CCK_B/gastrin receptor antagonists as potential drugs for peptic ulcer therapy

Francesco Makovec and Massimo D'Amato

Cholecystokinin (CCK) and gastrin are gastrointestinal polypeptide hormones that exert several regulatory functions on motility and secretion in the gastrointestinal tract. Their biological functions are mediated by different receptors located on the target tissues. Several selective and potent nonpeptide CCK_B/gastrin receptor antagonists have been synthesized and are currently being studied. This review describes the development of different classes of these antagonists, and looks at future potential therapeutic benefits of such agents.

In recent decades, considerable progress has been made in elucidating the pathophysiological role of gastrointestinal peptides. Our knowledge of their physiological role has progressed in parallel with advances in peptide chemistry. However, a decisive and important step has been the development of potent and specific receptor antagonists. Of the gastrointestinal peptides, only gastrin and cholecystokinin (CCK) antagonists include nonpeptidic molecules that have been tested in humans and which are therefore potential candidates for therapeutic application.

Gastrin was the first gastrointestinal peptide for which the structure was determined. Subsequently, several molecular forms have been identified and the gene that encodes gastrin has been isolated[1]. The major forms of gastrin are G-34, G-17 and G-14 (where 'G' stands for gastrin and the number indicates the constitutive amino acids). They all share the five-amino acid C-terminal sequence that is responsible for the biological activity of gastrin. The five-amino acid C-terminal of gastrin is also common to CCK, a structurally related gastrointestinal peptide, which, despite the similarity at the C-terminal pentapeptide sequence (Figure 1), exhibits different biological effects[2,3].

Gastrin is produced by G cells located in the gastric antral mucosa and upper small intestine. Its secretion is stimulated by increased vagal activity, the presence of amino acids in the gastric antrum and upper small intestine, local distension of the antrum and elevation of the intragastric pH. Gastrin mainly stimulates gastric acid secretion (GAS) from parietal cells and promotes the growth of gastric mucosa[4].

CCK is produced by the I cells of the duodenal and jejunal mucosa. The most prominent circulating bioactive form is an eight-amino acid peptide (CCK-8), which includes a sulphated tyrosine residue at position 7; it is also found in the gut in longer forms (e.g. 33, 39 and 59 amino acids), all possessing the C-terminal amino acid sequence of CCK-8. CCK has been recognized as the major regulator of gall bladder contraction[5] and pancreatic secretion[6]. In addition, considerable evidence now supports a physiological role for CCK in the regulation of motor function at various

CCK-8 Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
Pentagastrin Gly-Trp-Met-Asp-Phe-NH_2

Figure 1. *Amino acid sequences of cholecystokinin and pentagastrin.*

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levels of the alimentary tract[7]. More recently, it has been demonstrated that CCK is widely distributed in the brain, where it might act as a neurotransmitter or neuromodulator[8].

CCK/gastrin receptors as therapeutic targets

The biological functions of gastrin and CCK are mediated by receptors located on the target tissues. Different CCK/gastrin receptors have been identified. High-affinity CCK binding sites were initially demonstrated in rat pancreatic acini[9] and rat cerebral cortex[10] and showed distinct differences in their specificity for various CCK-related peptides[11,12]. Given their anatomical location, the CCK receptors were classified as type A (alimentary) and type B (brain)[13]. Extensive evidence now indicates that CCK_A receptors are also present in the brain and CCK_B receptors in the periphery[14]; however, the original nomenclature still holds. Moreover, very recently, human gall bladder (CCK_A)[15] as well as brain and gastric (CCK_B/gastrin) receptors[16] have been cloned and show discrete homology. This is not surprising given that the endogenous ligands of these receptors, gastrin and CCK, in all their circulating and/or bioactive molecular forms, possess the C-terminal pentapeptide.

Gastrin plasma levels are elevated in a variety of gastric disorders, although a clear-cut causal relationship between high gastrin plasma levels and a disease is recognized only for the Zollinger–Ellison syndrome, which is characterized by abnormal GAS and multiple gastric and duodenal ulcers. In atrophic gastritis, where hypergastrinaemia is associated with achlorhydria, hypergastrinaemia is the result of the normal response to reduced gastric acid output. However, gastrin excess may occur despite normal or elevated acid output, as in the case of G cell hyperplasia. Whichever is the underlying mechanism, hypergastrinaemia is somehow related to the process leading to malignancy[17]. CCK_B/gastrin receptor antagonists may therefore have a therapeutic potential not only in peptic ulcer disease as acid antisecretory drugs but they may also represent an adjuvant therapy in gastrin-receptor-sensitive gastrointestinal tumours, because they could also inhibit the trophic effect of gastrin on gastrin-dependent malignant tumour cells[18,19].

Before the recent progress in CCK/gastrin research, a number of weak and aspecific CCK/gastrin antagonists were used to investigate the biological role of these hormones. For example, proglumide (Figure 2), the first putative gastrin antagonist clinically available, has long been used in the treatment of peptic ulcers, especially in the pre-H_2-receptor antagonist era, because of its antisecretory and gastroprotective activities[20]. Several studies have subsequently demonstrated that proglumide is also a weak CCK_A receptor antagonist[21] and, despite its low potency, it has been the reference CCK and gastrin antagonist for several years.

