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CCC: 0377-8282/2002

Cizolirtine Citrate

Analgesic

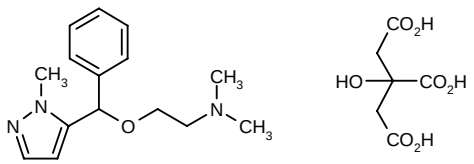
Prop INNM

E-4018
E-4960 ((*R*)-isomer)
E-4961 ((*S*)-isomer)

(±)-*O*-[2-(Dimethylamino)ethyl]-1-methyl-α-phenyl-1*H*-pyrazol-5-methanol citrate

(±)-5-[α-[2-(Dimethylamino)ethoxy]benzyl]-1-methyl-1*H*-pyrazole citrate

(±)-*N*,*N*-Dimethyl-2-[1-(1-methyl-1*H*-pyrazol-5-yl)-1-phenylmethoxy]ethanamine citrate



$C_{15}H_{21}N_3O.C_6H_8O7$

Mol wt: 451.4731

CAS: 142155-44-0

CAS: 148981-63-9 [as (*R*)-enantiomer]

CAS: 148981-64-0 [(*S*)-enantiomer, as free base]

CAS: 148981-65-1 ([as (*S*)-enantiomer]

CAS: 142155-43-9 (as free base)

CAS: 148981-62-8 [(*R*)-enantiomer, as free base]

EN: 170954

Abstract

There is still a need for novel analgesics with different mechanisms of action and improved safety and tolerability profiles. α_2 Adrenoceptors appear to play a role in modulation of sensory nociceptive processes of the spinal cord, activating noradrenergic pathways in the brain nuclei to the spinal cord. Selective α_2 agonists have been shown to have potent analgesic effects and inhibition of substance P and calcitonin-gene related peptide release, both involved in the transmission of nociceptive information, is an effective form of eliciting analgesic responses. Studies have shown that agonists acting at opioid receptors, some serotonin receptors, α_2 adrenoceptors, $GABA_B$ and adenosine receptors directly inhibit presynaptic primary afferent fiber transmission producing analgesic effects. Cizolirtine citrate is a new analgesic agent that is more potent than aspirin and other NSAIDs and was chosen for further development as a treatment for neuropathic pain.

Synthesis

The synthesis of (±)-cizolirtine citrate was performed according to the method shown in Scheme 1. The key compound 5-(α-hydroxybenzyl)-1-methyl-1*H*-pyrazole [(±)-I] was obtained by two related ways: 1) by condensation of *N*-methylpyrazole with benzonitrile and butyl lithium in tetrahydrofuran and reduction of the obtained ketone with sodium borohydride in methanol or 2) by condensation of *N*-methylpyrazole with benzaldehyde and butyl lithium in THF/toluene. The alkylation of [(±)-I] with 2-(dimethylamino)ethyl chloride hydrochloride was carried out using phase transfer conditions and reaction with citric acid using propanone as solvent (1).

The optical resolution of (±)-cizolirtine was accomplished by recrystallizing diastereoisomeric salts formed with the antipodes of di-*p*-toluoyltartaric acid in isopropanol (2). Scheme 2.

Another method for obtaining enantiopure cizolirtine involved the preparation of enantiomerically pure key compound (I) which was achieved by four different approaches: 1) making diastereomeric compounds with a covalent bond, 2) kinetic resolution, 3) enantioselective synthesis, and 4) enantiomerically pure compound synthesis starting from a suitable compound of the chiral pool which allowed the determination of the absolute configuration.

1) Resolution of alcohol [(±)-I] as its diastereomeric *O*-acetylmandelate esters and saponification of each of them in ethanol in the presence of catalytic amounts of sodium cyanide yielded the enantiomerically pure alcohols [(*R*)-(+)-I] and [(*S*)-(–)-I] (3). Scheme 3.

2) The transesterification of [(±)-I] with vinyl acetate enzymatically catalyzed (lipase) allowed the selective recovery of the enatiomer [(*R*)-(+)-I] and the hydrolysis and racemization of the undesired enantiomer [(*S*)-(–)-II] with hydrochloric acid (4). Scheme 4.

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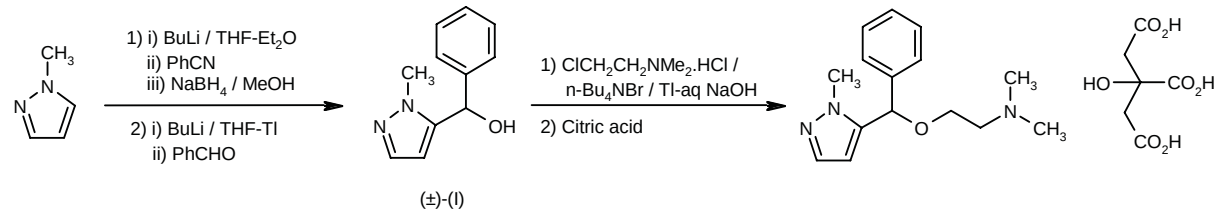
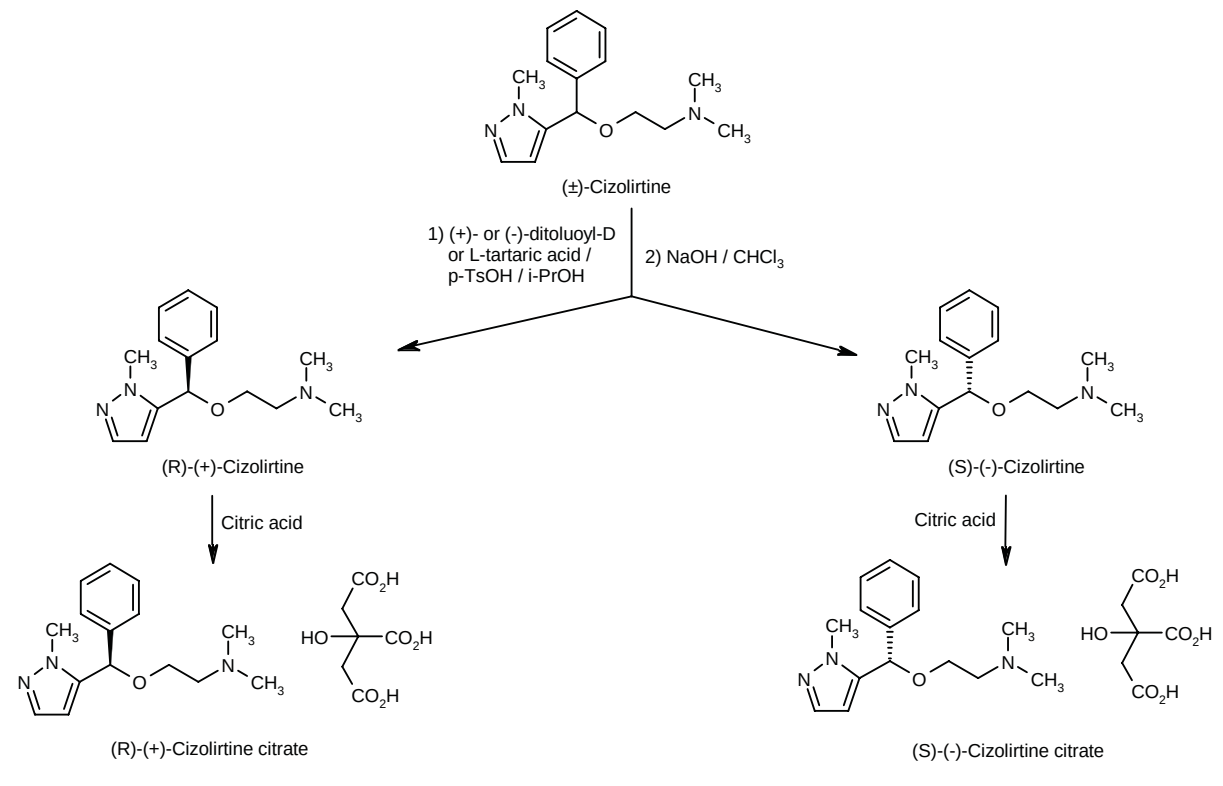
Scheme 1: Synthesis of Cizolirtine Citrate



Scheme 2: Resolution of Cizolirtine



3) Asymmetric reduction of ketone (III) with catecholborane or borane–methyl sulfide complex and CBS-oxazaborolidene as catalyst afforded [(*R*)-(+)-I] or [(*S*)-(–)-I] (5). Scheme 5.

