Biologically Active Ester Derivatives as Potent Inhibitors of the Soluble Epoxide Hydrolase

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Abstract

Substituted ureas with a carboxylic acid ester as a secondary pharmacophore are potent soluble epoxide hydrolase (sEH) inhibitors. Although the ester substituent imparts better physical properties, such compounds are quickly metabolized to the corresponding less potent acids. Toward producing biologically active ester compounds, a series of esters were prepared and evaluated for potency on the human enzyme, stability in human liver microsomes, and physical properties. Modifications around the ester function enhanced *in vitro* metabolic stability of the ester inhibitors up to 32-fold without a decrease in inhibition potency. Further, several compounds had improved physical properties.

Keywords

sEH; sEH inhibitors; substituted urea-ester derivatives; metabolic stability

Epoxyeicosatrienoic acids (EETs), which are metabolites of arachidonic acid by cytochrome P450 epoxygenases, have important roles in the regulation of hypertension,[1–6] inflammation,[7–11] and other cardiovascular related diseases.[12–13] Recently a role in modulating pain was also reported.[14] However, metabolism of EETs (**1**) to their corresponding hydrated products (**2**) by soluble epoxide hydrolase (sEH) generally reduces these biological activities (Figure 1).[1] Both *in vitro* and *in vivo* studies have demonstrated that the anti-hypertensive and cardio protective effects mediated by the EETs are reversely dependent on the extent of sEH hydrolysis of the EETs.[2–4,6–8,13,15] Thus, maintaining the *in vivo* concentration of EETs through sEH inhibition is a promising therapeutic pathway to treat cardiovascular and other diseases. Urea, amide, and carbamate 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

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Supplementary Data Syntheses and detailed analytical data for compounds **5–13**, IC_{50} assay conditions, *in vitro* metabolic stability in human liver microsomes, and water solubility are available.

properties of the early compounds, such as low solubilities and high melting points, likely result in limited *in vivo* availability.[17] The addition of a polar functional group on specific positions of one of the urea-substitution 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] Among a variety of polar functional groups explored previously, ester functionalities located on the fifth atom from the urea function were found to be among the most suitable polar groups for making potent inhibitors with improved physical properties.[18] However, as expected, the ester functionality is rapidly hydrolyzed both *in vitro* and *in vivo* producing the corresponding short chain acid metabolite that has dramatically reduced inhibition potency on the human sEH.[20] Although rapid enzymatic hydrolysis of the ester compound is not favorable for high *in vivo* efficacy, it offers some advantage. First, alcohol moieties of ester groups can be rapidly altered to explore binding properties of the right hand side of the inhibitors such as those shown in Scheme 1. Second, the introduction of a secondary pharmacophore roughly 7–8 Å from the urea or amide carbonyl of the primary pharmacophore often improves solubility while retaining high potency.[18,21–23] Finally, to date the urea inhibitors of the sEH appear highly selective with low toxicity. The epoxy-lipids stabilized by these inhibitors have numerous biological activities. Although the activities of the epoxy-lipids are so far found to be beneficial, it may be important to direct the activities to individual tissues. Administering short acting compounds may be also important for the treatment of pulmonary inflammation by inhalation. Thus for these latter two approaches it is important to develop esters of varying metabolic stability with increasing stability for classical systemic administration and decreasing stability for short term or local effects.[24] In the present study, we explored structural modifications of urea-ester inhibitors toward altering the enzymatic stability of the ester function in an *in vitro* metabolic system while maintaining sEH inhibition potency and appropriate physical properties.

