

ABSTRACT

GTS-21

GTS-21 (also known as DMXBA, DMBX-anabaseine, and 3-(2,4-dimethoxybenzylidene)anabaseine) is a small-molecule partial agonist of the homopentameric α -7 subtype of the neuronal nicotinic acetylcholine receptor. The compound is a synthetic derivative of the natural product anabaseine, a marine alkaloid originally isolated from nemertine ribbonworms and from certain ant species, characterized chemically and pharmacologically by William Kem at the University of Florida beginning in the late 1970s. Anabaseine itself is a non-selective nicotinic receptor agonist with substantial muscle-type α -1 nicotinic activity that produces neuromuscular toxicity at doses producing central nervous system effects. The 3-(2,4-dimethoxybenzylidene) substitution at the anabaseine scaffold (the structural feature defining DMXBA) substantially increases α -7 nicotinic receptor selectivity over the muscle-type receptor and produces the partial agonist functional profile that has supported clinical development. Functional intrinsic activity at human α -7 nicotinic receptors expressed in heterologous systems is approximately 30 to 50 percent of the acetylcholine maximum response. Selectivity over the α -4-beta-2 nicotinic subtype is incomplete: GTS-21 binds α -4-beta-2 with affinity comparable to α -7 and acts as a partial agonist at α -4-beta-2 in some functional assays and as an antagonist in others, contributing complexity to the *in vivo* pharmacology that distinguishes GTS-21 from the more selective subsequent candidates encenicline, bradanicline, and PHA-543613. The compound is the longest-studied α -7 nicotinic partial agonist in cognitive applications, with continuous research from the early 1990s through the 2020s,

and is the principal historical reference compound for the receptor class. GTS-21 was advanced through multiple Phase 1 studies in healthy volunteers in the 1990s and 2000s with positive cognitive signals (the original Kitagawa et al. 2003 healthy volunteer report demonstrated improvements on the Connors Continuous Performance Test), through several Phase 2 studies in schizophrenia cognitive impairment (Olincy et al. 2006 immediate-release proof-of-concept; Olincy et al. 2017 extended-release confirmatory), and through Phase 2 studies in Alzheimer disease and other exploratory cognitive indications. The clinical results have been consistent with a small effect size that produced significant signals in some early studies and not in subsequent larger studies. The compound is metabolized to two principal active metabolites (4-OH-GTS-21 and 2-OH-GTS-21) that retain alpha-7 nicotinic partial agonist activity and contribute to the pharmacodynamic profile; the metabolite contribution complicates the dose-response interpretation and was the principal motivation for the extended-release formulation tested in the Olincy 2017 study. The compound is not in active commercial development as of the most recent monograph revision; research-grade GTS-21 is widely available and continues to be used as a reference alpha-7 partial agonist in fundamental pharmacology. This monograph reviews the chemistry, anabaseine biosynthesis, structural class of GTS-21; the receptor pharmacology including the alpha-4-beta-2 cross-reactivity; the comprehensive human pharmacokinetic record including the active metabolite contribution; the clinical evidence base across schizophrenia, Alzheimer disease, healthy volunteer cognition, and selected inflammatory indications; and a structured comparative assessment of five alpha-7 nicotinic acetylcholine receptor candidates against GTS-21.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Names: GTS-21. The compound has been known by multiple synonyms reflecting its long research history: DMXBA (the most common chemistry-research designation, an acronym for 3-(2,4-Dimethoxybenzylidene)anabaseine); DMBX-anabaseine (an alternative formulation of the same chemistry name); GTS-21 dihydrochloride (the salt form supplied for research); ChemBridge ID and other catalog designations.

Trade names: None. The compound has not advanced to commercial registration.

Chemical Abstracts Service registry number: 156223-05-1 (dihydrochloride).

International Union of Pure and Applied Chemistry name: 3-[(3E)-3-[(2,4-dimethoxyphenyl)methylidene]-5,6-dihydro-4H-pyridin-2-yl]pyridine dihydrochloride.

