

**Supplemental information**

**Development of an  $\alpha$ -synuclein positron emission  
tomography tracer for imaging synucleinopathies**

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**Table S1: Candidate compound information, related to Figure 1.**

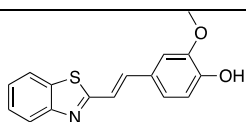
No.	Compound	Structure	No.	Compound	Structure
<b>A. Commercial compounds</b>					
1	Z644		13	96-9	
2	Z213		14	54-4	
3	Z922		15	83-4	
4	Z584		16	77-4	
5	Z864		17	01-7	
6	Z987		18	61-8	
7	Z257		19	19-1	
8	Z427		20	80-3	
9	Z819		21	83-4	
10	Z876		22	34-5	
11	Z398		23	68-7	

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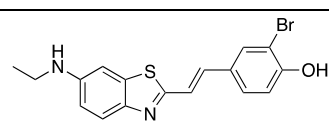
**B, Derivatives**

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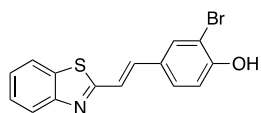
1 EU01-01A



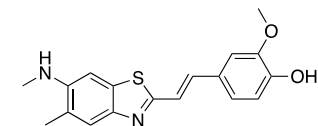
12 EU04-03B



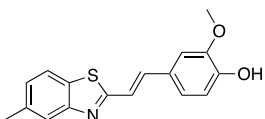
2 EU01-01B



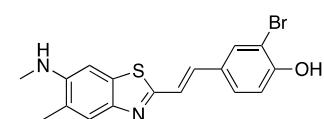
13 EU05-01A



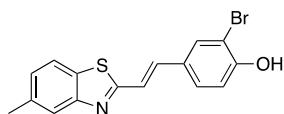
3 EU02-01A



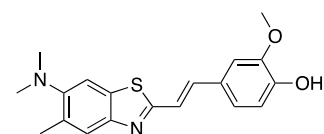
14 EU05-01B



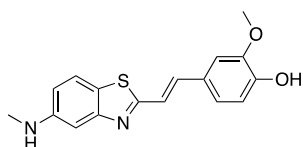
4 EU02-01B



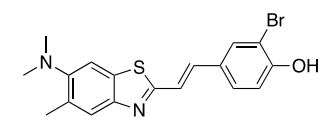
15 EU05-02A



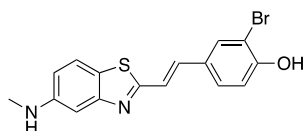
5 EU03-01A



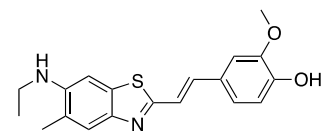
16 EU05-02B



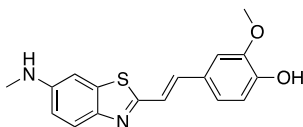
6 EU03-01B



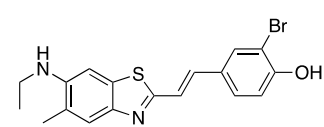
17 EU05-03A



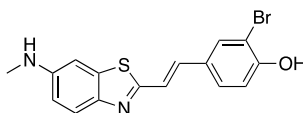
7 EU04-01A



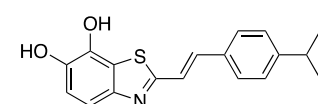
18 EU05-03B



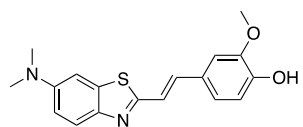
8 EU04-01B



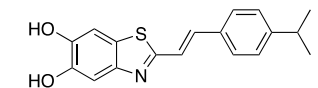
19 Compound 1



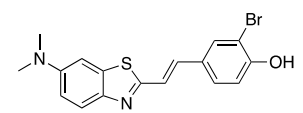
9 EU04-02A



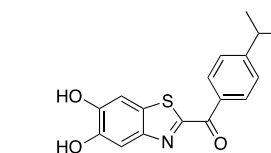
20 WT17008-001A



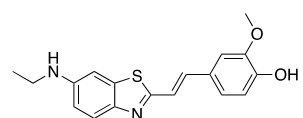
10 EU04-02B



21 WT17008-001B



11 EU04-03A



**Table S2: Plasma and brain concentrations and B/P ratio of F0502B in male ICR mice (i.v. injected with 5 mg/kg F0502B), related to Figure 2.**

