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## Effect of Xanthone Derivatives on Animal Models of Depression

Xu Zhao, MD<sup>1</sup>, Qunying Chen, MD<sup>2</sup>, Yuan Liu, BS<sup>1</sup>, Chao Xia, BS<sup>3</sup>, Jincheng Shi, MD<sup>1</sup>, Maqing Zheng, PhD<sup>1,\*</sup>



<sup>1</sup> College of Pharmacy, Nanjing University of Technology, Nanjing, China

<sup>2</sup> Department of Pharmacology, Jiangsu Institute of Materia Medica Co, Ltd, Nanjing University of Technology, Nanjing, China

<sup>3</sup> Plant Chemical Laboratory, Jiangsu Institute of Materia Medica Co, Ltd, Nanjing University of Technology, Nanjing, China

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## ABSTRACT

**Background:** Extracts of the plant *Hypericum perforatum* L. have been traditionally used in folk medicine for the treatment of depressive disorders. Xanthone, a component of *Hypericum perforatum* L., has been shown to be effective in animal models of depression.

**Objective:** We investigated if 2 xanthone derivatives (1101 and 1105) were as effective as venlafaxine, which is a serotonin–norepinephrine reuptake inhibitor and was used as a positive control, in animal models of depression.

**Methods:** A series of derivatives from xanthone were designed and synthesized. After preliminary experiments, 2 xanthone derivatives (1101 and 1105) were considered to be effective in our mouse depression model. To further determine their effects on depression, classical behavioral despair animal models (forced swim and tail suspension tests) were used to assess the efficacies of these derivatives, whereas venlafaxine hydrochloride was used as a positive control. Oral acute toxicity studies were used to determine if the derivatives were toxic in mice.

**Results:** The oral acute toxicity studies of 2 xanthone derivatives (1101 and 1105) did not show any toxic effect until the dose at 1000 mg/kg body weight, and xanthone derivatives 1101 and 1105 resulted in a significant decrease of the immobility period (in seconds) compared with the untreated control group during the forced swim test with rats (dose = 12 mg/kg;  $P < 0.05$ ) and mice (dose = 25 mg/kg;  $P < 0.001$ ). At lower doses, derivatives 1101 and 1105 also decreased the immobility period of rats and mice during the forced swim test but significant differences were only found in mice compared with the untreated control group ( $P < 0.05$ ). No difference was found between the groups treated with xanthone derivatives and the positive control group during the swimming period in both mice (dose = 25 mg/kg) and rats (dose = 12 mg/kg) ( $P > 0.05$ ). In the tail suspension test, derivatives 1101 and 1105 produced marked effects with regard to the motion of mice ( $P < 0.01$  or 0.001, respectively) and the derivatives were also noted to have some effects on rats at a dose of 12 mg/kg ( $P < 0.05$ ). Compared with the positive venlafaxine control group, no differences were found between those treated with either derivative 1101 or derivative 1105 and venlafaxine ( $P > 0.05$ ).

**Conclusions:** Within certain dose ranges, xanthone derivatives 1101 and 1105 have similar effects to venlafaxine hydrochloride in the treatment of depression as suggested by behavioral despair animal models using rats and mice.

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## Introduction

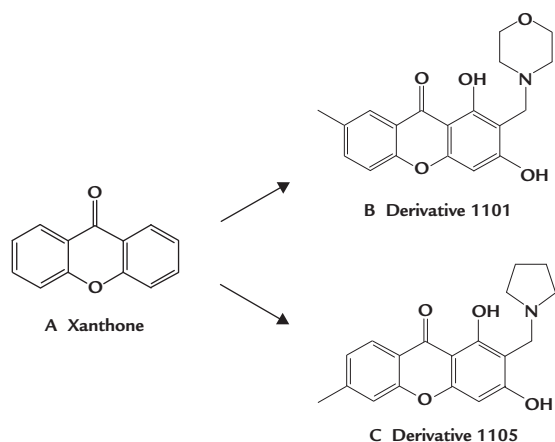
Depression is a common mental disorder characterized by pronounced and long-lasting depressed mood, as well as a variety of additional symptoms (behavioral, affective, and cognitive). The

number of patients with depression has increased dramatically in recent decades and, according to the World Health Organization, it is projected to become the second largest contributor to the global burden of disease by the year 2020.<sup>1</sup>

Drug treatment is 1 of the most effective tools used to deal with depression. At present, a variety of antidepressant medications are available that have shown beneficial effects. However, all are known to exert adverse side effects, and some are very expensive. Additional treatment modalities with little risk, credible benefit, and moderate costs would be useful additions to depression management.

