Discovery of novel 5-HT2A receptor modulators for the treatment of mood disorders

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Objective

Treatment-resistant depression impacts approximately 30% of people with major depressive disorder, and therapeutic approaches with robust and sustained efficacy remain a significant medical need [18].

Psilocybin, and its active metabolite psilocin, are naturally-occurring psychedelic compounds found in hundreds of species of hallucinogenic mushrooms with an extensive history of use in humans. These compounds exert functional activity at a variety of central nervous system receptors, including serotonin receptors, of which agonism at 5-HT2A receptors is believed to mediate the psychedelic effects [15].

Accumulating clinical research indicates that psilocybin demonstrates rapid and lasting antidepressant efficacy following single administration, including in treatment-resistant patients [7,9,10]. 5-HT2A receptor activation may play a role in the mood-improving effects of psilocybin [13].

Preclinical research also supports the therapeutic potential of psilocybin and psilocin, based on neuroplasticity-promoting and antidepressant-like behavioral effects [8,11,12,14,16,17]. Interestingly, some studies suggest 5-HT2A receptor activation-induced hallucinogenic activity may not be required for therapeutic-like effects.

A goal of Entheogenix Biosciences (EGX) research is to use a combination of artificial intelligence (AI)-based drug design, traditional medicinal chemistry and structure-based drug design to discover novel 5-HT2A receptor agonists with optimal CNS drug-like properties that show in vitro and in vivo pharmacological effects consistent with psilocybin/psilocin, including antidepressant-like activity.

Methods

AI-Enabled Hit Identification Methods

To generate initial hit molecules, we used our Ligand Design methodology, which uses a small molecule generator, Deriver [1], and assesses proposed molecules using MatchMaker [2] to select those predicted to preferentially bind targets over anti-targets. Briefly, MatchMaker is a deep learning model that predicts binding between a small molecule drug/binding site pair by using paired structural features of the small molecule with the 3D structural features of the protein binding sites. MatchMaker is trained with positive example complexes of a molecule within a pocket by threading drug-target interaction (DTI) data onto 3D structures of protein–ligand binding sites obtained from the Protein DataBank and SwissModel. Negative data is simulated by shuffling positive DTI pairs and designating them alternative proteins to avoid any small molecule structural bias.

To employ this methodology, we first collected protein structure models for all targets and anti-targets The lone target of interest was 5-HT2A, with anti-targets including 5-HT2B, DRD2/3, and HRH1. Both homology models and crystal structures were explored for each of the targets under consideration. For 5-HT2A, at the time of the study the PDB structures available (6A93, 6A94) were both co-crystallized with antagonist molecules, with these sites selected to represent 5-HT2A. It was deemed that this was an acceptable site given that this same site would accommodate agonists (a later paper would confirm agonists binding [3]). In the end, while several anti-targets were used in preliminary test runs, we elected to focus on 5-HT2B as the major anti-target, utilizing structure models already embedded in our pocketome (i.e., the proteins used within training) as well as several picked specifically with co-crystal structure (6DRX, 6DRY, 6DRZ).

With targets and anti-target sites selected, we ran Ligand Design to explore chemistry within the Enamine REAL Space. Initial generations used random combinations of synthons, with subsequent generations selecting molecules as parents for the next generation based on a weighted scoring function for target over anti-target. The method was run until convergence, which was defined as 2 consecutive generations where no new top scoring compounds were found. Chemistry explored was limited to those that had aliphatic amines, to bias selection for a well-established pharmacophore. Molecules were selected by balancing activity for target and selectivity over anti-target.

In vitro pharmacology methods

Calcium flux assays were performed using human 5-HT receptor subtype GPCR Biosensor Assays in agonist mode. Gq-mediated secondary messenger signaling of calcium mobilization is monitored with a calcium-sensitive dye and is used as a readout for GPCR activation. Stably-transfected cell lines (U2OS) expressing human 5-HT2A and 5-HT2C receptors were loaded with the calcium-sensitive dye in exchange of culture media prior to drug treatments. Reference and test compound agonist activity was measured on a FLIPR Tetra (MDS) via fluorescence detection of the calcium-sensitive dye. 5-HT was used as the assay reference agonist. Data were normalized to the maximal and minimal response observed in the presence of control ligand and vehicle.

β-Arrestin activity was determined using a stably-transfected cell line (U2OS) expressing human 5-HT2A receptors with the PathHunter assay (Eurofins DiscoverX), which utilizes an Enzyme Fragment Complementation technology with β-galactosidase as the functional reporter. The enzyme is split into two inactive complementary portions expressed as fusion proteins in the cell. Upon 5-HT2A activation and β-Arrestin recruitment, complementation occurs, restoring β-galactosidase activity. Cells were incubated with reference or test compound, with 5-HT as the reference agonist, and β-Arrestin recruitment, via 5-HT2A receptor activation and β-galactosidase complementation was measured using chemiluminescent PathHunter Detection Reagents. Data were normalized to the maximal and minimal response observed in the presence of control ligand and vehicle.

