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HIGHLY CONSTRAINED DIPEPTOID ANALOGUES CONTAINING A TYPE II' β-TURN MIMIC AS NOVEL AND SELECTIVE CCK-A RECEPTOR LIGANDS

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Abstract.
Conformationally constrained dipeptoid analogues containing the type II' β-turn mimic (2*S*,5*S*,11b*R*)-2-amino-3-oxohexahydroindolizino[8,7-*b*]indole-5-carboxylate framework in place of the α-MeTrp residue, show high binding affinity and selectivity for CCK-A receptors, suggesting that a turn-like conformation could contribute to the bioactive conformation at this CCK receptor subtype.

Keywords: Cholecystokinin; Peptoids; Antagonists

Cholecystokinin (CCK) is a peptide hormone and neurotransmitter regulating gastrointestinal function and behaviour by interacting with specific receptors [1]. The variety of possible therapeutic uses for CCK receptor agonists and antagonists has prompted an intensive research in this area and several potent and selective non-peptide CCK-A and CCK-B receptor antagonists have been reported over the past decade [2-4]. Among these compounds, dipeptoids **1** have been designed, following a deletion approach, from structure-activity relationship studies on the endogenous CCK-B receptor selective agonist CCK-4 (H-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2), which revealed the importance of the aromatic side-chains of Trp and Phe residues as key binding fragments[5-7]. A representative member of this series, **1a**, is a highly selective CCK-B receptor antagonist, while the related compound **1c** has good affinity and selectivity for the CCK-A receptor[8]. Following the discovery of dipeptoids, different constrained analogues have been prepared in an attempt to stablish three-dimensional structure-activity relationships and to develop pharmacological agents with improved properties. Restrictions by *N*-terminal cyclization[9], macrocyclization[10,11] and amide bond rigidification[12] resulted in dipeptoid analogues with reduced affinity for CCK-B receptors with respect to the corresponding acyclic parents. By contrast, conformational restriction of the *C*-terminal residue through a Pro ring[13] or by incorporation of a tetrahydronaphthyl group[14] have been reported to maintain or to increase, respectively, the affinity for CCK-B receptors. However, in both cases, the restricted derivatives showed

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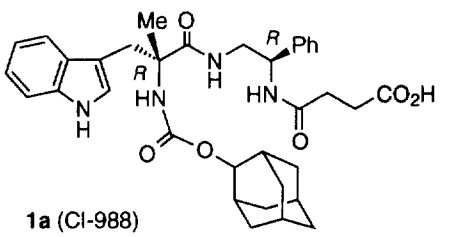
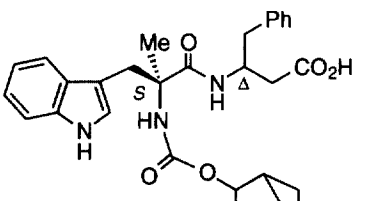
higher affinity for CCK-A receptors and, therefore, a lower selectivity than their parents. The enhancement of the affinity for the CCK-A receptor subtype was specially remarkable in constrained dipeptoid analogues incorporating dehydro- and cyclopropylPhe derivatives at *C*-terminus[15], for which conformational studies indicated the presence of a β-turn within the peptide backbone, although no preference in type was observed[16].

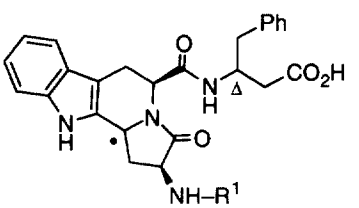
In order to investigate whether a turn-like conformation is that adopted at the CCK-A receptor site, we designed a new series of conformationally constrained dipeptoid derivatives **2** and **3**, in which the α-MeTrp residue of dipeptoids **1b** and **1c** (CCK-B- and CCK-A- selective, respectively) has been replaced with the (2*S*,5*S*,11b*S*)- and (2*S*,5*S*,11b*R*)-2-amino-3-oxohexahydroindolizino[8,7-*b*]indole-5-carboxylate skeleton[17]. These diastereomeric skeletons contain the indole side chain of the Trp residue and, as we have recently reported, are able to mimic a type II' β-turn conformation with a degree of accuracy that depends on the *C*-11b configuration[18]. For ease of synthesis, Z and Boc groups were initially selected as substituents of the 2-amino group of compounds **2** and **3**, instead of the 2-Adoc moiety present in dipeptoids **1**.



