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^{14}C-Labeling of a Novel Anxiolytic Agent Tandospirone

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SUMMARY

N-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]bicyclo-[2.2.1]heptane-2,3-di-exo-carboxyimide dihydrogen citrate (tandospirone), a novel anxiolytic agent, was labeled with carbon-14 individually at the imido carbonyl group and the pyrimidinyl ring. The synthesis of carbonyl-labeled tandospirone was achieved according to the scheme shown in Fig. 3. Diels-Alder reaction of maleic anhydride (2) with cyclopentadiene (3) afforded the endo anhydride (4), which was transformed into the imide (5) by treating with ammonia water. Thermal isomerization of the endo imide (5) and subsequent chromatographic separation gave the pure exo compound (6). Catalytic hydrogenation of 6 followed by alkylation with 1,4-dibromobutane (8) yielded the bromide (9a). Condensation of 9a with N-(2-pyrimidinyl)piperazine (10a) followed by treatment of the resulting disubstituted piperazine (11a) with citric acid afforded [carbonyl-^{14}C]tandospirone (1a). The overall yield of 1a was 22% from 2.

The similar method was applied to the synthesis of pyrimidinyl-labeled tandospirone as shown in Fig. 4. Condensation of 2-chloro[2-^{14}C]pyrimidine (12) with anhydrous piperazine gave the pyrimidinylpiperazine (10b). N-alkylation of 10b followed by treatment with citric acid afforded [pyrimidinyl-2-^{14}C]tandospirone (1b). The overall yield of 1b was 68% from 12.

Key words: carbon-14, anxiolytic agent, succinimide derivative, thermal isomerization, Diels-Alder reaction, pyrimidinylpiperazine

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INTRODUCTION

The pursuit of nonbenzodiazepine anxiolytic agent has stimulated the emergence of structurally diverse candidates which shows fewer or less intense side effects compared to what is normally observed with the benzodiazepines. Tandospirone, a succinimide derivative substituted with piperazinylalkyl group at the imido nitrogen[1], is one of the representatives of this kind because it lacks the sedative, muscle relaxant or anticonvulsant effects which accompany the benzodiazepines[2]. Its anxiolytic activity is equipotent to that of diazepam, but it does not appear to be directly actuated through benzodiazepine receptor[3]. In order to fully investigate the metabolism, radioactive forms of this compound were required. This report describes the synthesis of tandospirone labeled with carbon-14 individually at the imido carbonyl group and the pyrimidinyl ring.

RESULTS AND DISCUSSION

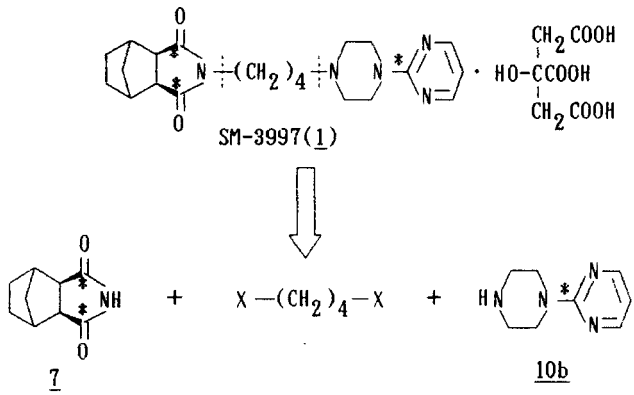

Fig. 1

Our retrosynthetic approach to tandospirone (1) is illustrated in Fig. 1. Disconnection of 1 at both ends of the butyl group provided three segments, two of which were considered suitable intermediates for the present labeling work. Therefore, we focused our attention on the preparation of the imide (7) and the piperazine (10b) in designing the potential routes to the carbonyl and the pyrimidinyl labeled tandospirone, respectively.

