Radiosynthesis, *in vitro* and *in vivo* evaluation of [^{18}F]Z-3540 for imaging 4R-tauopathies

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INTRODUCTION

- Human tau is comprised of six isoforms that possess three microtubule-binding repeats (3R) or four repeats (4R): Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are considered 4R-tauopathies, Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE) are mixed 3R/4R-tauopthies while Pick's disease (PiD) is considered a 3R-tauopathy.[1]
- Cryo-EM studies have demonstrated that tauopathies have different tau folding structures. AD & CTE share a common fold, where as PiD, PSP, or CBD are comprised of unique folds.[2]
- The development of tau PET radiotracers has been focused on AD. Of the radiopharmaceuticals reported to bind 4R-tau in non-AD tauopathies, [^{18}F]PM-PBB3 and [^{18}F]PI-2620 are the most advanced.[3]

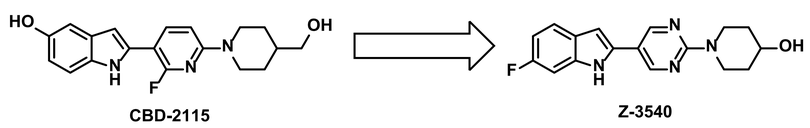


Figure 1. Structures of CBD-2115, Z-3540 and Z-2340.

- We recently reported the development of [^{18}F]CBD-2115 (**Figure 1**), a first-in-class PET radiotracer for imaging 4R-tauopathies based on a novel fluoro-pyridinyl indole structural scaffold.[4]
- [^{3}H]CBD-2115 binds to tau aggregates in PSP tissue *in vitro*. Unfortunately, [^{18}F]CBD-2115 had low brain uptake in rodent and non-human primate PET imaging studies.

AIMS

- To identify novel high-affinity analogs of CBD-2115 with suitable physicochemical properties for blood-brain barrier (BBB) permeability through high-resolution structural fingerprint searches.
- Radiolabel the lead candidates and evaluate them using *in vitro* binding assays as well as *in vivo* PET studies in rodents.

- Given the promising *in vitro* binding results of [^{3}H]/[^{18}F]CBD-2115 for non-AD tauopathies, a study to identify new analogs to improve brain uptake was initiated by chemical fingerprint-based method to identify closely related compounds to CBD-2115 from virtual screening libraries.
- An *in silico* search was performed by constructing a high-resolution structural fingerprint database of a 3.5B compound library from the Enamine REAL collection to identify promising leads.

METHODS & RESULTS

In Silico & *In Vitro* Evaluation

The current study utilized a variety of *in silico* approaches to identify high affinity candidates with higher probability of BBB permeability than CBD-2115:

- Cryo-EM structures of 4R-tau filaments were used to identify potential binding sites for structure-activity relationship (SAR) studies.
- A structural fingerprint based on CBD-2115 was then used to screen 3.5 Billion compounds virtually to identify candidates with similar SAR.
- Three computational methods, CNS MPO, CNS PET MPO and BBB score [5], were used to evaluate BBB permeability.
- Z-3540 showed improved scores in all three models compared to CBD-2115 (**Table 1**).

Table 1. *In silico* BBB permeability scores for Z-3540 compared to CBD-2115.

Compound	CNS MPO (6.0)	CNS PET MPO (6.0)	BBB Score (6.0)
CBD-2115	3.7	1.9	3.18
OXD-3540	**3.8**	**2.9**	**3.55**

- [^{3}H]Z-3540 affinity to tau filaments were evaluated in human AD, PSP, CBD, PiD and PD homogenous tissue (**Table 2**).
- K_d values showed that [^{3}H]Z-3540 binds with high affinity (≥5 nM) to mixed 3R/4R tau (AD tissue) as well as 4R tau (PSP and CBD tissue).

Table 2. Binding affinities (K_d) for [^{3}H]Z-3540 in human homogenous brain tissues.

Tissue	[^{3}H]Z-3540 K_d (nM)
AD	**4.0 ± 3.1**
PSP	**5.1 ± 1.2**
CBD	**4.5 ± 0.4**
PiD	9.7 ± 7.4
PD	89 ± 10

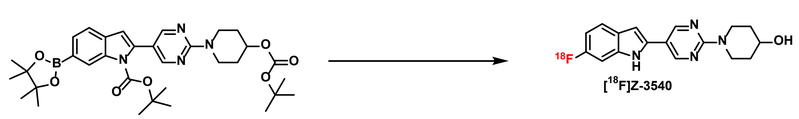
In Vitro Assays and Radiosynthesis

- In competitive binding assays against [^{3}H]PM-PBB3, [^{3}H]CBD-2115 and [^{3}H]PI-2620, Z-3540 blocked [^{3}H]PM-PBB3 but showed low capacity to block either [^{3}H]CBD-2115 or [^{3}H]PI-2620 (**Table 3**).
- Surprisingly, Z-3540 did not compete well for the same high affinity binding site as [^{3}H]CBD-2115, but did compete with [^{3}H]PM-PBB3.

