Discovery of Inhibitors of Soluble Epoxide Hydrolase: A Target with Multiple Potential Therapeutic Indications

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1. INTRODUCTION

Epoxide hydrolases have been detected in prokaryotes and eukaryotes ranging from plants to mammals.[1−3] In mammals these include the soluble epoxide hydrolase (sEH), microsomal epoxide hydrolase (mEH), cholesterol epoxide hydrolase, and leukotriene A_4 (LTA_4) hydrolase. These enzymes mediate the addition of water to both exogenous and endogenous epoxides, leading to the corresponding vicinal diols except for LTA_4 hydrolase, and they display different substrate selectivity. For example, the mammalian sEH is selective for aliphatic epoxides and particularly fatty acid epoxides, whereas mEH is more selective for cyclic and arene epoxides. Studies on the mEH have focused on its role in xenobiotic metabolism, but its distribution, particularly in the brain and adrenal gland, suggests a possible endogenous role.[4] Although its catalytic activity on fatty acid epoxides is low, the high level of the mEH in some brain regions may contribute to their hydrolysis. The catalytic activity of the sEH on arene oxides and other cyclic epoxides is so low that its contribution appears insignificant compared to the mEH as well as for chemical and glutathione S-transferase catalyzed conjugation of reactive epoxides. Although the sEH can metabolize some aliphatic natural products, the sEH is thought to be involved largely in the metabolism of regulatory epoxy lipids, particularly those of the arachidonic acid cascade (Figure 1). Titers of free arachidonic acid are very low, but when it is released, it is converted to a wide variety of biologically active metabolites. Most research has focused on the cyclooxygenase and lipoxygenase pathways, but increasing attention is being paid to the cytochrome P450 branch of the cascade. One set of P450 enzymes carry out allylic and ω and ω-1 oxidation. Another set of P450 enzymes form regioisomeric epoxides of arachidonic acid and other unsaturated lipids. In the arachidonate series these epoxides are called epoxyeicosatrienoic acids (EETs). The EETs are metabolized by incorporation into phospholipids, chain shortening, chain elongation, hydroxylation, and other pathways.[5] However, the dominant pathway is hydration of the epoxides to the corresponding 1,2-diols by sEH. It is noted that multiple drugs have already been discovered to act on the cyclooxygenase and lipoxygenase branches of the arachidonic acid metabolic cascade. For example, numerous nonsteroidal anti-inflammatory drugs (NSAIDs) are inhibitors of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2).[6] In addition, montelukast is a leukotriene (LT) receptor antagonist blocking the action of LTD_4 and secondary ligands LTC_4 and LTE_4.[7] Zileuton inhibits 5-lipoxygenase, an enzyme of the eicosanoid synthesis pathway for the production of LTs.[8] Both montelukast and zileuton are effective therapies for treatment of asthma. Lastly,

laropiprant, an antagonist of the DP1 receptor of prostaglandin D_2, is used in combination with niacin to suppress the niacin-induced vasodilation.[9]

As shown in Figure 2, sEH in human (hsEH, EPHX2, EC 3.3.2.10) is a bifunctional homodimeric enzyme located in both cytosol and peroxisomes with both epoxide hydrolase and phosphatase activity.[1] Specifically, the C-terminus epoxide hydrolase motif of sEH transforms four regioisomers of EETs, namely, 5,6-, 8,9-, 11,12-, and 14,15-EETs, which are the endogenous chemical mediators derived from arachidonic acid by cytochrome P450 epoxygenases, to the corresponding dihydroxyeicosatrienoic acids (DHETs), whereby the biological effects of EETs are diminished, eliminated, or altered.[10] Recent work has shown that some fatty acid diols have unique biological activities, but the diols are far more polar than the epoxides and thus quickly move out of cells and often are conjugated.[11,12] The sEH hydrates all fatty acids so far tested. For example, sEH converts linoleic acid epoxides to proinflammatory linoleic acid diols, which are proposed endogenous chemical mediators as well. Regarding the enzyme activity in tissues, it was found that human liver possesses the highest sEH specific activity followed by the kidney.[13,14] Of note, specific cell types in the heart, vasculature, brain, lung, and kidney have quite high levels of enzyme. However, much less is known about the N-terminal phosphatase regarding its endogenous substrates and physiological roles.

The catalytic mechanism of epoxide hydrolases was worked out by a series of biochemical studies in several laboratories based on the homology of the mEH and sEH to haloalkane dehalogenase. Similarly the hypothetical arrangement of binding sites in the enzyme was predicted by three-dimensional quantitative structure−activity relationship (3D QSAR).[15] The X-ray crystal structure of human sEH complexed with an sEH inhibitor (PDB code 1ZD3) and later structures revealed the catalytic pocket and the key structural features required to inhibit the epoxide hydrolase activity of this enzyme in great detail (Figure 3).[16] The epoxide hydrolase catalytic pocket consists of two tyrosine residues (Tyr381 and Tyr465) which activate the epoxide ring-opening by Asp333. The resulting ester is then rapidly hydrolyzed into DHETs. It has been recognized that amide, carbamate, and urea groups fit well in the hydrolase catalytic pocket. Specifically, the carbonyl oxygen of amide or urea is involved in a hydrogen bonding interaction with Tyr381 and Tyr465, and the NH of ureas or amides acts as a hydrogen bond donor to Asp333. Therefore, various ureas and amides (1 and 2) have been developed as competitive, reversible,
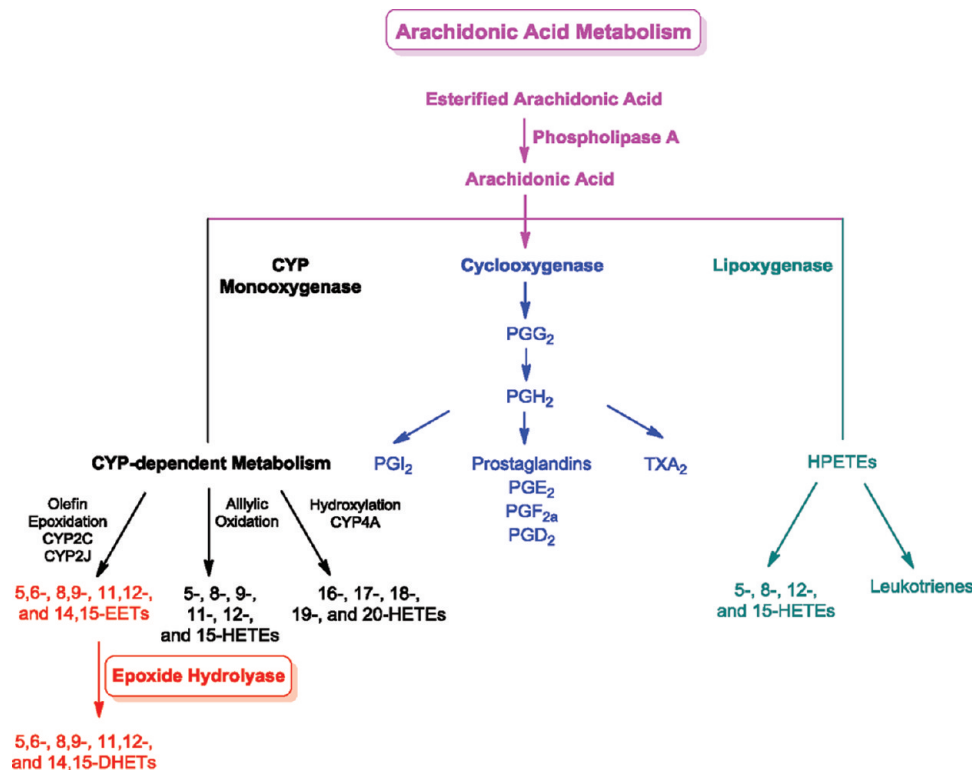
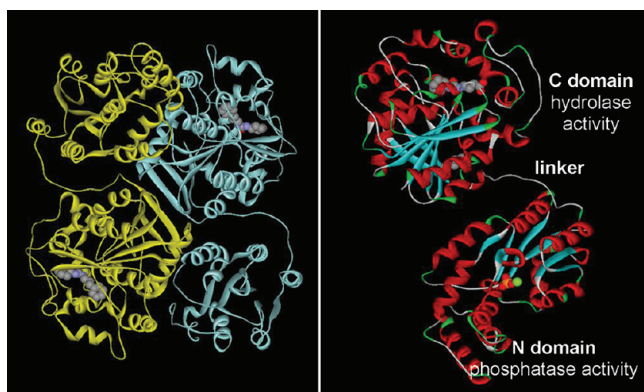**Figure 1.** Major pathways of arachidonic acid metabolism.

Figure 2. Left: X-ray cocrystal structure of murine sEH (PDB code 1CR6). One of the antiparallel monomers is shown in blue and the other in yellow. Right: X-ray cocrystal structure of a single subunit of the human sEH (PDB code 1ZD3).
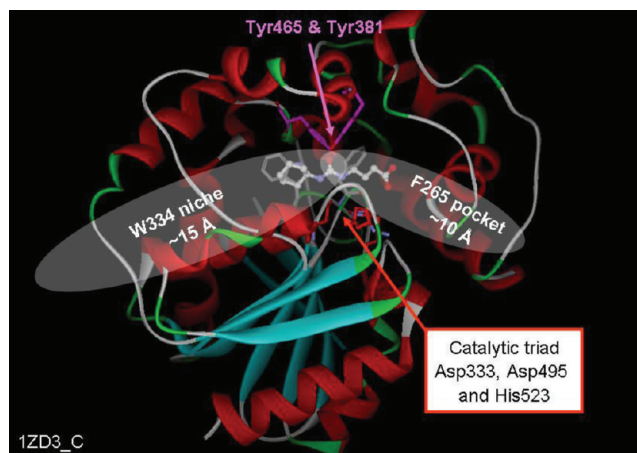

Figure 3. X-ray cocrystal structure of human sEH C domain showing the binding site that exists as an "L" shape hydrophobic tunnel (PDB code 1ZD3).

and often tight binding sEH inhibitors. Several of these inhibitors bind to the sEH in the picomolar range. The X-ray structure also showed that the two domains of the sEH were joined by a proline rich bridge, the enzyme in mammals is an antiparallel homodimer, and the putative catalytic site of the N-terminal domain suggested an active phosphatase of unknown role. The W334 niche and F265 binding pocket, as depicted in Figure 3, can each accommodate a variety of functional groups of sEH inhibitors. These structures not only have assisted with optimization of sEH inhibitors but have proven valuable to evaluate the biology associated with single nucleotide polymorphisms (SNPs) in the enzyme.

In mammals the mEH (EPHX1, EC 3.3.2.9) is membrane bound and largely in the endoplasmic reticulum.[17,18] In some pathological states the mEH dissociates from the membrane and appears in the blood where it is known as the preneoplatic

antigen and is a marker for tissue damage including cancer.[19] Activity and polymorphisms of the enzyme have been associated with a variety of diseases, and this is a quite active area of research.[20] With regard to this review it is important to note that although the mEH and sEH have similar catalytic mechanisms and are members of the α/β-hydrolase fold family of proteins, their evolutionary paths diverged at the level of prokaryotes. Thus, it is possible to differentially inhibit the mEH and sEH with high selectivity.[21]

Evidence is excellent that the enzyme referred to as hepoxilin epoxide hydrolase is in fact the sEH. The cholesterol 5,6-epoxide hydrolase and leukotriene A_4 hydrolase are in different enzyme families working by different catalytic mechanisms and will not be discussed further. In the study of the evolution of the sEH,

related genes were further found. These hypothetical products of these genes are known as EH3 and EH4. Their expression in vertebrates, particularly in man, is under investigation, and their biological activity is so far not clear.[18] In light of the recent progress in this field, this Perspective will provide biological rationales, medicinal chemistry approaches, and possible paths for sEH inhibitors to enter clinical trials.

