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Design, synthesis and evaluation of MCH receptor 1 antagonists—Part I: Optimization of HTS hits towards an in vivo efficacious tool compound BI 414

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ABSTRACT

Despite recent approvals of anti-obesity drugs there is still a high therapeutic need for alternative options with higher efficacy in humans. As part of our MCH-R1 antagonist program for the treatment of obesity, a series of biphenylacetamide HTS hits was evaluated. Several issues of the initial lead structures had to be resolved, such as potency, selectivity over related GPCRs and P-gp efflux limiting brain exposure in this series. We could demonstrate that all parameters can be significantly improved by structural modifications resulting in BI 414 as a potent and orally available MCH-R1 antagonist tool compound with acceptable in vivo efficacy in an animal model of obesity.

Obesity is a major risk factor for many serious diseases of the metabolic syndrome complex such as type 2 diabetes, dyslipidemia, coronary heart disease and stroke.[1] These diseases are a major burden for patients and health care systems worldwide. Consequently, both in academia and pharmaceutical industry research activities focus on understanding the underlying mechanism of the metabolic syndrome in order to develop strategies for efficacious treatments. As a potential target melanin-concentrating hormone (MCH) has been in the focus of obesity research in the recent years.[2] MCH is expressed in brain regions relevant for food intake and energy homeostasis and is the natural ligand for both the MCH-R1 and MCH-R2 receptor. Activation of the MCH-R1 with MCH results in an increase in food intake and body weight in rodents, therefore antagonizing this effect is considered as a new concept for the treatment of obesity. As MCH-R2 is expressed in humans only, its overall physiological role still needs to be clarified. Despite many efforts in the pharmaceutical industry, a clinical proof of concept has not been achieved yet. The few compounds advanced to phase 1 clinical stage so far had to be discontinued due to safety concerns.[3–6] To finally assess the potential of MCH-1R antagonists as a treatment option for obesity in humans the need for potent and safe compounds is still high.

An in house high throughput screening (HTS) campaign resulted in the identification of **1** as a moderately potent MCH-R1 antagonist (IC_{50} = 251 nM). A significant improvement in potency could be achieved by inverting the central amide moiety (**2**, IC_{50} = 15 nM). In order to avoid anilines as potential mutagenic metabolites replacements by non-aromatic amines were investigated. The use of phenethylamines in combination with a shorter benzylic left hand amine moiety yielded **3** as a potent MCH-R1 antagonist (IC_{50} = 2 nM). This series was accepted as a lead series and optimization efforts were initiated (see Fig. 1).

The synthesis of these compounds started with commercially available phenethylamine **4**. Amide coupling followed by hydrolysis gave acid **6**, which was further reduced to benzylic alcohol **7**. Activation of the alcohol as mesylate followed by substitution with primary or secondary amines yielded the desired derivatives (see Scheme 1).

Representative structure–activity relationships (SAR) in this series (amine-, left-hand aryl- and right-hand aryl moiety) are summarized in Table 1. The amine part can be modified in broad fashion (**9**, **10**) and additional functional groups (**11**, **12**) are

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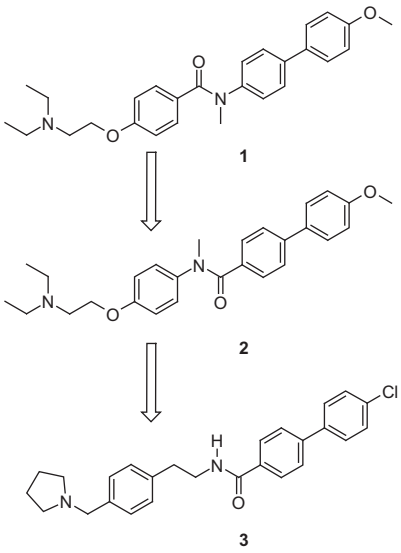


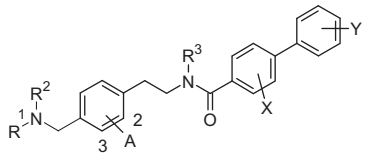
Figure 1. HTS hit series and lead series.

tolerated. Comparable potencies could be obtained with substituents in the left-hand aryl ring (**13**, **14**); polar moieties (**15**) led to reduced potency. For each right-hand aryl ring small lipophilic substituents proved to be advantageous, with compounds **17** and **18** being the most potent in this series.

