Bioorganic & Medicinal Chemistry Letters 21 (2011) 2641–2645

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery and evaluation of pyrazolo[1,5-*a*]pyrimidines as neuropeptide Y1 receptor antagonists

David A. Griffith [a], Diane M. Hargrove [b], Tristan S. Maurer [c], Charles A. Blum [d], Stéphane De Lombaert [d], John K. Inthavongsay [a], Lee E. Klade [a], Christine M. Mack [d], Colin R. Rose [a], Martin J. Sanders [b], Philip A. Carpino [a,*]

[a] Department of Worldwide Medicinal Chemistry, Pfizer PharmaTherapeutics, Groton, CT 06340, USA
[b] Department of Cardiovascular, Metabolic, and Endocrine Diseases, Pfizer Global Research & Development, Groton, CT 06340, USA
[c] Department of Pharmacokinetics, Dynamics & Metabolism, Pfizer PharmaTherapeutics, Groton, CT 06340, USA
[d] Neurogen Corporation, Branford, CT 06405, USA

ARTICLE INFO

Article history:
Received 18 October 2010
Revised 18 December 2010
Accepted 21 December 2010
Available online 7 January 2011

Keywords:
Obesity
NPY Y1R
Pyrazolo[1,5-*a*]pyrimidine

ABSTRACT

A novel series of pyrazolo[1,5-*a*]pyrimidine derivatives was synthesized and evaluated as NPY Y1R antagonists. High binding affinity and selectivity were achieved with C3 trisubstituted aryl groups and C7 substituted 2-(tetrahydro-2*H*-pyran-4-ylamino)ethylamine moieties. Efforts to find close analogs with low plasma clearance in the rat and minimal p-glycoprotein efflux in the mouse were unsuccessful. Compound **2f** (CP-671906) inhibited NPY-induced increases in blood pressure and food intake after iv and icv administration, respectively, in Sprague–Dawley (SD) rat models. Oral administration of compound **2f** resulted in a modest, but statistically significant, reduction in food intake in a Wistar rat model of feeding behavior. Small inhibitions of food intake were also observed in an overnight fasting/refeeding model in SD rats. These data suggest a potential role for Y1R in the regulation of food intake in rodents.

Obesity is a body weight disorder characterized by excess adipose tissue and a body mass index (BMI) greater than or equal to 30 kg/m^2. Accumulation of fat mass is protective during periods of starvation, but can otherwise lead to multiple co-morbidities including hypertension, diabetes mellitus, and dyslipidemia. The prevalence of obesity has steadily increased in the past decade to the point where it is now considered to be the second leading cause of preventable death after smoking.[1] Weight loss through diet, exercise, pharmacological treatment, or even bariatric surgery has been shown to lead to significant improvements in the co-morbidities associated with the disease. Diet and exercise are considered first line treatments for obesity while bariatric surgery is only prescribed for the morbidly obese. Diet and exercise are rarely successful over the long-term in maintaining weight loss, thus the need for pharmacotherapy. Only a few drugs, including Orlistat®, are currently approved to treat obesity, but these agents have limitations which prevent their widespread use.[2] A number of promising pharmacological agents have recently completed Phase III clinical trials and are awaiting regulatory approval (Qnexa®, Contrave®, and locaserin).[3] A growing need exists for new and effective weight loss drugs with improved toleration and excellent safety profiles.

The 36 amino acid polypeptide neuropeptide Y (NPY), a member of a family of pancreatic polypeptides, has been shown to play a role in the regulation of food intake, energy homeostasis, vascular tone, anxiety, and reproductive health.[4,5] NPY is primarily synthesized in the hypothalamus, a key area in the central nervous system (CNS) involved in the control of energy homeostasis. NPY stimulates food intake when injected centrally in satiated rats.[6] Furthermore, chronic administration of NPY induces a sustained increase in food intake and body weight.

