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# Design of bioavailable derivatives of 12-(3-adamantan-1-yl-ureido)dodecanoic acid, a potent inhibitor of the soluble epoxide hydrolase

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**Abstract**—The soluble epoxide hydrolase (sEH) plays an important role in the metabolism of endogenous chemical mediators involved in blood pressure regulation and vascular inflammation. 12-(3-Adamantan-1-yl-ureido)-dodecanoic acid (AUDA, **1**) is a very active inhibitor of sEH both in vitro and in vivo. However, its relatively high melting point and limited solubility in either water or oil-based solvents leads to difficulties in formulating the compound and often results in poor in vivo availability. We investigated the effect of derivatization of the acid functional group of inhibitor **1** on the inhibition potencies, physical properties, and pharmacokinetic properties. For human sEH, similar inhibition potency was obtained when the acid of compound **1** was modified to esters (**2**–**15**). The resulting compounds exhibited improved physical properties (23–66 °C lower melting point and 5-fold better solubility in oil). Pharmacokinetic studies showed that the esters possess improved oral bioavailability in mice. On the other hand, amide derivatives of AUDA **1** did not show significant improvement in inhibition potencies or physical properties (higher melting points and lower solubility). The esterification of **1** results in compounds that are easier to formulate in animal food and in triglycerides for gavage and other routes of administration, making it easier to study the biological effects of sEH inhibition in vivo.


## 1. Introduction

In mammals, the soluble epoxide hydrolase (sEH) is involved in the metabolism of endogenous mediators such as epoxides of arachidonic acid,[1–3] linoleic acid,[4] and other lipid epoxides.[5,6] Epoxides of arachidonic acid (epoxyeicosatrienoic acids or EETs) are effective regulators of blood pressure[7] and have anti-inflammatory properties in vivo.[8,9] However, the dihydroxyeicosatrienoic acids (DHETs), the sEH hydrolyzed metabolites of the EETs, often have reduced biological activity, and are more water soluble and more easily conjugated.[7] The blood pressure of spontaneous hypertensive and angiotensin II induced hypertensive rats treated with effective sEH inhibitors is dramatically reduced.[10–13] In addition, the EETs have further vascular protective effects such as anti-inflammatory properties in endothelial cells,[14–17] suppression of reactive oxygen species following hypoxia-reoxygenation,[18] attenuation of vascular smooth muscle migration,[19] and enhancement of a fibrinolytic pathway.[20] In cellular and animal models, the EET-mediated regulation of hypertension and cardioprotective effects are dependent in part on the extent of epoxide hydrolysis by sEH,[4,21] suggesting that the inhibitors of the sEH are worth exploring as pharmaceuticals for the treatment of hypertension, inflammation, and other disorders that can be induced by changing the in vivo concentration of EETs.

1,3-Disubstituted ureas and related compounds are very potent and stable inhibitors of sEH. These compounds

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efficiently reduce epoxide hydrolysis in several in vitro and in vivo models.[11,12,22] However, limited solubility in either water or organic solvents and high melting points of some of these inhibitors likely affect their in vivo efficacy and make formulation difficult.[10,23] Toward solving these problems, we previously showed that a polar functional group on one of the alkyl chains of the urea inhibitors improved solubility in water 2-4-fold while retaining inhibition potency.[24] A polar group such as an ester, alcohol, ether, or ketone located on the fifth/sixth atom from the carbonyl group of the urea function, or an acid function present on the thirteenth atom from the urea pharmacophore were both effective for making soluble inhibitors in either water or oil-based solvents often without a drop in inhibition potencies.[23,24] These functional groups incorporated into lipophilic compounds are also useful in improving the binding selectivity of the inhibitors to the enzyme possibly by establishing additional hydrogen bonding.[25,26] Such compounds, for example, 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA), were used to inhibit sEH in both cultured cells and animals.[27–29] However, AUDA is difficult to formulate, and was administered with a co-solvent (up to 10% of DMSO),[28,29] or in the drinking water with a sizable amount of 2-hydroxypropyl β-cyclodextrin.[27] Unless AUDA and similar compounds are in true solution when administered to animals, the availability is very low. Although a valuable improvement in the solubility was obtained from the inhibitors functionalized with an acid group or another polar group, their relatively high melting points (>100 °C) still limited solubility and speed of dissolution in both water and oil-based solvents, leading to relatively poor observed in vivo oral availability.[23,24,27,28] Therefore, in the present study, we investigated modifications of the acid function of AUDA on the inhibition potency and physical properties (e.g., melting point and solubility) with the goal of improving the availability of AUDA and its pharmacokinetic parameters in mice. We explored two kinds of modification: making alkyl esters to obtain compounds more soluble in oil, and various polar amides to obtain compounds more soluble in water.

## 2. Results and discussion

### 2.1. Synthesis

While the synthesis of compound **1** (12-(3-adamantan-1-yl-ureido)dodecanoic acid; AUDA) was previously described,[24] we report herein a simplified (see Scheme 1), more efficient method of producing this compound. Reaction of 1-adamantyl isocyanate with 12-aminododecanoic acid in 1,2-dichloroethane provided compound **1** in 95–100% yield. However, when this reaction was performed in DMF or THF, approximately 20% of the yield was 1,3-diadamantylurea, a crystalline by-product that has also been reported as a potent inhibitor of sEH.[23] Chlorinated solvents such as 1,2-dichloroethane used for the preparation of **1** effectively produced a pure compound in a high yield without contamination by the bis urea. It must be cautioned that adamantyl isocyanate contains up to 5% of the 1,3-diadamantylurea even when freshly opened under nitrogen. Low concentrations of the 1,3-diadamantyl urea could be removed from the AUDA derivatives by trituration in hexane. Careful recrystallization of the products removes the last traces of the diadamantyl urea. All products were examined for the presence of diadamantyl urea by LC-MS/MS and no products contained more than 0.01% w/w of this impurity.

