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Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 13 (2005) 5986–5995

Preparation of highly substituted γ-lactam follicle stimulating hormone receptor agonists

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Received 26 April 2005; revised 7 July 2005; accepted 7 July 2005
Available online 15 August 2005

Abstract—An unusual combination of Weinreb amidation and Mitsunobu lactam formation was used to prepare highly substituted γ-lactam analogues of a thiazolidinone follicle stimulating hormone receptor agonist. The analogue synthesis was stereoselective and the final products were chemically stable. Biological properties of the target molecules were nearly identical to those of the lead compound.

1. Introduction

Follicle stimulating hormone (FSH) is a 38 kDa protein that triggers maturation of ovarian follicles in women and spermatogenesis in men. It is released from the anterior pituitary gland, following stimulation by gonadotropin-releasing hormone (GnRH), and serves as the naturally occurring agonist of the FSH receptor (FSHR).[1] The receptors are located on granulosa cells in females and Sertoli cells in males. Natural or recombinant FSH is used as a fertility treatment in women.[2] Its expense and method of administration (injection in a clinical setting), however, make compliance difficult. A small molecule FSH agonist could offer considerable mitigation of these circumstances.[3,4]

A recent disclosure described the preparation and FSH agonist activity of thiazolidinones, such as **1**.[4a] Compounds were initially prepared using split–mix combinatorial synthesis technology and were tested as mixtures, following resin cleavage of the products, in a luciferase reporter gene assay dependent on FSHR activation (compound **1** EC_{50} = 14 nM).[5] Despite the ease of thiazolidinone preparation, little control over relative stereoselectivity at the 2,5-positions on the heterocyclic ring could be achieved. In our hands, this also occurred during resynthesis of the discrete compounds. In general, the *anti* isomer predominated, while additional testing of the separable diastereomers indicated that the more active species had the *syn* configuration. In addition to this, after separation to pure diastereomers both compounds tended to isomerize upon standing.

Chemical instability, observed in **1**, is presumed to originate from the activated hydrogen at the 5-position and/or the thioaminal carbon via a ring opening and closure equilibrium mechanism. In an attempt to overcome this situation, we designed a new compound, **2a** (Fig. 1), that has a methylene group in the place of the ring sulfur atom, as well as a methyl group replacing hydrogen at position 5. These changes were expected to impart chemical stability by locking the resulting γ-lactam in the active *syn* configuration. Structural similarity to compound **1** also suggests that biological activity should remain in this compound.

The proposed method of preparation for the target compound is shown in the retrosynthetic analysis (Fig. 2). The key step would be the proposed conversion of lactone **4** to highly substituted lactam **6** in a stereoselective fashion. An interesting way to implement this transformation would be amide formation (from the corresponding lactone) via Weinreb methodology,[6] followed by an intramolecular Mitsunobu cyclization of **5** to the lactam. It is desirable that this closure occur with inversion of configuration since the stereochemistry α to the carbonyl in lactone **4** will be opposite to that desired in the final compound **2**. Mitsunobu alkylation of primary and secondary amides generally occur with

Keywords: Follicle stimulating hormone; FSH; Mitsunobu reaction.
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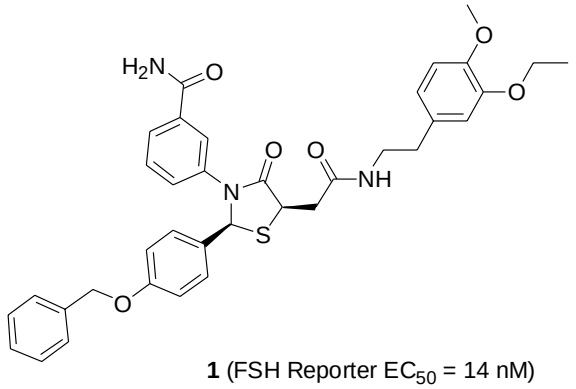
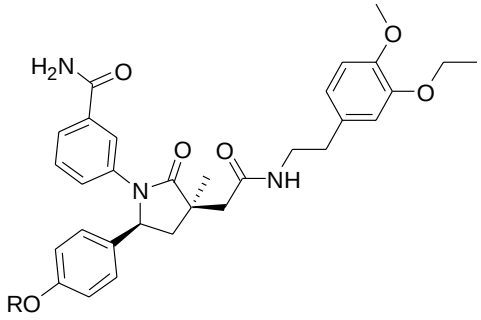
doi:10.1016/j.bmc.2005.07.025



1 (FSH Reporter EC_{50} = 14 nM)



2a, R = Bn

2b, R = n-Bu

Figure 1. FSHR agonist **1** and the proposed structure **2**.

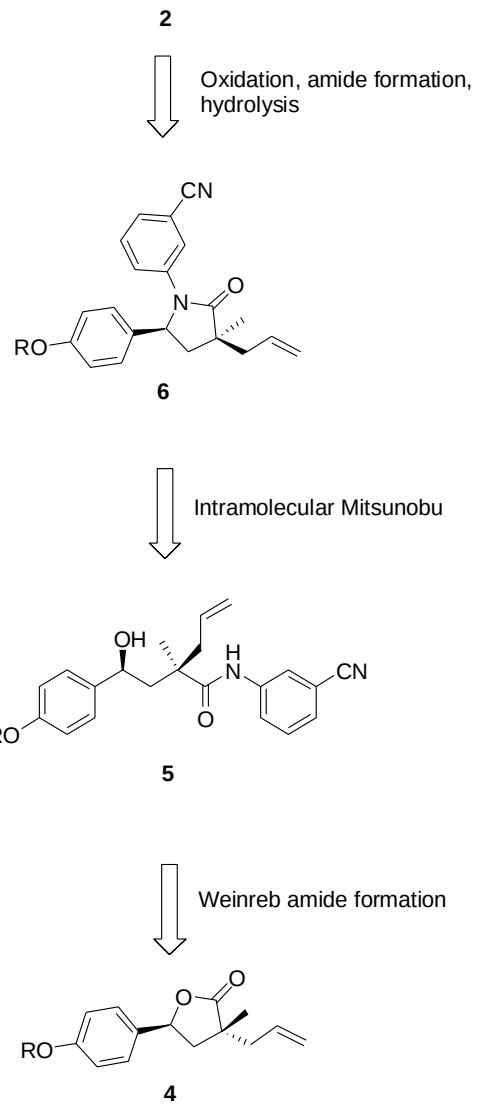


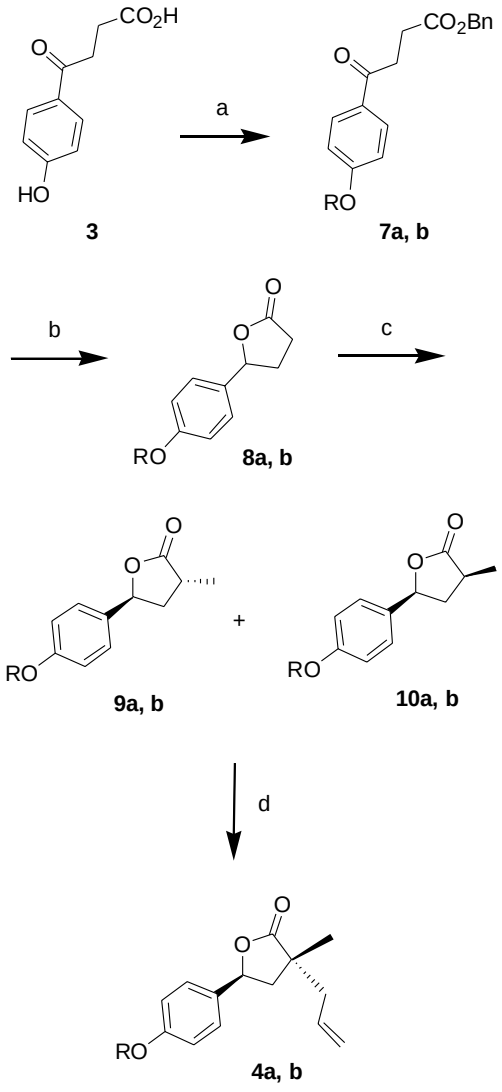
Figure 2. Retrosynthetic analysis of **2**.

the more acidic versions.[7] There are a few instances, however, of lactam formation via intramolecular cyclization,[8] while stereoselective examples are rare.[9] Conversion to the final product **2** should be accomplished by standard techniques.

