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Original article

Synthesis and biological evaluation of potential 5-HT$_7$ receptor PET radiotracers

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ABSTRACT

Brain serotonin 7 receptor (5-HT$_7$) is involved in several mood disorders and drug candidates targeting this subtype are currently in development. Positron emission tomography (PET) is a molecular imaging modality offering great promise for accelerating the process from preclinical discovery to clinical phases. As no PET radiopharmaceutical has yet been used successfully to study the 5-HT$_7$ receptor *in vivo*, our objective is to develop the first 5-HT$_7$ fluorine-18 labeled radiotracer.

Four structural analogs of **SB269970**, a specific 5-HT$_7$ receptor antagonist, divided in FP3 series and FPMP series were synthesized. Their antagonist effects were investigated by cellular functional assay. Nitro-precursors of these analogs were radiolabeled via a [^{18}F$^-$]nucleophilic substitution and *in vitro* autoradiographies were performed in rat brain.

Chemical and radiochemical purities of fluorine radiotracers were >99% with specific activities in 40–129 GBq/μmole range. The four derivates presented antagonism potencies toward 5-HT$_7$ receptors (pK$_B$) between 7.8 and 8.8. The four PET radiotracers had suitable characteristic for 5-HT$_7$ receptor probing *in vitro* even if the FP3 series seemed to be more specific for this receptor. These results encourage us to pursue *in vivo* studies.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a central neurotransmitter involved in physiological functions, as well as neurological and pathological disorders. Pharmacological studies allowed identification of numerous serotoninergic receptors families and subtypes which were classified by structural, functional and pharmacological criteria into seven distinct receptor classes (5-HT$_{1-7}$) [1].

The 5-HT$_7$ subtype is the most recently cloned serotonin receptor [2]. 5-HT$_7$ receptors (5-HT$_7$R) are coupled to Gs proteins [3] and are found in rodent, pig, non-human and human primate with relatively high concentrations in hippocampus, thalamus, and hypothalamus and, with lower levels, in cortex and amygdala [4].

The recent availability of selective 5-HT$_7$ receptor antagonists [5] and of 5-HT$_7$ receptor knockout mice [6] has considerably advanced the understanding of the physiological function of this receptor [7]. The 5-HT$_7$ receptor has been identified as important in circadian rhythms and sleep [8]. As in the case of the 5-HT$_6$ subtype, 5-HT$_7$ receptors also bind several antidepressants (*e.g.*, mianserin, maprotiline) and antipsychotics (clozapine, risperidone) with high affinity, indicating that this receptor may represent a therapeutic target for schizophrenia and mood disorders. In fact, selective 5-HT$_7$ antagonists were proposed as a potential treatment for schizophrenia or depression [9]. Several reports, based on receptor distribution and preliminary pharmacologic analyses, suggest that 5-HT$_7$ receptors might represent another serotoninergic target for memory enhancement, epilepsy and pain [10].

Therefore an ability to image serotonin 5-HT$_7$ receptors in human brain *in vivo* is needed to assess directly this receptor involvement in neuropsychiatric diseases and their possible therapies. With the development of positron emission tomography (PET) as a molecular imaging method, the opportunity has evolved to perform *in vivo* observations both in animal models and in humans [11]. These studies can be performed at "tracer concentrations" implying the intravenous administration of a "radiotracer" at a very small amounts (3–10 μg) and far away from levels at which pharmacological effects might occur. This implies the availability of a PET radiotracer which specifically labels the 5-HT$_7$ receptor.

If several selective antagonists exist [5], few have been radiolabeled for use in radioligand binding and autoradiography studies

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[12]. For example, [^{3}H]**SB269970**, a high affinity and selective ligand (K_d = 1.2 nM) [13], has been used to radiolabel *in vitro* the 5-HT$_7$ receptor in rodent, pig, marmoset and human brain [4b, 14]. It demonstrated high specific binding that was saturable and monophasic, with fast association and dissociation kinetics.

Notably, only one team developed a 5-HT$_7$ radiotracer for PET imaging. DR4446 is a PET tracer synthesized from tetrohydrobenzindole derivatives. It was shown to possess high selectivity for 5-HT$_7$ over the other 5-HT receptors, and moderate affinity (K_i = 9.7 nM). On this basis, [^{11}C]**DR4446** was radiosynthesised and tested in monkey [12c]. This preliminary PET study indicated that DR4446 had good brain penetrability, a specific binding component, and was metabolically stable. However, to our knowledge, no further reports have been published yet with this radiotracer. Furthermore, the use of ^{11}C radioisotope is not always optimal for further studies because of its short half-life ($T_{1/2}$ = 20 min).

We have recently reported a first attempt to modify the **SB269970** structure by introducing a *p*-[^{18}F]benzoïc ester moiety in 4-position of the piperidine (**1**, Fig. 1) [15]. However, this compound appeared to be too sensitive to *in vivo* hydrolysis and, consequently, was rapidly metabolized. Consequently, despite a suitable lipophilicity (logD), only a low brain penetration was measured *in vivo* [15].

Here we describe the synthesis, the radiolabeling and the *in vitro* distribution of a new improved series of potential 5-HT$_7$ receptor ligands derived from **SB-269970** and rationally designed for an eventual use as PET radiotracer.

2. Results and discussion

2.1. Chemistry

Because of the radioactive decay of positron emitters, the half-lives of radioisotopes constitute crucial parameters in the synthesis of radiolabeled compounds. Therefore, we envisaged to use fluorine-18 ($T_{1/2}$ = 109.7 min), more convenient for preclinical and clinical uses than carbon-11 radiolabeling ($T_{1/2}$ = 20 min). Because of its high specific radioactivity and its straightforward production via the ^{18}O(p,n) reaction, $^{18}F^-$ anion constitutes the most common source for ^{18}F introduction into molecules. We chose to introduce the radioisotope at the last step, to conserve the maximum of radioactivity, by an aromatic nucleophilic substitution (S_NAr) reaction using radioactive fluoride anions. Consequently, the structural modifications of **SB269970** and the synthesis strategy of targeted molecules take in consideration these constraints.

