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Synthesis of ^{14}C-Labelled ER-30346, a Novel Antifungal Triazole

Toshihiko Naito*, Akihiko Tsuruoka, Hideki Sakurai, and Ritsuko Sakai.

Tsukuba Research Laboratories, Eisai Co.,Ltd., 1-3 Tokodai 5-Chome

Tsukuba-shi, Ibaraki 300-26, Japan

SUMMARY

ER-30346 is a new orally active antifungal triazole which has potent, broad spectrum antifungal activity, and good safety profile. It was synthesized labelled in the phenyl ring of the benzonitrile moiety with carbon-14, starting from 4-acetyl[ring-U-^{14}C]benzonitrile, according to the method illustrated in Scheme 1. ^{14}C-labelled ER-30346 having a specific activity of 1.89 Bq/mmol was obtained in 65.4 % overall radiochemical yield, with a radiochemical purity of 99.7 %.

Key Words ; ^{14}C-labelled, ER-30346, antifungal agent, triazole, 4-acetyl[ring-U-^{14}C]benzonitrile, thiazole

INTRODUCTION

The therapy of systemic fungal infections, particular those caused by opportunistic pathogens such as Candida, Cryptococcus and Aspergillus in immumocompromised patients, remains a difficult therapeutic problems.[1] Since triazole antifungals are characterized by broad-spectrum activity and relatively low toxicity, they have been widely used for systemic fungal infections.[2] The azole antifungals act by inhibiting the cytochrome P-450 monooxgenase, lanosterol 14-α-demethylase, a key enzyme of fungal ergosterol biosynthesis.[3]

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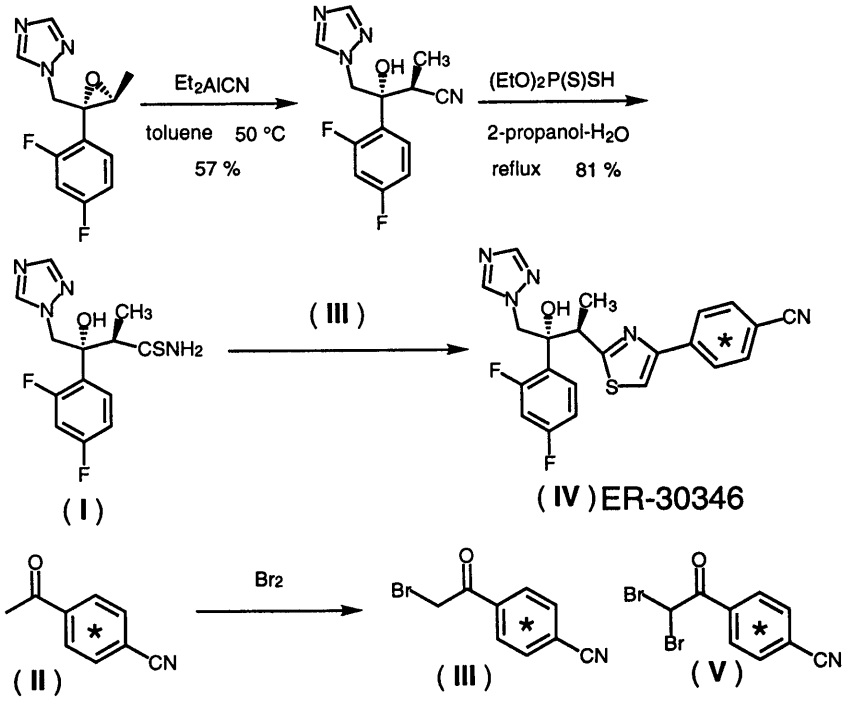
showed higher *in vitro* activity against candida, aspergillus, and cryptoccocus species than fluconazole and amphotericin B. ER-30346 was comparable to or more active than itraconazole against these species. After evaluations of experimental fungal infection models, ER-30346 was selected out.[4] ER-30346 may have potential efficacy in the therapy of aspergillosis, candidosis, and cryptococcosis, and merits further studies.

In this paper we report the synthesis of ^{14}C-labelled ER-30346, labelled in the benzonitrile moiety of the side chain. The compound was required for pharmacokinetic studies.



Scheme 1

RESULTS AND DISCUSSION

^{14}C-labelled ER-30346 was synthesized from (*2R,3S*)-2-(2,4-difluorophenyl)-3-methyl-2-(*1H*-1,2,4-triazol-1-yl)methyloxirane in three steps as shown in Scheme 1 according to the same method in the preparation of

non-radioactive ER-30346.[4b, 6a] The optically active oxirane was prepared by using known method.[5] Non-radioactive ER-30346 was synthesized by reaction of (*2R,3R*)-3-(2,4-difluorophenyl)-3-hydroxyl-2-methyl-4-(*1H*-1,2,4-triazol-1-yl)thiobutanamide (**I**)[6a, b] with 4-(2-bromo)acetylbenzonitrile.[6a, b] No epimerization reaction is observed in this thiazole ringformation, and ER-30346 can be obtained in excellent chemical yield and optical purity. The synthesis of ^{14}C-labelled ER-30346 was carried out according to this method, after optimizing experimental conditions suitable for handling radioactive materials.

