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Solid-phase combinatorial approach for the optimization of soluble epoxide hydrolase inhibitors

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Abstract—A 192-member library of *N*,*N*′-disubstituted urea inhibitors was synthesized by a solid-phase method. The ureas were tested for their inhibitory activities against recombinant human soluble epoxide hydrolase. Simple carbocyclic or *para*/*meta*-substituted phenyl groups showed inhibition potencies that were equal to or greater than adamantane-based sEH inhibitors, while the presence of bulky or ionizable groups close to the urea group dramatically decreased their activities.

Soluble epoxide hydrolase (sEH, EC 3.3.2.3) is involved in the metabolism of endogenously derived fatty acid epoxides, such as arachidonic acid, linoleic acid, and other lipid epoxides.[1] Epoxyeicosatrienoic acids (EETs), the cytochrome P450 epoxygenase products of arachidonic acid, act at vascular, renal, and cardiac levels of blood pressure regulation. Recent studies have showed that EETs are involved in antihypertensive effects as an endothelium-derived hyperpolarizing factor (EDHF) that mediates vasodilation by activating Ca^{2+}-activated K^+ channels in smooth muscle cells.[1,2] TRPV4, a Ca^{2+} entry channel belonging to the vanilloid subfamily of the transient receptor potential (TRP) channels, acts as an extracellular receptor for EETs.[3] The sEH enzyme catalytically hydrolyzes EETs into dihydroxyeicosatrienoic acids (DHETs) which show reduced biological activity.[1] We have demonstrated that sEH inhibition significantly reduces the blood pressure of the spontaneous hypertensive rats (SHRs) and angiotensin II-induced hypertensive rats.[4,5] EETs also possess anti-inflammatory properties in endothelial cells by inhibiting the expression of tumor necrosis factor-α (TNF-α)-induced vascular cell adhesion molecule-1 (VCAM-1) which is a pro-atherogenic mediator or by activating PPARγ which suppresses the NF-κB-mediated expression of molecules, that is, VCAM-1, intercellular adhesion molecule-1 (ICAM-1), and endothelin-1.[6] In addition, diols derived from epoxy-linoleate (leukotoxin) via sEH hydrolysis perturb membrane permeability and calcium homeostasis, which results in inflammation.[7] These results suggest that sEH inhibition may represent a novel approach for the treatment of hypertension and inflammatory diseases.

Earlier inhibitors developed for sEH were substrate-like compounds, such as chalcone oxides.[8] More recently, we have explored urea, amide, and carbamate-based transition state analog inhibitors of sEH.[9] Several recent studies have emphasized adamantane-based urea compounds due to their ease of synthesis, high potency, and ease of analysis. The recent development of fluorescent substrates[10,11] for sEH which both discriminate among low nanomolar and picomolar inhibitors and facilitate high-throughput analysis makes the development of inhibitors through a combinatorial approach attractive. In this report we illustrate this combinatorial approach in the further exploration of the role of R^1 (see compound **D**, Scheme 1) as it influences the potency of sEH inhibitors.

A focused library of 192-ureas was designed in order to optimize R^1 group as well as to avoid the tedious purification step caused by the main contaminant during urea formation, a symmetrical urea ($R^1NHC(=O)NHR^1$, by-product from the step b in Scheme 1), which also has significant inhibitory activity against the human sEH enzyme.

