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4-Acylamino- and 4-ureidobenzamides as melanin-concentrating hormone (MCH) receptor 1 antagonists

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Abstract—Synthesis, in vitro biological evaluation and structure–activity relationships of 4-acylamino-and 4-ureidobenzamides as novel hMCH1R-antagonists are disclosed. The nature of the amine side chains could be varied considerably in contrast to the central benzamide scaffold and aromatic substituents.

Melanin-concentrating hormone (MCH) is a nonadecapeptide found in rat[1] and human brain.[2] Expressed in particular in the lateral hypothalamus, evidence for involvement of MCH in feeding and body weight regulation is abundant,[3] and includes the observation of upregulated MCH mRNA in fasting rats and in obese *ob*/*ob* rats, and increased food consumption upon i.c.v.-injection of MCH in rats.[4] Also, deletion of the MCH-gene in mice results in a lean phenotype,[5] whereas overexpression of the MCH-gene in the lateral hypothalamus gives an obese and insulin resistant phenotype.[6] Furthermore, the hormone is reported to be involved in memory functions,[7] anxiety,[8] and depression.[9] Two seven transmembrane (7TM) G-protein coupled receptors, MCH1R (SLC-1) and MCH2R (SLT), have been identified for MCH,[3,10,11] and MCH1R-deficient mice are reported to be lean, hyperactive and hyperphagic.[12] Aminotetraline T-226296 (Fig. 1) was the first reported small molecule MCH1R antagonist.[13] T-226296 and SNAP-7941[9] (Fig. 1) were reported to suppress food intake induced by i.c.v.-injected MCH in rats. Thus, MCH1R antagonists are potentially interesting agents for treatment of metabolic or obesity-related disorders, as well as of disorders related to depression and anxiety.

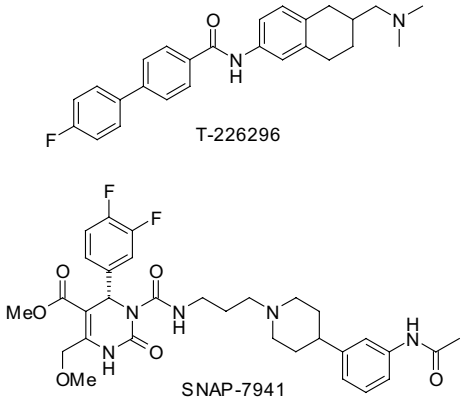

Figure 1. MCH antagonists.

A comparison of the physicochemical environment of the binding site (physicogenetic analysis[14]) of the MCH1 receptor with other 7TM receptors revealed a high similarity to several monoaminergic receptors, in particular dopamine D_2 and D_3. The combination of the plethora of small molecule ligand information on these receptors, especially the dopamine ligands exemplified in Figure 2,[15] with the aminotetraline T-226296, which was the only known MCH1R ligand at the time,[13] led to the design of novel benzamide ligands with a hydrophobic western appendage. Especially appealing was the presumption that an intramolecular hydrogen bond could lock the eastern amide side chain,[15] forming a coplanar arrangement similar to that in the aminotetraline. Furthermore, the 2-methoxybenzamide system may

