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# Antidepressant-like activity of adhyperforin, a novel constituent of *Hypericum perforatum L.*

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Adhyperforin is a novel constituent of *Hypericum perforatum L.*, but its antidepressant-like activity remains unclear. To explore that, several well-validated animal models of depression as well as neurotransmitter reuptake and transporter binding assays were conducted. The results showed adhyperforin could reduce the immobility time of mice in the forced swimming test and tail suspension assay, antagonize the behaviors induced by reserpine, and have no effect on locomotor activity. Furthermore, following establishment of a chronic unpredictable mild stress model, adhyperforin increased the number of crossings and rearings in rats in the open field test and increased the sucrose consumption. Finally, adhyperforin inhibited uptake of serotonin, norepinephrine, and dopamine, and displayed robust binding affinities for the serotonin and norepinephrine transporters. Overall, the current study provides the first evidence that adhyperforin is a novel, active ingredient of *Hypericum perforatum L.* with robust antidepressant-like activity.

Major depressive disorder (MDD) is a mental disorder characterized by episodes of all-encompassing low mood accompanied by low self-esteem and loss of interest or pleasure in normally enjoyable activities[1]. By 2020, it is estimated that MDD will be the second highest disease burden worldwide, which will impair people's quality of life, reduce productivity, and increase disability and mortality[2,3]. Several classes of antidepressants have been approved to treat MDD, but they are not satisfactory because of a variety of side effects and the poor therapeutic effectiveness[4,5]. Recently, botanical agents, such as Cimicifugaracemosa, Chaihushugansan, Cimicifuga foetida L., and Hypericum perforatum, have been reported to produce robust antidepressant-like effects in clinical and preclinical studies, with lower side-effect profiles than standard antidepressants[6~9].

*Hypericum perforatum L* (*H. perforatum*), known as St. John's wort (SJW), is widely used for the treatment of mild to moderate MDD. Clinical studies have demonstrated that *H. perforatum* extracts could show similar efficacy against moderate depression compared with tricyclic antidepressants and selective serotonin reuptake inhibitors, in which *H. perforatum* extracts produced fewer side effects and had a lower economic burden[8,10]. It is well known that *H. perforatum* extracts contain a wide variety of constituents, such as naphthodianthrones, phenylpropanes, flavonol derivatives, biflavones, proanthocyanidines, phloroglucinols, different amino acids and essential oil constituents, which might play a role in their antidepressant activity[11].

Recently, the hyperforin, one phloroglucinol derivative, has gained interest as a major monoamine uptake-inhibiting constituent of *H. perforatum* extract. It was well documented that hyperforin inhibited the synaptic uptake of serotonin (5-HT), norepinephrine (NE) and dopamine (DA), and hyperforin and hyperforin-enriched extracts exerted antidepressant-like effects in animal models of depression[12,13]. One hyperforin-free extract, however, has also shown *in vivo* antidepressant activity, which indicates that the anti-depressive properties of *H. perforatum* extracts do not depend exclusively on the hyperforin[14]. In this study, we explored the antidepressant-like activity of adhyperforin, another phloroglucinol derivative[15], using several well-validated animal models of depression, including the tail suspension test (TST), forced swimming test (FST), and chronic unpredictable mild stress (CUMS) model, as well as its effects on monoamine uptake and its binding affinity for monoamine transporters.
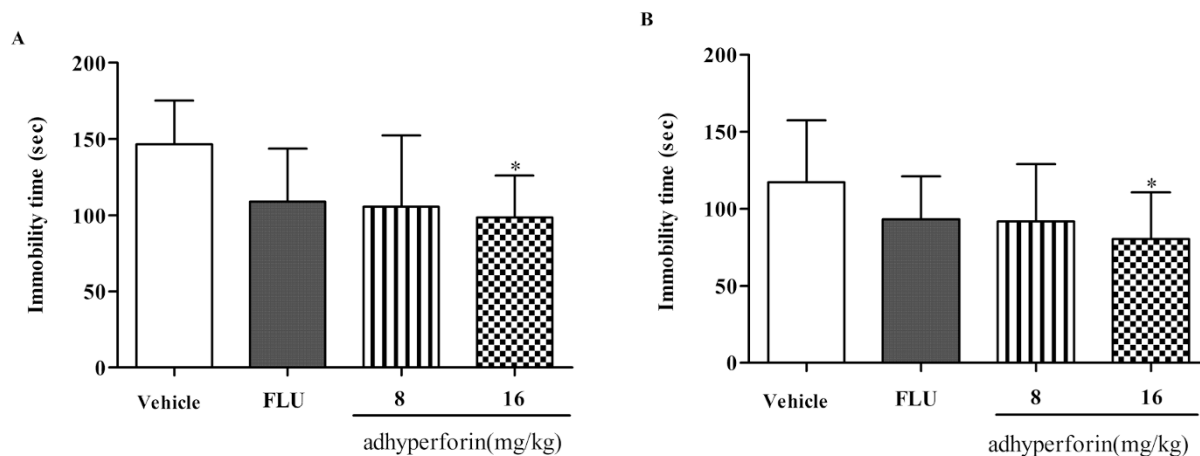
**Figure 1 | Effects of adhyperforin on the immobility times of mice in the forced swimming and tail suspension tests.** The animals were administered adhyperforin at doses of 8 and 16 mg/kg or FLU at 3.3 mg/kg by gavage, and then the immobility times of animals in the forced swimming test (A) and tail suspension test (B) were recorded. The data are presented as the mean ± SD (n = 8).

