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Soluble Epoxide Hydrolase

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Synonyms

EPHX2; Epoxide hydrolase 2; sEH

Definition

The epoxide hydrolase (EH) family of enzymes is responsible for converting the epoxide residue of various endogenous and exogenous compounds to the corresponding vicinal diols (Morisseau and ammock 2005). Soluble epoxide hydrolase (sEH) is an enzyme in the α-/β-hydrolase fold protein family, primarily found in the cytosolic and peroxisomal fractions of cells (Chiamvimonvat et al. 2007). It is encoded by the EPHX2 gene. It is a bifunctional homodimer that has EH activity at the C-terminal domain and phosphatase activity at the N-terminal domain (He et al. 2016). sEH is abundantly expressed in the liver, but it is also present in most organs including the kidney, brain, smooth muscle, intestines, and lungs (Morisseau and Hammock 2005). Its substrate selectivity is largely restricted to noncyclic 1,2-disubstituted epoxides, and hence, unlike the microsomal epoxide hydrolase (mEH), it is generally not involved in the metabolism or detoxification of xenobiotics (Morisseau and Hammock 2005). On the other hand, polyunsaturated fatty acid (PUFA)-derived bioactive lipid mediators are usually good sEH substrates and are subject to hydrolysis. These epoxy fatty acids (EpFAs) have demonstrated numerous tissue healing and organ-protective effects. sEH inhibitors (sEHI) help stabilize endogenous levels of EpFAs, thus enhancing their bioavailability and facilitating their biological action. Hence, targeting sEH with novel and potent small-molecule inhibitors is a pharmacological tool employed to capitalize on the beneficial effects of EpFAs and potentially treat several disease conditions (Morisseau and Hammock 2005). Alternative approaches involve (i) administration of EpFAs themselves, (ii) synthesis and application of EpFA mimics, and (iii) stimulation of EpFA biosynthesis.

Basic Characteristics

Metabolism of PUFAs

Arachidonic acid (20:4, ω-6) is a PUFA derived from the phospholipids of cell membranes and is metabolized by three main enzymatic pathways (Fig. 1) (Imig and Hammock 2009). The cyclooxygenase (COX) and lipoxygenase (LOX) routes generate largely pro-inflammatory mediators and are the target of several pharmaceuticals currently available on the market (e.g., nonsteroidal anti-

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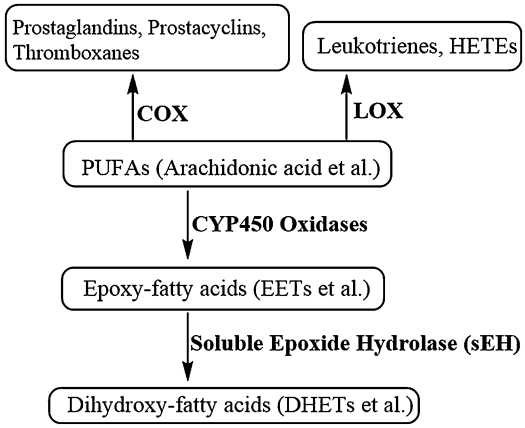


Soluble Epoxide Hydrolase, Fig. 1 Schematic depicting major pathways of polyunsaturated fatty acid (PUFA) metabolism

