opnMe by Boehringer Ingelheim

5-HT$_{2C}$ agonists

BI-4752 and BI-3234

Boehringer Ingelheim

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Summary

BI-4752 and BI-3234 are 5-HT_{2C} receptor agonists with good *in vitro* potency (EC_{50} 5-HT_{2C} = 11 nM and 35 nM, respectively) and excellent selectivity against 5-HT_{2a} and 5-HT_{2b}. They have different *in vivo* properties and hence, represent good tools to study 5-HT_{2c} receptor molecular signaling.

Chemical Structure

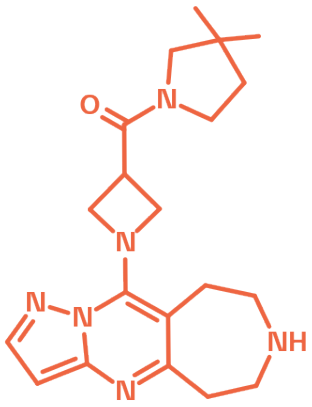

Figure 1: 2D structure of BI-4752, a potent 5-HT2C receptor agonist.

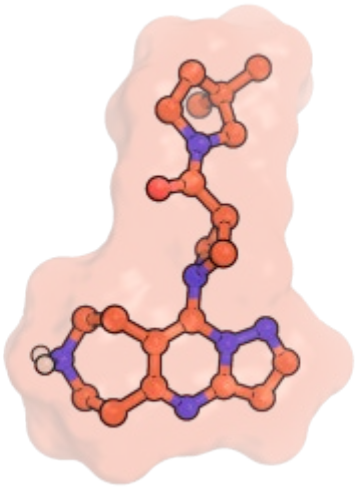

Figure 2: BI-4752, low energy conformation (bioactive conformation not known)

Highlights

BI-4752 and BI-3234 are potent and full 5-HT_{2C} receptor agonists and have high selectivity against the 5-HT_{2A} and 5-HT_{2B} receptors. They have shown good solubility and moderate permeability *in vitro*. In rats, both molecules have a good PK profile with high bioavailability, so they are suitable for *in vivo* experiments.

Target information

The 5-HT_{2C} receptor is a G-protein coupled receptor (GPCR). It belongs to the 5-HT_2 sub-family, which is one of 14 serotonin receptor subtypes[1]. This particular 5-HT_2 sub-family comprises of the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors, which all share considerable sequence homology. While the 5-HT_{2A} and 5-HT_{2B} receptors are expressed both peripherally and, in the CNS, 5-HT_{2C} is exclusively expressed in the CNS. To avoid potential side effects (e.g. hallucinations for 5-HT_{2A}[2], cardiac valvulopathy and pulmonary hypertension for 5-HT_{2B})[3], substantial selectivity is desired. Both molecules fulfill this criterion. There is pre-clinical evidence that targeting the 5-HT_{2C} receptor represents a potentially new treatment approach against a variety of diseases such as schizophrenia, obesity, sexual dysfunction, urinary incontinence, and diabetes[4,5]. A crystal structure of the human 5-HT_{2C} receptor in complex with the serotonin receptor antagonist ritanserin was reported in 2018 (Figure 3)[6].

BI-4752 and BI-3234 have been designed in a joint project of Boehringer Ingelheim and Evotec, whereby Evotec was involved in the synthesis and the design of the molecule.

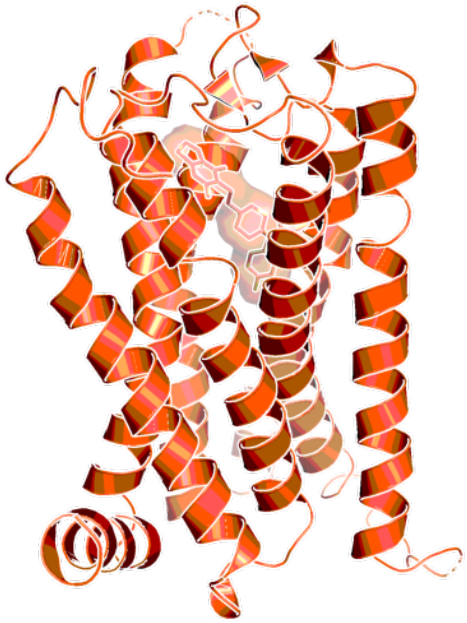

Figure 3: Crystal structure of human 5-HT2C receptor in complex with ritanserin (PDB code:6 6BQH, 2.7 Å resolution)