1.2 Structural class and chemistry

GTS-21 is a 3-arylidene-tetrahydropyridine with a pyridine substituent at the 2-position and a 2,4-dimethoxybenzylidene substituent at the 3-position of the 5,6-dihydro-4H-pyridine ring. The compound is a derivative of the natural product anabaseine (3-(3,4,5,6-tetrahydro-2H-pyridin-2-yl)pyridine), a non-selective nicotinic alkaloid first isolated from marine nemertine ribbonworms by William Kem and colleagues at the University of Florida in the late 1970s and subsequently characterized as a defensive toxin secreted by these animals [1].

The structural class is distinct from:

- The quinuclidine-amide class (encenicline, bradanicline, PHA-543613)
- The tropane-indole class (tropisetron)
- The isoxazole-acrylamide class (AVL-3288)

The 3-(2,4-dimethoxybenzylidene) substitution is the principal medicinal-chemistry feature that distinguishes GTS-21 from the parent anabaseine. The substitution increases alpha-7 nicotinic receptor selectivity over the muscle-type alpha-1 nicotinic receptor (essential for clinical safety) by introducing steric bulk that disfavors muscle-type receptor binding while preserving central neuronal nicotinic activity. The geometric isomerism of the benzylidene (the (E) configuration) is the stable thermodynamic form and is preserved through synthesis and formulation.

Molecular formula: C₁₉H₂₀N₂O₂ (free base); C₁₉H₂₂Cl₂N₂O₂ (dihydrochloride).

Exact mass: 308.15 g/mol (free base, monoisotopic).

Molecular weight: 308.38 g/mol (free base); 381.31 g/mol (dihydrochloride salt).

Physical properties: Yellow crystalline solid as the dihydrochloride. Soluble in water (as the salt), methanol, dimethyl sulfoxide; sparingly soluble in apolar solvents. Stable at room temperature in dry conditions. The benzylidene geometry is photo-stable under typical laboratory lighting but should be protected from prolonged ultraviolet exposure.

Stereochemistry: GTS-21 is not chiral; the compound contains no stereocenters in the conventional sense. The benzylidene geometric configuration (the (E) isomer is the active form) is the only stereochemical consideration.

1.3 Synthesis

The published synthesis of GTS-21 proceeds from anabaseine (commercially available or prepared by reduction-cyclization of nicotinic acid derivatives) through aldol-type condensation with 2,4-dimethoxybenzaldehyde under acidic or basic catalysis to form the (E)-3-arylidene product. The condensation is selective for the 3-position of the dihydropyridine ring owing to the activating effect of the adjacent imine. The free base is converted to the dihydrochloride salt by addition of stoichiometric hydrogen chloride in dioxane or diethyl ether. The synthesis is straightforward and does not require chirality control; the compound is therefore manufactured at lower cost than the chiral quinuclidine-amide alpha-7 candidates.

2. Abstract

GTS-21 (DMXBA, 3-(2,4-dimethoxybenzylidene)anabaseine) is a small-molecule alpha-7 nicotinic acetylcholine receptor partial agonist, derivative of the natural product anabaseine, originally synthesized and characterized in the laboratory of William R. Kem at the University of Florida in the early 1990s as a follow-up to extensive characterization of the parent anabaseine (a non-selective nicotinic alkaloid isolated from marine nemertine ribbonworms in the late 1970s). The compound is the longest-studied alpha-7 nicotinic partial agonist in cognitive applications, with research extending continuously from the early 1990s through the 2020s, and is the principal historical reference compound for the receptor class. The compound binds the alpha-7 nicotinic receptor at the orthosteric site with affinity in the low-micromolar range and exhibits partial agonist functional activity at approximately 30 to 50 percent of the acetylcholine maximum response in heterologous expression systems [1, 2]. Notably, GTS-21 also binds the alpha-4-beta-2 nicotinic receptor with affinity comparable to alpha-7 and acts as a partial agonist at alpha-4-beta-2 in some functional assays and as a competitive antagonist in others; this incomplete selectivity over alpha-4-beta-2 is the principal medicinal-chemistry distinction from the more selective subsequent candidates encenicline, bradanicline, and PHA-543613.

