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Rosiglitazone Maleate (BRL 49653-C); The Preparation of [^{14}C] and [^{3}H] Isotopomers

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Summary

The glitazone insulin sensitisers are an important class of pharmaceuticals for the treatment of Type 2 diabetes. Syntheses of [*methyl*-^{14}C] and [^{3}H]rosiglitazone maleate (BRL 49653-C), marketed by SmithKline Beecham Pharmaceuticals as *Avandia®* are described. [*Methyl*-^{14}C]BRL 49653-C was prepared in 5 steps in 12.6% overall radiochemical yield from K[^{14}C]CN. Catalytic reduction with tritium gas of a dibromo derivative gave [^{3}H]rosiglitazone with a specific activity of 58Ci/mmol.

keywords: glitazone, type 2 diabetes, rosiglitazone, BRL 49653, carbon-14, tritium, *Avandia®*.

Introduction

Type 2 diabetes[1] is a widespread metabolic disease; its characteristic insulin resistance in the liver and peripheral tissues giving rise to hyperglycemia and possibly other metabolic disorders such as obesity, hypertension and atherosclerosis. Currently, the sulfonylureas[2] are the most widely prescribed class of antidiabetic drugs for the treatment of type 2 diabetes, but have a modest success rate and suffer from undesirable side effects such as hypoglycemia. In 1982, scientists at Takeda[3] reported the discovery of ciglitazone the first of the glitazone series of antihyperglycemic agents (for some representative structures see Scheme 1), which improve glycemic control by enhancing insulin action in target tissues rather than by increasing insulin secretion. These thiazolidinone insulin sensitisers have since been the subject of much research[1], e.,g. Pfizer, Sankyo, Tanabe, SmithKline Beecham and Wyeth-Ayerst among others have all published work in this area, and a number are being or have been progressed clinically. We report here the syntheses of [^{14}C] and [^{3}H] isotopomers of rosiglitazone maleate[4], BRL 49653-C, a compound marketed by SmithKline Beecham Pharmaceuticals as a treatment for type 2 diabetes under the tradename *Avandia®*.

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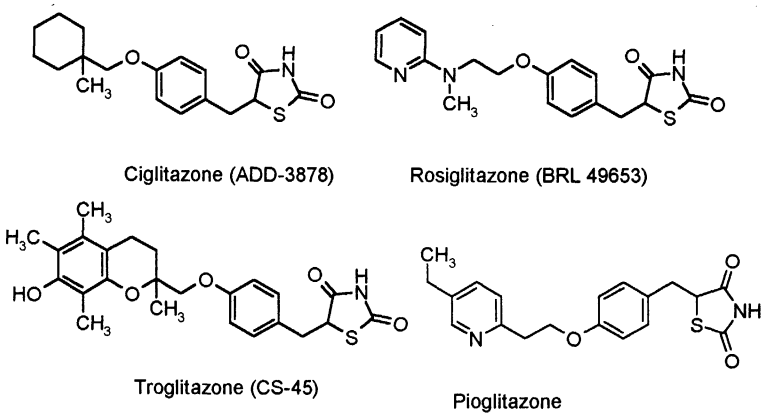
Scheme 1: Structures of some glitazone antidiabetic agents



