Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 538–541
Bioorganic & Medicinal Chemistry Letters

Identification of selective neuropeptide Y2 peptide agonists

Lynn B. DeCarr,[a] Thomas M. Buckholz,[b] Philip D. G. Coish,[c] Zahra Fathi,[a] Stephen E. Fisk,[b] Michelle R. Mays,[a] Stephen J. O'Connor[c] and Kevin J. Lumb[b,*]

[a]*Department of Metabolic Disorders Research, Bayer Pharmaceuticals Corporation, 400 Morgan Lane, West Haven, CT 06516, USA*
[b]*Department of Research Technologies, Bayer Pharmaceuticals Corporation, 400 Morgan Lane, West Haven, CT 06516, USA*
[c]*Department of Chemistry Research, Bayer Pharmaceuticals Corporation, 400 Morgan Lane, West Haven, CT 06516, USA*

Received 2 August 2006; revised 4 October 2006; accepted 5 October 2006
Available online 7 October 2006

Abstract—Activation of the NPY2 receptor to reduce appetite while avoiding stimulation of the NPY1 and NPY5 receptors that induce feeding provides a pharmaceutical approach to modulate food intake. The naturally occurring peptide PYY(3–36) is a non-selective NPY1, NPY2, and NPY5 agonist. N-terminal truncation of PYY to abrogate affinity for the NPY1 and NPY5 receptors and subsequent N-terminal modification with aminobenzoic analogs to restore NPY2 receptor potency results in a series of highly selective NPY2 receptor peptide agonists.

Pharmaceutical modulation of the activity of neuropeptide Y (NPY) receptors offers an approach to the regulation of food intake and provides a potential strategy for the control of obesity and associated disorders.[1] The gut hormone PYY(3–36) is a potent in vitro NPY2 receptor agonist that reduces food intake and body weight in rodents and rabbits, and reduces food intake in monkeys and humans.[2–11] Studies in NPY2 knockout mice and with NPY2 antagonists suggest that the anorexigenic effect of PYY(3–36) is mediated through the stimulation of the NPY2 receptor.[2,12] PYY(3–36) also activates the NPY1 and NPY5 receptor subtypes that stimulate feeding.[13,14] PYY variants that are selective for the NPY2 receptor would be expected to reduce appetite without the counteracting stimulatory effect on feeding associated with NPY1 and NPY5 activation.

Several selective NPY2 receptor peptides have been described previously.[15,16] These include NPY or PYY peptides with N-terminal deletions (**2**, Table 1),[17] NPY with an internal replacement of residues 5–24 with aminohexanoic acid (**8**),[18] and an N-terminal deletion variant of PYY with an internal lactam bridge between Lys 28 and Glu 32 (**10**).[19]

While N-terminal deletion of residues from PYY provides an effective approach to obtain NPY2-selective agonists such as **2**, this also leads to diminished affinity for the NPY2 receptor.[17] Here, we describe the restoration of NPY2 receptor potency while retaining selectivity by the use of novel N-terminal modifying groups.

Peptides derived from N-terminal deletions of PYY (**4**–**7**), as well as the previously reported selective NPY2 agonists **2**, **8**, and **10**,[16–18] were evaluated for binding and functional activity at the human NPY2 receptor and for binding at the human NPY1 and NPY5 receptors (Table 1).[20–24] The shortest peptide to exhibit moderate affinity for the NPY2 receptor and minimal potency (>1 μM) for the NPY1 or NPY5 receptors is PYY(25–36) (**4**) (Table 1). Further truncation of PYY beyond Arg 25 results in a significant loss of potency at the NPY2 receptor (e.g., **7**).

Acetylation of the N-terminal α-amino group of PYY(25–36) (**4**) results in a significant improvement (approximately 10-fold) in NPY2 receptor potency while maintaining NPY1 and NPY5 receptor selectivity (**5**). This is not a general effect with NPY or PYY peptides, since N-terminal acetylation of PYY(22–36) (cf. **2** and **3**) or NPY[Ahx5–24] (cf. **8** and **9**) does not improve NPY2 potency (Table 1).

