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14,15-Epoxyeicosa-5,8,11-trienoic Acid (14,15-EET) Surrogates Containing Epoxide Bioisosteres: Influence upon Vascular Relaxation and Soluble Epoxide Hydrolase Inhibition

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All-*cis*-14,15-epoxyeicosa-5,8,11-trienoic acid (14,15-EET) is a labile, vasodilatory eicosanoid generated from arachidonic acid by cytochrome P450 epoxygenases. A series of robust, partially saturated analogues containing epoxide bioisosteres were synthesized and evaluated for relaxation of precontracted bovine coronary artery rings and for in vitro inhibition of soluble epoxide hydrolase (sEH). Depending upon the bioisostere and its position along the carbon chain, varying levels of vascular relaxation and/or sEH inhibition were observed. For example, oxamide **16** and *N*-*i*Pr-amide **20** were comparable (ED_{50} 1.7 μM) to 14,15-EET as vasorelaxants but were approximately 10−35 times less potent as sEH inhibitors (IC_{50} 59 and 19 μM, respectively); unsubstituted urea **12** showed useful activity in both assays (ED_{50} 3.5 μM, IC_{50} 16 nM). These data reveal differential structural parameters for the two pharmacophores that could assist the development of potent and specific in vivo drug candidates.

Introduction

The oxidative metabolism of polyunsaturated fatty acids by the cytochrome P450 branch of the eicosanoid cascade generates, inter alia, one or more regioisomeric epoxides.[1] The best studied of these epoxanoids is all-*cis*-14,15-epoxyeicosatrienoic acid (14,15-EET[a]), which is derived from arachidonic acid and ascribed an impressive array of cardiovascular, pulmonary, renal, and CNS roles.[2] Activation of a guanine nucleotide-binding protein (G-protein) is thought to play a pivotal role in many of the responses to 14,15-EET and often involves ADP-ribosylation of the $G_s\alpha$ subunit. For instance, opening of vascular calcium-sensitive potassium channels by 14,15-EET is completely thwarted by guanosine 5′-*O*-(2-thio)diphosphate, a G-protein inhibitor, or by an antibody against $G_s\alpha$.[3] A reversible and abundant high-affinity binding site for 14,15-EET and its analogues has been identified[4] and shown to preferentially recognize the 14(*R*),15(*S*)-enantiomer.[5] The kinetic parameters of this binding site share many characteristics in common with the canonical prostanoid and leukotriene receptors, e.g., K_d values in the low nanomolar range;[4] however, characterization of the putative EET receptor at the molecular level has been elusive.[6,7]

14,15-EET, in common with most eicosanoids,[8] is chemically and metabolically labile (Figure 1).[9] Further transformations by enzymes of the cascade,[10] esterification,[11] conjugation,[12] β-oxidation,[13] chain elongation, and hydration by soluble epoxide hydrolase[14] (sEH) are well documented as inactivation or catabolic processes for 14,15-EET. The latter, in particular, appears to play a major role in regulating the intracellular levels of 14,15-EET,[15] whose in vivo half-life has been estimated as a few seconds to minutes.[16] Additionally, the proclivity of 14,15-EET toward auto-oxidation, a consequence of the 1,4-dienyl moieties present along the backbone,[17] introduces a further layer of complication and often necessitates storage and/or handling under an inert atmosphere.[9] Hence, a wide variety of factors combine to trammel the study of 14,15-EET and limit its potential therapeutic applications.

Structure−activity studies in the Falck and Campbell laboratories have addressed some of these limitations and led to the introduction of partially saturated 14,15-EET agonist analogues that obviate or minimize secondary metabolism as well as auto-oxidation.[18,19] More recently, Hammock and colleagues have pioneered soluble epoxide hydrolase inhibition (sEHi) as an alternative, albeit indirect, strategy for pharmacological intervention in EET-dependent events.[20] This approach ostensibly[21] prolongs the eicosanoid's half-life, thereby elevating steady state levels of endogenous EETs as well as other epoxides. From an artful series of studies, lipophilic 1,3-disubstituted ureas emerged as especially efficacious in vitro and in vivo sEH inhibitors.[20] Advanced members of this genre show promise as first-in-class therapeutics for a variety of diseases including diabetes, inflammation, and hypertension.[22] In the present studies, we sought to develop and evaluate chimeric analogues that combine the more robust backbone of the partially saturated EET mimics with

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[a] Abbreviations: 14,15-EET, *cis*-14,15-epoxyeicosa-5(*Z*),8(*Z*),11(*Z*)-trienoic acid; sEH, soluble epoxide hydrolase; sEHi, soluble epoxide hydrolase inhibition; VR, vascular relaxation.

