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Synthesis of an Unnatural Steroid as a Farnesoid X Receptor Antagonist

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Bile acids are major products of hepatic cholesterol catabolism and are natural ligands for the Farnesoid X receptor (FXR).[1] These acids play critical roles in the regulation of intestinal lipid absorption, bile flow, and biliary lipid secretion.[2] FXR, also called the bile acid receptor (BAR), is a member of the nuclear receptor superfamily, which includes ligand-modulated transcription factors and is involved in cholesterol homeostasis in mammals.[3] The role of FXR is to control the synthesis and transport of hepatic and intestinal bile acids. Bile acids activate nuclear receptors that induce action of the bile salt export pump (BSEP) but that inhibit cholesterol 7α-hydroxylase (CYP7A1) gene transcription in the liver.[4] CYP7A1 catalyzes the rate-determining step in bile acid biosynthesis, and thus, this system is feedback inhibited by bile acids returning to the liver *via* enterohepatic circulation. FXR has become a challenging target for the discovery of new drugs to treat hyperlipidemia, obesity, and hypercholesterolemia.[5]

Guggulsterone (Fig. 1(a)) is an active ingredient of the traditional hypolipidemic drug extracted from *Commiphora mukul* (guggulu in Sanskrit). The plant sterol guggulsterone has been widely used in ancient Indian Ayurveda medicine since at least 600 BC to treat obesity and hyperlipidemia in India. Recently, we have successfully prepared *E*-guggulsterone in 84% yield over two steps from 16,17-epoxypregnenolone *via* hydrazine reduction and Oppenhauer oxidation.[6] Several clinical trials have demonstrated that guggulipid effectively lowers LDL cholesterol and triglyceride levels and increases HDL cholesterol levels.[7] In a study by Urizar *et al*. published in *Science*, it was reported that the cholesterol-level in the human body was lowered at the molecular level through the antagonism of FXR by negative-feedback loop regulation.[8] Moreover, the hepatic cholesterol-lowering effect of guggulsterone was controlled by FXR through an experiment using FXR-null mice. In this study, we report a synthetic method to prepare *pseudo*-guggulsterone (PG, Fig. 1(b)) and its resulting antagonism for FXR. Compound **2** was obtained by reacting compound **1** (testosterone) with a chromium-based oxidant. In this oxidation process, the secondary alcohol at C-17 was easily transformed into a ketone group with pyridinium chlorochromate (PCC). To synthesize compound **2**, testosterone (0.2 mmol) was dissolved in CH_2Cl_2 (3 mL) at 0 °C, and then, 4Å molecular sieves (500 mg) and PCC (0.4 mmol) were added. The reaction mixture was stirred at 0 °C for 1.5 h and was then quenched with diethyl ether (1.5 mL). The crude compound was purified *via* column chromatography on silica gel using hexane:EtOAc (1:2) as the eluent to produce compound **2** as a white solid in good yield (532 mg, 93%).

To prepare compound **3**, steroid **2** was subjected to the Wittig reaction in the presence of *t*-BuOK as a strong base. To achieve this transformation, ethyltriphenyl phosphonium bromide (1.5 mmol) was dissolved in anhydrous THF (5.0 mL) at 0 °C, and then, potassium *t*-butoxide (1.5 mmol) was added slowly. After the addition was completed, the reaction temperature was slowly raised to room temperature, and then, a solution of compound **2** was slowly added for 30 min at rt. After 2 h, the reaction mixture was quenched with brine, and the organic layer was separated and dried with anhydrous $MgSO_4$. After filtration, the solvent was removed under vacuum. The crude compound was purified *via* chromatography on silica gel using hexane:EtOAc (10:1) as the eluent to produce compound **3** in 91% yield.

Compound **4** was prepared by reacting compound **3** with selenium dioxide (SeO_2) and hydrogen peroxide in an aprotic polar solvent. Selenium dioxide (0.5 mmol) was dissolved in CH_2Cl_2 (1.5 mL), and *t*-butyl peroxide (0.2 mL) was added dropwise. This mixture was stirred at room temperature for 1 hour. Next, compound **3** (1 mmol) was added to the reaction mixture as a solution in CH_2Cl_2 (1.5 mL). After 2 h, the solvent was removed under reduced pressure, and the product was extracted with ethyl acetate and distilled water. The combined organic layers were dried

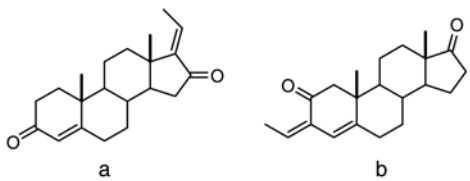

Figure 1. Chemical structures of guggulsterone (a) and *pseudo*-guggulsterone (b).

