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Enantioselective Synthesis of a Novel Thiazoline Core as a Potent Peroxisome Proliferator-Activated Receptor δ Agonist

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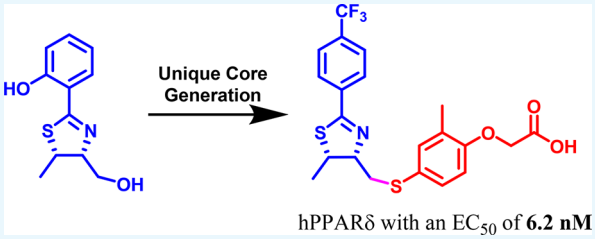
Supporting Information

ABSTRACT: The convergent and enantioselective synthesis of a highly potent human peroxisome proliferator-activated receptor delta agonist is presented. More specifically, the thiazoline structure, which constitutes the biosynthetically distinctive core structure of pulicatin (a secondary metabolite of symbiotic bacteria), was synthesized from a commercially available and inexpensive chiral pool of L-threonine.



1. INTRODUCTION

Pulicatin A is a secondary metabolite that was recently isolated from the cone snail-associated symbiotic bacteria, *Streptomyces* sp. CP32, and possesses a relatively simple but unique stereogenic 5-methyl-arylthiazoline core (see Figure 1).[1] Two similar core structures, namely the aryldihydrothiazole and the arylthiazole cores, are also present within the pulicatin series and are of particular importance in the discovery of novel bioactive scaffolds, as the symbiotic *Streptomyces* sp. CP32 isolated from *Conus pulicarius* biosynthesizes small-molecule allelochemicals as a defensive mechanism in marine bioenvironments.[2−4]

In addition, a further class of arylthiazole derivatives, namely the anithiactins (Figure 1), was identified from a different marine source, and the total syntheses of anithiactins A−C were investigated as part of the ongoing research into the discovery of novel secondary metabolites from the marine-derived *Streptomyces* sp.[5,6]

In the context of drug targets, the nuclear receptor peroxisome proliferator-activated receptor delta (PPARδ) has recently received attention as a re-emerging target for the treatment of diseases, such as metabolic syndromes,[7−12] as its activation alters glucose and lipid metabolism through transcriptional regulation and results in beneficial pharmacological effects.[13,14] For example, Endurobol (GW501516, **1**, Figure 1) is a well-known effective and potent PPARδ agonist, which is currently in late-phase clinical trials.[15−18] Because of its highly selective and potent activity compared to other PPARδ agonists, the development of novel synthetic routes toward target **1** and its analogs has received growing attention to allow its use in both structural and biological studies.[19−23] Indeed, our group previously reported one of the shortest synthetic routes toward compound **1** and generated a series of different analogs with the aim of discovering novel agonists.[20]

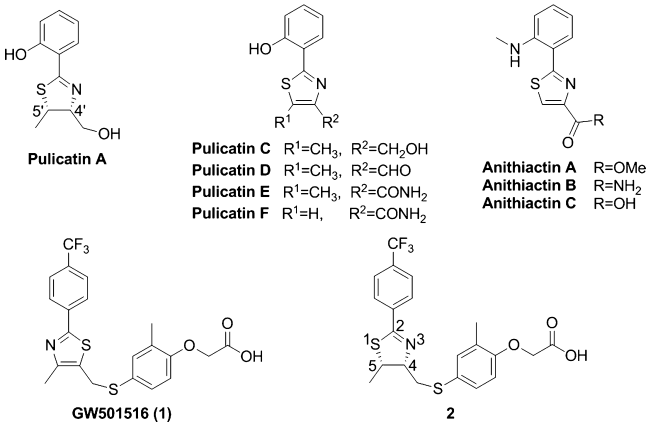


Figure 1. Structures of pulicatins A, C, D, E, and F, anithiactins A−C, and PPAR δ agonists **1** and **2**.

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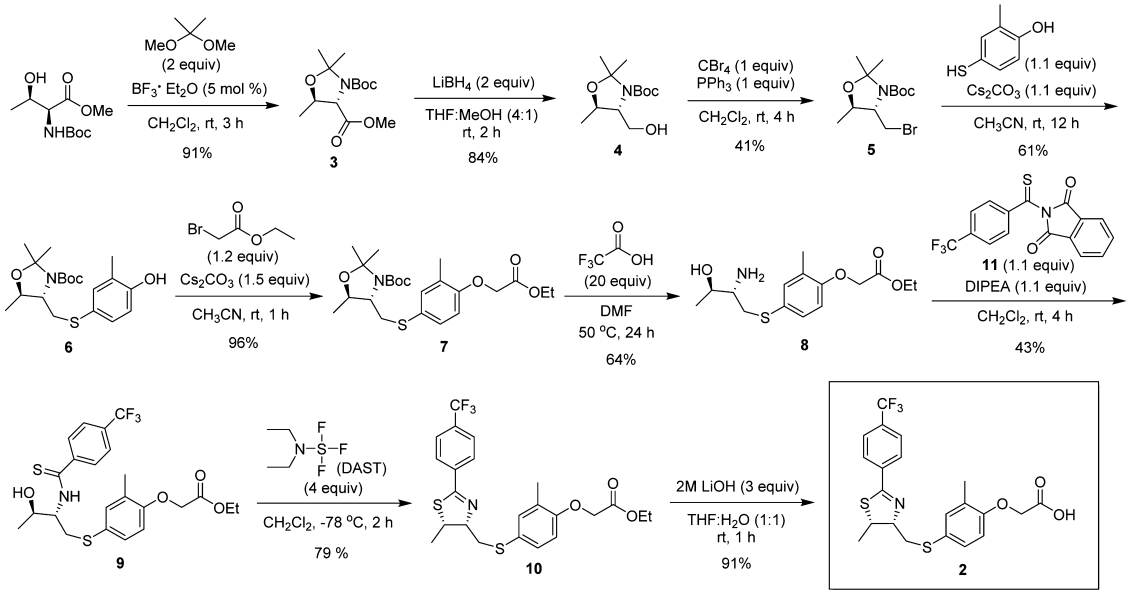
Scheme 1. Preparation of 2



