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Activation of Peroxisome Proliferator-Activated Receptor γ (PPARγ) by Nitroalkene Fatty Acids: Importance of Nitration Position and Degree of Unsaturation

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Nitroalkene fatty acids are potent endogenous ligand activators of PPARγ-dependent transcription. Previous studies with the naturally occurring regioisomers of nitrolinoleic acid revealed that the isomers are not equivalent with respect to PPARγ activation. To gain further insight into the structure−activity relationships between nitroalkenes and PPARγ, we examined additional naturally occurring nitroalkenes derived from oleic acid, 9-nitrooleic acid (*E*-9-NO_2-18:1 [**1**]) and 10-nitrooleic acid (*E*-10-NO_2-18:1 [**2**]), and several synthetic nitrated enoic fatty acids of variable carbon chain length, double bonds, and nitration site. At submicromolar concentrations, *E*-12-NO_2 derivatives were considerably more potent than isomers nitrated at carbons 5, 6, 9, 10, and 13, and chain length (16 versus 18) or number of double bonds (1 versus 2) was of little consequence for PPARγ activation. Interestingly, at higher concentrations ($> 2\ \mu$M) the nitrated enoic fatty acids (*E*-9-NO_2-18:1 [**1**], *E*-9-NO_2-16:1 [**3**], *E*-10-NO_2-18:1 [**2**], and *E*-12-NO_2-18:1 [**7**]) deviated significantly from the saturable pattern of PPARγ activation observed for nitrated 1,4-dienoic fatty acids (*E*-9-NO_2-18:2, *E*-10-NO_2-18:2, *E*-12-NO_2-18:2, and *E*-13-NO_2-18:2).

Introduction

Unsaturated nitrated fatty acids are naturally occurring electrophiles implicated in the modulations of several signaling pathways: they can activate PPARγ,[a] inhibit NFkB, and activate ARE-dependent gene transcription by influencing the Nrf2/Keap1 pathway.[1−4] As consequence of these and perhaps other activities, the nitroakene fatty acids can mediate vasodilation, inhibit inflammation, induce differentiation in susceptible cells, and may, via PPARγ-dependent mechanisms, influence fat and carbohydrate homeostasis.[2,4−6] The nitroalkenes, which include the abundant naturally occurring nitrated derivatives of linoleic and oleic acid, are among the most potent endogenous activators of PPARγ with significant PPARγ-dependent transcription observed at nanomolar concentrations.[3,7] Moreover, their physiological significance is suggested by publications reporting aggregate concentrations of these nitroalkenes that can approach $1-2\ \mu$M.[8]

Recently, we examined the four naturally occurring regioisomers of nitrolinoleic acid (NO_2-LA or NO_2-18:2). While all activate PPARγ in a concentration dependent and saturable manner, the 12-nitro derivative (*E*-12-NO_2-18:2) is by far the most potent with respect to maximum agonist activity and lowest EC_{50}.[7] We speculated that the superiority of *E*-12-NO_2-18:2 over the other regioisomers may be related to unique interactions suggested to occur between amino acid residues within the ligand binding pocket of PPARγ and the 12-NO_2 moeity of *E*-12-NO_2-18:2.[9] To investigate these structure−activity relationships further, we synthesized and tested, using PPARγ-dependent reporter gene and ligand-binding assays, the relative potencies of a number of both naturally occurring and model nitroalkene fatty acids (Figure 1).

The main goal of this study was to determine the structural features of nitroalkene fatty acids that influence their relative abilities to activate PPARγ. The results of these studies establish the general importance, for optimal agonist activity, of nitration at the 12-position. In addition, they demonstrate, for the first time, the unique properties of nitrated enoic fatty acids toward PPARγ activation at higher ligand concentrations that are not observed with the nitrated 1,4-dienoic fatty acids or the pharmaceutical ligand rosiglitazone.

Results

Synthesis and Characterization of Nitroalkene Fatty Acids. The nitrooleic acid isomers (**1**-**2**, *E*-9-NO_2-18:1 and *E*-10-NO_2-18:1) were regio- and stereoselectively (*E* isomer) prepared using our previously reported procedure.[10] Schemes 1−6 depict a similar synthetic strategy that produced the nitro-16:1 derivatives (**3**, **4**; *E*-9-NO_2-16:1 and

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[a] Abbreviations: ARE, antioxidant or electrophile response element; Keap1, kelch-like ECH-associated protein 1; NFkB, nuclear factor κ B; *E*-9-NO_2-16:1, (*E*)-9-nitrohexadec-9-enoic acid; *E*-10-NO_2-16:1, (*E*)-9-nitrohexadec-9-enoic acid; *E*-5-NO_2-18:1, (*E*)-5-nitrooctadec-5-enoic acid; *E*-6-NO_2-18:1, (*E*)-6-nitrooctadec-5-enoic acid; *E*-9-NO_2-OA or *E*-9-NO_2-18:1, (*E*)-9-nitrooctadec-9-enoic acid; *E*-10-NO_2-OA or *E*-10-NO_2-18:1, (*E*)-10-nitrooctadec-9-enoic acid; *E*-12-NO_2-LA or *E*-12-NO_2-18:2, (9*Z*,12*E*)-12-nitrooctadeca-9,12-dienoic acid; *Z*-9/10-NO_2-OA or *Z*-9/10-NO_2-18:1, (*Z*)-9-nitrooctadec-9-enoic and (*Z*)-10-nitrooctadec-9-enoic acid mixture; *E*-12-NO_2-18:1, (*E*)-12-nitrooctadec-12-enoic acid; *E*-13-NO_2-18:1, (*E*)-13-nitrooctadec-12-enoic acid; Nrf2, NF-E2-related factor 2; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator-activated receptor responsive element.

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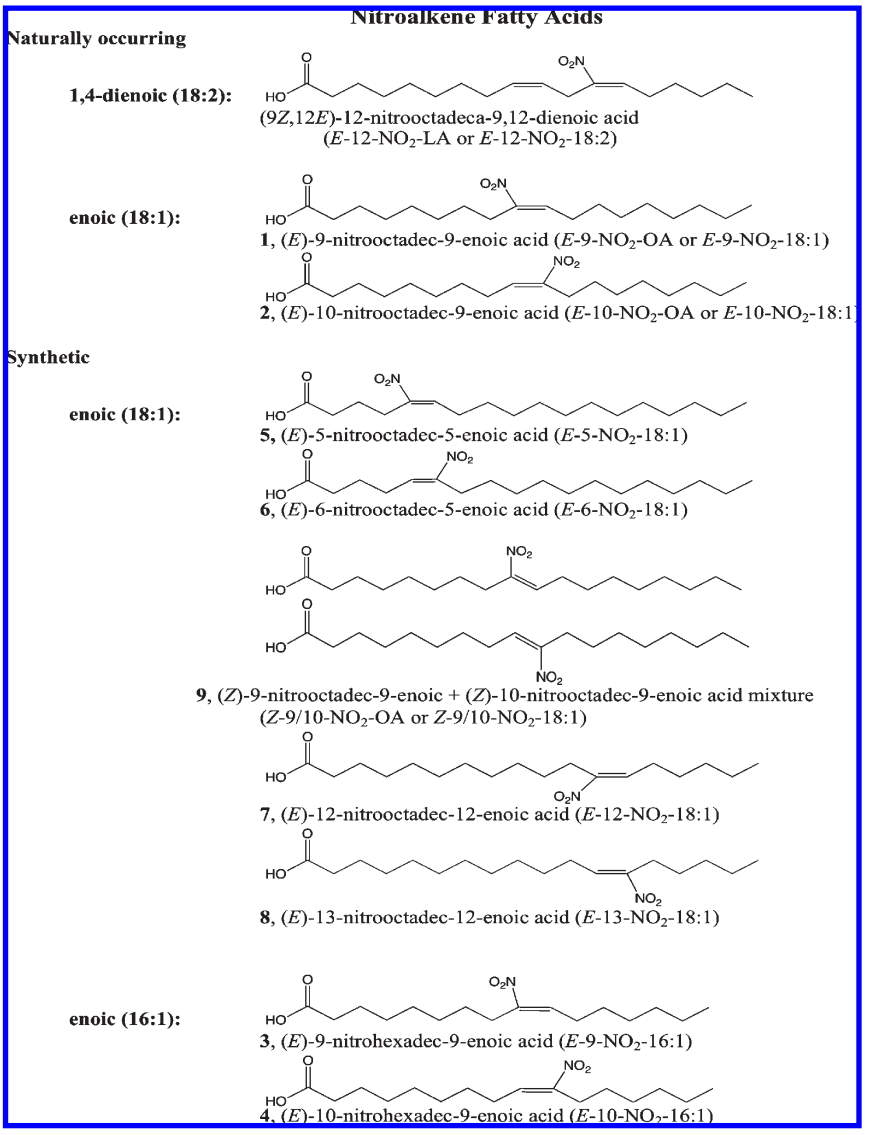
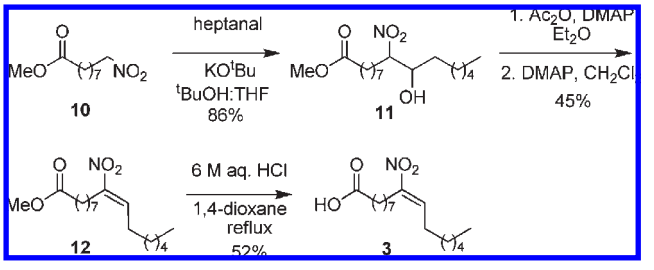