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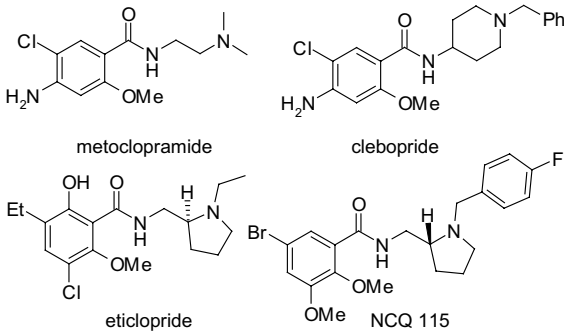
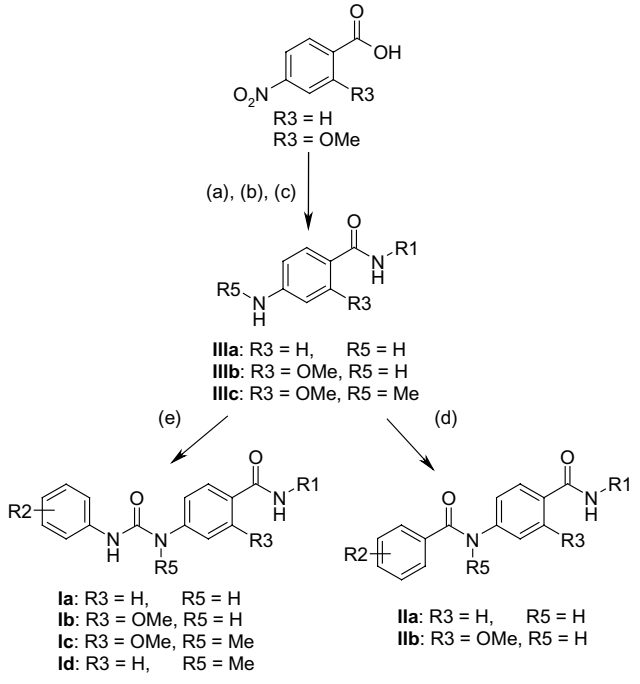


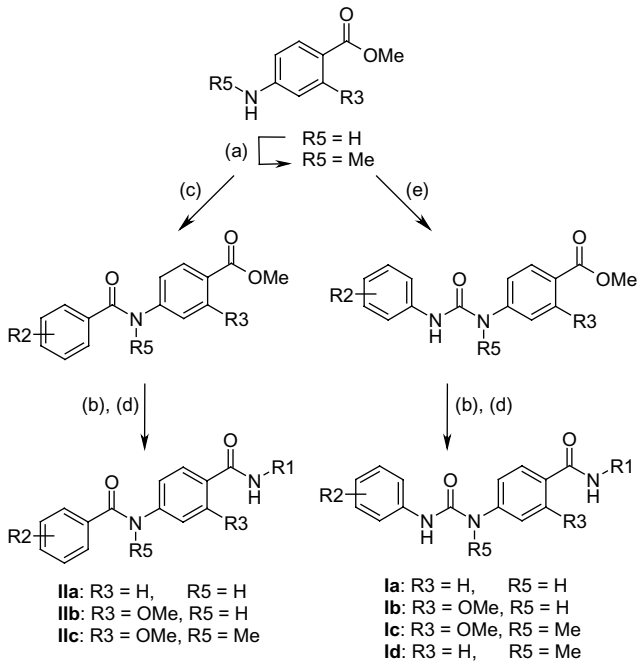
Figure 2. Benzamide dopamine D_2 ligands.

serve a dual purpose in promoting aqueous solubility by introducing potential hydrogen donor/acceptor sites available for interaction with water, while the intramolecular hydrogen-bond may promote transport over the blood–brain barrier by minimization of hydrogen bond interaction sites. Modeling of putative ligands in an MCH1 receptor homology model also corroborated the need of a hydrophobic western side.[16] Thus, the benzamides **I** and **II** (Schemes 1 and 2) appeared as potentially interesting structures in the search for novel MCH1R ligands.

The majority of the compounds were synthesized by one of the two equally efficient routes shown in Schemes 1 and 2, depending on which part of the molecule that was in focus for the exploration. Libraries with diverse



Scheme 1. Reagents and conditions: (a) (i) $(COCl)_2$, DCM, DMF (cat.), 0 °C, 1 h; (ii) amine ($R1NH_2$), rt 12 h; (b) H_2, Pd/C, EtOH, rt 12 h or H_2, Pt/C, EtOH, rt 12 h; (c) (i) paraformaldehyde, MeONa, MeOH, 40 °C, 12 h; then $NaBH_4$, rt to 50 °C 8 h; (ii) LiOH, THF/MeOH/water, rt 12 h; (d) acid chloride ($R2C_6H_4COCl$), DCM, rt,12 h; (e) isocyanate ($R2C_6H_4NCO$), DCM, 12 h; PS-trisamine, 12 h.



