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THE DESIGN OF POLAR β-TURN DIPEPTIDE MIMETICS

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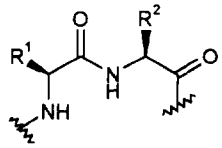
Abstract - The design and synthesis of conformationally constrained, non-peptide templates (3-substituted pyrrolidines) which allow the incorporation of two adjacent amino acid side-chains in an orientation similar to that found in the i+1 and i+2 positions of a β-turn are reported. The NK_2 tachykinin receptor affinity of a Trp-Phe mimetic (**7a**) which mimics the β-turn in MEN10627 (**8**), a constrained cyclic hexapeptide, high affinity NK_2 tachykinin receptor antagonist, was determined and shown to have no significant binding affinity (NK_2, IC_{50}> 10,000 nM).

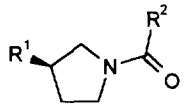
Our interest in the rational design of non-peptide drug candidates (peptoids), for neuropeptide receptors based on the structure of the endogenous neuropeptide has led us to devise a general design strategy.[1] As part of this strategy we are developing a family of 'rigid' templates with a range of physicochemical properties which mimic part of the common structural motifs found in proteins.[2] In these templates, the α-β C-C bonds of at least two of the 'amino acid side chains' should overlay the corresponding bonds in the secondary peptide structure of interest (see for example Figures 1 and 2). A suitable template can be selected based on the type of side chains to be attached and the *inferred binding conformation of the peptide*, and coupled with *key amino acid side chains* identified from the *alanine scanning* step.[1]

One of the targets we chose was polar dipeptide mimetics of a β-turn. Compounds of this type have been described by several groups but none meet the stringent requirements that we have set in terms of appropriate physicochemical properties.[3,4]

Figure 1: Groups which the β-turn template are mimicking in the peptide



part of a β-turn



β-turn mimetic

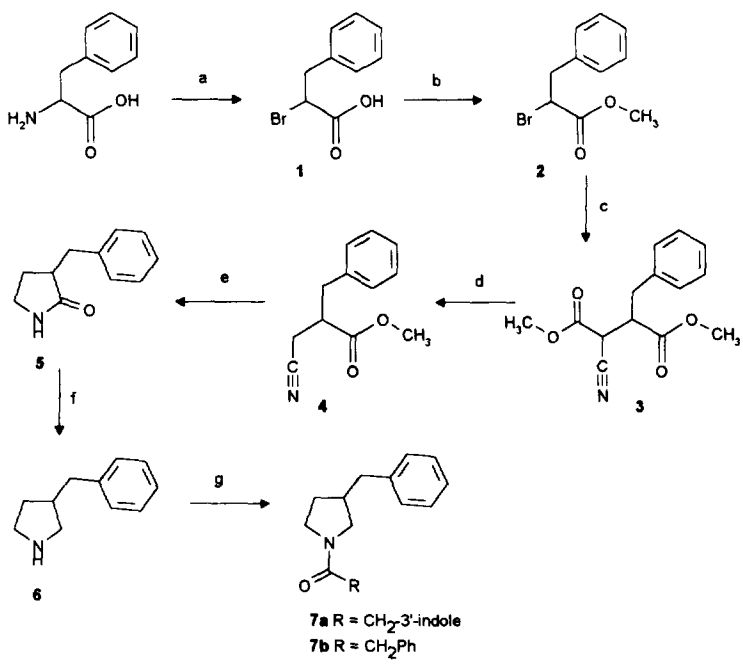
Here we report the design and synthesis of conformationally constrained, non-peptide templates which allow the incorporation of two adjacent amino acid side-chains in an orientation similar to that found in a dipeptide segment of a β-turn. The NK_2 tachykinin receptor affinity of the Trp-Phe mimetic (**7a**) prepared was also determined.

The compounds studied and their syntheses as racemic mixtures are outlined in Scheme 1.

Racemic phenylalanine was converted into 2-bromo-3-phenylpropionic acid **1** by treatment with sodium nitrite and potassium bromide in dilute sulfuric acid. The α-bromo acid **1** was esterified to give **2**, and the bromide then displaced by methyl cyanoacetate to give the dimethyl succinate **3** in a good yield (73%). Compound **3** was subjected to a Krapcho demethoxycarbonylation which furnished the β-cyanoester **4**.[5] Cyclization of **4** to pyrrolidinone **5** was achieved by reducing the nitrile with molecular hydrogen using 5% rhodium on alumina as a catalyst. The pyrrolidinone **5** was then reduced by $LiAlH_4$, and the resulting pyrrolidine **6** coupled to either 3-indoleacetic acid or phenylacetic acid, activated with bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), to give target molecules **7a** and **7b** respectively.

