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SYNTHESIS OF TRITIUM LABELLED 4P-PDOT, A SELECTIVE MELATONIN RECEPTOR ANTAGONIST

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SUMMARY

A selective MT2 melatonin receptor antagonist, 4-phenyl-2-propionamido-tetralin (4P-PDOT), was prepared as a tritium-labelled compound with high specific activity and radiochemical purity. Catalytic hydrogenation of a unique vinyl bromo precursor **10** was used to introduce the tritium.

Keywords: vinyl bromo precursor, catalytic halogen-tritium replacement, melatonin antagonist, MT2 antagonist, 4P-PDOT

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is the principal hormone of the pineal gland. This molecule elicits potent anti-stress, anti-aging, and oncostatic properties and influences various immunological and endocrinological functions (1,2). The melatonin-generating system is characterized by photosensitivity; a circadian rhythm such that the highest levels of the hormone are produced at night, in darkness; and decreased activity with age. At least three types of binding sites have been described for melatonin: the G-coupled, seven-*trans* membrane domain receptors mt1 and MT2, and a putative binding site called MT3 (3). Although the lack of specific, potent and subtype-selective agonists and antagonists has

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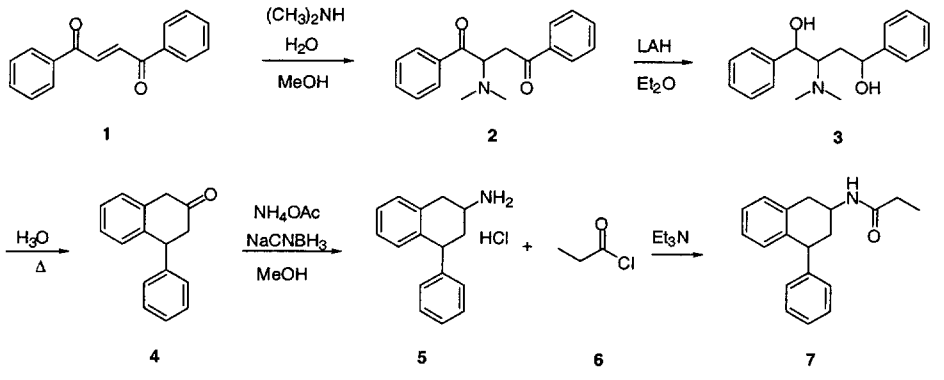
hampered studies of melatonin's physiological actions, the MT2 receptor is known to be involved in mediating phase advances of circadian activity rhythms by melatonin (4) and has been found to enhance the vascular response to melatonin in arterial segments (5). We have synthesized the selective MT2 receptor antagonist 4-phenyl-2-propionamidotetralin (4P-PDOT) (3,6,7) labelled with tritium for use in studies of melatonin's biological activity.

RESULTS AND DISCUSSION

The key intermediate **5** was prepared according to Scheme I, along with **7**, unlabelled 4P-PDOT, (7), for use as a reference material. Since a high specific activity was required for the biological studies, we planned to utilize the bromo compound **9** as a substrate for tritium incorporation as shown in Scheme II. Amine **5** was reacted with 2,3-dibromopropionyl chloride **8** to give a single compound in 23% yield.

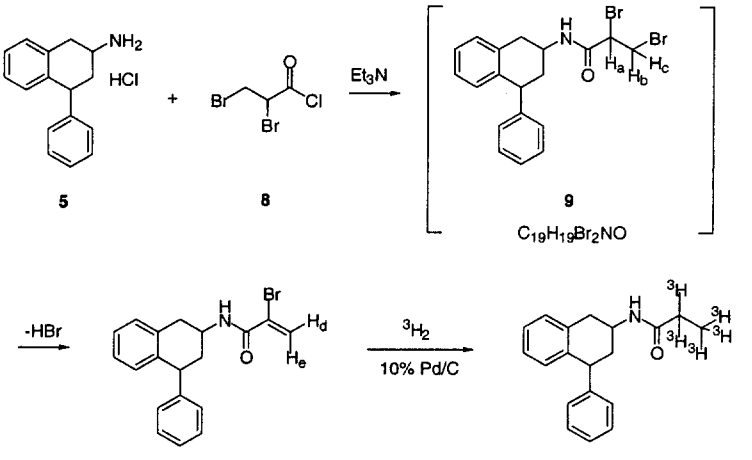
Scheme I



However, the NMR and mass spectra of this compound did not match that expected for compound **9**. The mass spectrum of the material showed the presence of one bromide and a molecular formula of $C_{19}H_{18}BrNO$ by elemental analysis. Integration of the NMR spectrum showed 18 protons instead of the 19 protons that would be expected for compound **9**. The product's NMR spectrum showed two protons at δ 6.02 and 7.00 with small coupling constants (J = 1.5 Hz), as would be expected with geminal protons such as those in compound **10** (H_d and H_e). Based on the data, the product of the reaction was determined to be **10**. In the presence of base, the dibromoamide **9** readily dehydrobrominates to give the more stable,

conjugated bromoamide **10**. Hydrogenation of the product **10** gave the desired product **7**, thus confirming its structure.

