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Identification of a novel series of benzimidazoles as potent and selective antagonists of the human melanocortin-4 receptor

Lydie Poitout,[a,*] Valérie Brault,[a] Carole Sackur,[a] Sonia Bernetière,[b] José Camara,[b] Pascale Plas[c] and Pierre Roubert[c]

[a]*Department of Medicinal Chemistry, Ipsen Research Laboratories, Institut Henri Beaufour, 5 Avenue du Canada, 91966 Les Ulis Cedex, France*
[b]*Department of Biopharmaceutical Profiling and Structural Chemistry, Ipsen Research Laboratories, Institut Henri Beaufour, 5 Avenue du Canada, 91966 Les Ulis Cedex, France*
[c]*Department of Endocrinology, Ipsen Research Laboratories, Institut Henri Beaufour, 5 Avenue du Canada, 91966 Les Ulis Cedex, France*

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Abstract—A novel series of benzimidazoles was identified and optimized, leading to the discovery of potent and selective antagonists of the human melanocortin-4 receptor. In addition, compound **5i** was shown to cross the blood–brain barrier after intravenous dosing in rats.

The melanocortin receptors are a family of five G protein-coupled receptor subtypes. They are activated by α-, β- and γ-melanocyte stimulating hormones (MSH) and adrenocorticotropin (ACTH), all of which are derived from a single precursor peptide, proopiomelanocortin (POMC).[1] Of these five subtypes, the melanocortin receptor subtype 4 (MC4-R), which is distributed in multiple sites in the brain, plays a key role in the regulation of food intake and energy homeostasis. Activation of MC4-R reduces food intake and body weight, whereas inhibition of MC4-R activity induces increased feeding and weight gain.[2]

Cachexia is a wasting syndrome and a major cause of morbidity and mortality that occurs in many chronic diseases such as cancer, AIDS, heart or renal failure, lung disease, liver cirrhosis or rheumatoid arthritis.[3] However, no effective treatment is currently available to reverse the loss of body weight.

Small molecule MC4 antagonists which cross the blood–brain barrier are believed to be potentially useful for the treatment of cachexia related syndromes.[4]

Several small molecule MC4-R antagonists have already been reported (Fig. 1). Compound **1** binds to the human MC4 (hMC4) receptor with high affinity (K_i = 7.9 nM).[5] The benzamidine **2a**, which exhibits a K_i of 160 nM on the hMC4 receptor, was shown to reduce tumour-induced weight loss in mice following peripheral administration.[6a] A related series with similar in vitro binding affinity, where the amidine is replaced by an imidazole such as **2b**, was also reported.[6b] Finally, a series of piperazinebenzylamines were found to be potent MC4 antagonists. For example, compound **3a** exhibited a K_i value of 3.2 nM.[7a] Further optimization led to **3b** (NBI-12i; K_i = 6.3 nM) which increased food intake in mice over a 24-h period, when orally administered.[7b,c] Recently, a related propionylpiperazine series was disclosed,[7d] **3c** for example, binds to the hMC4 receptor with a K_i value of 25 nM.

Screening of our compound collection, by competitive binding experiments[8] using the radiolabelled ligand [^{125}I]NDP-α-MSH in CHO-K1 cells, led to the identification of several leads. Among them, an amino-benzimidazole series (Fig. 2) was found to bind selectively to the hMC4 receptor with moderate affinity (**4a**, K_i = 1.0 μM; **5a**, K_i = 0.77 μM). These encouraging results prompted us to initiate a structure–activity relationship (SAR) study of the amide group, the basic

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* Corresponding author. Tel.: +33 1 6092 2000; fax: +33 1 6907 3802; e-mail: lydie.poitout@ipsen.com

