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NOVEL CHOLECYSTOKININ RECEPTOR LIGANDS: OXOPIPERAZINES DERIVED FROM BOC-CCK-4

Andrzej R Batt, David A Kendrick, Elizabeth Mathews, David P Rooker, Hamish Ryder*, Graeme Semple and Michael Szelke

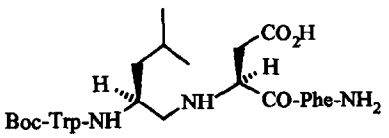
Ferring Research Institute, Southampton University Research Centre, Chilworth, Southampton SO1 7NP, U.K.

Abstract: Replacement of the C-terminal dipeptide amide of Boc-CCK-4 (1) with oxopiperazines yields a series of weak CCK-receptor ligands. Further modifications result in more potent and receptor subtype selective compounds.

The rational design of novel ligands for the cholecystokinin (CCK) and gastrin receptors, based upon the C-terminal tetrapeptide fragment common to the two hormones, has received substantial attention in recent years. Boc-CCK-4 (1) has high (nM) affinity for CCK-B/gastrin receptors but weaker (μM) affinity for CCK-A receptors.[1] Simple derivatives of this potent gastrin/CCK-B agonist, such as the phenethylamide 2[2] and the aminomethylene pseudopeptide 3[3], are moderately active gastrin antagonists. Replacement of the methionine residue of Boc-CCK-4 with lysine derivatives has, on the other hand, resulted in a series of potent and selective CCK-A agonists exemplified by A-71623 (4).[1, 4, 5] Several studies have indicated that gastrin and CCK peptides adopt a turn conformation at the C-terminus,[6-8] which brings the aromatic side chains of tryptophan and phenylalanine into close proximity. The best evidence that the bioactive conformation is non-extended comes from the work of Horwell et al.,[9] who have shown that modest CCK-B receptor binding affinity was retained by the dipeptide Boc-Trp-Phe-NH_2. This lead compound has subsequently been developed to yield the potent CCK-B receptor antagonist CI-988 (5).[10, 11]

Boc-Trp-Met-Asp-Phe-NH_2 **1**

Boc-Trp-Leu-Asp-NH-$(CH_2)_2$Ph **2**



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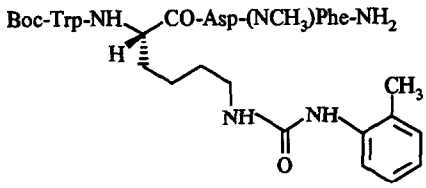

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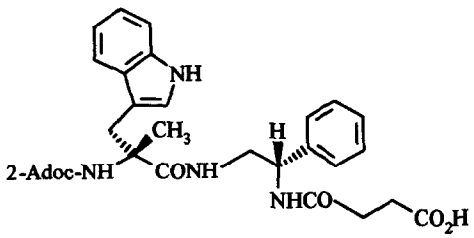

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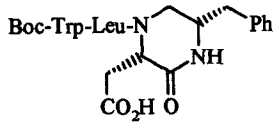

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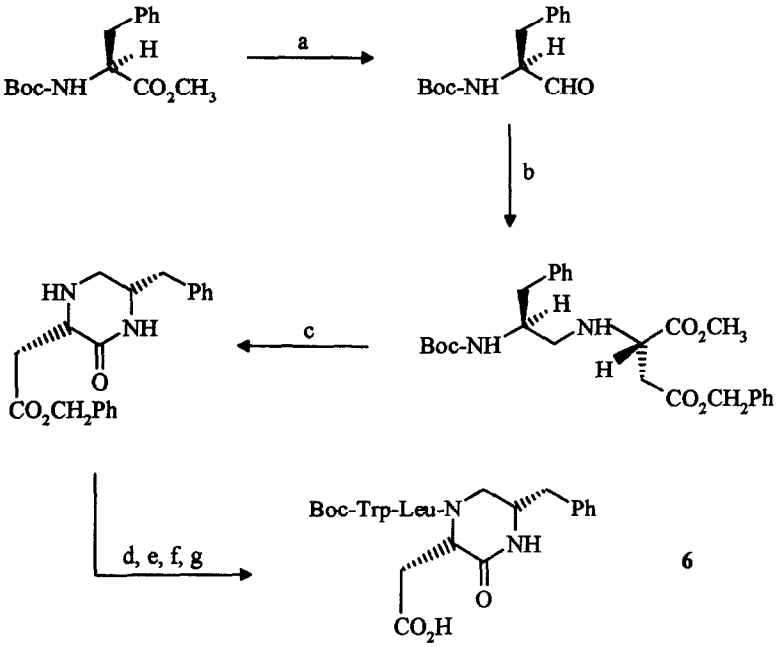
As part of our programme aimed at the development of non-peptidic gastrin and CCK antagonists we have introduced a series of conformational restrictions to the peptide backbone of Boc-CCK-4 analogues, with the expectation that one or more of these constrained compounds might mimic the bioactive conformation. It was found that compound **6**, obtained by replacement of the C-terminal dipeptide with an oxopiperazine moiety,[12] retained weak affinity for the CCK-B receptor and had rather stronger affinity for the CCK-A receptor. Herein we describe the manipulation of this lead to yield compounds of varying selectivity and potency for CCK-A and CCK-B receptors. The synthesis of compounds of this type was straightforward and utilised standard procedures. The synthesis of **6** (Scheme 1) is a typical example.

