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Carbon-14 radiosynthesis of 4-(5-chloro-2-hydroxyphenyl)-3-(2-hydroxyethyl)-6-(trifluoromethyl)-[4-^{14}C]quinolin-2(1*H*)-one (XEN-D0401), A novel BK channel activator†

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A method has been developed for the carbon-14 radiosynthesis of [^{14}C]XEN-D0401, a 4-(2-hydroxyphenyl)quinolin-2(1*H*)-one derivative. The radiosynthetic route involves a series of *ortho*-lithiations directed by both 2-methoxymethyl (MOM) and pivaloyl protecting groups. This is demonstrated in the first key radiochemical step between the reaction of 5-chloro-2-methoxymethoxy-[carboxyl-^{14}C]benzoic acid methyl ester [^{14}C]-3 and the *ortho*-lithiated intermediate generated from 2,2-dimethyl-*N*-(4-trifluoromethylphenyl)-propionamide (2) to afford the protected benzophenone [^{14}C]-4. The other key radiochemical step utilizes a variation of the Friedländer quinoline synthesis, which involves a base catalysed, one-pot condensation process between (2-amino-5-trifluoromethyl-phenyl)-(5-chloro-2-methoxymethoxy-phenyl)-[^{14}C]methanone [^{14}C]-5 and γ-butyrolactone to give MOM-protected [^{14}C]-6. On MOM deprotection, [^{14}C]XEN-D0401 was isolated with a radiochemical purity of >97%, with a specific activity of 55 mCi/mmol from seven radiochemical steps, starting from barium [^{14}C]carbonate in a radiochemical yield of 10.5%.

Keywords: 3-substituted-4-arylquinolin-2(1*H*)-one; carbon-14; [^{14}C]XEN-D0401; BK channel activator; overactive bladder (OAB); BMS-223131

Introduction

The drug candidate 4-(5-chloro-2-hydroxyphenyl)-3-(2-hydroxyethyl)-6-(trifluoromethyl)-quinolin-2(1*H*)-one, XEN-D0401, shown in Figure 1 has found application in the management of disease states arising from the dysfunction of cellular membrane polarization and conductance.[1] XEN-D0401 contains a 3-substituted-4-arylquinolin-2(1*H*)-one derivative and is a novel, selective small molecule activator to open the large-conductance calcium-activated potassium channel, known as the BK channel.[2] Compounds with this heterocyclic scaffold have also been used as inhibitors of acyl-coenzyme A and cholesterol acyltransferase.[1] This drug candidate is in Phase I development for the treatment of overactive bladder (OAB).[3] Currently, it is estimated that 200 million people worldwide suffer from this condition.[4] Treatments using anti-muscarinic-based drugs (which operate on the muscarinic acetylcholine receptors) have poor tolerability profiles and therefore new drugs for the management of OAB are needed.

This paper describes a method for the radiosynthesis of [^{14}C]XEN-D0401, having a single carbon-14 label (denoted by *) in the quinolin-2(1*H*)-one ring with a specific activity greater than 50 mCi/mmol, as illustrated in Figure 2. This strategy is based on a viable route published by Wang *et al.*[5] and a personal communication with Crispino *et al.* to produce multi-kilogram quantities of the drug candidate BMS-223131 for the treatment of male erectile dysfunction.[6]

The retro-synthetic analysis for the incorporation of a single carbon-14 label at position 4 on the substituted quinolin-2(1*H*)-one ring to give [^{14}C]XEN-D0401 is shown in Scheme 1. The disconnection of the amide bond in the 3-substituted-4-arylquinolin-2(1*H*)-one derivative of [^{14}C]XEN-D0401 gives the retro-target (**A**) with the aliphatic primary hydroxyl protected. Further disconnection provides the target benzophenone synthon (**B**), which can be synthesized from the building blocks (**D**) and (**E**). The key retro-synthetic step is the formation of the retro-target (**A**), which in turn can be synthesized from the condensation of 'synthons' (**B**) with (**C**). The carbon-14 radiolabel can be incorporated into (**E**) from reacting synthon (**F**) with [^{14}C]carbon dioxide.

The target [^{14}C]XEN-D0401 was prepared in seven radiochemical steps starting from barium [^{14}C]carbonate. This included

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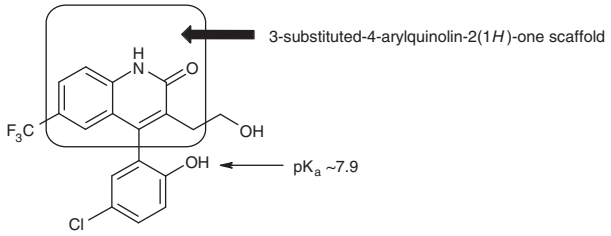


Figure 1. XEN-D0401.

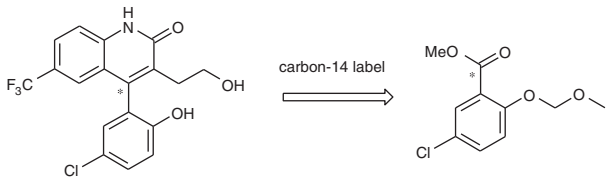


Figure 2. Position of carbon-14 label.

the intermediate 5-chloro-2-methoxymethoxy-[carboxyl-^{14}C]benzoic acid methyl ester **[^{14}C]-3**, shown in Scheme 2, which was prepared using in-house carboxylation radiochemistry and is outlined in Scheme 3.

