Boc-CCK-4 Derivatives Containing Side-Chain Ureas as Potent and Selective CCK-A Receptor Agonists

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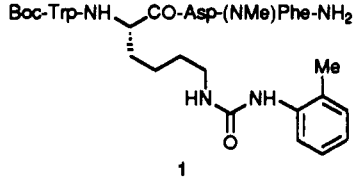
Novel Boc-CCK-4 derivatives were communicated recently as having high potency and selectivity for the CCK-A receptor (Shiosaki et al. *J. Med. Chem.* **1990**, *33*, 2950–2952). While Boc-CCK-4 binds selectively to the CCK-B receptor, replacement of the methionine with an N^{ϵ}-substituted lysine dramatically reversed receptor selectivity, leading to the development of this novel series of tetrapeptides. A detailed structure–activity analysis of a series of urea-substituted tetrapeptides, represented by the general structure Boc-Trp-Lys(N^{ϵ}-CO-NHR)-Asp-Phe-NH_2, revealed that a number of substituted phenyl, naphthyl, and aliphatic urea residues in the lysine side chain yielded potent and selective CCK-A ligands. These tetrapeptides elicit full agonist responses in stimulating pancreatic amylase release that are effectively blocked by a selective CCK-A receptor antagonist. Conversion of the urea to a thiourea significantly reduced CCK-A binding potency as did replacement of the lysine with the homologous ornithine or homolysine. Tetrapeptides that were partial agonists (<80% efficacy) in phosphoinositide (PI) hydrolysis relative to CCK-8 did not exhibit high-dose inhibition of amylase secretion in guinea pig acini.

Cholecystokinin (CCK) is a brain–gut peptide that is found in a number of mammalian species. In the periphery, CCK promotes gall bladder contraction and secretion of pancreatic enzymes[1,2] and may modulate CNS-linked behaviors associated with feeding[3] through activation of CCK receptors on peripheral vagal afferent fibers.[4] These effects appear to be mediated by the CCK-A (alimentary) receptors, which are found predominantly in peripheral tissues such as the pancreas.[5,6] The prevalent CCK receptors in the central nervous system (CNS) have been labeled CCK-B (brain) and possess a similar ligand binding profile as the peripheral gastrin receptor. These two CCK receptors can be differentiated by their relative affinity for CCK fragments, most notably by Boc-CCK-4, which possesses high affinity (nM) for the CCK-B receptor and low affinity (μM) for the CCK-A receptor.[7]

Recently, we communicated a series of CCK tetrapeptide analogues, typified by A-71623 (1), that possess



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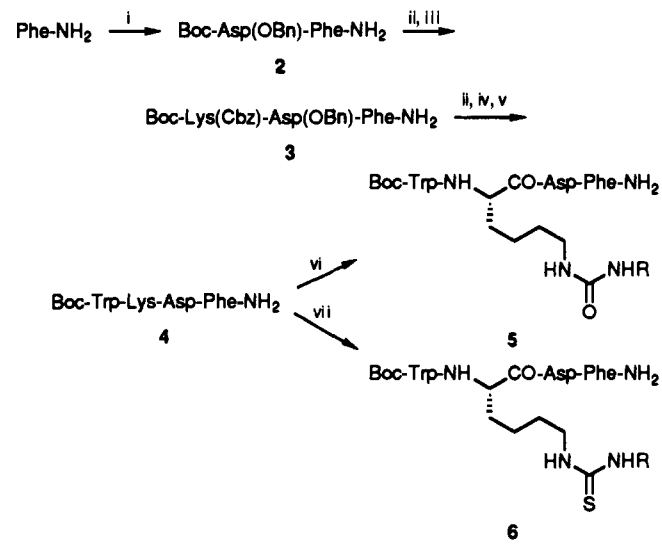
high affinity and selectively for the CCK-A receptor and elicit full agonist responses relative to CCK-8 in stimulating amylase release and phosphoinositide (PI) breakdown in pancreas.[7,8] These stimulatory effects were potently inhibited by the selective CCK-A antagonist, MK-329.[9] Acute intraperitoneal and intramuscular administration of A-71623 effectively suppressed food intake in rodents and cynomologous monkeys, respectively.[10] In addition, chronic administration of the tetrapeptide in rodents over an 11-day period maintained this suppression of food intake and resulted in significant reduction in body weight gains over control animals.

The reversal in receptor selectivity from Boc-CCK-4, which is 70-fold CCK-B selective, to these novel tetrapeptides possessing up to 1000-fold selectivity for the CCK-A receptor was achieved by substitution of methionine in Boc-CCK-4 with a modified lysine residue. In contrast, potent CCK-A agonists that have been reported to date require peptide lengths of seven or eight amino acids and an acidic moiety such as a sulfated tyrosine.[11,12] The corresponding non-sulfated hepta- and octapeptides

Scheme I[a]



[a] **Reagents:** (i) Boc-Asp(OBn)-OH, EDCI, HOBt; (ii) HCl in HOAc; (iii) Boc-Lys(Cbz)-OH, EDCI, HOBt, *N*-methylmorpholine; (iv) Boc-Trp hydroxysuccinimide ester, *N*-methylmorpholine; (v) H_2, 10% Pd–C; (vi) RNCO, *N*-methylmorpholine; (vii) RNCS, *N*-methylmorpholine.