Figure 1. Structures and abbreviations used for the nitroalkene fatty acids.

E-10-NO_2-16:1) and the nitrooleic acid isomers (**5**−**8**, *E*-5-NO_2-18:1, *E*-6-NO_2-18:1, *E*-12-NO_2-18:1, and *E*-13-NO_2-18:1). In general, the synthesis of these nitrated fatty acids involved four steps: a nitroaldol condensation (Henry reaction) between the appropriate nitroalkane and aldehyde, acetylation of the corresponding nitro alcohol, dehydroacetylation and hydrolysis of the methyl ester.[10] The Henry reaction generally proceeded in good yield to form the nitro alcohols (**11**, **15**, **19**, **24**, **29**, and **32**) and ultimately allows complete control of the position of the nitro group in the final product (Schemes 1−6).[11] Dehydroacetylation of the intermediate nitro alcohols gives the corresponding nitroalkene methyl esters (**12**, **16**, **20**, **25**, **30**, and **33**) in moderate yield (16−58%, Schemes 1−6).[11] Proton NMR chemical shift analysis indicates exclusive formation of the *E* isomer, as the alkene protons of *E*-nitroalkenes display a characteristic chemical shift of approximately δ 7.00 ppm.[12,13] Hydrolysis of the methyl esters provided the desired nitro fatty acid derivatives (**3**−**8**, Schemes 1−6) that were characterized by proton and carbon NMR spectroscopy, mass spectrometry, and elemental analysis.[10] A similar sequence (Henry reaction, dehydroacetylation followed by sodium borohydride reduction) afforded a variety of nitroalkanes required for subsequent Henry reactions in good yield (for example, **14**, Scheme 2)[12,14] Nitroselenation of eldaidic acid (*trans*-oleic acid) followed by elimination yielded a mixture (∼1:1) of the *Z*-nitroalkenes (**9**, *Z*-9/10-NO_2-18:1), and chemical shift analysis supports the formation of the *Z* isomer, as *Z*-nitroalkenes demonstrate a characteristic chemical shift of about δ 5.80 ppm.[12,13,15]

Scheme 1

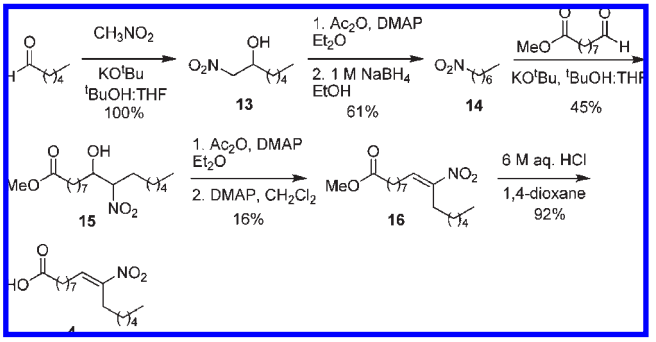


Affinities of the Nitroalkene Fatty Acids for the Ligand Binding Domain of PPARγ. A radioligand competition scintillation proximity assay was used to examine nitroalkene

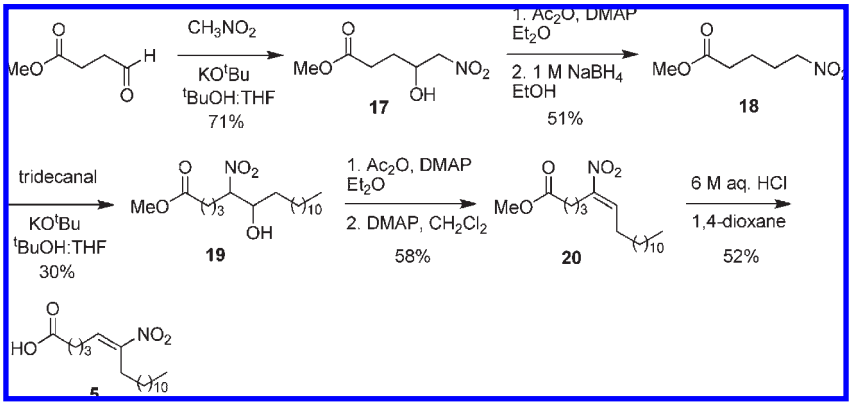
interactions with the ligand binding domains of PPARγ and PPARα. The results are presented in Table 1 as IC_{50} values that provide measures for the relative affinities of the various ligands for PPARs. The previously determined IC_{50} values for the pharmaceutical PPARγ ligand, rosiglitazone, and the 12-NO_2 isomer of nitrolinoleic acid (*E*-12-NO_2-18:2) are shown for reference.[7] All of the nitroalkenes are relatively high affinity ligands of PPARγ that substantially favor interactions with PPARγ over PPARα. Affinities toward PPARγ of the naturally occurring nitrated derivatives of oleic acid, *E*-9-NO_2-18:1 (**1**) and *E*-10-NO_2-18:1 (**2**), are comparable to most of the model synthetic nitroalkenes, with IC_{50} values ranging from 0.63 to 1.73 μM. Exceptions are the nitroenoic fatty acids, *E*-12-NO_2-18:1 (**7**) and *E*-13-NO_2-18:1 (**8**), which bind PPARγ with considerably greater affinity (IC_{50} values of 0.039 and 0.19 μM, respectively).

Differential Activation Profiles of PPARγ by the Various Nitroalkene Fatty Acids. In our earlier work, a major feature distinguishing *E*-12-NO_2-18:2 from its other regioisomers of nitrolinoleic acid was its ability to activate PPARγ at low