The pharmacological actions of NPY are mediated through four G-protein coupled receptors (Y1R, Y2R, Y4R, and Y5R).[7] A fifth cloned receptor called y6R is present in the mouse and rabbit, but is non-functional in rats and humans.[8] Y1R and Y5R are primarily localized in the hypothalamus and vasculature, Y2R in the gut and the hypothalamus, and Y4R in the nucleus of the solitary tract and the hypothalamus.[9] Studies with transgenic knockout animals and subtype-selective peptidic analogs (i.e., antagonists for Y1R and Y5R; agonists for Y2R and Y4R) have implicated the four functional NPY receptors in the regulation of feeding behavior.[4] NPY receptors represent potential drug targets for the treatment of obesity.

In the past few years, the discovery of non-peptidic, small molecule Y1R and Y5R antagonists has attracted considerable interest, especially in the pharmaceutical industry. Two Y5R antagonists, MK-0557 and S-2367, have been advanced into human clinical

* Corresponding author. Tel.: +1 860 441 5694; fax: +1 860 441 8067.
E-mail address: philip.a.carpino@pfizer.com (P.A. Carpino).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.12.116

trials, however MK-0557 was recently dropped because of a modest effect in preventing weight re-gain following a very low calorie diet.[10,11] Several novel series of Y1R antagonists have been identified that reportedly modulate feeding behavior in rodent models.[12,13] As part of an effort to validate Y1R as an anti-obesity target, we sought Y1R antagonists with high functional activities and CNS exposure to explore the role of the central Y1R on feeding behavior in rodent models.

N-(2-Aminoethyl)-*N*-ethyl-9-mesityl-2-methyl-9*H*-pyrimido[4,5-*b*]indol-4-amine **1** was identified as a Y1R antagonist during the counterscreening of compounds prepared as corticotrophin-releasing hormone receptor (CRHR) antagonists for another project (Fig. 1).[14] Compound **1** exhibited 29-fold selectivity for Y1R over CRHR. Concerns about the high lipophilicity of **1** [calculated *E*log*D* (*cE*log*D*) = 3.9][15] led to the removal of the *N*-ethyl group and the replacement of the tricyclic ring with bicyclic heterocyclic moieties found in other known CRHR antagonists. These changes resulted in the discovery of *N*-(2-aminoethyl)-3-mesityl-2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-amine **2a** which exhibited moderate Y1R binding affinity, selectivity for Y1R over CRHR, and reduced lipophilicity compared to **1**. Herein, we describe the structure–activity relationships and biological characterizations of compounds **2a**–**m** and report on our efforts to ensure target engagement and confirm specificity-of-action in behavioral studies measuring changes in food intake.

The pyrazolo[1,5-*a*]pyrimidine derivatives **2a**–**m** were synthesized from key intermediate **5** which was prepared in two steps from benzonitrile derivative **3** (Scheme 1). Cyclocondensation of **5** with ethyl acetoacetate afforded compound **6** which was treated first with $POCl_3$ and then with an appropriately substituted diamine derivative to give a family of analogs encompassed by **2a** or **7**. When substituted ethylenediamine derivatives were used, the predominant product came from the addition of the less sterically hindered amino group. Reductive amination of **7** with appropriately substituted aldehyde or ketone derivatives provided target compounds **2a**–**f** and **2i**–**m**. Methyl ether **2f** was transformed into ethyl and *n*-propyl ethers **2g** and **2h** in two steps that involved demethylation of the aryl ether group using aqueous HBr, followed by alkylation with an appropriately substituted alkyl halide (Scheme 2).[16]

The pyrazolo[1,5-*a*]pyrimidine derivatives were evaluated in ^{125}I-PYY and ^{125}I-sauvagine binding displacement assays using Sf9 membranes over-expressing recombinant human or rat Y1 receptors (hY1R or rY1R) or membranes from IMR-32 cells endogenously expressing CRHR.[14,17] In vitro functional activity was assessed in human neuroblastoma SK-N-MC cells by measuring the ability of test compounds to antagonize NPY-induced mobilization of intracellular Ca^{2+} concentrations using a Fluorometric Imaging Plate Reader (FLIPR).[18] In vivo functional activity was evaluated in Sprague–Dawley (SD) rats by measuring the ability of test compounds to antagonize NPY-induced increases in blood pressure (BP) (NPY pressor model).[5,19]