\* Address correspondence to: Maqing Zheng, PhD, College of Pharmacy, Nanjing University of Technology, Xinmofan Rd, No. 5, Gulou District, Nanjing 210009, China.

E-mail address: [qingm109@qq.com](mailto:qingm109@qq.com) (M. Zheng).



**Fig. 1.** Chemical structures of (A) xanthone, and the derivatives of (B) xanthone 1101 and (C) xanthone 1105 used in this study

Chinese herbal medicine has been considered a source from which such compounds could be selected for investigation.<sup>2</sup> In recent years, *Hypericum perforatum* L. (HPL) has become a focus of research interest and investigated for its therapeutic effects with regard to mood disorders, and its antidepressive action has been demonstrated in animals and humans.<sup>3</sup> Consequently, in Germany, extracts of HPL have been licensed for the treatment of depression. Indeed, HPL extracts have been found to be more effective than placebos in the treatment of mild to moderate depression,<sup>4</sup> and as effective as several tricyclic antidepressants and fluoxetine.<sup>5</sup> The antidepressant activity of HPL is thought to stem from the presence of flavonoids. Xanthone (Figure 1A) is 1 such flavonoid, and can be isolated from the aerial parts of HPL.<sup>6</sup> Furthermore, xanthone has been shown to be a prototype drug useful in the treatment of depression,<sup>7</sup> and its derivatives have been shown to have potential antidepressant activity via forced swim tests (FSTs).<sup>8</sup>

To modify the structure of xanthone and promote its activity to compete with selective serotonin reuptake inhibitors in terms of efficacy, a series of xanthone derivatives were designed and synthesized in this study. After preliminary experiments, 2 derivatives (1101 and 1105) (Figure 1B–1C) were considered to be effective following mice swimming test models. Subsequently, FSTs and tail suspension tests (TSTs) were used to detect their effects with regard to depression in rats and mice. Our results suggest that the 2 derivatives effectively improve behavioral despair symptoms in rats and mice compared with negative controls and may be as effective as venlafaxine at certain doses.

## Materials and Methods

### Animals

Male imprinting control region mice weighing between 18 and 22 g were commercially obtained from the Sino-British SIPPR/BK Lab (Shanghai, China). Male Wistar rats weighing between 150 and 170 g were obtained from the experimental animal center of the Military Medical Science Academy of the Chinese People's Liberation Army (Beijing, China). The animals were maintained under a standard 12-hour light/dark cycle (lights on at 8:00 AM) at a constant temperature of 22°C ( $\pm 1^\circ\text{C}$ ) and mean (SD) relative humidity of 45% (15%) with free access to food and water, except for periods of water and food deprivation. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. The experiments were performed between

9:00 AM and 3:00 PM. The procedures in this study were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Maximal effort was made to minimize animal trauma and the number of animals necessary for the acquisition of reliable data. All experiments were approved by our local ethics committee.

### Oral acute toxicity studies in mice

The derivative (1101 or 1105) was weighed and resuspended with 0.5% hydroxymethyl cellulose aqueous solution to obtain the desired concentrations. Mice were starved overnight (16 hours) before feeding with a single oral dose of derivative. Animals were divided into 7 groups of 10 (5 males and 5 females for each group). Mice in each group were fed derivatives (1101 or 1105) at a single oral dose of 250, 500, or 1000 mg/kg body weight for each derivative, respectively; the control group received a single oral dose of 0.5% hydroxymethyl cellulose aqueous solution. General behavior of each mouse was observed continuously for 1 hour after each dose, intermittently every 4 hours for three times, and thereafter over a period of 24 hours.<sup>9</sup> Animals were observed for up to 14 days for any sign of toxicity (eg, behavior change related to central nervous, cardiovascular, and gastrointestinal systems), body weight change, and water and food consumption. At the end of the observation period, all animals were sacrificed under ether anesthesia and vital organs (heart, lung, liver, spleen, and kidney) were removed from all animals for gross and histopathologic examination.

