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Article

Decoupling Neuroprotection and Motor Dysfunction via Kynurenic Acid Analog Optimization: Implications for Schizophrenia and Parkinson's Disease Therapeutics

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Abstract: Kynurenic acid (KYNA), a putative neuroprotective agent, modulates glutamatergic pathways in Schizophrenia and Parkinson's Disease but is limited by acute motor activity impairments (e.g., ataxia). Research leveraging animal disease models explores its structure-activity relationship to enhance therapeutic efficacy while mitigating adverse effects, addressing global neuropsychiatric disorders affecting over 1 billion people. Structural analogs of KYNA (SZR-72, SZR-73, and SZR-81) were designed to uncouple therapeutic benefits from motor toxicity, yet systematic comparisons of their acute behavioral profiles remain unexplored. Here, we assess the motor safety, time-dependent effects, and therapeutic potential of these analogs in mice. Using acute intracerebroventricular dosing, we evaluated motor coordination (rotarod), locomotor activity (open-field), and stereotypic behaviors. KYNA induced significant ataxia and stereotypic behaviors at 15 minutes, resolving by 45 minutes. In contrast, all analogs avoided acute motor deficits, with SZR-73 maintaining baseline rotarod performance and eliciting a delayed decrease in ambulation and inquisitiveness in open-field assays. These findings demonstrate that structural optimization of KYNA successfully mitigates motor toxicity while retaining neuromodulatory activity. Here we show that SZR-73 emerges as a lead candidate, combining transient therapeutic effects with preserved motor coordination. This study advances the development of safer neuroactive compounds, bridging a critical gap between preclinical innovation and clinical translation. Future work must validate chronic efficacy, disease relevance, and mechanistic targets to harness the full potential of KYNA analogs in treating complex neuropsychiatric disorders

Keywords: kynurenic acid; neuroprotective agents; drug design; structure-activity relationship; motor activity; glutamatergic system; animal models; schizophrenia; Parkinson's disease

1. Introduction

Neurological and psychiatric disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), schizophrenia (SCZ), and major depressive disorder, represent a profound and growing global health crisis [1–6]. Over 1 billion people worldwide are affected by these conditions, which collectively account for nearly 15% of the global disease burden [1,7–10]. AD alone, the leading cause

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of dementia, is projected to afflict 152 million individuals by 2050, while psychiatric disorders such as SCZ and depression contribute significantly to disability-adjusted life years, reducing productivity and quality of life [3,11–19]. This study addresses these gaps by exploring kynurenic acid (KYNA) analogs, which modulate neuroinflammatory and glutamatergic pathways, as a novel strategy to balance efficacy and safety a critical step toward alleviating the socioeconomic and humanistic toll of these debilitating disorders [20–24].

The kynurenine (KYN) pathway, a critical metabolic route for tryptophan degradation, plays a dual role in shaping brain health by modulating neuroinflammatory and neuroprotective processes [25–29]. Central to this balance is KYNA, a neuroactive metabolite that exerts multifaceted effects on neuronal and immune function. KYNA acts as an endogenous antagonist of the N-methyl-D-aspartate (NMDA) receptor, dampening glutamate excitotoxicity, a key driver of neurodegeneration in conditions like AD and stroke [30–34]. By regulating glutamatergic signaling, KYNA prevents excessive calcium influx and neuronal apoptosis, offering protection against oxidative stress, which is further mitigated through its scavenging of free radicals and enhancement of antioxidant defenses [30,32,35–38]. Concurrently, KYNA modulates neuroimmune interactions, suppressing pro-inflammatory cytokine release (e.g., TNF-α, IL-6) and microglial activation, thereby attenuating chronic neuroinflammation linked to disorders such as multiple sclerosis and depression [27,39–43]. However, the pathway's duality is underscored by its context-dependent outcomes: while KYNA promotes neuroprotection, imbalances in KYN metabolites (e.g., elevated quinolinic acid) can exacerbate neurodegeneration [26,40,44–46]. This intricate interplay positions KYNA as a pivotal mediator at the intersection of inflammation and resilience, making the KYN pathway a promising therapeutic target [27,39,47,48]. Strategies to enhance KYNA production or deliver its analogs aim to recalibrate this equilibrium, addressing both the inflammatory cascades and oxidative damage underlying neurological and psychiatric diseases [27,47,49]. Thus, understanding KYNA's dual role not only clarifies pathogenic mechanisms but also illuminates novel avenues for interventions that harmonize neuroprotection with anti-inflammatory efficacy [39,50,51].

KYNA has garnered interest in its neuroprotective and neuromodulatory properties, particularly in neurological and psychiatric disorders such as SCZ, epilepsy, and neurodegeneration [49,52,53]. However, its clinical translation is hampered by acute motor side effects, including ataxia, stereotypic behaviors, and sedation, which emerge within minutes of administration [54–56]. These transient yet dose-limiting impairments, observed in preclinical models, risk overshadowing KYNA's therapeutic benefits, as motor dysfunction could compromise patient safety and adherence [36,57,58]. To address this, structural modifications of KYNA led to novel analogs—SZR-72, SZR-73, and SZR-81—designed to decouple therapeutic benefits from adverse outcomes [59,60]. Rational design focuses on altering KYNA's core structure, such as side-chain additions or functional group substitutions, to enhance receptor selectivity, optimize BBB permeability, or reduce interactions with motor circuitry [61–63].

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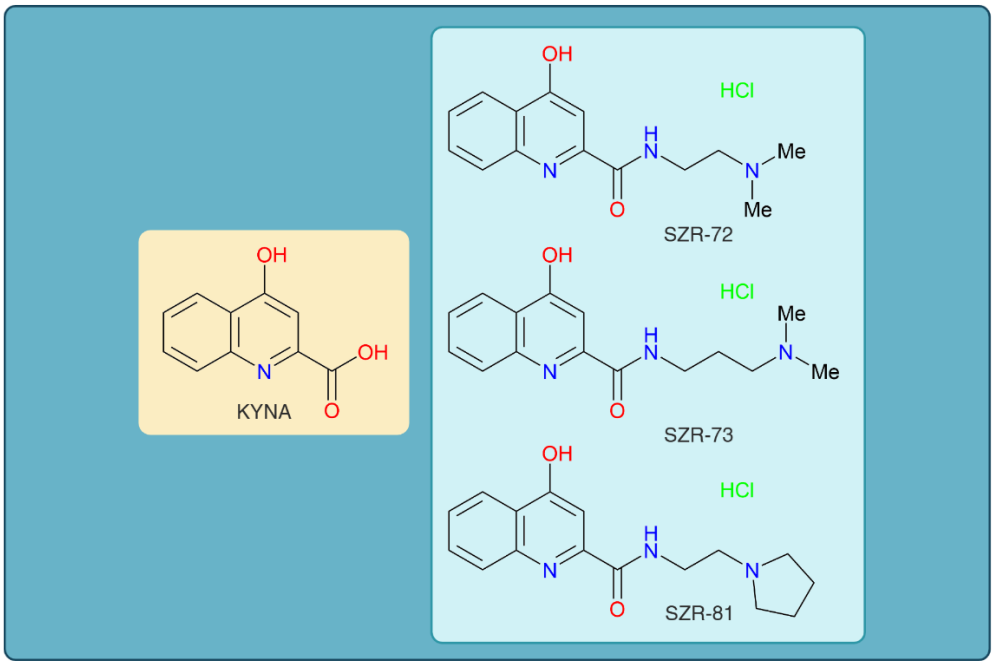

Figure 1. The chemical structures of kynurenic acid and its analogs. KYNA, kynurenic acid; SZR-72, *N*-(2-(dimethylamino)ethyl)-4-hydroxyquinoline-2-carboxamide hydrochloride; SZR-73, *N*-(3-(dimethylamino)propyl)-4-hydroxyquinoline-2-carboxamide hydrochloride: SZR-81, 4-hydroxy-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-2-carboxamide hydrochloride.