Results and discussion

The starting material 2,2-dimethyl-*N*-(4-trifluoromethylphenyl)-propionamide (**2**) was easily prepared in 83% isolated yield from the *N*-acylation of 4-trifluoromethylphenylamine (**1**) using pivaloyl chloride, as illustrated in Scheme 2.[7]

Directed *ortho*-lithiation on the substrate (**2**) was achieved with 2.5 equivalents of a solution of *n*-butyl lithium in tetrahydrofuran at −78°C. Progress of the reaction was monitored by quenching an aliquot of the reaction mixture in D_2O. Reaction completion was established when the proton-NMR spectrum in d_6-DMSO of the quenched reaction mixture showed replacement of the symmetrical AB quartet group of signals, integrating for four aryl ring protons, with a series of multiplets with an ABX splitting pattern, integrating for only 3 protons.

The introduction of the single carbon-14 label was achieved via the substrate **[^{14}C]-3**, which was prepared using in-house carboxylation radiochemistry, as outlined in Scheme 3. The carbon-14-labelled material **[^{14}C]-3** was synthesized from the commercially available 4-chloro-2-bromophenol (**7**) and proved capricious in terms of yield.

Phenol (**7**) was protected using chloromethyl methyl ether (MOMCl) to give the MOM ether (**8**).[8] Subsequent lithium–halogen exchange preferentially at the bromo atom, followed by carboxylation of the lithiated intermediate, with [^{14}C]carbon dioxide, was carried out to give benzoic acid **[^{14}C]-9**, after an aqueous basic quench and mild acidic work-up with 5% aqueous citric acid. [^{14}C]Carbon dioxide used for the carboxylation was generated *in situ* by the action of concentrated sulphuric acid on 250 mCi of barium [^{14}C]carbonate (specific activity >50 mCi/mmol) using high vacuum procedures. The [^{14}C]carbon dioxide generated was dried and stored in a glass bulb containing P_2O_5 until required. The crude product **[^{14}C]-9** underwent azeotropic-drying and the resultant oil was then taken up in acetone to react with methyl iodide in the presence of potassium carbonate.

On analysis of the reaction mixture by normal phase radio-TLC, formation of a major impurity was observed. This was isolated in 47% radiochemical yield and identified by proton-NMR spectroscopy as 5-chloro-2-hydroxy-[carboxyl-^{14}C]benzoic acid methyl ester **[^{14}C]-10**. The reason for this low radiochemical yield was during the methylation procedure, the MOM protecting group had been cleaved and resulted in *O*-methylation to give **[^{14}C]-11**. Consequently, the substrate **[^{14}C]-10** was successfully re-protected with MOMCl in 97% radiochemical yield by generating the phenoxide derivative using sodium hydride in anhydrous dimethylformamide. The labelled substrate **[^{14}C]-3** was isolated with a total activity of 86 mCi with a radiochemical yield of 35% from barium [^{14}C]carbonate.

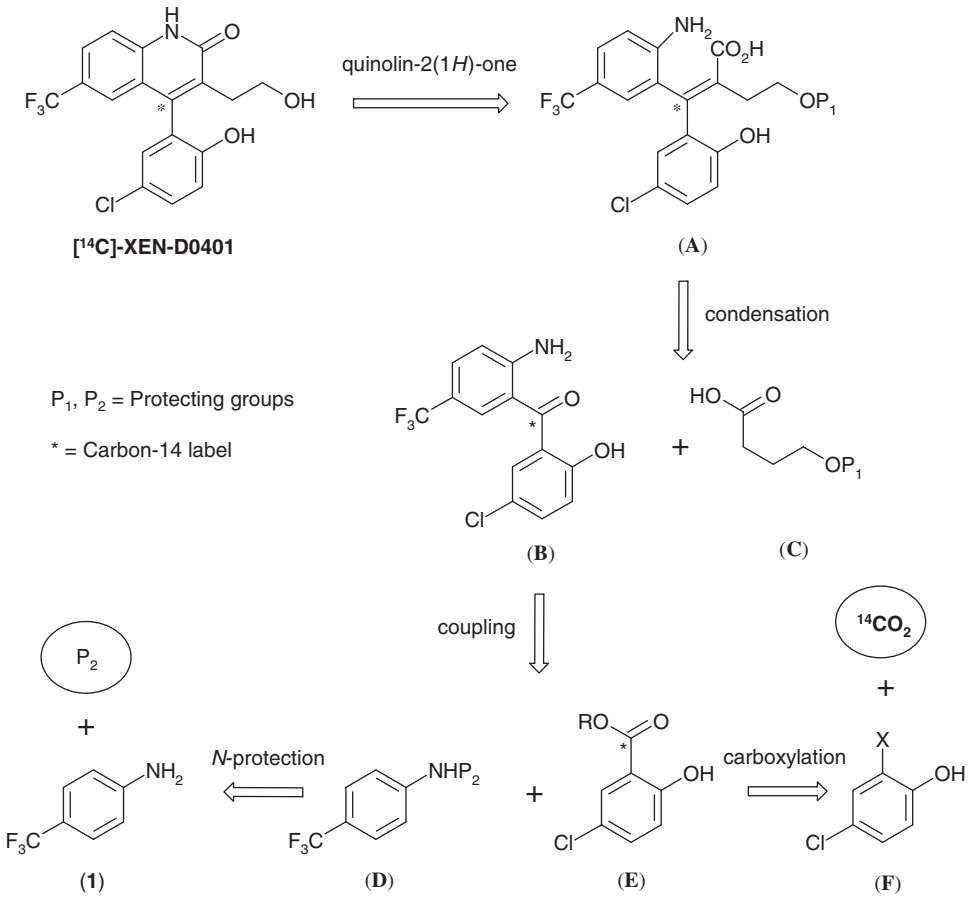
A key step in the radiosynthesis of [^{14}C]XEN-D0401 was the formation of the protected benzophenone **[^{14}C]-4.** This unsymmetrical benzophenone derivative was made from reacting the *ortho*-lithiated intermediate with **[^{14}C]-3** as illustrated in Scheme 2. This reaction was trialed and optimized several times due to the poor yields of **[^{14}C]-4** from the incomplete lithium–halogen exchange on pivaloyl protected (**2**). On further investigation an acceptable yield of 71% of the benzophenone derivative, **[^{14}C]-4**, was achieved after purification.

