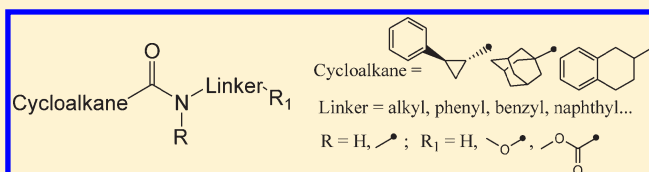


Structure–Activity Relationships of Cycloalkylamide Derivatives as Inhibitors of the Soluble Epoxide Hydrolase

In-Hae Kim,[†] Yong-Kyu Park,[‡] Bruce D. Hammock,[§] and Kosuke Nishi^{*,||}[†]Industry and Academic Cooperation Laboratory for Drug Development, Mokpo National University, Muan, Jeonnam 534-729, Republic of Korea[‡]Department of Medicinal Chemistry, Hyundai Pharma, Suwon, Gyeonggi 443-270, Republic of Korea[§]Department of Entomology and University of California Davis Cancer Center, University of California, One Shields Avenue, Davis, California 95616, United States^{||}Department of Applied Bioscience, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan**S** Supporting Information

ABSTRACT: Structure–activity relationships of cycloalkylamide compounds as inhibitors of human sEH were investigated. When the left side of amide function was modified by a variety of cycloalkanes, at least a C6 like cyclohexane was necessary to yield reasonable inhibition potency on the target enzyme. In compounds with a smaller cycloalkane or with a polar group on the left side of amide function, no inhibition was observed. On the other hand, increased hydrophobicity dramatically improved inhibition potency. Especially, a tetrahydronaphthalene (**20**) effectively increased the potency. When a series of alkyl or aryl derivatives of cycloalkylamide were investigated to continuously optimize the right side of the amide pharmacophore, a benzyl moiety functionalized with a polar group produced highly potent inhibition. A nonsubstituted benzyl, alkyl, aryl, or biaryl structure present on the right side of the cycloalkylamide function induced a big decrease in inhibition potency. Also, the resulting potent cycloalkylamide (**32**) showed reasonable physical properties.

**INTRODUCTION**

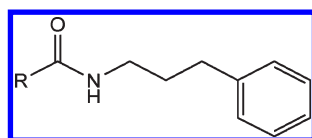
Epoxyeicosatrienoic acids (EETs), which are produced from arachidonic acid by cytochrome P450 epoxygenases, have important roles in the regulation of hypertension,^{1–6} inflammation,^{7–11} and other cardiovascular related diseases.^{12–14} However, metabolism of EETs to their corresponding hydrated products by soluble epoxide hydrolase (sEH) generally reduces these biological activities.¹ Both in vitro and In Vivo studies have indicated that the antihypertensive and cardioprotective effects mediated by the EETs are reversibly dependent on the extent of sEH hydrolysis of the EETs.^{2–4,6–8,14,15} Thus, maintaining the In Vivo concentration of EETs through sEH inhibition is a promising therapeutic pathway to treat cardiovascular inflammatory and other diseases.

Urea compounds substituted with hydrophobic groups are very potent and stable inhibitors of sEH with significant biological activities in both in vitro and In Vivo models.^{3,4,16} However, poor physical properties of the early compounds, such as low solubility and high melting points, likely resulted in limited In Vivo availability.¹⁷ The addition of a polar functional group on specific positions of one of the urea substituents is effective in increasing solubility in either water or organic solvents and also in improving In Vivo availability while maintaining the inhibition potency on the target enzyme.^{18–21} However, the positive effect on the solubility in water of the inhibitor is generally quite limited

and many of the resulting compounds still have relatively high melting points.¹⁹ Interestingly, dramatic improvement in melting points and/or solubility in water is obtained when the corresponding urea central pharmacophore is modified by a series of functional groups such as amides, carbamates, carbonates, and esters.^{18,19} Among them, alkylamide function with a polar group is effective for producing potent inhibitors with improved physical properties,¹⁹ suggesting that the amide structure is a very useful functionality as one of central pharmacophores for developing bioavailable potent inhibitors of human sEH. There is a strong correlation between the potency of sEH inhibitor with urea and amide central pharmacophores. However, the range of substituents for generating optimum amide sEH inhibitors appears more restricted and slightly different from that with a urea central pharmacophore.^{18–27} Thus, investigation of the relationships of the structure and inhibition potency of amide compounds is important to further develop highly potent inhibitors with improved physical properties and bioavailability. In the present study, we report structure–activity relationships of amide derivatives, specifically investigating the effect of various structural modifications of cycloalkylamide compounds on inhibition potency for human sEH to design potent inhibitors with cycloalkylamide function as a central pharmacophore.

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Table 1. Inhibition of Human sEH by Cycloalkylamide Derivatives



No.	Structure (R) ^a	Human sEH ^b IC ₅₀ (nM)	No.	Structure (R) ^a	Human sEH ^b IC ₅₀ (nM)
4		>1000	14		>1000
5		>1000	15		78
6		235	16		>1000
7		222	17		>1000
8		64	18		>1000
9		58	19		41
10		>1000	20		15
11		>1000	21		288
12		243	22		>1000
13		>1000	23		>1000

^a No steric isomers or mixtures of *cis/trans* or *R/S* unless otherwise indicated. ^b Human sEH (1 nM) was incubated with inhibitors for 10 min in 25 mM Bis-Tris/HCl buffer (200 μ L, pH 7.0) at 30 °C before fluorescent substrate (CMNPC) introduction ($[S] = 5 \mu$ M). Results are triplicate averages.