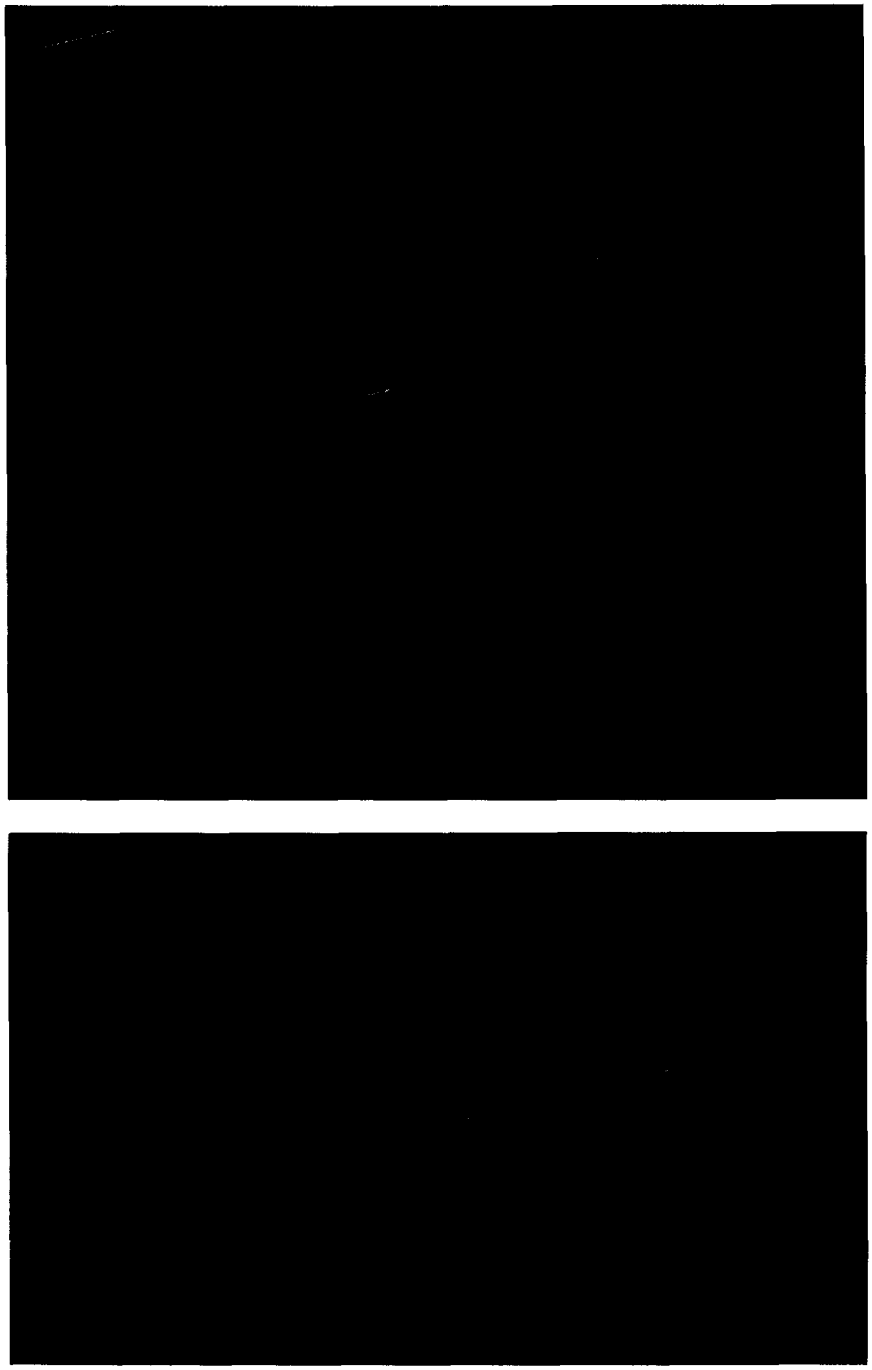
NMR experiments show that amides **7a** and **7b** exist as 1:1 mixtures of *cis* and *trans* conformers around the amide bond at room temperature.[6] The two conformers are not separable by chromatography. At elevated temperatures interconversion between the *cis* and *trans* conformers occurs.[6]

