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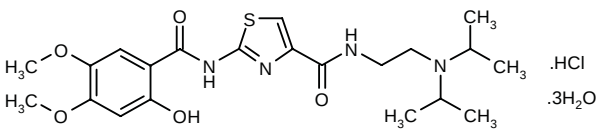
Z-338

Treatment of Non-Ulcer Dyspepsia

N-[4-[*N*-[2-(Diisopropylamino)ethyl]carbamoyl]thiazol-2-yl]-2-hydroxy-4,5-dimethoxybenzamide hydrochloride trihydrate
N-[2-(Diisopropylamino)ethyl]-2-(2-hydroxy-4,5-dimethoxybenzamido)thiazole-4-carboxamide hydrochloride trihydrate



$C_{21}H_{30}N_4O_5S.HCl.3H_2$
Mol wt: 541.0623
CAS: 185104-11-4 (anhydrous)
CAS: 185106-16-5 (as anhydrous free base)
CAS: 403651-06-9 (as trihydrate)
CAS: 211999-70-1 (as maleate)
EN: 262475

Abstract

Research efforts focusing on discovering gastroprokinetic agents with mechanisms of action different from available compounds have identified Z-338, a 2-(acylamino)thiazole-4-carboxamide derivative, as having potent gastroprokinetic activity and an excellent safety profile. Results from preclinical studies demonstrated that Z-338 enhances spontaneous contractions and electrically stimulated excitatory junction potentials and acetylcholine release, possibly through inhibition of muscarinic M_1 and M_2 autoreceptors and possibly an M5-like receptor. Z-338 has been shown to be safe in phase I trials involving healthy volunteers in Europe and Japan and in an early phase II trial conducted in patients with functional dyspepsia. Z-338 continues to undergo phase II development.

Synthesis

Z-338 can be prepared by two closely related ways:

1) Acylation of 2-aminothiazole-4-carboxylic acid ethyl ester (I) with 2,4,5-trimethoxybenzoyl chloride (II) gives the corresponding amide (III). The 2-methoxy group of (III) is then selectively cleaved by treatment with pyridine hydrochloride, to yield the 2-hydroxybenzamide (IV). Finally, the ethyl ester group of (IV) is displaced by *N,N*-diisopropylethanediamine (V) upon heating at 120 °C (1). Scheme 1.

2) Acid chloride (II), prepared by treatment of 2,4,5-trimethoxybenzoic acid (VI) with $SOCl_2$ in hot toluene, is condensed with aminothiazole (I) to provide amide (III). Displacement of the ethyl ester group of (III) by *N,N*-diisopropylethanediamine (V) at 135 °C gives diamide (VII), which by treatment with hydrochloric acid in isopropanol results in the simultaneous cleavage of the 2-methoxy group and formation of the hydrochloride salt (2). Scheme 2.

Introduction

Functional dyspepsia is an extremely common upper abdominal disease. Individuals suffering from the disorder experience chronic symptoms such as early satiety, upper abdominal pain, fullness, bloating, nausea and vomiting in the absence of organic disease. Pathologically, the symptoms of functional dyspepsia are thought to be due to delayed gastric emptying, impaired gastric accommodation and visceral hypersensitivity. At present, gastroprokinetic agents such as cisapride, domperidone, itopride and mosapride which accelerate gastric emptying, are the mainstay for treatment of the disorder. However, these agents have been associated with extrapyramidal syndrome, increased plasma prolactin levels and/or severe adverse cardiovascular effects.

Researchers have therefore focused efforts on discovering potent and safe gastroprokinetic agents with mechanisms of action distinct from available agents. After pharmacologically evaluating a number of newly synthesized compounds, a 2-(acylamino)thiazole-4-carboxamide derivative Z-338 was found to have excellent gastroprokinetic activity that was as potent as cisapride and more potent than other agents such as itopride or mosapride. Moreover, Z-338 did not induce severe adverse events (3, 4). The mechanisms of action involved in the gastroprokinetic effects of Z-338 are still not known, although the drug appears to increase contraction, increase release of acetylcholine (ACh) and inhibit

