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SYNTHESIS OF ^{3}H-SCH 51048 and ^{14}C-SCH 56592

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Summary

^{3}H-SCH 51048 and **^{14}C-SCH 56592** have been synthesized. ^{3}H-SCH 51048 was prepared in two steps by acid catalyzed tritium exchange while ^{14}C-SCH 56592 was prepared in three steps from ^{14}C-formamidine acetate in an overall 21% radiochemical yield.

Key Words: Candida, Aspergillus, antifungal, formamidine acetate, heptafluorobutyric acid.

Introduction

One of the most difficult problems in the medical profession today is the management of invasive mycoses in immunocompromised patients. Antifungal therapy is the only marginally effective means of combating system Candida, Aspergillus and meningeal Cryptococcus infections. Itraconazole and Fluconazole are two new compounds introduced for the treatment of systemic infections while Itraconazole is also used for the treatment of Aspergillosis.[1]

SCH 51048 (Fig. 1) was identified as a lead compound and in order to perform preliminary drug metabolism studies, tritium labelled material was requested. As a result of further studies, SCH 56592, a hydroxy analog of SCH 51048, was

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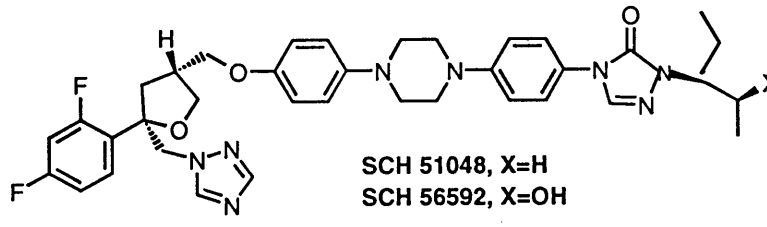
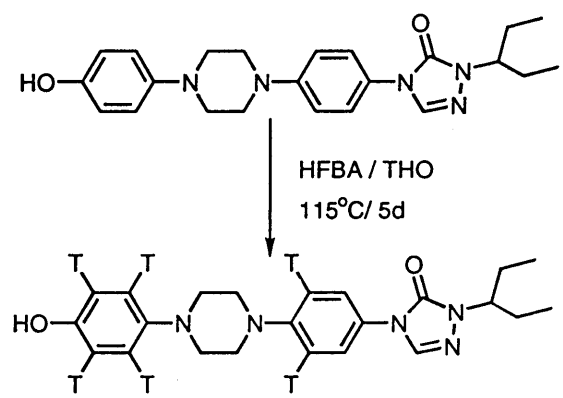
progressed to the development phase, where carbon-14 labelled material was required for the formal development program. Hence this paper describes the synthesis of ^{3}H-SCH 51048 and ^{14}C-SCH 56592.



SCH 51048, X=H
SCH 56592, X=OH

Discussion

^{3}H-SCH 51048 was prepared from the phenol intermediate (Scheme 1) by heptafluorobutyric acid catalyzed exchange.[2] ^{3}H-heptafluorobutyric acid was generated by the addition of an equivalent of tritiated water to heptafluorobutyric anhydride, which was directly reacted with the substrate under the conditions as shown in Scheme 1 to generate crude product at about 66% radiochemical purity. After silica gel purification, the phenol was reacted with the tosylate, 1, using 50% sodium hydroxide in DMSO as shown in Scheme 2. After hplc purification **^{3}H-SCH 51048** was isolated in 73% yield from the ^{3}H-phenol at a specific activity of 330 mCi/mmole. The sites of ^{3}H-labelling were confirmed by

