Discovery of 1-{2-[(1*S*)-(3-Dimethylamino-propionyl)amino-2-methylpropyl]-4-methyl-phenyl}-4-[(2*R*)-methyl-3-(4-chlorophenyl)-propionyl]piperazine as an Orally Active Antagonist of the Melanocortin-4 Receptor for the Potential Treatment of Cachexia

Chen Chen,* Wanlong Jiang, Fabio Tucci,† Joe A. Tran, Beth A. Fleck, Sam R. Hoare, Margaret Joppa, Stacy Markison, Jenny Wen, Yang Sai, Michael Johns, Ajay Madan, Takung Chen, Caroline W. Chen, Dragan Marinkovic, Melissa Arellano, John Saunders,‡ and Alan C. Foster

Department of Medicinal Chemistry, Department of Pharmacology, Department of Neuroscience and Department of Preclinical Development, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, California 92130

Received July 5, 2007

Abstract: A potent and selective antagonist of the melanocortin-4 receptor, 1-{2-[(1*S*)-(3-dimethylaminopropionyl)amino-2-methylpropyl]-6-methylphenyl}-4-[(2*R*)-methyl-3-(4-chlorophenyl)propionyl]piperazine (**10d**), was identified from a series piperazinebenzylamine attached with a *N*,*N*-dimethyl-β-alanine side chain. This compound possessed high water solubility and exhibited good metabolic profiles. In animals, **10d** showed moderate to good oral bioavailability and promoted food intake in tumor-bearing mice after oral administration.

Cancer cachexia significantly impairs quality of life and response to antineoplastic therapies and increases morbidity and mortality of cancer patients. Unfortunately, currently available drugs do not provide very effective treatment.[1,2]

The melanocortin-4 receptor (MC4R), a member of the class A G-protein-coupled receptor superfamily, plays a very important role in feeding behavior and energy homeostasis in animals and humans.[3] Human MC4R mutations are associated with binge eating and juvenile-onset obesity.[4,5] MC4R agonists have been demonstrated to suppress food intake and reduce body weight in animals and could potentially be used for the treatment of obesity.[6] In contrast, MC4R antagonists have been shown to promote food intake and increase weight gain.[7] Recent studies have also demonstrated that cachexia brought about by a variety of illnesses can be attenuated or reversed by blocking activation of the melanocortin-4 receptor within the central nervous system.[8] Centrally-administered peptide MC4R antagonists attenuate tumor-induced cachexia in animal models.[9−12]

Recently, small molecule MC4R antagonists have been identified that are suitable for peripheral administration.[12] A 2-phenylimidazoline **1** (ML00253764, Figure 1) is reported to be a functional MC4R antagonist (IC_{50} = 103 nM) and to improve cachexia symptoms in C57BL6 mice bearing Lewis lung carcinoma tumors.[13,14] However, this compound also has relatively weak affinity for MC4R and poor selectivity versus the MC3R, a subtype that is also believed to have a role in feeding regulation.[15] We have shown that a β-alanine-(2,4-Cl)-phenylalanine dipeptide derivative **2** is a potent functional MC4R antagonist with negligible affinity at MC3R.[16] Intraperitoneal (i.p.) administration of **2** effectively stimulates daytime (satiated) food intake as well as decreases basal metabolic rate in normal animals. Furthermore, this compound attenuates cachexia and preserves lean body mass in a murine cancer cachexia model.[17]

