

UC Davis

UC Davis Previously Published Works

Title

Structure-activity relationships of substituted oxyoxalamides as inhibitors of the human soluble epoxide hydrolase

Permalink

<https://escholarship.org/uc/item/1pz5w78z>

Journal

Bioorganic & Medicinal Chemistry, 22(3)

ISSN

0968-0896

Authors

Kim, In-Hae
Lee, In-Hee
Nishiwaki, Hisashi
et al.

Publication Date

2014-02-01

DOI

10.1016/j.bmc.2013.12.027

Peer reviewed

Published in final edited form as:

Bioorg Med Chem. 2014 February 1; 22(3): 1163–1175. doi:10.1016/j.bmc.2013.12.027.

Structure–activity relationships of substituted oxyoxalamides as inhibitors of the human soluble epoxide hydrolase

In-Hae Kim^a, In-Hee Lee^b, Hisashi Nishiwaki^a, Bruce D. Hammock^c, and Kosuke Nishi^{a,*}

^aDepartment of Applied Bioscience, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan

^bDepartment of Medicinal Chemistry, Hyundai Pharm Co., Ltd, Suwon, Gyonggi 443-270, Republic of Korea

^cDepartment of Entomology & UCD Comprehensive Cancer Center, University of California Davis, One Shields Ave, Davis, CA 95616, USA

Abstract

We explored both structure–activity relationships among substituted oxyoxalamides used as the primary pharmacophore of inhibitors of the human sEH and as a secondary pharmacophore to improve water solubility of inhibitors. When the oxyoxalamide function was modified with a variety of alkyls or substituted alkyls, compound **6** with a 2-adamantyl group and a benzyl group was found to be a potent sEH inhibitor, suggesting that the substituted oxyoxalamide function is a promising primary pharmacophore for the human sEH, and compound **6** can be a novel lead structure for the development of further improved oxyoxalamide or other related derivatives. In addition, introduction of substituted oxyoxalamide to inhibitors with an amide or urea primary pharmacophore produced significant improvements in inhibition potency and water solubility. In particular, the *N,N,O*-trimethyloxyoxalamide group in amide or urea inhibitors (**26** and **31**) was most effective among those tested for both inhibition and solubility. The results indicate that substituted oxyoxalamide function incorporated into amide or urea inhibitors is a useful secondary pharmacophore, and the resulting structures will be an important basis for the development of bioavailable sEH inhibitors.

Keywords

Substituted oxyoxalamides; Human soluble epoxide hydrolase; Inhibitors

1. Introduction

Arachidonic acid, a ω -6 polyunsaturated fatty acid, plays important roles in cellular signaling as a lipid second messenger and is also a precursor in the production of oxidative

© 2013 Elsevier Ltd. All rights reserved.

*Corresponding author: Tel./fax: +81 89 946 9818. knishi@agr.ehime-u.ac.jp (K. Nishi).

Supplementary data

Supplementary data (¹H NMR of compounds **6**, **26**, and **31**, LC–MS analyses, preparation of human sEH, and inhibition assays) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2013.12.027>.

metabolites known as eicosanoids by enzymes such as cyclooxygenase, lipoxygenase, and cytochrome P450. Prostanoids^{1,2} and leukotrienes³ are major metabolic products of arachidonic acid by cyclooxygenase and lipoxygenase, respectively. These two pathways are largely inflammatory and induce inflammation, pain, and asthma,¹⁻³ making the both enzymes current pharmaceutical targets for relief from the symptoms. The third branch of arachidonic acid cascade involves oxidation by cytochrome P450 to produce several inflammatory hydroxylated metabolites and the corresponding lipid epoxides formed at the olefinic centers and known as epoxyeicosatrienoic acids (EETs). EETs have been reported as a new class of lipid mediators with important biological functions.⁴ The endogenous epoxy lipids, EETs, influence blood pressure by modulating cardiac output, vascular resistance, and urinary composition.⁵⁻¹¹ In addition, vascular inflammation and pain are modulated by the action of EETs.^{9,10} However, the metabolism of the epoxy functionality of the EETs to the *vicinal* diols by soluble epoxide hydrolase (sEH) dramatically diminishes the biological activities.⁶ Many reports have shown that the treatment of potent human sEH inhibitors increases EET levels and reduces blood pressure and inflammatory responses in *in vitro* and *in vivo* experimental models,⁵⁻¹¹ suggesting that human sEH is a promising pharmacological target for the treatment of cardiovascular and other diseases.

A number of urea compounds with a variety of substituents are highly potent inhibitors of the human sEH.¹²⁻²¹ The best optimization of urea derivatives affords specific inhibition potency for the target enzyme in a range of less than 1 nM. Structure–activity relationship studies indicate that a carbonyl group and a single proton donating NH group of urea function are essential for making it an effective primary pharmacophore to inhibit the enzyme activity. Functionalities such as amides and carbamates with both a carbonyl group and an NH group are, therefore, known to produce potent inhibition as a primary pharmacophore, while ester or carbonate functions without a proton donating NH group yield no inhibition for the target enzyme.^{12,22-24} Many of these compounds are difficult to formulate because they are high melting lipophilic solids. These formulation problems can be solved by reducing the melting point and crystal stability, increasing water solubility, and increasing potency. On the other hand, when a variety of functionalities including amides, esters, ketones, and ethers are incorporated as a secondary pharmacophore remote from the catalytic site in potent urea inhibitors, dramatic changes in inhibition potency are not observed, rather significant improvement in physical properties is often obtained,¹² implying that primary inhibition of the human sEH depends on the structure of primary pharmacophores and secondary pharmacophores are useful for improving physical properties and potency. In the present study, we first investigated replacement of the primary pharmacophore with a series of substituted oxyxalamides and then used oxyxalamides as a second series to replace the secondary pharmacophore using the classical amide and urea primary pharmacophores. In both series, potent compounds were found with improved water solubility.

2. Results and discussion

2.1. Chemistry

Substituted oxyoxalamide derivatives (**3–15**) and *N*-(benzyloxy)-2-(adamant-2-ylamino)acetamide (**16**) in Tables 1 and 2 were synthesized as outlined in Scheme 1. Ethyl (chlorocarbonyl)formate was reacted with an alkyl- or a cycloalkyl-amine (Scheme 1A) or adamant-2-ylamine (Scheme 1B) in dichloromethane, followed by hydrolysis with 1 N NaOH in ethanol to provide the corresponding (carbamoyl)formic acid in approximately 80–95% yield. The formic acid was then coupled with benzyloxyamine (Scheme 1A) or with a substituted oxyamine (Scheme 1B) using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP) in dichloromethane to yield compounds **3–12**, **14**, and **15** in 50–85% yield.²³ As seen in Scheme 1B, substituted oxyamines for compounds **10–12** were prepared by the reaction of a corresponding bromide with *N*-hydroxyphthalimide in *N,N*-dimethylformamide (DMF), which was followed by the addition of hydrazine in trichloromethane (40–50%). *N*-Methylation of compound **6** with iodomethane (CH₃I) in the presence of potassium carbonate (K₂CO₃) in DMF afforded compound **13** in 55% yield (Scheme 1C). Substitution of methyl 2-bromoacetate with adamant-2-ylamine in dichloromethane and hydrolysis of the ester with 1 N NaOH in methanol gave the corresponding adamantylaminoacetic acid in approximately 85% yield. Then, coupling of this acid with benzyloxyamine using EDCI/DMAP in dichloromethane produced compound **16** in 70% yield (Scheme 1D).

Amide-oxyoxalamide derivatives **18–26** in Table 3 were synthesized by the procedure outlined in Scheme 2. Coupling reaction of 4-aminobenzylamine with adamant-1-ylcarboxylic acid (for compounds **18–20** and **22**) or with adamant-1-ylmethylcarboxylic acid (for compound **23**) using EDCI/DMAP in dichloromethane afforded an amide-amine intermediate, which was used for the reaction with ethyl (chlorocarbonyl)formate in the presence of triethylamine (Et₃N) in dichloromethane to make substituted carbonylformate in about 60% yield. Hydrolysis of the ester function of this intermediate with 1 N NaOH in ethanol gave the corresponding (carbamoyl)formic acid, which was further reacted with a substituted oxyamine in the presence of EDCI/DMAP coupling reagents in dichloromethane to produce compounds **18–20**, **22**, and **23** in a range of 50–90% yield (Scheme 2A). As depicted in Scheme 2B, coupling of adamant-1-ylmethylcarboxylic acid with mono-*N*-protected 4-aminoaniline using EDCI/DMAP and following *N*-de-protection using 4 N HCl provided the corresponding amide-amine intermediate in about 80% yield. This amide-amine was reacted with ethyl (chlorocarbonyl)formate in dichloromethane to afford the corresponding formate, which was hydrolyzed with 1 N NaOH in ethanol to give a formic acid intermediate in 85% yield. Coupling of the acid function with methoxymethylamine using EDCI/DMAP yielded compound **25** in 70% yield. In addition, compounds **21**, **24**, and **26** were synthesized by the alkylation of compounds **20**, **23**, **25**, respectively, with CH₃I in the presence of K₂CO₃ as a base in DMF in approximately 60% yield (Scheme 2C).

Urea compounds with substituted oxyoxalamide were synthesized as outlined in Scheme 3. The reaction of 4-aminobenzylamine with adamant-1-yl isocyanate in DMF gave 1-adamantyl-3-(4-aminobenzyl) urea in 100% yield.¹³ Compounds **28**, **29**, **30**, and **32** were

produced by the above reactions used for the syntheses of compounds **18**, **19**, **20**, and **22** from this urea-amine intermediate in a range of 40–70% yield (Scheme 3A). N-Methylation of compound **30** using CH₃I and K₂CO₃ as a base in DMF yielded compound **31** in 50% yield (Scheme 3B).

