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Research Article

Synthesis of ^{14}C-labeled E4010

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Summary

^{14}C-Labeled E4010, [4-(3-chloro-4-methoxybenzyl)amino-1-(4-hydroxy) -piperidino]-6-[4-^{14}C]phthalazinecarbonitrile monohydrochloride was synthesized for drug metabolism and pharmacokinetic studies using [1-chloro-4-(3-chloro-4-methoxybenzyl)amino]-6-[4-^{14}C]phthalazinecarbonitrile as the starting material. The radiochemical yield was 33.4%. The specific radioactivity and radiochemical purity, as determined by radio-HPLC and LSC, were 4.77 MBq/mg and 99.8%, respectively. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: ^{14}C-labeled E4010 ; PDE5 inhibitor ; synthesis

Introduction

E4010, [4-(3-chloro-4-methoxybenzyl)amino-1-(4-hydroxy)piperidino]-6-phthalazinecarbonitrile monohydrochloride, is a new compound which has potent and selective cGMP phosphodiesterase (PDE5) inhibitory activity.[1–3] A ^{14}C-labeled E4010, [4-(3-chloro-4-methoxybenzyl)amino-1-(4-hydroxy)-piperidino]-6-[4-^{14}C]phthalazinecarbonitrile monohydrochloride was synthesized for use in the studies of metabolic fate of E4010 in laboratory animals. The synthetic route for ^{14}C-labeled E4010 was the same as that used for unlabeled E4010.[1]

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Results and conclusion

^{14}C-Labeled E4010 was prepared by the route outlined in Figure 1.

Radiochemical purity of ^{14}C-labeled E4010, determined by radio-HPLC was 99.8%, as shown in Figure 2. 0.2% of a radiochemical impurity was observed. Its retention time relative to was 0.60, which was approximately the same as an impurity found in unlabeled E4010.

Radioactivity of the ^{14}C-labeled E4010 solution was 618 MBq/99.5 ml and its concentration was 129.5 mg/99.5 ml. Therefore, the specific radioactivity was 4.77 MBq/mg.

618 MBq (129.5 mg) of ^{14}C-labeled E4010 was finally obtained and its radiochemical yield was 33.4%.

The amount of ^{14}C-labeled E4010 obtained from this synthesis was sufficient to study the metabolic fate of E4010 in laboratory animals.

Experimental

Synthesis

[1-Chloro-4-(3-chloro-4-methoxybenzyl)amino]-6-[4-^{14}C]phthalazinecarbonitrile (**1**) was purchased from Amersham. All other reagents and solvents were of analytical reagent grade, purchased commercially, and used without any further purification. 4-Hydroxypiperidine hydrochloride, 1-methyl-2-pyrrolidinone and 1 N hydrochloric acid were obtained from Wako Pure Chemical (Osaka, Japan). Potassium carbonate, ethanol and *n*-hexane were obtained from Kanto Kagaku (Tokyo, Japan). Ethyl acetate was obtained from Junsei Kagaku (Tokyo, Japan). Silica gel (70–230 mesh) was obtained from Merck (Darmstadt, USA). NMR spectra were recorded using a JNM-GX400 spectrometer with CD_2HSOCD_3 as internal standard. MASS spectra

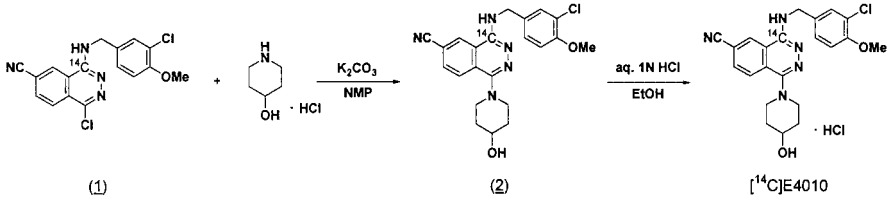
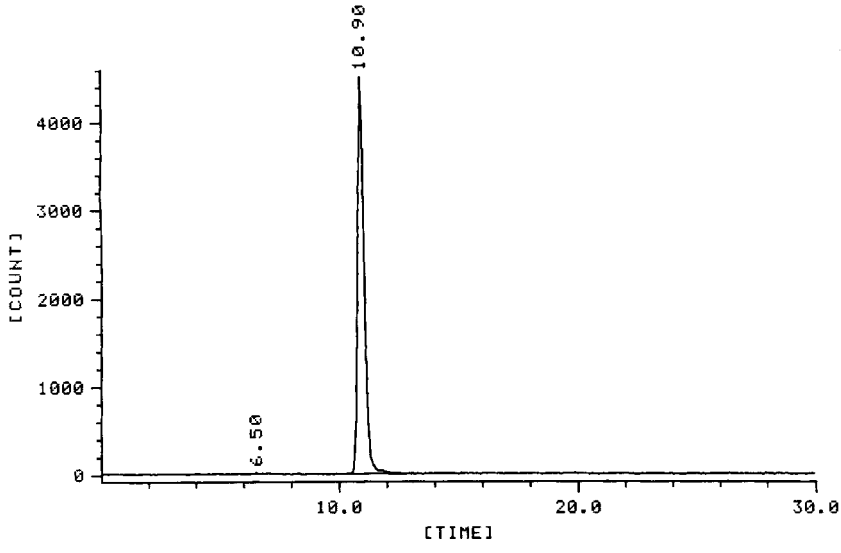


