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Design, synthesis, biological evaluation and docking studies of pterostilbene analogs inside PPARα

Cassia S. Mizuno,[a] Guoyi Ma,[b] Shabana Khan,[b] Akshay Patny,[c]
Mitchell A. Avery[c] and Agnes M. Rimando[a,*]

[a]USDA-ARS, Natural Products Utilization Research Unit, PO Box 8048, University, MS 38677, United States
[b]NCNPR, School of Pharmacy, University of Mississippi, University, MS 38677, United States
[c]Department of Medicinal Chemistry and Laboratory for Applied Drug Design and Synthesis, School of Pharmacy,
University of Mississippi, University, MS 38677, United States

Abstract—Pterostilbene, a naturally occurring analog of resveratrol, has previously shown PPARα activation in H4IIEC3 cells and was found to decrease cholesterol levels in animals. In this study, analogs of pterostilbene were synthesized and their ability to activate PPARα was investigated. Among analogs that was synthesized (*E*)-4-(3,5-dimethoxystyryl)phenyl dihydrogen phosphate showed activity higher than pterostilbene and control drug ciprofibrate. Docking of the stilbenes inside PPARα showed the presence of important hydrogen bond interactions for PPARα activation.
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1. Introduction

The stilbene scaffold is known to hold several biological activities considered beneficial for human health and for use in agriculture. The potential health benefits of this class of compounds have been extensively investigated in the past several years. Pharmacological properties of hydroxylated stilbenes include antioxidative,[1] anti-inflammatory,[2] antileukemic,[3] antibacterial,[4] antifungal,[5] antiplatelet aggregation,[6] vasodilator,[7] and antitumor[8] activities. Due to the variety of their beneficial effects, including chemopreventive[9] and chemoprotective[10] activities, this relatively structurally simple group of compounds has attracted attention as a novel potential therapeutic class. Resveratrol **1** [(*E*)-1-(4′-hydroxyphenyl)-2-(3,5-dihydroxyphenyl)ethene, Fig. 1], the most widely studied stilbene, is a naturally occurring polyphenol stilbenoid found in grapes,[11] peanuts, and berries.[12] This molecule is synthesized by plants in response to environmental stress and fungal infections and is known to be involved in defense mechanisms of plants.

Resveratrol has been extensively investigated because its occurrence in wine has been linked to lower risk of cardiovascular diseases among wine drinking population.[13,14] The antioxidant activity of resveratrol has been proven in a number of studies.[1,15–17] In addition to its antioxidant activity, resveratrol has also been demonstrated to reduce blood lipid levels in animals.[18] Pterostilbene **2**, a naturally occurring dimethylether analog of **1**, has shown in previous studies to lower plasma lipid levels when fed to hamsters.[19] Significant activation of PPARα was also observed with **2** in the same study, whereas none of the other naturally occurring stilbenes tested, including **1**, showed activation of PPARα.[19]

The peroxisome proliferator activated receptors (PPARs) are members of the nuclear receptor superfamily of ligand-activated transcription factors that play important role in carbohydrate and lipid metabolism. PPARα is expressed in the liver, heart, and muscles and is involved in the metabolism of fatty acids and lipids. PPARα is a known target for the treatment of dyslipidemia. Among the commonly used drugs for hypercholesterolemia (or hyperlipidemia), fibrates are known PPARα agonists. The triglyceride lowering and HDL-cholesterol increasing effects of fibrates are attributed to the activation of PPARα, which plays a role in lipoprotein catabolism.[20]

This study was undertaken to investigate the effect of chemical modification of pterostilbene on PPARα activation. We kept the methoxy groups at the 3 and 5 positions and introduced different groups at 4′ position. The docking of some of the natural and synthetic stilbenes into PPARα is also reported.

2. Chemistry

Pterostilbene derivatives **8a,b–10a,b** were synthesized through Wittig reaction according to Pettit et al.[21] (Scheme 1). Coupling of phosphonium salt **4**[21] and aromatic aldehydes **5**, **6**, and **7** gave a mixture of *cis* and *trans* isomers, which were isolated by column chromatography. The configurations of the molecules were determined by comparing their spectral data with those reported in the literature.[22] The nitro derivatives **8a** and **8b** were treated with sodium dithionite[22] to give compounds **11a** and **11b** (Scheme 2). Hydrolysis of esters **9a** and **9b** afforded acids **12a** and **12b** (Scheme 3). To study the role of the vinyl double bond of **2** in the activation of PPARα, reduction was accomplished with Pd/C in MeOH generating the saturated compound **13**. Phosphate derivative **15** was synthesized through reaction of **2** and dibenzylphosphite using DMAP, DIEA, followed by deprotection of the benzyl groups[21] (Scheme 4). Wittig reaction between aldehyde **17** and phosphonium salt **19**[21] afforded *cis* and *trans* isomers **21** and **22** (Scheme 5).
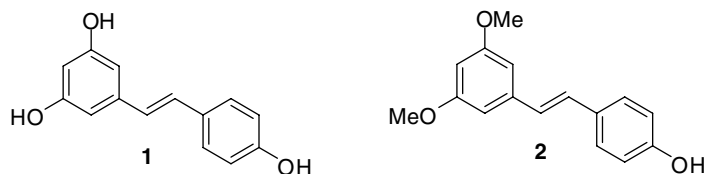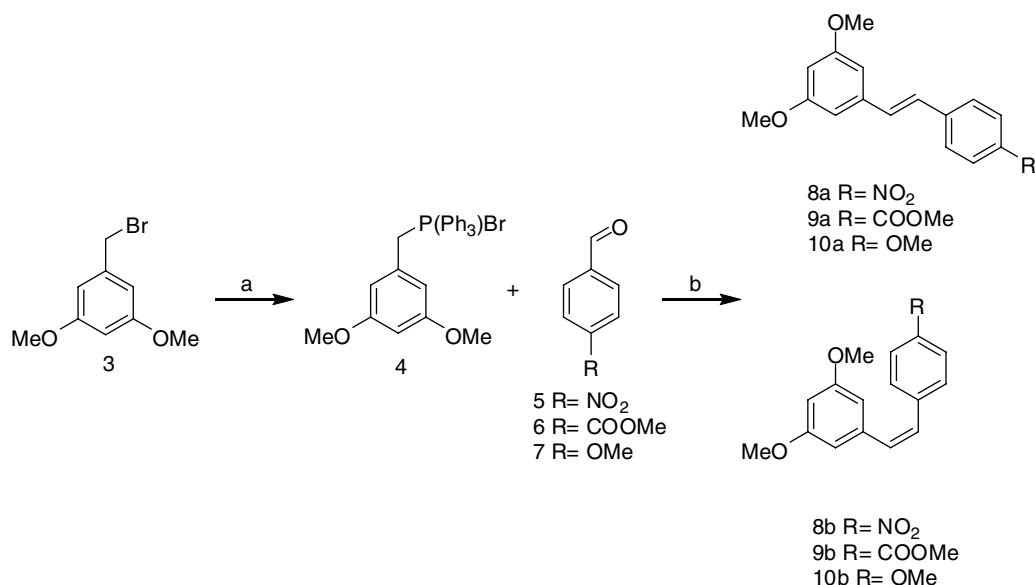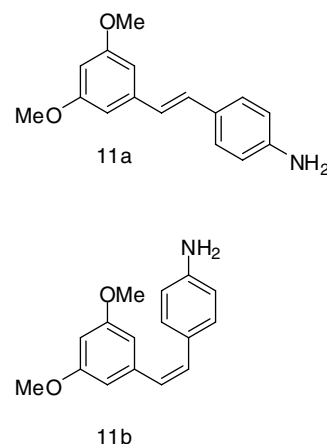

