Identification of Lead 5-HT2A Receptor Agonists for use in Treatment Resistant Depression with Non-hallucinogenic Potential and Low Valvulopathy Liability.

PT_251

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Introduction

5-HT2AR Agonists

- Psychedelic 5-HT2AR agonists (i.e. psilocybin) show **rapid** & **lasting antidepressant efficacy** following single administration[1].
- Non-hallucinogenic 5-HT2AR agonists (i.e. 2-Br-LSD) show pre-clinical antidepressant-like efficacy[2].

Challenges

- **Hallucinatory effects** of psychedelics limit accessibility of treatment and increase health care costs.
- Many 5-HT2AR agonists also **activate 5-HT2BR**, introducing **cardiac valvulopathy risk**[3].

Unknowns

- The pharmacology which differentiates psychedelics from non-hallucinogenic 5-HT2AR agonists.
- Data informed criteria for screening out compounds with valvulopathy risk using early *in vitro* functional activity assays.

atai has identified novel 5-HT2AR agonists with CNS drug-like properties and antidepressant-like efficacy that do not induce the head twitch response (HTR) in mice, indicating non-hallucinogenic potential.

Multiple *In Vitro* Readouts

Informs novel compound design

IP1 Accumulation

- ✓ Highly sensitive.
- ✓ Captures slow-binding agonists.
- – May saturate agonist efficacy.

Compound profiling

Ca^{2+} Flux

- ✓ Characterization of agonist efficacy with instantaneous readout.
- – May miss slow binding agonists.

Valvulopathy relevant screening

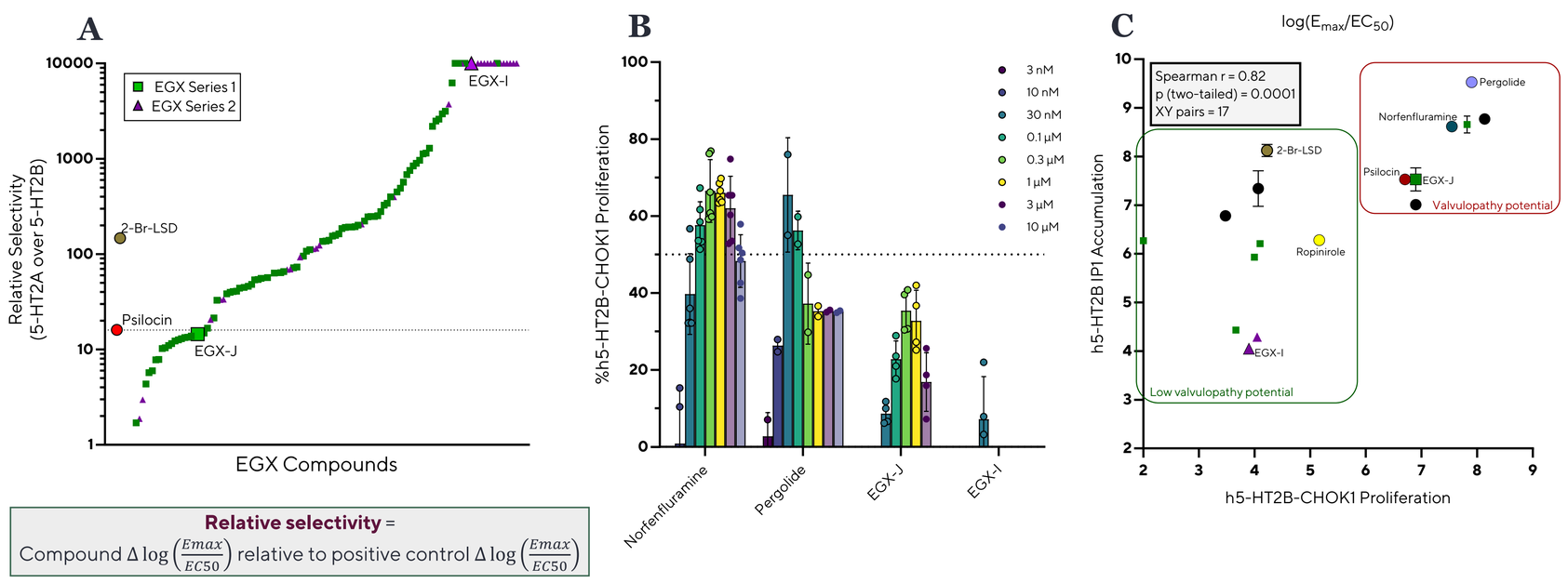
5-HT2B Proliferation

- ✓ Validated with valvulopathogens and non-valvulopathogen 5-HT2B agonists.
- – Does not translate chronic dosing.

