Antidepressant-like Effects of ZBH2012001, a Novel Potent Serotonin and Norepinephrine Reuptake Inhibitor

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Keywords

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SUMMARY

Aims: The present study was conducted to evaluate the antidepressant-like effects of ZBH2012001, a novel potential serotonin and norepinephrine reuptake inhibitor (SNRI). **Methods:** Competitive binding assays, calcium flow, and cAMP detection methods were used to determine the affinity of ZBH2012001 for serotonin transporters (SERTs) and norepinephrine transporters (NETs), as well as its selectivity over dopamine transporters (DATs) and 16 other G-protein-coupled receptors (GPCRs) or ion channels. The antidepressant-like effects of ZBH2012001 were determined using the tail suspension test, forced swim test, and learned helplessness paradigm. The pharmacokinetics and acute toxicity of ZBH2012001 were also assessed. **Results:** ZBH2012001 exhibited a moderate affinity to SERTs and NETs (Ki values were 35.3 ± 2.86 and 225 ± 26.0 nM, respectively); it had no effects on the DATs or the 16 other GPCRs or ion channels. Data from behavioral tests indicated that ZBH2012001 exhibited superior antidepressant-like effects compared with duloxetine (one of the most used SNRIs) in the three depression models. The pharmacokinetic evaluation of ZBH2012001 indicated that the absolute bioavailability value was 60.5%, and the acute toxicity test indicated that LD₅₀ of ZBH2012001 was 346 mg/kg. **Conclusion:** These findings suggest that ZBH2012001 is a novel SNRI with superior antidepressant-like effects, lower acute toxicity and a better pharmacokinetic profile compared with duloxetine. Thus, ZBH2012001 may have potential therapeutic effects in depression disorders.

Introduction

Major depressive disorder (MDD) is a severe psychiatric disease with a high prevalence and socioeconomic burden. According to the estimation by the World Health Organization, there are at least 350 million individuals who suffer from depression, and MDD may become the second leading cause of disability worldwide by 2020 [1–3].

Although the potential pathophysiological mechanisms of depression remain poorly understood, the current antidepressants for MDD mainly include tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), and serotonin and norepinephrine reuptake inhibitors (SNRIs) [4,5]. TCAs and MAOIs are currently less commonly used because of their undesirable serious side effects [6]. Undoubtedly, SSRIs, which were developed based on a single-target strategy, have successfully solved the tolerance problem and have been widely used in previous decades [7]. However, two of the primary issues in depression therapy, including efficacy and recurrence, have not substantially improved. MDD is a notoriously complicated disease with emotional and physical symptoms [8]. Furthermore, pain-related residual somatic symptoms have

been reported as an important cause of depression recurrence [9,10]. Serotonin (5-HT) and norepinephrine (NE), which represent the most crucial transmitters in depression treatment, are implicated in the modulation of not only different emotional symptoms [11–13] but also descending inhibitory pain pathways [7,14,15]. Accordingly, the dual reuptake inhibitors, including SNRIs such as duloxetine, are expected to represent a significant improvement in terms of better efficacy and lower recurrence compared with the SSRIs [16]. Duloxetine, one of the most used SNRIs, inhibits the reuptake of serotonin and norepinephrine (NE) in the central nervous system, and clinical evidence indicates that duloxetine exhibits superior efficacy in the control of emotional and physical symptoms [17]. Unfortunately, although duloxetine is recommended as a first-line antidepressant, preclinical and clinical data indicated that duloxetine may have greater risk of hepatotoxicity. The discovery of novel SNRIs with superior pharmacological and toxicological profiles represents a promising strategy in antidepressant development.

Therefore, taken duloxetine as the leading compound, we designed and synthesized a series of compounds, and the behavioral screening results subsequently demonstrated that a compound with a novel structure, ZBH2012001 (Figure 1; 5-(3-

Figure 1 Chemical structure of ZBH2012001.

(methylamino)-1-(thiophen-2-yl)propyl)benzo[d][1,3]dioxol-4-ol hydrochloride), exhibited strong antidepressant-like effects. Here, we performed a thorough preclinical evaluation of ZBH2012001, including target research, an evaluation of antidepressant-like effects, and preliminary pharmacokinetic and toxicological research.

