

RESEARCH MONOGRAPH . COMPOUND PROFILE

Unifiram

A hexahydropyrrolo[1,2-a]pyrazinone cognition enhancer synthesized at the University of Florence, approximately 1000-fold more potent than piracetam in rodent anti-amnesic assays, operating through AMPA receptor-dependent glutamatergic facilitation and cholinergic release enhancement without direct binding to any characterized central receptor.

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ABSTRACT

Unifiram (DM-232) is an experimental nootropic compound of the hexahydropyrrolo[1,2-a]pyrazinone structural class, synthesized in the late 1990s at the University of Florence by the research group led by Fulvio Gualtieri within the Department of Pharmaceutical Sciences. The compound was first disclosed in 2000 and characterized in a series of publications by Ghelardini, Galeotti, Romanelli, and colleagues between 2002 and 2006. Unifiram is approximately 1000-fold more potent than piracetam in the mouse passive avoidance test and the rat Morris water maze, the standard behavioral assays for anti-amnesic and procognitive activity in the racetam research tradition. The compound prevents amnesia induced by scopolamine (muscarinic antagonism), mecamylamine (nicotinic antagonism), baclofen (GABA-B agonism), and clonidine (alpha-2 adrenergic agonism) at intraperitoneal doses of 0.001 to 0.1 mg/kg and oral doses of 0.01 to 0.1 mg/kg in mice, with no impairment of motor coordination on the rota rod test at doses up to 10 mg/kg.

The mechanism of action is not fully elucidated, but the available evidence supports AMPA receptor-dependent glutamatergic facilitation as the principal pharmacological activity. Unifiram reverses amnesia induced by the selective AMPA receptor antagonist NBQX, increases the amplitude of field excitatory postsynaptic potentials (fEPSP) in rat hippocampal slices in a concentration-dependent manner, and stimulates acetylcholine release from rat cerebral cortex *in vitro*. Despite these functional effects, unifiram shows no measurable affinity for any of the principal central nervous system receptors, ion channels, or

transporters at concentrations up to 1 micromolar in standard radioligand binding panels, including glutamate (AMPA, NMDA, kainate), GABA, serotonin, dopamine, adrenergic, histamine, muscarinic, nicotinic, and opioid sites. The compound therefore appears to operate through an indirect or allosteric mechanism on AMPA receptor-mediated neurotransmission rather than through direct orthosteric binding.

Sunifiram (DM-235), the molecular simplification of unifiram produced by the same research group, retains comparable potency and shares the AMPA-dependent mechanism. A third analog, sapunifiram (MN-19), has also been characterized with similar activity. The structure-activity relationship program at Florence explored modifications of the piperazine and bicyclic ring systems and identified compounds with amnesic (pro-amnesic) activity of comparable potency to scopolamine, confirming the pharmacological specificity of the cognition-enhancing scaffold.

No human clinical trials of unifiram have been conducted. No formal toxicology studies beyond acute rodent dosing have been published. The compound was never patented, and by approximately 2012 it appeared on commercial websites as a consumer nootropic despite the absence of human safety or efficacy data. The 2015 commentary by Gualtieri in the *Journal of Enzyme Inhibition and Medicinal Chemistry* characterized the commercial availability of unifiram and sunifiram as an illustration of academic and industrial shortcomings in the translation of early-stage research compounds. The compound is not approved for human use in any jurisdiction. It is not scheduled as a controlled substance in most jurisdictions but is sold as a research chemical. Investigators should obtain analytical

confirmation of identity and purity on every lot and should not extrapolate rodent dose-response data to human applications without appropriate pharmacokinetic and safety characterization.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Common name: Unifiram.

Development code: DM-232. The development code is the canonical identifier in the original University of Florence publications and in all subsequent pharmacological characterizations [1, 2].

Chemical Abstracts Service registry number: 272786-64-8.

International Union of Pure and Applied Chemistry name: 2-(4-fluorophenylsulfonyl)-1,3,4,7,8,8a-hexahydropyrrolo[1,2-a]pyrazin-6-one. The compound is a bicyclic piperazinone bearing a 4-fluorophenylsulfonyl substituent at the 2-position of the hexahydropyrrolo[1,2-a]pyrazine ring system.

Other synonyms: 2-[(4-fluorophenyl)sulfonyl]hexahydropyrrolo[1,2-a]pyrazin-6(2H)-one.

1.2 Structural class and chemistry

Unifiram belongs to the hexahydropyrrolo[1,2-a]pyrazinone structural class, a bicyclic ring system incorporating a fused piperazine and pyrrolidinone. The compound was designed as a conformationally constrained analog within a broader medicinal chemistry program at the University of Florence that explored 1,4-diazabicyclo[4.3.0]nonan-9-ones as cognition-enhancing scaffolds structurally related to the 2-pyrrolidinone core of piracetam [2, 3]. The 4-fluorophenylsulfonyl group at the 2-position of the piperazine ring is a distinguishing structural feature that differentiates unifiram from sunifiram (DM-235), which is a simplified open-chain piperazine derivative of the same program [4].

Molecular formula: C₁₃H₁₅FN₂O₃S.

Molecular weight: 298.34 g/mol.

Canonical SMILES: C1CC(=O)N2C1CN(CC2)S(=O)(=O)c1ccc(F)cc1.

Physical properties: White to off-white crystalline powder. Soluble in dimethyl sulfoxide; sparingly soluble in water. Melting point data are not comprehensively reported in the primary literature.

Stereochemistry: The hexahydropyrrolo[1,2-a]pyrazinone ring system contains a stereocenter at the ring junction (position 8a). The published pharmacological studies do not specify enantiomeric resolution or the stereochemical assignment of the tested material; it is presumed that the racemic mixture was used in the original characterizations [1, 2]. Investigators acquiring research-grade unifiram should confirm whether the material is racemic or enantiomerically enriched.