In vitro activity

BI-4752 displays an EC_{50} (5-HT_{2C}) of 11 nM and is a weak partial 5-HT_{2B} (EC_{50} = 2037 nM, E_{max} = 58%) and 5-HT_{2A} (EC_{50} = 1881 nM, E_{max} = 50%) agonist. BI-4752 is selective against 5-HT_{1B} (IC_{50} > 10,000 nM). BI-3234 is also a potent full 5-HT_{2C} agonist (EC_{50} = 35 nM), with an even higher selectivity against the 5-HT_{2B} and 5-HT_{2A} receptors.

PROBE NAME / CONTROL MOLECULE	BI-4752	BI-3234
MW [Da, free base][a]	368.5	382.5
5-HT_{2C} (EC_{50}) [nM][b]	11	35
5-HT_{2B} (EC_{50}) [nM] / E_{max} [%][b/c]	2,037 / 58	>10,000
5-HT_{2A} (EC_{50}) [nM] / E_{max} [%][b/c]	1,881 / 50	>10,000
5-HT_{1B} (IC_{50}) [nM][c]	>10,000	>10,000

[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

[b] Agonism was monitored in whole cells by measuring the Ca^{2+} release using the Fluorimetric Imaging Plate Reader (FLIPR) using the FLIPR Calcium 3 no-wash assay kit (Molecular Devices). More details are available in our *Bioorg. Med. Chem. Lett.* publication[1]. Additional information can be obtained via the "Contact us" formular.

[c] Color code: green = selective compound; yellow = weak agonist.

In vitro DMPK and CMC parameters

BI-4752 and BI-3234 possess a good solubility and a moderate permeability. They display only minimal cytochrome and hERG inhibition.

PROBE NAME / CONTROL MOLECULE	BI-4752	BI-3234
logD @ pH 7.4	0.4	0.3
Solubility @ pH 6.8 [µg/mL]	>1,000	>87
Caco-2 permeability AB @ pH 7.4 [$*10^{-6}$ cm/s]	0.3	1.4
Caco-2 efflux ratio	13	28
hERG [inh. % @ 10 µM]	20	32
hERG (IC_{50}) [µM]	71	n.d.
CYP 3A4 (IC_{50}) [µM]	>50	>50
CYP 2D6 (IC_{50}) [µM]	>50	5
$t_{1/2}$ human / rat liver microsomes [min]	>60 / >60	>60 / 55

In vivo DMPK parameters

BI-4752 possesses good PK profiles in mice[a] and rats[b]. BI-3234 possesses a good PK profile in rat[b], with good bioavailability.

MOLECULE / PROPERTIES	MOUSE BI-4752	RAT BI-4752	RAT BI-3234
Clearance [% Q_H]	36.0	83.0	17.5
Mean residence time after *i.v.* dose [h]	1.80	3.8	2.0
t_{max} [h]	1.67	1.41	0.50
C_{max} [nM]	29	92	66
F [%]	14	87	37
V_{ss} [L/kg]	3.5	13.0	3.0

[a] Mouse doses: *i.v.* 6 µmol/kg; *p.o.* 25 µmol/kg The *i.v.* formulation contained a 0.9% NaCl solution; the *p.o.* was a standard natrosol solution, acidified with 0.1 N HCl to pH 7.
[b] Rat doses: *i.v.*6 µmol/kg; *p.o.* 25 µmol/kg The *i.v.* formulation contained a 0.9% NaCl solution; the *p.o.* was a standard natrosol solution, acidified with 0.1 N HCl to pH 7.

In vivo pharmacology

Our current *in vivo* data for both compounds come from diabetes research. The daily food intake of diabetic *db*/*db* mice was reduced upon administration of both BI-4752 and BI-3234 (100 mg/kg) (Figure 4a). The body weight decreased over the 28 days period (Figure 4b). It is worth noting that despite the high dose (100 mg/kg), the compound was well tolerated over 28 days. These first two pre-clinical results confirmed our hypothesis that signaling via the 5-HT2c receptor might play a role in body weight regulation. When we tested the effect of those compounds on glycemic control as measured by HbA1c levels (Figure 4c), we only observed a decrease in mice treated with BI-4752 (-1.1%). When mice were treated with BI-3234, no significant HbA1c lowering was observed.

