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Research Article

Synthesis of PHA-690509 labelled with ^{14}C

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Abstract: PHA-690509, a cyclin-dependent kinase A inhibitor, has been labelled with carbon-14. [^{14}C]PHA-690509 was obtained via a three-step procedure in 10% overall radiochemical yield starting from [^{14}C]thiourea **3**.

Keywords: PHA-690509; CDK2; cyclin-dependent kinase; carbon-14

Introduction

Inhibition of tumor growth by inhibiting kinases involved in cell-cycle progression is an active area of cancer drug development. Cyclin-dependent kinase 2 (CDK2) is one of the serine–threonine kinases that plays a crucial role in the molecular control of cell-cycle progression.[1,2] During a discovery project aimed at finding a specific and selective CDK2 inhibitor, (2*S*)-2-[4-(acetylamino)phenyl]-*N*-(5-isopropyl-1,3-thiazol-2-yl) propanamide (PHA-690509) was selected among a series of synthetic 2-aminothiazoles for its promising *in vitro* and *in vivo* profile.[3,4]

As the development of the drug candidate has progressed, the preparation of a radiolabelled version was required to fully investigate the absorption, distribution, metabolism, excretion and the mechanism of action of the compound. In addition, the first clinical study planned with PHA-690509 was a microdose study in healthy male volunteers. This study was proposed to better understand the pharmacokinetic and metabolic behavior of the compound before starting the phase I studies in patients. Due to its high efficiency of detection when using decay counting methods such as the accelerator mass spectrometry, the isotope of choice of a microdose study is carbon-14. Therefore, the preparation of a metabolically stable [^{14}C]PHA-690509 was needed. In the present paper the preparation of [^{14}C]PHA-690509 is reported.

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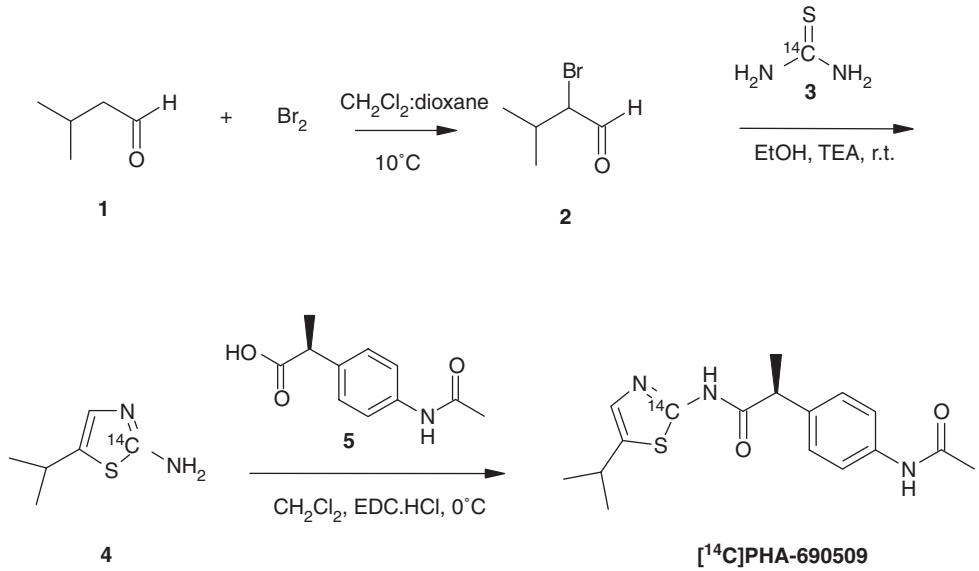
Results and discussion

The availability of ^{14}C-labelled thiourea at reasonable price as well as of suitable non-labelled intermediates, also GMP grade, prompted us to scale-down to the radiochemical scale an in-house procedure currently used to prepare gram quantities of PHA-690509. Moreover, following this procedure, carbon-14 can be introduced in the thiazole ring that, according to preliminary *in vitro* and *in vivo* studies, seemed not to be affected by metabolism. The synthetic pathway is shown in Scheme 1. The reaction of bromine with the aldehyde **1** in a mixture of dichloromethane:dioxane at 10°C for about 2 h gave the intermediate **2**. The bromoderivative **2** was immediately reacted with a slight molar excess of [^{14}C]thiourea **3** in ethanol (EtOH) in the presence of triethylamine (TEA). After about 20 h stirring at room temperature and work-up, the crude 5-isopropyl-1,3-[2-^{14}C]thiazol-2-amine **4** was obtained which was used without purification in the following step. The reaction of the amine **4** with (2*S*)-2-[4-(acetylamino)phenyl]propanoic acid **5** in dichloromethane in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) at 0°C for about 2 h afforded the crude [^{14}C]PHA-690509. After purification by preparative HPLC, [^{14}C]PHA-690509 was obtained with a radiochemical purity >98% and a specific activity of 2.15 GBq/mmol. The overall radiochemical yield was approximately 10% from **3**. The method of synthesis here described is suitable for the introduction of carbon-14 in PHA-690509 and the obtained compound was suitable for the planned studies.



Scheme 1

Experimental

Chemicals and materials

[^{14}C]Thiourea **3** (specific activity 2.15 GBq/mmol) was purchased from Perkin Elmer Life Sciences. All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated.

