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RESEARCH ARTICLE

# Brilaroxazine (RP5063), a Novel Serotonin-Dopamine Stabilizer, Displays Antipsychotic Efficacy in Rodents

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ABSTRACT

Introduction: Brilaroxazine (RP5063) displays high affinity for serotonin 5-$HT_{1A/2A/2B/7}$ and dopamine $D_{2/3/4}$ receptors and moderate affinity for $D_1$, serotonin transporter (SERT), and nicotinic acetylcholine receptor, $\alpha_4\beta_2$. These receptors are associated with multiple psychological disorders.

Methods: The pre-clinical assessment involved three standard models emulating human schizophrenia symptoms. The apomorphine climbing test (Protais et al., 1976), in 5 groups of 10 NMRI mice, compared brilaroxazine (1, 3, and 10 mg/kg i.p.), haloperidol (0.5 mg/kg i.p.), and vehicle. The apomorphine-induced deficit in prepulse inhibition (PPI) (Geyer et al., 2001), in 5 groups of 15 Wistar rats, compared brilaroxazine (3, 10, and 30 mg/kg i.p.), haloperidol (1 mg/kg i.p), and vehicle. The dizocilpine effect on locomotion, stereotypy, and rearing (Rung et al. 2005), in 6 groups of 10 Wistar rats, compared brilaroxazine (3, 10, and 30 mg/kg i.p.), olanzapine (6 mg/kg i.p.) and vehicle with (and without) induction.

Results: Brilaroxazine decreased apomorphine-induced climbing across the 1, 3, and 10 mg/kg doses versus controls ($p<0.001$). This compound dose-dependently reversed the apomorphine-induced PPI effects- 10 mg/kg at 87 dB ($p<0.05$) and 30 mg/kg at all levels ($p<0.01$). In the dizocilpine-induced model, it decreased versus vehicle controls: (1) spontaneous locomotor activity by 15% ($p<0.05$, 3 mg/kg), 40% ($p<0.001$, 10 mg/kg) and 30% ($p<0.01$, 30 mg/kg); (2) induced locomotion by 25% ($p<0.05$, 3 mg/kg), 49% ($p<0.01$, 10 mg/kg), and 47% ($p<0.01$, 30 mg/kg), (3) stereotypy by 51% and 58% ($p<0.001$, 10- and 30-mg/kg, respectively), and rearing (only 10 mg/kg, NS).

Conclusion: Brilaroxazine showed animal proof-of-concept activity by mitigating pharmacologically induced behaviors in rodents reflecting psychotic symptoms in humans.

**Keywords:** Antipsychotic, Brilaroxazine, Dizocilpine (MK-801)-induced model, Prepulse inhibition test, Rodent models of schizophrenia, RP5063, Schizophrenia

## Introduction

Schizophrenia is a complex, chronic, and debilitating psychiatric syndrome affecting approximately 1% of the world's population[1]. This disorder is characterized by a complex mix of positive symptoms, negative symptoms, mood symptoms, cognitive impairment and immune system abnormalities[2,3].

The pathobiology of schizophrenia involves an imbalance of dopamine (D) and serotonin (5-HT) levels in the brain due to a dysfunctional D/5-HT signaling system. Dysfunctional D/5-HT signaling system-derived dopamine and serotonin receptors in the brain can cause an imbalance in other key neurochemicals such as glutamate, GABA and nicotine levels through modulation of glutamate, GABA, and acetylcholine receptors, receptively via a cascade of the downstream signaling process. Serotonin signaling involving 5-$HT_{2B}$ and 5-$HT_7$ is implicated in inflammation and immune system abnormalities. The alteration of inflammatory cytokines and chemokines (e.g., C-reactive protein, IL-1β, IL-2. IL-4, IL-6, TNF-α, INF-γ) seen in the blood and cerebrospinal fluid of patients with this disorder is of particular interest[4–6]. Many of these are pro-inflammatory cytokines[4,7]. This presentation appears in deficit schizophrenia[7] and with negative symptoms, specifically motivational deficits[4]. Thus, key dopamine and serotonin receptors serve as primary upstream targets for the pharmacological treatment of schizophrenia. All the antipsychotics approved to date have either been dopamine receptor or dopamine and serotonin receptor selective compounds with varying degrees of functional activities[4].

Treatment involves using typical and atypical antipsychotic agents[8]. These options are far from achieving optimal efficacy and tolerability[8]. The primary mechanism comprises the attenuation of dopamine-mediated neurotransmission via an antagonistic or partial-agonist action at the dopamine $D_2$ receptor[8,9]. Typical agents (e.g., chlorpromazine, haloperidol) primarily block the $D_2$ receptor and improve positive symptoms. However, these agents lack efficacy against negative and other significant comorbid symptoms, possibly due to sub-optimal functional activity for key serotonin 5-HT receptors compared to primary target $D_2$ and produce neuroleptic and endocrine side effects (e.g., extrapyramidal symptoms [EPS] and hyperprolactinemia) due to $D_2$ antagonist activity that undermine compliance. Whereas, atypicals (e.g., clozapine, lurasidone, olanzapine, quetiapine, risperidone, and ziprasidone) affect both D and 5-HT receptors, particularly $D_2$ and 5-$HT_{2A}$. Like those in the typical class, these atypical agents mitigate positive symptomsbut are less effective in managing negative and comorbid mood and cognitive symptoms, possibly due to sub-optimal functional activities for key 5-HT receptors compared to primary target $D_2$ at their therapeutic doses. They are also limited by metabolic (e.g., weight gain, hyperglycemia, hypercholesterolemia), cardiac (e.g., QT interval prolongation), endocrine (e.g., prolactin increase, hypothyroidism), and reproductive (e.g., sexual dysfunction) issues possibly due to a relatively high binding affinity for off-targets compared to primary target $D_2$[10–13].

