Journal of Medicinal Chemistry

pubs.acs.org/jmc

Perspective

Discovery of Soluble Epoxide Hydrolase Inhibitors from Chemical Synthesis and Natural Products

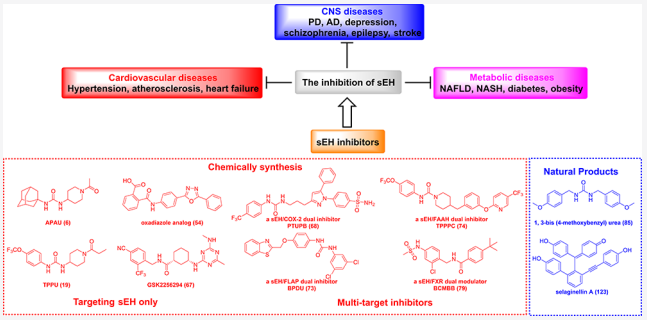
Cheng-Peng Sun,[‖] Xin-Yue Zhang,[‖] Christophe Morisseau,[‖] Sung Hee Hwang, Zhan-Jun Zhang, Bruce D. Hammock,* and Xiao-Chi Ma*

Cite This: *J. Med. Chem.* 2021, 64, 184−215

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Soluble epoxide hydrolase (sEH) is an α/β hydrolase fold protein and widely distributed in numerous organs including the liver, kidney, and brain. The inhibition of sEH can effectively maintain endogenous epoxyeicosatrienoic acids (EETs) levels and reduce dihydroxyeicosatrienoic acids (DHETs) levels, resulting in therapeutic potentials for cardiovascular, central nervous system, and metabolic diseases. Therefore, since the beginning of this century, the development of sEH inhibitors is a hot research topic. A variety of potent sEH inhibitors have been developed by chemical synthesis or isolated from natural sources. In this review, we mainly summarized the interconnected aspects of sEH with cardiovascular, central nervous system, and metabolic diseases and then focus on representative inhibitors, which would provide some useful guidance for the future development of potential sEH inhibitors.



1. INTRODUCTION

Epoxide hydrolases are widely distributed enzymes responsible for the rapid hydrolysis of epoxides to the corresponding vicinal diols.[1,2] In mammals, soluble epoxide hydrolase (sEH), an α/β hydrolase fold protein, is expressed in the cytosol, and sometimes also in the peroxisome, of numerous tissues, such as liver, kidney, lung, heart, brain, spleen, adrenal, and intestine.[3] Besides mammals, the sEH is present in all vertebrates; however, only the mammalian sEH has phosphatase activity.[4−14] Expression levels vary among species; for example, the sEH is much lower in rats than in mice.[15] Furthermore, the sEH expression can be induced by angiotensin II (Ang II) and peroxisome proliferator-activated receptor α (PPARα) and PPARγ agonists, such as fibrates and glitazones,[3,16] but also by sexual hormones.[17] Notably, the expression of sEH is upregulated by PPARγ agonists in adipose tissues, while the oppositive result is observed in cardiomyocytes.[18] Additionally, inflammation also increases its expression; therefore, sEH can be considered a marker of inflammation.[19]

The mammalian sEH comprises two 62.5 kDa monomers, containing 555 amino acids each, and it is a bifunctional enzyme that possesses a 25 kDa N-terminal phosphatase region and a 35 kDa C-terminal hydrolase domain (Figure 1A).[20] The C-terminal hydrolase catalyzes the hydrolysis of epoxy-fatty acids (EpFAs), such as epoxyeicosatrienoic acids (EETs), metabolized from arachidonic acid (AA) to the corresponding diols, such as dihydroxyeicosatrienoic acids (DHETs) (Figure 2).[20] The hydrolase catalytic pocket of the C-terminal hydrolase consists of two tyrosine residues (Tyr381 and Tyr465) that interact with the oxygen atom of the epoxide via two hydrogen bonds. The nucleophilic carboxylic acid Asp333 of the catalytic triad Asp333-Asp495-His523, located on the opposite side of Tyr381 and Tyr465, is oriented and activated by His523 and Asp495 and attacks the epoxide carbon backbone to form an ester bond with the opened epoxide (Figure 1C). The following hydrolysis of this ester results in the production of the corresponding diols.[21,22] Compared with the well-understood function of the C-terminal hydrolase domain, the role of the N-terminal phosphatase domain is still unclear.[21,22] Cronin et al. and Newman et al. independently discovered that the N-terminal domain of sEH exhibits phosphatase activity to hydrolyze diverse lipid phosphates *in vitro*, including farnesyl pyrophosphate, sphingosine 1-phosphate, and lysophosphatidic acid, whereas its physiological and possibly pathophysiological roles are still not well understood.[23,24]

Received: August 28, 2020
Published: December 28, 2020

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

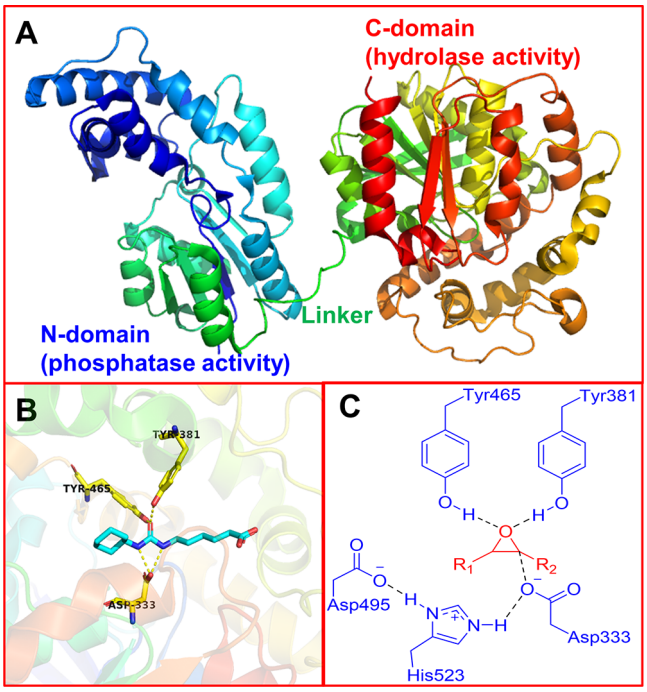


Figure 1. (A) X-ray cocrystal structure of a single subunit of the human sEH (PDB code 1ZD4). (B) X-ray cocrystal structure of human sEH C-domain showing the binding site (PDB code 1ZD4). (C) Potential interactions between epoxides and human sEH C-domain.

EPHX2, the human gene encoding sEH, is a 19-exon gene (45 kb) localized in the chromosomal region 8p21-12.[25] So far, seven single-nucleotide polymorphisms (SNPs) of sEH have been discovered, including K55R, R103C, C154Y, R287Q, R287Q/R103C, V422A, and E470G.[26] Compared with the wild-type (WT) protein, the mutant's strong reduction of its phosphatase activity in R103C and R287Q significantly decreased the phosphatase activity of sEH, and its hydrolase activity is increased in R287Q/R103C, while increasing its N-terminal activity in K55R and C154Y.[12,27] Among them, K55R, R103C, and R287Q have been verified in connection with several diseases,[28,29] including heart failure, ischemic stroke, anorexia nervosa, and nephropathy.

In response to infection or damage, the body generates an inflammatory response. Central to this response is the production of bioactive lipids from AA (Figure 2).[30] Eicosanoids possess a series of protective effects and are defined to be vital metabolites from AA via three metabolic pathways: (1) the cytochrome P450 (CYP) pathway forms EETs and hydroxy eicosatetraenoic acids (HETEs); (2) lipoxygenases (LOXs) catalyze the formation of lipoxins (LXs) and leukotrienes (LTs);[31] (3) cyclooxygenases (COXs) pathway produces prostanoids.[32] The above-mentioned pathways can also metabolize eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and other unsaturated fatty acids.[30,32−34]

All these bioactive lipids, called oxylipins, have strong roles in the inflammatory response.[35] For example, EETs, synthesized by CYP450s, such as CYP2C8, CYP2C9, and CYP2J2, activate guanine nucleotide-binding protein (G-protein) Gαs via ADP-ribosylation, resulting in the activation of the smooth muscle large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) to produce vasodilation and lowering blood pressure.[36−39] Furthermore, EETs increase Ca^{2+} influx, enhance fibrinolysis, and stimulate tube formation in the endothelium.[40,41] In addition, EETs prevent IκB degradation via suppressing of IκB kinase, inhibit nuclear transcription factor-κB (NF-κB) responsible for cytokine-mediated inflammation, and further inhibit NF-κB-mediated gene transcription, allowing the anti-inflammatory effect.[42,43] Compared with bioactive substrate EETs, DHETs are inactivation products of EETs[37,44] because their activities are reduced in many systems, such as relaxation of the preglomerular vasculature and the bovine coronary artery,[45,46] inhibition of

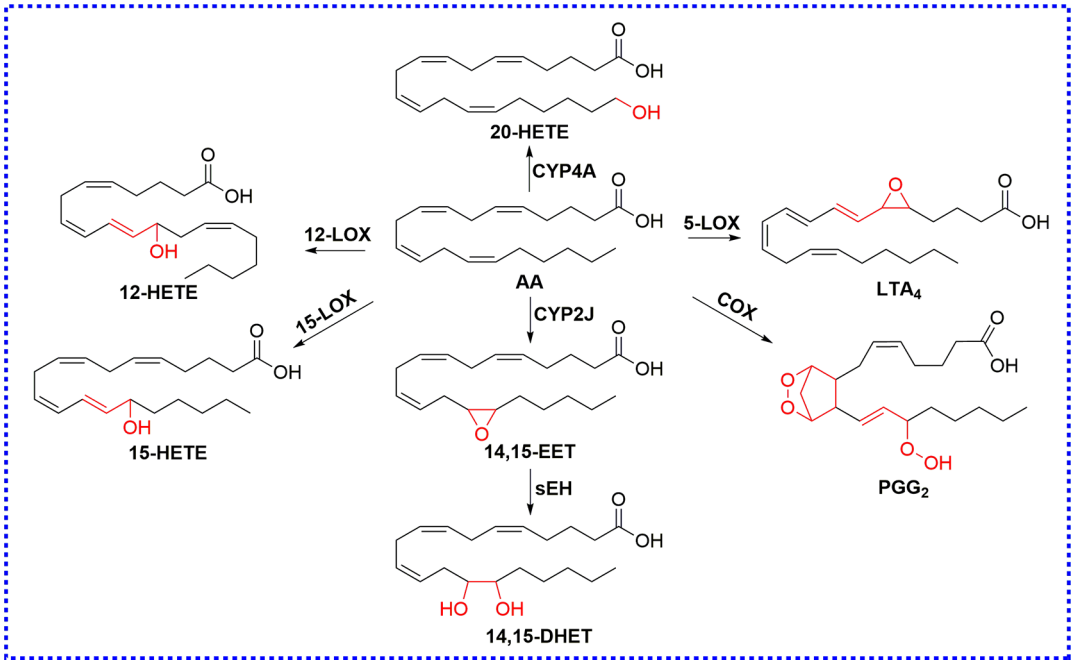


Figure 2. Metabolic pathways of AA.

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

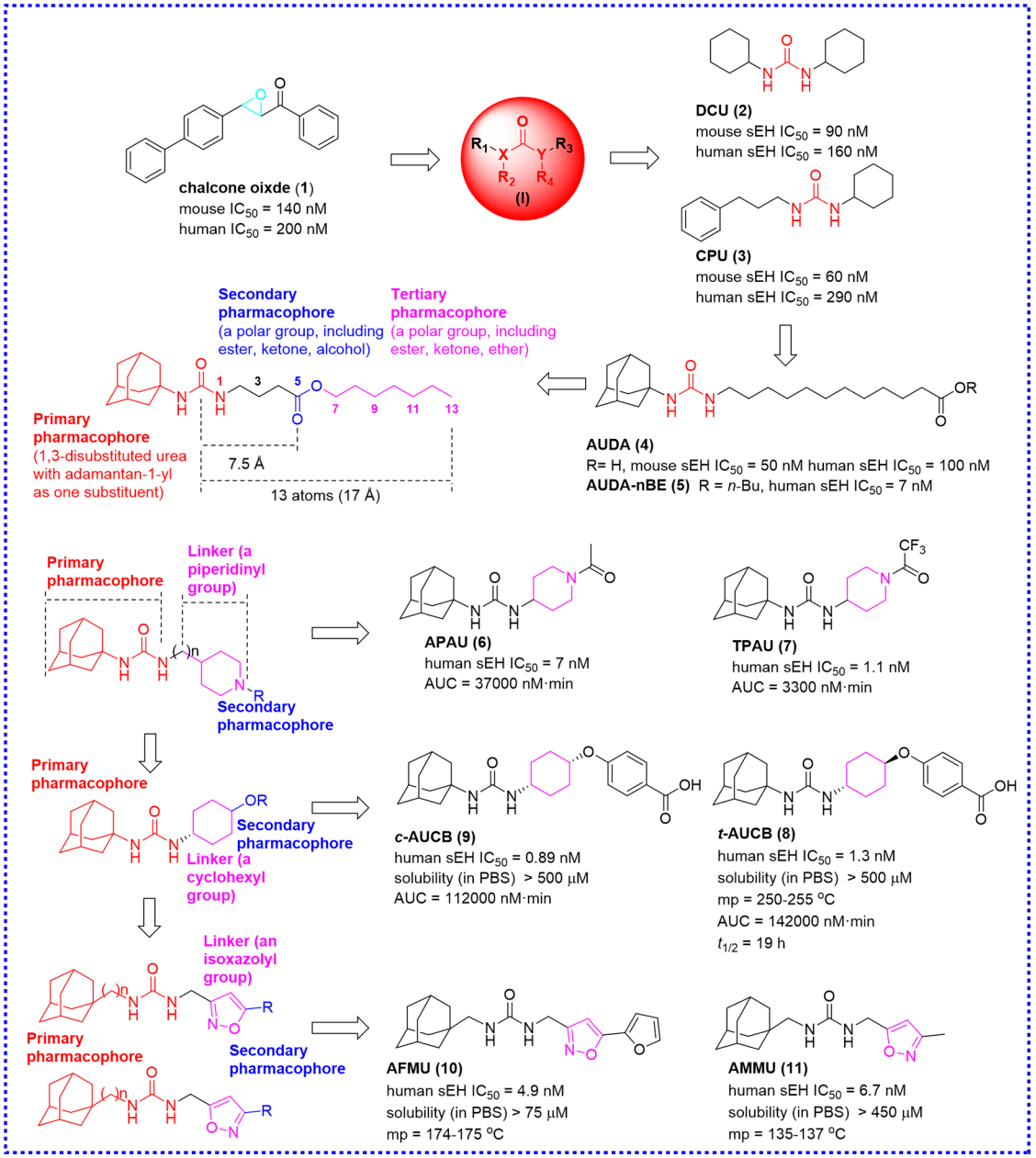


Figure 3. Representative sEH inhibitors and development of early sEH inhibitors.

Na^+ channel and cyclooxygenase,[47,48] and Ca^{2+} uptake.[49] In addition, DHETs produce functional effects in several other systems as well, including the inhibition of hydroosmotic action of arginine vasopressin,[50] relaxation of canine and porcine coronary arterioles,[51,52] and activation of the BK_{Ca} channel.[48−51]

Because sEH can rapidly metabolize or inactivate EETs to produce DHETs, nowadays, sEH inhibition or knockout has become an experimental approach to investigate the biological roles of EpFAs, especially EETs. For example, inflammatory pain can be ameliorated by increasing levels of EETs or inhibiting sEH to stabilize EETs.[53] Furthermore, the sEH expression level is upregulated in Ang II-induced lung injury mice, levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are significantly increased, and the *Ephx2* (the gene name of mouse sEH) knockout can remarkably alleviate the lung injury.[54] In addition, sEH is found to be expressed in the cerebral cortex and hippocampus as well as striatum (STR). Therefore, it is speculated that sEH is closely related to central nervous system (CNS) diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), depression, and stroke.[30] In this review, we summarized recent findings of sEH inhibitors from chemical synthesis and natural products, which provided useful guidance for the future development of potential sEH inhibitors, and the relationship between sEH and cardiovascular, CNS, and metabolic diseases.

2. sEH INHIBITORS

EETs exhibit potential biological functions in dilating blood vessels and protecting neurons. Numerous studies indicated that inhibiting sEH to stabilize EETs can significantly reduce inflammation and pain.[54,55] Therefore, sEH inhibitors are considered a promising and effective treatment for various diseases and its development has become a hot topic in the

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

research field since the beginning of this century. So far, sEH inhibitors have been discovered from a variety of pathways, such as synthesis and natural products;[56] therefore, we summarized the recent progress in the design and development of sEH inhibitors.

2.1. Targeting sEH Only. *2.1.1. Urea sEH Inhibitors.* The earliest selective sEH inhibitors reported are substituted chalcone oxides (such as compound **1**) and phenylglycidols rather than ureas. 4-Phenylchalcone oxide (**1**, Figure 3) is a sEH substrate and forms a covalent intermediate, allowing low turnover and transient inhibition. However, they are unstable in the presence of glutathione and slowly hydrolyzed by sEH; therefore, their use *in vivo* is limited.[57] The first potent and stable inhibitors of sEH were reported in 1999 based on the urea skeleton (**I**) (Figure 3), such as *N*,*N*′-dicyclohexyl-urea (**2**, DCU) and *N*-cyclohexyl-*N*′-(3-phenylpropyl)urea (**3**, CPU).[58] They display significant inhibitory potency against the mouse and human sEH with IC_{50} values in the tens of nanomolar. Their kinetic constants (K_i) are in the low nanomolar and are 20- to 150-fold better than **1** (K_d = 430 nM), revealing that compounds like DCU (**2**) or CPU (**3**)[59] act as competitive inhibitors at stoichiometric concentrations[56,60] and have a tight association with the target enzyme.[59] DCU (**2**) was the first to be used *in vivo* and could significantly inhibit the conversion from 14,15-EET to 14,15-DHET in spontaneously hypertensive rat, then leading to an antihypertensive effect.[61] In porcine coronary endothelial cells, DCU (**2**) led to the decrease of the EETs conversion rate by 3-fold.[62] While it demonstrated the usefulness of the urea inhibitors to affect the metabolism of EpFAs *in vitro* and *in vivo*, the early sEH inhibitors, such as DCU (**2**), had poor water solubility and high melting point (mp) due to its rigid structure and nonpolar groups, which affected its oral availability and bioavailability. To date, the urea derivatives are the most abundant and effective sEH inhibitors because the central urea group can bind strongly to the catalytic pocket of sEH (Figure 1B).[63] The oxygen atom on the urea group formed two hydrogen bonds with amino acid residues Tyr381 and Tyr465 in the catalytic pocket of sEH.[63] Moreover, the N−H of the urea group acts as the hydrogen bond donor of Asp333.[63] Because of their high potency, urea derivatives have been continuously developed, especially to improve solubility and bioavailability to apply them for the treatment of EpFA-related diseases.[56,64]

From DCU (**2**), the first improvement in the design of sEH inhibitors was the introduction on one side of the urea function of a flexible chain, such as 12-(3-adamantan-1-ylureido)dodecanoic acid (**4**, AUDA). The introduction of a flexible side chain significantly improved the water solubility of AUDA (**4**) and reduced the mp while maintaining the inhibitory effect.[65] AUDA (**4**) was continuously administered to mice via drinking water (25 mg/L) for 14 days and finally achieved the purpose of reducing blood pressure. Although AUDA (**4**) was easier to formulate and to dose animals with (it could be given in the drinking water at 25 mg/L),[66] it was extremely sensitive to β-oxidation, and therefore it was easily metabolized and excreted,[32] thus limiting its application in the clinic. Nevertheless, AUDA *n*-butyl ester prodrug (**5**, AUDA-nBE) has relatively good pharmacokinetics (PK)[67] allowing *in vivo* application. AUDA-nBE (**5**) and its *in vivo* metabolite AUDA (**4**) reduced ischemic cerebral infarct size in stroke-prone spontaneously hypertensive rats and prevented death and restored systolic blood pressure in lipopolysaccharide (LPS)-stimulated mice as well as the inhibition on the tumor growth.[68,69]

On the basis of the analysis of the structure−activity of 348 urea-based inhibitors[70] and in order to improve physical properties while reducing metabolism, polar functional groups were incorporated into one of the alkyl chains of 1,3-disubstituted ureas.[64] This study revealed that a representative sEH inhibitor could possess a secondary pharmacophore (a polar functional group) that incorporated at least five atoms (about 7.5 Å) from the primary pharmacophore (the central urea carbonyl) which effectively improved aqueous solubility and PK properties (Figure 3) while maintaining potency. Solubility could even be enhanced with a polar tertiary pharmacophore that is 13 atoms or ∼17 Å away from the primary pharmacophore, without affecting much the inhibitor potency.[64]

To reduce metabolism, in addition to the introduction of polar groups, Jones et al. proposed in 2006 conformationally constrained human sEH inhibitors,[71] which have the central pharmacophore and secondary pharmacophore connected by saturated rings, such as piperidine or other unsaturated rings. APAU (**6**) and TPAU (**7**) (Figure 3) are examples of the first series of piperidine-based urea inhibitors. These compounds are not only very potent inhibitors (IC_{50} values in the low nanomolar region) but have better PK characteristics, such as 10-fold higher area under the curve (AUC)[72] compared to AUDA (**4**) (Figure 3), underlying the benefits of conformationally constrained inhibitors.[71]

In order to further investigate the influence of conformationally constrained groups toward sEH inhibition, Hwang et al. used a cyclohexyl group as a linker instead of a piperidinyl group because of their structural similarity and designed a series of *cis*- or *trans*-1,4-cyclohexyloxy-*N*,*N*′-disubstituted urea derivatives based on the skeleton of APAU (**6**, Figure 3).[73] These inhibitors not only have good water solubility but also resolve the problem that flexible alkyl chains are easily oxidized and metabolized rapidly, increase bioavailability *in vivo*, and significantly improve metabolic stability.[73] Among them, a pair of epimers *trans*-4-[4-(3-adamantan-1-ylureido)-cyclohexyloxy]benzoic acid (**8**, *t*-AUCB) and *cis*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid (**9**, *c*-AUCB) show low nanomolar inhibitory effects (**8**, IC_{50} = 1.3 nM; **9**, IC_{50} = 0.89 nM) and desired PK properties, especially *t*-AUCB (**8**). It is notable that the 1,4-*trans* cyclohexane urea *t*-AUCB (**8**) is more stable than its isomer *c*-AUCB (**9**) in human liver microsome (HLM), and the AUC of the former in dogs increases by about 40-fold compared with that of AUDA (**4**). *t*-AUCB (**8**) at a dose of 1 mg/kg also shows the same therapeutic effect of returning blood pressure to normal as AUDA-nBE (**5**) at a dose of 10 mg/kg in the LPS-induced hypotension model. An analysis of their structures indicates that the introduced free carboxyl group forms an additional hydrogen bond with Met418 in addition to the classical hydrogen bonds of the urea function with Asp333 and Tyr381.[73] In addition, *t*-AUCB (**8**) displays remarkable biological effects, including antifibrosis and anti-inflammatory activities, as well as protective effects against liver and cardiovascular diseases.[74−78] *t*-AUCB (**8**) can downregulate muscle-specific miR-133 expression level in the myocardial infarction mouse established by ligating the coronary artery, allowing the reducing infarct size and preventing the development of cardiac arrhythmias.[74] Moreover, *t*-AUCB (**8**) reduces adipose tissue inflammation, prevents hepatic

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

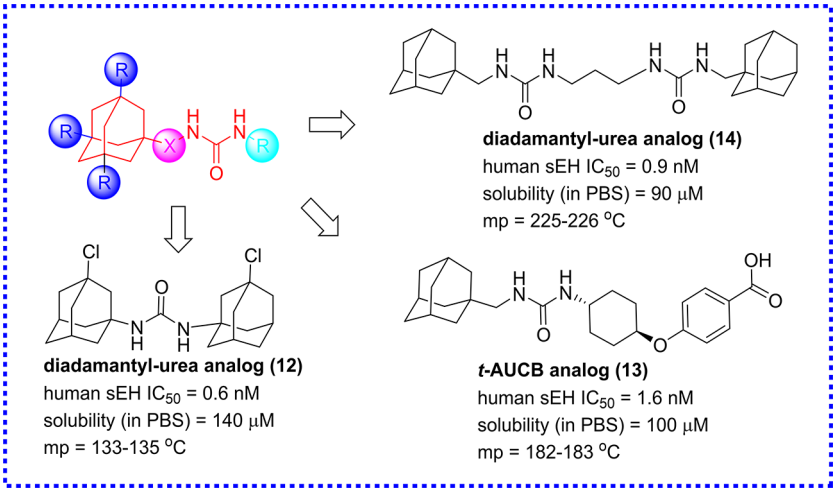


Figure 4. Optimization of adamantyl-ureas.

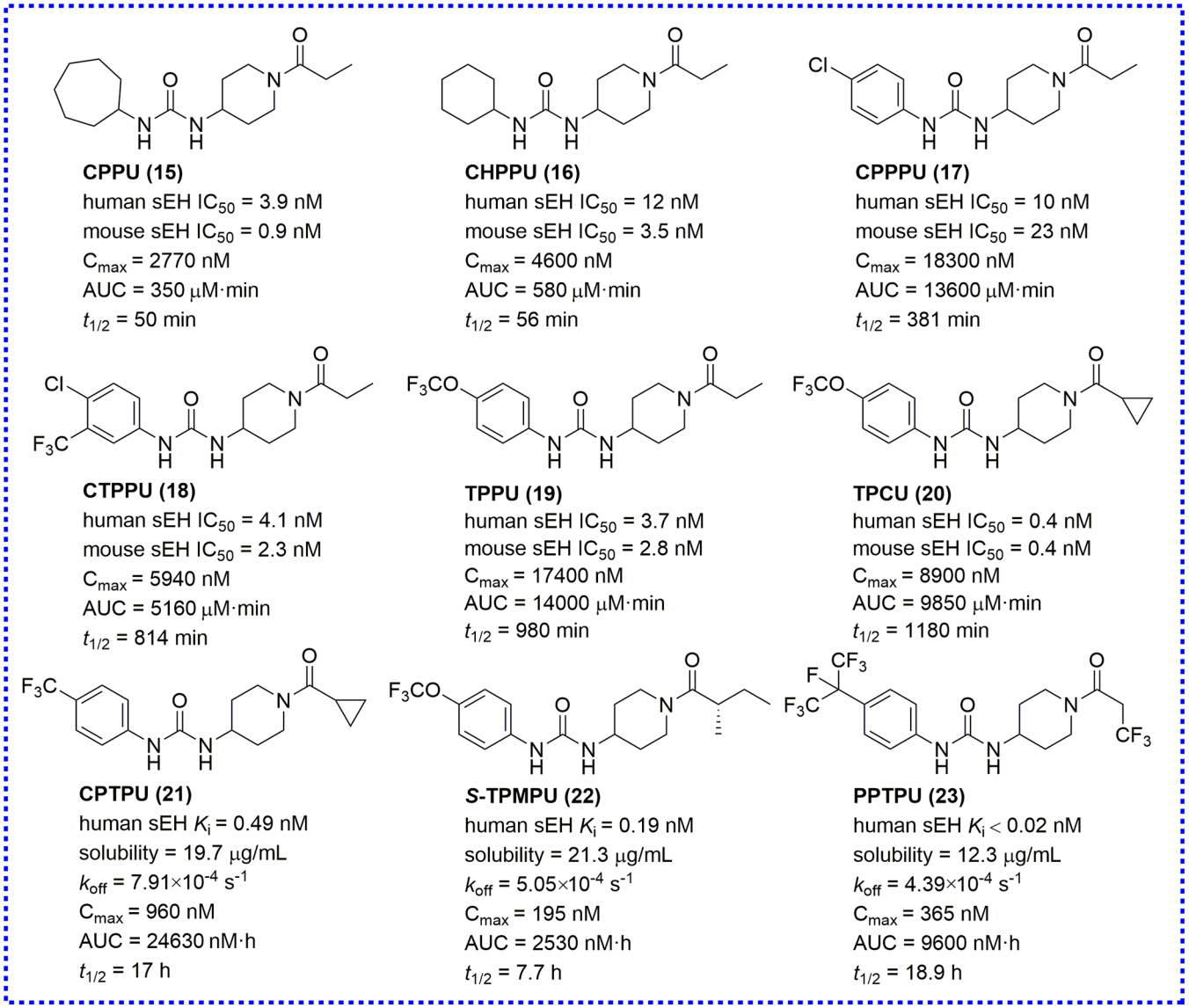


Figure 5. Representative urea sEH inhibitors.

