SYNTHESIS OF 5-[^{125}I]-IODO-ZACOPRIDE, A NEW PROBE FOR 5-HT$_3$ RECEPTOR BINDING SITES

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ABSTRACT

In an attempt to develop a specific probe of serotonin 5-HT$_3$ receptors, various methods have been investigated to synthesize a radioiodinated derivative of the potent 5-HT$_3$ antagonist zacopride. The direct iodination of 5-dechloro-zacopride **2** was performed using NaI in the presence of either chloramine T or iodo-beads. Irreproducible results or/and low yields were obtained by these methods. A third approach using a less oxidative medium allowed the synthesis of iodo-zacopride **4** from **2** with N-iodo-succinimide or with NaI and N-chloro-succinimide. Using this third condition, high yield was obtained (49%). However, the radioiodinated compound was contaminated by some "cold" zacopride also formed during the reaction. A two step synthesis was successful to eliminate "cold" zacopride but in lower yield (2-10%). Thus, 4-amino-2-methoxy-benzoate **5** was iodinated and then coupled with 3-aminoquinuclidine **8** to give 5-iodo-zacopride **4**. Radioactive synthesis was carried out in the same condition to give 5-[^{125}I]-iodo-zacopride **1** with a yield of 98%. The two enantiomers R- and S-5-[^{125}I]-iodo-zacopride were synthesized by direct iodination.

KEY WORDS : *125-Iodine, 5-iodo-zacopride, 5-Hydroxytryptamine, 5-HT$_3$ receptors, chloramine T, iodo-beads, N-halogeno-succinimide.*

INTRODUCTION

The neurotransmitter serotonin (5-Hydroxytryptamine; 5-HT) is involved in various physiological and pathological processes , via the stimulation of (at least) three main classes of receptors called 5-HT$_1$, 5-HT$_2$ and 5-HT$_3$ respectively. 5-HT$_3$ antagonists and among them zacopride **3**, are of special interest because recent studies have shown that they are potent antiemetic and potential anxiolytic drugs. To date, the distribution of 5-HT$_3$ receptors within the central nervous system has been examined by quantitative autoradiography using tritiated derivatives of selective 5-HT$_3$ antagonists (1,2,3). However, the density of 5-HT$_3$ binding sites is rather low and brain sections labelled by a

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tritiated probe have to be exposed for several months in order to yield autoradiograms. A radioiodinated probe would be a better tool for such studies because its high specific radioactivity would both reduce the exposure time and allow the detection of 5-HT_3 binding sites in brain areas where they are in low number (and possibly not detected by tritiated ligands).

We report here the synthesis of the first radioiodinated antagonist of 5-HT_3 receptors : 5-[^{125}I]-iodo-zacopride **1**. For this study we have chosen to introduce a iodine atom in a benzamide molecule derived from the potent antagonist zacopride **3**. We present here some iodination reactions leading to cold **4** and radioiodinated **1** ligands.

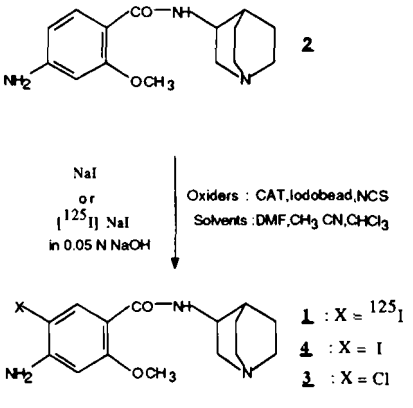
CHEMISTRY

All reactions were first studied with cold NaI using micromoles (μmol) or/and nanomoles (nmol) of precursor and then extended to [^{125}I]-NaI. Two approaches were investigated:

A) The direct iodination of 5-dechloro-zacopride **2** (using different oxidizing conditions (4,5))

B) A two step synthesis involving the iodination of benzoate ring followed by the coupling with 3-aminoquinuclidine **8**.

