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Synthesis of the [^{3}H] labelled potent and selective peripheral benzodiazepine receptor ligand CB 34

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Summary

The synthesis of the [^{3}H] labelled peripheral benzodiazepine receptor (PBR) ligand CB 34, useful for binding assay, tissue distribution studies, and elucidation of the physiological role of PBR, is described. Catalytic reduction with $^3H_2$ gas and 10% Pd/C catalyst of the key intermediate **2** afforded the product at a specific activity of 111 Ci/mmol. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: PBR ligand; 2-phenyl-imidazo[1,2-*a*]pyridines; CB 34; tritiation; binding assay

Introduction

The peripheral benzodiazepine receptor (PBR) is present in most tissues such as adrenals, kidney and heart, as well as in the brain.[1–3] It is pharmacologically distinct from the central benzodiazepine receptor

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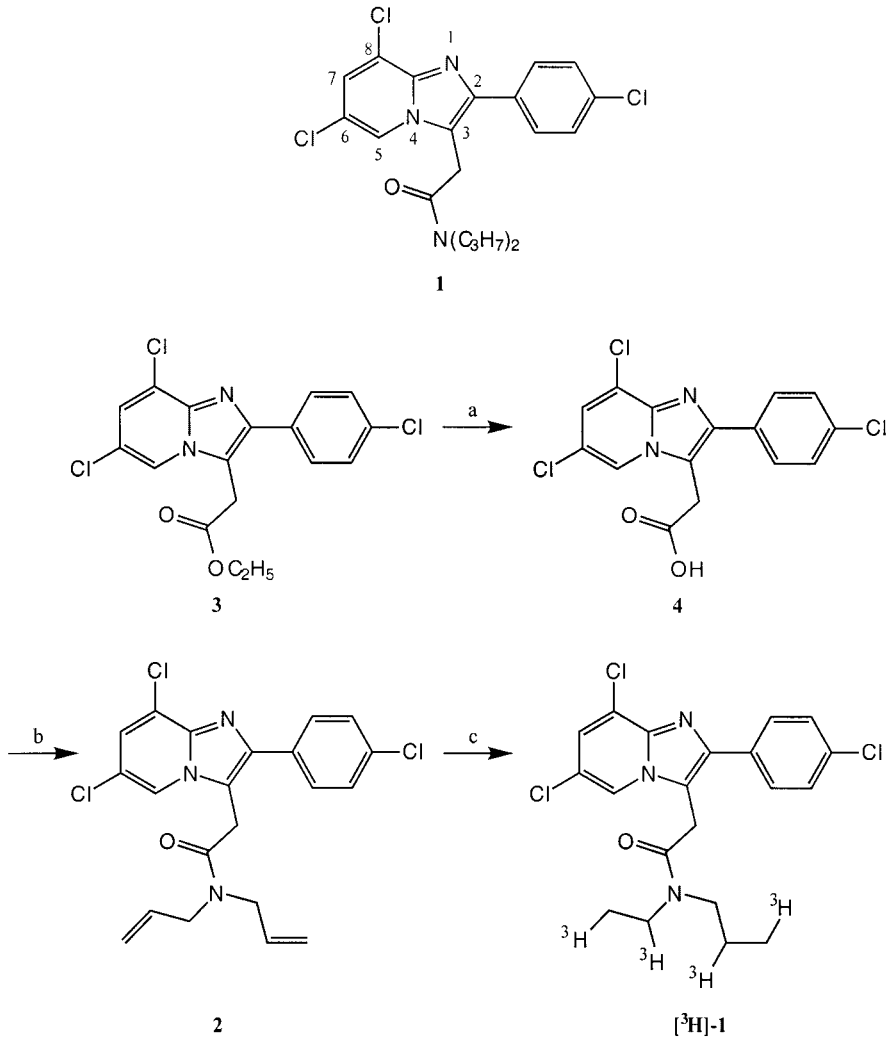
which is associated with $GABA_A$ receptors and mediates classical sedative, anxiolytic and anticonvulsant properties of benzodiazepines.[4] Conversely, the physiological role of PBR is still unclear, although several studies have shown that PBR may be involved in calcium homeostatis, lipid metabolism, mitochondrial oxidation, cell proliferation, and steroidogenesis.[5–7] Particularly interesting is the finding that PBR expression is selectively increased in human brain tumors.[8]

