

## RESEARCH MONOGRAPH . COMPOUND PROFILE

# Afobazole

*A 2-mercaptobenzimidazole derivative developed at the V.V. Zakusov Research Institute of Pharmacology and registered in Russia as a non-sedating anxiolytic, distinguished from benzodiazepines by absence of dependence liability and from buspirone by sigma-1 receptor chaperone agonism as the principal molecular mechanism.*

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| DOCUMENT       | KDC-MN-1462                                                                                                               |
| ISSUED         | 2026-05-13                                                                                                                |
| REVISION       | 1.0                                                                                                                       |
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| COMPOUND CLASS | Selective non-benzodiazepine anxiolytic with sigma-1 receptor chaperone agonism and multi-target neuroprotective activity |

## ABSTRACT

Afobazole (INN fabomotizole; development code CM-346; CAS 173352-21-1) is a 2-mercaptobenzimidazole anxiolytic synthesized and pharmacologically characterized at the V.V. Zakusov Research Institute of Pharmacology of the Russian Academy of Medical Sciences under the direction of Academician S.B. Seredenin. The compound was registered in the Russian Federation in 2006 for the treatment of generalized anxiety disorder and adjustment disorders, and was assigned the International Nonproprietary Name fabomotizole by the World Health Organization in 2012. Structurally, afobazole is 5-ethoxy-2-[2-(morpholino)-ethylthio]benzimidazole, a synthetic benzimidazole bearing a thioether-linked morpholine side chain and an ethoxy substituent on the aromatic ring. The molecular formula is C<sub>15</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>S (free base, molecular weight 307.41 g/mol), and the compound is supplied pharmaceutically as the dihydrochloride salt (C<sub>15</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>S, molecular weight 380.33 g/mol).

The pharmacological profile of afobazole is multi-target. Radioligand binding studies have identified the sigma-1 receptor chaperone protein (Sigma1R; K<sub>i</sub> approximately 5.9 micromolar), NRH:quinone reductase 2 (NQO2, also designated the MT3 melatonin binding site; K<sub>i</sub> approximately 0.97 micromolar), monoamine oxidase A (MAO-A regulatory site; K<sub>i</sub> approximately 3.6 micromolar), and the melatonin MT1 receptor (K<sub>i</sub> approximately 16 micromolar) as the principal molecular targets [1, 2]. The sigma-1 receptor interaction is the best-characterized mechanism: afobazole acts as a Sigma1R agonist, promoting dissociation of the Sigma1R-BiP complex at the endoplasmic

reticulum membrane and downstream modulation of calcium signaling, inositol 1,4,5-trisphosphate receptor function, and expression of neurotrophic factors including nerve growth factor and brain-derived neurotrophic factor [3, 4]. The anxiolytic effect is blocked by selective Sigma1R antagonists (BD-1047, NE-100) in rodent behavioral models, confirming the dependence of the therapeutic activity on Sigma1R engagement [5, 6]. Unlike benzodiazepines, afobazole does not produce sedation, myorelaxation, amnesia, tolerance, physical dependence, or withdrawal syndrome at therapeutic doses, a profile confirmed in both preclinical and clinical studies [7, 8].

Pharmacokinetics are characterized by rapid oral absorption (T<sub>max</sub> approximately 0.85 hours), pronounced first-pass hepatic metabolism (oral bioavailability approximately 44 percent), and a short plasma elimination half-life (approximately 0.82 hours) [9]. Despite the short systemic half-life, the anxiolytic effect persists substantially beyond the plasma residence of the parent compound, a pharmacodynamic feature attributed to the slow dissociation kinetics of the Sigma1R chaperone complex and to the sustained expression of downstream neurotrophic mediators. The recommended therapeutic dose is 30 mg per day in three divided administrations of 10 mg, with clinical effect emerging after 5 to 7 days of continuous dosing and maximal effect at 4 weeks [8, 10].

The clinical evidence base comprises several Russian multicenter trials. The principal registration study was a randomized, double-blind, active-controlled trial of 150 patients with generalized anxiety disorder or adjustment disorders comparing afobazole (30 mg/day) to diazepam (30 mg/day) for 30 days; afobazole produced greater reduction in Hamilton Anxiety Rating Scale total score than diazepam (mean difference 2.93 points;  $p = 0.01$ ), with 15

adverse events in the afobazole group versus 199 in the diazepam group, and no withdrawal syndrome on discontinuation [8]. Additional studies have evaluated afobazole in somatoform disorders, irritable bowel syndrome, premenstrual syndrome, alcohol withdrawal, and tobacco cessation, with consistent evidence of anxiolytic efficacy and favorable tolerability [10, 11, 12].

Preclinical research has extended the pharmacological profile well beyond anxiolysis. Afobazole produces Sigma1R-dependent neuroprotection in rat models of ischemic stroke (middle cerebral artery occlusion), with reduced infarct volume and improved neurological outcomes when administered up to 24 hours post-occlusion [13, 14]. In the 6-hydroxydopamine mouse model of Parkinson's disease, afobazole at 2.5 mg/kg intraperitoneally for 14 days restored striatal dopamine content, preserved tyrosine hydroxylase-positive neurons in the substantia nigra, and normalized motor coordination; these effects were abolished by the Sigma1R antagonist BD-1047 [15, 16]. Cardioprotective activity has been demonstrated in models of isoproterenol-induced myocardial injury, chronic heart failure, and alcoholic cardiomyopathy, with attenuation of pathological remodeling, preserved inotropic function, and reduced brain natriuretic peptide levels [17, 18, 19]. Angiogenic effects have also been reported [20].

This monograph reviews the chemistry, synthesis, and structural class of afobazole; the multi-target molecular pharmacology with emphasis on the Sigma1R chaperone mechanism; the comprehensive pharmacokinetic profile; the preclinical pharmacology across anxiolytic, neuroprotective, cardioprotective, and anti-inflammatory models; the clinical

evidence base in anxiety and related disorders; sourcing and quality considerations; reconstitution and handling; stack interactions; adverse events and safety signal; and a comparative assessment of five non-benzodiazepine anxiolytic candidates (buspirone, hydroxyzine, pregabalin, etifoxine, selank) against afobazole on five competency standards (novelty, effect size, promising potential, side-effect profile, and overall validation). The compound is not approved by the United States Food and Drug Administration or the European Medicines Agency. It is registered in the Russian Federation and is available as an over-the-counter pharmaceutical product within that jurisdiction and as a research-grade preparation from international chemical suppliers.

# 1. Compound Identification

## 1.1 Names, synonyms, and registry identifiers

**International Nonproprietary Name:** Fabomotizole (assigned by the World Health Organization in 2012).

**Marketed name:** Afobazole (Afobazol; OTCPharm / Pharmstandard; Russian Federation). The compound is sold as a non-prescription anxiolytic in Russian pharmacies in 10 mg tablet formulations.

**Development codes:** CM-346 (the identifier used in some international supplier catalogs and in early English-language literature). The compound is also referenced as Obenoxazine in some commercial databases.

**Chemical Abstracts Service registry numbers:** 173352-21-1 (free base), 173352-39-1 (hydrochloride salt), 189638-30-0 (dihydrochloride salt). The dihydrochloride is the pharmaceutical salt form used in the registered tablet formulation.

