5-(Tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine-Based Potent and Selective CCK_1 Receptor Antagonists: Structural Modifications at the Tryptophan Domain

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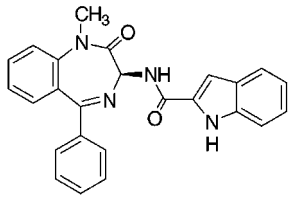
Analogues of the previously reported potent and highly selective CCK_1 receptor antagonist (4a*S*,5*R*)-2-benzyl-5-(*N*-Boc-tryptophyl)amino-1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine (**2a**) were prepared to explore the structural requirements at the Boc-tryptophan domain for CCK_1 receptor affinity. Structural modifications of **2a** involved the Trp side chain, its conformational freedom, the Boc group, and the carboxamide bond. Results of the CCK binding and in vitro functional activity evaluation showed three highly strict structural requirements: the type and orientation of the Trp side chain, the H-bonding acceptor carbonyl group of the carboxamide bond, and the presence of the Trp amino protection Boc. Replacement of this acid-labile group with 3,3-dimethylbutyryl or *tert*-butylaminocarbonyl conferred acid stability to analogues **14a** and **15a**, which retained a high potency and selectivity in binding to CCK_1 receptors, as well as an in vivo antagonist activity against the acute pancreatitis induced by caerulein in rats. Oral administration of compounds **14a** and **15a** also produced a lasting antagonism to the hypomotility induced by CCK-8 in mice, suggesting a good bioavailability and metabolic stability.

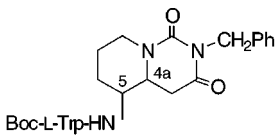
Introduction

The peptide Cholecystokinin (CCK) displays hormonal and neurotransmitter activities in the digestive tract and in the central nervous system (CNS)[1] and exerts its biological effects via at least two G protein coupled receptor subtypes, termed CCK_1 and CCK_2.[2] CCK_1 receptors are mainly found in the digestive tract, where they are involved in the diverse effects of CCK on the regulation of digestive processes[1] (pancreatic enzyme secretion, gut motility, and gallbladder contraction). They are also found in selected CNS areas, where they regulate dopamine release[1−3] and antagonize opioid analgesia.[1,4] Furthermore, CCK_1 receptors mediate the satiety effect of CCK in the CNS and in the peripheral nervous system[1,2,5] and are present in several tumoral cell lines.[2] CCK_2 receptors are predominantly found throughout the CNS[2] and are considered to be primarily involved in anxiety-related conditions,[6−9] memory processes,[1,10] and also in neuropsychiatric disorders.[1,11,12]

The variety of possible therapeutic utilities for CCK receptor agonists and antagonists has prompted an intensive research in this area and, over the past decade, a number of potent and selective non-peptide CCK_1 and CCK_2 receptor antagonists have been reported.[13] Some of these antagonists have been useful tools for characterizing the two CCK receptor subtypes and for gaining further insight into the functional significance of CCK in the periphery and in the CNS; nevertheless, the physiological effects of CCK mediated by CCK_1 or CCK_2 receptors are not completely established.[1,2] Despite the predominant role suggested for CCK_2 receptors in anxiety and in the control of nociception, anxiolytic-like effects and enhancement of morphine analgesia have been reported for the typical CCK_1 antagonist Devazepide[14,15] (**1**). It has been suggested[16] that these effects could be due to the blockade of CCK_2 receptors by Devazepide at high doses, since this



Devazepide (**1**)



2a: (4a*S*,5*R*)

2b: (4a*R*,5*S*)

compound is not completely devoid of affinity for this receptor subtype (selectivity for CCK_1 over CCK_2 varies from 170-[17] to 3000-fold[18]). Caution has also been recommended, in general, when attempting to differentiate between effects mediated by CCK_1 or CCK_2 receptors with Devazepide.[19] In view of these facts, the development of CCK receptor antagonists with higher selectivity for CCK_1 over CCK_2 receptors is of interest in order to shed further light on the functional roles of CCK receptor subtypes. In this regard, we recently reported the design, synthesis[20] and pharmacological properties[21] of the 5-(tryptophyl)amino-1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine derivative **2a** (IQM-95,333), a highly potent and selective CCK_1 receptor antagonist,

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both in vitro and in vivo. This novel compound showed a CCK_1 receptor affinity similar to that of Devazepide,[20] but it was virtually devoid of affinity at brain CCK_2 receptors. Interestingly, compound **2a** also showed a marked anxiolytic-like activity in animal models, and blocked the CCK-8-induced hypophagia and hypolocomotion effects after intraperitoneal administration.[21] From the first modifications performed on compound **2a**, the 4a,5-*trans* stereochemistry of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine skeleton and the L-configuration of the Boc-Trp residue emerged as essential features for CCK_1 binding affinity and subtype receptor selectivity.[20] Modifications of this residue have been now performed in order to define which functional groups are critical for binding to the receptor and to explore the tolerance of the receptor to conformational constraints. These modifications involve the Trp side chain, its conformational freedom, the Boc group and the carboxamide bond. Additionally, some of the modifications herein reported could lead to analogues with enhanced oral bioavailability and duration of action. The present paper deals with the synthesis, CCK receptor binding profile, and in vivo antagonist activity against the acute pancreatitis induced by caerulein in rats and against the hypomotility induced by CCK-8 in mice of this series of Boc-Trp modified analogues of the above indicated CCK_1 receptor antagonists.

