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Review Article

Update on nonpeptide CCK-B receptor antagonists

Laura Revel and Francesco Makovec*

*Rotta Research Laboratories S.p.A., Via Valosa di Sopra 7, 20052 Monza (MI), Italy. *Correspondence*

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Introduction

Since the original isolation and sequencing of cholecystokinin (CCK) from porcine intestine by Victor Mutt (1) about 30 years ago, considerable advances have been made in understanding the pathophysiological role of CCK and related peptides. The originally isolated CCK was identified as a COOH amidated-33 amino acid peptide, but sequences of 8-, 39-, 47- and 58-amino acids have also been isolated in various species (2, 3). Full biological activity resides in the COOH terminal-7 amino acids, although full potency requires the octapeptide to be sulfated at the tyrosine in the seventh position from the terminal COOH (Fig. 1).

Recent studies demonstrate that CCK immunoreactivity is widely distributed in the small intestine (duodenal I cells and enteric nerves), where CCK is secreted in response to meals and stimulates gallbladder contraction and pancreatic secretion (4). In addition, CCK seems to have a physiological role in the regulation of motor function in the alimentary tract (5) and a gastric secretory effect (stimulation of pepsin release from chief cells and somatostatin release from D-cells) (6). CCK has been found in the male reproductive system, suggesting a possible role in reproductive functions (7). More recently,

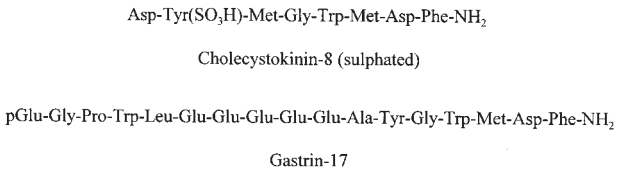


Fig. 1. Amino acid sequence of cholecystokinin-8 and gastrin-17.

CCK was also found widely in many areas of the central nervous system such as cerebral cortex, hippocampus, amygdala, septum, olfactory bulb, hypothalamus, thalamus and ventral mesencephalon (8). More specifically, CCK seems to coexist with substance P in central grey neurons projecting to the spinal cord (9), with enkephalin in the hippocampus (10), with corticotropin releasing hormone in the paraventricular nucleus (11), with oxytocin in the supraoptic and paraventricular nuclei of the hypothalamus (12) and with GABA in the cortex, hippocampus and amygdala (13-15). In addition, CCK has been localized in some specific neuronal pathways such as nigrostriatal dopaminergic pathways and corticostriatal pathways (16, 17).

Gastrin is closely related to CCK, sharing the same COOH terminal sequence Gly-Trp-Met-Asp-Phe-NH_2 (Fig. 1). Gastrin is synthesized as a large precursor molecule, preprogastrin, which is processed into a number of smaller bioactive peptides such as gastrin 71-, 52-, 34-, 17- and 5- (18); full biological activity resides in the COOH terminal pentapeptide. Gastrin is secreted by G-cells located in the gastric antral mucosa and upper small intestine. Gastrin has stimulatory effects on gastric acid secretion (GAS), trophic effects on gastric mucosa, particularly on enterochromaffin-like cells (ECL) (19), and growth stimulatory effects on gastrin-sensitive malignant cells (20).

Different CCK/gastrin receptor subtypes have been identified. Given their anatomical location, they were classified as type A (alimentary) and type B (brain) (21). High levels of CCK-A receptors are found in peripheral organs such as the gallbladder, pancreas and intestine. Extensive evidence now indicates that CCK-A receptors are also present in the brain and CCK-B receptors in the periphery, principally in the stomach; however, the original nomenclature still holds (22).

Therapeutic targets for CCK-B antagonists

Gastric secretory disorders

Gastrin has two major physiological functions: stimulation of acid secretion and stimulation of mucosal growth in the acid secreting part of the stomach. Gastrin is released in response to the presence of food in the stomach or in response to neutralization of the stomach content (23). The increase of circulating gastrin plasma levels stimulates DNA synthesis and proliferation of oxyntic mucosal cells (24), in particular parietal and ECL cells. Gastrin release is modulated by a negative feedback mechanism in which acidification inhibits the release of gastrin from antral G-cells.

Elevated fasting and postprandial gastrin levels have been described in several diseases such as diabetes mellitus, peptic ulcer, Zöllinger-Ellison syndrome, gastrinomas and G-cell hyperplasia (25). Moreover, chronic infection with *Helicobacter pylori* is associated with increased basal and gastrin-stimulated GAS (26). Therefore, CCK-B receptor antagonists may have therapeutic potential as antisecretory drugs in peptic ulcer disease as well as in all those pathological situations in which hypertrophy of mucosal gastric cells is present.

Gastrointestinal tumors

The role of gastrin in the growth of gastrointestinal tumors is complex, involving both endocrine and autocrine/paracrine pathways. Gastrin, via CCK-B receptors, stimulates oxyntic mucosa, which may lead to hyperplasia of ECL cells, resulting in ECL carcinoid tumors in rats (27) and in patients with chronic atrophic gastritis (28, 29). In humans, in addition to hypergastrinemia, genetic factors may be involved in the progression of ECL hyperplasia to carcinoids (25). The role of gastrin in colorectal cancer is still controversial (30, 31). Trophic effects of gastrin on colon malignant cells have been ascribed to interaction with gastrin receptors which are not uniformly present in many colorectal cancers (32, 33). Moreover, distinct receptors have been found to mediate the autocrine role of gastrin. Weinstock and Baldwin (34) showed that low-affinity receptors were expressed on a series of gastrointestinal cancer cell lines. This low-affinity receptor was identified as a distinct receptor subtype and was classified as the CCK-C/gastrin receptor.

The potential use of CCK-B receptor antagonists in gastrointestinal malignancy may be clarified when gastrin receptor subtyping confirms which isoforms play an important role in gastrin-mediated tumor growth.

Anxiety

Now there is good evidence to indicate that CCK mechanisms may be involved in the mediation of anxiety-related behavioral responses in animals and man.

In rats, CCK-8 microiontophoretically applied to pyramidal hippocampal neurons increased neuronal firing and this increase was suppressed by benzodiazepine anxiolytics such as diazepam and flurazepam (35, 36). Similarly, a 2-week benzodiazepine treatment in rats decreased the neuronal response to CCK (37). Furthermore, colocalization of GABA and CCK in rat cortical and hippocampal neurons has been reported (38). These neurophysiological and anatomical findings led to behavioral studies in animal models of anxiety, where CCK-B receptor agonists such as CCK-4 and pentagastrin showed an anxiogenic effect (39).