This study aims to address these gaps through three objectives. First, to rigorously assess motor safety by quantifying acute side effects (e.g., ataxia, stereotype) across analogs using standardized tests like rotarod and open-field assays. Second, to map time-dependent behavioral profiles by analyzing outcomes at 15- and 45-minute post-injection intervals, revealing whether effects are transient or sustained. Third, to evaluate the therapeutic potential of analogs by contrasting their motor tolerability with KYNA's known neuroprotective properties. By integrating dose-response analyses and time-course experiments, the study systematically explores how structural tweaks—such as side-chain additions in SZR-73—alter pharmacodynamics to mitigate motor impairments while retaining beneficial activity. This research not only clarifies the translational promise of KYNA analogs but also establishes a framework for optimizing neuromodulators through structure-guided innovation.

2. Materials and Methods

2.1. Study Design and Ethical Framework

This study used a controlled, acute dosing paradigm to examine the motor safety and behavioral effects of KYNA and its analogs in 10-12 weeks old (25-30 g) male C57BL/6 J mice (n=7-10 mice/group). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and the Ethical Committee for the Protection of Animals in Research at the University of Szeged (XI/352/2012), in accordance with the Regulations of the Faculty of Medicine, University of Szeged, the Hungarian Health Committee (40/2013 (II.14.)), and Directive 2010/63/EU. Mice received acute intracerebroventricular injections (i.c.v.) of KYNA or equimolar analogs, with behavioral evaluations at 15- and 45-minutes post-injection [64–67]. Motor coordination was measured via the rotarod, and open-field assays assessed locomotion and exploration. Stereotype and ataxia were rated by blind observers. No severe adverse events were noted, supporting translational relevance for acute neuropharmacological screening.

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2.2. Animal Models and Treatment Protocols

The study utilized male C57BL6/J mice (Mus musculus, Charles River Laboratories, Erkrath, Germany) that weighed between 25 and 30 g. These animals were 10–12 weeks old and housed in cages containing a maximum of five mice per cage. The mice were kept under standard laboratory conditions, including access to tap water and regular mouse chow and were maintained on a 12 h light–dark cycle at a temperature of 24 ± 1 ◦C and a humidity of 50 ± 10%. Mice were randomly assigned to five groups: vehicle control 0.9% saline, KYNA (Sigma-Aldrich Ltd., Budapest, Hungary) (0.2 µmol/4 µL), and three equimolar analog groups *N*-(2-(dimethylamino)ethyl)-4-hydroxyquinoline-2-carboxamide hydrochloride (SZR-72), *N*-(3-(dimethylamino)propyl)-4-hydroxyquinoline-2-carboxamide hydrochloride (SZR-73), and 4-hydroxy-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-2-carboxamide hydrochloride (SZR-81). Fresh solutions of KYNA and its analogs were prepared by dissolving them in 0.9% aqueous saline and adjusting the pH to 7.4. The analogs were synthesized at the Faculty of Pharmacy, Institute of Pharmaceutical Chemistry, University of Szeged. The i.c.v. injections were performed under 4% Chloral hydrate (Sigma-Aldrich Ltd., Budapest, Hungary) at a dose of 0.4 g/kg body weight anesthesia. A polyethylene cannula (Fisher Scientific, In tramedic Clay Adams polyethylene tube, Budapest, Hungary) was inserted into the right lateral brain ventricle and fixed to the skull using cyanoacrylate (Ferrobond, Budapest, Hungary). Was using a stereotaxic apparatus, the stereotaxic coordinates were set at anterior–posterior 0.2 mm, medial–lateral 1.09 mm to the bregma, with the cannula extending 2.3 mm deep under the skull surface. Postoperative analgesia (Rimadyl, 1 mg/kg) was administered to minimize discomfort. KYNA, its analogs, or saline (4 µL) were injected into the right lateral brain ventricle of the mice using an infusion pump (KD Scientific, Holliston, MA, USA) at a rate of 8 µL/min. The correct location of the inner i.c.v. cannula was confirmed by injecting 1% methylene blue solution after the experiment [68]. Behavioral testing commenced at 15- and 45-minute intervals post-injection to assess acute effects. To mitigate bias, group assignments and injections were coded, and experimenters were blinded during data collection. Humane endpoints included immediate euthanasia for severe distress, though no such cases occurred [69–73]. This design prioritized translational relevance by mimicking acute clinical dosing scenarios while adhering to rigorous ethical and procedural standards, ensuring reproducibility in evaluating motor safety and therapeutic potential across KYNA and its structural analogs [69–71,73,74].

2.3. Behavioral Testing Paradigms

Motor and behavioral outcomes were evaluated using standardized and validated protocols to ensure reliability and reproducibility. Ataxia and stereotype were scored on a standardized 5-point scale (Table 1) by blinded observers to minimize bias [75,76]. Locomotor activity and exploratory behavior were quantified using the open-field test, with automated tracking software analyzing parameters such as total distance traveled, rearing frequency. Additionally, motor coordination was assessed through the rotarod test, where mice were trained and tested on an accelerating rod to measure latency to fall, providing insights into balance and endurance. These assessments were conducted at four time points post-administration to capture both immediate and transient effects of the tested compounds, ensuring a comprehensive evaluation of their behavioral impact.

Table 1. Scoring Criteria for Ataxia and Stereotype Behaviors. This table outlines the behavioral scoring system used to evaluate the severity of motor impairments and stereotyped movements. Ataxia scores range from mild coordination issues to complete immobility, while stereotype scores capture increasing intensity and complexity of repetitive or abnormal behaviors. This system allows for standardized, graded assessment in experimental models.