Figure 1. Synthesis of ^{14}C-labeled E4010



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SAMPLE NO. 0 NAME: SERIAL NO. 0011

CHANNEL NO. 1 METHOD: NORMALIZATION WIDTH: 60

NO.	MK	TIME	AREA	HEIGHT	WIDTH	A-%	TP	RS
1	B	6.50	2.19000E+01	15.8	7.0	0.155	17041	12.83
2	B	10.90	1.41041E+04	4520.2	17.2	99.845	7997	

Figure 2.

were recorded using a JMS-HX100 spectrometer. NMR and MASS spectra were recorded at Daiichi Pure Chemical Co., Ltd.

^{14}C-Labeled E4010

A mixture of compound (**1**) (323 mg, 0.89 mmol, 1.85 GBq) and 4-hydroxypiperidine hydrochloride (860 mg, 6.25 mmol) and potassium carbonate (617 mg, 4.47 mmol), and 1-methyl-2-pyrrolidinone (5 ml) was stirred at 170°C for 2 h. After cooling, water (60 ml) was added and the mixture was extracted with ethyl acetate (15 ml). The organic layer was added to *n*-hexane (2 ml) and subjected to silicagel (32 g) column chromatography eluting with *n*-hexane:ethyl acetate (1:10). Compound (**2**) was obtained (353 mg, wet). To the suspension of (**2**) (353 mg, wet) in ethanol (4 ml) and water (3 ml), 1 N hydrochloric acid (1.0 ml) was added, and the contents warmed in an oil bath to achieve dissolution.

To the mixture, water (1 ml) was added and the whole mixture stirred at room temperature for 1 h. The precipitated solid was collected. The solid was dissolved in ethanol, and the latter removed *in vacuo* to give [^{14}C]E4010. [^{14}C]E4010 was dissolved in ethanol (150 ml) and filtered through a glass filter, and the filtrate was evaporated to about 85 ml volume and diluted to 99.5 ml with ethanol. ^{1}H-NMR (DMSO-d_6) δ; 1.62–1.73 (2H, m), 1.90–1.98 (2H, m), 2.97–3.08 (2H, m), 3.37–3.48 (2H, m), 3.72–3.80 (1H, m), 3.85 (3H, s), 4.72 (2H, d, $J = 5.6$ Hz), 7.16 (1H, d, $J = 8.4$ Hz), 7.47 (1H, dd, $J = 8.4, 2.0$ Hz), 7.59 (1H, d, $J = 2.0$ Hz), 8.23 (1H, br), 8.45 (1H, br), 9.19 (1H, br); MS *m/e* (FAB) 426 (MH^+).

Measurement of radiochemical purity and specific radioactivity

0.1 ml of the ethanol solution of ^{14}C-labeled E4010 was diluted to 1.1 ml with 0.1 N HCl:methanol = 1:1 (v/v).

Radiochemical purity. The radiochemical purity was determined by HPLC (column: YMC AM312 ODS, YMC Kyoto Japan, mobile phase: $CH_3CN/H_2O/HClO_4/NaClO_4 = 400/600/1/5$, flow rate: 1 ml/min). The ^{14}C-labeled E4010 was subjected to the HPLC and the peak area measured.

Specific radioactivity. The specific radioactivity was determined by liquid scintillation counting and quantitative determination of E4010 by HPLC-UV.

Measurement of radioactivity. Triplicate 50 µl aliquots of the ^{14}C-labeld E4010 solution were inserted into vials containing 15 ml of ACS-II (Amersham, UK) as the scintillator and the radioactivity was measured using a liquid scintillation counter system (model LSC-3500, Aloka, Tokyo, Japan).

Determination of E4010 concentration. To 0.1 ml of ^{14}C-labeled E4010 solution, 0.1 ml of internal standard solution (1.0 mg/ml) was added and 10 µl of the solution was subjected to HPLC. The concentration of E4010 was determined from peak area ratio to the internal standard. The HPLC instrument consisted of a CCPM pump (TOSOH, Japan), a WISP 712 autosampler (Waters), and a UV-8020 ultraviolet spectrometer (TOSOH, Japan).

The HPLC conditions were as follows:
Column: YMC AM312 ODS.
Mobile phase: $CH_3CN/H_2O/HClO_4/NaClO_4 = 400/600/1/5$ V/V/V/W).
Flow rate: 1 ml/min.
Detection: UV 254 nm.
Injection volume: 10 μl.

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