A more in-depth characterization of **3** revealed off-target affinities to both a serotonin receptor (5-HT2A) and a muscarinic receptor (M1) and a potent inhibition of the cytochrome P450 enzyme CYP 2D6 (see Table 2). Further structure–activity relationship studies indicate that the amine part has a strong influence on the selectivity profile. The introduction of an amide (**22**, **23**) or urea moiety (**26**) was beneficial for selectivity. In addition, N-methylation of the central amide further reduced 5-HT2A affinity compared to the secondary amide (**24** vs **23**). The CYP 2D6 interaction potential was also reduced by the introduction of the polar moieties (e.g., **24**). However, despite the increased polarity these compounds exhibited only low to moderate metabolic stability in human and rat liver microsomes.

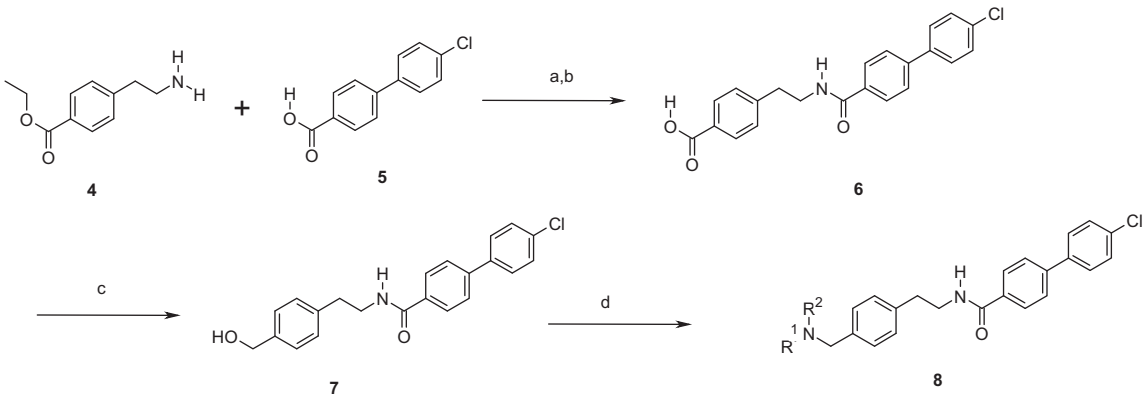
A rat pharmacokinetic (PK) investigation of **3** as a prototypical compound of this series revealed a low brain-to-plasma ratio which could be significantly increased with a P-glycoprotein (P-

Table 1
Representative SAR for lead series



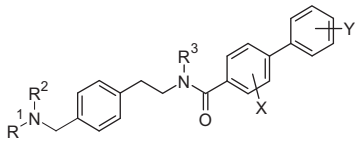
	R^1R^2N	A	R3	X	Y	MCH-R1[a] IC_{50} (nM)
3	Pyrrolidin-1-yl	H	H	H	4-Cl	2
9	Amino	H	H	H	4-Cl	68
10	Piperidin-1-yl	H	H	H	4-Cl	2
11	2-Hydroxyethylamin-1-yl	H	H	H	4-Cl	2
12	(Dimethylaminocarbonylmethyl)ethyl amin-1-yl	H	H	H	4-Cl	8
13	Pyrrolidin-1-yl	3-OMe	H	H	4-Cl	6
14	Pyrrolidin-1-yl	2-Br	H	H	4-Cl	2
15	Pyrrolidin-1-yl	3-$CONH_2$	H	H	4-Cl	470
16	Pyrrolidin-1-yl	H	Me	H	4-Cl	8
17	Pyrrolidin-1-yl	H	H	2-F	4-Cl	1
18	Pyrrolidin-1-yl	H	H	3-F	4-Cl	1
19	Pyrrolidin-1-yl	H	H	H	3-Cl	29
20	Pyrrolidin-1-yl	H	H	H	4-F	3
21	Pyrrolidin-1-yl	H	H	H	2,4-Cl_2	10

[a] For the description of biological methods, see Ref. 7.



Scheme 1. Reagents and conditions: (a) $SOCl_2$, cat. DMF, 60 °C; (b) 2 M NaOH, EtOH rt; (c) CDI, THF, $NaBH_4$, H_2O, rt; (d) NEt_3, CH_2Cl_2, $MeSO_2Cl$, 1 h 40 °C then R^1R^2NH, DMF, rt.

