SYNTHESIS OF [CARBONYL-^{14}C]- AND (METHOXY-d_3)-LABELED N-[(2RS,3RS)-1-BENZYL-2-METHYL-3-PYRROLIDINYL]-5-CHLORO-2-METHOXY-4-(METHYLAMINO)BENZAMIDE (YM-09151-2) A NEW POTENT NEUROLEPTIC AGENT

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SUMMARY

A new potent neuroleptic agent YM-09151-2, N-[(2RS,3RS)-1-benzyl-2-methyl-3-pyrrolidinyl]-5-chloro-2-methoxy-4-(methylamino)benzamide (10c), was labeled with carbon-14 and deuterium for biochemical studies such as metabolism and pharmacokinetics. The synthesis of [carbonyl-^{14}C]YM-09151-2 (10a) from 4-amino-2-hydroxy-[carboxyl-^{14}C]benzoic acid (1a) in six stages is described. Overall radiochemical yield was 79.1% at a specific activity of 21.18 mCi/mmol. (Methoxy-d_3)YM-09151-2 (10b) was prepared from methyl 2-(methoxy-d_3)-4-(N-methyl-N-tosylamido)benzoate (5b) which was obtained by the reaction of methyl 2-hydroxy-4-(N-methyl-N-tosylamido)benzoate (4c) with iodomethane-d_3. Overall yield of 10b was 60.8% from iodomethane-d_3.

Key words: Carbon-14, Deuterium, Benzamide, Pyrrolidine, YM-09151-2, Neuroleptic

INTRODUCTION

It is well known that some benzamide derivatives of alkane-1,2-diamines such as metoclopramide, sulpiride and sultopride

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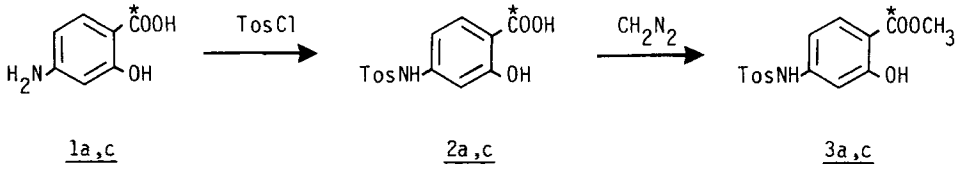
exhibit neuloleptic activities.[1] However, no benzamide derivative has been reported to be more potent than haloperidol, which is useful in clinical treatment of schizophrenia as well as phenothiazines. Recently, a number of benzamides of linear and cyclic 1,2-diaminoalkanes as potential neuroleptics were designed and synthesized in our laboratories by Iwanami et al.[2] They reported that N-[(2RS,3RS)-1-benzyl-2-methyl-3-pyrrolidinyl]-5-chloro-2-methoxy-4-(methylamino)benzamide (YM-09151-2) (10c) showed 13 and 408 times greater inhibitory effects on apomorphine-induced stereotyped behaviour in rats than haloperidol and metoclopramide, respectively. Moreover, YM-09151-2 (10c) exhibited a fairly high ratio of antistereotypic activity to cataleptogenecity compared with those drugs. It is therefore expected that YM-09151-2 (10c) will be used as a potent drug with few side effects in the treatment of psycosis.

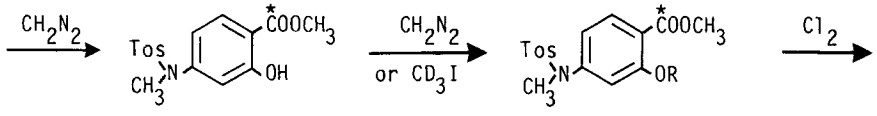
YM-09151-2 labeled with ^{14}C and d_3 (10a,b) were required for studies of the metabolic fate and the pharmacokinetics of YM-09151-2 (10c).[3] Scheme I shows the synthesis of [carbonyl-^{14}C]- and (methoxy-d_3)-labeled YM-09151-2 which was carried out by a modification of the reported method.[2] Details of the synthesis of the labeled compounds are described in this paper.