**International Union of Pure and Applied Chemistry name:** 4-{2-[(6-ethoxy-1H-benzimidazol-2-yl)sulfanyl]ethyl}morpholine. The compound is a 2-thioether-substituted benzimidazole bearing a morpholine ring connected through a two-carbon ethylene linker.

## 1.2 Structural class and chemistry

Afobazole is a synthetic benzimidazole derivative. The structural class places it within the 2-mercaptobenzimidazole family, a group of heterocyclic compounds that have been explored as pharmacological tools for sigma receptor ligands, antioxidants, and anti-inflammatory agents. The compound is distinguished from other members of the class by the morpholine-ethylthio side chain at position 2 and the ethoxy substituent at position 5 (or position 6, depending on numbering convention) of the benzimidazole ring.

**Molecular formula:** C<sub>15</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>S (free base); C<sub>15</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>S (dihydrochloride salt).

**Exact mass:** 307.1354 g/mol (free base, monoisotopic).

**Molecular weight:** 307.41 g/mol (free base); 380.33 g/mol (dihydrochloride salt).

**Physical properties:** White to off-white crystalline powder. Freely soluble in water as the dihydrochloride salt. Soluble in methanol and dimethyl sulfoxide. Sparingly soluble in apolar organic solvents. The compound is stable under standard storage conditions at room temperature in dry form.

**Stereochemistry:** Afobazole contains no stereocenters. The molecule is achiral; no enantiomeric separation is required for pharmaceutical or research use.

## 1.3 Synthesis

The synthesis of afobazole proceeds through a convergent route involving the benzimidazole core and the morpholine side chain. The general published approach involves: (a) preparation of 5-ethoxy-2-mercaptobenzimidazole by cyclization of 4-ethoxy-1,2-diaminobenzene with carbon disulfide or potassium ethyl xanthate under basic conditions; (b) alkylation of the 2-thiol with 2-(morpholino)ethyl chloride or the corresponding bromide in the presence of a base (typically potassium carbonate or sodium hydride) in a polar aprotic solvent (dimethylformamide, dimethyl sulfoxide, or acetonitrile); and (c) conversion to the dihydrochloride salt by treatment with hydrogen chloride in diethyl ether or isopropanol, followed by recrystallization [21]. The reaction sequence is straightforward and the compound is manufactured at scale in the Russian Federation for pharmaceutical use. Research-grade material from international suppliers is typically synthesized by analogous routes and characterized by nuclear magnetic resonance spectroscopy, mass spectrometry, and high-performance liquid chromatography.

## 2. Abstract

Afobazole (INN fabomotizole; CM-346; CAS 173352-21-1) is a selective non-benzodiazepine anxiolytic registered in the Russian Federation since 2006 for the treatment of generalized anxiety disorder and adjustment disorders. The compound was developed at the V.V. Zakusov Research Institute of Pharmacology under the direction of Academician Sergei Borisovich Seredenin through a systematic screening of benzimidazole derivatives for compounds that could prevent stress-induced reduction in benzodiazepine receptor binding without producing the sedation, myorelaxation, amnesia, tolerance, or dependence characteristic of the benzodiazepine class [7, 22].

The molecular pharmacology of afobazole is multi-target and has been progressively elucidated over two decades of post-registration research. The principal characterized targets are the sigma-1 receptor chaperone protein (Sigma1R), at which afobazole acts as an agonist with micromolar affinity ( $K_i$  approximately 5.9 micromolar); NRH:quinone reductase 2 (NQO2, also designated the MT3 melatonin binding site,  $K_i$  approximately 0.97 micromolar); the monoamine oxidase A regulatory site ( $K_i$  approximately 3.6 micromolar); and the melatonin MT1 receptor ( $K_i$  approximately 16 micromolar) [1, 2]. The Sigma1R interaction is the best-validated mechanism for the anxiolytic effect: selective Sigma1R antagonists (BD-1047, NE-100) abolish the anxiolytic activity of afobazole in the elevated plus maze, light-dark box, and Vogel conflict paradigms in rodents, while the selective Sigma1R agonist PRE-084 reproduces key pharmacological effects [5, 6]. Additional mechanistic contributions from GABA-A receptor modulation (indirect, through Sigma1R-mediated chaperoning of GABA-A receptor subunits), neurotrophic factor induction (NGF, BDNF), and MAO-A inhibition have been proposed but are less completely characterized [3, 4, 23].

The clinical evidence base is predominantly Russian-language and comprises several multicenter trials. The pivotal registration study compared afobazole (30 mg/day) to diazepam (30 mg/day) in 150 patients with generalized anxiety disorder or adjustment disorders over 30 days and demonstrated non-inferiority on the Hamilton Anxiety Rating Scale with a statistically significant advantage on the total score reduction (difference 2.93 points;  $p = 0.01$ ), markedly fewer adverse events (15 versus 199), and absence of withdrawal syndrome [8]. The compound has also been

studied in somatoform disorders, irritable bowel syndrome with anxiety, premenstrual dysphoric disorder, tobacco cessation, and alcohol withdrawal, with consistent anxiolytic effect and favorable tolerability across these populations [10, 11, 12].

The preclinical pharmacology extends well beyond anxiolysis. Sigma1R-dependent neuroprotection has been demonstrated in ischemic stroke models (reduced infarct volume and improved behavioral outcomes with delayed administration up to 24 hours post-occlusion) [13, 14]; in the 6-hydroxydopamine model of Parkinson's disease (restoration of striatal dopamine, preservation of nigral neurons) [15, 16]; and in models of oxidative stress, glutamate excitotoxicity, and cerebral hemorrhage [24, 25]. Cardioprotective effects have been demonstrated in chronic heart failure models, isoproterenol-induced cardiomyopathy, and alcoholic cardiomyopathy, mediated through Sigma1R activation and downstream reduction of pathological remodeling [17, 18, 19]. Angiogenic, anti-inflammatory, and cytoprotective properties have also been reported [20, 26].

Pharmacokinetics are characterized by rapid oral absorption (Tmax approximately 0.85 hours), moderate oral bioavailability (approximately 44 percent), and a short plasma half-life (approximately 0.82 hours) that stands in contrast to the sustained pharmacodynamic effect [9]. Metabolism is hepatic, with hydroxylation of the benzimidazole ring and oxidation of the morpholine moiety as principal biotransformation pathways.

The compound is not approved by the United States Food and Drug Administration, the European Medicines Agency, or any regulatory authority outside the Russian Federation and selected post-Soviet jurisdictions. It has had little clinical use outside Russia. Investigators acquiring afobazole for research should obtain material from established chemical suppliers and confirm identity and purity on every lot.

## 3. Discovery and Development History

### 3.1 Origin

Afobazole was developed at the V.V. Zakusov Research Institute of Pharmacology (formerly the Institute of Pharmacology of the Academy of Medical Sciences of the USSR, now the FSBI Zakusov Institute of Pharmacology) in Moscow during the 1990s. The research program was directed by Academician Sergei Borisovich Seredenin, who led a systematic effort to identify anxiolytic compounds that could reproduce the therapeutic effects of benzodiazepines without the characteristic liabilities of the class (sedation, myorelaxation, cognitive impairment, tolerance, physical dependence, and withdrawal syndrome) [7, 22].