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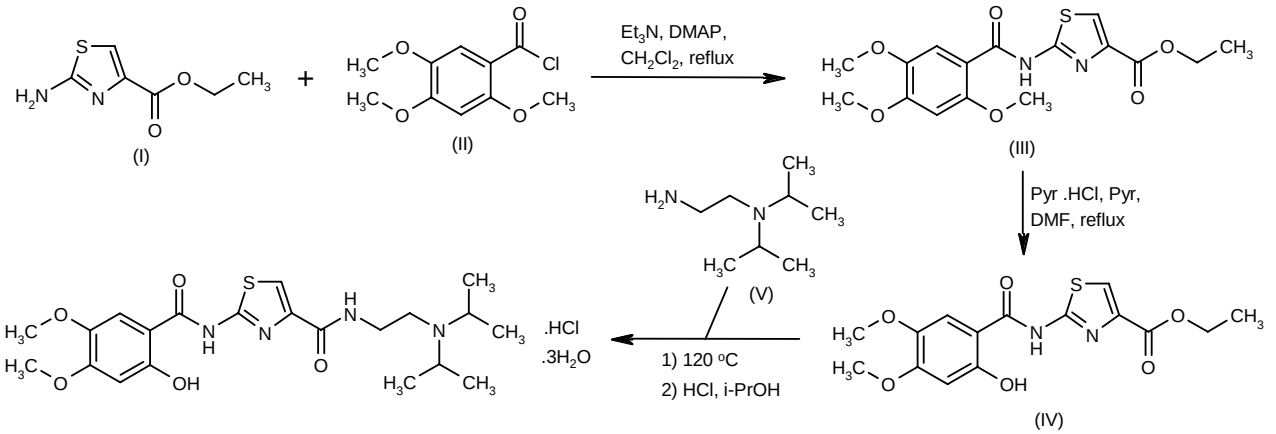
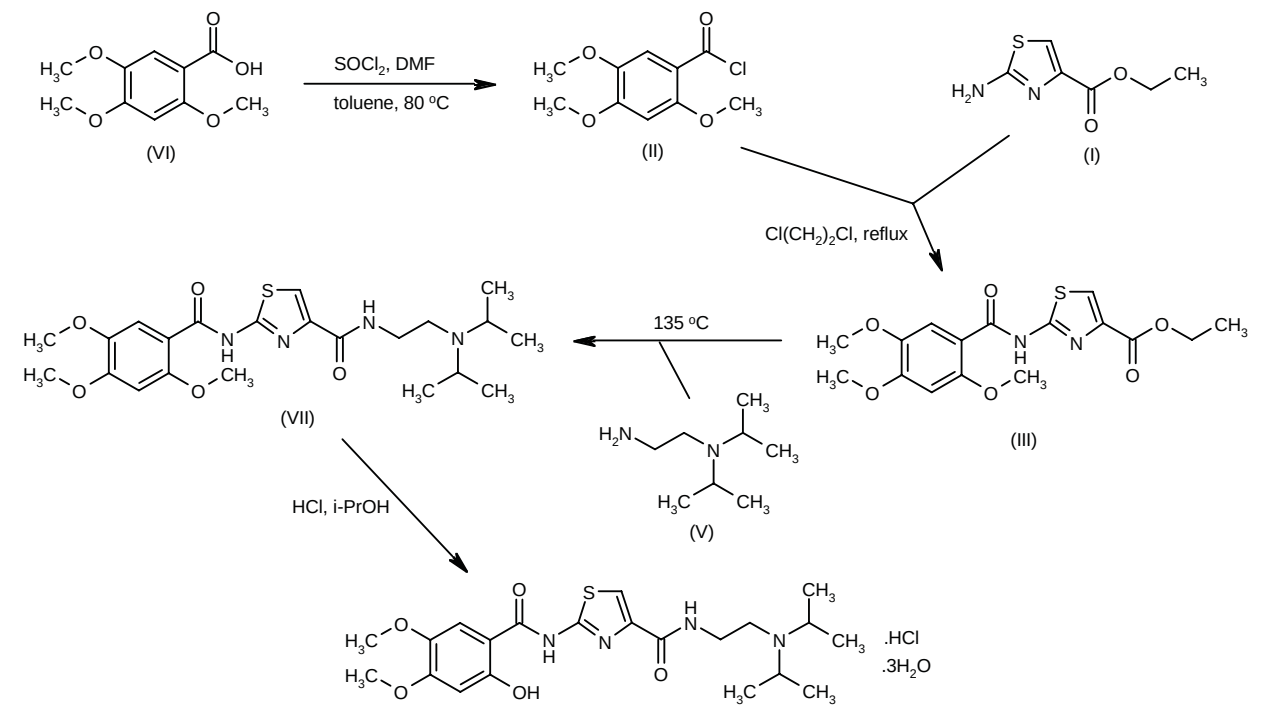
Scheme 1: Synthesis of Z-338



