Letters

Design and Synthesis of the Potent, Orally Available, Brain-Penetrable Arylpyrazole Class of Neuropeptide Y5 Receptor Antagonists

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Abstract: Novel arylpyrazole derivatives were synthesized and evaluated as neuropeptide Y (NPY) Y5 receptor antagonists. Compound (−)-**7**, which features a novel chiral 2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene moiety, showed good binding affinity and antagonistic activity for the Y5 receptor. After intracerebroventricular administration in SD rats, (−)-**7** significantly inhibited food intake that was induced by the centrally administered Y5-preferring agonist, bovine pancreatic polypeptide, but had only a negligible effect on NPY-induced feeding.
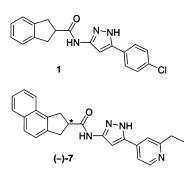
Introduction. Neuropeptide Y (NPY) is a highly conserved 36 amino acid peptide with potent, centrally mediated orexigenic effects.[1−4] Chronic administration of NPY into the brain induces hyperphagia and body weight gain.[5,6] Concentrations of NPY and its mRNA in the hypothalamus were markedly increased during food deprivation and in some genetic models of obesity in rodents.[7−11] In addition, NPY-deficient ob/ob mice are less obese and have reduced food intake when compared with ob/ob mice.[12] These data suggest that NPY may be one of the major regulators of physiological feeding behavior.

Five distinct types of G-protein coupled NPY receptors (Y1, Y2, Y4, Y5, and y6) have been cloned.[13] On the basis of the correlation between the in vitro functional and binding activity of different peptide agonists and their potent stimulation of food intake in rodent models, the Y5 receptor has been found to be a major feeding receptor.[14] In addition, a reduction in food intake induced by NPY and related peptides in Y5 receptor deficient (Y5 −/−) mice were observed,[15,16] thereby supporting the role of the Y5 receptor in food intake.
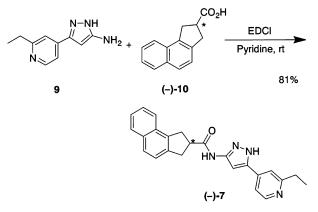
On the basis of these findings, the antagonism of the Y5 receptor may have considerable therapeutic benefits in treating obesity. CGP 71683 was the first Y5 antagonist reported in the literature, along with a description of its pharmacological data,[17,18] and many structural classes of compounds that target the Y5 receptor have
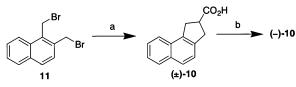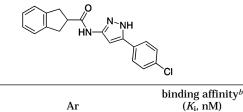
Chart 1

Scheme 1

only recently begun to appear in the literature.[19−23] Since in vivo specificity of those compounds is unclear, additional structurally diverse Y5 antagonists are important in order to probe the role of the Y5 receptor. We screened our in-house chemical collections for structurally diverse novel Y5 leads that have molecular weights in the neighborhood of 350 and found the arylpyrazole class compound, *N*-[5-(4-chlorophenyl)-1*H*-pyrazol-3-yl]-2-indanecarboxamide (**1**) (Chart 1). Consequent structural modification resulted in the potent, orally bioavailable, brain-penetrable, selective Y5 antagonist (−)-**7** (Chart 1). In the present study, we report the discovery of the novel arylpyrazole class of Y5 antagonist (−)-**7**, together with its pharmacokinetic profile and preliminary pharmacological effects on agonist-induced feeding in Sprague−Dawley (SD) rats.

Chemistry. Our target compounds were prepared by condensation of the corresponding 3-amino-5-arylpyrazoles and carboxylic acids, which is represented by the synthesis of (−)-**7** (Scheme 1). The 3-amino-5-arylpyrazoles represented by **9** were prepared following the literature procedure.[24] Herein, we describe the synthesis of *the novel optically active benzindanecarboxylic acid* (−)-**10**. Optically active acid (−)-**10** was synthesized from the dibromide **11** (Scheme 2). **11** was reacted with diethyl malonate in the presence of sodium hydride (NaH) in tetrahydrofurane (THF) to provide the corresponding diethyl cyclopentyldicarboxylate. The malonate cycloalkylation step required a dilute condition

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Scheme 2[a]

[a] Reagents: (a) (i) $CH_2(CO_2Et)_2$, NaH, THF, room temp, (ii) 2 N aqueous KOH−EtOH, 80 °C, (iii) 200 °C, neat, (77%); (b) (i) crystallization with (+)-1-(naphthyl)ethylamine from 1,4-dioxane, (ii) decomposition of the salt with a 1 N HCl solution.

