1,3-Disubstituted Ureas Functionalized with Ether Groups are Potent Inhibitors of the Soluble Epoxide Hydrolase with Improved Pharmacokinetic Properties

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Soluble epoxide hydrolase (sEH) is a therapeutic target for treating hypertension and inflammation. 1,3-Disubstituted ureas functionalized with an ether group are potent sEH inhibitors. However, their relatively low metabolic stability leads to poor pharmacokinetic properties. To improve their bioavailability, we investigated the effect of incorporating various polar groups on the ether function on the inhibition potencies, physical properties, in vitro metabolic stability, and pharmacokinetic properties. The structure−activity relationship studies showed that a hydrophobic linker between the urea group and the ether function is necessary to keep their potency. In addition, urea-ether inhibitors having a polar group such as diethylene glycol or morpholine significantly improved their physical properties and metabolic stability without any loss of inhibitory potency. Furthermore, improved pharmacokinetic properties in murine and canine models were obtained with the resulting inhibitors. These findings will facilitate the usage of sEH inhibitors in animal models of hypertension and inflammation.

Introduction

Cytochrome P450 epoxygenases oxidize polyunsaturated endogenous fatty acids, such as arachidonic acid,[1−5] to generate the corresponding epoxides (epoxyeicosatrienoic acids or EETs[a1]). These latter compounds have been reported as a new class of lipid mediators regulating blood pressure[6−11] and inflammation.[12−17] In addition, the EETs further have vascular protective effects such as suppression of reactive oxygen species following hypoxia-reoxygenation[18] or enhancement of a fibrinolytic pathway.[19] However, the metabolism of EETs to dihydroxyeicosatrienoic acids (DHETs) by the soluble epoxide hydrolase (sEH) often leads to reductions in these biological activities.[6] Thus, stabilizing the in vivo concentration of EETs through sEH inhibition represents a novel therapeutic avenue to treat hypertension, inflammation, and other cardiovascular disorders. This hypothesis is supported by numerous in vivo experiments in animal models. For example, the blood pressure of spontaneously hypertensive- or angiotensin II-induced hypertensive rats treated with sEH inhibitors is dramatically reduced.[7−9,11] Also, tobacco smoke-induced lung inflammation[12] or lipopolysaccharide (LPS)-induced acute inflammation[13] is attenuated by treating with sEH inhibitors. All of these studies support the hypothesis that anti-hypertensive and the cardio protective effects are mediated by EETs and are dependent on the extent of epoxide hydrolysis by sEH.[20,21]

1,3-Disubstituted ureas and related compounds are very potent inhibitors of sEH, and these compounds efficiently induce a reduction in epoxide hydrolysis in several cellular and animal models.[8,9,22] However, poor physical properties, especially limited solubility in either water or organic solvents of some of these urea inhibitors likely result in poor in vivo availability and difficulty in formulation.[23] We previously reported that a polar functional group located on specific positions of one of the alkyl chains of the urea inhibitors improves water solubility and generally decreases melting points without decreasing inhibitory potency.[24] Further, a carboxylic acid group present on the thirteenth atom or a polar group such as ester, sulfonamide, alcohol, ether, carbamate, or ketone located on the fifth/sixth atom from the urea group was effective for producing soluble inhibitors in either water or oil while retaining inhibition potency.[23−25] Such derivatives that have improved solubility and low melting point have enhanced pharmacokinetic properties in mice compared to the lipophilic inhibitor,[26,27] indicating that compounds having good physical properties result in a better inhibitor for in vivo study. Although significantly improved pharmacokinetic properties were obtained from these inhibitors, relatively low absorptions and short half-lives were still observed.[26,27] These inhibitors may not have adequate pharmacokinetic properties to be effective as pharmaceuticals. Toward obtaining compounds that have the better absorbance and the longer in vivo half-lives, we have previously explored the effect of ureas substituted with an adamantyl group at position 1 and a piperidine group at position 3 of the urea[28] and separately replaced the adamantane group with various groups.[29] In the present study, we investigated the effect of functionalizing 1,3-disubstituted ureas with various polar ether groups on physical properties, metabolic stability, and pharmacokinetic properties.

Chemistry

Scheme 1 outlines the syntheses of 1-adamantan-1-yl-3-(hydroxyalkyl)ureas and their aliphatic alkyl ether analogs. Reaction of 1-adamantyl isocyanate with a hydroxyalkylamine (2-hydroxyethylamine for compound **1**, 3-hydroxypropylamine for compound **3**, 4-hydroxybutylamine for compound **5**, 5-hydroxypentylamine for compound **7**, and 6-hydroxyhexylamine for compound **9**) in *N*,*N*-dimethylformamide (DMF) provided 1-adamantan-1-yl-3-(hydroxyalkyl)ureas in 95−100% yields.[24,25] Each hydroxyl group of compounds **1**, **3**, **5**, **7**, and **9** was alkylated with an alkyl bromide (1-bromoheptane for compound

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[a] Abbreviations: AEPU, 1-adamantan-3-(5-(2-(ethyl-ethoxy)ethoxy)-pentyl)urea; AUC, area under the curve; DMAP, *N*,*N*-4-dimethylaminopyridine; EDCI, 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride; EETs, epoxyeicosatrienoic acids; DHETs, dihydroxyeicosatrienoic acids; LPS, lipopolysaccharide; SAR, structure−activity relationship; sEH, soluble epoxide hydrolase.

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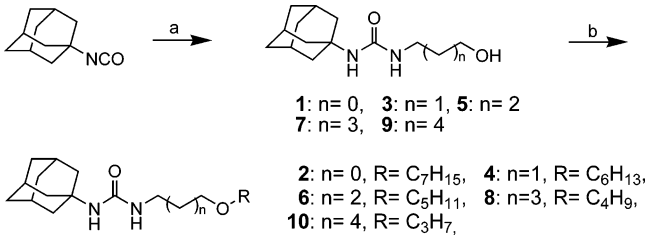
Scheme 1. Syntheses of 1-Adamantyl-3-hydroxyalkylureas (**1**, **3**, **5**, **7**, and **9**) and the Corresponding Ether Derivatives (**2**, **4**, **6**, **8**, and **10**)[a]



[a] Reagents and conditions: (a) 2-hydroxyethylamine (for compound **1**), 3-hydroxypropylamine (for compound **3**), 4-hydroxybutylamine (compound **5**), 5-hydroxypentylamine (for compound **7**), or 6-hydroxyhexylamine (for compound **9**), DMF, rt; (b) 1-bromoheptane (for compound **2**), 1-bromohexane (for compound **4**), 1-bromopentane (for compound **6**), 1-bromobutane (for compound **8**), or 1-bromopropane (for compound **10**), NaH, DMF, rt.

2, 1-bromohexane for compound **4**, 1-bromopentane for compound **6**, 1-bromobutane for compound **8**, and 3-bromopropane for compound **10**) to afford the corresponding ether compounds in a range of 30−55% yields.

The syntheses of alkyl ethers with a methyl branch (**11** and **12**) and ethylene glycol derivatives of compound **7** (**15**, **18**, and **19**) are described in Scheme 2A,B. As outlined in Scheme 2A, compound **7** was brominated with triphenylphosphine and carbon tetrabromide in DMF to produce 1-adamantan-1-yl-3-(5-bromopentyl)urea (**I**) in 87% yield, followed by etherification with the corresponding alcohol (2-hexanol for **11**, 2-methylpentanol for **12**, and triethylene glycol monomethyl ether for **18**) in the presence of sodium hydride in DMF in a range of 25−48% yields. Bromination of diethylene glycol monoethyl ether (for **15**) or 3-hydroxypropylmorpholine (for **19**) using triphenylphosphine and carbon tetrabromide in THF yielded the corresponding bromides **II** (87−95%), which were reacted with compound **7** in the presence of sodium hydride in DMF to afford compounds **15** and **19** in 52−65% yields, respectively.

Compounds **13**, **14**, **16**, and **17** were prepared by the procedures depicted in Scheme 3. Monoalkylation of 1,3-dihydroxypropane (for compound **13**) or 1,4-dihydroxybutane (for compound **14**) with 1-bromopropane in the presence of sodium hydride in DMF gave the corresponding monoalcohols 3-propoxy-1-propanol and 4-propoxy-1-butanol, respectively, in approximately 18% yield. Reaction of these alcohols with **I** in the presence of sodium hydride in DMF produced compounds **13** and **14** in 25−32% yields. Monoalkylation of di-(2-bromoethyl) ether with 2,2,2-trifluoroethanol (for compound **16**) or 4-ethylphenol (for compound **17**) using sodium hydride as a base in DMF afforded the corresponding 2-(2,2,2-trifluoroethoxyethoxy)ethyl bromide and 2-(4-ethylphenoxyethoxy)ethyl bromide in 60% yield. Each intermediate was reacted with compound **7** in the presence of sodium hydride in DMF to afford compounds **16** and **17**, respectively, in 45% yield.

