Note

Journal of Labelled Compounds and Radiopharmaceuticals

Received 13 August 2007, Accepted 7 October 2007 Published online 2 January 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1470

Synthesis of [^{3}H]fenobam, a radioligand for the mGlu5 receptor

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Fenobam is a clinically efficacious anxiolytic that acts as metabotropic glutamate receptor 5 (mGlu5) antagonist by binding to an allosteric site. Other mGlu5 receptor antagonists such as MPEP and MTEP bind to the same allosteric site and are efficacious in preclinical models of anxiety and depression. Consequently, the allosteric-binding site of the mGlu5 receptor is an attractive target for the discovery of novel psychiatric therapies. Radioligands of this binding site can be used for *in vitro* and *in vivo* pharmacodynamic studies. We report here a short synthesis of such a radioligand for the allosteric mGlu5 receptor-binding site, [^{3}H]fenobam.

Keywords: radioligand; fenobam; mGlu5; allosteric binding; tritium

Introduction

The G-protein-coupled metabotropic glutamate receptor 5 (mGlu5) is highly expressed in the limbic areas of the brain, which suggests a potential role of this receptor in psychiatric disorders, such as anxiety and depression.[1–6] Indeed, the selective mGlu5 antagonists MPEP and MTEP (Figure 1) have been shown to be active in a broad range of preclinical tests.[7] Recently, it was found that the clinically efficacious anxiolytic fenobam binds selectively and with high affinity ($K_i = 61$ nM) to the same allosteric site of the mGlu5 receptor as do MPEP and MTEP.[8–11] This finding makes fenobam an interesting pharmacological tool for preclinical studies, even if its clinical development was discontinued due to a highly variable PK profile in humans and unwanted side effects.[12] The recently published data about the identification of potent, metabolically stable fenobam analogs and their structure–activity relationship emphasized the current interest in the field of mGlu5 antagonists.[13–15] [^{14}C]Fenobam has been used for metabolism studies.[12] A preparation of [^{3}H]fenobam has not yet been described, although it might be a valuable tool for mGlu5 radioligand-binding assays or for mGlu5 distribution studies. We therefore devised a short synthesis of tritium-labeled fenobam, which is reported here.

Results and discussions

(1-Methyl-4-oxo-imidazolin-2-yl)-carbamic acid 2,5-dioxo-pyrrolidin-1-yl ester (3) as a precursor for [^{3}H]fenobam was obtained in analogy to the method of Takeda *et al.*[16] from creatinine and di(*N*-succinimidyl) carbonate (Scheme 1).

Precursor **3** was then reacted with commercially available 3-chloro-[2,6-^{3}H]aniline hydrochloride (4) according to Scheme 2. After high-performance liquid chromatography (HPLC) purification, [^{3}H]fenobam **5** was obtained in a satisfactory yield and high radiochemical purity.

Experimental

All reagents were of commercial quality unless otherwise stated. 3-Chloro-[2,6-^{3}H]aniline hydrochloride (23 Ci/mmol, 851 GBq/mmol) was prepared by the Tritium Custom Preparations Group, Amersham Biosciences, Cardiff, and was supplied as ethanolic solution.

Radioactivity measurements were carried out using a Wallac liquid scintillation counter 1414 WinSpectral™ instrument and Optiphase Hisafe 3 as scintillant. Analytical HPLC analyses were conducted using a Nucleosil® 100-5 C18 HD column (250 × 4 mm) with a mobile phase system composed of 30% acetonitrile in water (v/v) to 70% acetonitrile in water (v/v) over 10 min at 0.9 ml/min on an Agilent 1100 Series HPLC system equipped with quaternary pump, degasser, Rheodyne 7725i manual injector, temperature controller (30°C), multi-wavelength detector (250 nm), and a Raytest Ramona 2000 radioactivity flow detector with a solid scintillation cell (glass, 100 µl, 32–45 µm). Flash column chromatography was performed using Merck silica gel 0.04–0.063 mm. Thin-layer chromatography (TLC) was run on Merck Kieselgel silica gel 60 F_{254} glass plates. Radio-TLC was carried out on a Berthold TLC-Linear Analyzer Tracemaster 20 and on a Raytest Rita-Star instrument.