2. RESULTS AND DISCUSSION

During the course of our studies into the investigation of marine-derived natural products[24] and synthetic agonists toward PPARδ, where we aimed to develop safer and more effective drug leads, we discovered that the bioactive natural products pulicatins C−F and anithiactins A−C (see Figure 1) contain the same phenylthiazole core structure as PPARδ agonist **1**.

We also found that pulicatin A contains a biosynthetically distinctive thiazoline structure, which is essentially the enantiospecifically reduced form of the 5′-methylthiazole moiety. Thus, inspired by the fact that these thiazoline and thiazole analogs are produced from the same biological system, we designed a novel scaffold consisting of the stereogenic 4,5-dihydrothiazole core. More specifically, during our investigations into novel anti-obesity drugs, we synthesized **2** based on the structure of **1**.

Previously, the synthesis of a series of optically pure 2-aryl-4,5-dihydrothiazole analogs without the 5-methyl substituent was carried out using aryl nitriles and methyl cysteine as the starting materials, and the antibacterial activities of these compounds were examined.[25] However, to date, neither the enantiospecific synthesis of the 2-aryl-5-methyl-4,5-dihydrothiazole core nor the extended scaffold **2** has been explored.

Thus, we herein present the first enantiospecific synthesis of the highly potent PPARδ agonist **2** containing the novel thiazoline scaffold, in addition to the preliminary in vitro pharmacological studies of this compound toward PPAR subtypes.

From a synthetic point of view, the main challenge in the preparation of **2** involves the construction of the two thiazoline stereocenters. We therefore began the synthesis of **2** from L-threonine (Scheme 1), a readily available and cheap amino acid. Thus, one of the two stereocenters in the final product originates from L-threonine, whereas the other is constructed during the formation of the thiazoline ring using either diethylaminosulfur trifluoride (DAST) or methyl *N*-carbamate (Burgess reagent).

As indicated in Scheme 1, the initial stage of preparation involved acetonide protection of *N*-(*tert*-butoxycarbonyl)-L-threonine methyl ester with 2,2-dimethoxypropane (DMP) in the presence of a catalytic amount of boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) in CH_2Cl_2 at room temperature to give oxazolidine **3** in 91% yield. The ester group of **3** was then reduced by $LiBH_4$ in a mixture of tetrahydrofuran (THF)/methanol to afford alcohol **4** in 84% yield.[26] A subsequent Appel reaction of the hydroxy group of **4** with CBr_4 and PPh_3 in CH_2Cl_2 gave brominated product **5** in 41% yield.

The coupling of **5** with 4-mercapto-2-methylphenol was then carried out in the presence of Cs_2CO_3 in acetonitrile at room temperature over 12 h to give **6** in 61% yield. Etherification of the resulting phenol group with ethyl bromoacetate in the presence of Cs_2CO_3 in acetonitrile then afforded **7** in 96% yield. Subsequent deprotection of both acetonide and Boc groups of **7** was achieved in a single step using excess trifluoroacetic acid (TFA) in dimethylformamide (DMF) at 50 °C over 24 h followed by column chromatographic purification, to give alcohol-amine **8** in 64% yield.[27] Subsequently, the introduction of a thioamide group into **8** was carried out using 2-(4-(trifluoromethyl)phenylcarbonothioyl)isoindoline-1,3-dione (**11**) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in CH_2Cl_2 to give the desired thioamide **9** in 43% yield.[28]

To complete the novel synthesis of **2**, formation of the thiazoline ring from **9** was performed using DAST in CH_2Cl_2 at −78 °C over 2 h to provide the corresponding thiazoline **10** in 79% yield.[29] Use of Burgess reagent in the place of DAST was also possible in this step to give thiazoline **10** in 64% yield;[30] however, a longer reaction time and a higher temperature were required. Finally, saponification of **10** using 2 M LiOH in a mixture of THF/water provided the desired product **2** as a pale yellow solid in 91% yield. Thus, the first enantiospecific synthesis of the novel scaffold **2** was accomplished over nine steps in an overall yield of 3.6%. Further details regarding the synthetic route and characterization of the final product can be found in the Supporting Information.

