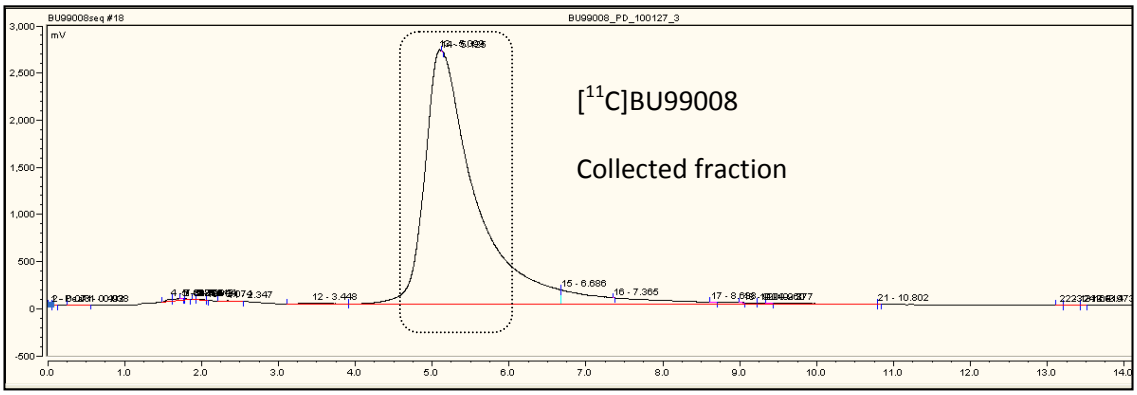
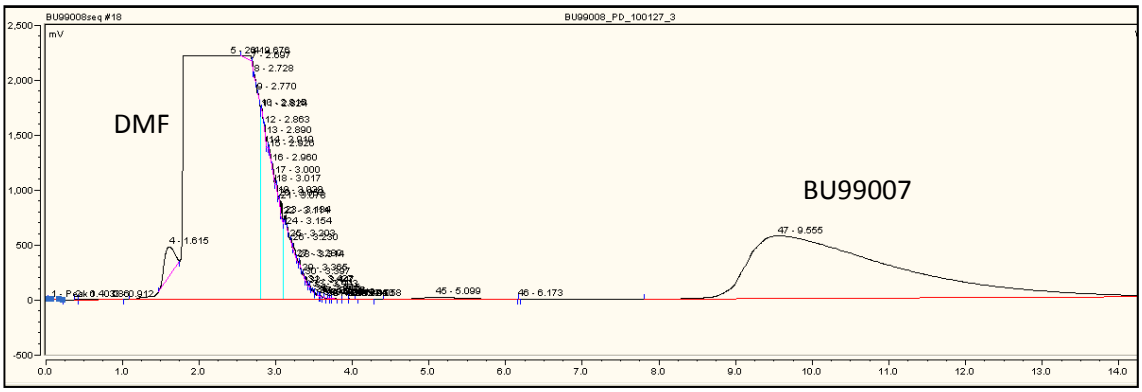
A