The key issue to be addressed in the radiosynthesis of the imide (7), which we hoped was readily accessible through catalytic hydrogenation of

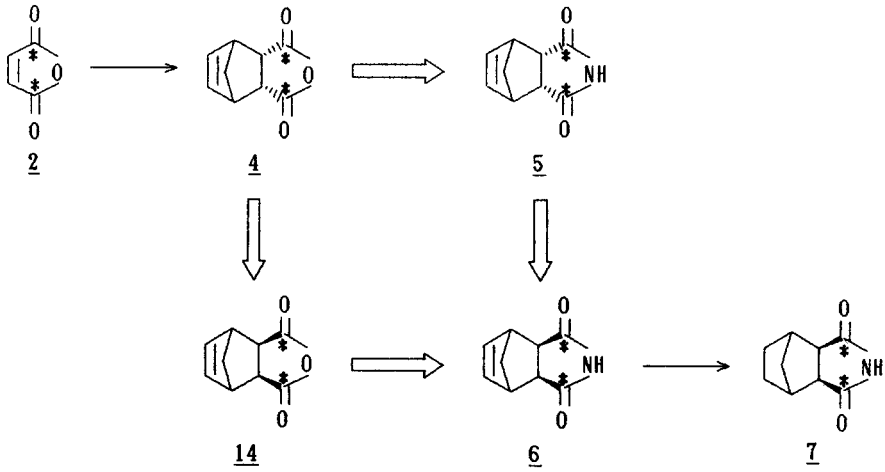

Fig. 2

the unsaturated imide (6), was obviously the construction of exo structure. As shown in Fig. 2, one plausible retrosynthetic analysis of 6 would require the exo anhydride (14), which would afford the desired imide (6) by treating with ammonia. The structure of 14 was quite similar to that obtained by Diels-Alder reaction of maleic anhydride (2) with cyclopentadiene[4]. The difference was that the Diels-Alder adduct (4) had the endo configuration as opposed to the exo configuration of 14. Synthesis of the exo anhydride (14) from the endo anhydride (4) has appreared in the literature[5][6]. These methods were, however, impractical for the present synthesis because of their tedious processes and predicted lower yields in the small scale syntheses. An alternate route was, therefore, explored. The feature of the new method was the use of the endo imide (5) instead of the anhydride (14). After several trials, it was found that the thermal isomerization of 5 afforded a 1:1 mixture of endo and exo compounds and that separation of the product by column chromatography gave the pure exo imide (6) in good yield. In addition, it was observed that 5 was recovered quantitatively from the mixture and could be converted to the exo compound (6) again. Based on these findings, we established the route illustrated in Fig. 3 for the synthesis of carbonyl-labeled tandospirone (1a).

Diels-Alder reaction of [1,4-^{14}C]maleic anhydride (2) with cyclopentadiene (3) gave 4 in 84% yield. The anhydride (4) was transformed into 5

