Supporting Information

Identification of a novel positron emission tomography (PET) ligand for imaging β-site amyloid precursor protein cleaving enzyme 1 (BACE-1) in brain

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1. ^{1}H NMR and ^{13}C NMR spectra and peak assignments for compound 3 (PF-06684511) and the tosylate precursor 18

^{1}H and ^{13}C NMR spectra were acquired using a VNMRS 400 MHz NMR spectrometer equipped with a 5.0 mm z-gradient OneNMR broadband probe with the proton and carbon coils tuned to 400.20 MHz and 100.64 MHz, respectively.

The ^{1}H NMR spectrum of **3** is shown in Figure 1-1 and the peak assignments are shown in Table 1-1.

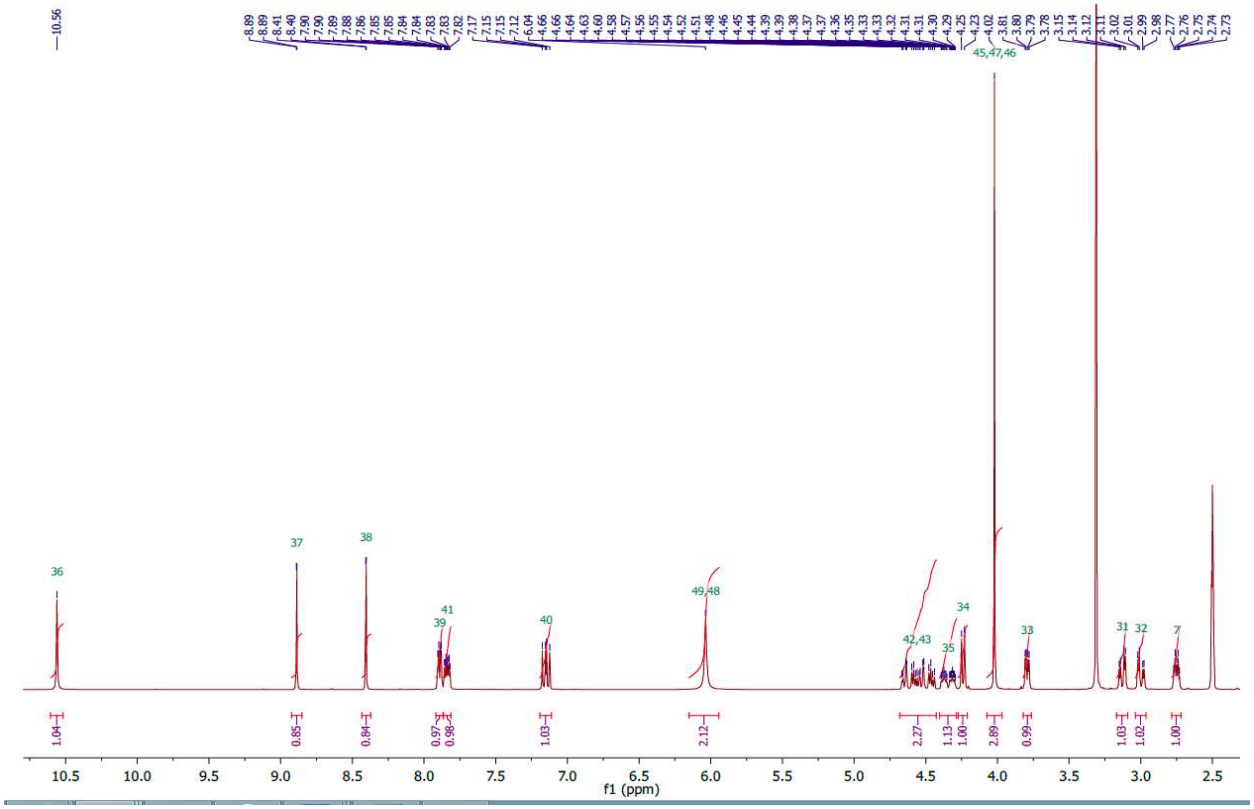


Figure 1-1. ^{1}H NMR spectrum of compound **3**, DMSO-d^{6}

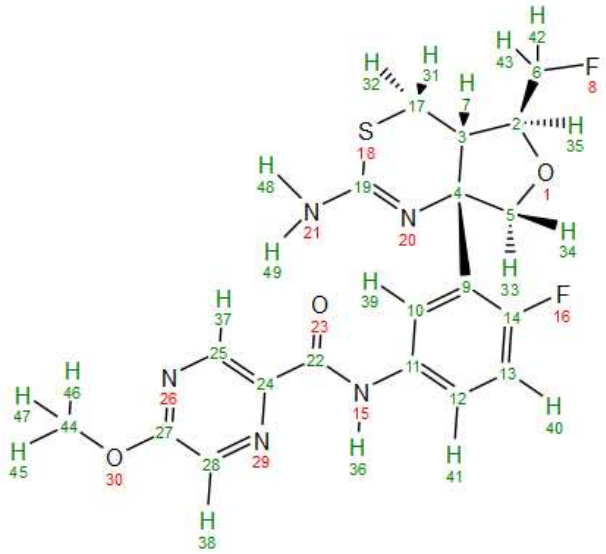


Table 1-1. ^{1}H assignments for compound **3**

Atom Number(s)	^{1}H chemical shift (ppm)	^{1}H multiplicity	^{1}H Coupling Constants (Hz)	Number of Protons
7	2.75	dt	3.7, 3.7, 7.8	1
32	3.00	dd	3.8, 13.5	1
31	3.13	dd	3.6, 13.5	1
33	3.79	dd	3.2, 8.1	1
45,46,47	4.02	s		3
34	4.24	d	8.2	1
35	4.34	dddd	2.4, 5.1, 7.7, 23.5	1
42,43	4.56 (4.43-4.68)	m		2
48,49	6.04	s		2
40	7.15	dd	8.8, 11.9	1
41	7.84	ddd	2.8, 4.0, 8.6	1
39	7.89	dd	2.6, 7.3	1
38	8.40	d	1.3	1
37	8.89	d	1.3	1
36	10.56	s		1

The ^{13}C NMR spectrum of **3** is shown in Figure 1-2 and the peak assignments are shown in Table 1-2.

