Potent and Selective 1,2,3-Trisubstituted Indole NPY Y-1 Antagonists

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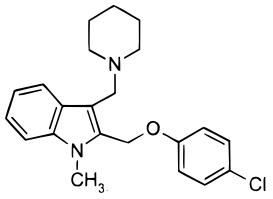
Received August 4, 1997

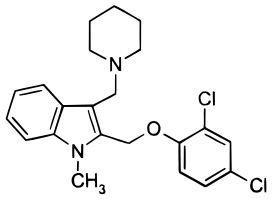
Neuropeptide Y (NPY) was originally isolated from extracts of porcine brain and is named for the presence of an N-terminal tyrosine (tyr = "Y") and a C-terminal tyrosine amide. NPY is a 36 amino acid peptide and a member of the family of neurotransmitter peptides that also includes peptide YY (PYY) and pancreatic polypeptide (PP).[1] NPY is the most abundant peptide neurotransmitter in the brain, and its receptors are prominent in both the central nervous system and peripheral tissues.[2,3] While several NPY receptor subtypes have been characterized at the molecular level (Y1, Y2, Y4, Y5, and Y6), the various pharmacological responses of NPY have not, in many cases, been definitively ascribed to a particular subtype.[4] However, based on NPY physiological responses and anatomical localization, NPY has been implicated in a variety of disorders including obesity and diabetes.[5−11]

One of the fundamental difficulties in defining the functional roles of NPY has been the absence of specific receptor ligands. The synthesis of peptide analogs or chimeric peptides provided selective agonists, but few useful peptide antagonists are available. Over the last several years, nonpeptidyl NPY Y1 receptor antagonists (BIBP3226,[12,13] SR120819A,[14] and PD160170[15]) have appeared in the literature. While these NPY Y1 tools do exist, the description of unique NPY Y1 receptor subtype pharmacology has been ambiguous due to lack of potency, selectivity, or *in vivo* efficacy of these agents. Considerable interest remains in the discovery of selective Y1 antagonists. Herein we disclose a totally novel series of selective and potent NPY Y1 antagonists that should help elucidate of the role of the Y1 receptor subtype.

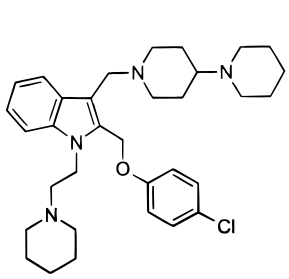
Biased library screening and follow-up similarity searching of the Lilly compound files uncovered the trisubstituted indole **1** (K_i = 2.1 μM). On the basis of this low molecular weight lead, a series of trisubstituted indoles were pursued using traditional medicinal chemistry. In this paper the effects of substituent pattern modifications at N-1, C-2, and C-3 will be reported. In addition to chemical synthesis, radioligand binding affinities for the cloned human Y1 receptor, *in vitro* functional activity, and selectivity data verses Y1, Y2, Y4 and Y5 receptor lines will be reported. Initial *in vivo* data showing antagonism by *S*-**18** of the feeding induced by intracerebroventricularly injected NPY is also presented.



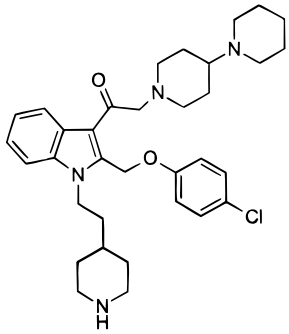
1
2315 ± 193nM



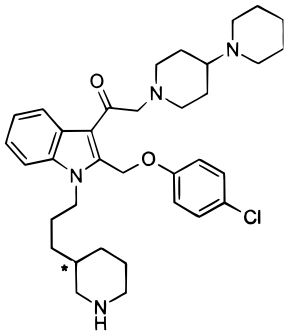
10
1260 ± 122 nM



16
98 ± 3.2 nM



17
1.9 ± 0.13 nM



18 (S); 0.75 ± 0.02 nM
18 (R); 1.4 ± 0.07 nM

Chemistry. Synthesis of compounds **1** and **10** through **18** was accomplished as described in Scheme 1. Deprotonation of **2** with sodium hydride followed by alkylation with the appropriate alkyl iodide or bromide gave **3**. Reduction with lithium aluminum hydride followed by nucleophilic aromatic substitution of a fluorobenzene yielded **5**. Alternatively the aryloxy group could be installed via Mitsunobu reaction of **4** and the appropriate phenol. Mannich reaction of **5** using paraformaldehyde and the appropriate amine gave **6**. Alternatively, **5** could be treated with oxalyl chloride in ether, followed by an amine to yield compounds such as **7**. Reduction of **7** with borane−dimethyl sulfide produced compound **8** in reasonable yield. Finally, compound **5** could also be treated with bromoacetyl bromide and powdered lithium carbonate in diethyl ether under ultrasound conditions, followed by treatment with the desired amine to give **9** in modest yield. Deprotection of tritylamino or *tert*-butyl carbamate groups, if necessary (see compounds **17**, *S*-**18**, and *R*-**18**), to yield free amino functionalities was accomplished via standard techniques. Chiral alkyl bromides necessary for compounds *S*-**18** and *R*-**18** were prepared via classical resolution techniques, and enantiomeric purities were verified (>99% ee) via intermediate Mosher amide

