Biological studies of clavine alkaloids targeting CNS receptors

Nikhil R. Tasker, Ethan J. Pazur and Peter Wipf*

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA, United States

In contrast to well established psychedelics such as lysergic acid diethylamide (LSD) and psilocybin, ergot alkaloids of the clavine subclass have not been thoroughly investigated, in spite of their broad occurrence in nature and their well-established potent physiological effects. This study presents the current knowledge on the biological properties of clavine alkaloids, draws comparisons to the pharmacology of ergolines and related psychedelics, and demonstrates opportunities to develop novel structure−activity relationship (SAR) profiles. The latter could usher in a new stage of medicinal chemistry studies that enable an expansion of the currently structurally limited portfolio of psychedelic therapeutics.

1 Introduction

Psychedelic compounds have been used by a variety of cultures for centuries due to their ability to elicit an altered state of consciousness, evoke transcendence, reopen the social reward learning critical period, and to heal a variety of health disorders (1). Traditionally used by communities in South America and Africa for religious ceremonies and rituals, these compounds share a potential for substance abuse but are now re-emerging as potential therapeutics in North America and Europe (2). The demand for psychedelic therapy (3) and psychedelic wellness retreats (4, 5) has seen tremendous growth in recent years, in part driven by microdosing applications and the combination with traditional psychotherapeutic counseling. Users experience an altered state that involves changes in sensory perception and mood and may undergo dissociation or a loss of self-identity (6–10). An ability to reset a social reward learning mechanism might be a shared feature across psychedelic drugs (11). Interestingly, some users claim that a psychedelic experience permanently changed their outlook on life and/or improved their quality of life long after the experience (12). Although these claims are subjective, there is increasing evidence that suggests psychedelics can be used to treat psychiatric diseases, including anxiety and major depressive disorders (13, 14).

Historically, scientific research into psychedelics and their therapeutic benefits has been slow and controversial. Classic psychedelics including lysergic acid diethylamide (LSD), psilocybin, dimethyltryptamine (DMT), and mescaline were used in the 1950s and 60s to treat alcoholism, addiction, anxiety, and trauma (Figure 1) (15, 16). LSD was also used to obtain a model for psychosis and schizophrenia due to its ability to elicit symptoms of these disorders at high doses (17–19). Unfortunately, there were growing concerns of abuse regarding psychedelics and these compounds became associated with counterculture. As a result, the Drug Abuse Control Amendments of 1965 and the Controlled Substances Act of 1970 were passed. These policies severely limited the availability of psychedelic compounds for researchers and clinical investigations into these substances were stalled. Twenty years later, many seminal and novel studies on

psychedelic compounds emerged. Among the earliest examples are Rick Strassman's studies on the dose dependent effects of DMT in humans (20, 21). These promising psychiatric and clinical investigations of

psychedelics have brought these compounds out of the dark ages (22). Indeed, this new wave of vibrant research into psychedelic compounds has ushered in a "psychedelic renaissance." (23).
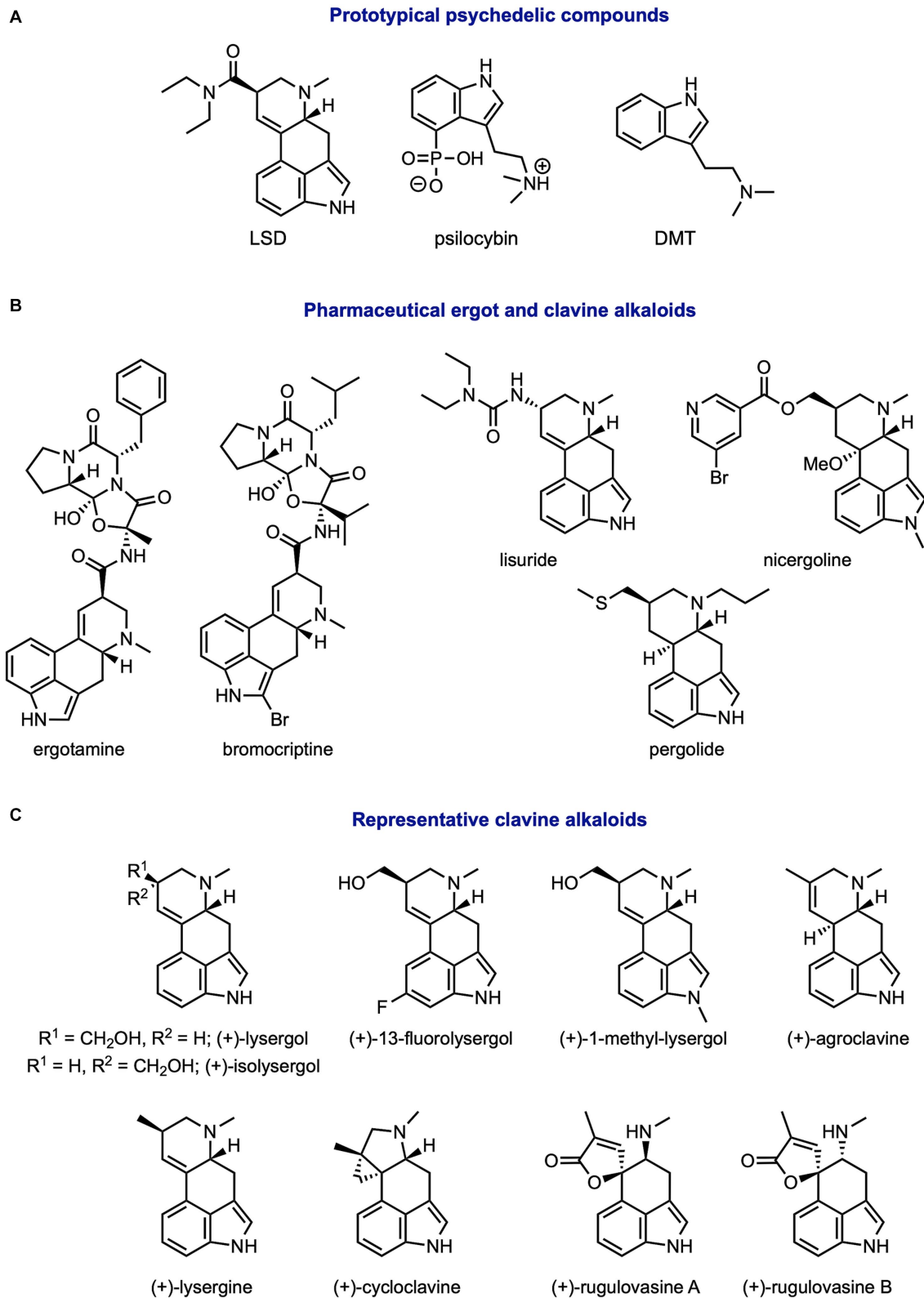

FIGURE 1
Structures of common ergot and clavine alkaloids. **(A)** Prototypical psychedelics, **(B)** pharmaceutical alkaloids, and **(C)** other studied clavine alkaloids.