Table 2
Optimization of lead series towards improved selectivity and reduced CYP 2D6 interaction potential



	R^1R^2N	R^3	X	Y	MCH-R1[a] IC_{50} (nM)	M1 IC_{50} (nM)	5HT2A IC_{50} (nM)	CYP 2D6 IC_{50} (μM)	HLM/RLM $t_{1/2}$ (min)
3	Pyrrolidin-1-yl	H	H	4-Cl	2	84	107	0.5	NT
22	4-Acetylpiperazin-1-yl	H	H	4-Cl	6	877	2330	NT	NT
23	4-Acetylaminopiperidin-1-yl	H	H	4-F	3	8620	360	6.5	>45/22
24	4-Acetylaminopiperidin-1-yl	Me	H	4-F	11	>10,000	>10,000	15	9/13
25	(4-Acetyl)methylaminopiperidin-1-yl	H	H	4-F	5	2520	528	2.4	12/7
26	Ethylaminocarbonyl-methylamin-1-yl	H	2-F	4-Cl	36	>10,000	8150	>50	NT/21

[a] For the description of biological methods, see Ref. 7; NT: not tested; HLM: human liver microsomes; RLM: rat liver microsomes.

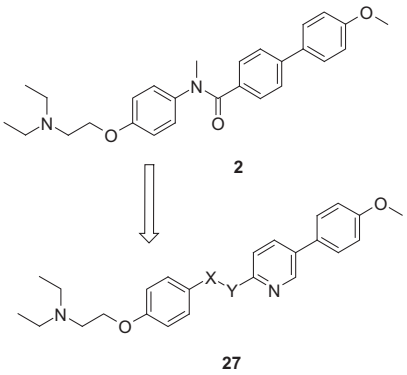
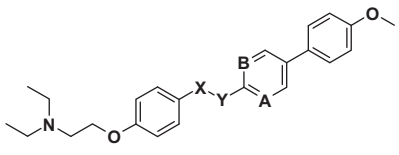


Figure 2. Spacer modifications.

gp) inhibitor (data not shown), thus, indicating **3** to be a substrate of the P-gp transporter. We felt that an optimization of this series would require substantial efforts to overcome all the described liabilities. Therefore we started a re-investigation of **2** in order to identify a new lead series (see Fig. 2).

Hypothesizing that the polar amide linker acts as a recognition element for P-gp (**2** is also a P-gp substrate), we decided to introduce less-polar linker moieties and re-balance the net polarity by incorporation of heteroatoms in the right hand aryl moiety (as indicated in **27**). The resulting structure–activity relationships are summarized in Table 3. As both the secondary and tertiary amide display comparable potency, we designed alkene analogs **29** and **30** to reflect both possible amide conformations. In addition, a switch to carbon linkers would avoid anilines as potential mutagenic metabolites. Unfortunately, a pronounced loss in potency was seen for both modifications, which also held true for the compound with a saturated spacer (**31**). Surprisingly, the potency could be regained with an alkyne moiety (**32**). The hydrogen bond acceptor (A=N) seems to be of importance as a 7-fold loss in potency was observed for the corresponding phenyl derivative **33** (A=CH). The addition of a second acceptor as in **34** resulted in a reduction of potency. The encouraging results observed for the alkyne spacer were the basis for further optimization efforts.

Table 3
Spacer modifications



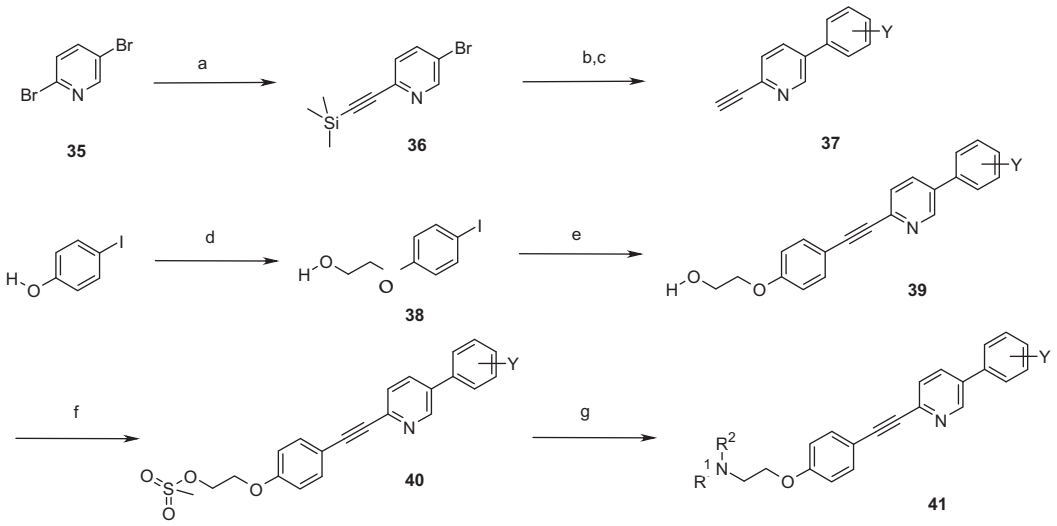
	–X–Y–	A	B	MCH-R1[a] IC_{50} (nM)
2	–NMe–CO–	CH	CH	15
28	–NH–CO–	CH	CH	33
29	–CH=CH– (*cis*)	N	CH	700
30	–CH=CH– (*trans*)	N	CH	420
31	$–CH_2–CH_2–$	N	CH	360
32	–C≡C–	N	CH	35
33	–C≡C–	CH	CH	247
34	–C≡C–	N	N	149