Scheme 2: Synthesis of Z-338



acetylcholinesterase (AChE). Z-338 has affinity for the muscarinic M_1 and M_2 receptors but not for the serotonin 5-HT_2, 5-HT_3 or 5-HT_4 receptors. In addition, Z-338 has been shown to enhance excitatory-neuro-effector transmission via prejunctional mechanisms. Due to its excellent preclinical safety and efficacy profiles, Z-338 was chosen for further development as a treatment for functional dyspepsia.

Pharmacological Actions

Several preclinical *in vitro* and *in vivo* studies have attempted to elucidate the mechanism(s) of action involved in the gastroprokinetic effects of Z-338.

A study examining guinea pig stomach motility using tension and microelectrode recordings of circular gastric muscle strips revealed that Z-338 (greater than 10 nM)

dose-dependently enhanced the amplitude of twitch-like contractions and excitatory junction potentials (EJPs) induced by single or repetitive electrical field stimulation. The agent had no effects on nonadrenergic noncholinergic (NANC) relaxation or on inhibitory junction potentials (IJPs). Z-338 (EC_{50} = 47 nM; Hill coefficient = 0.96) enhanced EJP amplitude in a manner similar to the muscarinic M_1 antagonist pirenzepine (greater than 10 nM; EC_{50} = 10 nM; Hill coefficient = 0.94). In contrast, the M_2 antagonist AF-DX116 and the M_3 antagonist 4-diphenylacetoxy-*N*-methylpiperine (4-DAMP) dose-dependently decreased EJP amplitude. These results suggest that the gastroprokinetic effects of Z-338 may be due, in part, to enhancement of excitatory neuro-effector transmission so that ACh release is enhanced via inhibition of a prejunctional M_1 receptor in the stomach (3).

Results from another *in vitro* study involving tension recordings of guinea pig circular gastric muscle strips and membrane binding studies concluded that Z-338 facilitates ACh release from cholinergic nerve terminals via blockade of muscarinic M_1 and M_2 autoreceptors. Z-338 (3-30 μM) was shown to enhance electrically evoked contractions and release of ACh. Binding studies revealed that Z-338 displaced [^{3}H]-pirenzepine binding to rat cortex membrane (*i.e.*, M_1 receptor; K_i = 8.4 μM) and [^{3}H]-*N*-methyl-scopolamine (NMS) binding to rat heart membrane (*i.e.*, M_2 receptor; K_i = 9.4 μM); the agent had no effect on [^{3}H]-NMS binding to rat submaxillary gland membrane (*i.e.*, M_3 receptor) (4).

An *in vitro* study using *Xenopus* oocytes heterologously expressing muscarinic M_1 and M_2 receptors showed that Z-338 inhibited ACh (100 nM)-induced Ca^{2+} activated Cl^- currents in oocytes expressing M_1 receptors (IC_{50} = 2 μM) and muscarinic inward K^+ currents in oocytes expressing M_2 receptors and Kir3.1 (GIRK1) (IC_{50} = 7 μM). Results further suggest that the agent acts as an antagonist at the M_1 and M_2 receptors (4, 5).

