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Research Article

Synthesis of PNU-243922 labelled with ^{14}C

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Summary

The arylpiperazine PNU-243922 (**1**) has been labelled with ^{14}C. A two-step sequence starting from the ^{14}C labelled alcohol **6a** led to radiochemically pure (> 98%) [^{14}C]PNU-243922 with a specific activity of 546 MBq/mmol.

Key Words: PNU-243922; arylpiperazine; carbon-14; anti-obesity agent

Introduction

PNU-243922 (8-[2-({3-[(2*R*)-2-methylpiperazinyl}-2-pyrazinyl}oxy)ethoxy]-2-quinolinylamine (**1**)) is a novel agonist at the $5HT_{2C}$ [1,2] receptor belonging to the arylpiperazine chemical class.

The compound was evaluated for its potential application as control calorie intake and nutrient balance, which should result in weight loss without inducing psychotropic side effects. The preparation of a

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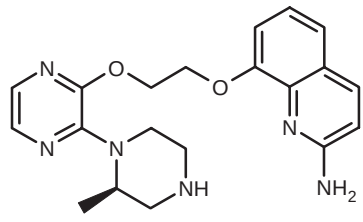

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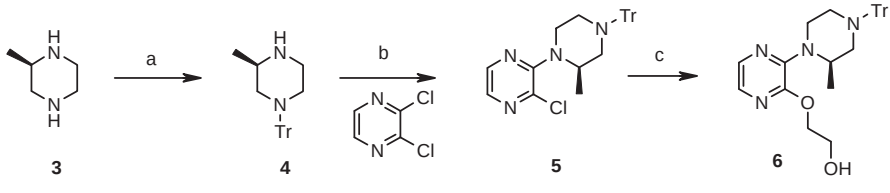
specifically labelled ^{14}C form of PNU-243922 was required at an early stage of the research program in order to help the selection of a compound with an adequate ADME profile. The piperazine ring seemed to be at first glance the most convenient group to introduce a radiolabel in the molecule. However, previous metabolism studies carried out with compounds belonging to the same chemical, class showed that this labelling position was not suitable. On the other hand, preliminary metabolism studies indicated that the $–O–CH_2–CH_2–O–$ chain was unlikely to be lost through metabolic activity. In this paper the synthesis of the ^{14}C labelled form of the title compound as well as its precursor is reported.

Discussion and results

During the preparation of a promising compound of the same chemical class, useful information was obtained about the Mitsunobu reaction.[3–5] Moreover, the radiolabelled intermediate **6a** was identified as a common precursor to prepare a series of ^{14}C compounds of potential interest for the $5HT_{2C}$ project. Compound **6** was synthesized according to Scheme 1, using trityl (Tr) as protecting group. Attempts

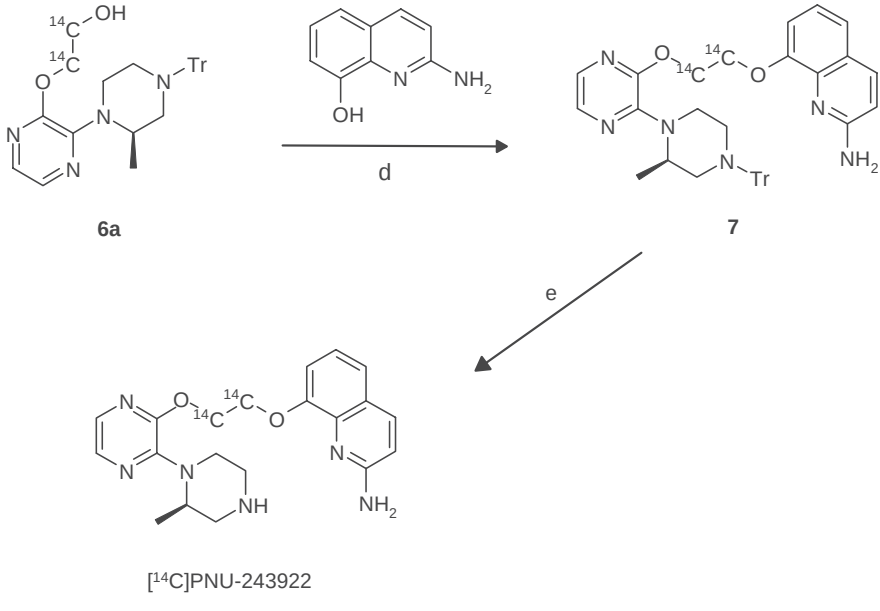


3 **4** **5** **6**

Scheme 1. Reagents and conditions: (a) $TrCl/CH_2Cl_2$; (b) K_2CO_3/DMF and (c) ethylene glycol, NaH/DMF