2. BIOLOGICAL RATIONALES AND POTENTIAL INDICATIONS FOR SEH INHIBITORS

It was envisaged that sEH inhibition may lead to elevated levels of EETs, which in turn could elicit various beneficial biological effects.[22] These effects may be translated into therapeutic treatment for hypertension, atherosclerosis, pulmonary diseases, diabetes, pain, inflammation, immunological disorders, and other indications.

The first major therapeutic area pursued for sEH inhibitors was hypertension. This target selection was based on extensive previous biology on the endothelium derived hyperpolarizing factor and the important roles of EETs in the regulation of renal tubular and vascular function.[23,24] It was an early target probably also because the role of EETs and other epoxy lipids in inflammation and pain was not widely appreciated. The hypertension target is supported by many preclinical studies and multiple models of hypertension, but a major limitation is that the vast majority of studies have been done in rodent species.[24] For example, the intra-arterial infusion of 14,15-EET transiently reduced arterial blood pressure in rats.[25] Furthermore, certain preclinical studies supported that sEH inhibitors could be used for hypertension treatment and disease-modifying end organ protection. It has been reported that sEH inhibitors could lower blood pressure in angiotensin II (AngII) treated rats[26] and spontaneously hypertensive rats (SHRs).[10] An sEH inhibitor in AngII-infused hypertensive rats successfully attenuated the afferent kidney arteriolar diameter and reduced urinary albumin secretion, a marker of compromised renal function. On the other hand, there are also conflicting reports that showed that highly potent sEH inhibitors failed to induce blood pressure lowering effects in some SHR strains which are often used as models of human hypertension by industry.[27,28] Most importantly, a clinical evaluation of an sEH inhibitor, 1-(1-acetylpiperidin-4-yl)-3-adamantan-1-ylurea (**28**,AR9281), most likely showed no efficacy in phase II studies.[29] Although compound **28** is not a highly potent inhibitor of the human sEH and it has a short blood half-life,[30] it showed a clear reduction of blood pressure in an AngII model of hypertension.[31]

There are multiple cautions about targeting human hypertension as an early clinical trial for sEH inhibitors in addition to the failure of compound **28** in human trials. There is of course the ongoing question in the field whether any rodent models predict efficacy in man. There are a variety of SHR strains. It was reported that beneficial effects could be obtained from one SHR strain without lowering hypertension.[32] Several studies from the Doris laboratory caution that there are haplotypes of SHR making them insensitive to the action of sEH inhibitors.[33,34] A second caution is that with hypertension and some other indications, EETs and other fatty acid epoxides appear to modulate blood pressure back toward the normotensive state rather than being true blood pressure lowering agents. Finally the actions of sEH inhibitors are likely to be influenced by the dietary status of the patient, with major effects from salt consumption and relative amounts of ω-3 and ω-6 dietary lipids as well as lipid chain length.

The most studied targets for intervention by sEH inhibitors are related to reduction in inflammation. sEH inhibitors are effective as an antiatherosclerosis therapy in murine models and for improving blood flow and reducing inflammation in models of stroke and myocardial infarction. This is supported by the roles of EETs in dilating coronary arterioles via the activation of BK_{Ca} ion channels.[35] In addition, EETs inhibited leukocyte adhesion to the vascular wall, presumably by inhibiting NF-κB and IKB kinases.[36] As an endothelium-derived hyperpolarizing factor (EDHF) to relax vascular smooth muscle,[37] 11,12-EET also displayed anti-inflammatory activity apparently by decreasing the expression of cytokine induced endothelial cell adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and E-selectin.[38] Preclinically, a short-term intracoronary infusion of EETs reduced infarct size in a canine ischemia—reperfusion model.[39] Thus, sEH inhibitors may elevate levels of EETs, thereby potentially ameliorating vascular inflammation, endothelial dysfunction, and ultimately atherosclerosis.[40] The utility of sEH inhibitors in cardiovascular animal models has been reported. For example, an sEH inhibitor 12-(3-adamantan-1-ylureido)dodecanoic acid butyl ester (AUDA-BE) reduced the infarct size after a middle cerebral artery occlusion (MCAO) in mice.[41] An sEH inhibitor was shown to prevent the development of cardiac hypertrophy and failure and to suppress cardiac arrhythmias in thoracic aortic constriction (TAC) mice.[42] Therefore, sEH inhibitors may be useful for treating cardiomyopathy and inhibiting cardiac arrhythmia. Furthermore, Arête disclosed that an sEH inhibitor effectively decreased total cholesterol, low density lipoprotein cholesterol (LDL-C), triglyceride (TG), and glucose in AngII-infused ApoE deficient mice.[43] Blood eicosanoids and particularly fatty acid epoxide to diol absolute levels and ratios are good indicators of in vivo target engagement in these and other studies. Despite these observations, the major challenges to pursue antiatherosclerosis and many other indications driven by inflammation with sEH inhibitors are the lack of disease-modifying efficacy biomarkers, absence of data in non-rodent models, and the high cost of outcome trials.

Arête disclosed several studies of sEH inhibitors in metabolic disease models.[44] One sEH inhibitor was shown to slow the weight gain of diet-induced obese (DIO) mice relative to vehicle. This compound reduced plasma cholesterol and serum glucose in an interperitoneal glucose tolerance test and decreased blood pressure at 60 mg/kg (mpk) (b.i.d.) in C57B1/6 mice fed on high-fat and high-fructose diet without significantly changing the heart rate. In another related patent application,[43] Arête also indicated that an sEH inhibitor could effectively reduce total cholesterol, LDL, TG, and glucose in AngII-infused ApoE deficient mice. Therefore, it appears that sEH inhibition may be useful in treating metabolic syndromes including obesity, hypertension, diabetes, and hypercholesterolemia. However, a mechanism for these effects remains elusive[45] and sEH inhibitors have not proven universally successful in reducing metabolic disease in rodent models.

Another therapeutic area of interest for sEH inhibitors is neuropathic and inflammatory pain.[46] An sEH inhibitor provided similar efficacy with respect to morphine (1 mpk) in a pain alleviation model and far greater potency in another model.[47] Interestingly, sEH inhibitors were also found to synergize activity of COX and 5-lipoxygenase (5-LOX) inhibitors.[48,49] In a pain model, efficacy of pain tolerance after lipopolysaccharide (LPS) exposure appeared to be similar for Vioxx (10 mpk) and

AUDA-BE (20 mpk). In addition, 12-[3-adamantane-1-ylureido]-dodecanoic acid (AUDA, **3**) analogues blocked LPS-elicited thermal hyperalgesia in rats.[50] Topical application of either an sEH inhibitor or EETs reduced inflammatory pain in rats, and the combination was far more effective.[51] Of particular interest, sEH inhibitors reduced neuropathic pain in a number of rodent models including nerve damage and diabetic neuropathic pain. This is a largely unmet medical need, and sEH inhibitors appeared to be superior to the gabapentin family of drugs while not causing changes in behavior or coordination associated with opiates.[52] Interestingly sEH inhibitors seemed to reduce the perception of pain in models where pain perception was enhanced (allodynia and hyperalgesia) but to not influence pain perception in normal animals. This may be due to cyclic nucleotides being needed for sEH inhibitors to act.[53] Interestingly sEH inhibitors were synergized in reducing neuropathic pain with COX inhibitors such as diclofenac.[54]

sEH inhibitors also displayed reasonable rheumatoid arthritis assessment score improvement in a mouse model.[55] One patent application claimed that the intraocular high pressure caused by inflammation could be attenuated by using EETs or sEH inhibitors.[56,57]

Boehringer Ingelheim discovered that pyrazole aniline derived amides were sEH inhibitors that may be effective in treating T-lymphocyte mediated immunological disorders in their preliminary in vitro and in vivo studies.[58]

Inhibitors of sEH reduced pulmonary infiltration by neutrophils and reduced leukotoxin diols that are toxic to pulmonary and vascular epithelium cells associated with adult respiratory distress syndrome.[59,60] The dosing of an EET and sEH inhibitor appeared to be synergistic in reducing the number of neutrophils in lung, which implies their potential utility to treat obstructive pulmonary diseases, restrictive airway diseases, and asthma.[59]

sEH inhibitors may also treat smooth muscle disorders such as erectile dysfunction, overactive bladder, uterine contractions, and irritable bowel syndrome.[61] A patent application from Roche claimed a method of treating genitourinary disorders and particularly overactive bladder by using sEH inhibitors.[62] They reported that an sEH inhibitor reduced the bladder pressure and decreased the bladder contraction frequency as well as amplitude in anesthetized SHRs. These data indicate that fatty acid epoxides and particularly EETs may be the hyperpolarizing factor of the urinary epithelium.

A common theme among sEH inhibitors in different models is that the compounds seem to act more to return a physiological system toward a normal state rather than being overtly hypotensive, hypoalgesic, or anti-inflammatory. For example, there is little change in the plasma oxylipin profile following administration of sEH inhibitors to normal animals. However, in inflamed animals there is a dramatic shift toward profiles indicating resolution of inflammation rather than its propagation.[63,64] The eicosanoid profile seen after administration of sEH inhibitors suggests that they should synergize with NSAIDs, COX-2 blockers (COXIBs), and inhibitors of the 5-LOX pathway. This was verified experimentally.[65−67] Co-treatment with sEH inhibitors also reduced the thrombotic events associated with the massive increase in 20-HETE by some COXIBs.[68] The additive to synergistic effect and the reduction of side effects of COXIBs with sEH inhibitors indicate that they might be attractive in drug combinations, and joint inhibitors have also been prepared.[69]

3. MEDICINAL CHEMISTRY

The potential therapeutic utility of sEH inhibition, based on the aforementioned biological rationales, prompted the discovery of sEH inhibitors with enhanced potency, improved solubility and pharmacokinetics (PK) properties, and high target selectivity. This is a case where a detailed understanding of the catalytic mechanism of the enzyme and later multiple crystal structures allowed the development of theoretical transition state mimic inhibitors of the enzyme from first principles based on the pioneering idea of Linus Pauling.[70] Several pharmaceutical companies and academic institutions, including Arête, Astellas, Boehringer Ingelheim, Chinese Academy of Sciences, Dainippon Sumitomo, GlaxoSmithKline, Merck, Roche, Taisho, Takeda, University of California—Davis, Columbia University, and Shanghai Institute of Biological Sciences, etc., have entered the arena of developing sEH inhibitors to target various therapeutic indications. As a result, more than 100 patent applications[71] and over 30 medicinal chemistry articles on sEH inhibitors have been published to date. Overviews of medicinal chemistry are presented in at least two comprehensive reviews.[72,73] The section Medicinal Chemistry of this Perspective is primarily devoted to the reported approaches to identify tool compounds with desirable properties for pharmacological studies. The biological data of benchmark sEH inhibitors will also be highlighted and used to indicate strengths and limitations of the approach of using sEH inhibitors in medicine.

