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LABELING OF A SEROTONINERGIC LIGAND WITH ^{18}F : [^{18}F] SETOPERONE

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SUMMARY

Setoperone has been labeled with ^{18}F to visualize the serotoninergic receptors by positron emission tomography. The synthesis is carried out by a nucleophilic substitution from a nitro derivative of setoperone with $^{18}F^-$. The synthesis of nitro-derivative is described as well as the different trials of recovery of ion $^{18}F^-$ from neon or aqueous target. The considered technique of labeling allow to obtain from 7.4 GBq (200 mCi) ^{18}F, 1.5 to 2 GBq (40-50 mCi) of [^{18}F]setoperone, 110 minutes after the end of bombardment. The specific activity obtained from this quantity of radioactivity is about 37 GBq/μMol (1 Ci/μMol).

Key words : Nucleophilic substitution reaction, [^{18}F]setoperone.

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INTRODUCTION

Positron emission tomography is a method used to visualize receptor sites in vivo in humans by injection of a ligand labeled with a $\beta+$ emitter. Ligand-receptor interactions have already been investigated on dopamine, benzodiazepine, acetylcholine and opiate receptors for example.

Serotoninergic sites (central 5 HT_2 receptor sites) have also been studied after injection of [^{11}C]ketanserine (1, 2). Unfortunately, however, this ligand has not proved selective enough for these sites and has a strong non-specific binding, so a more selective ligand with weaker non-specific binding had to be found. Recently another piperidine derivative, setoperone (3) : 6-[2-[4-(4-fluorobenzoyl)-1-piperidinyl] ethyl]-2,3-dihydro-7-methyl-5H-thiazolo [3,2-a] pyrimidin-5-one), was reported to have more potent serotoninergic properties than ketanserin with moderate dopamine antagonist properties. For this reason the labeling of this molecule with fluorine-18, a $\beta+$ emitter of half-life 110 minutes, was undertaken.

MATERIAL AND METHODS

Fluorine-18 production

Three methods were used to produce fluorine-18 (table I).

Nuclear reaction		Particle	Target
I	$Ne(^3He,\alpha n)^{18}Ne \rightarrow ^{18}F$	^3He 30 MeV	Neon + hydrogen
II	$^{16}O(^3He,p)^{18}F$	^3He 30 MeV	Natural water
III	$^{18}O(p,n)^{18}F$	p 16 MeV	50 % enriched water

Table I : Methods of ^{18}F-production

Only one change was made to the production method as already published (4) : the trap where fluorine-18 is trapped during irradiation is made of polyethylene instead of PTFE.

Natural or enriched water is irradiated in a target similar to that described by Kilbourn (5) (front foil : Titanium or havar foil 12

µM, rear foil : titanium 100 µM, target volume 2 ml). The target was irradiated for one hour with the 520 CGR-MeV cyclotron, with beam intensities ranging from 10 to 20 µA.

The neon-hydrogen mixture used was supplied by Air Liquide Company (neon N40 with hydrogen N55) ; 50 % enriched water was purchased from the ORIS-CEA Company (ref. ISO 18-2-50).

Natural water was obtained from a Millipore purification system (Milli Q system, water at 18 megohm x cm).

Resolubilization of 18-fluoride ion

In the neon method, the fluorine-18 was distilled from the polyethylene trap into a catheter tube kept at -15°C, 90 % of the fluorine produced was distilled. The catheter was then rinsed with 500 µl of solvent, in which 95 % of the distilled radioactivity was solubilized.

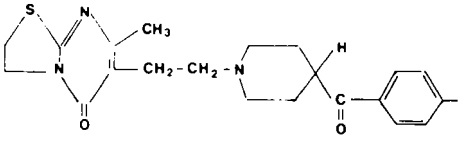
When a water target was used for production, after irradiation, the water was evaporated at 120°C under a current of nitrogen with a few mg of potassium carbonate into either a Pyrex flask (conical vial, V=1 ml from Aldrich) or a siliconized tube (Vacutainer ^{R}, Becton Dickinson, no additive, 5 ml volume). The solvents used, dimethyl sulphoxide (DMSO) and tetramethylene sulfone (sulfolane), obtained from the Aldrich Company, were previously distilled under vacuum onto calcium hydride and kept on 4 A molecular sieve.

