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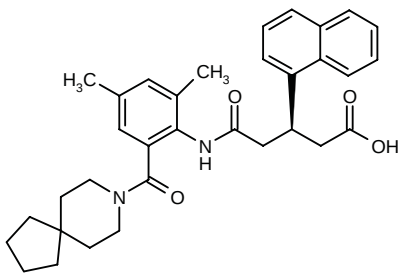
CR-2945

CCK_2 Receptor Antagonist

(*R*)-*N*-[2-(8-Azaspiro[4.5]decan-8-ylcarbonyl)-4,6-dimethylphenyl]-3-(1-naphthyl)glutaramic acid



$C_{33}H_{38}N_2O_4$ Mol wt: 526.6732

CAS: 201605-51-8

EN: 261157

Synthesis*

The condensation of the amine (I) with the chloride of 3(*R*)-(1-naphthyl)glutaric acid monomethyl ester (II) by means of triethylamine in hot toluene gives the glutaramic ester (III), which is finally hydrolized with NaOH in methanol/methylene chloride/water (1). Scheme 1

The intermediates (I) and (II) have been obtained as follows:

1) Intermediate (I) is obtained by condensation of 3,5-dimethylisatoic acid anhydride (IV) with 8-azabicyclo-[4.5]decane (V) by means of triethylamine in refluxing toluene.

2) Intermediate (II) is obtained by reaction of 3-(1-naphthyl)glutaric anhydride (VI) with methanol, yielding the racemic monomethyl ester, which without isolation is submitted to optical resolution with cinchonine, thus obtaining the 3(*R*)-monoester (VII). Finally, this compound is treated with $SOCl_2$ in refluxing ethyl ether.

Description

Crystals, m.p. 182 °C, $[\alpha]_D^{21}$ +31.5° (MeOH/$CHCl_3$ 75/25).

Introduction

The peptide hormone and neurotransmitter cholecystokinin (CCK) is widely distributed throughout the gastrointestinal tract and central nervous system, where it is involved in the regulation of various biological functions (2). The action of CCK is mediated by two distinct receptor subtypes: CCK_1 and CCK_2, identified by pharmacological action and molecular cloning. CCK_1 receptors are found in peripheral tissues such as gallbladder, pancreas and ileum, as well as in discrete brain areas (3, 4). CCK_2 receptors are present throughout the brain (5, 6) and are also found in the stomach, where they are indistinguishable from the gastrin receptors as demonstrated by the analysis of genomic DNA (7). The biological roles of the peripheral CCK_1 receptors are well characterized and include gallbladder contraction, enzyme secretion and gut motility (8). The peripheral CCK_2 receptors principally mediate the stimulation of gastric acid secretion and regulate gastric mucosal hypertrophia (9). In contrast, the exact function of the central CCK receptors is not yet fully understood, although there is experimental evidence suggesting that these receptors can mediate anxiety, panic attacks (10), satiety (11) and perception of pain (12), as well as having a possible role in the pathogenesis of dopaminergic-related movements and behavioral disorders such as Parkinson's disease, Huntington disease and schizophrenia (13).

Selective antagonists have been developed which discriminate between the two receptor subtypes and have been widely used in pharmacological studies on the functional significance of the CCK receptor subtypes. In the last decade several pharmaceutical companies have focused their research on the synthesis of potent and selective nonpeptide CCK_2 receptor antagonists. Several chemical structures have been synthesized, including dipeptoid, benzodiazepine, pyrazolidine, quinazolinone, ureidoacetamide, ureidobenzazepine and amino acid derivatives (14, 15). The CCK receptor antagonists have been studied in a variety of therapeutic areas related to brain dysfunction such as anxiety, schizophrenia and perception of pain, as well as gastrin-related pathologies

