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17(*R*),18(*S*)-Epoxyeicosatetraenoic Acid, A Potent Eicosapentaenoic Acid (EPA)-Derived Regulator of Cardiomyocyte Contraction: Structure-Activity Relationships and Stable Analogs

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Abstract

17(*R*),18(*S*)-Epoxyeicosatetraenoic acid [17(*R*),18(*S*)-EETeTr], a cytochrome P450 epoxygenase metabolite of eicosapentaenoic acid (EPA), exerts negative chronotropic effects and protects neonatal rat cardiomyocytes against Ca^{2+}-overload with an EC_{50} ~1–2 nM. Structure-activity studies revealed a *cis*-$\Delta^{11,12}$- or $\Delta^{14,15}$-olefin and a 17(*R*),18(*S*)-epoxide are minimal structural elements for anti-arrhythmic activity whereas antagonist activity was often associated with the combination of a $\Delta^{14,15}$-olefin and a 17(*S*),18(*R*)-epoxide. Compared with natural material, the agonist and antagonist analogs are chemically and metabolically more robust and several show promise as templates for future development of clinical candidates.

Introduction

The intake of omega-3 polyunsaturated fatty acids (ω-3 PUFAsa) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is associated with manifold health benefits,[1] especially for cardiovascular disease,[2] e.g., lower triglyceride levels, amelioration of ischemic injury, and reduction of sudden cardiac death resulting from fatal arrhythmia.[3] However, the mechanism(s) of action as well as the molecular specie(s) or metabolite(s) responsible for the protective effects of the ω-3 PUFAs remain largely obscure.[4] A leading hypothesis is that EPA and its congeners compete with arachidonic acid (AA) for enzymatic transformations and that the ω-3 PUFA metabolites have physiological consequences that differ from those of AA.[5]

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Supporting Information Available. Synthetic procedures, analytical data, and NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

[a]Abbreviations: ω-3 PUFAs, omega-3 polyunsaturated fatty acids; EPA, eicosa-5(*Z*),8(*Z*),11(*Z*),14(*Z*),17(*Z*)-pentaenoic acid; DHA, docosa-5(*Z*),8(*Z*),11(*Z*),14(*Z*),16(*Z*),19(*Z*)-hexaenoic acid; AA, arachidonic acid or eicosa-5(*Z*),8(*Z*),11(*Z*),14(*Z*)-tetraenoic acid; EETeTr, epoxyeicosatetraenoic acid; NRCM, neonatal rat cardiomyocytes; sEH, soluble epoxide hydrolase; EET, epoxyeicosatrienoic acid; EEZE, 14,15-epoxyeicosa-5(*Z*)-enoic acid.

In concert with this proposal, a series of *in vitro* studies demonstrated that many major cytochrome P450 (CYP) epoxygenase isoforms preferentially metabolize EPA to the 17,18-epoxide **1** (17,18-EETeTr)[6] and often favor the 17(*R*),18(*S*)-enantiomer **2**.[6b,c,7] A recent *in vivo* study revealed that dietary ω-3 PUFA supplementation causes a profound shift in the endogenous CYP-eicosanoid profile from AA- to EPA/DHA-derived metabolites.[8] In fact, **1** represented the predominant epoxy-metabolite in the heart, lung, kidney and several other organs of rats fed an EPA/DHA-rich diet, whereas this metabolite was produced only in trace amounts when the animals received normal chow rich in ω-6 PUFAs.[8] There is also initial evidence for the *in vivo* formation of EETeTrs in humans based on studies showing the occurrence of these EPA metabolites and/or their hydrolysis products in urine and plasma of volunteers ingesting increased amounts of ω-3 PUFAs.[9,10] Epoxide **2** is a potent vasodilator and stimulates large-conductance K^+ (BK) channels in rat cerebral arteries.[11] The vasodilator effects of epoxides **1** and **2** are not blocked by the epoxyeicosatrienoic acid (EET) antagonist 14,15-epoxyeicosa-5(*Z*)-enoic acid (EEZE; 10 μM), suggesting a recognition or binding site independent of the putative EET receptor.[12,13] In the human lung, **1** also hyperpolarizes and relaxes pulmonary artery and bronchial smooth muscle cells and exerts anti-inflammatory effects.[14,15]

A major open question concerning the whole field of CYP-eicosanoid research is the heretofore unidentified, primary targets of CYP-eicosanoid action. Although some studies indicate direct interactions with certain ion channels and transcription factors, most of the observed CYP-eicosanoid actions apparently rely on the ability of these metabolites to trigger distinct intracellular signaling pathways. These pathways, depending upon the specific CYP metabolite and physiological context, can involve protein kinase A, guanine nucleotide binding proteins, tyrosine kinases, mitogen-activated kinases, phosphoinositol-3 kinase, IkappaB kinase, Rho-kinase or the epidermal growth factor and a series of other well-known key components of intracellular signaling.[16] In general, these signaling components become specifically engaged in pathways that are primarily elicited by membrane receptors. Recent studies indeed revealed the presence of a high affinity EET-binding site in U937 cells, which is probably a G-protein coupled receptor.[17] These findings suggest that the various CYP-dependent metabolites of ω-6 and ω-3-PUFAs may exert their specific functions via a family of G-protein coupled receptors analogous with the cyclooxygenase- and lipoxygenase-generated eicosanoids.

Neonatal rat cardiomyocytes (NRCMs) have been introduced as an in vitro model to analyze the anti-arrhythmic mechanisms of ω-3 PUFAs.[3a,18] In this model, EPA (3–10 μM) reduces the contraction rate of the spontaneously beating heart cells, i.e., it exerts a strong negative chronotropic effect. Furthermore, EPA reduces the response of NRCMs to β-adrenergic agonists as well as increases extracellular Ca^{2+}-concentrations and terminates the arrhythmias induced by these agents.[18] We have shown previously that **1** and **2** (Table 1) exert the same effects as EPA on NRCMs, but in contrast are active in the low nanomolar range suggesting that these metabolites may function as mediators of the anti-arrhythmic mechanism of ω-3 PUFAs.[8] In the present study, we used the chronotropic effect on NRCMs as a bioassay to evaluate the relative “anti-arrhythmic potencies” of a series of synthetic analogs based on the structure of the natural EPA epoxy-metabolites. This SAR study was performed to (i) identify the structural determinants that mediate the unique biological activities of **1**, (ii) to find selective agonists[19] and antagonists as required for future mechanistic studies and for isolation of the putative receptor, and (iii) to generate agonists with improved chemical and metabolic stability as a first step toward the development of novel anti-arrhythmic drugs.

Results and Discussion

Whilst EPA required prolonged incubation and a ~1000-fold higher concentration, epoxide **2** was almost immediately effective with an EC_{50} ~1–2 nM (Fig. 1). Notably, the negative chronotropic activity resides with the 17(*R*),18(*S*)-enantiomer **2** while its antipode **3** had no significant effect (Table 1). The soluble epoxide hydrolase (sEH) metabolite of **1**, i. e., diol **4**, was completely inactive. To capitalize on these observations for the development of more efficacious anti-arrhythmic therapies,[20] we initiated a systematic structure-activity relationship study to characterize this pharmacophore and to simultaneously address the chemical and metabolic labilities of **1** and **2** that restrict their wider utility.[21,22] Thus, our first priority was the partial saturation of the polyenoic backbone with the intention of strategically disrupting the three 1,4-dienyl combinations. This would obviate both autooxidation and metabolism by lipoxygenases and cycloxygenases.[4c] Of the three possible tetrahydro-variants **5**–**7** that satisfied this criteria, only **7** was still competent to significantly reduce the beating rate. The mono-olefinic series **8**–**11** was consistent with the preceding observations and clearly established the presence of a $\Delta^{11,12}$-olefin, as found in analog **10**, confers strong negative chronotropic activity. The fully saturated analog **12** proved to be largely inactive. As might be anticipated from the differences between the 17,18-EETeTr enantiomers **2** and **3**, analog **13** was a powerful negative chronotrope whereas its mirror image, analog **14**, had the opposite, albeit more modest activity. The enantiomeric pair **15** and **16** presented a similar pharmacological profile that suggests that the absolute configuration of the epoxide can trump the influence of olefin position. Terminal hydroxylation is a common degradation pathway for fatty acids and eicosanoids,[23] so it was no surprise that this modification mostly abrogated biological activity in the arrhythmia assay, e.g., **17** and **18**.

