Design, Synthesis, and Biological Evaluation of Substituted 2-Cyclohexyl-4-phenyl-1*H*-imidazoles: Potent and Selective Neuropeptide Y Y5-Receptor Antagonists

Charles A. Blum,* Xiaozhang Zheng, and Stéphane De Lombaert

Neurogen Corporation, 35 Northeast Industrial Road, Branford, Connecticut 06405

Received October 1, 2003

Antagonizing the robust stimulation of food intake by neuropeptide Y represents a new potential therapeutic approach for the treatment of obesity. Earlier pharmacological studies have pointed to the Y1 and Y5 receptors as the most likely mediators of the NPY orexigenic response. In this paper, we describe a new series of small molecule Y5 antagonists derived from a 2,4-diaryl-1*H*-imidazole lead. The main objectives of our structural optimization efforts were to produce novel and potent Y5 antagonists with an improved oral pharmacokinetic profile and less affinity for the hERG potassium channel compared to the lead 2,4-diarylimidazole structures. These goals were accomplished by replacement of the 2-aryl ring with a cyclohexyl ring and subsequent elaboration of the 4-position of the cyclohexyl ring with a variety of hydrophilic functionalities. The resulting compound, *N*-(2-hydroxy-*tert*-butyl)(4-{4-[3-(trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)carboxamide (**20**), displayed good potency at the Y5 receptor ($K_i = 3$ nM), while interactions at the hERG channel were essentially eliminated (6% inhibition at a concentration of 3 μM). Importantly, the pharmacokinetic properties of **20** ($F =$ 36%) represented a marked improvement over that of the initial 2,4-diarylimidazole structures.

Introduction

Neuropeptide Y (NPY), a 36-amino acid peptide, was first isolated from porcine brain in the early 1980s. It is widely distributed in the mammalian central and peripheral nervous systems.[1] More specifically, NPY is highly expressed in the hypothalamus, an area of the brain critical for the regulation of energy balance.[2] The release of NPY from these neuronal stores results in a variety of physiological effects involved in the regulation of blood pressure, anxiety, reproduction, thermoregulation, circadian rhythms, control of food intake, and others.[3] Of these, one of the most remarkable effects of NPY, and the one which has stimulated the most interest in the scientific community, is the strong orexigenic effect that this peptide induces following injections into the hypothalamus or cerebral ventricles of animals.[4] Indeed, chronic administration of NPY into the brain induces hyperphagia and weight gain. In addition, it has been shown that food deprivation in rats results in a marked increase in the levels of both NPY and its mRNA in the hypothalamus. Collectively these and other data suggest an important role for NPY as a regulator of physiological feeding behavior, particularly during periods of starvation or food deprivation.

Considering that obesity is a major health problem in advanced nations, and is known to increase the risk of developing a variety of disorders including hypertension, diabetes mellitus, and coronary heart failure among others, new and more effective treatments are sought.[5] Antagonizing the stimulation of food intake by neuropeptide Y could represent a potential therapeutic approach for the treatment of obesity.

Although NPY interacts with at least six G-protein-coupled receptors (Y1, Y2, Y3, Y4, Y5, y6), earlier pharmacological studies have pointed to the Y1 and Y5 receptor subtypes as the most likely mediators of the NPY orexigenic response. While the relative importance of both receptor subtypes on feeding behavior remains a matter of debate, we embarked several years ago in a parallel research program aimed at producing small molecule antagonists of the Y1 or Y5 receptors.

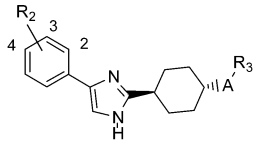
The initial evidence supporting the role of Y5 as an important regulator of feeding behavior include the positive correlation between the binding affinity of Y5 peptide agonists in vitro and their in vivo potency as orexigenic agents in animal feeding models,[6] the inhibition of NPY-stimulated food intake in rodents by oligodeoxynucleotides directed against the Y5 receptor,[7−9] and more recently, the reduction of food intake observed in ob/ob mice or Zucker obese rats following administration of small molecule Y5 antagonists.[10−13]

However, the importance of the Y5 receptor as a regulator in more natural feeding states has also been challenged recently. Indeed, despite reports of small molecule Y5 antagonists inhibiting Y5 agonist-induced feeding in rats, these same molecules have generally failed to block feeding in more natural food intake paradigms, or when feeding was elicited by exogenous centrally administered NPY.[14−18] Furthermore, in those other instances where a block of natural feeding could be demonstrated, the specificity of the compound toward the Y5 receptor was insufficient or had not been thoroughly evaluated.[12,13,19] Finally, in feeding experiments using Y5 knockout mice, NPY administration still evoked a vigorous feeding response, suggesting that the NPY feeding response is at least partially mediated through a receptor other than Y5, possibly Y1.[20]

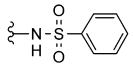
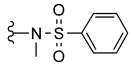
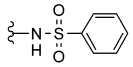
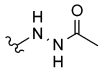
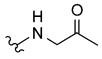
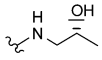
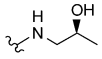
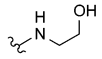
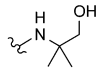
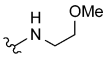
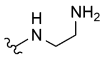
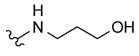
* To whom all correspondence should be addressed. Phone: (203) 488-8201. Fax: (203) 481-5290. E-mail: cblum@nrgn.com.

10.1021/jm030490g CCC: $27.50
Published on Web 03/20/2004

Table 1. In Vitro Data of Compounds **12**−**23**



12-23

Compound	R^2	A	R^3	NPY-5-Ki /nM[a]	hCl_{hep} (mL/min/kg[b]	Herg (% inh.@ 3μM[c] (SD)[d]
12	3-CF_3	CH_2		0.2 ± 0.01	4	87[e] (8)
13	3-CF_3	CH_2		1.3 ± 0.7	6	85[e] (13)
14	3-CF_3	C=O		31 ± 10	11	7 (3)
15	3-CF_3	C=O		1.4 ± 0.2	4	6 (1)
16	3-Br	C=O		10 ± 2	4	75 (5)
17	3-CF_3	C=O		8 ± 3	4	22 (6)
18	3-CF_3	C=O		31 ± 1	4	21 (11)
19	3-CF_3	C=O		11 ± 2	4	87 (2)
20	3-CF_3	C=O		2.8 ± 0.4	4	6 (8)
21	3-CF_3	C=O		99 ± 5	8	ND[f]
22	3-CF_3	C=O		915 ± 11	5	ND[f]
23	3-CF_3	C=O		185 ± 40	4	ND[f]
1	Chart 1	-	-	2.5 ± 0.01	18	60 (4)
2	Chart 1	-	-	6 ± 0.8	16	60 (10)
hERG reference compound (E-4031)[g]						IC_{50}= 10.2 ±0.2 nM

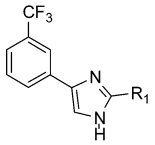
[a] Compounds were assayed for their ability to compete with [^{125}I]PYY at a recombinant human chimeric NPY_5/NPY_1 receptor expressed in SF9 cells (ref 32). Data are expressed as the mean (n = 2−3) ± SEM. [b] Hepatic clearance predicted from human microsomal half-life (n = 1). hCl_{hep} = 4 is the lower limit for this experiment and denotes a compound with no appreciable metabolism under the conditions used (see Experimental Section). [c] Percentage of block at hERG K channel determined in an in vitro electrophysiological whole cell assay using Cos7 cells ($n \geq 3$). [d] Standard deviation. [e] Percent inhibition at 300 nM. [f] ND = not determined. [g] *N*-[4-[[1-[2-(6-Methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl]methanesulfonamide (see ref 31).

