

Published in final edited form as:

Bioorg Med Chem Lett. 2012 January 1; 22(1): 601–605. doi:10.1016/j.bmcl.2011.10.074.

Design, synthesis and evaluation of non-urea inhibitors of soluble epoxide hydrolase

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Abstract

Inhibition of soluble epoxide hydrolase (sEH) has been proposed as a new pharmaceutical approach for treating hypertension and vascular inflammation. The most potent sEH inhibitors reported in literature to date are urea derivatives. However, these compounds have limited pharmacokinetic profiles. We investigated non-urea amide derivatives as sEH inhibitors and identified a potent human sEH inhibitor **14-34** having potency comparable to urea-based inhibitors.

Keywords

Soluble epoxide hydrolase; sEH; Non-urea inhibitors; Hypertension; Anti-inflammatory

Soluble epoxide hydrolase (sEH) is an enzyme that is involved in the metabolism of lipid epoxides.¹ This enzyme is found in various mammalian tissues and is mostly located in liver, kidneys and vascular tissues.² It converts endogenous substrate—epoxyeicosatrienoic acids (EETs)—to dihydroxy eicosatrienoic acids (DHETs), which show abolished or diminished or changed biological activity.³ EETs act as vasodilators in various arteries (renal, cerebral, and coronary),⁴ protect from ischemic injury⁵ and manifest anti-inflammatory properties.⁶ sEH inhibition increases cellular EETs levels and promote this activity. Several preclinical studies suggest that inhibition of sEH may represent a novel approach to treat hypertension, organ protection and inflammation.^{7–10} Furthermore, it has been shown that sEH inhibition reduces pain in several animal models.¹¹

Initial sEH inhibitors with IC₅₀ in the lower nanomolar range included *N,N'*-disubstituted ureas, *N,N'*-dicyclohexylurea (DCU) and *N*-adamantyl-*N'*-cyclohexylurea (ACU) (Fig. 1).¹² However, poor water solubility and limited *in vivo* studies¹³ led to a design of the second generation of sEH inhibitors, 12-(3-adamantan-1-ylureido) dodecanoic acid (AUDA) and 1-adamantan-1-yl-3-{5-[2-(ethoxyethoxy) ethoxy]pentyl}urea (AEPU) with improved water solubility and maintained inhibition.^{14,15} However, these inhibitors suffered from rapid metabolism *in vivo*.¹⁶ Recent studies have focused on piperidine-based di- and trisubstituted ureas, such as *N*-(1-(2,2,2-trifluoroethanoyl)piperidin-4-yl)-*N'*-(adamant-1-yl)urea (TPAU) and *N*-(1-acetylpiperidin-4-yl)-*N'*-(adamant-1-yl)urea (APAU).^{17,18} Some

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of these piperidine-based compounds possess low nano to picomolar activity and good pharmacokinetic properties in different animal models.^{17–21}

These extensive structure–activity relationship (SAR) studies suggested that the pharmacophore for sEH inhibitors should include a central urea moiety for hydrogen bonding to two tyrosine residues (Tyr381 and Tyr465) and one Asp333 residue—all three located in the hydrolase catalytic pocket of sEH.²²

Several common urea modifications such as thiourea, sulfonyl urea, amide and aminomethylene amide have been incorporated into central pharmacophore in order to maintain similar binding profile to that of urea but improve potential therapeutic applications.²³

We sought to identify potent, selective non-urea sEH inhibitors effective in vivo with good metabolic stability and pharmacokinetic and distribution properties, to test as an anti-hypertensive and anti-inflammatory drug candidate. A sensitive fluorescent based assay²⁹ was employed to determine IC₅₀ values. Using high throughput screening we have previously reported a series of non-urea sEH inhibitors with low micromolar to nanomolar potency.²⁴ From the compound collection provided by the NIH Road-map project we identified sulfonyl isonipecotamide **1**, a potent sEH inhibitor (IC₅₀ = 20 nM).²⁵ In addition, we synthesized a secondary library of compounds based on **1** and SAR revealed the most potent non-urea sEH inhibitor in that study, compound **2** with IC₅₀ = 7.9 nM (Fig. 2).²⁴ Herein we report design, synthesis and biological evaluation of non-urea sEH inhibitors based on compound **2**.

