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A modern approach to the synthesis of 2-(4-chlorophenyl)[2- ^{14}C]thiazol-4-ylacetic acid ([^{14}C] fenclozic acid) and its acyl glucuronide metabolite

David A. Killick and Nick Bushby*

An updated approach to the 1960s synthesis of [^{14}C] fenclozic acid from labelled potassium cyanide is presented. By employing modern synthetic methodology and purification techniques, many of the inherent hazards in the original synthesis are avoided or significantly reduced. The concomitant labelled stereoselective synthesis of the key acyl glucuronide metabolite (the 1-β-*O*-acyl glucuronide) is also described.

Keywords: fenclozic acid; acyl glucuronide; cyanide

Introduction

Fenclozic acid (ICI 54,450) (Figure 1) was one of a series of substituted phenylthiazoylacetic acids synthesised by ICI in the 1960s for evaluation in the non-steroidal treatment of rheumatoid arthritis. Early preclinical work showed the toxicology profile to be benign, with safety findings in multiple species indicating no evidence of overt liver injury. It was only when the compound progressed to man, where a high incidence of jaundice was recorded, that development was stopped.[1]

Drugs containing carboxylic acid moieties can be metabolised *in vivo* to form 1-β-*O*-acyl glucuronides following enzyme mediated reactions with uridine diphosphate-glucuronic acid. Because acyl glucuronides have the ability to undergo reactions resulting in covalent binding to endogenous proteins with the potential to form immunogenic, and therefore toxic haptens, they are of potential concern when developing a new drug, as highlighted by recent Food and Drug Administration guidelines.[2,3]

A key question from a current perspective is whether modern Drug Metabolism and Pharmacokinetics (DMPK) and Molecular Toxicology screens would have flagged up fenclozic acid **1** as a risk and stopped development earlier. This afforded the opportunity to review the original synthesis[4] and apply modern synthetic methodology and techniques to resynthesise [^{14}C] fenclozic acid **[^{14}C]-1** (Scheme 2). The subsequent synthesis of the [^{14}C] acyl glucuronide metabolite **[^{14}C]-2** (Figure 2) that has not been reported previously is also described (Scheme 3).

Results and discussion

The first step of the original synthetic approach (Scheme 1) to radiolabelled fenclozic acid **[^{14}C]-1** involved inorganic manipulation of commercially sourced potassium [^{14}C] cyanide **3** with copper sulfate and sodium metabisulfite to prepare labelled copper(I) cyanide **4**. The solid product could be readily isolated by filtration from the reaction mixture and used without further purification in the next step.

Coupling of the cyanide **4** to 4-bromochlorobenzene was achieved via a classical Rosenmund–von Braun reaction, heating the two components in dimethyl formamide at 150–155 °C for 7 h in a sealed tube. In addition to the harsh conditions used, the reaction was quite labour intensive – it was noted that higher yields were obtained if the reaction tube was removed every 20 min and thoroughly shaken.

The thioamide **6** was then prepared by passing dry hydrogen sulfide gas through a stirred solution of the chlorobenzonitrile **5** and triethylamine in absolute alcohol at 70 °C for 2 h before purifying by column chromatography. The Hantzsch thiazole synthesis was used to prepare the heterocycle **7**, reacting the thioamide **6** with 1,3-dichloro-2-propanone. The final steps involved displacement of the aliphatic chlorine with unlabelled potassium cyanide in dimethyl sulfoxide to afford the nitrile **8** that was hydrolysed, without further purification, in refluxing hydrochloric acid to give the carboxylic acid **[^{14}C]-1**. Purification by column chromatography afforded [^{14}C] fenclozic acid **[^{14}C]-1** with a radiochemical purity of 99.7% and specific activity of 12.6 μCi/mg (3.19 mCi/mmol), constituting an overall radiochemical yield of 22% from potassium [^{14}C] cyanide **3**.

A positive aspect to this synthetic approach was the minimal purification needed for many of the steps; many of the crude intermediates could be used directly in subsequent steps

DMPK IM, AstraZeneca UK Ltd, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

**Correspondence to: Nick Bushby, DMPK IM, AstraZeneca UK Ltd., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK.*
E-mail: Nick.Bushby@astrazeneca.com

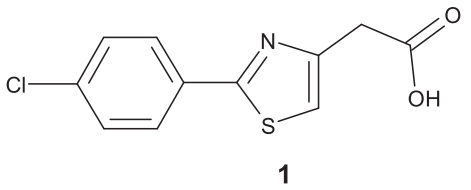

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Figure 1. Fenclozic acid.