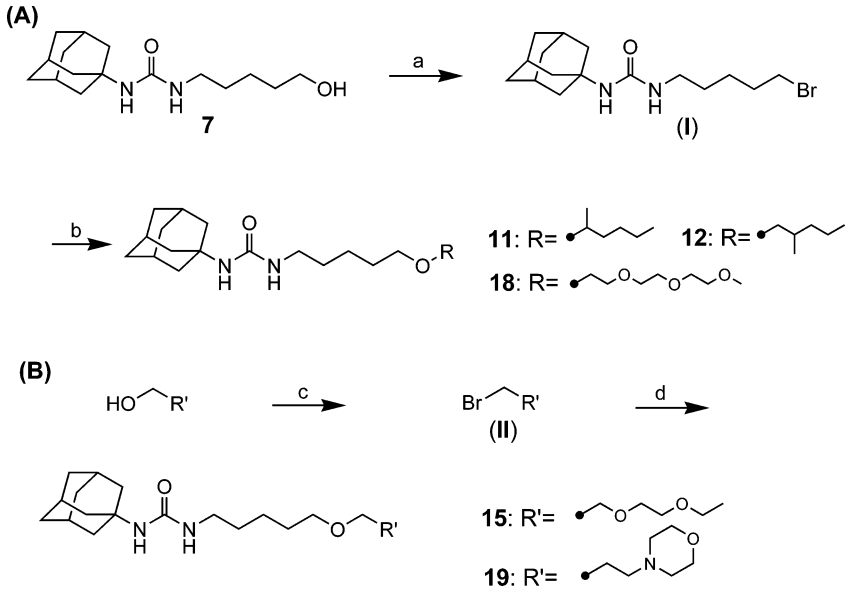
Scheme 4 shows the syntheses of methanesulfonamide ureas (**20** and **21**) and two corresponding amide derivatives (**22** and **23**) of compound **15**. As shown in Scheme 4A, reaction of 2-(2-aminoethoxy)ethyl alcohol (for compound **20**) and 5-amino-1-pentanol (for compound **21**) with methanesulfonic anhydride in acetonitrile gave 2-(2-hydroxyethoxy)ethylmethanesulfonamide and 5-hydroxypentylmethanesulfonamide, respectively, in 66% yield, which were reacted with **I** in the presence of sodium hydride in DMF to afford compounds **20** and **21** in 40−55% yield. As described in Scheme 4B, alkylation of 7-bromoheptanoic acid ethyl ester with diethylene glycol monoethyl ether in the presence of sodium hydride in DMF afforded substituted heptanoate in 40% yield, followed by hydrolysis. Coupling of the resulting acid with 1-adamantylamine using 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride (EDCI) in the presence of *N*,*N*-4-dimethylaminopyridine (DMAP) in dichloromethane provided compound **22** in 97% yield. For the preparation of the other amide analog **23** with the nitrogen on the "right side" of the carbonyl, the amino group of 5-amino-1-pentanol was protected in the reaction with di-(*tert*-butyl) dicarbonate in dioxane to give the corresponding protected intermediate in 97% yield, followed by alkylation of the hydroxyl group with 2-(ethoxyethoxy)ethyl bromide (**II**) in the presence of a catalytic amount of sodium iodide and sodium hydride in DMF (28%). Then the amine was deprotected with 4 N HCl in dioxane to afford amine salt, which was coupled with 1-adamantylacetic acid using EDCI and DMAP in dichloromethane to yield compound **23** (97%).

Scheme 5 outlines syntheses of analogs with a substituted cyclohexyl (**24**−**26**) or phenyl (**27**−**29**) group as a linker between the urea group and the oxygen atom in an ether group. Reaction of 1-adamantyl isocyanate with *trans*-4-aminocyclohexanol in DMF gave 1-adamantan-1-yl-3-(4-hydroxycyclohexyl)urea (**IV**) in 95% yield, followed by O-alkylation of **IV** with 1-bromopentane (for compound **24**), 2-(ethoxyethoxy)ethyl bromide (for compound **25**), or 3-morpholinopropyl bromide (for compound **26**) in the presence of sodium hydride in DMF to provide compounds **24**, **25**, and **26**, respectively, in 25−40% yields. Reaction of 1-adamantyl isocyanate with 3- or 4-aminophenol gave intermediates in 95% yield, which were alkylated with 2-(ethoxyethoxy)ethyl bromide (for compounds **27** and **28**) or 3-morpholinopropyl bromide (for compound **29**) to afford compounds **27**−**29** in a range of 80−90% yields.

Results and Discussion

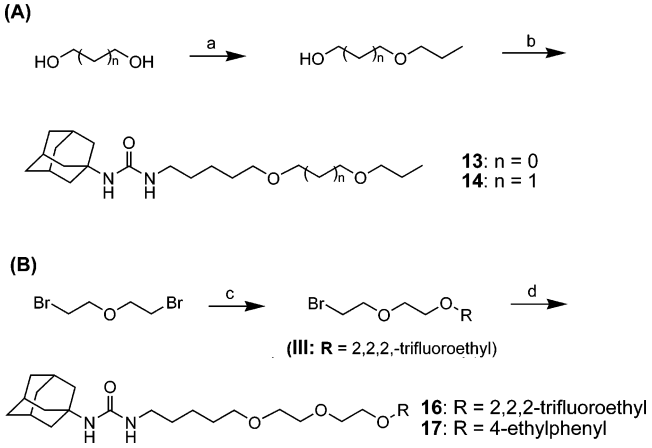
To synthesize soluble inhibitors with improved pharmacokinetic properties, a variety of urea derivatives functionalized with various polar groups were explored in this study. We previously showed that an alcohol or ether function present at least five atoms away from the urea carbonyl is very useful to make very potent soluble inhibitors.[24] This observation was extended to include many polar groups such as the secondary pharmacophore. Because ease and cost-effectiveness in synthesizing compounds are critical to facilitate extensive structure−activity relationship (SAR) studies, in this study an ether function was selected as the secondary functionality, which can be easily prepared in two reaction steps in a high yield, while five or six reactions are required for the preparation of the corresponding alcohol function, eventually resulting in a lower total reaction yield compared to that obtained in the preparation of the ether.[24] First, five ether derivatives were synthesized to determine the appropriate location for the incorporation of the ether or other hydrogen binding pharmacophore. As shown in Table 1, compared to 1-adamantyl-3-decylurea (IC_{50} =9.4 nM),[24] compounds **4**, **6**, **8**, and **10** showed potent inhibition on the target enzyme indicating that at least three methylene carbons between the primary urea and the secondary ether pharmacophores are necessary to produce potent inhibitory activity. In compounds with a shorter carbon chain length (**2**) or free alcohol functionality (**1**, **3**, **5**, **7**, and **9**), reduced inhibition potencies were observed, which is a similar result to that obtained previously with an ester functionality. It should be noted that when the free alcohol is sufficiently far from the central pharmacophore, as in **9**, the reduction in potency is minor. Thus, compounds such as **9** could be used to give enhanced water solubility or for the synthesis of prodrugs and soft drugs. Based

Scheme 2. Syntheses of 1-Adamantyl-3-substituted Alkyl-ureas[a]



[a] Reagents and conditions: (a) Ph_3P, CBr_4, DMF, rt; (b) 2-hexanol (for compound **11**), 2-methyl-1-pentanol (for compound **12**), or triethylene glycol monomethyl ether (for compound **18**), NaH, DMF, rt; (c) Ph_3P, CBr_4, THF, rt; (d) compound **7**, NaH, DMF, rt.

Scheme 3. Syntheses of 1-Adamantyl-3-substituted Alkyl-ureas[a]



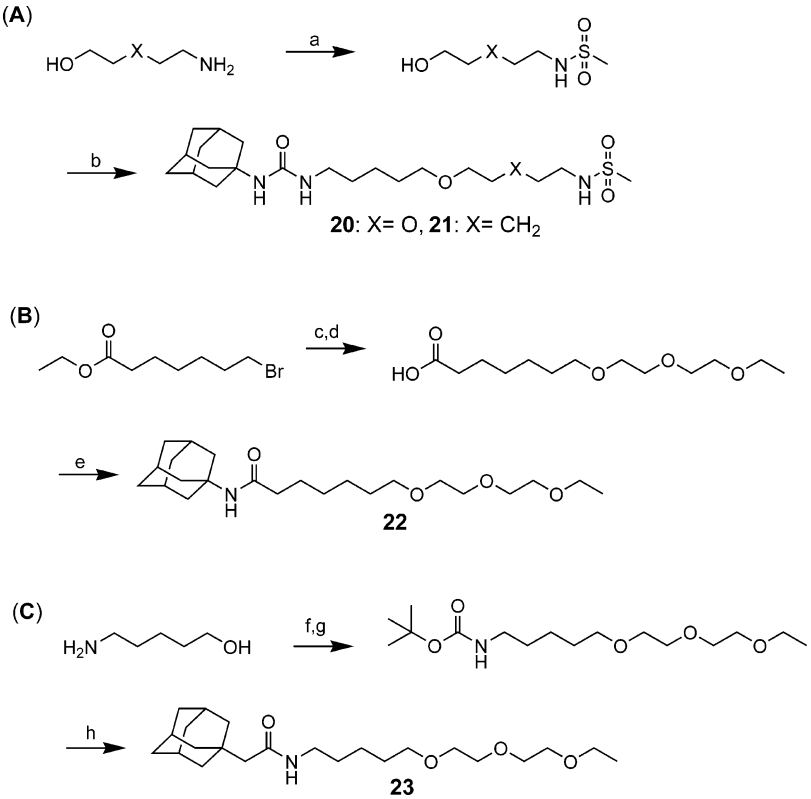
[a] Reagents and conditions: (a) 1,2-ethandiol (for compound **13**) or 1,3-propandiol (for compound **14**), 1-propanol, NaH, DMF, rt; (b) **I**, NaH, DMF, rt; (c) 2,2,2-trifluoroethanol (for compound **16**) or 4-ethylphenol (for compound **17**), NaH, DMF, rt; (d) compound **7**, NaH, DMF, rt.