Scheme 1 shows the syntheses of **1** and also the corresponding ester (**2**–**15**) and amide (**16**–**19**) derivatives. Alkylation of compound **1** with an alkyl or aryl bromide (**2**: methyl, **3**: ethyl, **4**: propyl, **5**: allyl, **6**: propagyl, **7**: isopropyl, **8**: butyl, **9**: 1-methylpropyl, **10**: 2-methylpropyl, **11**: benzyl, and **12**: 2-chlorobenzyl) in the presence of potassium carbonate as a base in DMF afforded the corresponding ester derivatives in 35–95% yield. Coupling of compound **1** with an alcohol or amine (**13**: *tert*-butanol, **14**: 1-adamantylmethanol, **15**: 1-naphthylmethanol, **16**: ethylamine, and **17**: isopropylamine) using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP) in dichloromethane or isobutyl chloroformate and triethylamine in THF yielded the corresponding ester and amide derivatives in 20–95% yield.[24,30]

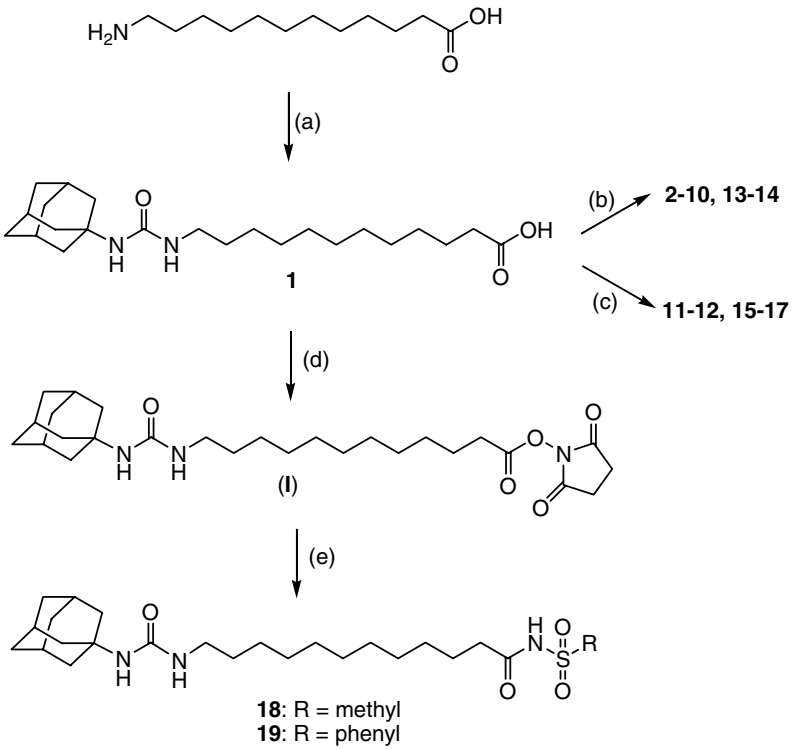
As outlined in Scheme 1, two sulfonamide derivatives (**18** and **19**) were prepared through the dioxopyrrolidinyl ester intermediate (**I**). Coupling of compound **1** with *N*-hydroxysuccinimide using 1,3-dicyclohexylcarbodiimide in THF provided **I** in 72% yield. This activated intermediate (**I**) was reacted with methanesulfonamide (for compound **18**) or benzenesulfonamide (for compound **19**) in the presence of DMAP to yield compounds **18** and **19** in 65–92% yield.

Scheme 2 describes the synthesis of 2-[12-3-(adamantan-1-yl-ureido)dodecanoylamino]-decanoic acid (**22**). Methyl 2-aminodecanoate (**II**) prepared by alkylation of diethyl acetamidomalonate was coupled with compound **1** using *O*-benzotrizol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and diisopropylethylamine (DIEA) in DMF to give the corresponding amide ester (**III**) in 66% yield. Hydrolysis of **III** in the presence of LiOH provided compound **22** in 45% yield.

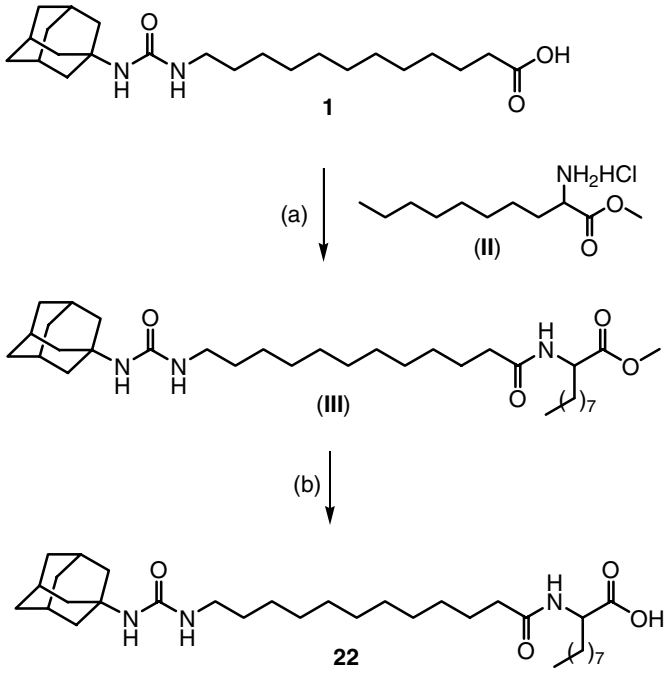
Compound **22** synthesized as shown in Scheme 2 was used for the preparation of compound **20** (Scheme 3). Azidation of protected glucopyranose with trimethylsilyl azide in the presence of tin (**IV**) chloride as a catalyst produced the corresponding azide (**IV**) in 82% yield. Reduction of **IV** under an atmosphere of hydrogen in the presence of palladium on carbon, followed by coupling with compound **22** using HBTU and DIEA in DMF, yielded **V** (59%). The acetyl protecting group of **V** was removed in the presence of sodium in methanol to afford compound **20** (95%). Synthesis of compound **21** was conducted in the same method as that described above from compound **1** instead of compound **22** (64%).

### 2.2. Biological activity

At first, we examined the inhibitory potency of the AUDA ester derivatives using our standard spectral

