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11,12-Epoxyeicosatrienoic Acid (11,12-EET): Structural Determinants for Inhibition of TNF-α-Induced VCAM-1 Expression☆

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Abstract—A series of 11,12-EET analogues were synthesized and compared using a human endothelial cell based TNF-α-induced VCAM-1 expression assay. The resulting data were used to map a putative recognition/binding domain for 11,12-EET.

Epoxyeicosatrienoic acids (EETs) are labile autacoids produced by the cytochrome P450 epoxygenase branch of the arachidonate cascade.[2] Their potent biological properties, ranging from regulation of ion channels[3] and stimulation of G-protein ADP-ribosylation[4] to modulation of the vasculature[5] and gap junctional communication,[6] have attracted wide and continuing interest. Recently, Liao et al.[7] demonstrated that amongst the four regioisomeric EETs, 11,12-EET is the most potent inhibitor of tumor necrosis factor-α (TNF-α) induced vascular cell adhesion molecule-1 (VCAM-1) expression in cytokine activated human endothelial cells. In addition, 11,12-EET inhibits VCAM-1 expression in response to other inflammatory mediators such as interlukin-1-α (IL-1 α) and bacterial lipopolysaccharide (LPS).[7] To help elucidate the spatial and functional determinants of 11,12-EET that contribute to inhibition as well as expedite the development of metabolically more stable anti-inflammatory agents, a series of 11,12-EET structural analogues were compared using a human endothelial cell based TNF-α-induced VCAM-1 expression assay. These data also provide the first insights into a putative macromolecular recognition and/or binding site[8] for 11,12-EET.[9,10] A representative sampling of these analogues is presented in Table 1 along with their bioassay results expressed as the percent inhibition of TNF-α-induced VCAM-1 expression elicited by 100 nM of eicosanoid. Under the assay conditions, *rac*-11,12-EET (**1**) and the individual antipodes **2** and **3** suppressed expression by ca. one-third. To ascertain the contributions of the olefins, a series of saturated or partially saturated analogues **4**–**8** were prepared and found to be comparable or less active than **1**. Tetrahydro-analogue **9**, in contrast, was nearly twice as potent as **1**. As a consequence of its simpler structure, **9** is easier to prepare and is chemically more stable than **1** since it does not contain the 1,4-diene subunits that are susceptible to autooxidation. Notably, conversion of the critical *cis*-$\Delta^{8,9}$-olefin in **9** to a linear acetylene, that is **10**, was detrimental. Alterations to the *cis*-epoxide were more or less well tolerated as illustrated by *trans*-epoxide **11**, furan **12**, cyclopropane **13**, episulfides **14**–**16**, and ether **17**. The latter is expected to be a useful alternative to **9** in systems where degradation by epoxide hydrolases must be minimized.[11] The substantial dip in activity shown by alcohol **18** suggests the presence of an oxygen is not sufficient and that this portion of the molecule should also remain hydrophobic. Alterations in the chain length at either end (analogues **19**–**22**), introduction of a heteroatom to obviate β-oxidation (analogue **23**), or the seemingly innocuous shift of the epoxide by just one carbon toward the ω-end (analogue **24**) proved undesirable.

☆See ref 1.