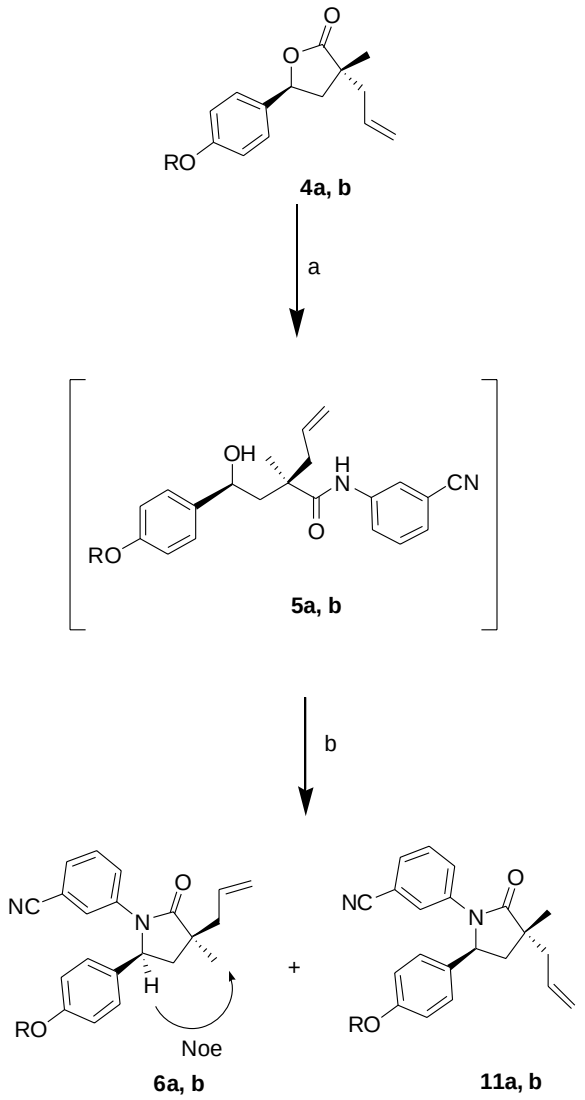
In addition to **2a**, compound **2b** was also prepared under the expectation (a priori) that biological activity of the *n*-butyl analogue of the benzyl compound **2a** would not differ significantly from the parent structure. With lower molecular weight (636 for **2a**, 602 for **2b**) and a C log *P* closer to 5.0 (5.99 for **2a**, 5.80 for **2b**), this compound may show better drug-like properties in future studies.

Preparation of intermediate **4** is shown in Scheme 1. The commercially available oxobutyric acid **3** reacted with benzyl bromide in the presence of potassium carbonate to give the ester **7a** in good yield, while **7b** was available commercially. Ketone reduction of **7a**, **b** with sodium borohydride provided the expected alcohols, which then spontaneously cyclized to lactones **8a**, **b** during workup procedures (see Section 2). The lactones were methylated under standard conditions to provide a separable mixture of diastereomers (**9a**:**10a**, 50:8 yield ratio and **9b**:**10b**, 67:9 yield ratio). The mixtures were allylated at the 3-position under conditions similar to those used for the previous reaction to provide the single isomers **4a**, **b** in 68% and 61% yields, respectively. The presumed relative stereochemistry of the products most likely resulted from phenyl group steric hindrance directing the nucleophilic attack from the opposite face of the lactones.

Key intermediate lactams **6a** and **b** were prepared, as shown in Scheme 2. Hence, lactones **4a** and **b** were treated with the aluminate of 3-cyanoaniline under Weinreb conditions.[6] Isolation of product **5a** from aluminum salts was difficult, so a one-pot procedure involving amidation and closure to the key intermediate lactam, via intramolecular Mitsunobu reaction, was attempted. Following amide formation, the intermediate **5a** was treated with triphenylphosphine (TPP) and diethylazodicarboxylate (DEAD) in toluene and stirred at room temperature. The reaction was followed by LC/MS and was complete in 1 h. Product isolation



Scheme 1. Reagents: (a) BnBr, K_2CO_3, H_2O, DMF (**7a**, R = Bn, 59%); (b) $NaBH_4$, NaOEt, EtOH (**8a**, R = Bn, 67%; **8b**, R = *n*-Bu, 64%); (c) LiHMDS, MeI, THF (**9a**, R = Bn, 50%; **10a**, 8%; **9b**, R = *n*-Bu, 67%; **10b**, 9%); (d) LiHMDS, allyl bromide, THF (**4a**, R = Bn, 68%; **4b**, R = *n*-Bu, 61%).

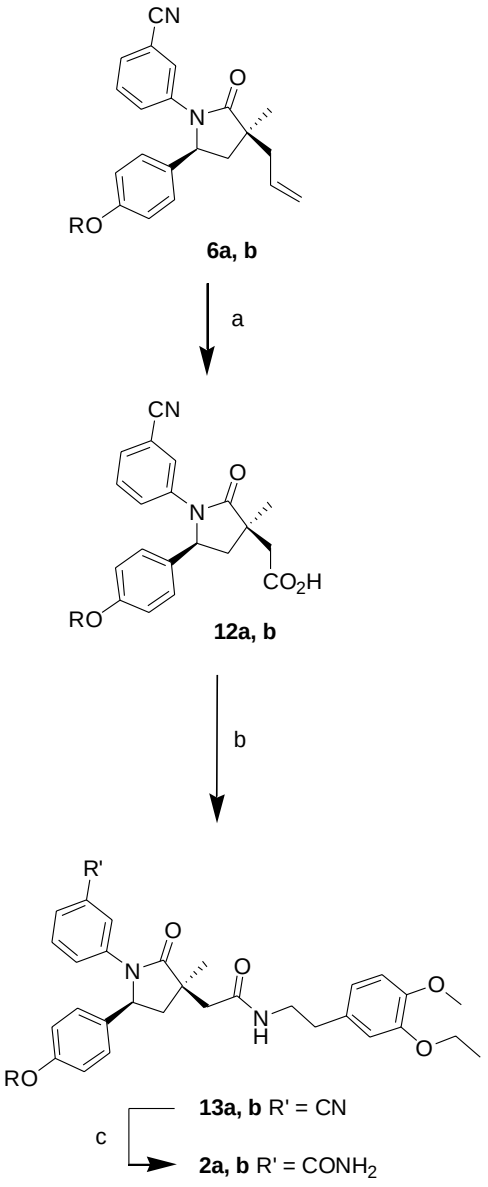


Scheme 2. Reagents: (a) $AlMe_3$, 3-cyanoaniline, toluene; (b) $(Ph)_3P$, DEAD, toluene (**6a**, R = Bn, 67%; **6b**, R = *n*-Bu, 39%; **11a**, R = Bn, 10%; **11b**, R = *n*-Bu, 0%).

and purification were considerably easier at this stage. The separable diastereomers **6a** and **11a** were isolated in a ratio of nearly 7:1 (total yield = 77%). NOE experiments confirmed the relative stereochemistry of lactam **6a**. This also confirmed that intramolecular Mitsunobu lactam formation can occur stereoselectively, even with a sensitive substrate, such as **5a**, which possesses an epimerizable benzylic alcohol *para* to the benzyloxy function. Transformation of the lactone **4b** to lactam **6b** under identical conditions occurred in modest yield but the diastereomer **11b** could not be detected.

Conversion of lactams **6a** and **b** to the final products **2a** and **b** (Scheme 3) began with oxidation of the olefins of **6** to the acids **11** (95% and 92% yields, respectively) under Sharpless conditions.[10] The acids were reacted with 3-ethoxy-4-methoxyphenethylamine using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) as the coupling reagent to provide the expected exocyclic amides **13a** and **b**. Finally, the nitriles were hydrolyzed under Evans conditions (LiOH, H_2O_2)[11] to give targets **2a** and **b** in 45% and 97% yields, respectively, over two steps. NMR analysis of the products indicated little change in structure and purity after standing for two weeks, thus confirming the expected increase in stability of **2a**, **b** in comparison to **1**.

