Bioorganic & Medicinal Chemistry Letters, Vol.2, No.12, pp. 1497-1502, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

Neuropeptide Y (NPY) Functional Group Mimetics: Design, Synthesis, and Characterization as NPY Receptor Antagonists

Michael B. Doughty*†, Shao Song Chu†, Gregory A. Misse¶, and Richard Tessel¶
Departments of Medicinal Chemistry† and Pharmacology and Toxicology¶, School of Pharmacy, University of Kansas, Lawrence, KS 66045-2506, U.S.A.

(*Received* 16 *June* 1992)

Abstract: N,N'-bis-[2-N-(O-2,6-dichlorobenzyl-L-tyrosyl)aminoethylguanyl]cystamine **3** and N,N'-bis-[2-N-(O-2,6-dichlorobenzyl-L-tyrosyl)aminoethyl]-1,6-hexanediguanidine **4** have been designed as neuropeptide Y (NPY) functional group mimetics. Both **3** and **4** displace N-[propionyl-^{3}H]-NPY from rat brain binding sites, and are NPY receptor antagonists in rat femoral artery ring segments.

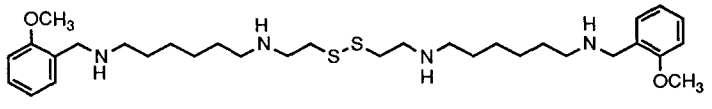
Neuropeptide Y (NPY,**1**, Chart), a 36 amino acid peptide neurotransmitter, is widely distributed with catecholamine-containing neurons in both the central and peripheral nervous systems of many animal species, including humans.[1] NPY's potent vasoconstrictive and neuromodulatory activities have stimulated interest in NPY antagonists as potential drugs for the treatment of hypertension and related cardiovascular disease states.[2] However, only benextramine[3] and PP56[4] have been reported as antagonists of NPY's vasoconstriction activity.

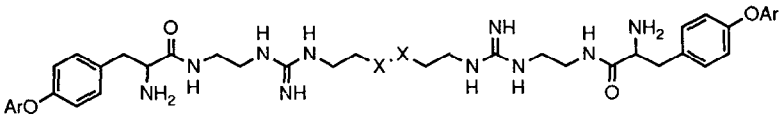
In a previous study, we reported that benextramine (**2**, Chart), a tetramine disulfide long known to be an irreversible antagonist of α-adrenoceptors, produces a long-acting antagonism of NPY's pressor activity, and irreversibly inhibits N-[propionyl-^{3}H]-NPY ([^{3}H]-NPY) specific binding to a sub-population (61%) of receptors in rat brain membranes.[3] The chemical similarities between benextramine and the N-terminal and C-terminal regions of NPY led us to hypothesize that

Chart

1 (NPY) Y-P-S-K-P-D-N-P-G-E-D-A-P-A-E-D-L-A-R-Y-Y-S-A-L-R-H-Y-I-N-L-I-T-R-Q-R-Y-NH_2

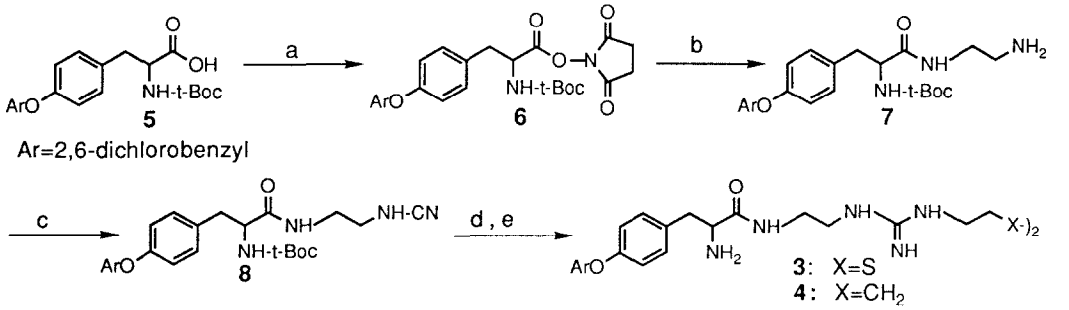
2 (benextramine)