2.2. Structure–activity relationships

In order to first investigate whether the oxyoxalamide function can act as an effective primary pharmacophore to inhibit the human sEH, a series of substituted oxyoxalamides were synthesized. Our previous studies show that potent inhibition of the target enzyme is obtained with the substitution of relatively hydrophobic alkyl or cycloalkyl groups and substituted alkyl or aromatic groups on the left side and the right side of urea or amide pharmacophores, respectively (**1** and **2** in Fig. 1).^{12,14,15,21,23} Thus, hydrophobic alkyls and substituted alkyl or aryl groups were incorporated in the both sides of the diketo of oxyoxalamide moiety, and inhibition potency of the oxyoxalamide derivatives for the human sEH was evaluated (Table 1 and Fig. 2A). As seen in compounds **3** and **4**, 3-phenylpropyl (**3**) and 1-adamantyl (**4**) groups in the left side of the diketo, which are useful for making potent inhibitors with urea or amide functions, were not effective for producing inhibition activity for the target enzyme with oxyoxalamide function. However, in replacing the substituent in the left side of the diketo of compounds **3** and **4** by an adamantylmethyl group (**5**), moderate inhibition was gained. Moreover, interestingly, introduction of a 2-adamantyl group (**6**) in the left side of the diketo moiety afforded a high improvement (16-fold) in inhibition potency compared to that of compound **5**. Comparing compound **6** with 2-adamantane to compound **4** with 1-adamantane, improvement in inhibition activity was dramatic, indicating that the 2-adamantyl group is particularly suitable for being the left side substituent of oxyoxalamide function to yield significant inhibition potency. It is also implied that slight changes in orientation or size of the alkyl groups in the left side of oxyoxalamide function as shown in compounds **3–6** result in a large variation in inhibition for the target enzyme, which is different from the results that similarly potent inhibition is observed in urea or amide derivatives substituted with the alkyl groups.^{14,15,22,23}

Based on the above results, the 2-adamantyl was fixed on the left side of the diketo moiety of the oxyoxalamide, and then a benzyl group in the right side of the diketo of compound **6** was further modified with phenyl and several arylalkyl groups. Because aryl containing groups in the right side of urea or amide pharmacophores (e.g., **2** in Fig. 1) provide much higher binding activity than aliphatic alkyl groups,^{15,21,23} compounds with aryl substituent (**7–13**) were synthesized. As seen in compound **7**, replacement of the benzyl group of compound **6** by a phenyl group resulted in no inhibition. This implies that the methylene benzyl carbon in the right side of the oxyoxalamide is necessary for producing inhibition of the target enzyme. When a functional group such as a methyl ester (**8**), nitro (**9**), or chloro (**10**) was incorporated on the 4-position of the benzyl group of compound **6**, >300-fold drop in inhibition was also observed, indicating that these substituents on the benzyl group of compound **6** are not effective for increasing inhibition potency. These results are not consistent with previous observations with urea or amide primary pharmacophores.^{21,23} Because we previously showed that a longer alkyl chain like an ethyl or a propyl between primary amide pharmacophore and benzene ring in the right side of the amide function

provides improved inhibition for the target enzyme,²³ 2-phenylethyl and 3-phenylpropyl were introduced instead of the benzyl group of compound **6**. As seen in compounds **11** and **12**, a propyl chain (**12**) resulted in approximately 8-fold better inhibition than an ethyl chain (**11**). However, this still gave approximately 10-fold drop in inhibition when compared to the potency of compound **6**, supporting that one methylene carbon of the benzyl group of compound **6** is important for yielding significant inhibition potency. N-Substitution of compound **6** with a methyl group (**13**) dropped inhibition potency approximately 60-fold. Inhibition was further lost in the presence of a dimethyl or a polar substituent as observed in compounds **14** and **15**, respectively, indicating that a benzyl group next to an unsubstituted NH function is effective as a right side substituent for yielding promising inhibitors with an oxyoxalamide function as a primary pharmacophore. Furthermore, when one carbonyl group of compound **6** was deleted as seen in compound **16** in Table 2, no inhibition was observed, suggesting that the carbonyl group of oxyoxalamide function plays an important role in inhibiting the target human enzyme like that of urea or amide functions.¹² The inhibition potency of compound **6** was approximately 15-fold lower when compared to that of a very potent urea-based inhibitor (AUDA),¹³ indicating that the oxyoxalamide **6** can be used as a new lead structure for structure-activity relationship studies to further produce as potent oxyoxalamide inhibitors as urea-based compounds. Overall, the results in Tables 1 and 2 show that the substituted oxyoxalamides are promising novel functionalities as primary pharmacophores to yield inhibitors of significant potency for the human sEH.

A functional group incorporated on around the 5th to 7th atom from the carbonyl of primary pharmacophores such as ureas or amides can play a role as a secondary pharmacophore to modify inhibition potency and/or physical properties of urea or amide inhibitors (Fig. 2B).^{12,14,22,23} In general, functions such as amides, esters, ketones, ethers, sulfonamides, sulfoxides, and sulfones are useful secondary pharmacophores for improving physical properties without decreasing inhibition potency of urea inhibitors.^{12,14,23} In order to investigate whether the oxyoxalamide function can act as an effective secondary pharmacophore for improving inhibition potency and/or physical properties, a series of amide and urea derivatives with substituted oxyoxalamide groups were synthesized as shown in Tables 3 and 4. We previously showed that incorporation of a functional group like an ester as a secondary pharmacophore on a benzene ring in the right side of amide inhibitors like compound **17** in Table 3 is highly useful for enhancing inhibition potency for the target enzyme (**2** in Table 2).²³ Thus, the oxyoxalamide function was introduced to compound **17** to see if it works as an effective secondary pharmacophore for improving inhibition potency or the physical properties of amide inhibitor. When an oxyoxalamide group was substituted on the 4-position of the benzene ring of compound **17** as seen in compound **18**, at least a 3-fold enhancement in inhibition was gained, indicating that the oxyoxalamide function is also a useful secondary pharmacophores for improving inhibitory potency of amide compounds. Because a polar functional group like an oxyoxalamide inserted as a secondary pharmacophore plays an important role in improving physical properties (e.g. water solubility) of hydrophobic inhibitors as well, oxyoxalamides with relatively less hydrophobic groups were incorporated on the amide inhibitors. As seen in compound **19**, substitution of a *t*-butyl group on the oxygen atom of the oxyoxalamide instead of a methyl group of compound **18** lowered inhibition a 1.5-fold, showing that a

smaller alkyl group on the oxygen atom is better for inhibition. In addition, oxyoxalamides with *N,O*-dimethyl (**20**) or *N,N,O*-trimethyl (**21**) yielded a further improved potency (1.5-fold) compared to that of *O*-methyl derivative (**18**), implying that the N-substitution of the oxyoxalamide is useful for enhancing inhibition for the target enzyme. On the other hand, an oxyoxalamide with a polar group on the oxygen atom (**22**) led to a complete loss in inhibition, suggesting that non-polar small alkyl groups such as a methyl group are optimal substituents for the oxyoxalamide function to produce significant inhibition potency. Interestingly, when the adamantyl group in the left side of the amide primary pharmacophore of compounds **20** and **21** was modified by an adamantylmethyl group (**23** and **24**), inhibition potency was enhanced up to 25-fold. In addition, replacement of the benzyl group in the right side of the amide function of compounds **23** and **24** with a phenyl group (**25** and **26**) improved inhibition potency approximately 2-fold. This is approximately 50-fold enhanced inhibition compared to that of compound **21**, indicating that optimizing around the amide pharmacophore is a useful approach for yielding potent amideoxyoxalamide inhibitors. Production of highly potent amideoxyoxalamide inhibitors depends on the structure of the primary pharmacophore more than that of secondary pharmacophore. It also was found that N-methylation of the both nitrogen atoms of the oxyoxalamide is especially important for affording potent amide-oxyoxalamide derivatives as shown by comparing compound **24** to **23**. Compound **26** with *N,N,O*-trimethylated oxyoxalamide had an 8-fold better inhibition than compound **25** with *N,O*-dimethylated oxyoxalamide, again showing the effectiveness of N-substitution of the both nitrogen atoms of the oxyoxalamide for the production of further improved amide-oxyoxalamide inhibitors. When water solubility of the potent compounds (**23–26**) was measured, a 2–15-fold enhancement was obtained in comparison with that of the amide inhibitor with no oxyoxalamide function (**17**). Furthermore, the solubility was comparable to those of previously reported soluble amide-(**2**)²³ and urea-based (**IK950**)¹⁴ inhibitors. It was also found that inhibitions obtained from compounds **24** and **26** were similar to those of potent inhibitors **2** and **IK950**. This implies that the incorporation of the substituted oxyoxalamide function as a secondary pharmacophore is effective for not only improving inhibition potency, but also significantly increasing water solubility of amide compounds. Comparing compound **24** to **23**, it was found that N-methylation of the both nitrogen atoms of the oxyoxalamide function provides better water solubility. A similar result was also observed between compounds **25** and **26**, suggesting that the trimethylated oxyoxalamide is especially effective for improving solubility in water.

The results in Table 3 show that attachment of a substituted oxyoxalamide as a secondary pharmacophore to amide inhibitors results in significant enhancements in inhibition potency and water solubility. Next, in order to see if the incorporation of the oxyoxalamide function to urea inhibitors affects their inhibition and water solubility, several urea-oxyoxalamide derivatives were synthesized as shown in Table 4 and Fig. 2B. As seen in compound **28**, an *O*-methyl-oxyoxalamide substitution resulted in a 2.5-fold better inhibitor when incorporated to urea compound **27**, indicating that the oxyoxalamide function is useful for further improving inhibition potency of potent urea compounds. On the other hand, *O-t*-butyl-oxyoxalamide (**29**) did not provide an increase in inhibition, implying that a smaller alkyl group like a methyl on the oxygen atom is better than a relatively bulky *t*-butyl group

for yielding improved urea inhibitors, which is similar to that observed in the corresponding amide derivatives **18** and **19** in Table 3. *N,O*-Dimethyl (**30**) and *N,N,O*-trimethyl (**31**) oxyoxalamides made a 3-fold and a 13-fold improvements in inhibition, respectively, in comparison with that of compound **27**. Interestingly, N-methylation of the both nitrogen atoms of the oxyoxalamide function was also found to be important for yielding highly potent inhibition in urea-oxyoxalamide derivatives as seen in compound **31**, which is the same result as that observed in the corresponding amide derivative (**26**). On the other hand, a polar substitution in the oxyoxalamide (**32**) was not effective for making an improved urea inhibitor, which is also similar to that gained with amide derivative **22** in Table 3. Furthermore, approximately 2–30-fold enhancement in water solubility was gained from the substituted urea compounds (**28–32**) when compared to that of compound **27**, making the substituted oxyoxalamide function an effective secondary pharmacophore for improving both inhibition and solubility. Among the derivatives tested, the most potent compound **31** with *N,N,O*-trimethyl-oxyoxalamide was most soluble in water, which is a 10-fold higher solubility than that of a urea inhibitor, AUDA. Moreover, the solubility of compound **31** was same as that of **IK950** known as a soluble urea inhibitor useful for various in vivo studies. In addition, a 3–12-fold enhancement in inhibition was observed in compound **31** when compared to those of AUDA and **IK950**. These results suggest that N-methylation of the both nitrogen atoms of the oxyoxalamide function is highly effective for both of inhibition and solubility of urea compounds as well, as that observed in the corresponding amide derivatives **24** and **26**.