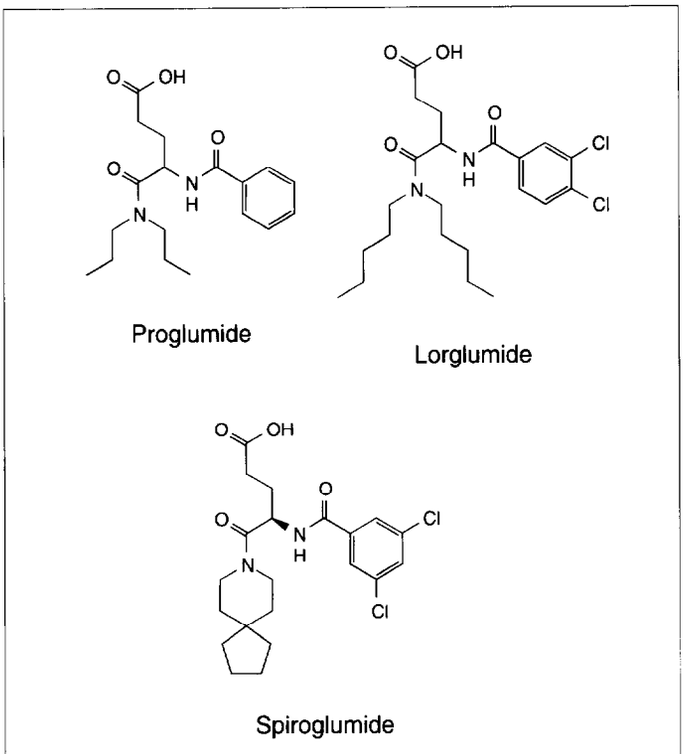


Figure 2. *Structure of early glutamic acid nonpeptide CCK_A and CCK_B receptor antagonists.*

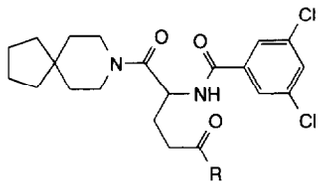
Nonpeptide antagonists of CCK_B/gastrin receptors

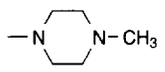
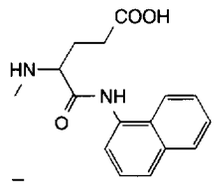
In recent years, many attempts have been made to discover potent and specific nonpeptide antagonists of CCK_B/gastrin receptors. As a result, a number of new chemical entities appeared, exhibiting high selectivity for specific populations of CCK_B/gastrin receptors.

The various compounds under development belong to the following main chemical classes:

- glutamic acid derivatives,
- benzodiazepine derivatives,
- tryptophan dipeptoid derivatives,
- pyrazolidinone derivatives,
- ureidoacetamido derivatives,
- dibenzobicyclo-octane and bicyclic heteroaromatic derivatives,
- ureidobenzazepine derivatives, and
- miscellaneous derivatives.

Table 1. Biological properties exhibited *in vitro* by selected glutamic acid derivatives (IC_{50}, 10^{-9} M)



Compound	R	Rabbit gastric glands[a] (gastrin) (1)	Guinea-pig cortex[b] (CCK_B) (2)	Rat pancreatic acini[c] (CCK_A) (3)	CCK_B selectivity (3)/(1)
Spiroglumide	–OH	1223	1400	13500	11.0
CR2345		1360	700	6600	4.9
CR2622		7.4	20	7380	1054
R-Lorglumide	–	–	5600	50	0.01[d]

[a]Inhibition of gastrin-induced cytosolic $[Ca^{2+}]$ elevation.
[b]Inhibition binding of [^{3}H][*N*-Me-*N*-Leu]CCK-8.
[c]Inhibition binding of ^{125}I-labelled CCK-8.
[d]CCK_B selectivity from (3)/(2).

This article will describe the development of these novel chemical entities, focusing in particular on the antagonism at the gastrointestinal level exerted by CCK_B/gastrin antagonists, because the potential therapeutic application of their antagonism at CNS level has been discussed elsewhere recently[22].

Glutamic acid derivatives

Appropriate chemical modification of proglumide led to lorglumide (Figure 2), the first nonpeptidic, potent, competitive and specific CCK_A receptor antagonist developed by the Rotta group. Further modifications to the structure of lorglumide produced CR2194 (spiroglumide) (Figure 2). This compound exhibited CCK_B/gastrin antagonism in the micromolar range in different models *in vitro* and was about 50 times more potent than proglumide in blocking pentagastrin-stimulated GAS in the *in vivo* perfused rat stomach[23].

In humans, spiroglumide, infused intravenously in the range of 1–7.5 mg/kg/h, dose-dependently antagonized gastrin-stimulated gastric acid and fluid responses with a competitive-like profile. Virtually complete inhibition was observed in the physiological dose range of gastrin-stimulated responses with 7.5 mg/kg/h spiroglumide[24]. In a subsequent Phase I randomized, double-blind, placebo-controlled trial in healthy male volunteers[25], the effect of spiroglumide on meal sham-feeding (MSF) and meal-stimulated intragastric acidity was evaluated. Infusion of spiroglumide at 7.5 mg/kg/h significantly decreased basal acid output as well as postprandial intragastric acidity. Moreover the MSF-stimulated acid responses were nearly abolished by spiroglumide. Taken together, these results suggest that the cephalic stimulation of gastric acid release under vagal cholinergic mechanism may also be regulated by endogenous gastrin.

However, the relatively low antigastrin potency exhibited *in vitro* (micromolar range) by spiroglumide, and its relative poor selectivity for CCK_B/gastrin receptors compared with CCK_A receptors (Table 1), raise doubts about the potential therapeutic usefulness of this compound, despite its excellent oral bioavailability.

