

Supporting Information

Selective Gelatinase Inhibitor Neuroprotective Agents Cross the Blood–Brain Barrier

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Table S1. Inter-day accuracy, precision, and relative error for SB-3CT and compounds 2-4 in spiked mouse brain samples

Day of Analysis	Low QC ^a				High QC (20 pmole/mg tissue)			
	SB-3CT	2	3	4	SB-3CT	2	3	4
1	0.0728	0.0541	0.0709	0.0605	21.0	21.4	18.1	20.5
	0.0875	0.0452	0.0862	0.0575	20.3	21.1	20.3	20.6
	0.0784	0.0481	0.0775	0.0513	20.9	21.4	18.7	20.9
2	0.0834	0.0594	0.0851	0.0595	17.2	18.1	19.3	18.5
	0.0758	0.0641	0.0759	0.0424	21.5	18.9	19.0	17.9
	0.0903	0.0493	0.0931	0.0379	19.2	19.4	18.1	18.5
3	0.0851	0.0422	0.0875	0.0552	18.2	19.6	18.9	20.2
	0.0763	0.0482	0.0766	0.0625	20.1	18.8	21.4	21.6
	0.0918	0.0546	0.0739	0.0555	20.9	18.2	19.5	20.2
mean	0.0824	0.0517	0.0807	0.0536	19.9	19.6	19.3	19.9
s.d.	0.0068	0.0070	0.0074	0.0084	1.4	1.3	1.1	1.4
Precision, CV%	8.30	13.5	9.21	15.6	7.17	6.72	5.53	6.80
Accuracy%	82.4	103	80.7	107	99.7	98.2	96.3	99.3
Relative error%	17.6	3.38	19.3	7.18	0.333	1.77	3.70	0.731

^a 0.10 pmole/mg tissue (for SB-3CT and compound **3**); 0.050 pmole/mg tissue (for compounds **2** and **4**)

Table S2. Inter-day accuracy, precision, and relative error for SB-3CT and compounds 2-4 in spiked mouse plasma samples

Day of Analysis	Low QC ^a				Medium QC (2 µM)				High QC (20 µM)			
	SB-3CT	2	3	4	SB-3CT	2	3	4	SB-3CT	2	3	4
1	0.0895	0.0436	0.102	0.0424	1.90	1.88	1.90	2.10	20.4	20.7	21.1	20.2
	0.0875	0.0452	0.113	0.0381	1.70	2.06	1.83	2.02	21.5	20.9	17.7	21.1
	0.0999	0.0444	0.121	0.0454	1.86	1.95	2.10	2.10	20.3	19.8	19.2	21.7
	0.0835	0.0458	0.114	0.0437	1.95	2.02	1.96	2.04	20.9	20.9	18.3	21.7
	0.0752	0.0439	0.0983	0.0384	1.89	2.01	1.93	1.94	18.7	19.5	18.3	21.6
2	0.114	0.0466	0.0912	0.0403	1.81	2.03	1.91	2.01	20.6	22.1	18.0	21.3
	0.0863	0.0439	0.0940	0.0460	1.90	2.17	1.93	1.97	19.1	21.4	19.4	21.4
	0.106	0.0409	0.0854	0.0557	1.96	2.07	1.88	2.07	19.2	19.4	20.8	21.2
	0.0793	0.0518	0.102	0.0324	1.97	1.98	2.04	2.05	21.4	21.8	20.0	19.5
	0.0952	0.0472	0.0794	0.0393	1.79	2.03	1.84	1.92	20.8	19.3	19.5	19.0
3	0.0894	0.0443	0.0897	0.0561	1.98	1.99	2.20	1.93	21.5	20.0	22.3	21.4
	0.0980	0.0430	0.105	0.0596	2.12	1.76	2.26	2.08	22.4	21.0	24.7	22.0
	0.0982	0.0447	0.0852	0.0550	2.18	1.76	1.97	1.61	19.5	20.0	22.9	21.8
	0.102	0.0438	0.125	0.0532	2.16	1.72	2.19	1.92	20.4	19.4	23.9	21.3
	0.0985	0.0444	0.121	0.0501	1.83	1.88	2.04	1.91	22.7	21.5	25.5	21.6
mean	0.0935	0.0449	0.102	0.0464	1.93	1.95	2.00	1.98	20.6	20.5	20.8	21.1
s.d.	0.0104	0.0024	0.015	0.0081	0.14	0.13	0.14	0.12	1.2	0.9	2.5	0.9
Precision, CV%	11.1	5.39	14.2	17.6	7.07	6.61	6.77	6.21	5.70	4.59	12.2	4.07
Accuracy%	93.5	89.8	102	92.8	96.7	97.7	99.9	98.9	103	103	104	106
Relative error%	6.50	10.2	1.75	7.24	3.33	2.30	0.0667	1.10	3.14	2.53	3.88	5.54

^a 0.10 µM (for SB-3CT and compound 3); 0.050 µM (for compounds 2 and 4)**Table S3. Extraction efficiency of SB-3CT and compounds 2-4 from mouse brain and plasma**

