A novel allosteric modulator of the cannabinoid CB₁ receptor ameliorates hyperdopaminergia endophenotypes in rodent models

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The endocannabinoid system (eCBs) encompasses the endocannabinoids, their synthetic and degradative enzymes, and cannabinoid (CB) receptors. The eCBs mediate inhibition of neurotransmitter release and acts as a major homeostatic system. Many aspects of the eCBs are altered in a number of psychiatric disorders including schizophrenia, which is characterized by dysregulation of dopaminergic signaling. The GluN1-Knockdown (GluN1KD) and Dopamine Transporter Knockout (DATKO) mice are models of hyperdopaminergia, which display abnormal psychosis-related behaviors, including hyperlocomotion and changes in pre-pulse inhibition (PPI). Here, we investigate the ability of a novel CB₁ receptor (CB₁R) allosteric modulator, ABM300, to ameliorate these dysregulated behaviors. ABM300 was characterized in vitro (receptor binding, β-arrestin2 recruitment, ERK1/2 phosphorylation, CAMP inhibition) and in vivo (anxiety-like behaviors, cannabimimetic effects, novel environment exploratory behavior, pre-pulse inhibition, conditioned avoidance response) to assess the effects of the compound in dysregulated behaviors within the transgenic models. In vitro, ABM300 increased CB₁R agonist binding but acted as an inhibitor of CB₁R agonist induced signaling, including β-arrestin2 translocation, ERK phosphorylation and CAMP inhibition. In vivo, ABM300 did not elicit anxiogenic-like or cannabimimetic effects, but it decreased novelty-induced hyperactivity, exaggerated stereotypy, and vertical exploration in both transgenic models of hyperdopaminergia, as well as normalizing PPI in DATKO mice. The data demonstrate for the first time that a CB₁R allosteric modulator ameliorates the behavioral deficits in two models of increased dopamine, warranting further investigation as a potential therapeutic target in psychiatry.

INTRODUCTION
Dysregulation of dopaminergic and glutamatergic signaling are thought to underpin the development of psychosis and schizophrenia [1]. Pharmacological treatment of schizophrenia and psychosis includes the use of antipsychotics, which act as orthosteric receptor antagonists/partial agonists of various GPCR targets, including dopamine receptor D₂ and serotonin receptor 5HT₁A. However, antipsychotics are associated with extrapyramidal side effects, sedation, metabolic syndrome, and weight gain [2, 3].

The endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are orthosteric agonists of the cannabinoid CB₁ receptor (CB₁/R). CB₁/R are expressed presynaptically on various neuronal types, including GABAergic, glutamatergic and serotoninergic neurons, where they mediate an inhibition of transmitter release. While not directly expressed on dopaminergic neurons, the endocannabinoid system acts as a crucial filter that integrates both inhibitory and excitatory signaling that modulates dopamine neuron signaling [4]. Furthermore, studies have shown that the endocannabinoid system is a negative modulator of both D₁ and D₂ receptor-mediated behaviors, implicating them in basal ganglia disorders [5]. As such, in combination with the complex dysregulation and circuit-based mechanisms for brain region-dependent alterations in dopaminergic signaling in psychiatry, this suggests that the CB₁R may be a more attractive, alternative therapeutic target to the classical D₂ receptor antagonism approaches of antipsychotics [4].

Furthermore, there is strong evidence from humans that both endocannabinoid levels and CB₁R are dysregulated in schizophrenia [6–9]. Serum and CSF levels of AEA are higher in patients with schizophrenia at all stages of the illness and are normalized after treatment with antipsychotics. CB₁R expression and binding is higher in post-mortem brain tissues of patients with schizophrenia [6, 9]. While the endocannabinoid system seems to be an important potential therapeutic target in psychiatry, targeting CB₁R at the orthosteric site has not yielded beneficial clinical outcomes. The CB₁R orthosteric inverse agonist, rimonabant, was effective treating obesity and metabolic syndrome, but caused suicidal ideation and was withdrawn from the market [10–12]. Here, we propose to investigate a novel pharmacological approach of targeting the CB₁R.
In 2005, we discovered the CB1R allosteric site and the original, prototype allosteric modulator, Org27569. This compound has served as a tool compound to characterize the allosteric site but is not a drug candidate. Org275, and related compounds, display an atypical, complex allosteric profile at CB1R [13, 14]. Org275 increases the $B_{max}$ of $[^{3}H]$CB1R agonist binding but functionally acts as an inhibitor of CB1R agonist-mediated signaling [13, 15]. Importantly, in October 2019, Shao et al. [16] elucidated the ternary crystal structure of CB1R in complex with agonist and Org275. The structure shows that Org275 binds to a cholesterol-binding site on the CB1R, suggesting that the compound works by partitioning into the bilayer and competing with endogenous cholesterol for this surface. Previous studies have demonstrated that cholesterol may act as an endogenous modulator of CB1R[17]. There is growing evidence that, instead of targeting the orthosteric site of CB1R, the allosteric site may have key advantages [15, 18, 19]. By modulating the effects of the endogenous ligand, normal physiological tone (spatial and temporal effects of ligand binding to the receptor) are maintained, as opposed to the non-physiological binding and distribution seen with exogenous direct ligands such as orthosteric agonists or antagonists.

