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A screening assay in the search of an α -synuclein PET radioligand : an update

Introduction

The accumulation of aggregated α -synuclein (α -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- β ($A\beta$) PET radiotracers have been developed, no selective α -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 α -Syn fibrils in vitro (1) and α -Syn glial inclusions in MSA patients (2).

Objective To develop a simple screening assay for identifying α -Syn ligands by competitive [¹⁸F]BF227 autoradiography and filter binding assay

Methods

α -Syn and $A\beta$ fibrils

- Obtained by incubating commercial recombinant human proteins at 200 μ 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 μ L of fibrils or PBS, and were euthanized soon thereafter
- Frozen brains were cryostat-cut into 20- μ m sections

Molecules

- For competitive autoradiography, positive and negative controls were obtained with 1 μ M PiB and 10 μ 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/ μ mol
- Brain slices were incubated with subnanomolar quantities of [¹⁸F]BF227 with or without unlabelled competitors at 1 and 10 μ 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)

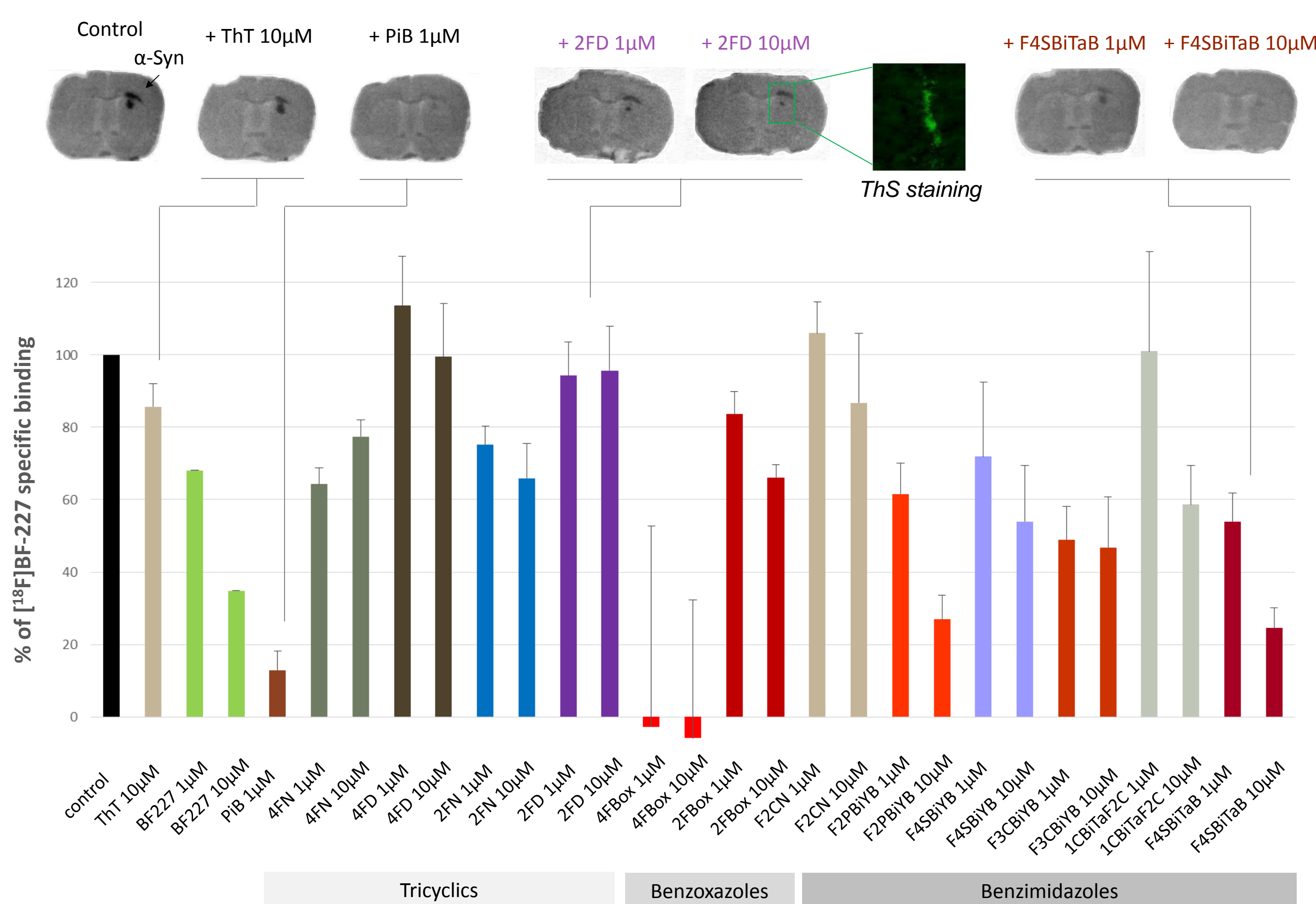
- α -Syn or $A\beta$ fibrils (200 nM) were incubated with 40 nM [¹⁸F]BF227 and increasing concentrations of selected candidate molecules (0.1-200 nM) for 60 min in 200 μ L of PBS
- Bound from free radioactivity was separated by filtration under reduced pressure, and filters underwent 3 washes
- K_i was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

