Synthesis of ^{14}C-Labelled (S)-(+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4',3':4,5]thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine (^{14}C-E6123)

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Summary

^{14}C-Labelled (S)-(+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4',3':4,5]thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine (^{14}C-E6123), a platelet activating factor receptor antagonist for studying the pharmacokinetic profile of E6123, was synthesized in three steps using [1-^{14}C] acetyl hydrazine fumarate as the labelled starting material. The final product has high chemical and radiochemical purity with a specific activity of 53.2mCi per mmol (1.97GBq per mmol). The overall radiochemical yield is 6.0%.

Key words: platelet activating factor (PAF), PAF antagonists, ^{14}C-E6123

Introduction

(S)-(+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4',3':4,5]thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine (E6123) has been developed as a platelet activating factor (PAF) receptor antagonist in our laboratories (1). E6123 has an asymmetric center in its molecule and shows very potent anti-PAF activities in in vitro and in vivo assay systems. In this paper, we describe the synthesis of ^{14}C-labelled E6123. This synthesis was carried out to study the pharmacokinetic profile of E6123.

Results and discussion

^{14}C-labelled **6** was prepared from [1-^{14}C] acetyl hydrazine fumarate **2** in three steps (2). The synthetic pathway is shown in Figure 1.

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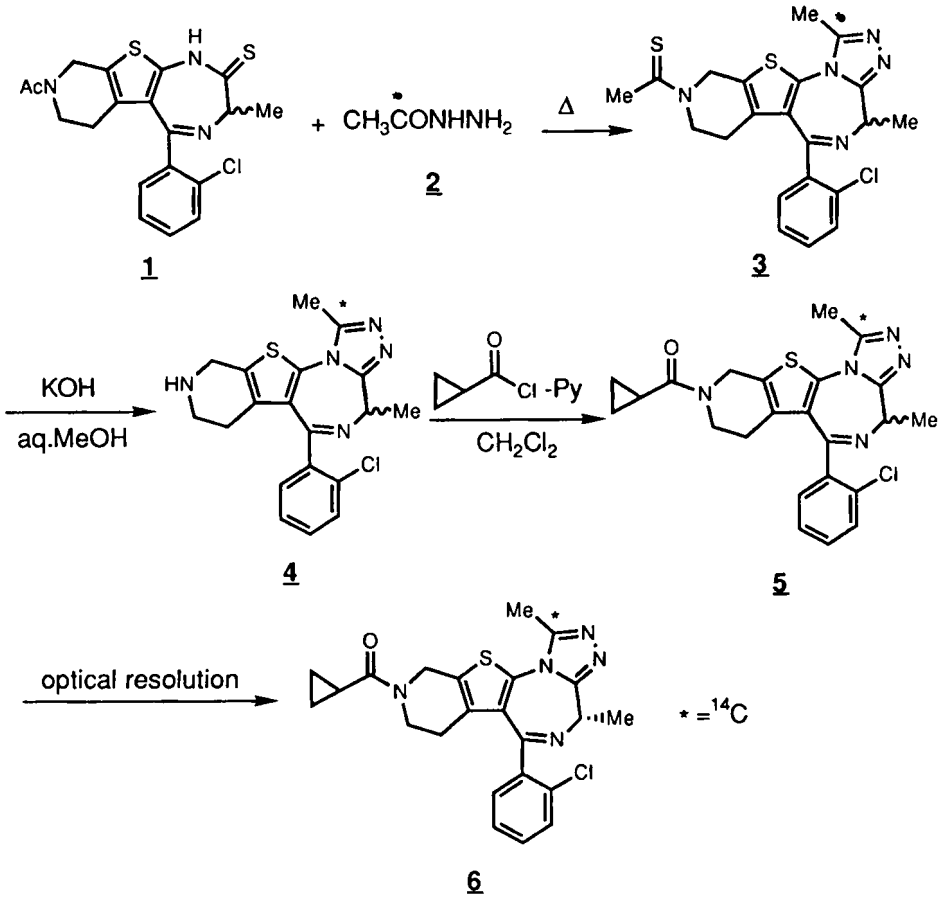

Figure 1. Synthesis of (S)-(+)-6-(2-chlorophenyl)-3-cyclopropane-carbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4',3':4,5]thieno [3,2-f][1,2,4][11-^{14}C]triazolo[4,3-a][1,4]diazepine (**6**).

[1-^{14}C] acetyl hydrazine fumarate **2** and 3-methyl-5-(2-chlorophenyl)-8-thioacetyl-6,7,8,9-tetrahydro-1H,3H-pyrido[4',3':4,5]thieno[3,2-f][1,4]diazepine-2-thione **1** in dioxane were heated together at 140°C with evaporation of the dioxane. Compound **3** was purified by column chromatography and then hydrolized with potassium hydroxide in aqueous methanol to give compound **4**. Compound **4** was acylated with cyclopropanecarbonyl chloride in the presence of pyridine to afford racemate **5**. Compound **5** was separated into in its enantiomers with a ChiraSpher HPLC column. The desired S-enantiomer was purified by thin-layer chromatography to give ^{14}C-labelled **6** in 6.0% radiochemical yield (based on **2**) and in 97% enantiomer excess. The structure of ^{14}C-labelled **6** was confirmed by

comparison (TLC and HPLC) with an unlabelled authentic sample of E6123. ^{14}C-labelled **6** had greater than 99.3% radiochemical purity and a specific activity of 53.2mCi per mmol (1.97GBq per mmol).

