Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3593–3596

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure–Activity Relationship Studies on 2-Heteroaryl-4-arylimidazoles NPY5 Receptor Antagonists

Richard L. Elliott,[a,*] Robert M. Oliver,[a] Janet A. LaFlamme,[a] Melissa L. Gillaspy,[a] Marlys Hammond,[a] Richard F. Hank,[a] Tristan S. Maurer,[a] Demetria L. Baker,[a] Paul A. DaSilva-Jardine,[a] Ralph W. Stevenson,[a] Christine M. Mack[b] and James V. Cassella[b]

[a]*Department of Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, CT 06340, USA*
[b]*Neurogen Corporation, Branford, CT 06405, USA*

Received 18 December 2002; accepted 4 June 2003

Abstract—A series of 2-heteroaryl-4-arylimidazoles with potent in vitro activity at the NPY5 receptor was developed. Introduction of electron-withdrawing groups on the 4-aryl ring led to a significant improvement of in vitro potency. Several analogues from this series had anorectic activity in rodent feeding models, but were also found to have undesired behavioral effects in spontaneous locomotor activity.

Neuropeptide Y (NPY) is a 36 amino acid peptide that has been implicated in modulating food intake in animals, including humans. NPY, which is found in both the periphery and CNS, mediates its biological effects via six receptor subtypes (NPY1-NPY6). It is currently believed that NPY's orexigenic effects are mediated by either the NPY1 or NPY5 receptor subtype, or possibly a combination of both receptor subtypes. Evidence supporting the role of the NPY5 receptor subtype in regulating food intake and body weight includes the finding that the orexigenic effect of peptide analogues of NPY correlate with affinity for the NPY5 receptor,[1] the anatomical location and physiological regulation of NPY5 receptors in areas of the CNS relevant to feeding,[2] and the determination that NPY5 antisense oligodeoxynulceotides have anorectic effects in rodents.[3] The robust orexigenic responses seen in animals when dosed with NPY and NPY agonists has led to great interest in identifying and developing NPY1 and/or NPY5 antagonists for the treatment of obesity.

Compound **1**, 2,5-diphenyl-1*H*-imidazole, was identified from a high-throughput screen for NPY5 antagonists. Although this compound had fairly good potency for the NPY5 receptor (34 nM, see Table 1) it was found to have poor metabolic stability and low water solubility. Herein, we report our SAR studies that were undertaken in an effort to improve the in vitro potency, physiochemical properties, and pharmacokinetic properties of this series.

The synthesis of the 2-heteroaryl-4-arylimidazoles utilized the condensation of a heteroaryl amidine **II** with an α-bromo arylketone **III** (Scheme 1). The requisite amidines **II**, when not commercially available, were generated by treatment of the corresponding heteroaryl nitrile **I** with lithium bis(trimethylsilyl)amide at room temperature, and subsequently used in situ in the condensation with the bromoketone **III** to afford the desired imidazole. The bromoketones **III** were either commercially available or synthesized via bromination of the corresponding aryl ketone.

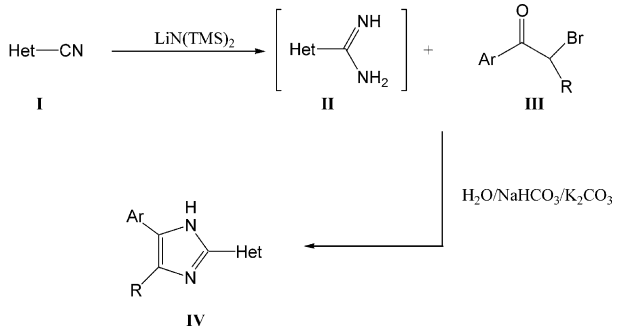
The NPY-5 receptor binding and functional antagonism activities for these compounds are shown in Table 1. Initial efforts to improve the potency and water solubility of these compounds led to the synthesis of **2**, **3**, and **4**, where the 2-phenyl moiety has been replaced by a 2-, 3-, and 4-pyridyl ring, respectively. Although compounds **2**–**4** had significantly lower binding potency

*Corresponding author. Fax: +1-860-715-8069; e-mail: richard_l_elliott@groton.pfizer.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00747-9

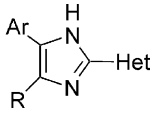
than **1**, the 3-pyridyl analogue **3** had the highest binding affinity of the three. Therefore, further 2-(3-pyridyl)-imidazole analogues with substituted aryl groups were synthesized, systematically exploring substitution at the *ortho*-, *meta*-, and *para*-position of the phenyl ring. Good binding affinity returned upon substitution at the *ortho*-position of the 4-aryl group with chloro, trifluoromethyl, and methyl substituents (compounds **5**–**7**). Also, these compounds were shown to be good functional antagonists in a whole cell Ca^{2+}-mobilization assay.

