

Supporting Information

Water-Soluble MMP-9 Inhibitor Prodrug Generates Active Metabolites that Cross the Blood-Brain Barrier

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Table S1. Concentrations of ND-478, ND-322 and ND-364 after a 30-min iv infusion of ND-478 to mice

Time (min) after end of infusion	ND-478 after dose of ND-478 Brain ^a	ND-478 after dose of ND-478 Plasma ^b	ND-322 after dose of ND-478 Brain ^a	ND-322 after dose of ND-478 Plasma ^b	ND-364 after dose of ND-478 Brain ^a	ND-364 after dose of ND-478 Plasma ^b
-15	NQ ^c	4.18 ± 0.840	0.0585 ± 0.0154	10.7 ± 0.860	2.26 ± 1.56	0.320 ± 0.0599
0	NQ ^c	6.81 ± 3.95	0.301	14.5 ± 2.81	2.17	0.365 ± 0.0688
10	NQ ^c	0.508 ± 0.0870	0.0275 ± 0.0198	4.36 ± 0.613	0.972 ± 0.703	0.229 ± 0.110
20	NQ ^c	0.274 ± 0.116	NQ ^c	2.15 ± 0.602	0.480 ± 0.367	0.142 ± 0.0617
30	NQ ^c	0.230 ± 0.167	NQ ^c	0.650 ± 0.432	0.178 ± 0.00937	0.0599 ± 0.0280
45	NQ ^c	0.0878 ± 0.0480	NQ ^c	0.120 ± 0.0236	0.0368 ± 0.00601	0.0167 ± 0.00410
60	NQ ^c	0.0573 ± 0.0263	NQ ^c	0.0729 ± 0.0104	NQ ^c	0.0111 ± 0.00325
120	NQ ^c	0.0309 ± 0.0140	NQ ^c	0.0502 ± 0.00471	NQ ^c	0.00827 ± 0.000808

^a Concentration in pmol/mg tissue

^b Concentration in μ M

^c NQ = not quantifiable

Table S2. Concentrations of ND-322 and ND-364 after a single sc dose to mice

Time (min)	ND-322				ND-364			
	ND-322 after dose of ND-322 Brain ^a	ND-322 after dose of ND-322 Plasma ^b	ND-364 after dose of ND-322 Brain ^a	ND-364 after dose of ND-322 Plasma ^b	ND-364 after dose of ND-364 Brain ^a	ND-364 after dose of ND-364 Plasma ^b	ND-322 after dose of ND-364 Brain ^a	ND-322 after dose of ND-364 Plasma ^b
10	0.685 ± 0.0363	7.29 ± 0.665	0.669 ± 0.0719	0.430 ± 0.0472	1.58 ± 0.406	6.60 ± 2.15	NQ ^c	0.215 ± 0.188
20	0.337 ± 0.0995	5.31 ± 1.51	0.468 ± 0.0550	0.322 ± 0.0920	1.36 ± 0.435	5.79 ± 1.93	NQ ^c	0.189 ± 0.0644
30	0.533 ± 0.0809	5.27 ± 1.20	0.553 ± 0.0235	0.319 ± 0.0377	1.39 ± 0.496	4.09 ± 1.44	NQ ^c	0.182 ± 0.0696
45	0.227 ± 0.0812	3.03 ± 0.0257	0.352 ± 0.0207	0.212 ± 0.0254	0.703 ± 0.357	3.46 ± 1.80	NQ ^c	0.156 ± 0.0774
60	0.254 ± 0.0298	1.95 ± 0.183	0.380 ± 0.0268	0.150 ± 0.0310	0.848 ± 0.542	1.61 ± 0.224	NQ ^c	0.133 ± 0.0328
90	0.163 ± 0.0222	1.34 ± 0.443	0.251 ± 0.0429	0.0952 ± 0.0433	0.437 ± 0.101	0.824 ± 0.267	NQ ^c	0.0667 ± 0.0327
120	0.156 ± 0.0295	0.812 ± 0.822	0.234 ± 0.100	0.0732 ± 0.0523	0.306 ± 0.180	0.343 ± 0.312	NQ ^c	0.0401 ± 0.0249
180	0.130 ± 0.0130	0.291 ± 0.211	0.169 ± 0.0365	0.0226 ± 0.00351	0.263 ± 0.0802	0.186 ± 0.120	NQ ^c	0.0197 ± 0.00668
240	0.117 ± 0.00244	0.0530 ± 0.0362	0.129 ± 0.00881	0.0146 ± 0.00283	0.135 ± 0.0228	0.0699 ± 0.0638	NQ ^c	NQ ^c

^a Concentration in pmol/mg tissue

^b Concentration in μM

^c NQ = not quantifiable

Table S3. Concentrations of ND-322 and ND-364 after a single ip dose of ND-364 to mice

Time (min)	ND-364 in Brain ^a	ND-364 in Plasma ^b	ND-322 in Brain ^a	ND-322 in Plasma ^b
5	6.01 ± 6.71	10.6 ± 10.9	0.0246 ± 0.0288	0.579 ± 0.560
10	15.5 ± 4.31	17.4 ± 6.89	0.578 ± 0.125	1.74 ± 0.530
20	2.16 ± 0.303	2.63 ± 0.497	0.0272 ± 0.00333	0.497 ± 0.0522
30	1.30 ± 0.958	1.82 ± 1.07	NQ ^c	0.465 ± 0.435
45	0.214 ± 0.0667	0.478 ± 0.0862	NQ ^c	0.137 ± 0.0458
60	0.0766 ± 0.0503	0.340 ± 0.170	NQ ^c	0.0338 ± 0.0269
90	0.0509 ± 0.0374	0.393 ± 0.238	NQ ^c	0.0159 ± 0.00829
120	0.0879 ± 0.0196	0.296 ± 0.0505	NQ ^c	0.0262 ± 0.0130
180	0.0430 ± 0.00187	0.210 ± 0.0165	NQ ^c	0.0128 ± 0.00543
240	0.0205 ± 0.00550	0.141 ± 0.00611	NQ ^c	0.0305 ± 0.0183

^a Concentration in pmol/mg tissue

^b Concentration in μM

^c NQ = not quantifiable

Table S4. Inter-day accuracy, precision, and relative error for ND-322 and ND-364 in spiked mouse plasma samples

Day of Analysis	Low QC (0.1 μ M)		Medium QC (8 μ M)		High QC (50 μ M)	
	ND-322	ND-364	ND-322	ND-364	ND-322	ND-364
1	0.0992	0.140	6.72	7.21	48.3	58.9
	0.129	0.0948	6.77	7.84	52.4	39.6
	0.120	0.0920	7.75	7.55	46.7	59.4
2	0.108	0.129	7.84	9.89	50.8	52.7
	0.104	0.120	9.63	7.82	46.5	55.8
	0.136	0.111	7.91	8.78	51.2	54.0
3	0.126	0.0992	6.75	7.23	50.5	45.6
	0.107	0.108	7.92	5.92	53.0	51.3
	0.0727	0.0784	6.62	7.74	55.7	45.3
Mean	0.111	0.108	7.55	7.78	50.6	51.4
s.d.	0.0191	0.0193	0.971	1.09	3.00	6.67
Precision, CV%	17.2	17.8	12.9	14.1	5.93	13.0
Accuracy%	111	108	94.3	97.2	101	103
Relative error%	11.2	8.03	5.67	2.80	1.14	2.82

Table S5. Inter-day accuracy, precision, and relative error for ND-322 and ND-364 in spiked mouse brain samples

Day of Analysis	Low QC (0.1 pmol/mg tissue)		Medium QC (8 pmol/mg tissue)		High QC (50 pmol/mg tissue)	
	ND-322	ND-364	ND-322	ND-364	ND-322	ND-364
1	0.135	0.112	7.26	7.99	50.5	50.7
	0.143	0.122	7.55	8.54	50.6	50.0
	0.104	0.118	7.87	8.41	50.4	50.6
2	0.136	0.125	7.50	7.56	50.0	50.0
	0.118	0.112	7.16	7.35	49.4	50.6
	0.119	0.116	7.69	8.29	51.0	49.5
3	0.133	0.0853	7.39	6.92	50.2	54.8
	0.109	0.130	8.34	7.48	48.1	48.7
	0.0899	0.136	7.35	8.52	49.1	46.2
Mean	0.121	0.117	7.57	7.89	50.0	50.1
s.d.	0.0174	0.0144	0.362	0.588	0.899	2.25
Precision, CV%	14.4	12.3	4.78	7.45	1.80	4.49
Accuracy%	87.9	88.3	94.6	98.7	99.8	100
Relative error%	12.1	11.7	5.42	1.33	0.194	0.262

Table S6. Intra-day accuracy, precision, and relative error for ND-322 and ND-364 in spiked mouse brain and plasma samples

Compound	Brain (n = 5)						Plasma (n = 5)					
	Low QC		Medium QC		High QC		Low QC		Medium QC		High QC	
	(0.1 pmol/mg tissue)		(8 pmol/mg tissue)		(50 pmol/mg tissue)		(0.1 μ M)		(8 μ M)		(50 μ M)	
	mean \pm s.d.	CV%	mean \pm s.d.	CV%	mean \pm s.d.	CV%	mean \pm s.d.	CV%	mean \pm s.d.	CV%	mean \pm s.d.	CV%
ND-322	0.111 \pm 0.0215	19.4	7.87 \pm 1.43	18.2	50.1 \pm 0.546	1.09	0.123 \pm 0.00347	2.83	7.78 \pm 1.09	13.9	51.3 \pm 3.03	5.91
ND-364	0.117 \pm 0.00502	4.29	8.55 \pm 0.389	4.55	50.0 \pm 0.710	1.42	0.119 \pm 0.0222	18.8	7.79 \pm 0.533	6.85	51.2 \pm 3.68	7.19

Table S7. Extraction efficiency of ND-322 and ND-364 from mouse brain and plasma

Compound	Brain Recovery% (n = 3)						Plasma Recovery% (n = 3)					
	Low QC		Medium QC		High QC		Low QC		Medium QC		High QC	
	(0.1 pmol/mg tissue)		(8 pmol/mg tissue)		(50 pmol/mg tissue)		(0.1 μ M)		(8 μ M)		(50 μ M)	
	mean \pm s.d.	CV%	mean \pm s.d.	CV%	mean \pm s.d.	CV%	mean \pm s.d.	CV%	mean \pm s.d.	CV%	mean \pm s.d.	CV%
ND-322	88.5 \pm 1.92	2.18	103 \pm 8.24	7.99	99.3 \pm 1.13	1.13	97.6 \pm 3.31	3.39	99.4 \pm 4.22	4.25	98.4 \pm 1.63	1.65
ND-364	95.5 \pm 3.69	3.87	89.0 \pm 2.97	3.34	99.3 \pm 0.974	0.980	95.1 \pm 18.7	19.6	102 \pm 10.4	10.2	100 \pm 20.5	20.4