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Pyrimidine-based antagonists of h-MCH-R1 derived from ATC0175: In vitro profiling and in vivo evaluation

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ABSTRACT

A series of pyrimidine analogues derived from ATC0175 were potent antagonists of human MCH-R1 in vitro. Significantly improved receptor selectivity was achieved with several analogues from this series, but no improvement in brain partitioning was noted. One example from this series was shown to inhibit food intake and decrease body weight in a chronic study. However no clear correlation between the pharmacodynamic effect and the pharmacokinetic data with respect to brain concentration was discernible leading us to conclude that the observed effect was most likely not due to interaction with the MCH-R1.

Melanin concentrating hormone (MCH) is a cyclic 19 amino acid neuropeptide first isolated from salmon pituitary.[1] MCH gene expression is restricted to the lateral hypothalamic area and zona incerta, indicating a possible role in feeding or metabolism.[2] MCH is over-expressed in a number of rodent genetic models of obesity and diabetes (e.g., ob/ob; db/db, kkAY mice and fa/fa rats), whereas prepro-MCH deficient mice are lean, hypophagic and display a slight increase in metabolic rate.[3] Melanin concentrating hormone receptor-1 (MCH-R1) knock-out mice have normal body weight but are lean and hyperphagic.[4] MCH exerts its effects via two receptors in higher species (MCH-R1 and MCH-R2), which share approximately 38% homology, whereas only a single receptor with high sequence homology (96%) to the human MCH-R1 has been identified in rodents.[5] Chronic (14-day, ICV) administration of a selective peptide MCH agonist to rats results in hyperphagia and an increase in fat mass, whereas central administration of a peptidic MCH-R1 antagonist reduces feeding and bodyweight gain over 14 days.[6] From these data it can be inferred that a centrally acting MCH-R1 antagonist would reduce food intake and bodyweight and hence may be useful for the treatment of obesity. This appeared to be confirmed with a non-peptide antagonist (SNAP-7941, Fig. 1), which when dosed twice daily (20 mg/kg, IP) reduced food intake and bodyweight in diet-induced obese (DIO) rats.[7] Moreover, there have been multiple reports that selective MCH-R1 antagonists decrease food intake and body weight in the rat after oral administration,[8] as well as a recent report of similar effects in the rhesus monkey.[9]

From our in-house collection of GPCR-biased ligands, we used high throughput screening with either a GTPγS binding or FLIPR assay[10] to identify a series of alkyl-amino quinazolines which were functional antagonists at human MCH-R1. Our initial screening hits for the receptor had IC_{50} values in the 100–500 nM range but lacked selectivity against a panel of other GPCRs, in particular the NPY Y5 receptor, and had somewhat poor in vitro metabolic stability. A rapid, parallel, 'hit-to-lead' type expansion of the SAR of this series led us into a number of sub-series with potent and selective MCH-R1 antagonist activity as well as significantly improved metabolic stability.[11,12] Lead optimization of two of the resulting series, again using a modular parallel synthesis approach,