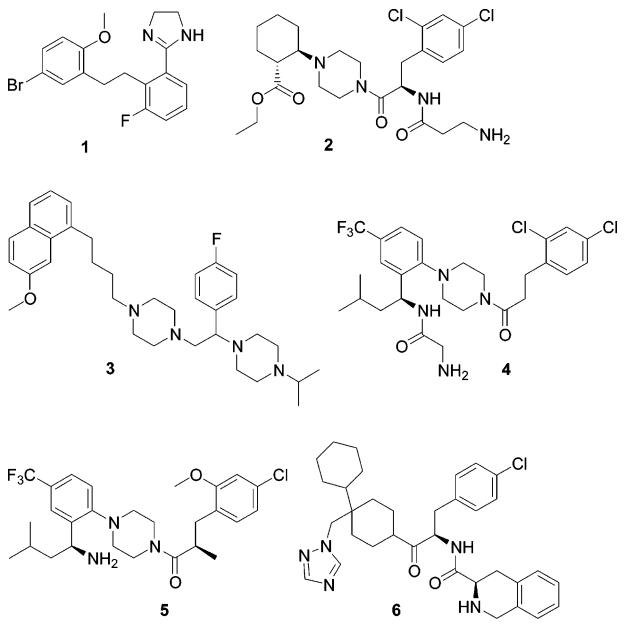


Figure 1. Chemical structures of MC4R antagonists **1**−**5** and agonist **6** (THIQ).

In addition, a series of bispiperazine derivatives exemplified by **3** have been reported as potent MC4R antagonists with in vivo effects in rodents.[18−20] These data provide evidence for the potential clinical utility of small molecule MC4R antagonists, particularly in the treatment of cachexia.[21]

We have previously reported the discovery of a series of piperazinebenzylamines such as **4** (K_i = 19 nM) and **5** (K_i = 6.5 nM) as MC4R antagonists.[22,23] Based on these leads, we started a research effort to improve their properties in several categories including potency and pharmacokinetics. Here we report the identification and characterization of 1-{2-[(1*S*)-(3-dimethylaminopropionyl)amino-2-methylpropyl]-6-methylphenyl}-4-[(2*R*)-methyl-3-(4-chlorophenyl)propionyl]piperazine (**10d**) as an orally active MC4R antagonist for the potential treatment of cancer cachexia.

The target compounds were synthesized from the protected piperazinebenzylamine **6**[24] as shown in Scheme 1. After a treatment of **6** with TFA, the resultant amine was coupled with (2*R*)-methyl-3-(2-Y-4-chlorophenyl)propionic acids to give the amides **7**, which was deprotected with HCl in methanol to afford the benzylamines **8**. Reductive alkylation of **8a** with methyl-(2-oxoethyl)carbamic acid *tert*-butyl ester provided the diamine **9** after Boc-deprotection. Alternatively, coupling reactions of **8** with *N*,*N*-dimethylamino-β-alanine gave the amides **10b**−**d** or, with *N*-Boc-amino acid, afforded **10a** and **10e**−**f** after TFA treatment.

Compounds **8**−**10** were tested for their binding affinity at the human melanocortin-4 receptor using a binding assay as previously described,[25] and results are summarized in Table 1. For the primary benzylamines **8**, the 2-methyl derivative **8b** had

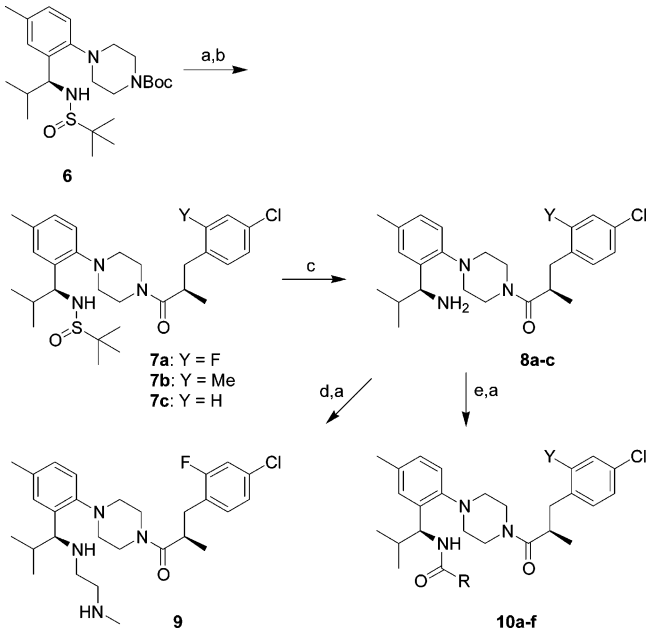
* To whom correspondence should be addressed. Phone: 858-617-7634. Fax: 858-617-7602. E-mail: cchen@neurocrine.com.