to use Boc protection was unsuccessful due to its instability under the reaction conditions. Compound **3** was treated with TrCl in CH_2Cl_2 to form only the isomer **4**,[6, 7] the second nitrogen acting as base to accept the proton. Condensation of **4** with excess 2,3-dichloropyrazine in DMF gave quantitative conversion to **5**. Finally, coupling with ethylene glycol in the presence of NaH in DMF gave **6**.

With the synthetic method to **6** in hand, it seemed most convenient to introduce ^{14}C in the –O–CH_2–CH_2–O– moiety of the title compound as shown in Scheme 2. However the Mitsunobu reaction conditions successfully applied during the preparation of similar compounds[5, 6] did not work in case of PNU-243922 and only unreacted **6a** was recovered. Several exploratory trials were carried out to optimize the reaction conditions such as temperature, solvents, reagents addition order, etc. The Mitsunobu reaction turned out to be extremely sensitive to the concentration gradient of the reactants while reaction temperature and reaction time did not substantially affect the yield. The best results were obtained when a 4.5 equivalents of diisopropyl azodicarboxylate (DIAD) and a 4.5 equivalents of triphenylphosphine (Ph_3P) were added in portions to the ^{14}C labelled alcohol **6a** and 2-amino-8-hydroxyquino-



Scheme 2. Reagents and conditions: (d) Ph_3P, DIAD, THF and (e) CH_3CN, TFA, then MeOH

line 2.5 equivalents in dry tetrahydrofuran (THF) under nitrogen. The obtained diether **7** was purified by flash-chromatography followed by preparative thin layer chromatography (TLC). The removal of the trityl group was achieved by treating **7** with trifluoroacetic acid (TFA) in acetonitrile. After purification by preparative high-performance liquid chromatography (HPLC), [^{14}C]PNU-243922 was obtained in > 98% radiochemically pure with a specific activity of 546 MBq/mmol. The overall radiochemical yield was about 2% from **6a**.

Experimental

General methods

2-({3-[(2*R*)-2-methyl-4-tritylpiperazinyl]-2-pyranizyl}oxy)-1-[U-^{14}C] ethanol **6a** was purchased from Amersham, synthesized according to our method for the unlabelled equivalent **6**. All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated. Radioactivity measurements were performed on a Tri-Carb 2100 TR liquid scintillation analyzer (Packard) using Rialuma (Lumac System) as liquid scintillation cocktail. TLC and radio-TLC measurements were made using a Packard Bioscan System 200 imaging scanner and silica gel Merck F254 plates (20 × 5 cm, 0.25 mm thick): toluene:ethyl acetate 7:3 (v:v) (system I), methylene chloride:methanol:triethylamine 2:8:0.1 (v:v) (system II). Preparative TLC were performed using silica gel Merck F254 plates (20 × 20 cm, 0.5 mm thick). Chemical purities were determined by HPLC performed at 25°C using a series-200 pump (Perkin-Elmer) equipped with a LC-295 UV/VIS detector (Perkin-Elmer) and PE-Nelson Turbochrom 4.0 software. Radiochemical purities were determined using an A-515TR radio-HPLC analyzer (Packard) equipped with a 0.5 ml homogeneous cell (liquid scintillation cocktail: Ultima Flo-M (Packard) ratio to HPLC effluent: 3:1), under the following conditions: YMC C8 column (100 × 4.6 mm ID; particle size 5 µm, supplied by YMC Europe-GmbH) eluting with H_2O:CH_3CN:TFA 94:6:0.1 (v:v) (A) and H_2O:CH_3CN:TFA 10:90:0.1 (v:v) (B) mixtures (isocratic at 100% A for 2 min, linear gradient from 100% A to 0% A over 8 min, isocratic at 100% B for 5 min, linear gradient from 0% A to 100% A over 0.5 min, isocratic at 100% A for 2.5 min); flow rate 1.5 ml/min; wavelength 254 nm. The