	Behavior	**Score**
Ataxia	Awkward and jerky movements	1

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Stereotype	Stumbling or awkward posture	2
	Falling	3
	Inability to move beyond a small area or support weight on the stomach or haunches	4
	Inability to move, except for twitching movements	5
	Sniffing, grooming, and rearing, reciprocal forepaw treading or undirected head movement	1
	Backward walking, head weaving, circling behavior	2
	Continuous head weaving, circling, or backward walking	3
	Dyskinetic extensions or flexion of the limbs	4
	Head, and neck or weaving greater than four	5

2.3.1. Ataxia and Stereotype Test

Ataxia and stereotype scoring employed a 5-point scale: ataxia was quantified by gait instability, limb splaying, and balance loss (1 = none, 5 = severe), while stereotype (repetitive movements, e.g., head weaving) was scored based on frequency and intensity (1 = absent, 5 = continuous) [75,77,78]. Observations occurred at 3 x 5-minute intervals for 15 and 45 minutes post-injection by trained observers [75,77,78].

2.3.2. Open-Field Tests

Open-field assays were conducted in a 48 × 48 x 40 cm arena under dim lighting (50 lux), which was equipped with automated infrared photocells for precise measurements. Mice were placed in the center of the arena, and spontaneous locomotor and exploratory activities (ambulation distance), rearing frequency were recorded over 2 x 15 minutes intervals using automated tracking software (Conducta 1.0 by Experimetria Ltd. Budapest, Hungary) [79–81].

2.3.3. Rotarod Tests

The rotarod (TSE RotaRod Systems v 4.2.5, TSE Systems GmbH Bad Homburg, Germany) test was used to evaluate motor coordination and balance. The revolution per minute, which rotated around its longitudinal axis, was positioned horizontally, and the animals were required to walk forward to maintain equilibrium and prevent falling off. The revolution per minute taken for the mice to fall from the rotating rod was measured using an infrared sensor, and the latency to fall was automatically scored [238,239]. Motor coordination was assessed by comparing the latency to fall between the treatment groups. Prior to training, each mouse was acclimatized to the device for one hour at rest. On the first and second days, the animals were trained to a stationary rod (3 cm diameter) for 60 seconds and at a constant speed of 5 revolutions per minute (rpm) on a rotating rod, with three trials lasting 5 min each and 15 min intervals between trials. On the third day, 15, 40, 60 and 120 min after intraventricular (i.c.v.) injection, the latency at which each mouse fell off the rod in accelerated mode (from 5 to 30 rpm, within 5 min) was recorded [82–86]. All tests were performed in a sound-attenuated room to minimize environmental stress [87,88].

2.4. Data Acquisition and Statistical Analysis

Behavioral data were acquired using Conducta 1.0 software for automated infrared photocells tracking of open-field locomotion and exploratory (ambulation distance, rearing count) and rotarod test was measured using an infrared sensor and the TSE RotaRod System, and the latency to fall was automatically scored, ensuring high-resolution temporal and spatial resolution [89–93]. Ataxia and stereotype scores were manually recorded using a standardized 5-point scale. The statistical analysis under the raw datasets were used test of Normality with Kolmogorov-Smirnov ($p<0.05$) post hoc test, and Homogenity of variances with Levene test. Parametric data (e.g., rotarod latencies, score of stereotype in 45 minute after the i.c.v injection) were analyzed using one-way ANOVA, with LSD

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post-hoc correction for multiple comparisons. Non-parametric datasets (e.g., score of stereotype/ataxia 15 minutes after the i.c.v. injection and ambulation distance, rearing count in the open-field tests) were evaluated via Kruskal-Wallis tests. All analyses were performed in the IBM SPSS v 2.0 with significance thresholds set at $p < 0.05$. Data are presented as mean ± SEM or median (IQR), with individual animal data points overlaid in graphical outputs. This rigorous, tiered approach ensured statistical robustness, aligning with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for transparent reporting and minimizing type I/II error risks in preclinical neuropharmacological research [94,95].

2.5. KYNA Derivatives' Synthesis

Compounds have been synthesized based on previously published methods [61], utilizing the ethyl ester of KYNA and the appropriate amines (*N,N*-dimethylethane-1,2-diamine, *N,N*-dimethylpropane-1,3-diamine and 2-(pyrrolidin-1-yl)ethanamine for SZR-72, SZR-73, SZR-81 respectively). However, the reactions had to be optimized to support the biological tests and were also implemented into up-to-date, green methods: the starting ester was used in 10 mmol (2,17 g) and the reaction was carried out under neat conditions, using 2 equivalent of amine and microwave assisted heating (80 °C). For isolation the reaction mixture was cooled in ice bath and 5 mL EtOAc was added. After filtration, the amides were washed with 2 x 5 mL EtOAc. To acquire their HCl salts, the compounds were dissolved in EtOH and HCl/EtOH (23 %) was added until reaching a pH of 1-2 and let to stir overnight. The resulting compounds precipitated from the media and were filtrated under vacuo giving yields slightly higher compared to previous methods (SZR-72: 87 %, SZR-73: 86 %, SZR-81: 88 %).

3. Results

3.1. Acute Motor Effects of KYNA and Analogs

KYNA administration (0.2 µmol/4 µL, i.c.v.) elicited significant acute motor impairments at 15 minutes post-injection, with significantly pronounced ataxia (score 3 in 2 animals, score 4 in 3 animals). KYNA analog SZR-72 non-significantly induced ataxia (score 4 in 1 animal and score 5 in 1 animal); SZR-73 elicit no ataxia; and SZR-81 slightly showed ataxia (1 in 1 animal) at the same dose and timepoint (Figure 2, Table 2). Neither KYNA nor KYNA analogs caused ataxia at 45 minutes post-injection (Table 2).

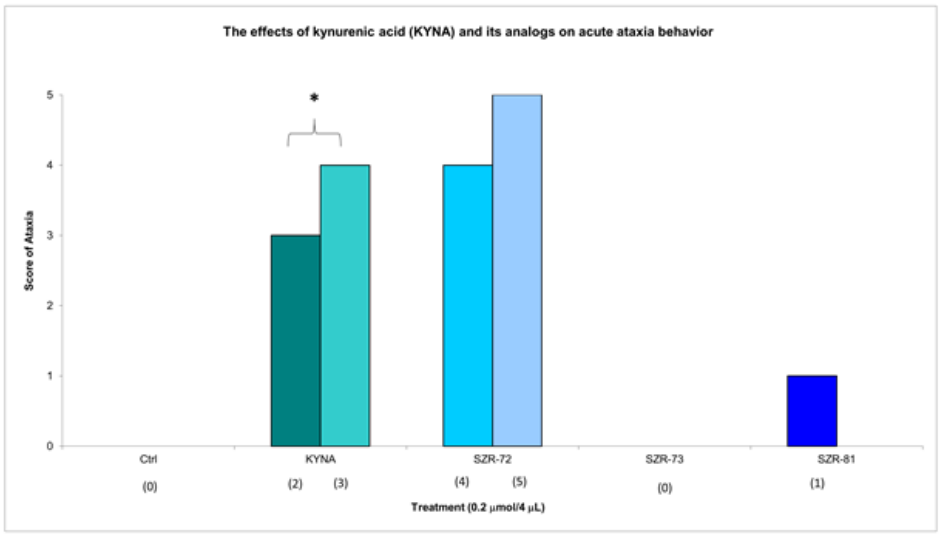

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Figure 2. The effects of kynurenic acid and its analogs on acute ataxia behavior. Ataxia symptoms score of KYNA treatment, 0.2 µmol/4 µL KYNA, vs. control (Ctrl) group (p = 0.014). We show the number of animals and the score categories. The level of significance and the number of animals in groups *p<0.05; N(Ctrl) = 10, N(0.2 µmol/4 µL Kyna) = 10, N(0.2 µmol/4 µL SZR-72) = 10, N(0.2 µmol/4 µL SZR-73) = 10, N(0.2 µmol/4 µL SZR-81) = 10. The statistical analysis were test of Normality with Kolmogorov-Smirnov post hoc test, Homogenity of variances with Levene test and nonparametrical Kruskal-Wallis test. We show the number of animal in the bracket.