To avoid the undesirable *in vivo* metabolization previously observed with **1** [15], ^{18}F labeling has been introduced onto a less reactive part of **SB269970**. Since we previously described the propensity of sulfonyl group to favorise S_NAr reaction with fluoride anion [16], we choose to introduce the radioisotope onto the phenylsulfonyl moiety. Then, two direct analogs of **SB269970** have been envisaged with a *ortho* or *para*-fluoro-phenylsulfonyl group grafted on the nitrogen of the pyrrolidine ([^{18}F]**2a** and [^{18}F]**2b**, Fig. 2). Because the (2-methoxyphenyl)piperazine core has contributed to bring good affinity for 5-HT$_7$R to certain ligands [12b], two others molecules bearing this moiety instead of the methyl-piperidine heterocycle have been also considered ([^{18}F]**3a** and [^{18}F]**3b**, Fig. 2).

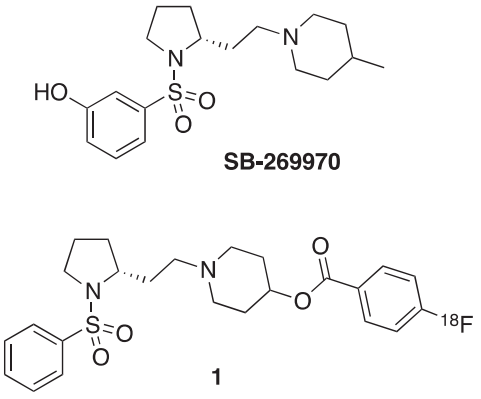


Fig. 1. Structure of **SB269970** and the first [^{18}F]radiolabeled derivated structure.

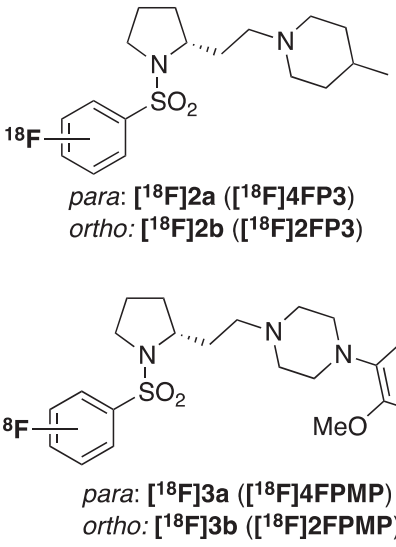
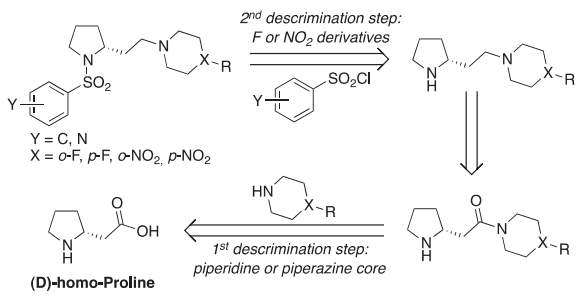


Fig. 2. Structures of the 4 new radioligands.

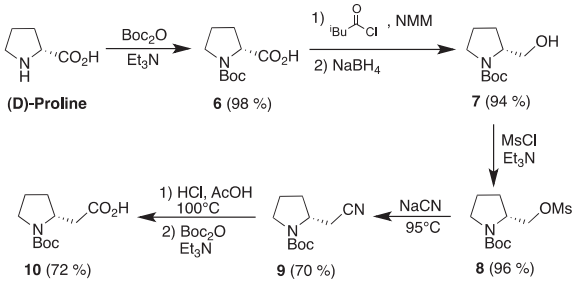
In each compound, the fluorine atom is in *ortho* or *para* position of electron-withdrawing group (required for S_NAr reaction) and could be inserted in the last step by the nitro group substitution by activated fluoride-18 anion. Each envisaged ligand required the synthesis of 2 compounds: the non-radioactive fluorinated molecules (**2**, **3**), required for *in vitro* affinity measurement and the nitro-derivatives (**4**, **5**), precursors for radiolabeling. Then, to optimize these syntheses, a divergent strategy has been elaborated via a common synthetic way, with discriminations at the late steps, as illustrated in the retrosynthetic Scheme 1. This strategy presents the interest to allow further modifications of the structures, if necessary.

The starting material is the *N*-Boc-(D)-homo-proline (**10**), which was easily obtained from (D)-proline with an overall yield of 44%, by a slightly modified multistep homologation procedure (Scheme 2) [15,17,18].

The *N*-Boc-(D)-homoproline **10** could be then coupled with the corresponding nitrogen heterocycle, depending on the expected



Scheme 1. Retrosynthetic analysis.



Scheme 2. Synthesis of *N*-Boc-(D)-homo-proline from (D)-proline.

series (**FP3** or **FPMP**). The subsequent reduction of the resulting amide (**11**–**12**) with $LiAlH_4$ gave **13**–**14** with good yield over the 2 steps. At this step, the arylsulfonyl group was chosen and this one was introduced, after Boc deprotection, with the corresponding arylsulfonyl chloride (Scheme 3).

The expected non-radioactive fluorinated ligands and their nitro-precursor for labeling have been obtained in 9 steps from (D)-proline with an overall yield in the range of 13–20% and 20–21% for **2**–**4** and **3**–**5** respectively.

2.2. Physico-chemical results and in vitro affinity

Since the lipophilicity is predictive of the blood-brain-barrier penetration, lipophilicity (logD) of compounds **2**–**3** has been calculated with the ACD/Labs software (Table 1). The values of estimated logD at physiological pH indicate a lipophilicity that should theoretically lead to good brain permeation.

Ligand affinities toward 5-HT_7 receptors (K_B) have been determined by cellular functional assays on expressed human recombinant receptors in CHO cells (Table 1). Because of some similarities between binding sites of 5-HT_7 receptors and 5-HT_{1A} and 5-HT_6 receptors [19], affinities toward these receptors have been also investigated (Table 1).

Table 1
Lipophilicity (logD) and 5-HT_7, 5-HT_{1A}, 5-HT_6 affinities (pK_B) of compounds **2**–**3**.