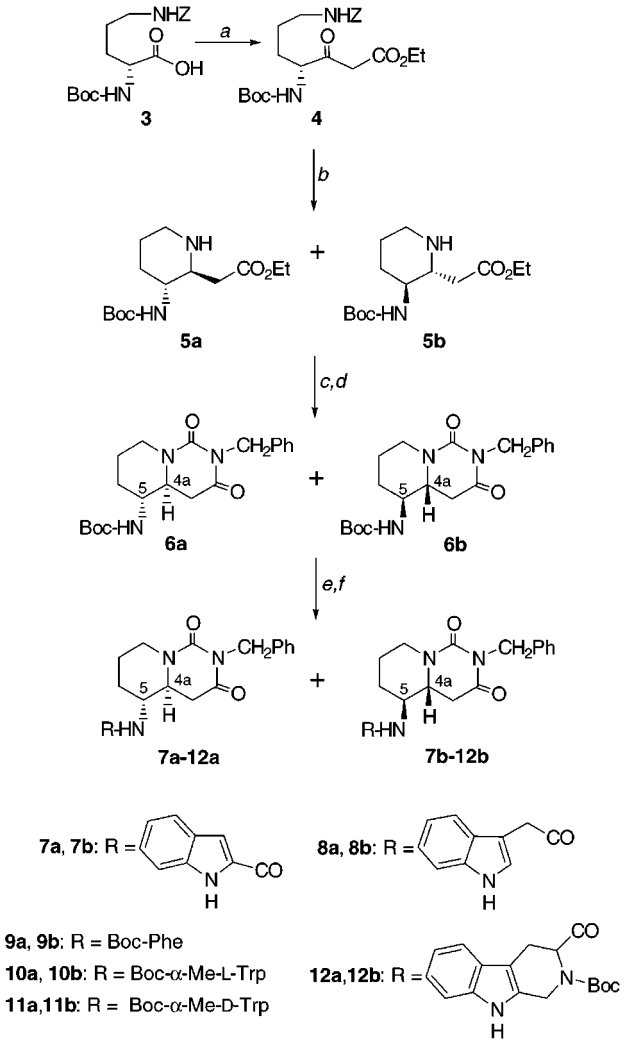
Chemistry

In an attempt to reduce the molecular weight of the parent compounds **2**, we initially synthesized analogues **7** and **8**, in which the Boc-Trp moiety was respectively replaced by indol-2-yl-carbonyl, the key group in Devazepide for binding to CCK_1 receptors,[22] and the indol-3-yl-acetyl residue.[23] Then, analogues **9**−**12**, containing Phe, α-Me-L-Trp, α-Me-D-Trp, and the tetrahydro-*β*-carbolin-3-yl-carbonyl in place of Trp, were prepared in order to study the influence of the type and conformational freedom of the side chain upon the binding affinity at CCK receptors. Compounds **7**−**12** were prepared following a synthetic route similar to that used for the synthesis of the model compounds **2**, as indicated in Scheme 1. This route involves sequential *N*-Boc removal in the 5-Boc-amino-1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine derivatives **6**, followed by coupling with the corresponding acid or Boc protected amino acid derivative. The starting 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidines **6** were obtained in four steps from Boc-D-Orn(Z)-OH, as a (4:1) racemic mixture of the (4a*S*,5*R*)- and (4a*R*,5*S*)-isomers, or as a (1:4) mixture from Boc-L-Orn(Z)-OH, as previously described.[20] Therefore, compounds **7** and **8** were obtained as racemic mixtures, and **9**−**12** were obtained as diastereoisomeric mixtures **a**,**b**, which could be chromatographically resolved only in the cases of **9** and **12**.

The ^{1}H NMR data for the lead compound **2a**, particularly the Trp $J_{\alpha,\beta}$ values (2.7 and 5.2 Hz) and the NOE effect between the *NH*-Boc and the indole 2-H protons, suggested a preferred *gauche* (−) conformation for the Trp side chain ($\chi_1 \approx -60$)[24,25] (Figure 1). We expected that the tetrahydro-*β*-carboline skeleton in compound **12a** could fix the Trp preferred conformation. However, the $J_{3,4}$ values in the ^{1}H NMR spectrum of **12a** (0 and 6 Hz) indicated an equatorial disposition for the tetrahydro-*β*-carboline 3-H proton and, therefore, a fixed *gauche* (+) conformation ($\chi_1 \approx 60$) for the restricted Trp side chain in this compound.

Taking into account the expected low stability of the *N*-Boc protection in the acid medium of the stomach,[26] our second objective was to change this protection in

Scheme 1[a]



[a] Reagents: (a) 1,1′-carbonyldiimidazole, $LiCH_2CO_2Et$, THF; (b) H_2, Pd(C), EtOH or MeOH; (c) benzyl isocyanate, THF; (d) NaH, THF; (e) TFA, CH_2Cl_2; (f) R-OH, BOP, Et_3N.

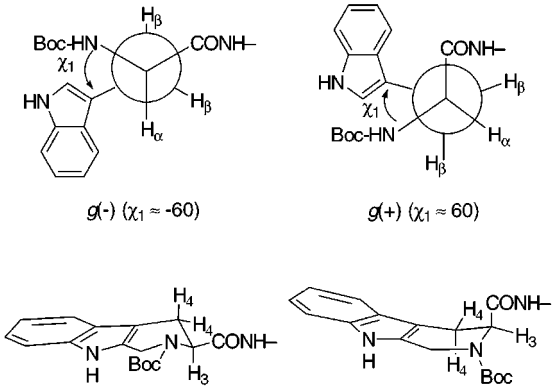
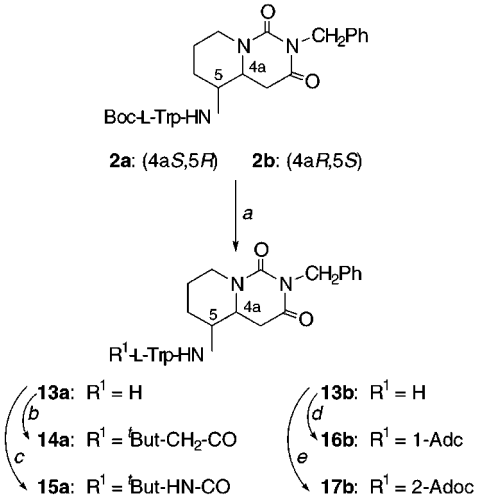


Figure 1. Newman projections of the *g*(−) and *g*(+) staggered ratational states of the Cα−C*β* bond in the Trp residue of compound **2a**, and the corresponding conformations of the tetrahydro-*β*-carboline skeleton in **12a**.

Scheme 2[a]



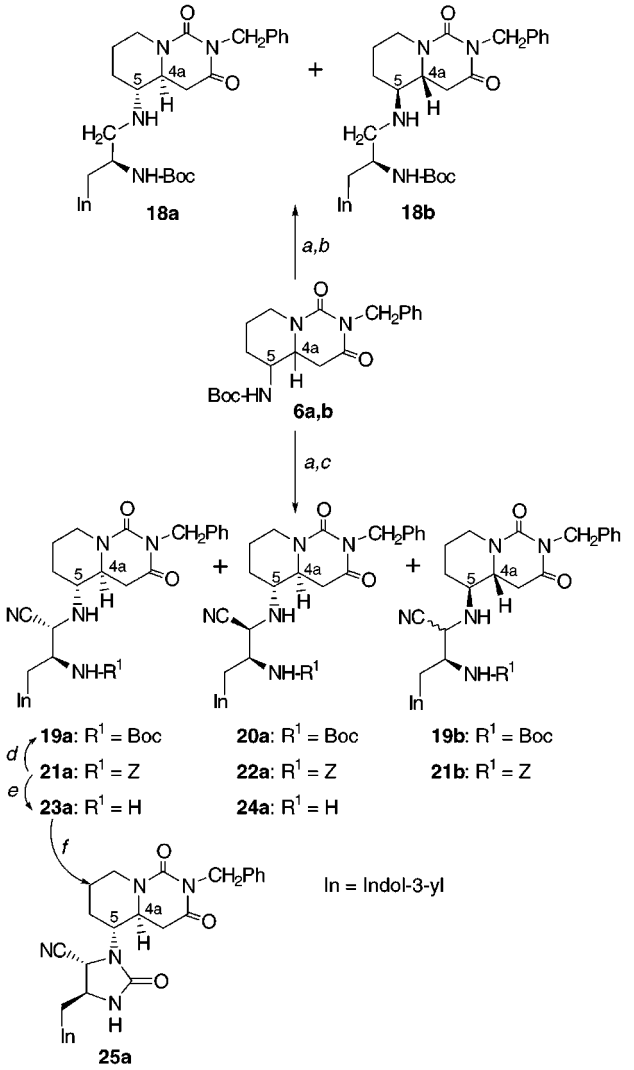
[a] Reagents: (a) TFA, CH_2Cl_2; (b) 3,3-dimethylbutyryl chloride, Et_3N, THF; (c) *t*-butyl isocyanate, Et_3N,THF; (d) 1-adamantylcarbonyl chloride, Et_3N, THF; (e) 2-adamantyl chloroformate, Et_3N, THF.

order to increase the stability in acid pH, without loss in the binding affinity. With this aim, the urethane Boc group was replaced in **2a** by its carboxamide and ureido bioisosters, the 3,3-dimethylbutyryl and *tert*-butylaminocarbonyl groups, respectively, giving compounds **14a** and **15a**, and in **2b** by the *N* protecting groups used in the series of dipeptoid CCK receptor antagonists,[27] 1-adamantylcarbonyl (1-Adc) and 2-adamantyloxycarbonyl (2-Adoc), obtaining analogues **16b** and **17b** (Scheme 2). A comparative RP HPLC study on the stability of compounds **2a**, **14a** and **15a** in acid medium showed that the half-life of **2a** in 2 N HCl solution in (1:1) H_2O−MeOH was 17 min, while that of **15a** was 11 days, and the analogue **14a** remained unaltered after that time.