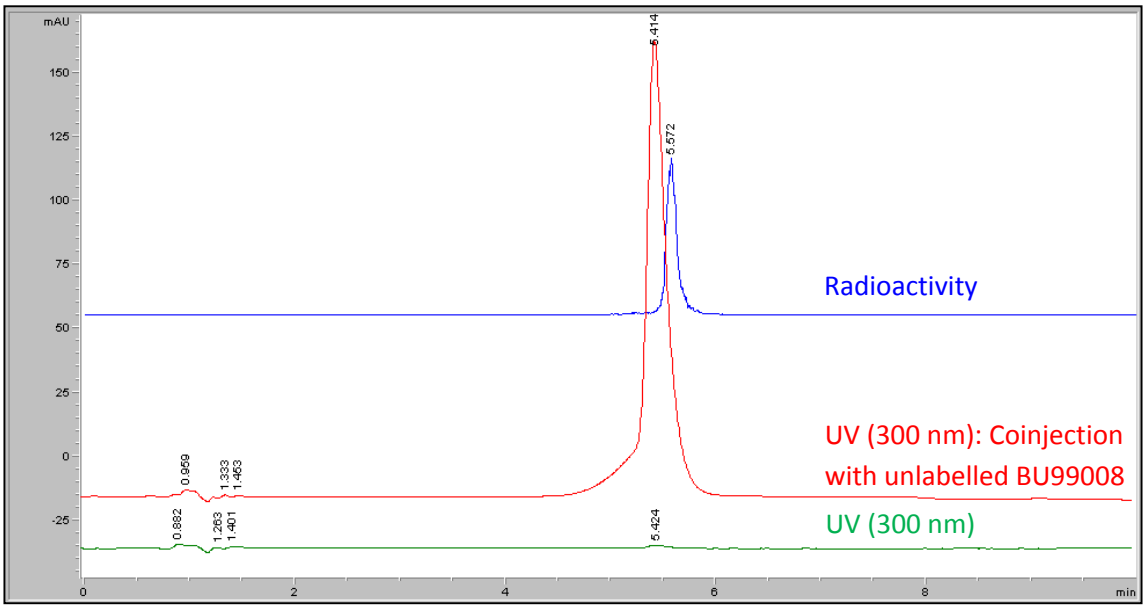


B

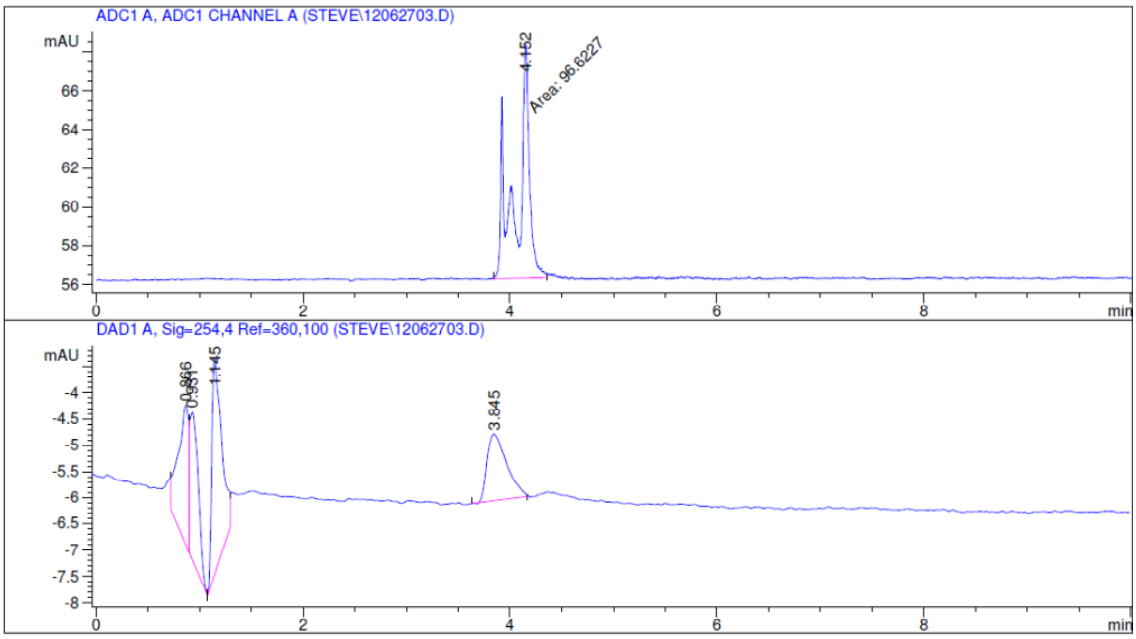


SUPPLEMENTAL FIGURE 1. **(a)** Semi-preparative HPLC chromatogram of crude [^{11}C]BU99008 (radioactivity). Agilent Eclipse SB-Phenyl column (250 x 9.4 mm); 50 % acetonitrile: 50% aqueous ammonium formate (50 mM, pH 9.9); 10 mL.min^{-1}**. (b)** Semi-preparative HPLC chromatogram of crude [^{11}C]BU99008 (UV, 300 nm).

