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Research Article

Synthesis of two non-peptidyl GnRH receptor antagonists via [^{14}C]carbonylation

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Summary

In support of a program to develop a new gonadotropin releasing hormone (GnRH) receptor antagonist, two ^{14}C labelled candidate tracers, **^{14}C-1** and **^{14}C-2**, were synthesized for utilization in metabolism studies. A slight modification of the Medicinal Chemistry route for the synthesis of the antagonists provided iodide **4**. Palladium (0) catalyzed **[^{14}C]**carbonylation of **4** proceeded in good chemical yield to afford acid **^{14}C-3** which served as a common precursor to **^{14}C-1** and **^{14}C-2**.

Key Words: [^{14}C]carbon monoxide; GnRH receptor antagonist; **[^{14}C]**carbonylation

Introduction

Gonadotropin releasing hormone (GnRH) is a decapeptide secreted by the hypothalamus which binds to GnRH receptors in gonadotropic cells of the pituitary gland; this binding activates the receptor and causes a G-protein coupled cascade that results in the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH).[1] LH

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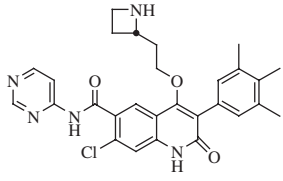
primarily regulates gonadal steroid biogenesis (estrogen and progesterone in females and androgen in males) while FSH stimulates spermatogenesis in males and follicular ripening in females. GnRH receptor antagonist and agonists have shown clinical efficacy for the treatment of several disease states including prostate cancer, breast cancer and endometriosis.[2] Chloroquinolones **1** and **2** were identified as potent and selective antagonists of the human GnRH receptor.[3,4] In order to perform preliminary drug metabolism and distribution studies, ^{14}C labelled tracers of each were required.



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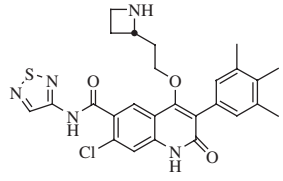

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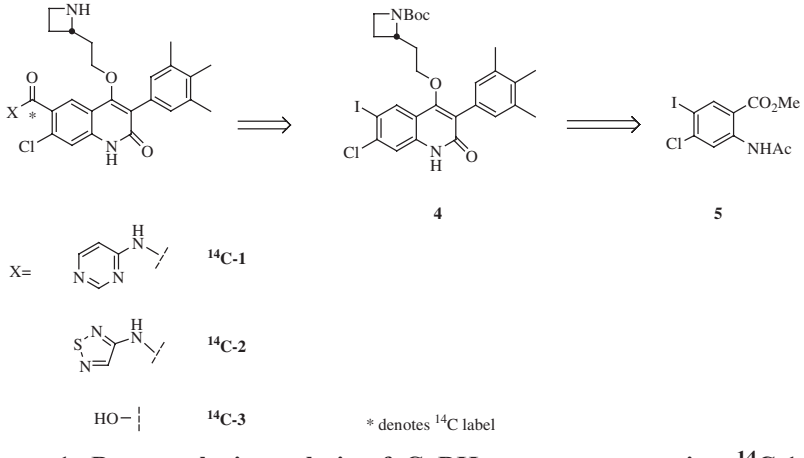
Results and discussion

DeVita *et al.* have reported the synthesis of quinolone **1** in 10 steps from methyl 2-(acetylamino)-4-chlorobenzoate[3]; however, this synthesis was not readily amenable to late stage incorporation of ^{14}C into the central core of the molecule. A simple modification of this route and analogous to that reported by Walsh[5] would provide iodide **4** in three steps from **5** (Scheme 1). A **[^{14}C]**carbonylation of **4** could be used to access **^{14}C-3**, a common labelled intermediate for synthesis of both **^{14}C-1** and **^{14}C-2**.



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Scheme 1. Retrosynthetic analysis of GnRH receptor antagonists ^{14}C-1 and ^{14}C-2

In order to prepare the iodo compound **4**, a four-step sequence shown in Scheme 2 was used. Methyl 2-(amino)-4-chlorobenzoate was regioselectively iodinated with silver sulfate and iodine to give aniline **5** which was coupled with 3,4,5-trimethylphenylacetyl chloride in 1, 2-dichloroethane to give amide **6** in 88% yield over 2 steps. Lithium hexamethyldisilazide initiated cyclization of **6** provided 4-hydroxy-quinolone **7**, and Mitsunobu coupling of **7** and azetidine **8** gave iodide **4** in 54% overall yield from methyl 2-(amino)-4-chlorobenzoate.