The observation that acetylation of PYY(25–36) (**4**) increases NPY2 receptor affinity while maintaining specificity against the NPY1 and NPY5 receptors suggests

Keywords: NPY2; NPY; PYY; Obesity; Peptide.
* Corresponding author. Tel.: +1 203 812 3783; fax: +1 203 812 2526; e-mail: kevin.lumb.b@bayer.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.10.007

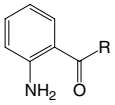
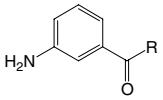
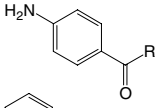
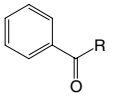
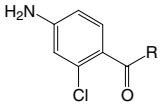
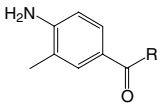
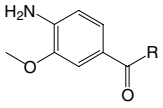
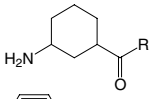
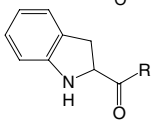
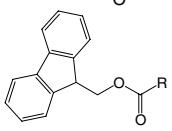
Table 1. Receptor activation (EC_{50}) and PYY-competitive binding (K_i) of reference compounds[20–24]

Compound	Peptide[a]	EC_{50}[b] (nM)	K_i[b] (nM)		
		NPY2	NPY2	NPY1	NPY5
1	PYY(3–36)	0.3 ± 0.5	0.4 ± 0.4	21 ± 2	20 ± 6
2	PYY(22–36)	11 ± 2	3 ± 0.5	390 ± 40	>1000
3	AcPYY(22–36)	13 ± 1	9 ± 1	118 ± 39	>1000
4	PYY(25–36)	240 ± 40	270 ± 40	>1000	>1000
5	AcPYY(25–36)	27 ± 6	30 ± 5	>1000	>1000
7	AcPYY(26–36)	430 ± 70	670 ± 99	>1000	>1000
8	NPY[Ahx5–24]	9 ± 2	5 ± 1	100 ± 54	>1000
9	AcNPY[Ahx5–24]	7 ± 1	7 ± 4	84 ± 28	>1000
10	AcNPY(25–36) lactam bridge	31 ± 6	75 ± 23	>1000	>1000

[a] PYY(X–Y) denotes a peptide corresponding to residues X–Y of human PYY. NPY[Ahx5–24] denotes a peptide corresponding to human NPY in which residues 5–24 are replaced with 6-aminohexanoic acid. AcNPY(25–36) lactam bridge denotes a peptide corresponding to residues 25–36 of human NPY with a lactam bridge between Lys 28 and Glu 32. Ac denotes acetylation of the N-terminal α-amino group. All peptides are amidated at the C terminus.

[b] Values are means of at least three experiments ± SEM.

Table 2. Receptor activation (EC_{50}) and PYY-competitive binding (K_i) of N-terminal α-amino analogs of **4**[20–24]

Compound	Peptide R = PYY(25–36)	EC_{50}[a] (nM)	K_i[a] (nM)		
		NPY2	NPY2	NPY1	NPY5
5		27 ± 6	30 ± 5	>1000	>1000
11		3 ± 1	4 ± 2	>1000	>1000
12		3 ± 1	4 ± 1	>1000	>1000
13		3 ± 1	6 ± 1	>1000	>1000
14		6 ± 1	6 ± 1	>1000	>1000
15		10 ± 1	6 ± 1	>1000	>1000
16		4 ± 1	6 ± 1	>1000	>1000
17		3 ± 1	5 ± 1	>1000	>1000
18		82 ± 13	110 ± 30	>1000	>1000
19		19 ± 4	18 ± 8	>1000	>1000
20		106 ± 9	95 ± 30	>1000	512 ± 25

[a] Values are means of at least three experiments ± SEM.

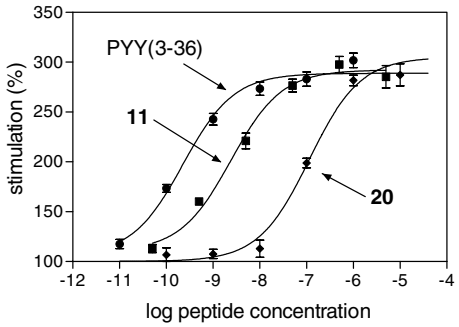


Figure 1. Stimulation of the human NPY2 receptor by human PYY(3–36) and the peptides **11** and **20** measured with GTPγS accumulation.[21] All peptides are full NPY2 agonists. Values are means of at least three experiments ± SEM.