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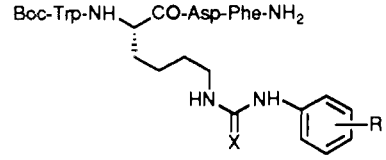
Table I. Physical Properties and Binding Data of Phenyl-Substituted Ureas and Thioureas 5 and 6



no.	R	X	mp, °C	formula	Anal.[a]	IC_{50}, nM[b,c] guinea pig pancreas	IC_{50}, nM[b,c] guinea pig cortex	C/P[d]
5a	H	O	197–198	$C_{42}H_{52}N_8O_9 \cdot H_2O$	C,H,N	26 ± 7.1 (3)	1100 ± 270 (3)	42
5b	2-Me	O	172–174	$C_{43}H_{54}N_8O_9 \cdot AcOH$	C,H,N	3.8 ± 0.49 (3)	1400 ± 490 (3)	370
5c	3-Me	O	189–191	$C_{43}H_{54}N_8O_9 \cdot 0.75H_2O$	C,H,N	16 ± 3.1 (3)	1900 ± 240 (3)	120
5d	4-Me	O	144–145	$C_{43}H_{54}N_8O_9 \cdot AcOH \cdot 1.75H_2O$	C,H,N	53 ± 24 (4)	1200 ± 50 (3)	23
5e	2-Cl	O	190–191	$C_{42}H_{51}N_8O_9Cl \cdot 0.5AcOH \cdot H_2O$	C,H,N	3.8 ± 1.2 (3)	1900 ± 360 (3)	500
5f	3-Cl	O	139–142	$C_{42}H_{51}N_8O_9Cl \cdot H_2O \cdot 0.5AcOH$	C,H,N	51 ± 11 (3)	2600 ± 710 (3)	51
5g	4-Cl	O	135–136	$C_{42}H_{51}N_8O_9Cl \cdot H_2O \cdot 0.5AcOH$	C,H,N	60 (2)	770 (1)	13
5h	2-CF_3	O	198–199	$C_{43}H_{51}N_8O_9F_3$	C,H,N	8.0 ± 1.6 (5)	1600 (1)	200
5i	3-CF_3	O	169–171	$C_{43}H_{51}N_8O_9F_3 \cdot H_2O \cdot AcOH$	C,H,N	59 (2)	3000 (1)	51
5j	4-CF_3	O	194–197	$C_{43}H_{51}N_8O_9F_3 \cdot H_2O \cdot AcOH$	C,H,N	170 (2)	850 (1)	5
5k	2,3-Cl_2	O	178–181	$C_{42}H_{50}N_8O_9Cl_2 \cdot 0.5H_2O$	C,H,N	4.5 ± 1.3 (3)	3000 (1)	670
5l	2,4-Cl_2	O	183–187	$C_{42}H_{50}N_8O_9Cl_2 \cdot H_2O$	C,H,N	8.5 (2)	2600 (2)	310
5m	2,6-Cl_2	O	199–200	$C_{42}H_{50}N_8O_9Cl_2 \cdot H_2O$	C,H,N	160 ± 32 (4)	7000 ± 710 (3)	44
5n	2,6-Me_2	O	205–206	$C_{44}H_{56}N_8O_9 \cdot H_2O$	C,H,N	800 ± 190 (7)	25,000 ± 600 (3)	31
5o	2-Br	O	211–212	$C_{42}H_{51}N_8O_9Br \cdot H_2O$	C,H,N	3.6 ± 0.3 (5)	1400 (1)	390
5p	2-MeO	O	140–142	$C_{43}H_{54}N_8O_8 \cdot 0.5AcOH$	C,H,N	23 ± 5.3 (4)	3400 ± 500 (4)	150
5q	2-*i*-Pr	O	158–160	$C_{45}H_{58}N_8O_9 \cdot H_2O \cdot 0.75AcOH$	C,H,N	23 ± 4.4 (3)	5700 (1)	250
5r	2-NO_2	O	123–124	$C_{42}H_{51}N_9O_{11} \cdot AcOH$	C,H,N	22 ± 3.9 (4)	990 (1)	45
5s	2-CO_2Me	O	191–192	$C_{44}H_{54}N_8O_{11} \cdot 0.5H_2O$	C,H,N	34 ± 4.5 (3)	3100 ± 590 (3)	91
5t	3-CO_2Me	O	200–202	$C_{44}H_{54}N_8O_{11} \cdot H_2O \cdot 0.75AcOH$	C,H,N	26 (2)	1400 (1)	54
5u	3-Ac	O	135–137	$C_{44}H_{54}N_8O_{10} \cdot H_2O$	C,H,N	19 ± 4.5 (3)	1300 (1)	68
6a	2-Me	S	135–136	$C_{43}H_{54}N_8O_8S \cdot H_2O$	C,H,N	220 (1)	2000 (1)	9
6b	2-Cl	S	129–131	$C_{42}H_{51}N_8O_8ClS \cdot H_2O$	C,H,N	100 ± 25 (3)	2900 (1)	29
6c	4-Cl	S	135–137	$C_{42}H_{51}N_8O_8ClS \cdot H_2O$	C,H,N	270 (1)	3700 (2)	14

[a] Compounds gave satisfactory analyses within ±0.4% of theoretical calculations. [b] Number of determinations is indicated in parentheses. Each determination was conducted in duplicate with <5% sample variability. Means ±SE are indicated for those compounds with three or more determinations. [c] IC_{50} was determined as the concentration of peptide that inhibited 50% of the specific binding of [^{125}I]BH-CCK-8 in each tissue. [d] IC_{50} cortex/IC_{50} pancreas.

were roughly 100-fold less potent for the pancreatic receptor.[2] Many of these longer peptides developed as CCK-A receptor agonists do not discriminate between the two CCK receptors. Only one example of a CCK-A-selective heptapeptide has been reported[13] while a number of CCK-B-selective peptides have been developed.[14-16]

The novel tetrapeptides described in this report represent a significant advancement toward developing CCK-A receptor agonists as therapeutic agents due to their considerably smaller, less complex structures over existing CCK-8 derivatives as well as their excellent receptor selectivity. These compounds also will contribute toward understanding the structural relationships among the various classes of CCK agonists and both the peptide and non-peptide series of antagonists. In this paper, we describe the details of the structure–activity analysis of these novel CCK-based tetrapeptides bearing a urea in the critical side chain.