Scheme II



5 **8** **9**

$C_{19}H_{19}Br_2NO$

10

$C_{19}H_{18}BrNO$

11

The tritiation of bromo compound **10** was accomplished using catalytic hydrogenation. The tritium-labelled material **11** has a total activity of 1.3Ci, a specific activity of 110 Ci/mmol, and 98% radiochemical purity as assessed by radio-HPLC (high performance liquid chromatography with radioactive detection). The higher specific activity (110 Ci/mmol) of the final product **11** provides additional evidence of the vinyl bromide **10** as the precursor. The ^{3}H-NMR spectrum shows the expected incorporation of tritium protons but also some exchange of the terminal methylene protons, which explains the higher specific activity observed (110 Ci/mmol instead of the 87 Ci/mmol expected for incorporation of three tritiums).

EXPERIMENTAL

Melting points (uncorrected) were determined on a Mel-Temp melting point apparatus. Infrared spectra were recorded on a Perkin Elmer 1600 FTIR spectrophotometer. Ultraviolet spectra were recorded on a Varian DMS-90 spectrophotometer. The NMR spectra were recorded on a Varian Gemini 300-MHz spectrophotometer using tetramethylsilane as the internal standard. In reporting the NMR

multiplicities, we use the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; apt, apparent; and br, broad. The mass spectra were obtained on a Ribermag R 10-10 GC/MS. The starting materials were purchased from Aldrich Chemical Company. The tritiation was carried out at the National Tritium Labeling Facility in Lawrence Berkeley National Laboratory, Berkeley, CA. Atlantic Microlab, Inc. (Norcross, GA) performed microanalyses.

2-(N,N-Dimethylamino)-1,4-diphenyl-1,4-butanedione (2). A solution of 40% aqueous dimethylamine (31.2 g, 0.27 mol) was added to a cooled suspension of *trans*-1,2 dibenzoylethylene **1** (33.9 g, 0.14 mol) in MeOH (250 mL). The yellow suspension became dark red after the addition. After standing for 6 h at RT and overnight in the refrigerator, the product was filtered and recrystallized from MeOH/H_2O (7/3) to give 21 g (53%) of the yellow solid **2** (8,9). ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 6H), 3.15 (dd, J = 3.2 Hz, J = 17.1 Hz, 1H), 3.83 (m, 1H), 4.94 (dd, J = 3.2 Hz, J = 9.7 Hz, 1H), 7.49 (m, 6H), 8.05 (apt dd, J = 7.02 Hz, J = 28.8 Hz, 4H).

2-(N,N-Dimethylamino)-1,4-diphenyl-1,4-butanediol (3). A solution of the dione **2** (50 g, 0.17 mol) in anhydrous ether (300 mL) was added to a stirred suspension of $LiAlH_4$ (21.2 g, 0.55 mol) in anhydrous ether (200 mL) under argon at 0 °C. After 2 h, an additional 100 mL of ether was added and the mixture was slowly warmed to RT and stirring was continued overnight. The mixture was cooled in an ice bath, and EtOAc (50 mL) was added dropwise, followed by the cautious addition of H_2O (50 mL). The mixture was filtered and washed with ether (2 × 800 mL). The combined filtrates were washed with H_2O, dried (magnesium sulfate), and filtered. The solvent was removed under reduced pressure and the residue recrystallized from benzene/hexane (1/2) to give 46.6 g (96%) of the white solid **3** (9).

4-Phenyl-2-tetralone (4). A solution of the diol **3** (46.6 g, 0.16 mol) in concentrated HCl (500 mL) was refluxed for 17 h and quickly formed an insoluble orange oil. The cooled mixture was extracted with ether (3 × 300 mL). The combined extracts were washed with H_2O (3 × 200 mL), saturated sodium carbonate solution, and H_2O (3 × 200 mL). The organic layer was dried (magnesium

sulfate), filtered, and evaporated under reduced pressure to give 30 g of orange oil. After an unsuccessful purification attempt by silica gel column chromatography (hexane/ EtOAc), the crude product was distilled (195 °C at 0.2 mm Hg) to give 4.2 g (11%) of 4-phenyl-2-tetralone, **4** (9). ^{1}H NMR ($CDCl_3$) δ, 2.91 (apt m, J = 16.5 Hz, 2H), 3.63 (dd, J = 8.2 Hz, J = 28.4 Hz, 2H), 4.46 (t, J = 6.5 Hz, 1H), 7.0-7.4 (m, 10H).

