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CCK-4 RESTRICTED ANALOGUES CONTAINING A 3-OXOINDOLIZIDINE SKELETON

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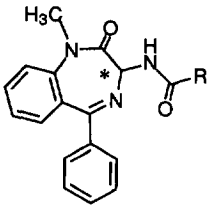
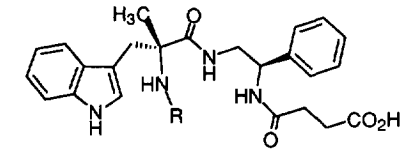
Abstract. A series of CCK-4 restricted analogues, incorporating a 3-oxoindolizidine bicyclic lactam as conformational constraint, is described. The 8-(Boc-tryptophyl)amino-2-benzyl derivatives behave as weak CCK-A or CCK-B receptor antagonists. The main factors for selectivity are the configuration at C-2 position of the 3-oxoindolizidine ring and at the Trp residue.

Cholecystokinin (CCK) is a peptide hormone involved in modulating gastrointestinal and behavioral activities by interacting with specific receptors.[1] A variety of non-peptide ligands for CCK-A and CCK-B receptors have been identified following different strategies.[2] However, the discovery of potent and selective antagonists for both types of receptors has been relied on broad screening and subsequent lead optimization. Thus, manipulation of asperlicin, a natural non-peptide product with modest affinity for CCK-A receptor, provided the potent benzodiazepine CCK-A and CCK-B antagonists **1** (Devazepide) and **2**, respectively,[3,4] as well as a series of quinazolidinones as selective CCK-B ligands.[5,6] Independent origin but similar basis led to diphenylpyrazolidinone derivatives, including selective CCK-A or CCK-B antagonists.[7]



1: R = 2-Indolyl; * *S*
2: R = 3-Me-phenyl-NH; * *R*



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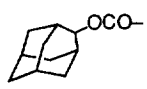

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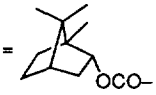
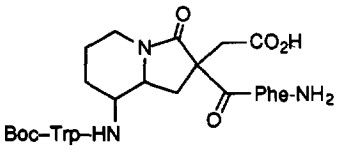
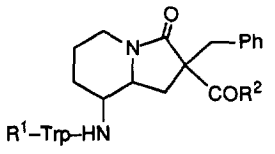


Concerning rational strategies, CCK receptor selective ligands have been designed from structure-activity relationship studies on the CCK-B receptor selective agonist CCK-4 (H-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2) which revealed the importance of the aromatic side chains of Trp and Phe as key binding fragments. Thus, "dipeptoids" **3** and **4** are highly potent and selective CCK-B antagonists discovered from a deletion approach.[8] Based on the template strategy, a series of CCK-A antagonists, in which mimetics of the amino acid side chains of Trp^{30}, Asp^{32} and Phe^{33} are attached to a pyrrolidinone ring, was designed.[9] The incorporation of conformationally restricted elements into the CCK-4 sequence has also led to selective ligands for CCK-B receptors. In particular, constrained analogues containing a 2,5-diketopiperazine ring and a *cyclo*-Trp nucleus are potent CCK-B agonists.[10,11] In spite of the structural diversity among the above mentioned CCK ligands the presence of a lactam function is a common feature of all of them, apart from the dipeptoids. This similarity and

the wide utility of lactams as conformational constraints in bioactive peptides,[12] focused our attention on the 3-oxoindolizidine bicyclic lactam, as a new template for carrying the Trp, Asp and Phe side chains. On this basis, we designed two series of CCK-4 restricted analogues, **5** and **6**, in which the Met [31] residue and the Met[31]-Asp[32] fragment have been replaced by suitably substituted 3-oxoindolizidines, as conformationally constrained spacers between Trp[30] and Phe[33]. Besides the synthetic accessibility to the 8-amino-3-oxoindolizidine bicyclic skeleton from ornithine derivatives,[13] the possibility to obtain various stereoisomers could allow us to study the influence of the different orientations of the appended substituents on the affinity for the CCK receptors.



