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# Synthesis of an Urea-substituted Selenoisobutyric Acid Isostere of the Peroxisome Proliferator-activated Receptor α Selective Agonist

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Peroxisome Proliferator-Activated Receptor α (PPARα), a major regulator of energy homeostasis discovered in 1990, is present at high density in the liver and regulates the expression of genes involved in fatty acid β-oxidation.[1] As research on its function has been localized to animal experiments, the function of PPARα in humans is still unclear. Past studies revealed PPARα participation in tumorgenesis,[2] inflammation[3,4] and atherosclerosis.[5] Therefore, selective agonists of PPARα are expected to be potential antitumor, anti-inflammatory and anti-atherosclerotic agents. Fibrates were the first generation of PPARα modulators (Figure 1) GlaxoSmithKline (GSK) then developed a series of urea-substituted thioisobutyric acids (ureido-TiBAs),[6] which were synthesized using a parallel-array synthetic method. Ureido-TiBA derivatives synthesized from the compounds GW7647 and GW9578 were found to be effective for heart disease caused by hypertension and high-cholesterol *in vivo* (Figure 2).

In particular, GW7647 demonstrated superior potency with ~200-fold selectivity over the other PPAR subtypes ($EC_{50}$ = 6 nM for human PPARα).[6] In addition, the administration of GW7647 to rats for 4 days decreased triglyceride and serum apolipoprotein CIII levels by 60% and 40%, respectively, and increased HDL-cholesterol levels by 60%. In this note, we shortly and efficiently synthesized a novel isosteric selenium substitution of ureido-selenoisobutyric acid of the PPARα agonist and compared its PPARα activity. Isosterism is a useful strategy for molecular modification and is a rational approach in drug design.[7] Isosteric analogs possess an equally well-established biological potency in terms of protein-receptor interactions.[8] As a proof-of-concept, sulfur-selenium bioisosterism was applied to GW7647 because molecular modeling study suggested a bulkier element at the sulfur fit the receptor better.[9]

During the course of one-pot synthetic studies of alkyl aryl selenides, we developed an *in situ* one-pot synthetic method that is used for the protection of the amine group in aryl bromides with alkylmagnesium bromide.[10,11] Thus, we set out to develop a method for the formation of an aryl alkyl selenide (such as compound **2**) with an amine substituent that could be used to prepare various ureido-selenoisobutyric acids via a simple and efficient synthetic route.

In our reaction, the starting material **1** is commercially available. 4-bromophenethylamine[6] is more expensive than 4-bromobenzeneselenol. But our protocol for preparing **2** is cheaper and shorter than that of the GSK protocol.[6] Although we could not directly detect the transition state during the reaction, intermediates for synthesis of the target