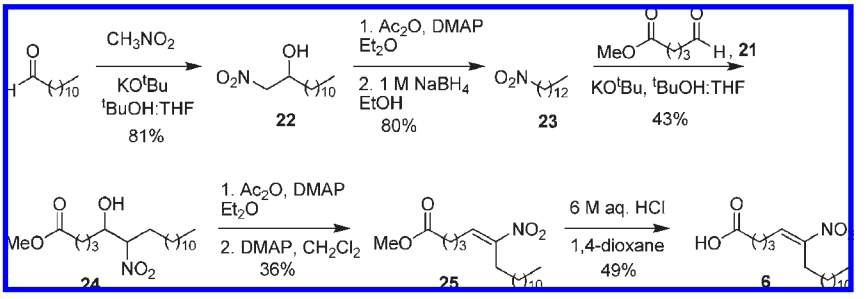
Scheme 2



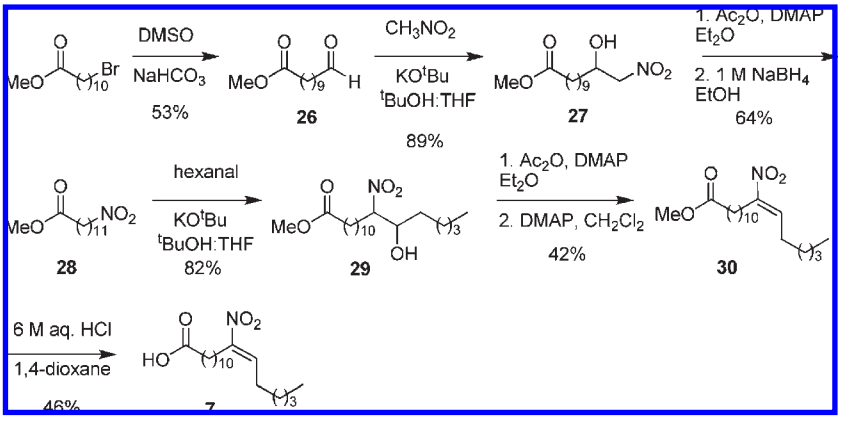
Scheme 3



Scheme 4



Scheme 5



Scheme 6

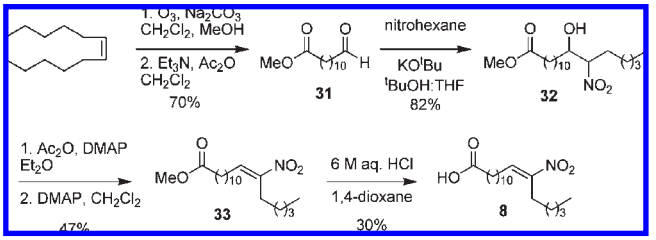


Table 1. Scintillation Proximity Competitive Binding Assay of Ligand−PPAR Interactions

compd	IC_{50} (μM) PPARγ	PPARα
rosiglitazone[a]	0.25	> 15
E-12-NO_2-18:2[a]	0.41	9.6
E-9-NO_2-18:1 (**1**)	0.98	> 15
E-10-NO_2-18:1 (**2**)	1.61	> 15
Z-9/10-NO_2-18:1 (**9**)	1.73	10.8
E-9-NO_2-16:1 (**3**)	0.83	> 15
E-10-NO_2-16:1 (**4**)	0.63	> 15
E-5-NO_2-18:1 (**5**)	1.68	> 15
E-6-NO_2-18:1 (**6**)	1.72	> 15
E-12-NO_2-18:1 (**7**)	0.039	1.6
E-13-NO_2-18:1 (**8**)	0.19	1.1

[a] IC_{50} values for rosiglitazone and *E*-12-NO_2-18:2 (12-NO_2-LA) were reported previously[7] and are included here for comparison.

concentrations (EC_{50} = 0.045 μM) whereas the others, *E*-9/10-NO_2-18:2 and *E*-13-NO_2-18:2, were considerably less active at low concentrations (EC_{50} = 0.41 and 0.62 μM, respectively). Hence, the nitroalkenes studied here were screened for PPARγ activation at low (0.1 μM) and high (3 μM) concentrations. These results are summarized in Figure 2 and include, for comparison, the pharmaceutical ligand rosiglitazone. At 3 μM, all nitroalkenes activated PPARγ-dependent transcription with induction levels ranging from a low of ~2.2-fold for the *Z*-9/10-NO_2-18:1 (**9**) *trans*-nitrooleic acid mixture and *E*-6-NO_2-18:1 (**6**) to a high of ~7.5-fold for the 12-nitroenoic fatty acid, *E*-12-NO_2-18:1 (**7**). More remarkable were differences in PPARγ activation between the nitroalkenes at low, 0.1 μM, concentrations. Only rosiglitazone and the 12-nitroalkenes, *E*-12-NO_2-18:2 and *E*-12-NO_2-18:1 (**7**), supported robust induction at low concentrations, whereas the other nitroalkenes, including the naturally occurring oleic acid derivatives *E*-9-NO_2-18:1 and *E*-10-NO_2-18:1 (**1**, **2**), supported little or no activation at these concentrations.

To examine the concentration dependencies of nitroalkene-mediated activation of PPARγ further, we selected five representative nitroalkene fatty acids for additional studies: the naturally occurring nitroalkenes, *E*-9-NO_2-18:1 (**1**), *E*-10-NO_2-18:1 (**2**) and *E*-12-NO_2-18:2; the 16 carbon chain analogue of *E*-9-NO_2-18:1 (**1**), *E*-9-NO_2-16:1 (**3**); and the enoic fatty acid analogue of *E*-12-NO_2-18:2, *E*-12-NO_2-18:1 (**7**). The results using natural nitroalkene fatty acids are shown in Figure 3. The 12-NO_2-18:2 isomer shows saturable induction of PPARγ-dependent transcription with robust induction at nanomolar concentrations (IC_{50} = 0.07 μM, similar to the value reported previously[7]). In contrast, the *E*-9-NO_2-18:1 (**1**) and *E*-10-NO_2-18:1 (**2**) isomers are relatively poor activators at submicromolar concentrations. Moreover, activation does not appear to saturate over the

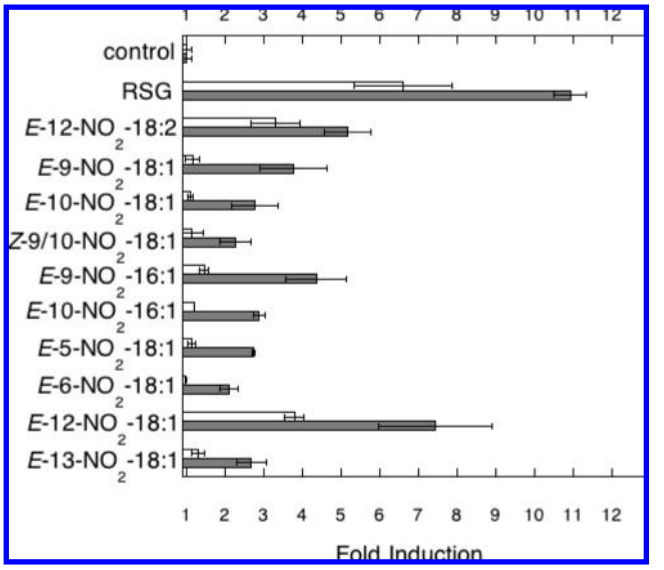


Figure 2. Activation of PPARγ-dependent transcription by nitroalkene fatty acids. The bar graphs show the PPARγ-dependent induction of PPRE-containing reporter gene expression by nitroalkene fatty acids in comparison with induction by rosiglitazone (RSG) or vehicle (control). Inductions were accomplished with 0.1 μM ligand (open bars) or 3 μM ligand (shaded dark bars) as described in Experimental Section. Fold induction is defined as (corrected PPRE-reporter activity + inducing agent)/(corrected PPRE-reporter activity − inducing agent). Bars represent the mean values of triplicate determinations ± 1 sd.

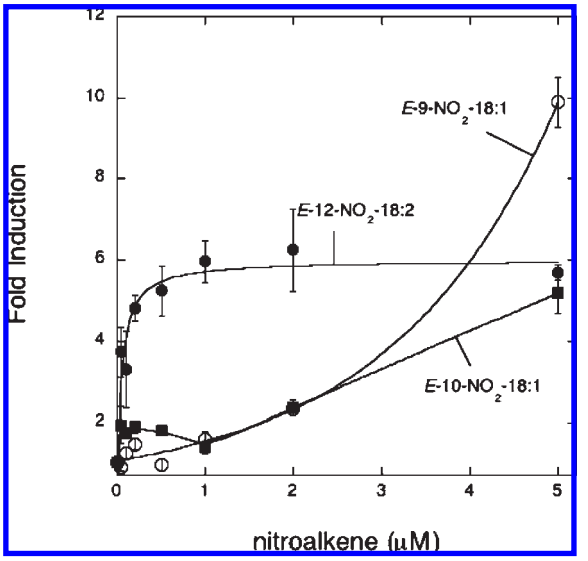


Figure 3. Dose−response analysis of PPARγ-dependent transcription induced by naturally occurring nitroalkene fatty acids. Concentration dependent induction of PPARγ-mediated PPRE-reporter gene expression was determined as described in Experimental Section for the 12-nitro derivative of linoleic acid, *E*-12-NO_2-18:2 (closed circles), and the 9- and 10-nitro derivative of oleic acid, *E*-9-NO_2-18:1 (**1**) (open circles) and *E*-10-NO_2-18:1 (**2**) (closed squares). Data points represent the mean values of triplicate determinations ± 1 sd. The dose−response curve for *E*-12-NO_2-18:2 was fitted to the equation described previously from which an EC_{50} value of 0.07 μM was determined.[7]

range of concentrations used but rather increases steadily for both and nearly exponentially for the *E*-9-NO_2-18:1 (**1**) isomer. In Figure 4, E-12-NO_2-18:2 activation is compared with the synthetic model nitroalkenes. *E*-9-NO_2-16:1 (**3**)