The solid-phase procedure was set up using a previously reported urea formation method starting from the commercially available acid-labile formylindole resin **A**.[12]

Keywords: Soluble epoxide hydrolase; Urea; Hypertension; Anti-inflammation.
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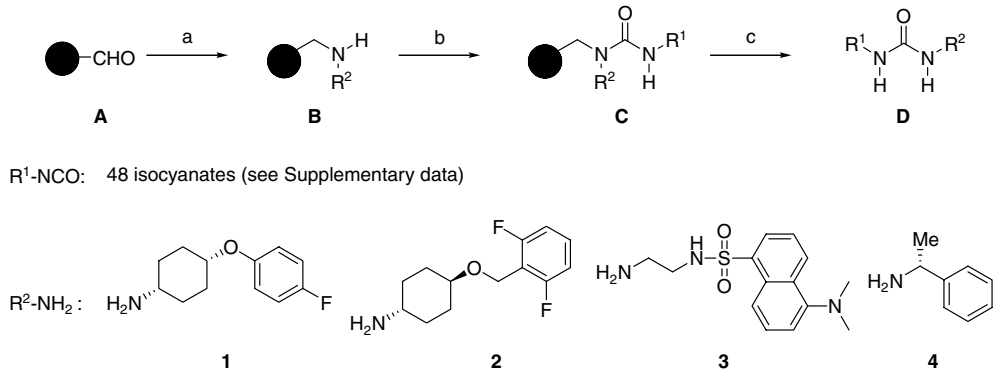


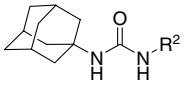
Scheme 1. Reagents and conditions: (a) i—R^2-NH_2, TEOF, THF, rt, 8 h; ii—1 M $NaBH_3CN$ in THF, AcOH, THF, rt, 4 h; (b) R^1-NCO, Et_3N, CH_2Cl_2, rt, 1 d; (c) 1% TFA in CH_2Cl_2, rt, 4 h.

The reactions were performed on an IRORI AccuTag™ Combinatorial Chemistry System[13] according to the general reaction pathway outlined in Scheme 1. Four amines **1**–**4** and commercially available 48 isocyanates were used to construct the 192-member urea library.

The four amines **1**–**4** were selected based on the following criteria. First, selection was based on inhibitory activities of the corresponding adamantane-based ureas (see IC_{50} values in Table 1). Second, three amines are relatively large (amine **1**, **2**, and **3**), thus driving their orientation in the catalytic tunnel, while one amine is relatively small (amine **4**).[14] Finally, amines containing a benzene ring were chosen in order to evaluate the purity of the products by UV detection on HPLC.

Based on the previous SAR analysis of the chalcone oxide derivatives[8] and other urea inhibitors,[15] R^1 groups in the isocyanates were selected as follows: (1) an adamantane as a control, (2) simple carbocyclic rings, (3) *para*-, *meta*-, and *ortho*-mono-substituted phenyl rings, (4) sterically hindered groups, such as *tert*-butyl or *ortho*-di-substituted phenyl rings, (5) 1- and 2-naphthyl group, and (6) phenylalkyl groups having various lengths of alkyl spacers.[16] The purity was assessed by HPLC and all mass spectra were consistent with the anticipated product structure. Out of the total 192-member library, 181 compounds having over 90% purity were tested for their inhibitory activity at a 100 nM concentration using recombinant human sEH by an end-point assay.[11,17]

Table 1. Inhibitory activities of the adamantane-based ureas **1**{**1**}, **2**{**1**}, **3**{**1**}, and **4**{**1**} derived from the amines **1** to **4**



Compound[a]	R^2-NH_2	IC_{50}[b] (nM)	% inhibition[c]
1{**1**}	**1**	0.5	86 ± 9
2{**1**}	**2**	0.5	75 ± 12
3{**1**}	**3**	30	19 ± 7
4{**1**}	**4**	100	10 ± 3

[a] The notation for the compound number: amine number {**isocyanate number**}, for example, **2**{**1**} indicates a compound made by the combination of amine **2** with isocyanate **1**.
[b] As determined via a kinetic fluorescent assay.[10]
[c] Determined via an end-point fluorescent assay, results are means ± SD of three separate experiments.[11]

Overall, compounds derived from amines **1** and **2** showed good inhibitory activity compared to those made from amines **3** and **4** (see Table 1 in Supplementary data). These results strongly suggest that the tested compounds orient in the same direction as the adamantane-based inhibitors do when they bind at the active site, which can be predicted from the recent X-ray crystal structure of human sEH with urea-based inhibitors.[14]

In order to determine the influence on the inhibitory activity by R^1 group itself, compounds having similar activity compared to the corresponding adamantane-based ureas (**1**{**1**}, **2**{**1**}, **3**{**1**}, and **4**{**1**}) are highlighted in black squares in Figure 1.

