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Looking for a 5-HT$_7$ radiotracer for positron emission tomography

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ABSTRACT

In search of a serotonin 5-HT$_7$ radiotracer for positron emission tomography, we developed 1-{2-[(2*S*)-1-(phenylsulfonyl)pyrrolidin-2-yl]ethyl}piperidin-4-yl 4-fluorobenzoate. After labeling in good yield with fluorine-18 via nitro for fluorine exchange, preliminary biological experiments with autoradiographies failed to evidence any specific 5-HT$_7$ receptor delineation.

Serotonin (5-hydroxytryptamine, 5-HT) is a central neurotransmitter involved in a great variety of physiological functions, as well as neurological and pathological disorders such as depression, eating disorders and Alzheimer's disease.[1] Pharmacological studies allowed identification of numerous serotoninergic receptors families and subtypes. These receptors have been classified by structural, functional and pharmacological criteria into seven distinct receptor classes (5-HT$_{1-7}$).[2] The 5-HT$_7$ receptor is one of the most recently identified serotonin receptors.[3] The recent availability of selective 5-HT$_7$ receptor antagonists[4] and of 5-HT$_7$ receptor knockout mice has considerably advanced the understanding of the physiological function of this receptor. The 5-HT$_7$ receptor is coupled to G_s proteins and, like 5-HT$_4$ or 5-HT$_6$ receptors, is positively linked to adenylate cyclase.[5] Recent results indicate that this receptor might be involved in mood regulation. For instance, 5-HT$_7$ receptor knockout mice exhibit antidepressant-like behaviors[6] and 5-HT$_7$ receptors are downregulated in hypothalamus after chronic antidepressant treatments.[7] Therefore, it is crucial for the therapeutic research to gain access to in vivo studies in order to understand the implication of this receptor in neurological and psychiatric diseases.

With the development of PET (positron emission tomography) as a molecular imaging method, the opportunity has evolved to perform in vivo observations both in animal models and in humans.[8] These studies can be performed at 'tracer concentrations' implying the intravenous administration of a molecule at a very low amount (3–10 μg). At these concentrations the in vivo measurements are usually far below levels at which pharmacological effects might occur.

An ability to image serotonin 5-HT$_7$ receptors in human brain in vivo is needed to assess directly 5-HT$_7$ receptors involvement in neuropsychiatric diseases and their possible therapies. By studying receptors in vivo, firstly in animal models and secondly in humans, many of the uncertainties attached to studies of post-mortem brain can be avoided,[8] helping to understand the role of the 5-HT$_7$ receptors particularly in mood disorders. Quantitative imaging of 5-HT$_7$ receptors would also allow to study of the interactions of established and new drugs with these receptors. This implies the availability of a PET radiotracer which specifically labels the 5-HT$_7$ receptor. Our objective is therefore to develop the required 5-HT$_7$ radioligands which will open new research directions in clinical research and in drug development.

According to the literature, several pharmacological compounds show good selectivity and affinity toward 5-HT$_7$ receptors.[9] In particular, arylsulfonamidoalkylamines exhibit one of the most potent and selective antagonist properties.[9c,10]

Because of the radioactive decay of positron emitters, the half-lives of the radioisotopes constitute crucial parameters in the synthesis of radiolabeled compounds. Therefore, we envisaged to use specifically fluorine-18 ($T_{1/2}$ = 109.7 min), more convenient for preclinical and clinical uses than carbon-11 radiolabeling. Because of its high specific radioactivity and its easy obtention via the $^{18}O(p,n)$ reaction, $^{18}F^-$ anion constitutes the most common source

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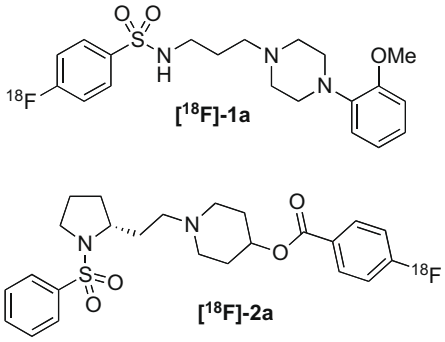


Figure 1. Targets for potential radiolabeled 5-HT_7 ligand.

for ^{18}F introduction into molecules. Consequently, we chose to introduce the radioisotope at the last step, to conserve the maximum of radioactivity, by a S_NAr reaction using radioactive fluoride anions.

