Published in final edited form as:

J Med Chem. 2011 March 24; 54(6): 1752–1761. doi:10.1021/jm101431v.

Structure activity relationships of cycloalkylamide derivatives as inhibitors of the soluble epoxide hydrolase

In-Hae Kim¹, Yong-Gyu Park², Bruce D. Hammock³, and Kosuke Nishi^{4,*}

¹Industry & Academic Cooperation Laboratory for Drug Development, Mokpo National University, Muan, Jeonnam 534–729, Republic of Korea

²Department of Medicinal Chemistry, Hyundai Pharma, Suwon, Gyeonggi 443–270, Republic of Korea

³Department of Entomology and University of California Davis Cancer Center, University of California, One Shields Avenue, Davis, California 95616, USA

⁴Department of Applied Bioscience, Faculty of Agriculture, Ehime University, 3–5-7 Tarumi, Matsuyama, Ehime 790–8566, Japan

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. Non-substituted benzyl, alkyl, aryl, or biaryl structure present in the right side of cycloalkylamide function induced a big decrease in inhibition potency. Also, a resulting potent cycloalkylamide (**32**) showed reasonable physical properties.

Introduction

Epoxyeicosatrienoic acids (EETs^a), 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 anti-hypertensive and cardio protective 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.

*Correspondence Address: Department of Applied Bioscience, Faculty of Agriculture, Ehime University, 3–5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan, +81-89-946-9818 (Phone/Fax), knishi@agr.ehime-u.ac.jp.

Supporting Information Available Detailed analytical data for compounds **3–10**, **12–14**, **17–30**, **33**, **34**, and **35**. Table of elemental analyses for compounds **19**, **20**, **29**, **32**, **33–34**, and **35**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

^aAbbreviations: EETs, epoxyeicosatrienoic acids; sEH, soluble epoxide hydrolase; EDCI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide; DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; CMNPC, cyano-(2-methoxynaphthalen-6-yl)-methyl *trans*-(3-phenyl-oxyran-2-yl)-methyl carbonate; AUDA, 12-(3-adamantan-1-yl-ureido)dodecanoic acid.

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 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 on 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.

Chemistry

Cycloalkyl- and substituted cycloalkyl-amide compounds in Tables 1 and 2 were synthesized as outlined in Scheme 1. Corresponding cycloalkanecarboxylic acid was coupled with 3-phenylpropylamine (**A** of Scheme 1) or with a substituted alkyl- or arylamine (**B** of Scheme 1) using 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide (EDCI) in the presence of 4-dimethyl-amino-pyridine (DMAP) in dichloromethane to provide corresponding non-substituted 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) to give compounds **30** and **31** in 90–95% yield (**B** of Scheme 1). For the syntheses of compounds **11** and **14** (**C** of Scheme 1), methyl 4-oxocyclohexane-carboxylate for compound **11** and methyl tetrahydro-2H-pyran-4-carboxylate for compound **14** were hydrolyzed with 1N 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 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 1N 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 **A** of Scheme 3. 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 **B** of Scheme 3, *N*-methylated analog 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 of IC₅₀ on human sEH). Generally, the corresponding alkylamide analogs (**2**) of urea inhibitors exhibit approximately a 10–20-fold decreased inhibition potency for human sEH.¹⁹ However, interestingly, a >200-fold drop in inhibition was observed when the similar substituents as that attached to compound **1** were incorporated to the amide function as seen in compound **3**. This suggests that cycloalkylamide structure like compound **3** is much less potent 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 in 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 in 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 in the left side of the urea function.^{17,18} However, at least a >5-fold improvement in inhibition was exhibited when the cycloalkane of compounds **4** or **5** was replaced by a cyclohexyl (**6**) or a cyclohexenyl (**7**) group. This indicates that at least six-membered cycloalkane structure in 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 *t*-butyl (**9**) on the cyclohexane moiety of compound **6** resulted in improved inhibitors (a 3.5–15-fold improvement) over the non-substituted 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 hydroxylmethyl 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 in 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 compounds **8** or **9** resulted. However, no inhibition was observed with the corresponding acid analog (**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 1.5–2-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 in 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 tetrahydro-naphthalene 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, implying that the presence of an aryl in the right side of the amide function is more effective to produce 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 in 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 presence of two (**25**) or four (**26**) methylene carbons between the amide and the benzene ring reduced inhibition potency (a 4–6-fold) for the target enzyme, implying that the phenylpropyl in 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 to improve 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 methyl 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 in 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 non-substituted 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 in 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 functionalized benzyl moiety is more effective than a biaryl structure to produce potent inhibitors for human sEH. Tetrahydronaphthalene (**20**) which was found as 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 in 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 naphthalene structure is very useful to produce highly potent inhibitions for human sEH. As shown in compound **33** with a 2-naphthalene in 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 analog (**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 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 be also 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) in 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**) as 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 that of cycloalkylamide compounds (**29** and **32**) was observed in a lower range (115–125°C). In addition, the inhibitors with an adamantane (**29**) or tetrahydro-naphthalene (**32**) group in the left side of amide pharmacophore showed higher solubility (4–8-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-yl-ureido)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 cycloalkylamide structure is highly promising to develop 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 provides an important base for developing valuable compounds which 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 as 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**) as 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 on the cycloalkane made improved inhibitors in inhibition potency. Furthermore, a sterically hindered cycloalkane (*e.g.* adamantane; **19**) present in the left side of the amide function was useful to enhance inhibition potency. We found a tetrahydronaphthalene (**20**) is the most promising left side substituent for cycloalkylamide derivatives. When a substituted benzyl linker with an ester group was present in the right side of the amide pharmacophore, highly enhanced inhibition potency for the target enzyme was obtained (**29** and **37**). In non-functionalized 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, in 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 to make potent inhibitors in cycloalkylamide compounds. In 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

