Second-Generation Benzodiazepine CCK-B Antagonists. Development of Subnanomolar Analogs with Selectivity and Water Solubility

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Received November 22, 1993

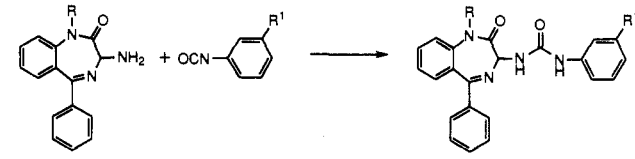
Isolated receptor preparations and sensitive bioassays afford a starting point for the identification of novel nonpeptide ligands for peptide receptors.[1] The selective cholecystokinin (CCK) antagonist, asperlicin, was deliberately sought out in this way and provided the catalytic spark for the ensuing development of the potent CCK-A- and CCK-B-selective agents MK-329 and L-365,260, respectively.[2] In the interim, additional examples of nonpeptide CCK receptor antagonists have emerged, expanding the structural diversity of agents binding to these receptors, especially those of the CCK-B subtype.[3] The recent molecular cloning and characterization of the CCK-A[4] and CCK-B/gastrin receptor subtypes[5,6] have manifestly intensified interest in the CCK area where the allure among medicinal chemists is to discover agents which control anxiogenesis/panic,[7,8] influence satiety,[9] and modulate dopamine-mediated behaviors.[10]

The archetypal nonpeptide CCK-B antagonist is L-365,-260 (1).[11] It displays high affinity for the human CCK-B receptor and is moderately selective compared with the CCK-A receptor (Table 1). A number of studies have been carried out in animals, including humans, which suggest promise for its possible therapeutic utility.[12,13] In spite of these encouraging results, L-365,260 suffers from limitations. Its chief deficit is low aqueous solubility (Table 1) of the crystalline form, necessitating the use of special formulations to obtain adequate oral bioavailability.[14] We therefore extended our search for compounds within the benzodiazepine manifold that would exceed the binding and selectivity attributes of 1 while overcoming some of its inherent physicochemical liabilities. In the discussion which follows we make our initial disclosure of a second generation of 1,4-benzodiazepine CCK-B receptor antagonists that meet these criteria.

We have previously identified several structural domains associated with the 3-(arylureido)-1,4-benzodiazepine core of 1 which can be modified without loss of CCK-B receptor binding affinity.[15] On this basis, we placed particular emphasis during this study on altering the N^1-substituent, R, and the nature of the phenylurea substituent, R^1 in 1.

The compounds shown in the table were prepared by combining either (3*R*)- or (3*S*)-1-alkyl-3-amino-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one with an aryl isocyanate (Scheme 1). The essential 1-alkyl-3-amino-1,4-benzodiazepines were synthesized according to previously described procedures.[16,17] The requisite aryl

Scheme 1



isocyanates were prepared *in situ* from the corresponding arylamines and triphosgene.[15] 3-(Aminophenyl)tetrazole and 3-(aminophenyl)oxadiazolone were synthesized from commercially available 3-aminobenzonitrile by employing conventional techniques.

IC_{50} values (nM) for half-maximal inhibition of binding of [^{125}I]Bolton Hunter CCK-8 to CCK receptors in rat pancreatic tissue and guinea pig cortical membranes were obtained as previously reported.[15,18]

Among the first analogs prepared which displayed measurable advances over 1 was the benzoic acid derivative 2 (Table 1). This compound retains high CCK-B receptor affinity and by virtue of its acidic functional group displays much improved aqueous solubility. Further increases in CCK-B receptor binding affinity and selectivity were subsequently achieved by incorporating carboxylic acid surrogates in the C^3-phenylurea appendage of 1. For example, the tetrazole-containing analog 3 shows an 8-fold increase in CCK-B receptor affinity and an enhancement in CCK-B versus CCK-A selectivity from 87 to 566; moreover, it has better water solubility than 1 by several orders of magnitude. The 1,2,4-oxadiazolone 7 also shows more favorable CCK-B receptor affinity/selectivity and solubility profiles than 1. To account for the enhanced CCK-B receptor potency of 3 and 7, we infer that the oxadiazolone and tetrazole rings (or other similar optimally placed polar phenylurea substituent) interact with a fundamental region of the CCK-B receptor in a manner unavailable to 1.[19]

CCK-B receptor potency and selectivity of the above-described analogs could be further augmented by replacing the N^1-methyl group with more lipophilic substituents. Analog 5 is approximately 7-fold more potent than 3, and it shows CCK-B/CCK-A selectivity which has now been enhanced by more than 2 orders of magnitude compared with 1. Other N^1-alkyl substituents were examined but the isobutyl, cyclopropylmethyl, and *n*-propyl (data not shown) groups were optimal. The further boost in receptor binding potency realized by increasing the lipophilic character of the N^1-substituent may be a consequence of the superior interactions of this substituent with that region of the CCK-B receptor, recently identified by site-directed mutagenesis,[20] which contains the critical aliphatic amino acid residue (Val^{319} in the human receptor) underlying non-peptide antagonist affinities.

Pharmacologically, both analogs 5 and 7 retain high affinity for the human CCK-B receptor from human cerebral cortex (5, IC_{50} 0.27 nM; 7, IC_{50} 0.61 nM) and for the guinea pig gastrin receptor ([^{125}I]gastrin: 5, IC_{50} 0.24 nM; 7, IC_{50} 0.17 nM, guinea pig gastric glands). The latter result supports the recently established identity between CCK-B and gastrin receptors.[21] As anticipated, neither 5 nor 7 have affinity for the GABA-A benzodiazepine binding site (IC_{50} > 10 mM) as measured by the specific binding of the antagonist [^{3}H]Ro 15-1788 to rat cortical membranes.