## Results

**Adhyperforin decreased the immobility of mice in the FST and TST.** The effects of adhyperforin on the immobility times of mice in the FST and TST were examined, and adhyperforin at doses of 16 mg/kg significantly decreased the immobility times of mice in the FST (Fig. 1A, $p = 0.049$). Similar results were observed in the TST, in which adhyperforin at a dose of 16 mg/kg significantly reduced the immobility times of mice (Fig. 1B, $p = 0.043$).

**Adhyperforin did not increase the locomotor activity of mice.** Next, the effects of adhyperforin on locomotor activity were explored using mice in the open field test (OFT), and adhyperforin had no significant effect on spontaneous locomotor activity in mice at any dose tested (Fig. 2, $p = 0.706$ and $p = 0.991$, respetively). Also, fluoxetine (FLU) at a dose of 3.3 mg/kg had no obvious effect on spontaneous locomotor activity ($p = 0.887$).

**Adhyperforin antagonized reserpine-induced ptosis, hypothermia, and akinesia.** The effects of adhyperforin on reserpine-induced ptosis, hypothermia and akinesia were determined in mice. As shown in Table 1, the animals administered reserpine at a dose of 4.0 mg/kg showed marked alterations of ptosis, hypothermia,
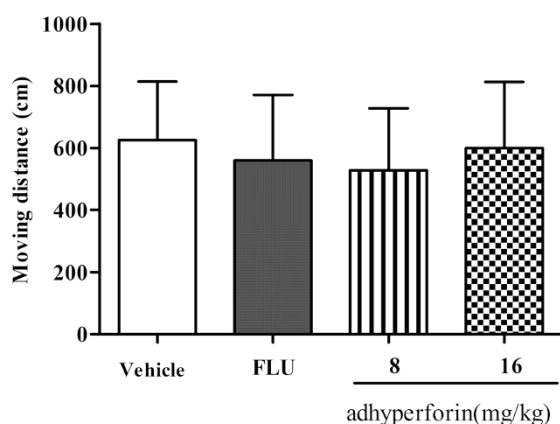


**Figure 2 | Effects of adhyperforin on spontaneous locomotor activity in mice.** The animals were administered adhyperforin at doses of 8 and 16 mg/kg or FLU at 3.3 mg/kg by gavage, and the OPT was used to detect the effects of the tested compounds on spontaneous locomotor activity. The data are presented as the mean ± SD (n = 8). *: $p < 0.05$, compared with the vehicle group.

and akinesia. However, administration of adhyperforin at 8–16 mg/kg for 7 days significantly antagonized these symptoms induced by reserpine. At the same time, FLU at 3.3 mg/kg also antagonized all these effects.