Materials and Methods

Animals

Male ICR mice that weighed 20–22 g and male Sprague Dawley rats that weighed 180–220 g (Beijing SPF Animal Technology Company, Beijing, China) were used in the current study. The animals were housed in standard conditions (room temperature: $21 \pm 1^\circ\text{C}$, humidity: 40–60%, 12-h: 12-h light/dark cycle, and lights on at 8:00 a.m.). Food and water were available *ad libitum*. The animal experiments were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Materials

Duloxetine hydrochloride was purchased from Zhejiang HaiXiang Pharmaceutical Corporation, Ltd. (Taizhou, China). ZBH2012001 hydrochloride was synthesized by our institute. Fluoxetine, 5-HT, and NE were purchased from Sigma Co. (St. Louis, MO, USA) and PerkinElmer Life Sciences (NEN, Boston, MA, USA). Dulbecco's modified Eagle's medium and fetal bovine serum were purchased from Invitrogen, Inc. (Grand Island, NY, USA) or HyClone Corp. (South Logan, UT, USA), respectively. The compounds were dissolved in distilled water and administered intravenously (i.v.) or orally (p.o.) via gavage at concentrations of 1.0 mL/kg for rats and 10 mL/kg for mice, unless otherwise specified.

Cell Membrane Preparation and Binding to hSERTs and hNETs

The membranes were prepared from HEK293 cells that stably expressed hSERTs and hNETs. Briefly, the cells were harvested via centrifugation at 110 g for 5 min at 4°C . The pellets were homogenized in an assay buffer (50 mM Tris–HCl buffer that contained 120 mM NaCl and 5 mM KCl, pH 7.4) and subsequently centrifuged twice at 36,000 g for 15 min at 4°C . The final pellet was resuspended in assay buffer and stored at 80°C until use.

The binding affinities for hSERTs and hNETs were determined via competition with [^3H] citalopram (2.0 nM) and [^3H]

nisoxetine (2.0 nM) at 25°C for 60 min. The nonspecific binding was determined using 10 μM fluoxetine and 10 μM duloxetine. The binding assays were performed in duplicate in three independent experiments [18,19].

Other Potential Binding Targets

The binding affinities for hDATs were determined via competition with [^3H] win35428 (5.0 nM) at 25°C for 60 min. The nonspecific binding was determined using 10 μM win35428 [18,19].

The calcium flow was determined using an FLIPR Calcium 4 Assay Kit (Molecular Devices, Sunnyvale, CA, USA) according to the manufacturer's recommendations. The cAMP detection was determined using HTRF technology (cAMP dynamic 2 100,000 tests kit; Cisbio Bioassays, Codolet, France). These two methods were used to determine whether ZBH2012001 (10 μM) had agonistic or antagonistic effects on 16 GPCRs or ion channels, including 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT₃, α_{1A} , α_{2A} , β_2 , D₁, D_{2S}, H₁, H₂, M₁, M₂, OPRD, and OPRM. The specific parameters are listed in Table S1.

Tail Suspension Test (TST)

The tail suspension test in mice was performed as previously described with minor modifications [20,21]. Prior to the test, the mice were randomly assigned to six treatment groups: vehicle, duloxetine (20 mg/kg), or ZBH2012001 (5, 10, 20, or 40 mg/kg). One hour after oral administration, the mice were suspended for 6 min at the top of the apparatus using adhesive tape placed approximately 1 cm from the tip of their tails, and the immobility time was measured for the last 4 min. The mice were considered immobile when they hung passively without moving.

Forced Swim Test (FST)

The experiment was performed with mice according to the protocol of Porsolt *et al.* [22] with minor modifications. Prior to the test, the mice were randomly assigned to six treatment groups: vehicle, duloxetine (40 mg/kg), or ZBH2012001 (5, 10, 20 or 40 mg/kg). One hour after oral administration, the mice were individually placed in cylindrical containers (diameter 12 cm, height 20 cm, contained 10 cm of water maintained at 25°C) for 6 min. The duration of immobility during the last 4 min of suspension was recorded by a blinded observer using a stopwatch. The immobile time was calculated as the time the mice floated motionless, with only movements necessary to maintain their heads above the water.

Locomotor Activity in Mice

The mice were randomly assigned to six treatment groups: vehicle, duloxetine (20 mg/kg), or ZBH2012001 (5, 10, 20, or 40 mg/kg). One hour after oral administration, each mouse was placed in separate chambers of the movement instrument (Shandong Medical Academy of Science, Jinan, China). The total distance of each mouse was automatically recorded during the 15-min test. The apparatus was cleaned during the test interval.