† Current address: Department of Medicinal Chemistry, Tanabe Research Laboratories, U.S.A., Inc., 4540 Towne Centre Ct., San Diego, CA 92121.

‡ Current address: Vertex Pharmaceuticals Inc., 11010 Torreyana Road, San Diego, CA 92121.

10.1021/jm070806a CCC: $37.00
Published on Web 10/05/2007

Scheme 1[a]



[a] Reagents and conditions: (a) TFA/CH_2Cl_2/rt, 1 h; (b) (*R*)-2-Y-4-$ClC_6H_3CH_2CH(Me)COOH$/EDC/HOBt/(*i*-Pr)$_2$NEt/CH_2Cl_2/rt, 8 h, 37% for **7c**; (c) HCl/MeOH/rt, 1 h, 50−60%; (d) *N*-Boc-NMeCH_2CHO/NaB-H(OAc)$_3$/CH_2Cl_2/rt, 2 h, 21%; (e) RCOOH/EDC/HOBt/CH_2Cl_2/rt, 8 h, 68% for **10a** and 78% for **10d**.

Table 1. SAR of MC4R Antagonists at the Human Melanocortin-4 Receptor[a]

cmpd	R	Y	K_i[b] (nM)	cAMP (%)	IC_{50}[d] (nM)
8a		F	35[e]	5	n.d.[f]
8b		Me	9.7	2	n.d.[f]
8c		H	69[e]	16	2400
9		F	6.0	7	660
10a	CH_2NHMe	F	2.8	12	410
10b	$CH_2CH_2NMe_2$	F	2.2	0	190
10c	$CH_2CH_2NMe_2$	Me	0.6	5	92
10d	$CH_2CH_2NMe_2$	H	2.8	7	35
10e	CH_2CH_2NHMe	H	5.7	8	87
10f	$CH_2CH_2NH_2$	H	5.9	5	280

[a] Data are average of 2−9 independent measurements. The affinity measurements for each compound differed by less than 4-fold, and the resulting coefficient of variance averaged 37% for the binding assay K_i values and 46% for the functional assay IC_{50} values. [b] Binding affinity using [^{125}I]-NDP−MSH as the radiolabeled ligand. [c] Stimulation of cAMP release at 10 μM concentration in comparison to α-MSH (100%). [d] Dose-dependent inhibition of α-MSH-stimulated cAMP release. [e] Single measurement. [f] Not determined.

improved affinity over **8c**, which was similar to the 2-fluoro analog **8a** in the binding assay. The X-ray crystal structure of **7c** was obtained (data not shown). In the solid state, this precursor of the antagonist **8c** possessed a very similar conformation to that of the MC4R agonist **6** (THIQ).[26] Thus, the 4-chlorophenyl ring of **7c** was almost parallel to the piperazine ring, suggesting that the framework of an antagonist structure is not much different from that of an agonist. This result indicates that the Tic-group of **6**, which is missing from the antagonist **8c**, is the primary contributor to its ability to activate the receptor.[27]

Incorporating a methylaminoethyl side chain to the benzylamine **8a** (K_i = 35 nM) increased its affinity by about 6-fold (**9**, K_i = 6.0 nM), while the amide derivative displayed further improvement (**10a**, K_i = 2.8 nM), suggesting the additional amino group in **9** and **10a** was able to function as the benzylamine of **8a** and further increase the affinity. The *N*,*N*-dimethylamino-β-alanine derivative **10b** (K_i = 2.2 nM) had 16-fold better affinity than its parent **8a**. Similar results were also obtained for **8b**/**10c** and **8c**/**10d** modifications. The *N*,*N*-dimethylamino-β-alanine derivatives **10b**−**d** were optimal, because the *N*-monomethyl compound **10e** and the primary amine **10f** showed no improvement in affinity compared with **10d**. These compounds were distinguished by their functional activity reflected by their IC_{50} values in the inhibition of α-MSH-stimulated cAMP release (Table 1). Thus, **10d** (IC_{50} = 35 nM) was 8-fold better than the primary amine **10f** (IC_{50} = 280 nM), while the functional inhibition produced by the secondary amine **10e** (IC_{50} = 87 nM) was between that of **10d** and **10f**. In parallel to binding affinity, **10d** was 70-fold better than its parent **8c** (IC_{50} = 2400 nM) in functional antagonist activity. The *N*,*N*-dimethyl-β-alanine derivative **10b** was also slightly better than the methylaminoethyl **9** and the sarcosine **10a** in terms of functional inhibition. None of these compounds significantly stimulated cAMP production at 10 μM concentration ($E_{max} \leq$ 16%).

