Electronic Supplementary Material

Imaging Sigma-1 Receptor (S1R) using Iodine-124 Labeled 1-(4-Iodophenyl)-3-(2-adamantyl)guanidine ([^{124}I]IPAG)

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Kishore K. Gangangari[1,2,3] András Váradi[4], Susruta Majumdar[4,7], Steven M. Larson[1,4,5], Gavril W. Pasternak[4], NagaVaraKishore Pillarsetty[1,6]

1. Department of Radiology, Memorial Sloan Kettering Cancer Center (MSKCC), New York, NY

2. Department of Chemistry, Hunter College, City University of New York, New York, NY

3. Ph.D program in Chemistry, The Graduate Center, City University of New York, New York, NY

4. Molecular Pharmacology and Chemistry Program, Department of Neurology, MSKCC, New York, NY

5. Molecular Imaging and Therapy Service, Department of Radiology, MSKCC, New York, NY

6. Department of Radiology, Weill Cornell Medical College, New York, NY

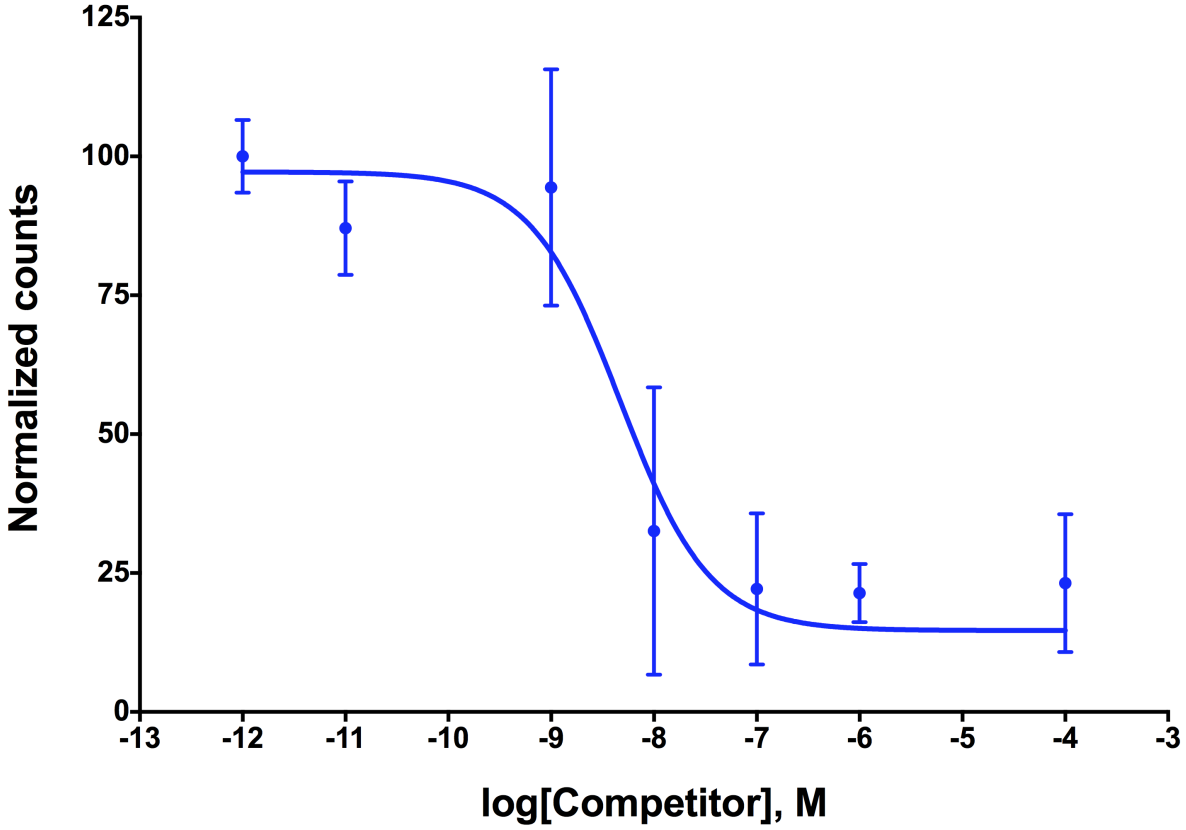
7. Current Affiliation: Center for Clinical Pharmacology, St. Louis College of Pharmacy and Washington University School of Medicine and, St. Louis, MO, USA.

