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-[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. The best studied of these epoxides is all-cis-14,15-epoxyeicosa-5,8,11-trienoic acid (14,15-EET), which is derived from arachidonic acid and ascribed an impressive array of cardiovascular, pulmonary, renal, and CNS roles. 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_α 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_α. A reversible and abundant high-affinity binding site for 14,15-EET and its analogues has been identified and shown to preferentially recognize the 14(R),15(S)-enantiomer. The kinetic parameters of this binding site share many characteristics in common with the canonical prostanoiid and leukotriene receptors, e.g., _K_α values in the low nanomolar range; however, characterization of the putative EET receptor at the molecular level has been elusive.

14,15-EET, in common with most eicosanoids, is chemically and metabolically labile (Figure 1). Further transformations by enzymes of the cascade, esterification, conjugation, β-oxidation, chain elongation, and hydration by soluble epoxide hydrolase (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, whose in vivo half-life has been estimated as a few seconds to minutes. Additionally, the proclivity of 14,15-EET toward auto-oxidation, a consequence of the 1,4-dienyl moieties present along the backbone, introduces a further layer of complication and often necessitates storage and/or handling under an inert atmosphere. 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. 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. This approach ostensibly 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 sEHi inhibitors. Advanced members of this genre show promise as first-in-class therapeutics for a variety of diseases including diabetes, inflammation, and hypertension. 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...
potential epoxide bioisosteres capable of functioning as stable 14,15-EET surrogates and/or as inhibitors of soluble epoxide hydrolase.

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 and LOX metabolism, (ii) a cis-4,8,9-olefin thought to be essential for EET agonist activity, and (iii) a sEH-resistant 1,3-disubstituted urea that we anticipated would function as a surrogate for the Δ14,15-epoxide. 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 on its affinity for sEH.

Table 1. Vasorelaxation of Precontracted Bovine Coronary Artery and In Vitro Inhibition of Recombinant Human Soluble Epoxide Hydrolase

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<th>Compd</th>
<th>Analog</th>
<th>Vascular Relax.</th>
<th>EC50 (μM)</th>
<th>IC50 (nM)</th>
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<th>Analog</th>
<th>Vascular Relax.</th>
<th>EC50 (μM)</th>
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At 10 mM, 14,15-EET induces 85% of maximum vasorelaxation and its ED50 is 2.2 μM. For recombinant human sEH, the IC50 for 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) is 3 nM. Bioassay determinations (n) = 3–5.

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

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 and LOX metabolism, (ii) a cis-Δ8,9-olefin thought to be essential for EET agonist activity, and (iii) a sEH-resistant 1,3-disubstituted urea that we anticipated would function as a surrogate for the Δ14,15-epoxide. 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 sEH activity.\textsuperscript{20} On the other hand, N-methylation of the distal urea nitrogen gave rise to regioisomer \textit{3} and significantly improved EET mimicry while sEH activity degraded sharply. The differences between these N-methylated regioisomers for sEH are similar to the differences observed previously with amide regioisomers.\textsuperscript{15} Substitution of both nitrogens as in \textit{4} did not prove additive with respect to VR but did further exacerbate the loss of sEH. These data were the first convincing indications that EET agonist activity and sEH 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 \textit{19} and \textit{23}. A lesser level of discrimination between the two activities could also be achieved by heteroatom replacement.\textsuperscript{20} Thiourea \textit{5} was equipotent with 14,15-EET across the entire concentration range of the vascular assay, while sEH declined by a factor of 16 with respect to VR. On the other hand, replacement of either urea nitrogen with oxygen, i.e., \textit{6} and \textit{7}, had relatively little influence on vessel tension but did blunt sEH. In light of these data, it is tempting to suggest that hydrogen bonding or coordination to a metal center (e.g., Cu\textsuperscript{2+}, Fe\textsuperscript{2+}, or Zn\textsuperscript{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 \textit{8} but not acetylene \textit{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 sEH, both \textit{8} and \textit{9} retained their low nanomolar potencies and were almost identical with that of the benchmark \textit{1}. Relocation of the cis-olefin to \textit{Δ2,6} of the carbon chain (analogue \textit{10}) only modestly perturbed EET agonist activity and not at all for the related acetylene \textit{11}. This is unexpected given the regiodependence observed\textsuperscript{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 sEH for \textit{11}, sans N=H, is consistent with established SAR for this pharmacophore.\textsuperscript{20} Shifting the urea one position right (analogue \textit{12}) or left (analogue \textit{13}) relative to its placement in \textit{1} improved VR with respect to \textit{1}, whereas displacement (accompanied by the cis-olefin) two positions further from the carboxylate, resembling an ω-3 fatty acid, was counterproductive (analogue \textit{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\textsubscript{50} for sEH declined steadily following the same series. This appears generally consistent with the proposal\textsuperscript{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.\textsuperscript{20}