The availability of homogeneous, recombinant sEH from human and model species was critical for the medicinal chemistry efforts described below. Modern sEH inhibitors are competitive, slowly reversible inhibitors acting at a low concentration and competing with a substrate likely present at a very low concentration. Thus, assay conditions may violate Michaelis−Menten assumptions, and it is difficult to compare inhibitor potencies among laboratories. The ability of assays to distinguish among the most potent inhibitors has improved with time. Early sEH assays were unable to distinguish among the most potent inhibitors.[74] As new assays were developed, still more active inhibitors followed that challenged the ability of enzymologists to distinguish among them. For example, the early colorimetric high throughput assay was unable to distinguish effectively among inhibitors in the nanomolar range.[74,75] Also, as the properties of these assays approach their limit to distinguish the most potent inhibitors, their reliability decreases. A high throughput fluorescent assay is commercially available.[76] With a highly purified substrate it can reach inhibitors acting in the subnanomolar range. Glutathione S-transferases and esterase interfere with this assay, and it is often used incorrectly. The development of assays to distinguish among the most potent inhibitors was driven by the synthesis of such potent inhibitors. The use of suspected endogenous substrates as substrates for assay is intellectually compelling and certainly should be used to verify the activity of lead inhibitors. However, these substrates are lipophilic, relatively water insoluble, and hard to detect; LC/MS/MS shows nanomolar limit of quantification (LOQ).[77,78] Even with these hypothetical endogenous substrates, it is a challenge to distinguish among inhibitors with picomolar IC_{50} values.[77]

The structural types of sEH inhibitors are extremely broad, which is consistent with the wide binding pocket of the enzyme (Figure 3). The major inhibitor chemotypes include urea, amide, carbamate, thioester, carbonate, ester, thiourea, thioamide, amidine, guanidine, heterocycles, aminoheterocycles, amino-heteroaryls, chalcone oxides, acylhydrazones, chalcone oxides,

and *trans*-3-phenylglycidols, of which the most advanced series in terms of potency and pharmacological studies are ureas and amides.[71] Interestingly even the early kinetically irreversible inhibitors including chalcone oxides and glycidols provided a structure—activity relationship (SAR) that gave a general indication of the shape of the catalytic site.[79]

3.1. University of California—Davis. The University of California—Davis (UCD) conducted pioneering research in the field of sEH inhibitors. Their early alternative substrate inhibitors were used to show in vivo effects of sEH inhibition, but these compounds were far too quickly metabolized to be useful drug leads. They subsequently reported a series of 1,3-disubstituted ureas and related amides and carbamates (**1**, Figure 4).[80] These
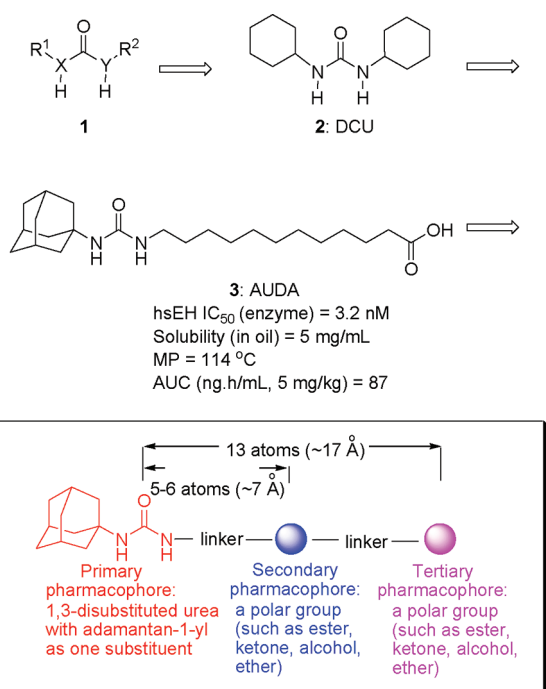

Figure 4. Evoluation of early sEH inhibitors and pharmacophores.

pharmacophores were thought to be mimics of EETs and possible mimics of a reaction transition state or transient intermediate to the diol. For example, their first lead was a common reaction side product in organic chemistry, dicyclohexyl urea (DCU, **2**). It elicited dose-dependent inhibition of sEH but no inhibition of the mEH. Since the mEH is involved in the metabolism of some dangerous xenobiotics, counterscreens on the mEH have been employed since the first papers in the field. By use of the tail cuff method to measure blood pressure, an average of 22 mmHg reduction of systolic blood pressure in SHRs was observed 6 h after the ip dose of 3 mpk of solubilized compound **2**, but no effect was observed in WKY rats. Similarly, compound **2** decreased the systolic blood pressure an average of 12 mmHg in SHRs 6 h after dosing.[10] With typical SAR approaches, the group optimized steric and lipophilic parameters for the substituents on the N and N′ positions of the urea as well as the amide pharmacophores. Aromatic groups with ortho substituents on the N and N′ positions of the urea dramatically reduced inhibitory potency. Aromatic groups with substituents in other than the ortho positions led to high activity with steric parameters generally dominating over electronic in the SAR. By introduction of a terminal solubilizing group to mimic the

carboxylic acid in a putative endogenous substrate, the benchmark compound **3** was discovered. It was reported that this compound improved renal hemodynamics, causing reduced sodium retention and vascular dilation in rats fed on high-fat diet.[81] This commercially available compound has been used in a wide variety of studies to knock out the sEH chemically and test for biological activity. The observation that compound **3** could dilate mesenteric arteries lacking the sEH provided evidence that these pharmacophores could mimic the epoxide at putative fatty acid oxide receptors.[82] This led to a new generation of EET mimics.[83]

Although compounds such as **3** were potent and competitive inhibitors with low nanomolar K_i values and subnanomolar binding, the lack of solubility in water and common formulation solvents and high melting points limited the use of compound **3** for pharmacodynamics (PD) studies. Compound **3** is also rapidly metabolized by cytochrome P450 action on the adamantyl substituent and particularly by β oxidation. Thus, the in vivo efficacy of compound **3** is brief in most systems. To improve physical properties, particularly solubility and PK of these disubstituted ureas, extensive SAR studies were carried out to introduce new polar pharmacophores and conformational constraints. For these studies druglike properties, high oral bioavailability, and good PK properties were seen as major drivers for improved structures. Since the adamantyl substituent gave a 10- to 50-fold increase in sensitivity of detection of the compounds on positive ion LC—MS, the adamantyl group was included in many derivatives during the optimization of structures on the right side of the central pharmacophore to fit into the F265 pocket. This allowed cassette dosing of animals at low drug levels and the determination of PK using only microliters of blood. This work designated the urea or amide moiety of sEH inhibitors as the primary pharmacophore that interacts with the catalytic triad of sEH (Figure 4).[84] Polar functional groups such as carbonyl, ester, ether, sulfonamide, or amide that are ~7 Å away from the urea carbonyl were defined as a secondary pharmacophore that typically improved aqueous solubility and PK properties while maintaining potency. In addition, a polar tertiary pharmacophore such as an ester, ether, acid, or amine that is 13 atoms or ~17 Å away from the urea carbonyl has also been identified. The linker that connects the primary and the secondary pharmacophores, or the secondary and the tertiary pharmacophores, can be either a conformationally flexible alkyl or restricted cyclic structures such as aryl, cycloalkyl, or cycloamino group.

The UCD group adopted multiple approaches to improve the physical properties and metabolic stability of the disubstituted urea sEH inhibitors (Figure 5). The first approach involved the derivatization of compound **3** to provide improved inhibition potencies and physical properties such as a 23−66 °C lower melting point and up to 5-fold better solubility in oil (triglyceride).[85] For example, the ester derivatives of compound **3** (**4**) gave appreciable increase of oral exposure compared to **3** when dosed in triglyceride formulations. The corresponding terminal amides, however, offered no advantage in potency or physical properties. It became clear that for the sEH inhibitors to be generally useful to biologists one needed material that had improved pharmacokinetic properties while preserving the high potency of compound **3**. The UCD group's next approach employed an amide to replace the urea moiety in compound **3**.[86] In earlier studies, the small advantage of the corresponding amides as central pharmacophore in terms of physical properties was offset by decreased potency. However, these studies showed
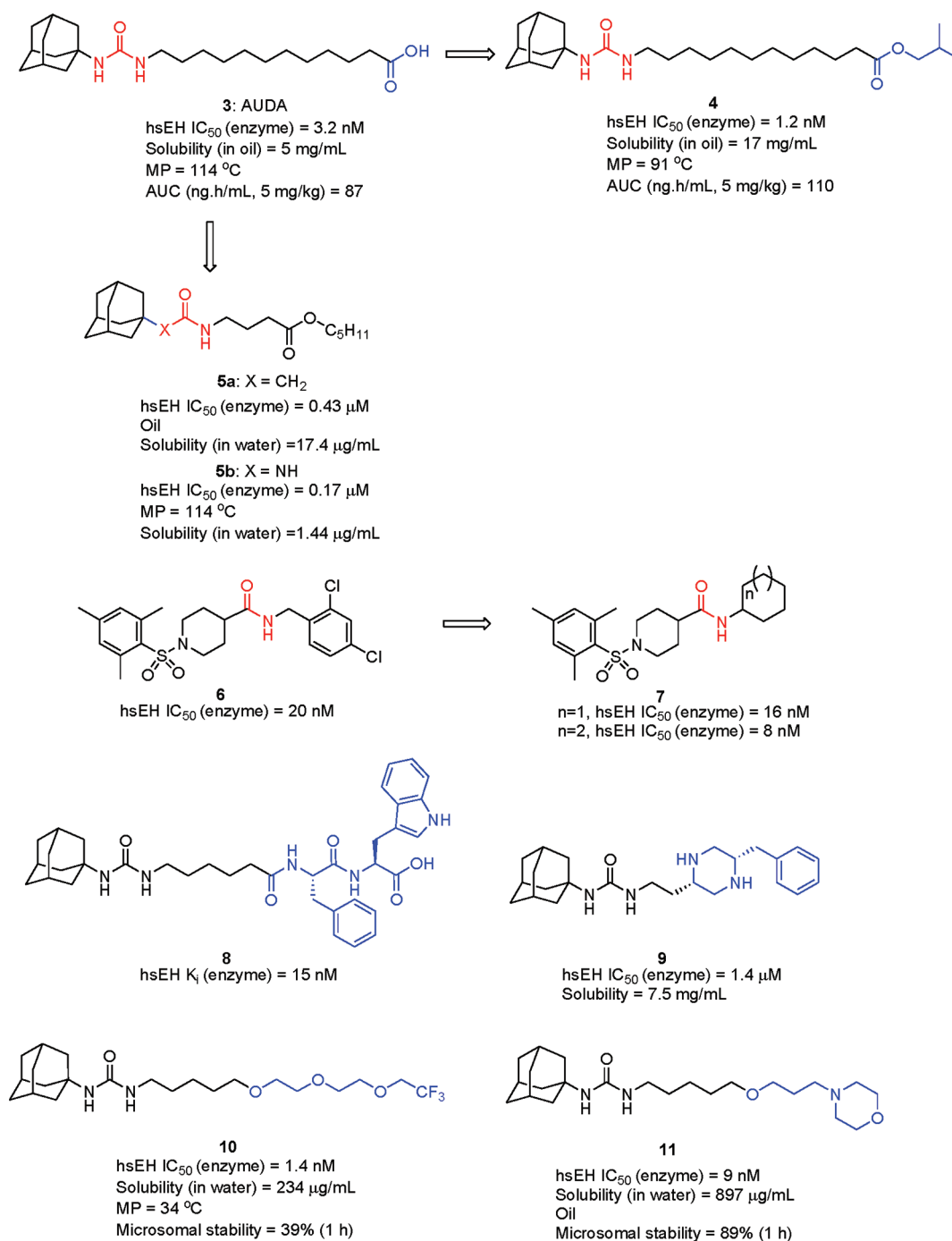
Figure 5. Various secondary urea-based sEH inhibitors.

that with the proper substituents amides could be remarkably powerful inhibitors. In general the SAR predicted from ureas applied as well to the corresponding amides. In this series the resulting amide analogue **5a** gave ∼2-fold less potency but 10-fold higher solubility in water than the corresponding urea analogue **5b**.

Approximately 300 hits were found following the screening of the 300 000 NIH "druglike" compound library. Most of these hits were amide or urea compounds that fit with published SAR work. This work also showed that the basic pharmacophore demonstrated high selectivity for the sEH, since the compounds were screened against numerous other targets. These data are in the public domain (http://pubchem.ncbi.nlm.nih.gov/).

Starting with a quite active hit (**6**) from this screen, the optimization of the amine partner on the right and the sulfonamide on the left led to modest success in terms of potency improvement.[87] The most active analogue resulting from such efforts contained a cycloalkylamine moiety on the left (**7**).