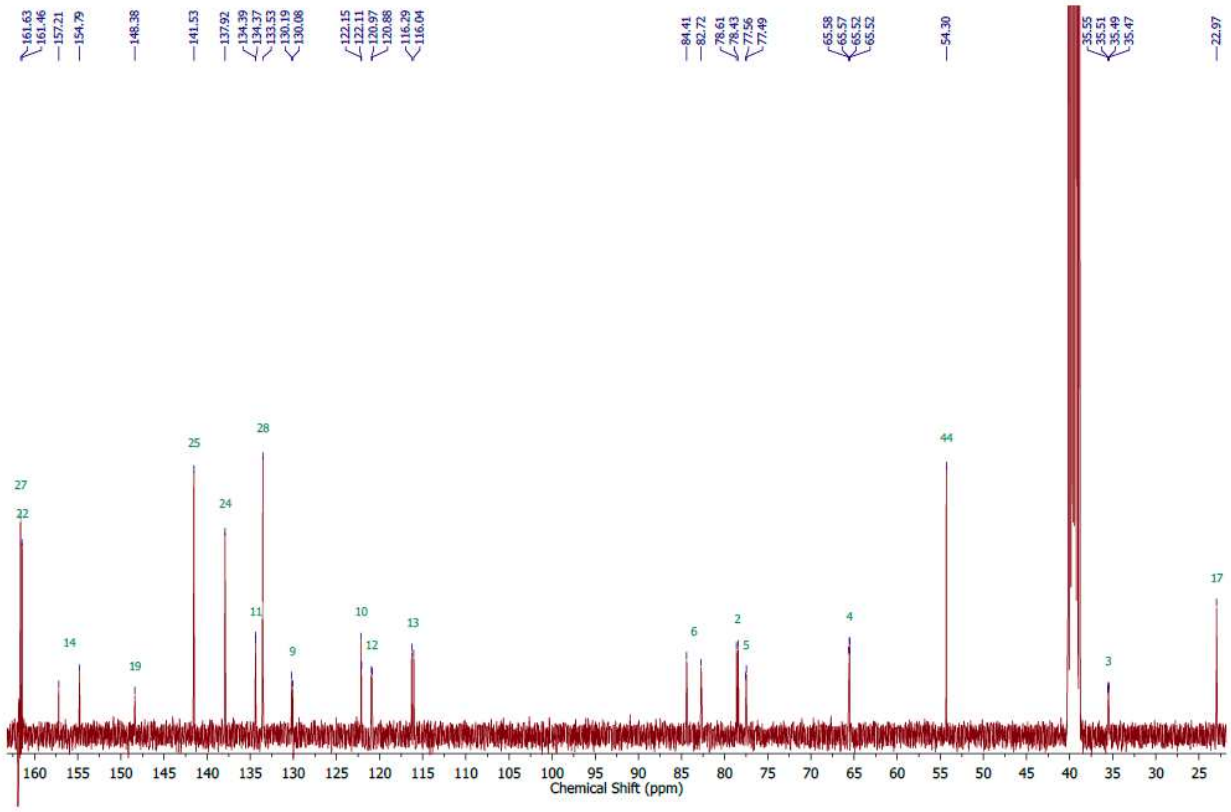


Figure 1-2. ^{13}C NMR spectrum of compound **3**, DMSO-d^6

Table 1-2. ^{13}C assignments for compound **3**

Atom Number	^{13}C chemical shift (ppm)
17	22.97
3*	35.51, *dd*, *J* = 5.1 Hz, 2.4 Hz
44	54.29
4*	65.80-65.36, m
5*	77.53, *d*, *J* = 6.1 Hz
2*	78.52, *d*, *J* = 18.2 Hz
6*	83.56, *d*, *J* = 169.7 Hz
13*	116.16, *d*, *J* = 25.0 Hz
12*	120.91, *d*, *J* = 8.4 Hz
10*	122.12, *d*, *J* = 4.1 Hz
9*	130.14, *d*, *J* = 12.4 Hz
28	133.53
11*	134.37, *d*, *J* = 2.3 Hz

24	137.92
25	141.53
19	148.36
14*	155.99, *d*, *J*= 243.6 Hz
22	161.45
27	161.63

**NMR signals are split due to coupling with ^{19}F (either atom 11 or 19 or both).*

The ^{1}H NMR spectrum of the tosylate precursor 1**8** is shown in Figure 1-3 and the peak assignments are shown in Table 1-3.