Despite the breadth of antipsychotic agents available, 30% of schizophrenia patients remain refractory to treatment[9,14–18]. Furthermore, these treatments are limited in managing major symptoms domains of schizophrenia[10,19]. Even with the newer drugs (e.g., aripiprazole, brexpiprzole and cariprazine) that claim broad efficacy against major symptom domains of schizophrenia, they still possess suboptimal effectiveness and significant side effects that impact patient adherence or increase the risk of neurological, cardiometabolic morbidity and mortality, particularly in elderly patients[19].

Current treatments are far from optimal. Clinical trial discontinuation rates in the short-term management of acute patients range from 30% to 50% and with long-term treatment span from 42% to 74% [20–29]. This poor treatment adherence is due to a lack of broad-spectrum efficacy in treating major symptoms (positive, negative, mood and cognitive symptoms, and inflammation) and intolerable adverse effects[30,31]. Accordingly, substantial unmet medical needs remain[29]. Therefore, the goal of current schizophrenia research is to identify novel agents that improve all major symptoms and enhance tolerability and adherence during acute and maintenance treatment[29].

Brilaroxazine (RP5063), a multimodal D and 5-HT receptor modulator stabilizing the D/5-HT system, represents a promising option for schizophrenia. This compound possesses a high binding affinity for $D_{2/3/4}$ and 5-$HT_{1A/2A/2B/7}$ and moderate binding affinity for 5-$HT_6$, serotonin transporter (SERT), and nicotinic acetylcholine receptor, $\alpha_4\beta_2$[32,33]. It possesses partial agonist activity at the $D_{2/3/4}$ and 5-$HT_{1A}$, and a weak partial agonist or neutral antagonist activity for 5-$HT_{2A}$ receptors and antagonist activity at the 5-$HT_{2B/6/7}$ receptors[32]. Its main points of pharmacologic differentiation from other antipsychotics are its combination of potent affinity (Ki, ≤6 nM) and selectivity, with <10-fold separation in its activity between $D_2$ and other key receptors $D_{3/4}$ and 5-$HT_{1A/2A/2B/7}$, along with its moderate activity (Ki, ≤50 nM) for 5-$HT_6$ and the $\alpha_4\beta_2$ nicotinic acetylcholine receptors, which play a

central role in the schizophrenia symptomatology and pathobiology[34–37].

Brilaroxazine possesses a broad *in vitro* pharmacology profile against key dopamine and serotonin receptors involved in schizophrenia and other neuropsychiatric disorder pathology [32,38]. In addition, pre-clinical work with brilaroxazine in pulmonary artery hypertension (PAH), idiopathic pulmonary fibrosis (IPF), and psoriasis provides initial evidence of this agent's effect on pro-inflammatory cytokines and chemokines[39–42]. Several of these pro-inflammatory cytokines are also found in patients with psychiatric disorders (e.g., schizophrenia, depression), which are also major comorbid conditions in patients with PAH, IPF and psoriasis[43]. Accordingly, the operant research question is whether brilaroxazine exerts pharmacologic activity in three standard translational rodent models for schizophrenia. This paper's purpose is to review brilaroxazine's antipsychotic activity profile in these rodent models: (1) apomorphine-induced climbing test in mice; (2) apomorphine-induced deficit in PPI deficit in rats; and (3) dizocilpine-induced hyperactivity and stereotypy in rats. This research's contribution offers the initial proof-of-concept activity with brilaroxazine in pre-clinical models of schizophrenia. The paper's flow starts methods of these three studies, then moves to their results, a discussion involving translational considerations, and conclusions.

## Methods

*Apomorphine-Induced Climbing Study in Mice*[44]

Animals and Handling: This experiment involved 50 Naval Medical Research Institute (NMRI) mice (Elevage Janvier, France; body weight 24-29 g) stabilized for at least five days after delivery in Makrolon cages (25 × 19 × 13 cm; 10 animals per cage) on wood litter and had free access to food and water. Directive 2010/63/EU. governed animal handling.

Treatment Groups and Experimental Procedure: Animal randomization involved five groups of ten animals: (1) vehicle (0.2% hydroxypropylmethylcellulose [HPMC] in physiologic saline); (2) brilaroxazine (1 mg/kg); (3) brilaroxazine 3 mg/kg; (4) brilaroxazine 10 mg/kg; or (5) haloperidol 0.5 mg/kg. Animals received treatments (i.p.) 30 minutes before apomorphine injection (1 mg/kg subcutaneously [s.c.]). After placement of each animal adjacent to a wire grid wall, evaluation of its behavior occurred every ten minutes using a five-point scale at each time point (10, 20, and 30 mins) over 30 minutes for the intensity of climbing. Evaluators were blind to treatments.

Assessment and Analysis: The intensity of climbing scoring involved a five-point scale (0 = normal behavior; 1 = excitation/sniffing; 2 = occasional climbing [2 paws]; 3 = occasional climbing [4 paws]; and 4 = permanent climbing [4 paws]). The total score comprised three measurement points of 10, 20, and 30 minutes. Data analysis compared treated groups with the vehicle control using the Kruskal-Wallis and Mann-Whitney U tests in the event of significant effect.

*Apomorphine-induced Deficit in Prepulse Inhibition (PPI) Study in Rats*[45,46]

Animals and Handling: This investigation involved 65 Wistar (Han) rats (Elevage Janvier, France; body weight, 260-304 g), stabilized for at least 5 days after delivery in Makrolon cages (25 × 19 × 13 cm; 5 animals per cage) on a wood litter with free access to food and water. Animals were handled per Directive 2010/63/EU.

Startle Chamber: The apparatus consisted of a commercially available, soundproofed startle chamber (San Diego Instruments, San Diego, CA). A computer program (San Diego Instruments) controlled all experimental events and data recording. Investigators placed the rats within a startle chamber in a small acrylic cylinder, slightly larger than the rat, attached to a base plate containing a strain gauge. Vertical movement of the rat occurred during a startle response resulting in the deformation of the base plate, which generated a current in the strain gauge proportional to the size of the movement (i.e., startle response). Directly above the rat was a loudspeaker to provide background sound and stimuli.