Discussion

1. The Synthesis of [^{14}C]Rosiglitazone Maleate (BRL 49653-C) (7)

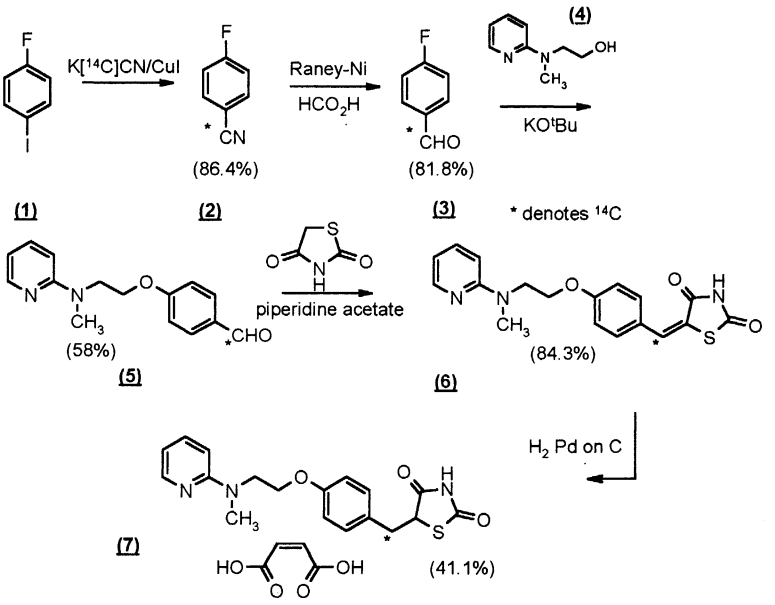
As part of its safety evaluation programme carbon-14 labelled rosiglitazone maleate was required for ADME studies. A number of potential sites for carbon-14 substitution can be envisaged; e.g. the N-methyl group, within the O-ethyl linker or phenyl ring, however we chose to label the methylene group, linking the phenyl and thiazolidinone rings, as this position should be metabolically stable and can be readily accessed by well established labelling strategies.

1.1 The Synthesis of [*methyl*-^{14}C]Rosiglitazone Maleate (BRL 49653-C) (7)

The carbon-14 label was introduced by cuprous iodide mediated[5] substitution of an aryl halide by cyanide. Reaction of 1-fluoro-4-iodobenzene (1) with K[^{14}C]CN and cuprous iodide in DMF at 160°C gave chemospecific substitution of iodide for cyanide in 86.4% radiochemical yield. Not suprisingly no evidence for substitution of fluoride was found. Palladium catalysed cross coupling of K[^{14}C]CN with 1-fluoro-4-iodobenzene also gave the desired nitrile but in lower yield. Selective reduction of the nitrile (2) to the corresponding rather volatile aldehyde (3) (Raney Ni, formic acid) proceeded in 81.8% yield. Substitution of the fluoride by the alkoxide of 2-(methyl-2-pyridylamino)ethanol (4)[6] and piperidine acetate catalysed condensation of the product with thiazolidine-2,4-dione (5) (49% over two steps) completed the molecular scaffold of rosiglitazone.

Catalytic reduction (10% Pd on C) of this olefin (6) (SB-200922) to BRL 49653 (7) at 1 atmosphere of hydrogen proved capricious and only a Johnson Matthey type 90 catalyst and very high catalyst loadings gave reproducible results, presumably poisoning of the catalyst by sulphurous impurities or degradation products is a major problem. Yields of less than 50% were typical. In subsequent syntheses the use of 80-100psi of hydrogen and a PMC1625C catalyst largely overcame these problems. Alternative reduction methods[7] ($LiBH_4$[8], activated Mg[9])

Scheme 2: The synthesis of [*methyl*-^{14}C]rosiglitazone maleate (BRL 49653-C) (7)



although successful offered little advantage in yield or purity of product. HPLC purification of crude rosiglitazone and crystallisation of the maleate salt from ethanol completed the synthesis in 12.6% overall yield from potassium cyanide.

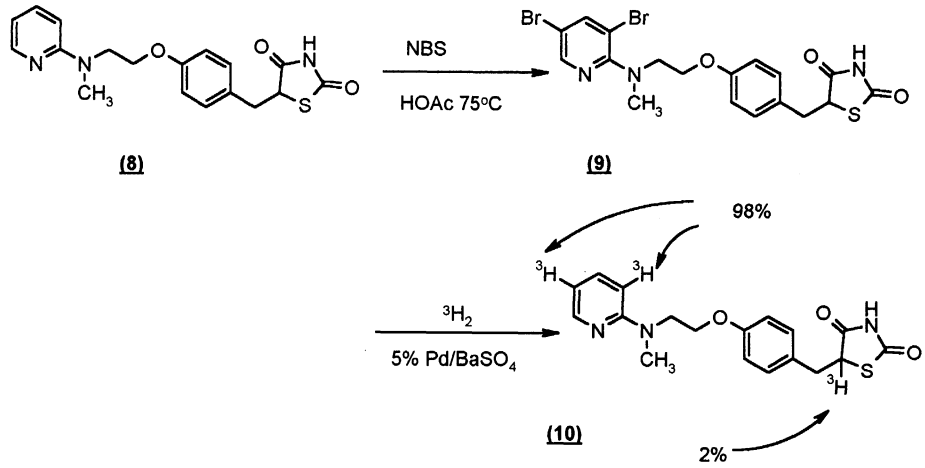
2. The Synthesis of [^{3}H]Rosiglitazone (BRL 49653)

