

Original Article

# SKF83959 is a novel triple reuptake inhibitor that elicits anti-depressant activity

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**Aim:** SKF83959 (3-methyl-6-chloro-7,8-hydroxy-1-(3-methylphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine) is an atypical dopamine receptor-1 (D<sub>1</sub> receptor) agonist, which exhibits many D<sub>1</sub> receptor-independent effects. In the present work, we examined the effects of SKF83959 on monoaminergic transporters *in vitro* and its anti-depressant activity *in vivo*.

**Methods:** Human serotonin transporter (SERT), norepinephrine transporters (NET) or dopamine transporters (DAT) were stably expressed in CHO cells. The uptake kinetics of SERT, NET, and DAT were examined using [<sup>3</sup>H]-serotonin, [<sup>3</sup>H]-norepinephrine or [<sup>3</sup>H]-dopamine, respectively. A triple reuptake inhibitor DOV21947 was used as the positive control. Tail suspension test and forced swimming test were conducted in mice. SKF83959 or DOV21947 (2–8 mg/kg) were intraperitoneally injected 30 min before the tests.

**Results:** SKF83959 was a competitive inhibitor of SERT ( $K_i=1.43\pm 0.45$   $\mu\text{mol/L}$ ), but a noncompetitive inhibitor of NET ( $K_i=0.60\pm 0.07$   $\mu\text{mol/L}$ ) and DAT ( $K_i=9.01\pm 0.80$   $\mu\text{mol/L}$ ). In contrast, DOV21947 was a competitive inhibitor of SERT ( $K_i=0.89\pm 0.24$   $\mu\text{mol/L}$ ) and DAT ( $K_i=1.47\pm 0.31$   $\mu\text{mol/L}$ ) and a noncompetitive inhibitor of NET ( $K_i=0.18\pm 0.04$   $\mu\text{mol/L}$ ). In mice, both SKF83959 and DOV21947 elicited anti-depressant activity in a dose-dependent manner.

**Conclusion:** SKF83959 functions as a novel triple reuptake inhibitor *in vitro* and exerts anti-depressant effects *in vivo*.

**Keywords:** SKF83959; monoamine transporter; serotonin; norepinephrine; dopamine; depression; DOV21947

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## Introduction

Depression is a severe and chronic mental disorder that affects a large population worldwide<sup>[1,2]</sup>. The development of effective anti-depressants with less adverse effects remains a challenge in pharmaceutical research. Within the past few decades, biogenic amine uptake inhibitors have been the mainstay for the treatment of depression, such as the prototypical tricyclic anti-depressants, the selective serotonin uptake inhibitors and the so-called “second” generation dual serotonin/norepinephrine uptake inhibitors with a “cleaner” side effect profile<sup>[3]</sup>.

Despite these advances, all these drugs often require 3 weeks to take effect. Moreover, nearly 30%–40% of patients

with depression were not responsive to these therapies<sup>[4]</sup>. Recent studies have shown that a compound producing an immediate increase in synaptic dopamine concentrations would result in a more rapid onset of relief and a shortening or elimination of the therapeutic lag<sup>[5]</sup>. Thus, triple uptake inhibitors have become a focal point in anti-depressant drug development. Furthermore, some of these drugs have shown promising responses in clinical trials<sup>[6–9]</sup>.

The atypical dopamine receptor-1 (D<sub>1</sub> receptor) agonist, 3-methyl-6-chloro-7,8-hydroxy-1-[3-methylphenyl]-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF83959) has shown various biological functions *in vitro* and in intact animals. Unlike the typical D<sub>1</sub> receptor agonists, SKF83959 does not stimulate the production of cyclic adenosine monophosphate (cAMP) via D<sub>1</sub>-like receptor-mediated activation of the G<sub>s</sub> protein<sup>[10–13]</sup>; instead, it selectively activates the G<sub>i</sub> protein via the D<sub>1</sub>-like receptor, which results in the production of inositol triphosphate<sup>[14–20]</sup>. In animals, this drug was found to increase

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eye blinking in monkeys and rats and to elicit excellent anti-Parkinsonism effects in a primate model as well as in a unilateral-lesioned rodent model<sup>[21–23]</sup>. The anti-Parkinsonism effects were shown to be independent of D<sub>1</sub> dopamine receptor-stimulated cAMP and may be associated with the drug-activated G<sub>q</sub>/phospholipase C pathway<sup>[23,24]</sup>.