Synthesis and purification

The synthesis method involves a nucleophilic substitution of an NO_2 group by $^{18}F^-$ ions (Scheme I).

$$R-NO_2 + K^{18}F \longrightarrow R-^{18}F$$

R:



Scheme I : Method of [^{18}F]setoperone synthesis

a) Synthesis of NO_2-derivative

The synthesis of the R-NO_2 derivative (scheme II) started from isonipecotic acid (commercialy available, JANSSEN CHIMICA). This compound was converted to N-benzoylisonipecotic acid 1 according to a modified procedure of the literature (6). Treatment of 1 by thionyl chloride gave the acyl chloride, which was reacted with acetanilide in dichloroethane. and $AlCl_3$ as the catalyst to give 3 in 42 % yield. Acidic hydrolysis of 3 followed by an oxidation with metachloroperbenzoic acid in chloroform gave the nitro precursor 5. The compound 7 was obtained by N-debenzoylation of 5 and alkylation of the resulting 6 by the suitable alkyl halide.

EXPERIMENTAL SECTION :

General methods : ^{1}H-NMR spectra were recorded on a BRUKER AW80 spectrometer in $CDCl_3$ or DMSO D_6 with TMS as the internal standard. Infrared spectra were recorded as KBr pellets or $CHCl_3$ solution on PERKIN-ELMER 257 spectrophotometer. Melting points were determined on a KOFLER apparatus.

1-Benzoyl-4-piperidinecarboxylic acid, 1

To a cooled (10°C) solution of nipecotic acid (129,2 g, 1 mol) in 250 ml of NaOH 4 N (1 mol) were added sequentially 250 ml of $CHCl_3$ and dropwise benzoylchloride (116 ml, 1 mol). The mixture was stirred for 30 mn and then evaporated in vacuo. The residue was extracted with $CHCl_3$ and the organic layer was washed with water. After drying over $MgSO_4$ and evaporation of the solvent 1 was left (156 g). Yield 67 % ; mp 140°C.
IR (KBr). 1710, 1620 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1,2-4 (m, 9H), 7.45 (s, 5H).

1-Benzoyl-4-piperidine carboxyl chloride, 2

A solution of 1 (49.9 g, 0.214 mol) in 250 ml of $SOCl_2$ was refluxed for 2 h and then the excess of $SOCl_2$ was distilled off to give 2 (53.9g), which was used without further purification.
IR ($CHCl_3$) 1740, 1780 cm^{-1}.

(4-acetamidophenyl)(1-benzoyl-4-piperidinyl) methanone, 3

To a solution of 2 (53.9 g, 0.214 mol) and acetanilide (34.7 g, 0.257 mol) in dichloroethane, $AlCl_3$ (85.6 g, 0.642 mol) was added at room temperature. After complete addition, the mixture was refluxed for 5 h. Then the mixture was cooled and poured on ice. The organic layer was dried over $MgSO_4$ and concentrated. The crude oil was chromatographied (Silicagel 70-230 mesh ; $CHCl_3$ 95/MeOH 5) to yield 30 g (42 %) of 3.mp 208°C.

IR (KBr) 3430, 3000, 1680 cm^{-1}. ^1H NMR (DMSO D_6)δ 1.1-4.6 (m, 9 H), 2.1 (s, 3H), 7.45 (s, 5H), 7.7 (d, 2H), 8 (d, 2H), 8.25 (s, 1H).

(4-aminophenyl)(1-benzoyl-4-piperidinyl) methanone, 4

A solution of 3 (15 g 0.045 mol) in 150 ml of HCl 2N and 75 ml of methanol was refluxed for 2 h. After distillation of the methanol the aqueous layer was basified with NaOH (pH 9-10) and extracted with $CHCl_3$. The organic extracts were dried over $Mg\ SO_4$ and evaporated to dryness to yield 12.4 g (89 %) of 4 mp 180°C.