High specific activity [^{3}H]rosiglitazone (**10**) was readily prepared by a bromination/debromotritiation sequence (Scheme 3). Bromination of rosiglitazone free base (**8**) with 2.1 equivalents of N-bromosuccinimide in glacial acetic acid for 45 minutes at 75°C gave a 43% yield of monobromo BRL 49653, obtained as a mixture of the 3 and 5 bromo isomers, and an 18% yield of 3,5-dibromo BRL 49653 (**9**).

The dibromo compound was subjected to tritiation with 2.7 Ci of tritium gas over 5% palladium on barium sulfate. Purification by HPLC gave the tritiated product at a radiochemical purity of 98.4% and a specific activity of 58 Ci/mmol[10]. Tritium NMR confirmed that 98% of the tritium label was at positions 3 and 5 in the pyridine ring while 2% of the label was at C-5 in the thiazolidinedione ring.

Tritiation was quite sluggish and required 400 weight percent catalyst to push the reaction to completion. Deuterium model studies showed that 100 weight percent catalyst left mostly unreacted starting material. Our favoured reduction conditions, which use 5% Pd/C in triethylamine/dimethylformamide, failed to give any product.

Scheme 3: The synthesis of [^{3}H]rosiglitazone, (BRL 49653) (16)



Experimental Section

Potassium [^{14}C]cyanide was supplied by Nycomed Amersham (Cardiff). $^3H_2$ gas was purchased from RC Tritec, Switzerland. Radioactivity was quantified by liquid scintillation counting using a Beckman LS6000A counter. Radiochemical purities were determined by HPLC, unless otherwise stated, on Kromasil 5u C18 (4.6 x 250mm) eluted at 1ml/min with A] 0.05M NaOAc (pH=5.0) B] CH_3CN 5-50%B over 15mins, holding for 25mins. UV detection was at 245nm and radioactive detection with a Packard 525TR or Berthold LB506 flow detector, Ultima Flo M scintillant at 3ml/min. Reactions were followed by HPLC on Spherisorb ODS2 4.6 x 125mm eluted with 0.05M Na_2HPO_4(pH=7.0)/CH_3CN 60:40 v/v at 1ml/min. NMR spectra were recorded at 400MHz or 200MHz on a Bruker ARX 400 or AC200 and chemical shifts are reported in parts per million downfield from tetramethylsilane. All commercial reagents were used as supplied unless otherwise stated. The identities of isotopically labelled intermediates were established by co-elution (TLC/HPLC) with authentic materials obtained in house or from commercial sources and where appropriate by ^{1}H NMR, and that of the final products by ^{1}H NMR.

4-Fluorobenz[*cyano*-^{14}C]nitrile **(2).**

Cuprous iodide (815mg, 4.29mmol) was ground finely and dried in the reaction flask under high vacuum by heating with a hot air gun and cooled under nitrogen. Potassium[^{14}C]cyanide (200mCi @57mCi/mmol, 3.51mmol) was added slurried in dry DMF (10ml), followed by 4-fluoro-iodobenzene (453ul, 873mg, 3.93mmol). The mixture was heated at reflux, under nitrogen, for 20hours. On cooling a solution of KCN (1.2g) in water (50ml) and ethyl acetate (20ml) were added and the mixture stirred vigorously for 15mins. The layers were separated, the aqueous thoroughly extracted with ethyl acetate, the combined organics washed with water (x3), dried over $MgSO_4$, filtered and the solvent carefully evaporated furnishing 4-fluorobenz[*cyano*-^{14}C]nitrile (172.8mCi, 86.4% yield) as an orange crystalline solid. (Caution this material is volatile and ~10mCi of volatile radioactivity was trapped in dry ice traps attached to the rotary evaporator). HPLC analysis showed this material to be of 96.2% radiochemical purity

