

Structural pharmacology and therapeutic potential of 5-methoxytryptamines

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Psychedelic substances such as lysergic acid diethylamide (LSD) and psilocybin show potential for the treatment of various neuropsychiatric disorders^{1–3}. These compounds are thought to mediate their hallucinogenic and therapeutic effects through the serotonin (5-hydroxytryptamine (5-HT)) receptor 5-HT_{2A} (ref. 4). However, 5-HT_{1A} also plays a part in the behavioural effects of tryptamine hallucinogens⁵, particularly 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT), a psychedelic found in the toxin of Colorado River toads⁶. Although 5-HT_{1A} is a validated therapeutic target^{7,8}, little is known about how psychedelics engage 5-HT_{1A} and which effects are mediated by this receptor. Here we map the molecular underpinnings of 5-MeO-DMT pharmacology through five cryogenic electron microscopy (cryo-EM) structures of 5-HT_{1A}, systematic medicinal chemistry, receptor mutagenesis and mouse behaviour. Structure–activity relationship analyses of 5-methoxytryptamines at both 5-HT_{1A} and 5-HT_{2A} enable the characterization of molecular determinants of 5-HT_{1A} signalling potency, efficacy and selectivity. Moreover, we contrast the structural interactions and in vitro pharmacology of 5-MeO-DMT and analogues to the pan-serotonergic agonist LSD and clinically used 5-HT_{1A} agonists. We show that a 5-HT_{1A}-selective 5-MeO-DMT analogue is devoid of hallucinogenic-like effects while retaining anxiolytic-like and antidepressant-like activity in socially defeated animals. Our studies uncover molecular aspects of 5-HT_{1A}-targeted psychedelics and therapeutics, which may facilitate the future development of new medications for neuropsychiatric disorders.

Recent scientific inquiry has demonstrated that serotonergic psychedelics such as psilocybin and LSD have both rapid and long-lasting anxiolytic and antidepressant effects³. Although the mind-altering, psychedelic effects of these compounds have been ascribed to actions at 5-HT_{2A} receptors, studies indicate that other 5-HT receptors have a modulatory role. The complex animal behavioural effects of the less-studied hallucinogen 5-MeO-DMT, found in the poison of the Colorado River toad (*Incilius alvarius*)⁶, are particularly reliant on the actions of the drug at 5-HT_{1A} receptors. The discriminatory stimulus of 5-MeO-DMT and its effects on exploratory behaviours and sedation are largely driven by 5-HT_{1A} agonist activity in vivo^{5,9}. Epidemiological surveys, which capture alternative medical uses of 5-MeO-DMT, indicate that 5-MeO-DMT generates a rapid and sustained reduction in depression and anxiety symptoms, as well as induction of meaningful and spiritually significant experiences¹⁰. 5-MeO-DMT is also clinically used in combination with the oneirogen ibogaine outside the United States. A recent survey of US Special Operation Forces veterans highlighted the therapeutic promise of this compound in the treatment of post-traumatic stress disorder (suicidal ideation and cognitive impairment), depression and anxiety². 5-MeO-DMT is currently in development as a therapeutic for a range of indications, including depression,

substance use disorders and neurological disorders¹¹. Although based largely on open-label trials and naturalistic surveys, the existing evidence suggests that 5-MeO-DMT produces rapid and strong effects across neuropsychiatric diagnostic indications. Given that 5-HT_{1A} is the primary target of approved anxiolytic and antidepressant medications, such as buspirone (BuSpar)⁸ and vilazodone (Viibryd)¹², this receptor may contribute to the reported therapeutic effects of 5-MeO-DMT.

Although much has recently been uncovered about the molecular mechanisms of LSD and other psychedelics at 5-HT_{2A} receptors^{13,14}, little is known about how 5-MeO-DMT, related tryptamines and classical psychedelics bind to and signal through 5-HT_{1A}. Most research and development of new probes of psychedelics have focused on 5-HT_{2A} receptors^{15,16}, whereas notably less effort has been dedicated to investigating the role of other 5-HT receptors in the polypharmacology of these compounds. This is despite the proposed complementary roles of 5-HT_{1A} and 5-HT_{2A} in moderating anxiety and stress¹⁷, which are some of the major areas for potential psychedelic-based therapies. Here we report a detailed structural and functional exploration of the mechanisms by which classical psychedelics, 5-methoxytryptamines (5-MeO-tryptamines) and prescription drugs bind to and activate 5-HT_{1A} at the molecular and atomic level. We also report on 5-HT_{1A}-selective

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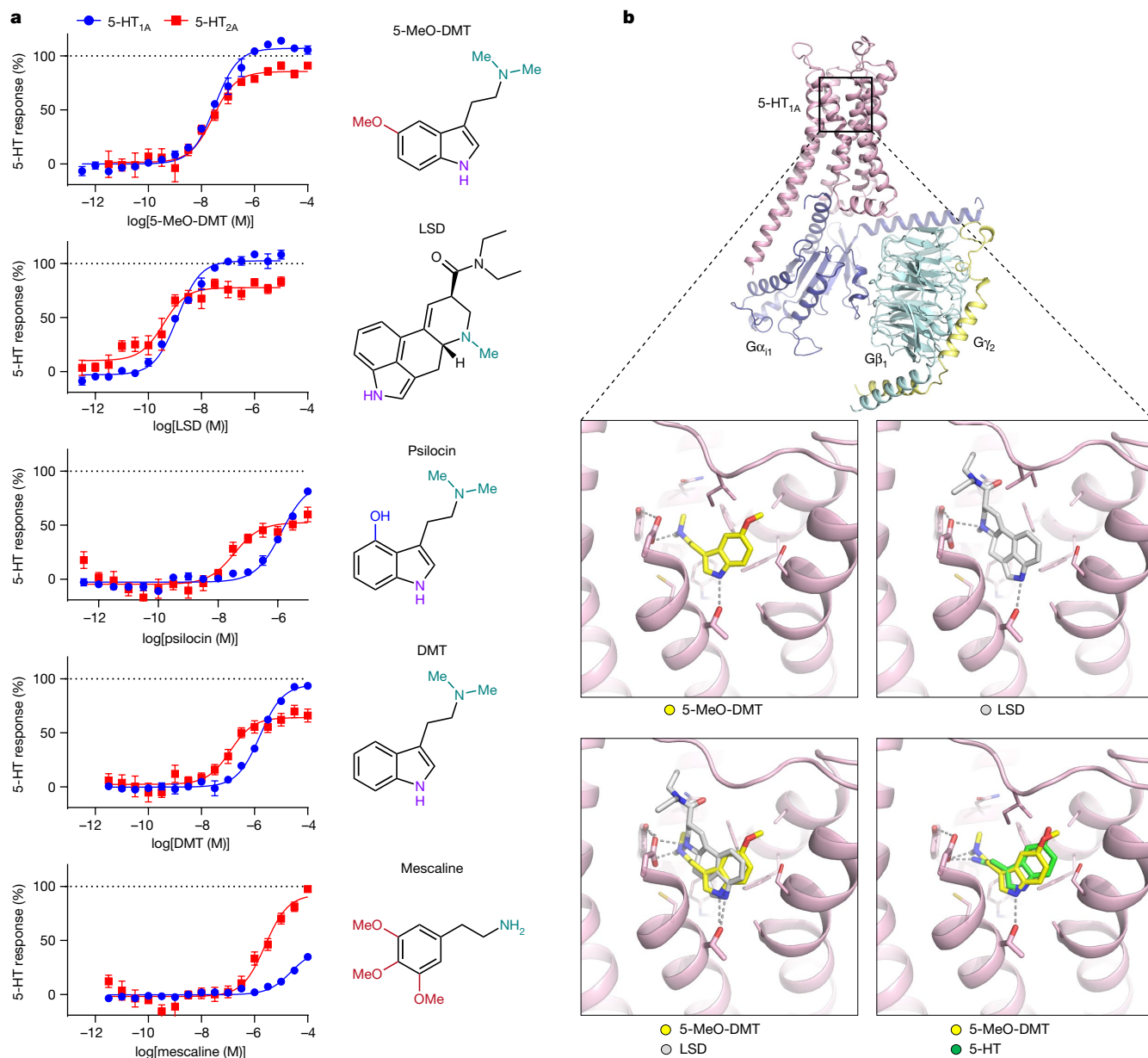


Fig. 1 | Activity of psychedelics at 5-HT_{1A} and 5-HT_{2A}, and cryo-EM structures of 5-MeO-DMT and LSD bound to the 5-HT_{1A} signalling complex. a, 5-HT_{1A}-mediated G_{ii} activation (blue) and 5-HT_{2A}-mediated G_q activation (red) by psychedelics determined by BRET. Concentration–response experiments were performed in triplicate and are averaged from two (mescaline) or more (all other compounds) independent experiments. Data are normalized against 5-HT and errors bars denote the s.e.m. **b**, Top,

cryo-EM structure of 5-HT_{1A}-Gα_{i1}-Gβ₁-Gγ₂ signalling complexes. 5-HT_{1A}, Gα_{i1}, Gβ₁ and Gγ₂ are shown in pink, dark blue, teal and yellow, respectively. Middle, zoom-in images show the 5-HT_{1A} orthosteric site bound to 5-MeO-DMT (yellow) and LSD (grey), with ionic interactions and hydrogen bonds indicated by dashed lines. Bottom, superposition of 5-HT_{1A} orthosteric binding sites comparing binding poses of 5-MeO-DMT and LSD, as well as 5-MeO-DMT and 5-HT (Protein Data Bank (PDB) identifier: 7E2Y).

tryptamines in a mouse model of depression, highlighting the potential of new tryptamine-based 5-HT_{1A} medications. Our studies provide crucial insights into an understudied class of psychedelics and related compounds that may facilitate the development of new neuropsychiatric therapeutics that target 5-HT_{1A}.

Psychedelics acting at 5-HT_{1A} and 5-HT_{2A}

To compare the actions of psychedelics at both 5-HT_{1A} and 5-HT_{2A}, we determined the signalling activities at both receptors using bioluminescence resonance energy transfer (BRET) G_{ii} and G_q activation reporters, respectively^{14,18} (Fig. 1a). In line with previous reports^{19,20}, most psychedelic drugs tested at 5-HT_{2A} showed partial agonism,

ranging from efficacies near 50% of that of serotonin for psilocin (the active metabolite of psilocybin) to nearly 100% as seen for mescaline (the main psychoactive alkaloid in peyote). *N,N*-dimethyltryptamine (DMT; the psychedelic compound in ayahuasca), psilocin and mescaline were more potent at 5-HT_{2A}, whereas LSD and 5-MeO-DMT were effectively equipotent at 5-HT_{1A} and 5-HT_{2A} (Fig. 1a). Despite the variation in selectivity and potency, the tryptamine psychedelics were full or near-full agonists of 5-HT_{1A}-mediated G protein signalling in our assays compared with serotonin (Fig. 1a and Supplementary Table 1). These findings validate the potency and efficacy of both LSD and 5-MeO-DMT at 5-HT_{1A}, complementing previous studies that have demonstrated the importance of 5-HT_{1A} in the in vivo pharmacology of both drugs^{5,21}.

Structures of psychedelic-bound 5-HT_{1A}

To elucidate the structural basis of how 5-MeO-DMT and LSD activate 5-HT_{1A}, we determined cryo-EM structures of drug-bound 5-HT_{1A}-G_i signalling complexes (Fig. 1b and Extended Data Fig. 1a). We used previously reported receptor and G protein constructs that we¹³ and others²² have used to determine 5-HT_{2A} and 5-HT_{1A} structures (see Methods for details). In brief, for 5-HT_{1A}, we replaced the first 24 residues with BRIL to facilitate expression and introduced a stabilizing L125^{3,41}W mutation (superscript numbers denote Ballesteros–Weinstein numbering²³). For the heterotrimeric G protein, we co-expressed Gβ₁ with a Gγ₂-Gα_{1i} fusion¹³ containing stabilizing mutations²².

The cryo-EM structures of 5-MeO-DMT-bound and LSD-bound 5-HT_{1A}-G_i signalling complexes were obtained at global nominal resolutions of 2.79 Å and 2.64 Å, respectively (Fig. 1b, Extended Data Fig. 1 and Extended Data Table 1). Our structures enabled us to unambiguously resolve backbones and side chains, elucidate sterols and lipids, and characterize drug–receptor interactions in molecular detail (Fig. 1b and Extended Data Fig. 2b). The structures displayed conserved features of active-state G protein-coupled receptors, such as an outward-rotated receptor TM6 and the carboxy-terminal α5 helix of the Gα_{1i} protein bound to the cytoplasmic transducer site of 5-HT_{1A} receptors^{22,24}. We observed a similar overall receptor conformation compared with previous 5-HT_{1A} structures²², with a root mean square deviation of 0.596 Å (Extended Data Fig. 2a), and we were able to elucidate additional 5-HT_{1A} residues such as a complete extracellular loop 2 (EL2). Notably, our structures uncovered distinct drug–receptor interactions in the orthosteric binding pocket (OBP) that may drive differences in drug pharmacology. Primarily anchored by a conserved ionic interaction with D116^{3,32}, 5-MeO-DMT and LSD bind 0.8 Å deeper in the pocket (measured from the indole nitrogen to the oxygen of T121^{3,37}) compared with the structurally similar serotonin²² (Fig. 1b). In addition, we observed a hydrogen bond between the indole nitrogen of the drugs and T121^{3,37} at the bottom of the 5-HT_{1A}-binding pocket, which is not observed for serotonin. This leads to a slight 8.5° rotation around an axis formed by the amine-D116^{3,32} bond for 5-MeO-DMT, a feature previously observed for ergoline compounds such as LSD at other 5-HT receptors^{14,25}. Indeed, LSD displays a 15.4° rotation towards the receptor core compared with the poses observed in our previous LSD-bound 5-HT_{2B} and 5-HT_{2A} structures^{13,14}, forming a similar interaction with T121^{3,37} as observed for 5-MeO-DMT (Fig. 1b and Extended Data Fig. 2e). Starting from the conserved interaction with D116^{3,32}, the diethylamide substituent of LSD extends towards the extracellular site forming hydrophobic interactions with I189^{EL2} in EL2. A similar interaction with L229^{EL2} of 5-HT_{2A} was suggested to be crucial for the slow binding kinetics and distinct pharmacological profile of LSD^{13,14}. Overall, we observed that the diethylamide substituent of LSD is accommodated 1 Å closer to TM3 (measured as the distance to the Cα of I^{3,29}) with one of the ethyl groups of LSD sandwiched between the side chains of F112^{3,28} and I113^{3,29} (Extended Data Fig. 2e). This configuration is different from that observed in LSD-bound 5-HT_{2A}, which is intriguing because the configuration of the ethyl groups of LSD seems to be pivotal for its pharmacology and psychedelic effects *in vivo*^{14,26}.

Our structural data revealed that LSD adopts distinct binding modes at 5-HT_{1A} and 5-HT_{2A}. Moreover, 5-MeO-DMT assumes a different binding pose from related serotonin at 5-HT_{1A}. However, it is unclear whether these differences are driven by the methylation of the amine, the methylation of the hydroxyl group or both.

Structure–activity relationship of 5-MeO-tryptamines at 5-HT_{1A}

Using 5-MeO-DMT as the starting point, we set out to systematically examine the structure–activity relationship (SAR) of 5-MeO-tryptamines and to uncover determinants of potency and selectivity

at 5-HT_{1A} and 5-HT_{2A}. The compounds were synthesized using indoles with the desired substitution and the oxalation–amidation–reduction sequence²⁷ to prepare the corresponding tryptamine analogues (Extended Data Fig. 3 and Supplementary Data 1).

As the first structural variable, we investigated the effect of different amine modifications, including comparisons of acyclic and cyclic amines on 5-HT_{1A} and 5-HT_{2A} signalling (Fig. 2 and Supplementary Table 1). Several of these compounds are known as ‘designer tryptamines’, with anecdotal reports of psychedelic activity in humans (Supplementary Table 2), including 5-methoxy-*N,N*-dipropyl-tryptamine (5-MeO-DPT), 5-methoxy-*N*-methyl-*N*-isopropyl-tryptamine (5-MeO-MIPT) and 5-methoxy-*N,N*-pyrrolidinyl-tryptamine (5-MeO-PyrT)^{28,29}. Extension of the methyl groups of 5-MeO-DMT (G_i BRET half-maximum effective concentration (EC₅₀) = 25.6 nM) to one ethyl group (5-MeO-MET; G_i BRET EC₅₀ = 25.9 nM) or two ethyl groups (5-MeO-DET; G_i BRET EC₅₀ = 37.1 nM) only marginally affected 5-HT_{1A} potency while retaining full efficacy, with similarly small effects at 5-HT_{2A} (Supplementary Table 1). By contrast, a cyclic pyrrolidine substituent increased potency at 5-HT_{1A} by about 12-fold (5-MeO-PyrT; G_i BRET EC₅₀ = 2.1 nM) and decreased 5-HT_{2A} potency by about 3-fold relative to 5-MeO-DMT. Cyclization of the amine moiety alone resulted in an approximately 38-fold increase in 5-HT_{1A} > 5-HT_{2A} selectivity. Next, we modestly decreased the steric demand of the pyrrolidine by removing two C-H bonds and installing a π bond. This change led to a further increase of around eightfold in potency at 5-HT_{1A} (5-MeO-3-PyrrolineT; G_i BRET EC₅₀ = 0.3 nM). By contrast, increasing the ring size to a six-membered piperidine (5-MeO-PipT; G_i BRET EC₅₀ = 88.5 nM) led to an approximate 42-fold loss of potency relative to 5-MeO-PyrT, which indicated a sensitivity to steric bulk at 5-HT_{1A} (Fig. 2b). Further elaboration to isoquinuclidine-containing tryptamines related to ibogaine led to a complete loss of 5-HT_{1A} activity (Supplementary Table 1 and Extended Data Fig. 3).

