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Discovery of Potent and Selective Small Molecule NPY Y5 Receptor Antagonists

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Abstract—The discovery of a new class of sulfonamide NPY Y5 receptor antagonists is described. Optimization of this series led to the identification of compounds with high affinity for the hY5 subtype and excellent selectivity over the other NPY receptor subtypes. The SAR for this series was examined and a model for understanding the ligand–receptor interactions was developed.

Neuropeptide Y (NPY) is a 36 amino acid peptide neurotransmitter widely expressed in both the peripheral and central nervous system.[1] Cloned receptors for NPY and related family members (peptide YY and pancreatic polypeptide) are classified as Y-type receptors (Y1, Y2, Y4, and Y5; note there is also a Y6 receptor gene that is absent in the rat and a pseudogene in human). NPY is considered to regulate a variety of physiological processes, including vasoconstriction, nasal congestion, blood pressure, intestinal motility, anxiety, depression, pain, feeding, reproductive endocrinology, neuronal excitability and memory retention.[2] As such, receptor-specific ligands to modulate NPY receptor signaling may have therapeutic value. In this report, we describe the discovery of potent and selective sulfonamide Y5 antagonists and provide a model of how these antagonist bind to the NPY Y5 receptor.[3]

When this project was initiated, limited information about small molecule NPY ligands was available. Our starting point was the weakly potent ligand benextramine **1** (Fig. 1).[4] Due to its flexible and polybasic structure, benextramine binds with little selectivity to multiple GPCRs and provides limited structural information to guide the discovery of more potent and selective ligands. Consequently, we used symmetrical polyamine benextramine analogues as probe molecules to explore shorter linking chains, different aromatic groups and to identify the number of basic amines required for binding. Symmetrical bis-2-naphthylmethylamines such as **2**–**4** with shorter (two, four, and six) methylene linker groups and containing either two or four basic sites were found to have similar potency to benextramine. In an effort to discover more potent and selective ligands, sulfonamides **5** and **6** were prepared. These arylsulfonamide amines were based on a common GPCR ligand motif or privileged structure[5,6] where an arylamide (or sulfonamide) functionality is separated by an alkyl spacer from a basic benzylic amine or an aryl piperazine. Both **5** and **6** demonstrate improved selectivity for the hY5 receptor. Sulfonamide **6**, containing the longer linker, has significant (120 nM) potency for the hY5 receptor in addition to good selectivity. It is interesting that the sulfonamide functionality is present in SR 120107A, a Sanofi NPY Y1 selective antagonist.[7] While we had hoped for an improvement in potency with the introduction of the sulfonamide, the significant enhancement in selectivity for Y5 versus Y1 was unexpected.

To improve the affinity of sulfonamide amine **6**, we examined more rigid linking chains that could orient the arylsulfonamide and benzylic amine functionalities correctly in the Y5 receptor's binding pockets. Overlays between sulfonamide **6** and multiple HTS hits suggested the more rigid bis-(1,4-aminomethylene)cyclohexane as a linker. Testing this hypothesis, lead to the synthesis and testing of sulfonamide **11**, a potent ($K_i = 8$ nM) Y5 receptor subtype antagonist with greater than 500-fold selectivity over the other NPY receptor subtypes. The