The compound was advanced through Phase 1 healthy-volunteer studies in the late 1990s and early 2000s. The Kitagawa et al. (2003) report demonstrated significant cognitive improvement on the Connors Continuous Performance Test after a single 50 milligram oral dose in healthy young men, supporting initiation of cognitive-disorder Phase 2 studies [3]. The Olincy et al. (2006) Phase 2 proof-of-concept study in 12 schizophrenia patients (a small randomized double-blind placebo-controlled cross-over design at 75 milligrams or 150 milligrams of GTS-21 immediate-release formulation taken three times daily for 28 days) reported significant improvement on attention measures and on P50 auditory evoked potential gating [4]. The Olincy et al. (2017) Phase 2 confirmatory study in 65 schizophrenia patients with extended-release formulation at 75 milligrams three times daily for 4 weeks did not produce significant improvement on the MATRICS Cognitive Battery, despite the earlier exploratory signals [5]. The discrepancy between immediate-release and extended-release formulation results, and between exploratory and con-

firmatory study results, has been attributed in the contemporary scientific commentary to pharmacokinetic limitations including the contribution of active metabolites (4-OH-GTS-21 and 2-OH-GTS-21) that produce a more complex dose-response than apparent from the parent compound concentrations alone.

The compound is metabolized in humans to two principal active metabolites, 4-OH-GTS-21 (the more abundant) and 2-OH-GTS-21, both retaining alpha-7 nicotinic partial agonist activity at the receptor. The metabolites are excreted as glucuronide conjugates in urine [6]. The metabolite contribution complicates the dose-response interpretation: at a given parent compound dose, the total alpha-7 receptor exposure includes contributions from both the parent and the active metabolites, with the metabolite-to-parent ratio varying with dosing schedule, formulation, and individual metabolic phenotype. This is one of the principal scientific lessons of GTS-21 development for the broader alpha-7 nicotinic receptor agonist class: pharmacokinetic complexity from active metabolites is not unique to GTS-21 but is a general consideration for compounds in this scaffold class.

The compound has also been studied in Alzheimer disease (early Phase 2 studies producing modest cognitive signals) and in selected exploratory indications (sepsis, cognitive enhancement in healthy volunteers, attention deficit hyperactivity disorder). None of these indications has produced replicated positive efficacy at the registration threshold. The compound is not in active commercial development as of the most recent monograph revision. Research-grade GTS-21 is widely available from chemical suppliers at moderate cost.

This monograph reviews the chemistry, anabaseine biology and biosynthesis, structural class of GTS-21; the receptor pharmacology including the important alpha-4-beta-2 cross-reactivity; the human pharmacokinetic record including the active metabolite contribution; the clinical evidence base across schizophrenia, Alzheimer disease, healthy volunteer cognition, and selected inflammatory indications; sourcing and quality verification; reconstitution and handling; stack-interaction considerations; the safety record; and a structured comparative assessment of five alpha-7 nicotinic acetylcholine receptor candidates against GTS-21 on the five competency standards.

3. Discovery and Development History

3.1 Anabaseine origin and characterization

The parent compound anabaseine was first isolated and characterized by William R. Kem and colleagues at the University of Florida in the late 1970s. Anabaseine is a defensive alkaloid secreted by certain marine nemertine ribbonworms (notably *Paranemertes peregrina*, the carnivorous Pacific Coast ribbonworm) and by selected ant species. The compound is a non-selective nicotinic acetylcholine receptor agonist with substantial activity at the muscle-type alpha-1 nicotinic receptor; in mammals, anabaseine is a potent neuromuscular and central nervous system stimulant with a narrow therapeutic window owing to the muscle-type receptor activity [1].

The Kem laboratory undertook a structure-activity relationship study of anabaseine derivatives in the late 1980s and early 1990s with the goal of identifying analogs with improved selectivity over the muscle-type receptor while preserving the central neuronal nicotinic activity. The 3-(2,4-dimethoxybenzylidene) substitution was identified as the key modification: it increased alpha-7 nicotinic selectivity over the muscle-type receptor by an order of magnitude while preserving the alpha-7 partial agonist activity. The compound was assigned the development code GTS-21 (GTS for Gemini Therapeutics, the small biotech company that initially licensed the anabaseine derivatives, with subsequent transfer through several owners) and the chemistry name DMXBA.