In recognition of the central role of the sEH enzyme in the metabolism of fatty epoxanoids,[24] replacement of the epoxide with an achiral epoxide bioisostere[21] was deemed crucial to the development of any robust, long-lived analogs intended for *in vivo* applications. Hence, we were delighted to find **19**,[21] a 1,3-disubstituted urea version of **10**, decisively suppressed the beating rate (Table 2). In contrast to prior experience with 14,15-EET analogs,[21] the introduction of N-methyls on to the urea, i.e., analog **20**, strongly dampened the anti-arrhythmic effect. 1,4-Disubstituted oxamide 21 proved to be the most efficacious of all the bioisosteres and, thus, was subjected to additional scrutiny. Amongst these, the *trans*-olefinic and acetylenic versions **22** and **23**, respectively, were the most informative. The strict stereochemical requirement for this portion of the molecule suggests the recognition or binding site contains a narrow pocket that best accommodates the hairpin configuration of a *cis*-olefin. The related amides **24**–**26** were disappointing; however, shifting the amide functionality just one position away from the ω-terminus as in **27** restored most of the negative chronotropic influence. Repositioning the olefin to the $\Delta^{14,15}$-position consistently reversed or annulled any negative chronotropic behavior irrespective of the epoxide surrogate: **28** *vs* **25**, **29** *vs* **21**, and **30** *vs* **19**.

Having identified several potent analogs containing both epoxide and bioisosteric replacements, it was of interest to evaluate the dose response profile of select examples versus 17(*R*),18(*S*)-EETeTr (**2**) on NRCM beating rates. The weakly active *trans*-olefinic analog **22** was included as a control. As evident in Figure 1, urea **19** and oxamide **21** outperformed **2** at most concentrations. Above ca. 5 nM, all of the test compounds began to plateau, except for analog **22** which did not display any significant efficacy until the low μM range.

In recognition of the ability of analog **16** to block the negative chronotropic effects of its antipode **15**, we sought to identify additional antagonists amongst the analogs in Table 1 that

were themselves largely devoid of activity. To this end, NRCMs were preincubated with the test antagonist (30 nM final concentration) for 5 min at 37 °C before the addition of analog **10** (30 nM final concentration). Only analogs **6** and **11** resulted in any useful antagonism (Figure 2). While both share a $\Delta^{14,15}$-olefin, this is not sufficient for antagonism since analog **5** also contains a $\Delta^{14,15}$-olefin, yet was not distinguishable from vehicle.

While analog **6** emerged as the most powerful antagonist in the foregoing study, there was no certainty that it could function as a universal antagonist against other negative chronotropes. Consequently, NRCMs were preincubated with several of the most efficacious negative chronotropes, *inter alia*, EPA, **1**, **7** and oxamide **21**, and then challenged with an equimolar amount of **6** (Figure 3). In all cases, blockades of 90% or better were achieved. Of particular note, antagonist **6** (30 nM) blocked not only the effects of epoxide **1** (30 nM) and its synthetic agonists, but also that of EPA (3.3 μM) suggesting that EPA and its natural metabolite act via the same mechanism.

To demonstrate the chemical modifications described above resulted in agonists with improved metabolic stability, we analyzed the metabolism of **1**, **12**, **13**, **19** and **21** by rat liver homogenates. The results are shown in detail in the Supporting Information section. As expected, epoxide **1** was rapidly hydrolyzed to *vic*-diol **4** and further attacked by epoxidation and hydroxylation reactions. In total, about 80% of **1** was metabolized within 30 min. Analog **21**, carrying an oxamide moiety instead of the epoxy-group and only the functionally essential 11,12-double bond, provided the currently best solution to overcome the metabolic instability of the natural compound. LC-MS analysis indicated that analog **21** was only slowly metabolized to regioisomeric hydroxy-metabolites and more than 85% of the original compound remained intact after 30 min incubation with liver homogenate (see Supporting Information).

It was also of interest to understand the consequences, if any, of the structural modifications on sEH activity. Other laboratories[25] have developed highly potent sEH-inhibitors that utilize ureas as the epoxide bioisostere. Similar functionality appears in some of our 17,18-EETeTr agonists, e.g., **19** (Table 2). Accordingly, the biological activity of our analogs might be attributable in part or entirely to inhibition of sEH and subsequent accumulation of endogenous epoxy-eicosanoids in the cardiomyocytes. To test this possibility, we analyzed selected compounds containing the urea (analog **19**) or oxamide (analogs **21** and **29**) moieties for their capacity to inhibit sEH. Analog **19** exerted weak dose-dependent sEH inhibition at 1 and 5 μM, i.e., concentrations 1000-fold higher than required for reducing the contractility of cardiomyocytes (see Supporting Information, Figure 2). Analogs **21** and **29** lacked any inhibitory effects up to 5 μM. Under the same conditions, AUDA (12-(3-adamantan-1-yl-ureido)dodecanoic acid), a representative of the urea-class of highly potent sEH-inhibitors, almost completely blocked sEH activity at 100 nM.[25] AUDA itself had no effect on the spontaneous beating rate of cultured cardiomyocytes. However, AUDA partially inhibited the negative chronotropic effect of **1** (see Supporting Information, Figure 3). Furthermore, combined administration of **19** or **21** with 17,18-EETeTr did not result in additive or synergistic effects (see Supporting Information, Figure 3). Taken together, these results show that our synthetic analogs do not or only weakly target sEH; hence, sEH inhibition can be excluded as a significant contributor to their effects on cardiomyocytes.

Chemistry

Syntheses of **7**, **8**, **10**, and **12** are summarized in Scheme 1 and are representative of the methodology used to prepare the other epoxide-containing analogs. Following literature precedent,[26] commercial octa-1,7-diyne (**31**) was alkylated with a limiting amount of 2-(4-bromobutoxy)-tetrahydro-2*H*-pyran[27] in THF/HMPA to give **32**. A second alkylation using

8-bromo-oct-3(*Z*)-ene[28] followed by acidic cleavage of the THP and Jones oxidation furnished acid **33** that was then subjected to Fischer esterification and selective epoxidation of the $\Delta^{17,18}$-olefin. The resultant epoxide **34** smoothly led to dienoate **35** via semi-hydrogenation of over P-2 nickel/hydrogen gas. Random hydrogenation of **35** using diimide generated *in situ* gave rise to a chromatographically separable mixture of unreacted **35**, mono-olefins **36** and **37**, and tetrahydro-epoxide **38**. LiOH hydrolysis of the individual methyl esters yielded the corresponding free acids **7**, **8**, **10**, and **12**.

Access to oxamide **21** (Scheme 2) began with alkylation of 12-(*tert*-utyldiphenylsilyloxy)dodec-1-yne[29] (**39**) with 2-(4-bromobutoxy)-tetrahydro-2*H*-pyran.[30] Subsequent desilylation evolved **40** which was advanced to **41** via P-2 nickel mediated semi-hydrogenation and Jones oxidation. Exposure of the product to acidic methanol simultaneously esterified the carboxylic acid and removed the THP ether. Replacement of the terminal alcohol with azide using diphenylphosphoryl azide (DPPA) under Mitsunobu-type conditions completed the transformation to **42**. Sequential Staudinger reduction, EDCI amidation with 2-(methylamino)-2-oxoacetic acid,[30] and hydrolysis delivered **21** and could be accomplished without the purification of intermediates.

