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A Synthesis of Tritium-Labeled Olanzapine

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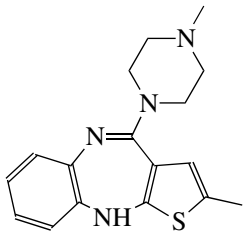
Abstract—A synthesis of olanzapine, 2-methyl-10-(4-methyl-1-piperazinyl)-4*H*-thieno[2,3-*b*][1,5]benzodiazepine, was carried out and the conditions for its tritium labeling were optimized to obtain a tritium-labeled olanzapine preparation with a specific radioactivity of 12 Ci/mmol.

Key words: olanzapine, synthesis, tritium

INTRODUCTION

Olanzapine, 2-methyl-10-(4-methyl-1-piperazinyl)-4*H*-thieno[2,3-*b*][1,5]benzodiazepine (**I**), is one of the most potent neuroleptics (antipsychotics) of a new generation. The characteristic features of these drugs are its efficiency in reducing both positive and negative symptoms of schizophrenia, lack of side effects incidental to the dysfunction of extrapyramidal system, and the mechanism of action related to their ability to bind to serotonin, dopamine, and other receptors [1, 2].



(**I**)

The goal of this study was the synthesis of tritium-labeled olanzapine. Unlabeled olanzapine (**I**) required for the preparation of the labeled compound was synthesized according to the modified procedure [3] and subsequently used for the optimization of the conditions for tritium incorporation into this compound.

RESULTS AND DISCUSSION

Olanzapine (**I**) was synthesized as proposed previously [3] starting from 2-amino-5-methylthiophene-3-carbonitrile (**II**) obtained by the Gewald reaction [4] (Scheme 1). While retaining the sequence of stages described in patent [3], we improved the procedures for isolation of intermediates and modified the ratios of reagents and reaction times.

Nitrile (**III**) was obtained by coupling (**II**) with *o*-fluoronitrobenzene in the presence of NaH in THF. Reduction of (**III**) with $SnCl_2$ in HCl and simultaneous cyclization led to 4-amino-2-methyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepine hydrochloride (**IV**). Its boiling with *N*-methylpiperazine in a DMSO–toluene mixture for 24 h gave the target olanzapine (**I**) in 65% yield. Physicochemical characteristics of (**I**) coincided with those described in patent [3]. More than 2 g of olanzapine (**I**) were obtained in this way, and it was used for the optimization of conditions for the incorporation of tritium label into olanzapine.

The labeled olanzapine with the retention of native structure of the unlabeled precursor can be obtained only by the method of isotope exchange. The exchange reaction is most often carried out using the liquid-phase procedure (*viz.*, the reaction between the solute and tritiated water) or a solid phase procedure (the reaction between gaseous tritium and the compound applied onto a catalyst surface) [5].

The generation of tritiated water directly in the reaction ampoule is considered to be the most efficient procedure for the isotope exchange with tritiated water. To this end, PdO, the starting compound, and a catalyst (usually, a palladium catalyst applied onto the surface of an inert support: Al_2O_3, activated charcoal, $BaSO_4$, $CaCO_3$, etc.) are placed in a reaction ampoule, which is then filled with gaseous tritium and kept at 70°C for 10–15 min. PdO is completely reduced under these conditions with the formation of tritiated water. The ampoule contents (the tritiated water formed, the compound, and the activated catalyst) are then frozen with liquid nitrogen, and the ampoule is evacuated for the removal of gaseous tritium and filled with argon. After the addition of a dioxane solution of triethylamine in the ampoule, it is sealed to prevent the influx of the tritium water vapor into the atmosphere and then kept under stirring either at room temperature (for 16 h in the case of olanzapine) or at an elevated temperature (100–170°C) in a thermostat.

