Radiosynthesis and biological evaluation of [^{18}F]R91150, a selective 5-HT$_{2A}$ receptor antagonist for PET-imaging

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ABSTRACT: Serotonergic 5-HT$_{2A}$ receptors in cortical and forebrain regions are an important substrate for the neuromodulatory actions of serotonin in the brain. They have been implicated in the etiology of many neuropsychiatric disorders and serve as a target for antipsychotic, antidepressant and anxiolytic drugs. Positron emission tomography imaging using suitable radioligands can be applied for *in vivo* quantification of receptor densities and receptor occupancy for therapy evaluation. Recently, the radiosynthesis of the selective 5-HT$_{2A}$R antagonist [^{18}F]R91150 was reported. However, the 6-step radiosynthesis is cumbersome and time-consuming with low radiochemical yields (RCYs) of <5 %. In this work, [^{18}F]R91150 was prepared using late-stage Cu-mediated radiofluorination to simplify its synthesis. The detailed protocol enabled to obtain RCYs of 14 ± 1 % and the total synthesis time was reduced to 60 min. In addition, autoradiographic studies with [^{18}F]R91150 in rat brain slices revealed the typical uptake pattern of 5-HT$_{2A}$ receptor ligands.

KEYWORDS: 5-HT$_{2A}$, autoradiography, PET imaging, fluorine-18, [^{18}F]R91150

Serotonin, or 5-hydroxytryptamine (5-HT), is an important neurotransmitter in the central nervous system and peripheral tissues that exerts its effects through a family of seven 5-HT receptors (5-HT$_{1-7}$). In the brain, 5-HT release from serotonergic fibers that originate in the raphe nuclei and innervate cortical and limbic structures of the medial temporal lobe system has long been recognized to modulate cognition, emotional processes and various forms of learning and memory[1, 2]. More recent findings indicate that serotonergic 5-HT$_{2A}$ receptors (5-HT$_{2A}$Rs), which are widely expressed in cortical and forebrain regions, may be an important substrate for these neuromodulatory actions[3]. In support of this assumption, changes in 5-HT$_{2A}$R expression or function have been implicated in the etiology of many neuropsychiatric disorders like depression, anxiety or schizophrenia and cognitive disturbances associated with Alzheimer's or Parkinson disease[4-7]. Based on these findings, pharmacological manipulation of 5-HT$_{2A}$Rs has emerged as a promising approach for the treatment of mental or cognitive disorders, as evidenced by various studies on the antidepressant[8], anxiolytic[9] and antipsychotic[10] effects of 5-HT$_{2A}$R antagonism.

Molecular imaging techniques like positron emission tomography (PET) or single photon emission computed tomography (SPECT) can be used for *in vivo* quantification of (changes in) receptor densities and measurement of receptor occupancy by novel therapeutic drugs. To date, various radioligands for PET-based visualization of 5-HT_{2A}Rs have been developed that can be broadly classified into radiolabeled antagonists like [^{18}F]setoperone[11, 12], [^{18}F]altanserin[13], [^{11}C]MDL100907[14, 15] or [^{18}F]MDL100907[16, 17], and radiolabeled agonists like [^{11}C]CIMBI-36[18-20] or [^{18}F]FETCIMBI-36[21]. Likewise, several ^{123}I-labelled compounds have been developed for SPECT imaging, although only the antagonist [^{123}I]iodo-R91150, a radiolabeled analog of the Janssen Research Foundation compound R91150 (Fig. 1), proved to exhibit suitable binding properties, selectivity and brain uptake for imaging of 5-HT_{2A}Rs in rats[22, 23]. Subsequent animal and human studies with [^{123}I]iodo-R91150 revealed a binding pattern consistent with the distribution of 5-HT_{2A}Rs observed in previous autoradiographic studies[24-27], and the tracer has since been used for imaging in patients with schizophrenia[28], depression[29], Parkinson's disease[30] or Asperger's syndrome[31]. A disadvantage of [^{123}I]iodo-R91150, especially with regard to preclinical imaging in small animal models, is that radioiodination increases the lipophilicity relative to the parent compound R91150 more than 10-fold[32] (Fig. 1), which results in a relatively low signal-to-noise ratio. Exploiting the presence of an aromatic fluorine atom in the parent compound (Fig. 1), this aspect has been addressed by preparation of the ^{18}F-labeled analog [^{18}F]R91150 for use in PET measurements[32]. Preliminary *in vitro* and *ex vivo* experiments indicated that [^{18}F]R91150 may have certain advantages over established 5-HT_{2A} antagonist radioligands for human use[32]. This includes a higher selectivity and lack of lipophilic, brain-permeant radiometabolites, if compared to [^{18}F]altanserin[33] or [^{18}F]setoperone[12], an increased 5-HT_{2A} affinity, reduced lipophilicity and improved signal-to-background ratio, if compared to [^{123}I]iodo-R91150[32, 34] and a longer-lived ^{18}F-label resulting in a better match between radionuclide and biological half-life, if compared to [^{11}C]MDL100907[35, 36]. On the other hand, a major disadvantage of [^{18}F]R91150 is the time-consuming 6-step radiosynthesis providing [^{18}F]R91150 in low RCYs of <5%. Furthermore, the radiosynthesis is not amenable to automation[32], and has prevented a more widespread use of this radioligand. However, a number of Cu-mediated radiofluorination methods developed in the last decade (for overview[37-41]) have provided new approaches for facile introduction of ^{18}F into aromatic compounds. In the present work, we describe a novel synthetic strategy for production of [^{18}F]R91150 via copper-mediated late-stage radiofluorination and present the results from autoradiographic studies performed with the tracer.

