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14,15-Epoxyeicosa-5,8,11-trienoic Acid (14,15-EET) Surrogates: Carboxylate Modifications

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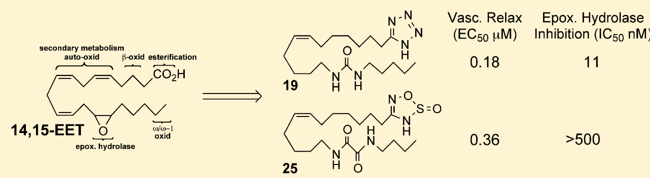
S *Supporting Information*

ABSTRACT: The cytochrome P450 eicosanoid 14,15-epoxyeicosa-5,8,11-trienoic acid (14,15-EET) is a powerful endogenous autacoid that has been ascribed an impressive array of physiologic functions including regulation of blood pressure. Because 14,15-EET is chemically and metabolically labile, structurally related surrogates containing epoxide bioisosteres were introduced and have become useful in vitro pharmacologic tools but are not suitable for in vivo applications. A new generation of EET mimics incorporating modifications to the carboxylate were prepared and evaluated for vasorelaxation and inhibition of soluble epoxide hydrolase (sEH). Tetrazole **19** (ED_{50} 0.18 μM) and oxadiazole-5-thione **25** (ED_{50} 0.36 μM) were 12- and 6-fold more potent, respectively, than 14,15-EET as vasorelaxants; on the other hand, their ability to block sEH differed substantially, i.e., 11 vs >500 nM. These data will expedite the development of potent and specific in vivo drug candidates.



INTRODUCTION

An imposing body of studies,[1] spanning more than three decades, has cogently elucidated the involvement of epoxyeicosatrienoic acids (EETs) in a wide array of critical physiological functions, inter alia, blood pressure regulation,[2] nociception,[3] adipogenesis,[4] anti-inflammatory activity,[5] organ regeneration,[6] insulin potentiation,[7] podocyte integrity,[8] and cellular responses to bacterial infection.[9] The most prominent regioisomer,[10] 14,15-epoxyeicosa-5(*Z*),8(*Z*),11(*Z*)-trienoic acid (14,15-EET), along with other members of this autacoid family, arise via metabolism of arachidonic acid by cytochromes P450, especially members of the 2C[11] and 2J[12] families. The ratio of EET isomers and their stereocomposition are CYP P450 isoform-dependent[13] and, thus, species- and tissue-specific.[14]

Numerous chemical and metabolic factors conspire to complicate investigations into the roles of 14,15-EET and restrain its potential therapeutic utility (Figure 1).[15] Its susceptibility toward aerial oxidation or auto-oxidation, i.e., a nonenzymatic, free radical process involving the 1,4-dienyl substructures characteristic of polyunsaturated fatty acids, necessitates handling and/or storage under strict conditions that minimize exposure to oxygen and trace transition metals.[16] A precis of inactivating enzymatic processes includes esterification,[17] metabolism by other pathways of the arachidonate cascade,[18] conjugation,[19] β-oxidation,[20] chain elongation,[21] and hydrolysis of the epoxide.[22] In biological milieu, hydration of the epoxide by soluble epoxide hydrolase (sEH) is a major determinant in maintaining the steady state levels of 14,15-EET,[23] whose half-life has been estimated at no more than seconds to minutes.[24] The pioneering studies of Hammock[25] and others[26] confirmed inhibition of sEH can elevate EET levels both in vitro and in vivo, thus offering an indirect means for pharmacologic intervention in EET-mediated processes. However, this strategy might prove less efficacious than an agonist replacement therapy whenever endogenous EET biosynthesis is compromised, for instance, as a consequence of disease, inflammation, radiotherapy, aging, and/or exposure to xenobiotics and drugs that inhibit cytochromes P450.[27]

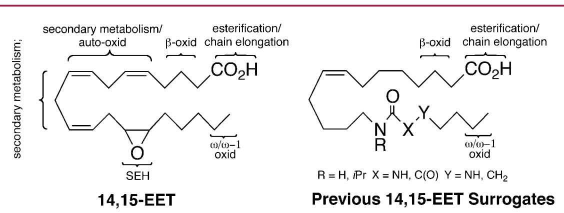


Figure 1. Major routes of metabolism/degradation (oxid = oxidation).

RESULTS AND DISCUSSION

To address some of the stability limitations of natural EETs, our laboratories previously prepared several iterations of EET surrogates with improved stability.[28,29] Advanced versions were