Table 2. Selectivity Profiles (K_i, nM) of MC4R Antagonists at Human Melanocortin Receptors[a]

cmpd	MC1R[b]	MC3R	MC4R	MC5R
10a	n.d.[c]	230[d]	2.8	180[d]
10d	(50%)	420	2.8	340
10e	(43%)	710	5.7	360
10f	(42%)	270	5.9	320

[a] Data are average of 2−3 independent measurements of binding affinity using [^{125}I]-NDP−MSH as the radiolabeled ligand. The affinity measurements for each compound differed by 2-fold at most, and the resulting coefficient of variance averaged 20%. [b] For results where a K_i value could not be determined (<50% inhibition at highest compound concentration tested), percentage inhibition at 10 μM concentration in parenthesis. [c] Not determined. [d] Single measurement.

Table 3. In Vitro Metabolism Data of **8c**−**10f**

cmpd	CYP3A4[a] IC_{50} (μM)	HLM Cl_{sys}[b] (mL/min·kg)	P_{app}[c,e] ($\times 10^{-6}$ cm/s)	ratio[d,e] (*b* to *a*/*a* to *b*)
8c	8.3	13.4	5.0	3.7
9	9.0	n.d.[f]	0.3	26
10a	6.9	12.4	0.8	7.3
10b	6.4	16.0	3.7	3.0
10c	6.8	16.6	1.9	2.7
10d	9.2	12.2	6.2	1.9
10e	6.0	4.8	1.2	7.2
10f	10	9.2	0.3	26

[a] Binding affinity at the cytochrome P450 3A4 enzyme. [b] Scaled systemic clearance in human liver microsomes. [c] Permeability in Caco-2 cell monolayer. [d] Ratio of P_{app} of basolateral to apical direction versus that of apical to basolateral direction. [e] Digoxin was used as a control in this assay, and its P_{app} was about 1.0, with a ratio of 10−30. [f] Not determined.

Compounds **10a** and **10d**−**f** were also tested for their selectivity at the other melanocortin receptor subtypes (Table 2). In general, these compounds had little interaction with the MC1 receptor and displayed much lower affinity at the MC3R and MC5R than at MC4R. For example, **10d** had over 100-fold selectivity at MC4R over MC3R and MC5R.

These potent antagonists were also tested in several in vitro assays measuring metabolism-related parameters, and the results are summarized in Table 3. A common liability for large and basic molecules is their inhibitory activity at the CYP3A4 enzyme.[28] In an in vitro binding assay, all of these compounds displayed IC_{50} values above 5 μM, and **10d** had an IC_{50} of 9.2 μM.[29]

When incubated with human liver microsomes in an in vitro assay, all compounds exhibited moderate metabolic stability, which might be associated with their high lipophilicity and high molecular weight. In a Caco-2 assay, which measures the cell permeability of a compound, the benzylamine **8c** showed good

Table 4. Pharmacokinetic Profiles of **9** and **10b**−**e** in Rats[a]

cmpd	CL (mL/min·kg)	V_d (L/kg)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	oral AUC (ng/mL·h)	*F*%	C_b (ng/g) at 1, 4 h	*b*/*p* ratio at 1, 4 h
9	60[b]	95	17	6	22	299	13	[c]	[c]
10b	22	4.2	2.2	4.7	134	1469	13	8, 31	0.2, 0.3
10c	3.7	4.3	13	4	208	1947	5.6	9, 42	0.1, 0.3
10d[b]	53	13	2.9	4.7	110	1398	43	20, 33	0.2, 0.4
10e	39	20	8.1	3.3	57	563	15	[c]	[c]

