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PD 165929 - The First High Affinity Non-peptide Neuromedin-B (NMB) Receptor Selective Antagonist

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Abstract : In this paper we describe the development of a novel series of non-peptide neuromedin-B (NMB) receptor ligands as exemplified by PD 165929. PD 165929, which exhibits nanomolar affinity for the NMB receptor (Ki=6.3nM), has been demonstrated to be a competitive antagonist at this receptor ($appK_B$=7.6nM) and is selective over the corresponding gastrin-releasing peptide (GRP) receptor type (Ki>10000nM).

INTRODUCTION

We have previously published on our interest in the development of non-peptide ligands for neuropeptide receptors as exemplified by the identification of non-peptide agonists[1] and antagonists for cholecystokinin[2] and tachykinin[3] receptors. This has been *via* the application of a so-called "peptoid" drug design strategy.[4] The basic premise behind the peptoid structure-based approach to drug design involves the rational development of non-peptide receptor ligands starting from, and utilising the information contained within, the endogenous peptide of the targeted receptor.In brief[5] this peptoid drug design strategy consists of the following discrete steps :

i) identification of a **minimum active peptide fragment** of the endogenous peptide,

ii) recognition of key residues contained within the **minimum active fragment** with respect to receptor binding - achieved by carrying out an alanine scan on the peptide fragment,

iii) incorporation of the side chains of the three[6] most important residues identified from the alanine scan onto a **small molecule template**

iv) **exploration of conformational space** of amino acid side chains and,

v) optimisation of the side chains to provide a **high affinity, non-peptide receptor ligand**.

In this paper we describe how the peptoid drug design strategy has been adapted to the development of a series of non-peptide neuromedin-B receptor selective antagonists.

BOMBESIN, NEUROMEDIN-B AND GASTRIN-RELEASING PEPTIDE

The amphibian tetradecapeptide bombesin (BB) belongs to a class of peptides which share structural homology in their C-terminal decapeptide region.[7] At present two mammalian bombesin-

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like peptides have been identified and characterised,[8] the decapeptide neuromedin- B (NMB) and a 27 residue amino acid, gastrin-releasing peptide (GRP). NMB and GRP peptides are believed to mediate a variety of biological actions such as on autocrine growth, satiety, thermoregulation and stereotyped behaviour[9] via an action upon the corresponding NMB-preferring (BB_1) and GRP-preferring (BB_2) receptors. Although there are a number of peptide-based selective ligands for both the NMB and GRP receptors,[10] the precise physiological roles of these neuropeptides remain unclear partly due to a lack of high affinity non-peptide bombesin antagonists.[11]

RESULTS AND DISCUSSION

i) Identification of a minimum active peptide fragment of the endogenous peptide.

In employing our peptoid drug design strategy to the development of non-peptide bombesin antagonists, we first carried out an alanine scan study on the previously published[12] bombesin peptide fragment AcBB (7-14). This octapeptide derivative exhibited nanomolar affinity (see table I) for both receptor types and thus, we felt, would serve as an appropriate **minimum active fragment** in the design of both NMB and GRP receptor ligands.

ii) Recognition of the key residues contained within the minimum active fragment.

The largest decreases in NMB and GRP receptor binding affinity ensuing from the alanine scan study occurred when the Trp-8 (>1000 fold decrease), Leu-13 (>5000 fold) and Val-10 (>100 fold) residues of the minimum active fragment were replaced by Ala.[13] These findings reveal the primary importance of the side chains of these residues in the molecular recognition of AcBB(7-14) at bombesin receptors.

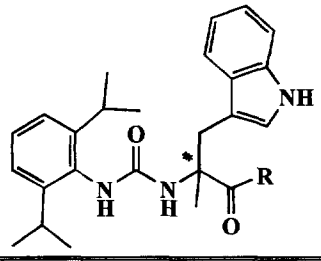
iii) Incorporation of the side chains of the three most important residues onto a small molecule template

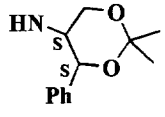
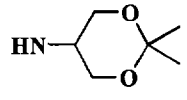
In accord with the peptoid design strategy, the next step was to append the side chains of the three most important residues, in this case Trp, Leu/Phe[14] and Val, onto appropriate **small molecule templates**. A variety of small molecule templates were investigated including, for example, urea and secondary amide moieties (unpublished results). The highest affinity lead, however, proved to be a mono amino acid ligand **[(S)-1]** identified from a limited search of a company compound collection using the Trp, Leu/Phe and Val side chains as search queries. Compound **(S)-1** (Table I) is a simple alanine derivative **small molecule template** on to which is appended Trp, Phe and Val side chains and exhibits excellent affinity and selectivity for the human NMB receptor type (NMB, Ki=95nM). As compound **1** contains the side chain of Phe as opposed to a Leu residue,[14] it is, as anticipated, selective for the NMB receptor type (GRP, Ki>10000nM).