4-Fluoro[*carbonyl*-^{14}C]benzaldehyde**(3).**

4-Fluoro-[*cyano*-^{14}C]benzonitrile (165mCi, 2.89mmol) was dissolved in DMF (1.5ml), Raney Nickel (420mg of a 50% aqueous slurry) in DMF (1.5ml) and 75% aqueous formic acid (2.5ml) were added. The mixture was heated at 90-95°C, and the course of the reaction followed by HPLC (system as for **(2)**). A further 395mg of catalyst and 2ml of formic acid were added after 2hours reaction, after 4.5h, a further 560mg of catalyst and 2.0ml of formic acid (in 1ml DMF), after 6.5hours a further 600mg and 2.0ml of formic acid (in 1ml DMF), after 7.5hours a further 640mg of catalyst and 2.0ml of formic acid . After 8.25h. reaction the mixture was allowed to cool and kept at ambient temperature for 72hours, when a further 190mg of catalyst were added and the mixture heated at reflux for 40min., and allowed to cool. Ethanol/water (4:1, 5ml) was added and the mixture filtered through a bed of Kieselguhr. The filter pad was washed with dichloromethane- the reaction flask was rinsed with dichloromethane, and water and the filter pad washed with the rinsings. The layers of the filtrate were separated and the aqueous layer extracted with dichloromethane. The combined organics were washed successively with saturated aqueous sodium bicarbonate, water, dried over magnesium sulphate and filtered. The filtrate was carefully evaporated furnishing the title compound as a yellow oil (1.8g, contains DMF, 135mCi, 81.8%) which was stored over 4A molecular sieves. (The aldehyde is volatile and so care must be taken when concentrating solutions).

4-(2-(Methyl-2-pyridylamino)ethoxy)[*carbonyl*-^{14}C]benzaldehyde **(5)**

2-(Methyl-2-pyridylamino)ethanol **(4)** (294mg, 1.93mmol) was dissolved in anhydrous DMF (1ml), under nitrogen, and sodium hydride (60mg of an 80% dispersion in oil) added portionwise. On cessation of reaction, 4-fluoro-[*carbonyl*-^{14}C]benzaldehyde (101mCi, 57mCi/mmol, 1.77mmol) in DMF (3ml) was added dropwise, with washings (2 x 0.5ml). Following 1hour at ambient temperature reaction had ceased but unreacted aldehyde remained. A further 190mg of 2-(methyl-2-pyridylamino)ethanol **(4)** and sodium hydride (38mg) were reacted separately in DMF (1ml) and the resulting alkoxide solution added to the main reaction mixture. Reaction was continued for a further 30min (2.5hours in total) and the mixture poured into water (30ml) and extracted with diethyl ether. The aqueous layer was saturated with salt and extracted further with ethyl acetate. The combined organics were washed with water (x3), dried over magnesium sulphate, filtered and evaporated to dryness, furnishing crude 4-(2-(methyl-2-pyridylamino)ethoxy)[*carbonyl*-^{14}C]benzaldehyde **(5)** (631mg) as a yellow oily solid, which was purified by column chromatography (silica 29g, eluted with ethyl acetate/hexane 1:3 (v/v)), giving 4-(2-(methyl-2-pyridylamino)ethoxy)[*carbonyl*-^{14}C]benzaldehyde **(5)** (264mg, 58%).

5-[[4-[2-(Methyl-2-pyridylamino)ethoxy]phenyl][*methylene*-^{14}C]methylene]-thiazolidine-2,4-dione **(6).**

4-(2-(methyl-2-pyridylamino)ethoxy)[*carbonyl*-^{14}C]benzaldehyde**(5)** (264mg 1.03mmol) was dissolved in anhydrous toluene (8ml), piperidine (14.5ul), glacial acetic acid (14.5ul), 4A molecular sieves (6g, oven dried) and thiazolidin-2,4-dione (140mg, 1.20mmol) added. The mixture was heated at reflux for 1.5hours, and allowed to cool, whereupon the 5-[[4-[2-(methyl-2-pyridylamino)ethoxy]phenyl][*methylene*-^{14}C]methylene]-thiazolidine-2,4-dione **(6).**(314mg, 84.3%) crystallised as a fluffy yellow solid.

