Involvement of adrenoceptors, dopamine receptors and AMPA receptors in antidepressant-like action of 7-O-ethylfangchinoline in mice

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Aim: 7-O-ethylfangchinoline (YH-200) is a bisbenzylisoquinoline derivative. The aim of this study was to investigate the antidepressant-like action and underlying mechanisms of YH-200 in mice.

Methods: Mice were treated with YH-200 (15, 30, and 60 mg/kg, ig) or tetrandrine (30 and 60 mg/kg, ig) before conducting forced swimming test (FST), tail suspension test (TST), or open field test (OFT).

Results: YH-200 (60 mg/kg) significantly decreased the immobility time in both FST and TST, and prolonged the latency to immobility in FST. YH-200 (60 mg/kg) was more potent than the natural bisbenzylisoquinoline alkaloid tetrandrine (60 mg/kg) in FST. Pretreatment with α₁-adrenoceptor antagonist prazosin (1 mg/kg), β-adrenoceptor antagonist propranolol (2 mg/kg), dopamine D₁/D₅ receptor antagonist SCH23390 (0.05 mg/kg), dopamine D₂/D₃ receptor antagonist haloperidol (0.2 mg/kg) or AMPA receptor antagonist NBQX (10 mg/kg) prevented the antidepressant-like action of YH-200 (60 mg/kg) in FST. In contrast, pretreatment with α₂ adrenoceptor antagonist yohimbine (1 mg/kg) augmented the antidepressant-like action of YH-200 (30 mg/kg) in FST. Chronic administration of YH-200 (30 and 60 mg/kg for 14 d) did not produce drug tolerance; instead its antidepressant-like action was strengthened. Chronic administration of YH-200 did not affect the body weight of mice compared to control mice.

Conclusion: YH-200 exerts its antidepressant-like action in mice via acting at multi-targets, including α₁, α₂ and β-adrenoceptors, D₁/D₅ and D₂/D₃ receptors, as well as AMPA receptors.

Keywords: depression; antidepressant; 7-O-ethylfangchinoline; tetrandrine; adrenoceptor; dopamine receptor; AMPA receptor; forced swimming test; tail suspension test

Introduction
Studies have demonstrated that numerous neural pathways are involved in the pathophysiology of depression[1]. The treatment of depression is one of the most challenging issues in contemporary psychiatry. Most of the currently available antidepressants harness monoaminergic mechanisms. The conventional and available antidepressants can provide a complete remission for only 50%-60% of the treated individuals[2], and it often takes more than 5-8 weeks for the patients to respond to the treatment. In addition, there is the presence of adverse effects[3]. Thus, pursuing new pharmacotherapy with elevated efficacy and fewer adverse side-effects is an utmost clinical need. Multi-targets have been emphasized as new antidepressant therapeutic strategies[4, 5]. The association of a clinically characterized antidepressant mechanism with a non-monoaminergic component of activity is an attractive strategy. For example, agomelatine (a melatonin agonist/5-HT₂C antagonist) has clinically proven activity in major depression[6].

It has been assumed that some bisbenzylisoquinoline (BBI) derivatives may possess potential antidepressant activity[7]. The total tertiary alkaloid fraction or hydroalcoholic leaf extract (containing warifteine, a type of BBI alkaloid) from Cissampelos sympodialis reduced the total immobility time in a forced swimming test (FST) and demonstrated an antidepressant effect[8]. Therefore, we hypothesized that 7-O-ethylfangchinoline (YH-200, Figure 1), a BBI derivative, may also possess antidepressant-like activity. In this study, we investigated the antidepressant-like effect of YH-200 in a mouse FST and tail suspension test (TST) and evaluated its possible mechanisms.

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Assays were conducted 24 h after YH-200 (15–60 mg/kg, ig) treatment. Different sets of mice were used in the TST, FST, OFT, and monoamines assay, and each mouse was used for only once.

In the experiments to investigate the drug interaction, mice were pre-treated with prazosin (1 mg/kg, ip), yohimbine (1 mg/kg, ip), propranolol (2 mg/kg, ip), SCH23390 (0.05 mg/kg, sc), haloperidol (0.2 mg/kg, ip) and NBQX (10 mg/kg, ip), respectively, and 15 min later animals received YH-200 (30 or 60 mg/kg, ig). The FST were performed after 60 min of YH-200 administration.