1.3 Synthesis

The synthesis of unifiram proceeds from commercially available starting materials through construction of the hexahydropyrrolo[1,2-a]pyrazinone ring system followed by sulfonylation with 4-fluorobenzenesulfonyl chloride [2, 3]. The bicyclic core is assembled by cyclization of an appropriately substituted piperazine precursor with a gamma-butyrolactone or equivalent pyrrolidinone-forming reagent. The sulfonylation is carried out under standard conditions (base, dichloromethane or tetrahydrofuran, 0 degrees Celsius to room temperature) and produces the product in moderate to good yield. Purification is by column chromatography or recrystallization. The published structure-activity relationship studies describe the synthesis of approximately 20 analogs using this general route, with modifications at the sulfonyl substituent, the ring size, and the ring fusion geometry [3, 5, 6]. Sunifiram (DM-235) is obtained by molecular simplification of the bicyclic ring system to a monocyclic piperazine while retaining the benzoyl acyl group [4]. Industrial synthesis details are not published; the compound was never advanced to process chemistry development.

2. Abstract

Unifram (DM-232) is an experimental cognition-enhancing compound of the hexahydropyrrolo[1,2-a]pyrazinone structural class, first disclosed in 2000 by the research group of Fulvio Gualtieri at the University of Florence and characterized in a series of publications between 2002 and 2006 [1, 2, 7, 8]. The compound is approximately 1000-fold more potent than piracetam in standard rodent anti-amnesic assays (mouse passive avoidance, rat Morris water maze, rat social learning), preventing amnesia induced by scopolamine, mecamylamine, baclofen, and clonidine at intraperitoneal doses of 0.001 to 0.1 mg/kg and oral doses of 0.01 to 0.1 mg/kg [1].

The mechanism of action involves AMPA receptor-dependent glutamatergic facilitation: unifram reverses NBQX-induced amnesia, enhances field excitatory postsynaptic potential amplitude in rat hippocampal slices, and stimulates cortical acetylcholine release [2, 7]. The compound does not bind directly to any of the principal central nervous system receptors or ion channels at concentrations up to 1 micromolar, suggesting an indirect or allosteric mode of action on glutamatergic neurotransmission [2].

Sunifram (DM-235), a molecular simplification of unifram, shares comparable potency and mechanism [4, 8]. A third analog, sapunifram (MN-19), has been characterized with similar activity [9]. The structure-activity relationship program at Florence confirmed the pharmacological specificity of the scaffold by identifying structural modifications that produce pro-amnesic rather than anti-amnesic activity [3].

No human clinical trials have been conducted. No formal toxicology beyond acute rodent studies has been published. The compound was never patented and appeared on consumer nootropic markets by approximately 2012, prompting a 2015 commentary by Gualtieri characterizing this trajectory as an illustration of translational failure [10]. Unifram is not approved for human use in any jurisdiction and is not scheduled as a controlled substance in most jurisdictions.

3. Discovery and Development History

3.1 Origin

Unifram was synthesized in the late 1990s at the Laboratory of Design, Synthesis and Study of Biologically Active Heterocycles (HeteroBioLab) within the Department of Pharmaceutical Sciences at the University of Florence (Universita degli Studi di Firenze), Italy. The research program, led by Fulvio Gualtieri and involving Maria Novella Romanelli, Dina Manetti, Carla Ghelardini, Nicoletta Galeotti, and others, was a systematic exploration of conformationally constrained analogs of the 2-pyrrolidinone ring system of piracetam, the prototypical nootropic compound [2, 10]. The program aimed to identify compounds with substantially greater potency than piracetam and the existing racetam class (aniracetam, oxiracetam, pramiracetam) while preserving the favorable tolerability profile characteristic of the 2-pyrrolidinone nootropics.

The medicinal chemistry strategy was to constrain the flexible 2-pyrrolidinone pharmacophore into a rigid bicyclic ring system, specifically the 1,4-diazabicyclo[4.3.0]nonan-9-one scaffold (a fused piperazine-pyrrolidinone), and to introduce arylsulfonyl substituents on the piperazine nitrogen as pharmacophoric elements [2, 3]. DM-232 (unifram) was one of the lead compounds from this series and was first disclosed alongside early pharmacological data in 2000.

3.2 Initial pharmacological characterization

The first full publication of unifram pharmacology was the 2002 report by Ghelardini, Galeotti, Gualtieri, and colleagues in Drug Development Research, which characterized the antiamnesic and procognitive activity of DM-232 in the mouse passive avoidance test, the rat Morris water maze, and the rat social learning test [1]. This publication established the approximately 1000-fold potency advantage of unifram over piracetam and demonstrated the compound's activity across multiple neurotransmitter-system-mediated amnesia models.

3.3 AMPA receptor mechanism identification

The 2003 publication by Galeotti, Ghelardini, Pittaluga, and colleagues in *Naunyn-Schmiedeberg's Archives of Pharmacology* identified AMPA receptor activation as a component of the anti-amnesic mechanism of both DM-232 (unifiram) and DM-235 (sunifiram) [7]. This was demonstrated by the reversal of NBQX-induced amnesia and by the hippocampal slice electrophysiology showing concentration-dependent enhancement of fEPSP amplitude by unifiram.