Compounds **1** and **2a**, **b** were tested in agonist mode on a Chinese hamster ovary (CHO) cell line that expresses recombinant human FSHR and a luciferase reporter gene regulated by a cAMP response element (CRE).[5] EC_{50}s of 14, 25, and 27 nM were obtained for these analogues, respectively (Table 1). All compounds clearly possessed cell-based agonist activity. No compounds were active when the luciferase assay was run in antagonist mode (data not shown). The three compounds were also tested on a CHO-cell line that overexpressed



Scheme 3. Reagents: (a) RuO_2, $NaIO_4$, CCl_4, AcCN, H_2O (**12a**, R = Bn, 95%; **12b**, R = *n*-Bu, 92%); (b) 3′-ethoxy-4′-methoxyphenylethylamine, HATU, DIPEA, DMF (R = Bn, 48%; R = *n*-Bu, 68%); (c) 30% H_2O_2, $LiOH{\cdot}H_2O$, H_2O, THF (**2a**, R = Bn, 45%; **2b**, R = *n*-Bu, 97%).

the FSHR for the ability to induce cAMP production.[12] The analogues **1** and **2a**, **b** had EC_{50}s of 166 nM (76% efficacy compared to FSH), 400 nM (87% efficacy compared to FSH), and 400 nM (80% efficacy compared to FSH), respectively, indicating similar functional potency and efficacy levels (Table 1). In antagonist mode, they were inactive (data not shown). In addition, analogues **1** and **2a**, **b** were tested on CHO cells that did not express the FSHR by measuring cAMP production. The compounds were inactive, indicating the lack of a nonspecific effect (data not shown).

Additional functional selectivity studies were performed in a CHO-cell line overexpressing the recombinant human thyroid stimulating hormone receptor (TSHR). The receptor shares significant homology with the FSHR, indicating cross-reactivity as a potential safety issue. The ability of lactams **1** and **2a**, **b** to induce or inhibit cAMP production in this TSHR cell line was negative (data not shown). Comparison of biological activity profiles for **1** and **2a**, **b** indicates similarity between them, which confirms that structural changes designed to enhance chemical stability have not been deleterious to desired FSH agonist activity.

To conclude, α-methyl lactam analogues of a thiazolidinone FSH receptor agonist were prepared using a novel, stereoselective, intramolecular Mitsunobu cyclization. Relative stereochemistry was confirmed by 2D NMR. Lactams **2a** and **b** possessed FSH agonist activity similar to compound **1** in functional potency, efficacy, and selectivity over the TSHR.

2. Experimental

2.1. General methods

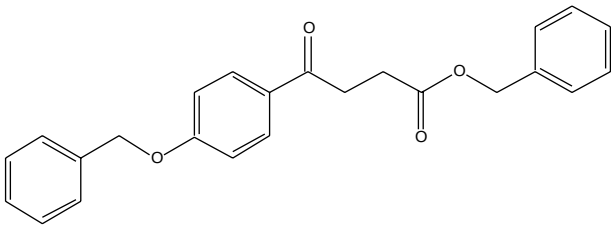
Appropriate safety practices were observed during all laboratory functions. General solvents and chemicals were purchased from VWR and used without further treatment. Anhydrous and deuterated solvents, as well as fine chemicals, were purchased from Aldrich Chemical Co. and used without further treatment. Microanalyses were performed by Robertson Microlit Labs (Madison, NJ). High-resolution mass spectra were taken on a Waters LC–TOFMS instrument. Accurate masses were measured to within 5 ppm of the calculated values. Proton ^1H and ^{13}C NMR were taken on a Bruker DPX300 (300 MHz) instrument and delta values (δ) were measured in parts per million using tetramethylsilane as an internal standard ($\delta = 0$ ppm). High performance liquid chromatography (HPLC) was performed with an Agilent 1100F series instrument with autosampler, thermoregulated column oven, and a diode array detector. The following method was used to determine the purity and retention times for intermediates and the final product:

Table 1. A comparison of functional FSH activity of **1** and **2a**, **b**

Compound	EC_{50} (nM) (% efficacy compared to FSH)	
	FSHR-dependent CRE LUC	cAMP production
1	14.4 ± 3.4 (*n* = 2; 92%)	166.7 ± 116.8 (*n* = 3; 76%)
2a	24.8 ± 11.8 (*n* = 2; 86%)	400 (*n* = 1; 87%)
2b	26.5 + 6.5 (*n* = 2; 111%)	400 (*n* = 1; 80%)

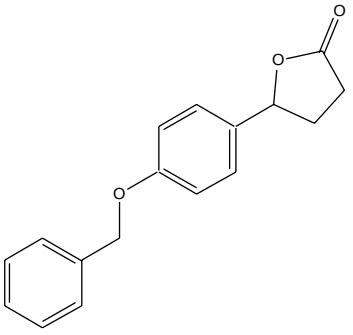
Data represent means ± SEM for replicate experiments as indicated in parentheses. EC_{50} values for luciferase and cAMP production for compound **1** and **2a**, **b** were not statistically significant as determined by *t*-test ($P > 0.05$).

Flow rate: 1.2 mL/min
Detection: 210–370 nm
Temperature: 30 °C
Column: Xterra RP18, 3.5 Um, 150 × 4.6 mm 85/15–5/95 (Ammonium formate buffer, pH 3.5/1:1 AcCN/MeOH) for 10 min, hold 4 min.



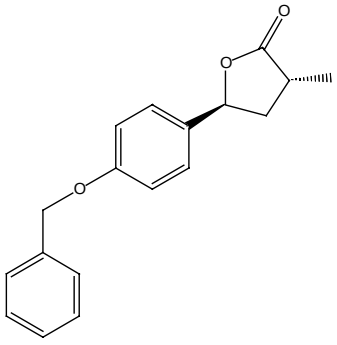
2.2. 4-(4-Benzyloxyphenyl)-4-oxo-butyric acid benzyl ester (7a)

Commercially available 4-(4-hydroxyphenyl)-4-oxo-butyric acid (**3**, 3.2 g, 17 mmol) was dissolved in DMF (200 mL). Potassium carbonate (8.83 g, 64 mmol) dissolved in water (60 mL) was added. Benzyl bromide (11.1 g, 64 mmol) was then added dropwise and after 1 h, the reaction was complete, as judged by TLC. The reaction mixture was partitioned between ethyl acetate (200 mL) and brine (200 mL). The organic layer was further washed with brine (3× 200 mL), dried ($MgSO_4$), and evaporated. The crude product was chromatographed on silica gel with 15% ethyl acetate/hexane to yield the title compound **7a** (3.8 g, 10.2 mmol, 59%). ^1H NMR ($CDCl_3$): $\delta = 7.96$ (d, $J = 8.8$ Hz, 2H), 7.32–7.44 (m, 10H), 7.01 (d, $J = 8.8$ Hz, 2H), 5.15 (s, 2H), 5.13 (s, 2H), 3.28 (t, $J = 6.6$ Hz, 2H), 2.80 (t, $J = 6.6$ Hz, 2H). ^{13}C NMR ($CDCl_3$): 196.52, 172.91, 162.69, 136.12, 135.90, 130.32, 129.81, 128.70, 128.54, 128.26, 128.19, 127.48, 114.58, 70.12, 66.47, 32.96, 28.36. MS (ESI-POS): $[M+H]^+ = 375$. Anal. Calcd for $C_{24}H_{22}O_4$: C, 76.99, H, 5.92. Found: C, 77.00, H, 5.73.



