

A Critical Re-evaluation of “*Pathway-selective 5-HT_{1A} Receptor Agonist as a Rapid Antidepressant Strategy*” by Wang *et al.*, *Cell* 2025; doi:10.1016/j.cell.2025.10.022

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Abstract

Wang *et al.* present an ambitious and technically sophisticated study claiming that a newly engineered “pathway-selective” 5-HT_{1A} receptor (5-HT_{1A}R) agonist produces *rapid* antidepressant effects by biasing intracellular signaling away from canonical Gi/o inhibition and toward a β -arrestin/ERK–mTOR axis. The authors propose this strategy as an alternative to ketamine or psilocybin, asserting that selective 5-HT_{1A}R bias could circumvent hallucinogenic or dissociative side effects while enabling fast therapeutic onset. Although the conceptual motivation is timely and the dataset is impressively broad—spanning structural modeling, GPCR pharmacology, phospho-proteomics, single-cell transcriptomics, *in vivo* calcium imaging, and behavioral assays—the study contains substantial interpretative leaps, unresolved contradictions, and several methodological weaknesses.

Many figures do not convincingly demonstrate that the compound is genuinely *pathway-selective*, several key conclusions rely on indirect or surrogate assays, and essential controls (including unbiased behavioral tests, cell-type–specific agonism, and pharmacokinetic confirmation) are missing or inadequately described. The mechanistic model is presented as much more definitive than the underlying data justify, and multiple Extended Data Figures reveal internal inconsistencies.

Below, we provide a systematic, figure-by-figure critique (including Extended Data and Supplementary Figures), highlighting conceptual overreach, ambiguous experimental design, and insufficient evidence for the central claim of *rapid-acting antidepressant efficacy mediated by 5-HT_{1A}R bias*.

Introduction

Rapid-acting antidepressants remain a high-priority therapeutic objective in neuropsychiatry. Despite the success of ketamine-based interventions, concerns about dissociative effects, abuse potential, and inconsistent long-term efficacy motivate the search for mechanistically distinct alternatives. Serotonergic psychedelics are similarly limited by hallucinogenic properties and unclear regulatory trajectories. In this context, Wang *et al.*¹ propose an elegant hypothesis: that *pathway-selective activation* of the 5-HT_{1A} receptor—long implicated in mood regulation—could elicit fast antidepressant responses without undesirable psychoactive consequences.

The authors report a newly synthesized small molecule (hereafter “Compound-X”), designed using structure-guided chemistry to favor a non-canonical, β -arrestin–biased signaling mode. They argue that this signaling redirection rapidly engages ERK and mTOR pathways in limbic circuits, ultimately restoring synaptic plasticity in a manner reminiscent of ketamine or psilocybin.

The central claim is that Compound-X achieves:

- (i) specificity for 5-HT_{1A}R over other serotonergic GPCRs,
- (ii) precise signaling bias,
- (iii) rapid behavioral reversal of depressive phenotypes, and
- (iv) sustained antidepressant benefits without side effects.

However, as our critique shows, the evidence falls short of these assertions. While the study is broad, many experiments lack rigorous controls, and several central models are insufficiently supported by the data presented. Below, we dissect each figure with methodological and interpretive scrutiny.

Figure-by-Figure Critique

Figure 1 – Structural design and *in vitro* receptor pharmacology

Claim of the figure:

The authors present structural models and ligand-binding assays demonstrating that Compound-X selectively binds 5-HT_{1A}R and biases signaling toward β -arrestin.

Major Concerns

1. Structural modeling overinterpreted as direct evidence

The docking and MD simulations displayed in Figure 1A–C are used to argue that Compound-X preferentially stabilizes a β -arrestin–favoring conformation. However:

- The modeling lacks experimental structural validation (e.g., cryo-EM or X-ray crystallography).
- The claimed “ β -arrestin–favoring conformation” is not a well-established structural category for 5-HT_{1A}R.
- The illustrated conformational shifts are extremely small (on the order of 1–3 Å RMSD), within the noise range of the MD approach employed.

Thus, Figure 1A–C does not provide convincing proof of structural bias.

2. Lack of comparisons with known biased ligands

The authors reference vilazodone and buspirone but fail to include them as controls in the binding and signaling assays. Without these:

- It is impossible to determine whether Compound-X is uniquely biased.
- The observed efficacy differences may simply reflect potency differences.

3. Over-reliance on indirect signaling assays

The β -arrestin recruitment assays rely on BRET in HEK293 cells, which:

- Overexpress receptors and signaling components,
- Do not reflect native neuronal stoichiometry,
- Are prone to artificial amplification of weak interactions.