The other challenging step was the de-protection of the pivaloyl group under basic conditions. It was found that the best procedure was to add all the sodium hydroxide in ethanol (25 wt%) in a single batch at the start of the reaction. After heating the reaction at reflux for 1 h, analysis of the reaction mixture, using normal phase radio-TLC, showed formation of **[^{14}C]-5** which was isolated with a radiochemical yield of 68% and radiochemical purity of 90.2%.

The most common route to quinoline derivatives utilizes the Friedländer 'quinoline' synthesis, which involves the condensation of aromatic *ortho*-amino aldehydes/ketones with an adjacent methylene aldehyde or ketone. This reaction step has been further explored (Crispino *et al.*, personal communication) to synthesize 4-aryl-quinolin-2(1*H*)-one derivatives. The limiting factor in using the Friedländer[9] strategy is the requirement of an activating group, which must be adjacent to an ester or carboxylic acid group. This condition is conveniently met by employing a lactone in the condensation with 2-aminobenzophenone derivatives. It has been demonstrated (Crispino *et al.*, personal communication) using a one-pot procedure that the required 4-aryl-quinolin-2(1*H*)-one can be produced by reacting γ-butyrolactone with the unlabelled substrate **(5)**. The advantage of using γ-butyrolactone is that it immediately gives the required side chain (2-hydroxyethyl) in the correct position on the 4-aryl-quinolin-2(1*H*)-one ring.

First the conversion from **[^{14}C]-5** into **[^{14}C]-6** was not successful due to the formation of **[^{14}C]-5** hydrate. The proton-NMR spectrum of the **[^{14}C]-5** hydrate gave the new resonance signals at 8.44 and 5.59–5.60 ppm. During unlabelled synthesis, the cyclized product was prepared successfully because the resultant unlabelled solid **(5)** was filtered off and dried under high vacuum at 40°C to remove any residual water.

For the cyclization step to be efficient, it was important to dry **[^{14}C]-5** prior to the reaction. Unfortunately, in the radiochemical synthesis the solid **[^{14}C]-5** was filtered off on a sinter funnel, and the solid dissolved in tetrahydrofuran and concentrated using rotary evaporator to obtain oil. The oil was azeotropically dried



Scheme 1. Retro-synthetic pathway of [^{14}C]XEN-D0401.

several times from tetrahydrofuran but residual water may still be present. The 'semi-dried' oil that was then subsequently used in the cyclization step and consequently did not give the desired product **[^{14}C]-6** but only starting material.

For the radiochemistry, success was finally achieved by prior, rigorous drying of **[^{14}C]-5** to ensure the absence of any residual water. This was accomplished by azeotropic drying from toluene, followed by pentane. Analysis of the proton-NMR spectrum of the rigorously dried **[^{14}C]-5** was consistent with the unlabelled material and showed the absence of the proton-NMR signals at δ 8.44 and 5.59–5.60 that were present in the wet product.

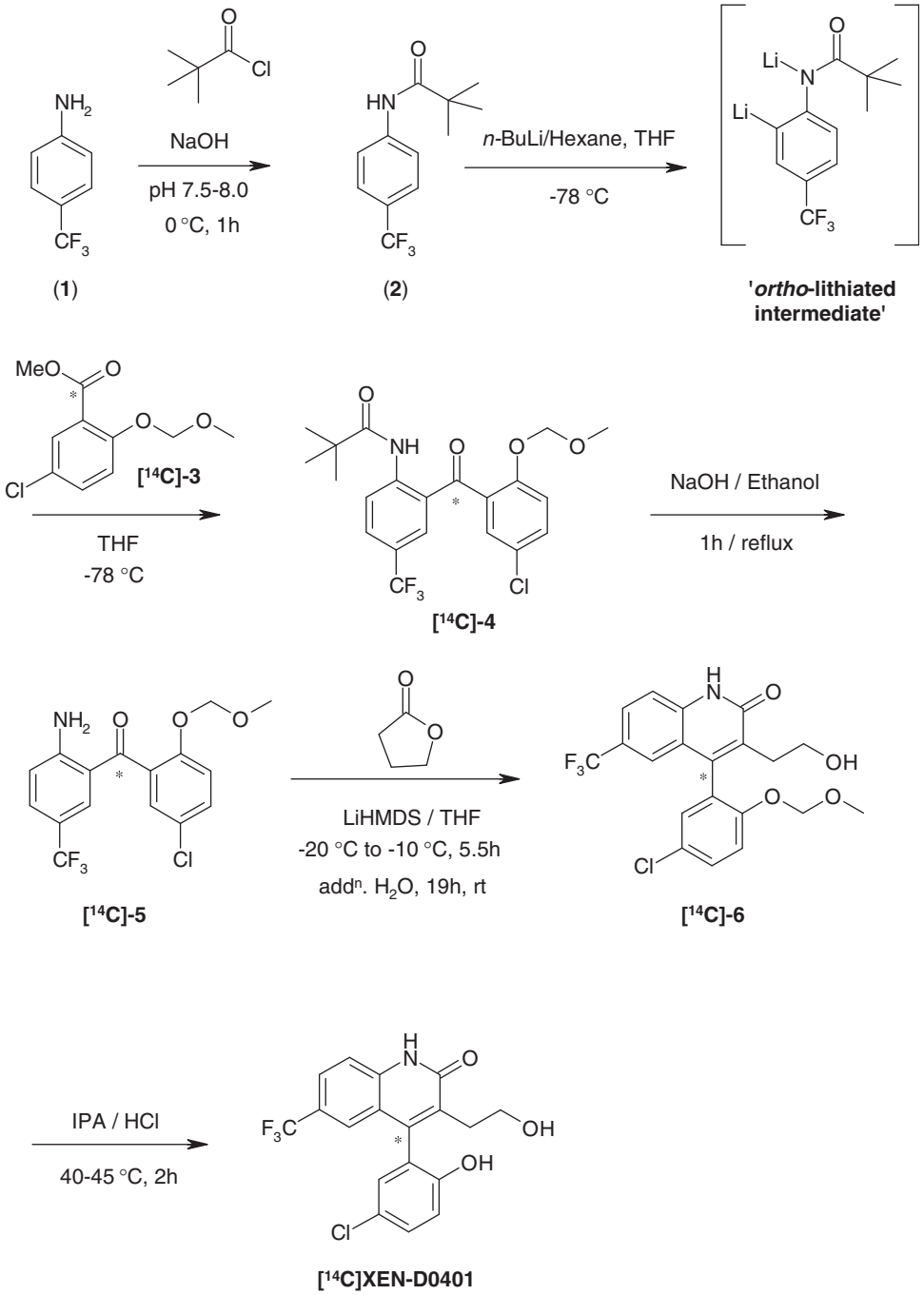
The anion at the amino moiety of the benzophenone derivative **[^{14}C]-5** was successfully achieved by using lithium *bis*(trimethylsilyl)amide (LiHMDS) in tetrahydrofuran. The generated anion was reacted with a solution of γ-butyrolactone in tetrahydrofuran and resulted in the formation of the cyclized MOM protected **[^{14}C]-6** after aqueous work-up, with a radiochemical purity of 78.8%. The proposed mechanism for the reaction of **[^{14}C]-5** with γ-butyrolactone is analogous to the one already described by Crispino *et al*.[5]