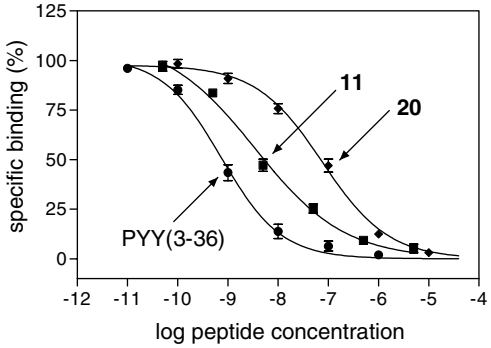


Figure 2. Competitive-binding displacement from the human NPY2 receptor of ^{125}I-labeled human PYY by human PYY(3–36) and by peptides **11** and **20**. Values are means of at least three experiments ± SEM.

that further improvements may be obtained with more complex modifications of the N-terminal α-amino group of PYY(25–36) (**4**).

N-terminal modification of PYY(25–36) (**4**) with aminobenzoic acid analogs provides potent and selective NPY2 receptor agonists (Table 2). Derivatization with 2-aminobenzoic acid (**11**) improves NPY2 receptor activation by 9-fold, increases NPY2 affinity and selectivity by 8-fold, and maintains selectivity against the NPY1 and NPY5 receptors (K_i > 1000 nM).

The connectivity or the presence of the amino moiety of the aminobenzoic acid analogs is not critical (**12**–**14**). The substituents Cl, CH_3, and OCH_3 are tolerated (**15**–**17**).

However, the use of a nonaromatic moiety (**18**) is very detrimental to NPY2 receptor binding and stimulation, the sterically larger N-terminal moiety of **19** offers no improvement over N-terminal α-amino acetylation, and the carbamate analog (**20**) is the least potent (Table 2). These results indicate that the effects of the N-terminal modification on NPY2 receptor binding and activation depend on the nature of the modifying group.

All of the peptides of Tables 1 and 2 are full agonists of the NPY2 receptor as compared with PYY(3–36) (Fig. 1), and changes in receptor binding (Fig. 2) correlate with changes in NPY2 receptor potency (Table 2).

We conclude that the combination of N-terminal truncation of PYY to minimize affinity for the NPY1 and NPY5 receptors and N-capping with aminobenzoic analogs to restore NPY2 receptor potency results in a series of highly selective NPY2 receptor agonists. This series of peptides exhibits both greater potency and NPY2 specificity, in terms of K_i ratios, compared to the previously described NPY2-selective peptide agonists **2**, **8**, and **10**.[17–19] Moreover, the series contains an amino group suitable for modification with fatty acids or PEG to improve in vivo efficacy.[25,26] Pharmaceutical modulation of the NPY2 receptor utilizing these highly selective and potent NPY2 receptor peptide agonists presents a potential approach for the management of obesity and associated disorders.

Acknowledgments

We thank B. R. Dixon, S. J. Gardell, J. N. Livingston, and P. P. Tamburini for valuable discussions and C. M. Pellegrino for mass spectrometry analysis.