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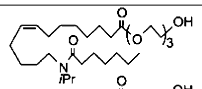
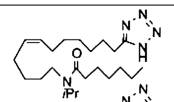
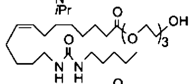
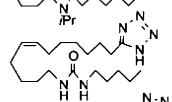
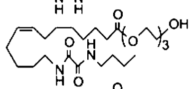
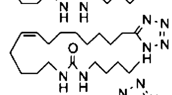
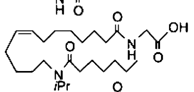
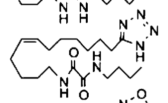
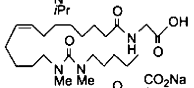
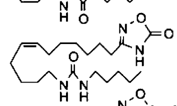
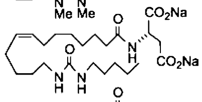
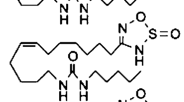
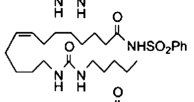
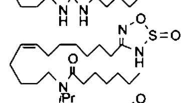
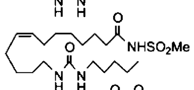
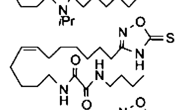
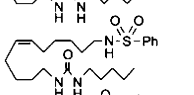
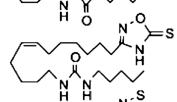
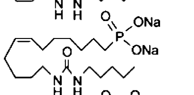
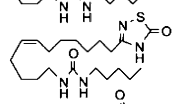
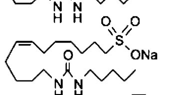
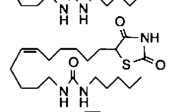
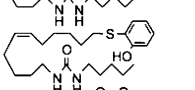
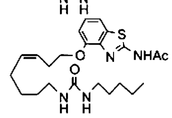
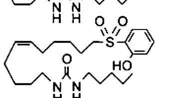
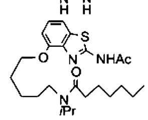
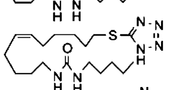
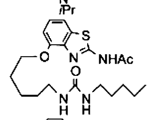
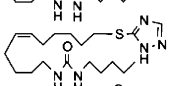
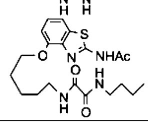
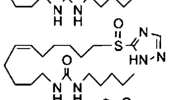
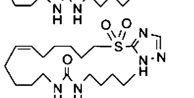
Table 1. Vasorelaxation of Precontracted Bovine Coronary Artery and in Vitro Inhibition of Recombinant Human Soluble Epoxide Hydrolase[a,b]

Compd	Analog	Vascular Relax. % (10 μM)	Vascular Relax. EC_{50} (μM)	sEHi IC_{50} (nM)	Compd	Analog	Vascular Relax. % (10 μM)	Vascular Relax. EC_{50} (μM)	sEHi IC_{50} (nM)
1		86	2.9	>500	**18**		74	1.0	>500
2		72	5.1	255	**19**		119	0.18	11
3		104	1.5	>500	**20**		110	1.1	32
4		95	1.9	>500	**21**		96	1.7	>500
5		92	2.75	>500	**22**		89	1.1	65
6		91	1.6	392	**23**		109	0.34	10
7		73	6.0	41	**24**		109	0.32	>500
8		61	6.7	71	**25**		116	0.36	>500
9		75	3.4	32	**26**		93	3.3	31
10		71	>10	>500	**27**		54	2.4	231
11		63	7.6	57	**28**		47	>10	57
12		48	>10	6	**29**		73	3.3	282
13		76	5.0	11	**30**		96	1.3	>500
14		85	3.7	22	**31**		73	0.9	>500
15		53	9.8	32	**32**		96	2.4	>500
16		92	3.5	96					
17		92	3.1	23					

[a]At 10 μM, 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.

evaluated for relaxation of precontracted bovine coronary artery rings and for in vitro inhibition of recombinant human sEH.[15] Depending upon the bioisostere and its position along the carbon chain, varying levels of vascular relaxation and/or sEH inhibition were observed. Generally, oxamides and *N*-*i*Pr-amides displayed useful 14,15-EET agonist activities but were modest to poor sEH inhibitors. Unsubstituted ureas proved to be both potent 14,15-EET agonists and sEH inhibitors. The in vitro success of this generation of analogues prompted us to consider further structural iterations.[30]

The surrogates described herein modify the free carboxylic acid of the previous generation of 14,15-EET mimics while, for the most part, retaining some key structural features of the pharmacophore identified earlier (i.e., *cis*-$\Delta^{8,9}$-olefin and an epoxide bioisostere). They were evaluated as described before in precontracted bovine coronary artery rings for (i) % vasorelaxation at 10 μM relative to a 14,15-EET control, (ii) EC_{50} for vasorelaxation, and (iii) IC_{50} for sEH inhibition (Table 1).[15] Interestingly, the simple expedient of conjugating the carboxylate with a short poly(ethylene oxide) (PEG) unit improved both % vasorelaxation and the EC_{50} somewhat compared with the parent free acids[31] regardless the type of epoxide bioisostere, viz., *N*-*i*Pr-amide **1**, urea **2**, and oxamide **3**; on the other hand, the soluble epoxide hydrolase (sEH) inhibitory activity of urea **2** was significantly compromised versus the parent free acid (EC_{50} 7.5 μM, IC_{50} 46 nM). Linkage of the carboxylate to the nitrogen of glycine (**4** and **5**) or aspartic acid (**6**) resulted in a modest boost to the vasoactivities while the sEH IC_{50} of **6** dropped by an order of magnitude compared to the parent free acid. Conversion to the *N*-phenyl and *N*-methylsulfonimides **7** and **8**, respectively, left the biological activities virtually unchanged in all categories while the simplified phenylsulfonamide **9** led to an improvement in the EC_{50} by a factor of ~2 and smaller improvements in the other parameters.