Large peptidyl moieties were also incorporated into sEH inhibitors. It was found that this modification was acceptable if the peptidyl group was located at a proper distance from the primary pharmacophore.[88] Despite the lack of advantage over compound **3** regarding PK, analogue **8** suggested that large terminal groups were tolerated in terms of sEH inhibitory activity. These groups could potentially be used for innovative delivery systems, and large reporter groups can fit into the right
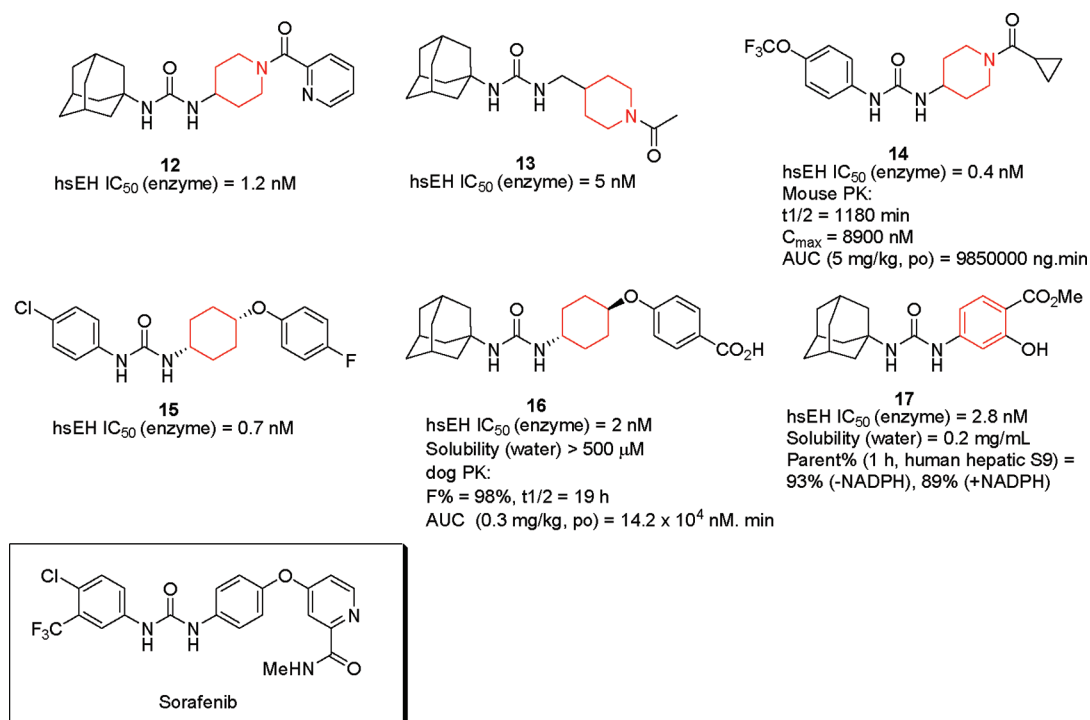
Figure 6. Conformationally constrained sEH inhibitors.

side cavity of the enzyme and even reach into the aqueous environment outside the catalytic tunnel.

The introduction of certain functional groups significantly altered the physical properties of sEH inhibitors. For example, a 5-substituted piperazine (**9**) was weaved by the Long group into a series of sEH inhibitors as a novel secondary pharmacophore that greatly enhanced the solubility of the urea-based analogues despite some loss of potency.[89] Urea ether analogues containing a diethylene glycol (**10**) or morpholine (**11**) motif exhibited good potency, markedly increased water solubility, and eliminated the β oxidation that led to rapid degradation of compound **3**.[90] Such derivatives could have utility in organ baths, intravenous applications, or oral hygiene.

Another strategy to improve physical properties of disubstituted ureas is to impose conformational constraints. Namely, the linker between the primary and secondary pharmacophores is no longer an alkyl chain but a saturated ring, in the case of piperidine or cyclohexane, or an unsaturated ring, in the case of a phenyl group (Figure 6). For instance, a piperidine moiety was identified to rigidify the linker region between the primary and secondary pharmacophores, resulting in highly potent analogues such as **12**, **13**, **27**, and **28**.[78,91] Although large substituents on the right side of the sulfonamide or amide secondary pharmacophore could result in increased potency as in **12**, they also resulted in much lower blood levels following oral administration to dogs.[78] This work also showed that the adamantyl moiety gave marginally acceptable pharmacokinetic properties with a piperidine acetamide group on the right as in compounds **12** and **28**. However, when the lipophilicity of the whole molecule was increased even slightly, the adamantane was so rapidly hydroxylated that these derivatives were not very useful in vivo.

Further modifications of the left side of the scaffold using aryl to replace admantyl resulted in several analogues with subnanomolar IC$_{50}$ against sEH.[47] The improved murine PK of analogue **14** allowed the evaluation of this compound in a rat

inflammatory pain model, in which this compound reduced local inflammatory pain caused by carrageenan at a far lower dose than morphine. These compounds gave good PK results in rats, dogs, and monkeys.[54,78,92] Besides piperidinyl, cyclohexyl could also be placed as an alternative conformational constraint for the linker region connecting the primary and secondary pharmacophores.[93] The solid-phase combinatorial approach successfully yielded highly potent inhibitors such as compound **15**. It is also noted that free carboxylic acid or ortho-substitution of the phenyl group on the left-hand side of the primary pharmacophore led to dramatic decrease of potency. In contrast, the addition of a carboxylic acid to the terminal phenyl group on the right led to an orally bioavailable and potent sEH inhibitor **16**.[94] Interestingly, the 1,4-trans cyclohexane isomers were more metabolically stable in human hepatic microsomes than the cis isomers although their in vivo AUCs were similar. The AUC of **16** in dogs was 40-fold higher than that of compound **3**, and the half time and oral bioavailability were both excellent.[78] Replacing the adamantane with other aliphatic or aromatic systems on the basic structure of **16** did not have a major effect on canine AUC but dramatically increased the blood levels in other species. These compounds represent another series with high potency on the enzyme and good exposure in multiple species following oral administration. Unlike the piperidines that appeared quite active on rodents and primates but poorly active on the sEHs from other species, the cyclohexyl ethers were active in multiple species. These compounds are remarkably similar to the commercial drug sorafenib, which is both a potent kinase and potent sEH inhibitor.[95] This observation allowed the synthesis of compounds that had no activity on known kinases and others that were selective inhibitors of both sEH and a subset of kinases. These studies suggested only limited structural space where compounds had both kinase and sEH inhibitory action. Sorafenib has a terminal carboxylic acid function converted to an amide. Although this increased the potency of compounds such as **16** into the low nanomolar range for sEH, it also decreased their
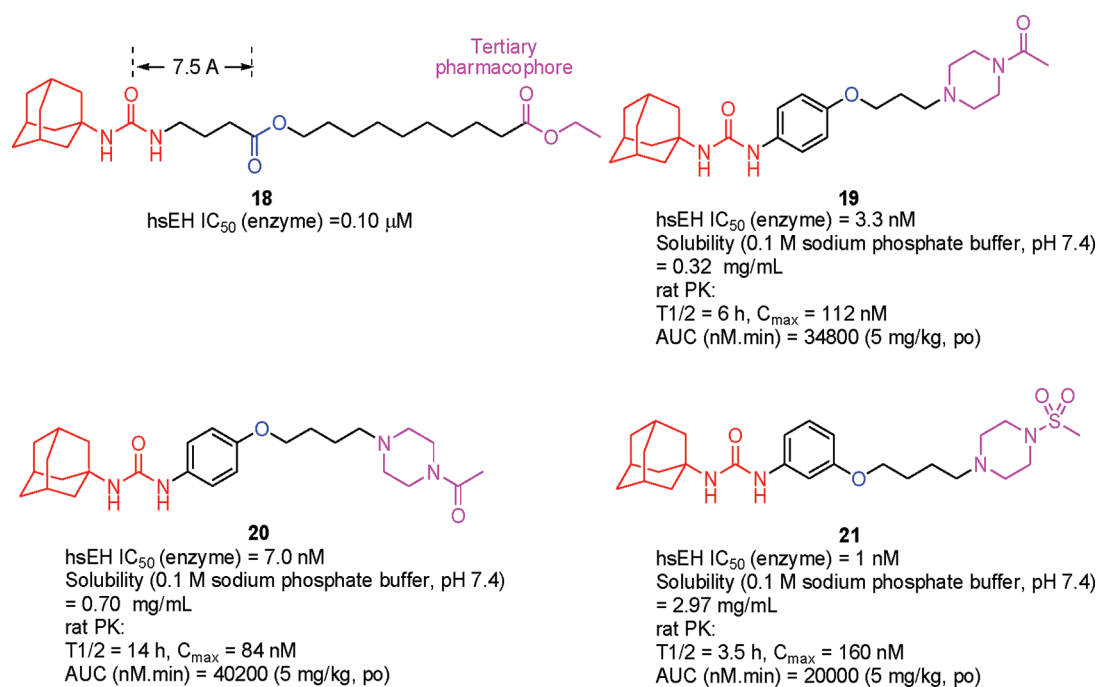
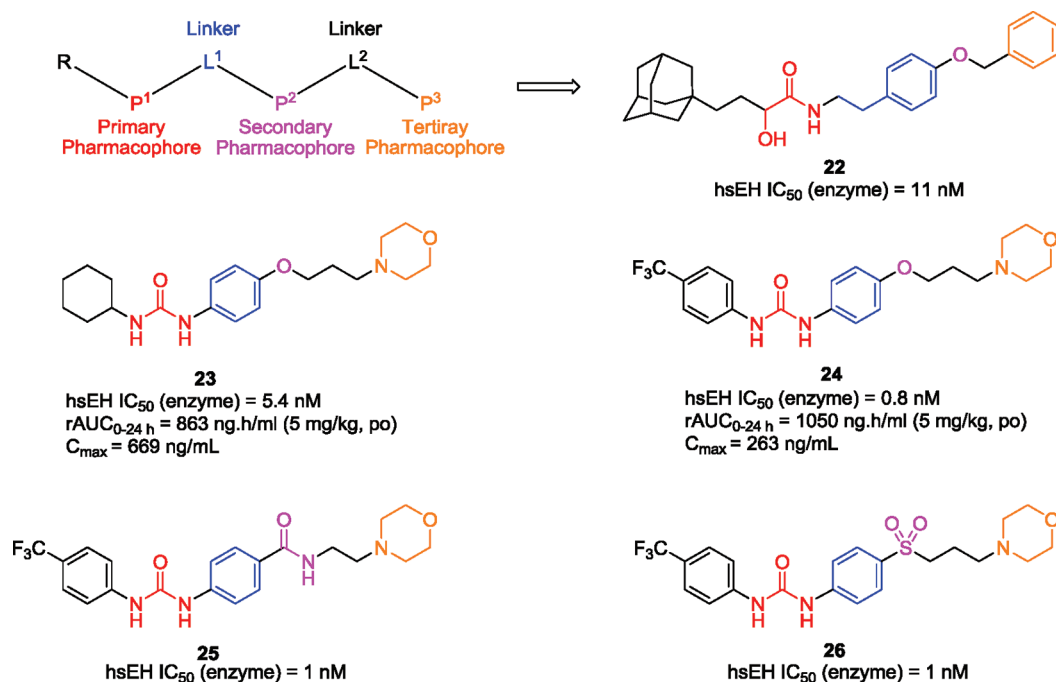**Figure 7.** Modifications of the tertiary pharmacophore to improve PK.