[a] For the description of biological methods, see Ref. 7.

The synthesis of the required alkyne derivatives started with commercially available 2,5 dibromo-pyridine **35**. Sonogashira coupling with trimethylsilylacetylene followed by a Suzuki coupling with substituted aryl-boronic acids and deprotection gave alkyne intermediates **37**. 4-Iodophenol was alkylated to furnish **38** which was used in a second Sonogashira reaction with **37** to yield **39**. Activation of the alcohol as mesylate (**40**) followed by a substitution with primary or secondary amines resulted in the desired alkyne derivatives **41** (Scheme 2).

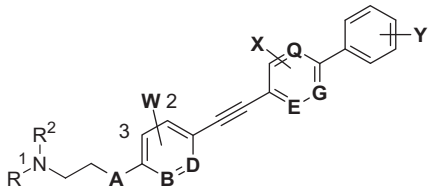
The structure–activity relationships for the alkyne derivatives are summarized in Table 4. Systematic modifications based on the structure–activity relationships observed in the amide series (pyrrolidine/4-chloro as in **3** instead of diethylamine/4-methoxy as in **2**) led to the identification of compound **43** as a potent MCH-R1 antagonist. The SAR of the amine moiety parallels that of the amide series; broad variations are possible including larger ring systems (**45**), introduction of polarity (**46**, **47**) and a second basic center (**48**). The attachment of the side chain via carbon (**49**) or nitrogen (**50**) is allowed, even a shorter linker (**51**) resulted in compounds of comparable potency. The introduction of nitrogen in the left hand aromatic system is allowed (**52**, **53**), whereas a pyridazine moiety led to an observed reduction in potency (**54**). Small substituents both in position 2 and 3 were tolerated (**55**, **56**). In the right hand aromatic system a different position for the hydrogen bond acceptor was not beneficial (**57**). On the other hand, increasing polarity by adding ring nitrogen atoms had only a small influence on potency (**58**, **59**). Substitution with small, lipophilic groups was tolerated, with fluorine being the most potent (**60**).

In-depth characterization of **43** as a key representative of the alkyne series revealed similar off-target affinities to the 5-HT2A receptor and the M1 receptor as seen for the amide series (Table 5). With modifications in the amine moiety an increase in



Scheme 2. Reagents and conditions: (a) trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, THF, 0 °C→rt; (b) (Y)ArB$(OH)_2$, $Pd(PPh_3)_4$, 2 M aq Na_2CO_3, dioxane, 90 °C; (c) TBAF*$3H_2O$, THF, rt; (d) 2-chloroethanole, K_2CO_3, DMF, rt; (e) **37**, $Pd(dppf)Cl_2$*CH_2Cl_2, CuI, piperidine, THF, rt; (f) $MeSO_2Cl$, NEt_3, THF, 0 °C→rt; (g) R^1R^2NH, DMF, 60 °C.