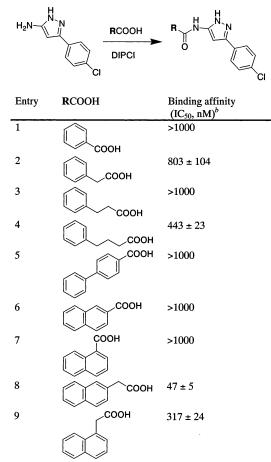**Table 1.** Y5 Binding Affinities of Compounds **1** and **2a−g** (Variation of the 5-Aryl Moiety)[a]

compd	Ar	binding affinity[b] (K_i, nM)
1	4-chlorophenyl	59 ± 1
2a	phenyl	260 ± 60
2b	3-chlorophenyl	120 ± 25
2c	2-chlorophenyl	450 ± 22
2d	4-methylphenyl	80 ± 16
2e	4-methoxyphenyl	62 ± 6
2f	4-isopropoxyphenyl	92 ± 14
2g	3,4-dimethoxyphenyl	33 ± 3
hNPY		1.2 ± 0.3

[a] The values represent the mean $\pm$ SE for $n = 3$. [b] Human recombinant Y5 receptors in LMtk⁻ cells, [^{125}I]PYY; see ref 27.

(<0.01 M substrate concentration) for good yield. Subsequent saponification and decarboxylation yielded (±)-**10** in 77% overall yield. The resulting racemate was resolved by crystallization with (+)-1-(naphthyl)ethylamine to give (−)-**10**. The diastereomeric excess of the amine salt was determined by HPLC analysis of the corresponding amide that was generated by treating a portion of the amine salt with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI). The optical purity of (−)-**10** was tentatively determined to be >98% ee, which was confirmed by the analysis of (−)-**7**. Determination of the absolute stereochemistry of (−)-**10** is currently underway. A mixture of amine **9** and carboxylic acid (−)-**10** in pyridine was treated with EDCI to give (−)-**7** in good yield (Scheme 1). Subsequently (−)-**7** was converted to its hydrochloride and used for pharmacological experiments. The optical purity of (−)-**7** was determined to be 98.6% ee by HPLC.

Results and Discussion. Screening of our in-house chemical collections against the human Y5 receptor resulted in the identification of the arylpyrazole class lead **1**. This lead has a K_i value of 59 nM, and its low molecular weight (MW = 337.80) is expected to be advantageous for oral bioavailability and brain penetrability. Modification of the 5-aryl moiety was made initially (Table 1). Although significant potency enhancement was not achieved, our preliminary modification of this site provided insights into structure−activity relationships (SAR). Removing the 4-Cl group as in **2a** reduced activity. Ortho- and meta-substituted phenyl derivatives **2b** and **2c** showed a decrease in potency. Para-substituted derivatives **2d−f** were generally equiactive to **1**. Since the methoxy and isopropoxy deriva-

Table 2. Y5 Binding Affinities of the Parallel Synthesis Library Samples[a]

Entry	RCOOH	Binding affinity (IC_{50}, nM)[b]
1		>1000
2		803 ± 104
3		>1000
4		443 ± 23
5		>1000
6		>1000
7		>1000
8		47 ± 5
9		317 ± 24

[a] The values represent the mean $\pm$ SE for $n = 3$. [b] Human recombinant Y5 receptors in LMtk⁻ cells, [^{125}I]PYY; see ref 27.

tives (**2e** and **2f**) are equipotent, more sterically demanding substituents might be tolerated at this position. The 3,4-dimethoxyphenyl derivative **2g** displayed slightly improved activity. At this point, we turned our attention to modification of the left-hand indane carbonyl moiety, aiming at significant potency improvement. For this purpose, an exploratory library was prepared using a parallel synthesis method, which allowed for rapid access to potent compounds by not employing purification methods (Table 2).[25] A 5-(4-chlorophenyl)-1*H*-pyrazole-3-amino group was chosen as the template because of its chemical accessibility and potency, and selection of the carboxylic acids was based on finding an optimum orientation of the left-hand aromatic groups. The 2-naphthylmethyl derivative (entry 8) appeared to be the most potent compound in this library. Thus, an analytically pure sample (compound **3**) was prepared, and its IC_{50} and K_i values were confirmed (Table 3). The corresponding 5-(3,4-dimethoxyphenyl) derivative **4** was also potent (K_i = 8.3 nM) (Table 3). We accomplished significant potency enhancement by the design of a benzindane structure (2,3-dihydro-1*H*-cyclopenta[*a*]-naphthalene group) as in (±)-**5** (Table 3). It was not a difficult task to realize the novel benzindane structure based on the given information. Since (±)-**5** showed very low oral bioavailability, we needed to examined the 5-aryl portion again and identified the orally available structure (±)-**6** (Table 3) (screening pharmacokinetic data not shown). Replacing the 4-pyridinyl group of

Table 3. Y5 Binding Affinities and Antagonistic Activities of Compounds **3**−**7**[a]

3 : Ar = 4-chlorophenyl
4 : Ar = 3,4-dimethoxyphenyl

(±)-**5** : Ar = 3,4-dimethoxyphenyl
(±)-**6** : Ar = 4-pyridinyl
(±)-**7** : Ar = 4-(2-ethylpyridinyl)

compd	binding affinity[b] (K_i, nM)	[Ca^{2+}]$_i$ response[c] (IC$_{50}$, nM)
3	16.0 ± 3.0 (16.3 ± 3.1)[d]	e
4	8.3 ± 1.5	e
(±)-**5**	2.8 ± 0.7	e
(±)-**6**	10 ± 2	e
(±)-**7**	7.3 ± 0.3	e
(+)-**7**	25 ± 0.3	28 ± 4
(−)-**7**	3.5 ± 0.3	14 ± 3

