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19-Hydroxyeicosatetraenoic acid analogs: Antagonism of 20-hydroxyeicosatetraenoic acid-induced vascular sensitization and hypertension

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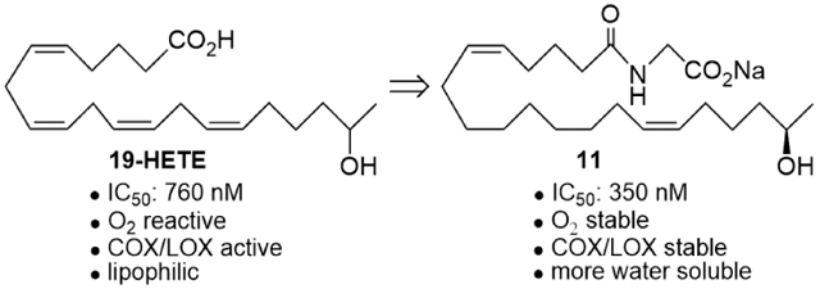
Abstract

19-Hydroxyeicosatetraenoic acid (19-HETE, **1**), a metabolically and chemically labile cytochrome P450 eicosanoid, has diverse biological activities including antagonism of the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE, **2**). A SAR study was conducted to develop robust analogs of **1** with improved *in vitro* and *in vivo* efficacy. Analogs were screened *in vitro* for inhibition of 20-HETE-induced sensitization of rat renal preglomerular microvessels toward phenylephrine and demonstrated to normalize the blood pressure of male Cyp4a14(−/−) mice that display androgen-driven, 20-HETE-dependent hypertension.

Graphical Abstract



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Keywords

Antagonist; Vascular sensitization; Hypertension; Eicosanoid; Bioisosteric replacement

19-Hydroxyeicosatetraenoic acid [19-HETE (**1**), Figure 1] is one of the principal hydroxylated metabolites of the cytochrome (CYP) P450 branch of the arachidonate cascade,[1] although relatively little is known about its physiological actions compared with the structurally related, proinflammatory 20-hydroxyeicosatetraenoic acid (20-HETE, **2**). Multiple CYP isoforms biosynthesize **1**, including the widely distributed CYP1A, CYP4A, and CYP2E1,[2-5] as well as organ specific isoforms such as brain CYP2J9.[6] Induction of select CYP isoforms can also influence endogenous levels of **1**.[2,7] In keeping with most of the CYP eicosanoids, **1** has diverse biological actions including inhibition of angiotensin II-induced hypertrophy in human and rat cardiac cells,[8] stimulation of renal Na^+-K^+-ATPase,[9] preglomerular blood vessel vasodilation,[10] blockade of recombinant P/Q-type Ca^{2+} channels,[6] and lowering of sodium-dependent phosphate uptake into renal cells.[11] Importantly, **1** also opposes many of the physiological actions of **2**. For instance, **1** reverses the effects of 20-HETE on NO and superoxide production, endothelial dysfunction,[12] and sensitization of renal arterioles toward vasoconstrictors such as phenylephrine, although only (*R*)-**1** is effective for the latter.[13] These and other observations led Nasjletti[13] to propose **1** can antagonize several biological activities of **2**. Herein, we describe the *in vitro* and *in vivo* evaluation of a family of robust 19-HETE analogs that will (i) expedite ongoing investigation into the physiologic role(s) of **1** and (ii) support proof-of-principle studies that a 19-HETE-inspired therapy could counteract the *in vivo* contributions of **2** to a variety of pathophysiologies including cardiac hypertrophy, cardiotoxicity, diabetic cardiomyopathy, cancer, and ischemia/reperfusion (I/R) injury.[14-19]

While **1** is known to be highly labile,[8] comparatively little is known about specific metabolic/catabolic pathways.[20,21] However, due to its close structural resemblance to **2**, it was reasoned that the same processes, *inter alia*, esterification, β-oxidation, alcohol dehydrogenase (ADH) oxidation, COX/LOX metabolism, and autooxidation, are also operative for **1** and similarly limit its utility as a research tool and/or clinical therapy. As our launching point for the development of more robust 19-HETE analogs, the carbon backbone of 20-hydroxyeicosa-5(*Z*),14(*Z*)-dienoic acid (20-5,14-HEDE, **3**), a widely used and fully functional agonist analog of **2**,[10] was adopted and the hydroxyl was relocated to C(19). This scaffold also obviates autooxidation as well as metabolism by the COX and LOX branches of the arachidonate cascade.

