Acta Pharmacologica Sinica (2015) 36: 949–956
© 2015 CPS and SIMM All rights reserved 1671-4083/15
www.nature.com/aps

Original Article

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

Zhao-fu SHENG, Xiang-yu CUI, Su-ying CUI, Bin YU, Xue-qiong ZHANG, Sheng-jie LI, Qing CAO, Yuan-li HUANG, Ya-ping XU, Jin-zhi SONG, Hui DING, Zhi-ge LIN, Guang YANG, Yong-he ZHANG*

Department of Pharmacology, School of Basic Medical Science, Peking University, Beijing 100191, China

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 α_1-adrenoceptor antagonist prazosin (1 mg/kg), β-adrenoceptor antagonist propranolol (2 mg/kg), dopamine D_1/D_5 receptor antagonist SCH23390 (0.05 mg/kg), dopamine D_2/D_3 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 α_2 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 α_1, α_2 and β-adrenoceptors, D_1/D_5 and D_2/D_3 receptors, as well as AMPA receptors.

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

Acta Pharmacologica Sinica (2015) 36: 949–956; doi: 10.1038/aps.2015.57

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-HT_{2C} 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.

* To whom correspondence should be addressed.
E-mail zhyh@hsc.pku.edu.cn
Received 2015-03-27 Accepted 2015-05-26

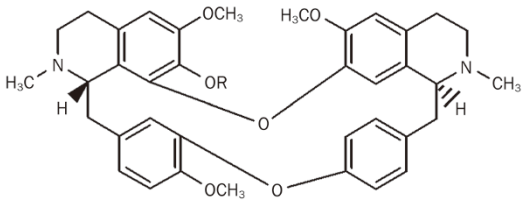

Figure 1. The structure of tetrandrine and 7-*O*-ethylfangchinoline (YH-200). Tetrandrine: R=CH_3; YH-200: R=CH_2CH_3.

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 ^{1}HNMR and ^{13}CNMR 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 α_1-adrenoceptor antagonist), yohimbine hydrochloride (an α_2-adrenoceptor antagonist), propranolol (a β-adrenoceptor antagonist), haloperidol (a dopamine D_2/D_3 receptor antagonist), *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepinehydrochloride (SCH23390, a dopamine D_1/D_5 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. 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 $mg \cdot kg^{-1} \cdot d^{-1}$, 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*[9, 10]. 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 (DigBehv-FS, Shanghai Jiliang Software Technology Co, Ltd, Shanghai, China). 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[11]. 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[12], 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 (separated 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.

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 NaH_2PO_4 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[13].

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 ($F_{(4,30)}=9.07$, $P<0.0001$; 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), the naturally occurring bisbenzylisoquinoline alkaloid.

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

Role of the adrenergic α_1, α_2, 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

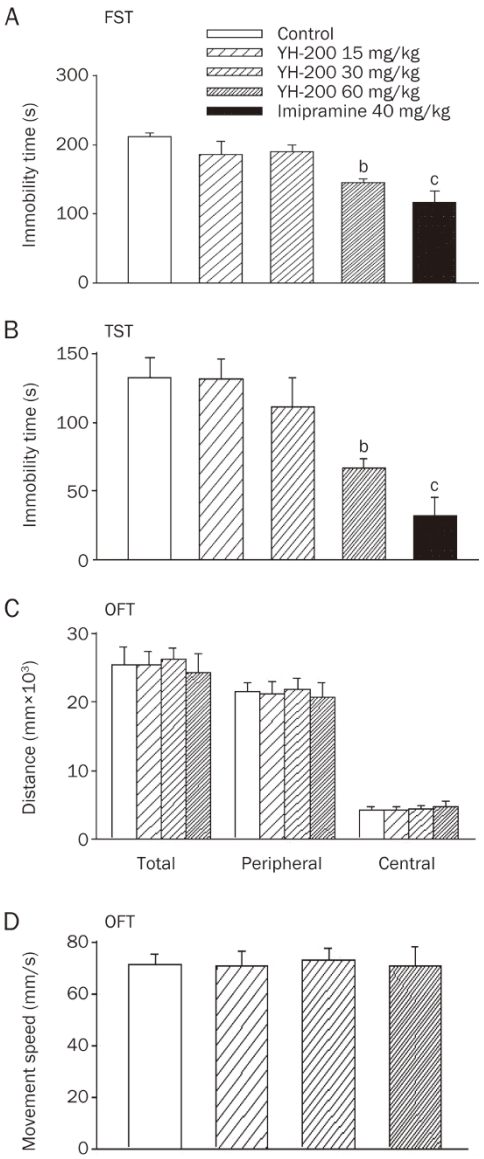

Figure 2. Effects of acute administration of YH-200 on immobility times in the forced swimming test (FST) (A, *n*=10/group) and tail suspension test (TST) (B, *n*=7/group). (C and D) Locomotor activity in the open field test (OFT) of mice treated with YH-200. *n*=10/group. Values are expressed as the mean±SEM. $^bP<0.05$, $^cP<0.01$ *vs* control group.

