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Synthesis of a potential M_1 muscarinic agent [^{76}Br]bromocaramiphen

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Summary

[^{76}Br]bromocaramiphen was prepared from the iodo-analogue by a Cu^+ nucleophilic bromodeiodination exchange. The radiolabelling yield was 40-45%. The radiochemical and chemical purities assessed by radio-TLC and HPLC were 98%. The precursor, iodocaramiphen, was synthesized from commercially available 1-phenylcyclopentanecarboxylic acid with a 10% overall yield in a 5 step procedure. This synthesis includes the formation of 1-(p-nitrophenyl)-, 1-(p-aminophenyl)- and 1-(p-iodophenyl) cyclopentane carboxylic acid. *In vivo* studies in rats showed high uptake in brain. A 10% decrease was observed by coinjecting with the radiotracer a cold load of QNB, a non subtype selective muscarinic ligand. The metabolite study performed in the pons tissues indicated that there was still 92% of unchanged radiotracer 30 min p.i. After coinjection of dextrometorphan, a sigma ligand, a reduction of the radioactivity uptake by 20 to 27% was observed in the pons, the cortex, the striatum and the cerebellum. These data suggest that [^{76}Br]bromocaramiphen is not a potential probe for investigating the status of central M_1 muscarinic receptors because of its high lipophilicity (log $P_{7.4}$ = 2.4) and its affinity for sigma sites.

Key Words: caramiphen, bromocaramiphen, bromine-76, PET, muscarinic cholinergic receptors, sigma receptors

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Introduction

There is recently considerable interest in centrally active muscarinic agents due to their therapeutic and diagnostic potential in Alzheimer's disease (AD). The deterioration in cognitive function is believed to be linked to the loss of cholinergic activity in the cortex and hippocampus (1) due to the degeneration of cholinergic neurons.

Molecular biology studies have demonstrated at least five gene products termed m_1 to m_5 (2-4). Muscarinic acetylcholine receptors (m-AChR) are heterogeneous and have been characterized pharmacologically as M_1, M_2 and M_3 subtypes based on the affinity of selective antagonists. M_1 muscarinic receptors, located primarily in the cerebral cortex and hippocampus, have a high affinity for pirenzepine (5,6). M_2 receptors have high affinity for AF-DX116 and are found in lower amounts in the cerebral cortex, as well as in various midbrain and brainstem nuclei (7). M_3 receptors, located particularly in glandular and smooth muscle tissue, have high affinity for 4-diphenylacetoxy-N-methylpiperidine (4-DAMP), hexahydrosiladifenidol (HHSiD), and its p-fluoro derivative (8). A M_4 subtype has been partially characterized (9).

Non-invasive measurement of total muscarinic receptor density in the brain may be clinically useful but the interest would be even greater if the relative proportions of receptor subtype population could be assessed *in vivo*. PET and SPECT images of muscarinic cholinergic receptors have thus far only been acquired by the use of non-subtype selective radioligands which therefore reflect the total concentration of binding sites rather than the density of a particular subtype population. These radioligands are [^{11}C]scopolamine (10), [^{11}C]QNB (11), [^{11}C]benztropine (12), [^{11}C]benzetimide and [^{123}I]iododexetimide (13).

The ability of caramiphen (2-(diethylamino)ethyl 1-phenylcyclopentanecarboxylic acid) to penetrate the blood brain barrier and to antagonize responses mediated by muscarinic receptors *in vivo* suggest that derivatives of this compound could be useful for the study of muscarinic subtypes in the central nervous system. *In vitro* binding studies have demonstrated that caramiphen is a selective M_1 antagonist (14). Para-substituted analogues of caramiphen were synthesized and

evaluated for their ability to bind to the M_1 and M_2 subtypes of muscarinic receptors (15). Para-substituted compounds with electron withdrawing groups showed M_1 selectivity. We report here the synthesis of [^{76}Br]bromocaramiphen and the preliminary pharmacological studies to evaluate its potential for the PET mapping of M_1 m-AChR.

Materials and methods

General

The synthetic route used to prepare the precursor iodocaramiphen from commercially available 1-phenylcyclopentanecarboxylic acid (Aldrich) is described in scheme 1 and follows mainly the procedure already published (14). Nitration of the aromatic ring in the para-position using a nitric/sulfuric acid mixture (16) gave the nitro-derivative. Catalytic reduction using palladium on charcoal and hydrogen gas or hydrazine procedure (17) cleanly afforded the amine. Diazotation ($HCl/NaNO_2$) followed by iodination with KI gave the desired iodinated phenylcyclopentanecarboxylic acid. Formation of the corresponding acyl chloride ($SOCl_2$) and esterification with N,N-diethylethanolamine afforded iodocaramiphen. A coupling procedure with carbonyldiimidazol was also tried.