As the second structural variable, we tested different modifications of the indole nucleus. We mapped the SAR of several positions of the indole to investigate the specificity of the 5-MeO-DMT core for potent 5-HT_{1A} activity (Fig. 2 and Supplementary Table 1). On the basis of previous studies showing that indole fluorination strongly affects 5-HT₁ and 5-HT₂ binding affinities^{30,31}, we introduced fluorine in the 4-position of 5-MeO-DMT (4-F,5-MeO-DMT). This modification led to a 14-fold increase in signalling potency at 5-HT_{1A} and a 3-fold decrease in potency at 5-HT_{2A}. This trend was consistent across different analogues, whereby 4-fluorination caused an approximately tenfold increase in potency at 5-HT_{1A} and about fivefold potency decrease at 5-HT_{2A} (except for 5-MeO-3-PyrrolineT). Consequently, combining amine cyclization and 4-fluorination generated highly potent 5-HT_{1A} compounds, such as 4-F,5-MeO-PyrT (G_i BRET EC₅₀ = 370 pM) and 4-F,5-MeO-3-PyrrolineT (G_i BRET EC₅₀ = 220 pM). 4-F,5-MeO-PyrT was the more selective compound, with a greater than 800-fold selectivity for 5-HT_{1A} > 5-HT_{2A}, an effect driven by both an increased potency at 5-HT_{1A} and a decreased potency at 5-HT_{2A} (Fig. 2).

Amine alkylation and 5-HT_{1A} activity

To investigate the structural determinants of the observed SAR effects, we determined a cryo-EM structure of the 5-HT_{1A} signalling complex bound to 4-F,5-MeO-PyrT at a global resolution of 2.85 Å (Fig. 3, Extended Data Fig. 4 and Extended Data Table 1). Ligand modifications did not alter the binding pose, and we observed almost indistinguishable interactions with D116^{3,32} and T121^{3,37} compared with 5-MeO-DMT but distinct from serotonin (Figs. 1 and 3 and Extended Data Fig. 4). Similar to the methyl groups in 5-MeO-DMT, the pyrrolidyl substituent of 4-F,5-MeO-PyrT is wedged between F361^{6,51}, Y390^{7,43} and N386^{7,39} (Fig. 3 and Extended Data Fig. 4c).

We first probed this interaction by mutating the conserved F361^{6,51} to leucine and successively smaller hydrophobic side chains. Tryptamines

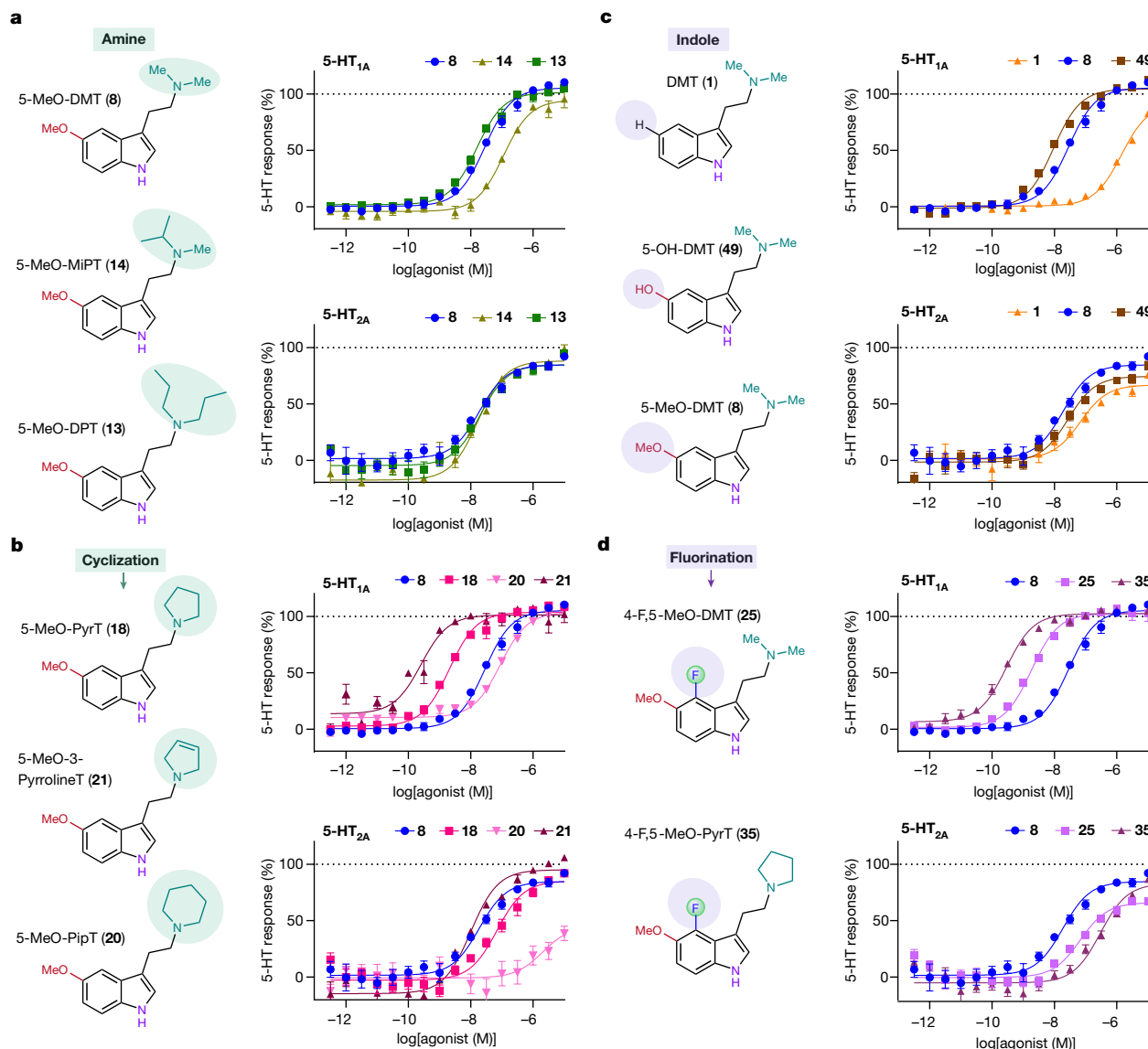


Fig. 2 | Differential pharmacological effects of amine and indole modifications to the 5-MeO-DMT scaffold at 5-HT_{1A} and 5-HT_{2A}. **a, b,** Schematic of modifications (left) and 5-HT_{1A}-Gi and 5-HT_{2A}-G_q BRET concentration–response activity (right) mediated by ‘designer’ tryptamines (**a**) and cyclized tryptamines (**b**). **c, d,** Schematic of modifications (left) and effects of indole modifications at the fifth position (**c**) and 4-fluorination (**d**)

on 5-HT_{1A}-Gi and 5-HT_{2A}-G_q BRET activity (right). All signalling experiments were performed in triplicate and are averaged from two (5-MeO-MiPT, 5-MeO-3-PyrrolineT, 5-MeO-PipT) or three (all other compounds) independent experiments. Data are normalized against 5-HT and errors bars denote the s.e.m.

with smaller or no amine substituents, such as 5-MeO-DMT and serotonin, showed greater loss of 5-HT_{1A} potency than 4-F,5-MeO-PyrT (Extended Data Fig. 4d and Supplementary Table 3). These results indicate that F361^{6,51} not only stabilizes the overall tryptamine scaffold, as shown in previous studies³², but also plays a key part in the potencies of different amine substitutions. We then investigated the interaction of N386^{7,39} by mutating it to the corresponding valine found at this position in 5-HT_{2A}. This modification led to a strong reduction in 5-HT_{1A} potency for 5-MeO-PyrT but not 5-MeO-DMT. Conversely, the V366N^{7,39} mutation in 5-HT_{2A} resulted in a small increase in potency only with cyclic amines. Notably, this mutation also led to an increase in signalling efficacy at 5-HT_{2A} across N-substituted tryptamines, including LSD. These findings highlight the importance of interactions with residues in position 7.39 for the activities of tryptamine ligands (Fig. 3). Our results also show that the residues F361^{6,51}, Y390^{7,43} and N386^{7,39} create a milieu that accommodates small amine rings that in turn leads to increased signalling potencies of these compounds.

Indole substitution and 5-HT_{1A} activity

Our SAR results confirmed that the introduction of fluorine in the 4-position or different groups in the 5-position of the indole nucleus substantially increases the signalling potency at 5-HT_{1A} and affects 5-HT_{1A} > 5-HT_{2A} selectivity (Fig. 2 and Supplementary Table 1). To investigate the mechanistic basis of these differences, we focused on A365^{6,55}, as this alanine is unique to 5-HT_{1A} among 5-HT receptors and is situated in proximity to the 4-indole and 5-indole substituents. We therefore mutated A365^{6,55} to the corresponding asparagine found at this position in 5-HT_{2A}. We observed a reduction in potency of all examined tryptamines, including fluorinated analogues, with the largest decrease of about 18-fold for 5-OH-DMT (bufotenine) (Supplementary Table 3). The A365^{6,55} of 5-HT_{1A} is therefore important for the high potency of 5-substituted tryptamines, an effect probably due to its ability to accommodate groups with various chemical properties. Meanwhile, specific interactions with A365^{6,55} are not responsible for increases in

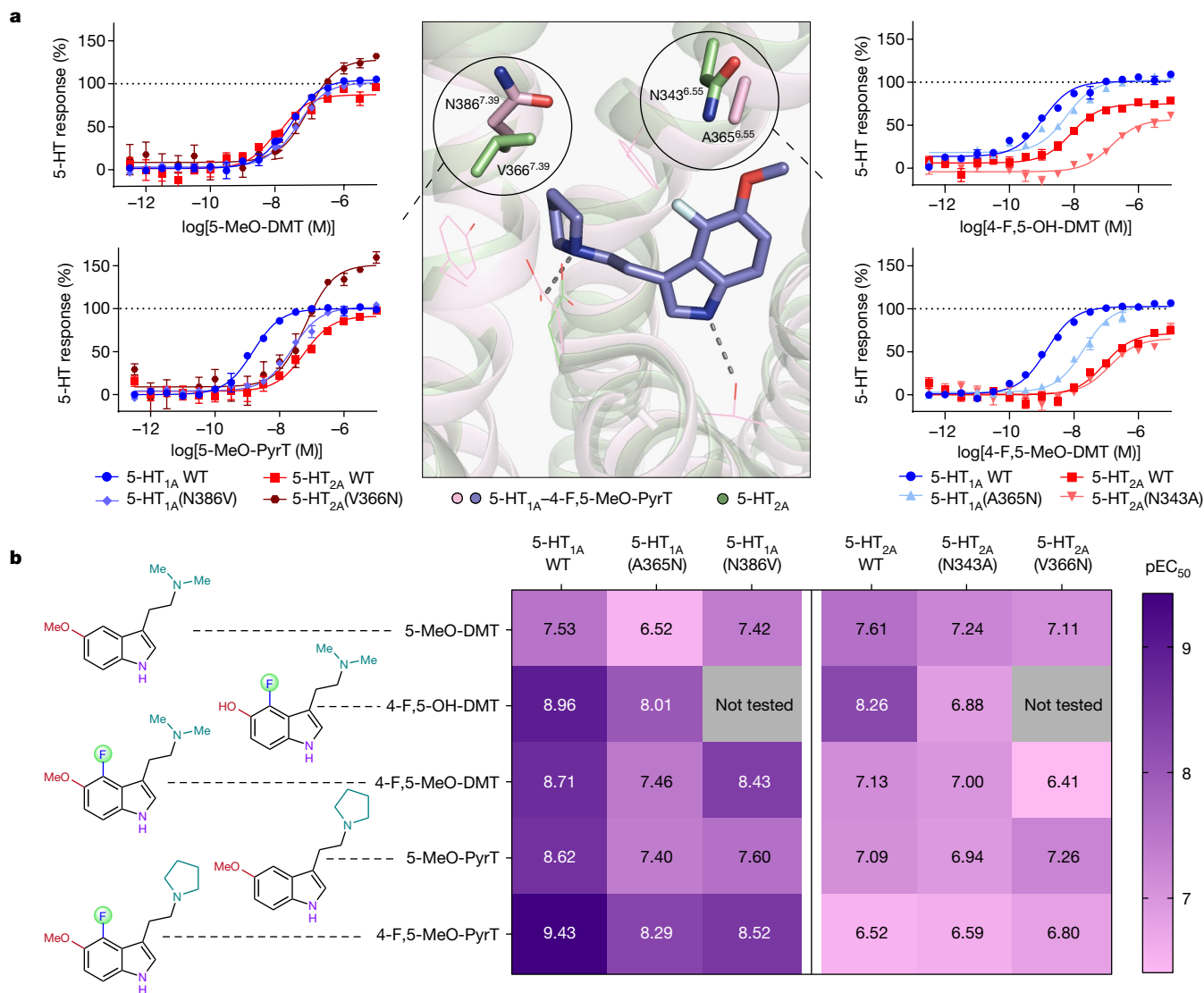


Fig. 3 | Elucidation of potency and selectivity determinants of tryptamines at 5-HT_{1A} and 5-HT_{2A}.

a, Left and right, 5-HT_{1A}-G_i and 5-HT_{2A}-G_q signalling mediated by tryptamine compounds at mutant and wild-type (WT) receptors according to BRET assays. Middle, residue differences in OBPs of 5-HT_{1A} (pink) and 5-HT_{2A} (green; PDB identifier 6WGT). **b**, Heatmap showing the effect of tryptamine modifications on the signalling potency (pEC₅₀) at mutant and

wild-type 5-HT_{1A} and 5-HT_{2A} receptors. Grey boxes indicate compound not tested. All signalling experiments were performed in triplicate and are averaged from two (5-HT_{1A}(N386V), 5-HT_{2A}(V366N), 5-HT_{1A}(A365N): 4-F,5-MeO-PyrT; 5-HT_{2A}(N343A): 4-F,5-MeO-PyrT) or three (all other compounds) independent experiments. Data are normalized against 5-HT and errors bars denote the s.e.m.

potency of fluorinated tryptamines, which are probably driven by the changes in electron density distribution in the fluorinated compounds and the resulting interactions with larger surfaces of the receptor.

Conversely, the N343^{6.55} mutation in 5-HT_{2A} to the alanine in 5-HT_{1A} had little effect on the potency of 4-F,5-MeO-tryptamines, but decreased the potency of serotonin and 4-F,5-OH-DMT by about 133-fold and 19-fold, respectively. As we only observed minor effects on DMT, we propose that N343^{6.55} of 5-HT_{2A} forms a hydrogen bond with the 5-hydroxyl group during activation. We further suggest that the decreased 5-HT_{2A} potency of 4-fluorinated tryptamines is due to an already reduced ability of 5-MeO-DMT to form hydrogen bonds with N343^{6.55}, as addition of a fluorine further changes the electronic character of the 5-position oxygen.

Structures of therapeutic-bound 5-HT_{1A}

Although psychedelics are useful tools to study 5-HT receptor structure and function, a renewed interest in their actions has been sparked by their therapeutic potential in the treatment of psychiatric disorders,

such as treatment-resistant depression and anxiety disorders^{1,33}. Even though the contribution of 5-HT_{1A} activity to the clinical efficacy of tryptamines and ergolines remains unclear, 5-HT_{1A} is a bona fide therapeutic target for the clinically used anxiolytic buspirone and the antidepressant vilazodone. To better understand the molecular pharmacology of 5-MeO-DMT in the context of prescribed medications, we performed structural and functional characterizations of buspirone and vilazodone and compared them to that of 4-F,5-MeO-PyrT, a 5-HT_{1A}-selective analogue of the psychedelic 5-MeO-DMT (Fig. 4). As it is unknown how buspirone and vilazodone bind to 5-HT_{1A}, we determined cryo-EM structures of drug-bound 5-HT_{1A}-G_i complexes at nominal resolutions of 2.62 Å and 2.94 Å, respectively (Fig. 4, Extended Data Fig. 5 and Extended Data Table 1). The binding pockets of all 5-HT_{1A}-drug complexes analysed here showed only subtle differences, with essentially all relevant side chains assuming similar states, except for an observed rotamer switch in the buspirone-bound receptor (described below). The indole core of vilazodone is bound to the OBP, forming a similar hydrogen bond with T121^{3.37} as observed for 5-MeO-DMT and 4-F,5-MeO-PyrT.

