"Dipeptoids": From the Chemical Structure of the Endogenous Peptide to the Design of Peptidomimetics

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Abstract: The present review details the rational multi-step process followed for the discovery of a family of non-peptide CCK receptor ligands ("dipeptoids"), starting from the structure of the endogenous peptide, CCK_8. Emphasis will be made on the *N*- and *C*-terminal modifications, on the singular effects of the stereochemical changes and the incorporation of conformational constraints into the structure of "dipeptoids", and on the modifications directed to improve the pharmacological profile of these compounds to afford valuable clinical candidates.

Keywords: Dipeptoids, cholecystokinin, peptidomimetics, rational design.

1. INTRODUCTION

Peptide hormones and neurotransmitters mediate a wide variety of biological processes, which suggest a tremendous potential in the development of new therapeutic agents. Although, in some cases, peptides or peptide analogues have proven to be suitable drug molecules [1], in general, peptide-based structures are not appropriate for use in therapy [2], due to poor oral bioavailability, lack of receptor subtype selectivity and high metabolic inestability. For these reasons, considerable efforts have been devoted to the search for peptidomimetics [3] or non-peptide small molecules with improved pharmacokinetic and pharmacodynamic profiles, able to bind to peptide receptors and capable of mimicking or antagonizing the biological effects of the parent peptide. The discovery of peptidomimetics can be undertaken by two basic strategies: empirical approaches based on screening of a large number of compounds and rational design from the chemical structure of the endogenous peptide.

This review will focus on a family of Cholecystokinin (CCK) receptors ligands called "dipeptoids", designed from the natural ligand CCK-8. The development of these compounds represents a good example of the steps needed for the rational design of peptidomimetics.

While it is beyond the scope of this review to provide a thoroughly report on CCK, some general remarks about its biological functions will be of interest for a better comprehension of the review. CCK is a gastrointestinal hormone and a neurotransmitter/neuromodulator that exists in multiple biologically active forms (CCK-58, CCK-39, CCK-33, CCK-8 and CCK-4) [4]. CCK actions are mediated by at least two distinct receptors subtypes, CCK_1 and CCK_2 receptors, belonging to the seven transmembrane G protein-coupled receptors superfamily [5-7]. An effective binding at CCK_1 receptors requires the sulphated octapeptide CCK-8, while the sulphate moiety is not needed for the interaction with CCK_2 receptors, for which the tetrapeptide CCK-4 is the minimum required sequence for binding and agonist activity. CCK receptors are widely distributed in the periphery and in the central nervous system (CNS), with CCK_1 receptor subtype predominating in the periphery, especially in the gastrointestinal tract, and CCK_2 receptors located mainly in the CNS. At the gastrointestinal level, CCK_1 receptors mediate pancreatic enzyme secretion, gallbladder and ileum contraction [6,8], whereas CCK_2 receptors regulate gastric-acid secretion [9]. At the central and peripheral nervous system CCK participates, through its action at CCK_1 and/or CCK_2 receptors [6,8,10], in the modulation of analgesia [11], anxiety [12-14], satiety [15,16], pain [17], memory processes [14,18] and dopamine-mediated behaviour [19,20].

As CCK is involved in different and important biological activities, the therapeutic potential of CCK receptor ligands seems to be broad and promising. Possible clinical applications concern the treatment of brain disorders (schizophrenia [21], Parkinson [22]) and/or pain [17], with CCK_2 receptor ligands, and of diseases involving food consumption [23], with CCK_1 receptor agonist or antagonist. These prospective therapeutic uses have prompted an intensive research in the area of CCK, and several potent and selective non-peptide CCK_1 and CCK_2 ligands, coming both from screening processes or rational design, have been reported [24-28]. This review will focus on a family of CCK receptor ligands, called "dipeptoids", mainly developed by Horwell's group at Parke Davis (now Pfizer) using rational criteria. Horwell uses the word "peptoid" in the spirit of Ariëns and Farmer [29], to describe monomeric non-peptide species able to mimic the three-dimensional display of the side-chain of key amino acids of the parent peptide. Attention has not only been devoted to enhance the affinity and selectivity, but also to improve the bioavailability of the designed derivatives, in order that they might be suitable for clinical development.

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1389-5575/04 $45.00+.00

2. "DIPEPTOID" CCK RECEPTOR ANTAGONISTS

The strategy followed to develop the "dipeptoid" CCK ligands [30,31] is a good illustration of the multi-step process applied to the discovery of peptidomimetics starting from the structure of the endogenous peptide [32,33]. The first step is the identification of the minimum fragment required for affinity and/or activity [34]. Once, the minimum active fragment is known, the importance of amide-bonds and side-chains is investigated. Subsequently, the insertion of conformational restrictions within the peptide backbone may provide information regarding the bioactive conformation. In this sense, the incorporation of mimetics of a particular secondary structure allows to enforce a determined peptidic backbone conformation [35,36]. Besides, the incorporation of conformational constraints if chosen properly, may lead to derivatives with increased affinity and/or selectivity.

2.1. Minimum Active Fragment and Preliminary Structure-Activity Relationships

To identify the minimum active fragment needed for interacting with CCK_2 receptors, Horwell *et al.* prepared a series of continuous and non-continuous fragments of the endogenous ligand, CCK_{26-33} **1**, ranging from 8 to 2 amino acid residues. From this first study, dipeptide **2** (Table 1) [37], a non-continuous fragment, was found to retain pentamolar affinity at CCK_2 receptors, suggesting a clear importance of the aromatic side-chains of Trp and Phe residues as key binding moieties. Subsequently, a series of α-MeTrp-Phe "dipeptoids" and their corresponding arylethylamino analogues were prepared (compounds **3-5**, Table 1) [38]. While the α-Me-Trp moiety was initially chosen because the α-substituent helps to stabilize the peptide bond against acid and enzymatic degradation *in vivo* [39], the slight increase in CCK_2 affinity showed by compounds **3-5** seems to indicate that the constrain imposed by the α-methyl group may serve to increase the population of conformations that are recognized by CCK_2 receptors.

Table 1. CCK_2 Receptor Binding Affinities of CCK-8 and Non-Continuous Fragments

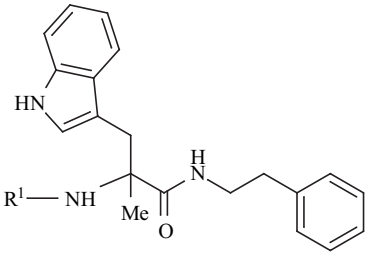
Compd.		Ki (μM)[a]
1	Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	0.003
2	Boc-Trp-Phe-NH_2	73
3	Boc-MeTrp-Phe-NH_2	67
4	Boc-D-MeTrp-Phe-NH_2	35
5	Boc-(D,L)MeTrp-NH-$(CH_2)_2$-Ph	12

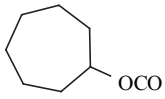
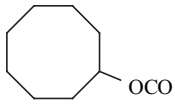
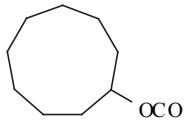
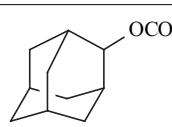
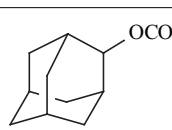
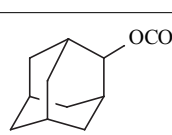
[a] Affinities are expressed as Ki values of the displacement of [^{3}H]-Boc-β-alanyl-CCK_{30-33} (pentagastrin) from mouse cerebral cortex.

Using compound **5** as the starting point, the next action was the independent optimization of the *N*- and *C*-terminal parts of the molecule. It was rationalized that the binding energies of the *N*- and *C*-terminal groups would likely be additive, as these molecules are only semi-rigid, and hence would allow the aromatic side-chains to explore and find their energy minima at the CCK_2 receptor. This assumption was justified using a Free-Wilson/Fujita-Ban (FW/FB) analysis [40]. The optimization process involved a successive series of *N*-SAR, *C*-SAR, *N*-SAR… studies, in which the group that had proven better in the previous study was kept in the following one.

The optimization of the *N*-terminal group was achieved in a stepwise manner. First, it was explored the replacement of the Boc group by simple alkyl and aryl carbamates, amides and ureas. Affinity was retained when the Boc group was replaced by other bulky branched groups [38], being the urethane moiety the most favourable linking group. Then, several cycloalkyl urethanes were prepared to establish the influence of ring size and lipophilicity on the affinity and selectivity at CCK_2 receptors. As shown in Table 2,

Table 2. CCK Receptors Binding Affinities of Modified *N*-Terminal Analogues



Compd.	R^1	α-Trp	IC_{50} (nM)	
			CCK_1	CCK_2
6		*RS*	ND	192
7		*RS*	ND	125
8		*RS*	ND	85
9		*RS*	ND	48
9a		*R*	650	32
9b		*S*	620	330

[a] IC_{50} represents the concentration producing half-maximal inhibition of specific [^{125}I]-labelled CCK-8S binding to CCK receptors in the rat pancreas (CCK_1) or the mouse cerebral cortex (CCK_2).