on this SAR result and cost-effectiveness, compound **8** with a pentyl linker between the two pharmacophores was selected as a lead structure for further modifications in this study. When water solubility of compound **8** was examined, a 4-fold better solubility was obtained compared to a nonfunctionalized lipophilic inhibitor. However, its solubility is still limited (<2 μg/mL) and similar to that obtained in the ester compound.[24] Moreover, only 25% of compound **8** remained when it was incubated with human liver microsomes for 60 min, suggesting that 75% was metabolized by microsomal enzymes such as P450s. These results suggest that compound **8** needs to be modified for making compounds with not only increased solubility but also improved metabolic stability, which can possibly result in inhibitors possessing enhanced pharmacokinetic properties.

As listed in Table 2, various ether compounds modified with a variety of functionalities such as a branched alkyl chain (**11** and **12**), polar ether groups (**13**−**18**), a morpholine (**19**), or a sulfonamide (**20** and **21**) were synthesized to investigate the effect of functionalization of compound **8** on inhibition potency on the target enzyme, physical properties, and in vitro metabolic stability. Incorporation of a methyl branch on the carbon alpha (**11**) or beta (**12**) to the ether oxygen atom of compound **8** decreased both inhibition potency and metabolic stability, while slightly enhanced physical properties were exhibited. Two derivatives with a propoxy group (**13** and **14**) showed increases in inhibition and physical properties compared to compound **8**. However, a 3-fold reduction in stability was observed in the compounds (**13** and **14**), implying that a polar propyloxy group or a branch chain incorporated in compound **8** does not effectively produce improved inhibitors in either metabolic stability or inhibition potency. Interestingly, an 80-fold better water solubility than that of compound **8** was observed when an ethoxyethoxy group was introduced in the place of the propoxy group of compounds **13** and **14** (**15**). Furthermore, a significant enhancement of in vitro metabolic stability was exhibited without a loss of inhibition potency, suggesting that the diethylene glycol group of compound **15** would be very useful for yielding inhibitors with improved pharmacokinetic properties. It was anticipated that compound **15** would be rapidly metabolized by ω-hydroxylation or by ω-1 hydroxylation alpha to the heteroatom. It was hypothesized that this metabolism would be blocked by a terminal trifluoromethyl substituent in **16**. To our surprise, compound **16** showed decreased stability in the S9 incubation, although dramatically improved water solubility was obtained with **16** without a decrease in the inhibition. An aryl ether derivative (**17**) also did not have significant improvement in either solubility or stability, while inhibition potency was maintained. Thus, neither a branch chain (**11** and **12**) nor a trifluoromethyl **(16)** group incorporated in the molecules provided a significant increase in metabolic stability. As expected, compound **17** containing a *p*-ethylphenyl group did not improve metabolic stability. On the other hand, comparing compounds **13** and **14** to **15**, the increased polarity of compound **15** seemed to be effective for enhancing stability as well as other physical properties.

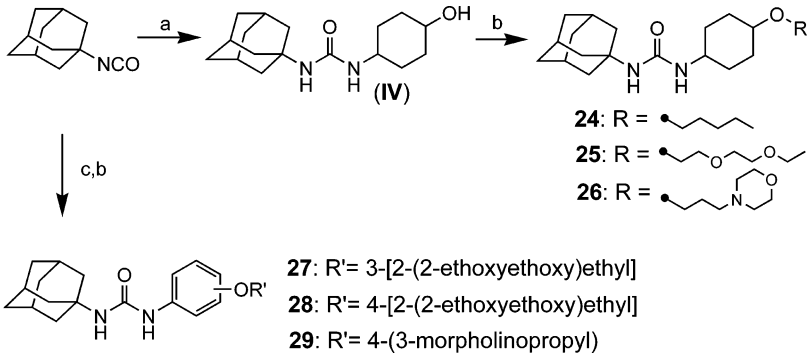
Based on the results above, compound **18** was prepared. It showed a dramatic improvement in physical properties and similar stability to that of compound **15**, while an approximately 5-fold drop in inhibition was obtained. This indicates that the

Scheme 4. Syntheses of 1-Adamantyl-3-substituted Alkyl-ureas with a Sulfonamide (**20** and **21**) and of Amide Derivatives (**22** and **23**) of Compound **15**[a]



[a] Reagents and conditions: (a) 2-aminoethoxyethanol (for compound **20**) or 5-amino-1-pentanol (for compound **21**), methanesulfonic anhydride, acetonitrile, rt; (b) **I**, NaH, DMF, rt; (c) diethylene glycol monoethyl ether, NaH, DMF, rt; (d) 1 N NaOH, ethanol, rt; (e) 1-adamantylamine, EDCI, DMAP, dichloromethane, rt; (f) di-(*tert*-butyl) dicarbonate, dioxane, rt; (g) 2-(ethoxyethoxy)ethyl bromide (prepared as described in Scheme **2** (**B**); **II**), NaH, DMF, rt; (h) (1) 4 N HCl in dioxane, rt; (2) 1-adamantylacetic acid, triethylamine, EDCI, DMAP, dichloromethane, rt.

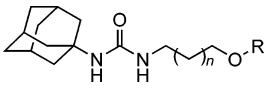
Scheme 5. Syntheses of 1-Adamantyl-3-substituted Cyclohexyl- or Phenyl-ureas[a]



[a] Reagents and conditions: (a) *trans*-4-aminocyclohexanol hydrochloride, triethylamine, DMF, rt; (b) 1-bromopentane (for compound **24**), 2-(ethoxyethoxy)ethyl bromide (**II**; for compound **25**), or 3-morpholinopropyl bromide (**II**; for compound **26**); (c) 3-aminophenol (for compound **27**) or 4-aminophenol (for compounds **28** and **29**), DMF, rt; (d) -(ethoxyethoxy)ethyl bromide (**II**), NaH, DMF, rt.

polar ethylene glycol tail of compound **18** would be very useful for producing soluble compounds in either water or organic solvents although decreased inhibition was observed in this series. On the other hand, a morpholino derivative (**19**) exhibited more desirable physical properties compared to compound **15**, while retaining reasonable inhibition. Moreover, a 3-fold improved stability in the microsomal or S9 incubation was gained in the polar morpholine compound (**19**), indicating that a morpholino group is a very effective functionality for producing enhanced sEH inhibitors not only with regard to physical properties but also with regard to metabolic stability. When the morpholino group of compound **19** was replaced by a methanesulfonamide function at least a 2-fold decrease in stability was observed (**20** and **21**). Also, unfortunately, a considerable drop in inhibition potency was shown with the sulfonamide (**20**), which is 12-fold less active than compound **15**. Although the other sulfonamide derivative (**21**) exhibited a reasonable inhibition on the enzyme, reductions in both solubility and stability were observed in this case, indicating that a sulfonamide group attached to the ether derivatives is not useful for either inhibiting the target enzyme effectively or stabilizing parent compounds against metabolism in this series. The result in Table 2 indicates that significantly enhanced improvements in physical properties and metabolic stability were exhibited with the introduction of a diethylene glycol or a morpholino group, as shown in compounds **15** and **19**. We have reported that modification of the urea pharmacophore of potent sEH inhibitors to the corresponding amide functionality does not

Table 1. Inhibition of Human sEH by 1-Adamantyl-3-hydroxyalkylureas or the Corresponding Ether Derivatives



No.	*n*	R	IC_{50} (nM)[a] human sEH
1	0	H	482
2	0	*n*-heptyl	29
3	1	H	746
4	1	*n*-hexyl	5.6
5	2	H	531
6	2	*n*-pentyl	4.4
7	3	H	84
8	3	*n*-butyl	4.1
9	4	H	12
10	4	*n*-propyl	3.7

[a] Human sEH (1 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 triplicate averages. The fluorescent assay as performed here has a standard error between 10 and 20%, suggesting that differences of 2-fold or greater are significant.[33]

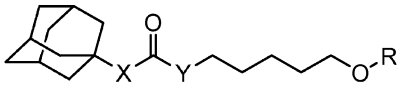
dramatically alter the inhibition potency and that an improvement in physical properties is observed in the amide derivatives.[25] This indicates that the physical properties of the ether derivatives (**15** or **19**) might be further improved with the modification of the urea pharmacophore to the amide function without a drop in inhibition. Thus, when two amide analogues of compound **15** were prepared (**22** and **23**), compound **22** with the nitrogen atom on the left side of the carbonyl of the amide function had approximately 50-fold decreased inhibition, while a similar stability to that of compound **15** was obtained. The presence of the nitrogen atom on the right side of the amide function did not drop inhibition potency, as shown in compound **23**, which is consistent with the previous report that the nitrogen on the right side of the carbonyl group of the amide pharmacophore is important for producing potent inhibitors.[25] However, a 6-fold decrease in metabolic stability was obtained in the amide compound **23**, indicating that an amide pharmacophore might not be effective in this ether series for making stable compounds in vivo while retaining potent inhibition unless the amide functionality could be stabilized.

