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Tritium labelling of several potent fluorinated antipsychotic drugs at high specific activity

David G. Ahern, Anne G. Laseter, Crist N. Filer*

PerkinElmer Life and Analytical Sciences, Inc., 549 Albany St., Boston, MA 02118, USA

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Abstract

Methods are presented to synthesize and characterize [methylene-^{3}H] haloperidol **2** and [*N*-methyl-^{3}H]trifluoperazine **6**.

Keywords: Antipsychotic; Haloperidol; Trifluoperazine; Tritium; Tritium NMR

1. Introduction

Psychotic episodes can be characterized by delusions, hallucinations, and disorganized speech patterns as well as by unusual behavior. A number of receptor systems have been implicated in this disabling mental illness, including the dopamine (Seeman, 2006), serotonin (Nakashima, 1999), sigma (Leonard, 2004; Mach et al., 2004), and others (Bolos, 2003). To target these binding sites and ameliorate psychotic symptoms, antipsychotic drugs have been carefully designed. Current efforts in this area have largely been focused on more selective drugs with reduced side effects (Silvestre and Prous, 2005). In advancing this important work, tritiated radioligands have been invaluable to elucidate the complex mechanism of these agents at the receptor level. We have had a long-term commitment to prepare tritiated radioligands in the neurochemical area and were called upon to label several fluorinated antipsychotic drugs with tritium.

2. Experimental

Evaporations were carried out on a Buchi rotary evaporator (model RE 111) at bath temperatures less than 40 °C. Analytical TLC was performed on Analtech plates coated with silica gel (250 μm). Autoradiography was performed at 0 °C after spraying with PPO and exposing the plates to X-ray film. TLC plates were also scanned for radioactivity (~370 kBq) using a Vanguard Autoscanner. Analytical HPLC was performed on a Waters instrument (model 510 pump) with peak detection done simultaneously by UV (280 nm, Waters 440 UV detector) and a IN/US Systems Beta RAM Model 3 radioactivity detector. Solution radioassays were conducted with a Beckman Model LS 3801 instrument. The tritium NMR spectra were recorded on a Bruker 300 MHz instrument with chemical shifts being reported as parts per million (ppm) downfield from internal TMS. The mass spectra were obtained on a Kratos Model MS25 RF instrument with direct injection. All chemicals used were of reagent grade.

2.1. [Methylene-^{3}H] haloperidol (2)

To a solution of 30 mg (0.08 mmol) of **1** (Sigma-RBI Cat. #H 1512) in 0.2 ml of dimethylformamide with 52 mg of 5% rhodium on alumina was added 3.7 TBq of tritiated water and the reaction was stirred overnight at 50 °C in the dark. After this time, labile tritium was removed by several vacuum evaporations of methanol and the reaction mixture was filtered free of catalyst to afford 56 GBq of crude product. A portion (3.7 GBq) of this was purified by preparative reverse phase HPLC eluted with ethanol:1% aqueous triethylammonium acetate; pH 4 (50:50) affording 1.85 GBq (an extrapolated 52% radiochemical yield based on **1**) of tritiated product **2**, which was >97%

*Corresponding author. Tel.: +1 617 350 9435; fax: +1 617 542 4330.
E-mail address: crist.filer@perkinelmer.com (C.N. Filer).

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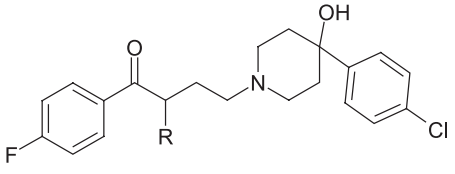
radiochemically pure and completely co-chromatographed with unlabelled **1** on HPLC (same system as above) and TLC (silica gel developed with chloroform:methanol:acetic acid (2:1:0.1)). The specific activity of product **2** was measured to be 0.67 TBq/mmol by radioassay with UV (ethanol:0.1 N HCl (9:1)) spectroscopy where $E_{247} =$ 11,900 for **1**. It also provided a proton decoupled tritium NMR (CD_3OD) with a multiplet at 3.20 ppm, showing exclusive tritium incorporation in the methylene group adjacent to the carbonyl of **2**. A mass spectrum of **2** showed a prominent MH^+ peak at 378 *m/e*.

