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RESEARCH ARTICLE

Optimized 5-HT$_{2b}$ inhibitors for neuropsychiatric syndromes with cognitive dysfunction

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Abstract

INTRODUCTION: Neuropsychiatric syndromes such as anxiety and agitation are clinical presentations common to diverse neurodegenerative diseases and brain injury sequelae. They are a concern due to the impact on cognition, social interactions, and non-pharmacological treatments. Cognitive or behavioral disturbances occur at early disease stages and increase with disease progression. Coincident pathologies include the loss of serotonin (5-HT) neurons and appearance of neurofibrillary tangles in the raphe nucleus. Brain 5-HT$_{2b}$ receptor (5-HT$_{2b}$R) levels are increased in Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and post-stroke morbidity. *HTR2B* gene variants are implicated in psychiatric disorders. 5-HTRs are associated with atypical neurotropic drug mechanisms and behavioral dysfunction in drug abuse. The accumulating body of evidence suggests that selective 5-HT$_{2b}$R inhibition might mitigate neuropsychiatric syndromes and the associated cognitive dysfunction. Atypical neurotropic drugs interact with a variety of monoamine receptors and outcomes are viewed as a combination of 5-HT and dopamine D2 receptor mediated actions. Clearly, there is a need for insight into precision 5-HT$_{2b}$R inhibition as a potential pharmacological mechanism for treatment of neuropsychiatric syndromes and cognitive dysfunction associated with dementia.

METHODS: Strategic optimization of an atypical neurotropic drug was used to develop MW073, a highly selective and orally bioavailable inhibitor of 5-HT$_{2b}$R activity and β-arrestin-1 recruitment that is devoid of dopamine receptor recognition and risk of 5-HT$_{2b}$R agonist activity.

RESULTS: MW073 ameliorates amyloid and tau induction of behavioral dysfunction in preventive or disease stage intervention paradigms. Using MW073 as a standard of comparison, risperidone was shown to be a dose-dependent inhibitor 5-HT$_{2b}$R activity and β-arrestin-1 recruitment.

Saktimayee M. Roy and Erica Acquarone contributed equally to this work.

DISCUSSION: Selective inhibition of 5-HT_{2b}R activity is a viable mechanism for the treatment of neuropsychiatric syndromes with synaptic dysfunction as a root cause and is a previously unrealized pharmacodynamic mechanism potentially embedded in current neurotherapeutics.

KEYWORDS

5-HT_{2B}, aggression, Alzheimer's disease, Anxiety, atypical neurotherapeutics, cognitive dysfunction, depression, G-protein–coupled receptor serotonin receptors, humans, irritability, memory, mental health, risperidone, synaptic plasticity, tauopathies

Highlights

- A new highly selective 5-HT_{2b}R antagonist, MW073, is described and used as a reference standard.
- MW073 attenuates synaptic and behavioral dysfunctions an animal models of neuropsychatric syndromes.
- Risperidone is a dose dependent inhibitor of 5-HT_{2b}R activity and arrestin recruitment.

1 | BACKGROUND

Neuropsychiatric syndromes such as anxiety and agitation are clinical presentations common to diverse neurodegenerative diseases, neurodevelopment complications, and brain injury sequelae.[1,2] The syndromes affect patient quality of life across the life span and are a clinical concern due to the impact on cognition, social interactions, and non-pharmacological interventions. In the case of Alzheimer's disease (AD), initial presentation to the clinic is usually for cognitive or minor behavioral disturbances that increase in intensity with disease progression and biomarker emergence.[3–6] Pathologies coincident with behavioral progression include the loss of serotonin (5-hydroxytryptamine; 5-HT) neurons in the raphe nucleus and appearance of neurofibrillary tangles in the dorsal raphe nucleus (DRN), a region rich in 5-HT neurons.[7–10] At disease stage, brain 5-HT_{2b} receptor (5-HT_{2b}R) levels are increased in AD, stroke, and amyotrophic lateral sclerosis (ALS).[11–13] Further, human *HTR2B* gene variants are implicated in psychiatric syndromes.[14] Pharmacologically, neuropsychiatric therapeutics include pleiotropic neurotropics that have 5-HTRs among their multiple targets and behavior-modifying recreational drugs (such as MDMA, ecstasy) include 5-HT_{2b}R as a molecular target. However, drug responsiveness varies broadly among individuals, the undesired side effects can impact patient health, and the potential for severe events requires close monitoring. Clearly, there is a need to directly test if selective 5-HT_{2b}R inhibition is a potential molecular mechanism of action that can be leveraged for clinical neuropsychiatric therapeutics.

The initial challenge for investigation is the absence of a standard of comparison. A reference standard should be a highly selective and pharmacologically derisked 5-HT_{2b}R inhibitor that is suitable for in vivo evaluations. Therefore, we developed MW073, a unique 5-HT_{2b}R inhibitor whose biochemical mechanism of action is concentration dependent inhibition of 5-HT_{2b}R activity and β-arrestin-1 recruitment. Its cellular mechanism of action is attenuation of stressor-induced synaptic dysfunction, a generally accepted mechanism of clinical cognitive and behavioral dysfunctions. MW073 attenuates amyloid beta (Aβ)- and tau-induced behavioral dysfunction in discrete animal models. Further, MW073 can serve as a standard of comparison to screen clinically effective drugs for a potentially embedded 5-HT_{2b}R mechanism of action, allowing a more informed use or identification of candidates for future repurposing using strategic optimization.

2 | METHODS

Due to publication space limitations, the details of MW073 discovery, production, and pharmacological activities as well as control behavioral studies and comparative drug analyses are provided in Data S1 (Figure S1, Tables S1–S7 in supporting information). Pharmacological qualification and analysis of in vivo risk are also presented along with detailed experimental results. Data include details of competitive 5-HT_{2b}R binding, large-scale cellular screening of G-protein–coupled receptors (GPCRs) for agonist and antagonist activity, GPCR activation and arrestin activity assays, kinome-wide inhibitor screening, acetylcholinesterase (AChE) and monoamine oxidase substrate risk, and metabolic stability. A comparison of 5-HT_{2b}R regulation by MW073, minaprine, and risperidone is summarized in Table S8 in supporting information.

2.1 | Animals

C57BL/6J, APP/PS1, and hTau/Mapt-KO mice (3–4 months of age; male and female) were kept on a 12 hour light/dark cycle in temperature- and humidity-controlled rooms with ad libitum food and

water in the Columbia University animal facility. APP/PS1 heterozygous double transgenic mice expressing both human amyloid precursor protein (APP; K670N:M671L) and human presenilin 1 (PS1; M146L; line 6.2)[15] were obtained by crossing heterozygous *APP* with *PS1* animals. hTau/Mapt-KO mice were obtained by crossing hTau mice in a murine tau-hemizygous background to generate hTau/Mapt-KO mice and siblings. The hTau animals express wild-type (WT), full-length human tau (2N4R) driven by the prion promoter. Animals were genotyped at weaning using polymerase chain reaction.[16,17] Methods for cannula implant surgery, electrophysiological recordings, behavioral studies, and statistical analyses are provided in Data S1.