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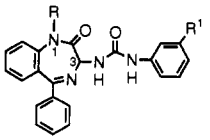
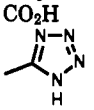
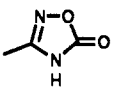
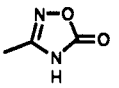
0022-2623/94/1837-0722$04.50/0

Table 1. Receptor Binding Affinities and Solubility Properties of 3-(Phenylureido)-1,4-benzodiazepines[a]



no.	R	R^1	3 stereo[b]	IC_{50} (nM) CCK-B	IC_{50} (nM) CCK-A	solubility,[c] mg/mL (pH)
1	CH_3	CH_3	*R*	8.5 (6.46; 11.2)	736 (585; 925)	<0.002 (7.4)
2	CH_3	CO_2H	*R*	6.69 (5.94; 7.54)	1555 (1331; 1816)	4.7 (7.4)
3	CH_3		*R*	1.02 (0.618; 1.68)	577 (473; 704)	3.74 (7.4)
4	CH_3		*S*	250 (115; 545)	6.16 (5.23; 7.26)	ND[d]
5	*i*-Bu		*R*	0.142 (0.070; 0.288)	1434 (1393; 1476)	1.4 (7.0) >11 (8.0)
6	*i*-Bu		*S*[e]	23.8 (17.9; 31.7)	379 (292; 491)	ND
7	CH_3		*R*	0.266 (0.174; 0.405)	983 (777; 1244)	0.41 (7.4) 1.66 (8.0)
8	CH_3		*S*	56.4 (45.5; 70.0)	15.4 (11.7; 20.2)	ND

[a] Receptor binding is expressed as IC_{50}, the concentration (nM) of compound required for half-maximal inhibition of the binding of [^{125}I]BH CCK-8s to receptors in rat pancreatic tissue (CCK-A) or guinea pig cortical membranes (CCK-B). The results represent the geometric mean of between two and six separate experiments. Statistical limits are given in parentheses. [b] Enantiomeric excess (ee) was assessed via HPLC employing a Pirkle covalent L-leucine column (Regis Chemical Co.) and was in excess of 99.5%. [c] Equilibrium solubility, determined after stirring compound in buffered solution for >5 h. [d] Not determined. [e] ee = 98.4%.

In order to assess the functional activity of **5** and **7** *in vitro*, electrophysiological studies were carried out in rat brain slices.[22] Both **5** and **7** potently block the pentagastrin-induced single cell firing rate of rat ventromedial hypothalamic (VMH) neurons (**5**, K_b 0.6 ± 0.4 nM (n = 5); **7**, K_b 0.46 ± 0.1 nM (n = 6); **1**, K_b 41 nM (n > 5)). As excitatory effects in the VMH are mediated through CCK-B receptors,[23] these results indicate that both **5** and **7** are potent and selective CCK-B receptor antagonists.

The ubiquitous distribution of CCK-B receptors throughout the central nervous system (CNS) implies that a clinically efficacious CCK-B antagonist should have the ability to cross the blood–brain barrier. Therefore, estimations of the ability of **5** and **7** to penetrate into the CNS after systemic administration were carried out, and their *in vivo* potency was assessed using an *ex vivo* binding model in the mouse.[24] In this model both compounds dose-dependently inhibit *ex vivo* binding (**5**, ED_{50} = 5.6 mg/kg (iv); **7**, ED_{50} = 6.5 mg/kg (iv), 23 mg/kg (oral)). However, when compared with the results obtained for **1** (ED_{50} of 13 mg/kg (iv)), the extent of *ex vivo* binding of **5** or **7** is not commensurate with the comparative differences in *in vitro* affinity between **1** and **5** (60-fold) or **1** and **7** (32-fold). This suggests that the brain penetration of L-368,-935 (**5**) and L-369,466 (**7**) is substantially less than that observed for L-365,260 (**1**). As such, these analogs complement those benzodiazepines presented in the companion paper that contain cationic solubilizing elements and display physicochemical characteristics which may be more compatible with brain penetration *in vivo*.[25]

The compounds disclosed in this work provide another indication of the tractability of the benzodiazepine core structure as a base for designing nonpeptide ligands for peptide receptors. The two principal structures to emerge from this work, L-368,935 (**5**) and L-369,466 (**7**), are CCK-B antagonists, with no agonist activity, that meet many of the prerequisites of therapeutic agents. As CCK-4 interacts selectively with CCK-B receptors and is unlikely to cross the blood–brain barrier, the relatively poor brain penetrability displayed by **5** and **7** could be advantageous in elucidating certain effects attributed to CCK-B receptors that may be peripherally mediated. Indeed, evidence to the existence of CCK and gastrin in the vagus nerve has been presented.[26] More recently, CCK-B receptors have been detected and characterized in rat vagal afferents[27] and in the rabbit vagus nerve.[28] The oral bioavailability,[29] potency, selectivity, and water solubility of **5** and **7** should therefore prove invaluable in explicating the relationship between CCK-B receptors and CCK in its various guises.

Acknowledgment. We are indebted to J. Lin for his valuable drug metabolism studies and his insightful comments, to G. Showell, M. Chambers, J. Castro, S. Fletcher, and V. Matassa for helpful discussions, and to Ms. J. Kaysen for manuscript preparation.

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