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Design and synthesis of substituted nicotinamides as inhibitors of soluble epoxide hydrolase

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ABSTRACT

A series of potent nicotinamide inhibitors of soluble epoxides hydrolase (sEH) is disclosed. This series was designed using structure-based deconstruction and a combination of two HTS hit series, resulting in hybrid analogs that retained the optimal potency from one series, and acceptable in vitro metabolic stability from the other. Structure-guided optimization of these analogs gave rise to nanomolar inhibitors of human sEH that had acceptable plasma exposure to qualify them as probes to determine the in vivo phenotypic consequences of sEH inhibition.

Soluble epoxide hydrolase (sEH) is an enzyme involved in the metabolism of chemical mediators derived from arachidonic acid.[1] In particular, sEH catalyzes the hydrolysis of epoxyeicosatrienoic acids (EETs) (derived from oxidation of arachidonic acid by CYP2 J and/or CYP2C) to the corresponding dihydroxyeicosatrienoic acids (DHETs) (Figure 1). Inhibition of sEH is expected to increase localized endogenous EETs levels, thereby potentiating their in vivo pharmacological effects which include anti-inflammatory and vasodilatory properties. For example, selective inhibition of soluble epoxide hydrolase has been invoked to account for the antihypertensive effect of dicyclohexyl urea (DCU) in the spontaneously hypertensive rat.[2] It is also reported that EETs elicit a vasodilatory response by acting as an endothelium derived hyperpolarizing factor (EDHF) that mediates vasodilatation through the stimulation of calcium-activated potassium channels in smooth muscle cells.[3] Selective sEH inhibitors have also shown beneficial effects in an angiotensin II-dependent model of hypertension in the Sprague–Dawley rat,[4] and protective action in models of hypertension-induced renal damage and failure.[5] An sEH inhibitor significantly decreased the total bronchoalveolar lavage cell number in tobacco smoke-exposed rats, with significant reductions noted in neutrophils, alveolar macrophages, and lymphocytes in a rat model of airway inflammation.[6] Combined, these reports suggest that inhibition of sEH represents a potentially novel method for the treatment of inflammatory and cardiovascular diseases.

To date, the majority of published sEH inhibitors can be depicted as urea derivatives, flanked on either side by lipophilic groups.[7] More recently, amide and carbamate inhibitors have also been disclosed.[8] The urea, amide, and carbamate moieties are thought to mimic the transition-state for the hydrolysis of endogenous epoxides within the active site of sEH. This mechanism is supported by the published crystal structure of sEH with a urea inhibitor, where the urea pharmacophore is involved in direct hydrogen bonding with the sEH catalytic triad of tyrosine 383,

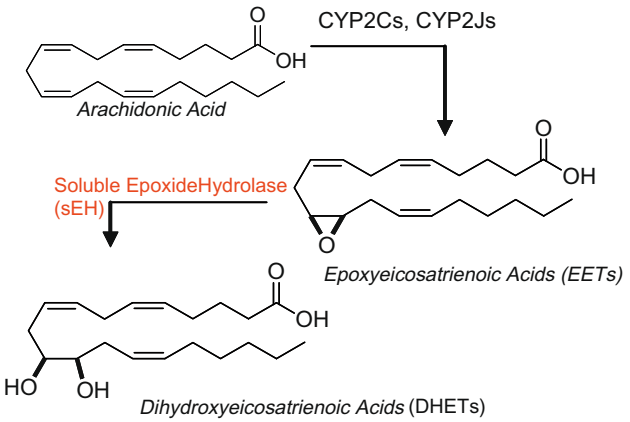

Figure 1. Role of soluble epoxide hydrolase in the conversion of EETs to DHETs.