Finally, the carboxamide bond between the Trp and the 1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine skeleton of compound **2a** was replaced by the reduced peptide bond Ψ[CH_2NH] in compounds **18a**,**b** and by the cyanomethyleneamino Ψ[CH(CN)NH] peptide bond surrogate in analogues **19**−**22** (Scheme 3). Both peptide bond modifications have been used in pseudopeptides to increase stability toward peptidases and to study if the replaced peptide bond has a functional role or merely serves to orient and align the side chains.[28−30] The Ψ[CH_2NH] peptide bond surrogate causes an increase of flexibility in the peptide backbone and a decrease in the H-bonding properties by loss of the H-bonding acceptor amide carbonyl group.[28,31] In contrast, the Ψ-[CH(CN)NH] was proposed as peptide bond surrogate based on the hypothesis that its cyano group keeps H-bonding acceptor properties and that it could impart higher backbone rigidity than the Ψ[CH_2NH].[32,33] On the other hand, both peptide bond surrogates have the additional advantage of introducing a basic NH, which could be protonated, enhancing the solubility in aqueous systems.[28]

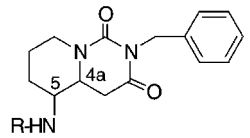
The reduced peptide bond analogues **18a**,**b** were obtained from the (4:1) racemic mixture of the 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives **6a**,**b** by *N*-Boc removal, followed by *N*-alkylation of the resulting deprotected compounds with Boc-L-tryptophanal, using $NaBH_3CN$ as reducing agent in the presence of $ZnCl_2$. Only the major isomer **18a** could be isolated from the diastereoisomeric mixture **18a**,**b**. With respect to the cyanomethyleneamino analogues **19**−**22**, these were prepared applying our methodology,[34] which involves reaction of the 1,3-dioxoperhydropyrido[1,2-*c*]-pyrimidine derivatives **6a**,**b**, after *N*-Boc deprotection, with Boc-L-tryptophanal or Z-L-tryptophanal and (trimethylsilyl)cyanide (TMSCN) in the presence of $ZnCl_2$. In this process a new chiral center is generated; therefore, in the reaction with Boc-L-tryptophanal from the (4:1) racemic mixture **6a**,**b**, two isomers with a (4a*S*,5*R*)-configuration [**19a** (31%) and **20a** (21%)] were obtained, and only one (**19b** (9%)) with the (4a*R*,5*S*)-configuration was obtained. The assignment of the absolute configuration of the new generated stereogenic center is usually made through the formation of an imidazolidin-2-one ring.[34] For this purpose it was necessary to remove the Trp amino-protection. However, the Boc removal was not possible in compounds **19** and **20** due to the extreme susceptibility of the indole ring of these compounds to oxidative degradation in acid media,

Scheme 3[a]



[a] Reagents: (a) TFA, CH_2Cl_2; (b) Boc-L-Trp-H, Et_3N, $ZnCl_2$, $NaBH_3CN$, MeOH; (c) Boc-L-Trp-H or Z-L-Trp-H, $ZnCl_2$, TMSCN, MeOH; (d) H_2, Pd(C), $(Boc)_2O$, MeOH; (e) H_2, Pd(C), MeOH; (f) bis(trichloromethyl)carbonate, Et_3N, CH_2Cl_2.

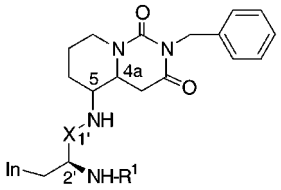
Table 1. Inhibition of the [^{3}H]pCCK-8 Specific Binding to Rat Pancreas (CCK_1) and Cerebral Cortex Membranes (CCK_2) by Compounds **7**−**12**



compd	stereochemistry	R	IC_{50} (nM)[a] CCK_1	IC_{50} (nM)[a] CCK_2
CCK-8			1.04 ± 0.08	5.60 ± 0.30
Devazepide			0.71 ± 0.05	696 ± 134
2a	(4a*S*,5*R*)	Boc-L-Trp	1.59 ± 0.10	>10000
2b	(4a*R*,5*S*)	Boc-L-Trp	22.7 ± 4.0	6153
7ab	(4:1) (4a*R*,5*S*):(4a*S*,5*R*)	In-2-yl-CO	>1000	>10000
8ab	(4:1) (4a*R*,5*S*):(4a*S*,5*R*)	In-3-yl-CH_2-CO	>1000	>10000
9a	(4a*S*,5*R*)	Boc-L-Phe	65.7 ± 22.6	>10000
10ab	(6:1) (4a*S*,5*R*):(4a*R*,5*S*)	Boc-α-Me-L-Trp	42.4	>10000
11ab	(6:1) (4a*S*,5*R*):(4a*R*,5*S*)	Boc-α-Me-D-Trp	1236	>10000
12a	(4a*S*,5*R*)	2-Boc-THBC-3-yl-CO[b]	226	>10000
12b	(4a*R*,5*S*)	2-Boc-THBC-3-yl-CO[b]	>1000	>10000

[a] Values are the mean or mean ± SEM of at least three experiments, performed with seven concentrations of test compounds in triplicate. [b] THBC = tetrahydro-β-carboline.

Table 2. Inhibition of the [^{3}H]pCCK-8 Specific Binding to Rat Pancreas (CCK_1) and Cerebral Cortex Membranes (CCK_2) by Compounds **13**−**22**



compd	stereochemistry	R^1	X	IC_{50} (nM)[a] CCK_1	IC_{50} (nM)[a] CCK_2
Devazepide				0.71 ± 0.05	696 ± 134
2a	(4a*S*,5*R*)	Boc	CO	1.59 ± 0.10	>10000
2b	(4a*R*,5*S*)	Boc	CO	22.7 ± 4.0	6153
13a[b]	(4a*S*,5*R*)	H	CO	>1000	>10000
13b[b]	(4a*R*,5*S*)	H	CO	>1000	>10000
14a	(4a*S*,5*R*)	^{t}But-CH_2-CO	CO	4.43 ± 0.36	>10000
15a	(4a*S*,5*R*)	^{t}But-HN-CO	CO	0.91 ± 0.08	>10000
16b	(4a*R*,5*S*)	1-Adc	CO	486	4390
17b	(4a*R*,5*S*)	2-Adoc	CO	340	3430
18a	(4a*S*,5*R*)	Boc	CH_2	>1000	>10000
19a	(4a*S*,5*R*)	Boc	[(*R*)CHCN]	7.69 ± 0.86	>10000
20a	(4a*S*,5*R*)	Boc	[(*S*)CHCN]	32.6 ± 5.26	>10000
21a	(4a*S*,5*R*)	Z	[(*R*)CHCN]	>1000	>10000
22a	(4a*S*,5*R*)	Z	[(*S*)CHCN]	>1000	>10000

