Route of Administration (e.g., Intraperitoneal Injection, Inhalation, Weight, Age) and Anesthetic Dosage or Dose. Additionally, provide the euthanasia method performed on the mice at the end of the study.

1. Establishment and Treatment of a Mouse Model of Alcohol Addiction

1.1 Route of Administration: Oral gavage
1.2 Administration Method:
1) Alcohol Addiction Induction: Two water bottles were placed in each cage, one containing pure water and the other containing 10% ethanol (v/v). Mice had free access to both bottles. Bottle positions were alternated daily, and consumption was measured by weighing the bottles. This continued for 3 weeks (days 0–21).
2) Post-Induction Treatment: Female mice from the induction phase were randomly divided into four groups (n=8): Control group, Model group, BI low-dose group, and BI high-dose group (0.1 and 1 mg/kg, p.o.). Treatment groups received oral administration once daily for 3 weeks (days 21–42). Ethanol and water consumption were measured daily, and individual ethanol intake and preference were calculated.
3) Drink-in-Dark (DID) Test: The Drink-in-Dark paradigm, widely used to model the binge-drinking stage of addiction, was employed to assess binge-like alcohol consumption. The DID test commenced on day 43, following the completion of the 42-day TBC test. After 4 hours of darkness, water bottles were removed and replaced with a single bottle containing 20% ethanol for 4 hours. Drug or vehicle treatment was administered 30 minutes prior to bottle presentation. Bottle weight before and after the session and mouse body weight were recorded. This procedure was repeated for 3 consecutive days.

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Ethanol intake (g/kg) was calculated as: (Ethanol consumed × 10% ethanol ma ss fraction) / Body weight, unit: g/kg.

Ethanol preference was calculated as: Ethanol solution consumption / (Ethanol solution consumption + Water consumption) × 100%.

1.3 Experimental Animals: 8–10-week-old female and male C57BL/6J mice (body weight 19-21 g)
1.4 Euthanasia Method: Cervical dislocation

2. Establishment and Treatment of a Mouse Model of Acute Alcohol Intoxication

2.1 Route of Administration: Intraperitoneal injection
2.2 Alcohol Induction Protocol: Intraperitoneal injection of a 2.5 g/kg alcohol solution (20%, v/v).
2.3 Administration Method: After acclimation, all mice were randomly divided into four groups: Vehicle (Control) group (n=6), EtOH (2.5 g/kg) Model group (n=9), EtOH + BI (1 mg/kg) low-dose treatment group (n=9), and EtOH + BI (3 mg/kg) high-dose treatment group (n=9). Following one week of acclimation, oral gavage

treatments were administered once every 24 hours. The Model and Control groups received the vehicle. The EtOH + BI (1 mg/kg) and EtOH + BI (3 mg/kg) groups received their respective drug doses. Thirty minutes after the third treatment, the Vehicle group received an intraperitoneal injection of saline, while the other three groups received an intraperitoneal injection of EtOH (2.5 g/kg). The duration of alcohol-induced loss of righting reflex (sleep time) was recorded, followed by open field test behavioral assessment to evaluate locomotor activity.

2.4 Experimental Animals: 8–10-week-old female C57BL/6J mice

2.5 Euthanasia Method: Cervical dislocation