<b>Plasma Concentrations (ng/mL) of F0502B in Male ICR Mouse After 5 mg/kg i.v. Dosed</b>						
Time Point (min) Concentrations (Mean ± SD)					T <sub>1/2</sub> (min)	CL (mL/min/kg)
1	5	15	30	60		
30396 ± 1290	4267 ± 507	1009 ± 47	324 ± 62	128 ± 22	6.9	33.6
<b>Brain Concentrations (ng/g) of F0502B in Male ICR Mouse After 5 mg/kg i.v. Dosed</b>						
Time Point (min) Concentrations (Mean ± SD)					T <sub>1/2</sub> (min)	CL (g/min/kg)
1	5	15	30	60		
3220 ± 88	3820 ± 526	2734 ± 349	2314 ± 198	1205 ± 83	35.8	24.1
<b>B/P Ratio of F0502B in Male ICR Mouse After 5 mg/kg i.v. Dosed</b>						
Time Point (h) Concentrations (Mean ± SD)						
1	5	15	30	60		
0.106 ± 0.006	0.894 ± 0.020	2.72 ± 0.44	7.32 ± 1.66	13.37 ± 2.64		

**Table S3: Complete cell count and liver/renal function test of C57BL/6 mice (i.v. injected with 1 mg/kg F0502B (10 folds of the dose in radioactive PET study), n = 3 mice per group), related to Figure 7.**

<b>Parameter</b>	<b>Mean</b>	<b>SD</b>	<b>Unit</b>	<b>Reference values</b>
Total white blood cell count (WBC)	5.20	0.44	10 <sup>9</sup> /L	0.8-6.8
Lymphocytes count	3.43	0.47	10 <sup>9</sup> /L	0.7-5.7
Monocytes count	0.30	0.10	10 <sup>9</sup> /L	0.0-0.3
Neutrophils count	1.47	0.42	10 <sup>9</sup> /L	0.5-3.8
mean corpuscular volume (MCV)	53.53	0.49	fL	48.2-58.3
red blood cell (RBC) count	8.63	0.23	10 <sup>12</sup> /L	6.36-12.42
hemoglobin concentration (HGB)	135.00	2.00	g/L	110-162
plateletcrit (PLT)	911.00	122.10	10 <sup>9</sup> /L	450-1590
alanine transaminase (ALT)	52.79	17.98	U/L	10.06-96.47
aspartate transaminase (AST)	135.84	16.76	U/L	36.31-235.48
total bilirubin (TBIL)	11.47	0.75	umol/l	0.45-33.89
blood urea nitrogen (BUN)	21.75	1.09	mg/dl	10.81-34.74
creatinine (CR)	15.69	0.45	umol/l	10.91-85.09
uric acid (UA)	54.54	2.37	umol/l	44.42-224.77

**Table S4. Statistics of cryo-EM data collection, refinement and validation, related to Figure 5, 6.**

Name	$\alpha$ -Syn fibril-F0502B complex	Apo- $\alpha$ -Syn fibril
PDB ID	7WMM	-
EMDB ID	EMD-32615	-
<b>Data Collection</b>		
Camera	BioContinuum K3	BioContinuum K3
Microscope	Krios G4	Krios G4
Magnification	105,000	105,000
Voltage (kV)	300	300
Defocus Range ( $\mu\text{m}$ )	-1.6 to -2.2	-1.6 to -2.2
Pixel size ( $\text{\AA}$ )	0.83	0.83
Exposure time (s/frame)	0.05	0.05
Number of frames	40	40
Total dose ( $\text{e}^-/\text{\AA}^2$ )	60	60
<b>Reconstruction</b>		
Micrographs	2,567	2,868
Manually picked fibrils	12,0504	29,120
Box size (pixel)	360	360
Inter-box distance ( $\text{\AA}$ )	29.88	29.88
Segments extracted	742,177	617,969
Segments after Class2D	155,475	116,827
Segments after Class3D	46,861	42,079
Resolution ( $\text{\AA}$ )	2.6	2.8
Map sharpening B-factor ( $\text{\AA}^2$ )	-74.94	-87.79
Half pitch (nm)	113.2	106.3
Helical rise ( $\text{\AA}$ )	2.41	2.42
Helical twist ( $^\circ$ )	179.62	179.59
<b>Atomic model</b>		
Non-hydrogen atoms	2,618	-

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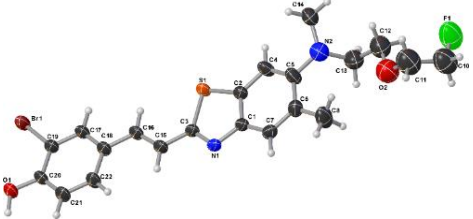
Protein residues	366	-
Ligands	2	-
Protein B-factors ( $\text{\AA}^2$ )	28-110 (avr. 50)	-
Ligand B-factors ( $\text{\AA}^2$ )	77	-
r.m.s.d. Bond lengths ( $\text{\AA}$ )	0.004	-
r.m.s.d. Bond angles ( $^\circ$ )	0.632	-
All-atom clash score	4.68	-
MolProbity score	1.70	-
Rotamer outliers	0.00%	-
Ramachandran Outliers	0.00%	-
Ramachandran Allowed	7.34%	-
Ramachandran Favored	92.66%	-