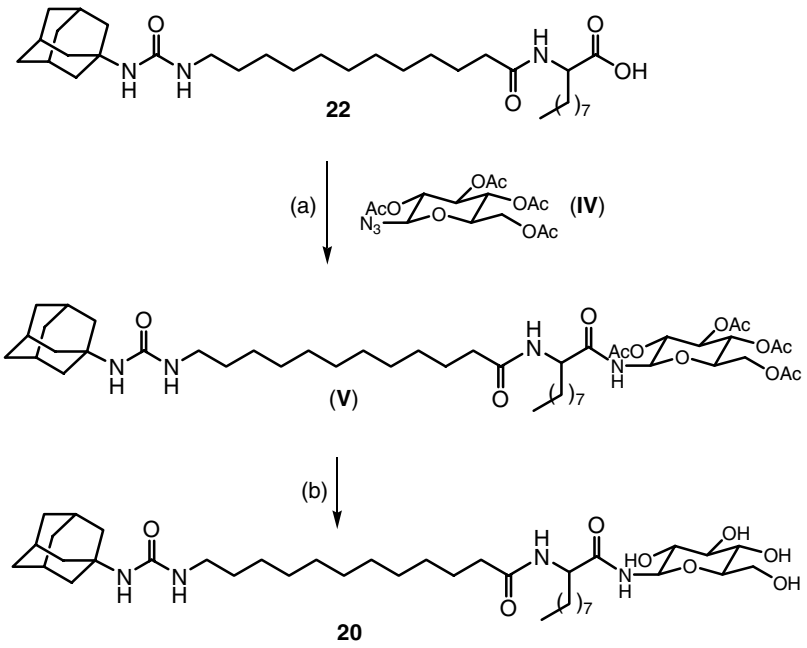



**Scheme 1.** Syntheses of alkyl ester (**2**–**17**) and sulfonamide (**18** and **19**) derivatives of 12-(3-adamantan-1-yl-ureido)dodecanoic acid. Reagents and conditions. (a) 1-adamantyl isocyanate, 1,2-dichloroethane, reflux; (b) alkyl bromide, $K_2CO_3$, DMF, rt; (c) EDCI/DMAP or isobutyl chloroformate/triethylamine, corresponding alcohol or amine; (d) *N*-hydroxysuccinimide, 1,3-dicyclohexylcarbodiimide, THF, rt; (e) methanesulfonamide (for compound **18**) or benzenesulfonamide (for compound **19**), DMAP, HMPA, 90 °C.




**Scheme 2.** Synthesis of 2-[12-3-(adamantan-1-yl-ureido)dodecanoylamino]decanoic acid (**22**). Reagents and conditions: (a) 1—methyl 2-amino decanoate (**II**) was prepared from diethyl acetamido malonate and 1-bromooctane; 2—**II**, HBTU, DIEA, DMF, rt; (b) $LiOH{\cdot}H_2O$, DME, $H_2O$, rt.

assay.[24] However, as seen in Table 1, $IC_{50}$ values for most of the derivatives on the recombinant murine and human enzymes were identical. Because such tight binding inhibitors approach stoichiometric interaction with the target enzyme, the observed $IC_{50}$s, which are in part dependent on enzyme concentration, approach [E]/2. Clearly the spectral assay was not able to distinguish relative potency among these inhibitors. Thus,



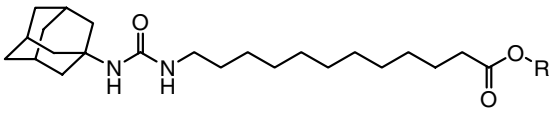

**Scheme 3.** Synthesis of 12-(3-adamantan-1-yl-ureido)dodecanoic acid [1-(2,3,4-trihydroxy-5-hydroxymethyl-cyclohexylcarbamoyl)nonyl]amide (**20**). Reagents and conditions. (a) 1—2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl azide (**IV**) was prepared from 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose in the presence of trimethylsilyl azide (4.24 mL, 32.1 mmol) and tin (**IV**) chloride, 2—**IV**, $H_2$, Pd/C, THF, rt, 3—compound **20**, HBTU, DIEA, DMF, rt. For the preparation of compound **21**, the same methods were used but from compound **1** instead of **22** in the first step.

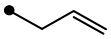
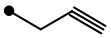
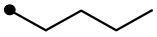
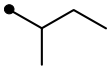
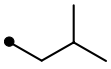
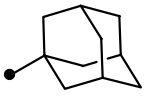
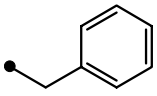
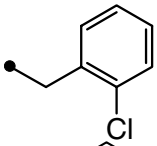
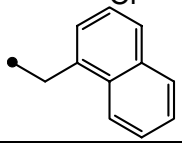
we reran the inhibitors using a recently reported fluorescent-based assay which uses a much lower enzyme concentration and can distinguish among $IC_{50}$s below 50 nM.[31] Like the spectral assay, the fluorescent assay as performed here has a standard error between 10% and 20%, suggesting that differences of twofold or greater are significant. The data in Table 1 illustrated the power of this new assay, and they also demonstrate that synthesis of esters with various alkyl groups (**2**–**15**) produces inhibitors that are as potent as the free acid (AUDA, **1**) on the human sEH. Even the presence of a bulky ester (**12**–**15**) did not influence the inhibitory potency in vitro. Furthermore, all of the ester derivatives in Table 1, except for compound **11** with a *tert*-butyl group, had 23–66 °C lower melting points than the corresponding acid. These results agreed with previous reports that a significant improvement in the inhibition potency and reduction in melting point are observed for esters of an acid functionality of urea inhibitors located around 5–6 atoms away from the urea.[23,24]

Based on the inhibition potency results, in a preliminary experiment we tested the small esters (**2**–**11**) for bioavailability in a fast in vivo assay (1 mouse per compounds). We found that 3–4 carbon ester alcohols (**4** and **8**) give a longer lasting AUDA concentration in the bloodstream than smaller one (**2** and **3**) (results are given in Supplementary data). Also, we observed that branch chain esters (**7**, **9**, and **10**) have beneficial effect compared to straight chain esters (**4** and **8**), while the presence of unsaturation (**5** and **6**) does not lead to major changes. Furthermore, the propargyl alcohol **6** could be toxic. From these results we selected four AUDA esters (compounds **7**–**10**) that are both more potent inhibitors and have a lower melting point than **1**. Their solubility in water and oil was first examined. The more hydrophobic ester inhibitors had 4- to 20-fold lower water solubility than the acid form compound **1** (Table 2), while 2- to 5-fold improvement in oil solubility was observed with the esters. The improved oil solubility would be useful for the formulation of the compounds for delivery for slow release by subcutaneous injection, by gavage, in the food, or following topical treatment. Furthermore, one could envision that the ester derivatives that have better solubilities could be used as prodrugs for AUDA. In general, esters are common functional groups used for the prodrug approach due to its rapid hydrolysis to active agents by esterases in vivo,[32] and prodrugs of numerous drugs, such as meperidine analgesics[33] and an anticancer agent CPT-11,[34,35] are used as esters. Thus, we examined the stability of the ester functionality of compounds **7**–**10** with two recombinant human carboxylesterases (hCE-1 and hCE-2). As shown in Table 2, both enzymes were able to hydrolyze the four esters tested to yield AUDA. Furthermore, the ester hydrolysis was time- and enzyme concentration-dependent as one could expect for a biocatalytic reaction. Overall these experiments suggest that the ester compounds could act as a prodrug-like inhibitor with improved physical properties that may be metabolized in vivo to afford the free acid (**1**) with increased bioavailability.