Selective ligands for PBR may be useful as tools for elucidating the physiological role of PBR and therapeutic agents, but only a few examples of such compounds are known. These include benzodiazepine compounds (Ro 5-4864),[9] isoquinoline carboxamides (PK 11195),[10] indoleacetamide derivatives (FGIN-1-27, FGIN-1-44),[11] phenoxyphenylacetamides (DAA1097 and DAA1106).[12] To examine the role of PBR, we studied the receptor binding and behavioral profiles of 2-phenylimidazo[1,2-*a*]pyridine derivatives as potent and selective ligands for peripheral benzodiazepine receptors.[13–15] Our binding data demonstrated that substitution at the C-8 position in the imidazopyridine compounds is a key factor for improving affinity and selectivity towards peripheral binding sites. In fact, members of the 8 and 6,8-disubstituted imidazopyridines show PBR selectivity $> 10^3$–10^5. Among these ligands, the 6,8-dichloro derivative (**1**, CB34) has been shown to be a particularly interesting compound because of its affinity, selectivity, and stimulation of the steroidogenesis.[15] In this paper we describe the synthesis of labelled CB34, a useful tool for radioligand binding assay, tissue distribution studies, and elucidation of the physiological role of PBR.

Results and Discussion

On the basis of a retrosynthetic analysis, the target compound [^{3}H]-**1** should be obtained *via* tritiation of the unsaturated diallylamide **2** (Scheme 1). This compound, in turn, can be derived by coupling the diallylamine with the carboxylic acid **4**. Therefore, the key intermediate of the synthesis is the diallylamide **2**, which was prepared in two steps starting from the ethyl ester **3**.[13] Hydrolysis of **3** with ethanolic NaOH gave the sodium salt of acid **4** from which this last compound was obtained, after the usual workup, in 85% yield. Amidification of the acid with diallylamine was attempted by using several condensing agents. We were able to obtain compound **2** in satisfactory yield (60%) by using carbonyl diimidazole (CODIm) as dehydrating agent.



Scheme 1. (a) NaOH, ethanol, RT, 4 h; (b) Diallylamine · HCl, CODIm, CH_2Cl_2, THF, RT; (c) $^3H_2$, ethyl acetate, 10% Pd/C RT

Compound **2** was obtained as a solid and its purity was checked by TLC and ^{1}H-NMR spectroscopy. To obtain the final labelled compound, the unsaturated amide **2** was tritiated by using $^3H_2$ in the presence of 10% Pd/C as catalyst.

Specific [^{3}H] CB34 binding to rat brain was characterized (Serra *et al.*, submitted); the data obtained indicated that [^{3}H] CB34 was rapid, reversible, saturable and of high affinity. Figure 1 shows that this binding exhibits discrete regional distribution in the rat central nervous system, being highest in the hypothalamus, whereas the cerebral cortex

