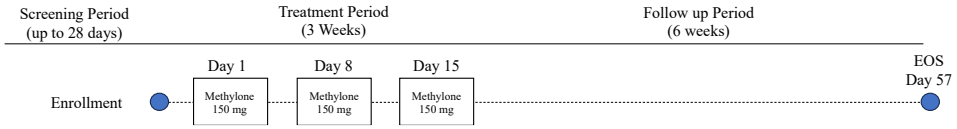
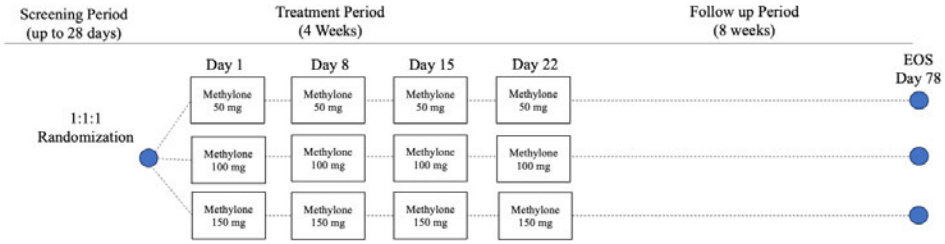
Protocol and Statistical Analysis Plan
Protocol #: TSND201-PTSD-202
Protocol Version Date: April 5, 2025
NCT#: NCT06215261

1 SYNOPSIS

Product Name / Number	TSND-201 / methylone
Protocol Number	TSND201-PTSD-202
Study Title	An Evaluation of the Safety and Efficacy of Methylone for the Treatment of PTSD IMPACT-2 (Investigation of Methylone for Post-Traumatic Stress Disorder [PTSD])
Indication	Post-Traumatic Stress Disorder (PTSD)
Objectives	Part A • To evaluate the efficacy of three doses of methylone, with each dose separated by 1 week, in participants with PTSD. • To evaluate the safety of three doses of methylone, with each dose separated by 1 week, in participants with PTSD. Part B • To evaluate the efficacy of three dose levels of methylone, given as 4 doses, with each dose separated by 1 week, in participants with PTSD. • To evaluate the safety of three dose levels of methylone, given as 4 doses, with each dose separated by 1 week, in participants with PTSD.
Study Design	This is a two-part study to evaluate the safety and efficacy of methylone for the treatment of PTSD. Part A is an open-label assessment in approximately 15 participants with PTSD; Part B is a randomized (1:1:1), single-blind assessment in approximately 45 participants. Selected site staff will be unblinded (eg, pharmacist) and participants, dosing session monitors and central raters will be blinded to the dose received in Part B. After completion of dosing in Part A, enrollment for Part B will begin. The open-label treatment in Part A is: • Methylone 150 mg x 3 doses, with each dose separated by 1 week. The three single-blinded treatments for Part B are: • Methylone 50 mg x 4 doses, with each dose separated by 1 week. • Methylone 100 mg x 4 doses, with each dose separated by 1 week. • Methylone 150 mg x 4 doses, with each dose separated by 1 week. Participants will be adults (age 18 to 70, inclusive) who meet the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) criteria for current PTSD, with a symptom duration of at least 6 months at Screening. Eligible participants will have severe PTSD symptoms (based on a Clinician-Administered PTSD Scale for DSM-5 [CAPS-5] total severity score of ≥32 at Screening). Eligible participants will attend the following study visits: • Screening visit (Day -28 to Baseline): Informed consent and eligibility assessments. For participants requiring additional time to washout of prohibited medications, the screening period can be up to 8 weeks. • Baseline/Preparatory Session (Day -4 to Day -1): Baseline assessments, confirmation of eligibility, and a preparatory psychoeducation session. • Treatment Period (Part A – 3 weeks, Part B – 4 weeks): Each participant will receive a dose of study drug at Day 1, Day 8, and Day 15 (also Day 22 for Part B). For each dose, the participant will remain in clinic for at least 8 hours after the dose and until all effects (physical and psychological) have resolved (whichever is longer). Each dose session will be followed by a safety phone call 1 day after dosing and efficacy assessments 2 days after dosing. Each dosing session may be recorded (eg, video and audio) for quality and training purposes. The recordings may be reviewed to ensure the dosing session monitor is adhering to their training. • Follow-Up Period (Day 22 to Day 57 for Part A; Day 36 to 78 for Part B): Following the final dosing session, follow-up visits will occur weekly for Part A