Considerable efforts have been expended to elucidate how psychedelics and structurally related compounds elicit their unique biological responses. LSD, an ergot alkaloid and a prototypical psychedelic, has strong interactions with aminergic G-protein-coupled receptors (GPCRs), including serotonin, dopamine, histamine, adrenergic and muscarinic receptors (24–30). Recently, Lewis et al. screened LSD at 33 human aminergic GPCRs using a G protein dissociation BRET-based assay as part of a pharmacological profiling of a non-hallucinogenic LSD analog (*vide infra*) (31). The receptors were ranked by calculating a relative activity (log E_{MAX}/EC_{50}), and the top-ranked GPCRs included all five of the 5-HT$_1$ and the three 5-HT$_2$ subtypes, 5-HT$_6$, and the α_{2C} adrenergic receptor. Dopamine receptors D$_{2–4}$ and the human serotonin receptors 5-HT$_{5A}$ and 5-HT$_{7A}$ were among the top 15 hits. LSD showed pan-agonism at 5-HT$_1$ ($EC_{50} = 0.62–2.38$ nM) and 5-HT$_2$ receptors ($EC_{50} = 0.30–0.66$ nM). At the remaining serotonin receptors, LSD demonstrated potent agonism at 5-HT$_6$ ($EC_{50} = 0.13$ nM), antagonism at 5-HT$_{7A}$, and only partial agonism at 5-HT$_{5A}$ ($EC_{50} = 2.33$ nM, $E_{MAX} = 11\%$ relative to 5-HT). LSD was an agonist at the D$_2$, and D$_4$ receptors ($EC_{50} = 2.17$ and 4.03 nM, respectively) and a partial agonist at D$_3$ ($EC_{50} = 7.57$ nM, $E_{MAX} = 75\%$ relative to dopamine). Finally, LSD showed partial agonism at α_{2C} ($EC_{50} = 0.56$ nM, $E_{MAX} = 80\%$ relative to norepinephrine). Ergotamine is a natural ergot alkaloid and a precursor of LSD with a similar pharmacological profile to LSD but is not generally considered as psychoactive. Ergotamine differs from LSD by its cyclic peptide moiety appended to the amide sidechain. Peng et al. profiled ergotamine's pharmacology and discovered that ergotamine has a good affinity ($K_i < 10$ μM) for ~70% of all human aminergic GPCRs (32). Furthermore, 15 of these receptors show low or sub-nanomolar affinity based on a radioligand competition binding assay. While ergotamine displayed agonism at 5-HT$_{2A–C}$ and most 5-HT$_1$ receptors, it did not display agonist activity at the D$_3$, α_{1B}, α_{1D}, or β_2 receptors despite high affinities, suggesting it acts as an antagonist at these receptors. Finally, ergotamine was an inverse agonist at 5-HT$_7$, and further profiling showed that it also has opioid receptor agonist activity.

Most targets of LSD and/or ergotamine have been implicated in numerous diseases and disorders. Many 5-HT$_1$ receptors show promise for the treatment of anxiety, depression, PTSD, and migraines (33–38). 5-HT$_{2A}$ activation promotes neuronal growth and dendritic arbor complexity, and these outcomes are linked to improvements in mood and addiction disorders (39–41). The 5-HT$_{2C}$ receptor contributes to mood regulation and is a common target for treating obsessive compulsive disorder (OCD) and the mode of action of antipsychotics (42–46). This mechanism has also been implicated in treating addiction and obesity (47). Targeting 5-HT$_6$ has been shown to improve learning and memory and may also treat cognitive impairment (48–50). Additionally, 5-HT$_7$ receptors regulate inflammation across the body, and agonists are associated with anti-inflammatory and neuroprotective effects (51–53). Finally, dopamine agonists are commonly used for treating involuntary movement disorders (54–56). However, activation of 5-HT$_{2A}$ is also believed to be a primary mechanism in which psychedelics produce their psychoactive effects (57–63). In contrast, activation of 5-HT$_{2B}$ is associated with cardiac valvulopathy, and thus targeting this receptor is generally undesirable (64, 65). Therefore, many studies aim to discover psychedelic analogs that are selective for other receptors over 5-HT$_{2A/2B}$ or demonstrate functional selectivity, in which a ligand

stabilizes distinct receptor conformations that can selectively activate or deactivate downstream signaling pathways while keeping others unchanged (66, 67).

Biochemical, structural biology, bioengineering, and docking studies have all aided in the pursuit of non-hallucinogenic psychedelic analogs by pinpointing key structural features of compounds that elicit biased agonism. In 2013, Wacker et al. demonstrated that LSD, ergotamine, and other ergolines have functional selectivity for β-arrestin signaling at the 5-HT$_{2B}$ while also being unbiased for the 5-HT$_{1B}$ receptor (68). A comparison of crystal structures of LSD and ergotamine in both receptors revealed that the peptide appended to ergotamine was the cause of ergotamine's superior biased signaling compared to LSD. An additional analysis of LSD complexed with 5-HT$_{2B}$ by Wacker et al. in 2017, in combination with molecular dynamics simulations, revealed a structural basis for LSD's slow binding kinetics at 5-HT$_{2A}$ and 5-HT$_{2B}$ (69). This work also revealed that the amide substituents in LSD play a significant role in the position of the ligand within the orthosteric pocket, the molecule's long residence time, and its ability to promote β-arrestin translation. Similarly, solved crystal structures of ergotamine and ritanserin bound to 5-HT$_{2C}$ by Peng et al. revealed distinctive binding modes for each compound and key receptor–ligand interactions that promote serotonin receptor-subtype selectivity (32). An analysis of serotonin, LSD, psilocin, and lisuride complexed with 5-HT$_{2A}$ by Cao et al. displayed a secondary binding pose for psilocin and serotonin in which their indole cores interact with the extended binding pocket of the receptor (70). Their data suggest that serotonin/psilocin may adopt two different positions, either in the orthosteric binding pocket or the extended binding pocket, and these would produce different biological effects. This group campaigned to synthesize a molecule that would favor the extended binding pocket, with the hypothesis that this would promote β-arrestin recruitment bias at 5-HT$_{2A}$. They successfully synthesized compounds IHCH-7079 and IHCH-7086 which displayed antidepressant effects without inducing hallucinogenic behavior in mouse models. Docking studies of tetrahydropyridines complexed with 5-HT$_{2A}$ performed by Kaplan et al. led to the synthesis and further testing of 17 molecules (71). Among these, two displayed antagonist activity at 5-HT$_{2B}$, one displayed antagonist activity at 5-HT$_{2A}$, and another displayed agonist activity at 5-HT$_{2B}$. Additional structure-based optimization led to the discovery of a selective 5-HT$_{2A}$ antagonist and two 5-HT$_{2A}$ partial agonists. The two agonists elicited antidepressant behavior in mouse models and low levels of head-twitch response when compared to LSD. Notably, these agonists were also quite selective and showed a 4.6–6.4-fold preference of 5-HT$_{2A}$ over 5-HT$_{2B}$ and a 29–51-fold preference over 5-HT$_{2C}$. Later, Dong et al. described a fluorescent biosensor which was capable of predicting a compound's hallucinogenic potential by detecting distinct GPCR conformations induced by hallucinogenic ligands *in vivo* (72). This biosensor was then used to identify a nonhallucinogenic DMT analog with neuroplasticity-promoting and antidepressant effects. Finally, 2-Br-LSD was demonstrated by Lewis et al. to be a non-hallucinogenic analog of LSD with neuroplasticity-promoting effects (31). Pharmacological profiling showed that 2-Br-LSD is a significantly more selective ligand than LSD and does not show activity at aminergic GPCRs known to affect blood pressure, heart

rate, and other autonomic functions (e.g., 5-HT$_{2B}$). Interestingly, while the compound was non-hallucinogenic, it still displayed agonism at 5-HT$_{2A}$. However, the authors had shown that while 2-Br-LSD has high potency at 5-HT$_{2A}$ (EC$_{50}$ = 0.81 nM), it only partially activates the receptor (E$_{MAX}$ = 60%, relative to serotonin); whereas LSD is nearly a full agonist (E$_{MAX}$ = 92%, relative to serotonin). They later tested 2-Br-LSD as an antagonist in 5-HT$_{2A}$ G$_q$ dissociation and β-arrestin2 recruitment assays, and the analog did indeed act as a potent partial antagonist.