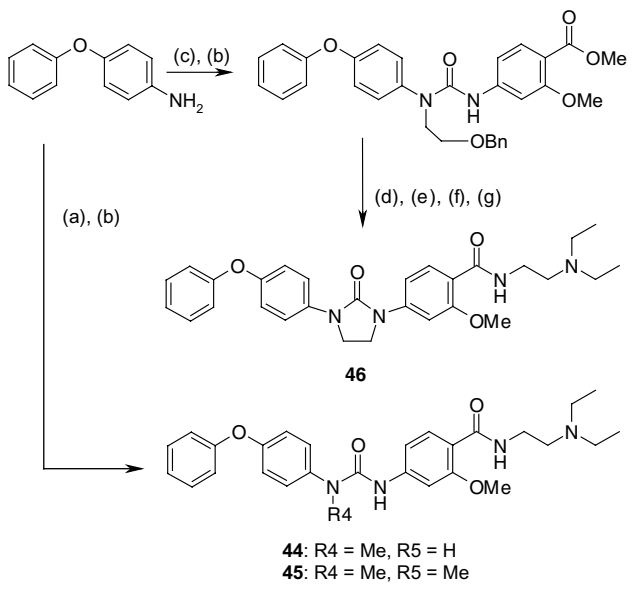
Scheme 2. Reagents and conditions: (a) paraformaldehyde, MeONa, MeOH, 40 °C, 12 h; then $NaBH_4$, rt to 50 °C 8 h; (b) LiOH, THF/MeOH/water, rt 12 h; (c) acid chloride ($R2C_6H_4COCl$), DCM, rt 12 h; (d) PS-DCC, HOBt, DCM, rt 30 min; then amine ($R1NH_2$) rt 12 h; (e) isocyanate ($R2C_6H_4NCO$), DCM, 12 h; PS-trisamine, 12 h.

western parts were constructed by coupling of 4-nitrobenzoic acids with an amine, hydrogenation of the nitro group, and reaction in a parallel fashion with either acid chlorides or isocyanates to provide amides **II** or ureas **I** (Scheme 1). Alternatively 4-aminobenzoic acids were reacted with acid chlorides or isocyanates, and the eastern amine part was introduced in the end to provide libraries with diverse eastern parts (Scheme 2).

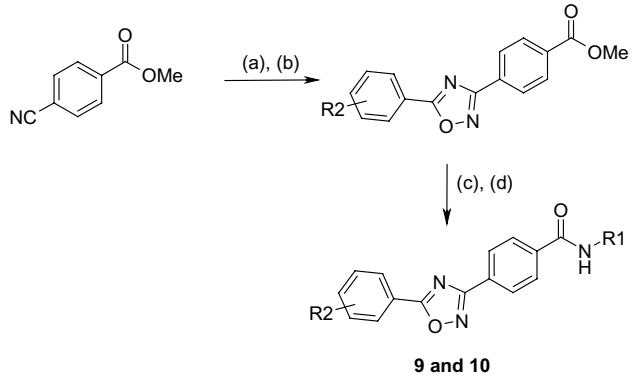
The *N*-methylated urea compounds were synthesized by similar routes incorporating reductive alkylation of the anilinic nitrogen prior to coupling with isocyanates (Schemes 1 or 2). Compounds with western *N*-methylated ureas (**44**, **45**) as well as the cyclic urea **46** were synthesized as outlined in Scheme 3.

Oxadiazoles were synthesized by the general route depicted in Scheme 4. Thus, the *N*-hydroxybenzamidine was formed by treatment of the nitrile with hydroxylamine and sodium methoxide in the presence of the methyl ester, and the oxadiazoles were obtained upon reaction with the appropriate acyl chloride and dehydration,[17] followed by hydrolysis of the methyl ester and coupling with the appropriate amine.

