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1,3-Disubstituted and 1,3,3-trisubstituted adamantyl-ureas with isoxazole as soluble epoxide hydrolase inhibitors

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Abstract

Adamantyl ureas are good soluble epoxide hydrolase (sEH) inhibitors; however they have limited solubility and rapid metabolism, thus limiti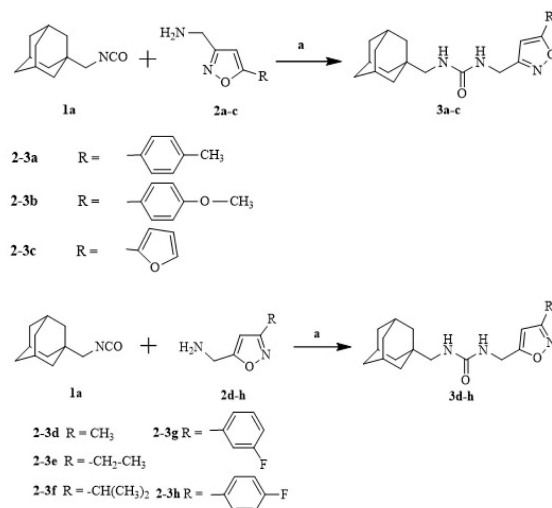ng their usefulness in some therapeutic indications. Herein, we test the hypothesis that nodal substitution on the adamantane will help solubilize and stabilize the compounds. A series of compounds containing adamantane derivatives and isoxazole functional groups were developed. Overall, the presence of methyl on the nodal positions of adamantane yields higher water solubility than previously reported urea-based sEH inhibitors while maintaining high inhibition potency. However, it did not improve microsomal stability.

Graphical abstract

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2-3a R = [benzene ring]—CH₃

2-3b R = [benzene ring]—O—CH₃

2-3c R = [furan ring]

2-3d R = CH₃   2-3g R = [benzene ring with F]

2-3e R = -CH₂-CH₃

2-3f R = -CH(CH₃)₂   2-3h R = [benzene ring]—F

Keywords

soluble epoxide hydrolase; inhibitor; adamantane; isocyanate; urea; isoxazole

The mammalian soluble epoxide hydrolase (sEH, E.C. 3.3.2.10) is involved in the metabolism of epoxy-fatty acids to vicinal diols through a catalytic addition of a water molecule.[1,2] Endogenous substrates for the sEH include epoxides of arachidonic acid, epoxyeicosatrienoic acids (EETs), and of docosahexaenoic acid, known as EpDPEs, and other fatty acid epoxides.[3,4] EETs are important lipid mediators that have key roles in blood pressure regulation by exerting vasodilatory effects through the activation of the Ca^{2+}-activated K^+ channels in endothelial cells, which are beneficial in many renal and cardiovascular diseases.[5,6] Furthermore, the EETs and EpDPEs have some anti-inflammatory and analgesic properties.[7] Recently, EETs have been reported to increase efficiency of organ transplant.[8] Their conversion to dihydroxyeicosatrienoic acids (DHETs) by sEH produces a molecule that is readily conjugated and removed from the site of action. The inhibition of sEH *in vivo* by highly selective inhibitors results in an increase in the concentration of EETs and EpDPEs, and is accompanied by a reduction in angiotensin driven blood pressure, but also reduction of inflammation and pain, thereby suggesting that sEH is a promising target for the treatment of hypertension, inflammatory diseases and pain.[9-11]

Early on, small N,N'-disubstituted symmetric ureas, such as 1,3-dicyclohexyl urea, were found to be very potent inhibitors of sEH.[12-16] However, these kinds of compounds have poor solubility in many solvents. To improve solubility, asymmetric ureas with a flexible side chain, such as AUDA (12-(3-adamantylureido)-dodecanoic acid) or AEPU (1-adamantanyl-3-{5-[2-(2-ethylethoxy)ethoxy]pentyl]}urea), were developed.[17] While this class of sEH inhibitor shows biological effects when tested *in vivo*, they are rapidly metabolized, limiting their utility.[18] Interestingly, a major site of metabolism for these compounds is on the adamantine, although beta oxidation of the side chains by CYPs is also important.[19] Therefore, to improve the metabolic stability, a third class of conformationally

