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Non-urea functionality as the primary pharmacophore in soluble epoxide hydrolase inhibitors

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ABSTRACT

Inhibition of soluble epoxide hydrolase has been proposed as a promising new pharmaceutical target for diseases involving hypertension and vascular inflammation. The most potent sEH inhibitors reported to date contain a urea or amide moiety as the central or 'primary' pharmacophore. We evaluated replacing the urea pharmacophore with other functional groups such as thiourea, sulfonamide, sulfonylurea, aminomethylene amide, hydroxyamide, and ketoamide to identify novel and potent inhibitors. The hydroxyamide moiety was identified as a novel pharmacophore affording potency comparable to urea.

Soluble epoxide hydrolase (sEH) has been proposed as a potential pharmaceutical target in a number of disease indications including hypertension,[1] stroke,[2] inflammatory disease,[3] and metabolic syndrome.[4] The sEH enzyme is found in a variety of mammalian tissues, with the highest activity in liver, kidney, intestinal and vascular tissue.[5] sEH metabolizes endogenously produced epoxides of arachidonic acid, epoxyeicosatrienoic acids (EETs), to the corresponding dihydroxyeicosatrienoic acids (DHETs), by catalyzing the addition of water to the epoxide moiety.[6] EETs produce vasodilation in various vascular beds such as renal, mesenteric, cerebral, pulmonary and coronary arteries.[7] Hydrolysis of EETs by sEH to the corresponding DHETs significantly diminishes this activity,[8] suggesting that inhibition of sEH may be a promising new therapy in the treatment of diseases involving hypertension and vascular inflammation.

sEH inhibitors based on epoxide,[9] urea,[10] carbamate,[10] amide,[11] and acyl hydrazone,[12] scaffolds have been reported. The most potent sEH inhibitors reported to date for which enzyme IC_{50} data have been reported contain a urea or amide as the central or 'primary' pharmacophore (Fig. 1).[13] Early epoxide based inhibitors that were designed based on the endogenous substrate proved to be of limited use for in vivo studies prompting research that led to the discovery of dicyclohexyl urea (DCU), a potent inhibitor of the sEH enzyme.[10] Evaluation of structural analogs of DCU including amide, thiourea, guanidine, carbamate, thiocarbamate, ketone, ester, and carbonate indicated that urea, amide, and carbamate showed the greatest potential as the required functionality for sEH inhibition. Subsequent attempts to improve solubility properties of such prototype molecules as dicyclohexyl urea (DCU), cyclohexyl dodecyl urea (CDU), and adamantyl dodecyl urea (ADU) led to incorporation of a solubilizing group at the end of the extended alkyl chain to afford molecules like 12-[3-adamantane-1-yl-ureido]-dodecanoic acid (AUDA). Additional studies targeting improved solubility and pharmacokinetic properties showed that polar functionality such as ether, ester, and amide moieties, designated as the 'secondary' pharmacophore,[13] could be incorporated into the alkyl chain ca. 7.5 Å distant from the 'primary' urea pharmacophore without loss in potency.[14] In some cases appropriate selection of the secondary pharmacophore and other structural features in the molecule could afford amide based scaffolds of comparable potency to urea based scaffolds.[11] Recent reports describe potent sEH inhibitors, which have improved pharmacokinetic profiles, containing conformationally restricted ligands such as a cyclohexane or piperidine ring linking the urea and the secondary pharmacophore.[15]

The ether scaffold **1** was chosen as a test scaffold for varying the primary pharmacophore P^1 based on relative ease of synthesis,

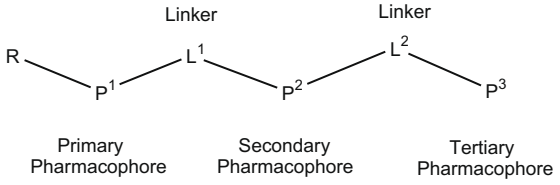

Figure 1. Pharmacophore model.

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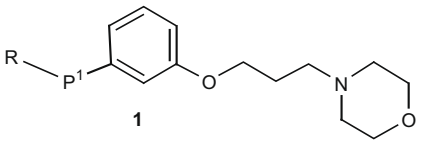
excellent potency of such compounds in the urea series (P^1 = NHCONH),[14] improved solubility, and potential for drugability.