Figure 8. Modifications of primary, secondary, and tertiary pharmacophores by Arête Therapeutics.

solubility. Aromatic systems such as a benzoic acid moiety may also be used as a conformationally restrained linker to increase the solubility of compounds.[78,85,86,96]

Because of its excellent activity in both human and other species, compound **16** was subsequently evaluated in the LPS-induced sepsis model in mice. At 1 mpk po dose, this compound ameliorated hypotension caused by LPS in mouse, which was equivalent to the effect of 10 mpk compound **3** under the same conditions. Furthermore, the increase in blood EETs/DHETs ratio correlated with the reversal of LPS-induced hypotension, suggesting that the observed efficacy may be mediated via

sEH inhibition. When the linker is a phenyl group in the case of a salicylate urea-based sEH inhibitor, compound **17** exhibited good potency and excellent stability in human hepatic S9 fraction, which was further reflected by its superior oral drug exposure compared with compound **3**.[96]

Lastly, tertiary pharmacophores of sEH inhibitors, in purple in Figure 7, were successfully explored to improve PK properties. For example, a second ester can be added to the long terminal alkane moiety (**18**) without loss of activity, but the PK was not acceptable.[97] The incorporation of a piperazine functionality into the 1,3-disubstituted urea series led to potent analogues **19**, **20**,

and **21**.[84] These compounds also demonstrated modest to good water solubility, excellent oral exposure, and good half-lives. Specifically, compound **20** provided a remarkable half-life and oral exposure, and compound **21** gave good solubility and potency. The extensive SAR developed with these compounds has allowed exploitation of biological observations that sEH inhibitors reduced the undesirable side effects and synergized with NSAIDs and COX2 inhibitors,[65,66] aspirin and FLAP inhibitors,[67] and some phosphodiesterase (PDE) inhibitors.[53] For example, Hwang et al. have prepared pyrazole derivatives that were both more potent and more efficacious in some in vivo assays than a combination of a good sEH inhibitor and celecoxib.[69]

3.2. Arête Therapeutics. Although most of the reported sEH inhibitors are amides and ureas, Arête Therapeutics investigated alternative primary pharmacophores such as sulfonamide, thiourea, sulfonylurea, aminomethylene, hydroxyamide, and ketoamide in order to identify chemotypes to cover potential intellectual property (IP) space. They found that almost all replacements led to substantial loss of sEH potency with the exception of α-hydroxyamides, which gave acceptable sEH inhibitory activity as shown by analogue **22** (Figure 8).[98] The hydroxy group in this position could be anticipated from earlier irreversible glycidol inhibitors.[99] To avoid the metabolic oxidation associated with the admantane ring of compound **3**, the Arête team reported unsymmetrical non-adamantyl N,N'-diarylurea and amide inhibitors such as disubstituted ureas **23** and **24**, which had excellent enzyme activity and significantly improved oral exposure.[100] Further SAR optimization of R, L^1, P^2, L^2, and P^3 moieties resulted in several potent sEH inhibitors including analogues **25** and **26** with low nanomolar IC_{50}.

Arête disclosed a series of studies of sEH inhibitors in metabolic disease models.[44] For example, compound **27** (Figure 9) caused

27

28: AR9281
hsEH IC_{50} (enzyme) = 8 nM
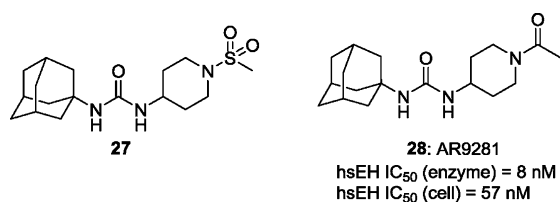hsEH IC_{50} (cell) = 57 nM

Figure 9. Key sEH inhibitors developed by Arête Therapeutics.

diet-induced obese (DIO) mice to gain less weight relative to vehicle. This analogue also reduced plasma cholesterol, decreased serum glucose relative to the control group in an interperitoneal glucose tolerance test, and reduced blood pressure at 60 mpk (b.i.d.) in C57B1/6 mice fed on high-fat and high-fructose diet without significantly changing the heart rate. Therefore, compound **27** may be useful in treating metabolic syndromes including obesity, hypertension, diabetes, and hypercholesterolemia.[45] However, the performance of sEH inhibitors in animal models of metabolic syndromes has been erratic. In another related patent application,[43] Arête disclosed for the first time an sEH inhibitor that effectively reduced total cholesterol, low-density lipoproteins (LDL), triglycerides (TG), and glucose in AngII-infused ApoE deficient mice.

The structure of Arête's phase IIa clinical candidate **28** has recently been revealed.[30] This previously published compound[91] exhibited good potency against sEH, good Caco-2 permeability, surprisingly high water solubility, and reasonable plasma protein binding across species. Regarding off-target profiles, compound **28** displayed excellent selectivity with extremely low inhibition of

the microsomal epoxide hydrolase and little inhibition or binding with a panel of about 150 other enzyme and receptor targets, and minimal hERG and cytochrome P450 (CYP) liability. Furthermore, compound **28** had good oral bioavailability ranging from 25% in cynomolgus monkey to 100% in rat. A robust correlation between the plasma concentration of compound **28** and inhibition of sEH, which was measured by the total EET/DHET and EpOME/DiHOME ratios, was observed in a murine model. When orally dosed at a relatively high 50 mpk (b.i.d.) in AngII-induced hypertensive rats, compound **28** provided 14−16 mmHg reduction of systolic blood pressure relative to vehicle control treatment.[31] It is noted that this compound was found to be only moderately efficacious at 300 mpk dose in SHRs, showing a lack of correlation between the target engagement and antihypertensive effects in this model. Compound **28** was also tested in DIO mouse for antidiabetic effects. At 100 mpk b.i.d. oral dose for 4 weeks, compound **28** reduced glucose AUC and maximal glucose excursion with respect to the vehicle-treated control group. In this study, blood sEH activity was inhibited over ∼90% up to 7 h after dosing and then ∼70% at the 12 h time point. Compound **28** is a better inhibitor of the rodent sEHs than the human and far less potent on canine and feline enzymes.[78] On the basis of its efficacy in rodent models and the fact that compound **28** was well tolerated in preclinical toxicology studies,[101,102] this compound was advanced to human clinical trial involving obese patients with stage 1 hypertension and impaired glucose tolerance. In a phase I trial involving healthy volunteers, compound **28** was well-tolerated in single and multiple oral doses with a mean terminal half-life of 3−5 h. 90% or greater of sEH inhibition was achieved over 8 h at the 250 mg dose and over 12 h at the 500 mg dose.[103] Multiple doses at 100−400 mg every 8 h resulted in ≥90% of sEH inhibition during the trough. Although a compound with a short half-life and used at high doses, compound **28** could be the first proof-of-concept molecule for this mechanism and could become a useful tool compound to test for other therapeutic indications. A caution is that the high throughput fluorescent screening assay for epoxide hydrolase inhibitors tends to overestimate the potency of some piperidines such as **28**, and this compound is rapidly metabolized in a variety of animal models including mice[47] and dogs.[78]

3.3. Boehringer Ingelheim. By performing a highthrough-put screening (HTS), researchers at Boehringer Ingelheim identified N-(3,3-diphenylpropyl)nicotinamide as a potent hit (**29**, Figure 10).[104] Interestingly, their lead compound was found in a cell based HTS for inflammation with a biological readout. In brilliant work the late Thomas Warren developed a photoaffinity label of the hit and identified the sEH as the biochemical target. It is likely that the left-side pyridine in the structure binds to a unique site in the W334 niche and the diphenyl group takes advantage of the large F265 pocket. This branched chain approach could likely be used in the generation of more selective sEH inhibitors. The compound also benefits from the increased solubility of the amide central pharmacophore. Further profiling of this compound revealed short metabolic half-lives in both human and rat liver microsomes as a result of extensive oxidation of the two phenyl groups of the benzhydryl moiety. The inclusion of fluorine atoms at the 4-position of the two aryl groups shifted the major metabolic route to the oxidation of the pyridyl group on the left-hand side. A trifluoroethoxy group was then introduced to the pyridyl (compound **30**), leading to significantly improved half-lives in liver microsomes. However, the increased microsomal stability
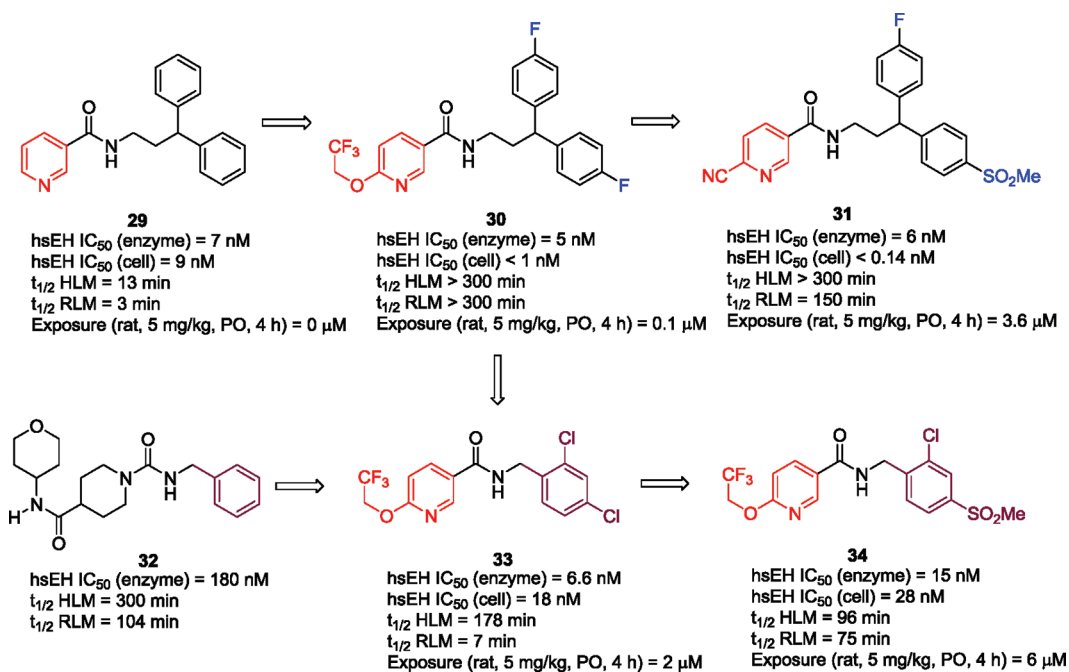
Figure 10. SAR development of an amide series by Boehringer Ingelheim.
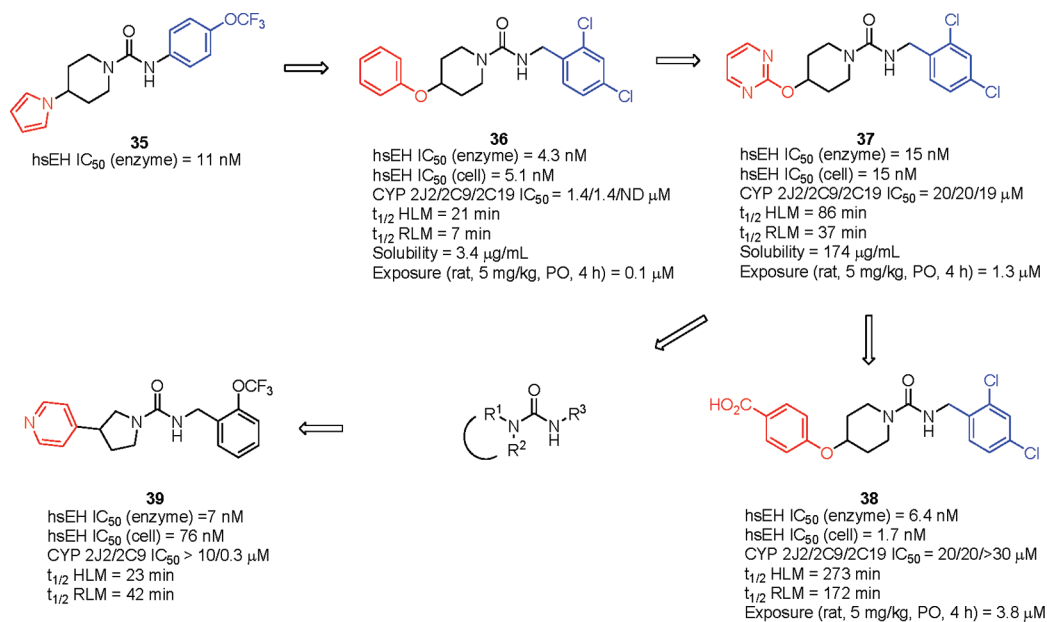

Figure 11. Piperidyl urea sEH inhibitors discovered by Boehringer Ingelheim.

did not translate into reasonable drug level at 4 h after dosing of 5 mpk **30** (po) presumably because of poor absorption. Inspired by the cocrystal structure of **30** with sEH, one of the two fluorine atoms in **30** was substituted with a polar group such as methylsulfonyl, and the trifluoroethoxy substituent of the pyridyl group was replaced by nitrile, allowing for the discovery of **31** as a potent and metabolically stable analogue with good oral exposure. In addition, this analogue displayed good selectivity for sEH over CYP enzymes as well as excellent cell permeability.