### FST

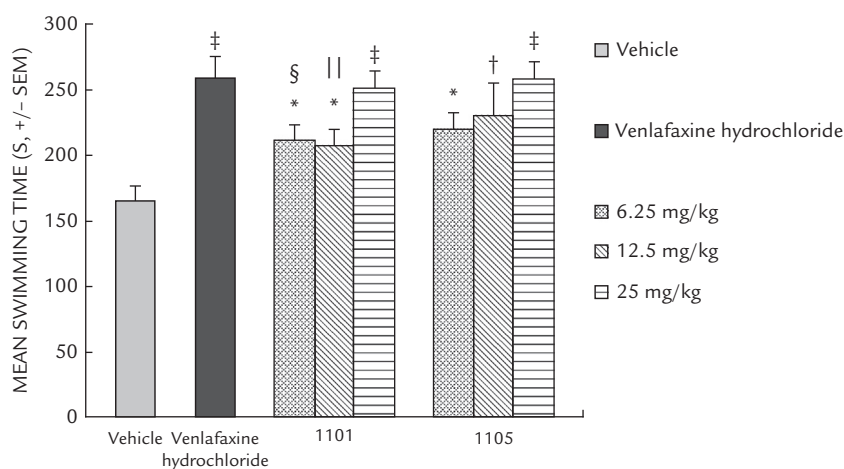
Mice were individually forced to swim inside a polycarbonate cylinder (height = 24 cm; diameter = 16 cm) containing 20 cm water at 25°C. Mice were allowed to swim for a total of 6 minutes; a camcorder (Sony Handycam; Sony Corporation of America, New York, New York) recorded the swim session. Digital video output was analyzed by a computer running SMART Video Tracking System software (Panlab, Barcelona, Spain). The swimming time during the experiment was measured with SMART software. It should be noted that the water was changed before the next animal was placed into the cylinder. Clean water was used for each behavior trial because used water has been shown to alter behavior due to an alarm signal.<sup>10</sup> Mice were returned to cages after testing and dried.

The modified rat FST described by Lucki<sup>11</sup> suggests that rats should swim for 15 minutes in a polycarbonate cylinder (height = 46 cm; diameter = 21 cm) containing 30 cm water at 25°C, with a 5-minute test period recorded. In our study we modified this protocol and allowed rats to swim for 12 minutes. The swimming sessions were recorded by camera but only the final 8 minutes were analyzed by a SMART Video Tracking system. In accordance with the mouse FST protocol, the water was changed after each animal was tested, and rats were returned to the cages after swimming and dried.

### TST

The TST was carried out according to the method of Steru et al.<sup>12</sup> Mice were suspended on a small metal hook fixed on the top of a box via adhesive tape placed approximately 1 cm from the tip of the tail. The duration of the immobility period was recorded with a SMART system. The recordings were performed for a total of 6 minutes and data were analyzed with SMART software.

The TSTs with rats were performed as described above for mice; however, the recording time was 9 minutes. The final 6 minutes of the process were analyzed with SMART software.



**Fig. 2.** Antidepressant-like effect of xanthone derivatives in mice following forced swim test. Data are represented as the mean seconds (SEM) of the total swim period during a 6-minute session. Xanthone derivatives 1101 and 1105 were administered 1 hour before testing and venlafaxine hydrochloride was administered 45 minutes before testing. Each group contained 8 to 9 mice. \* $P < 0.05$ ; † $P < 0.01$ ; ‡ $P < 0.001$  versus vehicle-treated mice; § $P < 0.05$ ; || $P < 0.01$  versus venlafaxine hydrochloride-treated mice.

### Drugs and treatment

Xanthone derivatives 1101 and 1105 were provided by the Plant Chemical Laboratory of Jiangsu Institute of Materia Medica Co, Ltd (Nanjing, China). Venlafaxine hydrochloride was obtained from Chengdu Kanghong Pharmaceutical Group Co, Ltd (Chengdu, China). All the derivatives were suspended in 0.5% hydroxymethyl cellulose aqueous solution and diluted to the desired concentration. Hydroxymethyl cellulose aqueous solution (0.5%) was used as a negative control.

Mice were given oral doses of 6.25, 12.5, or 25 mg/kg/d derivative 1101 or 1105, 16 mg/kg/d venlafaxine hydrochloride, or the negative control vehicle for 3 days. At Day 3, mice were given derivatives 1101 or 1105 or the negative control vehicle 1 hour before the swimming test. Positive control animals received venlafaxine hydrochloride 45 minutes before the test. SMART software was used for data processing.

Rats were given oral doses of 3, 6, or 12 mg/kg/d derivative 1101 or 1105, 8 mg/kg/d venlafaxine hydrochloride, or the negative control vehicle for 3 days. Other procedures were carried out in accordance with the mouse FST protocol.

All procedures were performed single-blind; that is, 1 researcher administered the drugs and another researcher performed data collection.

### Statistical analysis

Results are expressed as mean seconds (SEM). All data were analyzed using SPSS 13.0 software (IBM-SPSS Inc, Armonk, New York). The significance of the difference in the responses of the treatment groups in comparison to the control was determined by 1-way ANOVA. Fisher least squares difference test was used to further analyze significant differences. All analyses were 2-tailed and used an  $\alpha \leq 0.05$  to determine significance.