4) Ethyl (*R*)-mandelate [(*R*)-IV] was protected as its *tert*-butyldimethylsilyl ether and reduced with DIBAL to [(*R*)-V], which was treated with ethoxycarbonylmethylenetriphenylphosphorane, followed by reduction to give [(*R*)-VI]. Subsequent oxidation to [(*R*)-VII] and cyclization by reaction with methylhydrazine afforded a mixture of pyrazolines [(*R*)-VIII]. Oxidation to pyrazole and deprotection yielded [(*R*)-(+)-I] (3). Scheme 6.

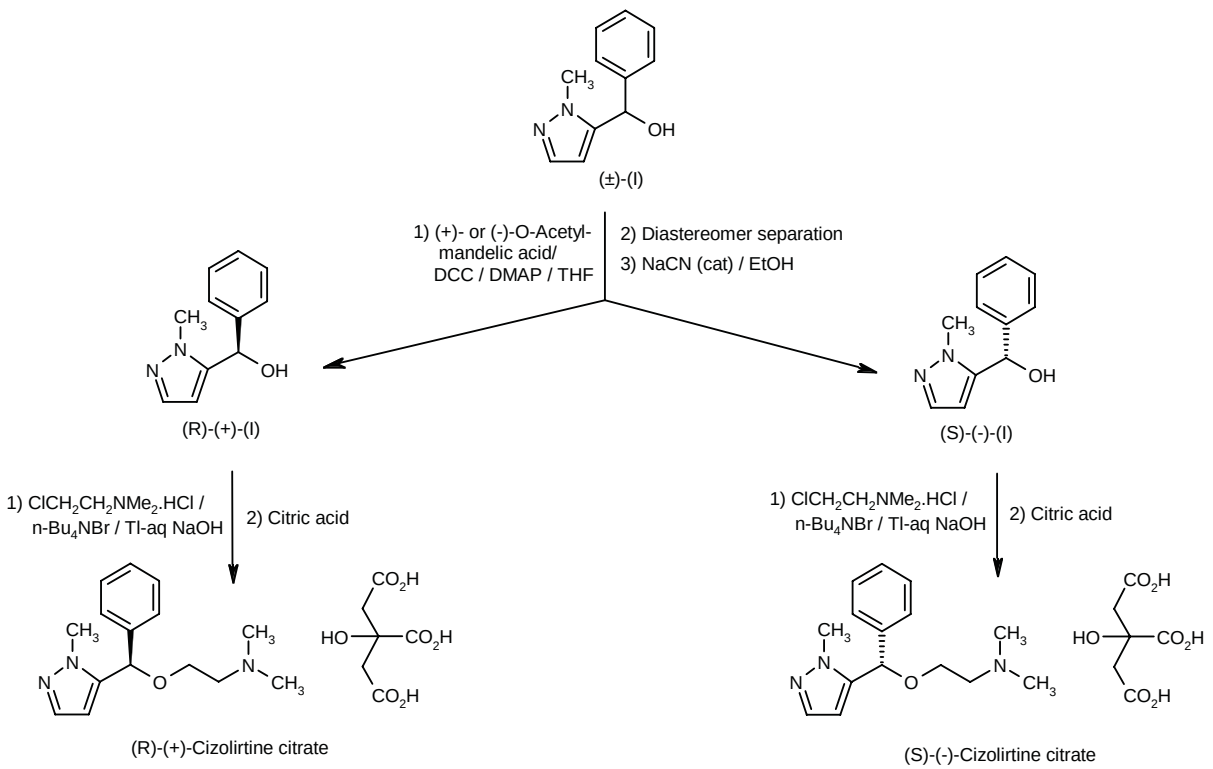
Description

(±)-Cizolirtine citrate, m.p. 138-142 °C; (*R*)-cizolirtine, $[\alpha]_D$ +40.2° (c 0.9, $CHCl_3$); (*R*)-cizolirtine citrate, m.p. 130-1 °C, $[\alpha]_D$ +12.3° (c 1, MeOH); (*S*)-cizolirtine, $[\alpha]_D$ –40.5° (c 1, $CHCl_3$); (*S*)-cizolirtine citrate, m.p. 129-131 °C, $[\alpha]_D$ –12.2° (c 1, MeOH).

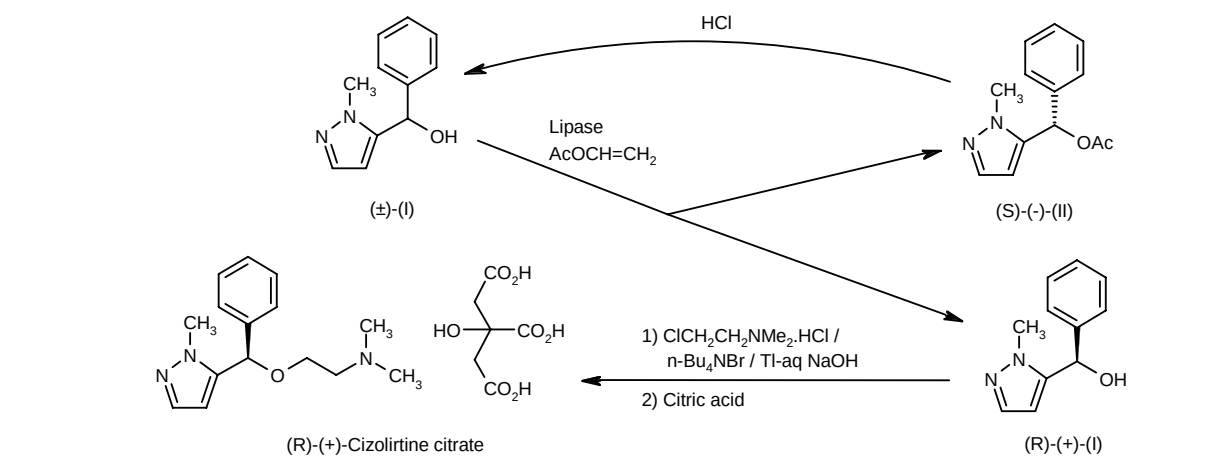
Introduction

There is still a need for new analgesics devoid of the side effects associated with opioids and nonsteroid anti-