[a] Three animals were dosed intravenously at 2.5 mg/kg, and orally (p.o.) at 10 mg/kg; brain concentrations were taken from p.o. dose. [b] Intravenous dose at 5 mg/kg. [c] Below detection limit (5 ng/mL).

permeability ($P_{app} = 5.0 \times 10^{-6}$ cm/s) and showed evidence of efflux transport (basolateral to apical direction versus its reverse direction (*b* to *a*/*a* to *b* = 3.7)), suggesting that this compound may be a substrate for the P-glycoprotein. In comparison, incorporating a methylaminoethyl (**9**) or sarcosine (**10a**) group into **8c** resulted in lower permeability (Table 3). The *N*,*N*-dimethylamino-β-alanine side chain (**10b**), however, did not significantly affect the permeability, and among the three analogs **10b**−**d**, **10d** displayed the best profile in this assay. The monomethylamine **10e** and the primary amine **10f** also had lower permeability than **10d**. The better permeability of **10d** might be associated with its high logD values (measured using a shake-flask method, 3.4) and its lower polar surface area (calculated using ACD software, 56 Å^2) compared to the secondary and primary amines **10a** and **10e**,**f**.

Compounds **9** and **10b**−**e** were studied in rats for their pharmacokinetic properties, and the results are summarized in Table 4. Whole brain concentrations were sampled at 1 and 4 h after oral administration. The diamine **9** had a very large volume of distribution (V_d), which might be associated with its dibasic structure,[30] resulting in a very long half-life ($t_{1/2}$) of 17 h. Oral bioavailability (*F*%) was moderate in this species. Despite its large V_d value, the compound was undetectable in the brain, presumably due to its P-glycoprotein activity, which prevented penetration through the blood−brain barrier.[31] The *N*,*N*-dimethyl-β-alanine **10b** penetrated into the brain with a brain/plasma (b/p) ratio of 0.2 and 0.3 at 1 and 4 h time points, respectively, after oral administration.

Among the three *N*,*N*-dimethyl-β-alanines **10b**−**d**, the 4-chlorophenyl analog **10d** had the best oral bioavailability (43%) in rats. It also had a high V_d value and a moderate $t_{1/2}$, resulting from a high plasma clearance (CL). In vitro incubation with liver microsomes suggested a major metabolite of **10d** from *N*-demethylation. This metabolite **10e** was also profiled in rats. Thus, **10e** had a lower plasma clearance and a higher V_d value than its parent **10d**, which resulted in a longer half-life of 8.1 h. However, this compound was unable to penetrate into the brain, which excluded it from further studies.

In a separate study in rats, the brain concentration−time relation of **10d** was studied after an oral administration at 10 mg/kg. The maximal whole brain concentration of **10d** was 41 ng/g, appearing at the 6 h time point, and the AUC was 254 ng/g·h. The brain to plasma ratio was 0.17 based on AUC values, suggesting low CNS penetration (Table 5). In mice, the brain/plasma ratio of **10d** based on AUC values was 0.15, which was very similar to that in rats.

Because of its favored pharmacological and pharmacokinetic properties, the effect of **10d** was then studied in a model of tumor-induced cachexia. Compound **10d** displayed good binding affinity at the mouse MC4R (K_i = 3.2 nM), similar to what was seen at the human receptor. In this study, male C57BL/6J mice, individually housed for at least one week, were subcutaneously injected into the upper flank with a subcloned line of Lewis lung carcinoma (LLC) cells as previously described.[17]

Table 5. Pharmacokinetic Properties of **10d** in Animals[a]

species	CD-1 mouse	Sprague−Dawley rat	beagle dog	rhesus monkey
i.v. dose (mg/kg)	2.5		5	5
CL (mL/min·kg)	29.6		10.3	8.1
V_d (L/kg)	4.1		5.9	2.0
$t_{1/2}$ (h)	1.6		6.7	2.9
AUC (ng/mL·h)	1410		8200	10 300
p.o. dose (mg/kg)	10	10	10	10
C_{max} (ng/mL)	946	279	262	761
T_{max} (h)	0.5	2.7	1.7	1.8
AUC (ng/mL·h)	2530	1520	2860	3860
F%	32	n/a	19	19
brain C_{max} (ng/g)	91	41	[b]	[b]
brain T_{max} (h)	0.3	6.0		
brain AUC (ng/g·h)	367	254		
brain/plasma ratio	0.15	0.17		

[a] Data are the average of three animals. [b] Brain concentration was not determined.