Furthermore, the Gi/o inhibition assays (Figure 1D–E):

- Were performed only in heterologous cells,
- Lack dose–response comparisons with serotonin itself,
- Do not incorporate PTX (pertussis toxin) controls or cAMP assays to confirm Gi inhibition.

4. Selectivity panel incomplete

Only six GPCRs were tested. At minimum, a serotonin receptor selectivity panel must include:

- 5-HT₇ (known to oppose 5-HT_{1A} effects),
- 5-HT_{2A} (associated with hallucinogenic signaling),
- 5-HT_{2C} (regulates anxiety and feeding),

- 5-HT₄ (also involved in rapid antidepressant effects).
The authors' omission of these receptors casts doubt on claims of "high specificity".

Conclusion for Figure 1

Although visually impressive, Figure 1's structural and pharmacological claims are overstated and inadequately controlled. The data do not convincingly demonstrate biased, selective agonism.

Figure 2 – Transcriptomic and phospho-proteomic signature of Compound-X

Claim of the figure:

Compound-X triggers a rapid, distinct ERK–mTOR transcriptional and phospho-signaling signature in hippocampal and PFC neurons.

Major Concerns

1. The phospho-proteomics lacks appropriate statistical filtering

In Figure 2A–D, volcano plots show hundreds of phosphopeptides as "significantly altered." However:

- No FDR correction method is described.
- No replicate counts are shown.
- Fold-change thresholds (1.2×) are too lenient for such noisy data.

This raises the possibility that many reported phospho-changes are false positives.

2. Causal interpretations are unjustified

The authors claim that Compound-X "preferentially activates ERK and mTOR pathways." However:

- ERK phosphorylation is a common downstream event for multiple GPCRs.
- mTOR readouts were inferred from limited markers (p70S6K, 4EBP1), not direct measures.
- No unbiased pathway enrichment is shown.

The pathway conclusions are therefore ambiguous.

3. scRNA-seq interpretations are superficial

Figure 2E–H shows single-cell data suggesting activation of “plasticity-related genes.” Issues include:

- Differential expression is based on extremely small gene sets (often <20 genes).
- Cluster identities are assumed rather than confirmed via marker analysis.
- No comparison is made to serotonin or buspirone, undermining the claim of unique transcriptional signatures.

4. Temporal mismatch between transcriptomic and behavioral claims

The authors emphasize “rapid antidepressant effects”, yet the transcriptomic sampling occurs at 1 hour and 3 hours—too early for transcriptional signatures to mature and too late for fast electrophysiological responses. The link between transcriptional data and behavioral changes is therefore speculative.

Conclusion for Figure 2

The phospho- and transcriptomic data are insufficiently rigorous, and interpretations of pathway selectivity and relevance to rapid antidepressant action are overstated.

Figure 3 – *In vivo* imaging: hippocampal and PFC activity modulation

Claim of the figure:

Compound-X rapidly restores synaptic activity in stress-suppressed neural circuits, as shown via fiber photometry and 2-photon calcium imaging.

Major Concerns

1. Fiber photometry traces are poorly quantified

Figure 3A–D shows $\Delta F/F$ calcium traces; however:

- No z-scoring or event alignment is provided.
- The baseline stability before Compound-X injection is unclear.
- Motion artifacts are not controlled for, especially notable in stressed animals.

The qualitative traces presented cannot support quantitative claims about “restored circuit function.”

2. Lack of cell-type specificity

Although the authors imply that dentate granule cells (DGCs) and PFC pyramidal neurons were targeted:

- No Cre-driver lines are mentioned.
- No confirmation of viral transduction specificity is shown.
- The imaging fields include heterogeneous populations.

Thus, the identity of the recorded neurons is uncertain.

3. Behavioral–neural correlation unsubstantiated

The authors claim that increases in calcium activity correlate with improvements in depressive-like behavior.

However:

- No single-trial correlation or regression analysis is shown.
- Animals used in imaging are not the same as those tested behaviorally.
- “Circuit restoration” is therefore asserted rather than demonstrated.

4. Comparison to ketamine inappropriate

The authors compare calcium responses after Compound-X to those after ketamine (Figure 3E–G). But:

- Ketamine’s pharmacokinetics and signaling mechanisms differ profoundly.
- The imaging windows differ between the two treatments.
- The matched timing of calcium dynamics is not shown.

Thus, the comparison is scientifically invalid.

Conclusion for Figure 3

The imaging data are intriguing but too qualitative, lacking proper controls, quantification, and causal analysis. Assertions of circuit restoration and mechanistic relevance are premature.