[a] Values are the mean or mean ± SEM of at least three experiments, performed with seven concentrations of test compounds in triplicate. [b] Tested as trifluoroacetate salt.

even by using different described conditions to minimize this problem.[35] In view of the difficulties in removing the Boc protection, the Z protected analogues **21a** (34%), **22a** (17%), and **21b** (10%) were prepared similarly. Then, after Z removal in **21a** and **22a** by catalytic hydrogenolysis, both of the resulting deprotected diastereoisomers **23a** and **24a** were subjected to reaction with bis(trichloromethyl)carbonate. However, only the isomer **23a** led to the corresponding imidazolidin-2-one derivative **25a**. The absolute configuration at the C_5 of the imidazolidin-2-one ring of this compound, and therefore at the Ψ[CH(CN)NH] chiral center of **21a** and **23a**, was established on the basis of its $J_{4,5}$ value (5 Hz) in its ^{1}H NMR spectrum, indicative of a *trans* disposition for the 4-H and 5-H imidazolidin-2-one protons.[34] Afterward, the configurational assignment in the Boc analogues was made through the catalytic hydrogenolysis of **21a** in the presence of di(*tert*-butyl) dicarbonate, which led directly to the major diastereoisomer **19a**. The low amount obtained of the minor isomers **19b** and **21b** did not allow their configuration assignment.

Biological Results and Discussion

The affinity of the new 1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives **7**−**22** at CCK_1 and CCK_2 receptors was determined by measuring the displacement of [^{3}H]propionyl-CCK-8 binding to rat pancreatic and cerebral cortex homogenates, respectively, as previously described.[36] The data are depicted in Tables 1 and 2. For comparative purposes CCK-8, Devazepide, and the model compounds **2a** and **2b** were also included in the assay. Like these model compounds, the new 1,3-

dioxoperhydropyrido[1,2-*c*]pyrimidine derivatives **7**−**22** bound preferentially to the CCK$_1$ receptor subtype. The replacement of Boc-Trp by the key Devazepide pharmacophoric group indol-2-yl-carbonyl and by its analogue indol-3-yl-acetyl in compounds **7** and **8**, respectively, led to the complete loss of affinity (Table 1). The phenylalanine analogue **9a** showed 1 order of magnitude lower CCK$_1$ binding potency than the lead compound **2a**. In contrast to the good results obtained in the dipeptoid CCK antagonist series by introducing an α-methyl substitution into their Trp residue,[27,37,38] the introduction of this substitution into **2a** or in its D-Trp analogue[20] to give **10** and **11** led to a decrease of 1 and 3 orders of magnitude, respectively, in the binding affinity. The conformational restriction introduced by the tetrahydro-*β*-carboline system into **12a** had a strong detrimental effect on the binding affinity. This effect could be due to the aforementioned fact that this skeleton fixes the *gauche* (+) Trp side chain conformation and not the preferred *gauche* (−) conformation of the prototype **2a**. In regard to the Trp amino protection (Table 2), it seems necessary for binding to CCK$_1$ receptors, as indicated by the inactivation of compounds **2a** and **2b** when deprotected to give **13a** and **13b**. The replacement of the urethane *N*-Boc protection of **2a** by its bioisosters, more stable to chemical degradation in acid media, the 3,3-dimethylbutyryl and *tert*-butylaminocarbonyl groups in **14a** and **15a** did not have a significant effect upon their affinity at CCK receptors. However, the exchange of this protection in **2b** for the usual[27] protections in dipeptoid antagonist 1-adamantylcarbonyl or 2-adamantyloxycarbonyl led to 1 order of magnitude lower CCK$_1$ binding potency in analogues **16b** and **17b**. This decrease, along with the fact that this replacement was the only modification which produced a slight increase in CCK$_2$ binding affinity, discouraged the preparation of the corresponding diastereoisomers **16a** and **17a**. Finally, in relation to the carboxamide bond (Table 2), its replacement by the reduced peptide bond in compound **18a** led to the complete loss of activity, while the incorporation of the [CH(CN)NH] group as a carboxamide surrogate was translated into a small decrease in the binding affinity for the (*R*)-epimer **19a** and into 1 order of magnitude lower potency in the case of the (*S*)-epimer **20a**. As the main difference between the [CH_2NH] and the [CH(CN)-NH] carboxamide surrogates lies in their H-bonding properties, rather than in their geometry,[31] these data indicate the importance of the H-bonding acceptor amide carbonyl or cyano groups for the binding of **2a** or **19a** to CCK$_1$ receptors. It also confirms the utility of the [CH(CN)NH] group as a carboxamide surrogate.[31] It is also interesting to note the complete loss of affinity when the Boc protection of **19a** and **20a** was exchanged for the Z protection in **21a** and **22a**.

Compounds with the higher affinity at CCK$_1$ receptors, **14a** and **15a**, were tested in vivo for their protective effect on the experimental acute pancreatitis induced by caerulein in rats, which is known to be sensitive to CCK$_1$ antagonists,[39] and for their antagonism to the hypomotility induced by CCK-8 in mice, which is also selectively reversed by antagonists at this CCK receptor subtype.[40] The model compound **2a**, as well as Devazepide, were comparatively included in these studies.

Table 3. Effect of Intraperitoneal Administration of CCK$_1$ Antagonists on the Increase in Plasma Amylase and Lipase Activities Induced by Caerulein in Rats[a]

treatment	plasma amylase (IU/mL)	plasma lipase (IU/mL)
vehicle	2073 ± 108	15 ± 1
caerulein	52101 ± 2221	3100 ± 146
Devazepide (0.1) + caerulein	8793 ± 1353	125 ± 26**
Devazepide (1) + caerulein	3650 ± 352**	26 ± 2**
2a (0.1) + caerulein	35985 ± 6387	1943 ± 505*
2a (1) + caerulein	2721 ± 275**	112 ± 21**
14a (0.1) + caerulein	61102 ± 3466	2956 ± 212
14a (1) + caerulein	28015 ± 4774**	1140 ± 218**
15a (0.1) + caerulein	27103 ± 3539**	1667 ± 224**
15a (1) + caerulein	5326 ± 434**	36 ± 5**

[a] Pancreatitis induced by four sc injections of caerulein (10 μg/kg) at hourly intervals. Test compounds (doses in mg/kg ip) given 30 min before the first caerulein injection *p < 0.05, **p < 0.01 vs caerulein (ANOVA followed by Sheffé's test).

