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C5-PIPERAZINYL-1,4-BENZODIAZEPINES, WATER-SOLUBLE, ORALLY BIOAVAILABLE CCK$_B$/GASTRIN RECEPTOR ANTAGONISTS

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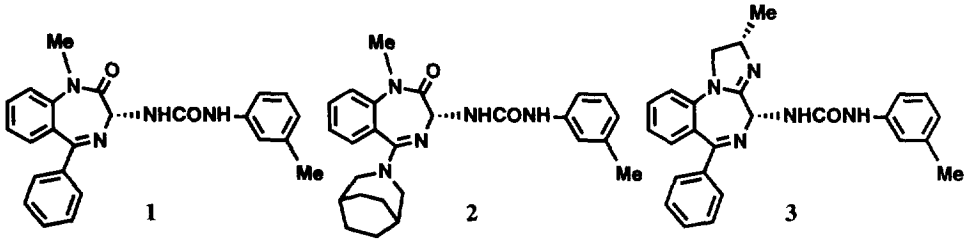
Abstract: A novel series of potent, water-soluble benzodiazepine based CCK$_B$/gastrin antagonists has been prepared which incorporate an N-methylpiperazine group at the C5 position of the benzodiazepine ring system. The N1-n-propyl analogue (**7b**) is a high affinity, selective and potent receptor antagonist *in vitro*, with good bioavailability and excellent oral absorption providing high plasma levels *in vivo*.

Cholecystokinin (CCK) is a member of a family of peptides which exerts effects on gut functions. At the present CCK receptors have been classed into two subtypes designated as CCK$_A$ and CCK$_B$.[1] The CCK$_B$ receptor subtype also displays ligand specificities similar to the gastrin receptor.[2] The majority of CCK receptors in the brain are of the CCK$_B$ subtype and these receptors are widely distributed throughout the brain[3] where it is postulated to modulate dopaminergic systems.[4]

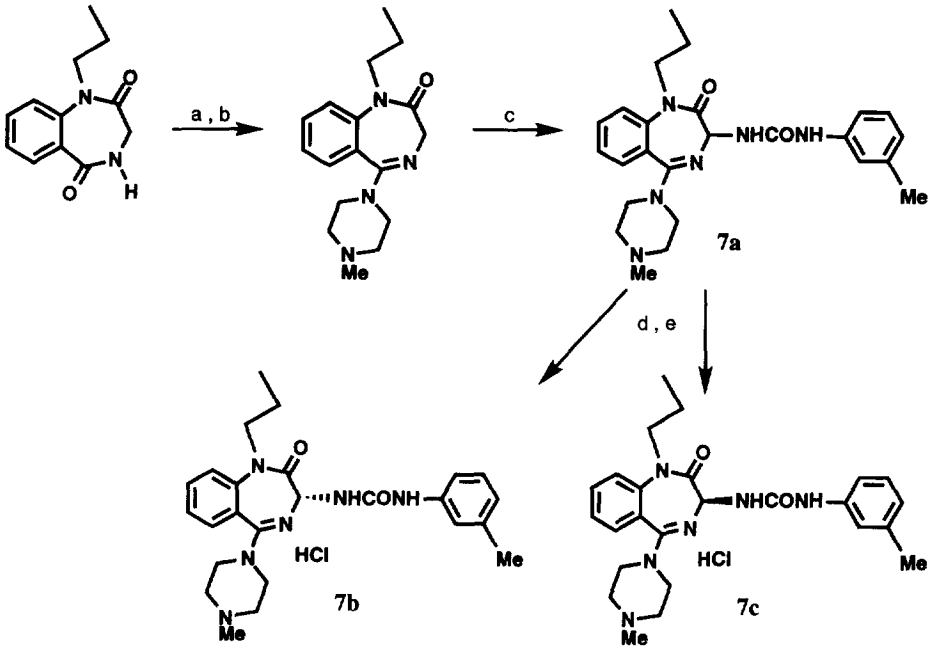
C3-Urea derivatives of 1,4-benzodiazepines (e.g **1**) have been known for some time to be antagonists of the CCK$_B$/gastrin receptor[5] and attempts to improve the aqueous solubility and bioavailability of such ligands, by incorporation of basic functionality, have recently been described.[6,7] These studies have led to the identification of high affinity, potent CCK$_B$/gastrin receptor antagonists **2** and **3**.



