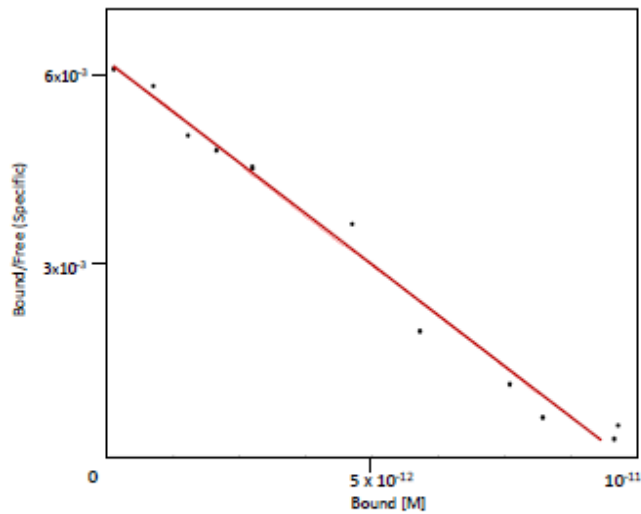


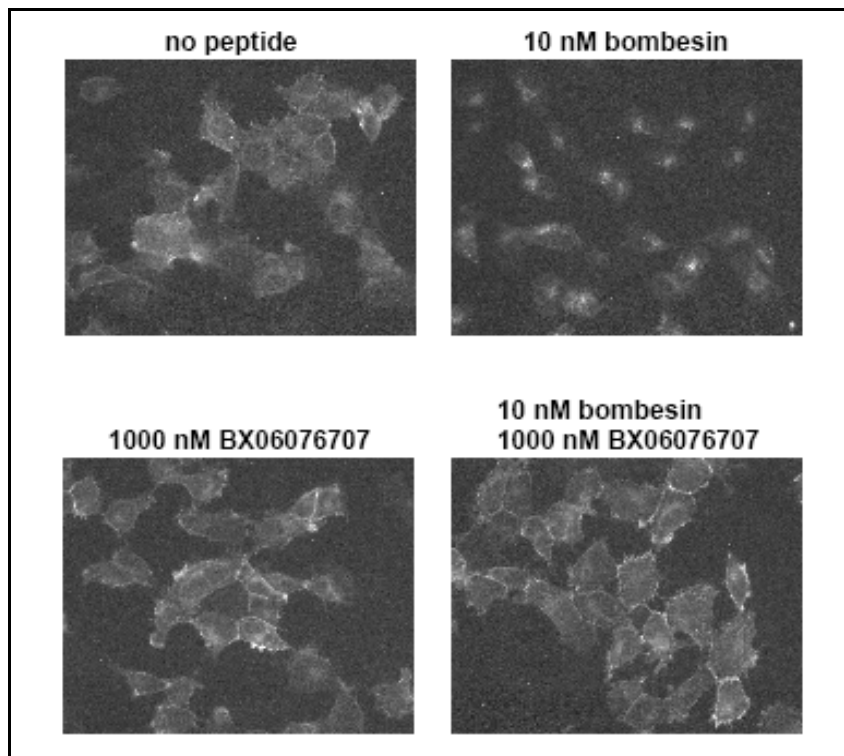
Supplemental Figure 1: Competitive binding of BAY 86-4367 against the radioligand ^{125}I -Tyr⁴-Bombesin in a scintillation proximity assay (SPA) using membranes of NMBr-transfected cells in comparison to NMB.



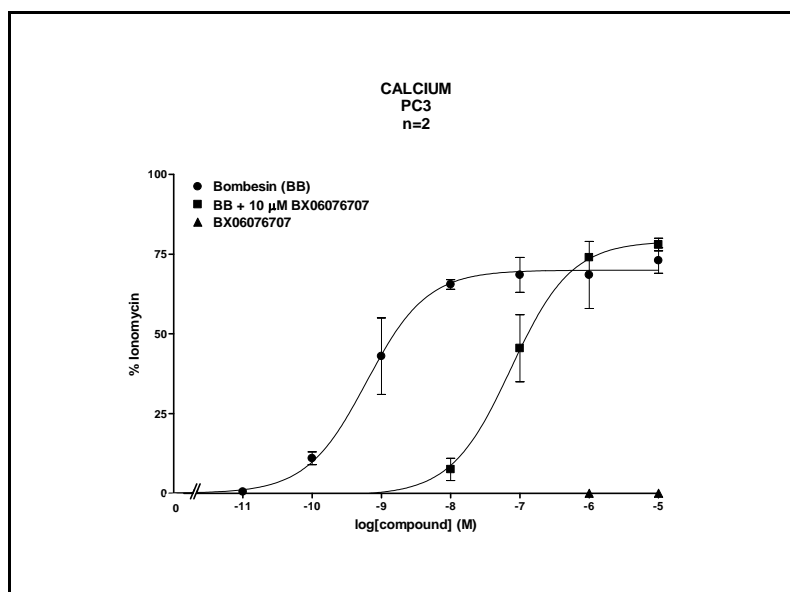
Supplemental Figure 2: Representative Scatchard plot of saturation binding of the ^{18}F -bombesin peptide to PC-3 cells.

