Radiosynthesis, in vitro and in vivo evaluation of [¹⁸F]Z-3540 for imaging 4R-tauopathies Anton Lindberg¹, Thomas J. A. Graham², Junchao Tong¹, Robert H. Mach², Chester A. Mathis³ and Neil Vasdev^{1,4}

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 Human tau is binding repeats (PSP) and c tauopathies, encephalopath (PiD) is conside Cryo-EM studi folding structur CBD are comp The developme radiopharmace [¹⁸F]PM-PBB3 	comprised of six s (3R) or four report orticobasal dege Alzheimer's dis y (CTE) are mixe ered a 3R-tauopat es have demonst es. AD & CTE sha rised of unique fol ent of tau PET rac uticals reported and [¹⁸ F]PI-2620 a	isoforms that possess three eats (4R): Progressive sup- eneration (CBD) are c sease (AD) and chro ed 3R/4R-tauopthies while thy.[1] trated that tauopathies hav are a common fold, where ds.[2] diotracers has been focused to bind 4R-tau in non-A are the most advanced.[3]	e microtubule- ranuclear palsy onsidered 4R- nic traumatic Pick's disease ve different tau as PiD, PSP, or	HO	-2115 -2115 CBD-2115, Z-3540 ar ported the deve radiotracer for indole structura binds to tau ago had low brain u S.	nd Z-2340. Iopment of [¹⁸ F imaging 4R-ta I scaffold.[4] gregates in PSI ptake in roden	FJCBD-2115 (Figuopathies base P tissue <i>in vitro.</i> t and non-huma	[■] yure 1), a first- d on a novel Unfortunately, in primate PET	 To identify novel hig physicochemical proper through high-resolution set Radiolabel the lead car assays as well as <i>in vivo</i> Given the promising <i>in v</i> AD tauopathies, a study was initiated by chemi- related compounds to CE An <i>in silico</i> search was structural fingerprint da Enamine REAL collection
	In Silico & I	<i>In Vitro</i> Evaluation		//	Ρ				
 The current study affinity candidates Cryo-EM structure binding sites for the structure of the structure of	y utilized a varied with higher proba- ctures of 4R-tau or structure-activity ngerprint based pounds virtually to	 In competitive binding assays against [³H]PM-PBB3, [³H]CBD-2115 and [³H]PI-2620, Z-3540 blocked [³H]PM-PBB3 but showed low capacity to block either [³H]CBD-2115 or [³H]PI-2620 (Table 3). Surprisingly, Z-3540 did not compete well for the same high affinity binding site as [³H]CBD-2115, but did compete with [³H]PM-PBB3. 					 [¹⁸F]Z-3540 showed init administration in rat PET Radioactivity cleared from during the duration of the 0-5 min 		
Three compute	ational methods, C	Table 3. Competitive b							
 [5], were used Z-3540 show CBD-2115 (Tal 	to evaluate BBB p ed improved sco ble 1).	Radioligand	Blocking Compound	AD tissue K _i (nM)	PSP tissue K _i (nM)	CBD tissue K _i (nM)			
Table 1. In silico BBB permeability scores for Z-3540 compared to CBD-2115.				[³ H]PM-PBB3	Z-3540	18	20	22	
Compound	CNS MPO (6.0)	CNS PET MPO (6.0)	BBB Score (6.0)	[³ H]CBD-2115	Z-3540	270	250	210	
CBD-2115	3.7	1.9	3.18		Z-3540	80	95	/	2.0 J Who
 OXD-3540 [³H]Z-3540 affin PiD and PD hor K_d values show 3R/4R tau (AD f 	3.8 ity to tau filaments nogenous tissue (ed that [³ H]Z-354 tissue) as well as 4	 [¹⁸F]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 >08% 					- 5.1 (l ₁₈ - 0.1 l ₁₈ - 0.5 - 0.5 - 0.0		
Table 2. Binding affinities (Kd) for [3H]Z-3540 in human homogenous brain tissues.Tissue[3H]Z-3540 Kd (nM)				 The logD_{7.4} value of [¹⁸F]Z-3540 were measured to 3.36±0.04, which is within the upper range of known brain penetrant PET radiotracers. 					Figure 2. PET summation images
	AD	4.0 ± 3.1							Imaging scans using [10F]Z-3540.
F	SP BD PiD PD	5.1 ± 1.2 4.5 ± 0.4 9.7 ± 7.4 89 ± 10		Cheme 1. Radiosynthemin followed by methan	 [¹⁸F]Z-3540 is a radiotracers develop Further work on assea identifying new ar 				
UNIVERSITY	of	Brain Health Imaging Centre Conn	e iversity of	References: (1) Goed Arai et al. <i>Acta Neuro</i> 2015 ; Shi et al. <i>Nature</i> 2021 (4) Lindberg et. <i>J. Med. Chem</i> . 2013	dert et al. <i>Annu. Rev. I</i> pathol. 2001 (2) Serge e 2021 (3) Malarte et a al. ACS Chem. Neuro	<i>Neurosci</i> . 2017 ; Ber eant et al. <i>J. Neuroc</i> al. <i>Eur. J. Nucl. Med</i> sci. 2021 (5) Gupta	riman et al. <i>Proc. Nat</i> chem. 1999 ; McKee e . <i>Mol. Imaging</i> 2021 ; et al. <i>J. Med Chem</i> . 2	<i>I. Acad. Sci</i> , 2003 ; t al. <i>Brain Pathol</i> . Tagai et al. <i>Neuron</i> 2019 ; Zhang et al.	Acknowledgements: We thank the radiochemistry, preclinical, Foundation, the Canada Research Chairs support. The National Institute on Neurolog NS110456), Human AD, PSP, CBD, and Pil the University of California, San Francisco