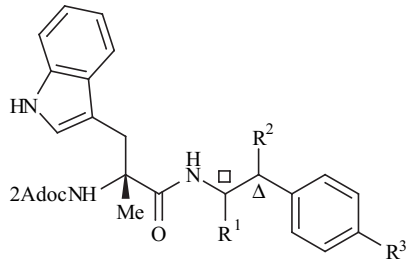
carbamate derivatives with ring sizes between 7 and 9 carbons, as compounds **6-8**, were preferred for CCK_2 affinity [41]. Finally, these rings were replaced by carbocycles containing two or more rings to ascertain the effect of "shape" on binding. The 2-adamantyloxycarbonyl (2-Adoc) group turned out to be the optimal one (compound **9**)[42]. Subsequent studies showed that not all the carbon atoms of the adamantine cage were employed in CCK_2 receptor binding [43]. Thus, derivative **26** (Table 6) with a (2-methyl)cyclohexyloxylcarbonyl group instead of the 2-Adoc group showed nanomolar affinity at CCK_2 receptors, although it was less selective than the corresponding 2-Adoc analogue. It is also worth mentioning that the *R*-configured Trp derivative **9a** has shown higher affinity than the *S*-Trp analogue **9b** (Table 2). As it will be discussed, the stereochemistry of the "dipeptoid" derivatives plays an important role in the receptor affinity and selectivity within this family of CCK receptor ligands.

In summary, the *N*-terminal SAR studies showed the requirement for a bulky and lipophilic *N*-terminal group, and that size, shape and lipophility may be important for "docking" this part of the molecule into a lipophilic pocket of the receptor. The 2-Adoc group imparted the best CCK_2 receptor binding affinity and selectivity, and was therefore the selected *N*-terminal group for ulterior modifications.

Regarding *C*-terminus, the most successful strategy was the incorporation of mobile chains with terminal COOH groups. The acid moiety was introduced as a topographical mimic of the Asp^{32} side-chain of CCK, to serve as an accessory binding group. On the basis of this rationale, a series of analogues with the carboxylic acid appended either directly or through spacers to the α or β position of the phenylethyl amide moiety of compound **9a** were prepared. These modifications resulted in a series of compounds with improved CCK_2 affinity, in the nanomolar range (compounds **10-12**, and **14-16**, Table 3) [42,44]. The study on the optimal orientation of the COOH chain showed that, in the α-phenylethylamide series, the *S* configuration was required at the new asymmetric center, and a distance from the phenethylamide backbone to the COOH group of 6.7-8.9 Å, whereas in the β-phenylethylamide series the *R* configuration and a distance of 4.3-7.3 Å proved to be the best possible [42]. Having established the importance of the carboxylic moiety, further studies were directed to replace the COOH group by several mimics. In general, all these modifications showed that the acid moiety should be planar and have a charge distribution similar to that of the carboxylic acid [45,46].

Due to the encouraging biological results found with derivative **15** (CI-988) and related analogues, a thoroughly exploration of the phenyl and indole rings was also investigated. Regarding the phenyl ring at *C*-terminus, different substituents were appended, at multiple ring positions, to incorporate systematic variation in lipophilic, electronic, steric and hydrogen bonding properties of the parent "dipeptoid" [47]. The subsequent QSAR analysis revealed that CCK_2 receptor affinity was governed by the overall size of the phenyl ring (small substituents were associated with increased affinity), and marginally by lipophilicity. These studies provided derivative **17** that showed an extraordinary high affinity and good selectivity at CCK_2 receptors.

Table 3. CCK Receptor Binding Affinities of Carboxylate-Containing *C*-Terminal "Dipeptoid" Analogues



Compd.	R^1	R^2	R^3	❒ Δ	IC_{50} (nM)[a]		CCK_1/CCK_2
					CCK_1	CCK_2	
10	CO_2H	H	H	*S*	120	39	3
11	CH_2CO_2H	H	H	*S*	25	0.15	167
12	$CH_2NHCO(CH_2)_2CO_2H$	H	H	*S*	950	4.2	226
13	CH_2OH	H	H	*S*	780	6.3	123
14	H	$NHCOCH_2CO_2H$	H	*R*	870	0.8	1087
15	H	$NHCO(CH_2)_2CO_2H$	H	*R*	4300	1.7	2529
16	H	$NHCO(CH_2)_3CO_2H$	H	*R*	1300	14	93
17	CH_2CO_2H	H	F	*S*	75	0.08	937

[a] Binding affinities defined in footnote a, Table 2.

Concerning the *N*-terminal indole moiety, several analogues in which this heterocycle was replaced by other aromatic rings were prepared [48, 49]. Several of these compounds, as the ones in which the 3-indolyl moiety was replaced by 2-naphthyl or 5,6,7,8-tetrahydro-2-naphthyl moieties, showed CCK_2 receptor binding values similar to that reported for CI-988 and were highly selective toward CCK_1 receptors.

On the whole, these SAR studies at *N*- and *C*-terminus of "dipeptoids" had succeeded in turning a weak CCK_2 receptor ligand, as the dipeptide Boc-Trp-Phe-NH_2 (**2**), into potent and highly selective CCK_2 "dipeptoid" receptor antagonists, exemplified by compound **15** (CI-988).

2.2. Optimization of the "Dipeptoids" Pharmacokinetic Profile

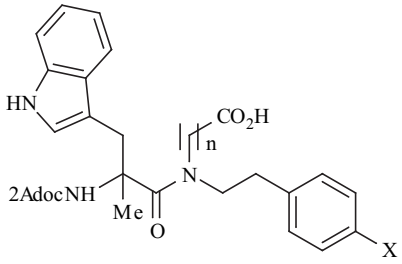
The good CCK_2 affinity and selectivity of the prototype compound in this series, CI-988, prompted the investigation of its pharmacological actions. It was shown that CI-988 could prevent morphine tolerance, thus having potential application for the treatment of chronic pain [50-52]. Regarding the gastric effects, "dipeptoid" **15** caused gastric gland degeneration and mucosal atrophy, being able to inhibit the growth of colonorectal cancer [53,54]. Besides, this compound was also able to inhibit growth of small cells lung cancer [55]. Another interesting activity of CI-988, and the first that was reported, was its anxiolitic profile in established *in vivo* paradigms [56-58], although these finding have not been consistently replicated [59]. Nevertheless, this molecule was developed as a clinical candidate due to its anxiolitic activity, and the lack of several adverse side effects (sedation or ataxia) found in the majority of compounds used to treat anxiety. However, during the preclinical and clinical development, it was determined that its bioavailability was very low (1-3%) [60-63], and that it had partial agonist properties, two facts that led to its final withdrawal. The low bioavailability of CI-988 was attributed to inefficient absorption and to high billiard excretion, in part due to the high molecular weight of this compound (MW = 614).

In order to circumvent these drawbacks several strategies were followed. Firstly, with the aim of increasing the lipophilicity, compounds **18**-**20** (Table 4), in which the mobile side-chain bearing the carboxylic acid functionality is appended directly onto the amide group, were prepared. This would also eliminate the chiral center at the *C*-terminus, and stabilize the backbone amide to base, acid and enzymatic degradation [64-66]. This approach led to compound **20a**, a potent and selective CCK_2 receptor ligand, with improved bioavailability and able to efficiently cross the blood-brain barrier. Thus, derivative **20a**, which has less peptide character than the parent dipeptoid **5a**, is an interesting tool for further investigating the physiopathological function of brain CCK_2 receptors.

Secondly, considering that the amide bond of peptides may constitute a major site for enzymatic intervention, the central amide bond in "dipeptoids" **5**, **13** and **15** was replaced by suitable amide bond replacements [67]. Unfortunately, the incorporation of different peptide bond surrogates led, in general, to marked decrease in CCK_2 affinity [68]. Since there was no correlation between binding affinities and physicochemical properties of the pseudodipeptoid derivatives, it seems that the amide bond does not directly participate in the interaction with the receptor, but a conformational role for appropriate positioning of the aromatic side-chains was presumed.

