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AMINO ACID-DERIVED PIPERIDIDES AS NOVEL CCK_B LIGANDS WITH ANXIOLYTIC-LIKE PROPERTIES

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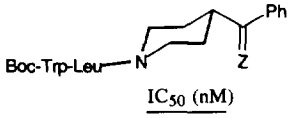
ABSTRACT: The development of a novel series of carbamoylamino acid benzoylpiperidides as CCK_B ligands is described. Selected members of the series antagonized CCK_8-induced calcium mobilization and showed efficacy in the mouse elevated-plus maze, a measure of potential anxiolytic activity.

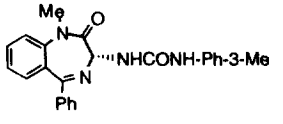
Receptors for the peptide hormone cholecystokinin (CCK) are currently classified into two major subtypes, namely CCK_A, located predominantly in the gut but also in discrete regions of the CNS, and CCK_B, which have widespread distribution in the CNS and which are identical to stomach gastrin receptors. Previous reports from these laboratories have described the identification of selective peptide agonist ligands for both subtypes of CCK receptors and also of non-peptide antagonists for CCK_A receptors.[2-4] CCK_B/gastrin receptor antagonists have recently emerged as agents with potential for the treatment of a number of conditions, including anxiety disorders and morphine tolerance.[5-7] The results of studies in this laboratory directed toward identification of non-peptide CCK_B antagonists with anxiolytic-like properties are reported in this Letter.

The initial lead compounds in this study were identified based on observation of the binding properties of the two dipeptide-4-substituted-piperidides **1** and **2**, which had been prepared during structure-activity explorations of CCK peptide analogues. The considerable enhancement in the affinity for the CCK_B receptor of the benzoylpiperidide **1** compared to the benzylpiperidide **2** suggested that the former might be advantageously elaborated to more selective CCK-B ligands. Initial approaches toward identification of improved ligands



IC_{50} (nM)

Cmpd	Z	CCK-B	CCK-A
1	O	912 ± 35	640 ± 120
2	H_2	>10000	200 ± 42



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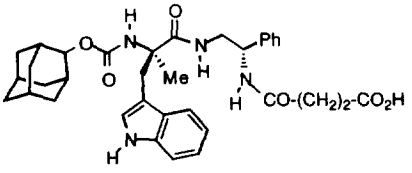

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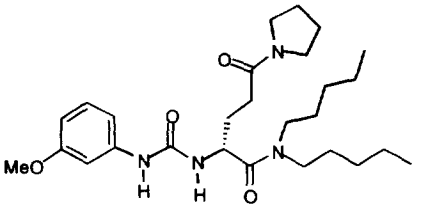

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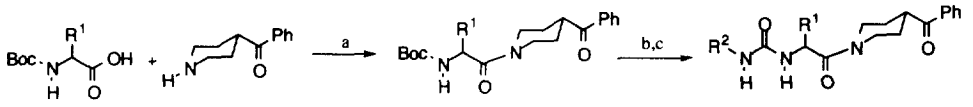
included size-reduction strategies and incorporation of moieties present in other known series of CCK-B antagonists, including L-365,260 (**3**),[8,9] CI-988 (**4**),[10] and a lorglumide[11]-derived series (e.g. **5**) reported by the Merck group.[12] Compounds were prepared using straightforward variants of the chemistry outlined in Scheme I.

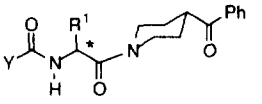
Scheme I



Reagents: a. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride, N-methylmorpholine, CH_2Cl_2. b. CF_3COOH. c. R^2NCO

The results in Table 1 indicate that replacement of the Boc-Trp- segment with m-toluylaminocarbonyl and changing the stereochemistry of the central residue to the *R*-configuration afford compound **9** with slightly improved affinity for CCK-B receptors, albeit with little selectivity between receptor subtypes. However, variation of the side chain to that derived from D-Glu(pyrrolidide) resulted in compound **10**, which exhibited substantial enhancement in CCK-B affinity and 36-fold selectivity for the CCK-B receptor; thus, it became apparent that the new series could be conceptually related to compound **5** (however, see below). In contrast, compound **11**, which incorporates the 2-Adoc and the α-Me-Trp moieties in common with compound **4**, showed affinity for both receptor subtypes only in the mid-micromolar range.

Table 1. Binding affinities of acylaminoacyl-4-benzoylpiperidides.



