Bioorganic & Medicinal Chemistry Letters, Vol.2, No.10, pp. 1251-1256, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

1,3,4-TRISUBSTITUTED PYRROLIDINONES AS SCAFFOLDS FOR CONSTRUCTION OF PEPTIDOMIMETIC CHOLECYSTOKININ ANTAGONISTS

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(*Received* 16 *June* 1992)

Abstract: A new series of cholecystokinin (CCK) antagonists are described which utilizes a new 1,3,4-trisubstituted pyrrolidinone as a scaffold for appending specific amino acid R group mimics (Figure 1). Compound 1A and 1E (SC-50998) exhibit potent nanomolar IC_{50} values in a CCK-A receptor binding assay. Compound 1E behaves as a competitive antagonist in vitro and is orally active.

Cholecystokinin (CCK) is an important brain/gut peptide which exerts profound pharmacology both in the central nervous system and in the periphery.[1] In the central nervous system cholecystokinin acts primarily on CCK-B receptors to modulate mesolimbic dopamine release[2], mediate anxiety/panic disorder[3,4], and control appetite[5]. CCK also functions as a physiological antagonist to endogenous opioids[6]. In the gastrointestinal system, CCK mediates physiological gall bladder contraction[7], pancreatic secretion[7,8], and controls motility through activation of CCK-A type receptors[8,9]. We have sought nonpeptidal antagonists to CCK at peripheral CCK-A receptor subtype for potential use in the treatment of irritable bowel syndrome, pancreatitis, eating disorders, and CCK-dependent pancreatic or colonic tumors.

Researchers from the Merck group[10] have reported the development of potent benzodiazepine CCK-A receptor antagonists based on the natural product lead asperlicin[11]. Rotta[12] and the Abbott[13] groups have reported CCK-A antagonists based on the structure of glutamic acid and benzotript. The Abbott group has also described conformationally constrained peptides wherein either the Met_{31} or Asp_{32} CCK residues are mimicked by gamma-lactams.[14] We now report on a new series of CCK-A antagonists which illustrates the use of 1,3,4-trisubstituted pyrrolidinones as scaffolds for appending specific amino acid side chain mimics in a regio- and stereo-defined manner.

Full agonist activity of CCK at peripheral CCK-A receptors may be mimicked by the C-terminal octapeptide Asp_{26}-$Tyr(SO_3)_{27}$-Met_{28}-Gly_{29}-Trp_{30}-Met_{31}-Asp_{32}-$Phe_{33}NH_2$. Lin et al[15] have recently reported that selective CCK-A agonists may be prepared with the short sequence: BOC-Trp_{30}-X-Asp_{32}-$Phe_{33}NH_2$, wherein X is a linker group. The end-capped Phe residue is essential for agonist activity. Even the slightest manipulation of the primary amide of Phe_{33} abolishes agonist activity. With this information in hand, we designed the pyrrolidinone scaffold shown in Figure 1. In its general sense, this pyrrolidinone scaffold could be used to prepare a variety of peptide mimics for specific receptors by choice of the R residues R_1, R_2, and R_3 (Figure 1A). For the specific case of CCK, we sought examples wherein R_1 mimicked the Phe_{33} side chain,

R_2 the Asp_{32} side chain, and R_3 the Trp_{30} side chain present in CCK-8 (Figure 1B).