3.4 Structure-activity relationship program

The 2003 publication by Manetti, Ghelardini, Bartolini, and colleagues in *Bioorganic and Medicinal Chemistry* explored systematic modifications of the unifiram and sunifiram scaffolds, examining changes to the ring system (bicyclic versus monocyclic), the sulfonyl substituent (fluorine position, alternative halogens, unsubstituted phenyl), and the ring fusion geometry [3]. Although none of the analogs exceeded the potency of the parent compounds, the study identified compounds with the opposite pharmacological activity (pro-amnesic, equipotent to scopolamine), confirming the structure-activity specificity of the scaffold. Two compounds from the series were identified with analgesic activity in a rat neuropathic pain model at 1 mg/kg [3].

3.5 Comprehensive review

The 2006 review by Romanelli, Galeotti, Ghelardini, Manetti, and colleagues in *CNS Drug Reviews* provided a comprehensive pharmacological characterization of both DM-232 and DM-235, integrating behavioral, electrophysiological, neurochemical, and radioligand binding data [2]. This review remains the principal reference for the pharmacology of both compounds.

3.6 Failure to advance to clinical development

Unifiram was never advanced beyond preclinical characterization. The compound was not patented by the University of Florence or by any pharmaceutical company. The Gualtieri (2015) commentary in the *Journal of Enzyme Inhibition and Medicinal Chemistry* attributed this failure to multiple factors: the academic setting of the discovery, the absence of industrial partnership, the modest commercial interest in nootropic compounds during the period of discovery (approximately

2000 to 2005), and the general difficulty of translating university research compounds to clinical development without venture or pharmaceutical-industry backing [10]. The compound remained a purely academic research tool until approximately 2012, when it began to appear on consumer nootropic websites alongside sunifiram, sold as a cognition-enhancing supplement despite the complete absence of human safety or efficacy data.

3.7 Subsequent analog development

The Florence group continued to explore the piperazine nootropic scaffold after the initial unifiram and sunifiram disclosures. The 2008 publication by Martini, Norcini, and colleagues described new analogs of DM-232 and DM-235 incorporating tetrahydropyrazinoisoindolone, diaminocyclohexane, and diaminobenzene ring systems [5]. The 2009 publication by Martini, Norcini, and colleagues explored analogs of sunifiram and sapunifiram (MN-19) with modifications to the acyl substituent [9]. These efforts produced compounds with moderate nootropic activity but did not identify a clinical candidate superior to the parent compounds.

4. Mechanism of Action

4.1 AMPA receptor-dependent glutamatergic facilitation

The principal mechanistic evidence for unifiram points to facilitation of AMPA receptor-mediated glutamatergic neurotransmission. Three lines of evidence support this conclusion.

First, unifiram (0.1 mg/kg intraperitoneal) fully reverses amnesia induced by the selective AMPA receptor antagonist NBQX (30 mg/kg intraperitoneal) in the mouse passive avoidance test [7]. This result demonstrates that intact AMPA receptor signaling is required for the anti-amnesic effect and that unifiram operates at or downstream of the AMPA receptor in the relevant circuitry.

Second, unifiram increases the amplitude of field excitatory postsynaptic potentials (fEPSP) in rat hippocampal slices in a concentration-dependent manner [2, 7]. The fEPSP in the CA1 region of the hippocampus is mediated predominantly by AMPA receptors at the Schaffer collateral-CA1 synapse, and enhancement of fEPSP amplitude is the canonical *in vitro* correlate of AMPA receptor positive modulation and of the ampakine drug class.

Third, the structurally related compound sunifiram (DM-235) has been shown to enhance long-term potentiation (LTP) in mouse hippocampal slices at nanomolar concentrations (10 to 100 nM) in a bell-shaped dose-response relationship, with the enhancement associated with increased phosphorylation of AMPA receptor subunits through calcium/calmodulin-dependent protein kinase II (CaMKII) activation and increased phosphorylation of NMDA receptor subunits through protein kinase C alpha (PKC-alpha) activation [11]. While this LTP enhancement has been demonstrated specifically for sunifiram rather than unifiram, the close structural relationship and shared behavioral pharmacology between the two compounds support the inference that unifiram engages the same signaling pathway.

4.2 Absence of direct receptor binding

A striking feature of the unifiram pharmacology is the absence of measurable affinity at any of the principal central nervous system receptor sites at concentrations up to 1 micromolar. The Romanelli et al. (2006) review reported that both unifiram and sunifiram were assayed against a comprehensive panel of receptors, ion channels, and transporters, including glutamate receptors (AMPA, NMDA, kainate, metabotropic), GABA-A and GABA-B receptors, serotonin receptors (5-HT1 through 5-HT7), dopamine receptors (D1 through D5), adrenergic receptors (alpha-1, alpha-2, beta), histamine receptors (H1, H2, H3), muscarinic acetylcholine receptors (M1 through M5), nicotinic acetylcholine receptors, opioid receptors (mu, delta, kappa), and voltage-gated sodium, potassium, and calcium channels [2]. No binding was detected at any site at the maximum tested concentration.

This result is pharmacologically significant. It indicates that unifiram does not operate through direct orthosteric binding at the AMPA receptor (as piracetam and classical racetams do weakly) or at any other characterized central receptor. The compound may instead act through an allosteric or modulatory mechanism on AMPA receptor function, through facilitation of presynaptic glutamate release, through modulation of receptor trafficking or phosphorylation, or through an as-yet-uncharacterized site. The precise molecular target remains unidentified.

4.3 Cholinergic facilitation

Unifiram and sunifiram both stimulate the release of acetylcholine from rat cerebral cortex in vitro [2]. This cholinergic facilitation is consistent with the anti-amnesic activity observed in the scopolamine (muscarinic antagonist) and mecamylamine (nicotinic antagonist) amnesia models and provides a second mechanistic axis for the cognition-enhancing effect. Whether the cholinergic facilitation is a direct effect on cholinergic terminals or is downstream of glutamatergic facilitation (cortical cholinergic neurons receive excitatory glutamatergic input) has not been definitively established. The ability to prevent mecamylamine-induced amnesia suggests that the nicotinic cholinergic pathway is involved in the overall procognitive effect, whether directly or indirectly.

