

RESEARCH MONOGRAPH . COMPOUND PROFILE

AF710B

A dual-target allosteric M1 muscarinic and sigma-1 receptor agonist developed at the Israel Institute for Biological Research and advanced by Anavex Life Sciences as ANAVEX 3-71, distinguished from earlier orthosteric muscarinic agonists by allosteric M1 selectivity, picomolar-range potency, sigma-1 chaperone engagement, and disease-modifying activity across amyloid, tau, and neuroinflammatory pathologies in transgenic rodent models of Alzheimer's disease.

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ABSTRACT

AF710B (ANAVEX 3-71) is a highly potent and selective allosteric agonist of the M1 subtype of the muscarinic acetylcholine receptor (M1 mAChR) and a concurrent agonist of the sigma-1 receptor (sigma-1R), first reported by Fisher et al. (2016) in *Neurodegenerative Diseases* as a next-generation candidate for cognitive enhancement and disease modification in Alzheimer's disease [1]. The compound emerged from a decades-long medicinal chemistry program at the Israel Institute for Biological Research (IIBR) that previously produced the orthosteric M1 agonists AF102B (cevimeline, approved for Sjogren's syndrome), AF267B, and AF292, each of which demonstrated procognitive and anti-amyloidogenic activity in preclinical and early clinical settings but was limited by insufficient M1 selectivity, dose-limiting cholinergic adverse events, or both. AF710B was designed to overcome these limitations through an allosteric mechanism of M1 receptor engagement: at nanomolar concentrations the compound potentiates the binding and efficacy of the endogenous agonist acetylcholine (and of the reference orthosteric agonist carbachol) at the M1 receptor, amplifying downstream signaling through phospho-ERK1/2 and phospho-CREB pathways without producing the gastrointestinal and cardiovascular cholinergic toxicity that constrained the earlier orthosteric series [1, 2]. Selectivity screening at 10 micromolar against 83 additional G-protein-coupled receptors, ion channels, and transporters produced no significant off-target hits, establishing a pharmacological selectivity profile substantially cleaner than that of xanomeline and other earlier M1-preferring agonists [1]. The sigma-1 receptor agonism adds a second, mechanistically

independent neuroprotective axis. Sigma-1R is an endoplasmic reticulum chaperone protein concentrated at the mitochondria-associated endoplasmic reticulum membrane (MAM), where it regulates calcium transfer between the endoplasmic reticulum and mitochondria, modulates the unfolded protein response, and activates anti-inflammatory and antiapoptotic signaling cascades [3, 4]. Activation of sigma-1R by AF710B has been linked to rescue of mushroom spine loss in presenilin-1 knock-in and APP knock-in neuronal cultures, reduction of neuroinflammatory markers, and normalization of brain-derived neurotrophic factor (BDNF) signaling in transgenic models [1, 5]. In the seminal Fisher et al. (2016) study, AF710B administered to female 3xTg-AD mice at 10 micrograms per kilogram intraperitoneally daily for two months mitigated cognitive impairment in the Morris water maze and reduced BACE1 expression, GSK3-beta activity, p25/CDK5 levels, neuroinflammation, soluble and insoluble amyloid-beta 40 and 42, amyloid plaques, and phosphorylated tau pathology [1]. A subsequent study by Hall et al. (2018) in Alzheimer's and Dementia extended these findings to the McGill-R-Thy1-APP transgenic rat model, demonstrating that chronic oral AF710B at 10 micrograms per kilogram daily for 4.5 months in 13-month-old (post-plaque) rats reversed cognitive deficits, reduced hippocampal amyloid plaque burden and cortical amyloid-beta 40 and 42 levels, decreased neuroinflammatory markers, increased cerebrospinal fluid amyloid clearance, and elevated the synaptic marker synaptophysin [6]. The disease-modifying character of the effect was underscored by its persistence through a five-week drug washout period following treatment cessation. A third study (Bhatt et al., 2024, Neurobiology of Aging) confirmed that early treatment with AF710B in the same rat model prevented cognitive decline when treatment was initiated before overt plaque

deposition [7]. Anavex Life Sciences Corporation acquired exclusive worldwide rights to the AF710B intellectual property in 2014 and advanced the compound under the designation ANAVEX 3-71 through a first-in-human Phase 1 single ascending dose study in 42 healthy volunteers (2020 to 2021), which demonstrated safety and tolerability at oral doses of 5 to 200 mg with no serious adverse events, no clinically significant electrocardiogram changes, and linear, dose-proportional pharmacokinetics with a mean terminal elimination half-life of approximately 3.56 hours [8, 9]. A Phase 1b study subsequently confirmed the bioavailability of a once-daily oral tablet formulation. In 2024, Anavex initiated a placebo-controlled Phase 2 study (ANAVEX3-71-SZ-001) in adults with schizophrenia, and in October 2025 reported positive topline results demonstrating safety, tolerability, reduction in the neuroinflammatory biomarker glial fibrillary acidic protein (GFAP), and encouraging trends in EEG and event-related potential biomarkers [10, 11]. The compound has received orphan drug designation from the FDA for frontotemporal dementia. No registration-enabling Phase 3 trial has been completed for any indication as of the most recent monograph revision. This monograph documents the chemistry, synthesis, dual-receptor pharmacology, pharmacokinetics, preclinical and clinical evidence, sourcing and handling considerations, stack interactions, adverse-event profile, and a structured comparative assessment of AF710B against five muscarinic and sigma-1 receptor candidates on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Research designation: AF710B.

Development name: ANAVEX 3-71 (Anavex Life Sciences Corporation).

Other designations: EVT-258416 (EvitaChem catalog designation).

Chemical Abstracts Service registry number: 1235733-73-9 (racemic form). The CAS number refers to the racemic mixture; the resolved enantiomeric form used in the Anavex clinical program is proprietary and the CAS for the single enantiomer has not been publicly disclosed in the peer-reviewed literature.

International Union of Pure and Applied Chemistry name: 1-(2,8-dimethyl-1-thia-3,8-diazaspiro[4.5]decan-3-yl)-3-(1H-indol-3-yl)propan-1-one.

PubChem compound identifier: CID 58516166.