3 (SC3117): X=S; Ar=2,6-dichlorobenzyl
4 (SC3199): X=CH_2; Ar=2,6-dichlorobenzyl

Scheme



a)DCC, N-hydroxysuccinimide; b) $H_2NCH_2CH_2NH_2$; c) BrCN, Et_3N, CH_2Cl_2; d) $H_2NCH_2CH_2S)_2/2HCl$ or $H_2N(CH_2)_6NH_2$ /2HCl, DIEA, n-butanol; e) 50% TFA/CH_2Cl_2.

benextramine mimics the functional groups of NPY involved in NPY receptor recognition, i.e., the phenolic side chain and α-amino group of Tyr^1, the guanidinium groups of Arg^{33} and Arg^{35}, and the phenolic side chain of Tyr^{36}. The importance of these functional groups for NPY's brain and peripheral activities has been demonstrated in previous structure-activity studies.[5]

Although benextramine does not fulfill the criteria of high affinity and specificity, it provides a "lead" for the development of non-peptide NPY antagonists with high potency and selectivity. In this study, two NPY functional group mimetics - **3** and **4** (Chart) - have been designed, synthesized, and characterized as NPY receptor antagonists. Principles applied to the design of **3** and **4** include: 1) the cystamine amine groups of benextramine were converted to guanidinium groups in order to more closely mimic the Arg^{33} and Arg^{35} guanidinium groups of NPY; 2) the *o*-methoxybenzylamine moieties of benextramine were converted to tyrosine residues in order to mimic Tyr^1 and Tyr^{36} of NPY; and 3) the disulfide of benextramine was converted to its carbon analog (in **4**) in order to avoid the toxic side effects observed with benextramine and to increase NPY versus α-adrenergic selectivity.

The synthetic protocol to **3** and **4** is outlined in the Scheme. Protected O-2,6-dichlorobenzyl-α-N-t-Boc-L-tyrosine **5** was reacted with DCC and N-hydroxysuccinimide in CH_3CN at room temperature to form the active ester **6** in 80% yield, which was then reacted with a 10-15 fold excess of ethylenediamine in CH_2Cl_2 to yield the tyrosine monoamide **7** in 81% yield.[6] Although the procedure reported by Buschaeur[7] using diphenyl N-benzoylcarbonimidate as the coupling reagent failed to convert **7** to the target disubstituted guanidines, a convenient synthetic method was developed. The N-alkyl-cyanamide **8** was synthesized in 70% yield by converting the amino group of **7** to a cyanimidyl group by reaction with cyanogen bromide.[8] Compounds **3** and **4** were obtained after refluxing two equivalents of **8** with one equivalent of the appropriate diamine dihydrochloride (cystamine or 1,6-hexanediamine, respectively) and 1 equivalent of DIEA in n-butanol overnight, followed by removal of the t-Boc protecting groups by treatment with a 50-fold excess of 50% TFA/CH_2Cl_2 at room termperature for 30 min. The products were purified by CM-

Sephadex C-25 ion-exchange chromatography and C-4 reverse phase HPLC, and subsequently converted to their hydrochloride salts.[9]

The receptor binding activities of **3** and **4** were assayed as the displacement of specifically-bound [^{3}H]-NPY from rat brain membrane homogenates obtained from male Sprague-Dawley (SD) rats (200-300 g) by a previously reported method.[3] The concentration-dependent displacement data were fit to a one- or two-site competitive binding isotherm by non-linear regression analysis using the program MINSQ (Micromath, Salt Lake City, UT). Compounds **3** and **4** maximally inhibited 63 and 100%, respectively, of [^{3}H]-NPY specific binding with IC_{50}'s of 18.5 and 45.4 μM, respectively (Table). In "paired tube" assays, **3** displayed selectivity for the benextramine-sensitive binding site population in rat brain in that it was unable to displace [^{3}H]-NPY from rat brain membrane binding sites remaining after treatment with 500 μM benextramine (data not shown). In contrast, **4** was not selective for the benextramine-sensitive sites since at 10^{-4} M it displaced 25±5% of specifically-bound [^{3}H]-NPY from binding sites remaining after treatment with 500 μM benextramine.