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tyrosine 466, and aspartic acid 335.[9] In October 2007, a phase 1 clinical trial for hypertension was initiated with a sEH inhibitor from the urea class, AR9281.[10]

In this report, we disclose our efforts towards designing novel synthetic inhibitors of sEH. Our goal was to identify a small, drug-like inhibitor of sEH that could be administered orally once daily. High-throughput screening (HTS) of a selection of our corporate library against sEH revealed three distinct chemical series, one of which is exemplified by the amide (**1**) shown in Figure 2. Initial SAR focused around the nicotinamide **1** which, albeit displaying excellent intrinsic potency using an assay that measures the displacement of a rhodamine-labeled probe from sEH,[15] suffered from a poor in vitro metabolic half-life. In vivo and in vitro metabolite identification revealed that oxidative metabolism was predominantly occurring on the benzhydryl motif,[11,12] most likely accounting for the rapid clearance of these analogs when dosed intravenously. Partial optimization of another of our HTS leads resulted in a class of urea inhibitors, exemplified by **2**, which displayed extended half life in the presence of human and rat microsomes, but resulted in lower potencies than the corresponding benzhydryl amides **1**.[12] We hypothesized that a combination of the right-hand side (RHS) dichlorobenzylamine present in the urea series coupled with the left-hand side substituents featured in the nicotinamides could provide inhibitors that retained the optimal properties from each of the parent series.

A crystal structure of inhibitor **1** bound to human sEH showed that the arylamide moiety occupies a large pocket that opens towards solvent (Figure 3, S1 pocket).[12] One aryl group of the benzhydryl motif is located in a deep pocket (S2) while the other occupies a narrow polar channel to solvent (S3). Docking (GLIDE) of compound **3** into the crystal structure of human sEH suggested that the dichlorobenzyl amine moiety of **3** could be used as an alternative to the benzhydryl motif of **1.** Figure 3 illustrates that both chloro groups of the dichlorobenzyl amine moiety were optimally positioned to allow for the addition of surrogate moieties to the two RHS aryl groups of **1**. The pocket occupied by the 2-chloro moiety was deeply buried in the protein matrix, and therefore severely limited the optimization in this area. In contrast, the 4-chloro vector allows access to solvent and more space for further structural modifications.

Based upon the docking data and the co-structure of the nicotinamide inhibitor (R = H), a panel of compounds were synthesized that combined the dichlorobenzyl scaffold **4** with a diverse selection of aromatic moieties that had previously provided potent sEH inhibitors with the benzhydryl RHS **5** (Table 1).[12] The selection criteria for the groups was driven by diversity of chemical structure with respect to polarity and substituent patterns on the heteroaromatic ring. Synthesis of the amides was performed under standard amide bond forming conditions, as shown in Scheme 1.

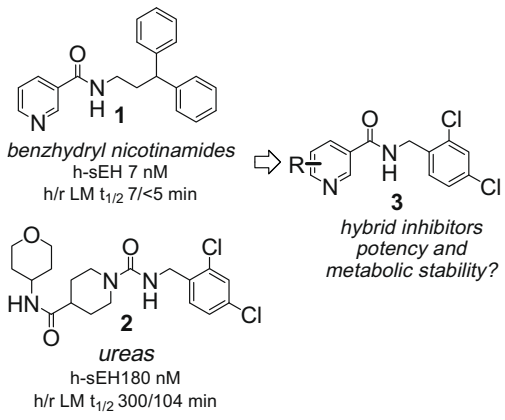


Figure 2. Representative examples of urea and benzhydryl nicotinamide series of sEH inhibitors.

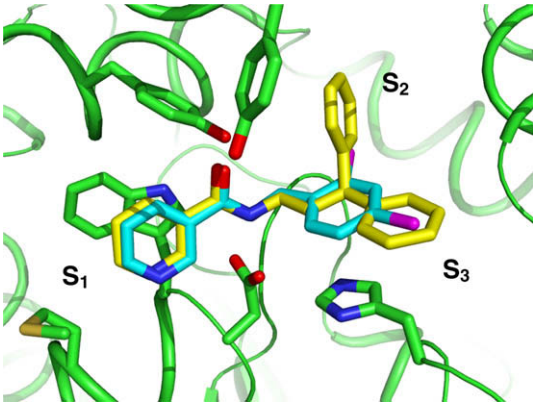


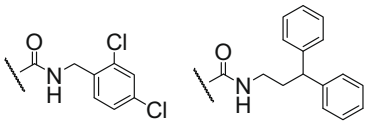
Figure 3. Compound **3** (cyan) containing a 2, 4-dichlorobenzyl motif docked into the co-crystal structure of human soluble epoxide hydrolase and compound **1** (yellow) PDB code 3I1Y.