Since discovering the CB1R allosteric site in 2005, and identification of Org275 as the first CB1R-negative allosteric modulator [13], we, and others, have worked to develop both CB1R-negative and -positive allosteric modulators. The positive allosteric modulators developed by us, and others, have shown efficacy in the treatment of neuropathic pain [20] and other therapeutic indications [21]. As Org275, and related compounds, have insufficient metabolic stability, in order to further investigate the potential of this unique class of CB1R allosteric modulator, we embarked on a chemistry campaign, with the goal of generating new molecules with improved drug-like characteristics that are more suitable for in vivo testing and clinical development. Our working hypothesis is that the unique pharmacological profile of CB1R allosterics provides a distinctive pharmacological approach for modulation of the endocannabinoid system in complex disorders, and offers an alternative to CB1R orthosteric antagonists [22]. The in vivo outcomes of this complex mechanism are yet to be elucidated, particularly in models in which the endocannabinoid system is dysregulated.

Here, we present data on the effects of a novel CB1R allosteric modulator, ABM300, in two distinct transgenic mouse models, both of which present with a state of hyperdopaminergia. Both the GluN1-Knockdown (GluN1KD) and Dopamine Transporter Knockout (DATKO) mice have increased synaptic dopamine in subcortical regions [23–25], which is implicated in their phenotypic behavioral changes [24, 26–28], as well as disrupted sensorimotor gating [29–31].

METHODS AND MATERIALS

Animal ethics
Animal housing and experimentation were carried out in accordance with the Canadian Council in Animal Care (CCAC) guidelines for the care and use of animals and following protocols approved by the Faculty of Medicine and Pharmacy Animal Care Committee at the University of Toronto and the University of Guelph Animal Care Committee, respectively.

Compound synthesis
See Supplementary Information for details.

Pharmacokinetic analyses
Microsomal stability assays were conducted by Cyprotex Ltd (Macclesfield, UK). The in vitro metabolic stability of ABM300 was measured in the presence of human or rat liver microsomes by determination of the rate of compound disappearance. Single dose in vivo PK studies were conducted by Sai Life Ltd (Pune, India) to investigate the plasma pharmacokinetics and brain distribution of ABM300 in male C57Bl6/J mice following a single intraperitoneal (i.p.) administration of a 10 mg/kg dose.

Predictive model of ABM300 bound to CB1R
The crystal structure of the CB1R-CP55940-Org275 complex (PDB code 6kqi) [16] was loaded into ICM (Molsoft, San Diego, CA), hydrogens were added, and roteameric states of hydroyx groups, histidine, asparagine, and glutamine side-chains optimized. ICM’s ligand editor was used to strip Org275 to the indole core scaffold shared with ABM300, and to incrementally grow the scaffold into ABM300, with a Monte Carlo-based energy minimization in the internal coordinate space at each step [32].

Equilibrium-binding assays
Binding assays in hCB, R CHO cells were performed by Eurofins Cereg with the CB1R agonist, $[^{3}H]$CP55,940 (0.5 nM, $K_{d}$ of 3.5 nM). Non-specific binding was defined in the presence of 10 μM WIN55,212-2.

PathHunter® β-arrestin assay
The PathHunter® β-Arrestin assay was conducted by Eurofins Pharma Discovery Services (further details can be found at https://www.eurofindsdiscoveryservices.com).

ERK1/2 phosphorylation assay
CB1R-mediated ERK1/2 phosphorylation was quantified using an AlphaLisa® Surefire® Ultra™ PERK1/2 Assay (PerkinElmer, Woodbridge, ON) according to the manufacturer’s protocol, in hCB1R CHO cells plated at a density of 40,000 cells/well (96-well). ABM300 IC50 values were determined in the presence of increasing concentrations of ABM300 at the EC50 for CP55,940 (40 nM). Results are presented as the percent stimulation of ERK1/2 phosphorylation by CP55,940 alone.