In addition to the receptor-mediated events, recent data also indicated that the D<sub>1</sub> receptor-independent pharmacological effects also played important roles in SKF83959-mediated biological responses. For example, we found that potent neuronal protection of the drug was only partially dependent on the D<sub>1</sub> receptor<sup>[25]</sup> and that SKF83959 blocked Na<sup>+</sup> channels<sup>[26]</sup>, modulated the delayed rectifier K<sup>+</sup> channels<sup>[27]</sup> and promoted the spontaneous release of glutamate in rat somatosensory cortical neurons<sup>[28]</sup>.

In the present work, we examined whether SKF83959 effectively inhibited the uptake activity of the serotonin transporter (SERT), norepinephrine transporter (NET) and dopamine transporter (DAT), by functioning as a potent triple uptake inhibitor. Moreover, we also examined the anti-depressant activity of SKF83959 *in vivo*.

## Materials and methods

### Animals

Male C57BL/6J mice, weighing 18–20 g, were purchased from Shanghai Laboratory Animal Co, Ltd (Shanghai, China) and were housed in plastic cages (temperature: 21±1 °C) with air exchange every 20 min and an automatic 12 h light/dark cycle (light on from 7:00 AM to 19:00 PM). The animals were fed a standard laboratory diet, and water was provided *ad libitum*. All the experimental protocols were approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Materia Medica, Chinese Academy of Sciences (SIMM-2011-06-ZXC-07) and were in compliance with the Guidelines for the Care and Use of Laboratory Animals (National Research Council, China, 1996).

### Drugs and chemicals

(±)-SKF83959 was synthesized in the Synthetic Organic & Medicinal Chemistry Laboratory, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China). (1*R*,5*S*)-(+)-1-(3,4-Dichlorophenyl)-3-azabicyclo[3.1.0]hexane hydrochloride (DOV21947) was supplied by the Novel Technology Center of Pharmaceutical Chemistry, Shanghai Institute of Pharmaceutical Industry. [<sup>3</sup>H]-serotonin, [<sup>3</sup>H]-dopamine and [<sup>3</sup>H]-norepinephrine were purchased from PerkinElmer Inc (Waltham, MA, USA). Pargyline, tropolone and ascorbic acid were obtained from Sigma-Aldrich Co (St Louis, MO, USA). SKF83959, pargyline, tropolone and DOV21947 were dissolved in dimethyl sulfoxide at a concentration of 100 mmol/L stock solution. Prior to the experiments, the stock solutions were diluted with Hanks' Balanced Salt Solution (HBSS) buffer (NaCl 140 mmol/L, KCl 5.4 mmol/L, KH<sub>2</sub>PO<sub>4</sub> 0.4 mmol/L, NaHCO<sub>3</sub> 4.2 mmol/L, Na<sub>2</sub>HPO<sub>4</sub> 0.3 mmol/L, D-glucose 5.5 mmol/L, pH 7.2–7.4) to the designated concentrations (0.1 nmol/L–0.1 mmol/L).

## The inhibitory effects of SKF83959 on SERT, NET, and DAT

Stably expressed transporter Chinese hamster ovary (CHO) cell lines were generated in our laboratory and have been previously used for compound activity tests<sup>[29]</sup>. These stably-expressed transporter cell lines were cultured in a mixture of Dulbecco's modified Eagle's medium (DMEM) and F12 (1:1, *v/v*) supplemented with 10% fetal calf serum (FCS) and antibiotics (10 units/mL penicillin, 100 µg/mL streptomycin, and 100 µg/mL G418). The culture dishes were maintained in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub>.