From the illustration in Figure 1, it is apparent that carbocyclic groups ({**2**–**6**}) and *para*-substituted phenyl groups ({**8**–**17**}) showed the best results, regardless of R^2 group. There was no strong correlation between the inhibition and the σ values. It is likely that the binding site in which the R^1 group resides is a narrow, hydrophobic tunnel. This is further supported by the fact that overall *para*-substituted phenyl groups are better than *meta*-substituted phenyl groups ({**18**–**26**}), the 2-naphthyl group ({**45**}) is better than the 1-naphthyl group ({**44**}), and the phenyl groups having longer alkyl spacers ({**47**–**48**}) gave the better activities. Meanwhile, sterically hindered derivatives, such as inhibitors from the isocyanates ({**27**–**44** and **46**}), showed poor inhibition. Based on these results, poor activities most likely came from steric effects of groups on R^1. In other words, the adamantyl group, which is generally considered as a bulky group, might be the marginal biggest group as the R^1.

In addition, as seen in Figure 2, ureas derived from amines **1** and **2** generally show similar inhibition; one can expect such results because of the similar IC_{50} values obtained for their corresponding adamantane-based ureas (Table 1). However, for ureas having sterically hindered groups on R^1 (R^1 = {**27**–**44**}), ureas derived

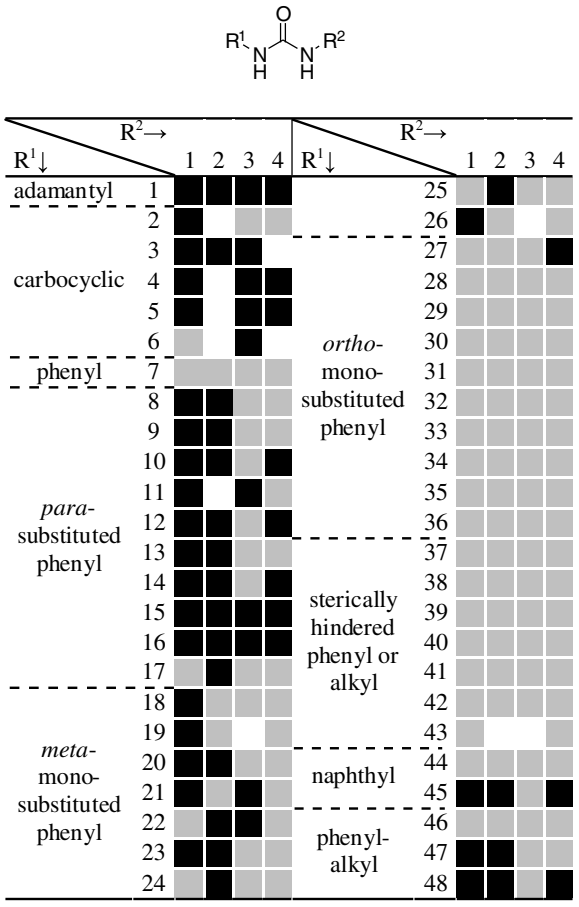
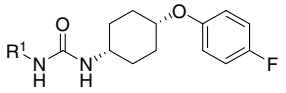


Figure 1. Relative inhibition of the 192-member urea library at 100 nM concentration compared to the corresponding adamantane-based ureas **1**{**1**}, **2**{**1**}, **3**{**1**}, and **4**{**1**}, respectively. Black square: equal to or greater than 80% of inhibition obtained when R^1 is an adamantyl group; grey square: less than 80% of the inhibition obtained when R^1 is an adamantyl group; white square: compounds were not tested due to the poor purity (less than 90%).

from amine **1** showed overall much better inhibition. It is likely that a repulsion caused by sterically hindered groups on R^1 influences the positioning of the R^2 group in the active site tunnel. Such altered positioning seems to result in the further destabilization effect for ureas derived from amine **2** than for those from amine **1**.