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

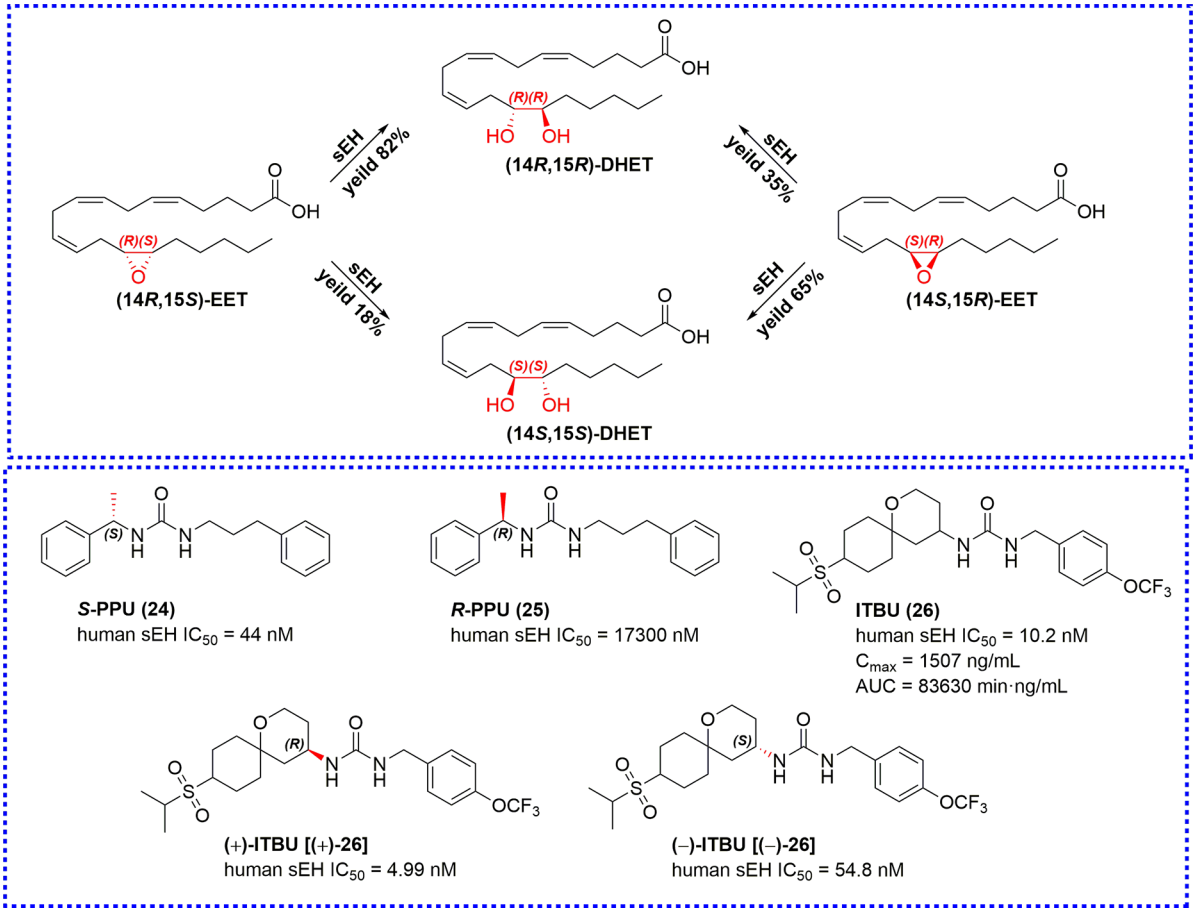


Figure 6. Influence of stereoconfigurations on inhibitory potentials and representative sEH inhibitors.

steatosis and the increased fasting glycemia, improves glucose tolerance, and decreases gluconeogenesis in high-fat diet (HFD) mice.[77]

In addition to the cyclohexyl group, an aromatic ring and an isoxazolyl group were also used as the replacement of the piperidinyl group at the right side of *N*-adamantyl-*N*′-piperidinyl-ureas such as APAU (**6**) in view of their limited solubility and rapid metabolism (Figure 3), which reduced the mp of the inhibitors and should enhance the stability.[79] Despite the potent inhibitory activity observed in this series of compounds, such as 1-(adamant-1-ylmethyl)-3-{[5-(furan-2-yl)isoxazol-3-yl]methyl}urea (**10**, AFMU) and 1-(adamant-1-ylmethyl)-3-[(3-methylisoxazol-5-yl)methyl]urea (**11**, AMMU), their stability was not improved in HLM with nicotinamide adenine dinucleotide phosphate (NADPH).[79]

The next step in sEH inhibitor development involved the optimization of the adamantyl-ureas via adding the substitution to the bridgehead of the adamantane to afford a library of this type compound (Figure 4).[80] Interestingly, the substitution influenced greatly the potency, physical properties, and metabolic stability of ureas and diureas. Interestingly, a methyl or chloride substitution on the bridgehead of the adamantane is to the benefit of the potency, and the adamantane and urea functions are linked via a methylene, which is in favor of potency and physical properties. These findings provide a new path to further design sEH inhibitors.[80]

Although the above-mentioned adamantyl-urea sEH inhibitors have excellent potency, this type of inhibitor is easily oxidized and metabolized, resulting in low drug concentrations and short *in vivo* half-life ($t_{1/2}$). Therefore, in the next iteration of compound design, aromatic or other aliphatic moieties were used instead of the adamantyl group based on the structure of *t*-AUCB (**8**).[81] A library of aromatic- and other aliphatic-ureas were synthesized (Figure 5; compounds **14**–**20**). While the replacement of the adamantyl group by aromatic groups did not significantly improve the inhibitory potencies, the PK properties were dramatically improved, especially for TPPU (**19**) and TPCU (**20**). The AUC/IC_{50} of TPCU (**20**), a metric of the inhibitory effect, is 5500-fold higher than that of APAU (**6**), and a similar result is obtained in the PK of TPPU (**19**) as well. These compounds have become commercial inhibitors of sEH and been used in a wide variety of studies to test for sEH biological activities.[81]

Although acyl piperidine ureas have good inhibitory effects, they show unsatisfactory PK properties in a dog model.[82] Hammock's team designed a series of piperidyl-urea derivatives for the treatment of diabetic neuropathic pain based on the skeleton of *N*-phenyl-*N*′-piperidyl-urea in 2014.[83] The potency of sEH inhibitors does not only consider IC_{50} or K_i, but also depend on how long the target is occupied by the inhibitor, because the catalysis is blocked only when the enzyme is occupied. In these 1,3-disubstituted ureas (Figure 5), the dissociation rate constants (k_{off}) of compounds **21**–**23** increase 2.4- to 4.4-fold compared with that of a previous inhibitor TPAU (**7**, $k_{off} = 19.2 \times 10^{-4}$ s^{-1}). An analysis of the interaction between their analog TPPU (**19**, $k_{off} = 10.2 \times 10^{-4}$ s^{-1}) and human sEH reveals a small secondary binding site next to the α-carbon of its amide, which provides the

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

possibility of additional binding. The hydrophobic 4-trimethoxyphenyl group binds closely to the right side of the binding pocket with a little extra binding space, which may be the reason why it stays on the target for a long time and shows excellent inhibitory effect.[83]

CYP enzymes can metabolize AA to produce four regioisomers, and each isomer has different proportions of *S*/*R*- or *R*/*S*-enantiomers (Figure 6). Because the production rate and ratio of stereoisomers of these diol products are closely related to the stereochemistry of substrates and epoxy ring substitution, stereoselectivity is also an important factor in the design of sEH inhibitors.[84] Manickam et al. designed a series of pairs of enantiomers with a chiral center close to urea nitrogen atoms.[84] There are significant differences in the inhibitory activity of enantiomers; for example, the inhibitory activity of (*S*)-1-(1-phenylethyl)-3-(3-phenylpropyl)urea (**24**, *S*-PPU) is 40-fold higher than that of its enantiomer (**25**). This similar result is also observed by Lukin et al., which indicates that the chirality of the α-carbon of urea nitrogen atoms can affect the inhibition of inhibitors on sEH.[60] Among the synthesized compounds, ITBU (**26**) possesses a potent sEH inhibitory activity and an excellent bioavailability in mice. However, the further analysis reveals that ITBU (**26**) is a racemic inhibitor and can be isolated to afford (+)-ITBU [(+)-**26**] and (−)-ITBU [(−)-**26**]. The inhibitory potency of the former is 11-fold higher than that of the latter. All the above-mentioned suggests that the selective sEH inhibition by different enantiomers provides more possibilities for the binding sites of sEH and a new direction for the development of sEH inhibitors.[60]

Except for the above-mentioned modification, the urea moiety was also altered by replacing the oxygen atom of urea with the sulfur atom to afford thiourea-based sEH inhibitors (Figure 7).[85] Among the thioureas, 1-(adamantan-2-yl)-3-

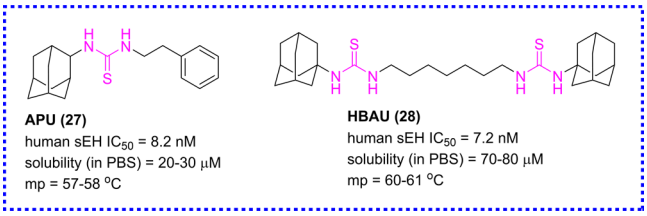


Figure 7. Representative thiourea-based sEH inhibitors.

phenethylthiourea (**27**, APU) and 1,1′-(heptane-1,7-diyl)bis(3-(adamantan-1-yl)thiourea) (**28**, HBAU) are found to be potent sEH inhibitors. Although their potencies are less than the previously described TPPU (**19**), they have better water solubility and are easier to be prepared, which is one of the ideal properties of an ideal inhibitor.[85]

2.1.2. Amide sEH Inhibitors. In addition to the urea-based sEH inhibitors, amides have been also widely developed as sEH inhibitors by removing one of the NH group of the ureas (Figure 8), yielding compounds **29**−**32**.[86] The primary structure−activity relationship (SAR) indicates that their inhibition potencies almost does not affect mouse sEH compared to the corresponding ureas, whereas this modification leads to the 2.5-fold decrease inhibition potency for human sEH. On the other hand, the amide function can significantly improve the physical property compared to the corresponding ureas, such as solubility and mp, for example, AABA-PE (**31**; mp, oil; solubility, 17.4 μg/mL).[86]

Eldrup and colleagues screened in-house compounds with drug-like characteristics for sEH inhibition and found that *N*-(3,3-diphenylpropyl)nicotinamide (**33**, DPN) could be regarded as a new starting point for an optimization process.[87] According to the X-ray crystal result of DPN (**33**) and sEH as shown in Figure 9, a library of amide inhibitors were designed and synthesized via the introduction of methanesulfonyl and fluorine at two benzene rings based on the structural core of DPN (**33**), yielding compounds **34**−**37** (Figure 9). They showed similar inhibitory potencies as DPN (**33**), whereas their metabolic stability was significantly improved in HLM and rat liver microsome (RLM).[88] CFMB (**36**) as a potential sEH inhibitor was found to have a low drug−drug interaction potential with selectivity for CYP enzymes.[87] In addition, DPN (**33**) was further optimized based on the structure of urea analog (**38**) with good metabolic stabilities ($t_{1/2}$, 185 min in HLM; 105 min in RLM), resulting in the production of a series of nicotinamide analogs.[89] Among them, nicotinamide analog (**39**) showed a low nanomolar inhibitory effect and a great metabolic stability and had also acceptable ($t_{1/2}$, 185 min in HLM and 105 min in RLM) plasma exposure.[89]

Anandan et al. analyzed previous urea inhibitors, such as CPU (**3**),[59] AUDA (**4**), and TPPU (**19**), and summarized the pharmacophore model comprising primary, secondary, and tertiary pharmacophores and two linkers; therefore, they developed a series of urea, amide, sulfonamide, thiourea, sulfonyl urea, hydroxyamide, and ketoamide inhibitors based on the skeleton (**II**, Figure 10).[90] A detailed analysis of SAR reveals that no loss of inhibitory potency is observed when the N atom on the left side of the carbonyl group of urea is replaced by a C atom, whereas the decrease of inhibitory potency is present when the urea group is substituted with other groups, such as sulfonamide, thiourea, sulfonyl urea, hydroxyamide, and ketoamide. A methylene group occurs between adamantyl and hydroxyamide or ketoamide group, which will be to the benefit of inhibitory potency except for amides (**40** and **45**). However, the inhibition potency is decreased when the adamantyl group is replaced by other groups, including cyclohexyl, 4-trifluoromethylphenyl, 4-chlorophenyl, and 4-trifluoromethoxyphenyl groups.[91]

On the basis of these findings, subsequent research focused on the secondary pharmacophore to optimize the inhibition potency of amides.[92−96] First, an oxyoxalamide functional group was used to design and develop sEH inhibitors as the secondary pharmacophore according to the skeleton (**III**) by Kim and co-workers (Figure 11).[94] Among the synthesized compounds, **50** (MMMO) and **51** (PMMO) show low nanomolar inhibitory activities similar to that of AUDA (**4**), and their physical property has been greatly improved compared to AUDA (**4**), especially their solubility. The new skeleton (**IV**) by modifying the secondary pharmacophore was constructed using the phosphonate function in place of the oxyoxalamide group[95] to afford potent sEH inhibitors, such as **52** (DPDP) and **53** (DAPDP). In addition, Zavareh et al. also tried to develop the novel skeleton (**V**) via using an oxadiazole ring as a novel secondary pharmacophore,[93,96] yielding a series of potent oxadiazole-base amide inhibitors, such as oxadiazole analogs **54** (IC_{50} = 0.91 nM) and **55** (IC_{50} = 0.83 nM). Molecular docking indicates that amino acid residues Asp335, Tyr383, and Tyr466 play a crucial role in the interaction with sEH, and Val498, His524, and Trp525 also form additional hydrogen bond interactions with an oxadiazole group. These

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

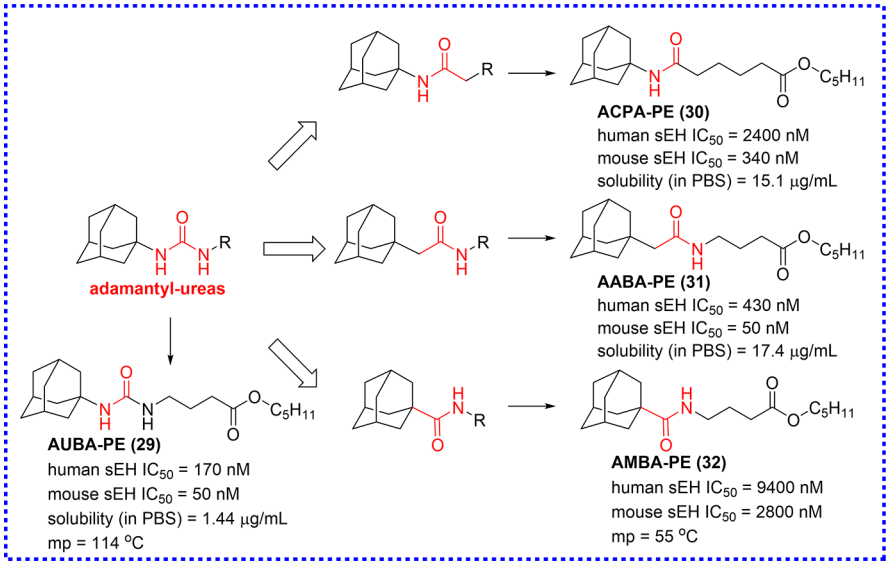


Figure 8. Modification of adamantyl-ureas to afford amide inhibitors.

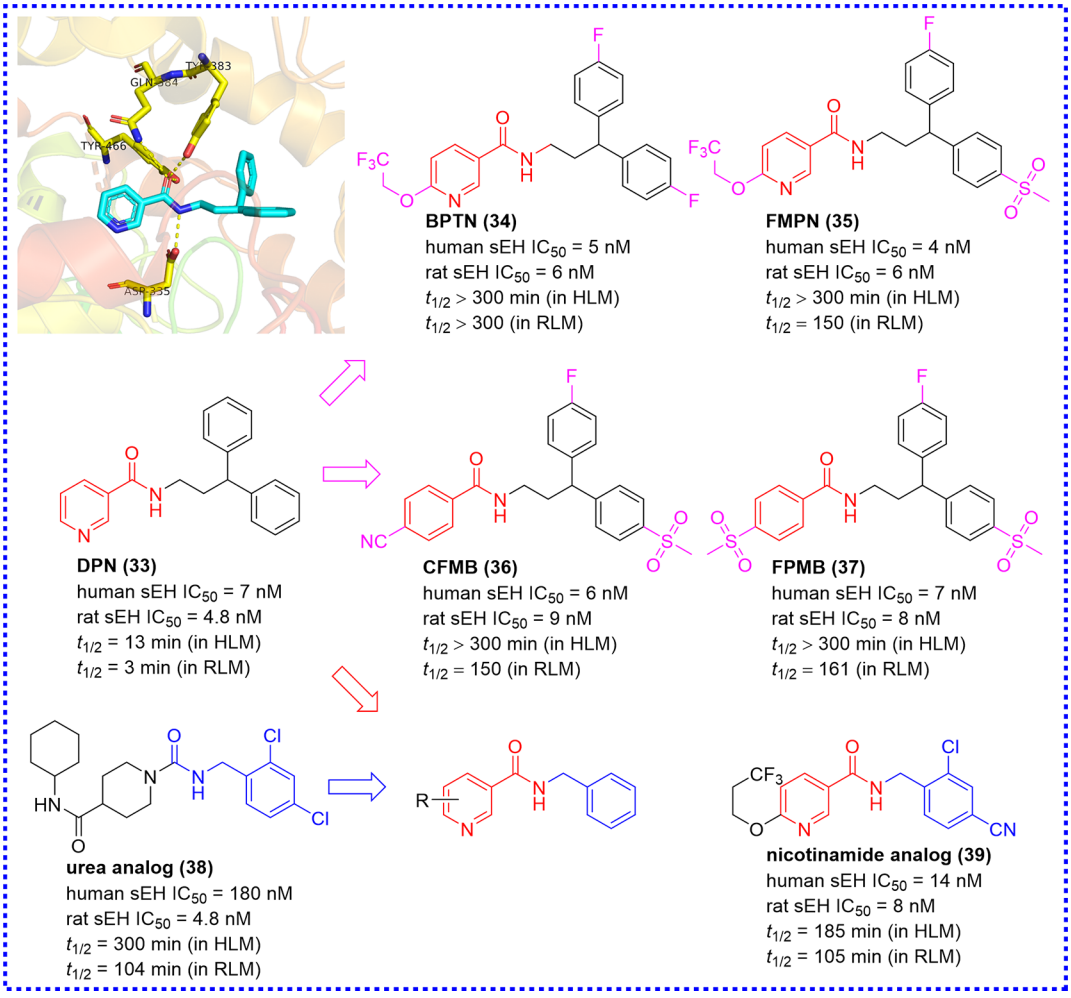


Figure 9. X-ray cocrystal structure of DPN (**33**) with sEH (PDB code 3I1Y) and its optimization.

findings have great importance in the design of sEH inhibitors.[96]

Kim and co-workers carried out the structural modification on both sides of the amide pharmacophore of 1,3-bicycloalkyl substituted amides to improve the inhibitory effect on sEH.[97] According to the SAR result (Figure 12), potent sEH inhibitors **56** (TPNC) and **57** (MTCMB) are obtained with reasonable physical properties. For example, the mp of MTCMB (**57**) is reduced (115 °C) and the water solubility (125 μM) is 2-fold higher than that of AUDA (**4**). This shows

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

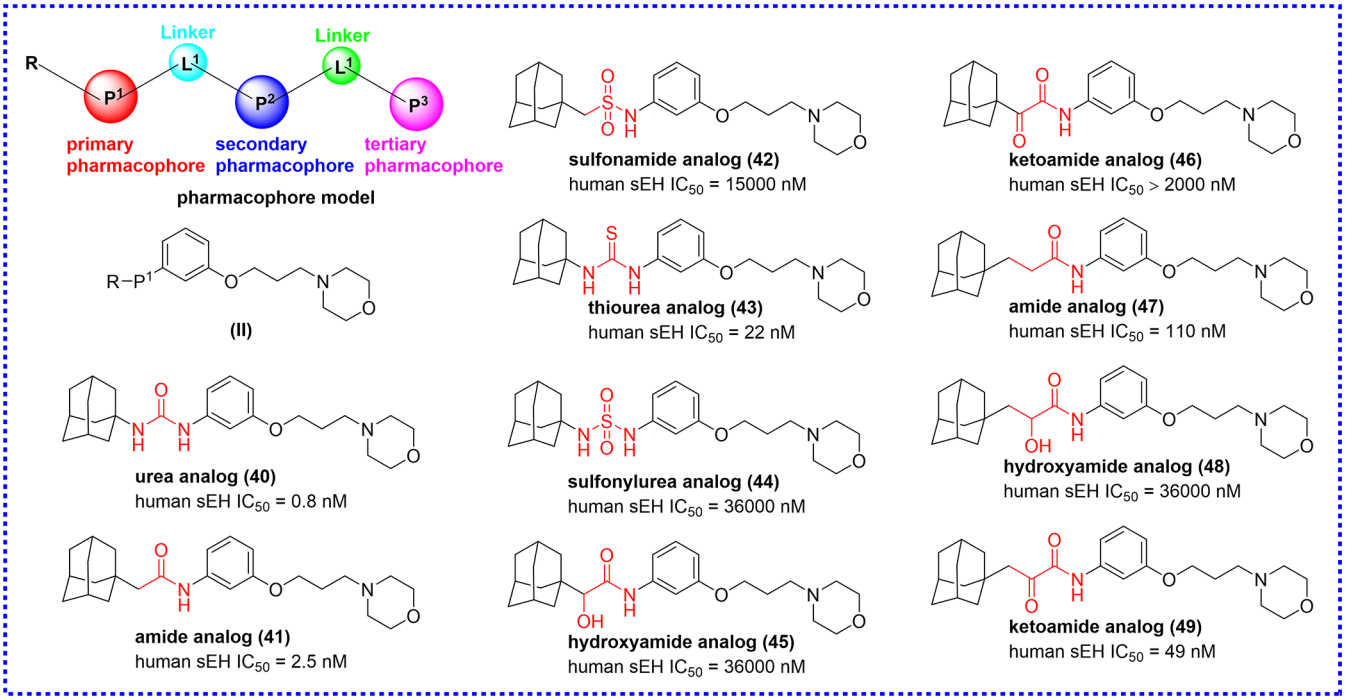


Figure 10. Modification based on the skeleton.

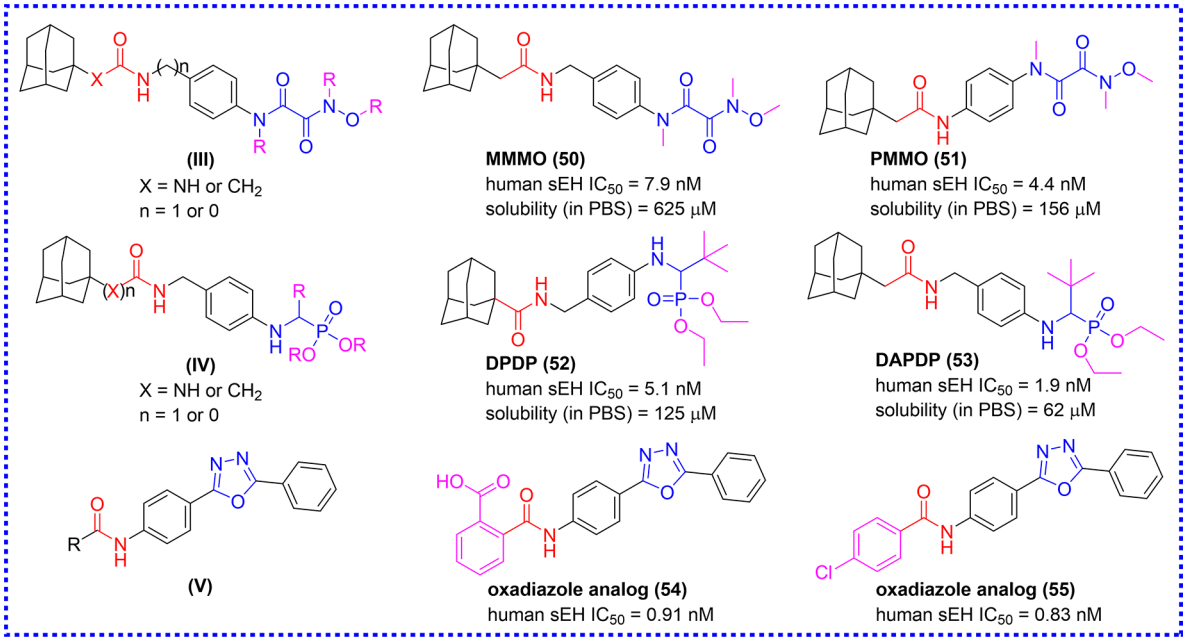


Figure 11. Modification of the secondary pharmacophore.

that amide inhibitors can achieve similar inhibitory potency as urea inhibitors while having better physical properties. In addition, this study opened the door to numerous possibilities for the design of potent amide inhibitors, especially through combinatorial chemistry.[97]

For finding new structures, Landry et al. tested a library of 130 000 chemicals, provided by the National Institutes of Health Roadmap project, for sEH inhibition. They obtained a lead compound **58** (Figure 13) with nanomolar potency that contains a sulfonyl isonipecotamide that is resembling APAU (**6**). Further optimization of **58** resulted in the production of three subnanomolar inhibitors (**59**−**61**).[98,99] Xing et al. designed a combinatorial library of benzoxazole derivatives and screened the potential sEH inhibitors via a virtual screening (Figure 14).[100] The X-ray crystal structure (PDB code 3PDC) of the human sEH with compound **62** revealed unique interactions between the enzyme and the benzoxazole group. In addition to the classic hydrogen bonds between the amide group and active site residues (Asp333, Tyr381, and Tyr465), the oxygen of the benzoxazole ring forms a hydrogen bond interaction with His523, one of the catalytic triad Asp333-Asp495-His523 (Figure 14). These additional hydrogen bonds may account for the enhanced potency of the amide-based inhibitors containing a benzoxazolyl group.[100]

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

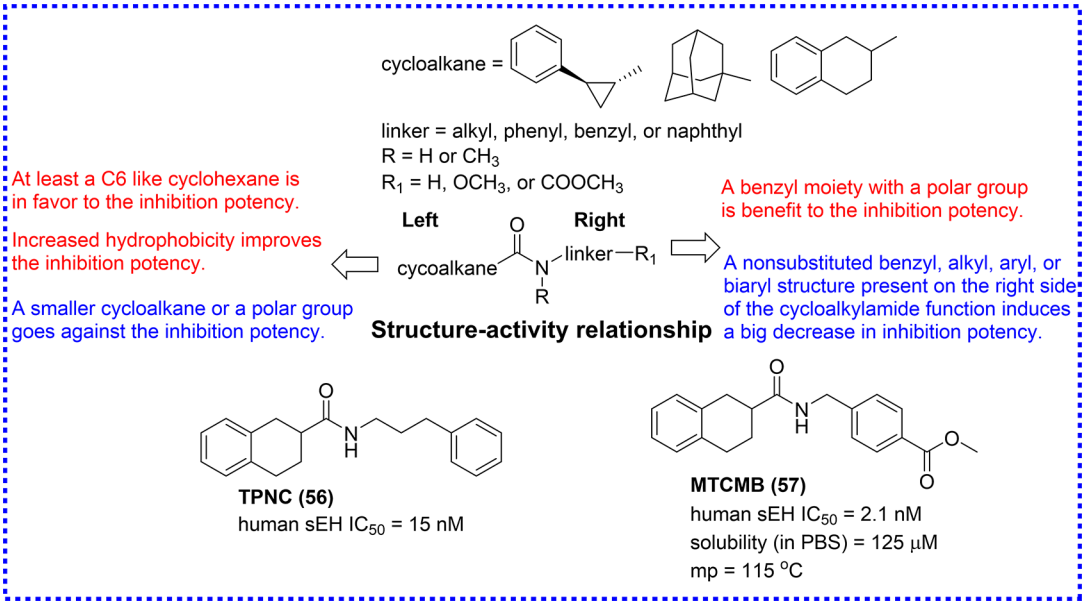


Figure 12. SAR of amide inhibitors summarized by Kim et al.

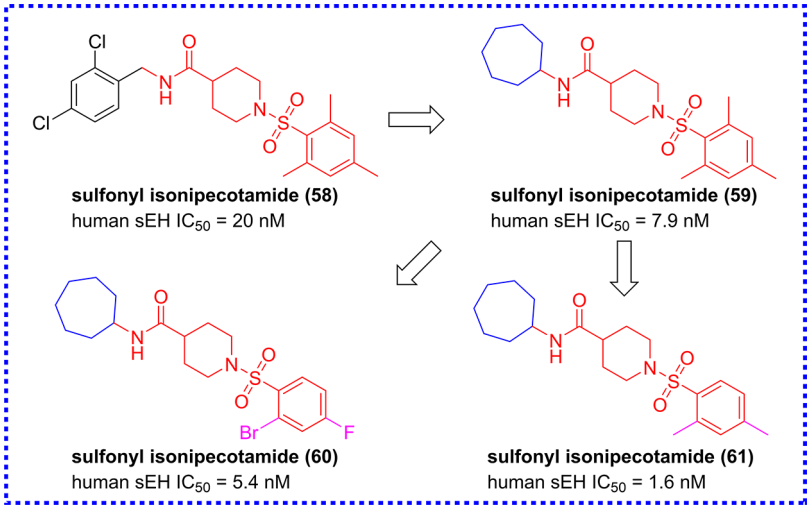


Figure 13. Chemical structures of sulfonyl isonipecotamides.