Compounds	logD (pH = 7.4) [a]	K_B 5-HT_7 [b]	K_B 5-HT_{1A} [b]	K_B 5-HT_6 [b]
4FP3 (**2a**)	1.92	14 nM	>10 μM	>10 μM
2FP3 (**2b**)	1.43	8.4 nM	>10 μM	>10 μM
4FPMP (**3a**)	3.89	4.8 nM	>10 μM	>10 μM
2FPMP (**3b**)	3.38	1.6 nM	>10 μM	>10 μM

[a] calculated with ACD/Labs V. 7.09 software.
[b] determined by CEREP (www.cerep.com)

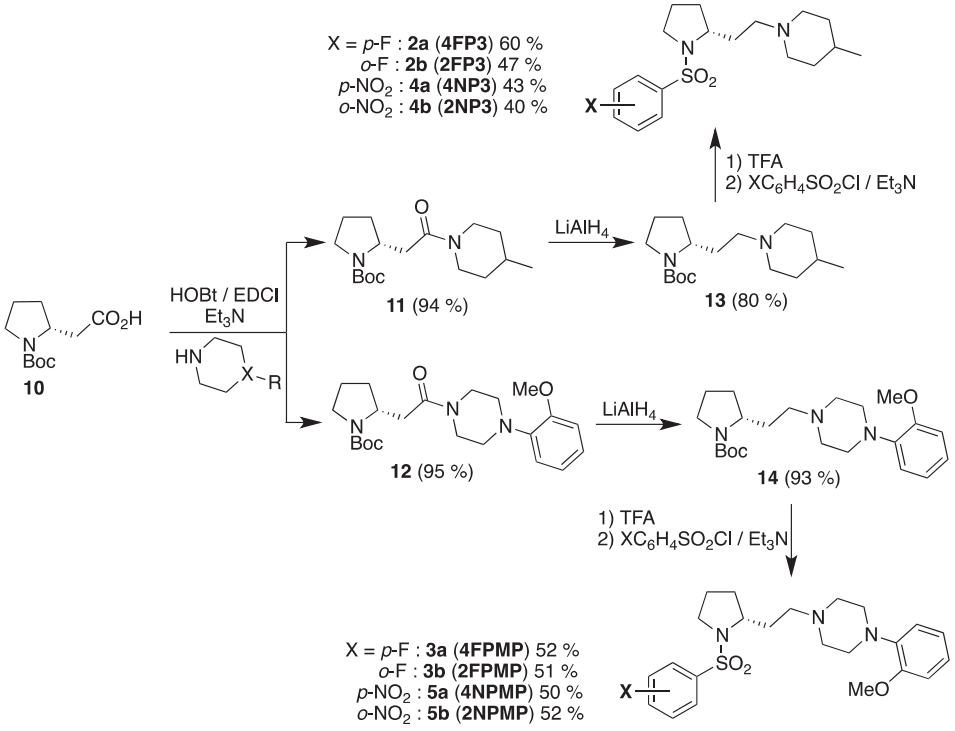
The apparent dissociation constant (K_B) demonstrated good affinities of the fluorinated molecules for 5-HT_7R, in particular for **2FPMP** with a value below 2 nM. Such affinities in the range of 1–10 nM constitute an important result because of the low 5-HT_7 receptors density observed in various brain areas [4b]. Furthermore, the K_B > 10 μM observed for 5-HT_6 and 5-HT_{1A} receptors indicated a low affinity of the series for these receptors.

These good lipophilicities, affinities and their probable selectivity toward 5-HT_7 receptor encouraged us to perform the radiolabeling of the nitro-precursors **4**–**5** to obtain the ^{18}F analogs of **2**–**3**.

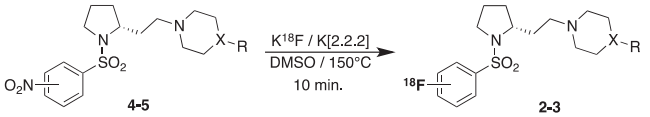
2.3. Radiochemistry

Labeling of **4**–**5** was realized by nucleophilic aromatic substitution of nitro group, in classical conditions at 150 °C, in dimethylsulfoxyde (DMSO) and in presence of kryptofix® (Scheme 4, Table 2) [20].

The radiolabeling of the various nitro-precursors (**4**–**5**) gave good radiochemical yields (EOB: End of Bombardment) in the range of 34–44% (Table 2, entries 1, 4, 6, 8). Furthermore, the *ortho* fluorination led to similar yields than the *para* (Table 2, entries 1,4 and 7,8), demonstrating that the steric hindrance was compensated



Scheme 3. Synthesis of non-radioactive ligands and their nitro-precursors for radiolabeling.



Scheme 4. Radiolabeling of **4**–**5**.

by the high reactivity of the aromatic ring due to the high electron-withdrawing character of the sulfonyl group. This high reactivity allowed us also to diminish significantly the amount of nitro-precursor without dramatic decrease of the radiochemical yield (Table 2, entries 3, 9).

The radiolabeled molecules were obtained with a chemical and radiochemical purity > 95% and with specific activities (EOS) in the range of 40–130 GBq.μmol^{-1}.

2.4. In vitro studies in rodent

The *in vitro* distributions of [^{18}F]4FP3, [^{18}F]2FP3, [^{18}F]4FPMP and [^{18}F]2FPMP were evaluated by semi-quantitative autoradiography in rat brains. Autoradiograms in the hippocampus were obtained after incubation with a constant *in vitro* concentration of the radiotracer. The binding pattern corresponded to regions described as rich in 5-HT$_7$ receptors, including the hippocampus (Fig. 3) and cerebellum (results not shown). The *in vitro* competition studies confirmed that the FP3 series specifically binds to 5-HT$_7$ receptors, since addition of **SB269970**, the 5-HT$_7$ antagonist, displaced radiotracer binding dose-dependently. Binding diminution in hippocampus for [^{18}F]4FP3 or [^{18}F]2FP3was −20%, −40% and −65% with 10 nM, 100 nM and 1 μM of **SB269970**, respectively (experiments were performed in triplicate for each radiotracer, Fig. 3). At the same concentrations of **SB269970** and in the same brain area, [^{18}F]4FPMP and [^{18}F]2FPMP binding remained unchanged (experiments were performed in triplicate for each radiotracer, Fig. 3).

3. Conclusion

The aim of the study was to select and evaluate a potential PET radioligand for *in vivo* imaging of brain 5-HT$_7$ receptors. The strategy was to synthesize four molecules and their nitro-precursors and to focus on *in vitro* studies.