To validate our high-throughput screening results, a subset of compounds having high percent inhibition, that is, **1**{**4**}, **1**{**12**}, **1**{**13**}, **1**{**14**}, **1**{**15**}, **1**{**16**} and having low percent inhibition, that is, **1**{**34**}, **1**{**38**}, **1**{**39**}, **1**{**40**}, were resynthesized. Their IC_{50} values were then determined using a continuous fluorescent assay.[10] In general, data from both methods showed good correlation as shown in Table 2. These results confirm that sterically less hindered *para*- or *meta*-substituted phenyl

Table 2. IC_{50} results for a selected subset of urea compounds from Schemes 1 and 2



Compound	IC_{50}[a] (nM)	Inhibition[b] (%)	R^1	Mp (°C)
1{**1**}	0.5 ± 0.1	86 ± 9	Adamantyl	242–245
1{**4**}	0.5 ± 0.1	87 ± 8	*c*-Hep	158–167
1{**12**}	1.0 ± 0.1	76 ± 7	4-I–Ph–	198–201
1{**13**}	0.7 ± 0.1	81 ± 8	4-Cl–Ph	177–182
1{**14**}	0.6 ± 0.1	80 ± 6	4-Br–Ph	192–194
1{**15**}	0.9 ± 0.1	84 ± 7	4-OCF_3–Ph	157–158
1{**16**}	1.2 ± 0.2	82 ± 5	4-CF_3–Ph	171–174
1{**34**}	100 ± 5	2 ± 3	2-OCF_3–Ph	158–160
1{**38**}	1200 ± 100	4 ± 3	2,6-Di-Me–Ph	196–199
1{**39**}	940 ± 60	1 ± 1	2,6-Di-Cl–Ph	181–187
1{**40**}	50500 ± 500	5 ± 3	2,6-Di-*i*-Pr–Ph	218–222
7	2.9 ± 0.2	62 ± 13	4-CO_2Me–Ph	162–163
8	220 ± 5	nd	4-CO_2H–Ph	269–285
9	2.0 ± 0.2	72 ± 6	3-CO_2Me–Ph	116–123
10	590 ± 60	nd	3-CO_2H–Ph	240–254

nd denotes not determined.

[a] Determined via a kinetic fluorescent assay, results are means ± SD of three separate experiments.

[b] Determined via an end-point fluorescent assay, results are means ± SD of three separate experiments.

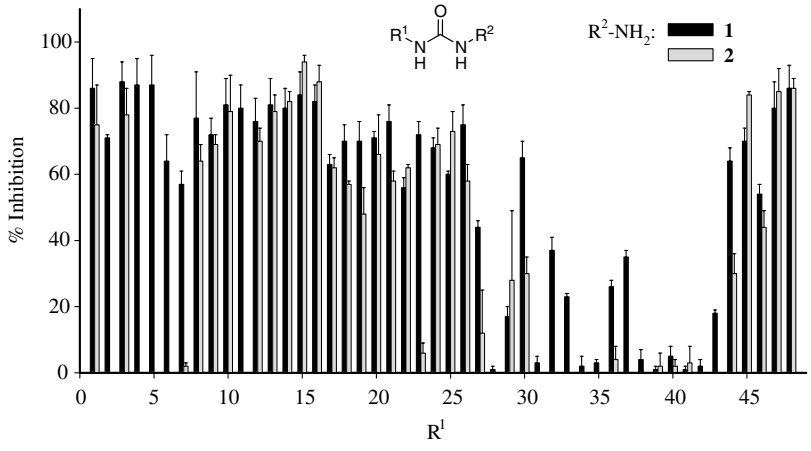
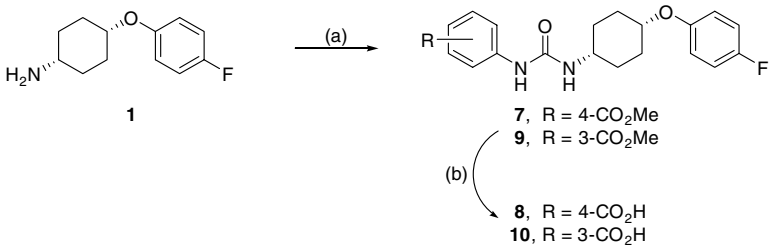