TLC were run on pre-coated plates of silicagel 60F254 (Merck). The compounds were visualized using a UV-lamp at 254 nm. Flash chromatography was run on silicagel 63-200 mesh (Merck) at 0.3 bar. HPLC were run on a C_{18} μ-Bondapak column (Millipore-Waters), flow-rate 1 ml/min.

NMR spectra were recorded on a Bruker AMX (300 MHz) using the deuteriated solvents ($CDCl_3$, d = 7.26 ppm or DMSO-d_6, d = 2.50 ppm) or TMS as internal standards for ^{1}H NMR and the solvents ($CDCl_3$, d = 77.0 ppm or DMSO, d = 39.5 ppm) as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm downfield from TMS. Mass spectra (MS, DCI/NH_4^+) were measured on a Nermag R10-10 apparatus; a ionization potential of 70 eV was used.

1-(p-nitrophenyl)cyclopentanecarboxylic acid **2**

^{1}H NMR (DMSO-d_6) : d : 12.62 (b, 1H, CO_2H) ; 8.19 (d, J = 9.0 Hz, 2H, Ar) ; 7.63 (d, J = 9.0 Hz, 2H, Ar) ; 2.60 (bd, $w_{1/2}$ = 15 Hz, 2H, cyclopentane ring) ; 1.89 (bd, $w_{1/2}$ = 15 Hz, 2H, cyclopentane ring) ; 1.70 (s, 4H, cyclopentane ring). ^{13}C NMR (DMSO-d_6) : d : 175.7 (C) ; 151.3 (C) ; 146.1 (C) ; 128.1 (CH) ; 123.3 (CH) ; 58.9 (C) ; 35.7 (CH_2) ; 23.3 (CH_2). MS : 253 [M + NH_4^+] ; 236 [M^+]. TLC : Rf = 0.7 (CH_2Cl_2/MeOH : 90/10). HPLC : Rt = 5.0 min (aq. CH_3COONH_4 0.1M / CH_3CN : 75/25).

1-(p-aminophenyl)cyclopentanecarboxylic acid **3**

^{1}H NMR (DMSO-d_6) : d : 7.01 (d, J = 9.0 Hz, 2H, Ar) ; 6.78 (b, $w_{1/2}$ = 15 Hz, 2H, NH_2) ; 6.52 (d, J = 9.0 Hz, 2H, Ar) ; 2.46 (bd, $w_{1/2}$ = 15 Hz, 2H, cyclopentane ring) ; 1.77 (bd, $w_{1/2}$ = 15 Hz, 2H, cyclopentane ring) ; 1.62 (s, 4H, cyclopentane ring). ^{13}C NMR (DMSO-d_6) : d : 177.4 (C) ; 147.1 (C) ; 130.8 (C) ; 127.3 (CH) ; 113.7 (CH) ; 57.7 (C) ; 35.7 (CH_2) ; 23.3 (CH_2). MS : 223 [M + NH_4^+] ; 206 [M^+]. TLC : Rf = 0.6 (CH_2Cl_2/MeOH: 90/10). HPLC : Rt = 3.9 min (aq. CH_3COONH_4 0.1M / CH_3CN : 75/25).

1-(p-iodophenyl)cyclopentanecarboxylic acid **4**

^{1}H NMR ($CDCl_3$/CD_3OD) : d : 7.62 (d, J = 9.0 Hz, 2H, Ar) ; 7.15 (d, J = 9.0 Hz, 2H, Ar) ; 2.58 (bd, $w_{1/2}$ = 15 Hz, 2H, cyclopentane ring) ; 1.90 (bd, $w_{1/2}$ = 15 Hz, 2H, cyclopentane ring) ; 1.74 (s, 4H, cyclopentane ring). ^{13}C NMR ($CDCl_3$/CD_3OD) : d : 177.7 (C) ; 143.3 (C) ; 137.0 (CH) ; 128.7 (CH) ; 91.5 (C) ; 58.4 (C) ; 35.9 (CH_2) ; 23.4 (CH_2). MS : 334 [M + NH_4^+]. TLC : Rf = 0.4 (CH_2Cl_2/MeOH : 90/10). HPLC : Rt = 14.0 min (aq. CH_3COONH_4 0.1M / CH_3CN : 75/25).