The screening strategy was based on the observation that chronic stress in rodents produces a measurable decrease in the specific binding of [3H]diazepam to central benzodiazepine receptors (GABA-A receptor benzodiazepine sites) and that compounds capable of preventing this stress-induced decrease in binding, without directly binding the benzodiazepine site themselves, might constitute a novel class of anxiolytics with a distinct mechanism and a differentiated side-effect profile. A library of benzimidazole derivatives bearing various substituents at the 2-position and on the aromatic ring was screened in this paradigm. The compound 5-ethoxy-2-[2-(morpholino)-ethylthio]benzimidazole was selected as the lead on the basis of potent prevention of stress-induced benzodiazepine binding reduction, robust anxiolytic activity in the elevated plus maze and Vogel conflict test, and absence of sedation, myorelaxation, and amnesia at behaviorally active doses [7].

### 3.2 Preclinical development and registration

Preclinical pharmacology and toxicology studies were conducted at the Zakusov Institute throughout the late 1990s and early 2000s. The compound demonstrated consistent anxiolytic activity across multiple rodent behavioral models (elevated plus maze, light-dark test, Vogel conflict test, open field) at doses of 1 to 10 mg/kg intraperitoneally and 5 to 30 mg/kg orally, without producing sedation or motor impairment at multiples of the effective dose. Acute and chronic toxicology studies demonstrated acceptable safety margins. The compound was advanced through

Phase 1 healthy volunteer pharmacokinetic studies and Phase 2 and Phase 3 clinical trials in generalized anxiety disorder and adjustment disorders conducted at multiple centers in the Russian Federation between 2001 and 2005 [8, 10].

### 3.3 Registration and marketing

Afobazole was registered in the Russian Federation in 2006 as a non-prescription (over-the-counter) anxiolytic under the trade name Afobazole (later also Afobazol). The registration holder was initially OTCPharm, with subsequent involvement of Pharmstandard. The compound is marketed in 10 mg tablets, with the recommended dose of 10 mg three times daily (30 mg/day) for courses of 2 to 4 weeks, with the possibility of extension to 3 months on physician recommendation.

### 3.4 International recognition and INN assignment

Data on the pharmacology and clinical evidence of afobazole were submitted to the World Health Organization for review, and in 2012 the WHO International Working Group on Drug Statistics Methodology assigned the International Nonproprietary Name "fabomotizole" to the compound. The compound was assigned ATC code N05BX04 (anxiolytics, other). This assignment was based on the review of the mechanism of action data and the clinical evidence base presented by the Zakusov Institute [27].

### 3.5 Post-registration research expansion

Following registration, the research program at the Zakusov Institute and collaborating laboratories expanded substantially into neuroprotection, cardioprotection, and the molecular characterization of the multi-target mechanism. The identification of Sigma1R as the primary molecular target for the anxiolytic effect was formally established in a series of publications from approximately 2009 to 2021, with the demonstration that selective Sigma1R antagonists abolish the anxiolytic effect and that the Sigma1R agonist PRE-084 reproduces key behavioral outcomes [1, 5, 6]. The ischemic stroke neuroprotection program, conducted in collaboration with Bhatt et al. at the University of South Florida, produced publications in 2013 and 2014 demonstrating clinically translatable delayed treatment windows [13, 14]. The Parkinson's disease program produced publications from 2019 to 2020 demonstrating Sigma1R-dependent dopaminergic neuroprotection [15, 16]. The cardioprotection program produced publications from 2018 to 2021 [17, 18, 19, 20].

The compound has not been submitted for regulatory approval outside the Russian Federation. It has had limited clinical use outside Russia and the former Soviet republics, and the clinical evidence base remains predominantly Russian-language. No international Phase 3 registration trials have been conducted.

## 4. Mechanism of Action

Afobazole is a multi-target ligand whose pharmacological activities have been progressively characterized over two decades of post-registration research. The principal molecular mechanisms, in order of current evidence strength, are: agonism at the sigma-1 receptor chaperone protein; binding to NRH:quinone reductase 2 (NQO2, the MT3 melatonin binding site); reversible inhibition of monoamine oxidase A; agonism at the melatonin MT1 receptor; and downstream modulation of GABA-A receptor function, neurotrophic factor expression, and calcium homeostasis.

### 4.1 Sigma-1 receptor chaperone agonism

The sigma-1 receptor (Sigma1R) is an endoplasmic reticulum (ER) resident chaperone protein that resides at the mitochondria-associated ER membrane (MAM). Under resting conditions, Sigma1R exists in a complex with the ER chaperone BiP (GRP78/HSPA5). Upon ligand binding by agonists, the Sigma1R-BiP complex dissociates; the liberated Sigma1R monomer translocates within the ER membrane and to the plasma membrane, where it modulates the activity of multiple client proteins including inositol 1,4,5-trisphosphate receptors (IP3Rs), voltage-gated ion channels, NMDA receptors, and GABA-A receptor subunits [28, 29].

Afobazole binds Sigma1R with a  $K_i$  of approximately 5.9 micromolar as determined by radioligand displacement of [3H]-(+)-pentazocine in rat brain membranes [1]. Functional agonism has been demonstrated by the following convergent lines of evidence: (a) afobazole promotes Sigma1R-BiP dissociation in cellular assays [5]; (b) the anxiolytic effect in the elevated plus maze and light-dark test is abolished by the selective Sigma1R antagonists BD-1047 and NE-100, administered systemically or intracerebroventricularly [5, 6]; (c) the neuroprotective effect in the 6-OHDA Parkinson's model is abolished by BD-1047 [15]; (d) the selective Sigma1R agonist PRE-084 reproduces the anxiolytic and neuroprotective effects at comparable doses [6, 15]; and (e) the anxiolytic effect is attenuated in Sigma1R knockout mice [6].

The downstream consequences of Sigma1R agonism relevant to the anxiolytic mechanism include: modulation of IP3R-mediated calcium release from the ER, which stabilizes intracellular calcium homeostasis under stress conditions; chaper-

oning of GABA-A receptor subunits (particularly alpha and gamma2 subunits) during trafficking from the ER to the plasma membrane, enhancing surface expression and GABAergic neurotransmission [23, 30]; and upregulation of NGF and BDNF expression in hippocampal and cortical neurons, supporting neuroplastic adaptation to stress [3, 4].

## 4.2 NQO2 (MT3 melatonin binding site) interaction

NRH:quinone reductase 2 (NQO2) is a cytosolic flavoprotein that was historically identified as the MT3 melatonin binding site on the basis of its high affinity for melatonin and for the melatonin agonist 2-iodomelatonin. Afobazole binds NQO2 with a  $K_i$  of approximately 0.97 micromolar, the highest affinity among its characterized targets [1, 2]. NQO2 catalyzes the two-electron reduction of quinone substrates using dihydronicotinamide riboside (NRH) as a cofactor; the enzyme has been implicated in cellular redox regulation, xenobiotic metabolism, and modulation of oxidative stress.

The functional consequences of afobazole binding to NQO2 are not fully elucidated. NQO2 inhibition by afobazole may contribute to the cytoprotective and antioxidant effects observed in vitro and in vivo, including protection against oxidative stress-induced neuronal death and glutamate excitotoxicity [24, 26]. The role of NQO2 in the anxiolytic mechanism is less well established than the Sigma1R contribution. A 2020 study from the Zakusov Institute examined the contribution of NQO2 to the antiparkinsonian activity of afobazole and found that NQO2 inhibition did not account for the dopaminergic neuroprotective effect, which was Sigma1R-dependent [31].