Scheme 2. Synthesis of **11a** and **11b**. Reagents and condition: (a) $Na_2S_2O_4$, acetone/H_2O, 50 °C.

3. Results and discussion

Based on previous observations of its significant PPARα activation, **2** was used as a lead compound for designing analogs with the aim of identifying molecules more active toward PPARα activation than the parent natural compound **2**.

The crystal structure of PPARα ligand-binding domain (LBD) (PDB code 1K7L) revealed that the ligand

Figure 1. Chemical structure of resveratrol **1** and pterostilbene **2**.

Scheme 1. Synthesis of **8a,b–10a,b**. Reagents and conditions: (a) PPh_3, toluene, reflux, overnight; (b) *n*-BuLi, THF, −78 °C → rt, overnight.
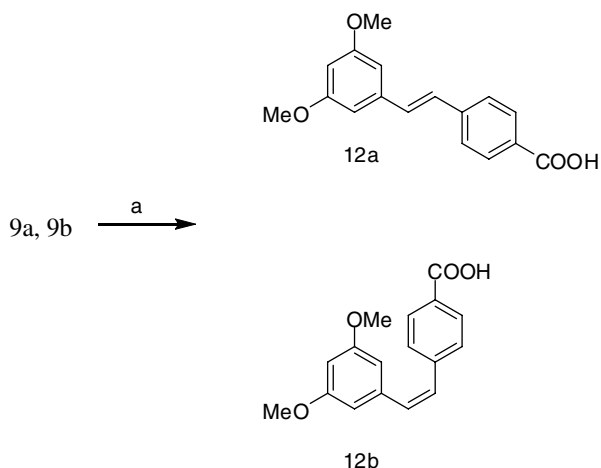
9a, 9b —a→

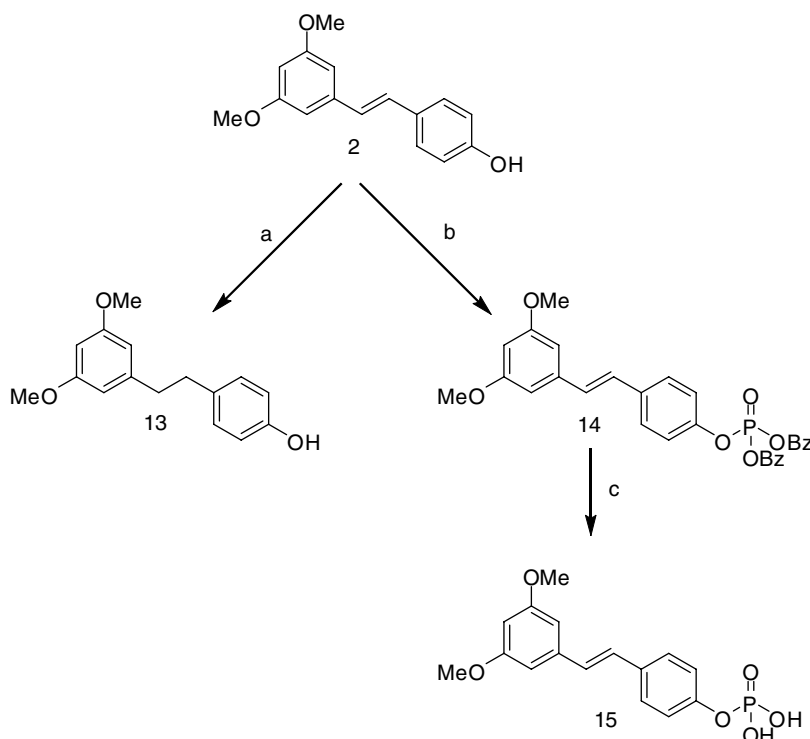**Scheme 3.** Synthesis of **12a** and **12b**. Reagents and condition: (a) NaOH, MeOH, reflux, 4 days.

GW409544 (Fig. 2) makes several hydrogen bond interactions with Ser280, Tyr314, Tyr464, and His440 that are known to be the key interactions for PPARα agonists.[23] GW409544 is a L-tyrosine analog of PPARγ agonist farglitazar (Fig. 2) and its synthesis was first described in 2000 by Collins et al.[24] for treatment of diabetes and cardiovascular diseases. This molecule is a full PPARα (EC$_{50}$ = 2.3 nM) and PPARγ (EC$_{50}$ = 0.28 nM) agonist.[23] GW409544 adopts a conformation where the acidic head group of the ligand functions as hydrogen bond donor and interacts with Tyr314 on helix 5 and Tyr464 on the AF-2 helix. These interactions stabilize the receptor in a conformation that leads to the tran-

scriptional activation of the receptor via recruitment of co-activator proteins.