## Results

### Acute oral toxicity study

The toxicity of derivatives (1101 and 1105) when given as a single oral dose in mice was investigated to define the optimal dose of derivatives to be used for evaluation of its *in vivo* antidepressant activity in behavioral despair animal models.

Results indicated virtually no toxicity at a single oral dose of 1000 mg/kg body weight for each derivative (1101 or 1105). All mice survived. There was neither sign of toxicity nor significant change in water and food consumption and body weights of mice in all groups during the 14-day observation period. The gross examination of vital organs (ie, heart, lung, liver, spleen, and kidney) in all treated (all dose levels) and control groups were similar either in size or cell morphology. Therefore, it was concluded that the dose up to 1000 mg/kg for each derivative (1101 or 1105) was safe.

### Effect of xanthone derivatives on FSTs

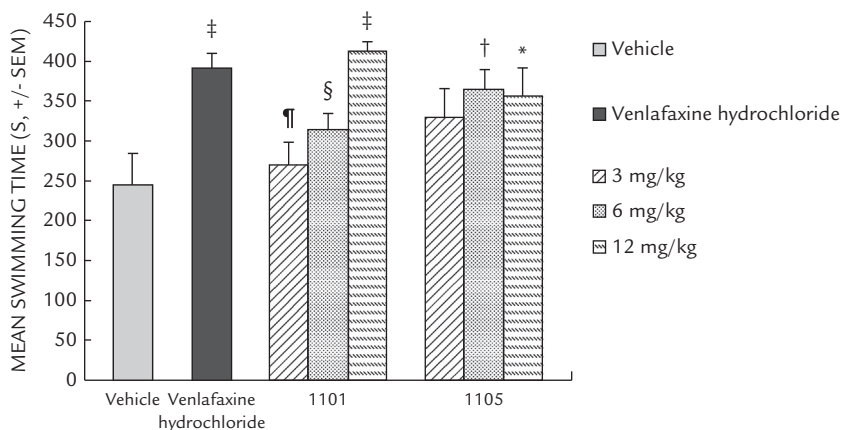
Our experiment was carried out according to a previously described method.<sup>13</sup> After the initial 2 to 3 minutes of vigorous activity, the animals displayed a period of immobility and floated with minimal movements. Some animals remained floating passively in the water in a slightly hunched but upright position with the nose above the surface of the water. All of the administered substances investigated in our study, including venlafaxine hydrochloride, increased the swimming period in mice (in seconds) compared with the negative control group in mouse FSTs. **Figure 2** and **Table I** show that venlafaxine hydrochloride (16 mg/kg) significantly increased the swimming period of mice ( $P < 0.001$ ). The tested substances 1101 and 1105 significantly prolonged the swimming period of mice also within a dose range of 6.25 to 25 mg/kg ( $P < 0.05$  or 0.001). No difference was found

**Table I**

ANOVA results for mice forced swim test (MFST) and mice tail suspension test (MTST) following administration of substances.

Test	Mouse groups			
	F	P	F	P
MFST	8.235	0.000	5.496	0.001
MTST	14.495	0.000	18.009	0.000

\* Vehicle (negative control) group received a single oral dose of 0.5% hydroxymethyl cellulose aqueous solution.



**Fig. 3.** Effects of xanthone derivatives in the rat forced swim test. Data are represented as the mean seconds (SEM) of the total swim period during the final 8 minutes of a 12-minute session. Xanthone derivatives 1101 and 1105 were administered 1 hour before testing and venlafaxine hydrochloride was administered 45 minutes before testing. Each group contained 8 or 9 rats.  $^*P < 0.05$ ;  $^{\dagger}P < 0.01$ ;  $^{\ddagger}P < 0.001$  versus vehicle-treated rats;  $^{\S}P < 0.05$ ;  $^{\P}P < 0.001$  versus venlafaxine hydrochloride-treated rats.

between the mice treated with substances 1105 or 1101 (25 mg/kg) and the positive control group ( $P > 0.05$ ).

To observe the effect of these substances in different animal models, male Wistar rats were also assessed. During the experiment, the recording time was modified to 8 minutes. Our results show that derivative 1101 and derivative 1105 at a dose of 12 mg/kg resulted in a decrease of the immobility period with respect to the negative control ( $P < 0.05$ ) (Figure 3 and Table II). In addition, derivative 1105 at a dose of 6 mg/kg resulted in a decrease of the immobility period with respect to the negative control ( $P < 0.01$ ) (Figure 3 and Table II). Lower doses were noted to decrease the immobility period, but no significant difference was found compared with the negative control group. Venlafaxine hydrochloride (8 mg/kg) increased the swimming period significantly in rats ( $P < 0.001$ ), which was in accordance with the FSTs performed with mice. No significant difference was found between rats that received derivative 1105 or 1101 (12 mg/kg) and the positive control ( $P > 0.05$ ).