restricted inhibitors, in which the adamantine was replaced by phenyl derivatives such as TPAU (1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl) urea) or *trans*-4-{4-[3-(4-trifluoromethoxyphenyl)-ureido] cyclohexyloxy} benzoic acid (*t*-TUCB*)*, were designed.[20,21] This latest series includes very potent and more metabolically stable sEH inhibitors that permit *in vivo* studies.[22] However, these compounds have in general poorer solubility than the corresponding adamantane containing compounds, and are expensive to synthesize since several steps (up to 5) are required.

A promising way to enhance the water solubility of the urea inhibitors of sEH is the introduction of heterocyclic moieties.[23] For example, ureas synthesized with amino-pyridazines, pyridines, pyrimidines, triazines, oxazoles and thiazoles containing amino groups showed high potency against epoxide hydrolases from *Mycobacterium tuberculosis*.[24] Thus, here as a continuation of previous work[25,26] we report the testing of adamantyl-ureas containing isoxazoles, however we also tested the hypothesis that nodal substitution on the adamantane will help solubilize and stabilize the resulting compounds because for adamantane containing urea inhibitors hydroxylation of nodal carbon is important metabolic pathway leading to a decrease of inhibitory potency.

Ureas were synthesized from 1-(isocyanatomethyl)adamantane (**1a**) or 1-isocyanato-3,5-dimethyladamantane (**1b**) and 3,5-disubstituted isoxazoles with aminomethyl or pyrrolidine-2-yl as one of the substituents. Compound **1a** was selected due to the flexibility-giving methyl spacer. The starting isoxazoles also contain a methylene bridge between the amino group and the isoxazole ring, in some cases this bridge included the pyrrolidine ring. Selected isoxazoles contain electron donor (Me, Et, *i*-Pr) as well as electron acceptor (4-MeC$_6$H$_4$, 4-MeOC$_6$H$_4$, 3(4)-FC$_6$H$_4$) substituents and amino methyl groups in the 3 and 5 positions.[27-33] Such a series of disubstituted isoxazoles will allow us to investigate the influence of its structure on the urea formation reaction and on the inhibition potency of corresponding ureas.

As described in scheme 1, simple (one step) and complementary approaches were used to obtain the desired compounds in high yield (> 95%).

The inhibition potency of the synthesized compounds was measured using recombinant purified human sEH and CMNPC (cyano(6-methoxynaphthalen-2-yl)methyl ((3-phenyloxiran-2-yl)methyl) carbonate) as a substrate as described.[34] For the isoxazole containing 1,3-disubstituted ureas, the best inhibitory potency (4.9 nM) was recorded for the compound **3c** with furyl group in 5th position of isoxasole ring (Table 1). Compound **3d** with the methyl substituent in 5th position of isoxasole ring has good inhibition potency (6.7 nM). Further increase of this substituent to the ethyl (**3e**) and *i*-propyl (**3f**) leads to the 30- and 45-fold decrease in potency (higher IC$_{50}$) and accompanied decrease in water solubility. Potency of the ureas with phenyl substitutents in 5th position of isoxasole ring is only slightly affected by the structure of those groups and ranges from 10.5 to 16.6 nM.

Due to the low reactivity of secondary amines in the reaction of nucleophilic addition to isocyanates reactions of isoxazoles **2i-m** were carried out at 80 °C in presence of Et$_3$N (Scheme 2).

Potency of 1,3,3-trisubstituted ureas clearly shows that availability of both NH in the urea group is vital for the satisfactory inhibition of sEH. Disruption of the ability to create one hydrogen bond leads to an up to significant decrease in activity in some cases. For example compound **3k** is >5-fold less active than the corresponding 1,3-disubstituted urea **3f**. Ureas synthesized from isoxazole with amino methyl group in 3rd position are more potent (up to 5-fold) than those with the amino methyl group in the 5th position. Probably this is due to the formation of the additional hydrogen bonds. The hydrogen bond acceptor in ureas synthesized from 5-amino isoxazoles is probably not able to reach the necessary distance to create such a bond.

