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Feasible Synthesis of Antagonist of GPR40 by Constructing 2-Thiouracil Ring via Acid Mediated Cyclization

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Abstract

GW1100 is an antagonist of GPR40 identified by high throughput screening recently. The synthesis of GW1100 has been developed. The key step involves cyclization of the 2-thiouracil heterocycle under acidic condition at room temperature.

Keywords

GPR40; GW1100; acid condition; cyclization; room temperature

1. Introduction

GPR40 is a G-protein coupled receptor and is highly expressed in the beta cell of pancreatic islets, and its activation by long-chain free fatty acids enhances glucose-stimulated insulin secretion¹. The gene knockout mice of GPR40 show resistance to high fat diet-induced diabetic symptoms². Therefore it has been identified as a possible novel target for the treatment of type 2 diabetes.

There is no clear understanding to date whether agonist and antagonist of GPR40 would provide beneficial effects for the treatment of type 2 diabetes. To learn more about the pharmacology of GPR40 and about the implications of receptor activation and inhibition, the development of a synthetic agonist and antagonist would be very helpful in GPR40 proof-of-concept studies as its functional mode.

Recently, the first selective antagonist, GW1100, was identified by high-throughput screening (HTS)³. This compound was shown to completely inhibit the enhancement of glucose-stimulated insulin secretion mediated by agonist and to partially inhibit the enhancement mediated by nature ligand (e.g. linoleic acid).

GW1100 is an attractive molecular tool to further study the biological activities of GPR40 *in vitro* and *in vivo*. The structure and activity (SAR) of GW1100 with its receptor GPR40 has not been explored. Large scale of compound is also demanded to study the pharmacological effects of GPR40 in animals. So facile synthetic route is urgently needed to prepare GW1100. However, there is no full report of GW1100 synthesis in the literatures.

To synthesize GW1100, the key step is to construct 2-thiouracil ring. Though there are many works show that substituted 2-thiouracil analogue have remarkably contributed to biological and medicinal chemistry, few work reported the synthesis of N-substituted 2-thiouracil ring, and strongly basic condition and high temperature were usually used⁴⁻⁶. In this work, we

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described the total synthesis of GW1100 using acidic condition for ring closure. To the best of our knowledge, this is the first paper reported feasible total synthesis of GW1100.

2. Results and discussion

The synthesis of GW1100 started with the inexpensive 5-bromo-2-chloropyrimidine **1** (Scheme 1). Thus, nucleophilic displacement of **1** afforded 2-ethoxy-5-bromopyrimidine **2** according to modification of literature procedures^{7, 8}. Our method for preparation of **2**, using freshly prepared sodium ethoxide and absolute alcohol as solvent to improve the solubility of reactant, considerably improve the yield to over 95 %. Coupling reaction of **2** with ethyl acrylate was conducted in the presence of a catalytic amount of the palladium diacetate-triphenylphosphine complex. The reaction mixture was heated at 140 °C in a sealed tube to afford ethyl 3-(2-ethoxypyrimidin-5-yl)acrylate **3** in 64 % yield^{9, 10}. The reduction of **3** was conducted in ethanol at room temperature to give ethyl 3-(2-ethoxypyrimidine-5-yl)propionate **4** in a yield of 80 %.

Compound **4** was subjected to formylation reaction using methyl formate and sodium ethoxide as a base^{11, 12}, then the resultant was methylated by dimethyl sulfate to get ester **5** in total yield of 53 %. We found that application of the freshly prepared sodium ethoxide can depress the unexpected by-products which showed high polarity comparing to product. Compound **5** was obtained as the mixture of 2:3 ethyl and methyl ester which was determined by ¹H NMR after isolation.

As shown in Scheme 2, the resulting ester mixture **5** was hydrolyzed directly under 2 M LiOH to give crystalline acid **6** in 88 % yield. The latter acid **6** was treated with oxalyl chloride in the presence of a drop of DMF to provide the acid chloride **7**^{13, 14}. The acid chloride **7** was then coupled with KSCN to give the corresponding isothiocyanate derivatives **8**^{15, 16}. After changing the solvent into acetonitrile, the compound **8** was reacted immediately with 4-aminobenzoate to give intermediate **9**. This intermediate then undergoes next step without isolation.

In literatures, strong basic condition and high temperature were usually applied for similar cyclization reaction^{3–5}. For this case (Scheme 3), we conducted ring-closure reaction under acidic condition. After acidifying the solution of intermediate **9**¹⁷, compound **10** was synthesized in total yield of 27 % from **6**. This step was the key step for synthesis of target molecule.

Finally, compound **10** was subjected to S-alkylation with 4-fluorobenzyl bromide to gave target product GW1100 in moderate yield⁴.

3. Conclusion

In conclusion, we have developed the acid mediated-cyclization route for the synthesis of GW1100. To the best of our knowledge this is the first report of full synthesis of GW1100. Significantly, the route requires very mild condition and utilizes inexpensive starting material 5-bromo-2-chloropyrimidine **1**. Application of this methodology to synthesize various pyrimidine derivatives can be explored in the future.