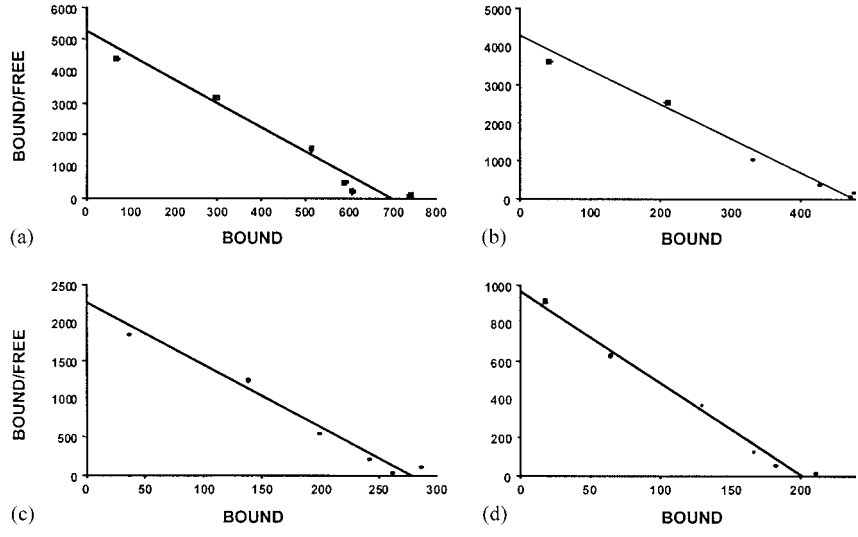


Figure 1. Representative plots of [^{3}H] CB34 binding to selected rat brain areas membranes. Membranes from hypothalamus (A), tuberculum olfactorium (B), hippocampus (C) and cerebral cortex (D) were incubated with [^{3}H] CB34 in concentrations from 0.05 to 16 nM for 120 min

showed the lowest amount of binding among those areas studied. Scatchard plot analysis indicate that the regional differences found in [^{3}H] CB34 binding are due primarily to variation in receptor density while the affinity of [^{3}H] CB34 for these recognition sites is approximately equal in all areas studied. Mean values of K_d and B_{max} are: hypothalamus 0.17 ± 0.02 nM and 700 ± 43 fmol/mg protein; tuberculum olfactorium 0.14 ± 0.03 nM and 524 ± 14 fmol/mg protein; hippocampus 0.19 ± 0.02 nM and 265 ± 12 fmol/mg protein; cerebral cortex 0.19 ± 0.02 nM and 188 ± 8 fmol/mg protein.

In summary, we have developed a short synthesis of [^{3}H] CB34 ([^{3}H]-**1**) starting from the ester **3** with the unsaturated amide **2** as key intermediate. Tritiation of **2** proceeds smoothly to provide the title compound. [^{3}H] CB34 ([^{3}H]-**1**) was used successfully to establish a radioligand binding assay at PBR.

Experimental

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. IR spectra were obtained on a

Perkin-Elmer 283 spectrophotometer (KBr pellets). ^{1}H NMR spectra were determined on a Varian or Bruker instrument, operating at 90 or 300 MHz, respectively. Chemical shifts are given in δ values downfield from Me_4Si as internal standard. Mass spectra were recorded on a Hewlett Packard 5995c GC-MS low-resolution spectrometer. All compounds showed appropriate IR, ^{1}H NMR and mass spectra. Elemental analyses were carried out with a Carlo Erba model 1106 analyser and results were within $\pm$ 0.40% of the theoretical values. Silica gel 60 (Merck 70–230 mesh) was used for column chromatography. HPLC analyses were performed under the following conditions: column: Beckman Ultrasphere C18 (250 $\times$ 10 mm); eluent: 0.1% trifluoroacetic acid in water: acetonitrile 1:1; flow rate: 3 ml/min; detection: 254 nM and radioactive; sample injection: 200 mCi in HPLC eluent (2 ml).

All the reagents were obtained from Aldrich, except for 10% Pd/C (from Fluka) and ethyl acetate (from Rathburn), and used as received.

*[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-a]pyridin-3-yl)] - acetic acid (**4**)*

To a solution of the ethyl ester **3**[13] (0.38 g, 1.0 mmol) in ethanol 95° (50 ml), an aqueous sodium hydroxide solution, freshly prepared by dissolving NaOH pellets (0.040 g, 1.0 mmol) in water (2 ml), was added dropwise. The mixture was stirred at room temperature and under a nitrogen atmosphere for 4 h. Then, the solvent was evaporated under reduced pressure, the resulting residue was dissolved in water (20 ml) and the aqueous solution washed with ethyl ether (6 $\times$ 30 ml). The water phase was cooled and acidified with dilute HCl. The resulting white solid was the essentially pure carboxylic acid **4**, which was collected by filtration. M.p. 260–262°C. IR (KBr) 3400–2500 broad absorption, 1695 cm^{-1}, ^{1}H-NMR (90 MHz) (DMSO d_6)δ: 3.90 (s, 2H, CH_2), 7.1–7.6 (m, 5H, Ar), 8.49 (s, 1H, Ar); MS *m/z* 310 (M^+-CO_2, base). Anal. ($C_{15}H_9Cl_3N_2O_2$) C, H, N.