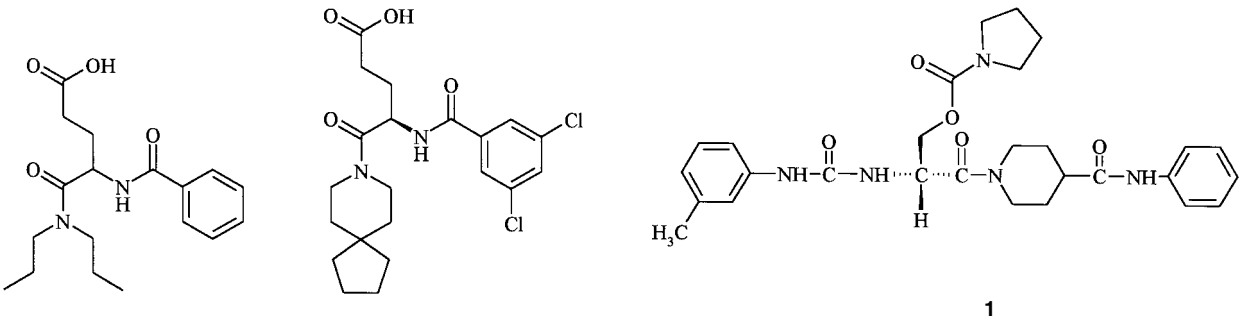
In humans, it has been demonstrated that CCK-4 administered intravenously (i.v.) produced short-lasting panic-like attacks in normal healthy volunteers (40, 41) and in patients with panic disorders (42). The efficacy of CCK-B receptor antagonists as anxiolytic or antipanic agents is still controversial. In fact, although CCK-B receptor antagonists appear to have anxiolytic-like effects in several animal models, their potency and efficacy vary markedly among paradigms and laboratories.

Analgesia

CCK has also been postulated to play a role in the perception of pain. Faris *et al.*, in 1983 demonstrated that CCK administered in the spinal cord antagonizes morphine and β-endorphin-induced analgesia in rats (43). Further evidence for the antagonistic interaction between CCK and opiates was the improvement of opiate analgesia by active immunization with CCK antiserum (44). Both CCK-A and CCK-B antagonists have been reported to enhance morphine analgesia (45, 46), and CCK-B antagonists have been shown to prevent the development of tolerance to morphine (47).

Schizophrenia

Hyperactivity of the dopamine system in brain seems to play a major role in the etiology of schizophrenia (48). CCK has been shown to coexist with dopamine in midbrain neurons, including ventral tegumental neurons projecting to the medial posterior nucleus accumbens (49). Although the interactions between CCK and dopamine are complex, some studies suggest that CCK can enhance dopamine function (50, 51). In schizophrenic patients the expression of CCK mRNA is increased compared to healthy subjects (52). There is experimental evidence that the activity of midbrain dopamine neurons may be inhibited by CCK-B but not by CCK-A receptor antagonists. CCK-B receptor antagonists may represent a novel class of antipsychotic drugs, having potential therapeutic effects in schizophrenia without the cataleptogenic effects induced by classical antipsychotic drugs.



Proglumide (Rotta) | Spiroglumide (Rotta) | **1** (Abbott)

Fig. 2. Structures of proglumide, spiroglumide and compound **1**.

Nonpeptide CCK-B receptor antagonists

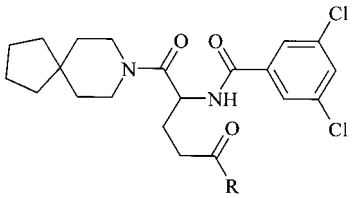
In the last decade, a number of nonpeptide CCK-B receptor antagonists have been synthesized. The approaches for designing new classes of CCK-B receptor antagonists by pharmaceutical companies have been different: modification of molecules that in the past had shown weak CCK antagonistic activity (proglumide, asperlicin), amino acid deletion studies on the tetragastrin molecule, modelling the shape of the tetragastrin molecule or broad screening exercise. In summary, compounds under development can be grouped into the following chemical classes: amino acid derivatives, benzodiazepine derivatives, dipeptoids, pyrazolidinone derivatives, quinazolinone derivatives, ureidoacetamido derivatives, dibenzobicyclo-octane and bicyclic heteroaromatic derivatives, ureidobenzazepine derivatives and miscellaneous structures.

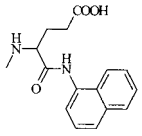
Amino acid derivatives

1) Glutamic acid derivatives

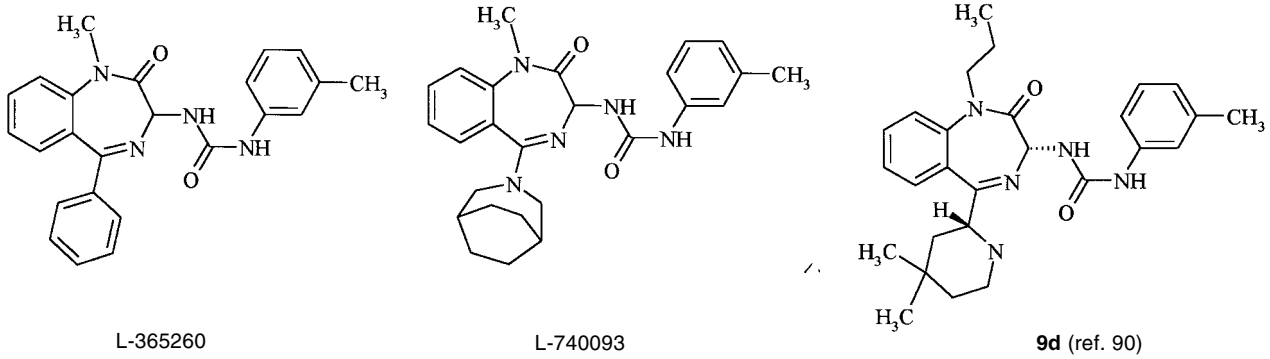
During the 1970s, the first putative gastrin antagonist, proglumide (Fig. 2A) was developed by Rotta and used as therapy for peptic ulcer (53, 54). Despite its low potency, proglumide has been the reference CCK antagonist compound for several years. To improve the potency and selectivity of proglumide, chemical modifications of its structure were investigated and led to a new series of CCK-A receptor antagonists whose representative, lorglumide, is a potent and selective CCK-A receptor antagonist (55). In order to obtain potent and specific CCK-B antagonists, a new series was designed conducting appropriate chemical manipulations of the structure of lorglumide. CR-2194 (spiroglumide; (*R)*-4-(3,5-dichlorobenzamido)-5-(8-azaspiro[4.5]decan-8-yl)-5-oxo pentanoic acid) (Fig. 2A) was the optimized CCK-B receptor antagonist in this series (56), showing a micromolar affinity for CCK-B receptor and discrete CCK-B over CCK-A selectivity (Table I). *In vivo* in anesthetized rats, spiroglumide antagonized pentagastrin-induced GAS with an ED_{50} of 11 mg/kg after i.v. administration, and was ineffective in antagonizing GAS induced by histamine or carbachol at doses 10 times higher than that required to antagonize the secretory response of pentagastrin. The antisecretory activity of spiroglumide did not significantly differ among species (ED_{50} = 11, 5.9 and 15.5 mg/kg in rats, dogs and cats, respectively). Moreover, in Heidenhain pouch dog, spiroglumide demonstrated good antisecretory activity as well as good absorption after oral administration (57). In humans, spiroglumide infused i.v. in the range of 1-7.5 mg/kg/h dose-dependently and competitively antagonized gastrin-stimulated GAS (58). In a phase II randomized, double-blind, placebo-controlled

Table I: CCK receptor binding affinities for glutamic acid derivatives.