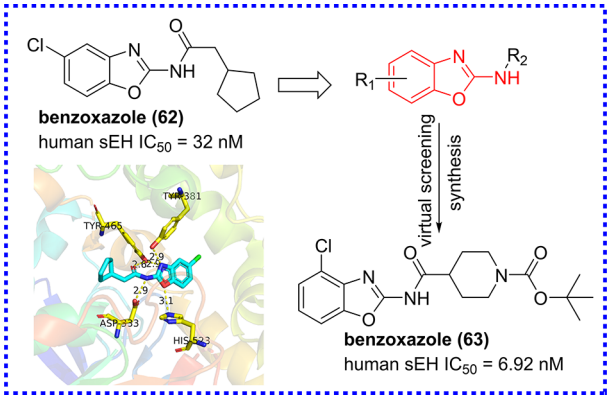


Figure 14. X-ray crystal structure of sEH complexed with the benzoxazole (**62**) (PDB code 3PDC) and its optimization.

Marino and co-workers used encoded library technology to discover a sEH inhibitor (**64**) featuring a piperidinecarboxamide skeleton via high throughput screening (HTS, Figure 15)[101,102] and optimized this skeleton to yield compounds **65** and **66** displaying low nanomolar inhibitory potencies.[103] The X-ray crystal structure of MTTP (**65**) with sEH (PDB code 4JNC) showed that **65** formed H-bonds with Asp335, Tyr383, and Tyr466 at the active site (Figure 15). The further optimization of this skeleton led to the production of a low picomolar sEH inhibitor GSK2256294 (**67**). The results of the *in vivo* study indicated that GSK2256294 (**67**) could dose-dependent reduce pulmonary leukocyte and keratinocyte chemoattractant levels in cigarette-smoke-induced mice, suggesting that GSK2256294 (**67**) would be an appropriate agent for the treatment of chronic obstructive pulmonary and cardiovascular diseases.[59] This compound has been tested in clinical trials.

2.2. Multitarget Inhibitors. Highly effective drugs with a single biological target are the mainstream of drug research and development.[104] However, they also have some limitations. By targeting a single enzyme or receptor only, a drug could lack efficacy on complex diseases and may cause additional adverse reactions when used in combination with other drugs.[104,105] In

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

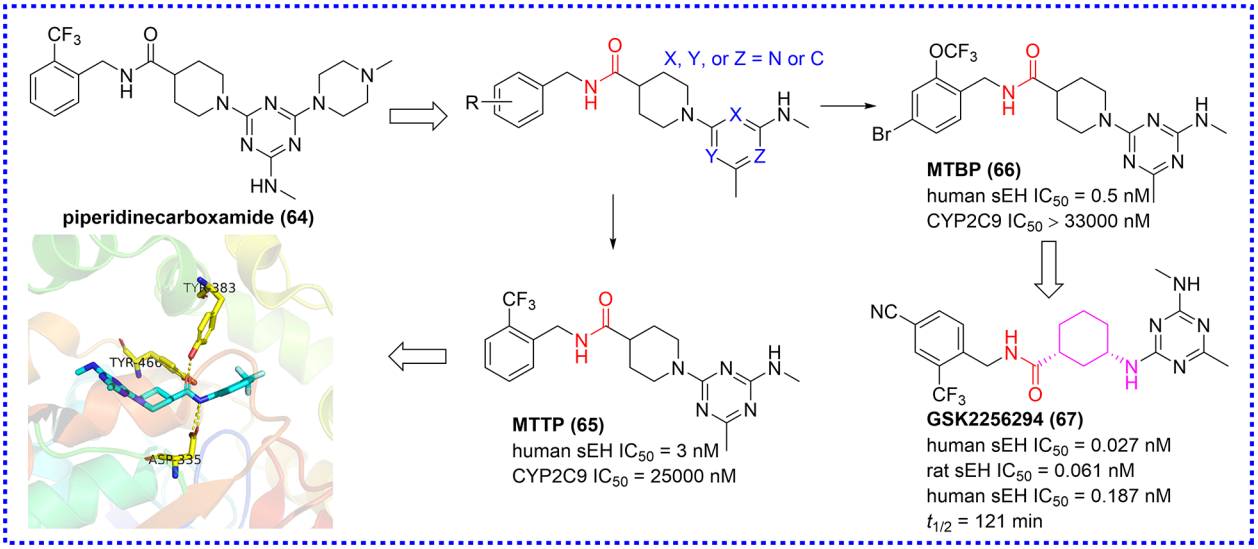


Figure 15. Modification based on the structure of the amide (**64**) and cocrystal structure of MTTP (**65**) and sEH (PDB code 4JNC).

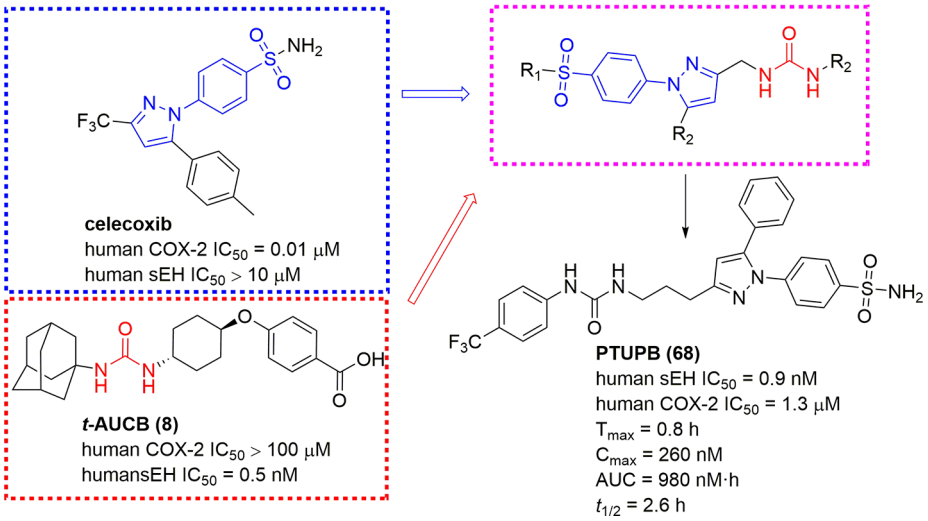


Figure 16. Design principle of an sEH/COX-2 dual inhibitor PTUPB (**68**).

order to solve these problems, research is focusing now on multitarget drugs with higher safety and effectiveness.[105,106] In recent years, several multitarget drugs have shown excellent therapeutic ability in meddling with the AA cascade. The sEH plays a crucial role in the AA cascade and participates in the pathological process of numerous diseases. In addition, the effectiveness of sEH inhibitors has been verified *in vivo*.[104] By combining the inhibition of sEH with the inhibition of another actor of the AA cascade, such as COX-2, 5-LOX, and 5-LOX-activating protein (FLAP), one could expect a greater effect for the treatment of complex diseases, such as neuroinflammation or cancer.[107,108]

2.2.1. sEH/COX-2 Dual Inhibitors. COX-2 is an enzyme in which expression is induced by the stimulation of inflammation, hormones, or growth factors.[109] COX-2 is highly expressed in inflammation and proliferative diseases, such as various cancers; therefore, it is a target to develop anti-inflammatory and antitumor agents.[104] A recent study has demonstrated that coadministration of EETs and COX inhibitors exerts a better effect than the single administration of COX inhibitors to reduce LPS-induced pain and hypotension.[79,84] Accordingly, a series of sEH/COX-2 dual inhibitors based on the structural skeletons of celecoxib and *t*-AUCB (**8**, Figure 16) were designed, and it was found that **68** (PTUPB) displayed potent inhibitory activities against both sEH and COX-2. Molecular stimulation shows that PTUPB (**68**) forms hydrogen bond interactions with amino acid residues Asp333, Tyr381, and Tyr465 of human sEH, and His90 and Tyr335 of mouse COX-2. In a nociceptive assay, PTUPB (**68**) enhances *in vivo* antiallodynia effects compared to both the individual and combination therapies of celecoxib and *t*-AUCB (**8**).[107,110]

PTUPB (**68**) was found to be beneficial to sepsis, nonalcoholic fatty liver disease (NAFLD), pulmonary fibrosis, and cancer.[33,107,111−116] PTUPB (**68**) inhibits tumor growth and metastasis by suppressing endothelial cell proliferation, and it also improves survival rate, delays the onset of debris-stimulated ovarian tumor growth, and reduces ascites.[107,115,116] In addition to the treatment of cancers, PTUPB (**68**) suppresses the activation of NLRP3 inflammasome,[111] allowing for improving the survival rate, reducing the clinical scores and systemic inflammatory response, and alleviating the multiple-

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

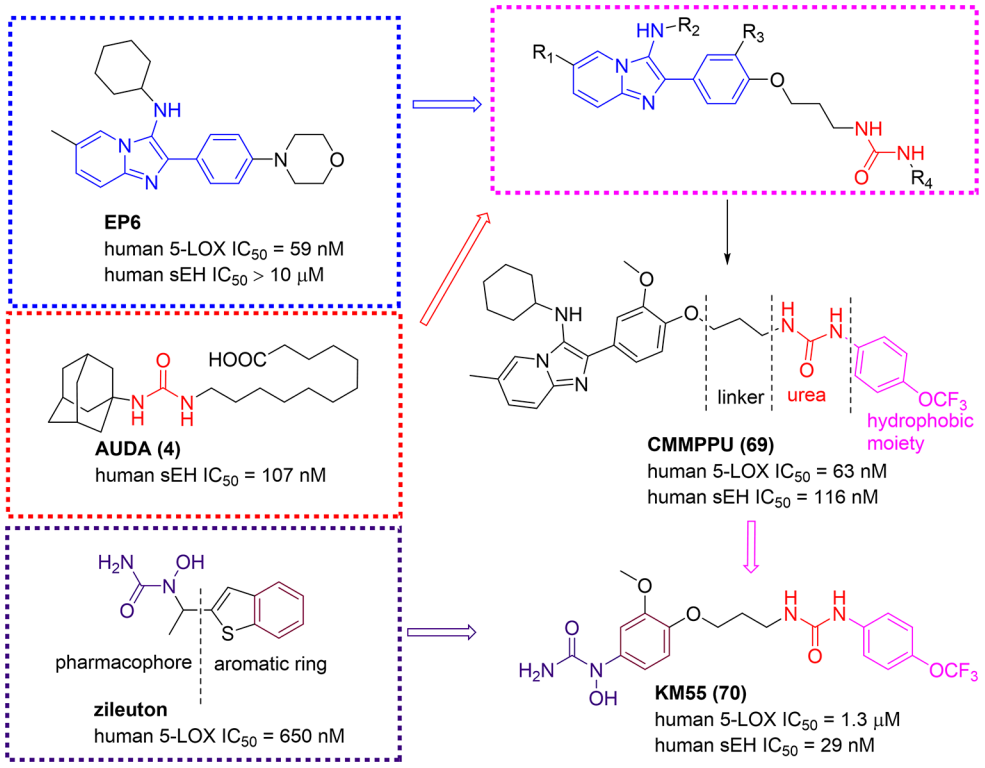


Figure 17. sEH/5-LOX dual inhibitors that linked two pharmacophores for sEH and 5-LOX.

organ injury in sepsis mice.[112] Therefore, sEH and COX-2 dual inhibition may be an approach to treat inflammation and cancer diseases.

2.2.2. sEH/5-LOX Dual Inhibitors. 5-LOX as a dioxygenase plays a catalytic role in two steps of the biosynthesis of LTs which regulate the innate immune response and play a pathophysiological role in chronic inflammatory diseases, such as asthma and atherosclerosis.[104] To date, a 5-LOX inhibitor zileuton is used in the clinic for the treatment of asthma with restrictions.[117] Coadministration of an sEH inhibitor *t*-AUCB (**8**) with a 5-LOX inhibitor showed a significant enhancement of the anti-inflammatory response.[109] Therefore, the sEH/5-LOX dual inhibitor has caught the attention of medicinal chemists. Moser and co-workers applied the pharmacophores of sEH and 5-LOX for screening 37 429 compounds *in silico* to afford a poor sEH/5-LOX dual inhibitor (sEH IC_{50} = 3.5 μM; 5-LOX IC_{50} = 36 μM), which nevertheless led to the new era of discovery dual inhibitors.[118] On the basis of the key pharmacophores, an imidazo[1,2-*a*]pyridine motif of a 5-LOX inhibitor EP6 (5-LOX IC_{50} = 59 nM) and a urea group of AUDA (**4**), a library of compounds were designed and synthesized by Meirer et al.[109] The SAR indicated that two pharmacophores required an *n*-propyl linker to maintain both sEH and 5-LOX inhibition. Among them, compound **69** showed high inhibitory potency against both 5-LOX and sEH (Figure 17). Although CMMPPU (**69**) exhibited high potency *in vitro*, it was not stable in plasma.[109] Subsequently, Meirer et al. designed KM55 (**70**), which showed a strong anti-inflammatory effect *in vitro*.[117]

Achenbach et al. used saturation transfer difference (STD)-NMR and activity-based assays (Figure 18) and found that a compound featuring an aminothiazole skeleton had better inhibitory activities toward sEH (IC_{50} = 170 nM) and 5-LOX (IC_{50} = 30 nM).[119] Nandha et al. designed and developed a

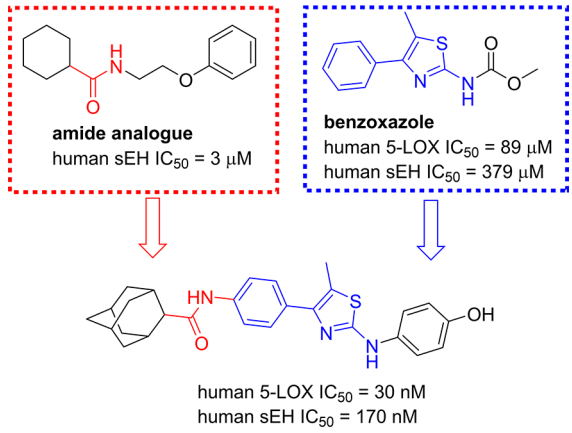


Figure 18. sEH/5-LOX dual inhibitor with an amide and an aminothiazole moiety.

potential sEH/5-LOX dual inhibitor (FFBMB, **71**) based on the structure of the 5-LOX inhibitor RWJ-63556 (Figure 19), a benzimidazole analog, and the sEH inhibitor *t*-TUCB. The *in vivo* study confirmed that FFBMB (**71**) possessed significant inhibition of carrageenan-induced rat paw edema.[120]

2.2.3. sEH/FLAP Dual Inhibitors. FLAP is a nuclear membrane-anchored protein responsible for transferring AA to 5-LOX that produces proinflammatory LTs. The inhibition of FLAP by FLAP inhibitors or genetic knockout can abolish the production of proinflammatory LTs. Meanwhile, a FLAP inhibitor, GSK2190915, has completed phase II trials for the treatment of asthma.[121,122] Recent studies have indicated that coadministration of a sEH inhibitor *t*-AUCB (**8**) and MK886, a FLAP inhibitor, enhances anti-inflammatory activities in a murine model, yielding interest in sEH/FLAP dual inhibitors.[123] Temml et al. used a pharmacophore-based virtual

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

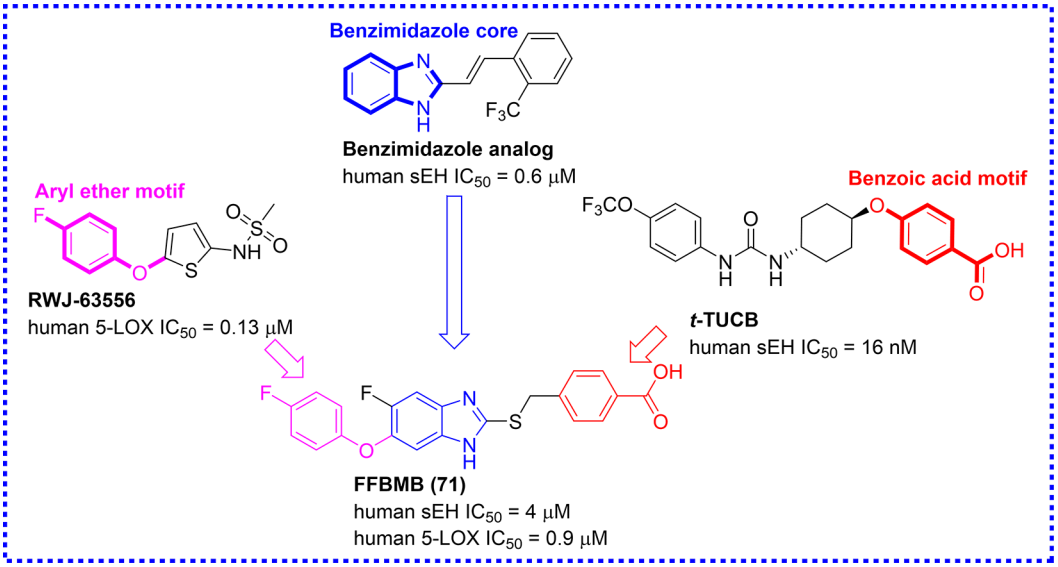


Figure 19. sEH/5-LOX dual inhibitor FFBMB (**71**) based on an aryl ether and a benzoic acid motif and a benzimidazole core.

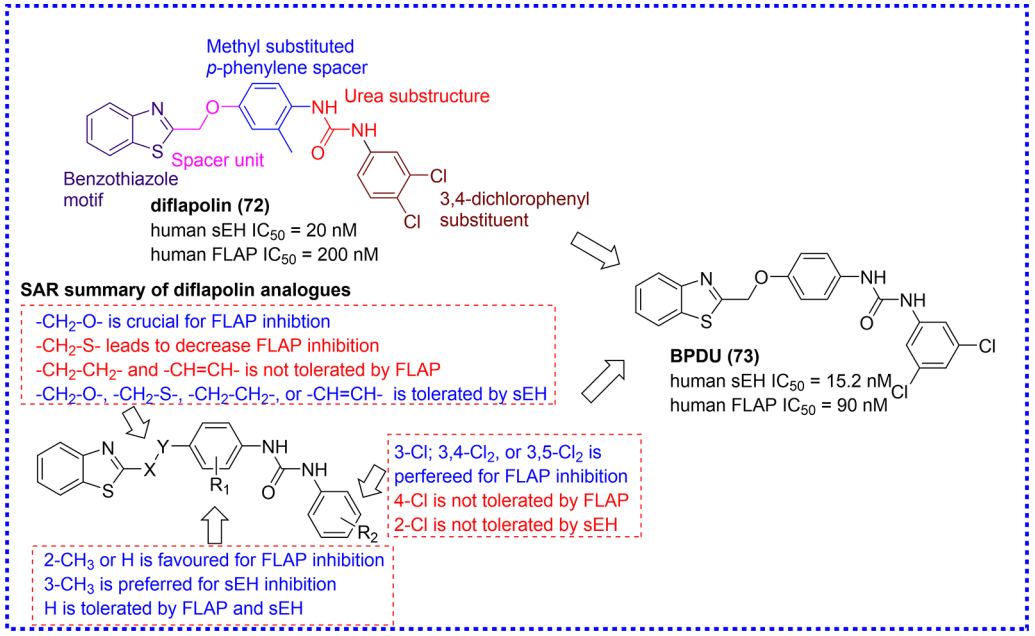


Figure 20. sEH/FLAP dual inhibitors diflapolin analogs.

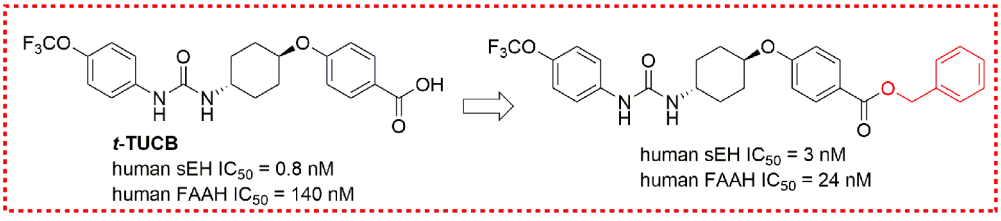


Figure 21. sEH/FAAH dual inhibitor derived from *t*-TUCB.

screening to afford a first sEH/FLAP dual inhibitor diflapolin (**72**) (Figure 20), which did not affect other enzymes of the AA cascade, suggesting that **72** is very selective for sEH and FLAP.[124] Investigation of its *in vivo* biological effects indicates that diflapolin (**72**) ameliorates vascular permeability, inhibits the formation of proinflammatory factors cysteinyl-LTs and leukotriene B_4 (LTB_4), and suppresses neutrophil infiltration in the zymosan-induced peritonitis mouse model.[124] Subsequently, Vieider and colleagues designed a library of diflapolin derivatives, leading to BPDU (**73**) (Figure 20), a more potent sEH/FLAP dual inhibitor.[125]

2.2.4. sEH/FAAH Dual Inhibitors. Fatty acid amide hydrolase (FAAH) is a membrane-bound serine hydrolase responsible for the deactivating metabolism of endogenous

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

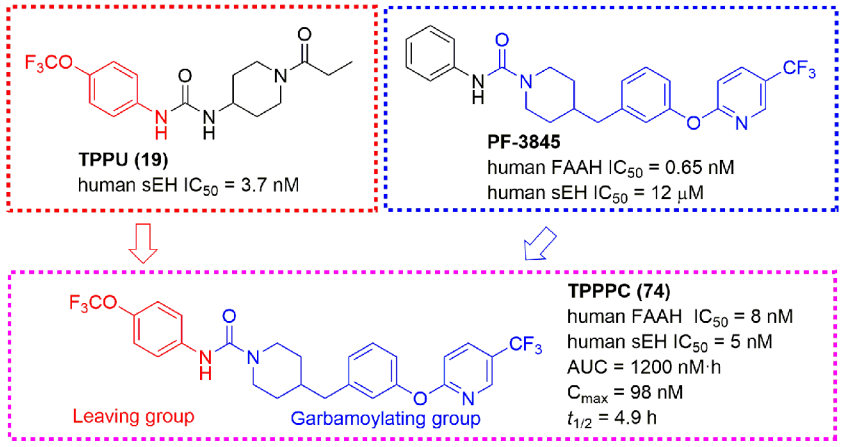


Figure 22. Selective sEH/FAAH dual inhibitor TPPPC (**74**) derived from TPPU (**19**) and PF-3845.

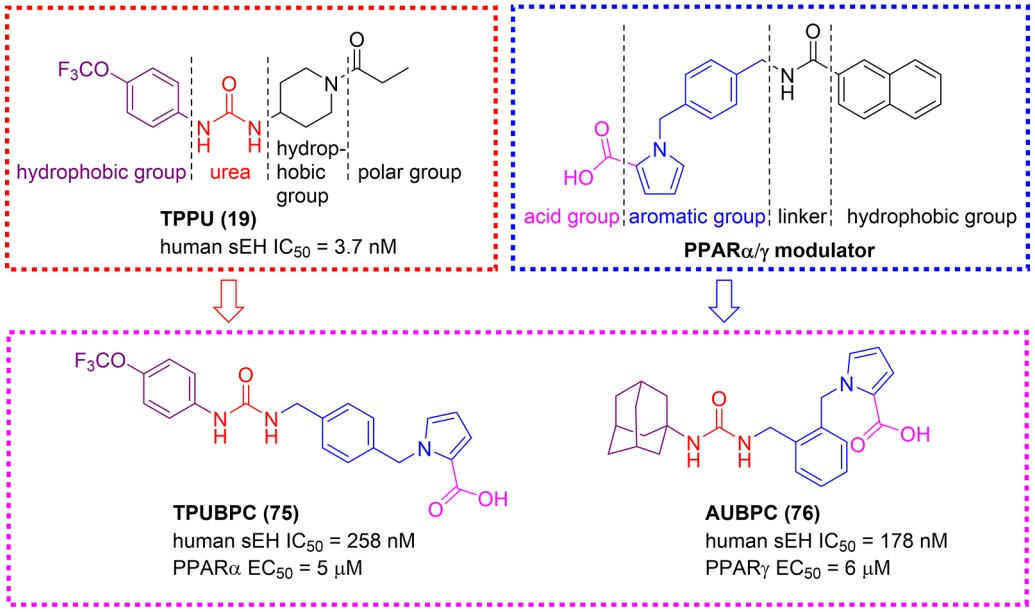


Figure 23. Selective sEH/PPAR dual modulators TPUBPC (**75**) and AUBPC (**76**).

cannabinoids, such as oleamide, anandamide, and myristic amide.[104] Both EpFAs and endocannabinoids exert anti-inflammatory effects; therefore, elevating their concentrations by inhibiting both sEH and FAAH should be a good method to treat inflammation and neuropathic pain.[104] Indeed, coadministration of a FAAH inhibitor and a sEH inhibitor elicited a synergistic analgesic response in acute inflammatory and chronic pain mice,[126] and thus, sEH/FAAH dual inhibitors had attracted more attention. Interestingly, because *t*-TUCB, a potent sEH inhibitor (IC_{50} = 0.4 nM), has a *N*-(3-trifluoromethoxyphenyl) moiety, which is a good leaving group for FAAH, it has also reasonable inhibitory potency against FAAH (IC_{50} = 260 nM).[105] Further chemical modifications resulted in a 6-fold increase potency against FAAH while retaining *t*-TUCB intrinsic sEH inhibitory effect (Figure 21).[105]

Separately, a series of sEH/FAAH dual inhibitors were developed based on the cores of a FAAH inhibitor PF-3845 and sEH inhibitor TPPU (**19**, Figure 22). Kodani et al. found that TPPPC (**74**) is a low nanomolar inhibitor for both FAAH and sEH inhibitions (Figure 22). Further investigation suggested that TPPPC (**74**) possesses a good target selectivity, PK property (AUC = 1200 nM·h; C_{max} = 98 nM; $t_{1/2}$ = 4.9 h in mice), and *in vivo* target engagement.[127]

2.2.5. sEH/PPARγ Dual Modulators. PPARγ is a nuclear receptor that can be activated by EETs allowing the inhibition of NF-κB pathway and anti-inflammatory effects.[128] Some studies have indicated that the sEH expression level is increased and the PPARγ transcriptional activity is decreased in obese patients, suggesting that simultaneous regulation of sEH and PPARγ has a therapeutic potential for metabolic diseases.[128] Recently, Imig and co-workers coadministrated thiazolidinedione (TZD), a PPARγ agonist, and *t*-AUCB(**8**), a sEH inhibitor, to spontaneously hypertensive obese rats, resulting in protective effects toward vascular function and kidney,[129] supporting the idea of sEH/PPAR dual modulators.[104] Proschak et al. designed a library of sEH/PPAR dual modulators based on the sEH pharmacophore 4-(trifluoromethoxy)phenyl ureas and PPAR modulator structures and obtained compounds, such as TPUBPC (**75**) and AUBPC (**76**), that display inhibition on sEH and activation on PPARα/γ (Figure 23).[108]

Subsequently, Blöcher et al. also designed a series of sEH/PPAR dual modulators with a benzylamide structure, a merged

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

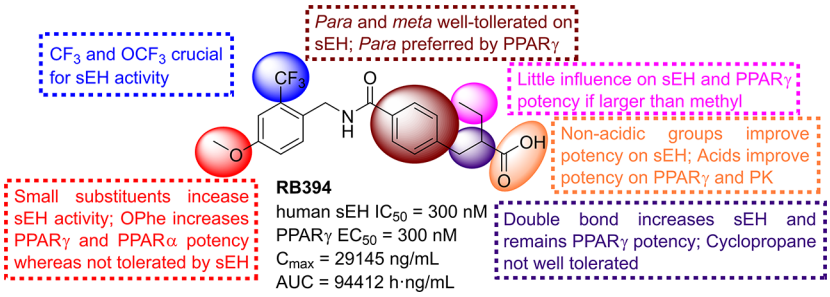


Figure 24. SAR of *N*-benzylbenzamides with sEH and PPARγ.

