Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2287–2290

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Pyrazolecarboxamide Human Neuropeptide Y5 Receptor Ligands with In Vivo Antifeedant Activity

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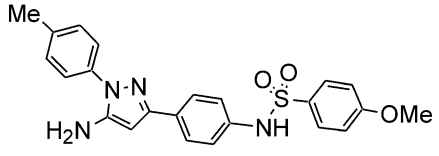
Received 26 March 2001; accepted 11 June 2001

Abstract—1-Aryl-3-carboxamido-5-alkylpyrazoles were prepared based on a hit found in high-throughput screening of our corporate compound library in an assay measuring affinity for the human neuropeptide Y5 receptor. 1-(3-Trifluoromethylphenyl)-3-[*N*-(5-quinolinyl)carboxamido]-5-methylpyrazole (**31**) bound to the human neuropeptide Y5 receptor with a 80 nM IC_{50} and was shown to inhibit cumulative food consumption 43.2% 2–6 h after ip dosing in a fasting-induced feeding model in rats.

Neuropeptide Y (NPY), a 36-amino acid C-amidated peptide, is abundantly expressed in the central nervous system and has been shown to robustly stimulate feeding.[1–4] A family of six NPY receptor subtypes belonging to the superfamily of G-protein coupled receptors has been described in the literature.[5–14] NPY Y1 and NPY Y5 are thought to be the most likely receptor subtypes responsible for centrally-mediated NPY-induced feeding responses.[13,15–17] In fact, antagonists at the NPY Y5 receptor have been shown to be effective in reducing food intake in animal models of feeding.[18] Consequently, there has been an impetus to discover small molecule NPY Y5 receptor antagonists in order to provide new treatments for obesity and other eating disorders.[19]

Our labs have recently identified several series of NPY Y5 antagonists.[20–22] We have also disclosed amino-pyrazoles as potent NPY Y5 receptor antagonists.[23] For example, pyrazoles **1** and **2** were shown to have 15 and 10 nM IC_{50}'s for the human NPY Y5 receptor, respectively (Fig. 1).

Screening our corporate chemical library in an assay measuring affinity for the human neuropeptide Y5 receptor identified triazole carboxamide **3**, obtained through a compound aquisition program, as having low micromolar binding affinity for the human Y5 receptor (Fig. 2). The first modification done to **3** was to replace the triazole core ring with a pyrazole ring, in order to impart novelty to the system and to simplify the synthesis of derivatives for SAR development. The



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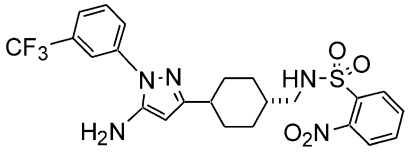

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Figure 1. Aminopyrazole NPY5 receptor antagonists.

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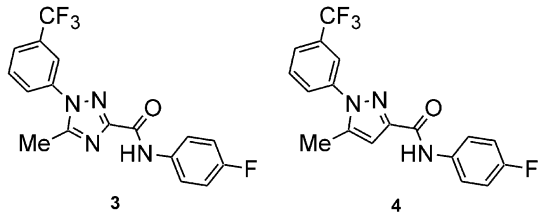


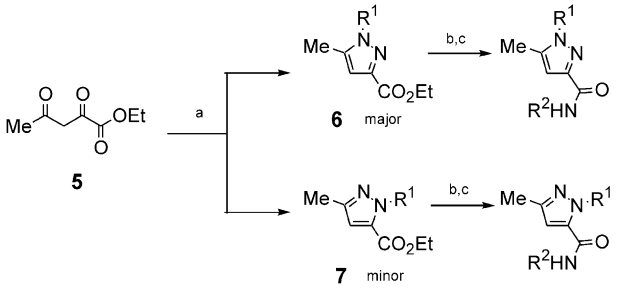
Figure 2. Lead structures after analysis of HTS.

corresponding pyrazole carboxamide **4** was equipotent to original triazole **3**. An intensive effort to elucidate structure–activity relationships based on pyrazole-carboxamide lead **4** was then undertaken.

The pyrazole carboxamides were synthesized in three steps from commercially available ethyl acetopyruvate **5** (Scheme 1). Reaction of **5** with an arylhydrazine under refluxing conditions in acetic acid afforded a 4:1 mixture of regioisomers **6** and **7**, which were generally separable by column chromatography on silica gel. Saponification of the ester followed by coupling of the resultant carboxylic acid with an amine resulted in the formation of the target pyrazole carboxamides in excellent yields.

