opnMe by Boehringer Ingelheim

NPY1R Antagonist

BIBO3304

Boehringer Ingelheim

Table of contents

Summary

BIBO3304 is a potent and selective *in vitro* and *in vivo* tool compound. It acts as an antagonist on the neuropeptide Y (NPY) receptor Y1. Its distomer BIBO3457 is available as negative control. BIBO3304 successfully demonstrated in a rodent assay that it could significantly inhibit food intake induced by fasting or application of NPY. It is our second NPY receptor antagonist, after our NPY2R antagonist launched in December, 2020.

Chemical Structure

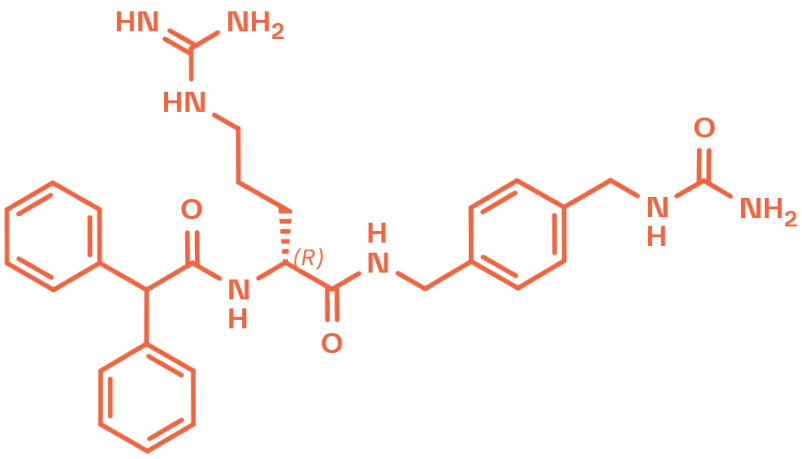

Figure 1: 2D structure of BIBO3304, a NPY-Y1 receptor antagonist

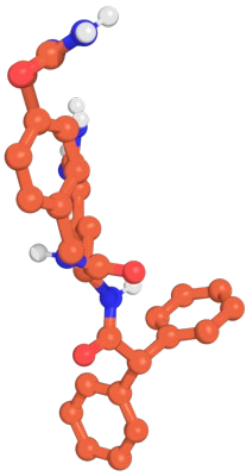

Figure 2: BIBO3304, 3D conformation

Highlights

BIBO3304 is a potent and selective NPY receptor Y1 antagonist. An (R)-argininamide derivative, it shows subnanomolar affinity for the human and rat Y1 receptor while displaying very low affinity for Y2, Y4 and Y5 receptors. In rodents, it has inhibited food intake induced by fasting or application of NPY. Its distomer, BIBO3457, is available as negative control.

Target information

Neuropeptide Y (NPY) is a 36 amino acid polypeptide that is abundantly expressed both in the peripheral and central nervous system, including the hypothalamus[1]. It is an endocrine and neuronal messenger that has been implied in the regulation of several physiological functions such as food intake and elevation of blood pressure[2-4]. The NPY-Y receptor system consists of three peptide ligands (NPY, pancreatic polypeptide and peptide YY) that interact in mammals with up to four receptors (Y1, Y2, Y4 and Y5) with different selectivity and affinity. These receptors are part of the G-protein coupled receptor superfamily that are coupled to the intracellular Ca^{2+} release and the inhibition of cAMP synthesis[5].

Research on the NPY receptors showed that the Y1 receptor could influence food intake[6].

Several small-molecule compounds and peptides have been characterised as NPY-Y1 antagonists with possible applications in oncology, cardio-metabolic desorders and bone loss[3,7-9]. Reported crystal structures of human NPY-Y1 receptor interacting with selective antagonists enable structure-based drug discovery[10]. Next to achieving sufficient potency, NPY-Y1 antagonists often struggle with selectivity, poor bioavailability, or brain penetration.