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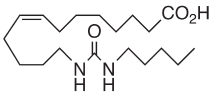
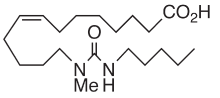
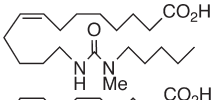
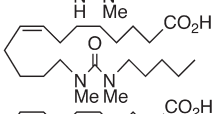
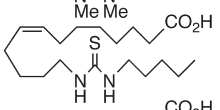
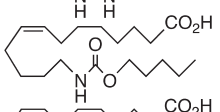
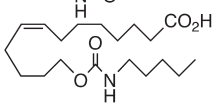
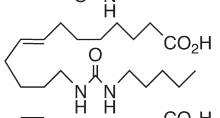
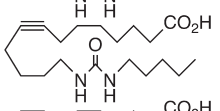
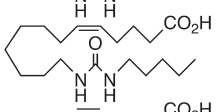
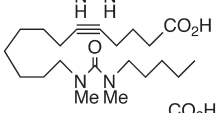
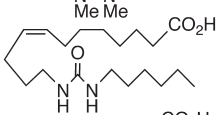
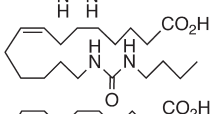
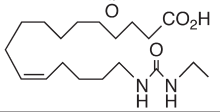
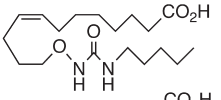
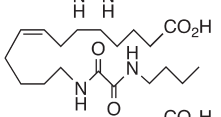
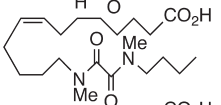
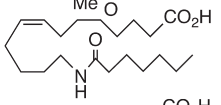
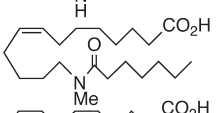
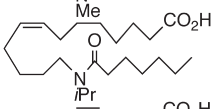
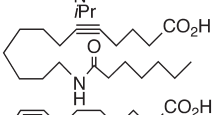
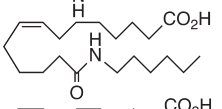
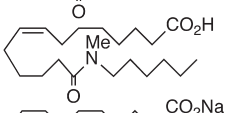
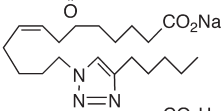
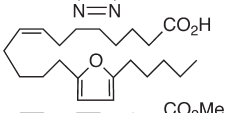
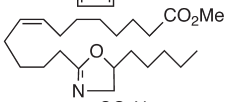
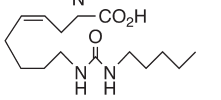
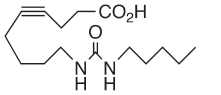
Table 1. Vasorelaxation of Precontracted Bovine Coronary Artery and in Vitro Inhibition of Recombinant Human Soluble Epoxide Hydrolase[a,b]

Compd	Analog	Vascular Relax. % (10 μM)	Vascular Relax. EC_{50} (μM)	SEHi IC_{50} (nM)
1		63	7.5	46
2		59	7.6	71.5
3		88	3.2	1451
4		83	3.2	8484
5		99	1.5	770
6		64	8.3	2834
7		53	9.3	3480
8		29	>10	48.5
9		62	3.1	43
10		66	5.6	793
11		77.5	2.3	11064
12		86	3.5	16
13		84	4.6	152
14		50	>10	2596
15		87	3.7	1374
16		89	1.7	58712
17		70	4.4	17622
18		64.5	6.1	79
19		64.5	4.3	11194
20		100	1.7	10712
21		69	5.7	272
22		74	4.9	66
23		12	>10	13877
24		65	6.2	17755
25		11	>10	7147
26		33	>10	688
27		16	>10	1355
28		46	>10	8110

[a] At 10 mM, 14,15-EET induces 85% of maximum vasorelaxation and its ED_{50} is 2.2 μM. For recombinant human sEH, the IC_{50} for 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) is 3 nM. [b] Bioassay determinations (n) = 3−5.

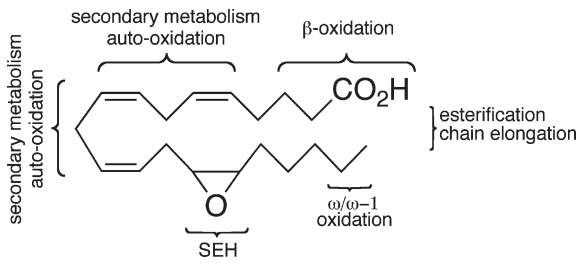


Figure 1. Metabolism/Degradation of 14,15-EET.

potential epoxide bioisosteres[23,24] capable of functioning as stable 14,15-EET surrogates and/or as inhibitors of soluble epoxide hydrolase.[25]

Results and Discussion

Drawing inspiration from the aforementioned studies, analogue **1** was deemed a suitable point of departure for our investigation. Notably, **1** contains several key structural features, inter alia: (i) a partially saturated carbon backbone to avoid auto-oxidation[17] and LOX metabolism,[26] (ii) a *cis*-$\Delta^{8,9}$-olefin thought to be essential for EET agonist activity,[18b] and (iii) a sEH-resistant 1,3-disubstituted urea that we anticipated would function as a surrogate for the $\Delta^{14,15}$-epoxide.[27] It was, thus, gratifying to find **1** mimics, albeit modestly, 14,15-EET as a vasorelaxant of precontracted bovine coronary artery rings (Table 1). Additionally, **1** proved to be a low nanomolar inhibitor of recombinant human sEH. Methylation of the proximal urea nitrogen of **1** provided **2** but had little influence

