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Unsymmetrical non-adamantyl *N,N′*-diaryl urea and amide inhibitors of soluble expoxide hydrolase

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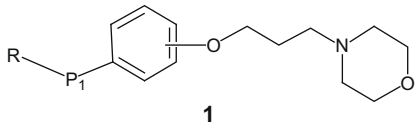
ABSTRACT

Incorporation of an adamantyl group in prototypical soluble expoxide hydrolase (sEH) inhibitors afforded improved enzyme potency. We explored replacement of the adamantyl group in unsymmetrical ureas and amides with substituted aryl rings to identify equipotent and metabolically stable sEH inhibitors. We found that aryl rings, especially those substituted in the *para* position with a strongly electron withdrawing substituent, afforded enzyme IC_{50} values comparable to the adamantyl compounds in an ether substituted, unsymmetrical *N,N′*-diaryl urea or amide scaffold.

Epoxides of arachidonic acids (epoxyeicosatrienoic acids or EETs) are known modulators of biological processes such as vasodilation in a range of vascular beds.[1] Soluble expoxide hydrolase (sEH) metabolizes EETs to the corresponding diols (dihydroxyeicosatrienoic acids or DHETs) by catalyzing the addition of water to the epoxide moiety.[2] Metabolism of EETs to DHETs by sEH often leads to reductions in beneficial biological effects.[3] Hence, inhibition of sEH leading to elevated levels of EETs has been proposed as a new therapeutic target for a number of disease indications including diabetes,[4] hypertension,[5] stroke,[6] and inflammatory diseases.[7] AR9281, an sEH inhibitor from Arete Therapeutics, is presently in a Phase 2 clinical trial program for the potential treatment of type 2 diabetes mellitus.

The most potent sEH inhibitors reported to date contain a urea or amide as the central or 'primary' pharmacophore (Fig. 1).[8] We have recently reported potent sEH inhibitors containing the hydroxyamide moiety as a replacement for urea.[9] Early animal studies with prototype sEH inhibitors such as dicyclohexyl urea (DCU) or adamantyl dodecyl urea (ADU) were hampered by poor aqueous solubility. Incorporation of a solubilizing group at the end of the extended alkyl chain afforded adamantyl ureido dodecanoic acid (AUDA). Although AUDA was found to be more soluble than ADU or DCU, it was still problematic to formulate for in vivo studies.[10] Subsequently, introduction of polar functionality such as an ether, ester, or amide as a 'secondary' pharmacophore ca. 7.5 Å from the 'primary' pharmacophore was found to afford improved aqueous solubility and pharmacokinetic properties.[11] Recent reports describe potent sEH inhibitors based on conformationally rigid structures with



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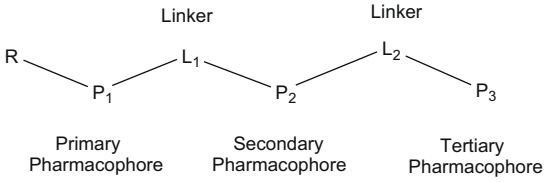

Figure 1. Pharmacophore model.

better pharmacokinetic profiles.[12] Non-adamantyl R groups have been reported with aliphatic L_1 linkers.[13]

Although incorporation of adamantyl as the R group afforded superior enzyme potency in prototypical sEH inhibitors,[14] adamantane rings can be readily metabolized in vivo to give rise to a variety of inactive, hydroxylated adamantyl derivatives.[15] Hence, we explored replacement of the adamantyl group with various substituted aryls to elucidate the SAR as well as to identify metabolically more stable compounds. The aryl ether scaffold **1** was selected as a convenient platform for the comparison of the effect of various R groups on potency since compounds with both urea and amide as the 'primary' pharmacophore P_1 could be prepared from a common amino precursor. Scaffold **1** also affords a reduction in the number of rotatable bonds seen in prototypical sEH inhibitors such as ADU or AUDA and incorporates both a secondary ether pharmacophore as well as a pendant morpholine as a tertiary pharmacophore for increased solubility.[11]

