

Study Title	<u>Example Final Report: Radioligand Binding Assay</u>
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Study Number	GBL_0000
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Report Reviewed by	Internal reviewer
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Signatures

The following were responsible for the overall conduct of this nonclinical laboratory study and for the data reported herein

Project Manager:

Name: Name of Principal Investigator, Ph.D.

Title: Principal Investigator / Chief Scientific Officer

Date:

Materials and Methods

Test Compound(s)	Compounds A – C; reference compound
Radiotracer	[³ H]Pentazocine (PerkinElmer)
Cells / Tissue	Guinea pig whole brain
Assay Buffer	50 mM Tris, 5 mM MgCl ₂ , 0.1 % EDTA, pH 7.4
Membrane Preparation	Frozen tissue maintained at -80 °C was partially thawed and homogenized in 10 volumes of cold lysis buffer (50 mM Tris, protease inhibitor cocktail) using a IKA homogenizer. The homogenate was centrifuged at 1,000 x g for 10 minutes at 4 °C to obtain supernatant. The supernatant was then centrifuged at 40,000 x g for 20 minutes at 4 °C to pellet the membranes, the supernatant replaced with fresh buffer and the pellet resuspended. Two additional rounds of centrifugation and resuspension were performed to ensure wash out of interfering endogenous ligands. The final pellet was resuspended in buffer containing 10% sucrose and stored at -80 °C. A sample of the homogenate was analyzed for protein content using the Pierce® BCA assay.
Compound Preparation	Compounds were dissolved in DMSO at 2 mg/ml and an aliquot of this solution further diluted with assay buffer to 0.1 mg/ml. A dilution series was prepared from the latter at 5x the final assay concentration. Remaining unused stock DMSO solutions were stored frozen at -20 °C in glass vials.
Radioligand Binding Assay	Filtration binding assay was carried out in 96-well plates in a final volume of 200 µL per well. To each well was added 120 µL of membranes, 40 µL of the test drug solution in binding buffer and 40 µL of radioligand solution in binding buffer. The plate was incubated at 30 °C for 90 minutes. The incubation was stopped by vacuum filtration onto 0.5% PEI presoaked 96-well GF/C filtermats using a 96-well Filtermate harvester followed by four washes with ice-cold wash buffer. Filters were then dried for 30 minutes at 50 °C. The filter was sealed in a polyethylene bag, scintillation cocktail (Beta Scint) added, and the radioactivity counted in a Wallac® TriLux 1450 MicroBeta counter.
Data Analysis	For each concentration of test compound, non-specific binding was subtracted from total binding to give specific binding. Data was fitted using the non-linear curve fitting routines in Prism® (Graphpad

Software Inc). For competition assays, K_i values were calculated from IC_{50} values using the formula $K_i = IC_{50} / (1 + ([S]/K_d))$ where $[S]$ is the radiotracer concentration used in the assay and K_d is the dissociation constant of the radiotracer. Values of 5×10^{-9} M and 15×10^{-9} M, respectively, were used for these parameters in K_i calculations.

Results

Summary Table

Compound	Log IC ₅₀	IC ₅₀ (nM)	K _i (nM)
Compound A	-7.03	93.3	71.2
Compound B	-7.20	63.1	48.1
Compound C	ND		
Reference	-8.05	8.9	6.8

Plate Counts

Assay date: 7.2.16
 WO: WO#1
 Plate ID: Plate 070217_A

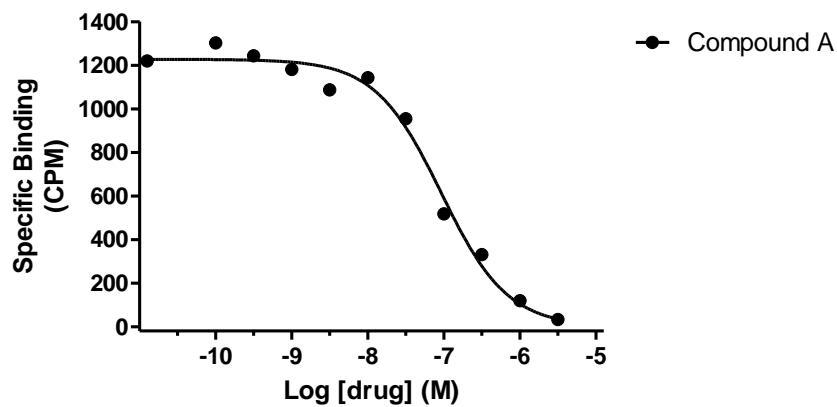
Drug Conc. (nM)	Log conc. (M)	CPM								Average CPM Cmpd A			Average CPM Cmpd B			Average CPM Cmpd C			Average CPM Ref.		
		Compound A		Compound B		Compound C		Reference Compound		Total	Non-specific		Total	Non-specific		Total	Non-specific		Total	Non-specific	
		CPM 1	CPM 2	CPM 1	CPM 2	CPM 1	CPM 2	CPM 1	CPM 2		spec.	Specific		spec.	Specific		spec.	Specific		spec.	Specific
0	-	1197	1341	1148	1053	1211	1158	1162	1147	1269	50	1219	1100	56	1044	1184	56	1128	1154	65	1089
0.1	-10	1394	1310	953	1164	1317	1214	1079	1131	1352	50	1302	1058	56	1002	1265	56	1209	1105	65	1040
0.3	-9.5	1355	1232	1035	1179	1160	1151	1184	1125	1293	50	1243	1107	56	1051	1155	56	1099	1154	65	1089
1	-9.0	1257	1205	1094	1187	1257	1038	1080	1148	1231	50	1181	1140	56	1084	1148	56	1092	1114	65	1049
3	-8.5	1191	1083	879	804	1295	1089	524	969	1137	50	1087	842	56	786	1192	56	1136	747	65	682
10	-8.0	1415	972	969	1082	1331	1077	704	632	1193	50	1143	1025	56	969	1204	56	1148	668	65	603
30	-7.5	1076	935	792	861	1352	1160	293	290	1005	50	955	827	56	771	1256	56	1200	291	65	226
100	-7.0	693	443	473	507	858	1139	137	101	568	50	518	490	56	434	998	56	942	119	65	54
300	-6.5	425	338	222	252	1400	1163	87	76	381	50	331	237	56	181	1281	56	1225	81	65	16
1000	-6.0	186	152	114	111	1181	1106	79	68	169	50	119	113	56	57	1143	56	1087	73	65	8
3000	-5.5	75	92	65	77	1062	855	63	74	83	50	33	71	56	15	959	56	903	69	65	4
Non-specific		50	50	63	48	47	66	61	69	50	50	0	56	56	0	56	56	0	65	65	0

