Discovery of Farnesoid X Receptor Antagonists Based on a Library of 3-O-Esters of Oleanolic Acid through Diverse Substitution Design and Molecular Docking Methods

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Synthesis of compound 4
To a stirred solution of 1a (200 mg, 0.44 mmol) in a mixed solvent of dioxane (2 ml) and water (2 ml) was added K₂CO₃ (121 mg, 0.88 mmol) and tetrabutylammonium bromide (112 mg, 0.35 mmol). After benzyl chloride (100 μl, 0.88 mmol) was added dropwise, the mixture was heated to 80ºC and maintained for 3 h. The excess K₂CO₃ was filtered off. The filtrate was concentrated under reduced pressure. And the residue was purified through a silica chromatography column (petroleum ether : acetone 10 : 1) to afford the product (4) as white solid (198 mg, 83.5 %).

Synthesis of compound 6
To a stirred solution of 5 (106 mg, 1.0 mmol) in a mixed solvent of MeOH (0.6 ml) and water (0.3 ml) was added Na₂CO₃ (58 mg, 0.55 mmol) and hydroxylammonium chloride (70 mg, 1.0 mmol). The mixture was stirred at room temperature for 3 h. Then water (2 ml) was added, and the pH of the solution was adjusted to 2. Diethyl ether (5 ml × 3) was used to extract the product from aqueous layer. Then the organic layer was collected, dried over Na₂SO₄ and concentrated. And the residue was purified through a silica chromatography column (petroleum ether : EtOAc 15 : 1) to afford the product (6) as white solid (74 mg, 61.2 %). ¹H NMR (300 MHz, CDCl₃): δ 8.16 (1H, s, C₆H₅NHOH), 7.57 (2H, m, H-2, 6 of Ph), 7.39 (3H, m, H-3, 4, 5 of Ph).

Synthesis of compound 7
To a stirred solution of 6 (20 mg, 0.17 mmol) in dry DMF (0.2 ml) was added N-chlorosuccinimide (NCS) (5 mg, 0.037 mmol). The mixture was heated to 40ºC, and two drops of 2M HCl (aq.) was added. After 10 min, the color of the solution turned to light blue. Then the reaction was moved to room temperature. After another portion of NCS (12 mg, 0.084 mmol) was added, the mixture was stirred at room temperature for 2 h. Then water (2 ml) was added, and the aqueous layer was extracted by diethyl ether (5 ml × 3). The organic layer was collected, dried over Na₂SO₄ and concentrated. And the residue was purified through a silica chromatography column (petroleum ether : EtOAc 15 : 1) to afford the product (7) as white solid (14.3 mg, 55.8 %). ¹H NMR (300 MHz, CDCl₃): δ 7.85 (2H, m, H-2, 6 of Ph), 7.44(3H, m, H-3, 4, 5 of Ph). ESI-MS: m/z [M+Na]⁺ 179.0.

Synthesis of compound 8
To a stirred solution of 7 (10 mg, 0.06 mmol) in dichloromethane (DCM) (0.2 ml) was added propargyl alcohol (5 μl, 0.09 mmol) and Et₃N (13 μl, 0.09 mmol) at room temperature. Then, the mixture was heated to 50 ºC for 2 h. After the removal of the solvent, the residue was purified through a silica chromatography column (petroleum ether : EtOAc 3 : 1) to afford the product (8) as white solid (10 mg, 89.0 %). ¹H NMR (300 MHz, CDCl₃): δ 7.78 (2H, m, H-2, 6 of Ph), 7.44 (3H, m, H-3, 4, 5 of Ph), 6.56 (1H, s, H of isoxazole), 4.81 (2H, s, H of methylene). ESI-MS: m/z [M+H]⁺ 176.2.

Synthesis of compound 2a
To a stirred solution of 8 (12 mg, 0.07 mmol) in acetone (0.4 ml) was added Jones’ reagent (50 μl) at room temperature. Then, the mixture was stirred for 3 h. Then water (2 ml) was added, and the aqueous layer was extracted by diethyl ether (5 ml × 3). The organic layer was collected, washed with water (5 ml × 3), dried over Na₂SO₄ and concentrated. The residue was recrystallized in CHCl₃ to afford the product (2a) as white solid (12 mg, 93.0 %). ¹H NMR (300 MHz, CDCl₃): δ 7.97 (2H,
m, H-2, 6 of Ph), 7.61 (1H, s, H of isoxazole), 7.54 (3H, m, H-3, 4, 5 of Ph). ESI-MS: \( m/z \ [M+H]^+ \) 190.3.

**Synthesis of compound 9**

2a (13.8 mg, 0.072 mmol) was stirred with SOCl\(_2\) (1 ml) at 80ºC for 6 h. Then the excess SOCl\(_2\) was evaporated under reduced pressure. And the residue was used directly for reactions below without any further purification.

**Synthesis of compound 2d**

After the mixture of 14 (164 μl, 2 mmol), hydroquinone (5 mg, 0.05 mmol) and 15 (0.2 ml, 2.3 mmol) was stirred at 100ºC for 10 h, another portion of 15 (0.34 ml, 4 mmol) was added, and the reaction mixture was heated to 140 ºC. After another 20 h, the reaction was stopped and concentrated. The residue was recrystallized in toluene to afford product (51 mg, 81.0 % based on 129 mg recovery of substrate). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) 7.27 (1H, d, H-4 of pyrazole, \( J = 1.8 \) Hz), 5.95 (1H, d, H-5 of pyrazole, \( J = 1.2 \) Hz), 4.32 (1H, dd, -N-CH\(_2\)-CH-, \( J = 6.3, 14.1 \) Hz), 4.10 (1H, dd, -N-CH\(_2\)-CH-, \( J = 5.4, 13.5 \) Hz), 3.04 (1H, m, -CH\(_2\)-CH-COOH), 2.22 (3H, s, CH\(_3\) of pyrazole), 1.14 (3H, d, \( J = 7.5 \) Hz, CH\(_3\)-CH-COOH). ESI-MS: \( m/z \ [M+H]^+ \) 169.1.
Figure S1 Binding modes of OA (green) and 3a (yellow) into FXR