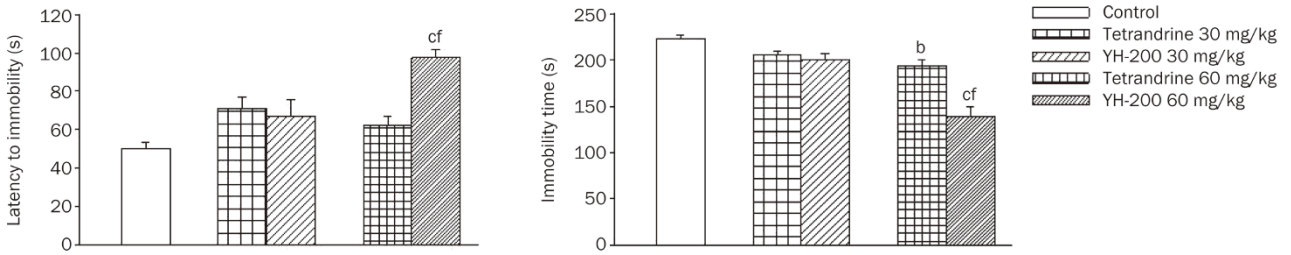

Figure 3. Antidepressant-like effects of YH-200 compared with that of tetrandrine in the FST. Values are expressed as the mean±SEM. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control. $^{f}P<0.01$ *vs* tetrandrine group at the same dose. $n=10$/group.