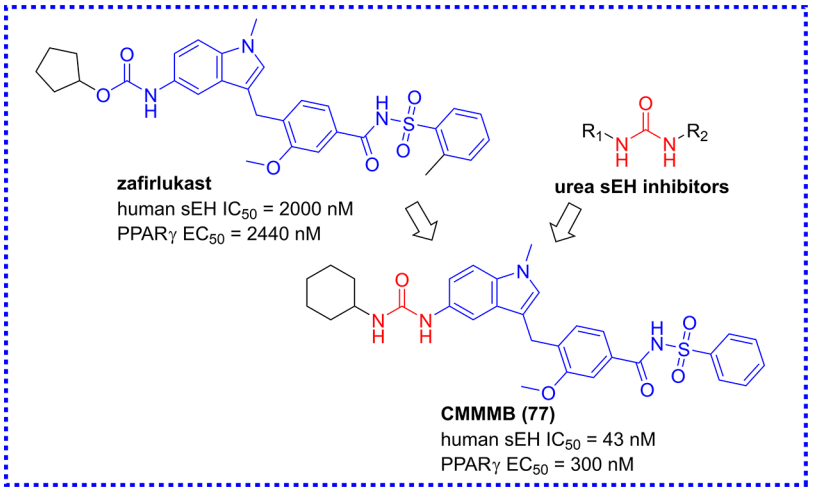


Figure 25. Selective sEH/PPAR dual modulator CMMMB (77) derived from zafirlukast.

pharmacophore for sEH and PPARγ, on the basis of the structure of the PPARγ agonist GSK1997132B, such as RB394 (Figure 24) with good potencies against sEH and PPARγ. In addition to its good PK and pharmacodynamic profile,[130] RB394 could also ameliorate the development of hypertension, insulin resistance, and hyperlipidemia in obese spontaneously hypertensive and obese diabetic Zucker fatty/spontaneously hypertensive heart failure F1 hybrid rats, which suggested that it could be regarded as a potential agent to treat metabolic syndromes.[131]

Afterward Schierle et al. found that zafirlukast, an antagonist of cysteinyl leukotriene receptor 1, also possesses an agonistic effect toward PPARγ and inhibitory effect toward sEH (Figure 25). Further structural optimization led to the production of compound **77**. Its potencies on sEH and PPARγ were increased by 46.5- and 8.1-fold compared to zafirlukast, respectively, and it also exerted an anti-inflammatory potency in the zymosan-induced paw edema.[132]

2.2.6. sEH/FXR Dual Modulators. Farnesoid X receptor (FXR), known as the bile acid receptor, is a ligand-activated nuclear receptor.[133] Clinical trials have reported that administration of FXR agonists could improve histological features and clinical markers of NAFLD as well as metabolic parameters, revealing that FXR can be served as a potential target for fatty liver disorders and metabolic diseases.

Interestingly, an antagonist of cysteinyl leukotriene receptor 1, zafirlukast, also has an agonistic effect against FXR (EC_{50} = 3.9 μM) and inhibitory effect against sEH (IC_{50} = 2.0 μM). The optimization of zafirlukast structure lead to a 15-fold increase of its agonistic effect against FXR with a similar potency toward sEH (Figure 26).[134]

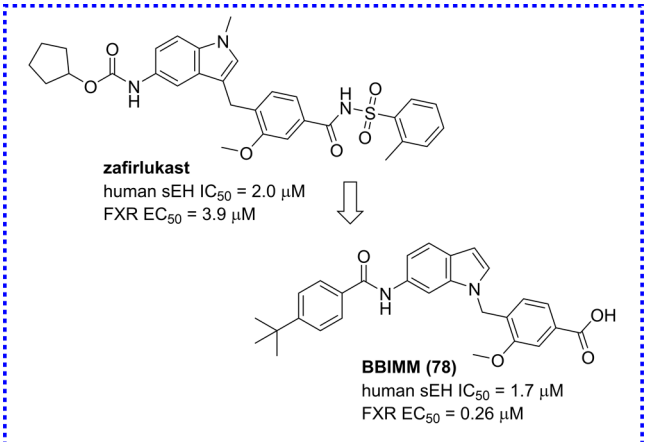


Figure 26. sEH/FXR dual modulator BBIMM (**78**) derived from zafirlukast.

Separately, on the basis of the structure of a sEH inhibitor (GSK2188931B, Figure 27) and a FXR agonist, Schmidt et al. extracted their similar structural characteristics and combined them in the dual pharmacophore *N*-benzylamide. This finding led to the production of a sEH/FXR dual modulator BCMBB (**79**).[135] Extensive *in vitro* characterization confirmed its potency and favorable PK (Figure 27).[134]

2.2.7. sEH/c-RAF Dual Inhibitors. RAF is a proto-oncogene serine/threonine-protein kinase (c-RAF or RAF1)[136] and is the target for antitumor drugs because its inhibition or knockout regulates the RAS-RAF-MEK-ERK pathway to suppress lung and ovarian tumors growth.[137] Sorafenib (**80**),

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

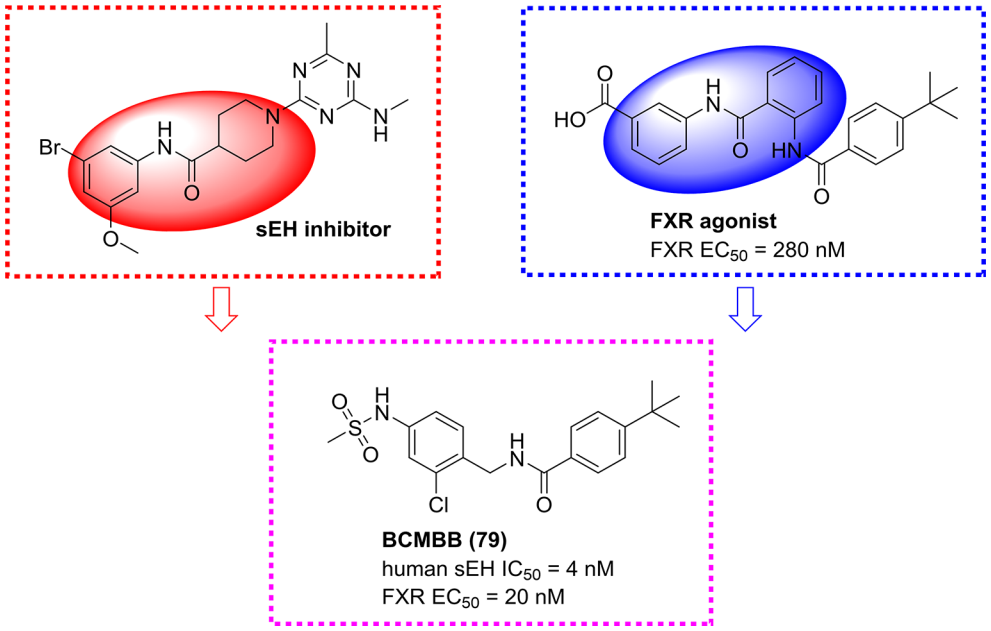


Figure 27. sEH/FXR dual modulator BCMBB (**79**).

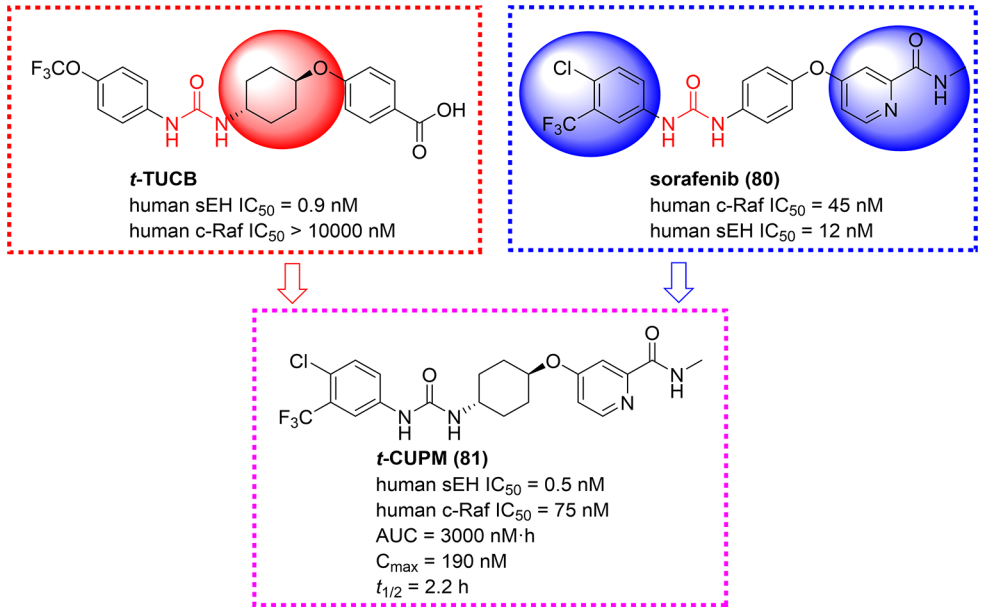


Figure 28. sEH/c-RAF dual modulator *t*-CUPM (**81**) derived from *t*-TUCB and sorafenib (**80**).

the first FDA-approved inhibitor of c-RAF,[138] possesses a similar urea pharmacophore as a selective sEH inhibitor *t*-TUCB, and it was found to exhibit potent inhibitory activity toward sEH as well (Figure 28).[139] Structural optimization leads to the design and synthesis of a sEH/c-RAF dual inhibitor *t*-CUPM (**81**, Figure 28).[140] Compared to sorafenib (**80**), *t*-CUPM (**81**) is 25-fold more potent toward sEH while displaying similar potency toward c-RAF, while having satisfying PK properties. The subsequent *in vivo* study in LSL-KrasG^{12}D/Pdx-1-Cre mice indicated that *t*-CUPM (**81**) significantly alleviated chronic pancreatitis by suppressing mutant Kras-transmitted phosphorylations of RAF/MEK/ERK.[140]

2.3. Natural (or Naturally Occurring) Products as sEH Inhibitors. Natural products play an important role in drug discovery, and about 50% of drugs in the clinic are natural products, such as artemisinin, paclitaxel (Taxol), andrographolide, and penicillin.[141,142] Accordingly, numerous compounds from natural resources also display inhibition of sEH, including natural ureas, triterpenoids, flavonoids, and phenylpropionic acids.[143−146] Interestingly, numerous compounds reported as sEH inhibitors have a weak potency with IC_{50} or K_i in the micromolar range, thus limiting their usefulness for eventually treating patients.

2.3.1. Natural Ureas and Amides. So far, as described above, ureas and amides have been most widely studied as sEH inhibitors. To date, natural urea-containing compounds also are discovered from the genera *Pentadiplandra*, *Salvadora*, *Lepidium*, and *Moringa* (Figure 29),[145,147−151] whereas only five ureas (**82**−**86**), isolated from *P. brazzeana* and *L. meyenii*, were screened for their sEH inhibitory activities and possess potent human and rat sEH inhibitory activities (Figure 29).[152]

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

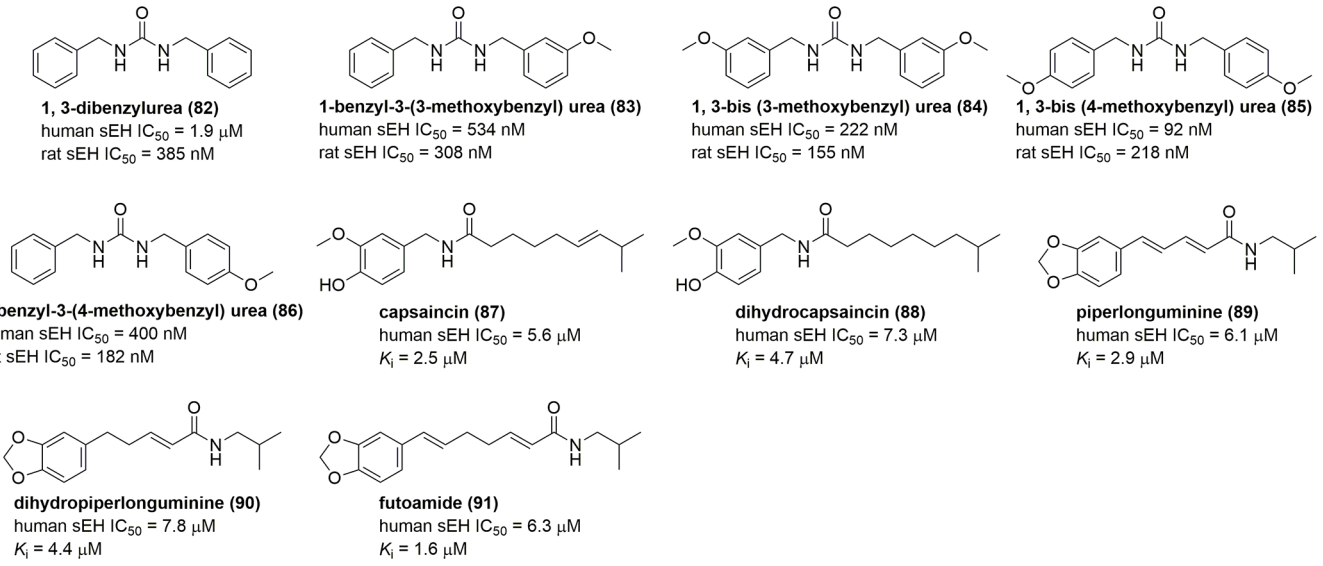


Figure 29. Chemical structures of naturally occurring sEH inhibitors: ureas (**82**–**86**) and amides (**87**–**91**).

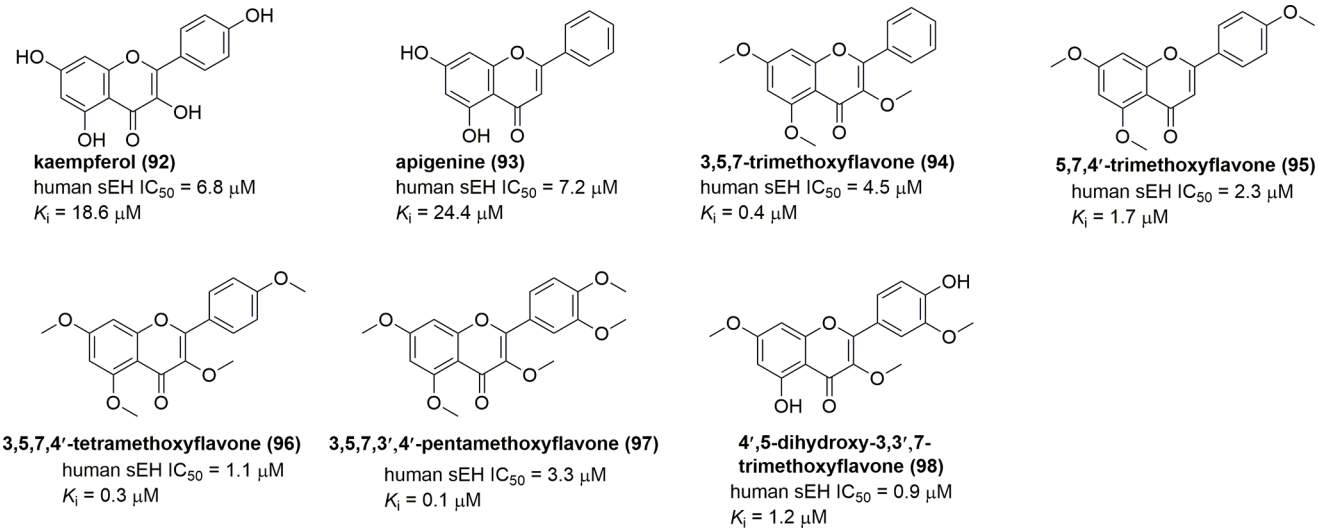


Figure 30. Chemical structures of naturally occurring sEH inhibitors: flavonoids (**92**–**98**).

These are the only reported natural compounds that inhibit sEH with IC_{50} in the nanomolar range.

Capsaicin (**87**) and dihyrocapsainin (**88**), isolated from *Capsicum chinense*, are competitive-type inhibitors with K_i values of 7.3 and 4.7 μM, respectively.[153] In addition, three amide derivatives piperlonguminine (**89**), piperlonguminine (**90**), and futoamide (**91**) from the traditional Chinese medicine *Scutellaria baicalensis*, are weak sEH inhibitors.[144]

2.3.2. Flavonoids. Some of flavonoids, isolated and identified from the genera *Scutellaria*, *Tetrastigma*, *Epimedium*, *Apios*, *Kaempferia*, *Boscia*, and so on, exhibited sEH inhibitory potencies (Figure 30).[144,145,154−158]

The investigation on *T. hemsleyanum* led to the isolation of kaempferol (**92**) and apigenine (**93**), and they were defined as noncompetitive-type inhibitors with K_i values of 18.6 and 24.4 μM, respectively.[145] In addition, 3,5,7-trimethoxyflavone (**94**), 5,7,4′-trimethoxyflavone (**95**), 3,5,7,4′-tetramethoxyflavone (**96**), 3,5,7,3′,4′-pentamethoxyflavone (**97**), and 4′,5-dihydroxy-3,3′,7-trimethoxyflavone (**98**), isolated from *K. parviflora*, displayed potent inhibitory potentials with K_i values ranging from 0.1 μM to 1.7 μM.[155]

2.3.3. Triterpenoids. Thao and co-workers studied the constituents of *Cimicifuga dahurica* to afford cycloartuane-type triterpenoids (**99**–**107**, Figure 31).[159,160] It was found that compounds **99**–**107** were mixed-type inhibitors and their IC_{50} values were 0.4–4.8 μM. Protostane-type triterpenoids are characteristic constituents of the genus *Alimsa*, and 25 of them were assayed for their inhibitory effects against sEH.[161] Among them, 11-deoxy-25-anhydroalisol E (**108**, IC_{50} = 3.4 μM) and 11-deoxyalisol B (**109**, IC_{50} = 5.9 μM) significantly suppressed sEH activity. As mixed-type inhibitors, 11-deoxy-25-anhydroalisol E (**108**, K_i = 12.6 μM) and 11-deoxyalisol B (**109**, K_i = 3.5 μM) are hypothesized to form hydrogen bonds with amino acid residues Asp335, Trp336, Tyr383, and Tyr466, respectively.

2.3.4. Phenylpropionic Acids. Six phenylpropionic acids from *T. hemsleyanum*, such as (1α,3*R*,4α,5*R*)-4-*O*-(*E*)-caffeoylquinic acid methyl ester (**110**) and caffeic acid (**111**, Figure 32), showed weak inhibitory activities toward sEH.[145]

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

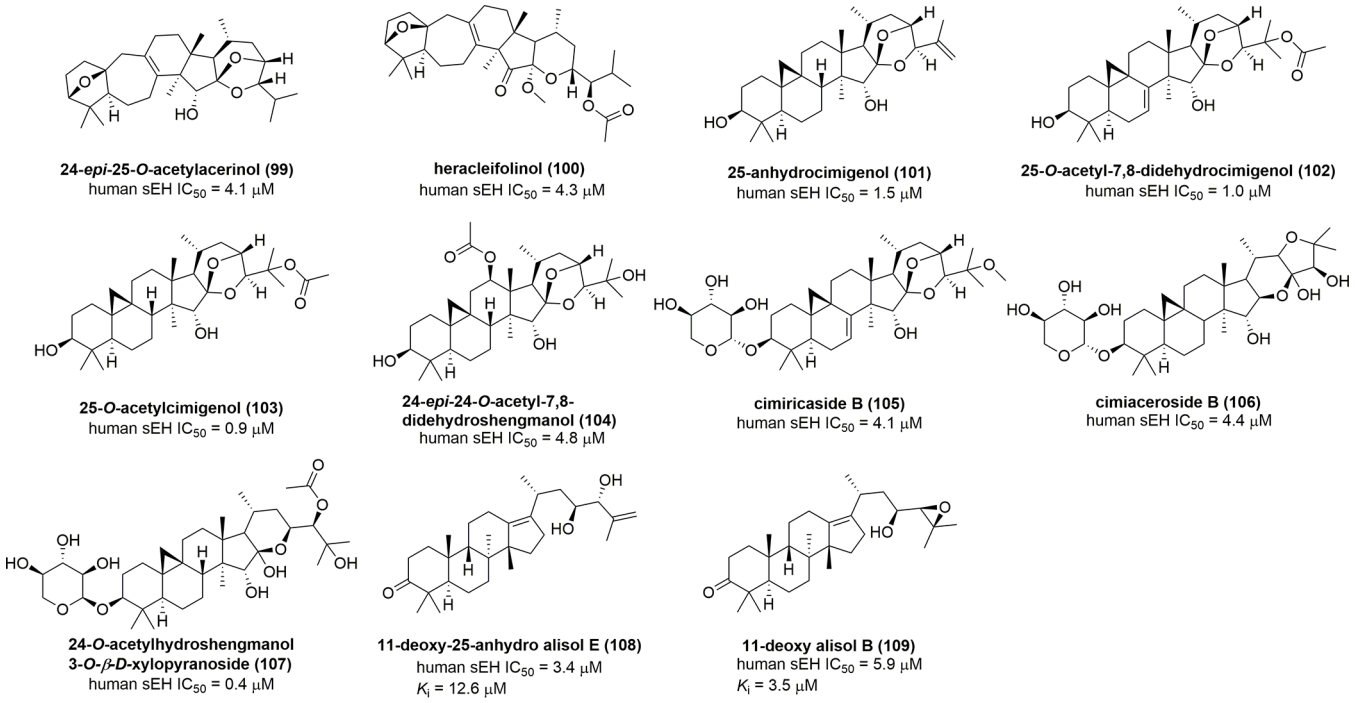


Figure 31. Chemical structures of naturally occurring sEH inhibitors: triterpenoids (**99**−**109**).

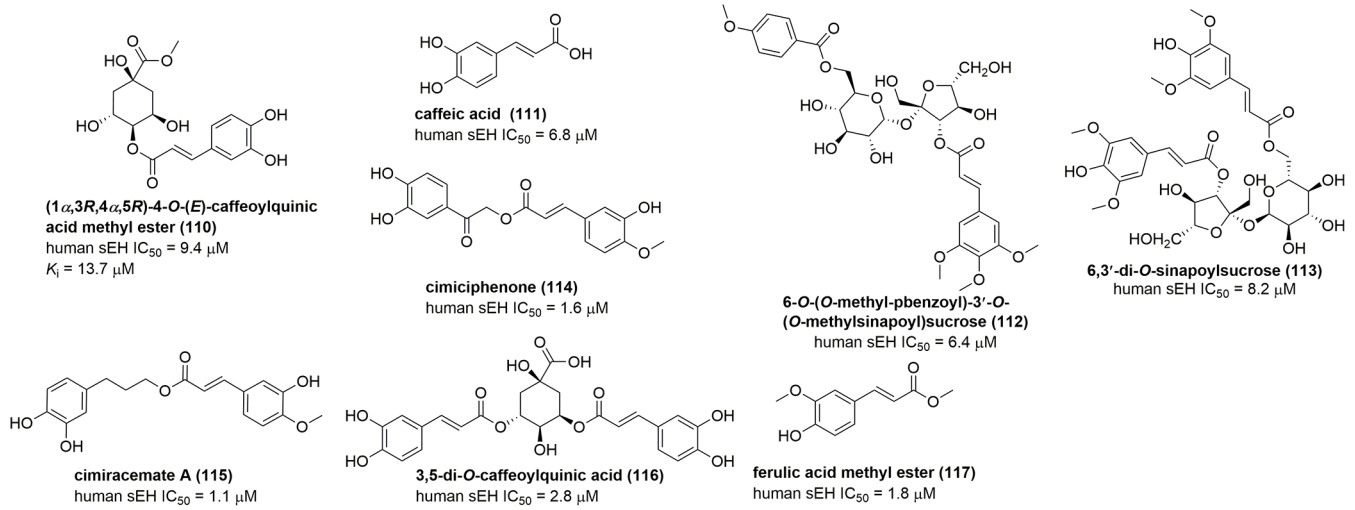


Figure 32. Chemical structures of naturally occurring sEH inhibitors: phenylpropionic acids (**110**−**117**).

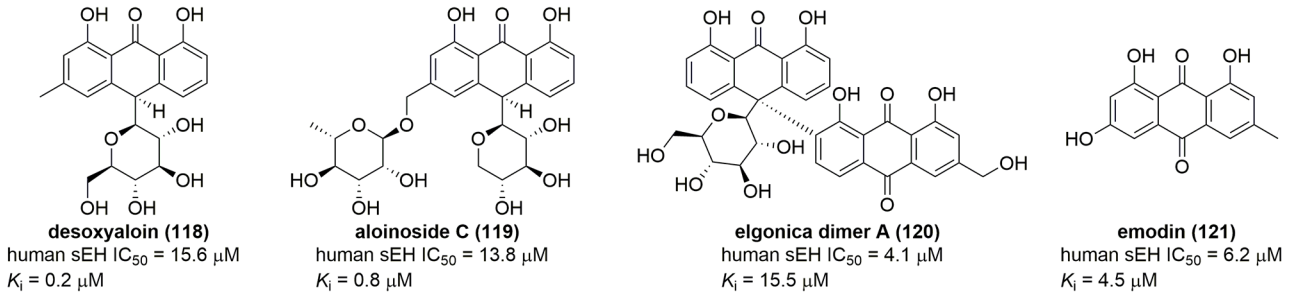


Figure 33. Chemical structures of naturally occurring sEH inhibitors: anthraquinones (**118**−**121**).

In order to discover more potent sEH inhibitors from natural resources, the subsequent investigation on *Polygala tenuifolia* led to the isolation of nine analogs, such as 6-*O*-(*O*-methylpbenzoyl)-3′-*O*-(*O*-methylsinapoyl)sucrose (**112**), and 6,3′-di-*O*-sinapoylsucrose (**113**).[146] Kim and co-workers investigated the constituents of *C. dahurica* and *Gentiana scabra* to obtain

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

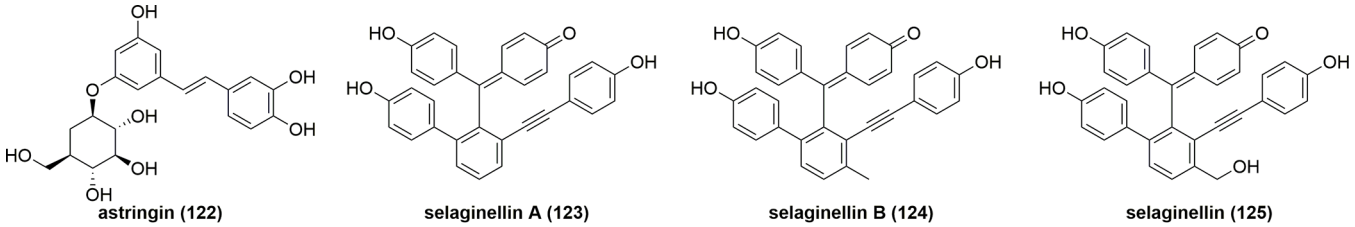


Figure 34. Chemical structures of naturally occurring sEH inhibitors: stilbenes (**122**−**125**).

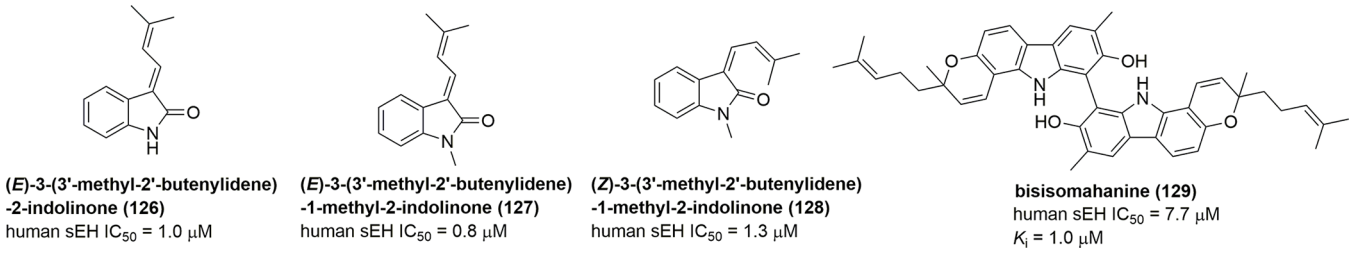


Figure 35. Chemical structures of naturally occurring sEH inhibitors: alkaloids (**126**−**129**).

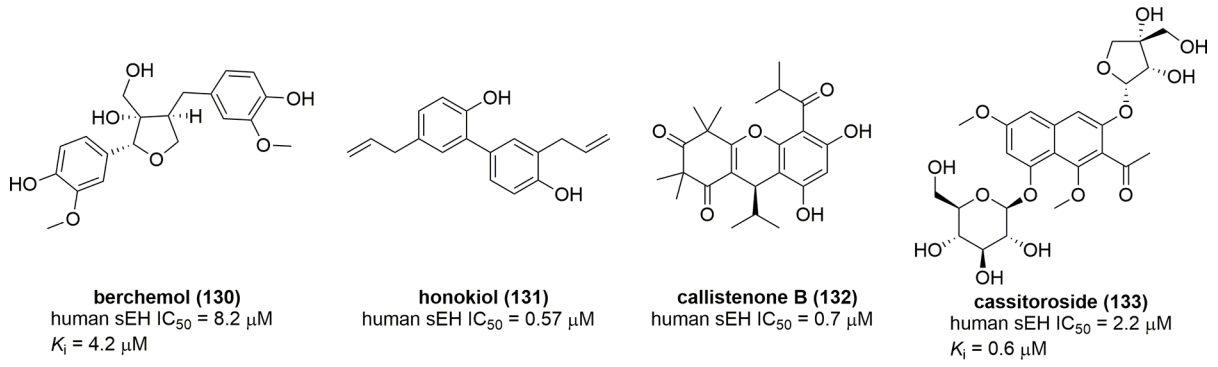


Figure 36. Chemical structures of naturally occurring sEH inhibitors: others (**130**−**133**).

cimiciphenone (**114**), cimiracemate A (**115**), 3,5-di-*O*-caffeoylquinic acid (**116**), and ferulic acid methyl ester (**117**).[162,163] Compounds **114**−**117** could significantly inhibit sEH activity (Figure 32).