## 4.3 Monoamine oxidase A inhibition

Afobazole binds a regulatory site on monoamine oxidase A (MAO-A) with a  $K_i$  of approximately 3.6 micromolar [1, 2]. The inhibition is reversible and has been characterized as interaction with a regulatory (allosteric) site rather than the catalytic site, distinguishing afobazole from classical MAO-A inhibitors (moclobemide, phenelzine, tranylcypromine). The functional consequence is modest reduction in the deamination of serotonin and norepinephrine, which may contribute to the anxiolytic and antidepressant-like effects observed in behavioral models. The clinical

cal significance of the MAO-A interaction at therapeutic doses (30 mg/day) has not been independently quantified, and the contribution is considered secondary to the Sigma1R mechanism.

## 4.4 Melatonin MT1 receptor agonism

Afobazole binds the melatonin MT1 receptor with a  $K_i$  of approximately 16 micromolar [1, 2]. At this affinity, meaningful MT1 receptor occupancy at the plasma concentrations achieved by the therapeutic dose is uncertain. The MT1 interaction may contribute to the reported anxiolytic and circadian-modulatory effects, particularly in patients with anxiety-related sleep disturbance, but this mechanism has not been dissected in isolation from the Sigma1R and NQO2 interactions in clinical studies.

## 4.5 Neurotrophic factor modulation

Seredenin et al. (2006, 2008) demonstrated that afobazole increases the expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in hippocampal and cortical neurons in vitro and in rodent brain in vivo [3, 4]. The neurotrophic induction is downstream of Sigma1R activation and is proposed as the molecular basis for the sustained anxiolytic effect that persists beyond the short plasma half-life of the parent compound. The neurotrophic mechanism is also the proposed basis for the neuroprotective effects observed in stroke and Parkinson's disease models.

## 4.6 Composite pharmacology and selectivity

The composite pharmacological profile of afobazole at the therapeutic dose of 30 mg/day (10 mg three times daily) produces peak plasma concentrations of approximately 0.13 micrograms per milliliter (approximately 0.42 micromolar for the free base) [9]. At this concentration, partial occupancy of NQO2 ( $K_i$  0.97 micromolar) is plausible; occupancy of Sigma1R ( $K_i$  5.9 micromolar) and MAO-A ( $K_i$  3.6 micromolar) is modest; and occupancy of MT1 ( $K_i$  16 micromolar) is marginal. The sustained pharmacodynamic effect despite the short half-life is attributed to the Sigma1R chaperone mechanism, in which agonist-induced dissociation of the Sigma1R-BiP complex initiates a signaling cascade (IP3R modulation, GABA-A receptor trafficking, neurotrophic factor expression) whose duration extends well beyond the period of receptor occupancy by the free drug.

## 5. Pharmacokinetics and Routes of Administration

### 5.1 Absorption

Afobazole is rapidly absorbed from the gastrointestinal tract after oral administration. Time to peak plasma concentration (T<sub>max</sub>) is approximately 0.85 +/- 0.13 hours [9]. Maximum plasma concentration (C<sub>max</sub>) after a single 10 mg oral dose is approximately 0.13 +/- 0.073 micrograms per milliliter. The compound undergoes a pronounced first-pass effect that limits oral bioavailability to approximately 43.6 percent [9].

### 5.2 Distribution

Afobazole is intensively distributed over well-vascularized organs and is transferred rapidly from the central compartment (blood plasma) to peripheral tissues. The compound crosses the blood-brain barrier; central nervous system penetration is sufficient to support the central pharmacological effects observed in behavioral models and in clinical studies. Specific data on volume of distribution and plasma protein binding in humans are limited in the English-language literature; the rapid redistribution from plasma to tissues is the principal determinant of the short apparent plasma half-life.

### 5.3 Metabolism

Afobazole undergoes extensive hepatic metabolism. The principal biotransformation pathways are hydroxylation on the aromatic ring of the benzimidazole nucleus and oxidation of the morpholine ring system [9, 32]. The cytochrome P450 enzymes involved include CYP2C9 and CYP1A2, as demonstrated by in vivo metabolic ratio studies in which afobazole at therapeutic doses did not alter the metabolic ratio of CYP2C9 or CYP1A2 probe substrates, but a five-fold dose increase produced induction of CYP2C9 [32, 33]. CYP2D6 involvement has not been specifically characterized in the available literature.

The principal identified metabolite is 4-hydroxy-afobazole (the product of aromatic hydroxylation). Metabolite pharmacological activity has not been fully characterized; preclinical data suggest that the hydroxylated metabolite retains some pharmacological activity but at reduced potency compared to the parent compound.

## 5.4 Elimination

The plasma elimination half-life of afobazole after oral administration is approximately 0.82 +/- 0.54 hours [9]. This short half-life reflects the combination of extensive first-pass metabolism and rapid tissue distribution. Elimination is predominantly through hepatic metabolism with subsequent renal excretion of conjugated metabolites. The short plasma half-life is pharmacodynamically dissociated from the duration of anxiolytic effect, which is sustained for hours after plasma concentrations have declined below measurable levels.

## 5.5 Routes of administration

The registered route of administration is oral (10 mg tablets, three times daily). Intravenous and intraperitoneal routes have been used in preclinical research but are not registered for clinical use. The compound is water-soluble as the dihydrochloride salt and is amenable to parenteral formulation for research applications.

## 5.6 Special populations

Pediatric pharmacokinetics have not been characterized; the compound is not approved for pediatric use. Geriatric data are limited. Hepatic impairment would be expected to increase exposure substantially given the extensive hepatic metabolism, but specific dose-adjustment guidelines for hepatic impairment have not been formally published. Renal impairment is not expected to significantly affect pharmacokinetics given the predominantly hepatic elimination pathway. Pregnancy and lactation data are limited; the compound is generally contraindicated in pregnancy and lactation in the Russian prescribing information.

## 6. Preclinical Pharmacology

### 6.1 Anxiolytic activity

Afobazole demonstrates consistent anxiolytic activity across multiple rodent behavioral models at doses of 1 to 10 mg/kg intraperitoneally and 5 to 30 mg/kg orally. In the elevated plus maze, afobazole increases open arm time and open arm entries without affecting total locomotor activity, distinguishing it from sedative anxiolytics [7]. In the Vogel conflict test (punished drinking), afobazole increases punished responding at doses that do not produce general behavioral disinhibition. In the light-dark test, afobazole increases time in the light compartment. In the open field test, afobazole reduces anxiety-related behaviors (thigmotaxis, freezing) without altering total ambulation [7, 22].

The anxiolytic profile is notable for the absence of sedation, myorelaxation, and amnesia at effective and supratherapeutic doses. In rotarod and traction test assays, afobazole does not impair motor coordination at doses up to 50 mg/kg in mice, contrasting with diazepam which produces significant motor impairment at 3 to 5 mg/kg [7]. In passive avoidance and Morris water maze paradigms, afobazole does not impair learning or memory at anxiolytic doses, and some reports suggest a modest procognitive effect [22].

### 6.2 Neuroprotection in ischemic stroke

Katnik et al. (2014) and Bhatt et al. (2013) at the University of South Florida demonstrated that afobazole produces significant neuroprotection in rat models of ischemic stroke. In the middle cerebral artery occlusion model, afobazole administered intraperitoneally at 0.3 to 3 mg/kg reduced infarct volume at 96 hours post-surgery and improved neurological deficit scores [13, 14]. The neuroprotective effect was preserved when treatment was initiated 24 hours after the ischemic event, a clinically relevant treatment window. The reduction in infarct volume was accompanied by preservation of myelin basic protein levels and normal histological distribution, indicating reduction of white matter injury. Both Sigma1R and sigma-2 receptor antagonists blocked the neuroprotective effect, indicating involvement of both receptor subtypes [14].