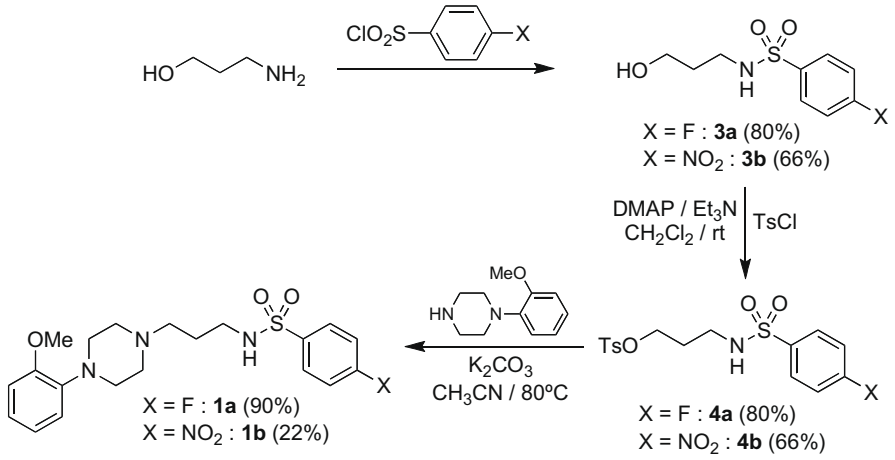
With this constraint in mind, two structures have been envisaged, inspired by the literature results (Fig. 1).[9c,10,11]

The first one (**[^{18}F]-1a**) is a piperazine derivative adapted from recent literature results.[11b] The second (**[^{18}F]-2a**) is based on **SB-269970** structure bearing an ester moiety in four-position of the piperidine core.[11a] In each compounds, the fluorine atom is in *para* position of electron-withdrawing group (required for S_NAr reaction) and can be inserted in the last step by the substitution of a nitro group by activated fluoride anion.

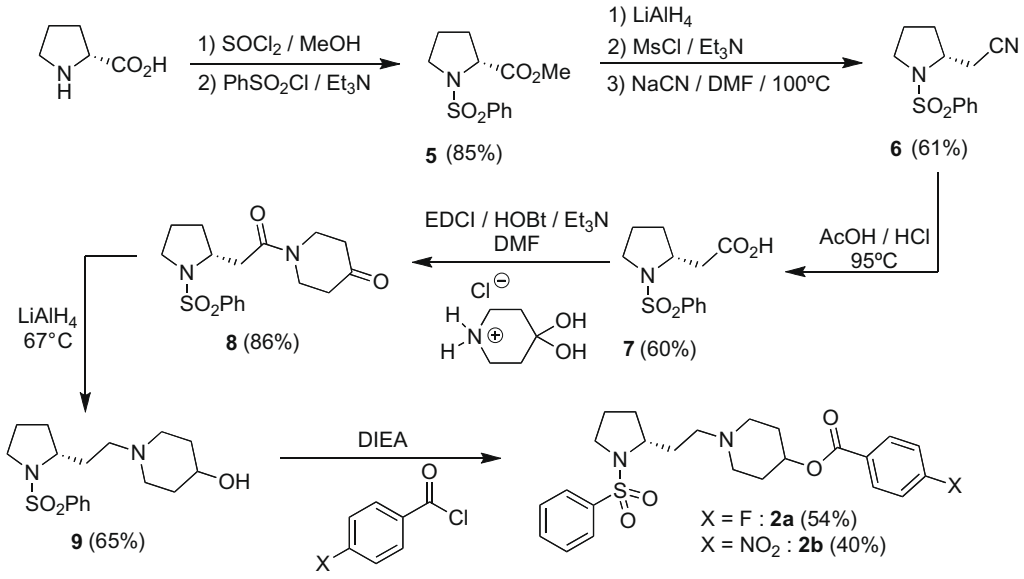
Non-radioactive compound **1a** and its nitro precursor for radiolabeling (**1b**) have been synthesized in three steps starting from 3-amino-propanol (Scheme 1).