Cyclic AMP (cAMP) assays
A DiscoverX HitHunter® cAMP Assay for Small Molecules (DiscoverX, Fremont, CA) was used to quantify cAMP levels as per the manufacturer’s instructions. hCB1R CHO cells were seeded at a density of 40,000 cells/well (white 96-well), and after 24 h were serum-starved in the presence of 0.1% Bovine Serum Albumin for 60 min prior to pretreatment for 30 min with increasing concentrations of ABM300 (10 $^{-10}$–$^{-10}$ M) and a final 30 min incubation with 40 nM CP55,940 and 5 μM forskolin. The cAMP BREX experiments were performed as previously described [33, 34]. Briefly, hCB1R HEK293 cells were transfected with 5 μg/10 cm dish of the CAMYEL biosensor using polyethyle

Drug administration
The following drugs were administered in a volume of 10 mL/kg via i.p. injections in a vehicle consisting of 95% ethanol, Tween80, and 0.9% NaCl in a 1:1:18 ratio, 30 min before behavioral testing, unless otherwise stated: ABM300 (10 mg/kg), rimonabant (RIM; 10 mg/kg), Cayman Chemical, Cat.# 9000484, Ann Arbor, MI), olanzapine (OLA; 1 mg/kg, gift from MedReleaf, Markham, ON, CA), and Δ9-tetrahydrocannabinol (THC; 10 mg/kg, gift from MedReleaf, Markham, ON, CA).

Cannabinoid-induced tetrad behaviors
Male C57Bl6/J mice (PD > 70) were tested on the cannabinoid-induced tetrad, as previously described [35].

Behavioral testing in murine models of hyperdopaminergia
The effect of ABM300 was compared to OLA. GluN1KD (F1 on C57Bl6/J x 129/SvImJ background) [26] and DATKO (C57Bl6/J background) [24] mice were used as murine models of...
In vitro pharmacology: ABM300 increases agonist binding but inhibits CB₁R orthosteric agonist signaling

In line with our previous studies using a related compound (Org275) [13], we find that ABM300 causes a significant and concentration-dependent increase in the specific binding of [³H]CP55,940 to hCB₁R CHO (Fig. 2a) with an $E_{\text{max}}$ value of 328 ± 47% and an EC₅₀ value of 132 nM (pEC₅₀ 6.90 ± 0.09) and an α value of 4.33 ± 0.79 (logα 0.622 ± 0.08) (Supplementary Table S2).

In the PathHunter® β-arrestin CB₁R assay, CP55,940-stimulated β-arrestin recruitment with an EC₅₀ value of 5.37 nM (pEC₅₀ 8.28 ± 0.06) and an $E_{\text{max}}$ of 104.3 ± 0.90%. In the presence of the EC₅₀ of CP55,940 (10 nM), ABM300 produced a concentration-related reduction in β-arrestin recruitment with an IC₅₀ value of 49.7 nM (pIC₅₀ 7.31 ± 0.02) (Fig. 2b).

Using an AlphaScreen® SureFire® ERK1/2 phosphorylation assay kit, we measured the effect of ABM300 on activation of ERK1/2 phosphorylation by CP55,940 in hCB₁R CHO cells. In the presence of vehicle, CP55,940 induced ERK1/2 phosphorylation with an EC₅₀ of 12.1 nM (pEC₅₀ 8.14 ± 0.23) (Fig. 2c). At concentrations of 100 or 1000 nM, ABM300 significantly decreased CP55,940 $E_{\text{max}}$ (efficacy), to 40.3 ± 5.57% and 14.7 ± 5.22%, respectively (Fig. 2c). ABM300 alone did not affect ERK1/2 phosphorylation at concentrations up to 10 µM (Fig. 2c). Additionally, 100 or 1000 nM ABM300 significantly decreased the $E_{\text{max}}$ (efficacy) of AEA from 100.3 ± 0.20% to 80.23 ± 14.4% and 31.88 ± 6.09%, respectively (Fig. 2d). In the presence of the EC₅₀ of CP55,940 (40 nM) of CP55,940, ABM300 produced a concentration-related reduction in ERK1/2 phosphorylation with an IC₅₀ value of 47.0 nM (pIC₅₀ 7.38 ± 0.12) (Fig. 2e). CP55,940 inhibited forskolin-stimulated cAMP accumulation in hCB₁R CHO cells (data not shown). At CP55,940 EC₅₀ (40 nM), ABM300 blocked this inhibition with an IC₅₀ value of 379 nM (pIC₅₀ 6.45 ± 0.08) (Fig. 2f).