Treatment Groups and Experimental Procedure: Investigators randomized animals into six groups of 15: (1) vehicle (0.2% HPMC in physiologic saline); (2) vehicle (0.2% HPMC in physiologic saline) and apomorphine (1 mg/kg s.c.); (3) brilaroxazine 3 mg/kg; and apomorphine (1 mg/kg s.c.); (4) brilaroxazine 10 mg/kg and apomorphine (1 mg/kg s.c.); (5) brilaroxazine 30 mg/kg and apomorphine (1 mg/kg s.c.); or (6) haloperidol 1 mg/kg and apomorphine (1 mg/kg s.c.). Treatment administration (i.p.) occurred 15 minutes before apomorphine induction.

Investigators placed the rats in the chamber to begin the session 15 minutes after apomorphine induction. The PPI protocol consisted of a 33-minute session. The first 10 minutes were for habituation, during which background noise of 70 dB intensity was provided within the chamber.

Following the habituation period was the presentation of eight trial types in pseudorandom

order, eight times each, separated by 15 to 25 seconds. The protocol involved four phases: (1) no stimulus to evaluate basal levels of movement; (2) prepulse, involving the presentation of a 20-millisecond burst of white noise at 87, 90, or 93 dB that should not produce a clear startle response; (3) 115 dB startle, involving the presentation of a 40-ms burst of white noise at 115 dB, resulting in a startle response; and (4) 115 dB with prepulse, involving an 87, 90, or 93 dB stimulus followed 80 ms later by the 115-dB stimulus.

Assessment, PPI Calculation, and Analysis: Observers, blinded to treatments, recorded startle platform output 100 ms starting from the onset of the startle stimulus. This effort involved the recording of three variables for each trial: (1) average response over the entire recording period; (2) peak response; and (3) time to peak response.
Calculating PPI for each rat involved averaging the eight trials of each type and calculating the percentage reduction in startle amplitude (average and peak values) caused by the 87-, 90-, or 93-dB prepulse. The time to peak response represented a measure of reaction time.
The qualitative analysis of brilaroxazine data involved comparing treated groups using a one-way analysis of variance (ANOVA), followed by planned comparisons against apomorphine alone. The analysis compared haloperidol data with apomorphine alone using an unpaired student's t-test.

*Dizocilpine-Induced Hyperactivity and Stereotypy Study in Rats*[47–49]

Animals and Handling: This experiment evaluated 60 Wistar rats (Raj Biotech Private Ltd, Maharashtra, India) with a body weight between 180 and 200 g and an age range between 8 and 9 weeks. Investigators housed the animals in groups of 4 in solid bottom polycarbonate cages with stainless steel grill tops and given bedding of clean, paddy husk. They suspended the cages on stainless steel racks; maintained the rats in air-conditioned rooms with 10 to 15 air changes per hour, 21 ± 3 °C, and a 30%-70% relative humidity; and provided pelleted rodent feed (Rayans Biotechnologies Pvt. Ltd., Hyderabad, India) and potable, filtered water ad libitum. Before the experiment, investigators handled the animals for two days per standard animal control guidelines.

Treatment Groups and Experimental Procedure: Investigators randomized animals to six experimental groups of 10 animals: (1) vehicle control (5% Pharmasolve [Ashland Inc., Lexington, KY] + 45% polyethylene glycol [PEG] 400 + 50% water for Injecttion [WFI] at 1 mL/kg, i.p); (2) vehicle control (5% Pharmasolve + PEG 400 + 50% WFI with dizocilpine (0.2 mg/kg, i.p.) induction; (3) brilaroxazine 3 mg/kg; (4) brilaroxazine 10 mg/kg; and (5) brilaroxazine 30 mg/kg; and (6) olanzapine (6 mg/kg). All treatments involved the same vehicle as used for the vehicle control.
The experiment proceeded over two days (i.e., five animals from each group on the first day and the remaining five on the next day). The procedures' setting involved an open field, a black-colored area 51 × 51 × 36 cm enclosed by black plastic walls of the same dimensions. The field contained an imaginary center 24 cm from the periphery of each side.
The experimental schedule occurred over 80 minutes. The protocol involved the following phases: (1) T=0 minute- animals received controls and treatments, then transferred to their home cages; (2) T=30 minutes- animals placed in the open field and tracked for their horizontal locomotion; (3) T=45 minutes- animals removed from the open field; (4) T=45 minutes- animals received dizocilpine or vehicle (i.p.), then transferred to the home cage; (5) T=60 minutes- animals placed in an open field; and (6) T=75- animals removed from the open area, transferred to a transparent cage, and observed for five minutes by two blinded observers.

Assessments and Analysis: Blinded observers assessed animals for the following behaviors: (1) spontaneous locomotor activity between 30 and 45 minutes in the distance measured (cm); (2) dizocilpine-induced locomotor activity between 60-75 minutes in the distance measured (cm); and (3) dizocilpine-induced stereotypy (sniffing, circling behavior, gnawing, and grooming (0 = absent, 1 = equivocal, 2 = present, 3 = intense, and 4 = intense and continuous) and number of rears between 70-75 minutes. Data analysis involved a 1-way ANOVA followed by the Newman-Keuls multiple comparison test.

## Results

*Apomorphine-Induced Climbing Study*

Figure 1 presents the results from the apomorphine climbing test evaluation. Apomorphine (1 mg/kg) administered s.c. to the vehicle control group induced climbing at 10, 20, and 30 minutes; the mean total score, which summed measurements on a five-point scale for the three-time points (10, 20, and 30 mins) measured, was 10.2 ± 0.7. Haloperidol 0.5 mg/kg significantly decreased climbing at each time point, with a similar effect on the mean total score, compared with the apomorphine controls

(p<0.001). Brilaroxazine at 1, 3, and 10 mg/kg decreased climbing at each time point, with a similar effect on the mean total score, as compared with apomorphine controls (p<0.001 at all 3 doses) (p<0.001 at all 3 doses).