4-Phenyl-2-aminotetralin HCl (5). To a stirred solution of 4-phenyl-2-tetralone **4** (4.0 g, 17.9 mmol) and ammonium acetate (14.17 g, 183.9 mmol) in dry MeOH (75 mL) under argon was added sodium cyanoborohydride (839 mg, 13.3 mmol) in one portion (8), and the solution was stirred at RT for 40 h. Concentrated HCl (30 mL) was added until pH<2. The MeOH was evaporated and the yellow residue dissolved in H_2O (200 mL) and washed with ether (200 mL). The aqueous phase was made basic (pH>10) with powdered KOH (15 g). The aqueous solution was extracted with ether (4 × 200 mL), dried (sodium sulfate), filtered, and concentrated to give 1.9 g of a dark liquid. The crude amine in ether (60 mL) was converted to the corresponding HCl salt by treatment with concentrated HCl (2 mL). The solid (1.6 g) was recrystallized from MeOH to give 1.06 g (27%) of product **5** (7). ^{1}H NMR (CD_3OD) δ 2.24 (m, 1H), 3.09 (m, 1H), 3.29 (m, 2H), 3.68 (m, 1H), 4.25 (m, 1H), 6.07 (d, J = 7.6 Hz, 2H), 7.19 (m, 9H).

4-Phenyl-2-propioamidotetralin (7). To a cooled and stirred mixture of 4-phenyl-2-aminotetralin HCl **5** (322 mg, 1.23 mmol) in toluene (10 mL) and triethylamine (0.2 mL, 1.43 mmol) under argon was added propionyl chloride **6** (0.1 mL, 1.15 mmol). The mixture was slowly warmed to RT and stirred for 14 h. Triethylamine HCl was removed by filtration and a rinse with toluene (10 mL). The filtrate was concentrated under reduced pressure to give 200 mg of solid residue. Recrystallization from acetone/hexane gave 88 mg (25%) of product **7** (7). Mp 164-165 °C [lit (7) 141-146 °C]; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, J = 7.6 Hz, 3H), 1.77 (q, J = 11.6 Hz, J = 23.9 Hz, 1H), 2.16 (m, 2H), 2.41 (m, 1H), 2.78 (apt q, J = 5.0 Hz, J = 11.0 Hz, 1H), 3.21 (apt q, J = 5.3 Hz, J = 10.5 Hz, 1H), 4.23 (apt q, J = 5.5 Hz, J = 6.0 Hz, 1H), 5.45 (apt d, J = 7.6 Hz, 1H), 6.79 (d, J = 7.7 Hz, 1H), 7.0-7.4 (m, 9H);

^{13}C NMR ($CDCl_3$) δ 9.86, 29.89, 36.95, 40.47, 45.67, 46.12, 126.27, 126.47, 128.60 128.61, 128.68, 129.08, 129.52, 135.01, 139.00, 146.06, 173.00; UV (EtOH) λ_{max} 217 nm (ε 7,853), 259 (405); FTIR (KBr) 3420, 3284, 1642, 1564 (amide I and amide II) cm^{-1}; TLC (silica gel) (hexane/EtOAc, 1/1) R_f= 0.52; MS (DCI-NH_3) m/e 280 (M+1); HPLC: Waters Nova C_{18} Column, 4.6 × 250 mm, solvent CH_3CN/H_2O (5/1), flow rate 1.3 mL/min, detection 254 nm, retention time 2.6 min, purity 94%; HPLC: Brownlee Spheri-5 RP-18 Column, 4.6 × 100 mm, solvent MeOH/H_2O (55/45); flow rate 1.0 mL/min, detection 254 nm, retention time 3.2 min, purity 98%. Anal. calcd. for $C_{19}H_{21}NO$•0.5 H_2O: C 79.13, H 7.69, N 4.88; found: C 79.02, H 7.57, N 4.81.

