} values of inhibitors with piperidyl linker is 10-20 fold higher while the values of the others are 1-3 fold higher in the radiolabeled assay. Although the ranking of compounds are still predictable (r^2=0.78) A serious caution is that potencies of compounds with a piperidyl linker are over estimated by this CMNPC assay possibly due to the lower affinity of the fluorescent substrate. As IC_{50} values approach 1 nanomolar, the ability of the assay to rank potency decreases. As currently formulated all highly potent compounds will have an IC_{50} in the fluorescent assay of 0.5 nM. Thus, it is possible that the potencies of compounds with an IC_{50} below 2 nanomolar, particularly those with a cyclohexyl ether linker are under estimated. Thus, more sensitive binding assays are needed to determine IC_{50}s of those potent inhibitors.



CMNPC

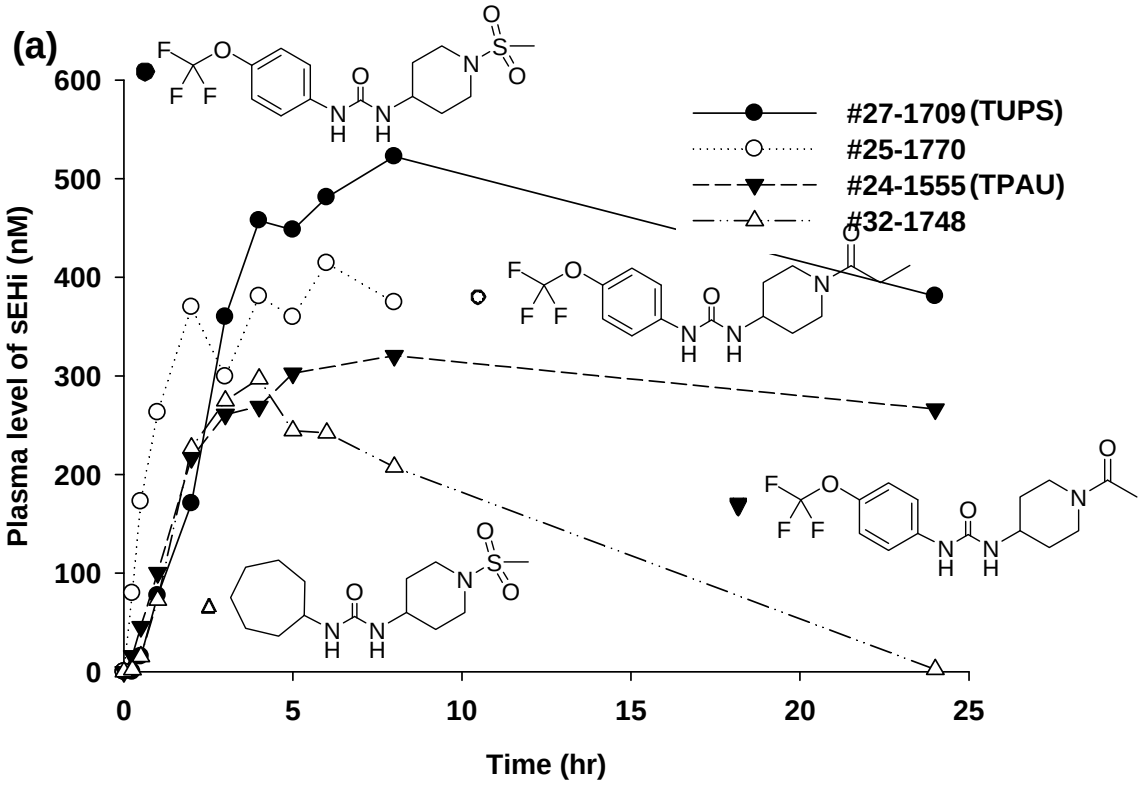


***t*-DPPO**



14, 15-EET

Fig. S2- Plasma concentration-time profiles of sEH inhibitors following an oralt dose of 0.3 mg/kg. For clarity the compounds are grouped according to their Cmax values. The maximum plasma concentration of inhibitors over 300 nM (a); 150 nM (b); 50 nM (c); 40 nM (d); 25 nM (e and f); 15 nM (g); 10 nM (h); and 2 nM (i). The legend refers to the number used in the text, a laboratory number used in previous publications, and an acronym used in the literature (ie #**27**-1709(TUPS)). The inhibitors are divided into four groups including a alkyl, a piperidyl, a cyclohexyl, or a benzyl linker (right side of the urea) and four sub-groups including an adamantyl, a 4-trifluoromethoxyphenyl, a 4-trifluoromethylphenyl, or a cycloheptyl group (left side of the urea). Among compounds with the piperidyl linker, inhibitors with a 4-trifluoromethoxyphenyl group have the highest oral exposure with different functional groups on the right side of urea (a) followed by inhibitors with a cycloheptyl group (a & b). The adamantane left side was used in earlier studies because of its high potency and very high sensitivity on LC-MS. However, the adamantane tends to give lower blood levels and shorter half-lives than the 4-trifluoromethoxyphenyl and cycloheptyl group particularly as right side groups become more lipophilic. The cyclohexyl linker moiety also shows good oral exposure (c & d). However, within this linker, the non-polar groups on the left side of urea have less effect on the plasma level. Most inhibitors with the alkyl or benzyl linker have less oral exposure (e, f, g, h & i). Furthermore, many inhibitors with the alkyl or benzyl linker were not detected in plasma. Each point represents one dog. Standard deviation of the repeated injection of the same sample is 5-10% of the reported value and the standard deviation of extraction and workup of the same sample is 10-30% of the reported value.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Plasma level of sEHi (nM)
Time (hr)
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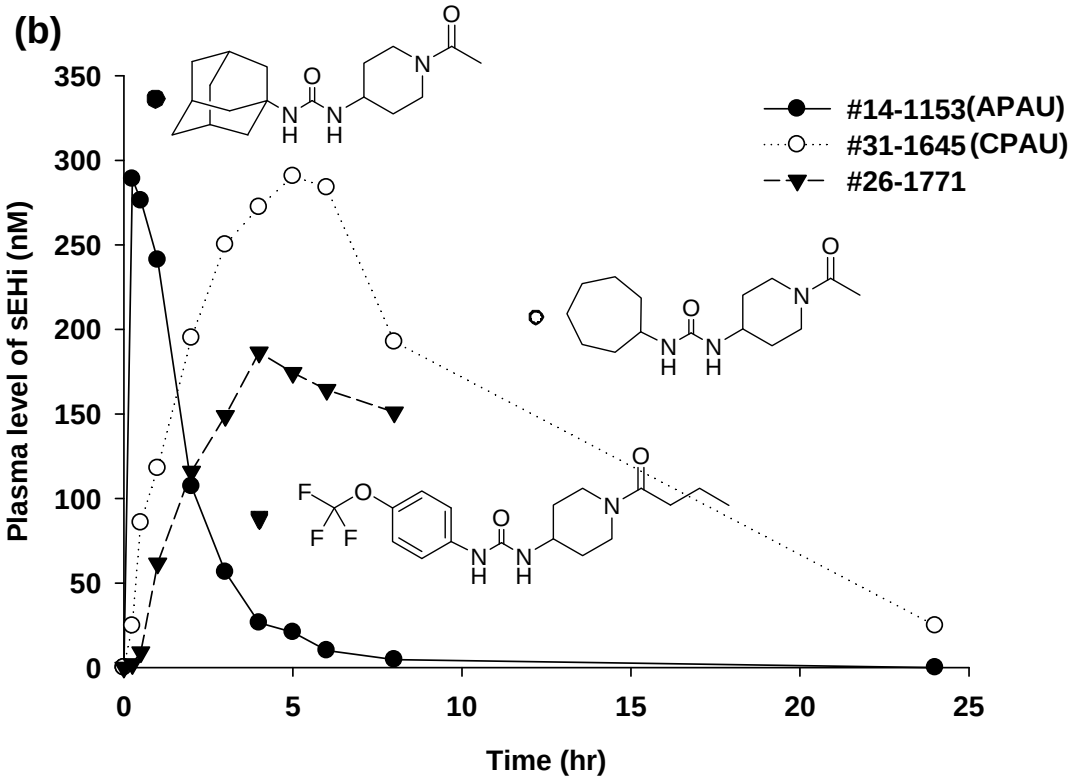
#25-1770
#24-1555 (TPAU)
#32-1748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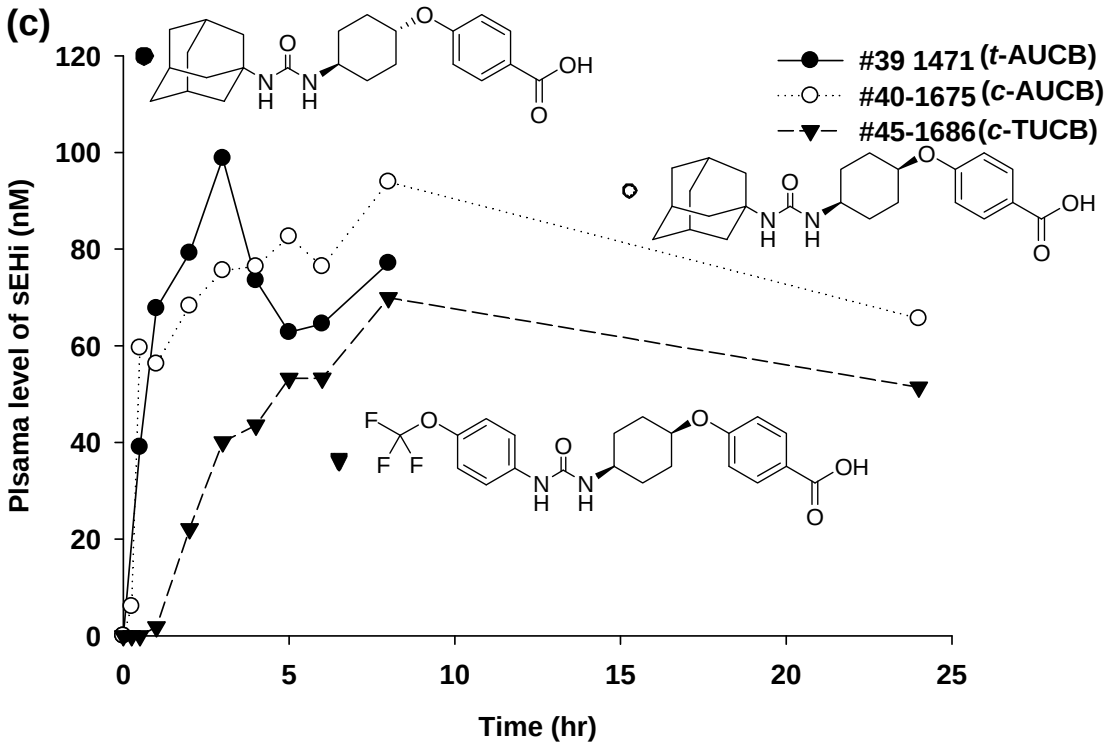
