Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 21 January 2009, Revised 7 May 2009, Accepted 18 May 2009 Published online 3 July 2009 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1620

Synthesis of carbon-14 labelled midazolam

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Cytochrome P450 (CYP) enzymes are responsible for much of the phase I oxidative metabolism observed *in vivo*. Many important pharmaceutical compounds are metabolized by CYP. Co-administration of a drug with another agent can alter the efficacy or the toxicity, especially in cases where drug clearance depends primarily on the CYP metabolism. Compounds that induce or inhibit the CYP activity are often used in drug–drug interaction studies. Midazolam is one such compound that is routinely used in drug–drug interaction studies because it is a known substrate for CYP3A enzymes. The synthesis of this important tool molecule has been documented, but unfortunately a detailed preparation of carbon-14-labelled midazolam has not been reported in the literature. This paper describes a two-step synthesis leading to [^{14}C]midazolam. A total of 4.5 mCi of [^{14}C]midazolam was obtained having a specific activity of 120.1 μCi/mg (39.12 mCi/mmol). The radiochemical purity as determined by HPLC was 99.8% and the overall radiochemical yield was 9%.

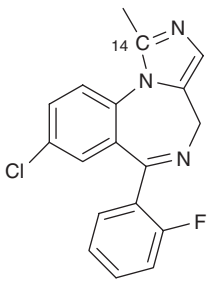
Keywords: midazolam; carbon-14; isotope labelling; drug–drug interaction

Introduction

As part of drug-candidate screening and the drug discovery and development process, investigators often conduct *in vitro* drug metabolism studies to assess the potential for Cytochrome P450 (CYP)-based drug interactions.[1] These studies characterize the metabolic pathway of the drug and the potential of other drugs to modify the metabolism.[1] Drug–drug interactions are thought to be one of the most important factors in severe adverse drug responses. Knowing this important fact, most pharmaceutical companies now consider it mandatory to minimize, as much as possible, the potential for drug–drug interactions.[2,3] Most drug–drug interactions occur when two drugs share a common clearance pathway involving a drug-metabolizing enzyme or a transporter.[4] Once the metabolic pathways of a drug candidate are understood, there is a need to also assess the potential impact of inhibition of these pathways by other drugs.

The largest group of enzymes known for the phase I oxidative metabolism is the CYP family of enzymes. CYP enzymes are located in various tissues throughout the body, with the liver being the largest source. Two intestinal CYP isoforms that account for 70% of the total intestinal CYP activity include CYP3A4 and CYP3A5. Since >50% of CYP-metabolized drugs are substrates for CYP3A, this enzyme is the source of numerous drug–drug interactions.[5] Several CYP3A probe drugs have been identified to detect and quantify potential CYP3A-mediated drug–drug interactions.[6] Midazolam, with its known CYP3A4- and CYP3A5-mediated metabolism, has been an important tool molecule for predicting drug–drug interactions.[6]

The syntheses of unlabelled, stable-labelled, and tritium-labelled midazolam have been detailed in the literature.[7–9] The use of carbon-14-labelled midazolam in metabolism studies has also been reported.[10–12] Surprisingly, a detailed synthesis of carbon-14-labelled midazolam was never reported. This paper describes the synthesis of carbon-14-labelled midazolam (**[^{14}C]-1**).



[^{14}C]-1

Discussion and results

The syntheses of unlabelled and stable isotope-labelled midazolam have been detailed in the literature.[7,8] These procedures were adopted for use in the carbon-14 synthesis using labelled ethyl acetimidate hydrochloride as shown in Scheme 1. Compound **2,** which was the starting material for our carbon-14-labelled synthesis, was prepared in seven steps from (2-amino-5-chlorophenyl)(2-fluorophenyl)methanone following a literature procedure.[13,14] A solution of the amine **2** in ethanol/tetrahydrofuran at 0°C

