Orally Bioavailable Potent Soluble Epoxide Hydrolase Inhibitors

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A series of N,N′-disubstituted ureas having a conformationally restricted *cis*- or *trans*-1,4-cyclohexane α to the urea were prepared and tested as soluble epoxide hydrolase (sEH) inhibitors. This series of compounds showed low nanomolar to picomolar activities against recombinant human sEH. Both isomers showed similar potencies, but the trans isomers were more metabolically stable in human hepatic microsomes. Furthermore, these new potent inhibitors show a greater metabolic stability in vivo than previously described sEH inhibitors. We demonstrated that *trans*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid **13g** (*t*-AUCB, $IC_{50} = 1.3 \pm 0.05$ nM) had excellent oral bioavailability (98%, $n = 2$) and blood area under the curve in dogs and was effective in vivo to treat hypotension in lipopolysaccharide challenged murine models.

Introduction

The soluble epoxide hydrolase (sEH, EC 3.3.2.3) belongs to the α/β hydrolase fold family of enzymes[1] and is involved in the metabolism of endogenously derived fatty acid epoxides and other lipid epoxides.[2] Epoxyeicosatrienoic acids (EETs),[3] the primary metabolites of cytochrome P450 epoxygenases of arachidonic acid, are known to act at vascular, renal, and cardiac levels of blood pressure regulation.[4] EETs have also been shown to possess anti-inflammatory properties.[5] The sEH enzyme catalytically hydrolyzes EETs into dihydroxyeicosatrienoic acids (DHETs), which show reduced biological activity.[6] We have demonstrated that sEH inhibition significantly reduces the blood pressure of the spontaneously hypertensive rats (SHRs)[7] as well as angiotensin II induced hypertensive rats.[8] Recently, we also demonstrated that sEH inhibitors not only dramatically synergize nonsteroidal anti-inflammatory drugs (NSAIDs) but also shift oxylipin metabolomic profiles away from propagation of inflammation.[9]

We initially reported conformationally restricted N,N′-disubstituted ureas, e.g., DCU or ACU (Figure 1) as simple sEH inhibitors.[10] Even though these compounds were very potent (K_i in the low nanomolar range), they were very difficult to use for in vivo studies because of their poor physical properties such as water solubility. Thus, a second homologous series of flexible sEH inhibitors such as AUDA,[a] AUDA-BE, and AEPU were investigated.[10b,11] These flexible compounds improved water solubility over DCU and ACU, thus facilitating efficacy studies in several animal models.[7−9] AEPU illustrates that a polar group can be added to the molecule roughly five carbons away from the central urea carbonyl group, increasing solubility without reducing potency.[11a] Similarly a series of polar residues such as esters, sulfones, amides, and carbamates can be placed roughly 5−7 Å from the central pharmacophore where there appear to be polar binding sites in the catalytic tunnel. However, the usefulness of these compounds was limited by the difficulties encountered during formulation as well as their rapid metabolism in vivo.[12] Because the flexible alkyl chain of these later compounds is susceptible to metabolism by β oxidation and cytochrome P450 oxidation,[13] we decided to investigate if more conformationally restricted compounds could be made that were more metabolically stable. To achieve this goal, we recently reported piperidine-based conformationally restricted sEH inhibitors such as TPAU, APAU, or AMAU that showed improved bioavailability in a canine model.[14] These piperidine-based inhibitors, however, still suffer from a short in vivo half-life. In addition, those compounds in piperidine-based series that showed optimal area under the curve (AUC) in a canine model did not have optimal potency on the human enzyme. The most potent compounds in this series, such as TPAU, did not show a good AUC.[14] Furthermore, we recently also reported conformationally restricted N,N′-disubstituted ureas harboring polar groups as potent sEH inhibitors.[15] Thus, in this study, we further explore conformationally restricted sEH inhibitors based on ACU as a simple scaffold in which a cyclohexane ring serves not only as a linker between a urea group and a polar group but also as a template to restrict the structure (Figure 2).

To be effective in vivo, in addition to potency, compounds need to have good metabolic stability and pharmacokinetic and distribution properties. Thus, the metabolic stability of synthesized potent sEH inhibitors was determined in human hepatic microsomes.[16] To determine the oral bioavailability of potent compounds, we screened the compounds in a canine model for the selection of compounds with good pharmacokinetic properties. Finally, the efficacy and the oral bioavailability of the best inhibitor **13g** in this series of compounds have been determined in mice and canines, respectively.

Chemistry

Scheme 1 outlines the general synthesis of N,N′-disubstituted ureas having a *cis*- or *trans*-1,4-cyclohexane ring between the carbonyl group on the urea and an oxygen atom in a benzyloxy or a phenoxy group. All compounds **3a**−**g**, **9a**−**g**, **13a**−**g**, **16a**−**k** were synthesized as a single isomer starting from commercially available *trans*-4-aminocyclohexanol hydrochloride **1** (Scheme 1).

The Mitsunobu coupling reaction[17] was used for the inversion of secondary alcohol configuration on the cyclohexane ring and

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[a] Abbreviations: *t*-AUCB, *trans*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid; AUDA, 12-(3-adamantan-1-ylureido)dodecanoic acid; AUDA-BE, 12-(3-adamantan-1-ylureido)dodecanoic acid butyl ester; DCU, *N*,*N*′-dicyclohexylurea; ACU, *N*-adamantyl-*N*′-cyclohexylurea; AEPU, 1-adamantan-1-yl-3-{5-[2-(2-ethoxyethoxy)ethoxy]pentyl}urea; APAU, *N*-(1-acetylpiperidin-4-yl)-*N*′-(adamant-1-yl)urea; TPAU, *N*-(1-(2,2,2-trifluoroethanoyl)piperidin-4-yl)-*N*′-(adamant-1-yl)urea; AMAU, *N*-((1-acetylpiperidin-4-yl)methyl)-*N*′-(adamant-1-yl)urea; *c*-FCTU, 1-[4-(4-fluorophenoxy)cyclohexyl]-3-(4-trifluoromethoxyphenyl)urea; *c*-FCUB, 4-{3-[4-(4-fluorophenoxy)-cyclohexyl]ureido}benzoic acid.

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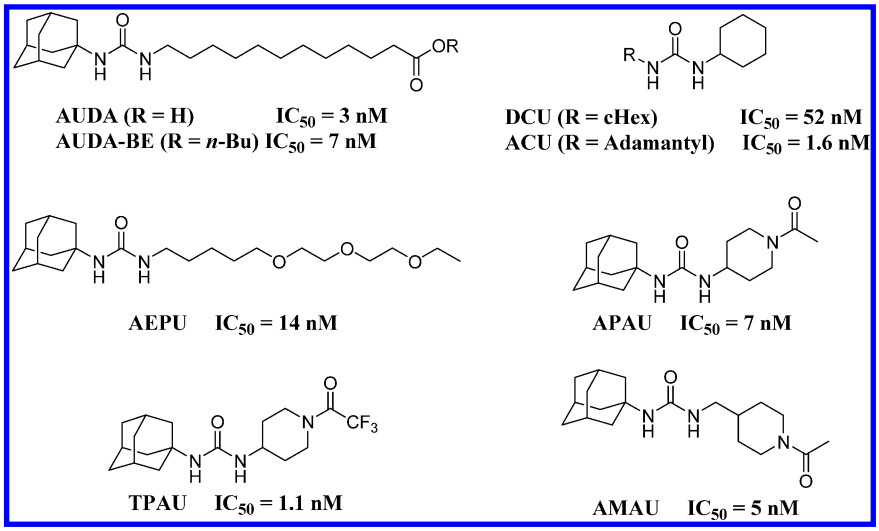


Figure 1. Common inhibitors of sEH. IC_{50} is for in vitro inhibition of the recombinant human sEH.

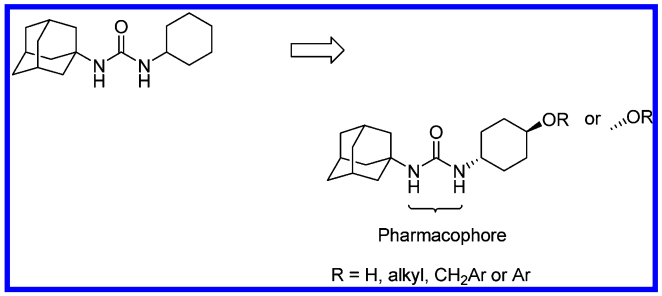


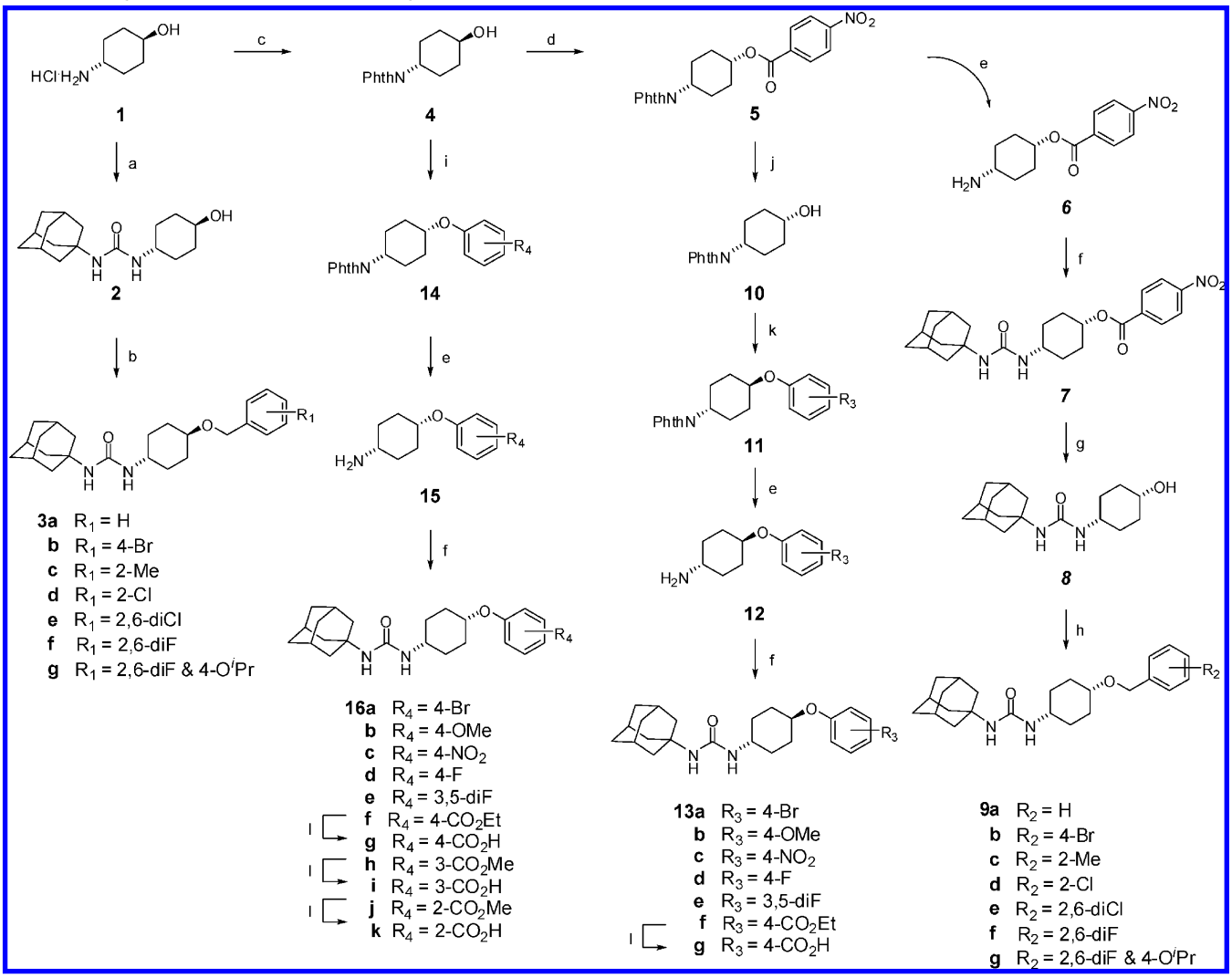
Figure 2. General structures of the new series of compounds.

allowed us to avoid tedious column chromatographic separations of a cis/trans mixture that are time-consuming and not practical for large-scale preparation of these compounds because of solubility limitations. The configurations of the isomers (**3a**−**g** vs **9a**−**g**; **13a**−**g** vs **16a**−**k**) and purity could be easily confirmed by comparisons of their NMR spectra. The relative configurations of trans and cis isomers were established by the comparison of peak assignments such as chemical shifts in ^{1}H and ^{13}C NMR with previous reported *cis*- and *trans*-1,4-cyclohexane isomers.[18] We also observed that ^{1}H NMR spectra of these compounds showed that trans isomers have the expected stable conformation in which both non-hydrogen substituents are equatorial, but cis isomers exist in rapid equilibrium between the two chair forms, which is consistent with the conformational mobility reported by Johnston et al.[19] and Hill et al.[20] Trans isomers **3a**−**g** were prepared by the reaction with various substituted benzyl bromides from the alcohol **2** that was obtained by the reaction of **1** with 1-adamantyl isocyanate in the presence of Et_3N in DMF.

Compounds **3h** and **3i** also were synthesized from **2** by reacting with iodomethane and cyclohexanemethyl methanesulfonate, respectively. Initial attempts to synthesize cis isomers **9a**−**g** and **16a**−**k** through the direct inversion of the configuration of the hydroxyl group from the *trans*-cyclohexanol **2** was problematic. In the presence of the urea group, the Mitsunobu coupling reaction gave a dehydrated product exclusively.[21] The protection of the amino group in **1** by a phthalic (Phth) group that is void of a hydrogen, however, minimized the dehydration problem, suggesting that a hydrogen on the urea group is likely responsible for the β-elimination of the hydroxyl group on the cyclohexane ring in the compound **2**.[22] Thus, compounds **9a**−**g**, **13a**−**g**, and **16a**−**k** were synthesized from the Phth-protected aminocyclohexanol **4** by alternating the configuration of the alcohol on a cyclohexane ring. A common intermediate **5** for the synthesis of cis isomers **9a**−**g** or trans isomers **13a**−**g** was obtained by the reaction of trans isomer **4** with *p*-nitrobenzoic acid in the presence of diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (PPh_3) in THF in excellent yield. Hydrazinolysis of the phthalic group in the intermediate **5**, followed by the reaction of amine **6** with 1-adamantyl isocyanate in DMF, provided compound **7** having the required cis configuration, which after hydrolysis under basic conditions afforded the desired cis alcohol **8**. The reaction of the cis alcohol **8** with various substituted benzyl bromides gave compounds **9a**−**g**. **9h** and **9i** were also synthesized from **2** reacting with iodomethane and cyclohexanemethyl methanesulfonate, respectively. By modification of a previously published method,[23] the cis alcohol **10** was obtained from **5** after saponification followed by a microwave-assisted phthalimide ring-closing reaction in the presence of excess triethylamine in DMF.[24] Mitsunobu coupling of the cis isomer **10** with various substituted phenols gave compounds **11a**−**f** having the desired trans configuration in moderate to excellent yields. This was followed by the hydrazinolysis of the phthalic group and urea formation resulting in trans isomers **13a**−**f**. Saponification of the ester **13f** led to the target acid **13g**. Compounds **16a**−**f**, **16h**, and **16j** were synthesized by reaction of 1-adamantyl isocyanate with amines **15a**−**f**, **15h**, and **15j** that were obtained by the Mitsunobu reaction of trans alcohol **4** with various substituted phenols followed by the removal of the phthalic group. Saponification of the esters **16f**, **16h**, and **16j** also led to the target acids **16g**, **16i**, and**16k**, respectively.

