

RESEARCH MONOGRAPH . COMPOUND PROFILE

ANAVEX 3-71

A spirocyclic thiadiazine-piperidine compound developed at the Israel Institute for Biological Research as a dual allosteric M1 muscarinic and sigma-1 receptor agonist, distinguished by sub-nanomolar allosteric potency at M1, disease-modifying preclinical efficacy in transgenic Alzheimer's disease models, and active clinical development across schizophrenia, frontotemporal dementia, and Alzheimer's disease indications.

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ABSTRACT

ANAVEX 3-71 (development code AF710B; CAS 1235733-73-9) is a synthetic spirocyclic thiadiazine-piperidine compound and a dual allosteric agonist at the M1 subtype of the muscarinic acetylcholine receptor and the sigma-1 receptor (SIGMAR1). The compound was invented by Abraham Fisher at the Israel Institute for Biological Research (IIBR) in Ness Ziona, Israel, as the fourth generation of a series of cholinergic agonists (AF102B, AF267B, AF292, AF710B) with progressively greater selectivity and allosteric character at the M1 muscarinic receptor. ANAVEX 3-71 emerged from high-throughput receptogram profiling as a compound with sub-nanomolar allosteric binding affinity at M1 muscarinic receptors (approximately 0.05 nM) and nanomolar affinity at the sigma-1 receptor (K_i approximately 1.3 nM), with no agonistic activity at M2 through M5 muscarinic subtypes, no significant binding at alpha-4-beta-2 or alpha-7 nicotinic receptors, and a clean off-target profile across 83 additional G-protein-coupled receptors, ion channels, and transporters screened at 10 micromolar. The preclinical pharmacology of ANAVEX 3-71 has been characterized principally in three studies using transgenic rodent models of Alzheimer's disease. Fisher et al. (2016) reported that AF710B at 10 micrograms per kilogram per day intraperitoneally for two months in 3xTg-AD mice reversed cognitive deficits in the Morris water maze, decreased BACE1, GSK3-beta activity, and tau phosphorylation at multiple epitopes (AT180, AT270, PHF-1), reduced soluble and insoluble amyloid-beta 40 and 42, and rescued mushroom dendritic spine loss in hippocampal neuronal cultures at 30 nanomolar. Hall et al. (2018) extended these findings to McGill-R-Thy1-APP transgenic rats,

demonstrating that oral treatment at 10 micrograms per kilogram per day for 4.5 months at 13 months of age (postplaque) reverted cognitive deficits, reduced amyloid pathology and neuroinflammation, and increased cerebrospinal fluid amyloid clearance, with effects maintained following a 5-week treatment interruption consistent with disease-modifying rather than symptomatic activity. Orciani et al. (2023) demonstrated that early (preplaque, 7 months of age) treatment at the same dose for 7 months prevented cognitive impairment, reduced hippocampal plaque burden and cortical amyloid-beta peptides, attenuated microglial and astrocytic neuroinflammation, and rescued pro-BDNF to mature BDNF conversion, with effects persisting through a 4-week washout. Human pharmacokinetics characterized in a Phase 1 single ascending dose study (NCT04442945; 36 healthy volunteers; 5 to 200 mg) demonstrate linear, dose-proportional, and time-invariant pharmacokinetics with a mean apparent terminal elimination half-life of 3.56 hours for the parent compound and 6.59 hours for the M8 metabolite. No clinically relevant effects on QTc or other electrocardiographic parameters were observed across the full studied dose range. Food had no effect on the pharmacokinetics of ANAVEX 3-71 or its M8 metabolite. Clinical development is ongoing across multiple indications. A Phase 2 study in schizophrenia (NCT06245213; 71 subjects) reported dose-dependent improvements in electroencephalographic biomarkers and favorable safety and tolerability with no serious treatment-emergent adverse events. A modified-release oral tablet enabling once-daily dosing was successfully developed in a Phase 1b study completed in October 2025. ANAVEX 3-71 is not approved by any regulatory authority and is supplied as a research-grade preparation. This monograph reviews the chemistry, dual-receptor pharmacology, preclinical

pharmacology in transgenic Alzheimer's models, human pharmacokinetics, the clinical evidence base, sourcing and quality verification, handling considerations, interaction profile, adverse event signal, and a comparative assessment of five mechanistically related compounds against ANAVEX 3-71 on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

International Nonproprietary Name: Not assigned. The compound is designated ANAVEX 3-71 by Anavex Life Sciences Corp. and AF710B in the original academic literature from the Israel Institute for Biological Research.

Development codes: AF710B (Israel Institute for Biological Research); ANAVEX 3-71 (Anavex Life Sciences Corp.). The development code AF710B is the canonical identifier in the preclinical pharmacology literature, including the seminal Fisher et al. (2016) characterization, the Hall et al. (2018) disease-modification study, and the Orciani et al. (2023) early-treatment study [1, 2, 3]. The designation ANAVEX 3-71 appears in the clinical development literature, including the Phase 1 and Phase 2 clinical trial registrations and the pharmacokinetic publications [4, 5].

Chemical Abstracts Service registry number: 1235733-73-9. This CAS number corresponds to the racemic form ((Rac)-AF710B). The pharmacologically active S-enantiomer is the material advanced in clinical development; the racemic mixture is also available from research-grade suppliers and has been used in some preclinical studies [1, 10].

International Union of Pure and Applied Chemistry name: 1-[(2S)-2,8-dimethyl-1-thia-3,8-diazaspiro[4.5]decan-3-yl]-3-(1H-indol-3-yl)propan-1-one [10].

1.2 Structural class and chemistry

ANAVEX 3-71 is a spirocyclic thiadiazine-piperidine compound bearing an indole pharmacophore linked through a propanone bridge. The central structural feature is a spiro junction between a thiazolidine ring and a piperidine ring, creating the 1-thia-3,8-diazaspiro[4.5]decane core. The thiazolidine sulfur and the piperidine nitrogen (N-8, methylated) are the heteroatoms defining the spirocyclic system. The indol-3-yl group is connected to the thiadiazine nitrogen via a three-carbon propanoyl linker. This architecture is structurally distinct from both the orthosteric M1 muscarinic agonists (xanomeline, cevimeline) and the conventional sigma-1 receptor ligands (PRE-084, pridopidine), reflecting the independent medicinal chemistry lineage from the Fisher laboratory at IIBR [1].

The compound belongs to a broader series of bicyclic heterocyclic spiro compounds covered by US Patent 9,034,891 B2, issued May 19, 2015, with claims encompassing compositions and methods for treating Alzheimer's disease, type 2 diabetes, and insulin resistance [9]. The medicinal chemistry progression within the Fisher laboratory moved from AF102B (an orthosteric M1 muscarinic agonist), through AF267B and AF292 (which introduced partial allosteric character), to AF710B (which achieved full allosteric agonism at M1 with concurrent sigma-1 receptor activity) [1, 6].

Molecular formula: C₂₀H₂₇N₃O₃S.

Molecular weight: 357.51 g/mol.

Canonical SMILES: C[C@H]1N(CC2(CCN(C)CC2)S1)C(=O)CCC3=CNC4=C3C=CC=C4 [10].

InChI: InChI=1S/C20H27N3OS/c1-15-23(14-20(25-15)9-11-22(2)12-10-20)19(24)8-7-16-13-21-18-6-4-3-5-17(16)18/h3-6,13,15,21H,7-12,14H2,1-2H3/t15-/m0/s1 [10].

InChIKey: XWMHYQATSBWUNT-HNNXBMFYSA-N [10].

Stereochemistry: The compound contains one defined stereocenter at carbon 2 of the thiazolidine ring (S-configuration in the pharmacologically active form). The S-enantiomer (AF710B) exhibits higher potency and selectivity for the M1 muscarinic receptor compared to the racemic mixture [1]. Investigators acquiring research-grade material should confirm enantiomeric identity, as both the racemic form ((Rac)-AF710B) and the single enantiomer are commercially available. Chiral high-performance liquid chromatography or polarimetry against a reference standard is appropriate for enantiomeric verification.

Physical properties: The compound is supplied as a white to off-white powder. Solubility data from research-grade suppliers indicate solubility in dimethyl sulfoxide (DMSO). Water solubility is limited for the free base form.