4-Acetyl[ring-U-^{14}C]benzonitrile (**II**: Amersham) was brominated by bromine in ethyl acetate in the presence of aluminum chloride to give 4-(2-bromo)acetyl[ring-U-^{14}C]benzonitrile (**III**) and a small amount of the corresponding dibrominated compound (**V**). Since the dibromide (**V**) does not react with the thioamide (**I**), the crude product can be used without further purifications. The bromide (**III**) was refluxed with the thioamide (**I**) in methanol to give ^{14}C-labelled ER-30346. The overall radioactive yields from 4-acetyl[ring-U-^{14}C]benzonitrile (**II**) was 65.4 %. The radiochemical and chemical purity were 99.7 % and 99.8 % respectively.

EXPERIMENTAL

All chemicals used in the synthesis were purchased, and were used without purification. Silica gel (Kiesegel 60 F_{254}, layer thickness 0.25 mm, Merck) was used for analytical thin layer chromatography (TLC). 4-Acetyl[ring-U-^{14}C]benzonitrile (**II**) was purchased from Amersham International plc. (Code: FCQ8227, Total activity: 3.70 GBq, Specific activity: 2.22 GBq/mmol, radiochemical purity : >99%). All solvents were either distilled or were of analytical reagent quality.

4-(2-Bromo)acetyl[ring-U-^{14}C]benzonitrile (III)

To a stirred solution of 4-acetyl[ring-U-^{14}C]benzonitrile (**II**: 245 mg, 1.54 mmol) and catalytic amount of aluminum chloride in ethyl acetate (3.5 ml) was added

0.6 ml of 3.0 M solution of bromine in ethyl acetate under ice cooling. After stirring for 30 minutes at room temperature, 3 ml of ethyl acetate was added and the reaction mixture was washed twice with water (5 ml). The organic layer was washed successively with a saturated aqueous solution of sodium thiosulfate (5 ml) and sodium chloride (5 ml), and dried over anhydrous sodium sulfate. After evaporation of the solvent under reduced pressure, 4-(2-bromo)acetyl[ring-U-^{14}C]benzonitrile (**III**: 480 mg) was obtained. This product was identical to authentic non-radioactive 4-(2-bromo)acetylbenzonitrile by TLC: R_f = 0.25 (n-hexane/ethyl acetate, 4:1). This product was used for the next step without purification.

^{14}C-labeled ER-30346 (IV)

A solution of 4-(2-bromo)acetyl[ring-U-^{14}C]benzonitrile (**III**: 480 mg) and (*2R,3R*)-3-(2,4-difluorophenyl)-3-hydroxyl-2-methyl-4-(*1H*-1,2,4-triazol-1-yl)thiobutanamide (**I**: 700 mg, 2.24 mmol) in methanol (6 ml) was refluxed for 40 minutes. After cooling to room temperature, dichloromethane (36 ml) and sodium hydrogen carbonate (145 mg, 1.73 mmol) in water (20 ml) were added to the reaction mixture and stirred for 5 minutes. The organic layer was separated and the water layer was extracted twice with dichloromethane (20 ml). The combined organic layers were dried over anhydrous sodium sulfate. The residue (1.08 g) obtained after evaporation of the solvent was chromatographed on silica gel (Wakogel C-200: 100-200 mesh, 45 g). After elution with 1 % methanol in dichloromethane, the fractions which contained the desired product were combined and evaporated to dryness. The residue was recrystallized from ethyl acetate (3.5 ml) and n-hexane (1.5 ml) to give ^{14}C-labelled ER-30346 (**IV**: 649 mg, 1.49 mmol). The R_f value was 0.57 (dichloromethane/methanol, 10:1). The HPLC retention time of this product was identical to that of authentic non-radioactive ER-30346. The total activity was 2.42 GBq (65.5 mCi), 65.4 % radiochemical yield based on 4-(2-bromo)acetyl[ring-U-^{14}C]benzonitrile (**III**) The specific activity was 1.89 MBq/mmol (51.2 mCi/mmol). The radiochemical and chemical purity were 99.7 % and 99.8 % respectively by HPLC analysis. Analysis of chemical and

radiochamical purity was performed on a reverse-phase column (YMC-Pack AM312 ODS, 4.6 mm (I.D.) x 150 mm). The mobile phase was acetonitrile and water (58 : 42 , v/v). The retention time was 15.2 min at a flow rate of 1.0 ml/min.

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