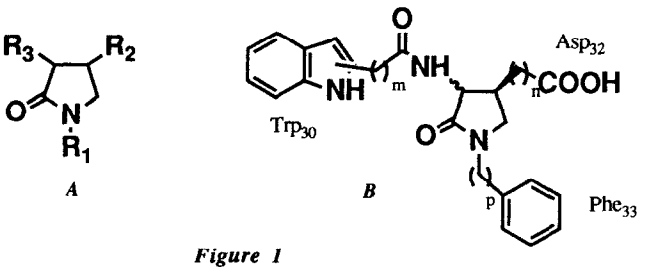


Figure 1

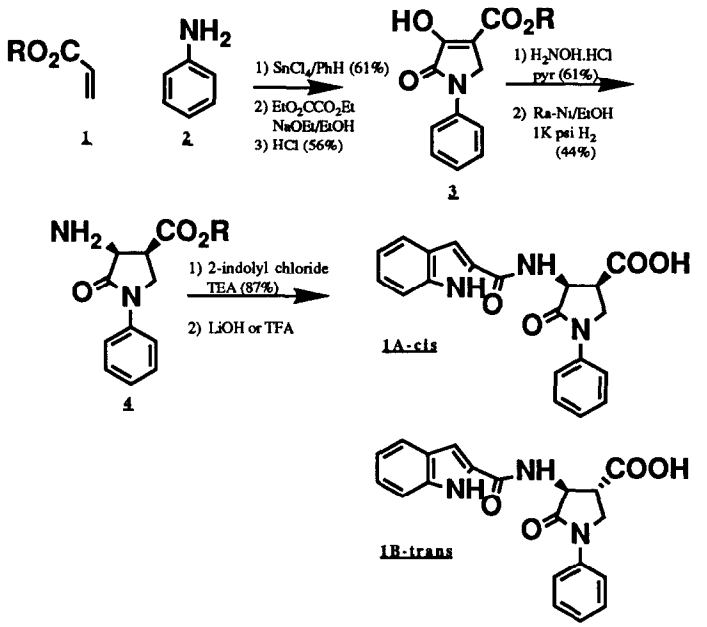
An initial series of compounds was prepared according to the synthesis outlined in Scheme 1. Ethyl or t-butyl acrylate was reacted with aniline to afford a Michael-adduct, which was treated with diethyl oxalate/NaOEt then HCl to give the 2,3-diketo-4-alkoxycarbonyl substituted pyrrolidinone **3**.[16] Oximation (hydroxylamine HCl/pyr) followed by hydrogenation (Ra-Ni, EtOH) gave the cis-3-amino-4-alkoxycarbonyl-1-phenylpyrrolidin-2-one **4**. Intermediate **4** was used to prepare a variety of desired agents using the sequence of amide formation (aroyl chloride/TEA) and hydrolysis (LiOH for R = Et; TFA for R = *t*-Bu). Scheme 1 illustrates the preparation of cis- and trans- **1A** and **1B** wherein R_1 is phenyl, R_2 is carboxyl, and R_3 is an indolyl moiety.

Table 1 illustrates initial structure-activity-relationships in this new series of CCK-A antagonists. Compound **1A** (R_1 = Phenyl; R_2 = COOH, R_3 = 2-indolylamide) is a potent CCK-A antagonist (IC_{50} = 19 nM) when evaluated in the rat pancreatic acinar membrane binding assay.[16] **1A** exhibits excellent selectivity as demonstrated by its weak IC_{50} value of 4.8 μM in the guinea pig brain cortex CCK-B binding assay[17] (CCK-A/-B selectivity of 250-fold). Interestingly, the cis-isomer is the more potent diastereomer, as shown by comparison of **1A** with the trans-isomer **1B** in the CCK-A binding assay (IC_{50} = 360 nM).

Substitution of the R_3 group by 3-quinolinyl affords **1D**, which is approximately 10-fold less active than **1A**. However, substitution by R_3 = 2-naphthoyl gives **1E**, which is equiactive (IC_{50} = 16 nM) to **1A** in the CCK-A binding assay. This compound, **SC-50998**, is 600-fold selective for CCK-A vs. CCK-B receptors. Again the cis-isomer **1E** is more more potent than its trans diastereomer **1F**. Finally, the presence of the amide carbonyl in the R_3 group appears quite important. Replacement by a sulfonamide group, as illustrated with **1K**, leads to a complete loss of activity.

Regarding R_1 structure-activity-relationships, substitution of fluorine in the ortho-position of the phenyl moiety affords active compounds (**1D** and **1G**). However, replacement of phenyl by benzyl (**1C**, **1H**) results in a precipitious drop in binding affinity at CCK-A receptors. This suggests that the Phe_{33} mimic in this

series is optimal when there is no alkylene spacer present in R_1 (Figure 1). Finally, initial variation of the R_2 substituent indicates that a carboxylic acid moiety is essential for binding affinity. The t-butyl ester **1J** [also the methyl ester and primary carbonol ($R_2 = CH_2OH$), not shown] does not bind to either CCK-A or -B receptors up to 1,000 nM. This suggests that the R_2 carboxylate is functioning as an aspartate mimic.

