# Design, Synthesis, and Biological Activity of 1,3-Disubstituted Ureas as Potent Inhibitors of the Soluble Epoxide Hydrolase of Increased Water Solubility

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*Received October 10, 2003*

The soluble epoxide hydrolase (sEH) is involved in the metabolism of endogenous chemical mediators that play an important role in blood pressure regulation and inflammation. 1,3-Disubstituted ureas are potent inhibitors of sEH that are active both in vitro and in vivo. However, their poor solubility in either water or lipid reduces their in vivo efficacy and makes them difficult to formulate. To improve these physical properties, the effect of incorporating polar functional groups into one of the alkyl chains was evaluated on their inhibitor potencies, water solubility, octanol/water partition coefficients (log *P*), and melting points. No loss of inhibition potency was observed when a polar functional group was incorporated at least five atoms (∼7.5 Å) from the central urea carbonyl. In addition, the presence of a polar group at least 11 atoms away from the urea carbonyl group for the mouse and human sEHs, respectively, did not alter the inhibitor potency. The resulting compounds have better water solubility and generally lower log *P* values and melting points than nonfunctionalized liphophilic ureas. These properties will make the compounds more bioavailable and more soluble in either water- or oil-based formulations.

## Introduction

Epoxide hydrolases (EHs, EC 3.3.2.3) catalyze the hydrolysis of epoxides or arene oxides to their corresponding diols by the addition of water.[1] There are two well-studied EHs in mammals, the microsomal epoxide hydrolase (mEH) and the soluble epoxide hydrolase (sEH). These enzymes are very distantly related, have different subcellular localization, and have different but partially overlapping substrate selectivities.[2,3] The microsomal EH is known to detoxify a wide array of mutagenic, toxic, and carcinogenic xenobiotic epoxides and it complements the sEH in substrate selectivity.[2,3]

The sEH is also involved in the metabolism of arachidonic acid,[4] linoleic acid,[5] and other lipid epoxides, some of which are endogenous chemical mediators.[6] Epoxides of arachidonic acid (epoxyeicosatrienoic acids or EETs) are known effectors of blood pressure[7] and modulators of vascular permeability.[8,9] Hydrolysis of the epoxides by sEH diminishes this activity.[7] We have reported that treatment with selective sEH inhibitors significantly reduces the blood pressure of spontaneous hypertensive rats (SHRs) or angiotensin II induced hypertension in rats.[10,11] In addition, male knockout sEH mice have significantly lower blood pressure than wild-type mice,[12] further supporting the role of sEH in blood pressure regulation. sEH hydrolysis of EETs also regulates their incorporation into coronary endothelial phospholipids, suggesting a regulation of endothelial function by sEH.[10] The EETs have also demonstrated antiinflammatory properties in endothelial cells.[13,14] In contrast, diols derived from epoxylinoleate (leukotoxin) perturb membrane permeability and calcium homeostasis,[5] which results in inflammation that is modulated by nitric oxide synthase and endothelin-1.[15,16] Micromolar concentrations of leukotoxin reported in association with inflammation and hypoxia[17] depress mitochondrial respiration in vitro[18] and cause mammalian cardiopulmonary toxicity in vivo.[15,19,20] Leukotoxin toxicity presents symptoms suggestive of multiple organ failure and acute respiratory distress syndrome (ARDS).[17] In both cellular and organismal models, leukotoxin-mediated toxicity is dependent upon epoxide hydrolysis,[5,21] suggesting a role for sEH in the regulation of inflammation. The bioactivity of these epoxy-fatty acids suggests that inhibition of *vic*-dihydroxy-lipid biosynthesis may have therapeutic value, making sEH a promising pharmacological target.

We reported 1,3-disubstituted ureas, carbamates, and amides as new, potent, and stable inhibitors of sEH. These compounds are competitive, tight-binding inhibitors with nanomolar $K_I$ values that interact stoichiometrically with purified recombinant sEH.[21] On the basis of the X-ray crystal structure, the urea inhibitors were shown to establish hydrogen bonds and to form salt bridges between the urea function of the inhibitor and residues of the sEH active site, mimicking features encountered in the reaction coordinate of epoxide ring opening by this enzyme.[22,23] These inhibitors efficiently reduced epoxide hydrolysis in several in vitro and in vivo models.[11,21,24] However, these dialkyl ureas have limited solubility in water and high melting points,[10] which likely affect their in vivo efficacy and certainly make formulation difficult. Therefore, we investigated the effect of changes in the structure of 1,3-disubstituted ureas, especially incorporating polar functional groups into one of the alkyl chains, to design potent sEH inhibitors with improved physical properties.

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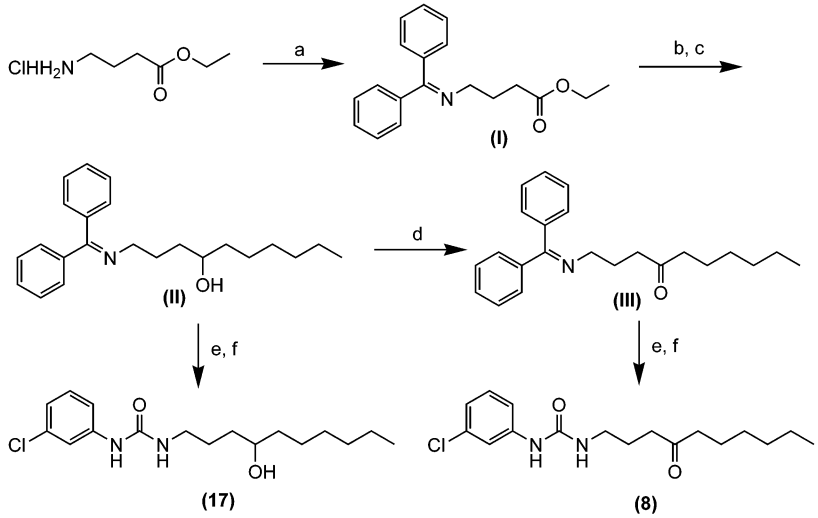
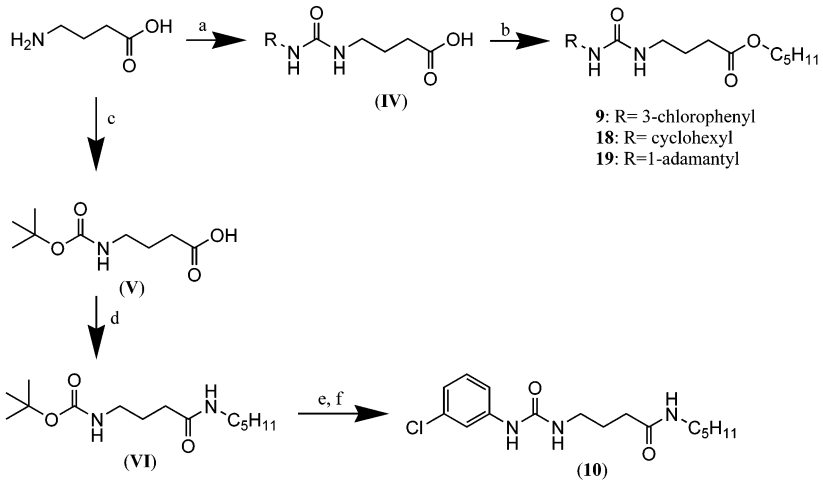
10.1021/jm030514j CCC: $27.50 
Published on Web 03/17/2004

**Scheme 1.** Synthesis of 1-(3-Chlorophenyl)-3-(4-oxo- or -hydroxydecyl)urea (8 and 17)[a]




[a] Reagents and conditions: (a) Benzophenone imine, $CH_2Cl_2$, rt; (b) DIBAL, THF, −78 °C; (c) $Mg/I_2$, bromohexane, THF, rt; (d) acetic anhydride, DMSO, rt; (e) 1 M HCl, rt; (f) 3-chlorophenyl isocyanate, TEA, DMF, rt.

**Scheme 2.** Syntheses of 4-[3-(Aryl or -Cycloalkyl)ureido]butyryl Compounds[a]




[a] Reagents and conditions: (a) aryl or cycloalkyl isocyanate, DMF, rt; (b) bromopentane, $K_2CO_3$, NaI, acetonitrile, reflux; (c) di-*tert*-butyl dicarbonate, dioxane, 50 °C; (d) pentylamine, isobutyl chloroformate, NMM, DMF, rt; (e) 4 M HCl, dioxane; (f) 3-chlorophenyl isocyanate, TEA, DMF, rt.

## Chemistry

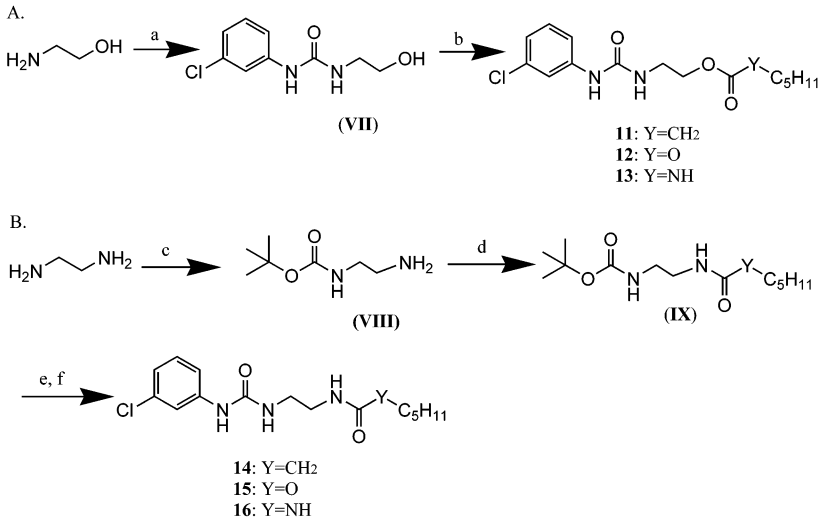
**Syntheses.** Compounds **1**−**7**, **20**, and **21** were prepared according to the procedures reported previously.[25]

Compounds **8** and **17** were synthesized by the procedure outlined in Scheme 1. The benzophenone Schiff base derivative (**I**) was prepared from ethyl 4-aminobutylrate hydrochloride using benzophenone imine in methylene chloride in 61% yield.[26] Treatment of the Schiff base with DIBAL solution in pentane at −78 °C under nitrogen,[27] followed by hexylmagnesium bromide in THF and warming to room temperature, provided the alkylated alcohol (**II**, 60%).[28] Oxidation of the alcohol with acetic anhydride in DMSO gave the corresponding ketone (**III**, 100%).[29] Hydrolyses of **III** and **II** with 1 N HCl aqueous solution yielded the *N*-deprotected amine salts, which reacted with 3-chlorophenyl isocyanate in the presence of TEA in DMF to afford **8** and **17** (30−50%), respectively.