Many studies of psychedelics and their analogs focus on lysergic acid amides and dimethyltryptamine derivatives and their biological effects. Clavine alkaloids differ from lysergic acid derivatives by the structure of the quinoline ring system fused onto the indole core and the absence of a carboxylic acid functional group at C8 (Figure 2). They often have a greater conformational flexibility and are therefore more tunable receptor ligands (73, 74). However, clavine alkaloids are still comparatively understudied despite their potential for high bioactivity and superior receptor selectivity. As testimony to their medicinal effectiveness, several semisynthetic ergolines and clavines have reached the market for treating disorders and diseases such as migraines, cancer, Parkinson's disease, and dementia (75, 76). For example, lisuride is structurally similar to LSD, yet it does not typically produce hallucinogenic effects and has often been used to treat Parkinson's disease and migraines (77). It also produces antidepressant behavior in mice (78). Lisuride displays nanomolar binding affinity for most aminergic GPCRs including serotoninergic, adrenergic, and especially dopaminergic receptors. Its ability to treat Parkinson's disease is believed to primarily stem from its agonist activity at the dopamine receptors (79). While lisuride has potent agonist activity at 5-HT$_{1A}$ (80), it is a strong antagonist at 5-HT$_{2B}$ and thus devoid of cardiac valvulopathy side effects (81). At 5-HT$_{2A}$, lisuride is a G-protein biased agonist and this may be the mechanism responsible for how the medicinal properties of lisuride and potential hallucinogenic side effects are decoupled (78). However, strong activation of 5-HT$_{1A}$ has been shown to block the head-twitch response in mice treated with a 5-HT$_{2A}$/5-HT$_{2C}$ agonist (82). Dopamine and adrenoreceptors may also play a significant role in modulating head twitch response. Bromocriptine, a 2-brominated derivative of ergocriptine is a medicinally relevant ergot alkaloid that has been used in combination with levodopa in the treatment of Parkinson's disease (83). Bromocriptine has also been implicated as a potential therapeutic agent for type 2 diabetes (84). Similar to lisuride, it is a potent D$_2$ agonist and has good binding affinity for a number of serotonin receptors. However, bromocriptine is an antagonist at D$_1$, an inverse agonist at D$_4$, and a partial agonist at 5-HT$_{2B}$ (80). It also behaves as an agonist at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and, interestingly, acts as an antagonist at the adrenoreceptors α$_{2A}$, α$_{2B}$, and α$_{2C}$. Nicergoline has been used for the treatment of dementia as well as cerebrovascular, peripheral vascular, and balance disorders (85, 86). The compound has high binding affinity for α$_1$ and 5-HT$_{1A}$ receptors and demonstrated antagonism at these receptors (87). While nicergoline only has appreciable affinity for α$_2$ and 5-HT$_2$ receptors, it has low affinity for the dopamine receptors D$_1$ and D$_2$ and the muscarinic receptors M$_1$ and M$_2$. As a final example, ergotamine (*vida supra*) has been used for the prevention and treatment of migraines (88). It should be noted that the toxicity of these

compounds and of all members of the ergot alkaloid class is a potential concern (89–92). Ergotism and ischemia can occur if the levels of ergot alkaloids in the body exceed a certain threshold. This typically occurs if a patient is co-treated with a cytochrome P450 inhibitor and the ergot alkaloid medication is not properly metabolized; therefore, prescribed dosages of ergot alkaloids are typically low (93). Potential toxicity should not discourage the development of these compounds into marketable drugs, but rather demonstrates the need for additional clinical and preclinical studies and investigations of natural and synthetic alkaloids before their full therapeutic potential can be unlocked.

2 Notable advancements

Most ergot alkaloid drugs are produced semi-synthetically from field cultivations or fermentations of ergot fungi (94). The clinically useful natural product precursors for derivatization are elymoclavine, lysergol, and lysergic acid, which can be isolated directly from the fungus or received from global saponification or reduction of the bulk mass of ergopeptines and lysergic acid amides. Recent studies to reconstitute the biosynthetic pathways of ergot alkaloids provide scalable access to single molecules. The biosynthesis of cycloclavine, a complex rearranged clavine alkaloid containing a cyclopropyl ring has been elucidated, and production of cycloclavine through heterologous expression in yeast, delivered titers of >500 mg L^{-1} (95). Microbial cell factories and cell-free systems, two unrelated systems for the biosynthesis of biomolecules, were combined to overproduce agroclavine in titers of 1,209 mg L^{-1} (96). The clavine oxidase (CloA) is a putative cytochrome P450 that catalyzes formation of lysergic acid from agroclavine in the ergot alkaloid biosynthetic pathway. A specific region between the F-G helices of CloA was identified as responsible for detecting and directing oxidation of agroclavine. An engineered, chimeric AT5 9Hypo CloA, which contained a clear substrate access channel and an enzyme surface cleft, increased levels of lysergic acid by 15-fold compared to the wildtype enzyme. Further discussion on scalable biosynthetic processes is beyond the scope of this review but remains an active field that holds potential to facilitate clavine and ergot alkaloid diversification. The biological implications of clavine alkaloids, closely related to LSD, have typically been evaluated through derivatizing natural products or investigations of readily derived stereoisomers.

2.1 Natural and substituted clavine alkaloids

Preliminary investigations into the SAR of the clavine alkaloids by Hofmann and Pertz established primary modalities pertinent for activity at CNS receptors. Notably, therapeutic investigations of clavine alkaloid derivatives were pioneered by Hofmann after his historic discovery of LSD (97). (+)-1-Methyl lysergol showed a 13-fold increase in serotonin inhibitory effect compared to naturally occurring (+)-lysergol, but 1-methyl-ergotamine was less potent than ergotamine, indicating this slight modification was mainly relevant to clavine alkaloids. Moreover, (+)-lysergol produces a pyretogenic effect, but the methylated analog initiates an inverse reaction and lowers the body temperature in rabbits. Finally, (+)-1-methyl lysergol

($LD_{50} = 18$ mg/kg) was over 50-fold less toxic than (+)-lysergol ($LD_{50} = 0.32$ mg/kg).

Furthermore, Pertz et al. investigated eight naturally occurring clavine alkaloids for antagonism/partial agonism at 5-HT_{2A} receptors and antagonism at α_1-adrenoreceptors in blood vessels of rat tail arteries and aorta (73). While CNS receptor structure differs between species, they demonstrated that clavine alkaloids can have different modes of action compared to 5-HT. This concept was later supported by cryo-EM structures of LSD-bound 5-HT_{2A}; the binding modes of the semi-synthetic ergot alkaloid differed from the agonist 25CN-NBOH (98). Clavines, with the exception of costaclavine containing the deactivating cis-CD ring junction, show moderate affinity to 5-HT_{2A} receptors and α_1-adrenoreceptors. They display partial agonism activity by themselves, and some are antagonists of 5-HT at micromolar concentrations. Moreover, N1-isopropylation can increase affinity and specificity for 5-HT_{2A} vs. α_1, which could improve the noxious effects associated with ergot alkaloids. In fact, a similar modification was previously demonstrated by Hofmann (vide supra). In a subsequent report, Pertz et al. investigated cycloalkanecarboxylic esters derived from lysergol, dihydrolysergol-I, and elymoclavine (74). As with the naturally occurring clavines, these derivatives displayed partial agonism and antagonism effects of the contractile properties of 5-HT. Thereby, SAR investigations into acyl group variation and N1-modification demonstrated that the tetracyclic indolo[4,3-fg] quinoline system was the primary pharmacophore, and hydroxy acylation with cycloalkyl groups coupled with N1-alkylation synergistically increased 5-HT_{2A} receptor selectivity over the α_1-adrenoreceptor.

In 2017, Luo et al. disclosed an elegant asymmetric synthesis of (+)-lysergol, (+)-isolysergol, (−)-isolysergol, and (+)-13-fluorolysergol featuring a Ciamician-Dennstedt-type rearrangement to form the piperidine C ring and a Rh-carbenoid formal [3 + 2] cycloaddition to construct the ergoline scaffold (99). These analogs were subjected to agonism assays at four serotonin receptors, $5\text{-HT}_{1A,2A-C}$. CHO-K1 cells expressing each receptor were loaded with a dye and analyzed by FLIPR during addition of compounds or control agonist. All of the compounds displayed a reduced maximal activation for each receptor compared to 5-HT (see Table 1). For instance, while (+)-lysergol activated 5-HT_{2A} with an EC_{50} value of 1.6 nM, it was limited to 51% of the maximum 5-HT stimulation. Similarly, (+)-lysergol activated 5-HT_{2C} to 43% maximum of 5-HT stimulation. The unnatural substituted analog (+)-13-fluorolysergol, while selective for 5-HT_{1A} and 5-HT_{2A}, only showed 17% maximal activation of 5-HT_{2A}

compared to 5-HT. While less effective than the natural ligand, 5-HT, partial agonism and receptor selectivity garnered by ring substitution could lead to effective therapeutic agents without deleterious hallucinogenic effects (vide supra).