Methods

The parent tetrapeptide 4 was prepared via standard solution-phase peptide chemistry techniques outlined in Scheme I. The side-chain amino group of the Lys in 4 was allowed to react with an aryl or alkyl isocyanate to produce the final urea-containing tetrapeptide **5**. No reaction of the free β-carboxyl of the aspartic acid in 4 was

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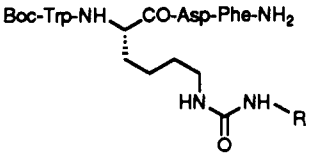
Table II. CCK Tetrapeptide Agonists Containing Aliphatic and Polyaromatic Ureas 5



no.	R	mp, °C	formula	Anal.[a]	IC_{50}, nM[b,c] guinea pig pancreas	IC_{50}, nM[b,c] guinea pig cortex	C/P[d]
5aa	1-naphthyl	175–177	$C_{46}H_{54}N_8O_9 \cdot 0.6H_2O \cdot AcOH$	C,H,N	16 ± 4.8 (9)	11000 ± 3500 (3)	690
5bb	2-naphthyl	207–210	$C_{46}H_{54}N_8O_9 \cdot H_2O$	C,H,N	16 ± 4.4 (5)	3200 ± 990 (3)	200
5cc	cyclohexyl	139–140	$C_{42}H_{58}N_8O_9 \cdot H_2O \cdot 0.75AcOH$	C,H,N	21 ± 5.6 (3)	2100 ± 390 (3)	100
5dd	*tert*-butyl	136–138	$C_{40}H_{58}N_8O_9 \cdot H_2O \cdot 0.75AcOH$	C,H,N	170 ± 50 (4)	5600 ± 200 (3)	33
5ee	benzyl	152–154	$C_{43}H_{54}N_8O_9 \cdot H_2O$	C,H,N	230 ± 52 (6)	3300 ± 350 (5)	14
5ff	H	203–204	$C_{36}H_{48}N_8O_9$	C,H,N	12000 ± 3400 (3)	1000 ± 270 (3)	0.083

[a] Compounds gave satisfactory analyses within ±0.4% of theoretical calculations. [b] Number of determinations is indicated in parentheses. Each determination was conducted in duplicate with <5% sample variability. Means ±SE are indicated for those compounds with three or more determinations. [c] IC_{50} was determined as the concentration of peptide that inhibited 50% of the specific binding of [^{125}I]BH-CCK-8 in each tissue. [d] IC_{50} cortex/IC_{50} pancreas.

observed under the reaction conditions. Reaction of tetrapeptide **4** with an isothiocyanate yielded thiourea **6**. The tetrapeptide containing an Orn in the place of Lys was prepared in an analogous manner. The L-homolysine was prepared from D-serine by the procedure described by Beaulieu and Schiller[17] and incorporated into the tetrapeptides as described.

The compounds were tested in a receptor binding assay described previously using guinea pig pancreas and cortex as tissues containing the CCK-A and CCK-B receptors, respectively, and [^{125}I]-Bolton-Hunter CCK-8 as the radioligand.[13] Protocols for assessing the ability of these compounds to stimulate amylase release and phosphatidylinositide (PI) breakdown in guinea pancreas have been described.[13] The compounds were initially evaluated by using a single determination performed in duplicate with less than 5% sample variability. Subsequent determinations were conducted on selected compounds of interest to obtain statistical limits.

Results and Discussion

As previously reported,[7] the development of this novel series of CCK-A agonists was initiated by our findings that, while Boc-CCK-4 (Boc-Trp-Met-Asp-Phe-NH_2), Boc-Trp-Lys-Asp-Phe-NH_2 (**4**), and Boc-Trp-Lys(Ac)-Asp-Phe-NH_2 were weak or inactive ligands for the CCK-A receptor, Boc-Trp-Lys(Cbz)-Asp-Phe-NH_2 had modest affinity for the pancreatic receptor and functioned as a full agonist relative to CCK-8 in stimulating amylase release. Since an appropriate side-chain function appeared to be critical for CCK-A receptor affinity and agonist activity, the ϵ-amino group of the lysine in tetrapeptide **4** was reacted with various reagents to explore which functional groups might suffice. From these studies, tetrapeptide derivatives bearing substituted ureas in the side chain were identified and further developed. The phenylurea **5a** improved pancreatic binding over the initial Cbz derivative (IC_{50} = 510 nM in pancreas)[7] by 20-fold (Table I). Systematic substitution about the phenyl ring with methyl (**5b–d**), chloro (**5e–g**), and trifluoromethyl (**5h–j**) demonstrated that the 2-substituted analogue in a given series was the most potent for pancreatic affinity. The 2-methyl (**5b**) and 2-chlorophenyl (**5e**) derivatives improved pancreatic receptor affinity by roughly 7-fold over the unsubstituted compound **5a**, while the effects of 3- or 4-substitutions were either not significant or decreased pancreatic affinity roughly 2- to 6-fold compared to that of **5a**. Each of the trifluoromethyl-substituted analogues had weaker affinity for the CCK-A receptor than the corresponding chloro and methyl compounds. In all three series, greatest CCK-A selectivity was achieved with 2-substitution (roughly 200- to 500-fold) and the least with 4-substitution (roughly 5- to 20-fold). Evaluation of several disubstituted series revealed high pancreatic binding affinity by the 2,3- (**5k**) and 2,4-dichlorophenyl (**5l**) analogues, suggesting that in a given compound the effects of 2-substitution appear to outweigh the contributions of substituents at the 3- and 4-positions. However, 2,6-disubstitution produced analogues **5m** and **5n** that were significantly less potent. All the disubstituted derivatives maintained CCK-A receptor selectivity.

