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Rapid synthesis of an array of trisubstituted urea-based soluble epoxide hydrolase inhibitors facilitated by a novel solid-phase method

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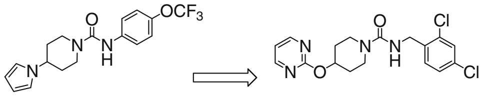
ABSTRACT

A 270-membered library of trisubstituted ureas was synthesized and evaluated for inhibition of soluble epoxide hydrolase. Library design and reagent selection was guided by the use of a pharmacophore model and synthesis of the array was enabled with a general solid-phase method. This array approach facilitated multi-dimensional SAR around this series and identified functionality responsible for binding affinity, as well as opportunities for modulating the overall in vitro profiles of this class of soluble epoxide hydrolase inhibitors.

Soluble epoxide hydrolase (sEH) is an enzyme involved in the metabolism of fatty acid epoxides derived from the cytochrome P450-mediated oxidation of arachidonic acid.[1] The initial epoxidation, mediated mainly by CYP2J2 and CYP2C9, generates a variety of epoxyeicosatrienoic acids (EETs), which act as chemical mediators of blood pressure at the vascular, renal, and cardiac levels, as well as modulators of inflammatory pathways.[2] Specifically, sEH hydrolyzes EETs to the corresponding dihydroxyeicosatrienoic acids (DHETs), which exhibit reduced biological effects. It is expected that selective inhibition of sEH would increase the local endogenous EET levels, thus potentiating their pharmacologically relevant in vivo phenotypes. As such, selective inhibition of sEH may represent a novel approach to treat hypertension and inflammatory diseases. Our goals in this area consist of identifying potent, selective, orally-active sEH inhibitors that ultimately possess anti-hypertensive activity comparable to existing therapies but which exhibit additional or superior end-organ protection benefits.[3]



1

h-sEH IC$_{50}$ = 12 nM
r-sEH IC$_{50}$ = 11 nM
IC$_{50}$ (cell) = 126 nM
HLM (t$_{1/2}$) = 56 min
RLM (t$_{1/2}$) = 25 min

2

h-sEH IC$_{50}$ = 18 nM
r-sEH IC$_{50}$ = 11 nM
IC$_{50}$ (cell) = 63 nM
HLM (t$_{1/2}$) = 86 min
RLM (t$_{1/2}$) = 37 min

Figure 1. Trisubstituted urea hit compound (**1**) and new lead compound (**2**).

We recently reported that screening of our proprietary compound collection against sEH identified several distinct chemical classes suitable for further optimization.[4,5] In addition, we described our efforts to optimize a urea lead (**1**) shown in Figure 1.[6] The initial strategy focused upon replacing the pyrrole and aniline structural alerts, while maintaining favorable molecular and cellular potencies. To this end, several ureas including **2** were generated, where the structural alerts were successfully replaced and the in vitro profile of the initial hit compound maintained.

In parallel with these efforts, we utilized a focused library approach as a tool to rapidly expand the SAR around compound **2**. Specifically, we sought to increase the structural diversity of the cyclic amine moiety and aniline-replacement within the trisubstituted urea context and identify combinations that afforded favorable molecular and cellular potencies, while addressing potential liabilities for off-target effects.

Library design and reagent selection of R^{1}R^2 cyclic amines and of R^3 aniline-replacements was guided by molecular modeling. The murine sEH crystal structure (PDB ID 1cr6) with the co-crystallized CPU ligand (*N*-cyclohexyl-*N′*-(propyl)phenyl urea)[7] was used to predict the binding modes of known sEH inhibitors including compound **1** (Fig. 2). Because no human sEH crystal structures were available at the onset of this study, we used a pharmacophore-based virtual screening approach rather than a structure-based docking approach to optimize the reagent selection for a