**Figure 1**

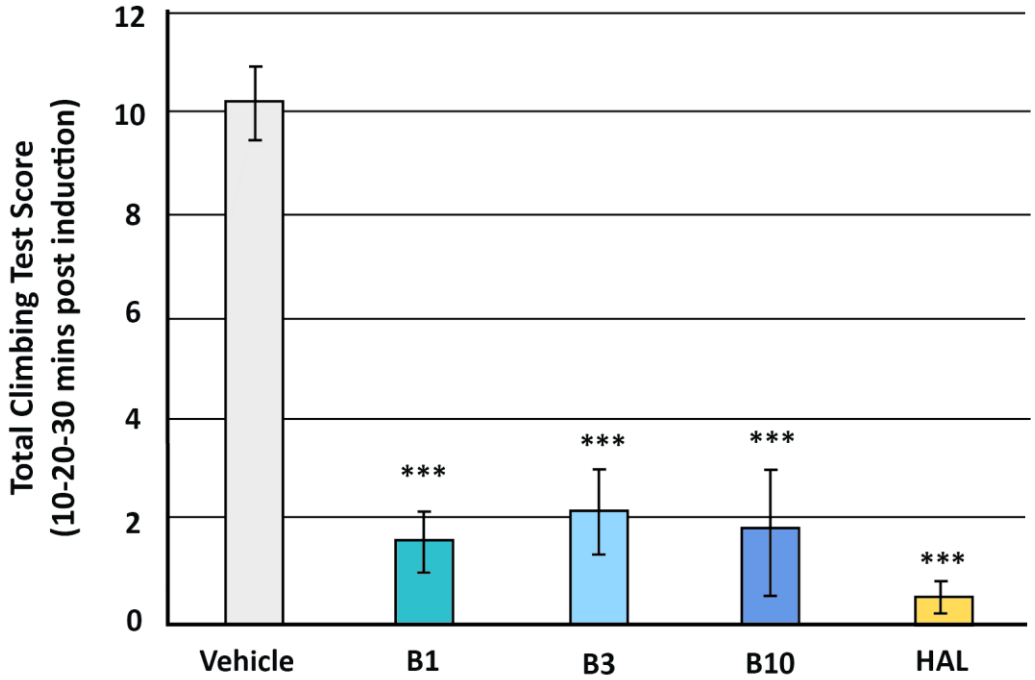


**Figure 1:** Effects of brilaroxazine at 1 mg/kg (B1), 3 mg/kg (B3), and 10 mg/kg (B10), haloperidol 0.5 mg/kg (H), and vehicle (V) in the apomorphine-induced climbing test in the mouse (n=10/group). Treatments administered i.p. 30 minutes before apomorphine (1 mg/kg s.c.). Mean ± SEM. ***p<0.001 (compared with apomorphine plus vehicle; Mann-Whitney U test).

*Apomorphine-Induced Deficit in the Prepulse Inhibition (PPI) Study*

Figure 2 illustrates the results from the apomorphine PPI evaluation. In the vehicle control group, the intensity of the startle response decreased when low-intensity prepulse preceded the startle (average intensity: 40.5%, 60.6%, and 69.4% reductions at prepulse intensities of 87, 90, and 93 dB, respectively). This decrease in the vehicle group indicated the presence of prepulse inhibition (PPI).

Apomorphine at 1 mg/kg decreased PPI compared with the vehicle control (average intensity: 19.9% [p<0.05], 42.0% [p<0.01], and 56.1% [p<0.05] at 87, 90, and 93 dB, respectively). It did not affect spontaneous movements in the absence of stimulus or the reaction to the pulse alone, but it increased the reaction to the prepulse alone (+49% [p<0.01], +46% [p<0.05], and +14% [p=not significant [NS]] at 87, 90, and 93 dB, respectively).

Concerning reference treatment, haloperidol 1 mg/kg decreased the apomorphine-induced deficit of PPI compared with the apomorphine controls (average intensity: 52.1%, 63.9%, and 74.8%, at 87, 90, and 93 dB, respectively [p<0.001]. It did not affect spontaneous movements without a stimulus, the reaction to the prepulse alone, or the pulse alone.

Brilaroxazine at 10 and 30 mg/kg i.p. 30 minutes before the test (i.e., 15 minutes before apomorphine induction) attenuated the apomorphine-induced PPI deficit in a dose-dependent fashion. The 3 mg dose had no effect. The 10 mg/kg dose increased PPI at an intensity of 87 dB compared with apomorphine controls (p<0.05). The 30 mg/kg increased PPI at all 3 prepulse intensities (p<0.01 in all cases). Brilaroxazine did not affect spontaneous movements without stimulus or the reaction to the pulse alone, but it slightly decreased the reaction to the prepulse (30 mg/kg at the intensity of 87 dB, p<0.01).