Additional phenylureas **5o–u**, particularly those substituted in the 2-position, were prepared and tested for CCK-A receptor affinity and selectivity. The 2-bromophenyl derivative **5o** exhibited comparable properties to the 2-methyl (**5b**) and 2-chloro (**5e**) analogues. Other functional groups at the 2-position, including 2-methoxy (**5p**), 2-isopropyl (**5q**), 2-nitro (**5r**), and 2-carbomethoxy (**5s**), as well as those at the 3-position, including 3-carbomethoxy (**5t**) and 3-acetyl (**5u**), did not affect pancreatic affinity with respect to the parent phenyl compound **5a**. All these derivatives maintained CCK-A selectivity.

Conversion of the urea to a thiourea decreased pancreatic binding affinity roughly 5- to 50-fold from the parent oxo compound in all the examples investigated (**6a–c**). The thiourea derivatives maintained pancreatic binding selectivity.

Replacement of the phenyl group with 1- and 2-naphthylene produced ureas **5aa** and **5bb**, respectively (Table II). The compounds were equipotent for the CCK-A receptor, although the 1-naphthyl derivative **5aa** was more selective for the pancreatic receptor. The presence of an aromatic urea is not mandatory for high CCK-A receptor affinity since the cyclohexyl urea **5cc** was equipotent with its unsaturated counterpart **5a**. Significantly weaker pancreatic affinity was observed for the *tert*-butyl (**5dd**) and benzyl (**5ee**) ureas. The monosubstituted urea **5ff** exhibited low affinity in pancreas while its affinity for CCK-B receptors, albeit weak, was improved slightly over many of the disubstituted examples. The presence of an N,N'-disubstituted urea appears to be critical for high affinity at the CCK-A receptor.

The effect of receptor binding affinity as a function of the carbon chain length connecting the critical urea moiety

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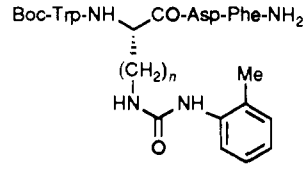
Table III. Effect of Chain Length on Potency of CCK Tetrapeptide Agonists



no.	n	mp, °C	formula	Anal.[a]	IC_{50}, nM[b,c] guinea pig pancreas	IC_{50}, nM[b,c] guinea pig cortex	C/P[d]
7	3	186–188	$C_{42}H_{52}N_8O_9 \cdot 1.5H_2O$	C,H,N	650 ± 140 (3)	15000 (2)	23
5b	4	172–174	$C_{43}H_{54}N_8O_9 \cdot AcOH$	C,H,N	3.8 ± 0.49 (3)	1400 ± 490 (3)	370
8	5	178–183	$C_{44}H_{56}N_8O_9 \cdot H_2O$	C,H,N	93 ± 16 (3)	2000 ± 310 (3)	21

[a] Compounds gave satisfactory analyses within ±0.4% of theoretical calculations. [b] Number of determinations is indicated in parentheses. Each determination was conducted in duplicate with <5% sample variability. Means ±SE are indicated for those compounds with three or more determinations. [c] IC_{50} was determined as the concentration of peptide that inhibited 50% of the specific binding of [^{125}I]BH-CCK-8 in each tissue. [d] IC_{50} cortex/IC_{50} pancreas.

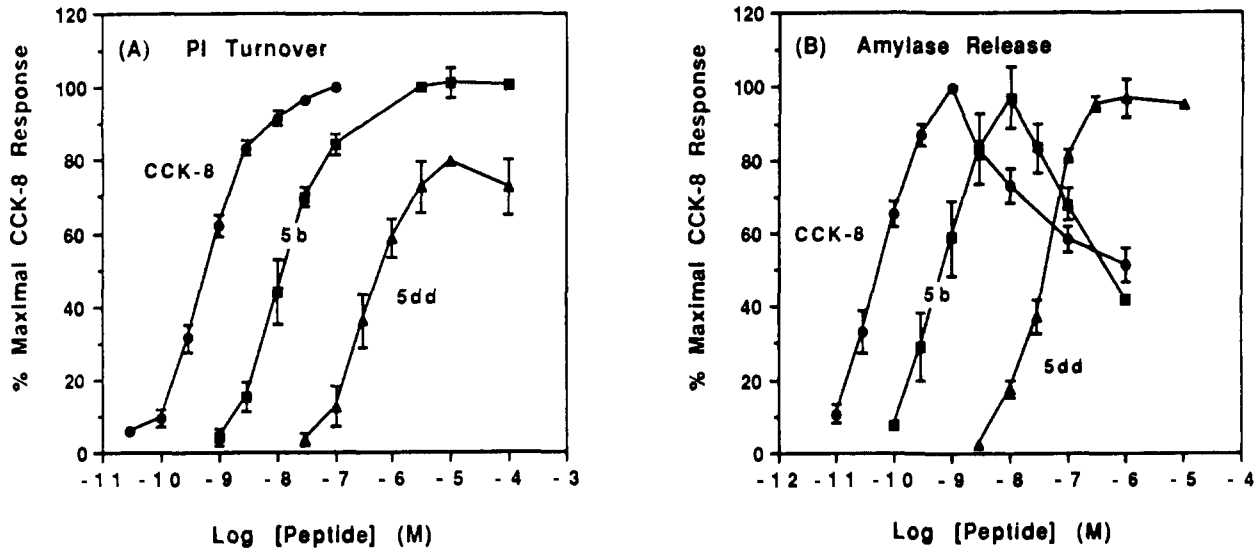


Figure 1. Comparison of the abilities of CCK-8, **5b**, and **5dd** to stimulate PI hydrolysis (A) and amylase release (B) in guinea pig pancreatic acini. The experimental protocols were as described previously.[13] Results shown are the mean ± SEM of three or more experiments conducted in duplicate. The amount of inositol phosphates was increased from 160 ± 40 dpm to 3700 ± 700 dpm in the presence of 100 nM CCK-8 (n = 4). The amount of amylase activity before the addition of the peptide was 2.9 ± 0.3% of the total cellular activity (n = 4). It was increased to 7.7 ± 1.1% and 26 ± 3.3%, respectively, in the absence and presence of 1 nM CCK-8 (n = 4).