Finally, we investigated the pharmacokinetics of these four esters in vivo. Each compound dissolved in olive oil was administered to mice via oral gavage at 5 mg/kg body weight. Compared to **1**, this dose corresponds to a molar dose 10% and 13% lower for **7** and **8**–**10**, respectively. The parent esters are undetectable or only

**Table 1.** Inhibition of murine and human sEH by 12-(3-adamantan-1-yl-ureido)dodecanoate derivatives.



| Compound | R | $IC_{50}$ (μM) | | $IC_{50}$ (nM) | Mp[c] (°C) |
|---|---|---|---|---|---|
| | | Murine sEH[a] | Human sEH[a] | Human sEH[b] | |
| **1** | H | 0.05 ± 0.01 | 0.1 ± 0.01 | 3.2 | 114 |
| **2** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 2.3 | 75 |
| **3** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 1.6 | 82 |
| **4** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 1.0 | 86 |
| **5** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 1.0 | 81 |
| **6** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 1.8 | 79 |
| **7** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 1.1 | 90 |
| **8** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 0.8 | 65 |
| **9** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 0.7 | 65 |
| **10** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 1.2 | 91 |
| **11** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 1.3 | 150 |
| **12** |  | 0.05 ± 0.01 | 0.23 ± 0.01 | 1.5 | 68 |
| **13** |  | 0.07 ± 0.01 | 0.13 ± 0.01 | 2.0 | 49 |
| **14** |  | 0.05 ± 0.01 | 0.29 ± 0.01 | 1.7 | 48 |
| **15** |  | 0.09 ± 0.01 | 0.21 ± 0.01 | 2.4 | 52 |

[a] Spectrometric-based assay: enzymes (0.12 μM murine sEH or 0.24 μM human sEH) were incubated with inhibitors for 5 min in 0.1 M sodium phosphate buffer (200 μL; pH 7.4) at 30 °C before spectrometric substrate (NEPC) introduction ([S] = 40 μM), results are means ± SD of three separate experiments.

[b] Fluorescent-based assay: human sEH (0.96 nM) was incubated with inhibitors for 5 min in 25 mM Bis-Tris/HCl buffer (200 μL; pH 7.0) at 30 °C before fluorescent substrate (CMNPC) introduction ([S] = 5 μM), results are averages of three separate measurements.

[c] Melting point.

**Table 2.** Physical properties, hydrolysis, and pharmacokinetic parameters of 12-(3-adamantan-1-yl-ureido)dodecanoic acid alkyl ester derivatives

| Compound | **1** | **7** | **8** | **9** | **10** |
|---|---|---|---|---|---|
| Solubility in water (μg/mL) | 35 | 7 | 5 | 5 | 1.7 |
| Solubility in oil (mg/mL) | 5 | 12 | 25 | 27 | 17 |
| hCE-1 hydrolysis (%)[a] | 100 | 72 | 41 | 50 | 55 |
| hCE-2 hydrolysis (%)[b] | 100 | 39 | 39 | 26 | 33 |
| $C_{max}$ (ng/mL)[c,f] | 26.9 | 29.6 | 39.5 | 13.1 | 42.9 |
| AUC (ng · h/mL)[d,f] | 87 | 84 | 96 | 47 | 110 |
| $T_{1/2}$ (h)[e,f] | 2.3 | 1.9 | 1.5 | 3.8 | 2.9 |
| Relative bioavailability[g] | 1.0 | 1.1 | 1.3 | 0.6 | 1.4 |

[a,b]Percentage (%) of compound **1** produced after 1 h incubation of compounds **1** and **7**–**10** with human carboxylesterase-1 (hCE-1) and human carboxylesterase-2 (hCE-2), respectively. Enzymes (333 nM) were incubated with inhibitors ([S] = 1.0 μM) for 0, 30, and 60 min with data shown for 60 min in sodium phosphate buffer (0.1 M; pH 7.4) at 37 °C. Results are averages of three separate measurements.

[c] Maximum concentration.

[d] Area under the concentration–time curve to terminal time. In each case AUDA **1** was the predominate metabolite detected with the esters only minor products.

[e] Elimination half-life.

[f] Because in each case AUDA **1** was the predominant metabolite with the parent esters only minor products, the parameters were calculated based on the blood concentration of AUDA released from the esters.

[g] Ratio of molar corrected AUC calculated after administration of each ester (**7**–**10**) to the AUC obtained after administration of AUDA **1**.

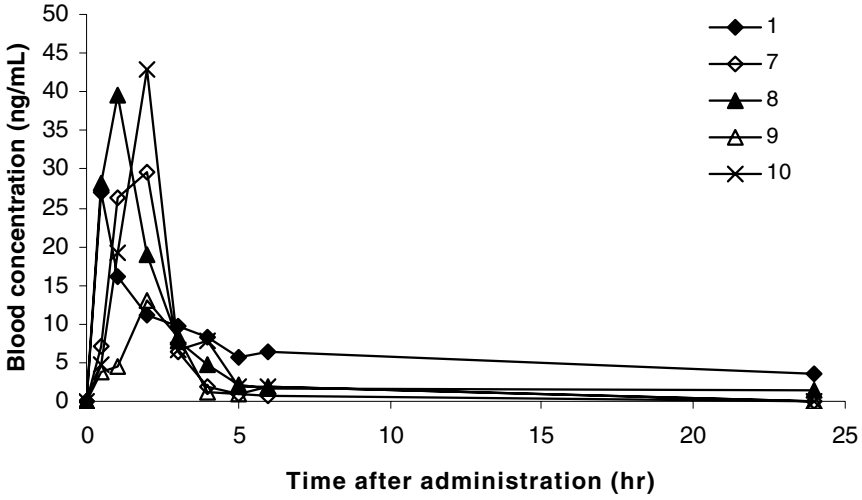


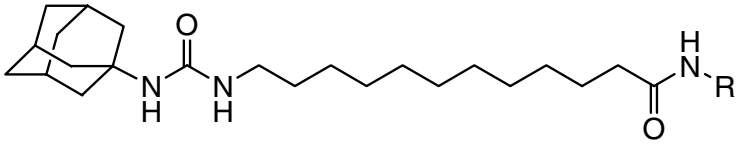

**Figure 1.** Blood concentration of AUDA **1** in mice as a function of time following oral gavage of sEH inhibitors **1** and **7**–**10**. All compounds were dissolved in olive oil and were given at 5 mg/kg body weight. Data are averages of results obtained from three different mice. Standard deviations were between 5% and 10%.

