Radiosynthesis and preclinical evaluation of serotonin 5-HT$_6$ receptor radioligands for PET imaging

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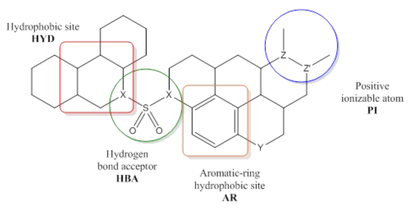
Introduction

According to their brain localization [1], the serotonin 5-HT$_6$ receptors are potent therapeutic targets for psychiatric and neurological diseases, i.e., schizophrenia and Alzheimer's disease [2, 3, 4]. However, the understanding of the serotonin 5-HT$_6$ pharmacology is currently partial and limited to animal models, particularly because of the lack of fluorinated PET 5-HT$_6$ radiopharmaceuticals [5]. In this context, we developed several 5-HT$_6$ radioligands and evaluated their suitability for PET imaging.

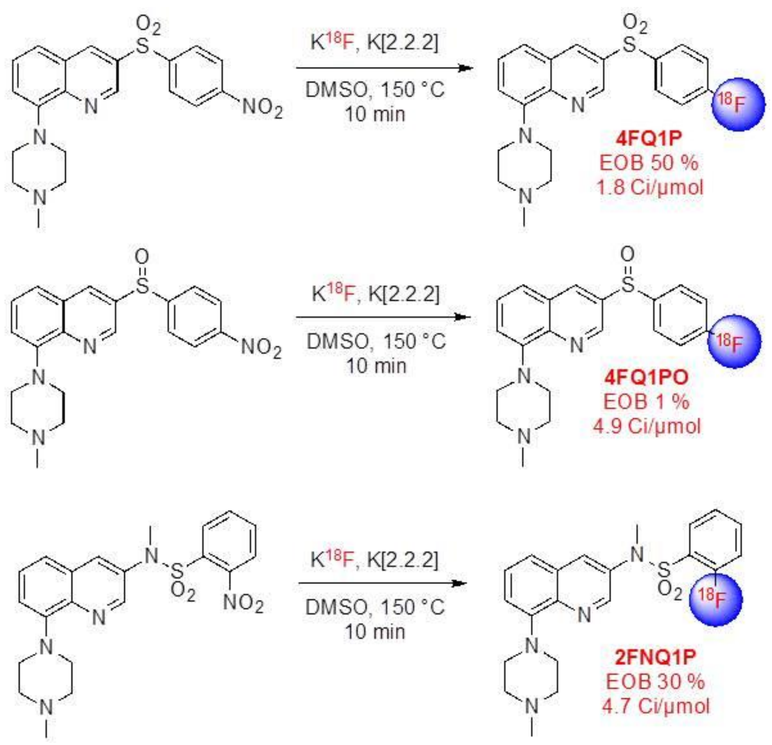
Method



Ten quinoline and pyridine-based ligands have been synthesized, inspired by the known 5-HT$_6$ receptor pharmacophore [5][6]. Only ligands with the higher in vitro affinities toward 5-HT$_6$ receptors and the lower affinities toward 5-HT$_{2A}$ receptors (a close pharmacophore) were radiolabeled via ^{18}F-nucleophilic aromatic substitution. These potential radiotracers were evaluated by in vitro autoradiography in rat brain. The most interesting radioligands of these were later evaluated by PETscans on anaesthetized cats (HR+ Siemens). Among these molecules, the selected 5-HT$_6$ radioligand was studied by high-resolution PET/CT (mCT Siemens)

Radioligand Synthesis

- 4FQ1P
  Ki (5-HT$_6$) = 0,21 nM
- 4FQ1PO
  Ki (5-HT$_6$) = 4,4 nM
- 4FNQNP
  Ki (5-HT$_6$) = >1 µM
- 2FNQNP
  Ki (5-HT$_6$) = >1 µM
- 4FQNP
  Ki (5-HT$_6$) = 7,6 nM
- 2FQNP
  Ki (5-HT$_6$) = 30 nM
- 4FQ1P5
  Ki (5-HT$_6$) = 53 nM
- 4FNQ1P
  Ki (5-HT$_6$) = 27 nM
- 2FNQ1P
  Ki (5-HT$_6$) = 0,9 nM