Derivatives of spiroglumide

Chemical manipulation of the structure of spiroglumide led to a new molecular entity (CR2622), exhibiting high affinity

(nanomolar range) and selective antagonism on CCK_B/gastrin receptors (Table 1). *In vivo*, in the *in situ* perfused rat stomach, CR2622 given intravenously reduced dose-dependently the GAS induced by 30 μg/kg/h of pentagastrin infusion with an ED_{50} effect of 2 mg/kg (Ref. 26). However, the relatively high molecular weight of CR2622 could greatly limit its oral absorption, as already demonstrated in animal studies (unpublished data).

Rotta also synthesized CR2345, a compound with basic characteristics structurally related to spiroglumide, to investigate whether the acidic character of the glutamic acid derivatives so far examined was essential for possessing CCK_B/gastrin receptor antagonism. The basic substitution obtained with the *N*-methylpiperazinyl group fully retains antagonistic activity of the acidic parent compound on CCK_B/gastrin receptors. Moreover, CR2345 also inhibits histamine- and carbachol-induced acid secretion[27]. Interestingly, CR2345 and its *N*-propyl analogue (CR2456) were shown to inhibit the growth *in vitro* of several human strains of *Helicobacter pylori*. These strains were isolated from patients with histological lesions of gastritis and can be considered as representative of this bacterial species, which is now recognized as the aetiological factor of peptic ulcers. The minimum inhibition concentrations (MIC_{50}) of the strains studied were in the range 1–16 μg/ml for CR2345 and CR2456 (unpublished data). Although the MICs for these basic derivatives of spiroglumide were relatively high in comparison to the most effective antibiotics (for example, the MIC_{50} range for clarithromycin is 0.03–0.3 μg/ml), these compounds deserve to be studied further because they are, to our knowledge, the first gastrin receptor antagonists endowed with bacteriostatic activity against *H. pylori*. An antisecretory drug with associated bacteriostatic activity against *H. pylori* might indeed represent a significant step towards the simplification of an effective eradication therapy that is currently achieved with a combination of different drugs (see below).

Benzodiazepine derivatives

The approach of Merck scientists to designing and optimizing an asperlicine structure resulted in the discovery of L365260 (Table 2), the first compound to exhibit CCK_B receptor antagonism in the nanomolar range *in vitro*[28].

L365260 was shown to interact stereoselectively and competitively with stomach gastrin and brain CCK_B receptors, and showed an affinity for the CCK_B/gastrin receptor that was about 100 times higher than that for the peripheral CCK_A receptor. In the pylorus-ligated rat, L365260 given intravenously inhibited pentagastrin-stimulated acid secretion with an ED_{50} of 0.7 mg/kg (Ref. 29), whereas higher doses were required to inhibit both histamine-stimulated and basal acid secretion (ED_{50} = 12.6 mg/kg). The potency *in vivo* of L365260 as a gastrin antagonist varied among species, being highest in mice and least in the dog.

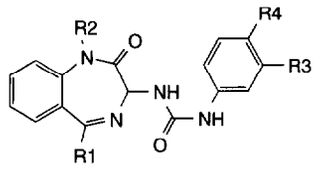
Phase I clinical trials[30,31] in healthy male volunteers have demonstrated that single oral doses (2.5–50 mg) of L365260 caused a dose-dependent inhibition of pentagastrin-stimulated GAS. A single oral dose of 50 mg of L365260 produced 50% inhibition of the gastric acid output response to 0.4 μg/kg/h of pentagastrin. The corresponding mean plasma concentration of L365260 in the 0–3 h period after dosing was about 500 ng/ml. The same single oral dose of L365260 did not inhibit basal gastric acid output. The relatively limited oral bioavailability of L365260 demonstrated in animals and humans could be explained by its very low aqueous solubility[32]. This suggests that L365260 would not be a suitable drug for oral administration in humans[33].

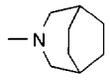
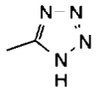
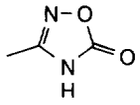
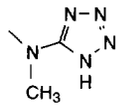
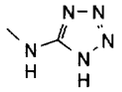
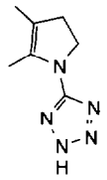
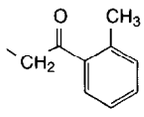
Second generation benzodiazepines

The objective of the Merck scientists, therefore, was to develop a second generation of CCK_B/gastrin receptor antagonists that would retain the binding potency and selectivity of L365260, and, by incorporating a water-solubilizing group in the structure, increase the oral bioavailability of new potential candidates. These efforts were very successful and led to a series of extremely potent and orally active CCKB/gastrin antagonists.

For instance, one of the most interesting compounds developed during optimization studies of the benzodiazepine C5 substituent, was the azabicyclo[3.2.2.]nonane derivative L740093 (Table 2 and Ref. 34), an amidine derivative having a basic character. L740093 showed very high CCK_B/gastrin receptor affinity (IC_{50} = 0.10 nM) while also displaying excellent receptor subtype selectivity. Its CCK_A/CCK_B ratio was about 16,000. L740093, at a 50% inhibitory dose of 0.01 mg/kg, given intraperitoneally, blocked pentagastrin-induced GAS in anaesthetized rats, showing 100-fold greater activity than L365260 (Ref. 35). Additionally, L740093 (as HCl salt) showed an aqueous solubility of 0.15 mg/ml, a value about 100 times higher than that exhibited by the parent compound L365260. Thus L740093 seems to possess physicochemical properties suitable for oral treatment in humans. Results from clinical trials with this new entity are still to be published.