2.2 | Chemicals and supplies

Chemicals were purchased from Aldrich or VWR International. Solvents were used as received unless stated otherwise. Water was obtained using a Milli-Q Biocel A10 purification system from Millipore Corporation. Synthesis of MW01-26-073SRM (MW073) was done using a Good Manufacturing Practice–compatible synthetic scheme presented in supporting information. Human Aβ_{42} oligomerization was done as described previously.[18,19] Tau oligomers were obtained as previously described.[20]

3 | RESULTS

The 5-HT$_{2b}$R is a member of the neurotransmitter serotonin receptor family that has been neglected as a potential therapeutic target, potentially due to the high toxicology risk associated with drugs that exhibit 5-HT$_{2b}$R agonist activity. However, serotonin stimulation of 5-HT$_{2b}$R activates the Gα_q protein subtype coupled to phospholipase C-β2 (PLC-β2), an important signaling pathway linked to clinical lipid profiles that are increasingly associated with neuropsychiatric conditions.[21] MW073 was developed to provide a pharmacologically qualified reference standard. It is a selective inhibitor of 5-HT$_{2b}$R activity and can also serve as a starting point for future development of more selective 5-HT$_{2b}$R candidates derisked of off-target liabilities, including 5-HT$_{2b}$R agonist activity. The MW073 molecular mechanism of action was elucidated for both human (h5-HT$_{2b}$) and mouse (m5-HT$_{2b}$) receptors to facilitate interpretation of mouse model outcomes in the context of its human counterpart. Further, MW073 was tested for selective dose-dependent 5-HT$_{2b}$R inhibition to prevent or attenuate synaptic and behavioral dysfunction induced by AD relevant stressors. Finally, MW073 was used as a standard of comparison to probe the previously unappreciated potential of dose-dependent inhibition of 5-HT$_{2b}$R activity by risperidone, a widely used atypical anti-psychotic approved for neuropsychiatric syndrome use for schizophrenia, autism, and AD patients.

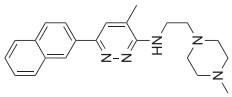
MW073 is a deliverable from the repurposing approach called strategic optimization, which allows retention or enhancement of a clinical drug's desired activities while minimizing off-target and toxicological risks (Table 1, Figure S1, Tables S1–S7). The atypical neurotherapeutic chosen for strategic optimization, CANTOR (CM 30038; minaprine), showed low risk for cardiotoxicity, drowsiness, or weight gain in neurology patients plus a suggestive trend in AD patient cognitive improvement. Like most atypical therapeutics, it is a pleiotropic drug that alters both serotonin- and dopamine-regulated pathways and exhibits both anti-depressive and anti-aggressive functions dependent on disease context and dosing. Selective optimization enhanced the weak 5-HT$_{2b}$R binding activity of the clinical drug from 863 ± 8 to 70 ± 26 nM for MW073 through conservative changes at two positions on the clinical drug (Figure S1). Pharmacological characterization of MW073 documented its highly selective 5-HT$_{2b}$R inhibitory activity, cellular and biochemical mechanisms of action, and dose-dependent functions in both preventive and disease stage intervention for animal models of AD-related pathophysiology (Table 1; Data S1).

MW073's biochemical mechanism of action for both h5-HT$_{2b}$R and m5-HT$_{2b}$R is dose-dependent inhibition of serotonin 5-HT$_{2b}$R activation and β-arrestin-1 recruitment (Figure 1A). The equilibrium dissociation constants (K_d) are similar across species, 89 nM for m5-HT$_{2b}$R and 125 nM for h5-HT$_{2b}$R. The equilibrium dissociation constants (K_B) for β-arrestin-1 recruitment were also similar between m5-HT$_{2b}$R (73 nM) and h5-HT$_{2b}$R (102 nM). Interestingly, MW073 also inhibits basal constitutive activity for both species of 5-HT$_{2b}$R, suggestive of inverse agonism potential.

RESEARCH IN CONTEXT

1. **Systematic review**: Neuropsychiatric syndromes affect cognition and patient quality of life and treatments are effective for only a subset of patients and exhibit clinical risk attributed to off-target activities. Literature searches (e.g., PubMed) for pharmacological management of neuropsychiatric syndromes in Alzheimer's disease, combined with US Food and Drug Administration information, revealed multi-target atypical anti-depressants and anti-psychotics often engage both dopaminergic and serotonergic mechanisms. The latter include activity at 5-HT$_2$ receptors. Relevant publications covering the prior art are cited.
2. **Interpretation**: Our findings support the hypothesis that selective, pharmacologically derisked 5-HT$_{2b}$ antagonists are viable drug development candidates for neuropsychiatric syndromes with associated cognitive dysfunction. The findings suggest strategic optimization is a tractable approach to mining precision medicine candidates embedded in clinically effective drugs.
3. **Future directions**: A planned phase 1 clinical trial and Good Manufacturing Practice clinical drug production are immediate next steps. The anticipation is addition of a critical therapeutic to an accumulating precision medicine armamentarium.

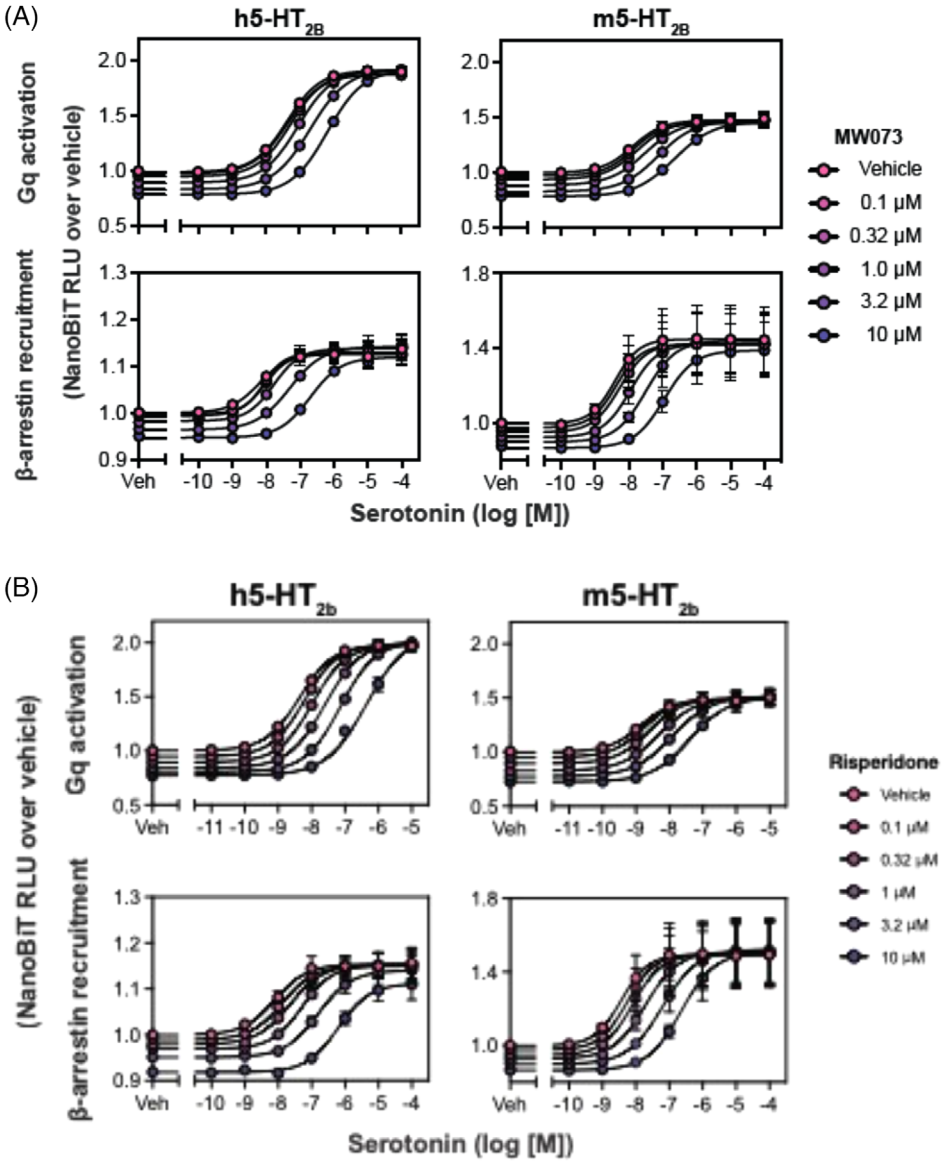
TABLE 1 Key features of MW073.[a]