Conclusions

The potency and rapid onset of 17(*R*),18(*S*)-EETeTr (**2**) are consistent with a prominent role for this cytochrome P450-dependent eicosanoid in mediating the anti-arrhythmic effects of EPA. Structure-activity correlations helped define the essential pharmacophore as well as led to the development of agonist and antagonist analogs that are more robust than natural material and that provide a sound foundation for the future development of potential clinical candidates. Additionally, these data are also consistent with the existence of a specific ω-3 epoxanoid receptor, albeit other mechanisms of actions cannot be excluded at present.

Experimental Section

General Procedures

Unless stated otherwise, yields refer to purified products and are not optimized. Final compounds were judged ≥95% pure by HPLC using a Zorbax Eclipse C18 (250 × 4.6 mm; Agilent) connected to an Agilent 1200 API/LC-MS with acetonitrile/water combinations as solvent; enantiomers were also analyzed by chiral phase HPLC using a Chiralcel OJ-H column (250 × 4.6 mm; Daicel) with hexane/isopropyl alcohol combinations as solvent and judged to be ≥95% e.e. All oxygen and/or 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. Flash chromatography (FC) was performed using E Merck silica gel 60 (240–400 mesh). Thin layer chromatography was performed using pre-coated plates purchased from E. Merck (silica gel 60 PF254, 0.25 mm). 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). Nuclear magnetic resonance (NMR) splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); the values of chemical shifts (δ) are given in ppm relative to residual solvent (chloroform δ = 7.27 for ^{1}H NMR or δ = 77.23 for proton decoupled ^{13}C NMR), and coupling constants (*J*) are given in Hertz (Hz). Melting points were determined using an OptiMelt (Stanford Research Systems) and are uncorrected. The Michigan State University Mass Spectroscopy Facility or Prof. Kasem Nithipatikom (Medical College of Wisconsin) kindly provided high-resolution mass spectral analyses.

32. *n*-BuLi (30 mL of 2.5 M soln in hexanes, 70 mmol) was added dropwise to a −78 °C solution of 31 (9.0 g, 84.9 mmol; G F Smith, Inc.) in dry THF/HMPA (350 mL, 6:1) under an argon atmosphere. After 30 min, the reaction mixture was warmed to −10 °C over 2 h and maintained at this temperature for 20 min, then re-cooled to −78 °C. To this was added a solution of 2-(4-bromobutoxy)-tetrahydro-2*H*-pyran[27] (15 g, 63.68 mmol) in dry THF (15 mL). The resulting mixture was warmed to room temperature over 3 h, maintained at this temperature for 12 h, then quenched with sat. aq. NH_4Cl (25 mL). After 20 min, the mixture was extracted with Et_2O (2 × 125 mL). The combined ethereal extracts were washed with water (2 × 100 mL), brine (100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by SiO_2 column chromatography using 5% EtOAc/hexanes as eluent to give **32** (10.85 g, 65%) as a colorless oil. TLC: 10% EtOAc/hexanes, R_f ≈ 0.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.57 (t, *J* = 2.5 Hz, 1H), 3.82–3.87 (m, 1H), 3.70–3.77 (m, 1H), 3.46–3.51 (m, 1H), 3.36–3.42 (m, 1H), 2.14–2.20 (m, 6H), 1.93 (t, *J* = 2.5 Hz, 1H), 1.46–1.72 (m, 14H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 98.78, 84.23, 80.31, 79.82, 68.50, 67.07, 62.25, 30.80, 30.76, 28.98, 28.07, 27.58, 25.96, 25.57, 19.68, 18.64, 18.31. HRMS calcd for $C_{16}H_{25}O_2$ $[M+1]^+$ 249.1855, found 249.1852.

33. Compound **32** (4.5 g, 17.2 mmol) was alkylated using *n*-BuLi (8.3 mL of 2.5 M soln in hexanes, 20.65 mmol) and 8-bromo-oct-3(*Z*)-ene[31] (4.1 g, 21.5 mmol) in THF/HMPA (180 mL, 6:1) as described above for the synthesis of **32** to give 2-[eicosa-17(*Z*)-en-5,11-diynyloxy]tetrahydro-2*H*-pyran (4.15 g, 65%) as a colorless oil. TLC: 10% EtOAc/hexanes, R_f ≈ 0.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26–5.41 (m, 2H), 4.58 (t, *J* = 2.5 Hz, 1H), 3.82–3.87 (m, 1H), 3.70–3.77 (m, 1H), 3.46–3.51 (m, 1H), 3.36–3.42 (m, 1H), 2.11–2.20 (m, 8H), 1.92–2.04 (m, 4H), 1.62–1.86 (m, 4H), 1.39–1.59 (m, 14H), 0.94 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.54, 128.42, 98.98, 80.47, 79.99, 68.56, 67.25, 62.49, 34.01, 32.49, 30.93, 29.12, 28.37, 28.21, 27.71, 26.34, 26.08, 25.69, 20.72, 19.83, 18.79, 18.46, 18.16, 14.53. HRMS calcd for $C_{24}H_{39}O_2$ $[M+1]^+$ 359.2950, found 359.2951.

A solution of 2-[eicos-17(*Z*)-en-5,11-diynyloxy]tetrahydro-2*H*-pyran (1.3 g, 3.49 mmol) and *p*-TSA (50 mg) in MeOH (50 mL) was stirred at room temperature for 4 h, then concentrated in *vacuo*. The residue was purified by SiO_2 column chromatography using 15% EtOAc/hexanes as eluent to give eicosa-17(*Z*)-en-5,11-diyn-1-ol (925 mg, 92%) as a colorless oil. TLC: 30% EtOAc/hexanes, R_f ≈ 0.35; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27–5.42 (m, 2H), 3.66 (t, *J* = 6.8 Hz, 2H), 2.00–2.19 (m, 12H), 1.43–1.72 (m, 12H), 0.95 (t, 3H, *J* = 7.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.56, 128.45, 80.42, 80.22, 62.69, 34.06, 32.51, 32.07, 28.39, 28.20, 27.73, 26.36, 25.51, 20.74, 18.73, 18.45, 18.16, 14.45. HRMS calcd for $C_{20}H_{33}O$ $[M+1]^+$ 289.2531, found 289.2534.

Jones reagent (5 mL of a 10 N aq. solution) in acetone (10 mL) was added slowly to a stirring, −40 °C solution of eicosa-17(*Z*)-ene-5,11-diyn-1-ol (1.0 g, 3.47 mmol) in acetone (50 mL). After 1 h, the reaction mixture was warmed to −10 °C and maintained at this temperature for 3 h, then quenched with excess isopropanol. The green chromium salts were removed by filtration, the filter cake was washed with acetone, and the combined filtrates were concentrated in *vacuo*. The residue was dissolved in EtOAc (100 mL), washed with water (50 mL), and concentrated in *vacuo*. The residue was purified by SiO_2 column chromatography using 15% EtOAc/hexanes as eluent to give **33** (920 mg, 88%) as a colorless oil. TLC: 30% EtOAc/hexanes, R_f ≈ 0.35; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24–5.41 (m, 2H), 2.41 (t, *J* = 6.9 Hz, 3H), 2.10–2.19 (m, 8H), 1.98–2.09 (m, 4H), 1.75–1.81 (m, 2H), 0.96 (t, *J* = 7.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.37, 132.54, 128.44, 84.43, 80.45, 80.26, 78.58, 34.08, 32.53, 32.09, 28.36, 28.23, 27.75, 26.38, 25.53, 20.76, 18.77, 18.48, 18.03, 14.59. HRMS calcd for $C_{20}H_{31}O_2$ $[M+1]^+$ 303.2324, found 303.2324.

