ACS Medicinal Chemistry Letters

Letter

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Discovery of a Novel Class of Bicyclo[3.1.0]hexanylpiperazines as Noncompetitive Neuropeptide Y Y1 Antagonists

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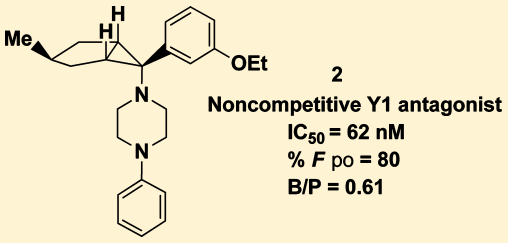
Supporting Information

ABSTRACT: A novel class of bicyclo[3.1.0]hexanylpiperazine neuropeptide Y (NPY) Y1 antagonists has been designed and synthesized. Scatchard binding analysis showed these compounds to be noncompetitive with [^{125}I]PYY binding to the Y1 receptor. The most potent member, 1-((1α,3α,5α,6β)-6-(3-ethoxyphenyl)-3-methylbicyclo[3.1.0]hexan-6-yl)-4-phenylpiperazine (**2**) had an IC_{50} = 62 nM and displayed excellent oral bioavailability in rat (% *F* po = 80), as well as good brain penetration (B/P ratio = 0.61). In a spontaneous nocturnal feeding study with male Sprague–Dawley rats, **2** significantly reduced food intake during a 12 h period.



KEYWORDS: *bicyclo[3.1.0]hexanylpiperazines, noncompetitive neuropeptide Y Y1 antagonists, brain penetration*

With obesity posing an ever-increasing threat to public health, the need to develop effective medicines for its prevention and treatment has become urgent. Becuase of the complex mechanisms involved in the regulation of food intake and energy expenditure, a number of neurological targets have been pursued for therapeutic intervention. Of interest to us was neuropeptide Y (NPY).[1−5] This molecule is highly expressed in the brain and has been shown to be a potent orexigenic agent.[6−9] Additionally, NPY and its mRNA are found at substantially higher levels in the brains of genetically obese rodents relative to their lean littermates, and concentrations of both increase in normal rats after fasting.[10−12] In other studies, NPY-deficient ob/ob mice showed less interest in food consumption and were less obese than WT ob/ob mice.[12] Additionally, chronic central nervous system administration of NPY in rodents results in hyperphagia and robust weight gain.[13]

Five NPY receptors (Y1−Y5) have been cloned and expressed. Of these, Y1 and Y5 are generally believed to be involved in both food intake and energy expenditure, although recent literature suggests the involvement of the Y2 and Y4 subtypes as well.[14] The pharmacology of the Y1 receptor has been studied using potent and selective Y1 receptor antagonists (Figure 1). The effect of NPY administration in Y5 knockout mice was completely blocked by coinjection of the peptidic Y1 antagonist, 1229U91.[15,16] Other peptidic antagonists[17] have been reported, as well as a number of small molecule Y1 antagonists such as BIBP3226,[18] SR-120819A,[19] LY357897,[20] BMS-193885,[21,22] and a novel carbazole series (structure not shown).[23] However, common characteristics of many of these agents are poor oral bioavailability and low brain penetration. Contrastingly, J-104870[24,25] showed the first reduced spontaneous food intake in Zucker fatty rats after oral administration (100 mg/kg). J-104870 is a Y1 antagonist that binds to a distinct but overlapping site to that of 1229U91.[26] Subsequently, Peterson et al. disclosed a novel class of Y1 antagonists (**1**) of smaller molecular weight, and the dihydrochloride salt of one compound from this series was found to have good oral bioavailability (% *F* po = 75).[27,28]

In the research presented here, we report our work on novel bicyclo[3.1.0]hexanylpiperazine NPY Y1 antagonists (Scheme 1). A preliminary structure−activity relationship (SAR) investigation is discussed, and the pharmacokinetic (PK) profile and in vivo effects of an early lead compound are reviewed.