Our evaluation of the SAR for **2** started with replacement of the sulfonamide moiety with both an amide and methylene group, in order to better understand what structural feature of the ‘secondary’ pharmacophore is important for sEH inhibition. Furthermore, amides have in general better properties for formulation. Additionally, we designed a compound in which the sulfonamide group was retained, but the central amide moiety was reversed. These three compounds were prepared by standard synthetic methods from commercially available starting materials (Scheme 1). Thus, per Scheme 1, cycloheptylamine **3** was condensed with Boc-isonipecotic acid **4** under standard peptide coupling conditions. The Boc group of the amide **5** was removed with TFA to afford amine **6**, which was reacted with 2,4,6-trimethylbenzoyl chloride to yield amide analog **7**. In order to synthesize the methylene analog of compound **2**, amine **6** was treated with mesitaldehyde under reductive amination conditions, which gave the final product **8**. The reversed amide analog was synthesized in similar fashion. Commercially available cycloheptanecarboxylic acid **9** and 4-amino-1-Boc-piperidine **10** were condensed under EDC coupling conditions to afford amide **11**, which was subsequently deprotected to furnish amine **12**. The amine **12** was sulfonylated with 2-mesitylenesulfonyl chloride to afford analog **13** (Scheme 2). This first set of compounds demonstrated that the sulfonamide group is important for recognition by sEH, since replacement with an amide group, as in compound **7**, resulted in a sixfold decrease in binding affinity (IC₅₀ = 46.1 nM). Compound **8**, which contains a methylene group, suffered a 15-fold loss of inhibitory potency (IC₅₀ = 120 nM). The effect of reversing amide group in compound **13** resulted in a small, 2.5-fold decrease in the binding affinity for sEH (IC₅₀ = 22 nM), supporting previous studies that the proton in NH (both in urea and amide sEH inhibitors) is important in sEH inhibition, ostensibly forming a salt bridge with the catalytic nucleophile Asp333.²⁶

These results returned our attention to the SAR of the right-hand side of the sulfonamide group of the original lead compound **2**. Thus, amine **6** was reacted with a variety of sulfonyl chlorides to yield products **14–1** to **14–51**. This group of analogs will allow us to evaluate

the role of different substituent on this part of the molecule. We introduced as well several different polar groups on the aromatic ring in order to obtain compounds that will be easier to formulate and administer. The results are summarized in Table 1. As illustrated in Table 1, the diverse right-hand side modifications led to improved or similar potency (**14–7**, **14–27**, **14–31** and **14–34**) over the original sulfonamide analog **2** and several structure–activity relationships can be discerned in this series of analogs. First, the nonpolar group in the *ortho*-position is important for potent inhibition. Great loss of activities was observed if *ortho*-group is deleted (**14–14**, **14–16**, **14–19**, **14–20**, **14–22**, **14–24**, **14–25**, etc.). *Para*-substitution is generally tolerated, but placement of any polar group at this position significantly diminished potency of the compounds (**14–11**, **14–12**, **14–15**, **14–21**, etc).

On the other hand, hydrophobic alkyl groups or halogen substitution at the *ortho*-position enhanced low nanomolar potency of the *para*- and *meta*- substituted analogs, suggesting that some of the substituents on the aromatic ring act synergistically (**14–27**, **14–28**, **14–32** and **14–34**).

Overall, polar groups were not tolerated in any position on the aromatic ring (**14–37**, **14–40**), even if a hydrophobic group was present in *ortho*-position (**14–38**, **14–43**). Furthermore, aromatic analogs appeared to be more favorable compared to the alkyl- and cyclo-sulfonamides (**14–1**, **14–2**, **14–3**, **14–4**, and **14–5**). Only the thiophene analog attached to the sulfonamide moiety via position 3 (**14–51**) had low nanomolar potency compared with other thiophene analogs that are linked via position 2 (**14–47**, **14–48**, **14–49** and **14–50**).

Although potency of amide analog **7** showed a sixfold decrease compared to lead compound **2**, the SAR on the right-hand side of the molecule was obvious and since the amide has better pharmacokinetic properties²⁸ compared to the corresponding sulfonamide, we decided to design a small library of amide analogs, **15–1** to **15–12**, as it is outlined in Scheme 1. However, the various modifications of the aromatic ring did not improve potency further (Table 2).

