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SEMAGACESTAT

Rec INN; USAN

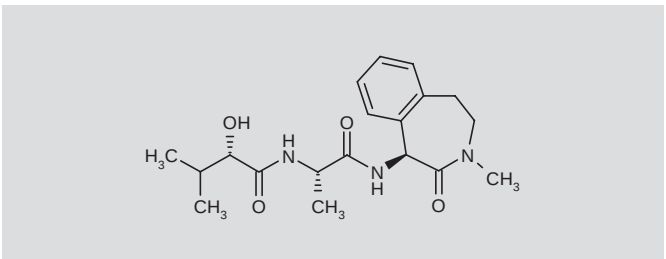
γ-Secretase Inhibitor
Treatment of Alzheimer's Disease

LY-450139

N^2-[2(*S*)-Hydroxy-3-methylbutyryl]-*N*^{1}-[3-methyl-2-oxo-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1(*S*)-yl]-L-alaninamide

2(*S*)-Hydroxy-3-methyl-*N*-[1(*S*)-methyl-2-oxo-2-[2,3,4,5-tetrahydro-3-methyl-2-oxo-1*H*-3-benzazepin-1(S)-ylamino]ethyl]butyramide

InChI=1S/C19H27N3O4/c1-11(2)16(23)18(25)20-12(3)17(24)21-15-14-8-6-5-7-13(14)9-10-22(4)19(15)26/h5-8,11-12,15-16,23H,9-10H2,1-4H3,(H,20,25)(H,21,24)/t12-,15-,16-/m0/s1



$C_{19}H_{27}N_3O_4$
Mol wt: 361.4354
CAS: 425386-60-3
EN: 322926

ABSTRACT

γ-Secretase has been identified as a therapeutic target for Alzheimer's disease (AD), as it plays a role in the production of β-amyloid (Aβ) peptide, a key component of AD plaques. Lilly is currently developing semagacestat (LY-450139), an inhibitor of γ-secretase, for the treatment of mild to moderate AD. In vivo and clinical studies have shown that semagacestat reduces Aβ levels and safety analyses have confirmed its tolerability in healthy volunteers and patients. Semagacestat has progressed to phase III clinical trials, which are currently ongoing in the United States.

SYNTHESIS

Semagacestat can be prepared as follows:

Racemic amine (I) can be resolved by the following alternative procedures: 1) treatment with (–)-*O,O'*-di-*p*-toluoyl-L-tartaric acid in refluxing MeOH and subsequent hydrolysis of the resulting salt with NaOH in CH_2Cl_2 affords the desired (*S*)-enantiomer as the free base (II) (1-5); and 2) treatment with (*R*)-(–)-mandelic acid in *i*-PrOAc/*i*-PrOH, and then with 5-nitrosalicylaldehyde at 45 °C, and final hydrolysis of the resulting salt with HCl in EtOAc yields the hydrochloride of compound (II) (1-4). The nondesired (*R*)-enantiomer (III) can be restituted to the corresponding racemate (I) by treatment with Et_3N in refluxing MeOH (5). Condensation of *N*-Boc-L-alanine (IV) with isopropyl chloroformate (V) by means of NMM in THF generates mixed anhydride (VI) (4). Without isolation, coupling mixed anhydride (VI) with 1(*S*)-amino-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (II) in the presence of NMM in THF followed by treatment with HCl in acetonitrile at 62 °C yields the hydrochloride salt (VII) (4), while treatment with MsOH in H_2O at 62 °C affords the corresponding mesylate salt (VIII) (4). Scheme 1.

An alternative procedure to compound (VII) begins by condensating amine (II) with *N*-Boc-L-alanine (IV) in the presence of HOBt.H_2O, DIEA and EDC in THF to yield the *N*-Boc-L-alaninamide derivative (IX) (1-4). Similarly, the hydrochloride of amine (II) reacts with *N*-Boc-L-alanine (IV) in the presence of HOBt.H_2O, DIEA and EDC in THF (6). Intermediate (IX) is then deprotected with either HCl in EtOAc or 1,4-dioxane (1-4), or concentrated HCl (4, 5), and the resulting amine (VII) is obtained as the free base or employed without isolation, respectively. Finally, either hydrochloride (VII) or the nonisolated species obtained from compound (IX) is condensed with 2(*S*)-hydroxy-3-methylbutyric acid (X) by means of HOBt.H_2O, NMM and EDC.HCl in H_2O to give semagacestat (4, 6). Under related conditions, the free base of (VII) couples with acid (XI) in the presence of HOBt.H_2O, DIEA and EDC in THF (1-4). Alternatively, silylation of 2(*S*)-hydroxy-3-methylbutyric acid (X) with HMDS in the presence of $(NH_4)_2SO_4$ at reflux gives trimethylsilyl 3-methyl-2(*S*)-(trimethylsilyloxy)butanoate (XI), which is first reacted with $(COCl)_2$ in the presence of DMF in CH_2Cl_2 and then with the mesylate salt (VIII) by means of NMM in CH_2Cl_2 (4). Scheme 1.