To investigate whether the drug resistance was developed, mice were repeatedly treated with YH-200 (30 and 60 mg·kg⁻¹·d⁻¹, ig) for 14 d. Sixty minutes after treatment on the last day, mice were subjected to the FST for 6 min. Body weight was measured daily before YH-200 administration.

**Forced swimming test**

The test was conducted using a slightly modified method described by Porsolt et al. Assays were conducted 24 h after a 15-min pretest session by placing the mice in transparent cylinders filled with water (25°C; diameter 10 cm, height 25 cm). The total duration of immobility during a 6-min test and the latency to the first bout of immobility were scored. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements that were necessary to keep its head above water. After the experiment, the mouse was removed from the cylinder, dried with a towel, and placed in its home cage to be dried under a heat lamp. The water in the cylinder was changed for each mouse.

**Tail suspension test**

The method is based on the fact that mice develop an immobile posture when they are suspended by their tail, which is an inescapable, short-term stressor. Briefly, mice were acoustically and visually isolated and then suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The immobility time was recorded for 6 min. Mice were considered to be immobile only when they hung passively and were completely motionless.

**Open field test (OFT)**

As described previously, locomotor activity was recorded and analyzed using a Mouse and Rat Spontaneous Activity Video Analysis System (JLBehv-LAG-4, Shanghai Jiliang Software Technology Co, Ltd). There were 4 adjacent enclosures (25 cm×25 cm×50 cm each) with video cameras on the ceiling. The activity of mice (separately in each enclosure) was recorded simultaneously for 6 min. Each enclosure was arbitrarily divided into a central region (12.5 cm×12.5 cm) and peripheral regions. The locomotor tracks in the open field were continuously recorded by a video camera and analyzed using a computer. After each testing session, the enclosures were thoroughly cleaned with 10% ethanol.

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**Materials and methods**

**Animals**

Male ICR mice (n=451; 20–25 g, purchased from the Animal Center of Peking University, Beijing, China) were used. The mice were maintained at 22–25°C with free access to water and food under a 12-h light/12-h dark cycle (lights on at 9:00 AM). All manipulations were carried out from 1:00 PM to 5:00 PM. All experiments were conducted in accordance with the European Community guidelines for the use of experimental animals and were approved by the Peking University Committee on Animal Care and Use.

**Drugs and treatment**

Tetrandrine and fangchinoline were isolated from the roots of the creeper Stephania tetrandrae S Moore of the Menispermaceae family. YH-200 was prepared from fangchinoline ethylation. Analysis of the ¹H NMR and ¹³CNMR spectra showed analytical and spectroscopic data that were in full agreement with these assigned structures. The chemical purities of these compounds (99.9%) were determined by high-performance liquid chromatography. Prazosin hydrochloride (an α₁-adrenoceptor antagonist), yohimbine hydrochloride (an α₂-adrenoceptor antagonist), propranolol (a β-adrenoceptor antagonist), haloperidol (a dopamine D₂/D₃ receptor antagonist) and imipramine were purchased from Sigma-Aldrich Co (St Louis, MO, USA); NBQX (an α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor antagonist) was purchased from Tocris Cookson Ltd (Bristol, UK). For administration, YH-200 and imipramine were dissolved in distilled water; haloperidol was dissolved in 5% ethanol/physiological saline. Other drugs were dissolved in saline. Acute and repeated (14 d) treatments were performed during the artificial lighting period. All drugs were administered in a constant volume of 10 mL/kg body weight.

In the experiments designed to verify the antidepressant-like effects of YH-200, mice were treated with vehicle, YH-200 (15, 30, and 60 mg/kg, ig) or imipramine (40 mg/kg, ig) as a positive control 60 min before the TST or FST. We also compared the effects of YH-200 (30 and 60 mg/kg, ig) with that of tetrandrine (30 and 60 mg/kg, ig) in the FST, and mice were treated in an identical manner. To further investigate whether the immobility time in the FST was related to alterations in locomotor activity, mice were subjected to an open field test (OFT) 60 min after YH-200 (15–60 mg/kg, ig) treatment.