In addition, the tyrosine moiety of GW409544 is oriented toward the hydrophobic pocket formed by helices 3, 6, and 10, adjacent to the C-terminal AF-2 helix.[23] Based on these observations we decided to introduce acidic and polar groups at the 4′ position of **2**. These polar groups are anticipated to form hydrogen bond and interact with the critical polar amino acid residues on the AF-2 helix such as Tyr464 considered important for PPARα activation.

Analogs (**8–22**) were evaluated for their activity as PPARα agonist in H4IIEC3 cells at concentrations ranging from 0.1 μM to 100 μM. Their activities were compared with ciprofibrate (Fig. 3).

The nitro compounds (**8a** and **8b**) and the amine compounds (**11a** and **11b**) did not exhibit a significant activation of PPARα. Among the *cis* isomers, compound **10b** had significant activity as PPARα agonists (>2-fold activation at 1–100 μM). Introduction of an ester group (**9a**) also resulted in increase in activity (>2-fold activation at 10–100 μM). Interestingly, reduction of the vinyl double bond in **2** resulted in loss of its activity as represented by **13**, indicating that the double bond is essential for PPARα activation for stilbenes. Saturation of the double bond causes conformation changes of the molecule resulting in loss of its activity. The most active compound was the phosphate derivative **15**, which exhibited a sixfold induction of PPARα at the highest concentration tested (100 μM) with twofold or more induction at
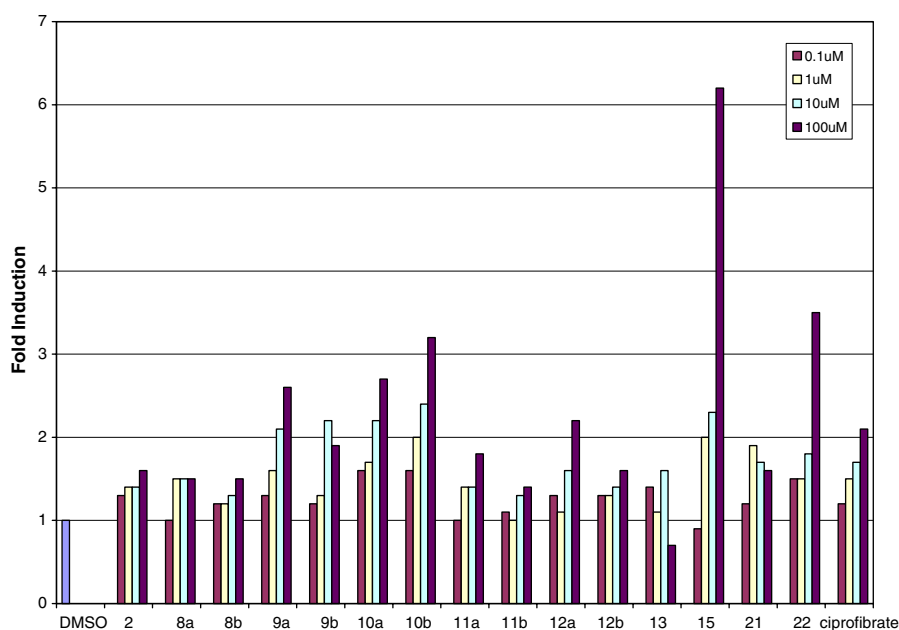

Scheme 4. Synthesis of **13** and **15**. Reagents and conditions: (a) Pd/C MeOH, overnight; (b) DMAP, DIEA, CH$_3$CN, dibenzyl phosphite, CCl$_4$, −10 °C → rt, 12 h; (c) BrSi(CH)$_3$, DCM 0 °C → rt, 2 h.
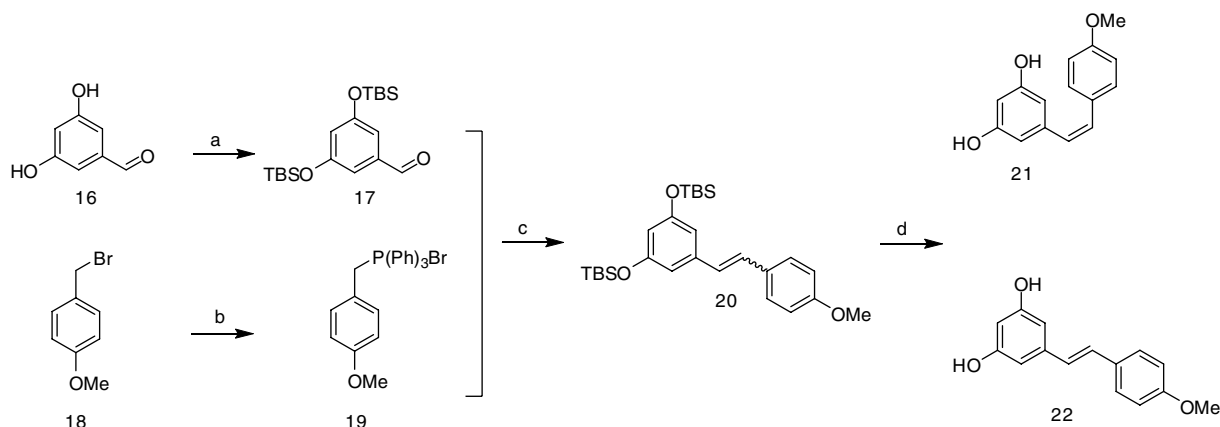
Scheme 5. Synthesis of **21** and **22**. Reagents and conditions: (a) TBDMSCl, DIEA, DMF, 18 h, rt, 60%; (b) PPh₃, toluene, reflux, overnight; (c) *n*-BuLi, THF, 15 °C → rt, 12 h; (d) TBAF, THF, 15 min.
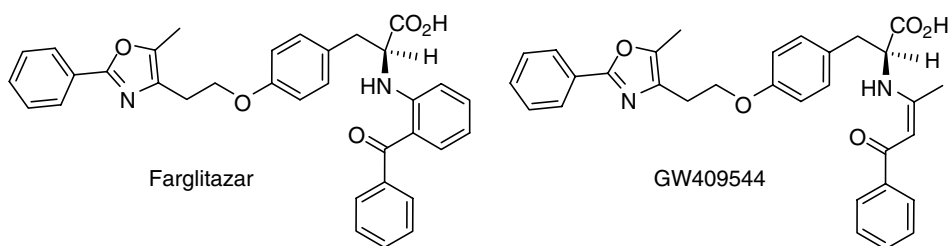
Figure 2. Chemical structure of Farglitazar and GW409544.

