2-Oxopyrrolidines and 6-Oxoperhydropyrrolo[1,2-*a*]pyrazines as Templates in the Search for Nonpeptide Cholecystokinin Ligands

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In order to find new classes of non-peptide cholecystokinin (CCK) ligands, the conformational restriction of a series of weak 3-oxoindolizidine-based CCK antagonists has been both decreased and increased. This tactic yielded a series of monocyclic 2-oxopyrrolidine derivatives 4 with selectivity for CCK-A or CCK-B receptors and with slightly improved binding affinity at the CCK-A receptor subtype with respect to the model 3-oxoindolizidines. In contrast, the incorporation of the Trp residue at the secondary amino group of a pyrrolo[1,2-*a*]pyrazine template 5, involving a drastic restriction in the conformational flexibility of the molecule, resulted in a series of bicyclic derivatives that did not bind to CCK receptors at concentrations up to 10^{-5} M.

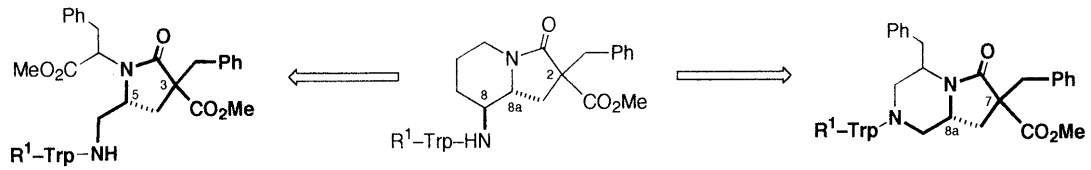
Key words cholecystokinin mimetic; 2-oxopyrrolidine; perhydropyrrolo[1,2-*a*]pyrazine

In a recent paper we showed that replacement of the Met^{31}–Asp^{32} dipeptide fragment in the *C*-terminal tetrapeptide of cholecystokinin (CCK-4, Trp–Met–Asp–Phe–NH_2) with the conformationally constrained (8*S*,8a*R*)-3-oxoindolizidine template led to a series of compounds that displayed moderate affinity at CCK-A or CCK-B receptors.[1] Within this series, compound **1** showed preference for the CCK-A over the CCK-B receptor subtype, while the selectivity was reversed in the case of its diastereoisomer **2** and the analogue **3**. The fact that these compounds bind to the CCK receptors suggests that they possess the appropriate pharmacophore groups, but their spatial disposition, fixed by the 3-oxoindolizidine skeleton, is not the most favorable for interacting with these receptors. Starting from this hypothesis, we thought that variations in the conformational flexibility of the appended aromatic side chains could serve to improve the CCK receptor binding, as described for other CCK-mimetics.[2] To this end, we selected two series of related analogues **4** and **5**, incorporating a 5(*R*)-2-oxopyrrolidine ring and a 8a(*R*)-6-oxoperhydropyrrolo[1,2-*a*]pyrazine nucleus, respectively, as templates for carrying the Trp and Phe side chains. On one hand, support for the selection for 5(*R*)-2-oxopyrrolidine comes from the successful use of a 1,3,4-trisubstituted-2-oxopyrrolidine ring as template for the construction of CCK-A antagonists.[3] On the other hand, the use of the perhydropyrido[1,2-*c*]pyrimidine skeleton, a nitrogen-bridged bicycle structurally related to **5**, as a template for appending the Trp and Phe side chains, has provided potent and highly selective CCK-A antagonists.[4]

When compared to the 3-oxoindolizidine model compounds, derivatives **4** and **5** keep unaltered the γ-lactam ring and, therefore, the available spatial dispositions of the Phe side chain, while the conformational flexibility of the Trp residue is enhanced and restricted, respectively. Since it has been reported that high binding affinity could be achieved with at least three appropriately oriented pharmacophores,[5] the additional benzyl and CO_2Me groups, present in **4** and **5**, could serve for increasing the affinity for the CCK receptors.