Learned Helplessness Paradigm

The learned helplessness paradigm was performed following the experimental procedure by Chourbaji *et al.* [23]. The protocol consisted of two sessions, which included inescapable foot-shock training and shuttle escape testing. In the first session, the mice were placed in the automated shuttle boxes (20 cm × 16.5 cm × 20 cm) and exposed to 360 inescapable shocks (intensity 0.30 mA, duration 2 s, 10 ± 2 s variable interval) for 1 h; the control mice [no shock (NS)] were placed over the course without exposure to foot shocks. Following 4 days of training, on day 5, the mice were screened and grouped according to the number of escape failures during the screening procedure (10 trials, 5-s light on, 10-s light + shock, 25-s interval). If a mouse failed to escape into the other compartment during the first 5 s of light or during the 5 s of light + shock, an escape failure was subsequently recorded by the computer. Only the mice that exhibited helplessness (more than six escape failures) were used for further evaluation.

In the following 3 days, the second session was performed. Briefly, the mice were individually placed in the shuttle box and subjected to 30 avoidance trials. During the first five trials, a light signal and a shock (0.3 mA) concurrently occurred, whereas in the following 25 trials, the light signal occurred in the first 5 s, followed by the delivery of the shock (0.3 mA). The shock was terminated only if the animal crossed to the other compartment or the trial ended. The number of escape failures and the escape latency were measured by the computer. The mice were orally administered different drugs 1 h prior to the shuttle escape testing procedure during the 3 days.

Pharmacokinetic Research

The rats were initially fasted for 16 h prior to the administration of ZBH2012001 (20 mg/kg, *p.o./i.v.*). At predosing, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h postdosing, blood samples were obtained from the fossa orbitalis vein into heparinized tubes. The samples were subsequently centrifuged at 1301 *g* for 15 min. The plasma was stored at −20°C until processing. A standard different solution of ZBH2012001 (50 μL), internal standard solution (50 μL, 100 ng/mL), and acetic ether were added to the blood samples (200 μL), and the mixtures were centrifuged at 14,000 *g* for 10 min. The supernatant was transferred and evaporated under N₂ atmosphere at 37°C for 30 min, and the material was subsequently redissolved in 200 μL of acetonitrile/deionized water (45/55, *v/v*). The high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) conditions followed Li *et al.* [24] with modifications. The detected ion pairs of ZBH2012001 and propranolol were *m/z* 291/44.1 (collision energy of 16 eV, fragmentation voltage of 117 V) and *m/z* 260.4/116.2 (collision energy of 17 eV, fragmentation voltage of 127 V), respectively.

Acute Toxicity Test

An acute toxicity test was performed using an up-and-down procedure as previously described by Bruce *et al.* [25]. The animals were dosed one at a time. If an animal survived, the dose for the subsequently animal was increased; if it died, the dose was

decreased. The toxic symptoms and mortality rates were recorded, and the median lethal dosage (LD₅₀) and 95% confidence interval values were calculated.

Statistical Analysis

Unless otherwise specified, statistical analysis was performed using GraphPad Prism (GraphPad Prism 5.0, version 2.0; GraphPad Software Inc., San Diego, CA, USA), and the data are expressed as the mean ± SEM. The transporter binding data were analyzed using one-site nonlinear regression of the concentration–effect curve. The *K_i* values were calculated from the Cheng–Prusoff equation: $K_i = IC_{50}/[(L/KD) + 1]$ [26]. In the behavioral assays, for the data with equal variances, Student's *t*-test or one-way analysis of variance (ANOVA) followed by Dunnett's test was performed; in contrast, the data that demonstrated unequal variances were analyzed using a Mann–Whitney *U*-test or Kruskal–Wallis test followed by Dunn's multiple comparison test. For the learned helplessness data, analyses were conducted using repeated-measures (mixed-model) ANOVA with different covariance structure. The LD₅₀ and 95% confidence interval values were calculated using AOT 425 StatPgm software (Organization for Economic Co-operation and Development, OECD). In the pharmacokinetic test, the non-compartmental pharmacokinetic parameters, that is, the *T*_{1/2}, *MRT*, *AUC*, *T*_{max}, and *C*_{max} values, were calculated using the pharmacokinetics software Phoenix WinNonlin 5.8 (Certara Company, Princeton, NJ, USA).