Scheme 1 : Synthesis of polar dipeptide mimetics of a β-turn



(a) $NaNO_2$ (1.8 eq), KBr (4.5 eq), 2.5 M H_2SO_4, -10°C, 4 h (70%); (b) conc. HCl, MeOH, 16 h (85%); (c) NaH (1.5 eq), $CNCH_2COOCH_3$ (1.6 eq), THF, 18 h (73%); (d) NaCl (1 eq), DMSO/H_2O, 9:1, 140°C, 2.5 h (77%); (e) Rh on alumina, H_2 (45 psi), 10% NH_3(g) in abs. EtOH, 18 h (75%); (f) $LiAlH_4$ (3 eq), Et_2O, reflux, 5 h (69%); (g) for R = CH_2-3'-indole: PyBroP (2 eq), Et_3N (4 eq), 3-IndCH_2COOH (2 eq), DMF, 18 h, (42%), for R = CH_2Ph: PyBroP (1.5 eq), iPr_2EtN (3 eq), $PhCH_2COOH$ (1.5 eq), CH_2Cl_2, 18 h (71%).

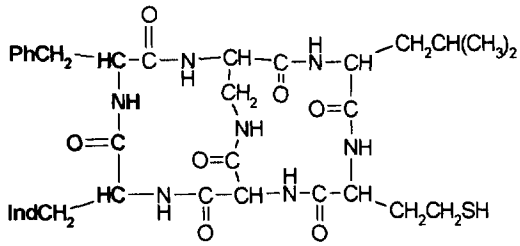
Figure 2: Overlay of the Trp-Phe mimetic **7a** with MEN 10627 (**8**)
MEN 10627 in yellow, Trp-Phe mimetic in blue



Computer assisted modelling[7] allowed us to identify a 3-substituted pyrrolidine-amide as a suitable template to mimic a β–turn. This indicated that the 3-pyrrolidine substituent and the $COCH_2$ bond of a low energy conformation of a model pyrrolidine-amide template (the TrpPhe mimetic **7a**) overlaid closely the Cα and Cβ carbon atoms of adjacent (i+1, i+2) side-chains in a β-turn. In fact the two Cα and the two Cβ carbon atoms of the model template overlay with the corresponding positions in a β-turn with a root mean square (rms) deviation of 0.16 Ångstroms (Figure 2). Furthermore, the template is orientated within the volume occupied by the β-turn peptide backbone.

Having designed a suitable template a synthesis was developed and illustrated with the preparation of Trp-Phe **7a** and Phe-Phe **7b** mimetics. The TrpPhe mimetic **7a** was chosen as the target compound because:
i.) we have reported an *alanine scan* for an analogue of the hexapeptide, high affinity tachykinin NK_2 receptor antagonist: L-659,874 (AcLeuMetGlnTrpPheGly)[8] which clearly indicates the critical importance of the Trp and Phe side chains of this peptide for tachykinin NK_2 receptor affinity[9] and,
ii.) Pavone *et al*[10] have designed a cyclic peptide, MEN 10627 (**8**), which is further constrained, but still binds to the NK_2 receptor with high affinity. MEN 10627 is described as consisting of two cyclo-tetrapeptides fused together, each containing a β-turn domain; based on preliminary structural analysis in solution, using NMR spectroscopy, and in the solid state, using X-ray diffraction techniques. The Trp and Phe amino-acids of this compound occupy the i+1 and i+2 positions of a β-turn.

Figure 3: MEN 10627 (**8**), with the Trp-Phe portion in bold (see also figure 2)



The affinity of the TrpPhe template **7a** for the tachykinin NK_2 (hamster urinary bladder, [^{125}I]-iodohistidyl-NKA (0.1 nM)) was determined,[9] and shown to be greater than ten micromolar (**7a**: NK_2 binding, IC_{50}> 10μM). This is clearly not comparable to either of the hexapeptides, L-659,874 (NK_2, IC_{50}= 19.8 nM)[8] or MEN 10627[10]. The lack of NK_2 receptor affinity observed for **7a** may be due to a number of reasons:-
i.) The solid state and solution conformation of MEN 10627 may not be the binding conformation of the peptide at the receptor.
ii.) The key binding groups for MEN 10627 are not Trp and Phe.