These hybrid compounds, and the initial HTS lead series analogs were profiled for their ability to inhibit human and rat sEH as well as their propensity to undergo in vitro oxidative metabolism. Achieving potency against the rat enzyme was also important, as the pharmacological effects of sEH inhibition were going to be assessed in this species. The baseline pyridone, amino pyridine, and pyrrolidine pyridine analogs were all significantly less potent (>50-fold), than the corresponding diphenyl propyl amine analogs (Table 1, entries 1–6).[13] This potency trend was observed for both the human and the rat isoforms of sEH. Despite their lack of intrinsic potency, the compounds in the dichlorobenzylamide series were, in general, less susceptible to in vitro oxidative metabolism compared to the benzhydryl analogs, supporting our initial hypothesis. We theorized that the potency of the compounds in this series could be increased by substitution of the nicotinamide moiety with lipophilic groups that would better fit the large, non-polar S1 pocket of sEH. Gratifyingly, the alkylated pyridone **12** was significantly more potent than the previously tested hybrid molecules **6**, **8**, and **10**, and retained the improved h-LM/r-LM data of these initial, less potent analogs (Table 1, entry 7). Furthermore, substitution of the pyridyl ring with a trifluoroethoxy group yielded compound **14**, that demonstrated high potency in both the human and rat molecular sEH assays and displayed a decreased susceptibility towards in vitro oxidative metabolism in the h-LM assay (Table 1 entry 9 and 10). However, albeit displaying a significant increase in potency, the dichlorobenzyl amides were still not as potent as the analogous compounds from the benzhydryl amide series.

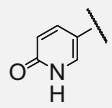
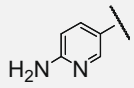
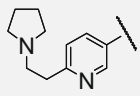
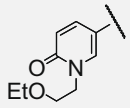
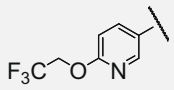
We then focused our optimization on the benzylamine portion of the molecule while keeping the (2,2,2)-trifluoroethoxy substituted pyridine constant. Based upon modeling of **3**, we explored substitution at the 2- and 4-position of the aromatic ring of the benzylamide, as these substituents would be engaging the sEH S2 pocket and S3 channel, respectively (Fig. 3).

Synthesis of differentially substituted benzyl amine reagents is shown in Scheme 2. Generation of the 2-chloro 4-methylsulfonamide benzylamine (**18**) was accomplished by treatment of 3-chloro-4-methylbenzenesulfonyl chloride (**16**) with methyl amine followed by bromination with NBS. The bromide **17** was subsequently converted to the benzylic azide which was then reduced under Staudinger conditions to furnish the benzylamine **18**. 2-Chloro-4-methylsulfonyl-benzylamine (**21**) was synthesized by conversion of 2-chloro-4-methylsulfonyl-benzoic acid **19** to the primary amide **20,** by generation of the mixed anhydride with BOC_2O, followed by displacement with hydroxylamine

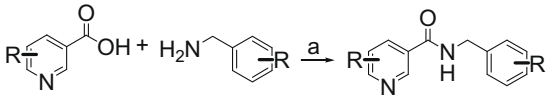
Table 1
Human and rat soluble epoxide hydrolase inhibition, and in vitro microsomal stability for hybrid compounds in comparison to compounds from the initial nicotinamide hit class



4 **5**

Entry	Compd	R	RHS	h-sEH IC_{50}[a] (nM)	r-sEH IC_{50}[a] (nM)	h-LM/r-LM ($t_{½}$ min)
1	**6**		4	400	330	78/191
2	**7**		5	8.1	10	96/7
3	**8**		4	520	430	46/44
4	**9**		5	9.1	8.5	33/5
5	**10**		4	1500	500	nt
6	**11**		5	18	9	46/41
7	**12**		4	16	6	24/19
8	**13**		5	2.6	4	4/5
9	**14**		4	6.6	7.3	178/7
10	**15**		5	5.4	7.8	38/<3