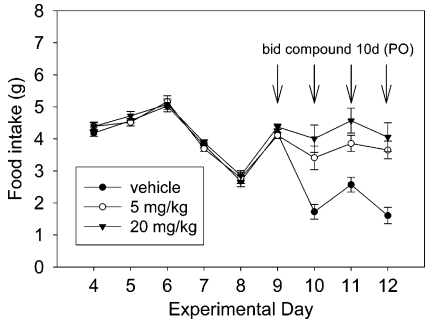


Figure 2. Effect of **10d** (5, 20 mg/kg, p.o.) on food intake in LLC tumor bearing mice. Daily food intake was significantly increased on days 10, 11, and 12 by treatment with 5 or 20 mg/kg of **10d** compared to tumor-bearing vehicle-treated controls. Values are mean ± SEM.

Four days after inoculation, food intake was measured daily. On day 9 after tumor inoculation, treatment with compound **10d** was begun. Mice were divided into three groups (balanced for body weight and tumor size) and given vehicle (sterile water), 5, or 20 mg/kg of **10d** twice a day over 4 days. Both the 5 and 20 mg/kg doses of **10d** significantly increased food intake on days 10, 11, and 12 (Figure 2, $p < 0.05$).

In addition to its low CYP3A4 inhibitory activity, compound **10d** also exhibited low affinity toward other major liver enzymes for drug metabolism: CYP1A2, 2D6, 2C9, and 2C19 (IC_{50} > 10 μM). Its pK_a was measured to be 7.8. As a hydrochloride salt, **10d** had very good aqueous solubility (33 mg/mL at pH 7, and >71 mg/mL at pH 3). This compound was also profiled in liver microsomes from several animal species. The scaled intrinsic clearances of **10d** were determined to be 46, 24, 15, and 39 mL/min·kg, respectively, for CD-1 mice, S-D rats, beagle dogs, and rhesus monkeys, predicting oral bioavailabilities of 49, 66, 40, and 13%, respectively, assuming complete absorption.

Compound **10d** was also studied in mice, dogs, and monkeys for its PK properties in these species (Table 5). In mice, **10d** exhibited a moderate plasma clearance of 29.6 mL/min·kg and its bioavailability was 32%. Its half-life of 1.6 h was short and its brain penetration was relatively low. The low brain penetration or short $t_{1/2}$ of **10d** might explain the requirement of a high dose for its in vivo efficacy in this species. In dogs and monkeys, **10d** displayed a moderate oral bioavailability of 19%. The half-life of this compound was 6.7 h in dogs, much longer than that in monkeys ($t_{1/2}$ = 2.9 h), which might be associated with its lower volume of distribution in this species.

In conclusion, we conducted a detailed study on a set of piperazinebenzylamines bearing an amine side chain as potent and selective antagonists of the human melanocortin-4 receptor. One analog, 1-{2-[(1*S*)-(3-dimethylaminopropionyl)amino-2-methylpropyl]-4-methylphenyl}-4-[(2*R*)-methyl-3-(4-chlorophenyl)propionyl]piperazine (**10d**) was identified to have good potency and selectivity. Despite its relatively high logD value (3.4), **10d** had good aqueous solubility. It was profiled for its metabolic and pharmacokinetic properties in several animal species. Finally, this compound demonstrated oral efficacy in a mouse tumor-induced cachexia model.

Acknowledgment. The authors would like to thank Dr. Daniel Marks of Oregon Health & Science University for his help in performing the mouse cachexia study.