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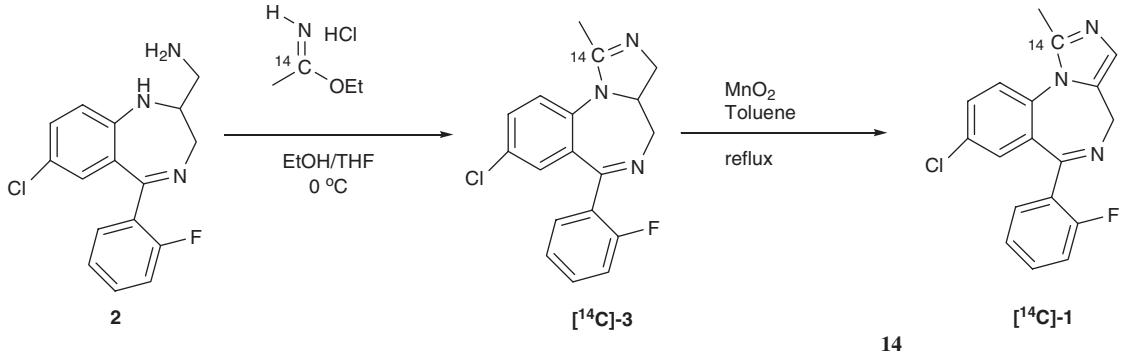

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Scheme 1. Synthesis of carbon-14-labelled midazolam ([**^{14}C]-1**).

was treated with 50 mCi (specific activity 56 mCi/mmol) of carbon-14-labelled ethyl acetimidate hydrochloride, followed by an equivalent portion of unlabelled ethyl acetimidate hydrochloride to give imidazoline **[^{14}C]-3** after 3 h at room temperature (RT). Conversion of imidazoline into imidazole was carried out using MnO_2 as the oxidizing agent. This conversion was very sensitive to the water content of the MnO_2 used. Sigma-Aldrich supplies several grades of manganese oxide. When high-purity, Reagent Plus (> 99%), or activated (85%) grades were used, little to no conversion to the desired midazolam was observed. However, the rate of extent of conversion increased when the activated (85%) MnO_2 was dried under vacuum at 125°C for 24 h. These results indicate that the water content of MnO_2 used dramatically affects the reaction.

A solution of **[^{14}C]-3** in dry toluene was treated with dried MnO_2 and refluxed for 3 h. After workup and purification by reversed-phase HPLC, 4.5 mCi of **[^{14}C]-1** was obtained having a specific activity of 120.1 μCi/mg (39.12 mCi/mmol). The radiochemical purity as determined by HPLC was 99.8% and the overall radiochemical yield from labelled acetimidate hydrochloride was 9%. Radiolabelled **[^{14}C]-1** was relatively unstable in the solid at high specific activity. At 120 μCi/mg (39.12 mCi/mmol), **[^{14}C]-1** decomposed at a rate of 6.8% per month into three primary impurities. Owing to the limited quantity synthesized, stabilization of radiolytic decomposition was not explored.

Experimental

All reagents were obtained from Aldrich Chemical Company and used without further purification. Carbon-14-labelled ethyl acetimidate hydrochloride was obtained from ViTrax Company. All experimental procedures were optimized using unlabelled materials. All glassware was dried and purged with nitrogen or argon before use. All reactions were monitored by HPLC using the following conditions: YMC Pack Pro C18 column s-3 μm (4.6 × 150 mm). Solvent A = water with 0.05% TFA; B = acetonitrile with 0.05% TFA. Gradient: 100% A 0–5 min, 100–0% A 5–20 min, 0% A 20–25 min, 0–100% A 25–30 min. Flow 1 mL/min, wavelength 215 nm. Chemical and radiochemical purity was determined by HPLC using a Varian ProStar system (Model 210) equipped with a Varian ProStar PDA (Model 330) and a Beta-Ram Detector (IN/US Systems, Inc.). Specific activity was determined by gravimetric analysis using liquid scintillation counting (Wallac Model 1409).