Further elaboration of the C5-substituent within the benzodiazepine framework has resulted in a complementary series of C5-piperazinyl-1,4-benzodiazepines providing water soluble CCK$_B$/gastrin antagonists with good oral bioavailability.

The benzodiazepine derivatives **4** - **11** were synthesised using the chemical procedures described previously.[6,8] An outline of the synthesis of **7b** is shown in Scheme 1. The enantiomers **7b** and **7c** were obtained from **7a** by using semipreparative HPLC, employing a Pirkle (dinitrobenzoyl)leucine column.[6]

Scheme 1[a]

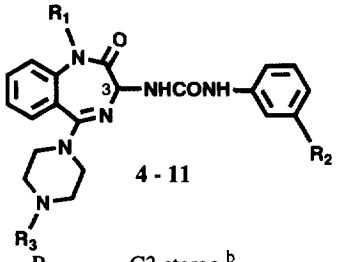


[a]Reagents: (a) PCl_5, CH_2Cl_2; (b) NEt_3, CH_2Cl_2, N-methylpiperazine; (c) See reference 6; (d) Pirkle DNBL column, nBuCl, MeOH, AcOH; (e) HCl.

Replacement of the C5-phenyl group in **1** by N-methylpiperazine provided compound **4**, which shows a fourteen fold reduction in affinity for the CCK_B receptor (Table 1) compared to **1**. In addition N-demethylation on the piperazine ring (**5**), or the presence of a carbamate group (**6**), gave analogues with poor receptor affinities. These results suggested that the CCK_B receptor is poorly tolerant of basic functionality or electron density at this portion of the receptor. It is well precedented[7,9] that addition of hydrophobicity to the N1 position of the benzodiazepine nucleus generally improves affinity at the CCK_B receptor. Substitution of the N1-methyl group with n-propyl afforded **7a** which exhibits an eleven fold increase in affinity and improved selectivity over the CCK_A receptor. An increase in lipophilicity on the phenylurea portion of the molecule provided compounds of comparable affinity and selectivity for the CCK_B receptor (compounds **8** and **9**). Neither the methoxy (**10**) or trifluoromethyl-phenylurea (**11**) offer any improvement in affinity *in vitro*.

The combination of good CCK_B affinity and water-solubility led **7a** to be the choice for resolution into its enantiomers. The R-enantiomer (**7b**) shows affinity for the CCK_B receptor comparable to **1** but with improved subtype selectivity (CCK_A/CCK_B >400), and displays high affinity for gastrin receptors (IC_{50} 2.7 nM, n = 2, guinea pig gastric glands). To support the high CCK_B affinity and subtype selectivity of **7b**, its functional activity was assessed *in vitro* using electrophysiological studies in rat brain slices. The pentagastrin-induced single cell firing rate of rat ventromedial hypothalamic (VMH) neurons is potently blocked by **7b** (K_b 1.8 nM (+/- 0.74 nM), n = 5). Excitatory effects in the VMH by pentagastrin are mediated through CCK_B receptors[10] indicating that **7b** is a potent and selective CCK_B receptor antagonist *in vitro*.