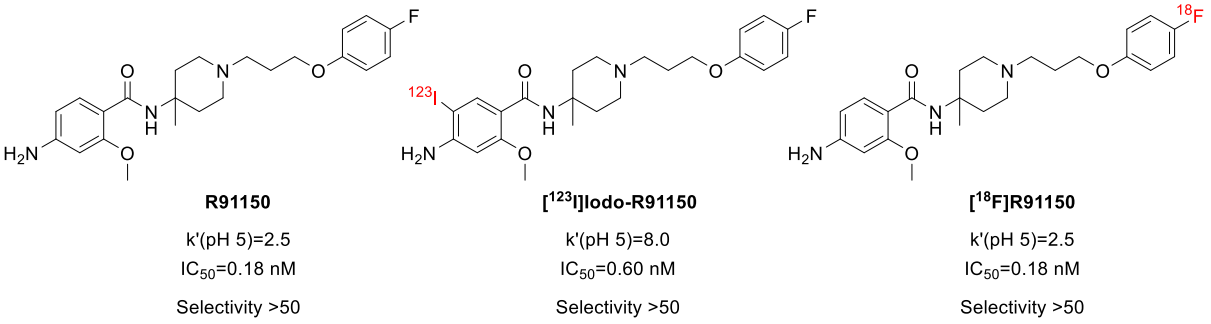
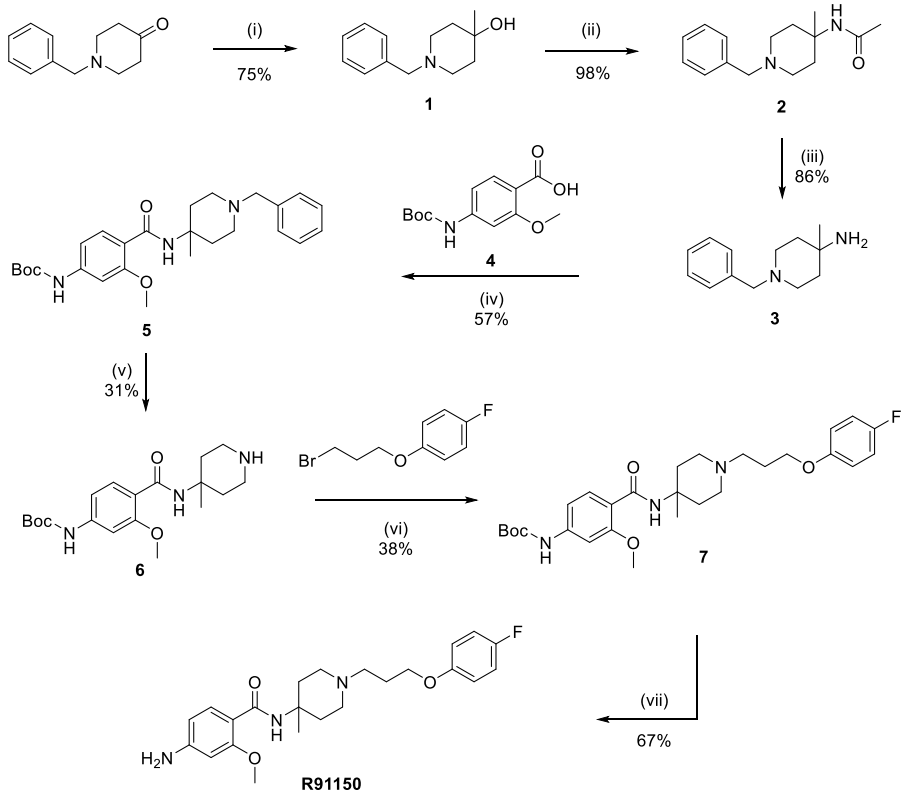