Figure 3. Effects of stilbenes on PPARα activation in a cell-based assay at different concentrations.

the lower concentrations. This observation shows that introduction of an acidic group leads to a compound more active than **2**. It is possible that phosphate **15** may serve as a prodrug with improved cell permeability that leads to the observed increase in its activity. In general, compounds **9a**, **10a**, and **10b** were also more effective than ciprofibrate in their ability to activate PPARα.

Considering that compounds **8–12** and **15** all have methoxy groups at 3 and 5 positions, the different groups at 4′ position determine the activity. Thus methoxy, ester, and phosphate are preferred. In the case of compounds with hydroxyl groups at 3 and 5 positions compound **21** did not show significant activation of PPARα at any concentration tested. Compound **22** showed activation

Figure 4. Proposed binding pose of *cis* isomer **21** (colored by atom type) inside PPARα ligand-binding domain. The protein backbone is shown as green ribbon. Important residues are shown in yellow.

of PPARα (>2-fold) only at the highest concentration indicating that methoxy groups at 3 and 5 positions are important for activity. Among the active compounds **9a**, **10a**, **10b**, and **15**, compound **10b** was the only *cis* isomer suggesting that the configuration *trans* is more favorable for activating PPARα.

To investigate in detail these molecules with different scaffolds from the known PPARα agonists, the stilbenes were docked into the PPARα ligand-binding domain.[23] Docking studies revealed that the inactive *cis* isomers were mostly docked in the hydrophobic pocket formed by helices 2′, 3, and β sheet and did not interact with polar amino acids located on the left most part of the T-shaped cavity known to be important for activation of the receptor (Fig. 4). This lack of polar interactions can possibly explain the lack of activity of the *cis* isomers. Compound **10b** was the only *cis* isomer that showed activity but the docking results are not consistent with the observed activity of this molecule in particular, as it was docked in very similar manner as the other inactive *cis* isomers. However, although active, **10b** showed only moderate activity and it may bind to the receptor in different mode.

In general, docking of the *trans* isomers revealed very similar interactions to **2** as shown in Figure 5. The two methoxy groups at 3 and 5 positions of **2** make hydrogen bond interactions with Ser280 and Tyr464 which were considered as key interactions for activation of the receptor. The remaining part of the molecule fitted very well into the hydrophobic pocket formed by helices 3, 6, and 10 adjacent to the AF-2 helix, also known as 'benzophenone' pocket.[25] Unlike GW409544 that forms favorable hydrophobic interactions with Phe351, the hydroxy group on the 4′ position of **2** displayed a hydro-

gen bond interaction with this amino acid residue (Fig. 5).

The phosphate derivative **15** was the most active compound of this series. Docking of this molecule showed that the phosphate group forms hydrogen bonds and interacts with Ser280 and His440, with the two phenyl rings docked into the hydrophobic pocket formed by helices 2′, 3, and β sheet (Fig. 6). As the compounds presented similar interactions with amino acids in the PPARα active site and the activities among the compounds were close, docking in this particular case could not explain the difference in activity between analogs but shall shed light on the structural basis for structure–activity relationship of stilbenes in general.

4. Conclusion

The stilbene comprises a unique template as PPARα agonist. Here we have described the synthesis of 14 stilbenes and their activities as PPARα agonists. Phosphate **15** was discovered as PPARα agonist with potency higher than ciprofibrate. In general, the *trans* analogs were more active than the *cis* isomers. Docking studies revealed that the *trans* isomers interact with amino acids residues essential for activation of the receptor while the *cis* isomers do not. Based on previous results with **2**, we succeeded to design a stilbene (**15**) which is more active as PPARα agonist.

5. Experimental

All solvents were redistilled prior to use. All reactions occurred under inert atmosphere. All round bottom

