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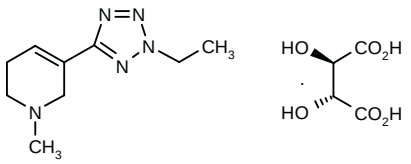
LU-25-109T

Cognition Enhancer
Muscarinic M_1 Agonist
Muscarinic M_2/M_3 Antagonist

2-Ethyl-5-(1-methyl-1,2,3,6-tetrahydropyridin-5-yl)-2*H*-tetrazole L-(+)-tartrate
5-(2-Ethyltetrazol-5-yl)-1-methyl-1,2,3,6-tetrahydropyridine L-(+)-tartrate



$C_9H_{15}N_5.C_4H_6O_6$; Mol wt: 343.3420

CAS: 159792-14-0
CAS: 120241-32-9 [as oxalate (1:1)]

EN: 216348

Synthesis*

The methylation of 3-cyanopyridine (I) with methyl iodide in acetone gives 3-cyano-1-methylpyridinium iodide (II), which is reduced with $NaBH_4$ in methanol/water yielding 1-methyl-1,2,5,6-tetrahydropyridine-3-carbonitrile (III). The reaction of (III) with ethyl chloroformate and K_2CO_3 in 1,1,1-trichloroethane affords 3-cyano-1,2,5,6-tetrahydropyridine-1-carboxylic acid ethyl ester (IV), which is cyclized with sodium azide by means of $AlCl_3$ in refluxing THF giving tetrazole (V). Alkylation of (V) with ethyl iodide and NaOH in refluxing acetone, followed by column chromatography, yields 3-(2-ethyltetrazol-5-yl)-1,2,5,6-tetrahydropyridine-1-carboxylic acid ethyl ester (VI). The decarboxylation of (VI) with HBr in acetic acid affords 2-ethyl-5-(1,2,5,6-tetrahydropyridin-3-yl)tetrazole (VII), which is methylated with formaldehyde/formic acid or with methyl iodide to give 2-ethyl-5-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)tetrazole (VIII) (1, 2). Finally, this compound is treated with L-(+)-tartaric acid (2). Scheme 1.

Description

Crystals, m.p. 143-5 ºC.

Introduction

Cholinergic neurotransmission is regulated by two different classes of receptors which have been termed nicotinic and muscarinic according to the alkaloids nicotine and muscarine, which have long been known to act as their respective nonphysiologic agonists. The muscarinic system consists of at least four subtypes (M_1, M_2, M_3, M_4) that can be distinguished pharmacologically and according to their macroscopic (tissue) and miscroscopic localization. While the postsynaptic M_1 receptor reconverts the chemical signal (embodied by the arrival of acetylcholine that has crossed the neuronal junction) back to an electrical one, M_2 autoreceptors are presynaptically located and act as part of a feedback loop that limits acetylcholine discharge from the “upstream” neuron. Ideally, a muscarinic receptor ligand intended as a potential treatment for cognitive impairment secondary to impaired cholinergic neurotransmission should act as a selective central M_1 agonist and M_2 antagonist, with no activity at M-type receptors that regulate smooth muscle function, blood pressure and salivary glands. While other such compounds in clinical development exert these activities mostly on a functional level (*i.e.*, by taking advantage of tissue-specific receptor reserve which varies over a wide range), LU-25-109T has a more pronounced intrinsic partial agonist profile and may be able to act on a pharmacological level. There is hope that this compound will outperform xanomeline, to which it is chemically related (both molecules are bioisosteres of the naturally occurring muscarinic agonist, arecoline).

While most of the attention of developers has focused on Alzheimer’s disease, the role of the cholinergic system in functional recovery from events such as stroke and traumatic brain injury has only recently drawn more attention. Lundbeck, the discoverer of LU-25-109T, is one of the few companies that are pioneering these new clinical indications.

