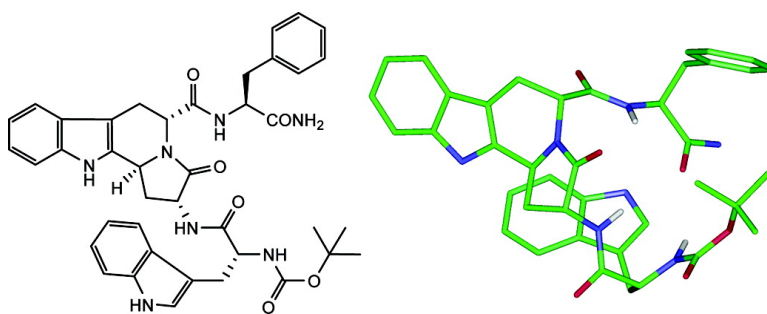


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Conformationally Constrained CCK4 Analogues Incorporating IBTM and BTD β -Turn Mimetics

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To test whether a turnlike arrangement is involved in the bioactive conformation of CCK4 analogues upon CCK₁ receptor recognition, we describe the preparation of two series of CCK4 derivatives, in which the central dipeptide Met-Asp has been replaced by recognized β -turn mimetics {(2*S*,5*S*,11*bR*)- and (2*R*,5*R*,11*bS*)-2-amino-5-carboxy-3-oxo-2,3,5,6,11,11*b*-hexahydro-1*H*-indolizino[8,7-*b*]indole (IBTM) and β -turn dipeptide, 2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (BTD)}. The incorporation of the indolizinoindole IBTM type II β -turn mimetic is preferred over its type II' counterpart for efficient CCK₁ receptor recognition, while BTD derivatives were completely inactive. The structure–conformation–activity relationship study in the IBTM series has shown some essential requirement of these CCK4 derivatives to favorably interact with CCK₁ receptors: (a) the adoption of turnlike conformations, (b) the presence of an *l*-Phe residue and a *C*-terminal carboxamide moiety, and (c) the indole ring of the IBTM skeleton. Moreover, the existence of π – π interactions between the phenyl ring of *D*-Phe residues and the indole ring of IBTM framework is detrimental for binding affinity. A series of potent and selective CCK₁ receptor antagonists, exemplified by compounds **8a** and **8b**, emerges among these IBTM-containing derivatives.

Introduction

Cholecystokinin (CCK) is a peptide hormone found in the periphery that plays a major role in gut function, in the digestive processes, and in the control of feeding behavior.^{1,2} CCK also occurs in brain, where it acts as a neurotransmitter or neuromodulator.^{1,3–7} At least two different receptors for CCK have been characterized, the CCK₁ and the CCK₂ receptors, both belonging to the class of G protein-coupled 7 TM receptors.^{8,9} CCK₁ binding sites predominate in the periphery but are also found in some discrete regions of the brain, whereas CCK₂ receptors are mainly located in the central nervous system (CNS). While there are different biologically active forms of CCK, the sulfated *C*-terminal octapeptide (CCK8) and the *C*-terminal tetrapeptide (CCK4) are the minimal sequences required for a complete biological activity at CCK₁ and CCK₂ receptors, respectively. Taking into account that CCK is involved in many different and important biological processes, the therapeutic potential of the CCK receptor ligands seems to be promising. This fact drove an intensive research in this area, where several potent and selective nonpeptide CCK₁ and CCK₂ receptor agonists and antagonists have been reported.^{10–13}

The *C*-terminal tetrapeptide CCK4 has previously been used as the chemical starting point for the rational design of novel ligands for CCK receptors. Modifications of this fragment showed that the *N*-terminal protection of the tetrapeptide, as in Boc-CCK4, increased CCK₂

selectivity and enzymatic stability.¹⁴ Previous reports also pointed out the importance of Trp and Phe side chains for high CCK₂ binding affinity.^{14–16} The first evidence indicating that the bioactive conformation of CCK4 is nonextended came from the work of Horwell et al., who have demonstrated that the dipeptide Boc-Trp-Phe-NH₂ was able to retain micromolar binding affinity at central CCK₂ receptors.¹⁷ Subsequent modifications of this dipeptide derivative led to an extensive series of α -MeTrp-containing dipeptoids with nanomolar affinity at both CCK₁ and CCK₂ receptors.¹⁸ In particular, the replacement of the α -MeTrp residue of dipeptoids by (2*S*,5*S*,11*bR*)- and (2*R*,5*R*,11*bS*)-2-amino-5-carboxy-3-oxo-2,3,5,6,11,11*b*-hexahydro-1*H*-indolizino[8,7-*b*]indole (IBTM) skeletons,^{19,20} type II' and II β -turn mimetics, respectively, led to derivatives **1** and **2** (Figure 1) that bound CCK₁ receptors with greater selectivity than the parent dipeptoids.^{21,22} We have also introduced the IBTM scaffold in a series of dipeptoids that have been shown to behave as CCK₁ receptor agonists. As expected, the incorporation of this conformational constraint increased the selectivity for CCK₁ receptors, but it was found that the modification prevented receptor activation and the new restricted dipeptoids behaved as antagonists.²³

Following our interest in CCK ligands, and the promising results of the IBTM skeleton in restricting the conformation of the series of dipeptoids, we decide to explore a different approximation in which the IBTM framework is used as a replacement of the Met-Asp residue of the tetrapeptide CCK4, to restrict the conformational freedom of its backbone (Figure 1). The conformational restraint of the *C*-terminal tetrapeptide has previously been explored by incorporation of im-

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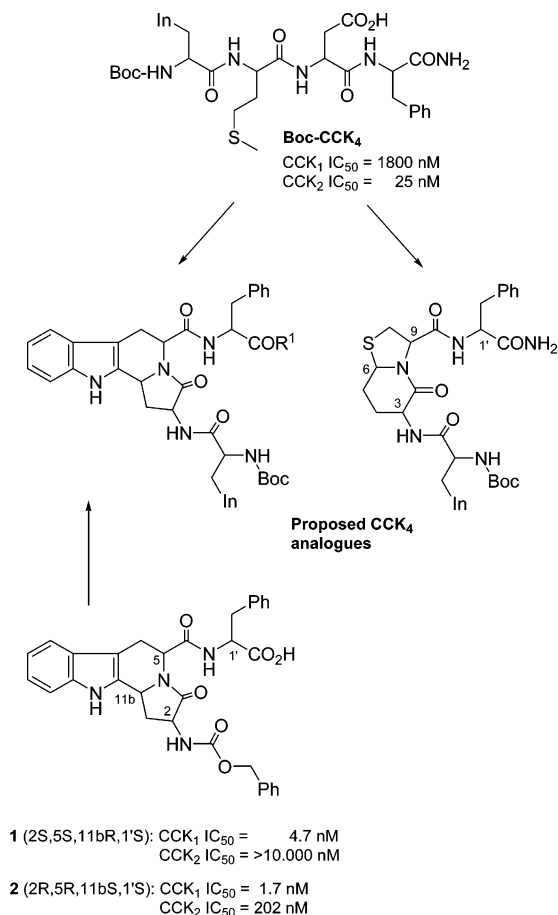
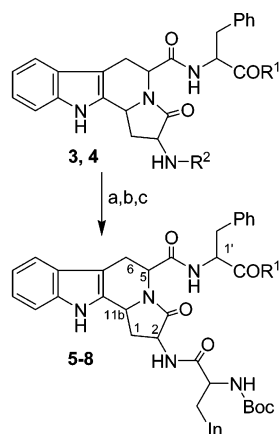


Figure 1. Proposed conformationally restricted CCK₄ analogues.

