Case Study

Comparison of hallucinogenic versus non-hallucinogenic psychedelics on 5HT2A receptors

Introduction

Serotonergic psychedelics, known for their impact on mood, perception, and cognitive processes, primarily target the serotonin (5HT) 2A receptor subtype. While these psychedelics have garnered attention for their therapeutic potential, their exploration has been hindered by their spectre of adverse effects. Issues such as disorientation, anxiety, hallucinations, seizures, and, in extreme cases, fatalities have tempered widespread investigation of their clinical application.

The 5HT2A receptor functions via various ntracellular signalling pathways, including the canonical Gq-coupled pathway and β-arrestin recruitment, each contributing to distinct physiological effects. It has been suggested that ligands showing functional bias towards specific signalling pathways may allow separation of the desired therapeutic effect from unwanted side effects, offering the potential to develop effective ligands that can be used to target specific neuropathological conditions.

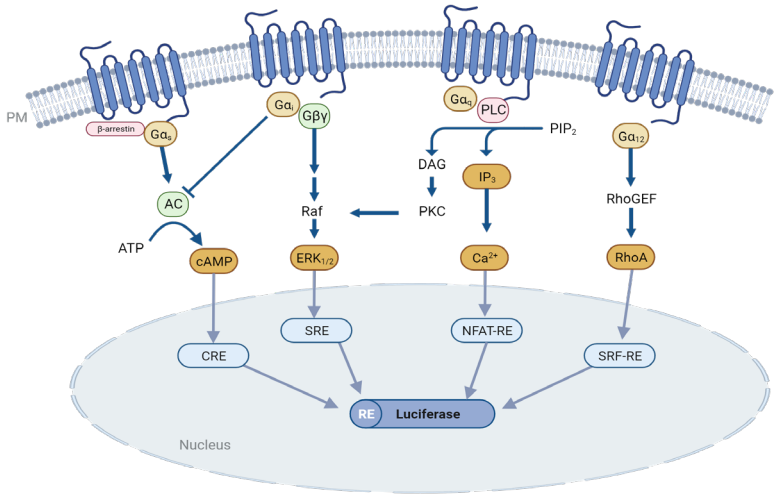


Figure 1: **5HT2A receptor signalling pathways.**

To facilitate further this understanding and drug development, SB Drug Discovery have developed a series of cell-based assays to meticulously identify and characterize 5HT2A receptor ligands. These assays enable precise evaluation of receptor bias towards signalling pathways and subtype selectivity and. Specifically, assays measuring calcium mobilization, β-arrestin interaction, receptor internalization, and pERK levels have been crafted and validated using standard reference compounds, including both psychedelic and non-psychedelic ligands. SB Drug Discovery's approach leverages a diverse suite of receptor-expressing cell lines, robust binding assays, and downstream functional assays catering to a spectrum of 5HT receptors. This suite includes selectivity assays for 5HT2B and 5HT2C receptors, as well as a broad range of additional subtype selectivity assays, species orthologs mouse and rat 5HT2A, and additional assays like SERT assays.

Additionally, SB Drug Discovery's 5HT receptor platform offers customizable tools for drug discovery, allowing the tailoring of screening cascades through our plug-and-play strategies. This customization includes various binding and cell-based assays optimized for high-throughput screening and lead-optimization formats. Orthogonal assays measuring β-arrestin recruitment, alternative signalling pathways, receptor internalization, and G-protein/GRK dissociation complement this suite of capabilties.

5HT Discovery Platform

- **Receptor Profiling Tools**
  Diverse cell lines and robust assays for various 5HT receptors, including core and broad selectivity assays, species orthologs, and SERT assessments.

- **Customizable Screening Cascades**
  Adopting a plug-and-play approach for tailored screening, covering β-arrestin recruitment, G-protein signaling, receptor internalization, and G-protein/GRK dissociation assays.

High-throughput 5HT2A assays

Cell-based assays (illustrated in Figure 2) have been developed for assessing 5HT2A signalling via two methods: (i) calcium flux assay and (ii) β-arrestin recruitment assay.

(i) calcium flux assay measures receptor activation, G-protein modulation and second messenger signalling via quantification of intracellular Ca^{2+} release.
(ii) β-arrestin recruitment assays employs structural complementation and luminescence to measure recruitment of β-arrestin by the 5HT2A receptor.