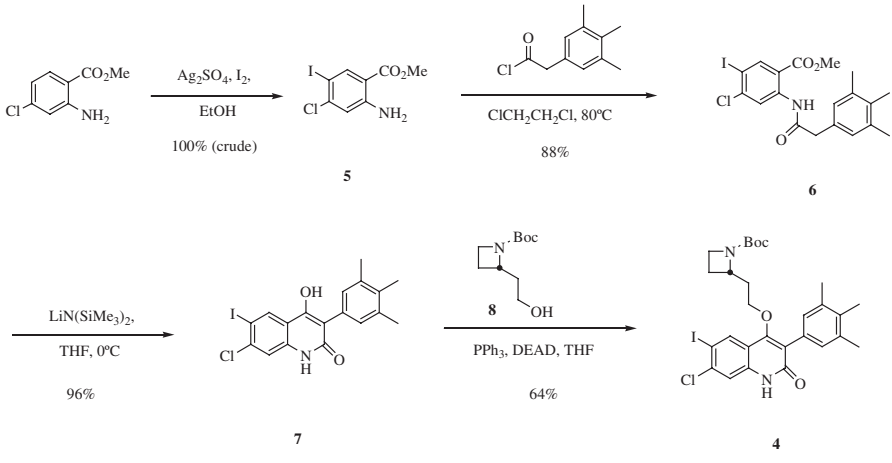
Palladium (0) catalyzed **[^{14}C]**carbonylation of **4** in the presence of KOAc using ca. 2.7 equivalents of ^{14}CO generated from lithium [^{14}C]formate[6] gave carboxylic acid **^{14}C-3** in 87% chemical yield (32% radiochemical yield, Scheme 3). An EDC-catalyzed coupling of **^{14}C-3** with either 4-aminopyrimidine or 2-aminothiazole followed by deprotection of the Boc group with trifluoroacetic acid provided **^{14}C-1** and **^{14}C-2** respectively. Purification was effected by preparative HPLC to give 150 μCi of **^{14}C-1** and 190 μCi of **^{14}C-2**.

The syntheses of these ^{14}C labelled tracers using a Pd-catalyzed [^{14}C]carbonylation of an aryl halide precursor as the key label incorporating step demonstrates the utility of this reaction for late stage label incorporation.

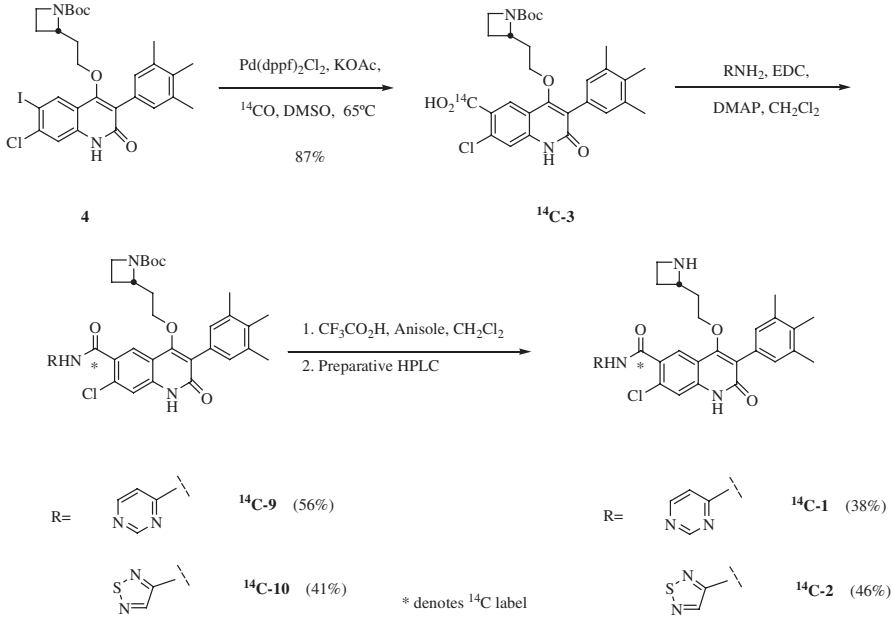
Experimental

General

$Ba^{14}CO_3$ was obtained from New England Nuclear. [1,1′-Bis(diphenylphosphino)ferrocene]dichloro-palladium(II) ($Pd(dppf)_2Cl_2$) was