Antagonist Characterization

Antagonistic properties were determined via receptor-internalization in hGRPr-transfected HEK-cells by immunofluorescence-microscopy (Ref. 24) or intracellular calcium-mobilization in PC-3 cells (Ref. 25) induced by the natural agonist bombesin and competitively inhibited by BAY 86-4367. Antagonist assays were carried out in the laboratory of Prof. J.-C. Reubi (University Bern, Switzerland).



Supplemental Figure 3a: Immunofluorescence of GRPr internalization in GRPr-transfected HEK-cells induced by the agonist bombesin and effectively inhibited by the antagonist BAY 86-4367. (top-left: non-complexed GRPr at the cell membrane, top-right: GRPr internalization after complexation with the agonistic ligand bombesin, down-left: GRPr complexed with BAY 86-4367 (=BX06076707) at the cell membrane without internalization, down-right: blocking of the Tyr⁴-bombesin induced GRPr internalization by BAY 86-4367)



Supplemental Figure 3b: Intracellular calcium mobilization induced in P-C3 cells (two independent experiments with each n=3; SD). Cells were loaded with Fluo-4 dye as described and analyzed for calcium mobilization in response either to Tyr⁴-bombesin, or to BAY 86-4367 (=BX06076707). Maximum calcium response was obtained by treating the cells with ionomycin (100% value). Results are shown as percentage of maximum calcium response induced by ionomycin.

GRPr Selectivity via autoradiography

Autoradiographic experiments were carried out in the laboratory of Prof. J.C. Reubi (University Bern, Switzerland). Frozen human cancer tissue samples (prostate cancer, ileal carcinoid, bronchial carcinoid) [according to Waser B, Eltschinger V, Linder K, Eur J Nucl Med Mol Imaging, 2007, 34: 95–100] were prepared and processed as described above in the binding affinity section. The selectivity of BAY 86-4367 was then tested in these human tumor tissue selectively expressing one of the three bombesin receptor subtypes using the universal radioligand [¹²⁵I]-[D-Tyr⁶,β-Ala¹¹,Phe¹³,Nle¹⁴]-bombesin-(6-14) competitively inhibited by BAY 86-4367 in a concentration range from 0.1 – 10,000nM.

Table 1: Bombesin receptor subtype profile as determined by receptor-autoradiography with human prostate cancer (GRPr), ileal carcinoid (NMBr) and bronchial carcinoid (BBR3) tissue samples. The binding affinities of BAY 86-4367 were measured competing against the radioligand [¹²⁵I]-bombesin 6-14 (binding to all bombesin-receptor subtypes)

Tracer:	¹²⁵ I-[D-Tyr ⁶ , β-Ala ¹¹ , Phe ¹³ , Nle ¹⁴]-bombesin (6-14)	
Tissue:	Human cancers selectively expressing one of the 3 bombesin receptor subtypes	
	BAY 86-4367 (for comparison)	
IC₅₀:	GRP-R :	7.8 - 13.0 nM
	BB 3 :	> 10,000 nM
	NMB – R :	> 1,000 nM

Further Biodistributions

Table 1: Biodistribution of ¹⁸F-BAY 86-4367 in PC-3-bearing mice (time points 0.5-4 h). Biodistribution data of various organs are expressed as % ID/ g values, recovery analysis as %ID. (mean ± SD, n = 3)