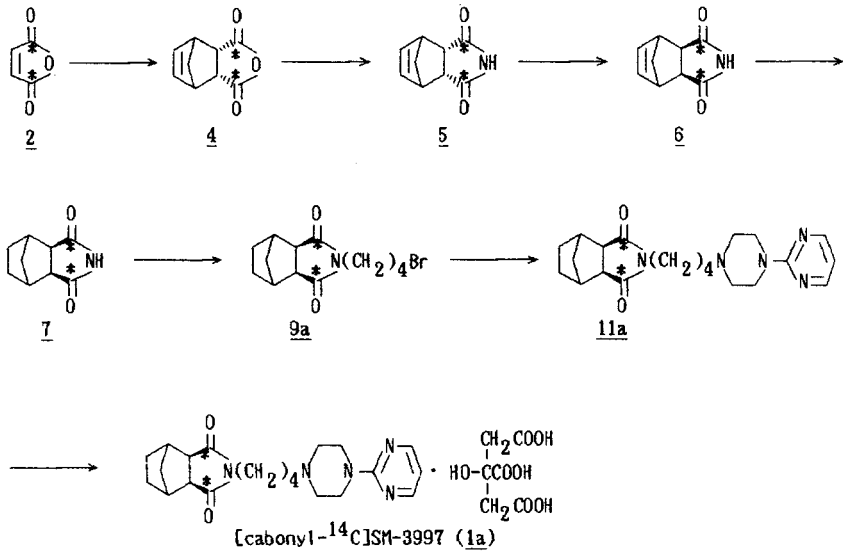

Fig. 3

in 72% yield by treating with ammonia water. Thermal isomerization of 5 at 190 °C for 15 h afforded a 1:1 mixture of the endo and exo forms (5 and 6). Column chromatography of the mixture gave the pure exo compound (6). The recovered endo/exo mixture was isomerized and chromatographed again in the same mannar as described above to give 6. Total yield of 6 was 59% from 5. Catalytic hydrogenation of 6 in the presence of palladium on carbon followed by alkylation of the resulting saturated imide (7) with 1,4-dibromobutane gave the bromide (9a) in 87% yield. Condensation of 9a with N-(2-pyrimidinyl)piperazine (10a) yielded the disubstitued piperazine (11a) in 87% yield. Subsequent treatment of 11a with citric acid afforded [carbonyl-^{14}C]Tandospirone (1a) in 90% yield.

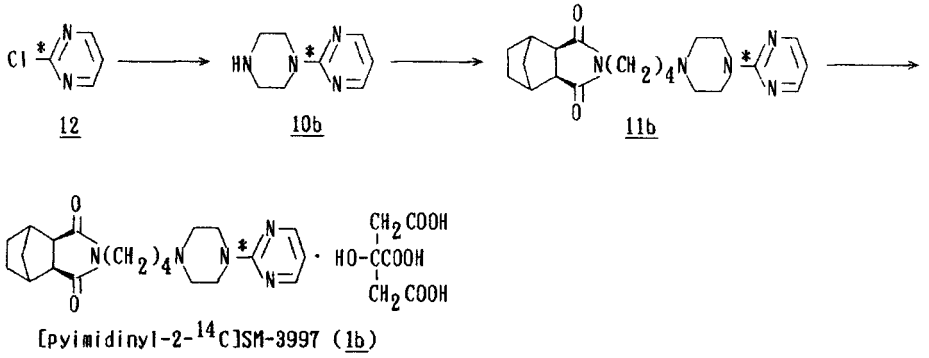

Fig. 4

The synthetic procedure of pyrimidinyl-labeled tandospirone (1b) is shown in Fig. 4. Howard reported the synthesis of pyrimidinylpiperazine through condensation of 2-chloropyrimidine (12) with anhydrous piperazine (13)[6]. Adaptation of this method, however, proved to be unsatisfactory for the radiosynthesis due to the formation of a disubstituted by-product (30%). In this reaction, it was considered that the yield of 10b depended on the molar ratio of 12/13. Subsequent reaction optimization studies revealed that condensation of 12 with a large excess of 13 resulted in an increase in yield. Derivatization of 10b to 1b was accomplished in a similar mannar to that adopted in preparation of the carbonyl-labeled compound (1a). Coupling reaction of 10b with the bromide (9b) afforded 11b in 78% yield. The disubstituted piperazine (11b) was treated with citric acid to give [pyrimidinyl-2-^{14}C]tandospirone (1b) in 97% yield.