portant amino acid side chains of CCK₄ into rigid structures, some of them designed on the basis of previous conformational studies.^{24–29} Therefore, the knowledge of the three-dimensional conformation of CCK fragments could be an important aid for the design of nonpeptide CCK receptor ligands. NMR and molecular modeling studies on CCK₈ and CCK₄ showed that, despite its intrinsic flexibility, they exist preferentially in folded forms, although a certain controversy persists. It has been shown that the tetrapeptide CCK₄ adopts a Z-shaped conformation with a relatively well-defined orientation of side chains, in which the hydrophobic groups (Trp, Met, Phe) clustered together on one side of the peptide backbone.³⁰ However, an alternative study placed the Met and Asp side chains on the same side of a similar Z-bend arrangement.¹⁶ On the other hand, conformational studies on the octapeptide CCK₈ and analogues evidenced the presence of differently positioned γ - and β -turns.^{31,32} In particular, an NMR conformational study on [Nle³¹]CCK₇, which shows nanomolar affinity at CCK₁ and CCK₂ receptors, suggested a β -turn centered on the Nle-Asp dipeptide fragment.³³

On the basis of all the above literature precedents, it is plausible that a turnlike conformation within the peptide backbone of the CCK₄ would be favorable for CCK₁ receptor recognition, as has previously been demonstrated for the dipeptoid derivatives. Thus, to test whether a turnlike arrangement is adopted at the CCK₁ receptor site, we describe here the preparation, NMR, and molecular modeling conformational analysis, as well

Scheme 1^a



Comp.	Config. 2,5,11b	Trp	Phe	R ¹	R ²
3a	SSR	-	L	OMe	Z
3b	SSR	-	D	NH ₂	Z
4a	RRS	-	L	OMe	Boc
4b	RRS	-	D	NH ₂	Boc
5a	SSR	L	L	OMe	-
5b	SSR	D	L	OMe	-
6a	SSR	L	L	NH ₂	-
6b	SSR	D	L	NH ₂	-
6c	SSR	L	D	NH ₂	-
6d	SSR	D	D	NH ₂	-
7a	RRS	L	L	OMe	-
7b	RRS	D	L	OMe	-
8a	RRS	L	L	NH ₂	-
8b	RRS	D	L	NH ₂	-
8c	RRS	L	D	NH ₂	-
8d	RRS	D	D	NH ₂	-

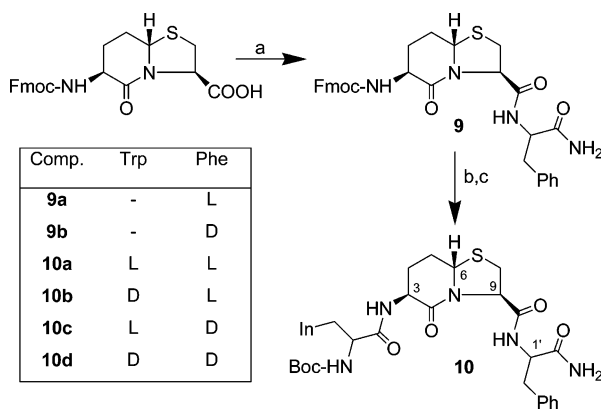
^a Reagents and conditions: (a) H₂/Pd-C, MeOH or TFA, CH₂Cl₂; (b) Boc-Trp-OH, BOP, Et₃N, CH₂Cl₂; (c) NH₃, MeOH.

as the binding affinity, of a series of conformationally restricted CCK₄ analogues in which the Met-Asp dipeptide fragment has been replaced by different β -turn mimetics, namely the IBTM and BTM (β -turn dipeptide, 2-oxo-7-thio-1-azabicyclo[4.3.0]nonane)^{34–36} scaffolds.

Results

1. Chemistry. The preparation of the IBTM-containing analogues **3** and **4** was performed by coupling of Z- and Boc-IBTM-OH derivatives with H-Phe-OMe or H-Phe-NH₂, following the previously described synthetic route.^{21,22} As indicated in Scheme 1, final CCK₄ constrained analogues **5–8** were prepared by condensation of derivatives **3** and **4** with Boc-L- and Boc-D-Trp-R¹, prior to elimination of the Z- and Boc protecting groups of the IBTM moiety by catalytic hydrogenation or by treatment with TFA, respectively. The C-terminal carbamoyl derivatives were obtained either by direct coupling of Phe-NH₂ residues to the IBTM skeletons or by ammonolysis of the corresponding methyl ester analogues. Standard BOP-mediated peptide couplings were used in all cases. In general, the formation of the amide bonds proceeded in good yields, except for the synthesis of compounds **7b** and **8d**, which incorporate the Boc-D-Trp-OH residue at the 2-amino group of the

Scheme 2



^a Reagents and conditions: (a) H-Phe-NH₂, BOP, Et₃N, CH₂Cl₂; (b) piperidine; (c) Boc-Trp-OH, BOP, Et₃N, CH₂Cl₂.

Table 1. Temperature Coefficients for Amide Protons in CCK4 Restricted Analogues [$\Delta\delta/\Delta T$ (10^{-3} ppm/K)]^a

compd	2-NH	1'-NH	compd	3-NH	1'-NH
6a, 8d	-3.9	-3.6	10a	-3.7	-3.1
6b, 8c	-5.3	-2.9	10b	-5.5	-2.4
6c, 8b	-4.6	-4.1	10c	-3.2	-4.8
6d, 8a	-5.7	-4.2	10d	-4.6	-5.0

^a Determined by least-squares linear regression analysis from measurements over the temperature range 30–60 °C (seven points) in DMSO-d₆.

corresponding (2*R*,5*R*,11*bS*)-IBTM derivative. These products were obtained in moderate yield (41 and 35%, respectively).

As depicted in Scheme 2, BTD-containing CCK4 restricted analogues **9** and **10** were prepared by a similar synthetic route to that described above for IBTM derivatives. Thus, the commercially available Fmoc-BTD-OH was coupled with H-L- or H-D-Phe-NH₂, followed by removal of the Fmoc protecting group with piperidine, and subsequent amide bond formation between the free 3-NH₂ group and Boc-L- or Boc-D-Trp-OH.

2. Conformational Studies. 2.1. NMR Studies. To evaluate the ability of the conformationally restricted CCK4 analogues **6**, **8**, and **10** to adopt β -turnlike conformations in solution, the ¹H NMR $J_{5,6}$ coupling constants (for IBTM derivatives) and the temperature coefficients of the amide protons were measured. As required for the adoption of β -turn conformations in IBTM-containing analogues,^{19,20} the spectra of compounds **6** and **8** exhibited no coupling between the downfield H-6 proton and the vicinal H-5 proton, which agrees with an axial disposition of the 5-carboxylate group. On the other hand, it has been described that small absolute values for the temperature coefficient ($\Delta\delta/\Delta T \leq 3 \times 10^{-3}$ ppm/K) of amide protons indicate their protection from solvent exchange either by involvement in an intramolecular hydrogen bond or by inaccessibility to solvent, whereas values higher than 4×10^{-3} ppm/K indicate exposure to the solvent.³⁷ As shown in Table 1, the $\Delta\delta/\Delta T$ values found for the 2-NH amide proton in all compounds, for the 3-NH proton in derivative **10b**, and for the 1'-NH or 3-NH protons in compounds **6c,d**, **8a,b**, and **10c,d** are indicative of the accessibility of these protons to bulk solvent. In contrast, the values obtained for the 1'-NH proton in derivatives **6b**, **8c** and **10b** indicated protection from the solvent,