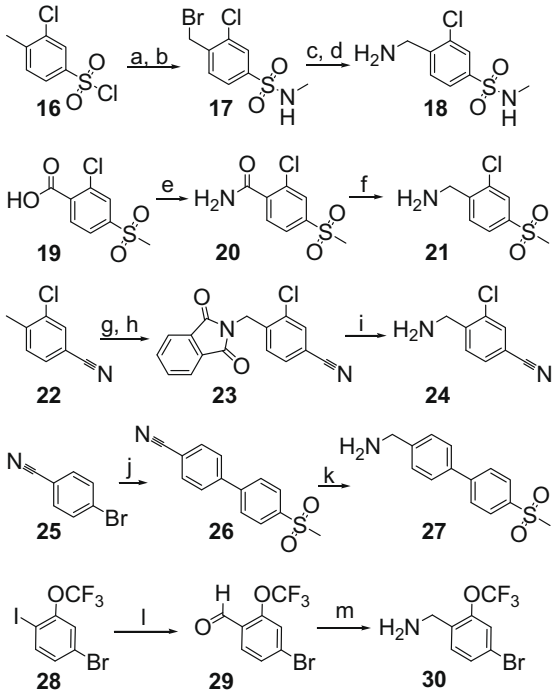
[a] Values are means of a minimum of two experiments (nt = not tested).
[b] h-LM (in vitro human liver microsomal half life), r-LM (in vitro rat liver microsomal half life) [12,16].



Scheme 1. General procedure for the **s**ynthesis of differentially substituted benzylamines. Reagents and conditions: (a) EDC-HCl (1.2 equiv), HOBT (1.2 equiv), DIEA (1.2–2.2 equiv), anhyd. DMF (0.2 M), rt 16 h.

hydrochloride. Selective reduction of the corresponding amide to amine **21** was accomplished with borane in THF. 2-Chloro-4-cyanobenzylamine (**24**) was synthesized by bromination of 3-chloro-4-methylbenzonitrile (**22**) with NBS, followed by displacement of the bromide with potassium phthalimide. Deprotection with hydrazine revealed the benzyl amine **24**. Synthesis of C-(4′-methylsulfonyl-biphenyl-4-yl)methylamine **27** proceeded via Suzuki–Miyaura coupling[14] of 4-bromobenzonitrile **25** with 4-methylsulfonyl boronic acid. Subsequent hydrogenation and deprotection of the intermediate carbamate provided compound **27**. 4-Bromo-2-trifluoromethoxy-benzylamine (**30**) was generated by selective lithium-halogen exchange of **28,** followed by in situ trapping of the aryl lithium species with morpholine-4-carbaldehyde. The resulting aldehyde (**29**) was converted to the benzylamine **30** via a reductive amination. These amines (and commercially available benzylamines) were coupled with 6-(2,2,2-trifluoroethoxy)-nicotinic acid using the general conditions outlined in Scheme 1.

The resulting nicotinamides were profiled in an sEH cellular assay to determine their activity in a more physiological relevant environment (Table 2), as well as in the human and rat sEH enzyme assays.[12,17] They were also profiled in human and rat microsomal preparations (h-LM/r-LM), as a predictor of in vivo oxidative metabolism. Sequential removal of the 2-and 4-chlorine atoms from the benzyl ring resulted in a 3- and 10-fold loss in potency, respectively, against the human isoform of sEH (Table 2, entries 2 and 3). This observation is consistent with the chlorine atoms occupying the large lipophilic S2 pocket of human sEH. Interestingly, removal of the halogen at the 2-position of the benzylamine resulted in a significant loss of inhibition of the human but not the rat isozyme (Table 2, entry 3). Complete removal of all substitution off the benzyl ring led to a much weaker inhibitor, especially of the human sEH (Table 2, entry 4). Based upon previous studies with the benzhydryl analogs **1,** we elected to replace the 4-chloro substituent with more polar functional groups in hope that this would give rise to additional protection against oxidative metabolism while maintaining acceptable potency. We hypothesized that substitution with a polar functional group would be tolerated at this site as the directionality of substitution off the *para* position engages solvent, and a number of hydrophilic amino acid side chains (i.e., serine 415). To this end, replacement of the *para*-chloro substituent with a methylsulfone (**34**) resulted in a slight decrease in potency, but this analog had a significantly longer half-life in the presence of rat liver microsomes (Table 2, entry 5). Replacing the 2-chlorine substituent of **14** with a methylsulfonyl group resulted in a 10-fold decrease in potency (Table 2, entry 6). The possibility that the methylsulfone in this position could compete



