Drugs of the Future 2007, 32(5): 399-407

CCC: 0377-8282/2007
DOI: 10.1358/dof.2007.032.05.1092901

Lecozotan Hydrochloride

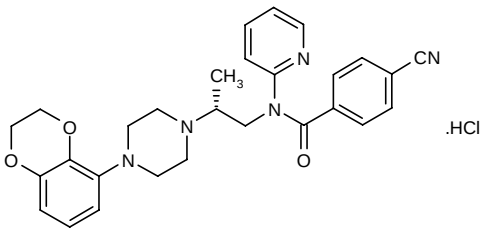
Rec INNM; USAN

Cognition Enhancer
Treatment of Alzheimer's Disease
Competitive 5-HT$_{1A}$ Receptor Antagonist

SRA-333

4-Cyano-*N*-[(2*R*)-2-[4-(2,3-dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]propyl]-*N*-2-pyridinylbenzamide monohydrochloride

InChI=1/C28H29N5O3.ClH/c1-21(31-13-15-32(16-14-31)24-5-4-6-25-27(24)36-18-17-35-25)20-33(26-7-2-3-12-30-26)28(34)23-10-8-22(19-29)9-11-23;/h2-12,21H,13-18,20H2,1H3;1H/t21-;/m1./s1



$C_{28}H_{30}ClN_5O_3$
Mol wt: 520.0224

CAS: 433282-68-9

CAS: 434283-16-6 (free base)

EN: 322323

Abstract

Lecozotan is a potent, selective, competitive, orally active antagonist of the serotonin 5-HT$_{1A}$ receptor in development for the symptomatic treatment of cognitive deficits such as those seen in Alzheimer's disease. The agent shows no intrinsic activity in a number of *in vitro* and *in vivo* assays, supporting the conclusion that it is a full antagonist under several conditions. *In vivo*, lecozotan enhances the potassium-stimulated release of both acetylcholine and glutamate, and it improves cognitive performance in multiple animal models of learning and memory. The compound was well tolerated in single- and multiple-ascending-dose (SAD and MAD) safety trials in both young and elderly subjects at total daily doses up to and including 10 mg (5 mg every 12 h) for the immediate-release formulation. Sustained-release formulations have been developed which provide favorable pharmacokinetics for once-daily dosing. Lecozotan is currently under investigation in advanced phase II clinical trials.

Synthesis

Lecozotan (IV) can be prepared in three steps from known starting materials. Condensation of (*S*)-4,5-dihydro-5-methyl-3-(2-pyridyl)-3*H*-[1,2,3]oxathiazole-2,2-dioxide (I) with 1-(2,3-dihydro-4,5-benzodioxan-5-yl)piperazine (II) in the presence of potassium carbonate gives an intermediate sulfamate, which is hydrolyzed to the amine (III) with warm aqueous HCl. The reaction proceeds with inversion of the configuration, as confirmed by X-ray crystallography, which shows the configuration of lecozotan to be (*R*). Acylation of amine (III) with 4-cyanobenzoyl chloride yields the final product (IV) in 75% overall yield, which is isolated as the monohydrochloride salt (1-3). Scheme 1.

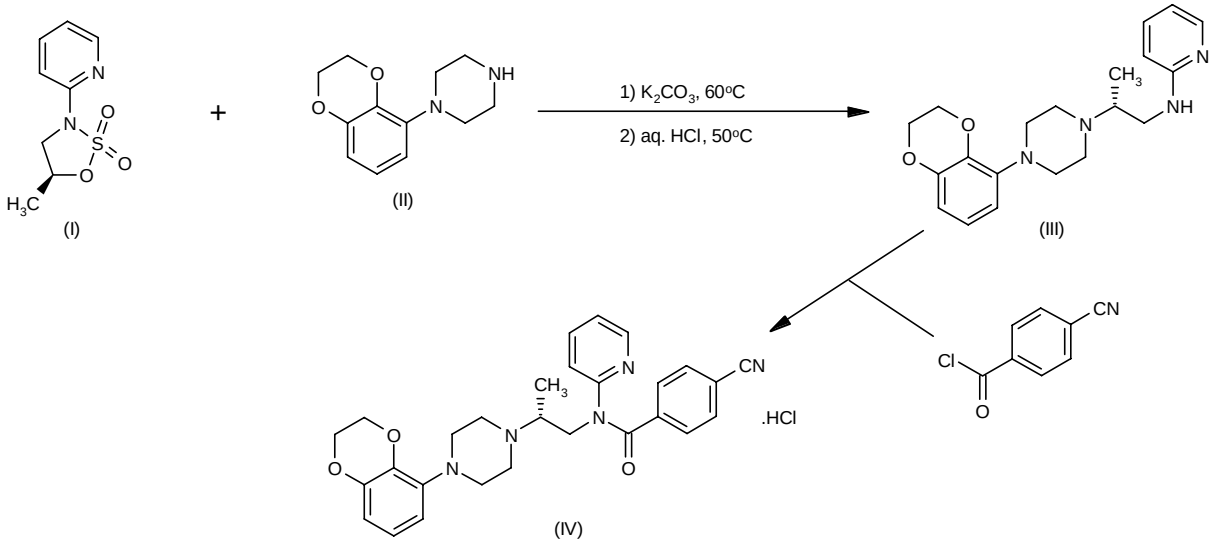
A number of patents and patent applications cover various aspects of the synthesis of lecozotan, as well as alternate synthetic routes (3-5).