1a (CI-988)



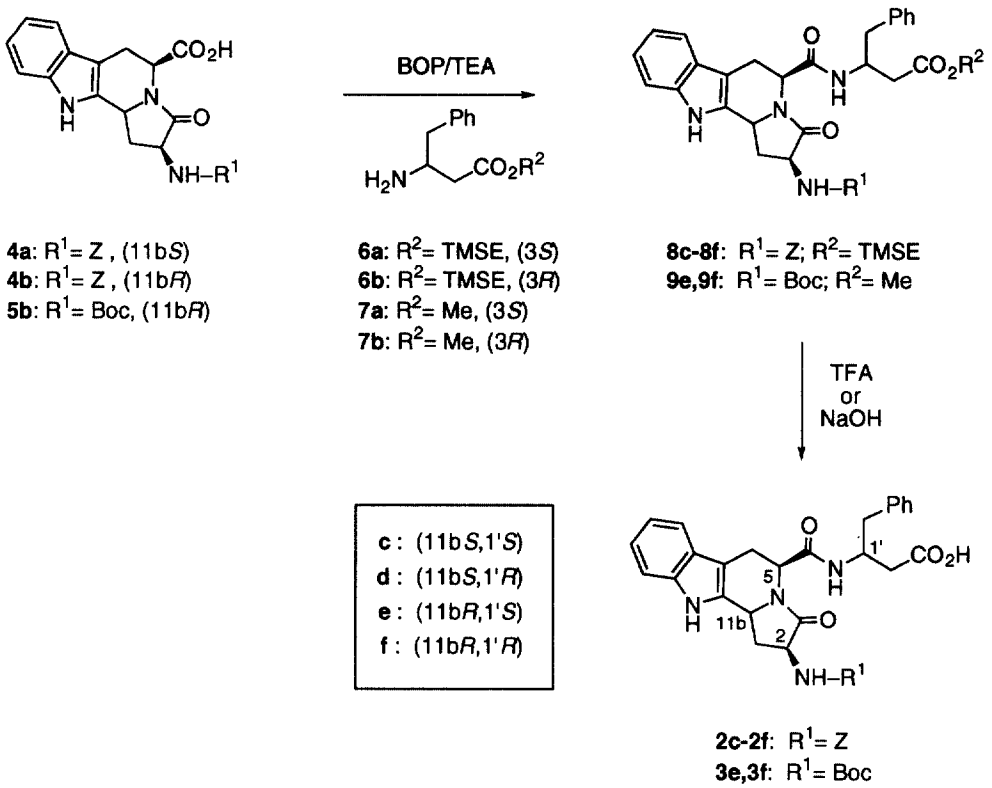
1b (PD-140547) (Δ,*S*)
1c (PD-140548) (Δ,*R*)



2: R^1= Z
3: R^1= Boc

RESULTS AND DISCUSSION

As indicated in Scheme 1, condensation of carboxylic acid derivatives **4a** and **4b**[17] with trimethylsilylethyl (3*S*)- and (3*R*)-3-amino-4-phenylbutanoate **6a** and **6b**[19], using BOP as coupling agent, provided the *C*-terminally protected dipeptoids **8c-8f**. Hydrolysis in acidic medium (TFA) of the trimethylsilylethyl (TMSE) ester in compounds **8c-8f** afforded the expected carboxylic acid derivatives **2c-2f**. Following a similar sequence of reactions, Boc-protected dipeptoids **3e** and **3f** were prepared by coupling the tetracyclic derivative **5b** with methyl phenylbutanoates **7a** and **7b**, respectively, followed by saponification of the resulting methyl ester derivatives **9e** and **9f**.

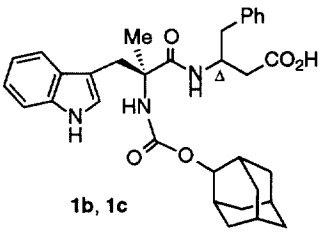


Scheme 1

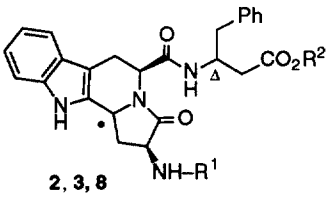
To evaluate the ability of the restricted dipeptoid analogues to adopt a type II' β-turn, the conformational behaviour in solution of the trimethylsilylethyl ester derivatives **8c-8f** was studied. The ^{1}H-NMR spectra in deuterated DMSO of compounds **8c-8f** showed that no coupling exists between the downfield H-6 proton and the vecinal H-5 proton ($J_{5,6}$= 0 Hz), in agreement with an axial disposition of the 5-carboxylate group, as required for the formation of type II' β-turn[17]. The temperature coefficients measured for the amide protons of the above indicated compounds was found to be dependent on the C-11b configuration. Thus, the 11b*S* derivatives **8c** and **8d** have large coefficients ($\Delta\delta/\Delta T \approx$ -4 ppb/°K), indicative of the accessibility of the respective amide protons to bulk solvent. On the contrary, the small absolute values of the temperature coefficients found for the 1'-NH proton in compounds **8e** and **8f** ($\Delta\delta/\Delta T$ = -1.8 and -1.9 ppb/°K, respectively), suggest that in the 11b*R*-configured derivatives the reverse turn is stabilized by an intramolecular hydrogen bond. Similar conformational behaviour was found for the free carboxylic acid derivatives **2e** and **2f**, showing small temperature coefficients for the 1'-NH (2.1 and -1.8 ppb/°K, respectively).

The affinity at CCK-A and CCK-B receptors of all the synthesized dipeptoid analogues was determined by measuring the displacement of [^{3}H]propionyl-CCK-8 binding to rat pancreatic and brain cortex homogenates, respectively, as previously described[20]. The obtained data, depicted in Table 1, were compared to those previously described for the parent dipeptoids **1b** and **1c**[4].