Results and Discussion

Compounds **4a—d** were synthesized from the corresponding 3,3-disubstituted γ-lactams **6a** and **6b**[6] in a sequence of reactions that includes *N*-Boc deprotection and condensation with Boc–L- or Boc–D-Trp–OH, using benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as the coupling agent. In order to establish the importance of the benzyl and the carboxylate substituents of the γ-lactam ring in the binding of these compounds to CCK receptors, compounds **4e** and **4f**, and **4g** and **4h** were prepared from the 3-monosubstituted and 3-unsubstituted analogues **6c** and **6d**, respectively. Finally, compounds **4i—l** were obtained by removal of the Boc-protecting group from derivatives



1: 2*S*, L-Trp, R^1= Boc
2: 2*R*, D-Trp, R^1= Boc
3: 2*R*, D-Trp, R^1= 1-Adc

Chart 1

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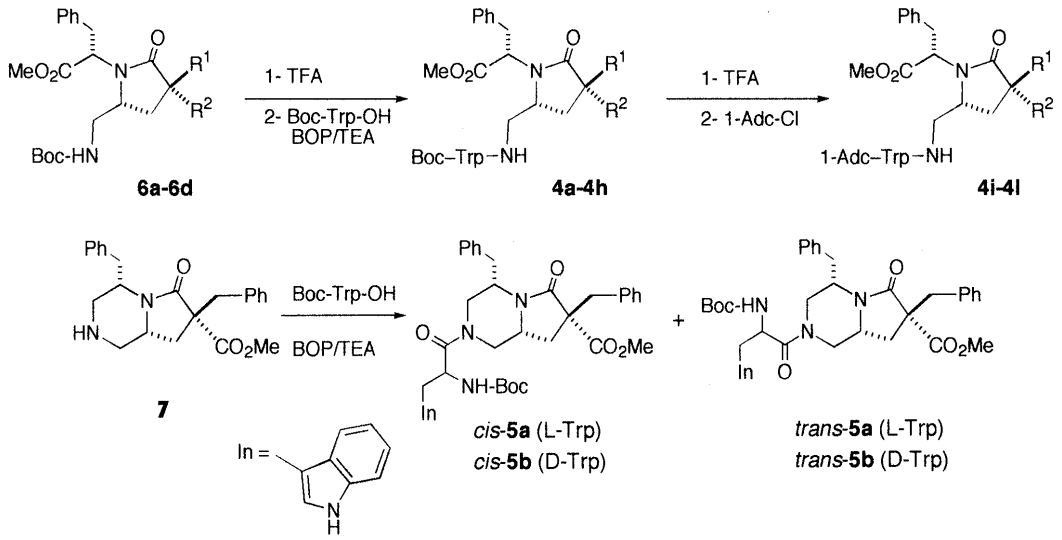


Chart 2

4a—**d**, followed by treatment of the resulting deprotected analogues with 1-adamantanecarbonyl chloride (1-Adc-Cl).

Coupling of Boc–L- or Boc–D-Trp with the secondary amino group of the 6-oxoperhydropyrrolo[1,2-*a*]pyrazine **7** afforded derivatives **5a** and **5b**, respectively.[7] As it has been reported for peptide derivatives containing alkylated peptide bonds,[8] the ^{1}H-NMR spectra of compounds **5a** and **5b** showed the presence of *cis*/*trans* isomerism around the CON bond of the secondary amide. The assignment of the signals corresponding to the *cis* and *trans* forms was based on the observation of a strong nuclear Overhauser effect (NOE) cross peak between the H-1eq and the Trp α-CH protons for the *cis* rotamer. The ratio of *cis*/*trans* rotamers in $CDCl_3$, calculated from the relative intensities of the Trp α-CH signals, was 1.2 : 1 for **5a** and 5 : 1 for **5b**.

All designed compounds, **4a**—**l**, **5a** and **5b**, were assayed for ability to displace [^{3}H]propionylCCK-8 binding to peripheral or central CCK receptors using rat pancreas or cerebral cortex homogenates, respectively (Table 1).[9] Model compounds **1**—**3** and Boc-CCK-4[10] were also included for comparative purposes.

As shown in the Table, the binding affinity and selectivity of compound **4a** were comparable to those of the model 3-oxoindolizidine **1**, just as the binding affinity and selectivity of compound **4d** were comparable to those of the corresponding model analogue **2**. Similarly to the 3-oxoindolizidine series, replacement of the D-Trp residue in **4d** with L-Trp to yield the diastereomer **4c**, led to a slight decrease in the binding affinity for CCK-B receptors. By contrast, while substitution of L-Trp by D-Trp in the oxoindolizidine **1** led to an analogue ineffective for binding to the CCK-A receptors, a similar substitution in the 2-oxopyrrolidine series improved the affinity for these receptors by one order of magnitude, yielding compound **4b**, the best ligand for the CCK receptors of this series. This divergence between the two series could be attributed to the enhanced flexibility of the Trp moiety in the oxopyrrolidines **4**. However, a certain participation of the additional benzyl and CO_2Me groups at the C-1′ position of the 2-oxopyrrolidine derivatives in the binding to CCK-A receptors cannot be ruled out.