We also compared the stereochemistry of some additional products based on that of compound **2** (see Figure 2 below). To synthesize a substance exhibiting the same stereochemical structure, L-threonine methyl ester hydrochloride was treated

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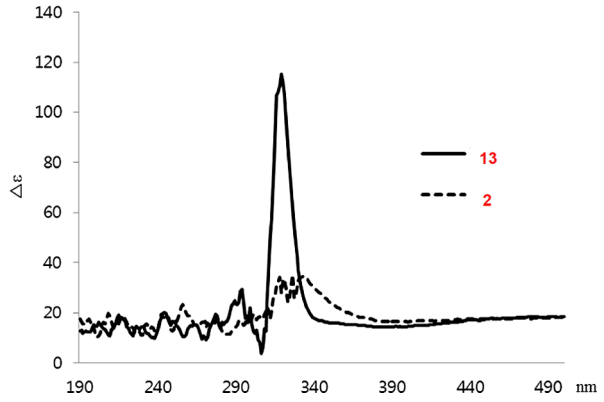
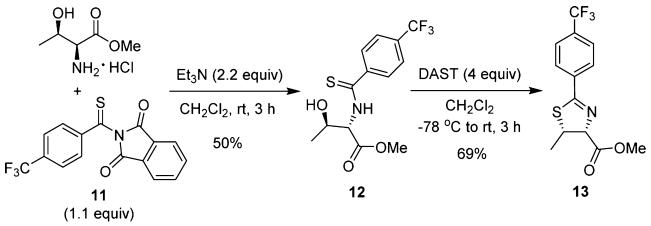


Figure 2. Experimental CD spectra of compounds **2** and **13**.

with triethylamine (TEA) and **11** in CH_2Cl_2 at room temperature to give thioamide **12** in 50% yield (Scheme 2). Subsequently, the thiazoline ring was generated using DAST in CH_2Cl_2 to give the cyclized product **13** in 69% yield.

Scheme 2. Preparation of Pulicatin Derivative 13



Thus, the absolute configurations of thiazolines **2** and the single isomer of **13** were determined by measuring the specific optical rotations of the two products and carrying out circular dichroism (CD) experiments (Figure 2). The obtained results were compared with the literature value for a similar compound bearing the (4*R*,5*S*)-pulicatin A configuration (see Figure 1).[1] The ^{1}H-^{1}H correlated spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), and ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC) nuclear magnetic resonance (NMR) spectra of compound **2** (see the Supporting Information and Figure 3) were also recorded to confirm the stereochemistry of this product.

The biological activities of the two key scaffolds, that is, GW501516 (**1**) and the synthesized **2**, were then examined through screening of their agonist activities toward PPARδ. As shown in the Supporting Information, the novel scaffold **2** displayed highly potent human peroxisome proliferator-activated receptor delta (hPPARδ) activity (EC_{50} = 6.2 nM) in a cell-based cotransfection assay.

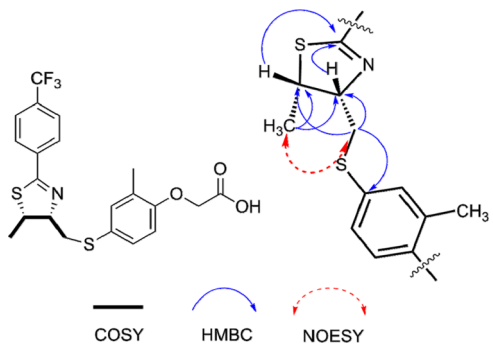


Figure 3. Key HMBC, COSY, and NOESY correlations for compound **2**.

3. CONCLUSIONS

We successfully synthesized the novel hPPARδ agonist **2** in a convergent and enantioselective manner using a chiral pool of L-threonine, a naturally occurring amino acid, as the starting material. The developed synthetic protocol for the preparation of the thiazoline structure involved simple and high-yielding chemical transformations. Following preliminary in vitro pharmacological studies, we could also conclude that scaffold **2** displayed highly potent hPPARδ activity. Further investigation into the preparation of synthetic derivatives of **2** and related structure−activity relationship studies are ongoing, and the results will be reported in due course, with the aim of developing a novel hPPARδ agonist for medicinal purposes.

4. EXPERIMENTAL SECTION

4.1. General. All reactions were monitored by thin-layer chromatography (TLC), performed using 0.2 mm silica gel plates (Merck 60 F_{254}) and visualized by UV light (254 nm) and stain solutions, such as $KMnO_4$ and *p*-anisaldehyde, with heating. Medium pressure liquid chromatography (MPLC) was carried out on a CombiFlash Rf system with RediSep Rf using flash column cartridges packed with silica gel. The purity of the target compounds was determined to be >95% by analytical high-performance liquid chromatography using dual different wavelength UV detector (254 and 280 nm). NMR spectra were recorded on a Bruker spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethylsilane as an internal standard, and coupling constants were expressed in hertz. Infrared (IR) spectra were obtained using a Shimadzu IRAffinity-1 spectrometer, and mass spectra were measured with a JEOL JMS 700 high-resolution mass spectrometer (HRMS) at the Korean Basic Science Institute (Daegu). Melting points were determined using MP90 Melting Point System with open capillaries, and optical rotations were measured on an AutoPOL I automatic polarimeter. CD spectra were obtained using a Jasco J-810 spectropolarimeter.

