Synthesis of ^{14}C-Labelled Satigrel

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SUMMARY

^{14}C-Labelled satigrel, or 4-cyano-5-(4'-methoxy [ring-U-^{14}C] phenyl)-5-(4"-methoxyphenyl)-4-pentenoic acid was synthesized for drug metabolism and pharmacokinetic studies using 4, 4'-dimethoxy [ring-U-^{14}C] benzophenone as the starting material. The radiochemical yield was 10.0%. The specific radioactivity and radiochemical purity, as determined by radio-HPLC analysis, were 10.3 MBq(277.2 μCi)/mg and 98.8%, respectively.

Keywords : anti-platelet aggregation agent, ^{14}C-labelled satigrel , 4-cyano-5-(4'-methoxy [ring-U-^{14}C] phenyl)-5-(4"-methoxyphenyl)-4-pentenoic acid, synthesis

INTRODUCTION

Satigrel, or 4-cyano-5,5-bis(4-methoxyphenyl)-4-pentenoic acid, is a new compound which has shown potent inhibitory effects on platelet aggregation in preclinical studies(1-3), and clinical studies are currently in progress to investigate its anti-thrombotic activity in patients under chronic haemodialysis.

A ^{14}C isotopically labelled form of satigrel, or 4-cyano-5-(4'-methoxy [ring-U-^{14}C] phenyl)-5-(4"-methoxyphenyl)-4-pentenoic acid was synthesized for use in the studies of metabolic fate of satigrel in laboratory animals. The synthetic route for ^{14}C- labelled satigrel was the same as that used for unlabelled satigrel(4).

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MATERIALS AND METHODS

1. Materials and reagents

4,4'-Dimethoxy[ring-U-^{14}C]benzophenone was purchased from Amersham International plc (Buckinhamshire, UK). Ethyl 4-bromo-4-cyanobutyrate was obtained from Eisai Chemical Co., Ltd. All other reagents and solvents were of analytical reagent grade, purchased commercially, and used without any further purification. Iodine was obtained from Nakarai Kagaku (Kyoto, Japan). Zinc powder was obtained from Kanto Kagaku (Tokyo, Japan). Tetrahydrofuran, ethylacetate, chloroform, methanol and benzene were obtained from Kanto Kagaku (Tokyo, Japan). Silica gel (70-230 mesh) was obtained from Merck (Darmstadt, USA). Ammonium chloride and 1N hydrochloric acid (titration grade) were obtained from Wako Pure Chemical (Osaka, Japan).

Unlabelled satigrel was obtained from Eisai Chemical Co., Ltd. 4-Cyano-6,6-bis(4-methoxyphenyl)-5-hexenoic acid for an internal standard in HPLC determination, and 4-cyano-5, 5-bis(4-methoxyphenyl)-4-pentenamide (AMD) for the HPLC reference standard were both synthesized at Tsukuba Research Laboratories, Eisai Co., Ltd.

2. Synthesis of ^{14}C-labelled satigrel

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

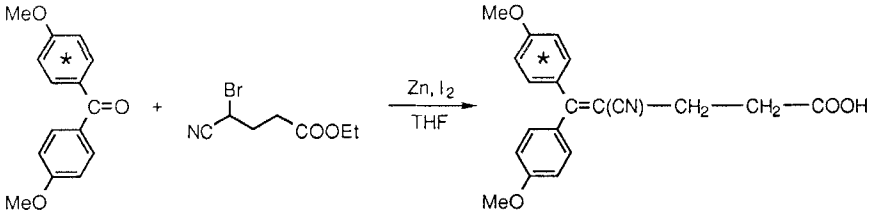


Figure 1 Synthesis of ^{14}C-labelled satigrel

4,4'-Dimethoxy [ring-U-^{14}C] benzophenone, 200 mCi (2.38 mmole), and zinc powder, 500 mg, were suspended in 10 ml dry tetrahydrofuran under nitrogen gas. After heating to 50°C, 1.24 ml of ethyl 4-bromo-4-cyanobutyrate and a catalytic amount of iodine were added, then the mixture was stirred for 3 hours at

50 to 60°C. A further 500 mg of zinc powder, 1.24 ml of ethyl 4-bromo-4-cyanobutyrate and iodine were then added, and the mixture was re-stirred for 7 hours at 50 to 60°C, then stirred overnight at room temperature. After dropwise addition of 5 ml of saturated ammonium chloride solution, the reaction liquid was stirred for 1 hour at room temperature, and filtrated under reduced pressure. The filtration residue was washed three times with 30 ml of ethylacetate (30 ml × 3), and the wash liquid was combined with the filtrate. The combined filtrate was acidified with 2 ml of 1N-hydrochloric acid, washed three times with 25 ml of salt saturated water (25 ml × 3) and once with 25 ml of water, and the organic layer was subjected to vacuum concentration.

The concentrate thus obtained was adsorbed to 50 g of silica gel and purified by silica gel chromatography.

Eluents

Ethylacetate	:	benzene		
0	:	100	150 ml	Fr. 1
2.5	:	97.5	400 ml	Fr. 2
25	:	75	400 ml	Fr. 3
Methanol	:	benzene		
5	:	95	500 ml	Fr. 4
10	:	90	500 ml	Fr. 5
25	:	75	500 ml	Fr. 6

Fr. 5 and Fr. 6 were combined, and subjected to vacuum concentration to obtain crude ^{14}C-labelled satigrel.