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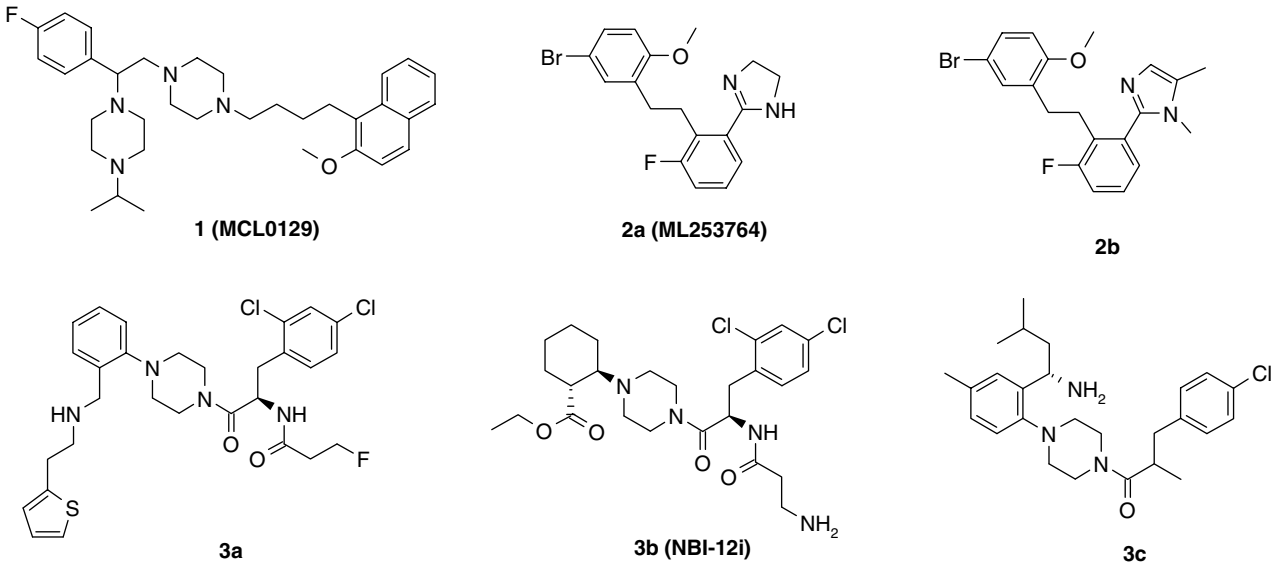


Figure 1. Small molecule antagonists of the human MC4 receptor.

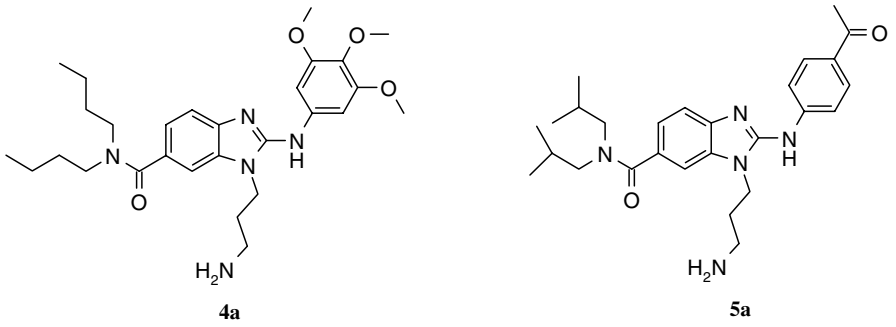


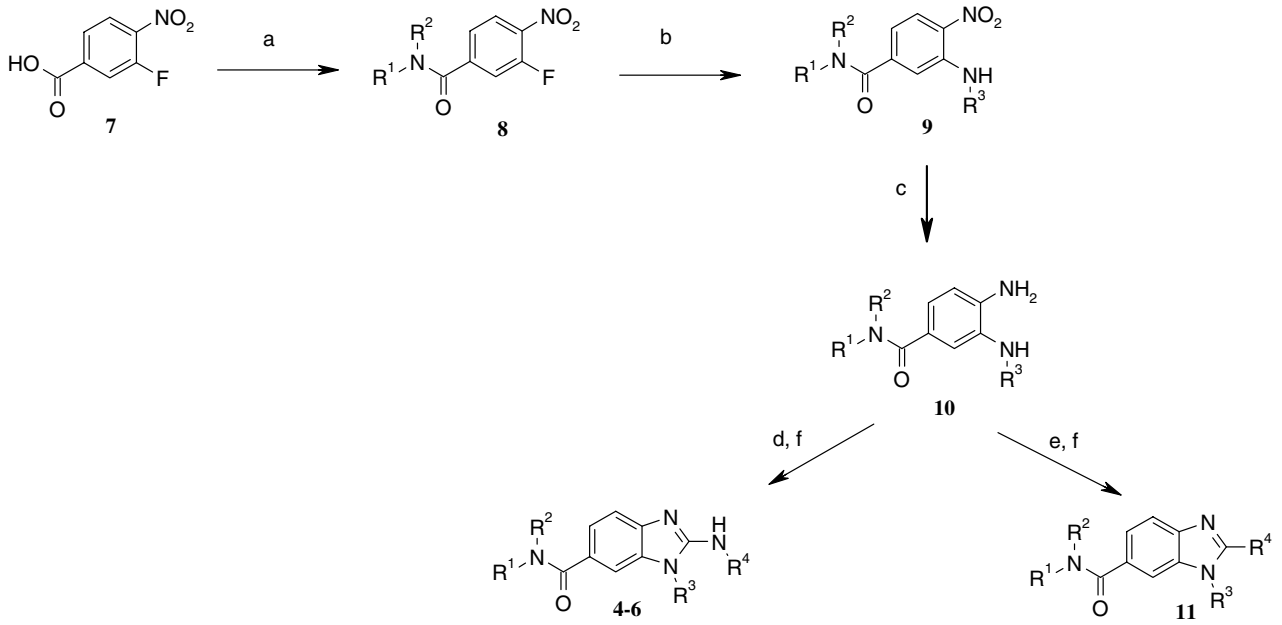
Figure 2. Lead compounds coming from screening.