IPOne assays were performed using stably-transfected cell lines (CHO-K1) expressing human 5-HT2A or 5-HT2B receptors. Upon activation of these receptors, Gq-mediated myo-Inositol 1 phosphate (IP1) production is detected by a Homogeneous Time-Resolved Fluorescence (HTRF) competitive immunoassay, whereby an IP1 analog coupled to a fluorophore (acceptor) competes with endogenous IP1 for binding to a labeled anti-IP1 antibody (donor). The resulting signal is inversely proportional to the concentration of IP1 in the sample. Cells were incubated with an IP1 inhibitor (to prevent degradation and allow detection) and either reference compound or test compound. α-Me-5-HT was used as the assay reference agonist. Activation of 5-HT2A or 5-HT2B receptors was measured via accumulation of IP1 detected by HTRF. Agonist activity of test compounds was expressed as a percentage of the activity of the reference agonist at its EC100 concentration.

In Vivo Pharmacology Methods

Mouse Head Twitch Response (HTR). Male C57BL/6J mice at 6-8 weeks (Jackson Laboratories) were group housed in a vivarium at UCSD. The room was operated on a reverse light cycle (1900h on; 0700h off) with food and water available ad libitum, except during testing. All testing was conducted between 1000h and 1800h. Mice were surgically implanted with a small neodymium magnet attached to the cranium and fixed with dental cement. After a minimum 2-week recovery period, the mice were injected intraperitoneally with drug or vehicle and immediately placed in a glass cylinder surrounded by a magnetometer coil and activity was recorded during a 30 min test [4]. Coil voltage was amplified, low pass filtered (2 kHz cutoff), and digitized (20 kHz sampling rate). Head twitches were identified in the recordings using a validated technique based on artificial intelligence [5]. Data were plotted as the average number of HTR recorded during the test for each treatment group and analyzed using a 1-way Analysis of Variance (ANOVA; GraphPad Prism). If there was a significant overall effect of treatment at the p<0.05 level, then a Dunnett's post hoc test was performed to compare each treatment group to the vehicle condition.

Rat Forced Swim Test (FST) . Male adult Sprague Dawley rats were used for FST experiments. Animals were grouped 3/cage and housed in a room operated on a light cycle (1900h off; 0700h on) with food and water available ad libitum, except during testing. Rats were handled daily for a minimum of 3 days prior to testing. On Day 1 following a 1 hr acclimation to the testing room, rats were subjected to a 15 min pre-swim in 24 ± 1°C water. Once dried, rats were administered vehicle, ketamine or imipramine, and EGX compounds. Eight hours following the pre-swim, rats received a second administration of test articles except for ketamine. A final third administration of test articles except for ketamine was given 15 or 30 min prior to the 5 min FST on Day 2. Data were plotted as the average % of vehicle response for frequency or duration of immobility recorded during the test for each treatment group and analyzed using a 1-way Analysis of Variance (ANOVA; GraphPad Prism). If there was a significant overall effect of treatment at the p<0.05 level, then a Dunnett's post hoc test was performed to compare each treatment group to the vehicle condition.

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Results

Figure 1: AI-enabled discovery of EGX-1, followed by traditional medicinal chemistry and structure-based drug design, led to identification of novel and potent 5-HT2A agonists with CNS drug-like properties

Initial hit molecules were discovered using a deep learning model to predict interactions between small molecules and targets of interest (5-HT2A) and anti-targets (i.e., 5-HT2B, DRD2/3 and HRH1).

EGX-1 was identified as an initial hit with 5-HT2A agonist activity and provided a starting point for a Medicinal Chemistry campaign to identify compounds with increased potency and selectivity at the 5-HT2A receptor over anti-targets.

18 months of traditional Medicinal Chemistry and Structure-based drug design led to the discovery of 2 novel series of potent 5-HT2A receptor agonists with CNS drug like properties.

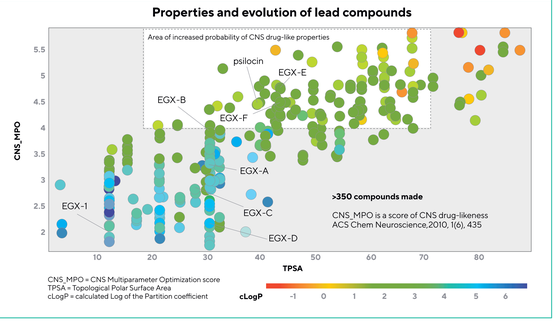
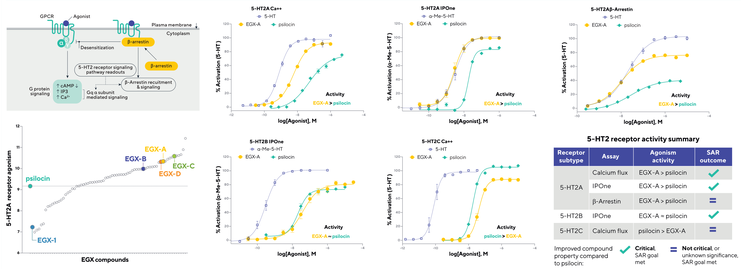