	Property
	MW = 361.48
	cLogP = 3.17
	PSA = 44.29
	Rotatable bonds = 5
	H-bond donor + acceptor = 6
Target class selectivity and activity	
Large-scale cellular GPCR screen	Negative for all agonist activity screens, including $5HT2_bR$ agonist activity. Negative for all antagonist screens except for $5HT_bR$.
5HT2bR competitive binding activity	$IC_{50} = 70 \pm 26$ nM
h5HT2bR affinity – Gq protein activation (human)	$K_D = 125 \pm 13$ nM
m5HT2bR affinity – Gq protein activation (mouse)	$K_D = 89 \pm 4$ nM
h5HT2bR affinity – arrestin recruitment (human)	$K_D = 102 \pm 5$ nM
m5HT2bR affinity – arrestin recruitment (mouse)	$K_D = 73 \pm 14$ nM
Human liver microsome stability	$T_{1/2}$ >60 min
Rodent liver microsome stability	$T_{1/2}$ >60 min
Oral bioavailability (rat)	%F: 38.8±16.1(M), 35.6±13.5(F) $T_{1/2}$ (h): 5.82 ± 1.52 Exposure (ng*h/mL): $AUC_{last} = 1400 \pm 307$, $AUC_{inf} = 1480 \pm 370$
Pharmacodynamics & efficacy (mice)	• Attenuates Aβ- & tau-oligomer induced synaptic dysfunction. • Attenuates (5 mg/kg) Aβ- & tau-oligomer induced errors in radial arm water maze (RAWM) and contextual fear conditioning (FC). • Dose-dependent amelioration of RAWM and fear conditioning in APP/PSI mice. • Repeat dosing (APP/PSI) using preventive or disease state intervention respectively abolishes or attenuates RAWM and fear conditioning. • Repeat dosing (hTau/Mapt-KO) using preventive or disease state intervention respectively abolishes or attenuates RAWM and fear conditioning.
Off-target, drug–drug interaction, tox risk screening	
Kinome-wide activity screen	Negative
Caco-2 permeability/P-gp substrate	Permeable/not a substrate; 65% recovery average in each direction
Panlabs spectrum screens for off-target safety	Negative, including key enzymes (monoamine oxidase, acetylcholinesterase, COX1, COX2, PDE3A, PDE4D2), transporters, and additional receptors.
Maximum tolerated dose screen (rat): 1) 20 and 80 mg/kg, PO, daily, 7 days, 2-day recovery. 2) 40 & 60 mg/kg, PO, daily, 28 days, no recovery	No Irwin test observational abnormalities. No MW073-related clinical pathology. No histopathology in heart, spleen, lung, thymus, parathyroid, epididymis (M), ovaries (F), and brain.
Transporter inhibition screen	Negative: OCT1, BSEP, BCRP, P-gp, MATE1, MATE2-K, MRP2, OAT1, OAT3, OATP1B1, OATP1B3. Needs follow-up analysis: OCT2, MATE1, MATE2-K
Inhibition of synaptosome monoamine uptake	Negative for serotonin, dopamine, & norepinephrine reuptake.
CYP substrate status screen	Negative: 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, 2A6, 2E1, 2J2,4F2.

[a]Details of methods, data, and study reports in Data S1 in supporting information.

MW073 was used as a reference standard to probe widely used extant clinical drugs for potential $5\text{-}HT_{2b}R$ inhibition mechanisms. Risperidone is a widely used atypical anti-psychotic approved for irritability or aggression use in schizophrenia, autism spectrum disorder, and AD patients. Like other atypical neurotropic drugs, risperidone is pleiotropic and associated with regulation of dopaminergic and serotoninergic pathways. Risperidone has estimated binding affinities ranging from 47 to 253 nM for $5\text{-}HT_{1C}$, $5\text{-}HT_{1D}$, $5\text{-}HT_{1A}$, D_2, α_1 and α_2 adrenergic, and H_1 histamine receptors.[22] No binding affinities are mentioned for $5\text{-}HT_{2b}$ nor is there reported cellular activity for inhibition of $5\text{-}HT_{2b}$ activation or arrestin recruitment. We found that increasing concentrations of risperidone attenuated serotonin-mediated $h5\text{-}HT_{2b}$ and $m5\text{-}HT_{2b}$ activation of the Gq-PLC-β2 pathway and reduced β-arrestin-1 recruitment (Figure 1B). Also, like MW073, risperidone inhibited basal $5\text{-}HT_{2b}R$ in the absence of an activator. Quantitative analysis (Figure 1C) revealed a risperidone pattern like



(C)

Ligand	Receptor	Gprotein EC_{50} (μM)	Gprotein E_{max}	β-Arrestin1 EC_{50} (μM)	β-Arrestin1 E_{max}
MW073	m5-HT_{2b}	6.7 ± 8.6	0.93 ± 0.06	0.6 ± 0.3	0.88 ± 0.01
	h5-HT_{2b}	1.1 ± 0.4	0.75 ± 0.04	2.4 ± 3.4	0.94 ± 0.04
Risperidone	m5-HT_{2b}	0.5 ± 2.0	0.81 ± 0.04	0.6 ± 0.3	0.87 ± 0.02
	h5-HT_{2b}	0.5 ± 0.06	0.74 ± 0.01	2.3 ± 0.9	0.90 ± 0.01

FIGURE 1 Inhibitor of 5-HT_{2b}R activity and β-arrestin-1 recruitment. A, Serotonin-mediated Gq activation and β-arrestin-1 recruitment are attenuated by increasing concentrations of MW073. Note that human (h)5-HT_{2b} and mouse (m)5-HT_{2b} are similar in their response to MW073. Increasing concentrations of MW073 also reduce Gq activation and β-arrestin-1 recruitment in the absence of an activator (inverse agonism). Data represent mean ± SEM from three to four independent experiments performed in duplicate or triplicate. B, Serotonin-mediated Gq activation and β-arrestin-1 recruitment through h5-HT_{2b} and m5-HT_{2b} are attenuated by increasing concentrations of risperidone. Increasing concentrations of risperidone also reduce Gq activation and β-arrestin-1 recruitment in the absence of an activator (vehicle). Data represent mean ± SEM from three to four independent experiments performed in duplicate or triplicate. C, Comparison of MW073 and risperidone EC_{50} and E_{max} values for h5-HT_{2b} and m5-HT_{2b}. SEM, standard error of the mean.