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On the basis of the X-ray crystal structure, the urea based inhibitors are postulated to establish hydrogen bonds between the urea moiety and residues of the sEH enzyme mimicking features of the transition state of epoxide ring opening.[16] Based on this rationale we decided to explore other functionality that could have a similar binding motif to that of urea. We first explored common urea modifications such as thiourea and sulfonyl urea followed by sulfonamide and amide as well as aminomethylene amide. We also prepared hydroxyamide and ketoamide modifications that have additional hydrogen binding possibilities.

The general synthetic route for the preparation of compounds with urea, amide and sulfonamide as the primary pharmacophore is shown in Scheme 1. Alkylation of morpholine with 1-bromo-3-chloropropane afforded *N*-(3-chloropropyl)-morpholine **2**.[17] Reaction of **2** with *m*-nitrophenol gave nitroether **3** which upon reduction yielded aniline intermediate **4.** Treatment of intermediate aniline **4** with the corresponding isocyanates resulted in urea analogs **5.** Treatment of intermediate **4** with various acids or sulfonyl chlorides afforded the desired amides **6** or sulfonamides **7**.

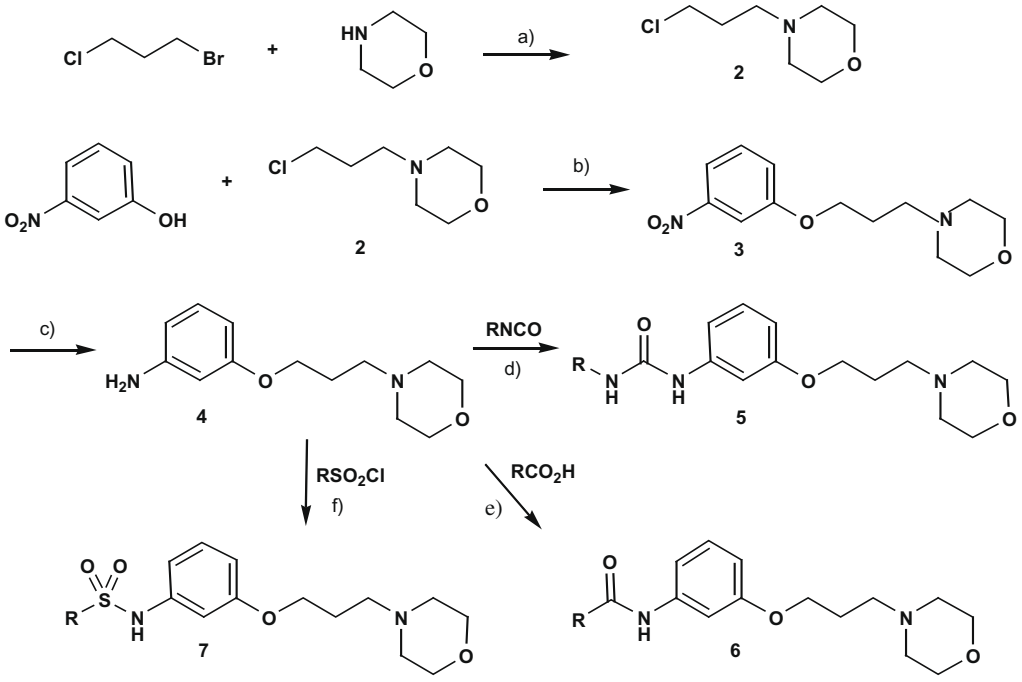
Thiourea analog **8** was prepared by reacting adamantyl isothiocyanate with aniline intermediate **4** under reflux. Sulfonyl urea **9** (P^1 = $NHSO_2NH$) was prepared by sequential reaction of the requisite amine with sulfuryl chloride followed by treatment with intermediate **4** (Scheme 2).

The aminomethylene amide analogs **11** were prepared by treating aniline intermediate **4** with chloroacetyl chloride followed by alkylation with the desired amine (Scheme 3).