Scheme 3: Preparation of Enantiopure Cizolirtine

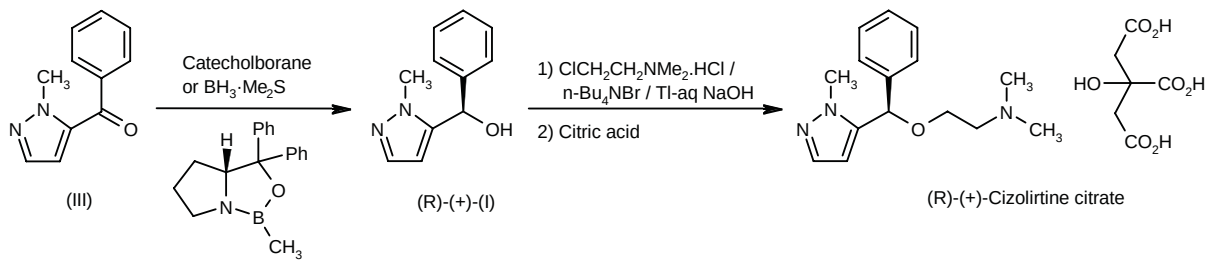


Scheme 4: Enzymatic Resolution and Preparation of (*R*)-Cizolirtine Citrate

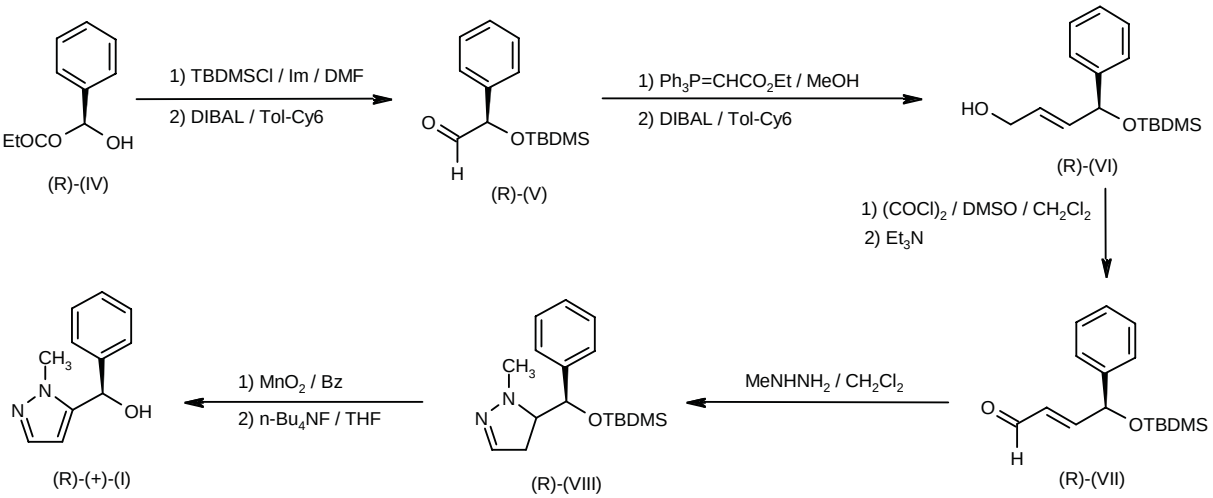


inflammatory drugs (NSAIDs). Opioids produce their effects by stimulation of specific receptors within the central nervous system, whereas NSAIDs are believed to act by inhibition of prostaglandin (PG) formation. Although opioid analgesics possess the liabilities associated with abuse, tolerance and dependence development, constipation and respiratory depression, they are generally considered to be more efficacious as analgesics than the antiinflammatory analgesics, and as a result are prescribed more extensively for the treatment of moderate to severe pain. Although the use of inhibitors of PG synthesis to produce analgesia is not limited by concerns of

Scheme 5: Enantioselective Synthesis of (*R*)-Cizolirtine Citrate



Scheme 6: EPC Synthesis of Intermediate (*R*)-(+)-(I)



abuse liability or development of tolerance and dependence, their use is often limited by lack of efficacy or the appearence of adverse effects, most notably gastrointestinal distress and renal damage.

Some substances modulate the sensory nociceptive processes of the spinal cord and activate the noradrenergic pathways from the brain nuclei to the spinal cord (6). The α_2 adrenoceptors appear to have a role in these functions, as selective α_2 agonists show clear analgesic effects in rodents after intrathecal injection (7). The analgesic activity of α_2 agonists is partly due to presynaptic effects on the afferent fibers, which are responsible for the transmission of the nociceptive messages to the spinal cord (8).

Descending noradrenergic neurons may also modulate nociception by preventing the release of neurotransmitters from the terminals of nociceptor primary afferent terminals in the spinal cord dorsal horn (9, 10). The release of substance P in the dorsal horn that is evoked by noxious stimulation can be reduced by noradrenaline (11). The inhibition of substance P release by noradrenaline appears to be mediated by α_2 adrenoceptors as such inhibition is blocked by the α_2-adrenoceptor antagonist yohimbine (11). These observations support the hypothesis that spinally projecting noradrenaline neurons can modulate nociception by reducing transmitter release from small diameter nociceptive afferents in the spinal cord dorsal horn.

The involvement of substance P and calcitonin-gene related peptide (CGRP) in the transmission of nociceptive information has been shown in different experimental

studies, including biochemical, electrophysiological and behavioral studies (12-17).

Primary afferent fibers, whose cell bodies are located in dorsal root ganglia, convey sensory information from the periphery to the dorsal horn of the spinal cord. The involvement of these fibers in nociception has been shown in experiments in which nociceptive stimuli enhanced the spinal release of substance P and CGRP, known neuroactive substances of primary afferent fibers (18-21).

The release of substance P and CGRP from the terminals of primary afferent fibers located in the superficial layers of the dorsal horn has been inhibited after administration of analgesic drugs (22). The direct presynaptic inhibition of primary afferent fibers that results in analgesic activity has been shown by agonists acting at opioid receptors (23, 24), some serotonin receptors (25, 26), α_2 adrenoceptors (6, 22), GABA-B receptors (27) and adenosine receptors (28).

Cizolirtine citrate (E-4018, E-3710 citrate) is a new analgesic agent that is more potent than aspirin and other NSAIDs and has shown activity in preclinical chemical, physical and thermal models of acute pain in mice and rats. Cizolirtine is also active in hyperalgesia and chronic neuropathic pain models in rats. Cizolirtine seems to require intact noradrenergic descending pathways to exert its analgesic effect as its activity is antagonized by pretreatment with idazoxan, a specific α_2 antagonist. More interestingly, the analgesic activity of cizolirtine has been associated with an inhibitory effect on substance P and CGRP.

Clinical studies have shown that cizolirtine is well tolerated, is not associate with severe adverse effects and has potential as an analgesic in various pain indications, including some neuropathic pain disorders.

Pharmacological Actions

Cizolirtine has been tested in several experiments of analgesic activity in comparison with other drugs.

Cizolirtine had significant analgesic activity in a number of tests, such as inhibition of phenylquinone- and acetic acid-induced writhing in mice and rats, and the tail-pinch and tail-flick tests in mice. The results showed that the analgesic effect of cizolirtine was not statistically different from the other compounds tested, except for codeine in the phenylquinone and tail-pinch tests and for *d*-propoxyphene in the rat acetic acid test. However, cizolirtine was more potent than aspirin in these tests, except in the acetic-acid writhing test in rats (29, 30) (Table I).

Cizolirtine had protective activity against formalin-induced pain in mouse paw. The first phase of this nociceptive response lasted for 5 min and was associated with the release of neuropeptides. The second phase was measured at 15-30 min after formalin injection. In both phases, cizolirtine was more active than diclofenac, and was not statistically different from morphine or amitriptyline (Table II).