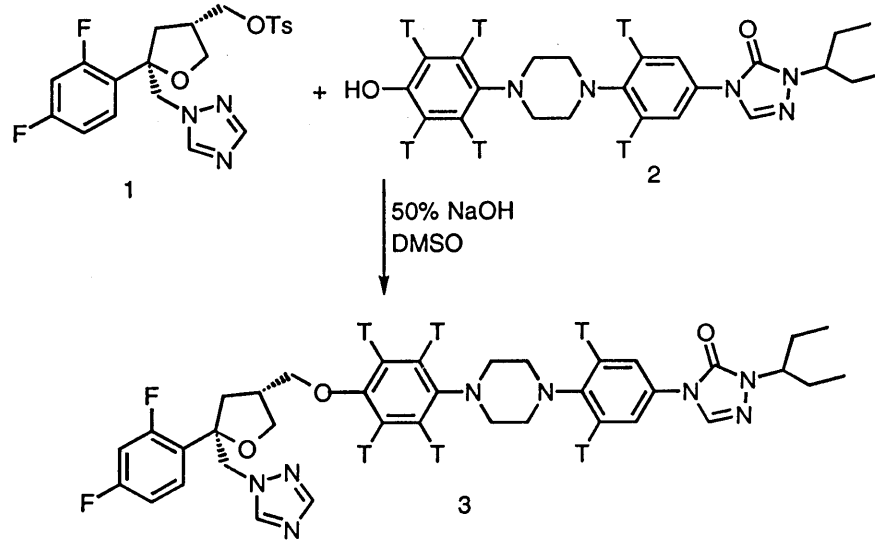


Scheme 1

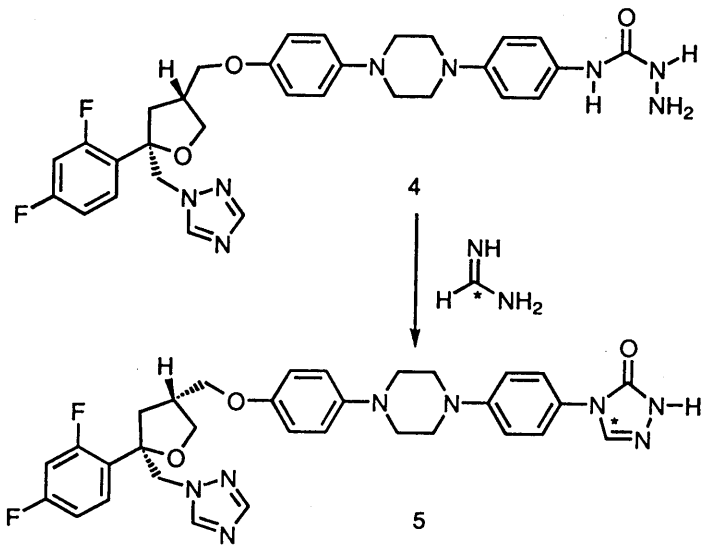
^{3}H NMR analysis[3], which showed equal incorporation in the three pairs of electrophilically active sites. As mentioned earlier, SCH 56592 was identified as a potent antifungal and carbon-14 labelled material was requested. A three step procedure to synthesize **^{14}C-SCH 56592** was employed using commercially available ^{14}C-formamidine acetate having a specific activity of 45.4 mCi/mmole as the starting material.

The first step in the synthesis was the construction of the radiolabelled triazolone ring. Semicarbazide (4) and ^{14}C-formamidine acetate were heated at 80°C in 2-methoxyethanol in the presence of triethylamine. After allowing the reaction to reflux for 16 hours, it was cooled and poured into water. A precipitate formed and was collected giving 93 mCi of triazolone, 5. Compound 5 required no purification and was used directly in the next step.

In general, N-alkylation of triazolones with alkyl halides or tosylates are usually carried out in the presence of sodium hydride in tetrahydrofuran or dimethyl sulfoxide. However, in some cases involving branched electrophiles, substantial



Scheme 2



amounts of by-products are formed. N-alkylations using cesium carbonate in DMF have been known to improve the yield as well as limit the amount of side products. Thus compound 5 was treated with cesium carbonate and 2-benzyl-oxy-3-pentanol p-bromobenzenesulfonate ester and heated at 80°C in DMF for 16 hours. Following work-up, the crude reaction product was chromatographed on silica gel using 40% acetone in methylene chloride as the eluent. This produced 43.6 mCi of compound 6. The thin layer chromatograph of the product was identical to that of an authentic sample.