EXPERIMENTAL

Radio-gas liquid chromatography (RGLC) was conducted on a Yanaco gas chromatograph G-180 (Yanagimoto Co., Ltd., Japan) equipped with a RD-4 gas flow counter (Aloka, Japan). Radio-thin layer chromatography (RTLC) was carried out on a Silica Gel F_{254} plate (Merck), and the radioactivity on the plate was determined by a Radiochromanizer (Aloka, Japan). Radio-high performance liquid chromatography (RHPLC) was conducted on a LC-3A liquid chromatograph (Shimadzu Co., Ltd., Japan) equipped with a SPD-2A UV detector (Shimadzu Co.) and a RLC-551 Radioanalyzer (Aloka). Radioactivity was measured by a TRI-CARB liquid scintilation counter (Packard Instrument Co., USA) by using Permafluor I (Packard) as the counting medium. An infrared spectrum (IR) was measured by a IR-810 grating infrared spectrophotometer (Jasco Co., Ltd., Japan), and the characteristic absorptions (ν_{max}) were reported in cm^{-1}. A proton nuclear magnetic resonance spectrum (NMR) was determined on a JNM FX-100 spectrometer (JEOL Ltd., Japan), and the chemical shifts (δ) for protons were quoted in ppm downfield from tetramethylsilane as the internal standard.

Bicyclo[2.2.1]hept-5-ene-2,3-endo-di[^{14}C]caboxylic anhydride (4)

To a solution of [1,4-^{14}C]maleic anhydride (2) (200 mCi, 745 mg, 7.60 mmol) in ethyl acetate (2.7 ml) was added freshly distilled cyclopentadiene (3) (0.76 ml, 9.20 mmol) at 0 °C. The mixture was stirred for 15 min at the same temperature. After addition of ligroin (4.0 ml), the solution was cooled to 0 °C and stirred for 1.5 h. The crystalline product was filtered, washed with ligroin and dried under reduced pressure to give 4 (168 mCi, 84.0%). NMR (δ, ppm, $CDCl_3$): 1.53-1.83 (2H, m, bicycloheptene ring), 3.47-3.61 (4H, m, bicycloheptene ring), 6.30-6.33 (2H, m, olefinic). IR (ν_{max}, cm^{-1}, nujol): 1840, 1770 (C=O).

Bicyclo[2.2.1]hept-5-ene-2,3-endo-di[^{14}C]carboxyimide (5)

To a suspension of the anhydride (4) (168 mCi, 1.05 g, 6.39 mmol) in tetrahydrofuran (2.1 ml) was added dropwise ammonia water (8% solution, 7.7 ml) at 0 °C. After complete addition, the mixture was stirred at the same temperature for 30 min. Tetrahydrofuran and water were distilled away, and the residue was heated at 140 °C for 10 min. After addition of toluene (4.2 ml), the solution was gradually cooled to 0 °C and stirred for 30 min. The crystalline product was filtered, washed with toluene and dried under reduced pressure to give 5 (121 mCi, 72.0%). The purity 87% on RTLC (benzene/ethyl acetate=1/1 v/v, Rf=0.29). NMR (δ, ppm, DMSO-d_6): 1.40-1.60 (2H, m, bicycloheptene ring), 3.19-3.26 (4H, m, bicycloheptene ring), 6.09-6.13 (2H, m, olefinic). IR (ν_{max}, cm^{-1}, nujol): 1760, 1700 (C=O).

Bicyclo[2.2.1]hept-5-ene-2,3-exo-di[^{14}C]carboxyimide (6)

A mixture of the endo imide (5) (121 mCi, 755 mg, 4.60 mmol), biphenyl (4.0 g) and diphenyl ether (4.0 g) was heated at 190 °C for 15 h. After cooling, the mixture was chromatographed on silic gel with benzene/ethyl acetate (1/1 v/v) to give 6. The fractions consisting of the endo and exo imides were collected, isomerized and chromatographed in the same mannar as described above to afford 6. The total yield of 6 was 71.0 mCi (58.7%) with the purity 98% on RTLC (benzene/ethyl acetate=1/1 v/v, Rf=0.38). NMR (δ, ppm, DMSO-d_6): 1.21-1.50 (2H, m, bicycloheptene ring), 2.63-2.66

(2H, m, bicycloheptene ring), 3.00-3.08 (4H, m, bicycloheptene ring), 6.21-6.27 (2H, m, olefinic). IR (ν_{max}, cm^{-1}, nujol): 1770, 1760 (C=O).