**Figure 2**

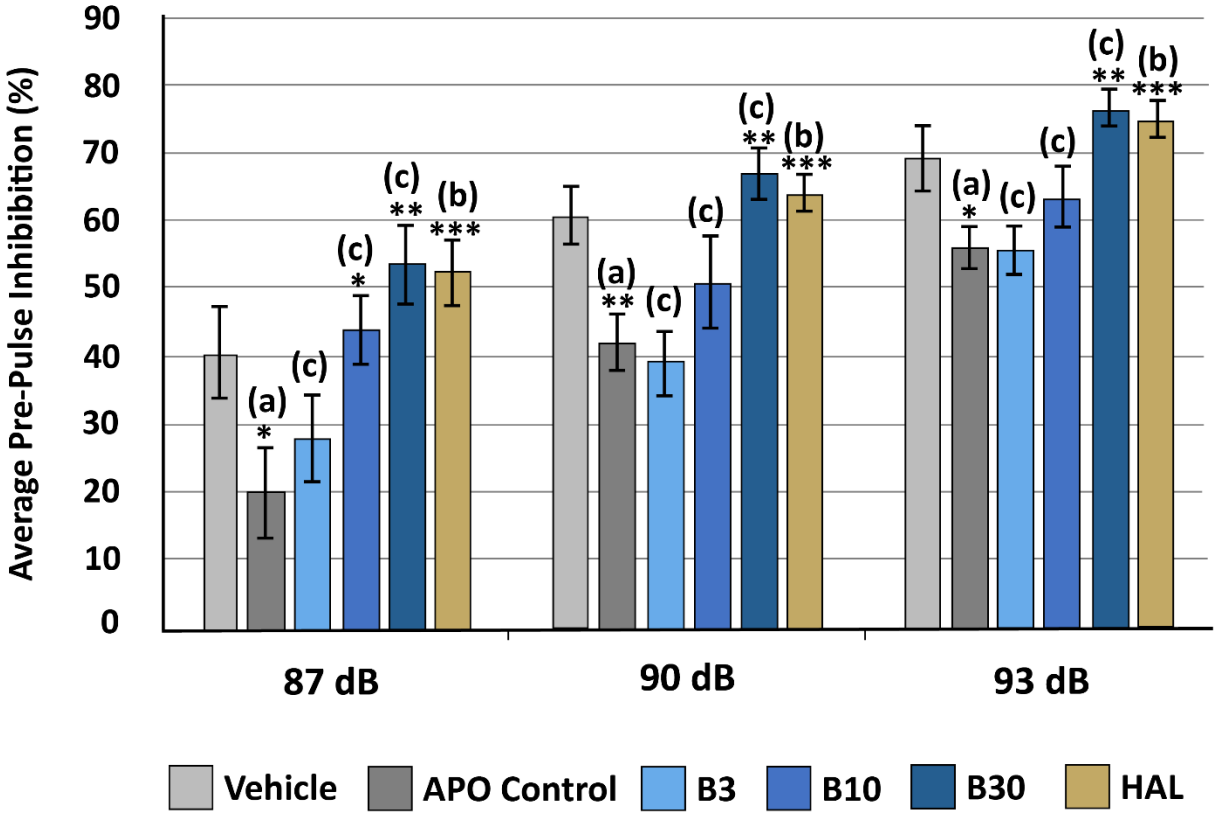


**Figure 2:** Mean response effects of brilaroxazine at 3 mg/kg (B3), 10 mg/kg (B10), and 30 mg/kg (B30), haloperidol at 1 mg/kg (H), and vehicle (V) in the apomorphine prepulse inhibition test at 87, 90, and 93 dB (n=14-15/group).
Notations: (a): Compared with vehicle control: * = $p < 0.05$; ** = $p < 0.01$. [Student's t-test]. (b): compared with apomorphine control: *** = $p < 0.001$. [Student's t-test]. (c): Compared with apomorphine control: no indication = not significant; * = $p < 0.05$; ** = $p < 0.01$. [One-way ANOVA followed by Dunnett's t-test in case of significant effect].

*Dizocilpine-Induced Hyperactivity and Stereotypy Study*

Spontaneous Locomotion (30-45 minutes)

Figure 3 describes spontaneous and dizocilpine-induced locomotor activity results.

Concerning spontaneous locomotor activity (Figure 3), rats in the non-induced vehicle group moved 29.65 m, and rats in the dizocilpine-induced group traveled 24.36 m ($P<0.05$). Olanzapine (6 mg/kg, i.p.) decreased spontaneous locomotor activity by 60% ($P<0.01$). Brilaroxazine (at 3, 10, and 30 mg/kg) reduced spontaneous locomotor activity in an asymptotic manner (not dose-dependent) by 30% and 40% in the 10 mg/kg and 30 mg/kg groups ($P<0.001$, 0.01, respectively).

Induced Hyperactivity or Locomotion (60-75 minutes)

Concerning dizocilpine-induced hyperactivity, as reflected by locomotor activity (Figure 3), animals in the vehicle without the dizocilpine group moved 26.41 cm (NS from spontaneous measurement). Animals in the control group induced with dizocilpine moved 79.16 cm. More than 3=three-fold higher than the spontaneous measurement, this observation reflected more extensive locomotion than the non-induced vehicle group animals ($p<0.001$).

Olanzapine and brilaroxazine reduced dizocilpine-induced locomotion. Olanzapine, the positive control, decreased dizocilpine-induced locomotion by 83% ($p<0.001$). Brilaroxazine also decreased dizocilpine-induced locomotion by 25% ($p<0.05$), 49% ($p<0.01$), and 47% ($p<0.01$) in the 3-, 10-, and 30-mg/kg groups, respectively. There was no significant difference in locomotion activities between the middle (10 mg) and highest (30 mg) doses of brilaroxazine.