Given the generally lackluster behavior of the esters and amides, our focus changed to replacement of the carboxylate with a variety of heterocyclic bioisosteres identified from literature sources.[32] For phosphonate **10** and sulfonate **11**, the increase in polarity did not improve potency as seen with the PEG esters. Interestingly, sulfonate **11** retained its ability to suppress sEH, in contrast to phosphonate **10** and PEG ester **2**. Vasorelaxation by *S*-alkylthiocatechol **12** was poor, but its IC_{50} for sEH was pushed down into the single digits.[33] The sulfone variant **13** regained some vasopotency, probably due to an increase in the acidity of the phenol but not sufficiently to be viable. Replacement of the phenol moiety in **12** with a tetrazole ring[34] produced **14** and was encouraging, but this trend was not continued in triazole **15**. Sequential oxidation of the sulfur to sulfoxamide **16** and sulfonamide **17**, as seen for **12** → **13**, incrementally improved the EC_{50}. The ED_{50} jumped in the tetrazole bioisostere series **18**–**21**, achieving a submicromolar value for urea **19** (12-fold better than 14,15-EET), while the % vasorelaxation versus 14,15-EET peaked. Factoring in a very respectable low nanomolar IC_{50}, **19** is the best dual activity analogue in the study and a leading candidate for further development. Notably, all three parameters began to erode, albeit minimally, in the one-carbon homologue **20** and further in oxamide **21**. The performance of another five-membered heterocycle,[35] oxadiazol-5-one **22**, while acceptable, was not comparable to urea **19**. The closely related oxathiadiazole-2-oxides[35] **23** and **24**, on the other hand, demonstrated good vasorelaxant activities; sEH inhibition potency was consistent with our previous observations that ureas ≫ *N*-*i*Pr-amides. The isomeric thioxo-1,2,4-oxadiazol[35] **25** registered a 6-fold improvement in ED_{50} over 14,15-EET yet was a comparatively poor sEH inhibitor and, thus, is a good option whenever an EET agonist with minimal impact on sEH function is required. The corresponding urea thioxo-oxadiazol,[36] **26**, could not sustain the vasopotency; its ED_{50} sagged by almost an order of magnitude versus **25** while at the same time its sEH IC_{50} improved by more than an order of magnitude. A final variant on this heterocyclic theme, 5-thioxothiadiazol[36] **27**, fell short of expectations on all levels and further study of this particular heterocycle was terminated. Despite its success in the glitazone series of antidiabetic drugs,[37] the 2,4-thiazolidinedione modification proved disappointing when incorporated into **28**. Given the larger size of the *N*-(4-hydroxy-2-benzothiazolyl)acetamide bioisostere,[38] the carbon chain utilized in most of the analogues (Figure 1) was trimmed by four carbons before attachment via the phenolic oxygen to generate **29**. The undistinguished results caused us to re-evaluate our assumptions. Following inspection of molecular models, the carbon chain was shortened even further and the olefin was deleted, leading to *N*-*i*Pr-amide **30**, urea **31**, and oxamide **32**, whose total chain length more closely resembled that of 14,15-EET. As hoped, the EC_{50} values increased relative to **29**, although the % vasorelaxation was variable. All were comparatively poor sEH inhibitors, including unexpectedly urea **31**.

Contrary to expectations, there was no evident correlation between the pK_a of the EET analogues and the ED_{50} for vasorelaxation (Figure 2), suggesting factors other than ionic interactions are involved in binding the carboxylate at the putative EET binding site.

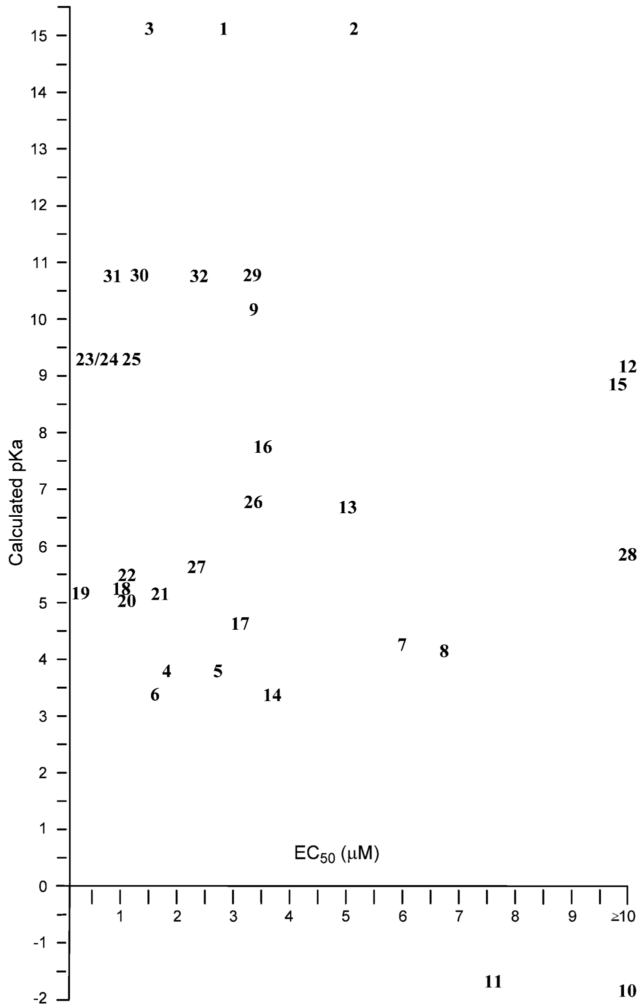


Figure 2. Plot of calculated pK_a vs ED_{50} (μM) of analogues in Table 1. The protonated form of analogues **6**, **11**, and **12** was used for the calculation.

Chemistry. The syntheses of tetrazole **19** and thioxo-1,2,4-oxadiazol **25** are summarized in Scheme 1 and illustrate the approach used to prepare the other analogues. Semihydrogenation of the known[39] acetylene **34** using P-2 nickel and H_2 led to *cis*-olefin **35** that was subjected to azidation using diphenylphosphoryl azide (DPPA) under Mitsunobu conditions. Staudinger reduction of the product, azide **36**, led to primary amine **37** that was reacted without purification with *n*-pentyl isocyanate to furnish urea **38**. An uneventful series of functional group interchanges proceeding through alcohol **39**, bromide **40**, and ending with nitrile **41** set the stage for the zinc

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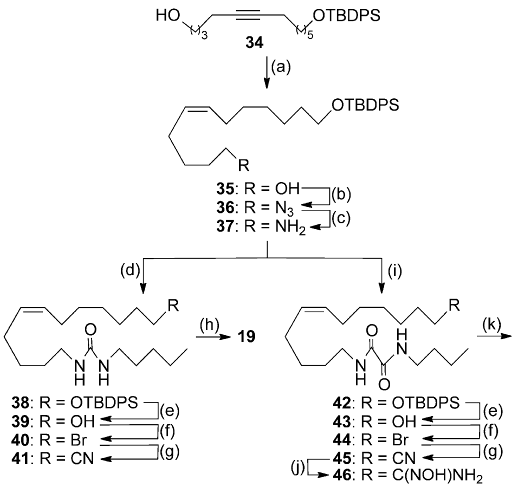
Scheme 1. Synthesis of Representative 14,15-EET Analogues[a]