iodocaramiphen **5**

^{1}H NMR ($CDCl_3$) : d : 7.58 (d, J = 9.0 Hz, 2H, Ar) ; 7.07 (d, J = 9.0 Hz, 2H, Ar) ; 4.06 (t, J = 6.0 Hz, 2H, -$CO_2C\underline{H}_2CH_2NEt_2$) ; 2.58 (t, J = 6.0 Hz, 2H, -$CO_2CH_2C\underline{H}_2NEt_2$) ; 2.57 (b, $w_{1/2}$ = 15 Hz, 2H, cyclopentane ring) ; 2.42 (q, J = 7.0 Hz, 4H, -$NC\underline{H}_2CH_3$) ; 1.83 (bd, $w_{1/2}$ = 15 Hz, 2H, cyclopentane ring) ; 1.68 (s, 4H, cyclopentane ring) ; 0.93 (t, J = 7.0 Hz, 6H, -$NCH_2C\underline{H}_3$). ^{13}C NMR ($CDCl_3$) : d : 175.1 (C) ; 142.8 (C) ; 137.1 (CH) ; 128.1 (CH) ; 92.1 (C) ; 63.1 (CH_2) ; 58.6 (C) ; 50.7 (CH_2) ; 47.2 (CH_2) ; 35.8 (CH_2) ; 23.4 (CH_2) ; 11.7 (CH_3). MS : 416 [M + NH_4^+].

TLC: Rf = 0.2 (heptane/CH_2Cl_2 : 80/20). HPLC : Rt = 14.0 min (aq. CH_3COONH_4 0.1M / CH_3CN : 25/75).

Bromine-76 was produced by irradiation of natural arsenic with a beam of 30 MeV [^{3}He] ions (18). Briefly, after 15h cooling to allow the ^{75}Br ($t_{1/2}$ = 1.6 h) decay, the target was dissolved in sulfuric acid followed by oxidation with chromic acid. The radioactive bromine was carried over with a nitrogen stream and trapped in ammonia which was evaporated to dryness and reconstituted in water. Radioactivity measurements were made using a Capintec CRC-12 radioisotope calibrator.

Preparation of [^{76}Br]bromocaramiphen

[^{76}Br]bromocaramiphen was prepared from the iodinated analogue using a Cu^+ assisted nucleophilic substitution reaction (18,19). Iodocaramiphen (1 mg, 2.4 µmol), gentisic acid (0.66 mg, 4.2 µmol), ascorbic acid (0.66 mg, 3.7 µmol), citric acid (1 mg, 5.2 µmol) were dissolved in 50 µl 1M acetic acid. $CuSO_4.5H_2O$ (0.14 µmol) was dissolved in 10 µl 1M acetic acid and 370 MBq [^{76}Br]NH_4 were added and the reaction vial was hermetically sealed. The exchange between bromine and iodine atoms was performed at 165°C for 60 min in a dry oven (Reacti-Therm, Pierce). The vial was cooled to room temperature and the reaction mixture was diluted with 1 ml water. The [^{76}Br]bromocaramiphen was purified using a C18 Sep-Pak with 3x5 ml of water and eluted from the column with 3 ml of methanol. Purification and isolation of the radiotracer was carried out by HPLC with a mixture of acetonitrile and ammonium acetate 0.1M (75/25) as mobile phase and a flow rate of 1 ml/min. The effluent from the column was monitored with a UV detector (254 nm, Waters module 440) and an in-line Geiger-Muller radioactivity detector. The radioactivity peak corresponding to [^{76}Br]bromocaramiphen was collected and evaporated to dryness. The residue was dissolved in sterile normal saline and the radioactivity measured. Radiochemical and chemical purities were assessed by radio-TLC (CH_2Cl_2:MeOH, 95:5) and HPLC.

Lipophilicity

The lipophilicity of the radiotracer was estimated by the shake flask method using a mixture of octanol and NaH_2PO_4/Na_2HPO_4 0.15 M (pH=7.4). [^{76}Br]bromocaramiphen (1.8 MBq) was shaken with a Vortex for 1 min in a test tube containing 1 ml of octanol and 1 ml of the phosphate mixture. After centrifugation, 100 µl of each phase was taken off and the radioactivity of each sample measured in a well γ-counter. A 600 µl aliquot of the octanol phase was transferred to another test tube containing 400 µl of octanol and 1 ml of the phosphate solution and was further shaken and centrifuged. This procedure was repeated 7 times. The radioactivity ratio of organic and aqueous phase (P) were calculated and the partition coefficient (log $P_{7.4}$) determined.