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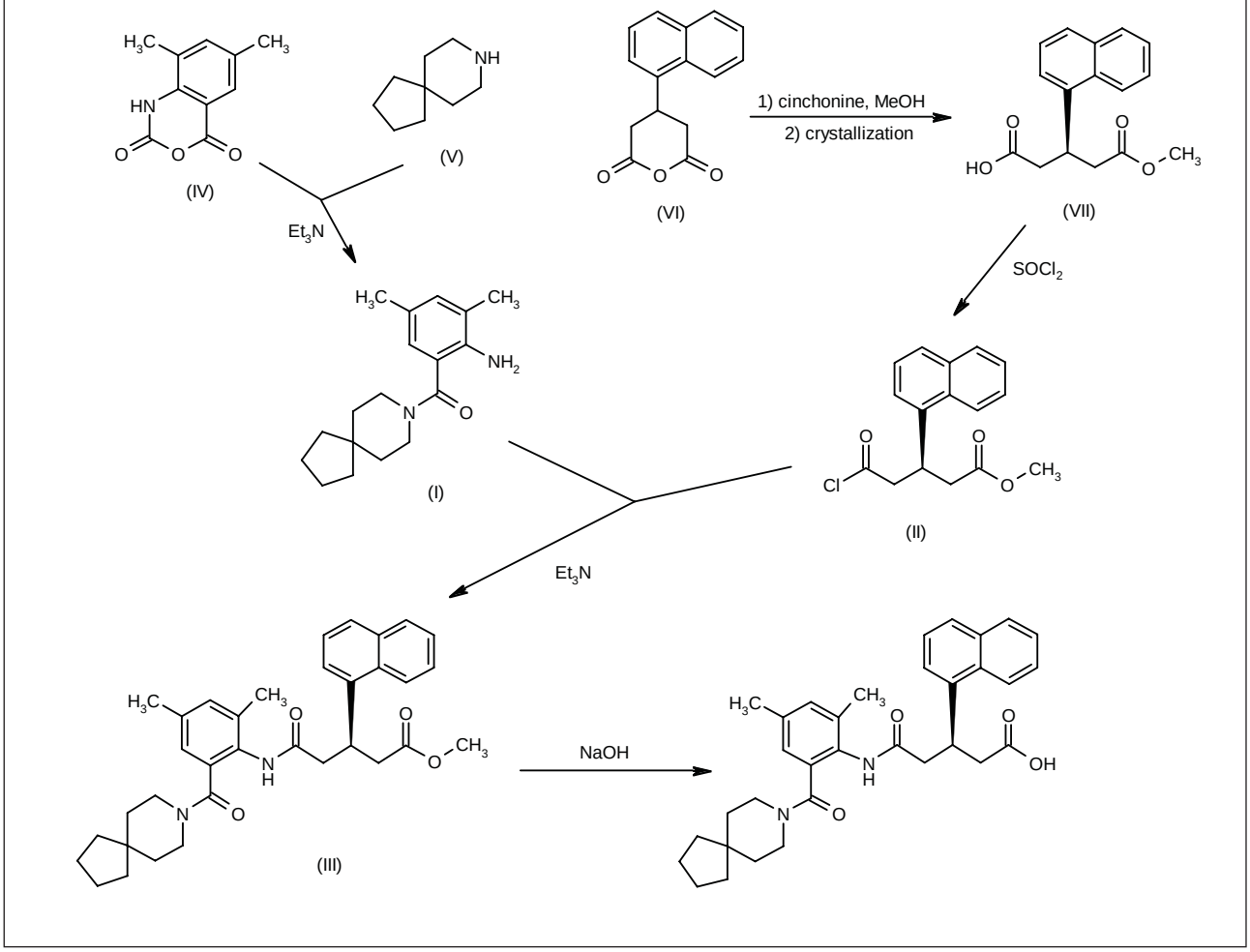
Scheme 1: Synthesis of CR-2945



such as gastric acid hypersecretion, gastric and duodenal ulcer, gastric hyperplasia and gastrointestinal malignancy. The search for potent and highly selective CCK_2 receptor antagonists led Rotta to synthesize a novel series of compounds which culminated in the discovery of the anthranilic acid derivative CR-2945, which demonstrated potent and highly selective activity *in vivo* (16).

Pharmacological Actions

CR-2945 exhibited *in vitro* nanomolar affinity for CCK_2 receptors, displacing [^{3}H]-pBC264 binding from rat cortex with a K_i of 2.3 nM. Further studies comparing the binding affinity of CR-2945 for rat brain CCK_2 receptors with pancreatic CCK_1 receptors revealed a 9000-fold selectivity for the CCK_2 receptor subtype (Table I) (17). *In vitro* functional studies have demonstrated the antagonistic activity of CR-2945. In enriched rabbit parietal cells preparation, the compound antagonized gastrin-induced cytosolic Ca^{2+} elevation with an IC_{50} of 5.9 nM. In contrast, in isolated guinea pig gallbladder, CR-2945 weakly antagonized CCK-8-induced contractions (CCK_1 receptor-mediated) only at micromolar concentrations, indicating a strong selectivity for CCK_2 receptor subtypes (18).