Compound	R	IC_{50} (nM)[a]		
		CCK-B	CCK-A	A/B
Spiroglumide	-OH	1400	13500	9.6
CR-2622		20	7380	369

[a]IC_{50} represents the concentration (nM) producing half-maximal inhibition of specific binding of [^{3}H](N-Me-N-Leu) CCK-8 in the guinea pig cerebral cortex (CCK-B) or of [^{125}I]-CCK-8 in the rat pancreas (CCK-A).



L-365260 L-740093 **9d** (ref. 90)

Fig. 3. Structures of Merck selected benzodiazepine derivatives.

study in healthy volunteers, the effects of spiroglumide on meal sham feeding and meal-stimulated intragastric acidity were evaluated. Spiroglumide infused at a dose of 7.5 mg/kg/h decreased both basal and meal-stimulated GAS.

Despite its excellent oral bioavailability, the relatively low affinity and selectivity for CCK-B receptor precluded further development of spiroglumide as a potential therapeutic tool for peptic ulcer. Chemical manipulation of the structure of spiroglumide resulted in CR-2622, a potent (IC_{50} = 7 nM in inhibiting gastrin-induced [Ca^{2+}] cytosolic elevation in rabbit parietal cells) and selective (CCK-A/CCK-B = 369) CCK-B receptor antagonist (Table I). *In vivo* in anesthetized rats, CR-2622 antagonized pentagastrin-induced GAS with an ED_{50} of 2 mg/kg after i.v. administration (59). In spite of its potency and selectivity, CR-2622 showed poor oral absorption which limited its development.

2) Piperidines

During structure-activity exploration of CCK peptide analogs, a group from Abbott designed a series of compounds from which compound **1** (Fig. 2) showed good affinity (IC_{50} = 34 nM) but weak selectivity (7.5-fold) for CCK-B over CCK-A receptor. This compound exhibited weak anxiolytic-like activity in the elevated plus maze in mice when administered intraperitoneally (i.p.) over a range of 0.001-1 mg/kg (60).

Benzodiazepine derivatives

The isolation and identification of asperlicin as a selective CCK antagonist (61) led to the subsequent development of the selective and potent CCK-A antagonist, devazepide, and the CCK-B antagonist, L-365260, both with nanomolar affinity for CCK-A and CCK-B receptors, respectively (62, 63). Due to their potency, these compounds have been important pharmacological tools in elucidating the physiological role of CCK-A and CCK-B receptors.

L-365260 (Fig. 3) binds stereospecifically and with high affinity (IC_{50} = 8.5 nM) to CCK-B receptor, showing selectivity (about 100-fold) over the CCK-A receptor (Table II) (63); the affinity for the cloned human brain receptor is similar (IC_{50} = 3.8 nM) (64).

In vivo in pylori-ligated rats, L-365260 antagonized pentagastrin-induced GAS, with an ED_{50} of 0.86 mg/kg after oral administration (65). Moreover, high doses of L-365260 were required to inhibit both histamine-stimulated and basal acid secretion (ED_{50} = 12.6 mg/kg). In the same dose range, L-365260 was effective in attenuating gastrointestinal damage induced by acid-dependent mechanisms (aspirin- and cysteamine-induced ulcer) (66). The potency of L-365260 as a gastrin antagonist varied among species (mouse>rat>guinea pig>dog) and correlated with differences in oral bioavailability (mouse>rat>guinea pig).

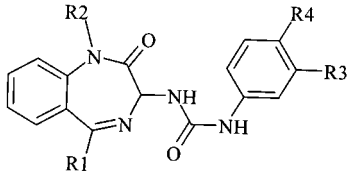
In a double-blind, crossover study, L-365260 at single oral doses of 2.5, 10 or 50 mg dose-dependently reduced pentagastrin-stimulated GAS in healthy volunteers (67). The dose of 50 mg induced a maximal inhibitory effect of 50% and was as effective as a 5 mg oral dose of famotidine.

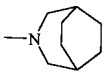
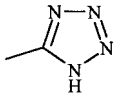
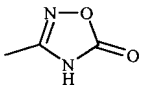
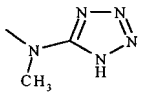
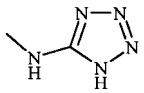
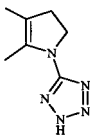
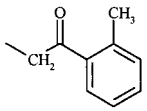
The anxiolytic-like effects of L-365260 in rodent models of anxiety are still under debate. Rataud (68) described an anxiolytic-like activity of L-365260 in mice at doses of 0.01-0.1 mg/kg. In contrast, Harro (69) and Dawson (70) found no effect in the rat elevated plus maze. This lack of activity could be due to poor bioavailability of the compound and as a consequence, insufficient occupation of central CCK-B receptors (71) to induce a robust anxiolytic effect.

In preliminary studies in humans, a single oral dose of L-365260 (50 mg/kg) blocked the anxiogenic effects of pentagastrin in healthy volunteers (72) and the panicogenic effect of CCK-4 in patients suffering from panic disorders (73). However, repeated dosing of L-365260 (30 mg q.i.d. x 6 weeks) in panic disorder patients did not induce any reduction in the frequency of panic attacks (74).

L-365260 has been evaluated for its ability to bind to CCK-B receptors expressed by gastrointestinal tumor

Table II: Benzodiazepine CCK-B antagonists: chemical structures and receptor binding affinities.



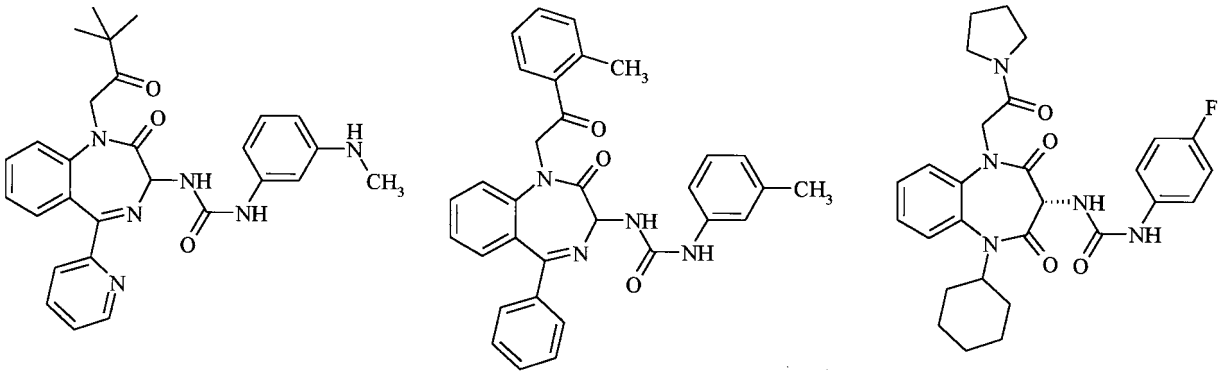
Compound	R^1	R^2	R^3	R^4	IC_{50} (nM)[a]	
					CCK-B	CCK-A
L-365260	Phenyl	$-CH_3$	$-CH_3$	-H	8.5	736
L-740093		$-CH_3$	$-CH_3$	-H	0.1	1604
L-368935	Phenyl	$-CH_2CH(CH_3)_2$		-H	0.14	1434
L-369466	Phenyl	$-CH_3$		-H	0.27	983
L-736380	Cyclohexyl	$-CH_3$		-H	0.05	400
L-737481	Cyclohexyl	$-CH_3$		$-CH_3$	0.07	802
L-738425	Phenyl	$-CH_3$			0.11	4080
YM-022	Phenyl		$-CH_3$	.H	0.11	150
YF-476	(2)-Pyridyl	$-CH_2COC(CH_3)_3$	$-NHCH_3$	-H	0.1[b]	502