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

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

A selection of nitrogen and oxygen heterocyclic bioisosteres, viz, triazole \textit{24}, furan \textit{25}, and 2-oxazoline \textit{26}, were prepared based upon the template in \textit{1}. They did not appear to offer any pharmacological advantage and were not pursued further. The loss of both VR and sEH activities in the tetranor-ureas \textit{27} and \textit{28} is noteworthy given prior reports that chain-shortened 14,15-EETs retain much of their biological activity in the vasculature.\textsuperscript{13} Formally, \textit{27} and \textit{28} can be envisioned as arising from \textit{1} and \textit{9}, respectively, via two cycles of β-oxidation.\textsuperscript{15} 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.

\textbf{Chemistry.} The syntheses of thiourea \textit{5}, oxamide \textit{16}, and \textit{N}-isopropylamide \textit{20} are summarized in Scheme 1 and are representative of the methodology used to prepare the other analogues. Following literature precedent,\textsuperscript{28} the dianion of commercial non-8-ynoic acid (\textit{29}) was alkylated with 1 equiv of 2-(4-bromobutoxy)-tetrahydro-2H-pyran\textsuperscript{29} (\textit{30}) in THF/HMPA (4:1). The resultant disubstituted acetylene \textit{31} gave rise to alcohol \textit{32} following acidic hydrolysis in methanol. Semi-hydrogenation over P-2 nickel created \textit{cis}-olefin \textit{33} that was subjected to azidation using diphenylphosphoryl azide (DPPA) under Mitsunobu conditions. Staudinger reduction of the product, azide \textit{34}, led to primary amine \textit{35}. Reaction of the latter with \textit{n}-pentylisothiocyanate or a combination of 2-(butylamino)-2-oxoacetic acid\textsuperscript{30} and 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) was unremarkable and furnished thiourea \textit{38} and oxamide \textit{40}, respectively. Alternatively, \textit{33} was converted with Ph3P/IB to iodide \textit{36} that was then displaced with excess isopropylamine at 80 °C. HATU-induced condensation of the product \textit{37} with \textit{n}-heptanoic acid delivered amide \textit{42} in good overall yield. Saponification of \textit{38}, \textit{40}, and \textit{42} afforded the corresponding free acids \textit{5}, \textit{16}, and \textit{20}, respectively.

\textbf{Scheme 1. Synthesis of Representative Analogues}
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 benzenophene ketyl, except for CH₂Cl₂, which was distilled from CaH₂. Extracts were dried over anhydrous Na₂SO₄ and filtered prior to removal of all volatiles under reduced pressure. Unless otherwise noted, commercially available materials were used without purification. Volatiles under reduced pressure. Unless otherwise noted, compounds were purified by SiO₂ column chromatography or preparative TLC. TLC plates consisted of 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 δ 7.26, CDCl₃ δ 7.29, MeOD δ 4.80, 31P NMR δ 0.00). Spectra were recorded at 298 K (300 MHz for 1H NMR and 75 MHz for 13C NMR). 1H NMR data are given in ppm relative to residual solvent (chloroform δ 7.26) 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(7)-enoate (38). 1-Methylbenzimidazole (62 mL of a 2.5 M solution in hexanes, 15.5 mmol) was added dropwise to a −20 °C solution of PPh₃ (241 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 diethylphosphoryl azide (DPPA; 364 mL, 1.68 mmol) was added dropwise. After stirring for 4 h at rt, the reaction mixture was quenched with saturated aq NH₄Cl (7.15 mmol), after 10 min, the combined extracts were washed with water (2 × 50 mL), after 10 min, the combined extracts were washed with brine (20 mL), dried, and concentrated under reduced pressure. The residue was purified by SiO₂ column chromatography eluting with 5% EtOAc/hexane to afford methyl 13-Aminotridec-8(7)-enoate (34). Dihydropyrimidinone (DEAD; 243 mg, 65%) was added to a 0 °C solution of non-8-ynoic acid (90 mg, 0.33 mmol) in THF (2 mL) containing 4 drops of denitrogenated water. After 12 h, the reaction mixture was diluted with CH₂Cl₂ (2 mL), dried, and concentrated in vacuo to give 35 (64 mg, 78%) as a colorless oil that was used directly in the next reaction without further purification. TLC: 20% MeOH/CH₂Cl₂, Rf 0.25. 1H NMR (300 MHz) δ 5.28–5.44 (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). 13C NMR (100 MHz) δ 174.24, 130.69, 129.17, 51.64, 51.55, 34.26, 29.66, 29.22, 28.61, 27.33, 26.96, 26.83, 25.22. LR (neat) 2985, 2954, 2845, 2106, 1754, 1250, 1104, 1029 cm⁻¹. HRMS calcd for C₁₄H₁₉O₂N [M + H]⁺ 267.3672, found 267.3680.