2.3. 5-(4-Benzyloxyphenyl)-dihydro-furan-2-one (8a)

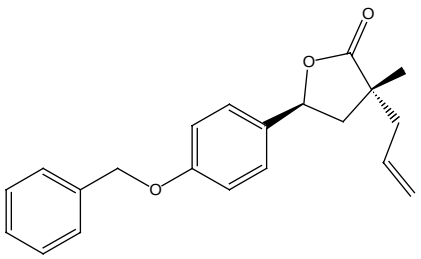
4-(4-Benzyloxyphenyl)-4-oxo-butyric acid benzyl ester (**7**, 3.8 g, 10 mmol) was dissolved in ethanol (200 mL). $NaBH_4$ (3.15 g, 83 mmol) and 21% NaOEt/EtOH solution (7 mL, 64 mmol) were added under nitrogen and the reaction mixture was heated to 50 °C. After 2 h, the reaction was complete, as judged by TLC. The reaction mixture was partitioned between ethyl acetate (200 mL) and brine (200 mL), the organic layer was washed further with brine (2× 200 mL), dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by silica gel chromatography (eluted with 10% ethyl acetate/hexane) to yield the title compound **8a** (1.8 g (67%), 6.7 mmol). ^1H NMR ($CDCl_3$): $\delta = 7.28$–7.49 (m, 7H), 7.06 (dd, $J = 6.8$, 1.9 Hz, 2H), 5.47 (dd, $J = 9.0$, 6.5 Hz, 1H), 5.12 (s, 2H), 2.52–2.75 (m, 3H), 2.14 (m, 1H) MS (ESI-POS): $[M+H]^+ = 269$. Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.1, H, 6.01. Found: C, 75.29, H, 6.31.



2.4. (*cis*)- and (*trans*)-5-(4-(Benzyloxy)phenyl)-3-methyldihydrofuran-2(3*H*)-one (9a and 10a)

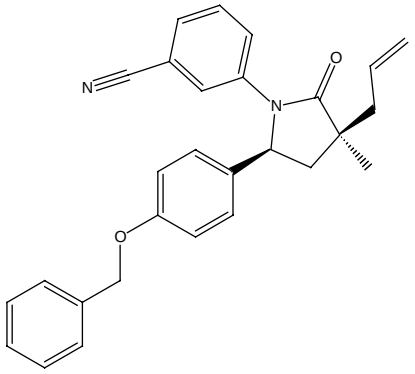
5-[4-(Benzyloxy)phenyl]dihydrofuran-2(*H*)-one (**8a**, 1.17 g, 4.3 mmol) was dissolved in THF (66 mL) and the solution was cooled to −78 °C under a nitrogen atmosphere. Lithium hexamethyldisilylamide (LiHMDS, 4.8 mL of a 1.0 M solution in tetrahydrofuran, 4.8 mmol) was added and the solution was allowed to stir for 5 min. Methyl iodide (0.68 g, 4.8 mmol) was added. After 1 h, the reaction was complete, as judged by TLC. Methanol (5 mL) was added to quench the reaction. The solvent was removed under vacuum and the crude mixture was subjected to column chromatography on silica gel (eluted with 15% ethyl acetate/hexane) to provide the title compound **9a** (600 mg (50%), 2.2 mmol): ^1H NMR (DMSO-d_6): $\delta = 7.28$–7.47 (m, 7H), 7.04 (dd, $J = 2.0$, 6.9 Hz, 2H), 5.57 (dd, $J = 7.3$, 5.9 Hz, 1H), 5.11 (s, 2H), 2.82 (m, 1H), 2.23–2.43 (m, 2H), 1.21 (d, $J = 7.3$ Hz, 3H). MS (ESI-POS): $[M+H]^+ = 283$. Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.57, H, 6.43. Found: C, 76.74, H, 6.49. A small amount of the *cis* isomer **10a**, 5-[4-(benzyloxy)phenyl]-3-methyldihydrofuran-2(3*H*)-one, was also obtained (92 mg (8%), 0.33 mmol): ^1H NMR (DMSO-d_6): $\delta = 7.31$–7.49 (m, 7H), 7.04 (d, $J = 8.7$ Hz, 2H), 5.35 (dd, $J = 10.9$, 5.4 5Hz, 1H), 5.12 (s, 2H), 2.92 (m, 1H), 2.70 (m, 1H), 1.81 (q, $J = 12.2$ Hz, 1H), 1.21 (d, $J = 7.0$ Hz, 3H). MS (ESI-POS): $[M+H]^+ = 283$. Anal. Calcd for

$C_{17}H_{16}O_3$: C, 76.57, H, 6.43. Found: C, 76.53, H, 6.50.



2.5. 3-Allyl-5-[4-(benzyloxy)phenyl]-3-methyldihydrofuran-2(3*H*)-one (4a)

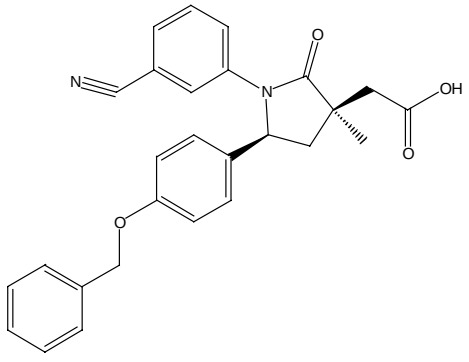
The isomeric mixture of 5-(4-(benzyloxy)phenyl)-3-methyldihydrofuran-2(3*H*)-one (**9a** and **10a**, 260 mg, 0.92 mmol) was dissolved in THF (20 mL), which was cooled to −78 °C. Lithium hexamethyldisilylamide (1.4 mL of a 1.0 M tetrahydrofuran solution, 1.4 mmol) was added slowly and the reaction mixture was allowed to stir for 5 min. Allyl bromide (890 mg, 7.4 mmol) was added and the reaction mixture was allowed to stir for 1 h. MeOH (3 mL) was added to quench the reaction and the solvent was removed. The crude product was chromatographed on silica gel (eluted with 12% ethyl acetate/hexane) to provide the title compound **4a** (220 mg (68%), 0.63 mmol). ^{1}H NMR (DMSO-d_6): δ = 7.33–7.49 (m, 7H), 7.03 (d, J = 11.5 Hz, 2H), 5.85 (m, 1H), 5.50 (dd, J = 10.0, 6.3, 1H), 5.18 (m, 2H), 5.12 (s, 2H), 2.58 (m, 1H), 2.38 (m, 2H), 2.01 (dd, J = 13.0, 10.1 Hz, 1H), 1.18 (s, 3H). ^{13}C NMR ($CDCl_3$): 180.13, 158.28, 136.85, 134.95, 133.06, 131.44, 128.33, 127.71, 127.57, 119.24, 114.71, 77.19, 69.09, 43.89, 41.93, 38.54, 22.28. MS (ESI-POS): $[M+H]^+$ = 323. Anal. Calcd for $C_{21}H_{22}O_3$: C, 78.23, H, 6.88. Found: C, 78.08, H, 6.89.



2.6. 3-Allyl-5-[4-(benzyloxy)phenyl]-3-methyl-2-oxopyrrolidin-1-yl benzonitrile (6a, 11a)

3-Aminobenzonitrile (**4a**, 142 mg, 1.2 mmol) was dissolved in toluene (12 mL) under nitrogen and trimethylaluminum (0.600 mL of a 2.0 M solution in heptane, 1.2 mmol) was added. This suspension was allowed to stir for 30 s. A solution of the lactone (150 mg, 0.43 mmol) in toluene (12 mL) was added with stirring. After 1 h, the reaction was complete, as judged by TLC. The solvent was removed, and the residue was partitioned between ethyl acetate (100 mL) and 3 N HCl (50 mL). The organic layer was washed further with brine, dried over $MgSO_4$, and evaporated to leave the crude product contaminated with aluminum salts. Toluene (15 mL) was added, followed by triphenylphosphine (524 mg, 2 mmol) and diethylazodicarboxylate (350 mg, 2 mmol). After 1 h, the reaction was judged complete by LC/MS. The solvent was removed and the crude product was purified by column chromatography on silica gel eluted with 20% ethyl acetate/hexane to yield the title compound **6a** (121 mg (67%), 0.28 mmol): ^{1}H NMR (DMSO-d_6): δ = 7.90 (d, J = 12.7 Hz, 1H), 7.65 (m, 1H), 7.28–7.56 (m, 7H), 7.20 (m, 2H), 6.90 (d, J = 8.7 Hz, 2H), 5.81–5.95 (m, 1H), 5.42–5.50 (m, 1H), 5.07–517 (m, 2H), 5.00 (s, 2H), 2.51 (m, 1H), 2.31 (m, 2H), 1.92 (dd, J = 12.7, 9.2 Hz, 1H), 1.20 (s, 3H). MS (ESI-POS): $[M+H]^+$ = 423.