[a]CCK-B binding was measured by displacement of [^{125}I]-CCK from guinea pig corticol membranes and CCK-A binding was measured by displacement of [^{125}I]-CCK from rat pancreatic tissue as described in references. [b]IC_{50} value for displacement of [^{125}I]-CCK-8 from rat brain.

cells. In a preliminary study (75) it was shown that L-365260 had no effect on the basal growth of AR4-2J cells, but reversed G17-stimulated growth both *in vitro* and *in vivo*. In agreement, in another study (76) L-365260 had no effect on the basal growth of human gastrointestinal cancer cell line AGS, but did inhibit gastrin-stimulated growth in a dose-dependent manner. This lack of effect on basal growth may be explained by the finding that L-365260 does not bind to the CCK-C/gastrin receptor purported to mediate the autocrine effect of gastrin. In a recent study (77) it was reported that L-365260 and devazepide reduced cell proliferation rate of the human colon cancer cell clone HT29-S-B6 dose-dependently and independent of the presence of exogenous gastrin or CCK. This effect seems to be independent of the CCK-A, CCK-B and CCK-C receptors.

Second generation benzodiazepines

The limited oral bioavailability of L-365260 demonstrated in animals and humans studies, probably due to its very low aqueous solubility, prompted a search for second generation CCK-B receptor antagonists. The initial strategy to increase water solubility was the inclusion of acidic solubilizing groups into the phenyl ring of the acylurea moiety of L-365260 (78). From this series, interesting biological activities were observed with a number of



YF-476
(Ferring Research; Yamanouchi)

YM-022
(Yamanouchi)

GR-199114X
(Glaxo Wellcome)

Fig. 4. Structures of selected benzodiazepine derivatives.

tetrazolyl urea derivatives. Two principal compounds emerged, L-368935, a tetrazol analog of L-365260 and the 1,2,4-oxadiazolone L-369446. Both compounds exhibited high affinity (CCK-B, IC_{50} = 0.1 nM and 0.27 nM, respectively) and selectivity (CCK-A/CCK-B = 10,000 and 3700, respectively) (Table II). However, both L-368935 and L-369466 demonstrated poor brain penetration, exhibiting an *ex vivo* binding inhibition (ED_{50}) of 5.6 and 6.5 mg/kg i.v., respectively; these values are similar to those obtained with L-365260 (13 mg/kg) (79). To improve this deficiency, another series of compounds was synthesized by incorporating an amino solubilizing group in the benzodiapin C5 substituent. Optimization of the benzodiazepine C5 substituents led to the azabicyclo [3.2.2]nonane derivative L-740093 (80).

L-740093 (Fig. 3) showed extremely high affinity for CCK-B receptors (IC_{50} = 0.1 nM), 90-fold higher than L-365260, and excellent receptor subtype selectivity (CCK-A/CCK-B = 16,000) (Table II). The aqueous solubility of L-740093 hydrochloride is 0.15 mg/ml. Results from *ex vivo* binding assays showed a 60-fold increase in potency compared with L-365260, demonstrating its excellent CNS penetration (71). *In vivo* in anesthetized rats, L-740093 antagonized pentagastrin-induced GAS with an ID_{50} of 0.01 mg/kg i.p., being 2-fold more potent than L-365260. Results from clinical trials with L-740093 are awaiting publication.

More recently, in order to increase brain penetration by modulating the pK_a of the acidic moiety, Merck scientists synthesized another series of benzodiazepine derivatives which incorporates a tetrazol-5-yl amino group into the phenyl ring of the acylurea moiety of L-365260. The indolinyltetrazole L-738425 and the cyclohexyl compounds L-736380 and L-737481 are the most prominent compounds of this series, showing excellent potency and high selectivity. In fact, L-738425 is the most selective (CCK-A/CCK-B = 37,000) CCK-B receptor antagonist thus far reported (81). *In vivo* in anesthetized rats, L-738425 inhibited pentagastrin-induced GAS with an ID_{50} of 0.064 mg/kg i.p. In spite of their potency and selectivity, the results from *ex vivo* binding studies showed ED_{50}s of 1.7 and 10 mg/kg for L-736380 and L-738425, respectively, indicating less brain penetration than L-365260.

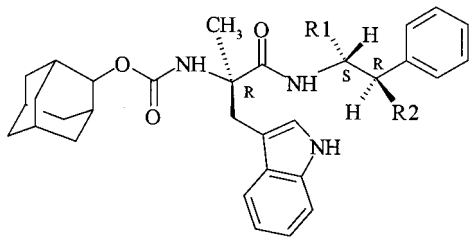
A novel series of 1-arylmethyl analogs of L-365260 was synthesized by Yamanouchi. YM-022 (Fig. 4) was the optimal compound of the series, eliciting very high CCK-B receptor affinity (IC_{50} = 0.11 nM) and good receptor subtype selectivity (CCK-A/CCK-B = 1300) (Table II) (82).

In vivo in anesthetized rats, YM-022 given i.v. inhibited pentagastrin-induced GAS with an ED_{50} of 0.0078 pmol/kg (about 0.04 mg/kg) and did not affect histamine- and bethanechol-induced acid secretion even at doses 1000-fold higher than the ED_{50} for inhibiting pentagastrin (83). Orally administered YM-022 dose-dependently inhibited basal GAS in pylorus-ligated rats, with an ED_{50} of 0.83 µmol/kg. Moreover, in rats YM-022 was as potent as famotidine in preventing gastric and duodenal ulcers induced by indomethacin and mepirizole (84).

In Heidenhain pouch dogs, YM-022 administered i.v. dose-dependently inhibited pentagastrin and peptone meal-induced acid secretion, with ED_{50}s of 0.0261 and 0.0654 µmol/kg, respectively, without affecting histamine- or metacholine-induced acid secretion at doses about 50 times higher than that required to inhibit pentagastrin (85). After 13 weeks of treatment, YM-022 increased plasma gastrin concentration similar to omeprazole, but prevented development of hyperresponse to pentagastrin and hyperplasia of gastric mucosa induced by omeprazole or famotidine (86). YM-022 may protect patients with peptic ulcers or esophagitis from hypergastrinemia caused by antisecretory agents. However, results from clinical trials have not yet been published.