purification of the final compound was performed by preparative HPLC under the following conditions: YMC J'sphere ODS-H80 column (150 × 100 mm ID; particle size 4 μm, supplied by YMC Europe-GmbH) eluting with H_2O:CH_3CN:TFA 94:6:0.1 (v:v) (A) and H_2O:CH_3CN:TFA 10:90:0.1 (v:v) (B) mixtures (isocratic at 93% A for 10 min, linear gradient from 93% A to 40% A over 3 min, isocratic at 40% A for 10 min, linear gradient from 40% A to 0% A over 1 min, isocratic at 100% B for 5 min, linear gradient from 0% A to 93% A over 1 min, isocratic at 93% A for 3 min); flow rate 8 ml/min; wavelength 254 nm.

*3-(R)-Methyl-1-tritylpiperazine (**4**)*

Solid TrCl (260 g, 0.935 mol) was slowly added to a well-stirred and cooled solution of **3** (95 g, 0.95 mol) in 1.2 l of CH_2Cl_2 (EXOTHERMIC!). After 45 min, the mixture was poured into an aqueous solution of K_2CO_3 (140 g in 0.5 l of water), the organic phase was separated and dried by K_2CO_3, and the solvent was evaporated. This gave 370 g oily, **4** which was used without purification. **4** can, however, be crystallized from heptane. ^{1}H-NMR ($CDCl_3$): 7.57–7.10 (m, 15H) Tr; 3.23–2.85 (m, 7H) piperazine; 0.95 (d, 3H, $J = 6.0$ Hz) Me.

*1-(3-Chloro-2-pyrazinyl)-2-(R)-methyl-4-trityl-piperazine (**5**)*

A mixture of **4** from above, 2,3-dichloropyrazine (154 g, 1.0 mol) and K_2CO_3 (160 g, 0.6 mol) in 1 l DMF was heated (100–110°C) for 20 h with intensive stirring (monitored by TLC in $CHCl_3$: EtOH 20:1). The mixture was then cooled and poured slowly into 6 l of water with stirring. Compound **5** was filtered, washed with water and dried under vacuum (3 mm/50°C, 2 days) to remove DMF completely. Yield 299 g (70.8%). For analysis, 3 g of **5** was dissolved in 10 ml of hot ethanol, and HCl (10% aqueous solution, 3 ml) was added slowly (to keep the compound in solution). TrOH begins to crystallize after 10–15 min. After 30 min, TrOH was filtered and ethanol was evaporated. The aqueous solution was extracted by ether (2 × 200 ml) and made basic by K_2CO_3 to pH 12. The compound was extracted by $CHCl_3$ (3 × 10 ml) dried with K_2CO_3, and then the solvent was evaporated to give 1.4 g of the detritylated product 1-(3-chloro-2-pyrazinyl)-2-(*R*)-methylpiperazine. ^{1}H-NMR ($CDCl_3$): 8.08 (d, 1H, $J = 2.7$ Hz) Ar; 7.83 (d, 1H) Ar; 4.11(m, 1H)

CH–Me; 3.32(m, 2H) + 3.13–2.72 (m, 5H) piperazine; 1.17 (d, 3H, $J = 6.6$ Hz) Me.

*2-({3-[(2R)-2-Methyl-4-tritylpiperazinyl]-2-pyrazinyl}oxy)-1-ethanol (**6**)*

The title compound was prepared using **5** (0.227 g, 0.5 mmol) which was added to glycol (0.310 g, 5.0 mmol) and NaH (0.097 g, 60% in mineral oil, 2.5 mmol) in DMF. The reaction was stirred at 65°C overnight. Purification by silica gel chromatography (20% ethylacetate in *n*-hexane) gave **6** as a yellow solid (0.120 g, 50% yield); mp 86–88°C; ^{13}H-NMR (DMSO) δ 7.67–7.65 (m, 1H), 7.50–7.45 (m, 5H), 7.45–7.42 (m, 1H), 7.32–7.27 (m, 5H), 7.20–7.15 (m, 3H), 4.67–4.61, (m, 1H), 4.38–4.28 (m, 2H), 4.16–4.10 (m, 1H), 3.95–3.69 (m, 2H), 3.65–3.58 (m, 1H), 2.95–2.89 (m, 1H), 2.85–2.80 (m, 1H), 2.04–1.97 (m, 1H), 1.73–1.65 (m, 1H), 1.50–1.46 (m, 3H).