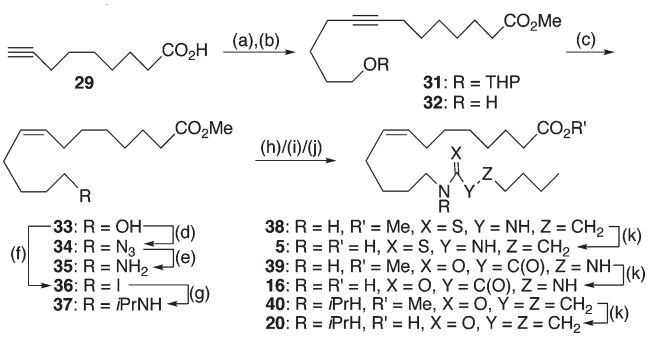
upon the vascular properties; as anticipated, methylation dramatically attenuated sEHi activity.[20] On the other hand, *N*-methylation of the distal urea nitrogen gave rise to regioisomer **3** and significantly improved EET mimicry while sEHi activity degraded sharply. The differences between these *N*-methylated regioisomers for sEHi are similar to the differences observed previously with amide regioisomers.[15] Substitution of both nitrogens as in **4** did not prove additive with respect to VR but did further exacerbate the loss of sEHi. These data were the first convincing indications that EET agonist activity and sEHi could be at least partially differentiated in this compound series. Such differences may also be indicative of restricted orientation in the putative EET and sEH binding sites; further evidence can be found later in the series, e.g., *N*-methylated amides **19** and **23**. A lesser level of discrimination between the two activities could also be achieved by heteroatom replacement.[20] Thiourea **5** was equipotent with 14,15-EET across the entire concentration range of the vascular assay, while sEHi declined by a factor of 16 with respect to **1**. On the other hand, replacement of either urea nitrogen with oxygen, i.e., **6** and **7**, had relatively little influence on vessel tension but did blunt sEHi. In light of these data, it is tempting to speculate that hydrogen bonding or coordination to a metal center (e.g., Cu^{2+}, Fe^{2+}, or Zn^{2+}) might be an important contributor to binding at the putative EET vascular receptor.

We next turned our attention to the olefinic moiety and found vascular activity was diminished in *trans*-olefin **8** but not acetylene **9**. Accommodation of a linear carbon chain or one that is bent in the natural *cis*-configuration, but not *trans*-geometry, is consistent with a shallow binding pocket for this portion of the molecule. For sEHi, both **8** and **9** retained their low nanomolar potencies and were almost identical with that of the benchmark **1**. Relocation of the *cis*-olefin to $\Delta^{5,6}$ of the carbon chain (analogue **10**) only modestly perturbed EET agonist activity and not at all for the related acetylene **11**. This is unexpected given the regiodependency observed[18b] in allied systems, e.g., *cis*-14,15-epoxy-eicosa-8(*Z*)-enoic acid is a 14,15-EET agonist while *cis*-14,15-epoxy-eicosa-5(*Z*)-enoic acid functions as a competitive antagonist. The dramatic slide in sEHi for **11**, sans N−H, is consistent with established SAR for this pharmacophore.[20] Shifting the urea one position right (analogue **12**) or left (analogue **13**) relative to its placement in **1** improved VR with respect to **1**, whereas displacement (accompanied by the *cis*-olefin) two positions further from the carboxylate, resembling an ω-3 fatty acid, was counterproductive (analogue **14**), thus it is difficult to discern a trend in the series **12** → **1** → **13** → **14** correlating urea chain position and VR. On the other hand, the IC_{50} for sEHi declined steadily following the same series. This appears generally consistent with the proposal[20] that "one hydrophobic group should be present on each side of the urea" and confirmed indications that a six-carbon *n*-alkyl chain is sufficient for low nanomolar inhibition.[20]

Bioisosteric epoxide replacement by *N*-hydroxyurea and 1,4-oxamide led to **15** and **16**, respectively, both of which showed acceptable VR at 10 μM. The latter was distinguished by a somewhat better ED_{50}, yet its IC_{50} for sEHi was more than 3 orders of magnitude greater than **1**. In contrast to the outcome from conversion of **1** into **4**, the *N*,*N*′-dimethylation of **16** to give **17** reduced its ability to relax the vessel while simultaneously moderating the loss of sEHi seen with **16**.

The regioisomeric amide bioisosteres **18** and **22** displayed potentially useful sEHi activities but were lackluster EET mimics. The analogous *N*-methylated derivatives **19** and **23**, respectively, as well as acetylene **21**, lost potency except for a small improvement in the EC_{50} of **19**. sEHi by *N*-isopropyl **20** was likewise depressed, but the trend toward a more robust response in the vascular assay portended in **19** was clearly evident in this example. Whether the enhancement can be attributed to a hydrophobic binding pocket remains unclear at present.

