RADIOLABELLING OPTIMIZATION OF 5-(4-[^{125}I]-IODOPHENYL)-2,3 DIHYDRO-5-HYDROXY-5H-IMIDAZO[2,1-a]ISOINDOLE OR [^{125}I]-IODO MAZINDOL : A POTENTIAL TOOL FOR SPECT EXPLORATIONS.

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SUMMARY

The radiolabelling of an iodinated analog of mazindol, 5-(4-[^{125}I]-iodophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]isoindole, was performed in order to develop a potential tool for SPECT exploration of the presynaptic dopamine transporter in the human brain. Radiosynthesis was performed by iodide for bromide nucleophilic exchange from a brominated precursor. The reaction was carried out in the presence of the copper I catalyst and reducing and complexing agents. In these radiolabelling conditions, [^{125}I] iodomazindol exhibited a tautomeric equilibrium. Therefore, in order to obtain the best labelling conditions, we studied variables such as copper I catalyst and brominated precursor concentrations, reaction temperature and heating time involved in the reaction .

This study of the kinetics could be used as a pattern for the radiosynthesis of other compounds which can be present in a tautomeric equilibrium under particular conditions. Indeed, we describe a convenient procedure to obtain each tautomeric form with high radiochemical purity in optimum radiolabelling conditions .

KEY WORDS : [^{125}I] iodo mazindol, copper I assisted nucleophilic exchange, high labelling efficiency, SPECT

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INTRODUCTION

Dysfunction of the central dopaminergic system is involved in numerous pathological conditions such as Parkinson's disease (1) and Schizophrenia (2). Therefore scintigraphic studies of the presynaptic dopamine transporter in the living human brain are of great value. For these explorations, it is important to synthesize new radiotracers, particularly radioiodinated products necessary for *in vivo* SPECT investigations. Indeed, compounds such as [^{125}I] GBR (3,4) and[$^{123/125}$I] RTI (5 - 7) were developed with the aim of evaluating this presynaptic function and better to understand the role and the disturbance of this process in neurological illnesses involving the central dopaminergic system (1,8). *In vitro* studies with [^{3}H] mazindol have shown that this ligand binds to the dopamine carrier with high affinity (9) and is suitable to detect modifications of the dopamine uptake system in these physiopathological situations.

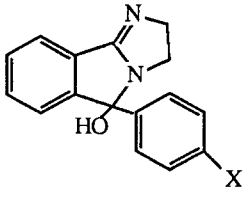
These results led us to carry out synthesis of the first radioiodinated analog of mazindol as a potential tool for SPECT explorations. For this goal, we prepared para brominated and para iodinated analogs of mazindol. The brominated product was used as precursor of the radioiodinated ligand, and then the iodinated compound was used to characterize the radioiodinated derivative and *in vitro* studies.

Radiolabelling from the brominated precursor was performed by [^{125}I] iodide for bromide nucleophilic exchange in the presence of the copper I catalyst. This paper describes a study developed to optimize the [^{125}I] iodo mazindol radiolabelling according to certain variables involved in this reaction.

This investigation was made more difficult by the presence in the radiolabelling conditions of the radioiodinated open tautomeric form of [^{125}I] iodo mazindol. It is necessary to take into account this side product to obtain the best labelling efficiency and high radiochemical purity of the radioligand.

RESULTS AND DISCUSSION

Several methods (10-13) have been described in the literature for the preparation of 5-(4-chloro)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a] isoindole or mazindol 1 and its congeners 2 and 3 (Fig. 1).



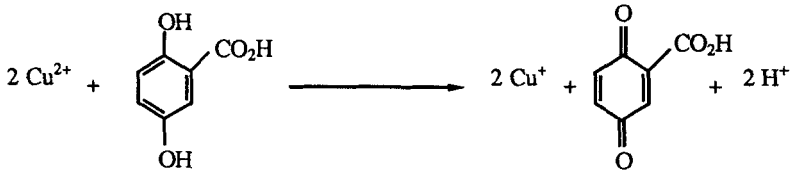
1 X = Cl
2 X = Br
3 X = I

Figure 1 : Mazindol 1, its para brominated 2 and para iodinated 3 analogs

We synthesized (14) unradioactive para brominated 2 and para iodinated 3 derivatives of mazindol according to the one-pot procedure described by Houlihan (13).