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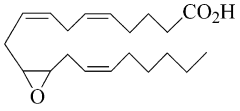
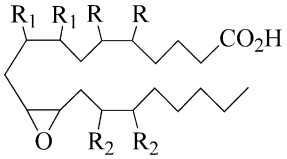
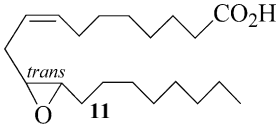
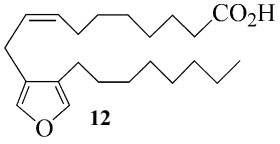
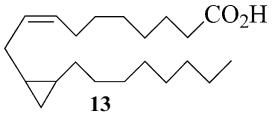
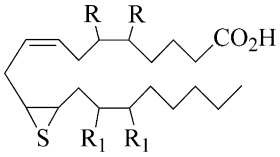
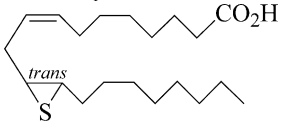
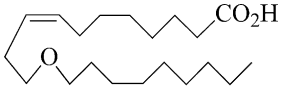
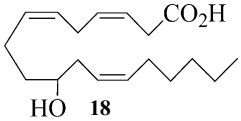
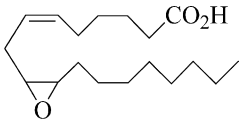
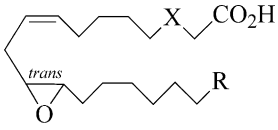
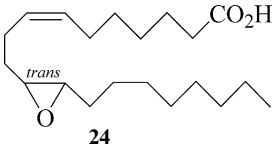
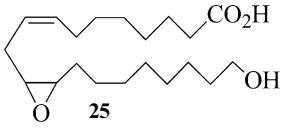
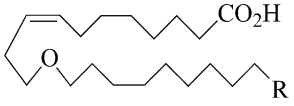
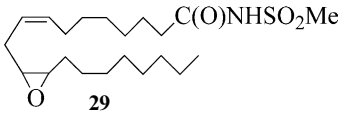
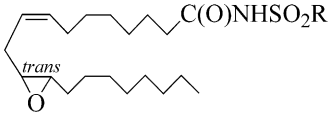
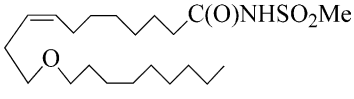
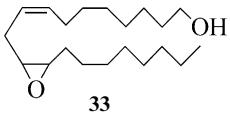
Table 1. Percent inhibition TNF-α-induced VCAM-1 expression

Eicosanoid (100 nM)	% (SEM/n[a])
	
1: *rac*-11,12-EET	39 (2.5/52)
2: 11(*R*),12(S)-EET	35 (7/6)
3: 11(*S*),12(R)-EET	31 (7/6)
	
4: R = R_1 = R_2 = H	36 (16/6)
5: R = H, R_1 = R_2 = *cis*-olefin	10 (12/6)
6: R = R_2 = *cis*-olefin, R_1 = H	30 (16/6)
7: R = R_1 = H, R_2 = *cis*-olefin	13 (16/6)
8: R = *cis*-olefin, R_1 = R_2 = H	31 (26/6)
9: R = R_2 = H, R_1-*cis*-olefin	69 (4/6)
10: R = R_2 = H, R_1-acetylene	28 (6/5)
 **11**	58 (4/23)
 **12**	50 (5/13)
 **13**	31 (8/13)
	
14: R = R_1 = *cis*-olefin	65 (5/13)
15: R = R_1 = H	49 (7/5)
 **16**	26 (7/13)
 **17**	48 (5/13)
 **18**	15 (6/7)

Eicosanoid (100 nM)	% (SEM/n[a])
	
19: R = H, 9(S),10(*R*)	13 (6/6)
20: R = H, 9(R),10(*S*)	17 (5/6)
	
21: X = CH_2, R = H	1 (3/6)
22: X = CH_2, R = $(CH_2)_3CH_3$	−1 (3/6)
23: X = O, R = CH_2CH_3	3 (2/6)
 **24**	13 (4/6)
 **25**	36 (6/13)
	
26: R = OH	27 (6/6)
27: R = MeCONH	63 (5/6)
28: R = 4-IC_6H_4O–	52 (5/6)
 **29**	29 (5/5)
	
30: R = Me	31 (3/13)
31: R = Ph	33 (5/13)
 **32**	1 (6/6)
 **33**	32 (5/5)

[a]Number of incubations

Hydroxylation of C(20) in **9** and **17** gave **25** and **26**, respectively, but abolished the gains in efficacy achieved in the parents. However, this could be largely restored using less polar derivatives as seen in acetamide **27** and iodophenoxy **28**, despite the latter's increased steric signature. Replacement of the carboxylic acid in **9**, **11**, and **17** with N-acylsulfonamides provided the ionizable isosteres[12] **29**–**31**, but failed to improve the level of inhibition and, in the latter case, was actually counterproductive. Likewise, reduction of **9** to alcohol **33** resulted in diminished activity compared to the free acid form.

Because of their anticipated chemical and/or metabolic stability and comparative potencies, tetrahydro analogue **9** and ether **17** were selected for further analysis. Their IC_{50} values (Fig. 1) were determined to be 60 nM and 45 nM, respectively.