5



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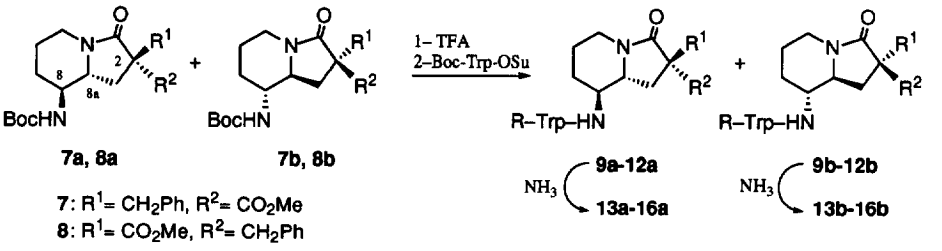
RESULTS AND DISCUSSION

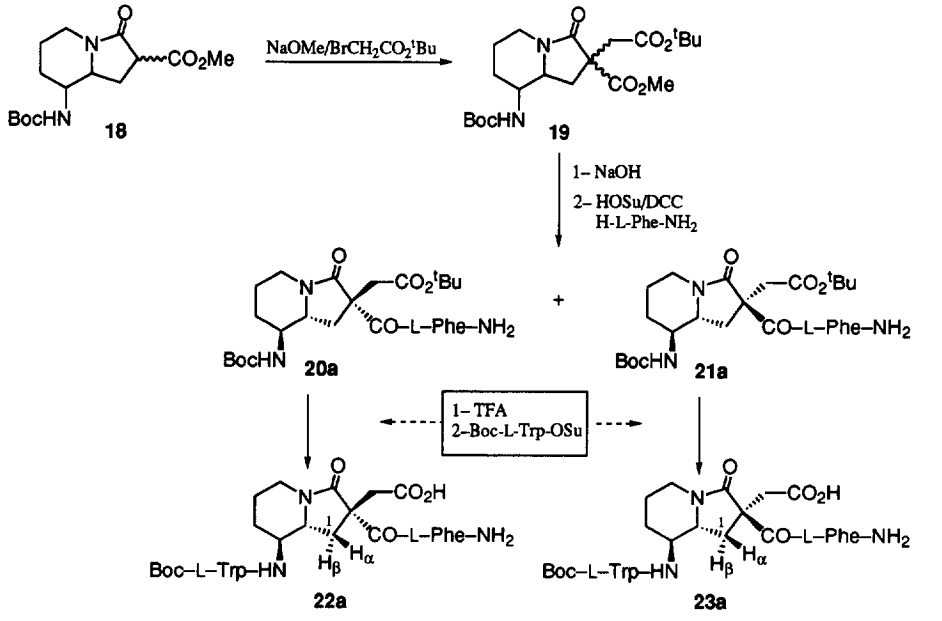
The CCK-4 conformationally restricted analogues **9-12**, in which the Met-Asp fragment have been replaced by a 3-oxoindolizidine bearing the Phe side chain, were synthesized from the enantiomeric mixtures **7ab** and **8ab** (**a/b** ≈ 6:1),[13,14] according to Scheme 1. Thus, *N*-deprotection of these compounds followed by coupling with Boc-L- or Boc-D-Trp-OSu afforded the 2-methoxycarbonyl derivatives **9-12** (48-60%, overall yield) as a mixture of two diastereoisomers **a** and **b**, that were then transformed into the corresponding amide analogues **13-16** (94-98%) by treatment with NH_3/MeOH.[15] The differences in the resulting diastereomeric **a/b** ratio can be attributed to the kinetic resolution due to the differential reaction rates during the condensation with Trp derivatives, as previously demonstrated.[14] Attempts to separate these diastereoisomeric mixtures using column chromatography were unsuccessful. However, separation of the diastereoisomeric pairs **10ab** and **11ab** was performed by semipreparative HPLC.[16] In a similar way, small amounts of compounds **12a-15a** were isolated in pure form, using the latter indicated technique. Compounds **17a** and **17b** were obtained by removal of the Boc-protecting group from derivative **10ab** followed by treatment of the resulting deprotected analogue with 1-adamantanecarbonyl chloride (1-Adc-Cl, 81%) and subsequent separation by semipreparative HPLC.[16]