Cizolirtine inhibited capsaicin-induced nociception with an ED_{50} of 7.14 mg/kg, which was not statistically different from morphine (ED_{50} = 5.59 mg/kg), while diclofenac (40 mg/kg) was not active in this test (Table II) (29). Capsaicin is an irritating agent that is capable of releasing neuropeptides such as substance P from the sensory neurons. The activity of cizolirtine in this test is an indication of a possible interaction with mechanisms related to substance P.

In a rat model of inflammatory pain such as carrageenan-induced paw inflammation, cizolirtine showed

Table I: Analgesic activity of cizolirtine in mice and rats.

	Analgesic activity ED_{50} (C.I.) mg/kg				
Compound	Phenylquinone[1] (mice)	Acetic acid[1] (mice)	Acetic acid[1] Rats	Tail pinch[2] (mice)	Tail flick[2] (mice)
Cizolirtine	33.7 (20.4-47.0)	24.4 (0-104)	21.3 (15.4-27.2)	68 (55.3-78.5)	46.0 (28.1-75.3)
Aspirin	218 (152-283)	>320	31.9 (14.6-49.2)	>640	>320
Diclofenac	82.2 (0-186)	106 (52.8-159)	33.9 (0-139)	>125	>120
Ibuprofen	107 (0-322)	157 (0-417)	10.8 (3.36-18.3)	258 (176-630)	>160
Codeine	7.64 (5.11-10.2)	11.4 (0-25.7)	6.76 (2.6-10.9)	28.0 (19.2-54.3)	14.0 (8.97-35.5)
d-Propoxyphene	29.5 (13.7-45.2)	19.0 (10.9-27.2)	6.97 (0.12-13.8)	>40	11.3

Each dose was tested in at least 10 animals. [1]Oral administration; [2]i.p. administration.

Table II: Antinociceptive effects of cizolirtine in mice.

	ED_{50} (C.I.) mg/kg i.p.		
	Formalin test		Capsaicin test
Compound	First phase	Second phase	
Cizolirtine	13.8 (1.53-2.61)	2.31 (0-7.17)	7.14 (0-24.2)
Diclofenac	56.3 (34.2-78.4)	48.1 (26.1-70.1)	(40 mg/kg: inactive)
Morphine	1.96 (0.54-3.39)	1.00 (0.41-1.59)	5.59 (4.39-6.79)
Amitriptyline	2.34 (0.62-4.07)	2.01 (1.32-2.70)	Not tested

Table III: Analgesic activity of cizolirtine in inflammatory pain in rats.

Compound	Mechanical stimulus ED_{50} (mg/kg p.o.)	Thermal stimulus ED_{50} (mg/kg p.o.)
Cizolirtine	31.4	26.8
Aspirin	43.0	25.5
Diclofenac	0.42	2.2
Indomethacin	0.78	0.55

analgesic activity against mechanical stimulus-induced pain (ED_{50} = 31.4 mg/kg p.o. in the Randall and Selitto test) and thermal stimulus-induced pain (ED_{50} = 26.8 mg/kg p.o. in the Hargreaves test) without modifying the inflammatory status of the paw. In comparison, the reference compounds showed similar or higher analgesic activity than cizolirtine (ED_{50} for aspirin = 43 and 25.5 in Randall and Selitto and Hargreaves test, respectively) that was associated with potent antiinflammatory activity (Table III).

Cizolirtine was also active in neuropathic pain models such as the rat chronic constriction injury model (described by Bennett) and the diabetic rat model. In the Bennett model, the rat undergoes irreversible ligature of the sciatic nerve of one limb and then the response to a hot, cold or mechanical stimulus on the injured limb is determined and compared to the contralateral limb. Cizolirtine exhibited analgesic activity against cold and mechanical stimulus at oral doses ranging from 1.44-5.75 mg/kg as well as against radiant heat stimulus (ED_{50} = 2.75 mg/kg p.o.). In the diabetic rat model, cizolirtine at a dose of 46 mg/kg i.p. gave full protection against the mechanical pressure -induced pain on the paw (Tables IV and V) (31, 32).

In a model of brewers yeast- or bacterial lipopolysaccharide-induced fever in rats, cizolirtine showed dose-dependent (46, 92 and 184 mg/kg p.o. and i.p.) antipyretic activity comparable to that of paracetamol and acetylsalicylic acid (40, 80 and 160 mg/kg p.o. and i.p.) (29).

Several pharmacological tests have shown that cizolirtine is a very safe compound. In the cold stress (–15 °C) test in rats, cizolirtine (23, 46 and 92 mg/kg p.o.), like codeine (20 mg/kg p.o.) and dextropropoxyphene (20 mg/kg p.o.), did not have ulcerogenic effects. In contrast, diclofenac, ibuprofen (40, 80 and 160 mg/kg) and acetylsalicylic acid (80, 160 and 320 mg/kg) induced different levels of gastric damage at all tested doses (Table VI) (29, 30).

Table V: Analgesic activity of cizolirtine in neuropathic pain models in rats.

Bennet model[a] Radiant heat stimulus	Diabetic rats[b] Paw pressure, AUC (g x min)		
		PWT	Voc
ED_{50} = 2.75 mg/kg p.o.	Control	100	100
E_{max} = 3.45 mg/kg p.o.	Cizolirtine (46 mg/kg i.p.)	500	550

[a]Chronic constriction injury to the sciatic nerve using radiant heat stimulus 1 h after cizolirtine treatment; [b]diabetic rats treated acutely with saline or cizolirtine: AUC calculated from the time-course changes in mechanical threshold for paw withdrawal (PWT) and vocalization (Voc).

The Irwin test was used to investigate the behavioral effects of cizolirtine (11.5, 23 and 46 mg/kg i.p.) in rats and mice. In rats, no abnormal behavior or depressive CNS effects such as passivity, sedation, prostration, ataxia, ptosis, muscle reflexes, reduced tonus, hypnosis, hypothermia or catalepsy were detected within 3 h after treatment; only a slight excitation was seen with the highest dose of 46 mg/kg, an effect which decreased at 23 mg/kg and disappeared at 11.5 mg/kg. Among peripheral nervous system effects, cizolirtine only had a slight effect on mydriasis at a dose of 46 mg/kg; this effect decreased at 23 mg/kg and disappeared at 11.5 mg/kg. In mice, cizolirtine only induced mild mydriasis, without depressor or stimulating effects at the dose of 46 mg/kg p.o.

Increasing bolus doses of cizolirtine (1.72, 5.75 and 17.2 mg/kg i.v.) were administered every 30 min to 6 urethane anesthetized rats to assess the compound's cardiovascular and respiratory effects. Cizolirtine was well tolerated up to 17.2 mg/kg in 3 of the 6 animals, with no effects on blood pressure, ECG, pulmonary evaluation or body temperature. Moreover, it had no hemolytic potential in human blood *in vitro* up to 0.57 mg/ml and did not show effects on coagulation or platelet aggregation in the rat at oral doses up to 172 mg/kg.

The addiction potential of cizolirtine was studied in the conditioned place preference test in mice. A dose of 23 mg/kg/day i.p. for 5 days did not induce addiction potential, whereas amphetamine at 10 mg/kg/day i.p. for 5 days

Table IV: Analgesic activity of cizolirtine in neuropathic pain in rats.[1]

Cizolirtine dose (mg/kg p.o.)	Mechanical stimulus Nerve injured paw AUC (g x min)	Mechanical stimulus Contralateral paw AUC (g x min)	Cold stimulus (10 °C) Nerve injured paw AUC (s x min)
Saline	7.7	7.7	1
1.44	11.5	21.5	10
2.88	14.6	33.8	22
5.75	23.1	33.8	50

[1]Struggle latencies were measured every 20 min until they had returned to baseline values.

