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THE DEVELOPMENT OF A NOVEL SERIES OF NON-PEPTIDE TACHYKININ NK_3 RECEPTOR SELECTIVE ANTAGONISTS

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Abstract : In this paper we describe the transformation of a series of modified dipeptide NK_3 receptor selective ligands, previously developed from a hit identified from the screening of a dipeptide chemical library, into non-peptide, nanomolar affinity NK_3 receptor selective antagonists eg. PD160946 and PD161182. PD 161182 blocks senktide-induced human NK_3 receptor mediated increases in intracellular calcium levels with a Ke of 0.88nM.

INTRODUCTION

The potentially important therapeutic indications[1] that have been associated with the tachykinins has provided a major stimulus for the development of a number of structurally diverse antagonists for both the NK_1 and NK_2 receptor types.[2] Notable examples of these antagonists include the piperidine based NK_1 receptor antagonists SR140333[3] and CP99994[4] and NK_2 receptor antagonists SR48968[5] and GR159897.[6] In contrast, however, only one non-peptide NK_3 receptor selective antagonist, SR142801 (see figure 1), has thus far been reported.[7]

Our interest in the design of non-peptide ligands for tachykinin receptors is illustrated by the development of "peptoid" antagonists for both the NK_1[8] and NK_2[9] receptor types. We have also published[10] on the development of small molecule NK_3 receptor selective antagonists eg. **2** and **3** (PD157672) (see figure 1). These modified dipeptide derivatives were developed from an initial micromolar affinity dipeptide lead, Boc(S)Phe(S)PheNH_2, which was identified from the screening of a dipeptide chemical library.[11]

In this paper we report on further studies conducted in this area and describe how we have converted this series of dipeptide NK_3 receptor ligands into nanomolar affinity, non-peptide NK_3 receptor selective antagonists.

RESULTS AND DISCUSSION

Having identified[10] modified dipeptide NK_3 receptor selective antagonists such as **2** and **3** (see figure 1), our next key objective was to develop lower molecular weight, non-peptide analogues of this class of compound. One chemical strategy that was followed in order to achieve this objective was to search for suitable replacements for the bulky Boc(S)Phe N-terminal moiety of the dipeptide series. A promising lead for this study proved to be a compound (**4**, see figure 1) which was initially prepared as part of our NK_1 receptor programme. In addition to having micromolar affinity (IC_{50}=1400nM) for the targeted NK_1 receptor, this mono amino acid derivative also exhibited similar affinity (IC_{50}=1200nM) for the NK_3 receptor type.