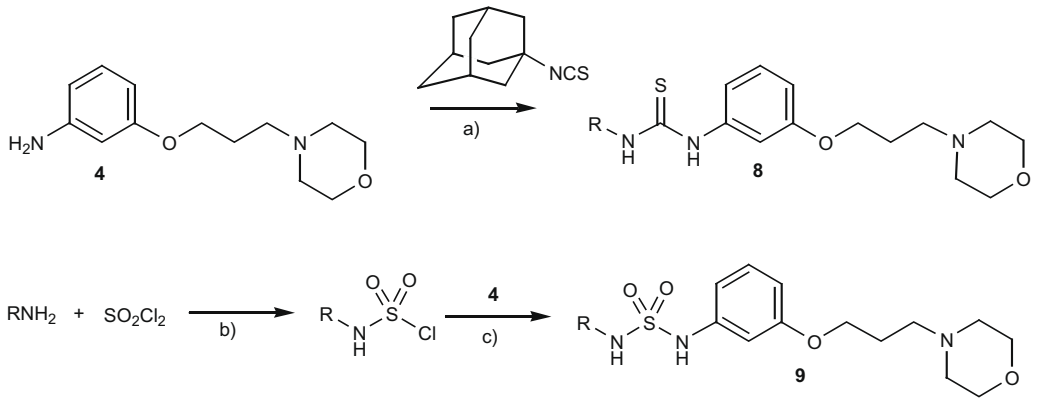
The synthesis of hydroxyamides **16** and ketoamides **17** is presented in Scheme 4. Adamantanol **12** was oxidized using Dess–Martin periodinane to the corresponding aldehyde **13** which on treatment with trimethylsilylcyanide gave cyanohydrin **14**. Intermediate **14** on treatment with hydrochloric acid in the presence of methanol resulted in methyl ester which upon treatment with aqueous lithium hydroxide yielded the hydroxy acid **15**. Coupling amine **4** with hydroxy acid **15** gave hydroxyamide **16.** Oxidation of hydroxyamide **16** with Dess–Martin periodinane afforded the desired ketoamide **17**.

A further series of hydroxyamides and ketoamides (**19** and **20**) with a more lipophilic benzyl ether on the right hand side and incorporating different spacer lengths on either side of the P^1 hydroxyamide or ketoamide were prepared (Scheme 5). Coupling of hydroxy acid **15** with 4-benzyloxyphenyl amine **18** afforded hydroxyamide **19,** which on further oxidation with Dess–Martin reagent yielded ketoamide **20**. Intermediate **15** was prepared from the corresponding adamantanol by sequential oxidation to aldehyde and cyanide addition followed by hydrolysis of nitrile to the corresponding acid. The amine intermediate **18** was prepared from the corresponding 4-benzyloxybenzyl alcohol by sequential mesylation, displacement with sodium azide and reduction to the corresponding amine.

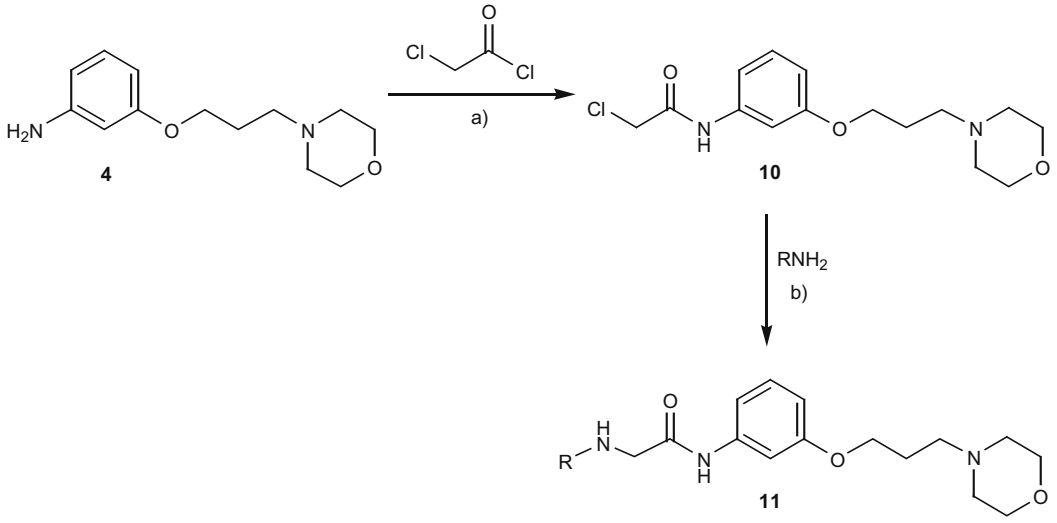
Comparison of sEH enzyme inhibitor potency for selected R groups in the amide series with the corresponding urea series is shown in Table 1. Amide analogs **6** in general were less potent than the corresponding urea **5** in a fluorescence-based enzyme assay.[18] While adamantyl urea analog **5a** was found to be very potent, the corresponding amide analog **6a** was found to be ca. two orders of magnitude less active. However, the potency could be improved in the amide series by inserting a methylene between the adamantyl group and the amide, essentially replacing the urea NH with a CH_2 (e.g., **6b**), maintaining a similar distance between the carbonyl and the left hand substituent as in the urea**.** A similar improvement was noted with the cyclohexylmethyl analogs **6d** and **6c** as well as with the 4-trifluoromethylphenylmethyl analogs **6f** and **6e**. Insertion of a methylene group between the urea NH and the urea carbonyl to afford the corresponding aminomethylene amide analog **11** resulted in diminished potency.