34. A solution of **33** (0.8 g, 2.63 mmol) and *p*-TSA (20 mg) in MeOH (30 mL) was stirred at room temperature for 10 h, then concentrated in *vacuo* and the residue was purified by SiO_2 column chromatography using 3% EtOAc/hexanes as eluent to give methyl eicos-17(*Z*)-en-5,11-diynoate (682 mg, 82%) as a colorless oil. TLC: 10% EtOAc/hexanes, $R_f \approx 0.60$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27–5.42 (m, 2H), 3.67 (s, 3H), 2.43 (t, 2H, *J* = 7.6 Hz), 2.12–2.21 (m, 8H), 1.99–2.09 (m, 4H), 1.76–1.82 (m, 2H), 1.42–1.58 (m, 8H), 0.95 (t, 3H, *J* = 7.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.30, 132.57, 128.48, 84.52, 80.46, 80.21, 79.97, 51.19, 33.09, 32.43, 32.19, 28.39, 28.27, 27.65, 26.36, 25.63, 20.73, 18.72, 18.49, 18.07, 13.76. HRMS calcd for $C_{21}H_{33}O_2$ $[M+1]^+$ 317.2481, found 317.2485.

m-CPBA (1.6 g, 4.76 mmol) was added in portions to a 0 °C solution of methyl eicosa-17(*Z*)-en-5,11-diynoate (1.15 g, 3.66 mmol) in CH_2Cl_2 (50 mL). After 2 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (25 mL), washed with sat. aq. $NaHCO_3$ (2 × 25 mL), brine (2 × 25 mL), water (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by SiO_2 column chromatography using 5% EtOAc/hexanes as eluent to give **34** (990 mg, 82%) as a colorless oil. TLC: 10% EtOAc/hexanes, $R_f \approx 0.3$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (s, 3H), 2.84–2.94 (m, 2H), 2.42 (t, 2H, *J* = 7.3 Hz), 2.14–2.23 (m, 8H), 1.74–1.83 (m, 2H), 1.42–1.61 (m, 12H), 1.03 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.21, 83.62, 80.35, 80.08, 62.96, 59.43, 52.32, 34.19, 32.28, 32.20, 29.17, 28.32, 28.25, 27.75, 26.43, 25.72, 20.75, 18.63, 18.28, 18.14, 14.53. HRMS calcd for $C_{21}H_{33}O_3$ $[M+1]^+$ 333.2430, found 333.2434.

35. $NaBH_4$ (33 mg, 0.88 mmol) was added portionwise to a stirring, rt solution of nickel(II) acetate tetrahydrate (190 mg, 0.76 mmol) in absolute ethanol (5 mL) under a hydrogen blanket (1 atm). After 15 min, freshly distilled ethylenediamine (200 mg, 3.24 mmol) was added followed by **34** (250 mg, 0.75 mmol) in absolute ethanol (5 mL) while maintaining the hydrogen blanket (1 atm). The heterogeneous mixture was stirred at rt for 90 min, then diluted with ether (40 mL), and filtered through a short pad of silica gel; the filtration pad was washed with ether (3 × 10 mL). The combined filtrates were dried over anhydrous Na_2SO_4 and concentrated in *vacuo* to give **35** (246 mg, 98%) as a colorless oil sufficiently pure to be used directly in the next step. TLC: 20% EtOAc/hexanes, $R_f \approx 0.65$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27–5.42 (m, 4H), 3.66 (s, 3H), 2.83–2.93 (m, 2H), 2.30 (t, 2H, *J* = 7.3 Hz), 1.92–2.09 (m, 8H), 1.63–1.72 (m, 2H), 1.25–1.58 (m, 12H), 1.03 (t, 3H, *J* = 7.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.29, 131.12, 130.20, 129.68, 128.64, 58.54, 57.45, 51.63, 33.62, 29.78, 29.53, 29.48, 27.79, 27.28, 26.71, 26.41, 25.05, 21.28, 10.80. HRMS calcd for $C_{21}H_{37}O_3$ $[M+1]^+$ 337.2743, found 337.2741.

7. An aqueous solution of LiOH (1 mL, 2 M soln) was added to a 0 °C solution of **35** (250 mg, 0.74 mmol) in THF (8 mL) and deionized H_2O (2 mL). After stirring at room temperature overnight, the reaction mixture was cooled to 0 °C, the pH was adjusted to 4 with 1 M aq. oxalic acid, and extracted with ethyl acetate (2 × 20 mL). The combined extracts were washed with water (30 mL), brine (25 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by SiO_2 column chromatography using 25% EtOAc/hexanes as eluent to give **7** (222 mg, 93%) as a colorless oil. TLC: 30% EtOAc/hexanes, $R_f \approx 0.3$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28–5.40 (m, 4H), 2.87–2.97 (m, 2H), 2.34 (t, 3H, *J* = 7.0 Hz), 1.97–2.12 (m, 8H), 1.63–1.74 (m, 2H), 1.30–1.60 (m, 12H), 1.02 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 180.06, 131.75, 130.03, 129.77, 128.66, 58.86, 57.87, 33.93, 29.93, 29.84, 29.81, 27.89, 27.68, 26.41, 26.36, 24.83, 21.26, 10.84. HRMS calcd for $C_{20}H_{35}O_3$ $[M+1]^+$ 323.4901, found 323.4901.

Syntheses of 8, 10, and 12

A stream of ethanol saturated air was passed through a stirring solution of hydrazine hydrate (400 mg, 12 mmol, 20 equiv), **35** (200 mg, 0.60 mmol), and $CuSO_4 \cdot 5H_2O$ (10 mg) in

ethanol (5 mL).[26] After 12 h, the reaction mixture was passed through a short pad of silica gel and the filter cake was washed with dichloromethane (3 × 10 mL). The combined filtrates were dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. The residue was resolved into its components by $AgNO_3$-impregnated PTLC using 2% CH_2Cl_2/benzene: R_f ≈ 0.2, 0.4, 0.55, and 0.85 for **35**, **37**, **36**, and **38**, respectively, isolated in a ratio of 2:3:3:2, respectively.

36: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27–5.42 (m, 2H), 3.66 (s, 3H), 2.84–2.92 (m, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.96–2.08 (m, 4H), 1.64–1.71 (m, 2H), 1.45–1.58 (m, 4H), 1.21–1.36 (m, 16H), 1.03 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.45, 131.88, 128.63, 58.64, 57.87, 51.96, 33.88, 29.99, 29.86, 29.74, 29.46, 27.98, 27.76, 26.88, 26.72, 25.88, 21.32, 10.48. HRMS calcd for $C_{21}H_{39}O_3$ $[M+1]^+$ 339.2899, found 339.2896. Hydrolysis of **36** as described above gave **8** (92%) as a colorless oil: TLC, 30% EtOAc/hexanes, R_f ≈ 0.3; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.27–5.43 (m, 2H), 2.85–2.93 (m, 2H), 2.34 (t, *J* = 7.6 Hz, 2H), 1.95–2.11 (m, 4H), 1.64–1.72 (m, 2H), 1.49–1.60 (m, 4H), 1.22–1.36 (m, 16H), 1.03 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.42, 131.54, 128.40, 60.08, 58.75, 57.73, 34.59, 31.86, 29.86, 29.74, 29.71, 29.45, 27.84, 27.42, 26.81, 26.64, 24.85, 21.28, 15.47, 10.81. HRMS calcd for $C_{20}H_{37}O_3$ $[M+1]^+$ 325.2743, found 325.2747.

