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Synthesis of isosteric selenium analog of the PPARβ/δ agonist GW501516 and comparison of biological activity

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ABSTRACT

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily. Herein, we describe an efficient synthesis of a novel isosteric selenium analog of the highly specific PPARβ/δ ligand 2-methyl-4-((4-methyl-2-(4-trifluoromethylphenyl)-1,3-thiazol-5-yl)-methylsulfanyl)phenoxy-acetic acid (GW501516; **1**). The study examined the efficiency of the novel selenium analog 2-methyl-4-((4-methyl-2-(4-trifluoromethylphenyl)-1,3-selenazol-5-yl)-methylsulfanyl)phenoxy-acetic acid (**2**) to activate PPARβ/δ and the effect of ligand activation of PPARβ/δ on cell proliferation and target gene expression in human HaCaT keratinocytes. The results showed that similar to GW501516, the Se-analog **2** increased expression of the known PPARβ/δ target gene angiopoietin-like protein 4 (*ANGPTL4*); the compound **2** was comparable in efficacy as compared to GW501516. Consistent with a large body of evidence, the Se-analog inhibited cell proliferation in HaCaT keratinocytes similar to that observed with GW501516. In summary, the novel Se-analog **2** has been developed as a potent PPARβ/δ ligand that may possess additional anti-cancer properties of selenium.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily, and exist in three isoforms, PPARα, PPARγ, and PPARβ (also referred to as PPARδ and PPARβ/δ). Each of these regulates tissue-specific target genes involved in many biological processes.[1,2] PPARα and PPARγ are the molecular targets of a number of marketed drugs, for example, the fibrate class of hypolipidemic drugs[3] (PPARα) and the thiazolidinedione class of insulin-sensitizing drugs[4] (PPARγ). In contrast to PPARα and PPARγ there are no marketed drugs that target PPARβ/δ and considerably less is known about the biological role of PPARβ/δ. Although ligand activation of PPARβ/δ is known to increase serum high-density lipoprotein cholesterol, increase skeletal muscle fatty acid catabolism, and improve insulin sensitivity,[5,6] its role in tumorigenesis, apoptosis, and cell proliferation remains controversial.[7–9] Extensive structure-based drug design and combinatorial chemistry studies have recently led to the identification of two highly selective PPARδ agonists GW501516 (**1**) and its analog GW0742.[10,11] Given the pharmacological potential of these PPARβ/δ agonists, which have been examined in clinical trials,[12] it is critical to design novel analogs that not only efficiently bind selectively to PPARβ/δ but have enhanced anti-cancer activity.

We hypothesized that isosteric substitution of the sulfur atom in GW501516 by selenium not only would retain the structural features required for its efficient binding to the receptor and hence its efficiency as a PPARβ/δ ligand, but would also possess additional anti-cancer properties of selenium. The hypothesis was based on our own recent studies[13,14] and literature reports[15] demonstrating that compared to sulfur structural analogs, selenium compounds are more potent anti-cancer agents. In addition, there is a large body of evidence showing selenium to be effective for inhibiting tumorigenesis in both animal models and epidemiological studies.[16–19] Furthermore, since both sulfur and selenium, exhibit similar oxidation states, the difference in size of the atom (from sulfur to selenium) was not expected to alter the geometry of the molecule significant enough to affect its binding to PPARβ/δ ligand. Therefore, we developed the selenium analog **2** (Fig. 1) and compared its efficacy as a PPARβ/δ ligand and as inhibitor of cell proliferation as compared to GW501516.

GW501516 used in this study was synthesized as described previously.[20] The selenium analog 2-methyl-4-((4-methyl-2-(4-trifluoromethylphenyl)-1,3-selenazol-5-yl)-methylsulfanyl)phenoxy-acetic acid (**2**) was synthesized as outlined in Scheme 1.[21] The key precursor **2** was synthesized by treating *o*-cresol (**3**) with potassium selenocyanate in the presence of potassium bromide and bromine, in methanol. Selenocyanate **4** was reduced with sodium borohydride to generate corresponding selenol in situ that was treated with thiazole **5**, synthesized following a reported method,[10] in a

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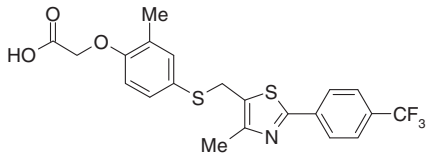
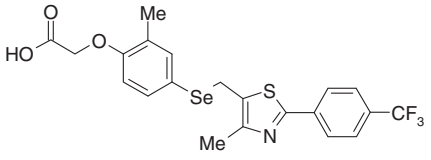


1. GW501516



2. Se-analog

Figure 1. Structures of GW501516 (**1**) and its novel selenium analog (**2**).