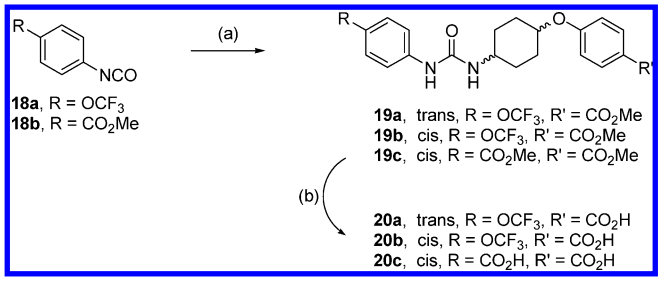
Compounds **20a**−**c** were synthesized by urea formation starting from the isocyanates **18a** or **18b** by reacting with amines **12f** or **15f** followed by soponification of corresponding esters **19a**−**c** (Scheme 2). Corresponding amides **22** and **23** of the urea-based inhibitors **16d** and **13d**, respectively, were synthesized as shown in Scheme 3. The EDC coupling of 1-adamantylacetic acid **21** with amines **24**[15] and **15d** gave amides **22** and **23**, respectively. Compounds having different linkers such as a *n*-butyl, acetylenyl, or phenyl group between a urea group and an oxygen atom were synthesized by the procedure outlined in Scheme 4. Compounds **27**, **29**, and **31** were synthesized starting from commercially available 4-fluorophenol **25**. Compounds **27** and **29** were synthesized by the reaction of 1-adamantyl isocyanate with the amines derived from compounds **26** and **28** that were obtained by Mitsunobu coupling of **25** with alcohols **32**[25] and **33**,[26] respectively. The reaction of 1-adamantyl

Scheme 1. Synthesis of *cis*- and *trans*-1,4-Cyclohexane Based Urea Derivatives[a]



[a] Reagents and conditions: (a) 1-adamantyl isocyanate, Et_3N, DMF, room temp, 6 h; (b) R_1-$PhCH_2Br$, NaH, DMF, 0 to room temp, 12 h; (c) Nefken's reagent, K_2CO_3, H_2O, room temp, 30 min; (d) PPh_3, *p*-nitrobenzoic acid, DIAD, THF, room temp, 12 h; (e) 35% hydrazine, CH_2Cl_2, MeOH, room temp, 1 day; (f) 1-adamantyl isocyanate, DMF, room temp, 12 h; (g) 1 N NaOH, CH_3CN, room temp; (h) NaH, R_2-$PhCH_2Br$, DMF; (i) R_4-PhOH, PPh_3, DIAD; THF, room temp, 12 h; (j) (i) 1 N NaOH; (ii) Et_3N, MW, 110 °C, DMF, 30 min; (k) R_3-PhOH, PPh_3, DIAD, THF, room temp, 12 h; (l) aqueous 1 N NaOH, CH_3CN, 90 °C, 6 h.

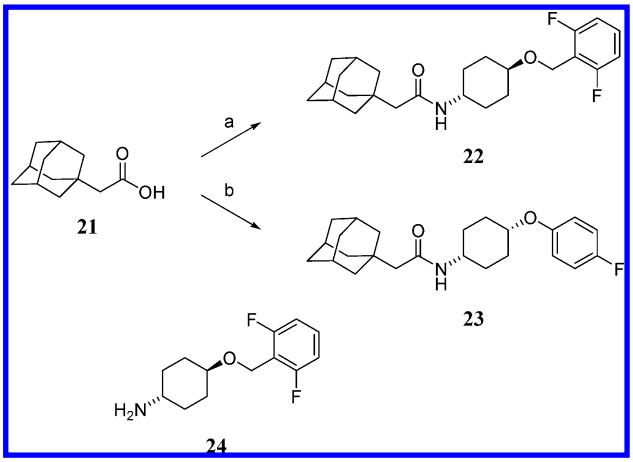
Scheme 2[a]



[a] Reagents and conditions: (a) **12f** (for **19a**) or **15f** (for **19b**,**c**), DMF, room temp, 12 h; (b) 1 N NaOH, acetonitrile, water, 90 °C, 6 h.

isocyanate with aniline **30**, which was obtained starting from **25** by following the procedure of Fotsch et al.,[27] gave the desired urea **31**. Scheme 5 shows the syntheses of cis/trans mixed ureas **36** having a methylene unit instead of an oxygen atom on the cyclohexane ring of either **13d** or **16d**. The ketone **34** was obtained by the PDC oxidation of the alcohol **2**. Wittig olefination of ketone **34** with 4-fluorobenzyltriphenylphosphonium bromide[28] afforded the olefin **35**, which was subjected to

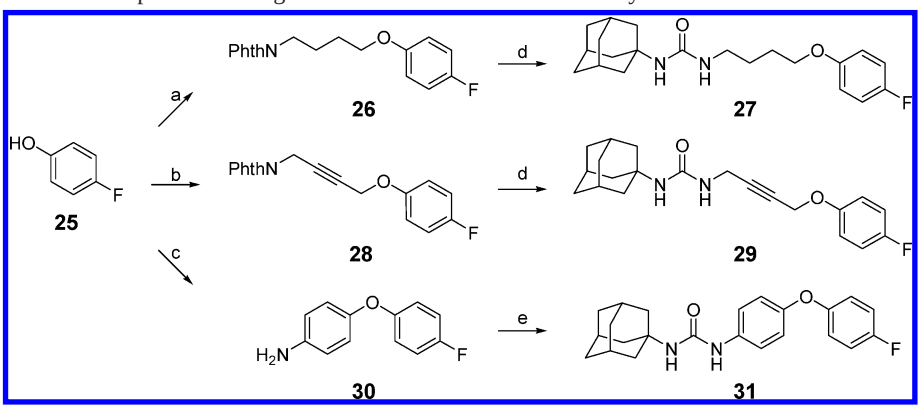
Scheme 3. Synthesis of Amide Derivatives **22** and **23**[a]



[a] Reagents and conditions: (a) **24**, EDC, CH_2Cl_2, room temp, 2 h; (b) **15d**, EDC, CH_2Cl_2, room temp, 2 h.

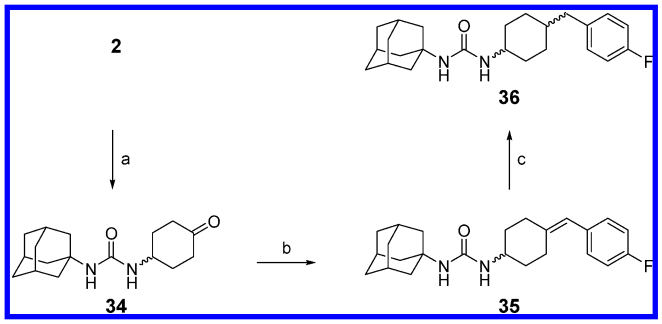
hydrogenation on 10% Pd/C generating desired compound **36** as an inseparable 1.25:1 (trans/cis) mixture.

Scheme 4. Synthesis of Urea Compounds Having Different Linker in Place of the Cyclohexane[a]



[a] Reagents and conditions: (a) $PhthNCH_2(CH_2)_2CH_2OH$ (**32**), DIAD, PPh_3, THF; (b) $PhthNCH_2C{\equiv}CCH_2OH$ (**33**), DIAD, PPh_3, THF; (c) (i) 1-fluoro-4-nitrobenzene, K_2CO_3, DMF, 150 °C; (ii) 10% Pd/C, H_2 (1 atm), EtOAc, room temp; (d) (i) 35% hydrazine, CH_2Cl_2, MeOH, room temp, 1 day; (ii) 1-adamantyl isocyanate, DMF; (e) 1-adamantyl isocyanate, DMF.

Scheme 5. Synthesis of Compounds Having a Carbon Isostere in Place of an Oxygen Atom[a]



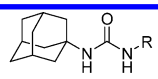
[a] Reagents and conditions: (a) PDC, DMF, room temp, 12 h; (b) *n*-BuLi, $(4\text{-F-Ph})CH_2PPh_3Br$, −78 °C to reflux, 12 h; (c) 10% Pd/C, H_2 (1 atm), MeOH, room temp, 2 h.

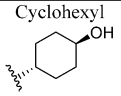
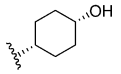
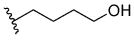
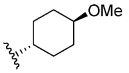
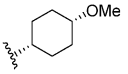
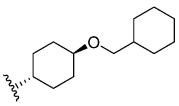
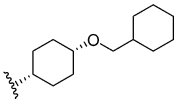
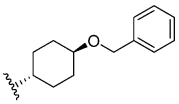
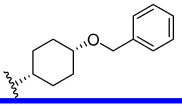
Results and Discussion

Incorporation of Ether Groups on ACU. We have previously shown that ureas with a linear alkyl chain having a variety of polar groups such as an ester, amide, or ether about five to seven atoms away from the urea moiety increase water solubility without changing the potency on the human sEH.[11] Because of the instability of most esters in vivo, they are most appropriate as "soft drugs". Thus, we turned our attention to ethereal derivatives. Incorporation of a hydroxyl group at position 4 on the cyclohexane ring in ACU such as *trans*-**2** resulted in higher IC_{50} value than the parent ACU (Table 1). However, compound **38**, which replaces the cyclohexane ring with a linear *n*-butyl chain, resulted in even greater reduction of inhibition activity, suggesting that restriction of the linear chain by a cyclohexane is beneficial. Because of the existence of conformational isomers of 1,4-disubstituted cyclohexane, the corresponding cis isomer **8** was also synthesized. Interestingly, this cis isomer **8** was 5-fold less potent compared to trans isomer **2**. Both isomers, however, became almost equally potent upon etherification of alcohol **(3h** vs **9h**, **3i** vs **9i**, and **3a** vs **9a**), and the cis isomers became more potent when etherified with large groups such as **3i**, **9i**, **3a**, and **9a**. These results suggested that the absence of a hydrogen donor at position 4 on the cyclohexane is crucial for obtaining highly potent inhibitors. In support of this hypothesis we observed that an ester analogue (**7**, 4-nitrobenzoic acid 4-(3-adamantan-1-ylureido)cyclohexyl ester) also showed excellent potency (IC_{50} = 1.3 nM).

Comparison of Cis and Trans Isomers. We previously showed that the addition of a third polar group about 11 atoms away from the urea carbonyl on a linear chain of a urea inhibitor resulted in increased solubility while maintaining or enhancing the inhibition potency.[11] Thus, we tested whether adding a third polar function will have a similar effect on these conformationally restricted urea inhibitors (Table 2). In general, as previously observed, the addition of a third polar group relatively far away from the urea carbonyl did not significantly influence the inhibition potency of the human sEH except for a carboxylic acid at the ortho position of the phenyl ring in **16k**, for which the IC_{50} is decreased ~100-fold. Furthermore, the cis isomers **9a**−**g** and **16a**−**g** were nearly as potent as the trans isomers **3a**−**g** and **13a**−**g**.

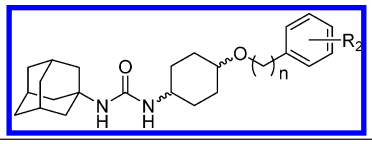
Table 1. SAR of Various Substituents on *cis*- or *trans*-Cyclohexane Ring



Compd	R_1	IC_{50} (nM)[a]
ACU	Cyclohexyl	1.6
2		14 ± 1
8		76 ± 11
38		153
3h		2.6 ± 0.2
9h		4.0 ± 0.5
3i		4.0 ± 0.3
9i		2.5 ± 0.1
3a		1.7 ± 0.1
9a		0.9 ± 0.1

[a] Values are the mean ± SD of three independent experiments. At least three concentrations above and below the repeated IC_{50} were used to generate the data.

Table 2



compd	*n*	R_2	IC_{50} [a] (nM)	stability[b] (% remaining)	solubility[c] (μM)
trans					
3a	1	H	1.7 ± 0.1	67	31 < *x* < 63
3b	1	4-Br	1.7 ± 0.2	41	31 < *x* < 63
3c	1	2-Me	1.6 ± 0.1	67	16 < *x* < 31
3d	1	2-Cl	2.7 ± 0.2	54	16 < *x* < 31
3e	1	2,6-diCl	1.7 ± 0.2	51	31 < *x* < 63
3f	1	2,6-diF	1.7 ± 0.1	43	16 < *x* < 31
3g	1	2,6-diF, 4-O^{i}Pr	3.5 ± 0.1	34	63 < *x* < 125
13a	0	4-Br	2.0 ± 0.1	23	31 < *x* < 63
13b	0	4-OMe	0.87 ± 0.03	82	16 < *x* < 31
13c	0	4-NO_2	0.64 ± 0.03	nd	31 < *x* < 63
13d	0	4-F	0.80 ± 0.05	69	16 < *x* < 31
13e	0	3,5-diF	1.0 ± 0.1	nd	31 < *x* < 63
13g	0	4-CO_2H	1.3 ± 0.05	>99	>500
cis					
9a	1	H	0.9 ± 0.1	nd	31 < *x* < 63
9b	1	4-Br	2.1 ± 0.1	20	31 < *x* < 63
9c	1	2-Me	3.4 ± 0.1	14	31 < *x* < 63
9d	1	2-Cl	2.0 ± 0.1	25	31 < *x* < 63
9e	1	2,6-diCl	1.5 ± 0.1	26	16 < *x* < 31
9f	1	2,6-diF	1.1 ± 0.1	19	31 < *x* < 63
9g	1	2,6-diF, 4-O^{i}Pr	3.5 ± 0.2	29	125 < *x* < 250
16a	0	4-Br	1.3 ± 0.1	35	31 < *x* < 63
16b	0	4-OMe	0.55 ± 0.06	14	63 < *x* < 125
16c	0	4-NO_2	0.72 ± 0.05	15	31 < *x* < 63
16d	0	4-F	1.0 ± 0.1	21	16 < *x* < 31
16e	0	3,5-diF	0.82 ± 0.01	20	31 < *x* < 63
16g	0	4-CO_2H	0.89 ± 0.04	98	>500
16i	0	3-CO_2H	1.9 ± 0.03	nd	>500
16k	0	2-CO_2H	330 ± 30	nd	>500

[a] Values are the mean ± SD of three experiments. [b] Metabolic stability in human hepatic microsomes. The remaining percentage of the parent compounds was measured after incubation for 60 min. nd, not determined. [c] Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO. The data present a range where the solubility is greater than the lower value. Results are the means of three separate experiments.

The metabolic stability of these compounds was determined against human hepatic microsomes fortified with NADPH, and their water solubility was determined in sodium phosphate buffer (Table 2). We previously showed that metabolism and water solubility are important factors contributing sEH inhibitor potency in vivo.[29] To produce more metabolically stable inhibitors, we first hypothesized that metabolism on the benzylic position is the most susceptible in these inhibitor skeletons.[30] We thus decided to take two approaches to improve the metabolic stability of the inhibitors: introduction of steric shields and removal of the metabolically susceptible position. Therefore, compounds were synthesized by introducing a methyl group or halogen atom(s) as steric shields on ortho position(s) of the phenyl ring, such as **3c**−**g** and **9c**−**g**, and by eliminating a methylene unit, such as **13a**−**g** and **16a**−**g**. Both approaches failed to stabilize the compounds except for **13g** and **16g**, suggesting that the benzylic position is not a susceptible metabolic group in this series of compounds. Moreover, phenoxy derivatives having electron-withdrawing group(s) such as **16c**−**f** showed metabolic instability, also supporting the hypothesis that the phenyl group is not susceptible to metabolism. In addition, despite the favorable in vitro activity and metabolic stability of compounds such as **13b** (0.87 ± 0.03 nM, stability = 82%), they were found to be poor candidates for in vivo studies because of minimal water solubility (in general less than 31 μM). Compounds such as the *p*-fluoro derivative **13d**, however, would be anticipated to be valuable physiological tools leading to constant exposure following subcutaneous injection in oil or administration as a wax plug. Surprisingly, the incorporation of a free carboxylic acid group in **13g** and **16g** not only dramatically increased the water solubility but also increased the metabolic stability of the compounds. The improved metabolic stability of the compounds **13g** and **16g** is most likely caused by the decreased lipophilicity of these compounds, which reduces their affinity for or accessibility to metabolic enzymes, particularly the cytochrome P450 family.[31] The relatively high inhibitor potency of the free carboxylic acid containing compounds at para and meta positions (**13g**, **16g**, **16i**) is partly due to having a sufficient distance between the urea group and the carboxylic acid.[32] Moving the carboxylic acid group to the ortho position (**16k**), however, dramatically decreased inhibitory activity. In general, cis isomers showed poorer metabolic stability against human hepatic microsomes than the corresponding trans isomers. Presumably, cis isomers were more susceptible to metabolism by CYP 450s than the trans isomers.