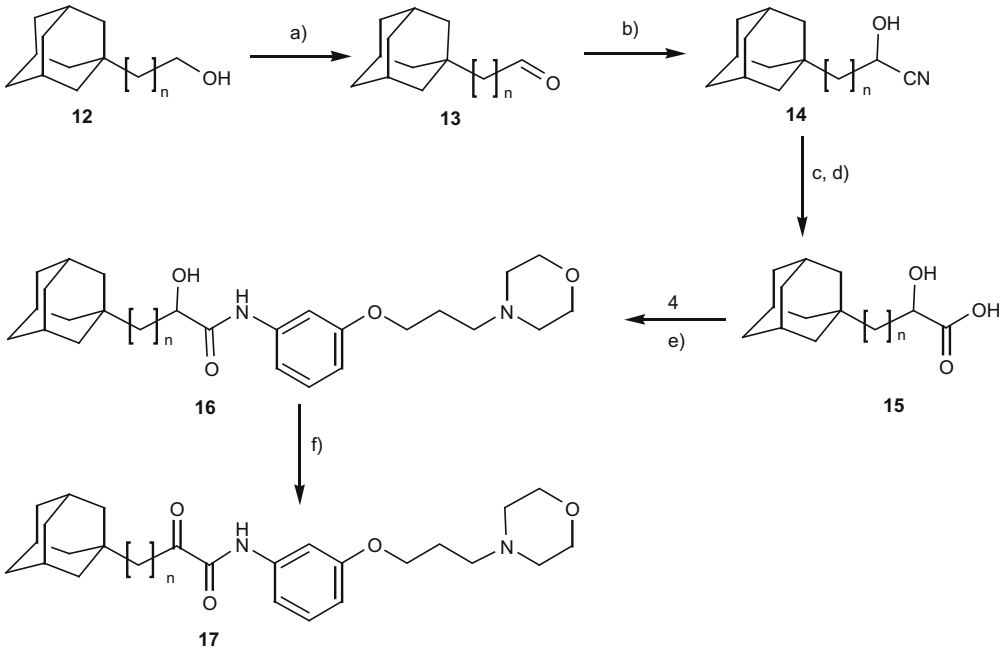
Scheme 1. General synthetic scheme for the preparation of ureas, amides, and sulfonamides. Reagents and conditions: (a) K_2CO_3, DMF, rt, 12 h, 83%; (b) NaH, THF, 4 h, 75%; (c) Fe, HCO_2NH_4, toluene, 12 h, 91%; (d) THF, reflux, 12 h, 58–73%; (e) Et_3N, DCM, 12 h, 60–83%; (f) EDCI, HOBT, DIEA, DMF, 4 h, 50–65%.