chain and the right hand side group of the lead compounds.

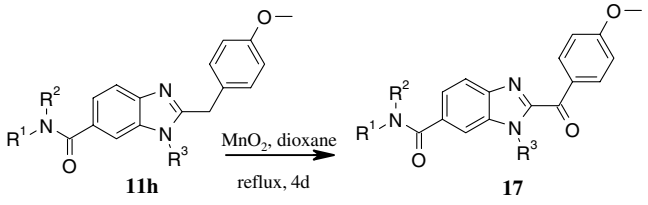
Benzimidazole derivatives were prepared according to Scheme 1. The synthesis started from 3-fluoro-4-nitrobenzoic acid **7**,[9] which was coupled with various amines under standard conditions (EDC, HOBt) to afford the corresponding amides **8**. Displacement of the fluorine atom by various primary amines led to the derivatives **9** followed by hydrogenation of the nitro group using Pd/C. Conversion of the dianilines **10** to amino-benzimidazoles **4**–**6** was performed using various isothiocyanates in the presence of cyclodesulfurization agents such as DCC[10a,b] or mercury(II) oxide.[10c] Preparation of the corresponding benzimidazole analogues **11** was carried out by condensation of dianilines **10** with aldehydes in nitrobenzene used as solvent and oxidant,[11a] or by acid-promoted cyclisation and dehydration in acetic acid[11b] of the amide intermediate, resulting from the condensation of dianilines **10** with acid chlorides or carboxylic acids using coupling reagents. When *N*-Boc protected amines were used for R^3, such as *N*-Boc propylamino group, the resulting compounds were deprotected under acidic conditions to generate the desired products. Oxidation of the benzyl group of **11h** using manganese dioxide gave the corresponding benzoyl derivative **17** (Scheme 2).

To explore the role of the amide group, an alternative synthetic route was used as depicted in Scheme 3. Thus, 3-fluoro-4-nitro-benzoic acid **7** was converted to the corresponding methyl ester **12** by treatment with a solution of trimethylsilyldiazomethane. Displacement of the fluorine atom by a primary amine followed by hydrogenation of the nitro group led to the dianilines **14**. Conversion to amino-benzimidazoles **15** was performed using the same conditions as described for Scheme 1, followed by saponification of the methyl ester to yield the corresponding carboxylic acid **16.** This, in turn, was subjected to coupling reactions with various amines to afford compounds **4**–**6**.

We first investigated the replacement of the *N*,*N*-dibutyl amide in compound **4a** (Table 1). Shortening the alkyl chains (compound **4c**) caused a drop in affinity, whereas extending it by one carbon maintained comparable activity (**4e**, $K_i = 0.64$ μM). Ramification of the lipophilic chains, for example, with diisopentyl groups led to the most active compounds in the series (**4b**, $K_i = 0.42$ μM). Introduction of this active pharmacophore in the other



Scheme 1. Reagents and conditions: (a) $R^1R^2NH_2$, EDC, HOBt, DCM, rt; (b) R^3NH_2, K_2CO_3, CH_3CN, reflux; (c) H_2, Pd/C, AcOEt/EtOH; (d) R^4NCS, •-DCC,[10b] THF, reflux; or R^4NCS, HgO, S, EtOH, reflux; (e) R^4CHO, nitrobenzene, 130 °C; or R^4COCl, AcOH, 100 °C; or R^4COOH, TBTU, DIEPA then AcOH, 100 °C; (f) for R^3 containing BocNH, TFA/DCM or HCl/dioxane.