From a chemical standpoint, the choice of FP3 and FPMP series structures was inspired by a pharmacophore model and by the structure of **SB269970**, the prototypical 5-HT$_7$ antagonist. Ortho and para positions were chosen for fluorine nucleophilic substitution, and two hydrophobic groups were selected, leading to the synthesis of the FP3 series (**4FP3** with ^{18}F in para, and **2FP3** with ^{18}F in ortho) and FPMP series (**4FPMP** with ^{18}F in para, and **2FPMP** with ^{18}F in ortho). Syntheses were realized starting from D-proline in ten steps. At the last stage, the labeling of the nitro-precursor was straightforward for each tracer candidate with excellent radiochemical purity and a satisfactory specific activity.

Table 2
Radiochemical yields of the radiolabeling of **4**–**5**.

Entry	Compounds	Quantity of precursor	RCY (EOB)[a]	Specific activity (EOS)[b]
1	**4FP3**	3.8–4.5 mg	44% ($n = 7$)	44–130 GBq.μmol^{-1}
2		2.1–2.5 mg	30% ($n = 2$)	
3		1.2–1.3 mg	15% ($n = 2$)	
4	**2FP3**	4.1–4.9 mg	37% ($n = 7$)	41–105 GBq.μmol^{-1}
5		2.7–3.0 mg	30% ($n = 3$)	
6	**4FPMP**	4.1–4.7 mg	43% ($n = 3$)	44–113 GBq.μmol^{-1}
7		3.2–3.8 mg	39% ($n = 4$)	
8	**2FPMP**	2.5–3.8 mg	34% ($n = 5$)	41–91 GBq.μmol^{-1}
9		1.8 mg	29% ($n = 1$)	

[a] based on the fluorine-18 activity recovered from the resin (EOB: end of bombardment). Mean of radiochemical yields of several radiolabeling (in parenthesis, number of radiolabeling experiments).
[b] EOS: End of synthesis.

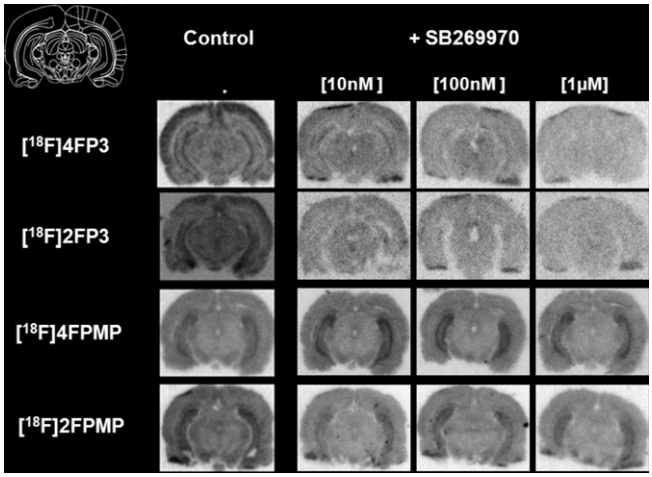


Fig. 3. *In vitro* autoradiography of brain sections of rat incubated with [^{18}F]4FP3 (SA = 80 GBq.μmol^{-1}), [^{18}F]2FP3 (SA = 70 GBq.μmol^{-1}), [^{18}F]4FPMP (SA = 74 GBq.μmol^{-1}) and [^{18}F]2FPMP (SA = 61 GBq.μmol^{-1}) in hippocampus region. Columns from the left side to the right side: the picture corresponding to the anatomic coronal slice in hippocampus; for each radiotracer, *in vitro* control autoradiography; corresponding autoradiography supplemented with **SB269970**, a selective 5-HT$_7$ antagonist, at 10 nM, 100 nM or 1 μM.

In vitro assay revealed the nanomolar affinity of the four molecules for the 5-HT7 receptor. *In vitro* studies in rodents revealed that only FP3 series (and not FPMP series) had a specific binding to 5-HT$_7$ receptors, reversed by a 5-HT$_7$ antagonist. These initial results encourage us to focus on future *in vivo* studies on the FP3 series ([^{18}F]**4FP3** and [^{18}F]**2FP3**) to investigate their potential as 5-HT$_7$ radiotracers for human brain imaging.

4. Experimental section

4.1. Materials and methods

The purity of the tested compounds **2**, **3** and their nitro-precursors **4**, **5** has been assessed by reverse phase high-performance liquid chromatography (RP-HPLC) and Elemental analyses. All compounds showed >95% purity. Column chromatography was performed with Macherey–Nagel silica gel 60 M (0.04–0.063 nm). Melting points were determined with Kofler bench. ^{1}H and ^{19}F NMR spectra were recorded, respectively, at 300 and 282 MHz on a Bruker ALS300 spectrometer. ^{13}C NMR spectra were recorded at 100 MHz on a Bruker DRX400 spectrometer. All spectra were recorded in $CDCl_3$. All chemical shift values are reported in ppm (δ) relative to tetramethylsilane (TMS) for ^{1}H and ^{13}C NMR spectra and to $CFCl_3$ for ^{19}F NMR spectra and coupling constant (J) in Hertz. The *N*-Boc-D-homoproline **10** and all the intermediates of the synthesis (**6**–**9**) were obtained according previously published procedures [15,17,18].

4.1.1. General procedure for the coupling of the N-boc-(D)-homoproline (*10*) with amines

To a solution of **10** (1 eq.) in DMF (2 ml per mmol of starting material) were added HOBt (1.1 eq.), EDCI (1.1 eq.) and triethylamine (1.6 eq.). The amine (4-methylpiperidine or 1-(2-methoxyphenyl)piperazine, 1.1 eq.) was added last to that mixture. After stirring overnight at room temperature AcOEt was added (15 ml for

1 ml of DMF) and the resulting solution was washed several times with 10% LiCl and brine. The organic layer was dried over $MgSO_4$ and concentred *in vacuo* to afford the expected product with a satisfactory purity, without further purification.

*4.1.2. Tert-butyl (2R)-2-[2-(4-methylpiperidin-1-yl)-2-oxoethyl] pyrrolidine-1-carboxylate (**11**)*

Yellow oil (Complex mixture of 4 rotamers). ^{1}H NMR: $\delta = 0.93$ (d, $J = 6.1$, 3H), 1.06 (m, 2H), 1.45 (s, 9H), 1.63 (m, 3H), 1.85 (m, 4H), 2.26 (m, 1H), 2.51 (m, 1H), 2.95 (m, 2H), 3.33 (m, 2H), 4.07 (m, 2H), 4.54 (m, 1H). ^{13}C NMR: $\delta = 21.3$, 21.4, 22.2, 23.1, 28.1, 29.5, 30.3, 30.6, 30.7, 33.4, 34.3, 34.4, 34.6, 37.2, 37.4, 37.9, 41.4, 41.5, 45.5, 45.7, 45.9, 46.2, 54.1(bs), 79.1, 79.6, 153.8, 153.9, 168.5, 168.7. Anal. Calcd for $C_{17}H_{30}N_2O_3$: C, 65.77; H, 9.74; N, 9.02. Found: C, 65.97; H, 9.91; N, 8.84.