	and biweekly for Part B. This will include 4 efficacy and safety assessments post-final study drug administration (Part A and Part B) and 2 telephone contacts at 3 and 5 weeks post-final study drug administration (Part A). Planned enrollment includes up to 60 participants (approximately 15 in Part A and approximately 45 in Part B). Participants in Part B will be stratified based on antidepressant medication use at Screening (e.g., participants undergoing tapering of antidepressant medications vs. those not receiving an antidepressant medication at Screening). In Part A, attempts will be made to enroll <50% of participants requiring an SSRI/SNRI washout. **Study Design Part A**  **Study Design Part B** 
Test Treatment	Part A: Methylone, 150mg, given orally during 3 dosing sessions, each dose separated by 1 week. Part B: Methylone, 50 mg, 100 mg or 150 mg, given orally during 4 dosing sessions, each dose separated by 1 week.
Dosing Regimen	Each participant will receive 3 doses (Part A) or 4 doses (Part B) of study drug at the following intervals. For Part B, the treatment the participant is randomized to at Day 1 will be the treatment administered at each study visit. Part A (open-label) • Day 1, 8, and 15: Methylone 150 mg (50mg capsules x3) Part B (single-blinded) • Day 1, 8, 15, and 22: Methylone 50 mg (50 mg capsules x1; placebo capsule x2), methylone 100 mg (50 mg capsules x2; placebo capsule x1), or methylone 150 mg (50 mg capsules x3)
Safety Evaluations	Safety will be evaluated by the following assessments: • Adverse events • Vital signs (blood pressure [BP], heart rate, respiratory rate, temperature) • Clinical labs (chemistry, hematology, urinalysis) • Columbia-Suicide Severity Rating Scale (C-SSRS) • Electrocardiogram (ECG) • Brief Psychiatric Rating Scale (BPRS) • Clinician-Administered Dissociative States Scale (CADSS)
Efficacy Evaluations	Efficacy will be assessed by the following measures: • CAPS-5 (total severity score; primary efficacy outcome measure) • PTSD Checklist for DSM-5 (PCL-5) • Montgomery-Åsberg Depression Rating Scale (MADRS) • Sheehan Disability Scale (SDS) • Clinical Global Impression of Improvement (CGI-I) • Clinical Global Impression of Severity (CGI-S)

	• Patient Global Impression of Change (PGI-C) • Patient Global Impression of Severity (PGI-S) • Generalized Anxiety Disorder – 7 (GAD-7) • Quick Inventory Depressive Symptoms (QIDS) • Pittsburgh Sleep Quality Index (PSQI) • Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS)
Exploratory Evaluations	• Visual Analog Scales (VAS) • Post-Traumatic Growth Inventory (PTGI) • Dose-level assessment questionnaire (DAQ) • Pharmacokinetic Evaluation
Study Duration	The total study duration for an individual participant will be up to 13 weeks for Part A (up to 17 weeks if additional washout period is needed) or 16 weeks for Part B (up to 20 weeks if additional washout period is needed). Screening Period: up to 4 weeks (for participants requiring additional time to washout of prohibited medications, the screening period can be up to 8 weeks) Treatment Period: 3 weeks for Part A, 4 weeks for Part B Follow up Period: 6 weeks for Part A, 8 weeks for Part B
Inclusion Criteria	1. Male or female participants aged between 18 to 70 years, inclusive. 2. Participant meets the DSM-5 criteria for severe PTSD diagnosis, with a symptom duration of at least 6 months at Screening, as assessed by the CAPS-5. Note: The PTSD diagnosis will be confirmed at Baseline by the central rater. 3. Participant must have a CAPS-5 score of ≥32 at Screening and ≥28 at Baseline. 4. Participant has tried at least one treatment for PTSD (either psychotherapy or pharmacological treatment). 5. Proficient in reading and writing in English language sufficient to complete questionnaires. 6. Free from any other clinically significant illness, disease, or social circumstance (eg, ongoing trauma) that may adversely affect the safety of the participant, or, the integrity of the study (in the opinion of the Investigator and Medical Monitor). 7. Willing to refrain from taking any psychiatric medications or interventions during the study, unless provided by the Investigator. Note: No participant will be asked to discontinue potentially beneficial treatment in order to participate in this study. 8. Willing to provide a contact for emergencies. 9. Willing to be contacted via telephone for all necessary telephone visit. 10. Ability to swallow and retain oral medications. 11. Males who engage in sexual activity that has the risk of pregnancy must agree to use one of the following acceptable contraceptive methods and agree to not donate sperm throughout the study and for at least 90 days after the last dose of the study medication: a. Use of a double barrier method (male condom with a diaphragm or a cervical cap) b. Female use of hormonal contraceptives (as described below) c. Either partner is surgically sterilized (as described below) d. Abstinence, if consistent with the participants preferred and usual lifestyle 12. Females of childbearing potential* must be willing to use one of the following acceptable contraceptive methods throughout the study and for at least 1 month after the last study drug administration. Additionally, they must not be pregnant, must not be breastfeeding and must not intend to breastfeed throughout the study. a. Hormonal contraceptives or intrauterine devices: at least 4 weeks before first dosing and must follow that product's package insert instructions including additional protection at times when hormonal contraceptive doses might be missed b. Male condom with a diaphragm or cervical cap: consistent use for at least 21 days prior to the first dosing