Scheme 1. Synthesis of Representative Analogues[a]



[a]Reagents and conditions: (a) *n*-BuLi, THF/HMPA (4:1), −78 to 0 °C, 2 h, then **30**, −78 to 23 °C, 12 h; (b) *p*-$MeC_6H_4SO_3H$ (cat), MeOH, 23 °C, 12 h, 55% (2 steps); (c) $Ni(OAc)_2/NaBH_4/H_2NCH_2CH_2NH_2$, H_2, EtOH, 23 °C, 1 h, 90%; (d) Ph_3P/DEAD/DPPA, THF, −20 to 23 °C, 4 h, 65%; (e) Ph_3P, THF/H_2O, 23 °C, 12 h; (f) Ph_3P/I_2, THF, 0 °C, 1 h, 83%; (g) *i*-$PrNH_2/K_2CO_3$, THF, 80 °C, 12 h, 70%; (h) **35**/*n*-pentylisothiocyanate, THF, 23 °C, 12 h, 70%; (i) **35**/HOC(O)C(O)NHC_4H_9/HATU, CH_2Cl_2, 23 °C, 12 h, 76%; (j) **37**/EDCI/*n*-heptanoic acid, DMF, 23 °C, 12 h, 77%; (k) LiOH, THF/H_2O, 23 °C, 12 h, 90−95%.

A selection of nitrogen and oxygen heterocyclic bioisosteres, viz, triazole **24**, furan **25**, and 2-oxazoline **26**, were prepared based upon the template in **1**. They did not appear to offer any pharmacological advantage and were not pursued further. The loss of both VR and sEHi activities in the tetranor-ureas **27** and **28** is noteworthy given prior reports that chain-shortened 14,15-EETs retain much of their biological activity in the vasculature.[13] Formally, **27** and **28** can be envisioned as arising from **1** and **9**, respectively, via two cycles of β-oxidation.[13] It will be of interest to determine if this is also a significant route of inactivation in vivo for the EET surrogates identified in this study.

Chemistry. The syntheses of thiourea **5**, oxamide **16**, and *N*-isopropylamide **20** are summarized in Scheme 1 and are representative of the methodology used to prepare the other analogues. Following literature precedent,[28] the dianion of commercial non-8-ynoic acid (**29**) was alkylated with 1 equiv of 2-(4-bromobutoxy)-tetrahydro-2*H*-pyran[29] (**30**) in THF/HMPA (4:1). The resultant disubstituted acetylene **31** gave rise to alcohol **32** following acidic hydrolysis in methanol. Semihydrogenation over P-2 nickel created *cis*-olefin **33** that was subjected to azidation using diphenylphosphoryl azide (DPPA) under Mitsunobu conditions. Staudinger reduction of the product, azide **34**, led to primary amine **35**. Reaction of the latter with *n*-pentylisothiocyanate or a combination of 2-(butylamino)-2-oxoacetic acid[30] and 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) was unremarkable and furnished thiourea **38** and oxamide **40**, respectively. Alternatively, **33** was converted with Ph_3P/I_2 to iodide **36** that was then displaced with excess *iso*-propylamine at 80 °C. HATU-induced condensation of the product **37** with *n*-heptanoic acid delivered amide **42** in good overall yield. Saponification of **38**, **40**, and **42** afforded the corresponding free acids **5**, **16**, and **20**, respectively.

Conclusions

Herein, we have shown *N*-substituted ureas, oxamides, and *N*-substituted amides are suitable bioisosteres for fatty acid epoxides, whereas the urethanes, triazole, 2-oxazoline, furan, and secondary amides described above are not. In concert with literature observations, secondary amides were powerful sEH inhibitors as were 1,3-disubstituted ureas if the appendages are sufficiently lipophilic. Some epoxide surrogates behaved as both good EET agonists and powerful sEH inhibitors. Overall, there was no correlation between vascular relaxation and sEHi potencies, suggesting differential structural parameters for the two pharmacophores. It is anticipated that these dual-activity analogues could function additively and provide a platform for the development of the next generation of analogues intended for in vivo applications.

Experimental Section

General Procedures. Unless stated otherwise, yields refer to purified products and are not optimized. Final compounds were judged ≥95% pure by HPLC. All moisture-sensitive reactions were performed under an argon atmosphere using oven-dried glassware and anhydrous solvents. Anhydrous solvents were freshly distilled from sodium benzophenone ketyl, except for CH_2Cl_2, which was distilled from CaH_2. Extracts were dried over anhydrous Na_2SO_4 and filtered prior to removal of all volatiles under reduced pressure. Unless otherwise noted, commercially available materials were used without purification. Silica gel chromatography was performed using E. Merck silica gel 60 (240−400 mesh). Thin layer chromatography was performed using precoated plates purchased from E. Merck (silica gel 60 F254, 0.25 mm). Nuclear magnetic resonance (NMR) splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); the value of chemical shifts (δ) are given in ppm relative to residual solvent (chloroform $\delta = 7.27$ for ^{1}H NMR or $\delta = 77.23$ for proton decoupled ^{13}C NMR), and coupling constants (J) are given in hertz (Hz). The Michigan State University Mass Spectroscopy Facility or Medical College of Wisconsin provided high-resolution mass spectral analyses.