whereas for compounds **6a**, **8d**, and **10a** and for the 3-NH amide proton of derivative **10a,c** the respective temperature coefficients are not conclusive in this regard. Although the $\Delta\delta/\Delta T$ values of some derivatives are within the range required for a hydrogen-bonded inverse turn, molecular modeling studies have shown that the protection from the solvent is not due to the adoption of this kind of turn, as discussed later. On the whole, these data indicate that the protection of the 1'-NH proton from the solvent is mainly dependent on the type of β -turn mimetic and on the configuration of the Phe residue. Thus, derivatives incorporating type II' β -turn mimetics [either BTD or (2*S*,5*S*,11*bR*)-IBTM frameworks] require an L-Phe residue at C-terminus, while a D-Phe amino acid is preferred for derivatives with the type II mimetic [(2*R*,5*R*,11*bS*)-IBTM skeleton]. In contrast, little influence of the configuration of Trp residue on the 1'-NH temperature coefficients was observed. Although both frameworks, IBTM and BTD, have shown good ability to induce β -turn secondary structures in other peptides,^{20,35,36} we have previously observed that the presence of a C-terminal carboxamide function in the closely related dipeptoids, which incorporate the IBTM scaffold, contribute to disrupt the β -turnlike conformation to some extent.²² The need for minute conformational changes to accommodate the appended hydrophobic residues (Phe and Boc-Trp) could also be responsible for the β -turn destabilization.

2.2. Molecular Modeling Studies. To get further insights into the 3D structure of this family of CCK4 analogues, we analyzed, by simulated annealing molecular dynamics, the conformational space of IBTM derivatives **8a–d**, which incorporate the type II β -turn mimetic, some of which show high affinity at CCK₁ receptors. The obtained minima, within 3 kcal/mol window above the global minimum, were compared and were clustered into families according to their heavy atom root mean square values and to the peptide backbone torsion angle values. On the whole, and based on the N–H orientations, we could make a broad division of the different conformers into two families. Each of these families could subsequently be subdivided in clusters depending on the Phe and Trp torsion angles. Figure 2 shows one representative conformer for each of these two broad families in derivatives **8a–d**.

It is interesting to note that, independent of the stereochemistry at the Phe and Trp residue, there is a preferred geometry that involves an interaction of the 1'-NH group with the indole ring of the central scaffold (Figure 2, conformers I). Moreover, this tendency is almost exclusive for derivatives that support a D-Phe residue, **8c** (100%) and **8d** (94%) (Table 2). The distances from the hydrogen of the 1'-NH to the centroid of the aromatic ring range from 3.4 to 3.8 Å, which are within the mean distances for described N–H/ π interactions.³⁸ Another feature of compounds with a D-Phe residue is that the distance between the centroids of the aromatic rings of Phe and IBTM residues is less than 5 Å (Table 2). This could be indicative of the existence of π – π interactions that are likely contributing to stabilize this spatial disposition. In contrast, only a few conformers of compounds with an L-Phe residue display this particularly short distance (4 and 19%). The conformational analysis of these latter compounds, **8a,b**, showed the

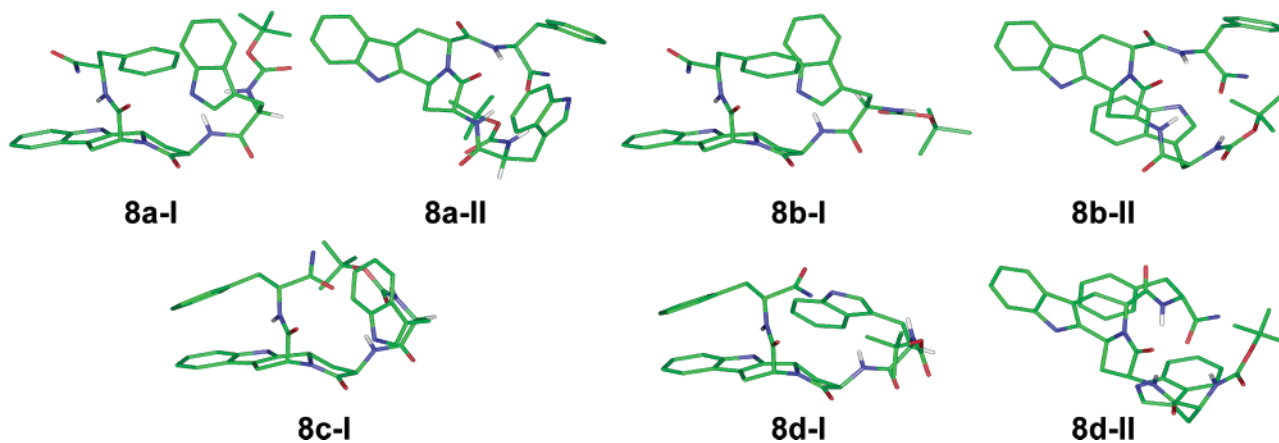


Figure 2. Representative conformers of derivatives **8a–d**. For clarity, only the NH amide hydrogens are shown.

Table 2. Statistical Data Obtained for the Conformers within a 3 kcal/mol Window above the Global Minimum

compd	IC ₅₀ CCK ₁ (nM)	D _{1'-NH-In} ^a < 4 Å	D _{1'-NH-CO} ^b < 4 Å	d _{Ph-In} ^c < 5 Å
8a	54.9	76	24	19
8b	25.9	66	34	4
8c	>1000	100	0	96
8d	582	94	5	100

^a *d*[1'-NH-In(IBTM)] (%). The distance refers to the centroid of the aromatic moiety, and to the hydrogen atom of the NH(Phe).
^b *d*[1'-NH-CO(3)] (%). ^c *d*[Ph(Phe)-In(IBTM)] (%). The distance refers to the centroids of the aromatic moieties.

presence of another family of conformers (Figure 2, conformers II), in which this amino group is pointing toward the CO(3). This spatial disposition is also present in derivative **8d**, albeit in very low percentage (Table 2). In conformers II, the distance between the hydrogen atom of the 1'-NH group and the oxygen of CO(3) is not within that required for a hydrogen bond. However, for these conformers in the most active derivatives **8a,b**, the values of the ϕ and ψ dihedral angles of the *i* + 2 residue are within the standard values ($\pm 30^\circ$) that define γ -turns. The molecular modeling conformational studies have shown that none of the studied derivatives have intramolecular H-bonds. However, the ¹H NMR study has shown that the 1'-NH is protected from the solvent in derivatives **8c** and **8d**. A possible explanation for this fact is the existence of the above-mentioned N-H/ π contacts, which together with the close interaction between the two aromatic moieties that surround this NH make it inaccessible to the solvent.

Although for compounds **8a,b** there is a small percentage of conformers (<10%) that show the existence of the H-bond characteristic of a β -turn, none of them have values of the torsion angles within that required for any type of β -turn.

3. Biological Results and Discussion. The binding affinities of all conformationally constrained CCK4 analogues at CCK₁ and CCK₂ receptors were determined by measuring the displacement of [³H]propionyl-CCK8 binding to rat pancreatic and cerebral cortex homogenates, respectively, as previously described (Table 3).³⁹ For comparative purposes, the described affinities for Boc-CCK4 and the representative CCK₁ dipeptoid antagonists **1** and **2**²² were also included.