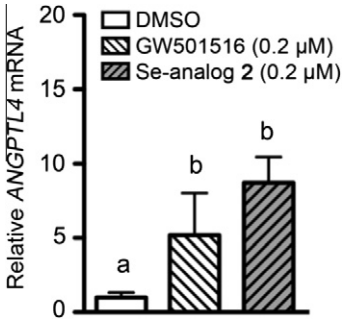


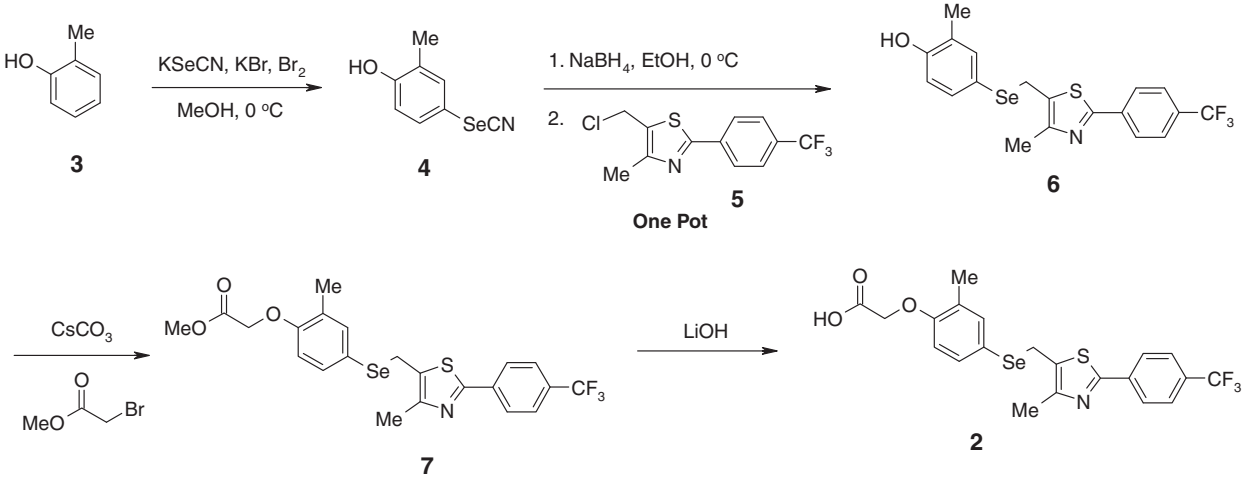
Figure 2. Modulation of gene expression by ligand activation of PPARβ/δ in HaCaT keratinocytes. HaCaT cells were treated for 8 h with the indicated concentration of GW501516 or selenium analog **2**. Quantitative real-time PCR was performed to examine the expression of mRNA encoding *ANGPTL4* normalized to mRNA encoding *GAPDH*. Values are the average-fold change compared with control treatment and represent means ± SEM. Values with different letters are significantly different, $P < 0.05$, as determined by ANOVA and Bonferroni's multiple comparison test.

one pot reaction to give the adduct **6** in excellent yield. Compound **6** on treatment with bromoethyl acetate in the presence of cesium carbonate resulted in the formation of ester **7** which was hydrolyzed following a literature method[20] using lithium hydroxide to yield the desired Se derivative **2**. The product was purified by silica gel column chromatography and characterized on the basis of NMR and high-resolution mass spectral data.

We and many other laboratories have previously demonstrated that GW501516 and other specific PPARβ/δ ligands increase the expression of *ANGPTL4*.[7–9,22] To evaluate if the Se-analog is a specific ligand for PPARβ/δ, we examined the expression of the known PPARβ/δ-dependent target gene *ANGPTL4*, and compared it with the highly specific PPARβ/δ ligand GW501516. HaCaT cells were treated for either 8 h with 0.2 μM GW501516 or its novel selenium analog **2**. Quantitative real-time PCR was performed to examine the expression of mRNA encoding *ANGPTL4* normalized to mRNA encoding *GAPDH*. The mRNA encoding the PPARβ/δ target gene *ANGPTL4* was induced by both GW501516 and selenium derivative **2** (Fig. 2). The selenium analog led to a similar induction of the target gene *ANGPTL4* as compared to GW501516. This suggests that the novel selenium derivative **2**, designed by isosterically replacing sulfur in GW501516 by selenium, retained the property of being a specific PPARβ/δ ligand.