37: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.25–5.35 (m, 2H), 3.61 (s, 3H), 2.79–2.89 (m, 2H), 2.25 (t, *J* = 7.3 Hz, 2H), 1.93–2.04 (m, 4H), 1.19–1.60 (m, 22H), 1.00 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) d174.48, 130.41, 129.54, 58.54, 57.45, 51.62, 34.27, 29.92, 29.81, 29.67, 29.63, 29.47, 29.46, 29.34, 27.80, 27.42, 27.27, 26.42, 25.14, 10.82. HRMS calcd for $C_{21}H_{39}O_3$ $[M+1]^+$ 339.2899, found 339.2900. Hydrolysis of **37** as described above gave **10** (92%) as a colorless oil: TLC, SiO_2, 30% EtOAc/hexanes, R_f ≈ 0.3; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28–5.40 (m, 2H), 2.84–2.94 (m, 2H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.96–2.04 (m, 4H), 1.02–1.62 (m, 22H), 1.01 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.10, 130.45, 129.57, 58.74, 57.67, 34.27, 29.92, 29.81, 29.66, 29.60, 29.46, 29.43, 29.25, 27.76, 27.43, 27.28, 26.41, 24.89, 21.27, 10.81. HRMS calcd for $C_{20}H_{37}O_3$ $[M+1]^+$ 325.2743, found 325.2745.

38: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (s, 3H), 2.84–2.94 (m, 2H), 2.31 (t, 2H, *J* = 7.4 Hz), 1.42–1.65 (m, 6H), 1.22–1.34 (m, 24H), 1.04 (t, 3H, *J* = 7.3 Hz). HRMS calcd for $C_{21}H_{41}O_3$ $[M+1]^+$ 341.3056, found 341.3056. Hydrolysis of **38** as described above gave **12** (94%) as white solid, mp 62.1–62.5 °C; TLC, 30% EtOAc/hexanes, R_f ≈ 0.35; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.86–2.94 (m, 2H), 2.34 (t, 2H, *J* = 7.3 Hz), 1.46–1.65 (m, 30H), 1.04 (t, 3H, *J* = 7.35 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.04, 58.83, 57.47, 34.24, 30.06, 30.03, 29.92, 29.81, 29.66, 29.60, 29.46, 29.43, 29.25, 27.76, 27.43, 27.28, 26.41, 24.89, 21.27, 10.89. HRMS calcd for $C_{20}H_{39}O_3$ $[M+1]^+$ 327.5219, found 327.5216.

Synthesis of 40

Alkylation of **39**[29] with 2-(4-bromobutoxy)tetrahydro-2*H*-pyran-2-yloxy[32] as described for **32** produced *tert*-butyldiphenyl-[16-(tetrahydro-2H-pyran-2-yloxy)hexadec-11-ynyloxy]silane (66%) as a colorless oil which was used without further purification. TLC: 10% EtOAc/hexane, R_f ≈ 0.5.

A solution of the above crude *tert*-butyldiphenyl-[16-(tetrahydro-2*H*-pyran-2-yloxy)hexadec-11-ynyloxy]silane (6 g, 10.42 mmol) and tetra-*n*-butylammonium fluoride (3.14 g, 12.5 mL of a 1 M soln in THF, 12.50 mmol) in THF (150 mL) was stirred at rt under an argon atmosphere. After 5 h, the reaction mixture was quenched with sat. aq. NH_4Cl (5 mL), washed with water (100 mL), and brine (75 mL). The aqueous layer was back-extracted with ether (2 × 75 mL). The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure, and the residue was purified by SiO_2 column

chromatography using 5–10% EtOAc/hexanes as eluent to give **40** (3.17 g, 80% overall) as a colorless oil. TLC: 40% EtOAc/hexanes, $R_f \approx 0.4$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.57–4.59 (m, 1H), 3.82–3.90 (m, 1H), 3.71–3.79 (m, 1H), 3.64 (t, 2H, J = 6.8 Hz), 3.46–3.53 (m, 1H), 3.36–3.44 (m, 1H), 2.10–2.22 (m, 4H), 1.20–1.80 (m, 26H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.90, 18.95, 21.51, 25.01, 25.63, 25.82, 27.85, 29.03, 29.15, 29.44, 29.61, 29.72, 29.96, 30.42, 63.88, 64.57, 66.65, 80.20, 80.55, 108.24. HRMS calcd for $C_{21}H_{39}O_3$ $[M+1]^+$ 339.2899, found 339.2897.

Synthesis of 41

Semi-hydrogenation of **40** as described above gave 16-(tetrahydro-2*H*-pyran-2-yloxy)hexadec-11(*Z*)-en-1-ol (99%) as a colorless oil. TLC: 20% EtOAc/hexane, $R_f \approx 0.30$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.33–5.37 (m, 2H), 4.58 (m, 1H), 3.83–3.90 (m, 1H), 3.73–3.77 (m, 1H), 3.65 (t, 2H, J = 6.7 Hz), 3.46–3.53 (m, 1H), 3.34–3.44 (m, 1H), 1.97–2.09 (m, 4H), 1.20–1.83 (m, 26H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.70, 23.41, 23.99, 24.90, 24.96, 28.45, 28.47, 29.06, 29.37, 30.13, 30.23, 30.46, 30.51, 31.26, 32.44, 61.81, 62.37, 68.14, 107.45, 130.56, 131.83. HRMS calcd for $C_{21}H_{41}O_3$ $[M+1]^+$ 341.3056, found 341.0360.

Jones oxidation of the preceding 16-(tetrahydro-2*H*-pyran-2-yloxy)hexadec-11(*Z*)-en-1-ol as described above gave **41** (68%) as a colorless oil. TLC: SiO_2, 40% EtOAc/hexanes, $R_f \approx 0.40$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.33–5.37 (m, 2H), 4.56–4.58 (m, 1H), 3.83–3.88 (m, 1H), 3.73–3.78 (m, 1H), 3.49–3.53 (m, 1H), 3.35–3.43 (m, 1H), 2.34 (t, J = 7.0 Hz, 2H) 1.97–2.09 (m, 4H), 1.20–1.84 (m, 24H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.54, 23.55, 25.71, 25.98, 26.41, 28.53, 28.67, 29.01, 29.14, 29.18, 29.22, 29.32, 30.04, 30.36, 32.56, 64.63, 67.89, 107.26, 130.85, 130.88, 180.48. HRMS calcd for $C_{21}H_{39}O_4$ $[M+1]^+$ 341.3056, found 341.0360.

Synthesis of 42

A solution of **41** (2.1 g, 5.93 mmol) and *p*-TSA (50 mg) in MeOH (30 mL) was stirred at room temperature for 10 h, then concentrated *in vacuo* and the residue was purified by SiO_2 column chromatography using 15% EtOAc/hexanes as eluent to give methyl 16-hydroxyhexadec-11(*Z*)-enoate (1.42 g, 83%) as a colorless oil. TLC: 20% EtOAc/hexanes, $R_f \approx 0.35$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.33–5.37 (m, 2H), 3.65 (s, 3H), 3.63 (t, J = 7.3 Hz, 2H), 2.29 (t, J = 7.0 Hz, 2H), 1.97–2.08 (m, 4H), 1.21–1.64 (m, 18H); ^{13}C NMR (75 MHz) δ 174.77, 130.30, 129.70, 62.95, 51.73, 34.28, 32.42, 29.64, 29.45, 29.17, 29.03, 27.29, 27.10, 27.08, 26.03, 25.07. HRMS calcd for $C_{17}H_{33}O_3$ $[M+1]^+$ 285.2430, found 285.2434.