Figure 1. Structure and properties of the selective 5-HT_{2A}R antagonist R91150 and its radiolabeled analogs. K_i values refer to inhibition of [^{3}H]ketanserin binding to rat frontal cortex membranes. Selectivity factors refer to 5-HT_{2A}R over serotonergic 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2C} and 5-HT_3, adrenergic α_1 and α_2, histaminergic H_1 and dopaminergic D_2 receptors. Capacity factors k' were determined at pH 5 and provide a measure for lipophilicity with excellent correlation to logP octanol/buffer values. Data from [22, 23, 32, 34].

For competition studies and to confirm the identity of [^{18}F]R91150, the reference compound R91150 was prepared according to the total synthesis described by Mülhausen *et al.*[32] (Scheme 1).

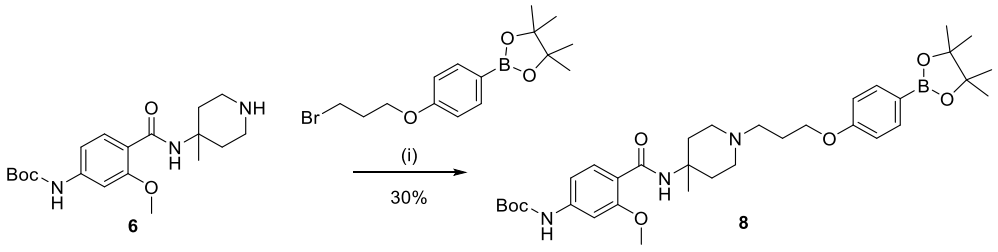


Scheme 1: Synthesis of **R91150**. Reaction conditions: (i) MeLi, Et_2O, -78 °C to r.t., 2 h. (ii) conc. H_2SO_4, MeCN, r.t., 2 h. (iii) conc. HCl, 100 °C, 48 h. (iv) PyBOP, Et_3N, **4**, DCM, r.t., 18 h. (v) NH_4HCO_3, palladium black, MeOH, 65 °C, 3.5 h. (vi) K_2CO_3, KI, DMF, 80 °C, 12 h. (vii) DCM/TFA (3:1), 80 °C, 2 h.

To this end, commercially available *N*-benzyl-piperid-4-one was reacted with methyllithium (MeLi) to introduce the quaternary methyl bearing carbon atom, which provided benzyl-4-

methyl-piperidin-4-ol (**1**) in good yields (75%). The hydroxyl group was almost quantitatively (98%) converted into the acetyl-protected amine by a *Ritter-Reaction* of **1** with MeCN and concentrated H_2SO_4 to obtain *N*-(1-benzyl-4-methylpiperidin-4-yl)acetamide (**2**), which was directly de-protected to 1-benzyl-4-methyl-piperidin-4-amine (**3**) in 86% yield by refluxing in concentrated hydrochloric acid (aq. HCl) for at least 2 days. Intermediate **3** was conjugated with 4-(*tert*-butoxycarbonylamino)-2-methoxybenzoic acid **4** (prepared from commercially available 4-amino-2-methoxybenzoic acid) using benzotriazol-1-yl-oxytripyrrolidino-phosphonium hexafluorophosphate (PyBOP) for carboxylic acid activation, which provided *tert*-butyl 4-(1-benzyl-4-methylpiperidin-4-ylcarbamoyl)-3-methoxyphenylcarbamate (**5**) in fair yield (57%), but still contaminated with pyrrolidine by-products according to $^{1}H/^{13}C$-NMR. Subsequent benzyl deprotection of **5** with palladium black was complete after 3.5 h (as indicated by TLC) and purification by flash column chromatography effectively removed all pyrrolidine from the previous step, affording **6** in 31% yield. The following amination of **6** with commercially available 1-(3-bromopropoxy)-4-fluorobenzene gave the *N*-Boc-protected reference compound **7** in 34% yield. R91150 was finally obtained in 67% yield by deprotection of **7** in a 3:1 mixture of dichloromethane (DCM) and trifluoroacetic acid (TFA) at 80 °C for 2 h. For optimal purity, the compound was purified by semi-preparative HPLC, followed by solid phase extraction (SPE) using a C18 cartridge.