Table: Activities of **2**, **3**, and **4** in displacing [^{3}H]-NPY from specific binding sites in rat brain.

Compound	IC_{50}[a] (μM)	K_i (μM)[b]	% MaxDisplacement	r^2
2	56.0±7.5[c]	-	61±3	0.999
3	18.5±2.6	-	63±6	0.9985
4	45.4±9.2	35.5	100	0.9997

a. IC_{50} (mean ± standard error; n=3) is the concentration required to inhibit ^{3}H-NPY specific binding by 50% from the binding site population(s) defined by % maximum displacement. b. K_i for **4** was calculated from the equation: $Ki=IC_{50}/(1+([[^3H]\text{-NPY}]/Kd_{NPY}))$. Ki's are not given for **2** and **3** because their respective IC_{50}'s contain a kinetic term for irreversible binding to the [^{3}H]-NPY binding sites. c. Data from reference 3.

The NPY-selective antagonistic activity of **3** and **4** relative to benextramine was evaluated as the inhibition of rat femoral artery constriction induced by near maximal concentrations of NPY or methoxamine. Briefly, 3-mm long femoral artery ring segments obtained from male SD rats (300-400 g) were mounted on two L-shaped metal holders. The artery rings were suspended in a water-jacketed organ bath containing 3 ml of modified Krebs buffer at 37°C, maintained at pH 7.4 by gassing with a mixture of 95% O_2-5% CO_2, and connected to a Grass T3 Force-Displacement Transducer with a 1000 mg resting tension (Time 0). The rings were subsequently exposed twice (30 and 60 min after Time 0) to 10 μM norepinephrine until maximal constriction occurred; norepinephrine was then removed by washing. In addition, the rings were washed with fresh buffer every 10 min except during exposure to vasoconstrictors. NPY (5 μM) or methoxamine (10 μM) was then applied (90 min after Time 0), and after maximal constriction was established, cumulative