Table VI: Ulcerogenic effects of cizolirtine in rats subjected to cold stress (–15 °C).

Compound	Dose (mg/kg p.o.)	Results	Statistical significance
Gum arabic	10	0.43 ± 0.17	–
Cizolirtine	46	0.10 ± 0.10	–
	92	1.00 ± 0.47	$p > 0.05$
Diclofenac	40	4.20 ± 0.92	$p < 0.001$
	80	8.00 ± 1.19	$p < 0.001$
	160	9.40 ± 0.60	$p < 0.001$
Ibuprofen	40	4.00 ± 1.12	$p < 0.001$
	80	7.00 ± 0.04	$p < 0.001$
	160	5.60 ± 0.32	$p < 0.001$
d-Propoxyphene	20	1.00 ± 0.26	$p > 0.05$
Codeine	20	0.10 ± 0.10	$p > 0.05$

Results are expressed as the mean ± SEM of gastric ulceration scores. Each dose was tested in 10 rats.

Table VII: Effect of pretreatment with naloxone on the analgesic activity of cizolirtine in mice.

Compound	Dose (mg/kg i.p.)	Pretreatment gum arabic Mean ± SEM	% Activity	Pretreatment naloxone Mean ± SEM	% Activity	Statistical significance
Control	10	23.8 ± 2.22	–	28.1 ± 4.71	–	$p > 0.05$
Codeine	5	13.5 ± 1.97	43.3	24.2 ± 3.38	0.00	$p < 0.001$
	10	6.80 ± 1.58	71.4	29.6 ± 3.41	0.00	$p < 0.001$
	20	1.80 ± 0.63	92.4	28.7 ± 2.92	0.00	$p < 0.001$
Cizolirtine	11.5	16.8 ± 3.10	29.4	16.5 ± 2.59	41.3	$p > 0.05$
	23	8.40 ± 1.43	64.7	10.5 ± 2.40	62.6	$p > 0.05$
	46	5.10 ± 1.49	78.6	3.90 ± 0.71	86.1	$p > 0.05$

The antinociceptive effects of cizolirtine and codeine after pretreatment with gum arabic (control) and 1 mg/kg naloxone are expressed. The results of both pretreatments were compared statistically. Each dose was tested in 10 mice.

Table VIII: Effect of pretreatment with naloxone on the antinociceptive effects of cizolirtine in mice.

Compound	Dose (mg/kg i.p.)	Pretreatment Gum arabic	Naloxone	Statistical significance
Gum arabic	10	2/54	2/54	$p > 0.05$
Codeine	5	5/10	0/10	$p < 0.01$
	10	7/10	1/10	$p < 0.001$
	20	9/10	1/10	$p < 0.001$
Cizolirtine	23	3/10	1/10	$p > 0.05$
	46	4/10	4/10	$p > 0.05$
	92	5/10	6/10	$p > 0.05$

Results are expressed as the number of active/treated mice. The antinociceptive effects of cizolirtine and codeine after pretreatment with gum arabic and 4 mg/kg of naloxone were compared.

induced a clear addictive behavior. Intravenous self-administration studies showed the lack of addictive properties of cizolirtine (0.72, 1.44, 2.87 mg/kg i.v.) in rats (29).

The analgesic activity of cizolirtine in the phenylquinone-induced writhing test and in the tail-flick test in mice was not antagonized by naloxone (Tables VII and VIII) (29) and was not related to inhibition of prostaglandin biosynthesis (PGE_2) or COX-1 or COX-2 enzymes. Cizolirtine did not modify the COX-2 enzyme expression or PGE synthesis in murine peritoneal macrophages (at 0.1-10 μM) and in rats it (184 mg/kg p.o.) did not inhibit the prostaglandin production in the inflammatory exudate (as measure of COX-2 inhibition) or in gastric mucosa (as measure of COX-1 inhibition) (Table IX). In addition, cizolirtine (46 mg/kg p.o.) did not inhibit castor oil-induced diarrhea in rats or carrageenan-induced edema (up to 115 mg/kg p.o.). Moreover, cizolirtine at 10 μM was unable to inhibit inducible nitric oxide synthase (iNOS), and it did not modify the production of proinflammatory cytokines, IL-6 or TNF-α in peritoneal macrophage culture. Cizolirtine (up to 10 μM) was inactive against a series of 83 receptors and 32 enzymes; only slight affinity was seen at higher concentrations for muscarinic M_5 and sigma receptors. No functional *in vitro* agonist or antagonist activity was found against a series of receptors, including muscarinic, histamine H_1 and H_2, α-adrenergic,

Table IX: Inhibitory effects of cizolirtine on PGE_2 production in rats.

Compound	Dose (mg/kg p.o.)	Inflammatory exudate % Activity (mean ± SEM)	ED_{50} (C.I.)	Gastric mucosa % Activity (mean ± SEM)	ED_{50} (C.I.)
Cizolirtine	46	5.04 ± 3.83	–	16.5 ± 6.37	–
	92	8.73 ± 6.45		20.1 ± 5.85	
	184	7.23 ± 4.71		5.97 ± 4.36	
Indomethacin	0.078	17 ± 10.6	0.29 (0.20-0.38)	27.3 ± 9.58	0.63 (0.16-1.10)
	0.156	39.2 ± 6.42		21.8 ± 6.98	
	0.313	42.2 ± 6.90		51.7 ± 9.35	
	0.625	76.9 ± 3.17		46.3 ± 9.59	
	1.25	81.5 ± 2.57		64.2 ± 5.52	
	2.50	93.6 ± 0.70		56.9 ± 10.5	
	5.00	96.6 ± 0.59		81.5 ± 4.39	
NS-398	0.156	24.9 ± 11.01	0.47 (0.26-0.68)	3.03 ± 2.77	
	0.313	34.9 ± 8.50		15.1 ± 6.24	
	0.625	68.3 ± 3.98		10.4 ± 6.16	
	1.25	69.6 ± 3.39		11.9 ± 6.23	
	2.50	84.7 ± 2.13		21.2 ± 8.03	
	5	82.4 ± 3.14		9.01 ± 4.90	
	10	84.2 ± 3.33		6.21 ± 3.81	
	20	94.4 ± 0.68		3.34 ± 2.63	

Each dose was tested in 6-18 rats. ED_{50} and C.I. (confidence interval) are expressed in mg/kg.

Table X: Effect of pretreatment with imipramine and idazoxan on the analgesic activity of cizolirtine in mice (50 min after cizolirtine).

	Pretreatment (20 min before cizolirtine)		
Cizolirtine (mg/kg i.p.)	Control (vehicle, 10 ml/kg i.p.)	Imipramine (20 mg/kg i.p.)	Idazoxan (1.25 mg/kg i.p.)
46	2/20 (10%)	2/10 (20%)	1/10 (10%)
69	7/25 (28%)	12/17 (71%)	1/10 (10%)
92	16/27 (60%)	12/15 (80%)	2/10 (20%)

Active/treated (protected mice)

β-adrenergic, serotonergic, dopaminergic or benzodiazepine and calcium channels.