Another strategy to increase the bioavailability of CI-988 consisted in the reduction of its molecular weight. Since the phenethyl and the acid side-chain moieties have a major

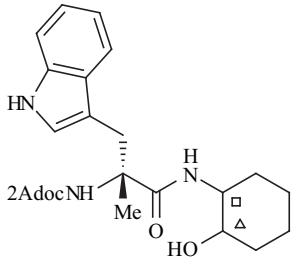
Tabla 4. CCK Receptor Binding Affinities of Derivatives with the Acid Side Chain on the Amide Nitrogen.



Compd.	X	n	Ki[a]		CCK_1/CCK_2
			CCK_1	CCK_2	
5a[b]	H	-	640	32	20
18	H	1	1518	14	108
19	H	2	1323	36	37
20	Cl	1	1129	6.5	174
20a[c]	Cl	1	1060	6.1	173
20b[d]	Cl	1	1039	36	29

[a] Ki values represent the means for determining CCK_1 and CCK_2 affinities on guinea pig pancreas membranas and cortex, respectively. [b] (R) Configuration at the α-Carbon atom, and lacks the acid side-chain on the *N* atom. [c] (R) Configuration at the α-Carbon atom. [d] (S) Configuration at the α-Carbon atom.

Table 5. CCK Receptor Binding Affinities of Second-Generation "Dipeptoids"



Compd.	Config. ❒	Config. Δ	IC_{50} (nM)[a]		1/2 [b]
			CCK_1	CCK_2	
21	*S*	*S*	2900	3.0	967
22	*R*	*R*	1950	14.2	137

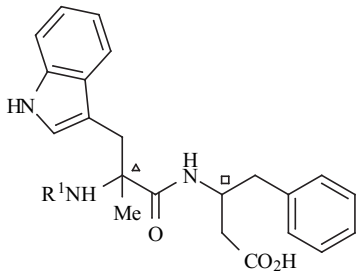
[a] Binding affinities as defined in footnote a, Table 2. [b] CCK_1/CCK_2.

contribution to the molecular weight, and SAR studies suggested that they could be manipulated while maintaining binding affinity, a series of *C*-terminal modified compounds were synthesized. Moreover, considering that analogues with a free carboxyl group were poorly absorbed, it was chosen not to pursue with these series of compounds despite their high affinity. The investigations turned on the simplification of the structure of the hydroxyderivative **13**, a "dipeptoid" with lower selectivity than CI-988 but with a good pharmacological profile. This study led to the discovery of derivatives **21** (CI-1015) and **22** (Table 5) [69,70]. Functional assays showed that compound **21** is a CCK_2 receptor antagonist, with anxiolytic profile. Besides, due to the decrease in molecular weight and absence of any ionizable group, this derivative is endowed with a bioavailability noticeably enhanced relative to CI-988, including an enhanced blood-brain penetration. Thus, on the basis of its overall improved pharmacokinetic profile, CI-1015 was chosen for further preclinical and clinical evaluation for the treatment of anxiety.

2.3. Influence of the Stereochemistry on the Selectivity: CCK_1 Receptor Ligands

In the family of "dipeptoids", not only considerable effort has been devoted to structure-activity relationships of CCK_2 receptors antagonists, but also in the search for new CCK_1 selective receptor ligands. The knowledge that in other series of CCK mimetics a change in the stereochemistry led to a reversion in CCK receptor subtype selectivity, as in the case of benzodiazepine derivatives Devazepide and L-365,260 [71,72], stimulated a series of studies directed to know the influence of the stereochemistry

Table 6. Influence of the Stereochemistry on the Selectivity



Compd.	R^1	Δ	❒	IC_{50} (nM)[a]		1/2 [b]
				CCK_1	CCK_2	
11	2-Adoc	*R*	*S*	25.5	0.15	170
23	2-Adoc	*S*	*S*	539	13.2	41
24	2-Adoc	*S*	*R*	2.8	260	0.01
25	2-Adoc	*R*	*R*	186	9.3	20
26	2-Mchoc[c]	*R*	*S*	18	0.34	53
27	2-Mchoc[c]	*S*	*R*	7.9	1160	0.007

[a] Binding affinities as defined in footnote a, Table 2. [b] CCK_1/CCK_2. [c] (1*R*,2*R*)-*trans*-(2-methyl)cyclohexyloxyl-carbonyl.

of "dipeptoids" on their binding affinity and selectivity at CCK receptors [42,43,73]. The results of these studies showed that by inverting simultaneously the two chiral centres in "dipeptoids" **11** and **26**, these selective CCK_2 receptor antagonists were converted into their enantiomers **24** and **27** (Table 6), respectively, that behave as selective CCK_1 antagonists. These data highlight the sensitivity to absolute configuration of "dipeptoids" in the interactions with CCK receptors, which have to be always considered in the design of new ligands for these receptors.

2.4 Conformationally Constrained "Dipeptoid" Analogues

2.4.1. Local Constraints

One advanced step in the design of peptidomimetics is the use of rigid or semi-rigid ligands, which may provide a deeper insight into the conformational, topographical, and dynamic properties that are critical for molecular recognition and biological activity. These rigid derivatives also help in the development of pharmacophore models from which novel molecules with enhanced receptor affinity and selectivity may be designed [2, 31].

Whilst CI-988 has excellent affinity and selectivity for the CCK_2 receptors, it remains a very flexible molecule. Since less flexible analogues could result in a rise in receptor selectivity, several conformationally constrained analogues of CI-988 and close analogues were explored.

The simplest restricted "dipeptoid" analogues were a series of α,β-dehidro derivatives, which could not only impose predictable conformation constrains to the aromatic side chains [74,75], but also could confer resistance to enzymatic degradation *in vivo* [76].

Regarding the Trp residue derivatives **28** and **29** were prepared as α,β-dehidro analogues of "dipeptoid" **11** (Table 7) [77,78]. Their biological evaluation showed that, as for

Table 7. CCK Receptors Binding Affinities of Dehidro- and Cyclopropyl-"Dipeptoids" N^{α}-(2-Adoc)-R

N.	R	IC_{50} (nM)[a]		1/2 [b]
		CCK_1	CCK_2	
28a	Δ^{Z}Trp-(*S*)-βHph-OH	550	13	42
28ab	$\Delta^{Z/E}$Trp-(*S*)-βHph-OH[c]	18	0.30	60
29a	Δ^{Z}Trp-(*R*)-βHph-OH	3.7	99	0.03
29ab	$\Delta^{Z/E}$Trp-(*R*)-βHph-OH[d]	3.9	60	0.06
30	(*R*)-α-MeTrp-Δ^{Z}Phe-OMe	ND	270	-
31	(*R*)-α-MeTrp-Δ^{Z}Phe-OH	60	54	1.1
32	(*R*)-α-MeTrp-∇^{Z}Phe-OMe	720	600	1.2
33	(*R*)-α-MeTrp-∇^{Z}Phe-OH	84	140	0.6
34	(*R*)-α-MeTrp-∇^{E}Phe-OMe	26	3.9	6.6

[a] Binding affinities defined in footnote a, Table 2. [b] CCK_1/CCK_2. [c] E/Z (35:65). [d] E/Z (40:60).

the parent "dipeptoid", the *S*-enantiomers preferentially bind to CCK_2 receptors. Since the mixture of Z and E isomers (**28ab**) showed increased affinity compared with the Z isomer alone (**28a**), likely the E isomer would possess exceptional affinity at CCK_2 receptors. Therefore, it seems that the restriction of the rotation around the C_α-C_β bond of the Trp residue imposed by the E-configured double bond is appropriate for an effective interaction of the indole ring with these receptors. On the other hand, the corresponding *R* enantiomer (**29a**), proved to be a potent and selective CCK_1 receptor ligand, thus reversing the receptor selectivity. This again highlights the importance of the absolute configuration for differentiating between CCK receptors subtypes.

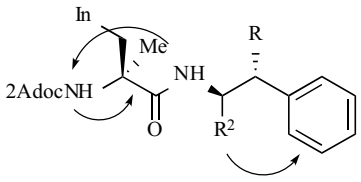
When the same C_α-C_β restriction was applied to the Phe side chain in compound **10**, the E-isomers were not obtained, but the corresponding Z-ΔPhe derivatives, **30**, **31** [79,80]. When compared to model compound **10**, dehydro-derivative **31** showed similar binding affinity at CCK_2 receptor and a two-fold increase in the CCK_1 affinity. As no information could be obtained for E isomers, and in order to gain further insights into the topographical requirements of the aromatic side-chains for affinity at CCK_1 and CCK_2 receptors, a series of cyclopropyl-Phe analogues of derivative **10** were also prepared. Once again, the stereochemistry proves to be important in determining CCK_1/CCK_2 ratio, with the E-isomer (derivative **34**) showing a considerable increment in CCK_1 and CCK_2 affinities over its diastereomeric Z-form (**32**) [79,80].