The radiolabeling precursor **8** was synthesized in an analogue manner in 30% yield by employing commercially available 2-(4-(3-bromopropoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane in the amination reaction (Scheme 2).

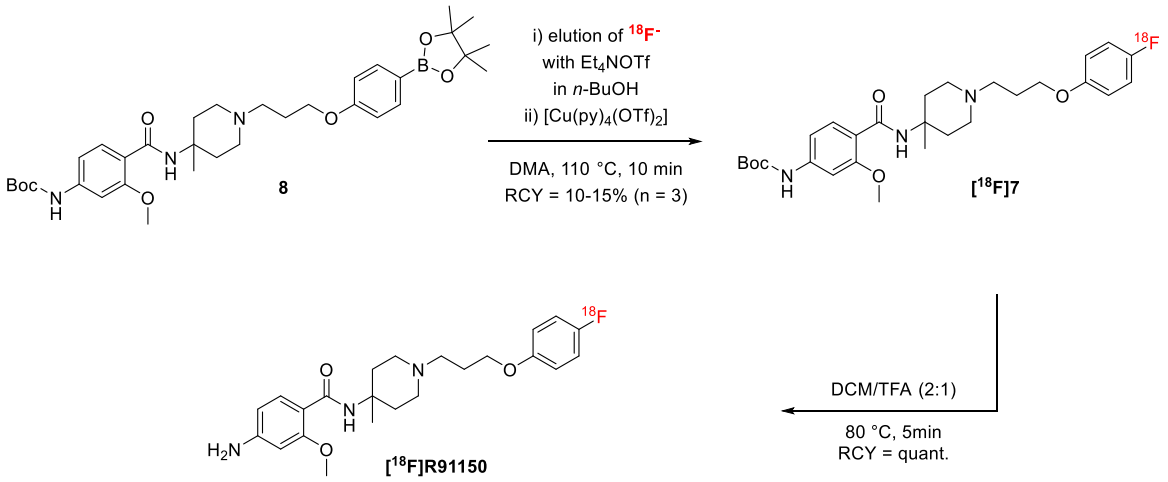


Scheme 2: Synthesis of labeling precursor **8**. Reaction conditions: (i) K_2CO_3, KI, DMF, r.t., 12 h.

Note: *Unless noted otherwise, all radiochemical yields (RCYs) described herein have been corrected for decay to the start of the synthesis, as recommended in the consensus nomenclature rules for radiopharmaceutical chemistry* [42].

[^{18}F]**R91150** was prepared in a two-step synthesis by Cu-mediated radiofluorination of the *N*-Boc-protected precursor **8** with subsequent deprotection followed by HPLC-purification. The

tracer was prepared within 60 min. Initially we attempted a direct labeling approach that involved deprotection of **8** (33% TFA in DCM at 80 °C for 5 min) with subsequent labeling in the same vial (after removal of TFA/DCM). Unfortunately, these attempts were not successful, potentially due to fast protodeboronation[43] of the precursor under the applied conditions. Assuming that the deprotected boronic acid pinacol ester might be prone to degradation upon long-term storage, we did not further attempt to implement a one-step radiosynthesis of [^{18}F]**R91150**.



Scheme 3: 2-Step radiosynthesis of [^{18}F]**R91150**.