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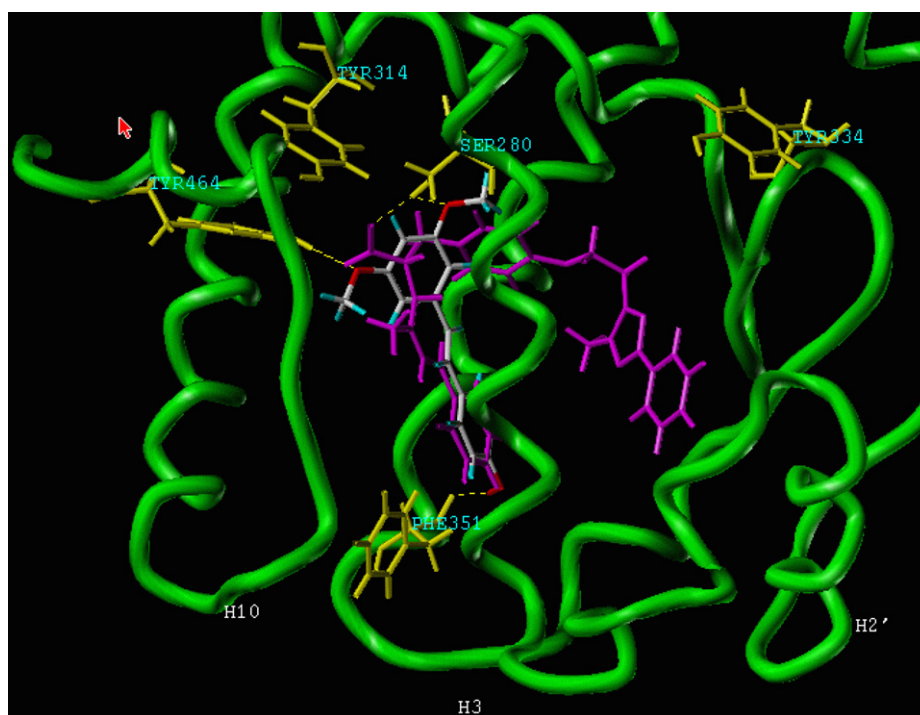
Figure 5. Proposed binding pose of **2** (colored by atom type) superimposed on the cocrystal structure of GW409544 (magenta) inside PPARα ligand-binding domain. The protein backbone is shown as green ribbon. Important residues are shown in yellow and hydrogen bond interactions are shown as yellow dotted line.
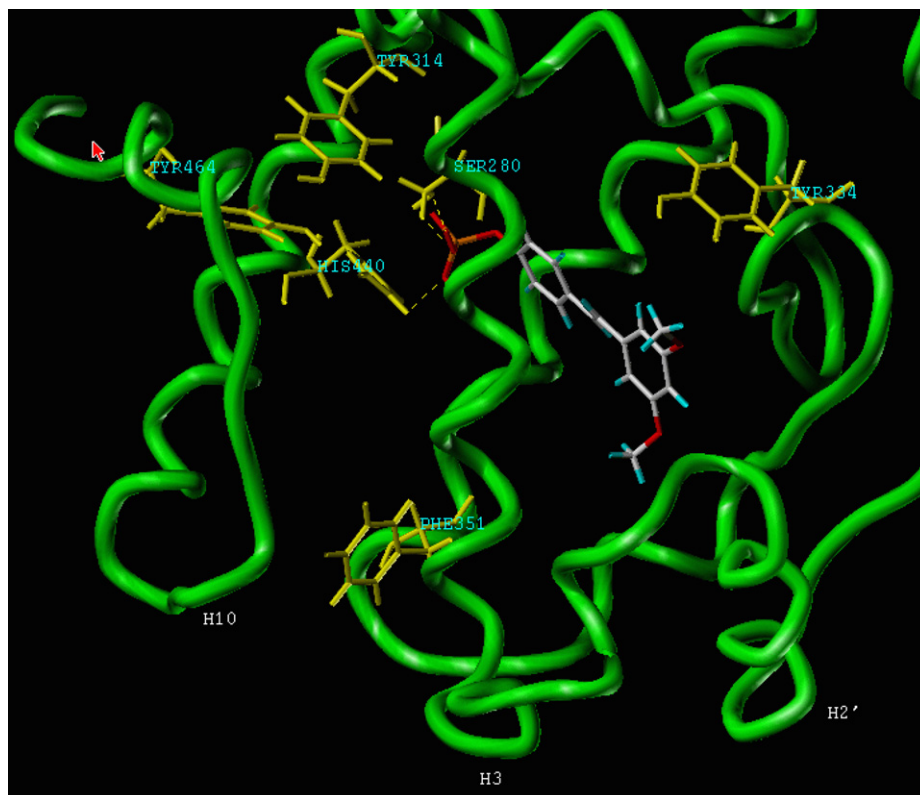
Figure 6. Proposed binding pose of **15** (colored by atom type) inside PPARα ligand-binding domain. The protein backbone is shown as green ribbon. Important residues are shown in yellow and hydrogen bond interactions are shown as yellow dotted line.

flasks were kept inside the oven or dried under vacuum and heated prior to use. Reactions were monitored by thin layer chromatography (TLC) using silica gel (Kieselgel 60 F$_{254}$, Merck). NMR data were recorded on a Bruker 400 MHz 50 MM, Bruker 400 MHz Ultra Shield or Varian AS400 and the analyses conducted in CDCl$_3$,

DMSO-d_6, or MeOD. Purification was performed using Automated Flash Purification (Biotage Horizon) and flash chromatography using silica gel (Sorbent Technologies 40–60 μM). Mass spectra were collected using a JEOL AccuTOF JMS-T100LC.

5.1. General procedure for stilbenes synthesis

To a cold solution ($-78\ °C$) of phosphonium salt (1.0 equiv) in THF was added *n*-butyllithium (1.6 mol in hexanes, 1.0 equiv) and the resulting solution was stirred under inert atmosphere for 2 h. A solution of aldehyde (1.0 equiv) in THF was added dropwise, and the mixture was stirred for 12 h at room temperature. The resulting suspension was poured into water and extracted with dichloromethane. The organic phase was combined and dried over MgSO$_4$ and concentrated under reduced pressure. The crude product was purified through automated flash purification eluting with hexanes/ethyl acetate (97:3). The *cis* isomer was eluted first followed by the *trans* isomer.

5.1.1. 1,3-Dimethoxy-5-(4-nitrostyryl)benzene (8a and 8b).
Reaction of (3,5-dimethoxybenzyl)triphenylphosphonium **4** (300 mg, 0.608 mmol) and 4-nitrobenzaldehyde (92 mg, 0.608 mmol) **3** afforded **8b** and **8a**. Compound **8a** as a yellow solid: 71 mg (41%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.83 (s, 6H); 6.45 (s, 1H); 6.68 (s, 2H); 7.07–7.16 (m, 1H); 7.23 (d, 1H, $J = 36$ Hz); 7.61 (d, 2H, $J = 8$ Hz); 8.20 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 55.4 (2C), 100.9, 105.0 (2C), 124.1 (2C), 126.7, 126.9 (2C), 133.2, 138.1, 143.6, 146.7, 161.0 (2C). HRMS: Calcd for [2M+Na] C$_{32}$H$_{30}$N$_2$O$_8$Na 593.18998, found 593.18332. Compound **8b** 56 mg (32% yield) ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.66 (s, 6H); 6.34–6.35 (m, 3H); 6.58 (d, 1H, $J = 12$ Hz); 6.74 (d, 1H, $J = 16$ Hz); 7.39 (d, 2H, $J = 8$ Hz); 8.07 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 55.4 (2C), 100.3, 106.9 (2C), 123.6 (2C), 128.5, 129.9 (2C), 134.1, 138.2, 144.2, 146.7, 161.0 (2C). HRMS: Calcd for [2M+Na] C$_{32}$H$_{30}$N$_2$O$_8$ Na 593.18998, found 593.18987.