Table 4. Effect of Oral Administration of CCK$_1$ Antagonists on the Increase in Plasma Amylase and Lipase Activities Induced by Caerulein in Rats[a]

treatment	plasma amylase (IU/mL)	plasma lipase (IU/mL)
vehicle	3308 ± 285	15 ± 1
caerulein	39293 ± 3522	2801 ± 288
Devazepide (0.1) + caerulein	7733 ± 1209**	871 ± 351**
Devazepide (1) + caerulein	4578 ± 516**	57 ± 9**
2a (0.1) + caerulein	37753 ± 1682	2837 ± 184
2a (1) + caerulein	31205 ± 1900	1948 ± 232
14a (0.1) + caerulein	51420 ± 3673	3089 ± 128
14a (1) + caerulein	46591 ± 2292	3375 ± 73
15a (0.1) + caerulein	28939 ± 2330	2200 ± 164
15a (1) + caerulein	19967 ± 552**	1062 ± 122**

[a] Pancreatitis induced like in Table 3. Test compounds (doses in mg/kg po) given 60 min before the first caerulein injection *p < 0.05, **p < 0.01 vs caerulein (ANOVA followed by Sheffé's test).

All of the tested compounds, at the same dose of 1 mg/kg ip, were able to significantly prevent in the acute pancreatitis model in rats the enormous rise in plasma amylase and lipase induced by caerulein. With a 10-fold lower dose, only Devazepide and **15a** showed a significant antagonism on the rise of both enzyme activities (Table 3). Since the affinity of **2a** and **15a** at CCK$_1$ receptors was approximately identical, it is supposed that the bioavailability of **15a** is higher. Such contention is supported by the results obtained in this pancreatitis model after oral administration of test compounds. As can be seen in Table 4, only Devazepide was effective at the lower dose of 0.1 mg/kg po while **15a**, but not **2a**, significantly prevented the rise in enzyme activity at the dose of 1 mg/kg po.

Spontaneous locomotor activity of mice was significantly reduced by CCK-8, 10 μg/kg, by approximately 60% (Figure 2). All of the tested compounds were able to significantly reverse the hypomotility when given ip at the dose of 0.1 mg/kg (not shown). The time course of the antagonism to CCK-8 was studied after oral administration of a 10-fold higher dose, 1 mg/kg, which did not produce on its own any significant effect on the locomotion. While the antagonism to the hypomotility was significant with all four compounds given 1 or 2 h before CCK-8, only Devazepide significantly antagonized the hypomotility when given 4 h before CCK (Figure 2). Nevertheless, at this time point the distance travelled by mice receiving CCK-8 or the combined treatment CCK-8 + **2a** was almost identical (Figure 2A),

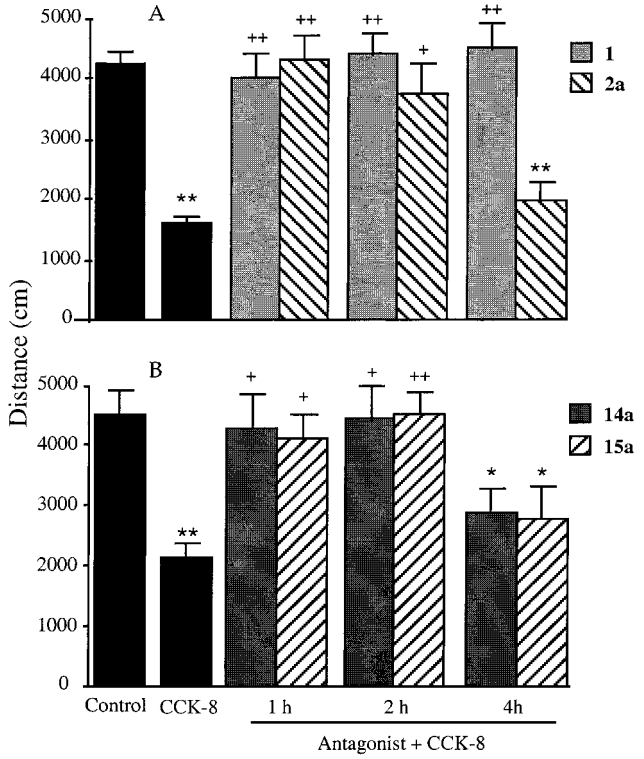


Figure 2. Effect of the CCK_1 antagonists devazepide (**1**) and **2a** (A), **14a** and **15a** (B) on the hypomotility induced by CCK-8 in mice. Antagonists (1 mg/kg po) were given at the times indicated before CCK-8 (10 μg/kg). Spontaneous locomotor activity (mean ± SEM of 8−12 animals) was measured 5 min after CCK-8 for 30 min $^{*}p < 0.05$ $^{**}p < 0.01$ vs control; $^{+}p < 0.05$, $^{++}p < 0.01$ vs CCK-8 (ANOVA followed by Scheffé's test).

whereas a certain trend toward an antagonism of the CCK-8 effect by either compound **14a** or **15a** was observed in Figure 2B, suggesting a slightly higher oral bioavailability in mice of compounds **14a** and **15a** as compared to compound **2a**.

In conclusion, the results herein described show the strong structural requirements at the tryptophan domain for CCK_1 receptor binding of this new family of highly selective CCK_1 antagonists based on the structure of 5-(tryptophyl)amino-1,3-dioxoperhydropyrido-[1,2-*c*]pyrimidine. Thus, both the indol of the Trp side chain and its orientation are important structural features, and the presence of the H-bonding acceptor carbonyl group of the carboxamide bond and the Boc group or some surrogate, such as the 3,3-dimethylbutyryl or the *tert*-butylaminocarbonyl, is essential. Compounds **14a** and **15a**, which incorporate these surrogates, with much higher chemical stability in acid media than the lead compound **2a**, showed similar CCK receptor in vitro activity to that of this prototype. These changes enhanced or tended to enhance their oral bioavailability. This improvement in the in vivo CCK_1 receptor antagonism profile was particularly significant in the case of the *tert*-butylaminocarbonyl derivative **15a**.

Experimental Section

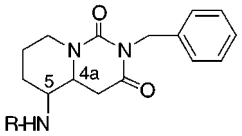
Chemistry. All reagents were of commercial quality. Solvents were dried and purified by standard methods. Amino acid derivatives were obtained from Bachem Feinchemikalien AG. Analytical TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F_{254}, Merck. Silica gel 60 (230−400 mesh), Merck, was used for flash chromatography. Melting points were taken on a micro hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian Gemini-200,Varian XL-300, or Unity-500 spectrometer, operating at 200, 300, or 500 MHz, using TMS as reference. ^{13}C NMR spectra were recorded with the Varian Gemini-200 spectrometer, operating at 50 MHz. Elemental analyses were obtained on a CH-O-RAPID apparatus. Analytical HPLC was performed on a Waters Nova-pak C_{18} (3.9 × 150 mm, 4 μm) column, with a flow rate of 1 mL/min, and using a tunable UV detector set at 214 nm. Mixtures of CH_3CN (solvent A) and 0.05% TFA in H_2O (solvent B) were used as mobile phase.