The identical substitutions at the *meta*-position of the aryl ring (i.e., chloro, trifluoromethyl, and methyl) led to a further increase in binding affinity, affording compounds **8**–**10** with K_i values of 8, 1.6, and 14 nM, respectively. Although the 3-methoxy analogue **11** had decrease binding affinity relative to **8**–**10**, the 3-trifluoromethoxy and 3-chloro analogue with a methyl group at the 5-position (compounds **12** and **13**) also had good binding potency. Overall, the functional potency of these *meta*-substituted compounds mirrored their binding potency at the NPY5 receptor. In general, substitution at the *para*-position led to compounds with decreased binding affinity relative the their *meta*-substituted counterparts, with K_i values ranging from 16 nM with the *para*-chloro analogue **14** to 3000 nM for the *para*-cyano analogue **17**. Introducing chlorine substituents at both the *meta*- and *para*-position afforded analogue **19**, which maintained good potency ($K_i = 4$ nM). Introduction of a *meta*, *para*-dichloro substituents on the 4-aryl group also significantly improved the binding potencies of the 2-pyridyl and 3-pyridyl compounds, with compounds **22** and **23** having K_i values of 30 and 3.5 nM, respectively. A brief survey of other non-pyridyl heteraryl replacements for the pyridyl ring was conducted, retaining the *meta*, *para*-dichloro substitution pattern on the aryl ring. Of the compounds investigated (**24**–**27**) the 2-pyrazinyl analogue **27** had the best binding affinity, with a K_i value of 40 nM. Compound **28**, in which the 4-aryl group was replaced with a cyclohexyl group, had a K_i value of 231 nM; better than



Scheme 1.

Table 1. Structure–activity relationships of 2-heteroaryl-4-arylimidazoles NPY5 antagonists[4]



Compd[a]	Het	Ar	R	Hu NPY5 binding K_i (nM)[b]	Ca^{2+} Mob. EC_{50} (nM)[c]
1	Phenyl	Phenyl	H	34±8.8 (4)	
2	2-Pyridyl	Phenyl	H	1449±569 (3)	
3	3-Pyridyl	Phenyl	H	525±164 (2)	
4	4-Pyridyl	Phenyl	H	1527±1074 (3)	
5	3-Pyridyl	2-Chlorophenyl	H	21±0.8 (2)	15±2.2 (2)
6	3-Pyridyl	2-Trifluoromethylphenyl	H	27±2.0 (2)	39 (1)
7	3-Pyridyl	2-Methylphenyl	H	40±3.1 (2)	52 (1)
8	3-Pyridyl	3-Chlorophenyl	H	8.0 (1)	
9	3-Pyridyl	3-Trifluoromethylphenyl	H	1.6±0.1 (2)	3.2±0.7 (2)
10	3-Pyridyl	3-Methylphenyl	H	14±2.2 (2)	24 (1)
11	3-Pyridyl	3-Methoxyphenyl	H	56±6.2(2)	45 (1)
12	3-Pyridyl	3-Trifluoromethoxyphenyl	H	4.9±1.4 (4)	13 (1)
13	3-Pyridyl	3-Chlorophenyl	Me	19±3.5 (2)	40±11 (2)
14	3-Pyridyl	4-Chlorophenyl	H	16±4.5 (2)	
15	3-Pyridyl	4-Trifluoromethylphenyl	H	37±3.5 (2)	
16	3-Pyridyl	4-Trifluoromethoxyphenyl	H	210±77 (2)	
17	3-Pyridyl	4-Cyanophenyl	H	3001±237 (2)	
18	3-Pyridyl	4-Chlorophenyl	Me	69±8.5 (2)	
19	3-Pyridyl	3,4-Dichlorophenyl	H	4.0±1.1 (2)	
20	3-Pyridyl	3,4-Difluorophenyl	H	34±4.5 (2)	
21	3-Pyridyl	3,4-Dimethoxyphenyl	H	148±41 (2)	723 (1)
22	2-Pyridyl	3,4-Dichlorophenyl	H	30±20 (2)	
23	4-Pyridyl	3,4-Dichlorophenyl	H	3.5±1.2 (3)	
24	3-Quinolyl	3,4-Dichlorophenyl	H	>10,000 (2)	
25	1-Isoquinolyl	3,4-Dichlorophenyl	H	116±51 (6)	17±4.2 (5)
26	3-Isoquinolyl	3,4-Dichlorophenyl	H	4769 (1)	
27	2-Pyrazinyl	3,4-Dichlorophenyl	H	40±.3 (2)	
28	3-Pyridyl	Cyclohexyl	H	231±8.5 (2)	36 (1)

[a]All final compounds have been fully characterized by ^1H NMR and mass spec, and are >95% pure.
[b]^{125}I-PYY binding in baculovirus-infected Sf9 cells expressing recombinat human NPY5 receptors.
[c]Ca^{2+} mobilization in NPY5 transfected human Bowes melanoma (HMCB) cell line. All values are means±SEM, number of replications in parentheses.