2.2 Clavine alkaloid stereoisomers

Wipf et al. reported the shortest synthesis of racemic lysergols to date, and after chiral resolution of the enantiomers, investigated the effects of all four stereoisomers on several serotonin receptors. Of note, binding data of (−)-lysergol were disclosed for the first time. All but (−)-isolysergol maintained excellent affinity for $5\text{-HT}_{1A,2A-C}$ (100). Agonism effects of (+)-lysergol, (−)-lysergol, and (+)-isolysergol were calculated as a percent of control response to a known reference (Table 2). The agonism activity of (+)-lysergol corroborated previous investigations (100). With 5-fold reduced activity at 5-HT_{2A}, the naturally occurring (+)-lysergol should have reduced psychoactive effects compared to LSD. Moreover, it displayed a similar profile to psilocin with about half of psilocin's activity at 5-HT_{2A}. Interestingly, (−)-lysergol was a selective agonist for 5-HT_{2C}. Many 5-HT_{2C} activators that are used to regulate mood are poorly selective between 5-HT_{2B} and 5-HT_{2A}, so further investigations and SAR would be justified to elaborate upon these findings. (+)-Isolysergol displayed very similar activation of 5-HT_{1A} compared to (+)-lysergol, but was less active towards 5-HT_{2C}, suggesting the configuration of C(8) plays a more significant role in agonism activity towards 5-HT_{2C} than 5-HT_{1A}.

Interestingly, while (+)-lysergol had a high affinity for 5-HT_{2B}, it produced no agonism effects within the tested range (101). Similarly, (+)-isolysergol showed high affinities for 5-HT_{2A} and 5-HT_{2B} without agonism effects. Since these ligands bind without inducing activation, further studies are required to determine if they act as antagonists, as findings from Pertz (vide supra) (73) suggest these clavines could also produce antagonism effects at other CNS receptors. Of particular note is also the stereospecificity of the binding/agonistic effects of the four lysergol stereoisomers. While not necessarily unexpected for rigid and selective compounds, this nonetheless highlights opportunities for future ligand designs at 5-HTRs.

Recently, investigations into the activation effects of rearranged clavine alkaloids, including (+)- and (−)-cycloclavine and all stereoisomers of rugulovasine have been reported (99, 100, 102, 103). Wipf et al. developed an asymmetric synthesis of cycloclavine and

TABLE 1 Lysergol analog activation of 5-HT receptors reported by Luo et al. (99).

Entry	Compound	5-HT_{1A} (nM)	5-HT_{2A} (nM)	5-HT_{2B} (nM)	5-HT_{2C} (nM)
1	5-HT	11 ± 2	0.96 ± 0.14	0.071 ± 0.010	0.14 ± 0.03
2	(+)-lysergol	73 ± 6	1.6 ± 0.5[a]	>10,000	6.6 ± 1.4[b]
3	(±)-lysergine	342 ± 23	2.7 ± 1.6[c]	145 ± 54[d]	103 ± 9[e]
4	(−)-isolysergol	481 ± 31	>10,000	>10,000	>10,000
5	(+)-13-fluorolysergol	424 ± 27	12 ± 3[f]	>10,000	>10,000

[a]51% max 5-HT stimulation.
[b]43% max 5-HT stimulation.
[c]57% max 5-HT stimulation.
[d]36% max 5-HT stimulation.
[e]42% max 5-HT stimulation.
[f]17% max 5-HT stimulation.

TABLE 2 Clavine alkaloid activation of 5-HT receptors.

Compound	5-HT$_{1A}$R	5-HT$_{2A}$R	5-HT$_{2B}$R	5-HT$_{2C}$R
(+)-lysergol[a]	76.5%[b]	50.4%[c]	–	61%[d]
(−)-lysergol[a]	–	–	–	70%[e]
(+)-isolysergol[a]	52.2%[b]	–	–	78.5%[b]
(+)-cycloclavine (μM)	0.14	~10	>20	0.016
(−)-cycloclavine (μM)	~5	>50	>20	3.2
(−)-rugulovasine A (nM)	37	n.t.[f]	n.t.	n.t.
(+)-rugulovasine A (nM)	47	n.t.	n.t.	n.t.
(+)-rugulovasine B (nM)	<2	46	58	339
(−)-rugulovasine B (nM)	116	n.t.	n.t.	n.t.
DMT[g] (μM)	0.075[h]	0.076	3.4	0.424[h]
Psilocin[g] (μM)	0.123[h]	0.721[h]	>20	0.094[h]
D-LSD[g] (μM)	0.003[h]	0.261[h]	12[i]	0.015[h]

[a]Cellular agonist effect was calculated as a % of control response to a known reference agonist for each target. CNS receptor assays were performed in singlicate at human receptors at Eurofins Cerep Panlabs. "–" indicates no agonism effects were detected at the tested concentrations.
[b]250 nM.
[c]1.3 μM.
[d]10 nM.
[e]2.0 μM.
[f]n.t. denotes "not tested".
[g]Data taken from ref. 30.
[h]Inhibition constants Ki.
[i]EC50 values.

investigated the specific binding and activation of both natural and unnatural enantiomers at several CNS receptors (104). The naturally occurring (+)-cycloclavine was an agonist for 5-HT$_{1A}$ (EC$_{50}$ = 0.14 μM) and 5-HT$_{2C}$ (EC$_{50}$ = 0.016 μM). (−)-Cycloclavine displayed comparably much smaller activation effects, and only nominally affected 5-HT$_{2C}$ (EC$_{50}$ = 3.2 μM). (+)-Cycloclavine displayed a remarkably similar profile to (+)-lysergol, albeit with ~8-fold lower 5-HT$_{2A}$ activation. Since both cycloclavine enantiomers were poor activators of 5-HT$_{2A}$, they may only exhibit weak hallucinogenic effects, but (+)-cycloclavine could display promising mood-regulating properties.

Piersanti et al. disclosed an asymmetric synthesis of all stereoisomers of rugulovasines and evaluated their CNS receptor activity (103). They were tested for specific binding at serotonin (5-HT$_{1A,2A,2C}$), adrenergic (α_2), and dopamine (D$_{1,2L,3}$) receptors, and were found to be specific solely for the first. Of the four isomers, (+)-rugulovasine B was the overall best ligand and (−)-rugulovasine B displayed poor affinity for all but 5-HT$_{1A}$. Moreover, among the targets that were investigated, the rugulovasines were selective for 5-HT receptors. Since all four isomers displayed a strong affinity for 5-HT$_{1A}$, they were subjected to an activation assay for that target. Each isomer was a strong activator of this receptor with nanomolar potencies. (+)-Rugulovasine was the most potent agonist (<2 nM) and was surprisingly more active than LSD. This significant enantiospecificity and potency translated to other 5-HT receptors. Among the seven other serotonin receptors tested (5-HT$_{1A,2B–C,5A,6,7}$), (+)-rugulovasine B displayed nanomolar activation for all but 5-HT$_{5A}$ (13.7 μM), in contrast to LSD which only displayed low activation of 5-HT$_{2B}$ (12.0 μM). (+)-Rugulovasine B is a potent activator of 5-HT$_{2A}$ and 5-HT$_{2B}$, suggesting hallucinogenic, cardiotoxic, and europhoric effects might occur in consumers.

Accordingly, further (ant)agonism assays of dopamine and adrenergic receptors of the rugulovasines are required to validate their therapeutic potential. Hopefully, the expedient synthetic approach will enable future investigations of the biologically understudied rugulovasine scaffold.