In considering the *N*′-aryl-*N*-benzylpiperazine scaffold present in **1**, we note that it is comparatively flexible; the cyclohexane ring is able to pseudorotate so that either the phenylpiperazine or the aryl group could occupy an axial position. We anticipated that this flexibility might contribute to the reduced potency observed with this compound class. Correspondingly, we expected that compounds with more rigid scaffolds would fix the substituents in either the axial or the equatorial position and may offer advantages in terms of potency and physiochemical properties. We concluded that this could be achieved by employing a bicyclo[3.1.0]hexane scaffold and investigated this hypothesis as described below.

The stereoselective syntheses of *endo* bicyclo[3.1.0]-hexanylpiperidines **5** was achieved using the methodology shown in Scheme 1. This chemistry was employed as it allowed the efficient introduction of substituents at the 6-postion of the bicyclo[3.1.0] hexanylpiperazine and facilitated the rapid development of an SAR at this sector of the chemotype.

Received: November 8, 2011
Accepted: January 4, 2012
Published: January 4, 2012

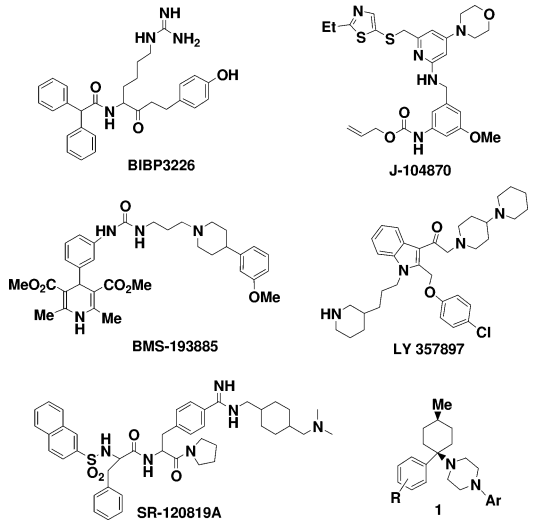
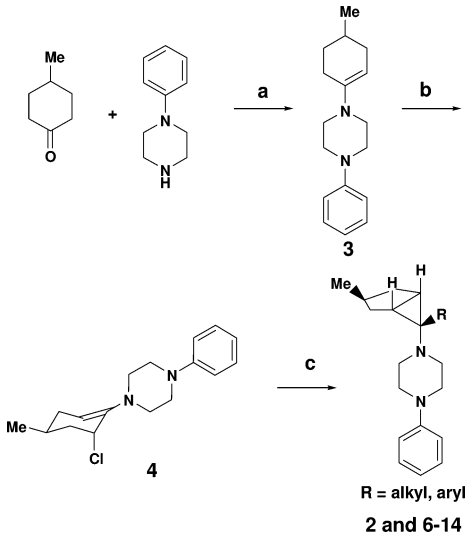


Figure 1. Reported NPY Y1 receptor antagonists.

Scheme 1. Synthesis of *endo* Bicyclo[3.1.0]hexanylpiperazines[a]



[a]Reagents and conditions: (a) Cat. TsOH, toluene, reflux. (b) NCS (1 equiv), CH_2Cl_2, −65 °C to room temperature. (c) Ether, 1.0 equiv of RMgBr, room temperature; quench with aqueous HCl (1 N, 1 equiv).

The synthesis involves condensation of 4-methylcyclohexanone with 1-phenylpiperazine in the presence of a catalytic amount of *p*-tolunesulfonic acid to form an intermediate, 1-(4-methylcyclohex-1-enyl)-4-phenylpiperazine (**3**). The latter can then be chlorinated stereoselectively using *N*-chlorosuccinimide to afford *trans*-1-(6-chloro-4-methylcyclohex-1-enyl)-4-phenylpiperazine (**4**). NOEs and the *J*-coupling constants observed in the NMR spectra of **4** were consistent with the proposed structural assignment with the chloro substituent *anti* to the pseudoequatorial methyl group. Vilsmaier[29] has shown that such intermediates can readily form fused cyclopropyl ring systems on treatment with nucleophiles, although his investigations were limited to cyanide and hydride. Here, we extended this reaction to aryl and alkyl Grignard nucleophiles, which add to the chloroenamine intermediate **4** in a stereoselective fashion to provide the *endo* bicyclo[3.1.0]hexanylpiperidines **5** in good yield. The stereochemistry of the products was again assigned based on extensive NMR investigations; subsequent work provided crystalline material from which we were able to confirm the assigned stereochemistry; see Figure 2. In the addition to the chloroenamine