4.4 Broad-spectrum antiamnesic profile

The antiamnesic profile of unifram is notably broad. The compound prevents amnesia induced by four pharmacologically distinct agents operating through different neurotransmitter systems [1]:

- Scopolamine (muscarinic acetylcholine receptor antagonist): effective dose 0.01 mg/kg intraperitoneal
- Mecamylamine (nicotinic acetylcholine receptor antagonist): effective dose 0.01 to 0.1 mg/kg intraperitoneal
- Baclofen (GABA-B receptor agonist): effective dose 0.01 to 0.1 mg/kg intraperitoneal
- Clonidine (alpha-2 adrenergic receptor agonist): effective dose 0.01 to 0.1 mg/kg intraperitoneal

This breadth of activity across cholinergic, GABAergic, and adrenergic amnesia models supports the interpretation that unifram operates at a point of convergence in the memory consolidation circuitry, plausibly the glutamatergic AMPA receptor-mediated synaptic transmission that is common to all four models, rather than through a single-neurotransmitter mechanism.

4.5 Composite pharmacological interpretation

The composite picture from behavioral, electrophysiological, neurochemical, and binding data is of a compound that enhances glutamatergic synaptic transmission through the AMPA receptor system, stimulates cortical acetylcholine release (directly or indirectly), and does not bind detectably to any major central receptor at pharmacologically relevant concentrations. The term "ampakine-like" is used in the literature to describe unifram, acknowledging the AMPA receptor dependence of the effect without the implication that the compound is a classical positive allosteric modulator of the AMPA receptor in the sense that CX516, CX717, or the biarylpropylsulfonamide ampakines are [2]. The molecular mechanism remains incompletely characterized, and identification of the direct molecular target would substantially advance the scientific positioning of the compound.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption and oral activity

Unifiram is orally active in mice and rats. The original Ghelardini et al. (2002) study demonstrated antiamnesic activity at oral doses of 0.01 to 0.1 mg/kg in mice, with the oral effective dose approximately 10-fold higher than the intraperitoneal effective dose (0.001 to 0.01 mg/kg) [1]. This 10-fold oral-to-intraperitoneal dose ratio suggests moderate oral bioavailability, consistent with a compound that undergoes first-pass hepatic metabolism but retains sufficient systemic exposure to produce central nervous system effects.

5.2 Distribution

No formal pharmacokinetic studies (plasma concentration-time profiles, volume of distribution, protein binding, tissue distribution) have been published for unifiram. The central nervous system activity at low oral doses implies adequate blood-brain barrier penetration. The molecular weight (298.34 g/mol), moderate lipophilicity (the 4-fluorophenylsulfonyl group confers intermediate polarity), and absence of permanent charge at physiological pH are consistent with passive transcellular blood-brain barrier penetration, though active transport has not been investigated.

5.3 Metabolism and elimination

No metabolic or elimination studies have been published for unifiram. The hexahydropyrrolo[1,2-a]pyrazinone ring system contains multiple potential sites for oxidative metabolism (ring hydroxylation, N-dealkylation, sulfonyl cleavage), and the 4-fluorophenyl group is resistant to the most common aromatic hydroxylation pathways. The absence of pharmacokinetic data is a significant gap in the characterization of the compound and a barrier to any rational dose extrapolation from rodent to human.

5.4 Routes of administration

Two routes have been used in published studies: intraperitoneal injection (the principal route in mouse studies, doses of 0.001 to 1 mg/kg) and oral gavage (mice, doses of 0.01 to 0.1 mg/kg) [1]. No intravenous, subcutaneous, or intracerebroventricular administration data have been published. For research applications, the compound is typically dissolved in dimethyl sulfoxide for in vitro work or in saline with a small proportion of dimethyl sulfoxide or Tween 80 for in vivo administration.

5.5 Special populations

No pharmacokinetic data exist for any special population (pediatric, geriatric, hepatic or renal impairment, polymorphic metabolism). The compound has been studied exclusively in adult male mice and rats of standard laboratory strains.

6. Preclinical Pharmacology

6.1 Mouse passive avoidance test

The mouse passive avoidance (step-through) test is the primary behavioral assay used to characterize unifram. In this paradigm, mice learn to avoid a dark compartment in which they previously received a mild foot shock; amnesia is induced by pharmacological agents administered before or after training, and the anti-amnesic compound is evaluated by its ability to restore avoidance behavior.

Unifram prevented scopolamine-induced amnesia at intraperitoneal doses of 0.01 mg/kg and above, mecamylamine-induced amnesia at 0.01 to 0.1 mg/kg, baclofen-induced amnesia at 0.01 to 0.1 mg/kg, and clonidine-induced amnesia at 0.01 to 0.1 mg/kg [1]. The effective doses are approximately three orders of magnitude lower than the effective doses of piracetam (30 to 100 mg/kg) and aniracetam (10 to 50 mg/kg) in the same assays. The compound also reversed NBQX-induced amnesia (AMPA receptor antagonist model) at 0.1 mg/kg intraperitoneal [7].

6.2 Rat Morris water maze

The Morris water maze evaluates spatial learning and memory by requiring rats to locate a submerged platform in a circular pool using distal visual cues. Unifram improved performance in the Morris water maze in rats with scopolamine-induced spatial memory impairment at oral doses of 0.01 to 0.1 mg/kg [1]. Latency to reach the platform was reduced and the number of platform crossings in the probe trial was increased relative to the scopolamine-alone group.