In conclusion, we have successfully improved the potency of non-urea sulfonamide analogs through SAR-guided modification. Compound **14–34**,³⁰ with an IC₅₀ of 1.6 nM, represents the most potent non-urea sEH inhibitor reported to date. Pharmacokinetic evaluation and pre-clinical studies of selected potent inhibitors are planned.

Acknowledgments

This work was supported in part by NIEHS R01 ES002710. B.D.H. is a George and Judy Senior fellow of the American Asthma Foundation.

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29. *IC₅₀ Assay Conditions*: Cyano(2-methoxynaphthalen-6-yl)methyl *trans*-(3-phenyloxyran-2-yl)methyl carbonate (CMNPC) was used as the fluorescent substrate. Human sEH (1 nM) or murine sEH (1 nM) was incubated with the inhibitor for 5 min in pH 7.0 Bis-Tris/HCl buffer (25 mM) containing 0.1 mg/mL of BSA at 30 °C prior to substrate introduction ([S] = 5 μM). Activity was determined by monitoring the appearance of 6-methoxy-2-naphthaldehyde over 10 min by fluorescence detection with an excitation wavelength of 330 nm and an emission wavelength of

465 nm. Reported IC₅₀ values are the average of the three replicates with at least two datum points above and at least two below the IC₅₀.

30. *Analytical data for the compound 14-34*: ¹H NMR (300 MHz, CDCl₃): δ 7.81–7.78 (d, *J* = 8.1 Hz, 1H) 7.12 (s, 2H), 5.35 (br, 1H), 3.92–3.89 (m, 1H), 3.72–3.68 (d, *J* = 12 Hz, 2H), 2.74–2.67 (t, *J* = 21 Hz, 2H), 2.57 (s, 3H), 2.37 (s, 3H), 2.13–2.05 (m, 1H), 1.88–1.84 (m, 2H), 1.78–1.69 (m, 6H), 1.62–1.39 (m, 8H), ¹³C NMR (75 MHz, CDCl₃) δ 172.6, 143.8, 138.1, 133.7, 133.0, 130.7, 126.8, 50.6, 44.8, 43.0, 35.5, 28.8, 28.3, 24.4, 21.6, 20.8; ESI-MS (M⁺+H): 393.

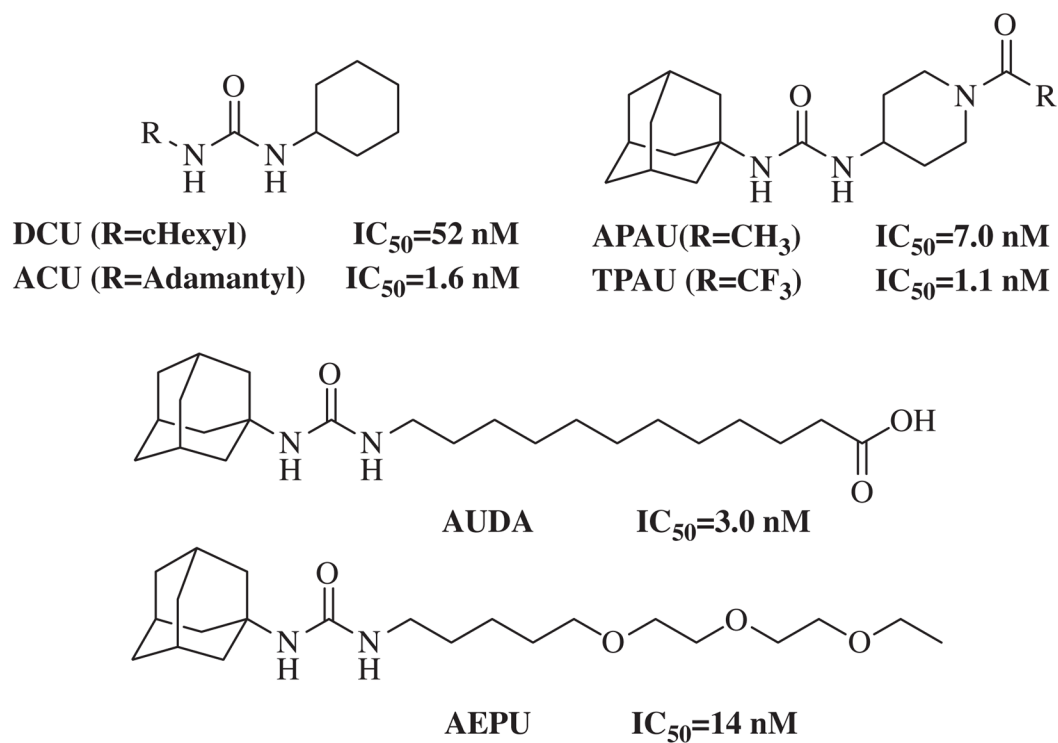


Figure 1.
Chemical structures of known sEH inhibitors.