We first investigated the inhibitory effects of SKF83959 on the uptake activity of SERT. The assays were performed as previously described<sup>[30]</sup>. Briefly, SERT-expressing CHO cells were seeded in 24-well cluster plates (Corning; NY, USA) at a density of 1.0×10<sup>6</sup> cells per well. After 12 h, the culture medium was removed, and 20 µL of SKF83959 or DOV21947 (final concentration: 0.1 nmol/L–0.1 mmol/L) and 160 µL HBSS buffer (containing 10 µmol/L pargyline, 10 µmol/L tropolone, and 10 µmol/L ascorbic acid) were added. After 10 min of incubation, [<sup>3</sup>H]-serotonin was added (final concentration: 2.4 nmol/L) for 10 min at 37°C. The uptake was terminated by rinsing the cells three times with ice-cold PBS buffer. The cells were lysed using 0.1 mL of 2 mol/L NaOH. Non-specific uptake was defined with Na<sup>+</sup>-free HBSS. The general procedures for norepinephrine and dopamine uptake were the same as those used for serotonin except for the different radiolabeled ligands<sup>[29]</sup>.

To further characterize the effects of SKF83959, we analyzed the kinetics of SKF83959 and DOV21947 on the corresponding transporters. For the K<sub>t</sub> measurement, the corresponding cells were incubated with different concentrations of radiolabeled ligand ([<sup>3</sup>H]-serotonin: 1.2–1280 nmol/L; [<sup>3</sup>H]-norepinephrine: 1.4–45 nmol/L; [<sup>3</sup>H]-dopamine: 1.6–100 nmol/L). The uptake was then performed as previously described. The K<sub>t</sub> values of the transporters were estimated using the Michaelis-Menten equation:

$$V = \frac{V_{\max} \cdot S}{K_t + S}$$

where *S* denotes the radiolabeled ligand concentration (nmol/L), *V*<sub>max</sub> denotes the maximal reuptake capacity (cpm/min·10<sup>6</sup> cell), and K<sub>t</sub> denotes the Michaelis-Menten constant for the transporter-mediated transport (nmol/L).

Next, the K<sub>i</sub> parameter was assayed. The saturation curves were re-examined in the presence of SKF83959 or DOV21947. Depending on the inhibitory pattern (competitive or non-competitive), the K<sub>i</sub> values were then calculated using the Michaelis-Menten competitive inhibition equation:

$$V = \frac{V_{\max} \cdot S}{K_t \left(1 + \frac{I}{K_i}\right) + S}$$

or noncompetitive inhibition equation:

$$V = \frac{V_{\max} \cdot S}{(K_t + S) \left(1 + \frac{I}{K_i}\right)}$$

where  $S$  denotes the radiolabeled ligand concentration (nmol/L),  $V_{\max}$  denotes the maximal reuptake capacity (cpm/min $\cdot 10^6$  cell),  $I$  denotes the concentration of SKF83959 or DOV21947 ( $\mu\text{mol/L}$ ), and  $K_i$  denotes the inhibition constant of SKF83959 or DOV21947 for the transporters ( $\mu\text{mol/L}$ ).

### Forced swimming test

The forced swimming test was performed as previously described<sup>[31]</sup>. The mice were placed in a transparent cylindrical beaker (height, ~25 cm; internal diameter, 10 cm) containing water (22–24°C) to a level of 15 cm. The water was always changed between trials. Thirty minutes before the test, SKF83959 or DOV21947 was intraperitoneally administered (2–8 mg/kg) to the mice. The immobility time was measured during a 5-min period by a trained observer who was blind to the treatment conditions. Each mouse was considered immobile if they were floating motionless or if they were only making the minimal amount of movements necessary to keep its head above the water surface.

### Tail-suspension test

The tail suspension test was performed as previously described<sup>[32]</sup>. C57BL/6J mice were individually suspended 75 cm above the tabletop with an adhesive tape placed approximately 1 cm from the tip of the tail. The immobility duration was recorded for 6 min. Thirty minutes before the test, SKF83959 or DOV21947 was intraperitoneally administered (2–8 mg/kg). The duration of the immobility was measured by a trained observer according to the double-blind test rule. The mice were considered immobile only if they were hung passively and demonstrated no motion.

### Data analysis

The  $IC_{50}$  values were expressed as the mean [95% confidence interval (95% CI)]. The difference between the  $IC_{50}$  was tested using weighted probit analysis. The other data were expressed as the mean $\pm$ SEM. For multiple comparisons of the parameters, one-way ANOVA and the Dunnett's *post-hoc* test were used. Differences were considered significant if  $P < 0.05$ . The data were analyzed using the Graphpad Prism software (GraphPad Software Inc, La Jolla, CA, USA, Version 5.0).