3. Conclusions

This study investigated whether the oxyoxalamide function works as an effective primary and/or secondary pharmacophore to inhibit the human sEH. In order to first see its potential to be a primary pharmacophore, a series of oxyoxalamides substituted with alkyl, cycloalkyl, aryl, or substituted aryl groups were synthesized (Table 1 and Fig. 2A). The inhibition results indicated that a 2-adamantyl group (**6**) is the most effective left side substituent of the oxyoxalamide function for producing significant inhibition potency for the target human enzyme. Interestingly, the 1-adamantyl group (**4**) or other hydrophobic groups (**3** and **5**), which are highly useful for yielding potent inhibition in amide or urea inhibitors, led to dramatically reduced or total lack of inhibition potency with the oxyoxalamide function. When the right side of the oxyoxalamide function was optimized with aryl containing substituents effective for affording potent amide or urea inhibitors, significant inhibition potency was gained from a non-substituent benzyl group (**6**). In the presence of a functional group on the benzene ring (**8–10**) or a longer alkyl group (**11** and **12**) between the benzene ring and the oxyoxalamide function, which are reported as necessary elements for the production of potent amide or urea inhibitors, a dramatic loss in inhibition was induced with the oxyoxalamide pharmacophore. In addition, N-substitution of the oxyoxalamide function of compound **6** was not effective for improving inhibition for the target enzyme (**13** and **14**). Overall structure–activity relationship (SAR) results demonstrate that the substituted oxyoxalamide function is a promising primary pharmacophore for human sEH inhibitors and the structural requirements for producing significant inhibition from oxyoxalamide pharmacophore are different from those for other primary pharmacophores such as amides

and ureas. Furthermore, compound **6** found in the present study can be a novel lead structure for the development of further improved oxyoxalamide and other related derivatives. Next, a series of substituted oxyoxalamides were incorporated into inhibitors with an amide or urea primary pharmacophore to investigate whether the oxyoxalamide function acts as an effective secondary pharmacophore for improving inhibition potency and/or water solubility of them. The SAR results in Tables 3 and 4 and Fig. 2B indicated that substituted oxyoxalamide function is a useful secondary pharmacophore for enhancing inhibition potency of amide and urea inhibitors. Substitution of the oxyoxalamide with smaller alkyl groups such as a methyl group was highly useful for improving inhibition potency (**18**, **20–21**, **23–26**, **28**, **30**, and **31**). However, oxyoxalamides with a larger alkyl group (**19** and **29**) or a polar group (**22** and **32**) led to a significant loss in inhibition. These data also indicate that a *N,N,O*-trimethylated oxyoxalamide is especially effective for making potent amide- or urea–oxyoxalamide derivatives (**24**, **26**, and **31**). In compounds with *N,O*-dimethylated (**23**, **25**, and **30**) or *O*-mono-methylated (**28**) oxyoxalamide, reduced inhibition of up to a 10-fold was observed, suggesting that the incorporation of three methyl groups on the oxyoxalamide is important for yielding highly potent amide- or urea–oxyoxalamide compounds. In addition, the potent compounds with oxyoxalamide function in Tables 3 and 4 had a 2–20-fold enhanced water solubility. Especially, the highest increase in water solubility was gained from compounds with the trimethylated oxyoxalamide function, suggesting that substituted oxyoxalamides incorporated into amide or urea inhibitors as a secondary pharmacophore are useful for improving not only inhibition but also solubility. The resulting compounds found in the present study will be the basis for the design of selective amide- or urea–oxyoxalamide inhibitors with improved physical properties, which will be useful for the development of intravenous or orally available compounds for hypertension, vascular inflammation, and other cardiovascular disorders related by endogenous mediators, including EETs and other epoxyfatty acids.

4. Experimental section

4.1. Chemistry

Unless otherwise noted, all materials were purchased from commercial suppliers and used without further purification. Purity and characterization of compounds were established by a combination of TLC, LC–MS, melting point, and NMR analysis 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 (TMS) as an internal standard. ¹³C NMR spectra were recorded on a JEOL JNM-EX400 spectrometer (JEOL Ltd, Japan), using TMS as an internal standard. High resolution mass spectra were measured by LC-MS (Xevo Q-TOFMS; Waters, UK) using positive mode electrospray ionization. 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.

4.1.1. *N*¹-(Adamant-2-yl)-*N*²-(benzyloxy)oxalamide (6)—To a solution of adamant-2-ylamine hydrochloride (1.00 g, 5.53 mmol) and triethylamine (1.47 g, 10.6 mmol) in dichloromethane (20 mL) was added ethyl (chlorocarbonyl)formate (0.73 g, 5.32 mmol) in dichloromethane (2 mL) at 0 °C. After stirring overnight at room temperature, the product was extracted with diethyl ether (50 mL), washed with an aqueous solution of 1 N HCl (30 mL) and water (50 mL × 2), and dried over MgSO₄. The ether solution was evaporated to dryness and the residue was used for the next reaction without purification. An aqueous solution of 1 N NaOH (2 mL) was added dropwise to a solution of the above residue in ethanol (10 mL) and the reaction mixture was stirred for 30 min at room temperature. After the reaction was acidified to pH 2 by adding an aqueous solution of 1 N HCl, the acid product was extracted with dichloromethane (50 mL × 2). The combined organic solution was washed with water (50 mL × 2), dried over MgSO₄, and evaporated to dryness. To the residue (0.19 g, 0.85 mmol) in dichloromethane (20 mL) was added 4-dimethylaminopyridine (DMAP; 0.10 g, 0.85 mmol) and benzyloxyamine hydrochloride (0.14 g, 0.85 mmol) at room temperature. After the reaction was stirred for 5 min, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI; 0.16 g, 0.85 mmol) was added to the reaction mixture at room temperature. After stirring overnight, the product was extracted with diethyl ether (50 mL). The organic layer was washed with an aqueous solution of 1 N HCl (30 mL × 2) and water (50 mL × 2), dried over MgSO₄, and evaporated. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 3:1) to afford compound **6** as a solid in 85% yield. ¹H NMR δ (CDCl₃): 1.65–1.68 (3H, m), 1.84–1.88 (9H, m), 1.94 (2H, s), 3.99 (1H, s), 4.97 (2H, s), 7.37–7.43 (5H, m), 7.70 (1H, s), 9.64 (1H, s). ¹³C NMR δ (CDCl₃): 26.9, 31.6, 36.9, 37.3, 53.8, 78.7, 128.7, 129.1, 129.2, 134.3, 156.9, 157.7. Purity: >90%. HRMS (ESI) *m/z* calcd for C₁₉H₂₄N₂O₃ [M+H]⁺ 329.1865, found [M+H]⁺ 329.1862, mp 137 °C.

Compounds **3–5** and **7–15** were synthesized in the same procedure used for the preparation of compound **6** by using a corresponding alkyl- or cycloalkyl-amine and a substituted oxyamine instead of adamant-2-ylamine and benzyloxyamine hydrochloride.

4.1.2. *N*¹-(Benzyloxy)-*N*²-(3-phenylpropyl)oxalamide (3)—¹H NMR δ (CDCl₃): 1.89 (2H, t, *J* = 7.3 Hz), 2.66 (2H, t, *J* = 7.3 Hz), 3.32 (2H, q, *J* = 7.3 Hz), 4.96 (2H, s), 7.16–7.26 (4H, m), 7.27–7.31 (3H, m), 7.37–7.40 (5H, m), 9.56 (1H, s). HRMS (ESI) *m/z* calcd for C₁₈H₂₀N₂O₃ [M+H]⁺ 313.1552, found [M+H]⁺ 313.1543, mp 123 °C.

4.1.3. *N*¹-(Adamant-1-yl)-*N*²-(benzyloxy)oxalamide (4)—¹H NMR δ (CDCl₃): 1.68 (6H, s), 2.00 (6H, s), 2.10 (3H, s), 4.86 (2H, s), 7.10 (1H, s), 7.37–7.42 (5H, m), 9.58 (1H, s). HRMS (ESI) *m/z* calcd for C₁₉H₂₄N₂O₃ [M+H]⁺ 329.1865, found [M+H]⁺ 329.1855, mp 178 °C.

4.1.4. *N*¹-(Adamant-1-ylmethyl)-*N*²-(benzyloxy)oxalamide (5)—¹H NMR δ (CDCl₃): 1.49 (6H, br s), 1.73 (6H, br s), 1.99 (3H, br s), 2.97 (2H, d, *J* = 6.8 Hz), 4.97 (2H, s), 7.38–7.42 (5H, m), 9.60 (1H, s). HRMS (ESI) *m/z* calcd for C₂₀H₂₆N₂O₃ [M+H]⁺ 343.2022, found [M+H]⁺ 343.2023, mp 195 °C.

4.1.5. *N*¹-(Adamant-2-yl)-*N*²-(phenyloxy)oxalamide (7)—¹H NMR δ (CDCl₃): 1.66–1.69 (3H, m), 1.87–1.90 (9H, m), 1.98 (2H, s), 4.04 (1H, s), 7.07–7.11 (3H, m), 7.31–7.35 (2H, m), 7.70 (1H, s), 10.14 (1H, s). HRMS (ESI) *m/z* calcd for C₁₈H₂₂N₂O₃ [M+H]⁺ 315.1709, found [M+H]⁺ 315.1711, mp 187 °C.