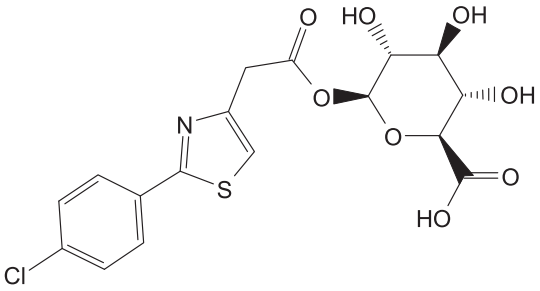

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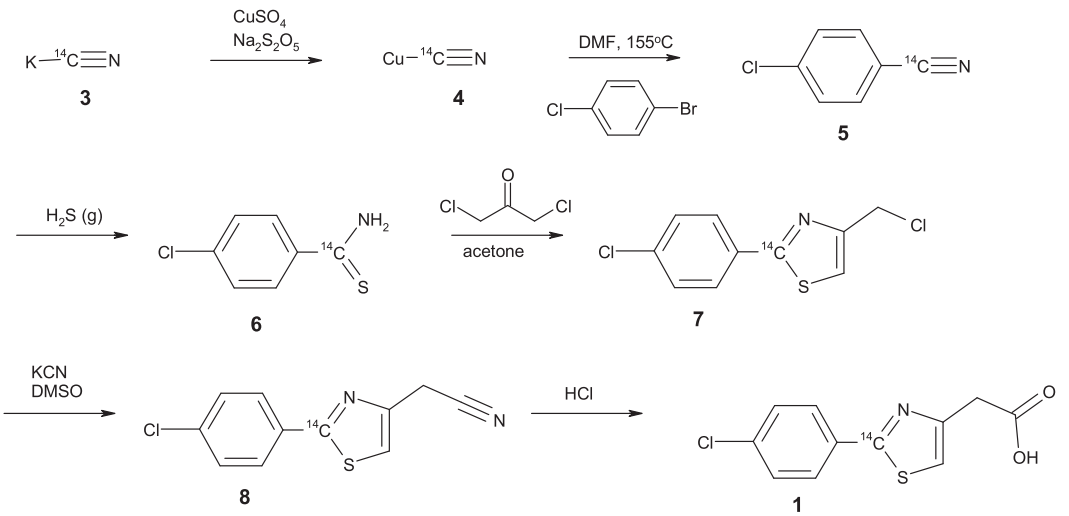
Figure 2. Fenclozic acid glucuronide.

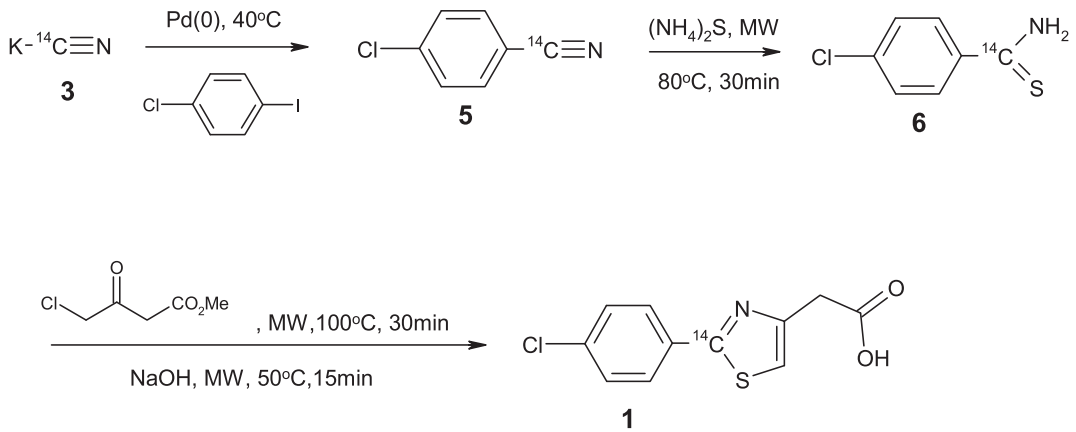
without further purification. Although recognising that the work was conducted using the best available methods at the time, a number of areas for potential improvement were quickly identified. The manipulation of inorganic potassium cyanide **3** to give copper cyanide **4** could be avoided by employing a suitable direct coupling method. In addition, some of the reaction conditions could be considered harsh, with high temperatures and strong acids and bases required. This was an important consideration as we were proposing to prepare fenclozic acid **[^{14}C]-1** at a much higher specific activity, which would exacerbate any stability issues. It was also hoped that the use of gaseous hydrogen sulfide could be avoided, and that the recurring use of potassium cyanide could also be avoided. In addition, many of the workup and purification procedures could be updated to reflect modern working practices, avoiding, for example, the use of benzene/chloroform mixtures for column chromatography as described in some of the original experimental procedures.