3.2 Phase 1 development

Phase 1 healthy-volunteer studies were conducted in the late 1990s and early 2000s. The Kitagawa et al. (2003) report consolidates the principal Phase 1 cognitive findings: a randomized double-blind placebo-controlled cross-over study in 18 healthy young men tested single oral doses of GTS-21 at 25, 50, 100, and 150 milligrams against placebo with cognitive testing including the Connors Continuous Performance Test and selected memory measures [3]. The 50 milligram dose produced significant improvement on the Connors Continuous Performance Test (the principal cognitive endpoint), with smaller and non-significant signals at the

higher doses (consistent with an inverted-U dose-response). The Phase 1 program also characterized the basic pharmacokinetic profile (rapid absorption with peak concentrations at approximately 1 to 2 hours, plasma elimination half-life of approximately 4 to 6 hours, extensive hepatic metabolism with active metabolite formation) and the safety profile (well tolerated with mild gastrointestinal and central nervous system adverse events at higher doses).

3.3 Phase 2 schizophrenia (Olinco 2006 proof-of-concept)

The Olinco et al. (2006) Phase 2 proof-of-concept study at the University of Colorado Health Sciences Center is the principal early Phase 2 schizophrenia readout for GTS-21 [4]. The study was a randomized double-blind placebo-controlled three-period cross-over design in 12 schizophrenia outpatients on stable antipsychotic background. Each subject received one week each of placebo, GTS-21 75 milligrams three times daily (immediate-release formulation), and GTS-21 150 milligrams three times daily, in random order, with cognitive and biomarker testing at the end of each treatment week.

Results showed:

- Significant improvement on attention measures (Connors Continuous Performance Test) at both active doses
- Significant improvement on P50 auditory evoked potential gating (a biomarker dysfunctional in schizophrenia, partially restored by alpha-7 nicotinic activation)
- Trend-level improvements on selected memory measures
- No significant effect on positive or negative symptom scales (consistent with the short treatment duration)
- Acceptable tolerability with no clinically noteworthy adverse events

The study supported continued development of GTS-21 in schizophrenia cognitive impairment.

3.4 Phase 2 Alzheimer disease

Several Phase 2 Alzheimer disease studies of GTS-21 were conducted in the early-to-mid 2000s. These studies tested GTS-21 as monotherapy and as add-on to acetylcholinesterase inhibitor background, with cognitive endpoints including the

Alzheimer Disease Assessment Scale-cognitive subscale. Results were modest (small effect sizes, mixed statistical significance across studies) and did not support further advancement to Phase 3 in this indication. Detailed publication of the Alzheimer disease Phase 2 results has been limited.

3.5 Phase 2 schizophrenia (Olinco 2017 confirmatory)

The Olinco et al. (2017) Phase 2 confirmatory study addressed the pharmacokinetic limitations of the immediate-release formulation by testing an extended-release formulation in a larger schizophrenia patient cohort [5]. The study randomized 65 schizophrenia outpatients on stable antipsychotic background to placebo or GTS-21 extended-release 75 milligrams three times daily for 4 weeks. The extended-release formulation was designed to maintain steady-state plasma concentrations across the dosing interval and to reduce the inverted-U dose-response observed in early Phase 1 and Phase 2 studies.

Results did not produce significant improvement on the MATRICS Cognitive Battery composite or on individual MATRICS subtest scores. The extended-release formulation, despite achieving the intended pharmacokinetic profile, did not translate to greater clinical efficacy than the immediate-release formulation in earlier studies. The Olinco 2017 readout is the principal contemporary scientific evidence that GTS-21 monotherapy at the formulations and doses studied does not produce clinically meaningful cognitive improvement in schizophrenia at the registration threshold.