Results

Effects of ZBH2012001 on hSERTs, hNETs, hDATs, and 16 GPCRs

The results demonstrated that ZBH2012001 possessed relatively moderate affinity for hSERTs and hNETs, and the *K_i* values were 35.3 ± 2.86 and 225 ± 26.0 nM, respectively. The positive control fluoxetine or duloxetine exhibited a high affinity for hSERTs (*K_i* = 3.96 ± 1.09 nM) or hNETs (*K_i* = 1.85 ± 0.65 nM), respectively, which indicates our experimental parameters and procedures were reliable.

As shown in Table 1, ZBH2012001 (10 μM) exhibited negligible effects on hDATs and the other 16 GPCRs or iron channels, which suggests that ZBH2012001 is a selective serotonin and norepinephrine reuptake inhibitor.

Effects of ZBH2012001 in the TST, FST, and Locomotor Activity in Mice

Figure 2A illustrates the effects of ZBH2012001 (*p.o.*) on the immobility time in the TST in the mice. Compared with the vehicle, the oral administration of duloxetine at 20 mg/kg significantly decreased the immobility time (Mann–Whitney test, *U* = 4.000, *P* = 0.0002), which indicates the behavioral tests were performed under normal conditions and were reliable. The oral administration of ZBH2012001 (10, 20, and 40 mg/kg) significantly reduced the immobility time in a dose-dependent manner (Kruskal–Wallis test, Kruskal–Wallis statistic = 34.36, *P* < 0.0001; Dunn's multiple

Table 1 Effects of ZBH2012001 on hDATs, 16 GPCRs, and iron channels

Targets	Detection method	Agonism (%)	Antagonism (%)
DATs	Radioligand receptor binding assays	31.38 (inhibition %)	
5HT _{1A}	Calcium	7.93	−9.07
5HT _{2A}	Calcium	−0.20	2.79
5HT _{2C}	Calcium	−0.64	4.62
5HT _{2B}	Calcium	−1.79	−12.89
5HT ₃	Calcium	−0.71	33.03
α_{1A}	Calcium	2.54	1.26 μ M (IC ₅₀)
α_{2A}	Calcium	2.89	5.65
β_2	cAMP	−8.53	−12.85
D ₁	cAMP	−0.55	2.82
D ₂₅	Calcium	3.79	4.10
M ₁	Calcium	1.27	32.39
M ₂	Calcium	2.53	9.70
H ₁	Calcium	0.75	−3.21
H ₂	cAMP	0.56	−0.69
OPRM	cAMP	1.70	0.49
OPRD	cAMP	12.55	5.71

comparison test, $P < 0.05$ for 10 mg/kg, $P < 0.01$ for 20 mg/kg, and $P < 0.001$ for 40 mg/kg).

Figure 2B illustrates the effects of ZBH2012001 on the immobility time in the FST in the mice. Compared with the vehicle, the acute administration of duloxetine at 40 mg/kg significantly reduced the immobility time (Student's t -test, $t = 4.345$, $P = 0.0004$), which indicates the behavioral tests were performed under normal conditions and were reliable. Acute oral treatment with ZBH2012001 (10, 20, and 40 mg/kg, p.o.) produced a main effect on the immobility time in the FST in the mice (Kruskal–Wallis test, Kruskal–Wallis statistic = 20.60, $P = 0.0004$). Furthermore, the post hoc analysis indicated that ZBH2012001 at doses of 10, 20, and 40 mg/kg significantly decreased the immobility time (Dunnett's test, $P < 0.05$ for 10 mg/kg, $P < 0.05$ for 20 mg/kg, and $P < 0.001$ for 40 mg/kg).

The acute oral administration of ZBH2012001 at various doses (one-way ANOVA, $F_{4, 45} = 0.7417$, $P = 0.5686$) and duloxetine (Student's t -test, $t = 0.8315$, $P = 0.4166$) had no effect on the locomotor activity in the mice (Figure 2C).