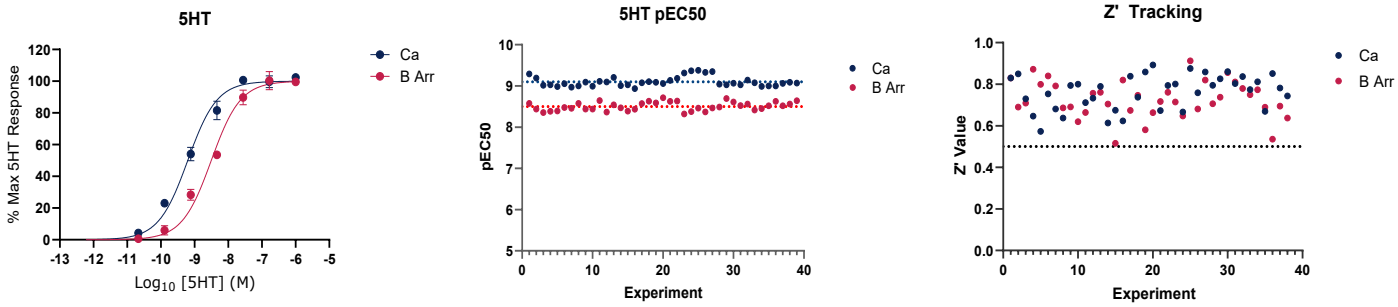


Figure 2: 5HT2A calcium and β-arrestin screening assays.
Both G_q and β-arrestin signalling play important roles in mediating 5HT2A agonist effects. HEK-5HT2A /β-Arrestin stable cell line was generated and validated for concentration dependent 5HT response using both calcium and nanoBiT assays. Data is presented as response relative to maximum 5HT for each assay (Left). Using the same cell line for each assay in parallel ensures comparable receptor expression levels and cellular conditions across both assays and contributes to optimal comparison of compound effect. Assessment of 5HT response across multiple experiments confirmed high levels of assay reproducibility. In the calcium and β-arrestin assays, 5HT showed average EC_{50} values of 0.8 and 3 nM respectively (middle) and Z' values greater than 0.5 (right) across all experments.

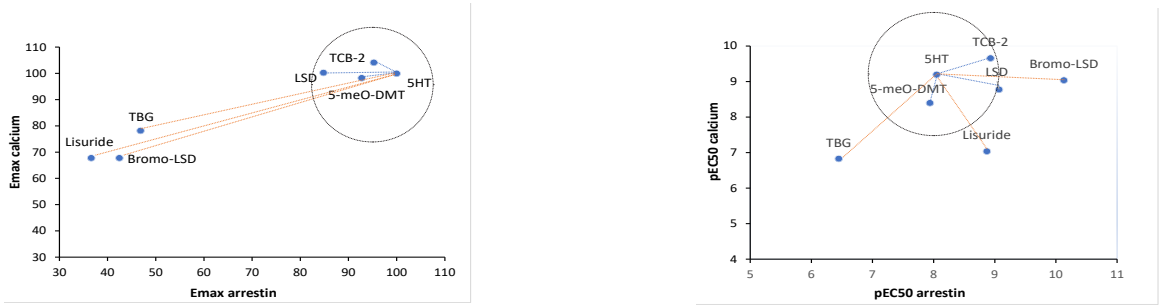


Figure 3: Comparison of hallucinogenic and non-hallucinogenic compounds in calcium and β-arrestin assays.
Orange and blue lines indicate activity of non-hallucinogenic and hallucinogenic compounds relative to 5HT respectively. Visual representation shows that non-hallucinogenic compounds exhibit reduced efficacy in the β-arrestin interaction assay compared to calcium assay and have reduced E_{max} relative to 5HT in both assays. pEC_{50} and E_{max} values of hallucinogenic compounds (depicted within black circle) are closest to 5HT with efficiency equivalent to 5HT, whilst non-hallucinogenic show greater window of activity relative to 5HT. Partial agonism was detected in the β-arrestin assay for all three non-hallucinogenic compounds.