A series of N2-alkylated derivatives of **2a** was initially investigated (Table 1). The 4-methoxyphenethylamino derivative **2b**

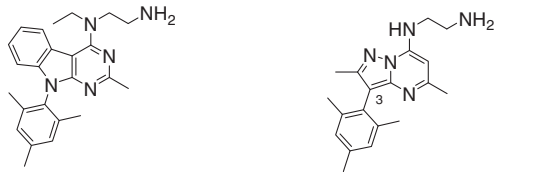
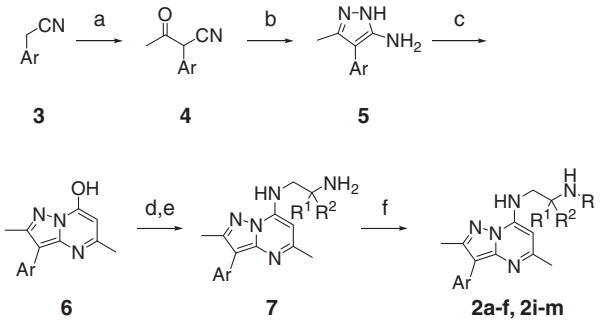
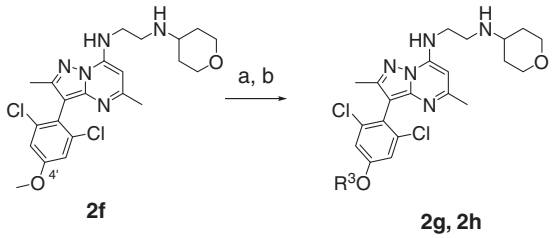


Figure 1. Y1R screening hit and selective bicyclic derivative.



Scheme 1. Reagents and conditions: (a) Na, EtOAc, 90 °C; (b) NH_2NH_2, HOAc, toluene, 130 °C, Dean–Stark trap; (c) ethyl acetoacetate, HOAc, 130 °C; (d) $POCl_3$, 130 °C; (e) $H_2NCH_2C(R^1R^2)NH_2$, CH_3CN, 80 °C; (f) R(=O), $NaBH(OAc)_3$, HOAc, room temperature.



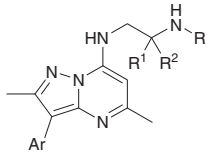
Scheme 2. Reagents and conditions: (a) aq HBr, 100 °C; (b) R^3I, aq NaOH, isopropyl alcohol, reflux.

and the *c*-pentylamino analog **2c** were significantly more potent than **2a** in the Y1R binding assay and exhibited high selectivity for Y1R over CRHR (data not shown). The mesityl group in **2c** could be replaced by a 2,6-di-Cl-phenyl moiety without resulting in a loss of binding affinity. Compounds **2b**–**d** exhibited high lipophilicities, outside the preferred range for drugs that act at pharmacological targets located in the CNS.[20] Replacement of the *c*-pentyl group in **2d** with a tetrahydropyran-4-yl (4-THP) group decreased lipophilicity and led to a surprising increase in binding affinity. Further fine-tuning of the lipophilicity of **2e** by incorporation of a methoxy substituent at the C4′ position in the 2,6-di-Cl-phenyl ring provided **2f** (CP-671906). Substitution of the methoxy group in **2f** with larger alkoxyl groups led to a slight decrease in binding affinity (**2g**, **2h**).

Compound **2f** was selected for further characterization based on its affinity for hY1R. This compound exhibited low nanomolar affinity for rY1R (K_i = 3.5 nM) in a binding displacement assay and showed selectivity versus the human Y2 and Y5 receptors (IC_{50}'s >10,000 nM). It did not display any significant activity across a panel of 42 receptors, ion channels, transporters, and enzymes (<50% inhibition at 1 μM)[21] or against CRHR (<50% inhibition at 10 μM).[22] Compound **2f** demonstrated potent Y1R functional antagonism in SK-N-MC cells (Table 2). In an anesthetized rat model, **2f** was shown to block the NPY-induced increase in blood pressure. Compound **2f** was the first compound in the pyrazolo[1,5-*a*]pyrimidine series to demonstrate functional activity in an animal model. This assay was introduced into the screening paradigm in order to confirm target engagement, albeit in the periphery and not in the CNS.