Corresponding author: NagaVaraKishore Pillarsetty, Ph.D email: pillarsn@mskcc.org
telephone: +1 (646) 888-2221 Fax: +1 (646) 888-3059

Short title: Imaging sigma-1 receptors using [^{124}I]IPAG

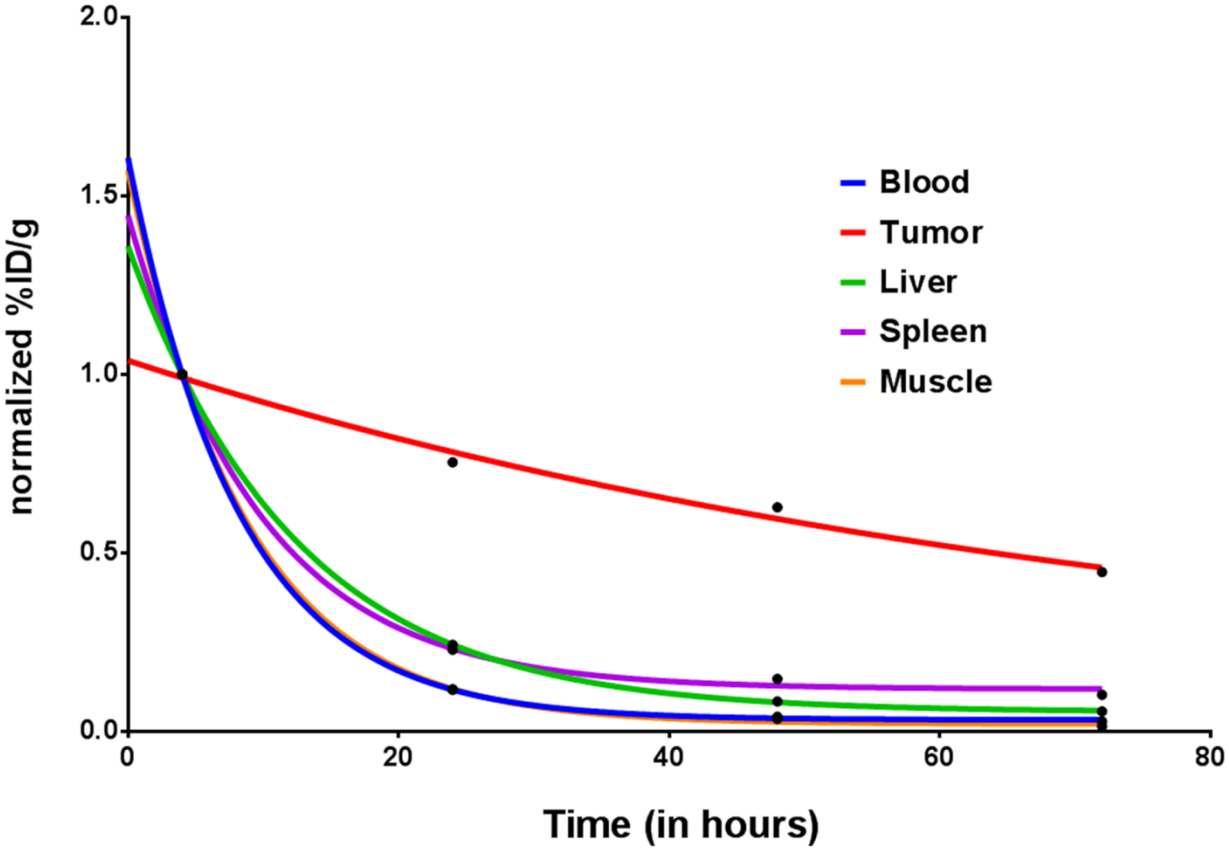
***In vitro* competitive binding assay:**

MCF7 cells were grown in monolayers to 70 % confluency in media suggested by the supplier and collected by trypsinization. Competitive binding studies of [^{131}I]IPAG were performed by co-incubating MCF-7 cells with IPAG or Haloperidol and adding [^{131}I]IPAG. Briefly, triplicate samples of MCF-7 cells (1×10^6) were incubated with [^{131}I]IPAG (1 μCi in phosphate buffered saline) and increasing amounts (10^{-12} - 10^{-3} M) of non-radiolabeled competitor were mixed with cells on an orbital shaker in an incubator (1 hour, 37 °C). Cells were then isolated by rapid filtration, washed with ice-cold TBS using a cell harvester (Brandel, Gaithersburg, MD), and counted, along with suitable blanks, using a γ-counter (Perkin Elmer 1480 Wizard 3, Waltham, MA). Nonspecific and specific uptake of [^{131}I]IPAG was determined. These data were plotted against the concentration of cold competitors, and sigmoidal displacement curves were analyzed by a least-squares fitting model using Graphpad Prism version 6.07 for Windows (Graphpad software, San Diego, CA, USA, www.graphpad.com).