[a]Reagents and conditions: (a) P2-Ni/$(H_2NCH_2)_2$, H_2 (1 atm), EtOH, rt, 1 h (96%); (b) DIAD/Ph_3P/$Ph_2P(O)N_3$, THF, −20 to 23 °C, 4 h (72%); (c) Ph_3P, H_2O/THF, rt, 12 h (76%); (d) $C_5H_{11}NCO$, THF, rt, 3 h (76%); (e) nBu$_4$NF, THF, rt, 12 h (82−89%); (f) CBr_4/Ph_3P, CH_2Cl_2, rt, 2 h (83−84%); (g) KCN, DMSO, rt, 12 h (78−81%); (h) NaN_3/$ZnBr_2$, *i*PrOH/H_2O (1:3), 110 °C, 18 h (76%); (i) HO-$(CO)_2NH(CH_2)_3CH_3$, EDCI, DMF, rt, 12 h (73%); (j) H_2NOH/Na_2CO_3, MeOH/H_2O (4:1), 60 °C, 18 h (62%); (k) $Im_2C(S)$, THF, rt, 45 min (63%).

bromide mediated annulation[40] with sodium azide that delivered tetrazole **19**.

Condensation of **37** with 2-(*n*-butylamino)-2-oxoacetic acid[15] gave rise to oxamide **42**. Following the same sequence of transformations as described above, **42** was converted into nitrile **45**. Condensation of hydroxyimine **46**, obtained by addition[41] of hydroxylamine to **45**, with 1,1′-(thiocarbonyl)-diimidazole yielded thioxo-1,2,4-oxadiazol **25** as a crystalline solid.

■ EXPERIMENTAL SECTION

General Methods and Materials. Final compounds were judged ≥95% pure by HPLC using a Zorbax Eclipse C18 column (250 mm × 4.6 mm; Agilent) connected to an Agilent 1200 API/LC-MS using acetonitrile/water combinations as eluent unless otherwise noted. Nuclear magnetic resonance (NMR) spectra were recorded on Varian 300, 400, or 500 spectrometers at operating frequencies of 300/400/500 MHz (^{1}H) or 75/100/125 MHz (^{13}C) in $CDCl_3$ with TMS as internal standard, unless otherwise stated. ^{1}H NMR data are reported as follows: chemical shift (ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, app q = apparent quartet, qn = quintet, app qn = apparent quintet, m = multiplet), and coupling constant (Hz). High resolution mass spectra (HRMS) were obtained at UT-Arlington using a Shimadzu IT-TOF mass spectrometer or at the Medical College of Wisconsin by Prof. Kasem Nithipatikom. Infrared (IR) spectra were obtained using a PerkinElmer Spectrum 1000 Fourier transform spectrometer. Melting points were measured using an OptiMelt from Stanford Research Systems and are uncorrected. Analytical thin layer chromatography (TLC) used EMD Chemicals TLC silica gel 60 F254 plates (0.040−0.063 mm) with visualization by UV light and/or $KMnO_4$ or phosphomolybdic acid (PMA) solution followed by heating. All oxygen and/or moisture sensitive reactions were performed under an argon atmosphere using oven-dried glassware and anhydrous solvents. Extracts were dried over anhydrous Na_2SO_4 and filtered prior to removal of all volatiles under reduced pressure. Chromatographic purifications utilized preparative TLC or flash chromatography using prepacked SiO_2 columns on a CombiFlash R_f200 chromatograph (Teledyne Isco). Unless otherwise noted, yields refer to isolated, purified material with spectral data consistent with assigned structures or, if known, were in agreement with published data. Reagents were purchased at the highest commercial quality available and used without further purification, unless otherwise noted. Anhydrous solvents were dried using a Glass Contours solvent system by passage through columns of activated packing material under argon immediately prior to use.

1-(12-(1*H*-Tetrazol-5-yl)dodec-5(*Z*)-en-1-yl)-3-pentylurea (19). A mixture of 1-(12-cyanododec-5(*Z*)-enyl)-3-*n*-pentylurea (**41**). (500 mg, 1.55 mmol), sodium azide (100 mg, 1.55 mmol), and zinc bromide (335 mg, 1.48 mmol) was heated at 110 °C in 2-propanol/H_2O (1:3, 8 mL) while stirring vigorously in a sealed tube.[40] After 18 h, the mixture was cooled to room temperature and the pH was adjusted to 1 using aq HCl (3 N, 4 mL). Ethyl acetate (10 mL) was added, and the stirring was continued until no solid was present. The organic layer was isolated and the aqueous layer extracted with EtOAc (2 × 25 mL). The combined organic fractions were washed with water (3 × 25 mL), dried, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give the tetrazole **19** (431 mg, 76%) as a colorless solid, mp 205.6−205.8 °C. TLC: 10% MeOH/CH_2Cl_2, R_f ∼ 0.30. ^{1}H NMR (CD_3OD, 300 MHz) δ 5.40−5.30 (m, 2H), 3.06−3.11 (m, 4H), 2.93 (t, J = 8.0 Hz, 2H), 1.98−2.10 (m, 4H), 1.70−1.82 (m, 2H), 1.24−1.50 (m, 16H), 0.90 (t, J = 7.6 Hz, 3H). ^{13}C NMR (CD_3OD, 75 MHz) δ 160.16, 156.81, 129.77, 129.47, 39.81, 39.68, 29.88, 29.80, 29.35, 28.99, 28.69, 28.55, 27.48, 26.85, 26.81, 26.68, 22.96, 22.31, 13.22. HRMS calcd for $C_{19}H_{37}N_6O$ [M + 1]$^+$ 365.3029, found 365.3030.