RESULTS AND DISCUSSION

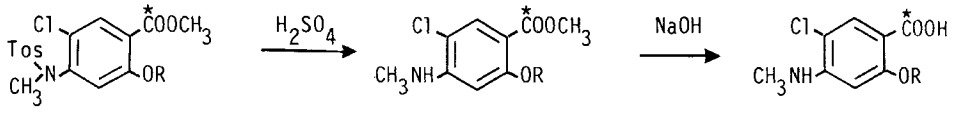
Among the various functional groups of YM-09151-2 (10c) the carbonyl group was chosen for labeling position with carbon-14 in view of the following facts. First, preliminary metabolic experiments using non-labeled YM-09151-2 (10c) showed that N-methyl and N-benzyl groups of 10c were eliminated from the molecule to give demethyl, debenzyl derivatives of 10c and the like.[3] Methoxy group may be transformed to hydroxy group by metabolism in analogy with sulpiride.[4] Second, a carbon-14 label in the pyrrolidine ring has a disadvantage in respect of



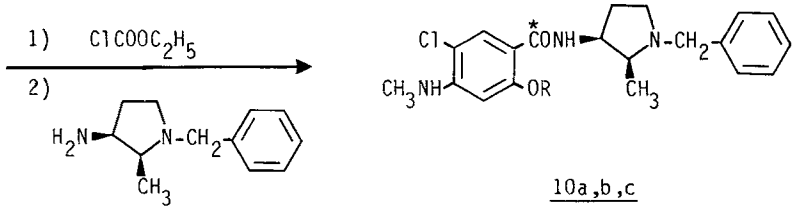
1a,c 2a,c 3a,c



4a,c 5a,b,c



6a,b,c 7a,b,c 8a,b,c



9 10a,b,c

a: * = ^{14}C, R = CH_3 b: * = non-label, R = CD_3 c: * = non-label, R = CH_3

Tos = p-tcluenesulfonyl

Scheme I

radiochemical yield, because cis-amine 9 is obtained by the separation of a mixture of cis and trans stereoisomers.[2] Furthermore, we observed that tritium atom labeled at 3-position of pyrrolidine ring in an analogue of 10c was partly converted to tritiated water in animals.[5]

Tosylamide (2a) was obtained by the reaction of 4-amino-2-hydroxy[carbonyl-^{14}C]benzoic acid (1a) with an excess of tosyl chloride in the presence of sodium carbonate in aqueous tetrahydrofuran under cooling. Protection of the acidic groups in 2 was required for chlorination of the benzene ring in the subsequent step. The reported method[2] seemed too drastic to prepare carbonyl-^{14}C labeled compound 5a, because the treatment of 2c with dimethyl sulfate and potassium hydroxide in boiling acetone resulted in decarboxylation in addition to the formation of the desired trimethyl compound 5c.[6] Use of diazomethane, a convenient reagent for methylation, was tried to obtain trimethyl compound 5a in a high yield. Although a phenolic group which forms part of a strongly chelated ortho-carbonylhydroxy system is not normally alkylated with a diazoalkane,[7] we succeeded in trimethylation of 2c in an excellent yield as described below. After addition of excess diazomethane, the reaction was followed by TLC analysis. The starting 2c was consumed immediately after the addition of diazomethane, and monomethyl compound 3c was formed as a major product with a small amount of dimethyl compound 4c. After a further 10 minutes, 3c was completely coverted to 4c , whereupon formation of a trace of trimethyl compound 5c was observed. After prolonged reaction for about 2 or 3 days, 5c which was derived from 4c was obtained in 85 to 90% yield. These results are of interest in connection with the fact that use of boron trifluoride etherate as a common catalyst did not accelerate the reactivity of the phenolic hydroxy group. This stepwise

methylation is a necessary and useful procedure to isolate each of the methylated products. The dimethyl compound 4c is especially useful as an intermediate for the preparation of methoxy-d_3 labeled YM-09151-2 (10b). Thus trimethyl compound 5a was obtained from 2a in 85.3% yield, and dimethyl compound 4c was prepared from 2c in 65.3% yield.

Chlorination of 5a with chlorine was completed within 30 minutes in chloroform on an ice-water bath. An excess of chlorine should be removed immediately to prevent side reactions. It is effective to bubble nitrogen gas into the reaction mixture before isolation of 6a.

