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CONVERSION OF ACYCLIC NONPEPTIDE CCK ANTAGONISTS INTO CCK AGONISTS

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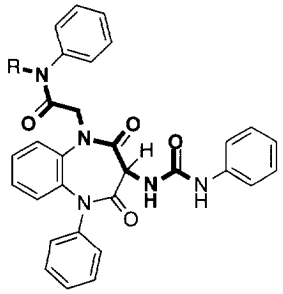
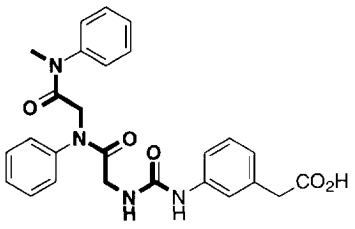
Abstract: The CCK antagonists RP 69758 and (*R*)-lorglumide were converted into CCK agonists by the introduction of an *N*-isopropylanilide agonist "trigger." The common structural features of these ligands suggest that nonpeptide agonists and antagonists bind to a common site in the CCK receptor.

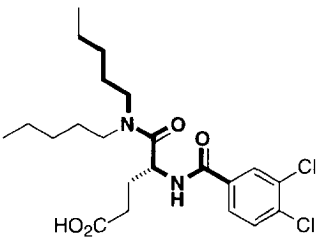
The octapeptide cholecystokinin (CCK) is a gastrointestinal hormone and CNS neurotransmitter.[1] The cognate receptors for CCK are found in two forms, named A and B, which belong to the superfamily of seven transmembrane G-protein coupled receptors. The identification of nonpeptide ligands for these receptors has been the focus of intense effort among several groups over the past decade due to the therapeutic potential of subtype selective agonists and antagonists.[2] The 1,5-benzodiazepine (**1a**) was recently identified as the first nonpeptide CCK agonist.[3] The *N*-isopropylanilide was termed the agonist "trigger", since the related *N*-methyl analog (**1b**) lacked agonist activity. Preliminary examination of **1** revealed an embedded polyamide backbone, elements of which were contained in the acyclic CCK-B antagonist RP 69758 (**2**)[4] and the acyclic CCK-A antagonist (*R*)-lorglumide (**3**)[5] (Figure 1). To probe the structural relationship between these cyclic and acyclic ligands, we have synthesized a series of hybrid structures of **1** with **2** and **3**. In addition to defining the minimal structural requirements for CCK agonist activity, the resulting analogs suggest that nonpeptide CCK agonists and antagonists bind to a common site in the receptor.



1a, R = *i*-Pr
1b, R = Me



2, RP 69758



3, (*R*)-lorglumide

Figure 1. Nonpeptide CCK ligands

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A series of hybrid molecules (**4-7**) were synthesized (Figure 2).[6] Introduction of the *N*-isopropylanilide into the RP 69758 (**2**) backbone gave the phenylacetamide (**4**). Remarkably, **4** demonstrated significant CCK agonist activity in the in vitro guinea pig gallbladder contraction assay (Table 1). Although **4** was not as efficacious as **1a**, it appeared to be more potent as measured by the ED_{50} in the functional assay. This was despite a large drop in receptor binding affinity. Unfortunately, the agonist activity of **4** did not translate to significant in vivo activity in the mouse gallbladder emptying assay. The reason for the lack of in vivo activity is unclear, but may be due to rapid metabolism or unfavorable pharmacodynamic properties of the ligand. However, **4** remains the smallest peptide-receptor agonist described to date, and it defines the minimal structural requirements for nonpeptide CCK mimetics.

Replacement of the *n*-pentyl chain of (*R*)-lorglumide (**3**) with the *N*-isopropylanilide gave the glutamic acid derivative (**5**). This hybrid compound was devoid of agonist activity. Notably, the related hybrid compound (**6**), in which the phenyl amide was replaced by a phenyl urea, showed agonist activity in vitro which translated to weak agonist activity in vivo. This result parallels our observations of the structure activity in the 1,5-benzodiazepine series, where phenyl amides show less agonist activity than the corresponding phenyl ureas,[7] and suggests that these substituents may bind to similar sites in the CCK receptor in both series of ligands.