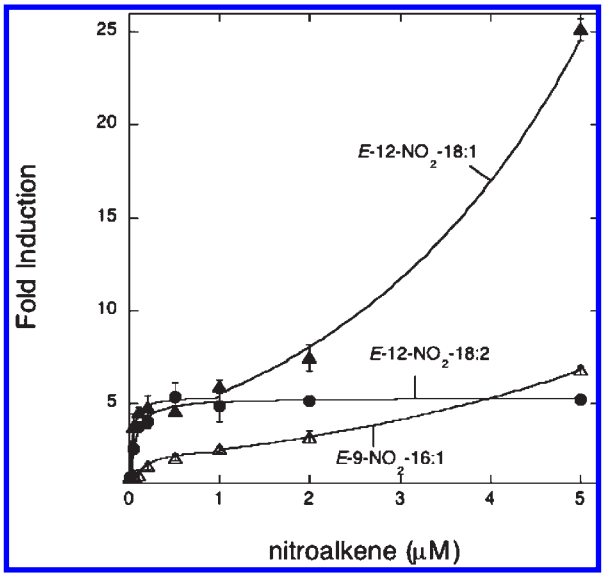


Figure 4. Dose−response analysis of PPARγ-dependent transcription induced by model synthetic nitroenoic fatty acids. Concentration dependent induction of PPARγ-mediated PPRE-reporter gene expression was determined as described in Experimental Section and Figure 3 for the model nitroenoic fatty acids *E*-12-NO_2-18:1 (**7**) (closed triangles) and *E*-9-NO_2-16:1 (**3**) (open triangles). The 12-nitro derivative of linoelic acid, *E*-12-NO_2-18:2 (closed circles), was included for comparison. Data points represent the mean values of triplicate determinations ± 1 sd.

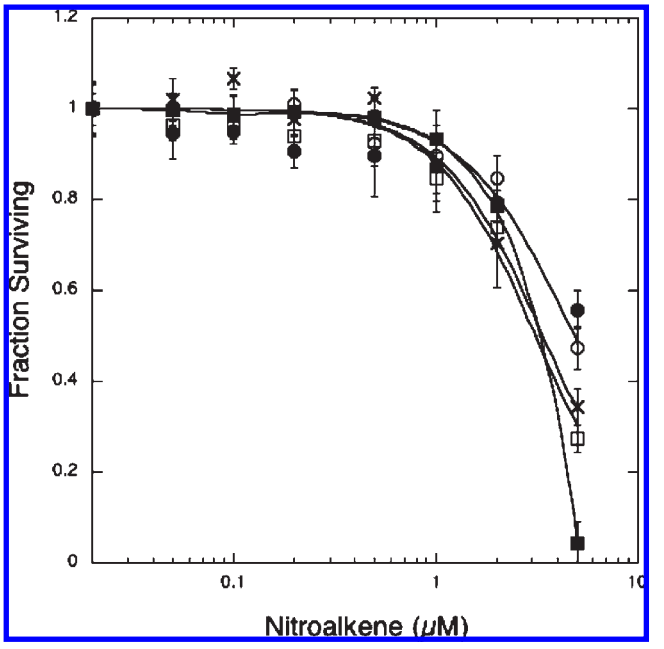


Figure 5. Cytotoxicity of nitroalkene fatty acids. The MCF7/RTO/PPARG1-4′ cells used for PPARγ-dependent reporter gene activation (Figures 2−4) were treated with varying concentration of nitroalkenes or vehicle control, and cytotoxicities were assessed as described in Experimental Section. Nitroalkene fatty acids used were 12-NO_2-18:2 (closed circles), 10-NO_2-18:1 (**2**) (open circles), 12-NO_2-18:1 (**7**) (crosses), 9-NO_2-16:1 (**3**) (open squares), and 9-NO_2-18:1 (**1**) (closed squares). Data points represent the mean values of eight replicate determinations ± 1 sd.

shows an activation profile similar to its 18-carbon analogue *E*-9-NO_2-18:1 (**1**): PPARγ activation is poor at submicromolar concentrations but steadily increases to the highest concentration used, 5 μM. The *E*-12-NO_2-18:1 (**7**) isomer has an activation profile that is, at low concentrations, similar to that of its 1,4-dienoic fatty acid analogue, *E*-12-NO_2-18:2. Induction of PPARγ-dependent transcription is robust at nanomolar concentrations and appears to begin to saturate at ~1 μM. However, at concentrations above 1 μM, the *E*-12-NO_2-18:1 (**7**) mediated activation profile deviates significantly from *E*-12-NO_2-18:2 mediated activation in that the former appears to increase exponentially from 1 to 5 μM. Collectively, these results indicate that the 12-position of nitration is the most important for optimal activation of PPARγ at low, submicromolar concentrations, and at high concentrations, the activation of PPARγ by the nitrated enoic fatty acids examined differs fundamentally from the activation of PPARγ by the nitrated 1,4-dienoic fatty acids.

Because all of the nitroalkene fatty acids are strong electrophiles, it was important to assess their potential toxicities over the range of concentrations used in the PPARγ activation studies. As shown in Figure 5, exposure of cells to the nitroalkenes at concentrations up to 1−2 μM was associated with little or no growth inhibition or cytotoxicity. However, at 5 μM, treatments with all five nitroalkenes were associated with significant cytotoxicities with 7-day survival ranging from 60% for *E*-12-NO_2-18:2 treated cells to < 5% for *E*-9-NO_2-18:1 (**1**) treated cells.

Discussion and Conclusions

The results described herein provide further evidence that the nitroalkene fatty acid isomers are *not* equivalent with respect to activation of PPARγ-dependent transcription. By examining a variety of naturally occurring and model synthetic nitroalkenes, we demonstrate both quantitative differences in their potencies of PPARγ activation and qualitative differences in their dose−response patterns of activation. These results reveal that the site of nitration plays an important role in optimal PPARγ activation at submicromolar concentrations. Previous work identifies the natural 12-nitrolinoleic acid *E*-12-NO_2-18:2 as the most potent of the nitrated linoleic acid regioisomers,[7] and results of the present studies with the synthetic compound *E*-12-NO_2-18:1 (**7**) verify the importance of nitration at C-12 for optimal activation of PPARγ. The recently reported X-ray structure of the PPARγ ligand-binding domain complexed with nitrated linoleic acid provides insight regarding the interaction of this compound with the protein.[9] As suggested for *E*-12-NO_2-18:2, specific noncovalent interactions of the nitro group of *E*-12-NO_2-18:1 (**7**) with Glu343 may induce conformational changes particularly favorable for transcriptional activation of PPARγ.[7] The relatively limited activation observed with both natural (**1** and **2**) and synthetic (**5**, **6**, and **8**) regioisomeric nitrated fatty acids at submicromolar concentrations further supports the importance of C-12 nitro group interactions with the protein for optimal PPARγ activation. The 9-enoic 16-carbon nitroalkene fatty acid homologues (**3** and **4**) and the *Z*-nitroalkenes (**9**) of nitrated oleic acid (**1** and **2**) were designed and prepared to examine chain length and alkene stereochemistry on activity. While the relatively lower level of PPARγ activation observed at low concentrations of **1**, **2**, **5**, **6**, and **9** makes structure−activity interpretations based on these compounds tentative at best, analysis of 9-enoic 16-carbon and 18-carbon nitroalkene fatty acid homologues suggests that carbon chain length, at least over this narrow range, has little affect on PPARγ activation. Importantly, in aggregate, our results show that

non-natural nitrated fatty acids, particularly if nitrated at C12, can potently activate PPARγ and provide a structural basis for the development of new compounds designed to interact with this transcription factor.