IP1 assays enable the prioritization of potent 5-HT2AR agonists with selectivity over 5-HT2BR.
Ca^{2+} flux 5-HT2AR assays may identify partial agonists, which may predict hallucinogenic potential[4].
5-HT2B proliferation assay provides a phenotypic, early-stage estimation of valvulopathy risk[6].

Screening for potent 5-HT2AR agonists with selectivity over 5-HT2BR

Figure 1: A) EGX series 1 and 2 compounds show improved agonist selectivity for 5-HT2AR over 5-HT2BR compared to reference compounds psilocin and 2-Br-LSD. **B)** EGX lead compounds show less potent, lower magnitude activity than known valvulopathogens in the 5-HT2B-CHOK1 cell proliferation assay. **C)** 5-HT2BR agonist activity in the IP1 accumulation assay significantly correlates with 5-HT2B-CHOK1 proliferation.



Relative selectivity =
Compound $\Delta \log\left(\frac{Emax}{EC50}\right)$ relative to positive control $\Delta \log\left(\frac{Emax}{EC50}\right)$

Table 1. Human 5-HT2A/2B IP1 accumulation data results for EGX leads, 5-HT2AR reference compounds and 5-HT2BR agonist valvulopathogens.

Target (Readout)	EGX-J	EGX-I	Psilocin	2-Br-LSD	Norfenfluramine	Pergolide
Human 5-HT2A (Gq-IP1); EC_{50} (nM) [E_{max}]	26.5 [99%]	19.4 [98%]	18.2 [83%]	0.68 [93%]		
Human 5-HT2B (Gq-IP1); EC_{50} (nM) [E_{max}]	29.3 [99%]	>10,000	21.6 [73%]	3.00 [40%]	2.43 [101%]	0.30 [101%]
Human 5-HT2B (Proliferation); EC_{50} (nM) [E_{max}]	43.9 [35%]	>10,000	29.8 [15%]	>10,000	18.4 [65%]	7.92 [63%]

Compounds are screened for agonist activity using IP1 HTRF assays with CHO-K1 cells expressing human 5-HT2AR or 5-HT2BR. IP1 degradation is blocked by LiCl, resulting in a cumulative readout of agonism over 1-hour of compound incubation.

This ensures any agonist activity at 5-HT2AR & 5-HT2BR is captured. Fixed end-point assays with non-accumulative readouts *may* not detect slow-binding agonists (i.e., Ca^{2+} flux).

Calculating **relative selectivity** for 5-HT2AR over 5-HT2BR enables prioritization of compounds and informs structural activity relationship (SAR) understanding (Figure 1A).

Known valvulopathogens (i.e., norfenfluramine, pergolide) induce proliferation of human cardiac valvular interstitial cells *and* h5-HT2BR-CHOK1 cells[5,6] (Figure 1B).

The h5-HT2BR-CHOK1 proliferation assay is a validated, pathology relevant, *in vitro* tool facilitating the screening out of compounds with high valvulopathy risk under conditions of chronic dosing.

We found that agonism in the h5-HT2BR IP1 assay significantly correlated with h5-HT2B-CHOK1 proliferation, informing our minimally acceptable target candidate criteria for lead optimization (Figure 1C). Only compounds with low valvulopathy risk are progressed.

Investigating the relationship between 5-HT2AR agonist efficacy & hallucinogenic potential

Figure 2: A) Lead EGX non-hallucinogenic compounds show lower h5-HT2A agonist efficacy (E_{max}) than psilocin, but similar efficacy to 2-Br-LSD in the Ca^{2+} assay. **B)** Correlation analysis of human 5-HT2AR agonist efficacy (activation %5-HT) versus mouse HTR magnitude (mean max HTR count over 30-minute recording).