6.3 Rat social learning test

The social learning test evaluates social recognition memory by measuring the reduction in investigatory behavior toward a previously encountered juvenile rat. Unifram improved social recognition memory in rats at doses consistent with those effective in the passive avoidance and Morris water maze paradigms [1].

6.4 Motor coordination and spontaneous motility

Unifiram did not impair motor coordination on the mouse rota rod test at intraperitoneal doses of 1 to 10 mg/kg, doses 100- to 10,000-fold higher than the effective anti-amnesic dose [1, 2]. The compound did not modify spontaneous motility or inspection activity at the effective anti-amnesic doses. This separation between the anti-amnesic effective dose and the dose at which motor impairment appears provides a favorable therapeutic index in the rodent behavioral context.

6.5 Hypnosis reduction

Unifiram reduced the duration (but not the induction time) of pentobarbital-induced hypnosis in mice, an effect shared with piracetam and several other nootropic compounds and interpreted as evidence of central nervous system arousal-promoting activity [1, 2]. The lack of effect on induction time distinguishes this activity from stimulant-type arousal and is consistent with a modulatory rather than direct activating mechanism.

6.6 Hippocampal electrophysiology

In rat hippocampal slices, unifiram increased the amplitude of field excitatory post-synaptic potentials (fEPSP) at the Schaffer collateral-CA1 synapse in a concentration-dependent manner [2, 7]. This effect, the canonical in vitro signature of positive AMPA receptor modulation, was observed at concentrations consistent with the estimated brain concentrations achievable at the effective in vivo dose range.

6.7 Acetylcholine release

Unifiram stimulated acetylcholine release from rat cerebral cortex slices in vitro, an effect shared with sunifiram and with several other nootropic compounds including piracetam and aniracetam [2]. The magnitude of the acetylcholine release enhancement has not been quantitatively compared across compounds in a single study.

6.8 Limitations of the preclinical database

The preclinical database for unifiram is limited in scope. All published behavioral studies originate from a single research group at the University of Florence. Independent replication by other laboratories has not been published. The behavioral assays are standard and well validated but represent a narrow range of cognitive domains (passive avoidance, spatial navigation, social recognition). Cognitive flexibility, working memory, attention, and executive function have not been tested. Chronic dosing studies, dose-response for efficacy and toxicity over extended periods, and formal preclinical safety pharmacology (cardiovascular, respiratory, gastrointestinal) have not been conducted.

7. Clinical Evidence Base

No human clinical trials of unifiram have been conducted [2, 10]. The compound has not been submitted for investigational new drug application in any jurisdiction, has not undergone Phase 1 safety or pharmacokinetic evaluation in human volunteers, and has no clinical efficacy data.

The absence of clinical data reflects the failure of the compound to transition from academic research to pharmaceutical or biotechnology development. As noted by Gualtieri (2015), unifiram was never patented, and no industrial partner was secured for clinical development [10]. The compound therefore occupies a position of strong preclinical signal in a limited set of rodent assays with no clinical translation.

Unvalidated human use reports are available in online nootropic communities at doses of approximately 2 to 10 mg per administration. These reports are not controlled, not blinded, not objectively measured, and cannot be used to draw conclusions about human efficacy or safety. The theoretical human-equivalent dose derived by allometric scaling from the mouse effective intraperitoneal dose (0.01 mg/kg) would be approximately 0.8 to 1.6 mg per administration for a 70 to 80 kg human, though this extrapolation is unreliable without pharmacokinetic data to support it.

8. Sourcing and Quality Verification

8.1 Sources

Unifram is not available as a pharmaceutical-grade product. No pharmacopoeial monograph exists. The compound is supplied exclusively as a research chemical by chemical and nootropic research suppliers, including MedChemExpress, Cayman Chemical, BenchChem, ChemScene, Smolecule, and various nootropic-specific vendors. Purity is typically stated as greater than 98 percent by HPLC. Supply is in milligram to gram quantities as the free base.

8.2 Quality verification

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

- High-performance liquid chromatography area-percent purity (target greater than 98 percent)
- Mass spectrometry confirmation of molecular weight (protonated molecular ion $[M+H]^+$ at m/z 299.09)
- Nuclear magnetic resonance spectroscopy (1H and ^{13}C) for structural confirmation
- Residual solvent analysis if the intended application involves in vivo administration

Third-party analytical services (Janoshik Analytical or equivalent) provide independent confirmation of vendor certificates of analysis. The research-grade unifram market is less well established than markets for registered pharmaceutical compounds, and lot-to-lot variability in purity and stereochemical composition should be anticipated.

8.3 Regulatory status

Unifram is not approved as a medicine or dietary supplement in any jurisdiction. It is not specifically scheduled as a controlled substance in the United States, the European Union, the United Kingdom, or most other jurisdictions as of the date of this monograph. Regulatory status may change; investigators should confirm the current status in their jurisdiction. The compound is marketed on some consumer websites as a "nootropic" or "cognitive enhancer"; these marketing claims are not supported by clinical evidence and are not authorized by any regulatory body.

9. Reconstitution and Handling

9.1 Solubility

Unifram is freely soluble in dimethyl sulfoxide (DMSO) at concentrations of 10 to 50 mg/mL. Solubility in water is limited; aqueous solutions at concentrations above 1 mg/mL may require cosolvent (DMSO, ethanol, or polyethylene glycol) or surfactant (Tween 80, Cremophor EL) to maintain dissolution. For in vitro work, concentrated stock solutions in DMSO (10 to 100 mM) are prepared and diluted into assay medium; the final DMSO concentration should be kept below 0.1 percent to avoid solvent effects.