Pharmacokinetic evaluation of **2f** in the SD rat revealed moderate plasma clearance (Table 2), a short half-life ($t_{1/2}$ = 1.2 h), and poor oral bioavailability (F = 13%).[23] A close analog with reduced plasma clearance was sought for in vivo studies. Studies with **2f** in rat microsomal preparations showed that a principle route of metabolism was O-demethylation of the alkoxy group on the C3 aryl ring. Plasma clearances of the ethoxy analog **2g** and the

Table 1
hY1R binding affinities and calculated lipophilicities



Compd	Ar	R^1, R^2	R	*cE* log *D*[15]	K_i[a,b] (nM)
2a	2,4,6-Me-Ph	H, H	H	2.4	2300
2b	2,4,6-Me-Ph	H, H	CH_2CH_2-4-MeO-Ph	4.1	29
2c	2,4,6-Me-Ph	H, H	*c*-Pentyl	3.3	21
2d	2,6-Cl-Ph	H, H	*c*-Pentyl	3.4	38
2e	2,6-Cl-Ph	H, H	4-THP[c]	2.8	7
2f (CP-671906)	2,6-Cl-4-MeO-Ph	H, H	4-THP	2.6	1
2g	2,6-Cl-4-EtO-Ph	H, H	4-THP	2.8	3
2h	2,6-Cl-4-*n*-PrO-Ph	H, H	4-THP	3.2	8
2i	2,6-Cl-4-MeO-Ph	H, Me[c]	4-THP	3.4	5
2j	2,6-Cl-4-MeO-Ph	Me, Me	4-THP	2.9	141
2k	2,6-Cl-4-MeO-Ph	H, H	2-F-cyclohex-1-yl[d]	4.2	5
2l	2,6-Cl-4-MeO-Ph	H, H	CH_2CH_2F	3.2	22
2m	2,6-Cl-4-MeO-Ph	H, H	CH_2CHF_2	3.4	383

[a] Human Y1R radioligand binding assay.[17]
[b] Data represent single determinations obtained in triplicate.
[c] Mixture of enantiomers.
[d] Mixture of cis/trans isomers.

Table 2
Y1R functional activities and plasma clearance and protein binding values

Compd	FLIPR IC_{50} (nM)[a]	Rat Clp (mL/min/kg)	Rat fu,p	Rat Clp,u (mL/min/kg)	NPY pressor response (%inh at 3 mg/kg iv)[19]
2f	7	45	0.059	763	92
2g	15	28	0.029	996	47
2h	207	25	0.011	2273	0

[a] FLIPR Ca^{2+} mobilization assay in SK-N-MC cells; IC_{50} curves were constructed using the logistic equation from eight or nine concentrations of test compound with four replicates per concentration.[18]

n-propoxy derivative **2h**, both of which showed good Y1R binding affinities and moderate half-lives in rat microsomal preparations (data not shown), were reduced, although the unbound clearance of **2h** was greater than that of **2g** or **2f**. Compounds **2g** and **2h** were 2- and 30-fold less potent in the FLIPR assay compared to **2f**. Compound **2g** was unable to completely block the NPY pressor response in anesthetized rats at the single dose tested, while **2h** was devoid of activity in this model, most likely as a result of weak functional potency and high unbound clearance (Table 2). Neither **2g** nor **2h** proved to be suitable tool compounds for assessing the role of Y1R. Pharmacokinetic properties of other close analogs of **2f**, including those lacking 4-methoxy groups on the C3 aryl ring, were not investigated due to either weak Y1R binding affinities or high microsomal clearance values (data not shown).