Background

Alzheimer's disease (AD) is a progressive brain disorder that gradually destroys a victim's memory and cognitive processes, ultimately making it impossible to carry out routine daily activities. It is believed that damage to the brain occurs years before symptoms begin to appear. Nearly 28 million people worldwide suffer from AD and other forms of dementia, and the cost of direct and indirect care is estimated to approach USD 248 billion annually. Seventy percent of AD patients live at home, placing

Wayne E. Childers, Jr.[1]*, Boyd L. Harrison[1], Magid A. Abou-Gharbia[1], Sangeeta Raje[2], Virginia Parks[3], Menelas N. Pangalos[4], Lee E. Schechter[4]. [1]Chemical & Screening Sciences, Wyeth Research, CN-8000, Princeton, NJ 08543-8000, USA; [2]Early Development and Clinical Pharmacology, Wyeth Pharmaceuticals, 500 Arcola Road, Collegeville, PA 19426-3982, USA; [3]Early Development and Clinical Pharmacology, Wyeth Pharmaceuticals, Coeur Defense - Tour A - La Defense 4, 92931 Paris la Defense Cedex, France; [4]Neuroscience, Wyeth Research, CN-8000, Princeton, NJ 08543-8000, USA. *Correspondence: e-mail: childew@wyeth.com.

Scheme 1: Synthesis of Lecozotan Hydrochloride



a tremendous burden on families and friends, especially in the later stages of the disease. As the current population continues to live longer than previous generations, the incidence of AD may exceed society's ability to absorb the added healthcare costs and care-giving resource demands (6, 7).

Although the exact causes of AD remain unknown, the pathophysiology of the disease has been extensively studied. The disease process is characterized by a progressive loss of neurons and synapses and the presence of large numbers of extracellular amyloid plaques and intracellular neurofibrillary tangles. Not all neurons are susceptible to the disease process. In fact, the initial discoveries of reduced levels of choline acetyltransferase in certain regions of the basal forebrain and a reduction in this population of cholinergic neurons in the brains of AD patients led to the "cholinergic hypothesis" of AD. A number of key findings have also led researchers to postulate a central role for β-amyloid (Aβ) and amyloid precursor protein (APP). These findings include the identification in familial AD patients of mutations in genes associated with APP processing, a correlation of AD with individuals carrying the apolipoprotein E4 gene (known to modulate Aβ aggregation and its clearance from the brain), and the neurotoxic effects of aggregated and soluble oligomeric species of Aβ *in vitro* and *in vivo*. Another hallmark of AD is the presence of neurofibrillary tangles. Tangles are generated after the aggregation and assembly of a hyperphosphorylated microtubule-binding protein, tau, into intracellular paired helical filiments. Abnormal accumulation of hyperphosphorylated tau is also seen in the neuritic processes found in association with senile plaques. The exact role of tangles and hyperphosphorylated tau in the neurodegenerative process is not completely understood. However, the number and density of tangles in AD patients are strongly correlated with the degree of cognitive impairment (8).

There are currently no approved treatments for AD that modify the disease process, although experimental drugs such as secretase inhibitors and amyloid-sequestering immunotherapeutics are currently being examined in clinical trials. The few symptomatic agents that are available target neurotransmitter systems that are known to decline during the progression of the disease. Cholinesterase inhibitors, which inhibit the breakdown of acetylcholine (ACh), have been available since the approval of tacrine (Cognex®) in 1993. Tacrine is rarely prescribed today owing to the drug's side effects; however, newer agents such as donepezil (Aricept®), rivastigmine (Exelon®) and galantamine (Razadine®) are commonly used. Cholinesterase inhibitors are generally characterized by a modest response rate and a relatively short period during which the drugs induce an improvement in symptoms, although recent analysis suggests that beneficial effects can be seen in some patients for up to 3 years. Memantine (Namenda®), a low-affinity uncompetitive inhibitor of the *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor, was approved for the symptomatic treatment of AD in 2003. It is currently approved for use in the later stages of the disease and may remain effective for longer periods compared to most cholinesterase inhibitors. However, its effect on cognitive decline and clinical deterioration has also been classified as modest, and the limited data from one clinical trial suggest that memantine may be more effective when given to patients who are already receiving and continue to receive donepezil (8-12).

