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Research Article

Synthesis of JTT-501 and its metabolite JTP-20604 labelled with ^{13}C

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Summary

JTT-501 specifically labelled with ^{13}C was obtained *via* a four-step synthesis at an isotopic enrichment level of 99% and in 14% overall chemical yield starting from 4-hydroxy-[ring-U-$^{13}C_6$]benzaldehyde **(3)**. The hydrogenation of [$^{13}C_6$]JTT-501 over Pd/C gave [$^{13}C_6$]JTP-20604 in 90% chemical yield.

Key Words: JTT-501; JTP-20604; oxazolidinedione; insulin-sensitizing agent; Carbon-13

Introduction

JTT-501 ((±)-(*RS*)-4-[4-[-2-(5-Methyl-2-phenyl-oxazol-4-ethoxy]benzyl] isoxazolidine-3,5-dione) is a novel insulin-sensitizing agent belonging to the isoxalidinedione class, that is currently being evaluated for its efficacy in the treatment of Type 2 diabetes mellitus.[1] Since an LC/MS method was developed to determine the unchanged drug and its metabolite JTP-20604 in biological matrices, a non-radioactive isotopically labelled version of JTT-501 and JTP-20604, with a molecular weight of at least three mass units higher than that of the compounds under investigation, was required for use as internal standard. A

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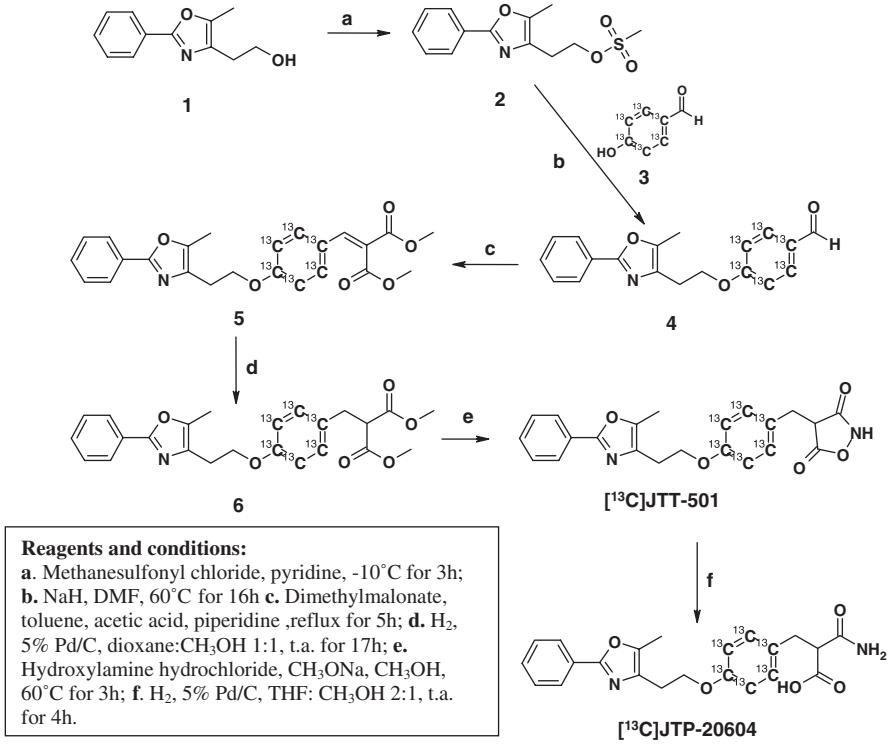
method to prepare JTT-501 is reported in the literature that foresees the use of 4-hydroxybenzaldehyde as starting material.[2] This synthetic approach seemed at first the most convenient way to prepare a suitable stable labelled version of JTT-501 and its metabolite due to the availability of 4-hydroxy-[ring-U-^{13}C]benzaldehyde as well as of the non-labelled intermediates. In the present paper, the preparation of [^{13}C]JTT-501 and [^{13}C]JTP-20604 is reported.