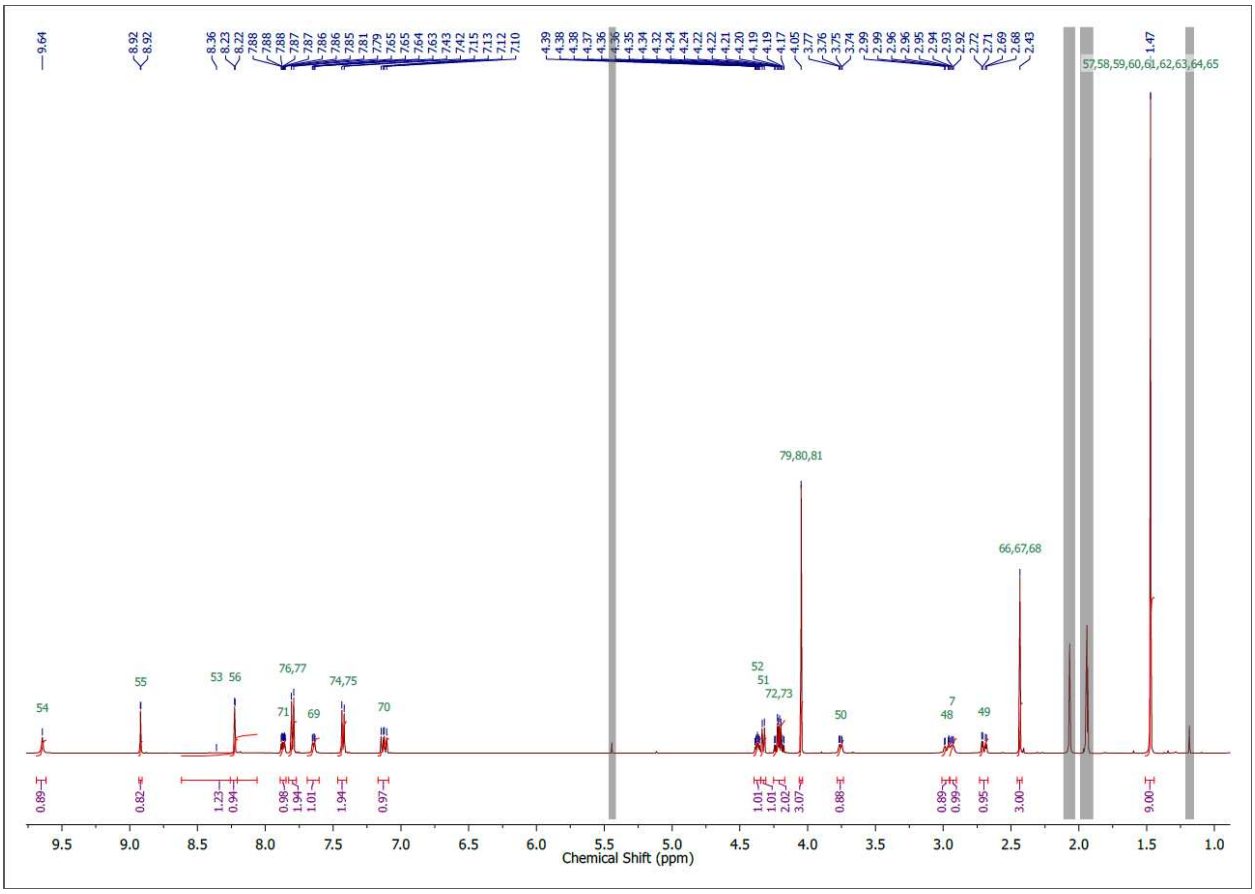


Figure 1-3. ^{1}H NMR Spectrum of Compound **18**, CD_3CN

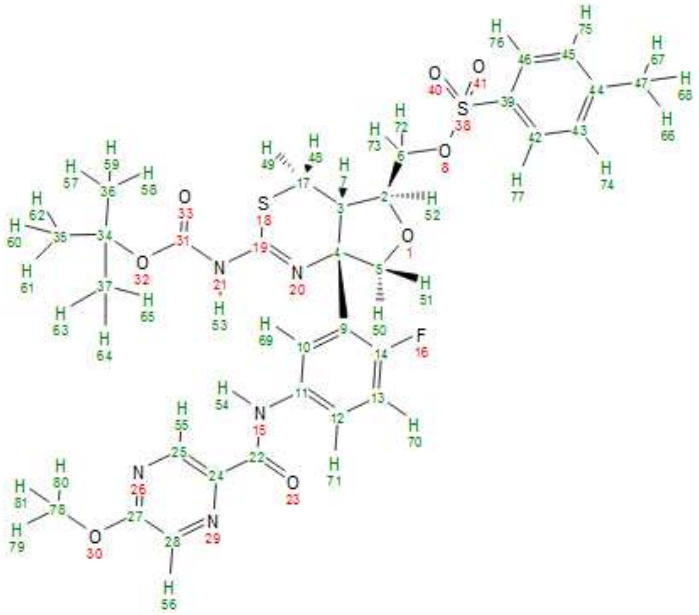


Table 1-3. ^{1}H assignments for compound **18**

Atom Number(s)	**^{1}H chemical shift (ppm)**	^{1}H multiplicity	^{1}H Coupling Constants (Hz)	Number of Protons
57,58,59,60,61,62,63,64,65	1.46	s		9
66,67,68	2.42	s		3
49	2.72-2.62	m		1
7	2.84-2.91	m		1
48	2.91-3.00	m		1
50	3.74	dd	2.5, 8.7	1
79,80,81	4.03	s		3
72,73	4.12-4.25	m		2
51	4.31	d	8.9	1
52	4.37	ddd	3.6, 5.2, 8.2	1
70	7.12	dd	8.9, 12.0	1
74,75	7.42	d	8.0	2
69	7.62	dd	2.6, 7.1	1
76,77	7.79	d	8.3	2
71	7.88	ddd	2.8, 4.1, 8.8	1
56	8.22	d	1.3	1
53	8.35	s (br)		1
55	8.91	s		1
54	9.67	s		1

The ^{13}C NMR spectrum of 1**8** is shown in Figure 1-4 and the peak assignments are shown in Table 1-4.