As indicated in Table 3, replacement of Met-Asp dipeptide fragment of CCK4 with IBTM skeletons led

Table 3. Inhibition of the [³H]pCCK8-specific Binding to Rat Pancreas (CCK₁) and Cerebral Cortex Membranes (CCK₂) by CCK4 Restricted Analogues

compd	config 2,5,11 ^b	Phe	Trp	R ₁	IC ₅₀ (nM) ^a	
					CCK ₁	CCK ₂
5a	SSR	L	L	OMe	>10000	>10000
5b	SSR	L	D	OMe	>10000	>10000
6a	SSR	L	L	NH ₂	625 ± 88.3	>10000
6b	SSR	L	D	NH ₂	817 ± 92.5	>10000
6c	SSR	D	L	NH ₂	>1000	524 ± 150
6d	SSR	D	D	NH ₂	>1000	938 ± 133
8a	RRS	L	L	NH ₂	54.9 ± 13.3	>10000
8b	RRS	L	D	NH ₂	25.9 ± 1.6	>10000
8c	RRS	D	L	NH ₂	>1000	>10000
8d	RRS	D	D	NH ₂	582.0 ± 59.2	>10000
10a	SSR ^b	L	L		>1000	>10000
10b	SSR ^b	L	D		>1000	>10000
10c	SSR ^b	D	L		>1000	>10000
10d	SSR ^b	D	D		>1000	>10000
1	SSR	L		OH	4.7 ± 1.30	>10000
2	RRS	L		OH	1.7 ± 0.30	202 ± 99
Boc-CCK4 ^c					1800	25

^a Values are the mean or mean ± SEM of at least three experiments, performed with seven concentrations of test compounds in duplicate. ^b Configuration at positions 3, 6, and 9 in the BTM skeleton. ^c From ref 22.

to a series of compounds with modest to good affinity for either CCK₁ or CCK₂ receptors. In agreement with the described importance of the carbamoyl moiety of CCK4 for binding and activation of CCK receptors,¹⁴ the C-terminal group of the restricted CCK4 analogues had a significant effect on the affinity. Thus, while derivatives **5a,b**, with a methoxycarbonyl group, were not recognized by either of the CCK receptor subtypes, the corresponding analogues **6a,b**, bearing a carboxamide group at the C-terminus, moderately bound to CCK₁ receptors.

With regard to the type of IBTM turn mimetic, some interesting conclusions can be drawn from the binding affinities of compounds **6** and **8**. CCK4 analogues **6a–d**, incorporating the type II' (2*S*,5*S*,11*bR*)-IBTM skeleton, bound CCK₁ or CCK₂ receptor subtype, depending on the configuration of the Phe residue. Thus, L-Phe-containing derivatives **6a,b** bound selectively to CCK₁ receptors, whereas their D-Phe counterparts **6c,d** preferred the CCK₂ receptor subtype. Related stereochemical changes within single structural types of other nonpeptide CCK ligands give rise to interconversions of CCK₁/CCK₂ or CCK₂/CCK₁ selectivity.^{13,18a} In con-

trast, derivatives **8a,b,d**, that incorporated the type II β -turn IBTM mimetic displayed selective CCK₁ receptor affinities and were unable to bind to CCK₂ receptors at 10⁻⁵ M concentrations. In compounds **8**, and in terms of CCK₁ binding potency, the presence of an L-Phe residue was again preferred (**8a,b** versus **8c,d**), and the configuration of the appending Trp residue has almost no influence (**8a** vs **8b**).

Compared to Boc-CCK4, some of the IBTM-containing analogues showed a significant increase of the affinity at CCK₁ receptors, while none of these derivatives were able to reach the nanomolar affinity showed by the CCK4 at the CCK₂ receptors. In contrast, none of the CCK4 analogues that incorporate the well-known type II' mimetic BTM were able to bind to either CCK₁ or CCK₂ receptors at all (Table 3). This points to the importance of the indole group of the IBTM framework for binding to CCK receptors. Thus, the IBTM skeleton appeared not to be just a scaffold for the proper disposition of the aromatic side chains of Phe and Trp residues but a part of the pharmacophore itself.

On the whole, the study of the binding data shows that the main factors that governs the affinity are, first, the stereochemistry of the central scaffold, where a type II β -turn mimetic is preferred, and, second, the configuration of the Phe residue, where an L-Phe residue is favored. In general, changes in the stereochemistry of the Trp residue lead to insignificant affinity variation.

To try to get deeper insights into the structure–conformation–activity relationships (SAR) of this series of restricted CCK4 derivatives, we proceeded to correlate low-energy conformers with CCK₁ receptor affinities. It is interesting to note that an increase in the conformers with Ph(Phe)–In(IBTM) stacking turns out to be detrimental for affinity, as observed by comparison of L-Phe and D-Phe derivatives (Table 2). In contrast, the increase in the percentage of conformers with a distance between 1'-NH and CO(3) of less than 4 Å correlates directly with an increase in the CCK₁ receptor binding potency. Although the latter distance is not within the value required for a H-bonded γ -turn, we have shown that derivatives **8a,b**, with higher CCK₁ receptor affinities, have a higher population of conformers with γ -turnlike conformations. This might suggest the existence of an inverse turnlike arrangement in the bioactive conformation recognized by CCK₁ receptors.

Following these results, it might be thought that the IBTM-containing CCK4 analogues described here could be conceptually related to the IBTM-restricted dipeptoids **1** and **2**,²² which differ only because the benzylloxycarbonyl group at position 2 has been replaced by Boc-Trp moieties in **5–8**. Thus, in both series the ability to adopt an inverse turn conformation seems to be directly related to the affinity at CCK₁ receptors. The decrease in affinity of compounds **8a,b** compared with the restricted dipeptoid **2**, might be due to the fact that the former is able to adopt γ -turnlike arrangements, whereas the latter prefers β -turn conformations. Moreover, in both series of IBTM-containing compounds the configuration of the Phe residue is determinant for high affinity, the optimal being an L-Phe residue. However, some divergence can be found between the CCK4 restricted analogues and the dipeptoid series. In the new CCK4 analogues described here, there are more re-

stricted stereochemistry requirements for the central scaffold, where only the incorporation of a type II β -turn mimetic leads to derivatives able to interact with CCK₁ receptors in the nanomolar range. Another point of discrepancy between the two series comes from the need for a carboxamide moiety in the CCK4 analogues, similarly to the endogenous peptide. In the dipeptoid series, a C-terminal methoxycarbonyl group or a free carboxylic acid is required in order to get high CCK₁ affinity, while a carboxamide moiety led to inactive compounds.²² These differences and similarities in the SAR of the two series might be explained through the interaction with distinct groups within a common binding site or, alternatively, they might only be sharing part of the binding site. Directed mutagenesis studies will be required to explore deeper the binding pockets of these compounds.

The functional activity of the best compounds in this series, **8a** and **8b**, was evaluated in the amylase release assay.^{40,41} These compounds were able to antagonize the CCK8-induced amylase secretion from pancreatic acini with IC₅₀ values of 130 ± 18 and 829 ± 402 nM, respectively, while they did not show any intrinsic effect on the amylase release at a 1 μ M concentration. Therefore, compounds **8a** and **8b** are representative members of this family of selective CCK₁ receptor antagonists.

Conclusions

In the present study, we have demonstrated that the incorporation of the β -turn inductor frameworks IBTM, as replacement for the Met-Asp central dipeptide fragment of CCK4, led to active CCK₁ compounds, whereas the related BTM-containing derivatives turn out to be inactive. The affinity of the IBTM derivatives was mainly dependent on the configuration of the Phe residue (L much better than D) and on the type of β -turn mimetic (type II preferred over type II'). A careful comparison of the conformational studies and the biological results revealed that the IBTM framework has a substantial contribution to the way in which these derivatives bind to the CCK receptors, suggesting that it is directly involved in the interaction with them. Although in this series of CCK4 derivatives the IBTM framework is not able to induce any typical β -turn conformation, molecular modeling studies have revealed the existence of γ -turnlike arrangements that are favorable for high binding affinity to CCK₁ receptors. On the whole, the introduction of conformational constraints into the CCK4 sequence by means of the IBTM skeleton resulted in a powerful way to obtain CCK₁ selective antagonists, illustrated by **8a** and **8b**. Further studies on these compounds, which proved to be relatively easy to synthesize, could serve to achieve a better definition of the structural and conformational requirements for CCK₁ receptor recognition.