4.1.6. *N*¹-(Adamant-2-yl)-*N*²-(4-methoxycarbonylbenzyloxy)oxalamide (8)—¹H NMR δ (CDCl₃): 1.63–1.68 (3H, m), 1.86–1.89 (9H, m), 1.94 (2H, s), 3.93 (3H, s), 4.01 (1H, s), 5.02 (2H, s), 7.50 (2H, d, *J* = 8.3 Hz), 7.71 (1H, s), 8.06 (2H, d, *J* = 8.3 Hz), 9.70 (1H, s). HRMS (ESI) *m/z* calcd for C₂₁H₂₆N₂O₅ [M+H]⁺ 387.1920, found [M+H]⁺ 387.1916, mp 175 °C.

4.1.7. *N*¹-(Adamant-2-yl)-*N*²-(4-nitrobenzyloxy)oxalamide (9)—¹H NMR δ (CDCl₃): 1.64–1.67 (3H, m), 1.86–1.88 (9H, m), 1.94 (2H, s), 4.00 (1H, s), 5.09 (2H, s), 7.52 (2H, d, *J* = 8.3 Hz), 7.76 (1H, s), 8.23 (2H, d, *J* = 8.3 Hz), 11.74 (1H, s). HRMS (ESI) *m/z* calcd for C₁₉H₂₃N₃O₅ [M+H]⁺ 374.1716, found [M+H]⁺ 374.1714, mp >210 °C.

4.1.8. *N*¹-(Adamant-2-yl)-*N*²-(4-chlorobenzyloxy)oxalamide (10)—To a solution of 4-chlorobenzyl bromide (4.00 g, 0.01947 mol) and *N*-hydroxyphthalimide (3.18 g, 0.01947 mol) in DMF (40 mL) was added triethylamine (3.94 g, 0.03894 mol) at 0 °C. After stirring overnight at room temperature, the product was extracted with diethyl ether (80 mL × 2). The organic layer was washed with water (80 mL × 2), dried over MgSO₄, and evaporated to dryness. To the residue in 10% methanol in trichloromethane (30 mL) was added hydrazine hydrate (0.93 g, 0.02912 mol) at room temperature. The reaction was stirred overnight and the product was extracted with diethyl ether (80 mL × 2). The combined organic layer was washed with water (80 mL × 2), dried over MgSO₄, and evaporated. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 5:1) to give (4-chlorobenzyl) oxyamine in 65% yield. Then, compound **10** was prepared in the same manner used for the preparation of compound **6** by using this oxyamine. ¹H NMR δ (CDCl₃): 1.65–1.68 (3H, m), 1.83–1.89 (9H, m), 1.94 (2H, s), 4.00 (1H, s), 4.93 (2H, s), 7.26 (2H, s), 7.36 (2H, s), 7.68 (1H, s), 9.60 (1H, s). HRMS (ESI) *m/z* calcd for C₁₉H₂₃ClN₂O₃ [M+H]⁺ 363.1475, found [M+H]⁺ 363.1472, mp 173 °C.

4.1.9. *N*¹-(Adamant-2-yl)-*N*²-(2-phenylethoxy)oxalamide (11)—(2-Phenylethyl)oxyamine was prepared in the same procedure used for the preparation of compound **10** by using 2-phenylethyl bromide instead of 4-chlorobenzyl bromide. Compound **11** was then synthesized in the same manner described in the preparation of compound **6** by using (2-phenylethyl)oxyamine. ¹H NMR δ (CDCl₃): 1.65–1.68 (3H, m), 1.83–1.89 (9H, m), 1.95 (2H, s), 3.02 (2H, t, *J* = 7.1 Hz), 4.01 (1H, s), 4.20 (2H, t, *J* = 7.1 Hz), 7.22–7.24 (3H, m), 7.26–7.33 (2H, m), 7.70 (1H, s), 9.71 (1H, s). Purity: >90%. HRMS (ESI) *m/z* calcd for C₂₀H₂₆N₂O₃ [M+H]⁺ 343.2022, found [M+H]⁺ 343.2015, mp >210 °C.

4.1.10. *N*¹-(Adamant-2-yl)-*N*²-(3-phenylpropyloxy)oxalamide (12)—(3-Phenylpropyl)oxyamine was prepared in the same procedure used for the preparation of compound **10** by using 3-phenylpropyl bromide instead of 4-chlorobenzyl bromide. Compound **12** was then synthesized in the same manner described in the preparation of

compound **6** by using (3-phenylpropyl)oxyamine. $^1\text{H NMR } \delta(\text{CDCl}_3)$: 1.66–1.69 (3H, m), 1.83–1.89 (9H, m), 1.98 (2H, s), 2.08 (2H, quint, $J = 7.3$ Hz), 2.74 (2H, t, $J = 7.3$ Hz), 4.06 (1H, s), 4.29 (2H, t, $J = 7.3$ Hz), 7.18–7.26 (3H, m), 7.28–7.30 (2H, m), 7.42 (1H, s), 9.70 (1H, s). HRMS (ESI) m/z calcd for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$ 357.2178, found $[\text{M}+\text{H}]^+$ 357.2178, mp 53 °C.

4.1.11. N^1 -(Adamant-2-yl)- N^2 -(benzyloxy)- N^2 -methyloxalamide (13**)**—A mixture of compound **6** (0.51 g, 1.55 mmol), potassium carbonate (0.43 g, 3.10 mmol), and iodomethane (0.33 g, 2.32 mmol) in DMF (20 mL) was stirred overnight at room temperature. The product was extracted with diethyl ether (80 mL). The organic solution was washed with water (80 mL \times 2), dried over MgSO_4 , and evaporated. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 4:1) to afford compound **13** as a solid in 55% yield. $^1\text{H NMR } \delta(\text{CDCl}_3)$: 1.61–1.66 (3H, m), 1.84–1.88 (9H, m), 1.92 (2H, s), 4.01 (1H, s), 4.10 (3H, s), 5.06 (2H, s), 6.93 (1H, s), 7.35–7.38 (5H, m). HRMS (ESI) m/z calcd for $\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$ 343.2022, found $[\text{M}+\text{H}]^+$ 343.2016, mp 110 °C.

4.1.12. N^1 -(Adamant-2-yl)- N^2 -methyl- N^2 -(methyloxy)oxalamide (14**)**— $^1\text{H NMR } \delta(\text{CDCl}_3)$: 1.64–1.67 (3H, m), 1.86–1.88 (9H, m), 1.96 (2H, s), 3.76 (3H, s), 3.79 (3H, s), 4.00 (1H, s), 6.81 (1H, s). Purity: >90%. HRMS (ESI) m/z calcd for $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$ 267.1709, found $[\text{M}+\text{H}]^+$ 267.1707, mp 148 °C.

4.1.13. N^1 -(Adamant-2-yl)- N^2 -(tetrahydro-2H-pyran-2-yloxy)oxalamide (15**)**— $^1\text{H NMR } \delta(\text{CDCl}_3)$: 1.62–1.70 (8H, m), 1.81–1.88 (10H, m), 1.95 (2H, s), 3.67 (1H, s), 3.99–4.04 (2H, m), 5.04 (1H, s), 7.72 (1H, s), 9.96 (1H, s). HRMS (ESI) m/z calcd for $\text{C}_{17}\text{H}_{26}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$ 323.1971, found $[\text{M}+\text{H}]^+$ 323.1973, mp 137 °C.

4.1.14. N -(Benzyloxy)-2-(adamant-2-ylamino)acetamide (16**)**—A mixture of methyl 2-bromoacetate (1.22 g, 7.99 mmol), adamant-2-ylamine hydrochloride (1.50 g, 7.99 mmol), and potassium carbonate (2.21 g, 15.9 mmol) in DMF (30 mL) was stirred overnight at room temperature. The product was extracted with diethyl ether (80 mL \times 2). The organic solution was washed with water (100 mL \times 2), dried over MgSO_4 , and evaporated to dryness. To the residue in methanol (15 mL) was added an aqueous solution of 1 N NaOH (4 mL) at room temperature, and the reaction was stirred for 1 h. After evaporating the reaction mixture to dryness, the residue was used for the next reaction without further purification. A mixture of the acid residue (0.79 g, 3.75 mmol), DMAP (0.46 g, 3.75 mmol), and benzyloxylamine hydrochloride (0.60 g, 3.75 mmol) in dichloromethane (30 mL) was stirred for 5 min at room temperature. To this reaction mixture was added EDCI (0.72 g, 3.75 mmol) at room temperature. After stirring overnight, the product was extracted with diethyl ether (80 mL \times 2). The combined organic solution was washed with an aqueous solution of 1 N HCl (40 mL) and water (80 mL \times 2), dried over MgSO_4 , and evaporated. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 1:1) to give compound **16** as a yellowish oil in 70% yield. $^1\text{H NMR } \delta(\text{CDCl}_3)$: 1.44–1.47 (2H, m), 1.61–1.72 (10H, m), 1.83–1.89 (4H, m), 2.53 (1H, s), 3.33 (2H, s), 4.95 (2H, s), 7.35–7.42 (5H, m). Purity:

>90%. HRMS (ESI) m/z calcd for $C_{19}H_{26}N_2O_2$ $[M+H]^+$ 315.2072, found $[M+H]^+$ 315.2071.

4.1.15. *N*-(4-(*N*²-Methyloxyoxalamido)benzyl)adamantanecarboxamide (18)

¹H NMR δ (CDCl₃): 1.68–1.71 (6H, m), 1.88 (6H, s), 2.05 (3H, s), 3.88 (3H, s), 4.42 (2H, s), 5.88 (1H, s), 7.27 (2H, d, J = 10 Hz), 7.57 (2H, d, J = 10 Hz), 9.09 (1H, s), 9.82 (1H, s). HRMS (ESI) m/z calcd for $C_{21}H_{27}N_3O_4$ $[M+H]^+$ 386.2080, found $[M+H]^+$ 386.2076, mp >210 °C.

4.1.16. *N*-(4-(*N*²-*t*Butyloxyoxalamido)benzyl)adamantanecarboxamide (19)

¹H NMR δ (CDCl₃): 1.35 (9H, s), 1.68–1.72 (6H, m), 1.88 (6H, s), 2.04 (3H, s), 4.43 (2H, s), 5.88 (1H, s), 7.29 (2H, d, J = 10 Hz), 7.57 (2H, d, J = 10 Hz), 9.16 (1H, s), 9.42 (1H, s). HRMS (ESI) m/z calcd for $C_{24}H_{33}N_3O_4$ $[M+H]^+$ 428.2549, found $[M+H]^+$ 428.2546, mp >210 °C.