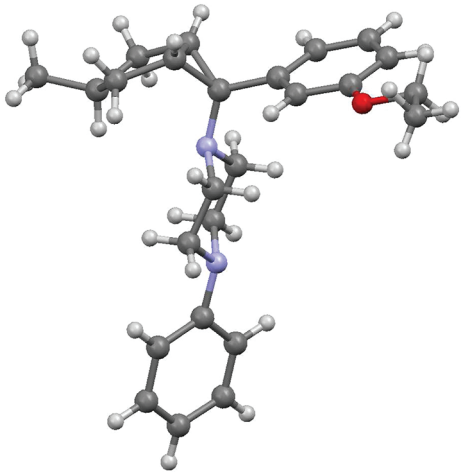


Figure 2. Crystal structure of 1-((1α,3α,5α,6β)-6-(3-ethoxyphenyl)-3-methylbicyclo [3.1.0]hexan-6-yl)-4-phenylpiperazine (**2**). Note the *cis*-disposition of methyl and aryl moieties.[30]

intermediate **4**, the Grignard reagents may initiate the cyclization by attacking the α-imine-carbon from *anti*-Cl direction and then simultaneously knocking off the chlorine on the C-6 to form the *endo*-bicyclic products.

Subsequently, with this chemistry optimized, we conducted the synthesis of a 45-membered library using a variety of different Grignard reagents. Compounds from the library were screened by measuring their ability to inhibit binding of [^{125}I]PYY (polypeptide YY) to the Y1 receptor in human neuroblastoma (SK-N-MC) cell membranes, and the IC_{50} values generated in this assay for select examples are provided in Table 1.

Table 1. Inhibition of [^{125}I]PYY Binding to NPY Y1 Receptor in Human Neuroblastoma (SK-N-MC) Cell Membranes

compd	R	IC_{50} (μM)[a]
2	3-ethoxyphenyl	0.062 (n = 10)
6	phenyl	2.2
7	2-methoxyphenyl	1.3
8	3-methoxyphenyl	0.30 (n = 4)
9	3-aminophenyl	0.19
10	3-chlorophenyl	>10
11	*p*-tolyl	0.46
12	4-hydroxyphenyl	0.17
13	4-methoxylphenyl	0.55
14	4-fluorophenyl	0.18
1a	3-ethoxy	0.12 (n = 3)

[a]IC_{50} values of the library members were obtained from a single experiment with each point being run in duplicate. BIBP 3226 displayed K_i of 15 nM in this Y_1 binding assay.

Early in our SAR investigations, we noted that all compounds with nonaromatic functionality depicted in Scheme 1 were devoid of Y1 binding affinity (IC_{50} > 10 μM), suggesting the necessity of an aromatic moiety at this position. However, the simple phenyl derivative **6** displayed moderate activity, IC_{50} = 2.2 μM. *ortho*-Substituents on the phenyl group as exemplified

dx.doi.org/10.1021/ml200265m | *ACS Med. Chem. Lett.* 2012, 3, 222−226

by **7** seemed to have little impact on binding affinity. Contrastingly, *meta* and *para* substituents as shown in **8**, **9**, and **11**−**14** significantly enhanced affinity except in **10** with a *meta*-chlorine (IC_{50} > 10 μM). Electron-donating functionality appeared to further advance the potency, and of the compounds examined, the ethoxy derivative, 1-((1α,3α,5α,6β)-6-(3-ethoxyphenyl)-3-methylbicyclo[3.1.0]hexan-6-yl)-4-phenylpiperazine (**2**) was found to be the most active with an IC_{50} of 62 nM. In the same [^{125}I]PYY binding assay, the corresponding member (**1a**) of chemical series **1** wherein R is an ethoxy had an IC_{50} of 120 nM. This result provided evidence that the increased rigidity indeed had a positive impact on potency. However, because the *cis* isomers in chemical series **1** (Me vs arylpiperidines) were reported to be more potent than the *trans* isomers,[27] it needs to be investigated in the future whether the *exo* bicyclo[3.1.0]-hexanylpiperazines, wherein the methyl is *cis* to arylpiperidines, would be more potent than the *endo* isomers, which are stereoselectively synthesized in this work. Compound **2** was further screened against other NPY receptors and did not exhibit affinity for the Y2 or Y5 receptor subtypes. With this result in hand, a more extensive profiling of compound **2** was undertaken.