ASSAY CONDITIONS:

Radioligand	[³ H]Pentazocine	Assay format	Filtration (filtermat)
Lot number	1729379 (PerkinElmer)	Incub. Buffer (mM)	50 Tris, 5 MgCl ₂ , 0.1 EDTA, pH 7.4
Spec. Act.	41.9 Ci/mmol	Incub. Vol (ml)	0.2
Cells / Tissue	Guinea pig whole brain	Incub. time / temp	60 min / 30 °C
Non Specific	Haloperidol (10 μM)	Wash buffer	50 Tris, 5 MgCl ₂ , 0.1 EDTA, pH 7.4
CPM/well	39,175	Wash volume	4 x 0.2 ml

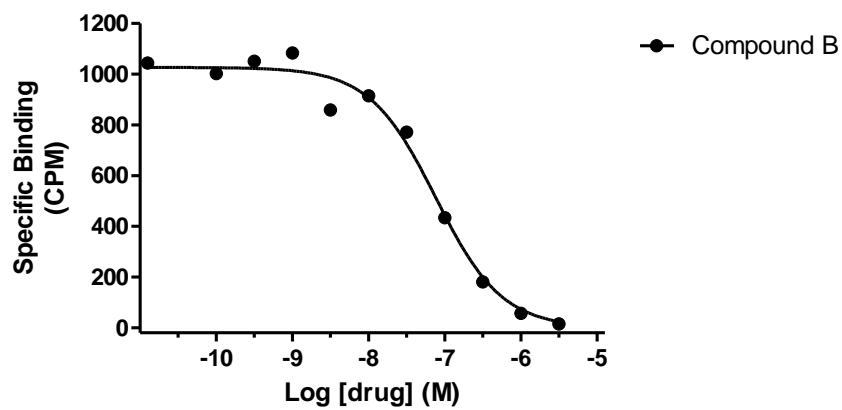
Graphical Plots

Compound A



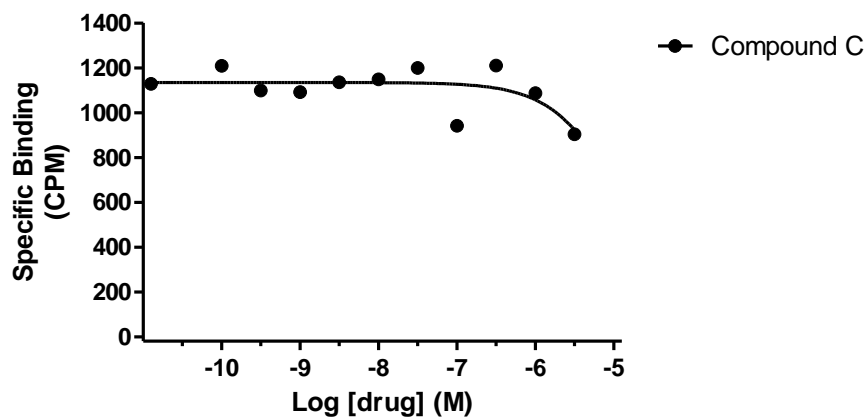
Log IC₅₀ (M): -7.03

Compound B



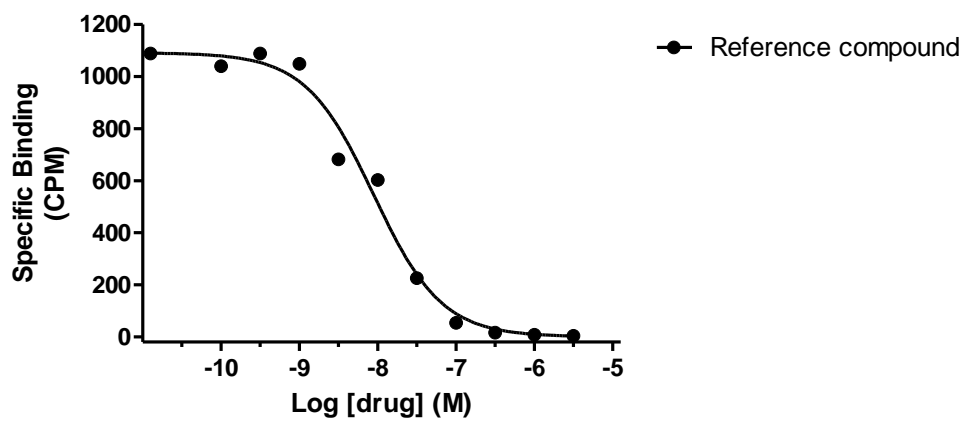
Log IC₅₀ (M): -7.20

Compound C



Log IC₅₀ (M): Not determined

Reference Compound



Log IC₅₀ (M): -8.05

Appendix

Data Files Included with this Report

Excel_spreadsheet.xls