Methyl 13-Aminotridec-8(7)-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 denitrogenated water. After 12 h, the reaction mixture was diluted with CH₂Cl₂ (2 mL), dried, and concentrated in vacuo to give 35 (64 mg, 78%) as a colorless oil that was used directly in the next reaction without further purification. TLC: 20% MeOH/CH₂Cl₂, Rf 0.25. 1H NMR (300 MHz) δ 5.22–5.36 (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). 13C 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 C₁₄H₂₅N₂O₂ [M + H]⁺ 261.3967, found 241.3705.

Methyl 13-(3-Pentylthioioureido)tridec-8(7)-enoate (39). 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-pentylthioisocyanate (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 SiO₂ column chromatography eluting with 30% EtOAc/hexane to afford 39 (80 mg, 70%) as a colorless oil. TLC: EtOAc/hexanes (1:1), Rf 0.65. 1H NMR (CDCl₃, 300 MHz) δ 0.88 (t, J = 6.8 Hz, 3H). 13C NMR (75 MHz) δ 174.54, 174.35, 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, 24.14, 13.41. HRMS calcd for C₁₅H₂₈N₂O₂ [M + H]⁺ 271.3663, found 271.3669.

Methyl 13-(3-Pentylthioioureido)tridec-8(7)-enoic Acid (40). LiOH (840 mg of a 1 M solution) was added to a 0 °C solution of methyl ester 38 (80 mg, 0.21 mmol) in THF/H₂O (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 H2O (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 SiO2 column chromatography eluting with EtOAc/hexane (1:1) to afford 5 (67 mg, 87%) as a colorless oil. TLC: 60% EtOAc/hexanes, Rf = 0.35; mp 84.4–85.2 °C. 1H NMR (300 MHz) δ 5.28–5.36 (m, 2H), 3.38 (brs, 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). 13C NMR (75 MHz) δ 181.05, 179.53, 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, 30.14, 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, 24.99, 24.9, 22.78, 21.54, 20.75, 14.29, ES-LC/MS m/z: 380 (M + H+)HRMS calc for C23H43NO3 [M + H+] 396.3478, found 396.3475.

13-(N-Isopropylheptanamido)-tridec-8(Z)-enanoic acid (20). Hydrolysis of 40 using LiOH as described for 38 afforded 40 (20% yield) as a colorless oil. TLC: 75% EtOAc/hexanes, Rf = 0.4. 1H 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 = 6.8 Hz, 6H). 13C NMR (75 MHz) δ 179.00, 178.24, 173.51, 173.08, 130.81, 130.08, 129.95, 129.15, 48.57, 45.66, 43.61, 41.26, 34.36, 34.28, 34.23, 30.14, 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, 24.99, 24.9, 22.78, 21.54, 20.75, 14.29, ES- LC/MS m/z: 380 (M + H+)HRMS calc for C23H43NO3 [M + H+] 396.3478, found 396.3475.

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.15a Synthesis 14-15 EET was used as 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 and was purified by affinity chromatography. Inhibition potencies (IC50) were determined using a fluorescent-based assay. Human sEH (IC50 < 1.0 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, cyanol[2-methoxyphenylthienyl-6-ylmethyl trans-(3-phenyl-oxo-xylyl-2-ylmethyl carbonate (CMNPC; [S]final = 5 μM), was added. Activity was assessed by measuring the appearance of the fluorescent 6-methoxyphenylthiazide product (λex = 465 nm) at 30 °C during a 10 min incubation (Spectramax M2, Molecular Device, Inc., Sunnyvale, CA). IC50 refer to the concentrations of inhibitor that reduced activity by 50% and are the averages of three replicates.
(5) Le Quere, V.; Plee-Gautier, A.; Mouton, P.; Madec, S.; Salama, J.-P. Human CVF4Ps are the Main Catalysts in the Oxidation of Fatty Acid Epoxides. J. Lipid Res. 2004, 45, 1446–1458.