4.2. Materials. Commercially available reagent grade chemicals, such as *N*-(*tert*-butoxycarbonyl)-L-threonine methyl ester and L-threonine methyl ester hydrochloride, were used as received without further purification unless noted otherwise.

4.3. Synthetic Procedure: Preparation of 2 (Scheme 1). *4.3.1. 3-(tert-Butyl) 4-Methyl (4S,5R)-2,2,5-Trimethyloxazolidine-3,4-dicarboxylate (**3**).* To a solution of *N*-(*tert*-butoxycarbonyl)-L-threonine methyl ester (5 g, 21.44 mmol) in CH_2Cl_2 (11 mL) were added DMP (5.3 mL, 42.9 mmol) and boron trifluoride diethyl etherate (0.14 mL, 1.072 mmol), and the resulting mixture was allowed to stir at room temperature for 3 h. After this time, the reaction mixture was extracted with CH_2Cl_2 (50 mL) and sat. $NaHCO_3$ (50 mL). The organic layer was then separated, washed with brine (50 mL), and dried over anhydrous $MgSO_4$. Following filtration and concentration to give crude residue, purification was by MPLC using hexane/ethyl acetate (9:1) as the eluant to give the desired product **3** as a colorless liquid (5.331 g, 91%), which was a mixture of two conformers (major/minor = 65:35); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.19−4.11 (m, 1H), 3.98 and 3.90 (minor: d, J = 7.7 Hz, major: d, J = 7.9 Hz, 1H), 3.76 (s, 3H), 1.64 and 1.56

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(major: s, minor: s, 3H), 1.58 (s, 3H), 1.48 (s, 3H), 1.39 and 1.38 (major: s, minor: s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) major conformer: δ 171.2, 150.9, 95.0, 80.2, 73.7, 66.1, 52.1, 28.1, 26.4, 23.9, 18.7, minor conformer: δ 170.7, 151.8, 94.4, 80.7, 73.4, 65.9, 52.3, 28.2, 27.7, 24.8, 18.9; FTIR (neat): 2982, 2936, 1757, 1712, 1380, 1367, 1259, 1202, 1172, 1131, 859 cm^{-1}.

*4.3.2. tert-Butyl (4R,5R)-4-(Hydroxymethyl)-2,2,5-trimethyloxazolidine-3-carboxylate (**4**).* To a solution of 3-(*tert*-butyl)-4-methyl (4*S*,5*R*)-2,2,5-trimethyloxazolidine-3,4-dicarboxylate **3** (5.242 g, 19.18 mmol) in THF/MeOH (4:1) (64 mL) was added $LiBH_4$ (0.836 g, 38.4 mmol) at 0 °C. The reaction mixture was then allowed to warm to room temperature and stirred for 2 h. Following complete consumption of the starting material (as determined by TLC), the reaction mixture was quenched using sat. $NaHCO_3$ (10 mL) prior to extracting with ethyl acetate (50 mL) and sat. $NaHCO_3$ (40 mL). The combined organic layers were then washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated to give the crude product. Finally, the obtained residue was purified by MPLC using hexane/ethyl acetate (8:2) as the eluant to give the desired product **4** as a colorless oil (3.971 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.98 (br s, 1H), 3.72−3.62 (m, 3H), 3.56−3.47 (m, 1H), 1.57 (s, 3H), 1.49 (s, 9H), 1.46 (s, 3H), 1.34 (d, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.2, 93.9, 81.1, 71.9, 67.1, 64.6, 28.3, 27.8, 25.8, 18.1; FTIR (neat): 3438, 2979, 2936, 1696, 1671, 1405, 1367, 1257, 1176, 1090, 1064 cm^{-1}.

*4.3.3. tert-Butyl (4S,5R)-4-(Bromomethyl)-2,2,5-trimethyloxazolidine-3-carboxylate (**5**).* To a solution of *tert*-butyl (4*R*,5*R*)-4-(hydroxymethyl)-2,2,5-trimethyloxazolidine-3-carboxylate **4** (3.90 g, 15.9 mmol) in CH_2Cl_2 (16 mL) were added CBr_4 (5.27 g, 15.9 mmol) and PPh_3 (4.17 g 15.9 mmol) at 0 °C. The reaction mixture was then allowed to warm to room temperature and stirred continuously for 4 h. After this time, the reaction mixture was directly absorbed onto silica gel and purified by MPLC using hexane/ethyl acetate (20:1) as the eluant to give the desired product **5** as a colorless liquid (2 g, 41%), which is a mixture of two conformers; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.21−4.15 (m, 1H), 3.85−3.48 (m, 3H), 1.62−1.49 (m, 15H), 1.36 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) major conformer: δ 152.0, 93.8, 80.5, 74.0, 63.2, 31.8, 28.3, 27.2, 26.3, 19.7, minor conformer: δ 151.2, 94.6, 80.3, 74.5, 63.1, 32.5, 28.3, 26.8, 26.8, 20.1; FTIR (neat): 2979, 2935, 1696, 1392, 1366, 1259, 1172, 1127, 1089, 863 cm^{-1}.