The radiolabelling was achieved from the brominated precursor 2 (scheme 1) by nucleophilic exchange of bromide for iodide. It is well known that this reaction is assisted by the copper I catalyst

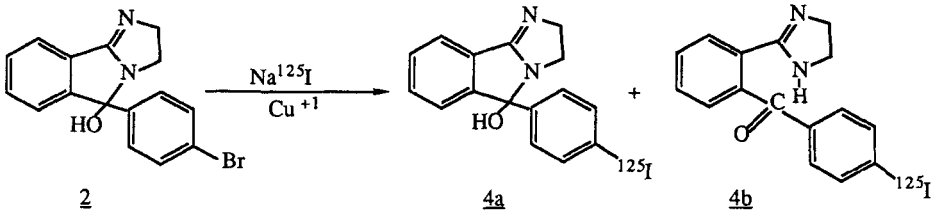
but it appeared that the presence of copper II was a poison catalyst for the reaction. To avoid oxidative formation of copper II, Mertens et al (15,16) and Gysemans et al (17) prepared copper I catalyst from cupric sulfate with an excess of reducing agents (stannous sulfate and gentisic acid) under nitrogen atmosphere (Equations 1 and 2).



Equation 1 : Oxydo-reduction reaction between Cu^{2+} and gentisic acid

Equation 2 : Oxydo-reduction reaction between Cu^{2+} and Sn^{2+}

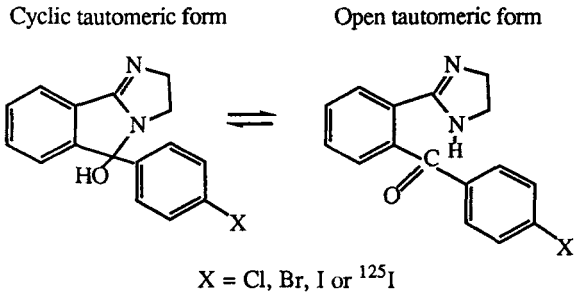
The reaction was performed in homogeneous catalyst conditions by forming copper I complex with acetic and citric acids. We applied these conditions to synthesize [^{125}I] iodo mazindol 4a.



Scheme 1 : Radiolabelling of bromo mazindol 2

The method of Mertens et al (18) permitted us to obtain the expected radioligand 4a which was isolated, purified and identified by co-injection with its unradioiodinated analog 3 in HPLC. The HPLC system was equipped with double detection which consisted of U V and radioactivity detectors.

Another radioiodinated compound with a higher retention time was separated by HPLC. This product was determined to be the open form 4b (scheme 1). This assignment is in agreement with the tautomeric equilibrium of mazindol reported by Barcza et al (19). These authors showed that in acid medium mazindol is preferentially under its open form. The presence of the two tautomeric forms (cyclic and open) was also observed by HPLC with para brominated 2 and para iodinated 3 derivatives when they were heated in acid medium. The chromatogram exhibited two signals, the first peak corresponding to starting material 2 or 3. According to Barcza et al (19), the second peak was assigned to the open tautomeric form (scheme 2).



Scheme 2 : Tautomeric equilibrium

Biological experiments (*in vitro* and *in vivo* studies) established that only the cyclic tautomeric form of radioiodinated derivative 4a had an affinity for the dopamine carrier. Consequently, only this radiolabelled cyclic form was usable for brain exploration by SPECT. Therefore the operatory conditions of radiosynthesis could be optimized to promote the formation of the cyclic tautomeric form 4a.