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**Figure 1.** The structure of tetrandrine and 7-O-ethylfangchinoline (YH-200). Tetrandrine: R=CH₃; YH-200: R=CH₂CH₃.
HPLC-ECD analysis of monoamines

To investigate neurochemical mechanisms, 60 min after YH-200 (15–60 mg/kg, ig) administration, the mice were decapitated. The hippocampus, prefrontal cortex, striatum and hypothalamus of the mice were separated rapidly and stored at -80 °C for the monoamines assay. Brain tissues were then homogenized by ultrasound in 0.2 mol/L perchloric acid homogenate. The mixture was centrifuged twice (18 000×g) for 50 min at 4 °C. Levels of norepinephrine (NE), dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were detected by HPLC-ECD (Dionex UltiMate 3000, Thermo Scientific, Santa Clara, CA, USA) using a C18 reverse phase column (3.0 mm id×75 mm; Capcell Pak C18 MG S3, Shiseido, Japan). The mobile phase of the HPLC system contained 0.85 mmol/L sodium octylsulfate, 0.5 mmol/L EDTA, and 0.1 mmol/L NaH2PO4 in 11% methanol at pH 3.25. The flow-rate was 0.6 mL/min. The electrochemical detector was set at 200 MV for the oxidizing potential and -175 MV for the reducing potential. The injection volume was 20 µL.

Statistical analysis

All values are expressed as the mean±SEM. The differences between groups were analyzed by a two-way or one-way analysis of variance (ANOVA) followed by Student-Newman-Keul’s test as a post hoc comparison. Probability values less than 0.05 (P<0.05) were considered statistically significant. All statistical procedures were carried out using SAS Software (version 9.1, SAS Institute Inc).

Results

YH-200 exhibited an anti-immobility effect without the influence of locomotor activity

As shown in Figure 2, YH-200 (60 mg/kg, ig) significantly decreased the immobility time of mice in the FST (F(4,45)=9.94, P<0.0001; Figure 2A) and TST (F(4,36)=9.07, P<0.0001; Figure 2B). The OFT showed that YH-200 (15–60 mg/kg, ig) did not influence the locomotor activity of mice (total distance: F(3,36)=0.11, P=0.9560; distance in peripheral area: F(3,36)=0.08, P=0.9721; distance in central area: F(3,36)=0.17, P=0.9140; mean movement speed: F(3,36)=0.11, P=0.9560; Figures 2C and 2D). In the comparison test, YH-200 (60 mg/kg, ig) showed the same potency by prolonging the latency to immobility (F(4,45)=8.75, P<0.0001; Figure 3A) and shortening the immobility time (F(4,45)=22.96, P<0.0001; Figure 3B) in the FST. The antidepressant-like activity of YH-200 was more potent than its derivative tetrandrine (Figure 3).

Antidepressant-like effect of YH-200 involved multi-receptors

Role of the adrenergic α1, α2 or β receptor in the antidepressant-like activity of YH-200

As shown in Figure 4A, a two-way ANOVA revealed significant differences in pre-treatment (saline or prazosin; F(1,28)=54.18, P<0.0001), treatment (vehicle or YH-200; F(1,28)=77.38, P<0.0001), and the pre-treatment×treatment interaction (F(1,28)=42.10, P<0.0001) in immobility times in the FST. Post hoc analysis indicated that pre-treating the mice with prazosin (1 mg/kg, ip) significantly abrogated the decrease in the immobility elicited by YH-200 (60 mg/kg, ig) in the FST.

As shown in Figure 4B, a two-way ANOVA revealed significant differences in pre-treatment (saline or yohimbine; F(1,28)=17.09, P=0.0003), treatment (vehicle or YH-200; F(1,28)=15.34, P=0.0005), and the pre-treatment×treatment interaction (F(1,28)=5.23, P=0.03) in immobility times in the FST. Post hoc analysis indicated that the antidepressant-like effect of a sub-threshold dose of YH-200 (30 mg/kg, ig) was significantly enhanced by pre-treatment with yohimbine (1 mg/kg, ip).