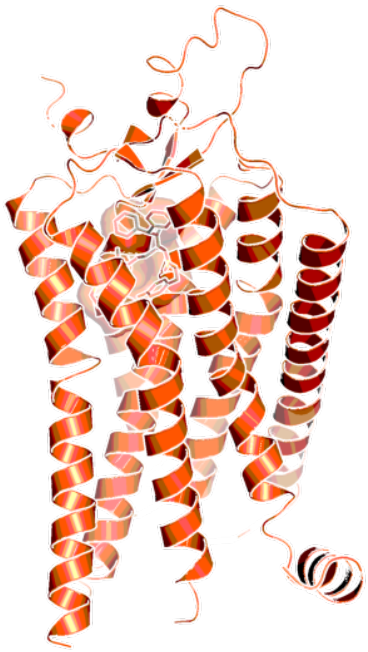

Figure 3: Crystal structures of NPY-Y1 complex with literature compound MK299 showing the location of the binding site[10].

In vitro activity

BIBO3304 displays an IC_{50} < 1nM for both the human and the rat Y1 receptor in cells stably transfected with the human or rat Y1 receptor (SK-N-MC cells)[9]. BIBO3304 binds with more than 1,000-10,000-fold lower affinity to the other subtypes (Y2, Y4 and Y5). Its (*S*)-enantiomer BIBO3457 exhibits an IC_{50} > 10,000 nM for the human and an IC_{50} > 1,000 nM for the rat Y1 receptor[9].

PROBE NAME / NEGATIVE CONTROL	BIBO3304	BIBO3457
MW [Da, free base][a]	529.6	529.6
Human Y1/BHK (IC_{50}) [nM]	0.7	> 10,000.0
Human Y1/SK-N-MC (IC_{50}) [nM]	0.4	1,300.0
Rat Y1/293 (IC_{50}) [nM]	0.7	> 1,000.0
Human Y2/SMS-KAN (IC_{50}) [nM]	> 10,000	> 10,000
Human Y2/BHK (IC_{50}) [nM]	> 1,000	> 10,000
Rat hippocampus (Y2)/CHO (IC_{50}) [nM]	> 10,000	n.a.

Human Y4/CHO (IC_{50}) [nM]	12,300	24,000
Rat Y4/CHO (IC_{50}) [nM]	> 10,000	> 10,000
Human Y5/293 (IC_{50}) [nM]	> 10,000	28,000
Rat Y5/CHO (IC_{50}) [nM]	21,000	23,000

[a] For the salt form you will get, please refer to the label on the vial and for the molecular weight of the salt, please refer to the FAQs

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BIBO3304	BIBO3457
logD @ pH 2 / 11	0.9 / >6.0	0.7 / 1.8
Solubility @ pH 6 [µg/mL]	374	96
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	0.1	0.1
Caco-2 efflux ratio	3	2
MDCK permeability P_{appAB} @ 1µM [10^{-6} cm/s]	0.6	0.5
MDCK efflux ratio	0.7	0.6
Microsomal stability (human/mouse/rat) [% Q_H]	45 / <23 / 81	35 / 34 / 78
Hepatocyte stability (human/mouse/rat) [% Q_H]	4 / 15 / 33	>4 / 37 / 31
Plasma Protein Binding (human/mouse/rat) [%]	90 / 91 / 84	75 / 90 / 84
hERG [inh. % @ 10 µM]	5	10
CYP 3A4 (IC_{50}) [µM]	>50	>50
CYP 2C8 (IC_{50}) [µM]	>50	31
CYP 2C9 (IC_{50}) [µM]	>50	>50
CYP 2C19 (IC_{50}) [µM]	n.a.	>50
CYP 2D6 (IC_{50}) [µM]	>50	>50

In vivo pharmacology

BIBO3304 is able to reduce food intake of rats that were stimulated either by endogenous (induced by fasting) or by exogenous NPY application[9]. The negative control BIBO3457 shows no significant influence on food intake in rats that were stimulated by exogenous NPY application[9].