iii.) The core template (the pyrrolidine and amide moiety) of **7a** interacts unfavourably with the receptor.
iv) The direction of the dipole moment of **7a** may cause it to approach the receptor in a unfavourable orientation. Computer assisted calculations of the dipole moments of **7a** and **8** suggest that when the molecules are oriented as shown in figure 2, the dipole moment of **7a** is at nearly right angles with the dipole moment of MEN 10627.[7]

Compounds **7a** and **7b** were also evaluated in NK_1 and NK_3 binding assays, as we have previously shown[11] that Boc-PhePheNH$_2$ (NK_3, IC_{50}= 1,600 nM) /Boc-TrpPheNH$_2$ (NK_3, IC_{50}= 3,000 nM) and Z-Trp-PheNH$_2$ (NK_1, IC_{50}= 9,300 nM; NK_2, IC_{50}= 5,200 nM) are micromolar affinity ligands for the NK_3 and NK_1/ NK_2 tachykinin receptors respectively; and as part of our overall strategy we intend to evaluate mimetics of these dipeptides constrained in a number of common protein structural motifs.[12] For the NK_1 receptor assay human IM9 lymphocytoma cells were used as the tissue source and [^{125}I]-BHSP (< 0.1 nM) as the radioligand,[11a] while for the NK_3 receptor assay the human NK_3 receptor expressed in CHO cells were used and [^{125}I]-[MePhe^7] NKB (< 0.1 nM) as the radioligand[11b]. The target compounds **7a** and **b** showed micromolar affinity for the NK_1 and NK_3 tachykinin receptors (**7a**: NK_3, IC_{50}= 4,400 nM; **7b**: NK_3, IC_{50}= 9,400 nM) comparable but not better than those determined for three dipeptide leads.

In conclusion we have described the design of a dipeptide polar β-turn template and illustrated its utility by the synthesis of two dipeptide mimetic examples containing lipophilic side chains. The synthesis of examples containing hydrophilic side chains and the development of polar β-turn templates with dipole moments in the direction of that of MEN 10627 is now in progress.

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6. ^{1}H-NMR data (DMSO, 400 MHz) for **7a**. δ = 10.82 (1H, s, br, aromatic), 7.55-6.94 (10 H, m, aromatics), 3.64 (3H, m, ind-C$\underline{H}_2$-CO, -NC$\underline{H}$HCH$_2$), 3.43 (1.5H, m, -NCH$\underline{H}$CH$_2$-, -NC$\underline{H}$HCHBz), 3.20 (1H, m, -NCH$\underline{H}$CH$_2$-, NCH$\underline{H}$CHBz), 2.97 (0.5H, m, -NCH$\underline{H}$CHBz), 2.61 (2H, m, -CH$_2$Ph), 2.41 (0.5H, m, -C$\underline{H}$Bz), 2.32 (0.5H, m, -C$\underline{H}$Bz-), 1.96 (0.5H, m, -C$\underline{H}$H-), 1.81 (0.5H, m, -C$\underline{H}$H-), 1.60 (0.5 H, m, -CH$\underline{H}$-), 1.45 (0.5H, m, -CH$\underline{H}$-). At 110^{0}C the double peaks at 1.60/1.45, 1.96/1.81 and 2.41/2.32 clearly coalesce to (broad) singlets and the multiplet at 2.61 is transformed into a doublet (J = 8 Hz).

7. All molecular modelling and systematic search analysis were performed using SYBYL (Tripos, Inc.: St. Louis, Missouri, USA) version 6.00 and 6.10 running on Silicon Graphics Iris 4D/310GTX. The molecules were built from the SYBYL fragment database and energetically optimized using the TRIPOS force field (Clark, M., Cramer, R.D. III, Van Opdenbosch, N., *J Comput. Chem.* **1989**, *10*, 982). MULTIFIT within the SYBYL (Labanovski, J., Motoc, I., Naylor, C.B., Mayer, D., Dammkoehler, R.A., *Quant. Struc-Act. Relat.*, **1986**, *5*, 138) was used to align the molecules by superimposing the Cαs and Cβs of Trp and Phe residues of MEN 10627 (**8**) and corresponding atoms of the template molecule (**7a**). The energy of **8** was 20.1 Kcal/mol and $DE_{template}$= 0.8 Kcal/mol (conformation that best fits **8** vs. local minimum conformation).

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