## Results

### Inhibition of SKF83959 on SERT, NET, and DAT

First, we tested the maximal inhibitory effect of SKF83959 on the three transporters. We found that SKF83959 (100  $\mu\text{mol/L}$ ) completely blocked the uptake activity. Moreover, the maximal inhibitory effect of SKF83959 was similar to that of DOV21947 ( $t$ -test,  $P > 0.05$ ).

Next, we studied the concentration-response relationship for SKF83959-inhibited uptake activity on the respective transporter. As shown in Figure 1 and Table 1, the  $IC_{50}$  of SKF83959 for SERT was similar to that of DOV21947 ( $t$ -test,  $P > 0.05$ ), but the  $IC_{50}$  values of SKF83959 for NET and DAT were greater than those of DOV21947 ( $t$ -test,  $P < 0.05$ ).

These data clearly demonstrated that SKF83959 functions as

a potent triple reuptake inhibitor.

### Effects of SKF83959 on the uptake kinetics of SERT, NET, and DAT

To further characterize the effects of SKF83959 on the three transporters, we analyzed the kinetics of the transporters and checked the effect of SKF83959 on the uptake kinetics. The relationship between the uptake activity and the substrate concentration was provided by the Michaelis-Menten equation.

As shown in Figure 2A and Table 2, SKF83959 and DOV21947 did not alter  $V_{\max}$  (one-way ANOVA,  $P > 0.05$ ), but significantly increased the value of  $K_i$  (one-way ANOVA,  $P < 0.05$ ), indicating that SKF83959 and DOV21947 were two competitive inhibitors for SERT. Moreover, the  $K_i$  value of SKF83959 was not significantly different from that of DOV21947 ( $t$ -test,  $P > 0.05$ ).

However, in contrast to the effect on SERT, both SKF83959 and DOV21947 reduced the  $V_{\max}$  value (one-way ANOVA,  $P < 0.05$ , compared to vehicle) but did not change the  $K_i$  value (one-way ANOVA,  $P > 0.05$ , compared to vehicle) in the NET uptake kinetics test, which was consistent with its noncompetitive pattern (Figure 2B, Table 2). The  $K_i$  of SKF83959 was greater than that of DOV21947 ( $t$ -test,  $P < 0.05$ ), indicating that the inhibitory activities of SKF83959 were weaker than that of DOV21947.

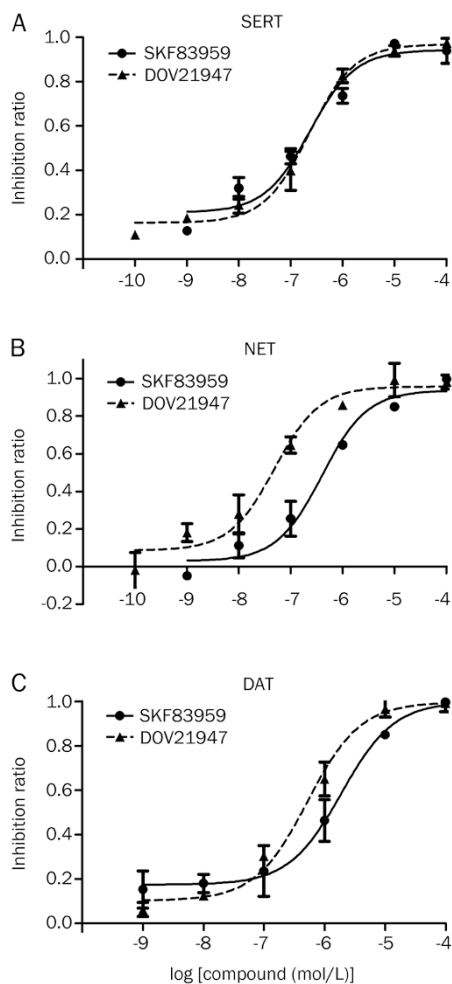
Similar to NET, SKF83959 also inhibited DAT in a noncompetitive manner. SKF83959 significantly decreased  $V_{\max}$  ( $t$ -test,  $P < 0.05$ ) without any change in  $K_i$  ( $t$ -test,  $P > 0.05$ ). Interestingly, in contrast to SKF83959, DOV21947 inhibited DAT in a competitive manner (Figure 2C, Table 2). The  $K_i$  of SKF83959 was greater than that of DOV21947 ( $t$ -test,  $P < 0.05$ ), indicating that the inhibitory activities of SKF83959 were weaker than that of DOV21947.