General Procedure for the Synthesis of the 1,3-Dioxoperhydropyrido[1,2-*c*]pyrimidine Derivatives 7−12. TFA (0.5 mL) was added to a solution of (4a*R**,5*S**)-2-benzyl-5-[(*tert*-butoxycarbonyl)amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine [**6a**,**b**, obtained as a (1:4) racemic mixture of **6a** and **6b**, from Boc-L-Orn(Z)-OH for **7a**,**b** and **8a**,**b**, and as a (4:1) mixture from Boc-D-Orn(Z)-OH for **9**−**12**] (75 mg, 0.2 mmol) in CH_2Cl_2 (1 mL); after 1 h at room temperature, the solvents were evaporated to dryness. The residue was dissolved in dry CH_2Cl_2 (5 mL), and the corresponding acid (indol-3-yl-acetic acid, indol-2-yl-carboxylic acid, Boc-L-Phe-OH, Boc-α-Me-L-Trp-OH, Boc-α-Me-D-Trp-OH, or 2-Boc-tetrahydro-β-carbolin-3-yl-carboxylic acid) (0.22 mmol), (benzotriazolyloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP; 97 mg, 0.22 mmol), and triethylamine (63 μL, 0.45 mmol) were added successively to that solution; stirring was continued at room temperature for 12 h. Afterward, the reaction mixture was diluted with CH_2Cl_2 (20 mL), and the resulting solution was washed successively with 10% citric acid (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL), H_2O (2 × 10 mL), and brine (10 mL), dried over Na_2SO_4, and evaporated. Flash chromatography of the residue with 20−50% gradients of EtOAc in hexane yielded in each case: the (1:4) racemic mixtures **7ab** (68%) and **8ab** (71%), the (6:1) diastereoisomeric mixtures **10ab** (90%) and **11ab** (94%), which could not be resolved, and the (4:1) diasteroisomeric mixtures **9ab** and **12ab**. Preparative TLC of **9ab** with (9:1) hexane−EtOAc yielded isolated **9a** (85%), while **12ab** was resolved by preparative TLC with (9:1) ethyl ether−EtOAc into **12a** (49%) and **12b** (7%). The most significant analytical and spectroscopic data of these compounds are summarized in Table 5.

Synthesis of (4a*S*,5*R*)- and (4a*R*,5*S*)-2-Benzyl-5-(L-tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine Trifluoroacetate (13a and 13b). TFA (0.5 mL) was added to a solution of (4a*S*,5*R*)- or (4a*R*,5*S*)-2-benzyl-5-(Boc-L-tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (**2a** or **2b**) (112 mg, 0.2 mmol) in CH_2Cl_2 (1 mL); after 1 h at room temperature, the solvents were evaporated to dryness. Flash chromatography of the residue with 1−5% gradient of MeOH in CH_2Cl_2 yielded the corresponding *N* deprotected tryptophyl derivative **13a** or **13b**, respectively, whose significant analytical and spectroscopic data are summarized in Table 6.

General Procedure for the Replacement of the *N*-Boc Protecting Group of 2a and 2b. Synthesis of Compounds 14a−17b. TFA (0.5 mL) was added to a solution of (4a*S*,5*R*)- or (4a*R*,5*S*)-2-benzyl-5-(Boc-L-tryptophyl)amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (**2a** or **2b**) (112 mg, 0.2 mmol) in CH_2Cl_2 (1 mL); after 1 h at room temperature, the solvents were evaporated to dryness. The residue was dissolved in dry THF (1 mL), and triethylamine (56 μL, 0.4 mmol) was added to that solution, which was stirred for 10 min. Then, the corresponding acylating agent (3,3-dimethylbutyryl chloride, *tert*-butyl isocyanate, 1-adamantylcarbonyl chloride, or 2-adamantyl chloroformate) was added (0.25 mmol), stirring was continued at room temperature for 1.5 h, and the reaction mixture was evaporated to dryness. The resulting residue was purified by preparative TLC with (1:2) EtOAc−ethyl ether in the tryptophyl derivatives **14a** and **15a**, and by flash chromatography in the case of **16b** and **17b**, using a (33−50%)

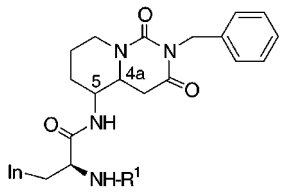
Table 5. Significant Analytical and Spectroscopic Data of Compounds **7**−**12**



	7ab	**8ab**	**9a**	**10ab**	**11ab**	**12a**	**12b**
R	In-2-yl-CO	In-3-yl-CH_2-CO	Boc-L-Phe	Boc-α-Me-L-Trp	Boc-α-Me-D-Trp	2-Boc-THBC-3-yl-CO[a]	2-Boc-THBC-3-yl-CO[a]
stereochemistry	(4a*R**,5*S**)	(4a*R**,5*S**)	(4a*S*,5*R*)	(4a*R**,5*S**)	(4a*R**,5*S**)	(4a*S*,5*R*)	(4a*R*,5*S*)
formula[b]	$C_{24}H_{24}N_4O_3$	$C_{25}H_{26}N_4O_3$	$C_{29}H_{36}N_4O_5$	$C_{32}H_{39}N_5O_5$	$C_{32}H_{39}N_5O_5$	$C_{32}H_{37}N_5O_5$	$C_{32}H_{37}N_5O_5$
yield (%)	71	68	85	90	94	49	7
mp (°C)	210−212	91−92	168−170			128−130	139−141
t_R (A:B)[c]	13.16 (45:55)	9.36 (45:55)	46.54 (33:67)	42.00 (33:67)	46.27 (33:67)	37.93 (37:63)	36.13 (37:63)
^{1}H NMR[d]							
α (R)[e]		3.64	4.15	1.53, 1.55	1.52, 1.54	5.16[f]	5.20[f]
2-CH_2	4.93	4.80, 4.90	4.92, 4.98	4.96	5.02	4.88, 4.96	4.92
4-H	2.76, 2.91	2.61	2.56, 2.72	2.54, 2.76	2.57, 2.69 2.57, 2.54	2.34	2.39, 2.49
4a-H	3.22	2.70	2.92	2.89, 2.96	2.87, 2.95	3.07	3.00
5-H	3.94	3.64	3.61	3.71	3.69	3.64	3.62
5-NH	5.88	5.47	5.59	6.18, 6.35	6.16, 6.35	5.83	5.76
6-H	1.20, 2.09	1.00, 1.81	1.08, 1.66	1.25, 1.88 1.25, 1.73	1.18, 1.81 1.25, 1.87	1.30, 1.94	1.40, 1.88
7-H	1.45−1.90	1.40−1.65	1.50	1.59, 1.73	1.44−1.59	1.56, 1.76	1.56, 1.73
8-H	2.65, 4.34	2.38, 4.18	2.51, 4.18	2.60, 4.33 2.60, 4.31	2.56, 4.30 2.56, 4.33	2.61, 4.32	2.60, 4.32

[a] THBC = tetrahydro-β-carboline. [b] Satisfactory analyses for C, H, N. [c] A = CH_3CN; B = 0.05% TFA in H_2O. [d] In $CDCl_3$, measured at 300 MHz. [e] H, except for **10ab** and **11ab** where it is CH_3. [f] $J_{3,4}$ in the tetrahydro-β-carboline skeleton 0 and 6 Hz.