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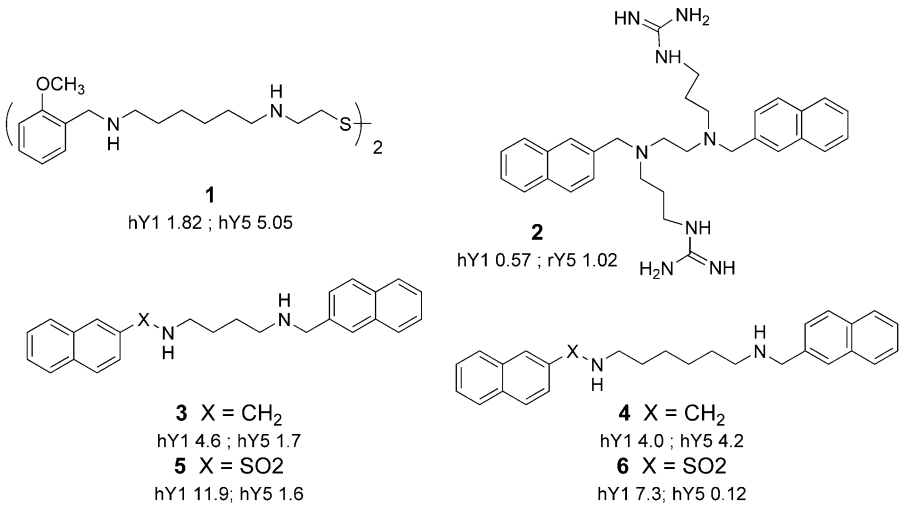
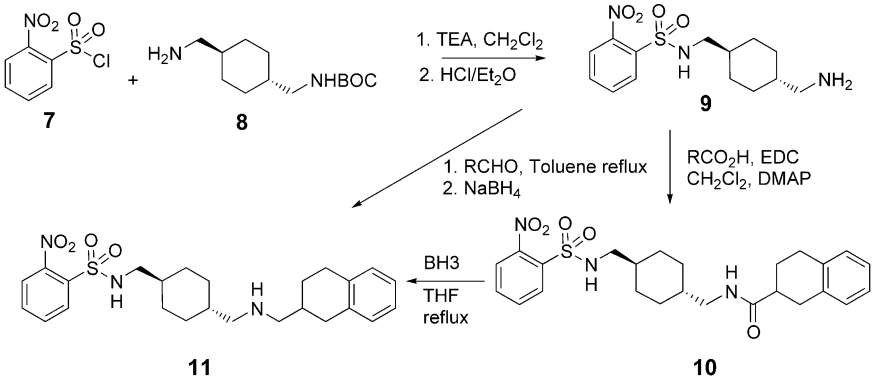


Figure 1. Binding affinities for benextramine and analogues (K_i in μM).

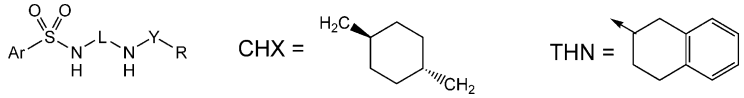


Scheme 1.

preferred stereochemistry of the 1,4-cyclohexane ring substituents is trans. Synthesis of sulfonamide **11** (Scheme 1) is illustrative of the synthetic route to a series of sulfonamide amines. Sulfonylation of the mono-protected diamine **8** followed by deprotection affords arylsulfonamides **9**. Reductive amination of **9** with 2-formyl-tetrahydronaphthalene yielded **11** directly. Alternatively, **9** was acylated to yield a sulfonamide amide **10.** Reduction of **10** with borane afforded the desired sulfonamide amine **11**.

Table 1 shows the binding affinities of selected sulfonamide analogues for human NPY receptor subtypes. Common to this set of potent hY5 ligands are two hydrophobic, preferably aromatic, groups. Naphthyl and 2-substituted phenyl groups are favored in the aryl sulfonamide binding region. The second aryl pocket is considerably more permissive and a range of different groups are compatible with good affinity. The ability to vary this second aromatic group allowed us to prepare a set of ligands with a range of physicochemical properties.

Table 1. Binding affinities for arylsulfonamide amines



Compd	Ar	L	Y	R	K_i hY1 (nM)	K_i hY5 (nM)
6	2-Naphthyl	$(CH_2)_6$	CH_2	2-Naphthyl	7300	123
11	2-$NO_2C_6H_4$	CHX	CH_2	THN	16,032	8
12	2-Naphthyl	CHX	CH_2	THN	5546	14
13	2-$NH_2C_6H_4$	CHX	CH_2	THN	17,179	10
14	2-$NO_2C_6H_4$	CHX	CH_2	2-Quinoline	23,714	14
15	1-Naphthyl	CHX	CH_2	THN	3548	11
16	2-$CF_3C_6H_4$	CHX	CH_2	1-Naphthyl	14,454	26
17	1-Naphthyl	CHX	$CH(CH_2OH)$	4-Cl-benzyl	3236	17
18	2-$CF_3C_6H_4$	CHX	CH_2	2-Naphthyl	12,981	10
19	2-$NO_2C_6H_4$	CHX	CH_2	4-Quinoline	48,697	28