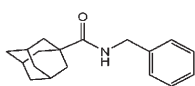
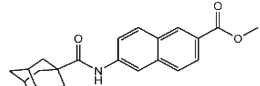
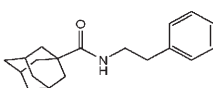
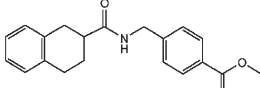
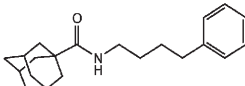
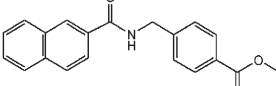
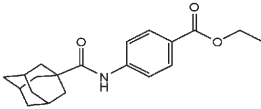
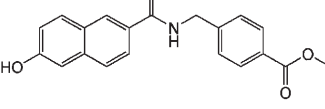
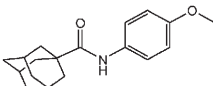
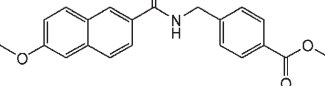
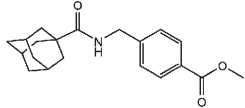
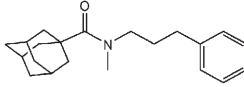
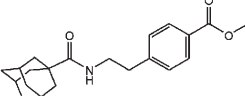
CHEMISTRY

Cycloalkylamide and substituted cycloalkylamide compounds in Tables 1 and 2 were synthesized as outlined in Scheme 1. The corresponding cycloalkanecarboxylic acid was coupled with 3-phenylpropylamine (Scheme 1A) or with a substituted alkyl- or aryl-amine (Scheme 1B) using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI) in the presence of 4-dimethylaminopyridine (DMAP) in dichloromethane to provide the corresponding nonsubstituted and substituted cycloalkylamide derivatives in approximately 40–85% yield.¹⁹ Alkylation of carboxylic acid with iodomethane in the presence of potassium

carbonate as a base in *N,N*-dimethylformamide (DMF) gave compounds **30** and **31** in 90–95% yield (Scheme 1B). For the syntheses of compounds **11** and **14** (Scheme 1C), methyl 4-oxocyclohexanecarboxylate for compound **11** and methyl tetrahydro-2*H*-pyran-4-carboxylate for compound **14** were hydrolyzed with 1 N NaOH in methanol to give the corresponding acids in 100% yield, followed by the above EDCI/DMAP coupling reaction with 3-phenylpropylamine (90%).

Compounds **15**, **16**, and **17** were prepared by the procedures depicted in Scheme 2. Alkylation of cyclohexane-1,4-dicarboxylic acid with iodomethane in the presence of potassium carbonate

Table 2. Inhibition of Human sEH by Adamantane- and Naphthaleneamide Derivatives

No.	Structure	Human sEH ^a IC ₅₀ (nM)	No.	Structure	Human sEH ^a IC ₅₀ (nM)
24		>1000	31		65
25		230	32		2.1
26		180	33		6.6
27		101	34		1.8
28		>1000	35		2.4
29		9.1	36		723
30		230			

^a Human sEH (1 nM) was incubated with inhibitors for 10 min in 25 mM Bis-Tris/HCl buffer (200 μ L, pH 7.0) at 30 °C before fluorescent substrate (CMNPC) introduction ([S] = 5 μ M). Results are triplicate averages.

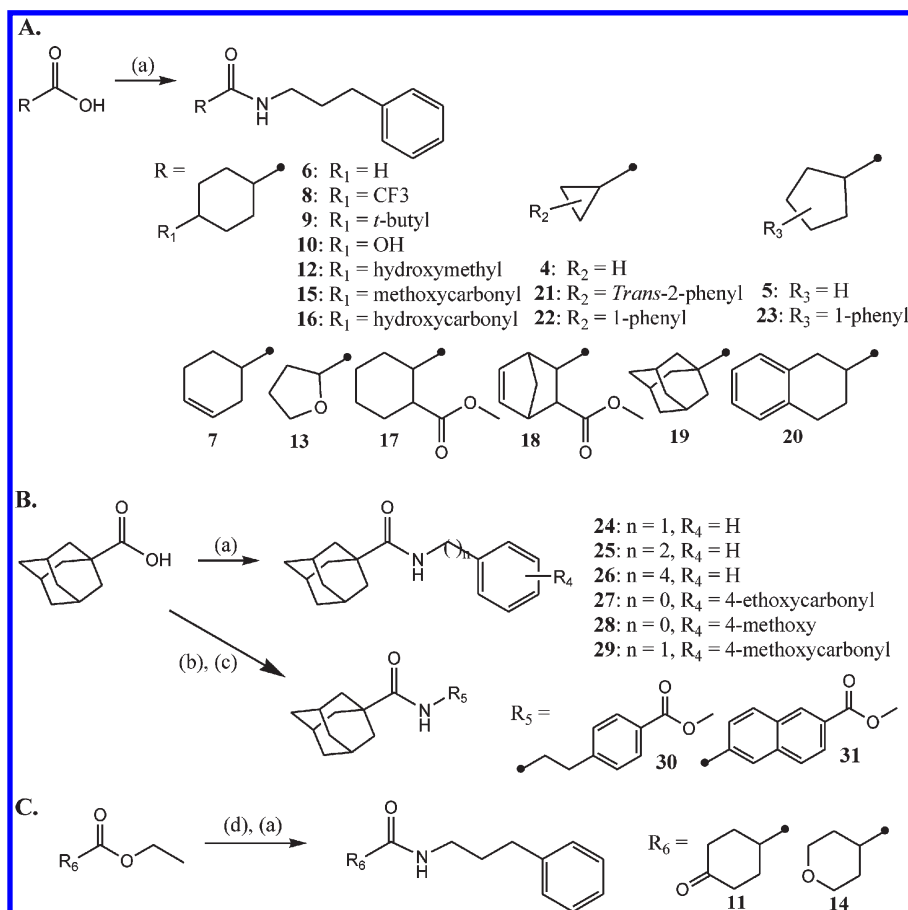
as a base in DMF yielded dialkylated ester in 100% yield. Monohydrolysis of the diester with barium hydroxide octahydrate in 80% methanol in H₂O produced 4-(methoxycarbonyl)cyclohexanecarboxylic acid in approximately 80% yield.²⁸ Coupling of this acid with 3-phenylpropylamine using EDCI and DMAP in dichloromethane provided compound 15 in 90% yield. Hydrolysis of the ester group of compound 15 with 1 N NaOH in methanol afforded the corresponding acid product (16) in 100% yield. For the synthesis of compound 17, cyclohexane-1,2-dicarboxylic acid was used instead of cyclohexane-1,4-dicarboxylic acid in the first step, which was followed by the monohydrolysis and coupling reaction to yield compound 17 in approximately 45% yield.