The relative proximity of these key amino acid side chain surrogates is consistent with previous modeling studies[13] carried out on AcBB(7-14) which indicate that the heptapeptide adopts a γ-turn conformation which acts to bring the Trp, Leu and Val side chains close together in space (distances between α-carbons <7 Angstrom).

In attempting to reduce the bulk and complexity of the C-terminal phenyl serinol moiety of **1**, we subsequently prepared a limited number of less structurally complex C-terminal derivatives (Table I). Removal of the whole of the phenyl serinol moiety (**2**, Ki=3700nM) from the C-terminal proved to have a detrimental effect on NMB receptor binding as did, to a lesser extent, exclusion of the phenyl (**3**, Ki=630nM) and dioxane (**4**, Ki=450nM) groups. Optimal among this data set of compounds proved to be the simple cyclohexyl derivatives **5** (Ki=310nM) and **6** (Ki=125nM) with the latter retaining much of the NMB receptor affinity and selectivity displayed by **1**. We felt the cyclohexyl methylamine derivative **6** was a more appropriate lead than **1** to carry onto the next step

Table I : SAR of C-Terminal Phenyl Serinol Moiety of Compound 1.



Compound No.	*	R	NMB, Ki(nM)[a]	GRP, Ki(nM)[a]
(S) - 1	S		95	>10000
(R) - 1	R		420	>10000
2	RS	NHMe	3700	>10000
3	RS		630	>10000
4	RS	$NH(CH_2)_2Ph$	450	>10000
5	RS	NHC_6H_{11}	310	>10000
6	RS	$NHCH_2C_6H_{11}$	125	>10000
NMB			0.068	56
GRP			9.1	0.040
Bombesin			2.0	0.15
AcBB(7-14)			2.1	0.70

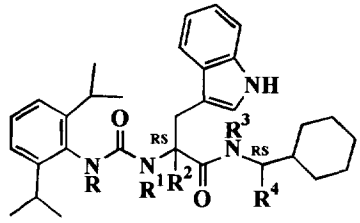
a) Values shown represent the geometric mean of at least 3 separate experiments carried out using [^{125}I][Tyr^4] bombesin to label cloned human NMB or GRP receptors stably expressed in CHO cells.[15]

of the peptoid design strategy since this NMB receptor ligand exhibited equal affinity to its parent (**1**) whilst, importantly, being less structurally complex.

iv) <u>Exploration of conformational space</u> of amino acid side chains.

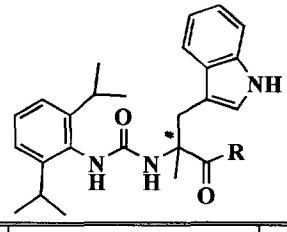
With this promising lead in hand the next priority was to **explore the spatial arrangement** of the amino acid side chains of the NMB receptor ligand **6**. One means of achieving this objective, and a strategy that has previously proved particularly successful,[2,3] is to incorporate a single methyl group at key positions, eg. on nitrogen and α-carbon atoms, on the molecule thus introducing conformational constraint. Table II lists the derivatives of compound **6** that were prepared in following this strategy. With the exception of the "non-methylated" compound (**11**, Ki=120nM), all of the methylated derivatives showed reduced NMB receptor affinity when compared to **6**. Although the optimal compound from this study with respect to NMB receptor binding affinity proved to be the non-methylated derivative **11**, we decided to proceed with the marginally lower

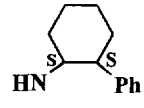
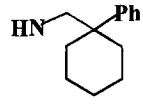
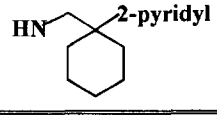
Table II : Exploration of Conformational Space of Amino Acid Side Chains of Compound 6.