IR (KBr) 3450, 1670, 1640 cm^{-1}. ^1H NMR ($CDCl_3$)δ 1.4-3.8 (m, 9 H) 4.5 (s, 2H), 6.7 (d, 2H), 7.75 (d, 2H), 7.45 (s, 5H).

(4-nitrophenyl)(1-benzoyl-4-piperidinyl) methanone, 5

To a refluxing solution of metachloroperbenzoic acid (technical grade 85 %) (20.2 g 0.1 mol) in 200 ml of $CHCl_3$ was added slowly a solution of 4 (6.1 g 0.0198 mol) in 100 ml of $CHCl_3$. After complete addition the mixture was heated at reflux for 10 mn, and then cooled to room temperature. After washing with $K_2\ CO_3$ (10 %), the chloroformic layer was dried over $MgSO_4$ and concentrated to dryness. The residue was purified by chromatography (Silicagel 35-70 mesh ; $CHCl_3$ 99.5/MeOH 5) to give 4.3 g (64 %) of 5.mp 134°C.

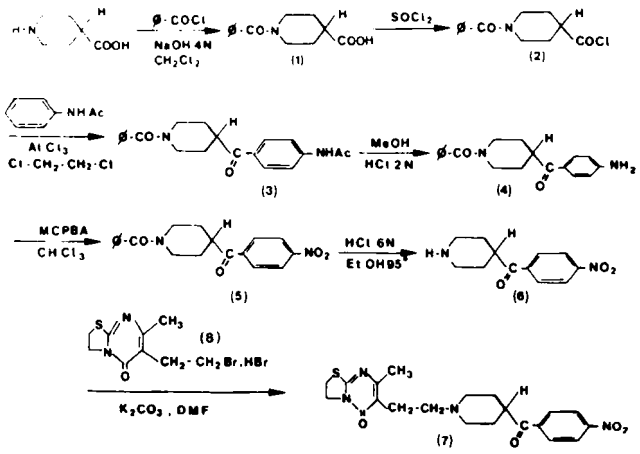
IR (KBr) 3060, 2960, 1720, 1680, 1630, 1530, 1350 cm^{-1}. 1H NMR ($CDCl_3$)δ 1.4-2.2 (m, 4H), 2.8-4.7 (m, 5H), 7.3 (s, 5H), 8.1 (d, 2H), 8.3 (d, 2H).

(4-nitrophenyl)-4-piperidinyl methanone hydrochloride, 6

A solution of 5 (8.45 g 0.025 mol) in 100 ml of HCl 6 N and 50 ml of EtOH was refluxed for 17 h. After evaporation of the solvents the residue crystallized from acetone to yield 4.2 g (62 %) of 6 mp 252°C. IR (KBr) 3400, 2940-2800, 1680, 1530, 1360 cm^{-1}. ^1H NMR(DMSO D_6) δ 1.6-3.6 (m, 9H), 8.3 (s, 4H).

6-[2-[4-(4-nitrobenzoyl)-1-piperidinyl]ethyl]-2,3-dihydro-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, 7

To a stirred solution of 6 (4.3 g 0.0159 mol) in 50 ml of DMF was added compound 8 (7) (6.8 g 0.019 mol) and K_2CO_3 (6.6 g 0.0477 mol). The mixture was heated at 60°C for 4 h. The solvent was evaporated, and the residue was extracted with $CHCl_3$ gave an oil which was purified by chromatography (silicagel 70-230 mesh, $CHCl_3$ 95/MeOH 5). The crude product was treated with an ethanolic solution of HCl and was recrystallized from methylethylketone to give 3.12 g of 7 (39 %).mp 203°C. IR (KBr) 3500-2500, 1700, 1685, 1530, 1350 cm^{-1}.