3 Conclusion

The exploration of clavine alkaloids as therapeutic agents is of continued and significant interest. Early work focused on improving the potency and decreasing the toxicity of natural clavines through minor structural modifications, such as N1-alkylation or O-acylation (73, 74, 97). More recently, efficient total syntheses of clavine alkaloids have enabled systematic investigations of stereoisomers (100, 103, 104) and analogs with significant structural modifications (99). (+)-Lysergol, (+)-isolysergol, and (+)-cycloclavine thus far bear the most potential as non-hallucinogenic "psychedelic" agents. While more CNS receptor profiling and in vivo studies are required, they maintain promising 5-HT receptor profiles, as they poorly activate receptors that cause deleterious effects [5-HT$_{2A}$ (hallucinations) and 5-HT$_{2B}$ (cardiotoxicity)], but strongly activate the mood-regulators 5-HT$_{1A}$ and 5-HT$_{2C}$. Moreover, (+)-lysergol displays a very similar profile to the clinically studied psychedelic psilocin at the 5-HT$_{1A,2A–C}$ receptors, and derivatization with deep-seated changes achievable through de novo synthesis could provide access to more selective ligands, as demonstrated by Luo et al. (99). The understudied rearranged clavine scaffolds, including cycloclavine and the rugulovasines, also demonstrate unique enantiospecific activation of the CNS receptors, similar to the

FIGURE 2
Structural overview of ergot and clavine alkaloids.
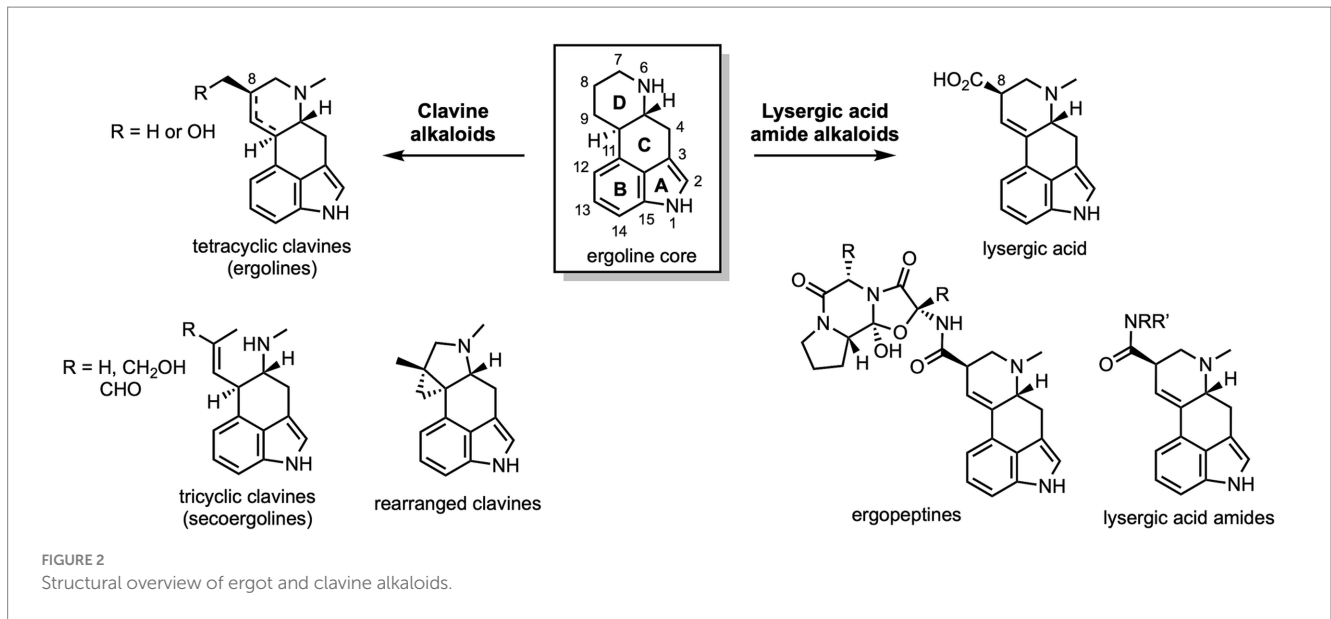
lysergols. Some clavines such as (+)-rugulovasine B can be potent 5-HT_{2A} activators; however, they are relatively unselective agonists and could produce detrimental side effects. Future SAR campaigns on the clavine alkaloid scaffold are well justified by their known biological profile and are likely to discover a broad range of novel and potentially therapeutic analogs, paving the way to decouple the development of new psychedelics from the study of the known natural product isolates.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

NT: Writing – original draft, Writing – review & editing. EP: Writing – original draft, Writing – review & editing. PW: Conceptualization, Supervision, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Schultes RE, Hofmann A, Rätsch C. *Plants of the gods: their sacred, healing, and hallucinogenic powers*. Rochester: Healing Arts Press (2001).

2. Tupper KW, Wood E, Yensen R, Johnson MW. Psychedelic medicine: a re-emerging therapeutic paradigm. *CMAJ*. (2015) 187:1054–9. doi: 10.1503/cmaj.141124

3. Gardner J, Carter A, O'Brien K, Seear K. Psychedelic-assisted therapies: the past, and the need to move forward responsibly. *Int J Drug Policy*. (2019) 70:94–8. doi: 10.1016/j.drugpo.2019.05.019

4. Lowe H, Toyang N, Steele B, Valentine H, Grant J, Ali A, et al. The therapeutic potential of psilocybin. *Molecules*. (2021) 26:2948. doi: 10.3390/molecules 26102948

5. Lutkajtis A. Four individuals' experience during and following a psilocybin truffle retreat in the Netherlands. *J Psychedelic Stud*. (2021) 5:22–32. doi: 10.1556/2054.2021.00162

6. Holze F, Vizeli P, Ley L, Müller F, Dolder P, Stocker M, et al. Acute dose-dependent effects of lysergic acid diethylamide in a double-blind placebo-controlled study in healthy subjects. *Neuropsychopharmacology*. (2021) 46:537–44. doi: 10.1038/s41386-020-00883-6

7. Vogt SB, Ley L, Erne L, Straumann I, Becker AM, Klaiber A, et al. Acute effects of intravenous DMT in a randomized placebo-controlled study in healthy participants. *Transl Psychiatry*. (2023) 13:172. doi: 10.1038/s41398-023-02477-4

8. Stoliker D, Egan GF, Friston KJ, Razi A. Neural mechanisms and psychology of psychedelic ego dissolution. *Pharmacol Rev*. (2022) 74:876–917. doi: 10.1124/pharmrev.121.000508

9. Forstmann M, Yudkin DA, Prosser AMB, Heller SM, Crockett MJ. Transformative experience and social connectedness mediate the mood-enhancing effects of psychedelic use in naturalistic settings. *Proc Natl Acad Sci U S A*. (2020) 117:2338–46. doi: 10.1073/pnas.1918477117

10. Nichols DE. Psychedelics. *Pharmacol Rev*. (2016) 68:264–355. doi: 10.1124/pr.115.011478

11. Nardou R, Sawyer E, Song YJ, Wilkinson M, Padovan-Hernandez Y, de Deus JL, et al. Psychedelics reopen the social reward learning critical period. *Nature*. (2023) 618:790–8. doi: 10.1038/s41586-023-06204-3

12. Nikolaidis A, Lancelotta R, Gukasyan N, Griffiths RR, Barrett FS, Davis AK. Subtypes of the psychedelic experience have reproducible and predictable effects on depression and anxiety symptoms. *J Affect Disord*. (2023) 324:239–49. doi: 10.1016/j.jad.2022.12.042

13. Plazas E, Faraone N. Indole alkaloids from psychoactive mushrooms: chemical and pharmacological potential as psychotherapeutic agents. *Biomedicine*. (2023) 11:461. doi: 10.3390/biomedicines11020461