The 19-HETE analogs were evaluated for their ability to inhibit 20-HETE-induced sensitization of rat renal preglomerular microvessels toward phenylephrine (Table 1). Analogs **4** and **5** proved to be moderate antagonists of 20-HETE vessel sensitization with the 19(*R*)-enantiomer being a little more potent. In anticipation of future click chemistry applications, an azide was appended to the terminal carbon of the chain to give the click-capable analogs **6** and **7**.[22] This resulted in a 6-8 fold boost in activity over the parent structures. The improvement in inhibitory activity might be due to additional hydrogen bonding to the azide at the receptor binding site.[23] To better understand this effect, the

hydroxyl and azide positions were exchanged. This not only abrogated the inhibition of 20-HETE vessel sensitization, the resultant 19-azido-20-hydroxyl analogs **8** and **9** modestly heightened the vessels' sensitivity to 20-HETE. Conversion of **4** and **5** to their *N*-glycinates **10** and **11**, respectively, led to a more robust inhibition and somewhat improved water solubility (**4** and **5** ≈ 0.25 mg/mL vs. **10** and **11** ≈ 0.5 mg/mL). On the other hand, the related *N*-aspartate **12** completely negated the preceding gains in potency whereas the drop in activity for **13** was less pronounced, but nevertheless was approximately half that of analog **11**.

Further exploration of the structural features of the scaffold revealed retention of just one olefin, i.e., analogs **14** and **15**, was sufficient to maintain biological activity, but the previously observed enhancement gained by conversion into a *N*-glycinate was blunted in analogs **16** and **17**. Analog **18**, in which the hydroxyl is shifted to C(18), was superior to the other simple eicosadienoic acids (*viz.*, **4** and **5**) as an inhibitor, yet the transition to the *N*-glycinate **19** was unhelpful. Analysis of the homologous series wherein the carbon chain was either lengthened to 21-carbons (analog **20**), branched (analog **21**), or shortened to 19-carbons (analogs **22** and **23**) led us to conclude the position of the hydroxyl along the chain is decisive in determining agonist or antagonist activity and not whether the alcohol is primary, secondary or tertiary. Replacement of the carboxylic acid with a 1*H*-tetrazole, a commonly used carboxylic acid bioisostere,[24] provided analog **24** whose activity was comparable to analogs **4** and **5**.

Blockade of 20-HETE-induced changes to the internal diameter of pressurized (100 mmHg) rat gracilis muscle arterioles by **13** in a concentration dependent manner was consistent with competitive inhibition of 20-HETE and demonstrated the broader utility of 19-HETE analogs in other vessel types (Figure 2). Importantly, **13** had no vasoactive effects alone, i.e., in the absence of **2** (data not shown). This contrasts with 20-hydroxyeicosa-6(*Z*),15(*Z*)-dienoic acid-based agents that heretofore have been utilized as inhibitors of **2**, but recently have been shown to have weak 20-HETE agonist activity in some assays [Prof. Susana Nowicki (CEDIE-Centro de Investigaciones Endocrinológica, Buenos Aires, Argentina), personal communication].

To validate the ability of 19-HETE analogs to mitigate the influence of 20-HETE *in vivo*, **13** (10 mg/Kg) was administered daily via interperitoneal (IP) injection to male Cyp4a14(−/−) mice which display androgen-driven 20-HETE-dependent hypertension.[25] IP saline was used as a control. Over a 10 day course, the animals' systolic blood pressure returned to normal levels (Figure 3).

The synthesis of analog **4** is summarized in Scheme 1 and is representative of the procedures used to prepare the remaining analogs in Table 1 (see Appendix A. Supplementary data for details). Alkynylation of 2-((13-bromotridec-5-yn-1-yl)oxy)tetrahydro-2*H*-pyran[26] (**25**) with the lithium anion of (*S*)-*tert*-butyl(hept-6-yn-2-yloxy)diphenylsilane[27] (**26**) led to adduct **27**. Selective deprotection using methanolic PPTS and semi-hydrogenation of the resultant primary alcohol **28** over P-2 nickel produced **29**. Following Jones oxidation, acid **30** was concomitantly esterified and desilylated via marination in acidic methanol affording **31** from

which **4** was obtained by saponification. Its enantiomer, **5**, was prepared by an identical route beginning with commercial (*R*)-hex-5-en-2-ol.