**Adhyperforin administration alleviated decreased sucrose intake in CUMS rats.** The sucrose intake assay is a popular experiment to detect the effect of drugs on the anhedonia in the animal models of depression. Sucrose intake volumes of CUMS-model rats were significantly less than those of the control group (Fig. 3, $p = 0.030$) after 3 weeks of consecutive CUMS. The administration of adhyperforin at 8 and 16 mg/kg, however, increased the sucrose volume consumed by CUMS animals ($p = 0.004$ and $p = 0.0001$, respectively), which was similar to the dramatic increase found in the FLU-treated group ($p = 0.0001$).

**Adhyperforin administration increased the numbers of crossings and rearings in CUMS rats.** Next, the OFT was conducted to explore the anxiolytic effects of adhyperforin. The numbers of crossings and rearings in CUMS-model rats were significantly less than those in the control group (Fig. 4, $p = 0.002$ and $p = 0.042$, respectively). Adhyperforin at doses of 8–16 mg/kg increased the numbers of crossings ($p = 0.118$ and $p = 0.207$, respectively) and rearings ($p = 0.059$ and $p = 0.056$, respectively) to a certain degree and was in a dose-dependent manner. At the same time, FLU at a dose of 3.3 mg/kg also significantly increased the numbers of crossings and rearings ($p = 0.014$ and $p = 0.011$, respectively).

**Adhyperforin inhibited the uptake of 5-HT, NE and DA in rat synaptosomes.** The effects of adhyperforin on the reuptake of 5-HT, NE, or DA were conducted using radio-labeled neurotransmitters. The results showed that adhyperforin potently blocked the uptake of [3H] 5-HT, [3H] NE or [3H] DA into the synaptosomes prepared from the different regions of rat brain (frontal cortex, hypothalamus or striatum, respectively). The $IC_{50}$ values for 5-HT, NE, or NA were $4.14 \pm 0.29$ μg/ml, $2.64 \pm 0.35$ μg/ml and $0.89 \pm 0.07$ μg/ml, respectively (Fig. 5).

**Adhyperforin bound to hSERT and hNET, but not hDAT.** The binding affinities of adhyperforin to hSERT, hNET or hDAT were detected using radioligand binding assay. As shown in the Fig. 6, adhyperforin had strong binding affinities to the hSERT and hNET with Ki values of $18.75 \pm 7.76$ μg/ml and $4.03 \pm 0.37$ μg/ml, respectively. However, no binding affinity was observed for adhyperforin to hDAT at the concentration of 50 μg/ml.

**Table 1 | Effects of adhyperforin on the reserpine-induced ptosis, hypothermia and akinesia in mice (n = 8)**

| Treatment | Dose (mg/kg) | Reserpine (4.0 mg/kg) | Ptosis (%) | Mean rectal temperature | Akinesia (%) |
|---|---|---|---|---|---|
| Vehicle | - | - | 0 | $36.83 \pm 0.54$ | 0 |
| Model | - | + | 95* | $34.59 \pm 1.23$# | 91* |
| Adhyperforin | 8 | + | 38# | $35.84 \pm 1.14$* | 14# |
| | 16 | + | 9.5# | $35.45 \pm 0.89$ | 25# |
| Fluoxetine | 3.3 | + | 20# | $35.39 \pm 0.39$ | 57# |

*$p < 0.05$ compared with vehicle group.
#$p < 0.05$, compared with model group ($\chi^2$-test).

## Discussion

Major depression disorder is a life-threatening mental illness with a high prevalence in the population, in which the quality of life can be significantly impacted[10]. The current state of therapy, which could improve the symptoms of depression but with poor tolerability, still cannot meet the medical needs of the patient population[16]. Botanical agents, such as C. racemosa and Cimicifuga foetida, have recently sparked interest because of their efficiency and safety[7,9]. In this study, we report for the first time that adhyperforin is a novel active constituent of *Hypericum perforatum L.* that displays robust antidepressant-like activity in several validated animal models of depression, which might be related to its inhibition of reuptake of 5-HT, NE, and DA.