SCHEME I: Direct radioiodation of 5-dechloro-zacopride 2



CAT = chloramine T
NCS = N- chloro-succinimide
DMF = dimethylformamide

A) DIRECT IODINATION of 5-dechloro-zacopride **2** (SCHEME I) :

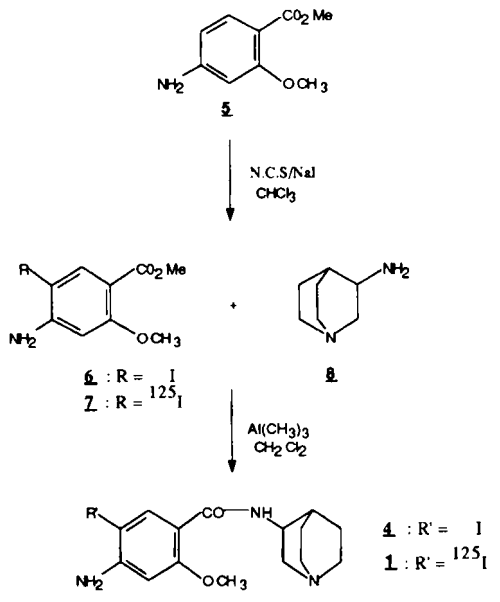
1) The iodination of the aromatic ring of 5-dechloro-zacopride **2** was first attempted with sodium iodide and chloramine T in a phosphate buffer (6). A limited amount of the iodinated derivative **4** was obtained under these standard conditions. The addition of acetone in the buffer increased the yield which, however, remained low (5-10%). When the reaction was conducted in dry DMF, the yield reached 20%. However, the radioactive synthesis performed on nanomolar scale did not lead to a pure compound. Since polar materials were formed during this reaction, we have hypothesized that chloramine T was a too strong oxidant. Therefore, we repeated the experiments using iodo-beads (7,8).

2) The iodination of **2** by NaI in presence of iodo-beads was conducted in various solvents. Better results were obtained in acetonitrile (30%) than in DMF (10%). However, in both cases the 5-iodo-zacopride **4** formed was contaminated by other products. The radiosynthesis with 2mCi of [^{125}I]-NaI gave only 20µCi of an unknown compound, which was found to have no affinity for 5-HT_3 sites. 76.6µCi (38% yield) of **1** were also obtained but they were contaminated by the previous unknown compound; attempts to purify **1** using different HPLC conditions were unsuccessful.

3) Since N-halogeno-succinimide derivatives can halogenate activated aromatic rings, we investigated the iodination of **2** by N-iodo-succinimide. The reaction of N-chloro-succinimide with sodium iodide in an organic solvent lead to N-iodosuccinimide (9,10). We have adapted this method for the radioiodination of **2**. This synthesis conducted with 5.3 mCi [^{125}I]-NaI led to 2.6mCi of a mixture of 5-[^{125}I]-iodo-zacopride **1** (49% yield) and a large amount of "cold" zacopride **3**. Indeed, "cold" zacopride **3** was formed from the reaction of the excess of N-chlorosuccinimide with 5-dechloro-zacopride **2**. However, two successive HPLC purifications were enough to eliminate completely "cold" zacopride **3** from 5-[^{125}I]-iodo-zacopride **1** (12). The same chemical pathway was used for the synthesis of (R) and (S) enantiomers of 5-[^{125}I]-iodo-zacopride. The specific radioactivity of **1**, estimated by U.V. absorption, ranged between 1000 and 1200 Ci/mmol.