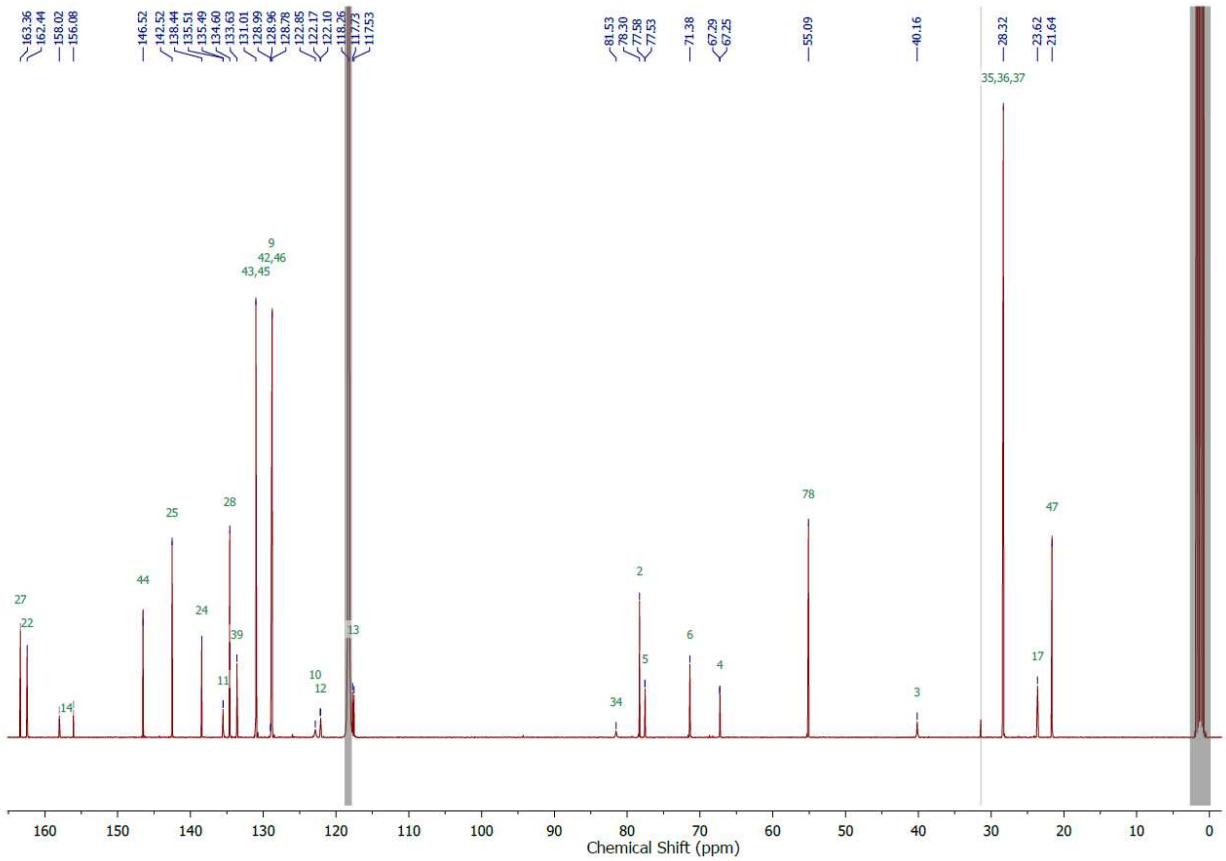


Figure 1-4. ^{13}C NMR Spectrum of Compound **18**, CD_3CN

Table 1-4. ^{13}C assignments for compound **18**

Atom Number(s)	^{13}C chemical shift (ppm)
47	21.67
17	23.64
35,36,37	28.35
3*	40.18, d, J = 3.1 Hz
78	55.12
4*	67.29, d, J = 5.1 Hz
6	71.40
5*	77.55, d, J = 6.5 Hz
2	78.31
34	81.55
13*	117.65, d, J = 25.0 Hz
12*	122.15, d, J = 8.7 Hz
10†	122.85, m (br)
42,46	128.81

9*	128.92, *d*, *J* = 11.5 Hz
43,45	131.03
39	133.64
28	134.63
11*	135.52, *d*, *J* = 2.5 Hz
24*	138.46
25	142.54
44	146.54
14*	157.06, *d*, *J* = 243.5 Hz
22	162.46
27	163.38
31	‡
19	‡

**NMR signals are split due to coupling with ^{19}F*

†Doublet expected due to coupling with ^{19}F but atom 10 defined as multiplet due to peak broadening and low intensity above baseline. Splitting into doublet can be seen upon zoom and location of chemical shift is as expected.