1.2 Structural class and chemistry

AF710B is a spirocyclic thiadiazadecane linked to an indole pharmacophore through a propanoyl bridge. The structural core is a 1-thia-3,8-diazaspiro[4.5]decane ring system, a spiro junction of a thiazolidine ring with a piperidine ring bearing methyl substituents at the 2- and 8-nitrogen positions. The indole-3-ylpropanoyl moiety provides the aromatic element required for receptor recognition at both the M1 muscarinic allosteric site and the sigma-1 receptor ligand-binding domain [1, 12].

Molecular formula: C₂₀H₂₇N₃OS.

Exact mass: 357.1875 g/mol (monoisotopic).

Molecular weight: 357.52 g/mol.

Physical properties: The compound is supplied as a white to off-white solid by research-grade suppliers. It is soluble in dimethyl sulfoxide (DMSO); aqueous solubility is limited and has not been quantitatively reported in the peer-reviewed liter-

ature. Storage is recommended at minus 20 degrees Celsius for long-term stability and at 2 to 8 degrees Celsius for short-term use, protected from light and moisture.

Stereochemistry: The spirocyclic junction at the spiro carbon introduces a stereocenter. The racemic form (rac-AF710B, CAS 1235733-73-9) is the material available from research-grade suppliers. The single-enantiomer form used in the Anavex clinical program has not been publicly characterized with respect to absolute configuration in the peer-reviewed literature; investigators acquiring research-grade material should note that the racemic form may differ from the clinical candidate in potency and selectivity.

1.3 Synthesis

The published synthesis of AF710B has not been described in full detail in a dedicated synthetic chemistry report. The structural class (spirocyclic thiadiazadecane indolepropanoyl amide) suggests a convergent synthesis involving: (a) construction of the 1-thia-3,8-diazaspiro[4.5]decane core, likely through cyclization of a suitably functionalized piperidone with a thiazolidine-forming reagent (thioglycolic acid derivative or equivalent); (b) preparation of 3-(1H-indol-3-yl)propanoic acid or its activated ester; and (c) amide bond formation between the secondary amine of the spirocyclic ring and the activated indolepropanoic acid [12]. Resolution of the racemate, if required for the clinical candidate, would proceed by chiral chromatography or by enantioselective synthesis at the spiro junction. The patents covering AF710B (assigned to Life Science Research Israel Ltd. and licensed to Anavex) contain synthetic details that are not reproduced in the peer-reviewed literature. Research-grade suppliers provide the racemic form at greater than 98 percent purity by high-performance liquid chromatography.

2. Abstract

AF710B is a dual-target allosteric M1 muscarinic acetylcholine receptor agonist and sigma-1 receptor agonist developed by Abraham Fisher and colleagues at the Israel Institute for Biological Research as the latest iteration in a multi-decade medicinal chemistry program that previously produced AF102B (cevimeline), AF267B, and AF292 [1, 2]. The compound is distinguished from its predecessors and from other muscarinic agonist candidates by three properties: allosteric (rather than orthosteric) engagement of the M1 muscarinic receptor, which preserves the spatiotemporal specificity of endogenous cholinergic signaling and avoids the dose-limiting gastrointestinal and cardiovascular cholinergic adverse events that constrained earlier orthosteric M1 agonists; concurrent sigma-1 receptor agonism, which engages an independent neuroprotective and anti-inflammatory signaling axis at the mitochondria-associated endoplasmic reticulum membrane; and exceptional potency, with procognitive activity in rats at oral doses as low as 1 microgram per kilogram [1].

The preclinical evidence base is substantial. In the 3xTg-AD triple-transgenic mouse model of Alzheimer's disease, AF710B at 10 micrograms per kilogram intraperitoneally daily for two months mitigated cognitive impairment and reduced amyloid plaques, soluble and insoluble amyloid-beta 40 and 42, BACE1, GSK3-beta activity, p25/CDK5, phosphorylated tau, and neuroinflammatory markers [1]. In the McGill-R-Thy1-APP transgenic rat model, chronic oral AF710B at the same dose reversed cognitive deficits and amyloid pathology in post-plaque aged animals, with effects persisting through a five-week drug washout period, supporting a disease-modifying rather than merely symptomatic mechanism [6]. Early-stage treatment in the same rat model prevented cognitive decline when initiated before overt plaque deposition [7]. The compound also rescued mushroom dendritic spine loss in presenilin-1 knock-in and APP knock-in neuronal cultures at 30 nanomolar, a potency substantially exceeding that of AF267B (1 micromolar) and donepezil [5].

Clinical development is conducted by Anavex Life Sciences Corporation under the name ANAVEX 3-71. A Phase 1 single ascending dose study in 42 healthy volunteers demonstrated safety and tolerability at oral doses of 5 to 200 mg, linear dose-proportional pharmacokinetics, a terminal elimination half-life of approximately 3.56 hours, and no clinically significant QT interval changes [8, 9]. A Phase 2 study

in schizophrenia (ANAVEX3-71-SZ-001) reported positive topline safety and biomarker results in October 2025 [10, 11]. The compound has received FDA orphan drug designation for frontotemporal dementia. No registration-enabling Phase 3 trial has been completed for any indication.

3. Discovery and Development History

3.1 Origin and the Fisher muscarinic agonist program

AF710B was developed by Abraham Fisher and colleagues at the Department of Medicinal Chemistry, Israel Institute for Biological Research (IIBR), Ness-Ziona, Israel. Fisher's laboratory conducted a multi-decade program of muscarinic receptor agonist medicinal chemistry beginning in the 1980s, producing a series of M1-preferring agonists designated with the "AF" prefix [2, 13].

The first clinically significant compound from this program was AF102B, an M1 selective orthosteric agonist that was developed as cevimeline and approved by the FDA in 2000 (marketed as Evoxac) for the treatment of dry mouth in Sjogren's syndrome. AF102B also demonstrated procognitive and anti-amyloidogenic activity in preclinical models, with chronic treatment reducing amyloid-beta levels in transgenic mice, but it was not developed for Alzheimer's disease [2, 13].

Subsequent compounds AF267B and AF292 were more potent orthosteric M1 agonists with improved selectivity profiles. AF267B demonstrated reduction of amyloid-beta and tau pathology in 3xTg-AD mice at doses of 1 to 5 milligrams per kilogram, but retained sufficient M2 and M3 cross-reactivity to produce dose-limiting cholinergic adverse events (salivation, lacrimation, gastrointestinal motility) at higher doses [2, 14]. The limitations of the orthosteric approach, specifically the high conservation of the orthosteric binding site across all five muscarinic subtypes (M1 through M5), motivated the shift to allosteric modulation as the design principle for the next generation.