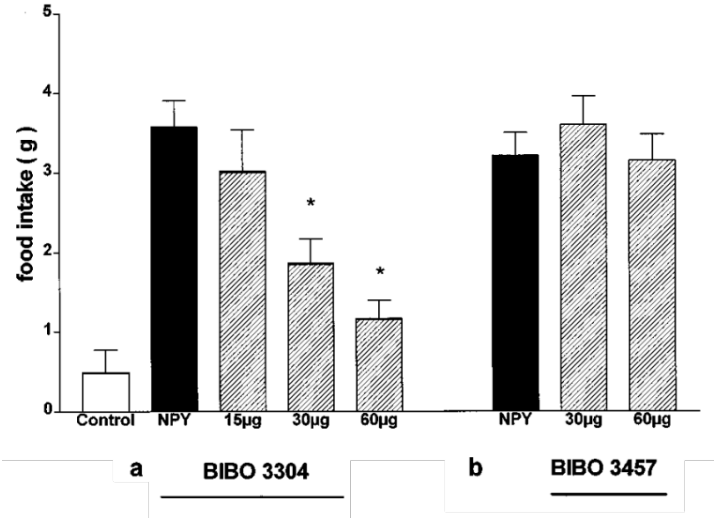

Figure 4: (a) Inhibition of NPY (1 µg, n = 25) induced feeding by BIBO3304 in adult male Chbb:Thom rats. (b) Lack of the effect of the inactive enantiomer BIBO3457 to inhibit NPY induced feeding (n = 8)[9].

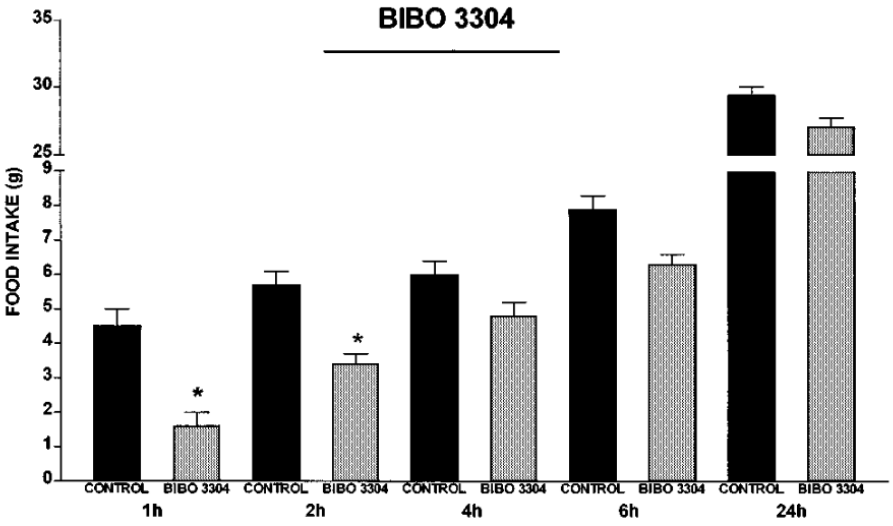

Figure 5: Effect of 30 µg of BIBO3304 (15 µg, bilateral) on food in 24 h fasted rats (n = 12)[9].

Negative control

BIBO3457 is the distomer of the active NPY-Y1 receptor antagonist BIBO3304. It displays low affinity towards both the human and the rat Y1 receptor[9].

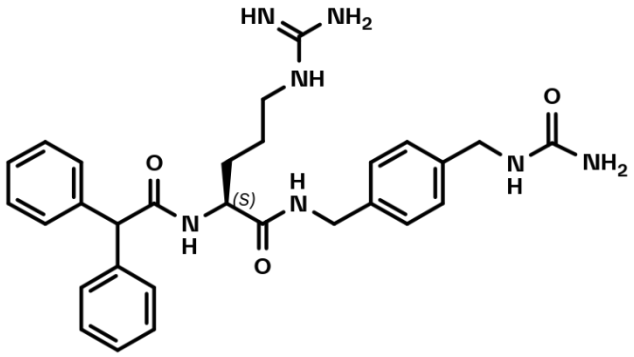

Figure 6: BIBO3457, which serves as a negative control

Selectivity

BIBO3304 was tested on 146 targets in a selectivity panel and showed ≥1,000-fold selectivity for 148 targets (≤50% inhibition @ 10 µM). In three assays (D2S(h), kappa KOP (h) and V1a (h)), the compound showed inhibition between 60-71%. The negative control BIBO3457 showed more than 50% inhibition @ 10 µM in 6 out of 44 targets.