In vivo studies

Wistar male rats (180-200g) were injected in the tail vein with 0.6 MBq of [^{76}Br]bromocaramiphen and sacrificed at 10, 30, 60, 120, 240 min p.i. Brain, liver, lungs, heart, kidney, muscle and blood samples were removed. The brain structures (cerebellum, hypothalamus, diencephalon, hippocampus, striatum, pons, frontal cortex and occipital cortex) were dissected (20) and weighed. Radioactivity of aliquot samples were measured and tissue radioactivity concentrations were expressed as percent of injected dose per gram of wet tissue (% ID/g).

The specificity of the in vivo brain uptake of [^{76}Br]bromocaramiphen was investigated in competition experiments by coinjecting with the labelled compound 5 mg/kg of QNB, a non selective subtype muscarinic ligand, and 10 mg/kg of dextrometorphan, a sigma ligand. The rats were sacrificed 1 h after iv administration of the radioligand. The brain structures were recovered and their radioactivity measured.

Metabolite study

The determination of unchanged [^{76}Br]bromocaramiphen in plasma and brain after 30 min p.i. was performed by HPLC analysis after elimination of protein by acetonitrile (21). Aliquots of plasma

(200 µl) and pons (100 mg) were added to acetonitrile (1 ml), exposed for 30 sec to an ultrasonic probe designed for cell disruption (Vibra-Cells, Sonics & Materials INC) and centrifuged. The radioactivity of the precipitates was measured to quantify the acetonitrile extraction efficiency. The supernatants were evaporated to dryness and the residues, dissolved in 20 µl of the mobile phase, were injected in a µ-Bondapack C18 column (300 mm x 7.8 mm). Radiolabelled compound was eluted with CH_3CN:ammonium acetate 0.1M buffer, 90:10 (flow rate 4 ml/min) from the column and monitored with a UV detector (254 nm, Shimadzu SPD-10A) and an in-line HPLC radioactivity monitor (Berthold LB506 C-1).

Results and discussion

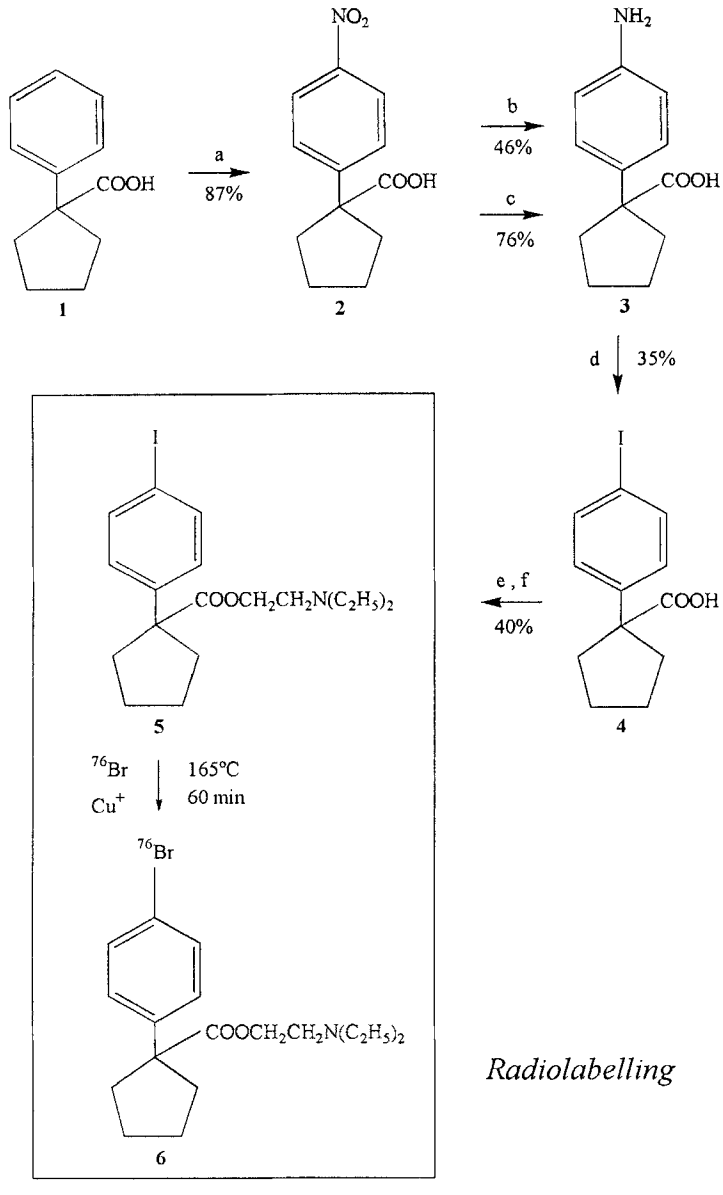
Precursor chemistry

Scheme 1 describes the 5 chemical steps and the corresponding reaction yields for preparing the iodocaramiphen precursor. The carbonyldiimidazol coupling is not described because of the very low yield (3%) obtained with this procedure. The total overall yield of iodocaramiphen was about 10%.