Supporting Information Available: The synthesis and characterization of compounds **7c**, **8a**−**c**, **9**, and **10a**−**f**, the protocols for pharmacokinetics, and in vivo efficacy studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Gordon, J. N.; Green, S. R.; Goggin, P. M. Cancer cachexia. *Q. J. Med.* **2005**, *98*, 779−788.
(2) Bossola, M.; Pacelli, F.; Tortorelli, A.; Doglietto, G. B. Cancer cachexia: It's time for more clinical trials. *Ann. Surg. Oncol.* **2007**, *14*, 276−285.
(3) Cone, R. D. The central melanocortin system and energy homeostasis. *Trends Endocrinol. Metab.* **1999**, *10*, 211−216.
(4) Tao, Y. X.; Segaloff, D. L. Functional analyses of melanocortin-4 receptor mutations identified from patients with binge eating disorder and nonobese or obese subjects. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 5632−5638.
(5) MacKenzie, R. G. Obesity-associated mutations in the human melanocortin-4 receptor gene. *Peptides* **2006**, *27*, 395−403.
(6) Boyce, R. S.; Duhl, D. M. Melanocortin-4 receptor agonists for the treatment of obesity. *Curr. Opin. Investig. Drugs* **2004**, *5*, 1063−1071.
(7) Foster, A. C.; Joppa, M.; Markison, S.; Gogas, K. R.; Fleck, B. A.; et al. Body weight regulation by selective MC4 receptor agonists and antagonists. *Ann. N. Y. Acad. Sci.* **2003**, *994*, 103−110.
(8) DeBoer, M. D.; Marks, D. L. Therapy insight: Use of melanocortin antagonists in the treatment of cachexia in chronic disease. *Nat. Clin. Pract. Endocrinol. Metab.* **2006**, *2*, 459−466.
(9) Wisse, B. E.; Frayo, R. S.; Schwartz, M. W.; Cummings, D. E. Reversal of cancer anorexia by blockade of central melanocortin receptors in rats. *Endocrinology* **2001**, *142*, 3292−3301.
(10) Marks, D. L.; Butler, A. A.; Turner, R.; Brookhart, G.; Cone, R. D. Differential role of melanocortin receptor subtypes in cachexia. *Endocrinology* **2003**, *144*, 1513−1523.
(11) Joppa, M. A.; Gogas, K. R.; Foster, A. C.; Markison, S. Central infusion of the melanocortin receptor antagonist agouti-related peptide (AgRP(83−132)) prevents cachexia-related symptoms induced by radiation and colon-26 tumors in mice. *Peptides* **2007**, *28*, 636−642.
(12) Foster, A. C.; Chen, C. Melanocortin-4 receptor antagonists as potential therapeutics in the treatment of cachexia. *Curr. Top. Med. Chem.* 2007, *7*, 1131−1136.
(13) Vos, T. J.; Caracoti, A.; Che, J. L.; Dai, M.; Farrer, C. A.; et al. Identification of 2-[2-[2-(5-bromo-2- methoxyphenyl)-ethyl]-3-fluorophenyl]-4,5-dihydro-1*H*-imidazole (ML00253764), a small molecule melanocortin 4 receptor antagonist that effectively reduces tumor-induced weight loss in a mouse model. *J. Med. Chem.* **2004**, *47*, 1602−1604.
(14) Nicholson, J. R.; Kohler, G.; Schaerer, F.; Senn, C.; Weyermann, P. et al. Peripheral administration of a melanocortin 4-receptor inverse agonist prevents loss of lean body mass in tumor-bearing mice. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 771−777.
(15) Marks, D. L.; Cone, R. D. The role of the melanocortin-3 receptor in cachexia. *Ann. N. Y. Acad. Sci.* **2003**, *994*, 258−266.
(16) Tucci, F. C.; White, N. S.; Markison, S.; Joppa, M.; Tran, J. A.; et al. Potent and orally active non-peptide antagonists of the human melanocortin-4 receptor based on a series of trans-2-disubstituted cyclohexylpiperazines. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4389−4395.