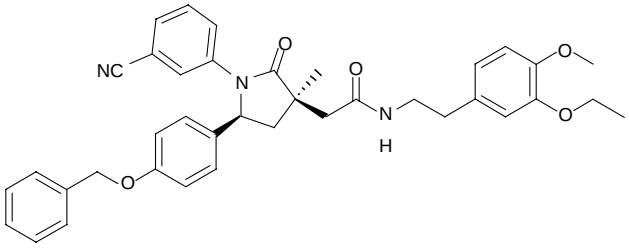
A small amount of the opposite diastereomer **11a** 3-allyl-5-[4-(benzyloxy)phenyl]-3-methyl-2-oxopyrrolidin-1-yl}benzonitrile was obtained (18 mg (10%), 0.043 mmol): ^{1}H NMR (DMSO-d_6): δ = 7.90 (m, 1H), 7.68 (m, 1H), 7.32–7.55 (m, 7H), 7.22 (d, J = 8.7 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 5.78–5.92 (m, 1H), 5.40–5.47 (dd, J = 7.7 Hz, 1H), 5.08–5.16 (m, 2H), 5.01 (s, 2H), 2.54–2.60 (m, 1H), 2.18–2.41 (m, 2H), 1.64–1.73 (dd, J = 13.1, 8.2 Hz, 1H), 1.22 (s, 3H). MS (ESI-POS): $[M+H]^+$ = 423. HRMS (ES-POS) calculated for $C_{28}H_{27}N_2O_2$: 423.2073. Found: 423.2058 (−3.4 ppm). Analytical HPLC: 75.5% purity at wavelengths 210–370, retention time: 10.9 min.



2.7. 5-[4-(Benzyloxy)phenyl]-1-(3-cyanophenyl)-3-methyl-2-oxopyrrolidin-3-ylacetic acid (12a)

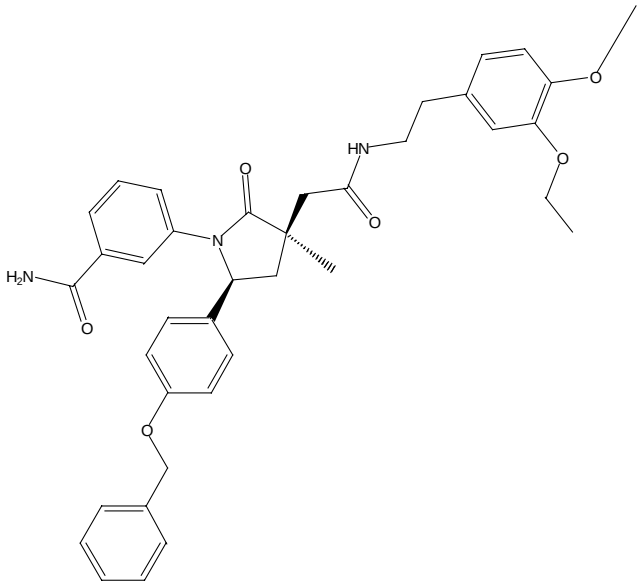
3-Allyl-5-[4-(benzyloxy)phenyl]-3-methyl-2-oxopyrrolidin-1-ylbenzonitrile (**6**, 80 mg, 0.19 mmol) in CCl_4 (1 mL) and acetonitrile (3 mL) under a nitrogen atmosphere was treated with $NaIO_4$ (490 mg, 2.3 mmol) in water

(4 mL) and stirred rapidly. RuO_2 (12 mg, 0.09 mmol) was added after a few minutes. After 4 h, the reaction was complete, as judged by LC/MS. The reaction mixture was dissolved in ethyl acetate (50 mL), washed with 3 N HCl (3× 25 mL), brine (25 mL), dried over $MgSO_4$, and evaporated to provide the title compound **12a** (80 mg, 0.18 mmol, 95%). ^{1}H NMR (DMSO-d_6): δ = 12.2–12.4 (br s, 1H), 7.78 (d, J = 8.6 Hz, 1H), 7.60–7.66 (m, 1H), 7.29–7.57 (m, 7H), 7.18 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.6 Hz, 2H), 5.45–5.52 (m, 1H), 5.00 (s, 2H), 2.54–2.83 (dd, J = 40.0, 4.2 Hz, 2H), 2.39 (m, 1H), 2.12 (m, 1H), 1.21 (s, 3H). MS (ESI-NEG): $[M-H]^-$ = 439. HRMS (ES-POS) calcd for $C_{27}H_{24}N_2O_4$: 441.1814. Found: 441.1820 (1.3 ppm). Analytical HPLC: 100% purity at wavelengths 210–370, retention time: 9.0 min.



2.8. 5-(4-Benzyloxyphenyl)-1-(3-cyano-phenyl)-3-methyl-2-oxo-pyrrolidin-3-yl]-*N*-[2-(3-ethoxy-4-methoxyphenyl)-ethyl]-acetamide (13a)

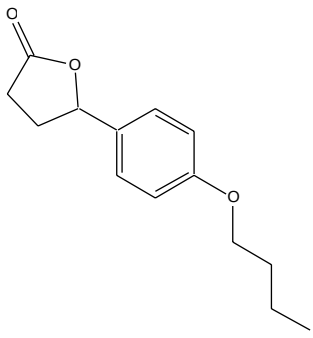
5-[4-(Benzyloxy)phenyl]-1-(3-cyanophenyl)-3-methyl-2-oxopyrrolidin-3- yl]acetic acid (**12**, 80 mg, 0.18 mmol) was dissolved in DMF (4 mL). Diisopropylethylamine (46 mg, 0.36 mmol) and 3-ethoxy-4-methoxyphenethylamine (70 mg, 0.36 mmol) were added. *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU, 137 mg, 0.36 mmol) was added under nitrogen. The reaction mixture was allowed to stir for 1 h and then the solution was partitioned between ethyl acetate (50 mL) and brine (25 mL). The organic layer was washed further with brine (2× 25 mL), 1 N HCl (25 mL), and brine again (25 mL). The solvent was dried over $MgSO_4$ and removed under reduced pressure. The crude product was purified by column chromatography on silica gel (eluted with 90% ethyl acetate/hexane) to provide the title compound (43 mg, 0.070 mmol, 48%). ^{1}H NMR (DMSO-d_6): δ = 8.01 (m, 1H), 7.78 (s, 1H), 7.62 (m, 1H), 7.28–7.50 (m, 8H), 6.92 (d, J = 8.7 Hz, 2H), 6.72–6.80 (m, 2H), 6.65–6.70 (m, 1H), 5.40–5.46 (m, 1H), 5.00 (s, 2H), 3.95 (q, J = 7.0 Hz, 4H), 3.69 (s, 3H), 2.62 (t, J = 9.2 Hz, 2H), 2.33–2.54 (dd, J = 41.2, 14.9 Hz, 2H), 2.26 (m, 1H), 2.12 (m, 1H), 1.28 (t, J = 7.0 Hz, 3H), 1.18 (s, 3H). MS (ESI-POS): $[M+H]^+$ = 618. HRMS (ES-POS) calcd for $C_{38}H_{39}N_3O_5$: 618.2968. Found: 618.2938 (−4.8 ppm). Analytical HPLC: 97% purity at wavelengths 210–370 nM, retention time: 10.4 min.