Docking Compounds 13g and 16g with sEH Enzyme. To understand the observation that the cis isomers were, in general, more potent than the trans isomers, we manually docked inhibitors **13g** and **16g** into the active site of sEH. For this, we used the published X-ray crystal structure of human sEH complexed with a urea-based ligand (4-(3-cyclohexylureido)-butyric acid, CU4, PDB accession number 1ZD3).[32]

Between two plausible binding modes for **13g** and **16g**, the orientation given in Figure 3 is more favorable. The other binding mode that is used to explain the increased activity of previous inhibitors, making an H-bond with Gln^{382}, resulted in steric clashes between the phenyl group of the inhibitors and the residues of the binding site, such as Met^{337}.

Our previous report also showed that this methionine residue plays an important role in binding of the inhibitor into the active site.[33] To test this hypothesis, we made the *trans*,*trans*-1,3-*bis*-(4-hydroxycyclohexyl)urea **39**. We found that the potency (IC_{50} = 1400 ± 200 nM) of **39** is dramatically decreased compared to DCU (IC_{50} = 52 nM) in which both hydroxyl groups of **39** are replaced by hydrogens. These results clearly demonstrated that a hydroxyl group on a cyclohexane in **39** interacts unfavorably with one of the active site residues, presumably with Met^{337}. The observation that **2** and **8**, which possess only one 4-hydroxycyclohexyl group, showed almost equal or greater inhibitory potency compared to DCU suggested that these compounds, and presumably other compounds in the same series (Table 2), orient themselves to avoid an unfavorable interaction with Met^{337} (Figure 3).

As seen in Figure 3, compounds **13g** and **16g** were bound primarily through interactions with Tyr^{381}, Tyr^{465}, and Asp^{333} with the urea pharmacophore. We also found that the carboxylate of compounds **13g** and **16g** could form hydrogen bonds with Met^{418} and with Arg^{408} and Trp^{524}, separately. Presumably, the presence of extra H-bonding in **16g** might explain the slightly increased activity of cis isomers compared to trans isomers.

Structural Contribution to Inhibition. Before moving onto the determination of the pharmacokinetic properties of these inhibitors, we evaluated the effect of structural components of each inhibitor by dividing them into four parts (P1, P2, P3, and P4, Table 3).

First, as we demonstrated previously,[15] replacement of the adamantyl group with a *p*-trifluoromethoxyphenyl group at P1 in *c*-FCTU, **20a**, and **20b** showed no changes in potency compared to **16d**, **13g**, and **16g**. In contrast to **20a** and **20b**, introduction of a free carboxylic acid close to the urea group

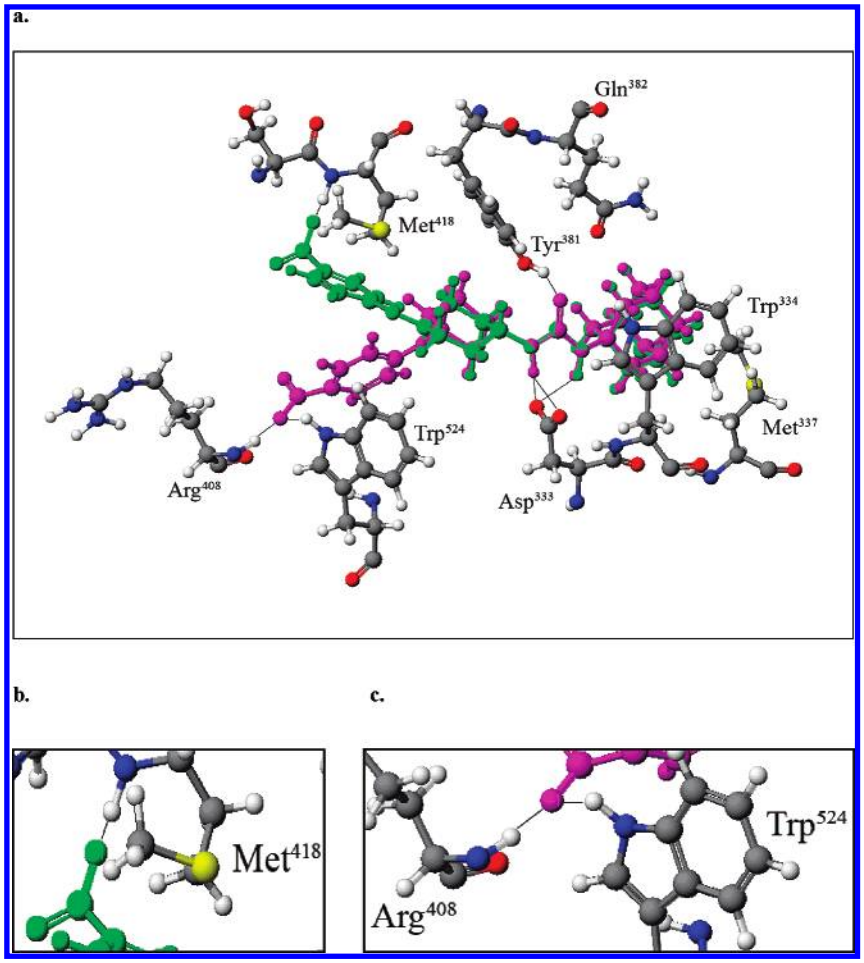
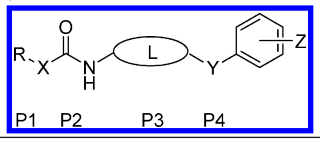


Figure 3. (a) Superposition of the compounds **13g** (green) and **16g** (magenta) docked into the active site of human sEH. The residues Tyr[465] and Leu[498] are omitted for clarity. (b) H-bonding of **13g** with residue Met[418]. (c) H-bondings of **16g** with residues Arg[408] and Trp[524]. Black lines indicate possible hydrogen bonds.

Table 3. Contribution of Each Portion (P1, P2, P3, and P4) in the Inhihitor



compd	R	X	L	Y	Z	IC_{50} [a] (nM)
c-FCTU[b]	*p*-CF_3O-Ph	NH	*cis*-*c*Hex	O	F	0.9 ± 0.1
20a	*p*-CF_3O-Ph	NH	*trans*-*c*Hex	O	*p*-CO_2H-Ph	0.9 ± 0.1
20b	*p*-CF_3O-Ph	NH	*cis*-*c*Hex	O	*p*-CO_2H-Ph	0.6 ± 0.1
c-FCUB[b]	*p*-CO_2H-Ph	NH	*cis*-*c*Hex	O	*p*-F-Ph	220 ± 5
20c	*p*-CO_2H-Ph	NH	*cis*-*c*Hex	O	*p*-CO_2H-Ph	8300 ± 200
22	adamantyl	CH_2	*trans*-*c*Hex	OCH_2	2,6-diF	3.5 ± 0.3
23	adamantyl	CH_2	*cis*-*c*Hex	O	4-F	9.1 ± 0.8
27	adamantyl	NH	*n*-Bu	O	4-F	3.9 ± 0.3
29	adamantyl	NH	$CH_2C{\equiv}CCH_2$	O	4-F	42 ± 2
31	adamantyl	NH	1,4-Ph	O	4-F	2.7 ± 0.2
36	adamantyl	NH	*c*Hex	CH_2	4-F	2.5 ± 0.3

[a] Values are the mean ± SD of three experiments. [b] Reference 15.

as in *c*-FCUB and **20c** dramatically decreased potency. These results also support the poor potency of **16k** compared to **16g** and **16i**. These data suggested that the potency of these compounds could be optimized by focused libraries at P1.[15]

Second, to test the importance of the urea group as a pharmacophore, amide derivatives **22** and **23** were synthesized. They showed 3.5- and 9-fold less potency compared to the corresponding urea compounds **3f** and **16d**, respectively, which was consistent with previous comparisons between amide-based and urea-based compounds,[11b] suggesting that the urea pharmacophore is generally more active than the amide group (Scheme 2). However, the amides generally show greater solubility and reduced melting point.

Third, to validate the choice of a cyclohexane group as a conformationally restricted spacer at the P3 region, compounds having different linkers such as a *n*-butyl, acetylenyl, or phenyl group between the urea group and the oxygen atom on a cyclohexane linker, were synthesized to evaluate the importance of the cyclohexane ring as a linker as illustrated by analogues **27**, **29**, and **31** (Scheme 3). When the cyclohexane linker is

Table 4. Comparison of Potencies of Selected Inhibitors for sEH from Various Animal Species[a]

compd	IC$_{50}$ (nM) mouse sEH[b]	rat sEH[b]	hamster sEH[c]	cat sEH[c]	dog sEH[c]	human sEH[b]	solubility (μM)	mp (°C)	AUC[d] ($\times 10^4$ nM·min)
AUDA	10	11	5	3	3	3	$63 < x < 125$	142−143	0.4
AEPU	3	5	2	27	86	14	$250 < x < 500$	74−75	1.9
APAU	9	6	2	450	500	15	>500	205−206	3.7
13g	8	8	2	6	1	2	>500	250−255	14.2

[a] Full data on the activity of TPAU on the sEH from other species are not included because of the instability of the compound on storage (IC$_{50}$ = 1.1 nM, AUC = 0.33 × 10^4 nM·min). [b] Measured with fluorescent assay. [c] Measured with radioactive assay. [d] Area under the curve (AUC) estimated from a plot of the inhibitor concentration in plasma (nM) versus time (minutes) following an oral dose of 0.3 mg/kg of the indicated compounds given to dogs in triglycerides.

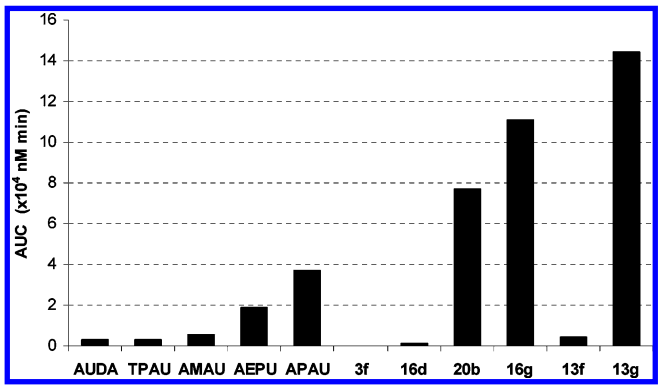


Figure 4. Pharmacokinetic profile data for selected compounds as obtained via oral administration in a canine model. Area under the curve (AUC) was estimated from a plot of inhibitor plasma concentration (nM) versus time (min) following an oral dose of 0.3 mg/kg of the indicated compounds in triglycerides.[34]

switched to a flexible linear *n*-butyl group, the inhibitory activity dropped by about 4-fold. Interestingly, enhancing the rigidity by incorporating a phenyl group or an acetylene group also decreased inhibitory activity by at least 3-fold and 40-fold, respectively.

The importance of the P4 region was tested by making analogue **36** as a mixture by changing an oxygen atom to its isostere, a methylene unit. Removal of an oxygen atom in either **13d** or **16d**, however, decreased potency, suggesting that the oxygen atom helps not only to increase water solubility but also to maintain potency (Scheme 4).

Pharmacokinetic Screening. At this point, we investigated in vivo properties of these inhibitors. This pharmacokinetic screening was performed following oral administration in dogs.[34] As can be seen in Figure 4, **13g**, **16g**, and **20b**, which have improved metabolic stability and water solubility, are more bioavailable than **16d** or **3f**. Meanwhile, ester **13f** was metabolized quickly to the corresponding acid **13g** as a its major metabolite, presumably by esterases.[35] Compound **13g** showed an almost 40-fold increase in AUC compared to AUDA (or TPAU) and a 4-fold increase compared to APAU. Since it has been demonstrated that the gastrointestinal absorption in humans and dogs is very similar, it is hoped that these results will be transferable to humans.[36] Compounds that are both poorly soluble in water and have a stable crystal structure as indicated by a high melting point are difficult to formulate. This situation is made even more difficult with urea structures such as AUDA that are also poorly soluble in common formulating reagents. The increased potency of **13g** over AUDA partially addresses the above issue because less material needs to be delivered. Although **13g** possessed a relatively high melting point, this problem is offset by the dramatically increased water solubility. In addition, the benzoic acid of **13g** was not susceptible to the β oxidation that causes rapid metabolism of AUDA.

Bioavailability of 13g in Dogs. Compound **13g** had the best blood levels from the pharmacokinetic screening, so it was chosen to assess oral bioavailability. The oral bioavailability of **13g** was determined after a single oral (po) and intravenous (iv) administration in the dogs. It was dosed at 0.3 mg/kg iv (n = 2) in 1% morpholine saline and 0.3 mg/kg po (n = 2) in 1% morpholine saline in two dogs via syringe. After a single administration of compound **13g** to female dogs, plasma samples were collected over 24 h and plasma concentrations were determined by HPLC−MS/MS. Its oral bioavailability in dogs under these conditions was 98% (n = 2) with a T_{max} of 8 h and a $T_{1/2}$ of 19 h.

Comparison of Inhibitory Activity for sEH from Different Animal Species. At this point, we decided to investigate the inhibitory activity of selected inhibitors to sEH enzyme from various animal species. AEPU, TPAU, and APAU, especially APAU, showed poor inhibitory activities against sEH enzymes from cat and dog. AUDA and **13g** were potent against sEH enzymes regardless of the species of origin, but **13g** showed better water solubility and metabolic stability than AUDA (Table 4).

Efficacy of 13g in Mice. With a compound having good pharmacokinetic property and good oral bioavailability in hand, our attention moved to its in vivo activity. The ability of **13g** to regulate murine blood pressure was evaluated. Administration of lipopolysaccharide (LPS) to mice causes profound and often fatal hypotension in addition to other symptoms. In this study, the administration of only 1 mg/kg of **13g** to mice treated with LPS returned the blood pressure to normal, while more than 10 mg/kg of AUDA-BE were required to have a similar effect. Even 0.5 mg/kg of **13g** returned blood pressure to 60% of normal values. These efficacy data strongly supported the increased efficacy of **13g** compared to AUDA-BE,[37] suggesting that our approach to optimize this lead was successful. To support the hypothesis that reversal of hypotension is directly derived from the inhibition of sEH, we determined the plasma EETs/DHETs ratio by LC−MS/MS. As seen in Figure 5, the increase in blood EETs/DHETs ratio caused by a low dose of **13g** supports the argument that **13g** is reducing hypotension by inhibiting the sEH.

Conclusion

We have described the synthesis and structure−activity relationships of a series of conformationally restricted sEH inhibitors using a *cis*/*trans*-cyclohexane spacer. Our efforts to optimize the cyclohexane portion of the lead ACU successfully improved pharmacokinetic properties and potency as well. We demonstrated that compound **13g**, which has excellent bioavailability, showed improved physical properties such as increased water solubility and metabolic stability compared to previously reported sEH inhibitors and enabled us to formulate the compound easily for animal studies. **13g** also showed good inhibitory activity against sEH enzymes from several different animal species, which will allow for the investigation of a variety of animal models using the same compound. In addition, **13g** was shown to be more than 10 times effective in vivo in

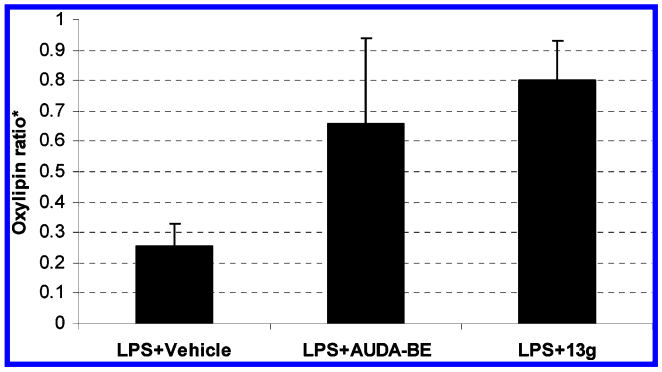


Figure 5. LPS exposure produced temporal changes in plasma oxylipins derived from epoxygenase and soluble epoxide hydrolase pathways. (∗) Results (average ± SD; $n = 4$) in LPS-exposed mice treated with vehicle, AUDA-BE, or **13g** are depicted as the ratio of $(\sum EETs/\sum DHETs)_{observed}/(\sum EETs/\sum DHETs)_{vehicle\ only}$. Doses are as follows: AUDA-BE, 10 mg/kg; **13g**, 1 mg/kg. Only the 8,9-, 11,12-, and 14,15-epoxides and corresponding diols were summed because the 5,6-regioisomer is a poor sEH substrate and spontaneously lactonized.

ameliorating hypotension than AUDA-BE in an LPS-sepsis model. Further studies on the pharmacology of these and related inhibitors of the sEH are underway.

