Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1771–1774

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

High-Throughput Synthesis Optimization of Sulfonamide NPY Y5 Antagonists

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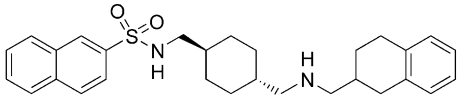
Received 7 December 2001; accepted 29 March 2002

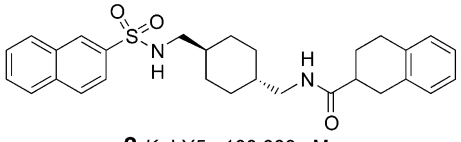
Abstract—A series of sulfonamide neuropeptide Y Y5 antagonists was optimized by preparation of sets of analogues using high-throughput synthesis and purification techniques. Testing of these compounds for their ability to bind to the human NPY Y5 receptor revealed separate SAR trends for sulfonamide amides versus sulfonamide ureas versus sulfonamide amines. By understanding these SAR trends, potent compounds were identified in all three series.

Neuropeptide Y (NPY), a 36 amino acid peptide, is one of the most potent feeding stimulating hormones known.[1] This peptide is found both in the peripheral and central nervous system and is one of the most abundant of the neuropeptides.[2] The physiological effects of NPY are mediated by a series of NPY receptor subtypes (Y1, Y2, Y4, Y5 and Y6) that are members of the G-protein coupled receptor (GPCR) family. 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.[3] NPY receptor-specific ligands may ultimately have value in several therapeutic areas including the treatment of obesity.[4]

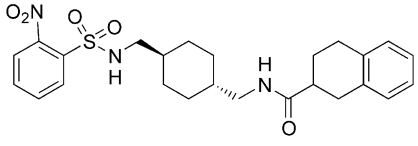
Our efforts focused on the discovery of potent and selective NPY Y5 antagonists.[5] This led to the discovery of a series of sulfonamide amines Y5 antagonists exemplified by **1** (K_i hY5 = 32 nM).[6] Replacing the basic amine functionality of **1** by an amide functionality resulted in a compound with no activity on the Y5 receptor (**2**, K_i hY5 > 100 μM). Remarkably, however, other amides containing different aryl sulfonamide groups (e.g., **3** K_i hY5 = 724 nM) and linking chains (e.g., **4**, K_i hY5 = 46 nM) have significant hY5 affinity. Consequently, we put into place a systematic program to explore and contrast the SAR of sulfonamide amides, sulfonamide ureas and sulfonamide amines. This program utilized combinatorial high throughput synthesis techniques to achieve results in a rapid and comprehensive fashion.[7]



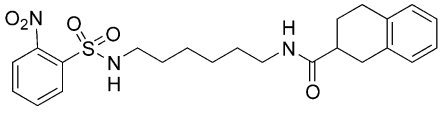
1 K_i hY5 32 nM



2 K_i hY5 >100,000 nM



3 K_i hY5 724 nM



4 K_i hY5 46 nM

We decided to derive global SAR trends by testing a set of 240 compounds where three different sections of the ligand were systematically varied. To ensure that receptor-binding assays could be used quantitatively, we

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0960-894X/02/$ - see front matter
PII: S0960-894X(02)00288-3

purified each product by HPLC. As shown in Figure 1, the synthetic sequence involved a straightforward solution-phase chemistry. In the first step, excess of a diamine was treated a sulfonyl chloride. These intermediates were prepared individually on a mmole scale. As sets, the sulfonamide amines were treated with acid halides to yield sulfonamide amides, isocyanates to yield sulfonamide ureas or reductively aminated to yield sulfonamide amines. In a standard acylation procedure, 1.2 equiv of the acylating reagent was individually added to 20 μmol solutions of the different sulfonamide amines in 200–500 μL DMF and 1.2 equiv of TEA in a 2-mL HPLC autosample vial. After shaking overnight in an orbital shaker, each reaction was quenched by adding 100–200 μL methanol. Half of the reaction mixture was injected directly onto a C18 reverse-phase column and purified by preparative HPLC. QC was performed by sampling 10–20% of the main peaks by NMR and all of the samples by mass spec. In every case, the major peak was highly pure ($>90\%$ as judged by NMR and HPLC) and contained the product of interest.

Component selection was designed both to answer subtle SAR issues and to provide structural diversity. The aryl sulfonyl chloride set included both the 1- and 2-naphthyl sulfonyl chloride (selected to understand the role of ligand shape in ligand binding) and three other aryl sulfonyl chlorides that were active in the sulfonamide amine series. Four different linking diamines were selected. Two of these, the 1,4-bis-aminomethylene-cyclohexane (W) and the 1,6-diaminohexane (X) were central to the potent sulfonamide amine series. The other two linkers (Y and Z) were selected to add diversity. Likewise, acylating groups were comprised of hydrophobic groups present in potent sulfonamide amines (b, d, e, h, and k) and other groups that add diversity (a, c, f, g, i, and j). Included, as control elements were sulfonamide amide **4** and a set of sulfonamide amines (l) that used a novel aldehyde expected to yield by the known SAR of this series[6] highly potent hY5 antagonists.