In vitro, afobazole protected cortical neurons against ischemia-induced death and acidosis through Sigma1R-dependent mechanisms, maintaining intracellular calcium homeostasis and reducing apoptotic signaling [34].

## 6.3 Neuroprotection in Parkinson's disease models

Zenina et al. (2019) demonstrated that afobazole at 2.5 mg/kg intraperitoneally for 14 days in ICR mice subjected to unilateral 6-hydroxydopamine (6-OHDA) striatal lesion restored dopamine content in the lesioned striatum, increased the number of tyrosine hydroxylase-positive neurons in the substantia nigra pars compacta, and normalized motor coordination on the rotarod test [15]. These effects were reproduced by the selective Sigma1R agonist PRE-084 (1.0 mg/kg) and were abolished by pre-administration of the selective Sigma1R antagonist BD-1047 (3.0 mg/kg), confirming Sigma1R dependence of the neuroprotective mechanism [15]. A subsequent study demonstrated that deferred administration of afobazole (beginning 7 days after the 6-OHDA lesion rather than concomitantly) also produced Sigma1R-dependent restoration of striatal dopamine, supporting a disease-modifying rather than merely neuroprotective mechanism [16].

## 6.4 Cardioprotective effects

The cardioprotective pharmacology of afobazole has been characterized in multiple models. In isoproterenol-induced myocardial injury in rats, afobazole reduced ST-segment depression, decreased serum creatine kinase and lactate dehydrogenase, and preserved myocardial histological architecture [17]. In a translational model of chronic heart failure (coronary artery ligation in rats), afobazole prevented pathological remodeling of the myocardium, maintained inotropic function, and reduced plasma brain natriuretic peptide levels [18]. In alcoholic cardiomyopathy, afobazole attenuated ethanol-induced cardiac fibrosis and contractile dysfunction [19]. In animals with endothelial dysfunction, afobazole reduced isoproterenol-induced ischemic changes [17]. These effects are attributed to Sigma1R agonism, with downstream modulation of calcium handling, mitochondrial function, and inflammatory signaling in cardiomyocytes.

## 6.5 Additional preclinical effects

Afobazole has demonstrated antimutagenic and antiteratogenic properties in mouse models [22]. Cytoprotective effects have been documented against oxidative stress-induced cell death, glutamate excitotoxicity, and hydrogen peroxide toxicity in neuronal and glial cultures, mediated through Sigma1R activation and NQO2 binding [24, 26]. Angiogenic effects have been reported, with afobazole promoting vascularization in ischemic tissue models [20]. Anti-inflammatory activity mediated through reduction of NF-kappaB-driven cytokine production and nitric oxide synthase inhibition has been documented in microglial and macrophage cultures [25]. Renal protective effects at doses of 10 to 20 mg/kg daily, reducing hyperglycemia-induced oxidative stress and inflammation in renal tissue, have been reported [35].

## 7. Clinical Evidence Base

### 7.1 Generalized anxiety disorder and adjustment disorders

The principal registration study was a multicenter randomized double-blind active-controlled trial of 150 patients (60 with generalized anxiety disorder and 90 with adjustment disorders) conducted at multiple centers in the Russian Federation between 2001 and 2003 [8]. Patients aged 18 to 60 years were randomized 2:1 to afobazole (30 mg/day in three divided doses; n = 100) or diazepam (30 mg/day in three divided doses; n = 50) for 30 days. The primary endpoint was reduction in Hamilton Anxiety Rating Scale (HAMA) total score.

Both afobazole and diazepam produced statistically significant reduction of HAMA total score from baseline. The reduction in anxiety in the afobazole group exceeded that in the diazepam group (mean difference in total score change: 2.93 points; p = 0.01). The proportion of patients with clinically meaningful reduction of disease severity was 72 percent in the afobazole group versus 58 percent in the diazepam group. After therapy completion, 69 percent of patients in the afobazole group had no or mild residual disorder compared to 44 percent in the diazepam group [8].

The safety differential was pronounced: 15 adverse events were recorded in the afobazole group compared to 199 in the diazepam group. No manifestations of withdrawal syndrome were observed in the afobazole group on discontinuation, whereas diazepam withdrawal syndrome was documented in 68 percent of patients [8].

### 7.2 Earlier phase studies

Neznamov et al. (2001) reported results of earlier clinical studies establishing the dose-response relationship and confirming the anxiolytic effect in patients with anxiety disorders [10]. These studies established the 30 mg/day dose as the optimal therapeutic dose and documented the onset of clinical effect at approximately 5 to 7 days, with maximal effect at 4 weeks of continuous dosing.

## 7.3 Somatoform disorders

Afobazole has been studied in patients with somatoform disorders (functional somatic symptom presentations with comorbid anxiety) in open-label and small randomized studies. Anxiolytic efficacy was demonstrated with reduction in both anxiety scores and somatic symptom burden, suggesting a role in the management of anxiety-somatization overlap presentations [10, 11].

## 7.4 Cardiovascular anxiety

Medvedev et al. (2007) reported on the use of afobazole in patients with cardiovascular conditions and comorbid anxiety, demonstrating anxiolytic efficacy without adverse cardiovascular effects and with some evidence of reduced cardiovascular symptom burden [36]. The absence of sedation and hemodynamic compromise was considered an advantage over benzodiazepines in this population.

## 7.5 Irritable bowel syndrome

Afobazole has been evaluated as adjunctive anxiolytic therapy in irritable bowel syndrome, where anxiety is a recognized driver of symptom severity. Open-label studies have reported reduction in both anxiety scores and gastrointestinal symptom scores, consistent with the dual anxiolytic and functional somatic benefit observed in somatoform disorder populations [11].

## 7.6 Alcohol and tobacco cessation

Afobazole has been studied as an adjunct to alcohol withdrawal management and tobacco cessation, where anxiety is a major contributor to relapse. Studies conducted in Russia have reported reduction in withdrawal anxiety and improved completion rates in cessation programs, though these were predominantly open-label or small controlled studies rather than large randomized trials [12].

## 7.7 Premenstrual dysphoric disorder

Open-label and small controlled studies have evaluated afobazole for premenstrual anxiety and dysphoria, with reports of reduced premenstrual anxiety scores and improved quality-of-life measures during the luteal phase [10].

## 7.8 Limitations of the clinical evidence base

The clinical evidence for afobazole has important limitations that must be acknowledged. The majority of clinical studies have been published in Russian-language journals, limiting independent verification and replication by the international research community. The pivotal registration study used diazepam as an active comparator rather than a placebo arm, which confounds the absolute efficacy assessment. Sample sizes in the available studies are modest by international registration standards. No international Phase 3 registration trials have been conducted, and the compound has not undergone independent regulatory review by the FDA, EMA, or other major regulatory authorities. The effect sizes, while statistically significant, are modest and the clinical significance of the observed differences requires contextualization within the broader anxiolytic evidence base [8, 10].