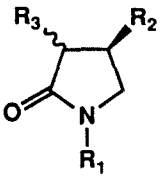


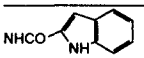
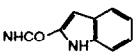
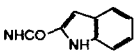
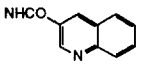
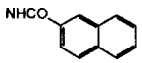
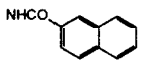
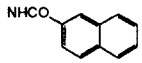
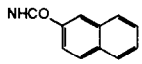
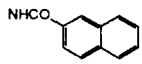
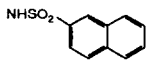
Scheme 1

Compound **1E** (SC-50998) was further evaluated in both in vitro and in vivo models of CCK-A antagonism. SC-50998 competitively blocked guinea pig ileal smooth muscle strip contraction to CCK-8 (pA2 = 8.2) and exhibited no agonist activity when evaluated in this preparation. SC-50998 also reversed CCK-8 induced delayed gastric emptying in the rat both by IP (ED_{50} = 0.41 mg/kg) and oral (ED_{50} = 1.8 mg/kg) routes of administration. The latter result demonstrates oral activity for this new series of peptidomimetic CCK-A antagonists. Details of these and other biological data will be reported in due course.

In summary, the preliminary structure activity relationships observed in this new chemical series is consistent with the original concept shown in Figure 1, where appending specific R side chain residues found in the natural ligand (CCK-8) onto a pyrrolidinone scaffold is able to produce antagonists with high potency.

TABLE 1
Structure-Activity-Relationships for Pyrrolidinone CCK-A Antagonists



ENTRY	3,4-stereo-chemistry	R1	R2	R3	CCK-A BINDING: IC50 (nM)	CCK-B BINDING: IC50 (nM)
A	cis	PHENYL	CO_2H		18.5 (18-19) n = 2	4,800 (4200-5400) n = 3
B	trans	PHENYL	CO_2H		360 (350-370) n = 2	>10,000 n = 2
C	cis	BENZYL	CO_2H		550 (510-590) n = 2	2,000 (1660-2300) n = 2
D	cis	2-F-PHENYL	CO_2H		170 (170-170) n = 2	>10,000 n = 2
E	cis	PHENYL	CO_2H		16 (15-17) n = 2	>10,000 n = 2
F	trans	PHENYL	CO_2H		290 (280-300) n = 2	>10,000 n = 2
G	cis	2-F-PHENYL	CO_2H		62 (59-63) n = 2	>10,000 n = 2
H	cis	BENZYL	CO_2H		1290 (1000-1580) n = 2	7350 (6500-8200) n = 2
J	cis	PHENYL	CO_2tBu		>>1,000 n = 2	>10,000 n = 2
K	cis	PHENYL	CO_2H		>>1,000 n = 2	>10,000 n = 3
Lorglumide					22 (19-25) n = 2	>>1,000 n = 3

Regarding the R_1 substituent, phenyl appears to mimic the Phe_{33} side chain better than benzyl; regarding R_2, carboxyl is essential for mimicking Asp_{32}; regarding R_3, indoyl, quinolinyl, or naphthoyl (fused aromatic groups) appears able to mimic Trp_{30}. In all cases, the 3,4-cis stereochemical arrangement is optimal for antagonist activity. This nonpeptidal approach to antagonists of peptide ligands also is able to generate agents exhibiting oral activity.

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17. Rat pancreas (CCK-A binding) or guinea pig (CCK-B binding) was homogenized in 50 mM Tris-HCl

buffer with a Brinkmann polytron homogenizer. The homogenates were centrifuged twice at 50,000 X g for 20 min with an intermediate rehomogenization in fresh buffer. The final pellets were resuspended in 20 volumes (brain) or 40 volumes (pancreas) of incubation buffer [50 mM Tris-HCl, 5 mM $MgCl_2$, 0.2% BSA, 5 mM dithiothreitol, 0.14 mg/ml bacitracin, pH 7.9 at 25 °C]. One mL of the final homogenate from brain or pancreas was diluted to 50 mL with incubation buffer to use for binding studies. Binding experiments were performed in a final volume of 1 mL. To duplicate tubes were added 890 µL of fresh resuspended pancreas or brain homogenate; ^{125}I-CCK-OP (73,000 dpm, 12 pM), and 10 µL of test compound in DMSO. Nonspecific binding was determined in the presence of 1 µM unlabeled CCK-OP. After Incubation for 30 min at 37° C the binding was stopped by rapid filtration under reduced pressure through Whatman GF/B filters on a Harvester (two washings with 5 mL of ice-cold Tris buffer). The radioactivity on the filters was counted in a gamma-counter. The ability of compounds to displace ^{125}I-CCK-OP binding was assessed in duplicate at concentrations varying from 10^{-5} M to 10^{-8} M.