Compound **3**, which was prepared as previously reported,[18,21] was coupled with a corresponding alcohol by using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI) in the presence of 4-dimethylaminopyridine (DMAP) in dichloromethane to afford compounds **5**, **6**, and **8** or was alkylated with 1-bromopentane in the presence of K_2CO_3 in DMF to afford compound **4** in a range of 64–95% yield (Scheme 1 (A)). For the synthesis of cyanoalkyl ester (**7**), 1-pentanal was first reacted with trimethylsilyl cyanide in the presence of zinc iodide in chloroform at 0°C, followed by hydrolysis with an aqueous solution of 3N HCl (80%). The resulting cyanohydrin intermediate was then coupled with compound **3** by using EDCI and DMAP in dichloromethane to provide **7** in 70% yield. In addition, ethoxyethanol with a phenyl group, which was prepared by the reaction of 2-ethoxyethanol with 2-phenyloxirane, was coupled with compound **3** in the above same method as that used for the preparation of compound **7** to afford compound **9** in 48% yield. Scheme 1 (B) shows syntheses of derivatives with a cyclohexyl linker (**10–13**) between the urea and ester pharmacophores.[21] Compound **10**, which was prepared from the coupling of 1-adamantyl isocyanate with 3-aminocyclohexanoic acid, was alkylated or coupled with a corresponding bromide or alcohol in the same reaction condition as that used for the preparation of compounds **4–9** in Scheme 1 (A) to give target compounds **11–13** in 65–95% yield.[21]

Five derivatives (**5–9**) were prepared to first explore the modification effect of the alcohol moiety of the ester function of compound **4** on inhibition potency. As shown in Table 1, the incorporation of a methyl group (**5**) on the *alpha* carbon of the alcohol moiety showed a 2.5-fold improvement in inhibition potency compared to compound **4**. An ethyl group (**6**) substituted on the *alpha* carbon of compound **4** exhibited a similar enhancement in potency, indicating that an alkyl substituent on the *alpha* position is effective for improving potency. On the other hand, the introduction of a cyano group (**7**) induced a 3-fold decrease in inhibition potency in comparison with that of methyl (**5**) or ethyl (**6**) groups. This suggests

that hydrophobic alkanes are better than a polar group to be incorporated on the *alpha* carbon of the alcohol moiety of the ester function for enhanced inhibition potency. In a previous report, we demonstrated that diethylene glycol groups attached to hydrophobic urea inhibitors are useful for improving water solubility and bioavailability without a loss in inhibition potency.[21] Two derivatives with a diethylene glycol group (**8** and **9**) were prepared to investigate whether the polar ethylene glycol group affects inhibition potency of urea-ester compounds. As illustrated by compound **8**, attachment of a diethylene glycol resulted in a 7–17-fold reduction in inhibition when compared to those of non-substituted (**4**) and alkyl substituted compounds (**5** and **6**). However, interestingly, a big improvement in inhibition was obtained by incorporating a phenyl group (**9**) on the *alpha* carbon of the alcohol moiety of compound **8**, which was as potent as that of the alkyl derivatives (**5** and **6**). This further indicates that a hydrophobic substituent on the *alpha* carbon of the ester function is highly effective to lead to potent urea-butyrate compounds as inhibitors of the human sEH. As described, the corresponding acid compound (**3**) of butyrate derivatives in Table 1 was poor as an inhibitor of the human enzyme. In addition, when a corresponding cyclohexylcarboxylic acid was synthesized as seen in compound **10** in Table 2, further reduced inhibition was exhibited. This indicates that a short chain acid attached to the urea function like urea-cyclohexylcarboxylic acid is a poor inhibitor and substitution of the acid could also result in potent compounds. When cyclohexylcarboxylic acid esters were prepared as seen in Table 2, as expected, a dramatic improvement in inhibition on the target human enzyme was observed in compound substituted with a pentyl group (**11**). The incorporation of an ethyl group (**12**) on the *alpha* carbon of the alcohol moiety of the pentyl ester **11** provided a highly potent inhibitor, as well. A diethyleneglycol group with a phenyl also made a potent inhibitor (**13**), although it was less potent than compounds **11** or **12**. The results in Table 2 imply that alkylation of the acid function of urea-cyclohexylcarboxylic acid (**10**) is effective for producing highly potent inhibitors like butyrate derivatives in Table 1.