(E)-8-chloro-6-(2-fluorophenyl)-1-methyl-3a,4-dihydro-3H-benzo[f-^{14}C]imidazo[1,5-a]diazepine ([^{14}C]-3)

To a solution of (E)-(7-chloro-5-(2-fluorophenyl)-2,3-dihydro-1H-benzo[e][1,4]diazepin-2-yl)methanamine[11,12] (**2**, 270 mg, 0.890 mmol) in ethanol (2 mL) and THF (1 mL) at 0°C was added [^{14}C-imino]ethyl acetimidate hydrochloride (110 mg, 0.890 mmol, 50 mCi), followed by unlabelled ethyl acetimidate hydrochloride (110 mg, 0.890 mmol).[7,8] The reaction was stirred for 30 min at 0°C, then warmed to RT and stirred for an additional 3 h. At that time the reaction was concentrated under reduced pressure. The residue was dissolved into 15 mL of dichloromethane and extracted with 20 mL of 5% NaHCO3. The layers were separated and the aqueous was extracted with 15 mL of dichloromethane. The combined dichloromethane extracts were washed with saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield crude **[^{14}C]-3** (298 mg, 0.909 mmol, 102% yield). The radiochemical purity as determined by HPLC was 65%.

(E)-8-chloro-6-(2-fluorophenyl)-1-methyl-4H-benzo[f][^{14}C]imidio[1,5-a][1,4]diazepine, [^{14}C]midazolam ([^{14}C]-1)

To a suspension of crude **[^{14}C]-3** (298 mg, 0.909 mmol) in toluene (15 mL) was added activated manganese(IV) oxide (1500 mg, 17.25 mmol) (dried under vacuum at 125°C for 24 h). The mixture was heated at 117°C for 3 h. After cooling to RT, the reaction mixture was filtered, rinsed with toluene (5 mL) and DCM (5 mL). The filtrate was concentrated under reduced pressure to afford crude carbon-14-labelled midazolam. The crude material was dissolved in 5 mL of acetonitrile. The solution was purified by semi-preparative HPLC in 16 × 0.3 mL injections on a Pack Pro C18 column (19 × 150 mm, Solvent A = 95:5 v/v 0.01 M ammonium acetate: acetonitrile; B = 5:95 v/v 0.01 M ammonium acetate:acetonitrile, gradient: 100% A 0–5 min, 100–0% A 5–25 min, 0–100% A 25–30 min, flow: 15 mL/min, wavelength: 215 nm). The pooled fractions of **[^{14}C]-1** were partially concentrated under reduced pressure to remove the acetonitrile. The remaining aqueous was extracted with 2 × 20 mL of DCM. The combined DCM extracts were washed with saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was dried under vacuum at RT to yield 4.5 mCi of **[^{14}C]-1**, having a specific activity of 120.1 μCi/mg (39.12 mCi/mmol). The radiochemical purity as determined by HPLC was 99.8% and the overall radiochemical yield from labelled acetimidate hydrochloride was 9%. NMR ($CDCl_3$) δ 2.95 ppm (S, 3), 4.08 ppm (dd, 1, J = 12.84 Hz), 5.18 ppm (dd, 1, J = 13.6 Hz), 7.0–7.69 (m, 8, Aromatic-H). NMR spectrum is consistent with the published spectrum of unlabelled midazolam.[7]

Conclusions

Carbon-14-labelled midazolam was synthesized in two steps from 50 mCi of radiolabelled precursor to afford 4.5 mCi (9%) of

the product with a specific activity of 120.1 μCi/mg and good radiochemical purity. The radiolabelled product was observed to be relatively unstable at high specific activity. At 120 μCi/mg (39.12 mCi/mmol), [^{14}C]-labelled midazolam decomposed at a rate of 6.8% per month. The importance of using activated and dried manganese oxide for the oxidation step in the preparation of [^{14}C]-labelled midazolam should also be noted.

Acknowledgement

The authors would like to thank the Bristol-Myers Squibb Discovery Chemical Synthesis group for the synthesis of the starting material **2**.

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