In light of this conflicting evidence, the evaluation of structurally diverse compound classes remains important in order to better understand the role of the Y5 receptor in the control of appetite, and hence the potential utility of these compounds for the treatment of obesity.

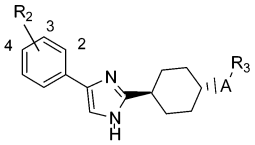
Early work in our laboratories to identify lead Y5 antagonists through compound library screening had led to the discovery of 2,4-diarylimidazoles, a structurally simple yet potent series of Y5 receptor antagonists.[18,21,22] The initial exploration of different substitution patterns within this framework quickly revealed that electron-withdrawing groups (e.g. CF_3 or CN) at the 3-position of the 4-aryl ring produced exceptionally potent compounds, exemplified by **1** (Chart 1, Table 1).

Although many of these compounds displayed high in vitro potency, the series was generally plagued by poor oral bioavailability in rats. For example, an early analogue of interest, 4-(4-chlorophenyl)-2-(4-fluorophenyl)imidazole, demonstrated an oral bioavailability in rats of only 3%. In addition, many compounds from the series significantly blocked the hERG potassium channel, thereby carrying a higher risk of adverse cardiac events due to QT interval prolongation. Standard approaches to create a more viable series, for example

Chart 1. NPY-5 Receptor Antagonists



1 R_1 = Phenyl
2 R_1 = Cyclohexyl



12-23

replacing the 2-aryl ring with a pyridine and incorporating a variety of polar ring substituents, were ultimately successful, leading to some potent Y5 antagonists with excellent pharmacokinetic properties.[18]

A parallel approach, exploiting the 2,4-diarylimidazole leads, was followed with the intent of improving on the structural novelty as well as the druglike properties of the series and reducing its interactions with the hERG channel to an acceptable level. Because the 2-aryl side of the molecule was much more amenable to the addition of polar functionalites,[18] this ring was the main focus of subsequent efforts to achieve these goals.

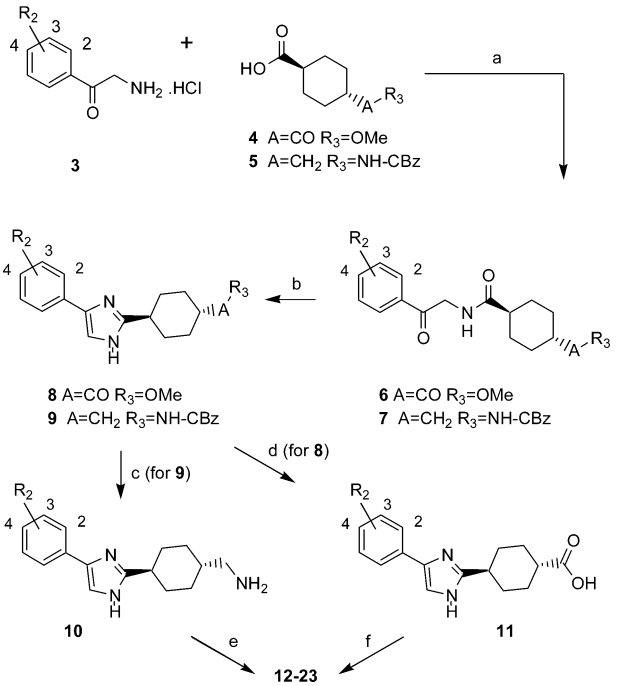
Our point of divergence from the diarylimidazole series came with the discovery that replacement of the 2-aryl ring with a saturated ring system produced compounds with only a modest loss of activity at the Y5 receptor (compare **1** and **2**, Table 1). Placement of a nitrogen atom into the cyclohexyl ring of **2** to generate the corresponding piperidine analogues would offer a handle from which to maximize potency and solubility. However, with this approach, we were unable to generate analogues with the desired level of potency.[23] Furthermore, a patent application disclosing similar analogues appeared during the course of our work.[24] Because the piperidines were no longer a viable option, efforts were then focused on substituted cyclohexane derivatives.

A common structural motif present in several series of small molecule Y5 antagonists is the *trans*-(1,4-disubstituted-cyclohexyl) ring.[13] In most examples, one or both of the 1,4-substituents contain a H-bond donor two atoms away from the ring carbon, usually in the form of an amine, a sulfonamide, or an amide functionality. In compound **2**, we envisioned that this H-bond donor would be represented by the imidazole N−H. The presence of this N−H in the diarylimidazole series was required for Y5 receptor affinity, as the replacement of the imidazole with a oxazole moiety or methylation of the N−H resulted in a complete loss of activity at Y5.[23] For the introduction of a diversity of 4-functionalized cyclohexyl substituents in **2**, the common precursors, **10** and **11,** were prepared (Scheme 1). A series of compounds were synthesized from these intermediates with the aim of achieveing the goals set forth above. In this article we report the results of this work and the optimization of a novel series of Y5-receptor antagonists derived from the diarylimidazoles.

Chemistry

Substituted α-aminoacetophenones (**3**) were prepared by reaction of α-bromoacetophenones with hexamethylenetetramine, followed by acidic hydrolysis of the resulting quaternary complex.[25] Coupling of **3** with *trans*-4-substituted cyclohexanecarboxylic acids **4** or **5** using the BOP reagent afforded keto-amides **6** and **7** in good yields. Cyclization of **6** or **7** to the 2-cyclohexyl-5-phenyl-1*H*-imidazoles **8** and **9** was accomplished upon reflux with an excess of NH_4OAc in acetic acid. Hydrolysis of **8** and the hydrogenolysis of **9** led to the acid and amino derivatives **11** and **10**, respectively. These key intermediates were used to prepare the target compounds **12**−**23** shown in Table 1. Specifically, compounds **12** and **13** were prepared by coupling **10** (R_2 = 3-CF_3) with benzenesulfonyl chloride and then selectively methylating the sulfonamide nitrogen. Coupling acid **11** with benzenesulfonamide, acetyl hydrazide, or substituted amines afforded compounds **14**, **15**, and **16**−**23**, respectively.

Scheme 1. Synthesis of Compounds **12**−**23**[a]



[a] Reagents: (a) BOP, TEA, DMF, 80 °C; (b) 30 equiv of NH_4OAc, HOAc, reflux (16 h); (c) **9**, 10% Pd/C, EtOH, 50 psi H_2; (d) **8**, LiOH−THF−H_2O, reflux; (e) 1.1 equiv of $ArSO_2Cl$, 2 equiv of TEA, CH_2Cl_2, room temp (compds **12**−**14**); (f) BOP, TEA, DMF, 80 °C (compds **16**, **18**−**21**) or EDCI/DMAP (1:1), CH_2Cl_2, room temp, 16 h (cmp **15**).

Biological Results and Discussion

Several other known Y5 receptor antagonists contain 1,4-disubstituted-cyclohexane spacers substituted with benzenesulfonamide groups.[12,13,26] Indeed, when **10** was coupled with benzenesulfonyl chloride, a very potent Y5 antagonist (**12**) was obtained (Table 1). Compound **12** was about 30-fold more potent at Y5 than the lead compound **2** and also showed excellent stability in in vitro human microsomal preparations as indicated by the low predicted plasma clearance value (4 mL/min/kg) shown in Table 1. The methylated analogue **13** revealed that the sulfonamide N−H was not critical for either Y5 receptor inhibition or hERG channel blockade. Although compounds containing the benzenesulfonamide group dramatically increased receptor potency (**12** and **13**), in vitro side-effect profiling showed that they consistently inhibit hERG channel currents at very low concentrations relative to lead compound **2** (Table 1). The blocking of this cardiac K^+ channel (IKr) has been linked to drug-induced long QT syndrome (LQT)[27] which can lead to torsades de pointes, a life threatening form of arrhythmia, and subsequent ventricular fibrillation. Compound **12**, for example, inhibited the hERG channel currents by 87% at a concentration of 300 nM.