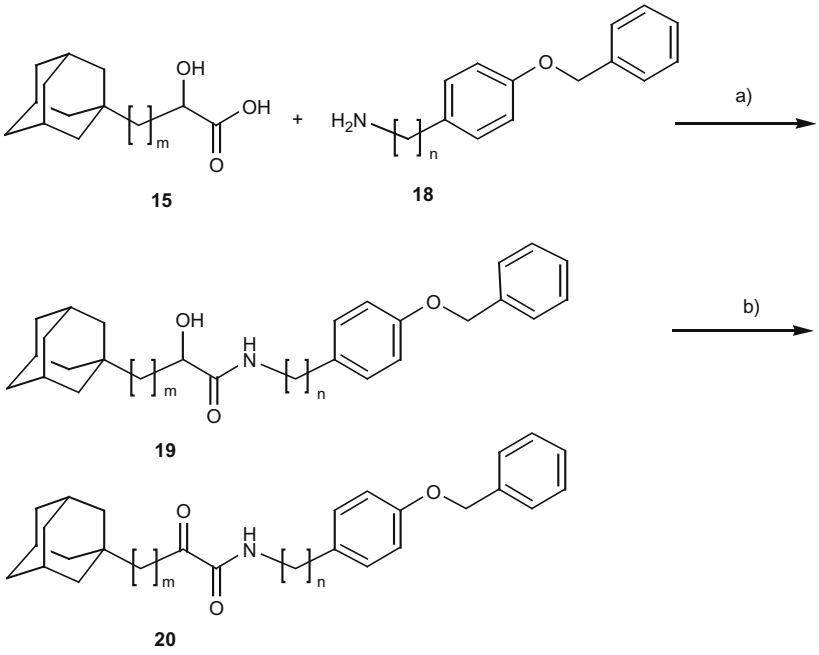
Scheme 2. Preparation of thioureas and sulfonylureas. Reagents and conditions: (a) THF, reflux, 12 h, 68%; (b) Et_3N, DCM, rt, 12 h, 65%; (c) Et_3N, DCM, rt, 16 h, 72%.



Scheme 3. Preparation of aminomethylene amides. Reagents and conditions: (a) Et_3N, DCM, rt, 12 h, 84% ; (b) Et_3N, DCM, rt, 16 h, 65–79%.



Scheme 4. Preparation of hydroxyamides and ketoamides. Reagents and conditions: (a) Dess–Martin periodinane, DCM, rt, 4 h, 52–84%; (b) TMSCN, THF, rt, 12 h, 58–83%; (c) HCl, MeOH, reflux, 12 h, 63–72%; (d) LiOH, MeOH, rt, 12 h, 56–79%; (e) EDCI, HOBT, DIEA, DCM, rt, 12 h, 60–75%; (f) Dess–Martin periodinane, DCM, rt, 6 h, 65%.



Scheme 5. Preparation of hydroxyamides and ketoamides. Reagents and conditions: (a) EDCI, HOBT, DIEA, DCM, rt, 12 h, 58–73%; (b) Dess–Martin periodinane, DCM, rt, 6 h, 54–68%.

Table 1
Enzyme IC_{50} values for urea, amide, and sulfonamides.

Compound	R Group	P[1]	IC_{50} (nM)[a]
5a	Adamantyl	Urea	0.8
5b	Cyclohexyl	Urea	3.9
5c	4-Trifluoromethylphenyl	Urea	1.2
5d	4-Trifluoromethoxyphenyl	Urea	8.2
6a	Adamantyl	Amide	88
6b	Adamantylmethyl	Amide	2.5
6c	Cyclohexyl	Amide	390
6d	Cyclohexylmethyl	Amide	28
6e	4-Trifluoromethylphenyl	Amide	93
6f	4-Trifluoromethylphenylmethyl	Amide	55
7	4-Trifluoromethylphenyl	Sulfonamide	15,000
8	Adamantyl	Thio urea	22
9	Adamantyl	Sulfonyl urea	3600
11	Adamantyl	Aminomethylene amide	110

[a] IC_{50} values for all s-EH inhibitors were determined using a fluorescence assay.[18]