CR-2945 was shown to have *in vivo* efficacy in gastrointestinal area, with antisecretive and antiulcer activity, as well as in the central nervous system where it exhibited anxiolytic-like activity in a number of behavioral paradigms in rats and mice.

CR-2945 antagonized gastric acid secretion induced by pentagastrin in anesthetized (*in situ* perfused stomach) and conscious (implanted with chronic gastric fistula) rats, as well as in conscious cats with chronic gastric fistula. In these models, the compound exhibited similar efficacy, with ED_{50}s of 1.3, 0.8 and 1.64 mg/kg i.v. in anesthetized rats, conscious rats and conscious cats, respectively. In the anesthetized rat, CR-2945 exhibited good absorption after intraduodenal administration, as demonstrated by an ED_{50} of 2.7 mg/kg (Table II) (19). In

Table I: Affinities of CR-2945, CCK-8 and selected CCK_2 antagonists for CCK receptors (from Prous Science MFLine database).

Compound	CCK_2 Rat cerebral cortex[1] K_i (nM)	CCK_1 Rat pancreas[2] K_i (nM)	Relative CCK_2 selectivity CCK_1/CCK_2	Ref.
CCK-8	2.4	0.5	0.2	17
CR-2945	2.3	20,700	9000	17
L-365260	8.0	1245	155	17
PD-135158	4.7	3970	850	17
RP-73870	0.48	1634[4]	3404	20
YM-022	0.11[3]	75	682	21-23

[1]Displacement of [^{3}H]-pBC264; [2]displacement of [^{125}I]-BH-CCK-8; [3]IC_{50} in rat cerebral cortex, displacement of [^{125}I]-CCK-8; [4]guinea pig pancreas.

Table II: Effects of CR-2945 and selected CCK_2 receptor antagonists on pentagastrin-stimulated gastric acid secretion in rat (from Prous Science MFLine database).

Compound	ED_{50} (mg/kg i.v.)	ED_{50} (mg/kg i.d.)	Ref.
CR-2945	1.3	2.7	18
L-365260	0.5-2.07	0.1->10	18, 20, 24
RP-73870	0.05		20
YM-022	4.0[1]	0.1	23, 25
Ranitidine	0.3	8.3	18
Omeprazole	0.6	5.9	18

[1]μg/kg

in situ perfused rat stomach, CR-2945 exhibited properties consistent with competitive antagonism, producing a rightward shift in the curve of pentagastrin with no reduction in the maximum response to agonist; the pA_2 calculated from Schild-plot analysis was 7.33 (18).

Separate studies have revealed the reversibility of this antagonism. In gastric fistula rats during concomitant infusion with CR-2945 and pentagastrin, gastric acid secretion was inhibited by CR-2945, but after stopping CR-2945 infusion, gastric acid secretion increased to reach normal values with a dose-related delay. CR-2945 did not antagonize gastric acid secretion stimulated by histamine or carbachol at doses of about 20-fold higher than its antigastrin ED_{50}. CR-2945 inhibited basal gastric acid secretion in conscious rats implanted with a chronic gastric fistula, but only at doses of about 3-fold higher than those able to antagonize pentagastrin-induced gastric acid secretion: a dose of 10 mg/kg i.v. significantly reduced basal gastric acid secretion by approximately 50% (18).

CR-2945 was effective in preventing gastric and duodenal lesions in both acid-dependent and acid-independent damage models in rats. Gastric and duodenal ulcers induced by both acetylsalicylic acid (or indomethacin) and cysteamine were associated with a loss of mucosal barrier and consequently an increased sensitivity to luminal acid (19). The protection that CR-2945 exerted on gastric or duodenal mucosa largely depended upon its inhibition of acid secretion. CR-2945 at doses of 3 and 10 mg/kg s.c. significantly inhibited the formation of gastric lesions induced by indomethacin; total remission of gastric ulcer was achieved with the 10-mg dose. The compound was also efficacious in the prevention of duodenal ulcer induced by cysteamine (ED_{50} = 8.8 mg/kg). CR-2945 also protected against non acid-dependent ethanol-induced gastric mucosal damage, with an ED_{50} of 7.6 mg/kg after i.v. administration (Table III) (18).