1.3 Synthesis

The published synthesis of ANAVEX 3-71 proceeds through construction of the 1-thia-3,8-diazaspiro[4.5]decane core followed by acylation with 3-(1H-indol-3-yl)propanoic acid or a suitably activated derivative. The spirocyclic core is assembled by condensation of an appropriately substituted piperidone with a thiazoli-

dine precursor bearing the S-configured methyl substituent. The propanoyl linkage to the indole pharmacophore is established through standard amide bond formation (carbodiimide or acid chloride coupling). The synthetic route and structural claims are described in US Patent 9,034,891 B2 [9]. Resolution of enantiomers, if the synthesis is racemic, is achieved by chiral chromatography. Industrial-scale synthesis details have not been published in the peer-reviewed literature as of the present date.

2. Abstract

ANAVEX 3-71 (AF710B) is an allosteric agonist at the M1 muscarinic acetylcholine receptor and at the sigma-1 receptor (SIGMAR1). The compound was developed by Abraham Fisher at the Israel Institute for Biological Research and licensed to Anavex Life Sciences Corp. for clinical development in Alzheimer's disease, frontotemporal dementia, and schizophrenia. At the M1 muscarinic receptor, ANAVEX 3-71 acts as an allosteric modulator with sub-nanomolar binding affinity, potentiating orthosteric agonist binding and downstream signaling (phospho-ERK1/2, phospho-CREB) without direct orthosteric agonism, and with no activity at M2 through M5 muscarinic subtypes [1, 7]. At the sigma-1 receptor, the compound binds with a K_i of approximately 1.3 nanomolar and engages neuroprotective signaling cascades involving calcium homeostasis, endoplasmic reticulum stress modulation, and mitochondrial function at the mitochondria-associated endoplasmic reticulum membrane (MAM) [1, 7].

Preclinical evidence from three principal studies in transgenic rodent models of Alzheimer's disease demonstrates that ANAVEX 3-71 at oral or intraperitoneal doses of 10 micrograms per kilogram per day reverses or prevents cognitive deficits, reduces amyloid and tau pathology, attenuates neuroinflammation, and rescues synaptic markers, with effects persisting through multi-week washout periods consistent with disease-modifying activity [1, 2, 3]. Phase 1 human pharmacokinetics demonstrate linear, dose-proportional kinetics with a parent half-life of approximately 3.56 hours and no clinically relevant cardiac safety signal [4, 5]. A Phase 2 study in schizophrenia has reported favorable safety and dose-dependent biomarker improvements [13, 14]. The compound is not approved by any regulatory authority.

3. Discovery and Development History

3.1 Origin

ANAVEX 3-71 (AF710B) was developed by Abraham Fisher and colleagues at the Israel Institute for Biological Research (IIBR) in Ness Ziona, Israel. The compound represents the fourth generation of a cholinergic agonist series that began with AF102B, an orthosteric M1 muscarinic agonist studied in the 1990s, and progressed through AF267B and AF292, which introduced progressively greater allosteric character and selectivity [1, 6]. AF710B was identified through high-throughput receptor profiling as a compound with an unprecedented combination of highly potent allosteric M1 muscarinic agonism and sigma-1 receptor agonism, with no agonistic activity at M2 through M5 muscarinic subtypes [1]. The compound's dual-target pharmacology distinguished it from both the conventional orthosteric muscarinic agonists (which are limited by peripheral cholinergic adverse events mediated by M2 and M3 receptor activation) and from single-target sigma-1 receptor agonists.

3.2 Intellectual property and licensing

The compound is covered by US Patent 9,034,891 B2, titled "Bicyclic heterocyclic spiro compounds," issued May 19, 2015 [9]. Patent claims encompass compositions and methods for treating Alzheimer's disease, type 2 diabetes, and insulin resistance. ANAVEX 3-71 was licensed by IIBR to Anavex Life Sciences Corp. (New York, USA) for clinical development. Anavex has subsequently been the sponsor for all registered clinical trials of ANAVEX 3-71.

3.3 Preclinical development arc

The first peer-reviewed characterization of AF710B was published by Fisher et al. (2016) in *Neurodegenerative Diseases* [1]. This paper reported the receptor binding profile, the allosteric mechanism at M1, the neuroprotective activity via sigma-1 receptor, and the therapeutic efficacy in 3xTg-AD mice and in cognitively impaired rats. Hall et al. presented preliminary McGill transgenic rat data at the 2016 Alzheimer's Association International Conference [8] and published the complete

disease-modification study in Alzheimer's and Dementia in 2018 [2]. Orciani et al. (2023) completed the preclinical characterization with the early-treatment prevention study in preplaque McGill rats [3]. Cuello, Hall, and Do Carmo (2019) reviewed the compound's experimental pharmacology in the context of transgenic rodent models of Alzheimer's disease [6].

3.4 Clinical development

Anavex Life Sciences initiated the first human study of ANAVEX 3-71 in July 2020 with a Phase 1 single ascending dose (SAD) trial in 36 healthy volunteers (NCT04442945), completed in October 2021 [11]. The Phase 1 data, published in two clinical pharmacology papers by Fadiran et al. (2023, 2024), characterized the pharmacokinetics, food effect, and cardiac safety profile [4, 5].

In March 2024, Anavex initiated a Phase 2 study (ANAVEX3-71-SZ-001, NCT06245213) evaluating ANAVEX 3-71 in adults with schizophrenia [12]. The two-part study enrolled 71 subjects: Part A (multiple ascending dose, 16 subjects at 30 mg or 60 mg three times daily or placebo for 10 days) and Part B (double-blind, placebo-controlled, 55 subjects at 60 mg three times daily or placebo for 28 days) [12]. Preliminary biomarker results from Part A were announced in October 2024 [14], and positive topline results were announced in October 2025 [13].

In October 2025, Anavex completed a Phase 1b study (ANAVEX3-71-002) comparing an immediate-release capsule with a modified-release oral tablet formulation, demonstrating that the modified-release tablet enables once-daily dosing with a safety profile consistent with prior studies [15].

The compound's stated clinical development indications include frontotemporal dementia, schizophrenia, and Alzheimer's disease [4, 5]. No clinical trial in frontotemporal dementia or Alzheimer's disease has been registered on ClinicalTrials.gov as of the present date; the clinical development in these indications is at the pre-registration or pre-IND stage. The schizophrenia indication represents the most advanced clinical program.

4. Mechanism of Action

ANAVEX 3-71 is a dual-target ligand whose principal molecular activities are allosteric agonism at the M1 subtype of the muscarinic acetylcholine receptor and agonism at the sigma-1 receptor (SIGMAR1). These activities operate at pharmacologically distinct concentrations and engage separable downstream signaling cascades. The dual mechanism is the pharmacological basis for the compound's observed effects on cognition, amyloid processing, tau phosphorylation, neuroinflammation, and synaptic integrity in transgenic Alzheimer's disease models.

4.1 M1 muscarinic receptor allosteric agonism

The M1 muscarinic acetylcholine receptor (CHRM1) is a Gq/11-coupled G-protein-coupled receptor expressed at high density on cortical and hippocampal pyramidal neurons, on basal forebrain cholinergic projection neurons, and on striatal medium spiny neurons. It mediates the principal procognitive effects of cholinergic neurotransmission in the central nervous system and is the therapeutic target for cognitive enhancement in Alzheimer's disease and schizophrenia [21, 25].

ANAVEX 3-71 binds the M1 receptor at an allosteric site with a reported affinity of approximately 0.05 nanomolar [7]. The compound does not produce direct orthosteric activation; instead, it potentiates the binding and efficacy of the endogenous orthosteric agonist acetylcholine (and the synthetic agonist carbachol in experimental systems). In PC12M1 cells, AF710B at 0.1 nanomolar decreased the high-affinity dissociation constant (K_H) for carbachol by approximately two orders of magnitude and increased the ratio of low-affinity to high-affinity binding (K_L/K_H) from 110 to 1650, indicating a substantial allosteric enhancement of agonist-receptor coupling [1]. At concentrations from 0.1 to 100 nanomolar, AF710B potentiated carbachol-induced phosphorylation of extracellular signal-regulated kinases 1/2 (ERK1/2) and cAMP response element-binding protein (CREB) in the same cell system [1]. These two downstream effectors (phospho-ERK1/2 and phospho-CREB) are canonical mediators of synaptic plasticity, long-term potentiation, and memory consolidation in hippocampal circuits.

The selectivity of ANAVEX 3-71 for M1 over the other muscarinic subtypes is a critical pharmacological property. The compound showed no agonistic activity at M2, M3, M4, or M5 muscarinic acetylcholine receptors [1]. This subtype selectivity is the principal advantage of the allosteric mechanism over orthosteric muscarinic agonists such as xanomeline, which activate M1 and M4 at the orthosteric site but also engage peripheral M2 and M3 receptors, producing dose-limiting gastrointestinal and cardiovascular cholinergic adverse events [18, 25].