2.3.5. Anthraquinones. *Aloe* is a short-stemmed succulent herb mainly used in traditional medicine to treat various diseases. Anthraquinones are considered as the major active constituents of the genus *Aloe* (Figure 33). Sun et al. obtained three anthraquinones, desoxyaloin (**118**), aloinoside C (**119**), and elgonica dimer A (**120**), from *Aloe*.[164] Among them, desoxyaloin (**118**) showed the most potent inhibitory activity against sEH with a mixed-type inhibitor behavior. Molecular docking suggested that desoxyaloin (**118**) could bond to sEH with hydrogen bond interactions with Tyr343 and Pro361. Besides, emodin (**121**), an anthraquinone derivative isolated from *P. multiflorum*, also displayed inhibitory potency against sEH.[165]

2.3.6. Stilbenes. The investigation on *Rheum undulatum* resulted in the isolation of astringin (**122**, Figure 34), which was found to inhibit sEH.[166] Molecular docking suggests seven hydrogen bonds between astringin (**122**) and Ser415, Leu417, Met419, Tyr466, Lys495, and His524 residues of sEH. In addition, three noncompetitive inhibitors of sEH, selaginellin A (**123**), selaginellin B (**124**), and selaginellin (**125**) were isolated from *Selaginella tamariscina*.[164] *In silico* investigation suggests that they bonded to a complex of sEH and substrate via hydrogen bond interactions.

2.3.7. Alkaloids. Investigation of the phytochemical constituents of traditional Chinese medicines *C. dahurica* and *Glycosmis stenocarpa* resulted in the isolation and identification of four alkaloids (**126**−**129**) (Figure 35) that inhibit sEH.[160,167]

2.3.8. Others. Besides all of the above-mentioned compounds, some other type of compounds isolated from *G. scabra*, *Magnolia officinalis*, *Callistemon citrinus*, and *Cassia tora*[163,168,169] also displayed some inhibition on sEH, such as berchemol (**130**), honokiol (**131**), callistenone B (**132**), and cassitoroside (**133**) (Figure 36).

2.4. Selectivity of sEH Inhibitors. High selectivity of pharmacological inhibitors as an important factor limits off-target nondesirable side effects in the clinic. Here, we are mostly concentrating on the urea-based sEH inhibitors. Because urea function is also the central pharmacophore for compounds that inhibit proteases and kinases[170] and bind to canabinoid receptors 1,[171] it is possible that inhibitors for sEH could also alter the function of these other proteins. It is interesting that the Raf-1 kinase inhibitor sorafenib (**80**), used to treat some cancer, was found to inhibit sEH also.[139] A commonly used sEH inhibitor, TPPU (**19**), was recently reported to also inhibit significantly the p38 kinase, influencing

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

its action in an AD model.[172] The selectivity of a particular compound is dependent not only on the central pharmacophore but also on the chemical groups present on both sides of the urea. As these groups are becoming more complex and establishing additional bonds with sEH, the likelihood of a compound to bind to another protein decreases. However, any compound developed as a pharmacological sEH inhibitor should be tested for it selectivity especially for its ability to inhibit or not proteases and kinases.[173]

3. BIOLOGICAL POTENTIALS FOR sEH INHIBITION

EpFAs play a crucial role in biological functions, such as reducing inflammation and pain, dilating blood vessels, and protecting neurons. A growing body of studies indicated that many cardiovascular, CNS, and metabolic diseases, such as hypertension, arteriosclerosis, AD, PD, NAFLD, and diabetes, are related to the level of EpFAs in the human body and the expression of sEH.[32,174−176] Moreover, chemical inhibition and genetic knockout sEH, which yields a steady state of EpFAs level in the body, have beneficial effects on the physiological environment and greatly influence the above-mentioned diseases.[177]

3.1. Cardiovascular Diseases. The abnormal function of vascular endothelial cells plays a crucial role in the occurrence and development of cardiovascular diseases. The prevention of endothelial cell dysfunction is one of the key problems related to cardiovascular diseases to solve. EETs synthesized in endothelial cells act as an autocrine and paracrine mediator of cardiovascular system, which has vasodilation and anti-inflammatory effects.[174] Therefore, sEH is considered in the treatment of cardiovascular diseases via improving the bioavailability of endothelium-derived hyperpolarization and relaxing factor EETs.[32]

Hypertension is caused by a complex interaction of genes and several environmental factors and is also one of the important causes of heart remodeling. Hypertension not only reduces the quality of life but also increases the risk of other cardiovascular diseases.[178] Extensive research has verified that endogenic EETs contribute to vasodilation in various organs, such as brain, kidney, intestine, and heart,[45,179,180] especially 14,15-EET. They activate BK_{Ca} in vascular smooth muscle cells to promote K^+ efflux and membrane hyperpolarization via modulation of cAMP-mediated protein kinase A and ADP ribosylation of the Gαs pathway, leading to the relaxation of blood vessels.[40,181−185] However, DHETs, sEH-mediated hydrolysates of EETs, exhibit a weak relaxant effect,[184] which suggests that inhibition of sEH to stabilize levels of EETs is a therapeutic strategy for hypertension.[179] The present series of studies provide evidence that the expression level of sEH is increased in hypertensive rats.[61,186] For example, the increasing of sEH expression was observed in the kidney of spontaneously hypertensive rats and Ang II-induced hypertension rodent model.[187,188] The similar result was also found in aortic specimens from saline-fed and Ang II-infused rats,[189,190] demonstrating the presence of the relationship between sEH and hypertension. The *Ephx2* abolishment significantly decreases systolic blood pressure and enhances the ratio of EETs/DHETs in a saline-fed-induced hypertensive model.[191] Moreover, a great increase of evidence demonstrated that administration of sEH inhibitors, such as AUDA (**4**), TPPU (**19**), and *c*-AUCB (**9**), lowered blood pressure in the hypertensive mice model.[67,192,193] An orally administered sEH inhibitor AR9281 (APAU, **6**) developed by Arête Therapeutics Inc. finished the phase I and phase II clinical trials. AR9281 is safe and well tolerated for healthy volunteers, even administration with a high dose (single oral dose, 1000 mg; multiple dose, 400 mg every 8 h for 7 days) in the phase I clinical trial, whereas it does not display thrilling efficacy in early-stage hypertension during the phase II clinical trial.[194]

Atherosclerotic diseases are the major causes of mortality worldwide. Atherosclerosis is a progressive disease characterized by the formation of foam cells, dyslipidemia, and accumulation of lipid plaques in the arterial wall.[195] Extensive epidemiological studies clearly revealed the correlation of the plasma cholesterol profile with atherosclerosis. In the course of atherosclerosis, low-density lipoprotein (LDL), especially oxidized LDL, activates inflammation and foam cell formation, but high-density lipoprotein (HDL) has a protective effect on atherosclerosis. sEH inhibitors reduced atherosclerotic lesion formation in the deficient apolipoprotein E ($ApoE^{-/-}$) or LDL receptor ($Ldlr^{-/-}$) mice; these mice exhibited advanced lesions and increases in the LDL level.[196] In $ApoE^{-/-}$ mice, administration of a sEH inhibitor significantly decreased serum LDL concentrations and secretion of inflammatory factors.[196] In order to reveal the protective mechanism for inhibition of sEH, a study by Xu et al. reported that administration of *t*-AUCB (**8**) could enhance CD36-mediated recognition and interpretation of oxidized-LDL through the activation of PPARγ and further improve cholesterol outflow by increasing the ATP cassette A1 expression level, which reduced the formation of atherosclerotic lesions in $Ldlr^{-/-}$mice.[197] Similarly, Shen et al. found that *t*-AUCB (**8**) treatment alleviated atherosclerosis symptoms in $Ldlr^{-/-}$ mice as well via modulation of the expression of ATP binding cassette transporter A1, cholesterol efflux, and plasma HDL levels.[198] In addition, inhibition of sEH with *t*-TUCB delayed the formation of hyperlipidemia and atherosclerosis in $Ldlr^{-/-}$ mice, and *Ephx2* genetic abolishment significantly alleviated the symptoms of atherosclerosis as well.[195]

Heart failure is caused by blocking blood flow to the heart, resulting in coronary heart disease, myocardial infarction, arrhythmia, viral myocarditis, and genetic cardiomyopathies.[199] A study by Seubert et al. has demonstrated the increasing of sEH activity in animal models of myocardial infarction, and the increasing of sEH expression was observed in the human hearts of patients with ischemic cardiomyopathy. Moreover, increasing EET levels protected against myocardial infarction and left ventricular dysfunction after ischemic injury in $Ephx2^{-/-}$ mice.[200] Inhibition of sEH with GSK2188931B and TUPS both protected heart failure.[201,202] For example, TUPS treatment reduced the thickness of the heart wall, down-regulated the expression of hypertrophy markers atrial and brain natriuretic peptides, and regulated negative autophagy via activation of mammalian target of rapamycin (mTOR) signaling, allowing prevention of myocardial hypertrophy induced by isoproterenol.[202] These data all suggested the therapeutic potential of sEH in heart failure.

Preclinical results have demonstrated that inhibition of sEH by inhibitors or *Ephx2* genetic knockout possesses beneficial effects, such as vasodilation, antihypertension, and antiatherosclerotic effects. Although inhibition of sEH to treat human diseases still remains to be demonstrated in clinical trials, the increasing of positive effects of sEH inhibitors in cardiovascular diseases strongly supports the clinical role of sEH in cardiovascular diseases.

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

3.2. CNS Diseases. EETs play an important role in CNS diseases. They can regulate angiogenesis and cerebral blood flow (CBF) and affect signal transduction process. Because of the abundant expression of sEH in various parts of the brain, the neuroprotective effect of sEH inhibitors has become an important research direction in the treatment of CNS diseases. In recent years, sEH inhibitors have been used in the treatment of CNS diseases, such as AD, PD, and depression with satisfactory performance.[175,203−205]

PD, one of the most common neurodegenerative diseases, leads to progressive motor deficits and nonmotor symptoms, including speech, motor, and mental disorders.[203,204] In the course of onset, dopaminergic neurons in substantia nigra (SN) prematurely die and Lewy bodies accumulate in the brain.[71] The current treatment works by increasing dopamine concentration and/or stimulating its receptors, but this treatment only targets basal ganglia and dopamine but does not involve more neurons associated with PD. Therefore, this therapy is generally not very effective. In human and animal PD models, the expression level of sEH in brain regions is associated with dopaminergic death, endoplasmic reticulum (ER) stress, and tyrosine hydroxylase positive cell death, which can be alleviated by administration of AUDA (**4**), TPPU (**19**), or 14,15-EET.[206,207] EETs stimulate astrocytes to secrete nerve growth factors (NGFs), including vascular endothelial growth factor and brain-derived neurotrophic factor (BDNF), which promotes the growth and differentiation of nerve cells and protects neurons.[177] Administration of AUDA (**4**) reduced MPTP-mediated loss of TH positive cells in SN.[207] Furthermore, the *Ephx2* abolishment protected against MPTP-induced neurotoxicity in STR, while overexpression of sEH in STR significantly enhanced MPTP-induced neurotoxicity.[206] Moreover, the expression of the sEH protein in STR from MPTP-treated mice was significantly higher than the control group. Interestingly, there was a positive correlation between sEH expression and the phosphorylation of an α-synuclein in STR, suggesting that sEH may play a role in the phosphorylation of α-synuclein in STR. In model animals, administration of TPPU (**19**) increased dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid levels and inhibited the apoptosis of Parkinson disease protein 2 (PARK2) neurons. Accordingly, it is likely that sEH can act as a potential target for the treatment of PD.

AD is a neurodegenerative disease characterized by impaired learning, memory, and cognitive abilities. Neuroinflammation is a critical factor causing AD and plays a crucial role in the development course of AD;[208−210] therefore, 16% of currently ongoing clinical trials for the treatment of AD is correlated with inflammation.[211] Neuroinflammation is intimately linked to the oxidative stress associated with AD[212,213] and controls the interactions between the immune system and the nervous system.[214] The senescence-accelerated mouse prone 8 (SAMP8) is a paradigm of late-onset AD and cognitive impairment in age, causing oxidative stress, neuroinflammation, tau hyperphosphorylation, and proamilodogenic APP processing.[215−219] Griñán-Ferré et al. found the increasing of sEH expression in the hippocampus of SAMP8 mice, and inhibition of sEH with TPPU (**19**), AS2586114, or UB-EV52 could reduce biomarkers of inflammation, oxidative stress, and ER stress.[220] Similarly, these positive results were also observed in a 5×FAD mouse model of early onset AD after administration of sEH inhibitors.[220] A recent study has revealed that the sEH level was increased in the brain and predominantly appeared in hippocampal astrocytes of *APP/PS1 Tg* mice.[221] Genetic deletion of sEH alleviates behavior outcomes and amyloid β (Aβ) accumulation, increases astrogliosis and the production of anti-inflammatory cytokines IL-4 and IL-10, and inhibits NF-κB signaling pathway, resulting in a protective effect against AD.[175] Additionally, compared with a healthy human, the analysis of clinical specimens demonstrated that sEH was overexpressed in Braak III and V phases of AD patients' brains,[220] which confirmed the correlation of sEH with AD.

Depression and schizophrenia both have high mortality rates and involve a wide range of severe chronic mental illness.[205] The proinflammatory cytokines in serum and cerebrospinal fluid are increased, and the sEH level is significantly increased in the parietal cortex of the brains of deceased patients suffering from these diseases.[222] It strongly suggests that sEH is likely involved in the pathophysiological process of depression and schizophrenia. In LPS-treated depressed mice, TPPU (**19**), a sEH inhibitor, can significantly improve depressive symptoms by reducing the level of TNF-α, upregulating the expression of BDNF in mouse hippocampus and PC12 cells and enhancing NGF-induced neuronal growth in PC12 cells. Moreover, sEH knockout ameliorates symptoms of depression as well.[223] Chemical inhibition on sEH by AS2586114 decreases levels of 11,12-DHET and 14,15-DHET and improves hyperactivity and prepulse inhibition defects in phencyclidin-induced schizophrenia.[224] Thus, the inhibition of sEH appears to exert antidepressant and antischizophrenic effects by increasing the concentration of EETs, regulating the BDNF-tropomyosin receptor kinase B (BDNF-TrkB) signaling pathway, and preventing oxidative stress.[222]

Additionally, sEH is also involved in epilepsy and stroke. Administration of sEH inhibitors could prevent neuroinflammation and reduce the number and duration of seizures of epilepsy.[55,225] After treatment with AUDA (**4**), the expression of inflammatory factors decreased and the EETs/DHETs ratio increased, while at the same time, the epileptic induction threshold was increased and the epileptic sensitivity was decreased. There is a correlation between the mutation of R287Q of sEH, which led to a decrease of sEH activity and a reduced risk of stroke.[30] Chemical inhibition can reduce astrocyte infiltration, glial scar formation, microglial activation, neuronal death, insufficient blood flow, and behavioral disorder after cerebral ischemia.[226] Put together, sEH has a clear role in CNS health and diseases and represents a potential target for treatment.

3.3. Metabolic Diseases. Besides cardiovascular and CNS diseases, sEH is closely related to metabolic diseases, including NAFLD, diabetes, and obesity. Many studies have shown overexpression of sEH in many liver diseases, such as NAFLD, nonalcoholic steatohepatitis (NASH),[227] and hepatic fibrosis.[227−231] NAFLD is caused by excessive fat accumulation in hepatocytes, affecting approximately 25% of the global adult population. Some of preclinical results verified that sEH inhibition was to the benefit of NAFLD and NASH. Iyer et al. investigated the role of sEH inhibition in a high-carbohydrate- and high-fat-diet (HCHFD)-induced rat model.[33] The inhibition of sEH with *t*-AUCB (**8**) alleviated HCHF-induced liver hypertrophy and steatosis, had significant improvements in plasma lipid levels and insulin sensitivity, and suppressed the increasing of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels.[33] Similarly, chemical inhibition of sEH or *Ephx2* genetic abolishment has a protective role in

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

high-fat-diet (HFD)-induced NAFLD.[232] *t*-AUCB (**8**) could reduce HFD-induced macrophage accumulation and down-regulate mRNA expression levels of proinflammatory cytokines TNF-α, IL-6, MCP-1, and IFN, leading to the improvement of HFD-induced inflammation via the activation of MAPK pathway (JNK and p38).[232] However, overexpression of sEH by the injection of adenoviruses encoding human sEH resulted in the aggravation of metabolic syndromes, such as the increasing of triglyceride and proinflammatory cytokine and the upregulation of JNK and p38.[232] Conversely, the *Ephx2* deletion alleviated hepatic steatosis and tissue inflammatory, described as administration of *t*-AUCB (**8**), which suggested the role of sEH-mediated hepatic steatosis. The role of sEH in NAFLD also was supported in high-methionine-diet-induced hyperhomocysteinemia and hepatic steatosis.[233] Accumulating evidence revealed that hyperhomocysteinemia disturbs lipid metabolism in the liver and is served as a significant risk factor. A study by Yao et al. demonstrated that sEH inhibition by TPPU (**19**) ameliorated hyperhomocysteinemia-induced lipid accumulation, and further mechanism investigation suggested that the increase of EETs through the inhibition of sEH by TPPU (**19**), especially 11,12-EET, elevated the expression levels of PPARα target genes and activated PPARα.[233] These findings suggested that the increase of sEH activity was a critical threat in hyperhomocysteinemia-induced hepatic steatosis, and sEH inhibition might be regarded as an effective means to treat hyperhomocysteinemia-induced hepatic steatosis.

Inflammasome is responsible for the modulation of the adaptive response of the liver to pathogenic challenge, and a library of evidence demonstrated that sEH inhibition could suppress inflammasome activation to reduce hepatic inflammation.[230] Administration of PTUPB (**68**), a sEH/COX-2 dual inhibitor, could block fibrotic progression by suppressing collagen deposition and down-modulating Col1a1, Col1a11a3, and α-SMA expression levels. The dual inhibition of sEH and COX-2 also suppressed the NLRP3 inflammasome activation through the decrease of Nlrp3/NLRP3 and Asc expression and reduced the downstream target protein of NLRP3, procaspase 1, pro-IL-1β, and pro-IL-18. Besides, PTUPB (**68**) treatment decreased the release of proinflammatory cytokines IL-6 and MCP-1.[230] Accordingly, it was worth noting that the role of sEH in inflammasome activation still needs to be further studied.

Diabetes is mainly due to the lack of insulin produced by pancreatic β-cells or the insensitivity of the target tissue to insulin, leading to the severe injuries to organs.[234] A growing body of evidence has demonstrated that the stabilization of EETs, inhibition of sEH by sEH inhibitors, or *Ephx2* genetic deletion plays a crucial role in diabetes. A study by Schaefer et al. verified that EETs enhanced insulin signaling through the regulation of Akt expression level rather than affecting levels of insulin receptor.[72] Furthermore, the insulin release was also promoted by 5,6-EET, while 8,9-EET, 11,12-EET, and 14,15-EET might promote the release of glucagon and increase the level of blood glucose, which suggested that increasing the concentration of EETs through inhibition of sEH might be a potential pathway to treat diabetes.[32] In streptozotocin (STZ)-induced diabetic mice, administration of *t*-AUCB (**8**) attenuated hyperglycemia and had protective effects against β-cell damage.[235] An investigation by Pardeshi et al. has been reported that TPPU (**19**) reduced the level of fasting blood glucose, improved the cognitive impairment and memory impairment by stabilizing EETs to dilate arterioles, and increased cerebral blood perfusion in STZ-induced diabetes.[236] A similar result was also observed after administration of *t*-AUCB (**8**).[237] In addition, inhibition of sEH or *Ephx2* knockout has been found to alleviate symptoms of hyperglycemia and insulin resistance in HFD-induced type 2 diabetes,[238] which might activate insulin signaling in the liver and adipose tissue.

Diabetes also causes many complications, such as retinopathy, nephropathy, and cardiovascular diseases, and seriously affects a patient's quality of life. A great number of studies have verified that inhibition of sEH by inhibitors or genetic knockout sEH is beneficial to diabetic complications.[228,239] Diabetic retinopathy is one of the diabetic complications caused by progressive loss of vascular cells and slow dissolution of vascular junctions.[240] The expression of sEH is increased in human retina and vitreous of diabetic retinopathy. Furthermore, overexpression of sEH in retinal Müller glial cells can result in vascular abnormalities in diabetic retinopathy.[239] Therefore, the overexpression of sEH is a key factor in the pathogenesis of diabetic retinopathy. Jouihan et al. found that STZ treatment promoted sEH mRNA expression and reduced EET level in cerebral vessels.[228] Furthermore, brain infarction was exacerbated in STZ-treated mice after middle cerebral artery occlusion, which was reversed through the inhibition of sEH by *t*-AUCB (**8**). Moreover, sEH is related to diabetic cardiomyopathy since sEH is highly expressed in the heart tissue of type 1 diabetes mice.[231] In addition, sEH genetic disruption reduced levels of blood urea nitrogen and creatinine as well as via suppressed renal tubular apoptosis and enhanced renal endothelial function, allowing the alleviation of diabetic nephropathy.[229,230]

So far, a selective sEH inhibitor GSK2256294 (**67**) has finished its phase I clinical trial, whose clinical experimental data demonstrated its safety and sustained inhibitory effect against sEH.[173] These results supported further clinical trials in patients with endothelial dysfunction, such as diabetes. In 2018, Vanderbilt University Medical Center sponsored the phase II clinical trial of GSK2256294 (**67**) to treat diabetes, endocrine system diseases, glucose metabolism disorders, and obesity and started volunteer recruitment.

Obesity is a global public health problem and a major risk factor for diseases, the same as cancer and diabetes. In metabolic disorders, chemical inhibition of sEH or *Ephx2* deletion is capable of lessening body weight gain, lowering blood pressure, and suppressing the production of inflammatory cytokines.[78,241,242] Moreover, the increasing of sEH expression has been found in adipose tissue of obese mice,[78,241,242] revealing the potential correlation of sEH with obese. A growing body of evidence indicates that ER is a known cellular consequence of metabolic diseases, such as obesity and NAFLD.[243] Importantly, sEH deficiency or inhibition in mice attenuated HFD-induced ER stress in liver and adipose tissue. Similarly, the inhibition of sEH in 3T3-L1 preadipocytes mitigated chemical-induced ER stress and activation of JNK, p38, and cell death.[54] A study by Lopez-Vicario et al. has reported that *t*-TUCB could stabilize the levels of EpFAs, 17,18-epoxyeicosatetraenoic acid (17,18-EEQ), and 19,20-epoxydocosapentaenoic (19,20-EDP) derived from ω-3.[244] Moreover, *t*-TUCB promoted the polarization of macrophage to anti-inflammatory M2-type cells, induced hepatic autophagy by increasing Atg12-Atg5 and LC3-II expression levels, and reduced p62 expression and ER stress

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

by suppressing p-IRE-1α and p-eIF2α expression levels.[244] The inhibition of sEH to exert a protective effect was also confirmed in palmitate-primed hepatocytes and adipocytes through the incubation of 19,20-EDP or 17,18-EEQ,[244] which suggested that sEH was a physiological modulator of ER stress and a potential target for the treatment of obesity. In short, the inhibition on sEH blocks the progression of obesity and is of great significance for obesity and related diseases.

4. CONCLUSION AND PERSPECTIVE

In this review, we described a complete picture of chemically synthesized or naturally obtained sEH inhibitors. Among these inhibitors, urea-based compounds are the most prominent because they can accommodate well the active pocket of sEH and form stable hydrogen bonds and have in general a good selectivity for sEH. Besides potency, the main drivers for improvement have been to increase water solubility and other physical properties as well as better bioavailability and PK parameters. Toward solving these problems, amides are good alternatives to ureas for sEH inhibition. In addition, the introduction of secondary polar groups and tertiary pharmacophore into the structure of the inhibitors would greatly help with the design of a "better" inhibitor. Nowadays, nature is providing more and more structural skeletons on which to build potent sEH inhibitors besides ureas and amides, such as flavonoids and triterpenoids. These compounds have their advantages and disadvantages, which provide more space for the development and optimization of future sEH inhibitors.

Currently, sEH is a potential therapeutic target in multiple diseases related to inflammation, such as cardiovascular (hypertension and arteriosclerosis), CNS (AD, PD, and depression), and metabolic (diabetes, NAFLD, NASH, and obesity) diseases. Moreover, some sEH inhibitors, such as AR9281, EC5026, and GSK2256294, are underway at clinical trials to treat heart failure, insulin resistance, glucose intolerance, hypertension, endothelial dysfunction, and pain, while future research in some key areas is needed to further elucidate its applications and action mechanisms. Compared with the function of C-terminal hydrolase, the role of the N-terminal phosphatase domain is still undetermined. Although Kramer et al. have first discovered *in vivo* active inhibitors of sEH phosphatase with favorable PK, its N-terminal physiological function needs to be further in-depth investigated. Future studies should consider that inhibition of sEH C-terminal activity remains its phosphatase activity, discovering any differences in phenotype between sEH hydrolase inhibitor-treated and *Ephx2* deficiency animals.

AUTHOR INFORMATION

Corresponding Authors

Bruce D. Hammock − *Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis, California 95616, United States;* orcid.org/0000-0003-1408-8317; Phone: +1 530 752-7519; Email: bdhammock@ucdavis.edu

Xiao-Chi Ma − *Dalian Key Laboratory of Metabolic Target Characterization and Traditional Chinese Medicine Intervention, College (Institute) of Integrative Medicine, College of Pharmacy, Dalian Medical University, Dalian 116044, People's Republic of China; College of Pharmacy, School of Medicine, Hangzhou Normal University, Hangzhou 311121, People's Republic of China;* orcid.org/0000-0003-4397-537X; Phone: +86-411-86110418; Email: maxc1978@163.com

Authors

Cheng-Peng Sun − *Dalian Key Laboratory of Metabolic Target Characterization and Traditional Chinese Medicine Intervention, College (Institute) of Integrative Medicine, College of Pharmacy, Dalian Medical University, Dalian 116044, People's Republic of China;* orcid.org/0000-0001-6801-3439

Xin-Yue Zhang − *Dalian Key Laboratory of Metabolic Target Characterization and Traditional Chinese Medicine Intervention, College (Institute) of Integrative Medicine, College of Pharmacy, Dalian Medical University, Dalian 116044, People's Republic of China*

Christophe Morisseau − *Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis, California 95616, United States*

Sung Hee Hwang − *Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis, California 95616, United States*

Zhan-Jun Zhang − *State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, Beijing 100875, People's Republic of China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.0c01507

Author Contributions

‖C.-P.S., X.-Y.Z., and C.M. contributed equally to this work.

Notes

The authors declare no competing financial interest.

Biographies

Cheng-Peng Sun graduated from Shenyang Pharmaceutical University in 2016 and received his Ph.D. in Natural Medicinal Chemistry under the guidance of Prof. Feng Qiu. Afterward, he joined the Department of Medicinal Chemistry in Dalian Medical University. In January 2019, he was promoted to Associate Professor of Medicinal Chemistry at Dalian Medical University. So far, he has published more than 40 papers on journals indexed by the Science Citation Index. His major research interests include extraction and isolation of chemical constituents from natural medicines, structural modification of active compositions, and natural modulators of sEH, FXR, and PXR. In addition, he is focusing on the prevention and treatment of neurodegenerative diseases.

Xin-Yue Zhang graduated from Dalian Medical University and received her Bachelor's degree in 2019. She is pursuing a Master's degree at the Dalian Medical University in the research group of Prof. Xiao-Chi Ma. Her research focuses on the active substances of traditional Chinese medicine and the discovery of sEH inhibitors.

Christophe Morisseau obtained his Ph.D. in Organic Chemistry in 1995 from the Universitée de la Mditerranée in Marseille, and he has since been working with Prof. Hammock at University of California, Davis. He is now a Professional Researcher faculty. He is working at understanding the mechanism and biological role of EHs and developing selective tight binding inhibitors for EHs. He developed the first stable highly potent inhibitor of human sEH, obtained the first crystal structures for sEH and the first high-throughput assays for the human sEH and mEH, and developed more biochemical tools to study EHs. Some of the inhibitors he obtained are in development for

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

the treatment of inflammation, cardiovascular disease, pain, and cancer. His work has resulted in more than 250 papers and 20 patents.