Table 3. Competitive binding assay comparing Z-3540

Radioligand	Blocking Compound	AD tissue K_i (nM)	PSP tissue K_i (nM)	CBD tissue K_i (nM)
[^{3}H]PM-PBB3	Z-3540	**18**	**20**	**22**
[^{3}H]CBD-2115	Z-3540	270	250	210
[^{3}H]PI-2620	Z-3540	80	95	71

- [^{18}F]Z-3540 was radiolabeled in a two-step fully automated reaction using alcohol enhanced copper-mediated radiofluorination followed by deprotection of the BOC groups in methanol at high temperature.
- The unactivated site for the radiofluorination limited the radiochemical yields to <1.0% with molar activities of 65.3±15.2 GBq/μmol and radiochemical purity of >98%.
- The log$D_{7.4}$ value of [^{18}F]Z-3540 were measured to 3.36±0.04, which is within the upper range of known brain penetrant PET radiotracers.



Scheme 1. Radiosynthesis of [^{18}F]Z-3540.Conditions: [^{18}F]F-, K_2CO_3, Kryptofix$_{2.2.2}$, DMSO, 160°C, 20 min followed by methanol, 130°C, 20 min.

PET Imaging in Rats

- [^{18}F]Z-3540 showed initial uptake of 1.7 SUV in brain following *iv.* administration in rat PET imaging studies (**Figure 2**).
- Radioactivity cleared from brain as expected in wild-type rats to 0.5 SUV during the duration of the PET scans.

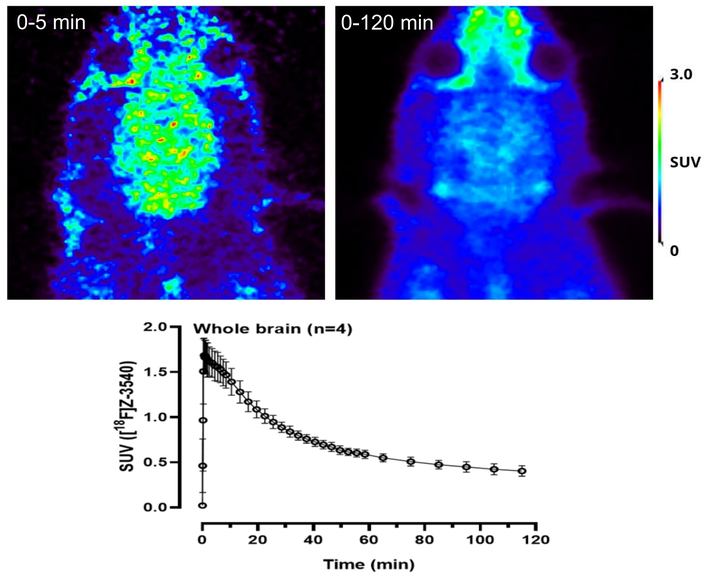


Figure 2. PET summation images (0-5 min and 0-120 min) and time-activity curve from rat PET imaging scans using [^{18}F]Z-3540.

CONCLUSIONS & FUTURE PERSPECTIVES

- [^{18}F]Z-3540 is a brain penetrant high-affinity 4R-tau PET radiotracers developed through *in silico* methodology.
- Further work on assessing their potential in higher species as well as identifying new analogs with higher affinities are ongoing.

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References: (1) Goedert et al. *Annu. Rev. Neurosci.* **2017**; Berriman et al. *Proc. Natl. Acad. Sci*, **2003**; Arai et al. *Acta Neuropathol.* **2001 (2)** Sergeant et al. *J. Neurochem.* **1999**; McKee et al. *Brain Pathol.* **2015**; Shi et al. *Nature* **2021 (3)** Malarte et al. *Eur. J. Nucl. Med. Mol. Imaging* **2021**; Tagai et al. *Neuron* **2021 (4)** Lindberg et. al. *ACS Chem. Neurosci.* **2021 (5)** Gupta et al. *J. Med Chem.* **2019**; Zhang et al. *J. Med. Chem.* **2013**

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