The foregoing studies confirm that 19-HETE analogs can function as a physiologically relevant counterbalance to 20-HETE (**2**). The recent correlation of higher 19-HETE levels with a better prognosis in acute coronary syndrome patients[28] suggests *in vivo* efficacious 19-HETE agonist analogs warrant further evaluation as possible therapeutics for a wide range of inflammatory and cardiovascular conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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22. In recent studies, analog **7** conjugated to a photoactivated cross-linker was exploited for the isolation and identification of the first 20-HETE receptor. As designed, click coupling of the azide played a critical role in the isolation of the cross-linked protein. See Garcia V, Gilani A, Shkolnik B, Pandey V, Zhang FF, Dakarapu R, Gandham SK, Reddy NR, Graves JP, Gruzdev A, Zeldin DC, Capdevila JH, Falck JR, Schwartzman ML. 20-HETE signals through G protein-coupled receptor GPR75 (Gq) to affect vascular function and trigger hypertension. Cir. Res 2017;120:1776.**7**
23. Tchertanov L Structural metrics relationships in covalently bonded organic azides. Acta Cryst 1999;B55:807.
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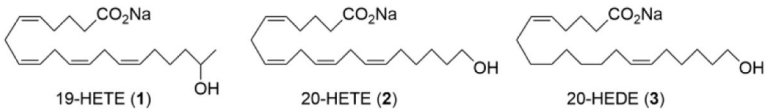


Figure 1.
Structures of 19-HETE (**1**), 20-HETE (**2**), and 20-5,14-HEDE (**3**).

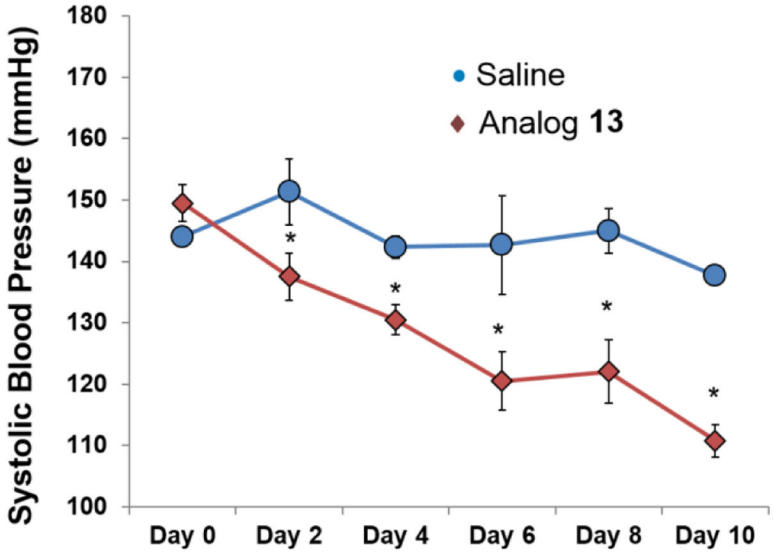


Figure 2.

Inhibition of 20-HETE-induced decrease (vasoconstriction) of pressurized (100 mmHg) rat gracilis muscle arteriole diameter by increasing concentrations of **13**.

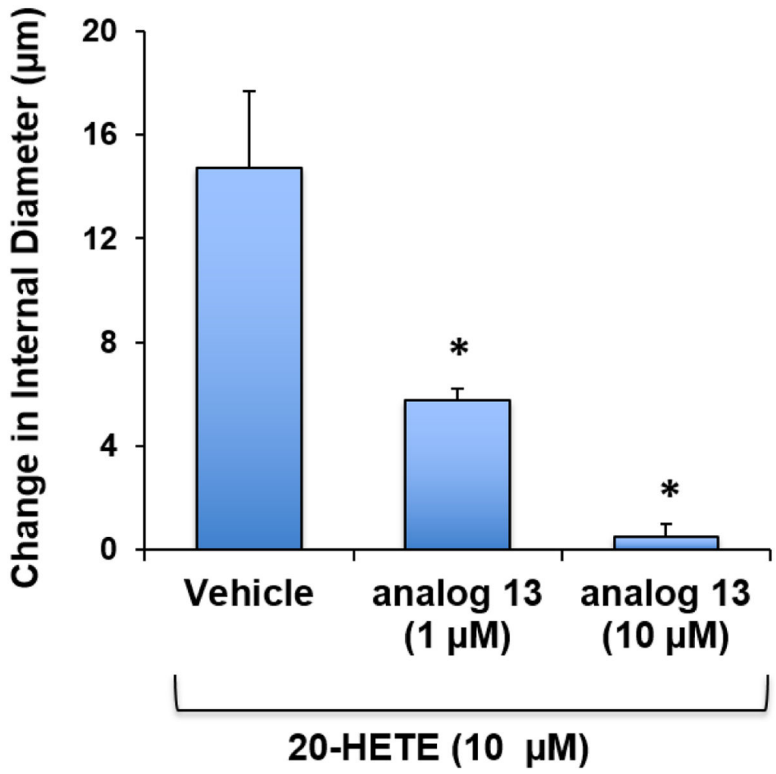
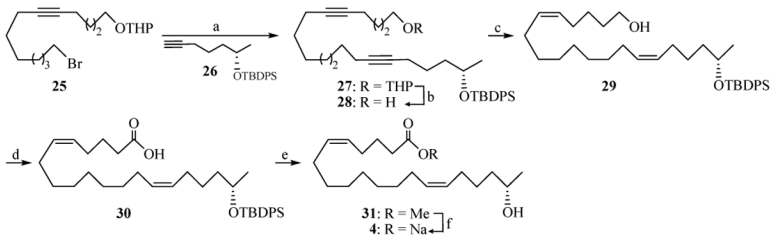