The introduction of basic amino substituents into the 3-position of the aryl urea portion of the YM-022 series provided an improvement in selectivity for the CCK-B over CCK-A receptor. From this new series the compound YF-476 emerged, a 3-methylamino substituent having excellent affinity for CCK-B receptors (0.1 nM) and selec-

Table III: CCK receptor binding affinities for dipeptoid derivatives (ref. 92).



Compound	R^1	R^2	IC_{50} (nM)[a] CCK-B	IC_{50} (nM)[a] CCK-A	A/B
11a	H	H	32	650	20
21e	$-CH_2NHCO(CH_2)_2COOH$	H	4.2	950	230
18	$-CH_2NHCOCH=CHCOOH$	H	0.8	440	550
29d	H	$-NHCO(CH_2)_2COOH$	1.7	4300	2500
27	H	-NHCOCH=CHCOOH	0.7	790	1100
CCK-8			0.3	0.1	0.33
Pentagastrin			0.8	600	750

[a]IC_{50} represents the concentration (nM) producing half-maximal inhibition of specific binding of [^{125}I]-CCK-8 to CCK receptor in the mouse cerebral cortex (CCK-B) or in the rat pancreas (CCK-A).

tivity (CCK-A/CCK-B = 5020) (Table II) (87). YF-476 (Fig. 4) inhibited the binding of [^{125}I]-CCK-8 to cloned human CCK-B receptors with a K_i value of 0.62 nM, resulting in a 45-fold higher affinity than that of L-365260 and 4-fold lower than that of YM-022. *In vivo* in anesthetized rats, YF-476 administered i.v. inhibited pentagastrin-induced GAS with an ED_{50} of 0.0086 µmol/kg. This effect was about 15-fold more potent than that of famotidine. However, YF-476 did not affect histamine- or bethanechol-induced GAS. In Heidenhain pouch dogs, YF-476 strongly inhibited pentagastrin-induced GAS with ED_{50}s of 0.018 and 0.020 µmol/kg after i.v. or oral administration, respectively (88).

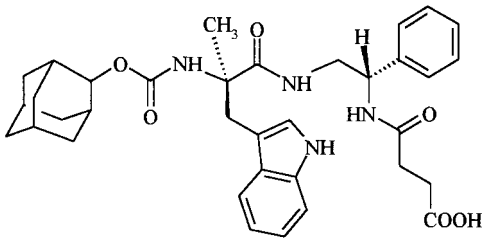
Because of its potency and good oral bioavailability, YF-476 was selected for further development and is currently under clinical investigation for the treatment of gastroesophageal reflux disease.

Starting from L-365260, Glaxo investigators synthesized several 1,5-benzodiazepindione derivatives. The saturation of the N-5 phenyl ring resulted in the cyclohexyl substituted compound GR-199114X (Fig. 4), which showed an increase in both potency and selectivity for CCK-B receptor in comparison with L-365260. Moreover, GR-199114X exhibited good bioavailability after oral administration (89).

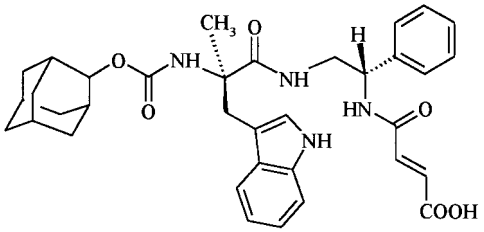
A novel series of CCK-B receptor antagonists was recently designed by scientists at Merck by incorporating a piperidin-2-yl or homopiperidin-2-yl group attached to C5 of a benzodiazepin core structure of L-365260. Compounds to emerge from this series, such as **9d** (Fig. 3), showed high affinity for the CCK-B receptor (IC_{50} = 1.5 nM) and very good selectivity over CCK-A receptor (CCK-A/CCK-B = 2060) (90).

Dipeptoids

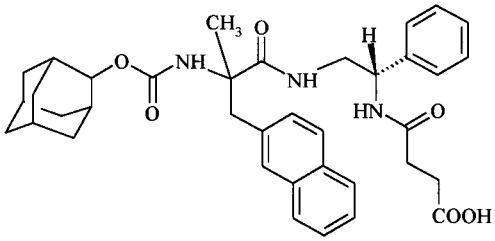
Amino acid deletion studies on CCK 30-33 have shown that the tryptophan and the phenylalanine residues of the CCK tetrapeptide were both necessary for the retention of micromolar binding affinity to CCK-B receptors. On this basis, the core molecule of the dipeptoid series was derived (91). The overall strategy for the development of the dipeptoids was to explore independently the SAR of the N- and C-terminal sites of the core molecule. A systematic SAR of the N-terminus revealed that bulky groups were preferred and optimization structure was reached with a 2-adamantyloxy-carbonyl group (**11a**, Table III) that showed a good CCK-B receptor binding affinity (IC_{50} = 32 nM), but weak selectivity for CCK-B receptor (CCK-A/CCK-B = 20). The strategy to improve the affinity and selectivity for CCK-B receptor was to explore the C-terminus of compound **11a**, leading to a series of highly selective CCK-B receptor antagonists (**21e**, **18**, **29d**, **27**, Table III) (92). Compounds **29d**, CI-988 (formerly PD-134308) and **27**, CAM-1189 (formerly PD-136450) (Fig. 5) showed nanomolar affinity (IC_{50} =1.7 and 0.7 nM, respectively) and good selectivity for CCK-B receptor (CCK-A/CCK-B = 2500 and 1100, respectively) (93). *In vivo* in anesthetized rats, CI-988 inhibited pentagastrin-induced GAS with an ED_{50} of 0.25 µmol/kg after subcutaneous (s.c.) administration, and its potency was comparable to that of the histamine H_2 antagonist, ranitidine. CI-988, at doses 100 times higher than its ED_{50} against pentagastrin, had no effect on basal acid secretion and did not inhibit the secretory response to histamine and bethanechol (94). *In vivo* in animal models for anxiety (mouse black and white test, rat elevated plus



CI-988
(Parke-Davis)



PD-136450
(Parke-Davis)



2
(Glaxo Wellcome)

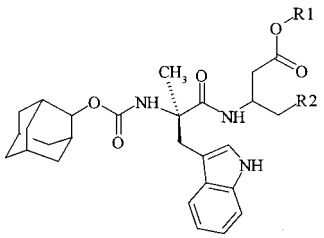
Fig. 5. Selected dipeptoid derivatives as CCK-B receptor antagonists.

maze and marmoset human threat test), CI-988 showed an anxiolytic profile at doses of 0.001-1 mg/kg after both oral and s.c. administration (95). In a comparative study with benzodiazepines, CI-988 was 50-fold more potent than chlorodiazepoxide (96) and as active as diazepam (97) without possessing sedative, ataxic or anticonvulsant actions at doses up to 3000-fold higher than those producing anxiolysis. On the contrary, in rat (96) and mouse (98) shock-motivated tests, where electric shock is used as an anxiogenic stimulus, and in a validated simulation of panic anxiety in rats (99), CI-988 failed to show any anxiolytic effect.