Figure 2: Structure activity relationship (SAR) analysis identifies highly active 5-HT2A receptor agonists with selectivity over 5-HT2B receptors

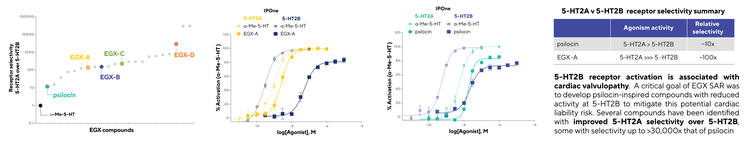
A. EGX SAR identifies compounds with increased 5-HT2A agonism activity compared to psilocin



Receptor subtype	Assay	Agonism activity	SAR outcome
5-HT2A	Calcium flux	EGX-A > psilocin	✓
	IPOne	EGX-A > psilocin	✓
	β-Arrestin	EGX-A > psilocin	=
5-HT2B	IPOne	EGX-A = psilocin	✓
5-HT2C	Calcium flux	psilocin > EGX-A	=

Improved compound property compared to psilocin: ✓ **Critical**, SAR goal met; = **Not critical**, or unknown significance, SAR goal met

B. EGX SAR identifies compounds selective for 5-HT2A over 5-HT2B receptors, with potential to reduce risk of cardiac valvulopathy associated with 5-HT2B agonism



5-HT2A v 5-HT2B receptor selectivity summary

	Agonism activity	Relative selectivity
psilocin	5-HT2A > 5-HT2B	~10x
EGX-A	5-HT2A >>> 5-HT2B	~100x

5-HT2B receptor activation is associated with cardiac valvulopathy. A critical goal of EGX SAR was to develop psilocin-inspired compounds with reduced activity at 5-HT2B to mitigate this potential cardiac liability risk. Several compounds have been identified with **improved 5-HT2A selectivity over 5-HT2B**, some with selectivity up to >30,000x that of psilocin

Figure 2. A. (Upper left) G protein dependent and independent signaling pathways and assay readouts associated with 5HT receptor activation (modified from [19]). (Lower left) EGX compounds rank-ordered by agonist activity (log(Emax/EC50); calcium flux assay; increasing activity left to right) from initial AI-enabled compound hit (EGX-1) to several EGX compounds with activity greater than psilocin. (Right panel) Representative concentration response curves of compound activity at 5-HT2A, 5-HT2B, and 5-HT2C receptors in the indicated assays (Ca++ (calcium flux), β-Arrestin recruitment, and IPOne (inositol phosphate (IP) 1 accumulation via IP3 stimulation)). Compound activity preference determined by comparing relative agonism (log(Emax/EC50), normalized to assay reference agonist). The reference agonist for each receptor/assay is indicated as either 5-HT or α-Me-5-HT. B. EGX compounds rank-ordered by relative agonist selectivity for 5-HT2A over 5-HT2B receptors (Δ Δlog(Emax/EC50); IPOne assay). Square symbols, compounds with selectivity ratio > 30,000. Assay reference (α-Me-5-HT) used for normalization at each receptor (5-HT2A/5-HT2B selectivity = 1).

Summary table

Table 1: In vitro and in vivo profiles of hallucinogenic and non-hallucinogenic EGX compounds

		in vitro profile					in vivo profile				
		5-HT2A EC50 (nM)			5-HT2B EC50 (nM)	5-HT2C EC50 (nM)	Mean HTR at Max Active or Tested Dose	HTR Min Effective Dose (mg/kg, IP)	HTR ED50 (mg/kg, IP)	Mean Mouse Brain/Plasma Ratio (IP)	Mean Rat Brain/Plasma Ratio (IP)
		Ca++	β-Arr	IPOne	IPOne	Ca++					
Hallucinogenic	Psilocin[δ]	51.0	28.0	18.2	21.6	1.1	39	0.3	0.15	12	NA
	EGX-1	7,700	4,200	NA	NA	>10,000	62*	30*	17*	NA	NA
	EGX-A	4.2	17.2	2.8	34.7	42.2	50	3	1.7	4.4	3.4
	EGX-B	9.0	25.8	9.2	81.1	73.52	41	10	3.4	5.7	3.8
Non-hallucinogenic	EGX-C	2.4	4.5	<1.0	19.3	280.0	5	>30	>30	2.6	NA
	EGX-D	3.0	0.7	1.1	143.0	70.0	8	>60	>60	4.6*	8.8