2.9. 5-[4-(Benzyloxy)phenyl]-3-(2-{[2-(3-ethoxy-4-methoxyphenyl) ethyl]amino}-2-oxoethyl)-3-methyl-2-oxopyrrolidin-1-yl]benzamide (2)

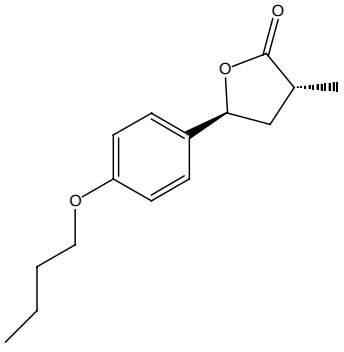
5-(4-Benzyloxyphenyl)-1-(3-cyano-phenyl)-3-methyl-2-oxo-pyrrolidin-3- yl]-*N*-[2-(3-ethoxy-4-methoxyphenyl)-ethyl]-acetamide from above (40 mg, 0.070 mmol) was dissolved in tetrahydrofuran (1 mL) under nitrogen. Hydrogen peroxide (0.032 mL of a 30% solution) and lithium hydroxidemonohydrate (9 mg, 0.21 mmol) were dissolved in water (0.400 mL). The peroxide solution was combined with the THF solution. The reaction mixture was allowed to stir for 18 h. The solution was partitioned between ethyl acetate (50 mL) and water (25 mL). The organic layer was further washed with brine (25 mL), dried ($MgSO_4$), and evaporated to provide the title compound **2** (20 mg, 45%, 0.030 mmol). ^{1}H NMR (DMSO-d_6): δ = 7.99 (br t, J = 5.5 Hz, 1H), 7.90 (br s, 1H), 7.85 (s, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.26–7.43 (m, 10H), 6.89 (d, J = 8.6 Hz, 2H), 6.72–6.80 (m, 2H), 6.63–6.69 (dd, J = 8.1, 1.7 Hz, 1H), 5.38 (m, 1H), 4.98 (s, 2H), 3.96 (q, J = 6.9 Hz, 2H), 3.69 (s, 3H), 3.15–3.40 (m, 2H), 2.63 (t, J = 7.1 Hz, 2H), 2.35–2.54 (dd, J = 40.8, 14.8 Hz, 2H), 2.29 (m, 1H), 2.12 (m, 1H), 1.32 (t, J = 6.9 Hz, 3H), 1.18 (s, 3H). ^{13}C NMR ($CDCl_3$): δ = 177.35, 169.63, 167.26, 157.46, 147.61, 147.15, 138.10, 136.84, 134.16, 133.45, 131.70, 128.39, 128.27, 127.82, 127.69, 126.31, 123.33, 122.93, 120.25, 114.44, 113.43, 111.74, 68.99, 63.34, 58.43, 55.27, 42.82, 42.55, 41.71, 22.71, 14.69. MS (ESI-POS): $[M+H]^+$ = 636. HRMS (ES-POS) calcd for $C_{38}H_{42}N_3O_6$: 636.3074. Found: 636.3070 (−0.6 ppm).

Analytical HPLC: 100% purity at wavelengths 210–370 nM, retention time: 9.6 min.



2.10. 5-(4-Butoxyphenyl)-dihydro-furan-2-one (8b)

Commercially available 4-(4-butoxyphenyl)-4-oxo-butyric acid methyl ester (**7b**, 264 mg, 1.0 mmol) was dissolved in ethanol (1 mL). $NaBH_4$ (44 mg, 1.2 mmol) and 21% NaOEt/EtOH solution (0.12 mL, 1.1 mmol) were added under nitrogen. After 2 h, there was no starting material observed by thin layer chromotography. The reaction mixture was partitioned between ethyl acetate and brine. The organic layer was washed twice with brine, dried over $MgSO_4$, and the solvent was removed. The crude product was purified by silica gel chromatography (10% ethyl acetate/hexane) to yield the title compound (170 mg, 0.64 mmol, 64%). ^{1}H NMR ($CDCl_3$): $\delta = 7.31$ (d, $J = 11$ Hz, 2H), 6.96 (d, $J = 11$ Hz, 2H), 5.44 (t, $J = 6$ Hz, 1H), 4.01 (t, $J = 5$ Hz, 2H), 2.85–3.02 (m, 3H), 2.08–2.15 (m, 1H), 1.65–1.90 (m, 2H), 1.40–1.53 (m, 2H), 0.97 (t, $J = 7$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 14.06$, 19.43, 29.46, 31.12, 31.46, 67.98, 81.64, 114.85, 127.13, 131.06, 159.56, 177.20. MS (ESI-POS): $[M+H]^+ = 235$. Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77, H, 7.74. Found: C, 71.86, H, 7.72.

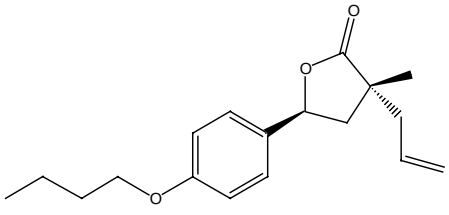


2.11. *trans*- and *cis*-5-(4-Butoxyphenyl)-3-methyldihydro-2(3*H*)-furanone (9b, 10b)

The 5-(4-*n*-butoxyphenyl)-3-methyl-2,3,4,5-tetrahydrofuran-2-one (**8b**, 300 mg, 1.3 mmol) was dissolved in dry THF (15 mL) in dry glassware, and the reaction vessel was cooled to −78 °C. LiHMDS (1.5 mL of 1.0 M solution in hexanes, 1.5 mmol) was added dropwise under nitrogen. This solution was allowed to stir for 10 min. Methyl iodide (2.84 g, 2.0 mmol) was added dropwise to the cooled solution. After 1 h, the reaction was complete. The solvent was removed under vacuum, and the residue was taken up in ethyl acetate. The organic solution was washed with a saturated solution of sodium bicarbonate (3×), brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude mixture was purified by silica gel chromatography (10% ethyl acetate/hexane) to yield the pure products as a *trans*-(**9b**, 170 mg, 67%), 0.86 mmol) and *cis*-(**10b**, 35 mg, 9%) isomers.

Compound **9b**: ^{1}H NMR ($CDCl_3$): $\delta = 7.33$ (d, $J = 11$ Hz, 2H), 6.94 (d, $J = 11$ Hz, 2H), 5.54 (t, $J = 6$ Hz, 1H), 4.01 (t, $J = 5$ Hz, 2H), 2.85–3.02 (m, 1H), 2.60–2.75 (m, 1H), 1.65–1.90 (m, 3H), 1.40–1.53 (m, 2H), 1.22 (d, $J = 7$ Hz, 3H), 0.97 (t, $J = 7$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 14.00$, 15.66, 19.38, 31.41, 36.67, 40.05, 67.92, 79.50, 144.77, 127.33, 131.05, 159.27, 179.41. MS (ESI-POS): $[M+H]^+ = 249$. Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55, H, 8.12. Found: C, 72.56, H, 8.34.

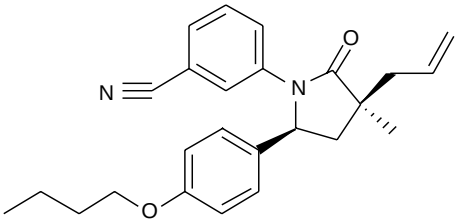
Compound **10b**: ^{1}H NMR ($CDCl_3$): $\delta = 7.43$ (d, $J = 11$ Hz, 2H), 6.94 (d, $J = 11$ Hz, 2H), 5.34 (dd, $J = 6$ Hz, 1H), 4.01 (t, $J = 5$ Hz, 2H), 2.85–3.02 (m, 1H), 2.60–2.75 (m, 1H), 1.65–1.90 (m, 3H), 1.40–1.53 (m, 2H), 1.22 (d, $J = 7$ Hz, 3H), 0.97 (t, $J = 7$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 14.04$, 15.15, 19.41, 31.45, 36.72, 40.05, 67.95, 79.50, 114.80, 127.36, 130.73, 159.58, 179.44. (ESI-POS): $[M+H]^+ = 249$. Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55, H, 8.12. Found: C, 72.30, H, 7.82.