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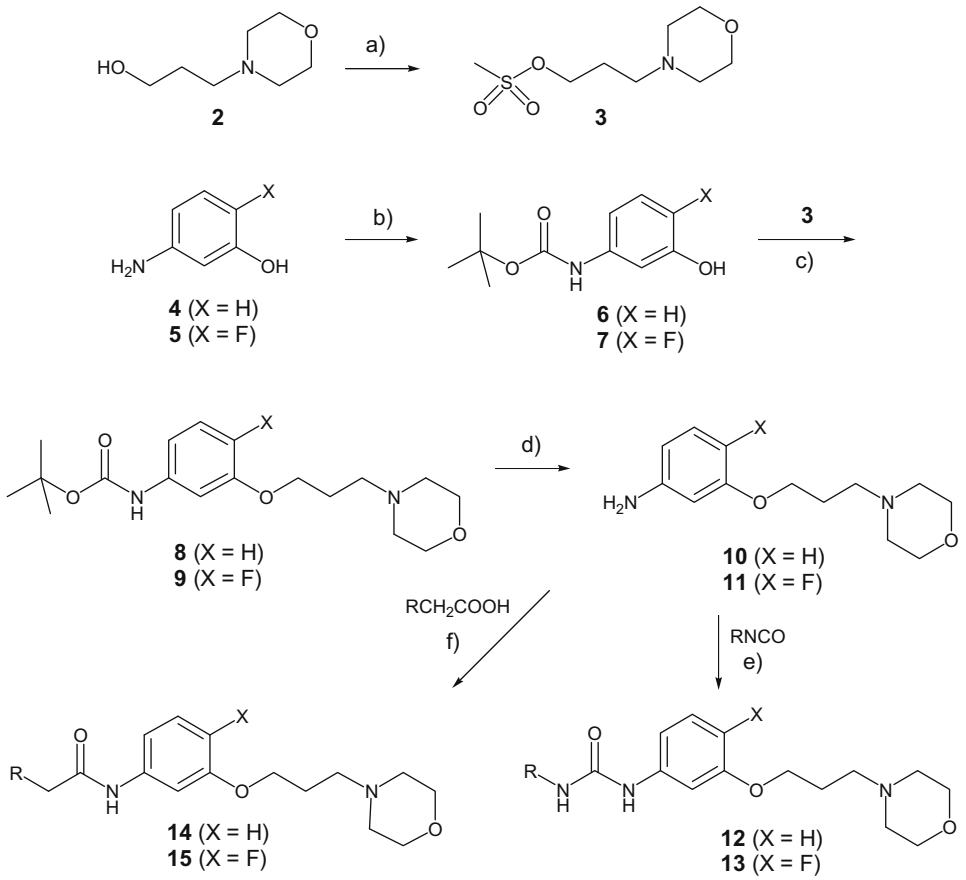


Scheme 1. General synthetic scheme for the preparation of ureas and amides. Reagents and conditions: (a) MsCl, Et_3N, DCM, 0 °C–rt, 3 h, 96%; (b) Boc_2O, THF, reflux, 18 h, 95%; (c) $CsCO_3$, DMF, 55 °C, 3 days, 82%; (d) 10% H_3PO_4, conc. HCl, rt, 2 h, 92%; (e) $CHCl_3$, rt, 2–3 days, 60–83%; (f) EDCI, HOBT, DMAP, $CHCl_3$, 19 h, rt, 50–65%.

