A SOLUTION-PHASE PROCEDURE FOR THE PREPARATION OF 4-AZASTEROID MIMETICS

Marcus C. Ruda*,†,‡, Jan Bergman‡,§, Konrad Koehler† and Ye Liu †

†*Karo Bio AB, Novum, SE-14157 Huddinge, Sweden*

‡*Unit for Organic Chemistry, CNT, Department of Biosciences at Novum, Karolinska Institute, Novum Research Park, SE-141 57 Huddinge, Sweden*

§*Södertörn University College, SE-141 04 Huddinge Sweden*

Abstract

A Diels-Alder approach has been employed to make 3-oxo-octahydro-benzo[f]quinoline derivatives as ligands for nuclear hormone receptors and steroid biosynthetic enzymes. The five-step synthesis is a model for the synthesis of libraries on solid-phase.

Introduction

Modified heterocyclic steroids have drawn increasing interest due to their synthetic challenge and interesting biological activities.[1-5] The goal of our project is to make biologically active aza-steroids targeted against nuclear hormone receptors and steroid biosynthetic enzymes. In order to prepare aza-steroid libraries we will apply parallel synthesis on solid phase.

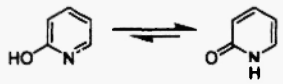


Figure 1. 2-Pyridone tautomers

We are interested in replacing the A-ring of a steroid with a bioisoster, such as 2-pyridone-ring system. An interesting feature of the 2-pyridone is that it has two tautomers (**Figure 1**) that can mimic either 3-keto-steroids (e.g., testosterone, glucocorticoids, mineralcorticoids) or phenol-steroids (e.g., estradiol) (**Figure 2**). The ligands will be tested in different receptor and enzymatic assays.

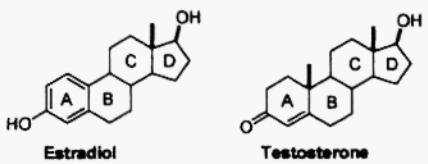


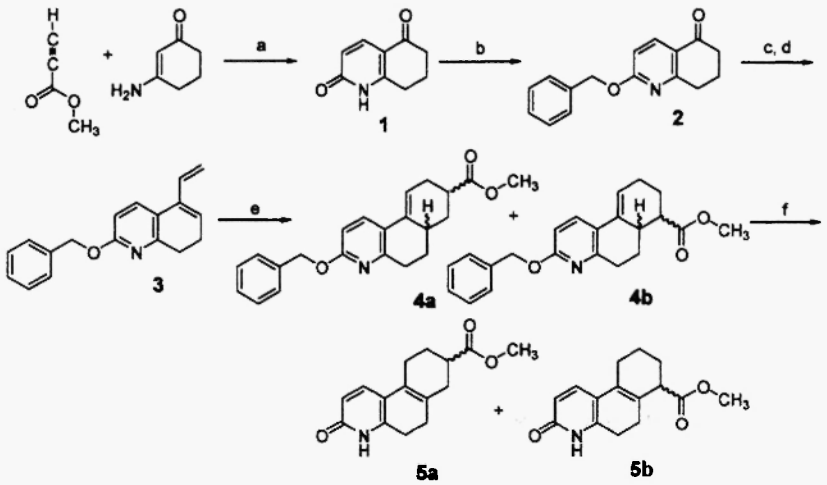
Figure 2. Phenol-steroid and 3-keto-steroid.

* To whom correspondence should be addressed. Phone: +46-8-608-6103. Fax: +46-8-774-8261. E-mail: marcus.ruda@karobio.se

The functionality of the 2-pyridone ring will serve as linking site to the solid support such as a Wang resin. A model solution-phase procedure had to be set up to confirm if the chosen protecting group (benzyl), expected to mimic the property of the resin, will survive the reaction conditions throughout the synthesis.

A Diels-Alder approach was chosen to construct the C-ring. Selection of different functionalized dienophiles would allow us to extensively elaborate the D-ring environment. Danishefsky *et. al.* have reported another method of constructing the parent C-ring, but unfortunately this procedure is not applicable for library synthesis.[6] We have previously reported a method to prepare N-alkylated pyridones via selective N-alkylation of 2-alkoxypyridines on solid-phase, which will be convenient to use in the preparation of future libraries.[7] However, as we then reported this method is not applicable for solution phase, and hence a traditional method with base and electrophile must be used for the model reactions.