Naphthalene derivatives (32–35) were prepared as depicted in Scheme 3A. 1,2,3,4-Tetrahydronaphthalene-2-carboxylic acid was coupled with methyl 4-aminomethylbenzoate in the presence of EDCI and DMAP in dichloromethane to give compound 32 in 95% yield. In addition, 2-naphthoic acid or 6-hydroxy-2-naphthoic acid was reacted with the same reagents used for the synthesis of compound 32 to afford compounds 33

and 34, respectively, in about 90% yield. Methylation of the hydroxyl group of compound 34 with iodomethane in the presence of potassium carbonate as a base in DMF provided compound 35 in 95% yield. As seen in Scheme 3B, the N-methylated analogue of compound 19 (36) was synthesized in the reaction of iodomethane with compound 19 in the presence of sodium hydride as a base in DMF in 95% yield.

RESULTS AND DISCUSSION

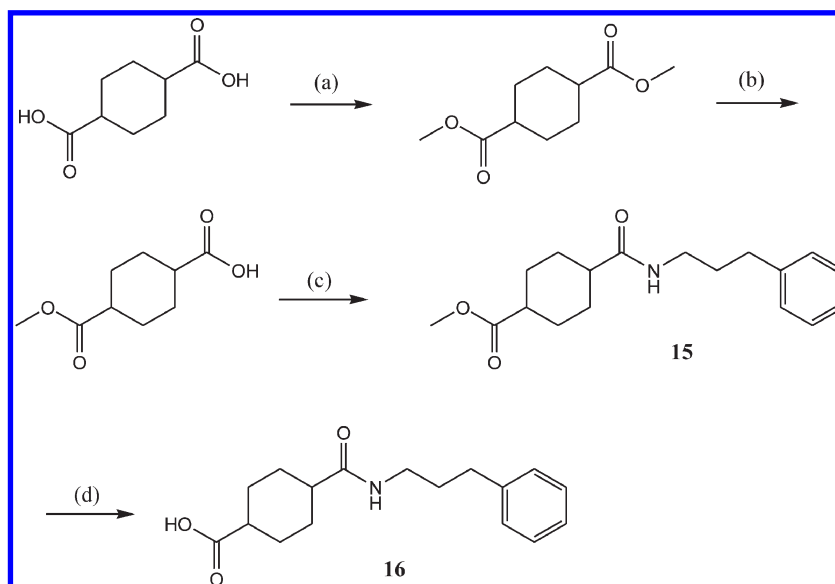
1,3-Dialkylureas have been found as highly potent inhibitors of human sEH.^{17–23} As seen in compound 1 in Figure 1, relatively large alkyl groups attached to the urea pharmacophore often lead to effective inhibitors (0.5–5 nM IC₅₀ on human sEH). Generally, the corresponding alkylamide analogues (2) of urea inhibitors exhibit approximately a 10- to 20-fold decreased inhibition potency for human sEH.¹⁹ However, interestingly, a >200-fold drop in inhibition was observed when substituents similar to that attached to compound 1 were incorporated into the amide function as seen in compound 3. This suggests that a cycloalkylamide structure like compound 3 is much less potent

Scheme 1. Syntheses of Cycloalkylamide Derivatives^a

^a (a) 3-Phenylpropylamine (in parts A and C) or substituted benzyl- or phenylamine (in part B), DMAP, EDCI, CH_2Cl_2 , room temp; (b) 4-(2-aminoethyl)benzoic acid (for 30) or 6-aminonaphthalene-2-carboxylic acid (for 31), DMAP, EDCI, CH_2Cl_2 , room temp; (c) CH_3I , K_2CO_3 , DMF, room temp; (d) 1 N NaOH, MeOH, room temp.

than alkylamide inhibitors in having the substituents effective for making potent inhibitors in ureas or alkylamides and that syntheses of a series of cycloalkylamides with various substitutions are necessary to improve inhibition potency of cycloalkylamide compounds against the target enzyme. In order to first investigate the effect of modifications in cycloalkyl substituents on the left side of the amide function on inhibition potency for the human enzyme, various cycloalkylamide structures were synthesized as listed in Table 1. The right side of the amide was fixed with a 3-phenylpropyl group, which is one of useful right side substituents of urea inhibitors for producing potent inhibitions and is also useful for monitoring chemical reactions quickly on a TLC plate. As seen in compounds 4 and 5, relatively small cycloalkyl groups such as cyclopropyl (4) or cyclopentyl (5) present on the left side of the amide function led to poor inhibitions, which is different from the result that urea derivatives show significant inhibition potency for human sEH (<500 nM) when a C5-carbon as a pentyl or a cyclopentyl is present on the left side of the urea function.^{17,18} However, at least a >5-fold improvement in inhibition was exhibited when the cycloalkane of 4 or 5 was replaced by a cyclohexyl (6) or a cyclohexenyl (7) group. This indicates that at least a six-membered cycloalkane structure on the left side of the amide is necessary for cycloalkylamide derivatives to produce potent inhibitors of the target enzyme. Further, the addition of a hydrophobic group such as trifluoromethyl