‡^{13}C signals for Atoms 31 and 19 not observed above the ^{13}C NMR baseline even with 12288 scans at a sample concentration of 15 mg/mL, presumably due to tautomerization and/or rotameric activity between the t-butyl carbamate and adjacent amidine functionality

2. Selectivity profile of compound 3 in CEREP® selectivity panel

The broad spectrum selectivity profile of **3** in CEREP® is shown in Table 2-1 and PDE selectivity profile is shown in Table 2-2.

Table 2-1. Selectivity profile of **3** in the CEREP® panel.

Functional Agonism	**% response at 10 µM**
Adenosine A1	10
Adenosine 2A	-1
Adrenergic Alpha1a	-4
Adrenergic Alpha2a	6
Adrenergic Alpha2b	0
Beta 1 adrenoceptor	-1
Beta 2 adrenoceptor	5
Angiotensin 1	-3
CB1	9
Cholecystokinin 2	0
D1	4
D2 short	6

Endothelin A	-3
H1	-2
H2	0
H3	-24
M1	-1
M2	7
M3	-1
Neurokinin NK1	-2
Delta opioid receptor	15
Kappa opioid receptor	18
Mu opioid receptor	17
Serotonin 1a	-19
Serotonin 1b	18
Serotonin 2a	0
Serotonin 2b	5
Serotonin 4e	5
Vasopressin 1a	-1
CRF1	0.4
MC2R	2.1
TRH1	-1.4

Functional Antagonism	**% inhibition at 10 µM**
Adrenergic Alpha1a	17
Adrenergic Alpha2b	-18
Beta 1 adrenoceptor	3
Beta 2 adrenoceptor	-8
Angiotensin 1	-4
CB1	2
D1	-31
D2 short	2
H1	-27
M1	22
M2	8
M3	17

CRF1	3.7
MC2R	-5.2
TRH1	-6.4

Ion Channel Binding	**% inhibition at 10 µM**
L-type Calcium (Verapmil)	24
L-type Calcium (Nifedipine)	14
L-type Calcium (Dittiazem)	14
GABAa (Cl channel)	46
GABAa1	-45
GABAa (benzodiazepine site)	-28
AMPA	5
NMDA	3
NMDA (PCP site)	3
nAChR Muscle	21
nAChR Neuronal	21
Serotonin 3	12
Sodium (Site 2)	24

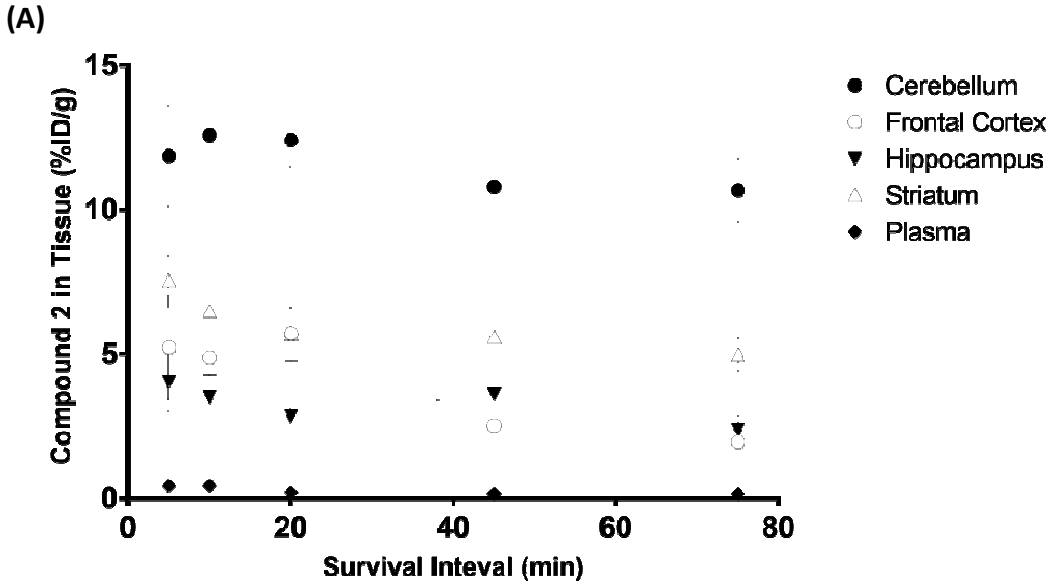
Transporter Binding	**% inhibition at 10 µM**
NET	3
DAT	-3
Serotonin transporter	40
Choline transporter	-14
GABA transporter	-6