14. Inserra A, de Gregorio D, Gobbi G. Psychedelics in psychiatry: neuroplastic, immunomodulatory, and neurotransmitter mechanisms. *Pharmacol Rev*. (2021) 73:202–77. doi: 10.1124/pharmrev.120.000056

15. Dyck E. LSD: a new treatment emerging from the past. *CMAJ*. (2015) 187:1079–80. doi: 10.1503/cmaj.141358

16. Calleja-Conde J, Morales-García JA, Echeverry-Alzate BKM, Giné E, López-Moreno JA. Classic psychedelics and alcohol use disorders: a systematic review of human and animal studies. *Addict Biol*. (2022) 27:e13229. doi: 10.1111/adb.13229

17. Cameron LP, Olson DE. Dark classics in chemical neuroscience: N,N-dimethyltryptamine (DMT). *ACS Chem Neurosci*. (2018) 9:2344–57. doi: 10.1021/acschemneuro.8b00101

18. Barber GS, Aaronson ST. The emerging field of psychedelic psychotherapy. *Curr Psychiatry Rep*. (2022) 24:583–90. doi: 10.1007/s11920-022-01363-y

19. Nichols DE. Dark classics in chemical neuroscience: lysergic acid diethylamide (LSD). *ACS Chem Neurosci*. (2018) 9:2331–43. doi: 10.1021/acschemneuro.8b00043

20. Strassman RJ, Qualls CR. Dose-response study of N,N-dimethyltryptamine in humans I. Neuroendocrine, autonomic, and aardiovascular effects. *Arch Gen Psychiatry*. (1994) 51:85–97. doi: 10.1001/archpsyc.1994.03950020009001

21. Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R. Dose-response study of N,N-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. *Arch Gen Psychiatry*. (1994) 51:98–108. doi: 10.1001/archpsyc.1994.03950020022002

22. Carhart-Harris RL, Goodwin GM. The therapeutic potential of psychedelic drugs: past, present, and future. *Neuropsychopharmacology*. (2017) 42:2105–13. doi: 10.1038/npp.2017.84

23. Hadar A, David J, Shalit N, Roseman L, Gross R, Sessa B, et al. The psychedelic renaissance in clinical research: a bibliometric analysis of three decades of human studies with psychedelics. *J Psychoactive Drugs*. (2023) 55:1–10. doi: 10.1080/02791072.2021.2022254

24. Nichols DE, Frescas S, Marona-Lewicka D, Kurrasch-Orbaugh DM. Lysergamides of isomeric 2,4-Dimethylazetidines map the binding orientation of the diethylamide moiety in the potent hallucinogenic AgentN,N-Diethyllysergamide (LSD). *J Med Chem*. (2002) 45:4344. doi: 10.1021/jm020153s

25. Passie T, Halpern JH, Stichtenoth DO, Emrich HM, Hintzen A. The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther*. (2008) 14:295–314. doi: 10.1111/j.1755-5949.2008.00059.x

26. Egan CT, Herrick-Davis K, Miller K, Glennon RA, Teitler M. Agonist activity of LSD and lisuride at cloned 5HT2A and 5HT2C receptors. *Psychopharmacology*. (1998) 136:409–14. doi: 10.1007/s002130050585

27. Watts VJ, Lawler CP, Fox DR, Neve KA, Nichols DE, Mailman RB. LSD and structural analogs: pharmacological evaluation at D1 dopamine receptors. *Psychopharmacology*. (1995) 118:401–9. doi: 10.1007/BF02245940

28. Giacomelli S, Palmerry M, Romanelli L, Cheng CY, Silvestrini B. Lysergic acid diethylamide (LSD) is a partial agonist of D2 dopaminergic receptors and it potentiates dopamine-mediated prolactin secretion in lactotrophs in vitro. *Life Sci*. (1998) 63:215–22. doi: 10.1016/s0024-3205(98)00262-8

29. Rickli A, Luethi RJ, Buchy D, Hoener MC, Liechti ME. Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). *Neuropharmacology*. (2015) 99:546. doi: 10.1016/j.neuropharm.2015.08.034

30. Rickli A, Moning OD, Hoener MC, Liechti ME. Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. *Eur Neuropsychopharmacol*. (2016) 26:1327–37. doi: 10.1016/j.euroneuro.2016.05.001

31. Lewis V, Bonniwell EM, Lanham JK, Ghaffari A, Sheshbaradaran H, Cao AB, et al. A non-hallucinogenic LSD analog with therapeutic potential for mood disorders. *Cell Rep*. (2023) 42:112203. doi: 10.1016/j.celrep.2023.112203

32. Peng Y, McCorvy JD, Harpsøe K, Lansu K, Yuan S, Popov P, et al. 5-HT2C receptor structures reveal the structural basis of GPCR polypharmacology. *Cells*. (2018) 172:719–730.e14. doi: 10.1016/j.cell.2018.01.001

33. Ramírez Rosas MB, Labruijere S, Villalón CM, Maassen VDBA. Activation of 5-hydroxytryptamine 1B/1D/1F receptors as a mechanism of action of antimigraine drugs. *Expert Opin Pharmacother*. (2013) 14:1599–10. doi: 10.1517/14656566.2013.806487

34. Xiang M, Jiang Y, Hu Z, Yang Y, Du X, Botchway BOA, et al. Serotonin receptors 2A and 1A modulate anxiety-like behavior in post-traumatic stress disordered mice. *Am J Transl Res*. (2019) 11:2288–303.

35. Garcia-Garcia A, Newman-Tancredi A, Leonardo ED. 5-HT1A receptors in mood and anxiety: recent insights into autoreceptor versus heteroreceptor function. *Psychopharmacology*. (2014) 231:623–36. doi: 10.1007/s00213-013-3389-x

36. Cameron LP, Benson CJ, Dunlap LE, Olson DE. Effects of N, N-dimethyltryptamine on rat Behaviors relevant to anxiety and depression. *ACS Chem Neurosci*. (2018) 9:1582–90. doi: 10.1021/acschemneuro.8b00134

37. Tiger M, Varnäs K, Okubo Y, Lundberg J. The 5-HT1B receptor – a potential target for antidepressant treatment. *Psychopharmacology*. (2018) 235:1317–34. doi: 10.1007/s00213-018-4872-1

38. Ruf BM, Bhagwager Z. The 5-HT1B receptor: a novel target for the pathophysiology of depression. *Curr Drug Targets*. (2009) 10:1118–38. doi: 10.2174/138945009789735192

39. Ly C, Greb AC, Cameron LP, Wong JM, Barragan EV, Wilson PC, et al. Psychedelics promote structural and functional neural plasticity. *Cell Rep*. (2018) 23:3170–82. doi: 10.1016/j.celrep.2018.05.022

40. Vargas MV, Dunlap LE, Dong C, Carter SJ, Tombari RJ, Jami SA, et al. Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science*. (2023) 379:700–6. doi: 10.1126/science.adf0435

41. Cameron LP, Tombari RJ, Lu J, Pell AJ, Hurley ZQ, Ehinger Y, et al. A non-hallucinogenic psychedelic analogue with therapeutic potential. *Nature*. (2021) 589:474–9. doi: 10.1038/S41586-020-3008-z

42. Papakosta VM, Kalogerakou S, Kontis D, Anyfandi E, Theochari E, Boulougouris V, et al. 5-HT2C receptor involvement in the control of persistence in the reinforced spatial alternation animal model of obsessive-compulsive disorder. *Behav Brain Res*. (2013) 243:176–83. doi: 10.1016/j.bbr.2013.01.005

43. Flaisher-Grinberg S, Klavir O, Joel D. The role of 5-HT2A and 5-HT2C receptors in the signal attenuation rat model of obsessive compulsive disorder. *Int J Neuropsychopharmacol*. (2008) 11:811–25. doi: 10.1017/s146114570800847x