Having reviewed the original synthesis, it was possible to simplify the reaction sequence to just four steps (Scheme 2), although still beginning from potassium [^{14}C] cyanide **3** and retaining the same site of labelling.

By employing suitable palladium coupling conditions[5] (Tris (dibenzylideneacetone) dipalladium(0)/1,1′-bis(diphenylphosphino)



Scheme 1. Original synthetic route to [^{14}C] fenclozic acid **[^{14}C]-1**.



Scheme 2. Revised synthetic route to [^{14}C] fenclozic acid **[^{14}C]-1**.

ferrocene), the labelled potassium cyanide **3** could be directly coupled to 4-chloroiodobenzene, exploiting the greater reactivity of the iodo substituent over the chloro to give the radiolabelled chlorobenzonitrile **5**. The modest temperature (40 °C) required for the reaction contrasts sharply to the elevated temperatures needed for the Rosenmund–von Braun reaction used in the original synthesis.

The thioamide **6** was then accessed in a microwave mediated reaction,[6] avoiding the use of gaseous hydrogen sulfide directly, by using solid ammonium sulfide. After heating for 30 min at 80 °C, the product was isolated as a solid with no further purification necessary.

The remaining stages were telescoped into a single pot procedure, again using a microwave approach, which allowed for some extremely quick reaction times. In a change to the previously reported method,[7] the thioamide **6** and methylchloroacetoacetate were mixed with methanol and heated in the microwave for 30 min at 100 °C to form the methyl ester of the parent compound. 2M Sodium hydroxide was added and the mixture reheated in the microwave for 15 min further at 50 °C to hydrolyse the ester. After acidification, the crude product was collected by filtration and washed with water. The material was purified by preparative HPLC to afford [^{14}C] fenclozic acid **[^{14}C]-1** in 24% overall radiochemical yield, with a radiochemical purity >99%, at a specific activity of 240 μCi/mg (61.8 mCi/mmol). Although this yield was comparable with that obtained through the original route, we believe that it could be improved further by using freshly prepared potassium [^{14}C] cyanide **3** rather than the aged batch we had available for this synthesis.

With the successful completion of the radiolabelled drug **[^{14}C]-1**, we turned our attention to the synthesis of a key metabolite **2** (Scheme 3). Recent AstraZeneca collaborations with academia have provided a viable approach to the routine synthesis of acyl glucuronide metabolites. Bowkett *et al*.[8] have shown that the synthesis of 1-β-*O*-acyl glucuronides can be achieved in high yield and excellent β-selectivity through the selective acylation of allyl or benzyl D-glucuronate, followed by deprotection. Benzyl protection was preferred for our work, as it avoided possible Pd residue contamination noted in the literature[9] when removing allyl protecting groups.

[^{14}C]-1 was coupled to benzyl D-glucuronate **9** using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate and *N*-methylmorpholine in acetonitrile. After stirring at ambient temperature for 24 h under nitrogen, the reaction mixture was neutralised with acid resin and the crude product **10** purified by silica cartridge chromatography.