Experimental Section

General. All reagents and solvents were obtained from commercial suppliers and were used without further purification. All reactions, unless otherwise described, were performed under an inert atmosphere of dry nitrogen. Melting points were determined on an OptiMelt melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. Elemental analyses were determined at Midwest Microlab, Indianapolis, IN. Microwave reactions were performed in an ETHOS SEL labstation (Milestone Inc., Shelton, CT). Mass spectra were measured by LC−MS equipped with a Waters 2790 and a Waters PDA 996 using electrospray (+) ionization. Flash chromatography was performed on silica gel. The following reagents were prepared by literature methods: benzyl bromide, methanesulfonic acid cyclohexylmethyl ester,[38] 4-fluorobenzyltriphenylphosphonium bromide,[29] 4-phthalylamino-1-butanol,[26] 4-phthalimidobut-2-yn-1-ol.[27]

***trans*-1-Adamantan-1-yl-3-(4-hydroxycyclohexyl)urea (2).** To a solution of 1-adamantyl isocyanate (5 g, 28 mmol) in DMF (35 mL) were added *trans*-4-aminocyclohexanol hydrochloride (6.4 g, 42 mmol) and Et_3N (5.9 mL, 42 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. After addition of 1 N HCl (40 mL) and water, the resulting white precipitates were collected by suction filtration. The collected solid was thoroughly washed with water. Recrystallization from methanol afforded 7.5 g (92%) of the title compound as a white solid. Mp 254−257 °C. ^{1}H NMR (DMSO-d_6): δ 5.48 (d. $J = 9$ Hz, 1H), 5.38 (s, 1H), 4.48 (d, $J = 5$ Hz, 1H), 3.42−3.28 (m, 1H), 3.28−3.13 (m, 1H), 2.02−1.93 (m, 3H), 1.87−1.68 (m, 10H), 1.63−1.54 (m, 6H), 1.24−0.93 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 156.48, 68.16, 49.32, 47.24, 42.05, 36.16, 33.97, 31.17, 28.96. MS (ESI) *m/z*: 293.2 (M + H$^+$). Anal. ($C_{17}H_{28}N_2O_2$) C, H, N.

General Procedure for the Synthesis of Trans Isomers 3a−h and 9h. To a solution of compound **2** (1 mmol) in DMF (10 mL) was added 60% sodium hydride in oil (1.5 equiv) at 0 °C. After 10 min, benzyl bromide (1.2 equiv) was added. The reaction mixture was allowed to slowly warm to room temperature overnight. The reaction was quenched by adding water, and the resulting white precipitates were collected and washed with water. The solids were chromatographed by a dry loading method.

***trans*-1-Adamantan-1-yl-3-(4-benzyloxycyclohexyl)urea (3a).** The general method above was used with benzyl bromide to afford a white solid (0.35 g, 91% yield). Mp 244−245 °C. ^{1}H NMR ($CDCl_3$): δ 7.40−7.23 (m, 5H), 4.52 (s, 2H), 4.10−3.92 (m, 2H), 3.58−3.41 (m, 1H), 3.37−3.24 (m, 1H), 2.11−1.81 (m, 13H), 1.50−1.33 (m, 6H), 1.50−1.33 (m, 2H), 1.17−0.99 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 156.65, 139.01, 128.49, 127.63, 127.57, 76.56, 70.14, 51.04, 48.64, 42.66, 36.57, 31.68, 30.82, 29.68. MS (ESI) *m/z*: 383.3 (M + H$^+$). Anal. ($C_{24}H_{34}N_2O_2$) C, H, N.

***trans*-1-Adamantan-1-yl-3-[4-(4-bromobenzyloxy)cyclohexyl]-urea (3b).** The general method above was used with *p*-bromobenzyl bromide to afford a white solid (0.42 g, 90% yield). Mp 250−251 °C. ^{1}H NMR ($CDCl_3$): δ 7.45 (d, $J = 8$ Hz, 2H), 7.20 (d, $J = 8$ Hz, 2H), 4.48 (s, 2H), 3.95 (s, 1H), 3.90 (d, $J = 8$ Hz, 1H), 3,-58−3.43 (m, 1H), 3.35−3.22 (m, 1H), 2.12−1.89 (m, 13H), 1.70−1.60 (m, 6H), 1.49−1.33 (m, 2H), 1.17−1.01 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 156.43, 138.77, 131.09, 129.37, 120.14, 76.03, 68.16, 49.32, 47.04, 42.04, 36.14, 30.70, 30.26, 28.93. MS (ESI) *m/z*: 461.2 (M + H$^+$).

***trans*-1-Adamantan-1-yl-3-[4-(2-methylbenzyloxy)cyclohexyl]-urea (3c).** The general method above was used with 2-methylbenzyl bromide to afford a white solid (0.34 g, 86% yield). Mp 247−248 °C. ^{1}H NMR ($CDCl_3$): δ 7.34−7.29 (m, 1H), 7.21−7.13 (m, 3H), 4.51 (s, 2H), 3.96 (s, 1H), 3.91 (d, $J = 8$ Hz, 1H), 3.57−3.43 (m, 1H), 3,38−3.25 (m, 1H), 2.32 (s, 3H), 2.13−1.87 (m, 13H), 1.69−1.62 (m, 6H), 1.51−1.35 (m, 2H), 1.19−1.03 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 156.59, 136.76, 130.36, 128.58, 127.83, 125.95, 76.80, 68.76, 51.12, 48.76, 42.70, 36.60, 31.75, 30.83, 29.72, 18.96. MS (ESI) *m/z*: 397.5 (M + H$^+$).

***trans*-1-Adamantan-1-yl-3-[4-(2-chlorobenzyloxy)cyclohexyl]-urea (3d).** The general method above was used with 2-chlorobenzyl bromide to afford a white solid (0.36 g, 86% yield). Mp 243−254 °C. ^{1}H NMR ($CDCl_3$): δ 7.50 (dd, $J = 7$ and 2 Hz, 1H), 7.35−7.17 (m, 3H), 4.62 (s, 2H), 3.95 (s, 1H), 3.90 (d, $J = 8$ Hz, 1H), 3.60−3.45 (m, 1H), 3.41−3.31 (m, 1H), 2.17−1.85 (m, 13H), 1.71−1.61 (m, 6H), 1.54−1.36 (m, 2H), 1.21−1.04 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 156.58, 136.77, 132.79, 129.30, 128.98, 128.60, 126.93, 77.40, 67.38, 51.11, 48.67, 42.67, 36.58, 31.68, 30.83, 29.70. MS (ESI) *m/z*: 417.9 (M + H$^+$).

***trans*-1-Adamantan-1-yl-3-[4-(2,6-dichlorobenzyloxy)cyclohexyl]-urea (3e).** The general method above was used with 2,6-dichlorobenzyl bromide to afford a white solid (0.39 g, 86% yield). Mp 259−260 °C. ^{1}H NMR ($CDCl_3$): δ 7.33−7.24 (m, 2H), 7.21−7.12 (m, 1H), 4.75 (s, 2H), 3.95 (s, 1H), 3.91 (d, $J = 8$ Hz, 1H), 3.58−3.31 (m, 4H), 2.17−1.82 (m, 13H), 1.76−1.56 (m, 6H), 1.52−1.34 (m, 2H), 1.22−1.04 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 156.57, 136.96, 133.98, 129.91, 128.56, 77.37, 65.14, 51.14, 48.73, 42.69, 36.60, 31.76, 30.79, 29.73. MS (ESI) *m/z*: 451.2 (M + H$^+$).

***trans*-1-Adamantan-1-yl-3-[4-(2,6-difluorobenzyloxy)cyclohexyl]-urea (3f).** The general method above was used with 2,6-difluorobenzyl bromide to afford a white solid (0.36 g, 86% yield). Mp 244−246 °C. ^{1}H NMR ($CDCl_3$): δ 7.33−7.20 (m, 1H), 6.96−6.83 (m, 2H), 4.59 (s, 2H), 3.94 (s, 1H), 3.88 (d, $J = 9$ Hz, 1H), 3.55−3.42 (m, 1H), 3.40−3.27 (m, 1H), 2.11−1.89 (m, 13H), 1.69−1.60 (m, 6H), 1.48−1.31 (m, 2H), 1.20−1.03 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 162.12 (d, $J = 250$ and 8 Hz), 156.54, 130.11 (t, $J = 10$ Hz), 114.46 (t, $J = 19$ Hz), 111.43 (dd, $J = 25$ and 10 Hz), 77.37, 57.57, 51.13, 48.71, 42.68, 36.58, 31.71, 30.73, 29.71. MS (ESI) *m/z*: 419.2 (M + H$^+$). Anal. ($C_{24}H_{32}F_2N_2O_2$) C, H, N.

***trans*-1-Adamantan-1-yl-3-[4-(2,6-difluoro-4-isopropoxybenzyloxy)cyclohexyl]urea (3g).** The general method above was used with 2-bromomethyl-1,3-difluoro-5-isopropoxybenzene **17** to afford a white solid (0.37 g, 78% yield). Mp 182−186 °C. ^{1}H NMR ($CDCl_3$): δ 6.39 (d, $J = 10$ Hz, 2H), 4.49 (s, 2H), 4.49−4.41 (m, 1H), 3.95 (s, 1H), 3.91 (d, $J = 8$ Hz, 1H), 3.54−3.39 (m, 1H), 3.35−3.23 (m, 1H), 2.08−1.89 (m, 13H), 1.67−1.61 (m, 6H), 1.45−1.24 (m, 2H), 1.31 (d, $J = 6$ Hz, 6H), 1.18−1.01 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 161.93 (dd, $J = 246$ and 12 Hz), 158.98 (t, $J = 15$ Hz), 156.43, 105.92 (t, $J = 21$ Hz), 99.16 (dd, $J = 29$ and 10 Hz), 76.03, 70.37, 56.41, 49.33, 47.00, 42.04, 36.15, 30.65, 30.11, 28.94, 21.53. MS (ESI) *m/z*: 477.4 (M + H$^+$).

***trans*-1-Adamantan-1-yl-3-(4-methoxycyclohexyl)urea (3h).** The general method above was used with iodomethane to afford a white solid (0.23 g, 75% yield). Mp 212−214 °C. ^{1}H NMR ($CDCl_3$): δ 4.12−3.98 (m, 2H), 3.58−3.42 (m, 1H), 3.34 (s, 3H),

3.18−3.06 (m, 1H), 2.13−1.88 (m, 13H), 1.74−1.58 (m, 6H), 1.41−1.24 (m, 2H), 1.20−1.03 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 156.43, 77.59, 55.06, 49.33, 47.08, 42.04, 36.14, 30.66, 29.83, 28.93. MS (ESI) *m/z*: 307.4 (M + H$^+$). Anal. ($C_{18}H_{30}N_2O_2$) C, H, N.

***trans*-1-Adamantan-1-yl-3-(4-cyclohexylmethoxycyclohexyl)-urea (9h).** The general method above was used with methanesulfonic acid cyclohexylmethyl ester to afford a white solid (0.21 g, 54% yield). Mp 249−250 °C. ^{1}H NMR ($CDCl_3$): δ 3.95 (s, 1H), 3.91 (d, J = 8 Hz, 1H), 3.56−3.41 (m, 1H), 3.22 (d, J = 6 Hz, 2H), 3.19−3.08 (m, 1H), 2.13−0.99 (m, 32H), 0.96−0.79 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 156.78, 77.30, 74.44, 50.98, 48.64, 42.67, 38.42, 36.58, 31.73, 30.83, 30.34, 29.68, 26.80, 26.00. MS (ESI) *m/z*: 389.5 (M + H$^+$). Anal. ($C_{24}H_{40}N_2O_2$) C, H, N.

***cis*-4-Nitrobenzoic Acid 4-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)cyclohexyl Ester (5).**[23] To a solution of *trans*-2-(4-hydroxycyclohexyl)isoindole-1,3-dione **4** (38 g, 155 mmol), triphenylphosphine (65 g, 248 mmol), and 4-nitrobenzoic acid (41 g, 248 mmol) in 1500 mL of THF was added dropwise diisopropyl azodicarboxylate (50 g, 248 mmol) at room temperature. The reaction mixture was stirred overnight. The solvent was evaporated, and the resulting solid was recrystallized from methanol to afford 53 g (87%) of the title compound as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.40−8.36 (m, 4H), 7.79 (ddd, J = 0.12, 0.02, and 0.02 Hz, 4H), 5.39 (s, 1H), 4.37−4.22 (m, 1H), 2.82−2.65 (m, 2H), 2.27−2.16 (m, 2H), 1.84−1.65 (m, 4H).

***cis*-4-Nitrobenzoic Acid 4-Aminocyclohexyl Ester (6).** An amount of 35 wt % hydrazine hydrate (0.93 g, 10.1 mmol) was added to a solution of compound **5** (2.0 g, 5.1 mmol) in CH_2Cl_2 (50 mL) followed by MeOH (50 mL) at room temperature. The reaction mixture was allowed to stir overnight. The resulting white precipitates were filtered off, and the solvent was removed in vacuo. The resulting white solids were dissolved in aqueous 1 N HCl solution and washed with CH_2Cl_2. The aqueous layer was basified with excess 1 N NaOH solution and then extracted with CH_2Cl_2. After the aqueous layer was dried with $MgSO_4$, the solvent was evaporated affording crude *trans*-4-nitrobenzoic acid 4-aminocyclohexyl ester **6** as a white solid (1.1 g, 89% yield), which was used in the next step without further purification. ^{1}H NMR (DMSO-d_6): δ 8.26 (dd, J = 44 and 9 Hz, 4H), 6.72 (d, J = 7 Hz, 2H), 5.08 (s, 1H), 2.00−1.36 (m, 9H).

***cis*-4-Nitrobenzoic Acid 4-(3-Adamantan-1-ylureido)cyclohexyl Ester (7).** To a solution compound **6** (0.66 g, 2.5 mmol) in DMF was added 1-adamantyl isocyanate (0.4 g, 2.3 mmol) followed by triethylamine (0.35 mL, 2.5 mmol) at 0 °C. The reaction mixture was stirred overnight. The reaction mixture was poured into water, and the resulting precipitates were collected and washed with water. The crude product was recrystallized from CH_2Cl_2/hexanes to afford 0.9 g (89%) of the title compound as a white solid. Mp 194−217 °C. ^{1}H NMR ($CDCl_3$): δ 8.24 (dd, J = 29 and 9 Hz, 4H), 5.23 (s, 1H), 4.13 (d, J = 7 Hz, 1H), 4.05 (s, 1H), 3.75−3.61 (m, 1H), 2.17−1.41 (m, 23H). MS (ESI) *m/z*: 442.2 (M + H$^+$).

***cis*-1-Adamantan-1-yl-3-(4-hydroxycyclohexyl)urea (8).** To a solution of ester **7** (1 g, 2.3 mmol) in THF (100 mL) was added 1 N NaOH solution (4.6 mL, 4.6 mmol) at room temperature. The reaction mixture was stirred overnight, at which time the reaction was quenched by addition of 1 N HCl solution (5.5 mL). The resulting white precipitate was collected by filtration and recrystallized from methanol/water to afford 0.63 g (95% yield) of the title compound as a white solid. Mp 205−207 °C. ^{1}H NMR (DMSO-d_6): δ 5.67 (d, J = 8 Hz, 1H), 5.45 (s, 1H), 4.41 (s, 1H), 3.63−3.51 (m, 1H), 3.46−3.36 (m, 1H), 2.00−1.92 (m, 3H), 1.87−1.78 (m, 6H), 1.62−1.53 (m, 6H), 1.51−1.36 (m, 8H). ^{13}C NMR (DMSO-d_6): δ 156.39, 65.51, 49.29, 45.04, 42.08, 36.16, 30.95, 28.95, 28.24. MS (ESI) *m/z*: 293.2 (M + H$^+$). Anal. ($C_{17}H_{28}N_2O_2$) C, H, N.

