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► **To cite this version:**

Mathieu Verdurand, Meriem Mendjel-Herda, Elise Levigoureux, Sophie Lancelot, Wael Zeinyeh, et al.. A screening assay in the search of an alpha-synuclein PET radioligand. World Molecular Imaging Congress (WMIC 2015), Sep 2015, Honolulu, United States. hal-01207698

**HAL Id: hal-01207698**

**<https://hal.science/hal-01207698>**

Submitted on 15 Jan 2024

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## 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)

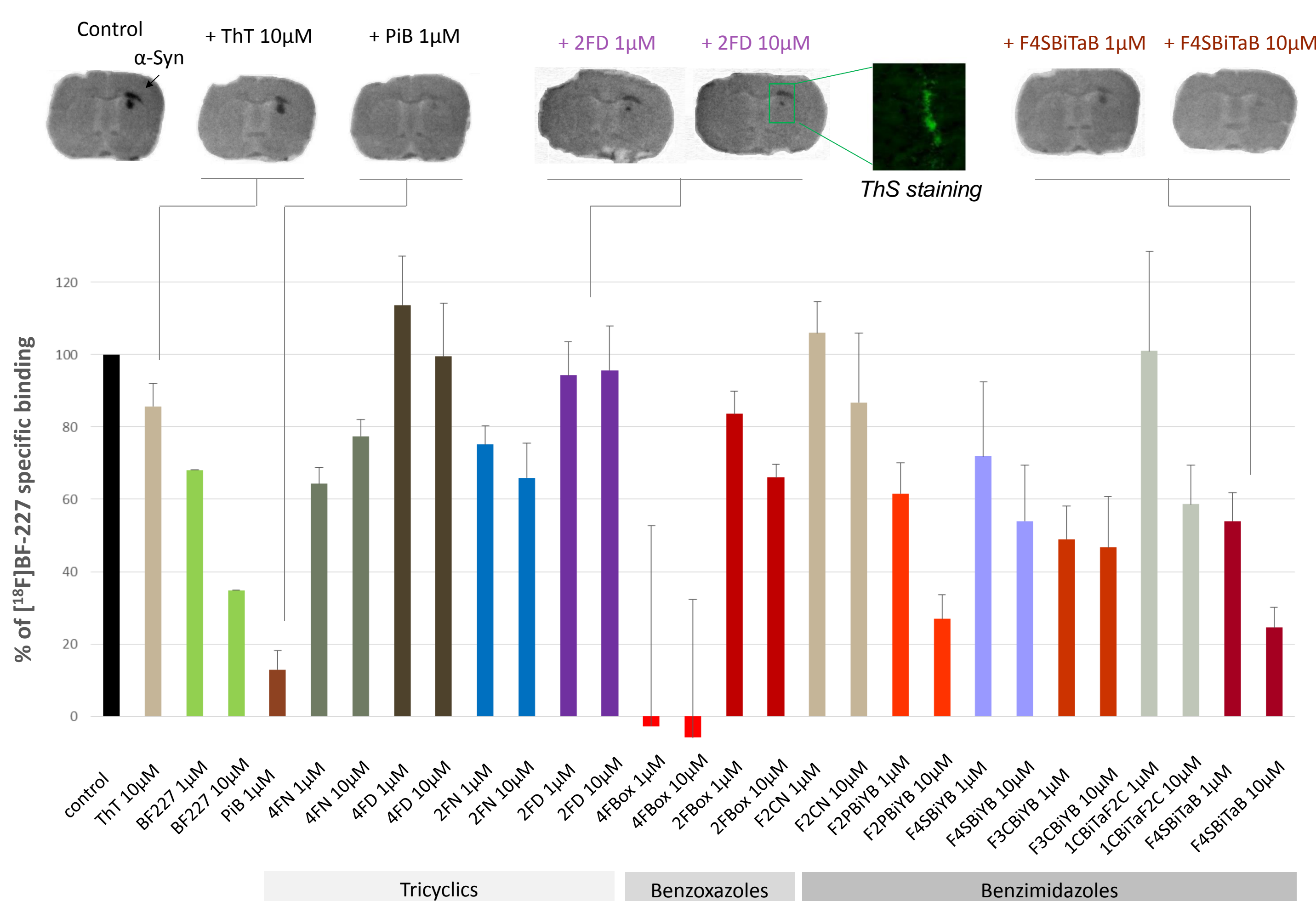
- $\alpha$ -Syn or  $A\beta$  fibrils (200 nM) were incubated with 40 nM [<sup>18</sup>F]BF227 and increasing concentrations of selected candidate molecules (0.1-200 nM) for 60 min in 200  $\mu$ L of PBS
- Bound from free radioactivity was separated by filtration under reduced pressure, and filters underwent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