***N*1-Butyl-*N*2-(12-(2-oxido-3*H*-1,2,3,5-oxathiadiazol-4-yl)-dodec-5(*Z*)-en-1-yl)oxalamide (25).** A mixture of N^1-(13-amino-13-(hydroxyimino)tridec-5(Z)-enyl)-N^2-*n*-butyloxalamide (**46**) (100 mg, 0.27 mmol) and 1,1′-(thiocarbonyl)diimidazole (57 mg, 0.32 mmol) in dry THF (5 mL) was stirred at room temperature for 45 min. The mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with water and dried, and the solvent was evaporated in vacuo. The residue was dissolved in acetonitrile (5 mL) to which was then added DBU (61 mg, 0.40 mmol). After stirring at room temperature for 1 h, the mixture was diluted with water (10 mL), adjusted pH ∼ 4 with 1 N HCl, and extracted with ethyl acetate (3 × 10 mL) to give thioxo-1,2,4-oxadiazol **25** (71 mg, 63%) as a white solid, mp 110.6−110.8 °C. TLC: MeOH/CH_2Cl_2 (1:9), R_f ∼ 0.55. ^{1}H NMR (400 MHz) δ 8.90 (br s, NH, 1H), 7.52 (br s, NH, 2H), 5.28−5.40 (m, 2H), 3.20−3.40 (m, 4H), 2.59 (t, J = 7.5 Hz, 2H), 1.98−2.10 (m, 4H), 1.21−1.70 (m, 16H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz) δ 160.12, 160.08, 153.31, 130.62, 129.46, 39.93, 39.85, 31.35, 29.33, 28.94, 28.89, 28.68, 27.02, 26.84, 26.69, 23.96, 20.23, 13.90. HRMS calcd for $C_{19}H_{35}N_4O_4S$ [M + 1]$^+$ 415.2379, found 415.2372.

12-(*tert*-Butyldiphenylsilyloxy)dodec-5(*Z*)-en-1-ol (35). $NaBH_4$ (82 mg, 2.28 mmol) was added in portions with vigorous stirring to a room temperature solution of $Ni(OAc)_2 \cdot 4H_2O$ (567 mg, 2.28 mmol) in absolute ethanol (20 mL) under a hydrogen atmosphere (1 atm). After 15 min, freshly distilled ethylenediamine (0.30 mL, 4.56 mmol) was added to the black suspension, followed after a further 15 min by a solution of 12-(*tert*-butyldiphenylsilyloxy)-dodec-5-yn-1-ol[39] (**34**) (4.0 g, 9.16 mmol) in absolute EtOH (10 mL). After 1 h, the reaction mixture was diluted with Et_2O (20 mL) and passed through a small bed of silica gel. The bed was rinsed with another portion of Et_2O (5 mL). The combined ethereal filtrates were concentrated under reduced pressure to afford alcohol **35** (3.85 g, 96%) as a colorless oil sufficiently pure to be used directly in the next step. TLC: EtOAc/hexanes (3:7), R_f ∼ 0.46. ^{1}H NMR (300 MHz) δ 7.64−7.68 (m, 4H), 7.34−7.42 (m, 6H), 5.42−5.28 (m, 2H), 3.63 (t, J = 6.4 Hz, 4H), 2.08−1.96 (m, 4H), 1.50−1.60 (m, 4H), 1.40−1.24 (m, 10H), 1.04 (s, 9H). ^{13}C NMR (100 MHz) δ 135.81, 134.40, 130.61, 129.71, 129.60, 127.80, 64.21, 63.14, 32.78, 32.60, 29.98, 29.27, 27.42, 27.14, 27.10, 26.08, 25.92, 19.48. HRMS calcd for $C_{28}H_{43}O_2Si$ [M + 1]$^+$ 439.3032, found 439.3027.

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1-*tert*-Butyldiphenylsilyloxy-12-azidododec-7(*Z*)-ene (36). Diisopropyl azodicarboxylate (DIAD; 1.46 mL, 7.35 mmol) was added dropwise to a −20 °C solution of PPh_3 (2.10 g, 8.0 mmol) in dry THF (45 mL) under an argon atmosphere. After 10 min, a solution of 12-(*tert*-butyldiphenylsilyloxy)dodec-5(*Z*)-en-1-ol (**35**) (3.20 g, 7.35 mmol) in dry THF (10 mL) was added dropwise. After 30 min, the mixture was warmed to 0 °C and diphenylphosphoryl azide (1.58 mL, 7.35 mmol) was added dropwise. After stirring 4 h at rt, the reaction mixture was quenched with water (150 mL) and extracted with EtOAc (2 × 100 mL). The combined organic extracts were washed with brine (100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by SiO_2 column chromatography eluting with 4% EtOAc/hexane to afford azide **36** (2.45 g, 72%). TLC: EtOAc/hexanes (1:9), $R_f \sim 0.55$. ^{1}H NMR (400 MHz) δ 7.64−7.68 (m, 4H), 7.34−7.42 (m, 6H), 5.28−5.42 (m, 2H), 3.70 (t, *J* = 5.8 Hz, 2H), 3.27 (t, *J* = 6.3 Hz, 2H), 1.96−2.10 (m, 4H), 1.24−1.64 (m, 12H), 1.04 (s, 9H). ^{13}C NMR (100 MHz) δ 135.84, 134.41, 130.93, 129.75, 129.12, 127.83, 64.22, 51.62, 32.81, 29.93, 29.30, 28.68, 27.46, 27.14, 27.02, 26.90, 25.96, 19.49. IR (neat) 2930, 2783, 2331, 2097, 1106 cm^{-1}. HRMS calcd for $C_{28}H_{42}N_3OSi$ $[M + 1]^+$ 464.3097, found 464.3099.