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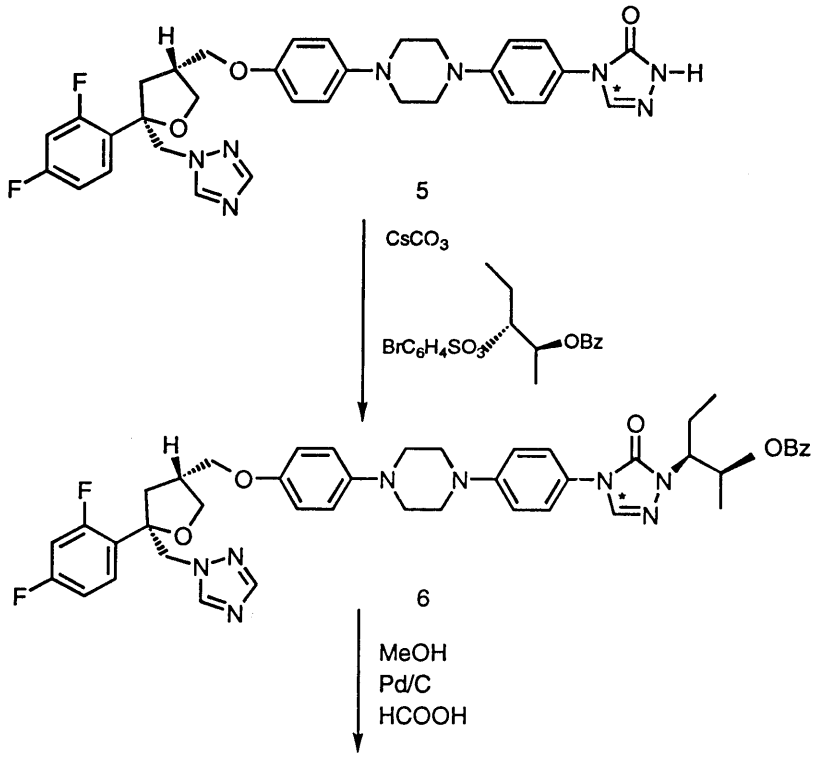
The final step of the synthesis is the removal of the benzyl ether protecting group by catalytic hydrogenation. Under a nitrogen atmosphere, a mixture of compound 6 and formic acid were added to methanol containing palladium black

and the suspension was stirred while heating at 60°C for 18 hours. Purification by silica gel chromatography using 2% methanol in methylene chloride as the eluent gave 21 mCi of **^{14}C-SCH 56592**. High pressure liquid chromatography of the product when compared to that of the reference standard was found to have chemical and radiochemical purities of 97.1% and 97.8%, respectively.



^{14}C-SCH 56592

Experimental

Materials

Tritiated water was obtained from New England Nuclear at a specific activity of 50 Ci/mL. Radiolabelled ^{14}C-formamidine acetate was obtained from Zeneca Chemicals at a specific activity of 45.5 mCi/mmole. All reagents and solvents

were used without purification. The reactions were carried out under an argon atmosphere.

Thin Layer Chromatography

Thin Layer Chromatography was performed on Whatman LK6DF (silica gel 60) 5 x 20 cm, 0.25 mm plates. Analysis of radioactive material was performed using a Bioscan System 200 imaging scanner. Column chromatography used 0.063 mesh silica gel 60 obtained from EM Science.

The following systems were used:

1) Methylene chloride: methanol (93:7)

2) Methylene chloride: isopropanol (92.5:7.5)

High Pressure Liquid Chromatography

High pressure liquid chromatography was used for radiochemical and chemical purity. A Waters 660E system controller was used with a Waters 712 WISP auto-injector. The chemical purity was determined using the Waters 490 programable multiwavelength detector while radiochemical purity was obtained from a Radiomatic Flow 1 detector. Radiomatic Flo-Scint III liquid scintillation cocktail was employed. The following systems were used:

1) Phenomenex Ultracarb ODS 5 (30); 15 cm x 4.6 mm ID maintained @ 50°C; 254 nm; H_2O:THF:H_3PO_4 (700:300:1) followed by a gradient to H_2O:THF:H_3PO_4 (350:650:1) at 1.0 mL/min.

2) Chiracel OD-R, 25 cm x 4.6 mm ID maintained @ 40°C, 254 nm; CH_3CN:H_2O (80:20) followed by a gradient to CH_3CN at 0.5 mL/min.

3) Rainin 5 C18, 25 cm x 4.6 mm I.D., 254 nm, methanol: water: trifluoroacetic acid (68:31.9:0.1) at 1 mL/min.

Liquid Scintillation Counting

Radioactivity measurements were performed using a Packard 2200CA TRICARB liquid scintillation analyzer.

Synthesis of ^{3}H-SCH 51048

(R-cis)-4-[4-(4-{4-hydroxy-2,3,5,6-^{3}H-phenyl}-1-piperazinyl)-2,6-^{3}H-phenyl]-2-(1-ethylpropyl)-2,4-Dihydro-3H-1,2,4-triazol-3-one (1) In a tritiation ampoule, unlabelled (R-cis)-4-[4-(4-{4-hydroxyphenyl}-1-piperazinyl)- phenyl]-2-(1-ethylpropyl)-2,4-Dihydro-3H-1,2,4-triazol-3-one (10 mg) was dissolved in a mixture of heptafluorobutyric anhydride (69 µL) and tritiated water (50 Ci/mL, 5 µL, 250 mCi). The ampoule was evacuated, flame sealed and placed in an oil bath at 115°C for 6 days. At the completion of the reaction, the reaction ampoule contents were partitioned between sodium bicarbonate (50g/L, 5 mL) and chloroform:isopropanol (1:1, 10 mL). The organic layer was run off and the aqueous layer extracted with 2 x 10 mL of chloroform:isopropanol (1:1). The organic extracts were combined and washed with brine (10 mL) and evaporated. The residue was dissolved in ethanol (10 mL) and re-evaporated to yield 39.8 mCi of ^{3}H-phenol, 1 at 67% RCP (TLC System 1). The compound was purified by chromatography on silica gel using 5% methanol in methylene chloride as eluent to yield 29 mCi of ^{3}H-phenol, 1 at 89% RCP, (TLC System 1). The compound was used directly in the next step.