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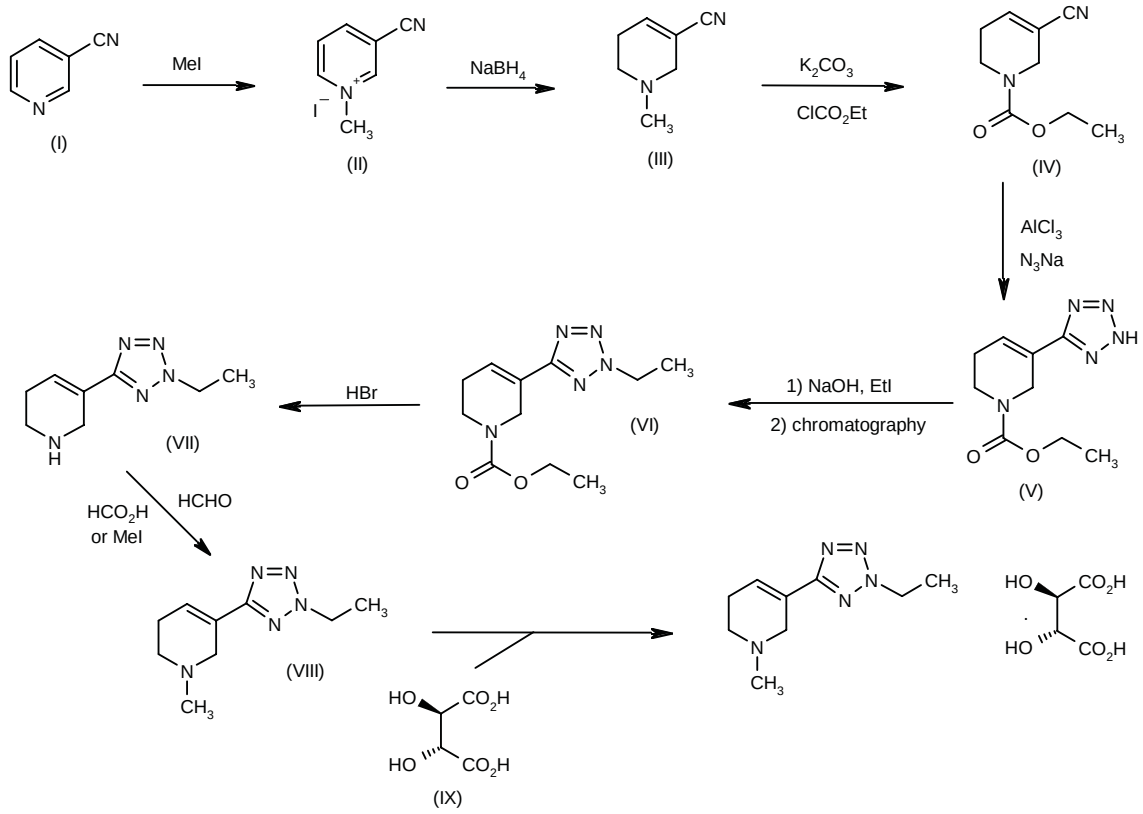
Scheme 1: Synthesis of LU-25-109T



Pharmacological Actions

In dedicated *in vitro* characterizations (3), LU-25-109T was found to have higher affinity for the M_1 receptor than most standard agonists, with the exception of xanomeline, which was 13 times as potent at M_1. LU-25-109T had the following K_i values: 36 nM (*vs.* [^{3}H]-pirenzepine) on M_1 receptors in rat brain; 130 nM on cloned human M_1 (hm1) receptors expressed on CHO-K1 cells; 340 nM (*vs.* [^{3}H]-quinuclidinyl benzylate) on M_2 receptors in rat brain stem; and 3100 nM (*vs.* [^{3}H]-*N*-methylscopolamine) on M_3 receptors in rat salivary glands. The selectivity ratio towards M_1 versus M_3 was 44; the M-agonist index (defined as the quotient of K_i values in oxotremorine-M and quinuclidinyl benzilate binding assays) of 47 was comparable to that of RS-86 and several times higher than that of xanomeline (8.1). LU-25-109T had no measurable affinity for phencyclidine, dopamine D_1, adrenergic α_1 and β, 5-HT_3, histaminergic H_1, nicotinic, kainate, $GABA_A$ and TBPS binding sites, or for monoaminergic uptake sites. It had very low affinity (13-49 μM) for σ_2, 5-HT_{1A} and 5-HT_{2A} receptors, and low affinity (4.1-7.4 μM) for σ_1, α_2, D_2 (agonist) and 5-HT_{2C} receptors.

In *ex vivo* tissue studies, LU-25-109T acted as a partial agonist at the rat superior cervical ganglion M_1 receptors (EC_{50} = 2 μM for agonism, IC_{50} = 15 μM for antagonism) and at the guinea pig ileum (M_1, M_2 and M_3), while it acted as an antagonist at the guinea pig left atrium M_2 receptors (EC_{50} = 2.7 μM, IC_{50} = 1.5 μM) and on cultured cerebellar granule cell M_3 receptors (IC_{50} = 3.6 μM). In drug discrimination studies, LU-25-109T behaved as an M_1 agonist, while no prominent effects of the compound were observed in other *in vivo* models, such as standard tests for anxiolytic potential, locomotor activity, isoniazid-induced convulsions and grid shock-induced nociception. It significantly improved performance of young and aged rats in the Morris maze, a cognition model of spatial learning and memory (4).

To test the hypothesis that activation of the muscarinic cholinergic system during the recovery period after traumatic brain injury will improve cognitive performance, rats received daily s.c. injections for 15 days with either 0.0, 3.6 or 15 μmol/kg of LU-25-109T beginning 24 h after delivery of a moderate (2.1 ± 0.1 atm shock force) central fluid percussion brain injury. Cognitive performance was assessed on days 11-15 postinjury in a Morris water maze. Injured rats treated with 15 μmol/kg, but not those treated with 3.6 μmol/kg, showed a significant improve-

ment ($p < 0.01$) in maze performance as compared with injured rats treated with vehicle (5).