Derivatives **22a** and **23a**, in which the indolizidine skeleton replaces the Met residue of CCK-4 and the Asp[32] side chain is directly situated on the bicyclic ring, were prepared by the synthetic route depicted in Scheme 2. Saponification of the diester derivative **19**, obtained by the alkylation of the corresponding 2-methyl ester analogue **18** with *tert*-butyl bromoacetate, followed by coupling to H-L-Phe-NH_2 provided compounds **20a** (66%) and **21a** (10%), that were separated by column chromatography. As expected, due to the lack of enantiospecificity in the formation of the 3-oxoindolizidine derivative **18**,[14] small amounts of the minor diastereoisomers **20b** and **21b** (< 10%), with 2*R*,8*R*,8a*S* and 2*S*,8*R*,8a*S* configuration, respectively, were detected in the crude reaction mixture, but they were not isolated. Finally, removal of the Boc and ^{t}Bu protecting groups from **20a** and **21a** and coupling with Boc-L-Trp-OSu provided the desired compounds **22a** (56%) and **23a** (58%), respectively. The absolute configuration at C-2 position in these CCK-4 restricted analogues was established by mean of bidimensional NOE experiments (ROESY). Thus, compound **22a** showed NOEs between H_{8a}-$H_{1\alpha}$, $H_{1\alpha}$-2-CH_2 and H_8-$H_{1\beta}$ protons, indicating that the H_{8a} proton and the Asp side chain at position 2 have a *cis*-relationship. In contrast, in compound **23a** the 2-CH_2 and the H-8 protons showed NOEs with the $H_{1\beta}$ proton indicating that the alkyl substituent at C-2 and the H-8 proton are located on the same face of the 3-oxoindolizidine ring.



Compd.	R	Trp	R^1	R^2	a/b Ratio
9ab	Boc	L	CH_2Ph	CO_2Me	10:1
10ab	Boc	D	CH_2Ph	CO_2Me	10:1
11ab	Boc	L	CO_2Me	CH_2Ph	4:1
12ab	Boc	D	CO_2Me	CH_2Ph	3:1
13ab	Boc	L	CH_2Ph	$CONH_2$	10:1
14ab	Boc	D	CH_2Ph	$CONH_2$	10:1
15ab	Boc	L	$CONH_2$	CH_2Ph	4:1
16ab	Boc	D	$CONH_2$	CH_2Ph	3:1
17ab	1-Adc	D	CH_2Ph	CO_2Me	10:1

Scheme 1



Scheme 2

The 3-oxoindolizidine-containing CCK-4 restricted analogues were studied as displacers of [^{3}H]propionyl-CCK$_8$ binding to peripheral or central CCK receptors using rat pancreatic or cerebral cortex homogenates, respectively (Table 1).[17] The CCK-A antagonist **1**, the CCK-B antagonist **4** and Boc-CCK$_4$[18] were also included in the assay for comparative purposes.

As indicated in Table 1, simplification of the CCK-4 structure by replacement of Met^{31}-Asp^{32} dipeptide fragment with conformationally constrained 3-oxoindolizidine building blocks, bearing the Phe side chain at C-2 position, led to a series of compounds that displayed modest affinity for CCK-A and CCK-B receptors. Thus, the CCK analogues **10a**, **13a**, **14a** and **17a**, in which the 2-benzyl group and the substituent at C-8 position have a relative *cis*-relationship, showed preference for brain CCK-B receptors. In this series, incorporation of D-Trp (**14a**) instead of L-Trp (**13a**), as well as the substitution of the Boc protecting group by a highly lipophilic adamantyl group (**17a**) improved the CCK-B binding by approximately one order of magnitude. A similar improvement in the CCK-B receptor affinity was observed when the 2-carboxamide derivative (**14a**) was compared with the corresponding methyl ester (**10a**). In contrast, derivatives **11a**, and **15a**, in which the Phe side chain and the 8-substituent are *trans*-orientated, preferentially bound to the pancreatic (CCK-A) receptors. In this case, the presence of L-Trp (**11a**) is preferred over the corresponding D-analogue (**12a**), while the change of methyl ester to amide has not a significant effect on CCK-A receptor binding. Diastereoisomers **10b** and **11b**, having 8*R*,8a*S* configuration, did not show significant affinity at any of the CCK receptors with concentrations up to 10^{-5} M. These results seem to indicate that the stereochemistry at positions 8 and 8a of the 3-oxoindolizidine ring has a substantial effect on CCK affinity, the 8*S*,8a*R* configuration being optimal for both CCK-A and CCK-B binding. However, the fact that the adamantyl derivative **17b** showed micromolar affinity for both binding sites indicated that not only the stereochemistry of the 3-oxoindolizidine is important for binding to CCK receptors, but also the hydrophobicity of the *N*-protecting group of the Trp residue.