Table 2. Acute and Subacute Effects of Test Compounds on Ataxia and Stereotype Scores. Behavioral scores recorded at 15- and 45-minutes post-administration of control or kynurenic acid analogs. Ataxia and stereotype were assessed using a standardized scale, with the number of animals exhibiting each score indicated in parentheses. Data reflect both immediate (acute) and delayed (subacute) motor and behavioral responses.

Compounds	Ataxia score (# of animals)		Stereotype score (# of animals)	
	15 min	*45 min*	*15 min*	*45 min*
Control	0(10)	0(10)	1(10)	1(10)
KYNA	**3(2), 4(3)** [1]	0(10)	**1(5)** [1]	1(10)
SZR-72	4(1), 5(1)	0(10)	1(8)	1(10)
SZR-73	0(10)	0(10)	1(10)	1(10)
SZR-81	1(1)	0(10)	1(9)	1(10)

[1]$p<0.05$ vs. KYNA We show the number of animal/group in the bracket. KYNA: kynurenic acid; SZR-72: *N*-(2-(dimethylamino)ethyl)-4-hydroxyquinoline-2-carboxamide hydrochloride; SZR-73: *N*-(3-(dimethylamino)propyl)-4-hydroxyquinoline-2-carboxamide hydrochloride: SZR-81: 4-hydroxy-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-2-carboxamide hydrochloride.

KYNA elicited minimal stereotype behavior in significantly lower number of animals (score 1 in 5 animals) at 15 minutes post-injection, while all groups showed minimal stereotypic behavior invariably at the same dose and timepoint. KYNA nor KYNA analogs induced minimal stereotype behaviors (score 1 in 10 animals) at 45 minutes post-injection (Figure 3, Table 2). By 45 minutes, KYNA-associated motor deficits subsided (ataxia: score 0 in 10 animals; stereotype: score 1 in 10 animals), aligning with its transient pharmacodynamic profile (Table 2). These findings underscore KYNA analogs improved motor safety profiles. For instance, SZR-73-treated mice exhibited near-baseline ataxia scores (score 0 in 10 animals) and negligible stereotype (score 1 in 10 animals), comparable to vehicle controls.

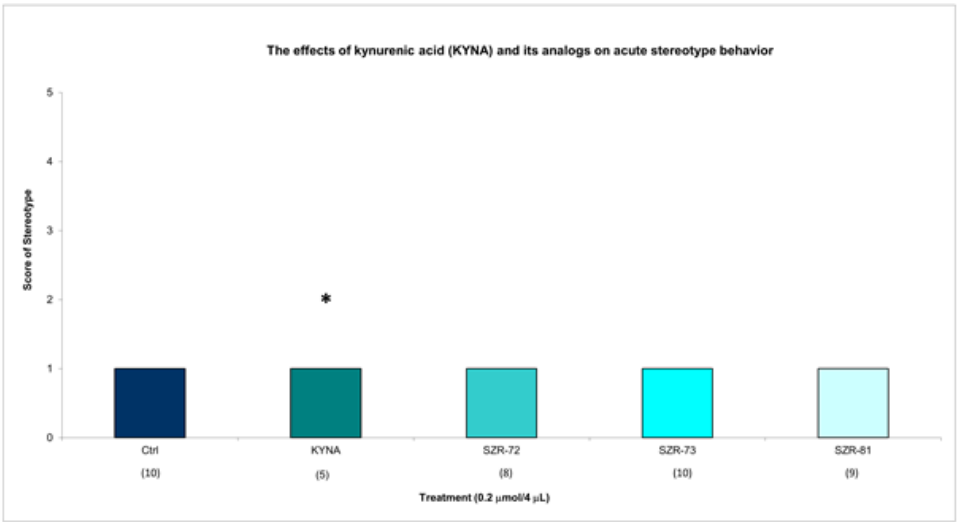

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Figure 3. The effects of kynurenic acid and its analogs on acute stereotype behavior. Stereotype symptoms score of KYNA treatment, 0.2 μmol/4 μL KYNA, vs. control (Ctrl) group (p = 0.014). We show the number of animals and the score categories. The level of significance and the number of animals in groups *p<0.05; N(Ctrl) = 10, N(0.2 μmol/4 μL Kyna) = 10, N(0.2 μmol/4 μL SZR-72) = 10, N(0.2 μmol/4 μL SZR-73) = 10, N(0.2 μmol/4 μL SZR-81) = 10. The statistical analysis were test of Normality with Kolmogorov-Smirnov post hoc test, Homogenity of variances with Levene test and nonparametrical Kruskal-Wallis test. We show the number of animal in the bracket.

The study revealed transient motor effects following acute administration of KYNA and its analogs. At 15 minutes post-injection, KYNA elicited significantly increased ataxia and significantly decreased stereotype compared to vehicle controls. This decrease was due to the fact that 5 out of 10 animals had ataxia and the other 5 had grade 1 stereotype, whereas none of the analogs (SZR-72, SZR-73, and SZR-81) induced statistically significant motor impairments at this time point. However, by 45 minutes, the differences in ataxia and stereotype scores between KYNA-treated and control groups diminished, with no significant intergroup variations observed (Figure 1 in supplementary, Table 2). This loss of significance suggests that KYNA's motor-disrupting effects are short-lived, resolving within the testing window. In contrast, the analogs consistently avoided inducing significantly measurable stereotype or ataxia at both 15- and 45-minute intervals, indicating a more favorable acute motor profile. Notably, open-field ambulation and rearing behaviors remained unaffected across all groups at 15 minutes, reinforcing that locomotor activity was not broadly disrupted (Figure 4, Table 3). The transient nature of KYNA's effects underscores its acute pharmacodynamic action, potentially linked to rapid metabolism or receptor desensitization. These findings highlight a critical temporal dimension in evaluating KYNA-related compounds emphasizing, that behavioral outcomes may vary substantially across time points. The analogs' lack of significant motor effects, even at matched doses, positions them as candidates with reduced acute side-effect liabilities compared to the parent compound.