	IPAG
IC_{50}	$4.71*10^{-9}$ M (95% confidence intervals (CI): $9.87*10^{-10}$ to $2.245*10^{-8}$M)

Supplementary Fig. 1: Competitive binding assays assessing the biochemical parameters and binding affinity of [^{124}I]IPAG to MCF-7 cells showing percent inhibition of [^{131}I]IPAG binding in a range of IPAG and haloperidol concentrations and IC_{50} values.



Supplementary Fig. 2: Exponential one phase decay curve of [^{124}I]IPAG showing preferential clearance for organs while being retained in the tumor.

Supplementary Table 1: Biodistribution of [^{124}I]IPAG in mouse bearing MCF-7 tumors at 4, 24, 48 and 72 h post injection.

	4h	24h	48h	72h
	%ID/g ± StDev	%ID/g ± StDev	%ID/g ± StDev	%ID/g ± StDev
Blood	0.5 ± 0.08	0.05 ± 0.01	0.02 ± 0	0.01 ± 0
Tumor	1.53 ± 0.23	1.09 ± 0.21	0.94 ± 0.2	0.68 ± 0.13
Heart	0.72 ± 0.06	0.11 ± 0.02	0.03 ± 0	0.01 ± 0
Lungs	4.19 ± 0.59	0.38 ± 0.06	0.11 ± 0	0.05 ± 0
Liver	7.68 ± 1.58	1.8 ± 0.19	0.62 ± 0.02	0.42 ± 0.05
Spleen	4.83 ± 0.5	1.09 ± 0.1	0.7 ± 0.06	0.48 ± 0.09
Stomach	3 ± 0.21	0.55 ± 0.26	0.24 ± 0.13	0.12 ± 0.05
Sm. Intestine	2.57 ± 0.52	0.81 ± 0.37	0.21 ± 0.02	0.06 ± 0.03
Lg. Intestine	6.28 ± 1.12	1.27 ± 0.32	0.29 ± 0.05	0.13 ± 0.02
Kidneys	2.9 ± 0.25	0.42 ± 0.16	0.15 ± 0.04	0.07 ± 0.03
muscle	0.75 ± 0.09	0.08 ± 0.02	0.02 ± 0	0.01 ± 0
Bone	0.97 ± 0.17	0.25 ± 0.07	0.06 ± 0	0.03 ± 0
Tail	2.51 ± 0.66	1.72 ± 0.35	1.05 ± 0.31	0.42 ± 0.18
Brain	0.28 ± 0.01	0.1 ± 0.01	0.03 ± 0	0.01 ± 0
Salivary Glands		3.34 ± 0.35	1.6 ± 0.37	0.42 ± 0.12

Supplementary Table 2: Tumor to organ ratio of [^{124}I]IPAG uptake in various organs showing clearance from selected organs.

	4h	24h	48h	72h
Blood	3.14 ± 0.71	21.86 ± 6.57	46.48 ± 9.97	49.75 ± 10.02
Tumor	1	1	1	1
Heart	2.15 ± 0.48	9.77 ± 1.8	29.46 ± 6.97	39.68 ± 7.4
Lungs	0.38 ± 0.1	2.86 ± 0.44	8.02 ± 1.48	12.83 ± 2.44
Liver	0.2 ± 0.05	0.61 ± 0.13	1.51 ± 0.33	1.68 ± 0.48
Spleen	0.32 ± 0.07	1.01 ± 0.2	1.38 ± 0.43	1.44 ± 0.2
Stomach	0.5 ± 0.05	2.42 ± 1.15	5.42 ± 3.21	7.07 ± 3.82
Sm. Intestine	0.62 ± 0.16	1.53 ± 0.49	4.54 ± 1.15	12.28 ± 3.93
Lg. Intestine	0.25 ± 0.05	0.97 ± 0.49	3.32 ± 0.84	5.34 ± 1.71
Kidneys	0.53 ± 0.12	2.81 ± 0.73	6.8 ± 2.28	11.81 ± 5.95
muscle	2.1 ± 0.53	12.88 ± 2.66	37.66 ± 7.42	67.18 ± 16.26
Bone	1.62 ± 0.38	4.49 ± 1.18	14.72 ± 3.21	17.85 ± 10.54
Tail	0.56 ± 0.22	0.53 ± 0.18	0.97 ± 0.3	1.79 ± 0.51
Brain	5.37 ± 0.71	10.76 ± 2.7	26.2 ± 2.86	57.99 ± 6.67
Salivary Glands	Organs were not collected	0.33 ± 0.08	0.59 ± 0.05	1.69 ± 0.4