***cis*-1-Adamantan-1-yl-3-(4-benzyloxycyclohexyl)urea (9a).** Compound **9a** was synthesized from compound **8** by the general procedure as that described for the synthesis of trans isomers **3a**−**h** and **9h** with benzyl bromide. Yield: 86% (0.33 g). Mp 181−182 °C. ^{1}H NMR ($CDCl_3$): δ 7.43−7.24 (m, 5H), 4.49 (s, 2H), 4.11 (d, J = 8 Hz, 1H), 4.02 (s, 1H), 3.66−3.51 (m, 2H), 2.23−1.07 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 156.70, 139.19, 128.45, 127.54, 127.50, 73.02, 69.81, 50.96, 47.63, 42.69, 36.58, 29.69, 28.64, 28.51. MS (ESI) *m/z*: 383.3 (M + H$^+$). Anal. ($C_{24}H_{34}N_2O_2$) C, H, N.

***cis*-1-Adamantan-1-yl-3-[4-(4-bromobenzyloxy)cyclohexyl]-urea (9b). 9b** was synthesized from compound **8** by the general procedure as that described for the synthesis of trans isomers **3a**−**h** and **9h** with *p*-bromobenzyl bromide. Yield: 85% (0.39 g). Mp 207−208 °C. ^{1}H NMR ($CDCl_3$): δ 7.46 (d, J = 8 Hz, 2H), 7.22 (d, J = 8 Hz, 2H), 4.44 (s, 2H), 4.09 (d, J = 6 Hz, 1H), 4.01 (s, 1H), 3.67−3.50 (m, 2H), 2.19−1.35 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 156.59, 138.26, 131.55, 129.16, 121.29, 73.20, 69.12, 51.03, 47.70, 42.70, 36.58, 29.70, 28.65, 28.47. MS (ESI) *m/z*: 461.1 (M + H$^+$).

***cis*-1-Adamantan-1-yl-3-[4-(2-methylbenzyloxy)cyclohexyl]-urea (9c). 9c** was synthesized from compound **8** by the general procedure as that described for the synthesis of trans isomers **3a**−**gh** and **9h** with 2-methylbenzyl bromide. Yield: 66% (0.26 g). Mp 156−158 °C. ^{1}H NMR ($CDCl_3$): δ 7.41−7.07 (m, 4H), 4.46 (s, 2H), 4.11 (d, J = 8 Hz, 1H), 4.03 (s, 1H), 3.66−3.51 (m, 2H), 2.33 (s, 3H), 2.18−1.43 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 156.68, 137.03, 136.50, 130.24, 128.30, 127.67, 125.89, 73.35, 68.44, 50.94, 47.63, 42.68, 36.57, 29.68, 28.69, 28.57, 18.98. MS (ESI) *m/z*: 397.2 (M + H$^+$).

***cis*-1-Adamantan-1-yl-3-[4-(2-chlorobenzyloxy)cyclohexyl]-urea (9d). 9d** was synthesized from compound **8** by the general procedure as that described for the synthesis of trans isomers **3a**−**h** and **9h** with 2-chlorobenzyl bromide. Yield: 82% (0.34 g). Mp 168−172 °C. ^{1}H NMR ($CDCl_3$): δ 7.31−7.20 (m, 1H), 6.93−6.83 (m, 3H), 4.54 (s, 2H), 4.04 (d, J = 7 Hz, 1H), 3.96 (s, 1H), 3.65−3.51 (m, 2H), 2.12−1.39 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 156.72, 136.93, 132.77, 129.28, 128.88, 128.52, 126.85, 73.72, 67.05, 50.98, 47.69, 42.69, 36.58, 29.69, 28.73, 28.54. MS (ESI) *m/z*: 417.1 (M + H$^+$).

***cis*-1-Adamantan-1-yl-3-[4-(2,6-dichlorobenzyloxy)cyclohexyl]-urea (9e). 9e** was synthesized from compound **8** by the general procedure as that described for the synthesis of trans isomers **3a**−**h** and **9h** with 2,6-dichlorobenzyl bromide. Yield: 58% (0.26 g). Mp 160−163 °C. ^{1}H NMR ($CDCl_3$): δ 7.40 (m, 3H), 4.71 (s, 2H), 4.08 (d, J = 8 Hz, 1H), 4.01 (s, 1H), 3.67−3.54 (m, 2H), 2.13−1.44 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 156.68, 136.92, 134.12, 129.82, 128.49, 74.22, 64.97, 50.94, 47.55, 42.66, 36.58, 29.68, 28.73, 28.49. MS (ESI) *m/z*: 452.1 (M + H$^+$).

***cis*-1-Adamantan-1-yl-3-[4-(2,6-difluorobenzyloxy)cyclohexyl]-urea (9f). 9f** was synthesized from compound **8** by the general procedure as that described for the synthesis of trans isomers **3a**−**h** and **9h** with 2,6-difluorobenzyl bromide. Yield: 63% (0.26 g). Mp 121−131 °C. ^{1}H NMR ($CDCl_3$): δ 7.31−7.19 (m, 1H), 6.93−6.84 (m, 2H), 4.54 (s, 2H), 4.04 (d, J = 7 Hz, 1H), 3.96 (s, 1H), 3.66−3.52 (m, 2H), 2.11−1.42 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 162.03 (dd, J = 250 and 8 Hz), 156.61, 130.03 (t, J = 10 Hz), 114.44 (t, J = 20 Hz), 111.37 (dd, J = 26 and 10 Hz), 73.56, 57.21, 50.98, 47.57, 42.68, 36.59, 29.70, 28.58, 28.36. MS (ESI) *m/z*: 419.2 (M + H$^+$).

***cis*-1-Adamantan-1-yl-3-[4-(2,6-difluoro-4-isopropoxybenzyloxy)cyclohexyl]urea (9g). 9a** was synthesized from compound **8** by the general procedure as that described for the synthesis of trans isomers **3a**−**h** and **9h** with 2-bromomethyl-1,3-difluoro-5-isopropoxybenzene **17**. Yield: 53% (0.25 g). Mp 78−80 °C. ^{1}H NMR ($CDCl_3$): δ 6.41 (d, J = 9 Hz, 2H), 4.54−4.42 (m, 1H), 4.46 (s, 2H), 4.20−3.97 (m, 2H), 3.65−3.50 (m, 2H), 2.11−1.45 (m, 23H), 1.32 (d, J = 7 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 162.68 (dd, J = 247 and 12 Hz), 159.41 (t, J = 14 Hz), 156.70, 106.28 (t, J = 21 Hz), 99.23 (dd, J = 29 and 10 Hz), 73.41, 70.83, 56.97 (t, J = 3 Hz), 50.88, 47.35, 42.63, 36.58, 29.68, 28.51, 28.39, 21.90. MS (ESI) *m/z*: 477.2 (M + H$^+$).

***cis*-1-Adamantan-1-yl-3-(4-methoxycyclohexyl)urea (3i). 3i** was synthesized from compound **8** by the general procedure as that described for the synthesis of trans isomers **3a**−**h** and **9h** with iodomethane. Yield: 85% (0.26 g). Mp 218−220 °C. ^{1}H NMR

($CDCl_3$): δ 4.11 (d, J = 7 Hz, 1H), 4.06 (s, 1H), 3.65−3.52 (m, 1H), 3.38−3.24 (m, 1H), 3.30 (s, 3H), 2.16−1.38 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 156.67, 75.07, 55.69, 50.97, 47.57, 42.70, 36.59, 29.70, 28.36, 28.22. MS (ESI) *m/z*: 307.2 ($M + H^+$). Anal. ($C_{18}H_{30}N_2O_2$) C, H, N.

***cis*-1-Adamantan-1-yl-3-(4-cyclohexylmethoxycyclohexyl)-urea (9i). 9i** was synthesized from compound **8** by the general procedure as that described for the synthesis of trans isomers **3a**−**h** and **9h** with methanesulfonic acid cyclohexylmethyl ester. Yield: 70% (0.27 g). Mp 210−217 °C. ^{1}H NMR ($CDCl_3$): δ 4.15 (d, J = 7 Hz, 1H), 4.07 (s, 1H), 3.67−3.50 (m, 1H), 3.43−3.34 (m, 1H), 3.18 (d, J = 7 Hz, 2H), 2.17−1.06 (m, 32H), 1.00−0.82 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 156.68, 73.86, 73.32, 50.97, 47.71, 42.69, 38.46, 36.59, 30.38, 29.70, 28.68, 28.51, 26.83, 26.05. MS (ESI) *m/z*: 389.2 ($M + H^+$). Anal. ($C_{24}H_{40}N_2O_2$) C, H, N.

***cis*-2-(4-Hydroxycyclohexyl)isoindole-1,3-dione (10).**[23] A 1 N NaOH solution (19 mL, 19 mmol) was added at room temperature to a solution of ester **5** (5 g, 12.7 mmol) in THF (100 mL). The mixture was stirred overnight at room temperature, at which time the reaction was quenched by addition of 1 N HCl solution (40 mL). The solvent was removed under reduced pressure, and the resulting white precipitate that formed was collected by filtration and dissolved in DMF. After adding triethylamine (6.5 g, 64 mmol) at room temperature, the reaction mixture was heated at 150 °C for 30 min in the microwave. After cooling to room temperature, the reaction mixture was poured into water and then extracted with ether. The organic layer was washed with water thoroughly. After the organic layer was dried with $MgSO_4$, the solvent was removed in vacuo. The resulting white solids were recrystallized from CH_2Cl_2/hexanes. Yield: 60% (1.9 g). ^{1}H NMR ($CDCl_3$): δ 7.76 (ddd, J = 38, 5, and 3 Hz, 4H), 4.21−4.07 (m, 2H), 2.72−2.55 (m, 2H), 1.96 (d, J = 14 Hz, 2H), 1.73−1.50 (m, 4H).

General Procedure for the Synthesis of Trans Isomers (Phenoxy Intermediates) 11a-f. *trans*-2-[4-(4-Bromophenoxy)-cyclohexyl]isoindole-1,3-dione (11a). Compound **11a** was prepared in 35% yield from compound **10** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.83 (dd, J = 5 and 3 Hz, 2H), 7.71 (dd, J = 5 and 3 Hz, 2H), 7.39−7.33 (m, 2H), 6.83−6.77 (m, 2H), 4.32−4.13 (m, 2H), 2.49−2.20 (m, 4H), 1.88−1.77 (m, 2H), 1.65−1.49 (m, 2H).

***trans*-2-[4-(4-Methoxyphenoxy)cyclohexyl]isoindole-1,3-dione (11b).** Compound **11b** was prepared in 38% yield from compound **10** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.85−7.77 (m, 2H), 7.74−7.65 (m, 2H), 6.92−6.76 (m, 4H), 4.28−4.11 (m, 2H), 3.77 (s, 3H), 2.47−2.19 (m, 4H), 1.88−1.75 (m, 2H), 1.64−1.45 (m, 2H).

***trans*-2-[4-(4-Nitrophenoxy)cyclohexyl]isoindole-1,3-dione (11c).** Compound **11c** was prepared in 28% yield from compound **10** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 8.21 (d, J = 9 Hz, 2H), 7.88−7.69 (m, 4H), 6.98 (d, J = 9 Hz, 2H), 4.53−4.40 (m, 1H), 4.32−4.18 (m, 1H), 2.56−2.20 (m, 4H), 1.95−1.82 (m, 2H), 1.73−1.52 (m, 2H).

***trans*-2-[4-(4-Fluorophenoxy)cyclohexyl]isoindole-1,3-dione (11d).** Compound **11d** was prepared in 40% yield from compound **10** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.77 (ddd, J = 38, 5, and 3 Hz, 4H), 7.00−6.84 (m, 4H), 4.30−4.15 (m, 2H), 2.48−2.31 (m, 2H), 2.26 (d, J = 11 Hz, 2H), 1.89−1.77 (m, 2H), 1.65−1.49 (m, 4H).

***trans*-2-[4-(3,5-Difluorophenoxy)cyclohexyl]isoindole-1,3-dione (11e).** Compound **11e** was prepared in 31% yield from compound **10** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.93−7.64 (m, 4H), 6.51−6.31 (m, 4H), 4.38−4.06 (m, 2H), 2.56−2.11 (m, 4H), 1.96−1.36 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 168.42, 165.59, 165.38, 162.33, 162.12, 159.72, 134.10, 132.04, 123.32, 99.64, 99.51, 99.39, 99.26, 96.76, 96.42, 96.08, 75.64, 49.51, 31.14, 27.44.

***trans*-4-[4-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)cyclohexyloxy]-benzoic Acid Ethyl Ester (11f).** Compound **11a** was prepared in 34% yield from compound **10** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.99 (d, J = 9 Hz, 2H), 7.85−7.81 (m, 2H), 7.75−7.69 (m, 2H), 6.93 (d, J = 9 Hz, 2H), 4.46−4.29 (m, 1H), 4.33 (q, J = 7 Hz, 2H), 4.28−4.18 (m, 1H), 2.61−2.19 (m, 4H), 1.88−1.79 (m, 2H), 1.69−1.56 (m, 2H), 1.38 (t, J = 7 Hz, 3H).

General Procedure for the Synthesis of Trans Isomers 13a−f. Hydrazine hydrate (35 wt %, 2 equiv) was added to a solution of compound **11** in CH_2Cl_2 followed by MeOH at room temperature. The reaction mixture was allowed to stir for 1 day. The resulting white precipitates were filtered off, and the solvent was removed in vacuo. The resulting white solids were dissolved in aqueous 1 N HCl solution and washed with CH_2Cl_2. The aqueous layer was basified with excess 1 N NaOH solution and then extracted with CH_2Cl_2. After the aqueous layer was dried with $MgSO_4$, the solvent was evaporated affording crude amine **12**, which was used in the next step without further purification. To a solution compound **12** in DMF was added 1-adamantyl isocyanate (0.9 equiv) followed by triethylamine (1 equiv) at 0 °C. The reaction mixture was stirred overnight. The reaction mixture was poured into water, and the resulting precipitates were collected and washed with water. The crude product was purified by column chromatography by a dry loading method.

***trans*-1-Adamantan-1-yl-3-[4-(4-bromophenoxy)cyclohexyl]-urea (13a).** 84% yield. Mp 205−210 °C. ^{1}H NMR ($CDCl_3$): δ 7.33 (d, J = 9 Hz, 2H), 6.75 (d, J = 9 Hz, 2H), 4.18−4.04 (m, 1H), 4.02−3.90 (m, 2H), 3.65−3.50 (m, 1H), 2.20−1.80 (m, 13H), 1.77−1.42 (m, 8H), 1.29−1.11 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 156.65, 156.45, 132.16, 117.93, 111.67, 74.48, 49.36, 46.79, 42.05, 36.15, 30.35, 29.71, 28.95. MS (ESI) *m/z*: 447.1 ($M + H^+$).

***trans*-1-Adamantan-1-yl-3-[4-(4-methoxyphenoxy)cyclohexyl]-urea (13b).** 82% yield. Mp 226−237 °C. ^{1}H NMR ($CDCl_3$): δ 6.89−6.80 (m, 4H), 5.59 (d, J = 7 Hz, 1H), 5.40 (s, 1H), 4.19−4.08 (m, 1H), 3.69 (s, 3H), 3.39−3.28 (m, 1H), 2.02−1.48 (m, 19H), 1.44−1.29 (m, 2H), 1.23−1.08 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 156.55, 154.18, 151.73, 117.89, 114.74, 76.62, 55.83, 51.13, 48.47, 42.68, 36.57, 31.47, 30.63, 29.70. MS (ESI) *m/z*: 399.26 ($M + H^+$).

***trans*-1-Adamantan-1-yl-3-[4-(4-nitrophenoxy)cyclohexyl]-urea (13c).** 95% yield. Mp 205−227 °C. ^{1}H NMR ($CDCl_3$): δ 8.16 (d, J = 9 Hz, 2H), 6.90 (d, J = 9 Hz, 2H), 4.35−4.23 (m, 1H), 4.17−3.90 (m, 2H), 3.68−3.54 (m, 1H), 2.20−1.87 (m, 13H), 1.71−1.51 (m, 8H), 1.34−1.16 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 162.95, 156.43, 140.44, 125.91, 115.70, 75.29, 49.36, 46.65, 42.04, 36.14, 30.22, 29.55, 28.95. MS (ESI) *m/z*: 414.24 ($M + H^+$).