As shown in Figure 4C, a two-way ANOVA revealed significant
differences in pre-treatment (saline or propranolol; $F_{(1,28)}=20.50$, $P<0.0001$), treatment (vehicle or YH-200; $F_{(1,28)}=16.86$, $P=0.0003$), and the pretreatment×treatment interaction ($F_{(1,28)}=21.65$, $P<0.0001$) in immobility times in the FST. Post hoc analyses indicated that the antidepressant-like effect of YH-200 (60 mg/kg, ig) was significantly attenuated by pre-treatment with propranolol (2 mg/kg, ip).

Role of the dopamine $D_1/D_5$ and $D_2/D_3$ receptors in the antidepressant-like activity of YH-200

As shown in Figure 4D, a two-way ANOVA revealed significant differences in pre-treatment (vehicle or SCH 23390; $F_{(1,28)}=35.30$, $P<0.0001$), treatment (vehicle or YH-200; $F_{(1,28)}=32.36$, $P<0.0001$), and the pre-treatment×treatment interaction ($F_{(1,28)}=28.77$, $P<0.0001$) in immobility times in the FST. Post hoc analyses indicated that pre-treating with SCH 23390 (0.05 mg/kg, sc) significantly abrogated the decrease in immobility times elicited by YH-200 (60 mg/kg, ig) in the FST.

As shown in Figure 4E, a two-way ANOVA revealed significant differences in pre-treatment (saline or haloperidol; $F_{(1,32)}=35.48$, $P<0.0001$), treatment (vehicle or YH-200; $F_{(1,32)}=36.96$, $P<0.0001$), and the pre-treatment×treatment interaction ($F_{(1,32)}=22.05$, $P<0.0001$) in immobility times in the FST. Post hoc analyses indicated that the antidepressant-like effect of YH-200 (60 mg/kg, ig) was significantly attenuated by pre-treatment with haloperidol (0.2 mg/kg, ip).

Role of the AMPA receptor in the antidepressant-like activity of YH-200

As shown in Figure 4F, a two-way ANOVA revealed significant differences in pre-treatment (saline or NBQX; $F_{(1,28)}=11.64$, $P=0.0020$), treatment (vehicle or YH-200; $F_{(1,28)}=6.50$, $P=0.0166$), and the pre-treatment×treatment interaction ($F_{(1,28)}=16.72$, $P=0.0003$) in immobility times in the FST. Post hoc analyses...
indicated that the antidepressant-like effect of YH-200 (60 mg/kg, ig) was significantly attenuated by pre-treatment with NBQX (10 mg/kg, ip).

Effects of YH-200 on the concentration of NE, DA, and DOPAC in the hippocampus, prefrontal cortex, striatum and hypothalamus

As shown in Table 1, YH-200 (30 and 60 mg/kg, ig) or imipramine (40 mg/kg, ig) significantly increased NE levels in the prefrontal cortex compared to the control group (P<0.01 for YH-200 30 mg/kg; P<0.01 for YH-200 60 mg/kg; P<0.01 for imipramine 40 mg/kg), and no significant differences in the DA and DOPAC levels were observed between the groups. In the striatum, YH-200 (60 mg/kg, ig) and imipramine (40 mg/kg, ig) significantly increased the NE levels (P<0.05 for YH-200 60 mg/kg; P<0.01 for imipramine 40 mg/kg) but did not influence the DA and DOPAC levels.

Table 1. Effects of YH-200 on the concentrations of NE, DA, DOPAC. Values are expressed as the mean±SEM. Data were analyzed by one-way ANOVA followed by the SNK test.