Another HTS hit series, exemplified by urea **32**, provided extended half-life in microsomes but lacked desirable sEH potency. The hybridization of the nicotinamide series and the urea series then led to another attractive class of sEH inhibitors with lower molecular weight. Specifically, the benzhydryl moiety

of hit **29** can be replaced with an aryl group which is one carbon linker away from the amide.[105] Despite the poor rat liver microsomal stability, compound **33** gave acceptable oral exposure and long half-life. The replacement of one of the two chlorine atoms in compound **33** with a methylsulfone then yielded analogue **34**, which had a 5-fold increase in plasma concentration compared with compound **34** at 1 h after dosing. As such, the combination of a metabolically more stable benzylamine and nicotinamide provided a new class of sEH inhibitors with balanced potency and metabolic profiles. The benzyl of **33** and **34** moved the ortho substituent far enough away from the amide NH to allow its hydrogen bonding with the catalytic Asp333 of the active site of sEH. In addition, the large ortho chlorine probably reduced benzylic hydroxylation by cytochrome P450 enzymes.

The Boehringer Ingelheim team also explored piperidylureas derived from a potent HTS hit (**35**) in their lead optimization efforts (Figure 11). The primary efforts were focused on the replacement of two potential toxicophores: the aniline and the pyrrole moieties. First, it was discovered that the aniline component could be replaced by 2,4-dichlorobenzyl (**36**), which is reminiscent of the aforementioned nicotinamide series, without loss of potency. Keeping the 2,4-dichlorobenzyl group constant, the team examined various substitutions of the piperidyl ring and ultimately found that aryloxy groups provided good potency. Although pyrimidyloxy analogue **37** was less active than the phenoxy analogue **36** in terms of enzyme and cell sEH inhibitory activity, analogue **37** was more selective against the CYP enzymes responsible for EETs production. Furthermore, compound **37** led to a prolonged half-life in liver microsomes and a 13-fold higher drug level at 4 h after oral dosing (5 mpk). The cocrystal structure of **37** and sEH revealed that the left-hand-side aromatic moiety occupied the W334 niche pocket. This observation prompted the SAR study of the aryl substitution in order to improve the PK while maintaining good sEH inhibitory activity. Despite similar enzyme inhibitory activity (IC$_{50}$ = 4−7 nM), compounds with polar aryl substituents including cyano, sulfonylmethyl, methylsulfonylamido, or methylamido were typically more potent in the cell-based assay (IC$_{50}$ < 1 nM) than those bearing halogen or trifluoromethyl groups. These analogues, however, did not have acceptable in vitro metabolic stability with the exception of analogue **38** containing a carboxylic acid group at the 4-position of the phenoxy moiety. This compound had high and sustained drug exposure over 6 h and was significantly more selective against sEH over several CYP enzymes.

The modular scaffold of the trisubstituted urea prompted the Boehringer Ingelheim team to utilize parallel solid-phase synthesis to enable rapid access of 270-member library analogues.[106] This approach started with a pharmacophore-based virtual screening based on a set of 287 known sEH ligands. The combined pharmacophore and shape model was then used in the context of a virtual screening software called PharmShape to screen a virtual library of 5510 compounds (29 R^{1}R^2 cyclic amines and 190 R^3 aniline replacements), of which 291 was removed. The similarity of the remaining virtual library compounds was compared with that of 93 known sEH inhibitors using 3D pharmacophore fingerprints. Each building block was assigned a consensus score by averaging the similarity scores of all enumerated compounds containing this building block. These building blocks were then clustered, and representatives of each cluster with the highest consensus scores were selected for parallel synthesis. The synthesis commenced with the conversion of solid-support amine (R^{3}NH-CH$_2$-solid support) reagents with 4-nitrophenyl chloroformate to generate activated carbamates, which then reacted with R^{1}R^2 cyclic amines followed by solid-support removal to afford the desired trisubstituted ureas without the need for chromatographic purification. As a result, several potent analogues such as compound **39** resulting from a modest variation of the lead structure were obtained.

Boehringer Ingelheim also described pyrazole aniline derived amide (**40**) as a lead to potent sEH inhibitors (Figure 12).[107] The structure of the cocrystallized sEH with inhibitor **40** suggested that both pyridyl groups were facing solvent and the ethyl group resides in a deep hydrophobic pocket of sEH. Three changes were adopted to provide more potent analogues such as **41**. First, the ethyl group was replaced with a trifluoromethyl group. Second, the left-hand-side 3-pyridyl was replaced by a
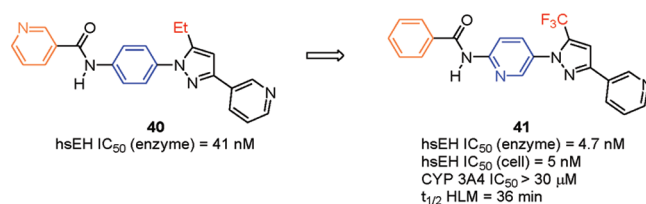

Figure 12. Pyrazole amides as sEH inhibitors developed by Boehringer Ingelheim.

phenyl group. Third, a pyridyl group was selected after an extensive exploration of heteroaryl replacement of the central benzene group due to the mutagenic concern related to the central aniline moiety of **40**. Eventually, analogue **41** offered good sEH inhibitory activity, a modest half-life in human liver microsomes, and no CYP3A4 inhibition, thus presenting an attractive lead for further optimization.

3.4. Dainippon Sumitomo. Ligand efficiency (LE) is an important parameter to ascertain the hit quality in lead identification. A Dainippon Sumitomo team used LE indices (LEI) in a virtual-screening-initiated lead generation study of sEH inhibitors.[108] High lipophilicity and high molecular weight were considered as risk factors of promiscuous association with off-target activity, causing undesired side effects. As such, the team selected compound **42** of low molecular weight (MW = 262) and less lipophilicity (AlogP = 2.76), but a high LEI value (0.43) during the hit triage process (Figure 13). The right-hand cyclopropylphenyl group was not optimized, since the preparation of substituted phenyl group was considered challenging. The conjugated cyclopropyl group offers a number of advantages in electronically stabilizing the urea, avoiding aniline as a possible metabolite, and avoiding a labile benzylic carbon. Thus, the left-hand moiety went through a hit-to-lead process, overcoming a series of issues including poor aqueous solubility, microsomal stability, and CYP inhibition while maintaining good LEI values. Ultimately, analogue **43** was identified as a desirable lead for further optimization. This fragment-based drug design approach was described as fragment-inspired medicinal chemistry by the authors.

3.5. Merck. The target validation efforts at Merck were aimed to establish whether sEH inhibitors can provide mechanism-based blood pressure lowering efficacy in SHR, a common preclinical hypertension model that responds to various antihypertensive drugs. This objective was rigorously pursued by using several series of highly selective and potent sEH inhibitors. The off-targets routinely monitored included CYP enzymes, particularly CYP2C8 and 2C9 which are involved in the production of EETs, ion channels with cardiac implications, and mEH, a significant player in xenobiotic detoxification[109] and steroid metabolism.[110]

The first series reported by Merck covered a range of 3,3-disubstituted piperidine-derived trisubstituted ureas (Figure 14). A highly potent analogue in both enzyme and cell-based assays, the lead compound **44** had some CYP and ion channel off-target activity and relatively high clearance and low oral bioavailability. Lead optimization studies were directed at the left-hand amine/aniline and two of the piperidinyl substituents colored as purple, blue, and red, respectively. 3,3-Disubstituted piperidine-derived trisubstituted urea *ent*-**45** was discovered ultimately as a highly potent and selective sEH inhibitor.[111] It is worth noting that the presence of a carboxylic acid with a proper linker length was key to the dramatically improved PK and selectivity against all of the off-targets. Despite the good compound oral exposure, excellent sEH inhibition in whole blood, and remarkable selectivity,
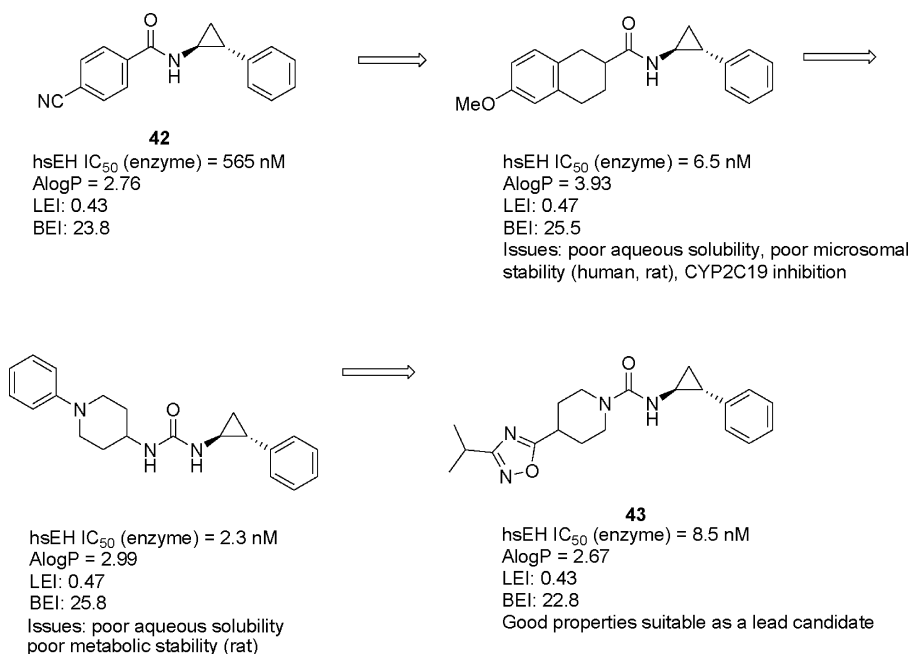
Figure 13. SAR evolution of sEH inhibitors with high ligand efficiencies by Dainippon Sumitomo.
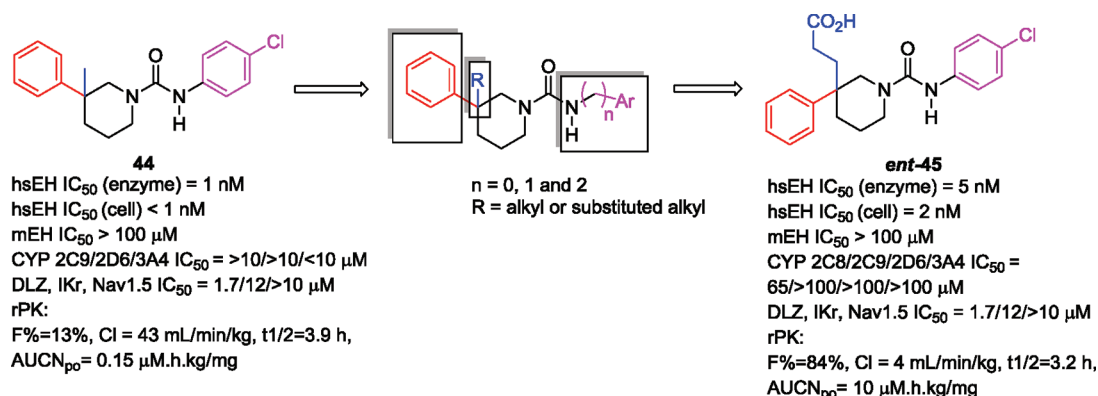

Figure 14. 3,3-Disubstituted piperidine-derived trisubstituted ureas as sEH inhibitors discovered by Merck.
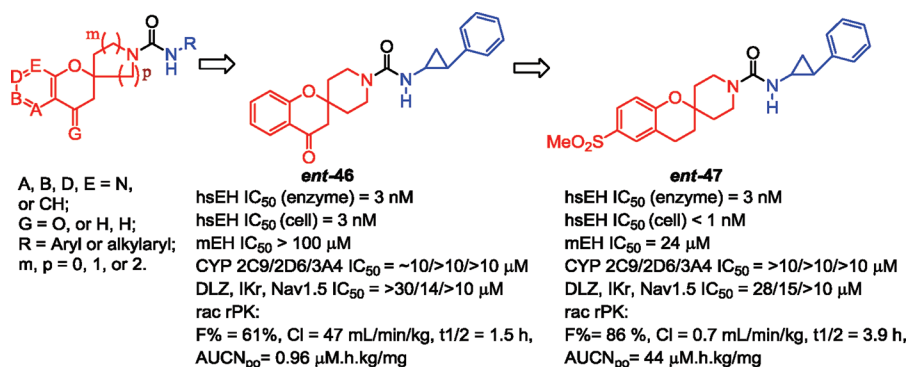

Figure 15. Spirocyclic secondary amine-derived trisubstituted urea inhibitors of sEH discovered by Merck.

compound *ent*-**45** failed to lower blood pressure acutely in SHRs at 50 mpk po dose.