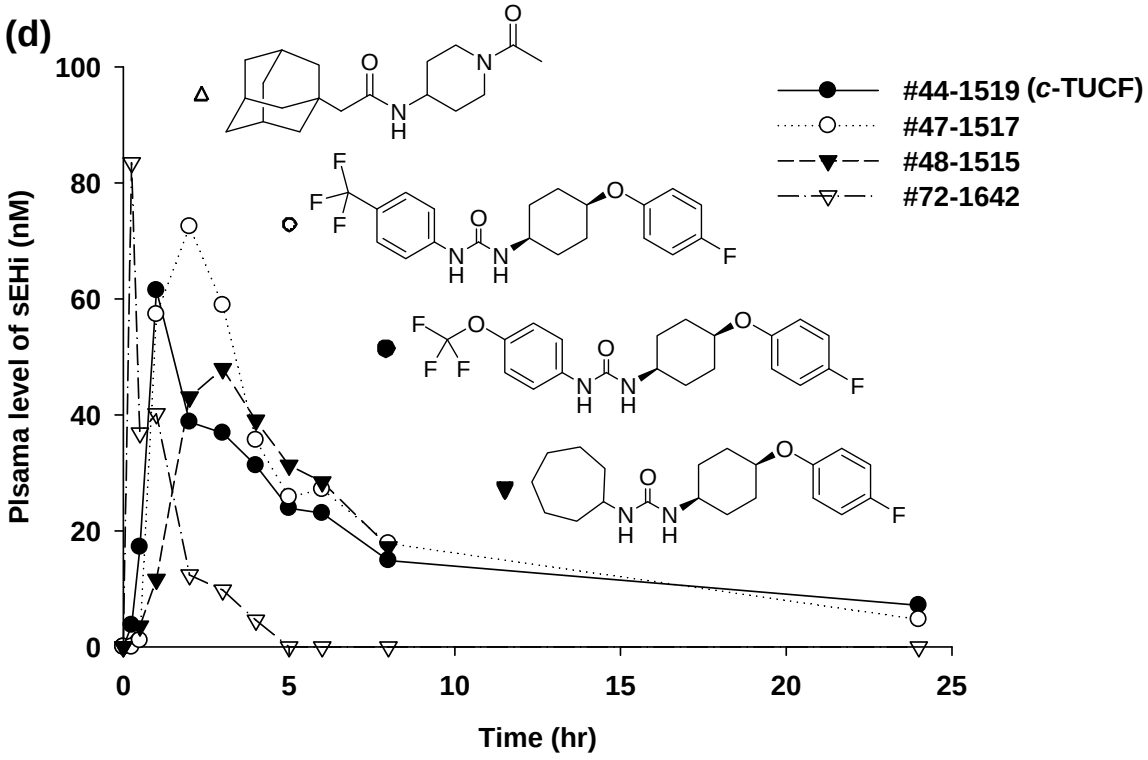

(c)
#39 1471 (t-AUCB)
#40-1675 (c-AUCB)
#45-1686 (c-TUCB)
Plsama level of sEHi (nM)
Time (hr)
0
20
40
60
80
100
120
0
5
10
15
20
25


(d)
#44-1519 (c-TUCF)
#47-1517
#48-1515
#72-1642
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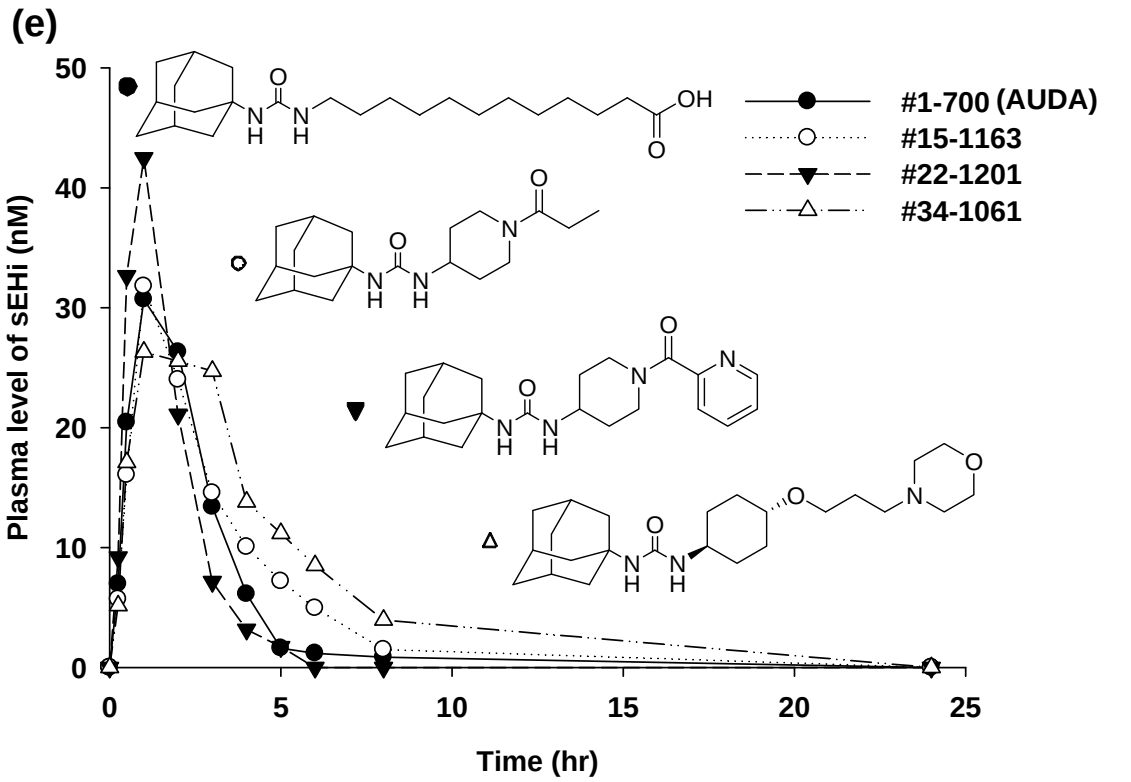
60
80
100
0
5
10
15
20
25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Plasma level of sEHi (nM)
Time (hr)
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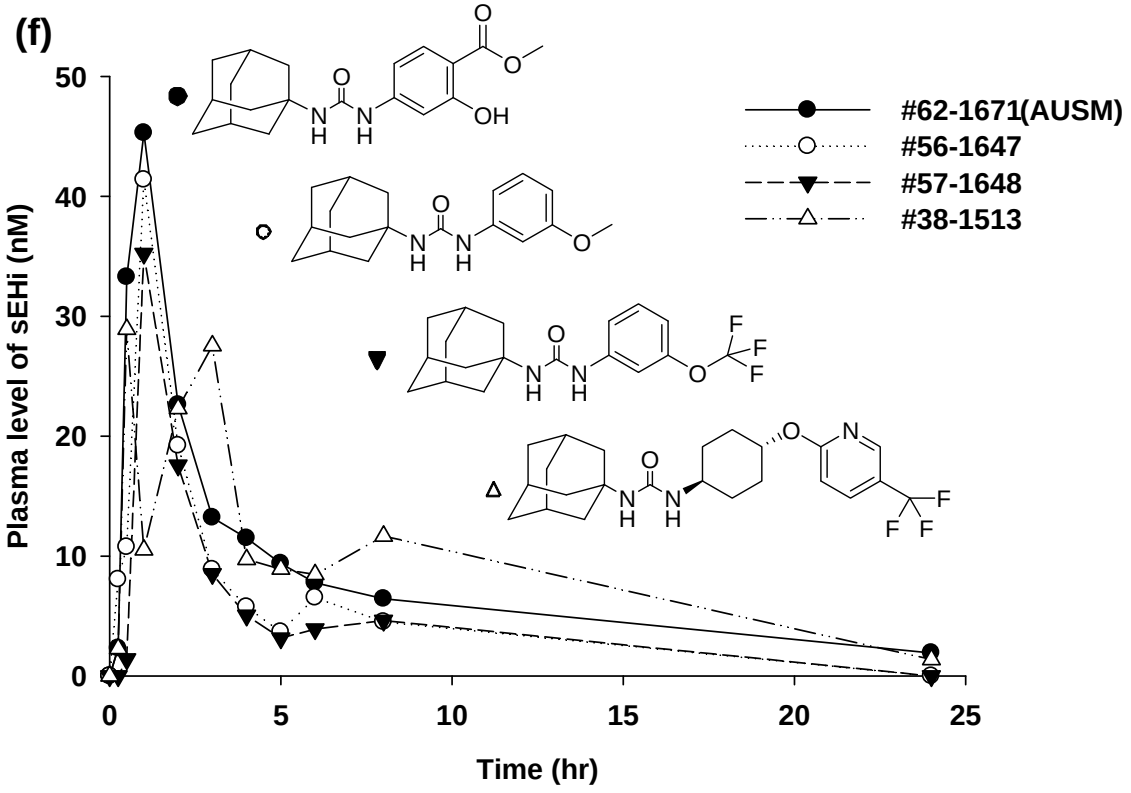
#15-1163
#22-1201