Scheme II : Synthesis of NO_2 derivative

^{1}H RMN ($CDCl_3$)δ 1.6-3.5 (m, 12H) 2.20 (s, 3H), 3.50 (t, 2H), 3.8 (m, 1H), 4.5 (t, 2H), 8 (d, 2H), 8.20 (d, 2H).

b) Synthesis of 18F-setoperone

The reaction takes place in a small Pyrex vial closed with septum plugs in the presence of potassium carbonate, the NO_2-derivative of setoperone, and a phase transfer catalyst : the kryptofix ^{R} 2.2.2. (8,9) and 500 µl of solvent (DMSO, sulfolane). The tests were run for 10 to 30 minutes at temperatures ranging from 130°C to 180°C.

c) Purification

Once the synthesis finished, the reaction mixture is diluted with 15 ml 0.IN NH_4OH and passed through a Sep-Pak C_{18} cartridge previously activated by 20 ml methanol and 20 ml 0.IN NH_4OH. The radioactivity is eluted by 6 ml of dichloromethane and the eluate is transferred onto a silica Sep-Pak cartridge. The silica is washed with 20 ml dichloromethane and then eluted with 30 ml dichloromethane containing 4 % ethanol. After evaporation of the solvent, the radioactivity is taken up in 500 µl of a CH_2Cl_2-2 % B mixture (B = ethanol 94, water 2, ethylamine 4) and is injected onto a HPLC column (Partisil 10, 500 mm x 9 mm), solvent CH_2Cl_2 + 2 % B, flow rate 8 ml/min. The radioactivity as well as the U.V. absorption at 254 nm (Waters Model 440) of the effluents from the HPLC columns were monitored. The setoperone retention time is about 15 minutes. The radioactive peak corresponding to ^{18}F-setoperone is recuperated. On leaving the column, the product is radiochemically pure but chemically impure, so a second HPLC chromatography is performed on a 7 µm Si 60 lichrosorb column (L = 250 mm, d = 9 mm), solvent = chloroform 968, methanol 30, water 2, flow rate 3 mL/min. The retention time of the ^{18}F product is 14 minutes.

RESULTS

The three nuclear reactions used to produce fluorine-18 give the following quantitites for a one hour irradiation.

20 µA ^{3}He - Neon-H_2 : 2.59 - 3.33 GBq I

15 µA ^{3}He - $H_2{}^{16}O$: 4.44 - 5.55 GBq II

15 µA p - $H_2{}^{18}O$: 14.8 - 16.65 GBq III

The first syntheses were tried with fluorine-18 from nuclear reaction I. After distillation, the fluorine-18 is obtained in 500 µl DMSO. 25 µmol of substrate (NO_2-derivative) and 25 µmol of potassium carbonate were used. The yields obtained according to temperature and quantity of kryptofix are given in table II for a 30 minutes reaction time.

Reaction temperature	Quantities of kryptofix 222	Radiochemical yield (EOB) (a)
180°C	50 µmol	54.7 %
150°C	50 µmol	43.5 %
130°C	50 µmol	39.7 %
180°C	0	8.6 %
130°C	0	4.1 %

Table II : Radiochemical yield according to temperature and quantity of kryptofix 2.2.2.

(a) : Each value given in the different tables of this paper represents the average of 3 to 5 experiments.

In view of the low specific radioactivities obtained (see below), the ^{18}F production method based on natural water was tried. When the irradiated water is evaporated into a pyrex tube and the reaction carried out directly in the tube itself, the synthesis yields are poor (10 to 15 %) with respect to the fluorine produced. An attempt was therefore made to solubilize the fluorine-18 before reaction. In case of evaporation into a pyrex or siliconized tube with nothing added, very little of the radioactivity produced passed into solution in the solvent

(10 %). Evaporation into a pyrex tube in the presence of potassium carbonate and kryptofix 2.2.2. gave mediocre results : 50 to 60 % of the radioactivity solubilized. When siliconized tube was used however, the total percentage of ^{18}F-recuperation and the reproductibility of the take-up were distinctly better : 80 to 90 % of the radioactivity solubilized as a result of evaporation into a Vacutainer ^{R} in the presence of 2 mg (18.5 µmol) $K_2 CO_3$ and 14 mg (37 µmol) kryptofix 2.2.2. After evaporation of the water the $K^{18}F$ is taken up in 500 µl DMSO or sulfolane. This is the recovery method adopted and applied also to ^{18}F produced from enriched water. In this latter case only 1 ml of the 2 ml irradiated water ($H_2{}^{18}O$-50 %) is used for the synthesis (7.4 to 8.14 GBq).