*4.1.3. Tert-butyl (2R)-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]-2-oxoethyl}pyrrolidine-1-carboxylate (**12**)*

Yellow oil (Complex mixture of 4 rotamers). ^{1}H NMR: $\delta = 1.40$ (s, 9H), 1.84 (m, 4H), 2.19 (m, 1H), 3.01 (m, 5H), 3.28 (m, 2H), 3.67 (m, 4H), 3.78 and 3.79 (2s, 3H), 4.03 (m, 1H), 6.90 (m, 4H). ^{13}C NMR: $\delta = 23.0$, 23.9, 28.8, 28.9, 30.2, 31.2, 38.1, 38.6, 42.1, 46.4, 46.7, 47.1, 50.9, 51.7, 52.0, 54.9, 55.0, 55.7, 55.8, 111.6, 118.6, 118.7, 121.4, 123.6, 123.7, 141.1, 141.2, 152.6, 152.6, 154.7, 154.8, 169.6, 169.8. Anal. Calcd for $C_{22}H_{33}N_3O_4$: C, 65.48; H, 8.24; N, 10.41. Found: C, 65.59; H, 7.87; N, 10.59.

4.1.4. General procedure for the reduction with $LiAlH_4$ of 11 and 12

To a solution of $LiAlH_4$ (2.1 eq.) in anhydrous THF (3 ml per mmol $LiAlH_4$), under inert atmosphere and at 0 °C, was slowly added a solution of the amide compound (**11** or **12**, 1 eq.) in anhydrous THF. The reaction was then allowed to reach room temperature, stirred for 210 min and then quenched at 0 °C with aqueous saturated NH_4Cl solution. The resulting mixture was filtered over Celite and extracted with dichloromethane. The combined organic layers were dried over $MgSO_4$ and evaporated to afford the expected product.

*4.1.5. Tert-butyl (2R)-2-[2-(4-methylpiperidin-1-yl)ethyl] pyrrolidine-1-carboxylate (**13**)*

Yellow oil (Mixture of 2 rotamers). ^{1}H NMR: $\delta = 0.89$ (m, 3H), 1.25 (m, 3H), 1.44 (s, 9H), 1.59 (m, 3H), 1.88 (m, 7H), 2.31 (m, 2H), 2.88 (m, 2H), 3.58 (m, 2H), 3.75 (bs, 1H). (*Conform to literature* [18a]).

*4.1.6. Tert-butyl (2R)-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl] ethyl}pyrrolidine-1-carboxylate (**14**)*

Yellow oil (Mixture of 2 rotamers). ^{1}H NMR: $\delta = 1.46$ (s, 9H), 1.68 (m, 1H), 1.87 (m, 5H), 2.44 (m, 2H), 2.67 (m, 3H), 3.08 (m, 5H), 3.32 (m, 2H), 3.75 (m, 1H), 3.86 and 3.87 (2s, 3H), 6.93 (m, 4H). Anal. Calcd for $C_{22}H_{35}N_3O_3$: C, 67.83; H, 9.06; N, 10.79. Found: C, 68.10; H, 9.34; N, 11.08.

*4.1.7. General procedure: synthesis of **2**–**5***

Deprotection of tert-butyloxycarbonyl (Boc) was done by dissolving the compound (**13** or **14**) in a Trifluoroacetic acid (TFA)/dichloromethane (1:3) mixture. The solution was stirred overnight at 50 °C and the volatile part was removed under reduced pressure. The resulting mixture was dissolved in dichloromethane and washed several times with sat. Na_2CO_3. The organic layer was dried over $MgSO_4$ and evaporated to give the crude product used as such in the next step. To a mixture of the previous crude (1 eq.) and triethylamine (3 eq.) in dichloromethane (30 ml per mmol of starting amine), under inert atmosphere, was added the proper sulfonylchloride (2- or 4-nitrobenzenesulfonylchloride, 2- or 4-fluorobenzenesulfonylchloride, 1.05 eq.) at 0 °C. After stirring at 0 °C for 3 h, the mixture was washed with 10% NaOH and brine. The organic layer was dried over $MgSO_4$, evaporated and purified by column chromatography on silica gel (pre-treated with triethylamine and using dichloromethane/MeOH (98:2) as eluant.

*4.1.8. 1-(2-{(2R)-1-[(4-Fluorophenyl)sulfonyl]pyrrolidin-2-yl} ethyl)-4-methylpiperidine (**2a**, **4FP3**)*

Beige solid. Mp < 40 °C. ^{1}H NMR: $\delta = 0.91$ (d, $J = 6.36$, 3H), 1.24 (m, 2H), 1.35 (m, 1H), 1.57 (m, 6H), 1.79 (m, 1H), 1.92 (m, 2H), 2.07 (m, 1H), 2.38 (m, 2H), 2.85 (bd, $J = 11.1$, 1H), 2.95 (bd, $J = 11.1$, 1H), 3.15 (m, 1H), 3.39 (m, 1H), 3.66 (m, 1H), 7.18 (t, $J = 8.7$, 2H), 7.84 (dd, $J = 5.13$, 8.7, 2H). ^{13}C NMR: $\delta = 22.0$, 24.2, 30.9, 31.0, 33.6, 34.4, 49.0, 53.9, 54.5, 55.7, 59.1, 116.3 (d, $J_{C-F} = 22.4$), 130.2 (d, $J_{C-F} = 9.2$), 134.1 (d, $J_{C-F} = 3.3$), 165.2 (d, $J_{C-F} = 254.4$). ^{19}F NMR: $\delta = -106$,1 (m). Anal. Calcd for $C_{18}H_{27}FN_2O_2S$: C, 60.99; H, 7.68; N, 7.90. Found: C, 61.13; H, 7.84; N, 8.12.