Methyl 13-Hydroxytridec-8-ynoate (32). *n*-Butyllithium (6.2 mL of 2.5 M solution in hexanes, 15.5 mmol) was added dropwise with stirring to a −78 °C solution of non-8-ynoic acid (**29**) (1 g, 6.5 mmol, G. F. Smith Chem. Co.) in THF/HMPA (4:1, 50 mL) under an argon atmosphere. After 30 min, the reaction mixture was warmed to 0 °C and maintained at this temperature for 2 h. After recooling to −78 °C, a solution of 2-(4-bromobutoxy)-tetrahydro-2*H*-pyran[29] (**30**) (1.68 g, 7.14 mmol) in THF (10 mL) was added and the reaction temperature was slowly raised over 3 h to 23 °C. After 12 h, the reaction mixture was quenched with saturated aq NH_4Cl solution (5 mL) and the pH was adjusted to ∼4 using 1 M oxalic acid. The mixture was extracted with EtOAc (2 × 50 mL), and the combined extracts were washed with water (2 × 10 mL), brine (10 mL), dried, and concentrated under reduced pressure. Crude **31** was dissolved in methanol (20 mL) to which was added *p*-toluenesulfonic acid (PTSA, 25 mg). After 12 h, the solvent was evaporated and the residue was purified by SiO_2 column chromatography to give **32** (700 mg, 55%) as a colorless oil. TLC: EtOAc/hexanes (1:1), $R_f \sim 0.55$. ^{1}H NMR (300 MHz) δ 3.68 (s, 3H), 3.66 (t, 2H, $J = 6.2$ Hz), 2.31 (t, 2H, $J = 7.3$ Hz), 2.10−2.24 (m, 4H), 1.30−1.75 (m, 12H). ^{13}C NMR (75 MHz) δ 174.55, 80.70, 80.14, 62.75, 51.73, 34.24, 32.13, 29.04, 28.84, 28.62, 25.56, 25.05, 18.85, 18.75. HRMS calcd for $C_{14}H_{25}O_3$ $[M + 1]^+$ 241.1804, found 241.1807.

Methyl 13-Hydroxytridec-8(*Z*)-enoate (33). $NaBH_4$ (16 mg, 0.416 mmol) was added in portions with vigorous stirring to a room temperature solution of $Ni(OAc)_2 \cdot 4H_2O$ (103.7 mg, 0.416 mmol) in absolute EtOH (7 mL) under a hydrogen atmosphere (1 atm). After 15 min, freshly distilled ethylenediamine (56 μL, 0.833 mmol) was added to the black suspension, followed after a further 15 min by a solution of **32** (400 mg, 1.66 mmmol) in absolute EtOH (2 mL). After 1 h, the reaction mixture was diluted with Et_2O (10 mL) and passed through a small bed of silica gel. The bed was rinsed with another portion of Et_2O (10 mL). The combined ethereal filtrates were concentrated under reduced pressure to afford methyl 13-hydroxytridec-8(*Z*)-enoate (**33**) (345 mg, 98%) as a colorless oil. TLC: EtOAc/hexane (1:1), $R_f \sim 0.42$. ^{1}H NMR (300 MHz) δ 5.32−5.48 (m, 2H), 3.66 (s, 3H), 3.64 (t, $J = 6.5$ Hz, 2H) 2.30 (t, $J = 7.4$, 2H), 1.96−2.10 (m, 4H), 1.54−1.66 (m, 4H), 1.20−1.44 (m, 10H). ^{13}C NMR (75 MHz) δ 174.77, 130.30, 129.70, 62.95, 51.73, 34.28, 32.42, 29.64, 29.17, 29.03, 27.29, 27.10, 26.03, 25.07. HRMS calcd for $C_{14}H_{25}O_3$ $[M + 1]^+$ 243.1960, found 243.1959.

Methyl 13-Azidotridec-8(*Z*)-enoate (34). Diethyl azodicaboxylate (DEAD; 243 μL, 1.54 mmol) was added dropwise to a −20 °C solution of PPh_3 (TPP; 405 mg, 1.54 mmol) in dry THF (10 mL) under an argon atmosphere. After 10 min, alcohol **33** (340 mg, 1.4 mmol) dissolved in dry THF (5 mL) was added dropwise. After 30 min, the reaction mixture was allowed to come to 0 °C and diphenylphosphoryl azide (DPPA; 364 μL, 1.68 mmol) was added dropwise. After stirring for 4 h at rt, the reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (20 mL), dried, and concentrated under reduced pressure. The residue was purified by SiO_2 column chromatography eluting with 5% EtOAc/hexane to afford **34** (240 mg, 65%). TLC: 10% EtOAc/hexanes, $R_f \sim 0.45$. ^{1}H NMR (300 MHz) δ 5.28−5.44 (m, 2H), 3.67 (s, 3H), 3.24 (t, $J = 7.3$ Hz, 2H), 2.30 (t, $J = 7.2$ Hz, 2H), 1.96−2.10 (m, 4H), 1.56−1.64 (m, 4H), 1.28−1.42 (m, 8H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.24, 130.69, 129.17, 51.64, 51.55, 34.26, 29.66, 29.22, 29.08, 28.61, 27.33, 26.96, 26.83, 25.09. IR (neat) 2985, 2954, 2845, 2106, 1754, 1250, 1104, 1029 cm^{-1}. HRMS calcd for $C_{14}H_{25}N_3O_2$ $[M + 1]^+$ 267.3672, found 267.3680.