3.2 Design rationale for allosteric M1 agonism

The allosteric binding site on the M1 muscarinic receptor is structurally distinct from the acetylcholine-binding orthosteric site and is less conserved across muscarinic subtypes, providing a pharmacological basis for M1 selectivity without the M2/M3 cross-reactivity that produces bradycardia, gastrointestinal hypermotility, and hypersalivation [15, 16]. Allosteric agonists additionally preserve the temporal dynamics of endogenous cholinergic neurotransmission by potentiating the response to acetylcholine rather than producing tonic receptor activation.

AF710B was identified from a medicinal chemistry campaign optimizing the spirocyclic thiadiazadecane scaffold for M1 allosteric agonism and sigma-1R agonism simultaneously. The dual-target profile was a deliberate design objective, not an incidental polypharmacology, reflecting Fisher's hypothesis that concurrent M1 muscarinic and sigma-1 receptor engagement would produce synergistic neuroprotective effects through mechanistically independent pathways [1].

3.3 Anavex licensing and clinical advancement

In March 2014, Anavex Life Sciences Corporation acquired exclusive worldwide rights to patents and patent applications covering AF710B and related compounds from Life Science Research Israel Ltd. (LSRI) [17]. Anavex designated the compound ANAVEX 3-71 and positioned it as a second clinical-stage sigma-1 and muscarinic receptor program parallel to ANAVEX 2-73 (blarcamesine), which targets the same receptors but through a distinct chemical scaffold and pharmacological profile.

In May 2020, Anavex initiated a first-in-human Phase 1 single ascending dose study in healthy volunteers at a clinical research unit in Australia [18]. The first participant was enrolled in July 2020. Positive Phase 1 results were announced in 2021, and the pharmacokinetic data were published by Fadiran et al. (2024) in *Clinical Pharmacology in Drug Development* [9]. A Phase 1b study subsequently confirmed the feasibility of a once-daily oral tablet formulation.

In January 2024, Anavex announced a U.S. Phase 2 clinical trial of ANAVEX 3-71 in schizophrenia, with first patient enrollment in mid-2024 [19]. The FDA had previously granted orphan drug designation for ANAVEX 3-71 in frontotemporal dementia. In October 2025, Anavex reported positive topline results from the Phase 2 schizophrenia study, demonstrating safety, tolerability, and encouraging biomarker trends [10, 11].

4. Mechanism of Action

AF710B is a dual-target ligand whose principal molecular activities are allosteric agonism at the M1 muscarinic acetylcholine receptor and agonism at the sigma-1 receptor. These two activities operate through mechanistically independent signaling cascades and converge on neuroprotective, procognitive, anti-amyloidogenic, anti-tau, and anti-inflammatory endpoints in preclinical models.

4.1 M1 muscarinic receptor allosteric agonism

The M1 muscarinic acetylcholine receptor (M1 mAChR) is a Gq/11-coupled G-protein-coupled receptor expressed at high density in the cerebral cortex, hippocampus, and striatum. It is the predominant postsynaptic muscarinic subtype in the cortical and hippocampal circuits that underwrite learning, memory, and executive function. Activation of M1 mAChR drives phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate, producing inositol trisphosphate (IP3) and diacylglycerol (DAG), which in turn activate protein kinase C and downstream signaling including the ERK1/2 mitogen-activated protein kinase and CREB transcription factor pathways [15, 16].

AF710B engages the M1 receptor at an allosteric site topographically distinct from the orthosteric acetylcholine-binding pocket. At very low concentrations (nanomolar range), AF710B significantly potentiates the binding and functional efficacy of the orthosteric agonist carbachol at M1 receptors, amplifying carbachol-stimulated phospho-ERK1/2 and phospho-CREB responses [1]. This positive allosteric modulator (PAM) activity with intrinsic agonism (ago-PAM) produces M1 activation that is both potentiated by and dependent on endogenous cholinergic tone, preserving the spatial and temporal dynamics of physiological neurotransmission. The allosteric mechanism is the structural basis for M1 selectivity: the allosteric site diverges across muscarinic subtypes, allowing AF710B to activate M1 with negligible engagement of M2, M3, M4, or M5 receptors at concentrations up to 10 micromolar in radioligand displacement assays [1].

The downstream consequences of M1 allosteric activation relevant to Alzheimer's disease include: promotion of alpha-secretase processing of amyloid precursor protein (APP), shifting the cleavage balance away from the amyloido-

genic BACE1/gamma-secretase pathway toward the non-amyloidogenic alpha-secretase pathway and increasing production of the neurotrophic soluble APP-alpha (sAPPalpha) [1, 14]; inhibition of GSK3-beta, the principal tau kinase, thereby reducing tau hyperphosphorylation and neurofibrillary tangle formation [1]; activation of CREB-dependent transcription, supporting synaptic plasticity and BDNF expression; and suppression of neuroinflammatory signaling through modulation of microglial activation state.

4.2 Sigma-1 receptor agonism

The sigma-1 receptor (sigma-1R, SIGMAR1) is an endoplasmic reticulum (ER) resident chaperone protein that is concentrated at mitochondria-associated ER membranes (MAMs), the specialized contact sites between the ER and the outer mitochondrial membrane [3, 4]. Sigma-1R functions as an inter-organelle signaling hub: it stabilizes IP3 receptors at MAMs, facilitating calcium transfer from ER to mitochondria; modulates the unfolded protein response (UPR) and ER stress signaling; regulates lipid transfer and membrane remodeling at the ER-mitochondria interface; and promotes cell survival through BDNF upregulation and suppression of pro-apoptotic cascades [3, 4].

AF710B activates sigma-1R with high affinity and selectivity. The functional consequences of sigma-1R activation by AF710B include: stabilization of mature BDNF through rescue of the conversion of pro-BDNF to its mature biologically active form [1, 5]; reduction of neuroinflammatory cytokine release through modulation of microglial and astrocytic activation; normalization of mitochondrial calcium homeostasis, which is disrupted in Alzheimer's disease and in presenilin-mutant models; and attenuation of ER stress responses that contribute to neuronal apoptosis [3, 4].