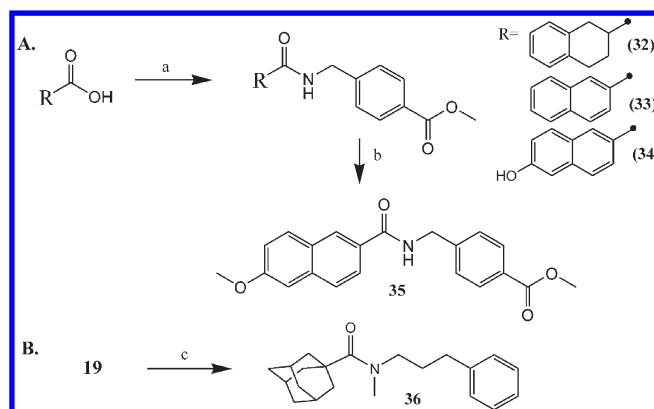
(8) or *tert*-butyl (9) on the cyclohexane moiety of compound 6 resulted in improved inhibitors (a 3.5- to 15-fold improvement) over the nonsubstituted derivatives (4–7), indicating that a hydrophobic substitution on cycloalkane is effective to further improve inhibition potency in cycloalkylamide compounds. On the other hand, incorporation of a hydroxyl group on the cyclohexyl (10) led to a poor inhibitor and conversion of the hydroxyl group of compound 10 to a ketone (11) also negatively impacted inhibition potency. However, when a methylene carbon was added between the hydroxyl group and the cyclohexane of compound 10, no drop in inhibition potency was observed (12). This indicates that direct incorporation of a polar group on the cyclohexane dramatically reduces inhibition potency, but a polar hydroxymethyl group on the cyclohexane does not interrupt the binding of the amide compound to the sEH. As shown in compounds 13 and 14, no significant inhibition was exhibited when an oxygen atom is incorporated into the cycloalkanes making cyclic ethers, implying that hydrophobicity yielded by a cycloalkane like cyclohexane on the left side of the amide function is highly important to produce inhibition for the target human enzyme, and the presence of a polar substitution incorporated into the cycloalkane dramatically decreases inhibition potency unless it is in the correct position as shown later. When an ester group was substituted on the 4-position of the cyclohexane (compound 15), inhibition similar to that of 8 or 9 resulted.

Scheme 2. Syntheses of Methyl 3-Phenylpropylcyclohexane-1,4-dicarboxylate (15) and Its Acid Analogue (16)^a

^a (a) CH_3I , K_2CO_3 , DMF, room temp; (b) $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, 80% MeOH in H_2O , room temp; (c) 3-phenylpropylamine, EDCI, DMAP, CH_2Cl_2 , room temp; (d) 1 N NaOH, MeOH, room temp. For the synthesis of compound 17, cyclohexane-1,2-dicarboxylic acid was used instead of cyclohexane-1,4-dicarboxylic acid in (a).

However, no inhibition was observed with the corresponding acid analogue (16). Also, the presence of an ester function on the 2-position of cycloalkanes as shown in compounds 17 and 18 provided no significant inhibition for the human sEH. This result indicates that an ester group on the 4-position of hydrophobic cyclohexane is effective for producing potent inhibition, while an ester on the 2-position close to the amide pharmacophore disturbs the binding of the amide compound to the target enzyme. Compound 19 with an adamantane in the left side of the amide function exhibited a 1.5- to 2-fold increase in inhibition potency when compared to compounds with substituted cyclohexanes (8, 9, and 15), which is similar to the previous results with urea central pharmacophores.^{17–19,21} Interestingly, when the adamantane group of compound 19 was replaced by a tetrahydronaphthalene (20), a 3-fold improvement in inhibition potency was exhibited, indicating that cycloalkanes fused with an aryl group would be useful to produce highly potent cycloalkylamide inhibitors. On the other hand, a >30-fold loss in inhibition potency was obtained in phenyl substituted cycloalkyl derivatives (21–23) compared to compound 20, implying that the fused structure of tetrahydronaphthalene as shown in compound 20 is necessary to retain highly potent inhibition for the target enzyme. Overall these results indicate that relatively bulky hydrophobic cycloalkyl substitution on the left side of the amide pharmacophore is important to yield significant inhibition potency in cycloalkylamide compounds. An adamantane (19) was an effective substituent for providing potent inhibition for the human sEH with the tetrahydronaphthalene in compound 20 still more effective.

In order to further optimize the right side of the amide pharmacophore, the 3-phenylpropyl group in compound 19 or 20 was modified with alkyl, substituted alkyl, aryl, or substituted aryl groups (Figure 1 and Table 2). Replacement of the 3-phenylpropyl of compound 19 by a dodecyl group (compound 3 in Figure 1) decreased inhibition potency approximately 2.0-fold,

Scheme 3. Syntheses of Naphthaleneamide Derivatives (32–35) and *N*-Methyl-*N*-(3-phenylpropyl)-1-adamantane-carboxamide (36)^a

^a (a) RCOOH, methyl 4-(aminomethyl)benzoate hydrochloride, EDCI, DMAP, CH_2Cl_2 , room temp; (b) compound 34, CH_3I , K_2CO_3 , DMF, room temp; (c) NaH, CH_3I , DMF, room temp.

implying that the presence of an aryl on the right side of the amide function is more effective for producing better inhibition for human sEH compared to the normal alkane. We found a similar result previously that aryls present in an optimal position of the right side of urea pharmacophore often lead to better inhibition potency than alkyls on the right side of the urea function.²¹ So cycloalkylamides with an aryl group in the right side of the amide function were synthesized to further increase inhibition potency for the target enzyme (Table 2). When the methylene carbon chain length between the amide function and the benzene ring of compound 19 was then modified, at least a 5-fold decrease in inhibition was observed in compounds 24–26. No significant activity was measured in compound 24 with one methylene carbon between the two moieties. Moreover, the

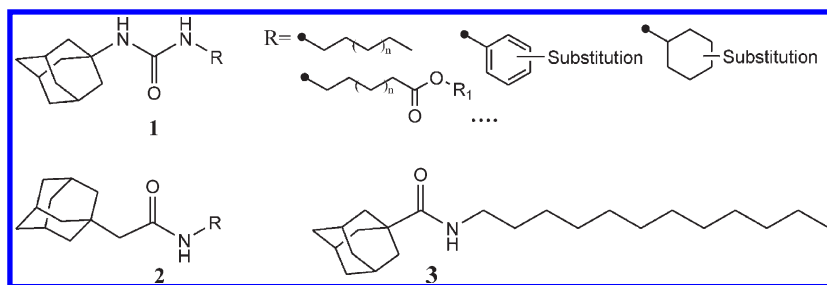


Figure 1. Structures of 1,3-disubstituted ureas (**1**) and the corresponding alkyl- (**2**) and cycloalkylamides (**3**): $n = 3-8$; $R_1 = H$, alkyl, or substituted alkyl. A variety of substituents, such as carboxylic acid, ester, or amide, can be placed on the benzene ring or cyclohexane in compound **1**. The IC_{50} of compound **3** on human sEH is 97 nM.