***trans*-1-Adamantan-1-yl-3-[4-(4-fluorophenoxy)cyclohexyl]-urea (13d).** 84% yield. Mp 242−245 °C. ^{1}H NMR ($CDCl_3$): δ 6.98−6.91 (m, 2H), 6.85−6.79 (m, 2H), 4.12−3.94 (m, 3H), 3.66−3.51 (m, 1H), 2.17−1.88 (m, 12H), 1.73−1.45 (m, 9H), 1.28−1.11 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 156.43, 155.81 (d, J = 236 Hz), 153.42 (d, J = 2 Hz), 117.17 (d, J = 8 Hz), 115.81 (d, J = 23 Hz), 74.91, 49.35, 46.81, 42.04, 36.14, 30.37, 29.82, 28.93. MS (ESI) *m/z*: 387.24 ($M + H^+$).

***trans*-1-Adamantan-1-yl-3-[4-(3,5-difluorophenoxy)cyclohexyl]-urea (13e).** 87% yield. Mp 255−258 °C. ^{1}H NMR (DMSO-d_6): δ 6.76−6.64 (m, 3H), 5.56 (d, J = 8 Hz, 1H), 5.32 (s, 1H), 4.43−4.30 (m, 1H), 3.45−3.31 (m, 1H), 2.06−1.73 (m, 13H), 1.69−1.35 (m, 8H), 1.32−1.15 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 163.91 (dd, J = 246 and 16 Hz), 159.85, 156.50, 99.67, 99.54, 99.42, 99.29, 96.42 (t, J = 26 Hz), 76.00, 51.29, 48.32, 42.75, 36.64, 31.38, 30.30, 29.77. MS (ESI) *m/z*: 405.24 ($M + H^+$).

***trans*-4-[4-(3-Adamantan-1-ylureido)cyclohexyloxy]benzoic Acid Ethyl Ester (13f).** 68% yield. Mp 187−189 °C. ^{1}H NMR (DMSO-d_6): δ 7.88 (d, J = 9 Hz, 2H), 7.04 (d, J = 9 Hz, 2H), 5.61 (d, J = 7 Hz, 1H), 5.40 (s, 1H), 4.49−4.38 (m, 1H), 4.27 (q, J = 8 Hz, 2H), 3.43−3.29 (m, 1H), 2.08−1.78 (m, 13H), 1.65−1.53 (m, 6H), 1.52−1.36 (m, 2H), 1.29 (t, J = 8 Hz, 3H), 1.33−1.15 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 166.59, 161.64, 156.73, 131.68, 122.75, 115.12, 75.21, 60.79, 51.03, 48.11, 42.65, 36.56, 31.26, 30.28, 29.66, 14.51. MS (ESI) *m/z*: 441.4 ($M + H^+$). Anal. ($C_{26}H_{36}N_2O_4$) C, H, N.

***trans*-4-[4-(3-Adamantan-1-ylureido)cyclohexyloxy]benzoic Acid (13g).** To a solution of urea in CH_3CN was added lithium hydroxide (3 equiv) followed by water at room temperature. The reaction

mixture was stirred overnight. The solvent was evaporated in vacuo and washed with EtOAc. The aqueous layer was acidified with 1 N HCl to give white precipitates. The resulting white solids were collected by suction filtration and washed with water. The crude product was recrystallized from MeOH to give **13g** (88% yield) as a white solid. Mp 250−255 °C. ^{1}H NMR (DMSO-d_6): δ 12.58 (s, 1H), 7.86 (d, J = 9 Hz, 2H), 7.02 (d, J = 9 Hz, 2H), 5.62 (d, J = 8 Hz, 1H), 5.41 (s, 1H), 4.48−4.36 (m, 1H), 3.63−3.54 (m, 1H), 2.11−1.34 (m, 21H), 1.32−1.11 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 166.99, 161.11, 156.43, 131.37, 122.61, 115.08, 74.39, 49.36, 46.75, 42.04, 36.14, 30.34, 29.73, 28.93. MS (ESI) *m/z*: 413.3 (M + H$^+$). Anal. ($C_{24}H_{32}N_2O_4$·CH_4O) C, H, N.

Synthesis of Cis Isomers (Phenoxy Intermediates) 14a−f,h,j. ***cis*-2-[4-(4-Bromophenoxy)cyclohexyl]isoindole-1,3-dione (14a).** Compound **14a** was prepared in 68% yield from compound **4** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.83 (dd, J = 5 and 3 Hz, 2H), 7.71 (dd, J = 5 and 3 Hz, 2H), 7.43−7.36 (m, 2H), 6.95−6.87 (m, 2H), 4.56 (s, 1H), 4.30−4.13 (m, 1H), 2.80−2.61 (m, 2H), 2.25−2.14 (m, 2H), 1.72−1.52 (m, 4H).

***cis*-2-[4-(4-Methoxyphenoxy)cyclohexyl]isoindole-1,3-dione (14b).** Compound **14b** was prepared in 52% yield from compound **4** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.83 (dd, J = 6 and 3 Hz, 2H), 7.71 (dd, J = 6 and 3 Hz, 2H), 7.00−6.73 (m, 4H), 4.51−4.46 (m, 1H), 4.26−4.14 (m, 1H), 3.77 (s, 3H), 2.84−2.61 (m, 2H), 2.29−2.10 (m, 2H), 1.72−1.46 (m, 4H).

***cis*-2-[4-(4-Nitrophenoxy)cyclohexyl]isoindole-1,3-dione (14c).** Compound **14c** was prepared in 44% yield from compound **4** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 8.21 (d, J = 9 Hz, 2H), 7.86−7.67 (m, 4H), 7.05 (d, J = 9 Hz, 2H), 4.78−4.69 (m, 1H), 4.30−4.17 (m, 1H), 2.78−2.60 (m, 2H), 2.29−2.17 (m, 2H), 1.80−1.53 (m, 4H).

***cis*-2-[4-(4-Fluorophenoxy)cyclohexyl]isoindole-1,3-dione (14d).** Compound **14d** was prepared in 80% yield from compound **4** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.84−7.80 (m, 2H), 7.71−7.67 (m, 2H), 6.98−6.94 (m, 4H), 4.51 (s, 1H), 4.26−4.12 (m, 1H), 2.76−2.60 (m, 2H), 2.18 (d, J = 13 Hz, 2H), 1.79−1.49 (m, 4H).

***cis*-2-[4-(3,5-Difluorophenoxy)cyclohexyl]isoindole-1,3-dione (14e).** Compound **14e** was prepared in 48% yield from compound **4** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.90−7.64 (m, 4H), 6.61−6.47 (m, 2H), 6.46−6.34 (m, 1H), 4.59−4.50 (m, 1H), 4.29−4.13 (m, 1H), 2.77−2.58 (m, 2H), 2.29−2.12 (m, 2H), 1.76−1.52 (m, 4H).

***cis*-4-[4-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)cyclohexyloxy]-benzoic Acid Ethyl Ester (14f).** Compound **14f** was prepared in 78% yield from compound **4** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 8.00 (d, J = 9 Hz, 2H), 7.86−7.65 (m, 4H), 7.01 (d, J = 9 Hz, 2H), 4.73−4.65 (m, 1H), 4.34 (q, J = 7 Hz, 2H), 4.28−4.15 (m, 1H), 2.80−2.62 (m, 2H), 2.28−2.17 (m, 2H), 1.75−1.52 (m, 2H), 1.38 (t, J = 7 Hz, 3H).

***cis*-3-[4-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)cyclohexyloxy]-benzoic Acid Ethyl Ester (14h).** Compound **14h** was prepared in 70% yield from compound **4** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.83 (dd, J = 6 and 3 Hz, 2H), 7.70 (dd, J = 6 and 3 Hz, 2H), 7.65−7.61 (m, 2H), 7.36 (t, J = 8 Hz, 1H), 7.23 (ddd, J = 8, 3, and 1 Hz, 1H), 4.70−4.65 (m, 1H), 4.22 (tt, J = 12 and 4 Hz, 1H), 3.92 (s, 3H), 2.86−2.58 (m, 2H), 2.28−2.16 (m, 2H), 1.75−1.53 (m, 4H).

***cis*-2-[4-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)cyclohexyloxy]-benzoic Acid Ethyl Ester (14j).** Compound **14j** was prepared in 68% yield from compound **4** using the procedure detailed for compound **5**. ^{1}H NMR ($CDCl_3$): δ 7.81 (dd, J = 5 and 3 Hz, 2H), 7.80−7.77 (m, 1H), 7.69 (dd, J = 5 and 3 Hz, 2H), 7.43−7.38 (m, 1H), 7.00−6.94 (m, 2H), 4.68−4.64 (m, 1H), 4.54 (q, J = 7 Hz, 2H), 4.19 (tt, J = 13 and 4 Hz, 1H), 2.74 (dq, J = 13 and 4 Hz, 2H), 2.30−2.22 (m, 2H), 1.72−1.55 (m, 4H), 1.43 (t, J = 7 Hz, 3H).

Synthesis of Cis Isomers (Phenoxyureas) 16a−f,h,j. ***cis*-1-Adamantan-1-yl-3-[4-(4-bromophenoxy)cyclohexyl]urea (16a).** Compound **16a** was synthesized from compound **14** through **15** by the general procedure for the synthesis of trans isomers **13a−f**. 82% yield. Mp 203−204 °C. ^{1}H NMR ($CDCl_3$): δ 7.35 (d, J = 9 Hz, 2H), 6.76 (d, J = 9 Hz, 2H), 4.43−4.36 (m, 1H), 4.17 (d, J = 7 Hz, 1H), 4.09 (s, 1H), 3.71−3.57 (m, 1H), 2.21−1.43 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 156.85, 156.62, 132.40, 117.86, 112.78, 71.86, 51.90, 47.46, 42.67, 36.56, 29.65, 28.40, 28.26. MS (ESI) *m/z*: 447.3 (M + H$^+$).

***cis*-1-Adamantan-1-yl-3-[4-(4-methoxyphenoxy)cyclohexyl]-urea (16b).** Compound **16b** was synthesized from compound **14** through **15** by the general procedure for the synthesis of trans isomers **13a−f.** 86% yield. Mp 188−189 °C. ^{1}H NMR (DMSO-d_6): δ 6.98−6.74 (m, 4H), 5.76 (d, J = 8 Hz, 1H), 5.42 (s, 1H), 4.34−4.24 (m, 1H), 3.69 (s, 3H), 3.55−3.41 (m, 1H), 2.05−1.33 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 156.57, 154.06, 151.51, 117.76, 114.78, 72.55, 55.83, 51.08, 47.82, 42.71, 36.59, 29.70, 28.63, 28.35. MS (ESI) *m/z*: 399.3 (M + H$^+$). Anal. ($C_{24}H_{34}N_2O_3$) C, H, N.

***cis*-1-Adamantan-1-yl-3-[4-(4-nitrophenoxy)cyclohexyl]urea (16c).** Compound **16c** was synthesized from compound **14** through **15** by the general procedure for the synthesis of trans isomers **13a−f**. 79% yield. Mp 213−216 °C. ^{1}H NMR ($CDCl_3$): δ 8.28 (d, J = 8 Hz, 2H), 8.19 (d, J = 8 Hz, 2H), 5.27−5.18 (m, 1H), 4.24 (d, J = 6 Hz, 1H), 4.1 (s, 1H), 3.75−3.60 (m, 1H), 2.18−1.44 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 162.90, 156.52, 141.29, 126.16, 115.44, 72.40, 51.13, 47.58, 42.69, 36.56, 29.69, 28.47, 28.17. MS (ESI) *m/z*: 414.2 (M + H$^+$). Anal. ($C_{23}H_{31}N_3O_4$) C, H, N.

***cis*-1-Adamantan-1-yl-3-[4-(4-fluorophenoxy)cyclohexyl]-urea (16d).** Compound **16d** was synthesized from compound **14** through **15** by the general procedure for the synthesis of trans isomers **13a−f**. 92% yield. Mp 206−209 °C. ^{1}H NMR ($CDCl_3$): δ 6.98−6.91 (m, 2H), 6.84−6.78 (m, 2H), 4.34 (s, 1H), 4.30 (d, J = 10 Hz, 1H), 4.20 (s, 1H), 3.71−3.56 (m, 1H), 2.13−1.44 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 158.89, 156.70, 155.74, 153.60, 153.57, 117.50, 117.39, 116.12, 115.82, 72.44, 51.00, 47.66, 42.69, 36.58, 31.72, 29.68, 28.52, 28.30. MS (ESI) *m/z*: 387.2 (M + H$^+$). Anal. ($C_{23}H_{31}FN_2O_2$) C, H, N.

***cis*-1-Adamantan-1-yl-3-[4-(3,5-difluorophenoxy)cyclohexyl]-urea (16e).** Compound **16e** was synthesized from compound **14** through **15** by the general procedure for the synthesis of trans isomers **13a−f**. 83% yield. Mp 193−198 °C. ^{1}H NMR ($CDCl_3$): δ 6.45−6.34 (m, 3H), 4.43−4.35 (m, 1H), 4.18−3.93 (m, 2H), 3.71−3.57 (m, 1H), 2.13−1.43 (m, 23H). ^{13}C NMR ($CDCl_3$): δ 164.52, 164.36, 162.10, 161.94, 159.45, 156.36, 99.68, 99.61, 99.48, 99.40, 96.14, 95.88, 95.62, 73.01, 49.33, 44.87, 42.06, 36.14, 28.94, 28.03, 27.19. MS (ESI) *m/z*: 405.5 (M + H$^+$).

***cis*-4-[4-(3-Adamantan-1-ylureido)cyclohexyloxy]benzoic Acid Ethyl Ester (16f).** Compound **16f** was synthesized from compound **14** through **15** by the general procedure for the synthesis of trans isomers **13a−f**. 69% yield. Mp 101−136 °C. ^{1}H NMR ($CDCl_3$): δ 7.89 (d, J = 9 Hz, 2H), 7.04 (d, J = 9 Hz, 2H), 5.78 (d, J = 8 Hz, 1H), 5.40 (s, 1H), 4.61−4.53 (m, 1H), 4.27 (q, J = 7 Hz, 2H), 3.56−3.46 (m, 1H), 2.02−1.93 (m, 3H), 1.88−1.39 (m, 20H), 1.30 (t, J = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 166.62, 161.46, 156.86, 131.70, 122.63, 115.22, 71.57, 60.80, 50.87, 47.35, 42.68, 36.58, 29.67, 28.47, 28.28, 14. 52. MS (ESI) *m/z*: 441.28 (M + H$^+$).

***cis*-4-[4-(3-Adamantan-1-ylureido)cyclohexyloxy]benzoic Acid (16g).** Compound **16g** was prepared from **16f** according to the same procedure described for compound **13g**. 88% yield. Mp 178−187 °C. ^{1}H NMR (DMSO-d_6): δ 12.60 (s, 1H), 7.87 (d, J = 9 Hz, 2H), 7.01 (d, J = 9 Hz, 2H), 5.80 (d, J = 7 Hz, 1H), 5.42 (s, 1H), 4.62−4.52 (m, 1H), 3.59−3.45 (m, 1H), 2.09−1.38 (m, 23H). ^{13}C NMR (DMSO-d_6): δ 166.97, 160.91, 156.36, 131.44, 122.63, 115.22, 72.24, 49.35, 44.90, 42.07, 36.15, 28.94, 28.09, 27.32. MS (ESI) *m/z*: 413.2 (M + H$^+$). Anal. ($C_{24}H_{32}N_2O_4$·0.5CH_4O) C, H, N.

***cis*-3-[4-(3-Adamantan-1-ylureido)cyclohexyloxy]benzoic Acid Methyl Ester (16h).** Compound **16d** was synthesized from compound **14** through **15** by the general procedure for the synthesis of trans isomers **13a−f**. 67% yield. Mp 153−156 °C. ^{1}H NMR (DMSO-d_6): δ 7.54−7.48 (m, 1H), 7.45−7.39 (m, 2H), 7.25−7.20 (m, 1H), 5.78 (d, J = 8 Hz, 1H), 5.41 (s, 1H), 4.56−4.47 (m,

1H), 3.84 (s, 3H), 3.57−3.44 (m, 1H), 2.01−1.94 (m, 3H), 1.86−1.82 (m, 6H), 1.76−1.54 (m, 12H), 1.52−1.39 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 165.94, 157.07, 156.27, 130.96, 129.95, 121.20, 120.77, 116.00, 72.11, 52.11, 49.24, 45.04, 41.97, 36.05, 28.86, 27.93, 27.29. MS (ESI) *m/z*: 427.3 (M + H$^+$).