Effects of ZBH2012001 in the LH Test in Mice

The escape latency and escape failures as indices of learned helplessness were recorded 1 h after drug administration for 3 consecutive days. As shown in Figure 2D, compared with the NIS group, IS significantly increased the escape latency in the mice (repeated-measures ANOVA, treatment factor $F_{1, 20} = 21.75$, $P = 0.0001$, time factor $F_{2, 20} = 8.43$, $P = 0.0022$, and interaction factor $F_{2, 20} = 0.96$, $P = 0.3983$; Bonferroni posttests, $P < 0.001$, $P < 0.01$, and $P < 0.001$ for day 1, day 2, and day 3, respectively). Fluoxetine at 10 mg/kg reversed the increased escape latency induced by IS (repeated-measures ANOVA, treatment factor $F_{1, 20} = 10.28$, $P = 0.0044$, time factor $F_{2, 20} = 12.34$, $P = 0.0003$, and interaction factor $F_{2, 20} = 0.04$, $P = 0.9635$; Bonferroni posttests, $P < 0.05$, $P = 0.0597$, and $P < 0.001$ for day 1, day 2, and day 3,

respectively). Similarly, the oral administration of ZBH2012001 (5, 10, and 20 mg/kg) significantly decreased the escape latency compared with the IS group (repeated-measures ANOVA, treatment factor $F_{3, 39} = 9.04$, $P = 0.0001$, time factor $F_{2, 39} = 27.23$, $P < 0.0001$, and interaction factor $F_{6, 39} = 0.93$, $P = 0.4812$; Bonferroni posttests, for 5 mg/kg, $P < 0.01$, $P < 0.01$, and $P < 0.01$ for day 1, day 2, and day 3, respectively; for 10 mg/kg, $P < 0.001$, $P < 0.01$, and $P < 0.01$ for day 1, day 2, and day 3, respectively; for 20 mg/kg, $P < 0.01$, $P < 0.01$, and $P < 0.001$ for day 1, day 2, and day 3, respectively).

As shown in Figure 2E, compared with NIS, IS significantly increased the escape failures in the mice (repeated-measures ANOVA, treatment factor $F_{1, 19} = 53.70$, $P < 0.0001$, time factor $F_{2, 19} = 31.04$, $P < 0.0001$, and interaction factor $F_{2, 19} = 7.10$, $P = 0.0050$; Bonferroni posttests, $P < 0.001$, $P < 0.001$, and $P < 0.001$ for day 1, day 2, and day 3, respectively). Fluoxetine (10 mg/kg, p.o.) reversed the increased number of escape failures in the IS group (repeated-measures ANOVA, treatment factor $F_{1, 19} = 15.19$, $P = 0.0010$, time factor $F_{2, 31} = 3.34$, $P = 0.0486$, and interaction factor $F_{2, 31} = 1.34$, $P = 0.2773$; Bonferroni posttests, $P < 0.001$, $P < 0.05$, and $P < 0.01$ for day 1, day 2, and day 3, respectively). Similarly, ZBH2012001 (5, 10, and 20 mg/kg, p.o.) significantly decreased the escape failures compared with the IS group (repeated-measures ANOVA, treatment factor $F_{3, 39} = 5.73$, $P = 0.0024$, time factor $F_{2, 61} = 8.31$, $P = 0.0006$, and interaction factor $F_{6, 61} = 0.57$, $P = 0.7502$; Bonferroni posttests, for 5 mg/kg, $P < 0.001$, $P < 0.05$, and $P < 0.05$ for day 1, day 2, and day 3, respectively; for 10 mg/kg, $P < 0.001$, $P < 0.05$, and $P < 0.05$ for day 1, day 2, and day 3, respectively; for 20 mg/kg, $P < 0.05$, $P < 0.05$, and $P < 0.05$ for day 1, day 2, and day 3, respectively).

Pharmacokinetic Study In Vivo

Table 2 illustrates the pharmacokinetic parameters. Following the intravenous and oral administration of ZBH2012001 in the rats, the terminal half-lives were 2.3 ± 0.6 and 1.9 ± 0.1 h, respectively. The peak concentration occurred at 0.0167 and 1.4 ± 1.0 h following the intravenous and oral administration, respectively, and the maximum concentrations were 6886.8 ± 2379.2 and 815.1 ± 309.9 ng/mL, respectively. The absolute bioavailability estimated based on the AUC_{0-∞} was 60.5%.

Acute Toxicity Test

The acute toxicity test in the mice was performed using an up-and-down method. According to the analysis results using AOT 425 StatPgm software (OECD), the LD₅₀ value following the p.o. administration of ZBH2012001 in the mice was 346 mg/kg, and the approximate 95% confidence interval (95% CI) value was 300–400 mg/kg (Table 3).