[a] The values represent the mean ± SE for $n = 3$. [b] Human recombinant Y5 receptors in LMtk⁻ cells, [^{125}I]PYY; see ref 27. [c] Antagonistic activities (human recombinant Y5 receptors/Gqi5 in CHO cells) at 100 nM NPY stimulation. [d] The value in parentheses is IC$_{50}$ (mean ± SE for $n = 3$). [e] Not determined.
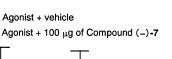
Table 4. Pharmacokinetic Parameters of (−)-**7** in SD Rats[a]

iv (1 mg/kg)	$t_{1/2} = 0.88 \pm 0.03$ h
	$Vd_{ss} = 0.62 \pm 0.03$ L kg^{-1}
	$CL_{tot} = 9.52 \pm 0.03$ mL min^{-1} kg^{-1}
po (10 mg/kg)	$t_{1/2} = 1.9 \pm 0.6$ h
	$T_{max} = 2.0$ h
	$C_{max} = 5.0 \pm 1.3$ mM
	$F = 70 \pm 9\%$

[a] The values represent the means ± SE for $n = 3$.

(±)-**6** with a 4-(2-ethylpyridinyl) group aided us in identifying the more potent compound (±)-**7** (Table 3). Hence, optically active (−)-**7** and (+)-**7** were synthesized. (−)-**7** proved to be a highly potent ($K_i = 3.5$ nM) and selective Y5 antagonist (IC$_{50}$ > 10 μM at human Y1, Y2, and Y4). A good pharmacokinetic profile of (−)-**7** was observed after oral (10 mg/kg) and intravenous (1 mg/kg) administration in SD rats (Table 4). The antagonistic activity of (−)-**7** was measured by its ability to inhibit NPY-induced [Ca^{2+}]$_i$ increases in LMtk⁻ cells, which expressed the recombinant human Y5 receptor. In this [Ca^{2+}]$_i$ functional assay, (−)-**7** dose-dependently inhibited the [Ca^{2+}]$_i$ increase (Table 3).

We evaluated the effects of (−)-**7** on NPY and bovine pancreatic polypeptide (bPP) induced food intake in SD rats.[26] After intracerebroventricular (ICV) administration, (−)-**7** significantly inhibited bPP-induced food intake (Figure 1). After oral administration, (−)-**7** (10 and 30 mg/kg) showed only moderate dose-dependent efficacy in suppressing feeding induced by bPP (data not shown). The brain concentration of (−)-**7** (10 mg/kg) in SD rats 2 h after oral administration was 0.45 μM. We surmised that the distribution of (−)-**7** in the brain after oral administration is not efficient. Therefore, the availability of the molecule to the target Y5 receptor may not be sufficient to show significant efficacy. We are currently searching for more potent oral compounds in this class. Interestingly, ICV-administered (−)-**7** did not affect NPY-induced food intake to a significant degree in SD rats (Figure 1), contrary to the previously reported observation by Synaptic and Novartis groups.[17] In addition, our xanthene class Y5 antagonist **8** (Chart 2) showed exactly the same behavior as did (−)-**7**.[27] The
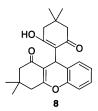

Figure 1. Effects of ICV-injected (−)-**7** on NPY and bPP-induced food intake in SD rats. Significant differences with respect to controls are indicated by an asterisk (*, $P < 0.05$). The data shown are expressed as the mean ± SE. The graph shows the cumulative food intake in SD rats up to 2 h after ICV injection of drugs. $n = 8$ rats/group. See ref 27 for details.

Chart 2

8

relative importance of the Y5 receptor in feeding regulation is still controversial; therefore, it is an extremely important finding that two structurally distinct Y5 antagonists, (−)-**7** and **8**, displayed similar profiles in feeding behaviors elicited by ICV-administered bPP and NPY. To address the role of the Y5 receptor, the evaluation of structurally diverse new classes of compounds could be critical. Our investigation into the role of the Y5 receptor using the arylpyrazole derivatives described herein, the xanthene derivatives, and related analogues is currently underway in physiological and pathophysiological feeding models.

In summary, a preliminary SAR of the novel arylpyrazole class of Y5 antagonists and the discovery of (−)-**7** are described. The novel benzindanecarbonyl portion of (−)-**7** has a significant effect on potency enhancement. This novel Y5 antagonist has good oral bioavailability and brain permeability. After ICV administration in SD rats, (−)-**7** significantly inhibited food intake induced by centrally administered bPP in SD rats but had only a negligible effect on NPY-induced feeding. The structurally distinct selective ligand (−)-**7** is a powerful tool that may be used to probe the pharmacologic properties of the Y5 receptor. The investigation into the role of the Y5 receptor and the search for improved analogues are ongoing. The updated results will be reported in due course.

Supporting Information Available: Synthetic procedures including the exploratory library. This material is available free of charge via the Internet at http://pubs.acs.org.

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