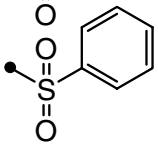
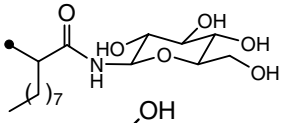
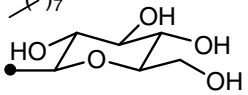
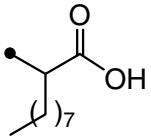
at trace levels in the blood of orally treated animals, but AUDA released from the esters was observed (Fig. 1), confirming that the esters could act as prodrugs for AUDA. In order to determine if the ester derivatives have improved bioavailability, pharmacokinetic parameters such as maximum concentration ($C_{max}$), area under the curve (AUC), and half-life ($T_{1/2}$) were determined using the blood concentrations of AUDA (Table 2). A 1.5-fold increase in $C_{max}$ was observed with a substituent of an *n*-butyl (**8**) or isobutyl group (**10**). Improved AUC results were also determined for these two compounds (**8** and **10**), while a similar or a 2-fold lower $C_{max}$ or AUC was observed for compounds **7** and **9**, respectively, compared to compound **1**. It resulted in a 30–40% increase of the relative bioavailability of AUDA for **8** and **10**. This implies that esterification of the acid function of compound **1** is useful in improving bioavailability, and that *n*-butyl (**8**) or isobutyl (**10**) esters are more effective than other alkyl groups tested for this purpose. Half-lives of AUDA released from the four esters (1.5–3.8 h) were similar to the one (2.3 h) obtained by directly giving AUDA to the animals, as one could expect. The short half-life of AUDA in the blood is likely due to β-oxidation and also movement of the lipophilic compound into refractory pools as both the free acid and biological esters. This may account for the long beta phase of blood clearance. The observed changes in $C_{max}$ and AUC for the various esters tested probably reflect differential absorption by the gut. The esters may encourage penetration of the gut epithelium and thus more rapid and complete movement into the bloodstream. Since no or very low levels of esters were found in the blood, ester hydrolysis could be almost immediate or one could have the more lipophilic esters move quickly from the blood into more refractory pools.

In a second part of this work, we investigated the effect of changing the acid function to an amide on inhibition

potency and physical properties, toward obtaining a more stable and bioavailable sEH inhibitor. For this purpose, seven amide derivatives, of compound **1** with various functionalities were synthesized. As shown in Table 3, alkyl, sulfonyl, lipoamino acid, and glucosyl amide derivatives were prepared, and their inhibition potency on sEHs, melting point, and solubility in water or oil were examined. For murine sEH, there was no change in the inhibition activity when the acid function of compound **1** was substituted by an alkyl (**16** and **17**), sulfonyl (**18** and **19**), glucosyl (**20** and **21**), and lipoamino acid (**22**) groups. Similarly, for human sEH, no big differences in inhibition potencies, which were measured by the spectrophotometric and fluorescent-based assays, were observed when replacing the acid of compound **1** by the substituents except for compound **22** where inhibition of the sEH decreased by 18-fold. When physical properties were examined, no decreased melting point was observed in the alkyl amides (**16** and **17**), while the esters **3** and **7** had 25–32 °C lower melting point than that of compound **1**, suggesting that alkyl amide function is not useful for improving physical properties of the free acid **1**. Because it was expected that a polar sulfonyl group can mimic the carboxylic acid function of compound **1** in binding to the target enzyme, resist β-oxidation, and is also useful for making water soluble compounds, two sulfonamide compounds with a methyl (**18**) or phenyl (**19**) group were prepared. However, in both compounds high melting points were measured, which led to a 3.5-fold decrease in water solubility of compound **18**, and no improvement in oil solubility in either case (**18** and **19**). This implies that the sulfonamides are

**Table 3.** Inhibition and physical properties of murine and human sEH by 12-(3-adamantan-1-yl-ureido)dodecanoic acid substituted amide derivatives



| Compound | R | $IC_{50}$ (μM) | | $IC_{50}$ (nM) | Mp[c] (°C) | Solubility in water (μg/mL) | Solubility in oil (mg/mL) |
|---|---|---|---|---|---|---|---|
| | | Murine sEH[a] | Human sEH[a] | Human sEH[b] | | | |
| **16** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 4.3 | 119 | ND[d] | ND |
| **17** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 2.9 | 115 | ND | ND |
| **18** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 2.1 | 103 | 10 | 4 |
| **19** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 1.9 | 100 | ND | 5 |
| **20** |  | 0.05 ± 0.01 | 0.1 ± 0.01 | 7.5 | 130–140 | 38 | 2 |
| **21** |  | 0.05 ± 0.01 | 0.22 ± 0.01 | 7.6 | 100–102 | ND | ND |
| **22** |  | 0.05 ± 0.01 | 0.21 ± 0.01 | 55 | oil | 63 | ND |

[a] Spectrometric-based assay: enzymes (0.12 μM murine sEH or 0.24 μM human sEH) were incubated with inhibitors for 5 min in 0.1 M sodium phosphate buffer (200 μL; pH 7.4) at 30 °C before substrate (NEPC) introduction ([S] = 40 μM), results are means ± SD of three separate experiments.

[b] Fluorescent-based assay: human sEH (0.96 nM) was incubated with inhibitors for 5 min in 25 mM Bis-Tris/HCl buffer (200 μL; pH 7.0) at 30 °C before substrate (CMNPC) introduction ([S] = 5 μM), results are averages of three separate measurements.

[c] Melting point.

[d] Non-determined.

not effective for improving physical properties of compound **1**. The sulfonamides were insoluble in the triglyceride formulation used for absorption, distribution, metabolism, and elimination experiments described here and in a separate protocol where the compounds were dosed in triglyceride at 0.1 mg/kg body weight. In both cases, the resulting blood levels were very low. We further investigated the use of glycosylation as a method of improving water solubility of compound **1**. The conjugation of sugar moieties such as glucose has the advantage of introducing a highly hydrophobic group in a compact, biologically compatible unit. In addition, the introduction of the glucose moiety at the opposite end of the molecule to the hydrophobic adamantyl group has the potential to provide the resulting molecule with surfactant properties because it now possesses a polar head group and a lipophilic tail. Surfactants are well known to exhibit improved membrane permeability, and thus this property may greatly enhance the delivery of the drug in vivo. The sugar moiety was conjugated to the AUDA (**1**) molecule via an amide linkage that is relatively stable in vivo. As shown in compounds **20** and **21**, higher melting points than that of **1** were observed. Interestingly, no improved water solubility was obtained from the glucosylated compound **20**, which might result from relatively high melting point of the compound although a very polar glucose moiety is present in the molecule. As with sulfonamides, a 2.5-fold decreased solubility in oil was observed in the polar glucose derivative (**20**), suggesting that highly polar functional groups like glucose are not effective for improving physical properties in this series of structures. On the other hand, a huge decrease in melting point was gained when the glucose moiety was removed from the high melting compound **20** (**22**). However, as mentioned above, a huge decrease in the inhibition potency was exhibited by the lipoamino acid compound (**22**), which is 18-fold less active than compound **1**, implying that a free acid function present around sixteen atoms away from the urea function is not alone effective for retaining the inhibition on the human enzyme. When compound **22** was incubated with human carboxylesterases (hCE-1 and -2), no hydrolysis by hCE-1 and -2 was exhibited, indicating that the sterically hindered amide linkage of compound **22** is stable to hydrolysis by esterases. Unless there is hydrolysis by an amidase, there is a very low chance for compound **22** to behave like a prodrug in vivo that is hydrolyzed to produce the active component compound **1**. Although no significant improvements in the physical properties of the amide derivatives listed in Table 3 were observed, their relative stability and inhibition potency are sufficient to encourage the further exploration of other amide compounds to develop bioavailable, potent inhibitors with improved physical properties. We have reported that modification of the urea pharmacophore of potent sEH inhibitors to an amide functionality does not dramatically alter the inhibition potency and that at least a 10-fold improvement in water solubility and a decrease in the melting points of these amide inhibitors are observed.[36] This suggests that the inhibition potency and physical properties of the amide derivatives in Table 3 might be improved with the modification of the urea function to the corresponding amide pharmacophore. The base structure of AUDA (**1**) with carboxylic acid as a tertiary pharmacophore raises the possibility of dramatically altering physical properties with salts, esters of varying hydrophobicity, prodrugs, and isosters.