Timepoint :	0.5 h		1 h		2 h		4 h	
% ID/ g	S.D.		S.D.		S.D.		S.D.	
Spleen	0.55	0.20	0.30	0.05	0.39	0.06	0.07	0.01
Liver	1.70	0.31	0.94	0.07	0.57	0.14	0.36	0.06
Kidneys	3.98	0.40	1.68	0.26	1.36	0.20	0.81	0.44
Lung	0.72	0.14	0.39	0.03	0.30	0.09	0.11	0.02
Bone*	0.91	0.08	1.22	0.12	2.20	0.39	2.75	0.79
Heart	0.42	0.10	0.20	0.02	0.14	0.02	0.07	0.03
Brain	0.04	0.01	0.03	0.01	0.03	0.00	0.02	0.00
Fat	0.15	0.03	0.11	0.07	0.04	0.02	0.05	0.05
Thyroid	0.74	0.16	0.50	0.04	0.52	0.06	0.62	0.22
Gallbladder	31.12	1.65	58.51	5.03	29.38	7.06	31.21	5.76
Muscle	0.19	0.01	0.10	0.02	0.06	0.04	0.03	0.01
Tumor	5.59	1.35	4.20	2.40	5.00	1.45	5.01	1.20
Skin*	0.74	0.17	0.33	0.10	0.24	0.13	0.13	0.03
Blood	0.80	0.13	0.34	0.03	0.23	0.03	0.13	0.02
Tail	2.20	1.80	1.15	0.21	1.31	0.85	0.68	0.08
Stomach	3.08	0.05	2.22	0.52	2.16	0.27	1.98	0.17
Prostate	7.15	6.95	0.71	0.90	1.29	1.86	0.03	0.03
Intestine	5.98	1.09	4.30	0.52	3.58	0.68	2.39	0.61
Pancreas	48.82	7.22	43.03	8.01	26.21	5.09	18.74	2.98
Adrenals	2.19	0.36	1.03	0.38	0.61	0.27	0.20	0.15
Summary % ID								
Recovery	90.75	3.31	97.48	0.94	93.24	17.04	92.57	4.34
Organs	43.75	1.64	31.30	1.05	24.55	3.53	16.16	1.04
Carcass	19.46	1.44	10.50	0.78	11.46	0.91	9.76	0.43
Urine	27.51	2.51	52.18	0.79	57.88	15.02	68.77	2.54
Faeces	-	-	3.49	1.44	1.91	2.13	1.95	1.60
Tumor / Tissue ratios								
T / Liver	3.38	1.01	4.49	2.54	8.70	0.84	14.13	3.45
T / Kidneys	1.40	0.28	2.59	1.75	3.64	0.70	7.31	3.05
T / Blood	7.32	3.07	12.75	8.08	21.91	3.62	39.19	6.04
T/ Muscle	29.87	6.36	47.91	38.32	90.59	41.21	164.11	66.27
T/ Bone	6.17	1.43	3.58	2.41	2.38	1.03	1.98	0.98

* Tissue aliquots only

Table 2: Biodistribution of ¹⁸F-BAY 86-4367 in LNCaP-bearing mice (timepoints 0.5-4 h). Biodistribution data of various organs are expressed as % ID/ g values, recovery analysis as %ID. (mean ± SD, n = 3)

Timepoint :	0.5 h		1 h		2 h		4 h	
% ID/ g	S.D.		S.D.		S.D.		S.D.	
Spleen	0.70	0.49	0.40	0.03	0.33	0.22	0.18	0.06
Liver	2.03	0.68	0.69	0.01	0.41	0.03	0.22	0.12
Kidneys	2.93	0.70	1.69	0.11	0.78	0.11	0.68	0.30
Lung	0.87	0.09	0.47	0.05	0.19	0.04	0.12	0.04
Bone*	0.81	0.23	1.42	0.14	1.60	0.52	1.15	0.27
Heart	0.48	0.11	0.23	0.05	0.10	0.02	0.05	0.02
Brain	0.04	0.01	0.03	0.00	0.03	0.01	0.02	0.01
Fat	1.39	0.41	0.59	0.62	0.11	0.18	1.62	2.72
Thyroid	0.57	0.18	0.57	0.12	0.28	0.10	0.41	0.27
Gallbladder	41.58	16.05	21.75	30.81	32.91	22.39	1.33	0.42
Muscle	0.20	0.03	0.13	0.03	0.04	0.01	0.04	0.02
Tumor	3.27	1.89	2.28	0.19	2.01	0.23	1.41	0.45
Skin*	1.47	1.07	0.54	0.23	0.20	0.05	0.27	0.23
Blood	0.97	0.17	0.38	0.04	0.19	0.02	0.09	0.02
Tail	1.88	0.43	0.89	0.04	1.14	0.23	0.65	0.12
Stomach	3.91	1.18	2.76	0.30	2.15	0.49	2.18	0.33
Prostate	0.75	0.29	0.46	0.19	0.13	0.15	3.05	4.95
Intestine	4.65	0.72	5.08	0.13	3.21	0.69	1.92	0.36
Pancreas	50.08	11.91	35.61	2.75	33.72	3.65	19.23	4.78
Adrenals	3.31	1.35	2.48	1.23	0.86	0.40	0.70	0.66
Summary % ID								
Recovery	90.19	2.66	90.89	3.78	91.09	3.27	94.10	3.21
Organs	43.63	4.98	33.88	2.33	22.15	3.49	14.38	3.23
Carcass	12.23	1.40	11.66	6.58	7.77	1.76	10.85	5.43
Urine	34.31	3.57	45.25	12.42	63.69	3.01	68.01	1.18
Faeces	-	-	0.10	0.15	0.83	0.63	3.66	3.32
Tumor / Tissue ratios								
T / Liver	1.73	1.06	3.31	0.25	4.83	0.39	7.40	3.68
T / Kidneys	1.09	0.54	1.35	0.19	2.58	0.09	2.67	2.08
T / Blood	3.21	1.49	6.03	0.95	10.43	0.49	15.31	5.40
T/ Muscle	15.87	7.58	19.22	6.85	53.06	19.50	34.49	4.02
T/ Bone	3.82	1.48	1.62	0.23	1.37	0.58	1.33	0.68

* Tissue aliquots only