5.1.2. Methyl 4-(3,5-dimethoxystyryl)benzoate 9a and 9b.
Reaction of (3,5-dimethoxybenzyl)triphenylphosphonium **4** (300 mg, 0.608 mmol) and methyl 4-formylbenzoate **6** (99 mg, 0.608 mmol) afforded **9b** and **9a**. Compound **9a** as a white solid: 50.2 mg (27%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.83 (s, 6H); 3.92 (s, 3H); 6.42 (s, 1H); 6.68 (s, 2H); 7.07–7.16 (m, 2H); 7.55 (d, 2H, $J = 8$ Hz); 8.02 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 52.3, 55.5 (2C), 100.7, 105.0 (2C), 126.6 (2C), 128.2, 129.1, 130.2 (2C), 131.4, 138.9, 141.8, 161.2 (2C), 167. HRMS: Calcd for [2M+Na] C$_{36}$H$_{36}$NaO$_8$ 619.23079, found 619.22594. Compound **9b** as a viscous liquid: 95 mg (52%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.63 (s, 6H); 3.88 (s, 3H); 6.33 (9s, 1H); 6.36 (s, 2H); 6.57 (dd, 2H, $J_{1,2} = 12$ Hz, $J_{1,3} = 8$ Hz); 7.32 (d, 2H, $J = 8$ Hz); 7.90 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 52.0, 55.1 (2C), 100.0, 106.7 (2C), 128.6, 128.9 (2C), 129.4 (2C), 129.5, 132.1, 138.4, 142.0, 160.6 (2C), 166.8. HRMS: Calcd for [2M+Na] C$_{36}$H$_{36}$NaO$_8$ 619.23079, found 619.22729.

5.1.3. 1,3-Dimethoxy-5-(4-methoxystyryl)benzene 10a and 10b.
Reaction of (3,5-dimethoxybenzyl)triphenylphosphonium **4** (300 mg, 0.608 mmol) and 4-methoxybenzaldehyde **7** (75 μL, 0.608 mmol) **10b** and **10a**. Compound **10a** as a white solid: 15 mg (9%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.83 (s, 9H); 6.39 (s, 1H); 6.67 (s, 2H); 6.89 (s, 2H); 6.92 (d, 1H, $J = 8$ Hz); 7.02 (d, 1H, $J = 8$ Hz); 7.45 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 55.3 (3C), 99.6, 104.3 (2C), 114.1 (2C), 126.5, 127.8 (2C), 128.7, 129.9, 139.7, 159.4, 161.0 (2C). HRMS: Calcd for [2M+Na] C$_{34}$H$_{36}$NaO$_6$ 563.24096, found 563.24419. Compound **10b** as a viscous liquid: 58 mg (35%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.69 (s, 6H); 3.78 (s, 3H); 6.37 (s, 1H); 6.46–6.49 (m, 3H); 6.55 (d, 1H, $J = 12$ Hz); 6.80 (d, 2H, $J = 8$ Hz); 7.26 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 55.1 (2C), 99.6, 106.6 (2C), 113.5 (2C), 128.7, 129.5, 130.2 (2C), 130.3 (2C), 139.5, 158.8, 160.6 (2C). HRMS: Calcd for [2M+Na] C$_{34}$H$_{36}$NaO$_6$ 563.24096, found 563.24396.

5.1.4. 5-(4-Methoxystyryl)benzene-1,3-diol 21 and 22.
Reaction of 3,5-bis(*tert*-butyldimethylsilyloxy)benzaldehyde **17** (408 mg, 1.11 mmol) and (4-methoxybenzyl)triphenylphosphonium bromide **19** (500 mg, 1.11 mmol) afforded 452 mg (86% yield) of mixture of *cis* and *trans* stilbenes **20**. Due to difficulties found in separating the two isomers, deprotection of TBS group was followed without isolation of the isomers. Tetrabutylammonium fluoride (2.5 mL, 2.5 mmol) was added to a mixture of (*E*) and (*Z*)-(5-(4-methoxystyryl)-1,3-phenylene)bis(oxy)bis(*tert*-butyldimethylsilane) **20** (452 mg, 0.96 mmol) in anhydrous tetrahydrofuran (10 mL). The solution was stirred for 45 min, poured into water, and extracted with ether. After removal of the solid, the resulting crude mixture was purified using flash chromatography (7:3 hexanes/ethyl acetate) and afforded *cis* (**21**) and *trans* (**22**). Compound **22** 23 mg (8%). ^{1}H NMR (MeOD, 400 MHz): δ 3.73 (s, 3 H); 6.19 (s, 1H); 6.47 (s, 2H); 6.79–6.85 (m, 3H); 6.96 (d, 1H, $J = 16$ Hz); 7.38 (d, 2H, $J = 12$ Hz). ^{13}C NMR (DMSO, 400 MHz): δ 58.5, 105.6, 108.7 (2C), 117.9 (2C), 130.5, 131.5 (2C), 131.9, 134.2, 144.0, 162.4 (2C), 163.5. HRMS: Calcd for [M−H] C$_{15}$H$_{13}$O$_3$ 241.08647, found 241.08863. Compound **21** 57 mg (22%). ^{1}H NMR (MeOD, 400 MHz): δ 3.70 (s, 3H); 6.18 (s, 1H); 6.32 (s, 2H); 6.33 (d, 1H, $J = 12$); 6.47 (d, 1H, $J = 12$); 6.73 (d, 2H, $J = 8$ Hz); 7.18 (d, 2H, $J = 8$ Hz). ^{13}C NMR (DMSO, 400 MHz): δ 58.4, 105.2, 111.1, 117.3 (2C), 132.5, 133.5, 133.7 (2C), 134.1 (2C), 143.9, 162 (2C), 162.9. HRMS: Calcd for [M−H] C$_{15}$H$_{13}$O$_3$ 241.08647, found 241.08735.