Discussion and results

[^{13}C]JTT-501 and its metabolite [^{13}C]JTP-20604 were prepared as outlined in Scheme 1. The methanesulfonyl derivative **2** was prepared immediately prior to use by treatment of the commercially available 2-(5-methyl-2-phenyl-4-oxazolyl)ethanol **1** with methanesulfonyl chloride in anhydrous pyridine. The reaction of **2** with the commercially



Scheme 1.

available 4-hydroxy-[ring-U-^{13}C]benzaldehyde **3** (1:1 molar ratio) in the presence of sodium hydride in anhydrous dimethylformamide (DMF) at 60°C for 16 h, afforded the intermediate **4**. The treatment of **4** with a slight excess of dimethyl malonate (1: 1,1 molar ratio) in the presence of piperidine and acetic acid in dry toluene at reflux (110°C) for 5 h gave the benzylidene **5**. The hydrogenation over 5% Pd/C in dioxane: methanol (1:1 v/v) at room temperature for about 17 h of **5** yielded the corresponding dimethyl malonate **6**. The reaction of **6** with hydroxylamine hydrochloride and sodium methoxide in anhydrous methanol afforded the crude ^{13}C labelled JTT-501. After purification by preparative high performance liquid chromatography (HPLC), [^{13}C]JTT-501 was obtained in >97% chemically pure form and an isotopic enrichment of 99%. The overall chemical yield was approximately 14% from **3**. The ^{13}C labelled metabolite JTP-20604 was accomplished via hydrogenation of [^{13}C]JTT-501 over 5% Pd/C in tetrahydrofuran: methanol (2:1 v/v) at room temperature for about 4 h. [^{13}C] JTP-20604 was obtained in a >99% chemically pure form with a chemical yield of 89% from [^{13}C]JTT-501. These compounds were suitable for use as internal standards in the LC-MS bioanalytical assay.

Experimental

General methods

4-hydroxy-[ring-U-$^{13}C_6$]benzaldehyde **(3)** (99% isotopic enrichment) was purchased from Cambridge Isotope Labs. All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated. Chemical purities were determined by HPLC using a series-200 pump (Perkin-Elmer) equipped with a series 200 solvent degasser (Perkin-Elmer), series AS-950 autosampler (Jasco) and a LC-295 UV/VIS or a LC-235 UV diode array detector (Perkin-Elmer) and Turbochrom 4.0 software (Perkin-Elmer). Preparative HPLC was performed using a PrepStar SD-1 pump (Varian) equipped with a ProStar 320 UV/Vis detector (Varian), a ProStar 701 fraction collector (Varian) and PrepStar 4.2 software (Varian).

*2-(5-Methyl-2-phenyl-4-oxazolyl)ethoxymethanesulfonyl (**2**)*

2-(5-Methyl-2-phenyl-4-oxazoly)ethanol **1** (1 g; 4.92 mmol) was dissolved in anhydrous pyridine (52.6 ml) and the solution was cooled to

–10°C. After 5 min stirring at –10°C, methanesulfonyl chloride (0.775 ml; 9.99 mmol) was added dropwise and the solution was stirred for 3 h at –10°C. At the end of reaction (as determined by HPLC[3]) methylene chloride (160 ml) was added, the solution was transferred into a 500 ml separating funnel and washed with water (3 × 150 ml), 0.01N HCl (2 × 20 ml) and water (2 × 20 ml). The organic layer was dried (Na_2SO_4) and, after solvent evaporation, the intermediate **2** was obtained (1.166 g; 4.14 mmol), 97% chemically pure (by HPLC[3]; Rt = 18.6 min). The yield of this step was 84%.

*4-[2-(5-Methyl-2-phenyl-4-oxazolyl)ethoxy]-[ring-U-^{13}C] benzaldehyde (**4**)*

A solution of 4-hydroxy-[ring-U-^{13}C]benzaldehyde **3** (300 mg; 2.34 mmol) in anhydrous DMF (2.5 ml) was added to sodium hydride (74 mg; 3.04 mmol) in anhydrous DMF (2.5 ml) at 0°C. The resulting suspension was added dropwise to a solution of **2** (990 mg; 3.51 mmol) in anhydrous DMF (1.5 ml) at 0°C. The reaction mixture was then stirred at 60°C for 16 h under nitrogen atmosphere. At the end of the reaction (as determined by HPLC[3]) the solution was added with water (15 ml), the mixture transferred into a 100 ml separating funnel and extracted with diethyl ether (3 × 20 ml). The organic layer was washed with brine (20 ml), dried (Na_2SO_4) and after solvent evaporation, the crude compound **4** was obtained. The compound was purified by flash-chromatography on a silica gel column (15 × 5 cm ID) using a mixture of hexane:ethyl acetate 2:1 by volume as eluting solvent system. The fractions containing the compound **4** were combined and after solvent evaporation to dryness, the intermediate **4** was obtained (943 mg, 2.83 mmol), 93% chemically pure (by HPLC [3]; Rt = 24.2 min). The yield of this step was 85%.