In general, liphophilicity of compounds causes limited solubility in water, which probably affects *in vivo* efficacy of compounds.[17,21,25] In addition, the stability of the crystals of compounds, indicated by their high melting points, leads 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. We examined physical properties of the above potent derivatives in Tables 1 and 2. As seen in Table 3, butyric acid (**3**) and the corresponding ester derivatives (**4** and **6**) had very low solubility in water, while dramatic improvement in solubility was observed in diethylene glycol compounds (**8** and **9**). Similar increase in solubility was obtained in cyclohexylcarboxylic acid ester with a diethylene glycol group (**13**). The diethylene glycol derivatives (**8**, **9**, and **13**) also possessed much lower melting points in comparison with the acids (**3** and **10**) or other ester compounds (**4**, **6**, and **12**). This result indicates that hydrophilic alcohol moiety of the ester function of compounds **9** and **13** is highly beneficial for improving physical properties while producing potent inhibition. In order to see *in vitro* metabolism of the urea-ester compounds we used human liver microsomes, which contain various kinds of enzymes such as esterases, amidases, and cytochrome P450s playing a primary role in drug metabolism. When compounds **3** and **10** were tested for microsomal incubation, >95% of the parent acids were determined. This implies that the adamantylurea-acid structure of compounds **3** and **10** are stable under the incubation conditions and the human liver microsomal incubation is useful to investigate relative *in vitro* metabolic stability of urea-ester compounds. As expected, pentyl ester of butyric acid (**4**) was all metabolized to the corresponding butyric acid (**3**) in the incubation. On the other hand, a little increased stability was shown in compound **6** with an ethyl branch on the *alpha* carbon of the ester function. Further, diethylene glycol compounds (**8** and **9**) had >2.5-fold

improved metabolic stability compared to compound **6**, suggesting that a polar alcohol moiety of the ester function is more effective for improving the ester stability over a hydrophobic alkyl group. Interestingly, cyclohexylcarboxylic acid ester derivatives (**12** and **13**) exhibited much higher metabolic stability than the corresponding butyric acid esters (**6** and **9**). Comparing both compounds with an ethyl branch on the *alpha* carbon (**6** and **12**) a 2.5-fold increase in stability was observed in the cyclohexane derivative (**12**). Approximately a 2-fold improved stability was also shown in compound **13** compared to that of the corresponding ethyleneglycol butyric acid ester (**9**), indicating that sterically rigid cyclohexane linker present between the urea primary pharmacophore and the ester secondary pharmacophore is highly effective for increasing the ester stability. The results in Table 3 showed that solubility and stability of ureaester compounds are varied up to 390-fold and 32-fold, respectively, through incorporation of a substituent and/or modification in the linker structure.

In conclusion, we synthesized a series of urea-ester compounds as inhibitors for the human soluble epoxide hydrolase (sEH) to produce compounds of varying metabolic stability by improving the biodegradable ester function while possessing potent inhibition and improved physical properties. The SAR study showed that the incorporation of an alkyl group on the *alpha* carbon of the alcohol moiety of the ester increased inhibition potency on the sEH, while a polar group in the alcohol moiety resulted in a big decrease in potency. However, interestingly, a substituted polar group with a hydrophobic function made potent inhibitors (**9** and **13**). Moreover, the potent polar compounds (**9** and **13**) exhibited approximately a 20–45-fold improved water solubility and low melting points when compared to those of alkyl substituted inhibitors (**4** or **6**). Because we found that adamantylureas with a short chain acid (**3** and **10** in Table 3) are not metabolized in incubation with human liver microsomal enzymes, this incubation was used to investigate relative ester bond stability of urea-ester compounds. Among the modified urea-ester derivatives, the highest improvement (32-fold) in stability was obtained from a cyclohexyl analogue with a polar substituent (**13**). Approximately a 2-fold lower stability was observed when the cyclohexyl linker of compound **13** was replaced by an alkyl chain linker (**9**). In compounds with a cyclohexyl linker (**12**) or polar group (**8**), a 3-fold decreased stability was shown compared to that of **13**. A dramatic decrease in stability was exhibited in butyrate derivatives with an alkyl substitution (**4** and **6**). The overall structural modifications altered the relative metabolism of the ester inhibitors up to 32-fold without a decrease in inhibition potency. Further, several compounds had improved physical properties. These findings will facilitate development of biologically active urea-ester compounds as potent inhibitors of the sEH. The ester compounds described in this study could be particularly applicable for administration directly to inflamed tissue by inhalation for chronic obstructive pulmonary disorder (COPD) or asthma. Alternatively they could be given by slow release formulations to address inflammatory bowel diseases.[26] sEH inhibitors have been shown to be active on a variety of cardiovascular, inflammatory, and pain related disorders in rodents.[27,28]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