4. Experimental Section

4.1. General

¹H NMR and ¹³C NMR were recorded on Varian VRX 300, 400 spectrometer. NMR spectra were recorded in CDCl₃ (¹H NMR at 500 MHz), and chemical shifts are expressed in parts per million (δ) relative to internal CDCl₃.

THF, CH₂Cl₂, toluene was distilled prior to use. DMF and CH₃CN was anhydrous solvent obtained from Aldrich. All other reagents and solvents were obtained from commercial sources and were used without any further purification. Reactions were routinely carried out under an atmosphere of dry argon with magnetic stirring. Flash chromatography was generally performed on silica gel. The yield was calculated after isolation.

4.2. 2-Ethoxy-5-bromopyrimidine (2)

Sodium (0.220 g, 9.56 mmol) was put into dry ethanol (40 mL) under 0 °C, and the mixture was stirred at rt until the sodium disappeared. To the resulting solution, 5-bromo-2-chloropyrimidine (1.5 g, 7.5 mmol) was added. The mixture solution was stirred at rt overnight. After removing of ethanol under reduced pressure, the residue was partition between water and ester. The organic phase was washed with brine and dried over anhydrous Na₂SO₄. After evaporation of solvent, the residue was purified by flash chromatography on silica gel (Hexanes: Ester = 5: 2) to give compound **2** as white solid (1.44 g, 7.1 mmol, 95 %). ¹H NMR (400MHz, CDCl₃, 25 °C) δ 1.43 (t, *J* = 7.2 Hz, 3H), 4.40 (q, *J* = 7.2 Hz, 2H), 8.51 (s, 2H).

4.3. Ethyl 3-(2-ethoxypyrimidin-5-yl)acrylate (3)

A mixture of **2** (0.300 g, 1.48 mmol), triphenylphosphine (0.0093 g, 0.0356 mmol), palladium (II) acetate (0.0043 g, 0.0192 mmol), ethyl acrylate (0.6 g, 5.25 mmol) and triethylamine (1 mL) were heated at 140 °C with stirring under argon for 16 h. After cooling to room temperature, the ethyl acrylate was removed and the residues were partitioned between ethyl acetate and water. The organic phase was washed with brine and dried over anhydrous Na₂SO₄. After evaporation of solvent, the residue was purified by flash chromatography on silica gel (Hexanes: Ester = 1: 1) to give compound **3** as white solid (0.210 g, 0.945 mmol, 64 %). ¹H NMR (400MHz, CDCl₃, 25 °C) δ 1.34 (t, *J* = 6.8 Hz, 3H), 1.45 (t, *J* = 7.2 Hz, 3H), 4.27 (q, *J* = 7.2 Hz, 2H), 4.45 (q, *J* = 6.8 Hz, 2H), 6.43 (d, *J* = 16.4 Hz, 1H), 7.56 (d, *J* = 16.4 Hz, 1H), 8.65 (s, 2H).

4.4. Ethyl 3-(2-ethoxypyrimidine-5-yl)propionate (4)

A suspension of **3** (0.210 g, 0.945 mmol) in ethanol (2 mL) and 10 % Pd/C (0.012 g), triethylamine (0.2 mL) was hydrogenated at 50 psi overnight. The catalyst was filtered off and the filtrate evaporated. The residue was dissolved in CH₂Cl₂, washed by saturated NH₄Cl and brine respectively, and dried over anhydrous Na₂SO₄. After evaporation of solvent, the residue was purified by flash chromatography on silica gel (Hexanes: Ester = 1: 1) to give compound **4** as colorless oil (0.170 g, 0.758 mmol, 80 %). ¹H NMR (400MHz, CDCl₃, 25 °C) δ 1.23 (t, *J* = 7.2 Hz, 3H), 1.42 (t, *J* = 6.9 Hz, 3H), 2.60 (t, *J* = 6.9 Hz, 2H), 2.86 (t, *J* = 7.2 Hz, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 4.39 (q, *J* = 7.2 Hz, 2H), 8.35 (s, 2H).

4.5. 2-(Methoxymethylene)-3-(2-ethoxypyrimidine-5-yl)propionic acid ethyl/methyl ester (5)

To a stirring suspension of freshly made NaOEt (0.118 g, 1.74 mmol) in THF (0.5 mL) was added dropwise a solution of **4** (0.100 g, 0.446 mmol) and methyl formate (0.100 g, 0.793 mmol) in THF (0.5 mL) at 0 °C. After the mixture was stirred at rt overnight and cooled to 0 °C, the dimethyl fufate (0.1 mL) was added slowly. After 4 h the solvent was evaporated and the residue was treated with water and extracted with ethyl acetate. The extracts were washed with water, dried over anhydrous Na₂SO₄. After evaporation of solvent, the residue was purified by flash chromatography on silica gel (Hexanes: EtOAc = 1: 1) to give compound **5** as colorless oil 2-(ethoxymethylene)-3-(2-methoxypyrimidine-5-yl)propionic acid ethyl ester (0.030 g, 0.0945 mmol, 21 %) and methyl ester (0.03 g, 0.142 mmol, 32 %). ¹H NMR (400MHz, CDCl₃, 25 °C) Ethyl ester: δ 1.25 (t, *J* = 6.9 Hz, 3H), 1.41 (t, *J* =