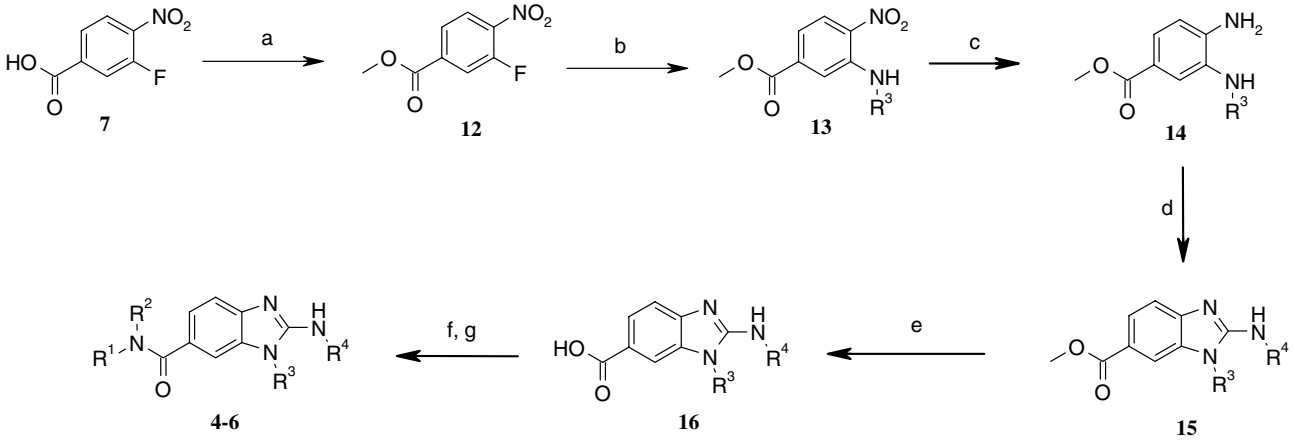


Scheme 2.

lead **5a** (Fig. 2) led to a more active compound **5b** with a K_i = 60 nM. Thus, further SAR was carried out on this compound.

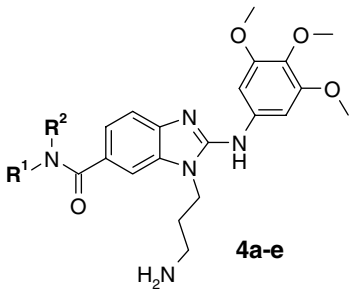
A study of the linker between the nitrogen and the benzimidazole ring (Table 2) revealed that the propylene chain provided optimal binding (**5b**), compounds with an aminoethyl or aminobutyl chain exhibiting lower binding affinity (**5c**, K_i = 220 nM; **5d**, K_i = 130 nM).

Substitution of the primary amine by a *tert*-butyloxycarbonyl group or its replacement by a hydroxy function resulted in a loss of activity, demonstrating that the basic site is crucial for binding. Incorporation of lipophilic alkyl groups on the primary amine (**5f** and **5g**) as well as introduction of heterocycloalkyl groups such as pyrrolidine or piperidine resulted in a significant increase of binding affinity, leading to potent compounds with nanomolar affinity for the hMC4 receptor (**5h**, K_i = 4.9 nM; **5i**, K_i = 2.0 nM). Morpholine and piperazine rings however gave less potent compounds (**5j** and **5k**). Introduction of conformational constraints to the

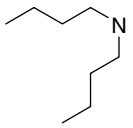
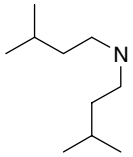
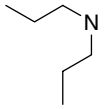
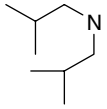
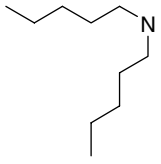


Scheme 3. Reagents and conditions: (a) $TMSCH_2N_2$, MeOH, 0 °C; (b) R^3NH_2, K_2CO_3, CH_3CN, reflux; (c) H_2, Pd/C, AcOEt/EtOH; (d) R^4NCS, •-DCC,[10b] THF, reflux; or R^4NCS, HgO, S, EtOH, reflux; (e) LiOH, THF, H_2O, reflux; (f) $R^1R^2NH_2$, EDC, HOBt, DCM, rt; (g) for R^3 containing BocNH, TFA/DCM or HCl/dioxane.