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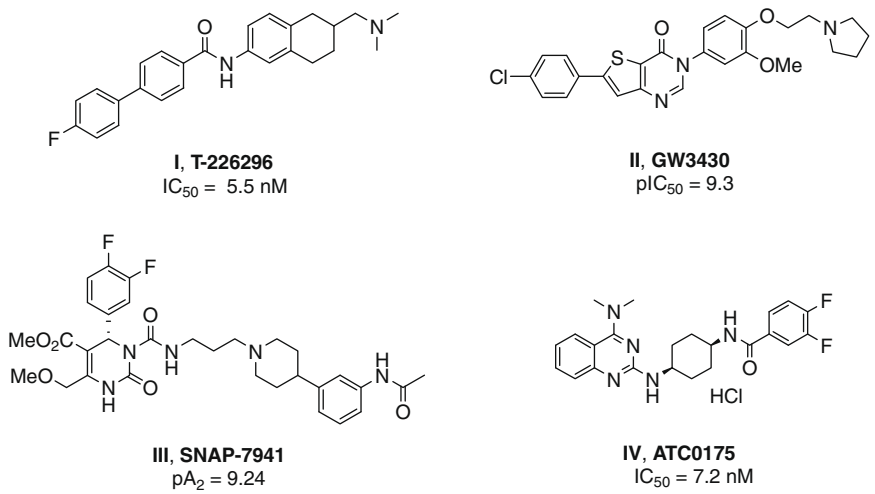
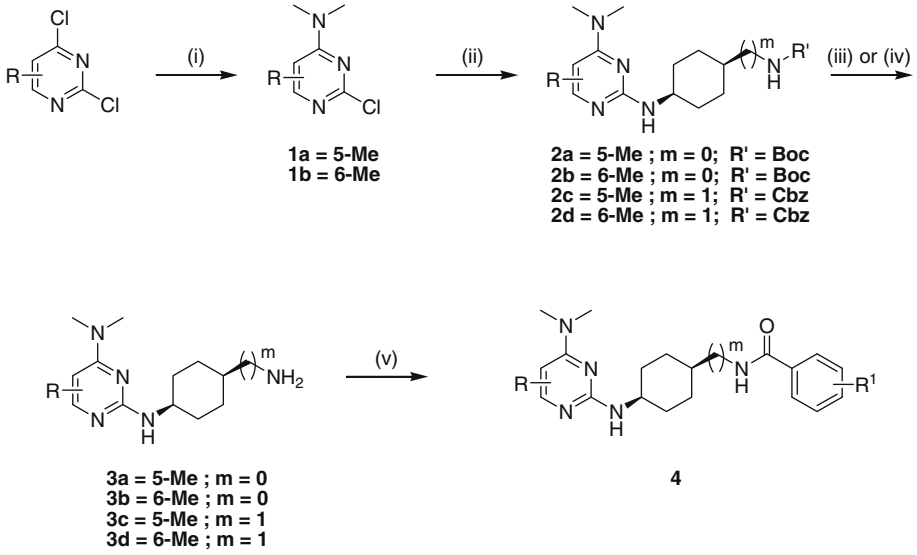


Figure 1. Known h-MCH-R1 antagonists.

confirmed further important aspects of the SAR. The inclusion of the dimethylamino substituent on the quinazoline portion of the lead series was found to be critical for optimal h-MCH-R1 activity, but a wide range of substituents were well tolerated at the other extreme of the molecule enabling us to manipulate the CNS partitioning properties of the series. This culminated in the identification of our early lead compound, ATC0175 (Fig. 1).[13] ATC0175 showed good oral bioavailability and moderate brain partitioning in rats (brain/plasma ratio 0.5–0.7 after oral administration) and was used as a pharmacological tool for proof of concept studies in vivo. For example, when dosed perorally (b.i.d.), ATC0175 dose-dependently decreased food intake and body weight over a four-day period in Sprague-Dawley rats fed on a high-fat diet.[14]

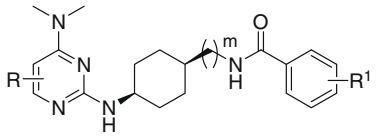
To further improve some of the properties of ATC0175 we focused in particular on trying to increase CNS partitioning so as to decrease the efficacious dose in vivo after oral administration, as well as improving the receptor selectivity profile. In an attempt to address both of these issues simultaneously, we turned our attention to analogues with lower molecular weight and reduced polar surface area. Hence, we targeted compounds in which the fused benzo ring of the quinazoline series was replaced by a methyl group, resulting in the preparation of a series of substituted pyrimidines **4** as shown in Scheme 1. Selective dimethylamination of the dichloropyrimidine starting materials (e.g., R = 5-Me or 6-Me) provided the 4-substituted intermediates **1a** and **1b** in good yield (>95%) with only a trace of the undesired 2-substitution products observed. A second nucleophilic substitution under more forcing conditions with a mono-protected diamine provided the intermediates **2** which were converted to the desired final products **4** by removal of the protective group to provide **3**, followed by acylation with the appropriate benzoyl chloride.