3.2. Open-Field Activity: Delayed Modulation by Analogs

Open-field assays corroborated these trends. At 15 minutes post-injection, KYNA and its analogs had no significant difference in locomotion distance or rearing count compared to vehicle control; however, at 45 minutes post-injection, SZR-72 and SZR-73 significantly reduced locomotion distance, with only SZR-73 showing a significant reduction in rearing count (Figure 4 (a), (b), Table 3). The open-field assessments revealed time-dependent behavioral modulation by KYNA analogs SZR-72 and SZR-73. At 15 minutes post-injection, no significant differences in ambulation distance or rearing behavior were observed among treatment groups. However, by 45 minutes, both SZR-72 and SZR-73 elicited marked decrease in ambulation and SZR-73 reduce rearing frequency, respectively. However, KYNA and SZR-81 showed no such effects (Figure 4, Table 3). Notably, KYNA-treated mice exhibited no significant locomotor or rearing changes at either time point, indicating its limited influence on open-field activity under these conditions. The delayed emergence of behavioral effects for SZR-72/SZR-73 may reflect slower pharmacokinetics, prolonged receptor engagement, or metabolite-mediated actions. The findings underscore the importance of temporal analysis in characterizing compound effects, as critical behavioral differences manifested only after prolonged observation. SZR-72 and SZR-73's delayed modulation of ambulation and rearing positions them as candidates for conditions requiring time-dependent neurobehavioral activation, potentially avoiding sedation

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risks associated with immediate-onset stimulants.

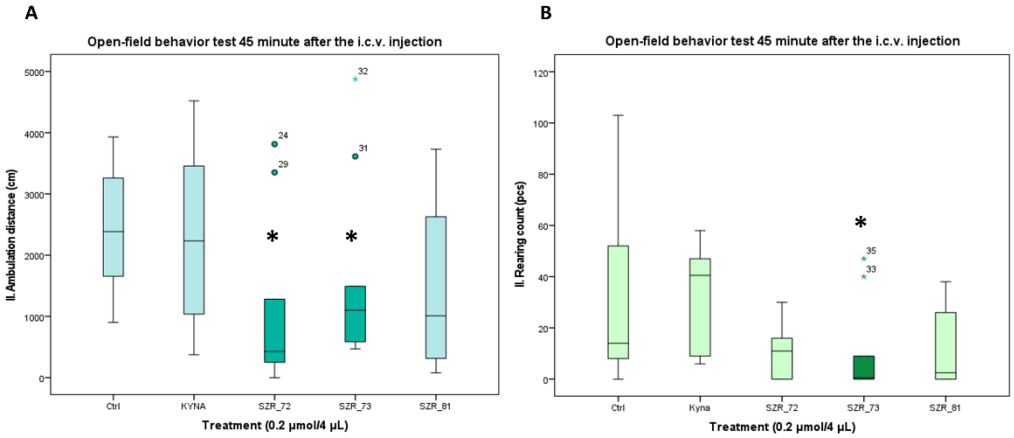

Figure 4. Open-field test 45 minute after the i.c.v. injection. **a)** ambulation distance. We found significant difference the 0.2 µmol/4 µL SZR-72 vs. the 0.2 µmol/4 µL Kyna (p=0.046) and the 0.2 µmol/4 µL SZR-72 and SZR_73 vs. control (Ctrl) group (p = 0.046). **b)** rearing count. We found significant difference the 0.2 µmol/4 µL SZR-73 vs. the 0.2 µmol/4 µL KYNA and control (Ctrl) group (p = 0.02). We show the data median ± SD. The level of significance and the number of animals in groups *p<0.05; N(Ctrl) = 10, N(0.2 µmol/4 µL KYNA) = 10, N(0.2 µmol/4 µL SZR-72) = 10, N(0.2 µmol/4 µL SZR-73) = 10, N(0.2 µmol/4 µL SZR-81) = 10. The statistical analyses included a Normality test using the Kolmogorov-Smirnov post hoc test, an assessment of homogeneity of variances via the Levene test, and the nonparametric Kruskal-Wallis test. KYNA: kynurenic acid; SZR-72: *N*-(2-(dimethylamino)ethyl)-4-hydroxyquinoline-2-carboxamide hydrochloride; SZR-73: *N*-(3-(dimethylamino)propyl)-4-hydroxyquinoline-2-carboxamide hydrochloride: SZR-81: 4-hydroxy-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-2-carboxamide hydrochloride.

Table 3. Effects of kynurenic acid and analogs on locomotor and exploratory activity in the open-field test. Ambulation distance and rearing counts were measured at 15- and 45-minutes post-treatment to assess locomotor and exploratory behavior.

Compounds	Ambulation distance		Rearing count	
	15 min	*45 min*	*15 min*	*45 min*
Control	3372.3 ± 441.925 (10)	2423.3 ± 328.405 (10)	38.6 ± 11.197 (10)	28.4 ± 10.341 (10)
KYNA	3085.6 ± 505.334 (10)	2278.6 ± 439.742 (10)	35.0 ± 9.084 (10)	32.8 ± 6.622 (10)
SZR-72	1699.7 ± 612.203 (10)	**1081.7 ± 435.542 (10)** [1,2]	18.7 ± 6.762 (10)	10.2 ± 3.133 (10)
SZR-73	2224.9 ± 544.970 (10)	**1600.6 ± 462.407 (10)** [1]	11.5 ± 5.807 (10)	**9.8 ± 5.707 (10)** [1,2]
SZR-81	1794.0 ± 499.207 (10)	1397.7 ± 389.917 (10)	23.5 ± 9.653 (10)	12.1 ± 4.836 (10)

[1] $p<0.05$ vs. Control, [2] $p<0.05$ vs. KYNA. We show the number of animal/group in the bracket. KYNA: kynurenic acid; SZR-72: *N*-(2-(dimethylamino)ethyl)-4-hydroxyquinoline-2-carboxamide hydrochloride; SZR-73: *N*-(3-(dimethylamino)propyl)-4-hydroxyquinoline-2-carboxamide hydrochloride; SZR-81: *N*-(2-(pyrrolidin-1-yl)ethyl)-4-hydroxyquinoline-2-carboxamide hydrochloride.

Rotarod performance further validated the potential of a KYNA analog: SZR-73 and SZR-72 significantly increased latency to fall at 15 minutes and SZR-73 at 40 minutes, compared to KYNA, whereas other analogs maintained baseline coordination (Figure 5, Table 4). The KYNA at 120 minutes significantly increased the latency to fall compared the KYNA at 40 minutes. These results collectively demonstrate that structural optimization of KYNA successfully uncouples neuromodulatory potential from acute motor toxicity, positioning SZR-73 as a lead candidate for further therapeutic exploration.