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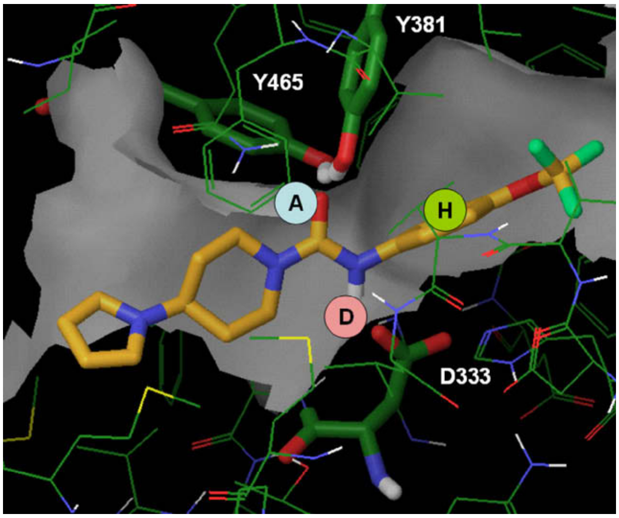


Figure 2. Murine sEH crystal structure (PDB ID 1cr6) with docked compound **1.** Part of the catalytic site surface is shown in grey. Hydrogen bond donor (D) acceptor (A) and a hydrophobic feature (H) of the pharmacophore model are shown.

combinatorial library. Therefore, a pharmacophore model was derived from the murine sEH crystal structure. The pharmacophore model consisted of a hydrogen bond acceptor (interaction with Y381 and Y465) and a hydrogen bond donor (interaction with D333) in the catalytic site, as well as a hydrophobic feature as illustrated in Figure 2. To include shape constraints in the pharmacophore-based virtual screen, a set of 287 known sEH ligands was docked using the program Glide[8] into a homology model of the human sEH built from the murine sEH structure (data not shown). Only the docked binding modes that matched the pharmacophore model outlined above were accepted. The composite shape of the docked ligands was then combined with the pharmacophore model to generate a pharmacophore and shape query for virtual screening.

The combined pharmacophore and shape model was used together with the in-house-developed virtual screening software PharmShape to screen a virtual library of 5510 compounds (29 R^1R^2 cyclic amine groups and 190 R^3 aniline-replacement groups). Of those compounds, 291 did not fit the query and were discarded. The similarity of the remaining virtual library compounds compared to 93 known sEH inhibitors was evaluated using 3D pharmacophore fingerprints.[9] A consensus score was assigned to each building block by averaging the similarity scores of all enumerated compounds that contained this building block. All building blocks were then clustered based on their pharmacophore fingerprints and the final reagents (16 R^1R^2 cyclic amine groups and 20 R^3 aniline-replacement groups) were selected by choosing representatives from each cluster with the highest consensus scores (Fig. 3).

In order to enable the synthesis of the desired compound array, a general solid-phase method was developed for incorporating a diverse set of R^1R^2 cyclic amines and R^3 aniline-replacements. This methodology eliminated the formation of symmetrical ureas, typical by-products of urea formation reactions, and obviated the need for chromatographic purification of final compounds. The solid-phase methodology employed a resin-bound *p*-nitrophenyl carbamate, as an isocyanate equivalent[10] and is outlined in Scheme 1.

R^3-substituted amine resins were readily synthesized by reductive amination of aldehyde resin[11] using standard procedures,[12] followed by capping with *p*-nitrophenylchloroformate. Excess R^1R^2 amine, in conjunction with base, was used to drive urea formation to completion. Cleavage from the resin using TFA afforded 270 compounds that met the >80% purity criteria by LC/MS (ELSD and UV_{254}). The new compounds were submitted within three - weeks of synthesis initiation and in multi-milligram quantities (average mass 7.5 mg) that allowed broad in vitro profiling. Figure 4 shows the results from the profiling of all library compounds for binding to h-sEH, as assessed by displacement of a rhodamine-labeled probe.[13] The color codes reflect their affinities.

The R^1R^2 cyclic amines and R^3 aniline-replacements that impart sEH binding affinity are easily identified by displaying the data in this array format. Generally, compounds derived from R^3 amines **4**, **16**, and **19** exhibited single digit or low double digit nanomolar affinities toward h-sEH, regardless of the R^1R^2 cyclic amine. In contrast, most compounds derived from the *meta*- or *para*-substituted benzylamine R^3 groups (**1**, **3**, **11**, **12**, **14**, **18**) and compounds from a variety of substituted phenethylamine R^3 groups (**2**, **5**–**10**, **13**, **17**), representing the remainder of the R^3 amines chosen for library synthesis, did not show acceptable binding to h-sEH. This apparent disconnect from the pharmacophore model was explained by the subsequent h-sEH co-crystal structure of related trisubstituted urea inhibitors.[14] This co-crystal structure demonstrated that the