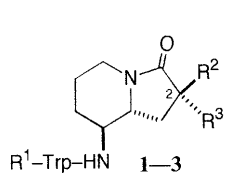
Removal of the 3-carboxymethyl or 3-carboxymethyl and 3-benzyl groups from compound **4a** or **4b** to yield 3-monosubstituted or 3-unsubstituted analogues **4e**—**h**, led to the total loss of affinity for CCK-A receptors. This fact indicated that both groups at position 3 of the γ-lactam ring are involved in the interaction with these receptors.

Although replacement of the Boc protecting group in **2** by the 1-Adc moiety (compound **3**) in the 3-oxoindolizidine series served to improve the CCK-B receptor binding affinity by one order of magnitude, the same modification of **4d** greatly reduced the binding affinity to CCK-B receptors in compound **4l**. The same results were found with analogues **4i**, **4j** and **4k** bearing the 1-Adc group as a protector of the L- or D-Trp residue. This difference between the related 3-oxoindolizidine and 2-oxopyrrolidine derivatives seems to indicate that the two series of compounds have different binding sites within the CCK receptors and that, in the last case, the hydrophobic pocket for the *N*-Trp substituent is unable to accommodate the bulky adamantane moiety.

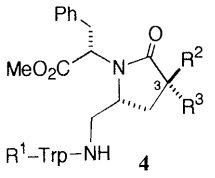
Finally, incorporation of the Trp residue at the secondary amino group of the pyrrolopyrazine template resulted in derivatives **5a** and **5b** that did not bind to CCK receptors at concentrations up to 10^{-5} M. Since in these compounds the conformational flexibility of the Trp residue is highly restricted, while the spatial arrangement of the 7-benzyl and 7-methoxycarbonyl groups must be very similar to those of the same substituents in compounds **4c**, **4d** and **2**, the lack of affinity of **5a** and **5b** could be mainly attributed to an inappropriate spatial disposition of the indole side chain and of the *N*-Boc group.

The above results imply that the spatial disposition of functional groups in **1** and **2** is conserved in compounds **4** for interaction with CCK receptors even though the conformational flexibility has been increased in **4**. However, the functional groups in the 6-oxoperhydropyrrolo[1,2-*a*]pyrazines **5a** and **5b** are not appropriately oriented for such an interaction. It was also shown that the decisive factor for receptor selectivity was the configuration at the C-3 groups in compounds **4** as at C-2

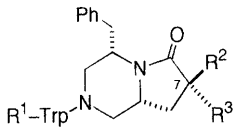
Table 1. Effect of Compounds **4** and **5** on Binding of [^{3}H]pCCK-8 to Rat CCK-A and CCK-B Receptors



1—3



4



5

Compd.	R^1	R^2	R^3	Config. C-2, C-3 or C-7	Config. Trp	IC_{50} (μM)[a]	
						CCK-A	CCK-B
4a	Boc	CO_2Me	CH_2Ph	*S*	L	6.8	>10
4b	Boc	CO_2Me	CH_2Ph	*S*	D	0.5	>10
4c	Boc	CH_2Ph	CO_2Me	*R*	L	>10	4.8
4d	Boc	CH_2Ph	CO_2Me	*R*	D	>10	1.5
4e	Boc	H	CH_2Ph	*R*	L	>10	>10
4f	Boc	H	CH_2Ph	*R*	D	>10	>10
4g	Boc	H	H	—	L	>10	>10
4h	Boc	H	H	—	D	>10	>10
4i	1-Adc	CO_2Me	CH_2Ph	*S*	L	>10	>10
4j	1-Adc	CO_2Me	CH_2Ph	*S*	D	>10	>10
4k	1-Adc	CH_2Ph	CO_2Me	*R*	L	>10	>10
4l	1-Adc	CH_2Ph	CO_2Me	*R*	D	>10	>10
5a	Boc	CH_2Ph	CO_2Me	*R*	L	>10	>10
5b	Boc	CH_2Ph	CO_2Me	*R*	D	>10	>10
1	Boc	CO_2Me	CH_2Ph	*S*	L	2.3	>10
2	Boc	CH_2Ph	CO_2Me	*R*	D	>10	3.7
3	1-Adc	CH_2Ph	CO_2Me	*R*	D	>10	0.2
Boc-CCK-4[b]						1.8	0.02

a) Rat pancreas (CCK-A) or cerebral cortex (CCK-B) homogenates were used for binding studies. The values given are the mean of at least three separate experiments. For all these values, standard errors were below 15% of the mean. *b*) From reference 10.