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 KMnO_4 . The purity of all final compounds was determined to be greater than 95% unless otherwise indicated. Synthetic methods are described for representative compounds.

Synthesis

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 1N NaOH (15 mL) at room temperature. After stirring overnight, the reaction mixture was acidified with an aqueous solution of 1N HCl to pH 2. The water solution was extracted with ethyl acetate (60 mL x 3) and dichloromethane (60 mL), respectively. The combined organic solution was washed with water (60 mL x 2), dried over MgSO_4 , and evaporated to dryness. To the residue (0.89 g, 6.25 mmol) in dichloromethane (50 mL) was added 4-dimethyl-aminopyridine (DMAP; 0.76 g, 6.25 mmol) and 3-phenylpropylamine (0.93 g, 6.88 mmol) at room temperature. After stirring five minute, 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide (EDCI; 1.31 g, 6.88 mmol) was added portionwise to the reaction mixture. Extraction and purification of the product were performed in the same method used for the preparation of compound **32** to afford **11** as a solid (85%). ^1H NMR δ (CDCl_3): 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 $\text{C}_{16}\text{H}_{21}\text{NO}_2$ $[\text{M} + \text{H}]^+$ 260.16, found $[\text{M} + \text{H}]^+$ 260.52, mp 70°C.

Compound **14** was synthesized in 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 x 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), 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 x 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)-cyclohexane-carboxylic acid (65%). Without further purification the residue was used for the next reaction.

To a solution of the above mono-acid (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 hours at room temperature. The product was extracted and purified in the same method used for the synthesis of compound **32** to yield **15** as a solid (90%). ¹H NMR δ (CDCl₃): 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 C₁₈H₂₅NO₃ [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 1N NaOH (3 mL). After stirring overnight at room temperature, the reaction mixture was acidified by adding an aqueous solution of 1N HCl to pH 2. The product was extracted with ethyl acetate (50 mL x 2). The organic layer was washed with water (50 mL), dried over MgSO₄, and evaporated. The residue was recrystallized in ethyl acetate to afford **16** as a solid in 95% yield. 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 C₁₇H₂₃NO₃ [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 in the same method used for the synthesis of compound **32** using the corresponding 1-adamantane-carboxylic 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 stirring overnight, the product was extracted with diethyl ether (60 mL). The organic solution was washed with water (60 mL) twice, dried over MgSO₄, 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 (CDCl₃): 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 C₂₃H₂₅NO₃ [M + H]⁺ 364.18, found [M + H]⁺ 364.77, mp >200°C.

Compounds **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 stirring for 4 hours, the product was extracted with diethyl ether (50 mL) twice. The ether solution was washed with an aqueous solution of 0.5N 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 $\text{C}_{20}\text{H}_{21}\text{NO}_3$ $[\text{M} + \text{H}]^+$ 324.15, found $[\text{M} + \text{H}]^+$ 324.54, mp 115°C.

Compounds **3–14**, **18–29**, **33**, **34**, and **35** were synthesized with the same procedure used for the preparation of **32** using the corresponding cycloalkanecarboxylic acid and alkyl- or aryl-amine 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 stirring for five minutes, 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 $\text{C}_{21}\text{H}_{29}\text{NO}$ $[\text{M} + \text{H}]^+$ 312.21, found $[\text{M} + \text{H}]^+$ 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 hours post-infection, the infected cells were homogenized and the recombinant protein was purified by immobilized metal affinity chromatography. After treating 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-phenyl-oxyran-2-yl)-methyl carbonate; 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 minutes on a fluorometer (Victor3; PerkinElmer).^{30,32} The IC₅₀ values were determined 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.