AD is characterized by multiple deficits in neurotransmitter function. Early research concentrated on the decline of the ACh system, but it has become apparent

that not all patients display deficits in the ACh system alone. Glutamate and its receptors play an important role in learning and memory, especially the NMDA subtype of the glutamate receptor, which is involved in neuronal plasticity and long-term potentiation. Recent research suggests that deficits in the glutamatergic system may occur prior to those observed in the ACh system, an event which could help explain the limited efficacy and short efficacy time window seen with cholinesterase inhibitors. In contrast to ACh and glutamate, the serotonin (5-HT) neurotransmitter system may be hyperactive in AD as a result of enhanced turnover of 5-HT. This finding led Bowen *et al.* to hypothesize in 1994 that 5-HT_{1A} antagonists might function as an effective treatment for the cognitive deficits associated with AD. Serotonergic neurons provide inhibitory tone to cholinergic and glutamatergic neurons which stimulate cortical and CA3 hippocampal pyramidal cells (Fig. 1). Serotonergic neurons also innervate and directly provide inhibitory tone to the cortical pyramidal pathways. 5-HT_{1A} receptors play a major role in this inhibitory signal. Thus, 5-HT_{1A} antagonists might be expected to reverse AD-associated cognitive deficits both by enhancing excitatory cholinergic and glutamate neurotransmission and by blocking direct inhibitory serotonergic input. In support of this theory, the 5-HT_{1A} full antagonist WAY-100635 demonstrates cognition-enhancing effects in numerous animal models of learning and memory, and similar efficacy has recently been described with the orally active analogue WAY-405 (13-15).

Preclinical Pharmacology

Lecozotan (SRA-333) is an orally administered, competitive 5-HT_{1A}-selective receptor antagonist developed as a cognition enhancer for the symptomatic relief of cognitive deficits such as those observed in AD. It competitively inhibited the binding of [^{3}H]-8-OH-DPAT and [^{3}H]-WAY-100635 to human 5-HT_{1A} receptors (Table I) stably transfected in Chinese hamster ovary (CHO) cells (IC_{50} = 1.6 and 4.5 nM, respectively). In a NovaScreen® assessment (Table II), lecozotan was > 100-fold selective for 5-HT_{1A} receptors compared to over 50 different receptors, ion channels and transporters, with the exception of dopamine D4 receptors, where lecozotan displayed a K_i value of 98 nM and concomitant 61-fold selectivity. *In vitro* antagonist activity was assessed using two models (Table I). In CHO cells stably transfected with the human 5-HT_{1A} receptor, lecozotan displayed a full antagonist profile by completely blocking the ability of the 5-HT_{1A} full agonist 8-OH-DPAT to inhibit forskolin-induced cAMP production, without eliciting any agonist-like effects on its own (IC_{50} = 25.1 nM). This result was confirmed in a [^{35}S]-GTPγS binding assay employing the same cell system, where lecozotan completely blocked 8-OH-DPAT-induced increases in GTPγS binding but did not display any agonist activity alone (IC_{50} = 36.7 nM) (1, 16).

The intrinsic activity of lecozotan was studied *in vivo* using several models which are indicative of functional activity. Both neurochemical and behavioral endpoints were examined. In an *in vivo* microdialysis assay using concious rats, 8-OH-DPAT (0.3 mg/kg s.c.) induced a significant decrease in extracellular levels of 5-HT in the hippocampus. Pretreatment with lecozotan (0.3 mg/kg s.c.) completely attenuated the response to 8-OH-DPAT. Treatment with lecozotan alone at doses up to 3 mg/kg s.c. did not produce any agonist-like effects, confirming the lack of intrinsic activity even at high doses. In a rat *in vivo* electrophysiology assay, 8-OH-DPAT (0.025 mg/kg