Table 3. Melting Point and Water Solubility of Adamantane- and Naphthaleneamide Derivatives

compd	melting point ($^{\circ}C$)	solubility in water (μM) ^a
29	152	125
32	115	125
33	151	31
34	>200	31
35	172	15
AUDA ^b	114	63

^a Water solubility was determined by adding varying concentrations of a test compound in DMSO to 0.1 M sodium phosphate buffer (pH 7.4) in a final ratio of 5:95 (v/v). The turbidity of the water solution was measured at 650 nm to determine solubility in water. Results are triplicate averages. ^b 12-(3-Adamantan-1-ylureido)dodecanoic acid, which was synthesized in the reaction of 1-adamantane isocyanate with 12-aminododecanoic acid in 1,2-dichloroethanol as previously described.²⁰ ¹H NMR δ (CDCl₃) 1.20–1.36 (16H, m), 1.42–1.48 (2H, m), 1.61 (6H, s), 1.86 (6H, s), 1.96 (3H, s), 2.18 (2H, t, $J = 6.9$ Hz), 2.90 (2H, t, $J = 6.9$ Hz), 3.45 (1H, s), 5.43 (1H, s), 5.58 (1H, s).

presence of two (**25**) or four (**26**) methylene carbons between the amide and the benzene ring reduced inhibition potency (4- to 6-fold) for the target enzyme, implying that the phenylpropyl on the right side of the amide function in compound **19** is more effective than phenethyl (**25**) or phenylbutyl (**26**) for inhibition potency. In previous reports, we indicated that introducing functional groups in specific positions in hydrophobic inhibitors is generally useful for improving physical properties such as solubility or melting point while maintaining inhibition potency on human sEH.^{18,20,21} This suggests that optimal incorporation of polar functional groups is important for increasing both potency and physical properties. Thus, a series of functionalized compounds were investigated to see if inhibition potency is affected by functionalization as observed previously. When an ester (**27**) or methoxy group (**28**) was incorporated on a benzene ring, a >10-fold better inhibition was obtained in the ethyl ester compound (**27**) in comparison with that of compound **28** with a methoxy group, indicating that an ester group is one of useful functionalities to be incorporated to improve inhibition potency for the target enzyme. Interestingly, a large improvement in inhibition was gained in adding an ester function on the benzene ring of compound **24** with poor inhibition potency (**29**). On the other hand, similar potency was observed in both compounds **25** and **30** with phenethyl on the right side of the amide function, implying that functionalization of the benzene ring is highly effective for increasing inhibition potency for the target enzyme. These results also show that the functionalized benzyl group on

the right position of the amide function is more effective than functionalized phenethyl to improve inhibition potency. Non-substituted phenethyl (**25**) provided better inhibition than nonsubstituted benzyl (**24**). Compound **29** with a benzyl linker between the amide pharmacophore and the ester on the benzene ring had approximately a 10-fold better inhibition than compound **27** with a one carbon chain shorter linker. This further supports that the presence of a benzyl linker functionalized with an ester group on the right side of the amide pharmacophore provides highly potent inhibitors for human sEH. On analogy with previous urea derivatives a variety of polar groups could be placed in this secondary pharmacophore position.^{18–21} Modifying the benzyl linker of compound **29** with a naphthalene (**31**) resulted in a 7-fold loss in inhibition potency. This suggests that the functionalized benzyl moiety is more effective than a biaryl structure for producing potent inhibitors for human sEH. Tetrahydronaphthalene (**20**), which was found to be a promising substitution for the left side of the amide function in Table 1, was then combined with the benzyl group, an effective right side substituent of the amide pharmacophore, to see if an improved inhibitor is produced. As expected, replacement of the adamantane group on the left side of the amide function of compound **29** by a tetrahydronaphthalene resulted in a highly potent inhibitor **32** (a 5-fold improvement), which is consistent with the results in Table 1. This suggests that the naphthalene structure is very useful for producing highly potent inhibitions for human sEH. As shown in compound **33** with a 2-naphthalene on the left side of the amide function, relatively potent inhibition was also obtained for the target enzyme. Compound **33**, however, was 3-fold less potent when compared to that of the tetrahydronaphthalene derivative (**32**). Interestingly, substituted 2-naphthalene derivatives with a hydroxy (**34**) or a methoxy (**35**) group exhibited an increase in inhibition potency that is similar to that of the tetrahydronaphthalene analogue (**32**). In general, polar groups on this side of the central pharmacophore dramatically reduce the potency of the resulting inhibitors. However, compound **34** in particular shows that the polar functionality present correctly on the left side of the primary pharmacophore can result in potent compounds. On the other hand, a dramatic decrease in inhibition potency was observed when the amide function was substituted with a methyl group, as seen in compound **36**. This indicates that a free NH of the amide function of cycloalkylamide compounds investigated in the present study plays an important role in inhibiting the target enzyme in the binding pocket. Analysis of the X-ray crystal structure shows that the urea central pharmacophore is bound by hydrogen bonding between the phenol groups of Tyr³⁸¹ and Tyr⁴⁶⁵ and the carbonyl of the urea while a salt bridge is formed between one of the urea NH and Asp³³³.^{19,29}

The free NH and the carbonyl of the amide pharmacophore may also be bound by the hydrogen bonding and the salt bridge in the pocket in inhibiting the enzyme.