Enzymes and Kinases	**% inhibition at 10 µM**
ACE	-3
AChE	19
Cyclooxygenase 2	20
HIV-1 protease	3
MAO	20

PDE3B	-1
PDE4D2	-1
AbI kinase	-4
Aurora A	2.1
EGFR	-1
LCK	5
P38 MAP	-3
SRC	8
VEGFR2	-17

NHR Binding	**% inhibition at 10 µM**
Androgen receptor	12
Glucocorticoid receptor	-1.8
PPAR gamma	-13.1

3. Uptake and tissue distribution of compounds 2 and 3 (5-75 minute survival interval) in male wild-type mice



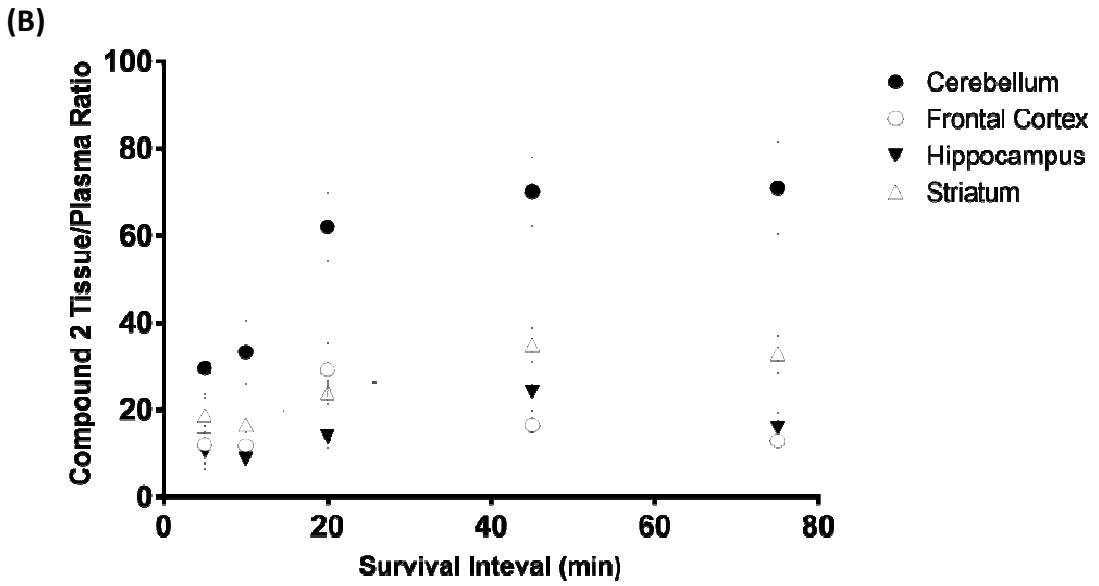
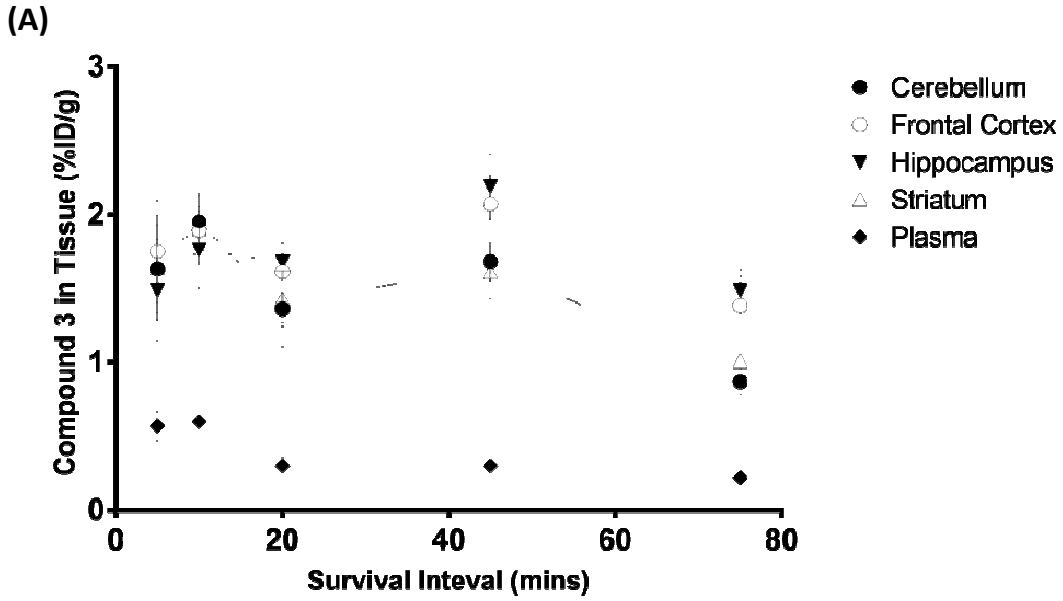