As the Y1R mediating the orexigenic activity of NPY is located within the hypothalamus, brain penetration of **2f** was measured. Brain-to-plasma (B/P) ratios were shown to be poor in SD rats and FVB mice (0.13 and 0.1, respectively).[24,25] The low B/P values suggested the compound might be a substrate for efflux transporters such as p-glycoprotein (PGP), an ATP-dependent drug transport protein widely distributed in the body including the brain capillary endothelial cells.[26] Efflux was confirmed in studies measuring brain and plasma concentrations in multidrug resistant Mdr1a/1b knockout (KO) mice and wild-type FVB mice following a subcutaneous (sc) dose (Note–the mdr gene encodes for PGP). The B/P ratio was ninefold higher in Mdr1a/1b knockout mice compared to the B/P ratio in FVB mice. The large ratio of B/P ratios showed that **2f** was actively transported from the CNS by PGP at the level of the blood–brain barrier.

Several strategies were investigated for decreasing PGP efflux of **2f** in the mouse and improving CNS penetration: (1) introduction of steric hindrance around the tetrahydro-2*H*-pyran-4-amino group in **2f**; and (2) reduction of the pK_a of this amino group.[27] The basic amino group was hypothesized to play a role in the recognition of the compounds by PGP efflux transporters as compounds from other series that lacked such a group were generally found to be weak PGP efflux substrates (data not shown). The methyl derivative **2i** showed similar PGP efflux to **2f** but the gem-dimethyl analog **2j** exhibited reduced efflux (Table 3). Unfortunately, **2j** was significantly less potent in the binding assay compared to **2f**. The pK_a of the amino group in **2f** was modulated by replacing the 4-THP group with fluorinated cycloalkyl and alkyl groups (Table 3).[27] The 2-F-cyclohex-1-yl derivative **2k** was shown to be a PGP efflux substrate while the 2-F-ethylamino analog **2l** and the 2,2-F-ethylamino compound **2m** exhibited reduced affinity for the PGP transporter. Unfortunately, **2l** and **2m** also displayed weaker binding affinities for Y1R (Table 1).

Since an analog of **2f** with reduced plasma clearance in the rat, reduced affinity for PGP efflux transporters in mouse models, and comparable pharmacological activity could not be readily identified, compound **2f** was selected for in vivo studies exploring the role of Y1R in the regulation of food intake.[28] To demonstrate

Table 3
Calculated pK_a values and ratios of B/P ratios in Mdr1a/1b KO and wild-type FVB mice

Compd	Calculated pK_a[a]	[B/P] in Mdr1a/1b knockout mice/[B/P] in FVB mice[b]
2f	8.9	9
2i	9.0	10
2j	9.0	4
2k	8.0	8
2l	7.7	4
2m	6.3	2

[a] Calculated using the software program ACD labs pK_a predictor.
[b] Brain and plasma levels were measured at 0.5 h following a 5 mg/kg sc dose.

target engagement in the CNS where Y1R is hypothesized to regulate feeding behavior, **2f** was co-injected with NPY into the hypothalamus of SD rats via an intracerebroventricular (icv) cannula.[29] Compound **2f** significantly suppressed NPY-induced feeding, confirming the ability of the drug to modulate the target in SD rats, albeit at very high concentrations as administered via the icv route (Fig. 2). The effects of oral administration of **2f** (40 mg/kg) were tested in lean rats subjected to two different feeding conditions. Compound **2f** induced a small, transitory decrease in food intake in fasting/refeeding SD rats (12% decrease versus vehicle treated controls at the 2 h timepoint following return of food or the 4 h timepoint post-dose; no change at the 4 h timepoint following return of food).[30] No significant changes were observed in locomotor activity, a signal of potential off-target activity. Compound **2f** also induced a modest, but statistically significant inhibition, of nocturnal feeding behavior in Wistar rats, again without affecting locomotor activity (Fig. 3).[31]

To confirm that **2f** was exposed at the target site in SD rats following a 40 mg/kg oral dose, concentrations of the drug in both cerebral spinal fluid (CSF) and brain interstitial fluid (ISF) were measured, although in a separate group of animals from the feeding studies.[32] Concentrations of **2f** were 26 nM at the 2 h timepoint, significantly higher than the K_i value measured in the radioligand binding assay, but notably lower than that expected from unbound plasma concentrations (95 nM), consistent with the impairment observed in the MDR1a/1b knockout and FVB mouse exposure studies. ISF concentrations of **2f** (collected at the 2 h timepoint from paraventricular nucleus using microdialysis probes) were found to be similar to the concentrations of **2f** in the CSF ([ISF] = 17 nM). Brain and CSF exposure of **2f** in the Wistar rat model of nocturnal feeding behavior was not measured.