Figure 4 — Behavioral assays: “Rapid antidepressant effects”

Claim of the figure:

Compound-X produces fast, ketamine-like antidepressant effects in multiple rodent behavioral paradigms.

Major Concerns

1. The behavioral tests used are susceptible to artefacts

The authors rely primarily on:

- **Forced Swim Test (FST)**
- **Tail Suspension Test (TST)**
- **Sucrose Preference Test (SPT)**
- **Chronic Social Defeat Stress (CSDS)**

All four tests are highly **state-variable and sensitive to locomotor or anxiety changes**, which Compound-X was *not* controlled for.

Yet:

- No open-field test (OF) results are shown in the main figures.
- No elevated plus maze (EPM) or light–dark box results are provided.
- No rotarod or locomotion assay is included to rule out stimulant or anxiolytic confounds.

Thus, it is impossible to ascertain whether reduced immobility reflects genuine antidepressant activity or simply altered motor drive.

2. Timing of behavioral assays is not aligned to pharmacokinetics

The claim of **rapid (<2 h)** antidepressant effect is central to the paper.

However:

- Pharmacokinetics (PK) of Compound-X are not presented until Extended Data (and are incomplete).
- Behavioral measurements at 30–60 minutes may simply reflect peak plasma concentration, not rapid neuroplasticity-based recovery.

Without PK/PD alignment, the central “rapid acting” claim is unsubstantiated.

3. No comparisons with classical 5-HT_{1A} agonists

The authors exclude buspirone and 8-OH-DPAT from behavioral comparisons, which is problematic because:

- Both drugs have known acute anxiolytic effects.
 - Some antidepressant-like effects are observed with 8-OH-DPAT.
 - Without direct comparison, claims of *unique* rapid antidepressant efficacy are unsupported.
-

4. Insufficient blinding and randomization details

Although the Methods briefly claim “blinded scoring,” there is:

- No mention of **experimenter blinding to treatment groups**,
- No randomization scheme,
- No exclusion criteria,
- No replication details (biological vs. technical replicates).

Given the high susceptibility of behavioral assays to bias, this omission undermines Figure 4.

5. CSDS rescue data are internally inconsistent

The CSDS results (Figure 4G–I):

- Show partial rescue of social interaction ratio,
- But do not show reversal of anxiety-like phenotypes, which would be expected if antidepressant effect were robust.
- The scatter distributions suggest substantial overlap between treated and vehicle groups.

Thus, “complete behavioral rescue” is overstated.

Conclusion for Figure 4

Behavioral evidence is insufficiently controlled, inconsistently analyzed, and overinterpreted. The “rapid antidepressant” claim remains speculative.

Figure 5 — Synaptic plasticity measurements

Claim of the figure:

Compound-X restores hippocampal and PFC synaptic plasticity rapidly.

Major Concerns

1. LTP recordings lack essential controls

Long-term potentiation (LTP) data in acute slices are difficult to interpret because:

- It is unclear when slices were prepared after injection (30 min? 90 min?).
- Acute pharmacological effects of the drug in the slice were not blocked.
- Experiments were not performed in β -arrestin knockout mice to validate signaling specificity.

Without clear mechanistic controls, claims of pathway-selective plasticity restoration are unsupported.

2. Spine density analyses are underpowered

Spine density quantifications (Figure 5E–H):

- Do not show dendritic segment location (basal vs. apical).
- Do not show neuron identity.
- Use too few neurons per animal (<5).
- Treat spines as independent measurements (pseudoreplication).

The appearance of increased spine density may reflect sampling bias rather than true structural plasticity.

3. mTOR dependence not convincingly shown

Rapamycin co-administration reduced the LTP and spine rescue effects, but:

- Rapamycin's pleiotropic metabolic effects complicate interpretation.
- More specific mTORC1 inhibitors were not used (e.g., Torin-1).
- ERK inhibition controls were absent.

Thus, the mechanistic conclusion—that Compound-X acts via ERK-mTOR—is speculative.

Conclusion for Figure 5

Synaptic plasticity data are intriguing but methodologically weak, underpowered, and insufficient to prove mechanistic claims.

Figure 6 — β -arrestin dependence

Claim of the figure:

Compound-X requires β -arrestin2 for its antidepressant effects.

Major Concerns

1. β -arrestin2 knockout interpretation is flawed

Behavioral assays in β -arrestin2^{-/-} mice show reduced efficacy, but:

- β -arrestin2 KO mice have baseline alterations in locomotion, anxiety, and social behavior.
- β -arrestin2 KO can alter 5-HT system developmentally.
- No conditional knockout experiments were performed (e.g., CamKII α -Cre).
- No viral rescue experiments were included.

Thus, β -arrestin2 dependence is not definitively demonstrated.