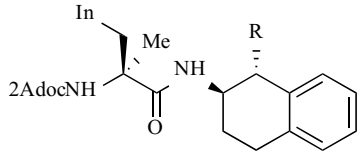
In general, it was observed that the incorporation of dehydro or cyclopropyl amino acids led to a decrease in CCK_2 receptor affinity, while an improvement in CCK_1 receptor binding was commomly observed. It appears that these restricted derivatives are able to adopt conformations more suitable for CCK_1 receptors than their flexible analogues. In this respect, molecular modelling studies have suggested β-turn-like conformations within the backbone of dehydro- and cyclopropyl-constrained dipeptoid analogues [80].

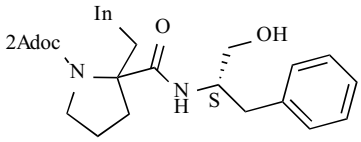
Another restriction of the mobility of the *C*-terminal aromatic side-chain was the linking of the phenyl ring with the α-carbon atom, in order to restrict free rotation of the aromatic moiety [81]. The resulting substituted naphthyl derivatives **35**-**37** (Table 8), analogues of "dipeptoids" **14**-**16**, respectively, were able to maintain nanomolar CCK_2 affinity, with *trans* isomers generally showing higher binding affinity than the corresponding cis-substituted analogues. Only compound **37** showed a significant enhanced affinity for CCK_2 receptors compared to the parent compound, **16**. Regarding CCK_1 receptor recognition, all these semi-rigid analogues showed an increase in CCK_1 affinity and, accordingly, compounds **35**-**37** are less selective than their acyclic parents.

In order to investigate the effect of diminishing the flexibility of the "dipeptoid" backbone, a serie of heterocycle scaffolds were selected based on the X-ray crystal structure of CI-988 [82], and aided by computer assisted modelling. In this regard, it was assumed the premise that the X-ray structure is the binding conformer at the CCK_2 receptor-binding site. Among these scaffolds, a proline moiety [83] was incorporated into the *N*-terminal residue, since this ring

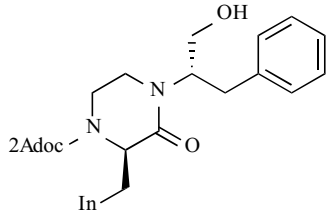
Table 8. CCK Receptors Binding Affinities of Conformational Constrained "Dipeptoids"







38



39

Compd.	R	IC_{50} (nM)[a]		1/2 [b]
		CCK_1	CCK_2	
35	$NHCOCH_2CO_2H$	357	1.48	241
36	$NHCO(CH_2)_2CO_2H$	460	2.31	199
37	$NHCO(CH_2)_3CO_2H$	437	1.51	289
38	-	2080	1050	2
39	-	ND	9120	-

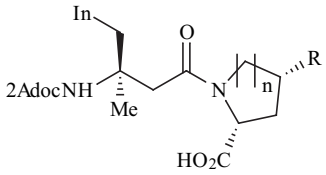
[a] Binding affinities as defined in footnote a, Table 2. [b] CCK_1/CCK_2.

could be able to mimic the eclipsed orientation of the α-methyl and the carbamate NH proton, observed in the X-ray crystal structure of CI-988 [82]. However, the resulting derivative (compound **38**, Table 8) had significantly weaker affinity than the model "dipeptoid". This decrease in affinity might reveal a role for the carbamate NH group or might be caused by an undesired steric interaction between the receptor and the extra atoms in the five-membered ring.

A combined restriction of ϕ and ψ dihedral angles of the *N*-terminal Trp residue was obtained by incorporation of a piperazinone skeleton [84]. Unluckily, this restriction of the backbone flexibility led to a considerable loss in CCK_2 receptor binding affinity (compound **39**, Table 8). This loss in affinity was attributed to the absence of the amide and/or urethane NH protons, which could potentially result in the loss of H-bonding centres, but the existence of a global inappropriate conformation, induced by the piperazinone ring, cannot be discarded.

To explore the validity of simultaneous restrictions at the backbone and at the side-chain of the C-terminal residue, a series of analogues of "dipeptoids" **18** and **20**, incorporating a methylene bridge, that linked the β carbon of the phenethyl side-chain and the α carbon bearing the carboxylate function, were prepared. The biological evaluation of the resulting proline derivatives **40-45** (Table 9) showed that all these compounds had lower affinities for CCK_1 than for CCK_2 receptors, although there was a decrease in the selectivity compared to the starting more flexible analogues **18** and **20** [85]. This suggested that this type of reduction of the conformational freedom might lead to relatively more favourable conformations for CCK_1 binding, as compared to the linear series. Regarding the influence of the stereochemistry of the chiral centers, an *R* (D) configuration of Pro and Trp residues was always preferred for high CCK_2 affinity, the latter in agreement with reported data on previous "dipeptoids". Moreover, a *cis* orientation of the substituents at C2 and C4 positions of the proline ring resultes more favourable for CCK_2 recognition. Although reduction of the conformational freedom, did not improve the affinity of linear "dipeptoid" **20**, the antagonist potency was increased in these proline-restricted derivatives. Moreover, it is expected that these new molecules possess higher stability toward enzymatic and acid degradation and increased lipophilicity, which would facilitate blood-brain barrier penetration. The fact that these analogues, behaved as selective CCK_2 antagonists, but with a decrease in affinity, indicated that the selected combination of constraints forced the two substitutuents on the pyrrolidine ring into a good, but not optimal, spatial arrangement for CCK_2 receptor recognition. Structure-affinity relationship studies on this proline-containing series indicated that lengthening the distance between the amide nitrogen atom and the phenyl ring was of little importance (compound **42** and **43**, Table 9), while the position of the carboxylate could not be modified. Therefore, new restrained compounds were designed, increasing the size of the pyrrolidine ring by one methylene, as in piperidine analogue **46**. This compound resulted in a ten-fold lower CCK_2 receptor affinity [86], indicating that the six-membered ring did not appear to force the essential features for CCK_2 recognition into an optimal fit.

Table 9. CCK_2 Receptors Binding Affinities of Constrained Pro-Derived "Dipeptoids"



Compd.	R	n	Ki(nM)[a]	1/2[b]
40	Ph	1	20	33
41	Ph-pCl	1	32	12
42	OPh	1	28	22
43	OCH_2Ph	1	24	68
44	OPh-pCl	1	24	65
45	OPh-o,pF_2	1	17.6	22
46	OPh-o,pCl_2	2	175.2	21

[a] CCK_1 and CCK_2 binding affinities on pancreas membranes and on guinea pig cortex, respectively. Ki for CCK_1 receptors is only expressed as its ratio versus Ki for CCK_2 receptors. [b] CCK_1/CCK_2.

In order to get a deeper insight into the reasons of the different affinity of pyrrolidine and piperidine rings constrained derivatives, a structural and conformational analysis was done. The results showed that the bioactive conformation of peptoid CCK_2 antagonists is probably W-shaped.

On the whole, the incorporation of local conformational constraints into the aromatic side chains of "dipeptoids" is well tolerated, especially at *C*-terminal level, providing analogues with good affinity at CCK_2 receptors. However, restrictions at the backbone skeleton are detrimental for CCK receptor recognition. In general, it was observed that conformationally restricted "dipeptoids" are less selective than linear analogues, as a consequence of their enhanced CCK_1 affinities. Nevertheless, the knowledge acquired with the comparison of these constrained structures and their related linear compounds have yielded valuable information about the topographical requirements for optimal recognition of the CCK receptor subtypes by this family of compounds.

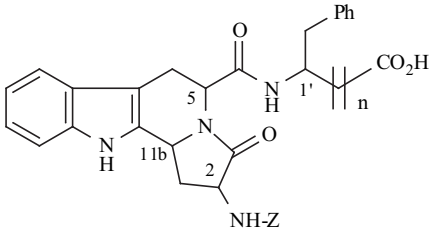
2.4.2 Secondary Structure Mimetics

As mentioned in the above section, the incorporation of conformational restrictions into "dipeptoids" led, in general, to increases in CCK_1 receptors affinity compared with their parents linear analogues. This enhancement of the CCK_1 affinity was especially remarkable in constrained "dipeptoid" analogues incorporating dehydro- and cyclopropyl-Phe derivatives at *C*-terminus [79,80], for which conformational studies indicated the presence of a β-turn within the peptide backbone, although no preference in type was observed.