Methyl 13-Aminotridec-8(*Z*)-enoate (35). Triphenylphosphine (TPP; 89 mg, 0.338 mmol) was added to a stirring, room temperature solution of azide **34** (90 mg, 0.33 mmol) in THF (2 mL) containing 4 drops of deionized water. After 12 h, the reaction mixture was diluted with CH_2Cl_2 (2 mL), dried, and concentrated in vacuo to give **35** (64 mg, 78%) as a viscous, colorless oil that was used directly in the next reaction without further purification. TLC: 20% MeOH/CH_2Cl_2, $R_f \sim 0.25$. ^{1}H NMR (300 MHz) δ 6.22 (bs, 2H), 5.24−5.36 (m, 2H), 3.64 (s, 3H), 2.79−2.88 (m, 2H), 2.27 (t, $J = 6.7$ Hz, 2H), 1.92−2.08 (m, 4H), 1.53−1.68 (m, 4H), 1.21−1.44 (m, 8H). ^{13}C NMR (100 MHz) δ 174.54, 130.46, 129.40, 51.69, 41.08, 34.30, 29.70, 29.25, 29.11, 27.34, 25.12, 24.83. HRMS calcd for $C_{14}H_{27}NO_2$ $[M + 1]^+$ 241.3697, found 241.3705.

Methyl 13-(3-Pentylthioureido)tridec-8(*Z*)-enoate (38). A solution of amine **35** (80 mg, 0.33 mmol) in THF (3 mL) was added dropwise to a stirring, 0 °C solution of *n*-pentylisothiocyanate (80 mg, 0.29 mmol) in THF (4 mL). After 12 h at room temperature, all volatiles were removed under reduced pressure and the residue was purified by SiO_2 column chromatography eluting with 30% EtOAc/hexane to afford **38** (90 mg, 70%) as a colorless oil. TLC: EtOAc/hexanes (1:1), $R_f \sim 0.65$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.72 (br s, NH), 5.29−5.35 (m, 2H), 3.66 (s, 3H), 3.40 (br s, NH), 2.30 (t, $J = 7.5$ Hz, 2H), 1.97−2.05 (m, 4H), 1.57−1.64 (m, 6H), 1.26−1.42 (m, 12H), 0.88 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (75 MHz) δ 174.54, 173.75, 130.17, 129.94. 51.67, 44.68, 34.30, 29.72, 29.64, 29.21, 29.26, 29.16, 27.55, 27.35, 27.31, 25.15, 26.98, 25.13, 22.74, 14.31. HRMS calcd for $C_{20}H_{38}N_2O_2S$ $[M + 1]^+$ 371.2663, found 371.2669.

13-(3-*n*-Pentylthioureido)tridec-8(*Z*)-enoic Acid (5). LiOH (840 mL of a 1 M soln) was added to a 0 °C solution of methyl ester **38** (80 mg, 0.21 mmol) in THF/H_2O (4:1, 10 mL). After stirring at room temperature for 12 h, the THF was evaporated under reduced pressure, the remaining reaction mixture was

diluted with H_2O (5 mL), recooled to 0 °C, and the pH adjusted to 4 using 1 M oxalic acid. The reaction mixture was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (20 mL), dried, concentrated under reduced pressure, and the residue was purified by SiO_2 column chromatography eluting with EtOAc/hexane (1:1) to afford **5** (67 mg, 87%) as a colorless oil. TLC: 60% EtOAc/hexanes, $R_f \sim 0.35$; mp 84.4−85.2 °C. ^{1}H NMR (300 MHz) δ 5.28−5.36 (m, 2H), 3.38 (br s, 2H), 2.34 (t, J = 7.3 Hz, 2H), 1.97−2.05 (m, 4H), 1.58−1.65 (m, 6H), 1.26−1.42 (m, 12H), 0.88 (t, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz) δ 181.05, 179.53, 130.72, 129.25, 44.43, 34.15, 29.45, 29.32, 29.23, 28.92, 28.83, 28.68, 27.16, 27.03, 24.75, 22.55, 14.17. LCMS $(M + 1)^+$ 357. HRMS calcd for $C_{19}H_{36}N_2O_2S$ $[M + 1]^+$ 357.2267, found 357.2572.