B) TWO STEP SYNTHESIS (SCHEME II) :
To avoid the contamination of 5-[^{125}I]-iodo-zacopride **1** by zacopride **3**, we performed a two step synthesis. In the first step, the iodination was conducted as previously described except that the starting material was no more **2**, but methyl-4-amino-2-methoxy-benzoate **5**. The yield of the

SCHEME II : Synthetic pathway of [^{125}I]Iodo-zacopride 1



N.C.S. : N-Chloro-succinimide

iodinated compound 6 was highly improved (97%) if trifluoroacetic acid was added in the reaction mixture. The iodinated ester 6 was purified in order to eliminate the starting ester 5 and the chlorinated ester. In a second step, the iodinated ester 6 was coupled with 3-aminoquinuclidine 8 previously complexed with trimethylaluminium (11). After hydrolysis and purification, 72% of 5-iodo-zacopride 4 were obtained. However, lower yields (2-10%) were obtained with the radioactive synthesis, probably because of the hydrolysis of the trimethylaluminium complex before its reaction. 5-[^{125}I]-iodo-zacopride 1 (1000-1200 Ci/mmol) was finally purified using HPLC up to a radiochemical purity of 99%.

Biological assays confirmed that both, the "cold" 5-iodozacopride 4 and the radioactive probe 1, prepared by the two step pathway, were not contaminated by any "cold" zacopride 3 left.

PHARMACOLOGICAL CHARACTERIZATION

Membranes from rat posterior cortex and NG108-15 clonal cells were labelled with [^{3}H]-zacopride as previously reported (13). 5-iodo-zacopride 4 inhibited [^{3}H]-zacopride binding to cortical and NG108-15

5-HT_3 sites with an IC_{50} of 6.1 nM and 8.3 nM respectively (12). In both cases, the Hill coefficient was close to unity. Saturation curves were constructed in both tissues with 5-[^{125}I]-iodo-zacopride **1**. The Scatchard plot indicated a Kd value of 2.6 nM (cortex) and 3.2 nM (NG108-15) for the specific binding of the radioiodinated ligand. A good correlation (r = 0.95) was also observed between the affinities of several drugs to inhibit the specific binding of [^3H]-zacopride or 5-[^{125}I]-iodo-zacopride **1** to NG108-15 cell membranes. Furthermore the distribution of 5-[^{125}I]-iodo-zacopride **1** specific binding sites in various areas of the central nervous system superimposed with that of 5-HT_3 sites labelled by [^3H]-zacopride. These results confirmed that 5-[^{125}I]-iodo-zacopride **1** and [^3H]-zacopride labelled the same 5-HT_3 sites. 5-[^{125}I]-iodo-zacopride **1** was then used for the autoradiographic visualization of 5-HT_3 binding sites in rat brain.

AUTORADIOGRAPHIC ANALYSIS

Fresh brain sections (20 µm thick) were incubated in 0.05 M HEPES, pH 7.4, supplemented with 100,000 cpm 5-[^{125}I]-iodo-zacopride **1** for one hour at room temperature. They were then rapidly dipped (5 s) into a cold water bath, air-dried and applied to tritium sensitive film for 1-2 days. Labelled 5-HT_3 sites were found in various nuclei of the amygdala, the entorhinal cortex and the primary olfactory cortex. In the brain stem, the nucleus of the solitary tract (NTS) was intensely labelled and at the level of the spinal cord, the dorsal horn also contained a high density of 5-HT_3 sites. Similar results were obtained with [^3H]-zacopride but after exposing the labelled brain sections to tritium sensitive film for 4-6 months.

EXPERIMENTAL

General

(R,S),(R) and (S) 5-dechloro-zacopride were kindly supplied by Laboratoires DELALANDE (Rueil Malmaison, France). Sodium [^{125}I]-iodide (carrier free in aqueous 0.05N sodium hydroxide) was purchased from Amersham International plc. All commercial chemicals were used without purification and solvents were freshly distilled before use.