It is possible that Z-338 may also activate a M_5-like receptor and modulate the M_3 receptor, according to results of an in vitro study using whole-cell patch clamped isolated guinea pig gastric myocytes to examine the effect of the agent on L-type Ca^{2+} currents. While ACh was found to both enhance (1-100 nM) and inhibit (1-100 μM) ICa, Z-338 (1 nM or more; ED_{50} = 120 nM) only exhibited an enhancing effect. Treatment with a combination of ACh and Z-338 resulted in nonadditive enhancing effects which were blocked by both intracellular GDPβS and extracellular 4-DAMP but not pertussis toxin (PTX), pirenzepine, AF-DX116 or oxybutynin. In addition, intracellular perfusion of cells with the protein kinase C activator phorbol-12,13-dibutyrate (PDBu; 150 μM) blocked the enhancing effects of both Z-338 and ACh on I_{Ca}. Results suggest that the M_3 receptor may be involved in the effects of Z-338. Moreover, because the actions of Z-338 were resistant to PTX and abolished by extracellular atropine or intracellular GDPβS or PDBu, its action on I_{Ca} in gastric myocytes may also involve activation of an M_5-like receptor (6).

Further electrophysiological studies involving nystatin perforated patch recording from freshly isolated rat paratracheal ganglion cells indicated that Z-338 acts on both M_1 receptors and neuronal nicotinic ACh receptors. Z-338 was found to competitively inhibit inward currents induced by oxotremorine-M, indicating antagonism of the M_1 ACh receptor. In addition, Z-338 noncompetitively abolished nicotine-induced inward currents. These inhibitory actions of Z-338 could contribute to the agent's gastroprokinetic effects (7).

The prokinetic effects of Z-338 were compared to cisapride on isolated circular segments of opossum lower esophageal sphinctor. Z-338 (10 nM to 10 μM) dose-dependently increased basal tone of segments in a manner more potent than cisapride (10 nM to 1 μM). In addition, Z-338 produced greater contractions than equidoses of cisapride. Z-338-induced contractions were unaffected by treatment with the selective 5-HT_4 antagonist GR-113808 but partially inhibited by atropine. Z-338-induced increases in basal tone were inhibited by TTX and TTX + ω-conotoxin GVIA (ω-CTX). In contrast, while atropine, TTX and TTX + ω-CTX had no effect on cisapride-induced contractions, GR-113808 was an effective blocker. Results suggest that the mechanism of action of the two agents is distinct. Cisapride appears to act directly on smooth muscle of the opossum lower esophageal sphinctor while Z-338 acts indirectly via modulation of cholinergic neurotransmission of excitatory postganglionic nerve endings (8).

The efficacy of Z-338 has been demonstrated *in vivo* with results indicating that the agent acts via antagonism of M_1 and M_2 autoreceptors.

Experiments conducted in conscious dogs with chronically implanted force transducers demonstrated that treatment with Z-338 (0.3-3 mg/kg i.v. or 3-30 mg/kg p.o.) enhanced postprandial gastric antral and duodenal motor activity. Z-338 at an oral dose of 30 mg/kg was more potent than cisapride (3 mg/kg p.o.), itopride (30 mg/kg p.o.) or mosapride (10 mg/kg p.o.). Atropine completely inhibited the enhancing activity of Z-338 while serotonin 5-HT_4 receptor antagonists had no effect. Z-338 had no effect on dopamine-induced suppression of gastric activity. Results from experiments using anesthetized dogs showed that Z-338 (0.3-3 mg/kg) significantly enhanced ACh release from the gastric antrum in a dose-dependent manner; comparable effects were observed with 3 mg/kg Z-338 and 0.3 mg/kg cisapride. Pirenzepine and AF-DX116 also increased ACh release from gastric antrum. These results suggest that the gastroprokinetic effects of Z-338 are unrelated to dopamine D_2 receptors or serotonin 5-HT_4 receptors but instead to enhancement of ACh release via antagonism of M_1 and M_2 autoreceptors (9-11).

The effects of Z-338 on gastric emptying were examined in dogs and rats treated with clonidine or cholecystokinin to suppress gastric motility and emptying. Both Z-338 (0.5 mg/kg i.v. and over) and cisapride (0.1 mg/kg) restored hypomotility to normal levels and improved clonidine-induced delayed gastric emptying in a similar manner. While Z-338 had no effect on normal gastric

Box 1: Efficacy and safety of Z-338 in functional dyspepsia (14) [from Prous Science Integrity®).