1-*tert*-Butyldiphenylsilyloxy-12-aminododec-7(*Z*)-ene (37). Triphenylphosphine (1.18 g, 4.50 mmol) was added to a stirring solution of 1-*tert*-butyldiphenylsilyloxy-12-azidododec-7(*Z*)-ene (**36**) (1.90 g, 4.10 mmol) in THF (12 mL) containing 10 drops of deionized water. After 12 h, the reaction mixture was diluted with CH_2Cl_2 (10 mL), dried, and concentrated in vacuo to give amine **37** (1.36 g, 76%) as a viscous, colorless oil that was used directly in the next reaction without further purification. TLC: MeOH/CH_2Cl_2 (1:4), $R_f \sim 0.25$. ^{1}H NMR (400 MHz) δ 7.62−7.68 (m, 4H), 7.32−7.40 (m, 6H), 5.30−5.40 (m, 2H), 3.63 (t, *J* = 5.2 Hz, 2H), 2.62 (t, *J* = 4.8 Hz, 2H), 1.92−2.06 (m, 4H), 1.40−1.58 (m, 4H), 1.20−1.40 (m, 8H), 1.03 (s, 9H). ^{13}C NMR (100 MHz) δ 135.79, 134.37, 130.42, 129.70, 127.78, 64.19, 42.28, 33.44, 32.77, 29.93, 29.28, 27.40, 27.21, 27.10, 25.92, 19.44. HRMS calcd for $C_{28}H_{44}NOSi$ $[M + 1]^+$ 438.3192, found 438.3186.

1-(12-(*tert*-Butyldiphenylsilyloxy)dodec-5(*Z*)-enyl)-3-*n*-pentylurea (38). A solution of 1-*tert*-butyldiphenylsilyloxy-12-aminododec-7(*Z*)-ene (**37**) (1.32 g, 3.0 mmol) in THF (5 mL) was added dropwise to a stirring solution of *n*-pentyl isocyanate (0.386 mL, 3.0 mmol) in THF (10 mL). After 3 h stirring at room temperature, all volatiles were removed under reduced pressure and the residue was purified by SiO_2 column chromatography eluting with 20% EtOAc/hexane to afford urea **38** (1.26 g, 76%) as a viscous oil. TLC: EtOAc/hexanes (2:3), $R_f \sim 0.40$. ^{1}H NMR (300 MHz) δ 7.60−7.70 (m, 4H), 7.35−7.42 (m, 6H), 5.28−5.42 (m, 2H), 5.16 (br s, −NH, 2H), 3.65 (t, *J* = 6.5 Hz, 2H), 3.08−3.20 (m, 4H), 1.96−2.08 (m, 4H), 1.22−1.60 (m, 18H), 1.02 (s, 9H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz) δ 159.23, 135.80, 134.24, 130.52, 129.74, 129.49, 127.82, 64.22, 40.62, 40.54, 32.80, 30.33, 29.95, 29.37, 29.32, 27.46, 27.34, 27.18, 27.11, 25.97, 22.71, 19.46, 14.29. HRMS calcd for $C_{34}H_{55}N_2O_2Si$ $[M + 1]^+$ 551.4033, found 551.4032.

1-(12-Hydroxydodec-5(*Z*)-enyl)-3-*n*-pentylurea (39). A mixture of 1-(12-(*tert*-butyldiphenylsilyloxy)dodec-5(*Z*)-enyl)-3-*n*-pentylurea (**38**) (1.12 g, 2.0 mmol) and tetra-*n*-butylammonium fluoride (2.20 mL of 1 M soln in THF, 2.2 mmol) in dry THF (10 mL) was stirred at room temperature under an argon atmosphere for 12 h and then evaporated to dryness in vacuo. The residue was dissolved in EtOAc (50 mL) and washed with water (30 mL), brine (30 mL), dried, and evaporated in vacuo. Purification of the residue via SiO_2 column chromatography gave alcohol **39** (0.56 g, 89%) as a colorless solid, mp 63.7−63.8 °C. TLC: EtOAc/hexanes (7:3), $R_f \sim 0.30$. ^{1}H NMR (300 MHz) δ 5.25−5.42 (m, 2H), 4.48 (br s, −NH, 2H), 3.64 (d, *J* = 6.5 Hz, 2H), 3.08−3.20 (m, 4H), 1.96−2.14 (m, 4H), 1.22−1.60 (m, 18H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz) δ 159.26, 130.23, 129.62, 63.72, 40.33, 40.29, 32.92, 30.30, 30.26, 29.74, 29.35, 29.13, 27.26, 27.20, 27.13, 25.82, 22.69, 14.27. HRMS calcd for $C_{18}H_{37}N_2O_2$ $[M + 1]^+$ 313.2855, found 313.2857.