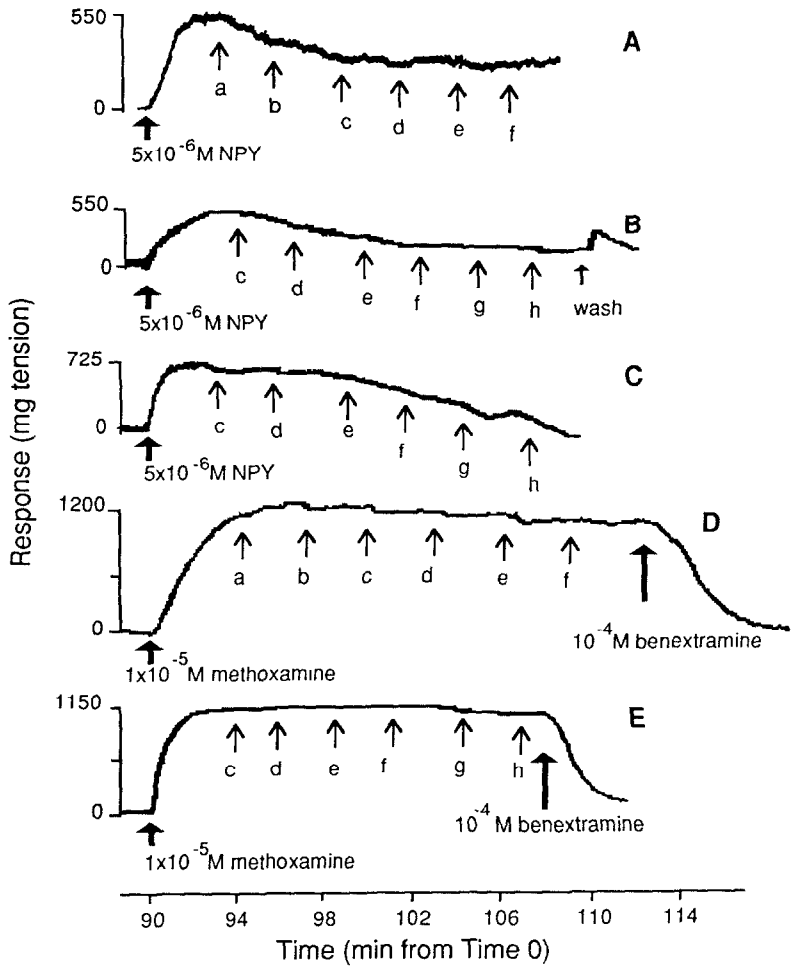


Figure: Representative experiments illustrating the inhibitory activities of **3**, **4** and benextramine at NPY and α-adrenergic receptors in rat femoral artery rings. **A.** The inhibitory effect of **3** after artery contraction with $5x10^{-6}$ M NPY. **B.** The inhibitory effect of **4** after contraction with $5x10^{-6}$ M NPY. **C.** The inhibitory effect of benextramine after contraction with $5x10^{-6}$ M NPY. **D.** The activity of **3** and benextramine ($1x10^{-4}$ M) after contraction with $1x10^{-5}$ M methoxamine. **E.** The activity of **4** and benextramine ($1x10^{-4}$ M) after contraction with $1x10^{-5}$ M methoxamine. Inhibitor concentrations (M): a, $3.2x10^{-8}$; b, $1x10^{-7}$; c, $3.2x10^{-7}$; d, $1x10^{-6}$; e, $3.2x10^{-6}$; f, $1x10^{-5}$; g, $3.2x10^{-5}$; h, $1x10^{-4}$.

doses of **3**, **4** or benextramine were applied every 3 min. As illustrated by the representative experiments given in the Figure, **3** (Figure, **A**), **4** (Figure, **B**) and benextramine (Figure, **C**) inhibited the vasoconstriction induced by NPY with IC_{50}'s of 43.1±4.1 nM, 414±38.6 nM and 2.88±0.18 μM, respectively (n=3). An interesting feature of the NPY vasoconstriction inhibitory activity of **3** and **4** is

that at maximum inhibition (10^{-6} and 10^{-5} M, respectively) the vessel was not fully relaxed. Although this may have been a mechanical effect since the vessel could not be further relaxed by washing and neither **3** nor **4** displayed partial agonist activity in these experiments in the rat femoral artery (Figure), we are currently determining the selectivity of **3** and **4** at post-synaptic Y_1- versus Y_2-like NPY receptor populations in this system.[10] Reasons for the increased potencies of **3**, **4** and benextramine in the in vitro vasoconstriction assay relative to their respective activities in the rat brain are currently unclear, but may represent structural differences between the NPY peripheral, post-synaptic receptors versus the rat brain [^{3}H]-NPY binding sites.

The activities of both **3** and **4** at α-adrenergic receptors are also illustrated in the Figure. Although **3** (Figure, **D**) and **4** (Figure, **E**) did not display a concentration-dependent inhibition of the vasoconstriction activity of 10μM methoxamine at concentrations up to 10^{-5} and 10^{-4} M, respectively, the constriction induced by methoxamine was antagonized completely by 10^{-4} M benextramine (Figure, **D** and **E**).

The reversibility of **3**, **4** and benextramine as NPY receptor antagonists was examined by a 10 min preincubation of the femoral artery ring segments with antagonist at Time 70 min, followed by washing the vessel with fresh buffer at Time 80 min prior to the application of 5 μM NPY at Time 90 min. In these experiments the NPY inhibitory activity of neither **3** nor benextramine could be washed away (data not shown), evidence suggesting that these antagonists are irreversible, or very slowly reversible. In contrast, preincubation with **4** at time 70 min followed by washing eliminated the effect of **4** on NPY-induced vasoconstriction.

In conclusion, a convenient synthetic method has been developed for the preparation of disubstituted guanidines using an N-alkyl-cyanamide as the precursor, and two NPY functional group mimetics have been successfully synthesized. The results of in vitro activity assays suggest that both **3** and **4** are NPY versus α-adrenergic receptor selective, irreversible and reversible antagonists, respectively, in rat femoral artery ring segments.

We acknowledge the American Heart Association, National Center, for providing financial support for this research.