The resulting compounds were tested for antagonism of the calcium response to MCH in cells stably transfected with a constitutively activated (CART) version of the human MCH-R1 in a FLIPR assay.[15] The resulting data is shown in Table 1. As can be seen, the pyrimidine analogue series followed a very similar SAR to that previously observed in the quinazoline series. As with the earlier series, activity was retained across a wide range of substitutions around the right hand side aromatic ring, but 3- or 4-substitution was favoured, as was substitution with halogen or methoxy



Scheme 1. Preparation of pyrimidine analogues of ATC0175. Reagents and conditions: (i) $NHMe_2$ (40% w/v in water), THF, 0 °C, (40–60% yield); (ii) Ŕ = Boc; *cis*-NH_2–cyclohexyl–CH_mNHBoc, ^{i}PrOH, DIEA, microwave, 175–185 °C or Ŕ = Cbz; *cis*-NH_2–cyclohexyl–CH_mNHCbz, ^{t}BuOH, DIEA, microwave 175–185 °C (40–86% yield); (iii) Ŕ = Boc; TFA, DCM, rt (70–80% yield); (iv) Ŕ = Cbz; H_2, Pd/C, EtOH, rt, (70–80% yield); (v) R^1PhCOCl, DCM, Et_3N, rt (20–60% yield).

Table 1
SAR of pyrimidinyl analogues of ATC0175



Compound	R	*m*	R^1	IC_{50} h-MCH-R1[a] (nM)
ATC0175	Fused phenyl	0	3,4-F_2	13.5[13b]
4a	6-Me	0	3-CH_3	9.6
4b	6-Me	0	4-Cl	16
4c	6-Me	0	3-Cl	6.5
4d	6-Me	0	4-F	25
4e	6-Me	0	4-OCF_3	230
4f	6-Me	0	3-OCH_3	7.6
4g	6-Me	0	3,4-F_2	2.5
4h	6-Me	0	3,4-Cl_2	4.8
4i	6-Me	0	3,5-$(CF_3)_2$	8
4j	5-Me	0	3-CH_3	2.9
4k	5-Me	0	4-CH_3	13
4l	5-Me	0	4-F	6
4m	5-Me	0	4-Cl	10
4n	5-Me	0	3,4-F_2	2
4o	H	0	3,4-F_2	n.e.
4p	5-Et	0	3,4-F_2	100
4q	5,6-Me_2	0	3,4-F_2	2.3
4r	6-NMe_2	0	3,4-F_2	58
4s	6-Me	1	3,4-F_2	210
4t	5-Me	1	3,4-F_2	120
4u	5-Me	1	3-CF_3	26
4v	6-Me	1	3,5-$(CF_3)_2$	81
4w	6-Me	1	3,5-Cl_2	64
4x	5-Me	1	3,5-$(CF_3)_2$	5
4y	5-Me	1	3,5-Cl_2	4

n.e. = no effect at any concentration up to 10 μM.
[a] Values are means of at least three determinations for all compounds with IC_{50} <10 nM and at least two determinations for all other compounds. Log S.D. <0.35 in all cases where $n \geqslant 3$.

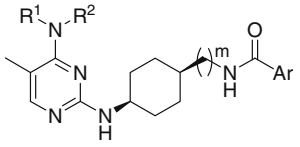
substituents. In the case where the methyl substitution on the pyrimidine ring was in the 5- or the 6-position, and where there was no spacer between the cyclohexyl ring and the amide linker (i.e., **4**, $m = 0$) the optimal aryl group was again 3,4-difluoro and essentially equal potency to ATC0175 was achieved with a significant reduction in molecular weight. At least one methyl substituent on the pyrimidine ring was required however, as **4o** showed no h-MCH-R1 antagonism in the FLIPR assay. In contrast, the dimethyl substituted analogue **4q** retained activity, but offered no improvement in potency over either the 5- or 6-monomethyl series. Substitution on the pyrimidine ring in the 5- or 6-position with larger alkyl or dimethyl amino groups was not well tolerated.

The SAR for the series containing a methylene spacer (**4**, $m = 1$) was somewhat different. In this case, with either 5- or 6-methyl substituted pyrimidines, the 3,4-difluorophenyl amide was not optimal. Instead we found that 3,5-substitution on the terminal aromatic ring was favoured, as was the incorporation of larger substituents such as chloro or trifluoromethyl. Another striking difference was the clear preference for the 5-substituted methyl pyrimidine in this elongated series that was not seen in the $m = 0$ series. With these differences in mind, we re-checked the previously observed absolute requirement for a dimethylamino substituent in the 4-position of the pyrimidine. However, even modest changes to this position resulted in significant loss of activity at h-MCH-R1 (Table 2), with only the methylethyl-amino group tolerated to any extent, thus confirming our previous observations.