We further characterized the real-time kinetic effect of ABM300 using a cAMP BRET sensor assay in HEK293 cells. Similar to...
previous observations with Org275 [38], ABM300 produced a complex, concentration and time-dependent modulation of agonist-mediated regulation of cAMP levels (Fig. 2g, h). Levels of cAMP were measured over time with a high concentration of CP55,940 (1 μM) in the presence of varying concentrations of ABM300. Consistent with Org275 observations, ABM300 did not affect the initial inhibition of cAMP by CP55,940 but, following a concentration-dependent “lag” in drug onset, inhibition of the agonist effect became apparent. At high concentrations, the ABM300 inhibitory effect overcame the CP55,940 effect and further enhanced cAMP levels above those produced by forskolin alone—reflecting inverse agonism.
ABM300 does not bind to the CB2 receptor and does not have off-target effects in a Safety Screen® panels. Off-target effects of ABM300 were assessed using the SafetyScreen® conducted at Eurofins Discovery Services. The screen assesses the selectivity of the compound on a diverse panel of targets that includes GPCRs, drug transporters, ion channels, nuclear receptors, kinases, and other non-kinase enzymes. The screen employs radioligand binding or enzyme assays for these targets. At 1 µM, ABM300 did not display any significant binding to these targets, including receptors CB2, Dopamine D1 and D2, NMDA, 5HT1A, 5HT1B, 5HT2A, 5HT2B, and 5HT3 (Supplementary Fig. S2).

ABM300 has no effect in the cannabinoid-induced tetrad alone and does not display anxiogenic-like effects.

We confirmed that ABM300 (10 mg/kg) did not have agonist activity via the cannabinoid-induced tetrad, compared to THC (10 mg/kg). In all four tetrad measures (Fig. 3), THC, but not ABM300, produced effects (p < 0.0001 for all outputs). Previous reports demonstrated adverse effects observed with CB1 receptor orthosteric agonists/inverse agonists [39, 40]. Possible anxiogenic effects of ABM300 (10 mg/kg) were investigated using the EPM and compared to rimonabant (10 mg/kg) (Supplementary Fig. S3). ABM300 did not affect time spent in the open arms (p = 0.3335).
ABM300 decreases novelty-induced hyperactivity, exaggerated stereotypy, vertical exploration, and normalizes pre-pulse inhibition (PPI) in DATKO mice. The DATKO mouse, another model of hyperdopaminergia, displays hyperactivity, increased stereotypy and vertical exploration, with an impairment in sensorimotor gating [24, 31]. Similar to the GluN1KD model, DATKO mice showed no change in Cnr1 mRNA expression (Supplementary Fig. S6). ABM300 normalized dysregulated hyperactivity, stereotypic movements, vertical exploration and sensorimotor gating in the DATKO mice (Fig. 5), a pattern of findings similar to what was seen in the GluN1KD model (Fig. 4). For the hyperactivity measure (Fig. 5a), a significant interaction was found between ABM300 and genotype (F[1,46] = 9.38, p = 0.004), indicating that ABM300 has genotype-specific effects on the exacerbated hyperactivity endophenotype in DATKO. There was an effect of genotype on exaggerated stereotypic movements and mania-like rearing behavior (F[1,46] = 40.18, p < 0.001; F[1,46] = 26.50, p < 0.001, respectively), which ABM300 had a beneficial effect in decreasing (F[1,46] = 14.22, p < 0.001; F[1,46] = 7.38, p = 0.009, respectively) (Fig. 5b, c). Furthermore, the actions of ABM300 extended to sensorimotor gating deficits present in DATKO (Fig. 5d), with a rescue of the PPI deficit at the 16 dB pre-pulse interval (p = 0.018). Neither genotype, nor treatment with ABM300,
had an effect on the acoustic startle response in the DATKO model (data not shown, genotype: $p = 0.214$, ABM300: $p = 0.516$).