References and Notes

1. Potter, E. K. *Pharmac. Ther.* **1988**, *37*, 251-273.
2. Lehmann, J. *Drug Dev. Res.* **1990**, *19*, 329-351.
3. Doughty, M. B.; Chu, S. S.; Miller, D. W.; Ke, L. ; Tessel, R. E. *Eur. J. Pharm.* **1990**, *185*, 113-114.
4. Edvinsson, L.; Adamsson, M. ; Jansen, I. *Neuropeptides* **1990**, *17*, 99-105.
5. Allen, J.; Novotny, J.; Martin, J. ; Heinrich, G. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 2532-2536.
6. Doughty, M. B.; Chaurasia, C. ; Li, K. *J .Med .Chem* .**1992**, in press.
7. Buschaeur, A. *J. Med. Chem.* **1989**, *32*, 1963-1970.
8. Cyanogen bromide (0.42 g, 4 mmol; **Caution**: cyanogen bromide is highly toxic and must be used in a well-ventilated fume hood) was added to a solution of **7** (1.93 g, 4 mmol) and triethylamine (0.5 g, 4 mmol) in methylene chloride (200 ml) at -78 °C with stirring. The solution was warmed to room temperature and stirred for an additional 30 min. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (250 ml). The ethyl acetate

solution was washed with 0.1 N HCl (3x 50 ml) followed by brine (3x 50 ml) and dried over $MgSO_4$. The solvent was removed by roto-evaporation and the crude product was purified by silica gel column chromatography (ethyl acetate/ methylene chloride/methanol 2:2:0.1) to afford 1.4 g of **8** in 70 % yield. Anal calcd for $C_{24}H_{28}N_4O_4Cl_2$: C,H,N; ^1H NMR ($CDCl_3$): δ 7.40 (d, J=6.5Hz, 2H), 7.30 (m, 1H), 7.15 (d, J=6 Hz, 2H), 7.00 (d, J=6 Hz, 2H), 6.25 (br, 1H), 5.25 (s, 2H), 4.50 (br, 1H), 4.20 (m, 1H), 3.38 (m, 2H), 3.16 (m, 2H), 3.02 (m, 2H), 1.40 (s, 9H); MS m/e: 507 ($M^{+\cdot}$ 50); 482 (M^+-CN, 20); IR (KBr) 2240 cm^{-1} (NHCN).

9. Crude product (300 mg) in 20% methanol at pH 5.5 was loaded onto a 1.5x30cm CM Sephadex C-25 column equilibrated in 0.1M NH_4OAc, 20% methanol, pH 5.5, and eluted with a pH gradient (pH 5.5 to 9.0 in 0.1M NH_4OAc, 20% methanol, 400 ml total volume) and a salt gradient (0.1 to 1.0 M NH_4OAc, 20% methanol, pH 6.0, 400 ml total volume). The last major peak was pooled and lyophilized. Final purification was accomplished by semi-preparative reverse phase HPLC on a Vydac C-4 column eluting with 40% CH_3CN in 0.1% TFA . Purification yields were generally 20-30%. For **3**: High-resolution FAB MS m/e 929.3342 [calcd for $C_{44}H_{56}N_{10}O_4Cl_4$ $(M+H)^+$: 929.3318]; ^1H NMR (CD_3OD): δ 7.32 (m, 2H), 7.24 (m, 1H), 7.08 (d, J=6 Hz, 2H), 6.92 (d, J=6 Hz, 2H), 5.14 (s, 2H), 4.04 (dd, J_1=9 Hz, J_2= 6 Hz, 1H), 3.42 (t, 4H), 3.12 (m, 1H), 3.05-2.80 (m, 3H), 2.74 (t, 2H). For **4**: High-resolution FAB MS m/e 965.2421 [calcd for $C_{42}H_{52}N_{10}O_4Cl_4S_2$ $(M+H)^+$: 965.2447];^1H NMR (CD_3OD): δ 7.22 (m, 2H), 7.14 (m, 1H), 7.08 (d, J=6 Hz, 2H), 6.88 (d, J=6 Hz, 2H), 5.08 (s, 2H), 4.08 (dd, J_1=9 Hz, J_2= 6 Hz, 1H), 3.45-2.80 (m, 8H), 1 38 (m, 2H), 1.10 (m, 2H).

10. Tessel, R. E.; Xu, D.; Miller, D. W. ; Doughty, M. B. *Soc. Neuroscience, Abst.* **1991**, *17*, 976.