4.2 Sigma-1 receptor agonism

The sigma-1 receptor (SIGMAR1) is an intracellular chaperone protein enriched at the mitochondria-associated endoplasmic reticulum membrane (MAM), where it modulates calcium signaling between the endoplasmic reticulum and mitochondria, regulates the unfolded protein response and endoplasmic reticulum stress, supports mitochondrial function and ATP production, and enhances autophagy through stabilization of nuclear pore proteins and promotion of TFEB nuclear translocation [7, 19, 22].

ANAVEX 3-71 binds the sigma-1 receptor with a K_i of approximately 1.3 nanomolar and the sigma-2 receptor with substantially lower affinity (approximately 10 micromolar), yielding a selectivity ratio of approximately 7,700-fold for sigma-1 over sigma-2 [7]. At 10 micromolar, the compound showed no significant binding at mu opioid receptors or serotonin receptors, and no significant activity across 83 additional GPCRs, ion channels, and transporters in a broad off-target screen [1].

The sigma-1 receptor agonism of ANAVEX 3-71 produces neuroprotective effects that are mechanistically separable from the M1 muscarinic activity. In hippocampal neuronal cultures, the neuroprotective effects of AF710B against amyloid-beta(25-35)-induced toxicity and against nerve growth factor (NGF) withdrawal were blocked by the sigma-1 receptor antagonist NE100, confirming sigma-1 mediation [1]. The compound was effective across a concentration range of 1 nanomolar to 1 micromolar in these neuroprotection assays [1]. Sigma-1 receptor knockdown by RNA interference abolished the dendritic spine rescue activity of AF710B, demonstrating that sigma-1 receptor engagement is required for the synaptic protective effect [1].

4.3 Dual-receptor requirement for synaptic protection

A distinctive finding from the Fisher et al. (2016) characterization is that both the M1 muscarinic and sigma-1 receptor activities are required for the compound's dendritic spine rescue effect. In hippocampal neuronal cultures from PS1-KI and APP-KI transgenic mice, AF710B at 30 nanomolar markedly rescued mushroom spine loss, restoring spine density nearly to the level of non-transgenic animals [1]. The earlier compound AF267B was only partially effective in PS1-KI cultures and ineffective in APP-KI cultures, suggesting that the dual-target mechanism of AF710B provides superior synaptic protection [1]. The M1-selective antagonist pirenzepine at 1 micromolar completely blocked the spine rescue by AF710B, confirming M1 dependence [1]. As noted above, sigma-1 receptor knockdown similarly abolished the effect. The compound's synaptic protective activity therefore requires concurrent engagement of both molecular targets.

4.4 Downstream effects on Alzheimer's disease pathology

Through M1 muscarinic and sigma-1 receptor activation, ANAVEX 3-71 modulates multiple pathological cascades relevant to Alzheimer's disease:

Amyloid processing: AF710B significantly diminished the expression of BACE1 (beta-site APP-cleaving enzyme 1), the rate-limiting enzyme in the amyloidogenic pathway, and reduced levels of the C99 fragment (the immediate precursor to amyloid-beta) in 3xTg-AD mice [1]. The compound increased secretion of soluble amyloid precursor protein alpha (sAPPalpha), the product of the non-amyloidogenic alpha-secretase pathway [1]. In McGill transgenic rats, long-term treatment increased cerebrospinal fluid amyloid-beta clearance [2].

Tau phosphorylation: AF710B reduced phosphorylated tau at the AT180 (Thr231), AT270 (Thr181), and PHF-1 (Ser396/Ser404) epitopes in 3xTg-AD mice [1]. The compound significantly increased inhibitory phosphorylation of GSK3-beta at the Serine-9 residue and diminished levels of the p25 fragment (an activator of CDK5), indicating modulation of two principal tau kinases [1].

Neuroinflammation: In both 3xTg-AD mice and McGill transgenic rats, AF710B reduced markers of neuroinflammation [1, 2, 3]. In the Orciani et al. (2023) study, treatment reduced microglia and astrocyte recruitment toward CA1 hippocampal amyloid-burdened neurons and altered the inflammatory cytokine profile [3].

Neurotrophic signaling: AF710B rescued the conversion of brain-derived neurotrophic factor (BDNF) precursor (proBDNF) to the mature, biologically active form in treated McGill transgenic rats [3]. The compound also increased BCL2 (anti-apoptotic) expression in mitochondria [1].

4.5 Composite pharmacology and selectivity

The composite pharmacological profile of ANAVEX 3-71 is defined by sub-nanomolar allosteric M1 muscarinic agonism, low-nanomolar sigma-1 receptor agonism, and absence of meaningful off-target activity at the 83-target panel at 10 micromolar [1, 7]. The allosteric M1 mechanism preserves the dependence on endogenous acetylcholine for receptor activation, avoiding the tonic cholinergic stimulation that limits orthosteric agonists. The sigma-1 receptor agonism provides a neuroprotective and neurotrophic complement that operates through an independent signaling axis. The requirement for both targets in the dendritic spine rescue assay suggests that the dual mechanism is not merely additive but synergistic in the context of synaptic protection [1].

5. Pharmacokinetics and Routes of Administration

5.1 Absorption and dose proportionality

The pharmacokinetics of ANAVEX 3-71 were characterized in a Phase 1 single ascending dose study (NCT04442945) in 36 healthy volunteers receiving oral doses of 5 to 200 mg [5, 11]. The pharmacokinetics are linear, dose proportional, and time invariant across the studied dose range, as determined by a power model for dose proportionality assessment [5]. Two-compartment and three-compartment linear pharmacokinetic models were tested; the population pharmacokinetic model was used to characterize the disposition of both the parent compound and its M8 metabolite [5].

Food effect was evaluated in 12 subjects who received 160 mg under fed and fasted conditions. Food had no effect on the pharmacokinetics of ANAVEX 3-71 or its M8 metabolite, indicating that the compound can be administered without regard to meals [5].

5.2 Distribution and elimination

The mean apparent terminal elimination half-life of ANAVEX 3-71 is 3.56 hours (standard deviation 4.09 hours), consistent with rapid elimination [5]. The M8 metabolite, which is the principal circulating metabolite, was eliminated with a mean apparent terminal elimination half-life of 6.59 hours (standard deviation 1.64 hours), approximately twice that of the parent compound [5]. The identity of the M8 metabolite (the metabolic transformation it represents and the enzymes responsible) has not been published in the peer-reviewed literature as of the present date. The cytochrome P450 isoforms responsible for ANAVEX 3-71 metabolism have not been formally characterized in published studies.

Population pharmacokinetic analysis indicated that age, body weight, and creatinine clearance were not significant explanatory covariates for the apparent clearance or apparent volume of the central compartment of ANAVEX 3-71 [5]. Formal characterization of metabolism by specific CYP isoforms, protein binding, volume

of distribution, and renal versus hepatic elimination routes remain unpublished. Investigators requiring these parameters should treat the available pharmacokinetic characterization as limited to the published Phase 1 data.

5.3 Cardiac safety

The cardiodynamic evaluation from the Phase 1 SAD study demonstrated no clinically relevant effect of ANAVEX 3-71 on heart rate, PR interval, QRS interval, or QTc interval at single ascending doses of 5 to 200 mg [4]. The estimated population slope of the concentration-QTcF relationship was small and slightly negative: -0.017 milliseconds per microgram per liter, with a treatment effect-specific intercept of -0.49 milliseconds [4]. An effect on the placebo-corrected, change-from-baseline QTc exceeding 10 milliseconds can be excluded within the full observed ranges of plasma concentrations of ANAVEX 3-71 (up to approximately 996 micrograms per liter) and M8 (up to approximately 58 micrograms per liter) [4].

5.4 Preclinical safety margin

In preclinical studies, the maximum tolerated dose of ANAVEX 3-71 exceeded 50 mg/kg by the oral route in rats [1]. Given that the pharmacologically efficacious dose in transgenic Alzheimer's disease models is 10 micrograms per kilogram, the preclinical safety margin exceeds 50,000-fold at the efficacious dose [1].

5.5 Routes of administration and formulations

The current clinical formulation is an immediate-release oral capsule. In the Phase 2 schizophrenia study, the compound was administered as oral capsules at 30 mg or 60 mg three times daily [12]. In October 2025, Anavex completed a Phase 1b study (ANAVEX3-71-002) comparing the immediate-release capsule with a modified-release oral tablet formulation [15]. The modified-release tablet demonstrated a pharmacokinetic profile supportive of once-daily dosing, with a safety profile consistent with prior studies [15]. The modified-release formulation is anticipated to be used in future clinical studies.