The Ca^{2+} flux FLIPR assay measures real-time fluctuations in cytosolic Ca^{2+} 2-minutes post-compound incubation, capturing the magnitude of Gαq-signaling in CHOK1 cells expressing human 5-HT2ARs. This permits characterization of partial agonist efficacy.

Human 5-HT2AR Ca^{2+} flux E_{max} is reported to correlate with mouse HTR magnitude, thus hallucinogenic potential[4]. Indeed, our HTR negative lead compounds & 2-Br-LSD showed lower 5-HT2AR efficacy than psilocin in our Ca^{2+} flux assays (Figure 2A).

However, our cross-species correlation analysis identified no significant relationship, notably, with a cluster of high E_{max} compounds with low hallucinogenic potential (Figure 2B).

While 5-HT2AR agonism is crucial, additional contributors may influence HTR (see below). These hypotheses are guiding the profiling our compounds to identify pharmacological profiles with low hallucinogenic potential, while preserving efficacy.

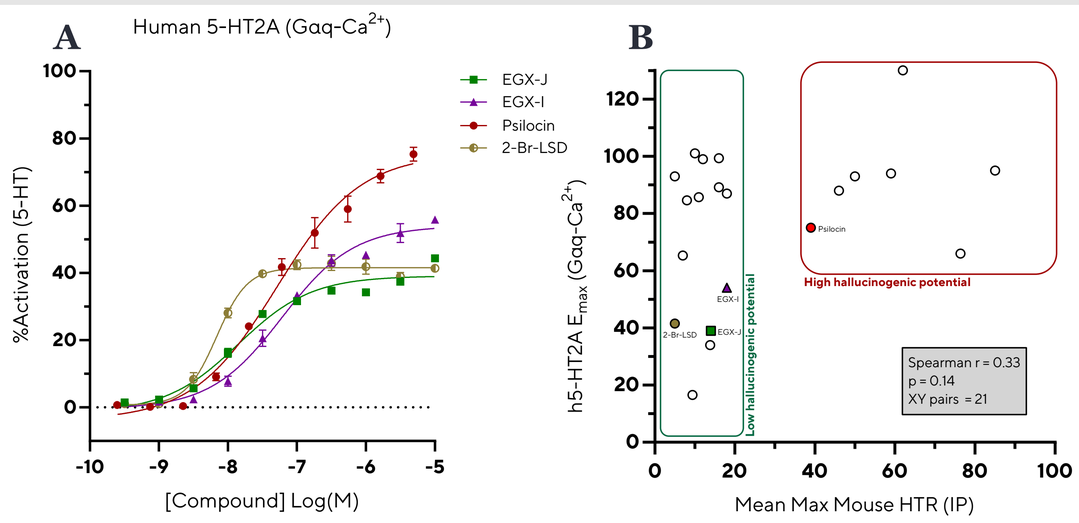


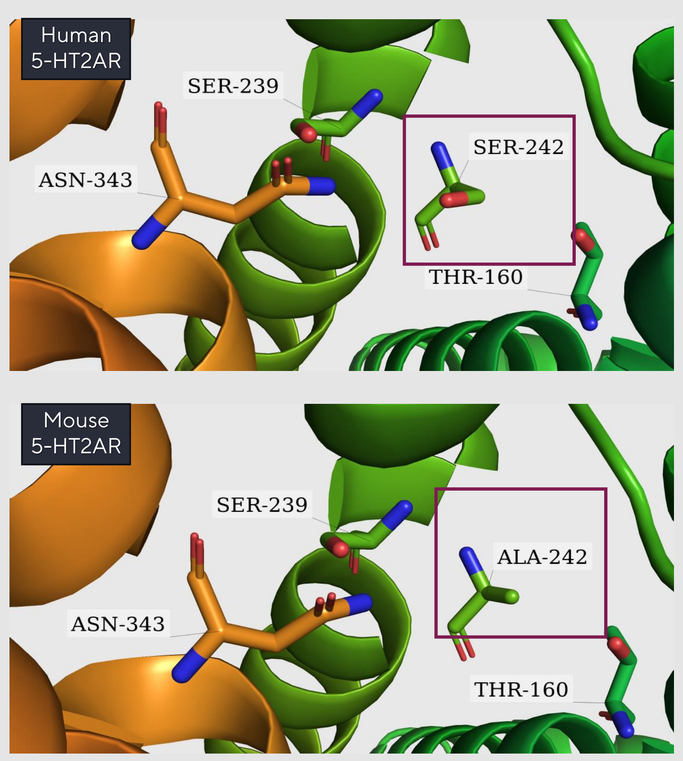
Table 2. Lead & reference compound human 5-HT2AR Ca^{2+} flux agonism data.

