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IDENTIFICATION AND BIOLOGICAL ACTIVITY OF NOVEL PEPTIDOMIMETIC GASTRIN/CCK-B RECEPTOR AGONISTS

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Abstract: The design, synthesis and biological activity of two novel series of compounds derived from the basic Boc-CCK-4 structure which provide potent ligands for the gastrin/CCK-B receptor is outlined. Within these series, new pseudopeptide compounds were discovered which unexpectedly were functional agonists *in vivo*, as shown by their ability to stimulate basal gastric acid secretion in rats, an effect which was blocked by the potent gastrin/CCK-B receptor antagonist YM022.

Cholecystokinin (CCK) and gastrin are peptide hormones which share a common tetrapeptide C-terminus and exist as families of isopeptides characterised by different N-terminal truncation and tyrosine sulphation.[1] Three receptors for these peptides, called CCK-A, CCK-B and gastrin, were originally defined on the basis of their localisation and the binding affinities of the various CCK isoforms. In the gut the major subtype is CCK-A, which tightly binds sulfated CCK-8 (CCK-8S) but has a weaker interaction with CCK-4. The CCK-B receptor is predominant in the CNS, although CCK-A receptors are found in some areas of the brain. CCK-B receptors are characterised by their ability to strongly bind CCK-4 and their weaker interaction with CCK-8S. Following the cloning of all three receptors, the brain CCK-B receptor was shown to be identical to the gastrin receptor found in the gastric mucosa.[2] More recently there has been convincing evidence for a second gastrin receptor, which can bind the glycine-extended form of gastrin-17.[3]

Figure 1: CCK-4 Based Gastrin/CCK-B receptor agonists

Boc-Trp-Met-Asp-Phe-NH_2

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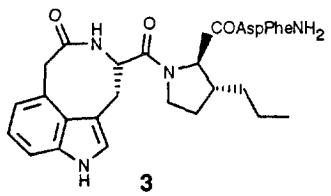

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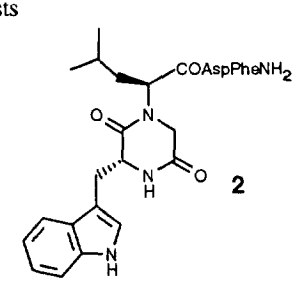

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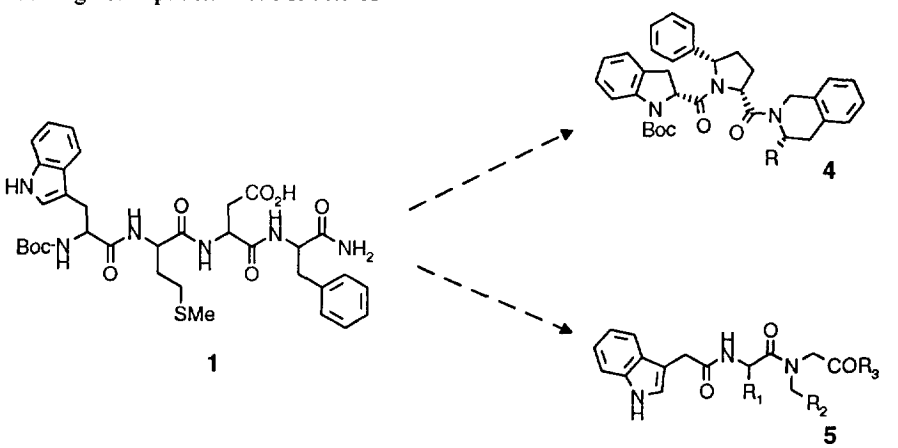
Replacement of the methionine residue of Boc-CCK-4 (**1,** Figure 1) with urea derivatives of lysine produces a series of compounds which are potent agonists of the CCK-A receptor [4] and cause a reduction in food intake in rats after central or peripheral administration. [5]

The most widely used agonists of the gastrin/CCK-B receptor, such as BC-197, BC-264 and SNF-8702 are stabilised derivatives of CCK-8S, [6,7] but smaller molecule agonists, again based on Boc-CCK-4, are also known. An N-terminal cyclisation to give the diketopiperazine analogue **2** (Figure 1), provides a compound with an IC_{50} of 37.7nM for the gastrin/CCK-B receptor, which has been shown to retain full agonist activity in stimulating calcium mobilisation in small cell lung cancer cells. [8] An alternative N-terminal cyclisation and incorporation of cis-3-n-propylproline in place of methionine provides A-63387 (**3,** Figure 1), which has an IC_{50} of 0.7nM for binding to the gastrin/CCK-B receptor, and again is a potent stimulator of calcium mobilisation. [9]

Most simple manipulations of Boc-CCK-4 or the closely related agonist BocTrpLeuPheAsp, such as truncation to the phenethylamide [10] or the incorporation of reduced isosteres [11] provide gastrin/CCK-B antagonists. We have previously shown that simple cyclisations of tetrapeptides related to CCK-4 to give a series of dipeptidyl oxopiperazines, provided modestly active receptor ligands with this expected functional activity. [12] We herein report however, on the synthesis and biological activity of two novel series of compounds derived from the basic CCK-4 structure which provide potent ligands for the gastrin/CCK-B receptor, and the discovery within these series of new pseudopeptide compounds which are agonists of that receptor *in vivo*.