in the series of 3-oxoindolizidines. Although the importance of benzyl and methoxycarbonyl groups on the γ-lactam was shown, the binding activities of the best derivatives in these series, 3-oxoindolizidines and 2-oxopyrrolidines, were still only moderate. These facts suggest that the substituents on these two skeletons do not possess the optimal spatial disposition for CCK receptor interaction.

Compounds **4a**, **4b** and **4c**, **4d** behave as CCK-A and CCK-B receptor antagonists, respectively, in the isolated longitudinal muscle myenteric plexus preparation from guinea pig ileum.[11] Compounds **4a** and **4b**, added at 10^{-5} M concentration, were able to inhibit the contractions induced by CCK-8 in the guinea pig ileum by 75% and 81%, respectively, values in accordance with their binding affinities at CCK-A receptors. Similarly, a good correlation was found between the binding affinities at CCK-B receptors of compounds **4c** and **4d** and the percentage of inhibition (63% and 72%, respectively) of the contraction induced by CCK-4.

In conclusion, our efforts to identify novel series of CCK ligands, structurally related to that of the 3-oxoindolizidine-based compounds **1**—**3**, led, on one hand, to the CCK-A antagonists **4a** and **4b** and, on the other, to the CCK-B antagonists **4c** and **4d**. Although, in general there does not appear to be a great difference between the two series, the 2-oxopyrrolidine **4b** was the best compound, showing a 4-fold improvement in the binding affinity at CCK-A receptors with respect to the model 3-oxoindolizidines.

Experimental

^{1}H-NMR spectra were recorded with a Varian Gemini-200, a Varian XL-300 or a Varian Unity-500 spectrometer operating at 200, 300 or 500 MHz, respectively, using tetramethylsilane (TMS) as an internal standard. Elemental analyses were obtained on a CHN-O-RAPID instrument. Analytical TLC was performed on aluminium sheets coated with a 0.2 mm layer of Silica gel 60 F_{254} (Merck).Compounds were detected under UV light or with ninhydrin spray. Silica gel 60 (230—400 mesh, Merck) was used for column chromatography. Compounds **6** and **7** were synthesized as described.[6,7]

5-[(N^{α}-(*tert*-Butoxycarbonyl)tryptophyl)amino]methyl-2-oxopyrrolidine Derivatives General Procedure: A solution of the corresponding 2-oxopyrrolidine (**6a**—**d**, 0.5 mmol) in CH_2Cl_2 (3 ml) was treated with trifluoroacetic acid (TFA) (1.5 ml). After 4 h of reaction at room temperature the solvents were evaporated to dryness. The resulting residue was dissolved in dry CH_2Cl_2 (3 ml) and then Boc-L-Trp-OH or Boc-D-Trp-OH (0.54 mmol), BOP (0.54 mmol) and triethylamine (TEA) (1.04 mmol) were succesively added. The mixture was stirred overnight at room temperature, then CH_2Cl_2 and H_2O were added. The organic layer was dried over Na_2SO_4 and evaporated. The resulting residue was purified on a silica gel column using a gradient from 9% to 20% acetone in hexane as the eluent.

(3*S*,5*R*,1′*S*)-3-Benzyl-5-[(N^{α}-(*tert*-butoxycarbonyl)-L-tryptophyl)-amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-3-methoxycarbonyl-2-oxopyrrolidine (4a) Yield, 73% (from **6a**), foam. *Anal.* Calcd for $C_{40}H_{46}N_4O_8$: C, 67.59; H, 6.52; N, 7.88. Found: C, 67.79; H, 6.79; N, 7.73.

(3*S*,5*R*,1′*S*)-3-Benzyl-5-[(N^{α}-(*tert*-butoxycarbonyl)-D-tryptophyl)-amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-3-methoxycarbonyl-2-oxopyrrolidine (4b) Yield, 99% (from **6a**), foam. *Anal.* Calcd for $C_{40}H_{46}N_4O_8$: C, 67.59; H, 6.52; N, 7.88. Found: C, 67.41; H, 6.22; N, 7.58.