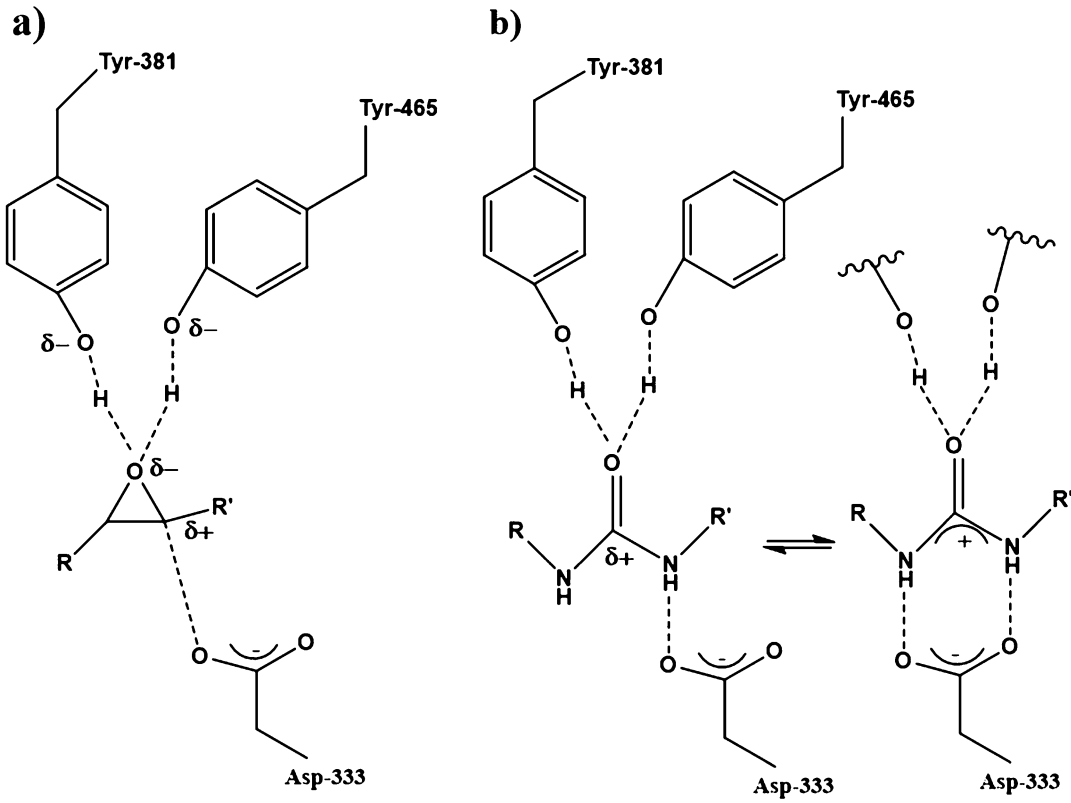
inflammatory drugs a.k.a. NSAIDs and selective COX-2 inhibitors a.k.a. COXIBs). The third pathway primarily involves cytochrome P450-mediated epoxidation to epoxy metabolites known as epoxyeicosatrienoic acids (EETs). Other ω-3 PUFAs such as docosahexaenoic acid (DHA, 22:6) and eicosapentaenoic acid (EPA, 20:5) are similarly metabolized, and the CYP450 branch generates epoxydocosapentaenoic acids (EDPs) and epoxyeicosatetraenoic acids (EEQs), respectively (He et al. 2016). EETs and other EpFAs are mono-epoxides that are chemical mediators and act in both autocrine and paracrine manners. However, the chemically stable EpFAs have transient metabolic half-lives due to rapid sEH-mediated degradation. Recently, epoxides of omega-3-derived endocannabinoids with anti-inflammatory, anti-tumorigenic, and analgesic function have been reported, suggesting a still broader role of sEH in regulatory biology (Das et al. 2019).

Hydrolysis of EpFAs

sEH hydrolyzes EETs and other EpFAs by an exothermic, two-step base-catalyzed mechanism (Morisseau and Hammock 2005). It involves the formation of a covalent hydroxyl-alkyl-enzyme ester intermediate via a catalytic aspartic acid, followed by the attack of water on the enzyme ester carbon that releases the 1,2-diol product. Within the hydrophobic active site, two tyrosine residues hydrogen bond to the epoxide oxygen, polarizing it. A nucleophilic aspartate acid conducts a backside attack on the epoxide, forming the covalent intermediate, while an additional aspartate residue acts as orienting and activating acid for the histidine general base. This activates a water molecule which conducts the described hydrolysis step. sEH metabolism of EpFAs is regioselective and stereoselective (Chiamvimonvat et al. 2007). For example, the EET regioisomer with the epoxide furthest from the carboxylic acid end (14,15-EET) is hydrolyzed the fastest, while the one closest is hydrolyzed most slowly (5,6-EET).

Mechanism of sEH Inhibition

Enzyme inhibitors acting to mimic reaction intermediates or transition states have been found as potent inhibitors in many α-/β-hydrolase fold enzymes (Morisseau and Hammock 2005). Based on the catalytic mechanism of sEH, the transition states of epoxide ring opening are effectively mimicked by the urea functional group (Fig. 2). The C=O moiety accepts hydrogen bonds from the two tyrosine residues and hence acts as a surrogate for the epoxide oxygen. One mechanism suggests that one of the urea NH groups donates a hydrogen bond to the nucleophilic aspartate, mimicking the electrophilic center (Fig. 2). Alternatively, both NH groups donate hydrogen bonds to the aspartate, stabilizing a partial salt bridge with the enzyme, and thus mimic the interaction between the enzyme and substrate during the transition states (Fig. 2). Accordingly, a series of small-molecule 1,3-disubstituted ureas as well as amides, carbamates, and heterocycles have been developed which act as slow, tight-binding, competitive, and highly potent (low nanomolar to picomolar IC_{50}) inhibitors of sEH (Morisseau and Hammock 2005). The functional groups attached to the primary urea pharmacophore are generally hydrophobic to allow effective interaction with the sEH active site, with a smaller group on one side and a typically larger group on the other. A polar moiety (e.g., ether, ester, alcohol, or ketone) added to the side with the larger group can act as a secondary pharmacophore and improve water



