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α,β-DIDEHYDROTRYPTOPHAN AS A SURROGATE FOR α-METHYLTRYPTOPHAN IN CCK 'PEPTOIDS' RELATED TO CI-988.

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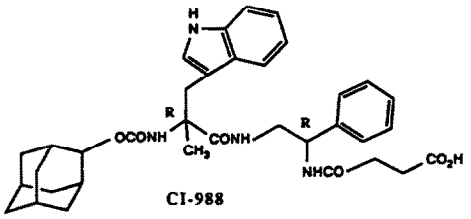
Abstract : The design and synthesis of high affinity α,β-didehydrotryptophan substituted CCK ligands is described. Ligands selective for both the CCK-A and CCK-B receptor subtypes have been prepared.

Introduction

The synthesis and utility of α,β-didehydro amino acids (DDAA) has been well documented in reviews[1,2] and original research articles[3,4] primarily due to their presence in biologically active fungal metabolites such as nisin and subtilin. In addition to their presence in natural products, DDAAs have also been shown to be versatile precursors for the asymmetric synthesis of amino acids and peptides.[5,6] However, their use has not solely been restricted to the role of chemical intermediates since several authors describe the incorporation of DDAAs into biologically active peptides to confer either resistance to in vivo enzymatic degradation[7] and/or impose predictable conformational constraints.[2,8] One recent example of this latter application has been the synthesis of derivatives of the brain/gut peptide cholecystokinin, containing a C-terminal α, β-didehydro Phe[33] residue.[9]

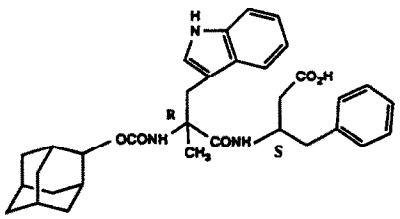
We have previously[10] published on the rational design and synthesis of small molecule, non-peptide ligands (peptoids) for the CCK receptor an example of which is the potent anxiolytic CCK-B receptor antagonist CI-988(1). The principal feature of this molecule is the α-methyl substituent on the Trp residue which is important for CCK-B receptor affinity and selectivity.[11] Our objective was to determine whether the α-methyl group of a closely related analogue (2)[12] (selected on the basis of its higher CCK-B receptor affinity) of CI-988 could be replaced by an achiral, planar, α,β-didehydro Trp residue.

This paper describes the synthesis and binding affinity of potent and selective CCK-A and CCK-B receptor subtype ligands incorporating an α,β-didehydro Trp residue.



CI-988

(1)



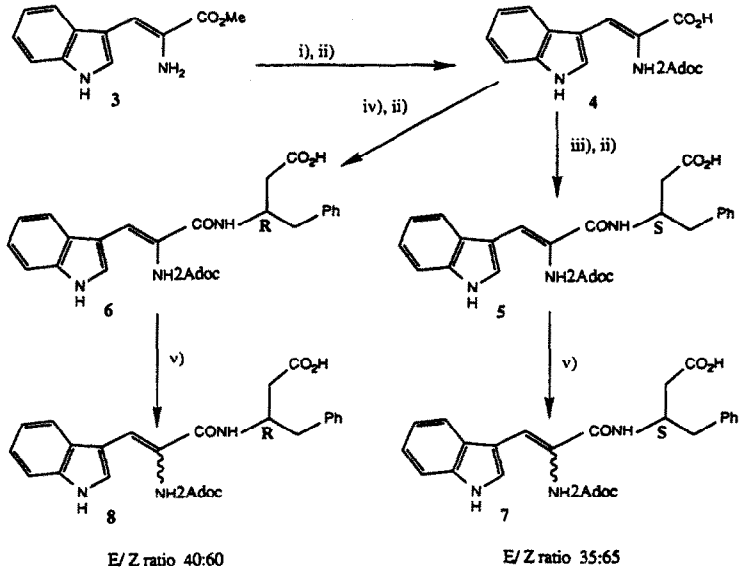
(2)

Figure 1

Results and Discussion

The target molecules in this study were a) the Z-configured DDAA derivative (5) of the parent α-methyl tryptophan substituted CCK ligand (2), b) the R enantiomer (6) of (5) and finally, c) their corresponding E-configured analogues (7) and (8). In preparing the R enantiomer (6) we hoped to probe the importance of stereochemistry with respect to CCK-A/B receptor selectivity in this series of compounds. There is good evidence in the literature underlining the importance of stereochemistry in differentiating between the CCK receptor subtypes.[13,14] Finally, synthesis of the E isomers (7) and (8) would enable us to determine the stereochemical preference of the CCK receptor toward the Trp residue.