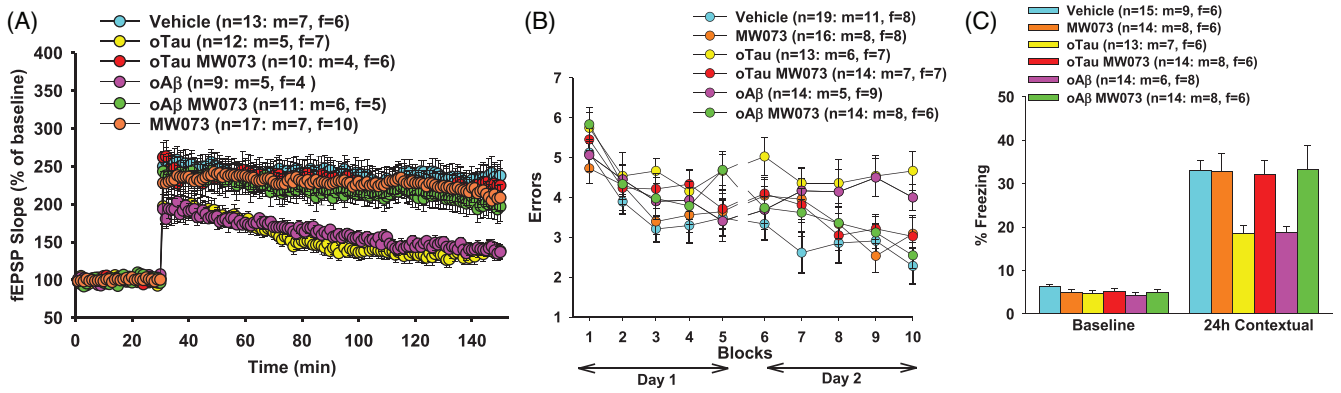


FIGURE 2 Acute MW073 administration of either Aβ or tau oligomers to C57Bl6 mice rescued the defects in LTP and memory deficits tested with the 2-day RAWM and contextual fear conditioning. A, MW073 attenuates both Aβ- and tau-oligomer–induced synaptic dysfunction. Perfusion with MW073 (1.9 μM) rescues LTP defect in hippocampal slices treated with Aβ or tau oligomers. The number of slices "n" and their sex (m = males, f = females) are indicated on the figure for this and the following LTP graphs. Overall ANOVA: $F_{(5,66)} = 6.240$, $p < 0.0001$. Two-way ANOVA among groups: $F_{(1,23)} = 19.71$, $p = 0.0002$ vehicle vs. tau; $F_{(1,20)} = 14.00$, $p = 0.0013$ vehicle vs. Aβ; $F_{(1,20)} = 15.31$, $p = 0.0009$ tau vs. tau + MW073; $F_{(1,18)} = 11.14$, $p = 0.0037$ Aβ vs. Aβ+MW073; $F_{(1,28)} = 0.2402$, $p = 0.6279$ vehicle vs. MW073. B, MW073 (5 mg/kg, i.p., 30 minutes prior to start of the test) attenuates both Aβ- and tau-oligomer–induced increase in the number of errors in the 2-day RAWM. Mice were infused with 200 nM Aβ or 500 nM tau oligomers or vehicle (intrahippocampal, 20 minutes prior to start the first and seventh trial of the RAWM for Aβ, and both 180 and 20 minutes prior to the first trial for tau). Two-way ANOVA for repeated measures among all groups (day 2): $F_{(5,84)} = 3.361$, $p = 0.0081$, one-way ANOVA for block 10: $F_{(5,84)} = 4.217$, $p = 0.0018$; one-way ANOVA for block 9: $F_{(5,84)} = 3.255$, $p = 0.0097$. C, MW073 (5 mg/kg, i.p., 30 minutes prior to start the test) attenuates both Aβ- and tau-oligomer–induced reduction in freezing in the contextual FC. Mice were infused with 200 nM Aβ or 500 nM tau oligomers or vehicle (intrahippocampal, 20 minutes prior to the electric shock for Aβ, and both 180 and 20 minutes prior to the electric shock for tau). Percent freezing corresponds to the percentage of freezing during contextual FC the day after training with the electric shock in this panel and the following ones. The number of animals "*n*" and their sex are indicated on the figure for this and the following behavior graphs. One-way ANOVA $F_{(5,78)} = 4.387$, $p = 0.0014$; Bonferroni: $p = 0.0215$ vehicle vs. Aβ, $p = 0.0365$, Aβ vs. Aβ+MW073, $p = 0.0364$ vehicle vs. tau, $p = 0.0309$ tau vs. tau+MW073. Aβ, amyloid beta; ANOVA, analysis of variance; FC, fear conditioning; i.p., intraperitoneal; LTP, long-term potentiation; RAWM, radial arm water maze.

MW073 in terms of Gprotein EC_{50} and E_{max} as well as β-arrestin-1 EC_{50} and E_{max}. Overall, risperidone mimics MW073 inhibition mechanism of action for mouse and human 5-HT_{2b}R.

The extensive pharmacological qualification of MW073 summarized in Table 1 and detailed in Data S1 qualifies MW073 for use as a selective in vivo probe of pharmacodynamic effects associated with 5-HT_{2b}R inhibition. For example, MW073 exhibited only 5-HT_{2b}R antagonist activity when subjected to large-scale cellular screening of 165 diverse GPCRs for agonist and antagonist activity. There was no agonist activity detected for any GPCR, including the 5-HT_{2b}R agonism associated with adverse events. Further, kinome-wide analysis of activity in optimized assays showed no off-target kinase inhibitor activity, removing complications of indirect cellular effects brought about by phosphorylation at regulated GPCRs, GPCR accessory proteins, or downstream signaling pathways. In addition, a battery of other secondary pharmacology testing outcomes added to the qualification profile. For example, maximal tolerated dose and drug–drug/drug–food interaction risk analysis allows a more informed interpretation of phenotypic responses and pharmacodynamic effects across efficacy doses under a variety of experimental conditions. In this regard, MW073 fills a critical gap in the field in that it provides the first in vivo molecular probe with the appropriate target selectivity and pharmacological profile for exploring the linkage of selective 5-HT_{2b}R inhibition, prevention of synaptic dysfunction, and attenuation of behavioral and cognitive disorders often associated with AD. Experimentally, Aβ and tau elevation are used as surrogates for induction of disease pathophysiology and related behavioral dysfunction. A cellular mechanism in neurological disease progression is synaptic dysfunction that is reflected phenotypically in behavioral and cognitive pathophysiology. Therefore, MW073 was used in a series of synaptic and behavioral dysfunction studies whose aggregate outcomes provided a close association between selective inhibition of 5-HT_{2b}R activation/β-arrestin-1 recruitment and prevention of synaptic and behavioral dysfunction.

First, MW073 protects against long-term potentiation (LTP) reduction by Aβ and tau oligomers (Figure 2A). The behavioral impact was confirmed by memory studies using contextual fear conditioning, a test that assesses associative memory, and 2-day radial-arm water maze (RAWM), a task that assesses short-term spatial memory (Figure 2B,C). The beneficial effect of MW073 in the behavioral experiments was genuinely due to an effect on memory because control experiments with the visible platform task (to rule out interference of sensory, motor, and motivational effects), the open field (to rule out exploratory behavior disorders), cued conditioning (to rule out amygdala involvement), and sensory threshold assessment (to rule out change in perception of the electric shock) did not show any difference among groups of mice (Figure S2A–F in supporting information).

Second, repeat daily administration of MW073 in the transgenic (Tg) APP/PS1 mouse, an animal model of amyloid elevation, protects against the deleterious effect of APP overexpression (Figure 3A,B), like Aβ- and tau-oligomer infusion models. The protection is dose depen-

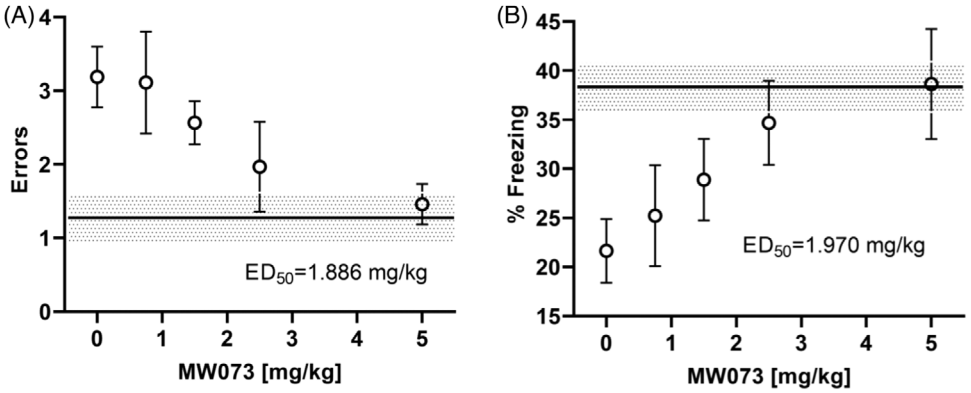


FIGURE 3 Repeat MW073 administration to APP/PS1 mice ameliorates the RAWM and FC defects in a dose-dependent manner. A, Repeat administration of increasing MW073 doses to APP/PSI mice proportionately reduced the number of RAWM errors. The ED_{50}s are indicated in the graphs. Mice were treated with increasing concentrations of MW073 (oral gavage, daily, for 30–45 days starting at the age of day 60–70). RAWM errors correspond to the number of errors that mice made at the last set of trials. B, Repeat administration of increasing MW073 doses (as in [A]) to APP/PSI mice ameliorated in a dose-dependent manner the contextual FC defect. For these experiments 9 to 16 mice per group were tested. The shaded area corresponds to the average number of errors in the RAWM or percent freezing in the FC (continuous lines) and the standard error range in vehicle treated WT mice. FC, fear conditioning; RAWM, radial arm water maze; WT, wild type.

dent. The results establish an initial link among synaptic function, in vivo pathophysiology, and MW073 pharmacodynamics.