With the change in scaffold from the dimethylamino quinazoline to dimethylamino pyrimidine, we observed a significant improvement in receptor selectivity for a number of analogues. ATC0175 had shown moderate to significant affinity for a range of monamine receptors and compounds with h-MCH-R1 activity were routinely tested against several of these receptors (Table 3). In general, it can be seen that the reduction in size led to an overall improvement in the compound profiles in vitro. From these data we identified compound **4x** as the analogue with the most promising selectivity profile. Of particular concern with ATC0175 was binding affinity for the serotonin 5-HT_{2B} and 5-HT_{1A} receptors. The former is present in the heart and its activation has been implicated in cardiac complications such as valvulopathy.[16] Binding to this receptor was reduced as a result of the scaffold switch, with all examples showing moderately lower affinity for 5-HT_{2B} than ATC0175, with **4x** being the most selective. To further alleviate the cardiovascular concerns, both ATC0175 and **4x** were shown to be inverse agonists of the 5-HT_{2B} receptor in an IP_3 functional assay. ATC0175 also showed high affinity for the 5-HT_{1A} receptor. Although this receptor was not screened routinely, compound **4x** proved to be significantly more selective than ATC0175 for h-MCH-R1 over h5-HT_{1A} (ATC0175: IC_{50} h5-HT_{1A} = 16.9 nM[13b]; **4x**: IC_{50} h5-HT_{1A} = 4.8 μM).

Compound **4x** proved efficacious in our 4-day screening model of food intake (dosed at 30 mg/kg PO qd). As a result of this positive effect, **4x** was tested in a chronic feeding study in diet-induced

Table 2
SAR of amino variants on 5-Me-pyrimidine core



Compound	R^1	R^2	*m*	Ar	IC_{50} h-MCH-R1[a] (nM)
4n	Me	Me	0	3,4-F_2-Ph	2
4ab	Me	Et	0	3,4-F_2-Ph	46
4ac	Me	H	0	3,4-F_2-Ph	n.e.
4ad	Et	H	0	3,4-F_2-Ph	n.e.
4ae	*i*Pr	H	0	3,4-F_2-Ph	n.e.
4x	Me	Me	1	3,5-$(CF_3)_2$-Ph	5
4af	Me	H	1	3,5-$(CF_3)_2$-Ph	200
4ag	H	H	1	3,5-$(CF_3)_2$-Ph	600
4ah	Et	H	1	3,5-$(CF_3)_2$-Ph	n.e.
4ai	Cyclo-$(CH_2)_4$		1	3,5-$(CF_3)_2$-Ph	n.e.

n.e. = no effect at any concentration up to 10 μM.
[a] Values are means of at least three determinations for all compounds with IC_{50} <10 nM and at least two determinations for all other compounds. Log S.D. <0.35 in all cases where $n \geqslant 3$.

Table 3
Receptor selectivity data for selected compounds

Compound	IC_{50} (μM) h-5-HT_{2B}[a] (*n*, log S.D.)	IC_{50} (μM) h-α_{2A}[b] (*n*, log S.D.)	IC_{50} (μM) h-α_{1A}[c](*n*, log S.D.)	IC_{50} (μM) h-H_1[d] (*n*, log S.D.)
ATC0175	0.15 (3, 0.07)*	0.104	0.3	0.15 (2, n.m.)
4b	0.17 (5, 0.20)	0.73 (4, 0.32)	3.0 (4, 0.45)	0.8 (3, 0.15)
4h	0.13 (5, 0.22)	0.13 (3, 0.42)	>10 (2, n.m.)	0.39 (4, 0.42)
4i	>10 (5, n.m.)	0.20 (4, 0.09)	1.32 (5, 0.22)	1.21 (4, 0.35)
4j	0.031 (4, 0.17)	0.58 (4, 0.17)	2.79 (5, 0.35)	1.6 (3, 0.21)
4k	0.12 (6, 0.14)	1.77 (3, 0.32)	>10 (7, n.m.)	0.17 (4, 0.09)
4l	0.3 (5, 0.11)	0.47 (4, 0.12)	7.5 (4, 0.95)	0.81 (4, 0.12)
4m	0.3 (3, 0.15)	1.59 (3, 0.45)	>10 (4, n.m.)	0.76 (3, 0.03)
4t	0.32 (3, 0.12)	0.21 (6, 0.29)	3.6 (5, 0.88)	0.92 (3, 0.41)
4x	3.1 (3, 0.31)	0.31 (4, 0.39)	3.2 (4, 0.5)	>10 (3, n.m.)