## 3. Conclusion

This investigation focused on producing potent and bioavailable inhibitors of human sEH by the chemical modification of the acid function of compound **1**. We found that esterification of the acid function of compound **1** did not change the inhibition potency for either murine or human sEHs. Further, the esterification reduced the melting point up to 66 °C and improved solubility in oil (Table 2). This increased solubility makes the resulting compound easier to formulate in oil for oral gavage, subcutaneous or intraperitoneal injection, or in food. Furthermore, in vitro and in vivo measurements clearly showed that compound **1** is readily released from the esters, suggesting that the esters reported herein could be good prodrugs for AUDA (**1**). The esters also slightly improved the pharmacokinetic properties of AUDA (higher $C_{max}$ and AUC) in mice following oral gavage. On the other hand, modification of the acid of compound **1** to an amide function did not provide significant improvement in inhibition potency, and it did not yield valuable improvements in physical properties. Overall, the results indicate that modification of the chemical structure of **1** to esters, such as the *n*-butyl (**8**) or isobutyl (**10**) derivatives is a very useful method to obtain potent, more bioavailable inhibitors for the human sEH. However, the half-life of AUDA was not improved for these two esters, and it may limit their therapeutic utilities for this particular inhibitor. Nevertheless, such derivatives should be of interest for inhibitors that have longer in vivo half-life but poor physical properties. Furthermore, understanding the metabolism of AUDA will highlight the source of its relative in vivo instability and allow the design of more stable derivatives.

The free acid AUDA in aqueous 2-hydroxypropyl β-cyclodextrin or an oil solution of *n*-butyl or other esters of AUDA are simple ways to administer these compounds orally, intraperitoneally, or subcutaneously (see Supplementary data for detailed instructions on formulation). These formulations were successfully used for the inhibition of sEH in vivo in recent work.[9,27,37,38] The *n*-butyl ester of AUDA **8** was used in a number of animal models for a combination of reasons. It has high potency on the human enzyme as both the ester and the acid metabolite. It is inexpensive and can be made in high yield. The melting point (65 °C) is high enough to facilitate purification by recrystallization, but low enough to facilitate rapid dissolution in oil-based formulations. Acceptable pharmacokinetic profiles for **8** were found following oral administration, intraperitoneal and subcutaneous injections in triglyceride. These findings are important for the ease of use of sEH inhibitors with in vivo models of hypertension and inflammatory diseases,

and further for designing inhibitors of improved physical properties and bioavailability for the treatment of the diseases.

## 4. Experimental

### 4.1. 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. Elemental analyses (C, H/N) were performed by Midwest Microlab (Indianapolis, IN); analytical results were within ±0.4% of the theoretical values for the formula given unless otherwise indicated (see Supplementary data for detailed results). $^1$H NMR spectra were recorded on QE-300 using tetramethylsilane as an internal standard. Signal multiplicities are represented as singlet (s), doublet (d), double doublet (dd), triplet (t), quartet (q), quintet (quint), multiplet (m), broad (br), and broad singlet (br s). Synthetic methods are described for representative compounds.

**4.1.1. 12-(3-Adamantan-1-yl-ureido)dodecanoic acid (1).** A mixture of 12-aminododecanoic acid (6.1 g, 28 mmol) and 1-adamantyl isocyanate (5.0 g, 28 mmol) in 1,2-dichloroethane (100 mL) was refluxed for 6 h under $N_2$. After cooling the reaction mixture to room temperature, the solid product produced was filtered, triturated and then washed with hexane (50 mL), and dried in the vacuum oven at 40 °C to afford pure compound **1** as a white solid in 95–100% yield. $^1$H NMR $\delta$ ($CDCl_3$) 1.20–1.36 (16H, m), 1.42–1.48 (2H, m), 1.57–1.65 (6H, m), 1.82–1.90 (6H, m), 1.94–1.98 (3H, m), 2.18 (2H, t, $J$ = 6.9 Hz), 2.90 (2H, q, $J$ = 6.9 Hz), 3.45 (1H, br s), 5.43 (1H, s), 5.58 (1H, s), LC-MS (ESI) $m/z$ calcd for $C_{23}H_{40}N_2O_3$ $[M+H]^+$ 393.30, found $[M+H]^+$ 393.28, mp 114 °C, Anal. ($C_{23}H_{40}N_2O_3$) C, H, N.

**4.1.2. 12-(3-Adamantan-1-yl-ureido)dodecanoic acid butyl ester (8).** To a solution of **1** (0.2 g, 0.51 mmol) in DMF (20 mL) were added potassium carbonate (84 mg, 0.61 mmol) and 1-bromobutane (86 mg, 0.61 mmol), and the reaction mixture was stirred for 24 h at room temperature. The product was extracted with diethyl ether (30 mL) and washed with water (30 mL). Then the organic solution was dried over magnesium sulfate and evaporated. The residue was purified using silica gel column chromatography eluting with hexane and ethyl acetate (5:1) to provide compound **8** as a white solid in 94% yield. $^1$H NMR $\delta$ ($CDCl_3$) 0.95 (3H, t, $J$ = 6.9 Hz), 1.23–1.35 (12H, m), 1.44–1.52 (4H, m), 1.57–1.61 (4H, m), 1.66–1.69 (6H, m), 1.96–2.00 (8H, m), 2.07–2.09 (3H, m), 2.30 (2H, t, $J$ = 6.9 Hz), 3.11 (2H, q, $J$ = 6.9 Hz), 4.02–4.10 (4H, m), LC-MS (ESI) $m/z$ calcd for $C_{27}H_{48}N_2O_3$ $[M+H]^+$ 449.37, found $[M+H]^+$ 449.36, mp 65 °C, Anal. ($C_{27}H_{48}N_2O_3$) C, H, N.