Third, MW073 was tested in APP/PS1 mice in a preventive paradigm as previously described.[19] Briefly, WT and APP/PS1 littermates were daily administered with MW073 or vehicle starting from the age of day 60 to 70 (when there are no amyloid plaques nor LTP or cognitive impairment) until euthanasia at the age of day 105 to 115 when hippocampal slices were harvested to record LTP. Behavioral assessment started at 90 days when amyloid plaques have appeared in the brain and both LTP and cognitive impairment are present.[16] MW073 prevented the appearance of electrophysiological and behavioral deficits (Figure 4A–C). The beneficial effect of MW073 in these experiments was also attributable to an effect on synaptic plasticity and memory because control experiments measuring the basal synaptic transmission, and behavioral controls did not show any difference among the four groups of mice (Figure S3A–G in supporting information).

MW073 was able to prevent electrophysiological and behavioral impairments when administered prior to disease pathophysiology appearance and restored them when administered in a disease state paradigm. In placebo-controlled experimental designs, treatment with MW073, starting from the age of ≈ 100 days throughout behavioral assessment that started at 130 days until euthanasia at the age of ≈ 145 days, protected the APP/PS1 mice against both electrophysiological and behavioral defects (Figure 4D–F). There was no difference among groups of mice in control experiments measuring basal synaptic transmission and control behavioral tasks (Figure S4A–G in supporting information).

Fourth, abnormal tau elevation is another hallmark of AD so we tested MW073 in the human tau (hTau)/Mapt-KO mouse model of tau elevation[17] both in the preventive and disease state paradigms. In these mice, the endogenous murine tau has been knocked out, but WT human tau is expressed at very similar levels as murine tau. Animals display tau oligomers, but not tangles, at the age of 8 months associated with age-dependent LTP impairment as well as reduction in performance with RAWM and contextual fear conditioning. Treatment with MW073 or vehicle protected hTau/Mapt-KO mice against both electrophysiological and behavioral defects in the preventive paradigm (Figure 5A–C) with no difference among groups of mice in control experiments measuring basal synaptic transmission, and behavioral controls (Figure S5A–G in supporting information). Moreover, MW073 protected hTau/Mapt-KO mice against both electrophysiological and behavioral defects when administered in a disease state paradigm (Figure 5D–F) with no difference among groups of mice in control experiments measuring basal synaptic transmission and behavioral controls (Figure S6A–G in supporting information).

Taken in aggregate, the convergent outcomes from distinct efficacy models demonstrate that MW073 can attenuate pathophysiology progression induced by AD-relevant stressors. Further, MW073 can be used in preventive or disease stage paradigms of intervention.

The linkage among cellular and molecular mechanisms with attenuation of behavioral and cognitive dysfunction facilitates the use of MW073 as a standard of comparison for 5-HT_{2b}R inhibition phenotype in disease models.

4 | DISCUSSION

Key outcomes from this study include (1) demonstration that 5-HT_{2b}R is a qualified molecular target for future neuropsychiatric intervention studies, especially in diseases such as AD, ALS, and post-stroke morbidity in which 5-HT_{2b}R levels are increased; and (2) documentation that clinically effective neuropsychiatric drugs might possess dose-dependent 5-HT_{2b}R inhibitory mechanisms not previously recognized. MW073 is an orally bioavailable 5-HT_{2b}R inhibitor with an exceptional target selectivity and pharmacological profile, defined molecular and cellular mechanisms of action, and efficacy in preventive