Sung Hee Hwang obtained his Ph.D. in Organic Chemistry in 2004 from the University of California, Davis. He is an accomplished medicinal chemist. He leads the development of highly potent inhibitors of epoxide hydrolase and cyclooxygenase enzymes. In addition, he has synthesized various fatty acids and their cytochrome P450-metabolite analogs to study their biological activities. His work has resulted in more than 100 publications.

Zhan-Jun Zhang received his Ph.D. in Medicine from Beijing University of Chinese Medicine in 2005 and is the Director of the Center for Beijing Aging Brain Rejuvenation Institute and the Vice President of State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University. He has been engaged in a long-term research of digging the pattern of varied human advanced cognitive functions decline, the degradation mode of the brain's structure and function network, the mechanism of genetic variation toward the brain and cognitive aging, and the contribution of Chinese medicine in protecting the brain from aging. He is the recipient of the National Science Fund for Distinguished Young Scholars and the New Century Excellent Talents Program of China's Ministry of Education.

Bruce D. Hammock received his Ph.D. in Entomology and Toxicology in 1974 from the University of California, Berkeley. He is now a Distinguished Professor at University of California, Davis. Dr. Hammock is an elected member of the National Academy of Sciences, U.S., since 1999. He discovered the sEH and has devoted his carreer studying the biological roles of this enzyme. He has over 30 years of extensive experience in the field of eicosanoids that has yielded over 1200 publications and 100 issued patents on synthesis, pharmacology, and biological action of eicosanoids and sEH inhibitors.

Xiao-Chi Ma received his Ph.D. in Phytochemistry from School of Traditional Chinese Medicine, Shenyang Pharmaceutical University, in July 2005, under the guidance of Prof. De-An Guo and Li-Jun Wu. He worked as a postdoc in School of Pharmaceutical Sciences, Peking Unversity, and mainly focused on biological fermentation, tissue culture of endangered plant medicine, biotransformation of natural products by microorganism and plant suspension cells and phytochemistry. In 2008, he joined the Dalian Medical University and was promoted to Professor of Natural Medicinal Chemistry at Dalian Medical University. He is the recipient of the National Science Fund for Excellent Young Scholars. His major research interests include effective substances from traditional Chinese medicine and drug metabolism, development, and application of small molecular probes.

■ ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (No. 81930112 and 81703769), National Key Research and Development Program of China (2018YFC1705900), Liaoning Provincial Key Research and Development Program (2019JH2/10300022), Distinguished professor of Liaoning Province, Dalian High Level Talents Innovation Support Plan (2019RD15), Revolutionizing Innovative, Visionary Environmental Health Research Program of the National Institute of Environmental Health Sciences (R35 ES030443), Superfund Basic Research Program of the National Institutes of Environmental Health Sciences (P42 ES04699), Liaoning Revitalization Talents Program, Natural Science Foundation of Liaoning Province (No. 2020-MS-256), and Dalian Young Star of Science and Technology (No. 2019RQ123).

■ ABBREVIATIONS USED

AA, arachidonic acid; Aβ, amyloid β; AD, Alzheimer's disease; AFIMU, 1-(adamant-1-ylmethyl)-3-{[5-(furan-2-yl)isoxazol-3-yl]methyl}urea; AMMU, 1-(adamant-1-ylmethyl)-3-[(3-methylisoxazol-5-yl)methyl]urea; Ang II, angiotensin II; APU, 1-(adamantan-2-yl)-3-phenethylthiourea; AUC, area under the curve; AUDA, 12-(3-adamantan-1-yl-ureido)dodecanoic acid; AUDA-nBE, AUDA *n*-butyl ester; BDNF-TrkB, BDNF-tropomyosin receptor kinase B; *c*-AUCB, *cis*-4-[4-(3-adamantan-1- ylureido)cyclohexyloxy]benzoic acid; CBF, cerebral blood flow; CNS, central nervous system; CPU, *N*-cyclohexyl-*N*′-(3-phenylpropyl)urea; COXs, cyclooxygenases; CYP, cytochrome P450; DCU, *N*,*N*′-dicyclohexylurea; DHA, docosahexaenoic acid; DHETs, dihydroxyeicosatrienoic acids; DPN, *N*-(3,3-diphenyl-propyl)nicotinamide; EETs, epoxyeicosatrienoic acids; EpFAs, epoxy-fatty acids; FAAH, fatty acid amide hydrolase; FLAP, 5-LOX-activating protein; HBAU, 1,1′-(heptane-1,7-diyl)bis(3-(adamantan-1-yl)thiourea); HCHFD, high-carbohydrate and high-fat diet; HDL, high-density lipoprotein; HETEs, hydroxy eicosatetraenoic acids; HFD, high-fat diet; HLM, human liver microsome; ITBU, *N*-[9-(isopropylsulfonyl)-1-oxa-9-azaspiro[5.5]undec-4-yl]-*N*′-[4-(trifluoromethoxy)benzyl]urea; K_i, kinetic constant; koff, dissociation rate constant; LDL, low-density lipoprotein; IL-1β, interleukin-1 beta; LOXs, lipoxygenases; LXs, lipoxins; LPS, lipopolysaccharide; LTB_4, leukotriene B_4; LTs, leukotrienes; mp, melting point; mTOR, mammalian target of rapamycin; NADPH, nicotinamide adenine dinucleotide phosphate; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor-kappa B; PD, Parkinson's disease; PK, pharmacokinetic; PARK2, Parkinson disease protein 2; PPARγ, peroxisome proliferator-activated receptor γ; RLM, rat live microsome; sEH, soluble epoxide hydrolase; *S*-PPU, (*S*)-1-(1-phenylethyl)-3-(3-phenylpropyl)urea; STZ, streptozotocin; *t*-AUCB, *trans*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid; TNF-α, tumor necrosis factor-α; TZD, thiazolidinedione

■ REFERENCES

(1) El-Sherbeni, A. A.; El-Kadi, A. O. The role of epoxide hydrolases in health and disease. *Arch. Toxicol.* **2014**, *88*, 2013−2032.
(2) Saini, P.; Sareen, D. An overview on the enhancement of enantioselectivity and stability of microbial epoxide hydrolases. *Mol. Biotechnol.* **2017**, *59*, 98−116.
(3) Newman, J. W.; Morisseau, C.; Hammock, B. D. Epoxide hydrolases: their roles and interactions with lipid metabolism. *Prog. Lipid Res.* **2005**, *44*, 1−51.
(4) Newman, J. W.; Denton, D. L.; Morisseau, C.; Koger, C. S.; Wheelock, C. E.; Hinton, D. E.; Hammock, B. D. Evaluation of fish models of soluble epoxide hydrolase inhibition. *Environ. Health Perspect.* **2001**, *109*, 61−66.
(5) Lauren, D. J.; Halarnkar, P. P.; Hammock, B. D.; Hinton, D. E. Microsomal and cytosolic epoxide hydrolase and glutathione S-transferase activities in the gill, liver, and kidney of the rainbow trout, *Salmo gairdneri*. Baseline levels and optimization of assay conditions. *Biochem. Pharmacol.* **1989**, *38*, 881−887.
(6) Schlezinger, J. J.; Parker, C.; Zeldin, D. C.; Stegeman, J. J. Arachidonic acid metabolism in the marine fish *Stenotomus chrysops* (Scup) and the effects of cytochrome P450 1A inducers. *Arch. Biochem. Biophys.* **1998**, *353*, 265−275.

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

(7) Ota, K.; Hammock, B. D. Cytosolic and microsomal epoxide hydrolases: differential properties in mammalian liver. *Science* **1980**, *207*, 1479−1481.
(8) Waechter, F.; Merdes, M.; Bieri, F.; Staubli, W.; Bentley, P. Purification and characterization of a soluble epoxide hydrolase from rabbit liver. *Eur. J. Biochem.* **1982**, *125*, 457−461.
(9) Meijer, J.; Lundqvist, G.; DePierre, J. W. Comparison of the sex and subcellular distributions, catalytic and immunochemical reactivities of hepatic epoxide hydrolases in seven mammalian species. *Eur. J. Biochem.* **1987**, *167*, 269−279.
(10) Miki, I.; Shimizu, T.; Seyama, Y.; Kitamura, S.; Yamaguchi, K.; Sano, H.; Ueno, H.; Hiratsuka, A.; Watabe, T. Enzymic conversion of 11,12-leukotriene A4 to 11,12-dihydroxy-5,14-*cis*-7,9-*trans*-eicosatetraenoic acid. Purification of an epoxide hydrolase from the guinea pig liver cytosol. *J. Biol. Chem.* **1989**, *264*, 5799−5805.
(11) Lakritz, J.; Winder, B. S.; Noorouz-Zadeh, J.; Huang, T. L.; Buckpitt, A. R.; Hammock, B. D.; Plopper, C. G. Hepatic and pulmonary enzyme activities in horses. *Am. J. Vet. Res.* **2000**, *61*, 152−157.
(12) Beetham, J. K.; Tian, T.; Hammock, B. D. cDNA cloning and expression of a soluble epoxide hydrolase from human liver. *Arch. Biochem. Biophys.* **1993**, *305*, 197−201.
(13) Pacifici, G. M.; Lindberg, B.; Glaumann, H.; Rane, A. Styrene oxide metabolism in rhesus monkey liver: enzyme activities in subcellular fractions and in isolated hepatocytes. *J. Pharmacol. Exp. Ther.* **1983**, *226*, 869−875.
(14) Newman, J. W.; Stok, J. E.; Vidal, J. D.; Corbin, C. J.; Huang, Q.; Hammock, B. D.; Conley, A. J. Cytochrome P450-dependent lipid metabolism in preovulatory follicles. *Endocrinology* **2004**, *145*, 5097−5105.
(15) Gill, S. S.; Hammock, B. D. Distribution and properties of a mammalian soluble epoxide hydrase. *Biochem. Pharmacol.* **1980**, *29*, 389−395.
(16) Liu, Y.; Zhang, Y.; Schmelzer, K.; Lee, T. S.; Fang, X.; Zhu, Y.; Spector, A. A.; Gill, S.; Morisseau, C.; Hammock, B. D.; Shyy, J. Y. The antiinflammatory effect of laminar flow: the role of PPARgamma, epoxyeicosatrienoic acids, and soluble epoxide hydrolase. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 16747−16752.
(17) Pinot, F.; Grant, D. F.; Spearow, J. L.; Parker, A. G.; Hammock, B. D. Differential regulation of soluble epoxide hydrolase by clofibrate and sexual hormones in the liver and kidneys of mice. *Biochem. Pharmacol.* **1995**, *50*, 501−508.
(18) Pang, W.; Li, N.; Ai, D.; Niu, X. L.; Guan, Y. F.; Zhu, Y. Activation of peroxisome proliferator-activated receptor-gamma downregulates soluble epoxide hydrolase in cardiomyocytes. *Clin. Exp. Pharmacol. Physiol.* **2011**, *38*, 358−364.
(19) Sjodin, M. O. D.; Checa, A.; Yang, M.; Dahlen, S. E.; Wheelock, A. M.; Eklund, A.; Grunewald, J.; Wheelock, C. E. Soluble epoxide hydrolase derived lipid mediators are elevated in bronchoalveolar lavage fluid from patients with sarcoidosis: a cross-sectional study. *Respir. Res.* **2018**, *19*, 236.
(20) Morisseau, C.; Hammock, B. D. Epoxide hydrolases: mechanisms, inhibitor designs, and biological roles. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 311−333.
(21) Kramer, J.; Proschak, E. Phosphatase activity of soluble epoxide hydrolase. *Prostaglandins Other Lipid Mediators* **2017**, *133*, 88−92.
(22) Gurung, A. B.; Mayengbam, B.; Bhattacharjee, A. Discovery of novel drug candidates for inhibition of soluble epoxide hydrolase of arachidonic acid cascade pathway implicated in atherosclerosis. *Comput. Biol. Chem.* **2018**, *74*, 1−11.
(23) Cronin, A.; Mowbray, S.; Durk, H.; Homburg, S.; Fleming, I.; Fisslthaler, B.; Oesch, F.; Arand, M. The N-terminal domain of mammalian soluble epoxide hydrolase is a phosphatase. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 1552−1557.
(24) Newman, J. W.; Morisseau, C.; Harris, T. R.; Hammock, B. D. The soluble epoxide hydrolase encoded by EPXH2 is a bifunctional enzyme with novel lipid phosphate phosphatase activity. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 1558−1563.
(25) Sandberg, M.; Meijer, J. Structural characterization of the human soluble epoxide hydrolase gene (EPHX2). *Biochem. Biophys. Res. Commun.* **1996**, *221*, 333−339.
(26) Przybyla-Zawislak, B. D.; Srivastava, P. K.; Vazquez-Matias, J.; Mohrenweiser, H. W.; Maxwell, J. E.; Hammock, B. D.; Bradbury, J. A.; Enayetallah, A. E.; Zeldin, D. C.; Grant, D. F. Polymorphisms in human soluble epoxide hydrolase. *Mol. Pharmacol.* **2003**, *64*, 482−490.
(27) Morisseau, C.; Hammock, B. D. Gerry Brooks and epoxide hydrolases: four decades to a pharmaceutical. *Pest Manage. Sci.* **2008**, *64*, 594−609.
(28) Scott-Van Zeeland, A. A.; Bloss, C. S.; Tewhey, R.; Bansal, V.; Torkamani, A.; Libiger, O.; Duvvuri, V.; Wineinger, N.; Galvez, L.; Darst, B. F.; Smith, E. N.; Carson, A.; Pham, P.; Phillips, T.; Villarasa, N.; Tisch, R.; Zhang, G.; Levy, S.; Murray, S.; Chen, W.; Srinivasan, S.; Berenson, G.; Brandt, H.; Crawford, S.; Crow, S.; Fichter, M. M.; Halmi, K. A.; Johnson, C.; Kaplan, A. S.; La Via, M.; Mitchell, J. E.; Strober, M.; Rotondo, A.; Treasure, J.; Woodside, D. B.; Bulik, C. M.; Keel, P.; Klump, K. L.; Lilenfeld, L.; Plotnicov, K.; Topol, E. J.; Shih, P. B.; Magistretti, P.; Bergen, A. W.; Berrettini, W.; Kaye, W.; Schork, N. J. Evidence for the role of EPHX2 gene variants in anorexia nervosa. *Mol. Psychiatry* **2014**, *19*, 724−732.
(29) Lee, J. P.; Yang, S. H.; Kim, D. K.; Lee, H.; Kim, B.; Cho, J. Y.; Yu, K. S.; Paik, J. H.; Kim, M.; Lim, C. S.; Kim, Y. S. *In vivo* activity of epoxide hydrolase according to sequence variation affects the progression of human IgA nephropathy. *Am. J. Physiol. Renal. Physiol.* **2011**, *300*, F1283−F1290.
(30) Zarriello, S.; Tuazon, J. P.; Corey, S.; Schimmel, S.; Rajani, M.; Gorsky, A.; Incontri, D.; Hammock, B. D.; Borlongan, C. V. Humble beginnings with big goals: Small molecule soluble epoxide hydrolase inhibitors for treating CNS disorders. *Prog. Neurobiol.* **2019**, *172*, 23−39.
(31) Afanador, L.; Roltsch, E. A.; Holcomb, L.; Campbell, K. S.; Keeling, D. A.; Zhang, Y.; Zimmer, D. B. The Ca^{2+} sensor S100A1 modulates neuroinflammation, histopathology and Akt activity in the PSAPP Alzheimer's disease mouse model. *Cell Calcium* **2014**, *56*, 68−80.
(32) Duflot, T.; Roche, C.; Lamoureux, F.; Guerrot, D.; Bellien, J. Design and discovery of soluble epoxide hydrolase inhibitors for the treatment of cardiovascular diseases. *Expert Opin. Drug Discovery* **2014**, *9*, 229−243.
(33) Dileepan, M.; Rastle-Simpson, S.; Greenberg, Y.; Wijesinghe, D. S.; Kumar, N. G.; Yang, J.; Hwang, S. H.; Hammock, B. D.; Sriramarao, P.; Rao, S. P. Effect of dual sEH/COX-2 inhibition on allergen-induced airway inflammation. *Front. Pharmacol.* **2019**, *10*, 1118.
(34) Hashimoto, K. Role of soluble epoxide hydrolase in metabolism of PUFAs in psychiatric and neurological disorders. *Front. Pharmacol.* **2019**, *10*, 36.
(35) Nayeem, M. A. Role of oxylipins in cardiovascular diseases. *Acta Pharmacol. Sin.* **2018**, *39*, 1142−1154.
(36) Harder, D. R.; Campbell, W. B.; Roman, R. J. Role of cytochrome P-450 enzymes and metabolites of arachidonic acid in the control of vascular tone. *J. Vasc. Res.* **1995**, *32*, 79−92.
(37) Roman, R. J. P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol. Rev.* **2002**, *82*, 131−185.
(38) Hu, S.; Kim, H. S. Activation of K^+ channel in vascular smooth muscles by cytochrome P450 metabolites of arachidonic acid. *Eur. J. Pharmacol.* **1993**, *230*, 215−221.
(39) Kroetz, D. L.; Zeldin, D. C. Cytochrome P450 pathways of arachidonic acid metabolism. *Curr. Opin. Lipidol.* **2002**, *13*, 273−283.
(40) Node, K.; Ruan, X. L.; Dai, J.; Yang, S. X.; Graham, L.; Zeldin, D. C.; Liao, J. K. Activation of Galpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. *J. Biol. Chem.* **2001**, *276*, 15983−15989.
(41) Munzenmaier, D. H.; Harder, D. R. Cerebral microvascular endothelial cell tube formation: role of astrocytic epoxyeicosatrienoic acid release. *Am. J. Physiol. Heart Circ. Physiol.* **2000**, *278*, H1163−H1167.

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

(42) Node, K.; Huo, Y.; Ruan, X.; Yang, B.; Spiecker, M.; Ley, K.; Zeldin, D. C.; Liao, J. K. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* **1999**, *285*, 1276−1279.
(43) Campbell, W. B. New role for epoxyeicosatrienoic acids as anti-inflammatory mediators. *Trends Pharmacol. Sci.* **2000**, *21*, 125−127.
(44) Zeldin, D. C. Epoxygenase pathways of arachidonic acid metabolism. *J. Biol. Chem.* **2001**, *276*, 36059−36062.
(45) Imig, J. D.; Navar, L. G.; Roman, R. J.; Reddy, K. K.; Falck, J. R. Actions of epoxygenase metabolites on the preglomerular vasculature. *J. Am. Soc. Nephrol.* **1996**, *7*, 2364−2370.
(46) Falck, J. R.; Krishna, U. M.; Reddy, Y. K.; Kumar, P. S.; Reddy, K. M.; Hittner, S. B.; Deeter, C.; Sharma, K. K.; Gauthier, K. M.; Campbell, W. B. Comparison of vasodilatory properties of 14,15-EET analogs: structural requirements for dilation. *Am. J. Physiol. Heart Circ. Physiol.* **2003**, *284*, H337−H349.
(47) Lee, H. C.; Lu, T.; Weintraub, N. L.; VanRollins, M.; Spector, A. A.; Shibata, E. F. Effects of epoxyeicosatrienoic acids on the cardiac sodium channels in isolated rat ventricular myocytes. *J. Physiol.* **1999**, *519*, 153−168.
(48) Fang, X.; Moore, S. A.; Stoll, L. L.; Rich, G.; Kaduce, T. L.; Weintraub, N. L.; Spector, A. A. 14,15-Epoxyeicosatrienoic acid inhibits prostaglandin E2 production in vascular smooth muscle cells. *Am. J. Physiol.* **1998**, *275*, H2113−H2121.
(49) Fang, X.; Weintraub, N. L.; Stoll, L. L.; Spector, A. A. Epoxyeicosatrienoic acids increase intracellular calcium concentration in vascular smooth muscle cells. *Hypertension* **1999**, *34*, 1242−1246.
(50) Hirt, D. L.; Capdevila, J.; Falck, J. R.; Breyer, M. D.; Jacobson, H. R. Cytochrome P450 metabolites of arachidonic acid are potent inhibitors of vasopressin action on rabbit cortical collecting duct. *J. Clin. Invest.* **1989**, *84*, 1805−1812.
(51) Weintraub, N. L.; Fang, X.; Kaduce, T. L.; VanRollins, M.; Chatterjee, P.; Spector, A. A. Potentiation of endothelium-dependent relaxation by epoxyeicosatrienoic acids. *Circ. Res.* **1997**, *81*, 258−267.
(52) Oltman, C. L.; Weintraub, N. L.; VanRollins, M.; Dellsperger, K. C. Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation. *Circ. Res.* **1998**, *83*, 932−939.
(53) Jones, R. D.; Liao, J.; Tong, X.; Xu, D. D.; Sun, L. Y.; Li, H. N.; Yang, G. Y. Epoxy-oxylipins and soluble epoxide hydrolase metabolic pathway as targets for NSAID-induced gastroenteropathy and inflammation-associated carcinogenesis. *Front. Pharmacol.* **2019**, *10*, 731.
(54) Harris, T. R.; Hammock, B. D. Soluble epoxide hydrolase: gene structure, expression and deletion. *Gene* **2013**, *526*, 61−74.
(55) Wagner, K. M.; McReynolds, C. B.; Schmidt, W. K.; Hammock, B. D. Soluble epoxide hydrolase as a therapeutic target for pain, inflammatory and neurodegenerative diseases. *Pharmacol. Ther.* **2017**, *180*, 62−76.
(56) Shen, H. C. Soluble epoxide hydrolase inhibitors: a patent review. *Expert Opin. Ther. Pat.* **2010**, *20*, 941−956.
(57) Mullin, C. A.; Hammock, B. D. Chalcone oxides - potent selective inhibitors of cytosolic epoxide hydrolase. *Arch. Biochem. Biophys.* **1982**, *216*, 423−439.
(58) Morisseau, C.; Goodrow, M. H.; Dowdy, D.; Zheng, J.; Greene, J. F.; Sanborn, J. R.; Hammock, B. D. Potent urea and carbamate inhibitors of soluble epoxide hydrolases. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 8849−8854.
(59) Podolin, P. L.; Bolognese, B. J.; Foley, J. F.; Long, E., 3rd; Peck, B.; Umbrecht, S.; Zhang, X.; Zhu, P.; Schwartz, B.; Xie, W.; Quinn, C.; Qi, H.; Sweitzer, S.; Chen, S.; Galop, M.; Ding, Y.; Belyanskaya, S. L.; Israel, D. I.; Morgan, B. A.; Behm, D. J.; Marino, J. P., Jr.; Kurali, E.; Barnette, M. S.; Mayer, R. J.; Booth-Genthe, C. L.; Callahan, J. F. *In vitro* and *in vivo* characterization of a novel soluble epoxide hydrolase inhibitor. *Prostaglandins Other Lipid Mediators* **2013**, *104-105*, 25−31.
(60) Lukin, A.; Kramer, J.; Hartmann, M.; Weizel, L.; Hernandez-Olmos, V.; Falahati, K.; Burghardt, I.; Kalinchenkova, N.; Bagnyukova, D.; Zhurilo, N.; Rautio, J.; Forsberg, M.; Ihalainen, J.; Auriola, S.; Leppanen, J.; Konstantinov, I.; Pogoryelov, D.; Proschak, E.; Dar'in, D.; Krasavin, M. Discovery of polar spirocyclic orally bioavailable urea inhibitors of soluble epoxide hydrolase. *Bioorg. Chem.* **2018**, *80*, 655−667.
(61) Yu, Z.; Xu, F.; Huse, L. M.; Morisseau, C.; Draper, A. J.; Newman, J. W.; Parker, C.; Graham, L.; Engler, M. M.; Hammock, B. D.; Zeldin, D. C.; Kroetz, D. L. Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ. Res.* **2000**, *87*, 992−998.
(62) Fang, X.; Kaduce, T. L.; Weintraub, N. L.; Harmon, S.; Teesch, L. M.; Morisseau, C.; Thompson, D. A.; Hammock, B. D.; Spector, A. A. Pathways of epoxyeicosatrienoic acid metabolism in endothelial cells. Implications for the vascular effects of soluble epoxide hydrolase inhibition. *J. Biol. Chem.* **2001**, *276*, 14867−14874.
(63) Gomez, G. A.; Morisseau, C.; Hammock, B. D.; Christianson, D. W. Human soluble epoxide hydrolase: structural basis of inhibition by 4-(3-cyclohexylureido)-carboxylic acids. *Protein Sci.* **2006**, *15*, 58−64.
(64) Kim, I. H.; Morisseau, C.; Watanabe, T.; Hammock, B. D. Design, synthesis, and biological activity of 1,3-disubstituted ureas as potent inhibitors of the soluble epoxide hydrolase of increased water solubility. *J. Med. Chem.* **2004**, *47*, 2110−2122.
(65) Morisseau, C.; Goodrow, M. H.; Newman, J. W.; Wheelock, C. E.; Dowdy, D. L.; Hammock, B. D. Structural refinement of inhibitors of urea-based souble epoxide hydrolases. *Biochem. Pharmacol.* **2002**, *63*, 1599−1608.
(66) Imig, J. D.; Zhao, X.; Capdevila, J. H.; Morisseau, C.; Hammock, B. D. Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. *Hypertension* **2002**, *39*, 690−694.
(67) Honetschlagerova, Z.; Sporkova, A.; Kopkan, L.; Huskova, Z.; Hwang, S. H.; Hammock, B. D.; Imig, J. D.; Kramer, H. J.; Kujal, P.; Vernerova, Z.; Chabova, V. C.; Tesar, V.; Cervenka, L. Inhibition of soluble epoxide hydrolase improves the impaired pressure-natriuresis relationship and attenuates the development of hypertension and hypertension-associated end-organ damage in Cyp1a1-Ren-2 transgenic rats. *J. Hypertens.* **2011**, *29*, 1590−1601.
(68) Gross, G. J.; Nithipatikom, K. Soluble epoxide hydrolase: a new target for cardioprotection. *Curr. Opin. Invest. Drugs* **2009**, *10*, 253−258.
(69) Schmelzer, K. R.; Kubala, L.; Newman, J. W.; Kim, I. H.; Eiserich, J. P.; Hammock, B. D. Soluble epoxide hydrolase is a therapeutic target for acute inflammation. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 9772−9777.
(70) McElroy, N. R.; Jurs, P. C.; Morisseau, C.; Hammock, B. D. QSAR and classification of murine and human soluble epoxide hydrolase inhibition by urea-like compounds. *J. Med. Chem.* **2003**, *46*, 1066−1080.
(71) Jones, P. D.; Tsai, H. J.; Do, Z. N.; Morisseau, C.; Hammock, B. D. Synthesis and SAR of conformationally restricted inhibitors of soluble epoxide hydrolase. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5212−5216.
(72) Schafer, A.; Neschen, S.; Kahle, M.; Sarioglu, H.; Gaisbauer, T.; Imhof, A.; Adamski, J.; Hauck, S. M.; Ueffing, M. The epoxyeicosatrienoic acid pathway enhances hepatic insulin signaling and is repressed in insulin-resistant mouse liver. *Mol. Cell. Proteomics* **2015**, *14*, 2764−2774.
(73) Hwang, S. H.; Tsai, H. J.; Liu, J. Y.; Morisseau, C.; Hammock, B. D. Orally bioavailable potent soluble epoxide hydrolase inhibitors. *J. Med. Chem.* **2007**, *50*, 3825−3840.
(74) Gui, Y.; Li, D.; Chen, J.; Wang, Y.; Hu, J.; Liao, C.; Deng, L.; Xiang, Q.; Yang, T.; Du, X.; Zhang, S.; Xu, D. Soluble epoxide hydrolase inhibitors, *t*-AUCB, downregulated miR-133 in a mouse model of myocardial infarction. *Lipids Health Dis.* **2018**, *17*, 129.
(75) Li, J.; Tang, C.; Li, L.; Li, R.; Fan, Y. Quercetin sensitizes glioblastoma to *t*-AUCB by dual inhibition of Hsp27 and COX-2 *in vitro* and *in vivo*. *J. Exp. Clin. Cancer Res.* **2016**, *35*, 61.
(76) Shen, L.; Peng, H. C.; Zhao, S. P.; Xu, D. Y. A potent soluble epoxide hydrolase inhibitor, *t*-AUCB, modulates cholesterol balance