4.1.17. *N*-(4-(*N*²-Methyl-*N*²-

(methyloxy)oxalamido)benzyl)adamantanecarboxamide (20)—¹H NMR δ (CDCl₃): 1.66–1.76 (6H, m), 1.88 (6H, s), 2.04 (6H, s), 3.29 (3H, s), 3.83 (3H, s), 4.41 (2H, s), 7.25 (2H, d, J = 8.3 Hz), 7.55 (2H, d, J = 8.3 Hz), 9.34 (1H, s). HRMS (ESI) m/z calcd for $C_{22}H_{29}N_3O_4$ $[M+H]^+$ 400.2236, found $[M+H]^+$ 400.2239, mp 181 °C.

4.1.18. *N*-(4-(*N*¹-Methyl-*N*²-methyl-*N*²-(methyloxy)oxalamido)-

benzyl)adamantanecarboxamide (21)—¹H NMR δ (CDCl₃): 1.68–1.72 (6H, m), 1.88 (6H, s), 2.04 (6H, s), 2.96 (3H, s), 3.31 (3H, s), 3.79 (3H, s), 4.43 (2H, s), 5.92 (1H, s), 7.24–7.26 (4H, m). HRMS (ESI) m/z calcd for $C_{23}H_{31}N_3O_4$ $[M+H]^+$ 414.2393, found $[M+H]^+$ 414.2386, mp 170 °C.

4.1.19. *N*-(4-(*N*²-(Tetrahydro-2*H*-pyran-2-yloxy)oxalamido)benzyl)

adamantanecarboxamide (22)—¹H NMR δ (CDCl₃): 1.61–1.65 (6H, m), 1.68–1.72 (6H, m), 1.88 (6H, s), 2.04 (6H, s), 3.66–3.70 (2H, m), 4.02 (1H, t, J = 6.8 Hz), 4.42 (2H, s), 5.90 (1H, s), 7.25 (2H, d, J = 8.3 Hz), 7.57 (2H, d, J = 8.3 Hz), 9.15 (1H, s), 9.98 (1H, s). Purity: >90%. HRMS (ESI) m/z calcd for $C_{25}H_{33}N_3O_5$ $[M+H]^+$ 456.2498, found $[M+H]^+$ 456.2495, mp 195 °C.

4.1.20. *N*¹-(4-((2-Adamantylacetamido)methyl)phenyl)-*N*²-methyl-*N*²-

methyloxyoxalamide (23)—¹H NMR δ (CDCl₃): 1.62–1.71 (12H, m), 1.96–1.97 (5H, s), 3.29 (3H, s), 3.83 (3H, s), 4.42 (2H, s), 5.58 (1H, s), 7.27 (2H, d, J = 8.3 Hz), 7.56 (2H, d, J = 8.3 Hz), 9.33 (1H, s). HRMS (ESI) m/z calcd for $C_{23}H_{31}N_3O_4$ $[M+H]^+$ 414.2393, found $[M+H]^+$ 414.2390, mp 203 °C.

4.1.21. *N*¹-(4-((2-Adamantylacetamido)methyl)phenyl)-*N*¹-methyl-*N*²-methyl-

***N*²-methyloxyoxalamide (24)**—¹H NMR δ (CDCl₃): 1.63–1.72 (12H, m), 1.96–1.97 (5H, s), 2.95 (3H, s), 3.33 (3H, s), 3.80 (3H, s), 4.43 (2H, s), 5.65 (1H, s), 7.23–7.30 (4H, m). ¹³C NMR δ (CDCl₃): 28.6, 31.2, 32.8, 35.7, 36.7, 42.6, 42.9, 51.7, 62.2, 126.3, 128.8, 138.6, 140.7, 164.3, 165.6, 170.9. HRMS (ESI) m/z calcd for $C_{24}H_{33}N_3O_4$ $[M+H]^+$ 428.2549, found $[M+H]^+$ 428.2545, mp 97 °C.

4.1.22. *N*¹-(4-(2-Adamantylacetamido)phenyl)-*N*²-methyl-*N*²-

(methoxy)oxalamide (25)—After a mixture of adamant-1-ylacetic acid (1.87 g, 9.60 mmol), DMAP (1.17 g, 9.60 mmol), and 4-(*tert*-butyloxycarbonyl)aminoaniline (2.00 g, 9.60 mmol) in dichloromethane (30 mL) was stirred for 10 min at room temperature, EDCI (1.84 g, 9.60 mmol) was added portionwise to the reaction mixture. The reaction was stirred overnight and the product was then extracted with ethyl acetate (80 mL ×2). The organic solution was washed with an aqueous solution of 1 N HCl (50 mL) and water (80 mL ×2), dried over MgSO₄, and evaporated. To the residue washed with diethyl ether (20 mL) in dichloromethane (30 mL) was added an aqueous solution of 4 N HCl (10 mL) at room temperature. After stirring overnight, the product was extracted with ethyl acetate (80 mL ×2). The organic layer was washed with water (80 mL ×2), dried over MgSO₄, and evaporated to dryness. The residue was used for the next reaction without further purification. To a solution of the residue (1.42 g, 5.27 mmol) and triethylamine (0.80 g, 7.90 mmol) in dichloromethane (30 mL) was added ethyl (chlorocarbonyl)formate (0.72 g, 5.27 mmol) at 0 °C. After stirring overnight at room temperature, the product was extracted with ethyl acetate (80 mL ×2). The organic solution was washed with an aqueous solution of 1 N HCl (40 mL) and water (80 mL ×2), dried over MgSO₄, and evaporated to dryness. An aqueous solution of 1 N NaOH (5 mL) was added to a solution of the residue in ethanol (10 mL) at room temperature. After the reaction was stirred overnight at room temperature and acidified to pH 2 by adding an aqueous solution of 1 N HCl, the product was extracted with ethyl acetate (80 mL ×2). The organic solution was washed with water (80 mL ×2), dried over MgSO₄, and evaporated to dryness. Continuously, to a solution of the residue (1.22 g, 3.42 mmol), DMAP (0.42 g, 5.13 mmol), methoxymethylamine hydrochloride (0.50 g, 5.13 mmol), and triethylamine (0.52 g, 5.13 mmol), which was stirred for 10 min, was added EDCI (0.98 g, 5.13 mmol) at room temperature. After stirring overnight, the product was extracted with ethyl acetate (80 mL ×2). The organic solution was washed with water (80 mL ×2), dried over MgSO₄, and evaporated. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 1:1) to afford compound **25** in 45% yield. ¹H NMR δ(CDCl₃): 1.63–1.69 (12H, m), 2.04 (3H, s), 2.09 (2H, s), 3.28 (3H, s), 3.82 (3H, s), 7.06 (1H, s), 7.51–7.57 (4H, m), 9.33 (1H, s). HRMS (ESI) *m/z* calcd for C₂₂H₂₉N₃O₄ [M+H]⁺ 400.2236, found [M+H]⁺ 400.2239, mp 208 °C.

Compounds **18–23**, **28–30** and **32** were prepared in the same manner used for the synthesis of compound **25** by using 4-aminobenzylamine, adamant-1-ylcarboxylic acid or adamant-1-yl isocyanate, and a corresponding substituted oxyamine instead of 4-(*tert*-butyloxycarbonyl)-aminoaniline and methoxymethylamine hydrochloride.

4.1.23. *N*¹-(4-(2-Adamantylacetamido)phenyl)-*N*¹-methyl-*N*²-methyl-*N*²-

(methoxy)oxalamide (26)—A mixture of compound **25** (0.30 g, 0.75 mmol), potassium carbonate (0.16 g, 1.12 mmol), and iodomethane (0.11 g, 1.12 mmol) in DMF (15 mL) was stirred overnight at room temperature. The product was extracted with diethyl ether (80 mL). The organic solution was washed with water (80 mL ×2), dried over MgSO₄, and evaporated. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 1:1) to give compound **26** in 60% yield. ¹H NMR δ(CDCl₃): 1.64–1.69 (12H, m), 1.99 (3H, s), 2.09 (2H, s), 2.98 (3H, s), 3.30 (3H, s), 3.80 (3H, s), 7.18 (2H, d, *J* =

8.3 Hz), 7.48 (2H, d, $J = 8.3$ Hz). ^{13}C NMR δ (CDCl₃): 28.6, 31.3, 33.3, 35.7, 36.6, 42.5, 52.7, 62.2, 120.2, 126.8, 136.9, 137.9, 164.2, 165.7, 169.6. Purity: >90%. HRMS (ESI) m/z calcd for C₂₃H₃₁N₃O₄ [M+H]⁺ 414.2393, found [M+H]⁺ 414.2391, mp 179 °C.

Compounds **21**, **24**, and **31** were synthesized in the same manner used for the preparation of compound **26** by using compound **20**, **23**, or **30**, respectively, instead of compound **25**.

4.1.24. 1-(4-(*N*²-Methyloxyoxalamido)benzyl)-3-adamantylurea (28)—Compound **28** was prepared in the same methods described in the syntheses of compounds **25** and **30**. ^1H NMR δ (CDCl₃): 1.65 (6H, s), 1.95 (6H, s), 2.06 (3H, s), 3.83 (3H, s), 4.09 (1H, s), 4.30 (2H, s), 4.44 (1H, s), 7.25 (2H, d, $J = 8.3$ Hz), 7.53 (2H, d, $J = 8.3$ Hz), 9.12 (1H, s), 9.35 (1H, s). HRMS (ESI) m/z calcd for C₂₁H₂₈N₄O₄ [M+H]⁺ 401.2189, found [M+H]⁺ 401.2187, mp 177 °C.

4.1.25. 1-(4-(*N*²-*t*-Butyloxyoxalamido)benzyl)-3-adamantylurea (29)—Compound **29** was prepared in the same methods described in the syntheses of compounds **25** and **30**. ^1H NMR δ (CDCl₃): 1.56 (9H, s), 1.66 (6H, s), 1.95 (6H, s), 2.05 (3H, s), 4.08 (1H, s), 4.30 (2H, s), 4.43 (1H, s), 7.30 (2H, d, $J = 8.3$ Hz), 7.57 (2H, d, $J = 8.3$ Hz), 9.16 (1H, s), 9.45 (1H, s). HRMS (ESI) m/z calcd for C₂₄H₃₄N₄O₄ [M+H]⁺ 443.2658, found [M+H]⁺ 443.2654, mp >210 °C.

