Article

Development of Fluorinated Non-Peptidic Ghrelin Receptor Ligands for Potential Use in Molecular Imaging

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Supplementary Materials

Antagonistic properties at the hGhrR

Molecules with the highest affinity in the binding assay were additionally tested for their antagonistic potency in an inositol phosphate accumulation assay [1] (Table S1). The receptor was first pre-stimulated with different concentrations of the compounds for 5 min and then 10^{-8} M ghrelin was added.

Table S1. Inositol phosphate accumulation assay to determine antagonistic potencies of candidate molecules with 10^{-8} M ghrelin.

Compound	IC_{50} (nM)	pIC_{50} [1]	E_{max} [1] (%)	n [2]
(*S*)-**9**	24.8	7.61 ± 0.20	38 ± 4	≥ 3
(*R*)-**9**	17.5	7.76 ± 0.24	45 ± 5	4
(*S*)-**10**	345	6.46 ± 0.48	22 ± 4	≥ 3
(*S*)-**11**	349	6.46 ± 0.29	56 ± 10	4
(*S*)-**13**	207	6.69 ± 0.29	31 ± 5	5
(*S*)-**14**	123	6.91 ± 0.18	57 ± 6	4
(*S*)-**16**	28.7	7.54 ± 0.15	65 ± 5	5

[1] Mean values ± SEM, [2] number of independent experiments in duplicates

Ghrelin was used as control with an EC_{50} of 0.9 nM (pEC_{50} = 9.05 ± 0.06, E_{max} = 100 ± 3%). Due to the partial agonism of the compounds, the effect of ghrelin cannot be completely antagonized. The assay provided IC_{50} values for (*R*)-**9** and (*S*)-**16** of 17.5 nM and 28.7 nM, respectively, which were similar to the antagonistic potency of the starting molecule *(S)*-**9** of 24.8 nM. (*S*)-**10**, (*S*)-**11**, (*S*)-**14,** and (*S*)-**13** were much less potent, as indicated by their IC_{50} values (Table S1). The highest antagonistic efficacy could be observed for (*S*)-**16** (E_{max} of 65 %).

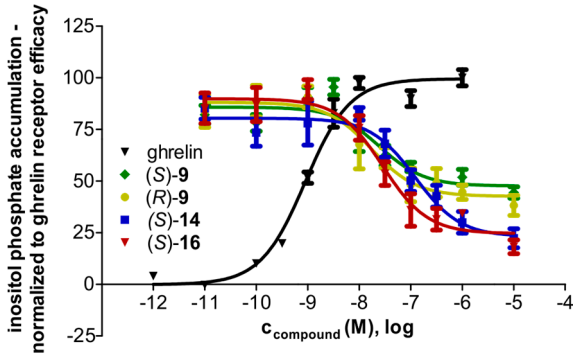

Figure S1. Antagonistic properties of the non-peptidic candidate compounds (*S*)-**9**, (*R*)-**9**, (*S*)-**14**, and (*S*)-**16**. Compounds were analyzed by inositol phosphate accumulation in COS7 cells stably transfected with hGhrR. Data were normalized to ghrelin (100% = maximal efficacy, 0% = constitutive receptor activity) and given in percent as means ± SEM of ≥ 3 independent experiments performed in duplicates.

Selectivity Studies

Table S2. Results of (*S*)-**9** screening by Eurofins Panlabs; for details on the methods see www.eurofinspanlabs.com.

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
203100	Adrenergic α_{1A}	395123	rat	2	1 µM	-3
203200	Adrenergic α_{1B}	395160	rat	2	1 µM	9
244530	Chemokine CXCR3	395320	hum	2	1 µM	0
252610	Muscarinic M_1	395145	hum	2	1 µM	14
252710	Muscarinic M_2	395239	hum	2	1 µM	11
265910	Potassium Channel hERG, [^{3}H]Dofetilide	395705	hum	2	1 µM	35
271710	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}, [^{3}H]Mesulergine	395157	hum	2	1 µM	4

1. Kostelnik, K.B.; Els-Heindl, S.; Kloting, N.; Baumann, S.; von Bergen, M.; Beck-Sickinger, A.G. High metabolic in vivo stability and bioavailability of a palmitoylated ghrelin receptor ligand assessed by mass spectrometry. *Bioorg. Med. Chem.* **2015**, *23*, 3925-3932.