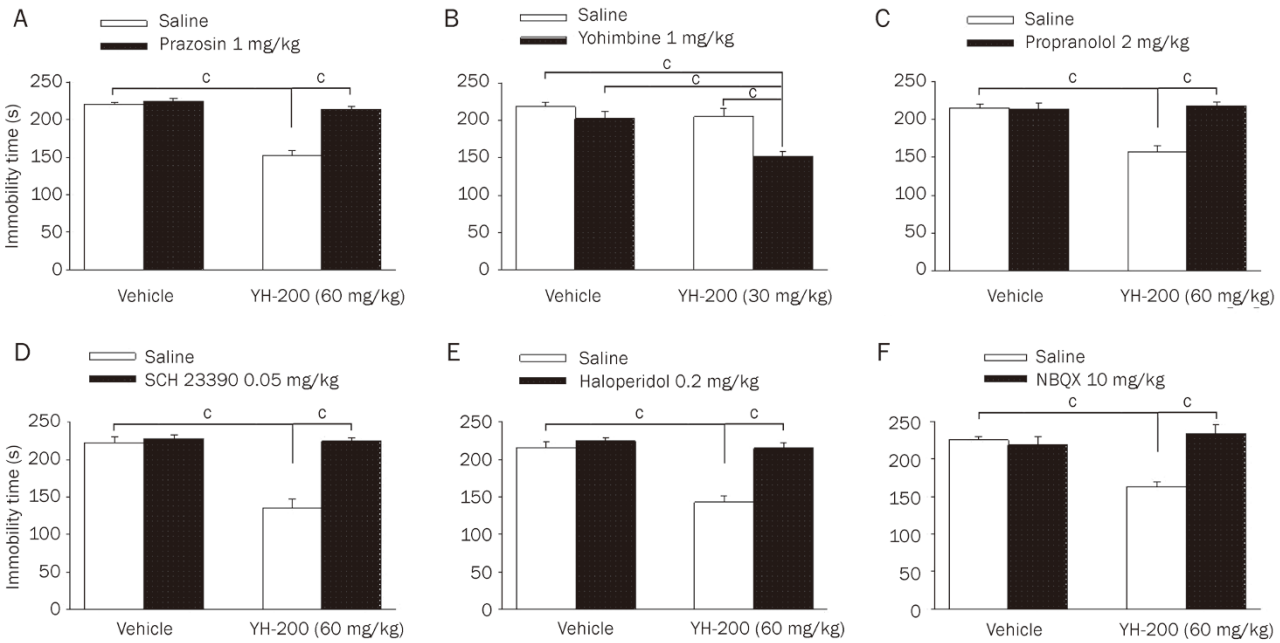

Figure 4. Effects of pretreatment with prazosin (A), yohimbine (B), propranolol (C), SCH23390 (D), haloperidol (E), or NBQX (F) on the anti-immobility effect of YH-200 in the mouse FST. $n=8$–9/group. The combined administration of subeffective dose of YH-200 and yohimbine showed synergistic antidepressant-like effect in the mouse FST. Values are expressed as mean±SEM. $^{c}P<0.01$.

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		372.0±18.7	79.3±26.6	88.5±19.4
YH-200	15 mg/kg	393.0±21.4	55.4±24.8	77.4±11.8
	30 mg/kg	944.6±34.3[c]	51.8±27.3	69.3±10.5
	60 mg/kg	995.8±32.1[c]	101.9±44.5	99.0±22.3
Imipramine	40 mg/kg	953.3±33.8[c]	80.9±39.4	69.6±10.6
		$F_{(4,45)}$=122.85	$F_{(4,45)}$=0.38	$F_{(4,45)}$=0.67
		P<0.0001	P=0.8230	P=0.6166
Hippocampus				
Vehicle		506.4±26.0	51.4±5.5	41.7±2.0
YH-200	15 mg/kg	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	40 mg/kg	491.2±39.3	58.2±7.0	42.9±4.0
		$F_{(4,45)}$=0.07	$F_{(4,45)}$=0.40	$F_{(4,45)}$=0.11
		P=0.9902	P=0.8073	P=0.89766
Striatum				
Vehicle		235.4±7.0	5782.5±314.1	1173.0±85.0
YH-200	15 mg/kg	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[b]	5718.4±315.4	1088.1±63.5
Imipramine	40 mg/kg	338.4±24.1[c]	6148.8±379.5	1170.5±73.9
		$F_{(4,45)}$=4.32	$F_{(4,45)}$=0.71	$F_{(4,45)}$=0.67
		P=0.0048	P=0.5886	P=0.6179
Hypothalamus				
Vehicle		2391.6±158.5	296.3±17.9	190.9±16.7
YH-200	15 mg/kg	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±36.6	205.7±21.5
Imipramine	40 mg/kg	2221.2±107.6	359.5±31.0	205.0±16.0
		$F_{(4,45)}$=0.43	$F_{(4,45)}$=0.50	$F_{(4,45)}$=0.36
		P=0.7891	P=0.7349	P=0.8376

[b]P<0.05, [c]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).

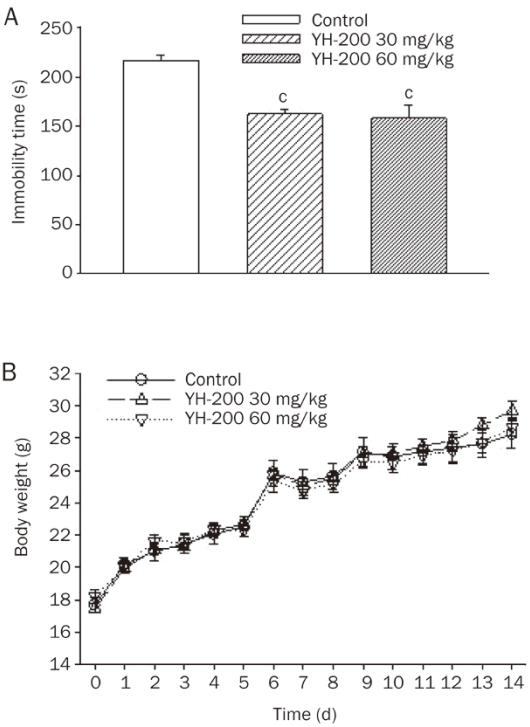

Figure 5. Effects of administration of YH-200 for 14 d on immobility time in the mouse FST (A) and body weight variation (B). Values are expressed as the mean±SEM. [c]P<0.01 *vs* control group. n=10/group.

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 LD_{50} value of 646 mg/kg (ig)[16], the LD_{50} value of

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 tetrandrine.