The convergence of M1 allosteric agonism and sigma-1R agonism on overlapping downstream endpoints (BDNF signaling, amyloid processing, tau phosphorylation, neuroinflammation, synaptic integrity) is the proposed mechanistic basis for the disease-modifying activity observed in transgenic rodent models [1, 6].

4.3 Selectivity and off-target pharmacology

AF710B was screened at 10 micromolar in a radioligand displacement panel covering 83 G-protein-coupled receptors, ion channels, and transporters known to mediate common drug adverse events [1]. No significant off-target activity was observed,

establishing a selectivity profile substantially cleaner than those of xanomeline (which activates M1, M2, M3, M4, and M5), of arecoline (a non-selective muscarinic agonist), and of the earlier orthosteric Fisher compounds AF267B and AF292 (which retained M2 and M3 cross-reactivity at higher doses). The clean off-target profile is the pharmacological basis for the absence of dose-limiting cholinergic adverse events in preclinical toxicology and in the Phase 1 human study [1, 8].

4.4 Composite pharmacology and dose-response

The composite pharmacology of AF710B at preclinical doses produces procognitive activity at remarkably low doses. In rats treated with the M1 antagonist trihexyphenidyl, AF710B at oral doses of 1 to 30 micrograms per kilogram reversed cognitive impairment, establishing a potency approximately 1000-fold greater than AF267B and donepezil in the same model [1]. The low effective dose is consistent with the allosteric mechanism, which amplifies endogenous cholinergic signaling rather than producing tonic receptor occupation and therefore operates at lower receptor occupancy than an orthosteric agonist.

In the 3xTg-AD model, the effective dose of 10 micrograms per kilogram intraperitoneal daily is approximately 100- to 1000-fold lower than the doses of xanomeline and AF267B required to produce comparable anti-amyloidogenic and anti-tau effects [1, 14]. This potency differential is a central distinguishing feature of the compound.

5. Pharmacokinetics and Routes of Administration

5.1 Preclinical pharmacokinetics

Detailed preclinical pharmacokinetic parameters (AUC, C_{max}, V_d, protein binding, tissue distribution) for AF710B have not been comprehensively published in the peer-reviewed literature. The published preclinical efficacy studies report the compound as orally bioavailable in rats and mice at microgram-per-kilogram doses, with efficacy observed after both intraperitoneal and oral administration [1, 6, 7]. Brain penetration is confirmed by the central nervous system pharmacodynamic effects (cognitive enhancement, reduction of central amyloid and tau pathology) observed after systemic administration.

5.2 Human pharmacokinetics

The Phase 1 single ascending dose study in 42 healthy volunteers (18 to 35 years) characterized the pharmacokinetics of oral ANAVEX 3-71 at doses of 5 to 200 mg [8, 9]. The Fadiran et al. (2024) population pharmacokinetic analysis published in Clinical Pharmacology in Drug Development reported the following findings [9]:

Absorption: Rapid oral absorption with time to peak plasma concentration consistent with an immediate-release oral formulation.

Linearity and dose proportionality: Pharmacokinetics were linear and dose proportional for doses up to 160 mg. AUC and C_{max} increased proportionally with dose across the 5 to 160 mg range.

Elimination: The mean apparent terminal elimination half-life of the parent compound was approximately 3.56 hours. The principal circulating metabolite (designated M8) was eliminated with a mean apparent terminal elimination half-life of 6.59 hours.

Food effect: Food had no effect on the pharmacokinetics of ANAVEX 3-71 or its M8 metabolite.

Demographics: Gender, age, weight, and creatinine clearance were not explanatory of variability in apparent clearance or apparent volume of the central compartment.

The short terminal half-life of the parent compound suggests that once-daily dosing may depend on pharmacodynamic duration (receptor occupancy kinetics, downstream signaling persistence) exceeding pharmacokinetic residence time, or on the contribution of the longer-lived M8 metabolite. A Phase 1b study confirmed the feasibility of a once-daily oral tablet formulation, suggesting adequate duration of pharmacodynamic effect with this dosing regimen.

5.3 Routes of administration

The preclinical literature reports both intraperitoneal and oral routes. The Fisher et al. (2016) study in 3xTg-AD mice used intraperitoneal administration at 10 micrograms per kilogram daily [1]. The Hall et al. (2018) study in McGill-R-Thy1-APP rats used oral administration at 10 micrograms per kilogram daily [6]. The human Phase 1 study used oral capsule and oral tablet formulations [8, 9].

6. Preclinical Pharmacology

6.1 Cognitive enhancement in pharmacological impairment models

AF710B reversed trihexyphenidyl-induced cognitive impairment in rats at oral doses of 1 to 30 micrograms per kilogram, establishing potent procognitive activity in a standard muscarinic antagonist challenge model [1]. This model isolates M1-dependent cognition by selectively impairing muscarinic cholinergic transmission and is the standard first-line preclinical assay for muscarinic agonist cognitive enhancement.

6.2 3xTg-AD triple-transgenic mouse model

The 3xTg-AD mouse expresses three mutant transgenes associated with familial Alzheimer's disease: APP Swedish (KM670/671NL), MAPT P301L, and PSEN1 M146V. The model develops progressive amyloid plaque deposition, neurofibrillary tangle-like tau pathology, neuroinflammation, and cognitive deficits that model the cardinal neuropathological features of human Alzheimer's disease [1].

In female 3xTg-AD mice, AF710B at 10 micrograms per kilogram intraperitoneally daily for two months produced the following effects compared to vehicle-treated controls [1]:

- Mitigation of cognitive impairment in the Morris water maze (spatial learning and memory)
- Reduction of BACE1 protein expression (the rate-limiting enzyme in amyloidogenic APP processing)
- Reduction of GSK3-beta activity (the principal tau kinase)
- Reduction of p25/CDK5 (a pathological activator of cyclin-dependent kinase 5 implicated in tau hyperphosphorylation)
- Reduction of soluble and insoluble amyloid-beta 40 and amyloid-beta 42
- Reduction of amyloid plaque burden
- Reduction of phosphorylated tau at multiple epitopes

- Reduction of neuroinflammatory markers

The breadth of these effects across the amyloid, tau, and neuroinflammatory axes is notable and distinguishes AF710B from compounds that act on only one of these pathological cascades.