Concerning the second ligand (**2**), a divergent strategy has been elaborated to obtain the precursor for radiolabeling (**2b**) or the non-radioactive fluorinated compound **2a** via a common synthetic way, with a discrimination step at the last stage (Scheme 2).

The *N*-phenylsulfonyl homoproline **7** has been easily synthesized from proline by modified literature processes.[12] The subsequent coupling between chlorhydrate of piperidone hydrate and **6** with CDI[13] and the over reduction with $LiAlH_4$ gave **8** with good yield. Finally, the expected compounds **2a and 2b** were obtained after esterification, in nine steps with an overall yield of 9% and 7%, respectively (Scheme 2).



Scheme 1. Synthesis of **1a** and its nitro precursor for radiolabeling **1b**.

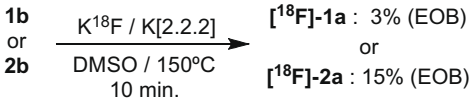


Scheme 2. Synthesis of **2a** and its nitro precursor for radiolabeling **2b**.

Table 1
Lipophilicity (log *D*) and 5-HT_7 affinity (pK_B) of **1a** and **2a** compounds

Compound	log *D* (pH = 7.4)[a]	pK_B[b]
1a	3.27	7.26
2a	4.13	7.10

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



Scheme 3. Radiolabeling of **1b** and **2b**.

Lipophilicity (log *D*) of compounds **1a** and **2a** have been then calculated with the ACD/Labs software and their respective affinity toward 5-HT_7 receptors (pK_B) have been determined by binding assays on expressed in CHO cells (Table 1).

The 5-HT_7 affinity values are intermediate although satisfactory and the calculated log *D* values at physiological pH suggest a relatively high lipophilicity for compounds. Since the lipophilicity of a compound is predictive of its blood–brain barrier penetration, these initial results were encouraging and led us to envisage the radiolabeling of **1a** and **2a** compounds and their first PET studies.

Fluorine-18 was obtained via the $^{18}O(p,n)\ ^{18}F$ nuclear reaction (IBA Cyclone 18/9 cyclotron). The nitro/fluoro exchange was realized by using a reprogrammed automated fluorination module (coincidence). Labeling of **1b** and **2b**, by nucleophilic aromatic substitution of nitro group, was realized in classical conditions at 150 °C, in presence of kryptofix® (Scheme 3).

Radiolabeling of **1b** did not give satisfactory results in term of radiochemical yield. This could be explained by a fast deprotonation of acidic hydrogen of sulfonamide by the very basic, naked fluoride anion. This deprotonation would quench the fluoride, which would not be further available for S_NAr reaction. On the other hand, the expected radiolabeled compound **[^{18}F]-2a** was obtained with a satisfactory radiochemical yield of 15% corrected for decay and 50 min radiosynthesis time (including purification step); the specific activities was in the range of 56–95 GBq/μmol corrected at the end of synthesis. No major radioactive by-products were observed and HPLC conditions chosen ensured good separation of **[^{18}F]-2a** from its nitro precursor **2b**.