### SKF83959 elicits potent anti-depressant activity in animal models

Because SKF83959 is a novel potent triple reuptake inhibitor, we tested the anti-depressant activity of this drug in mice. The forced swimming and tail suspension tests are two widely used methods to detect the anti-depressant efficacy of experimental drugs.

As shown in Figure 3A, SKF83959 decreased the immobility time in the forced swimming test in a dose-dependent manner. For the vehicle treated-mice, the immobility time was 158.40 $\pm$ 10.31 s. The immobility time was significantly decreased (one-way ANOVA,  $P < 0.001$ ) in mice treated with SKF83959. Importantly, the reductions in the immobility time produced by SKF83959 were comparable to that produced by DOV21947 at the same doses (Figure 3A).

Similarly, SKF83959 significantly reduced the immobility time in the tail suspension test (Figure 3B, one-way ANOVA,  $P < 0.001$ ). In the vehicle group, the immobility time was 135.00 $\pm$ 7.44 s. A significant decrease was observed with 2 mg/kg of SKF83959 treatment. Furthermore, the reductions in the immobility time produced by SKF83959 were comparable to those in the immobility time produced by DOV21947 at the same doses (Figure 3B).



**Figure 1.** The concentration-response curves of SKF83959 and DOV21947 on SERT, NET, and DAT. The uptake assays were performed as described in the Methods section. Various concentrations of SKF83959 or DOV21947 were incubated with [ $^3$ H]-radiolabeled ligand for 10 min. The final concentration of [ $^3$ H]-serotonin was 2.4 nmol/L, [ $^3$ H]-norepinephrine was 1.4 nmol/L and [ $^3$ H]-dopamine was 1.6 nmol/L. The nonspecific uptake activity was determined using  $\text{Na}^+$ -free HBSS solution and the specific uptake activity was normalized as 100%. Each experiment was replicated at least three times. The data were expressed as the mean  $\pm$  SEM.

## Discussion

In the present study, we found that SKF83959 was an effective inhibitor for the uptake activity of SERT, NET, and DAT. We further determined that SKF83959 functioned as a SERT competitive inhibitor, and behaved as a noncompetitive inhibitor for NET and DAT, which differed from the well-known triple uptake inhibitor DOV21947. Moreover, we provided convincing data demonstrating that SKF83959 was a potent anti-depressant agent in the forced swimming test and tail suspension test in mice. The present study showed, for the first time, that SKF83959 is a novel triple uptake inhibitor that elicits an anti-depressant effect. This finding may provide a novel pharmacological mechanism for the understanding of various biological functions of this drug.

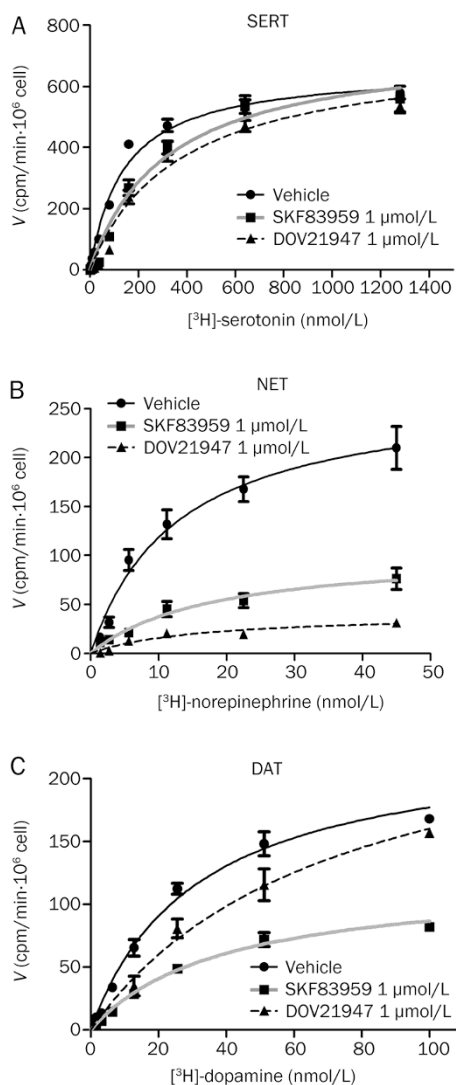
**Table 1.** Effects of SKF83959 and DOV21947 on the uptake activity of SERT, NET, and DAT.