Binding dynamics of **2** with the Y1 receptor were studied in equilibrium binding experiments (Figure 3). Scatchard analysis

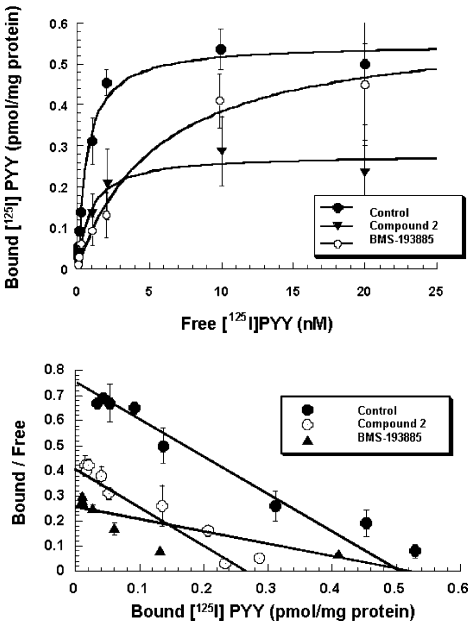


Figure 3. Equilibrium binding experiments and Scatchard analysis of compound **2** (50 nM) and BMS-193885 (5 nM). Data were analyzed by LIGAND program.

found that its inhibitory effect on [^{125}I]PYY binding to Y1 receptor in SK-N-MC cell membranes was noncompetitive. At the concentration of 50 nM, it reduced PYY binding capacity (B_{max} of 1.1 to 0.3 nmol/mg protein) but had little effect on PYY binding affinity (K_d = 1.1 ± 0.3 vs 1.2 ± 0.2 nM); meanwhile, a known competitive antagonist, BMS-193885,[21,22] at a concentration of 5 nM, did not affect PYY B_{max} but did reduce its binding affinity (K_d = 3.1 ± 0.9 nM).

To prove that **2** is not binding irreversibly to the receptor, we have preincubated SK-N-MC cell membrane expressing the Y1 receptor with the compound, removed it with subsequent washes, and performed competitive binding on the compound-treated and buffer-treated membrane. Receptor binding was identical (data not shown), indicating that **2** is a reversible ligand.

In a functional assay with CHO cells stably expressing the human neuropeptide Y1 receptor, **2** antagonized NPY-mediated inhibition of forskolin-stimulated cAMP accumulation; see Figure 4.

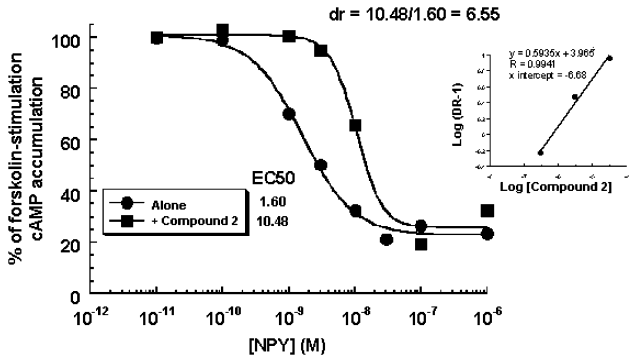


Figure 4. Effect of NPY and **2** on forskolin-stimulated (10 μM) cyclic AMP accumulation in CHO cells stably expressing the human NPY Y1 receptor.

Schild analysis yielded a K_b of 209 nM, which is larger than its affinity value as a result of the noncompetitive nature of **2**.