to the peptide backbone was investigated by substituting lysine in tetrapeptide **5b** with ornithine and homolysine, which shortened and lengthened the side chain, respectively. The binding data indicated significant loss of pancreatic affinity for both compounds **7** and **8** (Table III), with the ornithine derivative possessing weaker affinity for the CCK-A receptor than the homolysine compound. Both compounds maintained roughtly 20-fold selectivity for the CCK-A receptor. Despite their loss in pancreatic potencies, both compounds maintained their ability to stimulate pancreatic amylase release (Table IV). A related Boc-CCK-4 derivative (Boc-Trp-Orn(N^{γ}-Cbz)-Asp-Phe-NH_2) in which the methionine was replaced with an N^{γ}-Cbz-ornithine residue was reported by Roques and co-workers.[18] This derivative possessed similar binding affinity for the CCK-A receptor as the ornithine tetrapeptide **7**. However, in contrast to **7**, the N^{γ}-Cbz-ornithine derivative did not differentiate between the CCK receptors and functioned as an antagonist in the pancreatic amylase release assay. This striking contrast between the carbamate and urea derivatives further illustrates the importance of an appropriate pharmacophore extending from the side chain of the tetrapeptide for selectivity and efficacy at the CCK-A receptor.

The tetrapeptides we have described are full agonists relative to CCK-8 in eliciting pancreatic amylase release and function as either full or partial agonists in stimulating PI breakdown in guinea pig pancreas (Table IV). These responses can be potently inhibited by selective CCK-A antagonists used to block the effects of a related tetrapeptide agonist, A-71623.[8] Although exceptions are present, there appears to be a general trend correlating pancreatic affinity with functional potencies. Detailed characterization of a representative set of compounds reveals a relationship between the efficacy of a particular tetrapeptide to stimulate PI breakdown and its ability to effect high-dose inhibition of amylase release (Figure 1). Those tetrapeptides (represented by compound **5b**) that elicit a full or nearly full (≥90%) agonist responses in PI hydrolysis relative to CCK-8 also exhibit inhibition of amylase release at supramaximal concentrations, similar to the results observed with CCK-8 and with A-71623.[8] However, those tetrapeptides (represented by compound **5dd**) that are partial agonists in the PI assay (<80% of CCK-8 response) remain full agonists in amylase release

(18) Gonzalez-Muniz, R.; Bergeron, F.; Marseigne, I.; Durieux, C.; Roques, B. P. Boc-Trp-Orn(Z)-Asp-NH_2 and Derivatives: A New Family of CCK Antagonists. *J. Med. Chem.* **1990**, *33*, 3199–3204.

Table IV. Functional Data in Guinea Pig Pancreatic Acini of CCK Tetrapeptides 5–8

no.	amylase release[a,b] EC_{50}, nM	PI hydrolysis[a,c] EC_{50}, nM	% maximal response[d]
CCK-8	0.47 ± 0.15 (6)	1.2 ± 0.048 (4)	
A-71623	0.39 ± 0.06 (12)	3.4 ± 0.65 (4)	100
5a	4.0 ± 1.2 (3)	53 (1)	96
5b	1.1 ± 0.16 (3)	12 ± 3.1 (3)	101
5c	4.4 ± 1.3 (3)	51 ± 7.7 (3)	95
5d	13 ± 5.5 (3)	140 ± 35 (3)	91
5e	0.21 ± 0.047 (3)	4.6 (1)	91
5f	4.4 (1)	nd[e]	93
5g	9.6 (1)	nd	91
5h	0.77 (1)	6.2 (1)	100
5m	44 ± 15 (3)	310 ± 54 (3)	66
5n	120 ± 37 (3)	910 (1)	40
5p	0.4 ± 0.12 (3)	8.4 (2)	100
5q	4.4 (1)	nd	85
5r	1.9 (1)	21 (1)	100
5t	2.7 (1)	nd	88
5u	5.2 (1)	nd	95
6b	20 (1)	nd	83
5aa	1.6 (1)	14 (1)	103
5bb	7.0 (1)	140 (1)	79
5cc	4.2 ± 0.5 (3)	55 ± 10 (3)	97
5dd	42 ± 6 (3)	330 ± 90 (3)	73
7	420 (2)	nd	36
8	12 (2)	nd	83

[a] Number of determinations is indicated in parentheses. Each determination was conducted in duplicate with <5% sample variability. Means ±SE are indicated for those compounds with three or more determinations. [b] EC_{50} was determined in the amylase assay as concentration of peptide that produced 50% of maximal response. [c] EC_{50} was determined in the PI hydrolysis assay as concentration of peptide that produced 50% of maximal reponse. [d] Indicates % response of peptide in PI hydrolysis relative to maximal response elicited by CCK-8. [e] Not determined.

but do not exhibit the high-dose inhibition. The profiles of these latter tetrapeptides are comparable to our previously reported CCK tetrapeptide agonists A-57282 and A-70874.[8] Both A-57282 and A-70874 are partial agonists in the PI hydrolysis assay, eliciting 40% and 80% of the CCK-8 response, respectively, and neither compound exhibits high-dose inhibition of amylase release. The secretory activities of these tetrapeptides are similar to the heptapeptide CCK-JMV-180.[19-21] The mechanisms responsible for this high-dose inhibition are not understood at this time although the existence of low-affinity CCK receptors mediating this response has been proposed.[20,22,23] Further investigations utilizing these novel tetrapeptides may aid in understanding the basis for this observation.