## 8. Sourcing and Quality Verification

Pharmaceutical-grade afobazole (Afobazole brand, OTCPharm/Pharmstandard) is the highest quality source and is available within the Russian Federation as a non-prescription medication in 10 mg tablet formulations. Pharmaceutical sourcing outside Russia requires importation and may be subject to regulatory restrictions in the destination jurisdiction.

Research-grade fabomotizole is supplied by multiple international chemical and pharmaceutical research suppliers (Sigma-Aldrich, MedChemExpress, Selleckchem, Cayman Chemical, InvivoChem, BenchChem, TargetMol, BOC Sciences, others) as the free base (CAS 173352-21-1), hydrochloride salt (CAS 173352-39-1), or dihydrochloride salt (CAS 189638-30-0) at stated purity of greater than 98 percent by HPLC. Pricing is moderate, in the range of \$50 to \$300 per 100 milligrams of research-grade material at the time of monograph preparation, depending on supplier and quantity.

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

- High-performance liquid chromatography area-percent purity, targeting greater than 98 percent
- Mass spectrometry confirmation of molecular weight (308.14 [M+H]<sup>+</sup> for the protonated free base)
- Proton and carbon-13 nuclear magnetic resonance spectroscopy confirmation of structure
- Karl Fischer or loss-on-drying analysis for residual water content
- Residual solvent analysis by gas chromatography (common synthesis solvents: dimethylformamide, dimethyl sulfoxide, methanol, ethanol)

Third-party analytical laboratories provide independent confirmation of supplier certificates of analysis. The research-grade fabomotizole market is established but smaller than markets for more widely studied compounds; lot-to-lot variability in purity and salt form should be anticipated, and on-receipt analytical confirmation is appropriate for any investigation where dose-response precision is required.

## 9. Reconstitution and Handling

Afobazole dihydrochloride is freely soluble in water at room temperature. For research applications, the compound is supplied as a crystalline powder and is reconstituted as needed.

**Aqueous stock solutions:** Prepare at 10 to 50 mg/mL in sterile water, normal saline, or phosphate-buffered saline. The compound dissolves readily without heating or sonication.

**DMSO stock solutions:** For in vitro applications requiring higher concentrations, prepare at 50 to 100 mg/mL in dimethyl sulfoxide. Final DMSO concentration in cell culture or assay medium should not exceed 0.1 to 0.5 percent to avoid solvent cytotoxicity.

**Storage:** Store solid material at room temperature (15 to 25 degrees Celsius) in a dry, light-protected location. Refrigerated storage (2 to 8 degrees Celsius) is acceptable and may extend shelf life. Aqueous stock solutions should be prepared fresh or stored refrigerated and used within 7 to 14 days. DMSO stock solutions are more stable and may be stored at negative 20 degrees Celsius for up to 6 months. Aliquoting to avoid repeated freeze-thaw cycles is recommended for frozen stocks.

**Stability:** The solid compound is stable under standard storage conditions for at least 24 months (based on pharmaceutical product shelf-life data). Aqueous solutions are stable for at least 7 days at 2 to 8 degrees Celsius. Light protection is advisable but not strictly required for short-term handling.

**Formulation for in vivo research:** For intraperitoneal administration in rodent studies, dissolve in normal saline at the required concentration (typically 0.5 to 5 mg/mL for doses of 1 to 10 mg/kg in a standard injection volume of 10 mL/kg). Filter-sterilize through 0.22-micrometer membranes for parenteral use.

## 10. Stack Interactions and Combinations

Afobazole interacts with several drug classes through its multi-target pharmacology and hepatic metabolism. Investigators conducting research with afobazole should consider the following interactions.

**Benzodiazepines:** Concurrent administration with benzodiazepines produces additive anxiolytic effects without pharmacokinetic interaction. The combination has been used clinically in Russia during benzodiazepine tapering, with afobazole providing anxiolytic coverage while the benzodiazepine dose is reduced. No adverse pharmacodynamic interaction has been reported, and the absence of GABAergic sedation, myorelaxation, and respiratory depression from afobazole means that the combination does not produce the additive central nervous system depression characteristic of benzodiazepine-benzodiazepine or benzodiazepine-alcohol combinations.

**Selective serotonin reuptake inhibitors (SSRIs):** The MAO-A inhibitory activity of afobazole (Ki 3.6 micromolar) is modest and is at a regulatory (allosteric) site. At therapeutic doses, the risk of serotonin syndrome in combination with SSRIs is considered low; clinical use in Russia has included patients on concurrent SSRI therapy without reported serotonergic adverse events. However, the theoretical interaction should be monitored, particularly at supratherapeutic doses.

**CYP2C9 substrates:** At therapeutic doses (30 mg/day), afobazole does not alter CYP2C9 metabolic ratios [32, 33]. At doses five-fold above therapeutic (150 mg/day equivalent), CYP2C9 induction was observed. Investigators using high-dose afobazole in research should be aware of potential drug-drug interactions with CYP2C9 substrates (warfarin, phenytoin, tolbutamide, diclofenac) at supratherapeutic doses.

**CYP1A2 substrates:** At therapeutic doses, afobazole does not alter CYP1A2 metabolic ratios [32]. No clinically significant interaction with CYP1A2 substrates (caffeine, theophylline, clozapine) is expected at registered doses.

**Sigma-1 receptor ligands:** Concurrent administration with other Sigma1R agonists (PRE-084, dextromethorphan, fluvoxamine, donepezil) may produce additive or synergistic Sigma1R activation. Concurrent administration with Sigma1R antagonists (haloperidol, NE-100, BD-1047) may attenuate the anxiolytic and neuroprotective effects of afobazole.

**Ethanol:** No pharmacokinetic interaction with ethanol has been reported. Afobazole does not potentiate the sedative, motor-impairing, or respiratory-depressant effects of ethanol, in contrast to benzodiazepines. The compound has been used in alcohol withdrawal management on this basis [12].

**Melatonin and melatonergic agents:** Given the MT1 agonist and NQO2 (MT3 site) binding activities of afobazole, concurrent administration with melatonin or melatonin receptor agonists (ramelteon, tasimelteon, agomelatine) may produce additive melatonergic effects. The clinical significance of this interaction has not been formally studied.

# 11. Adverse Events and Safety

## 11.1 Common adverse events

The adverse-event profile of afobazole at the registered dose of 30 mg/day is favorable and contrasts markedly with both benzodiazepines and many other anxiolytic agents. In the pivotal registration study comparing afobazole to diazepam, only 15 adverse events were recorded in 100 patients over 30 days of afobazole treatment, compared to 199 adverse events in 50 patients on diazepam [8].

Reported adverse events include:

- Mild gastrointestinal discomfort (nausea, diarrhea; approximately 1 to 3 percent)
- Allergic reactions (rare; skin rash, pruritus)
- Dry mouth (occasional)
- Headache (infrequent)
- Insomnia or difficulty sleeping (infrequent, typically in the first days of treatment)

## 11.2 Absence of characteristic benzodiazepine adverse events

Afobazole does not produce the following adverse events that characterize the benzodiazepine class at anxiolytic doses:

- Sedation and daytime drowsiness
- Myorelaxation and impaired motor coordination
- Anterograde amnesia and cognitive impairment
- Tolerance (no evidence of diminishing effect with continued use at 4 to 12 weeks)
- Physical dependence and withdrawal syndrome (none observed on discontinuation in any published study)
- Respiratory depression

- Disinhibition or paradoxical reactions

The absence of dependence liability and withdrawal syndrome is the most clinically significant safety advantage. In the pivotal trial, diazepam withdrawal syndrome occurred in 68 percent of patients on discontinuation; no withdrawal was observed in the afobazole group [8].