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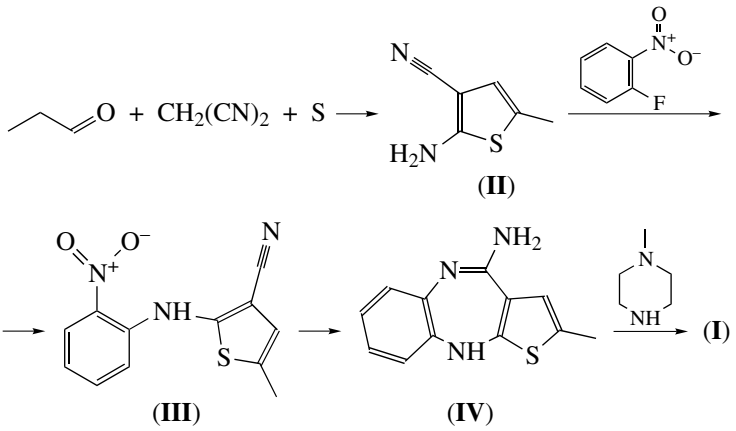

Scheme 1.

The principle of the solid phase procedure is that the mixture of the organic compound and the catalyst prepared as described above is heated in the atmosphere of gaseous tritium; the preparation procedures of these mixtures are given above.

The results of the experiments (table) showed that the exchange of protium by tritium between the gaseous tritium and olanzapine in the solid phase procedure was more efficient as compared to the labeling performed by the isotope exchange with tritiated water. In this case, the best result was achieved when the process was carried out at 200°C.

The labeled olanzapine was purified in two steps: first, the column chromatography on silica gel (Fig. 1) was used to obtain the target product with the radiochemical purity of 85% (Fig. 2); and, then, the radiochemical purity increased up to 98–99% by HPLC (Fig. 3). Thus, a highly tritium-labeled olanzapine with the specific radioactivity of 12 Ci/mmol and the radiochemical purity of 98–99% was prepared.

We are planning to apply the labeled olanzapine preparation for the further study of neurochemical mechanisms of schizophrenia and some other mental diseases. Moreover, the labeled olanzapine may be useful for the optimization of search for peptide preparations that have the pharmacological profile similar to that of neuroleptic (**I**).

EXPERIMENTAL

Catalysts, solvents, standards, and chemicals were commercial products. The melting points were measured on a Boetius heating microplate. ^{1}H NMR spectra were recorded using a Bruker VM-250 spectrometer (250.13 MHz), and mass spectra, on a Kratos MS instrument with direct inlet of sample into the ion source. TLC was carried out on Sorbfil plates (AO Sorbpolymer, Russia) in (A) 20 : 1 : 0.04 and (B) 20 : 1.5 : 0.1 chloroform–methanol–10 N ammonia mixtures.

HPLC of the labeled olanzapine was carried out on a packed with Kromasil 100C_{18}, 7 μm column (4 × 150 mm) at an elution rate of 1.0 ml min^{-1} in 1 : 3 methanol–ammonium phosphate buffer (pH 2.8); the olanzapine retention time (τ) was 5.13 min.

The acquisition and handling of chromatographic data were achieved using a MultiChrom system (OOO Ampersand, Russia) based on an IBM PC/AT. The radioactivity was measured on a scintillation counter with 30% efficiency of tritium registration in a dioxane scintillator. The conditions of tritium labeling were optimized using 1% tritium [6–8].

Chemical Synthesis of Olanzapine

2-Amino-5-methylthiophene-3-carbonitrile (II). Triethylamine (40 ml) was added to a mixture of sulfur (15 g, 0.47 mol) and propionic aldehyde (33 g, 0.56 mol) in DMF (95 ml) under vigorous stirring and

The dependence of olanzapine yield and specific radioactivity on the conditions of isotope-exchange reaction*

Temperature, °C	Yield, %	Relative specific radioactivity, %**
23	60	7
150	77	3
180	17	34
200	**10**	**100**

* The first row corresponds to the isotope exchange with tritiated water for 16 h in dioxane–triethylamine; the other rows, to the solid phase reaction: catalyst 5% Pd/$BaSO_4$, reaction time 10 min, the pressure of gaseous tritium 333 hPa.