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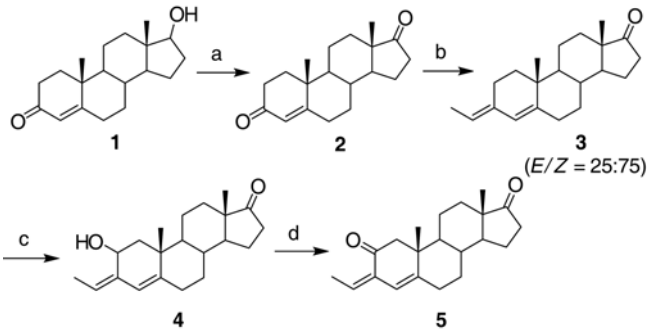

Scheme 1. Reaction conditions (a) PCC, CH_2Cl_2 (93%); (b) $Ph_3P^+CH_2CH_3Br^-$, *t*-BuOK, THF (91%); (c) SeO_2, *t*-BuOOH, CH_2Cl_2 (76%); (d) MnO_2, CH_2Cl_2 (90%).

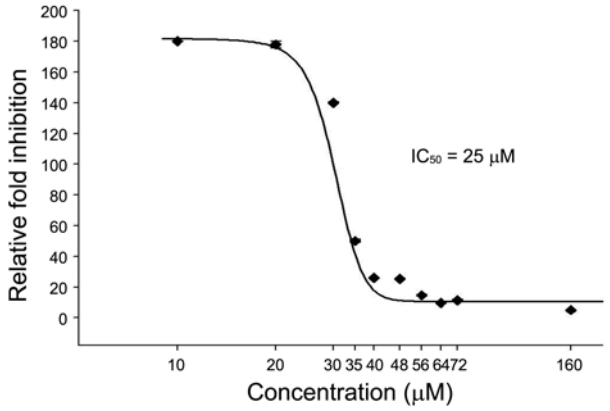

Figure 2. FXR antagonism of *pseudo*-guggulsterone.

with anhydrous $MgSO_4$, and the crude compound was purified *via* chromatography on silica gel using hexane: EtOAc (4:1) as the eluent to produce compound **4** as a yellow oil (236 mg, 76%).

Finally, compound **5** was obtained by reacting compound **4** with a selective oxidizing reagent. Activated manganese dioxide (MnO_2) can be used to selectively oxidize an alcohol group. In this reaction, compound **4** (0.1 mmol) was dissolved in CH_2Cl_2 (2.5 mL) at room temperature. Activated MnO_2 (10 mmol) was added to the above solution in one portion, and the mixture was stirred vigorously for 3 h. After the reaction was complete, excess manganese dioxide was removed *via* filtration. The solvent was evaporated under reduced pressure to produce compound **5** as a white solid (283 mg, 90%).

To investigate the biological effects of the synthetic *pseudo*-guggulsterone, we performed a co-transfection assay using full length FXR and its reporters. The plasmid containing FXR and the luciferase reporter plasmid were transfected into CV-1 (kidney cells of the African green monkey). The transfected cells were either left untreated or treated with *pseudo*-guggulsterone in presence of CDCA (chenodeoxycholic acid, 50 μM). As shown in Figure 2, the FXR transactivation by CDCA was inhibited by the *pseudo*-guggulsterone (Fig. 2) in a dose-dependent manner (IC_{50} = 25 μM). Next, an evaluation of *pseudo*-guggulsterone's

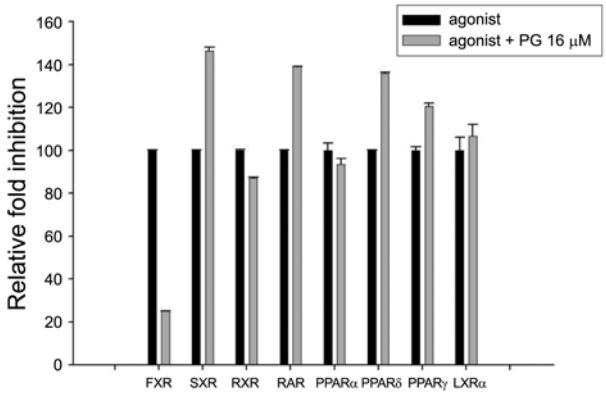

Figure 3. Selectivity of *pseudo*-guggulsterone. Cells were treated with the known agonist (FXR; CDCA (50 μM), SXR; lithocholic acid (100 μM), RAR; all-trans-RA (1 μM), PPARα; GW7647 (1 μM), PPARδ; GW501516 (1 μM), PPARγ; rosiglitazone (1 μM), LXRα; T0901317 (1 μM).

selectivity at the other nuclear receptors was also conducted, (Fig. 3) and our results demonstrated that *pseudo*-guggulsterone does not inhibit another receptor.

In summary, we have carried out the first efficient synthesis of the unnatural steroid *pseudo*-guggulsterone (PG) and investigated its selective inhibition of FXR activation through a co-transfection bioassay. Further studies to acquire more information about structure-activity relationships are in progress in our laboratory.

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9. The structure of compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$): 6.47 (1H, q), 6.21 (1H, s), 2.71-0.91 (26H, m); ^{13}C NMR (75.5 MHz, $CDCl_3$): 200.8, 199.8, 147.2, 133.2, 129.0, 117.1, 55.2, 53.0, 51.4, 47.9, 41.7, 36.1, 35.5, 32.1, 31.8, 31.2, 22.2, 21.2, 20.4, 14.1, 13.4; MS (EI) *m/z*: 312 (M^+).