The initial 2-methoxybenzamides were appended with a biphenyl carboxamide western moiety analogous with the aminotetraline T-226296 and in accordance with the receptor modeling.[16] Use of the metoclopramide *N*,*N*-dimethylethylamine side chain (**a**) immediately led to activity (**1**), albeit in the micromolar range (Table 1).[18] Slightly increasing the bulk to *N*,*N*-diethyl resulted in 10-fold improved affinity (**2**), as did homologation of



Scheme 3. Reagents and conditions: (a) paraformaldehyde, MeONa, MeOH, 40 °C, 12 h; then $NaBH_4$, rt to 50 °C 8 h; (b) phosgene (20% in PhMe), DIPEA, CH_2Cl_2, 0 °C, 15 min, followed by addition of aniline **IIIb–c**; (c) benzyloxy acetaldehyde, MeOH, 0 °C, 30 min, then $NaCNBH_3$, rt, 12 h; (d) H_2, $Pd(OH)_2$/C, MeOH, 30 °C, 5 h; (e) methanesulfonyl chloride, DIPEA, 0 °C, 2 h and then rt, 12 h, followed by Et_3N, CH_3CN, 70 °C, 2 h; (f) LiOH, THF/MeOH/water, 30 °C, 48 h; (g) PS-DCC, HOBt, DCM, rt, 30 min; then amine, rt 12 h.



Scheme 4. Reagents and conditions: (a) H_2NOH, MeONa, MeOH, rt, 48 h; (b) (i) acid chloride ($R2C_6H_4COCl$), DIPEA, DCM, 0 °C to rt, 12 h; (ii) TBAF, THF, rt 48 h; (c) LiOH, THF/MeOH/water (6:3:1), rt 12 h; (d) H_2N-R1, PS-DCC, HOBt, DCM, 48 h; then PS-trisamine, rt, 2 h.

the side chain (**3**). Further elongation of the eastern part with *N*-benzylated side chains, well known from the dopamine D_2 ligands (cf. Fig. 2), was generally well tolerated and in some cases resulted in improved affinity (**4**, **5**). Accordingly, for this series, there is a considerable freedom of operation around the eastern amine side chain. However, removal of the 2-methoxy group from this class of biphenyl carboxamides (**1**–**5**) resulted in the complete loss of affinity (**6**). Furthermore, the biphenyl western part seems to be somewhat superior to the phenoxyphenyl for the amide linker (A series), since insertion of an ether oxygen in **2** and **3** resulted in a slight loss of activity (**7** and **8**, respectively).

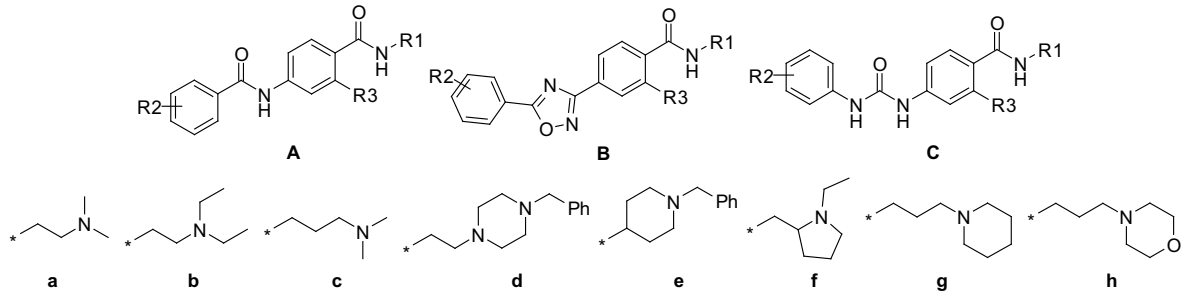
Replacement of the central amide linker with the bioisostere oxadiazole (B series) led to compounds (**9** and **10**) with activities comparable to the *para*-phenoxyphenyl derivatives **7** and **8**.

