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Discovery of 1-(1,3,5-triazin-2-yl)piperidine-4-carboxamides as inhibitors of soluble epoxide hydrolase

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ABSTRACT

1-(1,3,5-Triazin-yl)piperidine-4-carboxamide inhibitors of soluble epoxide hydrolase were identified from high through-put screening using encoded library technology. The triazine heterocycle proved to be a critical functional group, essential for high potency and P450 selectivity. Phenyl group substitution was important for reducing clearance, and establishing good oral exposure. Based on this lead optimization work, 1-[4-methyl-6-(methylamino)-1,3,5-triazin-2-yl]-*N*-{[[4-bromo-2-(trifluoromethoxy)]-phenyl]methyl}-4-piperidinecarboxamide (**27**) was identified as a useful tool compound for in vivo investigation. Robust effects on a serum biomarker, 9, 10-epoxyoctadec-12(*Z*)-enoic acid (the epoxide derived from linoleic acid) were observed, which provided evidence of robust in vivo target engagement and the suitability of **27** as a tool compound for study in various disease models.

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Inhibition of the enzyme soluble epoxide hydrolase (sEH) has recently emerged as a new approach to suppressing maladaptive functional and structural changes that are associated with cardiovascular disease.[1] Soluble epoxide hydrolase is a ubiquitously expressed enzyme responsible for the hydrolysis of the epoxides, epoxyeicosatrienoic acids (EETs), to their corresponding diols, dihydroxyeicosatrienoic acids (DHETs). In mammalian cells epoxyeicosanoids are formed through the cytochrome P450 oxidative metabolism (primarily CYP2C and CYP2J)[1] of arachidonic acid. EETs are known to function as autocrine and paracrine effectors producing a variety of cardiovascular and inflammatory effects.[2] In mesenteric and renal vessel beds EETs produce vasodilatory effects, and various reports suggest EETs may function as the endothelium-derived hyperpolarizing factor playing an integral role in the hyperpolarization of smooth muscle.[3] Based on the biochemical pathways affected by EETs, it is possible that EETs are cardio protective by preventing apoptosis (PI3K/AKT)[4,5], reducing inflammation (NF-κB)[6], and suppressing oxidative stress (K_{ATP}, NF-κB).[4,6,7]

To fully investigate the utility of a sEH inhibitor for cardiovascular and other disease indications our initial objective was to identify a tool compound with appropriate potency, selectivity, and oral pharmacokinetics. The first step on our critical path to a tool compound focused on cellular potency. Since sEH is an intracellular target we developed a cell assay overexpressing sEH to assess cell permeability and inhibition of EET to DHET enzymatic hydrolysis by sEH. Achieving good cardiac ion channel selectivity was also critical since chemical agents which block the L-type calcium channel (CaV1.2) or cardiac sodium channel (NaV1.5) would likely lower blood pressure, decrease cardiac output, and thus confound the interpretation of in vivo cardiovascular results. Selectivity

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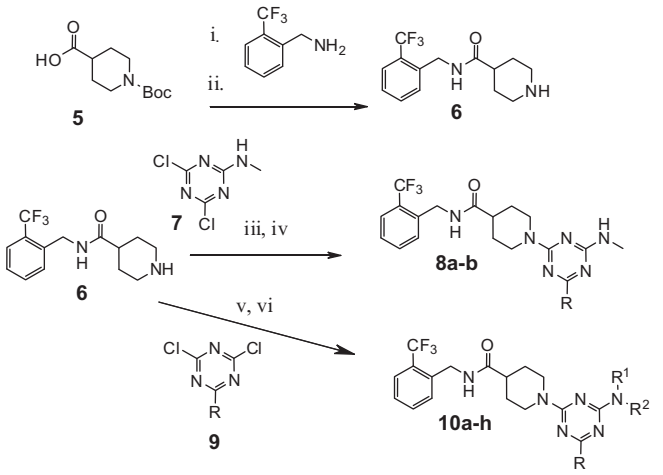
against microsomal epoxide hydrolase (mEH), an α/β-hydrolase fold enzyme with sequence and structural similarity to sEH was prioritized since mEH plays a key role in xenobiotic and extrahepatic metabolism.[8] Also, of concern was selectivity against the cytochrome P450 monooxygenases since these metabolic enzymes convert arachidonic acid to EET.[1a] Inhibition of P450 enzymes could reduce EET levels, and thus suppress the desired pharmacological effect gained by blocking sEH. In terms of the desired pharmacokinetic profile for a tool compound dosed chronically, good oral bioavailability was critical since drug exposure from chow is often targeted. Chow dosing was desired in order to maintain stable drug levels and avoid labor intensive oral gavage dosing on large groups of animals over multiple days. This article will describe the lead optimization of screening hit **4**, the developability profile of in vivo sEH tool compound **27**, and in vivo biomarker study of **27** in the rat.