#34-1061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Time (hr)
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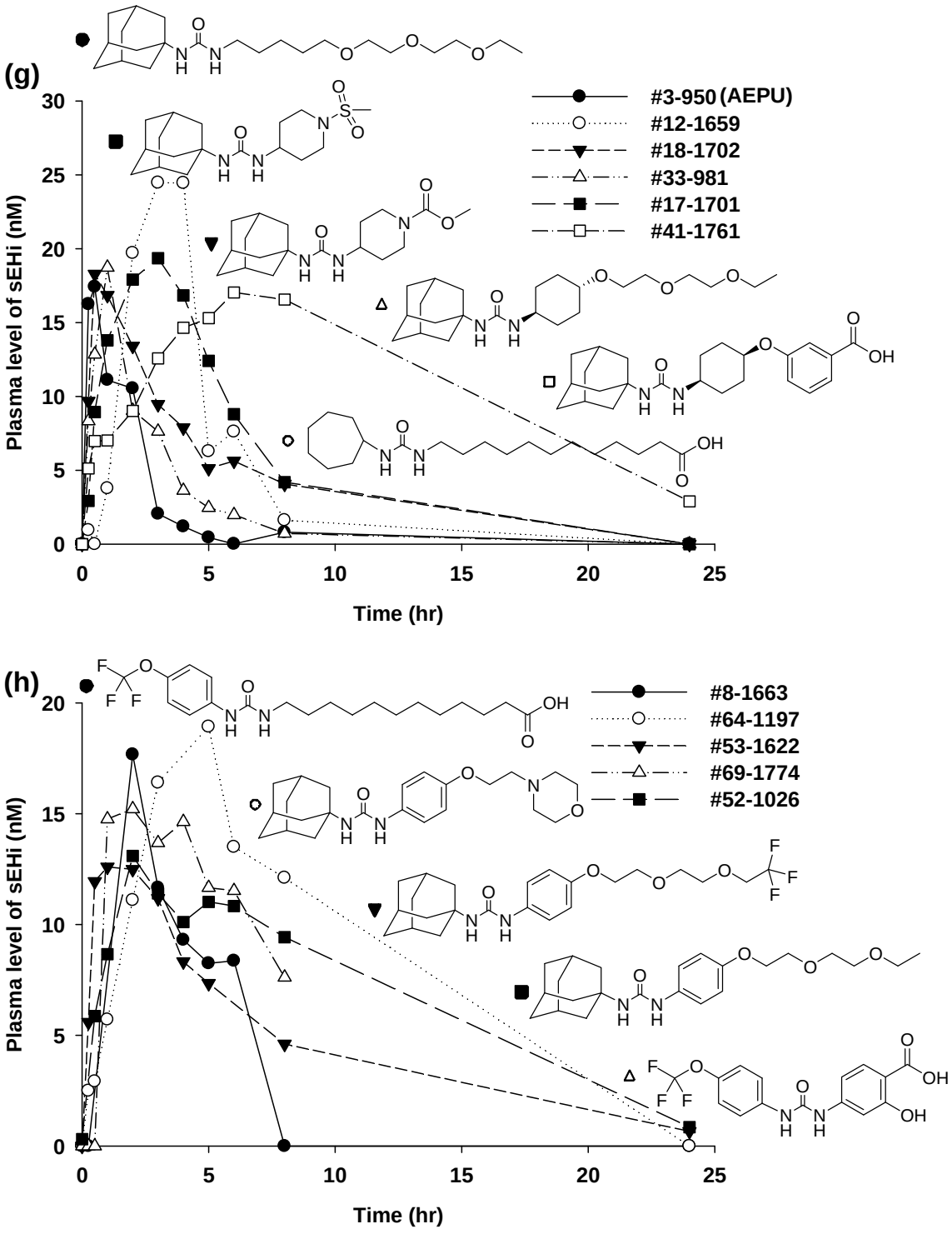

(g)
Plasma level of sEHi (nM)
Time (hr)
#3-950 (AEPU)
#12-1659
#18-1702
#33-981
#17-1701
#41-1761
(h)
Plasma level of sEHi (nM)
Time (hr)
#8-1663
#64-1197
#53-1622
#69-1774
#52-1026

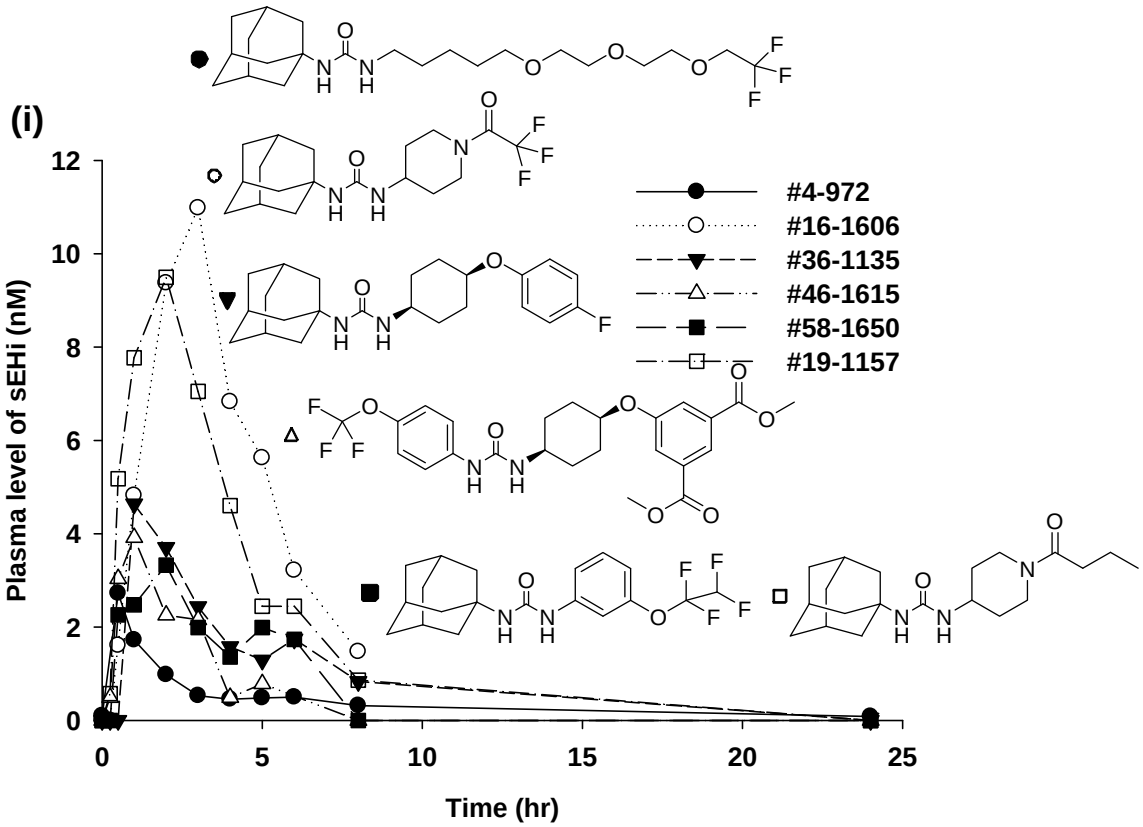

(i)
Plasma level of sEHi (nM)
Time (hr)
#4-972
#16-1606
#36-1135
#46-1615
#58-1650
#19-1157

Fig. S3 – SDS-PAGE of sEH-depleted dog liver cytosol. M: standard marker; C: crude cytosol (1 μg/well); U: unbound cytosol (10 μg/well); E: 1% SDS elution (4 μg/well).