44. Sard H, Kumaran G, Morency C, Roth BL, Toth BA, He P, et al. SAR of psilocybin analogs: discovery of a selective 5-HT2C agonist. *Bioorg Med Chem Lett*. (2005) 15:4555–9. doi: 10.1016/j.bmcl.2005.06.104

45. Chagraoui A, Thibaut F, Skiba M, Thuillez C, Bourin M. 5-HT2C receptors in psychiatric disorders: a review. *Prog Neuro-Psychopharmacol Biol Psychiatry*. (2016) 66:120–35. doi: 10.1016/j.pnpbp.2015.12.006

46. Pogorelov VM, Rodriguiz RM, Cheng J, Huang M, Schmerberg CM, Meltzer HY, et al. 5-HT2C agonists modulate schizophrenia-like behaviors in mice. *Neuropsychopharmacology*. (2017) 42:2163–77. doi: 10.1038/npp.2017.52

47. Higgins GA, Sellers EM, Fletcher PJ. From obesity to substance abuse: therapeutic opportunities for 5-HT2C receptor agonists. *Trends Pharmacol Sci*. (2013) 34:560–70. doi: 10.1016/j.tips.2013.08.001

48. Drop M, Canale V, Chaumont-Dubel S, Kurczab R, Satała G, Bantreil X, et al. 2-phenyl-1H-pyrrole-3-carboxamide as a new scaffold for developing 5-HT6 receptor inverse agonists with cognition-enhancing activity. *ACS Chem Neurosci*. (2021) 12:1228–40. doi: 10.1021/acschemneuro.1c00061

49. Woods S, Clarke NN, Layfield R, Fone KCF. 5-HT(6) receptor agonists and antagonists enhance learning and memory in a conditioned emotion response paradigm by modulation of cholinergic and glutamatergic mechanisms. *Br J Pharmacol*. (2012) 167:436–49. doi: 10.1111/j.1476-5381.2012.02022.x

50. Kendall I, Slotten HA, Codony X, Burgueño J, Pauwels PJ, Vela JM, et al. E-6801, a 5-HT6 receptor agonist, improves recognition memory by combined modulation of cholinergic and glutamatergic neurotransmission in the rat. *Psychopharmacology*. (2011) 213:413–30. doi: 10.1007/s00213-010-1854-3

51. Di Pilato P, Niso M, Adriani W, Romano E, Travaglini D, Berardi F, et al. Selective agonists for serotonin 7 (5-HT7) receptor and their applications in preclinical models: an overview. *Rev Neurosci*. (2014) 25:401–15. doi: 10.1515/revneuro-2014-0009

52. Quintero-Villegas A, Valdés-Ferrer SI. Central nervous system effects of 5-HT7 receptors: a potential target for neurodegenerative diseases. *Mol Medicine*. (2022) 28:70. doi: 10.1186/s10020-022-00497-2

53. Quintero-Villegas A, Valdés-Ferrer SI. Role of 5-HT7 receptors in the immune system in health and disease. *Mol Medicine*. (2020) 26:2. doi: 10.1186/s10020-019-0126-x

54. Brooks DJ. Dopamine agonists: their role in the treatment of Parkinson's disease. *J Neurol Neurosurg Psychiatry*. (2000) 68:685. doi: 10.1136/jnnp.68.6.685

55. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson disease. *Nat Rev Dis Primers*. (2017) 3:17013. doi: 10.1038/nrdp.2017.13

56. Scholz H, Trenkwalder C, Kohnen R, Kriston L, Riemann D, Hornyak M. Dopamine agonists for the treatment of restless legs syndrome. *Cochrane Database Syst Rev*. (2011) 2011:CD006009. doi: 10.1002/14651858.cd006009.pub2

57. López-Giménez JF, González-Maeso J. Hallucinogens and serotonin 5-HT2A receptor-mediated signaling pathways. *Curr Top Behav Neurosci*. (2018) 36:45–73. doi: 10.1007/7854_2017_478

58. Hesselgrave N, Troppoli TA, Wulff AB, Cole AB, Thompson SM. Harnessing psilocybin: antidepressant-like behavioral and synaptic actions of psilocybin are independent of 5-HT2R activation in mice. *Proc Natl Acad Sci U S A*. (2021) 118:e2022489118. doi: 10.1073/pnas.2022489118

59. Jaster AM, Elder H, Marsh SA, de La Fuente RM, Negus SS, González-Maeso J. Effects of the 5-HT2A receptor antagonist volinanserin on head-twitch response and intracranial self-stimulation depression induced by different structural classes of psychedelics in rodents. *Psychopharmacology*. (2022) 239:1665–77. doi: 10.1007/s00213-022-06092-x

60. Glatfelter GC, Pottie E, Partilla JS, Sherwood AM, Kaylo K, Pham DNK, et al. Structure–activity relationships for psilocybin, baeocystin, aeruginascin, and related analogues to produce pharmacological effects in mice. *ACS Pharmacol Transl Sci*. (2022) 5:1181–96. doi: 10.1021/acsptsci.2c00177

61. González-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R, et al. Hallucinogens recruit specific cortical 5-HT2A receptor-mediated signaling pathways to affect behavior. *Neuron*. (2007) 53:439–52. doi: 10.1016/j.neuron.2007.01.008

62. Canal CE, Morgan D. Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: a comprehensive history, a re-evaluation of mechanisms, and its utility as a model. *Drug Test Anal*. (2012) 4:556–76. doi: 10.1002/dta.1333

63. Willins DL, Meltzer HY. Direct injection of 5-HT2A receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. *J Pharmcol Exp Ther*. (1997) 282:699–706.

64. Rothman RB, Baumann MH. Serotonergic drugs and valvular heart disease. *Expert Opin Drug Saf*. (2009) 8:317–29. doi: 10.1517/14740330902931524

65. Hutcheson JD, Setola V, Roth BL, Merryman WD. Serotonin receptors and heart valve disease – it was meant 2B. *Pharmacol Ther*. (2011) 132:146–57. doi: 10.1016/j.pharmthera.2011.03.008

66. Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, et al. Functional selectivity and classical concepts of quantitative pharmacology. *J Pharmacol Exp Ther*. (2007) 320:1–13. doi: 10.1124/jpet.106.104463

67. Wootten D, Chrisopoulos A, Marti-Solano M, Babu MM, Sexton PM. Mechanisms of signaling and biased agonism in G protein-coupled receptors. *Nat Rev Mol Cell Biol*. (2018) 19:638–53. doi: 10.1038/s41580-018-0049-3

68. Wacker D, Wang C, Katritch V, Won Han G, Huang X-P, Vardy E, et al. Structural features for functional selectivity at serotonin receptors. *Science*. (2013) 340:615–9. doi: 10.1126/science.1232808

69. Wacker D, Wang S, McCorvy JD, Betz RM, Venkatakrishnan AJ, Levit A, et al. Crystal structure of an LSD-bound human serotonin receptor. *Cells*. (2017) 168:377–389.e12. doi: 10.1016/j.cell.2016.12.033

70. Cao D, Yu J, Wang H, Luo Z, Liu X, He L, et al. Structure-based discovery of nonhallucinogenic psychedelic analogs. *Science*. (2022) 375:403–11. doi: 10.1126/science.abl8615

71. Kaplan AL, Confer DN, Kim K, Barros-Álvarez X, Rodriguiz RM, Yang Y, et al. Bespoke library docking for 5-HT2A receptor agonists with antidepressant activity. *Nature*. (2022) 610:582–91. doi: 10.1038/s41586-022-05258-z

72. Dong C, Ly C, Dunlap LE, Vargas MV, Sun J, Hwang IW, et al. Psychedelic-inspired drug discovery using an engineered biosensor. *Cells*. (2021) 184:2779–2792.e18. doi: 10.1016/j.cell.2021.03.043

73. Pertz H. Naturally occurring clavines: antagonism/partial agonism at 5-HT2A receptors and antagonism at α1-adrenoceptors in blood vessels. *Planta Med*. (1996) 62:387–92. doi: 10.1055/s-2006-957922