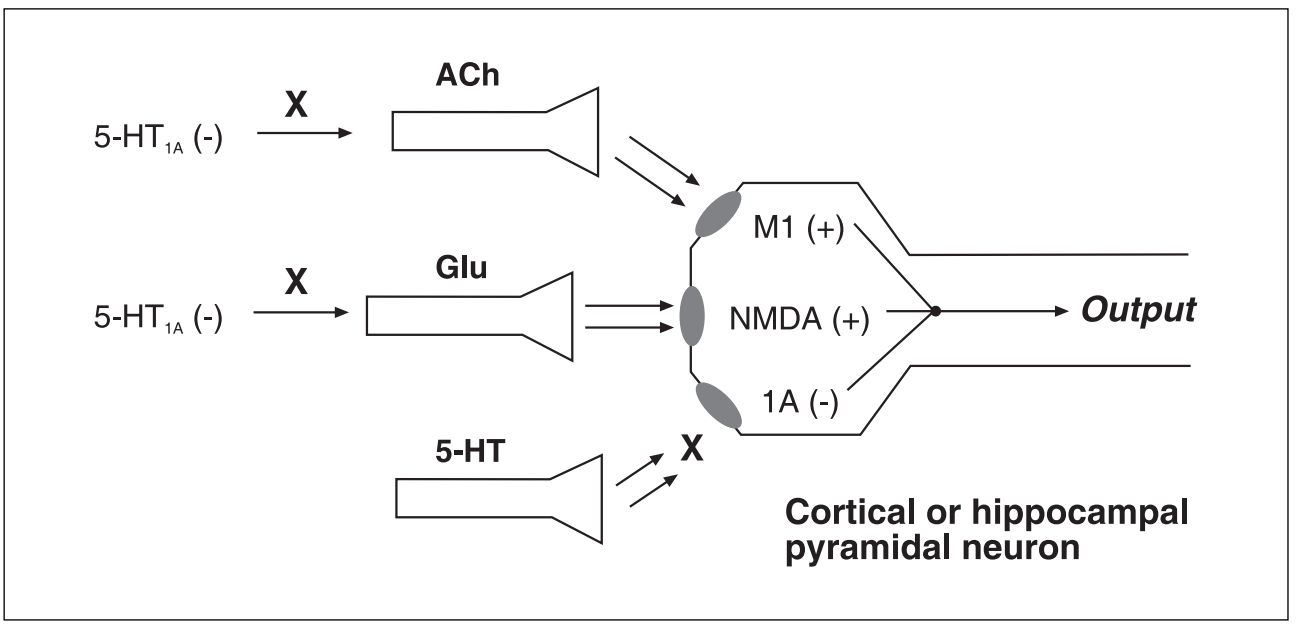


Fig. 1. Proposed mechanism of action for a 5-HT_{1A} antagonist in Alzheimer's disease. Reproduced (with modifications) with permission from the authors and Bentham Science Publishers, Ltd., from Ref. 13.

Table I: In vitro binding and functional data for lecozotan.

	[^{3}H]-8-OH-DPAT	5-HT$_{1A}$ [^{3}H]-WAY-100635	α_1	D2	D3	D4	cAMP	GTPγS
K_i (nM)	1.6 ± 0.3	4.5 ± 0.6	248	1548	320	98		
IC_{50} (nM)							25.1	36.7

cAMP = inhibition of 8-OH-DPAT-induced decrease in forskolin-stimulated cAMP production; GTPγS = inhibition of 8-OH-DPAT-induced increase in GTPγS binding.

Table II: Receptors examined via NovaScreen®.

Adenosine	**Norepinephrine**	**Dopamine**	**GABA$_A$**	**Glutamate**	
A1	1B	D1	Agonist site	Competitive NMDA	
A2	2A	D5	Benzodiazepine site	Kainate	
A3	2B	Transporter		AMPA	
Transporter	2C			MK-801	
	β1			PCP	
	β2			Strychnine-insensitive glycine	
	Transporter				
Histamine	**Cholinergic**	**Opiate**	**Serotonin**	**Sigma**	**Other**
H1	M1	Delta 1	5-HT1B	Sigma 1	Benzodiazepine (peripheral)
H2	M2	Delta 2	5-HT1D	Sigma 2	Glycine (strychnine-insensitive)
H3	M3	Kappa	5-HT2A		Imidazoline I2
	M4	Mu	5-HT2C		Melatonin
	M5		5-HT3		K-ATP site
	Nicotinic		5-HT4		Na channel (site 2)
			5-HT5A		
			5-HT6		
			5-HT7		
			Transporter		

s.c.) produced a significant inhibition of dorsal raphe neuronal firing. Lecozotan (0.3 mg/kg s.c.) produced no changes in dorsal raphe neuronal firing alone. However, pretreatment with lecozotan (0.3 mg/kg s.c.) significantly antagonized the 8-OH-DPAT effect. High doses of 8-OH-DPAT (0.625 mg/kg s.c.) were required to overcome the antagonist effect of lecozotan and restore inhibition of dorsal raphe neuronal firing to levels equal to those seen in the absence of lecozotan. The dorsal raphe firing assay is thought to be indicative of presynaptic 5-HT$_{1A}$ "autoreceptor" function and is particularly sensitive to low levels of intrinsic activity because of the high degree of receptor reserve. A lack of agonist activity in these two models, even at high doses, strongly suggests that lecozotan is a full antagonist at both pre- and postsynaptic 5-HT$_{1A}$ receptors (1, 16).

Behavioral assays confirmed that lecozotan is a potent 5-HT$_{1A}$ full antagonist. In a fixed-ratio operant responding model in rats, 8-OH-DPAT produced a dose-dependent decrease in response rate (lever press to obtain a food reward under an FR30 schedule of food presentation), with an ED_{50} value of 0.18 mg/kg s.c. Pretreatment with lecozotan (0.3-3.0 mg/kg s.c.) produced a rightward shift of the 8-OH-DPAT dose-effect curve. The maximum effect, a 20-fold shift in the 8-OH-DPAT dose-effect curve, was obtained at the dose of 3.0 mg/kg s.c. Oral pretreatment with lecozotan (3-30 mg/kg) also produced rightward shifts in the 8-OH-DPAT dose-effect curve, with the maximum effect (9.5-fold shift) seen at the highest dose. Similar results were obtained in a fixed-ratio operant responding model in squirrel monkeys (lever press to avoid a low-intensity electric shock to the tail under an FR10 schedule). 8-OH-DPAT (ED_{50} = 0.07 mg/kg i.m.) produced a dose-dependent decrease in the response rate. Intramuscular or oral pretreatment with lecozotan shifted the dose-effect curve of 8-OH-DPAT to the right, with maximum effects obtained at doses of 0.1 mg/kg i.m. (17-fold shift) and 1.0 mg/kg p.o. (10-fold shift). In a drug discrimination model using pigeons trained to an 8-OH-DPAT stimulus cue, pretreatment with lecozotan (0.01-1.0 mg/kg i.m.) dose-dependently decreased the percentage of responses on the agonist-appropriate key, with a complete shift to the saline-appropriate key seen at the dose of 1.0 mg/kg i.m. Response rates were unaffected by lecozotan when given alone at doses up to 1.0 mg/kg i.m. Finally, chronic treatment with lecozotan (10 mg/kg p.o. once daily for 7 days) inhibited the ability of 5-methoxy dimethyltryptamine (5-MeODMT) to induce the serotonin syndrome in rats. The serotonin syndrome was again induced with 5-MeODMT 24 h after the last dose of lecozotan and the agonist potency of lecozotan was determined. The ID_{50} value for lecozotan in the chronic drug-treated animals (4.26 mg/kg i.p.) did not differ significantly from the acute value or the potency after chronic vehicle treatment (4.92 mg/kg i.p.). In separate studies, the agonist potency of 5-MeOMDT was evaluated 24 h after the last dose of lecozotan. The ED_{50} value was similar in both chronic drug-treated animals