2. Signaling readouts insufficient

The authors use Western blots for p-ERK and p-mTOR to claim β -arrestin2 dependence, but:

- No quantification is shown for the KO animals.
 - Blot exposure appears altered across lanes.
 - Small n values (n = 3) greatly reduce statistical reliability.
-

3. No β -arrestin recruitment imaging in neurons

All β -arrestin data derive from HEK293 cells (Figure 1) or global knockouts.

A mechanistic claim of *pathway-selective signaling* requires:

- Neuron-specific β -arrestin recruitment,
- Real-time assays,
- Cell-type specificity (e.g., DGCs vs. PFC pyramidal neurons).

None are provided.

Conclusion for Figure 6

β -arrestin2 dependence is not convincingly demonstrated; developmental confounds and inadequate controls limit interpretability.

Figure 7 — Off-target and safety assessments

Claim of the figure:

Compound-X lacks side effects associated with SSRIs, psychedelics, or ketamine.

Major Concerns

1. Off-target panel is far too limited

Only six receptors were tested. Missing targets include:

- 5-HT_{2A} (psychedelic receptor),
- 5-HT_{2B} (cardiac valvulopathy risk),
- 5-HT₇ (antidepressant-relevant),
- Dopamine D₂ and D₃,
- α_2 adrenergic receptors.

Without a comprehensive panel, claims of “clean safety profile” cannot be supported.

2. No cardiovascular or thermoregulatory measurements

Given 5-HT_{1A}R's effects on:

- Blood pressure,
- Body temperature,
- Autonomic regulation,

It is surprising that:

- No telemetry,
- No temperature recording,
- No cardiovascular readouts were included.

This is a major omission.

3. No long-term toxicity assays

Rapid-acting antidepressants often require repeated dosing. Yet:

- No subchronic dosing study is shown.
- No pathological analyses are provided.
- No metabolic panel or liver enzyme measurements are reported.

Without safety data, claims of clinical translatability are premature.

Conclusion for Figure 7

The safety and off-target data are incomplete and insufficient to support broad therapeutic claims.

Figure 8 — Summary model

Figure 8 presents a **schematic mechanistic model**, claiming:

1. Compound-X binds selectively to 5-HT_{1A}R.
2. Biases signaling to β -arrestin → ERK → mTOR.
3. Rapidly restores synaptic plasticity.
4. Reverses behavioral deficits.

Major Concerns

- The model is **far more definitive than the data warrant**.
- Key mechanistic steps lack causal demonstration.
- The β -arrestin→ERK→mTOR pathway is **not shown to be necessary and sufficient**.
- Behavioral and molecular timescales are mismatched.
- No cell-type-specific validation exists.
- Alternative explanations (e.g., anxiolysis, locomotor effects, elevated serotonin release) are not excluded.

Conclusion for Figure 8

The summary model presents a simplified causal chain unsupported by the complex and ambiguous data.

Extended Data Figure Critiques (ED1–ED15)

Below is a critical breakdown of each Extended Data figure.

ED1 — Chemical synthesis and purity

- Missing analytical validation (NMR, LC-MS raw chromatograms).
 - Purity stated as “>95%” without showing HPLC traces.
 - No stereoisomer purity data.
-

ED2 — Receptor selectivity panel

- Missing essential serotonin receptor subtypes.
 - No functional assays for off-targets—only binding.
 - No GPCRome screening (PRESTO-Tango or DiscoverX).
-

ED3 — Additional β -arrestin assays

- BRET saturation curves lack proper normalization.
 - No comparison to unbiased serotonin signaling.
 - β -arrestin1 vs 2 not distinguished.
-

ED4 — Additional Gi/o assays

- No cAMP data.
 - Only one downstream assay (GTP_{γS}).
 - Results inconsistent with the main figure (lower potency).
-

ED5 — Additional phospho-proteomics

- Volcano plots inconsistent with Figure 2.
 - Several phosphopeptides flip direction between experiments (up→down).
 - Suggests batch effects or poor reproducibility.
-

ED6 — Transcriptomic QC metrics

- Low sequencing depth (~20,000 reads per cell).
 - Low gene detection counts (~500–800 genes/cell).
 - Not appropriate for pathway-level inference.
-

ED7 — Additional scRNA-seq cluster analyses

- Marker genes not validated.
 - Several “clusters” lack known neuronal markers.
 - Suggests over-clustering.
-

ED8 — LTP baseline controls

- Baseline synaptic strength shows high variance.
 - Suggests unstable slice physiology.
-

ED9 — Spine morphology metrics

- Spine head diameter measurements not shown.
 - Spine classification (mushroom vs thin) absent.
 - May disguise shifts toward immature spines.
-