2.12. 3-Allyl-5-(4-*n*-butoxyphenyl)-3-methyldihydrofuran-2(3*H*)-one (4b)

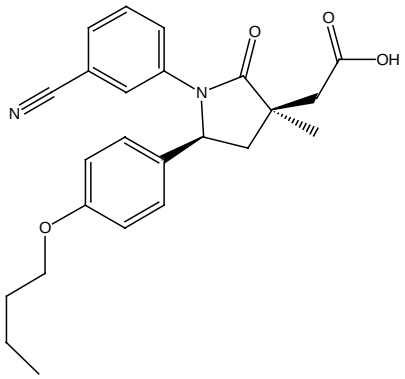
The 5-(4-*n*-butoxyphenyl)-3-methyldihydro-2(3H)-furanone mixture (**9b** and **10b**, 400 mg, 1.6 mmol) was dissolved in dry THF (20 mL) under a nitrogen atmosphere and cooled to −78 °C. LDA (0.900 mL of a 2 M solution, 1.8 mmol) was added dropwise and the reaction mixture was allowed to stir for 5 min. Allyl bromide (242 mg, 2 mmol) was added. After 2 h, the reaction was complete as judged by TLC. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and 3 N HCl.

The organic layer was washed with 3 N HCl and brine, followed by drying over $MgSO_4$ and solvent removal in vacuo. The crude product was purified by silica gel chromatography with 5% ethyl acetate/hexane to yield the title compound (280 mg, 0.97 mmol, 61%). ^1H NMR ($CDCl_3$): $\delta = 7.24$ (d, $J = 12$ Hz, 2H), 6.92 (d, $J = 12$ Hz, 2H), 5.76–5.95 (m, 1H), 5.16–5.40 (m, 2H), 3.98 (t, $J = 7$ Hz, 2H), 2.60–2.72 (m, 1H), 2.34–2.52 (m, 1H), 1.70–1.81 (m, 2H), 1.50–1.71 (m, 2H), 0.99 (t, $J = 7$ Hz, 3H). MS (ESI-POS): $[M+H]^+ = 289$. Anal. Calcd for $C_{18}H_{24}O_5$: C, 74.97, H, 8.39. Found: C, 73.17, H, 8.14. HRMS (ES-POS) calcd for $C_{18}H_{24}O_3$: 289.1800. Found: 289.1799 (−0.2 ppm). Analytical HPLC: 93.8% purity at 210 nm; 96.8% at 230 nm , retention time: 25.2 min. NMR (DMSO-d_6): 180.85, 159.41, 133.81, 131.81, 128.38, 119.93, 115.07, 77.97, 67.82, 44.62, 42.71, 41.05, 31.36, 23.08, 19.37, 14.32.



2.13. 3-Allyl-5-(4-*n*-butoxyphenyl)-3-methyl-2-oxo-pyrrolidin-1-yl]-benzonitrile (6b)

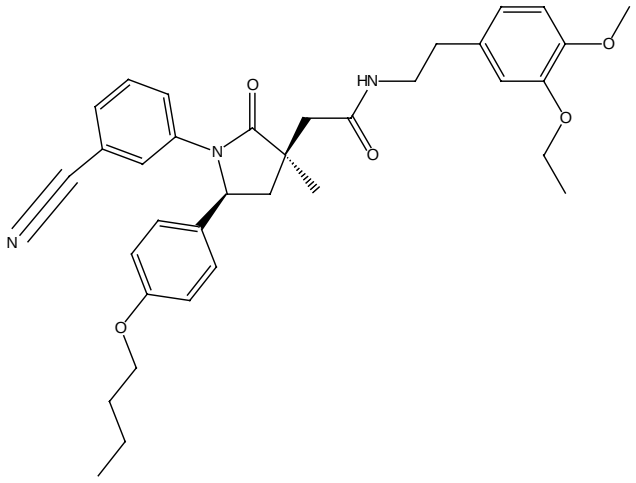
The 3-allyl-5-(4-*n*-butoxyphenyl)-3-methyldihydrofuran-2(3*H*)-one (**4b**, 670 mg, 2.3 mmol) was dissolved in toluene (60 mL) and set aside. 3-Aminobenzonitrile (390 mg, 3.3 mmol)) was dissolved in toluene (60 mL) under nitrogen and trimethylaluminum (1.7 mL of a 2 M solution, 3.3 mmol) was added and stirred several minutes. The two solutions were combined. After 5 h, the reaction was complete, as judged by TLC. The solvent was removed under vacuum and the residue was partitioned between ethyl acetate and 1 N HCl. The organic layer was washed with 1 N HCl (2×) and brine and then dried over $MgSO_4$ followed by solvent removal in vacuo. The residue was dissolved in toluene (60 mL). Triphenylphosphine (2.33 g, 8.8 mmol) was added to the reaction mixture, followed by diethylazodicarboxylate (1.44 g, 8.7 mmol). The reaction was complete after 2 h as judged by TLC. The solvents were removed in vacuo and the resulting crude product was chromatographed on silica gel with 15% ethyl acetate/hexane to provide the title compound as a single diastereomer (350 mg, 1.0 mmol, 39%). ^1H NMR ($CDCl_3$): $\delta = 7.69$ (s, 1H), 7.54–7.61 (m, 1H), 7.30 (m, 2H), 7.07 (d, $J = 10$ Hz, 2H), 6.80 (d, $J = 10$ Hz, 2H), 5.72–5.90 (m, 1H), 5.05–5.20 (m, 3H) 3.90 (t, $J = 4$ Hz, 2H), 2.50 (m, 1H), 2.23–2.40 (m, 2H), 2.01–2.10 (m, 1H), 1.67–1.81 (m, 2H), 1.38–150 (m, 2H), 0.98 (t, 3H). MS (ESI-POS): HRMS (ES-POS) calcd for $C_{18}H_{24}O_3$: 389.2240. Found: 389.2225 (−4.0 ppm). Analytical HPLC: 94.2% purity at wavelengths 210–370 nm, retention time: 11.0 min.



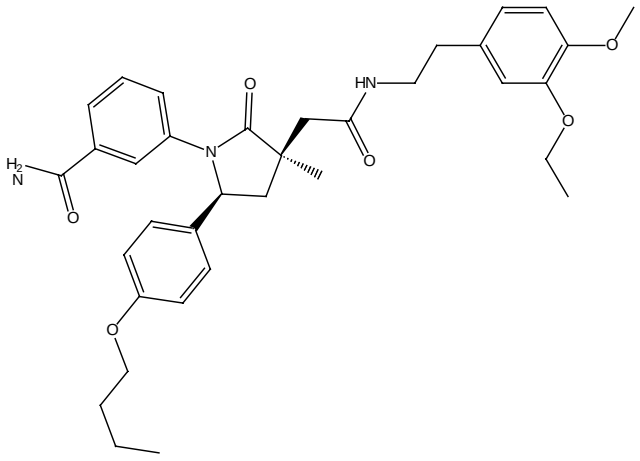
2.14. [5-(4-*n*-Butoxyphenyl)-1-(3-cyanophenyl)-3-methyl-2-oxopyrrolidin-3-yl]-acetic acid (12b)

The 3-[3-allyl-5-(4-*n*-butoxyphenyl)-3-methyl-2-oxo-pyrrolidin-1-yl]- benzonitrile (**6b**, 500 mg, 1.3 mmol) was dissolved in CCl_4 (6 mL) and acetonitrile (18 mL). A solution of $NaIO_4$ (3.41 g, 16 mmol) in water (22 mL) was added. To this heterogeneous mixture, RuO_2 (86 mg, 0.5 mmol) was added. The mixture was stirred at 40 °C for 20 h, followed by solvent removal in vacuo. The resulting crude mixture was partitioned between ethyl acetate and water. After separation, the organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure to provide the title compound (470 mg, 1.2 mmol, 92%). ^1H NMR ($CDCl_3$): $\delta = 11.0–13.0$ (br s, 1H), 7.72 (s, 1H), 7.54–7.63 (m, 1H), 7.32 (d, $J = 6$ Hz, 2H), 7.04 (d, $J = 10$ Hz, 2H), 6.80 (d, $J = 10$ Hz, 2H), 5.17 (m, 1H), 3.93 (t, $J = 4$ Hz, 2H), 2.50 (m, 3H), 2.23–2.40 (m, 2H), 2.01–2.10 (m, 2H), 1.67–1.81 (m, 2H), 1.38–150 (m, 2H), 0.98 (t, 3H). MS (ESI-POS): HRMS (ES-POS) calcd for $C_{24}H_{26}N_2O_4$: 407.1954. Found: 407.1974 (5.0 ppm). Analytical HPLC: 100% purity at wavelengths 210–370 nm, retention time: 12.8 min.