Table 1. Binding affinity of amides **4** at the human MC4 receptor[a]



4a-e

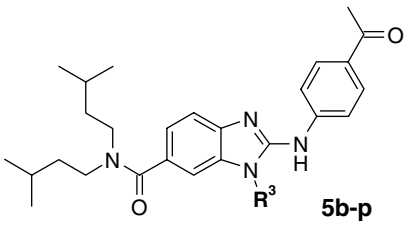
Compound	R^1R^2N	pK_i ± SEM[b]	K_i (μM)
4a		5.98 ± 0.23	1.0
4b		6.38 ± 0.12	0.42
4c		4.75 ± 0.14	17
4d		4.93 ± 0.094	11
4e		6.20 ± 0.033	0.64

[a] Binding affinity at the human melanocortin-4 receptor stably expressed in CHO-K1 cells, using [^{125}I]NDP-α-MSH as the radiolabelled ligand.
[b] pK_i are arithmetic means of three-independent measurements.

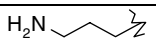
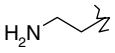
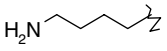
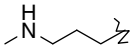
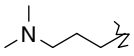
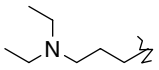
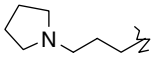
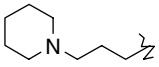
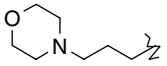
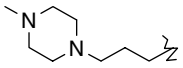
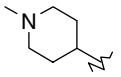
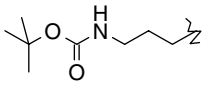
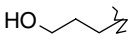
propyl linker, such as *gem*-dimethyl substituents, displayed lower potency (**5l**, K_i = 730 nM) and replacement of the acyclic chain by a piperidine ring decreased the activity (**5m**, K_i = 50 nM). The *N*-piperidine propyl chain gave the compound with the best binding activity in this series (**5i**, K_i = 2.0 nM).

A comprehensive survey of the substitution of the amino-benzimidazole **6** on the right hand side with different groups: substituted phenyls, aryls, heteroaryls, substituted alkyl chains (Table 3) showed that all compounds possess high binding affinity. However some slight differences can be noted. Investigation of the phenyl substituent revealed a preference for 4- and 3-substitution compared to 2-substitution as exemplified by compounds **5i** and **6a** versus **6b**. Similar binding activity was obtained either with electron donating substituents such as methoxy (**6d**, K_i = 5.0 nM) or electron-withdrawing groups such as carbonyl, ester or nitro. Thus, **5i**, **6i** and **6j** had K_i values of 2.0, 0.90 and 2.2 nM, respectively, on the hMC4 receptor. Halogenated phenyls gave compounds with slightly reduced potency (**6k**–**6m**). Increasing the size of the phenyl substituents such as phenyl, phenoxy, phenylketone, piperidine or

Table 2. SAR of the basic chain at the human MC4 receptor[a]

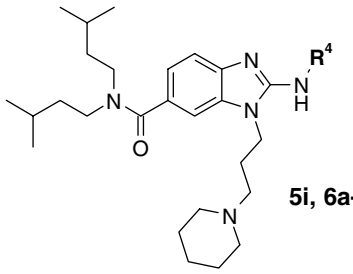


5b-p

Compound	R^3	pK_i ± SEM[b]	K_i (nM)
5b		7.22 ± 0.075	60
5c		6.66 ± 0.11	220
5d		6.89 ± 0.061	130
5e		7.29 ± 0.032	51
5f		7.93 ± 0.53	12
5g		8.10 ± 0.071	7.9
5h		8.31 ± 0.038	4.9
5i		8.71 ± 0.081	2.0
5j		7.49 ± 0.12	33
5k		7.34 ± 0.19	45
5l		6.14 ± 0.12	730
5m		7.30 ± 0.013	50
5o		4.07 ± 0.027	>10,000
5p		5.64 ± 0.18	2300

[a] Binding affinity at the human melanocortin-4 receptor stably expressed in CHO-K1 cells, using [^{125}I]NDP-α-MSH as the radiolabelled ligand.
[b] pK_i are arithmetic means of three-independent measurements.