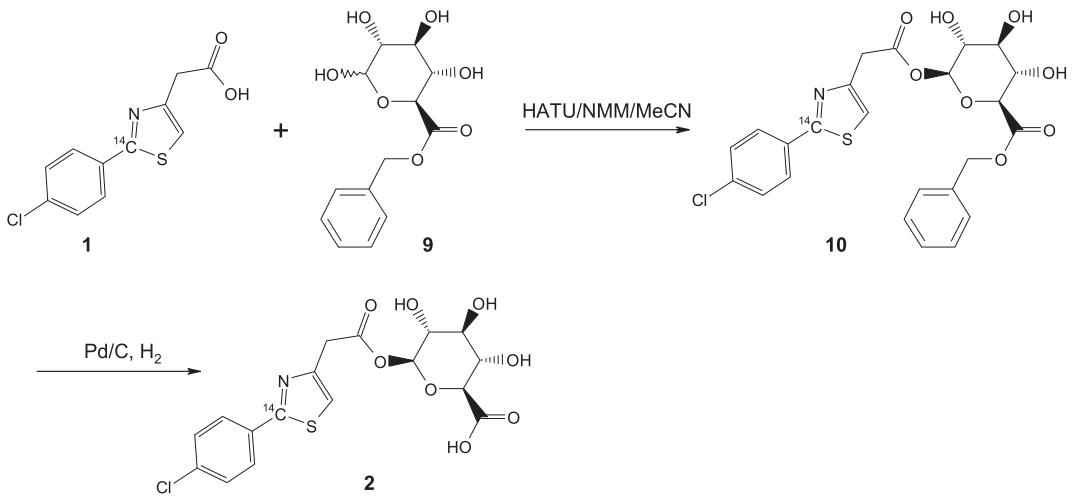
Removal of the benzyl protecting group from **10** proved more challenging. After several days of recharging the Pd/C catalyst, the reaction appeared to have stalled at 80% conversion. The mixture was worked up and the crude product purified by preparative HPLC to provide the acyl glucuronide metabolite **2** with a radiochemical purity >98% at a specific activity of 12.9 μCi/mg. NMR spectroscopy (Figure 3) confirmed the excellent selectivity of the synthetic method towards the required β-anomer, seen at δ 5.7 (^3J = 7.92 Hz). There was no evidence of the presence of the α-anomer (expected at approx. δ 6.1 with a coupling constant of ^3J = 3.7 Hz).[10]

Experimental

^{1}H-NMR spectra were recorded on either a Bruker (500 MHz) or a Bruker (600 MHz) in the deuterated solvent stated. Chemical shifts (δ) in ppm are quoted relative to DMSO-d6 (δ 2.50) or acetone-d6 (δ 2.09). Liquid chromatography-mass spectrometry (LC-MS) data was obtained on a Waters Alliance LC (Waters Corporation, MA, USA) with Waters ZQ mass detector. Analytical TLC was carried out on Merck 5785 Kieselgel 60F$_{254}$ fluorescent plates (Merck KGaA, Darmstadt, Germany). Microwave reactions were carried out in a CEM Explorer (CEM Corporation, NC, USA), heating at 100 W. Column chromatography was performed using ISCO (Teledyne Isco, NE, USA) Companions with pre-packed silica gel cartridges. The target compounds were purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 μm silica, 19 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 0.1% formic acid) and acetonitrile as eluents. Specific activities were measured gravimetrically with a Packard TriCarb 1900CA Liquid Scintillation Analyser (Packard Instrument Company Inc., IL, USA). Radiochemical purities were determined on an Agilent series 1100 HPLC system coupled to a β-Ram Flow Scintillation Analyser.

4-chlorobenzo[^{14}C]nitrile (5)

Tris(dibenzylideneacetone)dipalladium(0) (15.0 mg, 0.02 mmol) and 1,1′-bis(diphenylphosphino)ferrocene (30.4 mg, 0.06 mmol) were added to 1-chloro-4-iodobenzene (665 mg, 2.79 mmol) and potassium [^{14}C] cyanide (164 mCi, 875 μCi/mg, 187 mg, 2.79 mmol) in acetone (1 mL) under nitrogen. The resulting mixture was stirred at 40 °C for 4.5 h.



Scheme 3. Synthetic route to [^{14}C] fenclozic acid glucuronide **[^{14}C]-2**.