						<u>RODUCTIO</u>			
 Human tau binding rep (PSP) and tauopathies encephalop (PiD) is con Cryo-EM st folding strue CBD are co The develo radiopharm [¹⁸F]PM-PB 	i is comprised of size beats (3R) or four rep d corticobasal deg s, Alzheimer's d bathy (CTE) are mix hisidered a 3R-tauopa tudies have demons ctures. AD & CTE sl omprised of unique for pment of tau PET ra aceuticals reported B3 and [¹⁸ F]PI-2620	k isoforms that possess the peats (4R): Progressive supeneration (CBD) are isease (AD) and che ked 3R/4R-tauopthies while athy.[1] strated that tauopathies he hare a common fold, where olds.[2] adiotracers has been focus to bind 4R-tau in non- are the most advanced.[3]	hree microtubule- upranuclear palsy considered 4R- nonic traumatic le Pick's disease have different tau e as PiD, PSP, or ed on AD. Of the -AD tauopathies,	HO CBD Figure 1. Structures of • We recently rein-class PET fluoro-pyridiny • [³ H]CBD-2115 [¹⁸ F]CBD-2115 imaging studie	OH CBD-2115 f CBD-2115, Z-3540 a eported the deve radiotracer for d indole structura binds to tau age 5 had low brain to es.	nd Z-2340. elopment of [¹⁸ F imaging 4R-ta il scaffold.[4] gregates in PSF uptake in rodent	FICBD-2115 (Fi uopathies base tissue <i>in vitro</i> t and non-huma	$= N \longrightarrow -OH$ 40 gure 1), a first- ed on a novel b. Unfortunately, an primate PET	 AIMS To identify novel high-affinity and physicochemical properties for block through high-resolution structural finges Radiolabel the lead candidates and assays as well as <i>in vivo</i> PET studies Given the promising <i>in vitro</i> binding real AD tauopathies, a study to identify new was initiated by chemical fingerprinarelated compounds to CBD-2115 from An <i>in silico</i> search was performed structural fingerprint database of a Enamine REAL collection to identify prime
	In Silico &	In Vitro Evaluation			<i>n Vitro</i> Assa	PET Imagin			
 The current s affinity candida Cryo-EM s binding site A structure 3 5 Billion a 	study utilized a variant ates with higher prob structures of 4R-tau es for structure-activities al fingerprint based	ety of <i>in silico</i> approache bability of BBB permeability if filaments were used to ity relationship (SAR) studie on CBD-2115 was then to identify candidates with	 In competitive binding assays against [³H]PM-PBB3, [³H]CBD-2115 and [³H]PI-2620, Z-3540 blocked [³H]PM-PBB3 but showed low capacity to block either [³H]CBD-2115 or [³H]PI-2620 (Table 3). Surprisingly, Z-3540 did not compete well for the same high affinity binding site as [³H]CBD-2115, but did compete with [³H]PM-PBB3. 					 [¹⁸F]Z-3540 showed initial uptake of administration in rat PET imaging studie Radioactivity cleared from brain as explaned uring the duration of the PET scans. 	
 Three com 	putational methods.	CNS MPO, CNS PET MP	O and BBB score	Table 3. Competitive b	binding assay compari				
 [5], were used to evaluate BBB permeability. Z-3540 showed improved scores in all three models compared to CBD-2115 (Table 1). 				Radioligand	RadioligandBlockingAD tissuePSICompoundKi (nM)K		PSP tissue K _i (nM)	P tissue CBD tissue K _i (nM) K _i (nM)	
Table 1. In silico BE	BB permeability scores for 2	Z-3540 compared to CBD-2115.	[³ H]PM-PBB3	Z-3540	18	20	22		
Compound	d CNS MPO (6.0)	CNS PET MPO (6.0)	BBB Score (6.0)	[³ H]CBD-2115 г ³ н101-2620	Z-3540	270 80	250 05	210 71	
CBD-2115	3.7	1.9	3.18		Z-3340	00	90	/ 1	^{2.0} ך Whole brain (n=4)
OXD-3540	3.8	2.9	3.55	• [¹⁸ F]Z-3540 wa	as radiolabeled	in a two-step fu	ully automated	reaction using	Q 1.5-
• [³ H]Z-3540 a PiD and PD	affinity to tau filamen	its were evaluated in huma (Table 2)	alcohol enha deprotection of	anced copper- f the BOC group					
 K_d values sl 3R/4R tau (/ 	howed that [³ H]Z-35 AD tissue) as well as	40 binds with high affinity s 4R tau (PSP and CBD tiss	 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%. 						
Table 2. Binding af	finities (K _d) for [³ H]Z-3540 in	n human homogenous brain tissues	• The logD _{7.4} value of [¹⁸ F]Z-3540 were measured to 3.36 \pm 0.04, which is					0 20 40 60 Time (n	