7.5 Hz, 3H), 3.44 (s, 2H), 3.88 (s, 3H), 4.15 (q, $J = 7.5$ Hz, 2H), 4.37 (q, $J = 6.9$ Hz, 2H), 7.37 (s, 1H), 8.37 (s, 2H); Methyl ester: δ 1.40 (t, $J = 7.2$ Hz, 3H), 3.42 (s, 2H), 3.66 (s, 3H), 3.85 (s, 3H), 4.36 (q, $J = 7.2$ Hz, 2H), 7.35 (s, 1H), 8.35 (s, 2H).

4.6. 2-(Methoxymethylene)-3-(2-ethoxypyrimidine-5-yl)propionic acid (6)

A suspension of the ester **5** (0.23 g, 0.86 mmol) in 2 M aqueous lithium hydroxide (2 mL) was stirred at rt overnight. The resulting clear yellow solution was acidified by 1 M HCl to PH = 3 and extracted with CH₂Cl₂, and dried over anhydrous Na₂SO₄. White solid **6** (0.18 g, 0.756 mmol, 88 %) was afforded after evaporation of solvent. ¹H NMR (400MHz, CDCl₃, 25 °C) δ 1.41 (t, $J = 7.2$ Hz, 3H), 3.44 (s, 2H), 3.91 (s, 3H), 4.37 (q, $J = 7.2$ Hz, 2H), 7.48 (s, 1H), 8.39 (s, 2H).

4.7. 1-(4-Ethoxycarbonylphenyl)-5-(2-ethoxypyrimid-5-ylmethyl)-2-thiouracil (10)

To the solution of **6** (0.086 g, 0.361 mmol) in dry CH₂Cl₂ (2 mL) one drop of DMF was added, then oxalyl chloride (0.28 mL) slowly at 0 °C. After stirring at rt for 2 h, the solvent was removed and additional toluene was added and removed in vacuum. The solid was slurried in dry CH₃CN (0.5 mL), and the resulting mixture was dropped into the slurry solution of KSCN (0.070 g, 0.720 mmol) in CH₃CN (0.2 mL) at 0 °C. After the mixture was stirred at rt for 20 h, the solvent was removed in vacuum, and the resulting solid was dissolved in DMF (0.5 mL). The resulting solution was added to the solution of ethyl 4-aminobenzoate (0.074 g, 0.448 mmol) and triethylamine (0.096 mL) in DMF (0.5 mL). After stirring at rt for overnight, 2 M H₂SO₄ (4 mL) was added and the slurry solution was stirred at rt for 5 h. The resulting mixture was extracted with CH₂Cl₂ three times and the organic phase was dried over anhydrous Na₂SO₄. After evaporation of solvent, the residue was purified by flash chromatography on silica gel (Hexanes: EtOAc = 1: 2) to give compound **10** as pale yellow solid (0.040 g, 0.097 mmol, 27 %). ¹H NMR (400MHz, CDCl₃, 25 °C) Ethyl ester: δ 1.37 (m, 6H), 3.61 (s, 2H), 4.45 (m, 4H), 7.11 (s, 1H), 7.44 (d, $J = 6.6$ Hz, 2H), 8.23 (d, $J = 6.6$ Hz, 2H), 8.43 (s, 2H); MS (ESI⁺) m/z 413.13 [M + H]⁺.

4.8. 1-(4-Ethoxycarbonylphenyl)-2-(4-fluorobenzyl)thio-5-(2-ethoxypyrimid-5-ylmethyl) pyrimidin-4-one (11)

To the solution of **10** (0.040 g, 0.097 mmol) and 4-fluorobenzyl bromide (0.025 g, 0.13 mmol) in CH₂Cl₂ (0.5 mL) was added the diisopropylethylamine (0.025 mL). The solution was stirred at rt overnight. After evaporation of solvent, the residue was purified by flash chromatography on silica gel (EtOAc: Hexanes = 4: 1, then elute with EtOAc: MeOH = 10: 1) to get **11** (0.024 g, 0.0461 mmol, 48 %) as white solid. ¹H NMR (400MHz, CDCl₃, 25 °C) δ 1.40 (m, 6H), 3.67 (s, 2H), 4.39 (m, 6H), 6.95 (m, 3H), 7.26 (m, 2H), 7.37 (d, $J = 8.4$ Hz, 2H), 8.16 (d, $J = 8.4$ Hz, 2H), 8.45 (s, 2H); MS (ESI⁺) m/z 521.16 [M + H]⁺.

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Scheme 1.

Scheme 2.

Scheme 3.