Experimental Section

Chemistry. All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F₂₅₄. Silica gel 60 (230–400 mesh) was used for flash chromatography. ¹H NMR spectra were recorded with a Varian XL-300, operating at 300 MHz, using TMS as reference. Temperature coefficients were obtained from least-

squares fits to data of 30, 35, 40, 45, 50, 55, and 60 °C in DMSO-*d*₆. Elemental analyses were obtained on a CH-O-RAPID apparatus. Analytical HPLC was performed on a Waters Nova-pak C₁₈ column (3.9 × 150 mm, 4 μm) with a flow rate of 1 mL/min and using a tunable UV detector set at 214 nm. Mixtures of CH₃CN (solvent A) and 0.05% TFA in H₂O (solvent B) were used as mobile phase. Compounds **3a**, **b** and **4a** were prepared as described.²⁰

(2R,5R,11bS,1'R)-2-(tert-Butoxycarbonyl)amino-5-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (4b). A solution of (2R,5R,11bS)-2-(tert-butoxycarbonyl)amino-5-carboxy-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole¹⁹ (262 mg, 0.68 mmol) in dry CH₂Cl₂ (14 mL) was successively treated with H-D-Phe-NH₂ (146.2 mg, 0.89 mmol), BOP (393.6 mg, 0.89 mmol), and TEA (0.124 mL, 0.89 mmol). After stirring overnight, the solvent was evaporated, and the residue was dissolved in EtOAc and washed with citric acid (10%), NaHCO₃ (10%), and brine. The organic layer was dried (Na₂SO₄) and evaporated, leaving a residue that was purified on a silica gel column using a gradient from 15 to 67% acetone in hexane. The title compound (374.6 mg, 95%) was obtained as a syrup. Anal. (C₄₁H₄₄N₆O₇) C, H, N.

General Procedures for the Coupling of 2-Amino-hexahydroindolizino[8,7-b]indole Derivatives with Boc-Tryptophans. Method A. A solution of the corresponding 2-benzyloxycarbonyl-substituted hexahydroindolizinoindole **3** (139 mg, 0.25 mmol) in MeOH (25 mL) was hydrogenated at room temperature and 30 psi of pressure for 5 h, using 10% Pd-C as catalyst (20% w/w). After filtration of the catalyst, the solvent was evaporated. The resulting residue was dissolved in dry CH₂Cl₂ (6 mL), and Boc-L- or Boc-D-Trp-OH (97.4 mg, 0.32 mmol), BOP (141.5 mg, 0.32 mmol), and TEA (0.08 mL, 0.32 mmol) were successively added. After stirring overnight the solvent was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10%), NaHCO₃ (10%), and brine. The organic layer was dried (Na₂SO₄) and evaporated, leaving a residue that was purified on a silica gel column as specified in each case.

Method B. To a solution of the corresponding derivative **4** (139 mg, 0.25 mmol) in CH₂Cl₂ (2.5 mL) was added TFA (1.25 mL). The solution was stirred at room temperature for 1 h, and the solvent was removed by repeated coevaporations with CH₂Cl₂. The residue was dissolved in dry CH₂Cl₂ (6 mL); successively treated with Boc-L- or Boc-D-Trp-OH (97.4 mg, 0.32 mmol), BOP (141.5 mg, 0.32 mmol), and TEA (0.08 mL, 0.57 mmol); and stirred overnight. The workup was continued as above.

(2S,5S,11bR,1'S)-2-[N^α-(tert-Butoxycarbonyl)-L-tryptophyl]amino]-5-[(1'-methoxycarbonyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (5a). Yield: 71% (from **3a** and Boc-L-Trp-OH, method A) as a foam. Eluent: 3% of MeOH in CH₂Cl₂. HPLC: *t*_R = 18.33 min (A:B = 45/55). Anal. (C₄₁H₄₄N₆O₇) C, H, N.

(2S,5S,11bR,1'S)-2-[N^α-(tert-Butoxycarbonyl)-D-tryptophyl]amino]-5-[(1'-methoxycarbonyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (5b). Yield: 76% (from **3a** and Boc-D-Trp-OH, method A) as a foam. Eluent: 3% of MeOH in CH₂Cl₂. HPLC: *t*_R = 16.20 min (A:B = 45/55). Anal. (C₄₁H₄₄N₆O₇) C, H, N.

(2S,5S,11bR,1'R)-2-[N^α-(tert-Butoxycarbonyl)-L-tryptophyl]amino]-5-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (6c). Yield: 70% (from **3b** and Boc-L-Trp-OH, method A) as a foam. Eluent: gradient from 10 to 50% of acetone in hexane. HPLC: *t*_R = 5.08 min (A:B = 45/55). Anal. (C₄₀H₄₃N₇O₆) C, H, N.

(2S,5S,11bR,1'R)-2-[N^α-(tert-Butoxycarbonyl)-D-tryptophyl]amino]-5-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (6d). Yield: 70% (from **3b** and Boc-D-Trp-OH, method A) as a foam. Eluent: gradient from 10 to 50% of acetone in hexane. HPLC: *t*_R = 5.10 min (A:B = 45/55). Anal. (C₄₀H₄₃N₇O₆) C, H, N.

(2R,5R,11bS,1'S)-2-[N^α-(tert-Butoxycarbonyl)-L-tryptophyl]amino]-5-[(1'-methoxycarbonyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (7a). Yield: 71% (from **4a** and Boc-L-Trp-OH, method B) as a foam. Eluent: 2% of CH₂Cl₂ in MeOH. HPLC: *t*_R = 16.16 min (A:B = 45/55). Anal. (C₄₁H₄₄N₆O₇) C, H, N.

(2R,5R,11bS,1'S)-2-[N^α-(tert-Butoxycarbonyl)-D-tryptophyl]amino]-5-[(1'-methoxycarbonyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (7b). Yield: 41% (from **4a** and Boc-D-Trp-OH, method B) as a foam. Eluent: 2% of CH₂Cl₂ in MeOH. HPLC: *t*_R = 14.76 min (A:B = 45/55). Anal. (C₄₁H₄₄N₆O₇) C, H, N.

(2R,5R,11bS,1'R)-2-[N^α-(tert-Butoxycarbonyl)-L-tryptophyl]amino]-5-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (8c). Yield: 72% (from **4b** and Boc-L-Trp, method B). Eluent: gradient from 15 to 50% of acetone in hexane. HPLC: *t*_R = 10.33 min (A:B = 40/60). Anal. (C₄₀H₄₃N₇O₆) C, H, N.

(2R,5R,11bS,1'R)-2-[N^α-(tert-Butoxycarbonyl)-D-tryptophyl]amino]-5-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (8d). Yield: 35% (from **4b** and Boc-D-Trp, method B). Eluent: gradient from 15 to 67% of acetone in hexane. HPLC: *t*_R = 11.60 min (A:B = 40/60). Anal. (C₄₀H₄₃N₇O₆) C, H, N.

Preparation of C-Terminal Carbamoyl Derivatives. A solution of the corresponding 1'-methoxycarbonyl derivative (0.04 mmol) in MeOH saturated with NH₃ (10 mL) was stirred at room temperature for 2 days. After evaporation to dryness, the resulting residue was purified on a silica gel column using a gradient from 1 to 4% of MeOH in CH₂Cl₂.