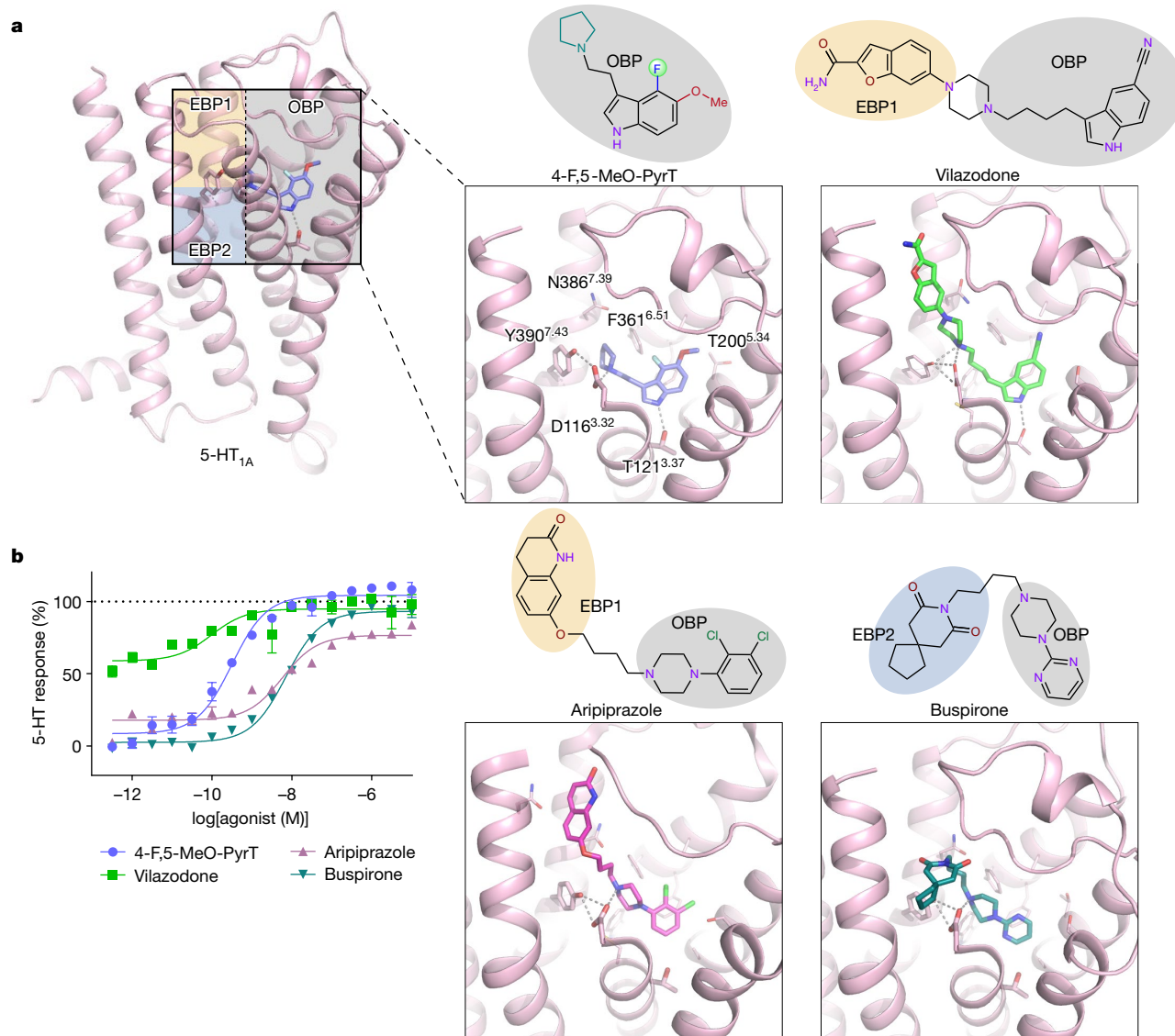


Fig. 4 | Structural and functional comparison of 4-F,5-MeO-PyrT and clinical 5-HT_{1A} medications at 5-HT_{1A}. **a**, Top, two-dimensional structures of 4-F,5-MeO-PyrT, vilazodone, aripiprazole and buspirone. Bottom, structural comparison of drug-binding poses of 4-F,5-MeO-PyrT (blue), vilazodone (green) and buspirone (teal) determined in this study, as well as a previous structure of aripiprazole (magenta; PDB identifier 7E2Z). Buspirone assumes a kinked conformation binding to a previously undescribed EBP (EBP2, blue shade), whereas vilazodone and aripiprazole stretch towards the extracellular

space forming interactions in a distinct EBP (EBP1, yellow shade). All compounds assume similar overall poses in the OBP (grey shade) of 5-HT_{1A}. **b**, 5-HT_{1A}-G_i BRET values of 4-F,5-MeO-PyrT, vilazodone, aripiprazole and buspirone. All signalling experiments were performed in triplicate and are averaged from two (aripiprazole) or three (all other compounds) independent experiments. Data are normalized against 5-HT and errors bars denote the s.e.m.

However, its benzofuran carboxamide group extends towards the extracellular site. Although this overall binding pose is reminiscent of aripiprazole (Abilify)²², an antipsychotic and antidepressant, the piperazine moiety of vilazodone is located farther towards the extracellular space than observed in any of the other compounds (Fig. 4). This feature is probably due to the differing binding configurations of vilazodone and aripiprazole. Specifically, the N-aryl substituent of aripiprazole and the N-alkyl substituent of vilazodone bind to the OBP, and D116^{3.32} and Y390^{7.43} therefore interact with the same piperazine amine in both compounds. By contrast, buspirone assumes an unusual overall binding mode, whereby its azaspirodecane-7,9-dione group does not extend towards the extracellular space, as observed for the piperazine substituents of aripiprazole or vilazodone. Instead, buspirone adopts a kinked conformation in which the azaspirodecane-7,9-dione group bends into a crevice between TM2 and TM3. There it seems to displace

the side chain of F112^{3.28}, which switches its rotamer conformation to face the membrane (Extended Data Fig. 5d). In the OBP, the piperazine of buspirone interacts with D116^{3.32} in a similar fashion as observed for aripiprazole, and its pyrimidine group is located near TM3 and TM5, forming mostly hydrophobic interactions. Overall, we note that despite their diverse scaffolds, vilazodone, aripiprazole and buspirone assume similar poses in the core OBP, with their aromatic piperazine substituents primarily being stabilized by phenylalanines in TM6 (F361^{6.51} and F362^{6.52}). 5-MeO-DMT and the 5-HT_{1A}-selective analogue 4-F,5-MeO-PyrT therefore exhibit similar binding modes as antidepressant medications in the OBP, which implies that these compounds have related pharmacological activities.

To test this hypothesis, we performed signalling assays to characterize and contrast the *in vitro* pharmacological activities of the different prescription drugs to those of 4-F,5-MeO-PyrT (Fig. 4). In BRET

assays determining 5-HT_{1A}-mediated activation of G_i, buspirone (maximum drug effect (E_{\max}) = 93.4% of 5-HT), vilazodone (E_{\max} = 97.4%) and 4-F,5-MeO-PyrT (E_{\max} = 102.8%) were all high-efficacy agonists, whereas aripiprazole (E_{\max} = 77.1%) showed modestly reduced efficacy. Note that vilazodone showed activity at concentrations as low as 30 pM, which we suspect is due to the slow binding kinetics of this drug. Vilazodone (G_i BRET EC₅₀ = 480 pM) showed the highest potency of the medications tested, which could be partially due to its atypical binding mode. In addition to its similar binding mode in the OBP to that of prescribed antidepressants, the 5-HT_{1A}-selective indoleamine 4-F,5-MeO-PyrT (G_i BRET EC₅₀ = 370 pM) showed comparable potency and efficacy to vilazodone. Although these findings underscore that 4-F,5-MeO-PyrT exhibits 5-HT_{1A} activity comparable to that of antidepressant medications, it also revealed subtle differences in their efficacies. This result is in line with our observation that vilazodone, buspirone and aripiprazole also bind to extended binding pockets (EBPs) and probably stabilize conformational ensembles distinct from that of 5-MeO-DMT and 4-F,5-MeO-PyrT. Thus, 4-F,5-MeO-PyrT as a 5-HT_{1A}-selective tryptamine could enable the exploration of 5-HT_{1A}-mediated behavioural aspects of 5-MeO-tryptamines.

In vivo activity of a 5-MeO-DMT derivative

We next tested the utility of 4-F,5-MeO-PyrT as a target-selective in vivo probe to interrogate the role of 5-HT_{1A} in both the hallucinogenic and the potential therapeutic effects of 5-MeO-tryptamines. Results from in vitro studies showed that 4-F,5-MeO-PyrT is more than 800-fold selective for 5-HT_{1A} over 5-HT_{2A} (Extended Data Fig. 6a and Supplementary Table 1). Moreover, the compound had substantially reduced activity at all other G protein-coupled 5-HT receptors, the serotonin transporter and other neurotransmitter transporters (Extended Data Fig. 6b,c). In mice, 4-F,5-MeO-PyrT brain penetration peaked 30 min following drug administration (total brain-to-plasma ratio of 3.3, unbound brain-to-plasma ratio of 0.91; peak plasma concentration ($C_{\max/\text{brain}}$) = 143 ng ml⁻¹ after 1 mg kg⁻¹ subcutaneous (s.c.) administration), and the compound was largely cleared within 2 h (Fig. 5a and Extended Data Table 2). At 82.5% brain tissue binding, the estimated free drug concentration in the brain at the time for the peak plasma concentration (T_{\max}) was about 100 nM following a s.c. dose of 1 mg kg⁻¹, which is anticipated to exert high 5-HT_{1A} engagement in vivo (G_i BRET EC₅₀ = 370 pM), with substantially reduced engagement of 5-HT_{2A} (G_q BRET EC₅₀ = 300 nM) (Extended Data Table 2 and Supplementary Table 1). To test these estimates in vivo, we examined in mice acute locomotor activity suppression (sedation) as a measure of 5-HT_{1A} activation and the head-twitch response (HTR) as a measure of 5-HT_{2A} activation³⁴. We observed dose-dependent locomotor suppression in the open-field test for both 4-F,5-MeO-PyrT and 5-MeO-MET (Extended Data Fig. 7), a non-scheduled, balanced 5-HT_{1A} and 5-HT_{2A} agonist with near-identical in vitro activity to 5-MeO-DMT (Extended Data Fig. 6a). In HTR tests, 5-MeO-MET showed strong dose-dependent activity following co-administration of the 5-HT_{1A}-selective antagonist WAY-100635. This result indicated that HTR was suppressed by 5-HT_{1A} activation in our experimental design, which is a well-documented effect of 5-HT_{1A} agonism^{35,36} (Fig. 5b). By contrast, 4-F,5-MeO-PyrT did not produce notable HTR at doses up to 3 mg kg⁻¹ in the presence or absence of WAY-100635 (Fig. 5b and Extended Data Fig. 7d). 4-F,5-MeO-PyrT therefore does not display apparent 5-HT_{2A} activity in vivo following comparatively high doses (up to 100-fold higher than the lowest dose that produced suppression of locomotion: 0.03 mg kg⁻¹). Together, these data suggest that in vivo, 4-F,5-MeO-PyrT is highly potent and selective for 5-HT_{1A}, readily enters the brain and does not functionally engage 5-HT_{2A} at the tested doses.

As 5-MeO-DMT is reported to have anxiolytic and antidepressant activity in humans^{10,37}, we next investigated 5-HT_{1A}-mediated behaviours in preclinical models using 4-F,5-MeO-PyrT. We used a chronic social

defeat (SD) stress model to induce a depressive-like phenotype in mice, which has been amply validated using selective serotonin reuptake inhibitors (chronic dosing) and ketamine (single administration)^{38–40}.

Male C57BL/6J mice were introduced to an unknown CD-1 aggressor mouse for 10 consecutive days of 10-min defeat bouts. Twenty-four hours after the last defeat bout, mice were tested in a two-part social interaction (SI) test (Fig. 5c). In the first phase, experimental mice were permitted to explore an open field (no target). In the second phase, a new unknown CD-1 aggressor mouse was placed into the enclosure (target). The interaction ratio (IR) was then determined by calculating the time that the experimental mouse spent in the interaction zone during the no-target and target phases. Given the social nature of rodents, control mice tend to spend more time with the new target mouse than with the empty enclosure, whereas stress-susceptible mice exhibit a generalized avoidance of conspecifics and spend less time interacting with the new mouse^{41,42}.

To test the effect of 4-F,5-MeO-PyrT in this experiment, we injected 1 mg kg⁻¹ of drug or vehicle (s.c.) 1 h after the last SD session on day 10, followed by the SI test 24 h after drug administration on day 11. This delayed readout reduced the confounding acute sedative effects of 4-F,5-MeO-PyrT and acts as a test of lasting therapeutic-like effects beyond initial drug exposure.

Results from the group that received vehicle confirmed that the SD paradigm induced social avoidance, as indicated by decreased IR values (Fig. 5d). This phenotype was rescued by 4-F,5-MeO-PyrT treatment, as indicated by an increased IR in the stress-exposed mice. Control mice that received either vehicle or drug did not show any distinguishable differences in IR, which indicated that the drug has measurable effects in a stress-experienced population. Results from mice co-administered with the selective 5-HT_{1A} antagonist WAY-100635 provided validation that the effects of 4-F,5-MeO-PyrT in this paradigm were indeed mediated by 5-HT_{1A}. WAY-100635 co-administration blocked the ability of 4-F,5-MeO-PyrT to ameliorate SI deficits in this model. Given the high affinity and selectivity of 4-F,5-MeO-PyrT and the inhibition of its behavioural effect by the established 5-HT_{1A} antagonist WAY-100635, these results suggest that the observed effects of 4-F,5-MeO-PyrT are mediated by 5-HT_{1A}.

We also recorded locomotor activity in a group of animals following SD and treatment with vehicle or 4-F,5-MeO-PyrT to eliminate the possibility of confounding factors due to decreased locomotion (Extended Data Fig. 7e). The amount of time each animal spent in the corners of the arena was also quantified. Vehicle-treated defeated mice spent more time in the corners, a result that confirmed previous observations that defeated mice not only show reduced SI but also engage in more vigilant, antisocial behaviours (Extended Data Fig. 7f,g). As expected, owing to the increased SI, this behaviour was no longer observed in drug-treated mice. It has been reported⁴² that approximately one third of mice seem to be stress-resilient in this paradigm, as defined by a SI ratio of greater than 1. This effect potentially leads to an underestimation of the effect of 4-F,5-MeO-PyrT in our experiments. To account for this resilience and to determine whether 4-F,5-MeO-PyrT treatment could increase the proportion of resilient mice, we compared vehicle-treated and drug-treated stressed animals. The results showed that 4-F,5-MeO-PyrT increased the number of resilient mice (Extended Data Fig. 7h). Together, these findings further support the conclusion that 4-F,5-MeO-PyrT treatment ameliorates stress-related social deficits.

To evaluate additional depressive-like behaviours induced by SD, we investigated anhedonia using a sucrose-preference test immediately following SI⁴² (Fig. 5e). Mice were allowed ad libitum access to two bottles: one containing water and the other 1% sucrose. Stressed mice treated with vehicle had a significantly reduced preference for sucrose compared with non-stressed mice, results that validate the depressive-like phenotype induced by SD. By contrast, stressed mice treated with 4-F,5-MeO-PyrT had significantly increased sucrose

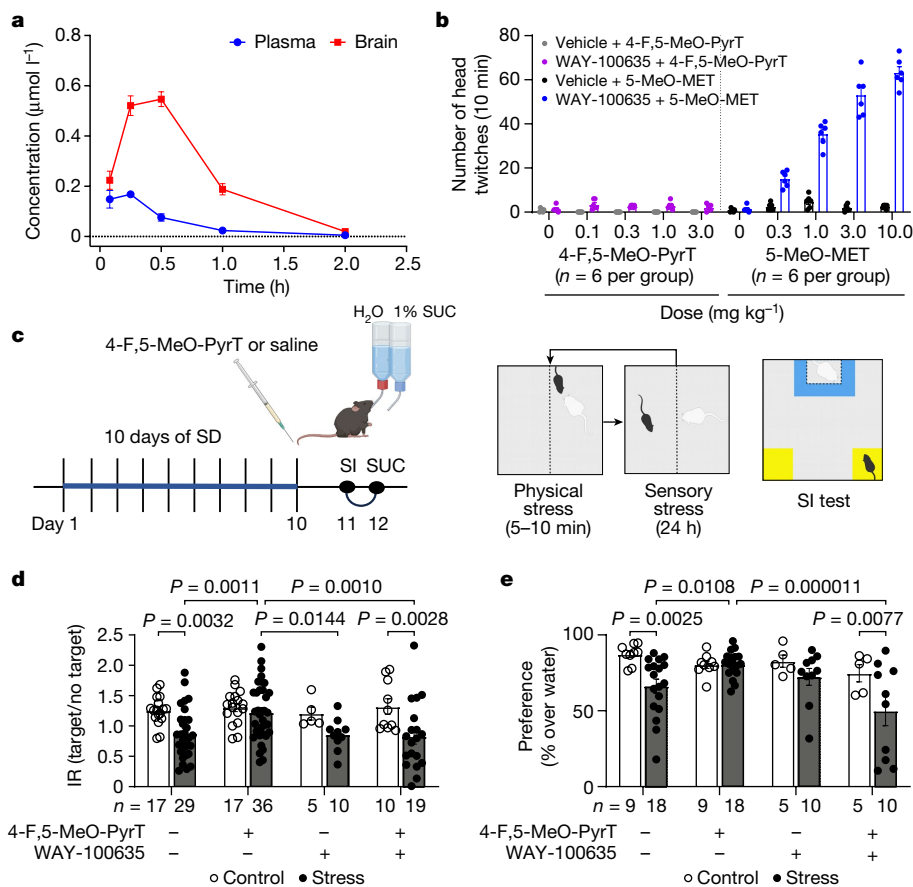


Fig. 5 | Effects of 5-MeO-DMT derivatives on mouse behaviour.

a, Pharmacokinetics profile of 4-F,5-MeO-PyrT following s.c. administration of 1 mg kg⁻¹. Data were obtained from three mice per time point (*n* = 3). **b**, HTR as a measure of 5-HT_{2A}-mediated hallucinogenic activity in mice in the presence and absence of the 5-HT_{1A}-selective antagonist WAY-100635 (1 mg kg⁻¹). **c**, Schematic of the chronic SD stress paradigm. **d,e**, Effects of saline, 4-F,5-MeO-PyrT and WAY-100635 administration on control mice and chronically defeated (stressed) mice. Determination of SI (**d**) and preference for 1% sucrose (SUC) solution over water in a two-bottle choice test (**e**) as a

measure of a depressive-like phenotype. Compounds were dosed at 1 mg kg⁻¹ unless otherwise indicated. Data are averaged from three (**d**) and two (**e**) independent experiments, and the number of mice for each group is indicated below the data for each respective group. Differences were determined by two-way analysis of variance with multiple comparisons using Fisher's least significance difference post hoc test, and exact *P* values are denoted in the figure. Error bars denote the s.e.m. Schematic in **c** was created using BioRender (<https://www.biorender.com>).

preference relative to vehicle-treated animals and were essentially indistinguishable from non-stressed mice treated with vehicle or drug. As observed for the SI experiment, co-administration of WAY-100635 reversed the effects of 4-F,5-MeO-PyrT in stressed animals, which suggests that the anti-anhedonic effect of 4-F,5-MeO-PyrT is mediated by 5-HT_{1A}. WAY-100635 treatment alone seemed to increase sucrose preference in stressed mice; however, this effect was not significant, and a similar effect was not observed in the SI experiment.