Morphine administration induces a spinal release of noradrenaline from supraespinal neurons (33, 34) which stimulates the α_2 receptors at the spinal level and is partially responsible for the analgesic activity of morphine. For this reason, the α_2 antagonists, and particularly idazoxan, antagonize morphine analgesia (35-37) and the noradrenaline reuptake inhibitors increase morphine analgesia (38). It is of interest that the inhibition of morphine analgesia by α_2 antagonists depends on the test used (yohimbine inhibits morphine analgesia in the tail-flick test but not in the hot plate test) (39) and also depends on the route of administration of the α_2 antagonists (40). The analgesia mediated by nonopioid compounds, like nicotine or S-12813-4 is also inhibited by administration of α_2 antagonists such as idazoxan or yohimbine (6, 41-43). Moreover, the importance of the noradrenergic pathways in pain transmission inhibition in the spinal cord dorsal horns has been reported (44).

Some *in vivo* studies showed that the descendent noradrenaline pathways must be intact for cizolirtine to exert analgesic activity. Cizolirtine had no affinity for the α_2 receptor, but its analgesic activity in the tail-flick test in mice and in the plantar test in rats was antagonized by pretreatment with idazoxan, a selective α_2 blocker, Furthermore, the analgesic activity of cizolirtine was increased after pretreatment with noradrenaline reuptake inhibitors such as imipramine (in the mouse tail-flick test) and desipramine (in the rat plantar test) (Tables X and XI) (29). *In vivo* microdialysis studies have demonstrated that cizolirtine (80 mg/kg i.p.) increases by up to 300% the release of noradrenaline and serotonin in the rat prefrontal cortex for 1.5 h, which corresponds to the duration of its analgesic activity.

Inhibition of spinal substance P release has been demonstrated after intrathecal or systemic administration of cizolirtine at therapeutic doses (45). *In vitro* and *in vivo* experiments assessed whether cizolirtine could affect the spinal release of two pain-related neuropeptides, substance P (SP) and calcitonin gene-related peptide (CGRP), in rats. Cizolirtine reduced the potassium-evoked overflow of both substance P (–25% at 0.1 μM-0.1 mM) and CGRP (–20% at 0.1-1.0 μM) from slices of the dorsal half of the lumbar enlargement of the spinal cord. Intrathecal perfusion of cizolirtine in halothane

Table XI: Effect of pretreatment with desipramine and idazoxan on the analgesic activity of cizolirtine in rats.

			% Activity ± SEM, statistical significance Pretreatment (i.p.)			
	Dose (mg/kg p.o.)	Gum arabic	Desipramine		Idazoxan	
Cizolirtine	1.44	0.00 ± 0.00	12.1 ± 4.92	$p > 0.05$	–	
Cizolirtine	2.87	0.00 ± 0.00	43.2 ± 9.32	$p < 0.001$	–	
Cizolirtine	5.75	0.00 ± 0.00	73.2 ± 9.06	$p < 0.001$	–	
Cizolirtine	11.5	17. 9 ± 9.58	73.7 ± 11.7	$p < 0.01$	10.9 ± 5.41	$p > 0.05$
Cizolirtine	23.0	66.8 ± 10.1	–		38.7 ± 11.6	$p > 0.05$
Cizolirtine	46.0	72.7 ± 9.42	–		29.8 ± 0.43	$p < 0.01$

The antinociceptive effects of cizolirtine after pretreatment with gum arabic were compared with the effects of pretreatment with desipramine or idazoxan.

anesthetized rats markedly reduced the spinal outflow of SP (up to –50% at 0.1 mM) but only marginally that of CGRP. Systemic administration of cizolirtine at an analgesic dose (46 mg/kg i.p.) also reduced spinal SP outflow (–50%) but not that of CGRP. Under both *in vitro* and *in vivo* conditions, idazoxan (10 μM) antagonized the effects of cizolirtine on SP and CGRP release, suggesting their mediation through α_2 adrenoceptors. In another study in rats with Freund's adjuvant-induced polyarthritis, cizolirtine (46 mg/kg i.p.) reduced the release of CGRP from the perfused spinal cord; idazoxan (10 μM) blocked the inhibitory action of cizolirtine on CGRP and SP release, suggesting α_2 adrenoceptor mediation.

In an *in vivo* study, the spontaneous spinal outflow of CGRP was significantly higher in diabetic rats than in healthy controls (32). Acute cizolirtine treatment (46 mg/kg i.p.) produced a significant reduction in spinal CGRP, suggesting a tonic activation of CGRP-containing primary afferent fibers. The effect of cizolirtine on diabetes associated neuropathic pain therefore may result from its inhibitory effect on activated CGRP-containing primary afferent fibers.

The analgesic activity of the two enantiomers of cizolirtine did not differ significantly from the racemate (3).

Pharmacokinetics and Metabolism

Following single i.v. or oral administration to rats and dogs (46, 47), cizolirtine was absorbed rapidly and pharmacokinetics were not affected by route of administration or sex. In rats cizolirtine had a bioavailability of 20% and a $t_{1/2}$ of 0.6-1.1 h; in dogs it showed higher bioavailability (> 60%) and a longer $t_{1/2}$ (1.6-2.6 h). In rats, tissue distribution studies using [^{14}C]-cizolirtine indicated higher affinities for liver, kidney, gastric mucosa and uveal tract tissues; 68% of radioactivity was excreted in the urine and 21% in the feces after both i.v. and p.o. administration. In dogs, the respective values were 93 and 6% after p.o. administration. Renal elimination of cizolirtine accounted for < 5% in rats and 20% in dogs. The clearance rate was approximately 35 ml/min for rats and 215 ml/min for dogs.

In rats and in dogs, metabolism takes place mainly through *N*-oxidation, *N*-demethylation and oxidative deamination. *In vitro* and *in vivo* metabolic studies of cizolirtine and its two enantiomers have been performed in rats. The results of these studies showed no differences between enantiomers, with no biotransformation between enantiomers.

In humans, the absorption of the unchanged substance was fast, linear and dose proportional. The maximum plasma levels (C_{max}) of cizolirtine were reached between 1.1 and 1.5 h. after administration. The C_{max} and AUC values increased dose-dependently whereas the $t_{1/2\beta}$ was not dose-related, ranging from 6.7-7.1 h at doses of 29, 57 and 86 mg. However, elimination was lower than that obtained in preclinical studies. The metabolism of cizolirtine in humans is similar to that in animal species and takes place mainly through oxidative deamination and *N*-oxidation.

Toxicity

Cizolirtine has been tested in acute toxicity studies in mice and rats after oral, i.p. and i.v. administration (47). Oral repeated-dose toxicity was measured in 13-week studies in mice, 26-week studies in rats and 52-week studies in dogs. Parenteral repeated-dose toxicity was measured in 4-week studies in rats and dogs. Cizolirtine had a remarkable safety margin after repeated administration in rats and dogs. No drug-related toxicities were observed at doses of 11 mg/kg/day p.o. in rats after 26 weeks, 34 mg/kg/day p.o. in dogs after 52 weeks (equivalent to 20 and 60 mg/kg/day of cizolirtine citrate, respectively) and 86 mg/kg/day p.o. in mice after 13 weeks.

The toxicity of the two cizolirtine enantiomers was compared after a 4-week repeated-dose study in rats, resulting in a toxicological profile similar to that of the racemic compound.

Reproductive toxicity studies of cizolirtine were performed after oral administration in rats and rabbits. Treatment with cizolirtine citrate is considered to have no effects on reproductive parameters at any of the tested doses. Treatment with cizolirtine had no effect on gestation in rats and rabbits, in terms of number of

implantations, number of postimplantation losses, fetal or placental weight and sex ratio of live fetuses. Also, treatment with cizolirtine had no effect on the incidence of fatal abnormalities or on the ossification status of skeletons. The highest administered doses were 115 mg/kg/day to rats and 46 mg/kg/day to rabbits. The observed no-effect level in the pre- and postnatal development study in rats was considered to be 11 mg/kg/day (equivalent to 20 mg/kg/day of cizolirtine citrate).