Substitution of common replacements for urea and amide such as thiourea and sulfonamide led to less potent compounds. Thiourea analog **8** was found to be about 10-fold less potent than the corresponding urea analog **5a**. Sulfonamide analog **7** was found to have substantially less potency in comparison to the corresponding amide analog **6e** or urea analog **5c**. Replacing the sulfonamide with sulfonyl urea (e.g., **9**) as the primary pharmacophore afforded improved potency relative to the sulfonamide, but the sulfonyl derivatives, in general, exhibited greatly diminished sEH enzyme activity compared to the urea and amide analogs**.**

Hydroxyamides **16** and ketoamides **17** were found to be less potent sEH inhibitors compared to urea analogs (Table 2). Based on the observation that improved potency was obtained with the insertion of a methylene group between the amide primary pharmacophore and the R group in compounds **6a** and **6b**, hydroxyamide **16b** with one methylene spacer between the adamantyl group and the hydroxyamide moiety was prepared and found to exhibit improved potency in the enzyme assay. This analog proved to be the most potent within the hydroxyamide series. Ketoamide analogs with one or two methylene spacers (**17b** or **17c**) afforded substantially improved and equally potent sEH enzyme inhibition.

Table 2
Enzyme IC_{50} values for hydroxyamides and ketoamides.

Compound	*n*	R Group	P[1]	IC_{50} (nM)[a]
16a	0	Adamantyl	Hydroxyamide	>2000
16b	1	Adamantyl	Hydroxyamide	23
16c	2	Adamantyl	Hydroxyamide	530
17a	0	Adamantyl	Ketoamide	>2000
17b	1	Adamantyl	Ketoamide	49
17c	2	Adamantyl	Ketoamide	48

[a] IC_{50} values for all sEH inhibitors were determined using a fluorescence assay.[18]

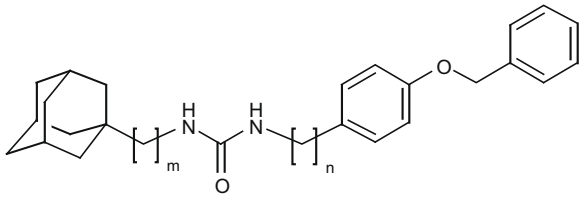
While hydroxyamides **16** and ketoamides **17** are novel sEH inhibitors, their in vitro potencies were somewhat inferior to the corresponding urea or amide analogs. In an attempt to improve the in vitro potency for the hydroxy- and ketoamide series, we prepared a further set of compounds containing a benzyl moiety on the right hand side. Incorporation of such a lipophilic group has been reported to afford improved potency in a series of benzamide sEH inhibitors.[19] sEH enzyme IC_{50} values for hydroxyamides **19** and ketoamides **20** are presented in Table 3.

Table 3
Enzyme IC_{50} values for hydroxyamides and ketoamides.

Compound	*m*	*n*	P[1]	IC_{50} (nM)[a]
19a	0	0	Hydroxyamide	310
19b	1	0	Hydroxyamide	270
19c	1	2	Hydroxyamide	185
19d	2	2	Hydroxyamide	11
20a	0	0	Ketoamide	210
20b	1	0	Ketoamide	1110
20c	1	2	Ketoamide	1540
20d	2	2	Ketoamide	685

[a] IC_{50} values for all sEH inhibitors were determined using a fluorescence assay.[18]

In this series the hydroxyamide analogs **19** were found to be generally more potent than the corresponding ketoamides **20**. Increasing the distance between the hydroxyamide pharmacophore and the adamantyl and benzyloxyphenyl group afforded improved sEH potency in the hydroxyamide series (**19a–d**), but no such trend was observed in the ketoamide series (**20a–d**). Hydroxyamide **19d** with two methylene spacer on both sides of the hydroxyamide primary pharmacophore was found to be the most potent sEH inhibitor in the series, exhibiting potency comparable to urea analog **21** (IC_{50} = 15 nM). The corresponding ketoamide **20d** was found to be ca. 60-fold less potent.



21 (m, n = 2)

In conclusion, we have identified the hydroxyamide moiety as a potent replacement for the urea primary pharmacophore in sEH inhibitors. The hydroxyamide analog **19d** was found to be the most potent sEH inhibitors in the benzyloxyphenyl series. Work is in progress to evaluate pharmacokinetic properties of these materials as well as incorporating other left and right hand side structural variations.

Acknowledgment

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