Consistent with these observations, recent studies have reported very modest effects of a high oral dose of CI-988 on CCK-4-induced panic symptoms in healthy volunteers (100) and inactivity on CCK-4-induced symptoms in panic disorder patients (101). Moreover, in a double-blind, placebo-controlled study, CI-988 administered for 4 weeks (300 mg/day) to patients with generalized anxiety disorders did not demonstrate anxiolytic effects (102). The controversial lack of marked effects observed following peripheral administration of CI-988 to rats is likely to reflect insufficient brain penetration of this molecule. Indeed, *ex vivo* binding experiments have shown that CI-988 has only weak central activity due to poor brain penetration in rodents (79). Therefore, the limited efficacy and poor absorption (100) in human trials, and a potential CCK-A agonist effect of CI-988 make it an unlikely candidate for further development.

Continued SAR efforts led to the discovery of a higher affinity compound (**1**, Table IV, IC_{50} = 0.15 nM), but less selective (CCK-A/CCK-B = 170) dipeptoid analog of CI-988 (103). Therefore, a series of compounds was synthesized in which the [(2-adamantyloxy)carbonyl]-α-methyl-(*R*)-tryptophan moiety of CI-988 was kept constant and the phenyl ring (R^2) of **1** was varied. These modifications led to the identification of a number of dipeptoids with high affinity and increased selectivity for binding to the CCK-B receptor. Compound **19** (Table IV) was the optimal compound of this series, exhibiting an extraordinarily high affinity (IC_{50} = 0.08 nM) and good selectivity (CCK-A/CCK-B = 900) (104).

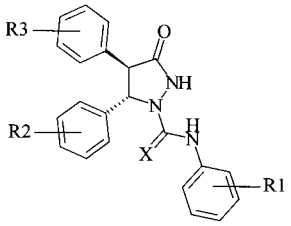
Table IV: CCK receptor binding affinities for C-terminal analogs of CI-988.



Compound	R^1	R^2	IC_{50} (nM)[a] CCK-B	CCK-A	A/B
1	H	Ph	0.15	22.5	150
19	H	Ph-4-F	0.08	75	930

[a]IC_{50} represents the concentration (nM) producing half-maximal inhibition of specific binding of [^{125}I]-CCK-8 to CCK receptor in the mouse cerebral cortex (CCK-B) or in the rat pancreas (CCK-A).

Table V: CCK receptor binding affinities for diphenylpyrazolidinone compounds.



Compound	X	R^1	R^2	R^3	IC_{50} (nM)[a] CCK-B	IC_{50} (nM)[a] CCK-A	A/B
LY-262684	O	4-Br	2-Cl	2-Cl	6	7900	1317
LY-191009	O	4-CF_3	2-Cl	H	10	>10000	>1000
LY-262691	O	4-Br	H	H	31	11600	374
LY-288513[b]	O	4-Br	H	H	16	>30000	>1870
LY-242040	O	4-CF_3	H	H	44	10600	240

[a]IC_{50} represents the concentration (nM) producing half-maximal inhibition of specific binding of [^{125}I]-CCK-8 to CCK receptor in the mouse cerebral cortex (CCK-B) or in the rat pancreas (CK-A). [b](+) optical isomer of LY-262691.

An important structural feature of CI-988 is the alpha-methyl-substituted Trp residue, considered essential for receptor affinity. The objective of the Glaxo investigators was to determine whether the indole moiety of the Trp residue could be replaced by a variety of aromatic rings. The best results were obtained with the naphthalene derivative **2** (Fig. 5), which retained similar affinity but improved selectivity for the CCK-B receptor in comparison with the parent compound CI-988 (105).

Pyrazolidinones

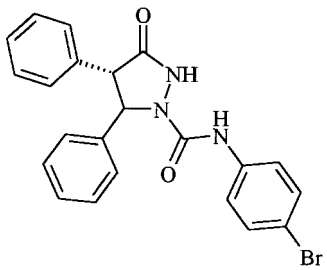
Through a broad screening and structure optimization at the CCK-B receptor, investigators at Lilly identified in the pyrazolidinone structure a valuable moiety for obtaining new chemical entities with promising CCK-B receptor antagonist activity. From this series, the most potent and selective compounds are shown in Table V. LY-288513 (Fig. 6), the active isomer of LY-262691, showed high selectivity for CCK-B (IC_{50} = 16 nM) over CCK-A receptor (IC_{50} >30,000) (106).

LY-288513 in rat elevated plus maze, at doses of 10 and 30 mg/kg (i.p. and oral, respectively) showed anxiolytic-like activity comparable to diazepam, but without side effects (107).

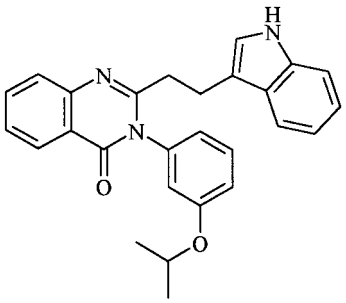
Moreover, LY-288513 seems to block the anxiogenic effects of diazepam withdrawal in auditory startle response test in rats (108).

Quinazolinones

Another series of CCK-B antagonists was designed at Lilly starting from the asperlicin molecule, yielding a 3-phenyl-4(3H)-quinazolinone nucleus, from which LY-247348 emerged (Fig. 6), exhibiting discrete CCK-B receptor affinity (IC_{50} = 32 nM) and selectivity (CCK-A/CCK-B >300) (109). LY-247348 showed anxiolytic-like activity on punished responding in squirrel monkeys (110).



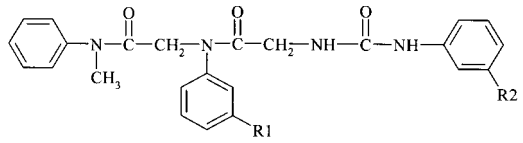
LY-288513



LY-247348

Fig. 6. Structures of Lilly's selected nonpeptide CCK-B antagonists.

Table VI: CCK receptor binding affinities for ureidoacetamides.



Compound	R^1	R^2	K_i (nM)[a] CCK-B	CCK-A	A/B
RP-69758	H	$-CH_2COOH$	9.0	1254	139
RP-72540	$-OCH_3$	$-CH(CH_3)-COOH$	2.4	2338	982
RP-73870	$-OCH_3$	$-CH(CH_3)-SO_3K$	0.48	1634	3404

[a]CCK-B binding was measured by displacement of [^{125}]-CCK-8 from guinea pig cortical membranes and CCK-A binding was measured by displacement of [^{3}H]-CCK-8 from guinea pig pancreatic membranes.

Acute administration of diphenylpyrazolidinones such as LY-262691 or quinazolinones such as LY-247348 dose-dependently decreased midbrain dopamine unit activity (111), indicating that CCK-B antagonists may represent a novel class of antipsychotic drugs with the potential for therapeutic effects in schizophrenic patients without a delayed onset of action. However, development of LY-288513 was discontinued due to adverse findings in preclinical toxicology, and results from clinical trials with LY-247348 have not yet been published (112).