Soluble Epoxide Hydrolase, Fig. 2 (**a**) Transition state of epoxide ring opening within the sEH active site. (**b**) Two possible binding modes of 1,3-disubstituted urea inhibitors mimicking multiple transition states and reaction intermediates along the reaction coordinate

solubility, pharmacokinetic parameters, and drug formulation properties, without lowering its potency (Imig and Hammock 2009).

Biological Action of EETs and EpFAs

The inhibition of sEH has yielded therapeutic potential in models for various inflammatory, hypertensive, metabolic, fibrotic, and neurodegenerative diseases. The physiological effects of sEH inhibitors stem from the upregulation and subsequent action of EpFAs. The lack of a defined receptor for EETs and other EpFAs makes the assessment of the specific mode of action more challenging. Nevertheless, EpFAs are known to exert several biological effects and are hypothesized to act through multiple pathways.

Inhibition of sEH attenuates and resolves chronic inflammation through multiple pathways (Imig and Hammock 2009). Certain EETs such as the 11,12-regioisomer prevent nuclear translation of NF-κB and thereby block transcription of genes that encode pro-inflammatory enzymes (e.g., iNOS, LOX-5, COX-2, PGE2 synthase). 11,12-EET reduces IKK activity, which decreases phosphorylation and degradation of IκB, allowing it to sequester NF-κB in the cytosol. Other pathways include STAT3 tyrosine-705 phosphorylation by 14,15-EET, PPAR-alpha and PPAR-gamma agonism, inhibition of cytokine-induced VCAM-1, reduced TNFα secretion and, as discussed later, the endoplasmic reticulum (ER) stress response.

sEH inhibition has also been associated with smooth muscle relaxation and vasodilatory function (Imig and Hammock 2009). EETs, specifically the 11,12- and 14,15-regioisomers, are

endothelium-derived hyperpolarizing factors (EDHFs). They stimulate calcium-activated potassium channels (K_{Ca}^{+}), triggering an efflux of K^{+} from vascular smooth muscle cells. This results in membrane hyperpolarization and subsequent dilation of blood vessels. cAMP activation of protein kinase A (PKA) and ADP ribosylation of the alpha subunit of G_s are mechanisms by which EETs have been demonstrated to activate K_{Ca}^{+} channels and promote vascular relaxation (Imig and Hammock 2009).

Disruptions in endoplasmic reticulum (ER) homeostasis caused by reactive oxygen species (ROS), high glucose, or xenobiotic perturbations result in ER stress. The ability of EpFAs to attenuate ER stress and downregulate the triggered unfolded protein response (UPR) pathways has been shown to underlie the amelioration of multiple metabolic disorders including fibrosis, inflammatory, and neuropathic pain (Fig. 3) (Inceoglu et al. 2017). sEHI modulation of the PERK/eif2α branch of the UPR enhances systemic insulin sensitivity and improves glucose tolerance (Inceoglu et al. 2017). It is also considered to play a role in ensuring proper pancreatic exocrine functionality and preventing pancreatitis. Mitigation of the IRE1α and ATF6 UPR pathways by sEH inhibition decreases the TGF-β-induced myofibroblastic differentiation of lung fibroblasts and helps prevent the development of lung fibrosis. The inhibition of sEH has also exhibited a promising analgesic action toward diabetic neuropathy and other types of chronic pain. One proposed mechanism suggests downregulation of the three UPR arms in peripheral nerves, which reduces predisposition to aberrant axonal firing and decreases apoptotic signaling and oxidative stress (Inceoglu et al. 2017).