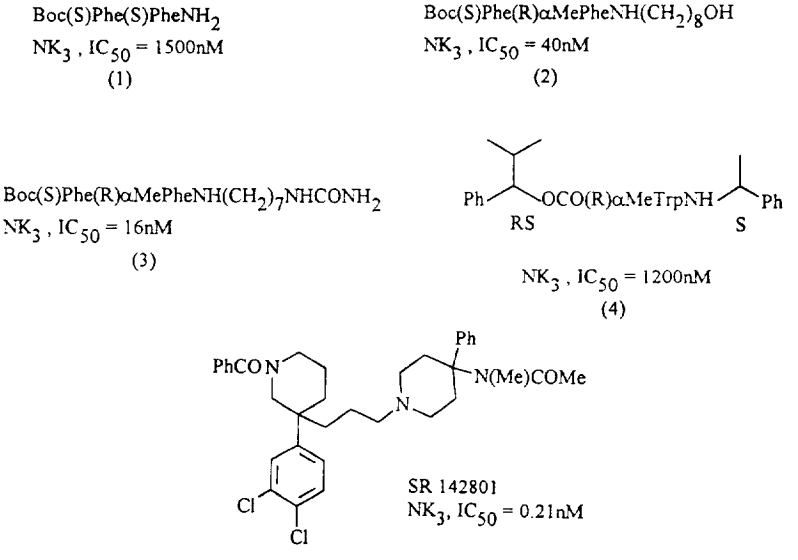
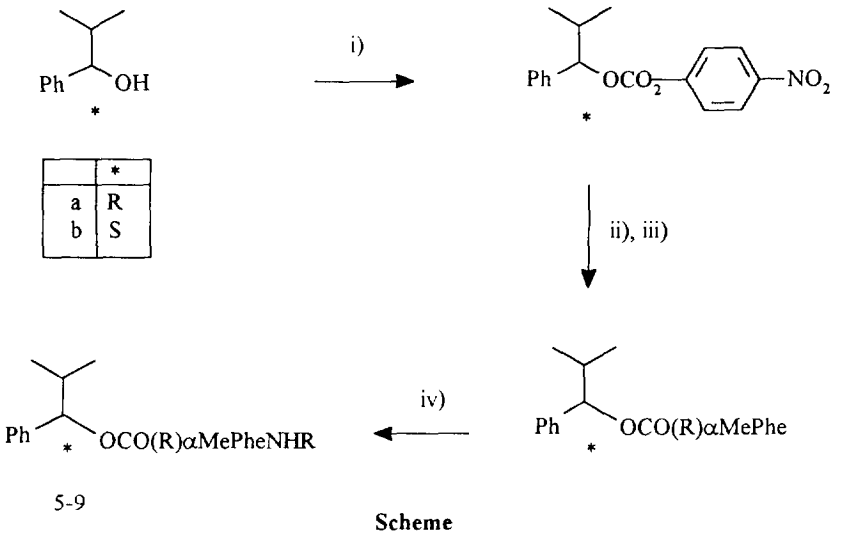


Figure 1

Since this mono amino acid derivative (**4**) bore some structural resemblance to the C-terminal segment of the dipeptide class of NK_3 receptor ligands (ie. both contained an α-methylated aromatic amino acid flanked by a lipophilic C-terminal), it was hypothesized that the i-propyl-benzyloxycarbonyl moiety of **4** may represent a potential replacement for the Boc(S)Phe moiety of the dipeptide compounds. In order to investigate this hypothesis we prepared compounds **5a** and **5b** (see scheme and table I) where the (R)αMeTrp moiety has been replaced by the (R)αMePhe residue present in the dipeptide series. As we wished to identify



5-9

Scheme

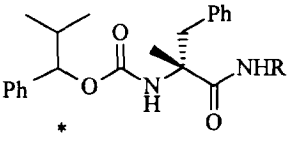
Reagents and conditions : i) p-nitrophenylchloroformate, DMAP, DMF; ii) (R)αMePheOMe, DMF; iii) LiOH, THF/H_2O; iv) RNH_2, HBTU, DIPEA, DMF.

the preferred stereochemistry at the substituted benzyloxycarbonyl N-terminal for optimal NK_3 receptor affinity (**4** is a mixture of diastereoisomers), we prepared both possible stereoisomers at this centre. Encouraged by the significant increase in affinity induced by these changes, the R,R isomer **5a** has an IC_{50} of 74nM at the NK_3 receptor (see table I), derivatives of **5a** and **5b** were prepared in which the preferred C-terminal segments that had previously been identified from the dipeptide series were appended. For example, appending an amino octanol moiety to the C-terminal provided compounds **6a** and **6b** (see table I) with the S,R stereoisomer (**6b**) showing very similar affinity (IC_{50}=52nM) to its dipeptide derived parent (**3**, IC_{50}=40nM). It is interesting to note that replacing the α-methyl benzylamine C-terminal group in **5** with the amino octanol moieties in **6** results in a change in stereochemical preference at the N-terminal substituted benzyl group with respect to NK_3 receptor binding affinity. Thus the R,R isomer **5a** has markedly higher affinity (IC_{50}=74nM) than its S,R diastereoisomer **5b** (IC_{50}>10000nM) whereas the S,R isomer **6b** in the C-terminal amino octanol series is clearly preferred for optimal NK_3 receptor binding. Further chemical manipulation of the C-terminal segment of **6a** yielded the urea derivative **7b** which exhibited marginally higher affinity (IC_{50}=43nM, see table I).