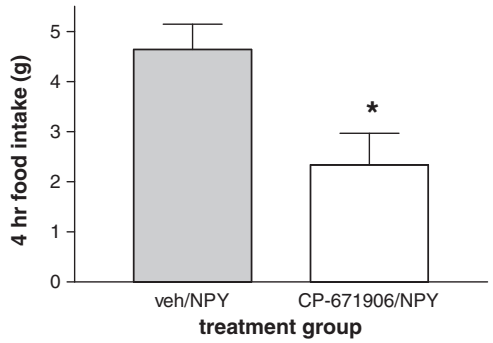


Figure 2. Icv inhibition by **2f** (CP-671906, 120 μg) of NPY(5 μg)-induced increase in daytime feeding in Sprague–Dawley rats [$^*p$ <0.05 for vehicle/NPY versus **2f**/NPY (paired two-tailed *t*-test)].

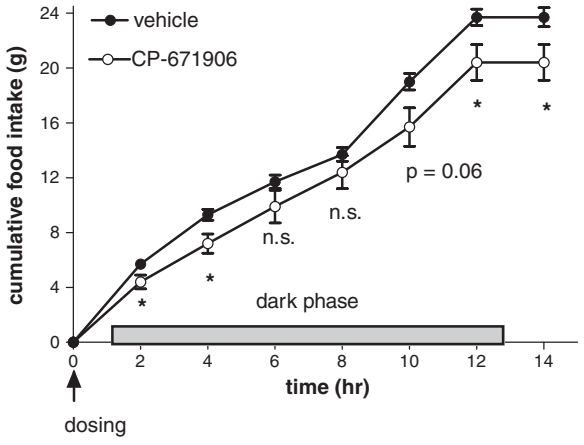


Figure 3. Time course of the effects of **2f** (CP-671906, 40 mg/kg po) on nocturnal feeding in Wistar rats in a continuous food intake monitoring system [$^*p$ <0.05 for **2f** versus time-matched vehicle point (two-tailed, unpaired *t*-test)].

In conclusion, pyrazolo[1,5-*a*]pyrimidines have been identified as a new chemotype with Y1R activity. One compound, **2f**, inhibited NPY-induced increases in blood pressure and food intake after iv and icv administration, respectively, confirming functional activity in an animal model. Analogs of **2f** with reduced plasma clearances in the rat or reduced PGP efflux liabilities in a mouse model were also discovered, but these derivatives were not suitable tool compounds. In studies of feeding behavior with **2f**, modest inhibitions of food intake were observed in several lean rodent models. Exposure of **2f** at the target site and direct modulation of the target were both demonstrated. Overall, the results suggest that Y1R antagonists may have modest effects on feeding behavior in rat models. Longer term studies will be required to determine if the small anorectic effects observed with an acute dose can be sustained during repeat dosing and translate into longer term reductions in body weight.

Acknowledgments

We thank David Hepworth for helpful discussions and Hans Rollema for the microdialysis data.