Compd.	R	R^1	R^2	R^3	R^4	NMB, Ki(nM)	GRP, Ki(nM)
7	Me					260	>10000
8		Me				760	1500
6			Me			125	>10000
9				Me		490	>10000
10					Me	440	>10000
11						120	>10000

Table III : C-Terminal Derivatives of Compound 6.



Compound No.	*	R	NMB, Ki (nM)	GRP, Ki (nM)
12	S		76	>10000
13	RS	(RS)-2-aminotetralin	310	>10000
14	RS	(RS)-1-aminotetralin	72	>10000
15	R	(S)-1-aminotetralin	14	>10000
16	RS		7.8	>10000
17 (PD165929)	S		6.3	>10000

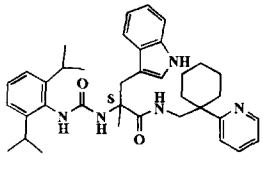
affinity parent compound **6** due to the advantageous properties the α-methyl group may confer on *in vivo* stability.[16]

v) Optimisation of the side chains to provide a high affinity, non-peptide receptor ligand.

Having decided upon the optimal spatial arrangement of the side chains surrounding the substituted alanine small molecule template, we then proceeded to optimise, individually, each of the three peripheral side chains. We first turned our attention towards investigating the SAR of the C-terminal cyclohexyl methylamine moiety of compound **6**. A selection of key compounds prepared for this study are listed in Table III. Compounds **12** through **14** were prepared in an attempt to further understand the dimensions of the NMB receptor binding pocket that interacts with the C-terminal of this class of compound. The similar NMB receptor binding affinity achieved with the 1,2-substituted cyclohexyl derivative **12** (Ki=76nM) in comparison to the dioxane derivative **1**, implied that the hydrophilic oxygen atoms were not contributing to NMB receptor binding to any great extent. This initial view was reinforced by the good receptor binding affinity exhibited by the racemic tetralin derivatives **13** (Ki=310nM) and, most notably, **14** (Ki=72nM). The preferred stereochemistry of the 1-substituted tetralin derivative was found to be R,S with this particular isomer (**15**) binding to the NMB receptor with an affinity of 14nM. Further high affinity compounds were obtained by appending aryl moieties to the 1-position of the cyclohexyl group in compound **6**. For example the simple racemic phenyl derivative **16** displayed excellent affinity and selectivity for the NMB receptor (Ki=7.8nM). However, our preferred compound from this series is the resolved 2-pyridyl variant **17** (**PD 165929**) since in addition to having good affinity for the NMB receptor (Ki=6.3nM) this S-configured derivative (S stereochemistry was found to be optimal in this series) has improved aqueous solubility over **16**.

In vitro functional assays demonstrate that compound **17** (**PD 165929**) acts as a competitive antagonist at the human NMB receptor exhibiting appK_B values in line with its binding affinity in two separate bioassays (Table IV).

Table IV : Species Selectivity and *In Vitro* Functional Activity of 17 (PD 165929).



NMB Receptor Binding Affinities		In Vitro Functional Assays	
Human	Rat[a]	Cytosensor[b]	*Xenopus Oocytes*[c]
Ki, nM	IC_{50}, nM	appK_B, nM	appK_B, nM
6.3	150	7.6	7.5
(3.5-11)	(130-170)	(5.3-11)	(4.2-10)

a) Values shown represent the geometric mean of 3 separate experiments carried out using [^{125}I][Tyr^4] bombesin in the presence of [D-Phe^6] bombesin (6-13) ethyl ester to label NMB receptor binding sites in rat olfactory bulb.[17]

b) Inhibition of NMB-induced acidification response at the human NMB receptor expressed in CHO cells. Values are the means of at least 3 determinations.[18]

c) Inhibition of NMB-evoked increases in chloride currents in xenopus oocytes expressing human NMB receptors. Values represent the mean of at least three separate experiments.[19]

CONCLUSIONS

In this paper we have described a novel series of high affinity, non-peptide NMB receptor selective antagonists that were identified through the application of a peptoid drug design strategy. To our knowledge these compounds represent the first known examples of high affinity non-peptide ligands that are selective for the NMB receptor.

Detailed synthetic procedures and further *in vitro* and *in vivo* pharmacology on this class of compound will be published elsewhere.

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