In order to investigate whether a turn-like conformation was that adopted by dipeptoids at CCK_1 receptor sites, a series of conformationally constrained derivatives were prepared (**47**-**56**, Table 10) [87,88]. In these compounds the α-MeTrp residue of "dipeptoids" **10** and **11** was replaced with the (2*S*,5*S*,11b*S*)-, (2*S*,5*S*,11b*R*)- and (2*R*,5*R*,11b*S*)-2-

amino-3-oxohexahydorindolizino[8,7-*b*]indole-5-carboxylate skeleton (IBTM) [89]. These diastereomeric skeletons contain the indole side-chain of the Trp residue and are able to mimic type II´ (2*S*,5*S*) and type II (2*R*,5*R*) β-turn conformations with a degree of accuracy that depends on the C-11*b* configuration [90].

Table 10. CCK Receptors Binding Affinities of Dipeptoids that Incorporate β-Turn Mimetics



Compd	Config	n	IC_{50} (nM)[a]		
	2,5,11b,1'		CCK_1	CCK_2	1/2 [b]
47	SSSS	1	635	>10000	>16
48	*SSSR*	1	1000	>10000	>10
49	*SSRS*	1	88	>10000	>113
50	*SSRR*	1	7.4	2700	365
51	*SSRS*	0	4.7	>10000	>2128
52	*SSRR*	0	54.6	>10000	>183
53	*RRSS*	1	97.8	>10000	>102
54	*RRSR*	1	>1000	>10000	-
55	*RRSS*	0	1.73	202	117
56	*RRSR*	0	>1000	3720	<4

[a] IC_{50} represents the concentration producing half-maximal inhibition of specific [^{3}H]-propionylCCK-8 specific binding to rat pancreas (CCK_1) or mouse cerebral cortex (CCK_2). [b] CCK_1/CCK_2.

The result of the biological evaluation of these highly restricted compounds showed that the affinity at CCK_2 receptors was negligible or very modest. In contrast, these derivatives showed an improved CCK_1 affinity in comparison with the parent "dipeptoids". These results seem to indicate that a turn-like conformation within the peptide backbone of "dipeptoids" is favourable for CCK_1 receptor recognition. This is backup by the fact that the 11b*R* configured isomers, **49** and **50**, that are better mimic of β-turn conformation than the corresponding 11b*S* isomers, **47** and **48**, showed higher CCK_1 binding potency [92].

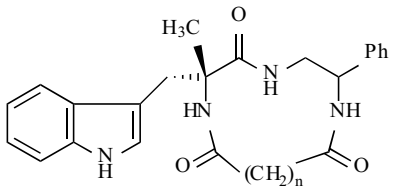
Structure-activity studies in this series of compounds showed that a bencyloxycarbonyl group (Z) was required at the *N*-terminal amino group, while a free carboxylic acid is preferred at *C*-terminus, this latter in agreement with previous requirements in the "dipeptoid" series. The interchange of βHph with Phe was well tolerated at the CCK_1 receptors (derivatives **49**-**52**, Table 10). Additionally, the β-turn type critically affects the selectivity for CCK_1 receptor subtype. Thus, while compounds **51** and **55** are both endowed with nanomolar affinity at CCK_1 receptors, restricted "dipeptoid" derivative **51**, incorporating the type II' mimetic, shows approximately 6-fold higher CCK_1 selectivity than analogue **55**, with the type II' inducer.

Derivatives **50**, **51** and **55**, the best compounds in this series, behave as potent and selective CCK_1 antagonists. The good affinity data of these constrained "dipeptoids" supported the hypothesis of the existence of β-turn conformations within the structure of "dipeptoids" in their binding to CCK_1 receptors. Moreover, this approximation has led to a family of highly constraint dipeptoids analogues, endowed with high binding affinity and selectivity for CCK_1 receptors, compound **51** being the prototype of this series.

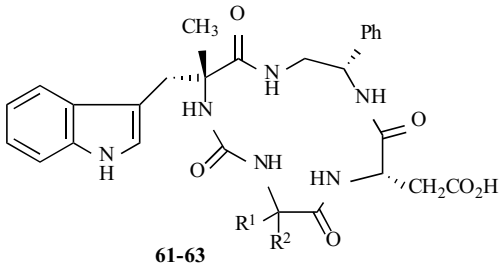
2.4.3 Global Constraints

Another way to reduce the conformational space available to peptides is the global cyclization of the peptide backbone leading to macrocyclic analogues. On the basis of the X-ray crystal structure and on the ^{1}H NMR nOe's of CI-988 [82], that provided evidence for a close through-space proximity of the adamantyl and succinic acid moieties, a series of 11 to 14-membered macrocyclic analogues were designed using computer assisted molecular modelling analysis [91,92]. An array of different sized ring was selected in order to prove the tolerance of the binding site and to aid in the development of a pharmacophore model. Moreover, these derivatives had lower molecular weight and reduced lipophilicity with respect to linear "dipeptoids", which could improve their bioavailability.

Table 11. CCK_2 Receptor Binding Affinity of Macrocyclic Analogues



57 n = 2 **58** n = 3
59 n =4 **60** n = 5



61-63

Compd.	R^1	R^2	IC_{50}[a] (μM)
61	H	H	>1000
62	cyclohexyl		4.1
63[b]	cyclohexyl		7.8

[a] Binding affinities as defined in footnote a, Table 2. [b] *R* configuration at Asp residue.

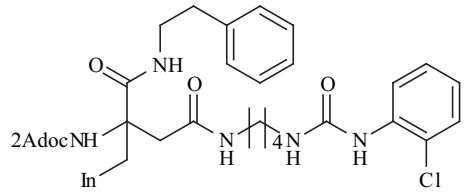
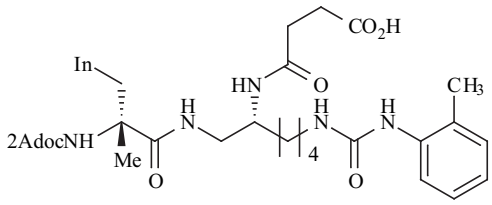
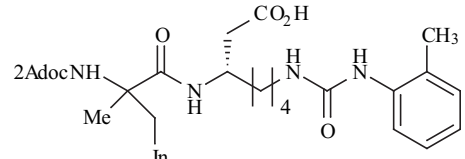
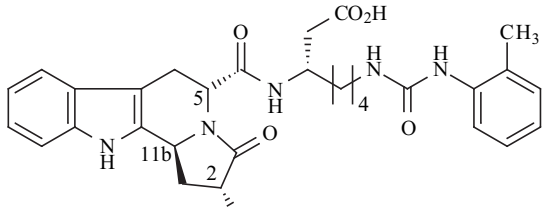
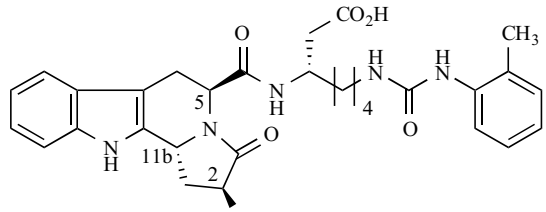
The CCK_2 receptor binding data showed that all the macrocycles were either inactive, as derivatives **57-61**, or had considerably lower binding affinity for CCK_2 receptors than the acyclic parent CI-988, as compound **62** and **63** (Table 11).

The biological results obtained with the macrocycles derivatives do not support the hypothesis that the conformation determined in solution and in the solid state for CI-988 was similar to the conformation adopted at the CCK_2 receptors. Alternatively, the rigidity built into these compounds could limit the conformational flexibility, which might be necessary to access the CCK_2 binding sites.

3. "DIPEPTOID" CCK_1 RECEPTOR AGONISTS

Before proceeding with the discussion of the development of CCK_1 receptor agonists, just a short note about CCK_2 receptor agonists. Although there was an

Table 12. CCK Receptor Binding Affinities of CCK_1 Ligands

Compound	IC_{50} (nM)[a]	
	CCK_1	CCK_2
 **64**	79[a]	>10000[a]
 **65**	12[b]	20[b]
 **66**	1.2[a]	6.7[a]
 **67**	8.2[c]	5799[c]
 **68**	324[c]	>10000[c]

[a] Binding affinities as defined in footnote a, Table 2. [b] Binding affinities were the pIC_{50} (CCK_1 = 7.92, CCK_2 = 7.70) of the concentration displacing 50% of [^{125}I] CCK-8 from membrane preparation isolated from CHO-K1 cells stably transfected with cDNA of human CCK_1 and CCK_2 receptors. [c] Binding affinities as defined in footnote a, Table 11.

approach to get such compounds [93], it was unsuccessful and it was not pursue any further, likely due to the severe side effects of a CCK_2 agonist, as enhanced gastric acid secretion or enhanced anxiety, which make these agonists unacceptable as therapeutic entities.

