Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 14, pp. 1679-1684, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00213-4

THE RATIONAL DEVELOPMENT OF SMALL MOLECULE TACHYKININ NK_3 RECEPTOR SELECTIVE ANTAGONISTS - THE UTILISATION OF A DIPEPTIDE CHEMICAL LIBRARY IN DRUG DESIGN

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Abstract : Boc(S)Phe(S)PheNH_2 (**1c**) was identified from the biological screening of an in-house dipeptide chemical library as a micromolar NK_3 receptor selective ligand (IC_{50}=1550nM). This lead structure has subsequently been developed into a series of potent and selective NK_3 receptor antagonists an example of which is the urea derivative Boc(S)Phe(R)αMePheNH$(CH_2)_7$NHCONH_2 (**11d, PD157672**) (IC_{50}=16nM).

INTRODUCTION

An important addition to chemical synthesis methodology has been the recent development of novel technology targeted toward the rapid synthesis of large peptide[1] and non-peptide[2] chemical libraries. The implementation of such technology could potentially lead to a significant increase in the size and molecular diversity of the existing compound collections accessible to biological evaluation. Our contribution to this chemical library concept has been the generation of a dipeptide library.[3] The construction of such a library was considered useful as a potential source of novel lead structures since

a) dipeptides have been shown, by us[4] and other groups[5], to be an excellent starting point for drug design,

b) sufficient quantity of compound, even for *in vivo* profiling, can easily be prepared at low cost and

c) a library containing only 256 compounds can provide a data set that spans a broad spectrum of physicochemical properties in a minimum number of compounds.[3]

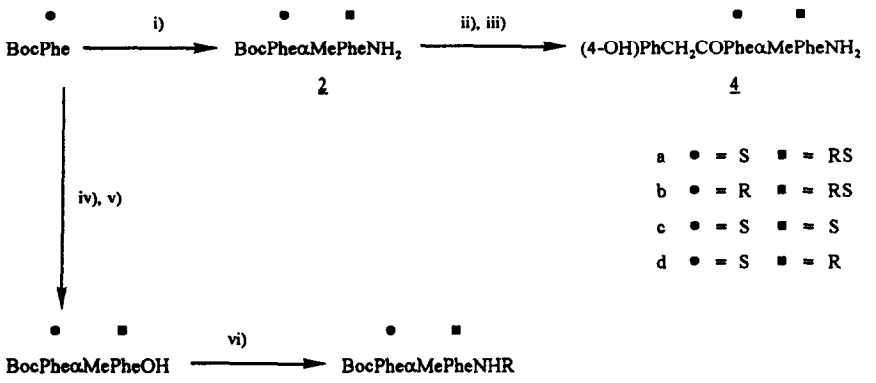
In this paper we describe the utilisation of a dipeptide library in identifying a novel, micromolar affinity NK_3 receptor lead and the subsequent development of this lead into potent, nanomolar NK_3 receptor selective antagonists.

RESULTS AND DISCUSSION

The broad ranging, and potentially important, therapeutic indications that have been associated with the tachykinins[6] is suitably reflected by the increasing number of publications describing non-peptide antagonists of tachykinin receptors.[7] Our interest in this competitive research area is illustrated by recent publications revealing "peptoid" antagonists for both the NK_1[8] and NK_2[9] receptor types. The successful application of our general peptoid strategy to the rational design of these NK_1 and NK_2 receptor antagonists (refer to scheme I in reference 9) prompted us to follow the same design strategy in our search for novel NK_3 receptor ligands.

In contrast to our experiences in the NK_1 and NK_2 programmes, an "alanine scan" on the most selective endogenous peptide (neurokinin B) for the NK_3 receptor subtype proved inconclusive as to the relative importance of the individual residues to NK_3 receptor binding affinity (unpublished data) Since therefore, unlike our NK_1 and NK_2 examples, a lead dipeptide could not be identified from this particular strategy, we chose to screen our dipeptide library in a cloned human NK_3 receptor binding assay as an alternative source of a low molecular weight lead. Of the compounds assayed, a number of dipeptides containing two aromatic residues exhibited higher than ten micromolar affinity for the NK_3 receptor. The optimum compound in this series was found to be the simple dipeptide Boc(S)Phe(S)PheNH_2 (**1c**). This compound, in addition to having low micromolar affinity for the NK_3 receptor (IC_{50}=1550nM, table I), was also selective over the NK_1 and NK_2 receptor subtypes (IC_{50}>10μM for both receptors).