References and notes

1. Mikdad, A. H.; Marks, J. S.; Stroup, D. F.; Gerberding, J. L. *J. Am. Med. Assoc.* **2004**, *291*, 1238.
2. Isidro, M. L.; Cordido, F. *Mini-Rev. Med. Chem.* **2009**, *9*, 664.
3. Klonoff, D. C.; Greenway, F. J. *Diabetes Sci. Technol.* **2008**, *2*, 913.
4. Kamiji, M. M.; Inui, A. *Endocr. Rev.* **2007**, *28*, 664.
5. Thorsell, A.; Heilig, M. *Neuropeptides* **2002**, *36*, 182.
6. Stanley, B. G.; Kyrkouli, S. E.; Lampert, S.; Leibowitz, S. F. *Peptides* **1986**, *7*, 1986.
7. Wieland, H. A.; Hamilton, B. S.; Krist, B.; Doods, H. N. *Expert Opin. Invest. Drugs* **2000**, *9*, 1327.
8. Blomqvist, A. G.; Herzog, H. *Trends Neurosci.* **1997**, *20*, 294.
9. Fetissov, S. O.; Kopp, J.; Hokfelt, T. *Neuropeptides* **2004**, *38*, 175.
10. Erondu, N.; Wadden, T.; Gantz, I.; Musser, B.; Nguyen, A. M.; Bays, H.; Bray, G.; O'Neil, P. M.; Basdevant, A.; Kaufman, K. D.; Heymsfield, S. B.; Amatruda, J. M. *Obesity* **2007**, *15*, 895.
11. Okuno, T.; Takenaka, H.; Aoyama, Y.; Kanda, Y.; Yoshida, Y.; Okada, T.; Hashizume, H.; Sakagami, M.; Nakatani, T.; Hattori, K.; Ichihashi, T.; Yoshikawa, T.; Yukioka, H.; Hanasaki, K.; Kawanishi, Y. *Abstract of Papers*, 240th National Meeting of the American Chemical Society, Boston, MA, 2010; Abstract MEDI-284.
12. (a) Sun, Z.-Y.; Zhu, Z.; Ye, Y.; McKittrick, B.; Czarniecki, M.; Greenlee, W.; Mullins, D.; Guzzi, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6801; (b) Cho, K.; Ando, M.; Kobayashi, K.; Miyazoe, H.; Tsujino, T.; Ito, S.; Suzuki, T.; Tanaka, T.; Tokita, S.; Sato, N. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4781; (c) Kameda, M.; Kobayashi, K.; Ito, H.; Miyazoe, H.; Tsujino, T.; Nakama, C.; Kawamoto, H.; Ando, M.; Ito, S.; Suzuki, T.; Kanno, T.; Tanaka, T.; Tahara, Y.; Tani, T.; Tanaka, S.; Tokita, S.; Sato, N. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4325.
13. (a) Lee, J.; Song, K.-S.; Kang, J.; Lee, S. H.; Lee, J. *Curr. Top. Med. Chem.* **2009**, *9*, 564; (b) MacNeil, D. J. *Curr. Top. Med. Chem.* **2007**, *7*, 1721.
14. Compound **1** was synthesized by the methods described in WO199951600 and the binding affinity against the CRH receptor was measured according to the assays described therein.
15. (a) *ElogD* was calculated from a model built according to the methods described in Ref. 15b, based on Cubist [Rulequest Research, www.rulequest.com]. The training set for the model was 33,000 *ElogD* values measured using methodology described in Ref. 15c.; (b) Gao, H.; Yao, L.; Mathieu, H. W.; Zhang, Y.; Maurer, T. S.; Troutman, M. D.; Scott, D. O.; Ruggeri, R. B.; Lin, J. *Drug Metab. Dispos.* **2008**, *36*, 2130; (c) Lombardo, F.; Shalaeva, M. Y.; Tupper, K. A.; Gao, F. *J. Med. Chem.* **2001**, *44*, 2490.
16. All compounds described in this manuscript gave satisfactory spectral data (^{1}H NMR, MS).
17. For details of this assay, see WO 2001023387.
18. Antagonism of NPY-induced mobilization of intracellular Ca^{2+} concentrations by **2f** was determined in Fluo-3 loaded SK-N-MC cells using a 96-well Fluorometric Imaging Plate Reader (FLIPR, Molecular Devices). SK-N-MC cells were maintained in culture under standard conditions and plated at a density of 25,000 cells per well 4 days prior to experimentation. On the day of the experiment, cells were washed with assay buffer (5 mM KCl, 115 mM NaCl, 0.96 mM NaH_2PO_4, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 1 mM carbachol, 5 mM D-glucose) and incubated in dye loading solution (assay buffer with 220 nM