5.1.5. General procedure for the reduction of nitro derivatives 8a and 8b.
A solution of *trans* nitro derivative **8a** (45 mg, 0.15 mmol) in acetone/water (10:5 mL) was heated to 50 °C for 30 min. Sodium dithionite (686 mg, 3.75 mmol) was slowly added and the mixture was heated to reflux for 1 h. After cooled to room temperature the mixture was poured into water and extracted with ethyl acetate. The organic phase was combined and dried over MgSO$_4$ and solvent was removed under reduced pressure. The crude mixture was purified using automated flash chromatography eluting with hexanes/

ethyl acetate (75:25). Compound **11a** as a yellow solid: 14.7 mg (36%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.82 (s, 6H); 6.37 (s, 1 H); 6.65–6.68 (m, 4H); 6.86 (d, 1H, $J = 16$ Hz); 7.01 (d, 1H, $J = 16$ Hz); 7.33 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 55.5 (2C), 99.6, 104.4 (2C), 115.5 (2C), 125.2, 128, 128.1 (2C), 129.5, 140.2, 146.4, 161.2 (2C). HRMS: Calcd for [M+H] C$_{16}$H$_{18}$NO$_2$ 256.13375, found 256.14321. Compound **11b** as a yellow solid (47%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.68 (s, 6H); 6.32 (s, 1H); 6.39 (s, 1H); 6.47 (m, 3H); 6.54 (d, 2H, $J = 8$ Hz); 7.11 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 55.4 (2C), 99.7, 106.8 (2C), 114.8 (2C), 127.6, 127.7, 130.4 (2C), 130.8, 140.1, 145.7, 160.7 (2C). HRMS: Calcd for [M+H] C$_{16}$H$_{18}$NO$_2$ 256.13375, found 256.10757.

5.1.6. General procedure for hydrolysis of esters 9a and 9b. To a solution of **9a** (50 mg, 0.16 mmol) in THF was added NaOH solution (1 N, 335 μL). The reaction was left stirring at reflux for 3 days and NaOH was added from time to time until starting material disappeared. The mixture was poured into water, the pH was brought to neutral and the mixture was extracted with ethyl acetate. The organic phase was combined and dried over MgSO$_4$ and concentrated. The crude material was purified using automated flash chromatography eluting with chloroform/methanol (9:1). Compound **12a** as a white solid: 19 mg (44%). ^{1}H NMR (DMSO, 400 MHz): δ 3.74 (s, 6 H); 6.41 (s, 1H); 6.78 (s, 2H); 7.29 (d, 2H, $J = 4$ Hz); 7.67 (d, 2H, $J = 8$ Hz); 7.91 (d, 2H, $J = 8$ Hz). ^{13}C NMR (DMSO, 400 MHz): δ 55.9 (2C), 101, 105.4 (2C), 127.8 (2C), 128.6, 130.2, 130.4 (2C), 131.6, 139.3, 142, 161.3 (2C), 167.8 HRMS: Calcd for [M−H]. C$_{17}$H$_{15}$O$_4$ 283.09703, found 283.09726. Compound **12b** as a white solid (24%). ^{1}H NMR (DMSO, 400 MHz): δ 3.56 (s, 6H); 6.31–6.34 (m, 3H); 6.64 (s, 2H); 7.30 (d, 2H, $J = 8$ Hz); 7.80 (d, 2H, $J = 8$ Hz). ^{13}C NMR (DMSO, 400 MHz): δ 55.4 (2C), 100, 107 (2C), 129.1 (2C), 129.7 (3C), 130.3, 132, 138.5, 141.7, 160.7 (2C), 167.8. HRMS: Calcd for [M−H] C$_{17}$H$_{15}$O$_4$ 283.09703, found 283.09681.

5.1.7. 4-(3,5-Dimethoxyphenethyl)phenol 13. Pd/C (catalytic) was added to a solution of pterostilbene **2** (50 mg, 0.195 mmol) in methanol. The mixture was left stirring overnight under H$_2$ at room temperature. The mixture was filtered and the solvent was evaporated under reduced pressure. The crude mixture was purified using flash chromatography eluting with hexanes/ethyl acetate (7:3) to afford 49 mg (98%) of 4-(3,5-dimethoxyphenethyl)phenol **13**. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 2.84 (s, 4H); 3.78 (s, 6H); 6.34–6.35 (m, 4H); 6.76 (d, 2H, $J = 8$ Hz); 7.05 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 36.7, 38.4, 55.2 (2C), 97.9, 106.6 (2C), 115.1 (2C), 129.5 (2C), 133.8, 144.2, 153.7, 160.6 (2C). HRMS: Calcd for [M−H] C$_{16}$H$_{17}$O$_3$ 257.11777, found 257.11889.