*4.3.4. tert-Butyl (4S,5R)-4-(((4-Hydroxy-3-methylphenyl)thio)methyl)-2,2,5-trimethyl-oxazolidine-3-carboxylate (**6**).* To a stirred solution of 4-mercapto-2-methylphenol (0.366 g, 2.61 mmol) in CH_3CN (7 mL) was added Cs_2CO_3 (0.850 g, 2.61 mmol) followed by a solution of *tert*-butyl (4*S*,5*R*)-4-(bromomethyl)-2,2,5-trimethyloxazolidine-3-carboxylate **5** (0.731 g, 2.372 mmol) in CH_3CN (5 mL). After stirring the reaction mixture at room temperature overnight, the solvent was evaporated from the mixture, and the mixture was poured into water (30 mL) prior to extracting with ethyl acetate (2 × 50 mL). The combined organic layers were then dried over anhydrous $MgSO_4$, filtered, and concentrated. The obtained residue was purified by MPLC using hexane/ethyl acetate (9:1) as the eluant to give the desired product **6** as a white solid (0.53 g, 61%), which was a mixture of two conformers (major/minor = 54:46). mp 110−111 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20−7.14 (m, 2H), 6.70 and 6.59 (minor: d, J = 7.5 Hz, major: d, J = 7.8 Hz, 1H), 5.97 and 5.59 (major: s, minor: s, 1H), 4.33−4.19 (m, 1H), 3.71 and 3.58 (major: s, minor: d, J = 5.6 Hz, 1H), 3.36 and 3.19 (major: d, J = 12.8 Hz, minor: d, J = 12.4 Hz, 1H), 3.01 and 2.88 (major: dd, J = 11.2, 9.9 Hz, minor: dd, J = 11.5, 10.4 Hz, 1H), 2.20 and 2.17 (minor: s, major: s, 3H), 1.61 and 1.58 (minor: s, major: s, 3H), 1.52 (s, 3H), 1.46−1.36 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) major conformer: δ 153.7, 152.2, 134.3, 130.3, 124.9, 124.8, 115.5, 93.8, 80.6, 74.8, 62.8, 36.5, 28.6, 28.3, 27.2, 20.6, 15.7, minor conformer: δ 153.9, 151.6, 135.4, 131.4, 125.0, 124.8, 115.5, 94.5, 80.0, 75.3, 62.6, 38.4, 28.3, 27.6, 27.0, 21.3, 15.7; FTIR (neat): 3381, 2989, 1672, 1598, 1492, 1398, 1234, 1146, 1117, 1046, 822 cm^{-1}; HRMS (EI): calcd for $[M]^+$ $C_{19}H_{29}NO_4S$, 367.1817; found, 367.1815.

*4.3.5. tert-Butyl (4S,5R)-4-(((4-(2-Ethoxy-2-oxoethoxy)-3-methylphenyl)thio)methyl)-2,2,5-trimethyloxazolidine-3-carboxylate (**7**).* To a solution of *tert*-butyl (4*S*,5*R*)-4-(((4-hydroxy-3-methylphenyl)thio)methyl)-2,2,5-trimethyloxazolidine-3-carboxylate **6** (1.545 g, 4.20 mmol) in CH_3CN (21 mL) was added Cs_2CO_3 (2.055 g, 6.31 mmol) followed by ethyl bromoacetate (0.56 mL, 5.04 mmol, dropwise). After stirring the reaction mixture at room temperature for 1 h, it was diluted with water (30 mL) and extracted with ethyl acetate (2 × 30 mL). The organic layers were then combined, washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated. The obtained residue was purified by MPLC using hexane/ethyl acetate (6:1) as the eluant to give the desired product **7** as a colorless oil (1.84 g, 96%), which was a mixture of two conformers (major/minor = 52:48); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24−7.20 (m, 2H), 6.62 (d, J = 8.0 Hz, 1H), 4.60 (s, 2H), 4.28−4.19 (m, 3H), 3.69 and 3.57 (minor: s, major: s, 1H), 3.39 and 3.22 (minor: d, J = 12.7 Hz, major: d, J = 12.7 Hz, 1H), 3.08 and 2.88 (minor: dd, J = 10.7, 9.5 Hz, major: dd, J = 10.9, 10.6 Hz, 1H), 2.25 (s, 3H), 1.60 and 1.56 (major: s, minor: s, 3H), 1.51 (s, 3H), 1.46−1.28 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) major conformer: δ 168.7, 155.7, 151.4, 134.8, 130.5, 128.3, 126.2, 111.6, 94.4, 79.8, 75.3, 65.6, 62.3, 61.2, 38.0, 28.3, 27.5, 26.9, 21.2, 16.0, 14.0, minor conformer: δ 168.8, 155.0, 152.0, 133.0, 128.5, 128.1, 127.0, 111.8, 93.6, 80.0, 74.6, 65.6, 62.6, 61.2, 35.4, 28.5, 28.3, 27.1, 20.4, 16.0, 14.0; FTIR (neat): 2980, 2933, 1762, 1696, 1491, 1387, 1366, 1303, 1258, 1186, 1141, 1088, 734 cm^{-1}; HRMS (EI): calcd for $[M]^+$ $C_{23}H_{35}NO_6S$, 453.2185; found, 453.2187.