** The maximal specific radioactivity in a given series of experiments was accepted as 100%.

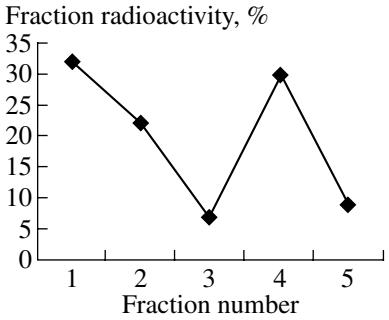

Fig. 1. Distribution of radioactivity in the fractions obtained by column chromatography on silica gel (the total radioactivity of five fraction was taken as 100%). A 3 : 2 chloroform–methanol system was used for elution; fraction volume, 4 ml.

cooling at such a rate so that the temperature of reaction mixture remained within the range of 5–10°C. The cooling was then stopped, the reaction mixture was stirred for about 1 h, and malonodinitrile (31.5 g, 0.47 mol) in DMF (60 ml) was added dropwise to the reaction mixture while keeping its temperature within the range of 18–20°C. The reaction mixture was stirred for additional 3 h and was poured into an ice–water mixture (800 ml), the water layer was discarded, and the residue was extracted with chloroform (3 × 200 ml). The organic layer was successively washed several times with water and with a saturated Na_2SO_4 solution, and dried with anhydrous Na_2SO_4. The removal of solvent and twofold coevaporation of the residue with dry toluene yielded 39 g (60%) of 2-amino-5-methylthiophene-3-carbonitrile (**II**) as a black-brown solid residue, which was used at the subsequent stage.

2-(2-Nitroanilino)-5-methylthiophene-3-carbonitrile (III). A solution of 2-fluoronitrobenzene (28.2 g, 0.2 mol) and 2-amino-5-methylthiophene-3-carbonitrile (**II**) (27.6 g, 0.2 mol) in dry THF (250 ml) was added dropwise under argon atmosphere to a vigorously stirred suspension of NaH (60% dispersion in oil, 14.5 g, 0.36 mol) in dry THF (50 ml). The reaction mixture was stirred at 25°C for 24 h, then poured into ice water (0.5 l), and extracted with chloroform (3 × 200 ml). The combined extracts were washed with 10% HCl and water, dried with anhydrous Na_2SO_4, and evaporated. The residue was twice recrystallized from isopropanol to yield 12.2 g (23%) of 2-(2-nitroanilino)-5-methylthiophene-3-carbonitrile (**III**); mp 97–100°C (lit. [3]: mp 99–102°C); ^{1}H NMR ($CDCl_3$, δ, ppm): 2.47 (3 H, s), 6.78 (1 H, s), 6.97 (1 H, t), 7.20 (1 H, d), 7.56 (1 H, t), 8.27 (1 H, d), 9.63 (1 H, br. s).

4-Amino-2-methyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepine hydrochloride (IV). A solution of tin chloride (13.6 g, 0.060 mol) in conc. HCl (45 ml) was added at 50°C for 10 min to a suspension of 2-(2-nitroanilino)-5-methylthiophene-3-carbonitrile (**III**) (5.0 g, 0.019 mol) in ethanol (55 ml). The mixture was refluxed under stirring for 1.5 h, evaporated to a half volume, and kept overnight in a refrigerator at +5°C. The precipitated solid was filtered, washed with a small volume of water, and recrystallized from ethanol to yield 6.9 g (65%) of 4-amino-2-methyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepine hydrochloride (**IV**); mp >250°C (lit. [3]: mp >250°C); ^{1}H NMR (DMSO-d_6,