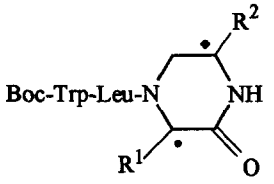
Scheme 1



Reagents: (a) DIBAL, $PhCH_3$, 80% (b) $NaCNBH_3$, MeOH, AcOH, H-Asp(OBzl)OMe, 77%
(c) 4M HCl, Dioxan then base work-up, 90% (d) BOP-Cl, Boc-Leu-OH, 65%
(e) 4M HCl, Dioxan, 100% (f) Boc-Trp-OH, HOBt, WSCI, 82% (g) H_2, Pd/C, 90%.

A brief investigation of structure-activity relationships at the C-terminal oxopiperazine moiety of **6** revealed compounds with improved potency for both CCK-A and CCK-B receptors (Table I). Stereochemical inversion of the carboxymethyl side-chain of **6** yields an 8-fold increase in CCK-B receptor affinity (compound **7**), whereas the removal of this side-chain (**12**) results in a compound with improved CCK-A binding affinity. Inversion of the remaining side-chain gave **13**, the most potent CCK-A ligand in this series and one which displays excellent selectivity for the CCK-A over the CCK-B receptor.

Table I: Effect on CCK Receptor Binding Affinities of Substitution and Stereochemistry of Oxopiperazine

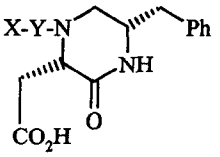


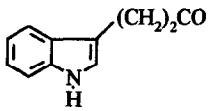
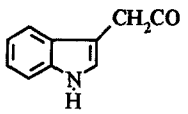
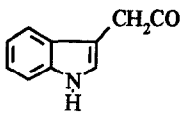
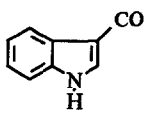
Compound	R^1	R^2	stereochemistry •	stereochemistry ◆	IC_{50} (μM)[a] CCK-A		IC_{50} (μM)[a] CCK-B		A/B ratio
6	CH_2CO_2H	CH_2Ph	S	S	0.84	(0.824 - 0.853)	61	(49.6 - 72.4)	0.014
7	CH_2CO_2H	CH_2Ph	R	S	2.8	(2.65 - 3.03)	7.4	(6.56 - 8.39)	0.38
8	CH_2CO_2H	CH_2Ph	S	R	3.6	(3.51 - 3.61)	17	(13.2 - 21.5)	0.21
9	CH_2CO_2H	CH_2Ph	R	R	6.3	(5.36 - 7.22)	70	(53.4 - 86.9)	0.09
10	CH_2CO_2H	Ph	S	S	9.4	(9.02 - 9.86)	9.4	(7.51 - 11.3)	1.0
11	CH_2CO_2H	$(CH_2)_2Ph$	S	S	0.63	(0.425 - 0.833)	8.2	(7.35 - 9.10)	0.077
12	H	CH_2Ph	-	S	0.27	(0.262 - 0.269)	37	(31.7 - 42.5)	0.0073
13	H	CH_2Ph	-	R	0.046	(0.0445 - 0.0480)	39	(36.2 - 42.7)	0.0012