3.6 Current status

The compound is not in active commercial development as of the most recent monograph revision. The substantial research literature continues to use GTS-21 as a reference alpha-7 nicotinic partial agonist for fundamental pharmacology, for combination-pharmacology research with other alpha-7 candidates and acetylcholinesterase inhibitors, and for preclinical model validation studies.

4. Mechanism of Action

4.1 Alpha-7 nicotinic acetylcholine receptor partial agonism

GTS-21 binds the alpha-7 nicotinic receptor at the orthosteric (acetylcholine) site with affinity in the low-micromolar range (approximately 0.5 to 2 micromolar at human alpha-7 expressed in heterologous systems). Functional intrinsic activity is approximately 30 to 50 percent of the acetylcholine maximum response, placing GTS-21 in the lower-intrinsic-activity stratum of alpha-7 partial agonists (similar to tropisetron at alpha-7, lower than encenicline and bradanicline) [1, 2].

4.2 Alpha-4-beta-2 cross-reactivity

GTS-21 also binds the alpha-4-beta-2 nicotinic acetylcholine receptor with affinity comparable to alpha-7. The functional activity at alpha-4-beta-2 is complex: in some assays GTS-21 acts as a partial agonist at alpha-4-beta-2; in others it acts as a competitive antagonist; the activity depends on the experimental conditions and on the specific alpha-4-beta-2 isoform tested [2]. The alpha-4-beta-2 cross-reactivity is the principal medicinal-chemistry distinction between GTS-21 and the more selective subsequent candidates encenicline, bradanicline, and PHA-543613 (all of which exhibit greater than 100-fold selectivity over alpha-4-beta-2). The complex alpha-4-beta-2 activity may contribute to the in vivo pharmacology in ways not predictable from the alpha-7 activity alone.

4.3 Active metabolites

GTS-21 is metabolized in humans to two principal active metabolites: 4-OH-GTS-21 (the major metabolite) and 2-OH-GTS-21 (a less abundant metabolite). Both metabolites retain alpha-7 nicotinic partial agonist activity at the receptor. The 4-OH-GTS-21 metabolite has higher functional intrinsic activity at alpha-7 than the parent (approximately 50 to 60 percent of the acetylcholine maximum) and somewhat greater alpha-7 selectivity over alpha-4-beta-2. Both metabolites cross the blood-brain barrier and contribute to central nervous system pharmacology [6].

The metabolite contribution is one of the principal pharmacokinetic features of GTS-21. The total alpha-7 receptor exposure at a given parent dose is the sum of parent and metabolite contributions, and the metabolite-to-parent ratio varies with dosing schedule (single dose versus chronic dosing), formulation (immediate-release versus extended-release), and individual metabolic phenotype. The metabolite contribution complicates the dose-response interpretation and the comparison of GTS-21 doses across studies and against other alpha-7 nicotinic receptor candidates that lack active metabolite contributions.

4.4 Composite pharmacology

The composite pharmacology of GTS-21 at clinical doses (75 to 150 milligrams three times daily) produces plasma concentrations of the parent in the low-micromolar range and concentrations of 4-OH-GTS-21 in a similar or modestly higher range. At these concentrations, the alpha-7 nicotinic receptor is partially occupied and partially activated by both parent and metabolite. Activity at alpha-4-beta-2 is also present and contributes to the pharmacology in ways not yet fully characterized. The composite mechanism therefore is more complex than the simple alpha-7 partial agonist mechanism that the more selective subsequent candidates exhibit, and is one of the reasons GTS-21 clinical results have been variable across studies.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

GTS-21 is absorbed from the gastrointestinal tract after oral administration. Time to peak plasma concentration is approximately 1 to 2 hours after the immediate-release formulation and approximately 3 to 5 hours after the extended-release formulation [3, 5]. Bioavailability has not been precisely characterized in published material; the dose-proportional plasma concentrations across the studied dose range support adequate absorption.

5.2 Distribution

Plasma protein binding has not been precisely characterized in published material; tissue distribution in rodent species supports moderate brain penetration with brain-to-plasma ratio of approximately 2 to 5.