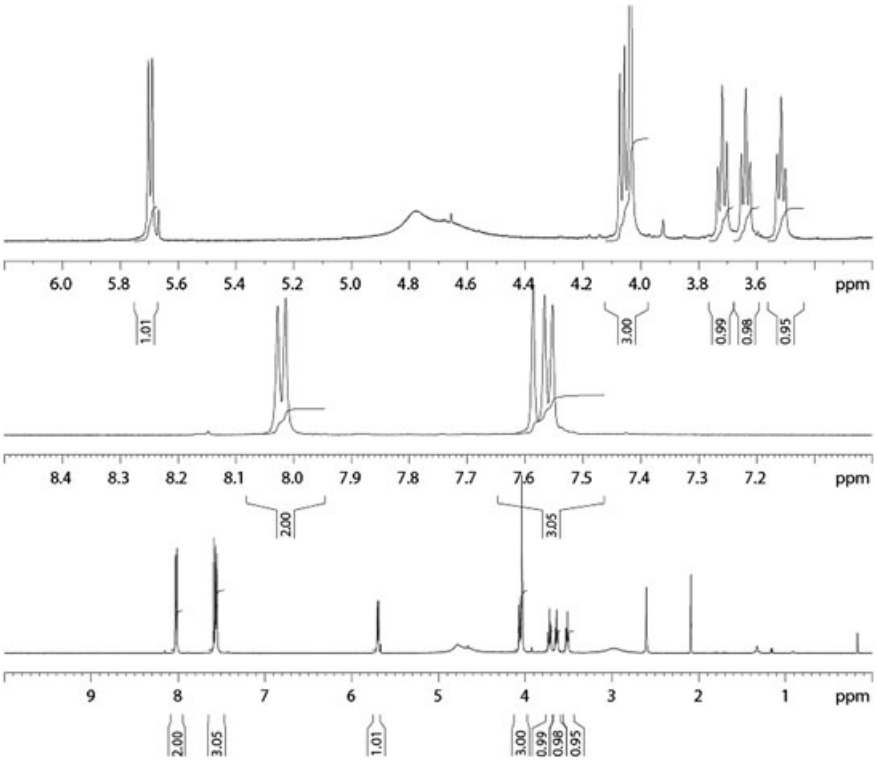


Figure 3. ^{1}H-NMR Spectra of [^{14}C] fenclozic acid glucuronide (acetone-d_6), showing the anomeric proton signal at δ 5.7 (^{3}J = 7.92 Hz).

The reaction mixture was evaporated to dryness and redissolved in ethyl acetate (30 mL) and washed with water (30 mL). The organic layer was dried over $MgSO_4$, filtered and the filtrate was evaporated.

The crude product was purified by flash silica chromatography, elution gradient 0–30% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford 4-chlorobenzo [^{14}C]nitrile (261 mg, 67%) as a yellow solid.

4-chlorobenzothio[^{14}C]amide (6)

4-Chlorobenzo[^{14}C]nitrile (201 mg, 1.44 mmol) and ammonium sulfide (0.983 mL, 7.21 mmol) were suspended in methanol (4 mL) and sealed into a microwave tube. The reaction was heated to 80 °C for 30 min in the microwave reactor and cooled at room temperature. The reaction mixture was diluted with water (8 mL) and the precipitate was collected by filtration, washed with water (10 mL) and dried under vacuum to afford 4-chlorobenzothio[^{14}C]amide (179 mg) as a yellow solid, which was used without further purification. LCMS *m/z* (ESI^-) $(M-H)^-$ = 172.

2-(2-(4-chlorophenyl)[2-^{14}C]thiazol-4-yl)acetic acid (Fenclozic acid, [^{14}C]-1)

4-Chlorobenzothio[^{14}C]amide (179 mg, 1.03 mmol) and methyl 4-chloroacetoacetate (121 µl, 1.03 mmol) were mixed with methanol (38 µl) and sealed into a microwave tube. The reaction was heated to 100 °C for 30 min in the microwave reactor and cooled at room temperature. Sodium hydroxide (2M, aqueous) (1902 µl, 3.8 mmol) was added and the mixture was heated in the microwave reactor to 50 °C for 15 min. The reaction mixture was acidified to pH 5–6 with 2M hydrochloric acid. The precipitate was collected by filtration, washed with water (2 mL) and dried under vacuum. The crude product was then purified by preparative HPLC; fractions containing the desired compound were evaporated to dryness before drying further in a desiccator at 40 °C to afford 2-(2-(4-chlorophenyl)[2-^{14}C]thiazol-4-yl)acetic acid (30 mCi, 125 mg) as a white solid in an overall radiochemical yield of 24% from potassium [^{14}C]cyanide. Radiochemical purity >99% by HPLC. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.92 (2H, d, ^{3}J = 8.22 Hz), 7.54 (3H, m), 3.78 (2H, s) LCMS *m/z* (ESI^+) $(M+H)^+$ = 256.