The number of double bonds, 1 versus 2, had little affect on PPARγ activation by 12-NO_2 derivatives at concentrations at or below 1 μM (Figure 4); however, at higher concentrations, the dose−response patterns for PPARγ activation of all nitroenoic fatty acids examined differed substantially from the patterns observed for the nitrated 1,4-dienoic fatty acids, including the *E*-12-NO_2-18:2 studied here as well as the *E*-9/10-NO_2-18:2 and *E*-13-NO_2-18:2 isomers studied previously.[7] While treatments with nitrated 1,4-dienoic fatty acids resulted in clearly saturable responses over the concentration range up to 5 μM (here, Figures 3 and 4, and previously[7]), treatments with nitroenoic fatty acids resulted in steady increases in the response profiles that, for some nitroenoic fatty acids, appeared exponential as concentrations approached 5 μM (Figures 3 and 4). The reason(s) for these deviations from saturation dose−response observed for nitroenoic fatty acids is (are) unclear. We can speculate that at high concentrations the PPARγ ligand binding domain may simultaneously bind two nitroenoic acid molecules (preferentially, over nitrated 1,4-dienoic fatty acids) as has been suggested by X-ray crystal structures solved for PPARγ in complex with the oxofatty acid ligand 9-(*S*)-hydroxyoctadeca-10,12-dienoic acid.[16] The binding of two such ligands could substantially modify the conformation of PPARγ and thereby its activation of transcription. Alternatively, the nitroenoic fatty acids may uniquely, or preferentially, interact with other signaling pathways that may then indirectly influence PPARγ activity. Indeed, for example, these nitroalkenes are potent electrophiles that, as a group, have the potential to influence a variety of other redox-sensitive signaling pathways and protein kinases[5,17−20] that may then secondarily modulate PPARγ-dependent transcription via phosphorylation of PPARγ or other ancillary transcription factors. Nevertheless, the physiological significance of these deviations from saturation at high nitroalkene concentrations is uncertain because, first, the nitroalkene fatty acids have significant cytotoxicities at concentrations above 2 μM (Figure 5) and, second, such high concentrations of nitroalkene fatty acids have yet to be reported in vivo.

Experimental Section

Chemistry: General. Heptanal, hexanal, methyl 4-oxobutanoate, tridecanal, δ-valerolactone, dodecylaldehyde, methyl 11-bromoundecanoate, cyclododecene, and 1-nitrohexane were purchased from Aldrich Chemical Co. and were used as received. Melting points (mp) were determined on a Mel-Temp capillary melting point apparatus and are uncorrected. Analytical TLC was performed on silica gel plates with QF-254 indicator. Visualization was accomplished with UV light, iodine, $KMnO_4$, bromocresol green, and/or dinitrophenylhydrazine. Solvents for extraction and purification were technical grade and were used as received. All reactions were performed in flame-dried glassware under an inert atmosphere of dry argon. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ using a BrukerAvance 300 MHz NMR spectrometer. Chemical shifts are given in ppm (δ), and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broadened). Mass spectrometry was performed by HT Laboratories (San Diego, CA). Data are reported in the form *m*/*z*. Purity of the compounds for biochemical assay was confirmed to be greater than 95% by elemental combustion analyses that were performed by Atlantic Microlab, Inc. (Atlanta, GA). Stock solutions of nitroalkene fatty acids were prepared with anhydrous dimethyl sulfoxide and stored at −80 °C.

Chemistry: Synthesis. General Method 1. Henry Reaction: Methyl 10-Hydroxy-9-nitrohexadecanoate (11). Powdered *t*-BuOK (57 mg, 0.51 mmol) was added to a solution of nitroalkane(**10**)[10] (1.32 g, 6.10 mmol) and heptanal (0.58 g, 0.71 mL, 5.09 mmol) in *t*-BuOH/THF (1:1, 5 mL) at 0 °C. After being stirred at room temperature for 24 h, the solution was diluted with Et_2O (25 mL) and water (25 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (25 mL) and brine (25 mL). The combined aqueous layers were back-extracted with Et_2O (2 × 50 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.21) to give **11** as a mixture of diastereomers (1.44 g, 86%): ^{1}H NMR 4.47−4.38 (m, 1H), 4.03−3.96 (m, 1H), 3.90−3.84 (m, 1H), 3.66 (s, 3H), 2.29 (t, J = 7.5 Hz, 2H), 1.80−1.18 (m, 22H), 0.84 (m, 3H); ^{13}C NMR 174.4, 93.0, 92.5, 72.5, 72.2, 51.6, 34.1, 33.8, 33.4, 32.1, 31.8, 30.6, 29.8, 29.5, 29.2, 29.1, 29.0, 28.9, 28.1, 26.0, 25.7, 25.4, 24.9, 22.7, 14.2.

General Method 2. Dehydration: (*E*)-Methyl 9-Nitrohexadec-9-enoate (12). A solution of **11** (1.44 g, 4.36 mmol), DMAP (54 mg, 0.44 mmol), and Ac_2O (0.55 g, 0.51 mL, 5.45 mmol) in Et_2O (10 mL) was stirred for 6 h at room temperature and concentrated. DMAP (0.64 g, 5.23 mmol) was added to a solution of the crude nitroacetates in CH_2Cl_2 (20 mL), and the solution was stirred at room temperature for 24 h, diluted with CH_2Cl_2 (50 mL), and washed with water (50 mL), 0.1 N aqueous HCl (50 mL), and brine (50 mL). The combined aqueous layers were back-extracted with CH_2Cl_2 (2 × 50 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.27) to give **12** as a yellow oil (0.61 g, 45%): ^{1}H NMR 7.06 (t, J = 7.8 Hz, 1H), 3.65 (s, 3H), 2.55 (t, J = 7.5 Hz, 2H0, 2.28 (t, J = 7.5 Hz, 2H), 2.20 (q, J = 7.5 Hz, 2H), 1.67−1.20 (m, 18H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR 174.3, 151.9, 136.5, 51.5, 34.1, 31.6, 29.14, 29.10, 29.08, 29.01, 28.6, 28.1, 28.0, 26.4, 25.0, 22.6, 14.1.

General Method 3. Hydrolysis: (*E*)-9-Nitrohexadec-9-enoic Acid (3, *E*-9-NO_2-16:1). A solution of **12** (0.60 g, 1.92 mmol), 1,4-dioxane (5 mL), and 6 M aqueous HCl (15 mL) was refluxed for 20 h. The solution was cooled to room temperature and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc → 1:1 hexanes/EtOAc, R_f = 0.08) to give **3** as a yellow oil (0.30 g, 52%): ^{1}H NMR 11.12 (bs, 1H), 7.04 (t, J = 8.1 Hz, 1H), 2.56 (t, J = 7.5 Hz, 2H), 2.32 (t, J = 7.2 Hz, 2H), 2.17 (q, J = 7.2, 7.5 Hz, 2H), 1.59−1.51 (m, 2H), 1.50−1.20 (m, 16H), 0.85 (t, J = 6.9 Hz, 3H); ^{13}C NMR 180.4, 151.8, 136.5, 34.1, 32.7, 31.6, 29.0, 28.9, 28.5, 28.3, 28.0, 27.9, 27.2, 26.3, 24.6, 22.5, 14.0; ESI MS *m*/*z* 298 (M-H$^-$). Anal. Calcd for $C_{16}H_{29}NO_4$: C, 64.18; H, 9.76; N, 4.68. Found: C, 64.36; H, 9.87; N, 4.47.

2-Hydroxy-1-nitroheptane (13). 13 was synthesized via general method 1 using nitromethane (2.44 g, 2.15 mL, 40.00 mmol) and hexanal (2.00 g, 2.46 mL, 20.00 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.30) to give **13** as an oil (3.22 g, 100%): ^{1}H NMR 4.46−4.29 (m, 3H), 2.74 (bs, 1H), 1.60−1.24 (m, 8H), 0.88 (t, J = 6.9 Hz, 3H).