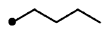
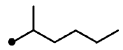
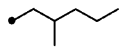
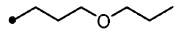
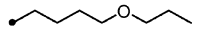
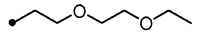
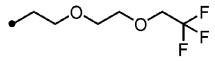
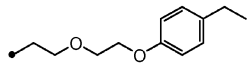
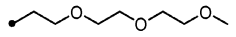
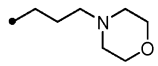
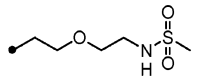
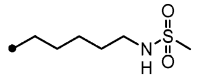
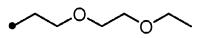
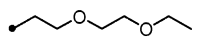
As shown in Table 3, the corresponding cyclohexyl (**24**−**26**) and phenyl (**27**−**29**) derivatives of compounds **15** and **19** were further synthesized. Interestingly, when the pentyl linker of compounds **8**, **15**, and **19** was replaced by a cyclohexyl or phenyl group (**24**−**29**), 4−8-fold increases in inhibition were obtained, indicating that the increased structural rigidity and increased hydrophobicity between the two pharmacophores is effective for producing highly potent inhibitors. This results in compounds binding to the target enzyme very specifically. Comparing compounds **8** and **24**, replacement of the pentyl linker (**8**) with a cyclohexyl (**24**) resulted in a poor inhibitor in terms of physical properties (higher melting point and lower water solubility), while similar stability was observed. However, a huge improvement in solubility was observed when the hydrophobic alkyl tail of compound **24** was replaced by a polar diethylene glycol (**25**) or 3-morpholinopropyl (**26**) group, which has similar solubility to that of the corresponding pentyl derivatives (**15** and **19**). Moreover, significantly greater stabilities were exhibited in the cyclohexyl derivatives with a polar tail (**25** and **26**) over the corresponding pentyl compounds (**15** and **19**), indicating that a cyclohexyl linker between the primary and the secondary pharmacophores, and a polar group attached to this linker are important for yielding very potent compounds with improved metabolic stability while retaining reasonable water solubility. Replacement of the cyclohexyl group by a phenyl group in **25** resulted in approximately a 10-fold drop in water solubility (**27** and **28**), while similar stability was exhibited with compound **28**. Interestingly, when the diethylene glycol group was present on the 3-position of the phenyl ring (**27**), stability was reduced suggesting that the *meta*-position on the phenyl ring is metabolically susceptible. A phenyl derivative with a morpholino group (**29**) also exhibited a 4-fold lower solubility, but no decrease in stability was observed. Overall, the results in Table 3 indicate that a cyclohexyl group as a linker is effective for increasing not only inhibition potency on the human sEH but metabolic stability without a drop in water solubility when compared to the corresponding pentyl analogs. Furthermore, a phenyl group as a linker between two pharmacophores is also effective for enhancing both inhibition and metabolic stability, although a decrease in solubility was observed. While in this study we concentrated on the human sEH, in previous studies we found over 90% correlation between the inhibition results observed with the human and the murine sEH.[21,23−25] Thus, the SAR conclusions drawn here for the human enzyme are probably valid for the rodent enzyme, and the best inhibitors herein are certainly also very potent inhibitor for the rodent sEH. However, there are occasional large differences in potency of certain compounds among model species.

We then investigated the pharmacokinetics of a series of urea-ether derivatives with improved physical properties and metabolic stability in mice, rats, and dogs. As shown in Table 4, the pharmacokinetic parameters (area under the curve (AUC) and half-life ($t_{1/2}$)) of eight derivatives were determined following oral administration at 5 mg/kg body weight for rodents and at 0.3 mg/kg body weight for dogs. A few sEH inhibitors such as compound **15** (also called AEPU (1-adamantan-3-(5-(2-(ethyl-ethoxy)ethoxy)pentyl)urea) or "950")[13] are bioavailable whether administered as a powder or a solution, however, many urea sEH inhibitors are poorly bioavailable if they are not in true solution. Thus, all compounds were administered by oral gavage in tristerate solution at body temperature. In a few cases a trace of ethanol was used to make a clear solution.

The AUC is an expression of how much and how long a drug stays in the body and it is related to the amount of drug absorbed systemically as well as the amount of drug metabolized, sequestered, and eliminated; while the $t_{1/2}$ is more indicative of the rates of degradation, distribution, and elimination. The experimental logP of each derivative was determined and compared to the calculated values (clogP). Although a similar trend was observed between the two values, there are significant differences between the calculated and experimental logP. When we compared these data to water solubility data (Tables 2 and 3), the changes in logP are related to their water solubility. In the mouse, little or no apparent absorption was observed with the compounds that have a pentyl group as a linker (**8**, **15**, and **19**). Interestingly, a significant increase in the AUC was obtained with the compounds that have a cyclohexyl group as a linker (**24** to **26**) compared to the corresponding pentyl compounds (**8**, **15**, and **19**). Furthermore, in the cyclohexyl series (**24** to **26**), the AUC decreased with an increase in the logP, while the half-lives increased, suggesting that the improved water solubility of the cyclohexyl derivatives is likely useful for enhancing absorption that consequently results in increased AUC. The replacement of the cyclohexyl by a phenyl group results in compounds that were not

Table 2. Inhibition of Human sEH, Physical Properties, and In Vitro Metabolic Stability of 1-Adamantyl-3-substituted Alkyl-urea Derivatives



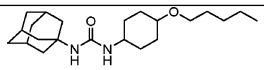
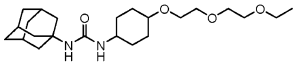
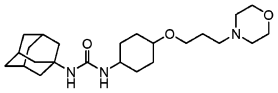
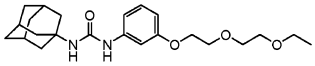
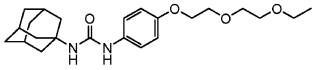
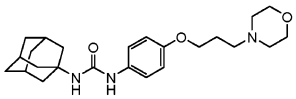
No.	X	Y	R	IC_{50} (nM)[a] Human sEH	Mp[b] (°C)	Solubility in water[c] (μg/mL)	Stability (%)[d]	
							microsomes	S9
8	NH	NH		4.1	71	1.6	25	ND[e]
11	NH	NH		11	45	2.3	12	ND
12	NH	NH		10	49	2.0	12	ND
13	NH	NH		2.5	oil	26	6.8	ND
14	NH	NH		2.3	oil	15	7.5	ND
15	NH	NH		14	78	120	30	11
16	NH	NH		1.4	34	234	39	1.1
17	NH	NH		1.7	71	0.38	21	ND
18	NH	NH		23	oil	3709	54	14
19	NH	NH		9.0	oil	897	89	34
20	NH	NH		58	oil	1082	42	12
21	NH	NH		11	93	75	59	<LD[f]
22	NH	CH_2		247	oil	ND	32	5
23	CH_2	NH		10	oil	ND	5	ND

[a] Human sEH (1 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. The fluorescent assay as performed here has a standard error between 10 and 20%, suggesting that differences of 2-fold or greater are significant.[33] [b] Melting point. [c] Solubility in sodium phosphate buffer (0.1 M, pH 7.0) at 25 ± 1.5 °C. [d] Percentage (%) of test compounds (**8**, **11**−**22**, and **23**) remaining after a 1 h incubation with enzymes in human liver microsomes or S9 fractions. Enzymes (0.125 mg for microsomes or 0.056 mg for S9 fractions) were incubated with inhibitors ([*S*] = 1.0 μM) for 0 and 60 min with data shown for 60 min in sodium phosphate buffer (0.1 M, pH 7.4) at 37 °C. [e] Nondetermined. [f] Limit of detection.

significantly absorbed in rodents (**28** and **29**). Overall, it implies that water solubility is an important factor for the absorption of this series of compounds in mice. In the rat, like in the mouse, the cyclohexyl derivatives (**24** to **26**) gave overall the best absorption and greatest metabolic stability. However, independent of the linker structure, compounds having a diethylene glycol group (**15**, **25**, and **28**) have better AUCs. Except for **15**, these latter compounds also have a longer half-life. Overall, for the series of chemicals tested herein, water solubility is probably not as important for absorption in rats as it is in mice. There appears to be an optimal value of logP, between 3.0 and 4.0, for good absorption.