9.2 Preparation for in vivo administration

For intraperitoneal injection in rodents, unifram is dissolved in saline with 1 to 5 percent DMSO or 0.5 percent Tween 80 as a cosolvent. For oral gavage, the compound is dissolved or suspended in 0.5 percent carboxymethylcellulose or in a DMSO/polyethylene glycol 400/saline vehicle. Injection volumes are typically 10 mL/kg in mice and 5 mL/kg in rats.

9.3 Storage

The solid compound should be stored at negative 20 degrees Celsius in a desiccated, light-protected container. Stock solutions in DMSO should be aliquoted for single use and stored at negative 20 degrees Celsius; stability under these conditions has not been formally characterized but is expected to be at least 6 months based on the chemical stability of the sulfonamide and lactam functionalities. Aqueous solutions should be prepared fresh for each experiment.

9.4 Handling precautions

Unifram is a pharmacologically active compound at microgram-per-kilogram doses. Standard laboratory precautions for handling potent compounds (gloves, laboratory coat, fume hood for weighing, contained waste disposal) should be observed. No specific occupational exposure limits have been established.

10. Stack Interactions and Combinations

No formal drug-drug interaction studies have been conducted for unifiram. The following considerations are derived from the known pharmacology and from general pharmacological principles.

Cholinergic agents: The combination of unifiram with acetylcholinesterase inhibitors (donepezil, rivastigmine, galantamine) or with direct cholinergic agonists could produce additive cholinergic facilitation through the cortical acetylcholine release mechanism. The combination is pharmacologically plausible for cognitive research applications but has not been studied.

Other AMPA receptor modulators: Concurrent administration with ampakines (CX717, CX516) or with other positive AMPA receptor modulators (aniracetam, IDRA-21) could produce additive or synergistic effects on glutamatergic neurotransmission. Excessive AMPA receptor activation carries a theoretical risk of excitotoxicity; the combination should be approached with caution in research settings.

NMDA receptor modulators: The relationship between AMPA and NMDA receptor function in synaptic plasticity (AMPA receptor activation depolarizes the postsynaptic membrane and relieves the voltage-dependent magnesium block of the NMDA receptor) suggests that unifiram could interact with NMDA receptor modulators. Concurrent administration with memantine (NMDA receptor antagonist) or with D-cycloserine (NMDA glycine site partial agonist) has not been studied.

Racetams: Combination with piracetam, aniracetam, oxiracetam, or pramiracetam is pharmacologically redundant if the mechanism of action converges on the same glutamatergic facilitation pathway, though the different potencies and potentially different molecular targets could produce complementary effects. No combination data have been published.

GABAergic agents: Unifiram prevents baclofen-induced amnesia, suggesting functional antagonism of GABAergic inhibition. Concurrent administration with benzodiazepines, barbiturates, or other GABAergic agents could reduce the anti-amnesic effect of unifiram.

Stimulants: Concurrent use with caffeine, amphetamines, or methylphenidate has not been studied. Unvalidated online reports suggest that the combination of unifiram with caffeine may produce additive arousal and, at higher doses, anxiety and cardiovascular stimulation; these reports are not controlled and should not be used to guide research design.

CYP450 interactions: In the absence of metabolic data, no specific cytochrome P450 interaction predictions can be made. Investigators combining unifiram with compounds known to be potent CYP inhibitors or inducers should consider the possibility of altered unifiram exposure.

11. Adverse Events and Safety

11.1 Preclinical safety

Formal preclinical safety pharmacology and toxicology studies have not been published for unifiram [2, 10]. The available safety data are limited to the behavioral pharmacology studies, which report:

- No impairment of motor coordination on the rota rod test at doses up to 10 mg/kg intraperitoneal (100- to 10,000-fold the effective anti-amnesic dose) [1, 2]
- No modification of spontaneous motility or inspection activity at effective anti-amnesic doses [1]
- No overt signs of toxicity (seizure, tremor, sedation, piloerection, diarrhea, death) at the doses used in published studies (up to 10 mg/kg intraperitoneal)

These observations provide a basic safety margin in the rodent behavioral context but do not constitute formal safety pharmacology. Cardiovascular safety (QT interval, blood pressure, heart rate), respiratory safety, renal and hepatic toxicity, genotoxicity, carcinogenicity, and reproductive toxicity have not been assessed.

11.2 Theoretical safety concerns

Three theoretical safety concerns arise from the mechanism of action:

Excitotoxicity. AMPA receptor potentiation, if excessive, can produce glutamatergic excitotoxicity through calcium overload of postsynaptic neurons. The bell-shaped dose-response relationship observed for sunifiram-induced LTP enhancement in hippocampal slices [11] is consistent with a ceiling effect that may be protective at lower doses but raises the question of excitotoxic potential at higher doses. No seizure activity has been reported in the published unifiram studies, but the maximum tested dose (10 mg/kg) is only 100-fold above the effective dose, and chronic high-dose exposure has not been studied.

Unknown long-term effects. The absence of chronic dosing studies means that the long-term effects of repeated AMPA receptor facilitation on synaptic remodeling, receptor expression, and neuronal survival are unknown. The racetam class

generally shows favorable chronic tolerability in humans, but unifiram operates at much lower doses and potentially at a different molecular target, so extrapolation from racetam safety data is not warranted.

Unknown metabolite toxicity. The metabolic fate of unifiram is completely uncharacterized. The 4-fluorophenylsulfonyl group could potentially be cleaved to release 4-fluorobenzenesulfonic acid or undergo other biotransformation producing reactive metabolites. This concern is speculative but cannot be dismissed in the absence of data.