Scheme 2 shows syntheses of 4-[3-(aryl or cycloalkyl)-ureido]butyryl compounds (**9**, **10**, **18**, and **19**). Reaction of 4-aminobutyric acid with 3-chlorophenyl, cyclohexyl, or 1-adamantyl isocyanate gave the corresponding urea acid (**IV**), which was alkylated with bromopentane in the presence of $K_2CO_3$ and NaI (catalytic amount) in acetonitrile to afford 4-[3-(3-chlorophenyl-, -cyclohexyl-, or -adamantan-1-yl)ureido]butyric acid pentyl esters (**9**, **18**, and **19**) in 60−70% yield. For the preparation of **10**, *N*-protection of 4-aminobutyric acid was first carried out by using di-*tert*-butyl dicarbonate and TEA in DMF at 50 °C to provide *tert*-butoxycarbonylated amino acid (**V**, 54%).[30] The acid was then coupled with pentylamine by using NMM and isobutyl chloroformate in DMF to give the amide (**VI**, 33%),[31] followed by *N*-deprotection of the amino group with 4 M hydrochloric acid in dioxane. Reaction of 3-chlorophenyl isocyanate with the amine in the presence of TEA provided 4-[3-(3-chlorophenyl)-ureido]-*N*-pentylbutyramide (**10**) in 100% yield.

The syntheses of 2-[3-(3-chlorophenyl)ureido]ethyl esters (**11**−**13**) are described in part A of Scheme 3 and 2-[3-(3-chlorophenyl)ureido]ethyl compounds with an amide, carbamate, or urea moiety (**14**, **15**, and **16**) were

**Scheme 3.** Syntheses of 2-[3-(3-Chlorophenyl)ureido]ethyl Compounds[a]

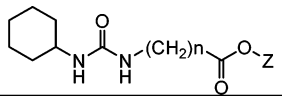



[a] Reagents and conditions: (a) 3-chlorophenyl isocyanate, DMF, rt; (b) heptanoic anhydride (**11**), chloroformic acid pentyl ester (**12**), or pentyl Isocyanate (**13**), TEA, DMF, rt; (c) di-*tert*-butyl dicarbonate, dioxane, rt; (d) heptanoic anhydride (**14**), chloroformic acid pentyl ester (**15**), or pentyl isocyanate (**16**), DMF, rt; (e) 4 M HCl, dioxane; (f) 3-chlorophenyl isocyanate, TEA, DMF, rt.

synthesized by the procedure outlined in part B of Scheme 3. Reaction of 2-aminoethanol with 3-chlorophenyl isocyanate in DMF gave urea alcohol **VII** (40%). Alkylation of the alcohol (**VII**) with heptanoic anhydride, chloroformic acid pentyl ester, or pentyl isocyanate in the presence of TEA afforded **11** (Y = $CH_2$), **12** (Y = O), and **13** (Y = NH) in 70−85% yield, respectively. For the syntheses of aminoethyl ureas (B in Scheme 3), first mono-*N*-protected diamine (**VIII**) was prepared by using di-*tert*-butyl dicarbonate in dioxane in 95% yield,[32] which was followed by alkylation with heptanoic anhydride, chloroformic acid pentyl ester, or pentyl isocyanate to provide **IX** (67%). *N*-Deprotection with 4 M HCl in dioxane and reaction with 3-chlorophenyl isocyanate afforded **14** (Y = $CH_2$), **15** (Y = O), and **16** (Y = NH) in 70−85% yield.

From 4-(3-adamantan-1-yl-ureido)butyric acid (**22**) prepared by the reaction of 4-aminobutanoic acid and 1-adamantyl isocyanate in DMF, various [4-(3-adamantan-1-yl-ureido)butyryloxy derivatives in Tables 5 and 6 were synthesized (Scheme 4). Alkylation of compound **22** with iodomethane in the presence of $K_2CO_3$ in DMF provided the methyl ester of compound **22** (**23**) in 95% yield. Five ethyl bromoalkanoates reacted with compound **22** to afford **29** (*n* = 1), **30** (*n* = 2), **31** (*n* = 3), **32** (*n* = 4), and **24** (*n* = 6) in a range of 30−95% yield. Coupling of compound **22** with 3,7-dimethyl-oct-6-en-1-ol in the presence of DMAP as a base by using ECDI gave compound **25** in 65% yield.[33] Ethyl 8-bromooctanoate, which was prepared by the coupling reaction of 8-bromooctanoic acid and ethanol using EDCI and DMAP, reacted with compound **22** in the presence of $K_2CO_3$ as a base in DMF to give compound **33** (75%). For the syntheses of compounds **34**, **35**, and **36**, 10-hydroxydecanoic acid, 11-hydroxyundecanoic acid, and 12-hydroxydodecanoic acid were used as starting materials, respectively. First, the $\omega$-hydroxyalkanoic acids were esterified with bromoethane in the presence of lithium carbonate as a base in DMF, which were coupled with compound **22** by using EDCI and DMAP in DMF to provide **34** (*n* = 9), **35** (*n* = 10), and **36** (*n* = 11) in 50−60% yield.

**Table 1.** Inhibition of Mouse and Human sEH by 1-cycloalkyl-3-alkylurea

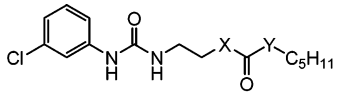


| No. | n | Z | $IC_{50}$ (μM)[a] Mouse sEH | $IC_{50}$ (μM)[a] Human sEH |
|---|---|---|---|---|
| **1** | 1 | H | >500 | >500 |
| **2** | 1 | $CH_3$ | 33 ± 2 | 70 ± 6 |
| **3** | 2 | H | 122 ± 2 | 358 ± 2 |
| **4** | 2 | $CH_3$ | 2.5 ± 0.1 | 78 ± 4 |
| **5** | 3 | H | >500 | >500 |
| **6** | 3 | $CH_3$ | 0.33 ± 0.03 | 6.2 ± 0.5 |
| **7** | 5 | H | 90 ± 3 | 253 ± 8 |

[a] Enzymes (0.12 μM mouse sEH and 0.24 μM human sEH) were incubated with inhibitors for 5 min in sodium phosphate buffer (pH 7.4) at 30 °C before substrate introduction ([S] = 40 μM). Results are means ± SD of three separate experiments.

Scheme 5 (A and B) describes the syntheses of 4-[4-(3-adamantan-1-yl-ureido)butyryloxy]aryl derivatives. Esterification of 4-formylbenzoic acid with bromoethane and $K_2CO_3$ in acetonitrile yielded ethyl 4-formylbenzoate, followed by reduction of the aldehyde by using sodium borohydride in ethanol. The reduced alcohol was coupled with compound **22** by using EDCI and DMAP

**Table 2.** Inhibition of Mouse and Human sEH by 1-(3-Chlorophenyl)-3-(2-alkylated ethyl)ureas



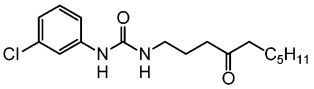
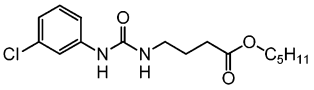
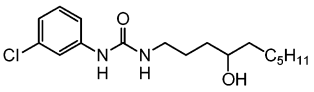
| No. | X | Y | $IC_{50}$ (μM) [a] | |
|---|---|---|---|---|
| | | | Mouse sEH | Human sEH |
| **8** | $CH_2$ | $CH_2$ | 0.41 ± 0.05 | 2.1 ± 0.2 |
| **9** | $CH_2$ | O | 0.37 ± 0.04 | 2.1 ± 0.07 |
| **10** | $CH_2$ | NH | 7.2 ± 0.9 | 32 ± 0.8 |
| **11** | O | $CH_2$ | 7.7 ± 0.6 | 26 ± 1 |
| **12** | O | O | 7.6 ± 0.3 | 22 ± 1 |
| **13** | O | NH | 5.3 ± 0.1 | 18 ± 0.9 |
| **14** | NH | $CH_2$ | 100 ± 10 | >100 |
| **15** | NH | O | 78 ± 6 | >100 |
| **16** | NH | NH | 110 ± 20 | >100 |

[a] Enzymes (0.12 μM mouse sEH and 0.24 μM human sEH) were incubated with inhibitors for 5 min in sodium phosphate buffer (pH 7.4) at 30 °C before substrate introduction ([S] = 40 μM). Results are means ± SD of three separate experiments.

to afford compound **27** (Scheme 5A) in 46% yield. 4-Hydroxybenzoic acid, 4-hydroxyphenylacetic acid, and 4-hydroxy acrylic acid were coupled with compound **22** by using EDCI and DMAP, respectively, which were alkylated with bromoethane and $K_2CO_3$ in DMF to give compounds **28** and **26** in 20−50% yield (Scheme 5B).