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**Table S5: F0502B crystal structure information, related to Figure 5, STAR**

**Methods.**



Compound	F0502B
Formula	C <sub>21</sub> H <sub>22</sub> BrFN <sub>2</sub> O <sub>2</sub> S
Structure	
<i>D</i> <sub>calc</sub> /g cm <sup>-3</sup>	1.477
$\mu$ /mm <sup>-1</sup>	3.846
Formula Weight	465.52
Color	yellow
Shape	plate
Size/mm <sup>3</sup>	0.45×0.20×0.05
<i>T</i> /K	101(2)
Crystal System	monoclinic
Space Group	<i>P</i> 2 <sub>1</sub> / <i>n</i>
<i>a</i> /Å	16.2164(4)
<i>b</i> /Å	14.8387(3)
<i>c</i> /Å	19.1152(5)
$\alpha$ /°	90
$\beta$ /°	114.474(3)
$\gamma$ /°	90
<i>V</i> /Å <sup>3</sup>	4186.4(2)
<i>Z</i>	8
<i>Z</i> '	2
Wavelength/Å	1.54184
Radiation type	CuK $\alpha$
$\theta_{min}$ /°	3.019
$\theta_{max}$ /°	68.249
Measured Refl.	37434
Independent Refl.	7618
Reflections with <i>I</i> > 2 $\sigma$ ( <i>I</i> )	6457
<i>R</i> <sub>int</sub>	0.0541

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Parameters	563
Restraints	517
Largest Peak	1.331
Deepest Hole	-1.304
GooF	1.062
$wR_2$ (all data)	0.2230
$wR_2$	0.2146
$R_1$ (all data)	0.0848
$R_1$	0.0760

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**Table S6: Human tissue information, related to STAR methods.**

<b>CASE NO.</b>	<b>PRIMARY NEUROPATHOLOGIC DIAGNOSIS</b>	<b>SECONDARY NEUROPATHOLOGIC DIAGNOSIS</b>	<b>AGE AT DEATH/BX</b>	<b>SEX</b>	<b>BRAIN REGIONS</b>
1	Control		65	m	cortex
2	Control		70	m	cortex
3	Control	Infarcts-hemorrhagic	69	m	cortex
4	Control	Microinfarct	61	m	cortex
5	Control		59	m	cortex
6	Control	Microinfarct-Hp	94	m	cortex
7	Control		87	m	midbrain, amygdala
8	Control		74	m	midbrain, amygdala
9	Control		89	m	midbrain, amygdala
10	AD		72	m	cortex
11	AD		68	m	cortex
12	AD		85	m	midbrain, amygdala
13	AD		77	m	midbrain, amygdala
14	AD		85	m	midbrain, amygdala
15	DLB-limbic	Status PostPallidotomy	74	m	cortex
16	DLB-limbic		67	m	cortex
17	DLB-limbic		72	m	cortex
18	DLB-limbic	TDP-43 incl-Hp, Ent	97	f	cortex
19	DLB-neocortical	Status Post Gamma-Knife Radiosurgery	69	m	cortex
20	DLB-neocortical	Status Post Pallidotomy	59	m	cortex
21	DLB-neocortical	Status postpallidotomy	78	m	cortex
22	DLB-neocortical		64	m	cortex
23	DLB-neocortical		64	m	cortex
24	DLB-neocortical	Marked loss of neurons in CA3	68	m	cortex, midbrain
25	DLB-neocortical	Braak III	75	m	cortex, midbrain
26	DLB-neocortical		72	f	cortex, midbrain
27	PD		83	m	midbrain, amygdala
28	PD		74	m	midbrain, amygdala
29	PD		85	m	midbrain, amygdala
30	PD-neocortical	Hemorrhage-pons	86	f	cortex
31	PD-neocortical	Status postpallidotomy	78	f	cortex
32	PD-neocortical		64	f	cortex
33	MSA(SND)		78	m	SN
34	MSA(SND)		68	m	SN
35	MSA (Corticonigral)	Lobar Atrophy w/o Pick Bodies	69	m	SN
36	MSA (SND and OPCA)		72	f	SN
37	MSA (SND and OPCA)	Sclerosis - Hp	75	m	SN