Table 2. Benzodiazepine CCK_B antagonists: chemical structures of compounds referred to in the text, and their receptor binding affinities



Code	R1	R2	R3	R4	IC_{50} (nM)[a] CCK_B	CCK_A
L365260	Phenyl	CH_3	$-CH_3$	–H	8.5	736
L740093		CH_3	$-CH_3$	–H	0.10	1604
L368730	Phenyl	CH_3		–H	1.0	577
L368935	Phenyl	$CH_2CH(CH_3)_2$		–H	0.14	1434
L369466	Phenyl	CH_3		–H	0.27	983
L736380	Cyclohexyl	CH_3		–H	0.05	400
L737481	Cyclohexyl	CH_3		$-CH_3$	0.07	802
L738425	Phenyl	CH_3			0.11	4080
YM022	Phenyl		$-CH_3$	–H	0.11	150

[a]CCK_B binding was measured by displacement of ^{125}I-labelled CCK from guinea-pig cortical membranes; CCK_A binding was measured by displacement of ^{125}I-labelled CCK from rat pancreatic tissue as described in Refs 28 and 34–38.

Another approach to increase the water solubility of L365260, in order to achieve adequate levels of oral bioavailability, was successfully performed by the Merck group by incorporating acidic solubilizing groups into the phenyl ring of the acylurea moiety of the parent compound[36]. Prominent compounds emerging from this new acidic series include the tetrazoles, L368730 and L368935, and the 1,2,4-oxadiazolone, L369466 (Table 2).

However, despite excellent potency and increased solubility, these compounds did not exhibit a satisfactory bioavailability. Therefore further modifications of tetrazole moiety of L368730 were performed in order to influence the

bioavailability of related compounds by modulating the pK_A of the acidic moiety by means of structural modifications around the tetrazole group[37]. The aminotetrazole group was chosen as the new acidic moiety, because its pK_A (6.0) is substantially higher than that of tetrazole itself. Compounds to emerge from this approach, such as the C5-cyclohexyl derivatives L736380 and L737481, are among the highest affinity and, in the case of compound L738425, most selective (CCK_A/CCK_B = 37,000) antagonists so far reported for CCK_B/gastrin receptors. For example, L736380, given by the intraperitoneal route, dose-dependently inhibited GAS in anaesthetized rats, with an ID_{50} of 0.064 mg/kg, and it is therefore one of the most potent acidic CCK_B/gastrin receptor antagonists reported until now (Table 2).

A novel series of 1-aroylmethyl analogues of L365260 was prepared and evaluated for activity as CCK_B/gastrin receptor antagonists by the Yamanouchi group. YM022 was the optimal compound of this series, exhibiting very high CCK_B/gastrin receptor affinity (IC_{50} 0.11 nM) and very good receptor subtype selectivity, the CCK_A/CCK_B ratio being about 1,300 (Ref. 38). *In vivo*, YM022, given intravenously, inhibited pentagastrin-induced GAS in anaesthetized rats, with an ED_{50} value of 0.0078 μmol/kg (about 0.04 mg/kg), whereas it did not affect histamine- and bethanechol-induced acid secretion in anaesthetized rats, even at doses 1,000-fold higher than the ED_{50} exhibited by inhibiting pentagastrin[39].

Oral YM022 dose-dependently suppressed acid secretion in pylorus-ligated rats with an ED_{50} of 0.83 μmol/kg (about 0.5 mg/kg). Its potency was comparable with that of famotidine and was about eight times greater than that of omeprazole. YM022, given orally, was also able to practically inhibit gastric damage in rats induced by restraint stress (about 50% inhibition at 15 mg/kg) or by acidified ethanol (ED_{50} = 15.9 μmol/kg, corresponding to about 8.2 mg/kg)[40].

In spite of its close structural similarity to the parent benzodiazepine derivative L365260 and its higher molecular weight, these results seem to suggest that YM022 may represent a useful therapeutic agent in the treatment of peptic ulcer disease.

Dipeptoids

The rational design of nonpeptide antagonists of CCK conducted by scientists at Parke-Davis, by examining the activity of CCK-30–33 fragments in binding experiments on CCK_B/gastrin receptors, led to tryptophan dipeptoid derivatives such as CI988 (formerly PD134308), with nanomolar affinity for CCK_B/gastrin receptors[41]. CI988 (Figure 3) exhibited a 1,600-fold selectivity for CCK_B/gastrin over CCK_A receptors. Given subcutaneously, it inhibited pentagastrin-induced GAS in *in situ* perfused stomach rat preparation with an ED_{50} of 0.25 μmol/kg. The relative potencies of CI988 versus ranitidine and omeprazole were 0.76 and 1.88, respectively.

CI988 had no effect on basal acid secretion and did not inhibit the secretory response to histamine and bethanechol[42].

C-terminal modifications of CI988 led to compounds with subnanomolar affinity for CCK_B/gastrin receptors. For example, **1** (Ref. 43) displays a very high affinity (IC_{50} = 0.3 nM) for the CCK_B/gastrin receptor, identical to that of the endogenous neuropeptide itself, CCK-26–33 (sulphated). Further exploration to optimize the substitution on the phenyl ring of **1** led to the 4-F