To add additional conformational constraint to urea structures, spirocyclic secondary amine-derived trisubstituted ureas were identified as highly potent, bioavailable, and selective sEH inhibitors (Figure 15).[112] Two major subseries, chromanones and chromans, represented by analogues *ent*-**46** and *ent*-**47**, are

both potent and reasonably clean in off-target screening. The presence of the ketone moiety in analogue *ent*-**46** might account for its higher clearance than *ent*-**47**, which had remarkably high oral exposure and low clearance. However, the in vitro DHET production was suppressed more by *ent*-**46** (93%) vs *ent*-**47** (68%) when tested at 1 μM compound in whole blood. In addition, it has been shown that compound *rac*-**46** suppressed

DHET production rate by >95% from 0.5 to 6 h after dosing (50 mpk, po). As such, this compound was subjected to a PD efficacy study in SHR. Despite good oral exposure and excellent ex vivo target engagement in blood, *rac*-46 failed to lower blood pressure acutely in SHRs at 300 mpk dose (po) over 8 h.

4-Substituted piperidine-derived trisubstituted ureas were also reported as highly potent and selective inhibitors for sEH (Figure 16).[113] By judiciously varying the heteroaryl substitution
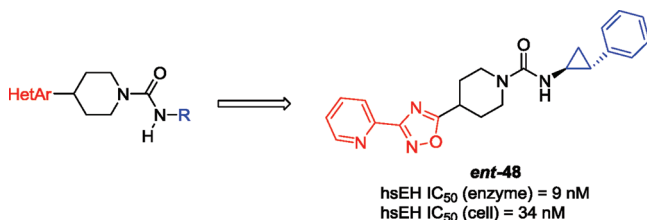

Figure 16. Optimization of the 4-heteroarylpiperidine-derived ureas by Merck.

of the piperidinyl group, compound *ent*-48 was discovered as a good inhibitor against sEH. In addition, this analogue has minimal mEH, ion channel, CYP, and other off-target liability against a panel of 166 counterscreening targets ($IC_{50} > 10\ \mu M$) including a subset involved in blood pressure regulation. The acute action of *ent*-48 on vascular tone was further evaluated by SHR mesenteric artery assay, in which *ent*-48 was able to reverse vascular contraction induced by methoxamine ($IC_{50} = 6.5\ \mu M$). No vasoconstriction was observed on basal tension with this compound up to 100 μM. These results were in line with the vasodilatory effect of EETs previously reported in literature.[114]

In part because of its excellent bioavailability (77%), moderate clearance (29 mL min^{-1} kg^{-1}), and good normalized oral exposure (1.2 $\mu M\cdot h\cdot kg/mg$), compound *ent*-48 also inhibited sEH activity effectively ex vivo and in vivo. After the treatment with *ent*-48 for 8 days (300 mg/pk, po, q.d.), the endogenous epoxide/diol ratios in SHR kidney were increased 2- to 9-fold (Figure 3). In telemetrized SHRs, *ent*-48 failed to lower systolic blood pressure acutely or chronically despite the excellent compound exposure and >95% sEH inhibition as reflected by the ex vivo measurement of DHET production over a time course of ~24 h. It has then been proposed that the lack of blood pressure lowering activity by *ent*-48 might be due to the observed 2.5-fold increase in 20-hydroxyeicosatetraenoic acid (20-HETE), a potent vasoconstrictor in kidney,[115] which could potentially negate the vasodilatory effect of elevated EET levels. The lack of robust blood pressure reduction effects of several potent, structurally distinct, and orally bioavailable sEH inhibitors 28, *ent*-45, *rac*-46, and *ent*-48 in SHRs suggests that sEH inhibitors may not be a valid target for hypertension if the strains of SHRs used in these

studies are a robust model for human hypertension. These data bring up the common problem in pharmacology of what animal models are predictive of human disease in drug development. This is particularly complex for hypertension, which appears to be a symptom of a number of biochemical imbalances.

Distinct from urea and amide inhibitors, novel amino-heteroaryl analogues were also prepared as potent sEH inhibitors (Figure 17).[116] It was envisioned that the NH group of this class of inhibitors interacted with Asp333, and the heteroatom of the heteroaryl group formed hydrogen bonds with Tyr381 and Tyr465. The SAR study revealed that aminobenzisoxazoles emerged were optimal in terms of their inhibitory activity against sEH. Analogue 49 was identified as a nanomolar inhibitor of sEH with a good PK profile. The compound is particularly attractive in being of low molecular weight, having excellent physical properties, and having a highly constrained structure. The strategy of employing such aminoheterocycles as amide replacements represents a useful approach to develop mimics of known hydrolase or protease inhibitors containing an amide moiety.

The aforementioned series developed by Merck demonstrated their judicious consideration of both intellectual property and physical properties of compounds. When they started the program, only disubstituted ureas were reported. Realizing that trisubstituted ureas often offer better physical properties and PK, the Merck team engineered some novel tertiary ureas bearing novel spirocycles (46, 47) or heteroaryls (48) and demonstrated that these are useful tool compounds with good potency, selectivity, and PK profiles. Their aminoheteroaryls represented a nice extension distinct from amide or urea type of common motifs for sEH inhibitors.

4. POSSIBLE APPROACHES TO EXPLOITING THE P450 BRANCH OF THE ARACHIDONATE CASCADE

The cytochrome P450 branch of the arachidonate cascade has so far not been exploited successfully by the pharmaceutical industry. Now that multiple natural eicosanoids in this cascade are commercial along with analytical methods for them and multiple pharmacological probes are available, knowledge about the biology of this branch of the cascade is expanding dramatically. There are many possible ways to apply the understanding of this cascade and these important regulatory lipids to improve human health. In addition, there are multiple pharmacological approaches including inhibition of synthesis of the predominantly inflammatory 20-HETE, but exploitation of the knowledge that the epoxides of fatty acids and particularly long chain ω-6 and ω-3 polyunsaturated lipids are powerful chemical mediators is the most advanced. Although diols of linoleate appear to be proinflammatory mediators of vascular permeability and diols of arachidonate appear to mediate stem cell mobility and other biologies, so far EETs and their ω-3 homologues appear to be the major anti-inflammatory, antihypertensive, and analgesic fatty acid epoxides with
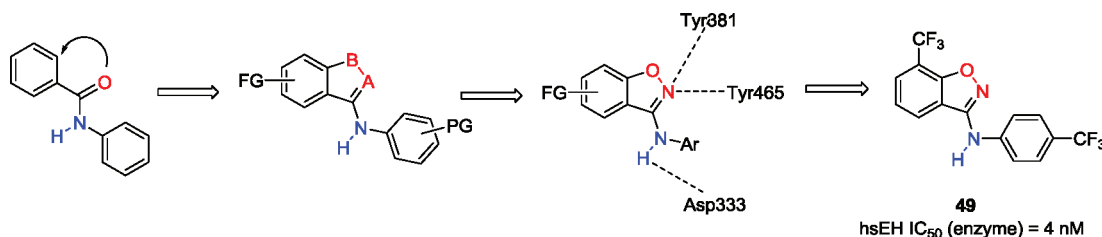

Figure 17. Aminoheteroaryls as novel sEH inhibitors discovered by Merck.

implications for treating diseases. In the indications discussed below the diols are simplistically regarded as highly polar inactivation products.

There are good pharmacological probes available to mimic and to inhibit the action of EETs and ω-3 epoxides, but so far mimics have not been exploited as druglike molecules. On analogy with angiotensin converting enzyme (ACE) inhibitors and AngII receptor blockers (ARBs), both EET mimics and sEH inhibitors are attractive as potential drug candidates. The concept of mimicking the bioactive eicosanoid is appealing in that the mimics will be active even in the absence of the natural eicosanoid. Thus, the maximal effects of these materials should be greater than the sEH inhibitors emphasized in this review. However, sEH inhibitors should stabilize epoxy lipids generally in the ratio of homologues and optical and regioisomers that are produced naturally. The increase in these chemical mediators also should be greatest near the site of production. This local effect could be pharmacologically important because lipid chemical mediators commonly have different ratios and activities in different tissues. sEH inhibitors have been repeatedly shown to increase the concentrations of epoxy lipids, since the sEH is the major route of metabolism of these compounds. However, since the sEH is only one of multiple pathways of degradation for epoxy lipids, the increase in epoxy lipids and decrease in corresponding diols will be limited. Thus, on the negative side, the maximal efficacy of sEH inhibition is thus restricted to stabilizing endogenous fatty acids. In contrast, theoretical mimics of these mediators would only be limited by receptor saturation and signal transduction. On the positive side, the possible side effects of dramatically increased EETs will also be limited with sEH inhibitors. This may explain the exceptionally positive therapeutic index so far observed with sEH inhibitors. As we learn more about the biological activities of the resulting diols, the reduction in diols might cause additional benefit or off-target effects. At this point reduction in diols seems to be generally positive in reducing the production of another inflammatory mediator. Of course sEH inhibitors will synergize the effects of exogenous epoxy fatty acids or other pharmaceutical agents such as NSAIDs and PDE inhibitors which increase them.

The stabilization of epoxy lipids with sEH inhibitors is the approach closest to the clinic which addresses this branch of the arachidonate cascade. As discussed above, the sEH inhibitors stabilize endogenous lipid epoxides and increasing these metabolites has generally proven beneficial in the reduction of hypertension, a variety of inflammatory disorders, and pain. Analogously, NSAIDs and COXIBs inhibit the biosynthesis of prostaglandins and thromboxanes. In general these eicosanoids have proven to increase pain and inflammation, so COXIBs are considered beneficial. However, a caution is that the effects of thromboxanes and prostaglandins are not in all cases detrimental. On analogy neither should we expect an increase in epoxy lipids caused by sEH inhibitors to be beneficial in all cases. As sEH inhibitors are investigated, it is critical that experiments into their biology are also designed in an attempt to elucidate possible detrimental and beneficial effects.