Solubility

Water solubility of compounds in Table 3 was determined experimentally by light scattering method in sodium phosphate buffer at $25 \pm 1.5^\circ\text{C}$. 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 $25 \pm 1.5^\circ \text{C}$. 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.

References

1. Capdevila JH, Falck JR, Harris RC. Cytochrome P450 and arachidonic acid bioactivation: molecular and functional properties on the arachidonate monooxygenase. *J Lipid Res.* 2000; 41:163–181. [PubMed: 10681399]
2. Yu Z, Xu F, Huse LM, Morisseau C, Draper AJ, Newman JW, Parker C, Graham L, Engler MM, Hammock BD, Zeldin DC, Kroetz DL. Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ Res.* 2000; 87:992–998. [PubMed: 11090543]
3. Imig JD, Zhao X, Capdevila JH, Morisseau C, Hammock BD. Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. *Hypertension.* 2002; 39:690–694. [PubMed: 11882632]
4. Zhao X, Yamamoto T, Newman JW, Kim I-H, Watanabe T, Hammock BD, Stewart J, Pollock JS, Pollock DM, Imig JD. Soluble epoxide hydrolase inhibition protects the kidney from hypertension-induced damage. *J Am Soc Nephrol.* 2004; 15:1244–1253. [PubMed: 15100364]
5. Jung O, Brandes RP, Kim I-H, Schweda F, Schmidt R, Hammock BD, Busse R, Fleming I. Soluble epoxide hydrolase is a main effector of angiotensin II-induced hypertension. *Hypertension.* 2005; 45:759–765. [PubMed: 15699457]
6. Imig JD, Zhao X, Zaharis CZ, Olearczyk JJ, Pollock DM, Newman JW, Kim I-H, Watanabe T, Hammock BD. An orally active epoxide hydrolase inhibitor lowers blood pressure and provides renal protection in salt-sensitive hypertension. *Hypertension.* 2006; 46:975–981. [PubMed: 16157792]
7. Smith KR, Pinkerton KE, Watanabe T, Pedersen TL, Ma SJ, Hammock BD. Attenuation of tobacco smoke-induced lung inflammation by treatment with a soluble epoxide hydrolase inhibitor. *Proc Natl Acad Sci USA.* 2005; 102:2186–2191. [PubMed: 15684051]
8. Schmelzer KR, Kubala L, Newman JW, Kim I-H, Eiserich JP, Hammock BD. Soluble epoxide hydrolase is a therapeutic target for acute inflammation. *Proc Natl Acad Sci USA.* 2005; 102:9772–9777. [PubMed: 15994227]
9. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, Busse R. Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature.* 1999; 401:493–497. [PubMed: 10519554]
10. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, Zeldin DC, Liao JK. Anti-inflammatory properties of cytochrome P450 epoxigenase-derived eicosanoids. *Science.* 1999; 285:1276–1279. [PubMed: 10455056]
11. Campbell WB. New role for epoxyeicosatrienoic acids as anti-inflammatory mediators. *Trends Pharmacol Sci.* 2000; 21:125–127. [PubMed: 10740283]
12. Yang B, Graham L, Dikalov S, Mason RP, Falck JR, Liao JK, Zeldin DC. Overexpression of cytochrome P450 CYP2J2 protects against hypoxia-reoxygenation injury in cultured bovine aortic endothelial cells. *Mol Pharmacol.* 2001; 60:310–320. [PubMed: 11455018]
13. Node K, Ruan X, Dai J, Yang S, Graham L, Zeldin DC, Liao JK. Activation of Gs mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. *J Biol Chem.* 2001; 276:15983–15989. [PubMed: 11279071]
14. Xu D, Li N, He Y, Timofeyev V, Lu L, Tsai H-J, Kim I-H, Tuteja D, Mateo RKP, Singapur A, Davis BB, Low R, Hammock BD, Chiamvimonvat N. Prevention and reversal of cardiac