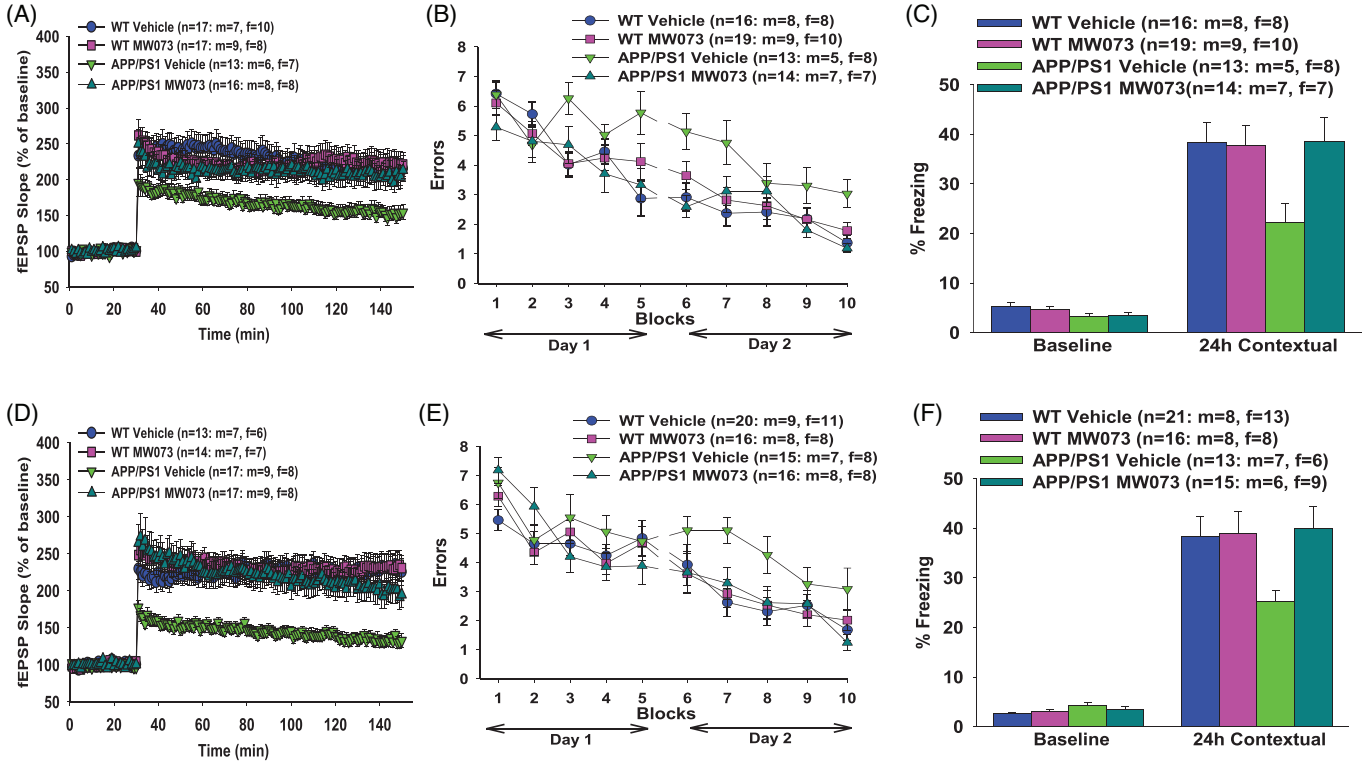


FIGURE 4 Repeat daily MW073 administration in a preventive paradigm (A–C) and during disease state (D–F) abolishes the impairment of LTP and both spatial and associative memory in APP/PS1 mice. A, Prevention of the LTP defect after treatment with MW073 of the APP/PS1 mouse. MW073 (5 mg/kg, oral gavage, daily, from day 60–70 for ≈ 45 days) prevents the LTP defect. MW073 alone in WT littermates does not affect potentiation. Overall ANOVA $F_{(3,59)} = 3.562$, $p = 0.0194$. Two-way ANOVA among groups: $F_{(1,32)} = 0.06575$, $p = 0.7993$ WT vehicle vs. WT + MW073; $F_{(1,28)} = 9.412$, $p = 0.0047$ WT vehicle vs. APP/PS1 vehicle; $F_{(1,27)} = 8.132$, $p = 0.0082$ APP/PS1 vehicle vs. APP/PS1 + MW073; $F_{(1,31)} = 0.4089$, $p = 0.5272$ WT + MW073 vs. APP/PS1 + MW073. B, Prevention of the defect of spatial memory tested through the RAWM after treatment with MW073 of the APP/PS1 mouse. MW073 (5 mg/kg, oral gavage, daily, from day 60 to 70 for ≈ 45 days) protects mice against the impairment of spatial memory, while MW073 alone in WT littermates does not affect performance. RAWM: two-way ANOVA for repeated measures among all (day 2): $F_{(3,58)} = 4.907$, $p = 0.0042$; one-way ANOVA for block 10: $F_{(3,58)} = 5.882$, $p = 0.0014$; one-way ANOVA for block 9: $F_{(3,58)} = 1.830$, $p = 0.1518$; one-way ANOVA for block 8: $F_{(3,58)} = 0.6642$, $p = 0.5775$; one-way ANOVA for block 7: $F_{(3,58)} = 3.468$, $p = 0.0218$; one-way ANOVA for block 6: $F_{(3,58)} = 4.539$, $p = 0.0063$. C, Prevention of the defect of associative memory tested through the contextual FC after treatment with MW073 of the APP/PS1 mouse. MW073 protects mice against the impairment of associative memory, while MW073 alone in WT littermates does not affect memory. ANOVA $F_{(3,54)} = 2.847$, $p = 0.0460$; Bonferroni $p = 0.0503$ WT vehicle vs. APP/PS1 vehicle, $p = 0.0547$; APP/PS1 vehicle vs. APP/PS1 MW073, $p > 0.999$; WT vehicle vs. WT MW073. D, MW073 (5 mg/kg, oral gavage, daily, from day ≈ 100 for ≈ 45 days) reverts the LTP defect in the APP/PS1 mouse. MW073 alone in WT littermates does not affect potentiation. Overall ANOVA $F_{(3,57)} = 9.256$, $p < 0.0001$; two-way ANOVA among groups: $F_{(1,25)} = 0.1559$, $p = 0.6963$; WT vehicle vs. WT + MW073; $F_{(1,28)} = 30.17$, $P\,p < 0.0001$; WT vehicle vs. APP/PS1 vehicle; $F_{(1,32)} = 16.03$, $p = 0.0003$; APP/PS1 vehicle vs. APP/PS1 + MW073; $F_{(1,29)} = 0.2725$, $p = 0.6065$; WT + MW073 vs. APP/PS1 + MW073. E, MW073 (5 mg/kg, oral gavage, daily, from day ≈ 100 for ≈ 30 days in this and the following graph) protects APP/PS1 mice against the impairment of spatial memory tested with the RAWM, while MW073 alone in WT littermates does not affect performance. Two-way ANOVA for repeated measures among all (day 2): $F_{(3,63)} = 3.217$, $p = 0.0287$. One-way ANOVA for block 10: $F_{(3,63)} = 2.973$, $p = 0.0399$; one-way ANOVA for block 9: $F_{(3,63)} = 0.8758$, $P\,p = 0.4585$; one-way ANOVA for block 8: $F_{(3,63)} = 2.753$, $p = 0.0498$; one-way ANOVA for block 7: $F_{(3,63)} = 4.876$, $p = 0.0041$; one-way ANOVA for block 6: $F_{(3,63)} = 1.108$, $p = 0.3526$. F, MW073 protects APP/PS1 mice against the impairment of associative memory tested through contextual FC, while MW073 alone in WT littermates does not affect memory. ANOVA $F_{(3,61)} = 2.999$, $p = 0.0374$; Bonferroni $p = 0.0466$ WT vehicle vs. APP/PS1 vehicle, $p = 0.0338$ APP/PS1 vehicle vs. APP/PS1 MW073, $p > 0.999$ WT vehicle vs. WT MW073. ANOVA, analysis of variance; FC, fear conditioning; LTP, long-term potentiation; RAWM, radial arm water maze; WT, wild type.

or disease stage administration of synaptic and behavioral dysfunction induced by disease-relevant surrogates. MW073 provides a previously unavailable reference standard for screening clinically effective neuropsychiatric therapeutics for inherent dose-dependent 5-HT_{2b}R inhibitor activity. Overall, our findings that MW073 attenuates synaptic and behavioral dysfunction with improved cognitive function when used in prevention or disease stage intervention indicates the potential of curated 5-HT_{2b}R inhibitors for intervention in AD-associated neuropsychiatric syndromes.

Calculations based on US Food and Drug Administration (FDA) guidance[23] reveal that the human equivalent dose for the MW073 used in the mouse model studies described in Figures 3 through 5 is comparable to the 32 mg/day for an average 60 kg person following FDA prescribing information for risperidone.[22] The computed and