Together, these data demonstrate that 4-F,5-MeO-PyrT can ameliorate social deficits and anhedonia in a SD mouse model in a similar fashion as has been shown for ketamine and selective serotonin reuptake inhibitors^{38,43,44}. Therefore, 5-HT_{1A} might play a key part in the observed therapeutic effects of 5-MeO-tryptamines.

Discussion

Recent preliminary clinical data suggest that 5-MeO-DMT is a promising transdiagnostic therapeutic with rapid and lasting effects. Our work and previous studies have shown that 5-MeO-DMT has comparable signalling potency and efficacy at 5-HT_{1A} and 5-HT_{2A} in vitro, and both receptors contribute to its in vivo pharmacology^{5,21,34}. In light of previous work showcasing 5-HT_{2A}-selective agonists that alleviate anxiety-like and depression-like states in preclinical models^{15,16},

we wanted to investigate the role of 5-HT_{1A} in both psychedelic and therapeutic effects of 5-MeO-tryptamines. Although 5-HT_{1A} is a validated therapeutic target for several approved medications, including vilazodone¹² and buspirone⁸, the importance of 5-HT_{1A} agonism to the therapeutic effects of tryptamine psychedelics has not been conclusively addressed.

Because 5-HT_{2A} is responsible for the visual and other sensory disturbances elicited by classical psychedelics, it is typically assumed that these receptors also mediate therapeutic effects. There is currently no clinical evidence to support this hypothesis, and the pre-clinical evidence is mixed. The 5-HT_{2A} antagonist ketanserin did not block psilocybin-mediated attenuation of anhedonia induced by chronic stress in mice⁴⁵, whereas in another study, ketanserin blocked 5-MeO-DMT-mediated effects in a forced-swim test⁴. In the latter study, psilocybin-mediated attenuation of anhedonia was abolished in 5-HT_{2A} knockout mice⁴. Similarly, synaptogenesis readouts considered relevant for the therapeutic effects of psychedelics were not mediated by 5-HT_{2A} in one study, whereas synaptic remodelling was 5-HT_{2A}-dependent in another study^{46,47}.

Our finding that 4-F,5-MeO-PyrT, a highly 5-HT_{1A}-selective agonist, rescued SI deficits and anhedonia in mice induced by a SD model is therefore of considerable interest and has potential implications for the therapeutic effects of 5-MeO-tryptamines. These data also support

the proposed complementary contributions of 5-HT_{1A} and 5-HT_{2A} to stress-coping mechanisms on a psychological¹⁷ and a cellular level⁴⁸, the role of 5-HT_{1A} in stress resilience⁴⁹, as well as the reported antidepressant efficacy of clinical¹² and preclinical⁵⁰ 5-HT_{1A} drugs. Furthermore, our results show that 5-HT_{1A}-selective tryptamines lack the preclinical indications of classical psychedelic effects (for example, HTR), which suggests that some of these compounds may not be hallucinogenic while retaining therapeutic effects.

With respect to acute psychedelic effects, studies suggest that receptors other than 5-HT_{2A} also modulate subjective experience. LSD has recently been reported to enhance emotional empathy and prosocial behaviour in healthy volunteers; however, the empathogenic effect was not blocked by ketanserin⁵¹. Other studies specifically point to a major role of 5-HT_{1A}, as the 5-HT_{1A} antagonist pindolol increased the subjective psychedelic effects of DMT⁵². Moreover, the 5-HT_{1A} agonist buspirone attenuates the visual hallucinogenic effects of psilocybin in healthy volunteers⁵³, which implies that the distinct subjective effects of the psychedelic experience of 5-MeO-DMT could be shaped by 5-HT_{1A}. Examination of the anecdotal reports on 5-MeO-DMT analogues studied herein reveals a myriad of psychoactive effects²⁸ (Supplementary Table 2). However, systematic human data are not available to enable meaningful correlation between the subjective experience of different 5-MeO-DMT derivatives and 5-HT_{1A} potency, and/or relative potency of 5-HT_{1A} and 5-HT_{2A}. It should be noted though that 5-MeO-PyrT, an analogue for which we report >38-fold selectivity for 5-HT_{1A} over 5-HT_{2A}, induces effects described as white-out and amnesia²⁸ (Supplementary Table 2). Although 5-MeO-DMT has some amnesic elements to its subjective experience⁵⁴, this effect seems to be amplified in 5-MeO-PyrT²⁸, which indicates that 5-HT_{1A} activation by 5-MeO-tryptamines has a possible role in the mediation of these brain states and effects.

Despite the importance of 5-HT_{1A} in the effects of psychedelics, comparatively little is known about the structural pharmacology of different psychedelics at 5-HT_{1A}. We addressed this important gap in knowledge in the current work by integrating cryo-EM with systematic receptor mutagenesis and medicinal chemistry. Together, we provide both a global comparative map of receptor structural pharmacology for different drug classes and detailed analyses of crucial binding areas and specific amino acid residues that determine the signalling potency and efficacy at 5-HT_{1A}, as well as the selectivity for 5-HT_{1A} over 5-HT_{2A}. We uncovered how receptor-specific subpockets determine both the potency and efficacy of tryptamine ligands at both receptors. Our findings provide a structure-guided framework that enables the development of tryptamine probes with finely tuned pharmacological activities and varying degrees of 5-HT_{1A} and 5-HT_{2A} selectivity, including potent and highly 5-HT_{1A}-selective compounds. Moreover, we elucidated how 5-MeO-DMT and a selective analogue engage 5-HT_{1A} in an almost identical way, thereby showcasing the usefulness of this probe in studying 5-HT_{1A}-mediated aspects of 5-MeO-DMT. These binding poses partially overlap with those of the 5-HT_{1A}-targeting medications buspirone, vilazodone and aripiprazole²². However, these medications occupy EBPs, which indicate the stabilization of distinct conformational ensembles and therefore the generation of signal outputs distinct from those of psychoactive tryptamines. These differences, as well as their engagement of other targets^{7,55}, probably have a role in the different physiological effects of these medications compared with the psychedelic 5-MeO-DMT, but further experiments are required to investigate the precise correlates in detail. Nonetheless, we demonstrated that modification of the 5-MeO-DMT scaffold can produce highly selective probes that engage 5-HT_{1A} in a structural and pharmacological manner that is similar to clinical drugs. Of note, the anxiolytic-like and antidepressant-like effects of these probes emphasize their utility in elucidating 5-HT_{1A}-mediated effects of tryptamine psychedelics in vivo and potentially facilitate the exploration of therapeutic applications in future work.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-024-07403-2>.

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Methods

Chemical synthesis

Compounds were synthesized as depicted in Supplementary Data 1. Compounds not synthesized in-house were purchased from the following sources: 5-HT (Sigma), 5-MeO-DMT (Cayman Chemicals), DMT (Cayman Chemicals), psilocin (Cayman Chemicals), 4-OH-MET (Cayman Chemical), LSD (Cayman Chemicals), mescaline (Cayman Chemicals), vilazodone (Sigma), buspirone (Alfa Aesar), bufotenine (Cayman Chemical), aripiprazole (Sigma), citalopram (TCI), FFN246 (Aobious), IDT307 (Sigma), ASP+ (Invitrogen), WAY-100635 (Biovision), 8-OH-DPAT (MedChemExpress), SEP-363856 (MedChemExpress), *N*-(1-naphthyl)piperazine (Alfa Aesar), tandospirone (Toronto Research Chemicals) and gepirone (Toronto Research Chemicals).

Most tryptamines were synthesized from commercially available indoles (unless otherwise specified) in a sequence of oxalation, glyoxy esterification, reduction, bromination and finally *N*-alkylation. In other cases, the Speeter–Anthony route was applied³⁰. 7-Azaindole tryptamines were obtained through acylation with bromoacetyl bromide, followed by reduction and *N*-alkylation. For the purification of the compounds, preparative TLC (1,000 micron plates) or silica gel chromatography for large-scale reactions was used. Compound characterization, including confirmation of the chemical identity and purity, was accomplished by ¹H, ¹³C and ¹⁹F nuclear magnetic resonance and high-resolution mass spectrometry. For detailed synthetic procedures and analytical data, see Supplementary Information.

Constructs and expression

Structural studies reported herein were performed with a 5-HT_{1A} construct based on previous work²². In brief, human 5-HT_{1A} (UniProtKB identifier P08908) was cloned into a modified pFastBac vector and a cleavable HA-signal sequence followed by a Flag tag, a 10×His tag and a TEV protease site was introduced at the amino terminus. In addition, the first 24 residues were replaced with BRIL, and a L125W^{3,41} mutation was introduced to increase expression levels. Heterotrimeric G protein was expressed from a single multibac virus following previous construct design¹³. In brief, *N*-terminally 6×His-tagged human Gβ₁ was cloned under the control of a polyhedrin promoter, whereas a Gγ₂–Gα_{1i} fusion construct was cloned under the control of a P10 promoter. Gγ₂ and Gα_{1i} were fused with a GSAGSAGSA linker. The 5-MeO-DMT–5-HT_{1A}–Gα_{1i}–Gβ₁–Gγ₂ and buspirone–5-HT_{1A}–Gα_{1i}–Gβ₁–Gγ₂ structures used a Gα_{1i} protein containing four individual point mutations (S47N, G203A, E245A and A326S) to enhance the stability of the heterotrimeric G protein (dominant-negative)²². Protein expression was done in Sf9 cells (Expression Systems) using the Bac-to-Bac Baculovirus expression system (Invitrogen), for which bacmid DNA was generated in DH10Bac cells (Invitrogen). Initial P0 virus was obtained through the addition of about 3 μg recombinant bacmid DNA with 3 μl FuGENE HD transfection reagent (Promega) in 100 μl Sf900 II medium (Invitrogen) to 500,000 Sf9 cells plated in 2 ml of SF900 II medium in wells of a 12-well plate. After 5 days at 27 °C, the supernatant was collected as viral stock, and high-titre recombinant P1 baculovirus (>10⁹ virus particles per ml) was generated by adding 300 μl P0 to 30 ml of 3 × 10⁶ cells per ml and incubating cells at 27 °C for 3 days. Approximate titres were estimated by flow cytometry analysis staining P1-infected cells with gp64-PE antibody (Expression Systems). Expression of both 5-HT_{1A} and heterotrimers was done separately by infecting Sf9 cells at a cell density of 2–3 × 10⁶ cells per ml with P1 virus at a multiplicity of infection of 5. After 48 h of shaking at 27 °C, cells expressing either receptor or G protein were collected by centrifugation (2,500g, 15 min, 4 °C) at 48 h after infection and stored at –80 °C until use.

5-HT_{1A} purification

For 5-HT_{1A} purification, insect cell membranes were disrupted by thawing frozen cell pellets in a hypotonic buffer containing 10 mM HEPES

pH 7.5, 10 mM MgCl₂, 20 mM KCl and home-made protease inhibitor cocktail (500 μM AEBSF, 1 μM E-64, 1 μM leupeptin and 150 nM aprotinin) (Gold Biotechnology). Total cellular membranes were spun down and washed twice by homogenization in a manual dounce homogenizer using around 30 strokes followed by centrifugation (125,000g, 40 min, 4 °C) in a high osmotic buffer containing 1 M NaCl, 10 mM HEPES pH 7.5, 10 mM MgCl₂, 20 mM KCl and home-made protease inhibitor cocktail. Purified membranes were directly flash-frozen in liquid nitrogen and stored at –80 °C until further use.

For the formation of 5-HT_{1A}–drug complexes, membranes were suspended in buffer containing 10 mM HEPES pH 7.5, 10 mM MgCl₂, 20 mM KCl, 150 mM NaCl, home-made protease inhibitor cocktail and 20 μM 5-MeO-DMT (Cayman Chemicals), LSD (Cayman Chemicals), 4-F,5-MeO-PyrT (synthesized in-house), vilazodone (Sigma) or buspirone (Alfa Aesar). Drug complexation was initiated through agitation for 1 h at room temperature followed by equilibration at 4 °C, addition of 2 mg ml^{–1} final concentration of iodoacetamide (Acros Organics) and subsequent solubilization through the addition of a final concentration of 1% (w/v) *n*-dodecyl-β-D-maltopyranoside (DDM; Anatrace), 0.2% (w/v) cholesteryl hemisuccinate (CHS; Anatrace) and home-made protease inhibitor cocktail for 2 h at 4 °C. Unsolubilized material was then removed by centrifugation (200,000g, 30 min, 4 °C), and imidazole was added to the supernatant to a final concentration of 20 mM. Proteins were bound to TALON IMAC resin (Clontech). Protein-bound TALON resin was washed with 10 column volumes (c.v.) of wash buffer I (50 mM HEPES, pH 7.5, 800 mM NaCl, 0.1% (w/v) DDM, 0.02% (w/v) CHS, 20 mM imidazole, 10% (v/v) glycerol and 10 μM drug). The detergent was then exchanged for lauryl maltose neopentyl glycol (LMNG) by incubating the protein-bound TALON with wash buffer I supplemented with 0.1% LMNG for 1 h at 4 °C followed by successive washes with the following buffers: wash buffer II (50 mM HEPES, pH 7.5, 800 mM NaCl, 0.05% (w/v) LMNG and 0.01% (w/v) CHS), wash buffer III (50 mM HEPES, pH 7.5, 800 mM NaCl, 0.01% (w/v) LMNG and 0.002% (w/v) CHS) and wash buffer IV (25 mM HEPES, pH 7.5, 500 mM NaCl, 0.005% (w/v) LMNG and 0.001% (w/v) CHS). After the final incubation step, the proteins were eluted with 25 mM HEPES, pH 7.5, 500 mM NaCl, 0.005% (w/v) LMNG, 0.001% (w/v) CHS, 10 μM drug and 250 mM imidazole. The eluted proteins were concentrated using Vivaspin 6 centrifugal concentrators (Sartorius), and imidazole was removed from the protein solution by applying the sample to a PD MiniTrap sample column (Cytiva) according to the manufacturer's protocol. The resulting protein sample was incubated with 200 μg of TEV protease overnight. The following day, the protein mixture was incubated with TALON resin for 10 min at 4 °C to remove TEV and uncleaved protein from the solution. The cleaved protein samples were further concentrated and immediately used for complexation. General protein purity and monodispersity was assessed by SDS–PAGE and analytical size-exclusion chromatography.

G protein purification

For G protein purification, Sf9 insect cells were homogenized in the following lysis buffer: 20 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM MgCl₂, 0.01 mM guanosine diphosphate, 10% glycerol, 5 mM β-mercaptoethanol, 30 mM imidazole, 0.2% Triton X-100 and home-made protease inhibitor cocktail (500 μM AEBSF, 1 μM E-64, 1 μM leupeptin and 150 nM aprotinin). The cytoplasmic and membrane fractions were separated by centrifugation (50,000g, 20 min, 4 °C). The resulting supernatant was subjected to an additional centrifugation step (200,000g, 45 min, 4 °C) to remove insoluble material and protein aggregate. The final supernatant was bound to HisPur Ni-NTA resin (Thermo Scientific) overnight at 4 °C. Protein-bound Ni-NTA resin was washed with 20 c.v. lysis buffer lacking 0.2% Triton X-100 followed by 20 c.v. lysis buffer lacking 0.2% Triton X-100 and 30 mM imidazole. Protein was eluted from the resin with lysis buffer lacking Triton X-100 and supplemented with 300 mM imidazole. The

eluent was concentrated using Vivaspin 6 centrifugal concentrators (Sartorius). Imidazole was removed from the concentrated eluent using PD MiniTrap sample preparation columns (Cytiva) according to the manufacturer's protocol. Eluted protein was further processed through the addition of PreScission Protease (Genscript) to remove the N-terminal His tag from the G β_3 subunit. Purified G protein and PreScission Protease were incubated overnight at 4 °C to facilitate cleavage. Cleaved G proteins were separated from PreScission Protease and uncleaved proteins by 15 min incubation with TALON resin at 4 °C. Cleaved G proteins were concentrated for immediate use or flash frozen and stored at -80 °C.

Formation of receptor-G protein complexes, purification and grid preparation

To assemble drug-bound 5-HT_{1A}-G_i complexes, we mixed purified receptor (500–1,000 μ g at a molecular weight of approximately 55.4 kDa) with purified heterotrimer (1,000–2,000 μ g at a molecular weight of approximately 86.4 kDa) at a molar ratio of 1:1.2 in a total volume of 110 μ l. The final buffer was adjusted to 20 mM HEPES, pH 7.5, 120 mM NaCl, 5 mM CaCl₂, 2.5 mM MgCl₂, 0.001% (w/v) LMNG, 0.0002% (w/v) CHS and 10 μ l drug, and supplemented with 25 mU per ml apyrase before incubation overnight at 4 °C. The next day, samples were concentrated and finally purified over a S200 size-exclusion chromatography column equilibrated in 20 mM HEPES, pH 7.5, 100 mM NaCl, 0.001% (w/v) LMNG, 0.0002% (w/v) CHS, 0.00025% GDN and 1–10 μ M drug. Peak fractions were then pooled, concentrated to about 15–30 mg ml⁻¹ and immediately used to prepare grids for cryo-EM data collection. To prepare cryo-EM grids for imaging, 3 μ l of the samples was applied to glow-discharged holey carbon EM grids (Quantifoil 300 copper mesh, R1.2/1.3) in an EM-GP2 plunge freezer (Leica). An EM-GP2 chamber was set to 95% humidity at 12 °C. Sample-coated grids were blotted for 3–3.3 s before plunge-freezing into liquid ethane and stored in liquid nitrogen for data collection.