Subsequently, PK studies of **2** were carried out in rats (Figure 5). After intra-arterial infusion (4 mg/kg), brain uptake appeared

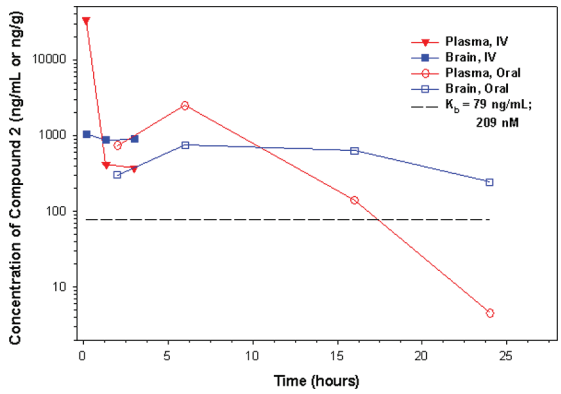


Figure 5. Concentration of compound **2** in brain and plasma after iv (4 mg/kg) and oral (10 mg/kg) administration to jugular vein-cannulated rats.

to be rapid, and the concentration in the brain remained relatively constant over 3 h. Following oral administration (10 mg/kg), a mean concentration of 2.0 μM (745 ng/mL) in brain was achieved at 6 h before declining to 0.64 μM (242 ng/mL) by 24 h. After oral administration, a brain to plasma ratio of 0.61 was estimated from the brain and plasma AUC0 → 24 h, and these results show that **2** had comparable or better brain penetration than J-104870.[24,25] Because the clearance of **2** from brain is lower than that from plasma, longer dosing intervals to maintain efficacy might be expected. In a separate PK study (graphics not shown), **2** exhibited an excellent PK profile with an oral bioavailability of 80%, half-life of 8.9 h, low clearance (10.1 mL min/kg), and a Vdss of 1.8 L/kg, which showed the drug was well distributed extravascularly.

Compound **2** was then evaluated for potential hepatotoxicity using immortalized human hepatocyte cell lines expressing the most four relevant human cytochrome isozymes: CYP1A2,

dx.doi.org/10.1021/ml200265m | ACS Med. Chem. Lett. 2012, 3, 222−226

CYP3A4, CYP2C9, and CYP2D6;[31] CYP3A4 is largely responsible for drug metabolism, and polymorphisms of the other three isozymes may contribute to hepatotoxicity. Toxicity in these cell lines was compared with their parent cell line, THLE-5B-c15, which does not express phase I-metabolizing activity. End points measured following 48 h of exposure to test compounds included ATP content, an indicator of cell viability. In these tests (Table 2), **2** was relatively nontoxic in the parent

Table 2. Effect of Compound 2 on Human Hepatocytes

	IC_{50} (μg/mL)[a]	
cell lines	ATP	n. red
THLE-5B-c15	30.3	61.2
THLE-5B-1A2	31.4	112.4
THLE-5B-3A4	267.6	347.9
THLE-5B-2C9	25.9	55.7
THLE-5B-2D6	63.7	290.9

[a]Cell lines were exposed to five test concentrations of 0, 5, 50, 100, 250, and 500 μg/mL. IC_{50} values were the average of four experiments.

and all recombinant cell lines, suggesting a low probability of hepatic necrosis of **2** or its metabolites. It is estimated that hepatic necrosis accounts for nearly 50% of all drug-related hepatic damage, more frequent than immune-mediated hepatic and cholestatic injury.[32]

Compound **2** was also evaluated for its mutagenicity in an Ames test with *Salmonella typhimurium* tester strains TA98 and TA100 in the absence and presence of induced rat liver S-9 metabolic activation. Compound **2** was not mutagenic in either tester strain when tested at concentrations up to 5000 μg/plate.[33]

Compound **2** was last tested in vivo in a spontaneous nocturnal feeding model with male Sprague–Dawley rats at oral dosing of 10 and 30 mg/kg. BMS-193885 was previously shown to reduce spontaneous nocturnal food intake measured 12–15 h after intraperitoneal administration (10 mg/kg) in rats. It also blocked centrally administrated NPY-induced food intake in rats and significantly reduced food intake and body weight in rats during chronic dosing of BMS-193885 (ip 10 mg/kg) for 44 days.[22] However, because BMS-193885 is not orally bioavailable, fenfluramine (1 and 3 mg/kg either orally or ip) was used instead, in these experiments, as a positive control; **2** was administrated 2–2.5 h before the onset of darkness, and the amount of food consumed during a 12 h period was recorded. As shown in Figure 6, the effect on food intake reached statistical significance for both doses tested during the 12 h period. The vehicle effect was observed up to the first 6 h, but the compound effect was significantly separated from the vehicle effect beginning 9 h after the administration.