2.15. 2-[5-(4-*n*-Butoxyphenyl)-1-(3-cyanophenyl)-3-methyl-2-oxo-pyrrolidin-3-yl]-*N*-[2-(3-ethoxy-4-methoxyphenyl)-ethyl]-acetamide (13b)



The [5-(4-*n*-butoxyphenyl)-1-(3-cyanophenyl)-3-methyl-2-oxopyrrolidin-3-yl]- acetic acid (**12b**, 210 mg, 0.5 mmol) was dissolved in DMF (16 mL). Diisopropylethylamine (129 mg, 1 mmol) and 3′-ethoxy-4′-methoxyphenethylamine (195 mg, 1 mmol) were added. *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′- tetramethyluronium hexafluorophosphate (HATU, 380 mg, 1 mmol) was added under nitrogen. The reaction mixture was allowed to stir for 1 h under nitrogen. The reaction solution was partitioned between ethyl acetate and brine, the organic layer was washed further with brine (2×), 1 N HCl, and brine again. The solvent was then dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was subjected to column chromatography and eluted with 60% ethyl acetate/hexane to yield the title compound (200 mg, 0.34 mmol, 68%). ^{1}H NMR ($CDCl_3$): 7.94 (s, 1H), 7.57 (s, 1H), 7.43 (m, 1H), 7.28 (d, J = 5 Hz, 2H), 7.05 (d, J = 9 Hz, 2H), 6.64–6.78 (m, 5H), 6.04 (m, 1H), 5.05 (m, 1H), 3.98 (t, J = 4 Hz, 2H), 3.80–3.86 (m,5H), 3.42–3.52 (m, 2H), 2.54 (m, 3H), 2.23–2.40 (m, 2H), 2.01–2.10 (m, 2H), 1.67–1.81 (m, 2H), 1.38–1.50 (m, 2H), 0.92 (t, 3H). MS (ESI-POS): HRMS (ES-POS) calcd for $C_{35}H_{31}N_3O_5$: 584.3124. Found: 584.3126 (0.3 ppm). Analytical HPLC: 100% purity at wavelengths 210–370 nm, retention time: 4.7 min.



2.16. 3-[5-(4-*n*-Butoxyphenyl)-3-{[2-(3-ethoxy-4-methoxyphenyl)-ethylcarbamoyl]-methyl}-3-methyl-2-oxopyrrolidin-1-yl]-benzamide (2b)

2-[5-(4-Butoxyphenyl)-1-(3-cyano-phenyl)-3-methyl-2-oxopyrrolidin-3- yl]-*N*-[2-(3-ethoxy-4-methoxyphenyl)-ethyl]-acetamide (**13b**, 181 mg, 0.31 mmol) was dissolved in THF (2 mL) under nitrogen. Hydrogen peroxide (0.068 mL of a 30% solution in water, 0.62 mmol) and LiOH monohydrate (15 mg, 0.37 mmol) were dissolved in water (0.5 mL). The peroxide solution was combined with the THF solution. The reaction mixture was allowed to stir for 18 h. The solution was partitioned between ethyl acetate and water. The organic layer was further washed with water, brine, dried ($MgSO_4$), and evaporated to provide the title compound (180 mg, 3.0 mmol, 97%). ^{1}H NMR (DMSO-d_6): 7.96 (m, 1H), 7.87 (s, 1H), 7.82 (m, 1H), 7.24–7.61 (m, 5H), 6.74–6.82 (m, 3H), 6.64 (m, 1H), 5.35 (m, 1H), 3.98 (t, J = 4 Hz, 2H), 3.80–3.86 (m, 5H), 3.42–3.52 (m, 2H), 2.54 (m, 3H), 2.23–2.40 (m, 2H), 2.01–2.10 (m, 2H), 1.67–1.81 (m, 2H), 1.38–1.47 (m, 2H), 0.92 (t, 3H). MS (ESI-POS): HRMS (ES-POS) calcd for $C_{35}H_{43}N_3O_6$: 602.3230. Found: 602.3233 (0.5 ppm). Analytical HPLC: 100% purity at wavelengths 210–370 nm, retention time: 16.4 min.

Acknowledgments

The authors thank Rebecca Dooley for obtaining 2D NMR, and Jamin Chi, Ed Meade, and Gi-Chung Chen for performing pharmacology experiments.

References and notes

1. Chappel, S.; Buckler, D.; Kelton, C.; El Tayer, N. *Hum. Reprod.* **1998**, *13*(Suppl. 3), 1998.
2. Out, H. J. *Excerpts in Human Reproduction*, 1st ed.; NV Organon: Oss, The Netherlands, 1997.
3. For examples of small molecule FSH antagonists see: Wrobel, J.; Green, D.; Jetter, J.; Kao, W.; Rogers, J.; Perez, M. C.; Hardenburg, J.; Deecher, D. C.; Lopez, F. J.; Arey, B. J.; Shen, E. S. *Bioorg. Med. Chem.* **2002**, *10*, 639–656.
4. (a) For examples of small molecule FSH agonists see: MacLean, D.; Holden, F.; Davis, A. F.; Scheuerman, R. A.; Yanofsky, S.; Holmes, C. P.; Fitch, W. L.; Tsutsui, K.; Barrett, R. W.; Gallop, M. A. *J. Comb. Chem.* **2004**, *6*, 196–206; (b) Guo, T.; Adang, A. E. P.; Dolle, R. E.; Dong, G.; Fitzpatrick, D.; Geng, P.; Ho, K.-K.; Kultgen, S. G.; Liu, R.; McDonald, E.; McGuinness, B. F.; Saionz, K. W.; Valenzano, K. J.; van Straten, N. C. R.; Xie, D.; Webb, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1713–1716; (c) Guo, T.; Adang, A. E. P.; Dong, G.; Fitzpatrick, D.; Geng, P.; Ho, K.-K.; Jibilian, C. H.; Kultgen, S. G.; Liu, R.; McDonald, E.; Saionz, K. W.; Valenzano, K. J.; van Straten, N. C. R.; Xie, D.; Webb, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1717–1720.
5. Albanese, C.; Christin-Mautre, S.; Sluss, P. M.; Crowley, W. F.; Jameson, J. L. *Mol. Cell. Endocrinol.* **1994**, *101*, 211–219.
6. Anwer, B.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, *18*, 4171–4174.
7. Defacqz, N.; Tran-trieu, V.; Cordi, A.; Marchand-Brynaert, J. *Tetrahedron Lett.* **2003**, *44*, 9111–9114.
8. (a) Townsend, C. A.; Nguyen, L. T. *Tetrahedron Lett.* **1982**, *23*, 4859–4862; (b) Kadow, J. F.; Vyas, D. M.; Doyle, T. W. *Tetrahedron Lett.* **1989**, *30*, 3299–3302; (c) Zegrocka, O.; Abramski, W.; Urbanczyk-Lipkowska, Z.; Chmielewski, M. *Carbohydr. Res.* **1998**, *307*, 33–43; (d) Bell, I. M.; Beshore, D. C.; Gallicchio, S. N.; Williams, T. M. *Tetrahedron Lett.* **2000**, *41*, 1141–1145.
9. Hadfield, P. S.; Galt, R. H. B.; Sawyer, Y.; Layland, N. J.; Page, M. I. *J. Chem. Soc., Perkin Trans. 1* **1997**, 503–509.
10. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936–3938.
11. Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141–6144.
12. Stevis, P. E.; Deecher, D. C.; Lopez, F. J.; Frail, D. E. *Endocrine* **1999**, *10*, 153.