6.3 McGill-R-Thy1-APP transgenic rat model

The McGill-R-Thy1-APP rat expresses human APP bearing the Swedish and Indiana mutations under the Thy1.2 promoter and develops progressive amyloid plaque deposition and cognitive deficits modeling Alzheimer's-like amyloid pathology [6, 7].

Hall et al. (2018) treated 13-month-old (post-plaque) McGill-R-Thy1-APP rats with oral AF710B at 10 micrograms per kilogram daily for 4.5 months, then discontinued treatment and performed behavioral testing five weeks after the last dose [6]. Results included:

- Reversal of cognitive deficits in spatial memory tasks
- Reduction of hippocampal amyloid plaque number
- Reduction of cortical amyloid-beta 40 and 42 levels
- Reduction of neuroinflammatory markers
- Increase in cerebrospinal fluid amyloid clearance (suggesting enhanced efflux rather than merely reduced production)
- Increase in synaptophysin (a presynaptic marker of synaptic integrity)

The persistence of cognitive and pathological improvements through a five-week washout period following treatment cessation is the principal evidence for disease-modifying (rather than purely symptomatic) activity. Symptomatic cognitive enhancers (such as acetylcholinesterase inhibitors) produce effects that dissipate within hours to days of treatment discontinuation; the sustained effect of AF710B through a five-week washout implies structural or epigenetic modification of the disease process.

Bhatt et al. (2024) extended these findings by demonstrating that early treatment with AF710B in the same rat model, initiated before overt plaque deposition, prevented the emergence of cognitive decline [7]. This prevention paradigm provides additional evidence for disease-modifying activity and supports the hypothesis that AF710B may be effective in prodromal or preclinical stages of Alzheimer's disease.

6.4 Dendritic spine rescue in neuronal cultures

Sun et al. demonstrated that AF710B at 30 nanomolar rescued mushroom dendritic spine loss in presenilin-1 knock-in (PS1-KI) and APP knock-in (APP-KI) neuronal cultures [5]. Mushroom spines are the morphological correlate of stable, potentiated synapses and their loss is an early feature of Alzheimer's disease neuropathology that precedes frank neuronal death. AF267B at 1 micromolar was less potent in PS1-KI cultures and ineffective in APP-KI cultures, establishing a potency advantage of approximately 30-fold for AF710B [5]. The *in vitro* spine rescue supports the *in vivo* synaptic marker (synaptophysin) findings from the Hall et al. (2018) study and implicates sigma-1R-mediated stabilization of ER-mitochondria calcium signaling as a contributor to the synaptic protective effect.

7. Clinical Evidence Base

7.1 Phase 1 single ascending dose study

The first-in-human Phase 1 study was a prospective, double-blind, randomized, placebo-controlled single ascending dose study in 42 healthy male and female volunteers aged 18 to 35 years, conducted at a clinical research unit in Australia beginning in 2020 [8, 9]. Subjects were divided into three cohorts receiving escalating single oral doses of ANAVEX 3-71 or placebo over a 30-day period. The first cohort received doses of 5 to 50 mg; the second cohort received doses of 55 to 200 mg; the third cohort received placebo.

Primary endpoint (safety and tolerability): ANAVEX 3-71 was well tolerated at all doses tested (5 to 200 mg). No serious adverse events were reported. No significant laboratory abnormalities were observed in any subject. Treatment-emergent adverse events were mild and similar in frequency and character to placebo [8].

Secondary endpoint (cardiac safety): Electrocardiogram parameters were not clinically significant in any subject. QT interval corrected for heart rate (QTc) measures were normal across all dose groups, with no differences between treatment and placebo groups [8]. This finding is notable given that some muscarinic agonists (xanomeline) have been associated with cardiac adverse events at higher doses.

Pharmacokinetics: As described in Section 5.2, the pharmacokinetics were linear and dose proportional up to 160 mg, with a terminal elimination half-life of approximately 3.56 hours and no food or gender effects [9].

7.2 Phase 1b oral tablet formulation

A Phase 1b study evaluated a once-daily oral tablet formulation of ANAVEX 3-71 and confirmed bioavailability and feasibility of the intended commercial dosing regimen. Results were reported by Anavex in corporate announcements; full peer-reviewed publication of the Phase 1b data had not appeared as of the most recent monograph revision.

7.3 Phase 2 schizophrenia study (ANAVEX3-71-SZ-001)

In January 2024, Anavex announced a U.S. Phase 2 clinical trial of ANAVEX 3-71 in adults with schizophrenia [19]. The study was a two-part design consisting of a multiple ascending dose part (Part A) and a 28-day treatment period in a larger cohort (Part B), placebo-controlled and double-blind.

In October 2025, Anavex reported positive topline results [10, 11]:

Primary endpoint: The study achieved its primary endpoint demonstrating safety and tolerability in adults with schizophrenia. The safety profile was consistent with that observed in healthy volunteers in the Phase 1 study. No serious treatment-emergent adverse events and no severe treatment-emergent adverse events were reported in either Part A or Part B.

Biomarker endpoints: Glial fibrillary acidic protein (GFAP), a biomarker of astrocytic activation and neuroinflammation, was reduced in participants receiving ANAVEX 3-71 compared to placebo. Encouraging trends were observed in electroencephalography (EEG) and event-related potential (ERP) biomarkers of schizophrenia, including markers of sensory gating and mismatch negativity.

Symptom-scale endpoints: Powered efficacy data on symptom scales (PANSS total score, negative symptom subscales, cognitive measures) were not reported in the October 2025 topline announcement. These data are anticipated in subsequent presentations and publications.

The schizophrenia indication reflects the role of M1 muscarinic hypofunction in schizophrenia pathophysiology, supported by the FDA approval of xanomeline-trospium (Cobenfy) for schizophrenia in September 2024 based on M1/M4 muscarinic agonism [20].

7.4 Frontotemporal dementia orphan drug designation

The FDA granted orphan drug designation for ANAVEX 3-71 in frontotemporal dementia (FTD). FTD is characterized by progressive degeneration of frontal and temporal lobes and involves proteinopathies (tau, TDP-43) and neuroinflammation for which both M1 muscarinic and sigma-1R mechanisms are potentially relevant. No clinical trial in FTD has been initiated as of the most recent monograph revision.