Table 6. Significant Analytical and Spectroscopic Data of Compounds **13**−**17**



	13a	**13b**	**14a**	**15a**	**16b**	**17b**
R^1	H	H	^{t}But-CH_2-CO	^{t}But-NH-CO	1-Adc	2-Adoc
stereochemistry	(4a*S*,5*R*)	(4a*R*,5*S*)	(4a*S*,5*R*)	(4a*S*,5*R*)	(4a*R*,5*S*)	(4a*R*,5*S*)
formula[a]	$C_{28}H_{30}F_3N_5O_5$[b]	$C_{28}H_{30}F_3N_5O_5$[b]	$C_{32}H_{39}N_5O_4$	$C_{31}H_{38}N_6O_4$	$C_{37}H_{43}N_5O_4$	$C_{37}H_{43}N_5O_5$
yield (%)	89	92	80	86	83	59
mp (°C)	foam	foam	138−140	132−134	117−119	137−139
t_R (A:B)[c]	5.33 (33:67)	14.34 (30:70)	35.13 (40:60)	57.67 (33:67)	31.20 (33:67)	23.67 (33:67)
^{1}H NMR[d]						
α-H (Trp)	4.29	4.19	4.66	4.53	4.71	4.48
NH-R^1			5.99	5.51	5.74	5.32
2-CH_2	4.82, 4.93	4.87, 4.97	4.89, 4.98	4.84, 4.96	4.91, 4.97	4.91, 4.98
4-H	2.75, 2.90	2.64, 2.78	2.51, 2.69	2.52, 2.64	2.23	2.16
4a-H	3.27	3.42	2.86	2.70	2.51	2.54
5-H	3.52	3.76	3.54	3.57	3.57	3.59
5-NH	8.27	7.32	5.98	5.51	5.74	5.38
6-H	1.20	1.48−1.89	0.97, 1.59	0.98, 1.50	1.14, 1.87	1.17, 1.89
7-H	1.56	1.48−1.89	1.43	1.50	1.48−1.84	1.41−1.84
8-H	2.62, 4.12	2.71, 4.25	2.50, 4.26	2.46, 4.21	2.50, 4.24	2.49, 4.24

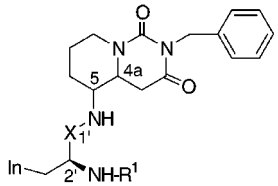
[a] Satisfactory analyses for C, H, N. [b] As trifluoroacetate salt. [c] A = CH_3CN; B = 0.05% TFA in H_2O. [d] Measured at 300 MHz in $CDCl_3$, except for **13a** and **13b** which were registered in $(CD_3)_2CO$.

gradient of EtOAc in hexane as eluant. Significant analytical and spectroscopic data of these compounds are summarized in Table 6.

Synthesis of (4a*S*,5*R*)-2-Benzyl-5-[(2*S*)-2-*N*-(*tert*-butoxycarbonyl)amino-3-(indol-3-yl)-propyl]amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (18a). TFA (0.5 mL) was added to a solution of (4a*R**,5*S**)-2-benzyl-5-[(*tert*-butoxycarbonyl)amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine [**6a**,**b**, obtained as a (9:1)[20] racemic mixture of **6a** and **6b**] (75 mg, 0.2 mmol) in CH_2Cl_2 (1 mL); after 1 h at room temperature, the solvents were evaporated to dryness. The residue was dissolved in MeOH (1.5 mL), and 4 Å molecular sieves and triethylamine (28 μL, 0.2 mmol) were added; after 15 min of stirring, Boc-L-tryptophanal (58 mg, 0.2 mmol), $ZnCl_2$ (14 mg, 0.1 mmol), and $NaBH_3CN$ (16 mg, 0.21 mmol) were added successively, and stirring at room temperature was continued for 15 h. Afterward, the reaction mixture was filtered and evaporated to dryness. The residue was dissolved in EtOAc (50 mL), and the resulting solution was washed successively with 0.1 N HCl (2 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL), H_2O (2 × 25 mL), and brine (25 mL), dried over Na_2SO_4, and evaporated. Flash chromatography of the residue with 4% of EtOAc in ethyl ether allowed the isolation of the major diastereoisomer **18a**, whose significant analytical and spectroscopic data are shown in Table 7.

Synthesis of the Cyanomethyleneamino Carboxamide Surrogate Containing Compounds 19−**22.** TFA (1 mL) was added to a solution of (4a*R**,5*S**)-2-benzyl-5-[(*tert*-butoxycar-

Table 7. Significant Analytical and Spectroscopic Data of Compounds **18**−**22**



	18a	**19a**	**19b**	**20a**	**21a**	**21b**	**22a**
R^1	Boc	Boc	Boc	Boc	Z	Z	Z
X	CH_2	[(*R*)CHCN]	[CHCN]	[(*S*)CHCN]	[(*R*)CHCN]	[CHCN]	[(*S*)CHCN]
stereochemistry	(4a*S*,5*R*)	(4a*S*,5*R*)	(4a*R*,5*S*)	(4a*S*,5*R*)	(4a*S*,5*R*)	(4a*R*,5*S*)	(4a*S*,5*R*)
formula[a]	$C_{31}H_{39}N_5O_4$	$C_{32}H_{38}N_6O_4$	$C_{32}H_{38}N_6O_4$	$C_{32}H_{38}N_6O_4$	$C_{35}H_{36}N_6O_4$	$C_{35}H_{36}N_6O_4$	$C_{35}H_{36}N_6O_4$
yield (%)	62	31	9	21	34	10	17
mp (°C)	80−82	99−101	102−104	106−108	68−70	foam	67−69
t_R (A:B)[b]	31.27 (40:60)	17.40 (45:55)	16.53 (45:55)	20.13 (45:55)	20.13 (45:55)	20.33 (45:55)	20.20 (45:55)
^{1}H NMR[c]							
1′-H	2.46, 2.98	3.57	3.64	3.79	3.49	3.64	3.81
2′-H	3.96	4.22	4.26	4.27	4.17	4.28	4.34
NH-R^1	4.60	4.74	4.80	4.86	5.07	5.03	5.20
2-CH_2	4.96	4.95	4.95	4.96, 4.90	4.87	4.93	4.97, 4.90
4-H	2.70, 3.02	2.60, 2.93	2.47, 2.87	2.50, 2.92	2.43, 2.76	2.33, 2.80	2.45, 2.84
4a-H	2.98	2.86	2.87	2.93	2.61	2.80	2.84
5-H	2.15	2.23	2.21	2.40	2.09	2.24	2.37
5-NH	2.17	1.69	1.71	1.72	1.69	1.62	1.68
6-H	1.10, 2.15	1.12, 2.40	1.06, 2.21	1.12, 1.95	1.03, 2.27	1.16, 2.24	0.98, 1.93
7-H	1.72, 1.47	1.44, 1.70	1.39, 1.73	1.44, 1.69	1.38, 1.65	1.25−1.76	1.36, 1.66
8-H	2.63, 4.31	2.53, 4.26	2.53, 4.27	2.60, 4.30	2.28, 4.21	2.56, 4.28	2.54, 4.27
^{13}CNMR[d]							
$C_{1'}$	50.60	53.17	53.94	52.93	54.34	54.75	53.94
CN		120.10	118.48	117.77	120.18	118.53	117.82

[a] Satisfactory analyses for C, H, N. [b] A = CH_3CN; B = 0.05% TFA in H_2O. [c] In $CDCl_3$, registered at 300 MHz. [d] In $CDCl_3$, measured at 50 MHz.

bonyl)amino]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine [**6a**,**b**, obtained as a (9:1)[20] racemic mixture of **6a** and **6b**] (160 mg, 0.43 mmol) in CH_2Cl_2 (2 mL); after 1 h at room temperature, the solvents were evaporated to dryness. The residue was dissolved in MeOH (3 mL), to this solution triethylamine (67 μL, 0.47 mmol) was added, and after 15 min of stirring, the reaction mixture was cooled at −20 °C. Boc- or Z-L-tryptophanal (1.29 mmol), $ZnCl_2$ (59 mg, 0.43 mmol), and TMSCN (0.161 μL, 1.29 mmol) were added successively, and stirring was continued, first at −20 °C for 1 h and then at 0 °C for 15 h. Afterward, the reaction mixture was evaporated to dryness. The residue was dissolved in EtOAc (50 mL), and the resulting solution was washed successively with H_2O (2 × 25 mL) and brine (25 mL), dried over Na_2SO_4, and evaporated. Preparative TLC of the residue using 1% of MeOH in CH_2Cl_2 yielded in each case, in decreasing order of R_f, the Boc protected compounds **19a**, **19b**, and **20a**, and the Z protected analogues **21a**, **21b**, and **22a**, whose significant analytical and spectroscopic data are summarized in Table 7.