(3*R*,5*R*,1′*S*)-3-Benzyl-5-[(N^{α}-(*tert*-butoxycarbonyl)-L-tryptophyl)-amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-3-methoxycarbonyl-2-oxopyrrolidine (4c) Yield: 88% (from **6b**), foam. *Anal.* Calcd for $C_{40}H_{46}N_4O_8$: C, 67.59; H, 6.52; N, 7.88. Found: C, 67.27; H, 6.73; N, 7.59.

**(3*R*,5*R*,1′*S*)-3-Benzyl-5-[(N^{α}-(*tert*-butoxycarbonyl)-D-tryptophyl)-

Table 2. Significant Chemical Shifts of 2-Oxopyrrolidine Derivatives **4** (^{1}H-NMR, 500 MHz, $CDCl_3$)

Compd.	R^1	R^2	Trp	H-1′	H-2′	H-3	3-CH_2	H-5	5-CH_2	5′-NH	Trp α-CH	Trp α-NH
4a	CO_2Me	CH_2Ph	L	3.12	3.12	—	3.21 3.32	2.67	2.37 3.39	5.85	3.94	5.22
4b	CO_2Me	CH_2Ph	D	3.67	3.18	—	2.89 3.28	2.72	2.63 3.34	6.22	4.35	5.04
4c	CH_2Ph	CO_2Me	L	3.82	3.09 3.21	—	2.90 3.26	2.60	2.90 3.43	6.40	4.37	5.14
4d	CH_2Ph	CO_2Me	D	3.93	3.15	—	2.83 3.19	2.47	2.90 3.15	6.13	4.26	5.01
4e	H	CH_2Ph	L	3.23	3.05	2.28	2.39 2.51	2.51	3.05 3.35	6.32	4.17	5.15
4f	H	CH_2Ph	D	3.41	3.07 3.34	2.30	3.30	2.30	2.72 3.29	6.51	4.37	4.96
4g[a]	H	H	L	3.49	3.10 3.29	1.83	—	2.47	2.70 3.49	6.79	4.33	5.04
4h[b]	H	H	D	4.30	3.17	1.97 2.12	—	2.66	2.99 3.32	7.51	4.08	6.86
4i[c]	CO_2Me	CH_2Ph	L	3.12	2.82 3.08	—	3.01 3.24	2.59	2.35 3.37	5.76	4.10	6.49
4j[a]	CO_2Me	CH_2Ph	D	3.33	3.03 3.33	—	2.77 3.31	2.61	2.61 3.33	6.30	4.72	6.33
4k[c]	CH_2Ph	CO_2Me	L	3.75	3.13 3.25	—	2.82 3.30	2.58	2.86 3.47	6.41	4.65	6.34
4l[a]	CH_2Ph	CO_2Me	D	4.03	3.23 3.26	—	3.23	2.52	2.92 3.30	6.10	4.60	6.28

a) Registered at 300 MHz. *b*) Registered at 300 MHz in DMSO-d_6. *c*) Registered at 200 MHz.

Table 3. Significant Chemical Shifts of the Perhydropyrrolo[1,2-*a*]pyrazine Derivatives **5** (^{1}H-NMR, 500 MHz, $CDCl_3$)

Compd.	Trp	Rotamer	H-1 Axial	H-1 Equatorial	H-3 Axial	H-3 Equatorial	H-4	4-CH_2	7-CH_2	H-8	H-8a	α-Trp
5a	L	*cis*	2.14	3.57	1.69	2.97	3.43	2.64 3.78	2.96 3.36	1.55 2.01	2.26	4.96
		trans	2.64	3.65	2.01	2.97	3.14	2.43 3.25	3.09 3.16	2.01	1.90	4.56
5b	D	*cis*	1.50	2.71	2.71	3.73	3.51	2.59 3.28	2.97 3.30	1.65	1.50	4.63
		trans	2.33	4.21	2.71	3.62	3.65	2.54 3.03	3.00 3.38	1.50	2.14	4.50

amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-3-methoxycarbonyl-2-oxopyrrolidine (4d) Yield, 93% (from **6b**). *Anal.* Calcd for $C_{40}H_{46}N_4O_8$: C, 67.59; H, 6.52; N, 7.88. Found: C, 67.23; H, 6.12; N, 7.56.