**Octanol/Water Partition Coefficients (*P*).** Partition coefficients were determined using the following procedure at 23 ± 1.5 °C. The test compound (**18**−**23**) was dissolved in buffer-saturated octanol (5 mL). Octanol-saturated sodium phosphate buffer, 0.1 M, pH 7.4 (5 mL), was then added to the solution of octanol, and the mixture was equilibrated during 24 h of shaking, followed by centrifugation (5 min, 200*g*). The octanol fraction (2.5μL) was dissolved in 0.5 mL of methanol, and to the methanol solution was added 0.5 mL of internal standard solution (1-cyclohexyl-3-tetradecylurea (CTU); 1000 ng/mL in methanol). A regression curve for each compound was obtained from five standard stock solutions ($r$ = 0.99) by using LC−MS/MS [a Waters 2790 liquid chromatograph equipped with a 30 × 2.1 mm 3 μm C18 Xterra column (Waters) and a Micromass Quattro Ultima triple quadrupole tandem mass spectrometer (Micromass, Manchester, UK)]. The absolute amount of each compound obtained by LC−MS/MS analysis was converted to a concentration (M), and the experimental log *P* value (eLogP) was obtained with the following equation: eLogP = log [octanol]/[water]. On the basis of the log *P* value, log *S* value (solubility in water) was also obtained with the following equation, which has been suggested by Banerjee et al.[34]: log $P$ = 6.5 − 0.89(log $S$) − 0.01(melting point). The cLogP value estimated by Crippen's method was generated by using CS ChemDraw Ultra version 6.0. The results reported in Table 4 are averages of triplicate analyses.

**Table 3.** Inhibition of Mouse and Human sEH by 1-(3-Chlorophenyl)-3-(4-hydroxydecyl)urea (17)

| No. | Structure | $IC_{50}$ (μM) [a] | |
|---|---|---|---|
| | | Mouse sEH | Human sEH |
| **8** |  | 0.41 ± 0.05 | 2.1 ± 0.2 |
| **9** |  | 0.37 ± 0.04 | 2.1 ± 0.07 |
| **17** |  | 0.14 ± 0.05 | 3.1 ± 0.3 |

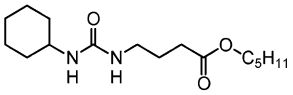
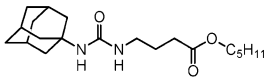
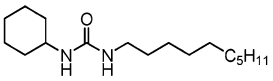
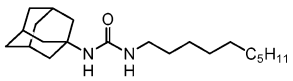
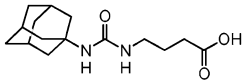
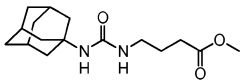
[a] Enzymes (0.12 μM mouse sEH and 0.24 μM human sEH) were incubated with inhibitors for 5 min in sodium phosphate buffer (pH 7.4) at 30 °C before substrate introduction ([S] = 40 μM). Results are means ± SD of three separate experiments.

**Water Solubility (*S*, mg/mL).** Water solubility was determined using the following procedure at 23 ± 1.5 °C. An excess of the test compound (**18**−**23**) was added to a vial containing sodium phosphate buffer, 0.1 M, pH 7.4 (1 mL), and a suspension of the mixture was equilibrated during 24 h of shaking, followed by centrifugation (5 min, 200*g*). The water supernatant (2.5μL) was dissolved in 0.5 mL of methanol, and to the methanol solution was added 0.5 mL of internal standard solution (CTU; 1000 ng/mL in methanol). Then, the absolute amount of each compound was obtained in the same procedure as described above.

**Enzyme Preparation.** Recombinant mouse sEH and human sEH were produced in a baculovirus expression system and purified by affinity chromatography.[35−37] The preparations were at least 97% pure as judged by SDS−PAGE and scanning densitometry. No detectable esterase or glutathione transferase activities, which can interfere with this sEH assay, were observed.[38] Protein concentration was quantified by using the Pierce BCA assay using Fraction V bovine serum albumin as the calibrating standard.

**$IC_{50}$ Assay Conditions.** $IC_{50}$ values were determined as described by using racemic 4-nitrophenyl-*trans*-2,3-epoxy-3-phenylpropyl carbonate as substrate.[38] Enzymes (0.10 μM mouse sEH or 0.20 μM human sEH) were incubated with inhibitors for 5 min in sodium phosphate buffer, 0.1 M, pH 7.4, containing 0.1 mg/mL

**Table 4.** Inhibition of Mouse and Human sEH by 1-Cylclohexyl or adamantyl-3-alkylureas

| No. | Structure | $IC_{50}$ (μM) [a] | | S [b] (mg/mL) | eLog P [c] (cLog P) [d] | MP (°C) | Log S [e] |
|---|---|---|---|---|---|---|---|
| | | Mouse sEH | Human sEH | | | | |
| **18** |  | 0.05 ± 0.01 | 1.02 ± 0.05 | 1.51 ± 0.28 | 0.82 ± 0.18 (2.49 ± 0.47) | 63 | 5.32 |
| **19** |  | 0.05 ± 0.01 | 0.17 ± 0.01 | 1.69 ± 0.37 | 0.44 ± 0.13 (2.77 ± 0.47) | 114 | 4.89 |
| **20** [f] |  | 0.05 ± 0.01 | 0.14 ± 0.01 | 0.44 ± 0.06 | 5.44 ± 0.30 (4.48 ± 0.47) | 85 | -0.21 |
| **21** [g] |  | 0.05 ± 0.01 | 0.10 ± 0.01 | 0.62 ± 0.05 | 5.25 ± 0.20 (4.76 ± 0.47) | 105 | -0.37 |
| **22** |  | 22 ± 1 | 37 ± 1 | 7.06 ± 0.26 | 0.14 ± 0.06 (0.85 ± 0.47) | 165 | 4.37 |
| **23** |  | 0.10 ± 0.01 | 1.6 ± 0.1 | 1.66 ± 0.14 | 0.18 ± 0.09 (1.12 ± 0.47) | 114 | 5.18 |

[a] Enzymes (0.12 μM mouse sEH and 0.24 μM human sEH) were incubated with inhibitors for 5 min in sodium phosphate buffer (pH 7.4) at 30 °C before substrate introduction ([S] = 40 μM). Results are means ± SD of three separate experiments. [b] Solubility in sodium phosphate buffer (0.1 M, pH 7.4) at 25 °C. Results are means ± SD of three separate experiments. [c] eLogP: experimental log *P* (log [octanol]/[water]). Sodium phosphate buffer (0.1 M, pH 7.4) was used as an aqueous phase. Results are means ± SD of three separate experiments. [d] cLogP: log *P* calculated by Crippen's method by using CS ChemDraw version 6.0. [e] Solubility in water. It was calculated according to the following equation suggested by Banerjee et al.: log $P = 6.5 - 0.89(\log S) - 0.015mp$, where mp is melting point. [f] $^1$H NMR δ ($CDCl_3$) 0.88 (3H, t, $J = 6.9$ Hz), 1.11−1.16 (2H, m) 1.26−1.37 (16H, m), 1.46−1.48 (2H, m), 1.58−1.60 (2H, m), 1.67−1.72 (2H, m), 1.91−1.95 (2H, m), 3.13 (2H, q, $J = 6.9$ Hz), 3.45−3.55 (1H, m), 4.19 (1H, s), 4.27 (1H, s); LC−MS (ESI) *m/z* calcd for $C_{17}H_{34}N_2O$ $[M + H]^+$ 283.27, found $[M + H]^+$ 283.27. [g] $^1$H NMR δ ($CDCl_3$) 0.88 (3H, t, $J = 6.9$ Hz), 1.16−1.36 (12H, m), 1.44−1.49 (2H, m), 1.58−1.59 (2H, m), 1.66−1.68 (6H, m), 1.90−1.96 (6H, m), 2.05−2.07 (3H, m), 3.09 (2H, q, $J = 6.9$ Hz), 3.98 (1H, s), 4.03 (1H, s); LC−MS (ESI) *m/z* calcd for $C_{21}H_{38}N_2O$ $[M + H]^+$ 335.30, found $[M + H]^+$ 335.29.

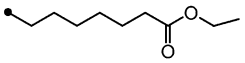
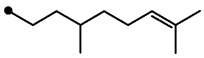
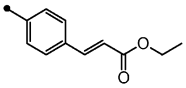
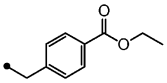
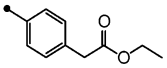
of BSA, at 30°C before substrate introduction ([S] = 40 μM). Activity was assessed by measuring the appearance of the 4-nitrophenolate anion at 405 nm at 30 °C during 1 min (Spectramax 340 PC, Molecular Devices). Assays were performed in triplicate. $IC_{50}$ is a concentration of inhibitor that reduces enzyme activity by 50% and was determined by regression of at least five datum points with a minimum of two points in the linear region of the curve on either side of the $IC_{50}$. The curve was generated from at least three separate runs, each in triplicate, to obtain the standard deviation (SD) given in Tables 1−7.

## Results and Discussion

Recently, we described that 1,3-disubstituted ureas (and related carbamates and amides) with various alkyl, cycloalkyl, and aryl groups are potent sEH inhibitors.[21,25,39] These urea inhibitors efficiently reduced epoxide hydrolysis in several in vitro and in vivo models. However, the lipophilicity of these ureas caused limited solubility in water, which probably affects their in vivo efficacy. Often high lipophilicity results in undesirable pharmacokinetic properties and a lack of specificity, since lipophilic molecules have high affinity for hydrophobic regions on many biological molecules. In addition, the stability of the crystal structure of our first-generation compounds, indicated by their high melting point, led to a general lack of solubility, even in organic solvents. These properties make it difficult to formulate the compounds in either an aqueous or oil base.