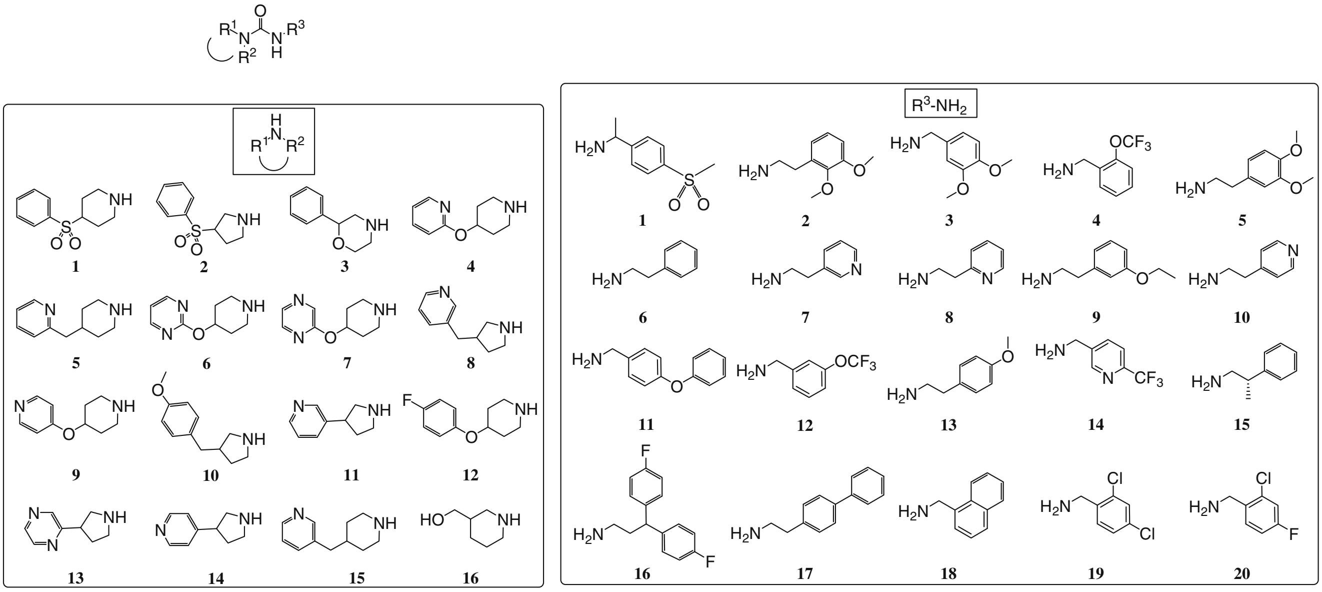
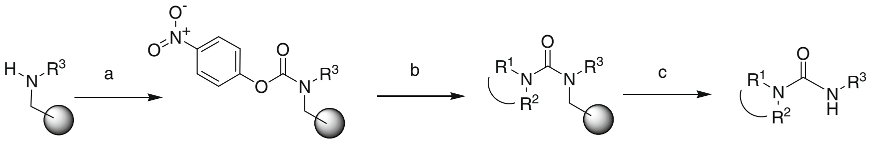


Figure 3. Sixteen R^1R^2 cyclic amine reagents and 20 R^3 aniline-replacement reagents chosen for library synthesis.



Scheme 1. Solid-phase synthesis of trisubstituted ureas. Reagents and conditions (a) *p*-nitrophenylchloroformate (3 equiv), *N*-methylmorpholine, CH_2Cl_2, room temperature, 15 h; (b) R^1R^2 amine (3 equiv), DBU, DMA, 100 °C, 36 h; (c) 20% TFA/CH_2Cl_2, room temperature, 2 h.