Instrumentation and equipment

Radioactivity measurements were performed on a Tri-Carb 2100 TR liquid scintillation analyzer (Packard) using Ultima Gold (Perkin Elmer Life Sciences) as liquid scintillation cocktail. Chemical purities were determined by HPLC using a series-200 pump (Perkin-Elmer) equipped with series 200 solvent degasser (Perkin-Elmer), series AS-950 autosampler (Jasco) and a LC-235 UV diode array detector (Perkin-Elmer) connected with Turbochrom Client/Server (PENelson) as integrator via link 600 interface (Perkin-Elmer). Radiochemical purities were determined using an A-515TR radio-HPLC analyser (Packard) equipped with a 0.5 ml homogeneous cell (liquid scintillation cocktail: Ultima Flo-M (Perkin Elmer Life Sciences); ratio to HPLC effluent: 2/1). Preparative-HPLC was carried out at 25°C using a PrepStar HPLC system (Varian).

Analytical methods

HPLC System A. Xterra MSC18 column (mm 100 × 4.6 ID, 5 μm) eluting with CH_3CN:H_2O:trifluoroacetic acid (TFA) 10:90:0.1 by volume (A) and CH_3CN:H_2O:TFA 90:10:0.1 by volume (B) mixtures: from 100%A to 0%A in 10 min; 5 min at 0%A. Flow rate: 1 ml/min. Column temperature: 40°C. Analytical wavelength: 254 nm.

HPLC System B. Chiralpak AD column (mm 250 × 4.6 ID, 10 μm) eluting with isopropanol: *n*-heptane 4:6 by volume. Flow rate: 0.7 ml/min. Column temperature: 25°C. Analytical wavelength: 254 nm.

HPLC System C. Zorbax SB-C18 column (mm 150 × 4.6 ID, 3.5 μm) eluting with 20 mM KH_2PO_4 at pH 2.5 with H_3PO_4:CH_3OH 420:580 by volume plus 2.16 g/l of sodium dodecyl sulfate. Flow rate: 1 ml/min. Column temperature: 25°C. Analytical wavelength: 254 nm.

2-Bromo-3-methyl-butanaldehyde (2)

A solution of bromine (0.165 ml, 3.16 mmol) in dichloromethane:dioxane 4:1 by volume (1.7 ml) was slowly dripped into a stirred and cooled (0°C) solution of **1** (0.34 ml, 3.16 mmol) in a mixture of dichloromethane:dioxane 4:1 by volume (2 ml). The reaction mixture was stirred at 10°C for 2 h and the obtained solution of the crude intermediate **2** (0.77 mmol/ml calculated) was immediately used in the next step.

2-Amino-5-isopropyl-1,3-(2-^{14}c)thiazole (4)

[^{14}C]Thiourea **3** (0.74 GBq, 0.344 mmol) was suspended in a mixture of dichloromethane:dioxane 4:1 by volume (1 ml) then triethyl amine (50 μl, 0.36 mmol), a solution of intermediate **2** (0.38 ml, 0.29 mmol) prepared as

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previously described and ethanol (100 μl) were added. The reaction mixture was stirred at room temperature for about 20 h then water (4 ml) was introduced into the reaction flask. The resulting mixture was made alkaline by adding 12 N NaOH up to pH 12 then was stirred at room temperature for about 1 h. The solution was transferred into a separating funnel and extracted with dichloromethane (3 × 5 ml). All the organic phases were combined and dried over Na_2SO_4. After evaporation to dryness, the crude intermediate **4** was recovered (0.18 GBq, 0.084 mmol), 56% radiochemically pure [by radio-HPLC; system A (see Analytical methods)]. The crude material was used without further purification in the next step.

(2S)-2-(4-(acetylamino)phenyl)-N-(5-isopropyl-1,3-(2-^{14}C)thiazol-2-yl)propanamide (^{14}C)PHA-690509)

The intermediate **5** (21.1 mg, 0.1 mmol) and EDC · HCl (20.7 mg, 0.1 mmol) were added to a stirred and cooled (0°C) solution of **4** (0.18 GBq, 27.9 mg, 0.084 mmol) in dichloromethane (1 ml). The reaction mixture was stirred at 0°C for about 2 h. At the end of the reaction [determined by radio-HPLC; system A (see Analytical methods)], the mixture was diluted with dichloromethane (5 ml) and transferred into a 50 ml separating funnel. The organic phase was washed with 1N $NaHCO_3$ (3 × 10 ml), water (3 × 10 ml), 1 N HCl (3 × 10 ml) and dried over Na_2SO_4. After filtration and evaporation to dryness, the crude [^{14}C]PHA-690509 was recovered (0.134 GBq, 0.062 mmol), with a radiochemical purity of 72% [by radio-HPLC; system A (see Analytical methods); R_t = 4.2 min]. The compound was purified by preparative HPLC (Symmetry Prep C18 column, mm 100 × 19 ID, 7 μm, eluting with CH_3CN:H_2O:TFA 10:90:0.1 by volume (A) and CH_3CN:H_2O:TFA 90:10:0.1 by volume (B) mixtures: from 100%A to 40%A in 14 min; from 40%A to 0%A in 1 min; at 0%A for 5 min; flow rate: 17 ml/min. Column temperature: ambient; analytical wavelength: 254 nm]. After work-up, [^{14}C]PHA-690509 (0.078 GBq, 0.038 mmol) was obtained as a white solid with a radiochemical purity >98% [by radio-HPLC; system C (see Analytical methods); R_t = 9.1 min]. The enantiomeric purity was >98% [by radio-HPLC; system B (see Analytical methods); R_t = 12.5 min].

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REFERENCES

1. Sherr CJ. *Science* 1996; **274**: 1672–1677.
2. Knockaert P, Greengard P, Meijer L. *Trends Pharmacol Sci* 2002; **23**: 417–425.
3. Vulpetti A, Casale E, Roletto F, Amici R, Villa M, Pevarello P. *J Mol Graph Model* 2006; **10**(5): 341–348.
4. Pevarello P, Amici R, Traquandi G, Villa M, Vulpetti A, Isacchi A. *PCT Int Appl WO00/26202*, 11 May 2000.