(1.68 mg/kg i.p.) and chronic vehicle-treated animals (1.83 mg/kg i.p.). These data suggest that 5-HT_{1A} receptor sensitivity was unaltered following chronic lecozotan treatment and that no tolerance developed (16).

Bowen's original hypothesis suggests that a 5-HT_{1A} antagonist may augment cortical pyramidal function by relieving the serotonergic inhibitory tone on cholinergic and glutamatergic pathways and enhancing the release of these two excitatory neurotransmitters. *In vivo* microdialysis techniques were employed to examine the effect of lecozotan on glutamate and ACh release. Lecozotan (0.3 mg/kg s.c.) produced a significant augmentation of K^+-induced increases in glutamate release in the rat dentate gyrus, enhancing extracellular glutamate levels by 471.5% over baseline levels and 318% over the maximum effect obtained with potassium chloride alone. By itself, lecozotan (1.0 mg/kg s.c.) had no effect on glutamate levels. In the rat hippocampal CA1 region, lecozotan (1.0 mg/kg s.c.) induced a small but significant increase in extracellular ACh (146% of baseline). Infusion of 100 mM potassium chloride stimulated ACh release to 200% of baseline values. Co-administration of lecozotan (1.0 mg/kg s.c.) further potentiated the potassium-stimulated ACh release to 275% of basal levels. These data indicate that lecozotan enhances stimulated excitatory neurotransmitter release in neural pathways thought to be important in AD (14, 16).

The cognition-enhancing effect of lecozotan was examined in a number of animal models of learning and memory. In one series of tests, marmosets were trained to perform tasks in order to receive a food reward. Two types of tasks were examined. The visual discrimination task, which is thought to be based in cortical brain regions, involves presenting two different objects to the animal in random positions. The food reward is always situated under the same object and the animal must learn to choose that object to receive the reward. The second task, a visuospatial discrimination task, is thought to involve hippocampal function. The animals must learn a conditional rule to receive a reward rather than acquire a simple association. For example, two pairs of identical objects are presented to the marmoset and the food reward is associated with each pair of objects. However, the identity of the object determines where the food reward is located (*e.g.*, a pair of key rings signifies that the food is under the leftmost object, while a pair of Eppendorf tubes means that the food reward is always under the rightmost object). The marmoset must learn to associate the location of the food reward with the object's identity. In both types of tasks, learning is expressed as a "mean learning score", the number of trials needed to successfully learn the task to criterion. Cognitive deficits in both the marmoset visual and visuospatial discrimination tasks can be induced by antagonists of the NMDA-specific glutamate receptor, such as the uncompetitive NMDA antagonist dizocilpine (MK-801). Treatment with lecozotan (2.0 mg/kg i.m.) prior to testing completely reversed the dizocilpine-induced cognitive deficits, returning the mean learning score to that of control animals that did not receive dizocilpine. Lecozotan alone had no effect on mean learning scores (16).

In marmosets, cholinergic input to the hippocampus can be interrupted by a specific lesion of the vertical diagonal band (VDB) induced by the neurotoxin saporin conjugated to an antibody that recognizes the p75 human low-affinity nerve growth factor receptor (NGFR). This lesion results in a significant impairment in learning the hippocampus-associated visuospatial discrimination task (*i.e.*, a higher mean learning score). Treatment with lecozotan (2.0 mg/kg i.m.) prior to the start of the session abolished the learning impairment caused by VDB lesion and returned the mean learning score to near that of non-lesioned animals. Lecozotan showed no effect in nonlesioned animals (16).

The effect of lecozotan on cognitive performance was also examined in aged rhesus monkeys using a delayed match-to-sample task. In this test, animals are presented with four press-keys that can be illuminated with a variety of colors. One of the four keys (the "sample" key) displaying a certain color is illuminated and then extinguished. A time interval passes and then two other keys are illuminated with the sample color and another random color. The animal must remember the original color and press the similarly colored key to receive a food reward. The time interval between sample presentation and trial is varied and categorized into intervals (zero interval, short interval, medium interval and long interval). Several doses of lecozotan (0..3-3.0 mg/kg i.m.) were examined and the effect on accuracy was analyzed based on the dose of the drug that provided the greatest cognition-enhancing effect for each of the time interval categories. The data were then analyzed across the four time interval categories. Treatment with lecozotan resulted in an average 16.5% improvement in task accuracy above baseline, which represents a very good level of efficacy compared with other compounds tested under similar conditions in this setting. The greatest effect was seen in trials associated with short time intervals, which may suggest that lecozotan has a significant effect on attentional aspects of cognition in this model. No untoward effects of the drug were noted before or after testing (16).