A key finding resulted when close-in modifications of **12** demonstrated that the hERG activity could in fact be substantially attenuated if the methylene linker between the cyclohexyl ring and the sulfonamide nitrogen was converted to a carbonyl group to give an acyl-

sulfonamide. The resulting compound (**14**, Table 1) exhibited a markedly reduced interaction with the hERG channel, but unfortunately a decreased affinity for the Y5 receptor. Acyl-sulfonamides have been used as bioisoteric replacements for carboxylic acids, and this reduction in hERG interaction could be attributable to the inherent acidity of **14**.

In parallel with this work, an effort was being made to replace the aryl-sulfonamide group with a more hydrophilic ring system. A series of analogues was synthesized in which five-membered ring heterocycles were directly attached to the 4-position of the cyclohexyl ring. Although these analogues were weak Y5 receptor binders,[23] the intermediate leading to an oxadiazole, i.e., the acyl hydrazide **15**, exhibited a very promising in vitro profile. Like the acyl-sulfonamide **14**, compound **15** displayed low hERG affinity, but this time with a much improved potency as a Y5 antagonist (Table 1). Furthermore, the acyl hydrazide was a more compact, hydrophilic functionality than the aryl-sulfonamides. Interestingly, during the course of our work a patent application appeared which disclosed a series of acyl-aminocarboxylic hydrazides as neuropeptide Y receptor ligands, wherein some of the examples utilized the 4-substituted-(acylhydrazido)cyclohexane ring system.[28] We were concerned, however, by the known propensity of acyl hydrazides to undergo hydrolysis in vivo with subsequent release of an acetylhydrazine moiety. In the liver, acetylhydrazine can be converted to a reactive metabolite leading to liver necrosis.[29] For this reason we began to investigate direct replacements for the acyl hydrazide group. Derivatives in which a hydrazide NH was replaced with a methylene yielded keto-amides, for example **16**, which were both less active at Y5 and more active at the hERG channel. However, reduction to the corresponding alcohols produced compounds with a more acceptable hERG profile (see **17** and **18**, Table 1). There was only a marginal chiral bias observed with regard to Y5 affinity, with the *R* enantiomer (**17**) exhibiting a 3-fold increase in potency relative to the *S* isomer (**18**) (Table 1). Preparation of the achiral amido-alcohol by removal of the terminal methyl group resulted in a substantial increase in the hERG activity (**19**, Table 1). Placement of the hydroxyl group β to the amide nitrogen turned out to be optimal for Y5 receptor affinity. Chain extension, for example, led to a much less potent Y5 antagonist, compound **23**. In addition, methylation of the β-hydroxyl group to give **21**, produced a 9-fold loss of activity (Table 1). An attempt to replace the hydroxyl group of **19** with NH_2 in order to maximize solubility resulted in a compound with significantly reduced affinity for the Y5 receptor (compound **22**). When a *gem*-dimethyl group was incorporated at the position α to the amide nitrogen in order to produce a more rigid side chain, the resulting compound, **20**, exhibited an in vitro profile that was favorable both in terms of Y5-receptor affinity (3 nM) and hERG activity (6% inhibition @ 3 μM). In addition, compound **20** was shown to be selective (<50% inhibition at 4 μM) in a battery of more than 20 in-house in vitro screens (including α1, D2, and GABA).

The pharmacokinetic properties of compound **20** were examined in rats at doses of 3.0 mg/kg (iv) and 20.0 mg/kg (po). The results shown in Table 2 suggest a compound with reasonable bioavailability (36%) and moderately high clearance (50 mL/min/mg) which limited exposure in vivo. Nevertheless, based on human liver microsomal data (Table 1), a lower clearance and hence a longer oral half-life was predicted for humans. The pharmacokinetic and in vitro pharmacological properties of **20** are such that it can now be assessed in pharmacological feeding models in rats. The results of these experiments will help determine the potential usefulness of these compounds as anorectic agents and may also help to clarify the role of Y5 as a regulator of feeding behavior in animals.

Table 2. Single Dose Rat Pharmacokinetics of Compound **20** after iv and po Administration[a]

C_{max} po,[b] ng/mL	AUC_{po},[c] (ng × h)/mL	F_{po},[d] %	CL[e] mL/min/kg	V_{ss},[f] L/kg	$T_{(1/2)po}$,[g] h
714	2380	36	50	6	13
SD = (362)[h]	(1103)[h]	(17)[h]	(4.8)[h]	(0.5)[h]	(10)[h]

[a] Rats were given either a single dose iv (3.0 mg/kg) (n = 2) or a single dose po (20.0 mg/kg) (n = 3) of **20**. [b] Maximum plasma concentration after oral dosing. [c] Estimated area under the plasma-concentration time curve after oral dosing. [d] Oral bioavailability. [e] Plasma clearance. [f] Volume of distribution at steady state. [g] Oral half-life. [h] Standard deviation.

Conclusion

We have succeeded in developing a low molecular weight (MW = 409.5) Y5 antagonist (**20**) with improved hydrophilicity (MlogP = 2.36) vs the lead compound **2** (MlogP = 3.34). An increase in structural novelty was achieved, and the hERG liability encountered with many of the compounds from the series was ultimately overcome by close-in structural modifications. Importantly, the pharmacokinetic properties of **20** represented a great improvement over that of initial 1,4-diarylimidazole structures, although the in vivo clearance in rats was substantially higher than predicted from in vitro microsomal assays. These improvements were achieved while maintaining low nanomolar potency at the Y5 receptor.

Experimental Section

Chemistry. Melting points were determined using a MEL-TEMP II melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini operating at 300 MHz or a Varian Unity 400 operating at 400 MHz. Atmospheric pressure chemical ionization (AP−CI) mass spectra were determined on a Sciex 150. Electrospray mass spectra were measured on a Waters single quad ZMD MKII. Microanalyses were performed by Robertson Microlit Laboratories, Inc. (Madison, NJ) for the elements indicated and are within 0.4% of the theoretical values unless indicated. Chromatographic separations were performed on silica gel (0.040−0.063 mm) columns by flash chromatography. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on precoated silica gel plates (Analtech, 0.25 mm). Amino-ketones (**3**, R = 3-CF_3 and R = 3-Br) were prepared according to procedures analogous to those outlined in the literature.[25]

2-Phenyl-4-[3-(trifluoromethyl)phenyl]imidazole (1). Compound **1** was synthesized according to general procedures A and B using **3** (R_2 = 3-CF_3) and benzoic acid as starting materials. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.61−7.65 (m, 3H, aromatic); 7.74−7.63 (m, 2H, aromatic); 8.27−8.36 (m, 3H, aromatic); 8.44 (s, 1H, N*H*). MS (CI): *m*/*z* (%) = 289 (100) [M + 1]$^+$. mp = 234−236 °C; Anal. ($C_{16}H_{11}N_2F_3$·HCl) C, H, N.

2-Cyclohexyl-4-[3-(trifluoromethyl)phenyl]imidazole (2). Compound **2** was synthesized according to general proce-

dures A and B using **3** (R_2 = 3-CF_3) and cyclohexanecarboxylic acid as starting materials. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.20−1.38 (m, 3H, CH_2); 1.65−1.81 (m, 5H, CH_2); 1.97−2.01 (m, 2H, CH_2); 3.06−3.13 (m, 1H, C*H*); 7.69−7.75 (m, 2H, aromatic); 8.23 (s, 1H, aromatic); 8.25 (s, 1H, aromatic); 8.32 (s, 1H, N*H*). MS (CI): *m*/*z* (%) = 295 (100) $[M + 1]^+$. mp = 245−247 °C; Anal. ($C_{16}H_{17}N_2F_3$·HCl) C, H, N.