While 1,3-disustituted ureas containing the adamantane moiety were widely investigated as soluble epoxide hydrolase inhibitors only 1-isocyanato adamantane was used for their formation. Introduction of methyl groups into nodal positions of adamantane significantly lowers melting point of the its derivatives. Moreover, the metabolism of adamantyl-containing 1,3-disubstituted ureas *in vivo* proceeds through the hydroxylation of nodal positions and the corresponding hydroxy derivatives have up to 50 fold less inhibitory activity.[23] In this case we decided to synthesize sEH inhibitor from 1-isocyanato-3,5-dimethyladamantane (**1b**).[24] The boiling point of compound **1b** (93 °C at 10 torr)[36] is lower than that of compound **1a** (98 °C at 10 torr)[36] so we assumed a corresponding reduction of melting points would occur with the appropriate 1,3-disubstituted ureas.

For the compounds **4a-4c** and **4g** the replacement of the adamantane part of the molecule did not result in noticeable change of the inhbitory potency. Surprisingly, compound **4d** is 10-fold less potent than **3d**, which is the second-best inhibitor of this series. However, we have mentioned the tendency of each new methyl group introduced into the aliphatic substituent in 5th position of isoxazole to reduce potency. In this case, we can conclude that the additional methyl groups for this series of ureas caused a decrease in the inhibitory activity as well as water solubility (comparing data in table 1 and 2). But presence of the phenyl or furyl rings negate this effect. Introduction of isocyanate **1b** instead of **1a** leads to the reduction of melting points of corresponding ureas at about 10 °C but additional methyl groups generally result in 2-fold decreased water solublity. Decreased water solubility is correlated with cLogP (Table 4).

To further assess the properties of the compounds, we measured their stability in human liver microsomes (Table 4).

However contrary to our assumption introduction of methyl groups to the nodal positions of adamantane did not improve microsomal stability of the corresponding ureas. This effect was observed probably due to the rapid metabolism of isoxazole ring, although isolated methyl groups often are rapidly oxidized by CYP enzymes.

We describe the synthesis and structure-activity relationship of a series of adamantylureas with isoxazole ring containing various substituents. Some ureas have methyl groups in nodal positions of adamantane. The data show that such ureas show very good inhibition potency along with high water solubility (compared to previously reported urea-based sEH inhibitors). We showed that both NH in urea group are essential for the inhibitor binding on