(17) Markison, S.; Foster, A. C.; Chen, C.; Brookhart, G. B.; Hesse, A.; et al. The regulation of feeding and metabolic rate and the prevention of murine cancer cachexia with a small-molecule melanocortin-4 receptor antagonist. *Endocrinology* **2005**, *146*, 2766−2773.
(18) Nozawa, D.; Okubo, T.; Ishii, T.; Kakinuma, H.; Chaki, S.; et al. Structure−activity relationships of novel piperazines as antagonists for the melanocortin-4 receptor. *Bioorg. Med. Chem.* **2007**, *15*, 1989−2005.
(19) Nozawa, D.; Okubo, T.; Ishii, T.; Takamori, K.; Chaki, S.; et al. Novel piperazines: Potent melanocortin-4 receptor antagonists with anxiolytic-like activity. *Bioorg. Med. Chem.* **2007**, *15*, 2375−2385.
(20) Shimazaki, T.; Chaki, S. Anxiolytic-like effect of a selective and non-peptidergic melanocortin 4 receptor antagonist, MCL0129, in a social interaction test. *Pharmacol. Biochem. Behav.* **2005**, *80*, 395−400.
(21) Foster, A. C.; Chen, C.; Markison, S.; Marks, D. L. MC4 receptor antagonists: A potential treatment for cachexia. *IDrugs* **2005**, *8*, 314−319.
(22) Jiang, W.; Tucci, F. C.; Chen, C. W.; Arellano, M.; Tran, J. A.; et al. Arylpropionylpiperazines as antagonists of the human melanocortin-4 receptor. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4674−4678.
(23) Chen, C. W.; Tran, J. A.; Jiang, W.; Tucci, F. C.; Arellano, M.; et al. Propionylpiperazines as human melanocortin-4 receptor ligands. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4800−4803.
(24) Jiang, W.; Chen, C.; Marinkovic, D.; Tran, J. A.; Chen, C. W.; et al. Practical asymmetric synthesis of alpha-branched 2-piperazinylbenzylamines by 1,2-additions of organometallic reagents to *N*-*tert*-butanesulfinyl imines. *J. Org. Chem.* **2005**, *70*, 8924−8931.
(25) Nickolls, S. A.; Cismowski, M. I.; Wang, X.; Wolff, M.; Conlon, P. J.; et al. Molecular determinants of melanocortin 4 receptor ligand binding and MC4/MC3 receptor selectivity. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 1217−1227.
(26) Sebhat, I. K.; Martin, W. J.; Ye, Z.; Barakat, K.; Mosley, R. T.; et al. Design and pharmacology of *N*-[(3R)-1,2,3,4-tetrahydroisoquinolinium- 3-ylcarbonyl]-(1*R*)-1-(4-chlorobenzyl)- 2-[4-cyclohexyl-4-(1*H*-1,2,4-triazol- 1-ylmethyl)piperidin-1-yl]-2-oxoethylamine (**1**), a potent, selective, melanocortin subtype-4 receptor agonist. *J. Med. Chem.* **2002**, *45*, 4589−4593.
(27) Pontillo, J.; Tran, J. A.; Fleck, B. A.; Marinkovic, D.; Arellano, M.; et al. Piperazinebenzylamines as potent and selective antagonists of the human melanocortin-4 receptor. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5605−5609.
(28) Tang, W.; Stearns, R. A. Heterotropic cooperativity of cytochrome P450 3A4 and potential drug−drug interactions. *Curr. Drug Metab.* **2001**, *2*, 185−198.
(29) Numata, A.; Kondo, Y.; Sakamoto, T. General synthetic method for naphthyridines and their N-oxides containing isoquinolinic nitrogen. *Synthesis* **1999**, 306−311.
(30) Bjorkman, S. Prediction of the volume of distribution of a drug: Which tissue-plasma partition coefficients are needed? *J. Pharm. Pharmacol.* **2002**, *54*, 1237−1245.
(31) Fromm, M. F. Importance of P-glycoprotein at blood-tissue barriers. *Trends Pharmacol. Sci.* **2004**, *25*, 423−429.

JM070806A