Scheme 1[a]

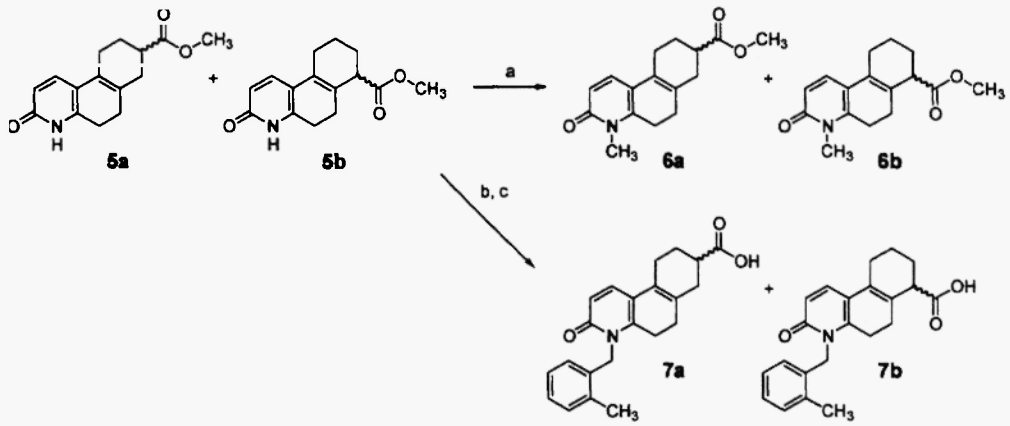


[a] Reaction conditions: (a) reflux in DMF, (37 %); (b) Ag_2CO_3, benzyl bromide, toluene, (93 %); (c) vinyl magnesium bromide, THF; (d) 2 M HCl, THF (61 %); (e) methyl acrylate, toluene, 120 °C, (62 %); (f) TMSCl, NaI, DCM, 60 °C, (95 %).

Result and Discussion

The synthesis of the target compounds is outlined in Scheme 1. The initial targets **5a,b**, were prepared from the tetrahydroquinolone derivivative **1**, which was prepared following a literature procedure starting from methyl propiolate and 3-amino-2-cyclohexen-1-one in refluxing DMF with repeated collection of the precipitate in 37% yield.[7,8] The amidic oxygen atom in **1** could be selectively protected with benzyl bromide and silver carbonate to yield **2** in 93 % yield.[9] The 2-benzyloxypyridine **2** was then treated with vinyl magnesium bromide followed by dehydration of the tertiary allylic alcohol with hydrochloric acid to generate the diene **3** in 61 % yield. The reaction conditions for the dehydration step had to be changed so it could be applicable for the solid phase synthesis as the swelling properties of the resin are poor with water in THF. Moreover, hydrochloric acid might cleave the substrate from the resin because the Wang resin has an increased acid lability, compared to the benzyl protecting group, due to the electron donating group on the phenyl ring. Treating with phosphorus oxychloride in pyridine at 0 °C and warming to room temperature, however, gave the desired product **3** cleanly and more rapidly than with hydrochloric acid.[10] A Diels-Alder reaction between methyl acrylate and **3** gave a diastereomeric mixture of the two regio isomers **4a,b** in 62 % yield. Cleavage of the protecting group and simultaneous migration of the double bond was achieved with trimethylsilyl iodide to yield a mixture of the regio isomers **5a,b** in 95 % yield. According to NMR (HMBC and HSQC) and HPLC-MS, the two regio isomers were obtained as 1:1 mixture, which was not separated since this was not necessary for the evaluation of the chemistry in the model reaction.

Scheme 2[a]



[a] Reaction conditions: (a) MeI, Li_2CO_3, DMF, 80 C, (quantitative yield); (b) 2-methyl benzyl bromide, Li_2CO_3, DMF, 100 C; (c) 1 M LiOH, THF, (quantitative yield).

A mixture of the tricyclic N-methylated derivatives **6a,b** was synthesized, in quantitative yield (Scheme 2), from **5a,b** by N-alkylation with methyl iodide in the presence of lithium carbonate . The pair **7a,b** was synthesized, in quantitative yield from **5a,b** *via* N-alkylation with lithium carbonate and 2-methylbenzyl bromide, followed by hydrolysis of the ester with lithium hydroxide.