(2S,5S,11bR,1'S)-2-[N^α-(tert-Butoxycarbonyl)-L-tryptophyl]amino]-5-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (6a). Yield: 97% (from **5a**) as a foam. HPLC: *t*_R = 11.60 min (A:B = 40/60). Anal. (C₄₀H₄₃N₇O₆) C, H, N.

(2S,5S,11bR,1'S)-2-[N^α-(tert-Butoxycarbonyl)-D-tryptophyl]amino]-5-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (6b). Yield: 65% (from **5b**) as a foam. HPLC: *t*_R = 10.33 min (A:B = 40/60). Anal. (C₄₀H₄₃N₇O₆) C, H, N.

(2R,5R,11bS,1'S)-2-[N^α-(tert-Butoxycarbonyl)-L-tryptophyl]amino]-5-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (8a). Yield: 95% (from **7a**) as a foam. HPLC: *t*_R = 9.52 min (A:B = 40/60). Anal. (C₄₀H₄₃N₇O₆) C, H, N.

(2R,5R,11bS,1'S)-2-[N^α-(tert-Butoxycarbonyl)-D-tryptophyl]amino]-5-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-3-oxo-2,3,5,6,11,11b-hexahydro-1H-indolizino[8,7-b]indole (8b). Yield: 72% (from **7b**) as a foam. HPLC: *t*_R = 9.80 min (A:B = 40/60). Anal. (C₄₀H₄₃N₇O₆) C, H, N.

Coupling of Fmoc-BTD-OH with H-Phe-NH₂. To a solution of Fmoc-BTD-OH (300 mg, 0.68 mmol) in dry CH₂Cl₂ (18 mL) were added successively H-L-Phe-NH₂ or H-D-Phe-NH₂ (146 mg, 0.89 mmol), BOP (393.3 mg, 0.89 mmol), and TEA (0.12 mL, 0.89 mmol). After stirring overnight the solvent was evaporated and the workup continues as in the coupling of IBTM derivatives (method A).

(3S,6S,9R,1'S)-3-[9'-Fluoromethyloxycarbonyl]-9-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (9a). Yield: 95% as a foam. Eluent: 3% of CH₂Cl₂ in MeOH. HPLC: *t*_R = 3.26 min (A:B = 30/70). Anal. (C₃₂H₃₂N₄O₅S) C, H, N, S.

(3S,6S,9R,1'R)-3-[9'-Fluoromethyloxycarbonyl]-9-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (9b). Yield: 90% as a foam. Eluent: 2% of CH₂Cl₂ in MeOH. HPLC: *t*_R = 11.20 min (A:B = 40/60). Anal. (C₃₂H₃₂N₄O₅S) C, H, N, S.

Coupling of H-BTD-Phe-NH₂ with Boc-Trp-OH. To a solution of the corresponding Fmoc derivative **9** (111 mg, 0.19 mmol) in THF (10 mL) was added piperidine (0.037 mL, 0.38 mmol). After stirring for 2 h, the solvent was evaporated to dryness and the residue precipitated from Et₂O. The resulting

residue was dissolved in dry CH_2Cl_2 (6 mL), and Boc-L- or Boc-D-Trp-OH (76.5 mg, 0.25 mmol), BOP (111 mg, 0.25 mmol), and TEA (0.034 mL, 0.25 mmol) were successively added. After stirring overnight, the solvent was evaporated, and the residue was treated as in the coupling of IBTM derivatives with Trp (method A).

(3S,6S,9R,1'S)-3-[(N^α-(tert-Butoxycarbonyl)-L-tryptophyl)amino]-9-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (10a). Yield: 74% (from **9a**) as a foam. Eluent: gradient from 1 to 10% of CH_2Cl_2 in MeOH. HPLC: t_R = 8.88 min (A:B = 40/60). Anal. ($\text{C}_{33}\text{H}_{40}\text{N}_6\text{O}_6\text{S}$) C, H, N, S.

(3S,6S,9R,1'S)-3-[(N^α-(tert-Butoxycarbonyl)-D-tryptophyl)amino]-9-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (10b). Yield: 86% (from **9a**) as a foam. Eluent: 1 to 5% of CH_2Cl_2 in MeOH. HPLC: t_R = 6.96 min (A:B = 40/60). Anal. ($\text{C}_{33}\text{H}_{40}\text{N}_6\text{O}_6\text{S}$) C, H, N, S.

(3S,6S,9R,1'R)-3-[(N^α-(tert-Butoxycarbonyl)-L-tryptophyl)amino]-9-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (10c). Yield: 82% (from **9b**) as a foam. Eluent: gradient from 10 to 50% of CH_2Cl_2 in MeOH. HPLC: t_R = 4.86 min (A:B = 40/60). Anal. ($\text{C}_{33}\text{H}_{40}\text{N}_6\text{O}_6\text{S}$) C, H, N, S.

(3S,6S,9R,1'R)-3-[(N^α-(tert-Butoxycarbonyl)-D-tryptophyl)amino]-9-[(1'-carbamoyl-2'-phenyl)ethyl]carbamoyl-2-oxo-7-thio-1-azabicyclo[4.3.0]nonane (10d). Yield: 75% (from **9b**) as a foam. Eluent: gradient from 10 to 50% of CH_2Cl_2 in MeOH. HPLC: t_R = 4.16 min (A:B = 40/60). Anal. ($\text{C}_{33}\text{H}_{40}\text{N}_6\text{O}_6\text{S}$) C, H, N, S.

Binding Assays. CCK₁ and CCK₂ receptor binding assays were performed using rat pancreas and cerebral cortex homogenates, respectively, according to the method previously described.³⁹

Amylase Release. Dispersed rat pancreatic acini were prepared by using a modification of the technique of Jensen et al.⁴⁰ A rat was decapitated and the pancreas was carefully cleaned. Tissue was injected three times with 5 mL of a solution of collagenase (Worthington) at a concentration of 70 U/mL (in mix buffer) and subjected to the digestion step consisting of three 10-min incubations at 37 °C in an atmosphere of pure O₂ and with agitation (200 cycles/min). The tissue was washed twice in mix buffer (composition: 98 mM NaCl, 6 mM KCl, 2.5 mM NaH₂PO₄, 0.5 mM CaCl₂, 5 mM theophylline, 11.4 mM glucose, 2 mM L-glutamine, 5 mM L-glutamic acid, 5 mM fumaric acid, 5 mM piruvic acid, 0.01% SBTI, 1% essential amino acid mixture, 1% essential vitamin mixture, pH 7.4) after each incubation. The tissue obtained after the last incubation was transferred to stop buffer (4% BSA) and shaken vigorously for 10 min. The homogenate was centrifuged twice (100g, 1 min, 4 °C); the pellet obtained in the first centrifugation was resuspended in wash buffer (0.2% BSA) and the final pellet was resuspended in incubation buffer (1% BSA). Cells were allowed to rest for 30 min and then they were centrifuged (100g, 1 min, 4 °C) and resuspended again in incubation buffer.

Amylase release was measured using the procedure of Peikin et al.⁴¹ Samples (2 mL) of the acini suspension were placed in plastic tubes and incubated for 30 min at 37 °C in an atmosphere of pure O₂ with agitation (70 cycles/min). Amylase activity was determined using the amyl kit reagent (Boehringer Mannheim). Release (S) was calculated as the percentage of amylase in the acini that was released into extracellular medium during the incubation period. The percentage of inhibition of amylase release by drugs elicited by a fixed CCK8 concentration (0.1 nM) was calculated according to the formula $\% I = [(S_{\text{CCK}} - S_C) - (S_T - S_C)] / (S_{\text{CCK}} - S_C) \times 100$, where S_C is the control release (vehicle), S_{CCK} is release elicited by CCK8, and S_T is release in the presence of increasing drug concentrations. IC₅₀ values were calculated for the drug tested.