Bicyclo[2.2.1]heptane-2,3-exo-di[^{14}C]carboxyimide (7)

A mixture of the exo imide (6) (71.0 mCi, 443 mg, 2.70 mmol) and 10% palladium on carbon (30 mg) in ethanol/tetrahydrofuran (1/1 v/v, 8.0 ml) was stirred under hydrogen atmosphere at room temperature for 1 h. The catalyst was filtered off and washed with ethyl acetate. The filtrate was concentrated to give 7 (71.0 mCi, 100%). The purity 98% on RTLC (benzene/ethyl acetate=1/1 v/v, Rf=0.38) and RGLC (column 3% Silicone OV-17 on chromosorb W AW DMCS, 60-80 mesh, 3 mm i.d. x 1 m; column temperature 150 °C; injection temperature 200 °C; carrier gas He 20 ml/min; retention time 14.8 min). NMR (δ, ppm, DMSO-d_6): 1.13-1.60 (6H, m, bicycloheptane ring), 2.46-2.58 (4H, m, bicycloheptane ring). IR (ν_{max}, cm^{-1}, nujol): 3200, 3080 (NH), 1780, 1700 (C=O).

N-(4-Bromobutyl)bicyclo[2.2.1]heptane-2,3-exo-di[^{14}C]carboxyimide (9a)

A mixture of the saturated imide (7) (71.0 mCi, 445 mg, 2.70 mmol), 1,4-dibromobutane (8) (1.61 ml, 13.5mmol) and potassium carbonate (560 mg, 4.05mmol) in acetone (4.5 ml) was refluxed for 3.5 h. After dilution with water, the mixture was extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed on silica gel with benzene/ethyl acetate (4/1 v/v) to afford (9a) (63.1 mCi, 86.5%). The purity 98% on RTLC (benzene/ethyl acetate=1/1 v/v, Rf=0.51). NMR (δ, ppm, $CDCl_3$): 1.13-1.86 (10H, m, methylene), 2.59-2.70 (4H, m, methine), 3.40-3.56 (4H, m, methylene). IR (ν_{max}, cm^{-1}, neat): 1770, 1700 (C=O).

N-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]bicyclo[2.2.1]heptane-2,3-exo-di[^{14}C]carboxyimide (11a)

A mixture of the bromide (9a) (63.1 mCi, 721 mg, 2.40 mmol), N-(2-pyrimidinyl)piperazine (10a) (429 mg, 2.62 mmol), potassium carbonate (361 mg, 2.62 mmol) and potassium iodide (87 mg, 0.52 mmol) in N,N-dimethylformamide (7.9 ml) was heated at 95 °C for 3.5 h. After dilution with water,

the mixture was extracted with benzene. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated. The residue was recrystallized from 2-propanol (2.7 ml) to afford 11a (47.8 mCi, 75.7%). The purity 98% on RTLC (chloroform/methanol/triethylamine=100/10/1 v/v/v, Rf=0.46). NMR (δ, ppm, $CDCl_3$): 1.13-1.81 (10H, m, methylene), 2.30-2.69 (10H, m, methylene and methine), 3.41-3.55 (2H, m, methylene), 3.81 (4H, t, J=5.0 Hz, piperazinyl), 6.46 (1H, t, J=5.0 Hz, pyrimidinyl), 8.29 (2H, d, J=5.0 Hz, pyrimidinyl). IR (ν_{max}, cm^{-1}, nujol): 1770, 1700 (C=O).