4.1.26. 1-(4-(*N*²-Methyl-*N*²-(methyloxy)oxalamido)benzyl)-3-adamantylurea (30)—To a solution of adamant-1-yl isocyanate (0.73 g, 4.09 mmol) in DMF (15 mL) was added dropwise 4-aminobenzylamine (0.50 g, 4.09 mmol) in DMF (2 mL) at 0 °C. After stirring overnight at room temperature, the product was extracted with diethyl ether (80 mL \times 2). The organic layer was washed with water (80 mL \times 2), dried over MgSO₄, and evaporated to give 1-(adamant-1-yl)-3-(4-aminobenzyl) urea in 100% yield. Compound **30** was synthesized in the same method described in the preparation of compound **25** by using 1-(adamant-1-yl)-3-(4-aminobenzyl)urea instead of the amide-amine intermediate. ^1H NMR δ (CDCl₃): 1.66 (6H, s), 1.95 (6H, s), 2.04 (3H, s), 3.28 (3H, s), 3.82 (3H, s), 4.09 (1H, s), 4.30 (2H, s), 4.40 (1H, s), 7.30 (2H, d, $J = 8.3$ Hz), 7.55 (2H, d, $J = 8.3$ Hz), 9.33 (1H, s). HRMS (ESI) m/z calcd for C₂₂H₃₀N₄O₄ [M+H]⁺ 415.2345, found [M+H]⁺ 415.2347, mp 202 °C.

4.1.27. 1-(4-(*N*¹-Methyl-*N*²-methyl-*N*²-(methyloxy)oxalamido)-benzyl)-3-adamantylurea (31)—Compound **31** was prepared in the same methods described in the syntheses of compounds **25** and **30**. ^1H NMR δ (CDCl₃): 1.66 (6H, s), 1.95 (6H, s), 2.04 (3H, s), 2.95 (3H, s), 3.30 (3H, s), 3.80 (3H, s), 4.08 (1H, s), 4.31 (2H, s), 4.44 (1H, s), 7.22–7.29 (4H, m). ^{13}C NMR δ (CDCl₃): 29.5, 31.2, 35.7, 36.4, 42.4, 43.5, 51.0, 62.2, 126.1, 128.3, 139.9, 140.4, 157.0, 164.3, 165.7. HRMS (ESI) m/z calcd for C₂₃H₃₂N₄O₄ [M+H]⁺ 429.2502, found [M+H]⁺ 429.2505, mp >210 °C.

4.1.28. 1-(4-(*N*²-(Tetrahydro-2*H*-pyran-2-yloxy)oxalamido)benzyl)-3-adamantylurea (32)—Compound **32** was prepared in the same methods described in the syntheses of compounds **25** and **30**. ^1H NMR δ (CDCl₃): 1.61–1.64 (12H, m), 1.93 (6H, s), 2.04 (3H, s), 3.69 (1H, s), 4.09–4.14 (2H, m), 4.25 (2H, s), 4.70 (1H, s), 5.19 (1H, s), 7.17

(2H, d, $J = 8.3$ Hz), 7.49 (2H, d, $J = 8.3$ Hz), 9.36 (1H, s), 10.56 (1H, s). HRMS (ESI) m/z calcd for $C_{25}H_{34}N_4O_5$ $[M+H]^+$ 471.2607, found $[M+H]^+$ 471.2608, mp 202 °C.

5. Biology

5.1. Enzyme preparation

Recombinant human sEH was prepared by using baculovirus expression system as previously reported.²⁵ Briefly, Sf9 insect cells were infected by recombinant baculovirus harboring human sEH gene fused with a 6xHis tag. At 72 h post-infection, the infected cells were homogenized and the recombinant protein was purified by immobilized metal affinity chromatography. After removing the 6xHis tag using the tobacco etch virus protease, human sEH was further purified by anion-exchange chromatography.

5.2. IC₅₀ assay conditions

Standard solutions of compounds in Tables 1–4 were prepared in DMSO. Fluorescent assays were performed by using a substrate (cyano-(2-methoxynaphthalen-6-yl)-methyl trans-(3-phenyl-oxyran-2-yl)-methyl carbonate; CMNPC; $[S] = 5 \mu M$) to determine IC₅₀ values of the derivatives.²⁶ Inhibition activity against human sEH (1 nM) was determined by measuring 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).^{25,26} The IC₅₀ values were gained by regression of at least six datum points with a minimum of three points in a linear region of the curve. IC₅₀ results are averages of three separate measurements. 12-(3-Adamantan-1-yl-ureido) dodecanoic acid (AUDA)¹³ in Table 2 was used as a positive control for the inhibition assay in the present study.

5.3. Solubility

Water solubility of amide and urea derivatives in Tables 3 and 4 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 a test compound prepared in DMSO to 0.1 M sodium phosphate buffer (pH 7.4) in a final ratio of 5:95 (v/v). Insolubility of the compound was shown 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by Hyundai Pharm Research Grant (HOB-024). Partial support was from NIEHS Grant R01 ES002710 and a Grant-in-aid for Young Scientists (B) 23710042 from Japan Society for the Promotion of Science.

Abbreviations

EETs	epoxyeicosatrienoic acids
sEH	soluble epoxide hydrolase
EDCI	1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
CMNPC	cyano-(2-methoxynaphthalen-6-yl)-methyl <i>trans</i> -(3-phenyl-oxyran-2-yl)-methyl carbonate

References and notes

- Robinson DR. *Am J Med.* 1983; 75:26. [PubMed: 6416064]
- Miller SB. *Semin Arthritis Rheum.* 2006; 36:37. [PubMed: 16887467]
- Peters-Golden M, Gleason MM, Togias A. *Clin Exp Allergy.* 2006; 36:689. [PubMed: 16776669]
- Pfister SL, Gauthier KM, Campbell WB. *Adv Pharmacol.* 2010; 60:27. [PubMed: 21081214]
- Imig JD, Zhao X, Capdevila JH, Morisseau C, Hammock BD. *Hypertension.* 2002; 39:690. [PubMed: 11882632]
- Zhao X, Yamamoto T, Newman JW, Kim IH, Watanabe T, Hammock BD, Stewart J, Pollock JS, Pollock DM, Imig JD. *J Am Soc Nephrol.* 2004; 15:1244. [PubMed: 15100364]
- Jung O, Brandes RP, Kim IH, Schweda F, Schmidt R, Hammock BD, Busse R, Fleming I. *Hypertension.* 2005; 45:759. [PubMed: 15699457]
- Imig JD, Zhao X, Zaharis CZ, Olearczyk JJ, Pollock DM, Newman JW, Kim IH, Watanabe T, Hammock BD. *Hypertension.* 2006; 46:975. [PubMed: 16157792]
- Schmelzer KR, Kubala L, Newman JW, Kim IH, Eiserich JP, Hammock BD. *Proc Natl Acad Sci USA.* 2005; 102:9772. [PubMed: 15994227]
- Inceoglu B, Wagner K, Schebb NH, Morisseau C, Jinks SL, Ulu A, Hegedus C, Rose T, Brosnan R, Hammock BD. *Proc Natl Acad Sci USA.* 2011; 108:5093. [PubMed: 21383170]
- Xu D, Li N, He Y, Timofeyev V, Lu L, Tsai HJ, Kim IH, Tuteja D, Mateo RKP, Singapuri A, Davis BB, Low R, Hammock BD, Chiamvimonvat N. *Proc Natl Acad Sci USA.* 2006; 103:18733. [PubMed: 17130447]
- Kim IH, Morisseau C, Watanabe T, Hammock BD. *J Med Chem.* 2004; 47:2110. [PubMed: 15056008]
- Kim IH, Nishi K, Tsai HJ, Bradford T, Koda Y, Watanabe T, Morisseau C, Blanchfield J, Toth I, Hammock BD. *Bioorg Med Chem.* 2007; 15:312. [PubMed: 17046265]
- Kim IH, Tsai HJ, Nishi K, Kasagami T, Morisseau C, Hammock BD. *J Med Chem.* 2007; 50:5217. [PubMed: 17894481]
- Kasagami T, Kim IH, Tsai HJ, Nishi K, Hammock BD, Morisseau C. *Bioorg Med Chem Lett.* 2009; 19:1784. [PubMed: 19216074]
- Jones PD, Tsai HJ, Do Z, Morisseau C, Hammock BC. *Bioorg Med Chem Lett.* 2006; 16:5212. [PubMed: 16870439]
- Hwang SH, Tsai HJ, Liu JY, Morisseau C, Hammock BD. *J Med Chem.* 2007; 50:3825. [PubMed: 17616115]
- Anandan SK, Gless RD. *Bioorg Med Chem Lett.* 2010; 20:2740. [PubMed: 20363133]
- Shen HC, Ding FX, Wang S, Xu S, Chen HS, Tong X, Tong V, Mitra K, Kumar S, Zhang X, Chen Y, Zhou G, Pai LY, Alonso-Galicia M, Chen X, Zhang B, Tata JR, Berger JP, Colletti S. *Bioorg Med Chem Lett.* 2009; 19:3398. [PubMed: 19481932]

20. Lo HY, Man CC, Fleck RW, Farrow NA, Ingraham RH, Kukulka A, Proudfoot JR, Betageri R, Kirrane T, Patel U, Sharma R, Hoermann MA, Kabcenell A, Lombaert SD. *Bioorg Med Chem Lett.* 2010; 20:6379. [PubMed: 20934334]
21. Kim IH, Nishi K, Kasagami T, Morisseau C, Liu JY, Tsai HJ, Hammock BD. *Bioorg Med Chem Lett.* 2012; 22:5889. [PubMed: 22901393]
22. Kim IH, Heirtzler FR, Morisseau C, Nishi K, Tsai HJ, Hammock BD. *J Med Chem.* 2005; 48:3621. [PubMed: 15887969]
23. Kim IH, Park YK, Hammock BD, Nishi K. *J Med Chem.* 2011; 54:1752. [PubMed: 21338111]
24. Eldrup AB, Soleymanzadeh F, Taylor SJ, Muegge I, Farrow NA, Joseph D, McKellop K, Man CC, Kukulka A, Lombaert SD. *J Med Chem.* 2009; 52:5880. [PubMed: 19746975]
25. Nishi K, Kim IH, Ma SJ. *Protein Expr Purif.* 2010; 69:34. [PubMed: 19782755]
26. Morisseau, C.; Hammock, BD. *Current Protocols in Toxicology.* John Wiley & Sons; New Jersey: 2007. p. 4.23.1-4.23.18.