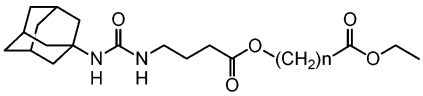
The addition of a polar functional group at the end of a long aliphatic chain of urea inhibitors made compounds less liphophilic while their inhibitory potencies were retained.[25] To further improve the physical property of urea inhibitors, we first investigated the effect of the addition of polar acid and corresponding methyl ester moieties onto short aliphatic chains (less than seven carbons) (Table 1). The free carboxylic acids (**1**, **3**, **5**, and **7**) failed to show valuable inhibition activity for either murine or human sEHs. However, the conversion of the carboxylic acid function to the methyl ester (**2**, **4**, and **6**) increased inhibition potency for both mouse and human sEHs, indicating that the free acid functionality at the end of short aliphatic chains negatively impacts inhibitor potency. In particular, the methyl ester of butanoic acid (**6**) showed 8−100-fold higher activity for both enzymes than the esters of acetic and propanoic acids (**2** and **4**), suggesting that a polar

**Table 5.** Inhibition of Mouse and Human sEH by 4-(3-Adamantan-1-ylureido)butyryloxy Compounds

| No. | R: | $IC_{50}$ (μM) [a] Mouse sEH | $IC_{50}$ (μM) [a] Human sEH | cLog P[b] |
|---|---|---|---|---|
| **24** |  | 0.05±0.01 | 0.12±0.01 | 2.81 ± 0.47 |
| **25** |  | 0.05 ± 0.01 | 0.11 ± 0.01 | 4.21 ± 0.47 |
| **26** |  | 0.05 ± 0.01 | 0.20 ± 0.01 | 3.28 ± 0.47 |
| **27** |  | 0.05 ± 0.01 | 0.10 ± 0.01 | 3.01 ± 0.47 |
| **28** |  | 0.05 ± 0.01 | 0.14 ± 0.01 | 2.88 ± 0.47 |

[a] Enzymes (0.12 μM mouse sEH and 0.24 μM human sEH) were incubated with inhibitors for 5 min in sodium phosphate buffer (pH 7.4) at 30 °C before substrate introduction ([S] = 40 μM). Results are means ± SD of three separate experiments. [b] cLogP: log *P* calculated by Crippen's method by using CS ChemDraw version 6.0.

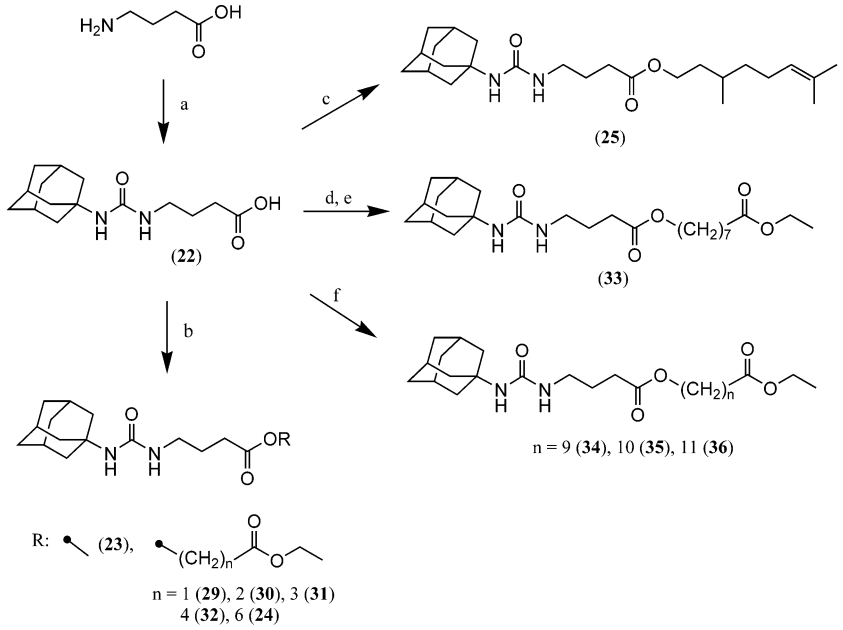
**Table 6.** Inhibition of Mouse and Human sEH by 4-(3-Adamantan-1-ylureido)butyryloxy Compounds



| No. | n | $T_A$[a] | Mouse sEH[b] $IC_{50}$ (μM) | Mouse sEH[b] $IC_{90}$ (μM) | Human sEH[b] $IC_{50}$ (μM) | Human sEH[b] $IC_{90}$ (μM) | MP (°C) | cLog P[c] |
|---|---|---|---|---|---|---|---|---|
| **29** | 1 | 8 | 0.05±0.01 | 0.11±0.01 | 0.39±0.01 | 9±2 | 123 | 0.98±0.47 |
| **30** | 2 | 9 | 0.05±0.01 | 0.63±0.02 | 0.54±0.05 | 9±2 | 95-97 | 1.27±0.47 |
| **31** | 3 | 10 | 0.05±0.01 | 0.16±0.01 | 0.12±0.01 | 5.0±0.1 | 89-91 | 1.55±0.47 |
| **32** | 4 | 11 | 0.05±0.01 | 0.10±0.01 | 0.13±0.01 | 1.5±0.1 | 84-86 | 1.97±0.47 |
| **24** | 6 | 13 | 0.05±0.01 | 0.13±0.01 | 0.12±0.01 | 0.81±0.01 | 65-67 | 2.81±0.47 |
| **33** | 7 | 14 | 0.05±0.01 | 0.16±0.02 | 0.11±0.01 | 0.72±0.01 | 58-59 | 3.22± .47 |
| **34** | 9 | 16 | 0.05±0.01 | 0.26±0.03 | 0.10±0.01 | 0.68±0.01 | 60-61 | 4.06±0.47 |
| **35** | 10 | 17 | 0.05±0.01 | 0.35±0.05 | 0.10±0.01 | 1.2±0.1 | 54-55 | 4.48±0.47 |
| **36** | 11 | 18 | 0.05±0.01 | 0.63±0.04 | 0.10±0.01 | 1.8±0.2 | 64-65 | 4.89±0.47 |

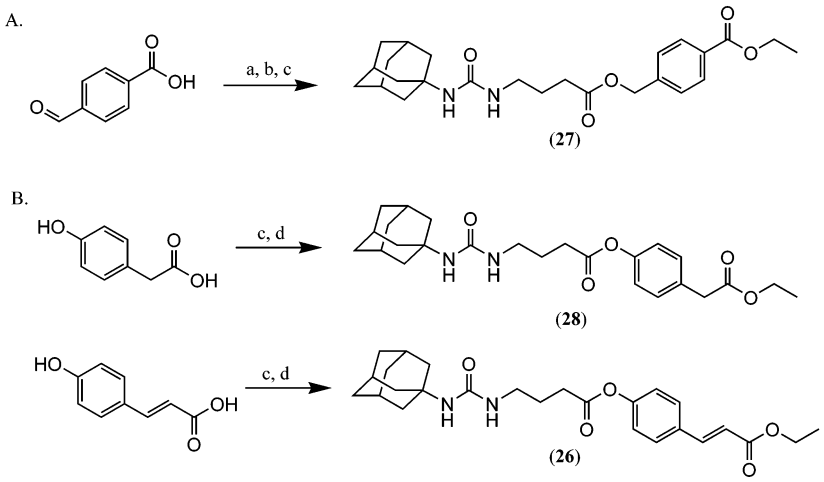
[a] The total number of atoms extending from the carbonyl group of the primary urea pharmacophore, $T_A = n + 7$. [b] Enzymes (0.12 μM mouse sEH and 0.24 μM human sEH) were incubated with inhibitors for 5 min in sodium phosphate buffer (pH 7.4) at 30 °C before substrate introduction ([S] = 40 μM). Results are means ± SD of three separate experiments. [c] cLogP: log *P* calculated by Crippen's method by using CS ChemDraw version 6.0.

**Scheme 4.** Syntheses of 4-(3-Adamantan-1-yl-ureido)butyryloxy Compounds[a]




[a] Reagents and conditions: (a) 1-adamantyl isocyanate, DMF, rt; (b) iodomethane (**23**) or bromoalkanoic acid ethyl esters, $K_2CO_3$, DMF, rt; (c) 3,7-dimethyl-oct-6-en-1-ol, DMAP, EDCI, DMF, rt; (d) 8-bromooctanoic acid, ethanol, DMAP, EDCI, DMF, rt; (e) bromoethane, $K_2CO_3$, DMF, rt; (f) 10-hydroxydecanoic acid (**34**), 11-hydroxyundecanoic Acid (**35**), or 12-hydroxydodecanoic acid (**36**), bromoethane, $Li_2CO_3$, DMF, 70 °C. The corresponding ethyl esters were coupled with **22** by Using DMAP and EDCI.

**Scheme 5.** Syntheses of 4-[4-(3-Adamantan-1-yl-ureido)butyryloxy]aryl Compounds[a]




[a] Reagents and conditions: (a) bromoethane, $K_2CO_3$, acetonitrile, reflux; (b) $NaBH_4$, ethanol, rt; (c) **22**, DMAP, EDCI; (d) bromoethane, $K_2CO_3$, DMF, rt.

functional group located on the fifth atom (approximately 7.5 Å) from the carbonyl group of the primary urea pharmacophore may be effective for preparing potent sEH inhibitors of improved water solubility (Figure 1).