***trans*-Cyclohexane-1,4-dicarboxylic Acid Monomethyl Ester (4).** A solution of *trans*-cyclohexane-1,4-dicarboxylic acid dimethyl ester (34.2 g, 0.171 mol) in dry methanol was brought to reflux. A solution of KOH (11.2 g, 0.171 mol) in dry methanol was added dropwise and the solution refluxed for an additional 5 h. The mixture was cooled and concentrated under reduced pressure, and the resulting solid was taken up in water. The mixture was washed with ether (3×) and brought to pH 6 with 6 N HCl. The precipitated white solid was collected via filtration, washed with water (1×), and air-dried to afford 16.2 g (51%) of **4**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.4−1.5 (m, 4H); 2.08 (m, 4H); 2.2−2.37 (m, 2H, 2 methines); 3.67 (s, OCH_3). MS (CI, negative ion mode): *m*/*z* (%) = 185 (100) $[M - 1]^-$.

General Procedure A: Amide Formation Mediated by BOP Reagent. *trans*-*N*-{2-Oxo-2-[3-(trifluoromethyl)phenyl]ethyl}(4-{[(phenylmethoxy)carbonylamino]methyl}cyclohexyl)carboxamide (7). To a solution of **3** (R_2 = 3-CF_3) (3.4 g, 0.0144 mol) and *trans*-4-{[(phenylmethoxy)carbonylamino]methyl}cyclohexanecarboxylic acid (**5**)[30] (4.2 g, 0.0144 mol) in DMF was added benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 6.4 g, 0.0144 mol) followed by triethylamine (4.0 mL, 0.0289 mol). The reaction was stirred overnight and then poured into water to produce a thick precipitate. The solid was collected on a sintered glass funnel and washed consecutively with H_2O, 3 N HCl (2×), H_2O, $NaHCO_3$ solution (2×), and H_2O. The filter cake was air-dried, added to a 500 mL round-bottom flask with toluene, and concentrated under reduced pressure. Additional toluene was added (200 mL), and the mixture was again concentrated under reduced pressure to yield *N*-{2-oxo-2-[3-(trifluoromethyl)phenyl]ethyl}(4-{[(phenylmethoxy)carbonylamino]methyl}cyclohexyl)carboxamide as a solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.8−0.93 (m, 2H); 1.2−1.37 (m, 3H); 1.71 (br s, 4H); 2.14 (t, 1H); 2.84 (br s, 2H); 4.57 (br s, 2H); 4.99 (s, 2H); 7.2−7.38 (m, 5H); 7.77(t, 1H); 8.01 (d, J = 8.0 Hz, 1H); 8.14−8.26 (m, 3H). MS 477 $(M + H)^+$.

***trans*-Methyl 4-{4-[3-(Trifluoromethyl)phenyl]imidazol-2-yl}cyclohexanecarboxylate (8, R_2 = 3-CF_3).** Compound **8** was synthesized according to general procedures A and B using **3** (R_2 = 3-CF_3) and **4** as starting materials. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.38−1.56 (m, 2H, CH_2); 1.63−1.80 (m, 2H, CH_2); 1.92−2.17 (m, 4H, CH_2); 2.30−2.51 (m, 1H, C*H*); 2.95−3.16 (m, 1H, C*H*); 3.60 (s, 3H, CH_3); 7.65−7.85 (m, 3H, aromatic); 8.16−8.36 (m, 3H, aromatic). MS (CI): *m*/*z* (%) = 354 (100) $[M + 2]^+$.

***trans*-Methyl 4-[4-(3-Bromophenyl)imidazol-2-yl]cyclohexanecarboxylate (8, R_2 = 3-Br).** Compound **8** was synthesized according to general procedures A and B using **3** (R_2 = 3-Br) and **4** as starting materials. ^{1}H NMR (300 MHz, CD_3OD): δ 1.41−1.81 (m, 4H, CH_2); 2.00−2.15 (m, 4H, CH_2); 2.48 (m, 1H, C*H*); 3.11 (m, 1H, C*H*); 3.68 (s, 3H, CH_3); 7.41 (m, 1H, aromatic); 7.60 (d, J = 4.1 Hz, 1H, aromatic); 7.780 (d, J = 4.1 Hz, 1H, aromatic); 7.95 (s, 1H, aromatic); 7.99 (s, 1H, aromatic). MS (CI): *m*/*z* (%) = 364 (100) $[M + 1]^+$.

General Procedure B: Cyclization of Keto-amides with Ammonium Acetate. *trans*-(Phenylmethoxy)-*N*-[(4-{4-[3-(trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)methyl]carboxamide (9). To a solution of **7** (5.4 g, 0.0113 mol) in acetic acid was added ammonium acetate (26 g, 0.34 mol). The resulting homogeneous mixture was refluxed overnight and then concentrated under reduced pressure. The residue was partitioned between 10% NaOH and EtOAc (1:1), and the EtOAc layer was washed with 10% NaOH (3×) and brine. The EtOAc was dried (Na_2SO_4) and concentrated under reduced pressure to afford the title compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.02−1.18 (m, 2H), 1.46−1.62 (m, 3H), 1.86−1.96 (m, 2H), 2.12−2.21 (m, 2H), 2.74 (m, 1H), 3.10 (t, J = 6 Hz, 2H), 4.91 (t, 1H, N−H), 5.10 (s, 2H), 7.22 (s, 1H), 7.32−7.37 (m, 5H), 7.45 (d, J = 4.9 Hz, 2H), 7.87 (m, 1H), 7.95 (s, 1H). MS 458 $(M + H)^+$.

***trans*-(4-{4-[3-(Trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)methylamine (10).** An ethanolic solution of **9** (4.15 g, 0.0091 mol) and 10% Pd/carbon (400 mg) was shaken overnight under 55 psi of hydrogen in a Paar apparatus. The mixture was filtered through Celite and concentrated under reduced pressure to give a foam. Trituration with EtOAc/hexanes/MeOH produced the title compound as a filterable solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.93−1.05 (m, 2H), 1.30−1.4 (m, 2H), 1.42−1.55 (m, 2H), 1.85 (d, J = 12.8 Hz, 2H), 1.99 (d, J = 11.6 Hz, 2H), 2.4−2.5 (m, 1H), 2.56−2.66 (m, 1H), 7.43−7.48 (m, 1H), 7.50−7.55 (m, 1H), 7.63 (s, 1H), 7.99 (d, J = 7.9 Hz, 1H), 8.02 (s, 1H). MS 324 $(M + H)^+$.

***trans*-4-{4-[3-(Trifluoromethyl)phenyl]imidazol-2-yl}cyclohexanecarboxylic acid (11, R_2 = 3-CF_3).** To a solution of **8** (5.2 g, 13.4 mmol) in THF−H_2O (1:1, 200 mL) was added LiOH−H_2O (3.4 g, 80.3 mmol). The resulting mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The residue was diluted with H_2O and acidified to pH 5−6. The aqueous layer was extracted with 3 × 100 mL of EtOAc, and the EtOAc layer was washed with brine. The EtOAc was dried (Na_2SO_4) and concentrated under reduced pressure to afford the title compound. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.38−1.6 (m, 4H, CH_2); 196−2.10 (m, 4H, CH_2); 2.20−2.32 (m, 1H, C*H*); 2.60−2.74 (m, 1H, C*H*); 7.64−7.60 (m, 2H, aromatic); 7.64 (s, 1H, aromatic); 7.96−8.08 (m, 2H, aromatic). MS (CI): *m*/*z* (%) = 339 (100) $[M + 1]^+$.