(3*R*,5*R*,1′*S*)-3-Benzyl-5-[(*N*$^\alpha$-(*tert*-butoxycarbonyl)-L-tryptophyl)-amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-2-oxopyrrolidine (4e) Yield, 77% (from **6c**), foam. *Anal.* Calcd for $C_{38}H_{44}N_4O_6$: C, 69.92; H, 6.79; N, 8.58. Found: C, 69.90; H, 7.10; N, 8.31.

(3*R*,5*R*,1′*S*)-3-Benzyl-5-[(*N*$^\alpha$-(*tert*-butoxycarbonyl)-D-tryptophyl)-amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-2-oxopyrrolidine (4f) Yield, 98% (from **6c**), foam. *Anal.* Calcd for $C_{38}H_{44}N_4O_6$: C, 69.92; H, 6.79; N, 8.58. Found: C, 70.21; H, 7.05; N, 8.28.

(5*R*,1′*S*)-5-[(*N*$^\alpha$-(*tert*-Butoxycarbonyl)-L-tryptophyl)amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-2-oxopyrrolidine (4g) Yield, 88% (from **6d**), foam. *Anal.* Calcd for $C_{31}H_{38}N_4O_6$: C, 66.17; H, 6.81; N, 9.96. Found: C, 65.82; H, 7.12; N, 9.63.

(5*R*,1′*S*)-5-[(*N*$^\alpha$-(*tert*-Butoxycarbonyl)-D-tryptophyl)amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-2-oxopyrrolidine (4h) Yield, 92% (from **6d**), white solid, mp 174—176 °C (acetone/hexane). *Anal.* Calcd for $C_{31}H_{38}N_4O_6$: C, 66.17; H, 6.81; N, 9.96. Found: C, 66.32; H, 6.53; N, 9.62.

Significant ^{1}H-NMR chemical shifts of these compounds are listed in Table 2.

5-[(*N*$^\alpha$-(1-Adamantanecarbonyl)tryptophyl)amino]methyl-2-oxopyrrolidine Derivatives General Procedure: A solution of the appropriate compound **4a—d** (50 mg, 0.07 mmol) in CH_2Cl_2 (1 ml) was treated with TFA (0.5 ml) and stirred at room temperature for 1 h. After evaporation of the solvents, the resulting residue was dissolved in dry tetrahydrofuran (THF) (1 ml) and treated with TEA (0.022 ml, 0.16 mmol) and 1-adamantanecarbonyl chloride (18 mg, 0.09 mmol). The mixture was stirred at room temperature for 1 h, then the solvent was evaporated off. The resulting residue was purified on a silica gel column using a gradient from 15 to 60% EtOAc in hexane as the eluent.

(3*S*,5*R*,1′*S*)-3-Benzyl-5-[(*N*$^\alpha$-(1-adamantanecarbonyl)-L-tryptophyl)-amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-3-methoxycarbonyl-2-oxopyrrolidine (4i) Yield, 89% (from **4a**), foam. *Anal.* Calcd for $C_{46}H_{52}N_4O_8$: C, 70.03; H, 6.64; N, 7.10. Found: C, 69.79; H, 6.51; N, 7.24.

(3*S*,5*R*,1′*S*)-3-Benzyl-5-[(*N*$^\alpha$-(1-adamantanecarbonyl)-D-tryptophyl)-amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-3-methoxycarbonyl-2-oxopyrrolidine (4j) Yield, 76% (from **4b**), foam. *Anal.* Calcd for $C_{46}H_{52}N_4O_8$: C, 70.03; H, 6.64; N, 7.10. Found: C, 69.84; H, 6.69; N, 7.07.

(3*R*,5*R*,1′*S*)-3-Benzyl-5-[(*N*$^\alpha$-(1-adamantanecarbonyl)-L-tryptophyl)-amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-3-methoxycarbonyl-2-oxopyrrolidine (4k) Yield, 58% (from **4c**), foam. *Anal.* Calcd for $C_{46}H_{52}N_4O_8$: C, 70.03; H, 6.64; N, 7.10. Found: C, 70.16; H, 6.41; N, 7.14.

(3*R*,5*R*,1′*S*)-3-Benzyl-5-[(*N*$^\alpha$-(1-adamantanecarbonyl)-D-tryptophyl)-amino]methyl-1-(2′-phenyl-1′-methoxycarbonyl)ethyl-3-methoxycarbonyl-2-oxopyrrolidine (4l) Yield, 65% (from **4d**). *Anal.* Calcd for $C_{46}H_{52}N_4O_8$: C, 70.03; H, 6.64; N, 7.10. Found: C, 69.95; H, 6.88; N, 6.87.