This crude ^{14}C-labelled satigrel was purified further by silica gel chromatography, using 25 g of silica gel.

Eluents

Methanol	:	benzene		
0	:	100	100 ml	Fr. 1
1.5	:	98.5	200 ml	Fr. 2
3	:	97	300 ml	Fr. 3
5	:	95	100 ml	Fr. 4
			100 ml	Fr. 5
10	:	90	100 ml	Fr. 6
			100 ml	Fr. 7
20	:	80	200 ml	Fr. 8

Fr. 5 through Fr. 8 were combined, and subjected to vacuum concentration to obtain ^{14}C-labelled satigrel. The residue was dissolved in exactly 50 ml of ethyl acetate and stored at -20°C.

3. Measurement of radiochemical purity and specific radioactivity

50 μl of the ethyl acetate solution of ^{14}C-labelled satigrel was diluted to 5.0 ml with acetonitrile.

Radiochemical purity: Radiochemical purity was determined by HPLC (column : Nucleosil 5C18, 4.6 mm i.d.× 250 mm long (Gasukuro Kogyo Inc., Japan), mobile phase : 1%acetic acid : acetonitrile=170:130, v/v, flow rate : 1.0 ml/min, detector : Radio detector). The ^{14}C-labelled satigrel solution was subjected to HPLC and the peak area was measured.

Specific radioactivity: Specific radioactivity was determined by liquid scintillation counting and quantitative determination of satigrel by HPLC-UV.

Measurement of radioactivity: Duplicate 50 μl aliquots of the ^{14}C-labelled satigrel solution were put in vials containing 5 ml of ACS-II (Amersham, UK) as the scintillator and radioactivity was measured by a liquid scintillation counter system (model LSC-3500, Aloka, Tokyo, Japan).

Determination of satigrel concentration: To 0.1 ml of ^{14}C-labelled satigrel solution, 0.1 ml of internal standard solution (20 μg/ml) was added and 50 μl of the solution was subjected to HPLC. The concentration of satigrel was determined from peak height ratio to the internal standard. The HPLC instrument consisted of a Model 6000A pump, a WISP 710 autosampler (Waters Assoc., USA) and a Model 875-UV ultraviolet spectrometer (Jasco, Japan). The HPLC conditions were as follows :

Column	:	Nucleosil 5C18, 4.6 mm i.d. × 250 mm long (Gasukuro Kogyo Inc., Japan)
Mobile phase	:	1% acetic acid : acetonitrile = 170:130, v/v
Flow rate	:	1.0 ml/min
Detection	:	UV283 nm
Injection volume	:	50 μl

RESULTS AND CONCLUSION

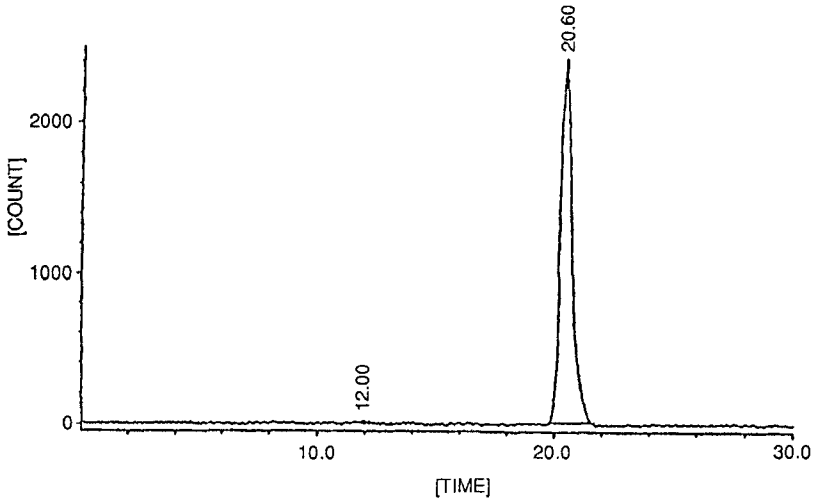
Starting from 4, 4'-dimethoxy [ring-U-^{14}C] benzophenone (200 mCi/2.38 mmole), ^{14}C-labelled satigrel was synthesized by the route outlined in Figure 1. This route is the same as that used for unlabelled satigrel.

Radiochemical purity

Radiochemical purity of ^{14}C-labelled satigrel, determined by radio-HPLC was 98.8%, as shown in Figure 2. Approximately 1% of a radiochemical impurity was observed. Its retention time relative to satigrel was 0.59, which was approximately the same as AMD(0.60) which was examined separately. AMD is an impurity found in unlabelled satigrel.

Specific radioactivity

Radioactivity of the ^{14}C-labelled satigrel solution was 20.0 mCi/50 ml and its concentration was 72.1 mg/50 ml. Therefore, the specific radioactivity was 10.3 MBq (277.2 μCi)/mg.



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WIDTH: 60

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2	B	20:50	1.35451E+04	2397.8	31.3	98.767	8627	

Figure 2 HPLC chromatogram of ^{14}C-labelled satigrel

Yield in amount and percentage

20.0 mCi (72.1 mg) of ^{14}C-labelled satigrel was finally obtained and its radiochemical yield of 10.0%.

The amount of ^{14}C-labelled satigrel obtained from this synthesis was sufficient to studies the metabolic fate of satigrel in laboratory animals.

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