Table 3. SAR of amino-benzimidazole **6** at the human MC4 receptor[a]



5i, 6a-z

Compound	R^4	pK_i ± SEM[b]	K_i (nM)
5i	4-Me(O)C–Ph–	8.71 ± 0.081	2.0
6a	3-Me(O)C–Ph–	8.35 ± 0.024	4.5
6b	2-Me(O)C–Ph–	6.96 ± 0.077	110
6c	Ph–	8.14 ± 0.11	**7.3**
6d	4-MeO–Ph–	8.30 ± 0.015	5.0
6e	4-Et(O)C–Ph–	8.57 ± 0.16	2.7
6f	4-MeNH(O)C–Ph–	8.48 ± 0.08	3.3
6g	4-H_2NC(O)–Ph–	8.19 ± 0.12	6.5
6h	4-MeC(O)NH–Ph–	8.43 ± 0.046	3.7
6i	4-MeOC(O)–Ph–	9.05 ± 0.089	0.90
6j	4-NO_2–Ph–	8.67 ± 0.061	2.2
6k	4-F–Ph–	8.08 ± 0.054	8.3
6l	4-Cl–Ph–	8.06 ± 0.066	8.8
6m	4-Br–Ph–	8.12 ± 0.10	7.5
6n	4-Ph–Ph–	8.28 ± 0.050	5.2
6o	4-PhO–Ph–	8.69 ± 0.12	2.1
6p	4-PhC(O)–Ph–	8.76 ± 0.066	1.7
6q	4-Piperidine–Ph–	8.01 ± 0.037	9.7
6r	4-(1H-Imidazolyl)–Ph–	8.40 ± 0.046	4.0
6s	3-Pyridyl–	8.38 ± 0.080	4.2
6t	4-Indolyl–	7.85 ± 0.11	14
6u	3-Thienyl	7.48 ± 0.030	33
6v	Ph–CH_2–	7.76 ± 0.11	18
6w	4-MeC(O)–Ph–CH_2–	8.24 ± 0.14	5.8
6x	Me(O)CNH–$(CH_2)_2$–	7.51 ± 0.13	31
6y	MeO–$(CH_2)_2$–	7.11 ± 0.13	78
6z	Pyrrolidinone–$(CH_2)_3$–	7.77 ± 0.13	17

[a] Binding affinity at the human melanocortin-4 receptor stably expressed in CHO-K1 cells, using [^{125}I]NDP-α-MSH as the radiolabelled ligand.
[b] pK_i are arithmetic means of three-independent measurements.

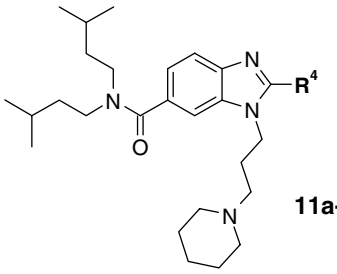
imidazolyl (**6n**–**6r**) gave rise to compounds with comparable activity to **6c** where the phenyl ring is unsubstituted.

Replacement of the phenyl ring by heteroaromatic rings such as indolyl (**6t**) or thienyl (**6u**) slightly lowered binding affinity, whereas pyridine exhibited a similar effect to the phenyl moiety in binding (**6c**, K_i = 7.3 nM; **6s**, K_i = 4.2 nM). Benzyl groups turned out to be slightly less potent than phenyl analogues (**6v**, K_i = 18 nM; **6w**, K_i = 5.8 nM vs **6c** and **5i**, respectively).

Changing the aryl substituent to alkyl groups bearing various functional groups such as amide, methoxy or pyrrolidinone (**6x**–**6z**) led to less potent compounds (K_i = 31, 78 and 17 nM, respectively).