Results

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

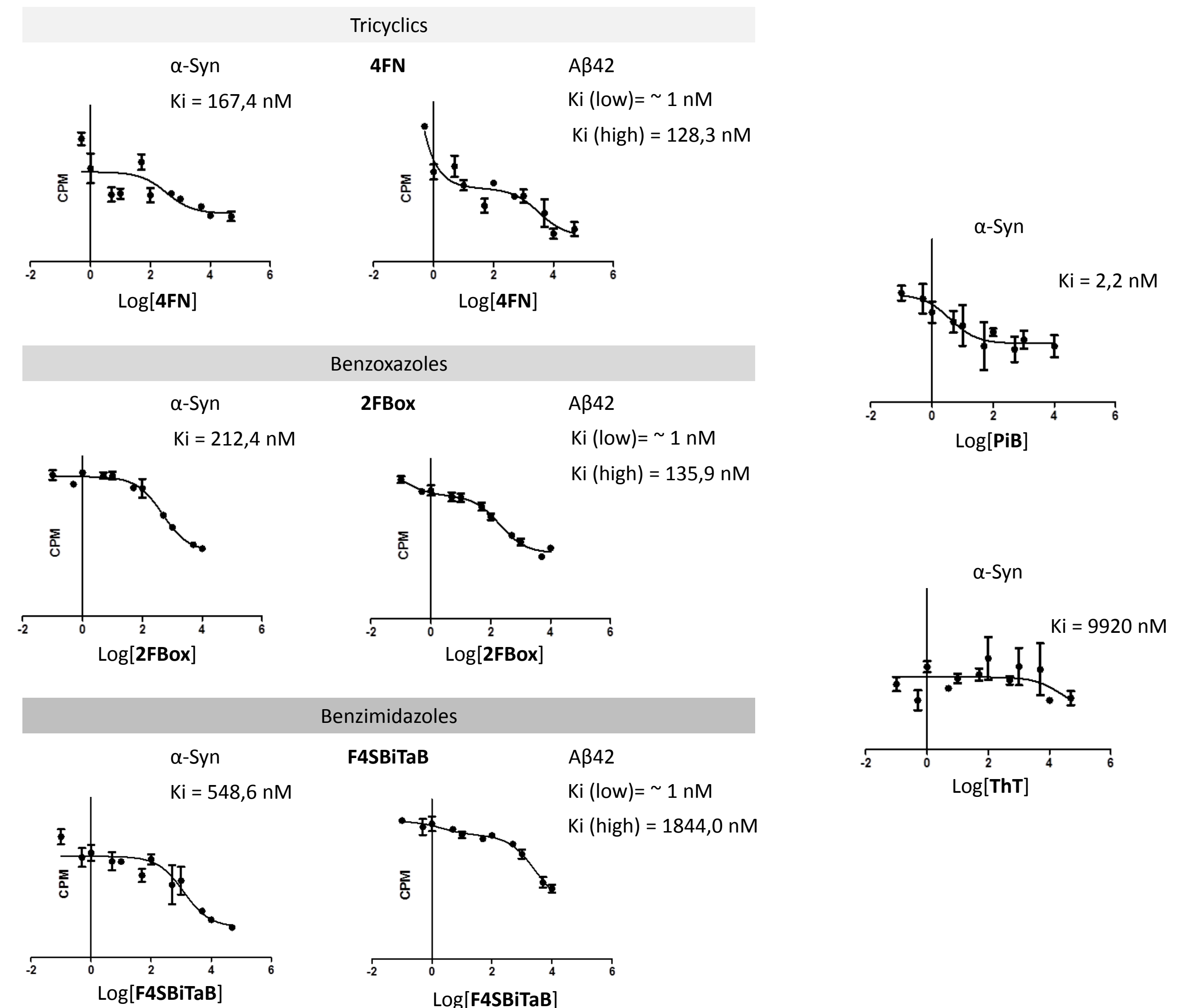
2. Competitive FBA allowed determining PiB affinity for α -Syn fibrils, which value was close to the one already reported (4). Some competitor molecules showed α -Syn fibrils affinity in the 100 nanomolar range but without displaying sufficient selectivity versus $A\beta$ fibrils. Experiments are still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

Competitive *in vitro* autoradiography



Histograms derived from competitive [¹⁸F]BF227 ARG analysis showing the ability of our original α -Syn candidate molecules to displace [¹⁸F]BF227 binding to recombinant human pre-aggregated α -Syn fibrils injected in rat striatum. Data represented as percentage of [¹⁸F]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 α -Syn candidate molecules through competitive filter binding assay with [¹⁸F]BF227 analysed with non-linear regression using Graphpad prism ("one site - fit K_i " model for α -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 [¹⁸F]BF227 binding sites on $A\beta$ and α -Syn fibrils
- > Identification of chemical scaffolds showing nanomolar affinity for α -Syn \rightarrow selectivity to be further tested versus other proteins forming beta-sheets ($A\beta$, Tau...)

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

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