Cryo-EM data collection and processing

All automatic data collection was performed on FEI Titan Krios instruments equipped with Gatan K3 direct electron detectors either operated by the Simons Electron Microscopy Center in the New York Structural Biology Center or the Laboratory of BioMolecular Structure at Brookhaven National Laboratory. The microscopes were operated at 300 kV accelerating voltage, at a nominal magnification of 64,000–81,000 corresponding to pixel sizes of 0.41–0.538 Å. For each dataset, at least 3,500 movies were obtained at a dose rate of 25–30 electrons per Å² per s with a defocus ranging from -0.5 to -1.8 μ m. The total exposure time was 2 s and intermediate frames were recorded in 0.05 s intervals, resulting in an accumulated dose of 50–60 electrons per Å² and a total of 40 frames per micrograph.

Movies were motion-corrected using MotionCor2 (ref. 56) and imported to cryoSPARC⁵⁷ for further processing. Contrast transfer functions were estimated using patchCTF in cryoSPARC. An initial model was produced from a subset of micrographs using blob picking, followed by extraction, 2D classification, selection of key classes and generation of a model ab initio. Subsequent map models were produced from a curated micrograph set using particles found by picking using the initial map model as a template. Particles were extracted, subjected to 2D classification and a final particle stack was obtained by iterative rounds of 3D classification generating several bad models from rejected particles as a sink in hetero-refinement. A final map was obtained using NU-refinement⁵⁸. Structures were built in Coot⁵⁹ based on a previous 5-HT_{1A}-G_i complex structure²², and further refined using PHENIX⁶⁰ and ServalCat⁶¹. Sterols were built as cholesterol hemisuccinate; however, we cannot fully rule out the possibility that some of these densities stem from cholesterol co-purified with the receptor. Final maps were imported to PyMOL⁶² and UCSF Chimera for generating figures shown in the manuscript.

5-HT_{1A}-mediated cAMP reduction assay

Reduction in cellular cAMP levels mediated by 5-HT_{1A} was determined in HEK293-T cells (American Type Culture Collection) using a cAMP biosensor (GloSensor, Promega)^{25,32}. First, HEK293-T cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) FBS and penicillin-streptomycin (Invitrogen). For transfection, cells were plated in 10 cm plates and medium was exchanged to DMEM supplemented with 1% (v/v) dialysed FBS (dFBS). Once cells reached 6–7 million in a 10 cm plate, transfection was done, forming particles in 500 μ l Opti-MEM (Invitrogen) using 4 μ g of human 5-HT_{1A} with a N-terminal HA signal sequence and Flag tag cloned into a pcDNA3 vector, 4 μ g of GloSensor DNA and 16 μ l of polyethyleneimine (PEI; Alfa Aesar). The following day, medium was aspirated, and cells were trypsinized and plated into each well of a poly-L-lysine-coated clear-bottom 384-well plate (Greiner Bio One) in DMEM supplemented with 1% (v/v) dFBS. Next, 40 μ l of cells at a density of 500,000 per ml were plated per well. The next day, medium was aspirated and D-luciferin (Gold Biotechnology) was loaded into cells by adding 30 μ l of a 1.2 mM solution in Hank's balanced salt solution (HBSS) supplemented with 20 mM HEPES pH 7.4, 0.1% BSA (w/v) and 0.01% ascorbic acid (w/v). Loading was performed at 37 °C for 1 h, followed by addition of 15 μ l of drug solutions at 3 \times concentration for 15 min at room temperature. To measure agonist activity for 5-HT_{1A}, we added 15 μ l of isoproterenol in HBSS supplemented with 20 mM HEPES pH 7.4 at a final concentration of 400 nM (to activate Gs through endogenous adrenergic receptors). Luminescence intensity was determined 15 min later in a MicroBeta TriLux liquid scintillation counter (Perkin Elmer). Data were plotted and analysed using GraphPad Prism 8.0.

5-HT_{7A}-mediated cAMP stimulation assay

HEK293-T cells were cultured and transfected as described above with 4 μ g of human 5-HT_{7A} with a N-terminal HA signal sequence and Flag tag cloned into a pcDNA3 vector and 4 μ g of GloSensor DNA^{25,32}. Transfected cells were split, plated and loaded with luciferin in the same manner as described above. Cells were stimulated with 15 μ l of drug solutions at 3 \times concentration for 30 min at room temperature. Assay plates were read immediately after following incubation in a MicroBeta TriLux liquid scintillation counter (Perkin Elmer). Data were plotted and analysed using GraphPad Prism 8.0.

BRET signalling assays

We determined both 5-HT_{1A}-mediated activation of heterotrimeric G₁₁ (G α_{11} -G β_3 -G γ_9) and 5-HT_{2A}-mediated activation of heterotrimeric G_q (G α_q -G β_3 -G γ_9) using previously published TRUPATH reporter constructs¹⁸. Assays were essentially performed as previously described. In brief, receptor as well as RLuc-G α , G β and eGFP-G γ constructs were transfected with PEI (Alfa Aesar) into HEK293-T cells using a ratio of 1:1:1:1 (5-HT_{1A}:G α_{11} :G β_3 :G γ_9) and 2:5:5:5 (5-HT_{2A}:G α_q :G β_3 :G γ_9). Typically, we transfected 6–7 million cells in DMEM supplemented with 1% (v/v) dFBS in a 10 cm plate with a total of 8 μ g of DNA. The next day, 20,000 cells were plated into each well of a poly-L-lysine-coated white-bottom 384-well plate (Greiner Bio One) in DMEM supplemented with 1% (v/v) dFBS. The following day, the day of the experiment, medium was aspirated and the cells were washed with HBSS supplemented with 20 mM HEPES pH 7.4. Next, 15 μ l of drug solutions at 3 \times concentration were added in 30 μ l HBSS supplemented with 20 mM HEPES pH 7.4, 0.1% (w/v) BSA and 0.01% (w/v) ascorbic acid. Cells were incubated for 30 min at 37 °C followed by addition of 15 μ l of freshly prepared 30 μ M coelenterazine 400a (Gold Biotechnology). Plates were then immediately read in a Victor NIVO plate reader (Perkin Elmer) with 395 nm (RLuc8-coelenterazine 400a) and 510 nm (GFP2) emission filters, at integration times of 1 s per well. BRET2 ratios were computed as the ratio of the GFP2 emission to RLuc8 emission. Data were plotted as a function of drug concentration, normalized to the per cent

of 5-HT stimulation, and analysed using 'log(agonist) vs. response' in GraphPad Prism 8.0.

Arrestin recruitment assay

To determine off-target effects, we performed PRESTO-Tango β -arrestin2 recruitment assays for all 5-HT receptors essentially as previously described⁶³. The receptor constructs were obtained from Addgene and contain the TEV cleavage site and the tetracycline transactivator (tTA) fused to the C terminus of the receptor. For the assay, constructs were transfected into HTLA cells (provided by B. Roth, University of North Carolina), which express TEV-fused β -arrestin2 and a tTA-driven luciferase and are necessary for this assay. A day before the assay, cells were cultured in DMEM supplemented with 1% dFBS and transfected with 8 μ g of DNA per 7 million cells in a 10 cm plate using PEI-mediated transfection. The next day, 20,000 cells in 40 μ l were then plated in poly-L-lysine-coated clear-bottom 384-well plates in the same medium and incubated for at least 5 h before stimulation. Next, 20 μ l drug solutions in HBSS supplemented with 20 mM HEPES pH 7.4, 0.1% (w/v) BSA and 0.01% (w/v) ascorbic were then added for overnight incubation. After 16–24 h of overnight incubation, medium and drug solutions were aspirated and 20 μ l per well of BrightGlo reagent (purchased from Promega, after 1:20 dilution) was added per well. The plate was incubated for 20 min at room temperature in the dark before being counted using a MicroBeta TriLux liquid scintillation counter. Relative luminescence units were then plotted as a function of drug concentration and compared to 5-HT stimulation.

Neurotransmitter transport assays

Serotonin transporter uptake inhibition. For determining inhibition of serotonin transporter (SERT) uptake, HEK293-T cells were cultured in DMEM supplemented with 10% (v/v) FBS and penicillin–streptomycin (Invitrogen). For transfection, cells were plated in 10 cm plates and medium was exchanged to DMEM supplemented with 1% (v/v) dFBS. Once cells reached 6–7 million in a 10 cm plate, a transfection mixture composed of 8 μ g of SERT cloned into a pcDNA3 vector, 500 μ l of OptiMEM and 16 μ l of PEI was added dropwise to the cells. The following day, transfected cells were trypsinized and plated at a density of 25,000 cells per well of a poly-L-lysine coated clear-bottom 384-well plate. The uptake inhibition assay was performed the following day. Medium was aspirated and cells were washed with HBSS supplemented with 20 mM HEPES pH 7.4. To each well, 30 μ l of HBSS containing 20 mM HEPES, 0.1% BSA and 0.01% ascorbic acid and 15 μ l of drug dilutions at 3 \times concentrations were added. Cells were then incubated at 37 °C for 45 min. A final concentration of 10 μ M FFN246 (Aobious)⁶⁴ was added to each well, and cells were incubated for an additional 1 h at 37 °C. The contents of each well were then aspirated, and each well was washed twice with HBSS supplemented with 20 mM HEPES pH 7.4. Next, 50 μ l of HBSS supplemented with 20 mM HEPES pH 7.4 was added to each well, and the assay plate was read using a Victor NIVO plate reader using 355 nm excitation and 435 nm emission filters, and measuring over 0.1 s. Data were plotted as a function of drug concentration, normalized to the per cent of citalopram inhibition and analysed using 'log(inhibitor) vs. response' in GraphPad Prism 8.0.

OCT1, OCT2 and PMAT uptake inhibition. Inducible cell lines stably expressing OCT1, OCT2 or PMAT were generated in-house using the Flp-In T-Rex cell line (ThermoFisher) according to the manufacturer's recommendations. Cells were maintained in DMEM supplemented with 10% FBS, 100 μ g ml⁻¹ hygromycin B (Gold Biotechnology) and 10 μ g ml⁻¹ blasticidin (Gold Biotechnology). To measure uptake inhibition, cells were seeded into poly-L-lysine-coated clear-bottom 384-well plates at a density of 25,000 cells per well in DMEM containing 10% FBS and 2 μ g ml⁻¹ tetracycline (Sigma) to induce transporter expression. At 24 h following induction, medium was aspirated and cells were washed with HBSS supplemented with 20 mM HEPES pH 7.4. To each well, 30 μ l of

HBSS with 20 mM HEPES pH 7.4, 0.1% (w/v) BSA and 0.01% ascorbic acid was added followed by 15 μ l of drug dilutions at 3 \times concentration. Cells were incubated with drug for 30 min at 37 °C. Fluorescent substrate was then added to the cells. To measure inhibition of OCT1 and PMAT, a final concentration of 12.5 μ M of IDT307 (Sigma) was used, whereas for OCT2, a final concentration of 5 μ M of the fluorescent substrate ASP+ (ThermoFisher) and 250 μ M trypan blue was added. Cells were incubated with respective substrates for 1 h at 37 °C. OCT2 cells were read directly in a Victor NIVO plate reader using 495 nm excitation and 580 emission filter for 0.1 s. OCT1 and PMAT cells were washed in HBSS supplemented with 20 mM HEPES pH 7.4 before reading in a Victor NIVO plate reader using 435 nm excitation and 480 nm emission filter for 0.1 s.

Cell lines

None of the cell lines used in this study were authenticated using independent methods. All cell lines were regularly tested for mycoplasma contamination.

Animal studies

The Icahn School of Medicine at Mount Sinai, Columbia University and Sai Life Sciences Limited are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All described experiments involving mice were approved by the Institutional Animal Care and Use Committee at the Icahn School of Medicine at Mount Sinai, Columbia University or Sai Life Sciences Limited to comply with ethical regulations. Male 8–15-week-old C57BL6/J mice were used as the experimental mice for all experiments. CD-1 retired breeders were used as aggressors and ranged from 4 to 6 months of age. Animals received regular veterinary care (weekly by institutional veterinarians), including daily health monitoring (by experimenters) of the animals (observing home cage behaviours, nesting and body weight). All procedures were designed to minimize any stress or distress. Mice were purchased from the Jackson Laboratory and housed 5 mice per cage with food and water available ad libitum. Mice were maintained on a 12-h light–dark cycle (lights on 7:00–19:00) and all testing was done in the light cycle. Temperature was kept constant at 22 \pm 2 °C, and relative humidity was maintained at 50 \pm 5%.

Drug preparation and administration for animal studies

All samples are prepared the same day testing was performed. Solids were weighed into small vials and dissolved in USP-grade 0.85% saline with the addition of 2 molar equivalents of glacial acetic acid. Sonication and gentle heating were applied until complete dissolution. The compounds were subsequently filtered through 0.45 μ m filters into a new glass vial. All compounds were administered at a selected s.c. dose.

Pharmacokinetics studies

Studies were conducted at Sai Life Sciences Limited. A total of 27 healthy C57BL/6 male mice were used in this study with 3 mice per time point. Animals were administered s.c. with a solution formulation of the compound at the indicated dose. Blood samples (approximately 60 μ l) were collected under light isoflurane anaesthesia (Surgivet) from the retro-orbital plexus from a set of 3 mice at 0.083, 0.25, 0.5, 1, 2, 4, 8 and 12 h. Immediately after blood collection, plasma was collected by centrifugation at 4,000 r.p.m. for 10 min at 40 °C and samples were stored at –70 \pm 10 °C until analyses. Following blood collection, animals were immediately euthanized and the abdominal vena cava was cut open. The whole body was then perfused from the heart using 10 ml of saline. Brain samples were collected from set of 3 mice at 0.083, 0.25, 0.5, 1, 2, 4, 8 and 12 h. After isolation, brain samples were rinsed 3 times in ice-cold saline for 5–10 s per rinse using 5–10 ml saline and subsequently dried on blotting paper. Brain samples were homogenized using ice-cold PBS (pH 7.4). Total homogenate volume was three times the tissue weight. All homogenates were stored below –70 \pm 10 °C until analyses.

Article

Concentrations of the compound in mouse plasma and brain samples were determined by LC–MS/MS. The non-compartmental-analysis tool of Phoenix WinNonlin (v.8.0) was used to assess the pharmacokinetics parameters. C_{\max} and T_{\max} were the observed values. The areas under the concentration time curve (AUC_{last}) was calculated using the linear trapezoidal rule. The terminal elimination rate constant, k_e , was determined by regression analysis of the linear terminal portion of the log plasma concentration–time curve. Tissue K_p values were calculated using Microsoft excel.

Plasma protein and brain tissue binding

Studies were conducted at Sai Life Sciences Limited. Previously frozen mouse plasma was thawed at room temperature. An aliquot of 1,000 μl was aliquoted into microcentrifuge tubes and incubated at 37 °C for 10 min. A volume of 5 μl of 4-F,5-MeO-PyrT or control drug from 1 mM DMSO stock was spiked into 995 μl of preincubated plasma to obtain 5 μM final concentration. Inserts were placed in a Teflon plate. Each insert was placed in the same orientation for easy recognition of the sample and buffer chamber. Next, 200 μl aliquots of 4-F,5-MeO-PyrT or control drug were added at 2 μM concentration ($n = 2$) in the red chamber and 350 μl of 50 mM PBS (pH 7.4) to the white chamber of the inserts. The red device was then sealed with an adhesive film and incubated at 37 °C with shaking at 100 r.p.m. for 4 h. For recovery and stability samples, 50 μl aliquots of 4-F,5-MeO-PyrT or control drug were added in 96-well deep-well plates and quenched with 400 μl acetonitrile (for 0 min). The remaining sample was incubated at 37 °C for 4 h. Following dialysis, 50 μl of aliquot was taken from each well (both red and white chamber) and diluted with equal volume of the opposite matrix to nullify the matrix effect. Similarly, 50 μl of PBS was added to recovery and stability samples. An aliquot of 100 μl was analysed by LC–MS/MS.

Open-field locomotion assay

Open-field locomotion evaluation was performed by trained observers who were blinded to both drug and dose. Male 10–15 week old C57BL/6J mice were allowed to habituate for 30 min. Mice were randomly administered with vehicle or WAY-100635 (1 mg kg^{-1} or 2 mg kg^{-1} , s.c.). Fifteen minutes after the pretreatment injection, mice were administered either 4-F,5-MeO-PyrT (0.1, 0.3, 1 or 3 mg kg^{-1} , s.c.) or 5-MeO-MET (0.3, 1, 3 or 10 mg kg^{-1} , s.c.) and immediately gently placed in a clear Plexiglass arena (27.31 \times 27.31 \times 20.32 cm, Med Associates ENV-510) lit with ambient light (about 330 lux) and allowed to ambulate freely for 30 min. A separate cohort was gently placed in the Plexiglass arena immediately after receiving a subcutaneous injection of 4-F,5-MeO-PyrT (0.03, 0.1, 0.3, 1 or 3 mg kg^{-1} , s.c.) and allowed to ambulate freely for 120 min. The locomotion of the animals was tracked by infrared beams embedded along the x, y and z axes of the area and automatically recorded. Data were collected using Activity Monitor by Med Associates.