11.3 Human safety data

No human safety data exist from controlled studies. Unvalidated reports from online nootropic communities describe occasional headache, irritability, anxiety, and insomnia at higher doses (above 10 mg). These reports are uncontrolled and subject to placebo effect, expectation bias, and confounding from concurrent substance use.

11.4 Pregnancy and lactation

No reproductive toxicity data exist. Use in pregnancy or lactation cannot be assessed and should be presumed contraindicated in the absence of data.

12. Comparative Assessment

This section presents a structured comparative assessment of five cognition-enhancing compounds against unifiram, evaluated on five standards: novelty, effect size, promising potential (current development state and trajectory), side-effect profile, and overall validation. The five comparators were selected to represent the principal pharmacological classes with mechanistic overlap to unifiram's AMPA receptor-dependent glutamatergic facilitation.

12.1 Piracetam

Mechanism: The prototypical nootropic, piracetam (2-oxo-1-pyrrolidineacetamide) is a weak positive modulator of AMPA receptor-mediated neurotransmission, with additional effects on membrane fluidity, cerebral blood flow, and platelet aggregation [12]. It binds weakly to a site on the AMPA receptor distinct from the glutamate binding site and produces a modest enhancement of receptor-mediated currents.

Novelty: Low. Piracetam was synthesized in 1964 and is the oldest nootropic compound in clinical or research use.

Effect size: Modest in animal models and modest to negligible in human clinical trials. The effective dose in the mouse passive avoidance test is approximately 30 to 100 mg/kg intraperitoneal, approximately 1000-fold higher than unifiram [1, 2]. Human clinical trials have produced inconsistent results; a Cochrane review found insufficient evidence for cognitive benefit in dementia.

Promising potential: Low. The compound is marketed as a prescription medicine in some European jurisdictions for cognitive complaints and as a dietary supplement in others. No major development program is active.

Side-effect profile: Excellent. Piracetam is well tolerated at therapeutic doses (1.2 to 4.8 g per day in humans) with a decades-long safety record. The principal adverse events are mild gastrointestinal disturbance, headache, and insomnia, all at low incidence.

Overall validation: Moderate. The compound has a large clinical literature but modest and inconsistent efficacy evidence. The safety database is extensive and favorable.

Comparison to unifiram: Unifiram is approximately 1000-fold more potent than piracetam in the same rodent assays and shares the AMPA receptor dependence of the mechanism. Piracetam has the overwhelming advantage of extensive human safety and efficacy data accumulated over five decades. Unifiram has the advantage of potency but the disadvantage of a completely absent human evidence base.

12.2 Aniracetam

Mechanism: Aniracetam (1-p-anisoyl-2-pyrrolidinone) is a pyrrolidinone nootropic structurally related to piracetam, with positive allosteric modulation of AMPA receptors at the cyclothiazide binding site and additional activity at metabotropic glutamate receptors [13]. It is more potent than piracetam but less potent than unifiram.

Novelty: Low. Aniracetam was developed in the 1970s and has been studied for approximately 50 years.

Effect size: Moderate in animal models. The effective anti-amnesic dose in the mouse passive avoidance test is approximately 10 to 50 mg/kg, approximately 100- to 1000-fold higher than unifiram. Human clinical trials have produced modestly positive results in some vascular and degenerative dementia populations.

Promising potential: Low. The compound is marketed in Japan and some European countries for cognitive impairment associated with cerebrovascular disease. No major new development program is active.

Side-effect profile: Good. The compound is well tolerated at therapeutic doses (1.5 g per day) with a safety profile similar to piracetam.

Overall validation: Moderate. The compound has a modest clinical literature with some positive efficacy signals in specific dementia populations.

Comparison to unifiram: Unifiram is substantially more potent than aniracetam and shares the AMPA receptor mechanism. Aniracetam has human safety and efficacy data; unifiram does not. Aniracetam's established positive allosteric modula-

tor mechanism at the AMPA receptor is better characterized than unifiram's indirect mechanism.

12.3 Sunifiram (DM-235)

Mechanism: Sunifiram is a molecular simplification of unifiram, sharing the AMPA receptor-dependent glutamatergic facilitation mechanism and the cholinergic release enhancement [4, 8, 11]. Sunifiram also enhances LTP through CaMKII-dependent AMPA receptor phosphorylation and PKC-alpha-dependent NMDA receptor phosphorylation [11].

Novelty: Moderate. The mechanism is shared with unifiram but the molecular simplification and the more detailed characterization of the LTP enhancement pathway distinguish sunifiram from the parent compound.

Effect size: Comparable to unifiram. Sunifiram is approximately equipotent with unifiram in the mouse passive avoidance test at intraperitoneal doses of 0.001 to 0.1 mg/kg [2, 4]. The LTP enhancement in hippocampal slices peaks at 10 nM in a bell-shaped dose-response [11].

Promising potential: Low. Like unifiram, sunifiram has not been advanced to clinical trials and was never patented. It shares the same translational gap.

Side-effect profile: Unknown. No formal safety data exist beyond the behavioral pharmacology observation of absence of motor impairment at high multiples of the effective dose [2].

Overall validation: Low. The preclinical database is slightly larger than that of unifiram (including the LTP enhancement characterization) but remains limited to a single research group and has not been independently replicated.

Comparison to unifiram: The two compounds are essentially equipotent and mechanistically equivalent in the published assays. Sunifiram has modestly more detailed mechanistic characterization (LTP, CaMKII, PKC-alpha pathway). Neither has any human data. For research applications, the choice between unifiram and sunifiram is primarily one of availability and investigator preference.