(*1-Methyl-4-oxo-imidazolin-2-yl*)-*carbamic acid 2,5-dioxo-pyrrolidin-1-yl ester*, ***3***: Under an atmosphere of nitrogen, a suspension

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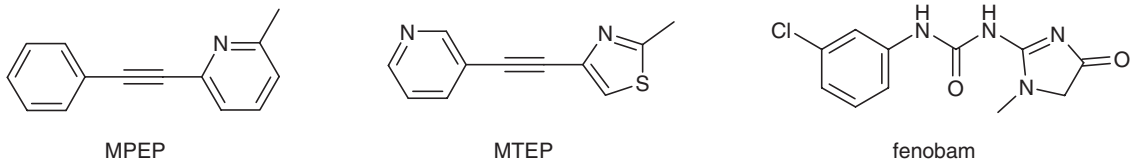
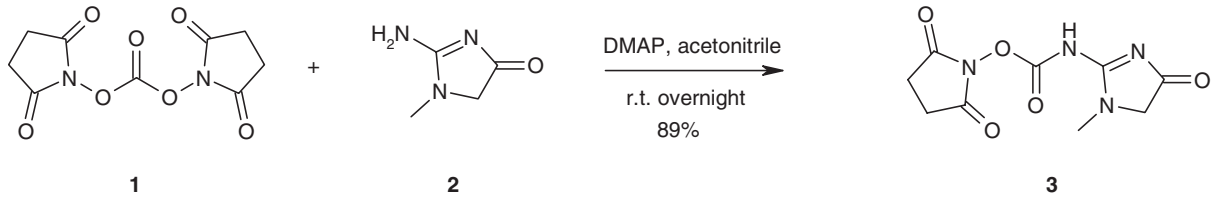
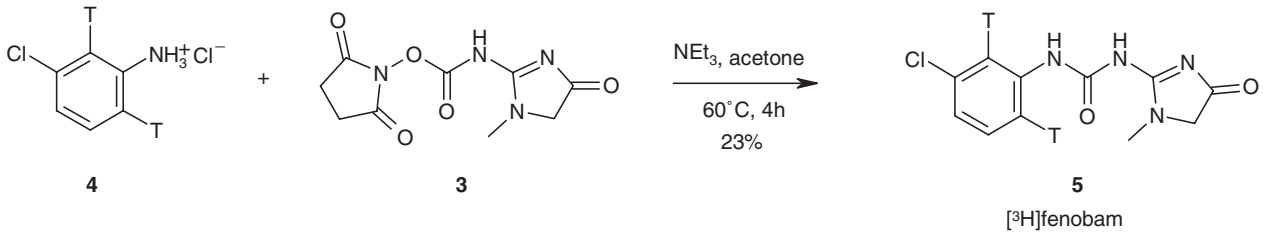


Figure 1. The anxiolytics MPEP, MTEP and fenobam bind to the same allosteric-binding site of the mGlu5 receptor.



Scheme 1



Schme 2

of creatinine (500 mg, 4 mmol) in acetonitrile (10 ml) was added slowly and dropwise over a period of 1.5 h to a solution of di(*N*-succinimidyl)-carbonate (2.26 g, 9 mmol) and DMAP (54 mg, 0.4 mmol) in acetonitrile (15 ml). The light brown reaction mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was suspended in dichloromethane, and stirred for 30 min. Filtration then provided the title compound (1.00 g, 89%). ^{1}H NMR (300 MHz, d^6 DMSO) δ 11.3 (1H, bs), 4.12 (2H, s), 2.98 (3H, s), 2.77 (4H, s).

1-(3-Chloro-[2,6-^{3}H]phenyl)-3-(1-methyl-4-oxo-2-imidazolin-2-yl) urea, [^{3}*H*]*fenobam*, ***5***: A solution of 150 mCi (5.55 GBq) of 3-chloro-[2,6-^{3}H]aniline hydrochloride (6.5 μmol, 1.08 mg) in 8 ml of ethanol was evaporated to dryness at 40°C under reduced pressure. After addition of 0.2 ml of toluene the solvent was removed again *in vacuo* to get rid of residual ethanol. The resulting brown residue was dissolved in 1.5 ml acetone and the solution was transferred into a heavy-wall pressure tube containing 5.2 mg (20.5 μmol) of (1-methyl-4-oxo-imidazolin-2-yl)-carbamic acid 2,5-dioxo-pyrrolidin-1-yl ester (3) and 3 μl (20.1 μmol) of triethylamine. The pressure tube was closed and the reaction mixture was heated at 60°C for 4 h. The product formation was confirmed by TLC analysis (silica, dichloromethane/methanol 95:5, $R_f = 0.56$). Subsequently, the reaction mixture was evaporated under reduced pressure and the remaining residue was purified by silica gel column chromatography with dichloromethane/methanol (98:2 to 97:3) as eluent. Radiochemically pure fractions were combined, the solvent was removed *in vacuo* and the resulting tritiated fenobam was dissolved in 25 ml of degassed *tert*-butyl methylether/toluene 1:1. Yield: 34 mCi (23%). Product specifications: radiochemical purity: > 99% (by analytical radio-HPLC analysis); specific activity (by MS): 23.5 Ci/mmol (869 GBq/mmol).

Conclusion

Tritium-labeled fenobam as a radioligand for the allosteric fenobam/MPEP-binding site of the mGlu5 receptor can be obtained easily from the commercially available tritiated precursor **4** and the carbamic acid ester reagent **3** in excellent radiochemical purity and sufficient high specific activity.

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