N-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]bicyclo[2.2.1]heptane-2,3-exo-di[^{14}C]carboxyimide dihydrogen citrate (1a)

To a mixture of the disubstituted piperazine (11a) (47.8 mCi, 698 mg, 1.82 mmol) in acetone (3.9 ml) was added dropwise a solution of citric acid monohydrate (388 mg, 1.82 mmol) in acetone (4.8 ml) at 50 °C. After cooling to 0 °C, the mixture was stirred for 2 h. The crystalline product was filtered, washed with ice-cold acetone and dried under reduced pressure to give 1a (43.0 mCi, 90.0%). The purity 99% on RTLC (chloroform/methanol/triethylamine=100/10/1 v/v/v, Rf=0.46; toluene/acetone/triethylamine=20/15/5 v/v/v, Rf=0.53; ethyl acetate/n-hexane/methanol/28% ammonia water=80/50/15/5 v/v/v/v, Rf=0.44) and RHPLC (column LiChrosorb RP-18, 4 mm i.d. x 30 cm, 5 μm; mobile phase 0.01M KH_2PO_4 (pH 7.0)/acetonitrile=4/6 v/v; flow rate 1.0 ml/min; detectors UV (240 nm) and radiodetector; temperature room temperature; retention time 7.3 min). NMR (δ, ppm, DMSO-d_6): 1.12-1.52 (10H, m, methylene), 2.48-2.37 (14H, m, methylene and methine), 3.29-3.43 (2H, m, methylene), 3.74-3.85 (4H, m, piperazinyl), 6.64 (1H, t, J=4.7 Hz, pyrimidinyl), 8.36 (2H, d, J=4.7 Hz, pyrimidinyl). IR (ν_{max}, cm^{-1}, nujol): 1740, 1690 (C=O).

N-(2-[2-^{14}C]Pyrimidinyl)piperazine (10b)

A mixture of 2-chloro[2-^{14}C]pyrimidine (12) (50.0 mCi, 192 mg, 1.68 mmol) and anhydrous piperazine (1.15 g, 13.3 mmol) in 95% ethanol (2.0 ml) was refluxed for 30 min. After dilution with water, the mixture was extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed

on silica gel with chloroform/methanol/triethylamine (200/5/2 v/v/v) to give 10b (47.9 mCi, 95.9%). The purity 98% on RTLC (chloroform/methanol/triethylamine=100/10/1 v/v/v, Rf=0.20). NMR (δ, ppm, $CDCl_3$): 2.94 (4H, t, J=5.2 Hz, piperazinyl), 3.79 (4H, t, J=5.2 Hz, piperazinyl), 6.46 (1H, t, J=4.6 Hz, pyrimidinyl), 8.29 (2H, d, J=4.6 Hz, pyrimidinyl). IR (ν_{max}, cm^{-1}, neat): 3300 (NH).

N-[4-[4-(2-[2-^{14}C]Pyrimidinyl)-1-piperazinyl]butyl]bicyclo[2.2.1]heptane-2,3-exo-dicarboxyimide (11b)

A mixture of the disubstituted piperazine (10b) (47.9 mCi, 264 mg, 1.61 mmol), the bromide (9b) (660 mg), potassium carbonate (304 mg, 2.20 mmol) and potassium iodide (66 mg, 0.40 mmol) in N,N-dimethylformamide (6.0 ml) was heated at 100 °C for 3 h. After dilution with water, the mixture was extracted with benzene. The organic phase was washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was recrystallized from 2-propanol (2.5 ml) to afford 11b (34.9 mCi, 72.8%). The purity 98% on RTLC (chloroform/methanol/triethylamine=100/10/1 v/v/v, Rf=0.46).

N-[4-[4-(2-[2-^{14}C]Pyrimidinyl)-1-piperazinyl]butyl]bicyclo[2.2.1]heptane-2,3-exo-dicarboxyimide dihydrogen citrate (1b)

To a mixture of the disubstituted piperazine (11b) (34.9 mCi, 449 mg, 1.17 mmol) in acetone (2.8 ml) was added dropwise a solution of citric acid monohydrate (245 mg, 1.17 mmol) in acetone (3.5 ml) at 50 °C. After cooling to 0 °C, the mixture was stirred for 2 h. The crystalline product was filtered, washed with ice-cold acetone and dried under reduced pressure to give 1b (34.0 mCi, 97.4%). The purity 99% on RTLC and RHPLC.

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