Intermediate (I) can be obtained by two different strategies.

Amination of 2-phenylethyl bromide (XII) with methylamine in THF gives *N*-methylphenethylamine (XIII) (5), which upon *N*-acylation with chloroacetyl chloride (XIV) in the presence of $NaHCO_3$ in CH_2Cl_2 (1-4) or methyl *t*-butyl ether (5) yields the chloroacetamide derivative

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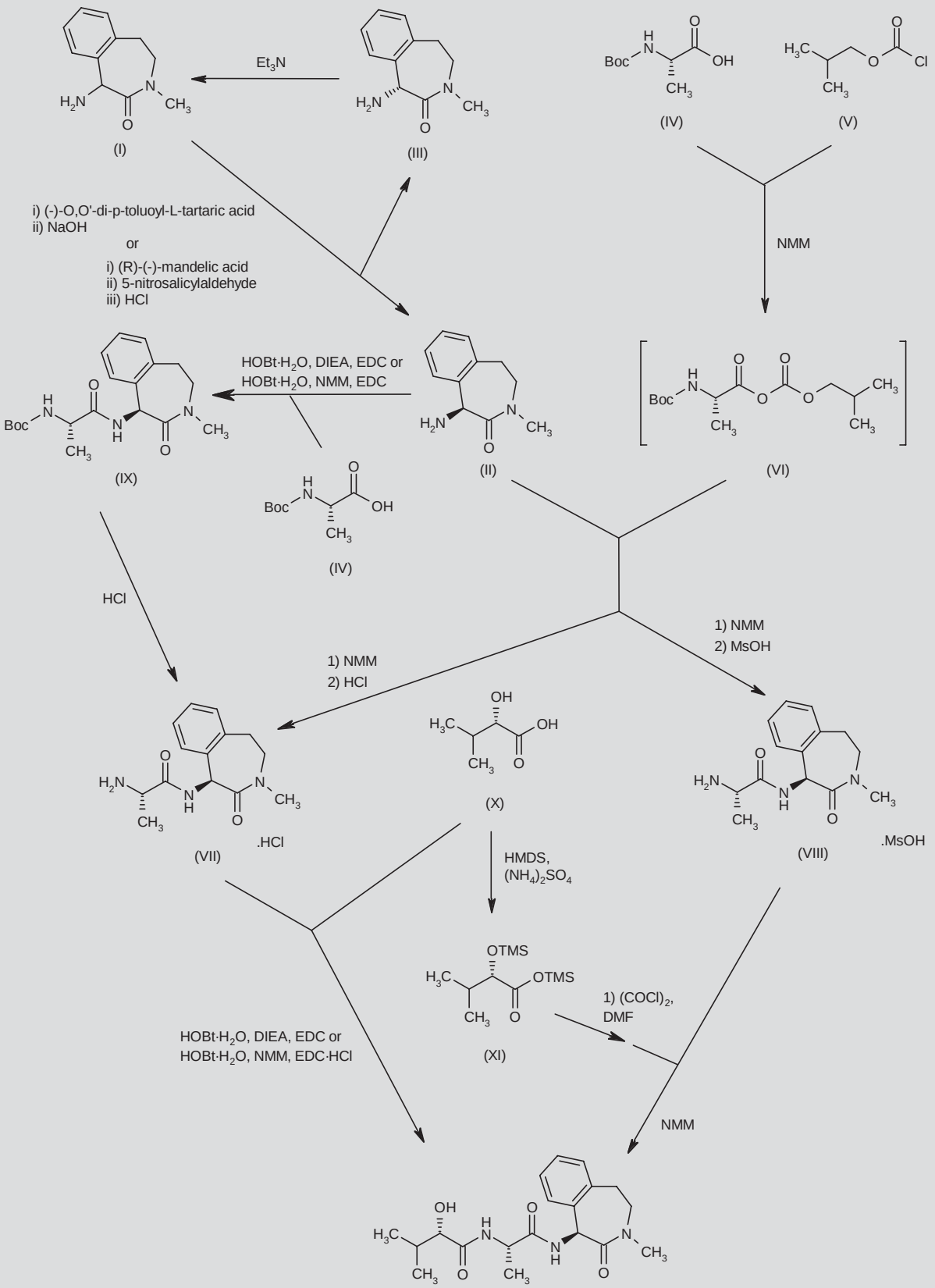
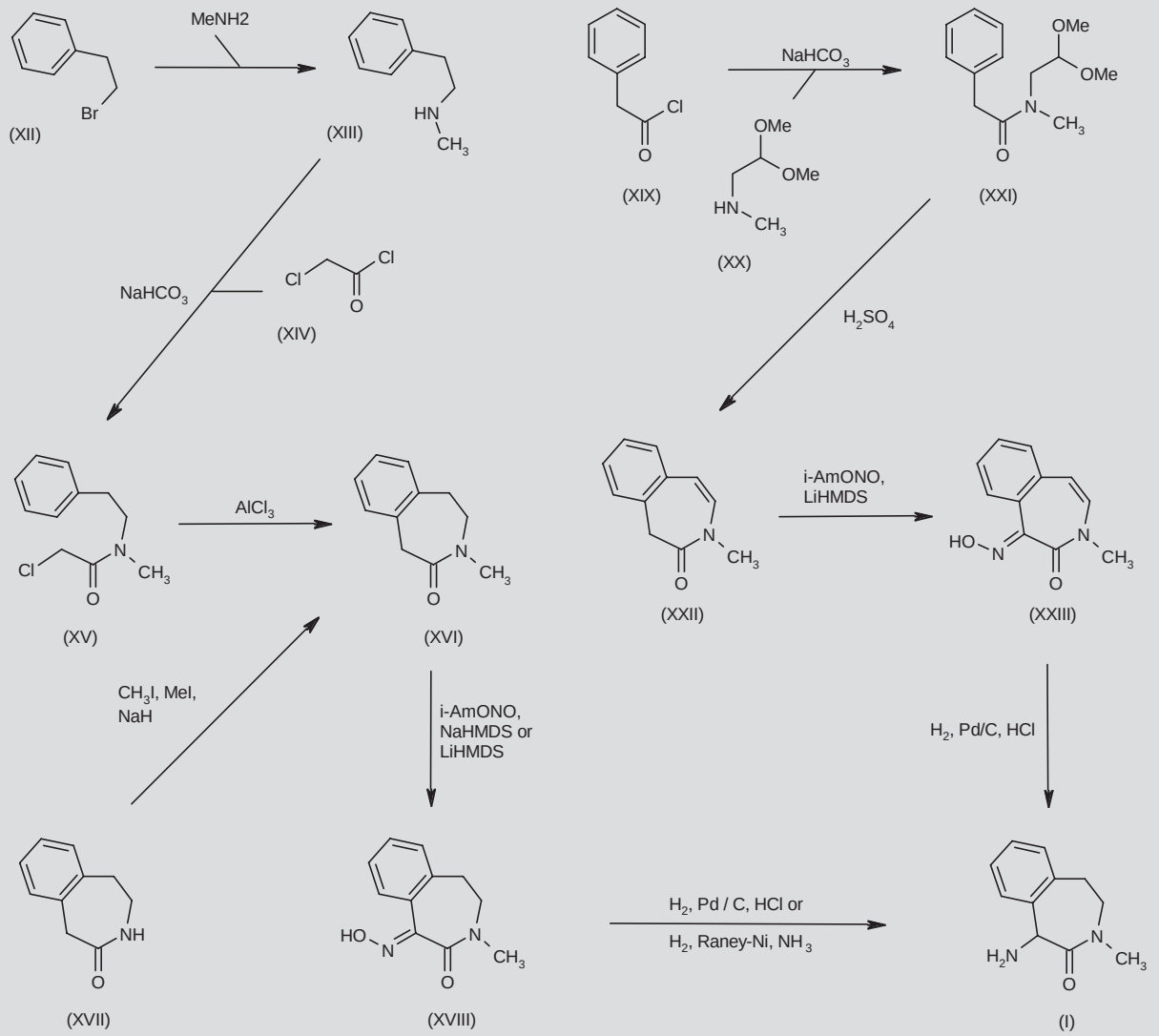
Scheme 1. Synthesis of Semagacestat