5.3 Metabolism

GTS-21 is extensively metabolized in the liver by cytochrome P450 enzymes; the principal isoforms responsible for the O-demethylation that produces 4-OH-GTS-21 and 2-OH-GTS-21 include CYP1A2, CYP2D6, and CYP3A4, with the relative contributions varying among individuals based on CYP genotype and concurrent medications. The two hydroxylated metabolites are subsequently glucuronidated by UGT enzymes and excreted in urine [6].

The metabolite-to-parent ratio at steady state on multiple dosing is approximately 1:1 to 2:1 for the 4-OH-GTS-21 metabolite, with the ratio higher in subjects with more active CYP-mediated metabolism. The 2-OH-GTS-21 metabolite is present at approximately 20 to 30 percent of the 4-OH-GTS-21 concentration.

5.4 Elimination

Plasma elimination half-life of the parent compound is approximately 4 to 6 hours, supporting three-times-daily dosing (the dosing schedule used in the Phase 2 studies). The 4-OH-GTS-21 metabolite has a similar half-life, contributing to a composite half-life that is approximately the same as the parent. The extended-release formulation tested in Olincy 2017 was designed to extend the apparent half-life through controlled release, achieving steady-state plasma concentrations across the dosing interval [5].

5.5 Routes of administration

Oral administration was the only route used in clinical development. The immediate-release formulation was the principal Phase 1 and Phase 2 administration form; the extended-release formulation was developed for the Olincy 2017 study.

6. Clinical Evidence Base

6.1 Healthy volunteer cognition (Kitagawa 2003)

The Phase 1 cognitive study in 18 healthy young men tested single oral doses of GTS-21 at 25, 50, 100, and 150 milligrams against placebo. The 50 milligram dose produced significant improvement on the Connors Continuous Performance Test (the principal cognitive endpoint), with smaller and non-significant signals at higher doses, consistent with an inverted-U dose-response [3].

6.2 Schizophrenia (Olinco 2006 proof-of-concept)

The 12-patient cross-over Phase 2 study at 75 and 150 milligrams three times daily for 1 week each produced significant improvements on attention measures and P50 auditory evoked potential gating compared to placebo [4]. The proof-of-concept study supported continued development.

6.3 Schizophrenia (Olinco 2017 confirmatory)

The 65-patient confirmatory Phase 2 study with extended-release formulation at 75 milligrams three times daily for 4 weeks did not produce significant improvement on the MATRICS Cognitive Battery composite or individual subtests [5]. The negative confirmatory readout was consistent with the broader pattern of alpha-7 nicotinic agonist Phase 2 confirmatory failures (bradanicline, encenicline) and indicates that GTS-21 monotherapy at the formulations and doses studied does not produce clinically meaningful cognitive improvement in schizophrenia at the registration threshold.

6.4 Alzheimer disease

Phase 2 studies of GTS-21 in Alzheimer disease produced modest cognitive signals that did not support advancement to Phase 3. Detailed publication of these results has been limited.

6.5 Inflammatory and other indications

GTS-21 has been studied in selected exploratory indications including sepsis (Phase 2 trial completed by Pavlov and colleagues; modest signals), attention deficit hyperactivity disorder (small Phase 2 study), and cognitive enhancement in healthy volunteers under stress. None of these indications has produced replicated positive efficacy at the registration threshold.

7. Reported Research Dose Ranges

7.1 Approved doses

None. GTS-21 has not received regulatory approval.

7.2 Investigational doses

The clinical-development doses spanned 25 milligrams to 150 milligrams three times daily by oral administration. The Phase 2 schizophrenia doses were 75 milligrams three times daily and 150 milligrams three times daily. The Phase 1 healthy volunteer doses were single doses of 25 to 150 milligrams.

For research applications using GTS-21 as an alpha-7 nicotinic partial agonist tool compound, in vitro concentrations are typically 0.1 to 30 micromolar in heterologous expression systems and primary neuronal culture, and rodent in vivo doses are typically 1 to 30 milligrams per kilogram by oral or intraperitoneal administration.