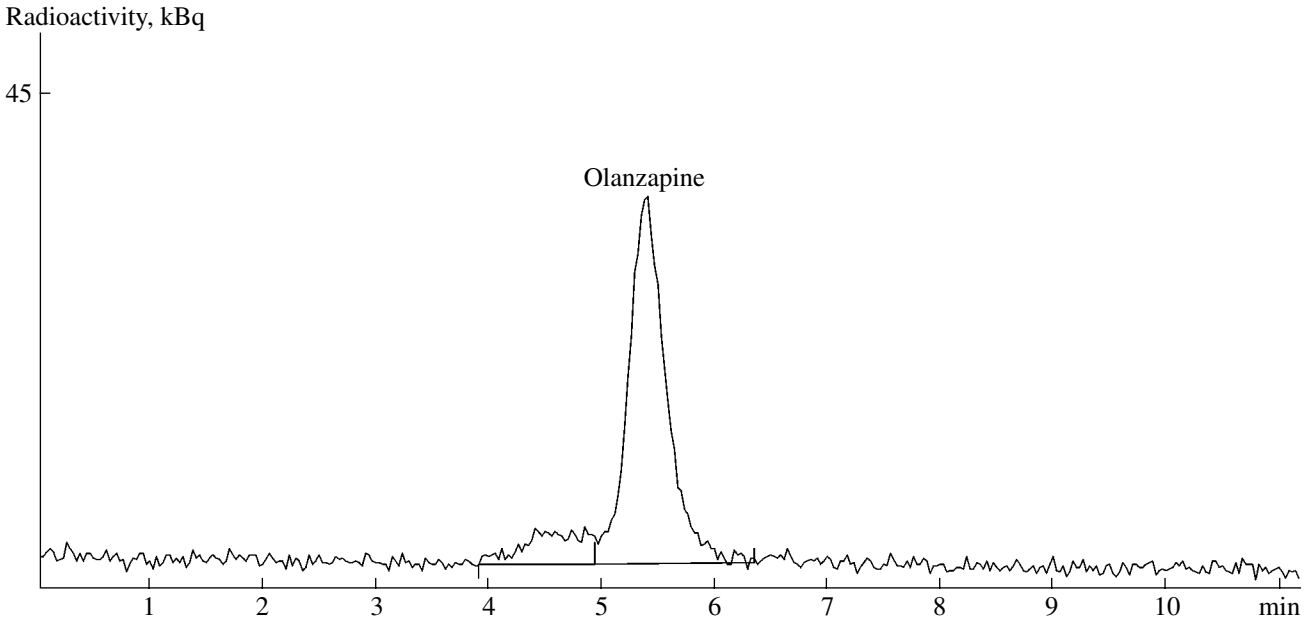

Fig. 2. Analytical HPLC of fraction 4 obtained by the chromatography on a silica gel column. Radioactivity profile of eluate.