Analysis

N.M.R. spectroscopy was performed using a 300 MHz spectrometer (Brücker); mass spectra were obtained on a quadripole Finnigan Mat 4600. I.R. and U.V. spectra were recorded using a Beckman 4250 and a Kontron (Uvikon 860) spectrometer respectively. Radioactive detection was performed with a Berthold detector (LB2040 electronic). Mass, N.M.R., U.V. and I.R. analyses were in agreement with the assigned structures. T.L.C. analyses were performed either on Merck silicagel (F254) or on Whatman KC18 (F254) plates. H.P.L.C. analyses were carried out using

a Shimadzu system (LC 5A pump) on either a Zorbax C8 column (25cm, 4.6mm, O.D., 5µm) using methanol/water/triethylamine (80/20/0.05; V/V/V) or a Zorbax C18 column (25cm, 4.6mm, O.D., 5µm) using acetonitrile/water (40/60; V/V) with U.V. detection at 265nm or 276nm (Waters, lambda Max 481).

Methyl-4-amino-5-iodo-2-methoxy-benzoate : 6

To a stirred solution of 0.55mmol (100mg) of **5** in 10ml of methylene chloride, 0.56mmol sodium iodide (84mg) and 0.56mmol N-chloro-succinimide (75mg) were added. 5.1mmol (390µl) of trifluoroacetic acid were then added and the coloured solution turned to purple. The reaction was carried out overnight, and subsequent concentration under vacuum, yielded a crude material which was purified by flash chromatography on silica gel (Si60 Merck, 15-25 µm). 164mg (97%) of pure methyl-4-amino-5-iodo-2-methoxy-benzoate **6** were isolated.

H.P.L.C. analysis : retention time : 30min at 0.5ml/min, C18 column.

M.S. (DCI/NH_3) : $(M+1)^+ = 308$ (100%), $(M+18)^+ = 325$ (30%), $(M+35)^+ = 342$ (13%).

^1H R.M.N.(CD_2Cl_2) : δ = 3.76 (s,3H), 3.80 (s,3H), 4.53 (s,2H), 6.30 (s,1H), 8.12 (s,1H).

U.V. (MeOH) : λmax_1 = 219nm (ε = 23 500), λmax_2 = 276nm (ε = 14 300), λmax_3 = 313nm (ε = 10 500), λmin_1 = 255nm (ε = 6 300), λmin_2 = 294nm (ε = 7 400).

4-amino-N-1-(azabicyclo[2.2.2]oct-3-yl)-5-iodo-2-methoxy benzamide (5-iodo-zacopride) : 4

A) Two step synthesis :

Under a nitrogen atmosphere, a complex was formed by reaction for 30min of a suspension of trimethylaluminium (1ml, 2mmol) with 3-aminoquinuclidine **8** (194mg, 0.98mmol) in suspension in 4ml of methylene chloride. Compound **6** (150mg, 0.49mmol) in 3ml methylene chloride was then added to the complex, and the reaction mixture was refluxed for two hours in an oil bath at 60°C. The crude mixture was subsequently hydrolyzed with an excess of methanol. After concentration under vacuum, an excess of potassium hydroxide in methanol (0.5M) was added and the mixture was filtered and concentrated under vacuum. Purification by flash chromatography on silica gel (Si60 Merck, 15-25 µm), gave 140mg of pure **4** (72% yield).

B) Direct iodination :

To a solution of **2** (150mg, 0.55mmol) in chloroform (40ml), 150mg of N-iodo-succinimide were added. After stirring for three days and

concentration under vacuum, water (30ml) was added and the organic layer was extracted with 3x10ml methylene chloride. The crude material was purified by flash chromatography on silica gel (Si60 Merck, 15-25 µm). 188mg (86%) of pure **4** were obtained.

HPLC :	retention time : 48min at 1ml/min, C8 column.
M.S. (DCI/NH_3) :	$(M+1)^+$= 402 (100%), $(M+18)^+$= 419 (7%),
^1H R.M.N. (D_2O) :	δ = 2.18-2.38 (m,4H), 2.58 (m,1H), 3.42 (m,1H), 3.5-3.65 (m,4H), 4.1 (t,1H), 4.8 (s,3H), 4.53 (m,1H), 6.55 (s,1H), 8.18 (s,1H).
U.V. (MeOH) :	λ max1 = 220nm (ε = 31 000), λ max2 = 276nm (ε = 17 200), λ max3 = 311nm (ε = 13 100), λ min1 = 253nm (ε = 6 700), λ min2 = 294nm (ε = 9 300).

