



**HAL**  
open science

## A screening assay in the search of an alpha-synuclein PET radioligand

Mathieu Verdurand, Meriem Mendjel-Herda, Elise Levigoureux, Sophie Lancelot, Waël Zeinyeh, Sébastien Alazet, Thierry Billard, Luc Zimmer, Fabien Chauveau

► **To cite this version:**

Mathieu Verdurand, Meriem Mendjel-Herda, Elise Levigoureux, Sophie Lancelot, Waël Zeinyeh, et al.. A screening assay in the search of an alpha-synuclein PET radioligand. 10th annual meeting of the European Society for Molecular Imaging (ESMI) - European Molecular Imaging Meeting (EMIM), Mar 2015, Tübingen, Germany. hal-01207682

**HAL Id: hal-01207682**

**<https://hal.science/hal-01207682v1>**

Submitted on 15 Jan 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## A screening assay in the search of an $\alpha$ -synuclein PET radioligand : an update

### Introduction

The accumulation of aggregated  $\alpha$ -synuclein ( $\alpha$ -Syn) in multiple brain regions is a neuropathological hallmark of synucleinopathies like Parkinson's disease, dementia with Lewy bodies and multiple system atrophy. A pre-mortem diagnosis tool would improve early diagnosis, monitor disease progression and therapeutic efficacy. While suitable amyloid- $\beta$  ( $A\beta$ ) PET radiotracers have been developed, no selective  $\alpha$ -Syn PET ligand has been identified to date. One PET tracer, BF227, labelled with carbon-11 or fluor-18, has demonstrated interesting

binding properties towards  $\alpha$ -Syn fibrils in vitro (1) and  $\alpha$ -Syn glial inclusions in MSA patients (2).

**Objective** To develop a simple screening assay for identifying  $\alpha$ -Syn ligands by competitive [<sup>18</sup>F]BF227 autoradiography and filter binding assay

### Methods

#### $\alpha$ -Syn and $A\beta$ fibrils

- Obtained by incubating commercial recombinant human proteins at 200  $\mu$ M, 37°C, 250 rpm
- Characterized by Transmission Electron Microscopy (TEM) and Thioflavine T (ThT) spectrofluorimetry

#### Animals

- Rats were bilaterally injected in the striata with 5  $\mu$ L of fibrils or PBS, and were euthanized soon thereafter
- Frozen brains were cryostat-cut into 20- $\mu$ m sections

#### Molecules

- For competitive autoradiography, positive and negative controls were obtained with 1  $\mu$ M PiB and 10  $\mu$ M ThT respectively
- 12 candidates with 3 distinct chemical scaffolds derived from tricyclics, benzoxazoles, and benzimidazoles were screened

#### Autoradiography (ARG)

- BF227 was radiolabelled according to (3) and obtained with radiochemical purity > 90 % and specific activity >2 Ci/ $\mu$ mol
- Brain slices were incubated with subnanomolar quantities of [<sup>18</sup>F]BF227 with or without unlabelled competitors at 1 and 10  $\mu$ M for 30 min, and then underwent 2 x 1-min washes
- Thioflavine S (ThS) staining was performed on the same slices