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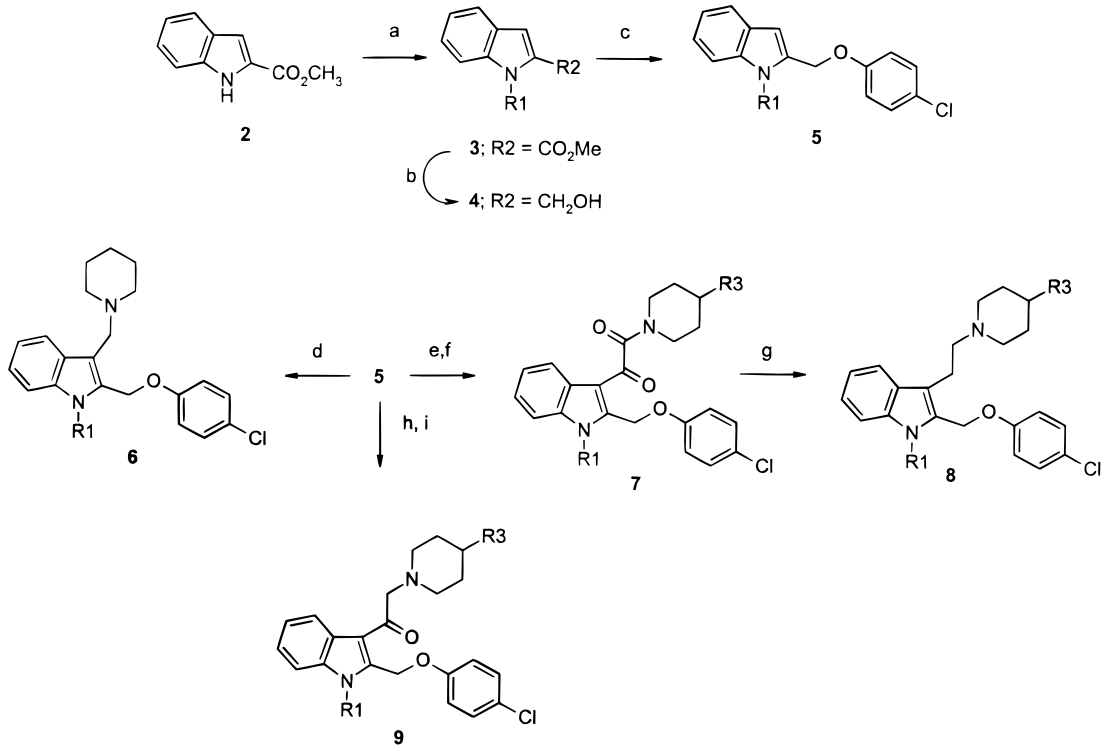
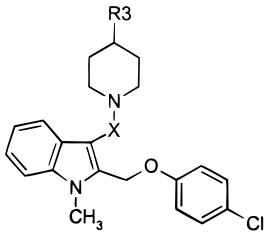
S0022-2623(97)00512-8 CCC: $14.00

Scheme 1.[a] General Synthetic Strategy for 1,2,3-Trisubstituted Indoles



[a] Reagents: (a) NaH, R1I or R1Br, DMF, 0 °C to room temperature; (b) LAH, THF, 0 °C to room temperature; (c) NaH, ArF, DMF, 80 °C; (d) piperidine, paraformaldehyde, concentrated aqueous HCl, NaOAc, EtOAc; (e) oxalyl chloride, diethyl ether; (f) amine, THF; (g) borane−dimethyl sulfide complex, THF; (h) bromoacetyl bromide, Li_2CO_3, diethyl ether; (i) amine, THF.

Table 1. Chemical Structures and Binding Affinities at Cloned NPY-1 Receptors Expressed in AV-12 Cells



compound	X	R3	Y1 binding affinity K_i, mean ± SEM (nM)
BIBP3226	NA	NA	4.6 ± 0.3
1	CH_2	H	2315 ± 193
11	CH_2CH_2	H	2875 ± 75
12	CH_2	1-piperidinyl	559 ± 83
13	CH_2CH_2	1-piperidinyl	93 ± 4
14	COCO	1-piperidinyl	440 ± 20
15	$COCH_2$	1-piperidinyl	26 ± 1.8

formation followed by capillary GC analysis. Absolute configurations of intermediates were determined via X-ray crystallographic analysis of mandelic acid salts.