5. POSSIBLE ROUTES FOR SEH INHIBITORS TO REACH THE CLINIC

As discussed in this review, a number of powerful inhibitors of the sEH have been prepared in several chemical series. Many of these compounds have druglike properties in terms of excellent potency, selectivity, and oral exposure. This class of drugs appears to have a massive therapeutic index with few side effects

in animals. These properties yield good target engagement as shown by an increase in epoxides in tissue fluids and often a decrease in the corresponding diols. In a variety of cases this has been coupled with demonstration of efficacy in numerous animal models of a variety of human diseases. These diverse series of inhibitors have effectively mapped the catalytic site of the enzyme. This knowledge coupled with several crystal structures of the murine and human enzyme will facilitate the synthesis of still other potent inhibitors.

sEH inhibitors have never been shown to reduce the blood pressure in normotensive animals. However, they are moderately to dramatically antihypertensive in a variety of animal models examined in many laboratories. They are particularly active in models where angiotensin is a major driver of hypertension and where renal damage is involved. Some SHR strains have proven particularly valuable for extrapolating from treating animal to treating human hypertension. It is worth noting that hepatic sEH levels in all rat strains examined so far are far lower than those in any other mammalian species reported.[14] In various SHR models the response from sEH inhibitors has been erratic. Some but not all of these observations can be attributed to documented genetic variations among the SHR strains used, as well as within strain variations in both the sEH titers and the sensitivity to treatment with sEH inhibitors. Some SHRs are even resistant to treatment with commercial antihypertensive compounds. Several extensive studies have shown good blood pressure reduction in SHR strains with sEH inhibitors. However, as discussed above the failure of potent sEH inhibitors with good exposure using their SHR models apparently led both Boehringer Ingelheim and Merck to put on hold their sEH inhibitors for blood pressure treatment. This appears to have been a good decision given the failure of Arête Therapeutics to control blood pressure in phase II clinical trials, the diversity of causes of hypertension in man, and the high cost and high risk of hypertension trials. One approach might be to segregate the human population into those with SNPs showing high sEH activity or those with angiotensin-driven hypertension, but such an approach is not now commercially attractive. The tendency of sEH inhibitors to reduce blood pressure could make them attractive add-on drugs for severe inflammation where extensive use of some NSAIDs exacerbates hypertension.

The major limitation in the field is that, in spite of these powerful chemical probes and a variety of attractive biological targets, there is not an obvious and inexpensive route to the clinic for a major market with these molecules. For example, diabetes is an attractive and timely target, and sEH inhibitors have been shown to reduce symptoms of diabetes in several models and to reduce symptoms dramatically in several. A caution is that in still other models there have been either marginal effects or no beneficial effects in overcoming insulin resistance. Furthermore, it is well-known that there is a lack of predictability of rodent diabetic model for humans. In addition, human genetic studies have not yet disclosed a strong correlation between sEH and diabetes. In the absence of a theoretical mechanism of action in treating diabetes, a compelling case for human clinical trials for diabetes has not been made. This caution was realized in a recent failure by Arête Therapeutics in a phase II trial to improve blood markers of metabolic syndromes. On the other hand, animal models show strong efficacy of sEH inhibitors for a number of comorbidities associated with diabetes, including in particular renal failure, vascular inflammation, atherosclerosis, fibrosis, heart failure (HF), neuropathic pain, stroke, and other indications. Compounds that offer these attributes and the ability to improve

insulin sensitivity and glucose tolerance even marginally in some models are attractive. However, it remains challenging to pursue these comorbidity indications without showing that sEH inhibitors can control blood sugar as a stand-alone therapy. This opinion could be reversed with demonstrated efficacy in other and particularly non-rodent models or a mechanistic understanding that allows extrapolation of rodent results to man.

On the other hand, even in SHRs resistant to blood pressure reduction with sEH inhibitors, other positive biological effects have been seen including a dramatic reduction in renal, cardiac ischemia—reperfusion, and stroke damage. Separate from hypertension, sEH inhibitors are effective with a variety of cardiovascular indications ranging from atherosclerosis through atrial fibrillation and fibrosis. For example, compound **3** was recently used in a small clinical trial to ascertain the effects of an sEH inhibitor on vascular tone.[117] It was found that compound **3** alone, or in the presence of urotensin II, increased flux in healthy controls and HF patients. However, most of the cardiovascular indications involve long and expensive outcome trials. This is in part due to a lack of biomarkers that indicate efficacy for anti-inflammatory drugs to treat atherosclerosis in both preclinical and clinical settings. As such, the pursuit of such indications for sEH inhibitors would be highly unlikely unless they could at least demonstrate utility in non-human primate disease models.

Diabetes and hypertension represent large potential markets. Multiple animal models suggest a role for sEH inhibitors as add-on or combination therapies. However, with the phase II failure of Arête Therapeutics with metabolic syndromes, the great expense of the trials for these indications, and the uncertainties with some of the animal models, to repeat trials in the near term is unlikely. There are a variety of potential short trials where animal models suggest a good chance of success. One example would be to test sEH inhibitors to reduce the renal toxicity of contrast agents. Other examples with similar limitations include treatment of restenosis, ateriovenous graft failure, inflammation from hemodialysis, or Raynaud's syndrome. A somewhat larger market suggested from rodent data is renal protection from the toxicity of chemotherapeutic agents. However, these potential markets seem of little interest to pharmaceutical companies because of their small size. Such potential utility of sEH inhibitors for minor indications may be better addressed with physician-initiated trials. A major limitation in the field is the absence of IND enabling work on an sEH inhibitor other than compound **28** which would permit clinical trials by small companies or in the public sector. Should such a public sector investigational new drug (IND) compound become available, there are a variety of indications ranging from psoriasis to eclampsia that could be approached in the public or private sector.

Numerous other indications appear attractive and represent large markets. However, they also involve high risk, long, and/or expensive trials. Examples of this dilemma include renal and vascular inflammation where evidence from animal models is very supportive. The sEH inhibitors dramatically reduce inflammation and damage associated with ischemia—reperfusion injury in a number of systems including stroke models, myocardial infarction (MI), and transplant. MI is one of the few indications where the rodent studies have been reproduced in canine systems. A caution is that some sEH inhibitors that are quite active on rodent and primate sEH are almost inactive with the feline and canine sEH. A variety of other inflammatory conditions such as chronic obstructive pulmonary disease (COPD), asthma, irritable bladder syndrome, pancreatitis, and inflammatory bowel disease (IBD) are attractive, but they also have long trials. Possibly the best

predictive evidence from both animal models and human genetics for a clinical trial is heart failure, but this trial is intimidating as well.

The positive effects of sEH inhibitors alone and in combination with NSAIDs and COXIBs to reduce both inflammatory and neuropathic pain could represent a relatively short trial to address a poorly met medical need. Since sEH inhibitors are known to reduce some of the toxicities associated with high dose use of NSAIDs and COXIBs and to synergize with them to reduce both inflammation and pain, a combination of a sEH inhibitor with a NSAID or COXIB appears attractive in increasing efficacy, reducing side effects, and possibly extending patent life. An example could be arthritis where long-term use of NSAIDs and COXIBs can be associated with hypertension, blood clotting, and other side effects. The ability to drop the NSAID—COXIB dose while reducing some deleterious side effects is reasonable. Along the same line PDE inhibitors have long been of therapeutic interest for several diseases. Since particularly PDEs 4 and 5 inhibitors are synergized by sEH, possibly these drug combinations could be used to avoid the severe dose-limiting side effects of PDE inhibitors.

The pain indication appears particularly attractive in representing a large potential market, an unmet medical need while offering phase II trials that are not prohibitively long and expensive. sEH inhibitors not only reduced inflammatory pain in a variety of animal models but surprisingly reduced neuropathic pain as well. When acting alone, they are far more active than the common standard for treatment of neuropathic pain, gabapentin, while not causing severe sedation as morphine does using either rotorod or open field experiments. Although NSAIDs and COXIBs are widely used for treating neuropathic pain, the literature shows them to be of variable and questionable benefit. For example, the mixed COX inhibitor diclofenac varies between slight analgesia and statistically significant enhancement of pain perception in rodent models depending on time after treatment and dose. However, when diclofenac is used with a low dose of sEH inhibitor, there is dramatic synergism in reducing pain resulting from induced type I diabetes in rodents. A problem with human pain trials is of course that there is often a major placebo effect. Therefore, careful controls are critical. On the other hand, patients often suffer from both inflammatory and neuropathic pain, and the sEH inhibitors may be able to reduce both. In addition, the trials can be relatively short, and neuropathic pain in particular represents an unmet medical need.

Numerous indications appear promising for treatment by sEH inhibitors varying from large markets such as comorbidities associated with diabetes to smaller markets such as periodontal disease or enhanced wound healing. One limitation with sEH inhibitors is that most published models have been based on rodents. It is not clear if these rodent data will apply to human disease. It would thus require a compelling biological rationale, cogent data in human genetics, and/or non-human primate data to support their entry to clinics. As far as tool compounds are concerned, these compounds should not have off-target liabilities that could contribute to observed PD effects in animals. As such, a thorough investigation of potential off-target activity that could confound the data would be imperative. Another aspect when validating indications of sEH inhibitors is a robust PK/PD correlation, which will enable the translation of target engagement in a proper compartment to observed PD effects.

Certainly for the field to advance one needs to see the compounds evaluated by either a pharmaceutical company or the availability of a compound with IND status from the FDA to allow physician-initiated clinical trials in the public sector.

Excellent druglike structures exist in both the private and public sectors which have properties indicating that they could be excellent candidates for INDs.

6. CONCLUSION

Early sEH inhibitors were conceived based on a knowledge of the enzyme's catalytic mechanism, in particular the transition state. They were optimized via classical SAR approaches coupled with moderate throughput enzyme assays and rodent pharmacokinetics. Three dimensional SAR helped to design a second generation of sEH inhibitors that were more sophisticated and with better physical properties and pharmacokinetics. The more recent structure-based drug design was possible because of the availability of several solid cocrystal structures of inhibitors bound to sEH. These cocrystal structures also elucidated the precise information on the key binding interactions of the inhibitor with the enzyme and suggested further structure modifications that could fit well in the binding pocket. In all cases, sEH inhibitors possess two key structural features in the hydrogen bond network. The first is a hydrogen bond acceptor, such as the carbonyl group of either an amide or a urea, which interacts with Tyr383 and Tyr466 mimicking the oxygen atom of an EET epoxide. The second structural feature is a hydrogen bond donor, such as the NH group of an amide or a urea, which engages a hydrogen bond with Asp335. The triple interaction is indispensable for inhibitors to achieve high activity, as the absence of either carbonyl or amide NH group typically resulted in significant loss of potency.

During the lead identification and optimization processes, appropriate balance of potency and physical properties, ligand efficiency, polarity, metabolic stability, PK, and off-target profiles are essential to provide tool compounds suitable for pharmacological proof of concept. Several useful approaches have been applied in the medicinal chemistry work on sEH inhibitors. One such is to introduce secondary or tertiary polar pharmacophores to increase solubility and polarity. Conformational restraint to boost potency while improving metabolic stability is particularly powerful.

The lack of efficacy of compound **28** to treat patients with mild to moderate hypertension and impaired glucose tolerance in a phase II clinical trial cautions whether sEH inhibition is a robust mechanism to treat hypertension and/or diabetes. However, it is conceivable that other promising therapeutic applications of sEH inhibitors, including for inflammation, pain, cardiovascular diseases, and comorbidities of diabetes, warrant further investigations. Possible routes do exist to explore the P450 branch of the arachidonate pathway and, in particular, to enable sEH inhibitors to be tested in the clinic.

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■ ABBREVIATIONS USED

sEH, soluble epoxide hydrolase; mEH, microsomal epoxide hydrolase; LTA_4, leukotriene A_4; EET, epoxyeicosatrienoic acid; NSAID, nonsteroidal anti-inflammatory drug; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; LT, leukotriene; DHET, dihydroepoxyeicosatrienoic acid; 3D QSAR, three-dimensional quantitative structure–activity relationship; SNP, single nucleotide polymorphism; AngII, angiotensin II; SHR, spontaneously hypertensive rat; EDHF, endothelium-derived hyperpolarizing factor; AUDA-BE, 12-(3-adamantan-1-ylureido)dodecanoic acid butyl ester; MCAO, middle cerebral artery occlusion; TAC, thoracic aortic constriction; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; mpk, mg/kg; 5-LOX, 5-lipoxygenase; LPS, lipopolysaccharide; AUDA, 12-[3-adamantane-1-ylureido]dodecanoic acid; COXIB, cyclooxygenase-2 blocker; PK, pharmacokinetics; LOQ, limit of quantification; SAR, structure–activity relationship; UCD, University of California—Davis; DCU, dicyclohexylurea; PD, pharmacodynamics; PDE, phosphodiesterase; IP, intellectual property; CYP, cytochrome P450; HTS, high throughput screening; LE, ligand efficiency; LEI, LE index; 20-HETE, 20-hydroxyeicosatetraenoic acid; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; IND, investigational new drug; MI, myocardial infarction; COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease

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