Compound	Brain Recovery % (n = 5)				Plasma Recovery % (n = 5)					
	Low QC ^a		High QC (20 pmole/mg tissue)		Low QC ^b		Medium QC (2 µM)		High QC (20 µM)	
	mean ± s.d.	CV%	mean ± s.d.	CV%	mean ± s.d.	CV%	mean ± s.d.	CV%	mean ± s.d.	CV%
SB-3CT	89.1 ± 15.1	16.9	98.6 ± 4.3	4.37	94.4 ± 6.6	6.95	105 ± 6	5.60	111 ± 5	4.88
2	91.4 ± 9.5	10.4	99.3 ± 2.2	2.17	109 ± 7	6.64	115 ± 5	4.11	116 ± 4	3.39
3	107 ± 18	16.8	94.7 ± 1.7	1.79	103 ± 9	9.11	110 ± 11	9.62	102 ± 7	7.14
4	91.8 ± 7.1	7.69	99.7 ± 4.0	3.98	104 ± 10	9.71	102 ± 4	4.08	111 ± 4	4.06

^a 0.10 pmole/mg tissue (for SB-3CT and compound 3); 0.050 pmole/mg tissue (for compounds 2 and 4)

^b 0.10 μ M (for SB-3CT and compound 3); 0.050 μ M (for compounds 2 and 4)

Table S4. Concentrations of SB-3CT and compound 2 after a single i.p. dose to female mice.

Time (min)	SB-3CT after Dose of SB-3CT		Compound 2 after Dose of SB-3CT		Compound 2 after Dose of Compound 2	
	Brain ^a	Plasma ^b	Brain ^a	Plasma ^b	Brain ^a	Plasma ^b
10	5.0 \pm 0.8	6.4 \pm 0.3	0.054 \pm 0.009	0.056 \pm 0.010	6.1 \pm 0.9	9.2 \pm 1.3
20	1.2 \pm 0.1	2.3 \pm 0.3	0.025 \pm 0.006	0.015 \pm 0.007	1.5 \pm 0.6	1.8 \pm 0.8
30	0.92 \pm 0.15	1.9 \pm 0.2	0.022 \pm 0.003	0.015 \pm 0.003	0.22 \pm 0.01	0.32 \pm 0.01
60	0.68 \pm 0.15	1.1 \pm 0.1	0.015 \pm 0.006	0.0048 \pm 0.0022	0.032 \pm 0.023	0.071 \pm 0.021
90	0.26 \pm 0.05	0.45 \pm 0.12	0.015 \pm 0.004	0.0036 \pm 0.0019	0.016 \pm 0.011	0.037 \pm 0.064
120	0.17 \pm 0.02	0.17 \pm 0.04	0.0081 \pm 0.0020	NQ ^c	0.0076 \pm 0.0004	0.019 \pm 0.010
180	0.067 \pm 0.043	NQ ^c	0.0052 \pm 0.0003	NQ ^c	0.0018 \pm 0.0032	NQ ^c

^a Concentrations in pmole/mg tissue

^b Concentrations in μ M

^c NQ = not quantifiable

Table S5. Concentrations and pharmacokinetic parameters of compounds 3 and 4 after a single i.p. dose to female and male mice.

Time (min)	Female Mice				Male Mice			
	Compound 3 after Dose of 3		Compound 4 after Dose of 3		Compound 3 after Dose of 3		Compound 4 after Dose of 3	
	Brain ^a	Plasma ^b	Brain ^a	Plasma ^b	Brain ^a	Plasma ^b	Brain ^a	Plasma ^b
10	4.2 \pm 0.2	0.81 \pm 0.16	0.85 \pm 0.10	0.33 \pm 0.13	3.9 \pm 0.4	0.50 \pm 0.15	0.50 \pm 0.02	0.085 \pm 0.010
30	2.7 \pm 0.7	0.40 \pm 0.09	0.22 \pm 0.18	0.078 \pm 0.024	1.3 \pm 0.2	0.31 \pm 0.03	0.10 \pm 0.03	0.027 \pm 0.015
60	0.61 \pm 0.36	0.14 \pm 0.12	0.036 \pm 0.015	0.015 \pm 0.001	0.58 \pm 0.05	0.16 \pm 0.02	0.073 \pm 0.037	0.010 \pm 0.006
120	0.33 \pm 0.06	0.071 \pm 0.026	0.016 \pm 0.003	0.0017 \pm 0.0030	0.35 \pm 0.06	0.14 \pm 0.03	0.016 \pm 0.003	0.0052 \pm 0.0032
180	0.19 \pm 0.03	0.045 \pm 0.009	0.014 \pm 0.001	0.0014 \pm 0.0024	0.23 \pm 0.05	0.080 \pm 0.039	0.011 \pm 0.004	0.0042 \pm 0.0013
300	0.079 \pm 0.088	0.023 \pm 0.027	0.012 \pm 0.001	NQ ^c	0.084 \pm 0.049	0.033 \pm 0.057	0.0051 \pm 0.0026	0.0031 \pm 0.0025
AUC_{0-last}^d	200	38.0	22.7	7.75	164	38.4	15.5	3.28
$AUC_{0-\infty}^d$	211	41.8	32.2	8.13	174	42.9	16.3	4.52
$t_{1/2\alpha}$ (min)	32	20	10	9.6	13	29	8.6	12
$t_{1/2\beta}$ (min)	96	114	559	187	87	94	110	277
Brain _{AUC} /Plasma _{AUC}	5.0		4.0		4.1		3.6	

^a Concentrations in pmole/mg tissue

^b Concentrations in μ M

^c NQ = not quantifiable

^d *AUC* in pmole·min/mg for brain and in $\mu\text{M}\cdot\text{min}$ for plasma