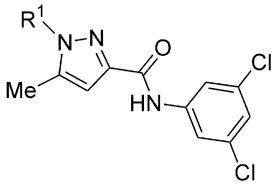
The pyrazole carboxamides were evaluated for binding affinity to the human neuropeptide Y Y5 receptor. The assay was executed using a stably transfected HEK293 cell line measuring competitive inhibition of binding of ^{125}I-PYY (Table 1).[20] Table 1 illustrates pyrazole carboxamides in which the substituent on the pyrazole core (R^1) was varied, and the *N*-(3,5-dichlorophenyl) group on the amide was held constant. In general, R^1 modifications were largely detrimental to NPY Y5 affinity except for the original 3-(trifluoromethyl)phenyl group (**11**) and also 3-methylphenyl (**13**). Unsubstituted phenyl (**8**), benzyl (**9**), other 3-substituted phenyls such as **14**, and 2- and 4-substituted phenyl groups (**10** and **12**) resulted in loss of activity. Compounds bearing a heteroaryl ring in R^1 such as pyridine **15** and thiophene **16** were inactive at the NPY Y5 receptor in our assay.[23]

In a similar manner, variations were made to R^2 (Table 2). In general, 2- and 3- substituted phenyl groups were well tolerated at the R^2 position (**17**–**25**). When 4-substituted phenyl groups are incorporated at this site activity is diminished (**4** and **26**). The 3,5-dichlorophenyl derivative **11** is one of the best compounds with a 150 nM IC_{50} for the human NPY Y5 receptor. 2,6-Difluorophenyl compound **27** is less active. From a set



Scheme 1. Reagents: (a) R^1NHNH_2, AcOH, reflux; (b) NaOH, EtOH, H_2O; (c) HATU, DIPEA, R^2NH_2, CH_2Cl_2.

Table 1. Y5 receptor binding affinity of pyrazole carboxamides with R^1 variations[a]



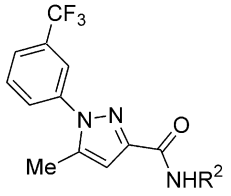
Compd	R^1	NPY Y5 IC_{50} (nM)
8	Phenyl	759
9	Benzyl	>1000
10	2-(Trifluoromethyl)phenyl	>1000
11	3-(Trifluoromethyl)phenyl	150
12	4-(Trifluoromethyl)phenyl	>1000
13	3-Methylphenyl	218
14	3-Methoxyphenyl	>1000
15	2-Pyridyl	>1000
16	2-Thienyl	>1000

[a]HEK 293 cells were stably transfected with the human NPY5 cDNA and were used according to the procedure in ref 20.

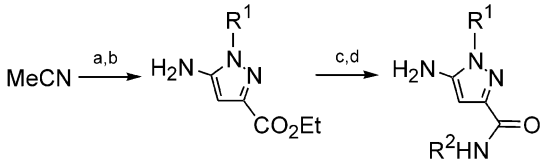
of bicyclic compounds (viz. **28**–**31**), the 5-isoquinolinyl derivative (**31**) emerged as the most active (80 nM IC_{50}). Other N-containing heteroaryls at R^2 are generally inactive, such as for pyridine **32** and benzimidazole **33**.

Several of the corresponding regioisomers derived from **7** were also prepared and evaluated in the NPY Y5 binding assay. These were completely inactive, indicating that the 1,3,5-substitution pattern on the pyrazole core structure is critical for activity.

Table 2. Y5 receptor binding affinity of pyrazolecarboxamides with variation of R^2



Compd	R^2	Y5 IC_{50} (nM)
17	2-Methylphenyl	573
18	2-Benzylphenyl	292
19	2-(*N*-Phenylamino)phenyl	267
20	2-(*N*-Pyrrolyl)phenyl	763
21	3-Chlorophenyl	520
22	3-Bromophenyl	359
23	3-Iodophenyl	670
24	3-Methylphenyl	622
25	3-(Trifluoromethoxy)phenyl	431
4	4-Fluorophenyl	800
26	4-Chlorophenyl	>1000
11	3,5-(Dichloro)phenyl	150
27	2,6-Difluorophenyl	233
28	1-Naphthyl	356
29	1-(5,6,7,8-Tetrahydronaphthyl)	197
30	5-Quinolinyl	232
31	5-Isoquinolinyl	80
32	2-Pyridyl	>1000
33	2-Benzimidazolyl	>1000



Scheme 2. Reagents: (a) NaOEt, diethyl oxalate, Et_2O, 0 °C; (b) R^1NHNH_2, H_2SO_4, H_2O, $CHCl_3$; (c) NaOH, EtOH, H_2O; (d) HATU, DIPEA, R^2NH_2, CH_2Cl_2.