Scheme 2. Synthesis of Intermediate (I)



(XV) (1-5). Cyclization of intermediate (XV) by Friedel-Crafts alkylation by means of $AlCl_3$ in 1,2-dichlorobenzene at 165 °C gives 3-methyl-1,3,4,5-tetrahydro-2*H*-3-benzazepin-2-one (XVI) (1-5). Alternatively, compound (XVI) can be obtained from 2,3,4,5-tetrahydro-1*H*-3-benzazepin-2-one (XVII) by *N*-methylation with methyl iodide by means of NaH in DMF (1-4). Treatment of lactam (XVI) with isoamyl nitrite (i-AmONO) in the presence of NaHMDS (1-4) or LiHMDS in THF (1-5) yields the 1-hydroxyiminobenzazepinone (XVIII), which is finally hydrogenated with H_2 over Pd/C in the presence of HCl in EtOH at 50 °C (1-5) or with H_2 over Raney-Ni in the presence of NH_3 in EtOH at 100 °C (1-4). Scheme 2.

Alternatively, phenylacetyl chloride (XIX) is condensed with *N*-(2,2-dimethoxyethyl)-*N*-methylamine (XX) by means of $NaHCO_3$ in methyl *t*-butyl ether/H_2O to furnish the phenylacetamide derivative (XXI), which upon cyclization in the presence of H_2SO_4 at 110 °C affords the benzazepin-2-one derivative (XXII). Treatment of intermediate (XXII) with i-AmONO in the presence of LiHMDS in THF gives the hydroxyimino derivative (XXIII), which is finally hydrogenated with H_2 over Pd/C in the presence of HCl in EtOH at 50 °C (1-4). Scheme 2.

BACKGROUND

According to 2009 figures released from a study based in the United States, an estimated 5.3 million Americans of all ages have Alzheimer's disease (AD), indicating that AD is the seventh leading cause of death, with an accompanying healthcare bill of over USD

148 billion each year (7). Investigations into the pathogenesis of AD have identified γ-secretase as a target for pharmaceutical intervention (8). γ-Secretase, mediated by presenilin, cleaves amyloid precursor protein (APP) to produce β-amyloid (Aβ) plaques in AD. These plaques are thought to impede nerve cell function and promote neuronal degeneration in AD brains and contribute to the formation of neurofibrillary tangles. These lesions are associated with neuronal cell death, which manifests as loss of memory (9). To date no γ-secretase inhibitors have been approved for the treatment of AD.

Lilly is currently investigating the safety and efficacy of semagacestat (LY-450139), an inhibitor of γ-secretase, for the treatment of mild to moderate AD. Two phase III clinical trials for semagacestat are currently ongoing in the U.S. (10, 11). Semagacestat was originally codeveloped by Lilly and Elan.

PRECLINICAL PHARMACOLOGY

The concentration-dependent modulation of Aβ by semagacestat has been investigated in a mouse embryonic stem cell-derived neuronal cell line and H4 cells overexpressing the Swedish mutation of APP (SWEAPP293). The results showed that, following drug exposure for 20 h, elevated levels of secreted Aβ were observed in both cellular models at subeffective concentrations, whereas concentration-dependent inhibition was observed at higher concentrations, with an IC_{50} of approximately 60 nM and almost complete inhibition achieved at higher concentrations (12).

In vitro assays have also shown that semagacestat does not inhibit cellular protein synthesis at concentrations over 100-fold its IC_{50} for Aβ lowering in HEK-293 cells overexpressing SWEAPP293 (13).

Studies have shown that semagacestat (0.2-60 mg/kg by oral gavage) differentially modulates plasma Aβ in a dose-dependent manner in guinea pigs (a nontransgenic model that has an Aβ sequence identical to humans). Male guinea pigs were treated with semagacestat and brain, cerebrospinal fluid (CSF) and plasma Aβ levels were characterized at 1, 3, 6, 9 and 14 h after dosing. Low doses significantly elevated plasma Aβ levels at early time points, with a return to baseline within hours. Higher doses inhibited Aβ levels in all compartments at early time points, but elevated plasma Aβ levels at later time points (12).