Table 2. Food intake and spontaneous locomotor activity effects of analogues in rats[a]

Compd	24-h food-deprived FI	Overnight FI	Spontaneous locomotor activity
8	+/+/−	—	NT
9	+/−	+	+
14	+/+	—	+
19	+/−	NT	NT

[a]+ Designates significant reduction in food intake relative to control rats observed; − designates no significant reduction in food intake relative to control rats observed. All compounds dosed orally at 40 mg/kg in male Sprague–Dawley rats.

the unsubstituted analogue **3** but not nearly as potent as many of the aryl-substituted analogues.

Our primary screening strategy for identifying compounds with in vivo anorectic effects utilized a feeding model in which 24-h food deprived rats were dosed with the test compound and food intake was measured for a 3-h period. As shown in Table 2, three of the four compounds evaluated showed highly variable results in this food intake model. Only compound **14** worked each time it was tested. Three of these compounds were further investigated in an overnight rat feeding model in which compounds were dosed 2 h prior to onset of the dark cycle, and overnight food intake was measured the following morning (13 h later). In this model only compound **9** caused a significant decrease in nighttime feeding.[5] However, it was disconcerting that the other compounds tested in vivo, most of which had very similar in vitro potency, did not demonstrate similar anorectic activity in this model at the doses tested.

In an attempt to explain this lack of efficacy, the systemic exposure and brain penetration of compound **8** was examined. Significant and sustained brain and plasma concentrations were achieved following a 40 mg/kg oral dose (Table 3). The unbound fractions in plasma and brain were determined to be 0.04 ± 0.005 and 0.0044 ± 0.0002 by equilibrium dialysis. As such, calculated free brain and plasma concentrations were greater than 100 nM for at least 4 h post dosing (>10-fold the K_i for NPY5). Even 8 h following dosing, free drug concentrations were in modest excess of the K_i. Consistent with these calculations, mean cerebrospinal fluid (CSF) concentrations were slightly greater than the K_i at 8 h post dosing (11 nM, Table 3). Lastly, the concordance of brain/plasma ratios (4.4–5.3) with the ratio of unbound plasma-to-unbound brain fractions (9.1 ± 0.5) also indicates that compound **8** has excellent penetration of the blood–brain barrier.[6] These results suggest that this compound's lack of anorectic activity is not the result of inadequate exposure or brain penetration.

Table 3. Plasma brain and CSF exposure of compound **8** in rats[a]

Hours	Plasma (μM)	Brain (μM)	CSF (μM)	$\frac{[Brain]}{[Plasma]}$
1	10 ± 2	45 ± 13	ND	4.4 ± 0.4
2	11.0 ± 1.4	56 ± 10	ND	5.1 ± 0.6
4	6.2 ± 1.9	29 ± 6	ND	4.8 ± 0.7
8	1.3 ± 1.1	4 ± 4	0.011 ± 0.008	5.3 ± 2.8

[a]Data represent the mean ± SD of exposures and exposure ratios determined in Sprague–Dawley rats by LC–MS/MS. ND represents values that were not determined.

These findings led us to further explore the specificity of compound **9**. It was subsequently determined that **9** did not have any significant activity at over 50 receptors, ion channels, and transporters (<50% inhibition @ 1 μM) tested. However, both compounds **9** and **14** showed decreased spontaneous locomotor activity (SLA) 1 h post-dosing, leading us to believe that perhaps non-specific effects were contributing to the anorectic effects observed in vivo with these compounds.

These issues arose not only within this series, but in other structurally distinct NPY-5 antagonist series as well, that is only a small percentage of NPY5 antagonists tested demonstrated anorectic activity in vivo at the doses tested, with no apparent correlation to in vitro potency or in vivo exposure. In some cases the 'active' compounds had confounding issues, for example activity at other neurotransmitter receptors or transporters, or undesirable behavioral effects such a decreased locomotor activity or increasing kaolin consumption in rodents, the latter being an indication of illness. In other cases 'active' compounds were identified with no apparent specificity issues. However, even with these compounds there was a disconnect between in vitro potency at the receptor (often single-digit nanomolar) and the high plasma, brain, and CSF levels required to observe anorectic effects (in some cases μM CSF levels were observed).[7] These findings are in line with other recent reports showing that potent and well-exposed NPY5 antagonists can block NPY5 agonist induced feeding in rodents, but are without effects in natural feeding models.[8–10]

In summary, we have developed a series of 2-heteroaryl-4-arylimidazoles with potent in vitro binding and functional activity at the NPY5 receptor. Several analogues from this series had anorectic activity in rodent feeding models, but were also found to have behavioral effects in spontaneous locomotor activity.