Experimental

Analytical and preparative thin-layer chromatography were developed using Kieselgel 60 F_{254} plate (Merck). Wakogel C-200 (Wako Chemical Industries) was used for silica gel chromatography. A ChiraSpher (Merck) column packed by SENSYU KAGAKU (Tokyo Japan) was used for optical resolution. The optical purity was determined on a ChiraSpher column (5μm, 4.6mmX250mm, mobile phase n-Hexane/Ethanol 85:15, flow rate 1mL/min, column temperature ambient, detector UV254nm). The measurments of radioactivity were carried out using an Aloka LSC-3500 type Liquid Scintillation Spectrometer. Thin-Layer radiochromatography was performed using a Radiochromanyzer JTC-601 (Aloka). [1-^{14}C] acetyl hydrazine fumarate was purchased from Amersham International plc.

6-(2-chlorphenyl)-3-thioacetyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4',3':4,5]thieno[3,2-f][1,2,4][11-^{14}C]triazolo[4,3-a][1,4]diazepine (3) [1-^{14}C] acetyl hydrazine fumarate **2** (0.25g, 1.9mmol, 3.7GBq) and 3-methyl-5-(2-chlorophenyl)-8-thioacetyl-6,7,8,9-tetrahydro-1H,3H-pyrido[4',3':4,5]thieno[3,2-f][1,4]diazepine-2-thione **1** (1.17g, 2.8mmol) in 10mL of dioxane were heated at 140°C with evaporation of the dioxane for 1h. The reaction mixture was evaporated under reduced pressure and the residue was purified by column chromatography ($CHCl_3$→98:2 $CHCl_3$/MeOH) to give **3** (0.57g, 68%) as a red-brown oil.

6-(2-chlorphenyl)-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4',3':4,5]thieno[3,2-f][1,2,4][11-^{14}C]triazolo[4,3-a][1,4]diazepine (4) A solution of compound **3** (0.57g, 1.3mmol) and potassium hydroxide (3.1g, 47.3mmol) in 80% aqueous methanol was heated at reflux. The reaction mixture was poured into brine and extracted four times with ethylacetate. The extract was concentrated under reduced pressure and the residue was purified by column chromatography ($CHCl_3$→98:2~95:5 $CHCl_3$/MeOH) to give **4** (0.28g, 57%) as a yellow colored oil.

6-(2-chlorphenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4',3':4,5]thieno[3,2-f][1,2,4][11-^{14}C]triazolo[4,3-a][1,4]diazepine (6) To a cooled (ice-bath) solution of 4 (0.28g, 0.74mmol) and pyridine (0.08g, 0.94mmol) in 15mL of dichloromethane was added cyclopropanecarbonyl chloride (0.09g, 0.85mmol) dropwise and the resulting solution was stirred at the same temperature for 30 min. The reaction mixture was poured into 25mL of sat.aqueous $NaHCO_3$ and extracted with dichloromethane, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$→98:2~97:3 $CHCl_3$/MeOH) to give 5 as a yellow colored oil. The racemic 5 thus obtained was optically resolved on a ChiraSpher column packed by SENSYU KAGAKU (Tokyo Japan) to give 6 (0.08g, 24%) in 97% enantiomeric excess. Preparative HPLC conditions: ChiraSpher 25μm column 20mmX500mm, mobile phase tetrahydrofuran/n-hexane 40:60, flow rate 20mL/min , detector UV254nm. This optically active compound 6 was purified by preparative thin-layer chromatography (solvent system: MeOH/$CHCl_3$ 20:80; eluent; EtOAc) to give the ^{14}C-labelled 6 as a white powder (52mg, 6.0%yield from 2, 97% enantiomeric excess, greater than 99.3% radiochemical purity, specific activity 53.2mCi per mmol or 1.97GBq per mmol). The identity of 6 was confirmed by comparison of its R_f-values on TLC developed by two different solvent systems and

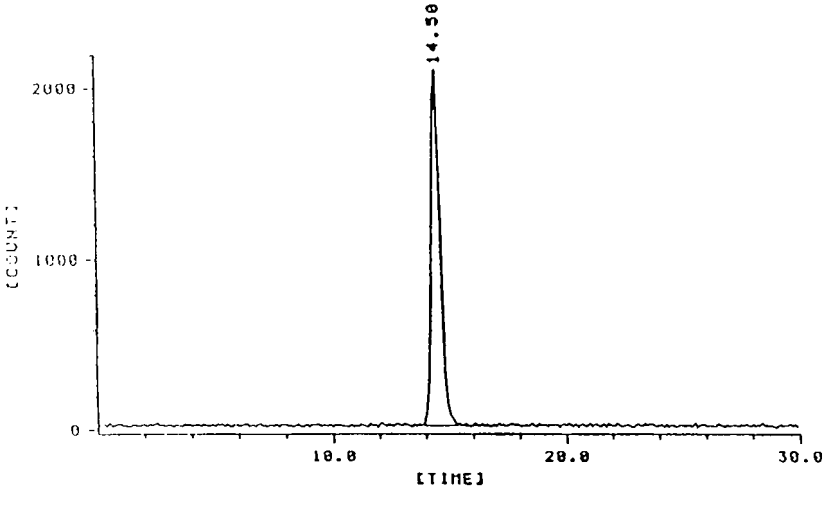

Figure 2. HPLC-radiochromatogram of ^{14}C-E6123 (6)

retention time on HPLC with those of an unlabelled authentic sample. R_f-values of the compound **6** were 0.66 in EtOAc/MeOH/diethylamine (100:100:1), 0.81 in $CHCl_3$/MeOH (8:2). ^{14}C-labelled **6** had greater than 99.3% radiochemical purity by HPLC (Figure 2).

HPLC conditions: column Wakosil $_5C_{18}$ 4.6mmX250mm, mobile phase 5mM sodium dodecylsulfate(pH3.0)/acetonitrile 145:70, flow rate 1.0mL/min, detector UV250nm, radio detector RS-8000 and CP8080 (TOSO).

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