*Hypericum perforatum L.*, also known as St. John's wort, has been shown to improve the symptoms of mild to moderate depression and is widely used throughout the world[17]. Some clinical and non-clinical experimental studies show that *H. perforatum*, like conventional antidepressants, potently inhibits the synaptosomal uptake of 5-HT, NE, and DA, in which hyperforin is regarded as the major active ingredient[18,19]. Recently, removal of hyperforin from the *H. perforatum* extract, however, did not result in the loss of its antidepressant-like activity, which indicates that another compound within the extract may also have anti-depressant-like activity[14]. In the present study, we first explored the antidepressant-like activity of adhyperforin using the FST and TST, which are two popular animal models that predict the efficacy of antidepressants. Our results clearly demonstrated that adhyperforin significantly decreased the immobility times of mice, and had no obvious effects on spontaneous locomotor activity, which indicates that adhyperforin has antidepressant properties.

The CUMS model of rat is a classic animal model of depression, in which animals are treated with a variety of unpredictable mild stressors to mimic the symptoms and pathogenesis of depression in humans[20]. The CUMS animals exhibited representative anhedonia, as indicated by decreased sucrose consumption and hypoactivity in OFT. In line with the findings of the TST and FST, administration of adhyperforin significantly improved anhedonia and hypoactivity in CUMS-model rats, which indicates that adhyperforin possesses antidepressant-like effects and may improve symptoms of depression.

To elucidate the mechanisms underlying the antidepressant-like activity of adhyperforin, the reserpine reversal test was conducted. As reserpine can irreversibly block the vesicular monoamine transporter and inhibit the vesicular uptake of monoamines, such as 5-HT, NE, and DA, the depletion of monoamine stores may stimulate the reuptake of monoamines and produce ptosis, hypothermia, and akinesia[21]. Chronic oral administration of adhyperforin (8–16 mg/kg) significantly antagonized the clinical observations induced by reserpine, which indicates that adhyperforin can inhibit the reuptake of the neurotransmitter and thereby increase the amount of monoamines in synaptic clefts.

Based on the findings, we used radioligand labeling methods to elucidate the effects of adhyperforin on the uptake of NE, 5-HT, and DA, as these transmitters play very important roles in the regulation of depression and are the targets of several approved antidepressants[22–24]. Consistent with previous reports[25,26], adhyperforin engendered concentration-dependent inhibitions of the uptake of [3H] NE, [3H] 5-HT, and [3H] DA in prepared synaptosomes, which indicates that adhyperforin may be a novel triple reuptake inhibitor. These results, however, were not consistent with the transported binding assay, in which adhyperforin showed high binding affinities for NET and SERT, but not for DAT. The exact mechanism for the inhibitory activity of adhyperforin on DA uptake remains unclear and should be further explored.

In summary, this study provides the first evidence that adhyperforin, a novel active ingredient of *Hypericum perforatum L.*, displays robust antidepressant-like activity in several validated animal models, which may be related to its inhibitory effects on the reuptake of NE, DA, and 5-HT. Collectively, all of these findings suggest that adhyperforin deserves further investigation as a potential antidepressant.
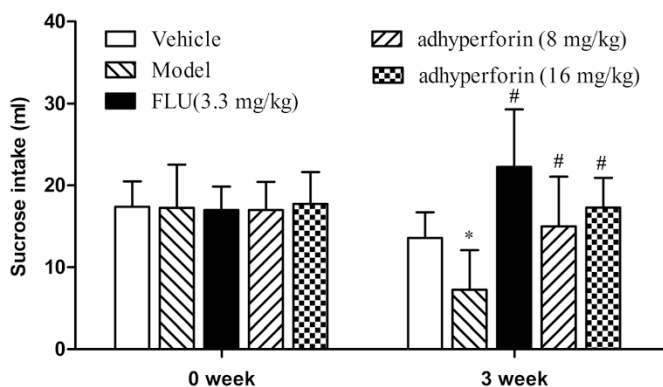
## Methods

**Figure 3 | Effects of adhyperforin on sucrose consumption in CUMS rats.** The CUMS model was established, the animals were treated with adhyperforin at doses of 8 and 16 mg/kg or FLU at 3.3 mg/kg by gavage, and then the sucrose consumptions of the animals were recorded and analyzed. The number of rats in each group was 12. The data are presented as the mean ± SD (n = 8). *: $p < 0.05$, compared with the control group; #: $p < 0.05$, compared with the CUMS-model group.

