High-Affinity and Potent, Water-Soluble 5-Amino-1,4-benzodiazepine CCK_B/Gastrin Receptor Antagonists Containing a Cationic Solubilizing Group

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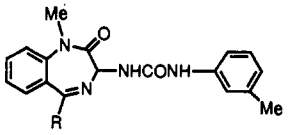
Cholecystokinin (CCK) is a polypeptide hormone which occurs in numerous molecular forms throughout the peripheral and central nervous systems. CCK exerts a variety of actions on peripheral organs, such as regulating pancreatic secretion, gut motility, and gall bladder contraction. In addition CCK may function as a neurotransmitter or neuromodulator in the central nervous system.[1,2] The actions of CCK are mediated by two receptor subtypes designated as CCK_A and CCK_B.[3] The CCK_B receptor subtype also displays ligand specificities similar to the stomach gastrin receptor.[4,5]

The benzodiazepine series of non-peptide CCK receptor antagonists, which was designed from the natural product asperlicin,[6] has been well documented. The high affinity and potent CCK_A receptor selective antagonist MK-329[7] and the CCK_B receptor selective antagonist L-365,260[8,9] (1) resulted from this work. One factor which determined CCK receptor subtype selectivity in this series was the C3-stereochemistry of the benzodiazepine ring system, the (3*R*)-enantiomer generally providing CCK_B receptor selectivity. Recent studies have shown that the C5-phenyl moiety of the core benzodiazepine structure could be replaced by C5-cycloalkyl groups, a modification which retained CCK_B affinity and selectivity.[10] In particular, the C5-cyclohexyl analog (2) displayed sub-nanomolar CCK_B receptor affinity (IC_{50}, 0.28 nM), with improved selectivity (CCK_A/CCK_B = 6500) compared to L-365,260.

The aqueous solubility of crystalline 1 has been measured as <0.002 mg/mL in the pH range 3–7.4, and pharmacokinetic studies[11] have shown that 1 displayed limited bioavailability in rat, dog, and monkey when dosed orally using 0.5% methylcellulose as a vehicle. This suggests that 1 would not be a suitable chemical entity for oral administration in humans as a simple tablet formulation.[12] The cyclohexyl analog 2 would be expected to suffer from the same drawback.

The objective of this present study, therefore, was the design, synthesis, and evaluation of CCK_B receptor antagonists which would retain the excellent binding and selectivity characteristics of 2, while embodying a water-solubilizing handle. In an accompanying Communication, we report on a series of acidic CCK_B receptor antagonists which fulfill these criteria. In the present work, we sought to complement the acidic series by incorporating an amine-based cationic solubilizing group within the benzodiazepine framework. We report herein the first example of a series of selective, high-affinity, non-peptide CCK_B receptor antagonists which contain overtly basic functionality.

Table 1. CCK_A and CCK_B Receptor Affinities for Benzodiazepine Ligands 1–6



compound[a]	R	C3 stereo	rat pancreas[b] CCK_A (IC_{50}, nM)[d]	guinea pig cortex[c] CCK_B (IC_{50}, nM)[d]
1		*R*	736 (585; 925)	8.50 (6.46; 11.2)
2		*R*	1797 (1205; 2115)	0.28 (0.13; 0.59)
3 (base)		*R,S*	480 (303; 759)	137 (137; 137)[e]
4 (base)		*R,S*	17 (15; 19)	5.7 (4.6; 7.0)
5a (base)		*R,S*	10.4 (5.8; 18.7)	1.25 (1.18; 1.33)
5b (HCl salt)		*R*	>3000 (38%)[f]	1.35 (1.13; 1.62)
5c (HCl salt)		*S*	7.9 (6.16; 10.2)	144 (101; 205)
6a (base)		*R,S*	19.9 (14.4; 27.4)	0.53 (0.38; 0.74)
6b (HCl salt)		*R*	1604 (1408; 1819)	0.10 (0.056; 0.187)
6c (HCl salt)		*S*	6.5 (4.83; 8.65)	26.7 (25.6; 27.8)

[a] All novel compounds gave satisfactory analytical data in full agreement with the proposed structure. [b] CCK_A binding was measured by the displacement of [^{125}I]BH CCK-8s from rat pancreatic tissues. [c] CCK_B binding was measured by the displacement of [^{125}I]BH CCK-8s from guinea pig cortical membranes. [d] Binding results are the geometrical mean of two to four independent determinations. Statistical limits are given in parentheses. [e] Geometric mean of two determinations, which were identical. [f] Full IC_{50} not obtained, percentage inhibition at a concentration of 3000 nM given in parentheses.