Clinical Studies

The safety, pharmacokinetics (PK) and pharmacodynamics (PD) of lecozotan have been examined in healthy volunteers (Table III). A summary of lecozotan PK data is presented in Table IV. Lecozotan was administered as an immediate-release (IR) formulation in a single-ascending-dose (SAD) study, a multiple-ascending-dose (MAD) study, a single-dose positron emission tomography (PET) study, an age-gender study and a MAD study in elderly subjects at single doses up to 10 mg and multiple doses up to 5 mg every 12 h. In an SAD study, oral doses of 2, 5 and 10 mg were examined in cohorts of 8 male subjects (6 actives and 2 placebos). Doses up to and including 5 mg were well tolerated. Dose-limiting mild to moderate CNS adverse events were observed in the 6 active sub-

Table III: Clinical studies of lecozotan.

Population	Design	Treatments	n	Formulation	Conclusions	Ref.
Healthy young male subjects	SAD Randomized Double-blind Placebo-controlled Sequential	Lecozotan, 2, 5, 10 mg p.o. Placebo	24	IR	Lecozotan was safe and well tolerated at doses up to and including 5 mg p.o. Its PK profile allowed for twice-daily dosing	17
Healthy young & elderly subjects (men and women)	MAD Randomized Double-blind Placebo-controlled Sequential	Lecozotan, 0.1, 0.25, 0.5,1, 5 mg p.o. 1x/12 h x 1 wk Placebo	49	IR	Lecozotan was safe and well tolerated at doses up to and including 5 mg p.o. for 14 days. Slightly higher exposure was seen in the elderly. No gender PK differences were noted. Its PK profile allowed for twice-daily dosing	18
Healthy young, young elderly and elderly men and women	Single-dose Randomized Double-blind Placebo-controlled Crossover	Lecozotan, 5 mg p.o. Placebo	48	IR	Lecozotan was well tolerated in all age groups. PK in the elderly were characterized by a small decrease in clearance that did not justify any dose adjustment	19
Healthy elderly subjects (men and women)	Multiple-dose Randomized Double-blind Placebo-controlled	Lecozotan, 5 mg p.o.1x/12 h x 14 d Placebo	16	IR	Lecozotan was safe in elderly subjects at multiple doses of 5 mg p.o. PK in the elderly were characterized by a small decrease in clearance that did not justify any dose adjustment	20
Healthy young male subjects	Single-dose Randomized Open-label Crossover	Lecozotan, 5 mg of one IR formulation and 3 SR formulations Placebo	20	IR, SR	Bioequivalence with the IR formulation suggests that the SR-fast and SR-medium formulations are suitable for further development. Low C_{max}/C_{24h} ratios indicate favorable properties for once-daily administration	21
Healthy young subjects, elderly subjects and AD patients	Single-dose Nonrandomized Sequential PET study	Lecozotan, 0.5, 1, 5 mg p.o. [^{11}C]-WAY-100635	20	IR	Receptor occupancy increased in a dose-dependent manner. A 5-mg dose resulted in an RO of 44% in young, 63% in elderly and 55% in AD patients. A once-daily dose of the SR formulation is predicted to produce ROs similar to those seen with a b.i.d. dose of the IR formulation	22

SAD = single ascending dose; MAD = multiple ascending dose; IR = immediate release; SR = sustained release; PET = positron emission tomography; RO = receptor occupancy.

jects at the higher dose of 10 mg, including paresthesia, euphoria (sensation of feeling high), sensorial disturbances and dizziness. No individual clinically significant sustained drug-related changes in vital signs, ECGs and routine laboratory tests were recorded. No clinically relevant impairments in attention, sensorimotor tasks and working and episodic memory were observed, as measured using the Cognitive Drug Research battery. The compound was rapidly absorbed (t_{max} = 1 h or less) and eliminated ($t_{1/2}$ = 6-8 h) and displayed linear kinetics. In a MAD study, oral doses of 0.1, 0.25, 0.5, 1.0 and 5.0 mg of lecozotan IR were administered every 12 h for 14 days to 41 young healthy subjects and 8 elderly healthy subjects (cohorts of 8 containing 6 actives and 2 placebos). Clinical signs and cognitive effects were recorded as described for the SAD study above. No dose-related CNS adverse events or effects on cognitive performance were observed. Lecozotan was rapidly absorbed (t_{max} = 1 h or less) and eliminated ($t_{1/2}$ = 5-7 h). Plasma concentrations exhibited approximately linear dose-proportionality over the entire dose range studied and multiple-dose PK were well predicted from the single-dose PK profile. In the elderly, lecozotan steady-state C_{max} and AUC values were moderately higher than for the young subjects. No gender differences were observed. The conclusions from these two studies were that lecozotan IR was safe and well tolerated up to daily doses of 10 mg (5 mg every 12 h) for 14 days, with the PK profile of the IR formulation supporting a twice-daily dosing regimen (17, 18).