**Figure 4 | Effects of adhyperforin on the numbers of crossings and rearings by CUMS rats in the open field test.** The CUMS model was established, the animals were treated with adhyperforin at doses of 8 and 16 mg/kg or FLU at 3.3 mg/kg by gavage, and then the numbers of crossings and rearings of animals were observed. The data are presented as the mean ± SD (n = 8). *: $p < 0.05$, compared with the control group; #: $p < 0.05$, compared with the CUMS-model group.
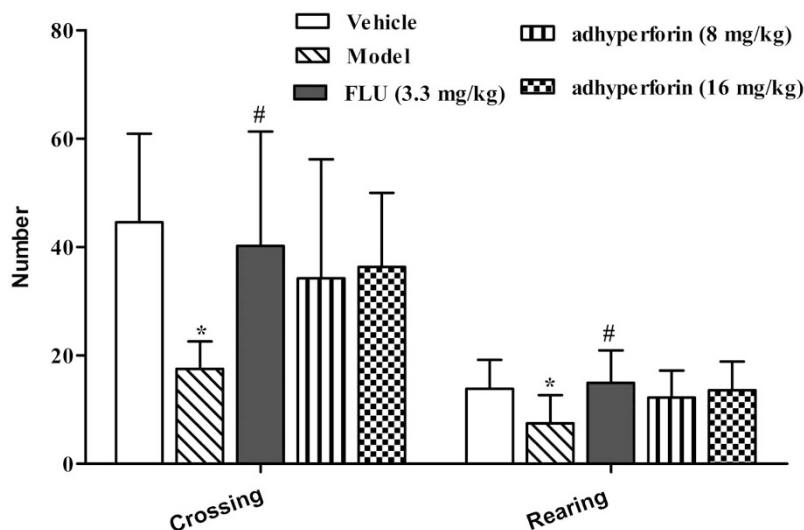
**FST in mice.** Thirty-two Swiss mice were randomly divided into four groups (8/group): The vehicle group, FLU group (3.3 mg/kg), and adhyperforin groups (8 and 16 mg/kg). The vehicle group was given an equal volume of vehicle to the other groups given different doses of the test compounds at 10 mL/kg. The animals were first administrated for the tested compounds for 6 days and fasted overnight with provision of water *ad libitum* before the test. On the 7th day, the animals were examined in the assay 1h post dosing according to published methods with minor modification[9]. Briefly, the mice were individually forced to swim in glass cylinders for 6 min (height: 20 cm, diameter: 10 cm) filled with 10 cm of water at 25°C. Each

animal was defined to be immobile whenever it remained floating in the water in an upright position and treading water with small movements to keep the head above water. The immobility times of the mice during the final 4 min of the 6-min test were recorded and analyzed.