		Compound	
		DOV21947	SKF83959
SERT	Maximal inhibiting ratio	95.10% $\pm$ 1.55%	97.35% $\pm$ 0.78%
	IC <sub>50</sub> (95% CI) (nmol/L)	187 (100–533)	242 (148–346)
NET	Maximal inhibiting ratio	99.65% $\pm$ 0.39%	98.14% $\pm$ 2.17%
	IC <sub>50</sub> (95% CI) (nmol/L)	93.5 (22.3–101)	381 (233–672) <sup>b</sup>
DAT	Maximal inhibiting ratio	99.45% $\pm$ 0.74%	99.20% $\pm$ 2.28%
	IC <sub>50</sub> (95% CI) (nmol/L)	533 (335–828)	1375 (1140–3170) <sup>b</sup>

The parameters were obtained from Figure 1. The values of IC<sub>50</sub> were expressed as mean [95% confidence interval (95% CI)]. The difference between IC<sub>50</sub> was tested by weighted probit analysis. Maximal Inhibiting ratio was expressed as mean  $\pm$  SEM. The nonspecific uptake activity was defined using  $\text{Na}^+$ -free HBSS. <sup>b</sup> $P < 0.05$  vs DOV21947.

Increasing the synaptic availability of biogenic amines is one important strategy used in anti-depressant therapy<sup>[33]</sup>. Following the application of dual uptake inhibitors (prototypic tricyclic anti-depressants and serotonin/norepinephrine uptake inhibitors) and the selective serotonin uptake inhibitor, triple uptake inhibitors were recently found to be promising chemical entities for the treatment of depression<sup>[34]</sup>, including DOV21947, PRC200SS, and GSK136070F. DOV21947 has demonstrated effective anti-depressant activity in a Phase III clinical study<sup>[34]</sup>. In addition, the most recent clinical studies have shown that DOV21947 begins to exert its anti-depressant activity after only one week of treatment<sup>[35]</sup>. The mean baseline score on the Montgomery-Åsberg Depression Rating Scale (MADRS) was significantly higher than that on the placebo-treated population. Our data indicated that SKF83959, with a benzazepine structure, is a novel and potent triple uptake inhibitor that elicits a comparable anti-depressant efficacy *in vivo* similar to DOV21947. Due to the difficulty in the design of triple uptake inhibitors<sup>[36]</sup>, the identification of a benzazepine-like structure as a novel category of triple uptake inhibitor may provide an alternative strategy in the development of anti-depressant drugs.

Analysis of the transporting kinetics of SKF83959 and DOV21947 revealed a significant difference between the two types of uptake inhibitors. We found that SKF83959 was a competitive inhibitor for SERT, but a noncompetitive inhibitor for NET and DAT. Consistent with previous reports<sup>[6]</sup>, we confirmed that DOV21947 was a competitive uptake inhibitor for DAT and SERT. Interestingly, we found that DOV21947 was a NET noncompetitive inhibitor, which has not been previously reported. Moreover, our data showed that the inhibitory potency of SKF83959 on SERT was similar to DOV21947, but was weaker on NET and DAT. Despite this difference, the same dose of SKF83959 and DOV21947 produced equivalent anti-depressant effects in different animal models (Figure 3). However, whether the differential inhibitory patterns in the kinetics between the two drugs contribute to the anti-depres-