To investigate the PK, PD and safety of lecozotan in subjects representing the likely target population, two studies were performed in healthy elderly subjects (Table III). In one study, a single 5-mg oral dose of lecozotan IR was examined in a 2-period, placebo-controlled, crossover study in 48 healthy subjects (8 men and 8 women in each of the following age groups: 18-45

Table IV: Pharmacokinetic profile of lecozotan after oral administration in humans.

	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	AUC (ng.h/ml)	Ref.
SAD study (single oral dose)					
Young males					17
2 mg	142	0.5	6.2	686[1]	
5 mg	232	0.92	6.2	1639[1]	
10 mg	476	0.79	8.2	3203[1]	
MAD study in healthy young & elderly (multiple oral dose b.i.d. for 14 days)					
Day 1 (single dose)					18
Young subjects					
0.1 mg	6.2	0.5	6.1	40[1]	
0.25 mg	12.2	0.54	5.3	68[1]	
0.5 mg	30.4	0.67	6.7	216[1]	
1 mg	50.3	0.5	6.0	315[1]	
5 mg	263.7	0.67	6.8	1666[1]	
Healthy elderly					
0.5 mg (males)	36	0.3	8.5	302[1]	
0.5 mg (females)	39	0.4	9.4	247[1]	
Day 14 (steady state)					
Young subjects					
0.1 mg	8.9	0.54	7.1	44[2]	
0.25 mg	11.7	0.42	5.9	55[2]	
0.5 mg	36.8	0.79	7.0	222[2]	
1 mg	74.7	0.46	7.9	382[2]	
5 mg	360	0.58	9.1	2001[2]	
Healthy elderly					
0.5 mg (males)	53	0.3	11	308[2]	
0.5 mg (females)	58	0.4	10.6	331[2]	
MAD study in healthy elderly (multiple oral dose of 5 mg b.i.d. for 14 days)					
Day 1 (single dose)	356	0.6	9.4	2924[1]	20
Day 14 (steady state)	527	0.6	11.0	3245[2]	
Gender study (single oral dose of 5 mg)					
Young men	267.8	0.75	9.8	2092[1]	19
Young women	313.9	0.53	9.3	2313[1]	
Young elderly men	273.8	0.5	9.7	2017[1]	
Youne elderly women	347.5	0.47	13.5	3215[1]	
Elderly men	316.1	0.41	14.4	2996[1]	
Elderly women	336.1	0.41	12.2	2748[1]	

[1]AUC = $AUC_{0-\infty}$; [2]AUC = AUC_{ss}.

[young], 65-74 [young elderly] and 75 years or more [elderly]). Evaluations consisted of the same parameters described for the SAD and MAD studies above. No clinically relevant effects on vital signs, ECG or routine laboratory tests or cognitive impairment were observed. The incidence of treatment-emergent adverse events (AEs) in elderly subjects was 50% less than in young subjects. Lecozotan was rapidly absorbed in all age groups (t_{max} < 1 h). The mean $t_{1/2}$ for lecozotan was longer in elderly subjects compared to young patients (14.8 h *versus* 9.7 h) due to a small reduction in body weight-adjusted oral clearance in elderly subjects (17% in men, 25% in women). The lecozotan AUC was approximately 30% higher in elderly subjects in comparison to young subjects. Lecozotan C_{max} values were 17-25% higher in women compared to men, which could be explained by weight differences (mean weight = 69 kg for women and 81 kg for men). These differences did not reach statistical significance based on the results of a 2-way ANOVA. In terms of the safety profile of lecozotan, the PK profile in elderly subjects did not justify any dose adjustment (19).

In the second study (MAD study in elderly), a 5-mg p.o. dose of lecozotan IR given every 12 h for 14 days was examined in 16 healthy elderly subjects (12 actives and 4 placebos). Tolerability was good, with few treatment-emergent AEs reported. The most common AEs were paresthesia, asthenia and headache, mainly reported by 1 subject. Lecozotan t_{max} was < 1 h and the $t_{1/2}$ was 9-11 h. Steady state was achieved by day 3 and the accumulation ratio was 1.8. Mean steady-state AUC (*e.g.*,

$AUC_{0\text{-}12h}$) was within 108% of the mean single-dose $AUC_{0\text{-}\infty}$, which suggests reliable multiple-dose predictability from single-dose PK. Lecozotan PK in AD patients are similar to those in healthy elderly subjects, and hence healthy elderly subjects can be used as surrogates for the target population (20).