The affinity for Androgen-, Estrogen-, Glucocorticoid-, Mineralocorticoid-, Progesterone- and Thyroid hormone receptor were in the range of 1 – 100 micro molar. However, further structure modifications might improve the affinity.

Acknowledgment.

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Experimental Section

General. All reactions were run under nitrogen atmosphere. Starting materials and solvents were obtained from commercial vendors and used without further purification. Proton NMR spectra were obtained on a JEOL 270 MHz and the 13C NMR spectra were measured on a JEOL 270 MHz at 67 MHz. The electro-spray mass were obtained on a Perkin-Elmer API 150ex.

7,8-Dihydro-1H,6H-quinoline-2,5-dione (1).

Methyl propiolate (2.69 g, 32 mmol) and 3-amino-2-cyclohexen-1-one (3.5 g, 32 mmol) were dissolved in DMF (20 mL) and heated to reflux. After 3 hrs the mixture was cooled on an ice bath, and the crystalline material was collected and washed with a small amount of DMF pre-cooled in dry ice. The filtrate was heated again for 16 hr after renewal addition of methyl propiolate (2.69 g, 32 mmol) and the solid precipitate was collected and washed with a small amount of DMF. This procedure was repeated once again. The crude solid was recrystallized from methanol to yield 1.93 g (37 %) of 1. ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.04 (d, 1H, J = 9.5 Hz), 6.45 (d, 1H, J = 9.5 Hz), 2.94 (t, 2H, J = 6.2 Hz), 2.57 (t, 2H, J = 6.2 Hz) and 2.12-2.21 (m, 2H); ^{13}C NMR (67 MHz, $CDCl_3$) δ 193.8, 165.9, 156.1, 139.1, 118.1, 115.0, 37.2, 27.3 and 21.4; ES-MS *m/z* 164 ($(M+H)^+$).

2-Benzyloxy-7,8-dihydro-6H-quinolin-5-one (2).

A mixture of 1 (1.138 g, 6.97 mmol), silver carbonate (1.138 g, 4.13 mmol) and benzyl bromide (1 mL) in toluene (50 mL) was stirred for 3 days in the dark at room temperature. The reaction mixture was filtered through Celite®, and the filtrate was evaporated to leave an orange oil, which was loaded on a silica gel column. Excess unreacted benzyl bromide was first removed by eluting with n-heptane and then the product was eluted with (2:8) EtOAc:heptane to yield 1.64 g (93 %) of **2**. ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.05 (d, 1H, J = 8.6 Hz), 7.19-7.40 (m, 5H), 6.58 (d, 1H, J = 8.6 Hz), 5.35 (s, 2H), 2.90 (t, 2H, J =

6.2 Hz), 2.48 (t, 2H, J = 6.0 and 7.2 Hz) and 1.94- 2.04 (m, 1H); ^{13}C NMR (67 MHz, $CDCl_3$) δ 196.7, 165.5, 163.8, 137.8, 136.8, 128.5, 128.2, 128.1, 123.0, 110.2, 68.2, 38.2, 32.5 and 22.0; ES-MS *m/z* 254 ($(M+H)^+$)

2-Benzyloxy-5-vinyl-7,8-dihydro-quinoline (3).
A solution of **2** (116 mg, 0.46 mmol) in dry THF (2 mL) was added slowly to a solution of vinyl magnesium bromide (4 ml, 1 M) in dry THF (10 mL) at 0 C. After 2 hrs the reaction mixture was poured on ice and 2M HCl (20 mL) was added and the reaction was stirred over night. The reaction mixture was then basified with $NaHCO_3$ and extracted with EtOAc. The organic phase was dried with Na_2SO_4, evaporated and loaded on a silica gel column and eluted with (1:9) EtOAc:heptane to yield 73.8 mg (61 %) of **3**. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.54 (d, 1H, J= 8.4 Hz), 7.30-7.50 (m, 5H), 6.62 (d, 1H, J = 8.4 Hz), 6.53 (dd, 1H, J = 10.9 and 17.3 Hz), 6.06 (t, 1H, J = 4.8 Hz), 5.49 (d, 1H, J = 17.3 Hz), 5.39 (s, 2H), 5.19 (d, 1H, J = 10.9 Hz), 2.87 (t, 2H, J = 8.3) and 2.36-2.44 (m, 2H); ^{13}C NMR (67 MHz, $CDCl_3$) δ 162.0, 155.6, 137.6, 135.2, 134.9, 134.1, 128.5, 128.1, 127.8, 124.3, 123.4, 115.4, 107.8, 67.7, 30.7 and 23.1; ES-MS *m/z* 269 ($(M+H)^+$)

