Study Title	Example final report; radioligand binding assay
Sponsor	Sponsor
Study Number	GBL_0000
Project Manager	Project lead scientist
Contributing Scientists	Contributing staff members
Testing Facility	Gifford Bioscience Limited The Biohub Birmingham Birmingham Research Park Vincent Drive Birmingham B15 2SQ United Kingdom
Report Issued	Issue 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^{0}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^{0}C to obtain supernatant. The supernatant was then centrifuged at 40,000 x g for 20 min at 4^{0}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 ^{O}C. A sample of the homogenate is 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 5 x the final assay concentration. Remaining unused stock DMSO solutions were stored frozen at -20 C in glass vials.
Radioligand binding assays	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 ^{O}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 fit 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 5E-9 M and 15E-9 M, respectively, were used for these parameters in K_i calculations.

RESULTS

Table 1. Data summary

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

Figure 1. Plate counts

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

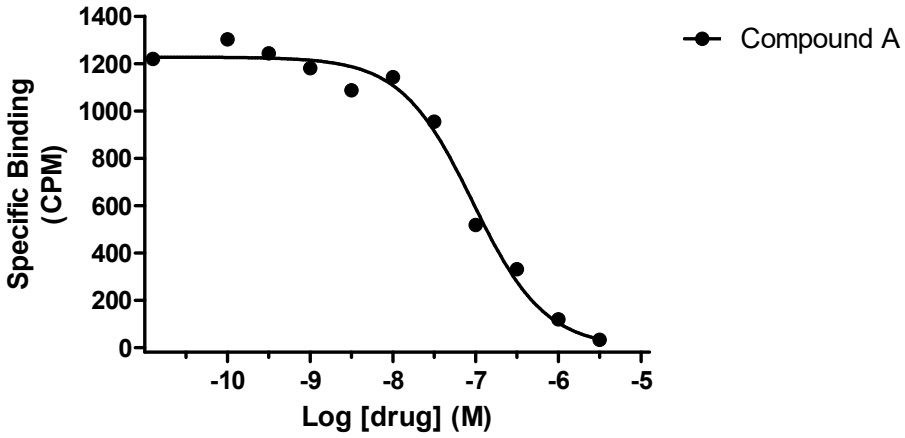
		CPM								Average CPM Cmpd A			Average CPM Cmpd B			Average CPM Cmpd C			Average CPM Ref.		
Drug Conc. (nM)	Log conc. (M)	Compound A		Compound B		Compound C		Reference Compound		Total	Non-spec.	Specific	Total	Non-spec.	Specific	Total	Non-spec.	Specific	Total	Non-spec.	Specific
		CPM 1	CPM 2	CPM 1	CPM 2	CPM 1	CPM 2	CPM 1	CPM 2												
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 MgCl2, 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 ^{0}C
Non Specific	Haloperidol (10 µM)	**Wash buffer**	50 Tris, 5 MgCl2, 0.1 EDTA, pH 7.4
CPM/well	39,175	**Wash volume**	4 x 0.2 ml

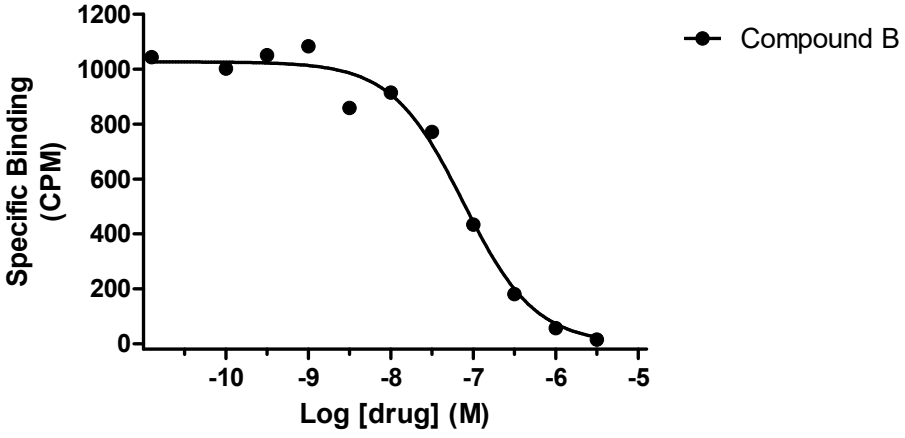
Figure 2. Graphical plots

Compound A



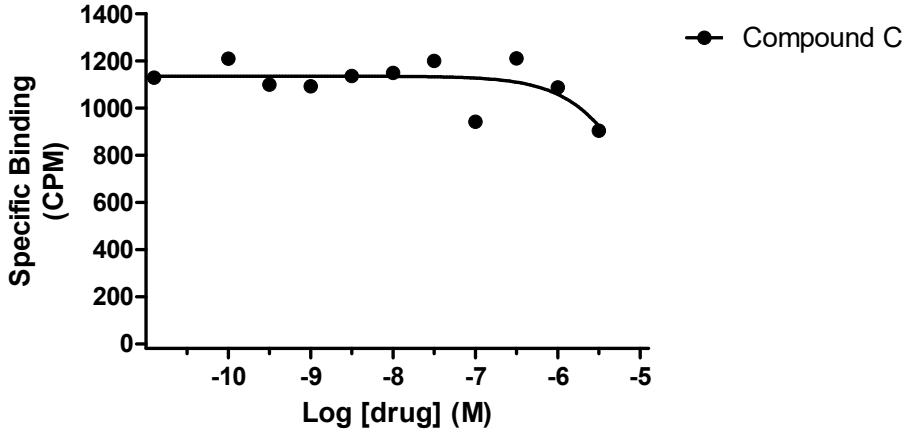
Log IC_{50} (M): -7.03

Compound B



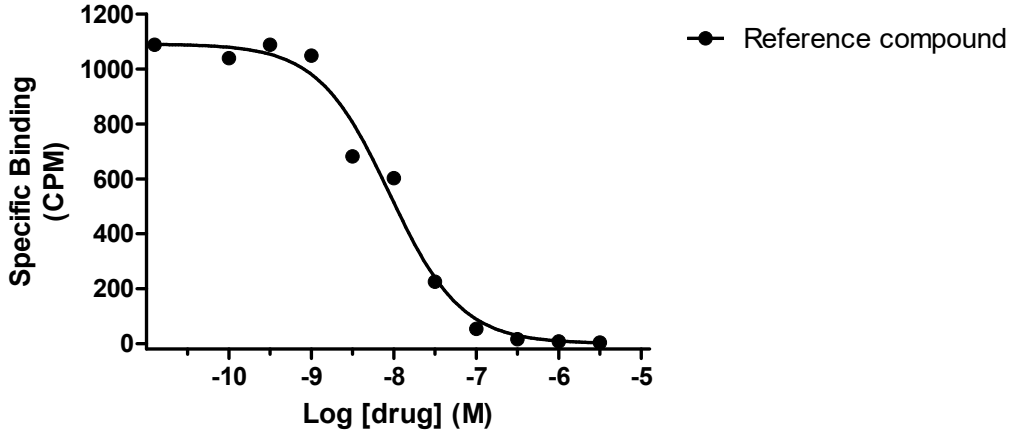
Log IC_{50} (M): -7.20

c) Compound C



Log IC_{50} (M): Not determined

d) Reference compound



Log IC_{50} (M): -8.05

SIGNATURES

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

Lead Scientist(s) _____

Title _____

Date _____

*** END OF REPORT ***