Benzimidazole analogues **11a**–**11c** (Table 4) displayed more than a 10-fold reduction in binding, compared to

Table 4. SAR of benzimidazoles **11** and **17** at the human MC4 receptor[a]



11a-h, 17

Compound	R^4	pK_i ± SEM[b]	K_i (nM)
11a	4-MeC(O)Ph–	7.70 ± 0.035	20
11b	4- MeO–Ph–	7.43 ± 0.13	37
11c	4-MeOC(O)–Ph–	7.35 ± 0.059	45
11d	3-Indolyl–	8.15 ± 0.083	7.0
11e	3-*N*-methyl-indolyl	8.49 ± 0.047	3.2
11f	2-Benzothienyl	8.53 ± 0.02	2.9
11g	4-MeC(O)Ph–CH_2–	8.41 ± 0.012	3.9
11h	4-MeO–Ph–CH_2–	7.55 ± 0.054	28
17	4-MeO–Ph–C(O)–	9.02 ± 0.015	0.96

[a] Binding affinity at the human melanocortin-4 receptor stably expressed in CHO-K1 cells, using [^{125}I]NDP-α-MSH as the radiolabelled ligand.
[b] pK_i are arithmetic means of three-independent measurements.

the amino derivatives **5i**, **6h** and **6i**. However, replacement of the NH group by a methylene group gave compounds with better binding affinity (**11g**, K_i = 3.9 nM) and replacement by a carbonyl group led to the most potent compound in this series (**17**, K_i = 0.96 nM).

Interestingly, all these results summarized in Tables 3 and 4 showed that this region of the molecule tolerated groups with different properties. Thus, it offers an opportunity for optimization of this series of compounds towards desirable physicochemical properties.

Selected compounds were tested for their selectivity over the other melanocortin receptor subtypes.[8] Compounds **5i**, **6f**, **6z**, **11g** and **17** exhibited a 40- to 1000-fold selectivity of MC4-R over the MC3 receptor subtype and in addition all compounds show weak binding activity for the other MC receptors (Table 5).

Functional antagonist and agonist assays were performed on the same set of compounds, using CHO-K1 cells stably transfected with the human MC4-R. While derivatives **5i**, **6f**, **6z**, **11g** and **17** were unable to stimulate cyclic AMP release at 10 μM, they inhibit NDP-α-MSH-stimulated cAMP production in a dose-dependent manner,[12] demonstrating their antagonist properties (Table 5). All the compounds tested increased the EC_{50} of NDP-α-MSH in a dose-dependent manner without affecting the maximal inhibition observed, indicating the competitive nature of the antagonism. Schild regression analysis of the data led to the determination of a K_B value (concentration of compound that shifts NDP-α-MSH dose–response twofold). Compound **6f** exhibited enhanced functional potency (K_B = 3.2 nM, Fig. 3) as compared to the other analogues (Table 5), however it

Table 5. Binding affinity (K_i, nM) at the melanocortin receptor subtypes[a,b] and functional antagonist potency (K_B, nM)[c] of selected compounds at the human MC4-R

Compound	K_i[b] (nM)				K_B[c] (nM)
	hMC1-R	hMC3-R	hMC4-R	hMC5-R	hMC4-R
5i	5200	400	2.0	2200	77
6f	9300	130	3.3	880	3.2
6z	>10,000	3800	17	>10,000	11
11g	>10,000	4500	3.9	5900	14
17	7500	540	0.96	1600	44

[a] Human melanocortin-1,-3,-4 and -5 receptors stably expressed in CHO-K1 cells and [^{125}I]NDP-α-MSH as radiolabelled ligand in the competitive binding assay.
[b] K_i are logarithmic means of three-independent measurements.
[c] K_B were determined using Schild regression analysis and are logarithmic means of three-independent measurements.

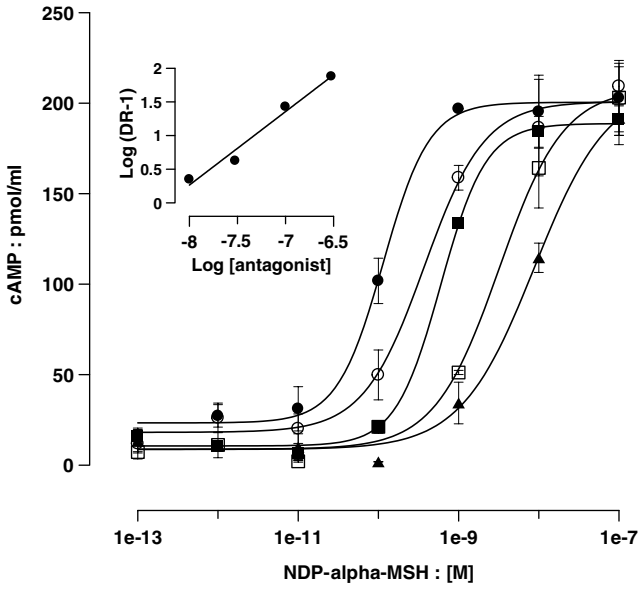


Figure 3. Effect of compound **6f** on NDP-α-MSH-stimulated cAMP production. cAMP levels were measured in presence of 10 nM (○), 30 nM (■), 100 nM (□), 300 nM (▲) or without (●) compound **6f**. The inset shows the Schild regression analysis of the data.

possessed lower selectivity towards the MC3 receptor subtype.