The results in Table 2 indicate that a benzyl linker substituted with a functional group (e.g., ester) on the right side of the amide function of cycloalkylamide compounds produces potent inhibitors of the target enzyme (29), and the presence of naphthalene structures (32–35) on the left side of the amide pharmacophore improves inhibition potency. In general, lipophilicity of compounds causes limited solubility in water, which probably affects their In Vivo efficacy.^{17,21,23} In addition, the stability of the crystals of compounds, indicated by their high melting points, led to a general lack of solubility even in organic solvents. These poor physical properties result in undesirable pharmacokinetic properties and difficulty in compound formulation in either an aqueous or oil base.^{21,23} So we continuously examined the physical properties of the above potent derivatives in Table 2. As seen in Table 3, relatively high melting points (>150 °C) were measured in the aryl derivatives (33–35), while those of cycloalkylamide compounds (29 and 32) were observed in a lower range (115–125 °C). In addition, the inhibitors with an adamantane (29) or tetrahydronaphthalene (32) group on the left side of amide pharmacophore showed higher solubility (4- to 8-fold) than the corresponding aryl derivatives (33, 34), suggesting that a cycloalkyl substituent is better than an aryl group in producing improved physical properties. When compared to that of 12-(3-adamantan-1-ylureido)dodecanoic acid (AUDA), a representative urea inhibitor used for sEH related biological experiments in vitro and In Vivo, a 2-fold better solubility was observed in compounds 29 and 32, indicating that the cycloalkylamide structure is highly promising for developing potent inhibitors with improved physical properties and bioavailability.

CONCLUSIONS

This work focused on investigating structure–activity relationships of cycloalkylamide derivatives as human sEH inhibitors, which provide an important base for developing valuable compounds that are not only highly potent inhibitors but show improved physical properties. First, these data indicate that sEH inhibitors with an cycloalkylamide central pharmacophore can be of the same order of potency as the best inhibitors with a urea central pharmacophore. The inhibition studies indicated that at least six-membered cycloalkyl groups on the left side of the amide function are necessary to produce reasonable inhibition potency for the target human enzyme (6 and 7). In compounds substituted with a smaller cycloalkyl group such as cyclopropane (4) or cyclopentane (5) on the left side of the amide pharmacophore no inhibition of the target enzyme was observed. In addition, direct incorporation of a polar group such as hydroxyl (10) or carbonyl (11) on the cycloalkane induced a dramatic decrease in inhibition potency. On the other hand, a hydrophobic (8 and 9) or an ester group (15) added to the cycloalkane made inhibitors with improved inhibition potency. Furthermore, a sterically hindered cycloalkane (e.g., adamantane, 19) present on the left side of the amide function was useful for enhancing inhibition potency. We found that a tetrahydronaphthalene (20) is the most promising left side substituent for cycloalkylamide derivatives. When a substituted benzyl linker with an ester group was present on the right side of the amide pharmacophore, highly enhanced inhibition potency for the target enzyme was obtained (29 and 37). In nonfunctionalized derivatives (19, 24, 25, and

26), a >5-fold decreased potency was yielded, suggesting that the presence of a functional group, such as an ester, on the right side of the amide pharmacophore is important to produce highly potent inhibition for human sEH in cycloalkylamide derivatives. Moreover, modification of the benzyl linker of compound 29 by ethylphenyl (30) or biaryl (31) induced a decrease in inhibition potency at least >7-fold, demonstrating that a benzyl group as a linker is important for making potent inhibitors in cycloalkylamide compounds. In a combined structure (32) with the optimized left and right units of the amide function in Tables 1 and 2 further improved inhibition was obtained while having improved physical properties. In general, either low water solubility or high melting point can be addressed with careful formulation. However, low water solubility and high melting point are hard to address by formulation unless a compound is highly potent. Amides are generally more soluble in a variety of reagents used for formulation and are generally lower melting than similar ureas. The results obtained from the present study will be the basis for the design of specific and selective sEH inhibitors containing cycloalkylamide pharmacophores. Furthermore, the results will be useful for the design of intravenous or orally available therapeutic agents for hypertension, vascular and renal inflammation, and other disorders that can be addressed by changing the In Vivo concentration of chemical mediators that contain an epoxide.

EXPERIMENTAL SECTION

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Purity and characterization of compounds were established by a combination of elemental analysis, TLC, LC–MS, melting point, and NMR analytical techniques described below. All melting points were determined with a Stuart SMP3 apparatus (A. H. Thomas Co.) and are uncorrected. ¹H NMR spectra were recorded on a Digital Avance 400 MHz spectrometer (Bruker Analytik GmbH), using tetramethylsilane as an internal standard. Mass spectra were measured by LC–MS/MS (EsquireHCT, Bruker Daltonics) using positive mode electrospray ionization. Elemental analyses (C, H, N) were performed for seven novel key compounds by Central Lab of Mokpo National University, Korea. Thin-layer chromatography (TLC) was performed on EMD precoated silica gel 60 F254 plates, and spots were visualized with UV light and stained with basic KMnO₄. The purity of all final compounds was determined to be greater than 95% unless otherwise indicated. Synthetic methods are described for representative compounds.

Synthesis of 4-Oxo-N-(3-phenylpropyl)cyclohexanecarboxamide (11). To a solution of ethyl 4-oxocyclohexanecarboxylate (2.0 g, 11.7 mmol) in ethanol (5 mL) was added an aqueous solution of 1 N NaOH (15 mL) at room temperature. After being stirred overnight, the reaction mixture was acidified with an aqueous solution of 1 N HCl to pH 2. The water solution was extracted with ethyl acetate (60 mL × 3) and dichloromethane (60 mL), respectively. The combined organic solution was washed with water (60 mL × 2), dried over MgSO₄, and evaporated to dryness. To the residue (0.89 g, 6.25 mmol) in dichloromethane (50 mL) was added 4-dimethylaminopyridine (DMAP; 0.76 g, 6.25 mmol) and 3-phenylpropylamine (0.93 g, 6.88 mmol) at room temperature. After the mixture was stirred for 5 min, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI; 1.31 g, 6.88 mmol) was added portionwise to the reaction mixture. Extraction and purification of the product were performed with the same method used for the preparation of compound 32 to afford 11 as a solid (85%). ¹H NMR δ (CDCl₃): 1.80–1.98 (4H, m), 2.05–2.17 (2H, m), 2.28–2.57 (5H, m), 2.63 (2H, t, J = 6.8 Hz), 3.32 (2H, t, J = 6.8 Hz), 5.56 (1H, s),

7.15–7.20 (3H, m), 7.26–7.30 (2H, m). LC–MS (ESI) m/z calcd for $C_{16}H_{21}NO_2$ $[M + H]^+$ 260.16, found $[M + H]^+$ 260.52. Mp 70 °C.