7.5 Alzheimer's disease

AF710B was originally developed for Alzheimer's disease and the preclinical evidence base is strongest in this indication. Anavex has indicated that ANAVEX 3-71 is in development for Alzheimer's disease, frontotemporal dementia, and schizophrenia, but no Alzheimer's-specific clinical trial has been publicly registered as of the most recent monograph revision. The compound's development for Alzheimer's disease is positioned as a second-wave indication following the schizophrenia proof-of-concept study.

8. Sourcing and Quality Verification

Research-grade AF710B is available from multiple chemical and pharmaceutical research suppliers in racemic form (rac-AF710B, CAS 1235733-73-9). Suppliers include MedChemExpress, Tocris, TargetMol, BenchChem, Smolecule, EvitaChem, Biorbyt, AbMole, and others. Supply is in milligram quantities (typically 5 to 50 mg vials) at greater than 98 percent purity by HPLC. Pricing is moderate to high for the compound class, reflecting the specialized spirocyclic synthesis and limited production scale.

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity, ideally greater than 98 percent
- Mass spectrometry confirmation of molecular weight (358.19 for the protonated molecule $[M+H]^+$)
- Proton and carbon-13 nuclear magnetic resonance spectroscopy for structural confirmation, with particular attention to the spirocyclic carbon environment
- Chiral HPLC or polarimetry to confirm enantiomeric composition, especially when comparing results to the single-enantiomer clinical candidate (investigators should note that results obtained with racemic AF710B may not be directly comparable to the Anavex clinical material)

The clinical-grade material (ANAVEX 3-71 single enantiomer, oral capsule and tablet formulations) is not commercially available and is accessible only through clinical trial participation or research collaboration with Anavex Life Sciences Corporation.

9. Reconstitution and Handling

AF710B is supplied as a solid powder by research-grade suppliers. For in vitro applications, the compound is reconstituted in dimethyl sulfoxide (DMSO) to prepare concentrated stock solutions, typically at 10 to 100 millimolar. DMSO stocks should be aliquoted for single-use to avoid freeze-thaw degradation. Final working concentrations in cell culture or receptor binding assays are typically in the nanomolar to low-micromolar range, with DMSO vehicle concentrations maintained below 0.1 percent (volume/volume) to avoid solvent cytotoxicity.

For in vivo applications in rodents, the compound has been administered in aqueous vehicle (normal saline or phosphate-buffered saline) at the very low doses used in the published studies (1 to 30 micrograms per kilogram orally, 10 micrograms per kilogram intraperitoneally), where the required concentrations are within the aqueous solubility range. For higher-concentration preparations, co-solvents (DMSO, polyethylene glycol 400, Tween 80) may be required; investigators should validate the formulation for solubility and stability before use.

Storage of the solid compound is recommended at minus 20 degrees Celsius for long-term preservation and at 2 to 8 degrees Celsius for short-term access. The compound should be protected from moisture and light. Reconstituted DMSO stock solutions are stable at minus 20 degrees Celsius for at least six months according to supplier specifications. Aqueous solutions should be prepared fresh and used within 24 hours unless stability data have been generated for the specific formulation.

10. Stack Interactions and Combinations

AF710B interacts with several drug classes through its M1 muscarinic and sigma-1R pharmacology. Investigators conducting research with AF710B, especially in combination with other neuroactive compounds, should consider:

Acetylcholinesterase inhibitors (donepezil, rivastigmine, galantamine): The allosteric M1 mechanism of AF710B potentiates the response to endogenous acetylcholine. Concurrent acetylcholinesterase inhibitors increase synaptic acetylcholine concentration, potentially producing additive or synergistic M1 activation. This combination is pharmacologically rational for Alzheimer's disease research but may amplify cholinergic adverse events (nausea, salivation, bradycardia) at higher doses.

Orthosteric muscarinic agonists (xanomeline, cevimeline, arecoline, pilocarpine): Concurrent orthosteric M1 agonists and AF710B would produce additive M1 activation through distinct binding sites. The combination has not been studied but is unlikely to be clinically translatable owing to the cholinergic adverse event burden of the orthosteric agonists. For in vitro research, the combination may be useful for characterizing allosteric-orthosteric cooperativity at the M1 receptor.

Muscarinic antagonists (atropine, scopolamine, trihexyphenidyl, benztropine): Muscarinic antagonists attenuate or abolish the procognitive effects of AF710B. Concurrent anticholinergic medications (including first-generation antihistamines with antimuscarinic activity) should be considered as confounders in any research application.

Sigma-1 receptor ligands (ANAVEX 2-73/blarcamesine, PRE-084, SA4503, haloperidol): Concurrent sigma-1R agonists would produce additive sigma-1R activation. Haloperidol is a sigma-1R antagonist and would oppose the sigma-1R component of AF710B activity. Investigators using antipsychotic regimens that include haloperidol should consider the sigma-1R antagonist interaction.

CYP-metabolized compounds: The specific cytochrome P450 enzymes responsible for AF710B metabolism have not been publicly identified. Until the metabolic pathways are characterized, caution is warranted with concurrent strong CYP inhibitors or inducers, particularly in human research applications.

QT-prolonging agents: The Phase 1 study demonstrated no clinically significant QT effects at doses up to 200 mg [8]. The cardiac safety profile is favorable compared to xanomeline; concurrent QT-prolonging medications are not expected to produce additive risk at the studied doses but should be monitored in any clinical research protocol.

11. Adverse Events and Safety

11.1 Preclinical safety

AF710B demonstrated a favorable safety profile in preclinical studies. The selectivity screening at 10 micromolar against 83 off-target receptors, ion channels, and transporters produced no significant hits [1]. The clean off-target profile and the allosteric mechanism predict a low liability for the cholinergic adverse events (gastrointestinal hypermotility, salivation, bradycardia, lacrimation) that constrained earlier orthosteric M1 agonists.

In the published rodent efficacy studies, AF710B at the effective dose of 10 micrograms per kilogram (intraperitoneal and oral) was described as safe and well tolerated, with no adverse behavioral or physiological observations noted [1, 6, 7].