As described for the "dipeptoids" and pyrrolidinone derived CCK ligands ,[9,19] the simultaneous inversion of two chiral centres in this series of 2-benzyl-3-oxoindolizidine CCK restricted analogues led to CCK ligands that bind preferentially to CCK-A or CCK-B receptors. Thus, derivatives having 2*R* configuration and D-Trp showed preference for CCK-B receptors, while a combination of L-Trp and 2*S* configuration led to CCK-A selective ligands. In addition, the substitution of the Boc group in the CCK-B selective analogues by the 1-Adc moiety improved binding to CCK-B receptors by one order of magnitude. It is expected that similar replacements in the CCK-A selective derivatives **11a** and **15a** could serve to enhance affinity at pancreatic receptors.

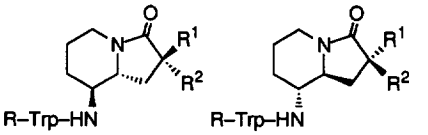
In contrast, substitution of the Met^{31} residue in CCK-4 by a 3-oxoindolizidine skeleton, in which the 2-acetate group could act as a mimic of the Asp^{32} side chain, resulted in compounds **22a** and **23a** that did not bind to CCK receptors at concentrations up to 10^{-5} M. This result seems to indicate that the structures of these CCK-4 restricted analogues possess, as a whole, an inappropriate side chain orientation for the recognition of CCK receptors.

Compounds with significant affinity for CCK receptors were tested for antagonism to CCK-8 or CCK-4 in the isolated longitudinal muscle myenteric plexus preparation of the guinea pig ileum.[20] In this preparation, CCK-8 produces a contractile effect by stimulation of CCK-A and CCK-B receptors whereas CCK-4 stimulates only the CCK-B receptor subtype. In this assay, compounds **11a** and **15a**, added at a 10^{-5} M concentration, were able to inhibit the CCK-8 (10^{-8} M) mediated contractions in 70 and 88%, respectively, which were in good agreement with their binding affinities at the CCK-A receptors. Moreover, compound **15a** (10^{-5} M) inhibited the amylase release from pancreatic acini induced by CCK-8 (10^{-9} M)[21] by 70%. These results indicate that derivatives **11a** and **15a** behave as CCK-A receptor antagonists. Similarly, a good correlation was found between the binding affinity at CCK-B receptors of

compound **17a** and the percentage of inhibition (67%) of the guinea pig ileum contraction induced by CCK-4 (10^{-6}M).

In conclusion, compounds such as **14a** and **17a**, which showed only one order of magnitude lower affinity than Boc-CCK-4 in the CCK-B receptor binding assay, may serve as useful leads to obtain new and more potent CCK-B receptor antagonists. The preparation of related analogues with enhanced conformational flexibility of the aromatic side chains is now in progress.

Table 1. Effect of 3-Oxoindolizidine Derivatives on Binding of [^{3}H]Propionyl-CCK-8 to Rat CCK-A and CCK-B Receptors



a **b**

Compd.	R	Trp	R^1	R^2	IC_{50} (μM)[a] CCK-A	CCK-B
10a	Boc	D	CH_2Ph	CO_2Me	>10	3.72
10b	Boc	D	CH_2Ph	CO_2Me	>10	>10
11a	Boc	L	CO_2Me	CH_2Ph	2.35	>10
11b	Boc	L	CO_2Me	CH_2Ph	>10	>10
12a	Boc	D	CO_2Me	CH_2Ph	>10	>10
13a	Boc	L	CH_2Ph	$CONH_2$	>10	3.53
14a	Boc	D	CH_2Ph	$CONH_2$	>10	0.82
15a	Boc	L	$CONH_2$	CH_2Ph	1.02	>10
17a	1-Adc	D	CH_2Ph	CO_2Me	>10	0.22
17b	1-Adc	D	CH_2Ph	CO_2Me	0.98	2.19
22a	Boc	L	CH_2CO_2H	$COPheNH_2$	>10	>10
23a	Boc	L	$COPheNH_2$	CH_2CO_2H	>10	>10
1					0.0007	1.09
4					1.12	0.009
Boc-CCK-4[b]					1.80	0.025

[a] Rat pancreas (CCK-A) or cerebral cortex (CCK-B) homogenates were used for binding studies. The values given are the mean of three separate experiments. For all these values, standard errors were below 15% of the mean. [b] From reference 18.

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