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

and oxidized low density lipoprotein metabolism in adipocytes *in vitro*. *Biol. Chem.* **2014**, *395*, 443−451.
(77) Roche, C.; Guerrot, D.; Harouki, N.; Duflot, T.; Besnier, M.; Remy-Jouet, I.; Renet, S.; Dumesnil, A.; Lejeune, A.; Morisseau, C.; Richard, V.; Bellien, J. Impact of soluble epoxide hydrolase inhibition on early kidney damage in hyperglycemic overweight mice. *Prostaglandins Other Lipid Mediators* **2015**, *120*, 148−154.
(78) Iyer, A.; Kauter, K.; Alam, M. A.; Hwang, S. H.; Morisseau, C.; Hammock, B. D.; Brown, L. Pharmacological inhibition of soluble epoxide hydrolase ameliorates diet-induced metabolic syndrome in rats. *Exp. Diabetes Res.* **2012**, *2012*, 758614.
(79) Burmistrov, V.; Morisseau, C.; Danilov, D.; Harris, T. R.; Dalinger, I.; Vatsadze, I.; Shkineva, T.; Butov, G. M.; Hammock, B. D. 1,3-Disubstituted and 1,3,3-trisubstituted adamantyl-ureas with isoxazole as soluble epoxide hydrolase inhibitors. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 5514−5519.
(80) Burmistrov, V.; Morisseau, C.; Harris, T. R.; Butov, G.; Hammock, B. D. Effects of adamantane alterations on soluble epoxide hydrolase inhibition potency, physical properties and metabolic stability. *Bioorg. Chem.* **2018**, *76*, 510−527.
(81) Rose, T. E.; Morisseau, C.; Liu, J. Y.; Inceoglu, B.; Jones, P. D.; Sanborn, J. R.; Hammock, B. D. 1-Aryl-3-(1-acylpiperidin-4-yl)urea inhibitors of human and murine soluble epoxide hydrolase: structure-activity relationships, pharmacokinetics, and reduction of inflammatory pain. *J. Med. Chem.* **2010**, *53*, 7067−7075.
(82) Tsai, H. J.; Hwang, S. H.; Morisseau, C.; Yang, J.; Jones, P. D.; Kasagami, T.; Kim, I. H.; Hammock, B. D. Pharmacokinetic screening of soluble epoxide hydrolase inhibitors in dogs. *Eur. J. Pharm. Sci.* **2010**, *40*, 222−238.
(83) Lee, K. S.; Liu, J. Y.; Wagner, K. M.; Pakhomova, S.; Dong, H.; Morisseau, C.; Fu, S. H.; Yang, J.; Wang, P.; Ulu, A.; Mate, C. A.; Nguyen, L. V.; Hwang, S. H.; Edin, M. L.; Mara, A. A.; Wulff, H.; Newcomer, M. E.; Zeldin, D. C.; Hammock, B. D. Optimized inhibitors of soluble epoxide hydrolase improve *in vitro* target residence time and *in vivo* efficacy. *J. Med. Chem.* **2014**, *57*, 7016−7030.
(84) Manickam, M.; Pillaiyar, T.; Boggu, P.; Venkateswararao, E.; Jalani, H. B.; Kim, N. D.; Lee, S. K.; Jeon, J. S.; Kim, S. K.; Jung, S. H. Discovery of enantioselectivity of urea inhibitors of soluble epoxide hydrolase. *Eur. J. Med. Chem.* **2016**, *117*, 113−124.
(85) Burmistrov, V.; Morisseau, C.; Pitushkin, D.; Karlov, D.; Fayzullin, R. R.; Butov, G. M.; Hammock, B. D. Adamantyl thioureas as soluble epoxide hydrolase inhibitors. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2302−2313.
(86) Kim, I. H.; Heirtzler, F. R.; Morisseau, C.; Nishi, K.; Tsai, H. J.; Hammock, B. D. Optimization of amide-based inhibitors of soluble epoxide hydrolase with improved water solubility. *J. Med. Chem.* **2005**, *48*, 3621−3629.
(87) Eldrup, A. B.; Soleymanzadeh, F.; Taylor, S. J.; Muegge, I.; Farrow, N. A.; Joseph, D.; McKellop, K.; Man, C. C.; Kukulka, A.; De Lombaert, S. Structure-based optimization of arylamides as inhibitors of soluble epoxide hydrolase. *J. Med. Chem.* **2009**, *52*, 5880−5895.
(88) Han, F.; Perrin, R. J.; Wang, Q.; Wang, Y.; Perlmutter, J. S.; Morris, J. C.; Benzinger, T. L. S.; Xu, J. B. Neuroinflammation and myelin status in Alzheimer's disease, Parkinson's disease, and normal aging brains: a small sample study. *Parkinson's Dis.* **2019**, *2019*, 7975407.
(89) Taylor, S. J.; Soleymanzadeh, F.; Eldrup, A. B.; Farrow, N. A.; Muegge, I.; Kukulka, A.; Kabcenell, A. K.; De Lombaert, S. Design and synthesis of substituted nicotinamides as inhibitors of soluble epoxide hydrolase. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5864−5868.
(90) Anandan, S. K.; Do, Z. N.; Webb, H. K.; Patel, D. V.; Gless, R. D. Non-urea functionality as the primary pharmacophore in soluble epoxide hydrolase inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1066−1070.
(91) Anandan, S. K.; Webb, H. K.; Do, Z. N.; Gless, R. D. Unsymmetrical non-adamantyl N,N'-diaryl urea and amide inhibitors of soluble expoxide hydrolase. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4259−4263.
(92) Piirainen, S.; Youssef, A.; Song, C.; Kalueff, A. V.; Landreth, G. E.; Malm, T.; Tian, L. Psychosocial stress on neuroinflammation and cognitive dysfunctions in Alzheimer's disease: the emerging role for microglia? *Neurosci. Biobehav. Rev.* **2017**, *77*, 148−164.
(93) Karami, L.; Saboury, A. A.; Rezaee, E.; Tabatabai, S. A. Investigation of the binding mode of 1, 3, 4-oxadiazole derivatives as amide-based inhibitors for soluble epoxide hydrolase (sEH) by molecular docking and MM-GBSA. *Eur. Biophys. J.* **2017**, *46*, 445−459.
(94) Kim, I. H.; Lee, I. H.; Nishiwaki, H.; Hammock, B. D.; Nishi, K. Structure-activity relationships of substituted oxyoxalamides as inhibitors of the human soluble epoxide hydrolase. *Bioorg. Med. Chem.* **2014**, *22*, 1163−1175.
(95) Kim, I. H.; Park, Y. K.; Nishiwaki, H.; Hammock, B. D.; Nishi, K. Structure-activity relationships of amide-phosphonate derivatives as inhibitors of the human soluble epoxide hydrolase. *Bioorg. Med. Chem.* **2015**, *23*, 7199−7210.
(96) Zavareh, E.; Hedayati, M.; Rad, L.; Kiani, A.; Shahhosseini, S.; Faizi, M.; Tabatabai, S. Design, synthesis and biological evaluation of some oxadiazole derivatives as novel amide-based inhibitors of soluble epoxide hydrolase. *Lett. Drug Des. Discovery* **2014**, *11*, 721−730.
(97) Kim, I. H.; Park, Y. K.; Hammock, B. D.; Nishi, K. Structure-activity relationships of cycloalkylamide derivatives as inhibitors of the soluble epoxide hydrolase. *J. Med. Chem.* **2011**, *54*, 1752−1761.
(98) Xie, Y.; Liu, Y.; Gong, G.; Smith, D. H.; Yan, F.; Rinderspacher, A.; Feng, Y.; Zhu, Z.; Li, X.; Deng, S. X.; Branden, L.; Vidovic, D.; Chung, C.; Schurer, S.; Morisseau, C.; Hammock, B. D.; Landry, D. W. Discovery of potent non-urea inhibitors of soluble epoxide hydrolase. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2354−2359.
(99) Pecic, S.; Deng, S. X.; Morisseau, C.; Hammock, B. D.; Landry, D. W. Design, synthesis and evaluation of non-urea inhibitors of soluble epoxide hydrolase. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 601−605.
(100) Xing, L.; McDonald, J. J.; Kolodziej, S. A.; Kurumbail, R. G.; Williams, J. M.; Warren, C. J.; O'Neal, J. M.; Skepner, J. E.; Roberds, S. L. Discovery of potent inhibitors of soluble epoxide hydrolase by combinatorial library design and structure-based virtual screening. *J. Med. Chem.* **2011**, *54*, 1211−1222.
(101) Deng, H.; O'Keefe, H.; Davie, C. P.; Lind, K. E.; Acharya, R. A.; Franklin, G. J.; Larkin, J.; Matico, R.; Neeb, M.; Thompson, M. M.; Lohr, T.; Gross, J. W.; Centrella, P. A.; O'Donovan, G. K.; Bedard, K. L.; van Vloten, K.; Mataruse, S.; Skinner, S. R.; Belyanskaya, S. L.; Carpenter, T. Y.; Shearer, T. W.; Clark, M. A.; Cuozzo, J. W.; Arico-Muendel, C. C.; Morgan, B. A. Discovery of highly potent and selective small molecule ADAMTS-5 inhibitors that inhibit human cartilage degradation via encoded library technology (ELT). *J. Med. Chem.* **2012**, *55*, 7061−7079.
(102) Belyanskaya, S. L.; Ding, Y.; Callahan, J. F.; Lazaar, A. L.; Israel, D. I. Discovering drugs with DNA-encoded library technology: from concept to clinic with an inhibitor of soluble epoxide hydrolase. *ChemBioChem* **2017**, *18*, 837−842.
(103) Thalji, R. K.; McAtee, J. J.; Belyanskaya, S.; Brandt, M.; Brown, G. D.; Costell, M. H.; Ding, Y.; Dodson, J. W.; Eisennagel, S. H.; Fries, R. E.; Gross, J. W.; Harpel, M. R.; Holt, D. A.; Israel, D. I.; Jolivette, L. J.; Krosky, D.; Li, H.; Lu, Q.; Mandichak, T.; Roethke, T.; Schnackenberg, C. G.; Schwartz, B.; Shewchuk, L. M.; Xie, W.; Behm, D. J.; Douglas, S. A.; Shaw, A. L.; Marino, J. P. Discovery of 1-(1,3,5-triazin-2-yl)piperidine-4-carboxamides as inhibitors of soluble epoxide hydrolase. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3584−3588.
(104) Hiesinger, K.; Wagner, K. M.; Hammock, B. D.; Proschak, E.; Hwang, S. H. Development of multitarget agents possessing soluble epoxide hydrolase inhibitory activity. *Prostaglandins Other Lipid Mediators* **2019**, *140*, 31−39.
(105) Kodani, S. D.; Bhakta, S.; Hwang, S. H.; Pakhomova, S.; Newcomer, M. E.; Morisseau, C.; Hammock, B. D. Identification and optimization of soluble epoxide hydrolase inhibitors with dual potency towards fatty acid amide hydrolase. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 762−768.

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

(106) Hefke, L.; Hiesinger, K.; Zhu, W. F.; Kramer, J. S.; Proschak, E. Computer-aided fragment growing strategies to design dual inhibitors of soluble epoxide hydrolase and LTA4 hydrolase. *ACS Med. Chem. Lett.* **2020**, *11*, 1244−1249.
(107) Zhang, G.; Panigrahy, D.; Hwang, S. H.; Yang, J.; Mahakian, L. M.; Wettersten, H. I.; Liu, J. Y.; Wang, Y.; Ingham, E. S.; Tam, S.; Kieran, M. W.; Weiss, R. H.; Ferrara, K. W.; Hammock, B. D. Dual inhibition of cyclooxygenase-2 and soluble epoxide hydrolase synergistically suppresses primary tumor growth and metastasis. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 11127−11132.
(108) la Buscato, E.; Blocher, R.; Lamers, C.; Klingler, F. M.; Hahn, S.; Steinhilber, D.; Schubert-Zsilavecz, M.; Proschak, E. Design and synthesis of dual modulators of soluble epoxide hydrolase and peroxisome proliferator-activated receptors. *J. Med. Chem.* **2012**, *55*, 10771−10775.
(109) Meirer, K.; Rodl, C. B.; Wisniewska, J. M.; George, S.; Hafner, A. K.; Buscato, E.; Klingler, F. M.; Hahn, S.; Berressem, D.; Wittmann, S. K.; Steinhilber, D.; Hofmann, B.; Proschak, E. Synthesis and structure-activity relationship studies of novel dual inhibitors of soluble epoxide hydrolase and 5-lipoxygenase. *J. Med. Chem.* **2013**, *56*, 1777−1781.
(110) Hwang, S. H.; Wagner, K. M.; Morisseau, C.; Liu, J. Y.; Dong, H.; Wecksler, A. T.; Hammock, B. D. Synthesis and structure-activity relationship studies of urea-containing pyrazoles as dual inhibitors of cyclooxygenase-2 and soluble epoxide hydrolase. *J. Med. Chem.* **2011**, *54*, 3037−3050.
(111) Sun, C. C.; Zhang, C. Y.; Duan, J. X.; Guan, X. X.; Yang, H. H.; Jiang, H. L.; Hammock, B. D.; Hwang, S. H.; Zhou, Y.; Guan, C. X.; Liu, S. K.; Zhang, J. PTUPB ameliorates high-fat diet-induced non-alcoholic fatty liver disease via inhibiting NLRP3 inflammasome activation in mice. *Biochem. Biophys. Res. Commun.* **2020**, *523*, 1020−1026.
(112) Zhang, Y. F.; Sun, C. C.; Duan, J. X.; Yang, H. H.; Zhang, C. Y.; Xiong, J. B.; Zhong, W. J.; Zu, C.; Guan, X. X.; Jiang, H. L.; Hammock, B. D.; Hwang, S. H.; Zhou, Y.; Guan, C. X. A COX-2/sEH dual inhibitor PTUPB ameliorates cecal ligation and puncture-induced sepsis in mice via anti-inflammation and anti-oxidative stress. *Biomed. Pharmacother.* **2020**, *126*, 109907.
(113) Zhang, C. Y.; Duan, J. X.; Yang, H. H.; Sun, C. C.; Zhong, W. J.; Tao, J. H.; Guan, X. X.; Jiang, H. L.; Hammock, B. D.; Hwang, S. H.; Zhou, Y.; Guan, C. X. COX-2/sEH dual inhibitor PTUPB alleviates bleomycin-induced pulmonary fibrosis in mice via inhibiting senescence. *FEBS J.* **2020**, *287*, 1666−1680.
(114) Lakkappa, N.; Krishnamurthy, P. T.; Yamjala, K.; Hwang, S. H.; Hammock, B. D.; Babu, B. Evaluation of antiparkinson activity of PTUPB by measuring dopamine and its metabolites in Drosophila melanogaster: LC-MS/MS method development. *J. Pharm. Biomed. Anal.* **2018**, *149*, 457−464.
(115) Li, J.; Zhou, Y.; Wang, H.; Gao, Y.; Li, L.; Hwang, S. H.; Ji, X.; Hammock, B. D. COX-2/sEH dual inhibitor PTUPB suppresses glioblastoma growth by targeting epidermal growth factor receptor and hyaluronan mediated motility receptor. *Oncotarget* **2017**, *8*, 87353−87363.
(116) Gartung, A.; Yang, J.; Sukhatme, V. P.; Bielenberg, D. R.; Fernandes, D.; Chang, J.; Schmidt, B. A.; Hwang, S. H.; Zurakowski, D.; Huang, S.; Kieran, M. W.; Hammock, B. D.; Panigrahy, D. Suppression of chemotherapy-induced cytokine/lipid mediator surge and ovarian cancer by a dual COX-2/sEH inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 1698−1703.
(117) Meirer, K.; Glatzel, D.; Kretschmer, S.; Wittmann, S. K.; Hartmann, M.; Blöcher, R.; Angioni, C.; Geisslinger, G.; Steinhilber, D.; Hofmann, B.; Fürst, R.; Proschak, E. Design, synthesis and cellular characterization of a dual inhibitor of 5-lipoxygenase and soluble epoxide hydrolase. *Molecules* **2017**, *22*, 45.
(118) Moser, D.; Wisniewska, J. M.; Hahn, S.; Achenbach, J.; Buscató, E. L.; Klingler, F. M.; Hofmann, B.; Steinhilber, D.; Proschak, E. Dual-target virtual screening by pharmacophore elucidation and molecular shape filtering. *ACS Med. Chem. Lett.* **2012**, *3*, 155−158.
(119) Achenbach, J.; Klingler, F. M.; Blöcher, R.; Moser, D.; Häfner, A. K.; Rödl, C. B.; Kretschmer, S.; Krüger, B.; Löhr, F.; Stark, H.; Hofmann, B.; Steinhilber, D.; Proschak, E. Exploring the chemical space of multitarget ligands using aligned self-organizing maps. *ACS Med. Chem. Lett.* **2013**, *4*, 1169−1172.
(120) Nandha, B.; Ramareddy, S. A.; Kuntal, H. Synthesis of substituted fluorobenzimidazoles as inhibitors of 5-lipoxygenase and soluble epoxide hydrolase for anti-inflammatory activity. *Arch. Pharm.* **2018**, *351*, No. 1800030.
(121) Klegeris, A.; McGeer, P. L. Toxicity of human monocytic THP-1 cells and microglia toward SH-SY5Y neuroblastoma cells is reduced by inhibitors of 5-lipoxygenase and its activating protein FLAP. *J. Leukocyte Biol.* **2003**, *73*, 369−378.
(122) Kent, S. E.; Boyce, M.; Diamant, Z.; Singh, D.; O'Connor, B. J.; Saggu, P. S.; Norris, S. The 5-lipoxygenase-activating protein inhibitor, GSK2190915, attenuates the early and late responses to inhaled allergen in mild asthma. *Clin. Exp. Allergy* **2013**, *43*, 177−186.
(123) Liu, J. Y.; Yang, J.; Inceoglu, B.; Qiu, H.; Ulu, A.; Hwang, S. H.; Chiamvimonvat, N.; Hammock, B. D. Inhibition of soluble epoxide hydrolase enhances the anti-inflammatory effects of aspirin and 5-lipoxygenase activation protein inhibitor in a murine model. *Biochem. Pharmacol.* **2010**, *79*, 880−887.
(124) Garscha, U.; Romp, E.; Pace, S.; Rossi, A.; Temml, V.; Schuster, D.; König, S.; Gerstmeier, J.; Liening, S.; Werner, M.; Atze, H.; Wittmann, S.; Weinigel, C.; Rummler, S.; Scriba, G. K.; Sautebin, L.; Werz, O. Pharmacological profile and efficiency in vivo of diflapolin, the first dual inhibitor of 5-lipoxygenase-activating protein and soluble epoxide hydrolase. *Sci. Rep.* **2017**, *7*, 9398.
(125) Vieider, L.; Romp, E.; Temml, V.; Fischer, J.; Kretzer, C.; Schoenthaler, M.; Taha, A.; Hernández-Olmos, V.; Sturm, S.; Schuster, D.; Werz, O.; Garscha, U.; Matuszczak, B. Synthesis, biological evaluation and structure-activity relationships of diflapolin analogues as dual sEH/FLAP inhibitors. *ACS Med. Chem. Lett.* **2019**, *10*, 62−66.
(126) Kodani, S. D.; Wan, D.; Wagner, K. M.; Hwang, S. H.; Morisseau, C.; Hammock, B. D. Design and potency of dual soluble epoxide hydrolase/fatty acid amide hydrolase inhibitors. *ACS Omega* **2018**, *3*, 14076−14086.
(127) Sasso, O.; Wagner, K.; Morisseau, C.; Inceoglu, B.; Hammock, B. D.; Piomelli, D. Peripheral FAAH and soluble epoxide hydrolase inhibitors are synergistically antinociceptive. *Pharmacol. Res.* **2015**, *97*, 7−15.
(128) Spector, A. A.; Norris, A. W. Action of epoxyeicosatrienoic acids on cellular function. *Am. J. Physiol. Cell. Physiol.* **2007**, *292*, C996−C1012.
(129) Imig, J. D.; Walsh, K. A.; Hye Khan, M. A.; Nagasawa, T.; Cherian-Shaw, M.; Shaw, S. M.; Hammock, B. D. Soluble epoxide hydrolase inhibition and peroxisome proliferator activated receptor γ agonist improve vascular function and decrease renal injury in hypertensive obese rats. *Exp. Biol. Med.* **2012**, *237*, 1402−1412.
(130) Blocher, R.; Lamers, C.; Wittmann, S. K.; Merk, D.; Hartmann, M.; Weizel, L.; Diehl, O.; Bruggerhoff, A.; Boss, M.; Kaiser, A.; Schader, T.; Gobel, T.; Grundmann, M.; Angioni, C.; Heering, J.; Geisslinger, G.; Wurglics, M.; Kostenis, E.; Brune, B.; Steinhilber, D.; Schubert-Zsilavecz, M.; Kahnt, A. S.; Proschak, E. *N*-Benzylbenzamides: a novel merged scaffold for orally available dual soluble epoxide hydrolase/peroxisome proliferator-activated receptor gamma modulators. *J. Med. Chem.* **2016**, *59*, 61−81.
(131) Hye Khan, M. A.; Kolb, L.; Skibba, M.; Hartmann, M.; Blocher, R.; Proschak, E.; Imig, J. D. A novel dual PPAR-gamma agonist/sEH inhibitor treats diabetic complications in a rat model of type 2 diabetes. *Diabetologia* **2018**, *61*, 2235−2246.
(132) Schierle, S.; Flauaus, C.; Heitel, P.; Willems, S.; Schmidt, J.; Kaiser, A.; Weizel, L.; Goebel, T.; Kahnt, A. S.; Geisslinger, G.; Steinhilber, D.; Wurglics, M.; Rovati, G. E.; Schmidtko, A.; Proschak, E.; Merk, D. Boosting anti-inflammatory potency of zafirlukast by designed polypharmacology. *J. Med. Chem.* **2018**, *61*, 5758−5764.
(133) Luan, Z. L.; Huo, X. K.; Dong, P. P.; Tian, X. G.; Sun, C. P.; Lv, X.; Feng, L.; Ning, J.; Wang, C.; Zhang, B. J.; Ma, X. C. Highly

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

potent non-steroidal FXR agonists protostane-type triterpenoids: structure-activity relationship and mechanism. *Eur. J. Med. Chem.* **2019**, *182*, 111652.
(134) Schierle, S.; Helmstädter, M.; Schmidt, J.; Hartmann, M.; Horz, M.; Kaiser, A.; Weizel, L.; Heitel, P.; Proschak, A.; Hernandez-Olmos, V.; Proschak, E.; Merk, D. Dual farnesoid X receptor/soluble epoxide hydrolase modulators derived from zafirlukast. *ChemMedChem* **2020**, *15*, 50−67.
(135) Schmidt, J.; Rotter, M.; Weiser, T.; Wittmann, S.; Weizel, L.; Kaiser, A.; Heering, J.; Goebel, T.; Angioni, C.; Wurglics, M.; Paulke, A.; Geisslinger, G.; Kahnt, A.; Steinhilber, D.; Proschak, E.; Merk, D. A dual modulator of farnesoid X receptor and soluble epoxide hydrolase to counter nonalcoholic steatohepatitis. *J. Med. Chem.* **2017**, *60*, 7703−7724.
(136) Nagao, M.; Ishikawa, F.; Tahira, T.; Ochiai, M.; Sugimura, T. Activation of rat and human c-raf(-1) by rearrangement. *Princess Takamatsu Symp.* **1986**, *17*, 75−84.
(137) Chang, F.; Steelman, L. S.; Shelton, J. G.; Lee, J. T.; Navolanic, P. M.; Blalock, W. L.; Franklin, R.; McCubrey, J. A. Regulation of cell cycle progression and apoptosis by the Ras/Raf/MEK/ERK pathway (review). *Int. J. Oncol.* **2003**, *22*, 469−480.
(138) Wilhelm, S.; Chien, D. S. BAY 43-9006: Preclinical data. *Curr. Pharm. Des.* **2002**, *8*, 2255−2257.
(139) Liu, J. Y.; Park, S. H.; Morisseau, C.; Hwang, S. H.; Hammock, B. D.; Weiss, R. H. Sorafenib has soluble epoxide hydrolase inhibitory activity, which contributes to its effect profile *in vivo*. *Mol. Cancer Ther.* **2009**, *8*, 2193−2203.
(140) Liao, J.; Hwang, S. H.; Li, H.; Yang, Y.; Yang, J.; Wecksler, A. T.; Liu, J. Y.; Hammock, B. D.; Yang, G. Y. Inhibition of mutant KrasG12D-initiated murine pancreatic carcinoma growth by a dual c-Raf and soluble epoxide hydrolase inhibitor t-CUPM. *Cancer Lett.* **2016**, *371*, 187−193.
(141) Baell, J. B. Feeling nature's PAINS: natural products, natural product drugs, and pan assay interference compounds (PAINS). *J. Nat. Prod.* **2016**, *79*, 616−628.
(142) Butler, M. S. The role of natural product chemistry in drug discovery. *J. Nat. Prod.* **2004**, *67*, 2141−2153.
(143) Lee, G. H.; Oh, S. J.; Lee, S. Y.; Lee, J. Y.; Ma, J. Y.; Kim, Y. H.; Kim, S. K. Discovery of soluble epoxide hydrolase inhibitors from natural products. *Food Chem. Toxicol.* **2014**, *64*, 225−230.
(144) Liu, Z. B.; Sun, C. P.; Xu, J. X.; Morisseau, C.; Hammock, B. D.; Qiu, F. Phytochemical constituents from *Scutellaria baicalensis* in soluble epoxide hydrolase inhibition: Kinetics and interaction mechanism merged with simulations. *Int. J. Biol. Macromol.* **2019**, *133*, 1187−1193.
(145) Wang, C. Y.; Lee, S.; Jang, H. J.; Su, X. D.; Wang, H. S.; Kim, Y. H.; Yang, S. Y. Inhibition potential of phenolic constituents from the aerial parts of *Tetrastigma hemsleyanum* against soluble epoxide hydrolase and nitric oxide synthase. *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 753−760.
(146) Vinh, L. B.; Kim, J. H.; Lee, J. S.; Nguyet, N. T. M.; Yang, S. Y.; Ma, J. Y.; Kim, Y. H. Soluble epoxide hydrolase inhibitory activity of phenolic glycosides from *Polygala tenuifolia* and in silico approach. *Med. Chem. Res.* **2018**, *27*, 726−734.
(147) Kitamura, S.; Morisseau, C.; Inceoglu, B.; Kamita, S. G.; De Nicola, G. R.; Nyegue, M.; Hammock, B. D. Potent natural soluble epoxide hydrolase inhibitors from *Pentadiplandra brazzeana* Baillon: Synthesis, quantification, and measurement of biological activities *in vitro* and *in vivo*. *PLoS One* **2015**, *10*, No. e0117438.
(148) Tsopmo, A.; Ngnokam, D.; Ngamga, D.; Ayafor, J. F.; Sterner, O. Urea derivatives from *Pentadiplandra brazzeana*. *J. Nat. Prod.* **1999**, *62*, 1435−1436.
(149) Ray, A.; Chand, L.; Dutta, S. C. Salvadourea: New urea derivative from *Salvadora persica*. *Chem. Ind.* **1975**, *12*, 517−518.
(150) Bahroun, A.; Damak, M. Contribution to the study of *Lepidium sativum* (Cruciferae). Structure of a new compound isolated from the seed: lepidine. *J. Soc. Chim. Tunis.* **1985**, *2*, 15−24.
(151) Sashidhara, K. V.; Rosaiah, J. N.; Tyagi, E.; Shukla, R.; Raghubir, R.; Rajendran, S. M. Rare dipeptide and urea derivatives from roots of *Moringa oleifera* as potential anti-inflammatory and antinociceptive agents. *Eur. J. Med. Chem.* **2009**, *44*, 432−436.
(152) Kitamura, S.; Morisseau, C.; Harris, T. R.; Inceoglu, B.; Hammock, B. D. Occurrence of urea-based soluble epoxide hydrolase inhibitors from the plants in the order Brassicales. *PLoS One* **2017**, *12*, No. e0176571.
(153) Kim, J. H.; Jo, Y. D.; Jin, C. H. Isolation of soluble epoxide hydrolase inhibitor of capsaicin analogs from *Capsicum chinense* Jacq. cv. Habanero. *Int. J. Biol. Macromol.* **2019**, *135*, 1202−1207.
(154) Kim, J. H.; Kim, H. Y.; Kang, S. Y.; Kim, Y. H.; Jin, C. H. Soluble epoxide hydrolase inhibitory activity of components isolated from *Apios americana* Medik. *Molecules* **2017**, *22*, 1432.
(155) Thao, N. P.; Luyen, B. T. T.; Kim, J. H.; Jo, A. R.; Yang, S. Y.; Dat, N. T.; Minh, C. V.; Kim, Y. H. Soluble epoxide hydrolase inhibitory activity by rhizomes of *Kaempferia parviflora* Wall. ex Baker. *Med. Chem. Res.* **2016**, *25*, 704−711.
(156) Morgan, A. M. A.; Kim, J. H.; Kim, S. K.; Lim, C.-H.; Kim, Y. H. A new flavonol glycoside from the leaves of *Boscia senegalensis*. *Bull. Korean Chem. Soc.* **2014**, *35*, 3447−3452.
(157) Su, X. D.; Li, W.; Kim, J. E.; Yang, S. Y.; Ma, J. Y.; Kim, Y. H. Prenyl-flavonoids from *Epimedium koreanum* Nakai and their soluble epoxide hydrolase and tyrosinase inhibitory activities. *Med. Chem. Res.* **2017**, *26*, 2761−2767.
(158) Bai, M. M.; Shi, W.; Tian, J. M.; Lei, M.; Kim, J. H.; Sun, Y. N.; Kim, Y. H.; Gao, J. M. Soluble epoxide hydrolase inhibitory and anti-inflammatory components from the leaves of *Eucommia ulmoides* Oliver (duzhong). *J. Agric. Food Chem.* **2015**, *63*, 2198−2205.
(159) Thao, N. P.; Luyen, B. T. T.; Lee, J. S.; Kim, J. H.; Dat, N. T.; Kim, Y. H. Inhibition potential of cycloartane-type glycosides from the roots of *Cimicifuga dahurica* against soluble epoxide hydrolase. *J. Nat. Prod.* **2017**, *80*, 1867−1875.
(160) Thao, N. P.; Kim, J. H.; Thuy Luyen, B. T.; Dat, N. T.; Kim, Y. H. In silico investigation of cycloartane triterpene derivatives from *Cimicifuga dahurica* (Turcz.) Maxim. roots for the development of potent soluble epoxide hydrolase inhibitors. *Int. J. Biol. Macromol.* **2017**, *98*, 526−534.
(161) Sun, C. P.; Zhang, J.; Zhao, W. Y.; Yi, J.; Yan, J. K.; Wang, Y. L.; Morisseau, C.; Liu, Z. B.; Hammock, B. D.; Ma, X. C. Protostane-type triterpenoids as natural soluble epoxide hydrolase inhibitors: Inhibition potentials and molecular dynamics. *Bioorg. Chem.* **2020**, *96*, 103637.
(162) Thao, N. P.; Luyen, B. T.; Lee, J. S.; Kim, J. H.; Kim, Y. H. Soluble epoxide hydrolase inhibitors of indolinone alkaloids and phenolic derivatives from *Cimicifuga dahurica* (Turcz.) Maxim. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 1874−1879.
(163) Li, W.; Kim, J. H.; Zhou, W.; Shim, S. H.; Ma, J. Y.; Kim, Y. H. Soluble epoxide hydrolase inhibitory activity of phenolic components from the rhizomes and roots of *Gentiana scabra*. *Biosci., Biotechnol., Biochem.* **2015**, *79*, 907−911.
(164) Sun, Y. N.; Kim, J. H.; Li, W.; Jo, A. R.; Yan, X. T.; Yang, S. Y.; Kim, Y. H. Soluble epoxide hydrolase inhibitory activity of anthraquinone components from *Aloe*. *Bioorg. Med. Chem.* **2015**, *23*, 6659−6665.
(165) Sun, Y. N.; Li, W.; Kim, J. H.; Yan, X. T.; Kim, J. E.; Yang, S. Y.; Kim, Y. H. Chemical constituents from the root of *Polygonum multiflorum* and their soluble epoxide hydrolase inhibitory activity. *Arch. Pharmacal Res.* **2015**, *38*, 998−1004.
(166) Jo, A. R.; Kim, J. H.; Yan, X. T.; Yang, S. Y.; Kim, Y. H. Soluble epoxide hydrolase inhibitory components from *Rheum undulatum* and in silico approach. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 70−78.
(167) Kim, J. H.; Morgan, A. M.; Tai, B. H.; Van, D. T.; Cuong, N. M.; Kim, Y. H. Inhibition of soluble epoxide hydrolase activity by compounds isolated from the aerial parts of *Glycosmis stenocarpa*. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 640−644.
(168) Khanh, P. N.; Duc, H. V.; Huong, T. T.; Son, N. T.; Ha, V. T.; Van, D. T.; Tai, B. H.; Kim, J. E.; Jo, A. R.; Kim, Y. H.; Cuong, N. M. Alkylphloroglucinol derivatives and triterpenoids with soluble epoxide