#### Filter Binding Assay (FBA)

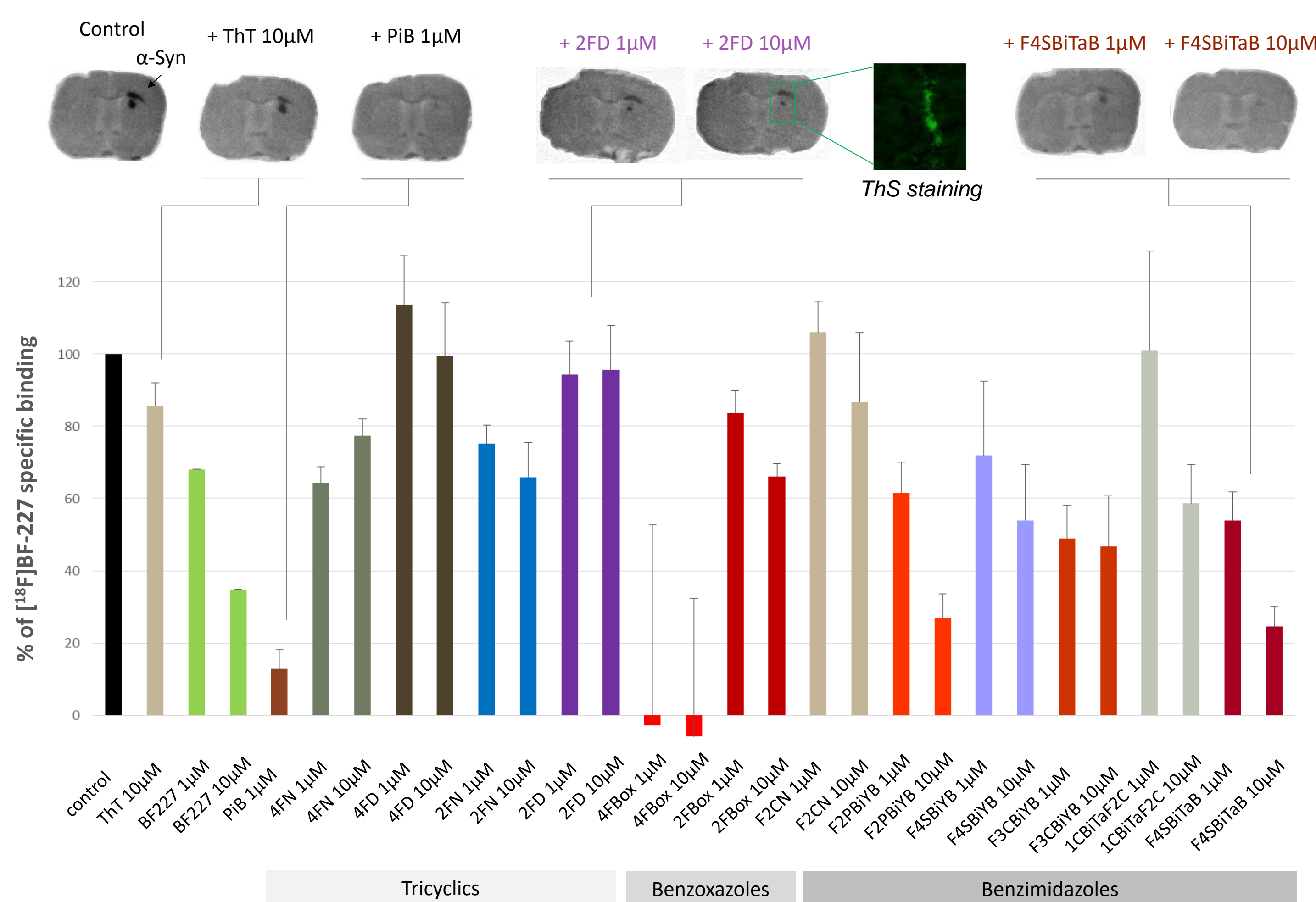
- $\alpha$ -Syn or  $A\beta$  fibrils (200 nM) were incubated with 40 nM [<sup>18</sup>F]BF227 and increasing concentrations of selected candidate molecules (0.1-200 nM) for 60 min in 200  $\mu$ L of PBS
- Bound from free radioactivity was separated by filtration under reduced pressure, and filters underwent 3 washes
- $K_i$  was determined using a previously measured  $K_d$  value of 30 nM for  $\alpha$ -Syn and 80 nM for  $A\beta$

### Results

1. In competitive ARG, PiB efficiently competed with [<sup>18</sup>F]BF227 binding to  $\alpha$ -Syn fibrils, in contrast to ThT. Several candidates were able to displace [<sup>18</sup>F]BF227 binding to  $\alpha$ -Syn injected fibrils at  $\mu$ M concentrations. One candidate (4FBox) showed promising  $\alpha$ -Syn binding characteristics that led us to quickly attempt its radiolabelling with fluorine-18 (experiments in progress).