Scheme 2. Synthesis of iodide 4



Scheme 3. Synthesis of GnRH receptor antagonist tracer ^{14}C-1 and ^{14}C-2

obtained from Aldrich. Anhydrous solvents were obtained from Aldrich and were dried over 4 Å molecular sieves for at least 24 h prior to use with the exception of THF and CH_2Cl_2 which were distilled from the appropriate drying agents. ^{1}H NMR spectra were recorded on a Varian XL series 400 MHz spectrometer, and ^{13}C NMR spectra were recorded on a Varian U-400 spectrometer. Low resolution mass spectral analyses were obtained with a LKB 9000 at an ionizing voltage of 70 eV. High resolution mass spectral analysis was obtained using a Finnigan New Star FT/ICR with electrospray ionization. LC/MS analyses were performed on an HP MSD-100 using a XDB-C8 column, with 5–95% gradient over 15 min with MeCN-2 mM ammonium formate buffer (pH 3.5) and electrospray ionization. Analytical HPLC was performed using a Shimadzu HPLC system with LC-10ATVP pumps, a SPD-10AVP UV detector, a CTO-10ASVP column oven heated to 40°C, a SCL-10A controller and a Packard Radiomatic^{TM} 150TR flow monitor. The radioactive products were identified by HPLC comparison with unlabelled reference material using either method A (30–100% MeCN–0.1% aqueous trifluoroacetic acid over 30 min, Zorbax SB C-18), method B (23% MeCN-0.1% aqueous trifluoroacetic acid over 30 min, Zorbax SB C-18), method C (40–100% MeCN-0.1% aqueous $HClO_4$ over 30 min, Zorbax RX C-8), or method D (45% MeOH-0.1%

aqueous $HClO_4$ over 45 min, Zorbax RX C-8). All HPLC analyses were conducted with the HPLC column heated to 30°C and were concluded with a 10 min wash of 100% organic. Normal phase column chromatography was carried out utilizing silica gel 60 (E. Merck).

*Methyl 2-amino-4-chloro-5-iodobenzoate (**5**):* To a mixture of 6.8 g (27 mmol) iodine and 8.4 g (27 mmol) silver (I) sulfate in 270 ml absolute ethanol was added 5.0 g (27 mmol) methyl 2-amino-4-chlorobenzoate. The resulting reaction mixture was stirred at room temperature for 45 min. The reaction mixture was filtered through a pad of Celite and the solvent was removed under vacuum. The residue was dissolved in 400 ml ethyl acetate and washed with saturated aqueous sodium bicarbonate (3×50 ml), water (3×50 ml) and once with brine. The organic layer was dried over magnesium sulfate, filtered and the filtrate concentrated under vacuum to afford 8.5 g ($\sim$100%) of an off-white solid. ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 3 H), 5.80 (s, 2 H), 6.80 (s, 1 H), 8.24 (s, 1 H). FAB-MS: calculated for $C_8H_7ClINO_2$ 311; found 312 (M + H, 100).

*Methyl 4-chloro-5-iodo-2-N-(3,4,5-trimethylphenyl)acetylaminobenzoate (**6**)*: To a solution of 3.28 g (10.5 mmol) methyl 2-amino-4-chloro-5-iodobenzoate (**5**) in 15 ml 1,2-dichloroethane was added 2.08 g (10.5 mmol) 3,4,5-trimethylphenylacetyl chloride. The resulting reaction mixture was heated at reflux for 4 hours. The reaction mixture was cooled to room temperature and the solvent was removed under vacuum. The resulting off-white solid was triturated in hot methanol. The mixture was then cooled in an ice bath, solids were filtered, washed with ice cold methanol and dried to afford 4.36 g (88%) of product as a white solid. ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 3 H), 2.27 (s, 6 H), 3.62 (s, 2 H), 3.85 (s, 3 H), 6.98 (s, 2 H), 8.39 (s, 1 H), 8.94 (s, 1 H).

*7-Chloro-4-hydroxy-6-iodo-3- (3,4,5-trimethyl) phenyl-1H-quinolin-2-one (**7**):* To a solution of 4.36 g (9.2 mmol) amide **6** in 5 ml dry tetrahydrofuran under nitrogen atmosphere at 0°C was added dropwise via syringe 23.1 ml (23.1 mmol, 2.5 equivalent) of a solution of lithium bis(trimethylsilyl)amide (1.0 M in THF). The resulting reaction mixture was stirred at 0°C for 1.5 h then acidified with 100 ml 6 N aqueous HCl/ice (1:1). The resulting solids were stirred vigorously, filtered, washed with ice cold water followed by ice cold acetonitrile. The resulting off-white solid was dried in a vacuum oven at 50°C for 16 h to afford 3.89 g (96%) of the product. ^{1}H NMR (DMSO-d_6): δ 2.14 (s, 3H), 2.24 (s, 6H),

3.85 (s, 3H), 6.93 (s, 2H), 7.37 (s, 1H), 8.43 (s, 1H). FAB-MS: calculated for $C_{20}H_{18}ClNO_4$ 371; found 372 (M + H, 100).