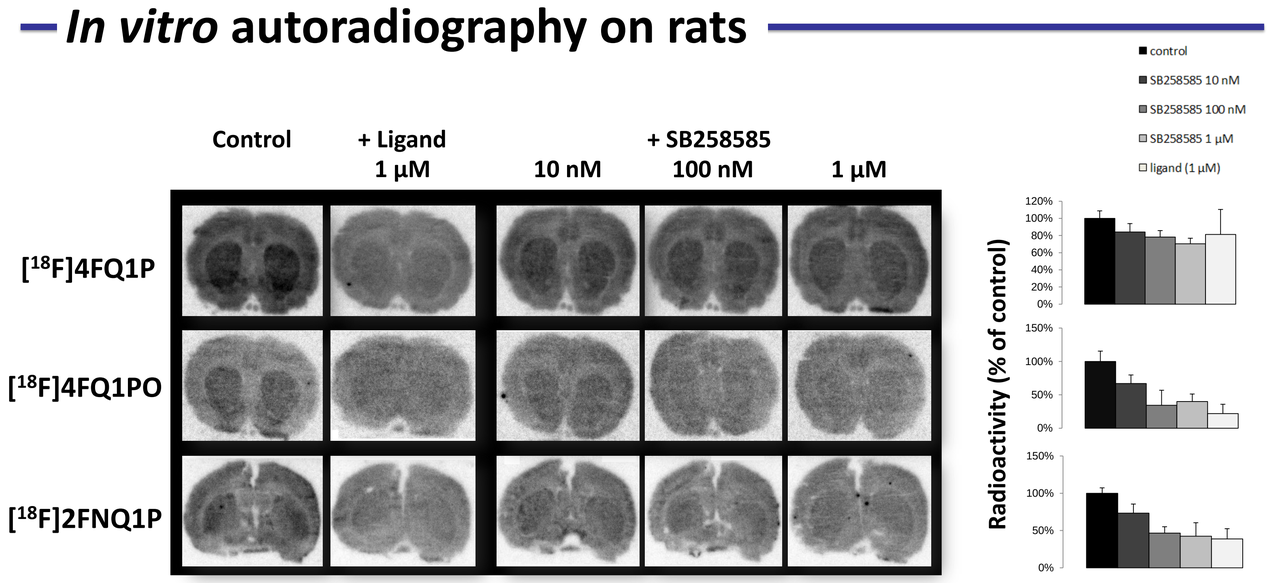


Ten molecules were synthesized and, according to their 5-HT$_6$ affinity, three of them (in red) were selected to be radiolabelled.

In vitro affinity and lipophilicity

Ligand	4FQ1P	4FQ1PO	2FNQ1P
Ki 5-HT$_6$ (nM)	0.21	4.4	0.9
Ki 5-HT$_{2A}$ (nM)	1.7	54	>10^{-6} M
Log D$_{7,4}$	3.01	2.00	1.86

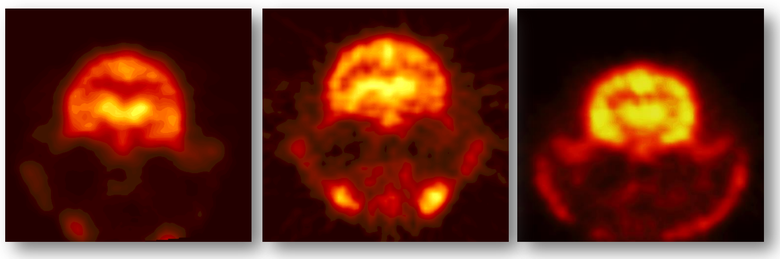
In vitro autoradiography on rats



Among the three ligands, [^{18}F]2FNQ1P has the higher 5-HT$_6$ selectivity and the more favorable *in vitro* profile, as shown by competition with a 5-HT$_6$ antagonist SB258585.