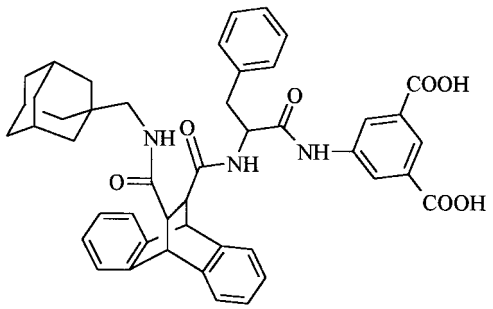
Ureidoacetamides

Rhône-Poulenc developed a series of nonpeptide CCK-B receptor antagonists with ureidoacetamide structure. Chemical structure, potency and selectivity are shown in Table VI, where the most representative molecules of this series are reported. RP-69758 and RP-72540 showed nanomolar affinity for CCK-B receptors (IC_{50} = 9 nM and 2.4 nM, respectively) and good selectivity (CCK-A/CCK-B = 139 and 982, respectively). *In vivo* in anesthetized rats, RP-69758 administered i.v. inhibited pentagastrin-induced GAS with an ED_{50} of 1.14 µmol/kg (113). Substitution of the acetic acid moiety in R2 (Table VI) with the ethyl sulfonate group led to RP-73870, a very potent (IC_{50} = 0.48 nM) and selective (CCK-A/CCK-B = 3404) compound. RP-73870 administered i.v. to anesthetized rats antagonized pentagastrin-stimulated GAS with an ED_{50} of 0.05 mg/kg. RP-73870 at a dose sufficient to completely block pentagastrin-stimulated secretion (0.3 mg/kg i.v.) was without effect on histamine-stimulated GAS. The compound also inhibited basal GAS in gastric fistula rats, with an ED_{50} of 25 mg/kg after oral administration. RP-73870 prevented acid-dependent gastric and duodenal damage in rats after both oral and i.v. administration, being as potent as other standard antiulcer compounds such as the histamine H_2 receptor antagonist famotidine or the proton pump inhibitor omeprazole (114).

Dibenzobicyclo[2.2.2]octane and bicyclic heteroaromatic derivatives

The James Black Foundation's approach to the search for CCK-B receptor antagonists was to design rigid structures that could be used to replace the peptide backbone of tetragastrin. These studies led to the discovery of a new series of potent and selective CCK-B receptor antagonists based on the dibenzobicyclo[2.2.2]octane (BCO) skeleton, which satisfied the stereoelectronic requirements for the putative pharmacophore of tetragastrin. From this series one compound emerged (**3**, Fig. 7), with submicromolar affinity for CCK-B receptor (pK_i = 8.8 in mouse cortical membranes) and at least 30-fold selectivity for CCK-B over CCK-A receptors (115, 116). Compounds from this series were found to show species-related behavior when examined in rat and dog models. In fact, when compound **3** was given i.v. at a dose of 0.025 µmol/kg in anesthetized rats, there was a 79% reduction in pentagastrin-stimulated GAS, but in gastric fistula dogs it was at least 700-fold less potent.

The design of molecules in which the BCO skeleton was replaced by bicyclic heteroaromatic frameworks led to compounds that maintained the activity and selectivity profile of **3**. The 5,6-disubstituted indole derivative (**4**, Fig. 7) emerged from this series (117). Compound **4** still maintained the excellent *in vitro* properties of compound **3** but



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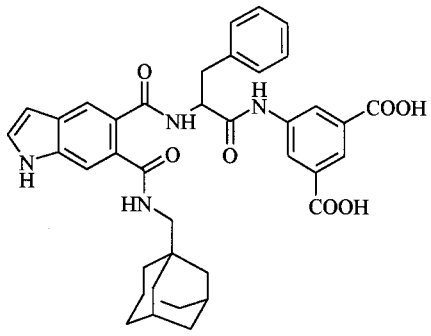

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Fig. 7. Structures of J.B. Foundations's selected dibenzobicyclo[2.2.2]octane (**3**) and bicyclic heteroaromatic (**4**) CCK-B antagonists.

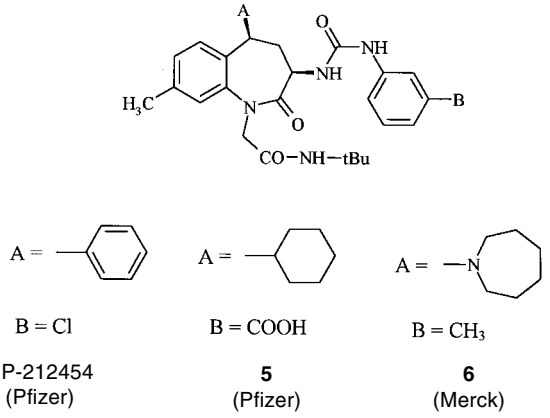

Fig. 8. Structures of selected ureidobenzazepine CCK-B receptor antagonists.

in vivo exhibited comparable activity in rat and dog assays: a dose of 0.025 µmol/kg reduced pentagastrin-induced GAS by 97% in anesthetized rats. Results of oral activities of these compounds have not been published.

Ureidobenzazepines

Modification of the benzodiazepine nucleus of L-365260 to a 3-ureido benzazepin-2-one, obtained by a group from Pfizer, was reported to afford relatively less potent and nonselective CCK-B receptor antagonism (118). Incorporation of a 5-phenyl ring into a series of ureido benzazepin-2-one structures led to compounds with potent and selective affinity for the CCK-B receptor. In particular, CP-212454 (Fig. 8) showed excellent affinity (IC_{50} = 0.48 nM) and selectivity (CCK-A/CCK-B = 370) for CCK-B receptor. *In vivo* in anesthetized rats, CP-212454 administered s.c. antagonized pentagastrin-induced GAS with an ED_{50} of 0.8 mg/kg. To increase the poor water solubility of this series, new molecules were synthesized incorporating a carboxylic acid, as well as other ionizable groups, into the 5-substituted 3-ureido benzazepin-2-one compounds, providing potent, selective and water-soluble CCK-B receptor antagonists. Compound **5** (Fig. 8), showed increased solubility (3 mg/ml compared to 0.0002 mg/ml for CP-212454), affinity for the CCK-B receptor and *in vivo* efficacy. Compound **5** showed nanomolar affinity for CCK-B receptor (IC_{50} = 0.1 nM) and increased selectivity with respect to CP-212454 (CCK-A/CCK-B = 14,000). *In vivo* in anesthetized rats, compound **5** antagonized pentagastrin-induced GAS with an ED_{50} of 0.03 mg/kg after s.c. administration (119).

Scientists from Merck synthesized a new series of benzazepines resulting from the incorporation of homopiperidine at the 5-position of the benzazepine, leading to compound **6** (Fig. 8), which showed good affinity (IC_{50} = 15.7 nM) but low selectivity for CCK-B over CCK-A receptors (120).

Miscellaneous

1) Anthranilic acid derivatives

CR-2945 (Fig. 9), an anthranilic acid derivative, was the result of a new series of compounds synthesized in Rotta with the objective of improving the oral bioavailability of CR-2622. CR-2945 showed excellent affinity (IC_{50} = 2.3 nM) and selectivity (CCK-A/CCK-B = 9000) for CCK-B receptor (121). *In vivo*, it antagonized pentagastrin-induced GAS with ED_{50}s of 1.3 and 3.2 mg/kg after i.v. and intraduodenal administration, respectively. CR-2945 was ineffective in antagonizing histamine-and carbachol-stimulated GAS up to doses 30 to 60 times higher than that required to antagonize pentagastrin stimulation. CR-2945 was also effective in the prevention of gastric and duodenal damage in several models (121). These results are encouraging for further development of the compound.