**Figure 3**

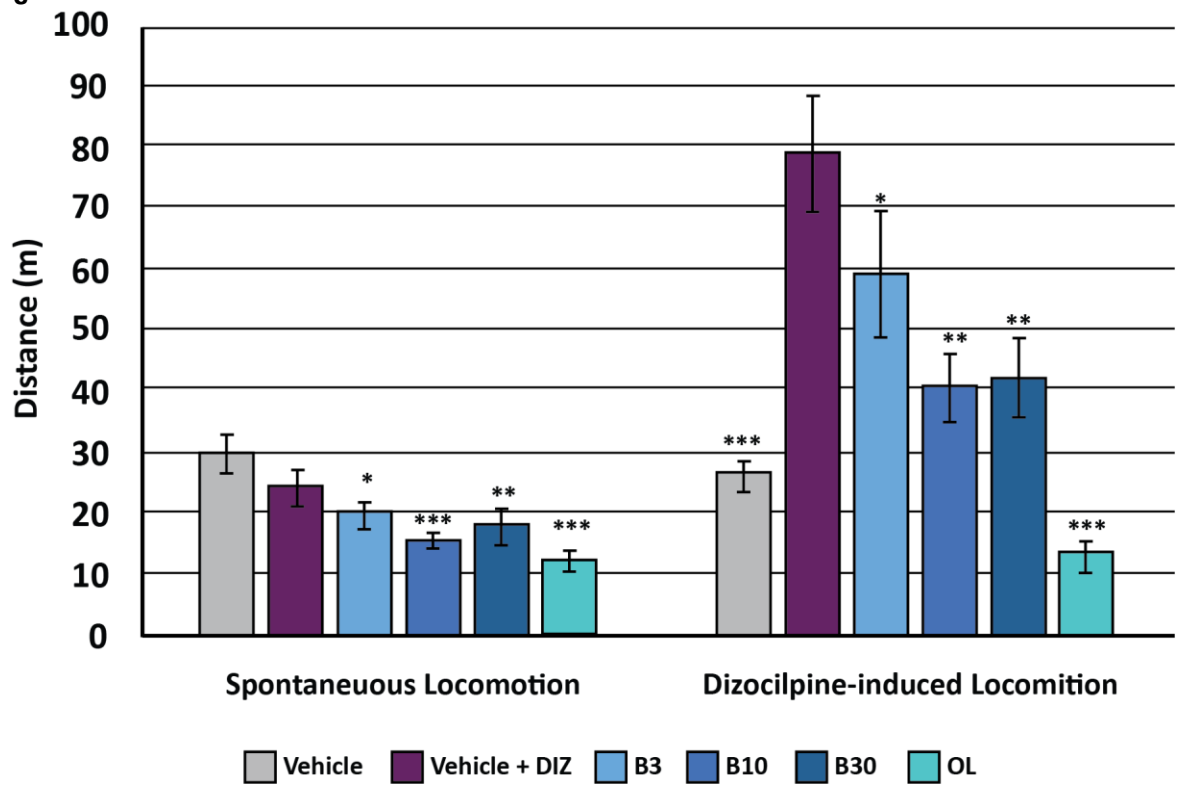


**Figure 3:** Effect of brilaroxazine at 3 mg/kg (B3), 10 mg/kg (B10), and 30 mg/kg (B30), olanzapine (6 mg/kg, i.p.) (O) on (A) spontaneous locomotion (T=30–45 minutes) and (B) dizocilpine-induced Locomotion (T=60–75 minutes). (n=9-10/group). *p<0.01; **p<0.05; ***p<0.001 versus dizocilpine-induced vehicle (group 1; 1-way ANOVA followed by the Newman- Keuls multiple comparison test).

Stereotypy (T= 60-75 minutes)

The vehicle group without dizocilpine induction (Figure 4) showed a mean stereotypy score of 0.00 (standard error of the mean [SEM] 0.00). In contrast, the control group with dizocilpine reflected a mean score of 4.3 (SEM 0.335; p<0.001 versus non-induced controls. Olanzapine showed a 70% reduction in stereotypic scores compared with the dizocilpine group (p<0.001). Brilaroxazine in the 3-, 10-, and 30-mg/kg groups showed reductions of 23% (p=NS), 51% (p<0.001), and 58% (p<0.001), respectively, in the stereotypic score as compared with the dizocilpine group but non-significant between dose groups.

Concerning rearing, animals on the vehicle had 9.3 (SEM 1.193) rears. Those induced with dizocilpine showed a significant reduction in rears to 0.6 (SEM 0.267) (p<0.001). Olanzapine 6 mg/kg i.p. and brilaroxazine 10 and 30 mg/kg i.p. did not attenuate dizocilpine-induced rearing at 0.000 (SEM 0.000) and 0.750 (SEM 0.000), respectively. Only the 3 mg/kg i.p. group influenced rearing with 2.9 (SEM 0.849) (p<0.05).

## Discussion

*Studies Provide Insight to Activity on Modelled Behaviors of Schizophrenia*

This paper's research question focused on whether brilaroxazine exerts pharmacologic activity in induced behaviors associated with schizophrenia in three standard translational rodent models- 1) apomorphine-induced climbing test, 2) apomorphine-induced deficit in PPI evaluation, and 3) dizocilpine-induced hyperactivity, stereotypy, and rearing model. This research provides evidence of brilaroxazine's ability to mitigate the induced behaviors in these models, providing the first findings of its treatment effect for schizophrenia in animal models.

Considering these findings, it is important to discuss the role of animal schizophrenia models in screening candidate compound activity involved with preventing or reversing specific pharmacologic-induced behavioral alteration against stimulated psychotic behaviors[8,45,46]. These studies use rodents as the test species because of their similarities in physiology and response to pharmacologic inducement.