4-(2-(N-t-Butoxycarbonylazetidin-2(S)-yl)ethoxy-7-chloro-6-iodo-3-(3,4,5-trimethyl)phenyl-1H-quinolin-2-one (**4**): To a vigorously stirred solution of 2.52 g (12.5 mmol) *N-t*-butoxycarbonylazetidin-2(S)-ethanol (**8**) in 50 ml dry tetrahydrofuran under nitrogen was added 6.6 g (15 mmol) quinolin-2-one **7**. To the resulting mixture was added 3.94 g (15 mmol) of PPh_3 then dropwise by syringe 2.43 ml (15 mmol) diethylazodicarboxylate. The resulting mixture was stirred at room temperature for 16 h at which time 50 ml silica gel was added to the reaction mixture. The excess solvent was removed under vacuum to provide a free flowing powder which was applied to the top of a prepacked silica gel column. The column was eluted with hexanes/ethyl acetate (65/35) to provide 4.97 g of the product contaminated with a minor amount of diethyl *N,N′*-hydrazinedicarboxylate. ^{1}H NMR (DMSO-d_6): δ 1.30 (s, 9H), 1.63 (m, 1H), 1.73 (m, 1H), 2.03 (m, 2H), 2.16 (s, 3H), 2.25 (m, 6H), 3.61 (m, 4H), 4.03 (m, 1H), 7.00 (s, 2H), 7.46 (s, 1H), 8.22 (s, 1H), 11.85 (s, 1H) and 1.16 (t, $J = 7.1$ Hz, 0.7H) and 4.02 (q, $J = 7.1$ Hz, 0.5H) arising from diethyl *N,N′*-hydrazinedicarboxylate. FAB-MS: calculated for $C_{28}H_{32}ClIN_2O_4$ 622; found 623 (M + H, 100).

4-(2-[1-[(tert-butyl)oxycarbonyl]azetidin-2-yl]ethoxy)-7-chloro-2-oxo-3-(3,4,5-trimethylphenyl)hydroquinoline-6-[^{14}C]carboxylic acid (***^{14}C-3***): A three-necked flask containing ca. 20 mCi of $LiO_2^{14}CH$ (54 mCi/mmol, 0.36 mmol) generated from 74 mg (0.38 mmol, 54 mCi/mmol) of $Ba^{14}CO_3$, 830 mg (2.99 mmol) of $PbCl_2$, and 0.6 ml (0.6 mmol) of 1 M $LiBEt_3H$ was connected to a vacuum manifold, a septum, and a round bottom flask via a 90° bent adapter.[6] The round bottom flask was charged with iodide **4** (93 mg, 0.13 mmol), $Pd(dppf)_2Cl_2$ (5 mg, 0.007 mmol), KOAc (42 mg, 0.43 mmol), and DMSO (1 ml). Both flasks were cooled in liquid nitrogen and evacuated to 0.05 mm Hg. The apparatus was isolated from the vacuum, and the cooling baths removed. Concentrated H_2SO_4 (10 ml) was added slowly through the septum on the three-necked flask to the solid at 0°C. The three-necked flask was heated at 70°C for 1 h, and the one-necked flask was heated at 70°C overnight after which the vacuum was released. The solution was diluted with CH_2Cl_2 (20 ml) and 1% NaOH (20 ml). The layers were separated and the organic layer was washed with NaOH (2×20 ml). The aqueous layer was acidified with 1 M HCl and was extracted with CH_2Cl_2 (3×20 ml). The organic layer was dried

($MgSO_4$) and filtered to give 10.2 mCi which had 64% radiochemical purity by HPLC (method A) (ca. 87% chemical yield; 32% radiochemical yield from $Ba^{14}CO_3$).