No intravenous, intramuscular, or subcutaneous formulations have been reported. Research-grade material is supplied as a powder for reconstitution in DMSO or other appropriate solvents for in vitro and in vivo investigations.

6. Preclinical Pharmacology

6.1 Cognitive enhancement in anticholinergic-impaired rats

The initial cognitive characterization of ANAVEX 3-71 was performed in Wistar rats treated with the M1-selective antagonist trihexyphenidyl to induce cholinergic cognitive impairment, using the passive avoidance paradigm [1]. AF710B at oral doses of 1, 3, 10, 30, and 100 micrograms per kilogram reversed the trihexyphenidyl-induced impairment in a dose-dependent manner. Retention latency values for rats treated with AF710B at 1, 3, or 10 micrograms per kilogram were significantly longer than those of trihexyphenidyl-treated control rats ($p < 0.001$) [1]. The effective oral dose range (1 to 30 micrograms per kilogram) in this model represents an extraordinarily low dose for a centrally active compound and is consistent with the sub-nanomolar allosteric binding affinity at M1.

6.2 Efficacy in 3xTg-AD transgenic mice

Fisher et al. (2016) evaluated AF710B in female 3xTg-AD mice, which express mutant human APP (Swedish), presenilin 1 (M146V), and tau (P301L) and develop progressive amyloid plaques, neurofibrillary tangles, and cognitive deficits recapitulating features of human Alzheimer's disease [1]. Treatment with AF710B at 10 micrograms per kilogram per day intraperitoneally for two months, initiated at 10 months of age, produced the following outcomes:

Cognition: AF710B improved performance in the Morris water maze, as demonstrated by decreased escape latency times in treated 3xTg-AD mice compared to vehicle-treated controls [1].

Amyloid pathology: AF710B treatment significantly reduced levels of amyloid-beta 40 and amyloid-beta 42 in both detergent-soluble and detergent-insoluble fractions [1]. The compound significantly diminished the expression of BACE1 and reduced C99 fragment levels, indicating reduced amyloidogenic processing of amyloid precursor protein [1].

Tau pathology: Phosphorylated tau was reduced in AF710B-treated 3xTg-AD mice at the AT180 (pThr231), AT270 (pThr181), and PHF-1 (pSer396/pSer404) epitopes [1]. The compound increased inhibitory phosphorylation of GSK3-beta at Serine-9 and diminished levels of the p25 fragment of CDK5, indicating modulation of two principal tau kinases [1].

Neuroinflammation: AF710B reduced markers of neuroinflammation in treated animals [1].

Dendritic spine rescue: In hippocampal neuronal cultures derived from PS1-KI (M146V) and APP-KI mice, AF710B at 30 nanomolar markedly rescued mushroom spine loss, restoring spine density nearly to the level of non-transgenic animals [1]. The earlier compound AF267B was only partially effective in PS1-KI cultures and ineffective in APP-KI cultures. Spine rescue was blocked by both the M1 antagonist pirenzepine (1 micromolar) and sigma-1 receptor knockdown, confirming the requirement for both targets [1].

6.3 Disease modification in McGill-R-Thy1-APP transgenic rats (postplaque treatment)

Hall et al. (2018) extended the preclinical characterization to the McGill-R-Thy1-APP transgenic rat, which expresses a single copy of human APP bearing the Swedish and Indiana mutations and develops progressive amyloid pathology with cognitive deficits [2]. This model was selected because its single-transgene design and the rat species context provide a closer translational approximation to human Alzheimer's disease than multi-transgenic mouse models.

Treatment with AF710B at 10 micrograms per kilogram per day by oral gavage was initiated in 13-month-old postplaque transgenic rats and continued for 4.5 months. Critically, treatment was interrupted 5 weeks before behavioral testing to permit a washout period that distinguishes disease-modifying from symptomatic effects [2].

AF710B long-term treatment reverted the cognitive deficits associated with advanced Alzheimer-like amyloid neuropathology in the treated rats [2]. These effects were accompanied by reductions in amyloid pathology and markers of neuroinflammation, increases in amyloid cerebrospinal fluid clearance, and increases in

levels of the synaptic marker synaptophysin [2, 6]. Cuello, Hall, and Do Carmo (2019) confirmed that pathology was "frankly diminished" and cognition improved after the washout period, supporting the disease-modifying interpretation [6].

The persistence of therapeutic effects through the 5-week washout period is the central disease-modification claim for ANAVEX 3-71. A purely symptomatic effect (such as that produced by acetylcholinesterase inhibitors) would be expected to reverse upon drug discontinuation. The sustained benefit in the Hall et al. design suggests modification of underlying disease biology rather than symptom masking.

6.4 Early (preplaque) treatment in McGill-R-Thy1-APP transgenic rats

Orciani et al. (2023) tested whether preventive treatment with AF710B, initiated before the onset of amyloid plaque deposition, could prevent the emergence of Alzheimer's disease-like pathology [3]. Daily oral administration of AF710B at 10 micrograms per kilogram was initiated in 7-month-old preplaque McGill-R-Thy1-APP rats and continued for 7 months, followed by a 4-week washout period before behavioral and histological assessment.

Long-term AF710B treatment prevented the cognitive impairment of McGill-R-Thy1-APP rats [3]. The effect was accompanied by a reduction in the number of amyloid plaques in the hippocampus and decreased levels of amyloid-beta 42 and amyloid-beta 40 peptides in the cerebral cortex [3]. AF710B treatment reduced microglia and astrocyte recruitment toward CA1 hippocampal amyloid-burdened neurons compared to vehicle-treated transgenic rats and altered the inflammatory cytokine profile [3]. The compound also rescued the conversion of brain-derived neurotrophic factor precursor (proBDNF) to the mature, biologically active form of BDNF [3].

The results support both preventive and disease-modifying properties of ANAVEX 3-71, extending the Hall et al. (2018) findings from postplaque rescue to preplaque prevention [3]. The consistent efficacious dose across all three principal preclinical studies (10 micrograms per kilogram per day) is notable for its low magnitude and supports the high in vivo potency expected from the sub-nanomolar allosteric M1 binding affinity.

6.5 Translational validity assessment

The preclinical evidence for ANAVEX 3-71 is concentrated in two transgenic models (3xTg-AD mice and McGill-R-Thy1-APP rats) studied by overlapping investigator groups (Fisher laboratory at IIBR and Cuello laboratory at McGill University). The compound has not been independently replicated by laboratories outside these collaborative groups in published peer-reviewed form. The disease-modification claim (persistence through washout) has been reproduced across the two distinct transgenic models and across both early-treatment and late-treatment paradigms, which strengthens the evidence; however, the absence of independent replication is a limitation that investigators should weigh. The pharmacologically active dose in rodent models (10 micrograms per kilogram) translates by interspecies allometric scaling to a human-equivalent dose that is substantially below the Phase 1 studied dose range (5 to 200 mg), providing a wide margin between the efficacious preclinical dose and the safe human dose range.

7. Clinical Evidence Base

The clinical evidence for ANAVEX 3-71 as of May 2026 consists of one completed Phase 1 single ascending dose study, one ongoing Phase 2 study in schizophrenia with preliminary results, and one completed Phase 1b formulation study. The compound is not approved by any regulatory authority and is not in clinical use for any indication. This section summarizes the published and publicly disclosed clinical data. Investigators should note that ANAVEX 3-71 is a research compound; the clinical evidence base presented below does not constitute evidence of therapeutic efficacy or a recommendation for human use.

7.1 Phase 1 single ascending dose study (NCT04442945)

A double-blind, randomized, placebo-controlled Phase 1 study was conducted in 36 healthy volunteers (ages 18 to 55, BMI 19 to 28 kg/m², non-smokers) from July 2020 to October 2021 [11]. Subjects received single ascending oral doses of ANAVEX 3-71 (up to four dose levels across the 5 to 200 mg range) or matching placebo, with quadruple masking (participant, care provider, investigator, outcomes assessor). The primary endpoints were safety and tolerability (adverse event monitoring and dose-limiting adverse events) and pharmacokinetic parameters (C_{max} and AUC). The secondary endpoint was electrocardiographic evaluation, specifically QT interval assessment [11].