In principle, one way in which cationic, water-solubilizing functionality could be introduced into L-365,260 (1) would be to replace the C5-phenyl ring by a cycloalkylamine, a maneuvre which would generate an amidine. The knowledge that the C5-phenyl ring could be successfully replaced by a cycloalkyl ring (cf. 2) gave considerable support to this notion. It was uncertain, however, if the CCK_B receptor site would tolerate the presence of a positive charge, since all reported high-affinity ligands for the CCK_B receptor were either neutral or acidic in nature.[13,14]

Compounds 3–6 (Table 1) were synthesized (Scheme 1) by forming the chloro imine from the bis-lactam (7), followed by reaction with the requisite amine, to afford the benzodiazepine nucleus (8). The C3-amino group was introduced via the oxime (9).[15] Reduction of the oxime using the conditions reported for the C5-phenyl analog[15] gave low, unreproducible yields. The use of other catalytic reduction methods and chemical reaction conditions (e.g., zinc/acetic acid) did not improve the yield significantly. The problem was solved by functionalizing the oxime to afford the carbamate (10), which was readily reduced at ambient temperature using 10% Pd on carbon under a hydrogen atmosphere. The electron-withdrawing nature of the carbamate group may activate the double bond to hydrogenation and subsequently the N–O bond to hy-

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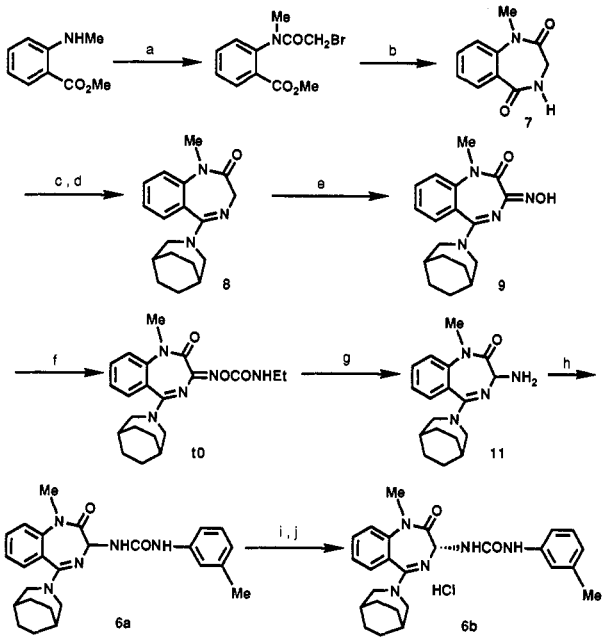
Scheme 1[a]



[a] Reagents: (a) $BrCH_2COBr$, NaOH, H_2O, CH_2Cl_2; (b) NH_3, MeOH; (c) PCl_5, CH_2Cl_2; (d) NEt_3, CH_2Cl_2, 3-azabicyclo[3.2.2]nonane; (e) KO^{t}Bu, toluene, isopentyl nitrite, −20 °C; (f) EtNCO, THF, NEt_3, 60 °C; (g) 10% Pd on C, MeOH, H_2, 40 psi; (h) *m*-tolyl isocyanate, THF; (i) Pirkle DNBL column, *n*-BuCl, MeOH, AcOH; (j) HCl.

drogenolysis. This methodology gave excellent, reproducible yields of the amine (11), which was then treated with *m*-tolyl isocyanate in THF, allowing the urea (**6a**) to crystallize in pure form from the reaction. Compounds **3–5** were synthesized in a similar fashion. The enantiomers were obtained by using semipreparative HPLC,[10] employing a Pirkle (dinitrobenzoyl)leucine column.

The CCK receptor affinities of the ligands **3–6** were evaluted in vitro using radioligand binding techniques[16] on rat pancreatic membranes (for CCK_A) and guinea pig cortical membranes (for CCK_B) (Table 1). The racemic C5-piperidino analog **4** showed a noteworthy 24-fold increase in CCK_B affinity compared to the pyrrolidine **3**. Employing a homopiperidine ring system to provide an increase in C5-substituent lipophilicity and bulk gave rise to a further improvement in CCK_B affinity (**5a**, CCK_B: IC_{50}, 1.25 nM). Resolution of **5a** confirmed the expected CCK_B selectivity of the series, the *R*-enantiomer (**5b**) showing much improved CCK_B receptor selectivity (CCK_A/CCK_B > 2300) when compared to the prototype CCK_B antagonist (**1**). Encouragingly, **5b** also displayed good aqueous solubility as its crystalline hydrochloride salt (0.6 mg/mL). Optimization of the benzodiazepine C5 substituent led to the azabicyclo[3.2.2]nonane derivative (**6b**).[17] Compound **6b** showed high CCK_B receptor affinity (IC_{50}, 0.10 nM) while also displaying excellent receptor subtype selectivity (CCK_A/CCK_B = 16 000). The aqueous solubility of the crystalline hydrochloride salt of **6b** was measured as 0.15 mg/mL, log *P* (octanol/pH 7.4 aqueous buffer) 4.7, and the pK_a was 7.1. These physicochemical parameters are consistent with a profile suitable for a simple tablet formulation while also in principle being compatible with good brain penetration in vivo, since 66% of **6b** would be un-ionized at physiological pH. The unusually low pK_a of the amidine is presumably due to the electron-withdrawing influence of the adjacent lactam and urea functional groups around the benzodiazepine ring.