Molecular Modeling Studies. Conformational Search. All calculations were run on an SGI workstation (Fuel, RP14000, 500 MB RAM) under an Irix 6.5 operating system.

The initial conformation of the IBTM derivatives **8a–d** were built using the library of fragments available in the molecular modeling program Insight II (version 2000.1, Biosym Technologies, San Diego, CA). The calculations were carried out within the molecular mechanics using the AMBER force field implemented in DISCOVER. They were conducted under vacuum with a distance dependent dielectric constant (4r) and a cutoff of 14 Å. In the simulations, a force constant of 500 kcal/mol/Å was used to restrain the amide plane in a trans configuration. The structure is heated to 1000 K and equilibrated during 10 ps. The structure is then cooled slowly to 300 K in steps; in each step the temperature was lowered by 100 K, and the system was allowed to stay at the new temperature for 100 ps. After cooling to 300 K, the final conformation obtained is energy-refined using a conjugated gradient algorithm with a final gradient of 0.001 kcal/mol as the convergence criteria. The conformer is stored and used to start a new simulation at high temperature. This procedure produced samples of 100 energy-minimized conformations, which were compared with each other to eliminate the identical ones. The protocol was run three times, employing different starting structures for derivatives **8a–d**. Independent of the starting structure, the percentage of the different families of conformers where equivalent.

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Supporting Information Available: ¹H NMR data and elemental analysis for all new compounds (**4–10**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Crawley, J. N.; Corwin, R. L. Biological actions of cholecystokinin. *Peptides* **1994**, *15*, 731–755.
- (2) Wank, S. A. G Protein-coupled receptors in gastrointestinal physiology I. CCK receptors: An exemplary family. *Am. J. Physiol.* **1998**, *37*, G607–G613.
- (3) Itoh, S.; Lal, H. Influences of cholecystokinin and analogues on memory processes. *Drug Dev. Res.* **1990**, *21*, 257–276.
- (4) Harro, J.; Vasar, E.; Bradwejn, J. CCK in Animal and human research on anxiety. *TIPS* **1993**, *14*, 244–249.
- (5) Dauge, V.; Lena, I. CCK in Anxiety and cognitive processes. *Neurosci. Biobehav. Rev.* **1998**, *22*, 815–825.
- (6) Griebel, G. Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders? *Pharmacol. Ther.* **1999**, *82*, 1–61.
- (7) Ritter, R. C.; Covasa, M.; Matson, C. A. Cholecystokinin: Proofs and prospects for involvement in control of food intake and body weight. *Neuropeptides* **1999**, *33*, 387–399.
- (8) Wank, S. A. Cholecystokinin receptors. *Am. J. Physiol.* **1995**, *269*, G628–G664.
- (9) Schalling, M.; Friberg, K.; Seroogy, K.; Riederer, P.; Bird, E.; Schifffmann, S. N.; Mailloux, P.; Vanderhaeghen, J. J.; Kuga, S.; Goldstein, M.; Kitahama, K.; Luppi, P. H.; Jouviet, M.; Hokfelt, T. Analysis of expression of cholecystokinin dopamine cells in the ventral mesencephalon of several species and in humans with schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8427–8431.
- (10) Dunlop, J. CCK receptor antagonists. *Gen Pharmacol.* **1998**, *31*, 519–524.
- (11) De Tullio, P.; Delarge, J.; Pirotte, B. Recent advances in the chemistry of cholecystokinin ligands (agonists and antagonists). *Curr. Med. Chem.* **1999**, *6*, 433–455.
- (12) Chambers, M. S.; Fletcher, S. R. CCK–B Antagonists in the control of anxiety and gastric acid secretion. In *Progress Medicinal Chemistry*; King, F. D., Oxford, A. W., Eds; Elsevier Science B. V.: Amsterdam, 2000; Vol. 37, pp 45–81.
- (13) Herranz, R. Cholecystokinin antagonists: Pharmacological and therapeutic potential. *Med. Res. Rev.* **2003**, *23*, 559–605.
- (14) Martínez, J. Gastrointestinal regulatory peptide receptors. In *Comprehensive Medicinal Chemistry*; Emmett, J. C., Ed.; Pergamon Press: Oxford, 1990; Vol. 3, pp 929–939.
- (15) Rolland, M.; Rodriguez, M.; Lignon, M.-F.; Gabas, M.-C.; Laur, J.; Aumelas, A.; Martínez, J. Synthesis and biological activity of 2-phenylethyl ester modified in Trp³⁰ region. *Int. J. Protein Res.* **1991**, *38*, 181–192.