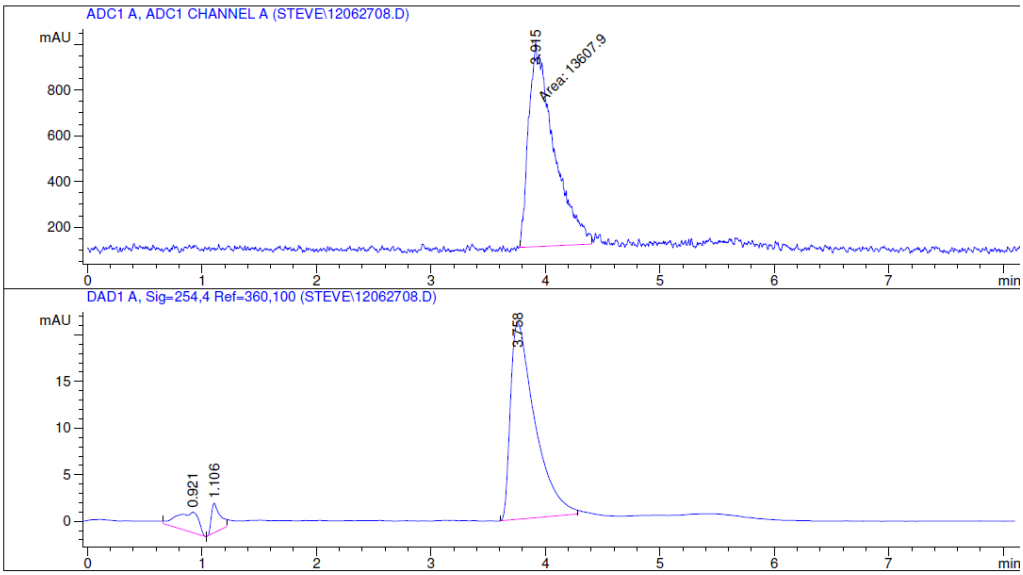
A


mAU
150
125
100
75
50
25
0
-25
5.414
5.572
Radioactivity
UV (300 nm): Coinjection
with unlabelled BU99008
UV (300 nm)
0.959
1.333
1.463
0.882
1.263
1.401
5.424
0
2
4
6
8
min

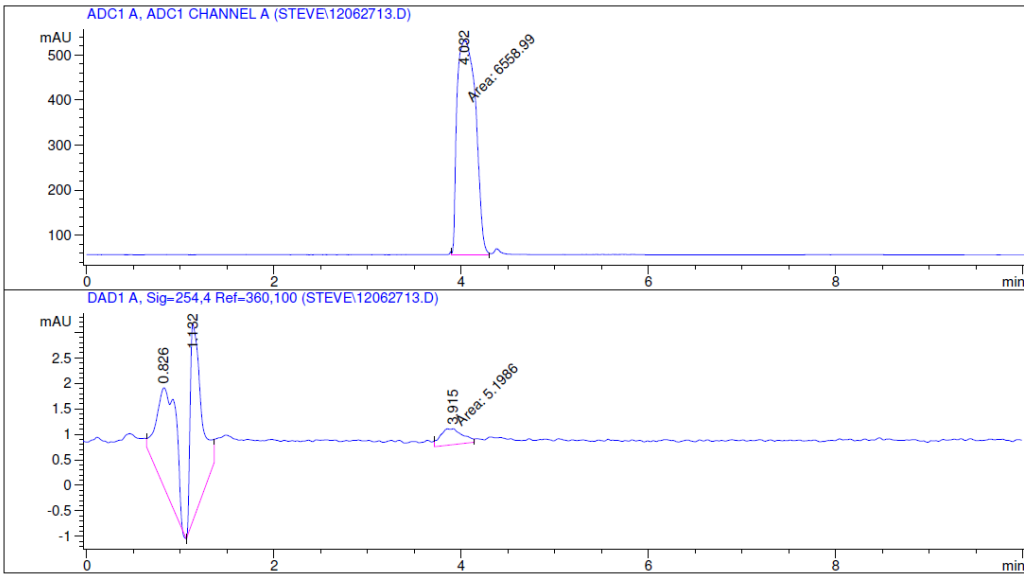
B


ADC1 A, ADC1 CHANNEL A (STEVE\12062703.D)
mAU
66
64
62
60
58
56
4.152
Area: 96.6227
0
2
4
6
8
min
DAD1 A, Sig=254,4 Ref=360,100 (STEVE\12062703.D)
mAU
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
1.145
3.845
0
2
4
6
8
min