Removal of the tosyl moiety of 6a with concentrated sulfuric acid in benzene at room temperature followed by hydrolysis with 1M-sodium hydroxide in methanol under reflux gave carboxylic acid 8a. Condensation of 8a with 9 by means of mixed anhydride technique afforded the desired benzamide 10a in a high yield, which was purified by recrystallization from isopropanol. Overall radiochemical yield was 79.1%. Specific activity was 21.18 mCi/mmol as determined by liquid scintillation counting. Radiochemical purity was greater than 99% as determined by radioactivity scanning on TLC.

It was found that the methoxy group of YM-09151-2 was intact in metabolism using ^{14}C-labeled YM-09151-2 (10a).[3] Therefore, for the pharmacokinetics of YM-09151-2 the methoxy group was labeled with deuterium. The key intermediate 4c was treated with iodomethane-d_3 under basic conditions to give 5b in 82.5% yield. (Methoxy-d_3)YM-09151-2 (10b), synthesized from 5b by a similar way as above, was recrystallized three times from isopropanol. The overall yield from iodomethane-d_3 was 60.8%.

EXPERIMENTAL

Carboxyl-^{14}C labeled 4-amino-2-hydroxybenzoic acid (1a, 95% radiochemically pure, custom preparation) was purchased from Amersham International plc, Amersham, England. Iodomethane-d_3 (minimum isotopic purity 99.5 atom % D) was obtained from Merck Sharp & Dohme Canada, Canada. The labeled compounds were used without further purification. All organic extracts were dried over anhydrous magnesium sulfate, unless otherwise specified, and evaporated *in vacuo*. The ^{14}C-labeled compounds were characterized by co-chromatography (TLC) with non-radioactive standards. Purity and identity of deuterium labeled compounds were established by normal spectra (IR, NMR, MS) and analytical (TLC, GC, elemental analysis) techniques. Melting points were determined on a Yanagimoto micro melting point apparatus and were uncorrected. IR spectra were obtanined in a potassium bromide disc using Hitachi 215 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a JEOL FX-90Q FT-NMR spectrometer using TMS as an internal standard in $CDCl_3$ unless otherwise noted. MS spectra were determined on a Hitachi RMU-6MG mass spectrometer and HR/MS on a Hitachi M-80 spectrometer. GC data were collected on a Hewlett Packard GC-5730A. TLC analyses were done on silica gel 60 F_{254} (Merck) glass plates (5 x 20 cm with 0.25 mm layer). Radioactivity measurement was made with a Packard Tri Carb Liquid Scintillation spectrometer, Model 3255. Radiochemical purity was determined by TLC with a Berthold Radio-TLC Scanner LB 2723. Elemental analysis was carried out with a Yanagimoto CHN Corder MT-2. UV monitoring of eluate of Lobar® column chromatograph was carried out with a Oyo-bunko UV-detector Ubilog 5III with 0.2 mm flow cell.

2-Hydroxy-4-tosylamido[carbonyl-^{14}C]benzoic acid (2a)

To a solution of 1a (20 mCi, 75.1 mg) in tetrahydrofuran (2 ml) were added 1M-NaOH (0.49 ml), water (1.5 ml), Na_2CO_3 (175 mg) and sodium 4-amino-2-hydroxybenzoate (Na salt of 1c) dihydrate (102.6 mg). Tosyl chloride (381 mg) was added to the mixture with stirring at 0-5 °C. After stirring for 23 hr at the same temperature and then for 1.5 hr at room temperature, the organic solvent was evaporated. Diluted hydrochloric acid (10% HCl, 1 ml) was added to the residue and crystallization was induced by scratching the flask. After standing for 30 minutes at 0 °C, the crystals were collected by filtration and washed with water (2 ml), and dried over anhydrous calcium chloride for 1.5 hr *in vacuo*. Yield: 320 mg (107%).