Supplementary Table 3: Biodistribution of [^{124}I]IPAG in selected organs showing the inhibition of tracer uptake in presence of 10-fold excess of IPAG in mice bearing MCF-7 tumors at 24 and 48 h.

	24h	24h IPAG Block	48h	48h IPAG block
Blood	0.055 ± 0.02	0.044 ± 0.02	0.020 ± 0.00	0.028 ± 0.00
Tumor	1.119 ± 0.24	0.211 ± 0.01	0.942 ± 0.22	0.106 ± 0.06
Heart	0.116 ± 0.02	0.044 ± 0.02	0.032 ± 0.00	0.033 ± 0.00
Lungs	0.395 ± 0.07	0.116 ± 0.05	0.117 ± 0.01	0.051 ± 0.00
Liver	1.838 ± 0.19	0.577 ± 0.19	0.623 ± 0.03	0.280 ± 0.04
Spleen	1.109 ± 0.10	0.189 ± 0.09	0.700 ± 0.07	0.218 ± 0.13
Stomach	0.561 ± 0.29	0.172 ± 0.07	0.241 ± 0.15	0.093 ± 0.01
Sm. Intestine	0.832 ± 0.41	0.113 ± 0.08	0.211 ± 0.02	0.206 ± 0.22
Lg. Intestine	1.300 ± 0.36	0.701 ± 0.74	0.291 ± 0.06	0.181 ± 0.11
Kidneys	0.436 ± 0.19	0.155 ± 0.08	0.150 ± 0.04	0.066 ± 0.03
Muscle	0.090 ± 0.02	0.028 ± 0.01	0.025 ± 0.00	0.026 ± 0.01
Bone	0.262 ± 0.08	0.047 ± 0.02	0.064 ± 0.00	0.048 ± 0.03
Tail	1.759 ± 0.39	0.189 ± 0.09	1.055 ± 0.34	0.220 ± 0.05
Brain	0.105 ± 0.01	0.026 ± 0.01	0.035 ± 0.00	0.011 ± 0.00
Salivary Glands	3.398 ± 0.38	0.244 ± 0.20	1.604 ± 0.42	0.075 ± 0.02

Supplementary Table 4: Biodistribution of [^{124}I]IPAG in selected organs showing the inhibition of tracer uptake in presence of 3.5-fold excess of haloperidol in mice bearing MCF-7 tumors at 72h. (n=3 for tumors)

	72h	72h Haldol Block
Blood	0.013 ± 0.00	0.016 ± 0.00
Tumor	0.676 ± 0.15	0.228 ± 0.01
Heart	0.017 ± 0.00	0.020 ± 0.00
Lungs	0.052 ± 0.00	0.038 ± 0.00
Liver	0.415 ± 0.05	0.391 ± 0.04
Spleen	0.473 ± 0.11	0.263 ± 0.16
Stomach	0.122 ± 0.05	0.096 ± 0.06
Sm. Intestine	0.064 ± 0.03	0.065 ± 0.06
Lg. Intestine	0.134 ± 0.03	0.067 ± 0.02
Kidneys	0.070 ± 0.03	0.037 ± 0.00
muscle	0.010 ± 0.00	0.009 ± 0.00
Bone	0.032 ± 0.00	0.036 ± 0.00
Tail	0.419 ± 0.20	0.225 ± 0.06
Brain	0.011 ± 0.00	0.005 ± 0.00
Salivary Glands	0.421 ± 0.14	0.051 ± 0.01