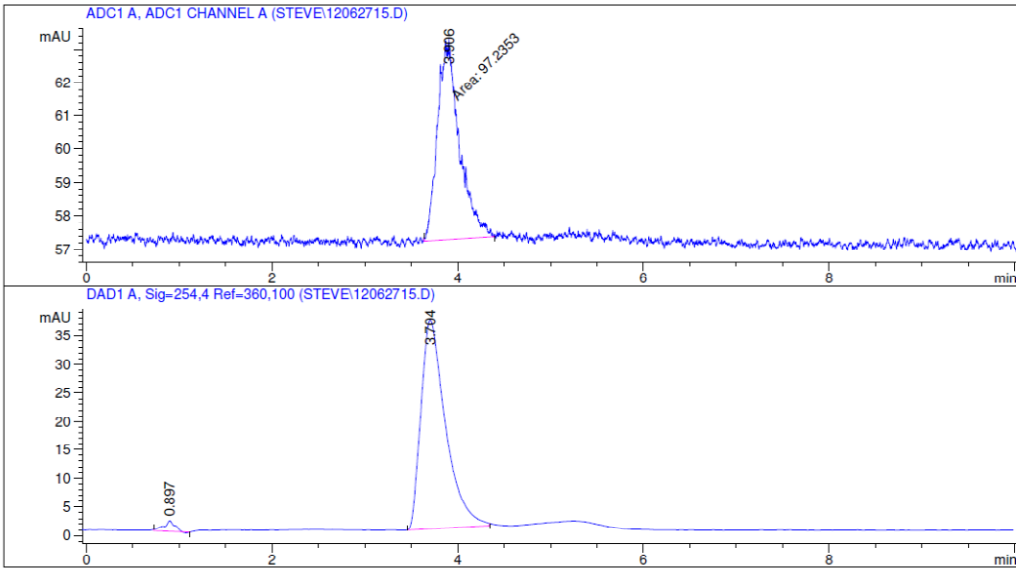
C


ADC1 A, ADC1 CHANNEL A (STEVE\12062708.D)
mAU
3.915
Area: 13607.9
min
DAD1 A, Sig=254,4 Ref=360,100 (STEVE\12062708.D)
mAU
3.758
0.921
1.106
min

D


ADC1 A, ADC1 CHANNEL A (STEVE\12062713.D)
mAU
4.082
Area: 6558.99
min
DAD1 A, Sig=254,4 Ref=360,100 (STEVE\12062713.D)
mAU
0.826
1.182
3.915
Area: 5.1986
min

E



SUPPLEMENTAL FIGURE 2. **(a)** Analytical HPLC chromatograms of purified [^{11}C]BU99008. Agilent Eclipse SB Phenyl column (150 x 4.6 mm); 50% acetonitrile : 50% ammonium formate (50 mM; pH 9.9); 1.5 mL.min^{-1}. **(b)** Analytical HPLC trace of purified [^{11}C]BU99008 (top: Radioactivity, bottom: UV). Agilent Eclipse XDB-C18 column (150 x 4.6 mm); 32% acetonitrile : 68% ammonium formate (50 mM; pH 9); 1.5 mL.min^{-1}. N.B. radioactivity peak appears as multiplet due to saturation of the radioactivity detector. Single peak observed for subsequent coinjection with unlabelled sample of BU99008 (below). **(c)** Analytical HPLC trace of purified [^{11}C]BU99008, coinjected with unlabelled sample of BU99008. Agilent Eclipse XDB-C18 column (150 x 4.6 mm); 32% acetonitrile : 68% ammonium formate (50 mM; pH 9); 1.5 mL.min^{-1}. **(d)** Analytical HPLC trace of purified [^{11}C]BU99008. Agilent Eclipse XDB-C18 column (150 x 4.6 mm); 32% acetonitrile : 68% ammonium formate (50 mM; pH 9); 1.5 mL.min^{-1}. **(e)** Analytical HPLC trace of purified [^{11}C]BU99008, coinjected with unlabelled sample of BU99008. Agilent Eclipse XDB-C18 column (150 x 4.6 mm); 32% acetonitrile : 68% ammonium formate (50 mM; pH 9); 1.5 mL.min^{-1}.

SUPPLEMENTAL TABLE 1. Comparative distribution of radioligand binding in selected ROIs in porcine and rat brain

	[^{11}C]BU99008 mL/cm^{-3}	**[^{3}H]2-BFI (1) fmol/mg tissue**	**[^{3}H]idazoxan (1) fmol/mg tissue**
Cerebellum	26.6	6.8	7.1
Frontal cortex (whole)	29.3	6.8	8.5
Hippocampus	36.2	9.7	11.5
Striatum	43.6	11.0	14.2
Thalamus/Hypothalamus	49.7	25.2	33.7

SUPPLEMENTAL TABLE 2. Correlation analysis statistics

	[^{3}H]2-BFI(28)	**[^{3}H]idazoxan (28)**
Number of XY Pairs	**5**	**5**
Pearson r	**0.8688**	**0.8804**
P value (one-tailed)	**0.0279**	**0.0244**
P value summary	*****	*****
Correlation significant? (alpha=0.05)	**Yes**	**Yes**
R square	**0.7548**	**0.7751**

References

1. Lione LA, Nutt DJ, Hudson AL. Characterization and localization of [3H]2-(2-benzofuranyl)-2-imidazoline binding in rat brain: a selective ligand for imidazoline I2 receptors. *Eur J Pharmacol.* 1998;**353**:123-135.