4-Phenyl -2-(2'-bromopropenoylamido)tetralin (10). To a cooled and stirred mixture of 4-phenyl-2-aminotetralin HCl **5** (541 mg, 2.08 mmol) and triethylamine (0.3 mL, 2.15 mmol) in toluene (20 mL) under argon was added dropwise 2,3-dibromopropionyl chloride **8** (0.3 mL, 2.61 mmol). The mixture was slowly warmed to RT and stirred for 14 h. The triethylammonium chloride was filtered off and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel eluted with an acetone/hexane gradient, and the isolated product was recrystallized from hexane/EtOAc to give 210 mg (23%) of **10-2**. Mp 155-158 °C; ^{1}H NMR ($CDCl_3$) δ 1.86 (q, J = 11.8 Hz, J = 24.1 Hz, 1H), 2.46 (m, 1H), 2.88 (dd, J = 11.0 Hz, J = 15.7 Hz, 1H), 3.27 (ddd, J = 1.7 Hz, J = 5.2 Hz, J = 15.7 Hz, 1H), 4.25 (q, J = 5.8 Hz, J = 11.5 Hz, 1H), 4.36 (m, 1H), 6.02 (d, J = 1.5 Hz, 1H), 6.62 (br d, J = 7.1 Hz, 1H), 6.80 (d, J = 7.5 Hz, 1H), 7.00 (d, J = 1.5 Hz, 1H), 7.05 (m, 1H), 7.15 (m, 4H), 7.28 (m, 3H); ^{13}C NMR($CDCl_3$) δ 36.54, 40.09, 46.13, 47.13, 123.00, 126.40, 127.46, 126.61, 127.64, 128.71, 129.10, 129.56, 134.40, 138.90, 145.80; UV (EtOH) λ_{max} 211 nm (ε 16,861); FTIR (KBr) 3304 (amide NH), 3058, 3028 (=CH_2), 1645, 1604 (amide I and II), 701 (C-Br) cm^{-1}; TLC (silica gel) (hexane/EtOAc, 1/1) R_f= 0.9; HPLC: Waters Nova C_{18} Column, 4.6 × 250 mm, solvent CH_3CN/H_2O (5/1), flow rate 1.3 mL/min, detection 254 nm, retention time 3.3 min, purity 99%; HPLC: Brownlee Speri-5 RP-18 Column, 4.6 × 100 mm), solvent MeOH/H_2O (55/45), flow rate 1.0 mL/min, detection 254 nm, retention time 4.4 min, purity 99%; MS (DCI-NH_3) m/e 356 (M+H). Anal. calcd.

for $C_{19}H_{18}BrNO$: C 64.05, H 5.09, N 3.93, Br 22.42; found: C 63.94, H 5.15, N 3.90, Br 22.54.

Alternative Synthesis of 4-Phenyl-2-(propioamido)tetralin (7). In a 100-mL round-bottom flask were placed 4-phenyl-2-(2'-bromopropenoylamido)tetralin **10** (9.96 mg, 0.028 mmol), 10% palladium on carbon, triethylamine (10 mg, 0.09 mmol), and EtOAc (10 mL). The mixture was degassed twice and hydrogenated at RT under hydrogen gas in a balloon. The progress of the reaction was checked by thin-layer chromatography (TLC) and HPLC, and after 1.5 h the mixture was degassed and passed through a pad of silica gel. The filtrate was concentrated to give 2 mg (64%) of a dark yellow solid. The product was identical with **7** prepared from 4-phenyl-2-aminotetralin hydrochloride **5** and propionyl chloride **6** according to Scheme I.

4-Phenyl-2-[2',3'(n)-^{3}H]-propioamidotetralin (11). In a tritiation flask were placed 4-phenyl-2-(2'-bromopropenoylamido)tetralin **10** (5 mg, 0.014 mmol), triethylamine (10 mg, 0.098 mmol), 10% palladium on carbon (8 mg), and EtOAc (2.5 mL). After a freezing and degassing cycle, the mixture was stirred under tritium gas (150 Ci) for 2.5 h at RT. The mixture was degassed again and the labile tritium was removed by washing with MeOH. Filtration and concentration of the filtrates gave the desired product, 4-phenyl-2-[2',3'(n)-^{3}H]-propioamidotetralin **11**. Radiochemical purity was determined on a Vydac Peptide C_{18} (4.6 × 250 mm) column with an IN/US β–ram detector, solvent acetonitrile/H_2O (6/4), flow rate 1.5 mL/min, retention time 3.5 min, radiochemical purity 98%. The product **11** has a total activity of 1.3 Ci with a specific activity of 110 Ci/mmol and a radiochemical purity of 98% by radio-HPLC.

CONCLUSIONS

This method allows the synthesis of tritium labelled 4P-PDOT with high specific activity and radiochemical purity, which will allow further investigation of melatonin's receptors and biological activity. The [^{3}H]-4P-PDOT is available for research from Dr. Linda Brady, National Institute of Mental Health, 6001 Executive Boulevard, 7N-7185, MSC 9641, Bethesda, MD 20857.

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