(*2S,3S,4S,5R,6S*)-benzyl 6-(2-(2-(4-chlorophenyl)[2-^{14}C] thiazol-4-yl)acetoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylate (10)

N-Methylmorpholine (0.173 mL, 1.58 mmol) was added dropwise over a period of 10 s to a stirring suspension of unlabelled fenclozic acid (180 mg, 0.71 mmol), [^{14}C] fenclozic acid (4.8 mCi 20 mg, 0.08 mmol) (*2S,3S,4S,5R,6R*)-benzyl 3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-carboxylate (224 mg, 0.79 mmol) and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (300 mg, 0.79 mmol) in acetonitrile (10 mL) at 22 °C. The resulting pale yellow solution was stirred under nitrogen for 24 h at room temperature.

The reaction mixture was acidified by the addition of Amberlyst A-15 (H+, 2 equiv., 1.53 mmol) and filtered. The filtrate was concentrated under reduced pressure to leave a pale yellow solid that was dissolved in dichloromethane:propan-2-ol (6:1.4, v:v) and purified by flash silica chromatography, elution gradient 5–10% propan-2-ol in dichloromethane. Fractions containing product were combined and concentrated under reduced pressure to give a white solid. The material was further dried at 40 °C under reduced pressure to leave (*2S,3S,4S,5R,6S*)-benzyl 6-(2-(2-(4-chlorophenyl)[2-^{14}C]thiazol-4-yl)acetoxy)-3,4,5-trihydroxy tetrahydro-2H-pyran-2-carboxylate (2.70 mCi, 231 mg, 56%) as a white solid. LCMS *m/z* (ESI^+) $(M+H)^+$ = 520.

(*2S,3S,4S,5R,6S*)-6-(2-(2-(4-chlorophenyl)[2-^{14}C]thiazol-4-yl) acetoxy)-3,4,5-trihydroxy tetrahydro-2H-pyran-2-carboxylic acid (Fenclozic acid acyl glucuronide, [^{14}C]-2)

(*2S,3S,4S,5R,6S*)-benzyl 6-(2-(2-(4-chlorophenyl)[2-^{14}C]thiazol-4-yl)acetoxy)-3,4,5-trihydroxy tetrahydro-2H-pyran-2-carboxylate (2.70 mCi, 231 mg, 0.44 mmol) was dissolved in propan-2-ol (10 mL) and tetrahydrofuran (5 mL). To this solution, palladium on carbon 10% (47.1 mg, 0.04 mmol)

was added and the mixture was stirred at room temperature under an atmosphere of hydrogen gas for 92 h, with regular recharging of the catalyst.

After filtration and evaporation of the filtrate, the crude product was purified by preparative HPLC; fractions containing the desired compound were evaporated to dryness. Dichloromethane (1–2 mL) was added to the product in a vial and the mixture was evaporated to dryness. This was repeated three times to give (*2S,3S,4S,5R,6S*)-6-(2-(2-(4-chlorophenyl)[2-^{14}C]thiazol-4-yl)acetoxy)-3,4,5-trihydroxytetra hydro-2H-pyran-2-carboxylic acid (0.66 mCi, 51.3 mg, 27%) as an off white solid with a radiochemical purity >98% by HPLC. ^{1}H-NMR (600 MHz, acetone-d_6) δ 8.03 (d 2H ^{3}J = 8.22 Hz), 7.59 (s 1H), 7.56 (d 2H ^{3}J = 8.22 Hz), 5.7 (d 1H ^{3}J = 7.92 Hz), 4.07 (d 1H ^{3}J = 9.39 Hz), 4.03 (s 2H), 3.73 (t 1H ^{3}J = 9.39 Hz), 3.64 (t 1H ^{3}J = 8.80 Hz), 3.51 (t 1H ^{3}J = 8.51 Hz). LCMS *m/z* (ESI^{+}) $(M + H)^{+}$ = 430.

Conclusion

[^{14}C] Fenclozic acid **[^{14}C]-1** has been successfully prepared using modern synthetic methodology, reducing the risks associated with the original synthesis. The radiolabelled acyl glucuronide **[^{14}C]-2** has also been chemically prepared for the first time, with excellent β-selectivity.

In vitro metabolism studies using the labelled fenclozic acid **[^{14}C]-1** have shown extensive covalent binding, whereas studies with the unlabelled acyl glucuronide **(2)** have demonstrated rapid transacylation kinetics.[11]

Future work will involve more detailed metabolism studies and the use of the radiolabelled acyl glucuronide **[^{14}C]-2** for further covalent binding studies.

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Conflict of Interest

The authors did not report any conflict of interest.

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