*4.3.6. Ethyl 2-(4-(((2S,3R)-2-Amino-3-hydroxybutyl)thio)-2-methylphenoxy)acetate (**8**).* To a solution of *tert*-butyl (4*S*,5*R*)-4-(((4-(2-ethoxy-2-oxoethoxy)-3-methylphenyl)thio)-methyl)-2,2,5-trimethyloxazolidine-3-carboxylate **7** (0.227 g, 0.5 mmol) in DMF (2.5 mL) was added dropwise TFA (0.77 mL, 10 mmol). After stirring the reaction mixture at 50 °C for 24 h, it was diluted with water (20 mL) and extracted with ethyl acetate (2 × 30 mL). The organic layers were then combined, dried over anhydrous Na_2SO_4, filtered, and concentrated. The obtained residue was purified by MPLC using dichloromethane/methanol (9:1) as the eluant to give the desired product **8** as a colorless gum (0.1 g, 64%); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 7.96 (s, 2H), 7.29 (s, 1H), 7.24 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 5.51 (br s, 1H), 4.81 (s, 2H), 4.16 (q, J = 7.0 Hz, 2H), 3.94−3.88 (m, 1H), 3.16−3.11 (m, 1H), 3.03−2.98 (m, 1H), 2.94−2.85 (m, 1H), 2.18 (s, 3H), 1.20 (t, J = 7.0 Hz, 3H), 1.11 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 169.1, 155.8, 133.9, 130.3, 127.8, 125.1, 112.7, 65.4, 64.6, 61.1, 55.5, 34.7, 20.2, 16.3, 14.4; FTIR (neat): 3419, 3139, 2984, 2930, 1594, 1490, 1305, 1212, 1136,

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ACS Omega 2018, 3, 1970−1976

1030, 800, 723 cm^{-1}; HRMS (EI): calcd for $[M]^+$ $C_{15}H_{23}NO_4S$, 313.1348; found, 313.1346.

4.3.7. Ethyl 2-(4-(((2S,3R)-3-Hydroxy-2-(4-(trifluoromethyl)phenylthioamido)butyl)thio)-2-methylphenoxy)acetate (9). To a stirred solution of ethyl 2-(4-(((2*S*,3*R*)-2-amino-3-hydroxybutyl)thio)-2-methylphenoxy)acetate **8** (0.1 g, 0.319 mmol) in anhydrous CH_2Cl_2 (1 mL) was added DIPEA (0.06 mL, 0.351 mmol) followed by a solution of 2-(4-(trifluoromethyl)-phenylcarbonothioyl)isoindoline-1,3-dione **11** (0.118 g, 0.351 mmol) in CH_2Cl_2 (0.6 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirring was continued for 4 h. After this time, the solvent was evaporated from the reaction mixture, and the obtained crude residue was purified by MPLC using hexane/ethyl acetate (4:1) as the eluant to give the desired product **9** as a yellow oil (0.070 g, 43%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.60 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 1.7 Hz, 1H), 7.24 (dd, J = 8.4, 2.2 Hz, 1H), 6.57 (d, J = 8.4 Hz, 1H), 4.78−4.73 (m, 1H), 4.56 (s, 2H), 4.47−4.39 (m, 1H), 4.25 (q, J = 7.1 Hz, 2H), 3.36−3.24 (m, 2H), 2.55 (s, 1H), 2.22 (s, 3H), 1.29 (t, J = 7.1 Hz, 3H), 1.25 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.7, 168.7, 155.6, 144.6, 133.8, 132.5 (q, $^2J_{CF}$ = 32.6 Hz), 129.7, 128.6, 126.9, 125.7, 125.3 (q, $^3J_{CF}$ = 3.7 Hz), 123.6 (q, $^1J_{CF}$ = 270.7 Hz), 111.7, 67.2, 65.4, 61.3, 59.6, 35.7, 20.9, 16.0, 14.0; FTIR (neat): 3443, 3366, 2977, 2930, 1740, 1492, 1413, 1325, 1189, 1131, 1068, 1015, 911, 849, 733 cm^{-1}; HRMS (EI): calcd for $[M]^+$ $C_{23}H_{26}F_3NO_4S_2$, 501.1255; found, 501.1258.