## 11.3 Overdose and toxicology

Acute toxicity data from preclinical studies indicate a wide therapeutic index. The LD50 in mice by intraperitoneal administration is substantially above the therapeutic dose range. No clinical reports of serious overdose have been published in the English-language literature. The compound is classified as non-prescription in the Russian Federation, reflecting the favorable safety profile.

## 11.4 Pregnancy and lactation

The compound is contraindicated in pregnancy and lactation in the Russian prescribing information. Animal reproductive toxicity data are limited; the compound has demonstrated antimutagenic and antiteratogenic properties in some mouse models [22], but formal developmental and reproductive toxicology studies meeting international regulatory standards have not been published.

## 11.5 Contraindications

Listed contraindications in the Russian prescribing information include hypersensitivity to the active substance or excipients, pregnancy, lactation, and age under 18 years.

## 12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five non-benzodiazepine anxiolytic candidates against afobazole, evaluated on five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation. The five comparators were selected as the principal non-benzodiazepine anxiolytics with substantive clinical evidence and research relevance, prioritizing those with differentiated mechanisms.

### 12.1 Buspirone

**Mechanism:** Selective partial agonist at the serotonin 5-HT<sub>1A</sub> receptor (both pre- and postsynaptic sites) with weak dopamine D<sub>2</sub> receptor antagonism at presynaptic autoreceptors. No activity at GABA-A benzodiazepine sites, sigma receptors, or melatonin receptors.

**Development trajectory:** Developed by Bristol-Myers Squibb and approved by the FDA in 1986 for generalized anxiety disorder. Marketed as BuSpar and in extensive generic availability worldwide. One of the most widely prescribed non-benzodiazepine anxiolytics globally.

**Novelty:** Low. The 5-HT<sub>1A</sub> partial agonist mechanism is well established and has been available for four decades. The mechanism is distinct from the Sigma<sub>1</sub>R agonism of afobazole but is fully explored pharmacologically.

**Effect size:** Moderate. Buspirone demonstrates consistent anxiolytic efficacy in generalized anxiety disorder with effect sizes comparable to benzodiazepines in meta-analyses, though with delayed onset (2 to 4 weeks to clinical effect). The delayed onset is a practical disadvantage for patients requiring rapid anxiolysis.

**Promising potential:** Stable. Buspirone has an established clinical position as a non-addicting anxiolytic and is recommended as a second-line treatment for generalized anxiety disorder in multiple international guidelines. No significant new indications are in development.

**Side-effect profile:** Favorable. Principal adverse events are dizziness, nausea, headache, and nervousness. No sedation, dependence, or withdrawal syndrome. No significant sexual dysfunction (in contrast to SSRIs). The side-effect profile is comparable in character and severity to afobazole.

**Overall validation:** High. Extensive clinical evidence base from multiple international randomized controlled trials, meta-analyses, and decades of post-marketing experience. FDA-approved, EMA-recognized, and included in all major treatment guidelines.

**Comparison to afobazole:** Buspirone has substantially greater regulatory validation (FDA-approved, internationally registered), a larger and more rigorous clinical evidence base, and longer clinical experience. Afobazole has a differentiated mechanism (Sigma1R agonism versus 5-HT1A partial agonism), additional neuroprotective and cardioprotective activities not shared by buspirone, and a marginally faster onset of clinical effect (5 to 7 days versus 2 to 4 weeks). The absence of international regulatory approval is the principal limitation of afobazole relative to buspirone.

## 12.2 Hydroxyzine

**Mechanism:** Histamine H1 receptor antagonist with secondary muscarinic anticholinergic activity. The anxiolytic effect is mediated through central H1 receptor blockade and is inseparable from the sedative mechanism.

**Development trajectory:** Developed by Pfizer (originally Union Chimique Belge) and marketed since the 1950s as Atarax and Vistaril. Approved for anxiety in multiple jurisdictions. One of the oldest non-benzodiazepine anxiolytics.

**Novelty:** Low. Antihistaminic anxiolysis is the simplest available mechanism and has been available for seven decades.

**Effect size:** Moderate. Randomized controlled trials demonstrate anxiolytic efficacy comparable to benzodiazepines and buspirone in generalized anxiety disorder. Effect is rapid (within hours), distinguishing hydroxyzine from buspirone. However, the effect is primarily short-term; hydroxyzine is not typically used for chronic anxiolysis.

**Promising potential:** Low. No significant new development is anticipated. The compound serves as a rescue or adjunctive anxiolytic for acute anxiety and as a comparator in clinical trials.

**Side-effect profile:** Moderate. Sedation is the principal adverse event and is inseparable from the anxiolytic mechanism at therapeutically effective doses. Dry mouth, constipation, and urinary retention (anticholinergic effects) are common. QT prolongation has been identified in post-marketing surveillance, leading to warnings against use in elderly patients and in patients with cardiac risk factors. Weight gain is minimal. No dependence or withdrawal syndrome.

**Overall validation:** Moderate. Established clinical history with supporting randomized controlled trial data. Limited primarily to short-term or adjunctive use by the sedative burden.

**Comparison to afobazole:** Afobazole has the advantage of absence of sedation, absence of anticholinergic burden, and absence of QT prolongation risk. Hydroxyzine has the advantage of faster onset (hours versus days), broader international regulatory approval, and wider clinical familiarity. The two compounds occupy different positions in the anxiolytic pharmacotherapy sequence: hydroxyzine as an acute, short-term agent; afobazole as a maintenance, non-sedating alternative.

## 12.3 Pregabalin

**Mechanism:** Binds the alpha-2-delta subunit of voltage-gated calcium channels (VGCC), reducing calcium influx at presynaptic terminals and decreasing release of excitatory neurotransmitters including glutamate, norepinephrine, and substance P. No direct GABA-A, sigma, or serotonin receptor activity.

**Development trajectory:** Developed by Pfizer as a successor to gabapentin. Approved by the EMA for generalized anxiety disorder (2006); FDA-approved for fibromyalgia, neuropathic pain, and epilepsy but not for anxiety. Marketed as Lyrica worldwide.

**Novelty:** Moderate. The alpha-2-delta calcium channel mechanism for anxiolysis was novel at the time of registration and remains mechanistically distinct from both benzodiazepine and serotonergic approaches.

**Effect size:** Moderate to high. Multiple large randomized controlled trials in generalized anxiety disorder demonstrate effect sizes comparable to or exceeding SSRIs and benzodiazepines (HAMA response rates of 50 to 60 percent versus 30 to 40 percent on placebo) [37]. Pregabalin is considered a first-line treatment for generalized anxiety disorder in European guidelines.

**Promising potential:** Moderate. Patent expiry and generic availability have reduced commercial interest. Pregabalin has been reclassified as a controlled substance (Schedule V in the United States, Class C in the United Kingdom) due to emerging evidence of abuse potential, particularly in populations with substance use disorders.

**Side-effect profile:** Moderate. Sedation and dizziness are the most common adverse events (20 to 30 percent at therapeutic doses). Weight gain (4 to 6 percent incidence) is a concern for chronic use. Peripheral edema, visual disturbance, and cognitive dulling occur. Dependence liability has been recognized, with withdrawal symptoms (insomnia, nausea, anxiety rebound, diaphoresis) reported on abrupt discontinuation. Abuse potential, particularly at supratherapeutic doses, has led to scheduling classification in multiple jurisdictions.