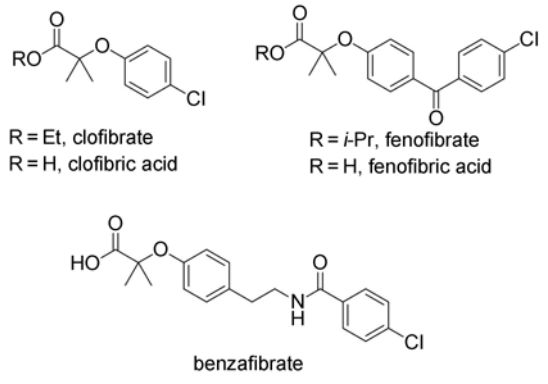



**Figure 1.** Chemical structures of fibrate compounds.

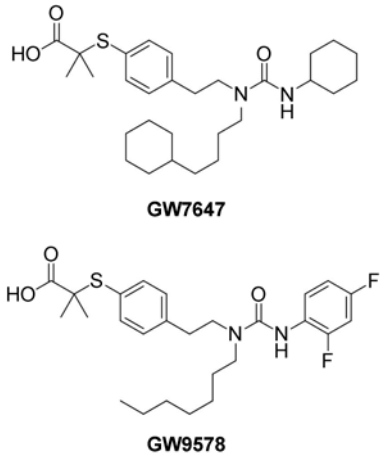



**Figure 2.** Chemical structures of GW7647 and GW9578, synthetic PPARα agonists.

**Table 1.** Selectivity of PPARa ligands

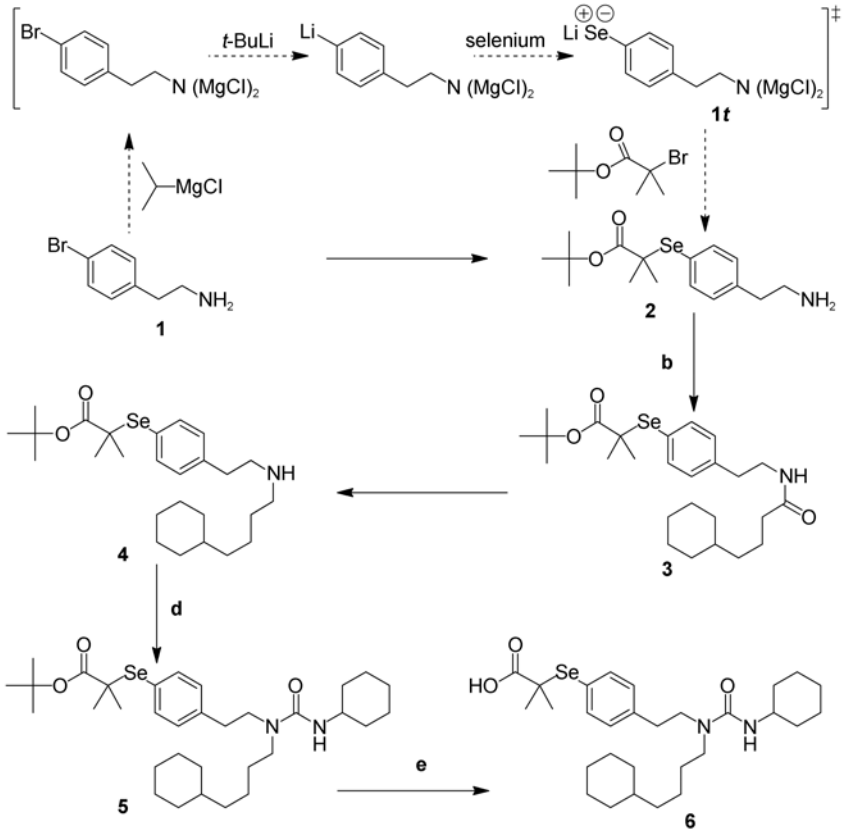
| Compounds | hPPARα ($EC_{50}$, μM) | hPPARδ ($EC_{50}$, μM) | hPPARγ ($EC_{50}$, μM) |
|---|---|---|---|
| **6** | 0.003 | 8.4 | i.a.[a] |
| **GW7647** | 0.007 | 6.8 | 1.5 |

[a]$EC_{50}$ values are higher than 1 μM.

compound **2** could be generated at each step of the reaction. Therefore, we proposed that the integrity of the *in situ* protected amine [-N($MgCl)_2$ moiety] in the reaction solvent is successfully maintained during both the lithium-halogen exchange and the selenium insertion reaction. As far as the nucleophilic reactivity of the selenium and amine anions in the intermediate **1t** are concerned, the selenium anion was more reactive to *t*-butyl bromoisobutyrate than the amine anion. The *t*-butyl bromoisobutyrate, however, did not react with the lithium selenolate **1t**, enabling us to successfully run the reaction with a base under traditional thermal reflux condition in one pot and to give of the titled compound **2**, which was directly used in the following step. Treatment of **2** with 4-cyclohexanebutanoic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriaole ($HOBT·H_2O$) in $CH_2Cl_2$ at room temperature for 12 h gave amide **3** in high yield. We improved the reaction yield using EDC as the coupling reagent instead of *N,N′*-Diisopropylcarbodiimide (DIC). The secondary alkyl amine **4** was prepared from amide **3** through the general reduction of borane, which was accomplished using excess 1 N $BH_3$·THF without any additional solvent at room temperature for 1 day. The rest of the transformation consisted of isourea-formation on the secondary amine, and hydrolysis of the *tert*-butyl ester. The final compound, ureido-selenoisobutyric acid (**6**), displayed higher potency ($EC_{50}$ = 3 nM) and selectivity than GW7647 (Table 1). In summary, we successfully synthesized 2-{4-[2-(4-Cyclohexyl-butyryl-amino)-ethyl]-phenylselanyl}-2-methyl-propionic acid *tert*-butyl ester, a key intermediate for the synthesis of ureido-selenoisobutyric acid, in 70% yield. From this intermediate, we obtained the desired compound **6** in 32% overall yield. We also report the in vitro activity of a novel isosteric selenium PPARa highly selective agonist.