Figure 2. Percent inhibition of ureas derived from amines **1** and **2** at 100 nM concentration. Note, ureas **2**{**2**}, **2**{**4**}, **2**{**5**}, **2**{**6**}, **2**{**11**}, and **2**{**43**} were not tested for inhibition because of their low purity.



Scheme 2. Reagents and conditions: (a) Ar-NCO, DMF, rt, 12 h; (b) LiOH, acetonitrile, water, 90 °C, 6 h.

groups, simple non-rigid carbocyclic groups, 2-naphthyl, and phenyl alkyl groups having at least an ethyl spacer are good alternatives to the adamantyl group. These results are also consistent with our previous SAR of chalcone oxide derivatives and other urea-based inhibitors.[8,15]

The coincidence between the current SAR with that of the chalcone oxide derivatives encouraged us to explore the carboxylated analogs to investigate the effect in the presence of the ionizable group on the R^1. The required acid compounds **8** and **10** were synthesized using amine **1** as outlined in Scheme 2.

In a similar manner as observed for chalcone oxide derivatives,[8] the introduction of the free carboxylic acid at the *para*-position of the phenyl ring dramatically decreased its activity about 440-fold compared to compound **1**{**1**}. Having a free carboxylic acid at the *meta*-position decreased inhibition potency even more dramatically. Corresponding ester compounds **7** and **9** are, however, only 2- to 6-fold less active compared to compound **1**{**1**}. The reason for the poor inhibitory activities of inhibitors having the free carboxylic acid on R^1 is at present unclear. Possible explanations include: (1) water solvation of the carboxylate might either prevent access of the inhibitor into the active site or cause the repulsion with the residues at the active site, (2) ionic interactions between the carboxylate anion and protonated imidazole on His^{523} preventing the optimal binding of the inhibitor at the active site. Recent X-ray crystal structure data of human sEH complexed with different dialkylurea inhibitors bearing pendant carboxylate of varying length supported the latter explanation.[14]

Due to the fact that some of these compounds are as potent as adamantane-based inhibitors in vitro on the recombinant human sEH enzyme, we propose that sterically less hindered lipophilic groups, such as the *para*-trifluoromethoxy phenyl group, are good replacements for adamantyl group. Such compounds are UV dense, have increased water solubility, and should lead to altered routes of metabolism and distribution.

In summary, we have demonstrated that several groups, such as simple non-rigid carbocylic rings or *para*/*meta*-substituted phenyl rings, can replace the adamantane ring found currently in the most potent urea-based sEH inhibitors. Compounds with sterically hindered groups as R^1, however, had significantly decreased potency. In addition, having ionizable free carboxylic acid on R^1 dramatically decreased the inhibitory activity. These observations strongly suggest that the side of the active site tunnel where R^1 of the *N*,*N*′-disubstituted urea binds favors sterically unhindered lipophilic groups.[9c]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.08.078.

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16. See Table 1 in Supplementary data for a complete list of these diversity reagents.
17. See Tables 1 and 2 in Supplementary data for the mass data, the purities, the yields, and the percent inhibition values at 100 nM concentration of the library.