1-(12-Bromododec-5(*Z*)-enyl)-3-*n*-pentylurea (40). CBr_4 (0.55 g, 1.66 mmol) and PPh_3 (0.43 g, 1.66 mmol) were added to a 0 °C solution of 1-(12-hydroxydodec-5(*Z*)-enyl)-3-*n*-pentylurea (**39**) (0.43 g, 1.38 mmol) in CH_2Cl_2 (20 mL). After 2 h at room temperature, the reaction mixture was concentrated in vacuo and the residue was purified via SiO_2 column chromatography to give 1-(12-bromododec-5(*Z*)-enyl)-3-*n*-pentylurea (**40**) (0.43 g, 83%) as a solid, mp 46.7−46.8 °C. TLC: EtOAc/hexanes (2:3), $R_f \sim 0.60$. ^{1}H NMR (300 MHz) δ 5.22−5.42 (m, 2H), 4.40 (br s, 2H), 3.42 (t, *J* = 9.3 Hz, 2H), 3.10−3.20 (m, 4H), 1.98−2.10 (m, 4H), 1.80−1.90 (m, 2H), 1.25−1.55 (m, 16H), 0.92 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz) δ 159.51, 130.14, 129.69, 40.48, 40.39, 34.20, 32.96, 30.34, 29.67, 29.36, 28.58, 28.25, 27.31, 27.27, 27.17, 22.68, 14.26. HRMS calcd for $C_{18}H_{36}BrN_2O$ $[M + 1]^+$ 375.2011, found 375.2014.

1-(12-Cyanododec-5(*Z*)-enyl)-3-*n*-pentylurea (41). A mixture of potassium cyanide (0.23 g, 3.54 mmol) and 1-(12-bromododec-5(*Z*)-enyl)-3-*n*-pentylurea (**40**) (0.90 g, 2.40 mmol) was stirred in DMSO (5 mL) at room temperature. After 12 h, the reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with water (2 × 25 mL), brine (25 mL), dried (Na_2SO_4), and passed through a silica gel column to give nitrile **41** (0.62 g, 81%) as a colorless solid, mp 56−57 °C. TLC: EtOAc/hexanes (2:3), $R_f \sim 0.45$. ^{1}H NMR (300 MHz) δ 5.29−5.40 (m, 2H), 4.27 (br s, −NH, 2H), 3.10−3.20 (m, 4H), 2.34 (t, *J* = 7.0 Hz, 2H), 1.98−2.08 (m, 4H) 1.24−1.70 (m, 18H), 0.89 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz) δ 159.41, 129.94, 129.86, 120.14, 40.45, 40.35, 30.30, 29.50, 29.33, 28.70, 28.51, 27.26, 27.16, 25.47, 22.66, 17.28, 14.24. IR (neat) 2930, 2281, 2184, 2042, 1936, 1613, 1197, 1042 cm^{-1}. HRMS calcd for $C_{19}H_{36}N_3O$ $[M + 1]^+$ 322.2858, found 322.2867.

***N*^{1}-*n*-Butyl-*N*^{2}-(12-(*tert*-butyldiphenylsilyloxy)dodec-5(*Z*)-enyl)oxalamide (42).** A mixture of 2-(*n*-butylamino)-2-oxoacetic acid[15] (0.40 g, 2.70 mmol), 1-*tert*-butyldiphenylsilyloxy-12-aminododec-7(*Z*)-ene (**37**) (1.20 g, 2.70 mmol), 1-hydroxybenzotriazole (HOBt; 0.44 g, 3.30 mmol), and [1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride] (EDCI: 0.63 g, 3.30 mmol) in dry DMF (5 mL) was stirred at room temperature overnight. The reaction mixture was quenched with water (30 mL) and extracted into ethyl acetate (3 × 20 mL). The combined organic extracts were washed with water (2 × 10 mL) and brine (10 mL), dried, and concentrated in vacuo. The residue was purified by SiO_2 column chromatography to give *N*^{1}-*n*-butyl-*N*^{2}-(12-(*tert*-butyldiphenylsilyloxy)dodec-5(*Z*)-enyl)oxalamide (**42**) (1.10 g, 73%). TLC: EtOAc/hexanes (2:3), $R_f \sim 0.55$. ^{1}H NMR (400 MHz) δ 8.05 (br s, −NH, 2H), 7.66−7.74 (m, 4H), 7.32−7.42 (m, 6H), 5.30−5.42 (m, 2H), 3.67 (t, *J* = 3.9 Hz, 2H), 3.31 (q, *J* = 5.2 Hz, 4H), 1.96−2.10 (m, 4H), 1.50−1.64 (m, 6H), 1.22−1.44 (m, 10H), 1.06 (s, 9H), 0.92 (t, *J* = 7.8 Hz, 3H). ^{13}C NMR (100 MHz) δ 160.33, 135.80, 134.35, 130.73, 129.74, 129.20, 127.83, 64.17, 39.89, 39.69, 32.79, 31.48, 29.94, 29.29, 29.07, 27.46, 27.23, 27.14, 27.0, 25.96, 20.29, 19.46, 13.96. HRMS calcd for $C_{34}H_{53}N_2O_3Si$ $[M + 1]^+$ 565.3826, found 565.3824.

***N*^{1}-*n*-Butyl-*N*^{2}-(12-hydroxydodec-5(*Z*)-enyl)oxalamide (43).** *N*^{1}-*n*-Butyl-N^2-(12-(*tert*-butyldiphenylsilyloxy)dodec-5(*Z*)-enyl)oxalamide (**42**) (1.20 g, 2.12 mmol) was desilylated as described above for **39** to give *N*^{1}-*n*-butyl-*N*^{2}-(12-hydroxydodec-5(*Z*)-enyl)oxalamide (**43**) (0.568 g, 82%) as a colorless solid, mp 102.8−102.9 °C. TLC: EtOAc/hexanes (7:3), $R_f \sim 0.55$. ^{1}H NMR (400 MHz) δ 7.69 (br s, 2H), 5.20−5.35 (m, 2H), 3.56 (t, *J* = 4.2 Hz, 2H), 3.26 (q, *J* = 5.6 Hz, 4H), 2.17 (br s, 1H), 1.95−2.02 (m, 4H), 1.44−1.56 (m, 6H), 1.20−1.40 (m, 10H), 0.87 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz) δ 160.15, 130.66, 129.21, 62.98, 39.80, 39.63, 32.93, 31.39, 29.77, 29.18, 28.95, 27.26, 27.0, 26.88, 25.80, 20.18, 13.85. HRMS calcd for $C_{18}H_{35}N_2O_3$ $[M + 1]^+$ 327.2648, found 327.2648.