Finally, we studied these eight derivatives in a canine model (Table 4). All compounds tested were absorbed quite well, and no direct relationships between structural features and pharmacokinetic parameters could be easily drawn. However, overall, the presence of a cyclic linker (cyclohexyl **24** to **26** or phenyl **28** and **29**) seems to increase the AUC in the dogs. For such linkers, the bioavailability is increased by the presence of polar groups such as diethylene glycol (**25** and **28**) or morpholino (**26** and **29**) groups. Overall, across the three species studied, the rigidification of the structure with cyclohexyl or phenyl group gives more bioavailable inhibitors than straight alkyl linkers such as the butyl or pentyl groups. Moreover, the presence of a polar group attached to this linker improves absorption. There are large differences among the three species with regard to the influence of structure on AUC and $t_{1/2}$. Overall, there is an apparent trend between AUC and the size of the species. However, even though the dose given to the small animals (5 mg/kg) was higher than the one given to the dogs (0.3 mg/kg), the total quantities received were larger for the dogs (6 mg instead of 0.1−1 mg for the rodents). There are

Table 3. Inhibitions of Human and Murine sEHs, Physical Properties, and In Vitro Metabolic Stability of 1-Adamantyl-3-substituted Cyclohexyl- or Phenyl-urea Derivatives

No.	R	IC_{50} (nM)[a] Human sEH	Mp[b] (°C)	Solubility in water[c] (μg/mL)	Stability (%)[d] microsomes	Stability (%)[d] S9
24		1.2	205	0.4	22	ND[e]
25		1.6	155	424	91	79
26		1.8	178	134	92	81
27		1.1	oil	17	56	9
28		1.8	105	19	88	70
29		1.3	191	34	93	97

[a] Human sEH (1 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. The fluorescent assay as performed here has a standard error between 10 and 20%, suggesting that differences of 2-fold or greater are significant.[33] [b] Melting point. [c] Solubility in sodium phosphate buffer (0.1 M, pH 7.0) at 25 ± 1.5 °C. [d] Percentage (%) of test compounds (**8**, **11**−**22**, and **23**) remaining after a 1 h incubation with enzymes in human liver microsomes or S9 fractions. Enzymes (0.125 mg for microsomes or 0.056 mg for S9 fractions) were incubated with inhibitors ([*S*] = 1.0 μM) for 0 and 60 min with data shown for 60 min in sodium phosphate buffer (0.1 M, pH 7.4) at 37 °C. [e] Nondetermined.

Table 4. Pharmacokinetic Parameters of 1-Adamantyl-3-substituted Pentyl (**8**, **15**, **19**)-, Cyclohexyl (**24**−**26**)-, or Phenyl (**28**, **29**)-urea Derivatives in Mouse, Rat, and Dog

		mouse		rat		dog	
No.	LogP[a] (cLogP)[b]	AUC[c]	$t_{1/2}$[d]	AUC[c]	$t_{1/2}$[d]	AUC[c]	$t_{1/2}$[d]
8	5.0 (3.1)	<LD[e]	<LD	<LD	<LD	5600	130
15	3.6 (1.9)	<LD	<LD	3000	39	8300	180
19	2.4 (1.5)	340	15	750	200	2500	90
24	>5.0 (3.4)	1200	170	560	120	5100	120
25	2.9 (1.7)	2800	160	5900	150	9800	90
26	2.2 (1.4)	10 000	80	2400	110	27 000	130
28	3.9 (2.6)	<LD	<LD	2900	580	34 000	320
29	2.9 (2.3)	<LD	<LD	<LD	<LD	8400	130

[a] Measured logP. [b] Calculate logP. [c] Area under the concentration-time curve to terminal time (min·g·nM·mg^{-1}). [d] Elimination half-life (min). [e] Limit of detection.

many variables involved in scaling from rodent to canine species, including total size, hepatic and renal blood flow, gut residence time, and many other factors. It must also be cautioned that blood levels correlate well with efficacy with only about half of the marketed pharmaceuticals. Furthermore, the in vivo behavior of the sEH inhibitors is not easy to predict from microsomal and S9. These suggest that further pharmacokinetic studies are needed to understand the mechanisms of absorption, disposition, metabolism, and elimination for this series of compounds and, thus, be able to predict their in vivo behaviors.

In conclusion, this work focused on producing inhibitors of human sEH with improved pharmacokinetic properties. This was done by analyzing the effects of structural changes of 1,3-disubstituted ureas with an ether function present at least three atoms away from the urea carbonyl on inhibition potency, physical properties (e.g., water solubility and melting point), and metabolic stability. The inhibition studies showed that a hydrophobic group as a linker between the primary urea and the ether function is necessary to yield potent inhibitors. Furthermore, the presence of polar groups on the other side of the ether does not affect the inhibition potency significantly. However, up to 80-fold and 3-fold increases in water solubility and in vitro metabolic stability, respectively, were observed. The best results were obtained in compounds functionalized with ethylene glycol or morpholine groups. Furthermore, the corresponding cyclohexyl or phenyl derivatives showed improved metabolic stability as well as enhanced inhibition potency while retaining reasonable water solubility. The improved water solubility and in vitro metabolic stability were useful for enhancing pharmacokinetic properties in animal models. Overall, across the three species studied, we found the bioavailability is enhanced by the presence of cyclohexyl as a linker between the urea and the ether and by a polar group, such as diethylene glycol or morpholine, on the other side of the ether. In summary, we were able to increase solubility and bioavailability of sEH inhibitors without any loss in potency. These findings are an important basis for the design of improved, orally available therapeutic agents for the treatment of hypertension and inflammation.

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/F) were performed by Midwest Microlab, IN; analytical results were within ±0.4% of the theoretical values for the formula given unless otherwise indicated. ^{1}H and ^{13}C NMR spectra were recorded on a QE-300 spectrometer, using tetramethylsilane as an internal standard. IR spectra were recorded on a Thermo Nicolet IR100 spectrometer. Synthetic methods are described for representative compounds.

1-Adamantan-1-yl-3-(5-hydroxypentyl)urea (7). To a solution of adamantyl isocyanate (0.20 g, 1.13 mmol) in DMF (15 mL) was added a solution of 5-amino-1-pentanol (0.17 g, 1.69 mmol) in DMF (15 mL) at 0 °C. After stirring for 12 h, an aqueous solution of 1 N HCl (40 mL) was added into the reaction at 0 °C, and the mixture was stirred for 30 min. The crystalline solid product was filtered and washed with water (40 mL) and ethyl acetate (20 mL). The resulting solid was dried in the vacuum oven to give **7** as a white solid (0.75 g, 100%). ^{1}H NMR δ ($CDCl_3$) 1.37−1.42 (2H, m), 1.46−1.53 (2H, m), 1.57−1.62 (2H, m), 1.66 (6H, brs), 1.96 (6H, brs), 2.06 (3H, brs), 3.13 (2H, q, J = 6.9 Hz), 3.65 (2H, t, J = 6.9 Hz), 4.14 (1H, s), 4.39 (1H, s). LC-MS (ESI) *m*/*z* calcd for $C_{16}H_{28}N_2O_2$ $[M + H]^+$, 281.22; found $[M + H]^+$, 281.33; mp 220 °C; Anal. ($C_{16}H_{28}N_2O_2$) C, H, N.

Compounds **1**, **3**, **5**, and **9** were synthesized with the same procedure used for the preparation of **7** using the corresponding aminoalkanol instead of 5-amino-1-pentanol.

1-Adamantan-1-yl-3-(5-butoxypentyl)urea (8). To a suspension of 60% sodium hydride (21 mg, 0.53 mmol) and **7** (150 mg, 0.53 mmol) in DMF (20 mL) was added dropwise 1-bromobutane (90 mg, 0.64 mmol) at room temperature. After stirring for 12 h, water (40 mL) was poured into the reaction mixture, and the product was extracted with ether (40 mL). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified using silica gel column chromatography (hexane/ethyl acetate = 3:1) to give **8** (80 mg, 45%) as a solid. ^{1}H NMR δ ($CDCl_3$) 0.92 (3H, t, J = 6.9 Hz), 1.35−1.38 (4H, m), 1.53−1.77 (12H, m), 1.946 (6H, brs), 2.06 (3H, brs), 3.10 (2H, q, J = 6.9 Hz), 3.36−3.43 (4H, m), 4.14 (1H, s), 4.26 (1H, s). LC-MS (ESI) *m*/*z* calcd for $C_{20}H_{36}N_2O_2$ $[M + H]^+$, 337.28; found $[M + H]^+$, 337.32; mp 71 °C; Anal. ($C_{20}H_{36}N_2O_2$) C, H, N.