2) Dual H_2 and CCK-B receptor antagonists

With the objective of alleviating the relapse problem frequently encountered with H_2 receptor antagonist thera-

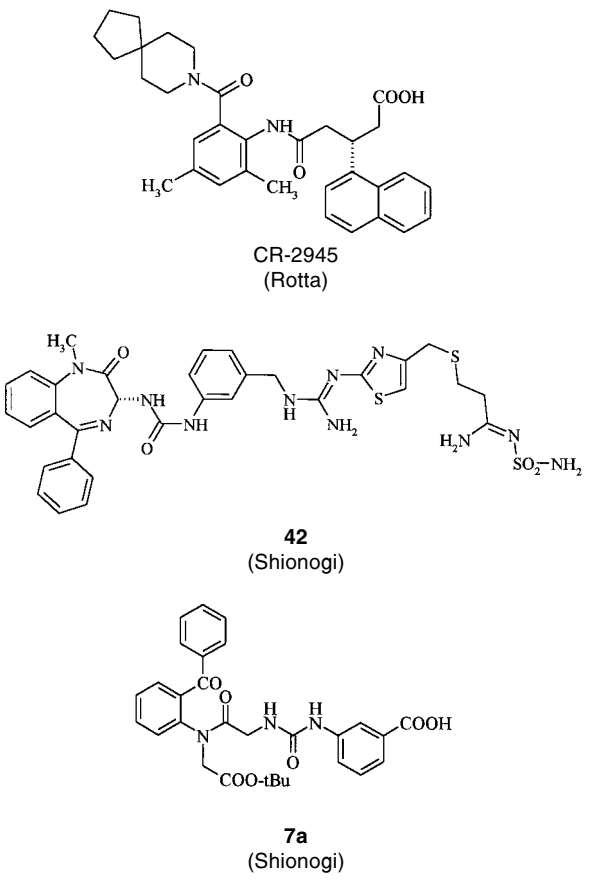

Fig. 9. Structures of miscellaneous selected nonpeptide CCK-B antagonists (refs. 123, 124).

py, investigators at Shionogi designed hybrid molecules with dual histamine H_2 and CCK-B receptor antagonism. These compounds were constructed from two basic pharmacophore moieties selected from H_2 antagonists (roxatidine and famotidine) and CCK-B antagonists (L-365260); the two pharmacophores were connected with a spacer. Compounds emerging from the roxatidine series exhibited only submicromolar affinity for CCK-B receptor and poor selectivity for CCK-B over CCK-A receptor (122). Moreover, the high molecular weight showed that improvement of the oral absorption of these hybrid molecules would be essential for their development as antiulcer agents. From the last published series, compound **42** (Fig. 9) which bears the famotidine moiety reversely connected to L-365260 at the guanidino group without a spacer, maintained a nanomolar affinity (IC_{50} = 38 nM) for CCK-B receptor and good H_2 receptor antagonist activity (pA_2 = 6.1). The reduced molecular weight improved oral absorption (123). Results from *in vivo* studies, however, were not completely satisfactory and other studies are still being conducted.

3) Ureidomethylcarbamoylphenylketone derivatives

Researchers at Shionogi have just published the results obtained from a new series of derivatives, with a ureido methylcarbamoyl phenylketone skeleton, exhibiting potent and selective CCK-B receptor antagonist activity. S-0509 (**7a**, Fig. 9) emerged from this series, having nanomolar affinity for CCK-B (IC_{50} = 23.5 nM) and good selectivity for CCK-B over CCK-A receptor (124). *In vivo* in anesthetized rats, S-0509 inhibited pentagastrin-induced GAS with an ED_{50} of 0.014 mg/kg after i.d. administration. For its chemical structure, compound S-0509 showed low blood-brain barrier permeability, suggesting *in vivo* selectivity for the peripheral over the central CCK-B receptor.

Conclusions

This article has reviewed recent progress in the identification of the major chemical classes of nonpeptide CCK-B gastrin receptor antagonists. As described above, CCK is implicated in a series of pathologies for which the CCK-B receptor antagonists can be used as promising alternatives to currently available therapeutic agents.

Various classes of CCK-B receptor antagonists have been shown to inhibit gastric acid secretion in animals. Therefore, these compounds may provide a new interesting approach for the treatment of gastric and duodenal ulcers and possibly in gastrin-sensitive malignancies. However, only a few CCK-B receptor antagonists have been evaluated in clinical trials and the results obtained thus far are not very encouraging. For instance, spiroglumide and L-365260 seem to have weak antisecretory activity when compared to histamine H_2 antagonists. These results may be explained by the relatively low potency of spiroglumide and the poor bioavailability of L-365260.

There is evidence that CCK-B receptor antagonists may have antiproliferative activity in gastrin-sensitive tumors but the therapeutic use of these compounds remains unclear until the tumor-specific receptors have been identified and characterized. Therefore, the therapeutic potential of these compounds in the gut will only be fully investigated when second-generation CCK-B antagonists are available to evaluate their effects on acid-related disease in controlled clinical trials.

Another interesting possible therapeutic indication for CCK-B gastrin receptor antagonists may be the treatment of generalized anxiety disorders (GAD). Animal studies have shown that CCK-B gastrin receptor antagonists can block the anxiogenic response induced by CCK-4 and exhibit anxiolytic properties.

As described above, results from phase I clinical trials showed that CI-988 attenuated CCK-4 induced panic attack in healthy volunteers (100) and L-365260 antagonized the panicogenic effects of CCK-4 in patients with panic disorders (73). On the contrary, the results of another phase I clinical trial (101) suggest that CI-988 in doses up to 100 mg was not effective in reducing symptoms of panic anxiety induced by CCK-4. Moreover, in a double-blind, placebo-controlled study in GAD patients, CI-988 (300 mg/day, twice daily for 4 weeks) did not demonstrate any anxiolytic effect superior to placebo (102). The possible reasons for this lack of effect are not clear, but the poor bioavailability of these compounds seems to be the most plausible explanation. Further studies with the new generation CCK-B/gastrin antagonists will probably answer some of the questions concerning the relevance of these compounds in the treatment of GAD.

Results from preclinical studies in midbrain dopamine neurons suggest that CCK-B antagonists may represent a novel class of antipsychotic drugs, with potential therapeutic effect in schizophrenia. Confirmation of the predicted antipsychotic activity in man awaits results from clinical trials that have not yet been published.

In conclusion, the first generation CCK-B receptor antagonists seem to have limitations as potential therapeutic tools; the novel potent and selective CCK-B receptor antagonists, with improved pharmaceutical properties, will facilitate studies on the therapeutic utility of this class of compounds in man. Only future clinical trials can demonstrate the validity of these compounds as therapeutic agents.

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