*N,N-Di-allyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-a]pyridin-3-yl)]acetamide (**2**)*

To a suspension of the acid **4** (1.5 g, 4.2 mmol) in an anhydrous mixture of dichloromethane–tetrahydrofuran (3:1 v/v) (40 ml), maintained under a nitrogen atmosphere, carbonyl diimidazole (CODIm, 0.80 g, 4.9 mmol) was added in small portions. Then, after 15 min, diallylamine hydrochloride (0.73 g, 5.5 mmol) was added dropwise. The mixture was

stirred for about 2 h at room temperature, then filtered and the filtrate evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography [light petroleum ether/ethyl acetate 8/2 (v/v) as eluent)] to give the amide **2** (1.10 g, 60% yield). M.p. 179–181°C. IR(KBr) 1650 cm^{-1}; ^{1}H-NMR (300 MHz) ($CDCl_3$) δ: 3.81 (d, 2H, CH_2), 4.00 (d, 2H, CH_2), 4.04 (s, 2H, CH_2), 5.0–5.2 (m, 4H, $=CH_2$), 5.5–5.8 (m, 2H, =CH), 7.3–7.6 (m, 5H, Ar), 8.12 (s, 1H, Ar); MS *m/z* 433 (M^+, 12), 309 (base). Anal. ($C_{21}H_{18}Cl_3N_2O$) C, H, N.

N,N-Di-2, 3-[^{3}H]-n-propyl-[2-(4-chlorophenyl)-6,8-dichloroimidazo[1,2-a]pyridin-3-yl)]acetamide. (^{3}H-1), ([^{3}H] CB34)

Compound **2** (4 mg) was transferred into a tritiation flask with ethyl acetate (2 ml) and 10% Palladium on charcoal catalyst (15 mg). The mixture was stirred under tritium gas (10 Ci) for approximately 30 min when the uptake of gas had slowed. The solution was filtered and the labile activity was removed by rotary evaporation with methanol (3×5 ml). The residue was dissolved in methanol (45 ml). Yield: 1094 mCi. Approximately 200 mCi of the crude product was rotary evaporated to dryness, reconstituted in HPLC eluent and purified by HPLC. The fractions corresponding to the major UV and radioactive peaks were collected, pooled and rotary evaporated to dryness and dissolved in ethanol. The solution was rotary evaporated and redissolved in ethanol twice more and the solution was counted. Yield: 26 mCi.

A sample of the product was analysed by HPLC and mass spectrometry. The radiochemical purity was shown to be 99.3%, the active material co-eluting with the inactive reference. The specific activity was determined as 111 Ci/mmol by mass spectrometry. The spectrum of the radioactive was consistent with the mass spectrum of the inactive reference. [^{3}H] CB34 could be diluted to 1 mCi/ml with ethanol and dispensed.

Binding assays

Females Sprague–Dawley CD rats (Charles River, Como, Italy) was used. After killing, the brain areas were rapidly removed, dissected and stored at −80°C until assayed. The tissues were thawed and homogenized in 50 volumes of Dulbecco's phosphate-buffered saline (PBS, pH 7.4) at 4°C with a Polytron PT 10 (setting 5 for 20 s). The homogenate was centrifuged at 40 000 g for 30 min, and the resulting

pellet was resuspended in 50 volumes of PBS and recentrifuged. The new pellet was resuspended in 10 volumes of PBS and used for the assay. [^{3}H] CB34 binding was determined in a final volume of 1000 µl, comprising 100 µl of membranes (0.35–0.40 mg of protein), 100 µl of [^{3}H] CB34 (127 Ci mmol^{-1}; Amersham Pharmacia Biotech) at a final assay concentrations of 0.05–16 nM and 800 µl of PBS. The incubations were performed at 25°C for 120 min, initiated by addition of membranes and terminated by rapid filtration through glass-fibre strips (Whatman GF/B) presoaked with 0.3% polyethyleneimine in a Cell Harvester filtration manifold (Brandel). The filters were washed five times with 4 ml of ice-cold PBS, and filter-bound radioactivity was quantified by liquid scintillation spectrometry. Nonspecific binding was defined as binding in the presence of 10 µM unlabelled PK 1195. Specific binding was determined by subtracting nonspecific from total binding and was approximately 70–80% of total binding.

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