The ability of these small, non-sulfated peptides to potently activate the CCK-A receptor is unprecedented and these tetrapeptides will prompt reevaluation of currently held tenets associated with structural requirements for high affinity and activity at the CCK-A receptor. Since the prototypic, tetrapeptide-based CCK-A receptor agonist A-71623 was shown to effectively suppress food intake under both acute and chronic administration, these related tetrapeptides represent a significant advancement for development into therapeutic agents over the larger, sulfated CCK peptides. In addition, the CCK-A selectivity of these tetrapeptides should minimize potential GI side effects that would be attributed to interactions at the CCK-B or gastrin receptors. We are currently conducting modeling studies directed toward understanding the structural correlations of these tetrapeptides with other classes of CCK ligands.

Experimental Section

Solvents and other reagents were reagent grade and used without further purification unless otherwise noted. Amino acids and *tert*-butoxycarbonyl (Boc) protected amino acids were purchased from Bachem, Inc., Torrance, CA, or Sigma Chemical Co., St. Louis, MO. The activated esters (*N*-hydroxysuccinimide) of Boc-protected amino acids were purchased from Chemical Dynamics, South Plainfield, NJ. Melting point are uncorrected and were obtained on a Buchi capillary melting point apparatus. ^1H NMR spectra were recorded at 300 or 500 MHz and expressed as ppm downfield from tetramethylsilane (TMS) as an internal standard. Column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck), using the following solvent system: ethyl acetate–pyridine–acetic acid–water (260:20:6:11). Pyridine used as the chromatography solvent was freshly distilled from barium oxide. Elemental analyses were performed by the Abbott Laboratories Analytical Department, North Chicago, IL, and are within ±0.4% of calculated values unless otherwise noted. The following abbrevations have been used: Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; EDCI, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole. Other peptide and amino acid abbreviations and conventions used are those recommended by the IUPAC-IUB Commission (*Biochem. J.* 1984, *219*, 345–373).

Boc-Asp(OBzl)-Phe-NH_2 (2).[18] To a solution of phenylalanine amide hydrochloride (19.4 g, 0.06 mol) in dimethylformamide (100 mL) cooled to 0 °C were added *N*-methylmorpholine (7.2 mL, 0.065 mol), a solution of Boc-Asp β-benzyl ester (12.0 g, 0.06 mol) in methylene chloride (80 mL), HOBt (12.2 g, 0.09 mol), and EDCI (12.4 g, 0.065 mol). The reaction was stirred for 12 h with warming to ambient temperature. The solvent was removed in vacuo and the resulting residue was dissolved in ethyl acetate and washed with 1 M H_3PO_4 (3×), saturated sodium bicarbonate ($NaHCO_3$) solution (3×), and brine. After drying ($MgSO_4$), the solvent was evaporated, the residue was dissolved in hot ethyl acetate, and the product was precipitated with dropwise addition of hexane. The product was collected and dried to yield **2** (25 g, 88%) as a white solid: MS (CI/NH_3) *m/e* 470 $(M + H)^+$, 487; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 9 H), 2.78 (dd, *J* = 18 Hz, 1 H), 2.92–3.05 (m, 2 H), 3.21 (dd, 1 H), 4.38–4.45 (m, 1 H), 4.65 (q, *J* = 6 Hz, 1 H), 5.36 (br s, 1 H), 5.49 (br d, *J* = 7.5 Hz, 1 H), 6.09 (br s, 1 H), 6.82 (br d, *J* = 7 Hz, 1 H), 7.21–7.40 (m, 10 H).

Boc-Lys(Cbz)-Asp(OBzl)-Phe-NH_2 (3). A solution of **2** (16.2 g, 34 mmol) in 50 mL of 1.5 M HCl (anhydrous) in acetic acid was stirred at ambient temperature for 1.5 h. The reaction was quenched with the addition of ether to precipitate the hydrochloride salt of the amine. The solid was collected, washed with fresh ether, and dried to yield 12.9 g of a white powder. To a solution of the hydrochloride salt (12.9 g, 32 mmol) in dimethylformamide (20 mL) and methylene chloride (20 mL) cooled to −10 °C were added *N*-methylmorpholine (3.9 mL, 35 mmol), Boc-Lys(Cbz) (12.1 g, 32 mmol), HOBt (6.5 g, 48 mmol), and EDCI

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(6.7 g, 35 mmol). The reaction was stirred for 12 h with warming to ambient temperature. The solvents were removed in vacuo, and the residue was dissolved in ethyl acetate and washed successively with solutions of 1 M H_3PO_4 (3×), saturated $NaHCO_3$ (3×), and brine. The solvent was removed in vacuo and the solid residue was dissolved in acetone with warming. The product was precipitated with the dropwise addition of water, collected, and dried to yield 3 (22.3 g, 90%) as a white powder: MS (CI/NH_3) m/e 732 $(M + H)^+$, 749, 632; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.13–1.54 (m, 6 H), 1.37 (br s, 9 H), 2.51–3.05 (m, 6 H), 3.86 (br s, 1 H), 4.38 (br s, 1 H), 4.61 (br s, 1 H), 5.00 (s, 2 H), 5.07 (s, 2 H), 6.87 (br d, J = 7 Hz, 1 H), 7.12–7.38 (m, 16 H), 7.85 (br d, 1 H), 8.15 (br d, 1 H). Anal. Calcd for $C_{39}H_{49}N_4O_9$: C, 64.00; H, 6.75; N, 9.57. Found: C, 63.92; H, 6.82; N, 9.54.

