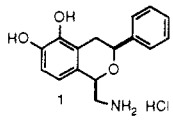
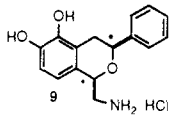
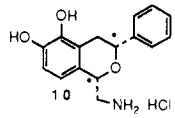
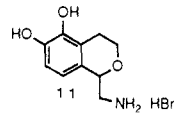
Table II. In Vivo Behavioral Data

structure	dose, μmol/kg (sc)	contralateral rotations/2 h
 1	0.41	192 ± 83
	1.2	895 ± 185
	4.1	1554 ± 194
	20.3[a]	326 ± 117
	40.6[a]	1066 ± 308
 9	0.20	348 ± 210
	1.02	1113 ± 227
	2.03	1525 ± 384
 10	2.03	-22 ± 13
	20.3	-24 ± 10
 11	65.0	93 ± 66
	130	286 ± 119
	260	584 ± 161

[a] Oral dose.

A standard behavioral method to access central dopaminergic receptor stimulation is the rat rotation model.[17] Rats with unilateral 6OHDA lesions of the nigro-striatal bundle rotate contralaterally to the lesion in response to exposure to a direct D1 or D2 agonist. The results from this assay using the isochromans are shown in Table II. Compound 1 was shown to be a very potent agonist in vivo by the subcutaneous (sc) route of administration. The compound was also demonstrated to be orally active, although the data suggests it has a low bioavailability. The activity of the enantiomers follow as predicted from the in vitro data. Compound **9** was potent in the whole animal while **10** was inactive in doses as high as 20 μmol/kg (sc).

In conclusion, A68930 (1) has been demonstrated to be a potent and selective D1 agonist. It is potent in vitro and is orally active in vivo. It has also been shown that all of the dopaminergic activity residues in the 1*R**,3*S** enantiomer. In addition to their therapeutic potential, these compounds represent useful probes of the D1 receptor. Further work on the SAR of this class of dopaminergic compounds will be disclosed in future publications.

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Development of CCK-Tetrapeptide Analogues as Potent and Selective CCK-A Receptor Agonists

Cholecystokinin (CCK) is a member of the brain-gut family of peptides that is found in a number of mammalian species including man. Various biological functions are attributed to this peptide including gall bladder contraction and release of pancreatic enzymes.[1,2] In addition, CCK may be involved with mechanisms linked to analgesia, appetite regulation, and dopamine modulation.[3,4] The receptors for CCK are classified into two sub-types: CCK-A (alimentary), found predominantly in peripheral tissues such as the pancreas and gall bladder, and CCK-B (brain), localized in the central nervous system and shown to have a similar ligand-binding profile to the gastrin receptor.[5,6] These receptors can be differentiated by their relative affinity for CCK and its fragments. While CCK and CCK-8 bind potently (low nanomolar) to both the peripheral and brain type receptors, BOC-CCK-4, the C-terminal tetrapeptide, exhibits high affinity only for the CCK-B receptor (Table I). The desulfated octapeptide (CCK-8-DS) is significantly less potent for the pancreatic receptor. The CCK-A receptor in the pancreas is coupled to phosphatidylinositol turnover (PI) while no such second messenger system has been definitely linked to the CCK-B receptor.

Recently, much attention has focused on the development of potent antagonists for the CCK-A receptor. A number of structurally diverse compounds is now available that exhibit high potency and selectivity for the CCK-A receptor and are being used to investigate the physiological roles of these receptors. Examples of these antagonists include the benzodiazepine MK-329, developed by Merck,[7] and the glutamic acid derivative A-65186, disclosed by Abbott.[8] Efforts to determine what structural relationships exist between these two series of compounds are underway. Recently, the Merck group has further modified the benzodiazepine structure to produce a derivative, L-365,260, with high potency and selectivity for the gastrin and CCK-B receptors.[9]

Although much progress has been made in developing non-peptide CCK-A antagonists, potent CCK-A agonists reported to date have remained sizeable peptides consisting of seven or eight amino acids.[10-12] In addition, an acidic function such as a sulfated hydroxyl on tyrosine was found to be necessary for high potency at the CCK-A receptor, the desulfated analogues being roughly 2-3 orders of magnitude less potent. Thus, the potent peptide agonists described in the literature do not differ significantly in structure from CCK-8 itself. Many of these peptides share another common feature to CCK-8 by binding with similar

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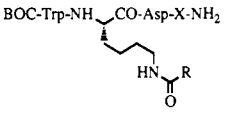
Table I. Receptor Binding Data of CCK Fragments for CCK-A (Pancreas) and CCK-B (Cortex) Receptors

	IC_{50},[a] nM	
	pancreas	cortex
CCK-8 (Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2)	0.4 ± 0.04 (27)	8.2 ± 1 (52)
CCK-8-DS (Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2)	190 ± 29 (4)	170 ± 29 (3)
BOC-CCK-4 (BOC-Trp-Met-Asp-Phe-NH_2)	1800 ± 630 (5)	25 ± 4.5 (6)

[a] Values are means ±SE of the number of determinations in parentheses. Each determination was conducted in duplicate with <5% sample variability. 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.