Significant ^{1}H-NMR chemical shifts of these compounds are listed in Table 2.

Perhydropyrrolo[1,2-*a*]pyrazine Derivatives General Procedure: A solution of the perhydropyrrolopyrazine **7** (30 mg, 0.079 mmol) in CH_2Cl_2 (3 ml) was treated with Boc-L-Trp-OH or Boc-D-Trp-OH (26.5 mg, 0.087 mmol), BOP (38.5 mg, 0.087 mmol) and TEA (0.12 ml, 0.087 mmol). The mixture was stirred overnight at room temperature, then CH_2Cl_2 and H_2O were added. The organic layer was dried over Na_2SO_4 and evaporated. The resulting residue was purified on a silica gel column using a gradient from 10% to 40% EtOAc in hexane as the eluent.

(4*S*,7*R*,8a*R*)-4,7-Dibenzyl-2-(*N*$^\alpha$-*tert*-butoxycarbonyl-L-tryptophyl)-7-methoxycarbonyl-6-oxoperhydropyrrolo[1,2-*a*]pyrazine (5a) Yield, 72%. *Anal.* Calcd for $C_{39}H_{44}N_4O_6$: C, 70.46; H, 6.67; N, 8.43. Found: C, 70.16; H, 6.95; N, 8.10.

(4*S*,7*R*,8a*R*)-4,7-Dibenzyl-2-(*N*$^\alpha$-*tert*-butoxycarbonyl-D-tryptophyl)-7-methoxycarbonyl-6-oxoperhydropyrrolo[1,2-*a*]pyrazine (5b) Yield, 80%. *Anal.* Calcd for $C_{39}H_{44}N_4O_6$: C, 70.46; H, 6.67; N, 8.43. Found: C, 70.85; H, 6.75; N, 8.35.

Significant ^{1}H-NMR chemical shifts of these compounds are listed in Table 3.

Receptor Binding Studies CCK-A and CCK-B receptor binding assays were performed in rat pancreas and cerebral cortex homogenates, respectively, according to a method previously described,[9] with minor modifications. Briefly, pancreatic membranes (0.2 mg protein per tube) were incubated with 0.5 nM [^{3}H]pCCK-8 in piperazinebis(ethanesulfonic acid) (PIPES) HCl buffer, pH 6.5, containing 30 mM $MgCl_2$, 0.2 mg·ml^{-1} bacitracin and 0.2 mg·ml^{-1} soybean trypsin inhibitor (SBTI), for 120 min at 25 °C. Brain membranes (0.45 mg per tube) were incubated with 1 nM

[^{3}H]pCCK-8 in 50 mM Tris–HCl buffer, pH 7.4, containing 5 mM $MgCl_2$ and 0.2 mg·ml^{-1} bacitracin, for 60 min at 25 °C. Final incubation volume was 0.5 ml in both cases. Non specific binding was determined with CCK-8 1 μM as the cold displacer.

Isolated Longitudinal Muscle-Myenteric Plexus (LMMP) Preparation Guinea-pigs were killed and bled. The ileum was excised approximately 10 cm from the ileocecal junction and longitudinal muscle strips with the myenteric plexus attached (LMMP) were prepared. These LMMP strips were placed in a 10 ml organ bath containing Krebs bicarbonate buffer, maintained at 37 °C and aerated with 95% O_2 5% CO_2. Tissues were equilibrated for 30 min at 0.5 mg applied force and then stimulated with square wave pulses (0.1 Hz) of supramaximal voltage (15 V) and 0.7 ms duration to stabilize base-line force. Stimulation was then discontinued and tissues were challenged with KCl (47 mM) to determine initial muscle contractility. After control responses to KCl had been obtained, noncumulative concentration–response curves to CCK-8 and CCK-4 were obtained by stepwise increases in concentration every 10 min. In the experiments designed to evaluate the effect of drugs on the contractile response to the peptides, each tissue was equilibrated for 20 min with drug (10^{-5} M) or vehicle before challenge with peptide (CCK-8, 10^{-8} M; CCK-4, 10^{-6} M). Responses after antagonists were compared with responses obtained in other tissues treated with the vehicle.

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