Cizolirtine has no genotoxic potential as assessed in experimental systems used including Ames test, mouse lymphoma assay, human lymphocyte and micronucleus test. Overall, these results showed that cizolirtine is safe and well tolerated in preclinical models and is a good candidate for clinical evaluations.

Clinical Studies

Clinical studies with cizolirtine administered p.o., i.m. or i.v. have been conducted in a total of 1059 subjects in Europe and the U.S. Of these, 240 were healthy volunteers and 819 were patients. Of the 240 healthy volunteers, 206 received cizolirtine and 34 received placebo. Of the 819 patients, 484 received cizolirtine and 198 received placebo. Cizolirtine was well tolerated, without severe adverse events or changes in vital signs, laboratory safety parameters or ECG findings. Cizolirtine citrate was administered at single escalating oral doses of 400, 600 and 800 mg and in a repeated oral dose tolerability study at 300 and 400 mg b.i.d. for 2 weeks. No severe adverse events were observed; the most frequent adverse events were mild drowsiness, dizziness and somnolence.

Systemic and local tolerability of i.m. injections of cizolirtine (20, 40, 60, 120, 180, 240 and 300 mg) was good, with no adverse effects reported.

A 15-min i.v. infusion of cizolirtine citrate (26, 260 and 400 mg) also had good local and systemic tolerability, with no adverse events reported.

Cizolirtine showed analgesic activity in two experimental pain studies, of tolerance to electrically and to thermally induced pain in healthy volunteers. In both studies, 100 mg of cizolirtine citrate was as active as 100 mg of diclofenac.

A series of inflammatory pain studies included two third molar extraction studies, one hip replacement study and one postepisiotomy study. In one of the third molar extraction studies, cizolirtine citrate was administered at doses of 50, 100 and 150 mg. Results showed that the delay in requesting rescue medication was significantly higher after doses of 100 and 150 mg. The other three studies failed to show efficacy of cizolirtine citrate at 200 mg due to a high placebo effect. In a migraine study, where the placebo effect was also very high, 400 mg of cizolirtine citrate and 800 mg of ibuprofen showed similar activity.

Two neuropathic pain studies showed interesting results after cizolirtine treatment. In a posttraumatic/postherpetic neuropathic pain study, no significant differences were detected between the control group and the cizolirtine citrate (200 mg b.i.d. for 21 days) group, but in a subgroup of patients with primary allodynia there was a significant marked improvement with cizolirtine citrate (48). This is an indication of the possible efficacy of cizolirtine in chronic pain after nerve and related tissue injury. In a diabetic neuropathy study, cizolirtine was administered at 200 mg b.i.d. for 10+4 days, showing a slight but consistent improvement in all variables.

The overall results of the above studies indicate that cizolirtine has analgesic activity, but probably due to the use of low doses, this activity was not statistically significant. The analgesic activity of cizolirtine has been demonstrated in 2 more recent phase II studies in which higher doses of the drug have been administered. In a renal colic study, cizolirtine citrate (350 mg i.v. infusion) and metamizol (2500 mg i.v. infusion) had similar significant analgesic activity, and in a cancer pain study cizolirtine (400 mg b.i.d.) and diclofenac (50 mg b.i.d.) had similar analgesic activity.

Source

Laboratorios Dr. Esteve S.A. (ES).

References

1. Colombo, A., Parés, J., Frigola, J. (Laboratorios del Dr. Esteve, SA). *Arylpyrazolylcarbinol compounds with analgesic activity.* EP 0289380, FR 2613720, JP 19882677761 , US 5017596.

2. Torrens, A., Frigola, J. (Laboratorios del Dr. Esteve, SA). *Resolution of amines.* ES 2130079, EP 1024137, US 6187930, WO 9902500.

3. Hueso, J., Berrocal, J., Gutierrez, B., Farré, A., Frigola, J. *Preparation of the enantiomers of the analgesic E-3710.* Bioorg Med Chem Lett 1993, 3: 269-72.

4. Frigola, J., Berrocal, J.M., Cuberes, M.R., Gotor, V. (Laboratorios del Dr. Esteve, SA). *Method for separating carbinols.* EP 0808308, ES 2128959, FR 2742147, US 5849931, WO 9720817.

5. Torrens, A., Frigola, J. (Laboratorios del Dr. Esteve, SA). *Process for obtaining enantiomers of cizolirtine.* ES 2130083, EP 1029852, US 6118009, WO 9907684.

6. Collin, E., Frechilla, D., Pohl, M., Bourgoin, S., Mauborgne, A., Hamon, M., Cesselin, F. *Differential effects of the novel analgesic, S-12813-4, on the spinal release of substance P- and CGRP-like materials in the rat.* Naunyn-Schmied Arch Pharmacol 1994, 349: 387-93.

7. Takano, Y., Yaksh, T.L. *Characterization of the pharmacology of intrathecally administered α_2-agonists and antagonists in rats.* J Pharmacol Exp Ther 1992, 261: 764-72.

8. Sullivan, A.F., Dashwood, M.R., Dickenson, A.H. α_2-*Adrenoceptor modulation of nociception in rat spinal cord: Location, effects and interactions with morphine.* Eur J Pharmacol 1987, 138: 169-77.

9. Pang, I.H., Vasko, M.R. *Morphine and norepinephrine but not 5-HT and GABA inhibit the K^+-stimulated release of substance P from rat spinal cord slices.* Brain Res 1986, 376: 268-79.

10. Fischbach, G.D., Dunlap, K., Mudge, A., Leeman, S. *Peptide and amine transmitter effect on embryonic chick sensory neurons in vitro.* Adv Biochem Psychopharmacol 1981, 28: 175-88.

11. Kuraishi, Y., Hirota, N., Sato, Y., Kaneko, S., Satoh, M., Takagi, H. *Noradrenergic inhibition of the release of substance P from the primary afferents in the rabbit spinal dorsal horn.* Brain Res 1985, 359: 177-82.

12. Jessell, T.L., Womack, M.D. *Substance P and the novel mammalian tachykinins: A diversity of receptors and cellular actions.* Trends Neurosci 1985, 8: 43-5.

13. Mantyh, P.W., Rogers, S.D., Honore, P., Allen, B.J., Ghilardi, J.R., Li, J., Daughters, R.S., Lappi, D.A., Wiley, R.G., Simone, D.A. *Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor.* Science 1997, 278: 275-9.

14. Micevych, P.E., Kruger, L. *The status of CGRP as an effector peptide.* Ann NY Acad Sci 1992, 657: 379-86.

15. Millan, M.J. *The induction of pain: An integrative review.* Prog Neurobiol 1999, 57: 1-164.

16. Weihe, E. *Neurochemical anatomy of the mammalian spinal cord: Functional implications.* Ann Anat 1992, 174: 89-118.

17. Wiessenfeld-Hallin, Z., Hökfelt, T., Lundberg, J.M., Forssmann, W.G., Reinecke, M., Tschopp, F.A., Fischer, J.A. *Immunoreactive CGRP and substance P coexist in sensory neurons to the spinal cord and interact in spinal behavioural responses of the rat.* Neurosci Lett 1984, 52: 199-204.

18. Duggan, A.W., Hendry, I.A., Morton, C.R., Hutchinson, W.D., Zhao, Z.Q. *Cutaneous stimuli releasing inmunoreactive substance P in the dorsal horn of the cat.* Brain Res 1988, 451: 261-73.