In summary, we have identified a novel series of bioavailable NPY Y1 antagonists (e.g., 80% with **2**). Despite being a noncompetitive Y1 antagonist, **2** showed significant oral efficacy in reducing food intake in a spontaneous nocturnal feeding model, thus contributing to the evidence that Y1 antagonists can potentially inhibit food intake. Lastly, our results indicate that bicyclo[3.1.0]hexane may be used, in general, as a conformationally constrained isosteric replacement of the cyclohexane ring in drug design and discovery.

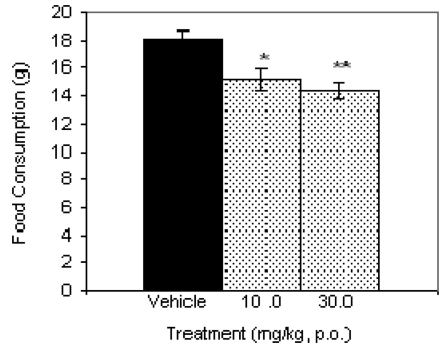


Figure 6. Oral administration of **2** at 10 and 30 mg/kg dose to male Sprague–Dawley rates (220–280 g) 2–2.5 h before the onset of darkness reduced 12 h spontaneous nocturnal food intake in rats. Results are presented as means ± SEMs and were analyzed using ANOVA (F = 6.8, p = 0.004) followed by Bonferroni/Dunn posthoc test (n = 9–11, *p = 0.01, and **p = 0.001 significantly different from vehicle). The vehicle used was 95% capmul and 5% water.

■ ASSOCIATED CONTENT

Supporting Information

Procedure for large scale synthesis of **2** and its full characterization; preparation of NPY Y1 expressed human neuroblastoma cells, SK-N-MC, and cell membranes; radioligand binding studies and library screening; in vitro functional assay; PKs and brain uptake; in vitro toxicity in cultures of immortalized human hepatocytes; Ames reverse-mutation study in *Salmonella*; and spontaneous nocturnal feeding studies upon oral administration. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Robert Gentles for helpful discussions of the manuscript.

■ REFERENCES

(1) Yulyaningsih, E.; Lei Zhang, L.; Herzog, H.; Sainsbury, A. NPY receptors as potential targets for anti-obesity drug development. *Br. J. Pharmacol.* **2011**, *163*, 1170–1202.

(2) Walther, C.; Morl, K.; Beck-Sickinger, A. G. Neuropeptide Y receptors: Ligand binding and trafficking suggest novel approaches in drug development. *J. Pept. Sci.* **2011**, *17*, 233–246.

(3) Zimanyi, I. A.; Poindexter, G. NPY-Ergic agents for the treatment of obesity. *Drug Dev. Res.* **2000**, *51*, 94–111.

(4) Sato, N.; Ogino, Y.; Mashiko, S.; Ando, M. Modulation of neuropeptide Y receptors for the treatment of obesity. *Expert Opin. Ther. Patents* **2009**, *19*, 1401–1415.

(5) Ishihara, A.; Moriya, M.; Macneil, D. J.; Fukami, T.; Kanatani, A. Neuropeptide Y receptors as targets of obesity treatment. *Expert Opin. Ther. Patents* **2006**, *16*, 1701–1712.

(6) Chao, P.-T.; Yang, L.; Aja, S.; Moran, T. H.; Bi, S. Knockdown of NPY expression in the dorsomedial hypothalamus promotes development of brown adipocytes and prevents diet-induced obesity. *Cell Metab.* **2011**, *13*, 573–583.