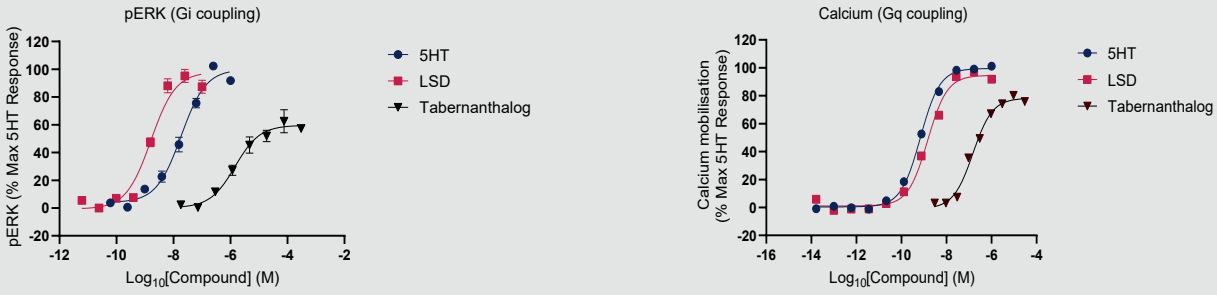


Figure 4: Cell based assays for assessing 5HT2A receptor signalling.
The effect of native ligand 5HT, hallucinogenic (LSD, TCB-2, 5-MeO-DMT) and non-hallucinogenic (Lisuride, Bromo-LSD and Tabernanthalog) compounds were examined in a range of cell-based assays indicative of G_q (calcium assay), G_i (pERK ELISA), $G\alpha_{12/13}$ RhoA (SRF-RE-luciferase reporter assay) pathway activation and β-arrestin recruitment. For each assay, data was expressed relative to maximum 5HT response. It was observed that LSD exhibits increased potency (>1 log) relative to 5HT in the pERK ELISA and SRF-RE reporter assays. Tabernanthalog showed consistent differential efficacy across all assay formats with partial agonism associated with pERK and β-arrestin recruitment assays.

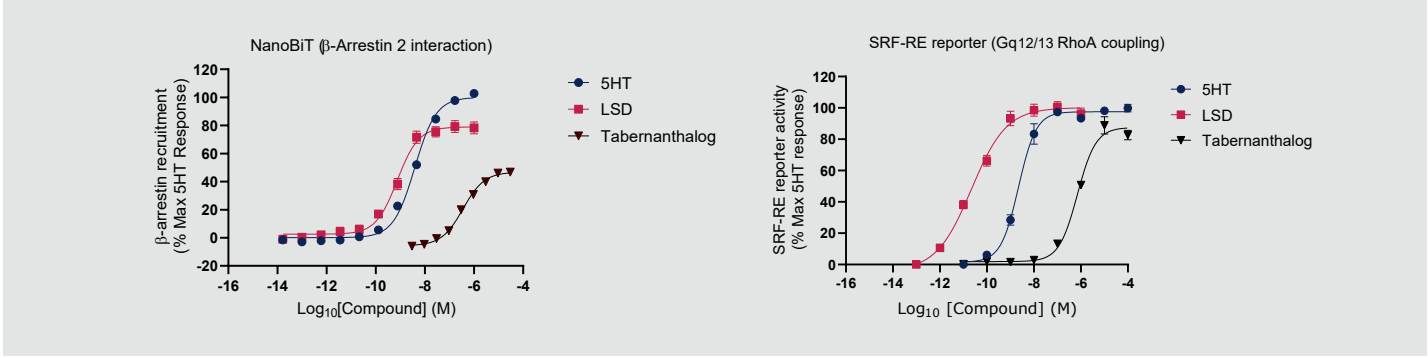


Figure 4: **(continued)**

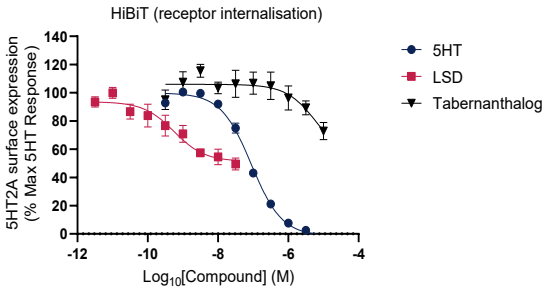
5HT2A Receptor Ligands Activate a Range of Signalling Pathways

Table 1: **Cell based assays for assessing compound effects on 5HT2A receptor signalling.**
A panel of reference compounds was assessed for concentration-dependent effect and data analyzed as percentage of maximum 5HT response. Table summarizes EC_{50} and E_{max} for all compounds in each of the different assay formats.

	EC_{50} (nM)				E_{max} (%)			
	Calcium	NanoBiT	pERK	SRF-RE	Calcium	NanoBiT	pERK	SRF-RE
5HT	0.69	3.9	18.6	2.12	100	100	100	100
LSD	1.45	0.75	1.61	0.02	95	79	98	100
TCB-2	0.22	1.26	0.44	4.47	104	97	75	93
5-meO-DMT	3.21	9.2	3.4	7.45	94	91	86	96
Lisuride	80.9	5.95	6.09	0.62	74	26	36	56
Bromo-LSD	0.59	0.14	0.78	0.03	76	31	32	53
Tabernanthalog	149	357	1446	708	78	47	60	88

5HT2A Receptor Internalization

HiBiT technology (Promega), is used to enable real-time measurement of receptor cell surface expression. Upon ligand binding, a reduction in luminescent signal is indicative of receptor internalization. SB Drug Discovery's receptor internalization assay enables investigation of ligand induced 5HT2A receptor internalization and has been used to assess the effect of a range of psychedelic and non-psychedlic ligands.