The synthesis of the target compounds (5) through (8) is outlined in Scheme 1. Preparation of the Z α,β-didehydro Trp amino acid (3) has been previously described.[15]

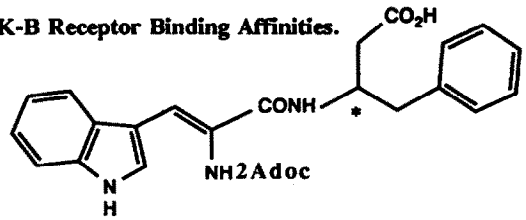


Scheme 1

Reagents and conditions: i) 2-adamantylchloroformate, pyridine, THF, 0°C (76%); ii) 1M LiOH, 1,4 dioxane/H_2O (87%); iii) S-homophenylalanine methylester [12], DCC, HOBT, EtOAc (54%); iv) R- homophenylalanine methylester [12], DCC, HOBT, EtOAc (65%); v) hv, 350nm, CH_3CN.

The binding affinity data (Table 1) illustrate that incorporation of a Z-configured DDAA Trp residue into (2) results in a high affinity (IC_{50} = 13 nM) and almost 50-fold selective CCK-B receptor selective ligand (5). Although the CCK-B affinity of (5) is in the 10^{-8}M range, it is nevertheless almost two orders of magnitude lower than that found for the parent (2) thus indicating a preference for the α,α-disubstituted Trp residue over the Z-configured DDAA moiety at the CCK-B receptor site. The difference in CCK-B receptor binding affinity between (2) and (5) is almost certainly due to the different conformational restraints induced by the α,α-disubstituted and α,β-didehydro Trp residues. However, due to the large number of

Table 1. CCK-A and CCK-B Receptor Binding Affinities.



No	Olefin Geometry[18]	*	IC_{50}, nM[17] CCK-B	CCK-A	A/B ratio
5	Z	S	13	550	42
6	Z	R	99	3.7	0.037
7	E/Z, 35:65	S	0.30	18	60
8	E/Z, 40:60	R	60	3.9	0.065
1	-	-	1.7	4300	2500
2	-	S	0.15	30	200
CCK-26-33(S)	-	-	0.30	0.10	0.33

freely rotatable bonds still remaining in (2) and (5), comparison of the possible conformations adopted by these CCK-B receptor ligands using computer assisted molecular modelling proved ambiguous and the results were inconclusive.

The corresponding R enantiomer (6) of (5) proved to be a potent (IC_{50} = 3.7 nM) and selective (CCK-B, IC_{50} = 99 nM) CCK-A receptor ligand thus 'reversing' the receptor selectivity found with (5). This finding is in line with previous publications on related[14] and structurally dissimilar[13] CCK receptor ligands which reveal a similar relationship between CCK receptor subtype selectivity and ligand stereochemistry.

Photolysis of the Z-configured (5) at 350 nm wavelength over a period of 2 hours gave a mixture (7) of the E and Z isomers in a ratio of ~35 : 65 in favour of the Z isomer as determined by HPLC.[16] Column chromatography (eluant:$MeOH/CH_2Cl_2$) on the crude photolysis mixture enabled us to remove any minor impurities present but we were unable to separate the E and Z isomers. This isomeric mixture displayed comparable CCK-B receptor binding affinity (IC_{50} = 0.3 nM) to the parent saturated analogue (2) despite containing only ~35% of the E isomer. From this result it was concluded that the E isomer shows exceptional affinity for the CCK-B receptor. Attempts to increase the proportion of the E isomer were unsuccessful primarily due to the inherent instability of the E isomer (interconverts to the Z isomer (5) on standing in solution at room temperature over a period of 48 hours).

Photolysis of the corresponding R enantiomer (6) under similar conditions also gave a mixture of isomers approximately 60 : 40 in favour of the Z isomer as assayed by HPLC. In contrast to the distinct differences in the CCK-A/B binding affinities found with (5) and (7), the corresponding R enantiomers (6)

and (8) displayed near identical binding affinities at both the CCK-A and CCK-B receptors (see Table 1). Taken together, these results provide evidence to support a marked dissimilarity in binding modes between the R and S enantiomers (5) and (6).

Conclusions

In this paper we have described the synthesis of a novel series of small molecule, non-peptide CCK receptor selective ligands containing an α,β-didehydro Trp residue. By altering either the configuration of the olefin or the homophenylalanine residue, we have prepared high affinity (10^{-9}M) selective CCK-B and CCK-A receptor ligands respectively. This series of compounds, in addition to demonstrating the successful inclusion of DDAAs in receptor active ligands, once again highlights the importance of stereochemistry with respect to differentiating between the CCK-A and CCK-B receptor subtypes.

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References and Notes

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16. Lichrosorb RP18 (5 μM) column using 60% CH_3CN/39.9% H_2O/0.1% TFA as eluant.
17. IC_{50} represents the concentration (nM) producing half-maximal inhibition of specific binding of [^{125}I]Bolton Hunter CCK-26-33 to CCK receptors in the mouse cerebral cortex (CCK-B) or the rat pancreas (CCK-A).
18. The Z olefin configuration was identified by an ^{1}Hnmr NOE between H-2 of the indole and the α-N-H of the amino acid. The E olefin was assigned on the basis of an NOE between the NH of the amide and H-2 of the indole.