Recognition/Binding Domain Map for 11,12-EET

The foregoing structure–activity relationships were used to map the recognition/binding domain responsible for the inhibition induced by 11,12-EET and its analogues. The representation presented in Figure 2 identifies five general regions and incorporates different binding motifs: (i) the ionic attraction of the carboxylate is important, although hydrogen bonding may also make a contribution since the corresponding alcohol retains modest agonist activity. However, this terminus is sterically constrained. (ii) The $\Delta^{5,6}$-olefin spans a mostly lipophilic region. While this section makes only a minor contribution to recognition, the replusion of a heteroatom in the chain can significantly disrupt binding. (iii) The $\Delta^{8,9}$-olefin seems to occupy a shallow pocket that conforms to the bent configuration of this *cis*-olefin, but poorly to a linear acetylene. The presence of an electron lone pair or π-bond in this vicinity is vital for inhibition. (iv) An oxygen or sulfur positioned between C(11) and C(12), irrespective of its nature (*cis*- or *trans*-epoxide/thiirane, ether, or furan, but not an alcohol), is also essential for maximum activity. The strong positional dependency suggests a single site of coordination. And (v), there is a lipophilic pocket that can accommodate the terminal methyl or other lipophilic group at the ω-terminus.

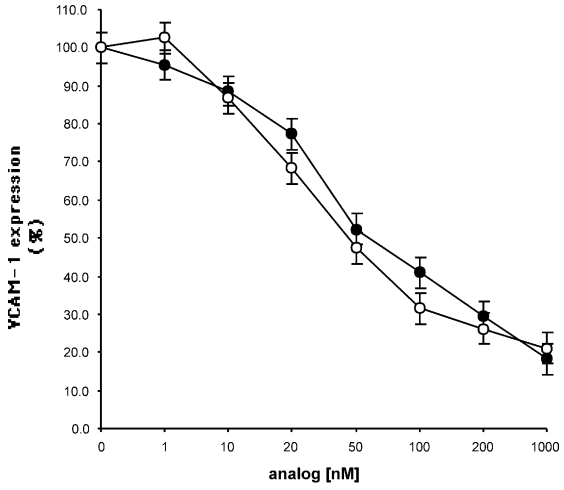


Figure 1. Inhibitory effects of analogues **9** (closed circles) and **17** (open circles) on TNF-α-induced VCAM-1 expression in human endothelial cells.[7] Data represent the mean ± standard deviation ($n = 6$).

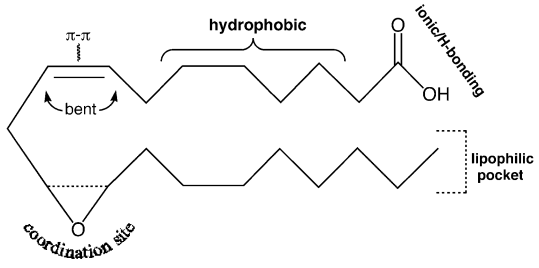


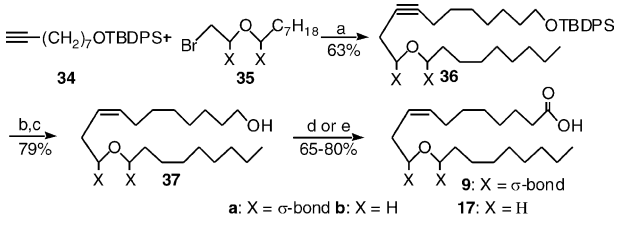
Figure 2. Recognition/binding domain map.

Analogue Syntheses

The chemical syntheses of analogues **9**[13] and **17** are outlined in Scheme 1 and are representative of the approach used for the other analogues. Protected acetylene **34**, prepared from 8-nonynol[14] (TBDPS-Cl/Im, CH_2Cl_2, rt, 4 h, 90%), was metalated using *n*-BuLi and then alkylated with either **35a** or **b**[15,16] to give **36a** and **b**, respectively. Desilylation and partial hydrogenation afforded **37a** which was oxidized to **9** using PDC whereas **37b** was transformed to **17** by Jones reagent.