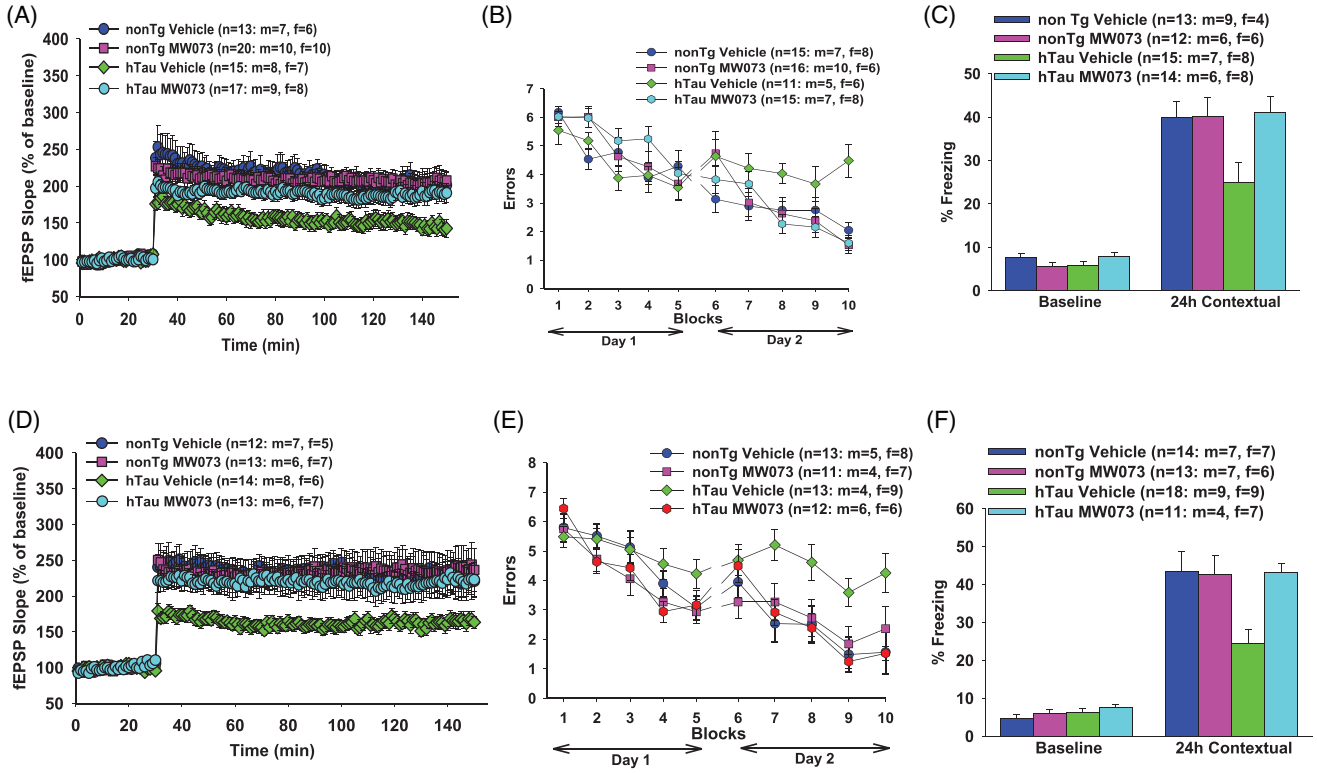


FIGURE 5 Repeat daily MW073 administration in a preventive paradigm (A–C) and during disease state (D–F) abolishes the impairment of LTP and both spatial and associative memory in hTau/Mapt-KO (hTau) mice. A, MW073 (5 mg/kg, oral gavage, daily, from the age of 7 months for ≈ 250 days) prevents the LTP defect in hTau mice. MW073 alone in non-transgenic (nonTg) control littermates does not affect potentiation. Overall ANOVA $F_{(3,61)} = 5.305$, $p = 0.0026$. Two-way ANOVA among groups: $F_{(1,31)} = 0.1241$, $p = 0.7270$ nonTg vehicle vs. nonTg + MW073; $F_{(1,26)} = 11,67$, $p = 0.0021$ nonTg vehicle vs. hTau vehicle; $F_{(1,30)} = 7.238$, $p = 0.011$ hTau vehicle vs. hTau + MW073; $F_{(1,35)} = 1.642$, $p = 0.2085$ nonTg + MW073 vs. hTau + MW073. B, MW073 (5 mg/kg, oral gavage, daily, from the age of 7 months for ≈ 180 days) re-establishes the normal number of errors with the RAWM in hTau mice, while MW073 alone in nonTg control littermates does not affect performance. Two-way ANOVA for repeated measures among all (day 2): $F_{(3,53)} = 3.537$, $p = 0.0207$. One-way ANOVA for block 10: $F_{(3,53)} = 13.35$, $p < 0.0001$; one-way ANOVA for block 9: $F_{(3,53)} = 1.458$, $p = 0.2365$; one-way ANOVA for block 8: $F_{(3,53)} = 2.666$, $p = 0.0571$; one-way ANOVA for block 7: $F_{(3,53)} = 1.450$, $p = 0.2386$; one-way ANOVA for block 6: $F_{(3,53)} = 1.620$, $p = 0.1956$. C, MW073 (5 mg/kg, oral gavage, daily, from the age of 7 months for ≈ 240 days) protects hTau mice against the impairment of associative memory tested with the contextual FC, while MW073 alone in nonTg control littermates does not affect memory. ANOVA $F_{(3,50)} = 3.432$, $p = 0.0238$; Bonferroni $p = 0.0465$ nonTg vehicle vs. hTau vehicle, $p = 0.0258$ hTau vehicle vs. hTau MW073, $p > 0.999$ nonTg vehicle vs. nonTg MW073. D, MW073 (5 mg/kg, oral gavage, daily, from the age of 11 months for ≈ 150 days) restores animal capability of undergoing potentiation in hTau mice. MW073 alone in control nonTg littermates does not affect potentiation. Overall ANOVA $F_{(3,48)} = 3.794$, $p = 0.0160$. Two-way ANOVA among groups: $F_{(1,23)} = 1.093e\text{-}005$, $p = 0.9974$ nonTg vehicle vs. nonTg + MW073; $F_{(1,24)} = 14.97$, $p = 0.0007$ nonTg vehicle vs. hTau vehicle; $F_{(1,25)} = 8.425$, $p = 0.0076$ hTau vehicle vs. hTau + MW073; $F_{(1,26)} = 0.2153$, $p = 0.6469$ nonTg + MW073 vs. hTau + MW073. E, MW073 (5 mg/kg, oral gavage, daily from the age of 11 months for ≈ 45 days) protects hTau mice against the impairment of spatial memory in the RAWM, while MW073 alone does not affect performance in nonTg littermates. Two-way ANOVA for repeated measures among all groups (day 2): $F_{(3,45)} = 4.732$, $p = 0.0059$. One-way ANOVA for block 10: $F_{(3,45)} = 4.862$, $p = 0.0052$; one-way ANOVA for block 9: $F_{(3,45)} = 5.728$, $p = 0.0021$; one-way ANOVA for block 8: $F_{(3,45)} = 3.179$, $p = 0.0329$; one-way ANOVA for block 7: $F_{(3,45)} = 4.624$, $p = 0.0067$; one-way ANOVA for block 6: $F_{(3,45)} = 0.9262$, $p = 0.4359$. F, MW073 (5 mg/kg, oral gavage, daily, from the age of 11 months for ≈ 140 days) protects hTau mice against the impairment of associative memory during contextual FC, while MW073 alone in control nonTg littermates does not affect memory. ANOVA $F_{(3,52)} = 8.834$, $p = 0.0001$; Bonferroni $p = 0.0003$ nonTg vehicle vs. hTau vehicle, $p = 0.0009$ hTau vehicle vs. hTau MW073, $p > 0.999$ nonTg vehicle vs. nonTg MW073. ANOVA, analysis of variance; FC, fear conditioning; LTP, long-term potentiation; RAWM, radial arm water maze.

clinical dosing similarity combined with the quantitative similarity in dose-dependent molecular mechanism indicate the potential utility of revisiting clinical dosing and exposure of existing neuropsychiatric therapeutics identified as possessing qualified 5-HT_{2b}R inhibitor activity. The risperidone data also identify it as a candidate for future strategic optimization in which 5-HT_{2b}R inhibitory activity could be retained while adverse off-target activities are reduced or eliminated. Strategic optimization is a repurposing strategy that complements the more widely used forms of drug repurposing such as re-formulation or alternative route of delivery. Both repurposing approaches leverage the portfolio of an existing drug or clinical candidate, but strategic optimization can avoid the pharmacological risks and intellectual property limitations of the parent drug that are inherent to the classical approach.[24] The feasibility for strategic optimization of risperidone is suggested by the recent approval of paliperidone,[25] an anti-psychotic drug that is simply 9-hydroxyrisperidone. In general, the multi-target

and pleiotropic nature of atypical anti-psychotics and anti-depressants are especially attractive starting points based on their depth and breadth of clinical utility. Dose-dependent comparison to MW073 as a standard can facilitate screening of existing drugs and focus Investigational New Drug (IND)–enabling development of new candidates.

Limitations of this report include the novelty of the aggregated outcomes. MW073 outcomes represent an initial phase of potential future neuropsychiatric therapeutic interventions focused on selective 5-HT_{2b}R inhibition. The availability of MW073 as a unique research reagent should facilitate the pursuit of critical next-step investigations that are not part of this report. For example, downstream lipidomic responses are important considering the linkage to the 5-HTR/Gα/PLC-β2 pathway and the association of clinical lipid profiles with neuropsychiatric conditions.[21] In addition, IND-enabling investigations are required for MW073 extension to clinical investigations, but these are straightforward based on the existing MW073 profile and its facile generation by strategic optimization of a clinical neurotropic drug. Taken in its entirety, the findings reported here provide a firm foundation and add to the accumulating body of evidence implicating selective 5-HT_{2b}R inhibition as a mechanism for treating neuropsychiatric syndromes and cognitive loss in AD and related disorders.