Some variables such as copper I and brominated precursor 2 concentrations, reaction temperature and heating time were investigated (Fig. 2 to 5) to determine the most suitable conditions for radiosynthesis.

The method used to optimize the labelling of the radioiodinated ligand 4a consisted of modifying one variable while the other three parameters were kept constant under conditions described by Mertens et al (15,16) and Gysemans et al (17).

Labelling efficiency as a function of copper I concentration

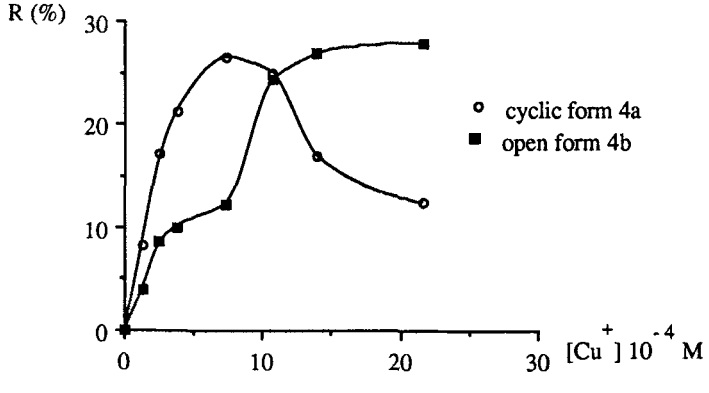


Figure 2 : Labelling yield (R %) as a function of copper I concentration

It is well known that without the copper I catalyst the iodide for bromide nucleophilic exchange is not possible from an aryl bromide. As expected, in the reaction conditions, the radiolabelling of the cyclic

form 4a increased with copper I catalyst concentration to reach a maximum of the radiochemical yield (26.5%) for [Cu^+] = 7.37 10^{-4} M.

The labelled open form 4b, formed in acid medium and heating conditions, is in tautomeric equilibrium with the cyclic form 4a. Up to [Cu^+] = 10.8 10^{-4} M, we observed that the ratio of the tautomeric forms was 4a > 4b. Increased values of copper I concentration from [Cu^+] = 10.8 10^{-4} M produced inversion of the proportion of tautomeric forms 4a < 4b.

While yield from the cyclic form 4a decreased, we noted that the yield from the open form 4b tended towards a constant value.

Moreover, the total yield of radiolabelling of the two isomers 4a and 4b remained under 50%. This phenomenon was probably due to the rate limiting effect of the copper I catalyst by formation of cuprous bromide and cuprous radioiodide which precipitated when the copper I concentration became higher. This saturation effect was described for copper I assisted radioiodination from bromophenyl-metyrapone by Mertens et al (20).

The highest labelling efficiency in the cyclic tautomeric form 4a was obtained for [Cu^+] = 7.37 10^{-4} M. This value must be selected for further radiolabelling studies.

Labelling efficiency as a function of para brominated precursor 2 concentration

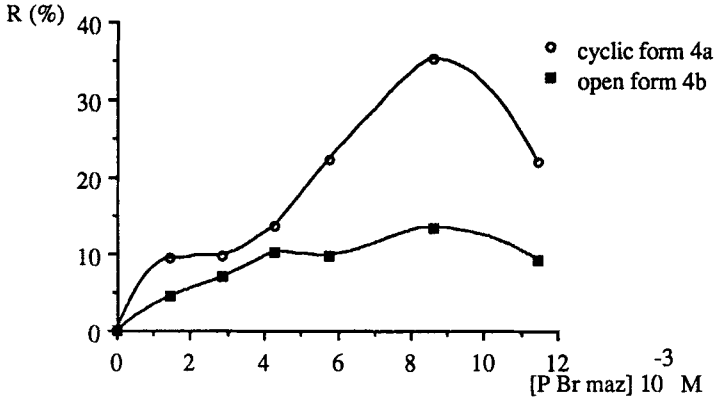
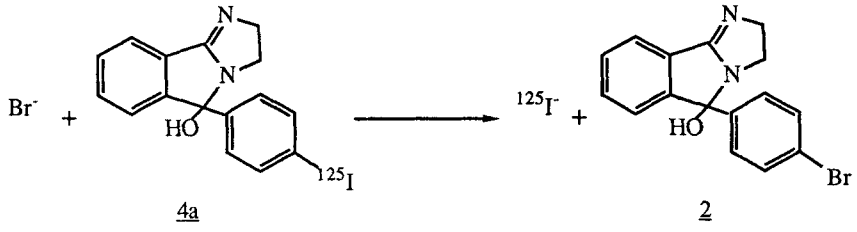