Scheme 2. Reagents and conditions: (a) methylamine, CH_2Cl_2, rt, 97%; (b) NBS, CCl_4, AIBN, reflux, 24 h, 27%; (c) NaN_3, DMF, 40 °C, 16 h, 50%; (d) PPh_3, THF/water, 24 h, 30%; (e) BOC_2O, NH_4HCO_3, pyridine, 84%; (f) BH_3 THF, reflux, 88%; (g) NBS, CCl_4, AIBN, reflux, 24 h, 86%; (h) potassium phthalimide, DMF, rt, 2 h, 71%; (i) N_2H_2, ethanol, reflux, 1 h, 63%; (j) 4-(methylsulphonyl)phenylboronic acid, $Pd_2(dba)_3$, KF, $[(t\text{-}Bu)_3PH]BF_4$, THF 80 °C, 18 h, 44%; (k) (i) BOC_2O, 50 psi H_2, 10% Pd/C, 18 h, 85%, (ii) HCl/dioxane, rt, 4 h, 55%; (l) *n*-BuLi, morpholine *N*-carbaldehyde −78 °C, 79%; (m) $NaBH_4$, NH_3, MeOH, rt, 16 h, 83%.

with the central amide pharmacophore for binding with the catalytic tyrosines 383 and 466 may explain the decrease in potency, as this substituent can be accommodated by the sheer size of the pocket. Mono substitution of the aromatic ring with a methylsulfone in either the 2- or 4-positions, resulted in a significant drop in potency compared to the monochloro derivatives **31** and **32** (Table 2, entries 7 and 8).

Replacement of the 4-chlorine in **14** with a nitrile (**40**) or a *N*-methylsulfonamide (**38**) gave similar results to the methylsulfone analog **34** (Table 2, entries 9 and 10). Likewise replacement of the 4-chlorine atom with a tetrazole yielded a potent inhibitor of human sEH, but not rat sEH (Table 2, entry 14). Introduction of a trifluoromethoxy group in the 2-position, even when lacking a complimentary substituent in the 4-position of the aryl ring, gave the most potent sEH inhibitor (4.6 nM), however **39** displayed a short in vitro microsomal half life (Table 2, entry 10). This short half life was attenuated by placement of a halogen atom at the 4-position (Table 2, entry 12), essentially doubling the in vitro half-life of the compound **41**, while maintaining acceptable intrinsic potency. Finally adding an additional aryl ring to the benzylamine (Table 2, entry 13), was tolerated as well.

Based upon the in vitro profile of these compounds we then assessed the in vivo exposure of a few representative analogs prior to initiating a full pharmacokinetic profile and evaluating these series further (Fig. 4).[14] Individual compounds were dosed orally as a suspension (5 mg/kg) in Sprague–Dawley rats ($n = 3$). Plasma samples were collected at 1, 2, 4, and 6 h post dosing. Compound **14** showed a 2.5 μM plasma level at 1 hour that was essentially maintained over 6 h. This result is in contrast to the relatively rapid predicted

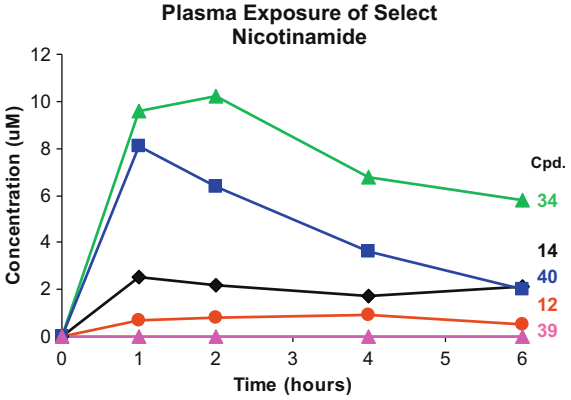
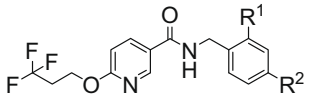