Among the most active compounds, a representative member of the amino-benzimidazole series, **5i**, was selected to be further evaluated for its ability to cross the blood–brain barrier, an important parameter to assess, since the MC4-R is centrally localized. Plasma and brain levels were determined following intravenous administration at 4.5 μmol/kg to rats.[13] Compound **5i** displayed a half-life of 5.0 h and was shown to penetrate into the brain yielding to an $AUC_{(brain/blood)}$ (area under the curve) ratio of 2.9.

In conclusion, a novel class of small molecule MC4-R antagonists has been identified and optimized for potency. Selected compounds display good to high selectivity over other melanocortin receptors and were shown to be potent antagonists at the MC4 receptor. Furthermore, one analogue **5i** showed a good brain penetration. Further optimization studies of compounds in this series as well as in vivo evaluation of analogues in food intake experiments will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.06.010.

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8. MC Receptor binding assay: membranes were prepared from CHO-K1 cells stably expressing the human melanocortin receptor subtypes MC1, MC3, MC4 and MC5: they were incubated at 1–10 μg protein/well in 50 mM Tris–HCl, pH 7.4, containing 0.2% BSA, 5 mM $MgCl_2$, 1 mM $CaCl_2$ and 0.1 mg/mL bacitracin, with increasing concentrations of the tested compound and 0.1–0.3 nM [^{125}I]NDP-α-MSH for 90–120 min at 37 °C, depending on the receptor subtype. Bound from free [^{125}I]NDP-α-MSH was separated by filtration through GF/C glass fibre filters presoaked with 0.1% (w/v) PEI. Filters were washed three times with 50 mM Tris–HCl, pH 7.4, at 0–4 °C and assayed for radioactivity using Perkin-Elmer Topcount counter. Binding data were analysed by computer-assisted non-linear regression analysis (XL fit; IDBS).
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12. Cyclic AMP assay: Intracellular cyclic AMP (cAMP) levels were determined by an electro-chemiluminescence (ECL) assay (Meso Scale Discovery, MSD). CHO-K1 cells stably expressing the human MC4 receptors were suspended in RMPI 1640 containing 0.5 mM IBMX and 0.2% protein cocktail (MSD). They were dispensed (7000 cells/well) in multi-array plates containing integrated carbon electrodes and coated with anti-cAMP antibody. Concentration–response experiments of NDP-α-MSH were carried out in the presence of increasing concentrations of the tested compound by incubating the cells for 40 min at 37 °C. Then, the cells were lysed and 2.5 nM TAG ruthenium-labelled cAMP was added. After 90 min, cAMP levels were determined by ECL detection using sector imager 6000 reader (MSD). cAMP data were analysed by computer-assisted non-linear regression analysis (XL fit; IDBS). The Kb values were determined by Schild regression analysis.
13. Fed male Sprague–Dawley rats were dosed in groups of three animals (for each kinetic point). A solution of 4.5 μmol/kg in 1 ml/kg of a 10% DMA/90% propanediol solution was given by intravenous administration via the penile vein. Then, rats were deeply anaesthetised under Isoflurane® and blood was taken from the vena cava (at 1, 15, 30, 60, 90 and 120 min) and centrifuged (2000*g*, 15 min, 4 °C). The supernatants were mixed with cold acetonitrile to precipitate the proteins. After a further centrifugation (2000*g*, 15 min, 4 °C), the samples were analysed by HPLC (UV detection) or frozen at − 80° pending analysis. After blood withdrawal, tissues were rapidly washed by intracardiac perfusion with 50 ml of saline. The brains were then removed, frozen in liquid nitrogen and weighed. After mixing the brains with methanol for 40 s with an Ultraturax®, brain homogenates were centrifuged (25,000*g*, 15 min, 4 °C) and the supernatants were analysed by HPLC.