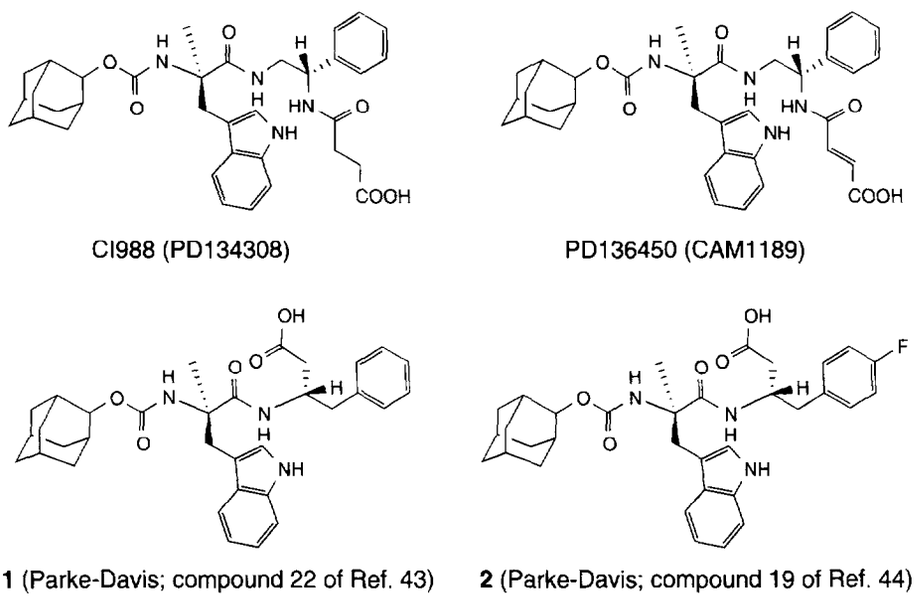


Figure 3. *Selected representatives of tryptophan 'dipeptoid' CCK_B receptor antagonists.*

analogue **2** (Ref. 44), which exhibited an extraordinarily high affinity at the CCK_B/gastrin receptor (IC_{50} = 0.08 nM) and was also very selective (940-fold over the CCK_A receptor) (Figure 3).

However, PD134308 and its didehydro analogue PD136450 (CAM1189) (Figure 3) act as CCK_B/gastrin partial agonists in a rat gastric acid secretion assay and as powerful agonists of pancreatic secretion in anaesthetized rats[45]. Furthermore, the relatively high molecular weight and the 'dipeptoid' structure of these derivatives seem to justify their apparent low bioavailability. These results lead to the speculation that perhaps these compounds would not be suitable drugs for oral therapy of gastroduodenal diseases.

Pyrazolidinones

Lilly's approach to designing and optimizing lead compounds through screening resulted in the discovery of diphenylpyrazidinones such as LY288513 (Figure 4), a potent CCK_B receptor antagonist, exhibiting a greater than 350-fold selectivity for CCK_B (IC_{50} = 16 nM) over CCK_A receptors[46,47]. However, development of LY288513 has been discontinued because of adverse effects in preclinical toxicological studies[22].

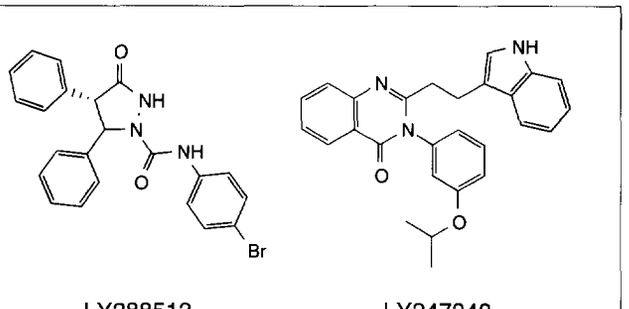


Figure 4. *Structures of Lilly's selected nonpeptide CCK_B antagonists.*

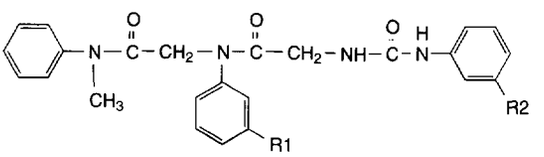
Ureidoacetamides

Nonpeptide ureidoacetamides, developed by Rhône-Poulenc, are potent and selective ligands for CCK_B/gastrin receptors. In binding experiments on guinea-pig brain and pancreas membrane preparations, the best representatives of this new series, for example RP69758 and RP72540 (Table 3), demonstrated nanomolar affinity for CCK_B/gastrin receptors and exhibited 100–1,000-fold selectivity for CCK_B/gastrin over CCK_A receptors[48]. RP69758 is also a potent CCK_B/gastrin receptor antagonist *in vivo*, and dose-dependently inhibited GAS induced by intravenous injection of pentagastrin in the rat. Its half-maximal inhibitory effect corresponded to a dose of 1.14 μmol/kg.

The substitution of an acetic acid moiety in R2 (Table 3) with the ethyl sulphonate group led to RP73870. This compound is a very potent inhibitor of pentagastrin-stimulated GAS in the perfused rat stomach. It reduced dose-dependently the effect induced by 30 μg/kg/h of pentagastrin infusion with an ID_{50} of 0.05 mg/kg (i.v.)[49]. The relative potencies of RP73870 versus famotidine and cimetidine, two H_2-receptor antagonists, were 0.34 and 18.8, respectively. This compound also inhibited basal GAS in the rat, although at doses higher than that required for inhibition of pentagastrin-stimulated GAS.