Figure 3 : Labelling yield (R %) as a function of para brominated precursor 2 concentration

Similarly to the previous study, we observed that the radiolabelling of the cyclic form 4a and the open form 4b increased with brominated precursor concentration to reach a maximum of the radiochemical yield. However in this case the proportion between the two tautomeric forms was always 4a > 4b. Moreover, the maximum of radiochemical yield was 35% for 4a and 13.4% for 4b with the same precursor concentration [2] = 8.6 10^{-3} M. Total radiolabelling yield of the two isomers 4a and 4b remained under 50% and decreased when precursor concentration increased from 8.6 10^{-3} M.

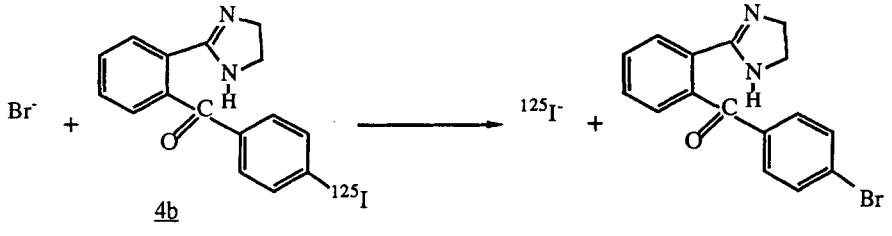
The cause of this phenomenon was probably the opposite reactions (Equations 3 and 4) when the

bromide concentration became higher due to greater precursor 2 concentration [Mertens et al (21)]. It is also possible that the rate limiting effect of the copper I catalyst was involved in the saturation of the labelling rate.



4a 2

Equation 3 : Opposite reaction of the cyclic tautomeric form by nucleophilic exchange of bromide for iodide



4b

Equation 4 : Opposite reaction of the open tautomeric form by nucleophilic exchange of bromide for iodide

Labelling efficiency as a function of reaction temperature

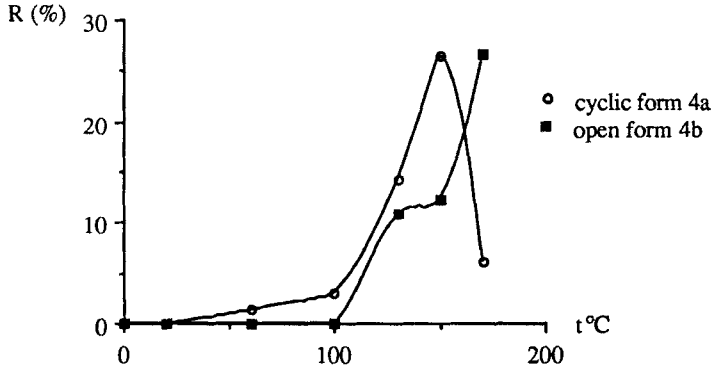


Figure 4 : Labelling yield (R %) as a function of temperature

Up to 100°C, radiolabelling of cyclic tautomeric form 4a slowly increased with temperature ; at 100°C we obtained 2.95% yield of radioiodinated derivative 4a. The open form 4b was virtually absent. However, from 100°C labelling of the two tautomeric forms quickly increased with temperature while the proportion of the two isomers was 4a > 4b. As anticipated, in acid medium and heating conditions, the labelling of the ligand 4a reached a maximum of 26.5% radiochemical yield observed at 150°C. The open form 4b continued to increase with temperature from 150°C, while the cyclic form 4a decreased according to tautomeric equilibrium in acid medium.