Diisopropyl azodicaboxylate (DIAD; 1.15 g, 5.70 mmol,) was added dropwise to a −20 °C solution of triphenylphosphine (1.49 g, 5.70 mmol) in dry THF (30 mL) under an argon atmosphere. After stirring for 10 min, a solution of methyl 16-hydroxyhexadec-11(*Z*)-enoate (1.35 g, 4.75 mmol) in anhydrous THF (5 mL) was added dropwise. After 30 min at −20 °C, the reaction mixture was warmed to 0 °C and diphenylphosphoryl azide (DPPA, 1.38 g, 5.70 mmol) was added dropwise. After stirring at room temperature for 6 h, the reaction was quenched with water (3 mL), diluted with ether (50 mL), and washed with brine (40 mL). The aqueous layer was back-extracted with ether (2 × 30 mL). The combined organic extracts were dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by SiO_2 column chromatography using 5% EtOAc/hexanes as eluent to give **42** (1.14 g, 78%) as a pale yellow oil. TLC: 10% EtOAc/hexanes, $R_f \approx 0.45$; ^{1}H NMR (300 MHz) δ 5.31–5.43 (m, 2H), 3.66 (s, 3H), 3.26 (t, J = 6.7 Hz, 2H), 2.30 (t, J = 7.1 Hz, 2H), 1.97–2.10 (m, 4H), 1.50–1.64 (m, 4H), 1.15–1.48 (m, 14H); ^{13}C NMR (100 MHz) δ 174.32, 130.84, 129.01, 51.09, 51.05, 34.21, 32.58, 32.18, 29.86, 29.61, 29.59, 29.54, 29.42, 29.31,

27.40, 26.94, 25.23; IR (neat) 2985, 2954, 2845, 2106, 1754, 1250, 1104, 1029 cm^{-1}; HRMS calcd for $C_{17}H_{32}N_3O_2$ $[M+1]^+$ 310.2495, found 310.2500.

Synthesis of 21

Triphenylphosphine (1.15 g., 4.41 mmol) was added to a room temperature solution of **42** (1.05 g., 3.4 mmol) in THF (25 mL). After 2 h, water (200 mL) was added and the stirring was continued for another 8 h. The reaction mixture was then diluted with EtOAc (20 mL), washed with water (20 mL) and brine (25 mL). Aqueous layers were back-extracted with EtOAc (2 × 30 mL). The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure and further dried under high vacuum for 4 h. The crude methyl 16-aminohexadec-11(*Z*)-enoate was used in the next step without additional purification.

2-(Methylamino)-2-oxoacetic acid[29] (79 mg, 0.77 mmol), 1-hydroxybenzotriazole (101 mg, 0.77 mmol; HOBt) and diisopropylethylamine (105 mg, 0.77 mmol; DIPEA) were added to a stirring solution of crude methyl 16-aminohexadec-11(*Z*)-enoate (180 mg, 0.64 mmol) from above in anhydrous DMF (20 mL) under an argon atmosphere. After 5 min, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (147 mg, 0.77 mmol; EDCI) was added as a solid. After stirring for 12 h at room temperature, the reaction mixture was diluted with EtOAc (30 mL), washed with water (3 × 20 mL), and brine (20 mL). The combined aqueous layers were back-extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure, and the residue was purified by SiO_2 column chromatography using EtOAc as eluent to give methyl 16-(2-(methylamino)-2-oxoacetamido)hexadec-11(*Z*)-enoate (160 mg, 68%). TLC: 100% EtOAc, $R_f \approx 0.4$; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.45 (br s, 1H), 5.26–5.42 (m, 2H), 3.66 (s, 3H), 3.27–3.35 (m, 2H), 2.90 (d, 3H, J = 5.2 Hz), 2.30 (t, 2H, J = 7.3 Hz), 1.96–2.08 (m, 4H), 1.24–1.66 (m, 18H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.60, 160.81, 159.94, 130.87, 129.08, 51.68, 39.79, 34.33, 29.91, 29.68, 29.63, 29.50, 29.46, 29.36, 29.02, 27.46, 27.08, 26.91, 26.40, 25.17. HRMS calcd for $C_{20}H_{36}N_2O_4$ $[M+1]^+$ 368.2675, found 368.2675.

Methyl 16-(2-(methylamino)-2-oxoacetamido)hexadec-11(*Z*)-enoate (150 mg, 0.40 mmol) was hydrolyzed using LiOH as described above to afford **21** (126 mg, 89%) as a white powder, mp 110.2–110.6 °C. TLC: 5% MeOH/CH_2Cl_2, $R_f \approx 0.4$; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.80 (br s, 1H), 7.66 (br s, 1H), 5.26–5.42 (m, 2H), 3.28–3.35 (m, 2H), 2.90 (s, 3H), 2.36 (t, 2H, J = 7.3 Hz), 1.97–2.08 (m, 4H), 1.51–1.64 (m, 4H), 1.22–1.42 (m, 14H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.98, 160.96, 159.93, 130.83, 129.22, 39.91, 33.91, 29.58, 29.25, 29.12, 29.01, 28.95, 27.21, 27.09, 26.93, 26.46, 24.89. HRMS calcd for $C_{19}H_{34}N_2O_4$ $[M+1]^+$ 354.2519, found 354.2516.

NRCM Bioassays

The biological activities of the metabolites and synthetic analogs were determined using a bioassay essentially as described previously.[33] Briefly, neonatal rat cardiomyocytes (NRCM) were isolated from 1–2 day old Wistar rats and cultured as monolayers on the bottom of Falcon flasks (12.5 cm) in 2.0 mL of Halle SM 20-I medium supplemented with 10% heat-inactivated fetal calf serum and 2 μM fluoro-deoxyuridine. Spontaneously beating cell clusters occurred after 5–7 days (120–150 beats/min, monitored at 37 °C using an inverted microscope). The beating rates were determined for 6 to 8 individual clusters before and five minutes after addition of the test substance(s). Based upon the difference between the basal and compound-induced beating rate of the individual clusters, the chronotropic effects (changes in beats/min) were calculated and are given as mean SE values; n = 18–40 clusters originating from at least three independent NRCM cultures. All test compounds were prepared as 1000-fold stock solutions in ethanol and tested at a final concentration of

30 nM. The exception was EPA that required a final concentration of 3.3 mM and 30 min preincubation.

Supplementary Material

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19. In the context of this anti-arrhythmic study, agonist activity refers to the ability of a species to reduce the compound-induced abnormal or pathogenic beating rate of NRCMs towards the normal, spontaneous beating rate of 120–150 beats/min. Inverse agonist activity refers to the ability of a species to increase the beating rate beyond the normal, spontaneous beating rate, i.e., it has a biological activity opposite to an agonist. An antagonist is a species capable of partially or completely blocking the activity of an agonist or inverse agonist, while having no significant effect of its own on receptor or physiological activity.

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22. To estimate comparative metabolic stability, EPA-epoxide 1 and several analogs were incubated for 30 min at 37 °C with rat liver homogenate in the presence of NADPH and analyzed by LC/MS (Supporting Information Section).

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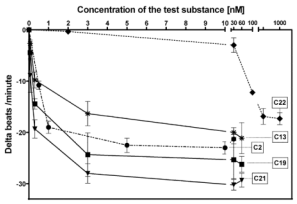


Figure 1.
Dose dependency of EPA-epoxide **2** and analogs **13**, **19**, **21**, and **22** on NRCM beating rates.