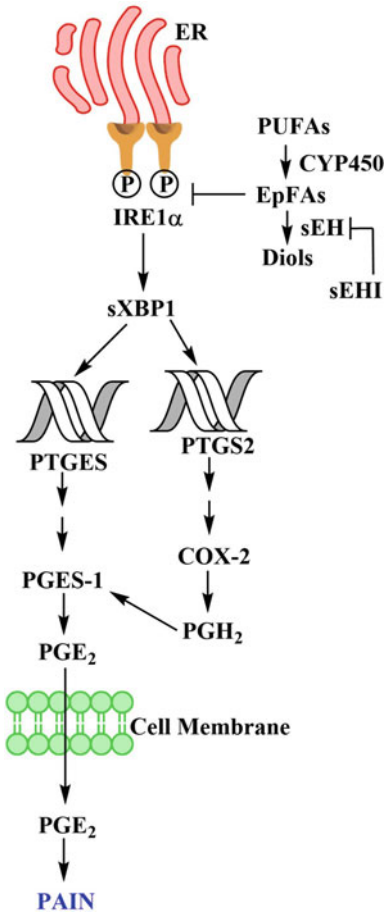
The inhibition of sEH and resulting elevated EpFA levels in the brain is considered to increase brain-derived neurotrophic factor (BDNF) levels in the prefrontal cortex and hippocampus (Hashimoto 2019). BDNF binding to tropomyosin receptor kinase B (TrkB) and enhanced BDNF-TrkB signaling promotes synaptogenesis and corrects dopaminergic dysfunction, leading to an improvement in depressive and schizophrenic symptoms. EpFAs also seem to play a role in preventing phosphorylation of α-synuclein and sequentially reducing the aggregation of α-synuclein in multiple brain regions (Hashimoto 2019). By blocking the deposition of phosphorylated α-synuclein aggregates (a.k.a. Lewy bodies), the loss of dopaminergic neurons from the substantia nigra is potentially mitigated, and the pathogenesis of Parkinson's disease and dementia with Lewy bodies (DLB) may be controlled.

For pain as an endpoint in animal studies, the inhibition of sEH forgoes the common adverse side effects of NSAIDs and COXIBs (e.g., GI ulceration, cardiovascular issues) (Wagner et al. 2017). Additionally, it displays none of the reward-seeking addictive potential associated with the use of opioid narcotics (Wagner et al. 2017). EETs can be angiogenic at very high doses, and sEHI upregulation of EETs is considered to slightly promote tumor growth. This phenomenon has been attributed to downstream COX metabolites of EETs (Rand et al. 2017). Combining sEH inhibitors and COX inhibitors has been shown to have significant anti-angiogenic effects and blunt tumor growth, as has the use of sEHI in animals with an enhanced omega-3 and depleted omega-6 diet.

Drugs

Representative sEH Inhibitors

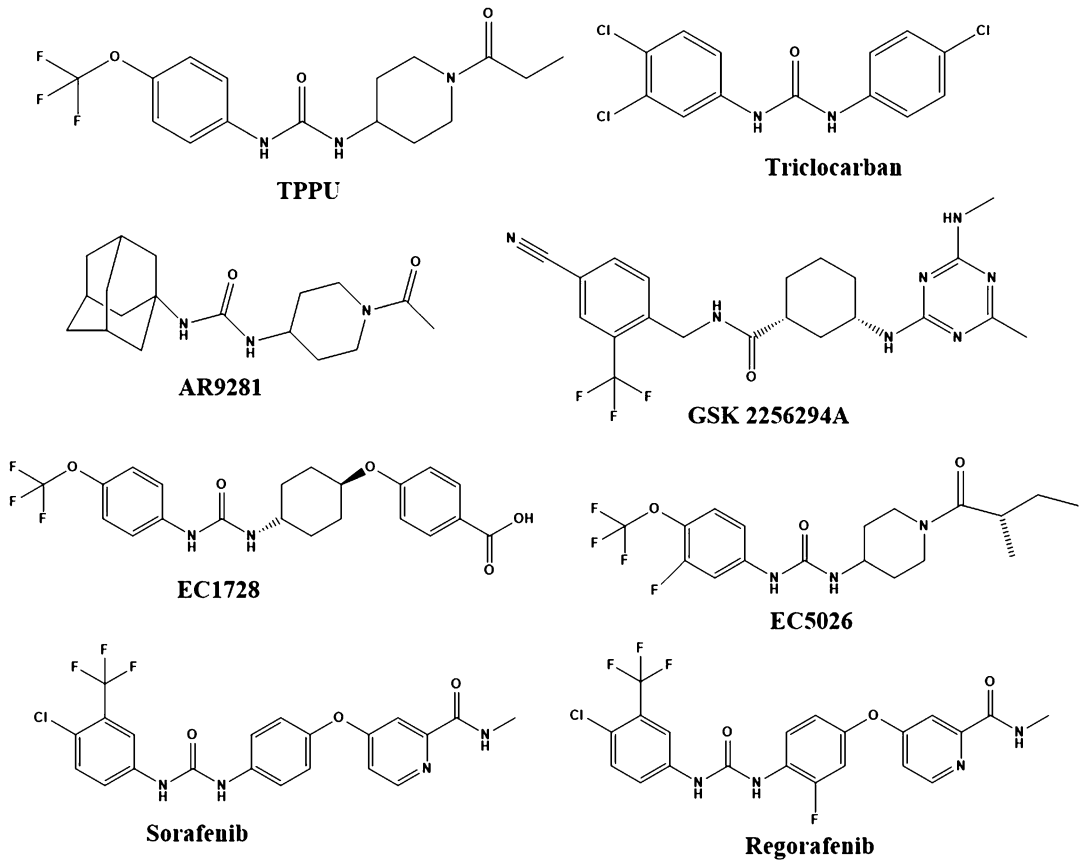
TPPU (i.e., UC1770) is extensively used in animal disease models because it is orally bioavailable and has favorable pharmacokinetic characteristics in addition to high potency (Fig. 4). Currently, no clinical drug on the market is used as an sEH inhibitor. However, antineoplastic agents sorafenib and regorafenib, inhibitors of vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and Raf family kinases are powerful sEH inhibitors (Fig. 4) (Liu 2019). This activity likely reduces severe toxicity associated with the use of protein kinase inhibitors. Triclocarban, a commonly used antibacterial, has also displayed sEH inhibitory potential (Fig. 4) (Liu 2019). These compounds contain the ideal disubstituted urea substructure which indicates the diversity of