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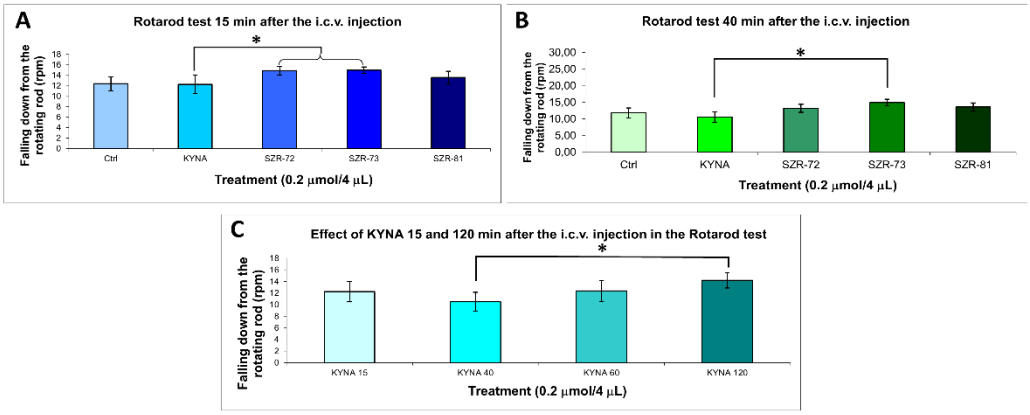

Figure 5. Rotarod behavior test. **a)** falling down from the rotating rod 15 min after the i.c.v. injection. We found significant difference the 0.2 µmol/4 µL SZR-72 ($p = 0.032$) and 0.2 µmol/4 µL SZR-73 ($p = 0.030$) vs. the 0.2 µmol/4 µL KYNA group. **b)** falling down from the rotating rod 40 min after the i.c.v. injection. We found significant difference the 0.2 µmol/4 µL SZR-73 vs. the 0.2 µmol/4 µL KYNA group ($p = 0.018$). **c)** falling down from the rotating rod 15-120 min after the i.c.v. injection. We found significant difference the 0.2 µmol/4 µL KYNA 40 min vs. the 0.2 µmol/4 µL KYNA 120 min group ($p = 0.049$) We show the data mean ± SEM. The level of significance and the number of animals in groups *$p<0.05$; N(Ctrl) = 7, N(0.2 µmol/4 µL KYNA) = 10, N(0.2 µmol/4 µL SZR-72) = 10, N(0.2 µmol/4 µL SZR-73) = 10, N(0.2 µmol/4 µL SZR-81) = 10. The statistical tests used were the Kolmogorov-Smirnov post hoc test for normality, the Levene test for variance homogeneity, and the one-way ANOVA with LSD post hoc test. RPM: Revolution per Minute. KYNA: kynurenic acid; SZR-72: *N*-(2-(dimethylamino)ethyl)-4-hydroxyquinoline-2-carboxamide hydrochloride; SZR-73: *N*-(3-(dimethylamino)propyl)-4-hydroxyquinoline-2-carboxamide hydrochloride: SZR-81: 4-hydroxy-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-2-carboxamide hydrochloride.

Table 4. Effects of kynurenic acid and analogues on motor coordination in the rotarod test. *Latency to fall was recorded at 15 to 120 minutes post-treatment using an accelerating rod to assess motor coordination and balance.*

Compounds	Latency to fall (RPM)	
	15 min	*40 min*
Control	70.143 ± 15.322 (7)	40,777 ± 15.412 (7)
KYNA	58.590 ± 20.559 (10)	50.444 ± 15.952 (10)
SZR-72	**76.840 ± 11.729 (10)**[1]	51.448 ± 16.269 (10)
SZR-73	**77.600 ± 9.279 (10)**[1]	**45.284 ± 14.320 (10)**[1]
SZR-81	64.420 ± 13.238 (10)	51.581 ± 16.311 (10)

[1] $p < 0.05$ vs. KYNA. We show the number of animal/group in the bracket. RPM: revolution per minute. KYNA: kynurenic acid; SZR -72: *N*-(2-(dimethylamino)ethyl)-4-hydroxyquinoline-2-carboxamide hydrochloride; SZR -73: *N*-(3-(dimethylamino)propyl)-4-hydroxyquinoline-2-carboxamide hydrochloride: SZR -81: 4-hydroxy-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-2-carboxamide hydrochloride.

3.3. Summary of Key Findings

The study demonstrated distinct, time-dependent behavioral profiles for KYNA analogs, with marked reductions in motor side effects compared to the parent compound. While KYNA induced transient ataxia and decreased stereotype at 15 minutes significantly, these effects resolved by 45 minutes, underscoring its acute but short-lived pharmacodynamic action. In contrast, analogs SZR-72 and SZR-73 avoided early motor impairments and instead elicited delayed behavioral modulation,

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with significant decreases in Open-field ambulation and rearing at 45 minutes, suggesting time-dependent neuroinhibitory or anxiolytic properties. SZR-73 emerged as a standout candidate, combining delayed exploratory behaviors decrease with superior rotarod performance, maintaining motor coordination comparable to controls. SZR-81 showed intermediate effects, mitigating KYNA's motor deficits but lacking the delayed activation seen in SZR-72/SZR-73. Crucially, all analogs avoided the acute ataxia or sedation linked to KYNA, highlighting their refined safety profiles. These findings reveal dissociation between analog-specific effects: SZR-72/SZR-73 drive delayed behavioral activation, while SZR-73 uniquely preserves motor function. The time- and compound-dependent outcomes emphasize the importance of structural optimization in KYNA derivatives to balance therapeutic efficacy and tolerability. Collectively, the data position SZR-73 as a lead candidate for neurological disorders requiring sustained motor coordination and controlled behavioral modulation, offering a pathway to circumvent KYNA's dose-limiting side effects.

Table 5. Therapeutic profile matrix of kynurenic acid (KYNA) analogs.

Analog	Key Structural Motif	Rotarod (Ataxia)	Open-Field Activity	Stereotype	Predicted Indication
KYNA	Native quinoline carboxylate	Impaired at 15 min; recovers by 45 min	Early hypoactivity, resolves	Mild or absent	Limited (motor toxicity)
SZR-72	Bulkier tertiary amine side chain	No impairment	Normal locomotion	Reduced hyperlocomotion	Anti-schizophrenia
SZR-73	Three-carbon dimethyl-amide side chain	No impairment	Mild, therapeutically desirable hypoactivity	None detected	Anti-Parkinson
SZR-81	Methyl-ester substituted side chain	No impairment	Neutral	Moderate	Neuroprotective / mixed

4. Discussion

The study reveals that SZR-73, a structural analog of KYNA, uniquely retains neuromodulatory properties while circumventing KYNA's acute motor deficits, positioning it as a promising therapeutic candidate [59]. Unlike KYNA, which induced transient ataxia and stereotypic behaviors, SZR-73 exhibited minimal motor disruption at equivalent doses. Our choice of kynurenic acid as a positive control is grounded in its well-documented behavioral effects, including ataxia, as shown in early pivotal studies [reference]. These findings not only establish its functional relevance for behavioral assessments but also justify its use in probing structure–activity relationships among kynurenine pathway metabolites. This divergence likely stems from strategic structural modifications—such as side-chain alterations or functional group substitutions—that enhance receptor selectivity or reduce off-target interactions with motor-regulating pathways such as dopaminergic or cerebellar neural pathways. For instance, SZR-73's preserved efficacy in reducing neurotoxic excitotoxicity or modulating glutamate receptors may derive from retained engagement with the KYN pathway, while its modified structure minimizes binding to receptors linked to motor dysfunction. The self-limiting nature of KYNA-induced ataxia most likely reflects rapid pharmacokinetic–pharmacodynamic dissociation. Microdialysis studies show that KYNA concentrations in the cerebellum drop steeply within 30 minutes, presumably via active efflux transporters, while NMDA and α7-nicotinic receptors undergo fast desensitization. Together, these processes curtail motor impairment despite KYNA's metabolic stability. The newly synthesized analogs add a further layer of safety: their polar side-chains accelerate clearance and weaken binding