8. Sourcing and Quality Verification

GTS-21 has not advanced to commercial registration and is not available as a pharmaceutical-grade preparation. Research-grade GTS-21 (greater than 97 percent purity) is widely supplied by multiple chemical suppliers (Sigma-Aldrich, MedChemExpress, Tocris, Cayman Chemical, MedKoo, BocSci, Xcess Biosciences, and others) at low to moderate prices, typically \$50 to \$200 per gram of the dihydrochloride salt at the time of monograph preparation. The compound is one of the more economical alpha-7 nicotinic receptor research tools owing to the simple non-chiral synthesis.

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

- High-performance liquid chromatography area-percent purity, ideally greater than 97 percent
- Mass spectrometry confirmation of molecular weight (309.16 ± 0.01 for the protonated free base $[M+H]^+$)
- Confirmation of the (E) geometric isomer (the (Z) isomer is inactive)
- Karl Fischer or loss-on-drying analysis for residual water in the dihydrochloride salt
- Residual solvent analysis

9. Reconstitution and Handling

GTS-21 dihydrochloride is freely soluble in water. For research applications, the compound is supplied as a yellow crystalline powder and is reconstituted as needed. Stock solutions are typically prepared at 10 to 50 millimolar concentrations in water or in dimethyl sulfoxide for in vitro work. Refrigerated storage of the solid is appropriate; the compound is stable at room temperature in dry conditions and at refrigerated conditions in solution for at least 30 days.

10. Stack Interaction Considerations

GTS-21 may interact with other drug classes through pharmacodynamic and pharmacokinetic mechanisms.

Acetylcholinesterase inhibitors: Concurrent administration produces additive cholinergic activity. The combination has been studied in some Alzheimer disease Phase 2 designs.

Other alpha-7 nicotinic agonists or PAMs: Concurrent administration with other alpha-7 candidates (tropisetron, encenicline, bradanicline, AVL-3288, PHA-543613) produces additive effects at the alpha-7 receptor; the alpha-4-beta-2 cross-reactivity of GTS-21 introduces complexity not present with the more selective candidates.

CYP1A2, CYP2D6, and CYP3A4 modulators: GTS-21 is a substrate of multiple CYP isoforms; concurrent strong inhibitors or inducers can alter parent-to-metabolite ratios and modify the pharmacodynamic profile.

Nicotine and tobacco: Tobacco status is a known interactor with alpha-7 nicotinic receptor pharmacology and was considered in the Phase 2 patient populations.

11. Adverse Events and Safety

11.1 Clinical safety profile

GTS-21 was generally well tolerated across the Phase 1 and Phase 2 clinical development. Common adverse events at the studied doses were mild and balanced or near-balanced with placebo, with some non-specific gastrointestinal and central nervous system events at higher doses [3, 4, 5]. No clinically significant safety findings were reported across the development program.

11.2 Comparison to encenicline gastrointestinal safety signal

GTS-21 did not produce the severe gastrointestinal toxicity that triggered the September 2015 FDA clinical hold on encenicline Phase 3 trials. The mechanism of the differential safety is incompletely characterized but may relate to the lower receptor selectivity of GTS-21 (the alpha-4-beta-2 cross-reactivity may modulate the pure alpha-7 effect) or to the lower dose used in GTS-21 development relative to total receptor exposure.

12. Comparable Compounds Assessment

Five alpha-7 nicotinic acetylcholine receptor candidates are evaluated against GTS-21 on the five competency standards.

12.1 Tropisetron

Mechanism: Multi-target ligand (5-HT3 antagonist plus alpha-7 partial agonist plus APP ectodomain binding). Alpha-7 intrinsic activity 25-40 percent (similar to GTS-21).

Novelty: Moderate. Multi-target mechanism distinct from pure alpha-7 strategies.

Effect size: Modest in cognitive endpoints; strong in fibromyalgia.

Promising potential: Moderate. Registered antiemetic and fibromyalgia indications.

Side-effect profile: Well characterized.

Overall validation: Moderate to high.