CR-2945 showed good penetration in the brain as demonstrated by its ability to inhibit [^{125}I]-BH-CCK-8 binding in rat cerebral cortex after systemic administration; the calculated ED_{50}s were 10.9 mg/kg i.v. and 13.5 mg/kg s.c. (Table IV). CR-2945 crossed the blood-cerebrospinal fluid barrier very rapidly, reaching a maximum concentration at 15 min after treatment. At this time, the ratio between cerebrospinal fluid and plasma concentration was approximately 0.5% (17). Taken together these results indicate the ability of CR-2945 to penetrate into the brain, a property consistent with the anxiolytic-like activity exhibited by the compound in behavioral experiments in rats and mice.

CR-2945 displayed potent anxiolytic activity in rodent paradigms that depend upon naturally aversive stimuli, such as permanence in an elevated and unprotected arm in elevated plus maze and zero maze tests in rats and intense light in the light/dark box in mice. In both the elevated plus maze and zero maze in rats, CR-2945 showed good anxiolytic activity at a dose-range of 0.1-10 mg/kg after oral administration. These effects were significant at doses of 1 and 10 mg/kg. When compared to diazepam, CR-2945 showed the same activity in the elevated plus maze in rats and was about 3-fold less potent in the zero maze in rats. In the elevated plus maze in rats, the administration of CR-2945 did not modify locomotor activity, indicating that the observed anxiolytic effects were not affected by motor impairment. In the light/dark box test in mice, CR-2945 exhibited anxiolytic activity at doses of 0.3 and 3 mg/kg i.p., being statistically significant at the 3-mg dose.

The anxiolytic activities of CR-2945 in mice were observed in the absence of sedation and ataxia, as it did not modify locomotor activity in the open field test and did not induce muscle relaxation in the rotarod test at doses up to 30 mg/kg i.p. Moreover, CR-2945 did not potentiate the sedative action of barbiturates and did not show anticonvulsant activity in mice at doses that induced anxiolytic activity (3-30 mg/kg p.o.). In conflict procedures, where electric shock was used as an anxiogenic stimulus, the

Table III: Effects of CR-2945 and selected CCK_2 receptor antagonists on gastric and duodenal ulcers. Data expressed as ED_{50} in mg/kg. (from Prous Science MFLine database).

Compound	Gastric ulcer				Duodenal ulcer		Ref.
	Indomethacin		Ethanol		Cysteamine		
	ED_{50}	Route	ED_{50}	Route	ED_{50}	Route	
CR-2945	1.0[1]	s.c.	7.6	i.v.	8.8	i.p. + s.c.	18
L-365260	11.5[2]	i.v.	-	-	17.8	p.o.	20
PD-134308	>10[2]	i.v.	-	-	>30	p.o.	20
RP-73870	2.9[2]	i.v.	>30	p.o.	3.5	p.o.	20
YM-022	2.38[2]	p.o.	8.2	p.o	4.5[3]	p.o	26
Ranitidine	1.0[1]	s.c.	10.0	i.v.	8.5	i.p. + s.c.	18

[1]Dose that reduced 50% of the damage, calculated as median ulcus index; [2]acetylsalicylic acid-induced gastric ulcer; [3]mepirizole-induced duodenal ulcer.

Table IV: Inhibitory effects of CR-2945 and selected compounds on ex vivo *binding in rat cortex (from Prous Science MFLine database).*

Compound	ID_{50} (mg/kg)[1]		Ref.
CR-2945	10.9	i.v.	17
	13.5	s.c.	17
L-365260	18.6	i.v.	17
L-736380	1.7[2]	i.v.	27
RP-101367	9.0[3]	i.p.	28
	12.0[3]	p.o.	

[1]Displacement of [^{125}]-BH-CCK-8; [2]displacement of [^{125}I]-BH-CCK-8 in mouse brain; [3]displacement of [^{3}H]-p-CCK-8 in mouse brain.

anxiolytic effect of CR-2945 was 3-fold less potent as compared with diazepam. These results were in agreement with previously published data, which showed a lack of potent anxiolytic effects for CCK_2 receptor antagonists in conflict procedures, indicating a different anxiolityc profile for CCK_2 receptor antagonists and benzodiazepines. Unlike diazepam, CR-2945 induced neither tolerance nor anxiety after withdrawal from chronic treatment (10 mg/kg b.i.d. for 7 days).

Pharmacokinetics

In a preliminary bioavailability study in healthy volunteers, CR-2945 administered orally at a dose of 1 mg/kg produced an $AUC_{0\text{-}24h}$ of 1200 ng/h/ml. In rats, a dose of 1 mg/kg i.v. produced a 6-fold lower AUC (unpublished data).

Manufacturer

Rotta Research Laboratorium SpA (IT).

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