Introduction of a urea moiety (C series) in the place of the central amide linker resulted in a slight loss of affinity for the biphenyl derivatives (**11** vs **3**), but significantly increased affinity and functional (IP3) activity for the *para*-phenoxyphenyl series (**12**,**13**). The potency was retained by the removal of the methoxy group (**14**,**15**) or by elongation to ethoxy (**16**). Extension of the eastern amine side chain gained another order of magnitude in potency (**17**). Notably, the corresponding *meta*-phenoxyphenyl compound **19** is equipotent with **17** and shows improved functional response whereas the *ortho*-analogue **18** is almost devoid of affinity (Table 1). The morpholine **20** was slightly less active than the piperidine **19**. In contrast to the amide series, the enhancement in affinity of the 2-methoxy group (**5** vs **6**) is not seen for the potent trifluorinated ureas (**21** vs **22** and **23** vs **24**).

The further influence of aromatic substituents was investigated in this series (Table 2). Removal of the phenoxy group resulted in complete loss of affinity (**27**) and the introduction of a *para*-fluoro substituent (**28**) only resulted in a weak affinity for the MCH1R. Likewise, the 3,5-dimethoxy (**30**) displays a weak affinity whereas the 3,5-dichloro compound **32** gives a moderate affinity, comparable to the 3-chloro-4-fluoro (**31**) and 3-trifluoromethoxy (**34**) derivatives. Introduction of a larger halogen (Br) in the *para*-position (**29**) leads to an appreciable affinity. The favored *para*-halogen is also evidenced in the 3,4-dichloro compound **33**. The lower affinity of **36**, **37**, and **38** indicates that hydrophilic properties in the *para* position are unfavorable. The softer thiomethyl **39** and especially its trifluoro analogue **41** have affinities comparable to the trifluoromethoxy derivative **21**. The favorable features of the latter are obviously optimal for the trifluoromethyl (**23**) derivative. The positive contribution by the *para*-positioned trifluoromethoxy group can be counteracted by a sterically demanding *ortho*-substituent like bromine (**35**). The rigid *para*-phenyl moiety (**11**) provides moderate affinity whereas the more flexible *para*-phenoxy **12** is 10-fold more active, which may be explained by better fit into the hydrophobic binding pocket (Tables 1 and 2).

The high binding affinity for the trifluoromethoxy or trifluoromethyl derivatives **21**–**23** is consistent with functional inhibition in the IP3 assay (Table 1). However, the high affinity for compound **24** (IC_{50} = 5 nM) was not reflected in the IP3 assay. Introduction of *meta*-phenylamine in the western part (**26**) resulted in another 10-fold increase in affinity (IC_{50} = 8 nM), as well as appreciable antagonistic functional activity (IC_{50} = 25 nM). In comparison with the phenoxy compounds, the corresponding *para*-phenylamine derivative **25** is surprisingly less active both in binding and functional assays (Table 1). This might indicate a pivotal interaction of the anilinic hydrogen in compound **26**.

Table 1.