References and notes

1. Small, C. J.; Bloom, S. R. *Expert Opin. Investig. Drugs* **2005**, *14*, 647.
2. Batterham, R. L.; Cowley, M. A.; Small, C. J.; Herzog, H.; Cohen, M. A.; Dakin, C. L.; Wren, A. M.; Brynes, A. E.; Low, M. J.; Ghatei, M. A.; Cone, R. D.; Bloom, S. R. *Nature* **2002**, *418*, 650.
3. Batterham, R. L.; Cohen, M. A.; Ellis, S. M.; Le Roux, C. W.; Withers, D. J.; Frost, G. S.; Ghatei, M. A.; Bloom, S. R. *N. Eng. J. Med.* **2003**, *349*, 941.
4. Challis, B. G.; Pinnock, S. B.; Coll, A. P.; Carter, R. N.; Dickson, S. L.; O'Rahilly, S. *Biochem. Biophys. Res. Commun.* **2003**, *311*, 915.
5. Cox, J. E.; Randich, A. *Peptides* **2004**, *25*, 985.
6. Pittner, R. A.; Moore, C. X.; Bhavsar, S. P.; Gedulin, B. R.; Smith, P. A.; Jodka, C. M.; Parkes, D. G.; Paterniti, J. R.; Srivastava, V. P.; Young, A. A. *Int. J. Obes. Relat. Metab. Disord.* **2004**, *28*, 963.
7. Chelikani, P. K.; Haver, A. C.; Reidelberger, R. D. *Endocrinology* **2005**, *146*, 879.
8. Degen, L.; Oesch, S.; Casanova, M.; Graf, S.; Ketterer, S.; Drewe, J.; Beglinger, C. *Gastroenterology* **2005**, *129*, 1430.
9. Koegler, F. H.; Enriori, P. J.; Billes, S. K.; Takahashi, D. L.; Martin, M. S.; Clark, R. L.; Evans, A. E.; Grove, K. L.; Cameron, J. L.; Cowley, M. A. *Diabetes* **2005**, *54*, 3198.
10. Moran, T. H.; Smedh, U.; Kinzig, K. P.; Scott, K. A.; Knipp, S.; Ladenheim, E. E. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2005**, *288*, R384.
11. Sileno, A. P.; Brandt, G. C.; Spann, B. M.; Quay, S. C. *Int. J. Obes.* **2006**, *30*, 68.
12. Abbott, C. R.; Small, C. J.; Kennedy, A. R.; Neary, N. M.; Sajedi, A.; Ghatei, M. A.; Bloom, S. R. *Brain Res.* **2005**, *1043*, 139.
13. 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.; Branchek, T. A.; Weinshank, R. L. *Nature* **1996**, *382*, 168.