General Method 4. Reduction: 1-Nitroheptane (14). A solution of **13** (3.22 g, 20.00 mmol), DMAP (0.24 g, 2.00 mmol), Ac_2O (2.55 g, 2.36 mL, 25.00 mmol), and Et_2O (40 mL) was stirred for 4 h at room temperature and concentrated. A solution of $NaBH_4$ (1.51 g, 40.00 mmol) in EtOH (40 mL) was added dropwise to the crude nitroacetates at 0 °C, and the solution was stirred for 2.5 h at room temperature and then acidified with

1 M aqueous HCl. The mixture was extracted with Et_2O (3 × 100 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude residue that was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.79) to give **14** as a pale-yellow oil (1.76 g, 61%): ^{1}H NMR 4.53 (t, J = 7.2 Hz, 2H), 2.25−2.11 (m, 2H), 1.78−1.37 (m, 10H), 1.01 (t, J = 6.9 Hz, 3H).

Methyl 9-Hydroxy-10-nitrohexadecanoate (15). 15 was Synthesized via general method 1 using 1-nitroheptane (**14**, 0.89 g, 6.10 mmol) and methyl 9-oxononanoate[10] (0.95 g, 5.09 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.21) to give **15** as a mixture of diastereomers (0.76 g, 45%): ^{1}H NMR 4.45−4.39 (m, 1H), 3.95−3.81 (m, 1H), 3.62 (s, 3H), 2.23 (t, J = 7.5 Hz, 2H), 1.80−1.18 (m, 22H), 0.82 (m, 3H); ^{13}C NMR 174.3, 93.4, 92.8, 72.7, 72.4, 51.9, 34.4, 33.9, 33.6, 31.8, 30.9, 30.1, 29.8, 29.5, 29.4, 29.1, 29.0, 28.5, 26.3, 26.1, 25.9, 25.6, 25.2, 23.1, 22.9, 14.4.

(*E*)-Methyl 10-Nitrohexadec-9-enoate (16). 16 was synthesized via general method 2 using **15** (0.76 g, 2.30 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.27) to give **16** as a yellow oil (0.12 g, 16%): ^{1}H NMR 7.05 (t, J = 7.8 Hz, 1H), 3.65 (s, 3H), 2.55 (t, J = 7.5 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 2.20 (q, J = 7.5 Hz, 2H), 1.68−1.18 (m, 18H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR 174.2, 152.1, 136.3, 51.5, 34.1, 31.6, 29.23, 29.08, 29.05, 28.99, 28.6, 28.1, 28.0, 26.5, 25.0, 22.6, 14.1.

(*E*)-10-Nitrohexadec-9-enoic Acid (4, *E*-10-NO$_2$-16:1). 4 was synthesized via general method 3 using **16** (0.10 g, 0.32 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc → 1:1 hexanes/EtOAc, R_f = 0.08) to give **4** as a yellow oil (0.09 g, 92%): ^{1}H NMR 10.62 (bs, 1H), 7.06 (t, J = 8.1 Hz, 1H), 2.56 (t, J = 7.5 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 2.20 (q, J = 7.2, 7.5 Hz, 2H), 1.68−1.20 (m, 18H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR 180.7, 152.4, 136.6, 34.5, 31.9, 29.5, 29.4, 29.31, 29.27, 29.12, 28.9, 28.4, 28.3, 26.8, 25.04, 24.96, 22.9, 14.4; ESI MS m/z 298 (M-H$^-$). Anal. Calcd for $C_{16}H_{29}NO_4$: C, 64.18; H, 9.76; N, 4.68. Found: C, 65.44; H, 10.02; N, 4.03.

Methyl 4-Hydroxy-5-nitropentanoate (17). 17 was synthesized via general method 1 using nitromethane (0.57 g, 0.50 mL, 9.32 mmol) and methyl 4-oxobutanoate (0.54 g, 4.66 mmol) to give a pale-yellow oil that was used without further purification (0.58 g, 71%): ^{1}H NMR 4.42−4.30 (m, 3H), 3.66 (s, 3H), 2.51 (t, J = 7.2 Hz, 2H), 1.82−1.73 (m, 2H); ^{13}C NMR 174.0, 80.7, 67.7, 52.0, 29.8, 28.7.

Methyl 5-Nitropentanoate (18). 18 was synthesized via general method 4 using **17** (0.71 g, 3.98 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.30) to give **18** as a yellow oil (0.33 g, 51%): ^{1}H NMR 4.38 (t, J = 6.9 Hz, 2H), 3.66 (s, 3H), 2.35 (t, J = 7.2 Hz, 2H), 2.07−1.97 (m, 2H), 1.74−1.64 (m, 2H); ^{13}C NMR 173.2, 75.2, 51.8, 33.0, 26.7, 21.6.

Methyl 6-Hydroxy-5-nitrooctadecanoate (19). 19 was synthesized via general method 1 using **18** (0.33 g, 2.04 mmol) and tridecanal (0.37 g, 0.44 mL, 1.85 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.07) to give **19** as a yellow oil (0.20 g, 30%): ^{1}H NMR 4.45−4.32 (m, 1H), 4.00 (bs, 1H), 3.65 (s, 3H), 2.35 (t, J = 7.2 Hz, 2H), 2.14−1.05 (m, 26H), 0.85 (t, J = 6.9 Hz, 3H); ^{13}C NMR 173.4, 92.0, 72.4, 72.05, 51.8, 33.2, 33.1, 32.0, 29.72, 29.7, 29.6, 29.5, 29.41, 29.39, 27.4, 26.7, 25.7, 22.8, 21.6, 21.4, 14.2.

(*E*)-Methyl 5-Nitrooctadec-5-enoate (20). 20 was synthesized via general method 2 using **19** (0.20 g, 0.56 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.58) to give **20** as a yellow oil (0.11 g, 58%): ^{1}H NMR 7.12 (t, J = 7.8 Hz, 1H), 3.66 (s, 3H), 2.63 (t, J = 7.5 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 2.23 (t, J = 7.5 Hz, 2H), 1.81 (quintet, J = 7.2 Hz, 2H), 1.50−1.43 (m, 2H), 1.42−1.07 (m, 18H), 0.86 (t, J = 6.9 Hz, 3H); ^{13}C NMR 173.4, 150.8, 137.7, 51.7, 33.1, 32.0, 29.71, 29.69, 29.6, 29.4, 28.6, 28.1, 25.7, 23.1, 22.8, 14.2.

(*E*)-5-Nitrooctadec-5-enoic Acid (5, *E*-5-NO$_2$-18:1). 5 was synthesized via general method 3 using **20** (0.11 g, 0.33 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc → 1:1 hexanes/EtOAc, R_f = 0.14) to give **5** as a white solid (0.056 g, 52% (63% brsm)): ^{1}H NMR 7.16 (t, J = 8.1 Hz, 1H), 2.67 (t, J = 7.5 Hz, 2H), 2.42 (t, J = 7.2 Hz, 2H), 2.23 (q, J = 7.5 Hz, 2H), 1.85 (quintet, J = 7.2 Hz, 2H), 1.52−1.10 (m, 20H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR 179.3, 150.7, 137.9, 33.1, 32.0, 29.77, 29.75, 29.72, 29.6, 29.5, 28.6, 28.2, 25.7, 22.9, 22.8, 14.2; ESI MS m/z 326 (M − H$^-$). Anal. Calcd for $C_{18}H_{33}NO_4$: C, 66.02; H, 10.16; N, 4.28. Found: C, 67.06; H, 10.29; N, 3.79.

Methyl 5-Oxopentanoate (21).[21] **21** was synthesized via a literature procedure in two steps from δ-valerolactone (50 mmol). The crude residue was purified by column chromatography (silica gel, 2:3 hexanes/EtOAc, R_f = 0.67) to give **21** as a colorless oil (5.86 g, 66%). ^{1}H and ^{13}C NMR matched the reported values.

1-Nitrotridecan-1-ol (22). 22 was synthesized via general method 1 using nitromethane (1.33 g, 1.17 mL, 21.7 mmol) and dodecylaldehyde (2.00 g, 10.85 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.39) to give **22** as a white solid (2.16 g, 81%): mp 26 °C; ^{1}H NMR 4.44−4.21 (m, 3H), 2.77 (bd, J = 4.5 Hz, 1H), 1.52−1.16 (m, 20H), 0.86 (t, J = 6.9 Hz, 3H); ^{13}C NMR 80.8, 68.8, 33.9, 32.0, 29.7, 29.6, 29.5, 29.4, 25.3, 22.8, 14.2.