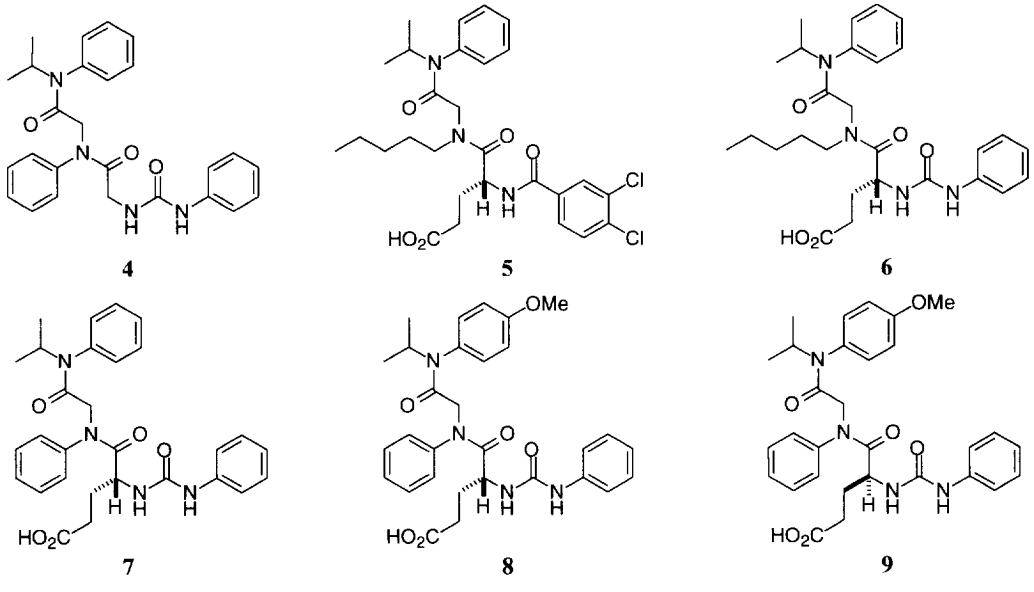
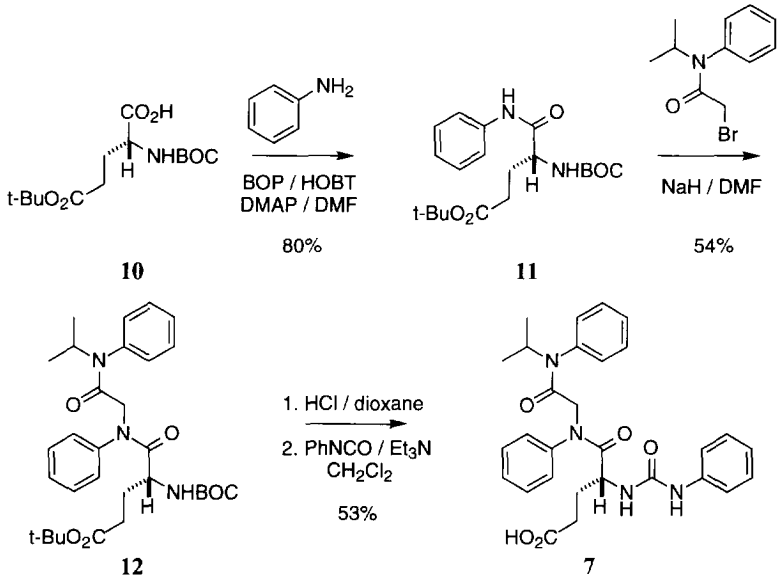


Figure 2. Hybrid CCK ligands

In the third iteration of hybrid molecules, the structural features of **4** and **6** were combined in the glutamic acid *N*-phenylamide analog (**7**). This compound retained the in vitro profile of **4**, demonstrating submicromolar CCK agonist activity. In addition, **7** demonstrated good in vivo activity in the mouse gallbladder emptying assay. The synthesis of **7** is outlined in Scheme 1. The *N*-Boc-d-glutamic acid ester (**10**) was coupled with aniline to generate the amide (**11**). Deprotonation of **11** and alkylation with 2-bromo-*N*-isopropylphenylacetamide[3] gave the tertiary amide (**12**) in moderate yield. Optimal conditions employed sequential anion formation at 0 °C for 5 min followed by addition of the alkylating agent. HCl deprotection

and coupling of the resulting amino acid hydrochloride salt with phenylisocyanate yielded the phenyl urea (**7**) as a white lyophile following HPLC purification.[6]



