Bioorganic & Medicinal Chemistry Letters, Vol.3, No.5, pp. 867-870, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

MULTIPURPOSE RECEPTOR LIGANDS: β-CARBOLINE CHOLECYSTOKININ ANTAGONISTS

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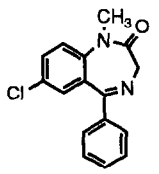
(*Received* 6 *July* 1992; *accepted* 4 *September* 1992)

Abstract: New amides and ureas of 3-amino-β-carboline are described which are selective ligands for CCK-A receptors. These compounds are developed by adaptation of benzodiazepine receptor-selective β-carboline-3-carboxylic esters. New CCK-A antagonists with 10^{-8}M receptor affinities are reported.

In earlier work[1-3], we described the adaptation of a single molecular structure to provide ligands selective for several different receptors. Specifically, we utilized the ring system represented by the selective, high-affinity benzodiazepine (BZD) receptor ligand, diazepam (**1**), a non-peptide, as the



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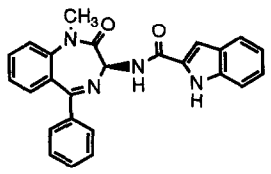

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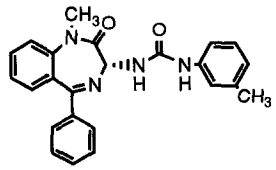

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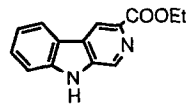

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base for development of potent and selective antagonists for the hormone, cholecystokinin (CCK), a peptide. These new CCK antagonists are exemplified by the CCK-A-selective amide, MK-329 (**2**)[1-3] and the CCK-B/gastrin-selective urea, L-365,260 (**3**)[4]. Two hypotheses underlay this effort: 1) peptides and non-peptides are fundamentally the same in their interactions with biological receptors: a suitable non-peptide should serve as an effective ligand for a peptide receptor; and 2) the concept of receptor specificity not withstanding, certain structures have the capacity to interact effectively with multiple receptors[3,5]. In the present work, we describe the adaptation of another non-peptide ligand selective for the benzodiazepine (BZD) receptor to obtain new ligands for the CCK-A peptide receptor. In this case, the 3-carboalkoxy-β-carbolines, (e.g., **4**), potent antagonists at benzodiazepine receptors, are adapted to provide selective antagonists for the CCK-A receptor.

The ß-carboline ring system occurs widely in nature, and compounds containing this nucleus exhibit numerous biological activities[6]. In 1980, it was reported that esters of ß-carboline-3-carboxylic acid (e.g., **4**) were high affinity ligands for brain benzodiazepine receptors[7]. ß-Carboline-3-

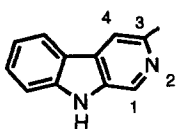
carboxylic acid ethyl ester (ß-CCE, **4**) was subsequently shown to antagonize the pharmacological actions of the anxiolytic benzodiazepines and to function as a potent anxiogenic in its own right[8]. The fact that the ß-carboline and benzodiazepine ring systems had in common the capacity to bind effectively to benzodiazepine receptors led us to consider whether this relationship extended to the CCK receptor as well. Since 3-amido and 3-ureido substitution had successfully changed a BZD receptor-selective benzodiazepine such as **1** into CCK-A- (**2**) and CCK-B/gastrin- (**3**) selective antagonists, the effect on CCK receptor affinity of similar 3-amido/ureido substitution of the β-carboline system was examined[9,10]. For these studies, 3-amino-ß-carboline was prepared according to published procedures[11]. From this precursor, a series of amides and ureas were prepared by conventional acylation techniques. The structures and CCK/gastrin receptor binding affinities of these compounds are summarized in Table 1.

As the data illustrate, the parent amines in each case, the β-carboline **5** and the benzodiazepines **6** and **7**, are ineffective as CCK receptor ligands. The 3-methyl ester in the β-carboline series (**8**), a compound with high affinity for benzodiazepine receptors[7], is similarly ineffective at CCK and gastrin receptors. As high affinity CCK-A receptor ligands, aroyl derivatives such as the p-chlorobenzoyl- (**11**) and 1-methyl-2-indolecarbonyl- (**13**) compounds had proven very effective in the benzodiazepine series[1-3]. In the β-carboline system, these substitutions are considerably less effective (**9**, **10**, **12**). Extension to a 3-aroylmethyl modification, such as phenylacetyl or 3-indolylmethylcarbonyl, on the other hand, is detrimental to CCK-A receptor affinity in the benzodiazepines (**16**)[1-3], but enhances affinity considerably in the β-carbolines (**14**, **15**). With further extension to the 3-phenylpropionyl amide (**17**), CCK-A receptor affinity in the β-carboline series recedes, however.