Since the aminopyrazoles such as **1** and **2** were very active,[24] we also prepared hybrid structures of that series and the one reported in this paper. These derivatives were obtained as illustrated in Scheme 2, involving the reaction of the anion of acetonitrile with diethyl oxalate, followed by formation of the pyrazole from the resultant intermediate by treatment with an aryl hydrazine. The pyrazole ester was then saponified with aqueous sodium hydroxide, and the acid that formed coupled to appropriate amines using standard peptide coupling procedures.

The best R^1 and R^2 groups from the pyrazole carboxamide series were incorporated into an aminopyrazole scaffold to provide **34** and **35** (Fig. 3). Both of these compounds had substantially *decreased* affinity for Y5 (>1000 and 400 nM IC_{50}'s, respectively).

Compounds **11** and **31** were tested in a fasting-induced feeding model in rats.[20,25] Both compounds were administered ip and compared to vehicle (PEG-200) and reference compound (fluoxetine). They caused statistically significant decreases in food consumption relative to fluoxetine as control (Table 3), and both were well tolerated. Since NPY was not added initially to enhance feeding, it is not possible to know whether this reduction of feeding is entirely mediated by an interaction with the NPY Y5 receptor. Neither compound was active upon oral administration.

We describe here another related class of compounds, pyrazole carboxamides, which modulate the human NPY5 receptor. The compound resulting from the combination of the best R^1 (3-trifluoromethyl) and R^2 substituents (5-isoquinolinyl) was **31**. This compound and an additional member of this series (**11**) decreased fasting-induced feeding in a rodent model upon ip administration, indicating that they may be useful for the treatment of human feeding disorders and obesity, especially if suitable compounds can be found that are orally bioavailable. The antifeedant activity seen for **11** and **31** are additional examples in which this effect can be seen for NPY Y5 receptor antagonists, even when exogenous NPY is not added to amplify NPY-induced feeding.

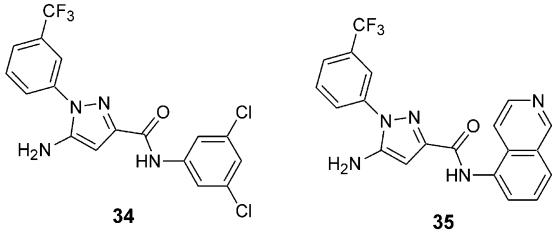


Figure 3. Aminopyrazole carboxamides.

Table 3. Data from rat feeding model[a]

Drug (IP)	Dose (mg/kg)		Experimental groups compared to control group: 2 h (g)	4 h (g)	6 h (g)	2–6 h (g)
PEG-200	**0**	AVG =	8.63	12.3	18.8	10.2
($N=24$)		SE =	0.398	0.53	0.756	0.59
Fluoxetine	**10**	AVG =	4.38*	7.25*	12.6*	8.25*
		SE =	0.294	0.479	0.648	0.512
($N=24$)		**%CH =**	**−49.2**	**−41.06**	**−32.98**	**−19.12**
		***p* =**	**<0.01**	**<0.01**	**<0.01**	**<0.05**
11	**30**	AVG =	5.31*	9.44*	13.9*	8.56
		SE =	0.538	0.652	0.856	0.612
($N=16$)		**%CH =**	**−38.47**	**−23.25**	**−26.06**	**−16.08**
		***p* =**	**<0.01**	**<0.01**	**<0.01**	**>0.05**
PEG-200	**0**	AVG =	10.4	16	23.4	13
($N=8$)		SE =	0.905	0.845	0.778	0.681
Fluoxetine	**10**	AVG =	4.50*	8.25*	13.4*	8.88*
		SE =	0.824	1.06	1.82	1.17
($N=8$)		**%CH =**	**−56.73**	**−48.4**	**−42.74**	**−31.69**
		***p* =**	**<0.01**	**<0.01**	**<0.01**	**<0.05**
31	**30**	AVG =	6.88*	9.13*	14.6*	7.38*
		SE =	0.718	0.934	1.37	1.22
($N=8$)		**%CH =**	**−33.85**	**−42.94**	**−37.61**	**−43.23**
		***p* =**	**<0.05**	**<0.01**	**<0.01**	**<0.01**

%CH, % change; * signifies statistical significance.

[a]Male Long–Evans rats were fasted for 18 h prior to testing. At the end of the fasting period, animals were administered either the test compound or vehicle 30 min intraperitoneally prior to the experiment. The test compounds were administered as a solution or suspension in PEG 200. Food consumption was determined at 2, 4, and 6 h after administration by weighing the special jar containing the food before the experiment and at each specified time.

Acknowledgements

We thank our colleagues for their helpful advice and encouragement, specifically Scott Dax, Carlos Plata-Salamán, Richard Shank, Jim McNally, Mark Youngman, Barry Dubinsky, and Coralie Hochman.

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