Compounds **2**–**7**, **9**, **10**, **13**, and **14** were prepared in the same method as that used for the preparation of compound **8** by using the corresponding alkyl bromide instead of 1-bromobutane.

**4.1.3. *N*-[12-(3-Adamantan-1-yl-ureido)dodecanoyl]methanesulfonamide (18).** To a solution of compound **1** (0.2 g, 0.51 mmol) and *N*-hydroxysuccinimide (60 mg, 0.56 mmol) in THF (10 mL) were added 1,3-dicyclohexylcarbodiimide (0.12 g, 0.56 mmol) at room temperature. The reaction mixture was stirred for 12 h and filtered. And then, the filtrate was purified by column chromatography (hexane/ethyl acetate = 1:1) to give 2,5-dioxopyrrolidinyl ester (**I**) (0.18 g, 0.37 mmol) in 72% yield. To this intermediate (**I**) dissolved in HMPA (10 mL) was added portionwise 4-dimethylaminopyridine (54 mg, 0.44 mmol; DMAP) and methanesulfonamide (0.35 g, 3.7 mmol). After stirring for 2 h at 90 °C, the product was extracted with ether (30 mL) and washed with water (30 mL). The organic solution was dried over magnesium sulfate and evaporated, and then the residue was purified using column chromatography eluting with hexane and ethyl acetate (1:1) to afford compound **18** (0.16 g, 0.34 mmol) in 92% yield. $^1$H NMR $\delta$ ($CDCl_3$) 1.23–1.35 (12H, m), 1.44–1.52 (4H, m), 1.57–1.61 (2H, m), 1.65–1.69 (6H, m), 1.92–1.98 (6H, m), 2.07–2.09 (3H, m), 2.38 (2H, t, $J$ = 6.9 Hz), 3.11 (2H, q, $J$ = 6.9 Hz), 3.20 (3H, s), 4.40 (1H, s), 4.48 (1H, s), 10.52 (1H, s), LC-MS (ESI) $m/z$ calcd for $C_{24}H_{43}N_3O_4S$ $[M+H]^+$ 469.30, found $[M+H]^+$, mp 103 °C, Anal. ($C_{24}H_{43}N_3O_4S$) C, H, N.

Compound **19** was synthesized with the same method used for the preparation of compound **18** using benzenesulfonamide instead of methanesulfonamide. $^1$H NMR $\delta$ ($CDCl_3$) 1.23–1.35 (12H, m), 1.44–1.52 (4H, m), 1.57–1.61 (2H, m), 1.65–1.69 (6H, m), 1.94–1.98 (6H, m), 2.06–2.09 (3H, m), 2.28 (2H, t, $J$ = 6.9 Hz), 3.10 (2H, q, $J$ = 6.9 Hz), 4.39 (1H, s), 4.93 (1H, s), 5.45 (1H, s), 7.50–7.55 (2H, m), 7.60–7.62 (1H, m), 7.80–7.83 (1H, m), 8.05–8.08 (1H, m), LC-MS (ESI) $m/z$ calcd for $C_{29}H_{45}N_3O_4S$ $[M+H]^+$ 532.31, found $[M+H]^+$ 532.34, mp 100 °C, Anal. ($C_{29}H_{45}N_3O_4S$) C, H, N.

**4.1.4. 2-[12-3-(Adamantan-1-yl-ureido)dodecanoylamino]decanoic acid (22).** Sodium metal (3.9 g, 0.17 mol) was dissolved in ethanol (100 mL) under an inert atmosphere in a round-bottomed flask fitted with a water condenser. Diethyl acetamido malonate (30.4 g, 0.14 mol) was then added followed by 1-bromooctane (36.7 g, 0.19 mol). The solution was refluxed overnight under an inert atmosphere. The reaction mixture was poured onto crushed ice (600 mL) and stirred. The aminodiester product precipitated and was collected by filtration. The crude product was then refluxed overnight in a solution of HCl/DMF (9:1, 200 mL). The precipitated product was collected by filtration, washed with ice water, and air-dried in a vacuum desiccator to afford the α-amino acid hydrochloride in >90% crude yield. The crude amino acid (3.0 g, 24.8 mmol) was then dissolved in methanol (100 mL) and cooled to 0 °C. Thionyl chloride (5.0 mL, 25.8 mmol) was added dropwise, and the reaction mixture was stirred at 0 °C for 10 min and then refluxed

overnight. The reaction mixture was cooled to room temperature and the volatiles removed under reduced pressure, and the crude product was triturated in methanol to afford racemic methyl 2-amino decanoate (**II**), 4.44 g, 89% yield.

Compound **1** (1.04 g, 2.65 mmol) and HBTU (1.0 g, 2.64 mmol) were dissolved in THF (60 mL). DIEA (0.5 mL, 2.87 mmol), DMF (~2 mL), and methyl 2-amino decanoate **II** (1.26 g, 5.30 mmol) were added, and the solution was stirred under $N_2$ at room temperature overnight. The yellow oil produced was diluted with 5% citric acid (100 mL) and extracted with ethyl acetate (3× 50 mL). Organic layers were combined and washed with 5% citric acid (2× 50 mL), saturated sodium bicarbonate ($NaHCO_3$) (2× 50 mL), and brine (1× 50 mL). The organic layer was dried over magnesium sulfate and evaporated to yield an oil. The crude product was purified by column chromatography with 1–2% methanol/dichloromethane to yield a yellow oil **III** (0.77 g, 66% yield). The methyl ester **III** (0.77 g, 1.34 mmol) was dissolved in DME (25 mL) and water (10 mL). Solid $LiOH{\cdot}H_2O$ (0.33 g, 7.86 mmol) was added, and the solution was stirred at room temperature overnight. Reaction mixture was acidified (pH 4) with 5% citric acid (~20 mL), and the product was extracted with ethyl acetate (3× 30 mL). Combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered, and evaporated to yield compound **22** as a yellow oil (0.34 g, 45% yield). $^1$H NMR $\delta$ ($CDCl_3$) 0.82 (3H, t, $J$ = 7 Hz), 1.16–1.23 (28H, br m), 1.41 (2H, m), 1.57 (2H, br m), 1.60 (6H, br s), 1.89 (6H, br s), 2.01 (3H, br s), 2.18 (2H, t, $J$ = 7 Hz), 2.98 (2H, m), 4.52 (1H, dd, $J$ = 7.3, 13.3 Hz), 6.46 (2H, d, $J$ = 7.6 Hz) 8.16 (1H, br s). $^{13}$C NMR $\delta$ ($CDCl_3$) 14.0, 22.5, 25.0, 25.6, 26.7, 28.85, 28.00, 29.06, 29.10, 29.13, 29.24, 29.31, 29.44, 29.56, 31.73, 32.22, 36.27, 36.41, 40.68, 42.29, 50.96, 52.31, 158.70, 173.65, 175.39. LC-MS (ESI) *m/z* calcd for $C_{33}H_{59}N_3O_4$ $[M+H]^+$ 562.45, found $[M+H]^+$ 562.51.