5-[[4-[2-(Methyl-2-pyridylamino)ethoxy]phenyl][*methyl*-^{14}C]methyl]-thiazolidine-2,4-dione **(7)**, Rosiglitazone maleate, BRL 49653-C[11]

5-[[4-[2-(Methyl-2-pyridylamino)ethoxy]phenyl][*methylene*-^{14}C]methylene]-thiazolidine-2,4-dione **(6)** (308mg) was dissolved in DMF (5ml,) and 10%Pd on C catalyst (Johnson Matthey Type 90, 300mg) added. The mixture was stirred under 1atm. of hydrogen for 2hours when HPLC showed 25% unreacted starting material. A further 50mg of catalyst were added and reaction continued for 1.75hours The mixture was filtered, the filtrate diluted with water (70ml) and extracted with ethyl acetate (x3). The combined organics were dried over magnesium sulphate, filtered and evaporated to dryness, giving crude [^{14}C]BRL 49653 (307mg) This was purified by HPLC on Techsphere 5 silica 22.5 x 250mm eluted with A] CH_2Cl_2 B] C_2H_5OH/c. NH_4OH (95:5 v/v) A:B 86:14 (v/v) at 10ml/min, UV detection at 254nm. The appropriate fractions were collected, combined and evaporated to dryness, giving [^{14}C]BRL 49653, (129mg) as a colourless solid of 97.8% radiochemical purity, which was dissolved in boiling anhydrous ethanol (4ml). To this was added, in ethanol (0.6ml) maleic acid (41.8mg) and the solution allowed to cool, and kept at 4°C overnight. The mother liquors were decanted and the crystal mass washed with ethanol (2 x 0.5ml) and thoroughly dried under vacuum (116.3mg). The mother liquors and washings were combined, evaporated to dryness and the residue crystallised from boiling ethanol (0.5ml), furnishing a further batch (28.2mg). ^{1}H NMR (d_6-DMSO) 12.0 (bs, 1, N**H**), 8.05 (ddd, 1, J=5.3,2.0,1, Ar**H** *o* to pyridine N), 7.62 (td, 1, J=8.9,7.1,1.9,Ar**H** *p* to pyridine N), 7.14 (d, 2, J=8.7, Ar**H** *o* to ArO), 6.86 (d, 2 J=8.8, Ar**H** *o* to $ArCH_2$, 6.82 (d, 1, J=8.7, Ar**H** *m* to pyridine N), 6.66 (ddd, 1, J=7.7,5.3,1, Ar**H** *m* to pyridine N), 6.21 (s, 2, maleate), 4.85 (dd, 1 J=8.9,4.5, SC**H**), 4.14 (t, 2, J-5.7, OCH_2), 3.93 (t, 2, J=5.7, NCH_2), 3.29 (dd, 1, J=14.2,4.4, $ArCH_2$ □), 3.11 (s, 3, NCH_3), 3.06 (dd, 1, J=14.2, 9.0, $ArCH_2$ □)

5-[[4-[2-(Methyl-2-pyridylamino)ethoxy]phenyl][*methylene*-^{14}C]methylene]-thiazolidine-2,4-dione **(6)** (207mg, 0.58mmol, 32.7mCi) was dissolved in anhydrous THF (10ml) containing pyridine (0.6ml). $LiBH_4$ (85mg, 3.86mmol) was added and the mixture heated at reflux for 3hours. Water was added and the pH adjusted to 7.0 (15% HCl) and the mixture extracted (x 2) with ethyl acetate. The combined extracts were dried over Na_2SO4, filtered and evaporated to dryness. Purification as described above gave 5-[[4-[2-(methyl-2-pyridylamino)ethoxy]phenyl][*methyl*-^{14}C]methyl]-thiazolidine-2,4-dione (92.8mg, 0.26mmol, 14.7mCi, 44.9%).