- hypertrophy by soluble epoxide hydrolase inhibitors. *Proc Natl Acad Sci USA*. 2006; 103:18733–18738. [PubMed: 17130447]
15. Moghaddam MF, Grant DF, Cheek JM, Greene JF, Williamson KC, Hammock BD. Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nat Med*. 1997; 3:562–567. [PubMed: 9142128]
16. Newman JW, Denton DL, Morisseau C, Koger CS, Wheelock CE, Hinton DE, Hammock BD. Evaluation of fish models of soluble epoxide hydrolase inhibition. *Environ Health Perspect*. 2001; 109:61–66. [PubMed: 11171526]
17. Morisseau C, Goodrow MH, Newman JW, Wheelock CE, Dowdy DL, Hammock BD. Structural refinement of inhibitors of urea based soluble epoxide hydrolase. *Biochem Pharmacol*. 2002; 63:1599–1608. [PubMed: 12007563]
18. Kim I-H, Morisseau C, Watanabe T, Hammock BD. Design, synthesis, and biological activity of 1,3-disubstituted ureas as potent inhibitors of the soluble epoxide hydrolase of increased water solubility. *J Med Chem*. 2004; 47:2110–2122. [PubMed: 15056008]
19. Kim I-H, Heitzler FR, Morisseau C, Nishi K, Tsai HJ, Hammock BD. Optimization of amide-based inhibitors of soluble epoxide hydrolase with improved water solubility. *J Med Chem*. 2005; 48:3621–3629. [PubMed: 15887969]
20. Kim I-H, Nishi K, Tsai H-J, Bradford T, Koda Y, Watanabe T, Morisseau C, Blanchfield J, Toth I, Hammock BD. Design of bioavailable derivatives of 12-(3-adamantan-1-yl-ureido)dodecanoic acid, a potent inhibitor of the soluble epoxide hydrolase. *Bioorg Med Chem*. 2007; 15:312–323. [PubMed: 17046265]
21. (a) Kim I-H, Tsai HJ, Nishi K, Kasagami T, Morisseau C, Hammock BD. 1,3-Disubstituted ureas functionalized with ether groups are potent inhibitors of the soluble epoxide hydrolase with improved pharmacokinetic properties. *J Med Chem*. 2007; 50:5217–5226. [PubMed: 17894481]
(b) Kasagami T, Kim IH, Tsai HJ, Nishi K, Hammock BD, Morisseau C. Salicylate-urea-based soluble epoxide hydrolase inhibitors with high metabolic and chemical stabilities. *Bioorg Med Chem Lett*. 2009; 19:1784–1789. [PubMed: 19216074]
22. Jones PD, Tsai H-J, Do Z, Morisseau C, Hammock BC. Synthesis and SAR of conformationally restricted inhibitors of soluble epoxide hydrolase. *Bioorg Med Chem Lett*. 2006; 16:5212–5216. [PubMed: 16870439]
23. Hwang SH, Tsai H-J, Liu J-Y, Morisseau C, Hammock BD. Orally bioavailable potent soluble epoxide hydrolase inhibitors. *J Med Chem*. 2007; 50:3825–3840. [PubMed: 17616115]
24. Rose TE, Morisseau C, Liu J-Y, Inceoglu B, Jones PD, Sanborn JR, Hammock BD. 1-Aryl-3-(1-acylpiperidin-4-yl)urea inhibitors of human and murine soluble epoxide hydrolase: structure-activity relationships, pharmacokinetics, and reduction of inflammatory pain. *J Med Chem*. 2010; 53:7067–7075. [PubMed: 20812725]
25. Anandan S-K, Gless RD. Exploration of secondary and tertiary pharmacophores in unsymmetrical *N,N'*-diaryurea inhibitors of soluble epoxide hydrolase. *Bioorg Med Chem Lett*. 2010; 20:2740–2744. [PubMed: 20363133]
26. Shen HC, Ding F-X, Wang S, Xu S, Chen H-S, Tong X, Tong V, Mitra K, Kumar S, Zhang X, Chen Y, Zhou G, Pai L-Y, Alonso-Galicia M, Chen X, Zhang B, Tata JR, Berger JP, Colletti S. Discovery of spirocyclic secondary amine-derived tertiary ureas as highly potent, selective and bioavailable soluble epoxide hydrolase inhibitors. *Bioorg Med Chem Lett*. 2009; 19:3398–3404. [PubMed: 19481932]
27. 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. Substituted pyrazoles as novel sEH antagonist: Investigation of key binding interactions within the catalytic domain. *Bioorg Med Chem Lett*. 2010; 20:6379–6383. [PubMed: 20934334]
28. Della EW, Tsanaksidis J. Decarboxylation of bridgehead carboxylic acids by the barton procedure. *Australian J Chem*. 1986; 39:2061–2066.
29. Argiriadi MA, Morisseau C, Goodrow MH, Dowdy DL, Hammock BD, Christianson DW. Binding of alkylurea inhibitors to epoxide hydrolase implicates active site tyrosines in substrate activation. *J Biol Chem*. 2000; 275:15265–15270. [PubMed: 10747889]