Methyl-4-amino-5-[^{125}I]-iodo-2-methoxy-benzoate : 7

2mCi of [^{125}I] sodium iodide were concentrated to dryness under a stream of argon in a "reacti-vial". Then, 170µl of chloroform, 15µl (180nmol, 55µg) of amino-ester **5** , 10µl (180nmol) of a N-chloro-succinimide solution (180nmol,13.3µg) and 5µl (0.435 µmol, 49.6µg) of trifluoroacetic acid were added to a final volume of 200µl (all reagents were solubilized in chloroform). After stirring for 20 hours at room temperature, 2µl of triethylamine were added; the solution was then dried under a stream of argon. The residue was dissolved in methylene chloride (1ml) and 1.3mCi of a crude material were obtained after a purification on a silica Sep-Pack cartridge. 472µCi (yield:13%) of **7** were then purified by H.P.L.C. on a reverse phase C18 column (retention times of **7** and **5**, 30min and 18min respectively at 0.5ml/min). **7** was used in the next step without any further purification.

4-amino-N-1(azabicyclo[2.2.2]oct-3-yl)-5-[^{125}I]-iodo-2-methoxy benzamide (5-[^{125}I]-iodo-zacopride) : 1

A) Two step synthesis :

A complex was first formed under a nitrogen atmosphere by the reaction (30min, room temperature) of trimethylaluminium (20µl, 26.6mg, 0.36mmol) and 3-aminoquinuclidine **8** (2mg, 10µmol) in methylene chloride (1ml). 10µl of the complex solution (100nmol) were added to 472µCi of 5-[^{125}I]-iodo-benzoate **7** in a "reacti-vial". The reaction mixture was refluxed overnight with the "reacti-vial" being screwed hermetically and heated in a oil bath at 45°C. The crude mixture was then dried under a stream of argon and purified by H.P.L.C.

on a C8 column. Retention times of 5-[^{125}I]-iodo-zacopride **1** and **7** were 48min and 7min respectively at 1ml/min. 10µCi (yield 2%) of **1** free of "cold" zacopride **3** were obtained with 98% radiochemical purity.

B) Direct radioiodination :
A solution of **2** (49µg; 180nmol) in chloroform (29µl) was added to 5.3mCi of [^{125}I]-NaI, 10µl of N-chloro-succinimide (32µg; 180nmol) and 5µl of trifluoroacetic acid (1mg, 9µmol) in chloroform to a final volume of 200µl. After stirring for 20 hours at room temperature, an excess of triethylamine was added and the solution was dried under a stream of argon. The residue was dissolved in methanol (500µl) and loaded on a Sep-Pack C-18 cartridge. The crude mixture was collected with methanol and concentrated under a stream of argon. The final product was purified at least twice by H.P.L.C. on a C8 column. Retention times were of 48min, 40min and 33min for **1**,**3**, and **2** respectively at 1ml/min. The identification and the purity (>99%) of **1** (2.4mCi : 45% radiochemical yield) were determined using coelution with the "cold" compound under the same conditions.

CONCLUSION

In this study, we showed that [^{125}I]-iodo-zacopride **1** can be obtained by different methods :
A) the two step synthesis, that gave pure radioactive compound, but with a low radioactive yield;
B) the direct radioiodination with N-chloro-succinimide as oxidant gave a good radioactive yield, but with some contamination by "cold" zacopride.
Therefore, for usual synthesis, we used the direct iodination method followed by successive HPLC purification. All batches of [^{125}I]-iodo-zacopride that we made had the same chemical and radiochemical purity, and specific radioactivity. Both enantiomers, R- and S-5-[^{125}I]-iodo-zacopride were also synthesized using the same chemical pathway.

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