Figure 3.

Effect of analog **13** on systolic blood pressure of Cyp4a14(−/−) mice. Statistical significance $p<0.05$ as compared to saline, n=4/group.

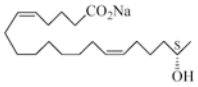
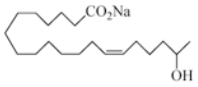
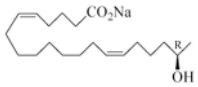
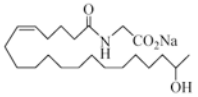
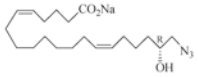
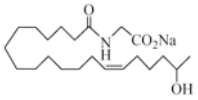
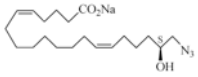
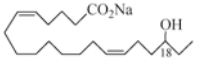
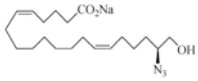
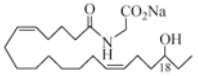
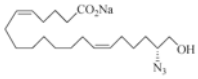
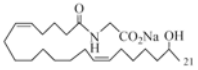
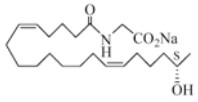
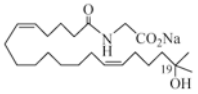
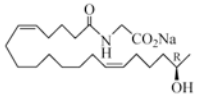
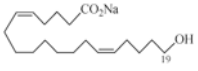
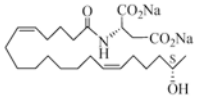
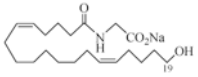
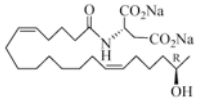
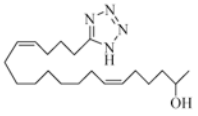
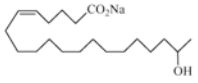


Scheme 1.

Reagents and conditions: (a) (i) *n*BuLi, **26**, THF/HMPA (4:1), −78 °C, 0.5 h, then 0 °C, 2 h. (ii) add **25** at −78 °C, stir at rt, 3 h, 72%; (b) PPTS, MeOH, 0 °C, 14 h, 68%; (c) P-2 Ni/H_2 (1 atm), EtOH, 1 h, 86%; (d) Jones, acetone, −20 °C, 2 h, 69%; (e) pTSA, MeOH, rt, 24 h, 62%; (f) NaOH, THF/H_2O, 12 h, 60%.

Table 1.

Effect of 19-HETE Analogs (1 μM) on 20-HETE (1 μM) Induced Vascular Sensitization to Phenylephrine.[a]

Analog Number	Structure	Fold Change from 20-HETE[b]	Analog Number	Structure	Fold Change from 20-HETE[b]
4		−0.68	**15**		−0.79
5		−0.85	**16**		−2.19
6		−5.69	**17**		−2.56
7		−5.45	**18**		−2.17
8		0.16	**19**		−1.13
9		0.50	**20**		0.58
10		−3.46	**21**		−1.16
11		−4.67	**22**		−0.68
12		−0.59	**23**		−0.74
13		−2.03	**24**		−0.78
14		−0.66			

[a] n = 3-5.

[b] Negative values indicate antagonism of 20-HETE sensitization and positive values indicate enhancement of 20-HETE activity.