The pharmacokinetic results, published by Fadirán et al. (2024), demonstrated linear, dose-proportional, and time-invariant pharmacokinetics with a mean apparent terminal elimination half-life of 3.56 hours for the parent compound and 6.59 hours for the M8 metabolite [5]. The cardiodynamic results, published by Fadirán et al. (2023), confirmed no clinically relevant effects on QT_c or other ECG parameters at any dose studied, with exclusion of a QT_c effect exceeding 10 milliseconds across the full observed plasma concentration range [4].

No serious adverse events were reported across all dose cohorts. No dose-limiting adverse events were identified, supporting the tolerability of single doses up to 200 mg [11]. The specific adverse event types and incidence rates from the Phase 1 study have not been published in detail beyond the aggregate safety statements in the pharmacokinetic papers; investigators seeking granular safety data should note

this gap. The study included a food-effect arm in which 12 subjects received 160 mg under fed and fasted conditions in a crossover design, confirming that food does not alter the pharmacokinetics of ANAVEX 3-71 or its M8 metabolite [5]. Formal results, including individual adverse event tabulations, have not been posted to ClinicalTrials.gov as of the present date [11].

7.2 Phase 2 study in schizophrenia (NCT06245213)

The ANAVEX3-71-SZ-001 study is a two-part Phase 2 study evaluating the safety, tolerability, efficacy, pharmacokinetics, and electrophysiology of ANAVEX 3-71 in adults with schizophrenia [12]. The study enrolled 71 participants at inpatient sites, with eligibility requiring primary schizophrenia diagnosis of at least one year, stable symptoms for at least 6 weeks, PANSS total score of 80 or less, cognitive impairment (BACS T-score below 50), and stable atypical antipsychotic background therapy [12].

Part A (Multiple Ascending Dose): Sixteen subjects were randomized to 30 mg three times daily (n = 6), 60 mg three times daily (n = 6), or placebo (n = 4) for 10 days. Preliminary biomarker results from Part A, announced in October 2024, reported dose-dependent improvements in two electrophysiological biomarkers: 40 Hz auditory steady-state response (ASSR) inter-trial coherence (ITC), indicating enhanced neural synchronization, and resting-state alpha power, reflecting improvements in thalamocortical circuits and sensory gating [14]. ANAVEX 3-71 was well tolerated in Part A, with no serious adverse events [14].

Part B (Double-Blind, Placebo-Controlled): Fifty-five subjects were randomized to 60 mg three times daily (n = 28) or placebo (n = 27) for 28 days. Secondary and exploratory endpoints included PANSS (symptom severity), BACS (cognitive assessment), CGI-SCH (clinical global impression), VRFCAT (functional capacity), biomarkers (YKL-40, GFAP, amino acid metabolites, genomic and transcriptomic analysis), and EEG/ERP measures [12].

Positive topline results were announced in October 2025 [13]. No serious treatment-emergent adverse events (TEAEs) and no severe TEAEs were reported in either part of the study. In Part A, one TEAE was reported (16.7% of the 30 mg group). In Part B, 39.3% of the treatment group versus 48.1% of the placebo group experienced TEAEs [13]. The study reported positive trends in objective EEG/ERP biomarkers and a reduction in glial fibrillary acidic protein (GFAP) in the ANAVEX 3-71 group versus placebo, which the sponsor characterized as suggestive of a po-

tential disease-modifying effect [13]. Specific PANSS total score changes and cognitive efficacy results (BACS, VRFCAT) have not been reported publicly as of May 2026.

The study is listed as active, not recruiting, with an estimated primary completion date of June 2025 [12].

7.3 Phase 1b formulation study (ANAVEX3-71-002)

An open-label, randomized Phase 1b study comparing the immediate-release oral capsule with a modified-release oral tablet formulation was conducted in healthy adults [15]. The study achieved its primary endpoint of demonstrating safety and tolerability in both male and female subjects [15]. The modified-release tablet exhibited a pharmacokinetic profile described by the sponsor as superior to the immediate-release capsule and supportive of once-daily dosing [15]. Specific pharmacokinetic parameters for the modified-release formulation have not been published. The study was completed in October 2025 [15].

7.4 Clinical development gaps

No clinical trials in Alzheimer's disease or frontotemporal dementia, the two indications with the strongest preclinical rationale, have been registered as of May 2026. The clinical efficacy of ANAVEX 3-71 on cognitive endpoints in any indication has not been formally reported. The Phase 2 schizophrenia biomarker data (EEG/ERP, GFAP) are promising signals but do not constitute evidence of clinical efficacy on primary disease endpoints. Investigators should treat the current clinical evidence base as early-stage safety and pharmacokinetic characterization with exploratory biomarker signals, pending publication of the full Phase 2 results.

8. Sourcing and Quality Verification

Research-grade ANAVEX 3-71 (AF710B) is supplied by multiple chemical research suppliers, including MedChemExpress, TargetMol, Smolecule, BenchChem, and others, at purity grades typically exceeding 98% by HPLC. The compound is available as both the racemic form ((Rac)-AF710B) and the pharmacologically active S-enantiomer, and investigators should specify the desired stereochemical form at the time of purchase. Pricing is in the range typical of research-grade pharmaceutical compounds (milligram to gram quantities).

Pharmaceutical-grade ANAVEX 3-71 is not commercially available; the clinical formulation (oral capsule and modified-release tablet) is accessible only through participation in Anavex-sponsored clinical trials.

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

- High-performance liquid chromatography (HPLC) area-percent purity, ideally greater than 98%
- Mass spectrometry confirmation of molecular weight (expected [M+H]⁺ at m/z 358.19 for C₂₀H₂₇N₃OS)
- Chiral HPLC or polarimetry to confirm enantiomeric purity if the S-enantiomer is specified
- Nuclear magnetic resonance (1H NMR) spectroscopy for structural confirmation
- Residual solvent analysis if the supplier's certificate of analysis does not include this assessment

Common impurities in spirocyclic thiadiazine-piperidine compounds include residual starting materials (the piperidone and thiazolidine precursors), incomplete acylation products (free amine intermediate), and the opposite enantiomer in preparations of the single enantiomer. The indole pharmacophore may undergo oxidative degradation during storage if light protection is inadequate, producing oxindole or other oxidation products detectable by HPLC as secondary peaks. Investigators observing unexpected pharmacological activity or anomalous dose-

response curves should consider compound degradation or enantiomeric contamination as potential confounders before attributing the observation to the compound's pharmacology.

Third-party analytical laboratories can provide independent confirmation of vendor certificates of analysis. The research-grade AF710B market is relatively small compared to more established compounds, and lot-to-lot variability is possible; on-receipt analytical confirmation is appropriate for all investigations where mechanism-of-action precision, dose-response characterization, or quantitative comparison to published literature values is important. For binding-affinity or functional-potency studies, where the reported allosteric activity of ANAVEX 3-71 at M1 is in the sub-nanomolar range [1, 7], even modest impurity levels can confound quantitative interpretation. Investigators should request a certificate of analysis from the vendor for every lot and retain an analytical sample for independent verification if the initial results warrant follow-up studies.

9. Reconstitution and Handling

ANAVEX 3-71 is supplied as a solid powder (free base) and requires reconstitution for research applications. Solubility in dimethyl sulfoxide (DMSO) is adequate for preparation of concentrated stock solutions for in vitro work; typical stock concentrations of 10 to 100 mM in DMSO are appropriate. Water solubility of the free base is limited; for aqueous applications, DMSO stock solutions are diluted into the assay buffer, maintaining the final DMSO concentration below 0.1% to avoid vehicle effects.

For in vivo research applications, the compound has been administered orally (by gavage) in aqueous vehicle in published preclinical studies at microgram-per-kilogram doses [1, 2, 3]. The specific vehicle composition used in the Fisher and Cuello laboratory studies has not been published in detail. Investigators designing in vivo dosing protocols should confirm dissolution and stability in the selected vehicle.

Storage: The solid powder should be stored at -20 degrees Celsius under desiccated, light-protected conditions for long-term stability. The indole pharmacophore is susceptible to oxidative degradation; inert atmosphere (nitrogen or argon) overlay in the storage vial is recommended for compounds stored for more than three months. Research-grade suppliers typically specify storage at -20 degrees Celsius and recommend protecting from light and moisture.

Aliquoting: Reconstituted DMSO stock solutions should be aliquoted into single-use volumes to avoid repeated freeze-thaw cycles. For a typical in vitro screening campaign, 10 to 50 microliter aliquots of 10 mM DMSO stock are appropriate. Aliquots should be stored at -20 degrees Celsius or -80 degrees Celsius and used within six months. Stability data for reconstituted solutions have not been published by the compound's developers; investigators should use freshly prepared solutions for dose-response studies or validate stability under their specific storage conditions by HPLC comparison of fresh and stored aliquots.