On the contrary, the identification of non-peptide agonists for the CCK_1 receptor subtype is an area of interest due to their potential utility for the treatment of obesity [94,95]. The approach to the design of such CCK_1 agonists has principally relied upon appending a phenylurea substituted Lys residue, the key feature of a series of peptide CCK_1 receptor selective agonists [96-99], onto a "dipeptoid" motif.

This strategy was successfully applied to a series of α,α-disubstituted "dipeptoids" [100,101], designed with the aid of computer assisted molecular modelling on the base of minimized conformation of CCK_{30-33} [102]. For instance, compound **64** (Table 12), with the Lys(Tac) side-chain appended at the α-carbon of Trp, showed quite good affinity at CCK_1 receptors and was selective over CCK_2 receptors. Since compound **64** is racemic, and considering the importance of the stereochemistry in CCK receptors subtype selectivity, an asymmetric synthesis was developed to prepare the two possible enantiomers [103]. Surprisingly, in this case the binding affinity values of the separated isomers were essentially equivalent to that of the racemic mixture.

It is well known that compound **15** (CI-988) is a potent CCK_2 receptor selective antagonist, but it retains weak CCK_1 agonist activity. Therefore, with the aim of increasing the CCK_1 affinity, the incorporation of Lys(Tac) side-chain into the structure of this "dipeptoid" as replacement for the phenyl ring was explored [104]. Once again, this approach proved worth and this replacement led to the potent CCK_1 agonist **65** (Table 12).

To explore further the optimal space disposition of the Lys(Tac) side-chain, the point of attachment of the substituted Lys was change to the α-carbon of the *C*-terminal homologated Phe derivative **11**. The target compound **66** (Table 12) behaved as an agonist at the high-affinity and as an antagonist at the low-affinity CCK_1 binding sites [105]. In addition to that, it was also a high affinity CCK_2 receptor antagonist.

On the bases of the rise in CCK_1 affinity and selectivity by the incorporation of the IBTM-derived β-turn mimetics within the "dipeptoid" structure, and with the aim of increasing the selectivity of agonist **66**, the (2*R*,5*R*,11b*S*)- and (2*S*,5*S*,11b*R*)-IBTM skeletons were incorporated into this "dipeptoid" to give conformationally restricted derivatives **67** and **68** [106]. Compound **67** (Table 12) maintains the nanomolar affinity at the CCK_1 receptors, but notably increased its selectivity comparing with the parent compound. Unfortunately, there is a change in the functional activity, and this new derivative behaves as an antagonist at CCK_1 receptors. This unexpected result, could be related to a reduced mobility of the βHly(Tac) side-chain in **67** and **68** with respect to **66**, although further studies will be needed to confirm this hypothesis.

In summary, the incorporation of Lys(Tac) side-chain into different positions of "dipeptoids" led to the development of non-peptide, full efficacy CCK_1 receptor agonists, albeit generally with low CCK_2 selectivity.

4. CONCLUSIONS

The family of "dipeptoids" represents one of the first rational designs of non-peptide ligands for neuropeptide receptors. Taking into account that the three dimensional structure of CCK receptors is unknown, the approach followed focused on the structure of the endogenous ligand CCK-8. In this sense, and in a stepwise manner, it was determined the minimum structural and topographical requirements for receptor recognition and activation. To get suitable drug candidates, the reduction of the peptide nature and the study of the best groups to enhance the bioavailability were also explored.

This approach has led to a family of potent and selective CCK_1 and CCK_2 receptors antagonists, and to low-selective CCK_1 agonists. Although several of these compounds have entered clinical trials, none of them is currently on the market. Nevertheless, these ligands have aided in better understanding the biological implications of CCK_1 and CCK_2 receptors, which will benefit future research in the field of CCK.

The "peptoid" drug design strategy [107] described in this review constitutes a general example of how the rational design starting from an endogenous peptide can lead to the development of potent non-peptide ligands for the corresponding peptide receptors. In this sense, this strategy has been adapted to the development of different non-peptide selective antagonists, for several neuropeptide receptors, as tachykinin (NK_1 [108,109], NK_2 [110], NK_3 [111,112]), neuromedin-B [113] and gastrin-releasing peptide receptors [114]. These compounds, also characterized by the presence of α-Me amino acid residues, proved that the lessons derived from the cholecystokinin series are applicable to other receptor-ligand systems.

REFERENCES

[1] Loffet, A. *J. Peptide Sci*., **2002**, *8*,1.
[2] McMartin, C. In Advances in Drug Research; Testa, B., Ed.; Academic Press: London, **1992**, Vol. *22*, pp. 41-106.
[3] Moore, G.J. *Trends Pharmacol. Sci.*, **1994**, *15*, 124.
[4] Rehfeld, J.F.; Nielsen, F.C. In Cholecystokinin and Anxiety; Bradwejn, J.; Vasas, E. ed.; Landes Company: Austin, **1995**, pp. 33-56.
[5] Wank, S.A. *Am. J. Physiol*, **1995**, *269*, G628.
[6] Noble, F.; Wank, S.A.; Crawley, J.N.; Bradwejn, J.; Seroogy, K.M.; Hamon, M.; Roques, B.P. *Pharmacol. Rev.*, **1999**, *51*, 745.
[7] Miyasaka, K.; Funakoshi, A. *J. Gastroenterology*, **2003**, *38*, 1.
[8] Crawley, J.N.; Corwin, R.L. *Peptides,* **1994**, *15*, 731.
[9] Schubert, M.L.; Shamburrek, R.D. *Gasterenterol. Clin. North. Am.*, **1990**, *19*, 1.
[10] An issue that gathers the topics of the first Neuronal Cholecystokinin Gordon Conference, *Peptides*, **2001**, Vol. *22*, Issue 8.
[11] Barber, N.D.; Dourish, C.T.; Hill, D.R. *Pain*, **1989**, *39*, 307.
[12] Ravard, S.; Dourish, C.T. *Trends Pharmacol. Sci.*, **1990**, *11*, 271.
[13] Harro, J.; Vasar, E.; Bradwejn, J. *Trends Pharmacol. Sci.*, **1993**, *14*, 244.
[14] Daugé, V.; Lena, I. *Neurosci. Biobehav. Rev.*, **1998**, *22*, 815.
[15] Smith, G.P.; Jerome, C.; Cushin, B.J.; Eterno, R.; Simansky, K.J. *Science*, **1981**, *213*, 1036.
[16] Dorish, C.T.; Ruckert, A.C.; Tattersall, F.D.; Iversen, S.D. *Eur. J. Pharmacol.*, **1989**, *173*, 233.