As one adds polar groups to lipophilic inhibitors, the biological activity is often dramatically reduced, because the water shell around the polar functionality reduces binding at the active site. However, if the new polar groups can hydrogen bond to the enzyme within the active site, without disturbing the binding of the primary pharmacophore, this offsets the energy required to strip the water shell. Thus, the addition of an additional polar group, which binds within the protein target, can be expected to dramatically increase the specificity of the resulting inhibitor. On the basis of the above results, we designed urea compounds with a polar carbonyl group on the fifth atom (approximately 7.5 Å) from the primary urea pharmacophore to improve the water solubility of lipophilic sEH inhibitors. Table 2 shows various functionalities, including ketone, ester, amide, carbonate, carbamate, and urea, that contribute a carbonyl group, and we term these groups as secondary pharmacophores (Figure 1). Because in this kind of time-consuming combinatorial chemistry, a fast monitor of chemical reactions is required; as shown in Table 2, we selected a 3-chlorophenyl group as one of substituents of the urea pharmacophore, which is useful for monitoring chemical reactions quickly on a TLC plate and produces sufficient inhibition to compare relative activity. For example, the 3-chlorophenyl urea is 2-fold less potent than the cyclohexyl urea with the recombinant murine and human enzymes. After optimizing the secondary pharmacophore, the aryl substituent can be

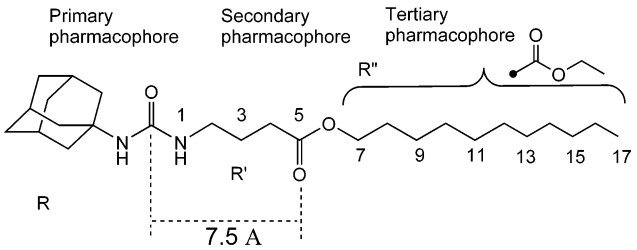



**Figure 1.** A representative sEH inhibitor with primary, secondary, and tertiary pharmacophores indicated. The trivial nomenclature used to describe the inhibitors in this study consists of these three polar pharmacophores and three R groups (R). The primary pharmacophore consists of a polar carbonyl group shown in this case as a urea with R = adamantane. The secondary pharmacophore consists of a polar carbonyl group located on the fifth atom (7.5 ± 1 Å) from the carbonyl group of the primary pharmacophore. A second R group, termed R′, joins these two pharmacophores. The tertiary pharmacophore used in this study is another carbonyl shown, above as an ethyl ester group. It is illustrated linearly from the carbonyl group of the primary pharmacophore. The tertiary pharmacophore is located between the eighth and 16th atoms from the primary phamacophore and isseparated from the secondary pharmacophore by R″. In the current study R″ consists of an aliphatic alkyl carbon or substituted aryl carbon chain.

replaced by a cyclohexyl, adamantyl or other group leading to more potent inhibitors.[25] When the left of the carbonyl (X) of the secondary pharmacophore is a methylene carbon, the best inhibition was obtained if a methylene carbon (ketone, **8**) or oxygen (ester, **9**) is present in the right position (Y). The presence of a nitrogen (amide, **10**) dropped the activity by >10-fold. In compounds with an oxygen to the left of the carbonyl group (X), a >10-fold drop in activity was observed and there was not any change in the activity even if the right position, Y, was modified with a methylene carbon (ester, **11**), oxygen (carbonate, **12**), or nitrogen (carbamate, **13**), respectively. All compounds (**14**, **15**, and **16**) with a nitrogen in the left position had >200-fold lower activities than **8** or **9**. Comparing compounds **9** and **11**, the presence of a methylene carbon around the carbonyl group showed a very different effect on the inhibition activity. The compound with a methylene carbon to the left of the carbonyl (**9**) showed a 20-fold better inhibition than that to the right (**11**). While the rank-order potency of this inhibitor series was equivalent with mouse and human sEHs, a 3−5-fold higher inhibition potency was observed for the murine enzyme. In addition, when the carbonyl group of compound **8** located on the fifth atom from the urea pharmacophore was modified to the corresponding hydroxyl group (**17** in Table 3), a 3-fold better inhibition potency was obtained for the mouse enzyme, while a 1.5-fold decreased inhibition was shown for the human enzyme.

The data in Tables 2 and 3 indicate that the best functionality for the secondary pharmacophore is a ketone (**8**), ester (**9**), or alcohol (**17**). Because among these three functionalities the ester functional group can be prepared more easily and in a higher yield than the ketone or alcohol, the ester functionality was maintained as the secondary pharmacophore in this study, and the 3-chlorophenyl group on the left side of the urea pharmacophore of the ester compound (**9**) was then replaced by a potent cyclohexyl or adamantyl group. As shown in Table 4, the substitution on the left side of the urea pharmacophore with a cyclohexyl (**18**) or adamantyl (**19**) group increased inhibitor potency 10-fold over the 3-chlorophenyl analogue (**9**), indicating that a bulky cycloalkyl group such as cyclohexyl or adamantyl is a more adequate substitutent of the urea pharmacophore than an aryl group for inducing potent inhibition among functionalized ureas. Furthermore, these compounds functionalized with a polar group were as active as potent nonfunctionalized lipophilic inhibitors (**20** and **21**) for both murine and human enzymes. Comparing the inhibition potency of the two functionalized compounds (**18** and **19**), the inhibitor with an adamantyl substituent (**19**) had a 10-fold better potency for human sEH than the cyclohexyl analogue (**18**). Also, the two adamantyl analogues with a polar group (**22** and **23** in Table 4) had 3−25-fold better potencies than the corresponding cyclohexyl analogues (**5** and **6** in Table 1).

When the water solubility of functionalized inhibitors (*S*) was measured, a 3−4-fold better solubility was shown in compounds **18**, **19**, and **23** with an ester group than in nonfunctionalized inhibitors (**20** and **21**), and in compound **22** with a carboxylic acid group, a 11−16-fold increased water solubility was obtained. As expected, decreased experimental log *P* values (between 0 and 1) were obtained in these functionalized inhibitors, indicating that a polar functional group incorporated into nonfunctionalized inhibitors improved solubility in water and that the log *P* value might be a useful factor to estimate water solubility. The log *P* values of neutral immiscible liquids generally run parallel with their solubility in water; however, for solids solubility also depends on the energy required to break the crystal lattice. Banerjee et al. have suggested an empirical equation [$\log P = 6.5 - 0.89(\log S) - 0.015\text{mp}$] to relate solubility, melting point (mp), and log *P*.[34] It is possible to have compounds with high log *P* values that are still relatively soluble because of their low melting point. Similarly it is possible to have a low log *P* compound with a high melting point that is very insoluble in both aqueous and organic solutions. As shown in Table 4, dramatically increased water solubility (log *S*), which was calculated according to the above equation, was obtained in functionalized compounds (**18**, **19**, **22**, and **23**), and a 1.5−9.5-fold better solubility was also observed in compound **18** than in the other functionalized inhibitors (**19**, **22**, and **23**), while there was no change in the experimental solubility of the compounds functionalized with an ester group (**18**, **19**, and **23**), indicating that the water solubility estimated through Banerjee's equation was not always consistent with the experimental solubility of urea compounds in water. On the other hand, when log *P* values calculated by Crippen's method (clogP) were generated, decreased values (between one and three) were observed in the functionalized compounds with an ester group, which was consistent with the improved experimental water solubility, indicating that the clogP value would be useful to estimate the solubility in water quickly.

Adding polar groups to compounds generally increases their water solubility, and this was the case when one compares compounds (**18** or **19** to **20** or **21** in Table 4). In addition, stripping water of hydration out of the

enzyme catalytic site requires about the same amount of energy that is gained by forming a new hydrogen bond between the inhibitor and the enzyme. Thus, addition of polar groups that hydrogen bond to a target enzyme may not dramatically increase potency if the inhibitor is already potent. However, the presence of an additional polar group can be expected to dramatically increase specificity by decreasing the hydrophobic binding to biological molecules other than the primary target (sEH). In this way, combining several active pharmacophores into a single molecule often has a massive increase in biological specificity in complex biological systems. Therefore, investigations were continued to identify additional positions within the sEH inhibitor structure that could allow the inclusion of another polar group (Table 5).

We have reported that ureas with an ester functional group on the 12th or 13th atom from the urea pharmacophore are potent sEH inhibitors.[25] In addition, as shown in Table 4, the adamantyl urea compound (**19**) with an ester carbonyl group on the fifth atom from the urea pharmacophore is also a potent inhibitor and has improved water solubility. Therefore, one ester carbonyl group on the fifth atom was maintained, and the right side of the carbonyl group was varied with aliphatic or aryl groups with a polar carbonyl group corresponding roughly to the 13th atom from the urea phamacophore (Table 5, Figure 1). This corresponds roughly to a secondary and tertiary pharmacophore in linear distance from the carbonyl of the primary pharmacophore. When two carbonyl groups were present on the fifth and 13th atoms from the primary pharmacophore (**24**), potencies were maintained for both the murine and human enzymes, and in the methyl-branched olefin (**25**), no loss of inhibition was also observed. A phenyl derivative (**26**) with an ethyl ester carbonyl group on the 13th atom showed a slightly decreased inhibition for human sEH. However, in two aryl derivatives with a carbonyl group on the 12th atom (**27** and **28**), respectively, no drop of inhibition was observed, indicating that in aryl derivatives the 12th position from the urea pharmacophore leads to better inhibition of the two enzymes tested. In addition, similar clogP values to those of compounds **18** and **19** in Table 4 were calculated, indicating that increased solubility in water may be also obtained in these compounds.

The presence of an ester carbonyl group on the 13th atom in the aliphatic compound (**24**) did not decrease the inhibition potency, while the potency was decreased in an aryl analogue (**26**). So investigations were continued to identify other possible positions for the tertiary pharmacophore in aliphatic inhibitors. All compounds shown in Table 6 have the secondary phamacophore (ester functionality) on the fifth atom from the urea primary pharmacophore, and the atom units between the secondary pharmacophore and tertiary pharmacophore were varied. As shown in Table 6, nine compounds tested had 6−90-fold higher inhibition for mouse sEH than for human sEH. For the mouse enzyme, a carbonyl group on the eighth (**29**) or 11th (**32**) atom from the primary pharmacophore provided the most potent inhibitors, while 15−90-fold reduced potencies were observed for the human enzyme. Compounds with a carbonyl group on the ninth (**30**) or 10th (**31**) and the 13th−18th (**24** and **33-36**) atom showed 1.3−25-fold decreased potencies. On the other hand, for the human enzyme a carbonyl group on the 13th (**24**), 14th (**33**), or 16th (**34**) atom induced 2−15-fold better inhibition than on the 8th−11th (**29-32**) and 17th or 18th (**35** and **36**) atom. In addition, compounds **24**, **33**, and **34** have lower melting points (58−67 °C) than compound **19** (114 °C) and similar or a little higher clogP values than that of compound **19**, indicating that improved solubility may result from these inhibitors as compound **19**.