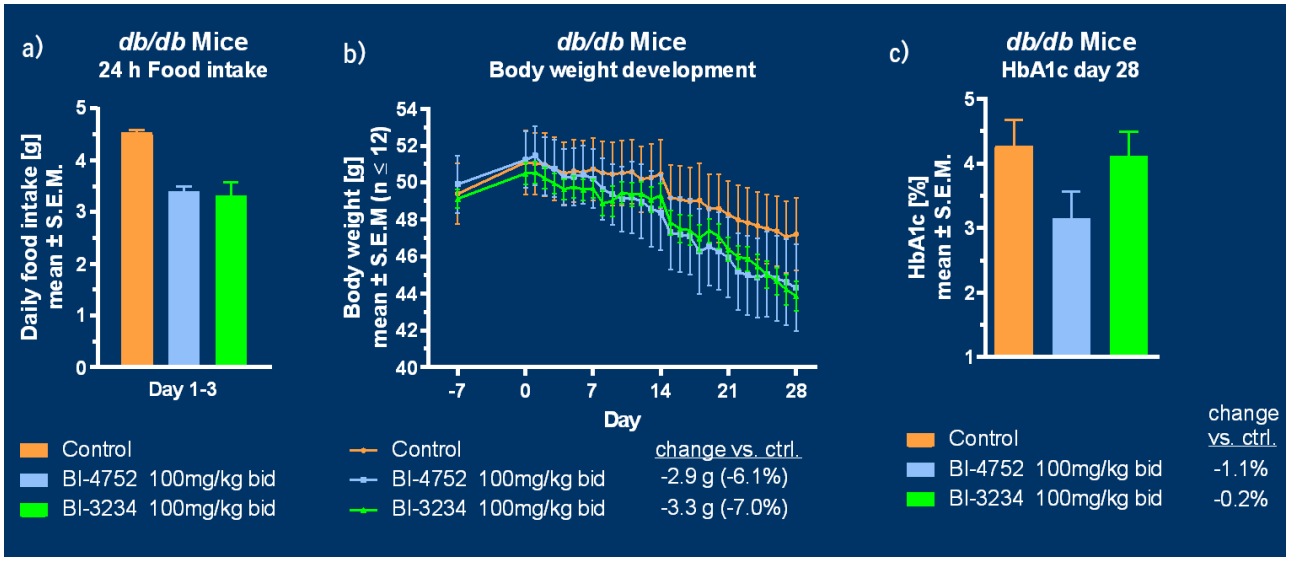

Figure 4: In vivo study in db/db mice. a) Daily food intake; b) Body weight development over 28 days; c) Glucose lowering on day 28 (as assessed by HbA1c measurement).

Control molecule for in vivo experiments

BI-3234 is offered as an active control compound. It is structurally closely related to BI-4752 (Figure 5), also a potent 5-HT_{2C} agonist (EC_{50} = 35 nM), and selective against the 5-HT_{2B} and 5-HT_{2A} receptors. It is therefore not suitable as an *in vitro* negative control. Unlike BI-4752 however, BI-3234 did not improve glucose control after 28 days treatment in a murine model (100 mg/kg, bid), and can therefore be used as a control molecule for *in vivo* experiments.

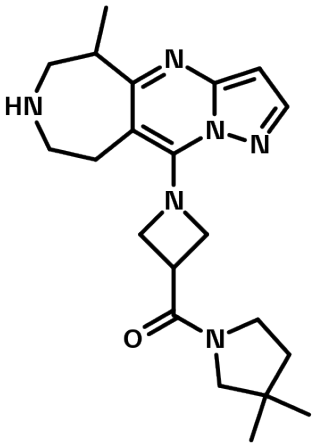

Figure 5: BI-3234 which serves as a control molecule for *in vivo* experiments.

Selectivity

BI-4752 inhibits the 5-HT_{1B} receptor in the low micromolar range (K_i = 0.75 µM) but was found to be selective against a panel of GPCRs. BI-3234 inhibits the 5HT2B (K_i = 1.14 µM) and 5-HT_{1B} (K_i = 0.75 µM) receptors in the low micromolar range but was found to be selective against a panel of GPCRs.

SELECTIVITY DATA AVAILABLE	BI-4752	BI-3234
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

lorcaserin (APD356), sibutramine

Supplementary data

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

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