Recently several structural types of *N*-benzyl amide inhibitors (**1**; Boehringer, **2**; Taisho, and **3**; Hammock and Landry labs) of sEH have been reported to possess good potency, aqueous solubility, and metabolic stability (Fig. 1).[9] Our own efforts in this area began with the discovery of lead **4** (Fig. 1) derived from high throughput screening employing encoded library technology (ELT).[10] Like previous amide inhibitors of sEH, it was anticipated that the amide functionality contained in **4** interacts with the catalytic residues (Tyr381/465 and Asp333) in the sEH active site.

The unique triamino-triazine functionality represented a good starting point for optimization of developability parameters such as bioavailability and clearance. Our goal for this series was to identify a tool compound with good cellular potency, selectivity, and oral PK for study in chronic models of cardiovascular and respiratory disease.

Soluble (human and rat) and microsomal (human) epoxide hydrolase enzyme activity was measured using a fluorescence-based detection assay employing 3-phenyl-oxiranyl-acetic acid cyano-(6-methoxy-naphthalen-2-yl)-methyl ester (PHOME) designed by Hammock and coworkers.[11] Cellular activity was determined by assessing a compounds ability to inhibit the conversion of EETs to DHETs in human embryonic kidney (HEK) cells over-expressing human sEH. This was accomplished using an ELISA readout which employed a radiolabeled DHET antibody.[12] In terms of oral PK, in general, early analogs from this series displayed good oral bioavailability however clearance was moderate to high. Lead optimization focused on identifying new leads with nanomolar sEH potency, greater than 1000-fold selectivity over CYP2C9 P450[13], and low iv clearance (with good oral %F).

The impact of substitution on the triazine ring was initially probed. Functionalization of the piperidine group was accomplished through *N*-benzyl-piperidine **6**, readily available from piperidine-4-carboxylic acid (Scheme 1). Reaction of piperidine **6** with the appropriate dichloro-triazine intermediate, followed by chloride displacement with the requisite amine gave rise to targets



Scheme 1. Preparation of triamino-triazines. Reagents and conditions: (i) EDC, DIEA, CH_2Cl_2/DMF; (ii) TFA, CH_2Cl_2; (iii) **7**, Hunigs base, CH_3CN, RT, 24 h; (iv) R-NH_2, Hunigs base, CH_3CN, RT, 24 h; (v) **9** (R = Me, Et, *c*-hexyl, phenyl), DIEA, CH_2Cl_2, rt. (vi) $MeNH_2$ or NHR^1R^2, MeOH, 40 °C.

4, **8a**–**b**, and **10a**–**h** (Table 1). Early analogs (**8a** and **8b**), in which the *N*,*N*-4-methyl-piperazine was replaced with other amine groups, did not show an improvement in iv clearance or potency, relative to **4**. However when the piperazine group was replaced with an alkyl or aryl substituent (**4** → **10a**–**h**), potency was improved by an order of magnitude. The most substantial change in clearance in the rat (iv) occurred where R = phenyl (**10d**). However the 10-fold reduction in clearance for **10d** may be due to the extremely high plasma protein binding observed for this analog (rat Fu = 1.5%). In most cases the CYP2C9 selectivity was 100-fold or better which indicates that inhibition of the CYP2C family may not be an issue for this chemical class.[13] SAR exploration around the methyl-amine group of lead **10a** identified several substitutions (**10g** and **10h**) that gave rise to improved cellular potency, however P450 selectivity was reduced.

The triazine core was another area that was examined to determine if this core offered advantages over other six-membered aromatic rings. Phenyl, pyridine, and pyrimidine core substitutions were synthesized in straight-forward fashion according to known procedures. In general, pyridines, pyrimidines, and phenyl core replacements led to a decrease in activity relative to triazine (Table 2). Examination of the iv clearance for the most potent pyrimidine (**13**) and pyridine (**16**) did not suggest a clear advantage for these two heterocycles over the triazine **10a**. Additionally, **13** displayed reduced CYP2C9 selectivity, indicating no advantage over triazine **10a**.

The crystal structure of **10a** bound in the active site of human sEH,[19] highlighted in Figure 2, suggested that there was space

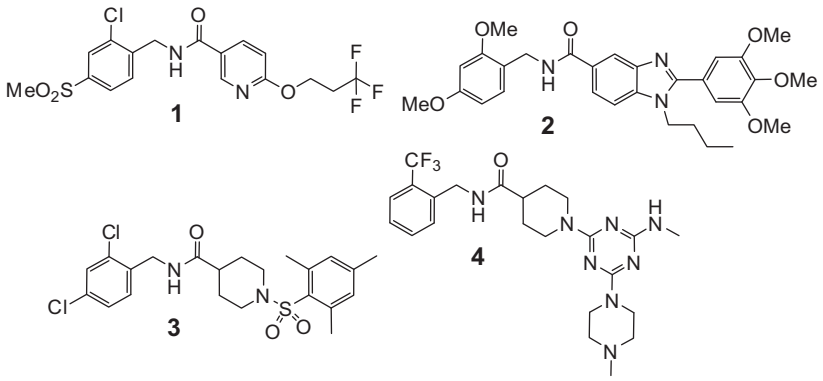


Figure 1. *N*-Benzylamide inhibitors of sEH.