We have obtained a number of new series of compounds by replacing the Boc-Trp-Met- portion of the parent CCK-4 structure with non-proteinogenic amino acid dipeptides and their mimetics. In one series we employed Boc-(2R)-carboxyindoline-(5R)-phenyl-D-proline for this purpose, and used tetrahydroisoquinoline derivatives to replace the C-terminal dipeptide portion to give **4** (Figure 2). A short series of C-terminal derivatives of this type were synthesised by standard peptide couplings of (5R)-phenyl-D-proline, prepared by a modification of the method of Ackermann *et al.*,[13] and resolved Boc-(2R)-carboxyindoline,[14] to the requisite C-terminal groups which were commercially available. The binding data for this series is shown in Table 1.

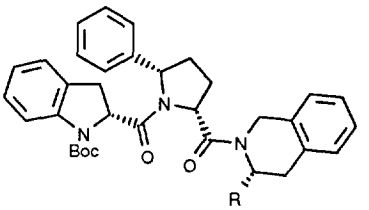
Figure 2: Design of Peptidomimetic structures



The carboxymethyltetrahydroisoquinoline compound **4a** showed good affinity for the gastrin/CCK-B receptor, and this activity was retained after shortening the acid side chain (**4b**). Conversion to the primary amide (**4c**) or removal of the side chain (**4d**) led to a decrease in potency. Affinity was significantly

increased by appending amino acid residues from the C-terminus of compound **4b**, with the addition of a proline residue providing a ligand with sub-nanomolar affinity for the gastrin/CCK-B receptor (**4f**) which maintained excellent selectivity for this receptor over CCK-A.

Table 1: Receptor binding data for the tetrahydroisoquinoline series



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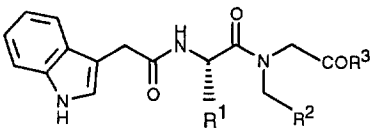
Compound	R	IC_{50} CCK-B (nM)[a]	IC_{50} CCK-A (nM)[b]
4a	CH_2CO_2H	35 (30-41)	>10,000
4b	CO_2H	39 (36-41)	>10,000
4c	$CONH_2$	170 (148-199)	>10,000
4d	H	52 (45-60)	>10,000
4e	COGlyOH	27 (15-48)	>10,000
4f	COProOH	0.25 (0.19-0.34)	>10,000
4g	CO-D-ProOH	36 (28.36-45.71)	>10,000

a) IC_{50} value for displacement of [^{125}I]-CCK-8 from Gastrin/CCK-B receptors from rat brain
b) IC_{50} value for displacement of [^{3}H]-L-364,718 from CCK-A receptors from rat pancreas. See Ref. 15 for full experimental details.

In a second series made up of branched amide derivatives, good affinity for the gastrin/CCK-B receptor was maintained by replacing the Boc-Trp-Met- portion of CCK-4 with the 3-indoleacetic acid amide of L-norleucine. In this case the C-terminal dipeptide portion was replaced by a non-peptidic secondary amine mimic (**5**, Figure 2). These compounds were again synthesised using standard peptide coupling procedures. The C-terminal portion was prepared by alkylation of the requisite amine with a bromoacetate ester. As in the tetrahydroisoquinoline series, potency in this branched series could be increased by adding an amino acid residue to the C-terminal position, but in this case a ten-fold advantage was obtained by the addition of the unnatural D-proline residue (**5c**, Table 2). Activity was further improved by reduction of the aromatic ring of the branched amide portion, which could be readily achieved by catalytic hydrogenation of the secondary amine intermediate, to provide the cyclohexyl analogue **5e**. Switching the central amino acid residue to L-phenylalanine (**5h**) provided the most potent gastrin/CCK-B ligand in this series.

Several of the compounds were examined for their ability to inhibit pentagastrin induced gastric acid secretion in rats. Compound **4a** showed the strongest antagonist effect in this model, inhibiting acid secretion by 60% at a dose of 3μmol/kg i.v. In contrast, compounds **4f** and **5h** both showed a dose dependent potentiation of acid secretion giving the appearance that these compounds were agonists of the gastrin/CCK-B receptor *in vivo*.