- (16) Low, C. M. R.; Black, J. W.; Broughton, H. B.; Buck, I. M.; Davies, J. M. R.; Dunstone, D. J.; Hull, R. A. D.; Kalindjian, S. B.; McDonald, I. M.; Pether, M. J.; Shankley, N. P.; Steel, K. I. M. Development of peptide 3D structure mimetics: Rational design of novel peptid cholecystokinin receptor antagonists. *J. Med. Chem.* **2000**, *43*, 3505–3517.
- (17) Horwell, D. C.; Beeby, A.; Clark, C. R.; Hughes, J. Synthesis and binding affinities of analogues of cholecystokinin-(30–33) as probes for central nervous system cholecystokinin receptors. *J. Med. Chem.* **1987**, *30*, 729–732.
- (18) (a) Higginbottom, M.; Horwell, D. C.; Roberts, E. Selective ligands for cholecystokinin receptor subtypes CCK-A and CCK-B within a single structural class. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 881–884. (b) Boden, P. R.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; Hughes, J.; Rees, D. C.; Roberts, E.; Singh, L.; Suman-Chauhan, N.; Woodruff, G. N. Cholecystokinin dipeptid antagonists: Design, synthesis, and anxiolytic profile of some novel CCK-A and CCK-B selective and mixed CCK-A/CCK-B antagonists. *J. Med. Chem.* **1993**, *36*, 552–565. (c) Boden, P. R.; Eden, J. M.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; Hunter, J. C.; Martin, K.; Pritchard, M. C.; Richardson, M. C.; Roberts, E. Rational designed dipeptid analogues of cholecystokinin (CCK): C-Terminal structure–activity relationships of α -methyl tryptophan derivatives. *Eur. J. Med. Chem.* **1993**, *28*, 47–61. (d) Padia, J. K.; Bolton, G. L.; Hill, D.; Horwell, D. C.; Roth, B. D.; Trivedi, B. K. Synthesis and SAR studies of novel CCK–B antagonists. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2805–2810. (e) Blommaert, A. G. S.; Weng, J.-H.; Dorville, A.; McCort, I.; Ducos, B.; Durieux, C.; Roques, B. P. Cholecystokinin peptidomimetics as selective CCK–B antagonists: Design, synthesis and in vitro and in vivo biochemical properties. *J. Med. Chem.* **1993**, *36*, 2868–2877. (f) Trivedi, B. K.; Padia, J. K.; Holmes, A.; Rose, S.; Wright, D. S.; Hinton, J. P.; Pritchard, M. C.; Eden, J. M.; Kneen, C.; Webdale, L.; Suman-Chauhan, N.; Boden, P.; Singh, L.; Field, M. J.; Hill, D. Second generation peptid CCK–B receptor antagonists: Identification and development of N-(adamantylloxycarbonyl)- α -methyl-(R)-tryptophan derivative (CI-1015) with an improved pharmacokinetic profile. *J. Med. Chem.* **1998**, *41*, 38–45.
- (19) De la Figuera, N.; Alkorta, I.; García-López, M. T.; Herranz, R.; González-Muñiz, R. 2-Amino-3-oxohexahydroindolizino[8,7-b]-indole-5-carboxylate derivatives as new scaffolds for mimicking β -turn secondary structures. Molecular dynamics and stereoselective synthesis. *Tetrahedron* **1995**, *51*, 7841–7856.
- (20) Andreu, D.; Ruiz, S.; Carreño, C.; Alsina, J.; Albericio, F.; Jiménez, M. A.; De la Figuera, N.; Herranz, R.; García-López, M. T.; González-Muñiz, R. IBTM-Containing gramicidin S analogues: Evidence for IBTM as a suitable type II' β -turn mimetic. *J. Am. Chem. Soc.* **1997**, *119*, 10579–10586.
- (21) De la Figuera, N.; Martín-Martínez, M.; Herranz, R.; García-López, M. T.; Latorre, M.; Cenarruzabeitia, E.; Del Río, J.; González-Muñiz, R. Highly Constrained dipeptid analogues containing a type II' β -turn mimic as novel and selective CCK-A receptor ligands. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 43–48.
- (22) Martín-Martínez, M.; De la Figuera, N.; Latorre, M.; Herranz, R.; García-López, M. T.; Cenarruzabeitia, E.; Del Río, J.; González-Muñiz, R. β -Turned dipeptid as potent and selective CCK₁ receptor antagonists. *J. Med. Chem.* **2000**, *43*, 3770–3777.
- (23) Martín-Martínez, M.; Latorre, M.; García-López, M. T.; Cenarruzabeitia, E.; Del Río, J.; González-Muñiz, R. Effects of the incorporation of IBTM β -turn mimetics into the dipeptid CCK₁ receptor agonists PD 170292. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 109–112.
- (24) Shiosaki, K.; Craig, R.; Lin, C. W.; Barrett, R. W.; Miller, T.; Witte, D.; Wolfram, C. A. W.; Nadzan, A. M. Torward development of peptidomimetics: Diketopiperazine templates for the Trp-Met segment of CCK-4. In *Peptides, Chemistry, Structure and Biology (Proceedings of the 11th American Peptide Symposium)*. Rivier, J. E., Marshall, G. R., Eds.; ESCOM: Leiden, 1990, pp 978–980.
- (25) Flynn, D. L.; Villamil, C. I.; Becker, D. P.; Gullikson, G. W.; Moumni, C.; Yang, D. C. 1,3,4-Trisubstituted pyrrolidinones as scaffolds for construction of peptidomimetic cholecystokinin antagonists. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1251–1256.
- (26) Holladay, M. W.; Bennett, M. J.; Bai, H.; Ralston, J. W.; Kerwin, J. F., Jr.; Stashko, M.; Miller, T. R.; O'Neil, A. B.; Nadzan, A. M.; Brioni, J.; Lin, C. W. Amino acid-derived piperidines as novel CCK_B ligands with anxiolytic-like properties. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 3057–3062.
- (27) Kalindjian, S. B.; Buck, I. M.; Cushnir, J. R.; Dunstone, D. J.; Hudson, M. L.; Low, C. M. R.; McDonald, I. M.; Pether, M. J.; Steel, K. I. M.; Tozer, M. J. Improving the affinity and selectivity of a nonpeptide series of cholecystokinin-B/gastric receptor antagonists based on the dibenzobicyclo[2.2.2]octane skeleton. *J. Med. Chem.* **1995**, *38*, 4294–4302.
- (28) González-Muñiz, R.; Domínguez, M. J.; Martín-Martínez, M.; Herranz, R.; García-López, M. T.; Barber, A.; Ballaz, S.; Del Río, J. CCK-4 restricted analogues containing a 3-oxoindolizidine skeleton. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 967–972.
- (29) Martín-Martínez, M.; Bartolomé-Nebreda, J. M.; Gómez-Monterrey, I.; González-Muñiz, R.; García-López, M. T.; Ballaz, S.; Barber, A.; Fortuño, A.; Del Río, J.; Herranz, R. Synthesis and stereochemical structure–activity relationships of 1,3-dioxopyridino[1,2-c]pyrimidine derivatives: Potent and selective cholecystokinin-A receptor antagonists. *J. Med. Chem.* **1997**, *40*, 3402–3407.
- (30) Kolodziej, S. A.; Nikiforovich, G. V.; Skeeane, R.; Lignon, M.-F.; Martínez, J.; Marshall, G. R. Ac-[3- and 4- Alkylthioprolinyl]-CCK4 analogs: Synthesis and implications for the CCK-B receptor-bound conformation. *J. Med. Chem.* **1995**, *38*, 137–149.
- (31) (a) Fournie-Zaluski, M. C.; Belleney, J.; Lux, B.; Durieux, C.; Gerard, D.; Maigret, B.; Roques, B. P. Conformational analysis of cholecystokinin CCK_{26–33} and related fragments by ¹H NMR spectroscopy, fluorescence-transfer measurements and calculations. *Biochemistry* **1986**, *25*, 3778–3787. (b) Kreissler, M.; Pesquer, M.; Maigret, B.; Fournie-Zaluski, M. C.; Roques, B. P. Computer simulation of the conformational behavior of cholecystokinin fragments: Conformational families of sulfated CCK8. *J. Computer-Aided Mol. Des.* **1989**, *3*, 85–94.
- (32) Nikiforovich, G.; Hruby, V. J. Models for the A- and B-receptor-bound conformations of CCK-8. *J. Biochem. Biophys. Res. Commun.* **1993**, *194*, 9–16.
- (33) Hurby, V. J.; Fang, S.; Knapp, R.; Kazmierski, W.; Lui, G. K.; Yamamura, H. I. Cholecystokinin analogues with high affinity and selectivity for brain membrane receptors. *Int. J. Pept. Protein Res.* **1990**, *35*, 566–573.
- (34) Nagai, U.; Sato, K. Synthesis of bicyclic dipeptide with the shape of β -turn-central part. *Tetrahedron Lett.* **1985**, *5*, 647–650.
- (35) Sato, K.; Nagai, U. Synthesis and antibiotic activity of a gramicidin S analogue containing bicyclic β -turn dipeptides. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1231–1234.
- (36) Bach, A. C., II; Markwalder, J. A.; Ripka, W. C. Synthesis and NMR conformational analysis of a β -turn mimic incorporated into gramicidin S. *Int. J. Pept. Protein Res.* **1991**, *38*, 314–323.
- (37) Kessler, H. Conformation and biological activity of cyclic peptides. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512–523.
- (38) Meyer, E. A.; Castellano, R. K.; Diederich, F. Interaction with aromatic rings in chemical and biological recognition. *Angew. Chem., Int. Ed.* **2003**, *42*, 1211–1250.
- (39) Ballaz, S.; Barber, A.; Fortuño, A.; Del Río, J.; Martín-Martínez, M.; Gómez-Monterrey, I.; Herranz, R.; González-Muñiz, R.; García-López, M. T. Pharmacological evaluation of IQM-95,333, a highly selective CCK-A receptor antagonist with anxiolytic-like activity in animal models. *Br. J. Pharmacol.* **1997**, *121*, 759–767.
- (40) Jensen, T. R.; Lemp, G. F.; Gardner, J. D. Interactions of COOH-terminal fragments of cholecystokinin with receptors on dispersed acini from guinea pig pancreas. *J. Biol. Chem.* **1982**, *257*, 5554–5559.
- (41) Peikin, S. R.; Rottman, A. J.; Batzri, S.; Gardner, J. D. Kinetics of amylase release by dispersed acini prepared from guinea pig pancreas. *Am. J. Physiol.* **1978**, *235*, E743–E749.

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