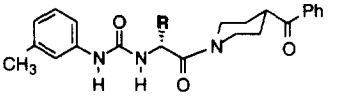
Compd[b]	Y	R^1	*Stereo-chemistry	IC_{50} (nM)[a] CCK-B	CCK-A
6	Boc-	isobutyl	S	>10,000	>2100
7	Boc-	-CH_2-3-indolyl	S	>10,000	4500 ± 230
8	3-Me-Ph-NH-	isobutyl	S	>10,000	5200 ± 2400
9	3-Me-Ph-NH-	isobutyl	R	5700 ± 2400	2000 ± 470
10	3-Me-Ph-NH-	-CH_2CH_2CO-N⟨ ⟩	R	53 ± 8	1900 ± 250
11	2-Adamantyl-O-	-CH_2-3-Indolyl; α-Me[c]	RS	3400 ± 480	1700 ± 370

a. CCK_B and CCK_A receptor binding assays were conducted using guinea pig cortical and pancreatic tissues, respectively, with ^{125}I-Bolton-Hunter-CCK-8 as the radioligand, as described in ref. 13. Binding data represent the mean of at least 3 determinations b. All new compounds exhibited NMR, MS and combustion analysis data consistent with the assigned structures. c. The central amino acid residue is α-Me-DL-Trp.

The results of further modifications of the central amino acid side-chain are shown in Table 2. These modifications led to decreased affinity and selectivity for the CCK_B receptor relative to **10**. The D-glutamate-derived benzyl ester **14** was more active than the corresponding free acid **13**; interestingly, this order of activity is reversed from that observed by Freidinger for compounds in the dipentylamide series.[12] Replacement of the

β-methylene of **10** with oxygen resulted in carbamate **24**, with 100 nM affinity and 14-fold selectivity for the CCK_B receptor. Carbamate analogs derived from alternative amines (**25-27**) were either less effective as CCK_B ligands or had reduced selectivity.

Table 2. Variation of the side chain in *m*-toluylaminocarbonyl-(R)-aminoacyl-4-benzoylpiperidides.



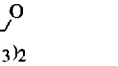
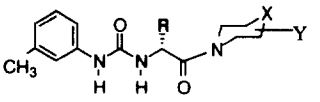
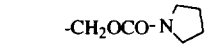
Compd	R	IC_{50} (nM) CCK_B	CCK_A
12	$-CH_2CH_2CH_2CH_3$	4800 ± 120	2500 ± 75
13	$-CH_2CH_2CO_2H$	2000 ± 380	1800 ± 110
14	$-CH_2CH_2CO_2CH_2Ph$	900 ± 220	900 ± 130
15	$-(CH_2)_2$-Ph	3900 ± 1300	2200 ± 500
16	$-CH_2$-3-indolyl	> 6000	1400 ± 280
17	$-(CH_2)_3$-Ph	1100 ± 180	1800 ± 450
18	$-CH_2CO_2CH_2Ph$	410 ± 100	1300 ± 160
19	$-CH_2OCH_2Ph$	300 ± 56	2400 ± 160
20	$-CH(R\text{-}CH_3)OCH_2Ph$	440 ± 63	1300 ± 260
21	$-CH_2SCH_2Ph$	390 ± 100	1300 ± 30
22	$-CH_2S(R,S\text{-}O)CH_2Ph$	170 ± 54	800 ± 280
23	$-CH_2S(O_2)CH_2Ph$	810 ± 210	1100 ± 260
24		100 ± 33	1400 ± 290
25		180 ± 30	1500 ± 230
26	$-CH_2OCO\text{-}N(CH_3)_2$	1500 ± 260	1300 ± 370
27	$-CH_2OCO\text{-}NHPh$	120 ± 18	290 ± 87

Table 3 shows structure-activity studies carried out in the region of the benzoylpiperidine moiety primarily in series based on D-glutamate benzyl ester **18** and D-serine benzyl ether **19**. Piperazine derivatives **28** and **29** were less active at the CCK B subtype than the corresponding benzoylpiperidide **18**, whereas anilide **31** possessed ca. 2-fold higher affinity for the CCK_B receptor than the corresponding benzoylpiperidide **19**. The substantially reduced activity and selectivity of 4-benzylpiperidide **32** relative to **19** reinforces the initial suggestion of an important role for the ketone oxygen in contributing to the affinity and CCK-B selectivity of the benzoyl piperidides. Combination of the anilido function with the D-serine-derived carbamate moiety of compound **24** afforded analog **36** with 34 nM affinity and 7-fold selectivity for CCK_B receptors.

Table 3. Binding affinities of m-toluylaminocarbonyl-(R)-aminoacyl amides.