***trans*-(Phenylsulfonyl)[(4-{4-[3-(trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)methyl]amine (12).** A solution of **10** (R_2 = 3-CF_3) (351 mg, 1.09 mmol) and triethylamine (110 mg, 1.09 mmol) in CH_2Cl_2 was treated with benzenesulfonyl chloride (192 mg, 1.09 mmol) via syringe in a dropwise fashion. The resulting mixture was stirred at room temperature for 2 h, diluted with CH_2Cl_2, and washed in a separatory funnel with aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a foam. Preparative plate chromatography (2 × 2 mm, 10% MeOH/CH_2Cl_2 eluent) yielded the title compound as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95−1.08 (m, 2H, cyclohex.); 1.40−1.58 (m, 3H, cyclohex.); 1.85 (d, J = 11.8 Hz, 2H); 2.09 (d, J = 11.5 Hz, 2H); 2.67−2.78 (m, 1H, cyclohex.); 2.78 (t, 2H, CH_2N); 7.21 (s, 1H, aromatic); 7.41 (m, 2H, aromatic); 7.50 (m, 2H, aromatic); 7.57 (m, 1H, aromatic); 7.8−7.9 (m, 4H, aromatic). MS (ES): *m*/*z* = 464 $[M + 1]^+$. The oxalate salt was prepared by adding a solution of oxalic acid (1 equiv) in EtOAc/MeOH to a solution of the product in ether. Anal. ($C_{23}H_{24}N_3O_2F_3S$. $C_2H_2O_4$) C, H, N.

***trans*-Methyl(phenylsulfonyl)[(4-{4-[3-(trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)methyl]amine (13).** A solution of **12** (73 mg, 0.158 mmol) in anhydrous THF was treated with sodium bis(trimethylsilyl)amide (1 N in THF, 158 μL) dropwise at room temperature. After 5 min, iodomethane (22.3 mg, 0.158 mmol) was added and the reaction stirred overnight. The reaction was partitioned between EtOAc and $NaHCO_3$ (aq) and the organic layer dried (Na_2SO_4) and concentrated. Preparative plate chromatography (2 × 2 mm, 10% MeOH/CH_2Cl_2 eluent) yielded the title compound as a clear oil in 40% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.06−1.08 (m, 2H, cyclohex); 1.54−1.72 (m, 3H, cyclohex); 1.97 (d, J = 11.2 Hz, 2H); 2.19 (d, J = 11.5 Hz, 2H); 2.74 (s, 3H, CH_3); 2.80 (m, 1H, C*H*); 2.85 (d, J = 7.51 Hz, 2H, CH_2−N); 7.26 (s, 1H, aromatic); 7.45 (d, J = 4.81 Hz, 2H); 7.52−7.62 (m, 3H, aromatic); 7.79 (d, J = 8.79 Hz, 2H); 7.89 (m, 1H); 7.96 (s, 1H). MS (CI): *m*/*z* (%) = 395 (54) $[M + 1]^+$; 147 (100). The oxalate salt was prepared by adding a solution of oxalic acid (1 equiv) in EtOAc/MeOH to a solution of the product in ether. mp = 220−221 °C; Anal. ($C_{24}H_{26}N_3O_2F_3S$. $C_2H_2O_4$) C, H, N.

***trans*-*N*-(Phenylsulfonyl)(4-{4-[3-(trifluoromethyl)-phenyl]imidazol-2-yl}cyclohexyl)carboxamide (14).** To an ice−water cooled mixture of **11** (100 mg, 0.30 mmol), benzenesulfonamide (47 mg, 0.30 mmol), and DMAP (36 mg, 0.30 mmol) in dichloromethane (10 mL) under nitrogen was added

EDCI (57 mg, 0.30 mmol). The mixture was stirred at room temperature for overnight, concentrated, and chromatographed on silica gel (19:1 EtOAc-MeOH eluent) to afford the title compound. ^{1}H NMR (400 MHz, CD_3OD): δ 1.35−1.75 (m, 4H, cyclohex); 1.82−2.14 (m, 4H, cyclohex); 2.20−2.38 (m, 1H, cyclohex); 2.65−2.80 (m, 1H, cyclohex); 7.30−7.55 (m, 5H, aromatic); 7.75−7.90 (m, 3H, aromatic); 7.98 (s, 1H, aromatic). MS (ES): m/z = 478 (100) $[M + 1]^+$. Anal. ($C_{23}H_{22}N_3O_3F_3S$): Calcd 57.85, 4.64, 8.80. Found 55.72, 4.40, 8.42

***trans*-*N*-[(4-{4-[3-(Trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)carbonylamino]acetamide (15).** A mixture of **11** (127 mg, 0.376 mmol), acetic hydrazide (28 mg, 0.376 mmol), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 166 mg, 0.376 mmol), and triethylamine (38 mg, 0.376 mmol) in DMF (4 mL) was heated at 60 °C for 1.5 h with stirring. The mixture was poured into water and extracted with EtOAc (3×). The combined organic layers were washed with 10% NaOH solution (2×) and brine (1×) and dried (Na_2SO_4). After concentrating under reduced pressure, the crude product was chromatographed using preparative TLC plates (2 × 2 mm, 10% MeOH/CH_2Cl_2 eluent) to afford the title compound. ^{1}H NMR (300 MHz, CD_3OD): δ 1.72 (m, 4H, cyclohex.); 1.98−2.15 (m, 4H, cyclohex.); 2.0 (s, 3H, CH_3); 2.37 (m, 1H, C*H*); 2.81 (m, 1H, C*H*); 7.42 (s, 1H, aromatic); 7.52 (m, 2H, aromatic); 7.92 (d, 1H, aromatic); 8.03 (s, 1H, aromatic). MS (CI): m/z (%) = 395 (54) $[M + 1]^+$; 147 (100). mp = 230−232 °C; Anal. ($C_{19}H_{21}N_4O_2F_3$): Calcd 57.86, 5.37, 14.21. Found 57.00, 5.06, 13.87.

***trans*-{4-[4-(3-Bromophenyl)imidazol-2-yl]cyclohexyl}-*N*−(2-oxopropyl)carboxamide (16).** To a cooled solution (−78 °C to −60 °C) of oxalyl chloride (2.0 M, 61.5 μL, 0.12 mmol) in CH_2Cl_2 (5 mL) was added DMSO (17.5 μL, 0.25 mmoL) dropwise. The mixture was stirred 2 min at the same temperature. *N*-(2-Hydroxypropyl)(4-[4-(3-bromophenyl)imidazol-2-yl}cyclohexyl]carboxamide (prepared from 1-aminopropan-2-ol and **11** [R_2 = Br] using procedures analogous to those used for **17** below) (50 mg, 0.12 mmol) in 2 mL of CH_2Cl_2 was added. The mixture was stirred for 15 min at the same temperature, and then TEA (85 μL, 0.60 mmol) was added. The mixture was stirred another 5 min, warmed to room temperature, and diluted with water. The organic layer was separated, dried over Na_2SO_4, concentrated under vacuum, and purified by flash chromatography (90:10:1 CH_2Cl_2/MeOH/NH_4OH) to afford the title compound. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.40−1.55 (m, 4H, CH_2); 1.78−1.86 (m, 2H, CH_2); 2.04 (s, 3H, CH_3); 2.22 (m, 1H, C*H*); 2.61 (m, 1H, C*H*); 3.21−3.39 (m, 2H, CH_2); 3.85 (d, J = 5.7 Hz, 2H, −COCH_2); 7.25−7.38 (m, 2H, aromatic,); 7.55 (s, 1H, aromatic); 7.77 (d, J = 4.5 Hz, aromatic); 7.89 (s, 1H, N*H*), 8.09 (m, 1H, aromatic) 1190 (br, 1H, CON*H*). MS (ES): m/z = 406 (100) $[M + 1]^+$. Anal. ($C_{19}H_{22}N_3O_3Br$) C, H, N.