Shelf life: The expected shelf life of the solid compound is approximately two years under recommended storage conditions, based on general stability expectations for small-molecule spirocyclic compounds. Vendor certificates of analysis

should specify a retest date. Investigators using material beyond the vendor-specified retest date should re-confirm purity by HPLC before use in quantitative studies.

Light sensitivity: The indole chromophore absorbs ultraviolet light and is subject to photodegradation. Working solutions should be prepared in amber glassware or wrapped in aluminum foil and protected from prolonged light exposure during experimental handling.

10. Stack Interaction Considerations

10.1 Metabolic interactions

The cytochrome P450 isoforms responsible for the metabolism of ANAVEX 3-71 have not been published. The principal circulating metabolite is designated M8; its structure, formation enzyme(s), and pharmacological activity have not been characterized in published form [5]. In the absence of formal drug-drug interaction studies, investigators should exercise caution when combining ANAVEX 3-71 with potent CYP inhibitors or inducers, as the metabolic pathway is unknown and unpredictable interactions are possible.

The population pharmacokinetic analysis indicated that creatinine clearance was not a significant covariate for ANAVEX 3-71 apparent clearance, suggesting that renal elimination of the parent compound is not a major pathway [5]. No formal hepatic or renal impairment studies have been reported.

10.2 Pharmacodynamic interactions

Cholinergic agents: Concurrent administration with acetylcholinesterase inhibitors (donepezil, rivastigmine, galantamine) would be expected to increase acetylcholine concentrations at M1 muscarinic synapses, potentially enhancing the allosteric effect of ANAVEX 3-71. This combination is pharmacologically plausible for cognitive research applications but has not been studied. Additive cholinergic adverse events (gastrointestinal, bradycardia) are a theoretical concern, though the allosteric M1-selective mechanism of ANAVEX 3-71 should limit peripheral muscarinic effects.

Other muscarinic agonists: Concurrent administration with orthosteric M1/M4 agonists (xanomeline) or M1 PAMs (TAK-071) would produce additive or synergistic effects at M1 receptors. The combination has not been studied and introduces the potential for excessive M1 activation. The combination of ANAVEX 3-71 with xanomeline-trospium (Cobenfy) is of particular theoretical interest given the overlapping schizophrenia indication; however, no data exist on this combination.

Sigma-1 receptor ligands: Concurrent administration with other sigma-1 agonists (blarcamesine, pridopidine, dextromethorphan) would produce additive sigma-1 receptor engagement. The pharmacodynamic consequences of sigma-1 receptor over-activation are not well characterized; preclinical data suggest that sigma-1 agonism is generally well tolerated across a wide dose range [19, 22].

Antipsychotics: The Phase 2 study in schizophrenia administered ANAVEX 3-71 as adjunctive therapy to stable atypical antipsychotic background medication [12]. The combination appears to be tolerated based on the reported safety profile, though formal pharmacokinetic interaction data between ANAVEX 3-71 and specific antipsychotics have not been published.

NMDA receptor modulators: Memantine (an NMDA receptor antagonist used in moderate-to-severe Alzheimer's disease) and ANAVEX 3-71 operate through independent receptor systems. No pharmacodynamic antagonism or synergy is expected, and the combination has not been studied. Investigators designing combination studies in Alzheimer's disease models should consider that memantine and ANAVEX 3-71 may produce complementary effects through non-overlapping mechanisms.

Benzodiazepines and GABAergic agents: No direct pharmacodynamic interaction between ANAVEX 3-71 and GABAergic compounds is expected based on the compound's receptor profile (no significant GABA receptor binding in the 83-target screen at 10 micromolar) [1]. In the Phase 2 schizophrenia study, subjects on stable background antipsychotic therapy (which may include concomitant benzodiazepine use) were enrolled without reported interaction signals [12].

QT-prolonging agents: ANAVEX 3-71 did not produce clinically relevant QTc prolongation at single doses up to 200 mg, with the concentration-QTcF relationship being slightly negative (-0.017 ms per microgram per liter) [4]. Combination with other QT-prolonging agents is unlikely to introduce an additive cardiac risk from ANAVEX 3-71. The QTc data are reassuring, though they derive from single ascending doses in healthy volunteers; multiple-dose cardiac safety in the context of QT-prolonging comedication has not been formally evaluated.

10.3 Summary of interaction evidence

The interaction profile of ANAVEX 3-71 is poorly characterized relative to the standard expected for an advanced clinical-stage compound. No formal drug-drug interaction studies have been conducted. The metabolic pathway (specific CYP isoforms, transporter substrates, inhibition and induction potential) is unpublished. All pharmacodynamic interaction statements above are theoretical, derived from the compound's known receptor profile and extrapolated from class pharmacology. Investigators planning combination studies should treat ANAVEX 3-71 as a compound with an undefined metabolic interaction profile and should include appropriate pharmacokinetic sampling to detect unexpected interactions.

11. Adverse Events and Safety Signal

11.1 Phase 1 safety profile

In the Phase 1 SAD study (NCT04442945) in 36 healthy volunteers at doses of 5 to 200 mg, no serious adverse events were reported [4, 5, 11]. No clinically relevant effects were observed on heart rate, PR interval, QRS interval, or QTc interval [4]. The concentration-QTcF slope was slightly negative (-0.017 ms per microgram per liter), providing strong evidence against a proarrhythmic effect at therapeutic concentrations [4]. Specific adverse event types and incidence rates from the Phase 1 study have not been published in detail.

11.2 Phase 2 safety profile (schizophrenia)

In the Phase 2 study (NCT06245213, 71 subjects with schizophrenia on background atypical antipsychotic therapy), the safety profile reported in October 2025 was as follows [13]:

- No serious treatment-emergent adverse events (TEAEs) in either study part
- No severe TEAEs in either study part
- Part A (10-day MAD): 1 TEAE reported in the 30 mg three-times-daily group (16.7% incidence); no TEAEs in the 60 mg group or placebo group
- Part B (28-day double-blind): 39.3% of the treatment group (60 mg three times daily) versus 48.1% of the placebo group experienced TEAEs

The lower TEAE incidence in the treatment group compared to placebo in Part B is notable, though the study was not powered for safety comparisons and the specific adverse event types have not been reported publicly. The safety profile was characterized by the sponsor as consistent with the healthy volunteer Phase 1 data [13].

11.3 Preclinical safety

The maximum tolerated dose of ANAVEX 3-71 exceeded 50 mg/kg by the oral route in rats, yielding a safety margin greater than 50,000-fold over the pharmacologically efficacious dose (10 micrograms per kilogram) [1]. This exceptionally wide

safety margin reflects the sub-nanomolar allosteric potency of the compound, which achieves therapeutic concentrations at doses far below those that produce toxicity.

11.4 Class-based safety considerations

The allosteric M1 muscarinic mechanism of ANAVEX 3-71 has favorable theoretical implications for cholinergic adverse events. Orthosteric muscarinic agonists (xanomeline, cevimeline) produce peripheral cholinergic adverse events (nausea, vomiting, diarrhea, salivation, diaphoresis, bradycardia) through activation of M2 and M3 muscarinic receptors in smooth muscle, exocrine glands, and cardiac conduction tissue [18, 21, 25]. The subtype selectivity of ANAVEX 3-71 (no M2 through M5 agonism) and its allosteric mechanism (which preserves dependence on endogenous acetylcholine tone rather than producing tonic receptor activation) would be expected to minimize these class effects [1]. The Phase 1 and Phase 2 data are consistent with this expectation: the TEAE profile shows no signal for cholinergic adverse events at the reported doses. However, formal comparison to orthosteric agonists at pharmacologically equivalent doses has not been performed.

Sigma-1 receptor agonists as a class have shown favorable tolerability in clinical development. Pridopidine, the most extensively studied sigma-1 agonist, exhibited safety comparable to placebo in studies spanning up to 6.5 years of open-label exposure [22]. Blarcamesine (ANAVEX 2-73) was associated with dizziness as the most common adverse event in its Phase 2b/3 trial, with serious TEAEs at 16.7% versus 10.1% placebo [16]. The sigma-1 class does not have identified dose-limiting or organ-specific toxicities.