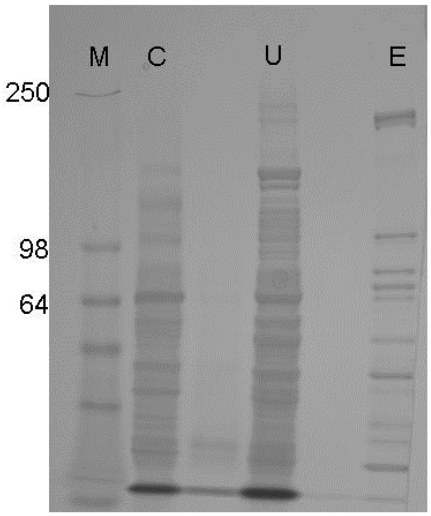
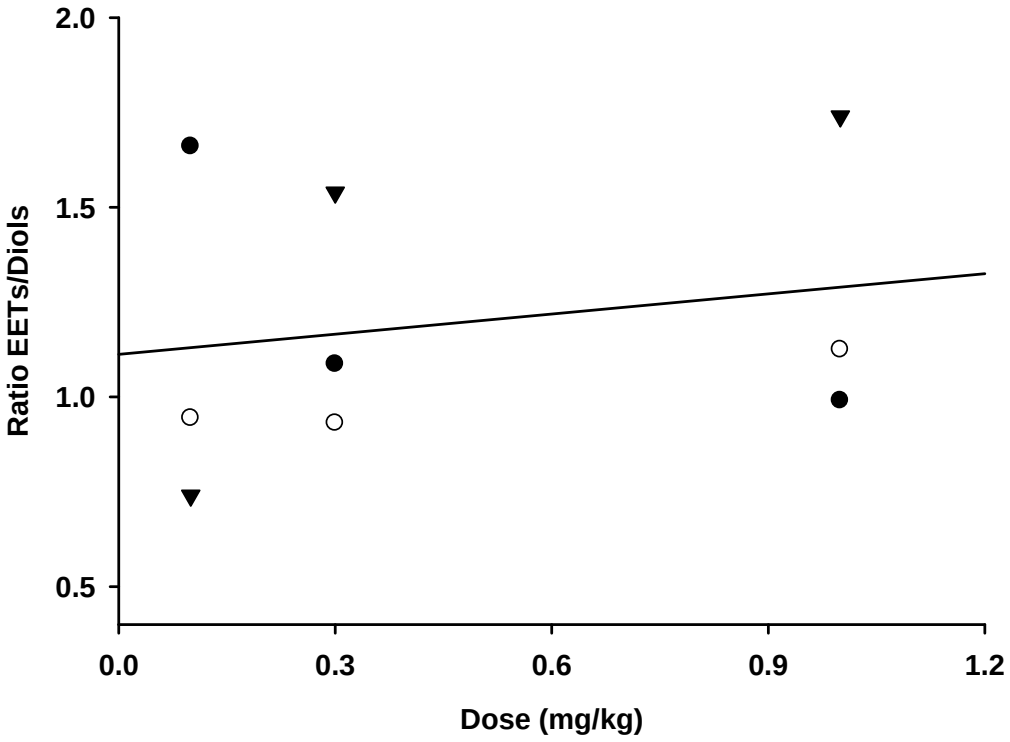


Fig. S4 - Ratios of epoxides and diols vs. dose of t-AUCB 39 at 30 minutes post an oral dose of 0.1, 0.3, and 1 mg/kg body weight approaching linearity (R2 = 0.04). Each datum point represents one dog. The curve approaches linearity with R2=0.04. These data show that epoxide to diol ratio is of limited value in showing target engagement with dogs not suffering from inflammation. This ratio has proven a valuable indicator of target engagement in other species particularly when in an inflammatory condition.



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