The final step in the radiosynthesis of [^{14}C]XEN-D0401 was the cleavage of the MOM protecting group using concentrated hydrochloric acid in propan-2-ol at elevated temperature. The crude [^{14}C]XEN-D0401 was isolated and purified by flash column chromatography on silica gel to give [^{14}C]XEN-D0401. Radio-TLC analysis established the radiochemical purity at 96%. Crystallization from a mixture of ethanol and water gave [^{14}C]XEN-D0401 as an off white solid. The radiochemical purity, as measured by analytical HPLC, was $>$97% with a gravimetric specific activity of 55 mCi/mmol. The 500 MHz proton NMR-spectrum of [^{14}C]XEN-D0401 was consistent with the molecular structure.[3] One feature of interest in the proton-NMR spectrum of [^{14}C]XEN-D0401 was that multiplets at δ 2.75 and 2.71 were assigned to the diastereotopic methylene protons ($C\mathbf{H_2}CH_2OH$) on the 2-hydroxyethyl moiety, an interpretation consistent with the reported atropisomerism of XEN-D0401.[3–10]

Experimental procedures

Materials and methods

The custom synthesis of [^{14}C]XEN-D0401 and all associated radioactive experiments were carried out in the Isotope Chemistry Laboratories at Almac Sciences in specially designed fume hoods dedicated to carbon-14 only. Reactions were performed under an inert atmosphere using filtered nitrogen gas unless specified differently. Chemical reagents and solvents used in the synthetic procedures were obtained from various chemical suppliers without further purification. Barium [^{14}C]carbonate was purchased from a commercial supplier with specific activity $>$50 mCi/mmol. High-purity HPLC solvents were used. Intermediates and products synthesized were characterized based on proton-NMR spectra and recorded on a Bruker DPX spectrometer operating at 500 MHz spectrometer. Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in either d_6-DMSO or $CDCl_3$ and coupling constants (*J*) are reported in Hertz. The NMR peak patterns are



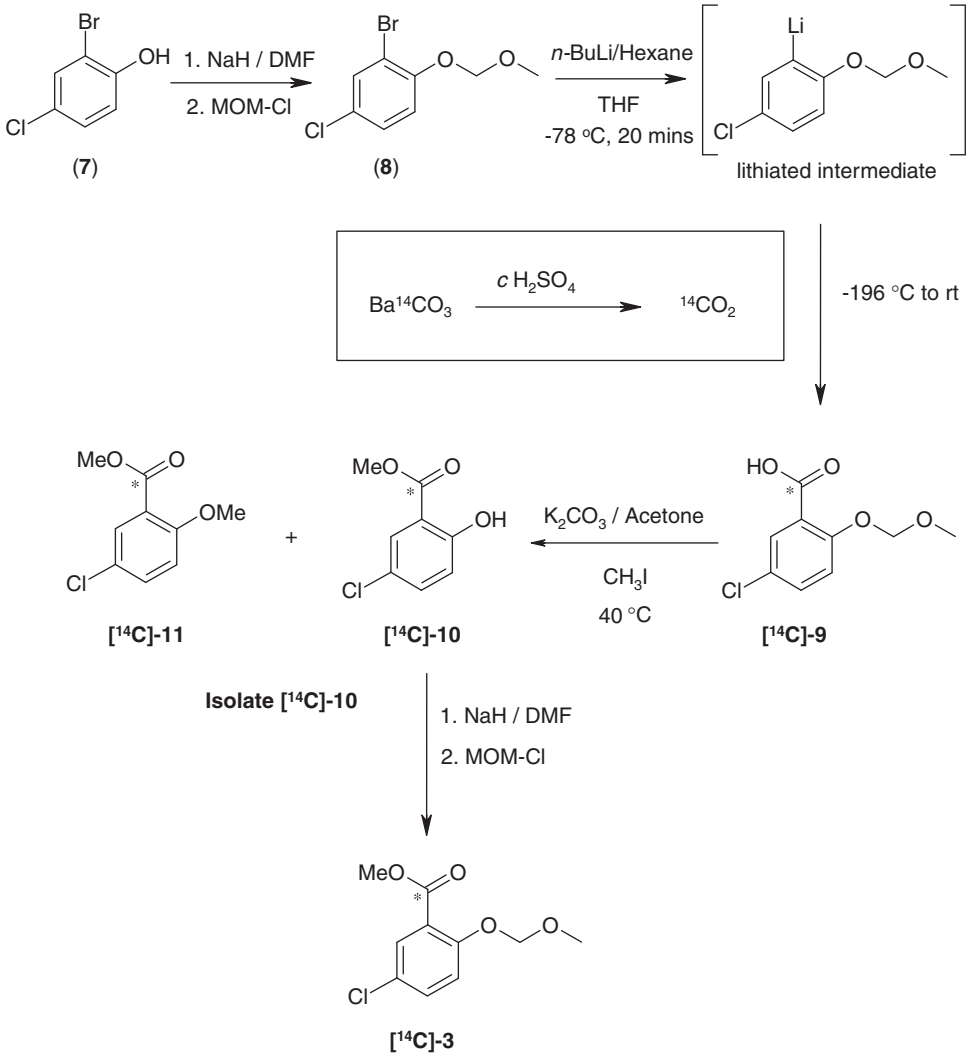
Scheme 2. Radiosynthetic route to [^{14}C]XEN-D0401.