***trans*-*N*-((2*R*)-2-Hydroxypropyl)(4-{4-[3-(trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)carboxamide (17).** Compound **17** was synthesized from **11** (R_2 = 3-CF_3) and 1-aminopropan-2-ol using general procedure A. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98 (d, J = 6.3 Hz, 3H, CH_3); 1.39−1.60 (m, 4H, CH_2); 1.76−1.85 (m, 2H, CH_2); 1.86−2.05 (m, 2H, CH_2); 2.12 (m, 1H, C*H*); 2.65 (m, 1H, C*H*); 2.93−3.05 (m, 2H, CH_2); 3.65 (m, 1H, C*H*); 4.65 (d, J = 5.1 Hz, 1H, −O*H*); 7.40−7.56 (m, 2H, aromatic); 7.67 (s, 1H, N*H*); 7.70−7.81 (m, 1H, aromatic), 7.95−8.09 (m, 2H, aromatic) 11.90 (s, 1H, CON*H*). MS (CI): m/z (%) = 396 (100) $[M + 1]^+$. The oxalate salt was prepared by adding a solution of oxalic acid (1 equiv) in EtOAc/MeOH to a solution of the product in ether. mp = 164−167 °C; Anal. ($C_{20}H_{24}N_3O_2F_3 \cdot C_2H_2O_4$) C, H, N.

***trans*-*N*-((2S)-2-Hydroxypropyl)(4-{4-[3-(trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)carboxamide (18).** Compound **18** was synthesized by the general procedure A. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.99 (d, J = 6.0 Hz, 3H, CH_3); 1.38−1.62 (m, 4H, CH_2); 1.78−1.90 (m, 2H, CH_2); 1.95−2.08 (m, 2H, CH_2); 2.20 (m, 1H, C*H*); 2.64 (m, 1H, C*H*); 2.90−3.08 (m, 2H, CH_2); 3.60 (m, 1H, C*H*); 4.65 (d, J = 4.8 Hz, 1H, −O*H*); 7.46−7.79 (m, 4H, aromatic, N*H*); 7.90−8.17 (m, 2H, aromatic) 11.88 (s, 1H, −CON*H*). MS (CI): m/z (%) = 396 (100) $[M + 1]^+$. Anal. ($C_{20}H_{24}N_3O_2F_3 \cdot 0.5H_2O$) C, H, N.

***trans*-*N*-(2-Hydroxyethyl)(4-{4-[3-(trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)carboxamide (19).** A mixture of **11** (R_2 = 3-CF_3) (50 mg, 0.148 mmol), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 65 mg, 0.148 mmol), and ethanolamine (27 mg, 0.444 mmol) in DMF was stirred at room temperature for 16 h. The mixture was diluted with water and extracted with EtOAc. The combined organics were washed with 10% NaOH solution (3×) and brine (1×), dried (Na_2SO_4), and concentrated under reduced pressure to give crude product. The residue was triturated with ether to give the title compound as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 1.46−1.64 (m, 4H, 2 × CH_2); 1.88−2.08 (m, 4H, 2 × CH_2); 2.17 (m, 1H, C*H*); 2.71 (m, 1H, C*H*); 3.31 (dd, 2H, CH_2); 3.62 (t, 2H, CH_2); 7.17 (s, 2H, aromatic); 7.40 (d, J = 4.7 Hz, 2H, aromatic); 7.77 (t, 1H, −N*H*); 7.86 (s, 1H, aromatic). MS (CI): m/z (%) = 382 (88) $[M + 1]^+$; 293 (100). Anal. ($C_{19}H_{22}N_3O_2F_3$) C, H, N.

***trans*-*N*-(2-Hydroxy-*tert*-butyl)(4-{4-[3-(trifluoromethyl)phenyl]imidazol-2-yl}cyclohexyl)carboxamide (20).** This compound was synthesized from 2-amino-2-methylpropan-1-ol and **11** (R_2 = 3-CF_3) using a method analogous to that used for **19**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (s, 6H, (CH_3)$_2$); 1.62 (m, 4H, 2 × CH_2); 1.94 (m, 2H, cyclohex); 2.10 (m, 3H, cyclohex); 2.78 (m, 1H, C*H*); 3.57 (s, 2H, CH_2O); 6.02 (s, 1H, −OH); 7.23 (s, 1H, aromatic); 7.42 (m, 2H, aromatic); 7.81 (t, 1H, aromatic); 7.91 (s, 1H, aromatic). MS (CI): m/z (%) = 410 (100) $[M + 1]^+$. The oxalate salt was prepared by adding a solution of oxalic acid (1 equiv) in EtOAc/MeOH to a solution of the product in ether. mp = 181−183 °C; Anal. ($C_{21}H_{26}N_3O_2F_3 \cdot C_2H_2O_4$) C, H, N.

***trans*-4-[4-(3-Trifluoromethylphenyl)-1*H*-imidazol-2-yl]cyclohexanecarboxylic Acid (2-Methoxyethyl)amide (21).** This compound was synthesized from 2-methoxyethylamine and **11** (R_2 = 3-CF_3) using a method analogous to that used for **19**. ^{1}H NMR (300 MHz, CD_3OD): δ 1.57−1.75 (m, 4H, CH_2); 1.90−1.97 (m, 2H, CH_2); 2.05−2.15 (m, 2H, CH_2); 2.30 (m, 1H, C*H*); 2.79 (m, 1H, C*H*); 3.25−3.40 (m, 2H, CH_2); 3.38 (s, 3H, OCH_3); 3.45−3.50 (m, 2H, CH_2); 7.41 (s, 1H, aromatic); 7.47−7.58 (m, 2H, aromatic), 7.91 (d, J = 6.8 Hz, 1H, aromatic); 8.00 (s, 1H, 11.90 aromatic). MS (CI): m/z (%) = 396 (100) $[M + 1]^+$. The oxalate salt was prepared by adding a solution of oxalic acid (1 equiv) in EtOAc/MeOH to a solution of the product in ether. mp = 176−178 °C; Anal. ($C_{20}H_{24}N_3O_2F_3 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

***trans*-4-[4-(3-Trifluoromethylphenyl)-1*H*-imidazol-2-yl]cyclohexanecarboxylic Acid (2-Aminoethyl)amide (22).** This compound was synthesized from ethylenediamine and **11** (R_2 = 3-CF_3) using a method analogous to that used for **19**. ^{1}H NMR (300 MHz, CD_3OD): δ 1.60−1.76 (m, 4H, CH_2); 1.96−2.08 (m, 4H, CH_2); 2.30 (m, 1H, C*H*); 2.64 (m, 1H, C*H*); 2.78−2.85 (m, 2H, CH_2); 3.25−3.35 (m, 2H, CH_2); 7.41 (s, 1H, aromatic); 7.50−7.58 (m, 2H, aromatic), 7.90 (d, J = 7.4 Hz, 1H, aromatic); 8.00 (s, 1H, 11.90 aromatic). MS (CI): m/z (%) = 389 (100) [M-1]$^+$. The oxalate salt was prepared by adding a solution of oxalic acid (1 equiv) in EtOAc/MeOH to a solution of the product in ether. Anal. ($C_{19}H_{23}N_4OF_3 \cdot 2C_2H_2O_4$) C, H, N.