Table II. Receptor Binding and Amylase Release Data of CCK-Based Tetrapeptides



no.	R	X	IC_{50},[a,b] nM: pancreas	IC_{50},[a,b] nM: cortex	EC_{50},[a,c] nM: amylase
1	OBn	Phe	510 ± 50 (5)	4900 ± 1200 (4)	370 ± 13 (6)
2	Me	Phe	11000 ± 3100 (3)	1900 ± 260 (3)	6300 ± 1600 (3)
3	*o*-Me-Ph-NH	Phe	3.8 ± 0.49 (3)	1500 ± 500 (3)	1.1 ± 0.16 (3)
4	Ph-NH	Phe	26 ± 7 (3)	1100 ± 270 (3)	4.0 ± 1.2 (3)
5	*m*-Me-Ph-NH	Phe	16 ± 3 (3)	1900 ± 240 (3)	4.4 ± 1.3 (3)
6	*p*-Me-Ph-NH	Phe	53 ± 24 (4)	1200 ± 45 (3)	13 ± 5.5 (3)
7	2,6-Me_2-Ph-NH	Phe	930 ± 300 (3)	27000 ± 1900 (3)	120 ± 37 (3)
8	*o*-Cl-Ph-NH	Phe	3.8 ± 1.2 (3)	1900 ± 360 (3)	0.21 ± 0.047 (3)
9	*o*-MeO-Ph-NH	Phe	23 ± 5.3 (4)	3800 ± 370 (3)	0.4 ± 0.12 (3)
10	*o*-Me-Ph-NH	(NMe)Phe	3.7 ± 0.85 (8)	4500 ± 770 (4)	0.39 ± 0.06 (12)

[a] Values are means ±SE of the number of determinations in parentheses. Each determination was conducted in duplicate with <5% sample variability. [b] 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. [c] EC_{50} was determined in the amylase assay as concentration of the peptide that produced 50% of maximal response.

potency to both the CCK-A and CCK-B receptors. An exception to this observation is a sulfated CCK-7 analogue in which substitution by an *N*-methyl-Asp at position 32 imparts selectivity for the CCK-A receptors.[13] In contrast, several CCK-8 and CCK-5 derivatives containing *N*-methyl-Nle at positions 28 and/or 31 are highly selective for the CCK-B receptors.[14]

We wish to report a novel series of tetrapeptides that bind potently and selectively to the CCK-A receptor and elicit full agonist responses in a number of CCK-based assays that are effectively blocked by selective CCK-A receptor antagonists. Our series represents a dramatic departure from what is currently known about the structural requirements for potent CCK-A agonist binding. These tetrapeptides are the shortest fragments possessing agonist activity that bind with high affinity to the pancreatic receptor. More interestingly, these compounds do not require an acidic moiety for potency as do the longer peptides. In addition, these tetrapeptides, in contrast to CCK-8, are roughly 1000-fold selective for the CCK-A receptor. Their selectivity should enhance their usefulness in understanding actions at the CCK-A receptor in complex biological systems.

The development of these tetrapeptide agonists was initiated by the observation that a derivative (**1**) of BOC-CCK-4, containing an (N^{ϵ}-Cbz)Lys residue in place of Met, significantly improved pancreatic binding over the parent tetrapeptide and reversed its binding selectivity in favor of the CCK-A receptor (Table II). The tetrapeptides were evaluated in a receptor-binding assay using guinea pig pancreas and cortex as tissues containing the CCK-A and CCK-B receptors, respectively, and iodinated Bolton–Hunter CCK-8 as the radioligand.[15] Both BOC-CCK-4 and tetrapeptide **1** functioned as full agonists relative to CCK-8, determined by measuring stimulation of amylase release in guinea pig pancreas.[15] Inactivity of the free amino compound (BOC-Trp-Lys-Asp-Phe-NH_2) and *N*-acyl derivative **2** suggested the importance of an appropriate appendage attached to the ϵ-amine of Lys for potency and efficacy.

On the basis of these initial observations, derivatives of compound **1** were prepared by reacting BOC-Trp-Lys-Asp-Phe-NH_2, synthesized under standard solution-phase techniques, with various isocyanates.[16] A number of tetrapeptide agonists were identified with significantly improved binding potencies. One series, typified by tetrapeptide **3**, exhibited high binding affinity for the pancreatic CCK-A receptor with an IC_{50} of 3.8 nM. Binding to the cortical CCK-B receptor was considerably weaker (IC_{50} = 1500 nM). Unsubstituted phenyl derivative **4** of compound **3** was nearly 10-fold less potent in the pancreas. Studies indicated that a single substitution at the 2-position of the phenyl ring was optimal, with preferred substitutents being methyl or chloro (Table II, **5**–**9**). In addition to their improved potencies over the lead tetrapeptide (**1**), these derivatives remained full agonists in the amylase release assay.