Table 1 CCK_A and CCK_B receptor affinities for Benzodiazepine ligands **1 - 11**



4 - 11

Compound [a]	R_1	R_2	R_3	C3 stereo [b]	IC_{50}, nM [c]	
					CCK_A rat pancreas	CCK_B guinea-pig cortex
1				R	736 (585; 925)	8.50 (6.46; 11.2)
2				R	1604 (1408; 1819)	0.10 (0.056; 0.187)
3				R	1559 (1119; 2171)	1.81 (1.2; 2.7)
4	Me	Me	Me	RS	4257 (3613; 5016)	121 (79; 184)
5	Me	Me	H	RS	>3000 (3%) [d]	880 (430; 1810)
6	Me	Me	CO_2CMe_3	RS	>3000 (0%)	>3000 (0%)
7a	nPr	Me	Me	RS	3200 (2700; 3900)	9.8 (6.6; 15)
7b	nPr	Me	Me	R	>3000 (11%)	7.6 (6.7; 8.6)
7c	nPr	Me	Me	S	1900 (1500; 2400)	270 (250; 298)
8	nPr	Et	Me	RS	>3000 (46%)	4.9 (4.6; 5.2)
9	nPr	nPr	Me	RS	5310 (5190; 5430)	18.3 (8.1; 41.4)
10	nPr	OMe	Me	RS	>3000 (15%)	31 (27; 37)
11	nPr	CF_3	Me	RS	3800 (3700; 4010)	11.8 (8.1; 17)

[a] All novel compounds gave satisfactory analytical data in full agreement with the proposed structure.

[b] The absolute configuration at the benzodiazepine C 3-position, see reference 6.

[c] Binding results are the geometric mean of between two to four independent determinations. Statistical limits are given in parentheses. Displacement of [^{125}I]BH CCK-8S, see reference 6 for details.

[d] Full IC_{50} not obtained, percentage inhibition at a concentration of 3000 nM given in parentheses.

Oral absorption has often been a problem with C3-urea substituted benzodiazepine CCK antagonists[11] and analogues such as **2**, **3** and **7b** have been designed to overcome such limitations. Rat pharmacokinetic studies established that the oral absorption of **7b**[12] is superior to that of both **1** and **2**. Compound **7b** (as its L-tartrate, dosed in water) has a bioavailability of 51%, and is rapidly absorbed (t_{max} = 5 minutes) with high plasma levels (C_{max} = 469 ng/mL) and a calculated plasma half-life of 55 minutes.

The C5-piperazinyl-1,4-benzodiazepine, **7b**[13], displays CCK_B receptor affinity comparable with that of **1**, but with improved CCK sub-type selectivity, and is a potent CCK_B receptor antagonist *in vitro*. In addition **7b** has good water solubility (0.6 mg/mL) and is absorbed efficiently in the rat when dosed orally in an aqueous vehicle. Compound **7b**, therefore, represents an additional example of structurally diverse CCK_B/gastrin receptor antagonists which can be added to our arsenal of ligands to aid our understanding of CCK_B/gastrin functions *in vivo*.

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12. Following overnight food deprivation, 36 male Sprague-Dawley rats (approx. 200 g body weight) were dosed with **7b** L-tartrate (3 mg/kg, eighteen i. v., eighteen p. o.) as an aqueous solution. Each rat was sacrificed at one of six predetermined time points (5, 15, 30, 60, 120, and 240 minutes) and a large blood sample was removed. Plasma was separated by centrifugation and kept at -20°C until analysis. Solid phase extracts of thawed plasma was analysed by HPLC using U. V. detection and an appropriate internal standard. Ref. 5 shows plasma levels of compound **1** measured in μg/mL (Table V). These units should read ng/mL.
13. **7b** L-tartrate: mp >165°C. Chemical purity: >99%, spherisorb phenyl column, 40% MeCN in 50 mM KH_2PO_4 (0.2% triethylamine, pH = 3), λ = 230 nm. Enantiomeric purity: >99.5% Pirkle DNBL column, 5% MeOH in dichloromethane (0.8% AcOH), λ = 250 nm. The aqueous solubility was measured as 0.6 mg/mL, logP (octanol/pH 7.4 buffer) 2.98, and the measured pKa was 7.6.

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