***trans*-4-[4-(3-Trifluoromethylphenyl)-1*H*-imidazol-2-yl]cyclohexanecarboxylic Acid (3-Hydroxypropyl)amide (23).** ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.42−1.5 (m, 6H, CH_2); 1.75−1.82 (m, 2H, CH_2); 1.90−2.04 (m, 2H, CH_2); 2.12 (m, 1H, C*H*); 2.63 (m, 1H, C*H*); 2.98−3.09 (m, 2H, CH_2); 3.35−3.42 (m, 2H, CH_2); 4.42 (t, 1H, O*H*); 7.40−7.56 (m, 2H, aromatic); 7.68 (s, 1H, N*H*); 7.75 (m, 1H, aromatic), 7.96−8.05 (m, 2H, aromatic) 11.93 (s, 1H, CON*H*). MS (CI): m/z (%) = 396 (100) $[M + 1]^+$. Anal. ($C_{120}H_{24}N_3O_2F_3$) C, H, N,: calcd, 60.75, 6.12, 10.63; found, 58.97, 6.36, 9.81.

Biology. Radioligand Binding Assay. S*f*9 cell pellets (ref 32) are resuspended in homogenization buffer (10 mM HEPES, 250 mM sucrose, 0.5 μg/mL leupeptin, 2 μg/mL Aprotinin, 200 μM PMSF, and 2.5 mM EDTA, pH 7.4) and homogenized using a Polytron homogenizer (setting 5 for 30 s). The homogenate

is centrifuged (536*g*/10 min/4 °C) to pellet the nuclei. The supernatant containing isolated membranes is decanted to a clean centrifuge tube, centrifuged (48 000*g*/ 30 min, 4 °C) and resuspended in 30 mL of homogenization buffer. This centrifugation and resuspension step is repeated twice. The final pellet is resuspended in ice cold Dulbecco's PBS containing 5 mM EDTA and stored in frozen aliquots until needed at −80 °C. The protein concentration of the resulting membrane preparation (P2 preparation) is measured using the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). By this measure, a 1-L culture of cells typically yields 50−75 mg of total membrane protein.

Purified P2 membranes are thawed, centrifuged, and washed with PBS and resuspended by homogenization in binding buffer (50 mM Tris-HCl, 5 mM KCl, 120 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% BSA, pH 7.4).

For competition analysis membranes (5−50 μg) are added to polypropylene tubes containing 0.050 nM [^{125}I]PYY (porcine) and 2 μL of test compound in DMSO (1− 4 μM final concentrated). Nonspecific binding is determined in the presence of 1 μM NPY (human, American Peptide Co., Sunnyvale, CA) and accounts for less that 10% of total binding. Following a 2-h incubation at room temperature, the reaction is terminated by rapid vacuum filtration. Samples are filtered over presoaked (in 1.0% polyethyeneimine for 2 h prior to use) GF/C Whatman filters and rinsed two times with 5 mL of cold binding buffer without BSA. Remaining bound radioactivity is quantified by gamma counting. K_i and Hill coefficient (n_H) are determined by fitting the Hill equation to the measured values with the aid of Sigmaplot software (SPSS Inc., Chicago).

For saturation binding analysis, membranes (5−50 μg) are added to polypropylene tubes containing 0.010−0.500 nM [^{125}I]PYY (porcine, New England Nuclear Corp., Boston, MA).

Electrophysiology Recordings. Standard whole-cell recording[31] was carried out on the COS cells transiently transfected with HERG K^+ channels. Microelectrodes were pulled from 1.5 mm glass (World Precision Instruments, TW100−4) on a horizontal puller (Sutter Instrument Company, Model P-87) and had resistances between 2 and 3 MΩ when filled with internal solution. The internal solution contains: 100 mM KF, 40 mM KCl, 5 mM NaCl, 10 mM EGTA, and 10 mM HEPES, adjusted to pH 7.4 with KOH. The cells were perfused with external solution containing 140 mM NaCl, 2 mM $CaCl_2$, 5 mM KCl, 2 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES, adjusted to pH 7.4 with NaOH. Currents were filtered at 5 kHz on an Axopatch-1D amplifier (Axon Instruments) and recorded onto a PC with sample rate of 1 kHz using pClamp 8 (Axon Instruments). Data were analyzed using Clampfit (Axon Instruments) and Origin softwares (Microcal). Stock solutions for compounds were made in DMSO at a concentration of 10 mM. Final test concentration was achieved by diluting the stock solution directly into the external solution.

Metabolic Stability. Compounds were incubated with pooled human liver microsomes. The rates of oxidative metabolism were measured under the following conditions: compound, 1 μM final concentration; final microsomal protein concentration approximately 1 mg/mL; NADPH, 0.5 mM; pH 7.4 sodium phosphate buffer. Incubations were performed at 39 °C and were initiated by the addition of NADPH. Samples (50 μL) were taken from each incubation well at about 0.25, 1, 5, 10, and 30 min and added to 75 μL of ice-cold acetonitrile. The aliquot mixtures were mixed and then centrifuged at 3000 rpm for 15 min. The supernatant was analyzed for parent compound using LC/MS, and the percent metabolized was calculated based on disappearance of parent compound.

Pharmacokinetics. The pharmacokinetics of **20** was investigated in Sprague−Dawley rats following a single intravenous dose of 3 mg/kg (n = 2 rats) and a single oral dose of 20 mg/kg (n = 3 rats). Preparation of iv dosing solution: 7.5 mg of **20** was weighed and transferred to a 5 mL volumetric flask. Vehicle (50% PEG/H_2O) was added and brought up to volume. Preparation of po dosing solution: 20 mg of **20** was weighed out and transferred to a 10 mL volumetric flask, 20% vitamin E added, and the solution brought up to volume with H_2O. The solution was sonicated for 15 min prior to dosing. Samples of iv and po dosing solutions were collected immediately after preparation and immediately before dosing and stored in polypropylene microcentrifuge tubes at 4 °C. The iv dosing solutions were administered via catheter (2 mL/kg) followed by a 1 mL saline wash. The oral dosing solutions were administered via oral gavage. Sample collection: blood samples (0.4 mL/animal) were collected at predose, 5, 10, 20, and 40 min, 1, 1.5, 2, 4, 6, 8, 10, and 24 h after iv dosing and at predose, 15, and 30 min, 1, 1.5, 2, 3, 4, 6, 8, 10, and 24 h after oral dosing. Blood samples from each animal were placed into tubes containing 20 units of sodium heparin. Each animal was injected intravenously with 0.4 mL of 0.9% saline after blood sample collection. Plasma samples were obtained by centrifugation of 0.4 mL of whole blood at 12000 rpm for 10 min and stored at −20 °C in polypropylene microcentrifuge tubes until needed. Sample preparation: Methanol/water 50/50 (20 μL) was added to a microcentrifuge tube containing 200 μL of plasma sample, followed by 200 μL of acetonitrile. The tubes were capped, vortexed, and centrifuged at 10 000 rpm for 20 min. The supernatant was transferred to injection vials for LC analysis.

Acknowledgment. The technical support of Chu Ngo, Charles Cheng, Jennifer Kwash, John Dessaint, Weifeng Yu (electrophysiology) and Melissa Hill (in vitro pharmacology) is gratefully acknowledged. The authors also thank Jean Simmermacher, Christine Orozco, Fengang Zhu, Phil Joyce, Yi Luo, and Du-Shieng Chien for DMPK support.