Each compound was evaluated for its ability to bind to the hY5 receptor. Binding affinity measurements were made by observing the amount of competitive displacement of ^{125}I-PYY using membranes from COS-7 cells transfected with the hY5 receptor.[8] The primary screen consisted of a single point displacement assay measuring amount of ligand binding at 1 μM. Compounds with greater than 70% binding were then tested in a single dose–response curve to yield a crude K_i. Table 1 shows the data from this process. It must be emphasized that due to inaccuracies in weighing as well as the single point nature of the K_i measurement, these values are only crude estimates. Any compounds deemed interesting were prepared on a larger scale and an accurate K_i ($n \geq 2$) determined. The results of these resynthesis and K_i determinations demonstrated that the Table 1 data is consistent but K_i values were overestimated by 3–10× (compounds are less potent than indicated in Table 1).[9]

The data in Table 1 clearly explains the SAR trends that led to the surprising differences in activity between sulfonamide amides **2**–**4**. First, the data for the sulfonamide amine set l fits the SAR we had observed for other sulfonamide amines. Set l compounds with the cyclohexyl linker (W) are very potent; typically 10+-fold better than the corresponding compound with the 1,6-diaminohexane (X) linker. As expected, compounds in

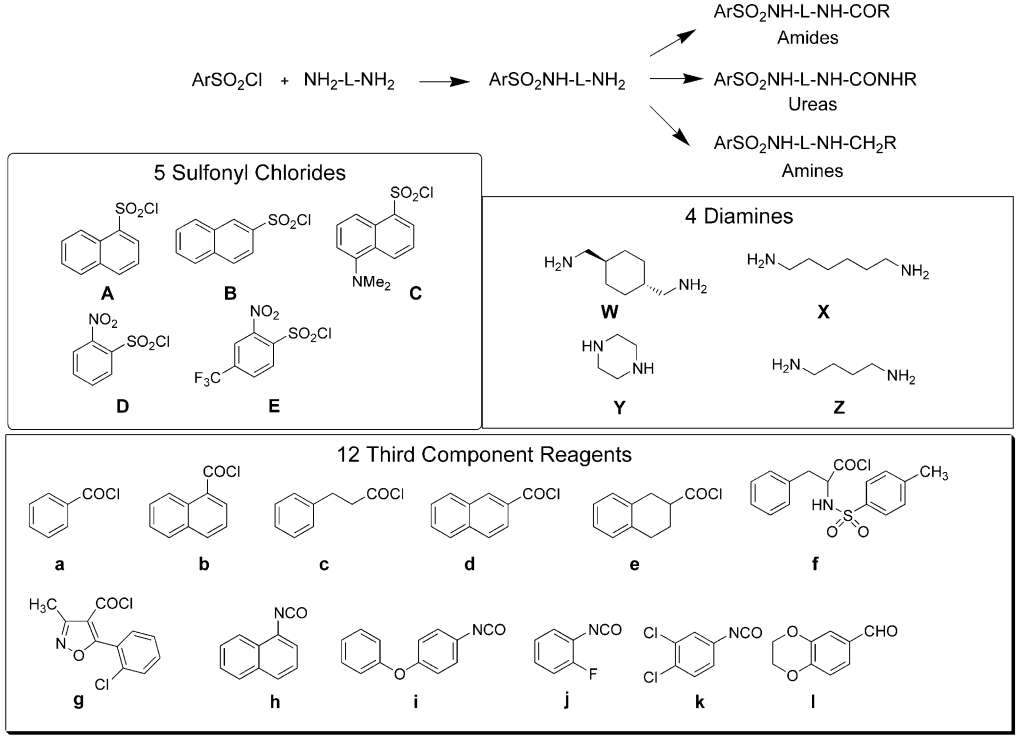


Figure 1. Synthetic strategy and list of components used in construction of the high-throughput synthesis array.

set l with the shorter linkers (Y and Z) are weak to inactive. In contrast, the amide and the urea sets show a different SAR. For amides, linker X is more active than linker W. There is a significant influence on binding by the aromatic group of the aryl sulfonamide. The 2-nitrophenyl group is usually the most favorable group and the 1-naphthyl group is significantly more potent than the 2-naphthyl group. Although the shorter linkers Y and Z produce less active compounds, unlike sulfonamide amines some submicromolar compounds were discovered. In the urea series, both the 2-nitrophenyl and 1-naphthyl groups are equally preferred with linker X. While with linker W, the 2-nitrophenyl group is favored. Comparisons of 2-nitrophenyl sulfonyl ureas show multiple sets (h, j and k) where both linker X and linker W yield compounds similar in potency. As with the amides, ureas demonstrate a clear preference for the 1-naphthyl sulfonamide over the 2-naphthyl sulfonamide. One of the diversity acylating groups, the *N*-tosylphenylalanine (set f) yielded compounds that lie outside these SAR tendencies. In particular, compound **BWf**, having both the disfavored cyclohexyl linker W and the disfavored 2-naphthyl group tested as the best compound in the set.