Labelling efficiency as a function of reaction time

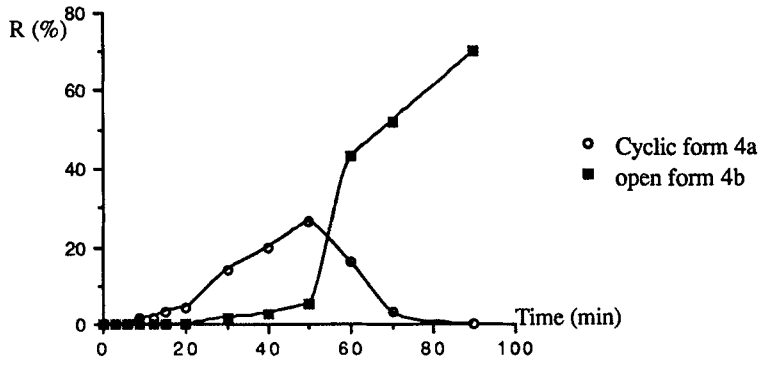


Figure 5 : Labelling yield (R %) as a function of time

Results showed that the labelling of the cyclic ligand 4a increased with reaction time up to 50 min (27% yield). From 50 min, the radiochemical yield of the compound 4a decreased while the labelling of the open form 4b quickly increased.
At 90 min, we noted that the cyclic tautomeric form 4a completely disappeared to the profit of the open tautomeric form 4b (70% yield).

It appeared that the best labelling conditions to obtain the radioiodinated ligand 4a consisted of using 1.5 mg of brominated precursor 2 and 30 µL (7.37. 10-4M) of cupric sulphate solution with reducing and complexing agents. It was then necessary to heat the reaction mixture with sodium [^{125}I] iodide for 50 min at 150°C. Each tautomeric form (cyclic 4a and open 4b) was isolated. They were obtained without starting material after HPLC purification allowing characterization by co-injection with their unradioactive tautomeric form.

CONCLUSION

We showed in this work that the radiolabelling of bromo mazindol 2 can be performed by nucleophilic exchange assisted by the copper I catalyst in acid medium. Optimum conditions were determined to obtain the radioiodinated mazindol cyclic form 4a with about 30 - 35% yield. HPLC

allowed the separation of the two tautomeric forms 4a and 4b. These two forms were collected without added carrier and with a radiochemical purity > 95%. Biological experiments proved that only the cyclic form 4a was valuable for SPECT explorations of the central dopaminergic system uptake.

This paper also describes a strategy for the optimization of the labelling of compounds which can exist under several tautomeric forms.

EXPERIMENTAL PART

1- GENERAL

HPLC analyses were performed on a LKB isocratic liquid chromatograph fitted with UV at 254 nm and radioactivity detectors, using a reverse phase column 10 RP 18 (25 cm x 4.6 mm) from Chrompack and MeOH/1% ET_3N-$CH_3CO_2NH_4$ buffer at pH 4.8 (40 : 60 v/v) as mobile phase flow : 1 mL/min.

Brominated 2 and iodinated 3 analogs of mazindol 1 were synthesized according described methodology (14).

2- METHODS OF MEASUREMENT OF LABELLING

Preparation of solution A and solution B

Solution A comprised $SnSO_4$ (1 mg), gentisic acid (25 mg) and citric acid (35 mg) in water (2.25 mL) and glacial acetic acid (25 µL).

Solution B comprised $CuSO_4$, 5 H_2O (32.5 mg) in H_2O (10 mL).