the active site of sEH. Introduction of methyl groups to the nodal positions of the adamantane did not affect inhibition potency (which indicates that there is still sufficient space in the cavity of the enzyme) as long as not affect microsomal stability but lead to the reduction of water solubility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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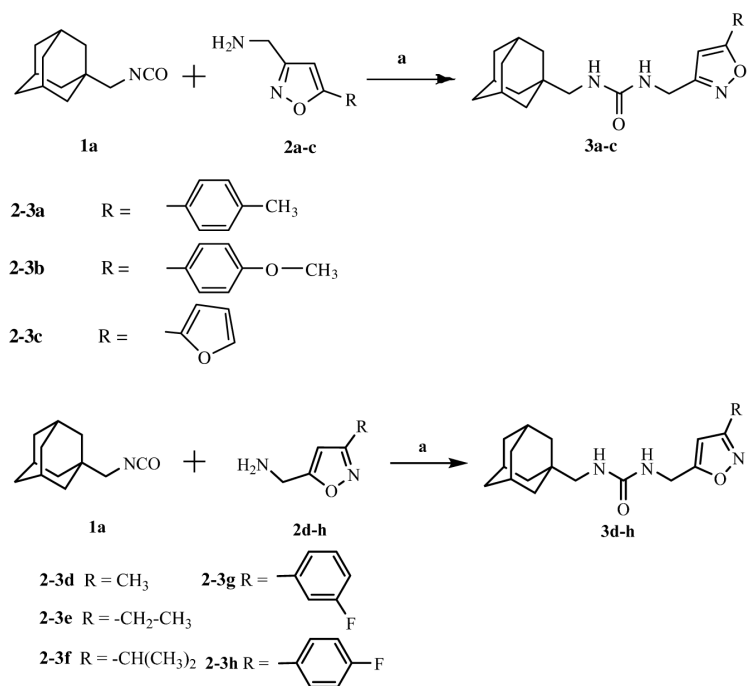
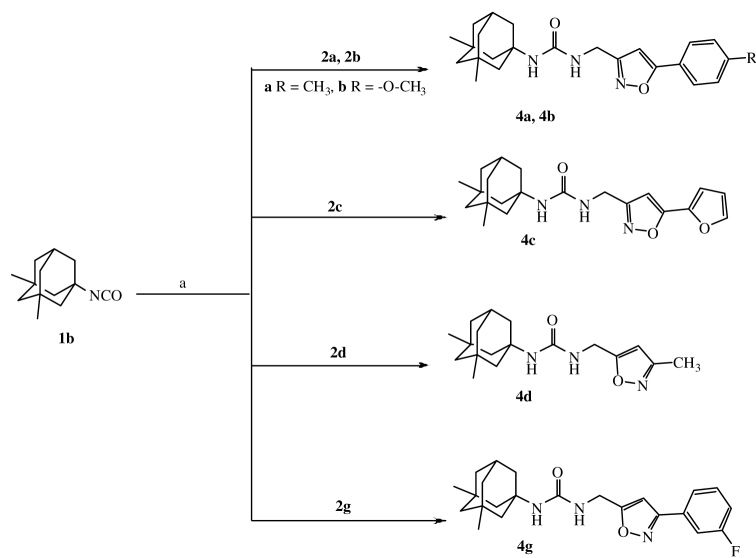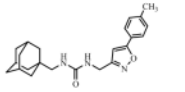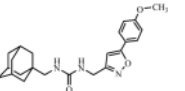**Scheme 1.**
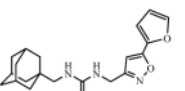Reagents and conditions: (a) DMF, rt, 12 h
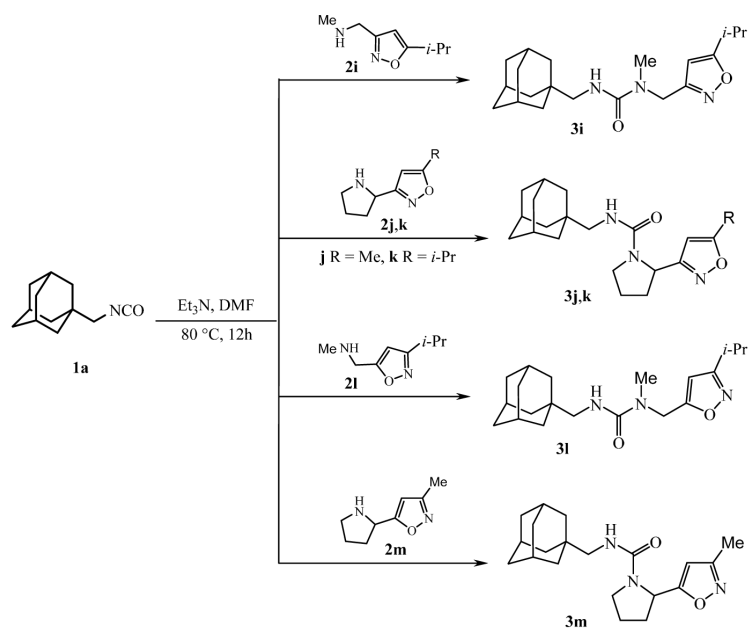
Scheme 2.
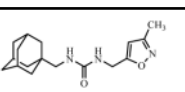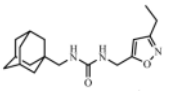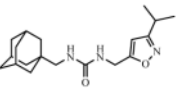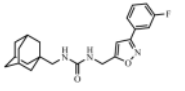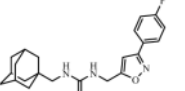Synthesis of 1,3,3-trisubstituted ureas with isoxazole part
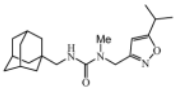
Scheme 3.
Reagents and conditions: (a) DMF, rt, 12 h