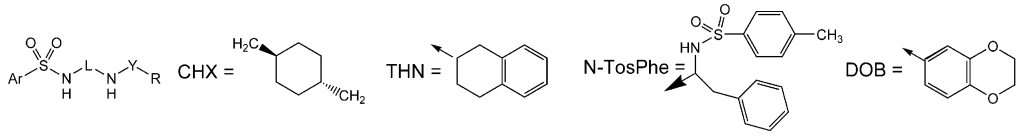
Table 2 shows hY5 binding affinities for a subset of compounds selected for resynthesis. Several compounds containing favorable combinations of arylsulfonamide,

Table 1. Estimated hY5 K_i values (nM) generated by testing the high-throughput synthesis array

	Amides							Ureas				Amines
	a	b	c	d	e	f	g	h	i	j	k	l
AW		8187	490	234	501		4786			447	31	1
BW						54						10
CW				6456	524	3235						
DW		229	400	288	114		912	25	93	151	112	8
EW			9332			3630						10
AX		645	X	218	91	69	2630	26	295	33	68	
BX		955	1445			501				468	389	110
CX					251	208		692		195	309	2630
DX		135	46	72	10	363	912	25	912	91	43	
EX			7079	1479	141					794	776	40
AY								1445		4897		
BY												
CY												
DY		316				3235			933		616	
EY											870	
AZ			691	239	1096	263				131	98	
BZ			X	3310		208						
CZ				3310	3890						346	
DZ			257	980	346	524		85	617	155	91	589
EZ			1348	1202	316				490		1585	186

Estimate binding affinity ($n=1$) measured by competitive displacement of ^{125}I-PYY using membranes from COS-7 cells transfected with the hY5 receptor. Only samples with $>70\%$ binding at 1 μM had est. K_i measured. X indicates compounds lost in processing.

Table 2. Binding affinities of resynthesized compounds



Compd	Code	Ar	L	Y	R	K_i hY5 (nM)
5	**DXd**	2-$NO_2C_6H_4$	$(CH_2)_6$	CO	2-Naphthyl	412
6	**AWe**	1-Naphthyl	CHX	CO	THN	4120
7	**AXe**	1-Naphthyl	$(CH_2)_6$	CO	THN	724
4	**DXe**	2-$NO_2C_6H_4$	$(CH_2)_6$	CO	THN	46
8	**BWf**	2-Naphthyl	CHX	CO	*N*-TosPhe	199
9	**AXf**	1-Naphthyl	$(CH_2)_6$	CO	*N*-TosPhe	426
10	**DWh**	2-$NO_2C_6H_4$	CHX	CONH	1-Naphthyl	103
11	**DXh**	2-$NO_2C_6H_4$	$(CH_2)_6$	CONH	1-Naphthyl	151
12	**DZh**	2-$NO_2C_6H_4$	$(CH_2)_4$	CONH	1-Naphthyl	724
13	**AXh**	1-Naphthyl	$(CH_2)_6$	CONH	1-Naphthyl	231
14	**AWk**	1-Naphthyl	CHX	CONH	3,4-$Cl_2C_6H_3$	95
15	**AWl**	1-Naphthyl	CHX	CH_2	DOB	12

Binding affinity measured by competitive displacement of ^{125}I-PYY using membranes from COS-7 cells transfected with the hY5 receptor and were done in duplicate.

linking chains and acylating groups have hY5 K_i values less than 250 nM. The SAR trends deduced by the array data are confirmed by examination of the Table 2 data. Thus, amides containing the linker X are more potent than those containing the linker W (compare **6** with **7**). Ureas containing the preferred 2-nitrophenyl sulfonyl group have similar potency with either linker W or X (compare **10** and **11**). As expected, sulfonamide amines from the l series are very potent (e.g., **15** hY5 K_i 12 nM). The outliers containing the *N*-tosyl phenylalanine group (series f) display surprisingly high hY5 affinity. These analogues (**8** and **9**) represent potent new structural types and present multiple new directions for optimization by modifications of both the benzyl and tosyl groups as well as examination of the chiral center.

In summary, high-throughput synthesis proved valuable in the optimization of sulfonamide Y5 antagonists. SAR trends vary with subtle changes to the ligand. Analysis of the global data for sets of compounds is extremely useful in identifying different SAR trends for series of closely related compounds. It is notable that the SAR from the sulfonamide amine series did not translate to either the amide or urea series. Consequently, if one kept the optimal aryl sulfonamide and linking groups constant, the optimal amide and urea derivatives would have been missed. Inclusion of diversity groups (such as set f) illustrated the power of preparing compounds in sets. The unusual SAR for set f indicates a different mode of receptor-ligand interactions and present starting points for the discovery of alternative NPY Y5 specific ligands.

Acknowledgement

We thank Jian Peng for his assistance in obtaining the mass spectra.

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9. The reasons for this inaccuracy in the crude estimate of K_i are unclear but are due in part to inaccuracies in weighing.