Measurement of labelling efficiency as a function of copper I concentration

To determine the effect of the copper I concentration on the labelling efficiency, 1.5 mg of parabrominated analog 2 of mazindol, acetic acid (45 µL), solution A (455 µL), various quantities (5 µL to 100 µL) of solution B and [^{125}I] NaI in 0.1 N NaOH from Amersham (60 µCi, no carrier added, specific activity 2000-2200 Ci/mmol) were added to the sealed vial. The reaction mixture was stirred for 5 min and heated in a sand bath for 50 min at 150 °C, then the reactor was put in an ice bath to stop the reaction and an aliquot was taken from the vial for measurement of radiochemical yield by HPLC.

Solution B µL	0	5	10	15	30	45	60	100
$[Cu^+]10^{-4}$ M	0	1.25	2.50	3.75	7.37	10.8	14.0	21.7
R(%) 4a	0	8.20	17.1	21.3	26.5	25.0	16.9	12.4
R(%) 4b	0	3.80	8.60	10.0	12.3	24.4	26.8	27.9

Measurement of labelling efficiency as a function of para brominated precursor 2 concentration

To determine the effect of the concentration of bromo precursor 2 on the labelling efficiency, various quantities (0.2 mg to 2.0 mg) of para brominated analog of mazindol, acetic acid (45 µL), solution A (455 µL, solution B (30 µL) and [^{125}I] NaI in 0.1 N NaOH from Amersham (60 µCi , no carrier added, specific activity 2000-2200 Ci/mmol) were added to the sealed vial. The reaction mixture was stirred for 5 min and heated in a sand bath for 50 min at 150 °C, then the reactor was put in an ice bath to stop the reaction and an aliquot was taken from the vial for measurement of radiochemical yield by HPLC.

Precursor (2) mg	0.2	0.5	0.7	1.0	1.5	2.0
Precursor (2) 10^{-3} M	1.43	2.86	4.30	5.73	8.60	11.50
R(%) 4a	9.40	9.75	13.7	22.1	35.3	21.9
R(%) 4b	4.40	6.94	10.2	9.75	13.4	9.16

Measurement of labelling efficiency as a function of reaction temperature

The variation of labelling efficiency as a function of reaction temperature was measured using the kits prepared as described below. In every kit, we introduced 1.5 mg of the bromo precursor 2 and 30 µL of the solution B. Each vial was placed in a sand bath and was heated to a chosen temperature. The vial was removed and put in an ice bath to stop the reaction after 50 min heating and the labelling efficiency was measured by chromatography.

Temperature °C	20	60	100	130	150	170
R(%) 4a	0	1.30	2.95	14.3	26.5	6.15
R(%) 4b	0	0	0	10.8	12.3	26.6

Measurement of labelling efficiency as a function of reaction time

The variation of labelling efficiency as a function of reaction time was measured using the kits prepared as described below. In every kit, we introduced 1.5 mg of bromo precursor 2 and 30 µL of the solution B. Each vial was placed in a sand bath and was heated to 150 °C. The vial was removed and put in an ice bath to stop the reaction after a chosen reaction time and the labelling efficiency was measured by chromatography.

Reaction Time (min)	0	3	6	9	12	15	20	30	40	50	60	70	90
R(%) 4a	0	0	0	1.3	1.8	3.2	4.3	14	20	27	16	3.0	0
R(%) 4b	0	0	0	0	0	0	0	1.5	2.7	5.4	43	52	70

3- RADIOSYNTHESIS

5-(4-[^{125}I]Iodophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]isoindole 4a