Acute dosing of semagacestat at 30 and 100 mg/kg elicited 20-55% inhibition of Aβ in brain and > 70% inhibition in CSF and plasma at both doses in CD-1 mice. Upon administration to guinea pigs at 3-100 mg/kg p.o. twice daily for 5 days, 60-95% inhibition of brain, CSF and plasma Aβ was observed at the highest dose 3 h after the final dose, while the lowest dose produced a 20% elevation in plasma Aβ and a 60% elevation in CSF Aβ (14).

Investigations in anesthetized dogs have shown that the absorption of a single oral dose of semagacestat (1 or 5 mg/kg) is variable and prolonged, with a plasma half-life estimated at between 5 and 14 h and a t_{max} of 1-10 h. Dogs receiving 5 mg/kg displayed significantly lower total Aβ and $Aβ_{1-40}$ levels in CSF samples versus those receiving vehicle. Aβ was reduced by a maximum of 60-70% from baseline at 10 h after dosing (15).

Semagacestat administration to beagle dogs confirmed that this agent can provide a persistent reduction in $Aβ_{1-40}$ and $Aβ_{1-42}$. Analysis of CSF samples taken from anesthetized animals receiving a single dose of 2 mg/kg semagacestat indicated that $Aβ_{1-40}$ and $Aβ_{1-42}$ levels were reduced by 30% and 60%, respectively, after 3 h and by 60% and 56%, respectively, after 6 (16).

Studies in PDAPP transgenic mice overexpressing the $APPV^{717F}$ familial AD mutation in brain tissue have shown that oral administration of semagacestat induces a dose-dependent reduction in hippocampal total Aβ, with a reduction of 40% observed at 1 mg/kg. These effects were sustained for up to 12 h following a single dose of 5 mg/kg (17). Further analyses also demonstrated that oral doses of 30 mg/kg/day semagacestat for 5 months provided a sustained decrease in Aβ levels in hippocampal tissue, without causing neurotoxic brain lesions secondary to increases in C-99 fragments of APP (18).

CLINICAL STUDIES

A randomized, placebo-controlled, double-blind, dose-escalation study with twice-daily dosing in healthy Japanese subjects has shown that semagacestat is well tolerated up to 140 mg for 14 days. No clinically significant Q-T_c prolongation or cardiovascular changes were observed in this study. Semagacestat was rapidly absorbed (t_{max} = 0.5-2 h) across doses of 40, 100 and 140 mg, with a mean half-life of 2.8 h. $Aβ_{1-40}$ levels were reduced by a maximum of 58% at 140 mg (19).

Stable isotope labeling kinetic techniques used in a double-blind, placebo-controlled study in 24 healthy men aged 21-50 years have shown that a single oral dose of 100, 140 or 280 mg of semagacestat dose-dependently decreases the absolute production rate of CNS Aβ (20).

The safety and tolerability of semagacestat have been investigated in healthy volunteers administered doses of 5-50 mg/day given over 14 days. Pharmacokinetic analyses conducted in 37 subjects indicated a plasma half-life of approximately 2.5 h, with a linear relationship between dose and plasma concentrations, and a C_{max} of 828 ng/mL following a dose of 50 mg. A dose-dependent reduction in plasma Aβ was demonstrated and changes in plasma Aβ concentrations were temporally related to the pharmacokinetic characteristics of the drug. CSF Aβ concentrations were unchanged. Adverse events reported by subjects taking 5, 20 or 40 mg were similar to those reported by subjects taking placebo; 29% of subjects taking 50 mg/day experienced adverse events that may have been drug-related (21).

A multicenter, randomized, double-blind, dose-escalating, placebo-controlled trial evaluated the safety, tolerability and Aβ response to semagacestat in patients with mild to moderate AD (N = 51). Semagacestat was generally well tolerated at doses of up to 140 mg/day for 14 weeks. There were reports of three possible drug-related rashes and a further three reports of hair color change in the treatment groups. A total of three patients discontinued the study due to adverse events, including one case of transient bowel obstruction. Plasma $Aβ_{1-40}$ concentrations were reduced by 58% and 65%, respectively, in the 100- and 140-mg groups; however, no significant reduction was seen in CSF Aβ levels. No significant difference in cognitive or functional measures was evident for any of the placebo or drug groups (22).

SOURCE

Eli Lilly and Company (US).

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