Therefore, the *N*-Boc-protected boronic acid pinacol ester **8** was radiofluorinated using the Cu(II)-mediated radiofluorination protocol according to Zischler et al.[44]. To this end, [^{18}F]fluoride was trapped on an anion-exchange (QMA) cartridge and eluted with tetraethylammonium bicarbonate (Et_4NHCO_3) in *n*-butanol with an elution efficacy of 95 ± 4 % (n = 10). A solution of precursor **8** and $Cu(OTf)_2(py)_4$ in DMA was added and the mixture was stirred at 110 °C for 10 min, which afforded [^{18}F]**7** in RCYs of 15% (n = 3) after isolation by SPE (Sep-Pak C18). Quantitative deprotection of [^{18}F]**7** was achieved with TFA in DCM at 80 °C for 5 min. Subsequently, the 5-HT_{2A}-radioligand [^{18}F]R91150 was isolated using an analytical HPLC column with 14 ± 1 % RCY (aliquots, n = 3) or in 13% RCY (n = 1) using a semi-preparative HPLC column. For *in vitro* studies, [^{18}F]**R91150** was isolated using an analytical HPLC column and the collected fraction (2-5 MBq/mL) was diluted in Tris HCl (pH 7.4) buffer (~2.5 kBq/mL).

The molar activity was determined to 70 GBq/µmol with a radiochemical purity of 97%, based on a starting activity of 4.4 GBq and a synthesis time of approx. 60 min. Cu-content was determined by ICP-MS of the isolated fraction (using semi-preparative HPLC) containing the tracer (10 mL, 174 MBq) and found to be 2.6 µg/L. This is far below any level of concern according to the ICH Guideline of Elemental Impurities (Q3D)[45].

In summary, the radiosynthesis of [^{18}F]**R91150** was considerably facilitated by reduction of synthesis time and improvement of radiochemical yield (Tab. 1). This enabled first *in vitro* studies of this radioligand and finally also allows for automation of the radiosynthesis.

Tab. 1: Comparison of the radiosynthesis of [^{18}F]**R91150** as published by Mülhausen *et al.*[32] with the two-step radiosynthesis in this work.

	reaction steps	synthesis time	RCY	n.d.c. yield	automation
This work	2 (facile)	60 min	14%	10±1%	possible
Mülhausen *et al.*[32]	6 (complex)	190 min	1.8-5.7%	0.5-1.7%	not applicable

The suitability of radioligand candidates for *in vivo* experiments strongly depends on a high ratio of specific to nonspecific binding and a lack of affinity for other, non-target receptors (unspecific binding). Here, *in vitro* autoradiography was used in competition with non-radioactive R91150 to determine nonspecific binding of the radioligand. To this end, transaxial and sagittal rat brain slices were either incubated with [^{18}F]R91150 alone (total binding, Fig. 2 A, Fig. 3 A & C, Fig. 4 A) or with [^{18}F]R91150 and an excess of the corresponding non-radioactive reference compound (nonspecific binding, Fig. 2 B, Fig. 3 B & D, Fig. 4 B-D).
In addition, the specific binding pattern of [^{18}F]R91150 was verified in competition assays with the 5-HT$_{2A}$ targeting drugs altanserin, (+)-lisuride[46] and (-)-lisuride (Fig. 4). In three independent experiments with molar activities between 1.5 and 70 GBq/µmol, [^{18}F]R91150 showed distinct specific binding throughout the cortex, with a higher binding in frontal compared to caudal cortical regions (Fig. 2). Binding was displaced by R91150, altanserin and (+)-lisuride to approximately the same extent, validating 5-HT$_{2A}$ specificity of [^{18}F]R91150. In direct comparison with other specific 5-HT$_{2A}$ ligands (e.g. (*R*)-[^{18}F]MH.MZ[47], [^{18}F]MDL100,907[16], [^{18}F]altanserin[48, 49]), a high degree of regional correspondence was observed for binding in neocortex (strongest labelling in lamina V), striatum (stronger signal in caudal parts of the nucleus) and the olfactory tubercle along with several of the brainstem nuclei (see Fig. 3)[50]. These regions of high 5-HT$_{2A}$R expression, as determined in previous *in situ* hybridization studies[51], are clearly visualized in the autoradiographic images (see Fig. 2 & Fig. 3).