AUTHOR CONTRIBUTIONS

Saktimayee M. Roy (conceptualization; data curation; investigation; funding acquisition; supervision; writing original draft); Erica Acquarone (conceptualization; data curation; formal analysis; investigation; writing original draft); Elentina Argyrousi (data curation; investigation); H. Zhang (data curation; investigation); A. Staniszewski (data curation; investigation); Asuka Inoue (data curation; formal analysis; investigation); Joshua J. Ziarek (conceptualization; data curation; formal analysis; investigation; writing original draft); Ottavio Arancio (conceptualization; data curation; funding acquisition; investigation; supervision; writing original draft); D. Martin Watterson (conceptualization; data curation; funding acquisition; investigation; supervision; writing original draft).

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CONFLICT OF INTEREST STATEMENT

Saktimayee M. Roy, Joshua J. Ziarek, Ottavio Arancio, D. Martin Watterson are investigators on a NIH peer-reviewed NIH award (AG066722) to Northwestern and Columbia Universities that supported the research reported. Saktimayee M. Roy, Erica Acquarone, Ottavio Arancio, and D. Martin Watterson are authors on patent applications filed by Northwestern and Columbia Universities that cover potential commercial use but not research use of MW073. The other authors have no relevant disclosures. Author disclosures are available in the supporting information.

HUMAN SUBJECTS

Consent was not necessary because no human subjects were included in this study.

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REFERENCES

1. Cummings J. Disease modification is not all—we need symptomatic therapies for Alzheimer disease. *Nat Rev Neurol*. 2022;18(1):3-4. doi:10.1038/s41582-021-00591-9
2. Sachdev PS. The neuropathological underpinnings of neuropsychiatric symptoms in dementia. *Am J Geriatr Psychiatry*. 2024;32(6):765-767. doi:10.1016/j.jagp.2024.01.024
3. Frank B, Walsh M, Hurley L, et al. Cognition mediates the association between cerebrospinal fluid biomarkers of amyloid and P-Tau and neuropsychiatric symptoms. *J Alzheimers Dis*. 2024;100(3):1055-1073. doi:10.3233/JAD-240125
4. Lyketsos CG, Carrillo MC, Ryan JM, et al. Neuropsychiatric symptoms in Alzheimer's disease. *Alzheimers Dement*. 2011;7(5):532-9. doi:10.1016/j.jalz.2011.05.2410
5. Masters MC., Morris JC, Roe CM. "Noncognitive" symptoms of early Alzheimer disease: a longitudinal analysis. *Neurology*. 2015, 84(6), 617–622
6. Veitch DP, Weiner MW, Miller M, et al. Alzheimer's Disease Neuroimaging Initiative. The Alzheimer's Disease Neuroimaging Initiative in the era of Alzheimer's disease treatment: a review of ADNI studies from 2021 to 2022. *Alzheimers Dement*. 2024;20(1):652-694. doi:10.1002/alz.13449
7. Grinberg LT, Rüb U, Ferretti RE, et al. The dorsal raphe nucleus shows phospho-tau neurofibrillary changes before the transentorhinal region in Alzheimer's disease. A precocious onset?. *Neuropathol Appl Neurobiol*. 2009;35(4):406-16. doi: 10.1111/j.1365-2990.2009.00997.x
8. Smith GS, Barrett FS, Joo JH, et al. Molecular imaging of serotonin degeneration in mild cognitive impairment. *Neurobiol Dis*. 2017;105:33–41.
9. Rüb U, Del Tredici K, Schultz C, Thal DR, Braak E, Braak H. The evolution of Alzheimer's disease-related cytoskeletal pathology in the human raphe nuclei. *Neuropathol Appl Neurobiol*. 2000;26(6):553-67. doi: 10.1046/j.0305-1846.2000.00291.x
10. Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol*. 2011;70(11):960-9. doi: 10.1097/NEN.0b013e318232a379
11. Buga AM, Ciobanu O, Bădescu GM, et al. Up-regulation of serotonin receptor 2B mRNA and protein in the peri-infarcted area of aged rats and stroke patients. *Oncotarget*. 2016;7(14):17415-30. doi: 10.18632/oncotarget.8277
12. Dentel C, Palamiuc L, Henriques A, et al. Degeneration of serotonergic neurons in amyotrophic lateral sclerosis: a link to spasticity. *Brain*. 2013;136(Pt 2):483-93. doi: 10.1093/brain/aws274
13. Acquarone E, Argyrousi EK, Arancio O, Watterson DM, Roy SM. The 5HT2b receptor in Alzheimer's disease: increased levels in patient brains and antagonist attenuation of amyloid and tau induced dysfunction. *J Alzheimers Dis*. 2024;98(4):1349-1360. doi: 10.3233/JAD-240063

14. Bevilacqua L, Doly S, Kaprio J, et al. A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature*. 2010;468(7327):1061-6. doi: 10.1038/nature09629
15. Holcomb L, Gordon MN, McGowan E, et al. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med*. 1998;4(1):97-100. doi:10.1038/nm0198-097
16. Trinchese F, Liu S, Battaglia F, Walter S, Mathews PM, Arancio O. Progressive age-related development of Alzheimer-like pathology in APP/PS1 mice. *Ann Neurol*. 2004;55(6):801-814. doi:10.1002/ana.20101
17. Puzzo D, Argyrousi EK, Staniszewski A, et al. Tau is not necessary for amyloid-β-induced synaptic and memory impairments. *J Clin Invest*. 2020;130(9):4831-4844. doi: 10.1172/JCI137040
18. Puzzo D, Vitolo O, Trinchese F, Jacob JP, Palmeri A, Arancio O. Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity. *J Neurosci*. 2005;25(29):6887-97. doi:10.1523/JNEUROSCI.5291-04.2005
19. Watterson DM, Grum-Tokars VL, Roy SM, et al. Development of novel in vivo chemical probes to address CNS protein kinase involvement in synaptic dysfunction. *PLoS One*. 2013;8(6):e66226. doi:10.1371/journal.pone.0066226
20. Argyrousi EK, Staniszewski A, Nicholls RE, Arancio O. Preparation of Tau oligomers after the protein extraction from bacteria and brain cortices. *Methods Mol Biol*. 2018; 1779:85-97. doi:10.1007/978-1-4939-7816-8_7
21. Mehdi SMA, Costa AP Swob, Pan C, et al. Depression and cognition are associated with lipid dysregulation in both a multigenerational study of depression and the National Health and Nutrition Examination Survey. *Trans Psychiatry*. 2024;14(1):142. doi: 10.1038/s41398-024-02847-6
22. *FDA. RISPERDAL® (risperidone) prescribing information*. U.S. Food and Drug Administration; 2010. Accessed August 2024. https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/020588s046lbl.pdf
23. FDA. *Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*. U.S. Food and Drug Administration; 2005. Accessed August 2024. https://www.fda.gov/media/72309/download
24. Liddicoat J, Hamidzadeh A, Liddell K, et al. New government drug repurposing programs: opportunities and uncertainties. *Sci Transl Med*. 2024;16(753):eadl0998. doi: 10.1126/scitranslmed.adl0998
25. FDA. 2006, INVEGA®. *INVEGA® (paliperidone) prescribing information*. U.S. Food and Drug Administration; 2010. Accessed December 2024. https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021999s018lbl.pdf

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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