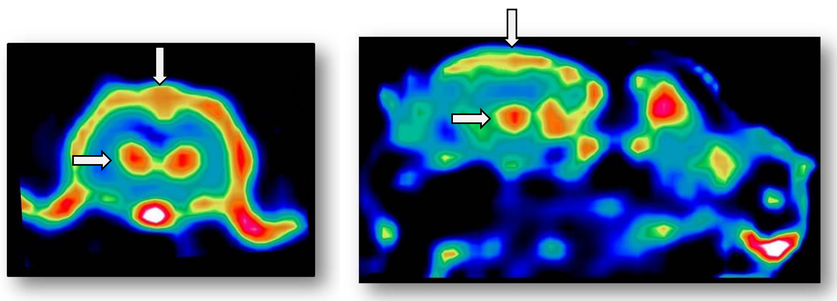
In vivo studies on cats

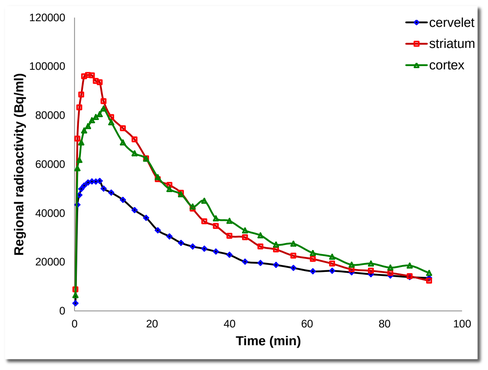
Brain penetration (PET camera)



[^{18}F]4FQ1P [^{18}F]4FQ1P0 [^{18}F]2FNQ1P

[^{18}F]2FNQ1P (high definition PET camera)

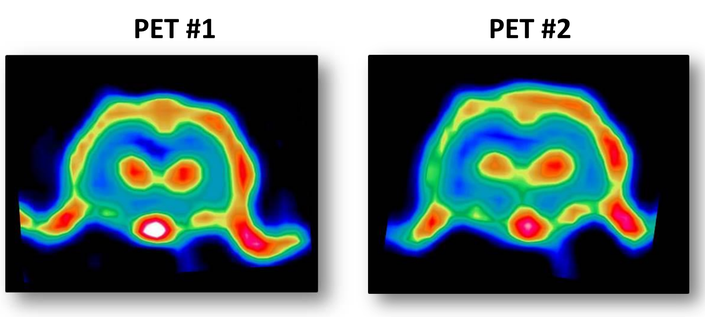


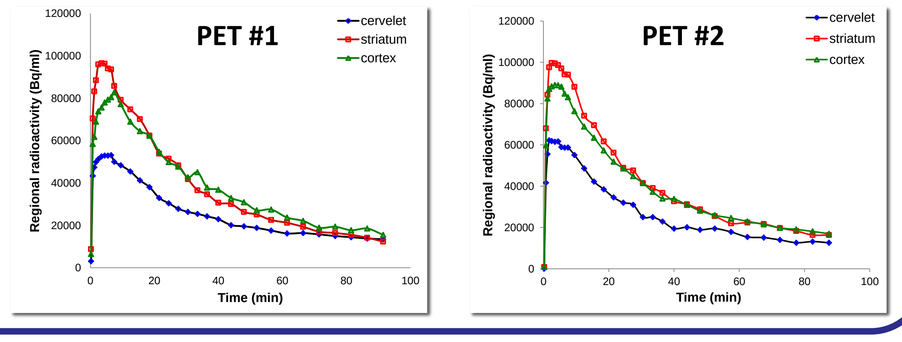


Despite the three radioligands cross the blood-brain barrier, [^{18}F]2FNQ1P reveals the most specific binding in the striatum (horizontal arrows) and in cortical areas (vertical arrows), in accordance with the 5-HT$_6$ receptor densities.

Test – retest reproducibility

[^{18}F]2FNQ1P display reliable reproducibility as shown by the test – retest study. Thus, PET #1 (97 MBq) and PET #2 (99 MBq) on the same cat, 3 days apart, have close radioactivity levels in ROIs.





Discussion and Conclusion

Ten quinoline-type ligands have be synthesized and, due to their in vitro 5-HT$_6$ receptor affinities and selectivity toward 5-HT$_{2A}$ receptors (a close pharmacophore), 3 of them were selected to be radiolabeled from their NO2-precursor. Those 3 molecules have led to encouraging results in autoradiography with a specific binding in the striatum and in cortical regions, rich in 5-HT$_6$ receptors. The PET-scans in anaesthetized cats revealed a good brain penetration of these radiotracers and a binding in the striatal region. Consequently, the development of these radiotracers is pursue in order to investigate the best 5-HT$_6$ PET radiotracer ([^{18}F]2FNQ1P).

[1] Wolley et al. Curr. Drug Targets, 2004 ; [2] Hamon et al. Neuropsychopharmacol, 1999 ; [3] Hirst et al. Brit. J. Pharmacol, 2000 ; [4] Codony et al. Curr. Op. Pharmacol, 2011[5] Parker et al. J Nucl Med, 2012 ; [6] Holenz et al. J med Chem, 2005