Bioassay

Human saphenous vein endothelial cells (HSVEC) grown to confluence were replated on low pyrogen fibronectin (1.5 μg/cm^2) at 2×10^4 cells/cm^2. After washing with PBS, the HSVEC monolayer was incubated with 11,12-EET (**1**) or an analogue (100 nM) for 1 h, and then stimulated with TNF-α for an additional 16 h. The cells were fixed in 96-well microtiter plates using 1% paraformaldehyde for 45 min, washed thrice with PBS, and then incubated with VCAM-1 antibody for 2 h (1:100 dilution in PBS). Following a PBS rinse, the cells were sequentially incubated with biotinylated horse anti-mouse IgG antibody (1:1000 dilution, Vector Labs, Inc., Burlingame, CA, USA) for 1 h, streptavidin-alkaline phosphatase (Zymed, South San Francisco, CA, USA) for 30 min, and *p*-nitrophenyl phosphate disodium (1.5 μg/mL) for 30 min at 22 °C. The final absorbance was measured at 410 nm to determine the cell surface expression of



Scheme 1. Synthesis of analogues **9** and **17**: (a) *n*-BuLi, THF/HMPA (4:1), −40 °C, 2 h; bromide, −40 °C to rt, 2 h; (b) *n*-Bu_4NF, THF, 0 °C, 4 h; (c) P-2 Ni/H_2, EtOH, rt, 2 h; (d) PDC, DMF, rt, 16 h; (e) Jones reagent, acetone, −20 °C, 4 h.

VCAM-1. Nonbinding control antibodies (OX 6, against MHC class II antigen) were used in each experiment.

Acknowledgements

The anti-VCAM-1 antibody (mouse IgG, E1/6) was kindly provided by Michael A. Gimbrone, Jr., Brigham & Woman's Hospital, Boston, MA, USA. Financial support provided in part by the Robert A. Welch Foundation and NIH (GM31278, DK38226, and HL48743).

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12. Drummond, J. T.; Johnson, G. *Tetrahedron Lett.* **1988**, *29*, 1653.
13. ^{1}H NMR (400 MHz) of analogue **9**: δ 0.88 (t, $J=6.8$ Hz, 3H), 1.20–1.63 (m, 22H), 1.99 (q, $J=6.8$ Hz, 2H), 2.14–2.22 (m, 1H), 2.27–2.38 (m, 3H), 2.64–2.70 (m, 2H), 5.31–5.40 (m, 1H), 5.42–5.51 (m, 1H). **11**: δ 0.88 (t, $J=6.7$ Hz, 3H), 1.20–1.44 (m, 17H), 1.45–1.58 (m, 3H), 1.59–1.70 (m, 2H), 2.05 (q, $J=6.7$ Hz, 2H), 2.13–2.24 (m, 1H), 2.29–2.42 (m, 3H), 2.89–2.97 (m, 2H), 5.38–5.47 (m, 1H), 5.48–5.56 (m, 1H). **14**: δ 0.88 (t, $J=7.0$ Hz, 3H), 1.20–1.41 (m, 6H), 1.72 (quintet, $J=7.6$ Hz, 2H), 2.05 (q, $J=6.4$ Hz, 2H), 2.13 (q, $J=6.7$ Hz, 2H), 2.20–2.45 (m, 4H), 2.48–2.61 (m, 2H), 2.80 (apparent t, $J=6.4$ Hz, 2H) 2.92–3.00 (m, 2H), 5.33–5.58 (m, 6H). **15**: δ 0.88 (t, $J=6.4$ Hz, 3H), 1.20–1.42 (m, 15H), 1.41–1.58 (m, 3H), 1.59–1.70 (m, 2H), 1.82–1.92 (m, 2H), 2.0–2.10 (m, 2H), 2.32–2.40 (m, 3H), 2.41–2.52 (m, 1H), 2.90–3.00 (m, 2H), 5.43–5.52 (m, 2H). **17**: δ 0.88 (t, $J=6.4$ Hz, 3H), 1.22–1.40 (m, 18H), 1.53–1.65 (m, 4H), 1.98–2.08 (m, 2H), 2.33 (quintet, $J=7$ Hz, 4H), 3.38–3.43 (m, 4H), 5.33–5.40 (m, 1H), 5.41–5.47 (m, 1H).
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