RP73870 was evaluated in a number of gastrointestinal damage models. It was effective in the prevention of aspirin-induced gastric damage in the rat after intravenous and

Table 3. CCK receptor binding affinities of selected ureidoacetamides (K_i, nM)



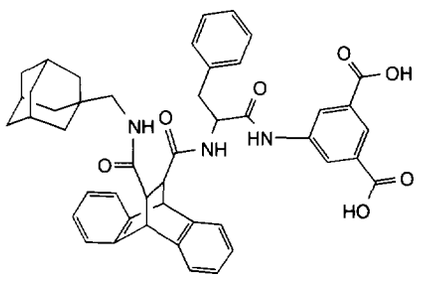
Compound	R1	R2	Guinea-pig cortex[a] (CCK_B) (1)	Guinea-pig pancreatic membranes[b] (CCK_A) (2)	CCK_B selectivity (2)/(1)
RP69758	H	$-CH_2-COOH$	9.0	1254	139
RP72540	$-OCH_3$	$-CH(CH_3)-COOH$	2.4	2338	982
RP73870	$-OCH_3$	$-CH(CH_3)-SO_3K$	0.48	1634	3404

[a]Inhibition binding of ^{125}I-labelled CCK-8.
[b]Inhibition binding of [^{3}H]CCK-8.

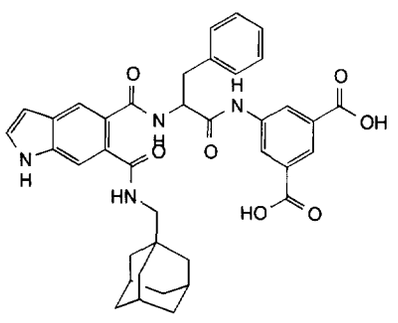
oral administration, with an ED_{50} of 2.9 and 31.1 mg/kg, respectively. Given orally, it prevented cysteamine-induced duodenal ulceration (ED_{50} = 3.5 mg/kg). In this model, despite its relatively poor oral bioavailability, RP73870 was as potent as other standard antiulcer compounds such as omeprazole and famotidine and five times more potent than the CCK_B antagonist L365260. On the other hand, RP73870, at the highest doses tested, was essentially inactive in gastric acid-independent damage models, such as ethanol-induced gastric ulcer and gastric damage induced by water immersion/restraint stress.

Dibenzobicyclo [2.2.2]octane and bicyclic heteroaromatic derivatives

The James Black Foundation has synthesized a novel series of potent and selective CCK_B/gastrin receptor antagonists based on the dibenzobicyclo[2.2.2]octane skeleton. The most potent representative of this series was compound **3** (Ref. 50) (Figure 5), with a pK_i of 8.8 at CCK_B/gastrin receptors in mouse cortical membranes. Its selectivity for these receptors over CCK_A receptors was about 1,200-fold.



3 (J.B. Foundation; compound 9 of Ref. 50)



4 (J.B. Foundation; compound 7 of Ref. 51)

Figure 5. *Structures of selected dibenzobicyclo[2.2.2]octane (**3**) and bicyclic heteroaromatic (**4**) CCK_B antagonists.*

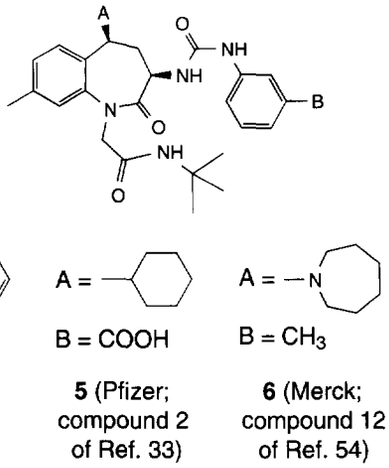
Compound **3**, given intravenously at the dose of 0.025 μmol/kg, gave a peak inhibition of 79% of the GAS observed for a submaximal infusion of pentagastrin (i.e. it was at least 40-fold more potent than L365260). However, when **3** was examined in conscious chronic gastric fistula dogs, it was at least 700-fold less potent than in the rat model. This result was clearly a problem, given the strong intraspecies nature of the variation.

Therefore new chemical entities were synthesized in which the dibenzobicyclo[2.2.2]octane skeleton was replaced with a bicyclic, heteroaromatic framework. Compound **4** (Ref. 51), a 5,6-disubstituted indole derivative, was the optimal compound of this new series (Figure 5). It totally inhibited (97%) pentagastrin-stimulated GAS in the rat when administered intravenously at the dose of 0.025 μmol/kg, and exhibited a comparable activity in the dog assay.

Results of experiments in which **4** was given orally have not yet been published.

Ureidobenzazepines

A group from Pfizer has modified the benzodiazepine nucleus of L365260 by the benzazepin-2-one moiety. CP212454 is representative of this new series (Figure 6). This compound demonstrated subnanomolar CCK_B/gastrin receptor affinity (IC_{50} = 0.48 nM) and good receptor subtype selectivity in guinea-pig, giving a CCK_A/CCK_B ratio of about 350 (Ref. 52). In the guinea-pig *in vivo*, CP212454 potently



A =	A =	A =
B = Cl	B = COOH	B = CH_3
CP212454 (Pfizer)	**5** (Pfizer; compound 2 of Ref. 33)	**6** (Merck; compound 12 of Ref. 54)

Figure 6. *Structures of selected ureidobenzazepine CCK/gastrin receptor antagonists.*

inhibited pentagastrin-induced GAS, with an ED_{50} value of 0.8 mg/kg s.c., compared with 1.5 mg/kg subcutaneously for L365260. Despite its potent and selective CCK_B/gastrin receptor affinity, the probable poor oral bioavailability of CP212454, resulting from its low water solubility, might become an insurmountable difficulty for its use in man. Tentative modification of the structure of CP212452, by inserting ionizable groups such as carboxylic acids or acid surrogates such as acyl sulfonamides and tetrazoles, led to the water-soluble **5** (Figure 6)[53]. Compared with CP212454, **5** showed both increased water solubility (3 mg/ml as the potassium salt, whereas CP212454 is practically insoluble) and *in vivo* efficacy (ED_{50} = 0.03 mg/kg s.c. in the pentagastrin-induced GAS model). Results from clinical trials with **5** have not been reported.