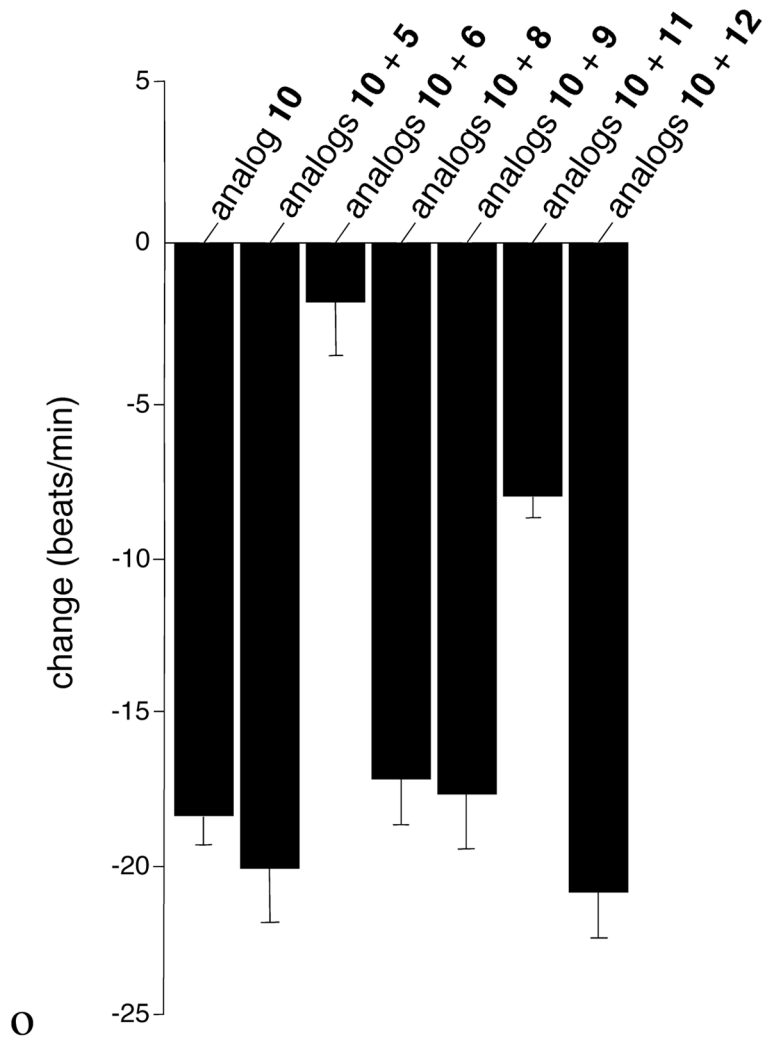


Figure 2.
Antagonism of **10** by Other Analogs.

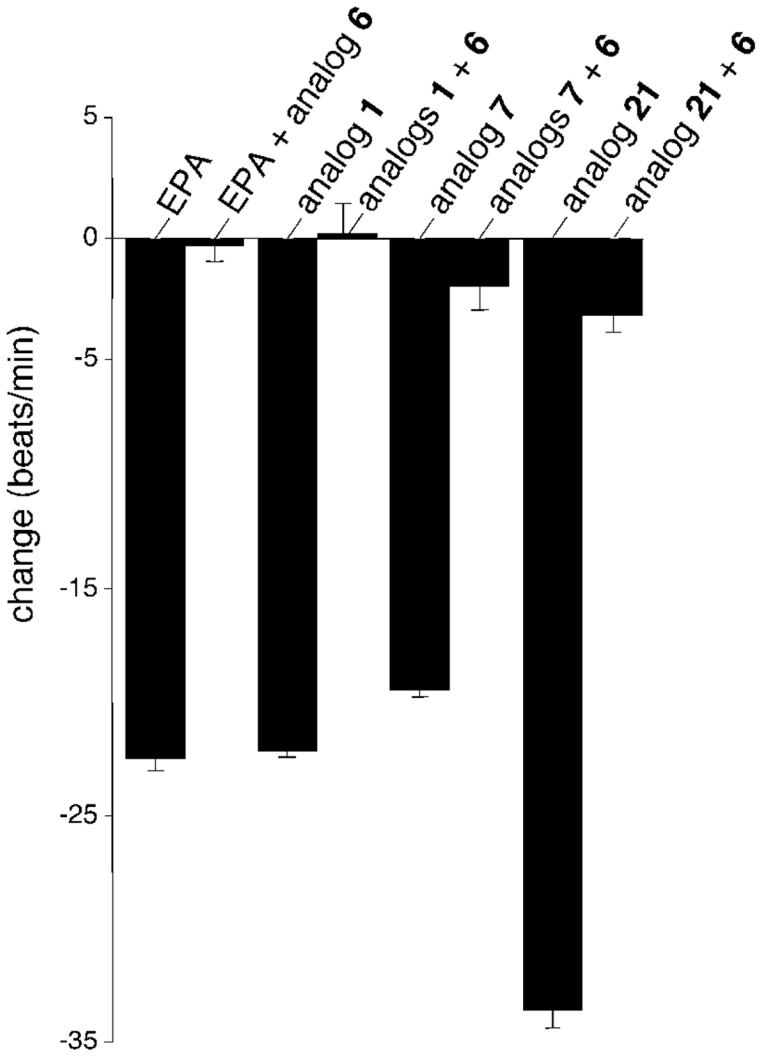
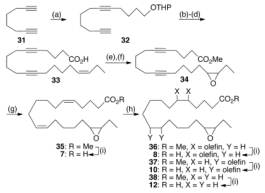


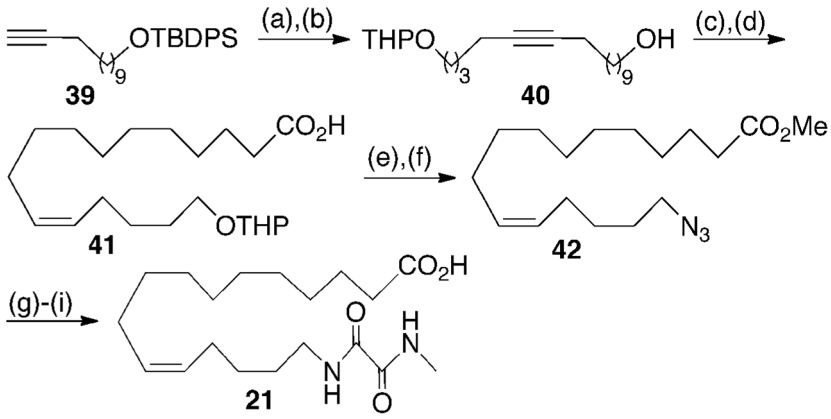
Figure 3.
Antagonism of Negative Chronotropes by **6**.



Scheme 1.

Synthesis of **7**, **8**, **10**, and **12**[a]

[a]Reagents and conditions: (a) *n*-BuLi (0.9 equiv), THF/HMPA (6:1), −78 °C to −10 °C, 2 h; $Br(CH_2)_4OTHP$, −78 °C to rt, 3 h; rt, 12 h, 65%; (b) *n*-BuLi, THF/HMPA (6:1), −78 °C to −10 °C, 2 h; $Br(CH_2)_4CH{=}CHCH_2CH_3$, −78 °C to rt, 3 h; rt, 12 h, 65%; (c) *p*-TSA, MeOH, rt, 4 h, 92%; (d) Jones reagent, acetone, −40 °C to −10 °C, 88%; (e) *p*-TSA, MeOH, rt, 10 h, 82%; (f) *m*-CPBA, CH_2Cl_2, rt, 2 h, 82%; (g) P-2 Ni/$(H_2NCH_2)_2$, H_2 (1 atm), EtOH, 98%; (h) $(H_2N)_2$, $CuSO_4$, O_2, EtOH, rt, 12 h; (i) LiOH, THF/H_2O (4:1), rt 10 h, 90–93%.