(7) Nguyen, A. D.; Herzog, H.; Sainsbury, A. Neuropeptide y and peptide YY: Important regulators of energy metabolism. *Curr. Opin. Endocrinol. Diabetes Obes.* **2011**, *18*, 56–60.

(8) Parker, E.; Wan Heek, M.; Stamford, A. Neuropeptide Y receptors as targets for antiobesity drug development: Perspective and current status. *Eur. J. Pharmacol.* **2002**, *440*, 173−187.
(9) Chamorro, S.; Della-Zuana, O.; Fauchere, J.-L.; Galizzi, J.-P.; Levens, N. Appetite suppression based on selective inhibition of NPY receptors. *Int. J. Obes.* **2002**, *26*, 281−298.
(10) Guan, X.-M.; Yu, H.; Van der Ploeg, L. H. T. Evidence of altered hypothalamic pro-opiomelanocortin/neuropeptide Y mRNA expression in tubby mice. *Mol. Brain Res.* **1998**, *59*, 273−279.
(11) Kesterson, R. A.; Huszar, D.; Lynch, C. A.; Simerly, R. B.; Cone, R. D. Induction of neuropeptide Y gene expression in the dorsal medial hypothalamic nucleus in two models of the *agouti* obesity syndrome. *Mol. Endocrinol.* **1997**, *11*, 630−637.
(12) Erickson, J. C.; Hollopeter, G.; Palmiter, R. D. Attenuation of the obesity syndrome of ob/ob mice mice by the loss of neuropeptide Y. *Science (Washington, DC)* **1996**, *274*, 1704−1707.
(13) Zarjevski, N.; Cusin, I.; Vettor, R.; Rohner-Jeanrenaud, F.; Jeanrenaud, B. Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. *Endocrinology* **1993**, *133*, 1753−1758.
(14) Lin, E.-J. D.; Zhang, L.; Sainsbury, A.; Herzog, H. Y2, Y4 receptors and obesity. *Expert Rev. Endocrinol. Metab.* **2007**, *2*, 163−173.
(15) Ishihara, A.; Tanaka, T.; Kanatani, A.; Fukami, T.; Ihara, M.; Fukuroda, T. A potent neuropeptide Y antagonist, 1229U91, suppressed spontaneous food intake in Zucker fatty rats. *Am. J. Physiol.* **1998**, *43*, R1500−R1504.
(16) Kanatani, A.; Ito, J.; Ishihara, A.; Iwaawa, H.; Fukaroda, T.; Fukami, T.; MacNeil, D. J.; Van der Ploeg, L. H. T.; Ihara, M. NPY-induced feeding involves the action of a Y1-like receptor in rodents. *Regul. Pept.* **1998**, *75*−*76*, 409−415.
(17) Balasubramaniam, A.; Dhawan, V. C.; Mullins, D. E.; Chance, W. T.; Sheriff, S.; Guzzi, M.; Prabhakaran, M.; Parker, E. M. Highly selective and potent neuropeptide Y (NPY) Y1 receptor antagonists based on [Pro(30), Tyr(32), Leu(34)]NPY(28−36)-NH2 (BW1911U90). *J. Med. Chem.* **2001**, *44*, 1479−1483.
(18) Rudolf, K.; Eberlein, W.; Engel, W.; Beck-Sickinger, A. G.; Wittneben, H.; Wieland, H. A.; Doods, H. In *Neuropeptide Y and Drug Development*; Grundemer, L., and Bloom, S. R., Eds.; Academic Press: San Diego, CA, 1997; pp 175−190.
(19) Serradiel-Le Gal, G. In *Neuropeptide Y and Drug Development*; Grundemer, L., and Bloom, S. R., Eds.; Academic Press: San Diego, CA, 1997; pp 157−174.
(20) Zarrinmayeh, H.; Zimmerman, D. M.; Cantrell, B. E.; Schober, D. A.; Bruns, R. F.; Gackenheimer, S. L.; Ornstein, P. L.; Hipskind, P. A.; Britton, T. C.; Gehlert, D. R. Structure-activity relationship of a series of diaminoalkyl substituted benzimidazole as neuropeptide Y Y1 receptor antagonists. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 647−652.