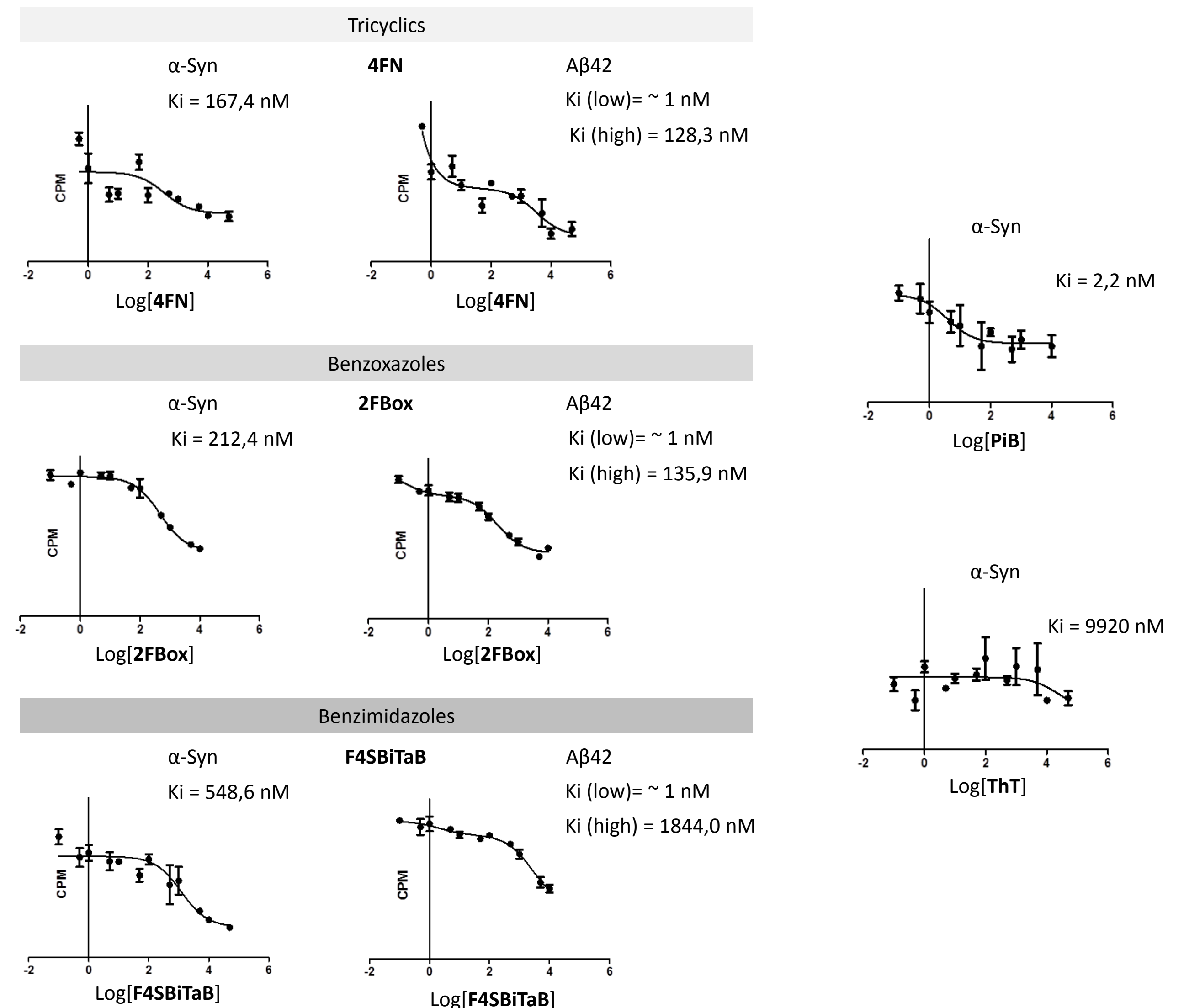
2. Competitive FBA allowed determining PiB affinity for  $\alpha$ -Syn fibrils, which value was close to the one already reported (4). Some competitor molecules showed  $\alpha$ -Syn fibrils affinity in the 100 nanomolar range but without displaying sufficient selectivity versus  $A\beta$  fibrils. Experiments are still underway to test all candidate molecules.

#### Competitive *in vitro* autoradiography



Histograms derived from competitive [<sup>18</sup>F]BF227 ARG analysis showing the ability of our original  $\alpha$ -Syn candidate molecules to displace [<sup>18</sup>F]BF227 binding to recombinant human pre-aggregated  $\alpha$ -Syn fibrils injected in rat striatum. Data represented as percentage of [<sup>18</sup>F]BF227 specific binding (mean  $\pm$  SEM; n  $\geq$  6 for each molecule and concentration).

#### Competitive Filter Binding Assays



Determination of the affinity inhibition constant ( $K_i$ ) of some of our  $\alpha$ -Syn candidate molecules through competitive filter binding assay with [<sup>18</sup>F]BF227 analysed with non-linear regression using Graphpad prism ("one site - fit  $K_i$ " model for  $\alpha$ -Syn and "two sites - fit  $K_i$ " for  $A\beta$ 42).

### Conclusion

- > Set-up of an original screening assay for testing the capacity of cold molecules to compete with [<sup>18</sup>F]BF227 binding sites on  $A\beta$  and  $\alpha$ -Syn fibrils
- > Identification of chemical scaffolds showing nanomolar affinity for  $\alpha$ -Syn  $\rightarrow$  selectivity to be further tested versus other proteins forming beta-sheets ( $A\beta$ , Tau...)

**Perspective** Radiolabelling (<sup>18</sup>F) of original candidates and *in vitro* testing on transgenic mice and human *post-mortem* tissues.

#### Acknowledgements

Dr Mathieu Verdurand is sponsored by Fondation Plan Alzheimer



#### References

1. Fodero-Tavoletti MT et al. In vitro characterisation of BF227 binding to  $\alpha$ -synuclein / Lewy bodies. *Eur J Pharmacol.* **2009**;617(1-3):54-58.
2. Kikuchi A et al. In vivo visualization of  $\alpha$ -synuclein deposition by carbon-11-labelled BF-227 PET in MSA. *Brain.* **2010**;133(Pt 6):1772-1778.
3. Levigoureux E, et al. Binding of the PET Radiotracer [<sup>18</sup>F]BF227 Does not Reflect the Presence of Alpha-Synuclein Aggregates in Transgenic Mice. *Curr Alz Res.* **2014**;11(10): 955-960.
4. Fodero-Tavoletti MT, et al. In vitro characterization of Pittsburgh compound-B binding to Lewy bodies. *J Neurosci.* **2007**;27(39): 10365-10371.