Compd	R	X	Y	IC_{50} (nM) CCK_B	CCK_A
28	$-CH_2CH_2CO_2CH_2Ph$	N	4-CO-Ph	>10,000	1500 ± 380
29	"	N	4-CO-NHPh	2000 ± 410	690 ± 79
30	"	CH	4-NHCO-Ph	>10,000	970 ± 270
31	$-CH_2OCH_2Ph$	CH	4-CONH-Ph	160 ± 40	500 ± 130
32	"	CH	4-CH_2-Ph	> 5900	3400 ± 1700
33	"	CH	4-CO-(p-F-Ph)	230 ± 92	420 ± 200
34	"	CH_2	3-CONH-Ph	>10,000	2200 ± 1300
35	"	CH	4-CO-Me	> 6900	1300 ± 440
36		CH	4-CONH-Ph	34 ± 11	250 ± 10

Compounds **10, 24**, and **36** were further evaluated for their ability to block CCK_8-induced calcium mobilization in NCI-H345 cells[13] and to elicit an anxiolytic-like effect in a mouse elevated plus maze paradigm[14] following i.p. injection. PD-135138,[15] a close analogue of CI-988 (1(S)-endobornyl in place of 2-adamantyl, FW 814 g/mol), also was tested in these assays for comparison. In the calcium assay, IC_{50} values for **10**, **24**, and **36** were 251 ± 34 nM, 220 nM, and 115 nM, respectively, compared to a value of 2 nM for PD-135138. This difference in *in vitro* potency did not translate to the *in vivo* anxiolytic assay, however (Fig. 1). The compounds were administered over a range of 0.001-1.0 mg/kg in log unit intervals, and an increase in the time spent by the animals in the open arms was used as an index of anxiolytic activity. Whereas no significant activity was observed for compound **10** (data not shown), significant activity was observed for compounds **24** and PD-135138 at an intermediate dose level and for compound **36** at the lowest dose tested. On a molar basis, the activity for **36** was found at a ca. 16-fold lower dose than for PD-135138.

In summary, modifications of amino acid-derived CCK antagonists to incorporate benzoylpiperidide or anilidopiperidide moieties at the carboxy terminus and an O-carbamoyl-D-serine-derived side chain has resulted in novel derivatives, some of which (e.g. compounds **24** and **36**) show midnanomolar affinity and 7- to 14-fold selectivity for guinea pig cortical CCK_B receptors. These compounds were shown to antagonize calcium mobilization in a human carcinoma cell line and to exhibit potent anxiolytic-like activity in mice.[16]

Acknowledgement: We thank Dr. John Hughes of Parke-Davis Research for PD-135138.

Fig. 1. Anxiolytic-like effects of test compounds in the elevated-plus maze during a 5 minute test. Mice were injected i.p. 30 min. before the test.

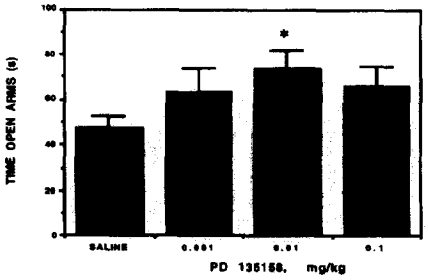


Fig. 1a: Anxiolytic-like effect of PD 135138. Data represent the mean ± SEM of 8 mice. * $p < 0.05$ against the control group.

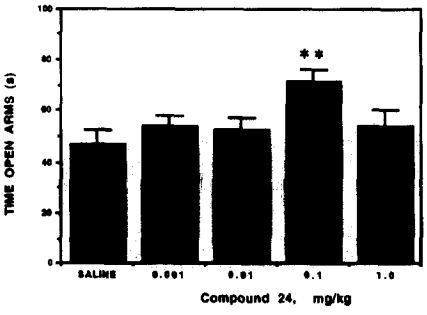


Fig. 1b: Anxiolytic-like effect of compound **24** . Data represent the mean ± SEM of 16 mice. ** $p < 0.01$ against the control group.

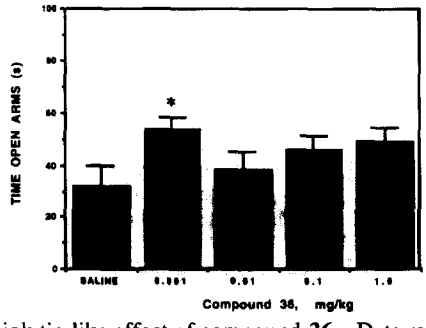


Fig. 1c: Anxiolytic-like effect of compound **36** . Data represent the mean ± SEM of 8 mice. ** $p < 0.05$ against the control group.

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