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BENZOLACTAMS AS NON-PEPTIDE CHOLECYSTOKININ RECEPTOR LIGANDS

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Abstract: A series of 1,3-substituted benzolactams are reported which are nonpeptidal receptor ligands of the peptide hormone cholecystokinin (CCK). These compounds are composites of potent, selective benzolactam CCK-A antagonists and unique structural elements which have been demonstrated to enhance the affinity of certain 1,4-benzodiazepine CCK-A antagonists for the CCK-B receptor.

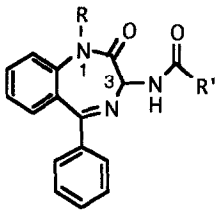
The gastrointestinal hormone cholecystokinin (CCK) is found in the gut and is also widely distributed in the central nervous system[1] where it may assume the function of a neurotransmitter.[2] Two principal receptor subtypes have been characterized for CCK and these are classified as CCK-A (alimentary) and CCK-B (brain).[3-5] A number of structurally distinct non-peptide ligands have now been uncovered which avidly bind to both CCK-A and CCK-B receptor subtypes with high selectivity.[6,7] These compounds have supported extensive investigations of the possible roles of CCK in behavior.[8-11]

Among the first and most versatile classes of CCK antagonists to be reported were the 1,4-benzodiazepines.[12] Apart from their high intrinsic affinity for the CCK-A receptor,[13] it was discovered that the 1,4-benzodiazepine selectivity for the CCK-B receptor could be modulated by chemical means.[14,15] We were intrigued by the prospect of applying similar design principles to close molecular analogues of 1,4-benzodiazepines, namely benzolactams, and to ascertain if by analogy with the 1,4-benzodiazepines, the CCK-A/CCK-B receptor binding affinities of selected benzolactams could likewise be reversed.

The compounds in this study were synthesized according to published procedures.[14,16] The methods employed for the determination of [^{125}I]CCK-8 binding to rat pancreas and guinea pig cortex and [^{125}I]gastrin binding to guinea pig gastric glands were also previously described.[13,17,18]

The starting point for this investigation was based on the observations that the 1,4-benzodiazepine **1** (MK-329) (Table 1) is a selective CCK-A antagonist and that it is more potent than its corresponding enantiomer. Additionally, substitution of the N-1 methyl group in **1** with an acetic acid ester and concomitant transformation of the 3-arylamide appendage to a 3-arylurea side chain gives compound **2** which, despite its racemic nature, is a selective CCK-B receptor ligand. Further refinement of potency and selectivity is possible by replacing the N-1 ester portion with various functional groups, optimally with the pyrrolidinyl and N-methylpiperidinyl amides, to afford analogues **3** and **4** respectively.

Table 1: 3-Substituted 1,4-Benzodiazepine Receptor Binding Affinities[a]

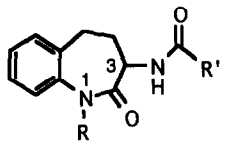


Compd	R	R'	3-Stereo	IC_{50} (nM) CCK-A	CCK-B	Gastrin
1	CH_3	2-indolyl	S	0.08	245	300
2	CH_2CO_2Et	4-chloroanilinyl	R S	370	1	3.3
3	CH_2CO-N⟨⟩	4-chloroanilinyl	R S	520	0.28	0.5
4	CH_2CO-N⟨⟩NCH_3	4-chloroanilinyl	R S	1200	8	2.6

[a] Procedures for receptor binding assay are contained in references 13 & 17.