1[4-(2-(N-t-butoxycarbonylazetidin-2(S)-ylethoxy)-7-chloro-2-oxo-3-(3,4,5-trimethylphenyl)(6-hydroquinolyl)]-N-pyrimidin-4-yl-[^{14}C]carboxamide (**^{14}C-9**): To a solution of acid **^{14}C-3** (1.0 mCi, 0.018 mmol, 54 mCi/mmol), 4-aminopyrimidine (18 mg, 0.19 mmol), dimethylaminopyridine (5.4 mg, 0.04 mmol), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (20 mg, 0.10 mmol) in CH_2Cl_2 (1 ml) was stirred under N_2 for 3 days. The solution was diluted with 4 ml of CH_2Cl_2 and the organic solution was washed with water (2×2 ml), dried ($MgSO_4$), and filtered to give a solution containing 0.90 mCi. The solution was concentrated to afford a yellow solid which was purified by column chromatography using 80:20 EtOAc:hexane as the eluant to give 0.56 mCi (95% radiochemical purity by HPLC method A, 56% yield). LC/MS (M/Z, % abundance): 618 (13), 620 (100, M + 1), 621 (36), 622 (38), 623 (12), 624 (2).

[4-(2-azetidin-2(S)-ylethoxy)-7-chloro-2-oxo-3-(3,4,5-trimethylphenyl)(6-hydroquinolyl)]-N-pyrimidin-4-yl-[^{14}C]carboxamide (**^{14}C-1**): A solution of 0.40 mCi of amide **^{14}C-9** and 20 μL of anisole in 2 ml of CH_2Cl_2 and 1 ml of CF_3CO_2H was stirred at rt under N_2 for 1 h. HPLC analysis (method A) showed the reaction to be complete; therefore, the volatiles were removed by passing N_2 gas over the solution. The residue was taken up in 5 ml of EtOH, and the solution was determined to contain 400 μCi (75% radiochemical purity by HPLC (method B)). The solution was concentrated to dryness under a N_2 stream, and the solid was taken up in 500 μL of EtOH, purified by preparative HPLC (21.2×250 mm Zorbax SB C-18, 23% MeCN-0.1% TFA, 20 ml/min), and isolated by elution from an Oasis™ HLB SepPak with MeOH to afford 150 μCi. HPLC (method B) radiochemical purity 99.0%. LC/MS (M/Z, % abundance): 518 (13), 520 (M + H, 100), 521 (32), 522 (34), 523 (11), 524 (2). ^{1}H NMR (CD_3OD): δ 2.07 (m, 2H), 2.20 (m, 2H), 2.25 (s, 3H), 2.34 (s, 6H), 3.74 (m, 1H), 3.78 (m, 2H), 3.98 (q, 1H, J = 9.4 Hz), 4.42 (quintet, 1H, J = 7.7 Hz), 7.08 (s, 2H), 7.49 (s, 1H), 8.11 (s, 1H), 8.34 (d, 1H, J = 5.8 Hz), 8.71 (m, 1H), 8.89 (s, 1H). ^{13}C NMR (CD_3OD): δ 15.4, 20.7, 25.8, 34.7, 44.4, 60.1, 70.2, 111.9, 117.3, 117.5, 122.7, 125.9, 130.3, 130.8, 134.8, 136.6, 137.6, 140.8, 159.1, 159.4, 159.6, 161.0, 165.9.

*[4-(2-(N-t-butoxycarbonylazetidin-2(S)-ylethoxy)-7-chloro-2-oxo-3-(3,4,5-tri-methylphenyl)(6-hydroquinolyl)]-N-(1,2,5thiadiazol-3-yl)[^{14}C]carboxamide (**^{14}C-10**)*: The procedure reported for **^{14}C-9** was followed using 1.0 mCi of acid [**^{14}C]-3** to give 0.62 mCi of **^{14}C-10** which assayed at 66% radiochemical purity by HPLC (method C).

*[4-(2-azetidin-2-ylethoxy)-7-chloro-2-oxo-3-(3,4,5-trimethylphenyl) (6-hydroquinolyl)]-N-(1,2,5thiadiazol-3-yl)[^{14}C]carboxamide (**^{14}C-2**)*: The procedure reported for the synthesis of **^{14}C-1** was followed using 0.62 mCi (66% radiochemical purity) of **^{14}C-10**. Purification by preparative HPLC (21.2 × 250 mm Zorbax RX C-8, 28% MeCN-0.1% $HClO_4$, 20 ml/min) afforded 0.19 mCi of antagonist **^{14}C-2** which was diluted with 3.6 mg of unlabeled **2**. HPLC analysis (method D) showed a 98.2% radiochemical purity, and the specific activity of 24 mCi/mmol was calculated from liquid scintillation counting and UV spectroscopic determination of concentration at three different wavelengths.

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