[a] [^{3}H]-rauwolsine binding; *Literature data; IC_{50} = 9.66 ± 1.58 nM in [^{125}I]-LSD binding.[13b]
[b] [^{3}H]-MK-912 binding.
[c] [^{3}H]-Prazosin binding.
[d] [^{3}H]-pyrilamine binding.

obese (DIO) female Wistar rats.[19] Once-daily oral doses (1, 5, 10 and 15 mg/kg/day) were selected and sibutramine (6 mg/kg/day PO) was included in this study as a positive control.

As previously demonstrated,[20] sibutramine caused a profound but transient effect on total energy intake on this modified cafeteria diet (Fig. 2), with daily intake returning to control levels within 8 days of dosing. This resulted in a decreased body weight relative to the control group. However, after 14 days the animals began to regain weight and their growth rate after this point essentially paralleled that of the control group. In contrast, the 10 and 15 mg/kg

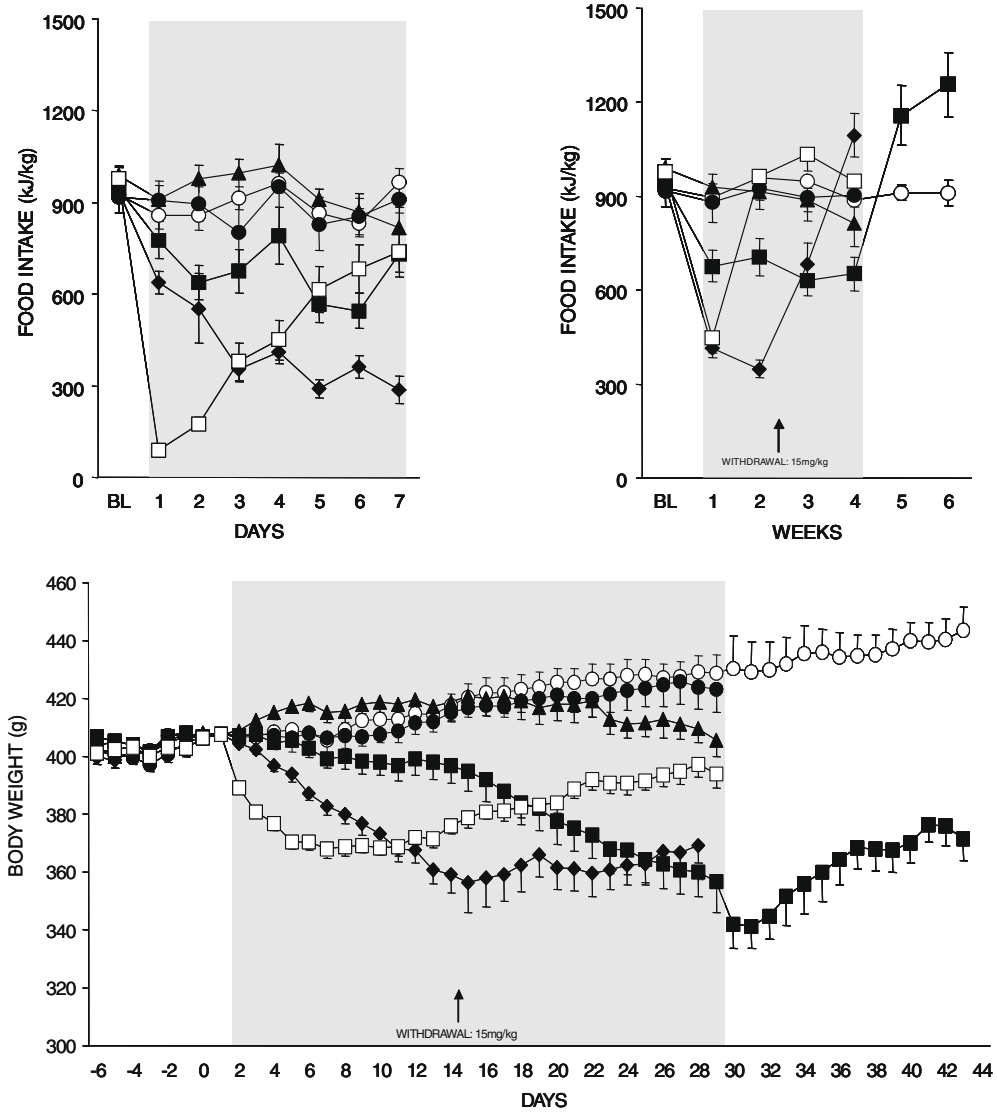


Figure 2. The dose–response effect of once daily oral doses of compound **4x** (1 mg/kg, ●; 5 mg/kg, ▲; 10 mg/kg, ■; 15 mg/kg, ♦) on food intake and body weight in DIO female Wistar rats compared to sibutramine (6 mg/kg, □) and vehicle (water, ○).