Compd		R1	R2	R3	hMCH1R		hMCH2R
					Binding (μM)[a]	IP3 (μM)[b]	(μM)[c]
1	A	a	4-Ph	OMe	2.41 ± 1.72 (3)	12	> 100[a]
2	A	b	4-Ph	OMe	0.37	—	—
3	A	c	4-Ph	OMe	0.64	5.29	> 100[a]
4	A	d	4-Ph	OMe	0.64	—	—
5	A	e	4-Ph	OMe	0.13	—	—
6	A	a	4-Ph	H	>100	—	—
7	A	b	4-PhO	OMe	1.97	6.81	—
8	A	c	4-PhO	OMe	1.92	—	—
9	B	b	4-PhO	H	2.45	—	—
10	B	f	4-PhO	H	1.90	—	—
11	C	c	4-Ph	OMe	1.48	—	—
12	C	b	4-PhO	OMe	0.14 ± 0.10 (2)	0.88	—
13	C	f	4-PhO	OMe	0.53	3.45	—
14	C	f	4-PhO	H	0.25 ± 0.16 (2)	1.30 ± 0.17 (2)	6.31[a]
15	C	d	4-PhO	H	0.14	5.82	2.88[a]
16	C	b	4-PhO	OEt	0.24	1.95	—
17	C	g	4-PhO	OMe	0.059 ± 0.021 (2)	0.24 ± 0.19 (2)	10[a], >100[b]
18	C	g	2-PhO	OMe	>10	—	
19	C	g	3-PhO	OMe	0.028 ± 0.002 (2)	0.032 ± 0.022 (4)	>100[a]
20	C	h	3-PhO	OMe	0.074 ± 0.008 (3)	0.112	>100[a,b]
21	C	b	4-CF_3O	OMe	0.059 ± 0.0014 (2)	0.26 ± 0.085 (2)	>100[a]
22	C	b	4-CF_3O	H	0.061 ± 0.012 (2)	0.25 ± 0.049 (2)	>100[a,b]
23	C	b	4-CF_3	OMe	0.027 ± 0.006 (2)	0.17 ± 0.060	>100[a]
24	C	b	4-CF_3	H	0.0053	0.17 ± 0.07 (2)	—
25	C	g	4-PhNH	OMe	0.85	1.44	
26	C	g	3-PhNH	OMe	0.0081 ± 0.0025 (2)	0.025 ± 0.011 (4)	10[a]

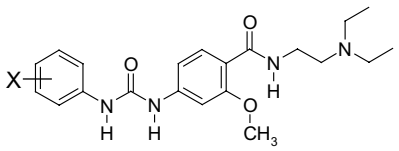
[a] Binding (IC_{50} CHO-whole cells).[18]
[b] Antagonism IP3 (IC_{50} CHO-whole cells).[18]
[c] Binding or antagonism IP3 (IC_{50} CHO-whole cells).[18]

To further investigate the binding mode of the urea-series, the hydrogen-bond donating groups were systematically modified. Hence, methylation of the easternmost nitrogen (R5) of the central urea of **12** (Table 3) was not affecting the affinity notably (**43**), whereas methylation at the westernmost nitrogen (R4) resulted in a significant drop in affinity (**44**). Di-methylation of the urea (**45**) resulted in a further drop to produce a virtually inactive compound. We reasoned that this could be due to repulsion between the methyl groups and disruption of the presumed planar *trans*,*trans*-configuration of the urea. To investigate this, the cyclic urea **46** was synthesized without achieving any enhancement in affinity. One possible explanation of these observations, that also would provide a partial rationale for the higher affinity of the urea compounds compared to amides and oxadiazoles, could be the involvement of the urea in a hydrogen-bond interaction with the receptor, preferably at the western nitrogen.

N-Methylation of the benzamide to form **48** only marginally reduced the receptor-binding compared to **47**. However, a corresponding *N*-methylation of the methoxybenzamide **12** to produce **49** resulted in a 100-fold loss of affinity. These observations are most likely explained by a disruption of the planar 2-methoxybenzamide system. Finally, introduction of a 5-chloro in the benzamide also resulted in a comparably inactive compound (**50**), indicating that a planar conformation of the urea in relation to the central benzamide is also required for activity.

In summary, by combining MCH1 receptor modeling and structural input from dopamine D_2 receptor ligands, which according to our analysis is one of the receptors with a binding site similar to MCH1R, with the known aminotetraline T-226296, we were able to identify new series of MCH1R antagonists. All compounds showed excellent selectivity over MCH2R

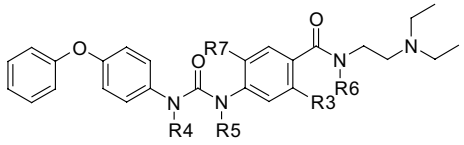
Table 2.