Compounds **2**, **4**, **6**, and **10** were synthesized with the same method used for the preparation of **8** using the corresponding 1-bromoalkane instead of 1-bromobutane.

1-Adamantan-1-yl-3-[5-(1-methylpentyloxy)pentyl]urea (11). To a solution of **7** (1.02 g, 3.64 mmol) in DMF (40 mL) was added portionwise triphenylphosphine (1.05 g, 4.01 mmol) and carbon tetrabromide (2.00 g, 4.01 mmol) at 0 °C. After stirring for 12 h, the product was extracted with ether (60 mL), and the ether solution was washed with water (60 mL), dried over $MgSO_4$, and concentrated. The residue was purified using silica gel column chromatography (hexane/ethyl acetate = 3:1) to give 1-adamantan-1-yl-3-(5-bromopentyl)urea **I** (1.08 g, 87%) as a solid. This bromide (0.10 g, 0.29 mmol) was added portionwise to a suspension of 60% sodium hydride (12 mg, 0.29 mmol) and 2-hexanol (41 mg, 0.35 mmol) in DMF (15 mL) at room temperature. After stirring for 12 h, water (30 mL) was poured into the reaction mixture, and the product was extracted with ether (40 mL). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified using silica gel column chromatography (hexane/ethyl acetate = 3:1) to afford **11** (5.8 mg, 52%) as a solid. ^{1}H NMR δ ($CDCl_3$) 0.89 (3H, t, J = 6.9 Hz), 1.12 (3H, d, J = 6.9 Hz), 1.30−1.40 (4H, m), 1.48−1.68 (14H, m), 1.96 (6H, brs), 2.06 (3H, brs), 3.09 (2H, q, J = 6.9 Hz), 3.32−3.35 (2H, m), 3.48−3.50 (1H, m), 4.12 (2H, brs). LC-MS (ESI) *m*/*z* calcd for $C_{22}H_{40}N_2O_2$ $[M + H]^+$, 365.31; found $[M + H]^+$, 365.31; mp 45−48 °C; Anal. ($C_{22}H_{40}N_2O_2$) C, H, N.

Compounds **12** and **18** were synthesized with the same procedure used for the preparation of **11** using 2-methyl-1-pentanol for **12** and triethylene glycol monomethyl ether for **18**, respectively, instead of 2-hexanol.

1-Adamantan-1-yl-3-{5-[2-(2-ethoxyethoxy)ethoxy]pentyl}-urea (15). To a solution of diethylene glycol monoethyl ether (13.0 g, 95 mmol) in THF (150 mL) was added portionwise triphenylphosphine (27.6 g, 0.11 mol) and carbon tetrabromide (35.0 g, 0.11 mol) at 0 °C. After stirring for 12 h at room temperature, hexane (100 mL) was added to the reaction mixture. This crude mixture was filtered to get rid of triphenylphospine oxide, and the organic solvent dissolving the product was washed with water (100 mL), dried over $MgSO_4$, and concentrated. The residue was purified using silica gel column chromatography (hexane only and hexane/ethyl acetate = 3:1) to give brominated product **II** as an oil (16.4 g, 87%). Product **II** (10.4 g, 53 mmol) was added dropwise to a suspension of 60% sodium hydride (2.83 g, 70mmol) and compound **7** (9.9 g, 35 mmol) in DMF (70 mL) at 0 °C. After stirring for 12 h, water (150 mL) was poured into the reaction mixture, and the product was extracted with ether (100 mL × 2). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified using silica gel column chromatography (hexane/ethyl acetate = 3:1) to afford **15** (52%) as a solid. ^{1}H NMR δ ($CDCl_3$) 1.22 (3H, t, J = 6.9 Hz), 1.37−1.43 (2H, m), 1.46−1.53 (2H, m), 1.56−1.61 (2H, m), 1.67 (6H, brs), 1.97 (6H, brs), 2.07 (3H, brs), 3.11 (2H, q, J = 6.9 Hz), 3.46 (2H, t, J = 6.9 Hz), 3.48−3.67 (10H, m), 4.21 (1H, s), 4.26 (1H, s). ^{13}C NMR δ ($CDCl_3$) 15.12, 23.36, 29.05, 29.53, 29.68, 36.43, 40.00, 42.51, 50.74, 66.64, 69.78, 69.97, 70.57, 70.60, 71.01, 157.2. IR 1631 cm^{-1}, 3299 cm^{-1}, 3379 cm^{-1}. LC-MS (ESI) *m*/*z* calcd for $C_{22}H_{40}N_2O_4$ $[M + H]^+$, 397.30; found $[M + H]^+$, 397.31; mp 78 °C; Anal. ($C_{22}H_{40}N_2O_4$) C, H, N.

Compound **19** was synthesized with the same procedure used for the preparation of **15** using 3-morpholinopropyl bromide prepared from the corresponding alcohol instead of 2-(ethoxyethoxy)-ethyl bromide.

1-Adamantan-1-yl-3-(5-{2-[2-(2,2,2-trifluoroethoxy)ethoxy]-ethoxy}pentyl)urea (16). To a solution of 60% sodium hydride (1.71 g, 42.8 mmol) and 2,2,2-trifluoroethanol (7.20 g, 71.4 mmol) in DMF (40 mL) was added di-(2-bromoethyl) ether (9.20 g, 35.7 mmol) at room temperature. The reaction mixture was stirred for 3 h, and water (50 mL) was poured into the reaction. The product was extracted with ether (30 mL × 2), and the organic layer was washed with water (30 mL × 2), dried over $MgSO_4$, and evaporated to give 2-(2,2,2-trifluoroethoxyethoxy)ethyl bromide in a crude mixture (~60% yield). Without further purification, this bromide intermediate was directly used for the next reaction. To a suspension of 60% sodium hydride (3.83 g, 95.7 mmol) and **7** (10.0 g, 39.3 mmol) stirred for 20 min in DMF (40 mL) was added this alkylated 2-bromoethyl ether intermediate at room temperature. After stirring overnight, the product was extracted with ether (50 mL × 2), and the organic layer was washed with water (80 mL), dried over $MgSO_4$, and evaporated. The residue was purified using column chromatography eluting with hexane and ethyl acetate (1:1) to provide **16** (0.36 g, 45%) as a solid. This purified product was further recrystallized in hexane and dried in the vacuum oven for 2 days to provide very pure compound. ^{1}H NMR δ ($CDCl_3$) 1.40 (2H, quint, J = 6.9 Hz), 1.50 (2H, quint, J = 6.9 Hz), 1.55 (2H, quint, J = 6.9 Hz), 1.65 (6H, brs), 1.95 (6H, brs), 2.05 (3H, brs), 3.09 (2H, t, J = 6.9 Hz), 3.45 (2H, t, J = 6.9 Hz), 3.57 (2H, t, J = 6.9 Hz), 3.64 (2H, t, J = 6.9 Hz), 3.68 (2H, t, J = 6.9 Hz), 3.78 (2H, t, J = 6.9 Hz), 3.90 (2H, q, J = 6.9 Hz), 4.23 (1H, s), 4.32 (1H, s). ^{13}C NMR δ ($CDCl_3$) 23.36, 29.20, 29.38, 29.52, 29.63, 29.92, 36.41, 40.07, 42.49, 50.71, 69.10, 69.97, 70.56, 70.70, 71.19, 71.89, 122.8, 157.3. IR 1631 cm^{-1}, 2909 cm^{-1}, 3352 cm^{-1}. LC-MS (ESI) *m*/*z* calcd for $C_{22}H_{37}F_3N_2O_4$ $[M + H]^+$, 451.27; found $[M + H]^+$, 451.27; mp 34 °C; Anal. ($C_{22}H_{37}F_3N_2O_4$) C, H, N, F.

Compound **17** was synthesized with the same procedure used for the preparation of **16** using 4-ethylphenol instead of 2,2,2-trifluoroethanol.

***N*-{5-[5-(3-Adamantan-1-yl-ureido)pentyloxy]pentyl}-methanesulfonamide (21).** To a solution of 5-amino-1-pentanol (0.59 g, 5.7 mmol) in acetonitrile (20 mL) was added methanesulfonic anhydride (0.50 g, 2.9 mmol) at room temperature. After stirring for 5 h, the solvent was evaporated. To this residue ethyl acetate (30 mL) and water (30 mL) were poured and partitioned.