Methyl 13-(2-(*n*-Butylamino)-2-oxoacetamido)tridec-8(*Z*)-enoate (39). A mixture of 2-(*n*-butylamino)-2-oxoacetic acid[30] (60 mg, 0.413 mmol), amine **35** (119 mg, 0.496 mmol), and 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU; 172 mg, 0.455 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature overnight and then all volatiles were removed in vacuo. The resultant residue was purified by SiO_2 column chromatography using 20% EtOAc/hexane as eluent to furnish oxamide **39** (112 mg, 76%) as a white solid, mp 112.5−112.9 °C. TLC: EtOAc/hexane (1:1), $R_f \sim 0.62$. ^{1}H NMR (400 MHz) δ 7.42−7.67 (m, 2H), 5.23−5.42 (m, 2H), 4.07−4.14 (m, 2H), 3.65 (s, 3H), 3.25−3.38 (m, 4H), 2.28 (t, 2H, J = 7.2 Hz), 1.96−2.08 (m, 4H), 1.49−1.62 (m, 4H), 1.21−1.41 (m, 10H), 0.92 (t, 3H, J = 7.1 Hz). ^{13}C NMR (75 MHz) δ 174.44, 160.15, 130.57, 129.22, 51.62, 39.78, 39.59, 34.23, 31.41, 29.65, 29.20, 29.06, 28.99, 27.32, 27.08, 26.90, 25.07, 20.18, 13.85. HRMS calcd for $C_{20}H_{36}N_2O_4$ $[M + 1]^+$ 369.2753, found 369.2757.

13-(2-(*n*-Butylamino)-2-oxoacetamido)tridec-8(*Z*)-enoic acid (16). Hydrolysis of **39** (100 mg, 0.272 mmol) using LiOH as described for **38** afforded **16** (76 mg, 90%) as a white solid, mp 101.4−102.2 °C. TLC: EtOAc/hexane (1:1), $R_f \sim 0.18$. ^{1}H NMR (500 MHz) δ 7.94 (br s, 1H), 7.63 (br s, 1H), 5.39−5.30 (m, 2H), 3.35−3.30 (m, 4H), 2.37 (t, 2H, J = 7.3 Hz), 2.09−2.01 (m, 4H), 1.68−1.52 (m, 6H), 1.41−1.34 (m, 10 H), 0.94 (t, 3H, J = 7.3 H). ^{13}C NMR (125 MHz) δ 178.9, 160.1, 160.0, 130.5, 129.2, 39.8, 39.7, 34.0, 31.2, 29.4, 28.9, 28.8, 27.0, 26.9, 26.8, 24.7, 20.1, 13.8. LC/API-MS m/z 376 $[M + Na]^+$. HRMS calcd for $C_{19}H_{34}N_2O_4$ $[M + 1]^+$ 355.2597, found 355.2594.

Methyl 13-Iodotridec-8(*Z*)-enoate (36). tetra-*n*-Butylammonium iodide (941 mg, 2.58 mmol) and DDQ (1.52 g, 2.58 mmol) were added in sequence to a solution of triphenylphosphine (676 mg, 2.58 mmol) and methyl 13-hydroxytridec-8(*Z*)-enoate (**33**) (520 mg, 2.15 mmol) in dry dichloromethane (30 mL) at room temperature. After 3 h, the reaction mixture was quenched with water (2 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined extracts were dried, concentrated under reduced pressure, and the residue was purified by SiO_2 column chromatography using 3−6% EtOAc/hexanes as eluent to give methyl 13-iodotridec-8(*Z*)-enoate (**36**) (514 mg, 83%) as a pale-yellow oil. TLC: 10% EtOAc/hexanes, $R_f \sim 0.6$. ^{1}H NMR (300 MHz) δ 5.28−5.42 (m, 2H), 3.66 (s, 3H), 3.18 (t, J = 7.0 Hz, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.98−2.08 (m, 4H), 1.79−1.87 (m, 2H), 1.24−1.62 (m, 10H). ^{13}C NMR (75 MHz) δ 174.46, 130.73, 129.16, 51.68, 34.28, 33.28, 30.72, 29.68, 29.25, 29.11, 27.36, 26.28, 25.12, 7.20. HRMS calcd for $C_{14}H_{25}IO_2$ $[M + 1]^+$ 353.1004, found 353.1010.

Methyl 13-(Isopropylamino)tridec-8(*Z*)-enoate (37). Isopropylamine (418 mg, 7.10 mmol) and K_2CO_3 (596 mg, 4.26 mmol) were added sequentially to a room temperature solution of methyl 13-iodotridec-8(*Z*)-enoate (500 mg, 1.42 mmol) in dry tetrahydrofuran (7 mL). The mixture was heated in a sealed tube at 90 °C for 12 h and then cooled to rt, diluted with water (2 mL), filtered, and the filtrate was extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were dried, concentrated under reduced pressure, and the residue was purified by SiO_2 column chromatography using a gradient from 2 to 5% MeOH/CH_2Cl_2 as eluent to give methyl 13-(*N*-isopropylamino)tridec-8(*Z*)-enoate (**37**) (314 mg, 78%) as a pale-yellow oil. TLC: 5% MeOH/CH_2Cl_2, $R_f \sim 0.6$. ^{1}H NMR (300 MHz) δ 5.30−5.40 (m, 2H), 3.66 (s, 3H), 2.72−2.84 (m, 1H), 2.58 (t, J = 7.2 Hz, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.98−2.08 (m, 4H), 1.22−1.68 (m, 12H), 1.05 (d, J = 6.4 Hz, 6H). ^{13}C NMR (75 MHz) δ 174.28, 130.57, 128.58, 51.43, 49.93, 44.69, 34.05, 29.43, 29.01, 28.87, 27.14, 26.64, 26.02, 24.86, 19.33. HRMS calcd for $C_{17}H_{33}NO_2$ $[M + 1]^+$ 284.2590, found 384.2592.