The highest affinity compounds in this non-peptide series were obtained by subsequent substitution of the phenyl ring of the αMePhe residue. Thus the 2-fluoro (**8b**, IC_{50}=16nM) and

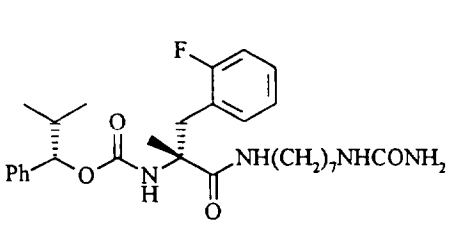
2,3-difluoro (**9b**, IC_{50}=7.3nM) derivatives exhibited equal or higher affinity than not only the parent dipeptide lead PD157672 (**3**, IC_{50}=16nM) but also the peptide ligands NKB (IC_{50}=10nM) and senktide (IC_{50}=21nM) (see table I and figure 2).

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



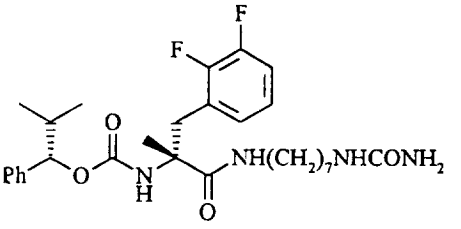
Compound No.	*	R	IC_{50}, nM[a]
5a	R	-(S)CH(Me)Ph	74 (45-180)
5b	S	-(S)CH(Me)Ph	>10000
6a	R	$-(CH_2)_8OH$	350 (220-870)
6b	S	$-(CH_2)_8OH$	52 (27-58)
7b	S	$-(CH_2)_7NHCONH_2$	43 (19-99)
8b[b]	S	$-(CH_2)_7NHCONH_2$	16 (14-20)
9b[c]	S	$-(CH_2)_7NHCONH_2$	7.3 (5.7-13)
NKB			10 (7.0-13)
Senktide			21 (12-31)

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) αMePhe residue has 2-fluoro substituent (see figure 2).
c) αMePhe residue has 2,3-difluoro substituents (see figure 2).



NK_3 , IC_{50} = 16nM

(8b)



NK_3 , IC_{50} = 7.3nM

(9b)

Figure 2

In vitro functional assays in human and Guinea pig paradigms demonstrate that this novel class of non-peptide NK_3 receptor ligands are competitive antagonists at the NK_3 receptor (see table II). For example compound **9b** exhibits a Ke of 0.88nM in blocking senktide induced responses at human NK_3 receptors expressed in CHO cells.

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 Hab.[b]	NK_1[c] (IM9)	NK_2[d] (HUB)	NK_3[e] (GP)
8b	2.2	13	2200	1500	14
(PD160946)	(1.6-11)	(4.8-21)	(750-3700)	(1300-1700)	(8.1-18)
9b	0.88	5.8	3000	790	3.7
(PD161182)	(0.50-1.5)	(4.4-7.2)	(2900-3100)	(480-1100)	(1.1-5.8)

a) Inhibition of senktide-evoked increases in intracellular calcium levels in CHO cells measured using the fluorescent indicator Fura2.[13] Equilibrium constants shown represent the mean of at least 3 separate experiments.
b) Inhibition of senktide-induced increases in spontaneous firing of Guinea pig habenula neurones *in vitro*.[14] Values are the mean of at least 3 determinations.
c) 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]
d) 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]
e)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

In this paper we have described the development of a novel series of high affinity, non-peptide NK_3 receptor selective antagonists. These ligands were derived from a series of modified dipeptide NK_3 receptor ligands which in turn originated from a weakly active dipeptide lead identified from the screening of a dipeptide chemical library.

We believe this study represents the first published example of the development of a high affinity non-peptide ligand for a membrane bound peptide receptor based upon the screening of a synthetic peptide chemical library.

More detailed studies delineating the SARs of this series of compounds will be published in full elsewhere.

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