[^{125}I] NaI in 0.1 N NaOH from Amersham (5.5 µL, 550 µCi, no-carrier-added, specific activity 2000-2200 Ci/mmol) was added to a mixture of brominated precursor 2 (1.5 mg) in glacial acetic acid (45 µL), solution A (455 µL) and solution B (30 µL) in a sealed vial. The reaction was allowed to proceed at 150 °C for 50 min. Radiolabelled compounds were purified on a SEP-PAK C18 cartridge from Waters, using H_2O for washing and MeOH (2 mL) for elution. The solvent was then removed under a stream of nitrogen and the residue was dissolved in the mobile phase MeOH / 1% Et_3N in $CH_3CO_2NH_4$ buffer at pH 4.8 (40:60 v/v) used for HPLC.
The appropriate fractions were collected and the mobile phase was eliminated on a SEP-PAK C18 cartridge, using an excess of H_2O. After elution with MeOH (2 mL) and evaporation of the solvent to dryness the no-carrier-added radiolabelled cyclic form 4a [R (%) = 20 à 25% - Rt = 35 min] and the open form 4b [R (%) = 10 à 15% - Rt = 55 min] were isolated with a radiochemical purity of >95%.

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REFERENCES

1. Pearce R.K.B., Seeman P., Jellinger K., Tourtelotte W.W. - Eur. Neurol. 30 9-14 (1990)
2. Lee T., Seeman P., Tourtelotte W.W., Farley I.J., Hornykiewicz O. - Nature 274 897-900 (1978)
3. Foulon C., Garreau L., Chalon S., Desplanches G., Frangin Y., Besnard J.C., Baulieu J.L., Guilloteau D. - Nucl. Med. Biol. 19 597-600 (1992)
4. Hanson R., Byon C., Matsushita T., Fischman A.J., Strauss H.W., Brill R., Elmaleh D.R. - In abstract of IXth International Symposium on Radiopharmaceutical Chemistry, Paris 246 (1992)
5. Boja J.W., Patel A., Carroll F. I., Rahman M. A., Philip A., Lewin A. H., Kopajtic T.A., Kuhar M.J., - Eur. J. Pharma. 194 133-134 (1991)
6. Shaya E.K., Scheffel U., Dannals R.F., Ricaurte G. A., Carroll F.I., Wagner H.N., Kuhar M. J., Wong D.F. - Synapse 10 169-172 (1992)
7. Baldwin R.M., Zea Ponce Y., Zoghbi S.S., Laurelle M., Al-Tikriti M.S., Sybirska E.H., Malison R.T., Neumeyer J.L., Milius R.A., Wang S., Stabin M., Smith E.O., Charney D.S., Hoffer P.B., Innis R.B. - Nucl. Med. Biol. 20 597-606 (1993)
8. Allard P., Alafuzoff I., Carlsson A., Eriksson K., Ericson E., Gottfries C.G., Marcusson J.O. - Eur. Neurol. 30 181-185 (1990)
9. Javitch J.A., Blaustein R.O., Snyder S.H. - Mol. Pharmacol. 26 35-44 (1984)

10. Aeberli P., Eden P., Gogerty J.H., Houlihan W.J., Penberthy C. - J. Med. Chem. 18 177-182 (1975)

11. Houlihan W.J., Parrino V.A. - J. Org. Chem. 47 5177-5180 (1982)

12. Houlihan W.J. - G.B. Pat 1,225,411 (1971)

13. Houlihan W.J. - U.S. Pat 3,932,450 (1976)

14. Galinier E., Garreau L., Dognon A.M., Ombetta-Goka J.E., Frangin Y., Chalon S., Besnard J.C., Guilloteau D. - Eur. J. Med. Chem. 28 927-933 (1993)

15. Mertens J. - American Chemical Society Meeting - Washington (1990)

16. Mertens J., Gysemans M.- Plenum Press Ed : AM Emran - New York (1991)

17. Gysemans M, Mertens J.- In abstract "E_1" of IXth International Symposium on Radiopharmaceutical Chemistry, Paris (1992)

18. Mertens J., Vanryckeghem W., Gysemans M., Eersels J., Finda-Panek E., Carlsen L. - Eur.J. Nucl. Med. 13 380-381 (1987)

19. Barcza S., Houlihan W.J. - J. Pharma. Sci. 64 829-831 (1975)

20. Mertens J., Gysemans M., Bossuyt-Piron C., Thomas M. - J. Lab. Compds. Radiopharm. 28 (6) 731-737 (1990)