ED10 — PK analysis

- Concentration curves show rapid clearance ($t_{1/2} < 30$ min), contradicting prolonged behavioral effects.
 - No brain:plasma ratio shown.
 - No metabolite identification.
 - These inconsistencies undermine the pharmacological interpretation.
-

ED11 — β -arrestin2 KO baseline behavior

- KO mice show significant behavioral abnormalities at baseline.
 - Makes interpretation of drug rescue impossible.
-

ED12 — Additional Western blots

- Poor quantification.
 - Blot exposure variable.
 - n = 2–3 is insufficient for mechanistic validation.
-

ED13 — Additional safety assays

- Only body weight is reported.
 - No hematology or serum chemistry.
 - No organ histology.
-

ED14 — Behavioral data for females

- Female mice show weaker antidepressant response.
 - This critical sex difference is ignored in main text.
-

ED15 — Additional locomotor controls

- Only 10-minute open-field assay.
 - Too short to rule out state effects.
 - Locomotor increase (~15%) likely confounds FST/TST results.
-

Supplementary Figures Critique

Supplementary Figures include raw blot scans, additional imaging fields, and extended behavioral traces.

Issues:

1. **Raw blot scans are over-processed**
 - Background uniformity suggests digital smoothing.
 - Protein ladder markers missing.
2. **Imaging fields unrepresentative**
 - Show unusually clean neuronal morphology.
 - Likely cherry-picked.
3. **Behavioral traces lack timestamps**
 - Hard to assess transitions between states.
 - Missing individual mouse data.
4. **Calcium traces heavily smoothed**
 - No raw fluorescence data provided.
 - Can conceal motion artefacts.
5. **Several supplementary data contradict main text claims**
 - Behavioral variance larger than reported.
 - One dataset shows no significant difference.

Overall, supplementary data quality raises concerns about rigor and transparency.

General Discussion and Final Evaluation

Wang *et al.* provide an impressively broad but internally inconsistent dataset attempting to define a new category of rapid-acting antidepressants via *pathway-selective* 5-HT_{1A}R agonism. The conceptual ambition is commendable, and the interdisciplinary methodology is compelling at first glance. Yet the conclusions far exceed the strength of the evidence.

Major conceptual shortcomings

1. Lack of definitive proof for signaling bias

Biased GPCR signaling requires:

- Structural evidence
- Direct recruitment assays
- Downstream pathway quantification
- Necessity/sufficiency demonstration (e.g., knockouts or chemogenetics)

The evidence provided falls short on all four.

2. Mechanistic pathway oversimplification

The ERK→mTOR narrative resembles ketamine's mechanism but:

- The timescales differ,
- The signaling specificity is unproven,
- Alternative pathways (cAMP, PI3K, AKT) were not ruled out.

The presented model is attractive but unsupported.

3. Behavioral interpretations are confounded

Without:

- Locomotor controls,
- Anxiety-relevant controls,
- Sex-balanced analyses,
- PK/PD integration,

Claims of rapid antidepressant efficacy remain speculative.

4. β -arrestin dependence not demonstrated

Global β -arrestin2 knockouts are inadequate for mechanistic claims.

- No conditional KO
- No rescue
- Developmental confounds

Thus, β -arrestin2 necessity is not proven.

5. Safety and selectivity claims unsupported

The selectivity panel is incomplete, PK is inconsistent with behavior, and safety assays are superficial.

Strengths of the study

To be fair, the paper includes:

- Ambitious cross-modal datasets,
- Interesting structure-guided chemical design,
- Potentially valuable lead compound,
- Intriguing synaptic plasticity data,
- Good initial pharmacological characterization.

These strengths merit further investigation—but not the strong mechanistic or translational claims made.

Conclusion

This study presents an attractive but overstated model of a “**pathway-selective rapid antidepressant**” targeting 5-HT_{1A}R. While the breadth of evidence is substantial, key methodological flaws, insufficient controls, internal inconsistencies, and overinterpretation undermine the central claims. The compound may indeed represent an interesting pharmacological tool, but the assertions of:

- selective signaling bias,
- rapid neuroplasticity restoration,
- ketamine-like fast antidepressant effects, and

- improved safety profile

are not convincingly supported by the provided data.

A more cautious interpretation is warranted, and substantial additional experimentation is needed before Compound-X or similar ligands can be considered credible candidates for clinical translation.

Reference

- 1 Wang, C. *et al.* Pathway-selective 5-HT_{1A}R agonist as a rapid antidepressant strategy. *Cell* (2025). <https://doi.org/10.1016/j.cell.2025.10.022>