**TST in mice.** Thirty-two Swiss mice were randomly grouped and administered the compounds under the same groupings as in the FST test. The protocol of the TST was adapted from a previously described method[27]. Briefly, the mice were suspended by a hook placed approximately 1 cm from the tip of the tail. The immobility time of the
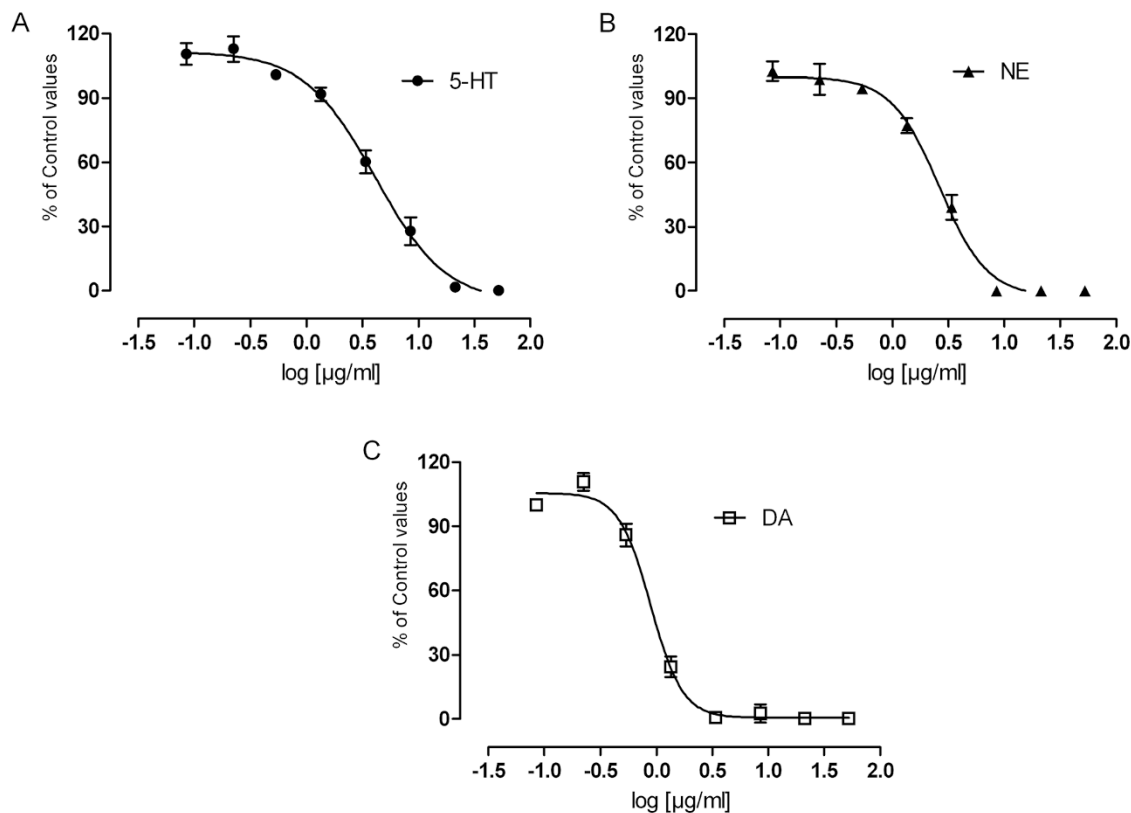


**Figure 5 | Effects of adhyperforin on the uptake of 5-HT, NE, and DA.** Synaptosomes were prepared and incubated with [3H] 5-HT, [3H] NE or [3H] DA in the absence or presence of the test or reference compounds. The samples were terminated and rinsed and then detected by a scintillation counter. The data are presented as the mean ± SD (n = 3).
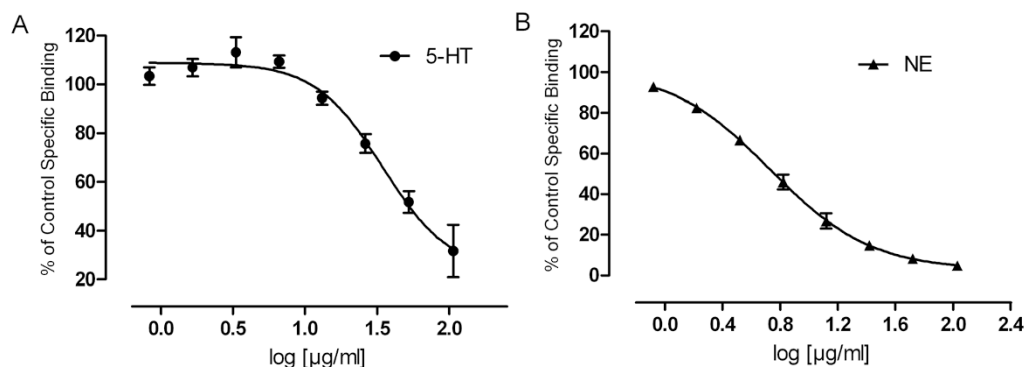
**Figure 6 | The binding affinities of adhyperforin for the hSERT, hNET and hDAT.** The cell membrane overexpressed hSERT, hNET, or hDAT were incubated with [3H] imipramine, [3H] nisoxetine, or [3H] BTCP, and then the samples were counted for radioactivity after complete rinsing. The data are presented as the mean ± SD (n = 3).

animals was recorded by a polygraph recorder during the final 4 min of the 6-min test and analyzed by a blinded observer.

**Locomotor activity in mice.** Thirty-two Swiss mice were randomly grouped and administered the compounds under the same groupings as in the FST test. The protocol to determine locomotor activity was adapted from a previously published method[28]. Briefly, the animals were individually placed in a custom-fabricated activity box (35 × 30 × 22 cm). The moving distance of the mice was recorded by a polygraph recorder during the final 6 min of the 10-min test and analyzed, in which the distance moved was the indicator of locomotor activity.