3-Benzyloxy-5,6,6a,7,8,9-hexahydro-benzo[f]quinoline-8-carboxylic acid methyl ester (4a) and 3-Benzyloxy-5,6,6a,7,8,9-hexahydro-benzo[f]quinoline-7-carboxylic acid methyl ester (4b).
A solution of **3** (73 mg, 0.28 mmol) and of methyl acrylate (2 mL) in toluene (5 mL) was added to a sealed reaction tube and heated to 120 C. The reaction was completed after 1.5 hrs as judged by TLC. The reaction mixture was evaporated and the crude was purified on a silica column (1:9) EtOAc:heptane to afford 62 mg (62 %) of **4a** and **4b** as 1:1 mixture. ES-MS *m/z* 350 ($(M+H)^+$)

3-Oxo-3,4,5,6,7,8,9,10-octahydro-benzo[f]quinoline-8-carboxylic acid methyl ester (5a) and 3-Oxo-3,4,5,6,7,8,9,10-octahydro-benzo[f]quinoline-7-carboxylic acid methyl ester (5b).
A solution of **4a** and **4b** (37 mg 0.11 mmol), trimethylsilyl chloride (46 mg, 0.42 mmol) and a large excess of NaI (0.5 mL) in DCM (5ml) were heated to 60 C in a sealed reaction tube. The reaction was followed with TLC. The reaction was quenched with MeOH after TLC (~1 hr) showed that all the starting material had been consumed. The reaction mixture was evaporated and the residue was extracted with DCM and water. The organic phase was evaporated and purified on a silica column eluted (95%:5%) DCM:MeOH to afford 26 mg (95 %) of **5a** and **5b** was as 1:1 mixture. ES-MS *m/z* 260 ($(M+H)^+$)

4-Methyl-3-oxo-3,4,5,6,7,8,9,10-octahydro-benzo[f]quinoline-8-carboxylic acid methyl ester (6a) and 4-Methyl-3-oxo-3,4,5,6,7,8,9,10-octahydro-benzo[f]quinoline-7-carboxylic acid methyl ester (6b).
5a and **5b** (10 mg, 0.04 mmol) were mixed with a large excess of lithium carbonate and a large excess of methyl iodide (100 µL) in DMF (1 mL) and then heated to 80 C. When TLC showed that all the starting material had been consumed after 8 hrs and the reaction was stopped. The product was purified by filtration trough dry silica plug in a syringe and eluted with (95%: 5%) DCM:MeOH to afford 10.9 mg (100 %) of **6a** and **6b** as 1:1 mixture. ES-MS *m/z* 274 ($(M+H)^+$)

2'-Methyl-4-benzyl-3-oxo-3,4,5,6,7,8,9,10-octahydro-benzo[f]quinoline-8-carboxylic acid methyl ester (7a) and 2'-Methyl-4-benzyl-3-oxo-3,4,5,6,7,8,9,10-octahydro-benzo[f]quinoline-7-carboxylic acid methyl ester (7b).
5a and **5b** (7.1 mg, 0.027 mmol) were mixed with lithium carbonate (4 mg, 0.055 mmol) and 2-methyl benzyl bromide (20 mg, 0.11 mmol) in DMF (1 mL) and then heated to 100 C. When TLC showed that all the starting material had been consumed after 8 hrs and the reaction was stopped. The solvent was evaporated and the residue was dissolved in a solution of THF (1 mL) and lithium hydroxide (0.5 mL, 1 M). When TLC showed that all the starting material had been consumed the reaction was extracted with DCM and then purified by filtration trough dry silica plug in a syringe and eluted with (95%: 5%) DCM:MeOH to afford 9.4 mg (100 %) of **7a** and **7b** as 1:1 mixture. ES-MS *m/z* 350 ($(M+H)^+$)

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