In addition, Merck scientists prepared a series of novel ureidobenzazepines containing a basic cationic substituent at the 5-position. Compound **6** was the optimal molecule of this series, exhibiting relatively high affinity at the CCK_B receptor (IC_{50} = 15.7 nM) but low selectivity (Figure 6)[54].

Miscellaneous structures

Quinazolinones

LY247348 (from Lilly) is a quinazolinone CCK_B/gastrin antagonist that demonstrated 300-fold selectivity for CCK_B/gastrin receptors (IC_{50} = 32 nM) over CCK_A receptors (Figure 4)[55]. However, no data on the peripheral activity *in vivo* of this compound are available.

Amino acid piperidines

Novel derivatives of amino acid piperidines were synthesized and evaluated as CCK_B/gastrin antagonists by the Abbott group. Some of these compounds, such as **7** (Figure 7), exhibited midnanomolar affinity (34 nM) and 7.5-fold selectivity for guinea-pig cortical CCK_B/gastrin receptors[56].

Dipeptoid analogue

The Glaxo group synthesized **8**, a dipeptoid analogue of PD134308, in which the indole moiety is replaced by the 2-naphthalene group (Figure 7)[57]. This compound exhibits CCK_B/gastrin receptor binding affinity similar to that of PD134308, with an improvement in selectivity over the CCK_A receptor. No *in vivo* data are available.

Dual H_2- and CCK_B receptor antagonists

Shionogi's approach to designing and synthesizing hybrid molecules with dual H_2- and CCK_B/gastrin receptor antagonism led to compounds such as **9** (Figure 7). This compound has the same pA_2 value as the parent compound cimetidine, but its CCK_B/gastrin receptor affinity is reduced. As regards *in vivo* gastric acid antisecretory activity, **9** showed quite marginal activity when administered by the intradermal route, suggesting poor oral absorbability of these hybrid compounds[58].

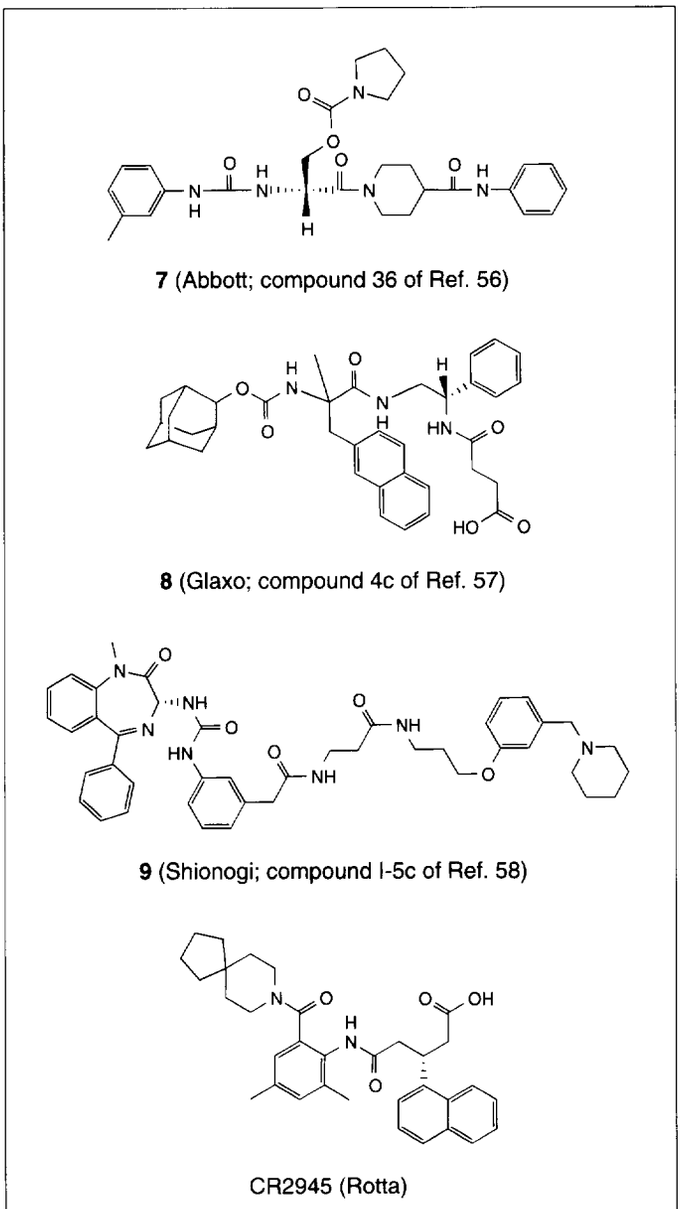


Figure 7. *Structures of miscellaneous selected nonpeptide CCK_B antagonists.*

CR2945

The objective of Rotta's scientists was the discovery of a compound that would retain the binding potency and

selectivity of CR2622 and the oral bioavailability of spiroglumide. These efforts led to an anthranilic acid derivative coded CR2945 (Figure 7). This compound showed high CCK_B/gastrin receptor affinity (IC_{50} = 4.0 nM) and excellent selectivity (5,470-fold) over the CCK_A receptor.