*4.1.9. 1-(2-{(2R)-1-[(2-Fluorophenyl)sulfonyl]pyrrolidin-2-yl} ethyl)-4-methylpiperidine (**2b**, **2FP3**)*

Yellow oil. ^{1}H NMR: $\delta = 0.95$ (d, $J = 5.3$, 3H), 1.41 (m, 3H), 1.79 (m, 7H), 2.11 (m, 3H), 2.60 (m, 2H), 3.05 (m, 2H), 3.39 (m, 2H), 3.93 (m, 1H), 7.24 (m, 2H), 7.57 (m, 1H), 7.91 (m, 1H). ^{13}C NMR: $\delta = 22.0$, 24.6, 30,7, 31.6, 32.7, 33.6, 48.7 (d, $J_{C-F} = 2.6$) 54.0, 54.2, 55.6, 59.1 (d, $J_{C-F} = 3.1$), 117.6 (d, $J_{C-F} = 22.3$), 124.8 (d, $J_{C-F} = 3,8$), 126.75 (d, $J_{C-F} = 15.2$), 132.1, 135.2 (d, $J_{C-F} = 8.5$), 159.2 (d, $J_{C-F} = 254$). ^{19}F NMR: $\delta = -107$,7 (m). Anal. Calcd for $C_{18}H_{27}FN_2O_2S$: C, 60.99; H, 7.68; N, 7.90. Found: C, 60.67; H, 7.40; N, 8.02.

*4.1.10. 4-Methyl-1-(2-{(2R)-1-[(4-nitrophenyl)sulfonyl]pyrrolidin-2-yl}ethyl)piperidine (**4a**, **4NP3**)*

Beige solid. Mp = 132–134 °C. ^{1}H NMR: $\delta = 0.95$ (d, $J = 6.4$, 3H), 1.36 (m, 3H), 1.61 (m, 6H), 1.90 (m, 3H), 2.13 (m, 1H), 2.39 (m, 2H), 2.87 (d, $J = 10.9$, 1H), 2.99 (d, $J = 10.9$, 1H), 3.21 (m, 1H), 3.48 (m, 1H), 3.76 (m, 1H), 8.04 (d, $J = 9.0$, 2H), 8.39 (d, $J = 9.0$, 2H). ^{13}C NMR: $\delta = 22.3$, 24.5, 31.2, 31.3, 33.8, 34.7, 49.4, 54.1, 54.9, 55.9, 59.7, 124.7, 129.0, 144.1, 150.4. Anal. Calcd for $C_{18}H_{27}N_3O_4S$: C, 56.67; H, 7.13. Found: C, 56.78; H, 6,84.

*4.1.11. 4-Methyl-1-(2-{(2R)-1-[(2-nitrophenyl)sulfonyl]pyrrolidin-2-yl}ethyl)piperidine (**4b**, **2NP3**)*

Yellow oil. ^{1}H NMR: $\delta = 0.94$ (d, $J = 6.3$, 3H), 1.31 (m, 3H), 1.64 (m, 3H), 1.77 (m, 2H), 2.00 (m, 5H), 2.39 (m, 2H), 2.90 (m, 2H), 3.46 (m, 2H), 4.00 (m, 1H), 7.60 (m, 1H), 7.70 (m, 2H), 8.04 (m, 1H). ^{13}C NMR: $\delta = 22.2$, 24.6, 31.1, 31.5, 33.1, 34.5, 49.1, 54.3, 54.5, 55.9, 59.8, 124.3, 131.29, 131.8, 132.7, 133.8, 148.9. Anal. Calcd for $C_{18}H_{27}N_3O_4S$: C, 56.67; H, 7.13. Found: C, 56.91; H, 6.87.

*4.1.12. 1-(2-{(2R)-1-[(4-Fluorophenyl)sulfonyl]pyrrolidin-2-yl} ethyl)-4-(2-methoxyphenyl) piperazine (**3a**, **4FPMP**)*

Yellow oil. ^{1}H NMR: $\delta = 1.54$ (m, 4H), 1.75 (m, 1H), 2.08 (m, 1H), 2.43 (m, 2H), 2.63 (bm, 4H), 3.09 (m, 5H), 3.35 (m, 1H), 3.67 (m, 1H), 3.80 (s, 3H), 6.88 (m, 4H), 7.13 (m, 2H), 7.79 (m, 2H). ^{13}C NMR: $\delta = 24.5$, 31.3, 33.7, 49.4, 51.0, 53.9, 55.6, 55.8, 59.2, 111.6, 118.6, 121.4, 123.4, 141.7, 152.7, 116.7 (d, $J_{C-F} = 22.4$), 130.5 (d, $J_{C-F} = 9.2$), 134.3 (d, $J_{C-F} = 3.3$), 165.49 (d, $J_{C-F} = 254.5$). ^{19}F NMR: $\delta = -106.0$ (m). Anal. Calcd for $C_{23}H_{30}FN_3O_3S$: C, 61.72; H, 6.76; N, 9.39. Found: C, 61.91; H, 7.03; N, 9.63.

*4.1.13. 1-(2-{(2R)-1-[(2-Fluorophenyl)sulfonyl]pyrrolidin-2-yl} ethyl)-4-(2-methoxyphenyl)piperazine (**3b**, **2FPMP**)*

Yellow oil. ^{1}H NMR: $\delta = 1.49–2.41$ (m, 6H), 2.67–3.57 (m, 12H), 3.88 (s, 3H), 4.02 (m, 1H), 6.99 (m, 4H), 7.28 (m, 2H), 7.60 (m, 1H), 7.93 (m, 1H). ^{13}C NMR: $\delta = 24.5$, 31.7, 31.9, 48.8 (d, $J_{C-F} = 2.2$), 49.0, 53.3, 55.4, 55.8, 58.6 (d, $J_{C-F} = 3.9$), 111.7, 117.7 (d, $J_{C-F} = 22.6$),

119.04, 121.50, 124.13, 124.9 (d, $J_{C-F} = 3.2$), 129.9 (d, $J_{C-F} = 16$), 132.25, 135.5 (d, $J_{C-F} = 7.3$), 149.0, 152.5, 159.2 (d, $J_{C-F} = 255.1$). ^{19}F NMR: $\delta = -107.55$ (m). Anal. Calcd for $C_{23}H_{30}FN_3O_3S$: C, 61.72; H, 6.76; N, 9.39. Found: C, 61.85; H, 6.55; N, 9.60.