To achieve once-daily dosing, sustained/modified-release (SR) formulations were developed for lecozotan and examined in 20 young healthy subjects in a single-dose, randomized, 4-period crossover study (Table III). The formulations were labeled based on the rate of drug release (slow, medium and fast). During the course of the study, all 20 subjects received a single 5-mg p.o. dose of the three SR formulations and a 5-mg p.o. dose of the IR formulation. Lecozotan t_{max} with the SR formulations was delayed to 4-6 h (t_{max} < 1 h for IR). Based on $AUC_{0\text{-}\infty}$, the SR-fast and SR-medium formulations were bioequivalent to the IR formulation (Table V). C_{max} values for the SR-fast and SR-medium formulations were approximately one-third those seen with the IR formulation. Lecozotan C_{max}/C_{12h} ratio for the IR formulation was approximately 5 in comparison to C_{max}/C_{24h} ratios of 2.3 for the SR-medium and 2.8 for the SR-fast formulations, which are intended to support once-daily administration. Treatment-emergent AEs were substantially less frequent with the SR formulations (1 *vs.* 14 for IR), presumably due to reduced C_{max}. The study concluded that the SR-fast and SR-medium formulations of lecozotan were suitable for further development and that the low C_{max}/C_{24h} ratios showed favorable properties for once-daily dosing (21, 22).

To guide dose selection for phase II clinical studies, the binding of lecozotan to 5-HT_{1A} receptors in the human brain was examined using PET and the PET tracer [^{11}C]-WAY-100635 (Table III). A single dose of 0.5, 1.0 or 5.0 mg p.o. of the IR formulation was administered to healthy young subjects and a dose of 5.0 mg p.o. of the IR formulation was administered to healthy elderly subjects and AD patients to access possible binding differences due to age or the disease state. The lecozotan plasma concentration-receptor occupancy relationship (RO) was described using an E_{max} pharmacodynamic model by means of a population approach. Peak and trough RO were predicted for various regimens of the IR and SR formulations. The average temporal cortex 5-HT_{1A} RO increased in a dose-dependent manner following single doses of the IR formulation (RO at 1-2 h postdose: 10% at 0.5 mg, 18% at 1 mg and 44% at 5 mg). After a single 5.0-mg dose of lecozotan, maximum RO observed at around lecozotan t_{max} (1-2 h) was 44% in young, 63% in healthy elderly and 53% in AD patients. Based on the E_{max} model, the lecozotan SR formulation is predicted to produce steady-state peak/trough ROs of approximately 37%/20%, 58%/38% and 71%/53% after a once-daily dose of 2, 5 and 10 mg, respectively. These results suggest that, at steady state, a once-daily regimen of the lecozotan SR formulation is predicted to achieve similar maximum and minimum RO throughout the dosing interval in comparison to the b.i.d. regimen of the IR formulation (23).

Conclusions

In conclusion, lecozotan is a potent, selective 5-HT_{1A} full antagonist that shows no intrinsic activity in numerous *in vitro* and *in vivo* assays. The compound enhances the stimulated release of ACh from the CA1 region of the rat hippocampus, as well as stimulated glutamate release from the rat dentate gyrus. These brain regions and neurotransmitter systems are thought to decline in the progression of AD. Lecozotan demonstrated cognition-enhancing effects in multiple animal models of learning and memory, including the performance of visual discrimination and visuospatial discrimination tasks in a marmoset monkey model, where deficits were induced in the cholinergic (VDB lesion) and glutamatergic (dizocilpine) neuronal pathways, and in a delayed match-to-sample task in aged rhesus monkeys. The compound was well tolerated by both young and elderly subjects during SAD and MAD safety trials at total daily doses up to and including 10 mg (5 mg every 12 h) for the IR formulation. A small decrease in clearance was observed in elderly subjects, but the difference did not justify any adjustment in dose. PK studies support a twice-daily dosing regimen for the IR formulation, but SR formulations have been devel-

Table V: Comparison of PK parameters for lecozotan SR formulations.

Formulation		C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0\text{-}\infty}$ (ng.h/ml)
IR	Mean	278.5	0.9	7.1	2059
	Geometric mean	269.3	0.8		1959
SR-fast	Mean	99.7	5.6	8.2	2247
	Geometric mean	97.4	5.0		2102
	Geometric mean ratio[1]	36.2			107.3
SR-medium	Mean	95.1	8.8	7.7	2222
	Geometric mean	84.3	6.2		2021
	Geometric mean ratio[1]	31.3			103.2
SR-slow	Mean	58.4	7.9	8.0	1565
	Geometric mean	55.3	4.5		1323
	Geometric mean ratio[1]	20.5			67.5

[1]Geometric mean ratio = ratio of geometric least square means for SR formulation *vs.* IR formulation.

oped which provide favorable PK for once-daily dosing. PET studies predict that the IR and SR formulations will achieve similar receptor occupancy in the brain. The frequency of treatment-emergent AEs was significantly less with the SR formulations, probably due to the lower C_{max}. Lecozotan is currently being examined in advanced phase II clinical trials for the treatment of cognitive dysfunction, such as that seen in AD.

Acknowledgements

The authors thank L.E. Schechter, L.A. Dawson and J.A. Harder and the publisher Bentham Science Publishers, Ltd., for their kind permission to reproduce (with modifications) Figure 1 from Ref. 13. The authors also thank Jeff Paul, Jonathan Bard and Phillip Vinall for their assistance in the preparation of this manuscript.

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