Boc-Trp-Lys-Asp-Phe-NH_2 (4). A solution of **3** (19 g, 26 mmol) in 80 mL of 1.5 M HCl (anhydrous) in acetic acid was stirred at ambient temperature for 1.5 h. The product was precipitated with the addition of ether, collected, and dried to yield 17.2 g of a white powder. To a solution of the hydrochloride salt (9.8 g, 15.5 mmol) in dimethylformamide (100 mL) cooled to 0 °C were added *N*-methylmorpholine (1.9 mL, 17 mmol) and Boc-tryptophan *N*-hydroxysuccinimide ester (6.15 g, 15.5 mmol). The reaction was stirred for 18 h with warming to ambient temperature. The solvent was removed in vacuo and the residue partitioned between a solution of 10% citric acid and ethyl acetate. The organic phase was further washed with solutions of saturated $NaHCO_3$ (3×) and water (3×). After drying over $MgSO_4$, the solvent was removed in vacuo. The solid residue was dissolved in ethyl acetate/acetone and the product precipitated with the addition of water. The product was collected and dried to yield 11.9 g of a white solid. A mixture of the fully protected tetrapeptide (5.0 g, 5.45 mmol) and 10% Pd/C (1.0 g) in acetic acid (100 mL) was hydrogenated for 18 h under 1 atm of hydrogen at ambient temperature. The catalyst was filtered and the solvent was removed in vacuo. The residue was triturated with ether to yield 3.95 g of **4** as a light pink powder: MS (FAB+) m/e 694 $(M + H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.11–1.62 (m, 6 H), 1.32 (br s, 1 H), 2.25–3.18 (m, 8 H), 4.10–4.43 (m, 4 H), 6.78–7.34 (m, 12 H), 7.51–7.62 (m, 2 H), 8.05 (br d, J = 7 Hz, 1 H), 8.17 (br d, J = 7 Hz, 1 H), 10.91 (br s, 1 H). Anal. Calcd for $C_{35}H_{47}N_7O_8 \cdot 1.75CH_3CO_2H$: C, 57.88; H, 6.81; N, 12.27. Found: C, 57.84; H, 6.92; N, 12.64.

General Procedure for the Preparation of Urea-Substituted Tetrapeptides 5. Boc-Trp-Lys(N^{ϵ}-phenylaminocarbonyl)-Asp-Phe-NH_2 (5a). A solution of tetrapeptide **4** (50 mg, 0.07 mmol), phenyl isocyanate (9 μL, 0.09 mmol), and *N*-methylmorpholine (12 μL, 0.11 mmol) in DMF (5 mL) was stirred at ambient temperature for 18 h. The DMF was evaporated in vacuo, and the residue was directly applied to a silica gel column and eluted with ethyl acetate–pyridine–acetic acid–water (260:20:6:11). After evaporation of solvents, the residue was dissolved in water–acetone (10:1, v:v), lyophilized, and further dried (50 °C) to yield **5a** (36 mg, 62%) as a white solid: MS (FAB+) m/e 827 $(M + H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.05–1.69 (m, 6 H), 1.30 (br s, 9 H), 2.16 (s, 3 H), 2.35–3.25 (m, 10 H), 4.16–4.40 (m, 3 H), 4.48 (q, 1 H), 6.78–7.42 (m, 16 H), 7.59 (br d, J = 7 Hz, 1 H), 7.75–8.05 (m, 4 H), 8.26 (d, 1 H), 8.58 (m, 1 H), 10.82 (br s, 1 H).

General Procedure for the Preparation of Thiourea-Substituted Tetrapeptides 6. Boc-Trp-Lys(N^{ϵ}-2-methylphenylamino(thiocarbonyl))-Asp-Phe-NH_2 (6a). A solution of the tetrapeptide **4** (160 mg, 0.23 mmol), *N*-methylmorpholine (38 μL, 0.34 mmol), and 2-methylphenyl isothiocyanate (37 μL, 0.28 mmol) in DMF (5 mL) was stirred at ambient temperature for 18 h. The DMF was removed in vacuo and the residue was chromatographed on silica gel, eluting with ethyl acetate–pyridine–acetic acid–water (260:20:6:11). The residue was dissolved in water–acetone (10:1, v:v) and lyophilized to yield **6a** (54 mg, 28%) as a white solid: MS (FAB+) m/e 843 $(M + H)^+$, ^1H NMR (DMSO-d_6, 300 MHz) δ 1.09–1.67 (m, 8 H), 1.30 (br s, 9 H), 2.44–2.66 (m, 2 H), 2.78–2.97 (m, 2 H), 3.01–3.19 (m, 2 H), 4.16–4.29 (m, 2 H), 4.30–4.39 (m, 1 H), 4.42–4.54 (m, 1 H), 6.82 (d, 1 H), 6.95 (t, 1 H), 7.10–7.37 (m, 7 H), 7.58 (d, 1 H), 7.87 (d, 1 H), 7.98 (d, 1 H), 8.22 (d, 1 H).

Boc-Trp-Orn(N^{γ}-2-methylphenylaminocarbonyl)-Asp-Phe-NH_2 (7). Tetrapeptide **7** was prepared in an analogous manner to the lysine-containing tetrapeptides. **7**: mp 186–188 °C; MS (FAB+) m/e 813 $(M + H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.09–1.72 (m, 4 H), 1.30 (s, 9 H), 2.17 (s, 3 H), 2.31 (dd, 1 H), 2.45 (dd, 1 H), 2.75–3.18 (m, 6 H), 4.16–4.47 (m, 4 H), 6.73–6.88 (m, 2 H), 6.95 (t, 1 H), 7.00–7.35 (m, 10 H), 7.59 (d, 1 H), 7.82 (d, 1 H), 8.05 (d, 1 H), 8.13–8.26 (m, 2 H).