Having selected **1c** as the **dipeptide lead**, the next step in our peptoid design strategy was to **explore the spatial arrangement of the amino acid side-chains and protecting groups**. One means of achieving this objective is to incorporate a single methyl group at key positions eg. on nitrogen atoms and α-carbons, along the dipeptide backbone thus introducing conformational constraint. This strategy had proved particularly successful in markedly improving the affinity of initial leads in our NK_1[8],NK_2[9] and CCK[4] receptor programmes. However, the only methylated analogue of **1c** to retain significant affinity for the NK_3 receptor was the C-terminal (RS)α-methyl Phe derivative **2a** (IC_{50}=1520nM, see scheme and table I). The corresponding N-terminal (R)Phe derivative (**2b**) was significantly less active (IC_{50}>10^{-5}M) thus indicating a preference for the S configuration at this centre. Compound **2a** superceeded our original dipeptide lead (**1c**) as the lead structure due to the advantageous properties the α-methyl group may confer on *in vivo* stability.[10]



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SCHEME

Reagents and conditions : i) HBTU, DIPEA, αMePheNH$_2$, DMF; ii)TFA; iii) HBTU, DIPEA, 4-hydroxyphenylacetic acid, DMF; iv) HBTU, DIPEA, αMePheOMe, DMF; v) LiOH, THF/H_2O; vi) HBTU, DIPEA, RNH_2 (see table I), DMF.

The next objective as part of our peptoid strategy was to improve upon the affinity of **2a** by **optimising both the N- and C-terminal groups** of the dipeptide lead. In selecting appropriate functional groups to append at the N- and C-termini, we utilised the working hypothesis that the PhePhe sequence of dipeptide **2a** mimics the binding characteristics of the same sequence present in the NK_3 receptor selective ligands NKB, senktide and 4-hydroxyphenylacetylPhePheGlyLeuMetNH_2 (**3**)[11](table I). Using the smaller of these peptide leads (**3**) as a template, we appended the 4-hydroxyphenylacetyl group, or suitable analogue, off either the N- or C-termini of dipeptide **2a** with the intention of mimicking the favourable binding properties imparted by the same moiety in the pentapeptide derivative **3**. [The phenylacetic acid group was appended to both N- and C-termini as a consequence of the symmetrical nature of the PhePhe sequence in dipeptide (**2a**) and template (**3**)]. Appending this 4-hydroxyphenylacetyl group to the N-terminus of **2a** resulted in a significant drop in affinity (**4a**, IC_{50}= 5890nM) whereas a similar substitution at the C-terminus resulted in an approximately 3-fold increase in NK_3 receptor binding affinity (**5a**, IC_{50}=425nM, table I).

Table I : Receptor Binding Affinities for Human NK_3 Receptor Expressed in CHO Cells.[12]

BocPhe(•)MePhe(■)NHR

Cmpd. No.	•	■	R	IC_{50},nM[a]
1c[b]	S	S	H	**1550** (1180-1800)
2a	S	RS	H	**1520** (787-2570)
2b	R	RS	H	**>10000**
4a[c]	S	RS	H	**5890** (4480-9180)
5a	S	RS	$-CH_2Ph(4-OH)$	**425** (328-625)
6a	S	RS	$-(CH_2)_8OH$	**92** (59-145)
7c	S	S	$-(CH_2)_8OH$	**1690** (1140-2380)
7d	S	R	$-(CH_2)_8OH$	**40** (26-61)
8a	S	RS	$-(CH_2)_8OMe$	**102** (75-135)
9a	S	RS	$-(CH_2)_7CO_2H$	**229** (160-303)
10d	S	R	$-(CH_2)_8CONH_2$	**39** (28-69)
11d(PD157672)	S	R	$-(CH_2)_7NHCONH_2$	**16** (8-24)
3	4-Hydroxyphenylacetyl**PhePhe**GlyLeuMetNH_2			**28** (15-38)
NKB	AspMetHisAsp**PhePhe**ValGlyLeuMetNH_2			**10** (7-13)
Senktide	Succ Asp**PheNMePhe**GlyLeuMetNH_2			**21** (12-31)

a) Values shown represent the geometric mean of 3-6 separate experiments carried out using [^{125}I]-[MePhe^7]NKB to label cloned human NK_3 receptors stably expressed in CHO cells.[12]
b) C-Terminal has no α-methyl.
c) Compound has 4-hydroxyphenylacetyl group in place of N-terminal Boc - see scheme.