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	c. Sterile male partner: vasectomized for at least 6 months prior to the first dose d. Abstinence, if consistent with the participant's preferred and usual lifestyle * A non-childbearing female is defined as: • Post-menopausal female (absence of menses for 12 months prior to drug administration, bilateral oophorectomy or hysterectomy with bilateral oophorectomy at least 6 months prior to drug administration); OR • Surgically sterile female (hysterectomy or tubal ligation at least 6 months prior to drug administration) Women of childbearing potential (WOCBP; see Appendix 1) must have a negative highly sensitive pregnancy test at Screening and Day 1, and prior to dosing. If a urine test cannot be confirmed as negative (e.g., an ambiguous result), a serum pregnancy test is required to be confirmed as negative prior to dosing. 13. Agree to not participate in any other interventional clinical trials for the duration of this study. 14. Willing to provide written informed consent, which includes compliance with the requirements and restrictions listed in the consent form. 15. Is able to understand the procedures and study requirements, is able to appropriately report on their PTSD symptoms, and agrees to abide by the study restrictions and return for the required study assessments.
Exclusion Criteria	1. Participant has a primary diagnosis of any other DSM-5 disorder, as assessed by the Mini International Neuropsychiatric Interview (MINI). 2. Participant has a body mass index (BMI) <18 kg/m^2 or ≥40 kg/m^2. 3. Participant is known to abuse illegal drugs or meets DSM-5 criteria for moderate or severe substance use disorder (mild substance use disorder, or moderate substance use disorder in early remission, may be allowed, after consultation with the sponsor, if use of the substance is unlikely to confound the study results) or has a positive urine test for illegal drugs at Screening, Baseline, or prior to dosing on Day 1. The Investigator must confirm the participant is not otherwise impaired prior to dosing on Day 1. 4. Known history of: Hepatitis B, Hepatitis C, or human immunodeficiency virus. 5. Participant smokes an average of >10 cigarettes and/or e-cigarettes per day. 6. Abnormal liver function tests (LFTs) at Screening defined as: a. AST, ALT or gamma-glutamyl transferase (GGT) >2 × the ULN, OR, b. Total bilirubin levels >1.5 × the ULN (participants with a diagnosis of Gilbert's syndrome with high unconjugated bilirubin are eligible provided they meet other LFT criteria). 7. Moderate to severe renal impairment at Screening, with an estimated glomerular filtration rate (eGFR) of ≤ 80 mL/min according to the Cockcroft-Gault equation. 8. Active suicidal ideation and/or intent within 2 months of Screening, indicated by a 'Yes' response to suicidal ideation question 4 or 5 on the C-SSRS. 9. Any history of suicidal behavior within the last 5 years (excluding suicidal ideation): a. Medical history of suicide attempt, or b. 'Yes' response to the following suicidal behavior questions on the C-SSRS: actual, interrupted, or aborted attempt. 10. Uncontrolled hypertension at Screening, defined as: a. SBP >140 mmHg, or b. DBP >90 mmHg. c. Resting heart rate at Screening of ≥90 bpm. 11. Have any history, physical or psychological symptoms, medication or other relevant findings that would make a participant unsuitable for the study based on the clinical judgement of study personnel. This would include (but is not limited to): a. A need to continue active psychotherapy or pharmacotherapy during the study period.