Boc-Trp-Homolysine(N^{ω}-2-methylphenylaminocarbonyl)-Asp-Phe-NH_2 (8). Boc-3,4-dehydrohomolysine (prepared from Boc-D-serine in the manner described by Beaulieu and Schiller[17]) was treated with 10% palladium-on-carbon in methanol under 1 atm of hydrogen. The catalyst was removed by filtration through Celite and the solvent evaporated in vacuo. The resulting product was dissolved in chloroform, cooled to 0 °C, and treated with an equivalent of benzyl chloroformate along with concomitant addition of sodium hydroxide solution to afford Boc-homolysine(N^{ω}-Cbz). The resulting amino acid was extended to the final tetrapeptide **8** via the procedures described for the lysine examples. **8**: mp 178–183 °C; MS (FAB+) m/e 841 $(M + H)^+$ m/e 863 $(M + Na)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.1–1.7 (m, 8 H), 1.31 (s, 9 H), 2.4–3.15 (m, 8 H), 4.15–4.45 (m, 3 H), 4.52 (m, 1 H), 6.52 (t, 1 H), 6.8–7.35 (m, 15 H), 7.55–7.65 (m, 2 H), 7.8–8.0 (m, 3 H), 8.29 (d, J = 7.5 Hz, 1 H), 10.8 (s, 1 H).

Registry No. **2**, 60058-69-7; **3**, 131450-39-0; **4**, 27168-83-8; **5a**, 130408-71-8; **5b** (free base), 130408-70-7; **5b**·AcOH, 131469-98-2; **5c**, 130408-72-9; **5d** (free base), 130408-73-0; **5d**·AcOH, 131449-98-4; **5e** (free base), 130408-75-2; **5e**·$^1/_2$AcOH, 131449-71-3; **5f** (free base), 131449-77-9; **5f**·$^1/_2$AcOH, 131449-78-0; **5g** (free base), 131449-70-2; **5g**·$^1/_2$AcOH, 131449-71-3; **5h**, 131449-88-2; **5i** (free base), 135257-12-4; **5i**·AcOH, 135257-18-0; **5j** (free base), 135257-13-5; **5j**·AcOH, 135257-19-1; **5k**, 135257-14-6; **5l**, 135257-15-7; **5m**, 131450-05-0; **5n**, 130408-74-1; **5o**, 131449-89-3; **5p** (free base), 130408-76-3; **5p**·$^1/_2$AcOH, 131449-99-5; **5q** (free base), 131449-95-1; **5q**·$^3/_4$AcOH, 135257-20-4; **5r** (free base), 131449-86-0; **5r**·AcOH, 131449-87-1; **5s**, 131450-01-6; **5t** (free base), 131450-03-8; **5t**·$^3/_4$AcOH, 135257-21-5; **5u**, 131449-92-8; **5aa** (free base), 131449-73-5; **5aa**·AcOH, 131449-74-6; **5bb**, 131450-00-5; **5cc** (free base), 131449-75-7; **5cc**·$^3/_4$AcOH, 135257-22-6; **5dd** (free base), 131449-81-5; **65dd**·$^3/_4$AcOH, 135257-23-7; **5ee**, 131450-09-4; **5ff**, 135257-17-9; **6a**, 131449-91-7; **6b**, 131449-90-6; **6c**, 135257-16-8; **7**, 131450-16-3; **8**, 131450-19-6; Phe-NH_2, 5241-58-7; Boc-Trp-Orn-Asp-Phe-NH_2, 129594-04-3; Boc-Trp-homoLys-Asp-Phe-NH_2, 135257-11-3; Boc-Asp(OBn)-OH, 7536-58-5; Boc-Lys(Cbz)-OH, 2389-45-9; Boc-Trp-OSu, 3392-11-8; PhNCO, 103-71-9; 2-MeC_6H_4NCO, 614-68-6; 3-MeC_6H_4NCO, 621-29-4; 4-MeC_6H_4NCO, 622-58-2; 2-ClC_6H_4NCO, 3320-83-0; 3-ClC_6H_4NCO, 2909-38-8; 4-ClC_6H_4NCO, 104-12-1; 2-$F_3CC_6H_4NCO$, 2285-12-3; 3-$F_3CC_6H_4NCO$, 329-01-1; 4-$F_3CC_6H_4NCO$, 1548-13-6; 2,3-$Cl_2C_6H_3NCO$, 41195-90-8; 2,4-$Cl_2C_6H_3NCO$, 2612-57-9; 2,6-$Cl_2C_6H_3NCO$, 39920-37-1; 2,6-$Me_2C_6H_3NCO$, 28556-81-2; 2-BrC_6H_4NCO, 1592-00-3; 2-$MeOC_6H_4NCO$, 700-87-8; 2-*i*-PrC_6H_4NCO, 56309-56-9; 2-$O_2NC_6H_4NCO$, 3320-86-3; 2-(MeO-CO)C_6H_4NCO, 1793-07-3; 3-(MeOCO)C_6H_4NCO, 41221-47-0; 3-AcC_6H_4NCO, 23138-64-9; *c*-$C_6H_{11}NCO$, 3173-53-3; *t*-BuNCO, 1609-86-5; BnNCO, 3173-56-6; HNCO, 75-13-8; 2-MeC_6H_4NCS, 614-69-7; 2-ClC_6H_4NCS, 2740-81-0; 4-ClC_6H_4NCS, 2131-55-7; 1-naphthyl isocyanate, 86-84-0; 2-naphthyl isocyanate, 2243-54-1.