11.5 Safety gaps

Formal characterization of organ-specific toxicity (hepatic, renal, cardiac, hematologic) from the Phase 1 study has not been published beyond the cardiac safety analysis of Fadiran et al. (2023) [4]. Long-term safety data beyond the 28-day Phase 2 dosing period do not exist; the compound has not been studied in open-label extensions or in treatment durations exceeding one month. Reproductive toxicology, genotoxicity, and carcinogenicity data have not been published. The safety of the modified-release tablet formulation beyond the single-dose Phase 1b study has not

been characterized [15]. No data exist on the safety of ANAVEX 3-71 in hepatic or renal impairment, in elderly populations, in pediatric populations, or in pregnant or lactating individuals.

The specific types of adverse events observed in the Phase 1 study have not been published; the available safety statements are limited to aggregate characterizations ("well tolerated," "no serious adverse events") [4, 5]. Similarly, the specific TEAE types in the Phase 2 study have not been publicly disclosed; only the aggregate incidence rates are available [13]. This gap limits the ability of investigators to assess specific organ-system risk. The total human safety exposure database for ANAVEX 3-71 comprises approximately 107 individuals (36 in Phase 1, 71 in Phase 2) with maximum treatment durations of 28 days, representing a limited exposure base for detection of uncommon adverse events. Investigators should treat the available safety data as preliminary and limited to short-term exposure in relatively young, healthy (Phase 1) or clinically stable (Phase 2) populations.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five compounds with mechanistic or indication-area overlap with ANAVEX 3-71, evaluated against five competency standards: novelty, effect size, promising potential (current development trajectory), side-effect profile, and overall validation. The five compounds were selected to represent the principal pharmacological strategies adjacent to ANAVEX 3-71's dual M1 muscarinic/sigma-1 mechanism: the sister sigma-1 compound (blarcamesine), the approved muscarinic agonist (xanomeline-trospium), the leading sigma-1 agonist in neurodegeneration (pridopidine), the M4 muscarinic PAM (emraclidine), and the M1 PAM (TAK-071).

12.1 Blarcamesine (ANAVEX 2-73)

Mechanism: Sigma-1 receptor agonist (K_i approximately 3,700 nM) and M1 muscarinic receptor agonist (K_i approximately 500 nM) [22]. Blarcamesine is the first-generation compound in the Anavex pipeline and the direct predecessor to ANAVEX 3-71 in mechanism, though it has substantially lower affinity at both sigma-1 and M1 targets compared to ANAVEX 3-71 (sigma-1: 3,700 nM vs 1.3 nM; M1: 500 nM vs 0.05 nM) [7, 22].

Development trajectory: Anavex Life Sciences advanced blarcamesine through a Phase 2b/3 randomized, double-blind, placebo-controlled trial (ANAVEX2-73-AD-004) in 508 participants with early Alzheimer's disease, with 48 weeks of daily oral dosing at 30 mg or 50 mg [16]. Sabbagh et al. (2025) reported that blarcamesine met the co-primary endpoint on ADAS-Cog13 (LSM difference -1.783; 95% CI, -3.314 to -0.251; $p = 0.0226$) and the secondary endpoint on CDR-SB (LSM difference -0.456; 95% CI, -0.831 to -0.080; $p = 0.0175$), but did not meet the co-primary endpoint on ADCS-ADL [16]. In the subgroup of subjects carrying the wild-type SIGMAR1 genotype, clinical progression was slowed by 49.8% at 48 weeks on the primary cognitive endpoint [16]. An earlier Phase 2a precision medicine analysis identified genomic biomarkers of response [17].

Novelty: 3/5. The dual sigma-1/M1 mechanism is non-novel within the Anavex platform but remains novel relative to the broader neurodegeneration drug landscape.

Effect size: 3/5. The ADAS-Cog13 effect (LSM difference -1.783) is modest but statistically significant. The SIGMAR1 wild-type subgroup analysis (49.8% slowing) is larger but is a post-hoc finding.

Promising potential: 4/5. The Phase 2b/3 positive cognitive readout in Alzheimer's disease, the precision medicine biomarker framework, and the genomic subgroup analysis represent the most advanced clinical package among sigma-1/M1 agents.

Side-effect profile: 3/5. Common TEAEs included dizziness (mostly mild to moderate). Serious TEAEs were higher in the blarcamesine group (16.7%) versus placebo (10.1%) [16].

Overall validation: 4/5. The largest and most rigorous clinical trial of any sigma-1/M1 agent, with positive primary cognitive endpoint and secondary clinical global endpoint.

Comparison to ANAVEX 3-71: ANAVEX 3-71 has dramatically higher receptor binding affinities at both targets (approximately 2,800-fold higher at sigma-1 and approximately 10,000-fold higher at M1) and a more selective allosteric mechanism at M1 compared to blarcamesine's orthosteric or mixed binding mode. Blarcamesine has the substantial advantage of the Phase 2b/3 Alzheimer's disease clinical data that ANAVEX 3-71 lacks. The two compounds are complementary within the Anavex pipeline: blarcamesine as the first-generation clinical candidate with late-stage data, and ANAVEX 3-71 as the next-generation compound with superior receptor pharmacology and earlier clinical development.

12.2 Xanomeline-trospium (KarXT, Cobenfy)

Mechanism: Xanomeline is an orthosteric M1/M4 muscarinic acetylcholine receptor agonist; trospium chloride is a peripherally restricted pan-muscarinic antagonist that mitigates the peripheral cholinergic adverse events of xanomeline [18, 21]. The combination was FDA-approved on September 26, 2024 for the treatment of schizophrenia in adults, the first new mechanism of action for schizophrenia since the introduction of chlorpromazine in the 1950s [20].

Development trajectory: The EMERGENT clinical trial program comprised three Phase 3 randomized, double-blind, placebo-controlled trials in schizophrenia. Kaul et al. (2024) reported the EMERGENT-2 results: in 252 subjects randomized to KarXT or placebo for 5 weeks, the PANSS total score favored KarXT (LSM difference -9.6; 95% CI, -13.9 to -5.2; $p < 0.0001$; Cohen's $d = 0.61$) [18]. Pooled analysis of the three EMERGENT trials (Kaul et al. 2024) showed an LSM PANSS difference of -9.9 (95% CI, -12.4 to -7.3; $p < 0.0001$; Cohen's $d = 0.65$) [19].

Novelty: 5/5. First FDA-approved non-dopaminergic antipsychotic; the muscarinic agonist/peripheral antagonist strategy is paradigmatic for the field.

Effect size: 4/5. PANSS improvements of 8.4 to 9.9 points over placebo across the three trials, with Cohen's d of 0.61 to 0.65, are clinically meaningful for schizophrenia [18, 19].

Promising potential: 5/5. FDA-approved and commercially marketed. Active clinical development in Alzheimer's disease psychosis and other neuropsychiatric indications.

Side-effect profile: 3/5. The combination mitigates the peripheral muscarinic adverse events of xanomeline but does not eliminate them: constipation (21%), nausea (19%), dyspepsia (19%), and vomiting (14%) were the principal adverse events in EMERGENT-2 [18]. Weight gain and extrapyramidal symptoms were absent [18]. Gastrointestinal tolerability limits dose optimization.

Overall validation: 5/5. Regulatory approval based on three positive Phase 3 trials, the highest level of validation in this comparator set.

Comparison to ANAVEX 3-71: Xanomeline-trospium is an orthosteric M1/M4 agonist paired with a peripheral antagonist, fundamentally different from ANAVEX 3-71's allosteric M1-selective mechanism with sigma-1 co-agonism. ANAVEX 3-71's allosteric selectivity for M1 (with no M2 through M5 activity) theoretically avoids the peripheral cholinergic adverse events that necessitate the trospium component of KarXT. ANAVEX 3-71 additionally engages the sigma-1 receptor, which may provide neuroprotective and neurotrophic activity absent from xanomeline's pharmacology. However, xanomeline-trospium has the definitive advantage of FDA approval, commercial availability, and validated clinical efficacy in schizophrenia, whereas ANAVEX 3-71 has only early-stage clinical data.

12.3 Pridopidine

Mechanism: Highly selective sigma-1 receptor agonist with K_i of 57 nanomolar [22]. Pridopidine achieves 28-fold selectivity over the alpha-2C adrenergic receptor and 96-fold selectivity over the sigma-2 receptor, and greater than 90% sigma-1 receptor occupancy in the human brain at the clinical dose of 45 mg twice daily [22]. Unlike ANAVEX 3-71, pridopidine does not engage muscarinic receptors.