References

(1) Stanley, B. G. Neuropeptide Y in Multiple Hypothalamic Sites Controls Eating Behavior, Endocrine, and Autonomic Systems for Body Energy Balance. *The Biology of Neuropeptide Y and Related Peptides*; Humana: Totowa, NJ, 1993; pp 457−509.
(2) Widdowson, P. S.; Williams, G. Regulation of appetite. *The Diabetes Annual*, 12th ed.; Elsevier Science: New York, 1999.
(3) *The Biology of Neuropeptide Y and Related Peptides*; Humana Press: Totowa, NJ, 1993.
(4) Stanley, B. G.; Magdalin, W.; Seirafi, A.; Nguyen, M. M.; Leibowitz, S. F. Evidence for Neuropeptide Y Mediation of Eating Produced by Food Deprivation and for a Variant of the Y1 Receptor Mediating This Peptide's Effect. *Peptides* **1992**, *13*, 581−587.
(5) Kordik, C. P.; Reitz, A. B. Pharmacological treatment of obesity: therapeutic strategies. *J. Med. Chem.* **1999**, *42*, 181−201.
(6) Gerald, C.; Walker, M. W.; Criscione, L.; Gustafson, E. L.; Batzl-Hartmann, C. et al. A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature* **1996**, *382*, 168−171.
(7) Schaffhauser, A. O.; Stricker-Krongrad, A.; Brunner, L.; Cumin, F.; Gerald, C. et al. Inhibition of food intake by neuropeptide Y Y5 receptor antisense oligodeoxynucleotides. *Diabetes* **1997**, *46*, 1792−1798.
(8) Tang-Christensen, M.; Kristensen, P.; Stidsen, C. E.; Brand, C. L.; Larsen, P. J. Central administration of Y5 receptor antisense decreases spontaneous food intake and attenuates feeding in response to exogenous neuropeptide Y. *J. Endocrinol.* **1998**, *159*, 307−312.
(9) Flynn, M. C.; Turrin, N. P.; Plata-Salaman, C. R.; Ffrench-Mullen, J. M. Feeding response to neuropeptide Y-related compounds in rats treated with Y5 receptor antisense or sense phosphothio-oligodeoxynucleotide. *Physiol. Behav.* **1999**, *66*, 881−884.
(10) Daniels, A. J.; Grizzle, M. K.; Wiard, R. P.; Matthews, J. E.; Heyer, D. Food intake inhibition and reduction in body weight gain in lean and obese rodents treated with GW438014A, a potent and selective NPY−Y5 receptor antagonist. *Regul. Pept.* **2002**, *106*, 47−54.
(11) Criscione, L.; Rigollier, P.; Batzl-Hartmann, C.; Rueger, H.; Stricker-Krongrad, A. et al. Food intake in free-feeding and energy-deprived lean rats is mediated by the neuropeptide Y5 receptor. *J. Clin. Invest.* **1998**, *102*, 2136−2145.
(12) Kordik, C. P.; Luo, C.; Zanoni, B. C.; Dax, S. L.; McNally, J. J. et al. Aminopyrazoles with high affinity for the human neuropeptide Y5 receptor. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2283−2286.
(13) Youngman, M. A.; McNally, J. J.; Lovenberg, T. W.; Reitz, A. B.; Willard, N. M. et al. alpha-Substituted N−(sulfonamido)-alkyl-beta-aminotetralins: potent and selective neuropeptide Y Y5 receptor antagonists. *J. Med. Chem.* **2000**, *43*, 346−350.

(14) Sato, N.; Takahashi, T.; Shibata, T.; Haga, Y.; Sakuraba, A. et al. Design and synthesis of the potent, orally available, brain-penetrable arylpyrazole class of neuropeptide Y5 receptor antagonists. *J. Med. Chem.* **2003**, *46*, 666−669.
(15) Kanatani, A.; Ishihara, A.; Iwaasa, H.; Nakamura, K.; Okamoto, O. et al. L-152, 804: orally active and selective neuropeptide Y Y5 receptor antagonist. *Biochem. Biophys. Res. Commun.* **2000**, *272*, 169−173.
(16) Block, M. H.; Boyer, S.; Brailsford, W.; Brittain, D. R.; Carroll, D. et al. Discovery and optimization of a series of carbazole ureas as NPY5 antagonists for the treatment of obesity. *J. Med. Chem.* **2002**, *45*, 3509−3523.
(17) Satoh, Y.; Hatori, C.; Ito, H. Novel potent antagonists of human neuropeptide Y−Y5 receptor. Part 4: tetrahydrodiazabenzazulene derivatives. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1009−1011.
(18) Elliott, R. L.; Oliver, R. M.; Hammond, M.; Patterson, T. A.; She, L. et al. In vitro and in vivo characterization of 3-[2-[6-(2-*tert*-butoxyethoxy)pyridin-3-yl]-1H-imidazol-4-yl]benzonitrile hydrochloride salt, a potent and selective NPY5 receptor antagonist. *J. Med. Chem.* **2003**, *46*, 670−673.
(19) Della Zuana, O.; Sadlo, M.; Germain, M.; Feletou, M.; Chamorro, S. et al. Reduced food intake in response to CGP 71683A may be due to mechanisms other than NPY Y5 receptor blockade. *Int. J. Obes. Relat. Metab. Disord.* **2001**, *25*, 84−94.
(20) Kanatani, A.; Mashiko, S.; Murai, N.; Sugimoto, N.; Ito, J. et al. Role of the Y1 receptor in the regulation of neuropeptide Y-mediated feeding: comparison of wild-type, Y1 receptor-deficient, and Y5 receptor-deficient mice. *Endocrinology* **2000**, *141*, 1011−1016.
(21) Thurkauf, A.; Yuan, J.; Hutchison, A.; Blum, C. A.; Elliot, R. L. et al. Preparation of diarylimidazoles as neuropeptide Y receptor ligands: PCT Int. Appl. WO 9901128, 1999; 46 pp.
(22) Thurkauf, A.; Yuan, J.; Hutchison, A.; Blum, C. A.; Elliot, R. L. et al. Pharmaceutical Compositions Containing Certain Diarylimidazole Derivatives: US 6515133, 2003.
(23) Blum, C. A.; Zheng, X. Unpublished Results.
(24) Stamford, A. W.; Boyle, C. D.; Huang, Y. Preparation of Substituted Imidazole Neuropeptide Y Y5 Receptor Antagonists: WO 0144201, 2000; 87 pp.
(25) Leclerc, G.; Bizec, J. C.; Bieth, N.; Schwartz, J. Synthesis and Structure−Activity Relationships Among -Adrenergic Receptor Agonists of the Phenylethanolamine Type. *J. Med. Chem.* **1980**, *23*, 738−744.
(26) Rueeger, H.; Rigollier, P.; Yamaguchi, Y.; Schmidlin, T.; Schilling, W. et al. Design, synthesis and SAR of a series of 2-substituted 4-amino-quinazoline neuropeptide Y Y5 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1175−1179.
(27) Curran, M. E.; Splawski, I.; Timothy, K. W.; Vincent, G. M.; Green, E. D. et al. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* **1995**, *80*, 795−803.
(28) Monge Vega, A.; Aldana Moraza, I.; Caignard, D.-H.; Duhault, J.; Boutin, J. et al. Preparation of Acylaminocarboxylic Hydrazides as Neuropeptide Y Receptor Ligands: EP 1010691, 2000; 38 pp.
(29) Vandenberghe, J. *Toxicology: Principles and Applications*; CRC Press: Boca Raton, 1996; p 710.
(30) Curtin, M. L.; Davidsen, S. K.; Heyman, H. R.; Garland, R. B.; Sheppard, G. S. et al. Discovery and evaluation of a series of 3-acylindole imidazopyridine platelet-activating factor antagonists. *J. Med. Chem.* **1998**, *41*, 74−95.
(31) Cheng, C. S.; Alderman, D.; Kwash, J.; Dessaint, J.; Patel, R. et al. A high-throughput HERG potassium channel function assay: an old assay with a new look. *Drug Dev. Ind. Pharm.* **2002**, *28*, 177−191.
(32) Bennett, M.; Brodbeck, R.; Krause, J. Preparation of Chimeric Neuropeptide Y Receptors and Application to Drug Screening Assay: PCT Int. Appl. WO 0155103, 2001; 72 pp.

JM030490G