4.1.14. 1-(2-Methoxyphenyl)-4-(2-{(2R)-1-[(4-nitrophenyl) sulfonyl]pyrrolidin-2-yl}ethyl)piperazine (**5a**, **4NPMP**)

Beige solid. Mp = 98–100 °C. ^1H NMR: $\delta = 1.64$ (m, 3H), 1.90 (m, 2H), 2.22 (m, 1H), 2.50–3.44 (m, 10H), 3.51 (m, 2H), 3.89 (s, 3H), 3.98 (m, 1H), 6.97 (m, 4H), 8.09 (d, $J = 8.5$, 2H), 8.41 (d, $J = 8.5$, 2H). ^{13}C NMR: $\delta = 24.4$, 31.5, 32.5, 49.5, 49.8, 53.6, 55.37, 55.81, 59.17, 111.66, 118.84, 118.88, 121.49, 123.89, 123.95, 124.79, 129.21, 140.71, 143.53, 150.57, 152.55. Anal. Calcd for $C_{23}H_{30}N_4O_5S$: C, 58.21; H, 6.37; N, 11.81. Found: C, 58.40; H, 6.49; N, 11.97.

4.1.15. 1-(2-Methoxyphenyl)-4-(2-{(2R)-1-[(2-nitrophenyl) sulfonyl]pyrrolidin-2-yl}ethyl)piperazine (**5b**, **2NPMP**)

Beige solid. Mp = 172 °C. ^1H NMR: $\delta = 1.69$ (m, 3H), 1.87 (m, 2H), 2.03 (m, 1H), 2.52 (m, 6H), 3.06 (m, 4H), 3.39 (m, 2H), 3.79 (s, 3H), 4.01 (m, 1H), 6.87 (m, 4H), 7.51 (m, 1H), 7.61 (m, 2H), 7.96 (m, 1H). ^{13}C NMR: $\delta = 24.7$, 31.5, 33.0, 49.1, 50.8, 53.8, 55.6, 55.8, 59.6, 111.6, 118.6, 121.4, 123.4, 124.3, 131.3, 131.8, 132.6, 133.9, 141.6, 148.9, 152.7. Anal. Calcd for $C_{23}H_{30}N_4O_5S$: C, 58.21; H, 6.37; N, 11.81. Found: C, 58.49; H, 6.64; N, 12.13.

4.2. Radiosynthesis

Fluorine-18 was produced via the $^{18}O(p,n)^{18}F$ nuclear reaction (IBA Cyclone 18/9 cyclotron). The nitro/fluoro exchange was realized on a standard Coincidence (GE Mx) synthesizer after reprogramming the automation sequence: after initial fluoride preparation (collection, drying and kryptofix activation), 1–5 mg of nitro precursors **4**–**5** were introduced, and the reaction mixture was heated at 150 °C for 10 min in DMSO. After dilution with 15 ml of water, the reaction mixture was passed through an activated C18 cartridge for pre-purification, and the crude product was eluted from the cartridge with 2 ml of ethanol. Pure **2**–**3** were obtained after separation on a preparative high-performance liquid chromatography (HPLC) (C18 Symmetry Prep Waters 7 μm 7.8 × 300 mm) eluted with H_3PO_4 20 mM/THF/TFA(0.1%) 3 ml min^{-1} (**2**: $\lambda = 240$ nm; **3** : $\lambda = 254$ nm). For biological use, the radiotracers were formulated via SPE techniques [21]. The dilution of the product was done with 40 ml of sterile water and loaded on a SEP-Pak Light C18 cartridge (Waters, Milford, MA, USA). The loaded cartridge was rinsed with water, eluted with 1 ml of ethanol, and the final product was diluted with isotonic saline and sterilized by filtration (sterile filter Millex-GS, 0.22 μm). The radiochemical purity and specific activity of [^{18}F]**2**–**3** were assayed by analytical HPLC (Macherey–Nagel EC 250/4.6 Nucleodur 100–5 C18 column; mobile phase H_3PO_4 20 mM/THF; flow rate, 0.9 mL/min (**2**) or 0.8 mL/min (**3**)). The identities of [^{18}F]**2**–**3** were confirmed by co-injection with an authentic non-radioactive sample.

4.3. In vitro Pharmacology

The respective affinity toward 5-HT_7, 5-HT_6, 5-HT_{1A} receptors (K_B) has been determined by the CEREP (experimental details are provided at http://www.cerep.fr). Briefly, cellular functional assays were performed to search a possible antagonist effect for the four compounds. Competition binding experiments were performed on expressed human recombinant receptors in CHO cells. The IC_{50} value (concentration causing a half-maximal inhibition of the control specific agonist response) were determined by non-linear regression analysis of the concentration-response curves generated with mean replicate values using Hill equation curve fitting. This analysis was performed using the commercial software SigmaPlot® 11.0. The apparent dissociation constant (K_B) was calculated using the modified Cheng Prusoff equation.

Lipophilicity (logD) of compounds was calculated with the software ACD/Labs® 7.0 which simulate the lipophilicity from the chemical structures.

4.4. In vitro studies in rodents

Male Sprague–Dawley rats were used. After euthanasia by an overdose isoflurane inhalation, the brain was carefully removed and immediately frozen in 2-methylbutane cooled with dry ice (−29 °C). Briefly, coronal sections (30 μm thick) cut across the hippocampus and the cerebellum were carried out using a −20 °C cryostat (Microm-Microtech), thaw-mounted on glass slides and allowed to air dry before storage at −80 °C until used. The day of radiotracers synthesis, the slides were allowed to reach ambient temperature and were then incubated for 20 min in Tris phosphate-buffered saline buffer (138 mM NaCl, 2.7 mM KCl, pH adjusted to 7.6) containing 37 kBq/ml (1 μCi/ml) of [^{18}F]**4FP3**, [^{18}F]**2FP3**, [^{18}F]**4FPMP** or [^{18}F]**2FPMP**. For competition experiments, the slides were placed in the same buffer supplemented with **SB269970**, a selective 5-HT_7 antagonist (10 nM, 100 nM or 1 μM). After incubation, the slides were dipped in cold buffer (4 °C) for 90 s, in distilled cold water (4 °C) for 90 s, then dried and juxtaposed to a phosphor imaging plate for 60 min (BAS-1800 II, Fujifilm).