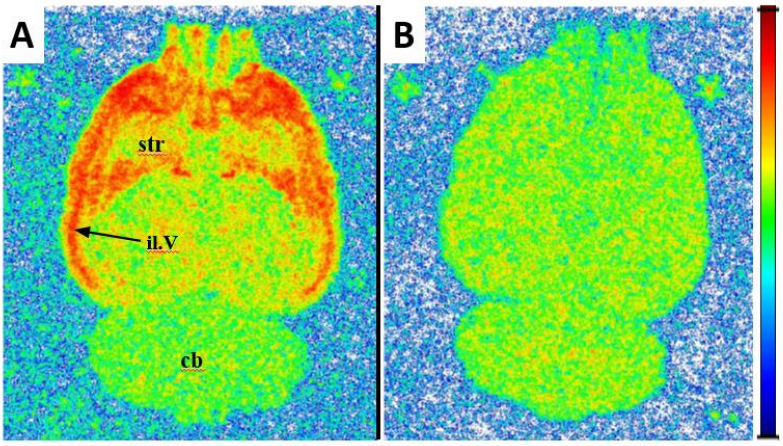


Fig. 2: *In vitro* autoradiography of transaxial rat brain slices with [^{18}F]R91150 (A: total binding) and in competition with R91150 (B: nonspecific binding). Major binding was detected in the lamina V (il.V) of the cortex and in caudal parts of the striatum (str). Abbreviations: cerebellum (cb), lamina V of the cortex (il.V), orbital cortex, striatum (str).

3).

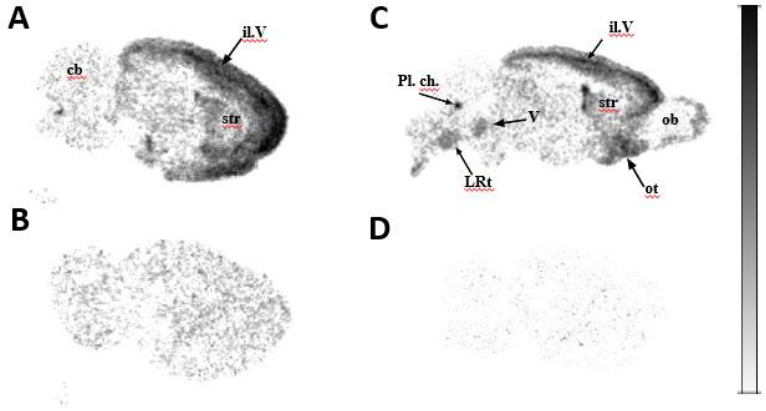


Fig. 3: *In vitro* autoradiography of sagittal rat brain slices with [^{18}F]R91150 (A & C: total binding) and in competition with R91150 (B & D: nonspecific binding). Abbreviations: cerebellum (cb), choroid plexus (Ph. ch.), lamina V of the cortex (il.V), olfactory bulb (ob), olfactory tubercle (ot), lateral reticular nucleus (LRt), striatum (str), trigeminal nerve (V). Each column shows different sets of experiments.

Almost no specific binding was observed throughout the cerebellum, which is consistent with several previous autoradiographic and immunohistological studies which confirmed low 5-HT_{2A}R abundance in the cerebellum.[52-54] Nonspecific binding was determined as the non-displaceable radioligand binding in the presence of 1 µM R91150, pointing to a low degree of nonspecific binding of 29% (Fig. 2B,

Tab. 2). A high [^{18}F]R91150 accumulation was found in the anterior forebrain, where tracer accumulation displayed a strong laminar pattern, similar to that observed in immunohistochemical localization studies on 5-HT_{2A}R expression in the middle layers of the cortex, that identified layer V as the region with most conspicuous 5-HT_{2A}R densities.[55] In the

competition assays with (-)-lisuride, (+)-lisuride and altanserin, (-)-lisuride displaced [^{18}F]R91150 binding in the cortex to a lower degree (53%) than (+)-lisuride (76%, Fig. 4), indicating a lower 5-HT$_{2A}$ affinity of this enantiomer as compared to (+)-lisuride.

