Radiosynthesis of a New Radiobrominated Ligand for $5HT_{2A}$ Receptors, a Potential Tracer for PET.

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SUMMARY.

4-Amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-2-methoxybenzamide, a compound with high affinity for $5HT_2$-receptors, was radiobrominated in the 5-position of the methoxybenzamide group by electrophilic substitution. Hydrogen peroxide / acetic acid, peracetic acid and a mixture of both were tried as oxidants. Radiobromination with the hydrogen peroxide method gave a labelling yield of 80 % in the 5-position and 20 % in the 3-position without any side products. On the other hand the labelling methods based on the use of peracetic acid gave a more selective radiobromination in the 5-position, but with low yields and moreover generated radioactive and non-radioactive side products. N.C.A. radiobromide of high radio-chemical purity was obtained by an ion-exchange procedure on the target material solution coupled to a purification step using methanol.

Keywords : electrophilic radiobromination, ^{77}Br-4-amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-5-bromo-2-methoxybenzamide, $5HT_2$ receptor ligand.

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INTRODUCTION.

Recently radioiodo-4-amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-5-iodo-2-methoxybenzamide was described as a radioligand showing high affinity and selectivity for $5HT_{2A}$ receptors (1,2). The ^{123}I labelled version is presently being used in clinical tests on healthy volunteers. The non-radioactive 5-bromo-2-methoxybenzamide analogue showed a pIC_{50} for the inhibition of [^{3}H]ketanserin binding to rat frontal cortex of 9.2 , which is similar to the values obtained for the parent compound and the 5-iodo-2-methoxybenzamide analogue (1). Therefore it was decided to develop the radiobrominated version as a potential $5HT_{2A}$ receptor tracer for PET. The longer lived ^{77}Br was chosen as isotope for the development work.

MATERIALS and METHODS.

Reagents:

4-Amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-2-methoxybenzamide.$2H_20$ **1**, 4-amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-5-bromo-2-methoxybenzamide **2** are original products of the Janssen Research Foundation (Beerse, Belgium) checked for identification and purity by routine control such as NMR and MS. Dowex AG1X8 (200-400 mesh) was purchased from Biorad. The other reagents used were p.a. grade (Merck) or HPLC grade (Lichrosolv quality Merck).

HPLC Equipment :

Analytical : The equipment consisted of a Rheodyne injector (50µl loop), a Hitachi 655A pump and L-6000 II controller, a 655A variable wavelength UV monitor at 275 nm, a NaI(Tl) detector (Harshaw QS) and appropiate electronics (Canberra), a D2000 Chromato integrator Hitachi and an Ankersmidt R40 one-channel recorder. Quality control was achieved on a Lichrospher 125x4 mm RP 8 (5µ) Merck column with a methanol/acetonitrile/water // trimethylamine/ acetic

acid (MeOH/ACN/H_2O // TMA/HOAc) : 19/26/55 // 1.5/2 (v/v) mixture as eluent at a flow rate of 1 ml/minute.

Semi-preparative : The equipment consisted of a rheodyne injector (2.5 ml loop), a Waters M6000A pump provided with a semi-preparative pumphead, a Waters Lambdamax UV-480 monitor at 275 nm, a NaI(Tl) detector (Harshaw QS) connected to Ortec electronics, a HP 3580 and a Intersmat ICR-18 integrator. A MeOH/ACN/H_2O//TMA/HOAc : 16/22/62//1,5/2 v/v mixture of pH = 4.8 was used as eluent at a flow rate of 6 ml/minute on a Lichrocart 250x10 mm Lichrosorb RP Select B (10μ) Merck column.

Production of ^{77}Br.

Bromine-77 was produced with a 560 CGR-MeV cyclotron by bombardment of a 400 mg As_2O_3 pellet (pressed at 5 tons in stainless steel target holders with shallow circular recess, depth 0.3 mm) covered with a 6μ Al foil and taking advantage of the ^{75}As (α,2n) ^{77}Br reaction (3).

With a medium energy accelerator a good yield of ^{77}Br with minimal ^{76}Br contamination is obtained by bombarding thick As_2O_3 targets during 3 hours with 30 MeV α-beams of 6μA. This yields about 170 Mbq EOB on target. The target is left for 12 hours allowing short lived contaminants, e.g. ^{18}F, to decay. Production of useful amounts of ^{76}Br needs higher energy. Irradiation with a 41 MeV α-beam (energy limit of the cyclotron) of 65μ thick targets results in a 10 % ^{77}Br contamination at EOB. For the production of ^{75}Br from a ^{75}As(α,4n) reaction at least 50 MeV α-particles are necessary.

Recovery of ^{77}Br.