reported as broad (br), singlets (s), doublets (d), triplets (t), quartets (q), septets (sep), and multiplets (m). Silica gel (200–400 mesh, 60 Å) was used for flash column chromatography. TLC plates (pre-coated glass plates with silica gel 60 F_{254}, 0.25 mm thickness) were used to monitor reactions and developed using the Cyclone Storage Phosphor System. Final products were dried in a high vacuum desiccator over phosphorous pentoxide. The radiochemical yield was determined by liquid scintillation counting using the Perkin–Elmer Tricarb 2900 Scintillation Analyzer. Purity of the final compound was further analysed using an Agilent 1100 HPLC system with UV and radiochemical detection; a Symmetry C18 analytical HPLC column 3.9 mm × 150 mm, 5 μm. *T* = ambient; eluents, solvent A, 0.1% trifluoroacetic acid in water/acetonitrile (90:10), solvent B, 0.1% trifluoroacetic acid in water/acetonitrile (10:90), gradient, 0–100% B over 0–30 min; flow rate, 1.0 mL/min; injection volume 10 μL; monitored at 230 nm wavelength. HPLC analyses are described as area%.

Carbon-14 radiosynthesis

*Synthesis of 2,2-dimethyl-N-(4-trifluoromethylphenyl)-propionamide (**2**)*

A 500 mL three-necked round bottom flask was charged with water (60 mL), acetone (30 mL) and **(1)** (15.2 g, 94.3 mmol). The



Scheme 3. Radiosynthetic route to labelled starting material **[^{14}C]-3**.

reaction mixture was cooled to 0°C and pivaloyl chloride (13.1 g, 108 mmol) was added dropwise via a pressure equalized dropping funnel with concurrent addition of aqueous sodium hydroxide (1M), so as to maintain the pH in the range of 7.5–8.0 using a pH probe. The reaction mixture was left stirring for 1 h at 0°C, filtered off and the solid washed with water (3 × 100 mL) and water/acetone (4:1, 4 × 100 mL). The white solid was dried in the high vacuum oven to constant weight to yield **(2)** (19.3 g, 83%): ^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 7.66 (m, 2H, *J* = 10.0 Hz), 7.57 (m, 2H, *J* = 10.0 Hz), 3.93 (s, 1H), 1.33 (s, 9H).

Synthesis of 5-chloro-2-methoxymethoxy[carboxyl-^{14}C]benzoic acid [^{14}C]-9

A two-necked 100 mL round bottom flask was charged with (**8**) (1.68 g, 6.70 mmol) and anhydrous tetrahydrofuran (9 mL) under nitrogen. The resultant solution was cooled down to −78°C and a solution of *n*-butyl lithium in hexane (2.0 M, 3.3 mL, 6.6 mmol) was added over 5 min via syringe. On complete addition, the reaction mixture was allowed to stir at −78°C for 20 min to give the *ortho*-lithiated product. The flask containing the *ortho*-lithiated product was connected to the high vacuum manifold via an H-piece and cooled down to −196°C using a liquid nitrogen Dewar. The [^{14}C]carbon dioxide was generated from the action of concentrated sulphuric acid on barium [^{14}C]carbonate (250 mCi, Specific Activity >50 mCi/mmol) on the high vacuum manifold and dried over P_2O_5. The [^{14}C]carbon dioxide was transferred to the flask containing the *ortho*-lithiated product and the reaction mixture was allowed to warm to ambient temperature. During this process the mixture went from orange to straw coloured. The reaction mixture was allowed to stir for 1 h and then re-cooled to −196°C and purged with nitrogen. This allowed for the addition of aqueous sodium hydroxide (1.0 M, 10 mL) and the quenched mixture was allowed to warm to ambient temperature overnight. The non-acidic organics were extracted into dichloromethane (5 × 10 mL) and the aqueous phase was made acidic to pH ~ 3–4 using aqueous citric acid (5%, 100 mL). The crude product was extracted into dichloromethane (2 × 10 mL), washed with brine, dried over magnesium sulphate, filtered and counted to give a total activity of 191 mCi and radiochemical yield of 76%. The dried organic layer was concentrated on the rotary evaporator to give the oil product **[^{14}C]-9**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 10.69 (s, 1H), 8.04

(d, 1H, $J = 3.0$ Hz), 7.47 (dd, 1H, $J = 11.5$, 3.0 Hz), 7.25 (d, 1H, $J = 11.5$ Hz), 5.34 (s, 2H), 3.54 (s, 3H).