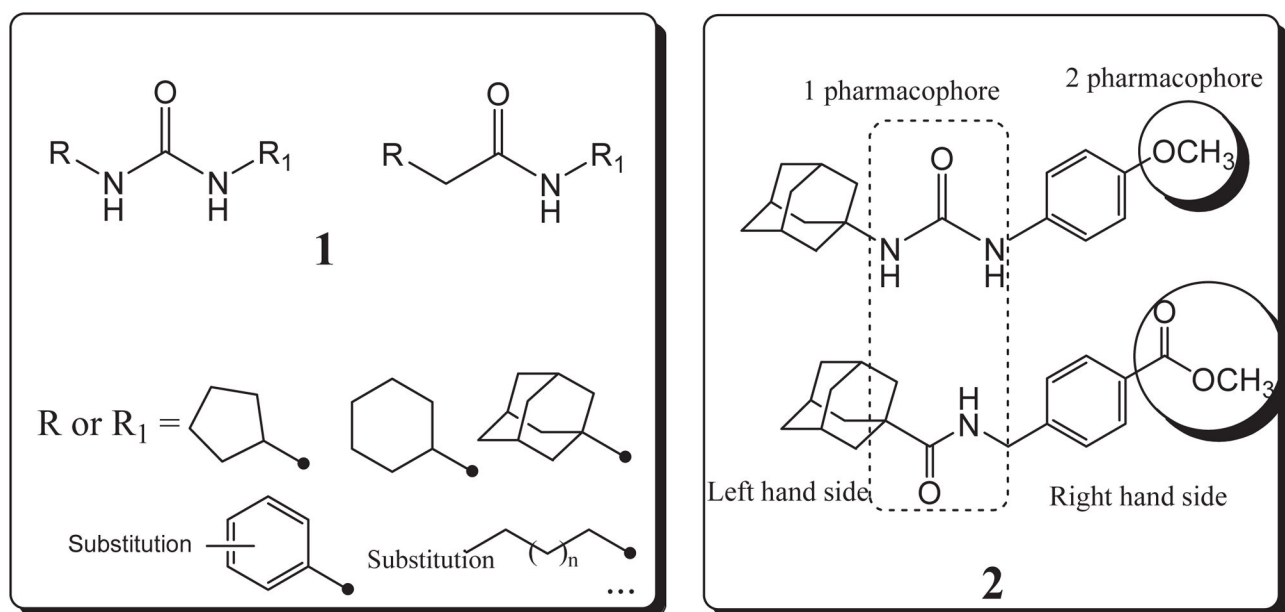


Figure 1.

Substituents of urea and amide primary pharmacophores (1) which yield potent inhibitors of the human sEH, and examples of potent urea and amide compounds with a secondary pharmacophore (2): $n = 0-10$, 1 pharmacophore = primary pharmacophore; 2 pharmacophore = secondary pharmacophore. The IC_{50} of urea and amide compounds in structure 2 for the human enzyme is 14 and 9.1 nM, respectively.

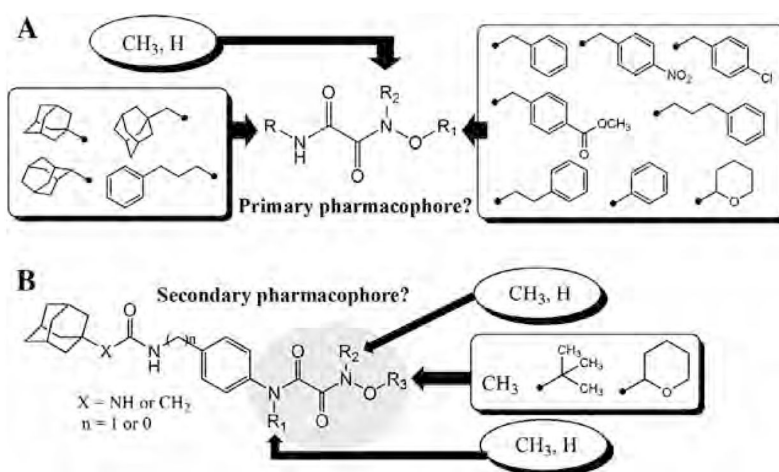
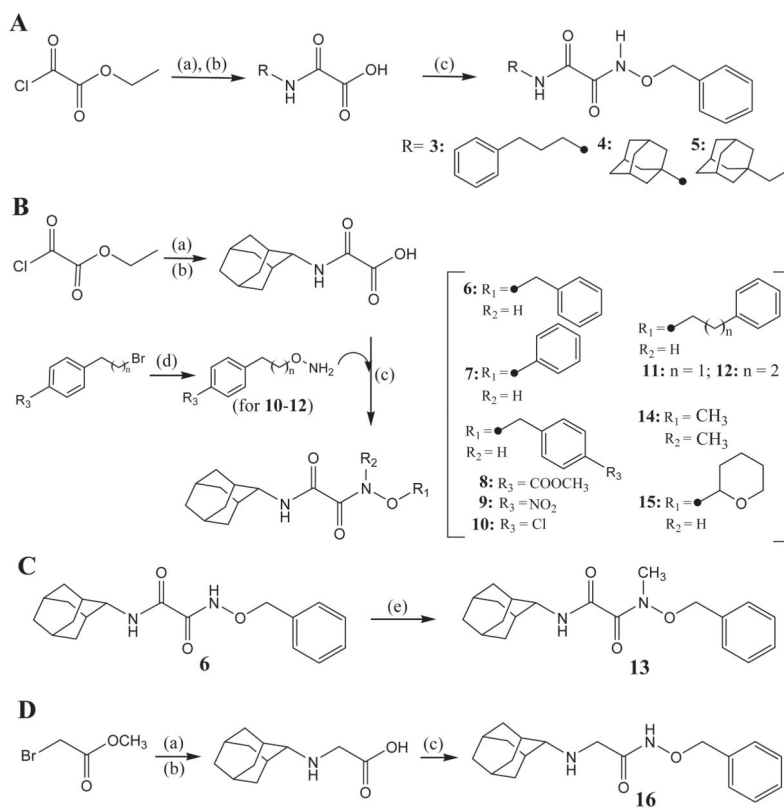
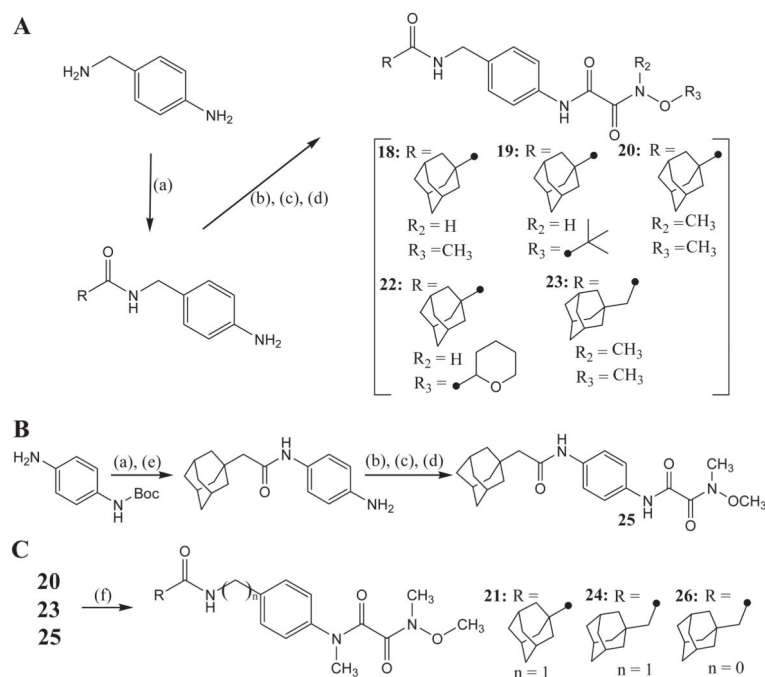


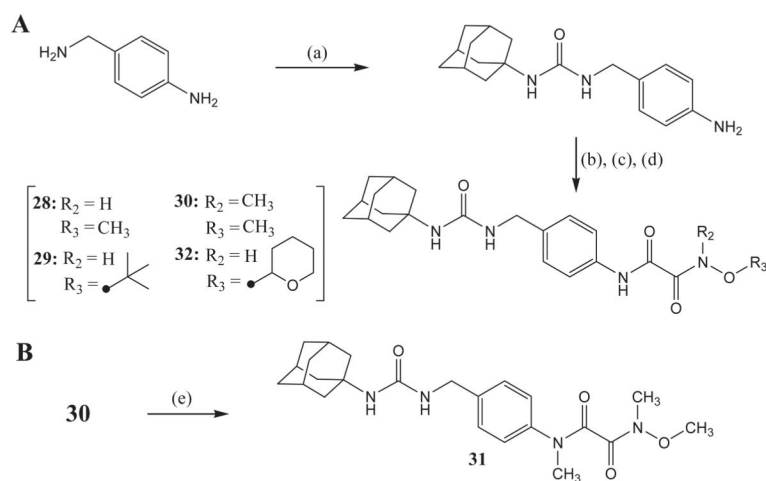
Figure 2. Structure–activity relationships of various oxyoxalamide derivatives as inhibitors of the human sEH were explored. In order to first investigate whether the substituted oxyoxalamides can be an effective primary pharmacophore to inhibit the target enzyme, various substituents were introduced into the oxyoxalamide function as shown in A (Tables 1 and 2). In addition, in order to see if it can be an effective secondary pharmacophore to improve inhibition and/or solubility of amide- or urea-based inhibitors, the substituted oxyoxalamides with a variety of groups were incorporated to amide and urea inhibitors as seen in B (Tables 3 and 4).