1-Nitrotridecane (23). 23 was synthesized via general method 4 using **22** (2.16 g, 8.83 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.69) to give **23** as a pale-yellow oil (1.62 g, 80%): ^{1}H NMR 4.42 (t, J = 7.2 Hz, 2H), 1.54−1.21 (m, 22H), 0.86 (t, J = 6.9 Hz, 3H); ^{13}C NMR 76.1, 32.3, 30.0, 29.9, 29.7, 29.5, 29.3, 28.9, 28.1, 27.8, 26.6, 23.1, 14.5.

Methyl 5-Hydroxy-6-nitrooctadecanoate (24). 24 was synthesized via general method 1 using **23** (0.71 g, 3.12 mmol) and **21** (0.34 g, 2.60 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.19) to give **24** as a pale-yellow oil as a mixture of diastereomers (0.40 g, 43%): ^{1}H NMR 4.47−4.36 (m, 1H), 4.13−3.86 (m, 1H), 3.66 (s, 3H), 2.36 (t, J = 7.5 Hz, 2H), 1.89−1.13 (m, 26H), 0.86 (t, J = 6.9 Hz, 3H); ^{13}C NMR 174.0, 93.1, 92.5, 72.0, 71.8, 51.8, 33.5, 33.4, 32.9, 32.6, 32.0, 30.5, 29.7, 29.5, 29.4, 29.3, 29.0, 28.4, 28.1, 26.1, 25.8, 22.8, 21.5, 20.9, 20.6, 14.2.

(*E*)-Methyl 6-Nitrooctadec-5-enoate (25). 25 was synthesized via general method 2 using **24** (0.36 g, 1.01 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.43) to give **25** as a pale-yellow oil (0.13 g, 36%): ^{1}H NMR 7.02 (t, J = 7.8 Hz, 1H), 3.66 (s, 3H), 2.55 (t, J = 7.2 Hz, 2H), 2.36 (t, J = 7.2 Hz, 2H), 2.27 (q, J = 7.5 Hz, 2H), 1.82 (quintet, 7.2, 7.5 Hz, 2H), 1.50−1.14 (m, 20H), 0.86 (t, J = 6.9 Hz, 3H); ^{13}C NMR 173.3, 152.7, 134.8, 51.8, 33.3, 32.0, 29.7, 29.6, 29.44, 29.36, 28.0, 27.3, 26.5, 23.8, 22.8, 14.2.

(*E*)-6-Nitrooctadec-5-enoic Acid (6, *E*-6-NO$_2$-18:1). 6 was synthesized via general method 3 using **25** (0.13 g, 0.38 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc → 1:1 hexanes/EtOAc, R_f = 0.14) to give **6** as a pale-yellow oil (0.06 g, 49% (98% brsm)): ^{1}H NMR 10.84 (bs, 1H), 7.03 (t, J = 7.5 Hz, 1H), 2.57 (t, J = 7.2 Hz, 2H), 2.43 (t, J = 7.2 Hz, 2H), 2.30 (q, J = 7.5 Hz, 2H), 1.83 (quintet, 7.2 Hz, 2H), 1.49−1.22 (m, 20H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR 179.0, 152.9, 134.5, 32.0, 29.8, 29.6, 29.5, 29.4, 28.1, 27.3, 26.5, 23.6, 22.8, 14.2; ESI MS m/z 326 (M-H$^-$). Anal. Calcd for $C_{18}H_{33}NO_4$: C, 66.02; H, 10.16; N, 4.28. Found: C, 65.60; H, 10.22; N, 3.68

Methyl 11-Oxoundecanoate (26).[22] **26** was synthesized via a literature procedure from methyl 11-bromoundecanoate (10.00 g, 35.81 mmol) to give **26** as a yellow oil (4.10 g, 53%): ^{1}H NMR 9.66 (t, J = 1.8 Hz, 1H), 3.56 (s, 3H), 2.32 (td, J = 7.2 Hz,

1.8 Hz, 2H), 2.20 (t, J = 7.5 Hz, 2H), 1.57−1.45 (m, 4H), 1.33−1.27 (m, 10H); ^{13}C NMR 202.6, 174.1, 51.3, 43.8, 33.9, 29.28, 29.19, 29.11, 29.07, 29.03, 29.00, 22.0.

Methyl 11-Hydroxy-12-nitrododecanoate (27). 27 was synthesized via general method 1 using nitromethane (1.11 g, 0.98 mL, 18.32 mmol) and **26** (1.96 g, 9.16 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.23) to give **27** as a white solid (2.24 g, 89%): mp 36−38 °C; ^{1}H NMR 4.45−4.22 (m, 3H), 3.65 (s, 3H), 2.76 (bs, 1H), 2.28 (t, J = 7.5 Hz, 2H), 1.64−1.18 (m, 16H); ^{13}C NMR 174.5, 80.8, 68.8, 51.6, 34.2, 33.9, 29.8, 29.4, 29.3, 29.2, 25.2, 25.0, 14.3

Methyl 12-Nitrododecanoate (28). 28 was synthesized via general method 4 using **27** (2.24 g, 8.15 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.41) to give **28** as a colorless oil (1.35 g, 64%): ^{1}H NMR 4.32 (t, J = 6.9 Hz, 2H), 3.60 (s, 3H), 2.23 (t, J = 7.5 Hz, 2H), 1.98−1.89 (m, 2H), 1.60−1.50 (m, 2H), 1.38−1.15 (m, 14H); ^{13}C NMR 174.6, 76.1, 51.7, 34.3, 29.7, 29.6, 29.5, 29.2, 27.7, 26.6, 25.3.

Methyl 13-Hydroxy-12-nitrooctadecanoate (29). 29 was synthesized via general method 1 using **28** (1.35 g, 5.20 mmol) and hexanal (0.44 g, 0.54 mL, 4.33 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes: EtOAc, R_f = 0.24) to give **29** as a mixture of diastereomers (1.27 g, 82%): ^{1}H NMR 4.48−4.39 (m, 1H), 4.04−3.80 (m, 1H), 3.65 (s, 3H), 2.29 (t, J = 7.2 Hz, 2H), 1.85−1.13 (m, 26H), 0.88 (t, J = 6.3 Hz, 3H); ^{13}C NMR 174.5, 93.0, 92.5, 72.5, 72.2, 51.6, 34.2, 33.7, 33.3, 31.6, 31.6, 30.6, 29.4, 29.3, 29.2, 29.1, 29.02, 28.92, 28.1, 27.5, 26.3, 26.1, 25.8, 25.4, 25.0, 22.6, 14.1.

(*E*)-Methyl 12-Nitrooctadec-12-enoate (30). 30 was synthesized via general method 2 using **29** (1.27 g, 3.54 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.53) to give **30** as a yellow oil (0.50 g, 42%): ^{1}H NMR 7.07 (t, J = 8.1 Hz, 1H), 3.65 (s, 3H), 2.56 (t, J = 7.5 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2H), 2.20 (q, J = 7.5 Hz, 2H), 1.64−1.53 (m, 2H), 1.52−1.40 (m, 4H), 1.36−1.18 (m, 16H), 0.86 (t, J = 6.9 Hz, 3H); ^{13}C NMR 174.4, 152.0, 136.5, 51.5, 34.2, 31.6, 29.52, 29.47, 29.33, 29.23, 28.32, 28.08, 28.02, 26.5, 25.1, 22.5, 14.0.

(*E*)-12-Nitrooctadec-12-enoic Acid (7, *E*-12-NO$_2$-18:1). 7 was synthesized via general method 3 using **30** (0.50 g, 1.47 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc → 1:1 hexanes/EtOAc, R_f = 0.11) to give **7** as a yellow oil (0.22 g, 46% (72% brsm)): ^{1}H NMR 11.24 (bs, 1H), 7.06 (t, J = 8.1 Hz, 1H), 2.56 (t, J = 7.5 Hz, 2H), 2.33 (t, J = 7.2 Hz, 2H), 2.18 (q, J = 7.2, 7.5 Hz, 2H), 1.66−1.17 (m, 22H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR 180.5, 152.0, 136.5, 34.2, 31.6, 29.48, 29.42, 29.30, 29.28, 29.10, 28.3, 28.04, 27.97, 26.4, 24.7, 22.5, 14.0; ESI MS m/z 326 (M − H$^-$). Anal. Calcd for $C_{18}H_{33}NO_4$: C, 66.02; H, 10.16; N, 4.28. Found: C, 65.97; H, 10.28; N, 4.16.