74. Pertz HH, Milhahn C, Eich E. Cycloalkanecarboxylic esters derived from lysergol, dihydrolysergol-I, and elymoclavine as partial agonists and antagonists at rat 5-HT2A receptors: pharmacological evidence that the indolo[4,3-fg]quinoline system of the ergolines is responsible for high 5-HT2A receptor affinity. *J Med Chem*. (1999) 42:659–68. doi: 10.1021/jm981092u

75. McCabe SR, Wipf P. Total synthesis, biosynthesis and biological profiles of clavine alkaloids. *Org Biomol Chem*. (2016) 14:5894–913. doi: 10.1039/c6ob00878j

76. Tasker NR, Wipf P. Biosynthesis, total synthesis, and biological profiles of ergot alkaloids. *Alkaloids Chem Biol*. (2021) 85:1–112. doi: 10.1016/bs.alkal.2020.08.001

77. Horowski R, Löschmann PA. Classic dopamine agonists. *J Neural Transm*. (2019) 126:449–54. doi: 10.1007/s00702-019-01989-y

78. Pogorelov VM, Rodriguiz RM, Roth B, Westel WC. The G protein biased serotonin 5-HT2A receptor agonist lisuride exerts anti-depressant drug-like activities in mice. *Front Mol Biosci*. (2023) 10:10. doi: 10.3389/fmolb.2023.1233743

79. McDonald RJ, Horowski R. Lisuride in the treatment of parkinsonism. *Eur Neurol*. (1983) 22:240–55. doi: 10.1159/000115567

80. Kvernmo T, Houben J, Sylte I. Receptor-binding and pharmacokinetic properties of dopaminergic agonists. *Curr Top Med Chem*. (2008) 8:1049–67. doi: 10.2174/156802608785161457

81. Hofman C, Penner U, Dorow R, Pertz HH, Jähnichen S, Horowski R, et al. Lisuride, a dopamine receptor agonist with 5-HT2B receptor antagonist properties: absence of cardiac valvulopathy adverse drug reaction reports supports the concept of

a crucial role for 5-HT2B receptor agonism in cardiac valvular fibrosis. *Clin Neuropharmacol*. (2006) 29:80–6. doi: 10.1097/00002826-200603000-00005

82. Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S, Millan MJ. (1-(2,5-dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT) 2A receptors: modulation by novel 5-HT2A/2C antagonists, D1 antagonists and 5-HT1A agonists. *J Pharmacol Exp Ther*. (1995) 273:101–12.

83. Zhuo C, Zhu X, Jiang R, Ji F, Su Z, Sue R, et al. Comparison for efficacy and tolerability among ten drugs for treatment of Parkinson's disease: a network meta-analysis. *Sci Rep*. (2017) 7:45865. doi: 10.1038/srep45865

84. Cincotta AH. Brain dopamine–clock interactions regulate cardiometabolic physiology: mechanisms of the observed cardioprotective effects of circadian-timed bromocriptine-QR therapy in type 2 diabetes subjects. *Int J Mol Sci*. (2023) 24:13255. doi: 10.3390/ijms241713255

85. Winblad B, Carfagna N, Bonura L, Rossini BM, Wong EHF, Battaglia A. Nicergoline in dementia: a review of its pharmacological properties and therapeutic potential. *CNS Drugs*. (2000) 14:267–87. doi: 10.2165/00023210-200014040-00003

86. Winblad B, Fioravanti M, Dolezal T, Logina I, Milanov IG, Popescu DC, et al. Therapeutic use of nicergoline. *Clin Drug Investig*. (2008) 28:533–52. doi: 10.2165/00044011-200828090-00001

87. Zajdel P, Bednarski M, Sapa J, Nowak G. Ergotamine and nicergoline – facts and myths. *Pharmacol Rep*. (2015) 67:360–3. doi: 10.1016/j.pharep.2014.10.010

88. Tfelt-Hansen P, Saxena PR, Dahlöf C, Pascual J, Láinez M, Henry P, et al. Ergotamine in the acute treatment of migraine: a review and European consensus. *Brain*. (2000) 123:9–18. doi: 10.1093/brain/123.1.9

89. Mulac D, Humpf HU. Cytotoxicity and accumulation of ergot alkaloids in human primary cells. *Toxicology*. (2011) 282:112–21. doi: 10.1016/j.tox.2011.01.019

90. Agriopoulou S. Ergot alkaloids mycotoxins in cereals and cereal-derived food products: characteristics, toxicity, prevalence, and control strategies. *Agronomy*. (2021) 11:931. doi: 10.3390/agronomy11050931

91. Korn AK, Gross M, Usleber E, Thom N, Köhler K, Erhardt G. Dietary ergot alkaloids as a possible cause of tail necrosis in rabbits. *Mycotoxin Res*. (2014) 30:241–50. doi: 10.1007/s12550-014-0208-0

92. Finch SC, Munday JS, Sprosen JM, Bhattarai S. Toxicity studies of Chanoclavine in mice. *Toxins*. (2019) 11:249. doi: 10.3390/toxins11050249

93. Fröhlich G, Kaplan V, Amann-Vesti B. Holy fire in an hiv-positive man: case of 21st-century ergotism. *CMAJ*. (2010) 182:378–80. doi: 10.1503/cmaj.091362

94. Jakubczyk D, Caputi L, Hatsch A, Nielsen CAF, Diefenbacher M, Klein J, et al. Discovery and reconstitution of the cycloclavine biosynthetic pathway – enzymatic formation of a cyclopropyl group. *Angew Chem Int Ed Engl*. (2015) 54:5117–21. doi: 10.1002/anie.201410002

95. Yu ZP, An C, Yao Y, Wang CY, Sun Z, Cui C, et al. A combined strategy for the overproduction of complex ergot alkaloid agroclavine. *Synth Syst Biotechnol*. (2022) 7:1126–32. doi: 10.1016/j.synbio.2022.08.003

96. Lim LR, Wong G, Go MK, Yew WS. Increasing lysergic acid levels for ergot alkaloid biosynthesis: directing catalysis via the F-G loop of clavine oxidases. *Front Microbiol*. (2023) 14:1–10. doi: 10.3389/fmicb.2023.1150937

97. Hofmann A., Troxler F. 1-substituted (+)-lysergol. U.S. patent no 3,232,942 (1966). United States Patent Office

98. Kim K, Che T, Panova O, DiBerto JF, Lyu J, Krumm BE, et al. Structure of a hallucinogen-activated Gq-coupled 5-HT2A serotonin receptor. *Cells*. (2020) 182:1574–1588.e19. doi: 10.1016/j.cell.2020.08.024

99. Yuan H, Guo Z, Luo T. Synthesis of (+)-lysergol and its analogues to assess serotonin receptor activity. *Org Lett*. (2017) 19:624–7. doi: 10.1021/acs.orglett.6b03779

100. Tasker NR, Wipf P. A short synthesis of ergot alkaloids and evaluation of the 5-HT1/2 receptor selectivity of lysergols and isolysergols. *Org Lett*. (2022) 24:7255–9. doi: 10.1021/acs.orglett.2c02569

101. Tasker NR. Photoflow preparation of PTP4A3 inhibitors, and concise total syntheses of ergot and clavine alkaloids. *PhD Dissertation* (2023). Pittsburgh (PA): University of Pittsburgh

102. McCabe SR, Wipf P. Eight-step enantioselective total synthesis of (−)-cycloclavine. *Angew Chem Int Ed*. (2017) 56:324–7. doi: 10.1002/anie.201608820

103. Bartoccini F, Regni A, Retini M, Piersanti G. Asymmetric total synthesis of all rugulovasine stereoisomers and preliminary evaluation of their biological properties. *Eur. J Org Chem*. (2022) 2022:e202200315. doi: 10.1002/ejoc.202200315

104. McCabe SR, Wipf P. Asymmetric total synthesis and biological evaluation of (+)-cycloclavine. *Synthesis*. (2019) 51:213–24. doi: 10.1055/s-0037-1610395