[17] Roques, B.P.; Noble, F. *Neurochem. Res.*, **1996**, *21*, 1395.
[18] Itoh, S.; Lal, H. *Drug Develop. Res.*, **1990**, *21*, 257.
[19] Crawley, N.J. *Trends Pharmacol. Sci.*, **1991**, *12*, 232.
[20] Derrein, M.; Durieux, C.; Dauge, V.; Roques, B.P. *Brain Res.*, **1993**, *617*, 181.
[21] Schalling, M.; Friberg, K.; Seroogy. K.; Riederer, P.; Bird, E.; Schiffmann, S.N.; Mailleux, P.; Vanderhaeghen, J.J.; Kuga, S.; Goldstein, M.; Kitahama, K.; Luppi, P.H.; Jouvet, M.; Hokfelt, T. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 8427.
[22] Boyce, S.; Rupniak, N.M.; Steventon, M.; Iversen, S.D. *Neurology*, **1990**, *40*, 717.
[23] Geracioti, T.D.J.; Liddle, R.A. *Engl. J. Med*., **1988**, *319*, 683.
[24] Trivedi, B.K. *Curr. Med. Chem.*, **1994**, *1*, 313.
[25] Wettstein, J.G.; Bueno, L.; Junien, J.L. *Pharmacol. Therapeut.*, **1994**, *62*, 267.
[26] Sugg, E.E.; Birkemo, L.; Gan, L.-S.L. *Pharm. Biotechnol.*, **1998**, *11*, 507.
[27] Revel, L.; Makovec, F. *Drugs Future*, **1998**, *23*, 751.
[28] De Tullio, P.; Delarge, J.; Pirotte, B. *Curr. Med. Chem.*, **1999**, *6*, 433.
[29] Farmer, P.S.; Ariëns, E.J. *Trends Pharmacol. Sci.*, **1982**, *3*, 362.
[30] Horwell, D.C. *Bioorg. Med. Chem.*, **1996**, *4*, 1573.
[31] Horwell, E.C. *Lett. Pept. Sci.*, **1998**, *5*, 115.
[32] Moore, G.J. *Trends Pharmacol. Sci.*, **1994**, *15*, 124.
[33] Olson, G.L.; Bolin, D.R.; Bonner, M.P.; Bos, M.; Cook, C.M.; Fry, D.C.; Graves, B.J.; Hatada, M.; Hill, D.E.; Kahn, M.; Madison, V.S.; Rusiecki, V.K.; Sarabu, R.; Spinwell J.; Vicent, G.P.; Voss, M.E. *J. Med Chem.*, **1993**, *36*, 3039.
[34] Marshall, G.R. *Tetrahedron*, **1993**, *49*, 3547.
[35] Holzemann, G. *Kontakte*, **1991**, 3.
[36] Holzemann, G. *Kontakte*, **1991**, 55.
[37] Horwell, D.C.; Beeby, A.; Clark, C.R.; Hughes, J. *J. Med. Chem.*, **1987**, *30*, 729.
[38] Horwell, D.C.; Birchmore, B.; Boden, P.R.; Higginbottom, M.; Ping, H.Y.; Hughes, J.; Hunter, J.C.; Richardson, R. *Eur. J. Med. Chem.*, **1990**, *25*, 53.
[39] Khosla, M.C.; Stackiwiak, K.; Smeby, R.R.; Bumpus, F.M.; Piriou, F.; Lintner, K.; Fermandjians, S. *Proc. Natl. Acad. Sci. USA*, **1981**, *78*, 757.
[40] Higginbottom, M.; Kneen, C.; Ratcliffe, G.S. *J. Med. Chem.*, **1992**, *35*, 1572.
[41] Eden, J.M.; Higginbottom, M.; Hill, D.R.; Horwell, D.C.; Hunter, J.C.; Martin, K.; Pritchard, M.C.; Rahman, S.S.; Richardson, R.S.; Roberts, E. *Eur. J. Med. Chem.*, **1993**, *28*, 37.
[42] Horwell, D.C.; Hughes, J.; Hunter, J.C.; Pritchard, M.C.; Richardson, R.S.; Roberts, E.; Woodruff, G.N. *J. Med. Chem.*, **1991**, *34*, 404.
[43] 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. *J. Med. Chem.*, **1993**, *36*, 552.
[44] Boden, P.R.; Eden, J.M.; Higginbottom, M.; Hill, D.R.; Horwell, D.C.; Hunter, J.C.; Martin, K.; Pritchard, M.C.; Richardson, R.S.; Roberts, E. *Eur. J. Med. Chem.*, **1993**, *28*, 47.
[45] Drysdale, M.J.; Pritchard, M.C.; Horwell, D.C. *J. Med. Chem.*, **1992**, *35*, 2573.
[46] Drysdale, M.J.; Pritchard, M.C.; Horwell, D.C. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 45.
[47] Augelli-Szafran, C.E.; Horwell, D.C.; Kneen, C.; Ortwine, D.F.; Pritchard, M.C.; Purchase, T.S.; Roth, B.D.; Trivedi, B.K.; Hill, D.; Suman-Chauhan, N.; Webdale, L. *Bioorg. Med. Chem.*, **1996**, *4*, 1733.
[48] Araldi, G.; Donati, D.; Oliosi, B.; Ursini, A.; van Amsterdam, F.; Natalini, B.; Pellicciari, R.; Tarzia, G. *Il Farmaco*, **1996**, *51*, 471.
[49] Araldi, G.; Donati, D.; Oliosi, B. ; Pasquarello, A.; Polinelli, S.; Tarzia, G.; Ursini, A.; van Amsterdam, F.T.M. *Eur. J. Med. Chem.*, **1996**, *31*, 215.
[50] Xu, X.J. ; Wiesenfeld-Hallin, Z.; Hughes, J.; Horwell, D.C.; Hokfelt, T. *Br. J. Pharmacol*, **1992**, *105*, 591.
[51] Coudore-Civiale, M.A.; Courteix, C.; Boucher, M.; Meen, M.; Fialip, J.; Eschalier, A.; Ardid, D. *Neurosci. Lett.*, **2000**, *286*, 37.
[52] Coudore-Civiale, M.A ; Courteix, C.; Fialip, J.; Boucher, M.; Eschalier, A. *Pain*, **2000**, *88*, 15.
[53] Dethloff, L.A.; Barr, B.M.; Bestervelt, L.L. *Food Chem. Toxiocol.*, **1999**, *37*, 105.
[54] Romani, R.; Howes, L.G.; Morris, D.L. *Clin. Exp. Pharmacol. Physiol.*, **1996**, *23*, 438.
[55] Moody, T.W.; Jesen, R.T. *J. Pharmacol. Exp. Ther.*, **2001**, *299*, 1154.
[56] Hughes, H.; Boden, P.; Costall, B.; Domeney, A.; Kelly, E.; Horwell, D.C.; Hunter, J.C.; Pinnock, R.D.; Woodruff, G.N. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 6728.
[57] Pande, A.C.; Greiner, M.; Adams, J.B. ; Lydiard, R.B. *Biol. Psychiatry,* **1999**, *46*, 860.
[58] Lodge, D.J.; Lawrence, A.J. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **2003**, *27*, 451.
[59] Wilson, J.; Watson, W.P.; Little, H.J. *Psychopharmacology*, **1998**, *137*, 120.
[60] Feng, R.; Hinton, J.P.; Hoffman, K.; Parker, T.D.; Wright, D.S. *Proc. Natl. Acad. Sci. USA*, **1991**, *8*, S-267.
[61] Hinton, J.P.; Rutkowskin, D.; Johnson, E.L.; Wright, D.S. *Pharm. Res.*, **1991**, *8*, S-267.
[62] Hinton, J.P.; Hoffman, G.; Poisson, A.; Klemisch, W.; Wright, D.S. *Pharm. Res.*, **1993**, *10*, S-330.
[63] Bradwejn, J.; Koszycki, D.; Paradis, M.; Reece, P.; Hinton, J.P.; Sedman, A. *Biol. Psychiatry*, **1995**, *38*, 742.
[64] Blommaert, A.G.S.; Weng, J.-H.; Dorville, A.; McCort, I.; Ducos, B.; Durieux, C.; Roques, B.P. *J. Med. Chem.*, **1993**, *36*, 2868.
[65] Bourne, G.T.; Horwell, D.C.; Pritchard, M.C. *Tetrahedron*, **1991**, *47*, 4763.
[66] Padia, J.K.; Bolton, G.L.; Hill, D.; Horwell, D.C.; Roth, B.D.; Trivedi, B.K. *Bioorg. Med. Chem. Lett.,* **1993**, *3*, 2805.
[67] Spatola, A.F. In Chemistry and Biochemistry of Amino Acids, Peptides and Proteins; Weinstein, B. Ed.; Dekker: New York, **1983**, Vol. *7*, pp. 267.
[68] Fincham, C.I.; Higginbottom, M.; Hill, D.R.; Horwell, D.C.; O'Toole, J.C.; Ratcliffe, G.S.; Rees, D.C.; Roberts, E. *J. Med. Chem.,* **1992**, *35*, 1472.
[69] 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. *J. Med. Chem.*, **1998**, *41*, 38.
[70] Trivedi, B.K.; Hinton, J.P. *Pharm. Biotechnol.*, **1998**, *11*, 481.
[71] Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardi, R.M.; Freidinger, R.M.; Whitter, W.L.; Lundell, G.F.; Veber, C.F.; Anderson, P.S.; Chang, R.S.L.; Lotti, V.J.; Cerno, D.J.; Chen, T.B.; Kling, P.F.; Kunkel, D.A.; Springer, J.P.; Hirshfield, J.J. *J. Med. Chem.*, **1988**, *31*, 2235.
[72] Bock, M.G.; DiPardo, R.M.; Evans, B.E.; Rittle, K.E.; Whitter, W.L.; Veber, D.F.; Anderson, P.S.; Freidinger, R.M. *J. Med. Chem.*, **1989**, *32*, 13.
[73] Higginbottom, M.; Horwell, D.C.; Roberts, E. *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 881.
[74] Cishah, E.; Grzeyorz, P.; Rzeszotaska, B. *Int. J. Peptide Protein Res.*, **1992**, *39*, 218 .
[75] Ciajolo, M.R.; Tuzi, A.; Pratesi, C.R.; Fissi, A.; Pieroni, E. *Int. J. Peptide Protein Res.*, **1991**, *38*, 539.
[76] English, M.L.; Stammer, C.H. *Biochem. Biophys. Commun.*, **1978**, *83*, 1964.
[77] Eden, J.M.; Horwell, D.C.; Pritchard, M.C., *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 989.
[78] The symbols Δ and ∇ are as defined by Stammer, C.H. *Tetrahedron*, **1990**, *46*, 2231, which states: "The symbol ∇^Z or ∇^E prefixed to the abbreviation for an amino acid residue as in ∇^Z Phe, means the Z-diasteromer of 2,3-methano- or cyclopropane-phenylalanine. It is used here only when the methanoamino acid appears in a peptide chain. The Δ^Z symbol indicates a dehydroamino acid, with Δ^Z Phe meaning (Z)-2,3-dehydrophenylalanine.
[79] Campbell, M.M.; Horwell, D.C.; Mahon, M.F.; Pritchard, M.C.; Walford, S.P. *Bioorg. Med. Chem. Lett.,* **1993**, *3*, 667.
[80] Walford, S.P.; Campbell, M.M.; Horwell, D.C. *J. Pharm. Pharmacol.*, **1996**, *48*, 188.
[81] Higginbottom, M.; Hill, D.R.; Horwell, D.C.; Mostafai, E.; Suman-Chauhan, N.; Roberts, E. *Bioorg. Med. Chem.*, **1993**, *1*, 209.
[82] Debaerdemaeker, T.; Heigl, T.; Steiner, K. *Acta Cryst.*, **1993**, *C49*, 565.
[83] Fincham, C.I.; Horwell, D.C.; Ratcliffe, G.S.; Rees, D.C. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 403.
[84] Horwell, D.C.; Lewthwaite, R.A.; Pritchard, M.C.; Ratcliffe, G.S.; Rubin, J.R. *Tetrahedron*, **1998**, *54*, 4591.