References and Notes

1. (a) Wyss, P.; Stricker-Krongrad, A.; Brunner, L.; Miller, J.; Crossthwaite, A.; Whitebread, S.; Criscione, L. *Reg. Peptides* **1998**, *75–76*, 363. (b) Gerald, C.; Walker, M. W.; Criscione, L.; Gustafson, E. L.; Batzl-Hartmann, C.; Smith, K. E.; Vaysse, P.; Durkin, M. M.; Laz, T. M.; Linemeyer, D. L.; Schaffhauser, A. O.; Whitebread, S.; Hofbauer, K. G.; Taber, R. I.; Branshek, T. A.; Weinshank, R. L. *Nature (London)* **1996**, *382*, 168.
2. Widdowson, P. S. *Brain Res.* **1997**, *758*, 17.
3. (a) Schaffhauser, A. O.; Stricker-Krongrad, A.; Brunner, L.; Cumin, F.; Gerald, C.; Whitebread, S.; Criscione, L.; Hofbauer, K. G. *Diabetes* **1997**, *46*, 1792. (b) Tang-Christensen, M.; Kristensen, P.; Stidsen, C. E.; Brand, C. L.; Larsen, P. J. *J. Endocrin.* **1998**, *159*, 307. (c) Flynn, M. C.; Turrin, N. P.; Plata-Salaman, C. R.; Ffrench-Mullen, J. M. *Physiol. Behav.* **1999**, *66*, 881.

4. The in vitro binding and functional assays were performed as described in patent WO9901128.
5. Very recently a series of 2,4-diarylimidazoles was reported on in which compound **9** was observed to cause weight loss in diet-induced obese rats when dosed over a 28-day period at 10 mg/kg/day po: Boyle, C. D.; Lankin, C; Chackalamannil, S.; Manna, M.; Stamford, A.; Mullins, D.; Guzzi, M.; Salisbury, B.; Hwa, J.; Ghibaudi, L.; Witten, M.; Gao, J.; Van Heek, M.; France, C.; Cook, J.; Bryant, M. 2,4-Diarylimidazoles as neuropeptide Y5 receptor antagonists. 224th ACS National Meeting (Boston, Aug. 18–22, 2002; Poster #335).
6. Kalvass, J. C.; Maurer, T. S. *Biopharm. Drug. Dispos.* **2002**, *23*, 327.
7. Elliott, R. L.; Oliver, R. M.; Hammond, M.; Patterson, T. A.; She, L.; Hargrove, D. M.; Martin, K. A.; Maurer, T. S.; Kalvass, J. C.; Morgan, B. P.; DaSilva-Jardine, P. A.; Stevenson, R. W.; Mack, C. M.; Cassella, J. V. *J. Med. Chem.* **2003**, *46*, 670.
8. Kanatani, A.; Ishihara, A.; Iwaasa, H.; Nakamura, K.; Okamoto, O.; Hidaka, M.; Ito, J.; Fukuroda, T.; MacNeil, D. J.; Van der Ploeg, L. H. T.; Ishii, Y.; Okabe, T.; Fukami, T.; Ihara, M. *Biochem. Biophys. Res. Commun.* **2000**, *272*, 169.
9. Turnbull, A. V.; Ellershaw, L.; Masters, D. J.; Birtles, S.; Boyer, S.; Carroll, D.; Clarkson, P.; Loxham, S. J. G.; McAulay, P.; Teague, J. L.; Foote, K. M.; Pease, J. E.; Block, M. H. *Diabetes* **2002**, *51*, 2441.
10. Block, M. H.; Boyer, S.; Brailsford, W.; Brittain, D. R.; Carroll, D.; Chapman, S.; Clarke, D. S.; Donald, C. S.; Foote, K. M.; Godfrey, L.; Ladner, A.; Marsham, P. R.; Masters, D. J.; Mee, C. D.; O'Donovan, M. R.; Pease, J. E.; Pickup, A. G.; Rayner, J. W.; Roberts, A.; Schofield, P.; Suleman, A.; Turnbull, A. V. *J. Med. Chem.* **2002**, *45*, 3509.