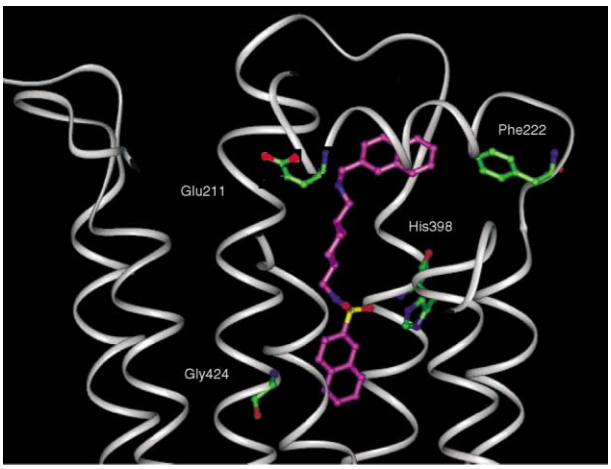


Figure 2. Model of sulfonamide **6** binding to hY5 receptor.

The sulfonamide amines were found to be very selective for the Y5 over the other NPY receptors (Y1, Y2, and Y4) and therefore represent excellent tools for studying the physiological roles of the Y5 receptor.

All of the sulfonamide amines in Table 1 were pharmacologically characterized in binding and functional assays ($n \geq 2$). Radioligand binding assays were performed with membranes from COS-7 cells transiently transfected with NPY receptor subtypes, labeled with ^{125}I-PYY plus or minus test compounds.[8] Functional assays were conducted with HEK-293 or LMTK- cells stably transfected with NPY receptor subtypes; intact cells were stimulated with forskolin and NPY plus or minus test compounds, then cAMP levels were detected by radioimmunoassay. All sulfonamides (**6** and **11**–**19**) are pure antagonists, in that they competitively attenuate the ability of NPY to inhibit forskolin-stimulated cAMP. These antagonists are potent inhibitors of the functional response to NPY with K_b values very similar to the K_i values (e.g., **11** hY5 $K_i = 8$ nM, hY5 $K_b = 26$ nM; **12** hY5 $K_i = 14$ nM, hY5 $K_b = 6$ nM). The compounds were tested in a panel of GPCRs and found to be selective for the NPY hY5 receptor (>100-fold for most receptors). The main cross reactivity being with the cloned human α2c and D2 receptors (e.g., **11** $K_i = 100$ nM on human α2c and $K_i = 63$ nM on human D2). The sulfonamide series of NPY antagonists are also potent and selective for the rat NPY Y5 subtype (e.g., **11** K_i rY5 = 18 nM, K_i rY1 = 22,000 nM).

Homology models of the different NPY subtypes were used to provide insights into the molecular basis for both potency and subtype selectivity for the sulfonamide series of ligands. These models are constructed based on data derived from structure-function studies using a set of receptors modified by site-directed mutagenesis.[9] Based on these results, we built a model consistent with both the SAR and structure–function data. As shown in Figure 2, the aryl sulfonamide orients the linking chain parallel to the transmembrane helices. This model suggests that a key residue responsible for high Y5 selectivity is His398. In the model, this residue forms a hydrogen bond to the sulfonamide NH. The Y5 receptor is the only NPY subtype to contain a histidine residue in TM6. Other important interactions highlighted by the model, include an acid-base pair formed with the amine of the sulfonamide amine and Glu211 and hydrophobic interactions between the aromatic groups and hydrophobic regions of the receptor.

In summary, we have shown that a weak and non-selective flexible molecule, benextramine, can be used as a starting point for the design of potent and selective ligands. Central to this improvement was the incorporation of a sulfonamide group that provides a bias towards the NPY Y5 receptor. Systematic exploration of this series led to a series of NPY Y5 antagonists of high potency. A model for how these ligands bind to the receptor is presented that is consistent with the SAR trends.

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