**Figure 4**

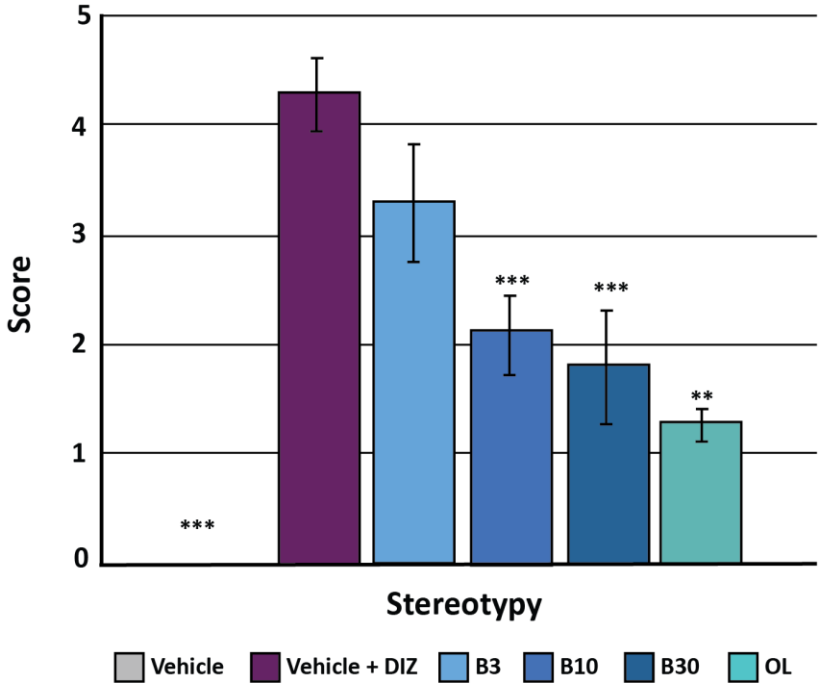


**Figure 4**: Effect of brilaroxazine at 3 mg/kg (B3), 10 mg/kg (B10), and 30 mg/kg (B30), olanzapine (6 mg/kg, i.p.) (O) on dizocilpine -induced stereotypy (n=9-10/group). **p<0.01; ***p<0.001 versus dizocilpine (1-way ANOVA followed by Newman-Keuls multiple comparison test).

Accordingly, this research's contribution is that these investigations provide pre-clinical proof-of-concept results supporting that brilaroxazine mitigates behaviors modeled to reflect those patients with schizophrenia experience. These studies used the most relevant translational rodent models to evaluate brilaroxazine's antipsychotic activity. These models take into consideration relevant signaling pathways and symptom presentation. In the pathophysiology of schizophrenia, both D and 5-HT receptor signaling pathways play a critical role[50–52]. Positive, negative, and cognitive symptoms define schizophrenia[53–56]. Positive symptoms are more dominant in the acute presentation of schizophrenia, and negative symptoms are more apparent in the chronic phase of this condition[50,53]. While both dopamine and serotonin contribute to positive and negative symptoms, a dysfunctional dopaminergic signaling pathway is reported to play a dominant role in positive symptoms, and the serotonergic signaling pathway is linked with negative, cognition and mood-related symptoms [50–52]. In addition, neuroinflammation via pro-inflammatory cytokines contributes to the disease pathophysiology emanating from immune responses or infection[4–7]. This pathology notably appears in deficit schizophrenia[7] and with negative symptoms, specifically motivational deficits[4].
Heterogeneity in the cluster of symptoms and their severities among schizophrenia patients exist. Hence, three models evaluated brilaroxazine's spectrum of antipsychotic activity. These experiments involved different surrogate models induced by the D agonist apomorphine and the N-methyl-D-aspartate receptor (NMDA) antagonist dizocilpine. Psychopharmacologists commonly use apomorphine-induced rodent models (reflective of a dopamine agonism) of schizophrenia[8,45,46,48,49] to evaluate the efficacy of an antipsychotic agent, especially its effectiveness in treating positive symptoms. Similarly, investigators also employ the NMDA receptor antagonist (dizocilpine- and phencyclidine-date-induced) rodent models widely in testing the efficacy of an antipsychotic, especially its effectiveness for negative symptoms and other comorbidities cognition, depression, and mood symptoms[8,48,49]. Dizocilpine-induced schizophrenia rodent models are routinely used in translational studies because they are reported to alter the dopamine and serotonin signaling pathways through allosteric modulation of both D and 5-HT receptors in the brain and cause psychotic symptoms representing a majority cluster of symptoms found in schizophrenia[51,57].
This analysis considered that the brilaroxazine studies reflected the triad of target receptor pharmacology, significant behavioral symptoms, and pre-

dictive data for translation to the clinic. The two apomorphine studies were used to model acute dopaminergic receptor stimulation. The dizocilpine experiment examined brilaroxazine's effects on the dopamine and serotonin systems through its interaction with the NMDA receptor. The tests chosen reflected a mix of significant behaviors seen in patients with schizophrenia that rodent behavior could mimic when induced by apomorphine stimulation of dopamine receptors or dizocilpine inhibition of NMDA receptors.

Brilaroxazine demonstrated pharmacologic activity in the three studies and prevented the expression of symptoms that mimic psychotic behaviors induced by apomorphine and dizocilpine. In the first apomorphine model, all brilaroxazine doses produced at all time points lowered climbing scores. Brilaroxazine provided an approximately five-fold reduction in climbing scores compared with the apomorphine control-induced group. Interestingly, this observation for brilaroxazine was similar across the dosing groups. This observation might reflect a ceiling effect due to $D_2$ partial agonist activity; however, this effect was robust and significant in all groups treated with brilaroxazine compared to the apomorphine-induced vehicle group.

Similarly, in the dizocilpine model in rats, brilaroxazine resulted in lower stereotypic scores in the 3-, 10-, and 30-mg/kg dose groups. Again, this effect was not dose-dependent, as to the degree of impact on stereotypic scores due to its dopamine $D_2$ partial agonist activity. It appeared to be much smaller, with the increase in brilaroxazine's dose from 10 to 30 mg/kg, compared with its increase from 3 to 10 mg/kg. Brilaroxazine also prevented dizocilpine-induced locomotion, a measure to reflect agitation as a positive symptom. All three brilaroxazine doses (3, 10, and 30mg/kg) mitigated the dizocilpine-induced locomotion, but the effect was significant with doses 10mg/kg and 30mg/kg. The effect with brilaroxazine plateaued at the 10-mg/kg dose, as the 30-mg/kg group did not show any further benefit, possibly due to $D_2$ partial agonist activity. The active control olanzapine also significantly decreased the dizocilpine-induced locomotion, but the effect was very close to its spontaneous locomotion. The difference between spontaneous and induced locomotion with brilaroxazine contrasts with the lack of difference observed with olanzapine, possibly due to its sedative effect.