**Overall validation:** High. Extensive international clinical evidence base, EMA approval for generalized anxiety disorder, multiple randomized controlled trials, and inclusion in major treatment guidelines. The abuse liability has modestly reduced the positioning relative to SSRIs and SNRIs.

**Comparison to afobazole:** Pregabalin has substantially greater regulatory validation, a larger clinical evidence base, and demonstrated larger effect sizes in generalized anxiety disorder. Afobazole has the advantages of absence of sedation, absence of dependence and withdrawal liability, absence of controlled-substance scheduling, and additional neuroprotective and cardioprotective activities. The two compounds target entirely different molecular mechanisms and occupy different pharmacological categories.

## 12.4 Etifoxine

**Mechanism:** Dual mechanism involving direct positive allosteric modulation of GABA-A receptors (at a site distinct from the benzodiazepine binding site) and stimulation of neurosteroid biosynthesis (increased production of allopregnanolone

and other GABA-A-active neurosteroids) through activation of the translocator protein (TSPO, formerly the peripheral benzodiazepine receptor) in mitochondrial membranes [38].

**Development trajectory:** Developed by Hoechst (now Sanofi) in the 1960s. Registered in France and approximately 40 countries (predominantly in Europe, Africa, and Asia) for the treatment of anxiety disorders, marketed as Stresam. Not FDA-approved. Limited clinical use in English-speaking countries.

**Novelty:** Moderate. The neurosteroidogenic mechanism, clarified in the 2000s, distinguishes etifoxine from both direct benzodiazepine site ligands and from non-GABAergic anxiolytics. The TSPO-mediated neurosteroid biosynthesis pathway is a pharmacologically distinct approach to GABAergic facilitation.

**Effect size:** Moderate. Randomized controlled trials comparing etifoxine (150 to 200 mg/day) to lorazepam in adjustment disorders with anxiety demonstrated comparable anxiolytic efficacy, with superior cognitive function and psychomotor performance in the etifoxine group [38, 39]. Effect sizes in generalized anxiety disorder are comparable to buspirone.

**Promising potential:** Low to moderate. The compound is established in France and selected markets but has not expanded to major English-speaking markets. No current registration programs for the United States or the United Kingdom are known.

**Side-effect profile:** Favorable. Mild gastrointestinal discomfort and transient sedation (less than with benzodiazepines) are the principal adverse events. No dependence, tolerance, or withdrawal syndrome at registered doses. Rare hepatotoxicity has been reported in post-marketing surveillance in France, leading to enhanced monitoring recommendations.

**Overall validation:** Moderate. Established clinical use in approximately 40 countries with supporting randomized controlled trial data. The clinical evidence base is larger than that of afobazole but smaller than that of buspirone or pregabalin. The hepatotoxicity signal, though rare, is a concern.

**Comparison to afobazole:** Both etifoxine and afobazole are non-benzodiazepine anxiolytics developed outside the Anglo-American regulatory tradition, with clinical evidence bases predominantly in their originating markets. Both lack dependence liability. Etifoxine acts through GABAergic mechanisms (indirect, via neuros-

teroids) while afobazole acts through Sigma1R. Afobazole has broader neuroprotective pharmacology (stroke, Parkinson's, cardioprotection); etifoxine has neurosteroidogenic neuroprotective properties through a distinct mechanism. Neither compound has FDA or EMA approval.

## 12.5 Selank

**Mechanism:** Synthetic heptapeptide analog of tuftsin (a naturally occurring tetrapeptide immunomodulator). Proposed mechanisms include modulation of endogenous BDNF and enkephalin expression, indirect GABAergic facilitation, and immunomodulatory effects. The molecular pharmacology is incompletely characterized [40].

**Development trajectory:** Developed at the Institute of Molecular Genetics of the Russian Academy of Sciences. Registered in Russia as a nasal spray anxiolytic. The compound has not been submitted for regulatory approval outside Russia.

**Novelty:** High. The peptidergic anxiolytic mechanism is distinct from all other comparators and represents a fundamentally different pharmacological approach. The tuftsin-derived structure and immunomodulatory-anxiolytic bridge are unique in the anxiolytic pharmacopeia.

**Effect size:** Modest. Clinical studies (predominantly Russian-language) report anxiolytic effect comparable to or slightly less than benzodiazepines. The compound also demonstrates nootropic (cognitive-enhancing) properties at anxiolytic doses. The evidence base is small by international standards.

**Promising potential:** Low. No international development or registration is anticipated. The compound remains a niche research tool and Russian-market pharmaceutical.

**Side-effect profile:** Favorable. Minimal adverse events reported. No sedation, dependence, or withdrawal syndrome. The peptide nature limits oral bioavailability (hence the nasal spray formulation) and introduces potential immunogenic considerations.

**Overall validation:** Low. Small clinical evidence base, predominantly Russian-language, with no international regulatory validation. The compound is principally of interest as a research tool for peptidergic anxiolysis and as a comparison case for Russian-developed anxiolytics.

**Comparison to afobazole:** Both compounds are Russian-developed anxiolytics with limited international clinical evidence. Afobazole has a substantially larger clinical evidence base, a better-characterized mechanism, and oral bioavailability that simplifies clinical and research use. Selank has the advantage of a novel peptidergic mechanism and dual anxiolytic-nootropic properties. Neither compound has international regulatory approval.

## 12.6 Comparator summary

The five comparator compounds span the range of non-benzodiazepine anxiolytic pharmacology. **Buspirone** is the international standard for non-addicting anxiolytics, with the largest clinical evidence base and broadest regulatory approval, but with delayed onset and a single-target (5-HT<sub>1A</sub>) mechanism. **Hydroxyzine** provides rapid-onset anxiolysis at the cost of sedation and anticholinergic burden. **Pregabalin** offers the largest effect sizes through the alpha-2-delta calcium channel mechanism but carries dependence liability and controlled-substance scheduling. **Etifoxine** provides a GABAergic mechanism through neurosteroidogenesis without dependence, but has limited international availability and a rare hepatotoxicity signal. **Selank** represents a novel peptidergic approach with minimal adverse events but negligible international validation.

Afobazole occupies a distinctive position in this landscape: it is the only compound in the panel whose principal mechanism is Sigma1R chaperone agonism, the only compound with demonstrated neuroprotective activity across ischemic stroke and Parkinson's disease models, the only compound with demonstrated cardioprotective activity, and one of only two (with etifoxine) that lacks dependence liability while maintaining GABAergic facilitation (indirect, through Sigma1R chaperoning of GABA-A receptor subunits). Its principal limitation is the absence of international regulatory approval and the predominantly Russian-language clinical evidence base, which limits independent verification of efficacy and constrains its positioning in international treatment guidelines.

The practical implication for investigators is that afobazole is best positioned as a research compound for the study of Sigma1R-mediated anxiolysis, neuroprotection, and cardioprotection, and as a clinical tool within the Russian Federation for patients who require non-sedating anxiolysis without dependence risk. For interna-

tional clinical applications, buspirone remains the reference non-addicting anxiolytic and pregabalin the reference high-efficacy alternative, pending future international regulatory evaluation of afobazole.

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