(21) Poindexter, G. S.; Bruce, M. A.; LeBoulluec, K. L.; Mokovic, I.; Martin, S. W.; Parker, E. M.; Iben, L. G.; McGovern, R. T.; Ortiz, A. A.; Stanley, J. A.; Mattson, G. K.; Kozlowski, M.; Arcuri, M.; Ildiko, A.-Z. Dihydropyridine Neuropeptide Y Y_1 Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 379−382.
(22) Antal-Zimanyi, I.; Bruce, M.; LeBoulluec, K.; Iben, L.; Mattson, G.; McGovern, R.; Hogan, J.; Leahy, C.; Flowers, S.; Stanley, J.; Ortiz, A.; Poundexter, G. Pharmacological characterization and appetite suppressive properties of BMS-193885, a novel and selective neuropeptide Y1 receptor antagonist. *Eur. J. Pharmacol.* **2008**, *590*, 224−232.
(23) Colin, P. L.; Romano, D. F.; Francesca, B.; Manuela, B.; Simone, B.; Giovanna, D. F.; Daniele, D.; Alessandro, F.; Damiano, G.; Riccardo, G.; Francesca, P.; Angelo, P.; Giorgio, P.; Domenica, A. P.; Giovanna, R.; Luigi, S. Novel carbazole derivatives as NPY Y1 antagonists. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1043−1046.
(24) Kanatani, A.; Kanno, T.; Ishihara, A.; Hata, M.; Sakuraba, A.; Tanaka, T.; Tsuchiya, Y.; Mase, T.; Fukuroda, T.; Fukami, T.; Ihara, M. The novel neuropeptide Y Y(1) receptor antagonist J-104870: A potent feeding suppressant with oral bioavailability. *Biochem. Biophys. Res. Commun.* **1999**, *266*, 88−91.
(25) Ishihara, A.; Kanatani, A.; Okada, M.; Hidaka, M.; Ianaka, I.; Mashiko, S.; Gomori, A.; Kanno, I.; Hata, M.; Kanesaka, M.; Iominaga, Y.; Sato, N.; Kobayashi, M.; Mural, I.; Watanabe, K.; Ishii, Y.; Fukuroda, I.; Fukami, I.; Ihara, M. Blockade of body weight gain and plasma corticosterone levels in Zucker fatty rats using an orally active neuropeptide Y Y1 antagonist. *Br. J. Phamacol.* **2002**, *136*, 341−346.
(26) Kannoa, T.; Kanatani, A.; Keen, S. L.; Arai-Otsuki, S.; Haga, Y.; Iwama, T.; Ishihara, A.; Sakuraba, A.; Iwaasa, H.; Hirose, M.; Morishima, H.; Fukami, T.; Ihara, M. Different binding sites for the neuropeptide Y Y1 antagonists 1229U91 and J-104870 on human Y1 receptors. *Peptides* **2001**, *22*, 405−413.
(27) Peterson, J. M.; Blum, C. A.; Cai, G.; Hutchison, A. U.S. Patent 5900415, 1999.
(28) Allen, D. J.; Brostron, L. R.; Kord, A. S.; Whipple, L. A. WO 9823603, 1998.
(29) Tetzlaff, F.; Vilsmaier, E.; Schlag, W.-R. Functionalized chloroenamines in aminocyclopropane synthesis—VI. Chlorotetrahydropyridines as a basis for the synthesis of 3-azabicyclo[3.1.0.]hexane derivatives. *Tetrahedron* **1990**, *46*, 8117−8130.
(30) Full crystallographic data have been deposited to the Cambridge Crystallographic Data Center (CCDC reference number 865396). Copies of the data can be obtained free of charge via the internet at http://www.ccdc.cam.ac.uk.
(31) Dambach, D. M.; Andrews, B. A; Moulin, F. New technologies and screening strategies for hepatotoxicity: Use of in vitro models. *Toxicol. Pathol.* **2005**, *33*, 17−26.
(32) Zimmerman, H. J. *Hepatotoxicity*, 2nd ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 1999; pp 3−10.
(33) Maron, D. M.; Ames, B. N. Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* **1983**, *113*, 173−215.