Methyl 2-hydroxy-4-(N-methyl-N-tosylamido)benzoate (4c)

An etheral solution of diazomethane was prepared from Diazald (Aldrich) and potassium hydroxide by the usual method.[7] To a suspension of 2c[2] (10g) in ether (20 ml) was added a solution of diazomethane (200 ml).[7] After 10 min the reaction mixture was evaporated to dryness and the residue was subjected to a silica gel column (53 mmϕ , Wako-gel C-200, 300 g/n-hexane) with dichloromethane (20 ml). The column was eluted with n-hexane/ethyl acetate (4:1, v/v) and the fraction from 800 to 2300 ml was collected. Evaporation followed by recrystallization from isopropanol gave pure 4c. Yield: 7.2 g (65.3%), mp. 101-102 °C MS: m/z= 335 (M^+), 304, 271, 148, 91. NMR: δ(ppm) 2.40 (s, 3H, tosyl-CH_3), 3.16 (s, 3H, NCH_3), 3.94 (s, 3H, $COOCH_3$), 6.5 - 7.8 (m, benzene ring), 10.47 (s, 1H, OH). IR: ν_{max}(cm^{-1}) 3300, 3100, 1660, 1340, 1260, 1160, 930, 790. GC: 3% OV-22 6 ft oven temperature 275° C, a single peak was observed at 9.07 min by 5 µg injection.

Methyl 2-methoxy-4-(N-methyl-N-tosylamido)[carbonyl-^{14}C]benzoate (5a)

To a solution of 2a (320 mg) in methanol (2 ml) was added 25 ml of a freshly prepared solution of diazomethane in ether. After standing overnight at room temperature, the reaction mixture was evaporated to about 2 ml and a further solution of diazomethane (25 ml) was added to the residue. After 6.5 hr, the reaction mixture was reduced to 2 ml and again treated with a solution of diazomethane (25 ml) and the resulting mixture allowed to stand overnight. At this time (40 hr after initial addition of diazomethane) 85% of radioactivity was detected at the spot of 5a (Rf value 0.37) on the TLC of the reaction mixture and no significant other spots with radioactivity were observed (solvent system: methanol/ethyl acetate (2:1, v/v)). The reaction mixture was evaporated to dryness and the residue was applied to a lobar® column (Si 60, B size, Merck) using dichloromethane (3x2 ml). The column was eluted with a mixture of n-hexane and ethyl acetate (2:1, v/v) at a flow rate of 11 ml/min with UV monitoring at 265 nm. The fraction (52-70 min) was collected and evaporated to give colorless crystals of 5a. Yield: 291.8 mg (85.3%).

Methyl 2-(methoxy-d_3)-4-(N-methyl-N-tosylamido)benzoate (5b)

To a mixture of 4c (19.6 g) and anhydrous potassium carbonate (8.1 g) in N,N-dimethylformamide (200 ml) was added dropwise iodomethane-d_3 (10 g) over a period of 10 minutes at room temperature. After stirring overnight, the reaction mixture was filtered and evaporated to dryness. The residue was dissolved in ethyl acetate and the resulting solution was filtered. Evaporation of the filtrate gave crystals which were recrystallized twice from isopropanol (60 ml) to afford pure colorless crystals of 5b (18.6 g). The combined mother liquors

were evaporated and the residue was applied to a Lobar® column to recover further crop of 5b (1.5 g) which was recrystallized from isopropanol (4.5 ml). Total yield: 20.1 g (82.8% from CD_3I), mp. 98-99 °C. MS: m/z= 352(M^+), 321, 288, 197, 91. (No peak at m/z=349 was observed and the fragmentation pattern was identical to that of non-labeled standard 5c). NMR: δ(ppm) 2.40 (s, 3H, Ph-CH_3), 3.16(s, 3H, N-CH_3), 3.88 (s, 3H, $COOCH_3$), 6.4-7.8 (m, 7H, benzene ring). IR: $\nu_{max}(cm^{-1})$ 2960, 1730, 1600, 1495, 1340, 1250, 1160, 805, 795. GC: 3% OV-22 6 ft oven temperature 275 °C, a single peak was observed at 10.84 min by 9 µg injection. 3% OV-17 3 ft oven temperature 245 °C, a single peak was observed at 4.68 min.