Compound **14** was synthesized with the same method used for the preparation of compound **11** using methyl tetrahydro-2H-pyran-4-carboxylate instead of ethyl 4-oxocyclohexanecarboxylate.

Synthesis of Methyl 4-(3-Phenylpropylcarbamoyl)cyclohexanecarboxylate (15). A mixture of 1,4-cyclohexanedicarboxylic acid (2.0 g, 11.6 mmol), potassium carbonate (4.82 g, 34.8 mmol), and iodomethane (4.95 g, 34.8 mmol) in DMF (10 mL) was stirred overnight at room temperature. The product was extracted with diethyl ether (60 mL). The organic solution was washed with water (60 mL \times 2), dried over $MgSO_4$, and evaporated. The residue was washed with cold hexane (20 mL) and concentrated to yield the corresponding diethyl ester (100%).

A mixture of the above diester (1.77 g, 8.84 mmol) and barium hydroxide octahydrate (1.39 g, 4.42 mmol) in 80% aqueous methanol (30 mL) was stirred overnight at room temperature. Then the reaction mixture was diluted with water (50 mL) and washed with hexane (50 mL \times 2) to remove remaining diester. The aqueous layer was acidified with concentrated HCl to pH 2, and the product was isolated by extracting with dichloromethane (50 mL). This organic layer was washed with water (50 mL), dried over $MgSO_4$, and evaporated to give 4-(methoxycarbonyl)cyclohexanecarboxylic acid (65%). Without further purification the residue was used for the next reaction.

To a solution of the above monoacid (0.89 g, 4.78 mmol), DMAP (0.58 g, 4.78 mmol), and 3-phenylpropylamine (0.64 g, 5.25 mmol) in dichloromethane (30 mL) was added EDCI (1.0 g, 5.25 mmol) at room temperature. The reaction mixture was then stirred for 4 h at room temperature. The product was extracted and purified with the same method used for the synthesis of compound **32** to yield **15** as a solid (90%). 1H NMR δ ($CDCl_3$): 1.52–1.68 (5H, m), 1.81 (2H, quint, $J = 6.8$ Hz), 2.03–2.16 (4H, m), 2.52–2.57 (1H, m), 2.62 (2H, t, $J = 6.8$ Hz), 3.23 (2H, t, $J = 6.8$ Hz), 3.64 (3H, s), 5.92 (1H, s), 7.15–7.20 (3H, m), 7.26–7.30 (2H, m). LC–MS (ESI) m/z calcd for $C_{18}H_{25}NO_3$ $[M + H]^+$ 304.18, found $[M + H]^+$ 304.53. Mp 63 °C.

Compound **17** was synthesized in the same manner used for the preparation of compound **15** using cyclohexane-1,2-dicarboxylic acid instead of cyclohexane-1,4-dicarboxylic acid.

Synthesis of 4-(3-Phenylpropylcarbamoyl)cyclohexanecarboxylic Acid (16). To a solution of compound **15** (0.20 g, 6.59 mmol) in methanol (5 mL) was added an aqueous solution of 1 N NaOH (3 mL). After being stirred overnight at room temperature, the reaction mixture was acidified by adding an aqueous solution of 1 N HCl to pH 2. The product was extracted with ethyl acetate (50 mL \times 2). The organic layer was washed with water (50 mL), dried over $MgSO_4$, and evaporated. The residue was recrystallized in ethyl acetate to afford **16** as a solid in 95% yield. 1H NMR δ ($CDCl_3$): 1.44–1.59 (2H, m), 1.67–1.73 (3H, m), 1.78–1.86 (3H, m), 2.06–2.19 (3H, m), 2.59–2.68 (3H, m), 3.28 (2H, t, $J = 6.8$ Hz), 5.44 (1H, s), 7.16–7.21 (3H, m), 7.26–7.31 (2H, m). LC–MS (ESI) m/z calcd for $C_{17}H_{23}NO_3$ $[M + H]^+$ 290.17, found $[M + H]^+$ 290.57. Mp 127 °C.

Synthesis of Methyl 6-(1-Adamantanecarboxamido)naphthalene-2-carboxylate (31). 6-(1-Adamantanecarboxamido)naphthalene-2-carboxylic acid intermediate was prepared with the same method used for the synthesis of compound **32** using the corresponding 1-adamantanecarboxylic acid (0.50 g, 2.77 mmol) and 6-aminonaphthalene-2-carboxylic acid (0.52 g, 2.77 mmol) in 60% yield. To a mixture of the acid intermediate and potassium carbonate (1.0 g, 6.93 mmol) was added iodomethane (1.0 g, 6.93 mmol) in DMF (25 mL) at room temperature. After the mixture was stirred overnight, the product was extracted with diethyl ether (60 mL). The organic solution was washed with water (60 mL) twice, dried over $MgSO_4$, and evaporated. The residue was purified using silica gel column chromatography (hexane/ethyl acetate = 3:1) to give **31** as a solid (95%). 1H

NMR δ ($CDCl_3$): 1.77 (6H, s), 2.01 (6H, s), 2.13 (3H, s), 3.97 (3H, s), 7.48–7.50 (2H, m), 7.80 (1H, d, $J = 6.8$ Hz), 7.89 (1H, d, $J = 6.8$ Hz), 8.01 (1H, d, $J = 6.8$ Hz), 8.36 (1H, s), 8.53 (1H, s). LC–MS (ESI) m/z calcd for $C_{23}H_{25}NO_3$ $[M + H]^+$ 364.18, found $[M + H]^+$ 364.77. Mp >200 °C.