The As_2O_3 is for the larger part dissolved (few particles remain in suspension) in 10 ml of boiling twice-distilled water within 10 minutes. After cooling the solution is filtered through a 0.22μ filter to remove the solid particles (no significant loss of radioactivity is observed) and the solution adjusted to pH 7.6 with dilute NaOH solution. Next the solution is passed through a small custom made anion-exchange column. The column consists of 30 mg Dowex AG1X8

(200 - 400 mesh OH^- form) kept between two small layers of a p.a. grade white sand, the underlayer being supported by glass-wool, in a 1 ml (∅ 4 mm) syringe. The column was successively rinsed with 3 ml of 2N NaOH and 10 ml of twice-distilled H_2O (pH needs to be neutral). The n.c.a. ^{77}Br is eluted with 600µl of 1M $NaHSO_4$ at a flow rate of 0.07 ml/minute. After neutralisation with 2N NaOH (~ 16µl / 100µl $NaHSO_4$) 1.5 ml of deep-freezer cooled MeOH was added and a suspension of Na_2SO_4 formed. After filtration on a Millipore (25 mm filter) filtration system connected to a protected vacuum line, the solution was evaporated under vacuum at room temperature. 200µl of twice-distilled H_20 was added to the residue to dissolve the n.c.a. ^{77}Br (pH of the solution 5-7). The recovery yield was > 80 %.

Quality Control of ^{77}Br.

The radiochemical purity was checked by both HPLC and thin layer chromatography (TLC). The HPLC analysis was performed using a Polyspher ICAN2 column (Merck) with 4mM salicylate pH 7.8 as eluent at a flow rate of 1.3 ml/minute and U.V. detection at 254 nm or using a RP18 Supersphere Lichrosorb column (Merck) with ACN / [4mM octylamine, 2mM NaCl adjusted to pH 7 with H_3PO_4] : 5 / 95 (v/v) as eluent at a flow rate of 1 ml/minute and U.V. detection at 222 nm.

Only 1 radioactive peak was detected with a retention time corresponding to that of the non-radioactive Br^- peak detected by U.V.

The TLC was performed as described by F. Lambert (4). Here also only 1 radioactive spot was observed coinciding with the non-radioactive Br^- spot.

Recovery experiments with ^{76}As-As_2O_3 have shown that > 99.9 % of the amount of arsenic is eluted in the initial and NaOH fractions and that less than 10^{-3} % is present in the $NaHSO_4$ fraction.

The radionuclidic purity of the ^{77}Br was checked by means of Ge spectrometry.

The specific activity was measured after labelling of the $5HT_2$ ligand by means of highly sensitive U.V. spectrometry coupled to the semi-preparative HPLC set-up.

N.C.A. ^{77}Br-4-amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-5-bromo-2-methoxybenzamide 3 .

0.7 mg 4-amino-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-2-methoxybenzamide.2H_2O 1 was dissolved in 500µl of glacial acetic acid. 50µl of the radiobromide solution was added while stirring followed by the addition of 100µl of 30 % hydrogen peroxide solution. The reaction was allowed to proceed for 15 minutes at room temperature. The reaction vial was transferred to a small ice-bath. To the reaction mixture 1.8 ml of 1M Na_2SO_3 was added while stirring and the pH was brought to 8 by addition of 2N NaOH. The obtained solution was passed through a Baker Bond Octadecyl 100 mg column. The column was consecutively rinsed with 5 ml of a diluted NaOH solution (pH 7.5) and 5 ml of H_20. The non-radioactive compound 1 and radioactive tracer were recovered in 500µl of MeOH. 1.3 ml of semi-preparative HPLC eluent was added and the complete mixture was injected for semi-preparative HPLC separation (Fig. 1).

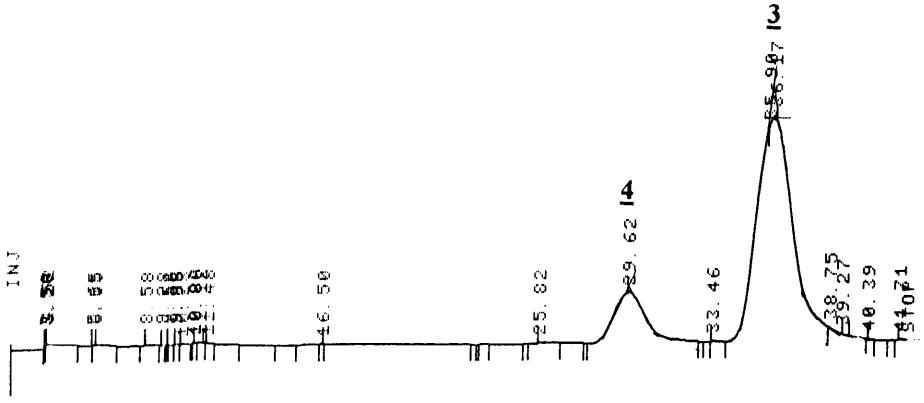

Fig. 1. RA chromatogram of the semi-preparative HPLC separation.

The pure radioactive tracer was recovered in about 25 ml of eluent, approximately 25 ml of H_20 were added and the solution brought to pH 11 with 2N NaOH. Preconcentration was performed on a Baker Bond Octadecyl column as described above. After blowing the column apparently dry, the radioactive tracer was recovered in 500µl EtOH. 5 ml of 9 ‰ NaCl was added and the mixture was filtered through a Millex GV-filter of 0.22µ into a vacuum ^{99m}Tc eluate collecting vial.