Soluble Epoxide Hydrolase, Fig. 3 Epoxy fatty acids (EpFAs) preserved by sEH inhibitors (sEHI) stabilize mitochondria against disruption, reduce reactive oxygen species (ROS), and shift the endoplasmic reticulum (ER) stress response away from initiating cell damage and inflammation and back toward maintaining homeostasis (Inceoglu et al. 2017). Downstream from mitochondrial stabilization and reduction in ROS, EpFAs alter ER stress-induced unfolded protein response (UPR) pathways. For one pathway acting through IRE1α (shown above), sEHI reduce protein and message for COX-2 (Schmelzer et al. 2005) and PGES-1 (Chopra et al. 2019), thus dramatically reducing PGE_2 and other inflammatory and pain-initiating eicosanoids

structures that can inhibit the sEH. Several pharmaceutical companies have been or are in the process of discovering and developing potent sEH inhibitors in order to treat several clinical indications. These include Arête Therapeutics, Boehringer Ingelheim, Taisho, Merck, GlaxoSmithKline (GSK), and EicOsis. One compound, AR9281 (i.e., UC1153 or APAU) from Arête, was designed to target moderate hypertension and impaired glucose tolerance (Fig. 4). It completed Phase I and Phase IIA clinical trials. AR9281 was well tolerated for both a single oral dose and multiple doses. However, as expected from the adamantane in the structure, AR9281 had poor pharmacokinetic and pharmacodynamic parameters suggesting at least a twice- or thrice-daily dosing regimen for effective sEH inhibition (Liu 2019). Not only was the inhibition of the human enzyme weak, but the target occupancy was also poor. Despite no safety concerns in clinical trials, AR9281 failed to show efficacy in early-stage hypertension indicating hypertension might not be a suitable primary clinical indication for sEHI and AR9281 was not appropriate for sustained inhibition of the target sEH. Another inhibitor, GSK2256294A from GSK, was targeted to treat chronic pulmonary obstructive disease (CPOD) and completed Phase I clinical trials (Fig. 4). It has shown no adverse effects and a dose-dependent increase in plasma drug levels and sEH inhibition (Liu 2019). EicOsis designs pain therapeutics, and the compound EC1728 (i.e., *t*-TUCB) has been shown to successfully treat equine laminitis as well as inflammatory pain in dogs and cats (Fig. 4). Currently, EC1728 is pending an FDA review to begin Phase 1 trials in horses in 2020. The inhibitor EC5026 has been selected for treating diabetic neuropathy and other forms of neuropathic pain in patients while promoting opioid-sparing effects (Fig. 4). It has displayed considerable efficacy in preclinical studies as well as ideal pharmacokinetic properties and stability. The IND has been filled, and it entered Phase 1 clinical trials in December of 2019.

So far, sEHI have generally demonstrated few to no adverse side effects even at relatively high doses, and they have an excellent therapeutic index. However, the broad range of biological effects observed with the use of sEHI, like other drugs acting on the arachidonate cascade, suggest they should be utilized with caution.



Soluble Epoxide Hydrolase, Fig. 4 Chemical structures of representative sEH inhibitors

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