The organic solution dissolving the product was washed with water (30 mL), dried over $MgSO_4$, and concentrated. The residue was purified using silica gel column chromatography (hexane/ethyl acetate = 1:3) to give 5-hydroxypentylmethanesulfonamide (0.34, 1.9 mmol) in 66% yield. To a suspension of 60% NaH (76 mg, 1.9 mmol) in DMF (20 mL), a solution of the above methanesulfonamide intermediate in DMF (2 mL) was added dropwise at room temperature. After stirring for 30 min, **I** (0.65 g, 1.9 mmol) was added to the reaction mixture and the reaction was further stirred for 12 h at room temperature. Water (50 mL) was poured into the mixture, and the product was extracted with ether (60 mL). The ether solution was dried over $MgSO_4$ and concentrated. The residue was purified by using silica gel column chromatography eluting with hexane and ethyl acetate (1:4) to afford **21** (0.46 g,1.0 mmol) in 55% yield. ^{1}H NMR δ ($CDCl_3$) 1.36−1.53 (6H, m), 1.59−1.66 (12H, m), 1.96 (6H, brs), 2.06 (3H, brs), 2.82 (3H, s), 3.11−3.19 (6H, m), 3.66 (2H, t, J = 6.9 Hz), 4.12 (1H, s), 4.22 (1H, s). LC-MS (ESI) *m*/*z* calcd for $C_{22}H_{41}N_3O_4S$ $[M + H]^+$, 444.28; found $[M + H]^+$, 444.32; mp 93 °C; Anal. ($C_{22}H_{41}N_3O_4S$) C, H, N.

Compound **20** was prepared with the same method used for the preparation of compound **21** using 2-aminoethoxyethanol instead of 5-amino-1-pentanol.

1-Adamantan-1-yl-3-(4-pentyloxycylclohexyl)urea (24). A mixture of 1-adamantyl isocyanate (0.30 g, 1.69 mmol), *trans*-4-aminocyclohexanol hydrochloride (0.38 g, 2.54 mmol), and triethylamine (0.37 mL, 2.54 mmol) in DMF (30 mL) was stirred at room temperature for 12 h, and to the reaction mixture was poured an aqueous solution of 1 N HCl (40 mL) at 0 °C. After 30 min stirring, the solid product crystallized was filtered and washed with water (50 mL) and ethyl acetate (30 mL). The resulting solid was dried in the vacuum oven at 50 °C to give 1-adamantan-1-yl-3-(4-hydroxycyclohexyl)urea **IV** (0.72 g, 100%) as a white solid. To a suspension of 60% sodium hydride (0.11 g, 2.74 mmol) in DMF (15 mL) was added a solution of **IV** (0.40 g, 1.37 mmol) in DMF (3 mL) at room temperature. After stirring for 30 min, 1-bromopentane (0.25 g, 1.64 mmol) was added to the reaction at room temperature. The reaction was stirred for 12 h and water (50 mL) was poured into the reaction mixture. The product was extracted with ether (60 mL), and the ether solution was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel, eluting with hexane and ethyl acetate (1:1) to afford **24** (0.12 g, 25%) as a solid. ^{1}H NMR δ ($CDCl_3$) 0.89 (3H, t, J = 6.9 Hz), 1.15 (2H, q, J = 6.9 Hz), 1.35 (2H, q, J = 6.9 Hz), 1.53−1.61 (6H, m), 1.66 (6H, brs), 1.96 (6H, brs), 1.99−2.11 (7H, m), 3.14−3.19 (1H, m), 3.41 (2H, t, J = 6.9 Hz), 3.50−3.52 (1H, m), 3.91 (2H, s). LC-MS (ESI) *m*/*z* calcd for $C_{22}H_{38}N_2O_2$ $[M + H]^+$, 363.29; found $[M + H]^+$, 363.31; mp 205 °C; Anal. ($C_{22}H_{38}N_2O_2$) C, H, N.

Compounds **25** and **26** were synthesized with the same method used for the preparation of compound **24** using 2-(ethoxyethoxy)-ethyl bromide and 3-morpholinopropyl bromide, respectively, instead of 1-bromopentane.

Enzyme Preparation. Recombinant human sEH was produced in a polyhedron positive baculovirus expression system following cloning and sequencing in this laboratory and was purified by affinity chromatography as previously reported.[30−32]

IC_{50} Assay Conditions. IC_{50} values were determined as described using a sensitive fluorescent-based assay,[33] and a brief description of the procedure is as follows: cyano(2-methoxynaphthalen-6-yl)-methyl *trans*-(3-phenyl-oxyran-2-yl) methyl carbonate (CMNPC) was used as a fluorescent substrate. Human sEH (1 nM) was incubated with inhibitors for 5 min in pH 7.0 Bis-Tris/HCl buffer (25 mM) containing 0.1 mg/mL of BSA at 30 °C prior to substrate introduction ([*S*] = 5 μM). Activity was measured by determining the appearance of the 6-methoxy-2-naphthaldehyde with an excitation wavelength of 330 nm and an emission wavelength of 465 nm for 10 min. IC_{50} results are averages of three replicates. The fluorescent assay as performed here has a standard error between 10 and 20%, suggesting that differences of 2-fold or greater are significant.[33]

Solubility. Water solubility was determined experimentally in 1.0 mL of sodium phosphate buffer (0.1 M, pH 7.4) as previously described at 25 ± 1.5 °C.[24,25,27]

LogP Measurement and Calculation. In a 20 mL glass vial, the test compound (4 mg) was dissolved in 5 mL of buffer-saturated octanol. Octanol-saturated sodium phosphate buffer (0.1 M, pH 7.4; 5 mL) was then added to the octanol solution. The vial content was mixed strongly for 24 h at 23 ± 1.5 °C. After, the phases separated, the concentration of the tested compound dissolved in the octanol, and the aqueous layers were measured by LC/MS-MS following the method described previously.[24] The logP value was obtained with the following equation: LogP = log([octanol]/[water]). The results reported in Table 4 are averages of triplicate analyses. The calculate LogP (cLogP) value estimated by Crippen's method was generated by using CS ChemDraw 8.0.

In Vitro Metabolic Stability in Human Liver Microsomes or S9 Fractions. Human liver microsomal (0.125 mg) or S9 (0.056 mg) protein was brought to 0.222 mL or 0.178 mL with sodium phosphate buffer (0.1 M, pH 7.4), respectively. The proteins were preincubated for 5 min in open glass tubes immersed in a shaking bath at 37 °C. After this preincubation, a solution of test compound (2.5 μL or 2.0 μL of 100 μM for microsome and S9) was added and the reaction was initiated by the addition of a NADPH generating system (25 μL or 20 μL for microsome and S9, respectively; NADP (2 mM), glucose 6-phosphate (57 mM), glucose 6-phosphate dehydrogenase (3.5 units), and magnesium chloride (50 mM) in 1 mL of sodium phosphate buffer (0.1 M, pH 7.4)). The incubation mixture (0.25 mL or 0.20 mL total volume for microsome and S9, respectively) was shaken in a water bath at 37 °C for 60 min. A control was prepared by the addition of ethanol (1 mL) immediately after adding the NADPH generating system. The reaction was terminated by the addition of cold ethanol (0.75 mL for microsomes or 0.60 mL for S9), and a 50 μL aliquot of 500 ng/mL 1-adamantyl-3-decylurea was added to the samples. The samples were then vortexed and centrifuged at 6000 rpm (4000 g) for 5 min. The extracts were transferred to a new glass tube and dried under nitrogen. The residue was reconstituted in methanol (0.5 mL). Aliquots (5 μL) were analyzed by LC-MS/MS.[34,35] The absolute amount of parent compounds remaining after the incubation was converted to a percentage. The results given are averages of triplicate independent analyses.

In Vivo Pharmacokinetic Studies. In vivo experiments were performed following protocols approved by the U.C.D. Animal Use and Care Committee. Two rodent species (mice and rats) and dogs were treated with test compounds orally at 5 mg/kg for rodents and 0.3 mg/kg body weight for dogs, respectively. Compounds were given by oral gavage in 3 mL of tristerate at body temperature for the dogs, in 0.1 mL of oleic oil solution for the mice, and in 1 mL of oleic oil solution for the rats. For the rodents, 10 μL of whole blood were collected at 0, 0.5, 1, 2, 4, 6, 8, and 24 h. For dogs, 1 mL of blood sample was collected into EDTA K_3 blood collecting tubes at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, and 24 h. The samples were centrifuged at 3000 rpm at 4 °C for 10 min and the plasma samples were collected for instrumental analysis. Blood sample preparation and LC/MS/MS analysis were performed as previously reported.[26,27] Pharmacokinetic parameters (AUC and $t_{1/2}$) were calculated by fitting the blood concentration−time data to a noncompartmental model with WinNonlin 5.0 (Pharsight, CA). Data are average results obtained from at least three different animals.

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Supporting Information Available: Syntheses and detailed analytical data for compounds **1**−**6**, **9**, **10**, **12**−**14**, **17**−**20**, **22**, **23**, **25**−**28**, and **29**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hammock, B. D.; Grant, D.; Storms, D. Epoxide hydrolase. In *Comprehensive Toxicology*; Sipes, I., McQueen, C., Gandolfi, A., Eds.; Oxford: Pergamon Press, 1977; pp 283−305.

(2) Fretland, A. J.; Omiecinski, C. J. Epoxide hydrolases: Biochemistry and molecular biology. *Chem.-Biol. Interact.* **2000**, *129*, 41−59.