sEH   soluble epoxide hydrolase

EET   epoxyeicosatrienoic acid

EDCI   1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide

DMAP   4-dimethylaminopyridine

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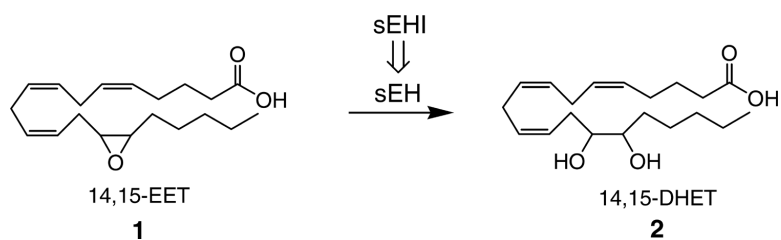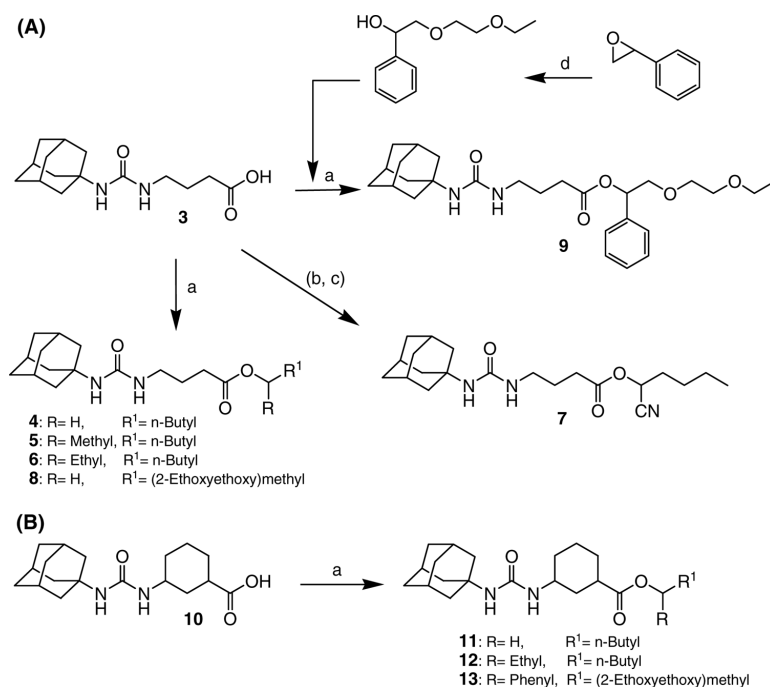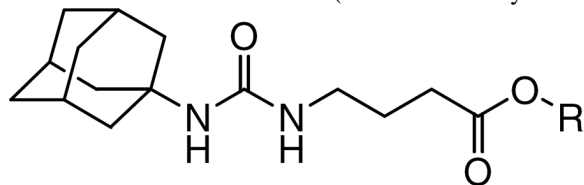
Figure 1.
Metabolism of biologically active EETs (**1**; *e.g.* 14,15-EET) to the less active DHETs (**2**; *e.g.* 14,15-DHET) by sEH. The sEH converts a variety of lipid epoxides to their corresponding diols. The sEHIs act by blocking this enzyme, increasing the *in vivo* concentrations of EETs which have direct actions on hypertension, inflammation, and pain. EET, epoxyeicosatrienoic acid; DHET, dihydroxyeicosatrienoic acid; sEHI, soluble epoxide hydrolase inhibitor.