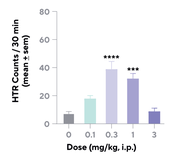
*Subcutaneous administration
NA: not available

Figure 3: The mouse Head Twitch Response (HTR) assay identified Entheogenix 5-HT2A agonist compounds with hallucinogenic and non-hallucinogenic potential

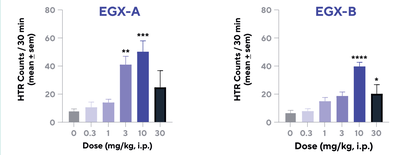
Head Twitch Response

Behavioral proxy for 5-HT2AR activation; Potency correlation with subjective effects in human

A: Psilocin induced a robust HTR

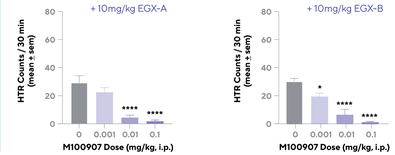


B: Examples of EGX 5-HT2A agonist compounds that induced HTR in a 5-HT2A dependent manner



5-HT2A antagonist M100907 blocks EGX-A-induced HTR
+ 10mg/kg EGX-A

5-HT2A antagonist M100907 blocks EGX-B-induced HTR
+ 10mg/kg EGX-B



C: Example of EGX 5-HT2A agonist compounds that did not induce HTR

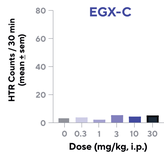
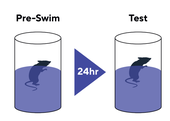


Figure 3. Mouse HTR measured over 30 min. (A) Psilocin[6] and (B) Compounds EGX-A and EGX-B induced robust HTR (upper panels). Blockade of HTR was accomplished with a 20 min pretreatment of a 5-HT2A antagonist, M100907 (0.001 to 0.1 mg/kg, i.p.), with 10 mg/kg i.p. of EGX-A or EGX-B. (lower panels). (C) Compound EGX-C does not induce the HTR, tested at doses 0.3-30 mg/kg, i.p. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 difference from vehicle, Dunnett's post hoc test.

Figure 4: EGX compounds showed antidepressant-like activity in the rat Forced Swim Test (FST)

Forced Swim Test



FST measures immobility behavior that follows an inability to escape from a cylinder of water. Traditional and novel antidepressant drugs reduce immobility in the test.

EGX compounds significantly reduced immobility, a behavioral readout of antidepressant-like activity in the FST

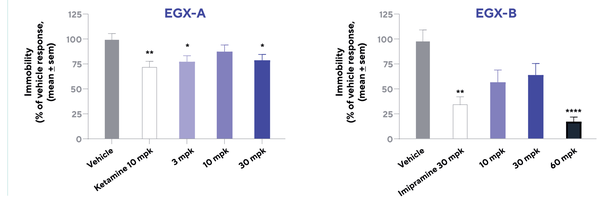


Figure 4. Effect of pro-hallucinogenic EGX compounds (HTR positive) on immobility during FST. Left: Illustration of the FST. Center: EGX-A (3, 10, and 30 mg/kg, i.p.) was dosed 3 times at 23.5, 16, and 0.5h before FST, and ketamine (10 mg/kg, i.p.) was dosed once at 23.5h before FST. Right: EGX-B (10, 30, 60 mg/kg, i.p.) and imipramine (30 mg/kg, i.p.) were dosed 3 times at 23.5, 16, and 0.25h before FST. * p<0.05, ** p<0.01, **** p<0.0001 difference from vehicle, Dunnett's post hoc test.

Summary

- AI-enabled drug design, followed by traditional medicinal chemistry and structure-based drug design, led to the discovery of two novel chemical series with potent 5-HT2A receptor agonism and optimal CNS drug-like properties.
- Novel compounds exhibited potent human 5-HT2A receptor activation of G protein-dependent and independent signaling, consistent with the in vitro functional activity profile of psilocin. In addition, compounds showed improved selectivity for 5-HT2A versus 5-HT2B receptor activation relative to psilocin.
- Novel compounds also induced HTR in mice with lower hallucinogenic potency than psilocin, as well as antidepressant-like activity in the rat FST. Studies are in progress to evaluate the activity of selected compounds in a rat model of treatment-resistant depression.
- These results demonstrate the potential for the discovery of novel, potent, small molecule 5-HT2A receptor agonists for the treatment of mood disorders with improved safety profiles relative to psilocybin/psilocin.
- As it remains unclear if a hallucinogenic subjective experience is necessary for antidepressant effects, novel non-hallucinogenic 5-HT2A receptor agonists currently are being explored for antidepressant-like activity as potential therapeutics that may offer more convenient dosing to a broader patient population.