***N*^{1}-(12-Bromododec-5(*Z*)-enyl)-*N*^{2}-*n*-butyloxalamide (44).** *N*^{1}-*n*-Butyl-*N*^{2}-(12-hydroxydodec-5(*Z*)-enyl)oxalamide (**43**) (330 mg, 1.0 mmol) was brominated as described above for **40** to give *N*^{1}-(12-bromododec-5(*Z*)-enyl)-*N*^{2}-*n*-butyloxalamide (**44**) (330 mg, 84%) as a white solid, mp 46.0−46.3 °C. TLC: EtOAc/hexanes (3:2), $R_f \sim 0.55$. ^{1}H NMR (400 MHz) δ 7.79 (br s, −NH, 1H), 7.77 (br s, −NH, 1H), 5.20−5.32 (m, 2H), 3.32 (t, *J* = 6.4 Hz, 2H), 3.22 (q, *J* = 7.2 Hz, 4H), 1.90−2.00 (m, 4H), 1.72−1.82 (m, 2H), 1.42−1.56 (m, 4H), 1.20−1.40 (m, 10H), 0.85 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz) δ

160.17, 160.15, 130.40, 129.34, 39.77, 39.59, 34.12, 32.93, 31.40, 29.62, 29.0, 28.54, 27.25, 27.24, 27.0, 26.91, 20.18, 13.85. HRMS calcd for $C_{18}H_{34}BrN_2O_2$ $[M + 1]^+$ 389.1804, found 389.1809.

***N*^{1}-*n*-Butyl-*N*^{2}-(12-cyanododec-5(*Z*)-enyl)oxalamide (45).** *N*^{1}-(12-Bromododec-5(*Z*)-enyl)-*N*^{2}-*n*-butyloxalamide (**44**) (250 mg, 0.642 mmol) was treated with potassium cyanide as described above for **41** to give *N*^{1}-*n*-butyl-*N*^{2}-(12-cyanododec-5(*Z*)-enyl)oxalamide (**45**) (168 mg, 78%) as a colorless solid, mp 83.0−83.3 °C. TLC: EtOAc/hexanes (3:2), $R_f \sim 0.35$. ^{1}H NMR (400 MHz) δ 7.45 (br s, −NH, 2H), 5.30−5.40 (m, 2H), 3.34 (q, J = 8.6 Hz, 4H), 2.32 (t, J = 7.6 Hz, 2H), 1.98−2.08 (m, 4H), 1.30−1.68 (m, 16H), 0.92 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz) δ 160.03 (2C), 130.03, 129.08, 120.10, 39.88, 39.42, 31.22, 29.40, 28.82, 28.60, 28.42, 27.07, 27.06, 26.82, 25.54, 20.06, 17.01, 13.80. HRMS calcd for $C_{19}H_{34}N_3O_2$ $[M + 1]^+$ 336.2651, found 336.2650.

***N*^{1}-(13-Amino-13-(hydroxyimino)tridec-5(*Z*)-enyl)-*N*^{2}-*n*-butyloxalamide (46).** To a suspension of *N*^{1}-*n*-butyl-*N*^{2}-(12-cyanododec-5(*Z*)-enyl)oxalamide (**45**) (420 mg, 1.29 mmol) in MeOH/H_2O (4:1; 12 mL) was added $H_2NOH\cdot HCl$ (228 mg, 3.28 mmol) and Na_2CO_3 (344 mg, 3.25 mmol).[41] The reaction mixture was heated at 60 °C for 18 h then cooled to room temperature, and all volatiles were removed in vacuo. The residue was diluted with water (30 mL) and extracted into ethyl acetate (2 × 25 mL). The combined organic extracts were washed with water (2 × 10 mL) and brine (10 mL), dried, and purified via silica gel column chromatography to give *N*^{1}-(13-amino-13-(hydroxyimino)tridec-5(*Z*)-enyl)-*N*^{2}-*n*-butyloxalamide (**46**) (287 mg, 62%) as a colorless solid, 116.3−116.4 °C. TLC: MeOH/CH_2Cl_2 (1:4), $R_f \sim 0.20$. ^{1}H NMR (CD_3OD, 400 MHz) δ 5.28−5.40 (m, 2H), 3.24 (t, J = 6.4 Hz, 4H), 1.98−2.00 (m, 6H), 1.50−1.60 (m, 6H), 1.26−1.40 (m, 10H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 160.55 (2C), 156.31, 130.05, 129.18, 39.23, 39.09, 31.18, 30.63, 29.51, 28.83, 28.69, 27.10, 26.87, 26.59, 19.88, 12.88. HRMS calcd for $C_{19}H_{37}N_4O_3$ $[M + 1]^+$ 369.2866, found 369.2864.

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.[42,43] 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[44] and was purified by affinity chromatography.[45] Inhibition potencies (IC_{50}s) were determined using a fluorescent-based assay.[46] 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 mM), 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).[46] IC_{50}s refer to the concentrations of inhibitor that reduced activity by 50% and are the averages of three replicates.

ASSOCIATED CONTENT

Supporting Information

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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Notes

The authors declare the following competing financial interest(s): JRF, JDI, and WBC authored patents describing the synthesis and clinical uses of the analogues described herein and assigned all rights to the Medical College of Wisconsin and the University of Texas Southwestern Medical Center.

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ABBREVIATIONS USED

14,15-EET, *cis*-14,15-epoxyeicosa-5(*Z*),8(*Z*),11(*Z*)-trienoic acid; sEH, soluble epoxide hydrolase

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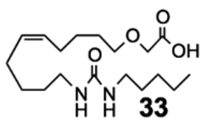

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