Several studies have shown that ligand activation of PPARβ/δ can induce terminal differentiation of keratinocytes and epithelium[7,8] and can inhibit cell growth in epithelium and other cell types, including keratinocytes, colonocytes, cardiomyocytes, lung fibroblasts, and cancer cell lines.[7–9] A few reports, however, also suggest that ligand activation of PPARβ/δ can potentiate cell growth.[7–9] We have previously demonstrated that ligand activation of PPARβ/δ inhibits keratinocyte proliferation through PPARβ/δ-dependent mechanisms.[22,23] To examine the effect of novel synthetic PPARβ/δ ligand **2** on cell growth, HaCaT keratinocyte cell proliferation was quantified in the presence of either GW501516 (**1**) or its selenium analog **2**.[24] Inhibition of HaCaT cell proliferation was observed with 10 μM concentrations of both GW501516 and its Se-analog **2** after 72 h of exposure. The inhibition was slightly more pronounced with the Se-analog **2** (Fig. 3A and B). These data support the hypothesis and suggests that the presence of selenium has the potential to make the compound more cytotoxic.

In summary, a novel isosteric selenium analog of GW501516 has been developed. The synthetic strategy is highly efficient and gave high overall yield. The new compound **2** is a specific PPARβ/δ ligand as it exhibited a marked induction of the target gene *ANGPTL4*. Furthermore, the new analog inhibited keratinocyte proliferation slightly better than GW501516. The new Se-analog could



Scheme 1. Synthesis of 2-methyl-4-((4-methyl-2-(4-trifluoromethylphenyl)-1,3-selenazol-5-yl)-methylsulfanyl)phenoxy-acetic acid (**2**).

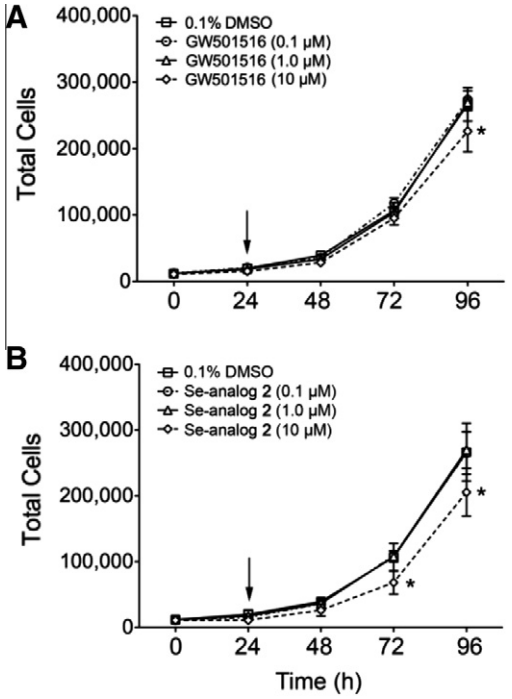


Figure 3. Ligand activation of PPARβ/δ inhibits cell proliferation of HaCaT keratinocytes. HaCaT cells were treated with either GW501516 (A) or selenium analog **2** (B) with the indicated concentration of ligand (arrow) in the presence of culture medium with serum and cell number was quantified. Values represent means ± SEM. *Significantly different values ($P < 0.05$) from vehicle (DMSO) at the particular time point, as determined by ANOVA and Bonferroni's multiple comparison test.

therefore be used a chemical tool to study the function of the ubiquitously expressed PPARβ/δ.