The CCK_B/gastrin receptor selectivity of **6b** was studied, but no discernible selectivity between gastrin and CCK_B receptors was observed ([^{125}I]gastrin: IC_{50}, 0.04 nM, n = 3, guinea pig gastric glands), not surprisingly in view of the proposed equivalence of the CCK_B and gastrin receptors.[18] Compound **6b** was inactive (IC_{50}, >10 000 nM, n = 2) at the $GABA_A$ benzodiazepine receptor in rat cortical membranes, as measured by displacement of the specific binding of the antagonist [^{3}H]Ro15-1788.

A measure of the functional antagonist activity of **6b** was obtained from the blockade of CCK_B receptor-mediated excitations of rat ventromedial hypothalamic (VMH) neurons,[19,20] recorded from brain slices in vitro. Compound **6b** blocked the extracellularly recorded increase in single-cell firing rate, which was induced by pentagastrin in a concentration-dependent manner. From the rightward shift of the pentagastrin concentration–response curve the estimated K_b was 0.06 nM (±0.01 nM, n = 6),[21] indicating that **6b** is an exceptionally potent CCK_B receptor-selective antagonist.

In conclusion, compound **6b** represents one of the most potent and selective CCK_B receptor antagonists reported to date. Additionally, **6b** displays water solubility suitable for simple tablet formulations and has physicochemical properties compatible with brain penetration in vivo.[22] **6b** (L-740,093) therefore represents an attractive molecule with which to study the pharmacology of CCK_B receptors within the central nervous system.

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Supplementary Material Available: Experimental procedure, including the analytical and spectral data, for the preparation of **6b** (4 pages). Ordering information is given on any current masthead page.

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(16) Radioligand binding to guinea pig cortical membranes was performed using 50 pM [^{125}I]-labeled Bolton Hunter CCK-8s in 20 mM HEPES buffer, pH = 6.5, containing 150 mM NaCl, 5 mM $MgCl_2$, 1 mM EGTA, and 0.025% bacitracin. For rat pancreatic membranes, assay buffer was supplemented with 0.01% trypsin inhibitor and 0.2% BSA. Guinea pig cortical membranes were prepared by homogenization in 0.32 M sucrose, centrifugation, and resuspension of the P2 pellet in assay buffer at 1 g wet weight in 120 mL. Rat pancreatic membranes were prepared in 10 mM HEPES/0.01% trypsin inhibitor, pH = 7.4, and centrifuged, and the pellet was resuspended in assay buffer at a 1 to 2000 dilution. Specific binding in all cases was defined using 1 μM CCK-8s, and the reaction was terminated by filtration through Whatman GF/C filters, using a Brandel cell harvester with 3 × 3-mL washes in ice-cold 100 mM saline wash buffer. Filters were counted on a LKB γ counter.

(17) The absolute configuration of **6b** was assigned as 3*R* on the basis of the comparison of circular dichroism spectra to other CCK-benzodiazepine ligands, where the absolute configuration was known by X-ray crystallographic or chemical means. All enantiomers described were >99% ee, as determined by chiral HPLC.

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(21) Coronal sections (from the brains of male Sprague–Dawley rats), 350 μm thick, containing the VMH were cut and placed in a small chamber, where they were continually perfused with oxygenated warmed artificial cerebrospinal fluid. Extracellular recordings of action potential firing rate were made from single VMH neurones using glass micropipettes filled with 3 M NaCl and having a resistance of 10–30 MΩ. CCK_B receptor-mediated responses were evoked with the CCK_B selective agonist pentagastrin, in the presence of the CCK_A receptor antagonist Devazepide (30 nM). Following the determination of a control concentration–response curve to pentagastrin, **6b** was continuously perfused until the level of block reached equilibrium (approximately 2 h). The antagonist affinity (K_b) was determined from the shift to the right (concentration ratio, CR) of the pentagastrin concentration–response curve produced by the antagonist, using the equation K_b = [antagonist]/(CR - 1). The value K_b = 0.06 nM was obtained using a concentration of 0.3 nM (n = 6).

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