Compound	% Internalization	EC50 (nM)
5HT	100	89
LSD	51	0.6
TCB-2	36	1.7
5-meO-DMT	54	36
Lisuride	0	n/d
Bromo-LSD	0	n/d
Tabernanthalog	27	n/d

Figure 5: **5HT2A receptor internalization.**
5HT2A receptor has been associated with both β-arrestin-dependent and -independent pathways. A panel of reference compounds were tested for their effect on 5HT2A receptor internalization. Lisuride and bromo-LSD did not elicit internalization whilst tabernanthalog induced only small levels of internalization. All hallucinogenic compounds elicited greater levels of internalization compared to non-hallucinogenic compounds and showed increased potency relative to 5HT.

Enabling 5HT Drug Discovery

SB Drug Discovery has developed an extensive array of tools for in-depth receptor profiling and customizable assay platforms. Their suite encompasses a diverse range of 5HT receptor-expressing cell lines, robust binding assays, and downstream functional studies, assessing a spectrum of 5HT-related targets 5HT1A, 5HT1B, 5HT2A, 5HT2B, 5HT2C, 5HT3A, 5HT4, 5HT5A, 5HT6, 5HT7, and the serotonin transporter. This includes core selectivity assays for key receptors (5HT2A, 5HT2B, and 5HT2C), broader subtype selectivity assessments, species orthologs, and studies focussing SERT.

Moreover, SB Drug Discovery's platform offers customizable profiling tools employing a plug-and-play strategy, facilitating tailored screening cascades. These tools encompass diverse assay formats such as β-arrestin recruitment, G-protein signalling assays (SRF-RE, SRE, CRE, pERK-ELISA, NFAT-RE), receptor internalization studies, and investigation into G-protein/ G-protein-coupled receptor kinase (GRK) dissociation dynamics. This diverse suite of tools allows researchers to conduct comprehensive receptor analysis and customise assays to meet specific research objectives effectively.

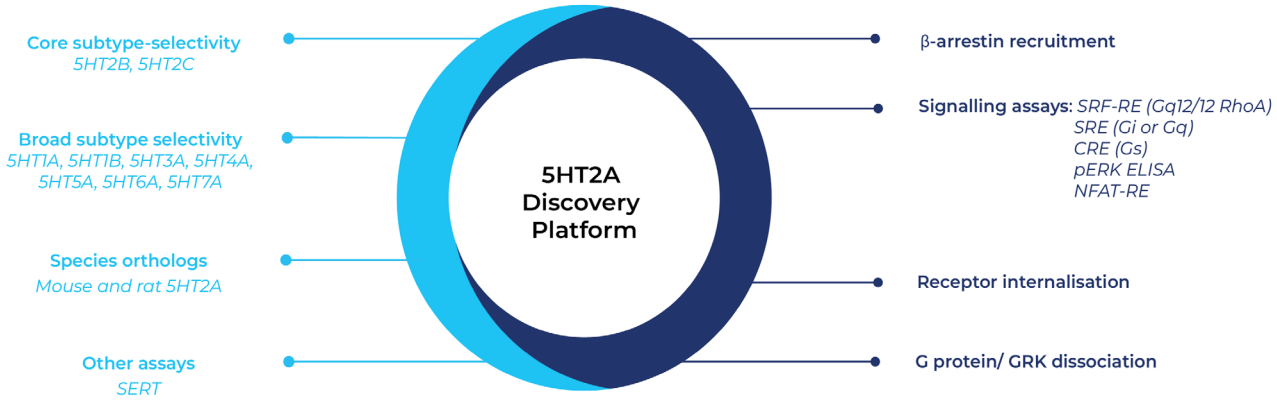


Figure 6: **Cascade of 5HT receptor screening assays**
Identification of 5HT2A receptor agonists and evaluation of mode of action requires a range of project driven tools. While both calcium and β-arrestin assays can support the identification of 5HT2A receptor agonists, additional pathways have also been associated with therapeutic effects of 5HT2A agonists. Compounds can be evaluated in a range of assays to provide a clearer indication of pathway activation which can contribute to more optimal compound design.

Summary

The exploration of serotonergic psychedelics and their therapeutic potential has been hampered by concerns surrounding adverse effects. To address these challenges, SB Drug Discovery has developed a robust series of cell-based assays meticulously designed to identify and characterize 5HT2A receptor ligands. These assays, evaluating receptor subtype selectivity and signalling pathway bias, offer understanding crucial for designing targeted therapeutics. The platform's versatility is underscored by the comprehensive suite of receptor-expressing cell lines, binding assays, and functional studies, covering a wide range of 5HT-related targets.

Notably, SB Drug Discovery's customizable tools, featuring diverse assays formats for β-arrestin recruitment, G-protein signalling pathways, receptor internalization, and GRK dissociation, empower researchers to tailor screening cascades. This sophisticated infrastructure encapsulates a comprehensive approach to understanding the complexities of 5HT receptor signalling, enabling precise assessment and development of safer, more targeted therapies.

Advance your research with SB Drug Discovery's cell line generation services. We offer unparalleled expertise in delivering premium, custom cell line generation solutions that provide a competitive advantage.

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