Table 4
Representative SAR of alkyne series



	R^1R^2N	A	B	D	W	E	G	Q	X	Y	MCH-R1[a] IC_{50} (nM)
32	Diethylamin-1-yl	O	CH	CH	H	N	CH	CH	H	4-OMe	35
42	Pyrrolidin-1-yl	O	CH	CH	H	N	CH	CH	H	4-OMe	20
43	Pyrrolidin-1-yl	O	CH	CH	H	N	CH	CH	H	4-Cl	7
44	Pyrrolidin-1-yl	O	CH	CH	H	N	CH	CH	H	4-Br	6
45	4-Methyl-piperidin-1-yl	O	CH	CH	H	N	CH	CH	H	4-Cl	5
46	4-Aminocarbonyl-piperidin-1-yl	O	CH	CH	H	N	CH	CH	H	4-Cl	8
47	4-Trifluoromethyl-4-hydroxy-piperidin-1-yl	O	CH	CH	H	N	CH	CH	H	4-Cl	6
48	4-Methylpiperazin-1-yl	O	CH	CH	H	N	CH	CH	H	4-Cl	10
49	Pyrrolidin-1-yl	CH_2	CH	CH	H	N	CH	CH	H	4-Cl	11
50	Pyrrolidin-1-yl	NH	CH	CH	H	N	CH	CH	H	4-Cl	5
51	Pyrrolidin-1-yl	—	CH	CH	H	N	CH	CH	H	4-Cl	26
52	Pyrrolidin-1-yl	O	N	CH	H	N	CH	CH	H	4-Cl	6
53	Pyrrolidin-1-yl	O	CH	N	H	N	CH	CH	H	4-Cl	10
54	Pyrrolidin-1-yl	O	N	N	H	N	CH	CH	H	4-Cl	77
55	Pyrrolidin-1-yl	O	CH	CH	2-Cl	N	CH	CH	H	4-Cl	20
56	Pyrrolidin-1-yl	O	CH	CH	3-Cl	N	CH	CH	H	4-Cl	5
57	Pyrrolidin-1-yl	O	CH	CH	H	C	N	CH	H	4-Cl	78
58	Pyrrolidin-1-yl	O	CH	CH	H	N	N	CH	H	4-Cl	12
59	Pyrrolidin-1-yl	O	CH	CH	H	N	CH	N	H	4-Cl	17
60	4-Methyl-piperidin-1-yl	O	CH	CH	H	N	CH	CH	3-F	4-Cl	4

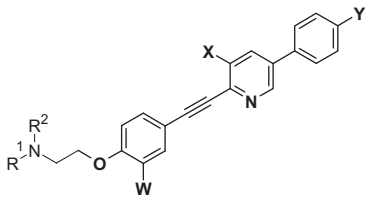
[a] For the description of biological methods, see Ref. 7.

selectivity was achievable. Polarity in the amine part appears to influence affinity to the 5-HT2A receptor more than to the M1 receptor (**46**, **61**), a substituent in the left hand aromatic system reduced affinity to the M1 receptor (**62** vs **61**) whereas a substituent in the right hand aromatic system seem to increase affinity to the M1 receptor (**63** vs **45**). But overall a clear structure–selectivity relationship could not be established in this series. In contrast to the amide series, the alkyne series showed a less pronounced interaction potential for CYP enzymes (e.g., CYP2D6) and an overall acceptable metabolic stability, thus qualifying this series for further in vivo investigations.

Further in vitro investigations of **43** revealed that it is not a P-gp substrate and therefore a reasonable brain exposure (as indicated by cerebrospinal fluid (CSF) levels) was detected in pharmacokinetic investigations (Fig. 3).

Based on these encouraging results we used **43** (=BI 414) to investigate the influence of pharmacological MCH-R1 antagonism on food intake and body weight in an animal model of obesity. Our model consists of three animal groups (rat, exbreeder, control group fed with standard rat chow diet, two groups with access to chow and high fat diet for the whole experiment) and two different compound treatment periods (**43**, 15 and 7.5 mg/kg *per oral*, once

Table 5
Optimization of alkyne series towards improved selectivity



	R^1R^2N	W	X	Y	MCH-R1[a] IC_{50} (nM)	M1 IC_{50} (nM)	5HT2A IC_{50} (nM)	CYP 2D6 IC_{50} (μM)	HLM/RLM $t_{1/2}$ (min)
43	Pyrrolidin-1-yl	H	H	Cl	7	170	500	6	>90/>68
45	4-Methyl-piperidin-1-yl	H	H	Cl	5	2210	1354	>10	>90/22
46	4-Aminocarbonyl-piperidin-1-yl	H	H	Cl	8	>10000	>10,000	>50	>90/21
61	(*R*)-2-Hydroxymethyl-pyrrolidin-1-yl	H	H	Cl	8	285	3290	NT	NT
62	(*R*)-2-Hydroxymethyl-pyrrolidin-1-yl	Me	H	Cl	4	2890	1640	>50	>90/>45
63	4-Methyl-piperidin-1-yl	H	F	Cl	4	665	>10,000	>50	NT/41

[a] For the description of biological methods, see Ref. 7; NT: not tested; HLM: human liver microsomes; RLM: rat liver microsomes.

	dose [μmol/kg]	C_{plasma}@2h [nM]	C_{CSF}@2h [nM]	Ki [nM]	C_{CSF}/Ki@2/8/24h
43	56.9	256	5.5	4	1.2/0.5/0.1

Figure 3. In vivo data **43** after oral application in Wistar rats.