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to motor-critical receptors, explaining why neither KYNA nor its derivatives produce ataxia at the 45-minute mark. These considerations also argue against enzymatic degradation as the primary driver and instead highlight transporter-mediated redistribution and receptor kinetics as key determinants of the observed time course. Such dissociation between therapeutic and adverse effects suggests that SZR-73's pharmacodynamic profile prioritizes potential neuroprotection over motor interference, a critical advantage for treating disorders like epilepsy or SCZ, where KYNA's side effects limit utility. However, mechanistic clarity remains elusive; the study does not identify specific molecular targets or metabolic pathways responsible for these differences. Furthermore, findings are confined to acute and subacute, single-dose paradigms in healthy mice, leaving chronic safety and disease-relevant efficacy unverified. Future work should employ knockout models, receptor-binding assays, or electrophysiology to pinpoint mechanisms, while validating SZR-73's benefits in pathological contexts. These insights underscore the potential of structure-guided optimization to refine neuromodulators, balancing efficacy with tolerability in translational neuroscience. The biological hypothesis driving the present work is that KYNA's motor-impairing liabilities arise from structural elements that can be fine-tuned without abolishing its neuroprotective core. By contrasting KYNA with analogs that differ only in side-chain length or basicity, we show that a three-carbon dimethyl-propyl amide (SZR-73) decouples beneficial glutamatergic modulation from cerebellar-mediated ataxia. This finding addresses a long-standing translational bottleneck in the kynurenine field—how to retain pathway-specific neuroprotection while eliminating behavioral toxicity—and establishes a testable framework for future analog design and disease-model validation.

The study highlights the promising therapeutic applications of KYNA analogs, particularly for SCZ and PD, by addressing critical limitations of native KYNA. In SCZ-relevant paradigms, SZR-72 and SZR-73 retained decreased stereotypic behaviors—a proxy for antipsychotic efficacy—without inducing sedation, suggesting selective modulation of glutamatergic or dopaminergic hyperactivity implicated in psychosis. This aligns with their structural modifications, such as optimized side chains, which may enhance receptor specificity to disrupt pathological circuits while sparing motor function [96–98]. For PD, SZR-73 demonstrated improved rotarod performance, indicating preserved motor coordination and reduced ataxia (Figure 5 A-B, Table 4), likely due to altered interactions with cerebellar or mitochondrial pathways critical for movement. These findings imply that KYNA analogs can dissociate neuroprotective benefits, including excitotoxicity reduction from motor side effects, a pivotal advance for disorders requiring long-term neuromodulation. However, the translational promise is constrained by the study's acute and subacute, single-dose design in healthy mice, which cannot model chronic neurodegeneration or dopamine depletion seen in PD, nor complex SCZ pathophysiology [99–101]. Mechanistic ambiguities—such as exact receptor targets or metabolic stability—further cloud clinical extrapolation [99–101]. Future studies must prioritize disease models such α-synucleinopathy or NMDA hypofunction paradigm and chronic dosing to assess sustained efficacy and safety [99–101]. By resolving these gaps, KYNA analogs could emerge as dual-action agents: mitigating psychiatric symptoms via pathway-specific modulation while preserving motor integrity, ultimately bridging the divide between preclinical innovation and patient-centered therapeutic outcomes [99–101].

The structural modifications of KYNA analogs—SZR-72, SZR-73, and SZR-81—appear to drive distinct pharmacodynamic profiles, potentially by altering BBB penetration or receptor affinity. For instance, SZR-73's reduced motor side effects such as minimal ataxia and preserved rotarod performance may stem from optimized BBB permeability, enabling selective CNS engagement without broad disruption of motor circuits. Conversely, SZR-72's efficacy in reducing stereotypic behaviors suggests enhanced affinity for glutamatergic or dopaminergic receptors implicated in SCZ, while its structural tweaks such as bulkier side chains might limit off-target binding to cerebellar pathways. SZR-81's improved motor coordination, meanwhile, could reflect preferential modulation of mitochondrial or synaptic plasticity pathways over receptors linked to sedation. These divergences imply subtle structural changes (e.g., functional group substitutions, side-chain additions) fine-tune receptor interactions or metabolic stability, decoupling therapeutic actions from adverse effects

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[30,62,102–104]. However, the study lacks direct evidence of BBB kinetics or receptor-binding specificity, leaving mechanistic explanations speculative. The acute behavioral differences observed—such as SZR-73's delayed effects at 45 minutes—may also reflect variable pharmacokinetics, with slower CNS uptake or prolonged receptor occupancy. While these profiles underscore the potential of medicinal chemistry to refine neuromodulators, the absence of mechanistic data, including receptor autoradiography, pharmacokinetic assays, etc. limits translational certainty [62,105–108]. Future studies must resolve whether structural modifications primarily influence target engagement, BBB dynamics, or metabolic pathways, a critical step toward rational design of analogs with tailored therapeutic windows for complex neurological disorders. For instance, recent reviews highlight alternative pathways for neuroinflammatory modulation, underscoring the importance of diversified therapeutic innovation alongside structural optimization of KYNA derivatives [38].

While this study provides critical insights into the acute behavioral profiles of KYNA analogs, several limitations temper translational optimism. First, findings derive from single-dose, acute experiments in healthy mice, precluding conclusions about chronic dosing effects, cumulative toxicity, or therapeutic durability—factors pivotal for disorders requiring sustained treatment, such as SCZ or PD. Second, the mechanistic ambiguity surrounding the analogs' actions remains unresolved: structural modifications (e.g., SZR-73's side chain) are hypothesized to alter receptor affinity, BBB penetration, or metabolic pathways, but direct evidence (e.g., receptor-binding assays, pharmacokinetic profiling) is absent. Without clarifying molecular targets, off-site interactions or unforeseen toxicities in humans cannot be ruled out. Third, the lack of disease-model validation limits clinical relevance. For instance, SZR-73's reduced motor deficits in healthy mice may not extrapolate to PD models with dopaminergic degeneration or SCZ models featuring glutamatergic hypofunction, where compensatory pathways could alter drug responses. Similarly, neuroinflammatory or aging-related changes in disease states might modulate analog efficacy or safety. These gaps underscore the preliminary nature of the findings and the need for caution in interpreting therapeutic potential. Future studies must prioritize mechanistic investigations (e.g., receptor autoradiography, metabolomics) and validation in pathophysiological contexts to confirm whether structural refinements truly decouple therapeutic benefits from adverse effects. Until then, the analogs' promise remains provisional, anchored to idealized preclinical conditions rather than the complexity of human neuropsychiatric disorders.