Synthesis of 5-chloro-2-methoxymethoxy-[carboxyl-^{14}C]benzoic acid methyl ester [^{14}C]-3

A 250 mL round bottom flask containing **[^{14}C]-9** (191 mCi) was taken up in anhydrous acetone (200 mL). To the resultant solution was added anhydrous potassium carbonate (2.36 g, 17.1 mmol, 5.0 eqv) and methyl iodide (0.318 mL, 5.11 mmol, 1.5 eqv). The mixture was heated to 40°C and left stirring for 18.5 h. Radio-TLC analysis on normal phase silica gel showed that the reaction under these conditions produced very little product. Another aliquot of methyl iodide (2.0 mL) was added and the mixture was left stirring at 40°C for 2.3 h. Radio-TLC analysis showed two products, without further progress of the reaction.

The reaction mixture was filtered and the filtrate concentrated on the rotary evaporator to give a residue. Proton-NMR spectroscopic analysis indicated that the MOM protecting group was cleaved and the dominate product was **[^{14}C]-10**. The [^{14}C]methyl ester was isolated on a column of normal phase silica gel, eluting the product off with a mixture of hexane/dichloromethane (4:1) to give 89.1 mCi. Radio-TLC analysis, on normal phase silica gel, eluting with diethyl ether/hexane/triethylamine (100:900:2) showed a radiochemical purity of 99.5%: $R_f = 0.42$ (silica gel, *n*-hexane/diethylether/triethylamine, 400:100:1); ^{1}H-NMR ($CDCl_3$, 500 MHz) $\delta = 10.67$ (s, 1H), 7.82 (m, 1H), 7.40 (m, 1H), 7.42 (m, 1H), 3.97 (s, 3H).

The substrate, **[^{14}C]-10** (89.1 mCi), was taken up in anhydrous *N,N*-dimethylformide (5.0 mL) and the resultant solution was cooled down to 0°C. Sodium hydride (60% dispersion in mineral oil, 67.2 mg, 1.68 mmol, 1.05 eqv) was then added under nitrogen, followed by the addition of MOMCl (0.128 mL, 1.68 mmol, 1.05 eqv). The reaction mixture was left stirring for 20 min at 0°C, followed by 20 min at ambient temperature. Radio-TLC analysis on normal phase silica gel, eluting with hexane/diethyl ether/triethylamine (400:100:1), gave a radiochemical purity of 98.4%. The reaction mixture was diluted with dichloromethane (20 mL) and the organic phase was washed with water (3 × 30 mL) and brine (1 × 50 mL). The product layer was concentrated on the rotary evaporator and dried azeotropically with tetrahydrofuran (3 × 100 mL) to give a colourless oil. The oil was taken up in a mixture of diethyl ether and hexane and applied to a column of silica gel and the product was eluted with a mixture of diethyl ether/hexane. The product fractions were combined and counted to give a total activity of (86 mCi). The solution was concentrated on the rotary evaporator to give **[^{14}C]-3** as an oil: $R_f = 0.15$ (silica gel, *n*-hexane/diethylether/triethylamine, 400:100:1); ^{1}H-NMR (d_6-DMSO, 500 MHz) $\delta = 7.65$ (m, 1H), 7.57 (m, 1H), 7.25 (m, 1H), 5.24 (s, 2H), 3.82 (s, 3H), 3.39 (s, 3H).

Synthesis of N-[2-(5-chloro-2-methoxymethoxy-[^{14}C-carbonyl]benzoyl)-4-trifluoromethyl-phenyl]-2,2-dimethyl-propionamide [^{14}C]-4

A 50 mL, three-necked round bottom flask was charged with **(2)** (414 mg, 1.69 mmol, 1.1 eqv) and anhydrous tetrahydrofuran (5 mL) under nitrogen. The solution was cooled to −78°C and held for 10 min. A solution of *n*-butyl lithium in hexane (2.19 M, 3.84 mmol, 1.75 mL, 2.5 eqv) was then added to the cold solution over 1 min and the reaction mixture was stirred at −78°C for a further 10 min, followed by stirring for 4.5 h at 0–5°C. Before quenching the slurry with water, an aliquot of the reaction mixture was quenched with D_2O and concentrated *in vacuo*. The residue was analysed by proton NMR spectroscopy to confirm that *ortho*-lithiation had taken place.

The reaction mixture was cooled to −78°C and a solution of **[^{14}C]-3** (86 mCi) in anhydrous tetrahydrofuran (2 mL) was added over 5 min. On complete addition, the reaction was left stirring at −78°C for 20 min. The mixture was warmed to 0–5°C and left stirring under nitrogen. An aliquot of the reaction mixture was quenched with a mixture of *tert*-butyl methyl ether and water. The organic phase was analysed by radio-TLC and showed the formation of **[^{14}C]-4**. The reaction mixture was quenched with water (5 mL), followed by the addition of *tert*-butyl methyl ether (5 mL). The organic components were extracted into *tert*-butyl methyl ether (2 × 50 mL) and concentrated *in vacuo* to afford an oil. The oil was dissolved in tetrahydrofuran (2 × 50 mL) and each time concentrated *in vacuo* to give a straw coloured oil. The oil was taken up in a mixture of dichloromethane/hexane and the active solution applied to a column of silica gel, which was neutralized with triethylamine. The product was eluted off first with hexane, hexane/dichloromethane (9:1), hexane/dichloromethane (8:2) and finally with hexane/dichloromethane (7:3). The product fractions were collected, combined and concentrated *in vacuo* to give a solid. The solid was dissolved in tetrahydrofuran and counted to give a total activity of 60.7 mCi and radiochemical yield of 71%. Activity from a previous batch of 8.4 mCi was combined with the 60.7 mCi batch to give a total activity of 69.1 mCi. Radio-TLC analysis on normal phase silica of the combined batches gave a radiochemical purity of 88.7%. Proton-NMR spectroscopic analysis was consistent with the titled compound: ^{1}H-NMR ($CDCl_3$, 500 MHz) $\delta = 11.85$ (bs, NH), 8.98 (d, 1H, $J = 9.0$ Hz), 7.78 (d, 1H, $J = 9.0$ Hz), 7.71 (s, 1H), 7.45 (dd, 1H, $J = 9.0$, 2.5 Hz), 7.35 (d, 1H, $J = 2.5$ Hz), 7.23 (d, 1H, $J = 9.0$ Hz), 5.02 (s, 2H), 3.28 (s, 3H), 1.39 (s, 9H).