doses of **4x** produced a strikingly opposing pattern of effect, with maximal decreases in food intake becoming apparent after only 2 and 3 days, respectively, although statistical separation from vehicle was achieved on day 2 at both doses, and continued throughout the dosing period. This pattern of food intake change resulted in progressive weight loss. After 14 days of dosing, the highest dose group was deemed to have lost too much weight too rapidly (>15% relative to control) and was allowed to recover. Food intake gradually returned to normal and the body weight decline was reversed. A similar effect was observed in the 10 mg/kg dose group where after 28 days of dosing a 17% decrease in body weight relative to the vehicle group was observed. A subgroup of these animals was allowed to recover after this period and in this case we observed a modest but statistically significant rebound in daily energy and water intake, resulting in a recovery of body weights to control values over the ensuing 2 two weeks. The third dose group of 5 mg/kg failed to achieve statistical significance in terms of decreasing food intake, although a trend was observed resulting, by day 28, in a statistically significant decrease in body weight relative to the vehicle group (−5.4%). The 1 mg/kg dose had no effect on any of the measured parameters.

The clear difference in the pattern of food intake and body weight changes between rats treated with sibutramine, which is known to act centrally, and those treated with **4x**, was immediately suggestive that the effect of the latter may not be centrally mediated. Indeed the pattern of effect on food intake and body weight much more closely resembled that observed with exendin-4, which is known to act by inhibiting gastric emptying. Indeed, when we examined the pharmacokinetic data for **4x** and related compounds, it was clear that the scaffold change we had engineered did not enhance the brain partitioning properties in rat for this series over ATC0175. For compound **4x** for example, the brain to plasma ratio remained significantly below unity at all time points tested (Table 4), and below concentrations previously believed to be required for in vivo efficacy of MCH-R1 antagonists. McBriar et al. demonstrated a requirement for 70% receptor occupancy at a 6 h time point with chronic efficacy on food intake and body weight in rats with MCH-R1 antagonists.[17] In addition, with another series of MCH-R1 antagonists, very high concentrations were required (>10 μg/mg in brain i.e., 1000-fold higher than in this study) to show a significant chronic effect on food intake and body weight following once daily oral dosing in rats.[18] Hence, for compound **4x** and related analogues we concluded, based on the low levels of brain concentrations observed, that the effect on feeding is most likely not mediated by the central MCH-R1.

In summary, we have demonstrated that the in vitro potency of ATC0175 at the h-MCH-R1 may be preserved by removing the fused ring of the quinazoline and replacing it with a methyl substituent, resulting in a series of pyrimidine analogues. Several examples from this series had greatly improved receptor selectivity profiles compared to ATC0175. However, although compounds from this series decreased food intake in vivo, this modification did not provide compounds with good brain partitioning. Given that central exposure appears to be a requirement for MCH-R1 antagonist-induced decreases in food intake, we believe that the profound and dose-dependent decrease in energy intake and body weight in a 28-day DIO rat model observed for **4x** was most likely an off-target effect. In 14-day toxicity studies, no significant effects (other than a decrease in food intake) were observed at doses up to 100 mg/kg/day. Although a non-specific toxicity effect can not be completely ruled out, we have since further investigated other possible mechanisms of action and these experiments will be described in due course.

In this present investigation we have again demonstrated the importance of correlating the pharmacokinetic and pharmacodynamic effects on food intake in rodents to try and confirm that the observed effects are specific to the molecular target thought to be responsible.

Table 4
Brain and plasma concentrations of **4x** compared to ATC0175 following a per oral dose of 10 mg/kg to fed male Sprague-Dawley rats

	PLASMA (ng/mL)		BRAIN (ng/g)	
	2 h	4 h	2 h	4 h
4x	50	63	9	10
ATC0175	109	101	55	70

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.003.

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