HTR assay

HTR evaluation was performed by trained observers who were blinded to both drug and dose. Male 10–15 week old C57BL/6J mice were habituated to the testing room for 30 min. The body weight of each mouse was recorded. HTR was defined as a rapid rotational movement of the head around the longitudinal axis of the animal's body. Mice were randomly administered with vehicle or WAY-100635 (1 mg kg^{-1} or 2 mg kg^{-1} , s.c.). Fifteen minutes after the pretreatment injection, mice were administered either 4-F,5-MeO-PyrT (0.1, 0.3, 1 or 3 mg kg^{-1} , s.c.) or 5-MeO-MET (0.3, 1, 3 or 10 mg kg^{-1} , s.c.) and placed in a new observation cage. An overhead camera (GoPro HERO9 at 120 Hz) recorded their movements. Observers scored HTR from recorded footage for the first 10-min period.

Chronic SD stress assay

The current set of experiments used the chronic SD stress paradigm to evaluate the antidepressant efficacy of the compounds of interest⁴¹.

In brief, male 8-week-old C57BL/6J mice were randomly assigned to either the control or stress condition. Mice in the stress condition were subjected to 10 consecutive days of subordination stress by being placed into the territorialized cage of a larger, aggressive CD-1 mouse. Stressed-exposed mice were housed overnight in the compartment adjacent to the recently encountered CD-1 mouse and introduced to a new aggressor each day for a 10-min defeat bout. Control mice were housed in pairs, one on each side of a perforated Plexiglas partition, and handled daily.

SI test

Twenty-four hours after the last defeat bout, mice were tested in the SI test to evaluate stress-induced social avoidance. For this experiment, 1 h after the last defeat bout (day 10), mice were injected with 4-F,5-MeO-PyrT (1.0 mg kg^{-1}) or vehicle and evaluated in the SI test 24 h later. Mice were all randomly assigned to receive either drug or vehicle.

The SI test is a two-session test consisting of a target and no-target session. In the no-target session, the experimental mouse is allowed to explore an open-field arena for 2.5 min. The mouse is then removed, and a new CD-1 male mouse is placed into a wire mesh cage, which is situated along one side of the arena. This area is the interaction zone. For the target session (SI phase), the test mouse is placed back into the arena for another 2.5 min and the amount of time spent in the interaction zone is measured. Experimenters were blinded to the drug pretreatment and behaviour during the SI test, which was automatically tracked using EthoVision analysis software (Noldus). We also calculated the ratio of time spent in the corners of the chamber during the SI phase. Socially defeated mice explored the interaction zone significantly less in the presence of the CD-1 mouse. Data are presented as the IR of time spent in the interaction zone between the target and no-target sessions, and locomotion data were measured during the no-target session. Data were averaged from three independent experiments. A total of 46 mice were administered vehicle treatment (17 control, 29 stress), 53 received 4-F,5-MeO-PyrT (17 control, 36 stress), 15 received WAY-100635 (5 control, 10 stress), and 29 received 4-F,5-MeO-PyrT + WAY-100635 (10 control, 19 stress). More animals were used for the stressed groups owing to the inherent resilience of mice to SD stress⁴². Analysis of different treatment conditions of control and stressed animals was done using GraphPad Prism with two-way analysis of variance. The interaction of all treatments with control and stressed animals showed a $P = 0.1778$, with a row (treatment) factor of $P = 0.0741$ and a column (control/stress) factor of $P = 0.0001$. Comparison of individual treatments, as shown in Fig. 5d, was done using multiple comparison post hoc analysis using Fisher's least significant difference test. We additionally analysed our data taking into account that a proportion of mice are resilient in this paradigm. Accordingly, we used a Fisher's exact test to analyse and compare the number of mice that are resilient (SI ratio > 1) and susceptible (SI ratio < 1) in the vehicle-treated and 4-F,5-MeO-PyrT-treated stressed cohorts.

Sucrose preference test

The sucrose preference test is a volitional two-bottle choice procedure in which mice are given the choice between consuming water and a 1% sucrose solution. This paradigm has been extensively used to assess the effects of stress-induced anhedonia. On day 10, chronic SD stressed mice were individually housed and habituated to two bottles containing just water. Twenty-four hours later, following the SI test, one bottle was replaced with a 1% sucrose solution (w/v) made with water from the animal vivarium. Mice had ad libitum overnight access to both bottles, and preference for sucrose over water was calculated as follows: (sucrose/(sucrose + water)) \times 100. Data were averaged from two independent experiments. A total of 27 mice were administered vehicle (9 control, 18 stress), 27 received 4-F,5-MeO-PyrT (9 control, 18 stress), 15 received WAY-100635 (5 control, 10 stress), and 15 received 4-F,5-MeO-PyrT + WAY-100635 (5 control, 10 stress). More animals were

used for the stressed groups owing to the inherent resilience of mice to SD stress⁴². Analysis of different treatment conditions of control and stressed animals was done using GraphPad Prism with two-way analysis of variance. The interaction of all treatments with control and stressed animals showed $P = 0.0773$, with a row (treatment) factor of $P = 0.0159$ and a column (control/stress) factor of $P = 0.0008$. Comparison of individual treatments, as shown in Fig. 5e, was done using multiple comparison post hoc analysis using Fisher's least significance difference test.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Density maps and structure coordinates have been deposited into the Electron Microscopy Data Bank (EMDB) and the PDB with the following accession identifiers: EMD-29560 and PDB 8FY8 for 5-MeO-DMT-5-HT_{1A}-G α_{i1} -G β_1 -G γ_2 ; EMD-29597 and PDB 8FYT for LSD-5-HT_{1A}-G α_{i1} -G β_1 -G γ_2 ; EMD-29571 and PDB 8FYE for 4-F,5-MeO-PyrT-5-HT_{1A}-G α_{i1} -G β_1 -G γ_2 ; EMD-29585 and PDB 8FYL for vilazodone-5-HT_{1A}-G α_{i1} -G β_1 -G γ_2 ; and EMD-29599 and PDB 8FYX for buspirone-5-HT_{1A}-G α_{i1} -G β_1 -G γ_2 . Source data are provided with this paper. Additional data from this study are available upon request.

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Author contributions D.S., A.C.K., M.J. Cunningham and D.L. conceived and initiated the project. A.L.W. designed experiments, expressed and purified protein for grid freezing, collected data, refined structures with help from M.J. Capper, performed signalling and uptake assays, and co-wrote the manuscript. D.L. designed, synthesized, purified and characterized compounds with assistance from V.H., and co-wrote the manuscript. M.J. Cunningham designed, synthesized, purified and characterized compounds. D.L., V.H. and D.S. designed and supervised the pharmacokinetics study. L.F.P. performed the chronic SD stress assay and subsequent behavioural analyses supervised by S.J.R. I.C.S. designed and performed in vivo pharmacology assays, including the open-field and HTR assays, with assistance from P.D. G.Z. prepared grids for structure determination and assisted with data collection. D.S. and D.W. conceptualized the overall project and designed experiments, analysed the data, supervised the project and management, and wrote the manuscript.

Competing interests The authors declare the following competing financial interests: D.S. and A.C.K. are co-founders of Gilgamesh Pharmaceuticals and Kures. M.J. Cunningham is a co-founder of Gilgamesh Pharmaceuticals. A.L.W., D.L., I.C.S., L.F.P., S.J.R., D.S. and D.W. are inventors on a patent application related to the featured compound class. D.W. has consulted for Otsuka Pharmaceutical, Longboard Pharmaceuticals and Ocean Bio on the design of psychedelic-based therapeutics. None of the companies listed herein contributed to the funding or experimental design. All other authors declare no competing interests.

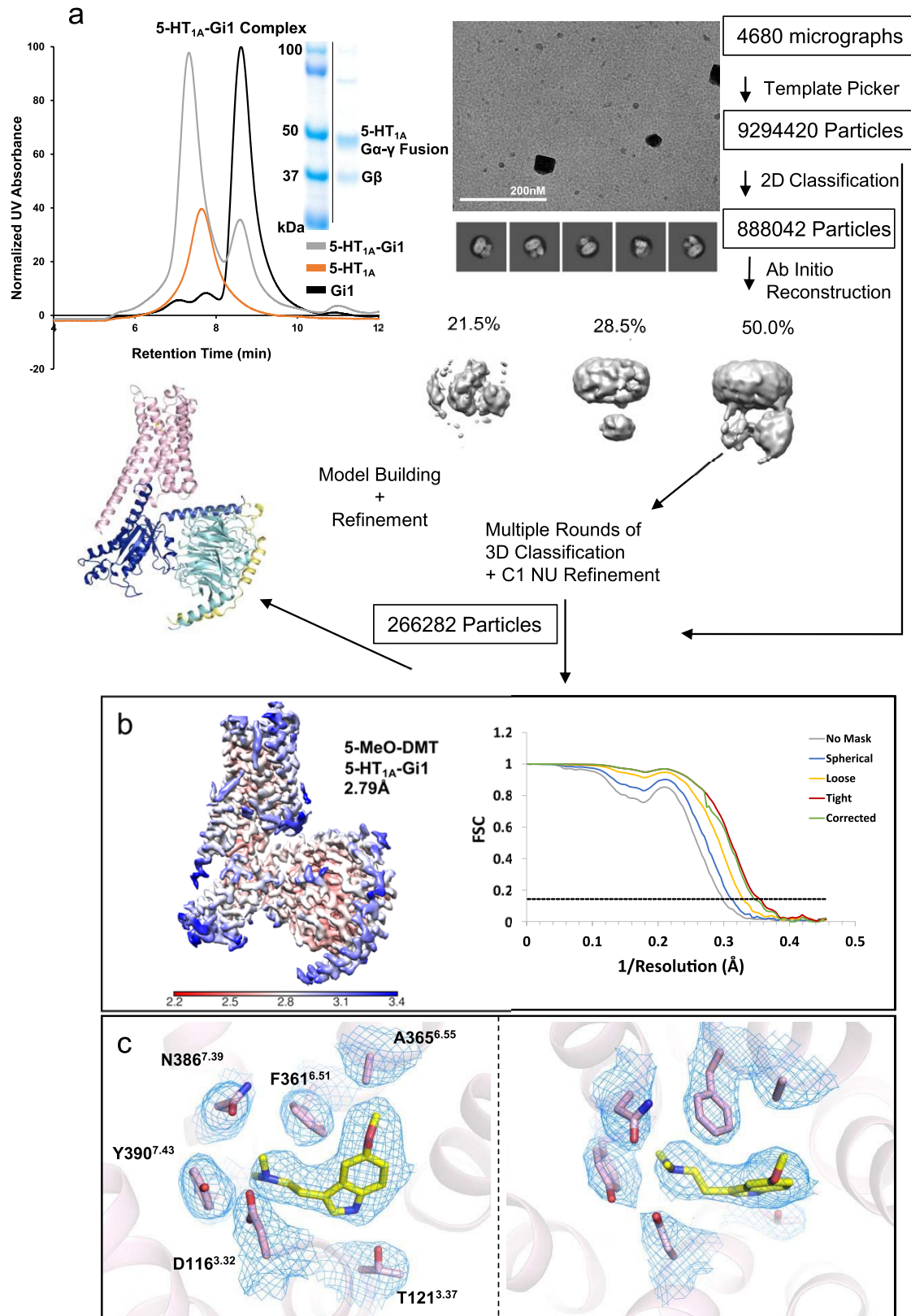
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-024-07403-2>.

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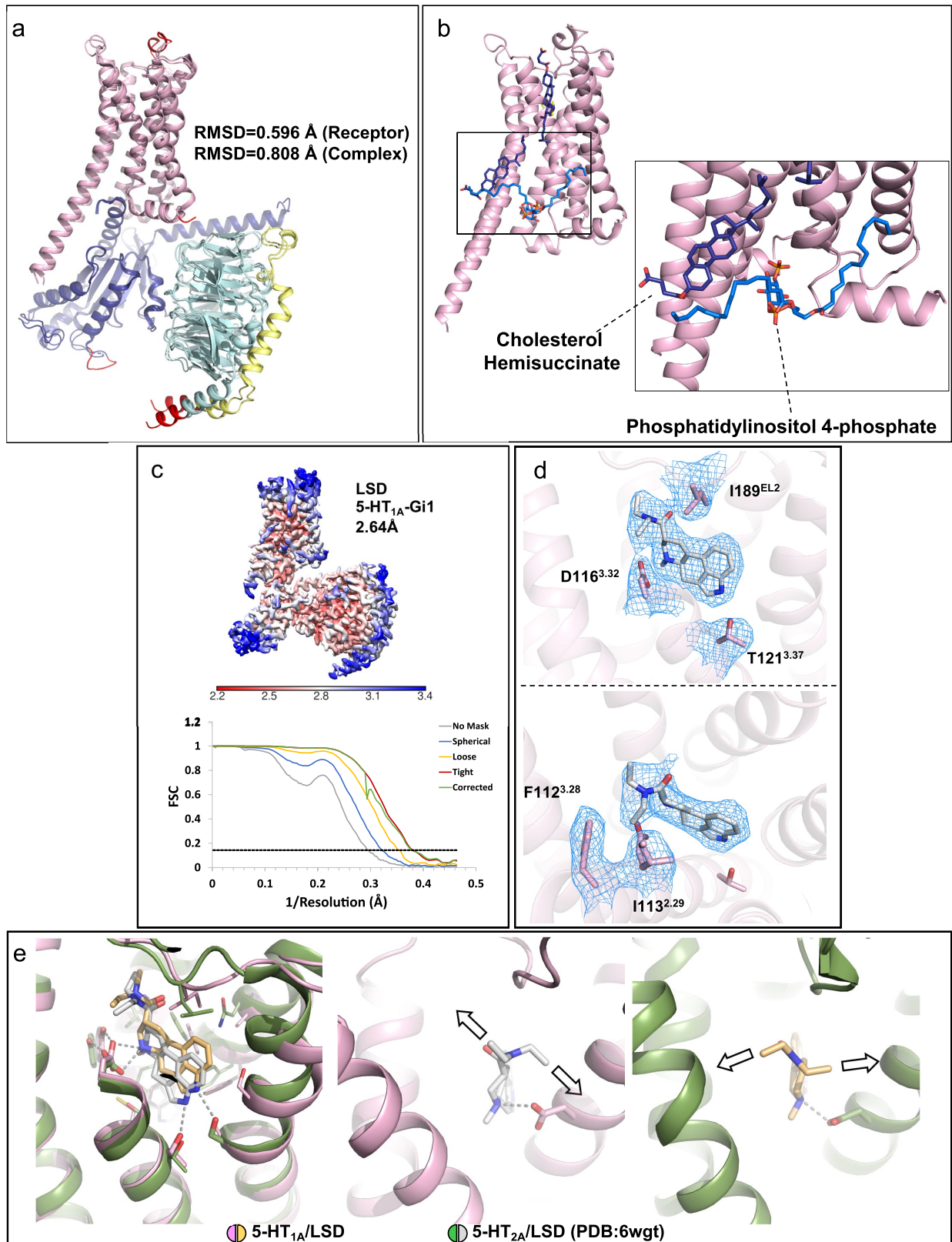
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Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Cryo-EM structure determination of drug-bound 5-HT_{1A}-Gαi1/Gβ1/Gγ2 complexes. **a**, Representative structure determination of 5-MeO-DMT-bound 5-HT_{1A} signalling complex. **Top Left**, Analytical size exclusion chromatography and SDS-PAGE show monodisperse and pure protein of intact complex and its components. **Right**, representative Cryo-EM micrograph (white bar indicates scale) of 4680 total micrographs and data processing schematic exemplified by 5-MeO-DMT-bound 5-HT_{1A}-Gi1 structure: After particle picking, 2D classification and multiple rounds of 3D classification, the final particle stack was refined using non-uniform refinement. A final map