11.2 Phase 1 human safety

In the Phase 1 single ascending dose study in 42 healthy volunteers [8]:

- No serious adverse events were reported at any dose (5 to 200 mg)
- No clinically significant laboratory abnormalities were observed
- Treatment-emergent adverse events were mild and similar in frequency and character to placebo
- QTc intervals were normal across all dose groups with no difference from placebo
- No dose-dependent adverse event signal was observed across the 5 to 200 mg range

11.3 Phase 2 schizophrenia safety

In the Phase 2 ANAVEX3-71-SZ-001 study [10, 11]:

- No serious treatment-emergent adverse events were reported
- No severe treatment-emergent adverse events were reported
- The safety profile was consistent with that observed in healthy volunteers

11.4 Comparison to other muscarinic agonists

The adverse event profile of AF710B compares favorably to that of xanomeline, which produces dose-limiting nausea, vomiting, salivation, and sweating through M2/M3 activation and requires co-administration with the peripheral muscarinic antagonist trospium (as in the Cobenfy formulation) to achieve tolerability [20]. AF710B does not require a peripheral antagonist co-formulation, reflecting the M1 selectivity conferred by the allosteric mechanism.

11.5 Anticipated safety considerations

Given the limited clinical exposure (42 healthy volunteers, undisclosed number of schizophrenia patients in Phase 2), the adverse event profile of ANAVEX 3-71 remains incompletely characterized. Potential safety signals that have not been excluded by the current data include: effects on gastrointestinal motility at doses higher than those studied (M1 receptors are expressed on enteric neurons); effects on cardiovascular parameters with chronic dosing; and interactions with concurrent cholinergic medications. Investigators should monitor for these signals in any research application involving chronic dosing or combination regimens.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five M1 muscarinic or sigma-1 receptor candidates against AF710B, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile (clinical or in vitro), and overall validation. The five candidates were selected as the principal ligands with substantive clinical or preclinical pharmacology programs in cognitive and neurodegenerative applications that share mechanistic overlap with AF710B.

12.1 Xanomeline-trospium (Cobenfy, KarXT)

Mechanism: Xanomeline is an orthosteric M1/M4-preferring muscarinic agonist with substantial M2, M3, and M5 cross-reactivity. Trospium chloride is a peripheral muscarinic antagonist that does not cross the blood-brain barrier and is co-administered to mitigate the peripheral cholinergic adverse events produced by xanomeline's non-selective muscarinic activation [20].

Development trajectory: Originally developed by Eli Lilly for Alzheimer's disease in the 1990s, xanomeline was abandoned owing to dose-limiting gastrointestinal and cardiovascular adverse events from M2/M3 activation. Karuna Therapeutics (later acquired by Bristol Myers Squibb) reformulated xanomeline with trospium and advanced through Phase 2 and Phase 3 trials in schizophrenia. In September 2024, the FDA approved Cobenfy as the first muscarinic agonist for schizophrenia [20].

Novelty: Moderate. The muscarinic agonist mechanism for schizophrenia is validated but xanomeline itself is an older compound (1990s development). The innovation is the trospium co-formulation strategy rather than the agonist molecule.

Effect size: Substantial in schizophrenia. The Phase 3 EMERGENT-2 trial reported a least squares mean PANSS total score reduction of 9.6 points versus placebo (Cohen's d approximately 0.61) at 5 weeks [20]. The effect size in Alzheimer's cognition (from the 1990s Eli Lilly trials) was modest.

Promising potential: High. The compound is FDA-approved for schizophrenia. Commercial availability and reimbursement are established.

Side-effect profile: Gastrointestinal adverse events remain the principal limitation despite trospium co-formulation: nausea (17 percent vs. 6 percent placebo), constipation (7 percent), vomiting (6 percent), indigestion (6 percent), hypertension (5 percent), tachycardia (5 percent) [20]. The peripheral antagonist co-formulation strategy reduces but does not eliminate these events.

Overall validation: High for schizophrenia (FDA-approved). Moderate for Alzheimer's disease (development abandoned).

Comparison to AF710B: AF710B and xanomeline share the M1 muscarinic target but differ fundamentally in binding mechanism (allosteric vs. orthosteric), in subtype selectivity (M1-selective vs. M1/M4-preferring with M2/M3/M5 cross-reactivity), and in the requirement for a peripheral antagonist. AF710B has the additional sigma-1R mechanism absent from xanomeline. AF710B has not reached Phase 3 or FDA approval; xanomeline-trospium has. The comparative question for future development is whether AF710B's allosteric selectivity and sigma-1R engagement translate to superior tolerability and disease-modifying activity in clinical trials.

12.2 Blarcamesine (ANAVEX 2-73)

Mechanism: Sigma-1R agonist with additional activity at M1 muscarinic receptors. The precise M1 mechanism (allosteric, orthosteric, or indirect) has not been fully characterized in the published literature. Blarcamesine represents the first-generation Anavex sigma-1/muscarinic program from which AF710B was the designed successor [21].

Development trajectory: Phase 2a completed in Alzheimer's disease. Phase 2 completed in Parkinson's disease dementia. Phase 2 completed in Rett syndrome. Phase 3 trials in Rett syndrome and Alzheimer's disease are in progress or planned. The compound is more clinically advanced than AF710B.

Novelty: Moderate. Sigma-1R agonism for neurodegenerative disease was a relatively novel therapeutic concept at the time of initial development.

Effect size: Phase 2a Alzheimer's disease data showed cognitive stabilization signals; effect sizes have not been reported with conventional metrics in peer-reviewed publications. Rett syndrome Phase 2 data showed promising clinical

signals.

Promising potential: Moderate to high. The compound is more clinically advanced than AF710B and is progressing toward Phase 3 in multiple indications.

Side-effect profile: Generally well tolerated in Phase 2 studies. The principal adverse events reported are dizziness and fatigue.

Overall validation: Moderate. Phase 2 data are available in multiple indications; Phase 3 data are pending.

Comparison to AF710B: Blarcamesine and AF710B share the sigma-1R agonist mechanism and are both Anavex compounds. AF710B has higher M1 muscarinic selectivity (allosteric mechanism) and greater preclinical potency. Blarcamesine has a more advanced clinical trajectory. The two compounds represent successive generations of the same therapeutic concept.

12.3 Cevimeline (AF102B, Evoxac)

Mechanism: Orthosteric M1/M3 muscarinic agonist. Originally developed by Fisher at IIBR as an M1-preferring agonist, cevimeline has significant M3 activity that produces salivation and is the basis for its approved indication [2, 13].

Development trajectory: FDA-approved in 2000 for treatment of dry mouth in Sjogren's syndrome. Approved in Japan in 2001. Not developed for Alzheimer's disease despite preclinical evidence of procognitive and anti-amyloidogenic activity.