Table1.– Inhibition of specific [^{3}H]propionyl-CCK-8 binding to rat pancreas (CCK-A) and rat cerebral cortex membranes (CCK-B) by compounds **2, 3** and **8**



1b, 1c



2, 3, 8

Compd.	R^1	R^2	Config. •	Config. Δ	IC_{50} (nM)[a] CCK-A	IC_{50} (nM)[a] CCK-B	B/A
2c	Z	H	*S*	*S*	635	>10000	>16
2d	Z	H	*S*	*R*	1000	>10000	>10
2e	Z	H	*R*	*S*	88	>10000	>113
2f	Z	H	*R*	*R*	7.4	2700	364
3e	Boc	H	*R*	*S*	>10000	>10000	–
3f	Boc	H	*R*	*R*	4480	>10000	>2
8c	Z	TMSE	*S*	*S*	460	>10000	>22
8d	Z	TMSE	*S*	*R*	1400	>10000	>7
8e	Z	TMSE	*R*	*S*	220	2400	11
8f	Z	TMSE	*R*	*R*	180	1500	8
1a[b]			–	–	4300	1.7	0.0004
1b[b]			–	*S*	539	13.2	0.02
1c[b]			–	*R*	2.8	259	92

[a] Values are the means of at least three experiments performed in triplicate (Standard errors within ±10-15% of the mean).

[b] Reported IC_{50} values at mouse cerebral cortex (CCK-B) and rat pancreas (CCK-A) receptors (Ref. 8).

As shown in Table 1, the affinity of these highly restricted compounds at CCK-B receptors was negligible or very modest. By contrast, the IC_{50}'s of the Z-protected derivatives **2e** and **2f** at the CCK-A receptor were 88 and 7.4 nM, respectively. A significant reduction in affinity was observed for the corresponding trimethylsilylethyl esters **8e** and **8f** when compared to the carboxylic acids **2e** and **2f**, demonstrating the importance of a free carboxylic acid at *C*-terminus. Replacement of the Z group in **2e** and **2f** with the Boc moiety led to a dramatic reduction in binding affinity (compounds **3e** and **3f**), suggesting that specific π-π interactions could exist between the phenyl moiety of the Z group and aromatic residues within the hydrophobic pocket accommodating the *N*-substituent at the receptor.

The most remarkable result obtained was that compounds **2e** and **2f**, having 11b*R* configuration showed a higher CCK-A binding potency, by one to two orders of magnitude, than the 11b*S*-configured isomers **2c** and **2d**. Since we have previously demonstrated that the 11b*R* isomer of the hexahydroindolizino[8,7-*b*]indole system is a better mimic of the type II' β-turn conformation than the 11b*S* isomer[18], this result seems to indicate that a turn-like conformation within the peptide backbone of dipeptoids is favorable for the CCK-A receptor recognition.

In comparison with the model dipeptoid **1c**, the highly restricted analogue **2f**, the more potent compound in this series, has a similar CCK-A binding affinity with an approximately 4-fold increased selectivity for this receptor subtype. Unlike this model, inversion of the C-1' configuration in **2f** (compound **2e**) did not reverse the sense of the selectivity. These facts indicate that the high degree of constraint imposed by the incorporation of the (2*S*,5*S*,11b*R*) 3-oxohexahydro-indolizino[8,7-*b*]indole skeleton into the dipeptoid series is not tolerated by the CCK-B receptor binding site. In contrast, this skeleton facilitates the correct orientation of the pharmacophoric side-chain residues to interact with the CCK-A receptor. This clear difference in conformational requirements for the interaction with CCK-A and CCK-B receptors could be helpful to design constrained dipeptoids with high selectivity for the CCK-A receptors.

Compounds with significant affinity for CCK-A receptors were tested for antagonism to CCK-8 in the isolated longitudinal muscle myenteric plexus preparation of guinea pig ileum[21]. In this assay, compounds **2e** and **2f**, added at a 10^{-5} M concentration, were able to inhibit the contractions induced by CCK-8 (10^{-8} M) by 72 and 89%, respectively, without producing any intrinsic contractile effect at the above mentioned concentration. The pK_B value[22] for compound **2f** was 7.8. These results indicate that constrained dipeptoids **2e** and **2f** behave as CCK-A receptor antagonists.

In summary, we have prepared two highly constrained dipeptoid analogues containing the (2*S*,5*S*,11b*R*) hexahydroindolizino[8,7-*b*]indole framework, which are endowed with high binding affinity and selectivity for CCK-A receptors. As this framework is a probed type-II' β-turn mimic, we propose that the presence of a β-turn within the peptide backbone of dipeptoid antagonists could contribute to the bioactive conformation at the CCK-A receptor subtype. The incorporation of other β-turn mimics, different from type II', and the conformational study of the new restricted dipeptoid analogues herein described could yield valuable information about the conformational requirements for optimal recognition of the CCK-A receptors by these antagonists.

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