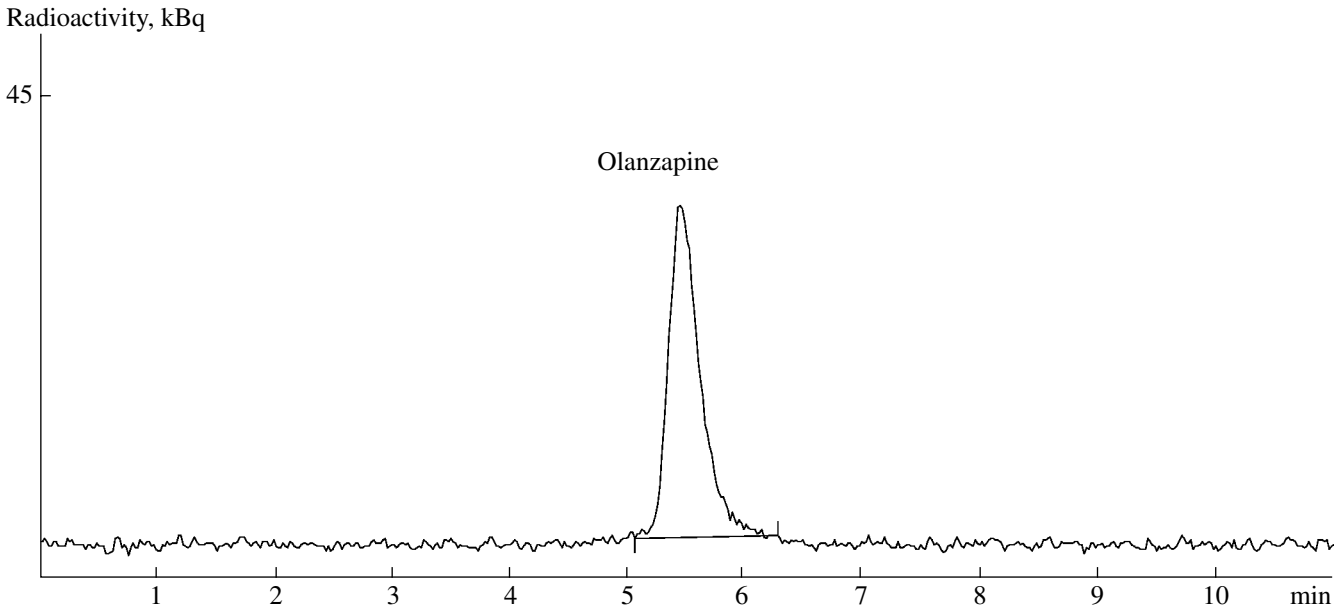

Fig. 3. The analysis of labeled olanzapine after purification by HPLC. Radioactivity profile of eluate.

δ, ppm): 2.24 (3 H, s), 6.80–7.15 (5 H, m), 8.90–9.15 (2 H, br. d), 9.58 (1 H, br. s), 11.27 (1 H, br. s).

2-Methyl-10-(4-methyl-1-piperazinyl)-4*H*-thieno-[2,3-*b*][1,5]benzodiazepine (I). A mixture of 4-amino-2-methyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepine hydrochloride (**IV**) (6.9 g, 0.012 mol), *N*-methylpiperazine (30 ml), DMSO (40 ml), and toluene (40 ml) was refluxed for 24 h, mixed with water (50 ml), and crystallized at +5°C. The crystals were filtered, recrystallized from acetonitrile, and dried in a vacuum at 60°C to yield 2.48 g (65%) of (**I**); mp 195–196°C (lit. [3]: mp 195°C); R_f 0.39 (A) and 0.90 (B); MS, *m*/*z* (*I*, %): 312 (26.3) $[M]^+$, 254 (9.4), 242 (100), 229 (68.4), 213 (53.7); ^{1}H NMR ($CDCl_3$, δ, ppm): 2.30 (3 H, s), 2.35 (3 H, s), 2.48 (4 H, m), 3.55 (4 H, m), 5.00 (1 H, br. s), 6.39 (1 H, s), 6.60 (1 H, d), 6.80-7.15 (3 H, m).

Preparation of Tritium-Labeled Olanzapine

(a) Olanzapine (**I**) (3 mg) applied onto 5% $Pd/BaSO_4$ (60 mg) was placed in a reaction ampoule, which was then evacuated to a residual pressure of 0.1 Pa, filled with gaseous tritium up to the pressure of 333 gPa and kept at 200°C for 10 min. Tritium excess was removed by evacuation. After cooling, the reaction products were extracted with methanol (6 × 1 ml), the catalyst was filtered, and the filtrates were evaporated. The residue was dissolved in methanol (3 ml) and evaporated once again. This procedure (removal of labile tritium) was repeated until the radioactivity of solution became constant. According to HPLC, the reaction mixture contained 0.6 mg (yield 20%) of labeled olanzapine with the radiochemical purity of approximately 6%.

A solution of the labeled olanzapine (**I**) preparation in 5 : 1 chloroform–methanol (0.5 ml) was applied onto a Silica gel L column (10 × 200 mm, 5 g, 100/250 μm; Chemapol, Czechia) successively eluted with chloroform (20 ml) and 10 : 1 (10 ml) and 7 : 3 (10 ml) chloroform–methanol mixtures. TLC (system A) indicated the absence of labeled olanzapine. The subsequent elution of the column with 3 : 2 chloroform–methanol (40 ml) and the collection of 4-ml fractions showed (TLC) that fraction 4 contained practically the whole labeled olanzapine (Fig. 1); yield of 0.45 mg (15%) and the radiochemical purity of 85% (Fig.2). This product was purified by preparative HPLC to give the labeled preparation in yield of 0.3 mg (10% from the initial unlabeled compound), the specific radioactivity of 12 Ci/mmol, and radiochemical purity of 98–99% (Fig. 3).

(b) The reaction was carried out as described in protocol (a) at 150°C for 15 min. The yield of labeled preparation reached 77%, its molar radioactivity was 0.3 Ci/mmol, and its radiochemical purity, 98–99%.

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