(3) Zeldin, D. C.; Kobayashi, J.; Falck, J. R.; Winder, B. S.; Hammock, B. D.; Snapper, J. R.; Capdevila, J. H. Regio- and enantiofacial selectivity of epoxyeicosatrienoic acid hydration by cytosolic epoxide hydrolase. *J. Biol. Chem.* **1993**, *268*, 6402−6407.

(4) Carroll, M. A.; McGiff, J. C. A new class of lipid mediators: Cytochrome P450 arachidonate metabolites. *Thorax* **2000**, *55*, S13−16.

(5) Newman, J. W.; Morisseau, C.; Hammock, B. D. Epoxide hydrolases: Their roles and interactions with lipid metabolism. *Prog. Lipid Res.* **2005**, *44*, 1−51.

(6) Capdevila, J. H.; Falck, J. R.; Harris, R. C. Cytochrome P450 and arachidonic acid bioactivation: Molecular and functional properties on the arachidonate monooxygenase. *J. Lipid Res.* **2000**, *41*, 163−181.

(7) Yu, Z.; Xu, F.; Huse, L. M.; Morisseau, C.; Draper, A. J.; Newman, J. W.; Parker, C.; Graham, L.; Engler, M. M.; Hammock, B. D.; Zeldin, D. C.; Kroetz, D. L. Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ. Res.* **2000**, *87*, 992−998.

(8) Imig, J. D.; Zhao, X.; Capdevila, J. H.; Morisseau, C.; Hammock, B. D. Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. *Hypertension* **2002**, *39*, 690−694.

(9) Zhao, X.; Yamamoto, T.; Newman, J. W.; Kim, I.-H.; Watanabe, T.; Hammock, B. D.; Stewart, J.; Pollock, J. S.; Pollock, D. M.; Imig, J. D. Soluble epoxide hydrolase inhibition protects the kidney from hypertension-induced damage. *J. Am. Soc. Nephrol.* **2004**, *15*, 1244−1253.

(10) Jung, O.; Brandes, R. P.; Kim, I.-H.; Schweda, F.; Schmidt, R.; Hammock, B. D.; Busse, R.; Fleming, I. Soluble epoxide hydrolase is a main effector of angiotensin II-induced hypertension. *Hypertension* **2005**, *45*, 759−765.

(11) Imig, J. D.; Zhao, X.; Zaharis, C. Z.; Olearczyk, J. J.; Pollock, D. M.; Newman, J. W.; Kim, I.-H.; Watanabe, T.; Hammock, B. D. An orally active epoxide hydrolase inhibitor lowers blood pressure and provides renal protection in salt-sensitive hypertension. *Hypertension* **2006**, *46*, 975−981.

(12) Smith, K. R.; Pinkerton, K. E.; Watanabe, T.; Pedersen, T. L.; Ma, S. J.; Hammock, B. D. Attenuation of tobacco smoke-induced lung inflammation by treatment with a soluble epoxide hydrolase inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 2186−2191.

(13) Schmelzer, K. R.; Kubala, L.; Newman, J. W.; Kim, I.-H.; Eiserich, J. P.; Hammock, B. D. Soluble epoxide hydrolase is a therapeutic target for acute inflammation. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9772−9777.

(14) Oltman, C. L.; Weintraub, N. L.; VanRollins, M.; Dellsperger, K. C. Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation. *Circ. Res.* **1998**, *83*, 932−939.

(15) Fisslthaler, B.; Popp, R.; Kiss, L.; Potente, M.; Harder, D. R.; Fleming, I.; Busse, R. Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* **1999**, *401*, 493−497.

(16) Node, K.; Huo, Y.; Ruan, X.; Yang, B.; Spiecker, M.; Ley, K.; Zeldin, D. C.; Liao, J. K. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* **1999**, *285*, 1276−1279.

(17) Campbell, W. B. New role for epoxyeicosatrienoic acids as anti-inflammatory mediators. *Trends Pharmacol. Sci.* **2000**, *21*, 125−127.

(18) Yang, B.; Graham, L.; Dikalov, S.; Mason, R. P.; Falck, J. R.; Liao, J. K.; Zeldin, D. C. Overexpression of cytochrome P450 CYP2J2 protects against hypoxia-reoxygenation injury in cultured bovine aortic endothelial cells. *Mol. Pharmacol.* **2001**, *60*, 310−320.

(19) Node, K.; Ruan, X.; Dai, J.; Yang, S.; Graham, L.; Zeldin, D. C.; Liao, J. K. Activation of Gs mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. *J. Biol. Chem.* **2001**, *276*, 15983−15989.

(20) Moghaddam, M. F.; Grant, D. F.; Cheek, J. M.; Greene, J. F.; Williamson, K. C.; Hammock, B. D. Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nat. Med.* **1997**, *3*, 562−567.

(21) Morisseau, C.; Goodrow, M. H.; Dowdy, D.; Zheng, J.; Greene, J. F.; Sanborn, J. R.; Hammock, B. D. Potent urea and carbamate inhibitors of soluble epoxide hydrolases. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8849−8854.

(22) Newman, J. W.; Denton, D. L.; Morisseau, C.; Koger, C. S.; Wheelock, C. E.; Hinton, D. E.; Hammock, B. D. Evaluation of fish models of soluble epoxide hydrolase inhibition. *Environ. Health Perspect.* **2001**, *109*, 61−66.

(23) Morisseau, C.; Goodrow, M. H.; Newman, J. W.; Wheelock, C. E.; Dowdy, D. L.; Hammock, B. D. Structural refinement of inhibitors of urea based soluble epoxide hydrolase. *Biochem. Pharmacol.* **2002**, *63*, 1599−1608.

(24) Kim, I.-H.; Morisseau, C.; Watanabe, T.; Hammock, B. D. Design, synthesis, and biological activity of 1,3-disubstituted ureas as potent inhibitors of the soluble epoxide hydrolase of increased water solubility. *J. Med. Chem.* **2004**, *47*, 2110−2122.

(25) Kim, I.-H.; Heirtzler, F. R.; Morisseau, C.; Nishi, K.; Tsai, H. J.; Hammock, B. D. Optimization of amide-based inhibitors of soluble epoxide hydrolase with improved water solubility. *J. Med. Chem.* **2005**, *48*, 3621−3629.

(26) Watanabe, T.; Schulz, D.; Morisseau, C.; Hammock, B. D. High-throughput pharmacokinetic method: cassette dosing in mice associated with minuscule serial bleedings and LC-MS-MS analysis. *Anal. Chim. Acta* **2006**, *559*, 37−44.

(27) Kim, I. H.; Nishi, K.; Tsai, H. J.; Bradford, T.; Koda, Y.; Watanabe, T.; Morisseau, C.; Blanchfield, J.; Toth, I.; Hammock, B. D. Design of bioavailable derivatives of 12-(3-adamantan-1-yl-ureido)dodecanoic acid, a potent inhibitor of the soluble epoxide hydrolase. *Bioorg. Med. Chem.* **2007**, *15*, 312−323.

(28) Jones, P. D.; Tsai, H. J.; Do, Z.; Moresseau, C.; Hammock, B. C. Synthesis and SAR of conformationally restricted inhibitors of soluble epoxide hydrolse. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5212−5216.

(29) Hwang, S. H.; Morisseau, C.; Do, Z.; Hammock, B. D. Solid-phase combinatorial approach for the optimization of soluble epoxide hydrolase inhibitors. *Bioorg. Med. Chem.* **2006**, *16*, 5773−5777.

(30) Grant, D. E.; Storms, D. H.; Hammock, B. D. Molecular cloning and expression of murine liver soluble epoxide hydrolase. *J. Biol. Chem.* **1993**, *268*, 17628−17633.

(31) Beetham, J. K.; Tian, T.; Hammock, B. D. cDNA cloning and expression of a soluble epoxide hydrolase from human liver. *Arch. Biochem. Biophys.* **1993**, *305*, 197−201.

(32) Wixtrom, R. N.; Silva, M. H.; Hammock, B. D. Affinity purification of cytosolic epoxide hydrolase using derivatized epoxy-activated sepharose gels. *Anal. Biochem.* **1988**, *169*, 71−80.

(33) Jones, P. D.; Wolf, N. M.; Morisseau, C.; Whetstone, P.; Hock, B.; Hammock, B. D. Fluorescent substrates for soluble epoxide hydrolase and application to inhibition studies. *Anal. Biochem.* **2005**, *343*, 66−75.

(34) Watanabe, T.; Hammock, B. D. Rapid determination of soluble epoxide hydrolase inhibitors in rat hepatic microsomes by high performance liquid chromatography with electrospray tandem mass spectrometry. *Anal. Biochem.* **2001**, *299*, 227−234.

(35) Watanabe, T.; Morisseau, C.; Newman, J. W.; Hammock, B. D. In vitro metabolism of the mammalian soluble epoxide hydrolase inhibitor, 1-cyclohexyl-3-dodecyl-urea. *Drug Metab. Dispos.* **2003**, *31*, 846−853.

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