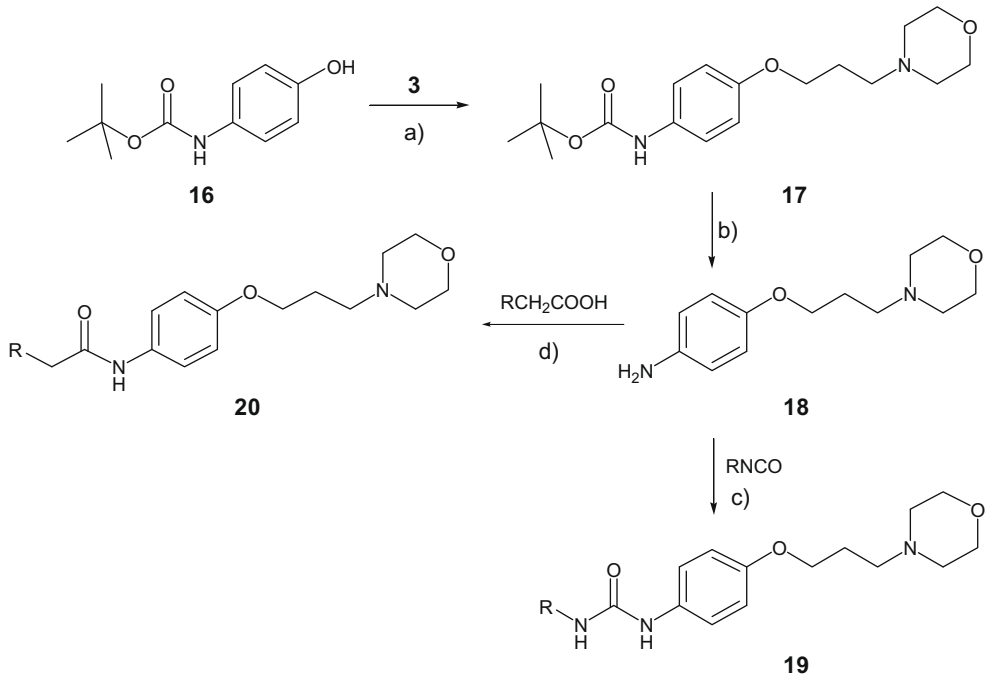
The general synthetic route for the preparation of urea and amide variations of scaffold **1** is shown in Scheme 1. Mesylation of *N*-(3-hydroxypropyl)-morpholine **2** resulted in mesylate **3**. Reaction of the Boc protected phenol intermediates **6** and **7** with mesylate **3** gave arylether intermediates **8** and **9**. Removal of the Boc group yielded the required amines **10** and **11** which upon treatment with isocyanate gave the urea ether analogs **12** and **13**. Reaction of amines **10** and **11** with the appropriate acid resulted in amide ether analogs **14** and **15**.

Synthesis of *para* substituted aryl urea analogs **19** and amide analogs **20** was carried out using similar chemistry (Scheme 2) as that used for the synthesis of the *meta* substituted analogs **12** and **14**. Treatment of phenol **16** with mesylate **3** resulted in Boc protected aniline **17** which was subsequently deprotected to afford the required aniline intermediate **18**. Aniline **18** on reaction with either isocyanate or acid resulted in urea ether analogs **19** or amide ether analogs **20**.

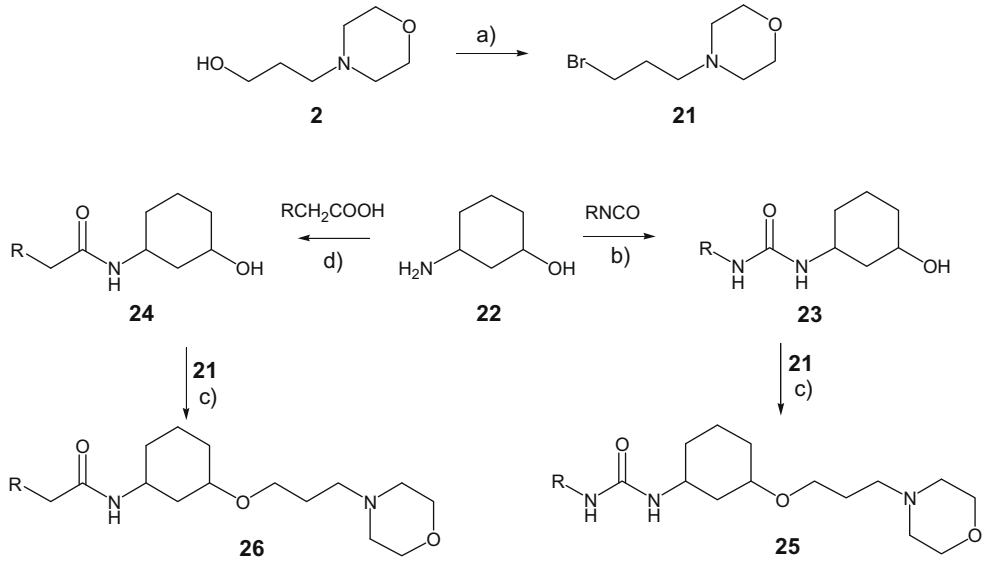
Cyclohexylethers **25** and **26** were prepared as isomeric mixtures starting from 3-aminocyclohexanol **22** as shown in Scheme 3. Aminocyclohexanol **22** on treatment with either isocyanate or acid resulted in urea **23** or amide **24** which upon reaction with bromomorpholine **21**, obtained by reacting *N*-(3-hydroxypropyl)-morpholine **2** with carbon tetrabromide in the presence of triphenylphosphine, yielded the desired urea ether analogs **25** or amide ether analogs **26.**