Figure 3-1. The uptake and distribution (A) in brain (ng/g) and plasma (ng/mL) and tissue/plasma ratio (B) of compound **2** (10 µg/kg, IV, survival interval 5-75 min) in male wild-type mice. Maximum tissue/plasma ratio was achieved at 45 min.



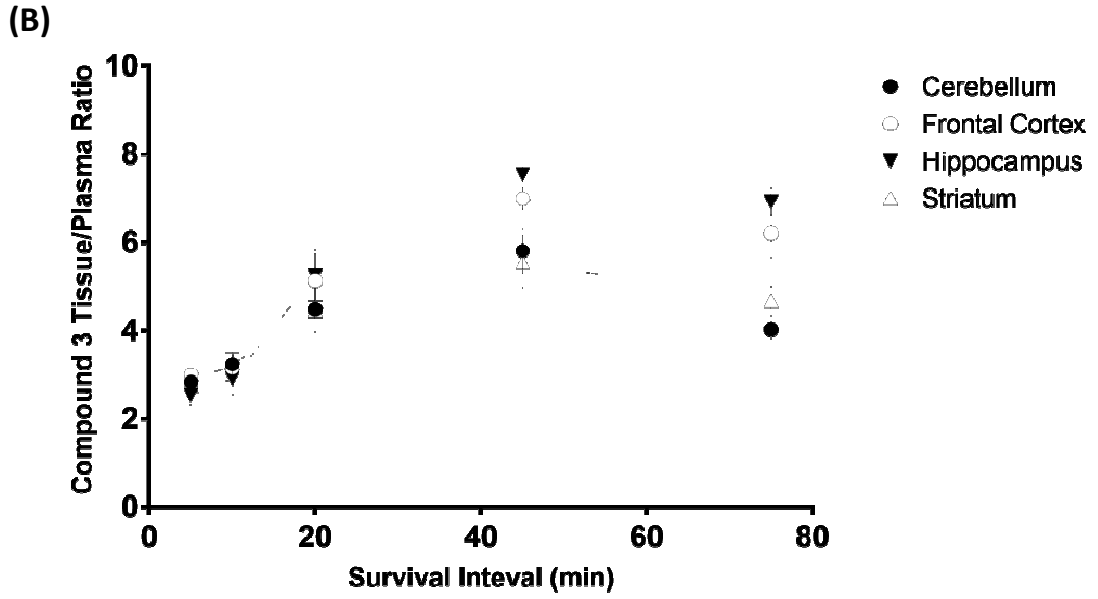


Figure 3-2. The uptake and distribution (A) in brain (ng/g) and plasma (ng/mL) and tissue/plasma ratio (B) of compound **3** (10 µg/kg, IV, survival interval 5-75 min) in male wild-type mice. Maximum tissue/plasma ratio was achieved at 45 min.