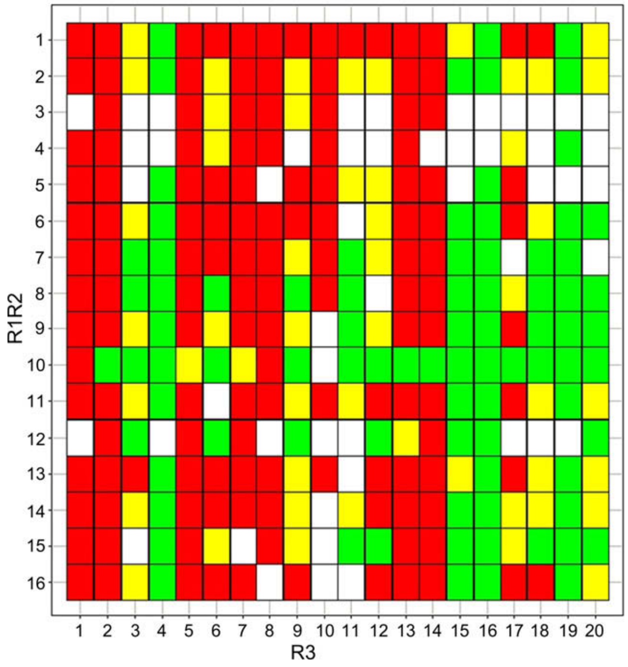


Figure 4. Relative binding affinity of the urea library resulting from 16 $R^1R^2 \times 20$ R^3 groups against h-sEH. Green square: $IC_{50} < 100$ nM; yellow square: 100 nM < $IC_{50} < 500$ nM; red square: $IC_{50} > 500$ nM; white square: compound not submitted for testing.

urea inhibitors bind in the sEH active site with the R^3 group oriented toward two well-defined hydrophobic pockets. Of the 20 residues that line the enzymatic pocket of sEH within a 5 Å distance of the CPU inhibitor in the m-sEH structure (1cr6), six are not conserved in h-sEH. Of those six residues, four are located in the two hydrophobic pockets that host the R^3 group. The residue differences between m-sEH and h-sEH include F406L, I416L, V418M, and I496F. In addition, the nearby F415V also contributes to the steric dissimilarities between the murine and human sEH structures in the R^3-binding pocket that were not captured adequately in the pharmacophore and shape model used to design this library. The *meta*- or *para*-substituted benzylamines and the substituted phenethylamines chosen for the library do not position functionality optimally to interact with these binding pockets, resulting in loss of binding affinity for compounds containing these R^3 groups. In light of the significant number of less active compounds in the library (Fig. 4), it is worth noting that there was no correlation observed between the binding affinities of library compounds to sEH and the calculated similarity consensus scores used to choose the reagents (data not shown).

The importance of this R^3 pocket is highlighted in the array data which shows that binding affinity is driven by the R^3 aniline-replacements. A variety of cyclic amines are tolerated in combination with the preferred aniline-replacements without loss of affinity. This is consistent with binding of the R^1R^2 group in a large, solvent exposed, and amphiphilic pocket as predicted by the co-crystal structure. While both 5- and 6-membered rings are tolerated as constituents of the urea linkage, R^1R^2 cyclic amines that possess a single carbon linker between pyrrolidine and aromatic moieties (**8** and **10**) afford good binders regardless of substitution around the aromatic ring. However, direct attachment of the aromatic group to a pyrrolidine ring, as with R^1R^2 amines **11**, **13**, and **14**, typically afford weaker binders. Similarly, R^1R^2 amines with sulfone linkages (R^1R^2 amines **1** and **2**) led to weaker binders.

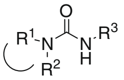
Synthesis of an array of compounds in this manner facilitated a basic understanding of the SAR for the series, demonstrating that, of the amines tested, affinity is clearly mediated by three preferred aniline-replacements and that a variety of functionality is tolerated within the cyclic amine portion of the urea. This provided an opportunity to modulate a variety of off-target effects and physical–chemical properties through modifications to the cyclic amine portion of the molecule.