^{3}H-SCH 51048, 3 ^{3}H-phenol, 1 (29 mCi, 6.96 mg, 0.017 mmole) and unlabelled 1 (25.2 mg, 0.062 mmole) were dissolved in DMSO (0.8 mL) and 50% sodium hydroxide (4.33 mL, 0.083 mmole) was added. After heating at 65°C for 90 minutes, tosylate 2 (39 mg, 0.087 mmole) was added and the reaction was continued at 80°C for 1 hour. The reaction was partitioned betwen sodium bicarbonate (50 g/L, 5 mL) and methylene chloride (15 mL). The organic layer was run off and the aqueous layer extracted with a further 2 x 10 mL portions of methylene chloride. The combined organic extracts were filtered and concentrated to dryness to yield 24 mCi of ^{3}H-SCH 51048 3 at 61% RCP (HPLC System 1). The compound was purified by chromatography on silica gel using

10-30% acetone in methylene chloride to yield 14.4 mCi of ^{3}H-SCH 51048, 3, at a specific activity of 331 mCi/mmole. The radiochemical purity as determined by HPLC System 1 and TLC System 2 was 98.7%.

Synthesis of ^{14}C-SCH 56592

(R-cis) 4-[4-(4-{4-[5-(2,4-difluorophenyl)-1-piperazinyl)phenyl]-2,4-dihydro-[3-^{14}C]-3H-1,2,4-triazol-3-one (5): To a 100 mL round bottom flask fitted with a nitrogen inlet tube was added semicarbazide, 4, (1.25g 2.07 mmol); ^{14}C-formamidine acetate (100 mCi, 0.226g, 2.18 mmol); triethylamine (0.35 mL, 1.2 eq.) and 2-methoxyethanol (80 mL). The mixture was heated at 80°C for 16 hours, cooled and poured into water (200 mL). The precipitate was collected and dried under vacuum to yield 1.19g (93%) for a total activity of 93 mCi.

(R-cis) 4-[4-(4-{4-[5-(2,4-difluorophenyl)-5-(1H-1,2,4-triazol-1-ylmethyl) tetrahydrofuran-3-yl)methoxy]phenyl}-1-piperazinyl)phenyl]-2,4-dihydro-2-[1-ethyl-2-(phenylmethoxy)propyl]-[3-^{14}C]-3H-1,2,4-triazol-3-one (6): To a 100 mL round bottom flask was added compound 5 (1.19g, 1.93 mmol) and cesium carbonate (0.64g, 1 eq.) in DMF (50 mL) and heated at 80°C for 18 hours. The mixture was cooled, diluted with ethyl acetate (30 mL) and transferred to a separatory funnel. The organic layer was washed with brine (1 x 30 mL), water (2 x 30 mL), dried over magnesium sulfate and filtered. The ethyl acetate was removed under reduced pressure to yield 2.6g of crude product. The crude product was purified by short path column chromatography (Kieselgel 60, acetone/methylene chloride, 2:3) to yield 0.618g 43.6 mCi, (47%), which co-chromatographed with an authentic sample on thin layer chromatography.

^{14}C-SCH 56592: To a 100 mL round bottom flask containing palladium black (0.19g) was added under a nitrogen atmosphere compound 6 (43.6 mCi, 0.618g, 0.78 mmol), formic acid (23.2g, 0.5 mmol) and methanol (50 mL). The flask was stoppered and the suspension stirred while heated at 60°C for 16 hours. The suspension was cooled and ice was added to the reaction flask. The pH was

adjusted to 5 with ammonium hydroxide. The reaction mixture was transferred to a separatory funnel and extracted with ethyl acetate (3 x 30 mL). The organic layer was dried with magnesium sulfate, filtered and the solvent removed under reduced pressure to yield 0.392g of crude product. The latter was purified by short path column chromatography (Kieselgel 60, methanol, methylene chloride, 1:49) to yield 0.326g, 21.1 mCi, (48%) of ^{14}C-SCH 56592. Radiochemical purity as determined in HPLC systems 1,2 and 3 was 98.3%.

Acknowledgements

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