Since the radiolabeled compound **[^{18}F]-2a** could be obtained in an efficient manner, its biological evaluation in rodents has been then envisaged. Firstly, **[^{18}F]-2a** binding to 5-HT_7 receptors was evaluated by in vitro autoradiographies using control rat brain. **[^{18}F]-2a** binding was homogenous throughout the brain, with a lack of specific binding in regions rich in 5-HT_7 receptors like hippocampus in comparison to other regions devoided of 5-HT_7 receptors like cerebellum.[14] No significant binding difference was found between control sections and sections pre-incubated with serotonin (10 μM) (results not shown). Therefore, these initial in vitro autoradiographies suggested a lack of selectivity of the **[^{18}F]-2a** compound in this animal model.

Since in vitro studies cannot be directly extrapolated to in vivo studies, it was crucial to perform ex vivo studies. Ex vivo autoradiographies experiments were conducted with the aim to evaluate the cerebral distribution of **[^{18}F]-2a** in alive animals. They imply the injection of the compound in the alive animal and subsequent brain removal and autoradiography study. The general observation was that **[^{18}F]-2a** showed a very low brain penetration. Hippocampus, the region known to be rich in 5-HT_7 receptors, displayed a low **[^{18}F]-2a** labeling. A similar low radiotracer uptake was seen in cerebellum suggesting a high level of non specific binding. The pre-injection of unlabeled compound **1** suppressed significantly the already low binding of **[^{18}F]-2a** in all regions of the brain studied showing that its fixation is saturable (Fig. 2).

The apparent discrepancy between the high value of log *D* (lipophilicity) and the low brain penetration measured ex vivo could be explained by the metabolic hydrolysis of ester moiety, generating [^{18}F]fluorobenzoate which should be eliminated by urinal way. Such reasonable hypothesis seemed to be confirmed by the high level of radioactivity we detected into rat kidneys suggesting a rapid renal elimination.

In summary, we reported in this study the radiolabeling of a new 5-HT_7 receptor antagonist. Our preliminary biological results, obtained with complementary in vitro and ex vivo approaches, demonstrate the inability of **[^{18}F]-2a** to visualize 5-HT_7 receptors in vivo. Because the discovery of a potent 5-HT_7 radiotracer is crucial for the exploration of brain serotoninergic function, other 5-HT_7 leads, without easy metabolisable parts, will be evaluated for future PET investigations.

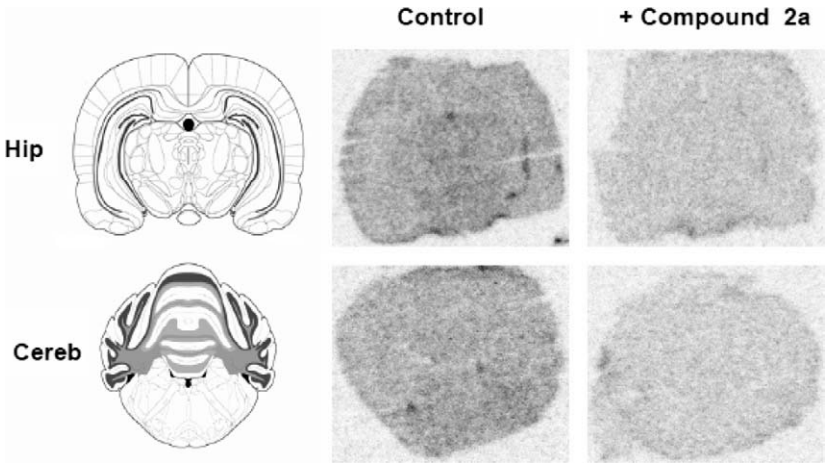


Figure 2. Ex vivo autoradiographies of rat brain sections (Hip, hippocampus; Cereb, cerebellum) in control rats (control; 20 min after the iv injection of 2 mCi (SA = 2 Ci/μmol) of **[^{18}F]-2a**) and in pretreated rats with the unlabeled compound **2a** (compound **2a**; 5 mg/kg iv, injected 30 min before the **[^{18}F]-2a** compound). On the left is reported the corresponding regions according to rat brain stereotaxic atlas.

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