30. Nishi K, Kim IH, Ma SJ. Expression of the human soluble epoxide hydrolase in *Escherichia coli* by auto-induction for the study of high-throughput inhibition assays. *Protein Expr Purif.* 2010; 69:34–38. [PubMed: 19782755]
31. Morisseau, C.; Hammock, BD. *Current Protocols in Toxicology*. John Wiley&Sons; New Jersey: 2007. Measurement of soluble epoxide hydrolase (sEH) activity. Techniques for analysis of chemical biotransformation; p. 4.23.1-4.23-18.
32. Jones PD, Wolf NM, Morisseau C, Whetstone P, Hock B, Hammock BD. Fluorescent substrates for soluble epoxide hydrolase and application to inhibition studies. *Anal Biochem.* 2005; 343:66–75. [PubMed: 15963942]

Figure 1. Structures of 1,3-disubstituted ureas (**1**) and the corresponding alkyl-(**2**) and cycloalkyl-amides (**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**. IC₅₀ value of compound **3** on human sEH is 97 nM.

Scheme 1.

Syntheses of cycloalkylamide derivatives. (a) 3-phenylpropylamine (in **A** and **C**) or substituted benzyl- or phenyl-amine (in **B**), DMAP, EDCI, CH_2Cl_2 , rt, (b) 4-(2-aminoethyl)benzoic acid (for **30**) or 6-aminonaphthalene-2-carboxylic acid (for **31**), DMAP, EDCI, CH_2Cl_2 , rt, (c) CH_3I , K_2CO_3 , DMF, rt, (d) 1N NaOH, MeOH, rt

Scheme 2.

Syntheses of methyl 3-phenylpropyl cyclohexane-1,4-dicarboxylate (**15**) and its acid analog (**16**). (a) CH₃I, K₂CO₃, DMF, rt, (b) Ba(OH)₂·8H₂O, 80% MeOH in H₂O, rt, (c) 3-phenylpropylamine, EDCI, DMAP, CH₂Cl₂, rt, (d) 1N NaOH, MeOH, rt. For the synthesis of compound **17**, cyclohexane-1,2-dicarboxylic acid was used instead of cyclohexane-1,4-dicarboxylic acid in the reaction (a).

Scheme 3.

Syntheses of naphthaleneamide derivatives (**32–35**) and *N*-methyl-*N*-(3-phenylpropyl)-1-adamantanecarboxamide (**36**). (a) RCOOH, methyl 4-(aminomethyl)-benzoate hydrochloride, EDCI, DMAP, CH₂Cl₂, rt, (b) compound **34**, CH₃I, K₂CO₃, DMF, rt, (c) NaH, CH₃I, DMF, rt.

Table 1

Inhibition of human sEH by cycloalkylamide derivatives.

No.	Structure (R) ^a	Human sEH ^b IC ₅₀ (nM)
4		>1000
5		>1000
6		235
7		222
8		64
9		58

No.	Structure (R) ^a	Human sEH ^b IC ₅₀ (nM)
10		>1000
11		>1000
12		243
13		>1000
14		>1000
15		78

No.	Structure (R) ^a	Human sEH ^b IC ₅₀ (nM)
16	 <chem>OC(=O)C1CCCC(C1)C</chem>	>1000
17	 <chem>COC(=O)C1CCCC(C1)C</chem>	>1000
18	 <chem>COC(=O)C12C=CC1CCC2C</chem>	>1000
19	 <chem>C12C=CC1CCC2C</chem>	41
20	 <chem>C1CCC2=CC=CC=C2CC1</chem>	15

No.	Structure (R) ^a	Human sEH ^b IC ₅₀ (nM)
21		288
22		>1000
23		>1000

^aNo steric isomers or mixtures of *cis/trans* or *R/S* unless otherwise indicated.

^bHuman 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.

Table 2

Inhibition of human sEH by adamantane- and naphthalene-amide derivatives.

No.	Structure	Human sEH ^a IC ₅₀ (nM)
24		>1000
25		230
26		180
27		101
28		>1000
29		9.1
30		230
31		65
32		2.1

No.	Structure	Human sEH ^a IC ₅₀ (nM)
33		6.6
34		1.8
35		2.4
36		723

^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.

Table 3

Melting point and water solubility of adamantane- and naphthalene-amide derivatives.

No.	Melting point (°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

^aWater 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.

^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 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).