Enzyme IC_{50} values against human sEH for compounds in both the urea and amide series **12–15** are shown in Tables 1 and 2. Use of an aryl ring as the R group, especially one substituted in the *para* position with a strongly electron withdrawing group, such as trifluoromethyl or chlorine, affords enzyme IC_{50} values comparable to the adamantyl group with CF_3 < Cl < CF_3O < F. Substitution at the *para* position appears to have the largest effect. While potency correlates reasonably well with the substituent Hammett σ value, it is not clear whether this effect is due to the inductive effect of the substituent or the lipophilicity of the binding site, that is, the more electron donating substituents tend to be the more polar. The presence of a polar, basic group as or on the aromatic ring, for example, 3-pyridyl (**12p** or **14p**) or 3-dimethylamino (**12o**), gives a substantial loss in potency. Substitution at the *ortho* position even by a small electronegative substituent such as fluorine gives a loss in potency (e.g., **12g**, **12h**), while 2,6-disubstitution with a bulkier group (e.g., **12m**) gives a dramatic decrease in potency. This latter result is likely due to steric hindrance near the sEH catalytic site.

As a primary pharmacophore, urea, in general, was found to be consistently more potent than amide across a range of R groups with one notable exception. Adamantyl urea **12c** is only slightly more potent than adamantyl amide **14c** while all other amides prepared are ca. 5–50-fold less potent than the corresponding ureas. The difference in activity between amide and the corresponding urea with various R groups became more pronounced, especially in less potent compounds. Replacement of the aryl L_1 ligand with 1,3-cyclohexyl, for example, urea **25** and amide **26** (Table 2), does not appear to offer any advantage in potency and introduces the additional complexities of chiral and cis/trans isomers as well as the potential for metabolic liability. Replacement of adamantyl with cyclohexyl as the R group gave enzyme potencies approaching that of adamantyl in the urea (**12b** vs **12c**) but not in the amide series (**14b** vs **14c**). Substitution of *t*-butyl for adamantyl (**12a** vs **12c**) as the R group gives dramatically reduced potency.



Scheme 2. General synthetic scheme for the preparation of ureas and amides. Reagents and conditions: (a) $CsCO_3$, DMF, 55 °C, 2 days, 99%; (b) 4 M HCl/dioxane, rt, 1 h, 98%; (c) DCM, rt, 18 h, 55–75%; (d) EDCI, HOBT, DMAP, DCM, rt, 18 h, 50–65%.



Scheme 3. General synthetic scheme for the preparation of ureas and amides. Reagents and conditions: (a) Ph_3P, CBr_4, THF, rt, 18 h, 87%; (b) DMF, rt, 1.5 h, 91%; (c) NaH, DMF, rt, 20 h, 58–70%; (d) EDCI, HOBT, DMAP, DMF, rt, 3 h, 50–65%.