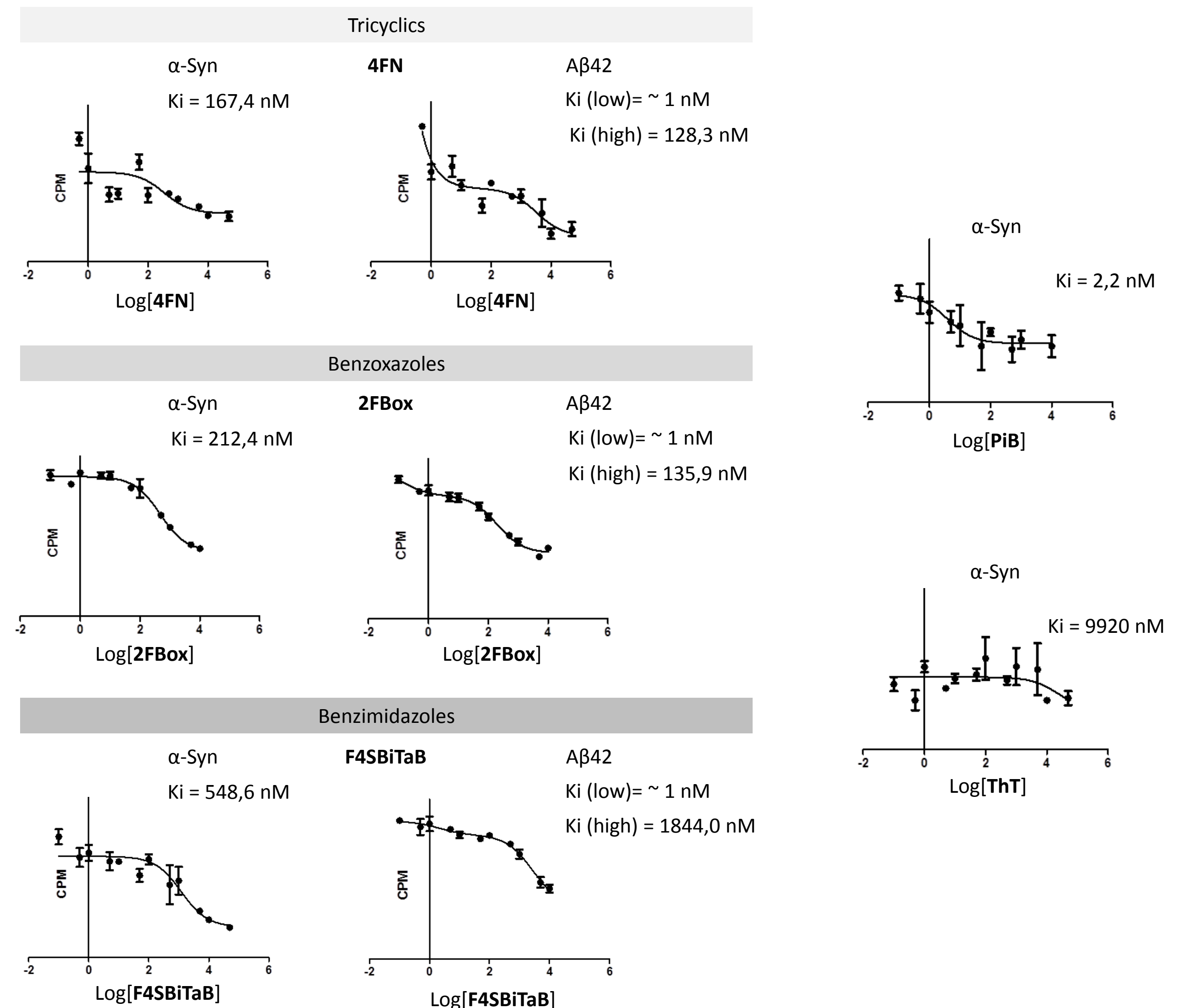
2. Competitive FBA allowed determining PiB affinity for  $\alpha$ -Syn fibrils, which value was close to the one already reported (4). Some competitor molecules showed  $\alpha$ -Syn fibrils affinity in the 100 nanomolar range but without displaying sufficient selectivity versus  $A\beta$  fibrils. Experiments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



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