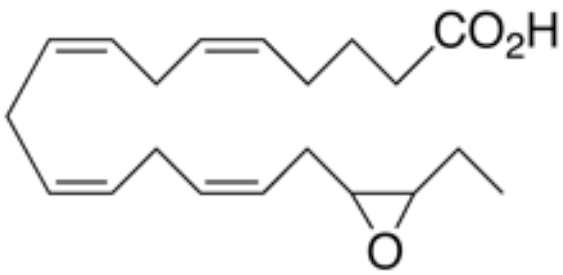
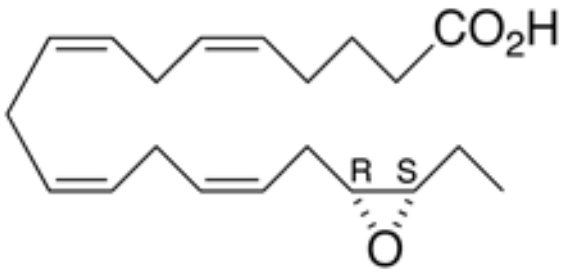
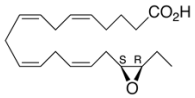
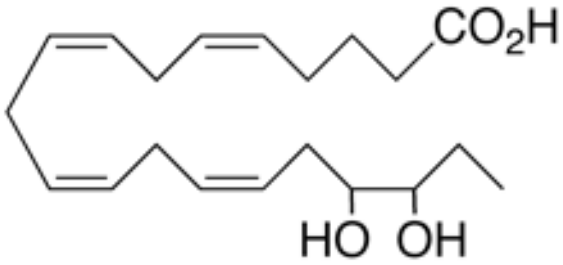
Scheme 2.

Synthesis of **21**[a]

[a]Reagents and conditions: (a) *n*-BuLi, THF/HMPA (6:1), −78 °C to −10 °C, 2 h; $Br(CH_2)_4OTHP$, −78 °C to rt, 3 h; rt, 12 h, 66%; (b) *n*-Bu_4NF, THF, rt, 5 h, 80%; (c) P-2 Ni/$(H_2NCH_2)_2$, H_2 (1 atm), EtOH, rt, 99%; (d) Jones reagent, acetone, −40 °C to −10 °C, 68%; (e) *p*-TSA, MeOH, rt, 10 h, 83%; (f) DEAD, Ph_3P, THF, −20 °C, 30 min; $(PhO)_2P(O)N_3$, 0 °C, 6 h, 78%; (g) Ph_3P, THF/H_2O, rt, 8 h; (h) EDCI, HOBt, *i*-$PrEt_2N$, MeNHC(O)C(O)OH, DMF, rt, 12 h, 68% over two steps; (i) LiOH, THF/H_2O (4:1), rt 10 h, 89%

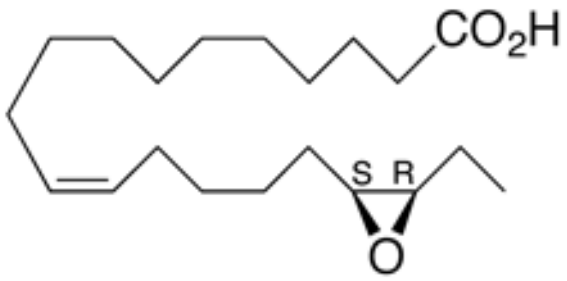
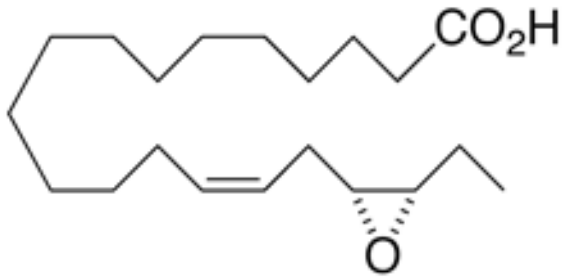
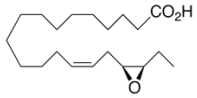
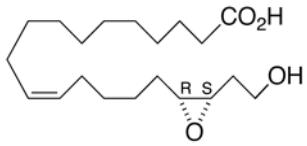
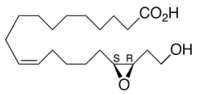
Table 1

Chronotropic Effects of 17,18-EETeTr and Analogs on NRCMs[a]

Compd	Analog	Change (beats/min)	n[b]
1		−22.5 ± 0.8	60
2		−21.3 ±0.9	12
3		+1.3 ±1.6	12
4		0.0 ±0.0	10

Compd	Analog	Change (beats/min)	n[b]
5		0.0 ±1.2	14
6		−2.7 ±1.4	22
7		−19.8 ±0.8	28
8		−0.6 ±1.0	14
9		−0.6 ±1.3	14

Compd	Analog	Change (beats/min)	n[b]
10		**−18.3 ±1.5**	21
11		−1.2 ±1.3	14
12		+0.8 ±1.6	14
13		**−20.3 ±1.2**	27

Compd	Analog	Change (beats/min)	n[b]
14		+3.3 ±1.1	29
15		−21.1 ±0.8	18
16		+0.7 ±0.9	18
17		−4.8 ± 0.26	18
18		−1.4 ±0.34	18

[a] All compounds were tested at a final concentration of 30 nM at 37 °C (control NRCM: 120–150 beats/min).

[b] n = number of determinations.

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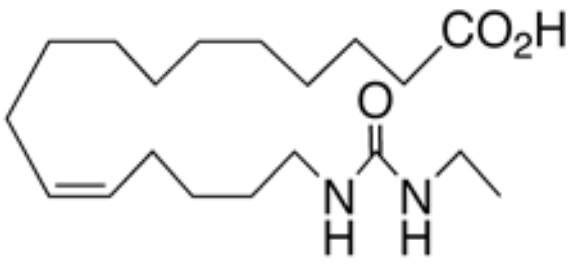
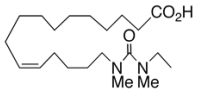
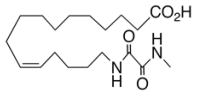
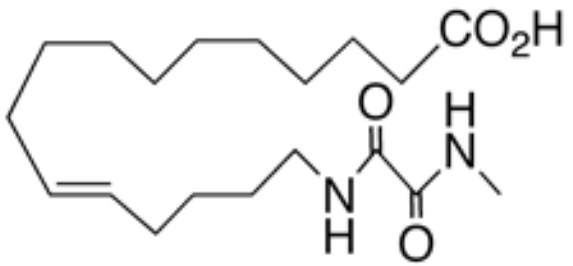
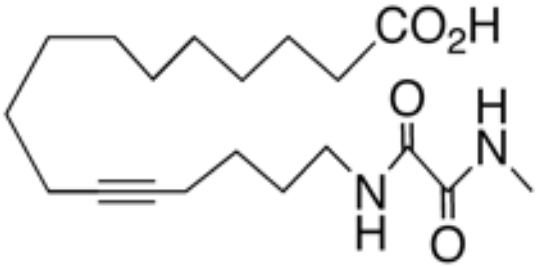
Table 2

Chronotropic Effects of Epoxide Bioisosteric Analogs on NRCMs[a]

Compd	Analog	Change (beats/min)	n[b]
19		−27.0 ± 1.2	27
20		−5.8 ± 0.4	6
21		**−33.7 ±1.3**	24
22		−4.7 ± 0.45	18
23		−2.6 ± 0.35	18

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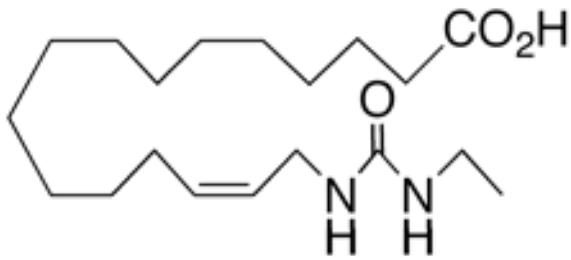
Compd	Analog	Change (beats/min)	n^b
24		+0.2 ±1.3	18
25		−1.6 ±0.9	18
26		−4.7 ±1.0	18
27		−22.4 ±1.7	18
28		+12.8 ±1.5	18
29		−1.3 ±1.0	18

Compd	Analog	Change (beats/min)	n^b
30		**+17.8 ±1.4**	18

[a] All compounds were tested at a final concentration of 30 nM at 37 °C (control NRCM: 120–150 beats/min)

[b] n = number of determinations.