4.3.8. Ethyl 2-(2-Methyl-4-((((4R,5S)-5-methyl-2-(4-(trifluoromethyl)phenyl)-4,5-dihydrothiazol-4-yl)methyl)-thio)phenoxy)acetate (10). To a solution of ethyl 2-(4-(((2*S*,3*R*)-3-hydroxy-2-(4-(trifluoromethyl)phenylthioamido)-butyl)thio)-2-methylphenoxy)acetate **9** (0.073 g, 0.146 mmol) in anhydrous CH_2Cl_2 (1 mL) was added DAST (0.077 mL, 0.582 mmol) dropwise at −78 °C, and the reaction mixture was stirred at −78 °C for 2 h. After this time, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and quenched with sat. $NaHCO_3$ (5 mL). The organic layer was then washed with sat. $NaHCO_3$ (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was purified by MPLC using hexane/ethyl acetate (9:1) as the eluant to give the desired product **10** as a yellow oil (0.056 g, 79%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (d, J = 8.1 Hz, 2H), 7.64 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 1.7 Hz, 1H), 7.28 (dd, J = 8.4, 2.2 Hz, 1H), 6.65 (d, J = 8.4 Hz, 1H), 4.62 (s, 2H), 4.41−4.35 (m, 1H), 4.26 (q, J = 7.1 Hz, 2H), 4.13−4.06 (m, 1H), 3.68 (dd, J = 13.3, 5.9 Hz, 1H), 3.16 (dd, J = 13.3, 9.8 Hz, 1H), 2.28 (s, 3H), 1.31−1.27 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.8, 166.5, 155.7, 136.5, 134.5, 132.7 (q, $^2J_{CF}$ = 32.2 Hz), 130.3, 128.5, 128.4, 126.3, 125.3 (q, $^3J_{CF}$ = 3.8 Hz), 123.7 (q, $^1J_{CF}$ = 270.9 Hz), 111.7, 78.2, 65.6, 61.3, 48.2, 35.4, 16.5, 16.1, 14.1; FTIR (neat): 2970, 2930, 1761, 1595, 1490, 1409, 1326, 1188, 1130, 1067, 1016, 976, 849, 618 cm^{-1}; HRMS (EI): calcd for $[M]^+$ $C_{23}H_{24}F_3NO_3S_2$, 483.1150; found, 483.1152.

4.3.9. 2-(2-Methyl-4-((((4R,5S)-5-methyl-2-(4-(trifluoromethyl)phenyl)-4,5-dihydrothiazol-4-yl)methyl)-thio)phenoxy)acetic acid (2). To a stirred solution of ethyl 2-(2-methyl-4-((((4*R*,5*S*)-5-methyl-2-(4-(trifluoromethyl)-phenyl)-4,5-dihydrothiazol-4-yl)methyl)thio)phenoxy)acetate **10** (34 mg, 0.070 mmol) in THF/H_2O (1:1) (0.7 mL) at 0 °C was added dropwise 2 M LiOH (0.105 mL, 0.211 mmol). The reaction mixture was then stirred at room temperature until TLC indicated that the reaction had reached completion (∼1 h). After this time, the reaction mixture was diluted with water (1 mL), acidified with sat. NH_4Cl (10 mL) to give a pH of 5, and extracted with ethyl acetate (3 × 20 mL). The organic layers were then combined, dried over anhydrous Na_2SO_4, filtered, and concentrated. The obtained residue was purified by MPLC using dichloromethane/methanol (10:1) as the eluant to give the desired product **2** as a pale yellow solid (29.6 mg, 91%); mp 88−90 °C; $[\alpha]_D^{21}$ −15.7 (*c* 0.166, $CHCl_3$); ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 7.93 (d, J = 8.0 Hz, 2H), 7.84 (d, J = 8.1 Hz, 2H), 7.32 (s, 1H), 7.27 (d, J = 8.3 Hz, 1H), 6.76 (d, J = 8.4 Hz, 1H), 4.52 (s, 2H), 4.35−4.30 (m, 1H), 4.23−4.18 (m, 1H), 3.51 (dd, J = 13.1, 7.1 Hz, 1H), 3.23 (dd, J = 13.0, 8.0 Hz, 1H), 2.17 (s, 3H), 1.22 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 165.4, 156.3, 136.8, 133.9, 131.6 (q, $^2J_{CF}$ = 31.6 Hz), 130.3, 129.0, 127.4, 126.2 (q, $^3J_{CF}$ = 3.7 Hz), 125.4, 124.3 (q, $^1J_{CF}$ = 270.7 Hz), 112.5, 78.5, 66.4, 48.6, 35.2, 29.4, 16.9, 16.4; FTIR (neat): 2923, 2852, 2360, 1592, 1490, 1409, 1323, 1230, 1168, 1127, 1066, 1015, 975, 845 cm^{-1}; HRMS (EI): calcd: for $[M]^+$ $C_{21}H_{20}F_3NO_3S_2$, 455.0837; found, 455.0836.

4.3.10. 2-(4-(Trifluoromethyl)phenylcarbonothioyl)-isoindoline-1,3-dione (11). To a stirred solution of 4-(trifluoromethyl)benzothioamide (3.0 g, 14.6 mmol) in THF (30 mL) at 0−5 °C were added K_2CO_3 (3.1 g, 21.9 mmol) and phthaloyl dichloride (2.53 mL, 17.5 mmol, dropwise). The resulting reaction mixture was stirred at 0−5 °C for 3 h, then diluted with water (20 mL) and ethyl acetate (30 mL). The organic layer was separated, washed with brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated. The resulting crude compound was purified by MPLC using hexane/ethyl acetate (8:2) as the eluant to give the desired product **11** as a purple solid (3.8 g, 78%); mp 125−126 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (dd, J = 5.5, 3.0 Hz, 2H), 7.90−7.85 (m, 4H), 7.64 (d, J = 8.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.1, 164.6, 145.0, 135.5, 134.0 (q, $^2J_{CF}$ = 32.5 Hz), 130.9, 128.5, 125.4 (q, $^3J_{CF}$ = 3.6 Hz), 124.7, 123.5 (q, $^1J_{CF}$ = 271.1 Hz); FTIR (neat) 2917, 1790, 1729, 1288, 1120, 1070, 1017, 871, 847, 789, 717 cm^{-1}; HRMS (EI): calcd for $[M]^+$ $C_{16}H_8F_3NO_2S$, 335.0228; found, 335.0228.