Synthesis of (4a*S*,5*R*)-2-Benzyl-5-[(4*S*,5*R*)-5-cyano-4-(indol-3-yl)methyl-2-oxoimidazolidin-1-yl]-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (25a). A solution of (4a*S*,5*R*)-2-benzyl-5-[(1*R*,2*S*)-2-*N*-(benzyloxycarbonyl)amino-1-cyano-3-(indol-3-yl)-propyl]amino-1,3-dioxoperhydropyrido[1,2-*c*]pyrimidine (**21a**) (60 mg, 0.1 mmol) in 10^{-5} N HCl in MeOH (20 mL) was hydrogenated at room temperature and 40 psi of pressure for 4 days, in the presence of 10% Pd(C) (28 mg). After filtering off the catalyst, the solvent was evaporated, and the resulting residue was dissolved in dry CH_2Cl_2 (6 mL). Triethylamine (28 μL, 0.2 mmol) was added to the resulting solution, and the mixture was stirred at room temperature for 15 min. Then, bis(trichloromethyl)carbonate (6 mg, 0.05 mmol) and triethylamine (17 μL, 0.12 mmol) were added at 0 °C, and the stirring was continued at this temperature for 12 h. Afterward, the reaction mixture was diluted with CH_2Cl_2 (25 mL), washed with water (10 mL) and brine (10 mL), and dried over Na_2SO_4. Removal of the solvent and preparative TLC of the residue, using 6% of MeOH in CH_2Cl_2 as eluant, gave the 2-oxoimidazolidine derivative **25a** as a foam (13 mg, 26%): RP HPLC t_R = 5.73 (A:B = 40:60); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.54 (m, 1H, 7-H), 1.80 (m, 3H, 6-H and 7-H), 2.63 (m, 1H, 4-H), 2.67 (m, 1H, 8-H), 2.94 (dd, 1H, 4-H, J = 6 and 17 Hz), 3.34 and 3.36 [2dd, 2H, 4-CH_2 (2-oxoimidazolidine), J = 6 and 15 Hz], 3.37 (m, 1H, 5-H), 3.79 (m, 1H, 4a-H), 4.16 [d, 1H, 5-H (2-oxoimidazolidine), J = 5 Hz], 4.25 [m, 1H, 4-H (2-oxoimidazolidine)], 4.35 (m, 1H, 8-H), 4.94 and 5.00 (2d, 2H, 2-CH_2, J = 14 Hz), 7.13−7.54 (m, 10H, aromatics), 8.27 [s, 1H, 1-H (In)]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.77 (C_7), 27.10 (C_6), 30.75 [4-CH_2 (2-oxoimidazolidine)], 33.59 (C_4), 44.14 (2-CH_2), 44.96 (C_8), 49.14 [C_5 (2-oxoimidazolidine)], 52.72 (C_{4a}), 55.47 [C_5, and C_4 (2-oxoimidazolidine)], 108.41 [C_3 (In)], 111.75 [C_7 (In)], 117.28 (CN), 117.95 [C_4 (In)], 120.39 [C_5 (In)], 122.91 [C_4 (Ph)], 123.18 [C_2 (In)], 126.97 [C_{3a} (In)], 127.36 [C_6 (In)], 128.40 and 128.46 [C_2 and C_3 (Ph)], 136.33 [C_{7a} (In)], 137.59 [C_1 (Ph)], 153.53 (C_1), 158.19 [C_1 (2-oxoimidazolidine)], 167.38 (C_3). Anal. ($C_{28}H_{28}N_6O_3$) C, H, N.

Binding Assays. CCK_1 and CCK_2 receptor binding assays were performed using rat pancreas and cerebral cortex homogenates respectively, according to the method described by Daugé et al,[36] with minor modifications. Briefly, rat pancreas tissue was carefully cleaned and homogenized in Pipes HCl buffer, pH 6.5, containing 30 mM $MgCl_2$ (15 mL/g of wet tissue), and the homogenate was then centrifuged twice at 4 °C for 10 min at 50000*g*. For displacement assays, pancreatic membranes (0.2 mg protein/tube) were incubated with 0.5 nM [^{3}H]pCCK-8 in Pipes HCl buffer, pH 6.5, containing $MgCl_2$ (30 mM), bacitracin (0.2 mg/mL), and soybean trypsin inhibitor (SBTI, 0.2 mg/mL), for 120 min at 25 °C. Rat brain cortex was homogenized in 50 mM Tris-HCl buffer pH 7.4 containing 5 mM $MgCl_2$ (20 mL/g of wet tissue), and the homogenate was centrifuged twice at 4 °C for 35 min at 100000*g*. Brain membranes (0.45 mg protein/tube) were incubated with 1 nM [^{3}H]pCCK-8 in 50 mM Tris-HCl buffer, pH 7.4, containing $MgCl_2$ (5 mM) and bacitracin (0.2 mg/mL) for 60 min at 25 °C. Final incubation volume was 0.5 mL in both cases. Nonspecific binding was determined using 1 μM CCK-8 as the cold displacer. The inhibition constants (K_i) were calculated

using the equation of Cheng and Prusoff from the displacement curves analyzed with the receptor fit competition LUNDON program.

Caerulein-Induced Acute Pancreatitis in Rats. Acute pancreatitis was induced in male Wistar rats (190−210 g) deprived of food for 24 h by four subcutaneous injections of caerulein (10 μg/kg) at hourly intervals as described.[41] CCK antagonists were administered by intraperitoneal or oral route (0.25 mL/100 g) 30 or 60 min, respectively, before the first caerulein injection. Three hours after the last caerulein injection, blood was collected and the pancreas was removed. Amylase and lipase activities in plasma were measured by means of commercially available kits (Boehringer Mannheim).

Spontaneous Locomotor Activity. Hypomotility was induced in mice by ip injection of CCK-8 (10 μg/kg) 5 min before recording the locomotion for 30 min.[40] Test compounds (0.1 mL/10 g) were given ip 30 min before CCK or po at different times before CCK. Locomotion was measured by placing the mice in a black wooden open-topped box (65 × 65 × 45 cm^3) and recording the distance travelled in centimeters by using a digital Videomex-V-system (Columbus Inst.) working with the appropriate computer program.

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