**4.1.5. 12-(3-Adamantan-1-yl-ureido)dodecanoic acid [1-(2,3,4-trihydroxy-5-hydroxymethyl-cyclohexylcarbamoyl)-nonyl]amide (20).** 1,2,3,4,6-Penta-*O*-acetyl-α-D-glucopyranose (5.00 g, 12.8 mmol) was dissolved in 10–15 mL dry $CH_2Cl_2$ under an inert atmosphere. Trimethylsilyl azide (4.24 mL, 32.1 mmol) and tin (**IV**) chloride (0.75 mL, 6.41 mmol) were added, and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was diluted with $CH_2Cl_2$ (30 mL) and washed twice with saturated $NaHCO_3$ (20 mL) and with brine (20 mL). The organic phase was dried, filtered, and concentrated to leave 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (**IV**) as a white solid (3.92 g, 82%). **IV** (0.30 g, 80 mmol) was then dissolved in dry THF (15 mL), and Pd/C was added (~5%) to this solution. The mixture was stirred under $H_2$ overnight. TLC revealed the reduction was completed ($Rf_{azide}$ = 0.80, $Rf_{amine}$ = 0.10). In a separate flask, compound **22** (0.17 g, 0.30 mmol) and 0.5 M HBTU in DMF (1.20 mL, 0.60 mmol) were combined, followed by the addition of DMF (~2 mL) and DIEA (104 μL, 0.60 mmol). This solution was then added to the reaction flask, and the reaction mixture was stirred under $N_2$ overnight. The resulting suspension was filtered through Celite, which was washed well with ethyl acetate (~100 mL), and the filtrate was washed with 5 M HCl (50 mL), saturated $NaHCO_3$ (2× 50 mL), and brine (1× 50 mL). The organic solution was dried over magnesium sulfate, filtered, and concentrated. The residue was purified using a silica gel column chromatography with 10% methanol/dichloromethane to yield **V** (0.16 g, 59.33). $^1$H NMR $\delta$ ($CDCl_3$) 0.78 (3H, t, $J$ = 7 Hz), 1.16–1.23 (32H, br m), 1.35 (2H, m) 1.52 (2H, m) 1.57 (6H, br s), 1.86 (6H, br s,), 1.91–2.00 (15H, m), 2.14 (2H, m), 2.99 (2H, t, $J$= 7 Hz), 3.75 (2H, m), 3.95–4.07 (3H, m), 4.15–4.29 (4H, m), 4.43–4.38 (1H, m) 4.80 (1H, dd, $J$ = 5, 11 Hz), 4.84–4.89 (2H, m), 4.95–5.02 (3H, m), 5.12–5.24 (3H, m), 5.35 (1H, d, $J$ = 3 Hz), 5.47 (1H, t, $J$ = 9.8 Hz), 6.28 (1H, dd, $J$ = 7.6, 19 Hz), 6.60 (1H, d, $J$ = 9.3 Hz), 7.18 (1H, d, $J$ = 9.1 Hz), 7.34 (1H, d, $J$ = 9.1 Hz). LC-MS (ESI) *m/z* calcd for $C_{47}H_{78}N_4O_{12}$ $[M+H]^+$ 891.56, found $[M+H]^+$ 891.69.

To a solution of **V** (0.16 g, 0.18 mmol) in methanol (15 mL) was added sodium metal (0.05 g), and the mixture was stirred at room temperature overnight. The reaction mixture was filtered through amberlite and washed with methanol (~150 mL). The solvent was evaporated under vacuum to produce compound **20** as a fine white powder (0.12 g, 95%). LC-MS (ESI) *m/z* calcd for $C_{39}H_{70}N_4O_8$ $[M+H]^+$ 724.50, found $[M+H]^+$ 724.6, mp 130–140 °C.

Compound **21** was prepared with a method similar to the one used for the preparation of compound **20**, but starting from compound **1** instead of compound **22**.

### 4.2. Enzyme preparation

Recombinant murine and human sEHs, as well as human carboxylesterases (hCE-1 and hCE-2), were produced in a baculovirus expression system and were purified as previously reported.[39–42]

### 4.3. $IC_{50}$ assay conditions

Spectrometric-based assay: enzymes (0.12 μM murine sEH or 0.24 μM human sEH) were incubated with inhibitors for 5 min in 0.1 M sodium phosphate buffer (200 μL; pH 7.4) at 30 °C before spectrometric substrate (4-nitrophenyl-*trans*-2,3-epoxy-3-phenylpropyl carbonate; NEPC) introduction ([S] = 40 μM). The $IC_{50}$s were calculated from at least three separate runs, each in triplicate, to obtain the standard deviation given in the results section.[43]

Fluorescent-based assay: human sEH (0.96 nM) was incubated with inhibitors for 5 min in 25 mM Bis-Tris/HCl buffer (200 μL; pH 7.0) at 30 °C before fluorescent substrate (cyano(2-methoxynaphthalen-6-yl)methyl *trans*-(3-phenyl-oxyran-2-yl)methyl carbonate; CMNPC) introduction ([S] = 5 μM) as that described in a previous report.[31] $IC_{50}$s results are averages of three replicates.

### 4.4. Solubility

Water solubility was determined experimentally in 1.0 mL of sodium phosphate buffer (0.1 M, pH 7.4) at 25 ± 1.5 °C as previously reported.[24,36] Solubility in oil was also determined experimentally (see Supplementary data for details).

### 4.5. Hydrolysis by esterases

Hydrolysis of ester compound (**7**–**10**; 100 μM) to its acid metabolite (**1**) was examined using purified hCE-1 (5 μg) or hCE-2 (5 μg). A percentage of free acid metabolite (**1**) was calculated using LC-MS/MS. Detailed experimental procedures are available in the Supplementary data.

### 4.6. In vivo pharmacokinetic study

Male Swiss Webster mice, 7 weeks old, were treated with test compounds orally at 5 mg/kg body weight. Blood sample preparation and LC-MS/MS analysis were performed as previously reported.[44] Pharmacokinetic parameters ($C_{max}$, AUC, and $T_{1/2}$) were calculated using AUDA blood concentrations. Data are average results obtained from at least three different mice.

## Acknowledgments

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## Supplementary data

Syntheses and detailed analytical data for compounds **2**–**7**, **9**–**17**, and **21**. Detailed experimental procedures on solubility measurement and esterase hydrolysis. This material is available in the online version at http://www.sciencedirect.com. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.09.057.

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