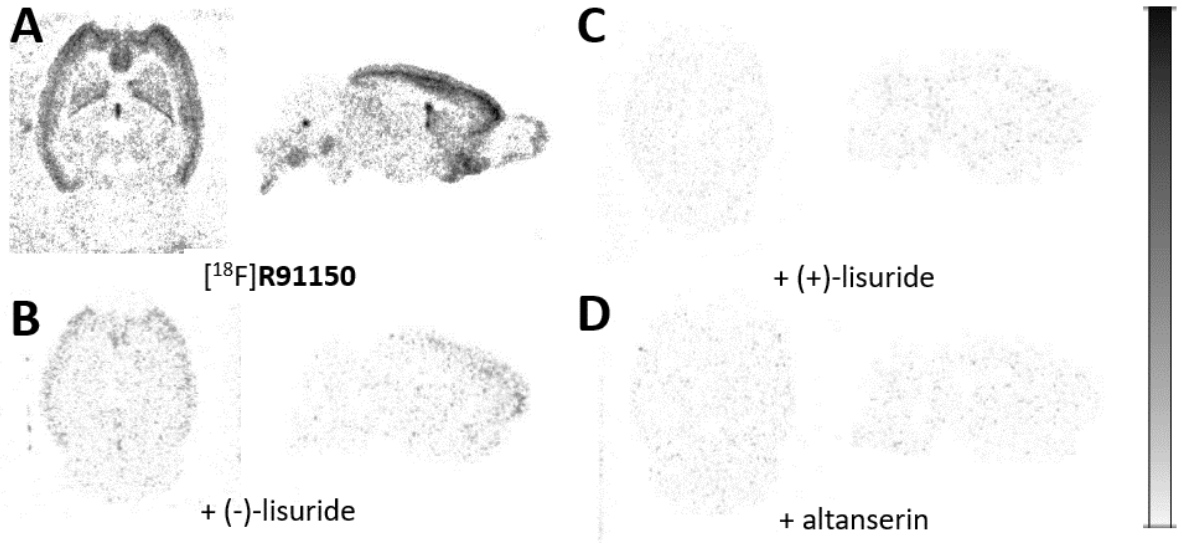


Fig. 4: Competition assay of [^{18}F]R91150 (A) with (-)-lisuride (B), (+)-lisuride (C) and altanserin (D). Only (-)-lisuride (B) could not completely block the binding of [^{18}F]R91150. Transaxial & sagittal orientation are shown for each set.

Tab. 2: Quantification of [^{18}F]R91150 binding. Displacement by known 5-HT$_{2A}$ targeting drugs indicates specific binding behavior of [^{18}F]R91150.

%-Displacement of [^{18}F]R91150 binding[a]

	R91150	**altanserin**	**(+)-lisuride**	**(-)-lisuride**
	1µM		2 µM	
cortex	70.5 ±3.8	70.6 ±3.2	75.6 ±5.5	52.7 ±2.6
striatum	34.3 ±12.5	32.9 ±12.6	49.9 ±13.7[b]	32.4 ±4.5
cerebellum	0	0	0	0

[a] Corrected for background, n=4. Regions of interest and data used for quantification are described in the supporting information. [b] Stronger displacement compared to R91150 due to higher concentration of the ligand.

In conclusion, the radiosynthesis of [^{18}F]R91150 was simplified by using late stage Cu-mediated radiofluorination, facilitating the production of [^{18}F]R91150 in fair RCY of 14% within 60 min. The *in vitro* evaluation of [^{18}F]R91150 revealed the potential of this tracer for PET imaging of 5-HT$_{2A}$R status in the human brain. Future studies should focus on the *in vivo* behavior of [^{18}F]R91150 and the transfer of its synthesis to an automated module for clinical applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: Experimental details for all compounds synthesized including radiosynthesis; determination of molar activity; spectroscopic data for standards and precursors; protocols for animal imaging studies; ^{1}H NMR and ^{13}C NMR spectra of all compounds; HRMS-spectra of compound 8 and R91150; *in vitro* autoradiography of [^{18}F]R91150; semi-preparative and analytical (Radio)-HPLC chromatogram of [^{18}F]R91150 (PDF).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

5-HT, 5-hydroxytryptamine; 5-HT$_{2A}$R, serotonergic 5-HT$_{2A}$ receptor; Boc, *tert*-butyloxy-carbonyl; Cu(OTf)$_2$(py)$_4$, tetrakis(pyridine)copper(II) triflate; DCM, dichloromethane; DMA, *N*,*N*-dimethylacetamide; DMF, *N*,*N*-dimethylformamide; HCl, hydrochloric acid; HPLC, high

performance liquid chromatography; ICP-MS, inductively coupled plasma mass spectrometry; MeLi, methyllithium; NMR, nuclear magnetic resonance, n.d.c., non-decay corrected; QMA, quaternary methyl ammonium; PET, positron emission tomography; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; R91150, 4-amino-*N*-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-2-methoxybenzamide; RCY, radiochemical yield; r.t., room temperature; SPECT, single photon emission computed tomography; SPE, solid phase extraction; TFA, trifluoroacetic acid; TLC, thin layer chromatography.

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