Synthesis of (2-amino-5-trifluoromethyl-phenyl)-(5-chloro-2-methoxymethoxy-phenyl)-[^{14}C]methanone [^{14}C]-5

A 250 mL round bottom flask was charged with **[^{14}C]-4** (69.1 mCi), ethanol (30 mL) and aqueous sodium hydroxide (25 wt%, 6 mL). The resultant yellow solution was heated to reflux for 1 h, then allowed to cool to ambient temperature. Radio-TLC analysis on normal phase silica of the reaction mixture showed product that co-eluted with an authentic sample and gave a radiochemical purity of 80%. The reaction mixture was quenched with water (70 mL) over 10 min and resulted in an off white slurry. The slurry was left stirring at 0–5°C for 2 h, then filtered off and washed with water until the pH of the filtrate was 6. The solid was taken up in tetrahydrofuran (250 mL) and counted to give a total activity of 60.6 mCi. Radio-TLC analysis on normal phase silica of the active solution gave a radiochemical purity of 90.2%. The solution was concentrated on the rotary evaporator to give a yellow oil. Analysis using proton-NMR spectroscopy indicated that the crude product may be a hydrate: ^{1}H-NMR ($CDCl_3$, 500 MHz)$\delta = 8.44$ (bs), 7.51–7.53 (m, 1H), 7.46–7.48 (m, 1H), 7.38–7.40 (m, 1H), 7.27–7.27 (m, 1H), 7.19–7.20 (m, 1H), 6.75–6.77 (m, 1H), 6.71 (bs, NH), 5.59–5.60 (m) 5.05 (s, 2H), 3.32 (s, 3H). The crude product was dried by azeotropic distillation from toluene (12 × 20 mL) and then from hexane (2 × 20 mL) to give ketone **[^{14}C]-5** (46.8 mCi) and radiochemical yield of 68%. Radio-TLC $R_f = 0.41$ (silica gel, dichloromethane/

methanol 9:1); ^{1}H-NMR ($CDCl_3$, 500 MHz) $\delta = 7.51–7.53$ (m, 1H), 7.46–7.48 (m, 1H), 7.39–7.40 (m, 1H), 7.26–7.27 (m, 1H), 7.19–7.20 (m, 1H), 6.75–6.77 (m, 1H), 6.70 (bs, NH) 5.05 (s, 2H), 3.32 (s, 3H).

Synthesis of 4-(5-chloro-2-methoxymethoxy-phenyl)-3-(2-hydroxyethyl)-6-trifluoromethyl-[4-^{14}C]-quinolin-2(1H)-one [^{14}C]-6

A 100 mL round bottom flask containing the white solid **[^{14}C]-5** (46.8 mCi) was dissolved in tetrahydrofuran (7 mL) and gave a total activity of 46.8 mCi. The resultant straw coloured solution was cooled to −20°C and a solution of lithium *bis*(trimethylsilyl)amide in tetrahydrofuran (1 M, 9 mL) was added slowly over 5 min to give an orange solution. The solution was allowed to warm to −10°C over 20 min then a solution of γ-butyrolactone (0.48 mL) in tetrahydrofuran (4 mL) was added slowly over 10 min. On complete addition the yellow/orange solution was allowed to warm to ambient and left to stir for 5.5 h. The reaction mixture was quenched with water (10 mL) and left stirring at ambient for 19 h. *tert*-Butyl methyl ether (30 mL) was added and the organics were extracted into *tert*-butyl methyl ether (2 × 50 mL), washed with brine (50 mL) and water (50 mL). The combined organic phases were concentrated on the rotary evaporator to give a yellow oil, which was dried using azeotropic distillation from toluene (6 × 20 mL), followed by pentane (6 × 20 mL) to give a yellow solid. Proton NMR spectroscopic analysis of the solid was consistent with the structure of the titled compound. The radiochemical purity was established as 78.8% by radio-TLC on normal phase silica gel: TLC $R_f = 0.41$ (silica gel, dichloromethane/methanol 9:1); ^{1}H-NMR (d_6DMSO, 500 MHz) $\delta = 12.27$ (bs, NH), 7.78 (dm, 1H, $J = 8.3$ Hz), 7.57 (dd, 1H, $J = 9.5$, 3.2 Hz), 7.52 (d, 1H, $J = 8.3$ Hz), 7.39 (d, 1H, $J = 3.2$ Hz), 7.34 (d, 1H, $J = 9.5$ Hz), 7.02–7.02 (m, 1H), 5.17 (d, 1H, $J = 7.3$ Hz), 5.05 (d, 1H, $J = 7.3$ Hz), 4.56 (m, OH), 3.38–3.45 (m, 2H), 3.06 (s, 3H), 2.52–2.63 (m, 1H), 2.38–2.44 (m, 1H).