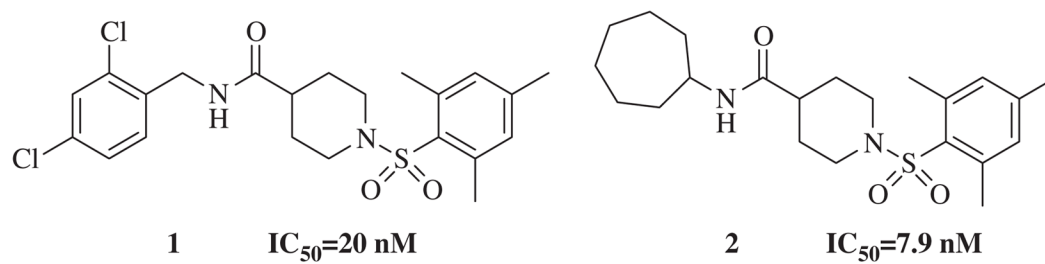
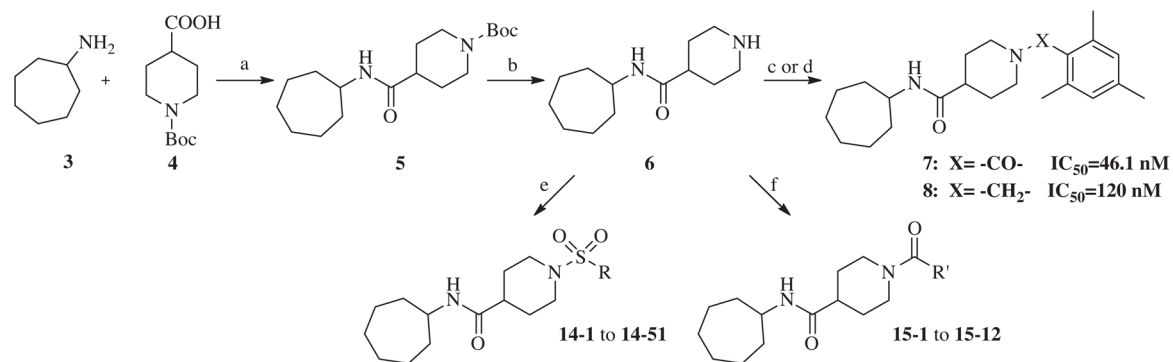
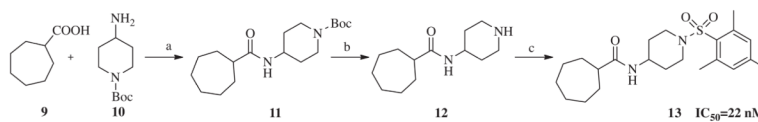


Figure 2.
Chemical structures of non-urea sEH inhibitors.

**Scheme 1.**

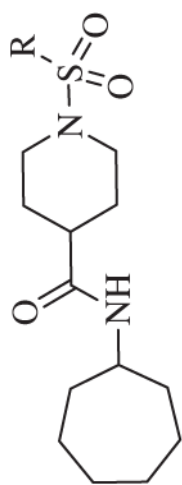
Reagents and conditions: (a) EDC, CH₂Cl₂, rt, 24 h, 68%; (b) TFA, CH₂Cl₂, rt, 24 h, 89%; (c) 2,4,6-trimethylbenzoyl chloride, Et₃N, CH₂Cl₂, rt, 24 h, 85%; (d) mesitaldehyde, NaBH(OAc)₃, CH₂Cl₂, rt, 12 h, 82%; (e) R-SO₂Cl, Et₃N, CH₂Cl₂, rt, 24 h, 70–91%; (f) R'OCl, Et₃N, CH₂Cl₂, rt, 24 h, 85–92%.