Scheme 1. Synthesis of d-glutamic acid CCK agonists

The studies that led to the identification of the hybrid CCK agonist (**7**) suggest that our original analysis of the relationship between CCK ligands (**1-3**) (Figure 1) was correct. Thus, exchange of an *N*-methylanilide for an *N*-isopropylanilide converted the benzodiazepine antagonist (**1b**) into the agonist (**1a**), and the acyclic antagonist (**2**) into the agonist (**4**). In the *N*-acylglutamic acid series, antagonist (**3**) yielded the agonists (**6** and **7**) when the *N*-isopropylanilide replaced the *n*-pentyl chain. These parallel changes in functional activity provide strong circumstantial evidence that these cyclic and acyclic nonpeptide ligands bind to similar sites in the CCK receptor.

Several groups have previously synthesized hybrid compounds between (*R*)-lorglumide (**3**) and the 1,4-benzodiazepine CCK-A antagonist MK-329 based on an alternate overlap of these ligands.[8] A critical component of these earlier models was the observation that (*R*)-lorglumide (**3**) and the (*S*)-isomer of MK-329 were more active as CCK-A antagonists than their corresponding enantiomers.[8a,b] In order to explore the effect of absolute configuration on the CCK activity of our *N*-acylglutamic acid agonists, the *N*-isopropyl-*p*-methoxyanilides (**8** and **9**) were synthesized from d-glutamic acid and l-glutamic acid, respectively (Figure 2).[6,9] The (*R*)-enantiomer (**8**) demonstrated CCK agonist activity comparable to **7**, both in vitro and in vivo (Table 1). By contrast, the (*S*)-enantiomer (**9**) was only a weak partial agonist in vitro. Thus, the enantioselectivity demonstrated by the *N*-acylglutamic acid CCK agonists (**8** and **9**) mirrored the selectivity reported for the related *N*-acylglutamic acid antagonists.[8a,b] The origin of the difference in the homology models may lie in the fact that, unlike the earlier antagonists, the agonist hybrid compounds now combine the structural features of both CCK-A and CCK-B selective ligands.[10] Notably, the observed preference for the (*R*)-enantiomers in both series of glutamic acid analogs provides further evidence of a common binding site for these nonpeptide agonist and antagonist CCK ligands.

Table 1. Biological data[a]

Compound	in vitro[b] EC_{50} (μM)	in vitro[b] RE	in vivo[c] RE	binding (pIC_{50})[d] CCK-A	binding (pIC_{50})[d] CCK-B
CCK-8	0.002	1.0	1.0	8.9	9.5
1a	1.6	0.8	0.8	7.3	7.6
4	0.30	0.6	<0.2	5.5	5.8
5	—	<0.1	—	—	—
6	—	0.5	0.3	—	—
7	0.21	0.6	0.6	5.8	5.0
8	0.11	0.6	0.8	6.6	4.5
9	5.6	0.2	—	—	—

[a] See reference 3 for general methods; all data represent the mean of duplicate tests ±10%; —, not tested.
[b] Contraction of guinea pig gallbladder tissue; EC_{50}, concentration of test compound that induced 50% of the maximal contraction; RE, relative efficacy following incubation with 1-30 μM test compound normalized to 1 μM CCK-8.
[c] Mouse gallbladder emptying following ip administration of 0.1 $\mu molkg^{-1}$ test compound; RE, relative efficacy of test compound normalized to 1.0 $nmolkg^{-1}$ CCK-8.
[d] Displacement of [^{125}I]-CCK-8 from membrane preparations isolated from CHO-K1 cells stably transfected with the cDNA of human CCK-A and CCK-B receptors; pIC_{50}, negative $\log_{10}$ of the concentration of test compound that displaced 50% of the radioligand.

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