hydrolase inhibitory activity from *Callistemon citrinus*. *Fitoterapia* **2016**, *109*, 39−44.
(169) Lee, G. Y.; Kim, J. H.; Choi, S. K.; Kim, Y. H. Constituents of the seeds of *Cassia tora* with inhibitory activity on soluble expoxide hydrolease. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 5097−5101.
(170) Li, H. Q.; Lv, P. C.; Yan, T.; Zhu, H. L. Urea derivatives as anticancer agents. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 471−480.
(171) Jagla, C. A. D.; Scott, C. E.; Tang, Y.; Qiao, C.; Mateo-Semidey, G. E.; Yudowski, G. A.; Lu, D.; Kendall, D. A. Pyrimidinyl biphenylureas act as allosteric modulators to activate cannabinoid receptor 1 and initiate beta-arrestin-dependent responses. *Mol. Pharmacol.* **2019**, *95*, 1−10.
(172) Liang, Z.; Zhang, B.; Xu, M.; Morisseau, C.; Hwang, S. H.; Hammock, B. D.; Li, Q. X. 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea, a selective and potent dual inhibitor of soluble epoxide hydrolase and p38 kinase intervenes in Alzheimer's signaling in human nerve cells. *ACS Chem. Neurosci.* **2019**, *10*, 4018−4030.
(173) Lazaar, A. L.; Yang, L.; Boardley, R. L.; Goyal, N. S.; Robertson, J.; Baldwin, S. J.; Newby, D. E.; Wilkinson, I. B.; Tal-Singer, R.; Mayer, R. J.; Cheriyan, J. Pharmacokinetics, pharmacodynamics and adverse event profile of GSK2256294, a novel soluble epoxide hydrolase inhibitor. *Br. J. Clin. Pharmacol.* **2016**, *81*, 971−979.
(174) Imig, J. D. Epoxyeicosatrienoic acids and 20-hydroxyeicosatetraenoic acid on endothelial and vascular function. *Adv. Pharmacol.* **2016**, *77*, 105−141.
(175) Lee, H. T.; Lee, K. I.; Chen, C. H.; Lee, T. S. Genetic deletion of soluble epoxide hydrolase delays the progression of Alzheimer's disease. *J. Neuroinflammation* **2019**, *16*, 267.
(176) Kodani, S. D.; Morisseau, C. Role of epoxy-fatty acids and epoxide hydrolases in the pathology of neuro-inflammation. *Biochimie* **2019**, *159*, 59−65.
(177) Inceoglu, B.; Bettaieb, A.; Haj, F. G.; Gomes, A. V.; Hammock, B. D. Modulation of mitochondrial dysfunction and endoplasmic reticulum stress are key mechanisms for the wide-ranging actions of epoxy fatty acids and soluble epoxide hydrolase inhibitors. *Prostaglandins Other Lipid Mediators* **2017**, *133*, 68−78.
(178) Marshall, I. J.; Wolfe, C. D.; McKevitt, C. Lay perspectives on hypertension and drug adherence: systematic review of qualitative research. *BMJ.* **2012**, *345*, No. e3953.
(179) Imig, J. D.; Hammock, B. D. Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases. *Nat. Rev. Drug Discovery* **2009**, *8*, 794−805.
(180) Gebremedhin, D.; Ma, Y. H.; Falck, J. R.; Roman, R. J.; VanRollins, M.; Harder, D. R. Mechanism of action of cerebral epoxyeicosatrienoic acids on cerebral arterial smooth muscle. *Am. J. Physiol.* **1992**, *263*, H519−H525.
(181) Li, P. L.; Zhang, D. X.; Ge, Z. D.; Campbell, W. B. Role of ADP-ribose in 11,12-EET-induced activation of K(Ca) channels in coronary arterial smooth muscle cells. *Am. J. Physiol. Heart Circ. Physiol.* **2002**, *282*, H1229−H1236.
(182) Li, P. L.; Chen, C. L.; Bortell, R.; Campbell, W. B. 11,12-Epoxyeicosatrienoic acid stimulates endogenous mono-ADP-ribosylation in bovine coronary arterial smooth muscle. *Circ. Res.* **1999**, *85*, 349−356.
(183) Archer, S. L.; Gragasin, F. S.; Wu, X.; Wang, S.; McMurtry, S.; Kim, D. H.; Platonov, M.; Koshal, A.; Hashimoto, K.; Campbell, W. B.; Falck, J. R.; Michelakis, E. D. Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK(Ca) channels. *Circulation* **2003**, *107*, 769−776.
(184) Fisslthaler, B.; Popp, R.; Kiss, L.; Potente, M.; Harder, D. R.; Fleming, I.; Busse, R. Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* **1999**, *401*, 493−497.
(185) Imig, J. D.; Inscho, E. W.; Deichmann, P. C.; Reddy, K. M.; Falck, J. R. Afferent arteriolar vasodilation to the sulfonimide analog of 11, 12-epoxyeicosatrienoic acid involves protein kinase A. *Hypertension* **1999**, *33*, 408−413.
(186) Zhao, X. Y.; Yamamoto, T.; Newman, J. W.; Kim, I. H.; Watanabe, T.; Hammock, B. D.; Stewart, J.; Pollock, J. S.; Pollock, D. M.; Imig, J. D. Soluble epoxide hydrolase inhibition protects the kidney from hypertension-induced damage. *J. Am. Soc. Nephrol.* **2004**, *15*, 1244−1253.
(187) Ashraf, J. M.; Ansari, M. A.; Fatma, S.; Abdullah, S. M. S.; Iqbal, J.; Madkhali, A.; Hamali, A. H.; Ahmad, S.; Jerah, A.; Echeverria, V.; Barreto, G. E.; Ashraf, G. M. Inhibiting effect of zinc oxide nanoparticles on advanced glycation products and oxidative modifications: a potential tool to counteract oxidative stress in neurodegenerative diseases. *Mol. Neurobiol.* **2018**, *55*, 7438−7452.
(188) Imig, J. D. Cardiovascular therapeutic aspects of soluble epoxide hydrolase inhibitors. *Cardiovasc. Drug Rev.* **2006**, *24*, 169−188.
(189) Ai, D.; Shyy, J. Y.; Zhu, Y. Linking an insect enzyme to hypertension: angiotensin II-epoxide hydrolase interactions. *Kidney Int.* **2010**, *77*, 88−92.
(190) Ai, D.; Fu, Y.; Guo, D. L.; Tanaka, H.; Wang, N. P.; Tang, C. S.; Hammock, B. D.; Shyy, J. Y. J.; Zhu, Y. Angiotensin II up-regulates soluble epoxide hydrolase in vascular endothelium *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 9018−9023.
(191) Sinal, C. J.; Miyata, M.; Tohkin, M.; Nagata, K.; Bend, J. R.; Gonzalez, F. J. Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J. Biol. Chem.* **2000**, *275*, 40504−40510.
(192) Bukhari, I. A.; Alorainey, B. I.; Al-Motrefi, A. A.; Mahmoud, A.; Campbell, W. B.; Hammock, B. D. 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU), a soluble epoxide hydrolase inhibitor, lowers L-NAME-induced hypertension through suppression of angiotensin-converting enzyme in rats. *Eur. Rev. Med. Pharmacol. Sci.* **2020**, *15*, 8143−8150.
(193) Imig, J. D.; Zhao, X.; Zaharis, C. Z.; Olearczyk, J. J.; Pollock, D. M.; Newman, J. W.; Kim, I. H.; Watanabe, T.; Hammock, B. D. An orally active epoxide hydrolase inhibitor lowers blood pressure and provides renal protection in salt-sensitive hypertension. *Hypertension* **2005**, *46*, 975−981.
(194) Aga, E. B.; Li, H. J.; Chen, J.; Li, P. Chemical constituents from the aerial parts of *Codonopsis nervosa*. *Chin. J. Nat. Med.* **2012**, *10*, 366−369.
(195) Li, D.; Liu, Y.; Zhang, X.; Lv, H.; Pang, W.; Sun, X.; Gan, L. M.; Hammock, B. D.; Ai, D.; Zhu, Y. Inhibition of soluble epoxide hydrolase alleviated atherosclerosis by reducing monocyte infiltration in Ldlr(−/−) mice. *J. Mol. Cell. Cardiol.* **2016**, *98*, 128−137.
(196) Zhang, L. N.; Vincelette, J.; Cheng, Y.; Mehra, U.; Chen, D.; Anandan, S. K.; Gless, R.; Webb, H. K.; Wang, Y. X. Inhibition of soluble epoxide hydrolase attenuated atherosclerosis, abdominal aortic aneurysm formation, and dyslipidemia. *Arterioscler., Thromb., Vasc. Biol.* **2009**, *29*, 1265−1270.
(197) Xu, D. Y.; Davis, B. B.; Wang, Z. H.; Zhao, S. P.; Wasti, B.; Liu, Z. L.; Li, N.; Morisseau, C.; Chiamvimonvat, N.; Hammock, B. D. A potent soluble epoxide hydrolase inhibitor, *t*-AUCB, acts through PPARgamma to modulate the function of endothelial progenitor cells from patients with acute myocardial infarction. *Int. J. Cardiol.* **2013**, *167*, 1298−1304.
(198) Shen, L.; Peng, H.; Peng, R.; Fan, Q.; Zhao, S.; Xu, D.; Morisseau, C.; Chiamvimonvat, N.; Hammock, B. D. Inhibition of soluble epoxide hydrolase in mice promotes reverse cholesterol transport and regression of atherosclerosis. *Atherosclerosis* **2015**, *239*, 557−65.
(199) Qiu, H.; Li, N.; Liu, J. Y.; Harris, T. R.; Hammock, B. D.; Chiamvimonvat, N. Soluble epoxide hydrolase inhibitors and heart failure. *Cardiovasc. Ther.* **2011**, *29*, 99−111.
(200) Seubert, J. M.; Sinal, C. J.; Graves, J.; DeGraff, L. M.; Bradbury, J. A.; Lee, C. R.; Goralski, K.; Carey, M. A.; Luria, A.; Newman, J. W.; Hammock, B. D.; Falck, J. R.; Roberts, H.; Rockman, H. A.; Murphy, E.; Zeldin, D. C. Role of soluble epoxide hydrolase in postischemic recovery of heart contractile function. *Circ. Res.* **2006**, *99*, 442−450.

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215

(201) Kompa, A. R.; Wang, B. H.; Xu, G.; Zhang, Y.; Ho, P. Y.; Eisennagel, S.; Thalji, R. K.; Marino, J. P., Jr.; Kelly, D. J.; Behm, D. J.; Krum, H. Soluble epoxide hydrolase inhibition exerts beneficial anti-remodeling actions post-myocardial infarction. *Int. J. Cardiol.* **2013**, *167*, 210−219.
(202) Zhang, H.; Zhang, K.; Liang, J.; Yan, W.; Wu, F.; Xu, W.; Wu, Z.; Chen, Y.; Pan, R.; Wu, G. Soluble epoxide hydrolase inhibitor, TUPS, attenuates isoproterenol/angiotensin II-induced cardiac hypertrophy through mammalian target of rapamycin-mediated autophagy inhibition. *J. Pharm. Pharmacol.* **2019**, *71*, 1291−1300.
(203) Lan, Y. L.; Zhou, J. J.; Liu, J.; Huo, X. K.; Wang, Y. L.; Liang, J. H.; Zhao, J. C.; Sun, C. P.; Yu, Z. L.; Fang, L. L.; Tian, X. G.; Feng, L.; Ning, J.; Zhang, B. J.; Wang, C.; Zhao, X. Y.; Ma, X. C. *Uncaria rhynchophylla* ameliorates Parkinson's disease by inhibiting HSP90 expression: insights from quantitative proteomics. *Cell. Physiol. Biochem.* **2018**, *47*, 1453−1464.
(204) Liang, J. H.; Luan, Z. L.; Tian, X. G.; Zhao, W. Y.; Wang, Y. L.; Sun, C. P.; Huo, X. K.; Deng, S.; Zhang, B. J.; Zhang, Z. J.; Ma, X. C. Uncarialins A-I, monoterpenoid indole alkaloids from *Uncaria rhynchophylla* as natural agonists of the 5-HT_{1A} receptor. *J. Nat. Prod.* **2019**, *82*, 3302−3310.
(205) Bosch, P.; van den Noort, M.; Staudte, H.; Lim, S. Schizophrenia and depression: a systematic review of the effectiveness and the working mechanisms behind acupuncture. *Explore* **2015**, *11*, 281−291.
(206) Ren, Q.; Ma, M.; Yang, J.; Nonaka, R.; Yamaguchi, A.; Ishikawa, K.-i.; Kobayashi, K.; Murayama, S.; Hwang, S. H.; Saiki, S.; Akamatsu, W.; Hattori, N.; Hammock, B. D.; Hashimoto, K. Soluble epoxide hydrolase plays a key role in the pathogenesis of Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, E5815−E5823.
(207) Qin, X.; Wu, Q.; Lin, L.; Sun, A.; Liu, S.; Li, X.; Cao, X.; Gao, T.; Luo, P.; Zhu, X.; Wang, X. Soluble epoxide hydrolase deficiency or inhibition attenuates MPTP-induced parkinsonism. *Mol. Neurobiol.* **2015**, *52*, 187−195.
(208) Krstic, D.; Knuesel, I. Deciphering the mechanism underlying late-onset Alzheimer disease. *Nat. Rev. Neurol.* **2013**, *9*, 25−34.
(209) Calsolaro, V.; Edison, P. Neuroinflammation in Alzheimer's disease: current evidence and future directions. *Alzheimer's Dementia* **2016**, *12*, 719−732.
(210) Fu, W. Y.; Wang, X. J.; Ip, N. Y. Targeting neuroinflammation as a therapeutic strategy for Alzheimer's disease: mechanisms, drug candidates, and new opportunities. *ACS Chem. Neurosci.* **2019**, *10*, 872−879.
(211) Kodamullil, A. T.; Zekri, F.; Sood, M.; Hengerer, B.; Canard, L.; McHale, D.; Hofmann-Apitius, M. Trial watch: tracing investment in drug development for Alzheimer disease. *Nat. Rev. Drug Discovery* **2017**, *16*, 819.
(212) Tonnies, E.; Trushina, E. Oxidative stress, synaptic dysfunction, and Alzheimer's disease. *J. Alzheimer's Dis.* **2017**, *57*, 1105−1121.
(213) Barnham, K. J.; Masters, C. L.; Bush, A. I. Neurodegenerative diseases and oxidative stress. *Nat. Rev. Drug Discovery* **2004**, *3*, 205−214.
(214) Reis-Dennis, S. Review of rethinking health care ethics by Stephen Scher and Kasia Kozlowska. *Monash. Bioeth. Rev.* **2020**, *38*, 83−86.
(215) Morley, J. E.; Farr, S. A.; Flood, J. F. Antibody to amyloid beta protein alleviates impaired acquisition, retention, and memory processing in SAMP8 mice. *Neurobiol. Learn. Mem.* **2002**, *78*, 125−138.
(216) Takeda, T. Senescence-accelerated mouse (SAM) with special references to neurodegeneration models, SAMP8 and SAMP10 mice. *Neurochem. Res.* **2009**, *34*, 639−659.
(217) Akiguchi, I.; Pallas, M.; Budka, H.; Akiyama, H.; Ueno, M.; Han, J.; Yagi, H.; Nishikawa, T.; Chiba, Y.; Sugiyama, H.; Takahashi, R.; Unno, K.; Higuchi, K.; Hosokawa, M. SAMP8 mice as a neuropathological model of accelerated brain aging and dementia: Toshio Takeda's legacy and future directions. *Neuropathology* **2017**, *37*, 293−305.
(218) Grinan-Ferre, C.; Palomera-Avalos, V.; Puigoriol-Illamola, D.; Camins, A.; Porquet, D.; Pla, V.; Aguado, F.; Pallas, M. Behaviour and cognitive changes correlated with hippocampal neuroinflammaging and neuronal markers in female SAMP8, a model of accelerated senescence. *Exp. Gerontol.* **2016**, *80*, 57−69.
(219) Pallàs, M. Senescence-accelerated mice P8: a tool to study brain aging and Alzheimer's disease in a mouse model. *ISRN Cell Biol.* **2012**, *2012*, 917167.
(220) Grinan-Ferre, C.; Codony, S.; Pujol, E.; Yang, J.; Leiva, R.; Escolano, C.; Puigoriol-Illamola, D.; Companys-Alemany, J.; Corpas, R.; Sanfeliu, C.; Perez, B.; Loza, M. I.; Brea, J.; Morisseau, C.; Hammock, B. D.; Vazquez, S.; Pallas, M.; Galdeano, C. Pharmacological inhibition of soluble epoxide hydrolase as a new therapy for Alzheimer's disease. *Neurotherapeutics* **2020**, DOI: 10.1007/s13311-020-00854-1.
(221) Hung, C. C.; Lee, Y. H.; Kuo, Y. M.; Hsu, P. C.; Tsay, H. J.; Hsu, Y. T.; Lee, C. C.; Liang, J. J.; Shie, F. S. Soluble epoxide hydrolase modulates immune responses in activated astrocytes involving regulation of STAT3 activity. *J. Neuroinflammation* **2019**, *16*, 123.
(222) Ren, Q. Soluble epoxide hydrolase inhibitor: a novel potential therapeutic or prophylactic drug for psychiatric disorders. *Front. Pharmacol.* **2019**, *10*, 420.
(223) Wu, Q.; Cai, H.; Song, J.; Chang, Q. The effects of sEH inhibitor on depression-like behavior and neurogenesis in male mice. *J. Neurosci. Res.* **2017**, *95*, 2483−2492.
(224) Ma, M.; Ren, Q.; Fujita, Y.; Ishima, T.; Zhang, J. C.; Hashimoto, K. Effects of AS2586114, a soluble epoxide hydrolase inhibitor, on hyperlocomotion and prepulse inhibition deficits in mice after administration of phencyclidine. *Pharmacol., Biochem. Behav.* **2013**, *110*, 98−103.
(225) Swardfager, W.; Hennebelle, M.; Yu, D.; Hammock, B. D.; Levitt, A. J.; Hashimoto, K.; Taha, A. Y. Metabolic/inflammatory/vascular comorbidity in psychiatric disorders; soluble epoxide hydrolase (sEH) as a possible new target. *Neurosci. Biobehav. Rev.* **2018**, *87*, 56−66.
(226) Tu, R.; Armstrong, J.; Lee, K. S. S.; Hammock, B. D.; Sapirstein, A.; Koehler, R. C. Soluble epoxide hydrolase inhibition decreases reperfusion injury after focal cerebral ischemia. *Sci. Rep.* **2018**, *8*, 5279.
(227) Finneran, D. J.; Nash, K. R. Neuroinflammation and fractalkine signaling in Alzheimer's disease. *J. Neuroinflammation* **2019**, *16*, 30.
(228) Jouihan, S. A.; Zuloaga, K. L.; Zhang, W.; Shangraw, R. E.; Krasnow, S. M.; Marks, D. L.; Alkayed, N. J. Role of soluble epoxide hydrolase in exacerbation of stroke by streptozotocin-induced type 1 diabetes mellitus. *J. Cereb. Blood Flow Metab.* **2013**, *33*, 1650−1656.
(229) Chen, G. Z.; Xu, R. F.; Wang, Y. N.; Wang, P. H.; Zhao, G.; Xu, X. Z.; Gruzdev, A.; Zeldin, D. C.; Wang, D. W. Genetic disruption of soluble epoxide hydrolase is protective against streptozotocin-induced diabetic nephropathy. *Am. J. Physiol. Endocrinol. Metab.* **2012**, *303*, E563−E575.
(230) Elmarakby, A. A.; Faulkner, J.; Al-Shabrawey, M.; Wang, M. H.; Maddipati, K. R.; Imig, J. D. Deletion of soluble epoxide hydrolase gene improves renal endothelial function and reduces renal inflammation and injury in streptozotocin-induced type 1 diabetes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *301*, R1307−R1317.
(231) Dewey, S.; Lai, X. Y.; Witzmann, F. A.; Sohal, M.; Gomes, A. V. Proteomic analysis of hearts from akita mice suggests that increases in soluble epoxide hydrolase and antioxidative programming are key changes in early stages of diabetic cardiomyopathy. *J. Proteome Res.* **2013**, *12*, 3920−3933.
(232) Liu, Y.; Dang, H.; Li, D.; Pang, W.; Hammock, B. D.; Zhu, Y. Inhibition of soluble epoxide hydrolase attenuates high-fat-diet-induced hepatic steatosis by reduced systemic inflammatory status in mice. *PLoS One* **2012**, *7*, No. e39165.
(233) Yao, L.; Cao, B.; Cheng, Q.; Cai, W.; Ye, C.; Liang, J.; Liu, W.; Tan, L.; Yan, M.; Li, B.; He, J.; Hwang, S. H.; Zhang, X.; Wang, C.; Ai, D.; Hammock, B. D.; Zhu, Y. Inhibition of soluble epoxide

hydrolase ameliorates hyperhomocysteinemia-induced hepatic steatosis by enhancing beta-oxidation of fatty acid in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2019**, *316*, G527−G538.
(234) Moreira da Silva, R.; Verjee, S.; de Gaitani, C. M.; Moraes de Oliveira, A. R.; Pires Bueno, P. C.; Cavalheiro, A. J.; Peporine Lopes, N.; Butterweck, V. Evaluation of the intestinal absorption mechanism of casearin X in Caco-2 cells with modified carboxylesterase activity. *J. Nat. Prod.* **2016**, *79*, 1084−1090.
(235) Chen, L.; Fan, C.; Zhang, Y.; Bakri, M.; Dong, H.; Morisseau, C.; Maddipati, K. R.; Luo, P.; Wang, C. Y.; Hammock, B. D.; Wang, M. H. Beneficial effects of inhibition of soluble epoxide hydrolase on glucose homeostasis and islet damage in a streptozotocin-induced diabetic mouse model. *Prostaglandins Other Lipid Mediators* **2013**, *104*, 42−48.
(236) Pardeshi, R.; Bolshette, N.; Gadhave, K.; Arfeen, M.; Ahmed, S.; Jamwal, R.; Hammock, B. D.; Lahkar, M.; Goswami, S. K. Docosahexaenoic acid increases the potency of soluble epoxide hydrolase inhibitor in alleviating streptozotocin-induced Alzheimer's disease-like complications of diabetes. *Front. Pharmacol.* **2019**, *10*, 288.
(237) Luo, P. C.; Chang, H. H.; Zhou, Y. Q.; Zhang, S. L.; Hwang, S. H.; Morisseau, C.; Wang, C. Y.; Inscho, E. W.; Hammock, B. D.; Wang, M. H. Inhibition or deletion of soluble epoxide hydrolase prevents hyperglycemia, promotes insulin secretion, and reduces islet apoptosis. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 430−438.
(238) Luria, A.; Bettaieb, A.; Xi, Y. N.; Shieh, G. J.; Liu, H. C.; Inoue, H.; Tsai, H. J.; Imig, J. D.; Haj, F. G.; Hammock, B. D. Soluble epoxide hydrolase deficiency alters pancreatic islet size and improves glucose homeostasis in a model of insulin resistance. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 9038−9043.
(239) Hu, J.; Popp, R.; Frömel, T.; Ehling, M.; Awwad, K.; Adams, R. H.; Hammes, H. P.; Fleming, I. Müller glia cells regulate Notch signaling and retinal angiogenesis via the generation of 19,20-dihydroxydocosapentaenoic acid. *J. Exp. Med.* **2014**, *211*, 281−295.
(240) Hu, J.; Dziumbla, S.; Lin, J.; Bibli, S. I.; Zukunft, S.; de Mos, J.; Awwad, K.; Fromel, T.; Jungmann, A.; Devraj, K.; Cheng, Z.; Wang, L.; Fauser, S.; Eberhart, C. G.; Sodhi, A.; Hammock, B. D.; Liebner, S.; Muller, O. J.; Glaubitz, C.; Hammes, H. P.; Popp, R.; Fleming, I. Inhibition of soluble epoxide hydrolase prevents diabetic retinopathy. *Nature* **2017**, *552*, 248−252.
(241) Morisseau, C.; Hammock, B. D. Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 37−58.
(242) Sodhi, K.; Inoue, K.; Gotlinger, K. H.; Canestraro, M.; Vanella, L.; Kim, D. H.; Manthati, V. L.; Koduru, S. R.; Falck, J. R.; Schwartzman, M. L.; Abraham, N. G. Epoxyeicosatrienoic acid agonist rescues the metabolic syndrome phenotype of HO-2-null mice. *J. Pharmacol. Exp. Ther.* **2009**, *331*, 906−916.
(243) Warner, J.; Hardesty, J.; Zirnheld, K.; McClain, C.; Warner, D.; Kirpich, I. Soluble epoxide hydrolase inhibition in liver diseases: a review of current research and knowledge gaps. *Biology* **2020**, *9*, 124.
(244) Lopez-Vicario, C.; Alcaraz-Quiles, J.; Garcia-Alonso, V.; Rius, B.; Hwang, S. H.; Titos, E.; Lopategi, A.; Hammock, B. D.; Arroyo, V.; Claria, J. Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: role for omega-3 epoxides. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 536−541.

https://dx.doi.org/10.1021/acs.jmedchem.0c01507
J. Med. Chem. 2021, 64, 184−215