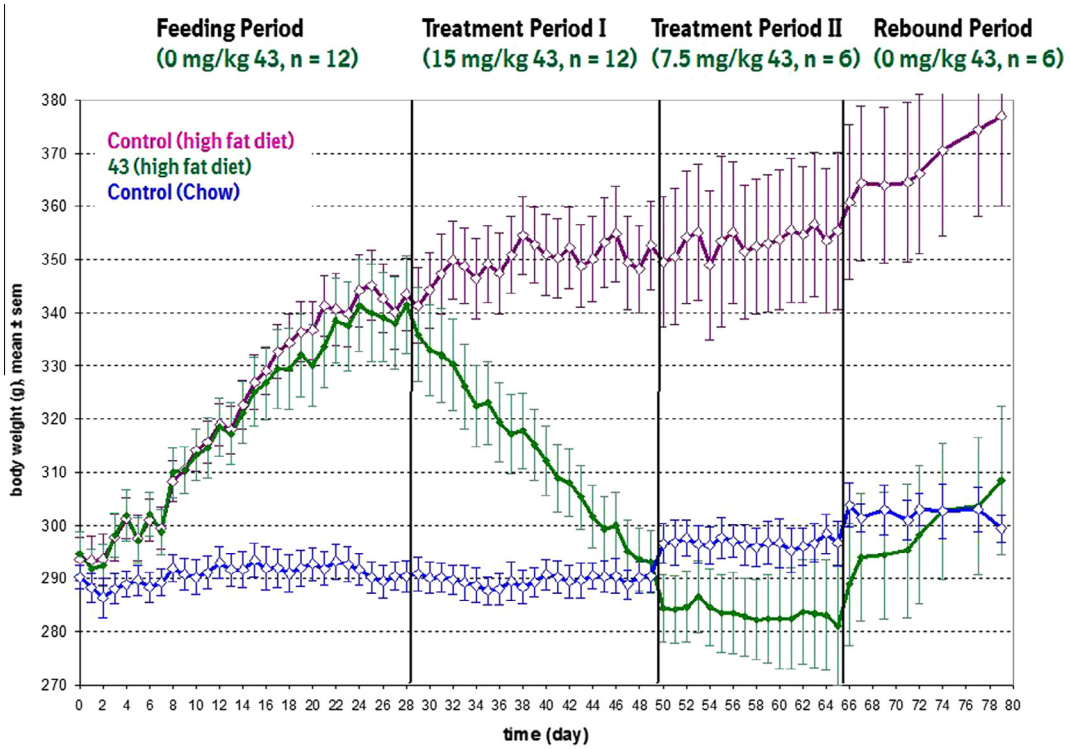


Figure 4. In vivo efficacy of compound **43** in animal model of obesity.

daily). The results obtained for the different groups are summarized in Figure 4. The weight gain in the animal groups on high fat diet (feeding period) could be reversed with compound treatment (period 1). The reduction of the dose resulted in a weight stable period for the animals in the treated group (period 2). A rebound in weight gain was seen after the treatment stopped. Compound **43** was well tolerated over the whole treatment period without any overt signs of adverse effects. To confirm these results the more selective[8] compound **45** was tested in the same setting (treatment period 1) resulting in a comparable outcome with respect to body weight reduction and tolerability (data not shown).

These results lend strong support to the concept of MCH-1R antagonism for the treatment of obesity. Unfortunately, **43**, **45** and other compounds from the alkyne series could not be pursued further, as a rather strong hERG interaction and phospholipidosis potential (both determined in vitro) were observed throughout

the series. The efforts to overcome these liabilities with new series of MCH-1R antagonists are described in the subsequent Letters.[9,10]

Acknowledgments

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8. >100 fold selective versus 43 GPCR's, 5 ion channels and 2 transporter (CEREP panel).
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