Another investigation (6) examined the effects of traumatic brain injury on basal forebrain choline acetyltransferase immunoreactivity (ChAT-IR) following daily administration of saline or 15 μmol/kg LU-25-109T. Rats were injured as described above or were surgically prepared (but not injured) and were injected (s.c.) with saline or drug on days 1-15 postinjury. Brain trauma caused a significant reduction in ChAT-IR neuronal density in saline- and LU-25-109T-treated rats with a 13% and 5% decrease in the medial septal nucleus (MSN), a 48% and 23% decrease in the vertical limb nucleus of the diagonal band (VDB), and a 51% and 28% decrease in the nucleus basilis magnocellularis (NBM), respectively. However, LU-25-109T significantly attenuated the injury-induced reductions in ChAT-IR. Loss in ChAT-IR-positive neuronal density was not thought to be a result of cell death as parallel cresyl violet-stained sections indicated no decrease in neuronal cell density in the MSN, VDB or NBM. These results support the hypothesis that increasing cholinergic tone during the recovery period after traumatic brain injury will restore cholinergic function impaired by brain trauma.

Pharmacokinetics

LU-25-109T readily permeated the blood-brain barrier in mice, with an oral bioavailability of 42% and a short half-life ($t_{1/2}$ = 41 min after oral administration of 116 μmol/kg) in brain. Maximum concentrations after i.v., s.c. and p.o. administration were reached within 5, 10 and 20 min, respectively. Radioactivity was eliminated from the brain 4 h after p.o. administration and 1 h after i.v. administration (3).Side effects normally associated with muscarinic agonists, such as hypothermia, tremor and salivation, were mild or absent. In anesthetized cats, no effects on blood pressure, heart rate and orthostatic reflex mechanism were observed, except for a short-lasting (< 2 min), dose-related decrease in both blood pressure and heart rate (4).

Clinical Studies

The safety and tolerability of LU-25-109T were evaluated in young and elderly healthy subjects in 1 open-label, single-dose study, 2 open-label, multiple-dose studies and 1 double-blind, randomized, placebo-controlled single- and multiple-dose study (7). More than 100 subjects have been given LU-25-109T in doses ranging from 0.5 mg as a single dose to 130 mg q.i.d. for 7 days. The drug was well tolerated. Adverse events were mostly mild and of short duration. Headache, leg pain and dizziness have been reported randomly across the dose levels. Salivation and gastrointestinal symptoms were seen only with the higher doses.

A double-blind, placebo-controlled, two-part, inpatient bridging study was designed to evaluate the safety and tolerability of multiple oral doses of LU-25-109T in patients with Alzheimer's disease, and to determine the maximum tolerated dose (MTD) in this population (8). In the first part of the study, the fixed-dose MTD was to be determined in five consecutive panels of 6 patients each (4 on LU-25-109T and 2 on placebo). Doses for the five panels were 100, 125, 150, 200 and 225 mg t.i.d. for 7 days. Cholinergic adverse events such as increased salivation, dizziness and gastrointestinal symptoms were observed at all doses studied. The dosing of fixed-dose panels was discontinued after 3 days at 200 mg t.i.d. due to unacceptable gastrointestinal adverse events. Thus, 150 mg t.i.d. was defined as the fixed-dose MTD. The second part of the study, conduced in a single panel of 8 patients (6 on LU-25-109T and 2 on placebo), was designed to determine if patients could tolerate higher doses of LU-25-109T when administered on a titration regimen. Patients were to receive doses that were 50%, 75%, 100%, 125% and 150% of the fixed dose MTD, with dose increases every 5 days. The first dose, 75 mg t.i.d., was very well tolerated; however, as in the first phase of the study, patients did not tolerate the 200 mg t.i.d. dose. Thus, the titration regimen employed did not improve the overall tolerability of LU-25-109T.

Meanwhile, approximately 30 centers in the United States, and more centers elsewhere, have participated in a double-blind, placebo-controlled, 32-week study which enrolled about 480 persons aged 45 and over who were diagnosed with mild to moderate Alzheimer's disease. Medication or placebo was given for 26 weeks. An optional open-label extension study will be offered after the initial study ends. Results have not yet been reported (9).

It is not known if plans for clinical development of LU-25-109T for the treatment of traumatic brain injury exist at this time.

Forest Laboratories has announced the discontinuation of LU-25-109T for Alzheimer's disease following unsatisfactory results of the completed phase II/III clinical trial. However, the company does plan to continue exploring other potential uses for LU-25-109T, such as urinary incontinence (10).

Manufacturer

Lundbeck A/S (DK), licensed to Forest Laboratories (US).

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