Comparison to GTS-21: Both compounds are alpha-7 partial agonists with similar intrinsic activity. Tropisetron has the additional 5-HT3 and APP mechanisms; GTS-21 has the alpha-4-beta-2 cross-reactivity. Tropisetron has registered clinical indications that GTS-21 lacks. The two compounds are mechanistically similar at alpha-7 but operationally distinct.

12.2 Encenicline

Mechanism: Selective alpha-7 partial agonist (60-70 percent intrinsic activity, higher than GTS-21).

Novelty: Moderate.

Effect size: Phase 2 cognitive signals; Phase 3 missed primary endpoints.

Promising potential: Low. Development terminated.

Side-effect profile: Phase 3 severe gastrointestinal toxicity.

Overall validation: Moderate. Phase 3 advancement.

Comparison to GTS-21: Encenicline was a medicinal-chemistry refinement over GTS-21 toward higher alpha-7 selectivity (versus GTS-21's mixed alpha-7/alpha-4-beta-2 profile) and higher functional intrinsic activity. The Phase 3 failure of encenicline despite these refinements has reframed the GTS-21 results: the GTS-21 lower selectivity and lower intrinsic activity may not have been the principal reasons for the modest clinical signals.

12.3 Bradanicline

Mechanism: Selective alpha-7 full agonist (80-90 percent intrinsic activity, substantially higher than GTS-21).

Novelty: Low.

Effect size: Phase 2 mixed.

Promising potential: Low for cognition; ongoing for Tourette syndrome.

Side-effect profile: Well tolerated.

Overall validation: Low to moderate.

Comparison to GTS-21: Bradanicline tested the medicinal-chemistry hypothesis that higher functional intrinsic activity at alpha-7 produces greater clinical benefit. The Phase 2 confirmatory failure has not supported this hypothesis. GTS-21 with lower intrinsic activity remains an active research tool; bradanicline does not demonstrate clear superiority despite higher intrinsic activity.

12.4 AVL-3288

Mechanism: Type I PAM at alpha-7 (allosteric, mechanistically distinct from GTS-21).

Novelty: High.

Effect size: Phase 1b modest exploratory.

Promising potential: Moderate. Novel mechanism.

Side-effect profile: Phase 1 favorable.

Overall validation: Low to moderate.

Comparison to GTS-21: AVL-3288 (allosteric) and GTS-21 (orthosteric) are mechanistically distinct in the most fundamental sense for the alpha-7 receptor. Combination of the two would test PAM augmentation of orthosteric partial agonist activity, a productive line for fundamental research.

12.5 PHA-543613

Mechanism: Highly selective alpha-7 full agonist. Preclinical only.

Novelty: Low to moderate.

Effect size: Substantial in preclinical models. No human data.

Promising potential: Limited as clinical candidate.

Side-effect profile: Preclinical only.

Overall validation: Low.

Comparison to GTS-21: Both are alpha-7 partial-or-full agonists of different selectivity profiles and clinical development stages. PHA-543613 is the more selective and more potent compound; GTS-21 is the more clinically validated. PHA-543613 serves as a research tool complementary to the GTS-21 historical reference.

12.6 Comparator summary

GTS-21 occupies the position of the historical reference compound for the alpha-7 nicotinic partial agonist class. Its long research history (early 1990s through present) and substantial published literature support its continued use as a research tool despite the modest clinical effect sizes and the lack of regulatory approval. The subsequent generation of more selective alpha-7 candidates (encenicline, bradanicline, AVL-3288, PHA-543613) was developed in part as medicinal-chemistry refinements over the GTS-21 baseline, and the Phase 3 and Phase 2 confirmatory failures of these refined candidates have reframed the GTS-21 results as not exceptionally limited within the broader receptor class. The principal scientific

lessons of GTS-21 development for the contemporary alpha-7 nicotinic receptor agonist field include the importance of active metabolite contribution to dose-response interpretation, the limitations of immediate-release versus extended-release formulations, and the persistent challenge of translating preclinical and exploratory clinical signals to confirmatory Phase 2 and Phase 3 efficacy in cognitive impairment.

13. References

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