Note a: IC_{50} represents the concentration producing half-maximal inhibition of specific binding of [^{125}I] CCK-8 to CCK receptors on mouse pancreatic membranes (CCK-A) and mouse forebrain membranes (CCK-B). Figures given are the mean and range of two determinations. Binding assays were carried out according to published methods.[13]

Modification of the N-terminal dipeptide portion of **6** yields compounds with improved potency and selectivity for the CCK-B receptor (Table II). In particular, removing the steric bulk of the N-terminal blocking group (**14**, **15**) reverses receptor selectivity. Optimisation of the length of the indole linker and replacement of the leucine residue with the aminohexanoic acid residue yields compound **17** which has micromolar affinity and displays moderate selectivity for the CCK-B receptor. The receptor binding affinities of the literature[2] antagonist **2** are included for comparison.

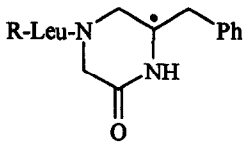
Table II: Effect on CCK Receptor Binding Affinities of N-terminal Dipeptide Modification



Compound	X	Y	IC_{50} (μM)[a] CCK-A	CCK-B	A/B ratio
6	Boc-Trp	Leu	0.84 (0.824 - 0.853)	61 (49.6 - 72.4)	0.014
14	H-Trp	Leu	51 (51.1 - 51.7)	15 (7.05 - 22.9)	3.4
15		Leu	56 (53.4 - 58.9)	8.9 (7.96 - 9.85)	6.3
16		Leu	13 (12.9 - 13.9)	2.5 (1.79 - 3.27)	5.2
17		Aha	7.2 (6.76 - 7.69)	1.2 (1.19 - 1.28)	6.0
18		Aha	1.4 (1.17 - 1.64)	>100	-
2			3.5	0.35	10

Variation of the N-terminus of the monosubstituted series (Table III) reveals that, in contrast to CCK-B binding, CCK-A receptor affinity increases with the size of the N-terminal blocking group. Thus benzyloxycarbonyl (**20**) is more potent than *tert*-butyloxycarbonyl (**12**), which is in turn more potent than ethoxycarbonyl (**21**). Interestingly, replacement of the tryptophan residue of **12** with phenylglycine (**23**) reverses receptor selectivity. Combination of the benzyloxycarbonyl with the R-oxopiperazine yields the most potent and selective CCK-A ligand in this series (**24**).

Table III: Modification of the N-terminus of Monosubstituted Oxopiperazines



Compound	R	Stereochemistry at •	IC_{50} (μM)[a] CCK-A	CCK-B	A/B ratio
12	Boc-Trp	S	0.27 (0.262 - 0.269)	37 (31.7 - 42.5)	0.0073
19	Boc-DTrp	S	5.5 (5.35 - 5.56)	>100	-
20	$PhCH_2OCO$-Trp	S	0.064 (0.0594 - 0.0687)	18 (16.8 - 18.8)	0.0036
21	CH_3CH_2OCO-Trp	S	0.43 (0.415 - 0.450)	38 (20.2 - 56.0)	0.011
22	PhCH=CHCO-Trp	S	0.52 (0.443 - 0.597)	>100	-
23	Boc-Phg	S	24 (22.7 - 25.3)	10 (9.37 - 10.6)	2.4
13	Boc-Trp	R	0.046 (0.0445 - 0.0480)	39 (36.2 - 42.7)	0.0012
24	$PhCH_2OCO$-Trp	R	0.014 (0.0131 - 0.0146)	15 (14.9 - 15.1)	0.0009

In summary, we have shown that rational manipulation of Boc-CCK-4 can lead to both CCK-A and CCK-B selective ligands with reduced peptide character.

The more potent and selective of these ligands are currently being utilised as the leads for the development of highly potent non-peptidic CCK-A and CCK-B receptor antagonists. This work will be reported in due course.

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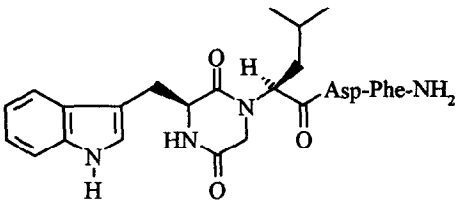

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