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_5 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 underlie some of the antidepressant-like effects of drugs observed in behavioral models of depression[23, 24]. In addition, the inhibition of α_1 adrenoceptor 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_1- 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_5) 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_5 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_5 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_2/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 anti-depressant 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 (α_1, α_2, and β receptors), dopaminergic (D_1/D_5 and D_2/D_3 receptors) and AMPAergic (AMPA receptor) systems. However, the direct actions of YH-200 with these receptors should be unraveled. Furthermore, pharmacokinetic 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.

Acknowledgements

This study was funded by grants from the National Natural Science Foundation of China (No 81173031, 81202511, and 81302746).

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 LI, Yuan-li HUANG, and Qing CAO 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.

References

1 Feder A, Nestler EJ, Charney DS. Psychobiology and molecular genetics of resilience. Nat Rev Neurosci 2009; 10: 446–57.
2 Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron 2002; 34: 13–25.
3 Páez-Pereda M. New drug targets in the signaling pathways activated by antidepressants. Prog Neuropsychopharmacol Biol Psychiatry 2005; 29: 1010–6.
4 Millan MJ. On 'polypharmacy' and multi-target agents, complementary strategies for improving the treatment of depression: a comparative appraisal. Int J Neuropsychopharmacol 2013; 17: 1009–37.
5 O'Leary OF, Dinan TG, Cryan JF. Faster, better, stronger: towards new antidepressant therapeutic strategies. Eur J Pharmacol 2015; 753: 32–50.
6 Demyttenaere K. Agomelatine: a narrative review. Eur Neuropsychopharmacol 2011; 21 Suppl 4: S703–9.
7 Protais P, Arbaoui J, Bakkali EH, Bermejo A, Cortes D. Effects of various isoquinoline alkaloids on *in vitro* ^{3}H-dopamine uptake by rat striatal synaptosomes. J Nat Prod 1995; 58: 1475–84.
8 Semwal DK, Semwal RB, Vermaak I, Viljoen A. From arrow poison to herbal medicine — the ethnobotanical, phytochemical and pharmacological significance of *Cissampelos* (Menispermaceae). J Ethnopharmacol 2014; 155: 1011–28.
9 Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977; 229: 327–36.
10 Gundersen BB, Blendy JA. Effects of the histone deacetylase inhibitor sodium butyrate in models of depression and anxiety. Neuropharmacology 2009; 57: 67–74.
11 Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl) 1985; 85: 367–70.
12 Zhang Q, Yu YP, Ye YL, Zhang JT, Zhang WP, Wei EQ. Spatiotemporal properties of locomotor activity after administration of central nervous stimulants and sedatives in mice. Pharmacol Biochem Behav 2011; 97: 577–85.
13 Wang ZJ, Zhang XQ, Cui XY, Cui SY, Yu B, Sheng ZF, *et al*. Glucocorticoid receptors in the locus coeruleus mediate sleep disorders caused by repeated corticosterone treatment. Sci Rep 2015; 5: 9442.
14 Castagné V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Curr Protoc Neurosci 2011; Chapter 8: Unit 8.10A.
15 Borsini F. Role of the serotonergic system in the forced swimming test. Neurosci Biobehav Rev 1995; 19: 377–95.
16 Chen Y, Chen M, Sun C, Li D. Study on acute toxicity of tetrandrine in male SD rats by intragastric administration. J Pract Med 2011; 27: 24–5. Chinese.
17 Elhwuegi AS. Central monoamines and their role in major depression. Prog Neuropsychopharmacol Biol Psychiatry 2004; 28: 435–51.
18 Millan MJ. The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. Eur J Pharmacol 2004; 500: 371–84.
19 Brunello N, Mendlewicz J, Kasper S, Leonard B, Montgomery S, Nelson J, *et al*. The role of noradrenaline and selective noradrenaline reuptake inhibition in depression. Eur Neuropsychopharmacol 2002; 12: 461–75.
20 Leonard BE. The role of noradrenaline in depression: a review. J Psychopharmacol 1997; 11 Suppl 4: S39–47.
21 Brunello N, Blier P, Judd LL, Mendlewicz J, Nelson CJ, Souery D, *et al*. Noradrenaline in mood and anxiety disorders: basic and clinical studies. Int Clin Psychopharmacol 2003; 18: 191–202.
22 Nutt DJ. The role of dopamine and norepinephrine in depression and antidepressant treatment. J Clin Psychiatry 2006; 67 Suppl 6: 3–8.
23 Danysz W, Kostowski W, Kozak W, Hauptmann M. On the role of noradrenergic neurotransmission in the action of desipramine and amitriptyline in animal models of depression. Pol J Pharmacol Pharm 1986; 38: 285–98.
24 Kaster MP, Raupp I, Binfaré RW, Andreatini R, Rodrigues ALS. Antidepressant-like effect of lamotrigine in the mouse forced swimming test: evidence for the involvement of the noradrenergic system. Eur J Pharmacol 2007; 565: 119–24.
25 Stone EA, Lin Y, Quartermain D. Immobility from administration of the alpha1-adrenergic antagonist, terazosin, in the IVth ventricle in rats. Neurosci Lett 2003; 353: 231–3.
26 Flugge G, van Kampen M, Meyer H, Fuchs E. Alpha2A and alpha2C-adrenoceptor regulation in the brain: alpha2A changes persist after chronic stress. Eur J Neurosci 2003; 17: 917–28.
27 Millan MJ, Newman-Tancredi A, Audinot V, Cussac D, Lejeune F, Nicolas JP, *et al*. Agonist and antagonist actions of yohimbine as compared to fluparoxan at alpha(2)-adrenergic receptors (AR)s, serotonin (5-HT)(1A), 5-HT(1B), 5-HT(1D) and dopamine D(2) and D(3) receptors. Significance for the modulation of frontocortical monoaminergic transmission and depressive states. Synapse 2000; 35: 79–95.
28 Goldberg MR, Robertson D. Yohimbine: a pharmacological probe for study of the alpha2-adrenoceptor. Pharmacol Rev 1983; 35: 143–80.
29 O'Donnell JM, Frith S, Wilkins J. Involvement of beta-1 and beta-2