Results and Discussion. A Topliss decision analysis of the C-2 side chain SAR lead to a modest improvement in activity over the 4-chloro substitution pattern with the 2,4-dichloro compound **10** (1.3 μM). All other substitution patterns lead to derivatives of lower affinity. For simplicity, the remaining structure−activity study is exemplified as the 4-monochloro derivative.

Structure−activity relationships for C-3 modifications are summarized in Table 1. Early concerns regarding the stability of "gramine" structures **6** motivated a desire toward additional C-3 modifications. Homologation of the C-3 side chain gave compound **11** (2.9 μM). While additional efforts toward higher homologs did not lead to improvement, further examination of the C-3 structure−activity relationships proved to be more fruitful. Studies of the substitution pattern around the C-3 piperidine ring revealed that additional basic substituents at the 4-position of this ring could improve affinity at the Y1 receptor significantly. Compound **12** was an early example wherein the affinity improved nearly 4-fold. Optimization of the C-3 chain length was achieved with compound **13** (93 nM).

During the synthesis of **13**, dicarbonyl compounds similar to **7** were employed as synthetic intermediates. Examination of their binding properties revealed they possessed reasonable affinity for the Y1 receptor (**14**, 0.44 μM). This variation of affinity based on differential oxidation states of the C-3 linker (**13** vs **14**) piqued our interest in other carbonyl variants that might act as acyclic conformational restraints at C-3. Among those compounds examined, **15** (26 nM) was uncovered, representing a 100-fold improvement over the original lead structure **1**.

Concurrent with the structure−activity studies at C-3, an analysis of the N-1 SAR was pursued. Early on in our efforts, it was hypothesized that additional basic substituents might further improve the potency of this series. This notion was supported by the reported importance of the -Arg^{33}-X^{34}-Arg^{35}- substructure in the carboxamide terminus of NPY.[17] The initial example of this type of compound was **16** which exhibited a 6-fold improvement in affinity over **12**. Optimal side chains for the N-1 position eventually proved to be alkylpiperidines with a free N-H. Combining this with our earlier findings within the C-2 and C-3 structure−activity studies resulted in the discovery of compounds **17** (1.3 nM) and *S*-**18** (0.75 nM). Apparently little

stereodifferentiation occurs at the binding site on the receptor as the opposite enantiomer, *R*-**18**, was only 2-fold less potent.

In addition to the Y1 binding assay, the binding affinity of all compounds at cloned human Y2, Y4, and Y5 receptors was examined. Affinities (K_i's) at these subtypes were in all cases greater than 10 μM. Further compound *S*-**18** reversed NPY-induced inhibition of forskolin-stimulated cAMP (K_i = 1.8 nM) and inhibited NPY-induced intracellular Ca^{2+} mobilization (K_i = 3.2 nM) in SK-N-MC cells.[16] All compounds in this series were found to be antagonists. No partial or full agonism was observed.

The ability of *S*-**18** to antagonize an *in vivo* NPY-induced (230 pmol, icv) food consumption effect was examined. When injected into the lateral ventricle of mice, very low doses of NPY (69−2300 pmol, icv) rapidly produced a specific and robust dose-dependent increase in food intake which peaks within 30 min of the injection and lasted for approximately 90 min. For example, a submaximal dose of NPY (230 pmol, icv), elicits a 200% increase in time spent feeding over vehicle control mice. The ability of *S*-**18** to block this central NPY-induced food consumption was tested by co-administering *S*-**18** with NPY (230 pmol, icv) into the lateral ventricle. *S*-**18** was found to block the increase in food consumption elicited by NPY [ED_{50} = 17 nmol (lower and upper confidence limits of 11−26 nmol)] in a dose dependent manner. Moreover, at doses found to antagonize NPY-induced feeding, icv-administered *S*-**18** did not exhibit neuromuscular dysfunction as measured by the horizontal screen test or general behavior malaise. Thus, the antagonism of central NPY-induced feeding behavior by *S*-**18** appears to be mediated by the Y-1 receptor. Unfortunately serum levels of *S*-**18** upon oral or subcutaneous administration were inadequate to evaluate the compound systemically in these models.

Biased library screening at cloned human NPY Y-1 receptors followed by a traditional medicinal chemistry strategy produced a novel series of 1,2,3-trisubstituted indole NPY Y1 antagonists. Structure−activity studies at N-1, C-2, and C-3 of the indole nucleus were examined, and the importance of a distally opposed diamine motif (N-1 *and* C-3 amine containing side chains) as well as homologation and optimal oxidation of the C-3 side chain was delineated. This resulted in the discovery of *S*-**18** (LY357897), the first selective, subnanomolar NPY Y1 antagonist. A more detailed description of our investigations within this class as well as complete pharmacological characterization of members of this and related series will be reported in subsequent papers.

Supporting Information Available: Experimental details and spectroscopic information (15 pages). Ordering information is given on any current masthead page.

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JM970512X