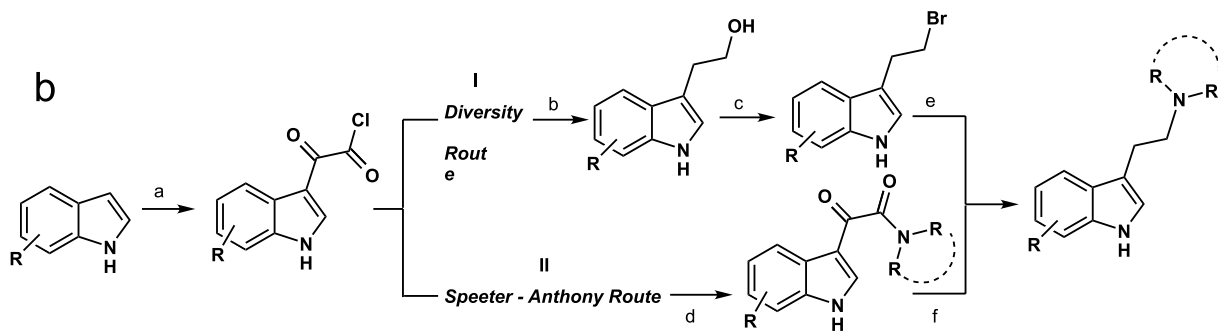
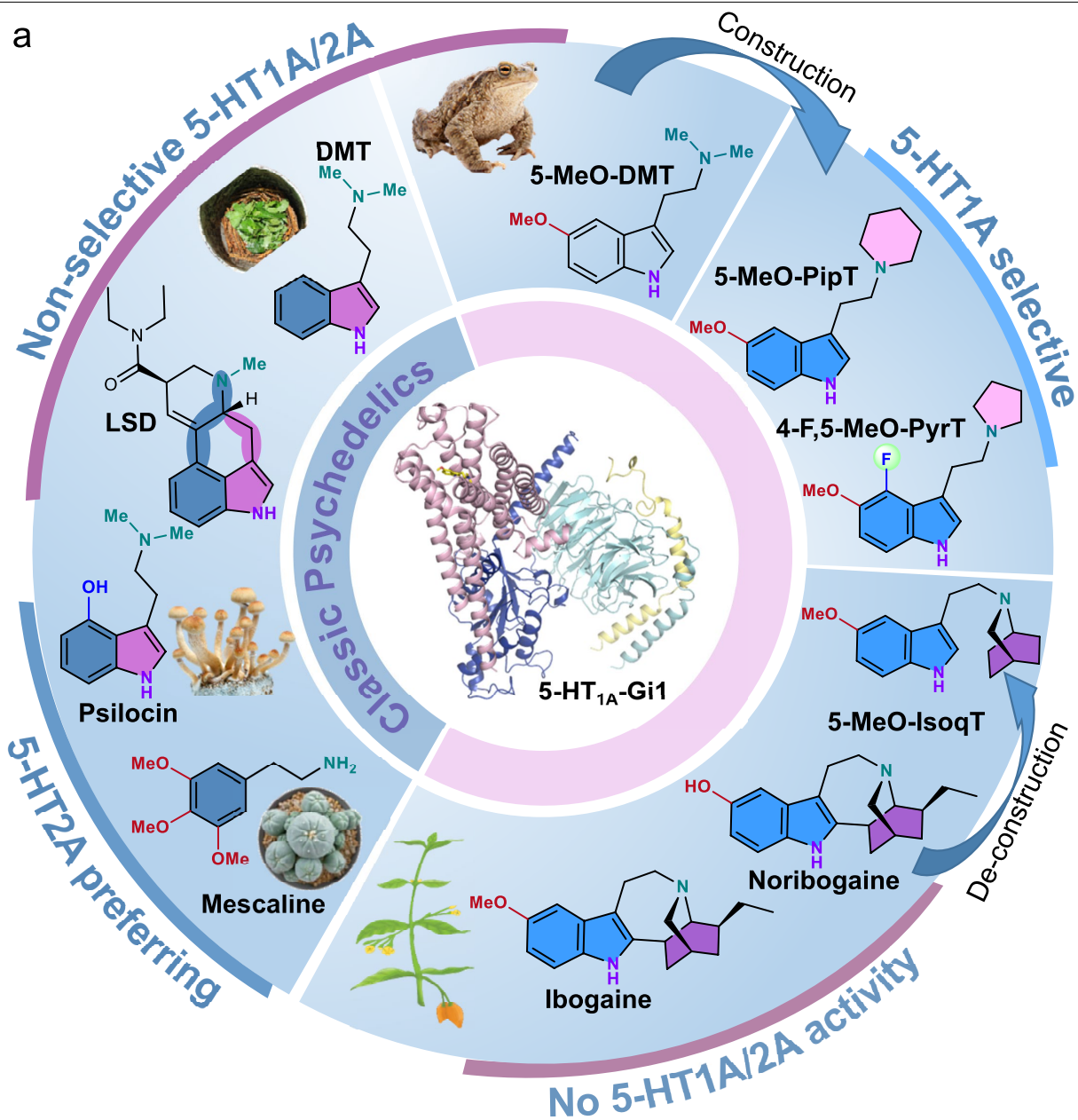
was obtained and resolutions were estimated applying the 0.143 cutoff in GS-FSC. Initial models were built in COOT, and then further refined in PHENIX for the generation of final coordinates shown in this manuscript. **b**, Local resolution map of a 5-MeO-DMT-bound 5-HT_{1A}-Gi1 complex (**left**) and FSC curves (**right**) calculated based on the final reconstruction in cryoSPARC. **c**, 5-MeO-DMT (yellow) in the orthosteric binding pocket from the side (**left**) and rotated 45° towards the top of the receptor (**right**) with the map of ligand and surround residue densities shown at 5σ.



Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Comparison of different 5-HT_{1A} structures and differences in binding of LSD to 5-HT_{1A} and 5-HT_{2A}. **a**, Superposition of herein reported 5-MeO-DMT-bound 5-HT_{1A}-Gi complex with the previously reported 5-HT-bound 5-HT_{1A}-Gi structure (PDB ID: 7E2Y) shows similar conformations. Additional residues in 5-HT_{1A}'s EL2 and G proteins not observed in previous structures are highlighted in red. **b**, Lipids (blue) and cholesterol hemisuccinate (dark blue) are bound to similar sites as observed before. **c**, Local resolution map of a LSD-bound 5-HT_{1A}-Gi1 complex (**left**) and FSC curves (**right**) calculated the final reconstruction in cryoSPARC. **d**, LSD (grey) in the orthosteric binding

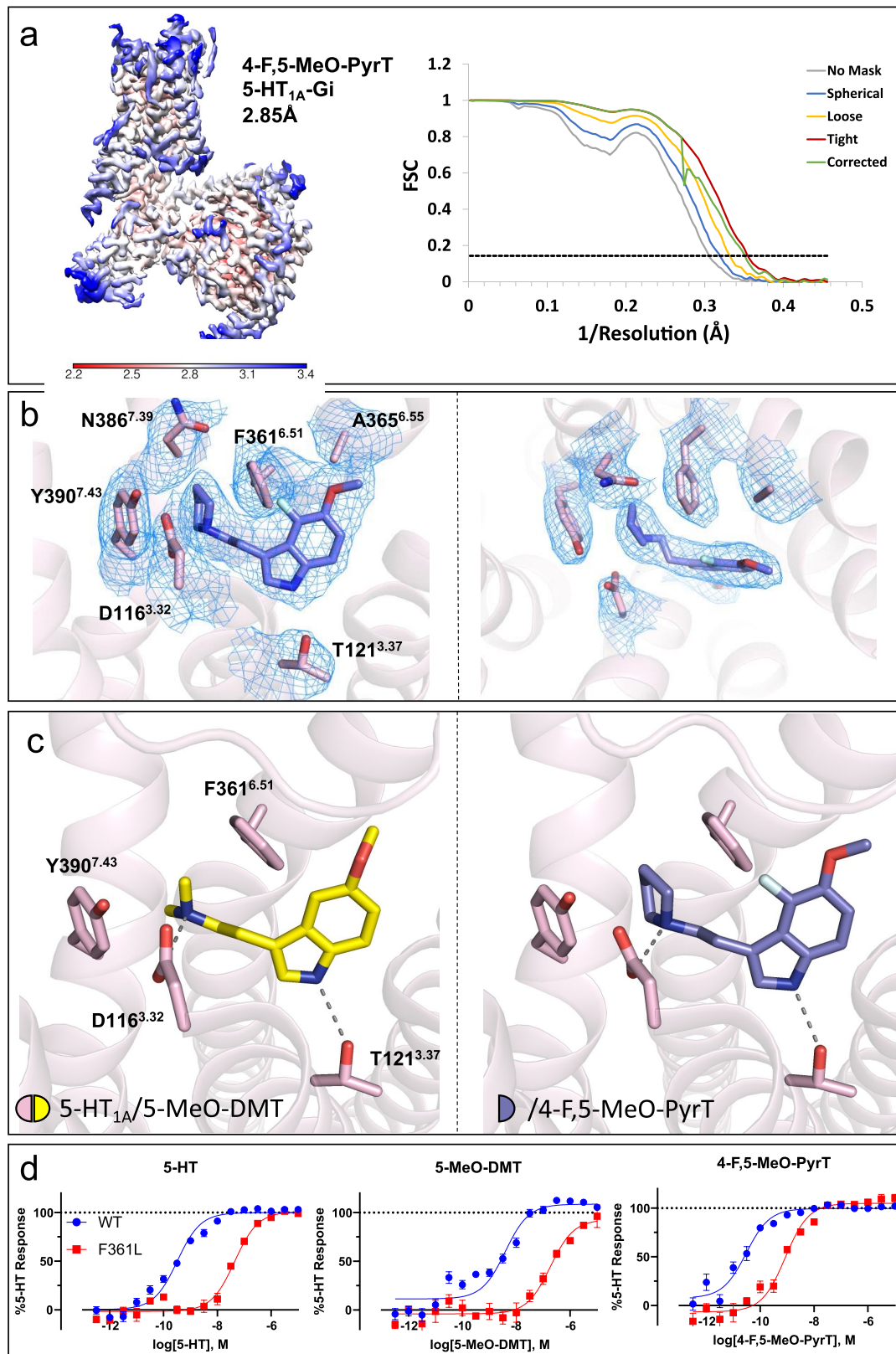
pocket from the side (**top**) and rotated 45° towards the top of the receptor (**bottom**) with the map of ligand and surround residue densities shown at 5σ. **e**, LSD shows distinct binding modes bound to 5-HT_{1A}-Gi signalling complex and 5-HT_{2A} (PDB ID: 6WGT). **Left**, 5-HT_{1A}-bound LSD (grey) sits deeper in the binding pocket compared to 5-HT_{2A}-bound LSD (orange). Zoom in of LSD in 5-HT_{1A}-Gi structure (**middle**) and 5-HT_{2A} structure (**right**) highlights differential stereochemistry and receptor-specific interactions of diethylamide moiety. Hydrogen bonds are indicated as grey dashed lines.



Extended Data Fig. 3 | See next page for caption.

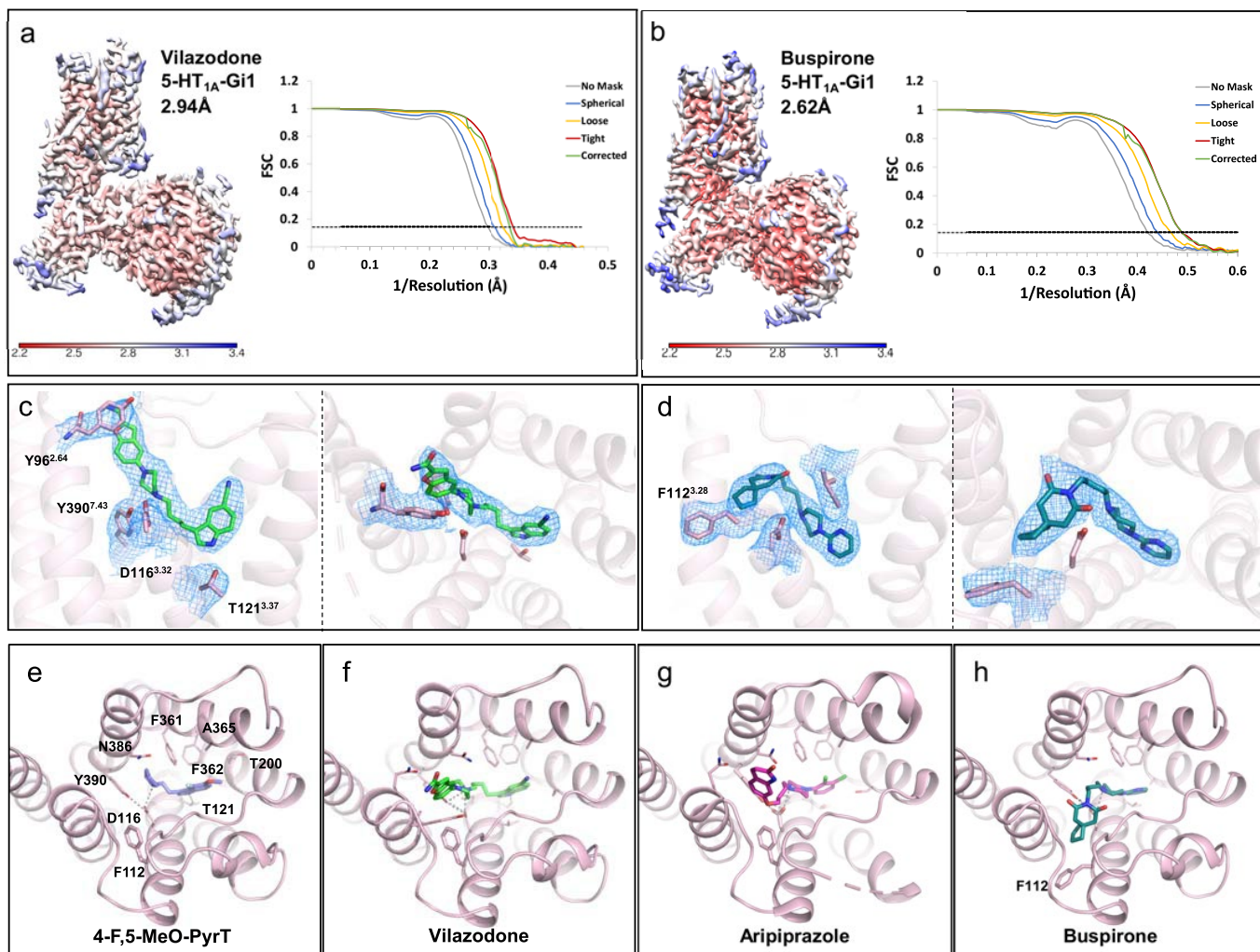
Extended Data Fig. 3 | Global structure-activity landscape of tryptamine psychedelics at 5-HT_{1A} and 5-HT_{2A} receptors and their synthesis. **a**, Overview of the cryo-EM structure of the 5HT_{1A} receptor-Gi signalling complex bound to 5-MeO-DMT (**center**). Classic psychedelics such as the prototypical compounds DMT and LSD are agonists of both 5-HT_{1A} and 5-HT_{2A} receptors (**left semi-circle**). 5-MeO-DMT (**top of the circle**), a major psychoactive compound found in toad secretions, shows comparable potency and efficacy at both 5-HT_{1A} and 5-HT_{2A} receptors. Systematic structural mapping via elaboration of the core 5-MeO-DMT structure identifies a class of 5-MeO-tryptamines with increasing 5-HT_{1A} selectivity (**right hemi-circle**). 5-MeO-DMT can be viewed as a

deconstruction of ibogaine, a oneirogen with a complex polycyclic tryptamine structure (**bottom of the circle**). Iboga compounds show no activity at 5-HT_{1A} and 5-HT_{2A} receptors, but this activity re-emerges by deconstruction of the isoquinuclidine core to simple mono-cyclic tryptamines such as 5-MeO-PipT (5-methoxypiperidinyl-tryptamine) and 4-F,5-MeO-PyrT (4-fluoro, 5-methoxypyrrolidinyl-tryptamine, right hemi-circle). Images of peyote, mushrooms, ayahuasca, and toad are from iStock and Shutterstock, and *Tabernanthe iboga* schematic is adapted from previous work⁶⁵. **b**, General synthesis methodology of tryptamines. a. oxalyl chloride, b. MeOH, LAH, c. PPh₃, CBr₄, d. Amine, TEA, e. Amine, f. LAH.



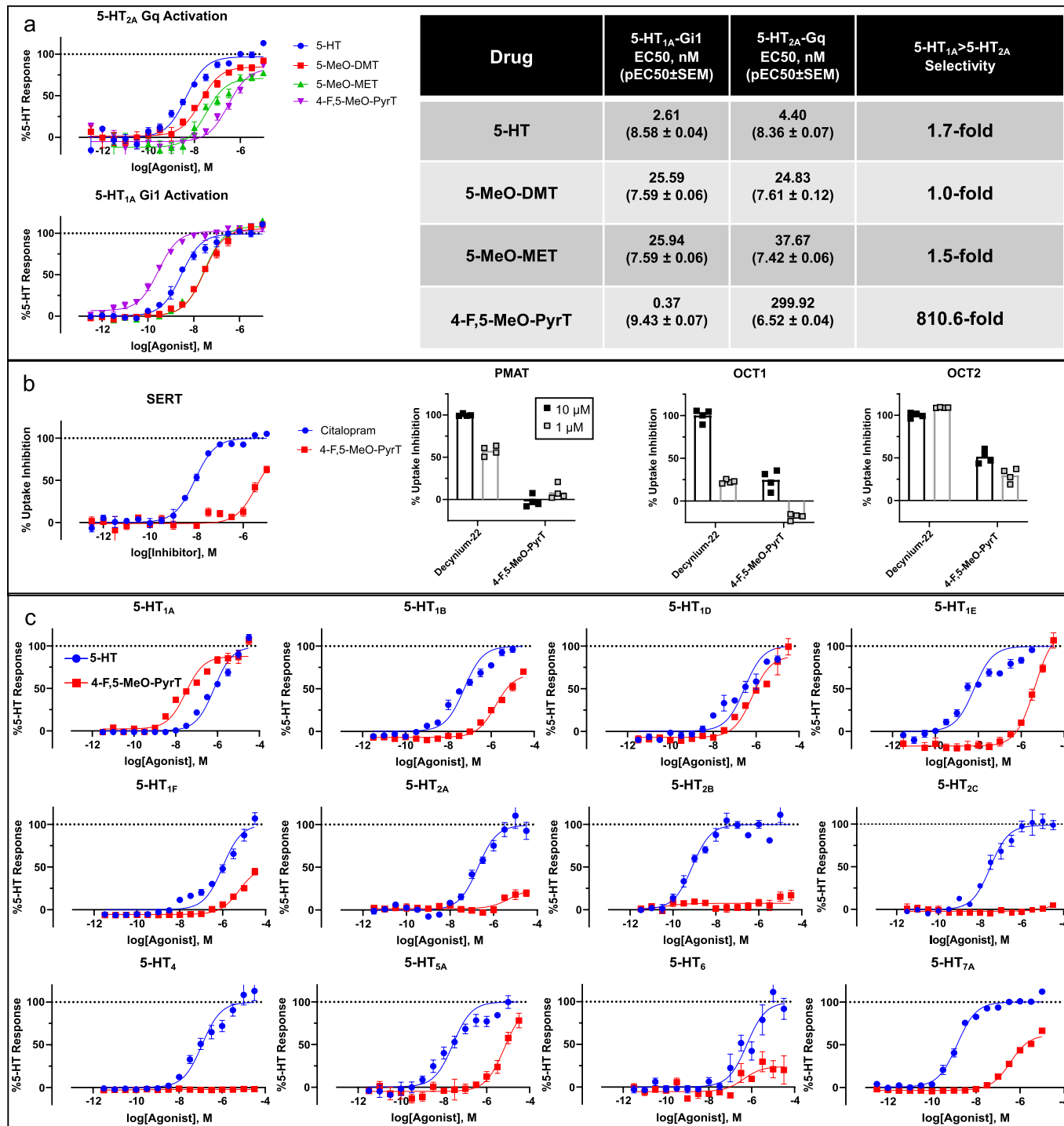
Extended Data Fig. 4 | Structural comparison of 5-MeO-DMT 5-HT_{1A}-selective analog 4-F, 5-MeO-PyrT bound to 5-HT_{1A}. **a**, Local resolution map of a 4-F,5-MeO-PyrT-bound 5-HT_{1A}-Gi complex (**left**) and FSC curves (**right**) calculated from the final reconstruction in cryoSPARC. **b**, 4-F,5-MeO-PyrT (dark blue) in the orthosteric binding pocket from the side (**left**) and rotated 45° towards the top of the receptor (**right**) with the map of ligand and surrounding residue densities shown at 5σ. **c**, structural side-by-side comparison of 5-HT_{1A}

orthosteric site bound to 5-MeO-DMT (yellow) and 4-F,5-MeO-PyrT (dark blue). **d**, cAMP accumulation assays using wildtype and mutant 5-HT_{1A}, and different drugs. Concentration-response experiments reveal different sensitivities of distinct drugs to F361L mutation. All signalling studies were performed in triplicates and are averaged from two to three independent experiments. Data have been normalized against 5-HT and errors bars denote s.e.m.