| Group          | Concentration (ng/g wet tissue) | NE    | DA      | DOPAC |
|----------------|--------------------------------|-------|---------|-------|
|                |                                |       |         |       |
|                | Prefrontal cortex              |       |         |       |
| Vehicle        |                                |       |         |       |
|                | 15 mg/kg                       | 372.0±18.7 | 79.3±26.6 | 88.5±19.4 |
| YH-200         |                                | 393.0±21.4 | 55.4±24.8 | 77.4±11.8 |
| 30 mg/kg       |                                | 944.6±34.3 | 51.8±27.3 | 69.3±10.5 |
| 60 mg/kg       |                                | 995.8±32.1 | 101.9±44.5 | 99.0±22.3 |
| Imipramine     |                                | 953.3±33.8 | 80.9±39.4 | 69.6±10.6 |
| 40 mg/kg       |                                | F_{(4,45)}=122.85 | F_{(4,45)}=0.38 | F_{(4,45)}=0.67 |
|                |                                | P<0.0001 | P=0.8230 | P=0.6166 |
|                | Hippocampus                    |       |         |       |
| Vehicle        |                                |       |         |       |
|                | 15 mg/kg                       | 506.4±26.0 | 51.4±5.5  | 41.7±2.0  |
| YH-200         |                                | 499.0±35.9 | 55.0±4.0  | 41.9±2.0  |
| 30 mg/kg       |                                | 485.2±30.6 | 50.2±4.0  | 41.9±3.0  |
| 60 mg/kg       |                                | 490.0±20.1 | 51.6±4.0  | 40.2±3.0  |
| Imipramine     |                                | 491.2±39.3 | 58.2±7.0  | 42.9±4.0  |
| 40 mg/kg       |                                | F_{(4,45)}=0.07 | F_{(4,45)}=0.40 | F_{(4,45)}=0.11 |
|                |                                | P=0.9902 | P=0.8073 | P=0.89766 |
|                | Striatum                       |       |         |       |
| Vehicle        |                                |       |         |       |
|                | 15 mg/kg                       | 235.4±7.0  | 5782.5±314.1 | 1173.0±85.0 |
| YH-200         |                                | 262.7±29.7  | 6367.5±536.3 | 1129.4±97.8 |
| 30 mg/kg       |                                | 285.1±14.2  | 5626.6±263.6 | 1024.0±54.6 |
| 60 mg/kg       |                                | 321.5±18.5  | 5718.4±315.4 | 1088.1±63.5 |
| Imipramine     |                                | 338.4±24.1  | 6148.8±379.5 | 1170.5±73.9 |
| 40 mg/kg       |                                | F_{(4,45)}=4.32 | F_{(4,45)}=0.71 | F_{(4,45)}=0.67 |
|                |                                | P=0.0048 | P=0.5886 | P=0.6179 |
|                | Hypothalamus                   |       |         |       |
| Vehicle        |                                |       |         |       |
|                | 15 mg/kg                       | 2391.6±158.5 | 296.3±17.9 | 190.9±16.7 |
| YH-200         |                                | 2412.1±98.2 | 373.8±73.4  | 240.3±33.8 |
| 30 mg/kg       |                                | 2395.1±129.0 | 324.1±32.8  | 218.5±12.3 |
| 60 mg/kg       |                                | 2405.8±117.6 | 340.7±36.8  | 205.7±21.5 |
| Imipramine     |                                | 2221.2±107.6 | 359.5±31.0  | 205.0±16.0 |
| 40 mg/kg       |                                | F_{(4,45)}=0.43 | F_{(4,45)}=0.50 | F_{(4,45)}=0.36 |
|                |                                | P=0.7891 | P=0.7349 | P=0.8376 |

*P<0.05, **P<0.01 vs control group. n=10/group.

Chronic (14 d) treatment of YH-200 did not reveal drug resistance

As shown in Figure 5A, 14-d administration of YH-200 significantly reduced the immobility time even at the lower dose of 30 mg·kg⁻¹·d⁻¹ and the 60 mg·kg⁻¹·d⁻¹ dose (F(2,27)=17.85, P<0.0001) in the FST. However, there were no significant differences in body weight among all groups (Figure 5B).

![Figure 5](image_url)

**Discussion**

The Porsolt forced swimming test, which is also known as the behavioral despair model, is a primary screening test for antidepressants[9, 14]. The basic idea of this model is that the co-administration of the test drug with specific receptor agonists or antagonists could be used to elucidate the mechanism of drug action[15]. The present study confirmed the antidepressant-like effect of YH-200 in the FST and TST. The oral administration of YH-200 exerted a significant antidepressant-like effect after acute treatment in mice, and this activity was more potent than its derivative tetrandrine. In addition, it is worth noting that 14-d administration of YH-200 did not develop drug resistance but instead revealed a more potent antidepressant effect at a lower dose than with acute treatment. In contrast to its BBI derivative tetrandrine, which has been reported to have a LD₅₀ value of 646 mg/kg (ig)[16], the LD₅₀ value of YH-200 60 mg/kg; P<0.01 for imipramine 40 mg/kg) but did not influence the DA and DOPAC levels.