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References

[1] D. Hoyer, J.P. Hannon, G.R. Martin, Pharmacol. Biochem. Behav. 71 (2002) 533–554.
[2] J.A. Bard, J. Zgombick, N. Adham, P. Vaysse, T.A. Branchek, R.L. Weinshank, J. Biol. Chem. 268 (1993) 23422–23426.
[3] R.M. Eglen, J.R. Jasper, D.J. Chang, G.R. Martin, Trends Pharmacol. Sci. 18 (1997) 104–107.
[4] (a) G. García-Alcocer, L.C. Segura, M. García Peña, A. Martínez-Torres, R. Miledi, Gene Expr. 13 (2006) 53–57;
(b) F.J. Martín-Cora, A. Pazos, Br. J. Pharmacol. 141 (2004) 92–104.
[5] (a) M. Leopoldo, F. Berardi, N.A. Colabufo, M. Contino, E. Lacivita, M. Niso, R. Perrone, V. Tortorella, J. Med. Chem. 47 (2004) 6616–6624;
(b) D.R. Thomas, J.J. Hagan, Curr. Drug Targets CNS Neurol. Disord 3 (2004) 81–90;
(c) V. Pittalà, L. Salerno, M. Modica, M.A. Siracusa, G. Romeo, Mini Rev. Med. Chem. 7 (2007) 945–960.
[6] P.B. Hedlund, L. Kelly, C. Mazur, T. Lovenberg, J.G. Sutcliffe, P. Bonaventure, Eur. J. Pharmacol. 487 (2004) 125–132.
[7] P.B. Hedlund, J.G. Sutcliffe, Trends Pharmacol. Sci. 25 (2004) 481–486.
[8] J.M. Monti, M. Leopoldo, H. Jantos, Behav. Brain Res. 191 (2008) 184–189.
[9] (a) U.L. Mullins, G. Gianutsos, A.S. Eison, Neuropsychopharmacology 21 (1999) 352–367;
(b) O. Mnie-Filali, L. Lambás-Señas, L. Zimmer, N. Haddjeri, Drug News Perspect. 20 (2007) 613–618;
(c) O. Mnie-Filali, L. Lambas-Señas, H. Scarna, N. Haddjeri, Curr. Drug Targets 10 (2009) 1109–1117.
[10] (a) L. Manuel-Apolinar, A. Meneses, Behav. Brain Res. 148 (2004) 179–184;
(b) P.B. Hedlund, Psychopharmacology 206 (2009) 345–354;
(c) X. Wang, Y. Fang, J. Liang, Z. Yin, J. Miao, N. Luo, Headache 50 (2010) 579–587;
(d) O. Yanarates, A. Dogrul, V. Yildirim, A. Sahin, A. Sizlan, M. Seyrek, A. Akgül, A. Kozak, E. Kurt, U. Aypar, Anesthesiology 112 (2010) 696–710.
[11] C.M. Lee, L. Farde, Trends Pharmacol. Sci. 27 (2006) 310–316.
[12] (a) C. Kikuchi, T. Ando, T. Watanabe, H. Nagaso, M. Okuno, T. Hiranuma, M. Koyama, J. Med. Chem. 45 (2002) 2197–2206;

(b) C. Kikuchi, H. Nagaso, T. Hiranuma, M. Koyama, J. Med. Chem. 42 (1999) 533–535;
(c) M.R. Zhang, T. Haradahira, J. Maeda, T. Okauchi, T. Kida, S. Obayashi, K. Suzuki, T. Suhara, J. Labelled Compd. Radiopharm. 45 (2002) 857–866.
[13] I.T. Forbes, S. Dabbs, D.M. Duckworth, A.J. Jennings, F.D. King, P.J. Lovell, A.M. Brown, L. Collin, J.J. Hagan, D.N. Middlemiss, G.J. Riley, D.R. Thomas, N. Upton, J. Med. Chem. 41 (1998) 655–657.
[14] (a) D.R. Thomas, P.J. Atkinson, P.G. Hastie, J.C. Roberts, D.N. Middlemiss, G.W. Price, Neuropharmacology 42 (2002) 74–81.
[15] J. Andriès, L. Lemoine, A. Mouchel-Blaisot, S. Tang, M. Verdurand, D. Le Bars, L. Zimmer, T. Billard, Bioorg. Med. Chem. Lett. 20 (2010) 3730–3733.
[16] S. Tang, M. Verdurand, B. Joseph, L. Lemoine, A. Daoust, T. Billard, G. Fournet, D. Le Bars, L. Zimmer, Nucl. Med. Biol. 34 (2007) 9995–10002.
[17] M. Rodriguez, M. Llinares, S. Doulut, A. Heitz, J. Martinez, Tetrahedron Lett. 32 (1991) 923–926.
[18] (a) P.J. Lovell, S.M. Bromidge, S. Dabbs, D.M. Duckworth, I.T. Forbes, A.J. Jennings, F.D. King, D.N. Middlemiss, S.K. Rahman, D.V. Saunders, L.L. Collin, J.J. Hagan, G.J. Riley, D.R. Thomas, J. Med. Chem. 43 (2000) 342–345;
(b) G. Cardillo, L. Gentilucci, A.R. Qasem, F. Sgarzi, S. Spampinato, J. Med. Chem. 45 (2002) 2571–2578.
[19] (a) A.J. Bojarski, Curr. Top. Med. Chem. 6 (2006) 2005–2026;
(b) A. Lepailleur, R. Bureau, S. Lemaître, F. Dauphin, J.-C. Lancelot, V. Contesse, S. Lenglet, C. Delarue, H. Vaudry, S. Rault, J. Chem. Inf. Comput. Sci. 44 (2004) 1148–1152.
[20] (a) D. Le Bars, C. Lemaire, N. Ginovart, A. Plenevaux, J. Aerts, C. Brihaye, W. Hassoun, V. Leviel, P. Mekhsian, D. Weissmann, J.F. Pujol, A. Luxen, D. Comar, Nucl. Med. Biol. 25 (1998) 343–350;
(b) D. Le Bars, J. Fluorine Chem. 127 (2006) 1488–1493.
[21] C. Lemaire, A. Plenevaux, J. Aerts, G. Del Fiore, C. Brihaye, D. Le Bars, et al., J. Labelled Compd. Radiopharm. 42 (1999) 63–75.