**The effects of adhyperforin on reserpine-induced ptosis, hypothermia, and akinesia.** The reserpine test was performed according to previously published methods[29]. Briefly, 40 Swiss mice were randomly grouped and administered the compounds under the same groupings as in the FST test. The mice were orally administered vehicle, FLU at 3.3 mg/kg, or adhyperforin at 8 or 16 mg/kg for 6 days. On the 7th day, the animals were injected with reserpine (4 mg/kg, i.p.) post-dose, and the rectal temperature was measured with a thermistor thermometer 1 h post injection. At same time, akinesia and ptosis were also observed, in which the animal was judged akinetic if they did not walk out of the edge of the disk within 15 seconds, and the animal was recorded to be ptosis if its eyes had one of the following reactions: one-quarter closed, half closed, or completely closed.

**Effects of adhyperforin on rat behavior and sucrose consumption in the CUMS model.** The rat CUMS model was developed according to a published method with minor modifications[30]. Briefly, the animals were first divided into two groups based on screening by the sucrose intake test: one group was exposed to various unpredictable stressors for 21 days, in which the animals were treated to three periods of food deprivation, water deprivation, overnight illumination, forced cold swimming (4°C, 5 min), exposure to an oven (45°C, 5 min), tail pinch (1 min), or horizontal shaking at a high speed (30 min). The other animals were housed in a separate room. Throughout the whole experiment, the test compounds were administered chronically once daily for 21 days. On day 23, with the exception of those that underwent the sucrose test, all animals were conducted the OFT, which was performed according to a previously described[31]. Briefly, the animals were placed in a black square plastic box (80 × 80 × 40 cm), in which the floor was divided into 25 equal squares with a black marker. After that, each animal was observed for 5 min and the number of crossings over the sector line with four paws and the number of rears with hind limbs on the floor during the 5-min period were recorded.

**Effects of adhyperforin on the uptake of 5-HT, NE, and DA.** The effects of adhyperforin on the uptake of 5-HT, NE, or NA were examined according to a published method[32,33]. Briefly, synaptosomes were prepared from the frontal cortex, hypothalamus, or striatum of the rat brain, and then the synaptosomes were incubated for 15 min at 37°C with 0.1 μCi [3H] 5-HT, 0.1 μCi [3H] NE or 0.1 μCi [3H] DA in the absence or presence of the test articles or reference compound. Basic control activity was determined in the presence of 10 μM imipramine, 10 μM protriptyline, or 1 μM GBR12909, respectively. The samples were terminated rapidly under vacuum using glass fiber filters (GF/B, Packard) and rinsed twice with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). After the filters were dried, the radioactivity of the samples in a scintillation cocktail (Microscint, Packard) was detected using a scintillation counter (Topcount, Packard), and the IC50 was calculated.

**The affinities of adhyperforin for hSERT, hNET, and hDAT.** The affinities of adhyperforin for hSERT, hNET and hDAT were determined according to previous report[34]. Briefly, cell membrane homogenates were prepared from CHO cells transfected with hSERT, hNET or hDAT, and then the transporters were incubated with [3H] imipramine, [3H] nisoxetine, or [3H] BTCP for hSERT, hNET or hDAT,

respectively. Nonspecific binding was determined by the presence of unlabeled 10 μM imipramine, 1 μM desipramine, or 10 μM BTCP, respectively. The samples were terminated rapidly under vacuum using glass fiber filters (GF/B, Packard) presoaked with PEI and rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters were dried and then counted for radioactivity, and the Ki value was calculated.

**Statistical analyses.** For in-vitro tests, the data were analyzed by LIGAND (Munson and Rodbard) to provide the IC50 and Ki values. For in-vivo studies, the values were expressed as mean ± standard deviation (mean ± SD). Differences were analyzed by Chi-square test or one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Significance levels were set at $p < 0.05$.

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## Author contributions

H.W. and J.T. designed the research and wrote the manuscript; F.Z. performed the majority of the experiments; F.Z., J.C., P.L. and S.G. supported several experiments. H.W. and J.T. contributed materials and revised the manuscript.

## Additional information

**Competing financial interests**: The authors declare no competing financial interests.

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