This investigation was implemented with the fundamental goal of producing sEH inhibitors with improved physical properties (e.g. solubility and melting points). We have found that urea sEH inhibitors possessing an ester carbonyl group (secondary pharmacophore) on the fifth atom (approximately 7.5 Å) from the urea pharmacophore produced excellent inhibition (**18** and **19** in Table 4) and also had improved solubility. In addition, a tertiary pharmacophore did not lower the inhibition potency when it was located 12th atom from the urea pharmacophore in an aryl derivative (**27**). In aliphatic derivatives, selectivity between two enzymes was observed: a carbonyl group on the eighth and 11$^{th}$ atoms (**29** and **32** in Table 6) from the carbonyl group of the primary phamacophore provided potent inhibition for the murine enzyme, while a carbonyl group on the 13th (**24**), 14th (**33**), or 16th (**34**) atom induced potent inhibition for the human enzyme. These results will be the basis for the design of specific and selective sEH inhibitors. Furthermore, all results obtained from the present study will be useful for the design of intravenous or orally available therapeutic agents for hypertension, vascular and renal inflammation, and other disorders that can be addressed by changing the in vivo concentration of chemical mediators that contain an epoxide.

## Experimental Section

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; analytical results were within ±0.4% of the theoretical values for the formula given, unless otherwise indicated. $^1$H NMR spectra were recorded on a QE-300 spectrometer, 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 (brs). Synthetic methods are described for representative compounds.

**Synthesis of 1-(3-Chlorophenyl)-3-(4-oxodecyl)urea (8).** Benzophenone imine (1.00 g, 5.52 mmol), ethyl 4-aminobutyrate hydrochloride (0.94 g, 5.52 mmol), and methylene chloride (20 mL) were stirred at room temperature for 24 h. The reaction mixture was filtered to remove $NH_4Cl$ and evaporated to dryness. The benzophenone Schiff base of ethyl 4-aminobutyrate (**I**) was extracted with ether (20 mL), and the ether solution was washed with water (20 mL), dried over sodium sulfate ($Na_2SO_4$), and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (5:1) to give **I** (1.00 g, 61%) as an oil. To the solution of the benzophenone Schiff base (**I**) in tetrahydrofuran (THF, 20 mL) was added 1 M diisobutylaluminum hydride (DIBAL, 3.7 mL) solution in pentane (3.73 mmol) at −78 °C under nitrogen, and the reaction was stirred for 2 h at this temperature. To magnesium turnings (0.10 g, 4.07 mmol) and $I_2$ (catalytic amount) in THF (10 mL) was added hexyl bromide (0.48 mL, 3.39 mmol) at room temperature under nitrogen. After stirring for 1 h, this reaction solution

was added dropwise to the above reaction mixture at −78 °C, and the solution was allowed to warm to room temperature with stirring. After stirring for 5 h at room temperature, 5% aqueous $NaHCO_3$ aqueous solution (10 mL) was added to the reaction, then the alkylated alcohol (**II**) was extracted with ether (20 mL), and the ether solution was washed with water (20 mL), dried over $Na_2SO_4$, and concentrated to give 0.26 g (60%) of the alcohol product (**II**).

Acetic anhydride (2 mL) was added to a solution of **II** (0.77 mmol) in dimethyl sulfoxide (DMSO, 5 mL). The mixture was allowed to stand at room temperature for 12 h and concentrated under reduced pressure. The residue was extracted with ether (20 mL), and the ether was washed with water (20 mL), dried over $Na_2SO_4$, and evaporated to provide 0.26 g (100%) of the ketone compound (**III**). To a solution of **III** in diethyl ether (5 mL) was added 1 N HCl (1 mL) aqueous solution at room temperature. The reaction mixture was stirred for 2 h, the water phase was concentrated to dryness, and then the residue was dissolved in dimethylformamide (DMF, 5 mL) and treated with triethylamine (TEA, 0.27 mL, 1.95 mmol) and a solution of 3-chlorophenyl isocyanate (0.10 mL, 0.78 mmol) in DMF (3 mL) at room temperature. After stirring for 5 h, the product was extracted with ether (30 mL), and the ether was washed with water (30 mL), dried over $Na_2SO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate (3:1) to afford 75 mg (30%) of **8** as a solid. $^1$H NMR $\delta$ ($CDCl_3$) 0.88 (3H, t, $J$ = 6.9 Hz), 1.21−1.29 (6H, m), 1.53−1.58 (2H, m), 1.81 (2H, quint, $J$ = 6.9 Hz), 2.43 (2H, t, $J$ = 6.9 Hz), 2.49 (2H, t, $J$ = 6.9 Hz), 3.23 (2H, q, $J$ = 6.9 Hz), 5.10 (1H, s), 6.93 (1H, s), 6.98−7.02 (1H, m), 7.10−7.23 (2H, m), 7.49 (1H, s); LC−MS (ESI) $m/z$ calcd for $C_{17}H_{25}ClN_2O_2$ $[M + H]^+$ 325.16, found $[M + H]^+$ 325.21; mp 62−64 °C. Anal. ($C_{17}H_{25}ClN_2O_2$) C, H, N.

Compound **17** was synthesized with the same procedure as that used for the preparation of compound **8** by using **II** and 3-chlorophenyl isocyanate instead of **III**.

**Synthesis of 4-[3-(3-Chlorophenyl)ureido]butyric Acid Pentyl Ester (9).** To a suspension of 4-aminobutyric acid (1.41 g, 13.7 mmol) in DMF (25 mL) was added 3-chlorophenyl isocyanate (0.70 g, 4.56 mmol; cyclohexyl isocyanate for **18** and 1-adamantyl isocyanate for **19**) at room temperature. The reaction mixture was stirred for 24 h. Then, 1 N HCl aqueous solution (30 mL) was added into the reaction, and the solution was stirred for 30 min. The solid product was filtered and washed with water (20 mL) and ethyl acetate (20 mL). The resulting solid was dried in the vacuum oven to give 1.17 g (100%) of urea acid (**IV**). A mixture of **IV** (0.50 g, 1.95 mmol), potassium carbonate ($K_2CO_3$, 0.54 g, 3.90 mmol), bromopentane (0.37 mL, 2.92 mmol), and sodium iodide (60 mg, 0.39 mmol) in DMF (20 mL) was stirred at room temperature for 20 h. Then the product was extracted with ether (20 mL), and the ether was washed with 1 N NaOH aqueous solution (20 mL) and brine (20 mL), dried over $Na_2SO_4$, and evaporated to afford 0.59 g (92%) of **9** as a solid: $^1$H NMR $\delta$ ($CDCl_3$) 0.90 (3H, t, $J$ = 6.9 Hz), 1.26−1.34 (4H, m), 1.62−1.65 (2H, m), 1.88 (2H, quint, $J$ = 6.9 Hz), 2.41 (2H, t, $J$ = 6.9 Hz), 3.30 (2H, q, $J$ = 6.9 Hz), 4.08 (2H, t, $J$ = 6.9 Hz), 4.96 (1H, s), 6.62 (1H, s), 7.01−7.04 (1H, m), 7.18−7.22 (2H, m), 7.47 (1H, s); LC−MS (ESI) $m/z$ calcd for $C_{16}H_{23}ClN_2O_3$ $[M + H]^+$ 327.14, found $[M + H]^+$ 327.12; mp 98 °C. Anal. ($C_{16}H_{23}ClN_2O_3$) C, H, N.

Compounds **18** and **19** were synthesized in the same manner as that described above using cyclohexyl isocyanate and adamantyl isocyanate instead of 3-chlorophenyl isocyanate, respectively.

**Synthesis of 4-[3-(3-Chlorophenyl)ureido]-*N*-pentylbutyramide (10).** To a suspension of 4-aminobutyric acid (2.84 g, 27.5 mmol) in DMF (30 mL) was added TEA (3.86 mL, 27.5 mmol). To this mixture, di-*tert*-butyl dicarbonate (2.00 g, 9.17 mmol) was added with stirring. The reaction mixture was heated to 50 °C for 12 h, and then stirred with ice-cold dilute hydrochloric acid (15 mL) for 10 min. The *tert*-butoxycarbonylated amino acid (**V**) was immediately extracted with ether (2 × 30 mL). The organic extract was dried over $Na_2SO_4$ and evaporated to give 1.00 g (54%) of **V** as an oil.

A solution of **V** and 4-methylmorpholine (NMM, 0.54 mL, 4.92 mmol) in DMF (10 mL) was treated at room temperature with isobutyl chloroformate (0.64 mL, 4.92 mmol). After 30 min, pentylamine (0.57 mL, 4.92 mmol) was added. The reaction mixture was stirred for 12 h. The solvent was evaporated, and the residue was partitioned between ethyl acetate (25 mL) and water (25 mL). The ethyl acetate layer was washed with 5% $NaHCO_3$ (10 mL) and brine (20 mL), dried over $Na_2SO_4$, and evaporated. The residue was chromatographed on silica gel eluting with hexane and ethyl acetate (2:1) to give 0.33 g (33%) of *tert*-butoxycarbonylated amino amide (**VI**). A solution of **VI** in dioxane (10 mL) was treated with 4 M hydrochloric acid (2 mL) in dioxane, and the mixture was stirred for 1 h at room temperature. Then the solvent was evaporated to dryness, and the residual solid was dissolved in DMF (10 mL) and treated with TEA (0.51 mL, 3.63 mmol) and 3-chlorophenyl isocyanate (0.15 mL, 1.21 mmol) at room temperature. After stirring for 5 h, the product was extracted with ether (30 mL), and the ether was washed with water (30 mL), dried over $Na_2SO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (3:1) to afford 0.39 g (100%) of **10** as a solid: $^1$H NMR $\delta$ ($CDCl_3$) 0.89 (3H, t, $J$ = 6.9 Hz), 1.26−1.28 (4H, m), 1.46−1.50 (2H, m), 1.86 (2H, quint, $J$ = 6.9 Hz), 2.30 (2H, t, $J$ = 6.9 Hz), 3.23 (2H, q, $J$ = 6.9 Hz), 3.30 (2H, q, $J$ = 6.9 Hz), 5.87 (1H, s), 6.06 (1H, s), 6.93−6.97 (1H, m), 7.12−7.23 (2H, m), 7.49 (1H, m), 7.73 (1H, s); LC−MS (ESI) $m/z$ calcd for $C_{16}H_{24}ClN_3O_2$ $[M + H]^+$ 326.16, found $[M + H]^+$ 326.16; mp 109 °C. Anal. ($C_{16}H_{24}ClN_3O_2$) C, H, N.