Compound **5** (Table 2) is a construct derived from conformational and structural resemblances between the 1,4-benzodiazepine and benzolactam core ring systems.[15] In our attempt to alter the CCK receptor selectivity displayed by **5** we modified its N-1 and C-3 side chains approximately paralleling the changes required to convert the CCK-A antagonist **1** to the CCK-B receptor ligands **3** and **4**. Derivative **6**, the first analogue based on this approach, is the linchpin between the 1,4-benzodiazepine and benzolactam series for it demonstrated that replacement of the 3-arylamide linkage in **5** with a 3-arylurea linkage resulted in an enhancement of CCK-B receptor affinity at the expense of the CCK-A binding potency. This reversal in selectivity could be duplicated by replacing the 4-chlorophenyl ring in **6** with a 1-naphthyl unit (cf **7**). However, no further improvement in potency or selectivity was realized by transforming the N-1 side chain in **7** to give either carboxylic acid or amide side chains (cf. **8-10**). Replacement of the 4-chloroanilinyl unit in **6** with 3-methoxy- and 3-methylanilinyl groups gave **11** and **15**, respectively. The latter displayed only a modest increase in affinity for the CCK-B receptor relative to **6**. Unlike the example cited in Table 1, however, conversion of the N-1 ester side chain in **15** to yield N-1 tertiary amides **17** and **19** provided no benefit with regard to CCK-B receptor binding affinity and selectivity. One proposal previously put forth which may account for this result is that the 5-phenyl ring of the benzodiazepine core structure and the N-1 functional group of the benzolactams share some of the same space on the receptor.[16] The more polar functional groups (acids, amides) may consequently not be as readily accomodated by a site to which the lipophilic phenyl ring binds. An alternative view posits that the benzolactams are missing the key 5-phenyl ring of the 1,4-benzodiazepines as is evident by superposing the core structures (e.g. N-1 of compound **3** with N-1 of compound **17**, etc.). Whatever the explanation, clearly the analogy between 1,4-benzodiazepines and benzolactams does not extend to this structural change.

Table 2: Receptor Binding Affinities for 3-Substituted Benzolactams[a]



Compd	R	R'	3-Stereo	IC_{50} (μM) CCK-A	CCK-B	Gastrin
5	CH_2CO_2-t-Bu	2-naphthyl	R S	0.0075	>10	ND[b]
6	CH_2CO_2-t-Bu	4-chloroanilinyl	RS	0.35	0.18	0.12
7	CH_2CO_2-t-Bu	1-aminonaphthyl	R S	0.96	0.15	0.48
8	CH_2CO_2H	1-aminonaphthyl	R S	20	17	5.5
9	CH_2CO-N⟨⟩	1-aminonaphthyl	R S	1.7	0.65	0.46
10	CH_2CO-N⟨⟩NCH_3	1-aminonaphthyl	R S	32	13	5.4
11	CH_2CO_2-t-Bu	3-methoxyanilinyl	RS	0.062[c]	0.270[c]	0.026
12	CH_2CO_2H	3-methoxyanilinyl	RS	2.1	8.6	3.1
13	CH_2CO-N⟨⟩	3-methoxyanilinyl	S	0.335[c]	1.19[c]	ND
14	CH_2CO-N⟨⟩	3-methoxyanilinyl	R	0.028[c]	0.103[c]	ND
15	CH_2CO_2-t-Bu	3-methylanilinyl	RS	0.140[c]	0.110[c]	0.018
16	CH_2CO_2H	3-methylanilinyl	RS	0.94	2.2	2.5
17	CH_2CO-N⟨⟩	3-methylanilinyl	RS	0.130[c]	0.160[c]	0.037
18	CH_2CO-N⟨⟩	3-methylanilinyl	S	1.00[c]	0.878[c]	ND
19	CH_2CO-N⟨⟩	3-methylanilinyl	R	0.030[c]	0.109[c]	ND
20	CH_2CO-N⟨⟩NCH_3	3-methylanilinyl	RS	0.97	5	0.61

[a] Procedures for receptor binding assays are contained in references 13 & 17. [b] Not determined.
[c] Reference 18.

The stereochemistry at the 3-position of previously disclosed 1,4-benzodiazepine and benzolactam CCK antagonists plays a decided role in receptor binding potency and selectivity.[13,14,16] This phenomenon does not translate to the benzolactam ureas which were examined in this study. Little difference in binding affinity and selectivity was observed among the R and S enantiomers **13**, **14**, **18**, and **19**. Indeed, of the four, all but compound **18** displayed a slight preference for the CCK-A receptor. We note also that the compounds in this study do not significantly discriminate between CCK-B and gastrin receptors. This result is consistent with the similar ligand requirements between the two receptor types further supporting the notion that these receptors may in fact be identical.[19,20]

In summary, we have shown that when a prototypical benzolactam CCK-A receptor ligand is structurally modified to more closely conform with CCK-B 1,4-benzodiazepine receptor ligands the selectivity profiles of the resulting analogues are significantly altered. While an optimal 3-dimensional substitution pattern, yielding CCK-B selective agents per se, has not yet been uncovered for these benzolactam analogues,[21] our preliminary results indicate that the benzolactam core structure should justifiably be considered as a candidate template in the general design of nonpeptide receptor ligands.

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