Novelty: Low. The compound is over 25 years old and the orthosteric M1 mechanism is well established.

Effect size: Strong for the Sjogren's indication (salivation). Preclinical cognitive enhancement data exist but have not been translated to clinical cognitive endpoints in Alzheimer's disease trials.

Promising potential: Low for cognitive or neurodegenerative indications. The compound is clinically available for Sjogren's syndrome but is not in development for Alzheimer's disease.

Side-effect profile: Dose-dependent salivation (from M3 activation), nausea, sweating, rhinitis. These cholinergic adverse events are intrinsic to the orthosteric mechanism and are the reason cevimeline was not developed for Alzheimer's

disease.

Overall validation: High for Sjogren's syndrome. Low for cognitive indications.

Comparison to AF710B: Cevimeline and AF710B are both products of the Fisher IIBR muscarinic agonist program but represent different generations and different mechanistic strategies (orthosteric vs. allosteric). AF710B was designed specifically to overcome the M3-mediated cholinergic adverse events that limited cevimeline's development for cognitive indications. AF710B has the additional sigma-1R mechanism absent from cevimeline.

12.4 Emraclidine (CVL-231)

Mechanism: M4-selective positive allosteric modulator. Emraclidine enhances M4 receptor signaling in response to endogenous acetylcholine without direct agonist activity and without M1, M2, M3, or M5 engagement [22].

Development trajectory: Cerevel Therapeutics (later acquired by AbbVie) advanced emraclidine through Phase 1b and into two Phase 2 trials (EMPOWER-1 and EMPOWER-2) for schizophrenia. In October 2024, AbbVie reported that the EMPOWER-1 trial did not meet its primary endpoint (change from baseline in PANSS total score at Week 8) [22]. The EMPOWER-2 trial continued enrollment.

Novelty: High. The M4-selective PAM mechanism is distinct from both orthosteric M1 agonism and allosteric M1 agonism and represents a different cholinergic approach to schizophrenia.

Effect size: Phase 2 EMPOWER-1 did not meet primary endpoint. Phase 1b data showed numerical improvements on PANSS but were not statistically powered.

Promising potential: Low to moderate following the Phase 2 failure. EMPOWER-2 results are pending.

Side-effect profile: Phase 1b data showed favorable tolerability. The M4-selective allosteric mechanism avoids the cholinergic adverse events associated with M1/M2/M3 agonism.

Overall validation: Low to moderate. Phase 2 failure on primary endpoint substantially reduces validation.

Comparison to AF710B: Emraclidine and AF710B are both allosteric modulators within the muscarinic system but target different subtypes (M4 vs. M1) and different indications. AF710B has the additional sigma-1R mechanism and the disease-modifying preclinical data in Alzheimer's disease models that emraclidine does not have. Emraclidine's Phase 2 failure in schizophrenia contrasts with AF710B's positive Phase 2 safety and biomarker data, though the endpoints differ (efficacy vs. safety).

12.5 PRE-084

Mechanism: Selective sigma-1R agonist. PRE-084 is a reference sigma-1R agonist widely used in preclinical research. It does not have muscarinic receptor activity [23].

Development trajectory: PRE-084 is a research tool compound that has not been advanced to clinical development. It is used extensively in preclinical sigma-1R pharmacology for mechanism-of-action studies.

Novelty: Low. The compound is well established as a sigma-1R reference agonist.

Effect size: Substantial in preclinical models of neurodegeneration, stroke, and pain. PRE-084 has demonstrated neuroprotection, cognitive enhancement, and anti-inflammatory effects in multiple rodent models. Effect sizes in animal models do not predict human clinical outcomes.

Promising potential: Low as a clinical candidate (no clinical development). High as a research tool for sigma-1R mechanism studies.

Side-effect profile: Preclinical only. No human safety data.

Overall validation: Low as a therapeutic candidate. High as a research tool.

Comparison to AF710B: PRE-084 isolates the sigma-1R component of AF710B's dual mechanism. Comparison of AF710B and PRE-084 effects in the same models can inform the relative contributions of M1 allosteric agonism and sigma-1R agonism to the composite pharmacological profile. AF710B's disease-modifying effects in Alzheimer's models exceed what has been reported for PRE-084 alone, supporting the hypothesis that the dual M1/sigma-1R mechanism produces synergistic neuroprotection.

12.6 Comparator summary

The five comparators occupy distinct positions in the muscarinic/sigma-1R pharmacology space. **Xanomeline-trospium** represents the most clinically validated muscarinic approach, with FDA approval for schizophrenia, but relies on orthosteric agonism with a peripheral antagonist co-formulation to manage tolerability. **Blarcamesine** shares the sigma-1R/muscarinic dual-target concept and is more clinically advanced than AF710B but represents an earlier-generation pharmacology. **Cevimeline** is the historical predecessor from the same medicinal chemistry program, approved for Sjogren's syndrome, and illustrates the tolerability limitations of orthosteric muscarinic agonism. **Emraclidine** represents an alternative allosteric approach targeting M4 rather than M1, with a recent Phase 2 failure. **PRE-084** isolates the sigma-1R mechanism for research comparison.

AF710B occupies a distinctive position in this landscape: it is the only compound that combines allosteric M1 selectivity (avoiding the cholinergic adverse event burden of orthosteric agonists) with sigma-1R agonism (engaging an independent neuroprotective axis) at picomolar-to-nanomolar potency in vivo. The disease-modifying preclinical data across amyloid, tau, and neuroinflammatory pathologies in two independent transgenic rodent models, with effects persisting through drug washout, are not matched by any of the comparators. The principal limitation is clinical maturity: AF710B has completed Phase 1 and an initial Phase 2 safety study, while xanomeline-trospium has achieved FDA approval and blarcamesine is approaching Phase 3.

The practical research question for AF710B is whether the superior preclinical potency, selectivity, and disease-modifying profile translate to clinical efficacy and whether the allosteric M1 mechanism permits doses sufficient for cognitive and disease-modifying effects without the cholinergic adverse events that limited the orthosteric class. The Phase 1 human safety data at doses up to 200 mg and the Phase 2 biomarker signals are encouraging; powered Phase 2 efficacy data and Phase 3 trials are required to establish the clinical positioning of AF710B relative to the validated comparators.

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