***cis*-3-[4-(3-Adamantan-1-ylureido)cyclohexyloxy]benzoic Acid (16i).** Compound **16i** was prepared from **16h** according to the same procedure described for compound **13g**. 91% yield. Mp 219−222 °C. ^{1}H NMR (DMSO-d_6): δ 12.98 (s, 1H), 7.54−7.35 (m, 3H), 7.22−7.16 (m, 1H), 5.78 (d, J = 8 Hz, 1H), 5.41 (s, 1H), 4.56−4.45 (m, 1H), 3.57−3.43 (m, 1H), 2.02−1.94 (m, 3H), 1.90−1.37 (m, 20H). ^{13}C NMR (DMSO-d_6): δ 167.14, 157.09, 156.36, 132.25, 129.85, 121.49, 120.51, 116.14, 72.17, 49.34, 45.08, 42.06, 36.15, 28.95, 28.07, 27.38. MS (ESI) *m/z*: 413.2 (M + H$^+$). Anal. ($C_{24}H_{32}N_2O_4$) C, H, N.

***cis*-2-[4-(3-Adamantan-1-ylureido)cyclohexyloxy]benzoic Acid Ethyl Ester (16j).** Compound **16d** was synthesized from compound **14** through **15** by the general procedure for the synthesis of trans isomers **13a**−**f**. 88% yield. Mp 157−160 °C. ^{1}H NMR ($CDCl_3$): δ 7.74 (dd, J = 8.00 and 2 Hz, 1H), 7.42−7.35 (m, 1H), 6.95−6.89 (m, 2H), 4.57−4.50 (m, 2H), 4.46 (s, 1H), 4.31 (q, J = 7 Hz, 2H), 3.73−3.58 (m, 1H), 2.10−1.90 (m, 11H), 1.76−1.60 (m, 12H), 1.35 (t, J = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 166.69, 157.00, 156.92, 133.18, 131.70, 121.62, 120.06, 114.76, 72.16, 60.84, 50.89, 47.64, 42.61, 36.58, 29.66, 28.66, 28.17, 14.50. MS (ESI) *m/z*: 441.3 (M + H$^+$).

***cis*-2-[4-(3-Adamantan-1-ylureido)cyclohexyloxy]benzoic Acid (16k).** Compound **16k** was prepared from **16j** according to the same procedure described for compound **13g**. 85% yield. Mp 215−221 °C. ^{1}H NMR (DMSO-d_6) δ 12.49 (s, 1H), 7.62−7.58 (m, 1H), 7.47−7.40 (m, 1H), 7.11 (d, J = 8 Hz, 1H), 6.95 (t, J = 8 Hz, 1H), 5.67 (d, J = 7.62 Hz, 1H), 5.51 (s, 1H), 4.58−4.49 (m, 1H), 3.52−3.37 (m, 1H), 2.02−1.74 (m, 11H), 1.71−1.44 (m, 12H). ^{13}C NMR (DMSO-d_6): δ 167.14, 157.09, 156.36, 132.25, 129.85, 121.49, 120.51, 116.14, 72.17, 49.34, 45.08, 42.06, 36.15, 28.95, 28.07, 27.38. MS (ESI) *m/z*: 413.2 (M + H$^+$). Anal. ($C_{24}H_{32}N_2O_4$) C, H, N.

2-Bromomethyl-1,3-difluoro-5-isopropoxybenzene (17). To a solution of 2,6-difluoro-4-hydroxylbenzyl alcohol[39] (0.55 g, 2.72 mmol) in THF (27 mL) were added PPh_3 (1.07 g, 4.08 mmol) and CBr_4 (1.35 g, 4.08 mmol) at 0 °C. The reaction mixture was warmed to room temperature and the stirred for 5 h. The solvent was evaporated in vacuo and the residue was purified by column chromatography to give 0.63 g (88%) of the titled compound as colorless oil. ^{1}H NMR ($CDCl_3$): δ 6.42 (d, J = 10 Hz, 2H), 4.51 (s, 2H), 4.54−4.44 (m, 1H), 1.34 (d, J = 6 Hz, 6H).

General Procedure for the Synthesis of 19a−c. To a solution of the appropriate amine (1.1 equiv) in DMF was added the indicated isocyanate (1 mmol) followed by triethylamine (1.1 equiv) at 0 °C. The reaction mixture was stirred overnight. The reaction mixture was poured into water, and the resulting precipitates were collected and washed with a 1 N HCl solution followed by water. The crude product was recrystallized from CH_2Cl_2/hexanes or was purified by silica gel chromatography using 30% EtOAc in hexanes as an eluent.

***trans*-4-{4-[3-(4-Trifluoromethoxyphenyl)ureido]cyclohexyloxy}benzoic Acid Ethyl Ester (19a).** ^{1}H NMR ($CDCl_3$): δ 7.97 (d, J = 9 Hz, 2H), 7.34 (d, J = 9 Hz, 2H), 7.15 (d, J = 9 Hz, 2H), 6.88 (d, J = 9 Hz, 2H), 6.52 (s, 1H), 4.67 (d, J = 8 Hz, 1H), 4.34 (q, J = 7 Hz, 2H), 4.30−4.20 (m, 1H), 3.84−3.69 (m, 1H), 2.21−2.08 (m, 4H), 1.69−1.54 (m, 2H), 1.38 (t, J = 7 Hz, 3H), 1.33−1.23 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 166.69, 161.61, 154.67, 137.51, 131.74, 122.89, 122.16, 121.35, 115.17, 74.99, 60.90, 48.48, 31.03, 30.16, 14.53. MS (ESI) *m/z*: 467.2 (M + H$^+$).

***cis*-4-{4-[3-(4-Trifluoromethoxyphenyl)ureido]cyclohexyloxy}benzoic Acid Ethyl Ester (19b).** ^{1}H NMR ($CDCl_3$): δ 7.98 (d, J = 9 Hz, 2H), 7.35 (d, J = 9 Hz, 2H), 7.13 (d, J = 9 Hz, 2H), 6.87 (d, J = 9 Hz, 2H), 6.85 (s, 1H), 5.01 (d, J = 8 Hz, 1H), 4.57−4.51 (m, 1H), 4.36 (q, J = 7 Hz, 2H), 3.89−3.72 (m, 1H), 2.07−1.52 (m, 8H), 1.39 (t, J = 7 Hz, 3H). MS (ESI) *m/z*: 467.2 (M + H$^+$).

***cis*-4-{4-[3-(4-Methoxycarbonylphenyl)ureido]cyclohexyloxy}benzoic Acid Ethyl Ester (19c).** ^{1}H NMR ($CDCl_3$): δ 7.96 (t, J = 9 Hz, 1H), 7.42 (d, J = 9 Hz, 1H), 7.16 (s, 1H), 6.87 (d, J = 9 Hz, 1H), 5.21 (d, J = 8 Hz, 1H), 4.57−4.51 (m, 1H), 4.36 (q, J = 7 Hz, 1H), 3.92−3.77 (m, 1H), 3.88 (s, 1H), 2.07−1.95 (m, 1H), 1.88−1.56 (m, 1H), 1.39 (t, J = 7 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 167.09, 167.01, 161.49, 154.39, 143.88, 131.78, 131.12, 123.95, 122.63, 118.00, 115.31, 71.31, 61.07, 52.12, 47.81, 28.41, 28.01, 14.52. MS (ESI) *m/z*: 441.2 (M + H$^+$).

General Procedure for the Hydrolysis of 19a−d To Synthesize 20a−c. To a solution of urea in CH_3CN was added lithium hydroxide (3 equiv for **20a**,**b**, 6 equiv for **20c**) followed by water at room temperature. The reaction mixture was stirred overnight or warmed to 90 °C for 6 h. The solvent was evaporated in vacuo and washed with EtOAc. The aqueous layer was acidified with 1 N HCl to give white precipitates. The resulting white solids were collected by suction filtration and washed with water. The crude product was recrystallized from MeOH.

***trans*-4-{4-[3-(4-Trifluoromethoxyphenyl)ureido]cyclohexyloxy}benzoic Acid (20a)**. Mp 244−273 °C. ^{1}H NMR (DMSO-d_6): δ 12.59 (s, 1H), 8.51 (s, 1H), 7.86 (d, J = 9 Hz, 1H), 7.47 (d, J = 9 Hz, 1H), 7.22 (d, J = 9 Hz, 1H), 7.03 (d, J = 9 Hz, 1H), 6.19 (d, J = 9 Hz, 1H), 4.52−4.38 (m, 1H), 3.61−3.45 (m, 1H), 2.12−1.87 (m, 1H), 1.58−1.28 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 167.07, 161.10, 154.47, 141.98, 139.87, 131.43, 122.74, 121.69, 118.55, 115.10, 74.31, 47.17, 30.01, 29.66. MS (ESI) *m/z*: 439.1 (M + H$^+$). Anal. ($C_{21}H_{21}F_3N_2O_5$) C, H, N.

***cis*-4-{4-[3-(4-Trifluoromethoxyphenyl)ureido]cyclohexyloxy}benzoic Acid (20b).** Mp 210−212 °C. ^{1}H NMR (DMSO-d_6): δ 12.60 (s, 1H), 8.48 (s, 1H), 7.87 (d, J = 8 Hz, 1H), 7.47 (d, J = 9 Hz, 1H), 7.21 (d, J = 9 Hz, 1H), 7.03 (d, J = 8 Hz, 1H), 6.35 (d, J = 8 Hz, 1H), 4.66−4.57 (m, 1H), 3.73−3.60 (m, 1H), 1.87−1.50 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 167.04, 160.90, 154.36, 141.99, 139.84, 131.48, 122.77, 121.67, 118.57, 115.28, 71.95, 45.66, 27.71, 27.36. MS (ESI) *m/z*: 439.1 (M + H$^+$). Anal. ($C_{21}H_{21}F_3N_2O_5$) C, H, N.

***cis*-4-{4-[3-(4-Carboxyphenyl)ureido]cyclohexyloxy}benzoic Acid (20c)**. Mp 250−257 °C. ^{1}H NMR (DMSO-d_6): δ 13.04 (br s, 1H), 8.69 (s, 1H), 8.07−8.05 (m, 1H), 7.80 (d, J = 9 Hz, 1H), 7.66 (d, J = 1 Hz, 1H), 7.48 (d, J = 9 Hz, 1H), 6.46 (d, J = 8 Hz, 1H), 4.70−4.62 (m, 1H), 3.73−3.60 (m, 1H), 1.93−1.50 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 167.14, 167.02, 160.89, 154.05, 144.79, 131.48, 130.57, 122.79, 122.74, 116.52, 115.29, 71.95, 45.64, 27.66, 27.33. Anal. ($C_{21}H_{22}N_2O_6 \cdot {}^1/_3CH_4O$) C, H, N.

General Procedure for the Synthesis of Amide Derivatives 22 and 23. To a solution of 1-adamantineacetic acid (1 mmol) in CH_2Cl_2 (10 mL) were added an appropriate amine (1 mmol), Et_3N (1.5 mmol), and EDCI (1.1 mmol) at 0 °C. The reaction mixture warmed up to room temperature and was stirred overnight. The solvent was evaporated, and the remaining residue was dissolved in EtOAc and washed with water. The organic layer was dried with $MgSO_4$ and filtered. After the solvent was evaporated, the residue was purified with column chromatography with 20−30% EtOAc in hexanes.

***trans*-2-Adamantan-1-yl-*N*-[4-(2,6-difluorobenzyloxy)cyclohexyl]-acetamide (22).** The general method was used with **24** to afford a white solid (0.26 g, 62% yield). Mp 169−171 °C. ^{1}H NMR (DMSO-d_6): δ 7.54 (d, J = 8 Hz, 1H), 7.49−7.38 (m, 1H), 7.15−7.05 (m, 2H), 4.51 (s, 2H), 3.58−3.43 (m, 1H), 3.35 (s, 2H), 3.35−3.25 (m, 1H), 2.04−1.47 (m, 19H), 1.29−1.09 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 169.01, 161.20 (dd, J = 248 and 8 Hz), 130.81 (t, J = 10 Hz), 114.09 (t, J = 20 Hz), 111.56 (d, J = 25 Hz), 111.55 (d, J = 13 Hz), 76.52, 56.66, 49.98, 46.67, 42.13, 36.50, 32.21, 30.22, 30.05, 28.07. MS (ESI) *m/z*: 418.3 (M + H$^+$). Anal. ($C_{25}H_{33}F_2NO_2$) C, H, N.

***cis*-2-Adamantan-1-yl-*N*-[4-(4-fluorophenoxy)cyclohexyl]acetamide (23).** The general method was used with **15d** to afford a white solid (0.27 g, 70% yield). Mp 151−152 °C. ^{1}H NMR (DMSO-d_6): δ 7.64 (d, J = 8 Hz, 1H), 7.09 (t, J = 9 Hz, 2H), 6.97−6.90 (m, 2H), 4.46−4.38 (m, 1H), 3.74−3.62 (m, 1H), 1.95−1.78 (m, 9H), 1.70−1.48 (m, 16H). ^{13}C NMR (DMSO-d_6): δ

169.02, 156.37 (d, $J = 236$ Hz), 153.38 (d, $J = 2$ Hz), 117.29 (d, $J = 8$ Hz), 115.88 (d, $J = 23$ Hz), 71.91, 49.88, 45.60, 42.14, 36.51, 32.24, 28.08, 27.57, 27.12. MS (ESI) *m/z*: 386.3 ($M + H^+$). Anal. ($C_{24}H_{32}FNO_2$) C, H, N.

2-[4-(4-Fluorophenoxy)butyl]isoindole-1,3-dione (26). Compound **26** was prepared in 85% yield from compound **32** using the procedure detailed for compound **5**. Mp 84−87 °C. ^{1}H NMR ($CDCl_3$): δ 7.83 (dd, $J = 5$ and 3 Hz, 2H), 7.71 (dd, $J = 5$ and 3 Hz, 2H), 6.97−6.89 (m, 2H), 6.83−6.76 (m, 2H), 3.94 (t, $J = 6$ Hz, 2H), 3.76 (t, $J = 7$ Hz, 2H), 1.94−1.75 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 168.49, 157.19 (d, $J = 238$ Hz), 155.05 (d, $J = 2$ Hz), 134.01, 132.13, 123.26, 115.78 (d, $J = 23$ Hz), 115.46 (d, $J = 8$ Hz), 67.80, 37.687, 26.67, 25.36. MS (ESI) *m/z*: 314.1 ($M + H^+$). Anal. ($C_{18}H_{16}FNO_3$) C, H, N.

1-Adamantan-1-yl-3-[4-(4-fluorophenoxy)butyl]urea (27). Compound **27** was synthesized from compound **26** by the general procedure for the synthesis of trans isomers **13a**−**f**. Mp 138−141 °C. ^{1}H NMR (DMSO-d_6): δ 7.09 (dt, $J = 9$ and 2 Hz, 2H), 6.92 (ddd, $J = 9$, 4, and 2 Hz, 2H), 5.66 (t, $J = 5$ Hz, 1H), 5.44 (s, 1H), 3.92 (t, $J = 6$ Hz, 2H), 3.01−2.92 (m, 2H), 2.01−1.94 (m, 3H), 1.86−1.82 (m, 6H), 1.70−1.56 (m, 8H), 1.52−1.40 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.37 (d, $J = 235$ Hz), 157.07, 154.96 (d, $J = 2$ Hz), 115.71 (d, $J = 31$ Hz), 115.65, 67.79, 49.35, 42.06, 38.45, 36.15, 28.96, 26.74, 26.22. MS (ESI) *m/z*: 361.23 ($M + H^+$).

2-[4-(4-Fluorophenoxy)but-2-ynyl]isoindole-1,3-dione (28). Compound **28** was prepared in 90% yield from compound **33** using the procedure detailed for compound **5**. Mp 119−121 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90−7.86 (m, 2H), 7.76−7.73 (m, 2H), 6.97−6.84 (m, 4H), 4.62 (t, $J = 2$ Hz, 2H), 4.49 (t, $J = 2$ Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 167.05, 157.74 (d, $J = 239$ Hz), 153.71 (d, $J = 2$ Hz), 134.33, 132.00, 123.64, 116.30 (d, $J = 8$ Hz), 115.88 (d, $J = 23$ Hz), 81.17, 78.05, 56.82, 27.33. MS (ESI) *m/z*: 310.1 ($M + H^+$). Anal. ($C_{18}H_{12}FNO_3$) C, H, N.