**Scheme 2.**

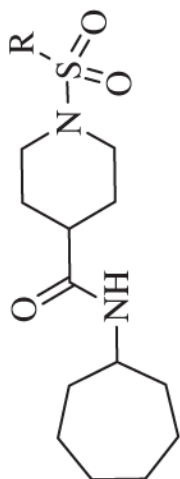
Reagents and conditions: (a) EDC, CH₂Cl₂, rt, 24 h, 65%; (b) TFA, CH₂Cl₂, rt, 24 h, 90%; (c) 2,4,6-trimethylbenzoylsulfonyl chloride, Et₃N, CH₂Cl₂, rt, 24 h, 85%.

Table 1

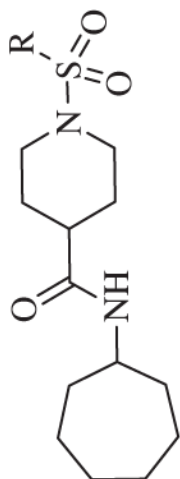
The biological results for sulfonamide analogs 14-1 to 14-52



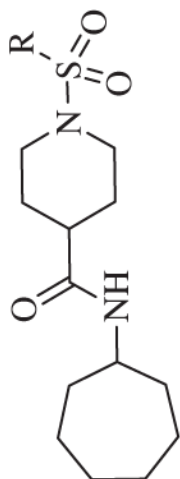
Compound	R	IC ₅₀ ^{a,b} (nM)	Compound	R	IC ₅₀ (nM)
14-1		340	14-27		5.4
14-2		960	14-28		15
14-3		490	14-29		18
14-4		450	14-30		27
14-5		950	14-31		7.7



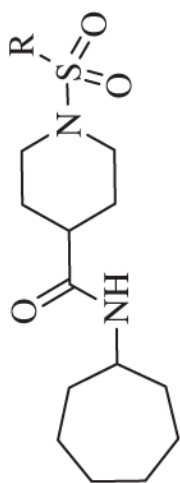
Compound	R	IC ₅₀ ^{a,b} (nM)	Compound	R	IC ₅₀ (nM)
14-6		510	14-32		12
14-7		6.7	14-33		56
14-8		78	14-34		1.6
14-9		24	14-35		1700
14-10		12	14-36		120
14-11		450	14-37		150



Compound	R	IC ₅₀ ^{a,b} (nM)	Compound	R	IC ₅₀ (nM)
14-12		340	14-38		32
14-13		62	14-39		17
14-14		2100	14-40		360
14-15		240	14-41		2000
14-16		2100	14-42		16
14-17		560	14-43		6000



Compound	R	IC ₅₀ ^{a,b} (nM)	Compound	R	IC ₅₀ (nM)
14-18		150	14-44		9.6
14-19		50000	14-45		224
14-20		1100	14-46		35
14-21		140	14-47		870
14-22		13000	14-48		4200
14-23		570	14-49		710



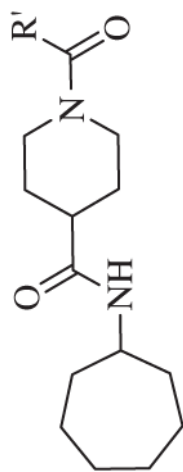
Compound	R	IC ₅₀ ^{a,b} (nM)	Compound	R	IC ₅₀ (nM)
14-24		1300	14-50		6000
14-25		1900	14-51		21
14-26		470			

^aReported IC₅₀ values are the average of three replicates. The fluorescent assay as performed here has a standard error between 10 and 20% suggesting that differences of two fold or greater are significant.²⁷

^bt-AUCB that has an IC₅₀ between 1 and 2 nM was used as positive control.¹³

Table 2

The biological results for amide analogs 15-1 to 15-12



Compound	R'	IC ₅₀ ^{a,b} (nM)	Compound	R'	IC ₅₀ (nM)
15-1		1200	15-7		780
15-2		1400	15-8		1200
15-3		1200	15-9		200
15-4		630	15-10		1100
15-5		950	15-11		2000
15-6		92	15-12		590

^aReported IC₅₀ values are the average of three replicates. The fluorescent assay as performed here has a standard error between 10 and 20% suggesting that differences of twofold or greater are significant.²⁷

^b*t*-AUCB that has an IC₅₀ between 1 and 2 nM was used as positive control.¹³