Psychopharmacologists widely accept the PPI test to evaluate psychotic symptoms of schizophrenia and associated comorbid cognition deficits in translational rodent species[58,59]. Other investigators have used variations of this method in the clinic[60,61]. All brilaroxazine at doses (3, 10, and 30 mg/kg) significantly increased PPI response scores ($p<0.001$) at each of the three intensity levels compared with the scores observed with induced controls. Furthermore, these improvements were dose-dependent, which contrasted the dose effects in the tests, thus reflecting the impact on positive symptoms.

Brilaroxazine's cognitive activity effect might be explained by its effects on multiple 5-HT receptors—particularly the 5-$HT_{1A/2A/2B/6/7}$ receptors—and its partial agonist effect on $D_{2/4}$ receptors[32,62]. This behavior is predominantly mediated through the dorsolateral prefrontal cortex region of the brain through the 5-$HT_{1A/2A/6/7}$ receptors[63,64]. Furthermore, pre-clinical data in the rodent models of PAH, IPF and psoriasis indicate that brilaroxazine impacts the release of pro-inflammatory cytokines and chemokines, such as TNF$\alpha$, IL-1$\beta$, IL-6, and MCP -1[39–42]. These observations suggest that brilaroxazine possesses a multifaceted basis for impacting the-se symptoms and distinguishes it from other atypical antipsychotics.

*Clinical Translation of Brilaroxazine's Activity*

These pre-clinical findings set the stage for clinical evaluation. Additionally, investigators at Northwestern University found that brilaroxazine displayed a multifaceted basis for enhancing cognition that distinguishes it from other atypical antipsychotic drugs, which depend on functional activities of 5-$HT_{1A/2A/2B/6/7}$ receptors to improve cognitive impairment associated with schizophrenia (CIAS)[62]. The brilaroxazine administration at 3 and 10 mg/kg blocked acute phencyclidine (PCP)- as well as amphetamine-induced hyperactivity-induced hyperactivity- as noted in its effects on locomotion ($p<0.001$). It also reversed subchronic phencyclidine-induced impairment in novel object recognition (NOR) and learning deficits, as compared with controls ($p<0.001$). Finally, this agent increased cortical dopamine efflux ($p<0.01$), which is potentially critical to its cognitive-enhancing properties.

Phase 1 studies investigated brilaroxazine in the clinic by assessing its performance in ascending single doses of 10 mg and 15 mg in normal healthy males and multiple doses ranging from 10 mg to 100 mg/day over ten days in patients with stable schizophrenia. In the multiple-dose study, an initial pharmacodynamic evaluation of brilaroxazine showed improvements with brilaroxazine ($p<0.05$) over placebo in a PANSS secondary analysis of patients with a baseline PANSS score >50 for Positive Symptom Subscale Scores. Also, improvements in Trails A and Trails B Test results for cognitive and executive functions assessment were observed for

patients treated in the 50 mg dose group for Days 5, 10, and 16[38,65].

These initial clinical experiences led to phase 2 investigation (REFRESH) in patients with acute schizophrenia or schizoaffective disorder[32]. This phase 2 trial produced substantial evidence for translating the signals for activity in the pre-clinical schizophrenia models examined in this paper. This 28-day, randomized, double-blind, placebo-controlled study evaluated brilaroxazine at 15, 30, and 50 mg in 233 adults with acute exacerbation of schizophrenia or schizoaffective disorder. Results from this study were extremely positive on efficacy, safety, and tolerability measures [32]. Compared with the placebo, all dose groups were numerically superior, and the 15- and 50-mg doses of brilaroxazine were statistically significant ($p<0.05$) in improving the primary endpoint, the PANSS total score[32]. The median rates of improvement in PANSS total score over baseline in the 15- and 50-mg groups were 23% and 22%, respectively. Both doses were superior to placebo concerning PANSS subscales for positive symptoms, negative symptoms and social functioning, and Clinical Global Impressions-Improvement scores.

## Conclusions

Brilaroxazine demonstrated significant antipsychotic effects on pharmacologic-induced behaviors associated with psychosis and schizophrenia in three standard translational surrogate rodent models. The findings from the apomorphine-induced climbing test, a dopaminergic model, reflected the presence of antipsychotic activity for brilaroxazine across 1-, 3-, and 10-mg/kg doses that were significant compared with the vehicle control. The observations in another dopaminergic model, the apomorphine-induced PPI test, pointed to a dose-dependent capacity to reverse the PPI deficits, which were significant versus the vehicle control at the 3-, 10-, and 30-mg/kg doses. In the NMDA-induced psychosis model, brilaroxazine significantly decreased dizocilpine-induced hyperlocomotion and stereotypy behaviors in a dose-dependent manner across the 3-, 10-, and 30-mg/kg doses. Such observations of brilaroxazine's schizophrenia efficacy in these animal models are due to this compound's multimodal effects involving critical dopamine and serotonin targets and influencing the release of pro-inflammatory cytokines. Another NMDA animal model using phencyclidine provides further supportive evidence of brilaroxazine's efficacy in schizophrenia and supports the findings described in this current paper [62]. Later work in the clinical setting translated the observations from these three pre-clinical studies. The phase 1 multiple ascending dose study results in patients with stable schizophrenia[38,65] and the phase 2 REFRESH study in patients with acute schizophrenia or schizoaffective disorder[32] reflect this connection. Brilaroxazine is currently undergoing evaluation in a larger population of patients with schizophrenia in a more extensive phase 3 trials, culminating the cycle from pre-clinical to clinical proof-of-concept.

## Disclosures

*Conflict of Interest:* Laxminarayan Bhat, Kouacou Adiey, Seema R Bhat, and Prabhu Mohapatra are employees of Reviva Pharmaceuticals, Inc.

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## Author Contributions

Study design (LB). Study oversight (LB, SB), Brilaroxazine synthesis (KA PM, SB). Data analysis and review (LB, KA, SB, PM). Manuscript development (LB, SB).

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