5-[[4-[2-(Methyl-2-[3',5'-dibromopyridyl]amino)ethoxy]phenyl]methyl]-thiazolidine-2,4-dione **(9)**

BRL 49653 **(8)** (100 mg, 0.28 mmol) was dissolved in 4 ml of glacial acetic acid. To this was added 105 mg (0.59 mmol) of N-bromosuccinimide. The reaction was heated at 75°C under a nitrogen atmosphere for 45 minutes. The solvent was removed under vacuum, the crude product was partitioned between dilute aqueous ammonium hydroxide and ethyl acetate. The organic layer was dried over $MgSO_4$, filtered and concentrated to a yellow oil under vacuum. Purification by flash chromatography gave 53 mg (43%) of the monobominated product as a white glass and 26 mg (18%) of the dibrominated product, also as a white glass. Dibromo BRL 49653: ^{1}H-NMR($CDCl_3$) 8.19 (d,1, J=2.1 Hz, Ar**H** *o* to pyridine N), 7.87 (d, 1, J=2.1 Hz, Ar**H** *p* to pyridine N), 7.11 (d, 2, J=8.7 Hz, Ar**H** *o* to ArO), 6.80 (d, 2, J=8.7Hz, Ar**H** *o* to $ArCH_2$-),

4.49 (dd, 1, J=3.9 Hz, 9.4 Hz, SC**H**), 4.21 (t, 2, J= 5.8Hz, OC$\mathbf{H}_2$), 3.77 (t, 2, J=5.8 Hz, NC$\mathbf{H}_2$), 3.43 (dd, 1, J=3.9 Hz, 14.2 Hz, ArC**H**), 3.09 (s, 3, N**Me**), 3.05-3.17 (m, 1, ArC**H**). Mass Spectrum (CI, NH_3) 518 (13), 516 (26), 514 (15, M+H$^+$),291 (2), 293 (4), 295 (2).

5-[[4-[2-(Methyl-2-[3',5'-^{3}H]pyridylamino)ethoxy]phenyl]methyl]-thiazolidine-2,4-dione **(10)**

The dibromo starting compound **(9)** from above (2.4 mg) was dissolved in DMF/triethylamine (9:1v/v, 1ml). To this was added 9.6 mg of 5% Pd/$BaSO_4$. The mixture was tritiated with 2.7 Ci of tritium gas for 72 hours. Unusued tritium was removed, the reaction filtered throught a 0.2 μm PTFE filter, 1 ml of methanol was added and the reaction taken to dryness by vacuum transfer. Methanol (1 ml) was again added and the solution taken to dryness by vacuum transfer. This was repeated. The crude product was purified by semi-preparative HPLC (Beckman Ultrasphere ODS column, 10mm x 250mm, eluted at 3 mL/min with 75:25:0.1 water/acetonitrile/trifluoroacetic acid, UV at 254 nm, retention time = 11.4 minutes). The product fraction was lyophilised and the residue re-dissolved in 20 ml of 95% ethanol. This gave 136 mCi of tritiated product with a radiochemical purity of 98.4% (by analytical HPLC using the same conditions as above on a 4.6mm x 250mm Beckman Ultrasphere ODS column, retention time = 7.4 minutes). Specific activity by mass spectrometry (CI with ammonia reagent gas) was 58 Ci/mmol. Tritium NMR shows 98% of the label in positions 3 and 5 in the pyridine ring and 2% of the tritium label at C-5 in the thiazolidinedione ring.

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10. This specific activity compares favourably to that achieved (32Ci/mmol) by the group from Ligand Pharmaceuticals who prepared [^{3}H] BRL 49653 by catalytic reduction of the olefinic precursor with tritium gas. D. E Mais, L. G Hamann, K. U Klausing, J. R Paterniti and R. Mukherjee; *Med. Chem. Res* 7 (5) 324 (1997).
11. C14 is a chiral centre and all the syntheses described herein give racemic product. The proton at this chiral centre, in common with other benzyl-thiazolidine-2,4-diones, is known to be labile and so the enantiomers interconvert. See B. C. Cantello, M. A. Cawthorne, D. Haigh, R. M. Hindley, S. A. Smith and P. L. Thurlby, *Bioorgan. Med. Chem. Letts.* **4** 1181 (1994) and D. J. Parks, N. C. O. Tomkinson, M. S. Villeneuve, S. G. Blanchard, T. M. Willson; *Bioorgan. Med. Chem. Letts* **8**: 3657-3658 (1998).