14. Mullins, D.; Kirby, D.; Hwa, J.; Guzzi, M.; Rivier, J.; Parker, E. *Mol. Pharmacol.* **2001**, *60*, 534.
15. Cabrele, C.; Beck-Sickinger, A. G. *J. Pept. Sci.* **2000**, *6*, 97.
16. Balasubramaniam, A. *Curr. Pharm. Des.* **2003**, *9*, 1165.
17. Balasubramaniam, A.; Servin, A. L.; Rigel, D. F.; Rouyer-Fessard, C. R.; Laburthe, M. *Pept. Res.* **1988**, *1*, 32.
18. Beck-Sickinger, A. G.; Hoffmann, E.; Gaida, W.; Grouzmann, E.; Dürr, H.; Jung, G. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 937.
19. Rist, B.; Zerbe, O.; Ingenhoven, N.; Scapozza, L.; Peers, C.; Vaughan, P. F.; McDonald, R. L.; Wieland, H. A.; Beck-Sickinger, A. G. *FEBS Lett.* **1996**, *394*, 169.
20. Peptides were synthesized with solid-phase Fmoc/HBTU chemistry with an ABI 433A synthesizer. N-terminally modified peptides were prepared by coupling a 10-fold molar excess of DIEA/HBTU-activated Fmoc-protected modifying compound to the N-terminal amine prior to cleavage. Final purification was performed by C_{18} HPLC using a linear H_2O/CH_3CN gradient containing 0.1% (v/v) TFA. Purity (>99%) was confirmed with analytical C_{18} HPLC and identity was confirmed with electrospray mass spectrometry.
21. NPY2 receptor [^{125}I]-labeled PYY competitive binding assays were performed in a SPA format with membrane prepared from human KAN-TS cells.[27] Reagents and beads were diluted with binding buffer (50 mM Hepes, 10 mM $CaCl_2$, 5 mM $MgCl_2$, pH 7.4, and supplemented with 0.1% BSA). Increasing concentrations of peptide were incubated with 50 pM [^{125}I]-labeled human PYY (Perkin-Elmer), 200 μg wheatgerm agglutinin beads (Amersham), and membrane (10 μg) in a final volume of 200 μl. Nonspecific binding was defined with 1 μM PYY. Plates were incubated for 30 min at room temperature while shaking, removed from shaker, and kept at room temperature for an additional 2.5 h. The amount of radioactivity in the samples was quantified with a Wallac 1450 Microbeta Trilux liquid scintillation counter (Perkin-Elmer). Data were fit to a single-site binding model with Prism 3.03 (GraphPad Software).
22. NPY2 receptor [^{35}S]GTPγS incorporation assays were performed in a SPA format with membrane prepared from human KAN-TS cells.[27] Reagents and beads were diluted with binding buffer (50 mM HEPES, 100 mM NaCl, 1 mM $MgCl_2$, 1 μM GDP, 10 μg/ml saponin, and 0.1% BSA, pH 7.4). KAN-TS membranes (10 μg), increasing concentrations of peptide, 300 μg wheatgerm agglutinin beads (Amersham RPNQ0001), and 100 pM [^{35}S]GTPγS (Perkin-Elmer) were incubated in a final volume of 100 μl for 60 min at room temperature and then centrifuged for 1 min at 2 krpm (Beckman GS-6KR). Nonspecific binding was determined using 10 μM GTPγS. The amount of agonist-stimulated [^{35}S]GTPγS incorporated into KAN-TS membranes was quantified with a Wallac 1450 Microbeta Trilux liquid scintillation counter (Perkin-Elmer). Data were fit to a single-site binding model with Prism 3.03 (GraphPad Software).
23. NPY1 receptor [^{125}I]-labeled PYY competitive binding assays were performed in a filter-binding format with membrane prepared from human SK-N-MC cells.[28] Reagents were diluted with binding buffer (20 mM HEPES, 137 mM NaCl, 5.4 mM KCl, 0.44 mM KH_2PO_4, 1.26 mM $CaCl_2$, 0.81 mM $MgSO_4$, and 0.3% BSA, pH 7.4). Increasing concentrations of peptide were incubated with 75 pM human [^{125}I]-labeled PYY (Perkin-Elmer), and membrane (20–30 μg) in a final volume of 200 μl for 2 h at room temperature. Nonspecific binding was defined with 1 μM PYY. Following incubation, total content of the wells was transferred to Millipore HV plates (pretreated with 0.1% BSA), rapidly filtered, and washed thrice with 200 μl ice-cold binding buffer. Filter plates were air-dried, 15–20 μl scintillant added (Microscint O, Perkin-Elmer Life Sciences), and radioactivity quantified with a Wallac 1450 Microbeta Trilux liquid scintillation counter (Perkin-Elmer Life Sciences). Data were fit to a single-site binding model with Prism 3.03 (GraphPad Software).
24. NPY5 receptor [^{125}I]-labeled PYY competitive binding assays were performed in a filter-binding format with membrane from a HEK 293 cell line expressing recombinant human NPY5 receptor.[29] Reagents were diluted with binding buffer (25 mM Tris, 120 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 0.1% BSA, pH 7.4). Increasing concentrations of peptide were incubated with 75 pM [^{125}I]-labeled human PYY (Perkin- Elmer), and membrane (10 μg) in a final volume of 200 μl for 2 h at room temperature. Nonspecific binding was defined with 1 μM PYY. Following the incubation, total content of the wells was transferred to Millipore HV plates (pretreated with 0.1% BSA and aspirated prior to transfer), rapidly filtered, and washed thrice with 200 μl ice-cold binding buffer. Filter plates were then air-dried, 15–20 μl scintillant added (Microscint O), and radioactivity quantified with a Wallac 1450 Microbeta Trilux liquid scintillation counter (Perkin-Elmer Life Sciences). Data were fit to a single-site binding model with Prism 3.03 (GraphPad Software).
25. Kurtzhals, P.; Havelund, S.; Jonassen, I.; Kiehr, B.; Larsen, U. D.; Ribel, U.; Markussen, J. *Biochem. J.* **1995**, *312*, 725.
26. Harris, J. M.; Chess, R. B. *Nat. Rev. Drug Disc.* **2003**, *2*, 214.
27. Rimland, J. M.; Seward, E. P.; Humbert, Y.; Ratti, E.; Trist, D. G.; North, R. A. *Mol. Pharmacol.* **1996**, *49*, 387.
28. Larhammar, D.; Blomqvist, A. G.; Yee, F.; Jazin, E.; Yoo, H.; Wahlested, C. *J. Biol. Chem.* **1992**, *267*, 10935.
29. Hu, Y.; Bloomquist, B. T.; Cornfield, L. J.; DeCarr, L. B.; Flores-Riveros, J. R.; Friedman, L.; Jiang, P.; Lewis-Higgins, L.; Sadlowski, Y.; Schaefer, J.; Velazquez, N.; McCaleb, M. L. *J. Biol. Chem.* **1996**, *271*, 26315.