The NMR data was in agreement with the proposed structures and is detailed in the Materials and Methods section.

Radiolabelling

[^{76}Br]bromocaramiphen was prepared from iodocaramiphen by a Cu^+ nucleophilic bromodeiodination reaction (scheme 1): iodine is able to coordinate with copper that initiates halogen exchange (22). After 1 h at 165°C, the radiolabelling yield, measured by TLC, was 40-45 %. For the purification of the radiotracer, the unreacted [^{76}Br]bromide, the radiolabelled by-products and the excess of unreacted organic acids were eliminated using a solid phase extraction. By using an analytical reverse phase column for HPLC purification, [^{76}Br]bromocaramiphen (Rt =



Reagents and conditions: (a) HNO_3/H_2SO_4 1/1, 0°C, 2 h then RT, 1 h; (b) NH_2NH_2 (excess), Pd/C (10%), EtOH, 80-100°C, 24h; (c) H_2 (1 atm), Pd/C (10%), EtOH, RT, 24 h; (d) $NaNO_2$/HCl, H_2O, 0°C, 3 h then KI, RT, 16 h; (e) $SOCl_2$ (excess), toluene, 110°C, 1 h; (f) $HOCH_2CH_2NEt_2$, toluene, 110°C, 2 h.

Scheme 1

12 min) eluted before the precursor (Rt = 13.5 min) avoiding any risk of contamination of the radiopharmaceutical with the iodo compound. The total synthesis time including [^{76}Br]bromide preparation was 3 to 3.5 h. The radiochemical and chemical purities checked by TLC were 98%. The lipophilicity of the radiotracer was log $P_{7.4}$ = 2.4.

Pharmacological evaluation

In the cortex, where muscarinic M_1 receptors are abundant, the uptake of radioactivity was 1.1% ID/g 10 min after injection and decreased to 0.4% ID/g after 120 min. In the other brain structures studied, the radioactive concentrations are not significantly different from that in the cortex. Significant uptakes in lung and liver (1% ID/g) were observed 30 min post injection. At this time, there was only 0.10% ID/g in plasma and 0.26% ID/g in the heart.

Competition studies (figure 1) were carried out by coinjecting a cold load of QNB and dextrometorphan. With QNB, the uptake in the pons, the striatum and the cortex were reduced by 7%, 11% and 9% respectively. With dextrometorphan, a reduction of the radioactivity by 20 to 27% appeared in these structures and in the cerebellum. The remaining non displaceable activity could be attributed to non specific binding.

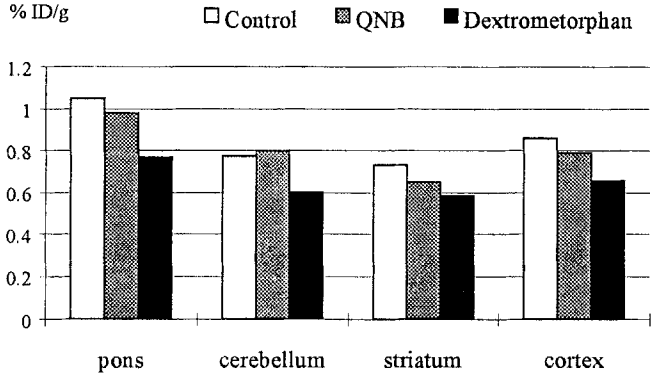


Figure 1: competition studies

In the metabolite study, the radioactive peak corresponding to [^{76}Br]bromocaramiphen has a retention time of 11.6 min. 30 min p.i., 92% and 40% of the radioactivity represented unchanged [^{76}Br]bromocaramiphen in the pons and the plasma, respectively.

Conclusion

[^{76}Br]bromocaramiphen was prepared by copper assisted bromodeiodination of iodocaramiphen with no carrier added [^{76}Br]NH_4. The radiolabelling and purification resulted in radiochemical and chemical pure products. The pharmacological evaluation of this radiotracer indicates that [^{76}Br]bromocaramiphen, due to its rapid kinetic, its high lipophilicity and its affinity for sigma sites in the brain, has a limited potential to be used as an *in vivo* muscarinic ligand.

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