## Experimental Section

**General.** All reactions were performed in oven- and flame-dried glassware under nitrogen atmosphere. Air and moisture sensitive reagents and solvents were transferred *via* syringes or cannula, and they were introduced into the reaction vessel through a rubber septum. Chemicals obtained from commercial sources were used without further purification. Flash column chromatography was carried out on




**Scheme 1.** Reagents and conditions: (a) (i) $^{i}$PrMgCl (2.0 equiv), THF, rt for 15 min.; (ii) −78 °C, *t*-BuLi (2.0 equiv) for 30 min.; (iii) selenium powder, −78 °C to −10 °C for 2 h.; (iv) vacuum, MeOH, KOH, $BrC(CH_3)_2CO_2t$-Bu, 80 °C for 2 h.; (b) 4-cyclohexanebutanoic acid, EDC, HOBT, rt for 15 h (70%); (c) 1 M $BH_3$·THF, rt for 1 day (65%); (d) cyclohexylisocyanate, $CH_2Cl_2$, rt for 18 h (85%); (e) 50% TFA/$CH_2Cl_2$, rt for 4 h (82%).

silica gel (230-400 mesh). Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F254. TLC plates were visualized with UV light and 5% ammonium dimolybdate or *p*-anisaldehyde in ethanol with heat. $^1$H-NMR (300 MHz) in $CDCl_3$ was recorded on a Bruker Avance III 400 MHz NMR spectrometer and chemical shifts (ä) were expressed in ppm downfield from the internal tetramethylsilane or with reference to residual $CHCl_3$. The purity of compounds was assessed by HPLC/MS spectra, which were recorded on a Finnigan LTQ LC/MS system.

**2-{4-[2-(4-Cyclohexyl-butyrylamino)-ethyl]-phenylselanyl}-2-methyl-propionic Acid tert-butyl Ester, Compound 3.** To a solution of 4-bromophenethylamine (400 mg, 2.0 mmol) in anhydrous THF (20 mL) was slowly added $^i$PrMgCl (2.0 M solution in diethyl ether, 2.0 mL, 4.0 mmol) at 0 °C for 10 min under $N_2$. After 30 min, *t*-BuLi (1.7 M solution in pentane, 2.4 mL, 4.0 mmol) was slowly added at −78 °C for 20 min and the reaction mixture was stirred for an additional 30 min. Selenium powder (158 mg, 2.0 mmol) was added at once and the reaction mixture was slowly warmed to −10 °C for 2 h. After the reaction was complete, the solvent was completely removed by evaporation under atmospheric conditions. To a solution of the residual product in MeOH (20 mL) was added KOH (117.8 mg, 2.1 mmol) at rt and then heated at 60 °C for another 2 h. After this time, the reaction mixture was poured into a saturated $NH_4Cl$ solution (35 mL) and was extracted with EtOAc (3 × 30 mL). The combined organic layer was washed with $H_2O$, dried ($MgSO_4$), filtered off and then concentrated on a rotary evaporator. To crude compound 2 (342 mg) and cyclohexanebutanoic acid (171 mg, 1.0 mmol) in dried $CH_2Cl_2$ was added 1-hydroxybenzotriaole (HOBT·$H_2O$) (205 mg, 1.5 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 576 mg, 3.0 mmol). The reaction mixture was then stirred at room temperature for 12 h. Next, the reaction mixture was sequentially washed with saturated $NaHCO_3$, 1 N HCl and brine solution, and then the organic layer was dried over anhydrous $MgSO_4$. After the solvent was evaporated under reduced pressure, the residue was purified by silica gel chromatography using 30% ethyl acetate in hexane to afford as a white solid (350 mg, 70%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, 2H, $J$ = 7.9 Hz), 7.14 (d, 2H, $J$ = 7.9 Hz), 5.43 (s, brs, 1H), 3.53 (q, 2H, $J$ = 6.7 Hz), 2.82 (t, 2H, $J$ = 6.9 Hz), 2.10 (t, 2H, $J$ = 7.5 Hz), 1.70-1.53 (m, 7H), 1.51 (s, 6H), 1.43 (s, 9H), 1.28-1.14 (m, 6H), 0.87 (m, 2H).

