CARBON-14 LABELLING OF FCE 24578, AN IMMUNOMODULATING AGENT

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SUMMARY

Two syntheses of radiocarbon labelled FCE 24578, namely 2-cyano-3-(1,4-dihydro-1-phenyl-[1]-benzothiopyrano[4,3-c] pyrazol-3-yl)-3-oxo-N-phenylpropanamide, are reported.
The former gave, employing [1-^{14}C]acetonitrile as labelled starting material and with an overall radiochemical yield of 39%, [cyano-^{14}C]FCE 24578, 97% radiochemically pure and with a specific radioactivity of 299 MBq/mmol.
The latter was carried out with [ring-U-^{14}C]benzoic acid to give [phenyl-U-^{14}C]FCE 24578, 96% radiochemically pure, with specific radioactivity of 400 MBq/mmol in an overall radiochemical yield of 40%.

Key words: FCE 24578, [1-^{14}C]acetonitrile, [ring-U-^{14}C]phenyl isocyanate, immunomodulating agent.

INTRODUCTION

FCE 24578, namely 2-cyano-3-(1,4-dihydro-1-phenyl-[1]-benzothiopyrano[4,3-c]pyrazol-3-yl)-3-oxo-N-phenylpropanamide, is a new synthetic compound endowed with immunomodulating activity or rather enhancing macrophage cytotoxicity and stimulating host mediated antibacterial defences in mice [1] [2] [3]. In order to perform "in vivo" and "in vitro" studies with this new immunomodulating agent, a radiolabelled form was required.

Two separate choices were possible to obtain the radiocarbon labelled FCE 24578. The former (upper side of the scheme), employing [1-^{14}C]acetonitrile as labelled precursor, enables to

0362–4803/90/101213–07$05.00

Received May 30, 1990

SCHEME

CH_3-[^{14}C]CN + R-CO-OC_2H_5 $\xrightarrow[(DMF)]{NaH}$ R-CO-CH_2-[^{14}C]CN

1 2 3

[3] + C_6H_5-NCO $\xrightarrow[(DMF)]{TEA}$ R-CO-CH(CO-NH-C_6H_5)-[^{14}C]CN

4

[cyano-^{14}C]FCE 24578

[ring-U-^{14}C]C_6H_5-COOH $\xrightarrow[(acetone)]{DPPA,TEA}$ [[ring-U-^{14}C]C_6H_5-CON_3]

5 6

[6] + R-CO-CH_2-CN $\xrightarrow[(benzene)]{24\ hrs,reflux}$ R-CO-CH(CO-NH-[U-^{14}C]C_6H_5)-CN

7 8

[phenyl-U-^{14}C]FCE 24578

R =

easily prepare the [cyano-^{14}C]FCE 24578 according to a modified procedure of the laboratory synthesis [4] [5].

Metabolic studies with the unlabelled drug do not absolutely permit to exclude the presence of aniline and its derivatives (as products of metabolic attack of FCE 24578) in animal biological fluids. Therefore the latter choice (lower side of the scheme) was necessary to prepare the appropriate radioactive compound, that is labelled in the phenyl group of propanamide side chain ([phenyl-U-^{14}C]FCE 24578).

RESULTS AND DISCUSSION

According to the scheme, [1-^{14}C]acetonitrile 1 was acylated by the ethyl ester 2 in presence of NaH to give the [^{14}C]nitrile derivative 3. This intermediate was reacted with phenylisocyanate to give, without purification, the [cyano-^{14}C]FCE 24578 4, 97% radiochemically pure (specific radioactivity 299 MBq/mmol), with a radiochemical yield of 39% from [1-^{14}C]acetonitrile.

The simple one-step conversion of carboxylic acids to urethanes, under mild conditions using a modified Curtius reaction in presence of diphenyl phosphoroazidate (DPPA), triethylamine (TEA) and appropriate hydroxyl component [6], prompted us to consider this synthetic route (see lower side of the scheme) very convenient to prepare the compound labelled in the phenyl group of propanamide side chain. In fact the "in situ" preparation of [ring-U-^{14}C]phenylisocyanate, "via" benzoic acid azide 6 from [ring-U-^{14}C]benzoic acid 5 [7], combined with the procedure already described to prepare the above labelled drug, was successful to synthesize the expected [phenyl-U-^{14}C]FCE 24578 8. It was obtained, 96% radiochemically pure with a specific radioactivity of 400 MBq/mmol in an overall radiochemical yield of 40% from [ring-U-^{14}C]benzoic acid.

EXPERIMENTAL

Thin layer chromatography (TLC)

TLC was carried out using Merck silica gel F 254 20x5 cm, 0.25 mm thick or 20x20 cm, 1 mm thick plates. The eluting solvent systems were:

A) n-hexane : ethylacetate (80 : 20 by volume)

B) chloroform : methanol (80 : 20 " ")

Ultraviolet spectra were determined on a Beckman DU50 spectrophotometer. Measurements of radioactivity were carried out with a Packard 300C liquid scintillation counter using Rialuma (Lumac System A.G.) as liquid scintillation cocktail. Radiochemical analyses of TLC plates were performed with a Berthold 3832 automatic linear analyzer.

[1-^{14}C]acetonitrile and [ring-U-^{14}C]benzoic acid were purchased from Amersham International p.l.c.