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YH-200 was higher than 1 g/kg in rats (ig). These data suggested that YH-200 may be a safer and more potent antidepressant than tetrahydrodralin.

The monoaminergic system is one of the most important targets in the pathophysiology and treatment of depression\(^{[17, 18]}\). In this study, the antidepressant-like effect elicited by YH-200 was reversed by pretreatment with SCH23390 (a dopamine D\(_1\)/D\(_3\) receptor antagonist), haloperidol (a dopamine D\(_2\)/D\(_3\) antagonist), prazosin (an α\(_1\)-adrenoceptor antagonist), propranolol (a non-selective β-adrenoceptor antagonist) and NBQX (an AMPA receptor antagonist), but was augmented by yohimbine (an α\(_2\)-adrenoceptor antagonist).

Depression is associated with hypofunction of the noradrenergic system\(^{[19]}\), and there is substantial preclinical and clinical evidence that NE plays a key role in the etiology of depressive disorders\(^{[20-22]}\). Moreover, α\(_1\) and α\(_2\)-adrenoceptors have been shown to undergo some of the antidepressant-like effects of drugs observed in observational models of depression\(^{[23, 24]}\). In addition, the inhibition of α\(_1\) adrenoceptors mimics a depressive state, which, similar to chronic stress, is associated with α\(_1\) desensitization\(^{[25]}\). Furthermore, chronic antidepressant treatment gradually downregulates α\(_2\)-adrenoceptor autoreceptors and their level is elevated both in depressed patients and by long-term stress\(^{[26]}\). In pharmacological doses, yohimbine is not exclusively selective for the α\(_2\) receptor, as it has been reported to bind with moderate or weak affinity to other receptors in vitro, such as D\(_2\), α\(_1\), and 5-HT\(_{1A}\) receptors, albeit with 5- to 10-fold lower affinity\(^{[27]}\). However, in tracer concentrations, yohimbine is highly selective for α\(_2\) sites in vivo. Yohimbine can produce an excessive release of NE by antagonizing presynaptic α\(_2\)-adrenoceptors\(^{[28]}\). The data obtained in this study indicated that the NE contents in the prefrontal cortex and striatum were significantly elevated by YH-200. These results indicated that the NE-elevating activities of YH-200 and yohimbine may underlie the augmentative effect of yohimbine on YH-200 in the FST.

In addition, β-adrenoceptors seem to play a role in the mechanism of antidepressant therapies. Isoproterenol, which is a non-selective β-adrenoceptor agonist, caused an antidepressant-like effect. This effect of centrally administered isoproterenol was similar to the effects produced by the administration of proven antidepressant drugs. Propranolol antagonized the effect of centrally administered isoproterenol, which suggested that the antidepressant-like effect of this agonist was mediated by beta adrenergic receptors\(^{[29]}\). In the present study, pre-treatment with prazosin and propranolol was able to block the antidepressant-like effect of YH-200. These results suggested that the antidepressant-like effect of YH-200 in the FST may be dependent on the activation of postsynaptic α\(_2\) and β-adrenoceptors.

Dopamine receptors are divided into two groups, depending on their pharmacological and structural properties: the D\(_1\)-like dopamine receptors (D\(_1\) and D\(_3\)) and the D\(_2\)-like dopamine receptors (D\(_2\), D\(_3\), and D\(_4\)). The dopaminergic system is strongly implicated in the regulation of mood\(^{[30]}\). A deficiency of mesolimbic dopamine is a leading candidate for the etiology of certain symptoms of depression (eg, anhedonia and loss of motivation\(^{[31]}\)). Several previous reports have suggested that depression may often be accompanied by a relative hypodopaminergic state and that some DA receptor agonists have beneficial effects in the treatment of refractory and bipolar depression\(^{[32]}\). In addition to involvement in the pathophysiology of depression, dopaminergic neurotransmission is known to mediate the effects of antidepressants. It has been reported that the potentiation of dopaminergic neurotransmission induced by chronic antidepressant treatments might contribute to their therapeutic effect\(^{[33]}\). In humans, this role was confirmed by the benefit of adding DA receptor agonists to standard antidepressants for treating resistant depression\(^{[34]}\).