Compound **30** was synthesized with the same procedure used for the preparation of **31** using the corresponding cycloalkanecarboxylic acid, alkylamine, and iodomethane.

Synthesis of Methyl 4-((1,2,3,4-Tetrahydronaphthalene-2-carboxamido)methyl)benzoate (32). To a solution of 1,2,3,4-tetrahydronaphthalenecarboxylic acid (0.4 g, 2.26 mmol) in dichloromethane (30 mL) was added DMAP (0.28 g, 2.26 mmol) and methyl 4-(aminomethyl)benzoate hydrochloride (0.46 g, 2.26 mmol) at room temperature. To this mixture was then added portionwise EDCI (0.44 g, 2.26 mmol) at room temperature. After the mixture was stirred for 4 h, the product was extracted with diethyl ether (50 mL) twice. The ether solution was washed with an aqueous solution of 0.5 N HCl (50 mL) and water (80 mL), dried over $MgSO_4$, and evaporated. The residue was recrystallized in hexane to afford compound **32** as a solid in 95% yield. 1H NMR δ ($CDCl_3$): 1.93–1.94 (1H, m), 2.09–2.10 (1H, m), 2.55–2.57 (1H, m), 2.82–3.04 (4H, m), 3.90 (3H, s), 4.50 (2H, s), 6.12 (1H, s), 7.06–7.12 (4H, m), 7.30 (2H, d, $J = 6.8$ Hz), 7.97 (2H, d, $J = 6.8$ Hz). LC–MS (ESI) m/z calcd for $C_{20}H_{21}NO_3$ $[M + H]^+$ 324.15, found $[M + H]^+$ 324.54. Mp 115 °C.

Compounds **3–14**, **18–29**, and **33–35** were synthesized with the same procedure used for the preparation of **32** using the corresponding cycloalkanecarboxylic acid and alkyl- or arylamine instead of 1,2,3,4-tetrahydronaphthalenecarboxylic acid and methyl 4-(aminomethyl)benzoate hydrochloride.

Synthesis of N-Methyl-N-(3-phenylpropyl)-1-adamantanecarboxamide (36). To a suspension of sodium hydride 60% (60 mg, 1.61 mmol) in DMF (10 mL) was added compound **19** (0.4 g, 1.34 mmol) at room temperature. After the mixture was stirred for 5 min, iodomethane (0.23 g, 1.61 mmol) was added to the reaction mixture at the same temperature. The product was extracted with diethyl ether (50 mL) after stirring overnight. The ether solution was washed with water (50 mL) twice, dried over $MgSO_4$, and evaporated. The residue was purified by using column chromatography (hexane/ethyl acetate = 5:1) to give compound **36** as an oil. 1H NMR δ ($CDCl_3$): 1.69 (6H, s), 1.88 (2H, t, $J = 6.8$ Hz), 1.93 (6H, s), 1.97 (3H, s), 2.61 (2H, t, $J = 6.8$ Hz), 3.02 (3H, s), 3.41 (2H, t, $J = 6.8$ Hz), 7.17–7.19 (3H, m), 7.26–7.29 (2H, m). LC–MS (ESI) m/z calcd for $C_{21}H_{29}NO$ $[M + H]^+$ 312.21, found $[M + H]^+$ 312.34.

Enzyme Preparation. Recombinant human sEH was produced in baculovirus expression system as previously reported.³⁰ Briefly, Sf9 insect cells were infected by recombinant baculovirus harboring human sEH gene fused with a 6xHis tag. At 72 h postinfection, the infected cells were homogenized and the recombinant protein was purified by immobilized metal affinity chromatography. After treatment with tobacco etch virus protease to remove the 6xHis tag, human sEH was further purified by anion-exchange chromatography.

IC₅₀ Assay Conditions. Standard solutions of compounds in Figure 1 and Tables 1 and 2 were prepared in DMSO. Fluorescent assays were performed by using a substrate (cyano(2-methoxynaphthalen-6-yl)methyl *trans*-(3-phenyloxiran-2-yl)methylcarbonate, CMNPC) to determine IC₅₀ values of the human sEH inhibitors according to a published protocol³¹ except for the final enzyme concentration (1 nM).^{30,32} Activity was measured by determining the appearance of the 6-methoxy-2-naphthaldehyde with an excitation wavelength of 330 nm and an emission wavelength of 465 nm for 10 min on a fluorometer (Victor3, PerkinElmer).^{30,32} The IC₅₀ values were determined by regression of at least six data points with a minimum of three points in a linear region of the curve. IC₅₀ results are averages of three separate measurements.

Solubility. Water solubility of compounds in Table 3 was determined experimentally by light scattering method in sodium phosphate buffer at 25 ± 1.5 °C. In brief, aqueous solubility was determined by adding varying concentrations of inhibitor in DMSO to 0.1 M sodium phosphate buffer (pH 7.4) in a final ratio of 5:95 (v/v). Insolubility of the inhibitor was indicated by the increase in turbidity of the water solution. The turbidity was measured as optical density at 650 nm on a SH-8000 microplate reader (Corona Electric, Ibaraki, Japan) at 25 ± 1.5 °C. Results are averages of three separate measurements.

■ ASSOCIATED CONTENT

S Supporting Information. Analytical data for compounds 3–10, 12–14, 17–30, and 33–35; table of elemental analyses for compounds 19, 20, 29, 32, and 33–35. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Phone/fax: +81-89-946-9818. E-mail: knishi@agr.ehime-u.ac.jp.

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■ ABBREVIATIONS USED

EET, epoxyeicosatrienoic acid; sEH, soluble epoxide hydrolase; EDCl, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide; DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; CM-NPC, cyano(2-methoxynaphthalen-6-yl)methyl *trans*-(3-phenyloxiran-2-yl)methylcarbonate; AUDA, 12-(3-adamantan-1-ylureido)dodecanoic acid

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