1-Adamantan-1-yl-3-[4-(4-fluorophenoxy)but-2-ynyl]urea (29). Compound **29** was synthesized from compound **28** by the general procedure for the synthesis of trans isomers **13a**−**f**. Mp 135−136 °C. ^{1}H NMR ($CDCl_3$): δ 7.02−6.85 (m, 4H), 4.64 (dd, $J = 2$ and 2 Hz, 2H), 4.29−4.06 (m, 2H), 4.01−3.94 (m, 2H), 2.13−2.02 (m, 3H), 2.01−1.90 (m, 6H), 1.71−1.62 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 156.76 (d, $J = 239$ Hz), 156.24, 153.67 (d, $J = 2$ Hz), 116.11 (d, $J = 8$ Hz), 115.82 (d, $J = 23$ Hz), 85.82, 76.58, 56.27, 49.60, 41.94, 36.10, 28.95, 28.48. MS (ESI) *m/z*: 357.2 ($M + H^+$). Anal. ($C_{21}H_{25}FN_2O_2$) C, H, N.

1-Adamantan-1-yl-3-[4-(4-fluorophenoxy)phenyl]urea (31). Compound **31** was prepared in 85% yield from compound **30** using the procedure detailed for compound **2** except the product was purified by column chromatography. Mp 169−207 °C. ^{1}H NMR (DMSO-d_6): δ 8.24 (s, 1H), 7.34 (d, $J = 9$ Hz, 2H), 7.18 (t, $J = 9$ Hz, 2H), 6.99−6.86 (m, 4H), 5.82 (s, 1H), 2.09−1.85 (m, 9H), 1.66−1.59 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 158.70 (d, $J = 241$ Hz), 155.11, 153.50 (d, $J = 2$ Hz), 153.33, 134.59, 122.61, 119.92 (d, $J = 8$ Hz), 119.43, 116.34 (d, $J = 23$ Hz), 51.37, 42.39, 36.49, 29.63. MS (ESI) *m/z*: 381.2 ($M + H^+$). Anal. ($C_{23}H_{25}FN_2O_2$) C, H, N.

1-Adamantan-1-yl-3-(4-oxocyclohexyl)urea (34). To a solution of **34** (1.5 g, 5.1 mmol) in DMF (50 mL) was added PDC (4.8 g, 12.8 mmol) at room temperature. After being stirred overnight, the reaction mixture was poured into water. The resulting white precipitates were filtered and washed with water thoroughly. The resulting solid was purified by recrystallization from DCM/hexanes to give 1.26 g (85%) of the titled compound. Mp 240−460 °C. ^{1}H NMR (DMSO-d_6): δ 5.95−5.71 (br s, 1H), 5.55−5.33 (br s, 1H), 3.88−3.68 (m, 1H), 2.47−2.30 (m, 2H), 2.28−2.14 (m, 2H), 2.11−1.77 (m, 11H), 1.70−1.45 (m, 8H). ^{13}C NMR (DMSO-d_6): δ 210.03, 156.48, 49.42, 45.22, 42.02, 38.50, 36.14, 31.92, 28.94.

1-Adamantan-1-yl-3-[4-(4-fluorobenzylidene)cyclohexyl]urea (35). To a solution of 4-fluorobenzyltriphenylphosphonium bromide (2.33 g, 5.2 mmol) in THF (15 mL) was added 1.6 M *n*-butyllithium in THF (3.25 mL, 5.2 mmol) at −78 °C, and the mixture was warmed to room temperature. A solution of **34** (0.5 g, 1.72 mmol) in THF (5 mL) was added dropwise over 10 min to the reaction mixture at room temperature and heated to reflux overnight. After the mixture was cooled to room temperature, the reaction was quenched by adding water. THF was removed in vacuo. The remained aqueous layer was extracted with EtOAc (2×). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by column chromatography (3:7 EtOAc/hexanes) to give 0.56 g (70%) of the titled compound as a white solid. Mp 193−199 °C. ^{1}H NMR ($CDCl_3$): δ 7.15−7.08 (m, 2H), 7.02−6.94 (m, 2H), 6.20 (s, 1H), 4.12−4.02 (m, 2H), 3.82−3.66 (m, 1H), 2.79−2.66 (m, 2H), 2.42−2.22 (m, 2H), 2.15−1.88 (m, 9H), 1.74−1.59 (m, 6H), 1.37−1.07 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 162.96, 159.71, 156.72, 141.02, 134.08, 130.49, 130.39, 122.17, 115.18, 114.90, 51.01, 48.59, 42.70, 36.57, 35.27, 34.97, 34.23, 29.68, 27.05.

1-Adamantan-1-yl-3-[4-(4-fluorobenzyl)cyclohexyl]urea (36). To a solution of **35** (0.3 g, 0.78 mmol) in EtOAc was added 10% palladium on carbon. The solution was filled with H_2, and the reaction mixture was stirred for 2 h. After the solution was filtered through Celite, the filtrate was concentrated in vacuo. Purification by column chromatography (3:7 EtOAc/hexanes) gave the title compound, 0.28 g (95%), as a white solid. Mp 185−187 °C. ^{1}H NMR ($CDCl_3$): δ 7.10−6.88 (m, 4H), 5.20 (d, $J = 8$ Hz, 0.56H), 4.85 (s, 0.56H), 4.72 (d, $J = 8$ Hz, 0.44H), 4.66 (s, 0.44H), 3.83−3.74 (m, 0.56H), 3.50−3.34 (m, 0.44H), 2.46 (d, $J = 7$ Hz, 1.12H), 2.42 (d, $J = 7$ Hz, 0.88H), 2.07−1.90 (m, 9H), 1.72−1.44 (m, 11H), 1.29−1.13 (m, 2H), 1.10−0.95 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 161.33 (d, $J = 243$ Hz), 156.92, 156.84, 136.74 (d, $J = 3$ Hz), 136.59 (d, $J = 3$ Hz), 130.42 (d, $J = 8$ Hz), 130.40 (d, $J = 8$ Hz), 115.01 (d, $J = 21$ Hz), 114.95 (d, $J = 21$ Hz), 50.90, 50.86, 49.36, 45.84, 42.72, 42.68, 41.60, 39.31, 38.18, 36.58, 33.90, 31.80, 30.20, 29.68, 27.64. MS (ESI) *m/z*: 385.3 ($M + H^+$). Anal. ($C_{24}H_{33}FN_2O$) C, H, N.

1-Adamantan-1-yl-3-(4-hydroxybutyl)urea (38). Compound **38** was prepared in 90% yield from 1-adamantyl isocycnate and 4-amino-1-butanol using the procedure detailed above for **7** except that the titled compound (93%) was recrystallized from MeOH. Mp 149−152 °C. ^{1}H NMR (DMSO-d_6): δ 5.60 (t, $J = 5$ Hz, 1H), 5.44 (s, 1H), 4.39 (t, $J = 5$, 1H), 3.41−3.33 (m, 2H), 2.94−2.86 (m, 2H), 2.01−1.94 (m, 3H), 1.88−1.80 (m, 6H), 1.62−1.56 (m, 6H), 1.42−1.27 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 157.09, 60.56, 49.34, 42.08, 38.73, 36.18, 30.01, 28.97, 26.75. MS (ESI) *m/z*: 267.2 ($M + H^+$). Anal. ($C_{15}H_{26}N_2O_2$) C, H, N.

***trans,trans*-1,3-Bis(4-hydroxycyclohexyl)urea (39).** To a solution of 1,1′-carbonyldiimidazole (0.2 g, 1 mmol) in CH_3CN (10 mL) were added *trans*-4-aminocyclohexanol (0.76 g, 5 mmol) and triethylamine (0.7 mL, 5 mmol) followed by water (5 mL) at room temperature. The reaction mixture was stirred overnight, and the organic solvent was evaporated in vacuo. The resulting white precipitate was filtered and washed with water thoroughly. The resulting sample was purified by recrystallization from methanol to give the titled compound (30%) as a white solid. Mp 285−290 °C. ^{1}H NMR (DMSO-d_6): δ 5.51 (d, $J = 8$ Hz, 2H), 4.49 (d, $J = 4$ Hz, 2H), 3.41−3.17 (m, 4H), 1.81−1.66 (m, 8H), 1.25−0.95 (m, 8H). ^{13}C NMR (DMSO-d_6): δ 156.86, 68.19, 47.54, 33.99, 31.19. MS (ESI) *m/z*: 257.2 ($M + H^+$). Anal. ($C_{13}H_{24}N_2O_3$) C, H, N.

Molecular Modeling. Molecular modeling was performed using "BioMedCAChe 5.0" software (Fujitsu Computer Systems Corporation). The atomic coordinates of the crystal structure of human sEH complexed with CU4 were retrieved from Protein Data Bank (PDB) (entry 1ZD3).[33] Compounds **13g** and **16g** were docked into the ligand-binding pocket manually by superposition with the parent molecule (CU4). The ligand and the amino acid residues within 8.0 Å from the ligand were minimized on MM geometry (MM3).

Metabolic Stability Assay. Human hepatic microsomes were purchased from BD diagnostics (Sparks, MD). The incubation mixture consisted of microsomal protein in potassium phosphate buffer (100 mM, pH 7.4) and 1 μM test compound in a final volume of 1 mL. The concentration of hepatic microsomal protein was 0.05 mg/mL. An NADPH-generating system containing 50 mM $MgCl_2$, 57 mM glucose 6-phosphate, 2 mM β-$NADP^+$, and 5 unit/mL

glucose 6-phosphate dehydrogenase (Sigma, St. Louis) was prepared and added to the incubation mixture with a 10% volume of the reaction mixture. After the addition of the NADPH-generating system, the mixture was incubated at 37 °C for 0 and 60 min. The reaction was terminated by the addition of one volume of ethanol. All incubations were made in triplicate. The concentrations of the test compound in the reaction mixture were measured by LC/MS−MS, using a Micromass Quattro Premier triple quadrupole tandem mass spectrometer (Micromass, Manchester, U.K.) equipped with an atomospheric pressure ionization source [atomospheric z-spray pressure chemical ionization (APcI) or electrospray ionization (ESI) interface]. The separation was performed by Ultra Performance LC (UPLC; Waters Corporation, Milford, MA) with an Aquity column (BEH 2.1 mm × 50 mm, 1.7 μm; Waters Corporation) at a flow rate of 0.3 mL/min at ambient temperature. Solvent A was 10% acetonitrile and 90% water containing 0.1% formic acid, and solvent B was acetonitrile containing 0.1% formic acid. Mobile phases were mixed with a linear gradient from 30% B to 100% B for 5 min and then isocratic for an additional 8 min with 100% B. Five microliters of standard and the extracted microsome samples were injected onto the column. Data were analyzed with MassLynx software (version 4.0).

Bioavailability Protocol. All in vivo experiments were done following protocols approved by the Animal Use and Care Committee of University of California—Davis. Two dogs weighing between 19 and 22 kg were used in the exposure studies ($n = 2$ per time point). For pharmacokinetic screening, oral doses of 0.3 mg/kg were administered via syringe in 1 mL of tristerate at 40 °C. For the determination of the bioavailability, **13g** was separately administered in saline containing 1% morpholine at 0.3 mg/kg orally and intravenously. Dogs were bled via the retro-orbital route at 0, 15, 30, 60, 120, 180, 240, 300, 360, 480, and 1440 min after oral dosing and at 0, 5, 10, 15, 30, 45, 60, 120, 150, 180, 240, 300, 360, and 1440 min after intravenous dosing. All blood samples were collected into EDTA tubes. Plasma was separated by centrifugation and stored at −78 °C until used. Plasma was assayed by electrospray LC−MS/MS as described above. Least-squares regression of the standard concentrations was used for sample quantitation.

Enzyme Preparation. Recombinant murine, rat, and human sEHs were produced in a baculovirus expression system[40,41] and were purified by affinity chromatography as previously reported.[42]

Liver samples from hamster, dog, and cat are generous gifts from Dr. W. Yokoyama (USDA), Dr. G. Gross (University of Wisconsin), and Dr. A. Hill (University of California, Davis), respectively. Cytosolic enzyme extracts were prepared in sodium phosphate buffer (100 mM, pH 7.4) as described.[43] The preparations were frozen at −80 °C until used. Protein concentration was quantified using the Pierce BCA assay (Pierce, Rockford, IL) using bovine serum albumin (BSA) as the calibrating standard.

IC_{50} Assay Conditions. For the spectrometric-based assay, enzymes (0.12 μM murine sEH or 0.24 μM human sEH) were incubated with inhibitors for 5 min in 0.1 M sodium phosphate buffer (200 μL, pH 7.4) at 30 °C before spectrometric substrate (4-nitrophenyl-*trans*-2,3-epoxy-3-phenylpropyl carbonate, NEPC) introduction ([S] = 40 μM).[40]

For the recombinant affinity purified sEHs (human, mouse, and rat), we used a fluorescent-based assay to determine IC_{50} values.[5] Enzymes (∼2nM mouse sEH, ∼1nM rat sEH, or ∼1 nM human sEH) were incubated with inhibitors for 5 min in 25 mM bis-Tris-HCl buffer (200 μL, pH 7.0) at 30 °C before substrate (cyano(2-methoxynaphthalen-6-yl)methyl *trans*-(3-phenyloxyran-2-yl)methylcarbonate, CMNPC) was added ($[S]_{final} = 5$ μM). Activity was assessed by measuring the appearance of the fluorescent 6-methoxynaphthaldehyde product ($\lambda_{em} = 330$ nm, $\lambda_{ex} = 465$ nm) at 30 °C during a 10 min incubation (Spectramax M2; Molecular Device, Inc., Sunnyvale, CA).[44] The IC_{50} values that are the concentrations of inhibitors that reduce activity by 50% were calculated from at least three separate runs, each in triplicate, to obtain the standard deviation given in the results section.

For the cytosolic preparation (hamster, cat, and dog), we used a radioactive based assay to determine IC_{50} values.[45] Enzyme extracts were incubated with inhibitors for 5 min in pH 7.4 sodium phosphate buffer at 30 °C prior to substrate (racemic [^{3}H]-*trans*-1,3-diphenylpropene oxide) introduction ($[S]_{final} = 50$ μM; ∼12000 dpm/assay). The enzymes were incubated at 30°C for 5−10 min. The reaction was quenched by addition of 60 μL of methanol and 200 μL of isooctane, which extracts the remaining epoxide from the aqueous phase. The activity was followed by measuring the quantity of radioactive diol formed in the aqueous phase using a liquid scintillation counter (Wallac model 1409; Gaithersburg, MD). Conditions used gave rates that were linear with both time and enzyme concentration and that resulted in at least 5% but not more than 30% hydrolysis of the substrate. Assays were performed in triplicate. IC_{50} results are the averages of three replicates.

Blood Pressure Determination and Oxylipin Profile. Male mice (C57BL/6, 8 weeks old, Charles River Laboratories) between 22 and 25 g were used in all treatments. The LPS was from *Escherichia coli* serotype 0111:B4 purchased from Sigma-Aldrich. **13g** was dissolved in trioleine containing 1% ethanol and AUDA-BE was dissolved directly in trioleine. **13g** and AUDA-BE were administered by oral gavage immediately after ip injection of LPS (10 mg/kg) in saline. Blood pressure was determined with a Visitech BP-2000 (Visitech Systems, Apex, NC) described by Schmelzer et al.[35]

Blood was collected by cardiac puncture with an EDTA-rinsed syringe. An amount of 10 μL of a mixture of triphenyphosphine, butylated hydroxytoluene, and indomethacin (0.2% for each, w/w) was added to each collection tube. Each sample was spun immediately and the plasma separated. All samples were stored at −80 °C until analysis. The ratio of EETs to DHETs was determined using a previously reported method.[46]

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Supporting Information Available: Elemental analysis data for the selected compounds and HPLC and ^{1}H NMR data for the evaluation of isomeric purity of compounds **13f** and **16f**. This material is available free of charge via the Internet at http://pubs.acs.org.

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