As shown in Figure 2, the dopamine D\(_1\)/D\(_3\) receptor antagonist SCH23390 and the dopamine D\(_2\)/D\(_3\) receptor antagonist haloperidol reversed the antidepressant effect elicited by YH-200. This result indicated that D\(_1\)/D\(_3\) and D\(_2\)/D\(_3\) receptors might play a role in the antidepressant-like effect elicited by YH-200. Moreover, it has been reported that dopamine D\(_1\)/D\(_3\) receptor stimulation is required for the rapid antidepressant-like effects of ketamine\(^{[35]}\). Thus, it should be hypothesized that YH-200 may rapidly exert its antidepressant effect.

Evidence from postmortem and in vivo brain imaging studies has implicated amino acid neurotransmitter systems in the pathophysiology of major depressive disorder\(^{[36]}\). A fast (within 2 h), robust, and relatively sustained antidepressant effect (lasting one to two weeks) was found after the infusion of ketamine in patients with treatment-resistant major depression\(^{[37]}\). There is emerging evidence that ketamine’s antidepressant properties rely on increased AMPA signaling and rapidly induced synaptogenesis\(^{[38]}\). However, ketamine has the potential of drug abuse and has been associated with cognitive impairments and alterations in brain imaging measures when abused for prolonged periods of time\(^{[39]}\). In addition, it is well known that ketamine has a short systemic half-life and is extensively metabolized in the liver, which results in a high first-pass effect and thus makes the drug unsuitable for oral delivery. Interestingly, studies have demonstrated that AMPA receptor agonists may also produce antidepressant effects\(^{[40, 41]}\). Thus, the development of promising novel agents that target the AMPA receptor is currently possible. The data obtained in the present study showed that the AMPA receptor may be involved in the antidepressive effects of YH-200; further, it could be presumed that YH-200 has rapid onset potential in treating depression patients. Interestingly, the AMPA receptor antagonist DNQX abolished the anti-immobility effect of ketamine in the TST, in agreement with reports in the literature that showed a similar effect using NBQX, but did not affect the anti-immobility effect of fluoxetine\(^{[42]}\). From these results, it could be presumed that YH-200 may perform its antidepressant effect as quickly as ketamine. In addition, this finding suggests that the antidepressant mechanism of YH-200 is different from that of fluoxetine.

Many researchers pay attention to the search for new compounds and treatment strategies for depression that could improve conventional therapies by utilizing multiple targets,
and natural product structure modifications are alternatives for the development of antidepressants with reduced or no adverse effects. The results presented herein demonstrated for the first time that YH-200 is able to produce an antidepressant-like effect. Moreover, the antidepressant-like effect of YH-200 seems to be mediated by an interaction with multi-targets including noradrenergic (α1, α2, and β receptors), dopaminergic (D1/D3 and D2/D3 receptors) and AMPAergic (AMPA receptor) systems. However, the direct actions of YH-200 with these receptors should be unraveled. Furthermore, pharmako-kinetic interactions cannot be completely ruled out at present. Further studies, including clinical trials with depressed patients, will be necessary to determine whether YH-200 produces similar therapeutic efficacy, as observed in the mouse behavioral tests in the present study.

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Author contribution
Yong-he ZHANG conceived and designed the experiments; Ya-ping XU, Jin-zhi SONG, Hui DING, Zhi-ge LIN, and Guang YANG isolated natural product; Yong-he ZHANG synthesized YH-200 and analyzed its structure; Zhao-fu SHENG, Sheng-Jie YANG performed the experiments; Zhao-fu SHENG, Bin YU, Xue-qiong ZHANG, and Suying CUI conducted the data analysis; Zhao-fu SHENG and Xiang-yu CUI wrote the first-draft manuscript and Yong-he ZHANG revised the manuscript.

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