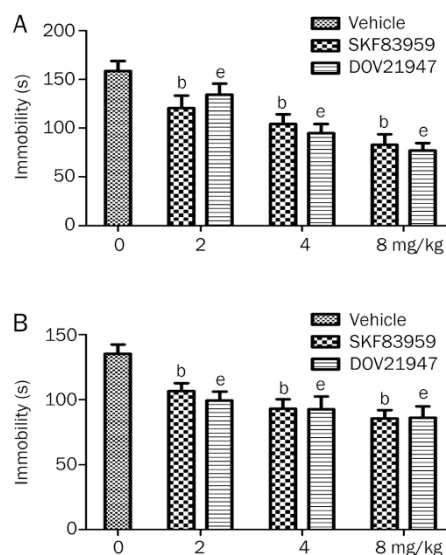


**Figure 2.** The effects of SKF83959 and DOV21947 on the transporting kinetics of SERT, NET, and DAT. The kinetics was assayed using [<sup>3</sup>H]-serotonin, [<sup>3</sup>H]-norepinephrine, or [<sup>3</sup>H]-dopamine in the presence of vehicle, SKF83959 or DOV21947. The nonspecific uptake activity was determined using Na<sup>+</sup>-free HBSS solution. Each experiment was replicated at least three times. The data were expressed as the mean±SEM.

**Table 2.** Effects of SKF83959 and DOV21947 on the transporting kinetics of SERT, NET, and DAT.

		Vehicle	Compound DOV21947	SKF83959
SERT	V <sub>max</sub> (cpm/min·10 <sup>6</sup> cells)	6581±305	7216±732	7426±580
	K <sub>i</sub> (nmol/L)	104.50±21.09	362.20±23.32 <sup>b</sup>	316.70±64.13 <sup>b</sup>
	K <sub>i</sub> (μmol/L)	-	0.89±0.24	1.43±0.45
NET	V <sub>max</sub> (cpm/min·10 <sup>6</sup> cells)	246.30±23.61	40.70±9.00 <sup>b</sup>	104.5±11.05 <sup>be</sup>
	K <sub>i</sub> (nmol/L)	13.24±3.13	13.69±2.21	13.30±2.19
	K <sub>i</sub> (μmol/L)	-	0.18±0.04	0.60±0.08 <sup>e</sup>
DAT	V <sub>max</sub> (cpm/min·10 <sup>6</sup> cells)	224.00±14.6	265.20±29.29	116.00±7.45 <sup>b</sup>
	K <sub>i</sub> (nmol/L)	29.65±4.73	68.05±14.11 <sup>b</sup>	30.81±3.37
	K <sub>i</sub> (μmol/L)	-	1.47±0.31	9.01±0.80 <sup>e</sup>

The parameters were obtained from Figure 2. Data were expressed as mean±SEM. <sup>b</sup>P<0.05 vs vehicle. <sup>e</sup>P<0.05 vs DOV21947.



**Figure 3.** Effects of SKF83959 on immobility in the forced swimming test and the tail suspension test. The behavioral assays were performed as described in the Methods section. Each experimental group contained 10 animals. (A) Forced swimming test. (B) Tail suspension test. The data were presented as the mean±SEM and analyzed using one-way ANOVA analysis followed by Dunnett's *post-hoc* test. <sup>b</sup>P<0.05, <sup>e</sup>P<0.05 vs the vehicle group.

sant response remains unknown.

It appears that the IC<sub>50</sub> and K<sub>i</sub> values in our experiments were greater than those previously reported by Skolnick *et al*. In fact, our experiment was completely different from that of Skolnick<sup>[6]</sup>. Our experiment was based on uptake kinetics, whereas that of Skilnick was based on receptor binding. Thus, the K<sub>i</sub> values between the two studies were completely different although they share a common symbol (K<sub>i</sub>). In addition, the difference in the cell lines used (CHO *vs* HEK293) may also account for the discrepancy. It is well known that the IC<sub>50</sub> value is closely dependent on the transporter density in the cell membrane as well as the expression level of the transporters. In addition, the difference in the transporters of the species employed (human transporter and rat transporter) also

produced some distinct potency in the *in vitro* cell models<sup>[10]</sup>.

In summary, the present data indicated that SKF83959 displayed potent anti-depressant effects. The identification of SKF83959, a benzazepine structure, as a triple uptake inhibitor may provide a new avenue for the discovery of novel anti-depressant drugs.

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### Author contribution

Xing FANG, Lin GUO, Xue-chu ZHEN, and Bin ZHAO designed the research; Lin GUO, Xing FANG, and Jia JIA performed the research; Yong-yong ZHENG, Jian-qi LI, and Ao ZHANG contributed new reagents and discussed the results; Guo-zhang JIN and Xing FANG analyzed the data; and Lin GUO and Xue-chu ZHEN wrote the manuscript.

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