Synthesis of 4-(5-chloro-2-hydroxyphenyl)-3-(2-hydroxyethyl)-6-trifluoro-methyl-2(1H)-quinolinone [^{14}C]XEN-D0401

A 250 mL round bottom flask was charged with the **[^{14}C]-6** and propan-2-ol (20 mL). To the resultant yellow solution was added dropwise concentrated hydrochloric acid (36%, 5 mL). The mixture was heated to 40–45°C and after 2 h radio-TLC on normal phase silica gel showed the formation of product with a radiochemical purity of 86.7%. The reaction mixture was allowed to cool to ambient temperature and quenched with ethyl acetate (100 mL), followed by the addition of water (50 mL). The lower aqueous layer was separated and the organic layer washed with water (3 × 100 mL) and concentrated on the rotary evaporator to give an oil. The oil was taken up in toluene (3 × 20 mL), followed by pentane (3 × 20 mL) and each time concentrated on the rotary evaporator to give a yellow solid. The solid was dissolved in a mixture of dichloromethane/methanol and the active solution applied to a column of silica. The product was eluted off with dichloromethane/methanol (95:5) and the product fractions combined and analysed by radio-TLC on normal phase silica gel to give a radiochemical purity of 97.8%, with a total activity of 33 mCi. The solution was concentrated on the rotary evaporator to give an off white solid. Radio-TLC on normal phase silica gel of the solid gave a radiochemical purity of 96.6%. The solid was taken up in hot absolute ethanol (5 mL) and water was added dropwise until the point of precipitation. Then on heating gave a solution which was allowed to cool to ambient temperature over 18 h. The resultant crystals were filtered under vacuum and washed with water/ethanol (9:1, 3 × 2 mL). The solid was dried in the high vacuum dessicator to constant weight to give [^{14}C]XEN-D0401 (207.4 mg, 142.4 μCi/mg, 29.5 mCi) which co-eluted with an authentic unlabelled sample: radiochemical purity (HPLC): 97.1%; specific activity 55 mCi/mmol; radio-TLC $R_f = 0.32$ (silica gel, dichloromethane/methanol 9:1) radiochemical purity: 97.3%; ^{1}H-NMR (d_4MeOH, 500 MHz) $\sigma = 7.72$ (dm, 1H, $J = 3.4$ Hz), 7.51 (d, 1H, $J = 3.4$ Hz), 7.40 (dd, 1H, $J = 3.1$, 0.9 Hz), 7.24 (m, 1H), 7.16 (d, 1H, $J = 0.9$ Hz), 7.01 (d, 1H, $J = 3.1$ Hz), 3.66 (dd, 2H, $J = 7.5$, 7.5 Hz), 2.75 (dt, 1H, $J = 12.5$, 7.5 Hz), 2.71 (dt, 1H, $J = 12.5$, 7.5 Hz).

Conclusion

At Almac Sciences we have utilized a route to the radiosynthesis of [^{14}C]XEN-D0401, developing the chemistry from (Crispino *et al*., personal communication) which allowed incorporation of a single carbon-14 radiolabel in the quinolin-2(1*H*)-one scaffold. The main features to highlight in this radiosynthesis of [^{14}C]XEN-D0401 are the formation of methyl ester **[^{14}C]-3** and the base catalysed, one-pot condensation process involving the MOM-protected benzophenone **[^{14}C]-5** with γ-butyrolactone to give the cyclized MOM-protected **[^{14}C]-6**. Other features to this radiosynthesis are the *ortho*-lithiation reactions and removal of MOM and pivaloyl protecting groups at the various stages in the radiochemical synthesis to release [^{14}C]XEN-D0401. The synthesis was completed in seven radiochemical steps and gave an overall radiochemical yield of 10.5% from barium [^{14}C]carbonate.

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References

[1] E. S. Hamanaka, 4-aryl-3-(heteroarylureido)-1,2-dihydro-2-oxo-quinoline derivatives as antihypercholesterolemic and antiatherosclerotic agents, US 5,565,472, **1996**. Available from: www.freepatentsonline.com/5565472.html.
[2] P. Hewawasam, J. E. Starrett, S. G. Swartz, 4-aryl-3-aminoquinoline-2-one derivatives as potassium channel modulators, US 5,972,961, **1999**. Available from: www.freepatentsonline.com/5972961.html.
[3] Xention Limited, *Investigational Medicinal Product Dossier XEN-D0401-CL-02 IMPD: XEN-D0401 Phase Ib Clinical Studies*, Cambridge, UK, **2007**.
[4] Xention Limited, Fact Sheet-Overactive Bladder, Available from: http://www.xention.com/wp-content/uploads/Fact-sheet-Overactive-bladder.pdf, **2009** [Friday 11 September 2009].
[5] J. Wang, R. P. Discordia, G. A. Crispino, J. Li, J. A. Grosso, R. Polniaszek, V. C. Truc, *Tetrahedron Lett.* **2003**, *44*, 4271–4273.
[6] D. V. Sivarao, K. Newberry, S. Langdon, A. V. Lee, P. Hewawasam, M. J. Plym, L. Signor, R. Myers, N. J. Lodge, *J. Pharmacol. Exp. Ther.* **2005**, *313*, 840–847.
[7] E. L. Elliott, S. M. Bushell, M. Cavero, B. Tolan, T. R. Kelly, *Org. Lett.* **2005**, *7*, 2449–2451.
[8] P. J. Kocieński, *Protecting Groups*, 3rd ed, Thieme, New York, **2005**.
[9] J. I. Ubeda, M. Villacampa, C. Avendano, *Synthesis* **1998**, 1176–1180.
[10] V. M. Vrudhula, B. Dasgupta, J. Qian-Cutrone, E. S. Kozlowski, C. G. Boissard, S. I. Dworetzky, D. Wu, Q. Gao, R. Kimura, V. K. Gribkoff, J. E. Starrett, *J. Med. Chem.* **2007**, *50*, 1050–1057.