Fluo-3 AM, 1 mM probenecid and an additional 10 mM HEPES) for 1 h at 37 °C in a 5% CO_2 atmosphere. After a 1 h incubation period, plates were washed and re-suspended in assay buffer containing test compound at the desired concentration. Fluorescence responses were elicited by an EC_{50} concentration of NPY (range 0.5–1.5 nM), which was determined just prior to each experiment.
19. SD rats, fasted overnight, were anesthetized with inactin (100 mg/kg ip), surgically prepared with tracheal (PE205) and femoral arterial and venous cannula (PE50) and placed on a water-jacketed heating pad. The arterial pressure and heart rate signals were fed into a PONEMAH Physiology Platform (Gould Instrument Systems, Valley View OH) and data were recorded every 5 seconds during the experiment. NPY (5 μg in 0.25 mL of saline), was administered to the rats at 15 min intervals as an iv bolus. Following the third bolus injection of NPY, the test compound (3 mg/kg in a saline vehicle, 1 mL/kg) was dosed iv and three subsequent NPY injections were administered.
20. Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. D.; Verhoest, P. R.; Villalobos, A.; Will, Y. *ACS Chem. Neurosci.* **2010**, 1.
21. Selectivity assays (42 receptors, ion channels and enzymes) were performed by MDS Pharma (Taipei, Taiwan).
22. CRH receptor binding activity was measured at Cerep (France); www.cerep.fr.
23. For the determination of pharmacokinetic parameters, jugular vein cannulated SD rats were dosed with **2f** by oral gavage (3 mg/kg in 0.5% methyl cellulose at 2 mL/kg, $n = 2$) or via the jugular vein (1.0 mg/kg in 50% PEG vehicle at 1 ml/kg, $n = 2$). Prior to dosing, rats were fasted overnight with water available ad libitum.
24. B/P ratios were calculated from unbound plasma - to - brain fraction ratios; equilibrium dialysis was used to measure unbound fraction of compound in brain homogenates and whole plasma from rats and mice.
25. Kalvass, J. C.; Maurer, T. S. *Biopharm. Drug Dispos.* **2002**, *23*, 327.
26. Schinkel, A. H. *Adv. Drug Delivery Rev.* **1999**, *36*.
27. Morgenthaler, M.; Schweizer, E.; Hoffmann-Roder, A.; Benini, F.; Martin, R. E.; Jaeschke, G.; Wagner, B.; Fischer, H.; Bendels, S.; Zimmerli, D.; Schneider, J.; Diederich, F.; Kansy, M.; Muller, K. *ChemMedChem* **2007**, *2*, 1100.
28. All animal protocols were reviewed and approved by the Pfizer or Neurogen Institutional Animal Care and Use Committees.
29. SD rats with icv cannula were fed powdered rat chow ad libitum. Between 10–11 am during the light cycle, **2f** (120 μg) or vehicle (1 μL of propylene glycol) were co-infused with NPY (5 μg in 4 μL sterile water) via the icv cannula (total volume 5 μL over 1 min). After dosing, food intake over the next 4 h was measured. Rats received two treatments in a cross-over experiment separated by 2 days.
30. SD rats were individually housed in suspended wire cages with powdered chow and water provided ad libitum. Before the experiment, rats were fasted overnight. On the day of the experiment, rats were dosed with **2f** (40 mg/kg, po) or vehicle (0.5% methyl cellulose in water, dosing volume 1.5 mL). Food, in pre-weighed jars, was restored to the cages 2 or 4 h post dose. Food intake was recorded 2 and 4 h after restoring food.
31. Wistar rats that had been acclimated to a continuous food intake monitoring system (Columbus Instruments, Columbus OH) were dosed with **2f** (40 mg/kg, po) or vehicle, 30 min prior to the dark cycle. The system recorded food intake every 10 min for 18 h post-dose.
32. CSF samples were obtained 2 h post dose ($n = 2$). Rats were anesthetized with metofane. CSF was drawn directly from the cisterna magna through a 26-gauge needle.