• 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-tauopathies 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, [18F]PM-PBB3 and [18F]PI-2620 are the most advanced.[3]

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

• 

\[
\text{K}_d \text{ values showed that [3H]Z-3540 binds with high affinity}
\]

\[
(18F)Z-3540
\]

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 (18F)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.

In Silico & In Vivo 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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CNS MPO (6.0)</th>
<th>CNS PET MPO (6.0)</th>
<th>BBB Score (6.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBD-2115</td>
<td>3.7</td>
<td>1.9</td>
<td>3.18</td>
</tr>
<tr>
<td>OXD-3540</td>
<td>3.8</td>
<td>2.9</td>
<td>3.55</td>
</tr>
</tbody>
</table>

• [1H]Z-3540 affinity to tau filaments were evaluated in human AD, PSP, CBD, PiD and PD homogenous tissue (Table 2).

\[
\text{K}_d \text{ values showed that [1H]Z-3540 binds with high affinity (≤5 nM) to mixed 3R/4R tau (AD tissue) as well as 4R tau (PSP and CBD tissue).}
\]

Table 2. Binding affinities (Kd) for [1H]Z-3540 in human homogenous brain tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>[1H]Z-3540 Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>4.0 ± 3.1</td>
</tr>
<tr>
<td>PSP</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>CBD</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>PiD</td>
<td>9.7 ± 7.4</td>
</tr>
<tr>
<td>PD</td>
<td>89 ± 10</td>
</tr>
</tbody>
</table>

• [18F]Z-3540 was radiolabeled in a two-step fully automated reaction using alcohol enhanced copper-mediated radiofluorination followed by purification of >98%.

• The logD7.4 value of [18F]Z-3540 were measured to 3.36 ± 0.04, which is within the upper range of known brain penetrant PET radiotracers.

• 

\[
\text{In competitive binding assays against [1H]PM-PBB3, [1H]CBD-2115 and [1H]PI-2620 (Table 3).}
\]

• Surprisingly, Z-3540 did not compete well for the same high affinity binding site as [1H]CBD-2115, but did compete with [1H]PM-PBB3.

• [18F]Z-3540 is a brain penetrant high-affinity 4R-tau PET radiotracer for imaging 4R-tauopathies based on a novel fluoro-pyridinyl indole structural scaffold.[4]

• A structural fingerprint database of a 3.5B compound library from the Enamine REAL collection to identify promising leads.

• [18F]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.

Table 3. Competitive binding assay comparing Z-3540

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>Blocking Compound</th>
<th>AD tissue Ki (nM)</th>
<th>PSP tissue Ki (nM)</th>
<th>CBD tissue Ki (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1H]PM-PBB3</td>
<td>Z-3540</td>
<td>18</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>[1H]CBD-2115</td>
<td>Z-3540</td>
<td>270</td>
<td>250</td>
<td>210</td>
</tr>
<tr>
<td>[1H]PI-2620</td>
<td>Z-3540</td>
<td>80</td>
<td>95</td>
<td>71</td>
</tr>
</tbody>
</table>

• [18F]Z-3540 was shown to have a low Ki (nM) in all tissues and a high Ki (nM) in PSP tissue.

• [18F]Z-3540 showed high 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.

CONCLUSIONS & FUTURE PERSPECTIVES

• [18F]Z-3540 is a brain penetrant high-affinity 4R-tau PET radiotracer 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.

References:

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