	X	hMCH1R binding (μM)[a]
27	H	>100
28	4-F	15.8
29	4-Br	0.25
30	3,5-$(OMe)_2$	18
31	3-Cl, 4-F	3.9
32	3,5-Cl_2	4.8
33	3,4-Cl_2	0.71
34	3-CF_3O	2.5
21	4-CF_3O	0.15
35	2-Br, 4-CF_3O	8.8
36	4-CH_3CO	13
37	4-CF_3CO	5.9
38	4-CN	3.2
39	4-SMe	0.96
40	3-SMe	4.7
41	4-CF_3S	0.20
23	4-CF_3	0.052
42	3-CF_3	7.9
11[b]	4-Ph	9.1
12	4-PhO	0.72

[a] SPA-binding (IC_{50} CHO-membranes),[18] usually giving higher IC_{50} values than whole cell assay used in Table 1.
[b] Three carbon amine side chain **c** (Table 1).

Table 3.



	R3	R4,R5	R6	R7	hMCH1R binding (μM)[a]
12	OMe	H,H	H	H	0.72
43	OMe	H,Me	H	H	0.54
44	OMe	Me,H	H	H	5.8
45	OMe	Me,Me	H	H	>100
46	OMe	CH_2CH_2	H	H	>100
47	H	H,H	H	H	3.5
48	H	H,H	Me	H	11
49	OMe	H,H	Me	H	>100
50	OMe	H,H	H	Cl	>100

[a] SPA-binding (IC_{50} CHO-membranes).[18]

(Table 1). Despite the structural input from the dopamine D_2 and D_3 ligands it was the serotonin 5-HT_{2A} and 5-HT_{2C}, histamine H_2 and the dopamine transporter that posed the largest selectivity issues for this class of compounds.[19] The activity of the compound series in general exhibited a considerable structural tolerance around the amine in the eastern part, but was generally quite sensitive to modifications in the western part. Substituents that disrupted the planarity of the system around the central benzamide were not tolerated. A urea derivative having a western *meta*-anilinic phenyl moiety (**26**) exhibited a single digit nanomolar activity that warrants further investigations.

Acknowledgements

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18. Radioligand binding assay was conducted with stably transfected CHO cells, expressing human MCH1 receptor or MCH2 receptor, by competition binding using [^{125}I]-MCH. Alternatively, binding was conducted as scintillation proximity assay (SPA) by incubating membranes from Euroscreen (ES-370-M) and PEI-treated WGA-coupled PVT SPA beads, type B from Amersham Pharmacia Biotech, with tracer in the presences of various concentrations of test compounds. Nonspecific binding was determined as the binding in the presence of 1 μM MCH. In the phosphatidylinositol assay, the cells were incubated with 5 μCi of [^{3}H]-myo-inositol in inositol-free culture medium. Phosphatidylinositol turnover was stimulated by submaximal concentrations of MCH in the presence of increasing amounts of ligand and the generated [^{3}H]-inositol phosphates were purified on Bio-Rad AG 1-X8 anion-exchange resin. Data were analyzed and IC_{50} values determined by nonlinear regression. For details of binding and functional assays see: Högberg, T.; Bjurling, A. E.; Receveur, J.-M.; Little, P. B.; Elling, C. E.; Nørregaard, P. K.; Ulven, T. *Int. Pat. Appl.* **2003**, WO 03/087045 A1.
19. Receptor profiling was conducted on representative compounds at CEREP on 75 receptors, transporters and ion channels at 10 μM. Typical results on the nearest receptors (IC_{50}-value or percentage inhibition at 10 μM): Compound **20**: adenosine A_3 48%, dopamine D_1 57%, histamine H_2 1.8 μM, muscarinic M_1 64%, opiate mu 48%, serotonin 5-HT_{2A} 0.67 μM, serotonin 5-HT_{2C} 1.3 μM, NE transporter 68%, DA transporter 1.5 μM. Compound **21**: adenosine A_3 36%, dopamine D_1 78%, dopamine D_2 79%, histamine H_2 2.2 μM, muscarinic M_1 72%, muscarinic M_2 46%, opiate mu 44%, serotonin 5-HT_{2A} 1.3 μM, serotonin 5-HT_{2C} 0.31 μM, Sigma 65%, Na+ channel (site 2) 76%, NE transporter 67%, DA transporter 6.9 μM.