4.4. Synthetic Procedure: Preparation of Pulicatin Derivative 13 (Scheme 2). *4.4.1. Methyl (4-(Trifluoromethyl)phenylcarbonothioyl)-L-threoninate (12).* To a stirred solution of L-threonine methyl ester hydrochloride (0.170 g, 1 mmol) in anhydrous CH_2Cl_2 (5 mL) was added TEA (0.31 mL, 2.2 mmol) followed by a solution of 2-(4-(trifluoromethyl)phenylcarbonothioyl)isoindoline-1,3-dione **11** (0.369 g, 1.1 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was then allowed to warm to room temperature, and stirring was continued at this temperature for 3 h. After this time, the solvent was evaporated from the reaction mixture, and the obtained crude residue was purified by MPLC using hexane/ethyl acetate (3:1) as the eluant to give the desired product **12** as a yellow oil (0.166 g, 50%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 8.1 Hz, 2H), 7.67 (d, J = 8.2 Hz, 2H), 5.45 (dd, J = 8.6, 2.2 Hz, 1H), 4.61−4.55 (m, 1H), 3.83 (s, 3H), 2.08 (d, J = 4.2 Hz, 1H), 1.35 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.3, 170.3, 144.3, 132.8 (q, $^2J_{CF}$ = 32.4 Hz), 127.2, 125.5 (q, $^3J_{CF}$ = 3.6 Hz), 123.6 (q, $^1J_{CF}$ = 271.0 Hz), 68.0, 63.1, 52.9, 20.3; FTIR (neat): 3385, 3317, 2956, 1739, 1734, 1521, 1413, 1325, 1130,

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1068, 1016, 910, 850 cm^{-1}; HRMS (EI): calcd for $[M]^+$ $C_{13}H_{14}F_3NO_3S$, 321.0646; found, 321.0649.

4.4.2. Methyl (4R,5S)-5-Methyl-2-(4-(trifluoromethyl)-phenyl)-4,5-dihydrothiazole-4-carboxylate (13). To a solution of methyl (4-(trifluoromethyl)phenylcarbonothioyl)-L-threoninate **12** (0.159 g, 0.495 mmol) in anhydrous CH_2Cl_2 (3.3 mL) was added DAST (0.262 mL, 1.979 mmol) dropwise at −78 °C, and the reaction mixture was stirred at −78 °C for 2 h. After this time, the reaction mixture was allowed to warm to room temperature, and stirring was continued for 1 h. After completion of the reaction, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and quenched with sat. $NaHCO_3$ (10 mL). The organic layer was then washed with sat. $NaHCO_3$ (20 mL), brine (30 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was purified by MPLC using hexane/ethyl acetate (9:1) as the eluant to give the desired product **13** as a pale yellow solid (0.104 g, 69%); mp 60−62 °C; $[\alpha]_D^{21}$ −58.5 (*c* 0.156, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 8.0 Hz, 2H), 5.16 (d, J = 7.6 Hz, 1H), 4.32−4.25 (m, 1H), 3.85 (s, 3H), 1.30 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 168.8, 136.0, 133.2 (q, $^2J_{CF}$ = 32.6 Hz), 128.8, 125.4 (q, $^3J_{CF}$ = 3.7 Hz), 123.6 (q, $^1J_{CF}$ = 270.9 Hz), 81.7, 52.3, 47.6, 18.2; FTIR (neat): 2929, 1749, 1599, 1323, 1195, 1169, 1144, 1064, 1018, 919, 846 cm^{-1}; HRMS (EI): calcd for $[M]^+$ $C_{13}H_{12}F_3NO_2S$, 303.0541; found, 303.0544.

4.5. In Vitro Transfection Assay. The monkey kidney cells, CV-1, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% resin-charcoal-stripped fetal bovine serum (FBS), 100 U/mL penicillin, and 100 g/mL streptomycin in a humidified incubator (5% CO_2 in air) at 37 °C. CV-1 cells were seeded at 6 × 103 cells per well in 96-well culture plates and then grown to 70% confluence before transfection. The cells were washed with serum-free medium and then transfected with a plasmid mixture containing human PPAR expression vector, β-galactosidase, and TK-PPRE-Luc vector by SuperFect reagent (QIAGEN). The 24 h post-transfected cells were washed with serum-free DMEM and incubated with freshly delipidated 5% FBS DMEM supplemented with either compounds or DMSO vehicle for 24 h. After incubation, cell lysates were obtained using cell lysis buffer, and a luciferase activity was determined upon substrate addition using a Microlumat Plus Luminometer (Berthold). The luciferase activity was normalized with β-galactosidase activity using an ONPG buffer. All of the assays were performed in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01689.

NMR and high-resolution electron impact mass spectrometry data of all compounds and in vitro activities of compound **2** (PDF)

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Notes

The authors declare no competing financial interest.

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