Target (Readout)	EGX-J	EGX-I	Psilocin	2-Br-LSD
Human 5-HT2A (Gq-Ca^{2+}); EC_{50} (nM) [E_{max}]	14.1 [39%]	60.9 [54%]	51.8 [75%]	7.00 [42%]

Hypothesized additional determinants of HTR results for 5-HT2AR agonists.

- Off-target pharmacology (i.e. 5-HT1AR agonism, D2R antagonism) which attenuates HTR[7,8].
- Non-canonical signaling at 5-HT2AR (i.e. Gα isoform & β-arrestin2 signaling[9,10])
- Compound metabolism & pharmacokinetic profile.
- 5-HT2AR ortholog differences (Figure 3).

Figure 3: The orthosteric binding site differs at human and mouse 5-HT2AR. The human contains a serine at **5.46 (242)** whereas the mouse contains an alanine residue. This may affect agonist efficacy of some compounds[11]. Visualized in PyMol.



Conclusions & Next Steps

- Novel compounds from two distinct chemical series exhibited >10,000-fold selectivity for 5-HT2AR over 5-HT2BR agonism (>1,000x psilocin) in the IP1 assays.
- Lead compounds showed less potent & lower magnitude 5-HT2B-CHOK1 proliferation than known valvulopathogens.
- Other compounds within both series did not exhibit significant 5-HT2B agonism-mediated proliferation at concentrations <10µM.
- 5-HT2BR IP1 agonism positively correlates with 5-HT2BR-CHOK1 proliferation.
- Lead HTR-negative compounds are partial 5-HT2AR agonists in the Ca2+ flux assay, akin to 2-Br-LSD but unlike psilocin.
- The Emax of the tested compounds in the human 5-HT2AR Ca2+ flux assay did not correlate significantly with mouse HTR magnitude.
- Optimized lead compounds, with improved selectivity for 5-HT2AR over 5-HT2BR agonism, are advancing to in vivo efficacy assays.
- Further in vitro functional and kinetic profiling is ongoing to understand the relationship between compound structure, pharmacology & hallucinogenic potential.

References: 1). Goodwin, G. M., et al. (2022). N Engl J Med 387, 1637-1648; 2). Lewis, V., et al. (2023). Cell Reports 42(3); 3). McIntyre, R. S. (2023). Expert Opin Drug Saf 22(10), 881-883; 4). Wallach, J., et al. (2023). Nat Commun 14(1), 8221; 5). Setola, V., et al. (2003). Mol Pharmacol 63(6), 1223-1229; 6). Huang, X.-P., et al. (2009). Mol Pharmacol 76(4), 710-722; 7). Glatfelter, G. C., et al. (2024). ACS Chem Neurosci 15(24), 4458-4477; 8). Halberstadt, A. L., & Geyer, M. A. (2013). Psychopharmacology 227(4), 727-739; 9). Kossatz, E., et al. (2024). Nat Commun 15(1), 4307; 10). Gumpper, R. H., et al. (2025). Nat Commun 16(1), 2734; 11). Canal, C. E., et al. (2013). J Pharmacol Exp Ther 347(3), 705-716.

Abbreviations: 5-HT2AR = Serotonin 2A receptor, HTR = Head twitch response, IP1 = Inositol monophosphate, HTRF = Homogeneous Time-Resolved Fluorescence, CHOK1 = Chinese hamster ovary K1. IP = Intraperitoneal, D2R = Dopamine 2 receptor.