Figure 4. Rapid assessment of compound exposure (RACE) for select hybrid molecules. Average plasma concentration in three discrete animals per compound after 5 mg/kg dose, dosed PO as a homogeneous suspension in CMC-Tween.

Table 2
Potency and in vitro metabolic stability of compounds with differentially substituted benzylamine P2-3 motifs



Entry	Compd	R^1	R^2	h-sEH IC_{50}[a] (nM)	r-sEH IC_{50}[a] (nM)	h-LM/r-LM ($t_{½}$ min)	h-sEH HepG2 (nM)
1	**14**	Cl	Cl	6.6	7.3	178/7	18
2	**31**	Cl	H	21	22	nt	nt
3	**32**	H	Cl	77	7	nt	nt
4	**33**	H	H	1200	50	nt	nt
5	**34**	Cl	SO_2Me	15	16	96/75	28
6	**35**	SO_2Me	Cl	66	11	300/99	nt
7	**36**	H	SO_2Me	110	25	156/173	nt
8	**37**	SO_2Me	H	270	160	nt	nt
9	**38**	Cl	SO_2NMe	14	19	81/25	31
10	**39**	OCF_3	H	4.6	6	78/7	21
11	**40**	Cl	CN	14	8	188/105	12
12	**41**	OCF_3	Br	12	7	300/17	0.1
13	**42**	H	4-SO_2Me (phenyl)	12	4.3	nt	24
14	**43**	Cl	tetrazole	21	180	300/227	38

[a] Values are means of a minimum of two experiments (nt = not tested), compound synthesis and purity reported in Ref. 16.
[b] h-LM (in vitro human liver microsomal half life), r-LM (in vitro rat liver microsomal half life)[12,16].
[c] Cellular assay for inhibition of sEH in human Hep G2 Cells, ELISA readout [12].

(in vitro) clearance for this analog. The pyridone analog **12** showed very low levels in the plasma, however these were sustained over the course of 6 h, implying absorption rather than metabolically driven clearance could be a problem for this class of inhibitors. The 2-trifluoromethoxy benzylamine analog **39** failed to show any plasma exposure in these experiments. Compound **34** showed almost a 5-fold increase in plasma concentration in rat compared to **14,** and this exposure was sustained over a 6 h period. In addition, the nitrile analog **40** showed micromolar exposure throughout the experiment. This study highlighted that, for several derivatives, microsomal half-life was not predictive of their in vivo exposure (extent and duration), as highlighted by **14** which showed sustained exposure despite a high in vitro predicted clearance (80% Qh).

These studies also demonstrated that combination of the dichlorobenzylamine with the arylamide of **1** resulted in low molecular weight, potent sEH inhibitors, that show extended plasma exposure in rats when dosed orally. A variety of substituents were tolerated on the RHS of the sEH inhibitor, whereas extension off the phenylamide with large lipophilic groups was required for potency against human and rat sEH. By combining a potent aryl amides with a metabolically stable dichlorobenzyl amines, hybrid molecules were generated that retained the optimal properties associated with each series. While low nanomolar potencies were achieved, the compounds in this hybrid series were still not as potent as the analogous compounds within the benzhydrylamide series. These studies serve as the foundation for extended chemical elaboration of the phenyl moiety to further increase potency, and to address additional target-independent profiles. However, these compounds, structurally distinct from the urea-based inhibitors, still have acceptable profiles such that they can be used as alternative tools to assess the in vivo pharmacological effect of sEH inhibition in appropriate animal models of cardiovascular and inflammatory diseases.

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17. An alignment of the rat and human protein sequences reveals that there are several amino-acid differences within the ligand binding site. Many of these changes are close to the region of the protein that would influence the SAR described in Table 2.