Table 1

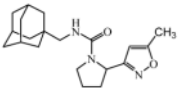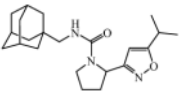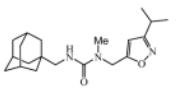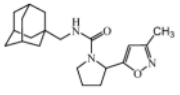IC$_{50}$ values for the isoxazole 1,3-disubstituted urea-based sEH inhibitors 3a-h

#	Structure	IC$_{50}$ (nM)^a	Solubility^b (µM)	mp (°C)
3a		15.4	75 < S < 100	198–199
3b		12.9	100< S < 125	180–181
3c		4.9	75 < S < 100	174–175
3d		6.7	450 < S < 475	135–137
3e		29.4	300 < S < 325	120–121
3f		44.6	125 < S < 150	108–109
3g		16.6	75 < S < 100	160–161
3h		10.5	50 < S < 75	186–187

^{a}As determined via a kinetic fluorescent assay.[34, 35]

^{b}Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.

Table 2

IC$_{50}$ values for the isoxazole 1,3,3-trisubstituted urea-based sEH inhibitors 3i-m

#		IC$_{50}$ (nM)^a	Solubility^b (µM)	mp (°C)
3i		575.8	200 < S < 225	119-120
3j		524.3	525 < S < 550	176–177
3k		247.2	250 < S < 275	128–129
3l		190.6	350 < S < 375	146–148
3m		91.0	575 < S < 600	157–158

^{a}As determined via a kinetic fluorescent assay.[34, 35]

^{b}Solubility was measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.

Table 3

IC$_{50}$ values for the isoxazole 1,3-disubstituted urea-based sEH inhibitors with 1,3-dimethyladamantane moiety 4a-d and 4g.

#		IC$_{50}$ (nM)^a	Solubility^b (μM)	mp (°C)
4a		25.8	$125 < S < 150$	185-186
4b		15.0	$25 < S < 50$	189-190
4c		4.1	$75 < S < 100$	149-150
4d		123.0	$200 < S < 225$	130-131
4g		17.0	$75 < S < 100$	146-147

^{a}As determined via a kinetic fluorescent assay.[34, 35]

^{b}Solubilities were measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.

Table 4

IC$_{50}$, water solubility and microsomal stability of selected compounds.

#	IC$_{50}$ nM[a]	Solubility[b] (μM)	cLogP[c]	Microsomal stability (%)[d] NADPH	
				−	+
3a	15.6 ± 0.7	75 < S < 100	4.24	97 ± 4	0.4 ± 0.2
4a	28.8 ± 4.5	125 < S < 150	4.50	115 ± 11	0.4 ± 0.2
3b	12.5 ± 1.8	100< S < 125	3.85	87 ± 6	0.8 ± 0.2
4b	18.0 ± 2.9	25 < S < 50	3.89	102 ± 11	0.4 ± 0.3
3c	4.9 ± 0.1	75 < S < 100	2.59	91 ± 8	0.4 ± 0.1
4c	4.8 ± 0.6	75 < S < 100	2.63	95 ± 1	0.3 ± 0.1
3d	9.9 ± 2.9	450 < S < 475	2.36	72 ± 4	2.9 ± 0.5
4d	111.1 ± 10.3	200 < S < 225	2.62	83 ± 3	0.1 ± 0.1
3g	13.7 ± 3.0	75 < S < 100	3.91	97 ± 10	0.3 ± 0.1
4g	14.2 ± 2.5	75 < S < 100	4.17	92 ± 6	0.4 ± 0.1

[a] As determined via a kinetic fluorescent assay.[34, 35] Results are average ± standard deviation of three separate measurement.

[b] Solubility was measured in sodium phosphate buffer (pH 7.4, 0.1 M) containing 1% of DMSO.

[c] Calculated using ChemBioDraw Ultra v12.0 (PerkinElmer, Waltham, MA).

[d] Percent of compound (1 μM) remaining after 30 minutes incubation with human liver microsomes (1 mg/mL) at 37°C with or without NADPH generating system. Results are the average of triplicates ± standard deviation.