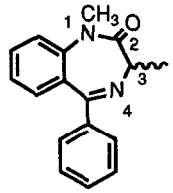
In the benzodiazepines, the urea modification adds CCK-B/gastrin receptor affinity, with R enantiomers in particular serving as highly effective CCK-B/gastrin ligands[4]. The corresponding S enantiomers are generally effective CCK-A receptor ligands, but retain considerable CCK-B/gastrin affinity as well[4]. The p-chlorophenylureas **19** and **20** illustrate these trends. In the β-carboline class, however, this same modification (**18**) virtually annihilates CCK-B/gastrin receptor affinity, but provides effective CCK-A receptor ligands. All these differences suggest that, in the binding of compounds of Table 1 to CCK receptors, the β-carboline ring is not a simple mimic for the benzodiazepine ring. How these two ring systems may interact with CCK receptors is suggested by the hybrid compounds, **21** and **22**. The structures and effective receptor binding capacities of these compounds suggest a model in which the benzodiazepine and β-carboline rings interact not with the same locus, but with adjacent loci within the CCK and gastrin receptor binding sites. The possibility that the β-carboline ring in compounds such as **21** and **22** may bind the portion of the receptor site occupied by the amido or ureido substituents in compounds such as **11**, **13**, **19**, and **20**, however is difficult to support given the divergent structure/activity profiles of the amides and ureas reviewed above.

Recently, two reports have appeared describing β-carboline-based compounds selective for CCK-B and gastrin receptors[14,15]. In these cases, amides of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid functionalized at N-1 with lipophilic aryl ureas or alkyl carbamates have been developed which show 10^{-7}-10^{-8}M affinity for CCK-B and gastrin receptors. These compounds as well

Table 1: Receptor Binding Affinities for 3-Substituted β-Carbolines (**A**) and Benzodiazepines (**B**)[a]



= **A**



= **B**

Compd	3-Substituent	A/B	3-Stereo (B)	IC_{50} (μM) CCK-A	CCK-B	Gastrin
5	NH_2	**A**	-	>100	>100	>100
6	NH_2	**B**	R	>100[b]	>100[b]	>100[b]
7	NH_2	**B**	S	100[b]	>100[b]	>100[b]
8	$COOCH_3$	**A**	-	>100[c]	>100	150
9	NHCO-phenyl	**A**	-	7.1	223	>100
10	NHCO-4-chlorophenyl	**A**	-	3.5	72	>100
11	NHCO-4-chlorophenyl	**B**	RS	0.0083[b]	40[b]	6.7[b]
12	NHCO-(1-methyl-2-indolyl)	**A**	-	0.71	>100	>100
13	NHCO-(1-methyl-2-indolyl)	**B**	RS	0.0014[b]	15[b]	2.0[b]
14	$NHCOCH_2$-phenyl	**A**	-	0.04	34	14
15	$NHCOCH_2$-3-indolyl	**A**	-	0.024	67	>100
16	$NHCOCH_2$-3-indolyl[e]	**B**	RS	1.0[b]	11[b]	32[b]
17	$NHCO(CH_2)_2$phenyl	**A**	-	0.33	68	35
18	NHCONH-4-chlorophenyl	**A**	-	0.085	>100	>100
19	NHCONH-4-chlorophenyl	**B**	R	1.1[d]	0.0055[d]	0.012[d]
20	NHCONH-4-chlorophenyl	**B**	S	0.026[d]	0.41[d]	0.54[d]
21	NHCONH—**B**	**A**	R	0.089[c]	0.27[c]	0.4
22	NHCONH—**B**	**A**	S	0.11[c]	5.9[c]	>10

[a] IC_{50} for half-maximal inhibition of binding of ^{125}I-CCK-33 to CCK receptors in rat pancreatic (CCK-A) or guinea-pig brain (CCK-B) tissues, or of ^{125}I-gastrin to guinea-pig gastric glands (Gastrin), determined as described in references 12 and 13. [b] Data from reference 3. [c] Reference 13. [d] Data from reference 4. [e] N-1 is unsubstituted in this compound.

reveal no close relationship between the β-carboline and benzodiazepine rings in ther modes of binding to CCK and gastrin receptors.

β-Carboline methyl ester (β-CCM, **8**) has been reported to antagonize various functions of CCK in mouse and guinea-pig (whole animal: ip dose levels 0.3-3 mg/kg; isolated tissue assays: concentrations 10^{-8}-10^{-4}M)[16]. The data in Table 1 suggest these effects are not mediated by direct action of β-CCM at CCK receptors: ester **8** shows very low affinity for the CCK-A or CCK-B receptor preparations used in this work (Table 1). Compounds such as **15** and **18** represent a novel class of effective CCK-A receptor ligands with 10^{-8} M CCK-A receptor affinities and high selectivies vs. CCK-B and gastrin receptors. In vivo, compound **15** is an orally effective antagonist of mouse gastric emptying[17] with an EC_{50} of < 4.0 mg.kg. The pharmacological advantages of these compounds over existing agents remain to be elucidated.

Acknowledgements. We are delighted to acknowledge the assistance of J. F. Kaysen in preparation of the manuscript. We are indebted as well to Drs. Paul S. Anderson, Ralph F. Hirschmann and Daniel F. Veber for their continuing support and encouragement throughout this work.

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13. For selected compounds, the published radioreceptor binding protocol for rat pancreas and guinea-pig cerebral cortex membranes was modified according to the following procedure: assays were conducted in a modified Hepes Krebs' buffer, pH 6.5, containing 20 nM Hepes, 1 mM EGTA, 5 mM $MgCl_2$ and 150 mM NaCl. The pancreas assay also contained 0.25 mg/ml bacitracin, 0.1 mg/ml soy bean trypsin inhibitor, and 2 mg/ml BSA. Tissue was resuspended at 1g (original wet weight) to 2000 ml (pancreas) and 120 ml (brain). Following a 90 min incubation, samples were filtered over GF/C filters and washed with ice cold 100 mM NaCl.
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