*8-[2-({3-[(2R)-2-methyl-4-tritylpiperazinyl]-2-pyrazinyl}oxy)[U-^{14}C]ethoxy]-2-quinolinylamine (**7**)*

To a cooled (0°C) solution of **6a** (195.36 MBq, 0.264 mmol, with a radiochemical purity of about 75%) and 2-amino-8-hydroxyquinoline (105.9 mg, 0.66 mmol) in dry THF (1 ml) a first portion of Ph_3P (43.3 mg, 0.172 mmol) was added. When the mixture of reaction became clear, a freshly prepared solution of DIAD in dry THF (330 mg/ml, 100 µl, 33.4 mg, 0.172 mmol) was added. After removing the cold bath and stirring at room temperature for about 30 min, 90% of the unreacted precursor **6a** was still present in the reaction mixture (determined by radio-TLC, systems I and II). At a distance of about 30 min, the Mitsunobu reagents were added again at 0°C as follows: Ph_3P (129.8 mg, 0.488 mmol) and DIAD in dry THF (330 mg/ml, 300 µl, 100 mg, 0.488 mmol) and twice Ph_3P (43.3 mg, 0.172 mmol) and DIAD in THF (330 mg/ml, 100 µl, 33.4 mg, 0.172 mmol). The reaction mixture was then stirred at room temperature for 24 h. As the conversion of **6a** was still not complete (29% of unreacted precursor, checked by radio-TLC, systems I and II) further Ph_3P (43.3 mg, 0.172 mmol) and a solution of DIAD in THF (330 mg/ml, 100 µl, 33.4 mg, 0.172 mmol) were added. At the end of the reaction (determined by radio-TLC, systems I and II), the solution

was evaporated to dryness and the non-radioactive yellow impurities were eliminated by a flash chromatography on SiO_2 eluting with toluene:ethyl acetate 7:3. The fractions containing the product (determined by radio-TLC, system I and by radio-HPLC) were combined and evaporated to dryness giving the crude intermediate **3** (77.6 MBq, 0.1422 mmol, about 20% radiochemically pure by radio-HPLC). The above material was then purified twice by preparative TLC eluting with a mixture methylene chloride:metha-nol:triethylamine 2:8:0.1 (v:v) and the chromatographic bands corresponding to **7** were extracted with a mixture ethyl acetate:toluene 1:1 (v:v). After organic phases evaporation to dryness, the compound **3** was obtained (5.66 MBq, 0.0104 mmol, 88% radiochemically pure by radio-HPLC). The overall radiochemical yield was 4% from **6a**.

8-[2-({3-[(2R)-2-methylpiperazinyl]-2-pyrazinyl}oxy)[U-^{14}C] ethoxy]-2-quinolinylamine([^{14}C]PNU-243922)

A solution of TFA (10 µl, 0.13 mmol) in dry acetonitrile (1.1 ml) was added dropwise into a cold (4°C) solution of **7** (7.43 MBq, 0.0136 mmol) in dry acetonitrile (1.1 ml) in about 5 min under nitrogen. The cold bath was removed and the reaction mixture was stirred under nitrogen at room temperature for about 1 h. After the disappearance of the precursor (determined by radio-HPLC), the solution was evaporated to dryness, methanol (1.5 ml) was added and the reaction mixture stirred at room temperature for three days. At the end of the reaction (determined by radio-HPLC) the solution was evaporated to dryness and the crude product was dissolved in the mobile phase A (2 ml) and purified by preparative HPLC. The fractions containing the pure product (checked by radio-HPLC) were combined and evaporated to small volume until the organic phase was removed. The aqueous phase was adjusted to pH 8.5 with 1 N NaOH and extracted with methylene chloride (3×20 ml). The organic phases were collected and washed with brine (2×10 ml). After drying over anhydrous sodium sulfate and solvent evaporation to dryness, [^{14}C]PNU-243922 (4.37 MBq) was obtained in 98% radiochemically pure form (by radio-HPLC, $R_t = 6.70$ min), with a specific activity of 546 MBq/mmol. The radiochemical yield of this step was about 60%.

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