Discussion

In the present study, we performed a thorough preclinical evaluation of ZBH2012001, a structurally novel compound, and demonstrated that ZBH2012001 was a novel serotonin and norepinephrine reuptake inhibitor with superior antidepressant-like

Figure 2 Effects of ZBH2012001 (5–40 mg/kg) and DLX in three depression animal models. **(A)** Immobility time in the tail suspension test. **(B)** Immobility time in the forced swim test. **(C)** Total distance in locomotor activity in mice. **(D)** Escape latency in the learned helplessness paradigm. **(E)** Number of escape failures in the learned helplessness paradigm. Results are expressed as the mean $\pm$ SEM. ($n = 10$ – 12). For statistical significance, in the TST and forced swim test (FST), $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ compared with the vehicle group. In the LH, $##P < 0.01$ and $###P < 0.001$ compared with the NIS group, and $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ compared with the IS group.

effect activity and pharmacokinetic profiles and lower acute toxicity compared with duloxetine.

First, to determine the targets of ZBH2012001, we evaluated the affinity or function of ZBH2012001 for three monoamine

transporters, including SERTs, NETs, and DATs, and 16 GPCRs or iron channels *in vitro*. Our data demonstrated that ZBH2012001 exhibited moderate affinities for SERTs and NETs with a 6.4-fold higher selectivity for SERTs. Duloxetine and venlafaxine,

Table 2 Pharmacokinetic parameters in rats following intravenous and oral administration of ZBH2012001 (20 mg/kg)

Parameter	Unit	i.v.	p.o.
AUC _(0-∞)	ng/mL*h	8236.2 ± 422.8	4977.0 ± 683.9
MRT	h	2.8 ± 0.3	4.8 ± 1.0
T _{1/2}	h	2.3 ± 0.6	1.9 ± 0.1
T _{max}	h	0.0167	1.4 ± 1.0
CL _Z	mL/h/kg	2165.1 ± 114.3	3618.6 ± 460.7
V _Z	mL/kg	7192.0 ± 1608.2	9601.0 ± 591.7
C _{max}	ng/mL	6886.8 ± 2379.2	815.1 ± 309.9
F	%	–	60.5

i.v., intravenously; p.o., orally; AUC_(0-∞), area under the concentration–time curve from zero to infinite time; MRT, mean residence time; T_{1/2}, terminal half-life; T_{max}, time to reach the maximum plasma concentration; CL_Z, total clearance; V_Z, volume of distribution at steady state; C_{max}, maximum plasma concentration; F, absolute bioavailability.

Table 3 Results in acute toxicity test in mice

Animal ID	Dose (mg/kg)	Short-term result	Long-term result	LD ₅₀ (mg/kg)	95% CI (mg/kg)
1	400	X	X	346	300–400
2	300	O	O		
3	400	X	X		
4	300	O	O		
5	400	X	X		
6	300	O	O		

X, Died; O, Survived; LD₅₀, the median lethal dosage; 95% CI, 95% confidence interval.

belonging to SNRIs, are dissimilar from one another in their pharmacological profiles. Duloxetine exhibits the most potent affinity for SERTs and NETs (K_i values are 0.79 and 5.34 nM, respectively) with a 7-fold higher selectivity for SERTs [19], whereas venlafaxine exhibits an absolutely weaker and unbalanced affinity for SERTs and NETs (K_i values are 82 and 2480 nM, respectively) with a 30-fold higher selectivity for SERTs [27]. Thus, ZBH2012001 exhibited moderate target potency, which was greater than venlafaxine (2-fold or 11-fold stronger affinity for SERTs or NETs, respectively) but weaker than duloxetine, and it was a balanced SNRI similar to duloxetine. Furthermore, ZBH2012001 exhibited no or very weak effects on the DAT and the other 16 GPCRs or ion channels, including 5-HT_{1A}, 5-HT_{2A},

5-HT_{2B}, 5-HT_{2C}, 5-HT₃, α_{1A}, α_{2A}, β₂, D₁, D_{2S}, H₁, H₂, M₁, M₂, OPRD, and OPRM; thus, ZBH2012001 may not have adverse effects on the cardiovascular, cholinergic, histamine, and opioid systems. We subsequently evaluated the antidepressant-like effects of ZBH2012001 using three classic depression models: the tail suspension test, the forced swim test, and the learned helplessness paradigm. In all three models, ZBH2012001 exhibited more potent and stable antidepressant-like effects in a dose-dependent manner. Furthermore, in Table 4, we summarized the pharmacological and toxicological profiles of ZBH2012001, duloxetine, and venlafaxine according to the literature and data obtained from our present study. As shown in Table 4, in contrast to duloxetine, ZBH2012001 or venlafaxine exhibited similar sensitivities in both the TST and FST. Compared with duloxetine, ZBH2012001 exhibited a similar efficacy in the TST, whereas it was more potent in the FST. Compared with venlafaxine, ZBH2012001 was more potent in both the TST and FST.