19. Morton, C.R., Hutchison, W.D. *Release of sensory neuropeptides in the spinal cord: Studies with CGRP and galanin.* Neuroscience 1989, 31: 807-815.

20. Pohl, M., Benoliel, J.J., Bourgoin, S., Clot, A.M., Hamon, M., Cesselin, F., Le Bars, D. *In vivo release of CGRP-like material from the cervico-trigeminal area in the rat. Effects of electrical and noxious stimulations of the muzzle.* Neuroscience 1992, 50: 697-706.

21. Skilling, S.R., Smullin, D.H., Beitz, A.J., Larson, A.A. *Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation.* J Neurochem 1988, 51: 127-32.

22. Bourgoin, S., Benoliel, J.J., Collin, E., Mauborgne, A., Pohl, M., Hamon, M., Cesselin, F. *Opioidergic control of the spinal release of neuropeptides. Possible significance for the analgesic effects of opioids.* Fundam Clin Pharmacol 1994, 8: 307-21.

23. Pohl, M., Lombard, M.C., Bourgoin, S., Carayon, A., Benoliel, J.J., Mauborgne, A., Besson, J.M., Hamon, M., Cesselin, F. *Opioid control of the in vitro release of CGRP from primary afferent fibres projecting in the rat cervical cord.* Neuropeptides 1989, 14: 151-9.

24. Pohl, M., Mauborgne, A., Bourgoin, S., Benoliel, J.J., Hamon, M., Cesselin, F. *Neonatal capsaicin treatment abolishes the modulation by opioids of substance P release from rat spinal cord slices.* Neurosci Lett 1989, 96: 102-7.

25. Arvieu, L., Mauborgne, A., Bourgoin, S., Oliver, C, Feltz., P., Hamon, M., Cesselin., F. *Sumatriptan inhibits the release of CGRP and substance P from the rat spinal cord.* NeuroReport 1996, 7: 1973-6.

26. Scott, M.A., Fone, K.C.F. *Modulation of CGRP release from dorsal thoraco-lumbar spinal cord slices by 5-HT_3 receptors.* Neurosci Lett 1994, 175: 5-8.

27. Malcangio, M., Bowery, N.G. *$GABA_B$, but not $GABA_A$ receptor activation, inhibits electrically evoked substance P-like immunoreactivity release from the rat spinal cord in vitro.* J Pharmacol Exp Ther 1993, 266: 1490-6.

28. Santicioli, P., Del Bianco, E., Tramontana, M., Maggi, C.A. *Adenosine inhibits action potential dependent release of CGRP and substance P-like immunoreactivities from primary afferents in rat spinal cord.* Neurosci Lett 1992, 144: 211-4.

29. Alvarez, I., Andreu, F., Buxens, J., Colombo, M., Dordal, A., Fort, M., Gutiérrez, B., Farré, A.J. *Pharmacology of cizolirtine: A new analgesic agent.* Methods Find Exp Clin Pharmacol 2000, 22: 211-21.

30. Farré, A.J., Colombo, A., Colombo, M., Fort, M., Gutierrez, B. *E-3710, a new product with original analgesic activity.* Rev Farmacol Clin Exp 1989, 6(3): Abst C-66.

31. Kayser, V., Christensen, D., Farré A.J. *Cizolirtine alleviates pain related behavior to mechanical and cold stimuli in neuropathic pain.* Life Sci, submitted.

32. Aubel, B., Kayser. V., Mauborgne, A., Farré, A.J., Hamon, M., Bourgoin S. *Effects of the non-opioid analgesic cizolirtine on diabetes-induced neuropathy in the rat: Behavioral and biochemical studies.* Fundam Clin Pharmacol 2001, 15: 142.

33. Bouaziz, H., Tong, C., Yoon, Y., Hood, D.D., Eisenach, J.C. *Intravenous opioids stimulate norepinephrine and acetylcholine release in spinal cord dorsal horn. Systematic studies in sheep and an observation in a human.* Anesthesiology 1996, 84: 143-54.

34. Xu, Z., Tong, C., Pan, H.L., Cerda, S.E., Eisenach, J.C., *Intravenous morphine increases release of nitric oxide from spinal cord by an α-adrenergic and cholinergic mechanism.* J Neurophysiol 1997, 78: 2072-8.

35. Song, H.K., Pan, H.L., Eisenach, J.C. *Spinal nitric oxide mediates antinociception from intravenous morphine.* Anesthesiology 1998, 89: 215-21.

36. Thomas, D.A., Anton, F., Kenshalo, D.R. Jr., Williams, G.M., Dubner, R. *Noradrenergic and opioid systems interact to alter the detection of noxious thermal stimuli and facial scratching in monkeys.* Pain 1993, 55: 63-70.

37. Tseng, L., Tang, R. *Differential actions of the blockade of spinal opioid adrenergic and serotonergic receptors of the tail-flick inhibition induced by morphine microinjected into dorsal raphe and central gray in rats.* Neuroscience 1989, 33: 93-100.

38. Taiwo, Y.O., Fabian, A., Pazoles, C.J., Fields, H.L. *Potentiation of morphine antinociception by monoamine reuptake inhibitors in the rat spinal cord.* Pain 1985, 21: 329-37.

39. Suh, H.H., Fujimoto, J.M., Tseng, L.L.F. *Differential mechanisms mediating beta-endorphin and morphine induced analgesia in mice.* Eur J Pharmacol 1989, 168: 67-70.

40. Hylden, J.L., Thomas, D.A., Iadarola, M.J., Nahin, R.L., Dubner, R. *Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: Possible*

involvement of noradrenergic mechanisms. Eur J Pharmacol 1991, 194: 135-43.

41. Henry, J.L. *Substance P and inflammatory pain: Potential of substance P antagonists as analgesics.* Agents Actions Suppl 1993, 41: 75-87.

42. Miampamba, M., Chery-Croze, S., Detolle-Sarbach, S., Guez, D., Chayvialle, J.A. *Antinociceptive effects of oral clonidine and S-12813-4 in acute colon inflammation in rats.* Eur J Pharmacol 1996, 308: 251-9.

43. Rogers, D.T., Iwamoto, E.T. *Multiple spinal mediators in parenteral nicotine-induced antinociception.* J Pharmacol Exp Ther 1993, 267: 341-9.

44. Kuraishi, Y. *Neuropeptide mediated transmission of nociceptive information and its regulation. Novel mechanisms of analgesics*. Yakugaku Zasshi 1990, 110: 711-26.

45. Ballet, S., Aubel, B., Mauborgne, A., Poliénor, H., Farre, A.J., Cesselin, F., Hamon, M., Bourgoin, S. *The novel analgesic, cizolirtine, inhibits the spinal release of substance P and CGRP in rats.* Neuropharmacology 2001, 40: 578-89.

46. Martinez, L., Serafini, M.T., Pretel, M.J., Garcia-Soret, A., Moragon, T., Puig, S. *Absorption, distribution, metabolism and excretion of cizolirtine, a new analgesic compound, in rats and dogs.* Xenobiotica 1999, 29: 859-71.

47. Monck, N. *Cizolirtine evaluation.* Curr Opin Invest Drugs 2001, 2: 1269-72.

48. Shembalkar, P., Täubel, J., Abadias, M., Arezina, R., Hammond, K., Anand, P. *Cizolirtine citrate (E-4018) in the treatment of chronic neuropathic pain.* CDurr Med Res Opin 2001, 17: 262-6.