Since inserting an electron withdrawing substituent in the R group *para* to the urea nitrogen led to increased potency, a similar strategy was investigated in the linker L_1. Placing a fluoro substituent at the *para* position of L_1 in the urea or amide series **12** and **14** resulted in a new series of compounds **13** and **15**. Incorporation of fluorine typically enhances the metabolic stability of an aryl ring as well as of an ether side chain at the *ortho* position leading to improved pharmocokinetics.[17] Enzyme IC_{50} values for the fluoroaryl ureas **13** and amides **15** are summarized in Table 2. The potency for the fluorophenyl urea compounds **13** appear, in general, to be comparable to that of the desfluoro analogs **12** with the possible exception of **13e** which is about threefold less potent. Amides in the L_1-fluoro series **15** with different R groups were found to be about threefold less potent compared to desfluoro analogs **14**.

The enzyme IC_{50} values for the *para* substituted urea and amide analogs **19** and **20** are presented in Table 3. The regiochemistry of the ether attachment was found to have no significant effect on the relative potency. Both the *meta* substituted (**12** and **14**) and *para* substituted (**19** and **20**) series gave comparable potency. The urea analogs **19** are typically more potent than the amide analogs **20** again except where R is adamantyl. Replacement of the 1,4-substitued aryl ring at L_1 with 1,4-cyclo-

Table 1
Enzyme IC_{50} Values[a] for compounds **12** and **14**

Compd	R	P_1	L_1	IC_{50} (nM)
12a	*t*-Butyl	NHCONH	1,3-Ph	1040
12b	Cyclohexyl	NHCONH	1,3-Ph	3.9
12c	Adamantyl	NHCONH	1,3-Ph	0.8
12d	Phenyl	NHCONH	1,3-Ph	38
12e	4-Fluorophenyl	NHCONH	1,3-Ph	14
12f	3,4-Difluorophenyl	NHCONH	1,3-Ph	11
12g	2,4-Difluorophenyl	NHCONH	1,3-Ph	121
12h	2,4,6-Trifluorophenyl	NHCONH	1,3-Ph	1880
12i	4-Trifluoromethylphenyl	NHCONH	1,3-Ph	1
12j	3-Trifluoromethylphenyl	NHCONH	1,3-Ph	13.8
12k	4-Trifluoromethoxyphenyl	NHCONH	1,3-Ph	8
12l	4-Chlorophenyl	NHCONH	1,3-Ph	2
12m	2,6-Dichlorophenyl	NHCONH	1,3-Ph	10,000
12n	3,4-Methylenedioxyphenyl	NHCONH	1,3-Ph	33
12o	3-Dimethylaminophenyl	NHCONH	1,3-Ph	240
12p	3-Pyridyl	NHCONH	1,3-Ph	5000
14a	*t*-Butyl	CH_2CONH	1,3-Ph	1550
14b	Cyclohexyl	CH_2CONH	1,3-Ph	28
14c	Adamantyl	CH_2CONH	1,3-Ph	2.5
14e	4-Fluorophenyl	CH_2CONH	1,3-Ph	550
14f	3,4-Difluorophenyl	CH_2CONH	1,3-Ph	321
14i	4-Trifluoromethylphenyl	CH_2CONH	1,3-Ph	55
14k	4-Trifluoromethoxyphenyl	CH_2CONH	1,3-Ph	43
14l	4-Chlorophenyl	CH_2CONH	1,3-Ph	116
14p	3-Pyridyl	CH_2CONH	1,3-Ph	14,000
14q	3,4-Methylenedioxyphenyl	CH_2CONH	1,3-Ph	365

[a] IC_{50} values for all sEH inhibitors were determined using a fluorescent assay.[16]

Table 2
Enzyme IC_{50} Values[a] for compounds **13**, **15**, **25**, and **26**

Compd	R	P_1	L_1	IC_{50} (nM)
13b	Cyclohexyl	NHCONH	1,3-Ph-4-F	6.5
13c	Adamantyl	NHCONH	1,3-Ph-4-F	0.8
13d	Phenyl	NHCONH	1,3-Ph-4-F	81
13e	4-Fluorophenyl	NHCONH	1,3-Ph-4-F	40
13i	4-Trifluoromethylphenyl	NHCONH	1,3-Ph-4-F	1.3
13k	4-Trifluoromethoxyphenyl	NHCONH	1,3-Ph-4-F	0.8
13l	4-Chlorophenyl	NHCONH	1,3-Ph-4-F	4.8
15c	Adamantyl	CH_2CONH	1,3-Ph-4-F	6.6
15i	4-Trifluoromethylphenyl	CH_2CONH	1,3-Ph-4-F	156
15k	4-Trifluoromethoxyphenyl	CH_2CONH	1,3-Ph-4-F	145
25c	Adamantyl	NHCONH	1,3-Cyclohexyl	2.5
26c	Adamantyl	CH_2CONH	1,3-Cyclohexyl	5.2