**Synthesis of Heptanoic Acid 2-[3-(3-Chlorophenyl)-ureido]ethyl Ester (11).** To a solution of 2-aminoethanol (2.98 g, 48.8 mmol) in DMF (30 mL) was added 3-chlorophenyl isocyanate (2.50 g, 16.3 mmol) at 0 °C. The reaction mixture was stirred for 5 h at room temperature. The solvent was evaporated, the residue was partitioned between ether (30 mL) and 1 N hydrochloric acid (20 mL), and the ether layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (1:1) to provide 1.49 g (40%) of urea alcohol (**VII**) as a white solid.

To a solution of **VII** (1.00 g, 4.60 mmol) and TEA (0.97 mL, 6.90 mmol) in DMF (15 mL) was added a solution of heptanoic anhydride (2.23 g, 9.20 mmol) in DMF (5 mL) at room temperature. The reaction was stirred for 12 h, and the solvent was evaporated. The residue was partitioned between ether (30 mL) and cold 1 N hydrochloric acid (20 mL). The ether layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residual solid was purified using silica gel column chromatography (hexane:ethyl acetate = 3:1) to afford 1.05 g (70%) of **11** as an oil: $^1$H NMR $\delta$ ($CDCl_3$) 0.87 (3H, t, $J$ = 6.9 Hz), 1.20−1.29 (6H, m), 1.60−1.62 (2H, m), 2.22−2.29 (2H, m), 3.50−3.55 (2H, m), 4.09−4.20 (2H, m), 5.32 (1H, s), 7.01−7.06 (2H, m), 7.16−7.22 (2H, m), 7.40 (1H, s); LC−MS (ESI) $m/z$ calcd for $C_{16}H_{23}ClN_2O_3$ $[M + H]^+$ 327.14, found $[M + H]^+$ 327.15. Anal. Calcd for $C_{16}H_{23}ClN_2O_3$: C, 58.80; H, 7.09; N, 8.57. Found: C, 60.99; H, 8.50; N, 7.09.

Compounds **12** and **13** were prepared in the same manner as that used for compound **11** from chloroformic acid pentyl ester and pentyl isocyanate instead of heptanoic anhydride.

**Synthesis of Heptanoic Acid {2-[3-(3-Chlorophenyl)-ureido]ethyl}amide (14).** A solution of di-*tert*-butyl dicarbonate (0.50 g, 2.29 mmol) in dioxane (20 mL) was added over a period of 1 h to a solution of 1,2-diaminoethane (1.10 g, 18.3 mmol) in dioxane (20 mL). The mixture was allowed to stir for 22 h and the solvent was evaporated to dryness. Water (30 mL) was added to the residue and the insoluble bis-substituted product was removed by filtration. The filtrate was extracted with methylene chloride (3 × 30 mL) and the methylene chloride was evaporated to yield **VIII** as an oil (0.35 g, 95%).

A solution of heptanoic anhydride (0.91 g, 3.75 mmol; chloroformic acid pentyl ester for **15** and pentyl isocyanate for **16**) and **VIII** (0.50 g, 3.13 mmol) in DMF (20 mL) was stirred for 2 h at room temperature. Then the solvent was evaporated. The residue was partitioned between ether (30 mL) and water (30 mL). The ether layer was dried over $Na_2SO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (1:1) to yield 0.57 g (67%) of alkylated *N-tert*-butoxycarbonylamine (**IX**).

A solution of **IX** in dioxane (10 mL) was treated with 4 M hydrochloric acid (2 mL) in dioxane, and the mixture was stirred for 1 h at room temperature. Then the solvent was evaporated to dryness, and the residual solid was dissolved in DMF (10 mL) and treated with TEA (0.58 mL, 4.19 mmol) and 3-chlorophenyl isocyanate (0.32 g, 2.10 mmol) at room temperature. After stirring for 5 h, the product was extracted with ether (30 mL), and the ether was washed with water (30 mL), dried over $Na_2SO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (1:1) to afford 0.68 g (100%) of **14** as a solid: $^1$H NMR $\delta$ ($CDCl_3$) 0.84 (3H, t, $J$ = 6.9 Hz), 1.16−1.25 (6H, m), 1.55−5.61 (2H, m), 2.21−2.24 (2H, m), 3.31−3.40 (4H, m), 6.27 (1H, s), 6.90−6.95 (2H, m), 7.18−7.20 (2H, m), 7.56 (1H, s), 8.07 (1H, s); LC−MS (ESI) *m/z* calcd for $C_{16}H_{24}ClN_3O_2$ $[M + H]^+$ 326.16, found $[M + H]^+$ 326.25; mp 133−135 °C. Anal. Calcd for $C_{16}H_{24}ClN_3O_2$: C, 58.98; H, 7.42; N, 12.90. Found: C, 58.18; H, 7.37; N, 12.64.

Compounds **15** and **16** were prepared in the same manner as that described above using chloroformic acid pentyl ester and pentyl isocyanate instead of heptanoic anhydride, repectively.

**Synthesis of 4-(3-Adamantan-1-yl-ureido)butyric Acid Methyl Ester (23).** To a suspension of 4-aminobutyric acid (2.79 g, 27.1 mmol) in DMF (40 mL) was added 1-adamantyl isocyanate (1.20 g, 6.77 mmol) at room temperature. The reaction mixture was stirred for 24 h. Then 1 N HCl aqueous solution (40 mL) was added into the reaction, and the mixture was stirred for 30 min. The solid crystalline product was filtered and washed with water (20 mL) and ethyl acetate (20 mL). The resulting solid was dried in a vacuum oven to give 1.90 g (100%) of 4-(3-adamantan-1-yl-ureido)butyric acid (**22**) as a white solid: $^1$H NMR $\delta$ ($CD_3OD$): 1.66−1.75 (8H, m), 1.94−1.97 (6H, m), 2.05−2.07 (3H, m), 2.30 (2H, t, $J$ = 6.9 Hz), 3.08 (2H, q, $J$ = 6.9 Hz), 3.32 (2H, s); LC−MS (ESI) *m/z* calcd for $C_{15}H_{24}N_2O_3$ $[M + H]^+$ 281.18, found $[M + H]^+$ 281.25; mp 165 °C. Anal. ($C_{15}H_{24}N_2O_3$) C, H, N.

A mixture of **22** (0.15 g, 0.54 mmol), $K_2CO_3$ (0.09 g, 0.64 mmol), and iodomethane (0.04 mL, 0.59 mmol) in DMF (20 mL) was stirred at room temperature for 20 h. Then the product was extracted with ether (20 mL), and the ether was washed with 1 N NaOH aqueous solution (20 mL) and brine (20 mL), dried over $Na_2SO_4$, and evaporated to afford 0.15 g (95%) of **23**: $^1$H NMR $\delta$ ($CDCl_3$) 1.66−1.68 (6H, m), 1.81 (2H, quint, $J$ = 6.9 Hz), 1.94−1.97 (6H, m), 2.05−2.07 (3H, m), 2.37 (2H, t, $J$ = 6.9 Hz), 3.16 (2H, q, $J$ = 6.9 Hz), 3.68 (3H, s), 4.09 (1H, s), 4.25 (1H, s); LC−MS (ESI) *m/z* calcd for $C_{16}H_{26}N_2O_3$ $[M + H]^+$ 295.19, found $[M + H]^+$ 295.24; mp 114 °C. Anal. ($C_{16}H_{26}N_2O_3$) C, H, N.

Compounds **29**, **30**, **31**, **32**, and **24** were prepared in the same manner using the corresponding ethyl bromoalkanoates instead of iodomethane to yield 30−95%.

**Synthesis of 4-(3-Adamantan-1-yl-ureido)butyric Acid 3,7-Dimethyl-oct-6-enyl Ester (25).** To a solution of **22** (0.10 g, 0.36 mmol), 4-(dimethylamino)pyridine (DMAP; 44 mg, 0.36 mmol), and 3,7-dimethyl-oct-6-en-1-ol (61 mg, 0.39 mmol) in methylene chloride (20 mL) was added 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDCI; 75 mg, 0.39 mmol) at room temperature. After stirring for 12 h, the reaction mixture was washed with 1 N NaOH aqueous solution (15 mL) and water (30 mL), and the organic layer was dried over $Na_2SO_4$ and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (3:1) to give **25** (97 mg, 65%) as a solid: $^1$H NMR $\delta$ ($CDCl_3$) 0.91 (3H, d, $J$ = 6.9 Hz), 1.34−1.37 (2H, m), 1.56−1.60 (9H, m), 1.67−1.69 (8H, m), 1.81 (2H, quint, $J$ = 6.9 Hz), 1.94−1.97 (6H, m), 2.05−2.07 (3H, m), 2.35 (2H, t, $J$ = 6.9 Hz), 3.16 (2H, q, $J$ = 6.9 Hz), 4.05 (1H, s), 4.11 (2H, t, $J$ = 6.9 Hz), 4.21 (1H, s), 5.09 (1H, t, $J$ = 6.9 Hz); LC−MS (ESI) *m/z* calcd for $C_{25}H_{42}N_2O_3$ $[M + H]^+$ 419.32, found $[M + H]^+$ 419.22; mp 49 °C. Anal. Calcd for $C_{25}H_{42}N_2O_3$: C, 71.73; H, 10.11; N, 6.69. Found: C, 70.27; H, 9.83; N, 6.39.