In vivo, CR2945 blocked pentagastrin-induced GAS in anaesthetized rats with a 50% inhibitory dose of 0.3 mg/kg given intravenously and 4.0 mg/kg given intraduodenum. Its binding affinity for CCK_B/gastrin receptors was about 300 times higher than that of spiroglumide, whereas the oral bioavailability in the rat was about 10 times lower. CR2945 inhibited basal GAS in the rat and was also effective in the prevention of gastric damage in several models (unpublished data). These results suggest that CR2945 might be useful in the treatment of peptic ulcer disease.

Conclusions

The aim of this article has been to review the major chemical classes of nonpeptide CCK_B/gastrin antagonists under development, without any pretence to be exhaustive. These compounds may provide a novel interesting approach for therapy of gastric and duodenal ulcers and possibly in gastrin-sensitive malignancies. Although peptic ulcer has, in the last decade, been recognized as being aetiologically related to the presence of *H. pylori* infection, the pharmacological control of GAS is still essential for obtaining symptom relief and a quicker healing rate. The main endogenous mediators of GAS are acetylcholine, gastrin and histamine. Each of these acts through its own receptor located on parietal cells, but the final step in each case involves the activation of the enzyme H^+/K^+-ATPase, which is responsible for acid production in the stomach.

Therapeutic potential of CCK_B/gastrin receptor antagonists

Early pharmacological control of GAS consisted of antiacids and anticholinergic agents. However, these therapies were never particularly effective and anticholinergics produced a number of side effects such as dry mouth and mydriasis. More recently, the discovery and development of a range of new drugs such as H_2-receptor antagonists and proton pump inhibitors revolutionized the medical treatment of peptic ulcer disease. Clinical trials performed in a wide range of acid-related diseases demonstrated that these drugs were able to produce up to 80–85% healing rates. It could therefore be deduced that the current spectrum of drugs could largely satisfy the clinical need to treat peptic ulcers[59].

The pharmacological properties of CCK_B/gastrin receptor antagonists remain to be clarified. It has been suggested that gastrin also causes GAS indirectly via histamine release from the enterochromaffin-like (ECL) cells. Hyperplasia of ECL cells after long-term treatment with proton pump inhibitors produces increased capacity of GAS by gastric mucosa[60]. Therefore, hypergastrinaemia may be a risk factor in both the relapse of peptic ulcers and the production of gastric cancer.

Interestingly, PD136450 was shown to prevent hyperplasia of ECL cells in animals produced by an irreversible proton pump inhibitor[19]. Hence, if CCK_B/gastrin antagonists are able to inhibit GAS effectively, they would be expected to be safer antiulcer drugs than H_2-receptor antagonists and proton pump inhibitors, and may prevent the potential hazards associated with long-lasting hypergastrinaemia.

Although CCK_B/gastrin antagonists represent an alternative novel therapeutic approach for the treatment of peptic ulcer, for only two compounds clinical studies have been published, and the results obtained so far are not very encouraging. For instance, the potential therapeutic role of L365260 in patients with ulcer disease would appear to be minor, since a 50 mg oral dose of L365260 had an antisecretory efficacy equivalent to a 5 mg oral dose of famotidine. Furthermore, at the same doses, L365260 seems to be ineffective in inhibiting basal acid secretion. Spiroglumide, at a dose of 7.5 mg/kg/h i.v., significantly reduced intragastric acidity in premeal and postprandial periods, and reduced GAS during sham-feeding. However, a dose of 1 mg/kg/h i.v. was ineffective. These results may be explained by the poor bioavailability of L365260 and the relatively low potency of spiroglumide.

Therefore, the therapeutic potential of this class of compounds in the gut will only be fully investigated when second generation CCK_B/gastrin antagonists, such as L740093, RP73870, YM022, CP212454, CR2945, etc., are available for study in randomized controlled clinical trials for the therapy of acid-related diseases.

Role in H. pylori *eradication therapy*

Another intriguing possible therapeutic indication for the CCK_B/gastrin receptor antagonists may be their potential role in *H. pylori* eradication therapy. Peptic ulceration is now recognized as a sequela of mucophilic *H. pylori* infection, and it is now accepted that the most important cause of the inappropriate hypergastrinaemia in patients with gastroduodenal disease is the infection provoked by this

bacterium[61]. Eradication studies have indicated that both basal gastrin and the gastrin response to meal are increased in *H. pylori*-infected patients[62]. Thus, there has been considerable interest in the mechanism, the 'gastrin-link', by which infection with *H. pylori* stimulates gastrin release[63]. One interesting hypothesis[64] is that an excessive release of plasma peptides and amino acids into antral mucosa damaged by *H. pylori* causes activation of G cells and inhibition of D cells. This imbalance may provoke high and inappropriate gastrin release in patients with peptic ulcer. In any case, *H. pylori* eradication is highly effective in promoting ulcer healing and preventing subsequent ulcer recurrence, and has thus become a primary goal of physicians treating patients with gastric or duodenal ulcers[65]. Despite the essential role of antimicrobial therapy against *H. pylori*, the pharmacological control of GAS is still necessary to achieve maximal healing rate and, moreover, symptom relief. In fact, the most recent guidelines for the treatment of peptic ulcer[66] recommend the association of antibiotics with inhibition of GAS. As CCK_B/gastrin receptor antagonists have been shown to inhibit GAS, they may represent, in association with antibiotics, another promising approach for the treatment of peptic ulcer disease. In addition, CCK_B/gastrin antagonists might have an important advantage over the existing pharmacological treatment because of their ability to antagonize the potential hazards linked to the hypergastrinaemia associated with both the inhibition of GAS and the *H. pylori* infection.

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