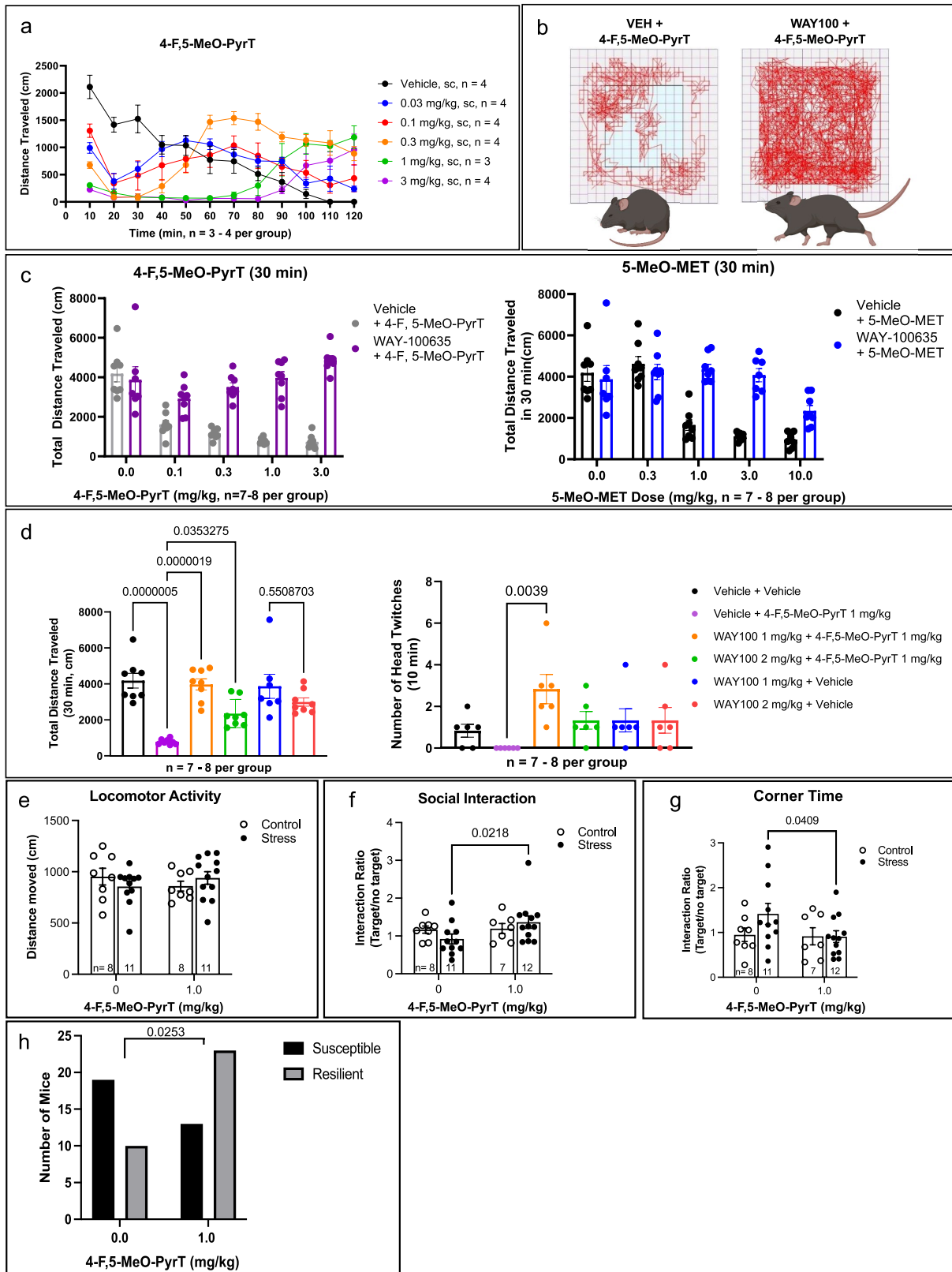
Extended Data Fig. 5 | Comparison of 4-F,5-MeO-PyrT binding pose to that of different clinical 5-HT_{1A} drugs. **a, b.** Local resolution maps of vilazodone-bound (**a**) and buspirone-bound (**b**) 5-HT_{1A}-Gi1 complexes and corresponding FSC curves calculated from the final reconstructions in cryoSPARC. **c, d.** Vilazodone (**c**, green) and buspirone (**d**, teal) in the orthosteric

binding pocket from the side (**left**) and rotated 45° towards the top of the receptor (**right**) with the density map of ligand and surrounding residues shown at 5σ. **e-h.** Extracellular view of 4-F,5-MeO-PyrT (**e**, dark blue), Vilazodone (**f**, green), Aripiprazole (**g**, magenta, PDB ID: 7E2Z), and Buspirone (**h**, teal) binding poses in 5-HT_{1A}'s orthosteric site.



Extended Data Fig. 6 | Selectivity of different 5-MeO-DMT analogs and off-target activity of 4-F,5-MeO-PyrT. **a**, 5-HT_{1A}-Gi and 5-HT_{2A}-Gq BRET of 5-HT, 5-MeO-DMT, 5-MeO-MET, and 4-F,5-MeO-PyrT with respective potencies and 5-HT_{1A} > 5-HT_{2A} selectivities. **b**, Off-target inhibition of transporters SERT, PMAT, OCT1, and OCT2 by 4-F,5-MeO-PyrT and known inhibitors. SERT uptake was performed in triplicates and data was averaged from two independent experiments showing data as mean±s.e.m. PMAT, OCT1, and OCT2 uptake was

performed once in quadruplicate. **c**, Arrestin-recruitment of 5-HT and 4-F,5-MeO-PyrT at all human 5-HT receptor subtypes except for 5-HT_{7A}, whose activation was measured via cAMP stimulation. All functional studies were performed in triplicates and are averaged from two to three independent experiments. Data have been normalized against 5-HT, Citalopram, and Decynium-22, and errors bars denote the s.e.m.



Extended Data Fig. 7 | See next page for caption.

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Extended Data Fig. 7 | Effects of 5-MeO-DMT derivatives on rodent behavior.

a, Evaluation of 4-F,5-MeO-PyrT in open field for two hours (n = 3-4/group).
b, Exemplary traces of the ambulatory distance traveled in open field following 4-F,5-MeO-PyrT (1 mg/kg, s.c.) administration and with and without WAY-100635 pre-treatment (1 mg/kg, s.c., 15 min prior). Panel was created with BioRender.com. **c**, Effect of WAY-100635 (1 mg/kg, s.c., 15 min prior) on 4-F,5-MeO-PyrT's and 5-MeO-MET's effect on total locomotion (n = 7-8/group, 30 min). **d**, Determination of optimal inhibitory WAY-100635 dose via administration of 1 mg/kg and 2 mg/kg WAY-100635 prior to studying 4-F,5-MeO-PyrT's effects on total locomotion (n = 7-8/group) and HTR (n = 6/group). Analysis was done using one-way ANOVA with multiple comparisons with Tukey's post hoc test, and exact p values have been denoted in the Figure.
e-g, Effects of saline or 4-F,5-MeO-PyrT administration on control (Control)

or chronically defeated mice (Stress). Determination of **e**, distance moved as a measure of locomotor activity, **f**, social interaction as a measure of anxiety- and depression-related phenotype, **g**, corner time as a measure of anxiety-like behavior. Analysis was done in a sub-cohort of the animals reported in Fig. 5d. Number of mice for each group is indicated below the data for each respective cohort. Differences were determined by two-way ANOVA with multiple comparisons using Fisher's LSD post hoc test, and exact p values have been denoted in the Figure. **h**, Vehicle- and drug-treated stressed mice shown in Fig. 5d were divided into susceptible (SI ratio < 1) and resilient (SI ratio > 1) populations. Significance in the population shift was determined by a two-sided Fisher's exact test and p value and number of mice have been denoted in the Figure. Error bars denote the s.e.m.

Extended Data Table 1 | Cryo-EM Data Collection, Model Refinement and Validation

	5-HT1A Gi1 DNG 5-MeO-DMT	5-HT1A Gi1 LSD	5-HT1A Gi1 4-F, 5-MeO- PyrT	5-HT1A Gi1 Vilazodone	5-HT1A Gi1 DNG Buspirone
<i>Data Collection and Processing</i>					
Magnification	64000	81000	64000	81000	105000
Voltage (kV)	300	300	300	300	300
Electron Exposure (e-/A2)	52.13	53.61	52.23	54.3	50
Defocus Range (µm)	-0.5 to -1.8	-0.5 to -1.8	-0.5 to -1.8	-0.5 to -1.8	-0.5 to -1.8
Pixel Size	1.076	1.058	1.076	1.069	0.825
Symmetry Imposed	C1	C1	C1	C1	C1
Total Micrographs	4680	4952	3071	7206	2489
Initial Particle Images	9294420	12502189	4565297	18088270	4167166
Final Particle Images	266282	318050	265975	785536	586489
Map Resolution	2.79	2.64	2.85	2.94	2.62
FSC Threshold	0.143	0.143	0.143	0.143	0.143
Sharpening B-factor	120.5	91.6	110.2	167.3	105.1
<i>Refinement</i>					
<i>Model Composition</i>					
Non-hydrogen Atoms	7309	7321	7321	7330	7325
Protein Residues	914	914	914	914	914
Ligands	4	4	4	4	4
<i>RMS Deviations</i>					
Bond Lengths (Å)	0.008	0.005	0.004	0.004	0.005
Bond Angles (°)	1.161	0.686	0.630	0.593	1.030
<i>Clash Score</i>					
Clash Score	5.35	5.75	6.10	5.14	6.71
Rotamers Outliers (%)	0.13	0	0	0	0
<i>Ramachandran Plot (%)</i>					
Favored	97.45	97.78	97.67	98.12	97.45
Allowed	2.55	2.22	2.33	1.88	2.55
Outliers	0	0	0	0	0
<i>Data Availability</i>					
PDB	8FY8	8FYT	8FYE	8FYL	8FYX
EMDB	EMD-29560	EMD-29597	EMD-29571	EMD-29585	EMD-29599

Report of data collection and refinement statistics from the structures presented in the manuscript. DNG indicates use of a dominant negative G protein further described in the methods.

Article

Extended Data Table 2 | AUC_{last} and C_{max} of 4-F,5-MeO-PyrT at 1mg/kg SC administration, and Plasma and Brain Tissue Binding of 4-F,5-MeO-PyrT

Pharmacokinetics										
Tissue	AUC _{last} (hr*ng/mL)		Tissue	C _{max} (ng/mL)						
Plasma	26.40		Plasma	44.02						
Brain	128.34		Brain	143.35						
Mouse Brain Tissue Binding										
Sr.no	Compound	Species	% Bound			STDV	% Unbound	fu	% Recovery	% Compound remaining at 4 h
			R1	R2	Mean					
1	Carbamazepine	Mouse	89.0	87.4	88.2	1.1	11.8	0.118	72	96
2	4-F, 5-MeO-PyrT	Brain	84.3	80.7	82.5	2.5	17.5	0.175	88	96
Mouse Plasma Protein Binding										
1	Warfarin	Mouse	93.9	93.3	93.6	0.4	6.4	0.064	90.9	103.3
2	4-F, 5-MeO-PyrT	Plasma	32.04	42.68	37.36	7.5	62.64	0.626	84.7	105.6
Human Plasma Protein Binding										
1	Warfarin	Human	98.0	98.5	98.3	0.3	1.7	0.017	108.1	108.8
2	4-F, 5-MeO-PyrT	Plasma	22.19	29.83	26.01	5.4	73.99	0.740	81.8	97.9

Summary of the pharmacokinetic and in vivo binding properties of 4-F,5-MeO-PyrT.

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- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
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- A description of any restrictions on data availability
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Density maps and structure coordinates have been deposited in the Electron Microscopy Data Bank (EMDB) and the Protein Data Bank (PDB): 5-MeO-DMT/5-HT1A-G α i1-G β 1-G γ 2 (EMD- 29560, PDB ID: 8FY8), LSD/5-HT1A-G α i1-G β 1-G γ 2 (EMD-29597, PDB ID: 8FYT), 4-F,5-MeO-PyrT/5-HT1A-G α i1-G β 1-G γ 2 (EMD-29571, PDB ID: 8FYE), Vilazodone/5-HT1A-G α i1-G β 1-G γ 2 (EMD-29585, PDB ID: 8FYL), Bupirone/5-HT1A-G α i1-G β 1-G γ 2 (EMD-29599, PDB ID: 8FYX).

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was not predetermined. Sample sizes were chosen based on years of experience with in vitro signaling, transport and in vivo (behavioral) assays, as well as based on sample sizes generally used and accepted in the field. For instance, for signaling studies we used a sample size of 2 or more independent experiments done in triplicates (technical repeats) to show reproducibility.
Data exclusions	No data was excluded
Replication	Experimental findings were reproduced, and number of technical repeats and independent experiments has been provided in the methods and figure legends.
Randomization	Mice were obtained from the same vendor and randomly assigned to all experimental conditions to ensure proper unbiased testing. In previous and preliminary in vitro signaling and transport experiments we observe low variability in the expression of receptor, transporter and reporter between cells cultured in neighboring wells of 96-well and 384-well plates. Based on these results we did not perform randomization.
Blinding	Mouse behavior (time spent in zone, locomotion) was tracked using an automated video-tracking system and data was populated by Noldus software. Further analysis was done under blinded conditions. No blinding was required for the in vitro experiments performed in this study, as all measures rely on instrument-based quantification of fluorescence and no subjective assessment of data that influences the outcome of the analysis.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.

Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.
Research sample	Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i> , all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

Did the study involve field work? Yes No

Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
Access & import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

Methods

- n/a Involved in the study
- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Clinical data
- Dual use research of concern

- n/a Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Antibodies

Antibodies used

Validation

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Authentication

Mycoplasma contamination

Commonly misidentified lines (See [ICLAC](#) register)

Palaeontology and Archaeology

Specimen provenance

Specimen deposition

Dating methods

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Wild animals

Reporting on sex

findings apply to females as the dosing schedule for psychedelic and antidepressant medications could differ between sexes. As such, the main goal of this work was to characterize the pharmacodynamics of these new compounds and testing a detailed dosing schedule in relevant disease contexts, would be part of future endeavors.

Field-collected samples

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

Study protocol

Data collection

Outcomes

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

- | No | Yes | |
|--------------------------|--------------------------|----------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | Public health |
| <input type="checkbox"/> | <input type="checkbox"/> | National security |
| <input type="checkbox"/> | <input type="checkbox"/> | Crops and/or livestock |
| <input type="checkbox"/> | <input type="checkbox"/> | Ecosystems |
| <input type="checkbox"/> | <input type="checkbox"/> | Any other significant area |

Experiments of concern

Does the work involve any of these experiments of concern:

- | No | Yes | |
|--------------------------|--------------------------|---|
| <input type="checkbox"/> | <input type="checkbox"/> | Demonstrate how to render a vaccine ineffective |
| <input type="checkbox"/> | <input type="checkbox"/> | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| <input type="checkbox"/> | <input type="checkbox"/> | Enhance the virulence of a pathogen or render a nonpathogen virulent |
| <input type="checkbox"/> | <input type="checkbox"/> | Increase transmissibility of a pathogen |
| <input type="checkbox"/> | <input type="checkbox"/> | Alter the host range of a pathogen |
| <input type="checkbox"/> | <input type="checkbox"/> | Enable evasion of diagnostic/detection modalities |
| <input type="checkbox"/> | <input type="checkbox"/> | Enable the weaponization of a biological agent or toxin |
| <input type="checkbox"/> | <input type="checkbox"/> | Any other potentially harmful combination of experiments and agents |

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

Files in database submission

Genome browser session
(e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument

Identify the instrument used for data collection, specifying make and model number.

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI Used Not used

Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: Whole brain ROI-based Both

Statistic type for inference (See [Eklund et al. 2016](#))

Correction

Models & analysis

n/a | Involved in the study

Functional and/or effective connectivity

Graph analysis

Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis