Supporting Information

Optimization of 8-Hydroxyquinolines as Inhibitors of Catechol *O*-Methyltransferase

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Table S1. Mouse Exposure time course of 5,7-substituted 8-hydroxyquinolines.[a]

	time 5 minutes		time 15 minutes		time 60 minutes	
Example	mean Plasma (ng/ml)	mean Brain (ng/g)	mean Plasma (ng/ml)	mean Brain (ng/g)	mean Plasma (ng/ml)	mean Brain (ng/g)
11	7929 ± 2226	715 ± 109	2776 ± 984	144 ± 43	173 ± 60	BLQ*
12	5458 ± 53	1090 ± 203	689 ± 87	167 ± 3	68 ± 69	10 ± 15
15	5457 ± 1562	311 ± 115	935 ± 69	60 ± 6	250 ± 296	11 ± 16
16	706 ± 155	184 ± 55	402 ± 379	162 ± 134	235 ± 51	109 ± 16
17	3894 ± 2208	BLQ**	2904 ± 1690	510 ± 36	1329 ± 1363	256 ± 23
18	3052 ± 4211	531 ± 698	5035 ± 6046	1568 ± 1957	2542 ± 2486	1094 ± 901
19	16725 ± 11844	3336 ± 2918	25700 ± 1131	6930 ± 509	16650 ± 5020	5655 ± 784
20	17200 ± 1697	1584 ± 184	3575 ± 304	319 ± 81	5720 ± 1428	527 ± 44
21	3638 ± 1500	525 ± 198	4929 ± 1229	992 ± 328	1744 ± 120	518 ± 77
22	8570 ± 1307	901 ± 366	13449 ± 404	2736 ± 156	8612 ± 1304	2661 ± 213
25	14060 ± 8400	3915 ± 1209	17000 ± 1838	3810 ± 721	6160 ± 1343	1914 ± 1497
26	15756 ± 331	2348 ± 667	19282 ± 3442	3912 ± 956	14669 ± 607	2792 ± 439
28	32066 ± 4847	2981 ± 580	10286 ± 5853	948 ± 598	1819 ± 307	110 ± 38
29	32850 ± 2050	1321 ± 788	24650 ± 1202	506 ± 71	16250 ± 494	1154 ± 189
30	3186 ± 116	509 ± 0	2316 ± 350	289 ± 16	576 ± 406	55 ±38

[a] n=2 mice per timepoint, data is average ±SEM.
* LOQ = 10 ng/ml
** LOQ = 50 ng/ml

Figure S1. Mouse brain concentrations

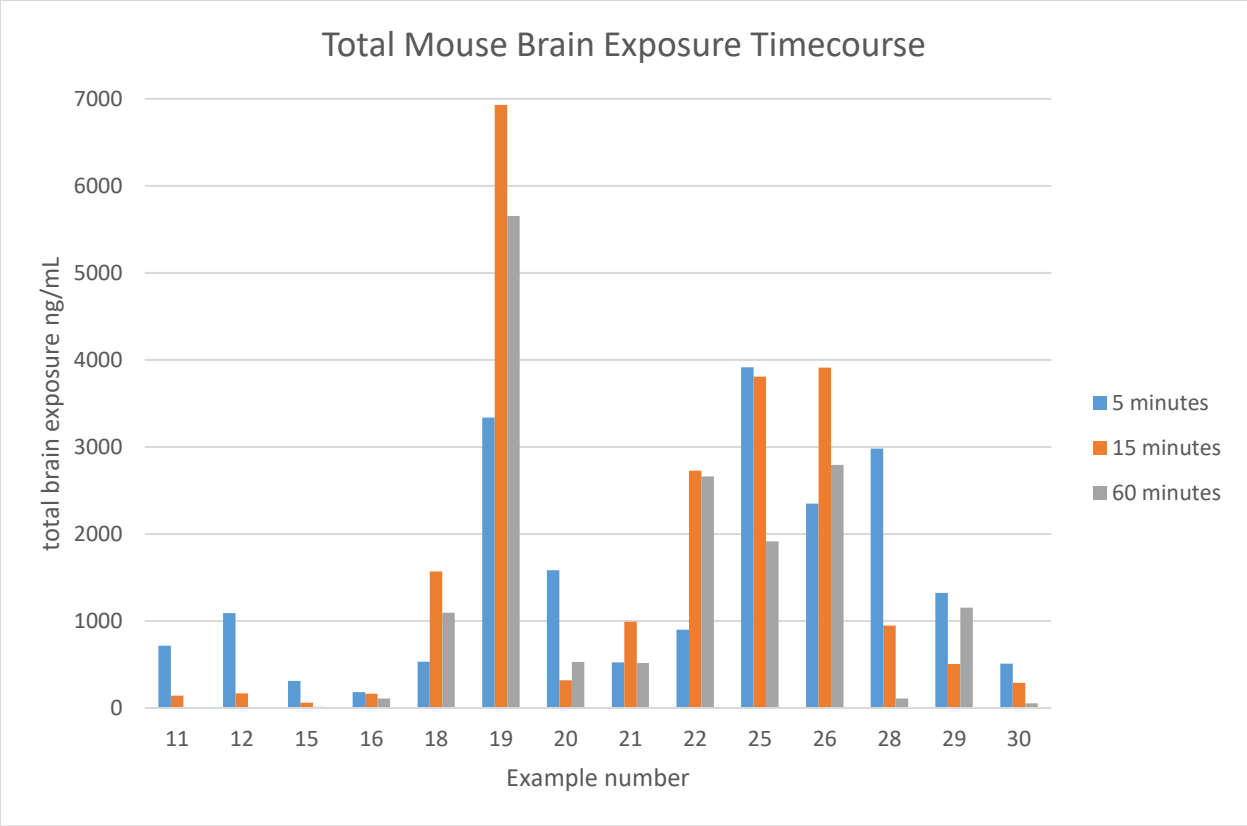

Table S2. Rat Biomarker Details[a]

Example	Number of rats (vehicle)	Number of rats (drug)	total plasma (nM)	total brain (nmol/g)	total CSF (nM)
1	8	8	3805 ± 1424	BLQ*	BLQ**
20	10	10	4005 ± 1010	321.0 ± 79.4	127.3 ± 33.9
25	10	10	9719 ± 1368	305 ± 111	321 ± 58
26	9	7	6471 ± 1211	353 ± 103	358 ± 64
28	5	5	3888 ± 563	437.8 ± 128.4	157.3 ± 26.0

[a] Rats dosed orally at 10mg/kg, samples collected 4 hours after dosing
* LOQ= 200 ng/mL
** LOQ= 50ng/mL

Co-Crystal structure of S-COMT with compound 21

The soluble part of rat COMT from M44 to S264 with N-terminal His-tag was recombinantly produced in *E. coli*. Cells were lysed (lysis buffer: 100 mM Tris/HCl pH 8.0, 300 mM NaCl, 10% glycerol, 5 mM 2-mercaptoethanol, 5 mM $MgCl_2$, protease inhibitor cocktail (Sigma-Aldrich)) and after centrifugation (15 min, 30'000 x g, 4 °C) Ni-NTA resin (ThermoFisher Scientific) added to supernatant and incubated for 2 hours at 4 °C. After washing with 100 mM Tris pH 7.5, 200 mM NaCl, 10% glycerol, 5 mM 2-mercaptoethanol, 7 mM imidazole protein was eluted with 100 mM Tris pH 7.5, 200 mM NaCl, 10% glycerol, 5 mM 2-mercaptoethanol, 100 mM imidazole. N-terminal His-tag was removed by TEV protease cleavage using His-tagged TEV (Sigma-Aldrich) which was subsequently separated from cleaved protein using Ni-NTA resin (ThermoFisher Scientific). Protein was further purified by size exclusion chromatography (HiLoad Superdex 75 prep grade (GE Healthcare Life Sciences), 50 mM MES pH 6.5, 10 mM DTT, 1 mM EDTA, 10% glycerol) and concentrated to around 5 mg mL^{-1}. Following the protocol from M. Ellermann et al.[1] 1 µM rat S-COMT was incubated overnight at 4 °C with 10 µM compound **21** and 1 mM SAM (Sigma-Aldrich) in 50 mM Tris-HCl pH 7.6, 50 mM NaCl, 10 mM DTT, 2 mM $MgCl_2$, concentrated to 13 mg mL^{-1} and cleared by centrifugation. Crystallization was set up at 18 °C with hanging drop vapour diffusion and crystals grew in 100 mM Bis-Tris propane pH 5.5-7.0, 100 mM NaCl, 1.2-1.8 M $(NH_4)_2SO_4$. The crystal was cryo-protected in 25 mM Bis-Tris propane pH 7.3, 40% (w/v) PEG3350, 150 mM sodium formate and flash-cooled in liquid nitrogen for data collection. Diffraction data were collected at 100 K using a MicroMax-007 HF rotating anode X-ray generator (Rigaku) and mar345 image plate detector (marXperts). Data were integrated and scaled using MOSFLM[2] and SCALA[3]. The structure was determined by molecular replacement using PHASER[4] (CCP4 program suite[5]) and the published S-COMT structure (PDB accession

code: 3S68)[1], without ligand, as search model. Structural refinement was performed with PHENIX[6] with altering cycles of manual model building with COOT[7]. The crystallographic statistics are summarized in Table 1. The protein structure was validated using MolProbity[8]. The coordinates and structure factors have been deposited in the Protein Data Bank (entry code 6GY1). Molecular pictures are prepared using program PyMol[9].

Table S3. Data collection and refinement statistics.

	S-COMT/compound **21** (PDB ID 6GY1)
Data collection	
Space group	$P3_221$
Resolution range (Å)	56.11-2.10 (2.21-2.10)[a]
Unit cell dimensions	
a,b,c (Å)	50.4, 50.4, 168.3
α,β,γ (°)	90, 90, 120
Wavelength (Å)	1.54179
No. molecules in asymmetric unit	1
Completeness (%)	99.3 (99.5)
Multiplicity	3.4 (3.5)
R_{merge}	0.073 (0.303)
Mean I/σI	8.0 (2.5)
Refinement	
Resolution range (Å)	42.26-2.10 (2.26-2.10)
No. reflections	14913
R_{work}/R_{free}	0.2123/0.2489
No. non-H atoms overall	1767
protein	1698
ligands	27
water	42
Average B-factor (Å^2) overall	30.30
protein	30.32
ligands	32.70
water	27.77
Geometry	
R.m.s. deviation bond lengths (Å)	0.008
R.m.s. deviation bond angles (°)	0.987
Ramachandran favored (%)	96
Ramachandran allowed (%)	4
Ramachandran outliers (%)	0

[a]Statistics in the highest resolution bins are shown in parenthesis.

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