**2-{4-[2-(4-Cyclohexyl-butylamino)-ethyl]-phenylselanyl}-2-methyl-propionic Acid tert-butyl Ester, Compound 4.** To a solution of 3 (494 mg, 1.0 mmol) was added a 1M solution of borane in THF (20 mL, 20.0 mmol), and the reaction mixture was allowed to stand for 1 day without stirring. Excess borane was destroyed by the careful addition of methanol and the resulting solution heated at reflux for 30 min. After the addition of *n*-butanol (5 mL) the solvent was evaporated and the residue was purified by silica gel chromatography using 10% methanol in ethyl acetate to afford a yellow viscous liquid (312 mg, 65%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, 2H, $J$ = 8.0 Hz), 7.17 (d, 2H, $J$ = 8.0 Hz), 3.13-2.67 (m, 7H), 1.69 (m, 6H), 1.50-1.43 (m, 15H), 1.28-1.16 (m, 6H), 0.82 (m, 2H).

**2-(4-{2-[3-Cyclohexyl-1-(4-cyclohexyl-butyl)-ureido]-ethyl}-phenylselanyl)-2-methyl-propionic Acid tert-butyl Ester, Compound 5.** To a solution of 4 (240 mg, 0.5 mmol) in dried $CH_2Cl_2$ (3 mL) was added cyclohexylisocyanate (126 mg, 1.0 mmol) and then the reaction mixture was stirred at room temperature for 18 h. Then, the solvent was evaporated and the residue was purified by silica gel chromatography using 5% methanol in $CH_2Cl_2$ to afford as a white solid (257 mg, 85%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, 2H, $J$ = 8.0 Hz), 7.16 (d, 2H, $J$ = 8.0 Hz), 4.05 (d, 1H, $J$ = 7.7 Hz), 3.63 (m, 1H), 3.42 (t, 2H, $J$ = 7.1 Hz), 3.05 (t, 2H, $J$ = 7.2 Hz), 2.84 (t, 2H, $J$ = 7.5 Hz), 1.90 (m, 2H), 1.67 (m, 8H), 1.42-1.01 (m, 30H), 0.86 (m, 2H).

**2-(4-{2-[3-Cyclohexyl-1-(4-cyclohexyl-butyl)-ureido]-ethyl}-phenylselanyl)-2-methyl-propionic Acid, Compound 6.** To a solution of 5 (302 mg, 0.5 mmol) in $CH_2Cl_2$ (3 mL) was slowly added trifluoroacetic acid (3 mL) and then the reaction mixture was stirred at room temperature for 4 h. On completion of the reaction, the solvent was evaporated and the residue was purified by silica gel chromatography using 5% methanol in $CH_2Cl_2$ to afford **6** as a white solid (225 mg, 82%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, 2H, $J$ = 7.7 Hz), 7.18 (d, 2H, $J$ = 7.8 Hz), 4.07 (d, 1H, $J$ = 7.6 Hz ), 3.54 (m, 1H), 3.43 (t, 2H, $J$ = 7.0 Hz), 3.04 (t, 2H, $J$ = 7.3 Hz), 2.79 (t, 2H, $J$ = 6.9 Hz), 1.87 (m, 2H), 1.68-0.95 (m, 23H), 1.54 (s, 6H), 0.86 (m, 2H). LC/MS (ESI+) Calcd for $C_{29}H_{46}N_2O_3Se$ [M+H] +: *m/z* 548.26. Found: *m/z* 549.28.

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