The translational promise of KYNA analogs hinges on addressing critical gaps between preclinical observations and clinical realities [27,108–111]. While acute studies in healthy mice highlight reduced motor side effects and retained neuromodulatory activity, chronic toxicity studies are urgently needed to evaluate long-term safety, cumulative dosing effects, and potential withdrawal or tolerance—issues paramount for disorders like SCZ or PD requiring lifelong therapy [112–114]. Furthermore, receptor-binding assays are essential to resolve mechanistic ambiguities: structural modifications in SZR-73 or SZR-81 may alter affinity for NMDA, α7-nACh, or AHR receptors, but without empirical validation, off-target interactions or interspecies variability in drug metabolism remain unaddressed [115–119]. Equally pressing is the need to test analogs in comorbid disease models, such as PD with depression or SCZ with cognitive deficits, where overlapping pathologies could modulate drug efficacy or safety [120–123]. For instance, neuroinflammation in AD or dopamine depletion in PD might amplify or negate analog effects observed in healthy mice. Additionally, pharmacokinetic profiling, including BBB penetration and metabolite stability, must complement behavioral data to predict human dosing regimens [124–127]. A further limitation is the lack of pharmacokinetic data describing the elimination of KYNA and its structural analogs from the brain to the periphery. Because our paradigm relied on acute intracerebroventricular administration and a short observation window, no plasma or cerebrospinal-fluid samples were obtained. Consequently, the current dataset cannot determine whether the analogs are cleared more rapidly than native KYNA or whether active efflux transporters contribute to their transient behavioral profile. Future studies should integrate serial plasma sampling, determination of brain-to-plasma

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ratios, and transporter-blockade experiments to quantify peripheral clearance and establish exposure–response relationships, thereby informing dose selection for chronic and systemic delivery. These steps are not merely incremental but foundational: they transform structure-activity correlations into actionable insights for clinical trial design. Only by integrating chronic safety data, mechanistic clarity, and disease complexity can KYNA analogs transition from promising preclinical candidates to viable therapies, ensuring their benefits withstand the multifaceted challenges of human neuropsychiatric care [128,129].

To advance SZR-73 as a lead KYNA analog, future research must prioritize three strategic pillars: pharmacogenomics, nanodelivery systems, and human neuron assays. Pharmacogenomic studies could elucidate genetic variants influencing SZR-73's efficacy or toxicity, enabling personalized dosing strategies for neuropsychiatric disorders with diverse genetic underpinnings (e.g., SCZ - linked *COMT* polymorphisms) [130–133]. Concurrently, nanodelivery systems—such as lipid nanoparticles or polymer-based carriers—should be explored to enhance SZR-73's BBB penetration, prolong half-life, or target specific brain regions (e.g., striatum in PD), potentially reducing systemic exposure and off-target effects [134–138]. Integrating these systems with real-time biodistribution imaging could refine delivery precision. Finally, human neuron assays—using iPSC-derived neurons or 3D organoids—are critical to validate SZR-73's effects on synaptic plasticity, neuroinflammation, or excitotoxicity in human-relevant contexts, circumventing limitations of rodent models [139–143]. For example, testing SZR-73 in dopaminergic neurons from PD patients or cortical circuits from SCZ cohorts could reveal disease-specific synergies or vulnerabilities. Together, these approaches would bridge mechanistic insights from acute mouse studies to human pathophysiology, addressing gaps in target specificity, metabolic stability, and interspecies translatability. By coupling structural innovation with translational tools, SZR-73 could evolve from a preclinical candidate to a precision therapeutic, tailored to the genetic, anatomical, and molecular complexities of neurological and psychiatric diseases.

Building on our comparative analysis of SZR-72/73/81 and legacy KYNA data, we distilled four predictive SAR principles that can guide the design of next-generation KYNA analogs for movement and psychotic disorders. First, three-carbon dimethyl-amide side chains (as exemplified by SZR-73) preserve neuroprotective glutamatergic modulation while abrogating cerebellar ataxia, whereas bulkier, more basic side chains (such as those in SZR-72) preferentially damp stereotypy via dopaminergic and glutamatergic hyperactivity suppression. Second, by integrating published 3D-QSAR and molecular docking data for NMDA and α7-nicotinic antagonists with our behavioral profiles, we have constructed a heuristic table that predicts whether future analogs will favor an anti-Parkinson'or anti-schizophrenia profile based on key structural motifs. Third, we propose cheminformatic filters—namely clogP ≤ 2.5 and H-bond donors ≤ 2—to ensure robust blood–brain-barrier penetration without off-target motor-circuit spill-over, as supported by our BBB-permeability discussion. Finally, we outline an in-silico screening workflow (LigandScout pharmacophore modeling → AutoDock Vina docking → ADMET Predictor assessment) that can be readily adopted by other researchers to rank novel KYNA scaffolds prior to synthesis and in vivo validation. To facilitate translation, we recommend that all lead candidates be benchmarked using our rotarod and open-field pipelines alongside microdialysis-based pharmacokinetics, allowing iterative refinement of efficacy–toxicity indices before embarking on rigorous disease-model trials and ultimately clinical assessment in downstream phases.

5. Conclusions

The structural optimization of KYNA into its analogs—notably SZR-73—demonstrates a pivotal success in mitigating KYNA's dose-limiting motor side effects, such as acute ataxia and stereotypic behaviors, while preserving its potential neuroprotective and neuromodulatory potential. SZR-73 emerges as a lead candidate, uniquely balancing therapeutic efficacy with motor safety, as evidenced by its reduced rotarod deficits and retained anxiolytic properties, likely due to strategic modifications like side-chain alterations that enhance receptor selectivity or BBB dynamics. However, this promise

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remains provisional without resolving key gaps: mechanistic studies to pinpoint molecular targets, chronic dosing trials to assess long-term safety, and validation in disease models replicating neurodegeneration or psychiatric pathophysiology. Bridging these gaps demands interdisciplinary collaboration, integrating medicinal chemistry, systems pharmacology, and clinical neuropsychiatry to refine pharmacokinetics, optimize delivery systems, and validate outcomes in human-derived models. By uniting these efforts, the field can advance KYNA-based therapies beyond preclinical promise into clinical trials, ensuring that structural innovation translates into safer, effective treatments for disorders like PD or SCZ. The journey from bench to bedside hinges on sustained, collaborative rigor—a call to action for researchers and clinicians alike to transform molecular potential into patient-centered breakthroughs [144].

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Abbreviations

The following abbreviations are used in this manuscript:

AD	Alzheimer's disease
BBB	blood-brain barrier
KYN	kynurenine
KYNA	kynurenic acid
NMDA	N-methyl-D-aspartate
PD	Parkinson's disease
SCZ	schizophrenia

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