RESULTS AND DISCUSSION.

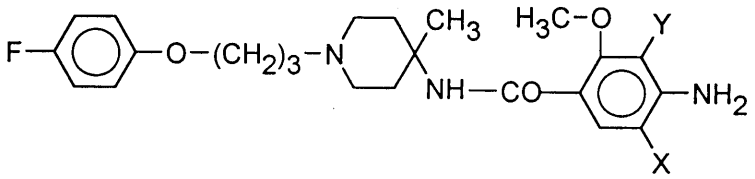

Fig. 2. Structure of 4-amino-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-2-methoxybenzamide

X	Y	Compound
H	H	1
Br	H	2
^{77}Br	H	3
H	^{77}Br	4

Choice of the labelling method.

In the 4-amino-2-methoxybenzamide group the 3-position is more activated than the 5-position for direct electrophilic substitution but this position is also hindered by the freely rotating methoxy at the 2-position (Fig. 2.). This is reflected in the kinetics as shown in figure 3. After 15 minutes the radiobromide is quantitatively consumed for substitution. HPLC shows 80 - 85 % of compound 3 and 20 - 15 % of compound 4.

The overall radiochemical yield of compound 3 obtained with the proposed method varied from 75 to 80 %. Analytical and semi-preparative HPLC control of the final product revealed a purity of at least 99 % and no starting or any other non-radioactive product could be detected (t_R compound 1 : 13 minutes, t_R compound 3 : 37 minutes) .

When using peracetic acid as oxidizing agent, the complex $(CH_3COOBrH)^+$ is thought to be the attacking electrophile (5) and direct the substitution to the 5-position due to the steric hinderance of the 3-position. When applying the peracetic

acid method, 88 % of the ^{77}Br is consumed. 29 % is substituted in the 5-position, 2 % in the 3-position, but 47 % of more lipophilic radiobrominated side products are produced. These compounds are probably nitro analogues resulting from oxidation of the amino-function. 10 % of the radioactive side products are less lipophilic than the required compound and can be radiobrominated benzoic acid analogues caused by degradation of the amide function.

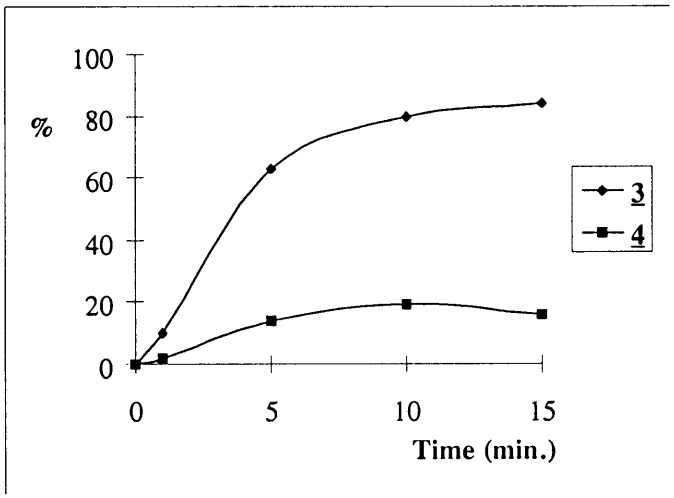

Fig. 3. Labelling yield as function of time.

To perform the labelling in less agressive conditions the peracetic radiobrominated electrophile was first generated in a separate vial and added to an excess of acetic acid containing the substrate. In these conditions only compound **3** was found, but the labelling yield reached not more than 23 %. Addition of supplementary H_2O_2 did not increase the labelling yield considerably (30 % in the 5-position, 0 % in the 3-position). Therefore the easy hydrogen peroxide method was proposed as the method of choice.

Specific activity of the radioligand.

As the anion-exchange resin is originally in the Cl^- form non-radioactive Br^-, for which the resin shows a higher affinity, can be present as contaminant on the column. The amount of Br^- present in 30 mg of resin (purchased in the OH^- form)

and determined by neutron activation using NH_4Br as reference, was estimated at no higher than 4.6 10^{-14}mol, which corresponds to 10 % and 1 % respectively of the theoretical specific activity of $^{75}Br^-$ and $^{76}Br^-$, the bromine isotopes suitable for PET.

In the case of $^{77}Br^-$, used by us for development of the labelling method, the specific activity of the radiobrominated ligand estimated by U.V. spectrometry corresponds to the theoretical activity (5.6 10^4 Ci/mmol) allowing us to obtain the tracer under n.c.a. conditions.

CONCLUSION.

As HPLC is already required for purification and recovery of the n.c.a. radiobrominated ligand direct electrophilic substitution using H_2O_2 / HOAc was proven to be a suitable method to obtain the tracer substituted on the required 5-position of the benzamide group with an overall yield of about 75 - 80 %. The radiobromide used for labelling was obtained with high purity and in n.c.a. conditions applying ion-exchange recovery followed by a final purification step in cooled methanol.

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