3-(1,4-Dihydro-1-phenyl-[1]-benzothiopyrano[4,3-c]pyrazol-3-yl) -3-oxo-propane[^{14}C]nitrile (3)

[1-^{14}C]Acetonitrile 1 (185 MBq, 90 μmoles), after dilution with about 28 ul (about 530 μmoles) of "cold" product, was transferred, with the aid of a vacuum manifold, into a 10 ml two necked flask containing a suspension of NaH (48 mg of 55-60% dispersion in oil; about 1 mmol) in 2 ml of N,N-dimethylformamide (DMF). The suspension was stirred at room temperature, under nitrogen, for about one hour. The ethyl ester 2 (1,4-dihydro-1-phenyl-[1]-benzothiopyran[4,3-c]pyrazol-3-carboxylic acid, ethyl ester ; 168 mg, 500 umoles) was added, under nitrogen into the suspension and the reaction mixture was stirred for one hour. At the end of the reaction (checked by radio-TLC; system A: RF of 3 = 0.25), water was added (5 ml) and the solution adjusted to pH 4 with aqueous 5% citric acid. The resulting precipitate was filtered through a D_2 sintered glass-filter, washed with water and dissolved in acetone. The acetonic solution was then evaporated to dryness to give 151 mg of crude compound 3, 95% radiochemically pure (radio-TLC ; system A), but very strong colored. Therefore the crude substance was dissolved in a mixture (5 ml) of chloroform:n-hexane in the ratio 1:2 (by volume) and charged over a column (20 x 2 ID cm) containing silicagel 60 Merck (230-400 mesh ASTM). The fractions eluted with the above solvent

mixture and containing the expected product were combined, evaporated to dryness to give 111 mg (86 MBq) of colorless 3, which was used in the next step.

2-[^{14}C]Cyano-3-(1,4-dihydro-1-phenyl-[1]benzothiopyrano[4,3-c] pyrazol-3-yl-3-oxo-N-phenyl-propanamide (5) [cyano-^{14}C]FCE 24578)

Phenylisocyanate (0.04 ml; 0.35 mmoles) and triethylamine (TEA 0.05 ml) were added to compound 3 (111 mg; 0.34 mmoles) dissolved in DMF (1 ml). The mixture was stirred at room temperature for one hour. When the conversion was complete (checked by radio-TLC, system B; RF = 0.35), water (5 ml) was added. The solution was adjusted to pH 5 with 2N HCl giving a precipitate which was filtered through a D_2 sintered glass-filter. The solid was washed with water (3x5 ml), ethanol (3x5 ml) and then dissolved in chloroform. The organic solution was evaporated to dryness to yield [cyano-^{14}C]FCE 24578 5 (72.5 MBq) with radiochemical purity 97% (by radio-TLC; system B) and specific radioactivity of 299 MBq/mmol (664 kBq/mg). The UV/VIS spectrum (in chloroform λ_{max} at 328 nm ; $E_{1cm}^{1\%}$ = 692.5) was concordant with that of the standard sample.

The overall radiochemical yield from 1 was 39%.

2-Cyano-3-(1,4-dihydro-1-phenyl-[1]-benzothiopyrano[4,3-c]pyrazol -3-yl)-3-oxo-N-[U-^{14}C]phenyl-propanamide (8) [phenyl-U-^{14}C]FCE 24578

[ring-U-^{14}C]Benzoic acid 5 (37 MBq; 8.3 µmoles), dissolved in acetone (1 ml), was added to 11.2 mg (91.7 µmoles) of benzoic acid. Acetone (1.6 ml), TEA (14 ul; 148 µmoles) and DPPA (22 µl; 102 µmoles) were added and the mixture was stirred at room temperature for 24 hours. The solvent was then distilled and recovered. A noteworthy amount (about 15%) of the initial radioactivity was present in this volatile fraction. The remaining solid was dissolved in benzene (2.7 ml) and

3-(1,4-dihydro-1-phenyl-[1]-benzothiopyrano[4,3-c]pyrazol-3-yl)--3-oxo-propanenitrile 7 (39.2 mg; 118 μmoles) was added. The solution was refluxed for 24 hours under stirring. At the end of the reaction (checked by radio-TLC; system B ;RF of 8 = 0.35) the mixture was evaporated to dryness and the resulting solid was dissolved in chloroform (15 ml). This solution was washed with 1N HCl (10 ml) and water up to neutrality to give, after evaporation under vacuum, 29 MBq of crude solid 8. It was dissolved in DMF (1.5 ml) and then precipitated with 0.5N HCl (4 ml). The precipitate was filtered through a D_3 sintered glass-filter, washed with water (4x3 ml) and ethanol (4x3 ml). The resulting compound 8 (19 MBq) had a radiochemical purity of 91% (checked by radio-TLC; system B). It was therefore submitted to a further purification by preparative TLC (silicagel 1 mm thick) using the mixture B as chromatographic eluent. The chromatograpic band corresponding to 8 was removed and the product extracted from silica gel with aqueous methanol (10% water) and successively several times with methanol/chloroform (1:4 by volume) until disappearance of radioactivity. The combined extracts were washed with 1N HCl, with water up to neutrality, and evaporated to dryness to yield [phenyl-U-^{14}C]FCE 24578 8 (14.8 MBq) with a 96% radiochemical purity and specific radioactivity of 400 MBq/mmol (888 kBq/mg). The UV/VIS spectrum was concordant with that of the standard sample. The overall radiochemical yield from 5 was 40%.

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