*Dimethyl[4-[2-(5-methyl-2-phenyl-4-oxazolyl)ethoxy]-[ring-U-^{3}C]benzylidenemalonate (**5**)*

The intermediate **4** (943 mg, 2.83 mmol) was dissolved in anhydrous toluene (36 ml), the solution was transferred into a round-bottomed flask equipped with a reflux condenser and a Dean-Stark trap. Dimethylmalonate (0.39 ml; 3.4 mmol), acetic acid (0.11 ml) and piperidine (0.11 ml) were then added and the solution refluxed for 5 h. At the end of reaction (as determined by HPLC[3]) the reaction mixture

was cooled to room temperature, water (30 ml) was added and the solution, then transferred into a 100 ml separating funnel and extracted with diethyl ether (3 × 10 ml). The organic layer was washed with 1 N HCl aqueous solution (10 ml), a 0.1% $NaHCO_3$ aqueous solution (10 ml), brine (10 ml) and dried (Na_2SO_4). After solvent evaporation, the crude compound **5** was obtained which was purified by flash-chromatography on a silica gel column (20 × 3 cm ID) using a mixture of hexane:ethyl acetate 7:2 by volume as eluting solvent system. After combining the fractions containing the compound **5** and solvent evaporation to dryness, the intermediate **5** was recovered (919.4 mg, 2.18 mmol), 97.7% chemically pure (by HPLC[3]; Rt = 27.2 min). The yield of this step was 77%.

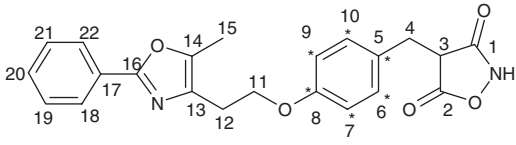
*Dimethyl 4-[2-(5-methyl-2-phenyl-4-oxazolyl)ethoxy]-[ring-U-^{13}C] benzylmalonate (**6**)*

The intemediate **5** (919.4 mg, 2.18 mmol) was dissolved in a mixture dioxane:methanol 1:1 by volume (18 ml), then 5% Pd/C (103 mg) was added. The reaction mixture was stirred under hydrogen gas at room temperature at atmospheric pressure until the reaction was complete (about 17 h; determined by TLC on silica gel F254 plates (20 × 5 cm, 0.25 mm thick; Merck) eluting with ethyl acetate 7:3 by volume). After catalyst removal by filtration through a D4 sintered-glass filter, the filtrate was evaporated to dryness. The residue was washed with *n*-hexane (2 × 5 ml) and the compound **6** was obtained (731 mg, 1.73 mmol) 88% chemically pure (by HPLC[3]; Rt = 27.2 min). The yield of this step was 79%.

(±)-(RS)-4-[4-[-2-(5-Methyl-2-phenyl-oxazol-4-yl)ethoxy]-[ring-U-^{13}C]benzyl] isoxazolidine-3,5-dione ([^{13}C]JTT-501)

Sodium methoxide (281.2 mg, 5.2 mmol) in anhydrous methanol (4 ml) was added to a solution of hydroxylamine hydrochloride (182 mg, 2.61 mmol) in anhydrous methanol (4 ml) at 0°C. The obtained solution was added dropwise to a solution of **6** (731 mg, 1.73 mmol) in anhydrous methanol (8 ml) then stirred at 60°C for 3 h. At the end of reaction (as determined by HPLC [4]), water (10 ml) was added to the mixture which was then washed with diethyl ether (2 × 25 ml). The aqueous layer was transferred into a 100 ml separating funnel, adjusted to pH 2 with a 1 N HCl aqueous solution. A white precipitate was formed which was