2.2. *N-desmethyltrifluoperazine* (**5**)

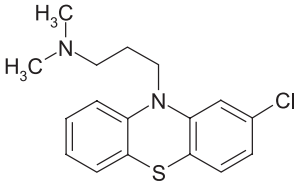
To a solution of 407 mg (1 mmol) of **4** in 10 ml of chloroform was added 132 mg (1.25 mmol) of cyanogen bromide in 5 ml of chloroform and the reaction was stirred for 2 h at ambient temperature. After this time, it was heated at 60 °C under nitrogen for 1 h and allowed to stand at room temperature overnight. The solution was then rotary evaporated to dryness and purified by preparative TLC on two 1000 μm silica gel plates developed with benzene:ethyl acetate (1:1). The major UV visualized bands were scraped and eluted with five 10 ml portions of ethanol affording 175 mg of the intermediate *N*-cyano adduct. This was dissolved in 4.5 ml of a water:acetic acid (1:2) solution with 775 mg of zinc dust and heated at 100 °C under nitrogen for 3 h. It was then cooled to room temperature and quenched with 30 ml of a 1% aqueous tartaric acid solution and filtered. The filtrate was extracted with five 15 ml portions of chloroform, dried over sodium sulfate, filtered and evaporated to yield 227 mg of crude product. It was purified on two 1000 μm silica gel plates developed with methanol:acetone:ammonium hydroxide (5:5:0.1). The major UV visualized bands were scraped, eluted with ethanol and rotary evaporated to afford 78 mg (20% yield) of **5** as a solid that was homogeneous on silica gel TLC (same system as above): ^{1}H NMR (DMSO-d_6): 7.40–6.90 (m, 7), 4.00 (t, 2), 3.50–3.00 (m, 10), 2.30 (m, 2) ppm; ^{13}C NMR (DMSO-d_6): 145.30, 143.62, 128.98, 128.55, 127.83, 127.48, 127.08, 123.01, 122.37, 118.70, 118.59, 116.25, 111.72, 55.12, 54.08, 45.36, 44.52 and 23.24 ppm.

2.3. *[N-methyl-^{3}H]trifluoperazine* (**6**)

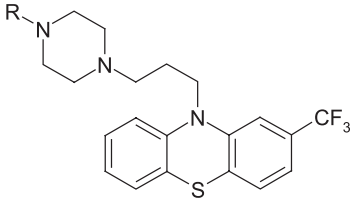
To a solution of 14.7 mg (0.038 mmol) of precursor **5** in 3 ml of methanol with 20 mg (0.24 mmol) of sodium carbonate was added 165 GBq of [^{3}H] methyl iodide (0.07 mmol at 2.3 TBq/mmol) and the reaction was heated at 60 °C in a sealed vessel for 1.5 h. After this time, labile tritium was removed by several vacuum evaporations of methanol. This crude product was purified by preparative TLC on two 500 μm silica gel plates eluted with acetone:-methanol:concentrated ammonium hydroxide (5:5:1). Authentic **4** was allowed to migrate at the side of each plate to facilitate UV visualization of the product. The visualized and scraped bands were eluted with seven 5 ml portions of ethanol, affording 22 GBq (a 25% radiochemical yield based on precursor **5**) of tritiated product **6**, which was >98% radiochemically pure and completely co-chromatographed with unlabelled **4** on reverse phase HPLC eluted with tetrahydrofuran:water:diethylamine (50:50:0.1)) and silica gel TLC (chloroform:methanol:acetic acid (3:1:0.1)). The specific activity of product **6** was measured to be 2.3 TBq/mmol by radioassay with UV (ethanol) spectroscopy where $E_{260} = 33{,}990$ for **4**. It also provided a proton decoupled tritium NMR (CD_3OD) as seen in Fig. 1 with a sharp singlet at 2.93 ppm, showing exclusive tritium incorporation in the *N*-methyl group of **6**.



1 R=H
2 R = ^{3}H



3



4 R = CH_3
5 R = H
6 R = C^3H_3

3. Results and discussion

Haloperidol is a member of the butyrophenone class of antipsychotics and one of the many pharmaceutically useful agents discovered by Janssen et al. (1959) at Janssen Pharmaceuticals. However, nothing in its synthesis path recommended an approach to labelling **1** with tritium. A single earlier report of its tritiation had appeared, but only achieving low specific activity and without any product characterization by HPLC or proof of labelling position (Muccino and Serico, 1978). Although a halogenation-catalytic tritium dehalogenation approach might have worked, another convenient strategy appeared more appealing and related to our approach to tritium labelling of colforsin and its analogues (Egan et al., 2004).

We discovered that simply heating **1** in the presence of a metal catalyst and tritiated water overnight was sufficient to routinely afford **2** in the specific activity range of