Methyl 5-chloro-2-methoxy-4-(N-methyl-N-tosylamido)[carbonyl-^{14}C]benzoate (6a)

Chlorine was passed into a solution of 5a (291.8 mg) in chloroform (7.5 ml) at 0° to 5 °C until the the solution became pale yellow. After a few minutes, nitrogen gas was bubbled at the same temperature for 1 hr to remove excess chlorine and hydrogen chloride formed. The solvent was evaporated to dryness and the crystaline residue was applied to a Lobar® column (Si 60, size B, Merck) using a mixture of benzene and ethyl acetate (10:1 v/v, 5 ml). The column was eluted with the same solvent at a flow rate of 10 ml/min with UV monitoring at 280 nm. The fraction (26-33 min) was collected and evaporated to dryness. The residue (338.9 mg) was recrystallized from isopropanol (6.5 ml) to yield colorless crystals of 6a. Yield: 313.4 mg (98.0%).

Methyl 5-chloro-2-(methoxy-d_3)-4-(N-methyl-N-tosylamido)benzoate (6b)

A solution of 5b (19.9 g) in chloroform (100 ml) was treated

with chlorine in a similar manner as above. Pure crystals of 6b were obtained by recrystallization from isopropanol (170 ml). Yield: 21.06 g (98.8%), mp. 139-140 °C. MS: m/z= 386 (M^+), 231. NMR: δ(ppm) 2.41 (s, 3H, ph-CH_3), 3.20 (s, 3H, N-CH_3), 3.86 (s, 3H, $COOCH_3$), 6.88 (s, 1H, 3-H), 7.39 (ABq, 4H, tosyl), 7.73 (s, 1H, 6-H); IR: ν_{max}(cm^{-1}) 1725, 1595, 1485, 1380, 1340, 1240, 1150, 1100, 1020, 810, 780, 685. GC: 3% OV-17 3 ft oven temperature 245 °C, a single peak was observed at 5.59 min by 18 μg injection.

5-Chloro-2-methoxy-4-methylamino[carbonyl-^{14}C]benzoic acid (8a)

To a solution of 6a (313.4 mg) in benzene (6 ml) was added concentrated sulfuric acid (0.38 ml) for 5 minutes at room temperature. After stirring for 40 minutes benzene was evaporated and crushed ice (5 g) was added to the residue. After standing for 1.5 hr at 4 °C, crystals were collected by filtration and washed with water. The wet filter cake was subjected to saponification with 1M-NaOH (2 ml) and methanol (3 ml). After refluxing for 1.5 hr, methanol was evaporated off and the residue was dissolved in water (5 ml) followed by filtration. The filtrate acidified with 1M-HCl (2.5 ml) was allowed to stand for 20 minutes at 0 °C. Colorless crystals of 8a were collected by filtration, washed with chilled water and dried over anhydrous calcium chloride under reduced pressure. Yield: 170 mg (97.1%). The radiochromatogram of 8a showed that the radiochemical purity was greater than 99%. Solvent system: chloroform/methanol (9:1 v/v).

5-Chloro-2-(methoxy-d_3)-4-methylaminobenzoic acid (8b)

To concentrated sulfuric acid (25 ml) were added the crystals of 8b (20.94 g) with stirring at room temperature. After the

crystals were dissolved, the reaction mixture was poured into crushed ice (40 g) with vigorous stirring. The precipitate (7b) was collected by filtration and washed with water. The wet crystals of 7b were heated at 80 °C in 1M-NaOH (94 ml) until they were completely dissolved (about 1 hr). To the cooled reaction mixture were added ice-water (300 ml) and concentrated HCl (7.8 ml) with stirring at a temperature below 10 °C. The precipitate was collected by filtration, washed with chilled water, and recrystallized from ethanol (220 ml) to yield pure colorless crystals of 8b. Yield: 10.38 g (87.7%), mp. 186-187 °C. MS: m/z= 218 (M^+), 201. NMR (in DMSO-d_6): δ(ppm) 2.48 (d, J=5.4Hz, 3H, N-CH_3), 6.16.(s and t, J=5.4Hz, 2H, 3-H, NH), 7.61 (s, 1H, 6-H), 10.57 (s, 1H, COOH). IR: ν_{max}(cm^{-1}) 3420, 3100-2300, 1660, 1575, 1550, 1390, 1350, 1250, 1224, 1100, 906, 810, 680.