By following a strategy used to improve in vivo potency and duration of action reported for a CCK heptapeptide series,[10] (*N*-Me)Phe was incorporated into compound **3**

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(16) All new compounds reported here were homogenous by TLC and HPLC and were characterized by their NMR and mass spectral data and satisfactory elemental analyses.

without loss of affinity or activity to produce A-71623 (**10**), which binds to the pancreatic receptor with an IC_{50} of 3.7 nM and to the cortical receptor with an IC_{50} of 4500 nM, thus making the compound greater than 1000-fold selective for the CCK-A receptor. A-71623 functioned as a full agonist in potentiating amylase release with an EC_{50} of 0.39 nM and exhibited a biphasic dose–response curve similar to that of CCK-8.[17] The response was effectively blocked by the CCK-A selective antagonist MK-329. On the basis of these results, A-71623 was selected as the prototypic, tetrapeptide-based CCK-A receptor agonist and is presently undergoing evaluation in additional biological assays to establish the therapeutic potential of these compounds.

Thus, we have demonstrated that a tetrapeptide can bind potently to the CCK-A receptor and elicit full agonist activity. We are currently conducting modeling studies to establish how the key structural elements of the tetrapeptide series map against the sulfated octa- and heptapeptides as well as with the various classes of CCK-A receptor antagonists. The results of these investigations along with further details concerning the development of this series will be reported.

(17) The percent maximal response for A-71623 in the amylase assay at 10^{-8} M was 100%; at 3×10^{-8} M, 85%; at 10^{-7} M, 80%; and at 10^{-6} M, 60%. For CCK-8, the percent maximal response at 10^{-9} M was 100%; at 10^{-8} M, 72%; and at 10^{-7} M, 60%. A detailed characterization regarding this aspect of amylase secretion, as well as other biological properties of these analogues, will be addressed in a separate paper submitted for publication by Lin, C. W. et al.

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Phosphorus-Containing Inhibitors of HMG-CoA Reductase. 1. 4-[(2-Arylethyl)hydroxyphosphinyl]-3-hydroxybutanoic Acids: A New Class of Cell-Selective Inhibitors of Cholesterol Biosynthesis

Herein we report a rationale for design and synthesis of a new class of hydroxyphosphinyl-containing 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) inhibitors of general structure **3** which are as effective in inhibiting cholesterol biosynthesis in vitro and in vivo as compounds with proven therapeutic efficacy (e.g., **1a–c**[1]) as hypocholesterolemic agents.[2] In vitro inhibition of cholesterol biosynthesis in whole cells indicates that these compounds exhibit a remarkably high degree of selectivity for hepatic cells compared to nonhepatic cell types.

On a molecular level, it has been demonstrated[3] that the 3,5-dihydroxyheptanoic acid side chain of the fungal metabolites **1** interacts with the 3-hydroxy-3-methylglutaryl (HMG) binding domain of the enzyme's active site. It has been postulated[3] that the tight binding of reductase inhibitors such as **1** is the result of the compounds ability to simultaneously interact with the HMG binding domain of the enzyme and an adjacent hydrophobic pocket which is not utilized in substrate binding. Previous synthetic studies have focused on replacing the highly functionalized decalin nucleus of the fungal metabolites by a variety of aromatic and heteroaromatic nuclei (e.g., **2**).[4] The design of the hydroxyphosphinyl-containing inhibitors was based on mechanistic consideration of the enzymatic reduction of HMG-CoA by HMG-CoA reductase. Kinetic studies have shown[5] that the enzymatic reaction follows the general chemical mechanism postulated for dehydrogenase catalysis in which a group on the enzyme acts as an acid–base catalyst to assist in direct transfer of a hydride ion between nucleotide and substrate. The pK_a of this catalytic group is dependent on whether reduced or oxidized cofactor (NADPH or $NADP^+$, respectively) is bound at the active site. The dihydroxyheptanoic acid inhibitors **1** and **2** apparently owe their inhibitory activity to their ability to mimic the half-reduced substrate mevaldate hemithioacetal. Therefore, by analogy, the 5-hydroxyl group of **1** or **2** must interact with the unprotonated form of this catalytic group. The hydroxyphosphinyl-containing inhibitors **3**, as the corresponding phosphinate anions, were designed to ion pair with the protonated form of the catalytic group[6] which normally serves to activate substrate carbonyl groups toward reduction.

With the goal of developing a general synthetic route to the hydroxyphosphinyl-containing compounds **3** which would allow maximum flexibility with respect to the "hydrophobic anchor" portion of the inhibitors, we envisioned the retrosynthetic disconnection shown in Figure 1. The required phosphonochloridate **5** in homochiral form[7] was prepared by a multistep route from isoascorbic acid (**6**) via known[8] bromohydridin ester **7** by the route

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