Methyl 13-(Isopropyl-*n*-heptanamido)tridec-8(*Z*)-enoate (40). Solid EDCI [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] (75 mg, 0.39 mmol) was added in portions to a room temperature solution of amine **37** (110 mg, 0.39 mmol), DMAP (48 mg, 0.39 mmol), and *n*-heptanoic acid (51 mg, 0.09 mmol) in dry DMF (3 mL). After 12 h, the reaction mixture was diluted with water (10 mL) and extracted with Et_2O (3 × 10 mL). The combined organic extracts were washed with brine, dried, and evaporated in vacuo. The residue was purified via SiO_2 column chromatography to give methyl 13-(isopropyl-*n*-heptanamido)tridec-8(*Z*)-enoate (**40**) (115 mg, 77%) as a colorless oil. TLC: EtOAc/hexanes (1:1), $R_f \sim 0.6$. ^{1}H NMR (300 MHz, ~3:2 mixture of rotamers) δ 5.25−5.38 (m, 2H), 4.61−4.67 and 3.99−4.04 (m, 1H for two rotamers), 3.66 (s, 3H), 3.04−3.13 (m, 2H), 2.20−2.36 (m, 4H), 1.90−2.06 (m, 4H), 1.20−1.64 (m, 20H), 1.15 and 1.09 (d, J = 6.7 Hz, 6H for two rotamers), 0.85 (t, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz) δ 176.96, 174.32, 172.76, 130.65, 129.99, 129.73, 129.04, 51.52, 48.36, 45.63, 43.54, 41.06, 34.32, 34.16, 33.94, 33.87, 31.82, 31.80, 31.63, 31.14, 29.82, 29.64, 29.58, 29.32, 29.15, 29.02, 27.71, 27.38, 27.31, 27.24, 27.08, 26.83, 25.84, 25.68, 25.03, 22.68, 22.62, 21.49, 20.61, 14.18. HRMS calcd for $C_{24}H_{45}NO_3$ $[M + 1]^+$ 396.3478, found 396.3475.

13-(*N*-Isopropylheptanamido)tridec-8(*Z*)-enoic acid (20). Hydrolysis of **40** using LiOH as described for **38** afforded 20 (92%) as a colorless oil. TLC: 75% EtOAc/hexanes, $R_f \sim 0.40$. ^{1}H NMR (300 MHz, mixture of rotamers) δ 5.25−5.39 (m, 2H), 4.63−4.68 and 4.02−4.07 (m, 1H for two rotamers in 55/45 ratio), 3.06−3.11 (m, 2H), 2.23−2.36 (m, 4H), 1.98−2.06 (m, 4H), 1.20−1.71 (m, 20H), 1.18 and 1.11 (d, J = 7.0 Hz, 6H for two rotamers in 55/45 ratio), 0.87 (t, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz) δ 179.00, 178.24, 173.51, 173.08, 130.81, 130.08, 129.95, 129.95, 129.15, 48.57, 45.66, 43.61, 41.26, 34.36, 34.28, 34.23, 34.10, 31.92, 31.86, 31.66, 31.26, 29.67, 29.60, 29.44, 29.40, 29.31, 29.17, 29.10, 28.97, 28.85, 28.49, 27.93, 27.47, 27.40, 27.32, 26.94, 26.81, 25.81, 25.93, 25.75, 24.99, 24.9, 22.78, 21.54, 20.75, 14.29. ESI-LC/MS m/z 380 $(M - H)^+$. HRMS calcd for $C_{23}H_{43}NO_3$ $[M + 1]^+$ 382.3321, found 382.3324.

Bioassays. The influence of eicosanoids and analogues on coronary vascular tone was measured by the induced changes in isometric tension of bovine coronary artery rings precontracted with the thromboxane-mimetic, U46619, as previously described.[18a,31] Synthetic 14,15-EET was used as a control. All assays were conducted in triplicate or greater and are means ± 10% SD of the reported value.

Recombinant human sEH was produced in a baculovirus expression system[32] and was purified by affinity chromatography.[33] Inhibition potencies (IC_{50}s) were determined using a fluorescent-based assay.[34] Human sEH (~1 nM) was incubated with inhibitors ($0.4 < [I]_{final} < 100000$ nM) for 5 min in 25 mM bis-tris/HCl buffer (200 mL, pH 7.0) at 30 °C before the substrate, cyano(2-methoxynaphthalen-6-yl)methyl *trans*-(3-phenyl-oxyyran-2-yl]methyl carbonate (CMNPC; $[S]_{final} = 5\ \mu$M), was added. Activity was assessed by measuring the appearance of the fluorescent 6-methoxynaphthaldehyde product (λ_{em} = 330 nm, λ_{ex} = 465 nm) at 30 °C during a 10 min incubation (Spectramax M2, Molecular Device, Inc., Sunnyvale, CA).[34] IC_{50}s refer to the concentrations of inhibitor that reduced activity by 50% and are the averages of three replicates.

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Supporting Information Available: Experimental procedures and copies of the $^{1}H/^{13}C$ NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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