adrenergic receptors in the antidepressant-like effects of centrally administered isoproterenol. J Pharmacol Exp Ther 1994; 271: 246–54.
30 Dailly E, Chenu F, Renard CE, Bourin M. Dopamine, depression and antidepressants. Fundam Clin Pharmacol 2004; 18: 601–7.
31 Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. Arch Gen Psychiatry 2007; 64: 327–37.
32 Takamura N, Nakagawa S, Masuda T, Boku S, Kato A, Song N, *et al*. The effect of dopamine on adult hippocampal neurogenesis. Prog Neuropsychopharmacol Biol Psychiatry 2014; 50: 116–24.
33 D'Aquila PS, Collu M, Gessa GL, Serra G. The role of dopamine in the mechanism of action of antidepressant drugs. Eur J Pharmacol 2000; 405: 365–73.
34 Takahashi H, Yoshida K, Higuchi H, Shimizu T, Inoue T, Koyama T. Addition of a dopamine agonist, cabergoline, to a serotonin-noradrenalin reuptake inhibitor, milnacipran as a therapeutic option in the treatment of refractory depression: two case reports. Clin Neuropharmacol 2003; 26: 230–2.
35 Li Y, Zhu ZR, Ou BC, Wang YQ, Tan ZB, Deng CM, *et al*. Dopamine D_2/D_3 but not dopamine D_1 receptors are involved in the rapid antidepressant-like effects of ketamine in the forced swim test. Behav Brain Res 2015; 279: 100–5.
36 Salvadore G, Singh JB. Ketamine as a fast acting antidepressant: current knowledge and open questions. CNS Neurosci Ther 2013; 19: 428–36.
37 Zarate CA Jr, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, *et al*. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 2006; 63: 856–64.
38 Machado-Vieira R, Salvadore G, DiazGranados N, Zarate CA. Ketamine and the next generation of antidepressants with a rapid onset of action. Pharmacol Ther 2009; 123: 143–50.
39 Lapidus KA, Soleimani L, Murrough JW. Novel glutamatergic drugs for the treatment of mood disorders. Neuropsychiatr Dis Treat 2013; 9: 1101–12.
40 Alt A, Nisenbaum ES, Bleakman D, Witkin JM. A role for AMPA receptors in mood disorders. Biochem Pharmacol 2006; 71: 1273–88.
41 Nations KR, Dogterom P, Bursi R, Schipper J, Greenwald S, Zraket D, *et al*. Examination of Org 26576, an AMPA receptor positive allosteric modulator, in patients diagnosed with major depressive disorder: an exploratory, randomized, double-blind, placebo-controlled trial. J Psychopharmacol 2012; 26: 1525–39.
42 Koike H, Iijima M, Chaki S. Involvement of AMPA receptor in both the rapid and sustained antidepressant-like effects of ketamine in animal models of depression. Behav Brain Res 2011; 224: 107–11.