Acknowledgments

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21. Synthesis of **2-Methyl-4-[[[4-methyl-2-[4-(trifluoromethyl)phenyl]-thiazol-5-yl]methyl]selenyl]phenoxy]acetic acid (2)**. To a stirred solution of *o*-cresol (**3**) (5.4 g, 50 mmol), potassium selenocyanate (23.0 g, 160 mmol), and methanol (40 mL) at 0 °C was added a solution of potassium bromide (5.95 g, 50 mmol) and bromine (8.0 g, 2.65 mL, 50 mmol) in methanol (60 mL). The mixture was stirred for 3 h and then diluted with saturated $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (3 × 150 mL), and the organic phases were combined, washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/hexanes 1:4) to give 2-methyl-4-selenocyanatophenol (**4**) as a light yellow solid (8.8 g, 83%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.33 (d, 1H, J = 2.4 Hz), 7.26 (dd, 1H, J = 8.4, 2.4 Hz), 6.79 (d, 1H, J = 8.4 Hz), 6.00 (br s, 1H), 2.23 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 156.3, 135.1, 131.5, 126.9, 116.6, 112.6, 112.5, 15.8. To a solution of **4** (2.12 g, 10 mmol) in ethanol at 0 °C under nitrogen atmosphere, was added sodium borohydride (1.9 g, 50 mmol) portion wise. The solution turns yellow and then colorless within 5 min. To this mixture at 0 °C was then added **5** (2.91 g, 10 mmol), the reaction was warmed to room temperature, and stirred for 3 h. The solvent was concentrated to about 10 mL under reduced pressure, diluted with ethyl acetate and washed twice with water. The organic layer was dried ($MgSO_4$), filtered, and the solvent was evaporated to give a pale yellow solid which was further purified by silica gel column chromatography (ethyl acetate/hexanes 1:3) to yield 4.1 g (92%) of 2-methyl-4-[[[4-methyl-2-[4-(trifluoromethyl) phenyl]thiazol-5-yl]methyl]selenyl]phenol (**6**) as a white solid. Mp138–139 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 2.08 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 4.11 (s, 2H, $SeCH_2$), 6.63 (d, J = 8.0 Hz, 1H), 7.12 (dd, J = 8.0 and 1.5 Hz, 1H), 7.30 (d, J = 1.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H); HRMS (ESI) m/z calcd for $C_{19}H_{16}F_3NOSSe{\cdot}H^+$, 444.0143; found: 444.0137. To a stirred solution of **6** (1.33 g, 3.0 mmol) in CH_3CN at room temperature was added Cs_2CO_3 (1.47 g, 4.5 mmol) followed by methyl bromoacetate (0.6 g, 0.36 mL, 3.9 mmol) and the reaction mixture was stirred for 2 h. Water was added to the reaction mixture and it was extracted with EtOAc (2× 100 mL). The organic layers were combined and washed with water again, dried over $MgSO_4$ and concentrated. The crude solid thus obtained was purified by silica gel column chromatography (ethyl acetate/hexane 1:4) to yield 1.44 g (93%) of Methyl [2-methyl-4-[[[4-methyl-2-[4-(trifluoromethyl)-phenyl]thiazol-5-yl]methyl]selenyl]phenoxy]acetate (**7**) as a white solid. Mp107–108 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 2.19 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 4.15 (s, 2H, $SeCH_2$), 4.67 (s, 2H, OCH_2), 6.59 (d, J = 8.5 Hz, 1H), 7.26 (dd, J = 8.5 and 1.5 Hz, 1H), 7.33 (d, J = 1.5 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H); HRMS (ESI) m/z calcd for $C_{22}H_{20}F_3NO_3SSe{\cdot}H^+$, 516.0354; found: 516.0373. To a solution of **7** (1.0 g, 1.95 mmol) in 30 mL of THF and 20 mL of H_2O at 0 °C was added slowly 2.0 mL (4.0 mmol) of 2.0 M LiOH and the reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was diluted with water (30 mL), acidified with 0.5 M $NaHSO_4$ (7 mL), and extracted with a mixed solvent of EtOAc and THF (3:1, 4× 50 mL). The combined organic fractions were briefly dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH (95:5) to yield 0.89 g (91%) of **2** as a white solid. Mp 144–145 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 2.08 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 4.13 (s, 2H, $SeCH_2$), 4.70 (s, 2H, OCH_2), 6.61 (d, J = 8.5 Hz, 1H), 7.21 (dd, J = 8.5 and 2.0 Hz, 1H), 7.29 (d, J = 2.0 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H); HRMS (ESI) m/z calcd for $C_{21}H_{18}F_3NO_3SSe{\cdot}H^+$, 502.0197; found: 502.0222.
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24. Cell proliferation analyses: Cell proliferation was examined as previously described.[21] Briefly, HaCaT keratinocytes were plated on a 12-well plate at a density of 20,000 cells/well 24 h before cell counting at time 0. Cell proliferation was determined using a Z1 Coulter particle counter (Beckman–Coulter, Hialeah, FL). Cells were then cultured for 24 h before ligand treatment. After this 24-h period, cells were maintained in DMEM with or without serum and treated with control (DMSO), GW0742 or GW501516 for up to an additional 72 h period. The concentration of GW0742 and GW501516 used for all experiments ranged from 0.1 to 10.0 μM, because these concentrations have been shown to specifically activate PPARβ/δ in case of GW501516.[22] Cell number was quantified every 24 h. Triplicate samples for each treatment were used for each time point, and each replicate was counted three times.