12.4 CX717 (Farampator)

Mechanism: CX717 is a "low-impact" ampakine, a positive allosteric modulator of AMPA receptors developed by Cortex Pharmaceuticals (later RespireRx Pharmaceuticals) [14]. The compound binds directly to the AMPA receptor at the cyclothiazide/aniracetam binding site and prolongs the time course of AMPA receptor-mediated synaptic currents. Unlike "high-impact" ampakines (such as CX614), CX717 does not substantially increase the peak amplitude of AMPA currents and is therefore classified as a low-impact modulator with a broader safety margin.

Novelty: Moderate. The ampakine class represents a well-characterized approach to AMPA receptor modulation, and CX717 is a second-generation compound optimized for safety.

Effect size: Moderate. CX717 improved cognitive performance in sleep-deprived humans (Porrino et al. 2005) and in a Phase 2A ADHD trial (2025) at 800 mg twice daily, demonstrating superiority to placebo on inattentive and hyperactivity subscales [14]. The effect in the ADHD trial is the most advanced clinical evidence for any ampakine compound in a cognitive application.

Promising potential: Moderate. The compound has advanced to Phase 2 clinical trials and has demonstrated tolerability and preliminary efficacy in ADHD and in respiratory depression. Active development may continue.

Side-effect profile: Favorable. CX717 was well tolerated in Phase 1 and Phase 2 studies without significant cardiovascular or other safety signals. The low-impact classification reflects a deliberate design choice to avoid the excitotoxic potential associated with high-impact AMPA receptor potentiation.

Overall validation: Moderate. The compound has Phase 2 clinical data, a characterized molecular mechanism, and a favorable safety profile. The clinical database is modest but growing.

Comparison to unifiram: CX717 has a characterized molecular target (direct AMPA receptor positive allosteric modulation), human safety and preliminary efficacy data, and an active clinical development program. Unifiram has greater potency in rodent antiamnesic assays (effective at microgram-per-kilogram versus milligram-per-kilogram doses) but has no molecular target identification, no hu-

man data, and no development program. CX717 represents the current clinical translation of the AMPA receptor modulation strategy that unifiram preclinically exemplifies.

12.5 IDRA-21

Mechanism: IDRA-21 (7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide) is a positive allosteric modulator of AMPA receptors, structurally related to the benzothiadiazide diuretics, that enhances AMPA receptor-mediated currents by slowing desensitization [15]. The compound is considerably more potent than aniracetam and CX516 in electrophysiological assays and in rodent cognitive models.

Novelty: Low to moderate. The compound was disclosed in the mid-1990s and has been studied for approximately 30 years.

Effect size: Moderate to high in animal models. IDRA-21 improved delayed matching-to-sample performance in young and aged rhesus monkeys at oral doses of 3 to 10 mg/kg [15]. Rodent cognitive improvements are robust across multiple assays.

Promising potential: Low. The compound was never advanced to human clinical trials. No development program is active. The compound is available as a research chemical.

Side-effect profile: Partially characterized in preclinical models. IDRA-21 has been associated with proconvulsant activity at high doses in some rodent studies, reflecting the theoretical excitotoxic risk of potent AMPA receptor facilitation. The compound has a narrower apparent safety margin than CX717.

Overall validation: Low to moderate. The compound has a substantial preclinical literature including primate data but no clinical validation.

Comparison to unifiram: Both compounds are preclinical AMPA receptor facilitators without clinical data. IDRA-21 has a characterized molecular target (positive allosteric modulation at the benzothiadiazide site on the AMPA receptor) and primate cognitive data; unifiram does not. Unifiram has greater potency in the mouse passive avoidance test (effective at 0.01 mg/kg versus 3 to 10 mg/kg for IDRA-21 in primates), though cross-species dose comparison is not straightforward. The safety profiles of both compounds are incompletely characterized.

12.6 Comparator summary

The five comparators occupy distinct positions in the AMPA receptor-associated cognition enhancement space. **Piracetam** is the historical reference with extensive human safety data, minimal efficacy, and a half-century clinical record. **Aniracetam** is a modestly more potent racetam with some human clinical evidence and a well-characterized AMPA receptor positive allosteric modulator mechanism. **Sunifiram** is the closest structural and pharmacological analog of unifiram, sharing potency, mechanism, and the complete absence of human data. **CX717** represents the most clinically advanced AMPA receptor modulator, with Phase 2 human data and a deliberately safety-optimized profile. **IDRA-21** is a potent preclinical AMPA modulator with primate data but no clinical development and a narrower safety margin.

Unifiram occupies a distinctive but problematic position in this landscape. It is the most potent compound in the panel by rodent anti-amnesic assay potency (effective at 0.01 mg/kg versus 30 to 100 mg/kg for piracetam, 10 to 50 mg/kg for aniracetam, and 3 to 10 mg/kg for IDRA-21 in the relevant species). It is mechanistically linked to AMPA receptor facilitation but without a characterized molecular target. It has no human safety or efficacy data. It was never patented, never entered clinical development, and now exists in an unusual regulatory and commercial space as an unvalidated consumer nootropic.

The principal research value of unifiram is as a high-potency tool compound for investigating the pharmacological limits of the cognition-enhancing piperazinone scaffold and for comparison with mechanistically characterized AMPA receptor modulators. The principal limitation is the absence of molecular target identification and the complete absence of translational (human pharmacokinetic and safety) data. Investigators selecting a cognition-enhancing AMPA receptor facilitator for research should consider CX717 if human data and a characterized mechanism are priorities, piracetam or aniracetam if an established safety database is the priority, and unifiram or sunifiram if high rodent potency and a structurally distinct scaffold are the priority for preclinical mechanistic investigation.

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