	b. Moderate or severe alcohol use disorder (as assessed by the MINI) OR the inability to refrain from alcohol use for 24 hours before Screening and each scheduled dose session. Note: Moderate alcohol use disorder in early remission is not exclusionary. c. Medical or psychiatric condition that is incompatible with establishment of rapport with the dosing session monitor, or safe exposure to study drug. 12. Prior treatment within the 90 days prior to Baseline, with: a. deep brain stimulation, b. vagus nerve stimulation, c. treatment with electroconvulsive therapy, or d. transcranial magnetic stimulation. 13. Use of a psychedelic (e.g., lysergic acid diethylamide [LSD], psilocybin, dimethyltryptamine [DMT], mescaline), or entactogens such as MDMA, within 12 months of Screening. 14. Use of prohibited concomitant medications or therapies. 15. Current or previous history of clinically significant cardiovascular / cerebrovascular conditions, including, but not limited to: a. Presence of underlying cardiovascular or cerebrovascular conditions where an acute rise in blood pressure would pose a clinical concern, including but not limited to, aneurysms or arteriovenous malformations, a history of cardiac or cerebral ischemia. b. Preexisting valvulopathy or pulmonary hypertension. c. History of clinically significant cardiac arrhythmias or cardiac abnormalities that could be exacerbated by increased heart rate or blood pressure. 16. Any clinically significant abnormal 12-lead ECG findings at Screening or other potential risk for QT prolongation, including, but not limited to: a. Resting QT interval corrected for heart rate using Fridericia's formula (QTcF) ≥450 msec (≥470 msec for females) at Screening, or inability to determine QTcF interval. b. Presence of risk factors for Torsades de Pointes. c. Congenital prolongation of the QT interval, family history of prolonged QT syndrome, or other risk factors for prolonged QT interval (including symptomatic bradycardia, hypokalemia, hypomagnesemia, or hypocalcemia). 17. Any history of head injury with loss of consciousness for more than 30 minutes within 12 months of Screening. 18. Clinically relevant history of schizophrenia, psychotic disorder, bipolar disorder, delusional disorder, paranoid personality disorder, antisocial personality disorder, schizoaffective disorder, borderline personality disorder or panic disorder, as assessed by the MINI and SCID-5-SPQ (and SCID-5-PD, if needed). 19. Have a first-degree relative with: a. schizophrenia spectrum, OR b. other psychotic disorders (except substance/medication-induced or due to another medical condition), OR c. bipolar I disorder. 20. Participant has participated in a clinical study with an unapproved investigational medication within 3 months, or 5 half-lives (whichever is longer), prior to Screening. 21. Has an allergy or intolerance to any of the materials contained in the investigational formulations (methylone or placebo capsules [Part B]). 22. Donation of blood or plasma of >400 mL within 3 months prior to Screening. 23. Family member of a member of the investigating team or Sponsor. 24. Any history of seizures, serotonin syndrome, or psychosis. 25. Previous exposure to TSND-201. 26. Family history of cardiac death from presumed or proven arrythmia.

Concomitant Medications	The following medications are prohibited within 14 days, or 5 half-lives, whichever is longer of Day 1: • Any chronically administered serotonergic medications or supplements (eg, SSRIs, SNRIs, tricyclic/tetracyclic antidepressants, 5 hydroxy-tryptophan or St. John's wort). NOTE: medications used on an as-needed basis (eg, anti-migraine medications) may be allowed pending review by the Medical Monitor. • Other medications to treat PTSD • Lithium and other mood stabilizers • Antipsychotics • Stimulants • Efavirenz • Strong CYP2D6 inhibitors or inducers • QT-prolonging medications (such as antiarrhythmics and some antibiotics) • CNS-acting medications unless as-needed use reviewed by the Medical Monitor Note: Participants who are receiving a prohibited medication at Screening may be tapered off the medication. The Screening period can be extended (up to 8 weeks) to allow for adequate time to taper off medications. The following are restricted within 24 hours of a dosing session: • Benzodiazepines (also within 24 hours of a central rater call) • Opioids • Alcohol
Analysis Populations	• Safety population – all participants who received at least one dose of study medication. • Intent-to-treat (ITT) population – all participants who receive at least one dose of study drug. • Per protocol population – all participants in the ITT population who received at least one dose of study drug and have no important major protocol deviations
Sample Size	No formal sample size calculation was performed for the study. A sample size of approximately 15 participants per dose group is based on medical and clinical considerations.
Statistical Considerations	All continuous efficacy endpoints will also be summarized by descriptive statistics (n, arithmetic mean, SD, median, minimum and maximum) by study part, overall, by treatment (for Part B) and study period. All categorical efficacy endpoints will be summarized by descriptive statistics (n, percentage). [redacted]