Development trajectory: Prilenia Therapeutics (formerly Teva) advanced pridopidine through Phase 2 and Phase 3 clinical development in Huntington's disease. The PROOF-HD Phase 3 trial (65 weeks) did not meet its primary endpoint of change in Total Functional Capacity (TFC) or the key secondary endpoint (composite UHDRS score) in the overall population [23]. In the pre-specified subgroup of patients not treated with antidopaminergic medications, pridopidine showed nominally significant benefits on composite UHDRS ($p = 0.004$ to 0.035 at weeks 26 to 52) and cognitive measures [22]. In ALS, the HEALEY ALS Platform Trial (Phase 2, 24 weeks) did not meet the primary endpoint; post-hoc analysis in early, rapidly progressing patients showed slowing of ALSFRS-R decline ($p = 0.03$) [22].

Novelty: 3/5. Selective sigma-1 agonism is mechanistically clean but is a single-target approach in a class with several clinical-stage compounds.

Effect size: 2/5. The Phase 3 Huntington's primary endpoint was not met. Subgroup analyses showed modest effects on motor and cognitive measures. The ALS signal is exploratory.

Promising potential: 3/5. The subgroup data maintain clinical interest, but the negative primary analyses in both Huntington's and ALS limit the regulatory trajectory. The compound remains in development.

Side-effect profile: 5/5. Safety and tolerability comparable to placebo across all double-blind trials and open-label extensions spanning up to 6.5 years, the most extensive long-term safety dataset among sigma-1 agonists [22].

Overall validation: 3/5. Extensive Phase 3 data in Huntington's disease but primary endpoints not met. The clinical pharmacology research literature on pridopidine is the largest among sigma-1-selective agonists.

Comparison to ANAVEX 3-71: Pridopidine is a sigma-1-selective agonist without muscarinic activity, whereas ANAVEX 3-71 engages both sigma-1 and M1 muscarinic receptors. ANAVEX 3-71 has approximately 44-fold higher sigma-1 receptor

binding affinity (1.3 nM vs 57 nM). The dual-target mechanism of ANAVEX 3-71 may provide cognitive benefits through M1 muscarinic activation that pridopidine lacks. Pridopidine has the advantage of a substantially more extensive clinical safety database and the largest body of sigma-1 clinical data, though without positive primary efficacy outcomes.

12.4 Emraclidine (CVL-231)

Mechanism: Positive allosteric modulator (PAM) of the M4 muscarinic acetylcholine receptor. Emraclidine selectively targets M4 receptor activation, resulting in reduced dopaminergic activity without direct dopamine receptor antagonism [25]. The M4 PAM mechanism is mechanistically distinct from both the M1-directed activity of ANAVEX 3-71 and the M1/M4 orthosteric agonism of xanomeline.

Development trajectory: Cerevel Therapeutics (acquired by AbbVie for approximately \$8.7 billion) advanced emraclidine through Phase 1b and Phase 2 clinical development for schizophrenia. The Phase 1b trial demonstrated clinically meaningful and statistically significant improvement in PANSS total score at week 6 compared to placebo. Both doses of emraclidine (30 mg once daily, $p = 0.023$; 20 mg twice daily, $p = 0.047$) showed significant PANSS improvement [25]. Phase 2 trials (NCT05227690, NCT05227703) evaluated 10 mg, 15 mg, and 30 mg doses over 6 weeks in subjects with acute schizophrenia exacerbation.

Novelty: 4/5. The M4-selective PAM mechanism is a novel therapeutic approach for schizophrenia, distinct from both M1 allosteric agonism and M1/M4 orthosteric agonism.

Effect size: 3/5. Phase 1b PANSS improvements were statistically significant. Full Phase 2 results have not been published as of May 2026.

Promising potential: 4/5. The AbbVie acquisition indicates substantial commercial confidence. Phase 2 results pending.

Side-effect profile: 4/5. Generally well tolerated in Phase 1b studies, without the prominent gastrointestinal adverse events observed with xanomeline-trospium.

Overall validation: 3/5. Phase 1b positive signal; Phase 2 data pending. The M4 PAM mechanism has strong preclinical support.

Comparison to ANAVEX 3-71: Emraclidine targets M4 rather than M1 muscarinic receptors and has no sigma-1 activity, making it mechanistically complementary rather than overlapping with ANAVEX 3-71. Both compounds share the allosteric modulatory approach to muscarinic pharmacology, avoiding the peripheral cholinergic liabilities of orthosteric agonists. Emraclidine is more advanced in schizophrenia clinical development (Phase 2 complete), while ANAVEX 3-71 has the additional sigma-1 neuroprotective mechanism that may be advantageous in neurodegenerative indications.

12.5 TAK-071

Mechanism: Positive allosteric modulator of the M1 muscarinic acetylcholine receptor with low cooperativity. The low-cooperativity design is intended to reduce the risk of cholinergic adverse events by limiting the maximal degree of M1 receptor potentiation [20, 24]. Unlike ANAVEX 3-71, TAK-071 does not engage sigma-1 receptors.

Development trajectory: Takeda developed TAK-071 through Phase 1 clinical studies. Yin et al. (2022) reported a first-in-human study in healthy volunteers demonstrating favorable safety, long mean terminal half-life (46.3 to 60.5 hours), excellent brain penetration, and quantitative EEG modulation effects (increased posterior alpha power at low doses, increased beta power and reduced delta power at high doses) [24]. No serious adverse events were reported. TAK-071 was also studied in combination with donepezil, with no pharmacokinetic interaction between the two drugs [24]. A subsequent study evaluated TAK-071 in patients with mild cognitive impairment and mild Alzheimer's disease. The current development status of TAK-071 is uncertain; no Phase 2 trials have been registered.

Novelty: 3/5. The M1 PAM with low cooperativity concept is a refinement of the M1 allosteric modulator approach. The long half-life (46 to 60 hours) is a distinctive pharmacokinetic feature.

Effect size: 2/5. EEG modulation effects in healthy volunteers are pharmacodynamic signals only. No clinical efficacy data in patients with cognitive impairment have been published.

Promising potential: 2/5. Development appears to have stalled after Phase 1. No Phase 2 trials registered.

Side-effect profile: 5/5. Well tolerated with no serious adverse events and no cholinergic adverse effects when administered without donepezil [24].

Overall validation: 2/5. Phase 1 safety and pharmacokinetic data only. The clinical pharmacology is well characterized but no efficacy data exist.

Comparison to ANAVEX 3-71: Both TAK-071 and ANAVEX 3-71 are allosteric M1 modulators, but they differ in mechanism (PAM with low cooperativity versus allosteric agonist), in the presence of sigma-1 co-agonism (absent in TAK-071), and in pharmacokinetics (TAK-071 half-life of 46 to 60 hours versus ANAVEX 3-71 half-life of 3.56 hours). TAK-071's long half-life permits once-daily dosing without formulation modification. ANAVEX 3-71's dual-target mechanism provides a broader pharmacological coverage. ANAVEX 3-71 is more advanced clinically (Phase 2 with preliminary results versus Phase 1 only for TAK-071).

12.6 Comparator summary

The five compounds occupy distinct positions in the muscarinic and sigma-1 receptor pharmacology space. **Blarcamesine** is the most clinically advanced sigma-1/M1 agent, with Phase 2b/3 Alzheimer's disease data, but has lower receptor affinities than ANAVEX 3-71. **Xanomeline-trospium** is the only FDA-approved muscarinic agent for schizophrenia, validated by three Phase 3 trials, but requires a peripheral antagonist to manage cholinergic side effects. **Pridopidine** is the most extensively studied sigma-1-selective agonist with the longest safety database, but has not met primary efficacy endpoints in Phase 3. **Emraclidine** targets M4 rather than M1 and represents a mechanistically distinct allosteric approach for schizophrenia. **TAK-071** is an M1 PAM with favorable pharmacokinetics but stalled clinical development.

ANAVEX 3-71 occupies a unique position in this landscape as the only compound combining sub-nanomolar allosteric M1 selectivity with nanomolar sigma-1 agonism, providing both procognitive and neuroprotective mechanisms in a single molecule. Its principal advantages are the dual-target mechanism, the exceptional M1 selectivity (no M2 through M5 activity), the strong disease-modification preclinical evidence (effects maintained through washout in two transgenic models), and the clean safety margin. Its principal limitations are the early clinical stage (Phase 2, no efficacy data published), the absence of registered trials in the neurodegenerative indications with the strongest preclinical rationale, and the reliance on a small number of preclinical studies from closely collaborating investigator groups.

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dence summarized herein does not constitute evidence of therapeutic efficacy. Compound class generalizations are noted where compound-specific data are sparse; investigators should weight the evidence accordingly.