ABM300 has no effect on the conditioned avoidance response (CAR)

The CAR task has been used as a test to infer antipsychotic efficacy via the selective suppression of the avoidance response [41, 42]. Administration of olanzapine (1 mg/kg), but not ABM300 (10 mg/kg), attenuated avoidance behavior in CAR testing (Supplementary Fig. S7). A main effect of drug was observed ($p = 0.007$). Furthermore, olanzapine, but not ABM300, enhanced escape responding during CAR (Supplementary Fig. S7B), with a main effect of drug treatment ($p = 0.011$) and an interaction between drug treatment and testing day ($p = 0.034$). Lastly, neither ABM300 nor olanzapine affected escape failures (Supplementary Fig. S7C). ABM300 did not induce catalepsy, or changes in body temperature (Supplementary Fig. S7D,E).

DISCUSSION

There is growing interest in the possible therapeutic potential of CB$_1$R allosteric molecules [21]. Here, we show for the first time that a novel CB$_1$R allosteric modulator ameliorates select disrupted behaviors in two distinct models of hyperdopaminergia. The rescue of these phenotypes by ABM300 occurred without adverse anxiogenic-like or cannabimimetic effects traditionally observed with orthosteric CB$_1$R inverse agonists. Thus, these findings represent the first study demonstrating the potential for the use of a CB$_1$Ra allosteric modulator as a therapeutic strategy for the treatment of hyperdopaminergic states, such as psychosis and mania.

Our molecular docking data indicate that ABM300 binds to the recently elucidated binding site for the original allosteric modulator Org275 [16]. The Org275-binding site on CB$_1$R overlaps with a cholesterol-binding site, which is an extrahelical site within the inner leafllet of the membrane. The model is broadly in agreement with literature demonstrating that Org275 apparently stabilizes a high affinity, agonist bound CB$_1$R [43], which may impede activation of selected downstream signaling pathways. The structure proposes a mechanism by which Org275, binding to the cholesterol site, captures an intermediate conformation that binds the orthosteric agonist and also inhibits G protein coupling. The model accommodates the complex effects of Org275 on CB$_1$ orthosteric ligand binding, including the increase in $B_{max}$ of agonists. ABM300 displays a similar in vitro pharmacological profile to Org275 [13, 15], whereby it increases CB$_1$R agonist binding with an $\alpha$ value $> 1$ ($4.33 \pm 0.79$) [44], but acts as a...
functional inhibitor of CB₁R agonist-mediated β-arrestin recruitment (IC₅₀, 50 nM), ERK phosphorylation (IC₅₀, 47 nM), and cAMP inhibition (IC₅₀, 380 nM).

As previously described [38], the unique time-dependent mechanism of action of ABM300 (Fig. 2g) posits that at moderate concentrations (high nM/low µM), early agonist signaling may remain unaffected; with inhibition initiating after a lag. These observations indicate that the molecular mechanism of action of ABM300 is related to previously characterized CB₁R allosteric modulators such as Org275 and PSNCBAM-1 [38]. This may introduce potential for a unique kinetic profile of modulation of endocannabinoid signaling. The consequences of such effects at a network level and in a disease state are highly complex but may underlie the beneficial effects observed with ABM300 in the genetic models of hyperdopaminergia. Furthermore, expression levels, affinity, and pre-coupling of CB₁R can significantly differ in various neuronal cell types; CB₁R on GABAergic interneurons have significantly higher agonist affinity than those found on glutamatergic terminals, but the coupling efficacy of glutamatergic CB₁R is significantly higher [45, 46]. This expression and affinity profile leads to the complex nature in the function of the endocannabinoid system, which explains the diverse effects of certain cannabionoids, and the opposing effects in different illnesses [46]. We hypothesize that, in the genetic murine models of hyperdopaminergia, ABM300 acts as a modulator of endogenous CB₁R signaling in vivo and, potentially, selectively modulates the endocannabinoid system in specific neurotransmitter system pathways to ameliorate the dysregulated behaviors observed in these mice.

Studies have demonstrated that administration of compounds that increase endocannabinoid levels modulate a number of schizophrenia-like responses in mice [47]. Studies in cultured cells have shown that the related compound, Org275, causes migration of CB₁R to the soma, while cholesterol, which binds to the same site as ABM300 and acts as a positive modulator, allows for the enrichment of CB₁R at the axon [48]. Thus, in addition to complex effects on endocannabinoid affinity and signaling, there is the potential for the modulation of topological CB₁R membrane localization by CB₁R allosterics. The consequences of this in a complex neuronal network, and pathological states, are yet to be fully elucidated.