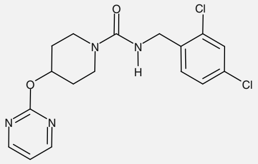
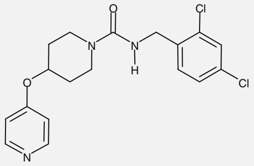
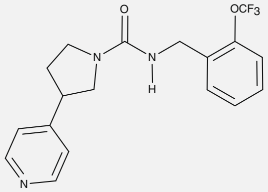
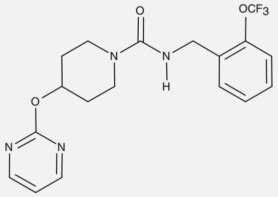
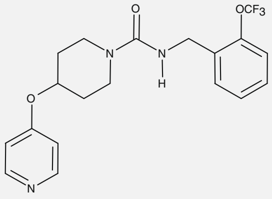
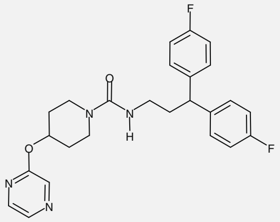
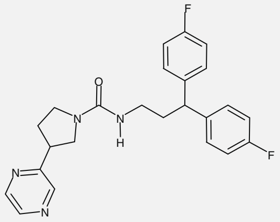
The compounds with the greatest affinity (<10 nM) were also assessed against h-sEH in a cellular assay measuring the ability of sEH to hydrolyze [2-^{3}H]-*trans*-1,3-diphenyl propylene oxide (*t*-DPPO),[15] as well as for their ability to inhibit CYP2J2 and CYP2C9 owing their role in the generation of EETs. Compounds were also incubated with human and rat liver microsomes as a means to predict in vivo oxidative clearance. In turn, this allowed the establishment of multi-dimensional SAR for the series and further distinction between the best three aniline-replacements based on their in vitro profiles. Data for compounds representing the best R^3 groups are shown in Table 1.

The CYP inhibition data shows that compounds possessing the top three potent aniline-replacements inhibit the CYP enzymes, CYP2J2 and CYP29C, to varying extents. The CYP activity can be modulated depending upon the cyclic amine substitution (Table 1, entries **1** and **2**), suggesting that none of these particular aniline-replacements generically confer CYP inhibition. As such, the three aniline-replacements are not differentiated by CYP inhibition potential. However, the three aniline-replacements distinguish themselves with respect to in vitro metabolic stability. Overall, compounds possessing the 3,3-bis-(4-fluorophenyl)-propyl moiety (Table 1, entries **6** and **7**) lack in vitro metabolic stability regardless of cyclic amine functionality. In contrast, acceptable in vitro microsomal half-lives for compounds possessing the 2,4-dichlorobenzyl and *o*-trifluoromethoxybenzyl moieties in combination with a variety of cyclic amines were consistently realized. The equipotent aniline-replacements were thus differentiated based on metabolic stability.

In conclusion, rapid exploration of SAR around a series of trisubstituted urea inhibitors of sEH was accomplished using a focused library approach. A general solid-phase method enabled the rapid synthesis of 270 analogs without the need for chromatographic purification. The library design and array approach was successful in generating multi-dimensional SAR relating binding affinity, cellular activity, selectivity against EETs-forming CYPs, and microsomal stability. Toward the specific goals of increasing cyclic amine and aniline-replacement diversity, two additional aniline-replacements (R^3 = **4** and **16**) were found, in addition to the previously identified 2,4-dichlorobenzylamine moiety. Compounds incorporating these aniline-replacements possess excellent affinities for h-sEH and cellular potencies. In combination with these

Table 1
Results of in vitro assays for representative compounds



#	R^1R^2	R^3	Compound	h-sEH (IC_{50}, nM)	h-sEH *t*-DPPO (nM)	CYP2J2 (μM)	CYP2C9 (μM)	h-LM/r-LM (*t* – ½, min)[a]
1	6	19		18	63	>20	25.5	86/37
2	9	19		9	22	2.7	0.1	42/25
3	14	4		7	76	>10	0.3	23/42
4	6	4		4	40	2	10.2	54/10
5	9	4		14	nd	>10	0.8	34/42
6	7	16		1	13	nd	2.9	7/2
7	13	16		3	4	nd	8.1	2/2

[a] h-LM, in vitro human liver microsomal half life; r-LM, in vitro rat liver microsomal half life; nd, not determined.

preferred aniline-replacements, a variety of diverse cyclic amines were identified that are well-tolerated. In addition to affording compounds with excellent molecular and cellular potencies, these cyclic amines offer an opportunity to modulate a variety of off-target effects and physical–chemical properties. Additional in vitro profiling further differentiated the three best aniline-replacements based on microsomal stability.

During the preparation of this manuscript, Shen and co-workers reported the properties of a class of sEH inhibitors that incorporate a regiospecific piperidine motif.[16–19]

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