Synthesis from aqueous 18F

Except $K^{18}F$, the 500 µl of solvent contain 14 mg of kryptofix 2.2.2. and an unknow proportion of the 2 mg K_2CO_3 used during evaporation of the water. This volume of solvent was made to react in a pyrex vial with various quantities of kryptofix 2.2.2. and K_2CO_3. Table III gives the synthesis yields obtained with respect to the ^{18}F solubilized. All these syntheses were performed at 180°C for 30 minutes.

Reagents used	Yield obtained
5 mg R-NO_2 5 mg k_2CO_3 37 mg kryptofix	7.5 %
5 mg R-NO_2 5 mg k_2CO_3 16 mg kryptofix	16.7 %
5 mg R-NO_2 2,7 mg $k_2 CO_3$ 10 mg kryptofix	22.4 %
5 mg R-NO_2 1.35 mg k_2CO_3 4 mg kryptofix	42 %
5 mg R-NO_2 1.35 mg $k_2 CO_3$	55.2 %
5 mg R-NO_2	< 5 %

Table III : Synthesis yield versus reagents concentration

It is found that increasing amount of added kryptofix cause the yield to drop sharply and the quantity taken in the evaporation tube seems adequate. On the other hand, it is essential to add potassium carbonate. The synthesis yield seems not to vary greatly with reaction time (Table IV ; synthesis conditions : 180°C, 5 mg R-NO_2, 1.35 mg K_2CO_3).

Reaction time	Yield
10 min	51.2 %
20 min	50.6 %
30 min	55.2 %

Table IV : Synthesis yield versus time

Specific radioactivity determination

The specific radioactivity of the ^{18}F-setoperone obtained was calculated from the HPLC chromatogram (Lichrosorb 7 µM). The mass of the product formed during the synthesis is determined by a U.V. detector at 254 nm. Before injection of the radioactive product, a standard curve is made with three concentrations of setoperone : 20, 30 and 50 nmol. For the synthesis with aqueous ^{18}F, it seems only to depend on the quantity of radioactivity involved (Table V, synthesis conditions : 5 mg R-NO_2, 1.3 mg K_2CO_3, 500 µl DMSO ou sulfolane , 30 minutes, 180°C).

Target	Activity produced (GBq - EOB)	Activity taken up in synthesis (GBq - EOB)	Specific activity GBq/µmol (EOB + 110 min)
Neon	2.59 - 3.33	2.22 - 2.77	< 3.7
$H_2{}^{16}O$	4.44 - 5.55	3.7 - 4.81	22.2
$H_2{}^{18}O$	14.8 - 16.65	5.55 - 7.03	30 - 41

Table V : Summary of the results obtained

The determination of F^- in [^{16}O]-H_2O with a specific electrode (Tacussel electrode PF 4L) gave a F^- concentration of 2 to 3 10^{-7}M. Into 2 ml of this water, there is less than 1 nmol of F^-. Now the mass of product formed at the end of the synthesis with aqueous fluoride is 25 to

30 nmol. Then there is a contamination with stable fluoride during the irradiation and may be also during the first step of the synthesis (DMSO reaction).

With the fluoride from the neon target, the specific activity obtained is very low. The target used for this production had been passivated, several years ago, with fluorine at elevated temperature. We can suppose that the nickel fluoride which is yet on the walls of the target, is partially decomposed during the irradiation and gives stable HF with hydrogen.

Finally the solvent of choice seems to be sulfolane, which gives fewer decomposition products at 180°C than DMSO. Starting with 7.4 GBq (200 mCi), it is possible to obtain 110 minutes after the end of bombardment between 1.5 and 2 GBq (40 - 50 mCi) of [^{18}F]setoperone ready for injection with a specific activity of 37 GBq/µmol (≃ 1 Ci/µmol). The product is solubilised into an isotonic solution pH 4 (100 µl CH_3 COOH 10 N, 1 ml NaOH 1N, 7 ml H_2O) and sterilised on a millipore filter (Millex FG, 0.2 µm).

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