To assess psychosis-like behaviors, and therapeutically efficacious of ABM300, we focused on two main behavioral categories: exploratory behavior (hyperactivity) and sensorimotor gating (disruption in PPI). The literature related to psychosis-like behaviors in genetically modified mouse models has classically focused on the same two categories [49]. Although these behaviors do not directly translate to human symptoms present in the disease, they do mimic the neurotransmitter changes that are involved in psychotic symptoms. It has been accepted that subcortical hyperdopaminergia is implicated in psychosis, confirmed further by the neuropsychopharmacological action of antipsychotic drugs currently available; all licensed pharmacological treatments of psychosis (antipsychotics) require interactions with the dopamine D₂ receptor [50]. Therefore, by assessing locomotor behavior within this study, we can infer that, driven by subcortical dopamine levels, an increase in dopamine leads to enhanced motor activity (either horizontal, rearing, and/or stereotypy) [49]. Meanwhile, disruption in PPI allows for a more straightforward phenotypic analysis of psychosis, as it has been reported in a number of psychiatric diseases, particularly schizophrenia and psychosis [51].

Here, we show that ABM300 restores dysregulated dopamine-mediated exploratory activity in both genetic models: decreasing exaggerated hyperactivity, stereotypy and rearing. Furthermore, ABM300 rescues PPI deficits in the DATKO model. Since psychosis symptomology is never present alone in a disease state such as schizophrenia, it would be intriguing to investigate the effects of ABM300 in other symptomatic domains, such as cognition. CB₁R antagonism and loss of function may enhance some forms of learning and memory, and it is possible that CB₁R-negative allosteric modulators may be pro-cognitive in preclinical schizophrenic models [52, 53].

We saw no effect of the compound in CAR in rat even though olanzapine produced significant suppression of CAR as has been shown in previous studies [54]. CAR is an extensively validated preclinical test used to predict therapeutic efficacy of antipsychotics that directly target the dopaminergic and serotonergic receptor systems [42, 55]. This experiment further supports our assertion that ABM300’s antipsychotic effects are not mediated via dopamine D₂ receptors, as considerable occupancy of striatal D₂ receptors is required to see suppression of CAR (65–80% for typical antipsychotics, ~50% for atypical antipsychotics like clozapine) [42].

It is important to note that we do not have direct evidence for the involvement of the CB₁R in the in vivo effect of ABM300 in genetic models. Directly implicating CB₁R is challenging. One approach would be to create a double knockout of CB₁R/-/- and DAT-/- or GluN1-/-/. However, given the crucial role of the CB₁R in dopaminergic and glutamatergic signaling, there is a strong likelihood that the double knockouts could have a novel phenotype (such as seizures) or that previous phenotypes would be exacerbated [56, 57]. Furthermore, genetic manipulation of the CB₁R has been shown to have effects on dopamine signaling [58, 59]. Another approach would be to investigate whether a CB₁R orthosteric antagonist would block the effects of ABM300, thereby implicating CB₁R. However, there is also doubt as to whether an orthosteric CB₁R antagonist would block the effects of a negative allosteric inhibitor (antagonist blocking inhibitor); it is conceivable that a synergistic effect might be observed. Taken together, these limitations highlight the complexity of directly implicating CB₁R in the mechanism of action of ABM300 in vivo in these genetic models. We plan to make this the subject of future investigations involving a variety of in vivo and ex vivo approaches. The pharmacological profiling and assessment of the potential for off-target interactions of ABM300 in binding screens suggest that the compound is CB₁R-selective. Taken together, the potent inhibitory effect of the compound on CB₁R signaling at nM concentrations in vitro and the favorable PK and brain penetration data, there is substantial support for the hypothesis that the CB₁R mediates the effects of ABM300 in vivo in the genetic mouse models of hyperdopaminergia. It is also notable that the present study only included acute administration of one dose of ABM300. Future experiments will focus on various dose regimens, chronic dosing, development of tolerance and the effects of ABM300 in combination with an antipsychotic.

The data presented here offer the first evidence that acute administration of a CB₁R allosteric modulator, with a unique pharmacological profile, effectively ameliorates certain behavioral deficits in two distinct models of increased dopamine. These data highlight that the allosteric-binding pocket on the CB₁R warrants further investigation as a potentially important therapeutic target in psychiatry.

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AUTHOR CONTRIBUTIONS

All authors included in this manuscript have contributed to at least one of the following ICMJE guidelines: (1) Substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data for the work; (2) Drafting the work or revising it critically for important intellectual content; (3) Final approval of the version to be published; (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ADDITIONAL INFORMATION

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