	lissue	[°H]Z-3540 K _d (nM)		within the uppe	er range or know	n brain penetrai			Figure 2. PET summation images (0-5 min and 0-
		4.0 ± 3.1							
	P3P	5.1 I 1.2			• [¹⁸ F17-3540 is a brain penet				
		4.3 ± U.4 0 7 ± 7 4		$\begin{array}{c} \begin{array}{c} \begin{array}{c} \\ \\ \end{array} \end{array} \end{array} \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \end{array} \end{array} \\ \begin{array}{c} \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \end{array} \end{array} \\ \begin{array}{c} \\ \\ \end{array} \end{array} \\ \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \end{array} \\ \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \end{array} \\ \begin{array}{c} \\ \end{array} \end{array} \\ \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \end{array} \\ \end{array} \end{array} $ \\ \begin{array}{c} \\ \end{array} \end{array} \end{array} \\ \begin{array}{c} \\ \end{array} \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \end{array} \\ \end{array} \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \end{array} \\ \\ \end{array} \\ \end{array} \\ \\ \end{array} \\ \end{array} \\ \end{array} \\ \\ \end{array} \\ \end{array} \\ \\ \\ \end{array} \\ \\ \\ \end{array} \\ \\ \end{array} \\ \\ \\ \end{array} \\ \\ \\ \\					radiotracers developed through <i>ir</i>
	PID	9.7 ± 7.4 89 + 10		Scheme 1. Radiosvnt	, thesis of [¹⁸ F17-3540.0	Conditions: [¹⁸ F]F-, k	CO ₂ Kryptofix	DMSO, 160°C, 20	 Further work on assessing their particular providentiation and a sessing their provident of the session of the se
				min followed by metha	anol, 130°C, 20 min.	······································	2 3, · · · J P · · · · · 2.2.2	, ,	A showled a structure of the structure o
UNIVERS TORO	annh ITY OF NTO	Brain Health Imaging Cent Penn NIVERSITY of PENNSYLVANIA	TC University of Pittsburgh	References: (1) Goe Arai et al. <i>Acta Neuro</i> 2015; Shi et al. <i>Natur</i> 2021 (4) Lindberg et. <i>J. Med. Chem</i> . 2013	edert et al. <i>Annu. Rev.</i> Spathol. 2001 (2) Serg re 2021 (3) Malarte et al. ACS Chem. Neurc	<i>Neurosci</i> . 2017 ; Berr Jeant et al. <i>J. Neuroc</i> al. <i>Eur. J. Nucl. Med.</i> osci. 2021 (5) Gupta e	riman et al. <i>Proc. Na</i> <i>hem</i> . 1999 ; McKee <i>Mol. Imaging</i> 2021 ; et al. <i>J. Med Chem</i> .	<i>tl. Acad. Sci</i> , 2003 ; et al. <i>Brain Pathol</i> . Tagai et al. <i>Neuron</i> 2019 ; Zhang et al.	Acknowledgements: We thank the radiochemistry, preclinical, and methodology tea Foundation, the Canada Research Chairs Program, Canada Fou support. The National Institute on Neurological Disorders and Stro NS110456), Human AD, PSP, CBD, and PiD tissue samples were the University of California, San Francisco, the Rainwater Char Human PD tissue was provided by the Michael J. Fox Foundation.

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AIMS

h-affinity analogs of CBD-2115 with suitable erties for blood-brain barrier (BBB) permeability structural fingerprint searches. ndidates and evaluate them using in vitro binding

o PET studies in rodents.

vitro binding results of [³H]/[¹⁸F]CBD-2115 for nony to identify new analogs to improve brain uptake ical fingerprint-based method to identify closely BD-2115 from virtual screening libraries.

as performed by constructing a high-resolution atabase of a 3.5B compound library from the n to identify promising leads.

PET Imaging in Rats

itial uptake of 1.7 SUV in brain following iv. imaging studies (**Figure 2**). m brain as expected in wild-type rats to 0.5 SUV PET scans.





DNS & FUTURE PERSPECTIVES brain penetrant high-affinity 4R-tau PET bed through in silico methodology. essing their potential in higher species as well nalogs with higher affinities are ongoing.

and methodology teams at CAMH Brain Health Imaging Centre; the Azrieli Program, Canada Foundation for Innovation and the Ontario Research Fund for jical Disorders and Stroke (NINDS) for supporting this research collaboration (U19 D tissue samples were provided by the Neurodegenerative Disease Brain Bank at o, the Rainwater Charitable Foundation, and the Bluefield Project to Cure FTD.