**Synthesis of 8-[4-(3-Adamantan-1-yl-ureido)butyryl-oxy]octanoic Acid Ethyl Ester (33).** To a solution of 8-bromooctanoic acid (0.20 g, 0.89 mmol), DMAP (0.12 g, 0.99 mmol), and ethanol (0.05 g, 0.99 mmol) in methylene chloride (20 mL) was added EDCI (0.19 g, 0.99 mmol) at room temperature. After stirring for 12 h, the reaction mixture was washed with 1 N NaOH aqueous solution (15 mL) and water (30 mL), and the organic layer was dried over $Na_2SO_4$ and evaporated to give 8-bromooctanoic acid ethyl ester (0.17 g, 75%). This bromide reacted with **22** in the same manner as that used for the preparation of **23** to provide **33** (0.19 g, 65%) as a solid: $^1$H NMR $\delta$ ($CDCl_3$) 1.26 (3H, t, $J$ = 6.9 Hz), 1.32−1.35 (6H, m), 1.59−1.66 (10H, m), 1.82 (2H, quint, $J$ = 6.9 Hz), 1.94−1.97 (6H, m), 2.05−2.07 (3H, m), 2.28 (2H, t, $J$ = 6.9 Hz), 2.36 (2H, t, $J$ = 6.9 Hz), 3.16 (2H, q, $J$ = 6.9 Hz), 4.05−4.14 (5H, m), 4.31 (1H, s); LC−MS (ESI) *m/z* calcd for $C_{25}H_{42}N_2O_5$ $[M + H]^+$ 451.31, found $[M + H]^+$ 451.20; mp 58−59 °C. Anal. ($C_{25}H_{42}N_2O_5$) C, H, N.

**Synthesis of 10-[4-(3-Adamantan-1-yl-ureido)butyryl-oxy]decanoic Acid Ethyl Ester (34).** A mixture of 10-hydroxydecanoic acid (0.25 g, 1.33 mmol; 11-hydroxyundecanoic acid for compound **35** and 12-hydroxydodecanoic acid for compound **36**), ethyl bromide (0.16 g, 1.46 mmol), and lithium carbonate (0.11 g, 1.46 mmol) in DMF (25 mL) was stirred at 70 °C for 6 h. Then the product was extracted with ether (30 mL), and the ether solution was washed with 1 N NaOH aqueous solution (20 mL) and water (30 mL), dried over $Na_2SO_4$, and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (3:1) to give 10-hydroxydecanoic acid ethyl ester (80 mg, 28%). This alcohol was coupled with **22** by using EDCI/DMAP coupling reagent to give **34** (0.11 g, 60%) as a solid: $^1$H NMR $\delta$ ($CDCl_3$) 1.24−1.32 (13H, m), 1.62−1.68 (10H, m), 1.80 (2H, quint, $J$ = 6.9 Hz), 1.94−1.97 (6H, m), 2.05−2.07 (3H, m), 2.28 (2H, t, $J$ = 6.9 Hz), 2.36 (2H, t, $J$ = 6.9 Hz), 3.16 (2H, q, $J$ = 6.9 Hz), 4.05−4.14 (5H, m), 4.25 (1H, s); LC−MS (ESI) *m/z* calcd for $C_{27}H_{46}N_2O_5$ $[M + H]^+$ 479.34, found $[M + H]^+$ 479.29; mp 60−61 °C. Anal. Calcd for $C_{27}H_{46}N_2O_5$: C, 67.75; H, 9.69; N, 5.85. Found: C, 68.33; H, 9.92; N, 5.97.

Compound **22** was coupled with 11-hydroxyundecanoic acid ethyl ester and 12-hydroxydodecanoic acid ethyl ester prepared from corresponding acids to get compounds **35** and **36**, respectively.

**Synthesis of 4-[4-(3-Adamantan-1-yl-ureido)butyryl-oxymethyl]benzoic Acid Ethyl Ester (27).** A mixture of 4-formylbenzoic acid (1.00 g, 6.66 mmol), bromoethane (1.09 g, 9.99 mmol), and $K_2CO_3$ (1.10 g, 7.99 mmol) in acetonitrile (30 mL) was refluxed for 6 h. After evaporation of the solvent, 4-formylbenzoic acid ethyl ester was extracted with ether (30 mL), and the organic solution was washed with 1 N NaOH aqueous solution (20 mL) and water (30 mL), dried over $Na_2SO_4$, and concentrated to give the ethyl ester product (0.65 g, 55%). Without further purification, to a solution of the ester was added sodium borohydride ($NaBH_4$; 0.05 g, 3.65 mmol) in ethanol (20 mL) at 0 °C. After stirring for 5 h at room temperature, the product was extracted with ether (30 mL), and the ether solution was washed with water (30 mL), dried over $Na_2SO_4$, and concentrated. The residue was purified by using column chromatography on silica gel eluting with hexane and ethyl acetate (3:1) to give 4-hydroxymethylbenzoic acid ethyl ester (0.30 g, 46%) as an oil.

To a solution of **22** (1.23 g, 0.83 mmol), DMAP (0.05 g, 0.42 mmol), and the above alcohol (0.15 g, 0.83 mmol) in methylene chloride (30 mL) was added EDCI (0.16 g, 0.83 mmol) at room temperature. After stirring for 12 h, the reaction mixture was washed with 1 N NaOH aqueous solution (15 mL) and water (30 mL), and the organic layer was dried over $Na_2SO_4$ and

concentrated. Then the residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate (5:1) to provide **27** (0.28 g, 75%) as a white solid: $^1$H NMR $\delta$ ($CDCl_3$) 1.40 (3H, t, $J$ = 6.9 Hz), 1.66−1.68 (6H, m), 1.84 (2H, quint, $J$ = 6.9 Hz), 1.94−1.96 (6H, m), 2.05−2.07 (3H, m), 2.44 (2H, t, $J$ = 6.9 Hz), 3.17 (2H, q, $J$ = 6.9 Hz), 4.02 (1H, s), 4.17 (1H, s), 4.38 (2H, q, $J$ = 6.9 Hz), 5.17 (2H, s), 7.40 (2H, d, $J$ = 7.8 Hz), 8.00 (2H, d, $J$ = 7.8 Hz); LC−MS (ESI) $m/z$ calcd for $C_{25}H_{34}N_2O_5$ $[M + H]^+$ 443.25, found $[M + H]^+$ 443.25; mp 96−99 °C. Anal. ($C_{25}H_{34}N_2O_5$) C, H, N.

**Synthesis of 4-(3-Adamantan-1-yl-ureido)butyric Acid 4-Ethoxycarbonylmethylphenyl Ester (28).** To a solution of **22** (0.15 g, 0.54 mmol), DMAP (0.07 g, 0.54 mmol), and 4-hydroxyphenylacetic acid (0.09 g, 0.59 mmol) in methylene chloride (20 mL) was added EDCI (0.11 g, 0.59 mmol) at room temperature. After stirring for 12 h, the reaction mixture was washed with water (20 mL), and the methylene chloride solution dissolving the product was dried over $Na_2SO_4$ and concentrated to give conjugated product. This crude mixture in DMF (30 mL) was treated with bromoethane (0.15 g, 1.34 mmol) and $K_2CO_3$ (0.18 g, 1.34 mmol) at room temperature and stirred for 12 h at room temperature. The ethyl ester product was extracted with ether (30 mL), and the ether solution was washed with 1 N NaOH aqueous solution (20 mL) and water (30 mL), dried over $Na_2SO_4$, and concentrated. The residue was purified by column chromatography on silica gel eluting hexane and ethyl acetate (5:1) to give **28** (47 mg, 20%) as a white solid: $^1$H NMR $\delta$ ($CDCl_3$) 1.40 (3H, t, $J$ = 6.9 Hz), 1.66−1.68 (6H, m), 1.89−1.95 (8H, m), 2.05−2.07 (3H, m), 2.62 (2H, t, $J$ = 6.9 Hz), 3.25 (2H, q, $J$ = 6.9 Hz), 3.60 (2H, s), 4.07 (1H, s), 4.16 (2H, q, $J$ = 6.9 Hz), 4.29 (1H, s), 7.08−7.10 (2H, m), 7.28−7.30 (2H, m); LC−MS (ESI) $m/z$ calcd for $C_{25}H_{34}N_2O_5$ $[M + H]^+$ 443.25, found $[M + H]^+$ 443.25; mp 95−97 °C. Anal. ($C_{25}H_{34}N_2O_5$) C, H, N.

Compound **26** was prepared in the same manner by using 4-hydroxyphenylacrylic acid instead of 4-hydroxyphenylacetic acid.

**Acknowledgment.** This work was supported, in part, by NIEHS Grant R37 ES02710, NIEHS Center for Environmental Health Sciences P30 ES05707, NIH/NIEHS Superfund Basic Research Program P42 ES04699, UC Systemwide Biotechnology Research and Education Training Grant #2001-07, NIEHS Center for Children's Environmental Health & Disease Prevention P01 ES11269, and NIH/NIDDK UC Davis Clinical Nutrition Research Unit P30 DK35747, Pilot Project.

**Supporting Information Available:** Detailed analytical data (NMR, LC−MS, elemental analysis, and melting point) for **12, 13, 15**−**19**, **24**, **26**, **29**−**32**, **35**, **36**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM030514J