5.1.8. (E)-Dibenzyl 4-(3,5-dimethoxystyryl)phenyl phosphate 14. To a cold mixture (−10 °C) of **2** (200 mg, 0.67 mmol) and *N*,*N*-(dimethylamino)pyridine (10.2 mg, 0.083 mmol) in anhydrous acetonitrile (10 mL) was added carbon tetrachloride (323 μL, 3.35 mmol) and

DIEA (245 μL, 1.4 mmol). The mixture was left stirring at −10 °C for 30 min and dibenzyl phosphate (224 μL, 1 mmol) was added. The solution was stirred for 12 h at room temperature and poured into 0.5 M monobasic potassium phosphate solution. The mixture was extracted with ethyl acetate and the organic phase was combined and dried over MgSO$_4$. The solvent was evaporated, the crude mixture was purified by column chromatography eluting with hexanes/ethyl acetate (7:3) and gave 360 mg (80%) of (E)-dibenzyl 4-(3,5-dimethoxystyryl)phenyl phosphate **14**. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.83 (s, 6 H); 5.13 (d, 4H, $J = 8$ Hz); 6.41 (s, 1H); 6.67 (s, 2H); (dd, 2H, $J_{1,2} = 16$ Hz, $J_{1,3} = 33$ Hz); 7.15 (d, 2H, $J = 8$ Hz); 7.34 (s, 10H); 7.43 (d, 2H, $J = 8$ Hz). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 55.6 (2C), 70.2 (2C), 100.3, 104.8 (2C), 120.5 (2C), 128 (2C), 128.2 (4C), 128.8 (4C), 128.9 (2C), 129, 134.5, 135.6, 135.7, 139.4, 150.2, 150.3, 161.2 (2C). HRMS: Calcd for [M+H] C$_{30}$H$_{30}$O$_6$P 517.17800, found 517.17444.

5.1.9. (E)-4-(3,5-dimethoxystyryl)phenyl dihydrogen phosphate 15. Bromotrimethylsilane (80 μL, 0.61 mmol) was added to a solution of dibenzyl phosphate **14** (150 mg, 0.29 mmol) in anhydrous dichloromethane (10 mL) at 0 °C. After 2 h stirring at room temperature, water was added and the solution was stirred for 1 h. The mixture was extracted with ethyl acetate and the organic phase was combined and dried over MgSO$_4$. The solvent was removed to afford **15**, 38.6 mg (39%). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.63 (s, 6H); 6.23 (s, 1H); 6.40 (s, 2H); 6.60 (d, 1H, $J = 16$ Hz); 6.71 (d, 1H, $J = 16$ Hz); 7.02–7.14 (m, 4H); 9.11 (br s, 2H). ^{13}C NMR (CDCl$_3$, 400 MHz): δ 58.6 (2C), 103.5, 108.2 (2C), 124.4, 131.4 (3C), 131.8, 132.1, 137.2, 143.6, 156, 165.1 (2C). HRMS: Calcd for [M−H] C$_{16}$H$_{17}$O$_6$P 335.06845, found 335.07300.

5.1.10. 3,5-Bis(*tert*-butyldimethylsilyloxy)benzaldehyde 17. To a solution of 3,5 dihydroxybenzaldehyde **16** (500 mg, 3.62 mmol) in DMF (10 mL) was added DIEA (126 μL, 7.24 mmol). The solution was stirred for 15 min when TBDMSCl was added dissolved in DMF. After stirring for 18 h, the mixture was poured into water and extracted with ethyl acetate. The organic phase was combined and dried over MgSO$_4$ and the solvent removed under reduced pressure. The crude mixture was purified using column chromatography eluting with hexanes/ethyl acetate (95:5) and afforded 800 mg, (60%) of 3,5-bis(*tert*-butyldimethylsilyloxy)benzaldehyde **17**. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 0.21 (s, 12H); 0.98 (s, 18H); 6.59 (s, 1H); 6.94 (d, 2H, $J = 4$ Hz); 9.85 (s, 1H). ^{13}C NMR (CDCl$_3$, 400 MHz): δ −4.20 (4C), 18.4 (2C), 25.8 (6C), 114.6 (2C), 118.6, 138.5, 157.4, 192 (2C). IR (neat) 2956, 2931, 2887, 2859, 1704 cm^{-1}.

5.2. Molecular docking

The compounds were sketched and converted into three-dimensional structures using the program Sybyl. Minimization was carried out for these compounds, which employed 1000 steps each of steepest descents followed by conjugate gradients and finally BFGS method up to a gradient of 0.01 kcal/mol/Å. Docking of pterostil-

bene analogs was performed in the PPARα ligand-binding domain (PDB code: 1K7L). Protein preparation was done using the program Maestro, which involved addition of hydrogen atoms, minimization of the protein and assigning of formal charges to the amino acid residues. The X-ray crystal bound pose of GW409544 was used to define binding site during protein preparation. Docking of the compounds was performed using the program GOLD. The binding site for docking was defined using the sulfur atom of Cys276 and all amino acid residues within 12 Å were defined as part of the binding cavity. The 'standard mode' settings were used and no artificial restraints were defined during docking. The 'early termination' criterion was set off. For each compound, a total of 25 different docking poses were collected. Gold scoring function was used to identify docking poses as well as to rank these poses. The various docking poses of each compound were visually inspected for anticipated ligand–receptor interactions and unreasonable conformation.

5.3. Cell-based reporter gene assay for PPARα in rat liver cells

Rat hepatoma cells (H4IIEC3) were cultured in DMEM/ F12 medium supplemented with FBS (10%) and antibiotics (penicillin G sodium 100 U/mL and streptomycin 100 μg/mL) at 37 °C in an atmosphere of 95% humidity and 5% CO_2. At about 75% confluency, cells were harvested by trypsinization and transfected with firefly Luc reporter gene construct containing PPRE-AB (peroxisome proliferator response element with rat fatty acyl CoA β-oxidase AB promoter region sequence). For transfection, 25 μg of DNA was added to 500 μL cell suspension (5×10^6 cells) and incubated for 5 min at room temperature in BTX disposable cuvettes (4 mm). The cells were electroporated at 190 V and a single 70 ms pulse in a BTX Electro Square Porator T 820 (BTX I, San Diego, CA). Transfected cells were plated in 96-well plate at a density of 5×10^4 cells/well and grown for 24 h. The cells were treated with different concentrations of test compounds for 24 h followed by addition of 40 μL 1:1 mixture of Luc-Lite reagent and PBS containing 1 mM calcium and magnesium. Luciferase activity was determined in terms of Light output measured on a TopCount microplate reader (Packard Instrument Co. Meriden, CT) in a single photon counting mode.

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