N-[(2RS,3RS)-1-Benzyl-2-methyl-3-pyrrolidinyl]-5-chloro-2-methoxy-4-methylamino[carbonyl-^{14}C]benzamide ([Carbonyl-^{14}C]YM-09151-2) (10a)

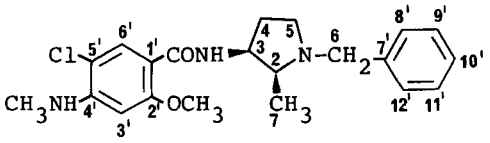
To a solution of 8a (170.8 mg) and trimethylamine (82.5 mg) in dichloromethane (0.36 ml) was added a solution of ethyl chloroformate (88 mg) in dichloromethane (0.36 ml) for 5 minutes at -20° C. After stirring for 1 hr at -10° to -15 °C, a solution of (2RS,3RS)-3-amino-1-benzyl-2-methylpyrrolidine (9)[2] (155 mg) in dichloromethane (0.62 ml) was added to the mixture at -20 °C. The resulting mixture was stirred for 1 hr at -10° to -15 °C and then for 3 hr at room temperature. The reaction mixture was washed successively with water (2 ml), 1M-NaOH (2 ml) and water (2 ml), and dried. Removal of the solvent gave pale yellow crystals which were recrystallized from isopropanol (1.9 ml) to afford pure 10a as white crystals.

Yield: 290.7 mg (94.6%). Specific activity: 21.18 mCi/mmol. Radiochemical purity was greater than 99% by TLC (Rf value: 0.52). Solvent system for TLC: benzene/acetone/diethylamine (80:20:5, v/v).

N-[(2RS,3RS)-1-Benzyl-2-methyl-3-pyrrolidinyl]-5-chloro-2-(methoxy-d_3)-4-methylaminobenzamide ((Methoxy-d_3)YM-09151-2) (10b)

The carboxylic acid 8b (10.0 g) was treated with ethyl chloroformate (5.07 g) and cis-amine 9 (8.88 g) in a similar manner as above. Three times recrystallization from isopropanol (60 ml) were carried out for purification of 10b. Yield: 15.15 g, mp. 154.5-155.5 °C. Analysis: Calculated for $C_{21}H_{23}N_3O_2ClD_3$ C, 64,52; H, 5.93; N, 10.75: Cl, 9.07: D, 1.55. Found C, 64.49; H, 6.03; N, 10.66; Cl, 9.26; D,1.57. HR/MS: Calculated for $C_{21}H_{23}N_3O_2ClD_3$; 390.19001. Found 390.18811. MS: m/z= 391 (M^++1), 390 (M^+), 389, 375, 301, 201, 173, 91, 56 (No peak at m/z 387).

Assignment of NMR signals based on a tentative numbering for 10b shown below.



10b

^{1}H-NMR: δ(ppm) 1.12 (d, J=6.5Hz, 3H, 7-CH_3), 1.60 (m, 1H, 4-H), 2.10 (m, 2H, 4-H and 5-H), 2.60 (m, 1H, 2-H), 2.93 (d, J=5Hz, 3H, 8-CH_3), 3.00 (m, 1H, 5-H), 3.16 (d, J=13Hz, 1H, 6-H), 4.02 (d, J=13Hz, 1H, 6-H), 4.66 (m, 2H, 3-H and CH_3NH), 6,09 (s, 1H, 3'-H), 2,77 (s, 5H, 7' to 12'-H), 8.07 (s, 1H, 6'-H).

^{13}C-NMR: δ(ppm) 164.0 (C=O), 158.0 (2'), 139.7 (7'), 132.2 (6'), 128.5 (8' and 12'), 128.1 (9' and 11'), 126.7 (10'), 111,3(5'), 110.6 (1'), 93.2 (3'), 61.7 (2), 57.5 (6), 52.4 (3), 51.6 (5), 31.1 (4), 30.1 (8), 14.1 (7), 55.2 (multiplet, O$\underline{C}D_3$). IR: ν_{max}(cm^{-1}) 3370, 2960, 2780, 1620, 1600, 1510, 1440, 1330, 1280, 1250, 1110, 800, 730, 690.

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