Scheme 1.
Syntheses of 4-(3-adamantan-1-yl-ureido)-butyric acid or -cyclohexanecarboxylic acid derivatives: (a) EDCI, DMAP, a corresponding alcohol (2-hexanol for compound **5**, 3-heptanol for compounds **6** and **12**, and diethylene glycol monoethyl ether for compounds **8** and **13**) in MC or 1-bromopentane for compounds **4** and **11** in DMF, rt, (b) pentanal, TMSCN, ZnI, chloroform, rt / 3N HCl (c) coupling of compound **3** with the product of reaction (b) by using EDCE/DMAP in MC, rt, (d) 2-ethoxyethanol, NaH, DMF, rt.

Table 1

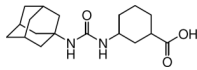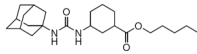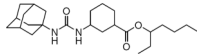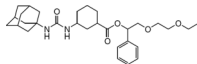Inhibition of human sEH of 4-(3-adamantan-1-yl-ureido)-butyric acid derivatives.

Compound	R	Human sEH IC$_{50}$ (nM)^{a}
3	H	684
4		10
5		4
6		4
7		12
8		67
9		4

^{a}Human sEH (0.96 nM) was incubated with inhibitors for 5 min in 25 mM Bis-Tris/HCl buffer (200 μL; pH 7.0) at 30°C before fluorescent substrate introduction ([S] = 5 μM). Results are averages of three separate measurements. The fluorescent assay as performed here has a standard error between 10 and 20%, suggesting that differences of 2-fold or greater are significant.

Table 2

Inhibition of human sEH of 3-(3-adamantan-1-yl-ureido)-cyclohexylcarboxylic acid derivatives.

Compound	Structure	Human sEH IC$_{50}$ (nM)[a]
10		1160
11		2
12		3
13		9

[a]Human sEH (0.96 nM) was incubated with inhibitors for 5 min in 25 mM Bis-Tris/HCl buffer (200 μL; pH 7.0) at 30°C before fluorescent substrate introduction ([S] = 5 μM). Results are averages of three separate measurements. The fluorescent assay as performed here has a standard error between 10 and 20%, suggesting that differences of 2-fold or greater are significant.

Table 3

Physical properties and *in vitro* metabolic stability of 4-(3-adamantan-1-yl-ureido)-butyric acid or -cyclohexylcarboxylic acid derivatives.

Compound	Solubility in water[a] (μg/mL)	MP (°C)	Stability[b] (% remaining)
3	7	165	97
4	2	114	<1
6	3	72	4
8	878	oil	10
9	89	oil	14
10	ND[c]	>250	95
12	1	124	10
13	41	oil	32

[a]An excess of the test compound was added to a vial containing sodium phosphate buffer, 0.1 M pH 7.4 (1 mL), and a suspension of the mixture was equilibrated during 1 hr of sonication and 24 hrs of shaking, followed by centrifugation. The water supernatant was analyzed by LC-MS/MS. A regression curve for each compound was obtained from five standard stock solutions by using LC-MS/MS. Then, the absolute amount of each compound was calculated.[19]

[b]Human liver microsomal protein (0.125 mg) was incubated with a solution of test compound under a NADPH generating system. Incubation mixture was shaken at 37°C for 60 min. The absolute quantity of parent compounds remaining after the incubation was analyzed by LC-MS/MS and was converted to a percentage. The corresponding acid metabolites (**3** and **10**) of ester compounds were analyzed in all determinations (data not shown). The results given are averages of triplicate independent analyses.

[c]Not-determined because the crystal of compound **10** was not dissolved in water which seems to be due to the high melting point (> 250°C).