SELECTIVITY DATA AVAILABLE	BIBO3304	BIBO3457
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No
DiscoverX®	No	No
Dundee	No	No

Supplementary data

2D structure files can be downloaded free of charge from opnMe.

References

1. Tomaszuk A., Simpson C., Williams G. Neuropeptide Y. The Hypothalamus and the Regulation of Energy Homeostasis *Horm. Res.* **1996**, 46, 53-58. DOI: 10.1159/000184996, PubMed: 8871182.

2. Stanley B. G., Magdalin W., Seirafi A., Nguyen M. M., Leibowitz S. F. Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y_1 receptor mediating this peptide's effect *Peptides* **1992**, 13, 581-587. DOI: 10.1016/0196-9781(92)90093-I, PubMed: 1326105.

3. Zhang L., Bijker M. S. Herzog H. The neuropeptide Y system: pathophysiological and therapeutic implications in obesity and cancer *Pharmacol. Ther.* **2011**, 131, 91–113. DOI: 10.1016/j.pharmthera.2011.03.011, PubMed: 21439311.

4. Edvinsson L., Håkanson R., Wahlestedt C., Uddman R. Effects of neuropeptide Y on the cardiovascular system Trends *Pharmacol. Sci.* **1987**, 8, 231-235. DOI: 10.1016/0165-6147(87)90069-1.

5. Aakerlund L., Gether U., Fuhlendorff J., Schwartz T. W., Thastrup O. Y_1 receptors for neuropeptide Y are coupled to mobilization of intracellular calcium and inhibition of adenylate cyclase *FEBS Lett.* **1990**, 260, 73-78. DOI: 10.1016/0014-5793(90)80069-U, PubMed: 2153577.

6. Yulyaningsih E., Zhang L., Herzog H., Sainsbury A. NPY receptors as potential targets for anti-obesity drug development *Br. J. Pharmacol.* **2011**, 163, 1170-1202. DOI: 10.1111/j.1476-5381.2011.01363.x, PubMed: 21545413.

7. Brennauer A., Dove S., Buschauer A. Structure-Activity Relationships of Nonpeptide Neuropeptide Y Receptor Antagonists *Handbook of Experimental Pharmacology* **2004**, 162. DOI: 10.1007/978-3-642-18764-3_17.

8. Rudolf K., Eberlein W., Engel W., Wieland H. A., Willim K. D., Entzeroth M., Wienen W., Beck-Sickinger A. G., Doods H. N. The first highly potent and selective non-peptide neuropeptide Y Y1 receptor antagonist: BIBP3226 *Eur. J. Pharmacol.* **1994**, 271, 11-13. DOI: 10.1016/0014-2999(94)90822-2, PubMed: 7705422.

9. Wieland H. A., Engel W., Eberlein W., Rudolf K., Doods H. N. Subtype selectivity of the novel nonpeptide neuropeptide Y Y1 receptor antagonist BIBO 3304 and its effect on feeding in rodents *Br. J. Pharmacol.* **1998**, 125, 549-555. DOI: 10.1038/sj.bjp.0702084, PubMed: 9806339.

10. Yang Z., Han S., Keller M., Kaiser A., Bender B. J., Bosse M., Burkert K., Kögler L. M., Wifling D., Bernhardt G., Plank N., Littmann T., Schmidt P., Yi C., Li B., Ye S., Zhang R., Xu B., Larhammar D., Stevens R. C., Huster D., Meiler J., Zhao Q., Beck-Sickinger A. G.,

Buschauer A., Wu B. Structural basis of ligand binding modes at the neuropeptide Y Y1 receptor *Nature* **2018**, 556, 520-524. DOI: 10.1038/s41586-018-0046-x, PubMed: 29670288.