Design	Multicenter, dose-finding, placebo-controlled, double-blind, randomized clinical study
Population	Patients with functional dyspepsia symptoms and a negative endoscopy (n = 62)
Treatments	Z-338, 50 mg p.o. t.i.d. x 3 wks (n = 16) Z-338, 100 mg p.o. t.i.d. x 3 wks (n = 14) Z-338, 300 mg p.o. t.i.d. x 3 wks (n = 17) Placebo (n = 15)
Conclusions	Z-338 was safe and well tolerated, with headache being the most frequent adverse event. The 100-mg dose appeared to improve symptoms and quality of life in patients with functional dyspepsia

emptying, a dose of 10 mg/kg i.p. improved this parameter in clonidine- or cholecystokinin-treated rats. These results indicate that Z-338 may be effective in the treatment of symptoms such as hypomotility and delayed gastric emptying in patients with functional dyspepsia (11).

Pharmacokinetics

A novel HPLC method has been described to determine Z-338 in dog plasma. The limit of detection was 2.5 ng/ml. The method was effective in monitoring plasma Z-338 concentrations in dogs orally administered the agent (30 mg/kg). The agent was found to be rapidly absorbed from the gastrointestinal tract with a C_{max} of 2643.3 ng/ml obtained at 0.4 h postdosing; the AUC value was 5997.3 ng·h/ml. Z-338 was eliminated from plasma within 24 h of dosing ($t_{1/2}$ = 2.9 h). It was concluded that this method may be used for therapeutic monitoring of plasma Z-338 concentrations (12).

The pharmacokinetics, safety and tolerability of oral single doses (25-800 mg in the fasted state and 200 mg given 35 min before or after food) and multiple doses (100 and 300 mg t.i.d. 35 min before food for 6 days) of Z-338 were examined in a phase I, double-blind, placebo-controlled trial conducted in healthy male subjects. Z-338 was safe and concluded to be generally well tolerated. The agent was rapidly absorbed displaying biphasic disposition; absorption was more rapid when administered before food intake. AUC and C_{max} values were dose-proportional after single doses of 50-400 mg. Steady-state was reached after 24 h with multiple dosing with slight accumulation noted; AUC and C_{max} values increased in a greater than dose-proportional manner. Less than 5% of the administered dose was excreted in urine. A minor metabolite, C1 deisopropyl Z-338, was detected in both plasma and urine (13).

Clinical Studies

A phase II, multicenter, randomized, double-blind, placebo-controlled, parallel-group pilot study involving 62 patients with symptoms of functional dyspepsia for more than 3 months, examined the efficacy, safety and tolerability of oral Z-338 (50, 100 or 300 mg t.i.d. for 3 weeks). Z-338 was safe and well tolerated. The most common adverse event was headache. A significant benefit over placebo was observed with the 300 mg dose of Z-338 for mean and maximum postmeal gastric relaxation. In addition, relaxation was significantly improved in 4 patients with impaired meal accommodation. Z-338 had no significant effect on gastric sensitivity or gastric emptying at any of the doses tested. Significant improvements over placebo in bloating and global symptom scores were seen at week 2 and in bloating, heartburn and postprandial fullness at week 3 in patients given the 100 mg Z-338 dose. In this treated group, symptom scores improved from baseline at week 2 and 3 (respectively) as follows: from 5.21 to 2.08 and 2.56 for bloating; from 5.21 to 2.43 and 2.93 for fullness; from 3.96 to 1.96 and 2.34 for early satiety; from 4.04 to 1.69 and 1.57 for pain; from 2.19 to 0.88 and 0.87 for heartburn; and from 5.79 to 2.8 and 3.27 for global symptom score. Patients treated with 100 mg Z-338 also showed significant improvements in the quality of life over placebo for physical and social functioning. One Z-338-treated patient experienced a worsening of upper abdominal discomfort (14) (Box 1).

Z-338 has completed phase I trials in Europe and Japan and an early phase II trial in Europe. A late phase II trial in patients with functional dyspepsia is planned in North America (15).

Source

Discovered at Zeria Pharmaceutical Co., Ltd. (JP); licensed to Yamanouchi Pharmaceutical Co., Ltd. (JP) for the U.S. and Canada.

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