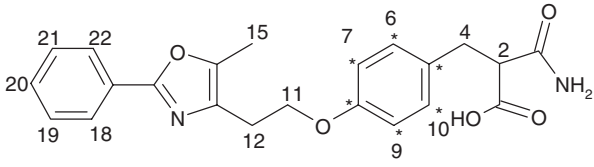
extracted with ethyl acetate (3×25 ml) .The organic layer was washed with brine (25 ml), dried (Na_2SO_4) and after solvent evaporation, the crude [^{13}C]JTT-501 (326 mg) was obtained. The compound was purified by preparative HPLC.[5] The fractions containing the compound of interest were collected under nitrogen in the dark, cooled immediately to about 4°C. After pooling, sodium chloride was added to saturation, pH was adjusted to 2 with hydrochloric acid and the resulting aqueous phase was extracted with cold ethyl acetate and toluene (3×25 ml). The organic layers were pooled and dried (Na_2SO_4). After filtration and solvent evaporation to dryness, [^{13}C]JTT-501 was obtained (182.8 mg, 0.46 mmol) 99% chemically pure (by HPLC[4]; Rt = 19.7 min). The yield of this step was approximately 27%. Mass spectrum (ESI-MS/MS): *m/z* 399 ([MH]$^+$). ^{1}H NMR (DMSO d6; 500 MHz) 2.35 δ (singlet, 15 CH_3); 2.89 δ (multiplet, 12 CH_2); 3.0–3.1 δ (multiplet, 4 CH_2); 4.14 δ, (multiplet, 11 CH_2); 6.74 δ, (multiplet, 7 CH + 9 CH); 7.07 δ (multiplet, 6 CH + 10 CH); 7.45 δ (multiplet, 19 CH + 20 CH + 21 CH); 7.91 δ (multiplet, 18 CH + 22 CH,); 8.45 δ (singlet, CONH-O).



2-{4-[2-(5-Methyl-2-phenyl-1,3-oxazol-4-yl)ethoxy]-[ring-U-^{13}C]benzyl}-3-oxo-beta-alanine ([^{13}C]JTP-20604)

[^{13}C] JTT-501 (75.8 mg, 0.19 mmol) was dissolved in a mixture of tetrahydrofuran: methanol 2:1 by volume (1.5 ml), after which 5% Pd/C (8.3 mg) was added. The reaction mixture was stirred under hydrogen gas at room temperature at atmospheric pressure until the reaction was complete (about 4 h) (determined by HPLC [4]). After catalyst removal by filtration through a Uniprep PTFE 0.2 mm filter (Wathman), the filtrate was evaporated to dryness. After solvent evaporation, [^{13}C] JTP-20604 was obtained (68 mg, 0.169 mmol) in 99% chemically pure form (by HPLC[4]; Rt = 12.6 min). The overall yield was about 89%. Mass spectrum (ESI-MS/MS): *m/z* 401 ([MH]$^+$). ^{1}H NMR (DMSO d6; 500 MHz); 2.34 δ (singlet, 15 CH_3); 2.90 δ (multiplet, 4 CH_2 + 12 CH_2); 3.39 δ (triplet of doublets, 2 CH); 4.16 δ, (triplet of doublets, 11 CH_2); 6.81 δ (multiplet, 7 CH + 9 CH); 7.08 δ (multiplet, 6 CH + 10 CH); 6.95

and 7.40 δ (two singlets $CONH_2$); 7.48 δ (multiplet, 19 CH + 20 CH + 21 CH); 7.90 δ (multiplet, 18 CH + 22 CH); 12.39 δ (broad signal, COOH).



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References

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2. Shinkai H, Onogi S, Tanaka M, *et al. J Med Chem* 1998; **41**:1927–33.
3. Symmetry C18 column (mm 150 × 4.6 ID, 5 µm, by Waters) eluting with CH_3CN (A) and 25 mM K_2HPO_4 at pH 2.5 with H_3PO_4 (B) mixtures: 10 min at 30%A; from 30%A to 50%A in 10 min; 10 min at 50% A. Flow rate: 1 ml/min. Column temperature: 40°C. Analytical wavelength: 254 nm.
4. Eclipse XDB C18 column (mm 150 × 4.6 ID, 5 µm, by Zorbax) eluting with 20 mM K_2HPO_4, 5mM tetrabutylammonium hydroxide at pH 6.5: THF 5:1 (v/v) (A) and 20 mM K_2HPO_4, 5 mM tetrabutylammonium hydroxide at pH 6.5: THF 4:6 (v/v) (B) mixtures: 40 min at 100% A; from 100% A to 0% A in 15 min; 4 min at 100% B. Flow rate: 0.8 ml/min. Column temperature: 40°C. Analytical wavelength: 280 nm.
5. Combi HT XDB C18 column (mm 150 × 21.2 ID, 5 µm, by Zorbax) eluting with 20 mM K_2HPO_4, 5 mM tetrabutylammonium hydroxide at pH 6.5: THF 5:1 (v/v) (A) and 20 mM K_2HPO_4, 5 mM tetrabutylammonium hydroxide at pH 6.5: THF 4:6 (v/v) (B) mixtures: 25 min at 90% A; from 90% A to 0% A in 15 min; 5 min at 100% B. Flow rate: 24 ml/min. Column temperature: ambient. Analytical wavelength: 280 nm.