To further evaluate the potential antidepressant-like effects of ZBH2012001, the learned helplessness paradigm was conducted. In the LH paradigm, ZBH2012001 at doses from 5 to 20 mg/kg significantly reversed the depressive behavior induced by inescapable shock, and the MED value was <5 mg/kg, which was only 0.25-fold less than duloxetine (20 mg/kg, L. Chen, unpublished data). These findings suggested that ZBH2012001 demonstrates more potent antidepressant-like effects compared with duloxetine in this model.

It was interesting and confusing that ZBH2012001 exhibited more potent antidepressant-like effects than duloxetine in these depression models, whereas the target affinity of ZBH2012001 was substantially weaker compared with duloxetine. In addition to the target affinity, the pharmacokinetic profiles are also a key contributing factor for pharmacological activity. Thus, we investigated the pharmacokinetic characteristics of ZBH2012001. Our results demonstrated that the absolute bioavailability of ZBH2012001 was as high as 60.5%, which is substantially higher than most antidepressants, including duloxetine (21%) [17] and venlafaxine (12.6%) [30]. This finding provided a reasonable explanation for the potent efficacy of ZBH2012001. The discovery of ZBH2012001 promoted us to reconsider the screening pattern of antidepressants, and we must keep in mind that the target affinity is not the only key factor that affects drug efficacy and future development.

Furthermore, in addition to the therapeutic benefit, toxicological profiles also play an important role in new drug development. Thus, we conducted an acute toxicity test using an up-and-down procedure to obtain preliminary toxicological

Table 4 Summary of pharmacological and toxicological profiles of ZBH2012001 compared with duloxetine and venlafaxine

Drugs	MED (mg/kg)		ED ₅₀ (mg/kg)		LD ₅₀ (mg/kg) p.o.	TI (LD ₅₀ /ED ₅₀)		F (%)
	TST	FST	TST	FST		TST	FST	
ZBH2012001	10	10	9.2	9.5	346	37.7	36.5	60.5
Duloxetine	5 [28]	40 [28]	20.0	20.1	177.1 [28]	8.86	8.81	21
Venlafaxine	30 [29]	30 [29]	20–30 [29]	30–40 [29]	350 [31]	11.7–17.5	8.7–11.5	12.6

MED, minimal effective dose; ED₅₀, 50% effective dose; LD₅₀, the median lethal dosage; TI, therapeutic index; F, the absolute bioavailability value; p.o., orally; TST, tail suspension test; FST, forced swim test.

information following the p.o. administration of ZBH2012001. An up-and-down method is widely used to estimate acute toxicity (LD_{50}) because it requires substantially fewer numbers of animals. Our findings indicated that the LD_{50} value following the p.o. administration of ZBH2012001 was 346 mg/kg, which indicates its acute toxicity was substantially lower than duloxetine (LD_{50} value was 177.1 mg/kg) [28]. We independently calculated the therapeutic index (TI, LD_{50}/ED_{50}) using the ED_{50} data from the TST (TI_{TST}) and FST (TI_{FST}), and we summarized the pharmacological and toxicological parameters (including the ED_{50} , LD_{50} , and TI values) of ZBH2012001, duloxetine, and venlafaxine [31] in Table 4. The TI_{TST} or TI_{FST} of ZBH2012001 was 4-fold higher than duloxetine (Table 4), which suggests that ZBH2012001 may have better tolerability and a lower risk of overdose toxicity. Certainly, this hypothesis requires further validation via additional preclinical and clinical data in the future.

In conclusion, the present study demonstrates that ZBH2012001 is a novel and selective serotonin and norepinephrine reuptake inhibitor with superior antidepressant-like effects, lower acute toxicity, and a better pharmacokinetic profile compared with duloxetine. Thus, ZBH2012001 may have potential therapeutic effects in MDD treatment.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Table S1 Specific parameters in other potential binding target research.

Figure S1 ZBH2012001 competed for the binding of radioligands specific to hSERTs (A) and hNETs (B).

Figure S2 Plasma concentration–time profiles in mice following intravenous and oral administration of ZBH2012001.