[85] Bellier, B.; McCort-Tranchepain, I.; Ducos, B.; Danascimento, S.; Meudal, H.; Noble, F.; Garbay, C.; Roques, B.P. *J. Med. Chem.*, **1997**, *40*, 3947.
[86] Bellier, B.; Da Nascimento, S.; Meudal, H.; Gincel, E.; Roques, B.P.; Garbay, C. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 1419.
[87] De la Figuera, N.; Martín-Martínez, M.; Herranz, R.; García-López, M.T.; Latorre, M.; Cenarruzabeitia, E.; Del Río, J.; González-Muñiz, R. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 43.
[88] Martín-Martínez, M.; De la Figuera, N.; Latorre, M.; Herranz, R.; García-López, M.T.; Cenarruzabeitia, E.; Del Río, J.; González-Muñiz, R. *J. Med. Chem.*, **2000**, *43*, 3770.
[89] De la Figuera, N.; Alkorta, I.; García-López, M.T.; Herranz, R.; González-Muñiz, R. *Tetrahedron*, **1995**, *51*, 7841.
[90] 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. *J. Am. Chem. Soc.*, **1997**, *119*, 10579.
[91] Didier, E.; Horwell, D.C.; Pritchard, M.C. *Tetrahedron*, **1992**, *48*, 8471.
[92] Bolton, G.L.; Roth, B.D.; Trivedi, B.K. *Tetrahedron*, **1993**, *49*, 525.
[93] Davey, A.E.; Horwell, D.C. *Bioorg. Med. Chem.*, **1993**, *1*, 45.
[94] Smith, G.P.; Gibbs, J. *Ann. N. Y. Acad. Sci.*, **1994**, *713*, 236.
[95] Szewczyk, J.K.; Laudeman, C. *Curr. Top. Med. Chem.*, **2003**, *3*, 837.
[96] Shiosaki, K.; Lik, C.W.; Kopecka, H.; Craig, R.; Wagenaar, F.L.; Bianchi, B.; Miller, T.; Witte, D.G.; Nadzan, A.M. *J. Med. Chem.*, **1990**, *33*, 2950.
[97] Shiosaki, K.; Li, C.W.; Kopecka, H.; Tufano, M.D.; Bianchi, B.; Miller, T.R.; Witte, D.G.; Nadzan, A.M. *J. Med. Chem.*, **1991**, *34*, 2837.
[98] Holladay, M.W.; Kopecka, H.; Miller, T.R.; Bednarz, L.; Nikkel, A.L.; Bianchi, B.; Witte, D.G.; Shiosaki, K.; Li, C.W.; Asin, K.E.; Nadzan, A.M. *J. Med. Chem.*, **1994**, *37*, 630.
[99] Elliot, R.L.; Kopecka, H.; Bennett, M.J.; Shue, Y.-K.; Craig, R.; Lin, C.W.; Bianchi, B.R.; Miller, T.R.; Witte, D.G.; Stashko, M.A.; Asin, K.E.; Nikkel, A.; Bednarz, L.; Nadzan, A.M. *J. Med. Chem.*, **1994**, *37*, 309.
[100] Bourne, G.T.; Horwell, D.C.; Pritchard, M.C. *Tetrahedron*, **1991**, *26*, 4763.
[101] Bourne, G.T.; Horwell, D.C.; Pritchard, M.C. *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 889.
[102] Pincus, M.R.; Murphy, R.B.; Carty, R.P-; Chen, J.; Shah, D.; Scherage, H.A. *Peptides*, **1988**, *9*, 145.
[103] Burgaud, B.G.M.; Horwell, D.C.; Pritchard, M.C. *Tetrahedron Asymetry*, **1995**, *6*, 1081.
[104] Dezube, M.; Sugg, E.E.; Birkemo, L.S.; Croom, D.K.; Doughterty, R.W.; Ervin, G.N.; Grizzle, M.K.; James, M.K.; Johnson, M.F.; Mosher, J.T.; Queen, K.L.; Rimele, T.J.; Sauls, H.R.; Triantafillou, J.A. *J. Med. Chem*., **1995**, *38*, 3384.
[105] Bernad, N.; Burgaud, B.G.M.; Horwell, D.C.; Lewthwaite, R.A.; Martinez, J.; Pritchard, M.C. *Bioorg. Med. Chem. Lett*., **2000**, *10*, 1245.
[106] Martín-Martínez, M.; Latorre, M.; García-López, M.T.; Cenarruzabeitia, E.; Del Río, J.; González-Muñiz, R. *Bioorg. Med. Chem. Lett*., **2002**, *12*, 109.
[107] Horwell, D.C. *Trends Biotechnol*., **1995**, *13*, 132.
[108] Boyle, S.; Guard, S.; Higginbottom, M.; Horwell, D.C.; Howson, W.; McKnight, A.T.; Martin, K.; Pritchard, M.C.; O'Toole, J.; Raphy, J.; Rees, D.C.; Roberts, E.; Watling, K.J.; Woodruff, G.N.; Hughes, J., *Bioorg. Med. Chem.*, **1994**, *2*, 357.
[109] Ashwood, V.A.; Field, M.J.; Horwell, D.C.; Julien-Larose, C.; Lewthwaite, R.A.; McCleary, S.; Pritchard, M.C.; Raphy, J.; Singh, L. *J. Med. Chem*., **2001**, *44*, 2276.
[110] Boyle, S.; Guard, S.; Hodgson, J.; Horwell, D.C.; Howson, W.; Hughes, J.; McKnight, A.; Martin, K.; Pritchard, M.C.; Watling, K.J.; Woodruff, G.N. *Bioorg. Med. Chem.*, **1994**, *2*, 101.
[111] Boden, P.; Eden, J.M.; Hodgson, J.; Horwell, D.C; Howson, W.; Hughes, J.; McKnight, A.T.; Meecham, K.; Pritchard, M.C.; Raphy, J.; Ratcliffe, G.S.; Suman-Chauhan, N.; Woodruff, G.N., *Bioorg. Med. Chem.*, **1994**, *4*, 1679.
[112] Boden, P.; Eden, J.M.; Hodgson, J.; Horwell, D.C; Pritchard, M.C.; Suman-Chauhan, N., *Bioorg. Med. Chem.,* **1995**, *5*, 1773.
[113] Eden, J.M.; Hall, M.D.; Higginbottom, M.; Horwell, D.C.; Horwson, W.; Hughes, J.; Jordan, R.E.; Lewthwaite, R.A.; Martin, K.; McKnight, A.T.; O'Toole, J.C.; Pinnock, R.D.; Pritchard, M.C.; Suman-Chauhan, N.; Williams, S.C. *Bioorg. Med. Chem.*, **1996**, *6*, 2617.
[114] Ashwood, V.; Brownhill, V.; Higginbottom, M.; Horwell, D.C.; Hughes, J.; Lewthwaite, R.A.; McKnight, A.T.; Pinnock, R.D.; Pritchard, M.C.; Suman-Chauhan, N.; Webb, C.; Williams, S.C. *Bioorg. Med. Chem.*, **1998**, *8*, 2589.

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