[a] IC_{50} values for all sEH inhibitors were determined using a fluorescent assay.[16]

hexyl is reported to afford comparable enzyme IC_{50} values for the urea series when R is adamantyl.[11]

Pharmacokinetics of selected urea analogs **12** and **19** and amide analog **14** were evaluated in healthy male Sprague Dawley rats following oral administration of compound at 5 mg/Kg (Table 4). The

Table 3
Enzyme IC_{50} Values[a] for compounds **19** and **20**

Compd	R	P_1	L_1	IC_{50} (nM)
19b	Cyclohexyl	NHCONH	1,4-Ph	5.4
19c	Adamantyl	NHCONH	1,4-Ph	1.3
19d	Phenyl	NHCONH	1,4-Ph	39
19i	4-Trifluoromethylphenyl	NHCONH	1,4-Ph	0.8
19k	4-Trifluoromethoxyphenyl	NHCONH	1,4-Ph	0.8
19l	4-Chlorophenyl	NHCONH	1,4-Ph	1.5
20c	Adamantyl	CH_2CONH	1,4-Ph	1.3
20d	Phenyl	CH_2CONH	1,4-Ph	870
20i	4-Trifluoromethylphenyl	CH_2CONH	1,4-Ph	54
20k	4-Trifluoromethoxyphenyl	CH_2CONH	1,4-Ph	42
20l	4-Chlorophenyl	CH_2CONH	1,4-Ph	140

[a] IC_{50} values for all sEH inhibitors were determined using a fluorescent assay.[16]

Table 4
AUC and C_{max} values for selected compounds

Compd	R	P_1	$AUC_{0-24\ h}$ (ng h/mL)	C_{max} (ng/mL)
12b	Cyclohexyl	NHCONH	131	102
12d	Phenyl	NHCONH	3570	1570
12e	4-Fluorophenyl	NHCONH	491	123
12f	3,4-Difluoro	NHCONH	3	3
12i	4-Trifluoromethylphenyl	NHCONH	8	5
12l	4-Chlorophenyl	NHCONH	9	6
12n	3,4-Methylenedioxyphenyl	NHCONH	753	218
14c	Adamantyl	CH_2CONH	0	0
14e	4-Fluorophenyl	CH_2CONH	57	48
19b	Cyclohexyl	NHCONH	863	669
19i	4-Trifluoromethylphenyl	NHCONH	1050	263

meta substituted ether compounds **12** exhibited a broad range of oral exposure values (AUC), however the more potent inhibitors containing electron withdrawing substituent on the aromatic ring (e.g., **12f**, **12i,** and **12l**) gave lower exposure. LC/MS/MS of plasma samples indicated that the primary route of metabolism involved hydroxylation of the morpholine ring[18] and cleavage of the aromatic ether. The best exposure was seen with the unsubstituted phenyl analog **12d**. Both the *para* substituted ether analogs **19b** and **19i** exhibited superior exposure to the *meta* substituted ethers **12b** and **12i**. The amide analogs **14c** and **14e** were found to have lower exposure than the urea analogs.

In conclusion, we have explored the SAR of the left hand side R group in the aryl ether scaffold **1** and shown that aryl rings bearing 4-CF_3, 4-Cl, and 4-CF_3O afford enzyme potency comparable to that afforded by the adamantyl group. We have identified a number of potent urea and amide analogs with improved pharmacokinetics over prototypical sEH inhibitors. Further work is in progress to evaluate sEH inhibitors **12d, 12n, 19b,** and **19i,** which exhibit good exposure, in animal efficacy models of type 2 diabetes.

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