Table 2: Receptor Binding Data for the branched amide series



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No.	R^1	R^2	R^3	IC_{50} CCK-B (nM)[a]	IC_{50} CCK-A (nM)[b]
5a	nBu		OH	165 (95-250)	n.d.
5b	nBu		OH	370 (240-567)	>10,000
5c	nBu		D-ProOH	34 (29-40)	n.d.
5d	nBu		ProOH	300 (190-495)	n.d.
5e	nBu		D-ProOH	2.3 (2.2-2.4)	>10,000
5f	nBu			146 (137-156)	>10,000
5g	nBu		D-ProOH	30.2 (27.85-32.75)	>10,000
5h	CH_2Ph		D-ProOH	0.94 (0.60-1.45)	>10,000
5i	$(CH_2)_2Ph$		D-ProOH	17 (15-20)	>10,000

n.d.=not determined

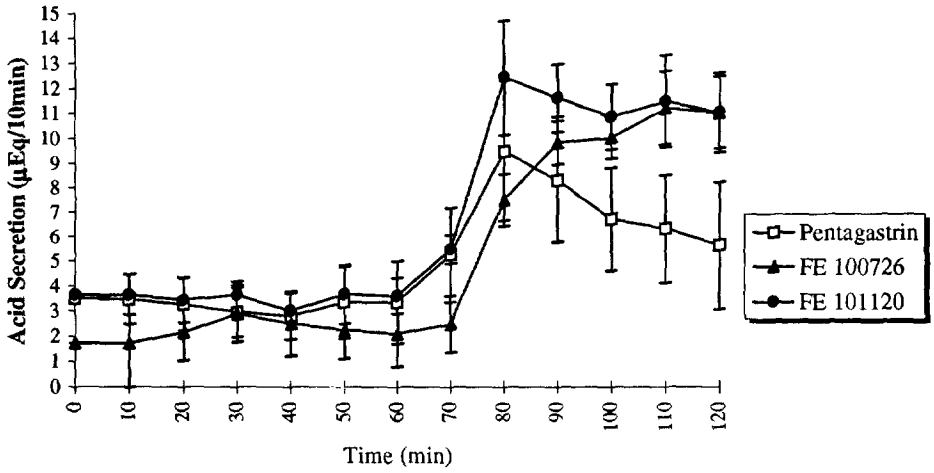
a) IC_{50} value for displacement of [^{125}I]-CCK-8 from Gastrin/CCK-B receptors from rat brain

b) IC_{50} value for displacement of [^{3}H]-L-364,718 from CCK-A receptors from rat pancreas. See Ref. 15 for full experimental details.

To further evaluate this activity we examined the effect of **4f** (FE 100726) and **5h** (FE 101120) on basal gastric acid secretion in rats (Figure 3). Both compounds induced secretion in this model at a dose of 3μmol/kg i.v, giving similar levels of gastric acid production to those observed after an i.v. dose of pentagastrin of 10nmol/kg. In addition it appears on preliminary inspection that the acid secretory effect may be longer lasting with these pseudopeptide agonists than with pentagastrin itself.

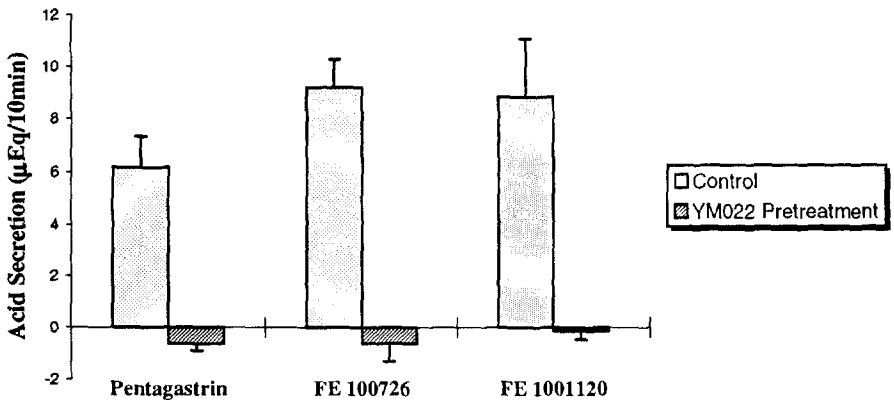
To examine whether these compounds were exerting their effect through interaction with the gastrin/CCK-B receptor, we examined their *in vivo* properties in the presence of the potent benzodiazepine gastrin/CCK-B antagonist YM022.[15] The induction of acid secretion by either pentagastrin, FE 100726 or FE 101120 was completely inhibited by pretreatment with an i.v. dose of 0.3μmol/kg of YM022 (Figure 4).

Figure 3: Effect of i.v. administration of FE 100726 (**4f**, 3 μmol/kg), FE 101120 (**5h**, 3 μmol/kg) and Pentagastrin (10 nmol/kg) at t=60 min, on basal acid secretion (in μEq/10min) in anesthetized rats.



We find it remarkable that two such structurally diverse compounds should both show a potent agonist effect in stimulating gastric acid secretion *in vivo*. The fact that the two compounds are also several steps removed from the natural agonist on which the two series described above were originally based, and that those series contain closely related compounds which are potent receptor antagonists, makes the discovery of their functional properties all the more unexpected and merits further investigation.

Figure 4: Effect of YM022 (0.3μmol/kg i.v.) on gastric acid secretion (μEq/10min) in rats induced by Pentagastrin (10nmol/kg i.v.), FE 100726 (**4f**, 3μmol/kg i.v.) and FE 101120 (**5h**, 3μmol/kg i.v.).



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