**Scheme 1.**

Syntheses of substituted oxyoxalamides (**3–15**) and *N*-(benzyloxy)-2-(adamant-2-ylamino)acetamide (**16**). (a) An alkyl- or a cycloalkyl-amine, Et_3N , CH_2Cl_2 , room temp; (b) 1 N NaOH, EtOH or MeOH, room temp; (c) benzyloxyamine (in parts A and D) or a substituted oxyamine (in part B), EDCI, DMAP, CH_2Cl_2 , room temp; (d) (i) *N*-hydroxyphthalimide, Et_3N , DMF, room temp, (ii) H_2NNH_2 , 10% MeOH in CHCl_3 , room temp; (e) CH_3I , K_2CO_3 , DMF, room temp.

**Scheme 2.**

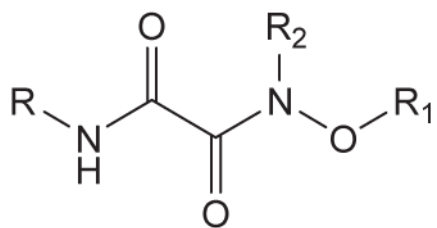
Syntheses of amide derivatives with substituted oxyoxalamide. (a) adamant-1-ylcarboxylic acid (for compounds **18**–**20** and **22**) or adamant-1-ylmethylcarboxylic acid (for compounds **23** and **25**), EDCI, DMAP, CH_2Cl_2 , room temp; (b) ethyl (chlorocarbonyl)formate, Et_3N , CH_2Cl_2 , room temp; (c) 1 N NaOH, EtOH, room temp; (d) a corresponding oxyamine (in parts A and B), EDCI, DMAP CH_2Cl_2 , room temp; (e) 4 N HCl, CH_2Cl_2 , room temp; (f) CH_3I , K_2CO_3 , DMF, room temp.

**Scheme 3.**

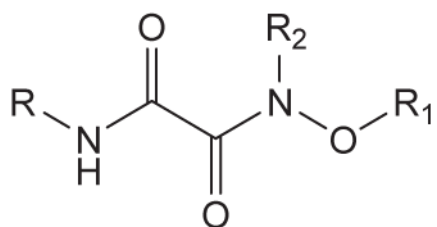
Syntheses of urea derivatives with substituted oxyoxalamide. (a) adamant-1-yl isocyanate, DMF, room temp; (b) ethyl (chlorocarbonyl)formate, Et_3N , CH_2Cl_2 , room temp; (c) 1 N NaOH, EtOH, room temp; (d) a corresponding oxyamine (in part A), EDCI, DMAP CH_2Cl_2 , room temp; (e) CH_3I , K_2CO_3 , DMF, room temp.

Table 1

Inhibition of human sEH by substituted oxyoxalamide derivatives



No.	R	R ₁	R ₂	Human sEH IC ₅₀ (nM) ^a
3			H	>10,000
4			H	>10,000
5			H	838
6			H	50
7			H	>10,000
8			H	1500
9			H	>10,000
10			H	2300

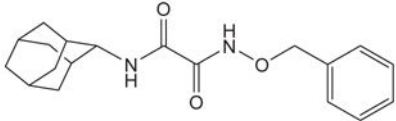
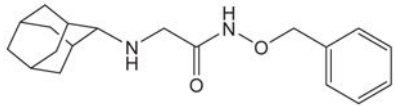
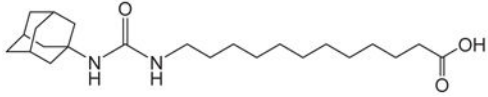


No.	R	R ₁	R ₂	Human sEH IC ₅₀ (nM) ^a
11			H	4400
12			H	550
13			CH ₃	3200
14		CH ₃	CH ₃	>10,000
15			H	>10,000

^aTest compounds prepared in DMSO were reacted with human sEH (1 nM) for 10 min in 25 mM Bis-Tris/HCl buffer (202 μL; pH 7.0) at 30 °C. The fluorescent substrate (CMNPC; [S] = 5 μM) was then introduced to the incubation mixture. Inhibition potency against the human sEH was determined by measuring 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. Results are averages of three separate measurements. See the Supplementary data for the detailed procedures.

Table 2

Inhibition of human sEH by *N*¹-(adamant-2-yl)-*N*²-(benzyloxy)oxalamide (**6**) and *N*-(benzyloxy)-2-(adamant-2-ylamino)acetamide (**16**)

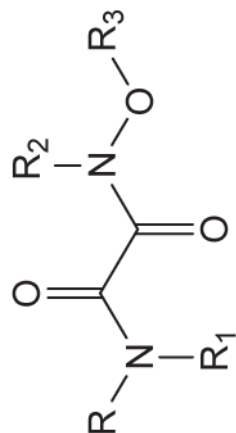
No.	Structure	Human sEH IC ₅₀ (nM) ^a
6		50
16		>10,000
AUDA ^b		3.2

^aTest compounds prepared in DMSO were reacted with human sEH (1 nM) for 10 min in 25 mM Bis-Tris/HCl buffer (202 μL; pH 7.0) at 30 °C. The fluorescent substrate (CMNPC; [S] = 5 μM) was then introduced to the incubation mixture. Inhibition potency against the human sEH was determined by measuring 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. Results are averages of three separate measurements. See the Supplementary data for the detailed procedures.

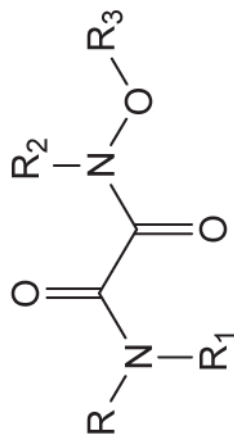
^b12-(3-Adamantan-1-yl-ureido)dodecanoic acid, which was synthesized in the reaction of 1-adamantane isocyanate with 12-aminododecanoic acid in 1,2-dichloroethanol as previously described¹³ and was used as a positive control for the inhibition assay in this study.

Table 3

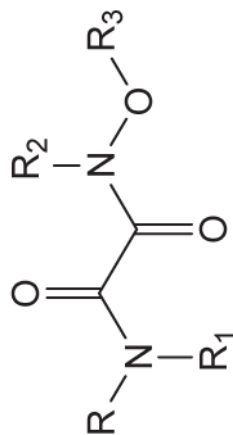
Inhibition of human sEH by amide derivatives substituted with oxoxalamide function



No.	R	R ₁	R ₂	R ₃	Human sEH IC ₅₀ ^a (nM)	Solubility ^b (μM)
17 ^d					>1000	40
18		H	H	CH ₃	280	ND ^c
19		H	H		408	ND
20		H	CH ₃	CH ₃	190	ND



No.	R	R ₁	R ₂	R ₃	Human sEH IC ₅₀ ^a (nM)	Solubility ^b (μM)
21		CH ₃	CH ₃	CH ₃	204	ND
22		H	H		>1000	ND
23		H	CH ₃	CH ₃	69	156
24		CH ₃	CH ₃	CH ₃	7.9	625
25		H	CH ₃	CH ₃	35	78



No.	R	R ₁	R ₂	R ₃	Human sEH IC ₅₀ ^a (nM)	Solubility ^b (μM)
26		CH ₃	CH ₃	CH ₃	4.4	156
2 ^c					9.1	125
IK950 ^e					14	625

^a Test compounds prepared in DMSO were reacted with human sEH (1 nM) for 10 min in 25 mM Bis-Tris/HCl buffer (202 μL; pH 7.0) at 30 °C. The fluorescent substrate (CMNPPC; [S] = 5 μM) was then introduced to the incubation mixture. Inhibition potency against the human sEH was determined by measuring the appearance of the 6-methoxy-2-naphthaldehyde with an excitation wavelength of 330 nm and an emission wavelength of 465 nm on a fluorometer. Results are averages of three separate measurements. See the Supplementary data for the detailed procedures.

^b Water solubility was determined by adding a variety of concentrations of a test compound prepared 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 the average of triplicate determinations.

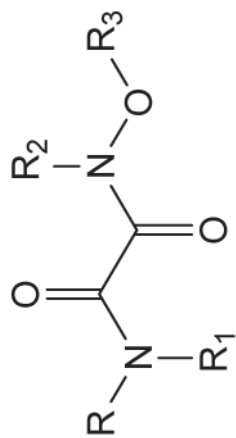
^c Not-determined because the inhibition results were not potent enough compared to that of other derivatives.

^d Amide inhibitor with no substitution by oxyxalamide function.

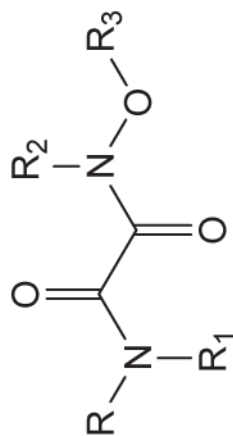
^e Previously reported potent and soluble inhibitors.^{1,4,23} **IK950** was used as a control compound for the measurement of water solubility in this study.

Table 4

Inhibition of human sEH by urea derivatives substituted with oxoxalamide function



No.	R	R ₁	R ₂	R ₃	Human sEH IC ₅₀ ^a (nM)	Solubility ^b (μM)
27 ^c		H	H	H	16	20
28		H	H	CH ₃	6.6	312
29		H	H		19	39
30		H	CH ₃	CH ₃	5.0	312



No.	R	R ₁	R ₂	R ₃	Human sEH IC ₅₀ ^a (nM)	Solubility ^b (μM)
31		CH ₃	CH ₃	CH ₃	1.2	625
32		H	H		19	78
AUDA ^d					3.2	63
IK950 ^d					14	625

^aTest compounds prepared in DMSO was reacted with human sEH (1 nM) for 10 min in 25 mM Bis-Tris/HCl buffer (202 μL, pH 7.0) at 30 °C. The fluorescent substrate (CMNFC; [S] = 5 μM) was then introduced to the incubation mixture. Inhibition potency against the human sEH was determined by measuring 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. Results are averages of three separate measurements. See the Supplementary data for the detailed procedures.

^bWater solubility was determined by adding a variety of concentrations of a test compound prepared 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 the average of triplicate determinations.

^cUrea inhibitor with no substitution by oxoxalamide function, which was synthesized by the reaction of 1-adamantyl isocyanate with benzyl amine in DMF in 100% yield.¹³

^dPotent urea-based inhibitors.^{13,14} **IK950** with improved water solubility was developed based on the structure of **AUDA**. **IK950** was used as a control compound for the measurement of water solubility in this study.