Methyl 12-Oxododecanoate (31). Ozone was bubbled into a mixture of cyclododecene (5.00 g, 5.75 mL, 30.06 mmol) and anhydrous Na_2CO_3 (0.82 g, 7.74 mmol) in CH_2Cl_2 (90 mL) and methanol (18 mL) at −78 °C until a faint blue color appeared. Argon was then bubbled into the mixture until the blue color was discharged. The cooling bath was removed and the mixture slowly warmed to room temperature. After filtration, benzene (30 mL) was added and the mixture concentrated to ∼20 mL with the resulting viscous liquid being diluted with CH_2Cl_2 (80 mL). After the mixture was cooled to 0 °C, anhydrous Et_3N (4.50 g, 6.20 mL, 44.48 mmol) and anhydrous Ac_2O (8.55 g, 7.90 mL, 83.73 mmol) were sequentially added dropwise and the mixture was stirred at 0 °C for 0.5 h and then at room temperature overnight. The organic phase was washed with 0.1 M aqueous HCl (2 × 60 mL), 10% aqueous NaOH (2 × 60 mL), water (60 mL) and dried over $MgSO_4$. The solution was filtered and concentrated in vacuo to give **31** as a oil (4.78 g, 70%): R_f = 0.44 (silica gel, 4:1 hexanes/EtOAc); ^{1}H NMR 9.74 (t, J = 1.8 Hz, 1H), 3.65 (s, 3H), 2.40 (td, J = 7.2, 1.8 Hz, 2H), 2.28 (td, J = 7.5 Hz, 1.5 Hz, 2H), 1.68−1.55 (m, 4H), 1.37−1.20 (m, 12H).

Methyl 12-Hydroxy-13-nitrooctadecanoate (32). 32 was synthesized via general method 1 using 1-nitrohexane (1.05 g, 1.12 mL, 8.00 mmol) and **31** (0.91 g, 4.00 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.15) to give **32** as a mixture of diastereomers (1.18 g, 82%): ^{1}H NMR 4.44−4.36 (m, 1H), 3.96−3.93 (m, 1H), 3.86−3.81 (m, 1H), 3.62 (s, 3H), 2.26 (t, J = 7.5 Hz, 2H), 1.80−1.17 (m, 26H), 0.84 (t, J = 6.9 Hz, 3H); ^{13}C NMR 174.5, 93.1, 92.6, 72.4, 72.1, 51.5, 34.1, 33.6, 33.3, 31.2, 31.1, 30.9, 30.4, 29.5, 29.4, 29.3, 29.2, 29.1, 28.2, 25.7, 25.6, 25.4, 25.3, 25.0, 22.3, 13.9.

(*E*)-Methyl 13-Nitrooctadec-12-enoate (33). 33 was synthesized via general method 2 using **32** (1.18 g, 3.29 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc, R_f = 0.46) to give **33** as a yellow oil (0.53 g, 47%): ^{1}H NMR 7.07 (t, J = 7.8 Hz, 1H), 3.66 (s, 3H), 2.56 (t, J = 7.5 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 2.20 (q, J = 7.5 Hz, 2H), 1.67−1.18 (m, 22H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR 174.4, 152.0, 136.5, 51.6, 34.2, 31.5, 29.52, 29.48, 29.43, 29.34, 29.24, 28.6, 28.1, 27.7, 26.4, 25.1, 22.5, 14.1.

(*E*)-13-Nitrooctadec-12-enoic Acid (8, 13-NO$_2$OA). 8 was synthesized via general method 3 using **33** (0.53 g, 1.54 mmol). The crude residue was purified by column chromatography (silica gel, 4:1 hexanes/EtOAc → 1:1 hexanes/EtOAc, R_f = 0.11) to give **8** as a yellow oil (0.15 g, 30% (61% brsm)): ^{1}H NMR 11.34 (bs, 1H), 7.07 (t, J = 8.1 Hz, 1H), 2.56 (t, J = 7.5 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 2.20 (q, J = 7.2, 7.5 Hz, 2H), 1.68−1.20 (m, 22H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR 180.5, 152.3, 136.8, 34.5, 31.8, 29.80, 29.75, 29.72, 29.61, 29.43, 28.9, 28.4, 28.0, 26.7, 25.1, 22.8, 14.4; ESI MS m/z 326 (M-H$^-$). Anal. Calcd for $C_{18}H_{33}NO_4$: C, 66.02; H, 10.16; N, 4.28. Found: C, 65.48; H, 10.14; N, 3.99.

(*Z*)-9 and 10-Nitrooctadec-9-enoic Acid (9, Z-9/10-NO$_2$-18:1 or Z-9/10-NO$_2$-OA). 9 was synthesized from elaidic acid (Aldrich, 0.282 g, 1.0 mmol) using a previously published nonselective nitroselenation-elimination sequence.[15] The crude residue was purified by column chromatography (silica gel, 70:30:1 hexanes/Et_2OAc/AcOH, R_f = 0.48) to give **9** as a yellow oil (mixture of regioisomers, 0.125 g, 38%): ^{1}H NMR 11.11 (bs, 1H), 5.65 (m, 1H), 2.49 (t, J = 7.2 Hz, 2H), 2.34 (t, J = 7.5 Hz, 4H), 1.64−1.26 (m, 22H), 0.87 (t, J = 6.3 Hz, 3H); ^{13}C NMR 180.5, 151.1, (131.9, 131.5), (40.0, 39.9), (32.7, 32.6), (31.8, 31.7), (29.4−28.1, multiple peaks), (27.2, 27.1), (24.6, 24.5), 22.6, 14.1.

Nitroalkene−PPARγ Interactions. Binding Assays. Relative binding affinities of test ligands for PPAR were determined by a radioligand competition scintillation proximity assay using purified recombinant GST-hPPAR receptor,[23] the radioligand [^{3}H$_2$]nTZD3, and varying concentrations of unlabeled test ligands (nitroalkene fatty acids) as described previously.[7,24] IC_{50} values were calculated from the average of duplicate data points obtained at each ligand concentration.

Nitroalkene−PPARγ Interactions: Activation of PPARγ-Dependent Transcription. Reporter gene analysis of ligand-dependent PPARγ transcription activation was accomplished as described previously.[7] Briefly, the studies used the MCF7/RTO/PPARG1-4′ cell line which expresses a doxycycline-repressible human PPARγ transgene. MCF7/RTO/PPARG1-4′ cells, grown in the absence of doxycycline, were plated in replicate 12-well tissue culture plates at a density of 4 × 10^4 cells/well. Twenty-fours hours later, cells were co-transfected with 0.4 μg of the PPRE-containing firefly luciferase reporter gene, pPPREx3-TK-LUC,[25] and 20 pg of control CMV-*Renilla* luciferase reporter gene, pGL4.75 (Promega, Madison, WI) using Superfect reagent (Qiagen, Valencia, CA) according to the manufacturer's recommendations. Twenty-four hours after the start of transfections, medium was replaced with medium containing vehicle (control) or varying concentrations of inducing

agent (nitroalkene fatty acids or rosiglitazone, Cayman, Ann Arbor, MI). Cells were harvested after 24 h of continuous exposure to vehicle or inducing agent. Luciferase assays were accomplished using the Dual-Luciferase Assay System (Promega, Madison, WI), and values were corrected for variations in transfection efficiencies and nonspecific induction as described previously.[26] All incubations, including transfections and inductions, were done in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum.

Cytotoxicity of Nitroalkene Fatty Acids. Cytotoxicity was determined using the sulforhodamine B microtiter plate assay as described.[27] Cells were plated at a density of 300 cells/well in 96-well plates. Twenty-four hours later, the cells were exposed to varying concentrations of nitroalkene fatty acids or vehicle control for 24 h in Dulbecco's modified Eagle medium containing 10% fetal calf serum. The medium was subsequently replaced, and the plates were incubated for 7 days, then fixed and stained.

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