The key finding that the C-terminal phenol moiety in **5a** increased binding affinity subsequently led, eventually, to the identification of the optimum compound in this series - the n-octanol derivative **6a** (IC_{50}=92nM)

All of the peptides described above are equal mixtures of two diastereoisomers. The significantly higher affinity of the S,R diastereoisomer (**7d**, IC_{50}=40nM) in comparison to its S,S isomer (**7c**, IC_{50}=1690nM) highlighted the necessity of retaining an R configuration at the C-terminal α-methyl Phe residue for optimum binding (table I)

Comparison of the NK_3 receptor binding affinities of the hydrogen-bond accepting C-terminal methyl ether derivative **8a** (IC_{50}=102nM) and the corresponding hydrogen-bond

donating carboxylic acid derivative **9a** (IC_{50}=229nM), suggests that the principal role of the C-terminal functional group of these ligands is that of a hydrogen bond acceptor.[13] Consequently, the C-terminal primary amide (**10d**) and urea (**11d, PD157672**) derivatives were then prepared as examples of compounds containing functional groups with greater hydrogen bond accepting[13] capacity than that of the parent alcohol derivative **7d**. This increase in hydrogen-bond accepting ability led to a corresponding increase in NK_3 receptor affinity with the urea derivative (**11d, PD157672**) exhibiting a higher binding affinity (IC_{50}=16nM) for the human NK_3 receptor than either the pentapeptide derivative **3** (IC_{50}=28nM) or senktide (IC_{50}=21nM).

In vitro functional assays in human and Guinea pig paradigms are consistent with these small molecules being potent and competitive NK_3 receptor selective ligands eg. compound **11d** exhibits a Ke of 7nM in blocking senktide responses at human NK_3 receptors expressed in CHO cells (table II).

Table II : *In Vitro* Functional Data and Tachykinin Receptor Selectivity.

	In Vitro Functional Assays, Ke (nM)			Binding Affinities, (IC_{50},nM)		
Cmpd. No.	CHO Cells[a]	GP Ileum[b]	GP Hab.[c]	NK_1[d] (IM9)	NK_2[e] (HUB)	NK_3[f] (GP)
7d	**29**	**14**	**54**	**>10000**	**6160**	**22**
	(25-36)	(8-40)	(34-108)		(3840-9990)	(12-51)
10d	**15**	**13**	**25**	**>10000**	**5020**	**15**
	(8-44)	(6-30)	(14-45)		(4260-5610)	(14-16)
11d	**7**	**42**	**16**	**>10000**	**6540**	**9**
(PD157672)	(2-22)	(26-130)	(12-21)		(4100-9700)	(5-13)

a) Inhibition of senktide-evoked increases in intracellular calcium levels in CHO cells measured using the fluorescent indicator Fura2.[14] Equilibrium constants shown represent the mean of at least 3 separate experiments.
b) Isometric contractions were recorded from longitudinal muscle myenteric plexus preparations with responses to the NK_3 receptor selective agonist senktide.[15] Data represent the geometric means of individual values in at least 3 separate experiments.
c) Inhibition of senktide-induced increases in spontaneous firing of Guinea pig habenula neurones *in vitro*.[16] Values are the mean of at least 3 determinations.
d) Values shown represent the geometric mean of 3 separate experiments carried out using [^{125}I]Bolton-Hunter substance P to label NK_1 binding sites in human lymphoma IM9 cells.[8]
e) Values shown represent the geometric mean of 3 separate experiments carried out using [^{125}I]NKA to label NK_2 binding sites in membranes prepared from hamster urinary bladder.[9]
f) Values shown represent the geometric mean of 3-6 separate experiments carried out using [^{125}I]-[MePhe^7]NKB to label NK_3 binding sites in Guinea pig cortical membranes.[12]

CONCLUSIONS

We have described the rational development of small molecule tachykinin NK_3 receptor selective ligands by utilising a dipeptide chemical library as a source for our initial lead structure. Modification of the C-terminal of the dipeptide lead has been shown to produce NK_3 receptor antagonists comparable in binding affinity to the peptide ligands neurokinin B, senktide and pentapeptide derivative **3**.

Future publications will elaborate on the strategy and results highlighted in this communication in addition to revealing SAR studies carried out toward identifying non-peptide NK_3 receptor ligands.

ACKNOWLEDGEMENTS

The authors wish to thank Miss H. Chilvers, Mrs R. Franks, Miss P. Grimson, Dr. P. D McDonnell, Mr S. Purbrick and Mrs L Webdale for their excellent technical assistance.

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(*Received in Belgium* 4 *May* 1994; *accepted* 24 *May* 1994)