#### Effect of xanthone derivatives on TSTs

In the experiments, oral doses of 6.25, 12.5, or 25 mg/kg derivatives 1101 or 1105 significantly increased the activity period of mice ( $P < 0.01$  or 0.001, respectively) compared with the negative control group. Venlafaxine hydrochloride (16 mg/kg) also resulted in a decrease of the immobility period with respect to the negative control group ( $P < 0.001$ ) (Figure 4 and Table I). No significant difference was found between substances 1105 or 1101 (25 mg/kg) and the positive control group with regard to the activity period during the mouse TST ( $P > 0.05$ ).

**Table II**

ANOVA results for rat forced swim test (RFST) and rat tail suspension test (RTST) following administration of substances.

Test	Rat groups		Rat groups	
	F	P	F	P
RFST	8.671	0.000	3.197	0.024
RTST	1.628	0.190	2.519	0.060

\* Vehicle (negative control) group received a single oral dose of 0.5% hydroxymethyl cellulose aqueous solution.

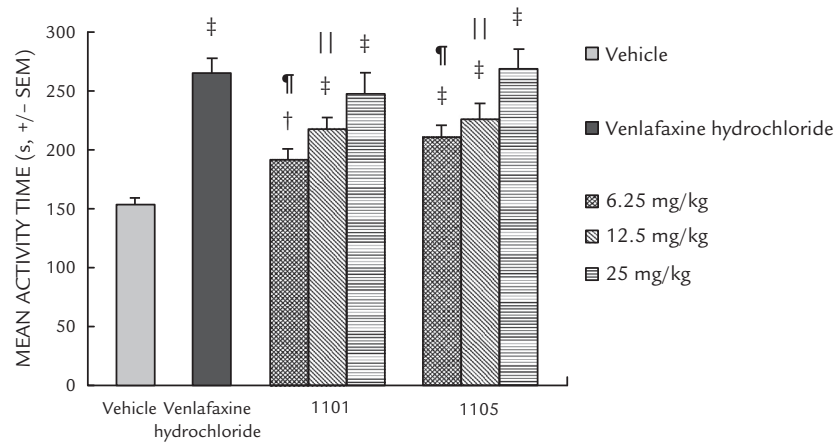
In the TSTs of rats all therapeutic agents decreased the immobility period, but only a dose of 12 mg/kg 1101 or 1105 resulted in a significant difference ( $P < 0.05$ ) compared with the negative control group. Although venlafaxine hydrochloride (8 mg/kg) decreased the immobility period of rats, no difference was found between the venlafaxine group and the control group (Figure 5 and Table II). No significant difference was found between substances 1105 and 1101 and the positive control group with regard to the activity period of rats during the suspension tests ( $P > 0.05$ ).

#### Discussion

Clinical depression is a common, chronic, recurrent, and debilitating condition associated with severe morbidity and significant mortality.<sup>14</sup> Traditional and current pharmacotherapies for depression have targeted monoaminergic systems, especially the noradrenergic and serotonergic systems. Although many antidepressants have been used in clinical settings, a number of them lack efficacy in more severe forms of depression.<sup>15</sup> Consequently, the discovery of effective antidepressants is of great importance.

HPL, commonly known as St John's wort, is a member of the *Hypericaceae* family and is a herbaceous perennial plant that is native to Europe, Northern Africa, Northern America, and China.<sup>16</sup> HPL extracts contain at least 10 constituents or groups of components that may contribute to its pharmacologic effects. These include naphthodianthrone (ie, hypericin, the content of which is standardized in most available preparations), flavonoids (ie, quercetin), xanthones, and biflavonoids.<sup>4</sup> HPL extracts may modulate the activity of several neurotransmitters in the brain in a specific manner depending on the concentrations of the active derivatives contained within the extract, especially the flavonoids, which have been shown to effectively inhibit monoamine oxidase activity.<sup>17</sup> Previous research has suggested that acute administration of large doses of HPL extracts can change the content of neurotransmitters; that is, 5-hydroxytryptamine, noradrenaline, and dopamine, which are involved in the pathophysiology of mood disorders.<sup>18</sup> The more the extract was administered, the more obvious and effective the influence was throughout the brain, which involved the diencephalon and brainstem, which has recently been implicated in the pathophysiology of depression.<sup>18</sup>

A component of HPL, xanthone has been shown to be effective in mood disorders, such as depression.<sup>7</sup> According to literature



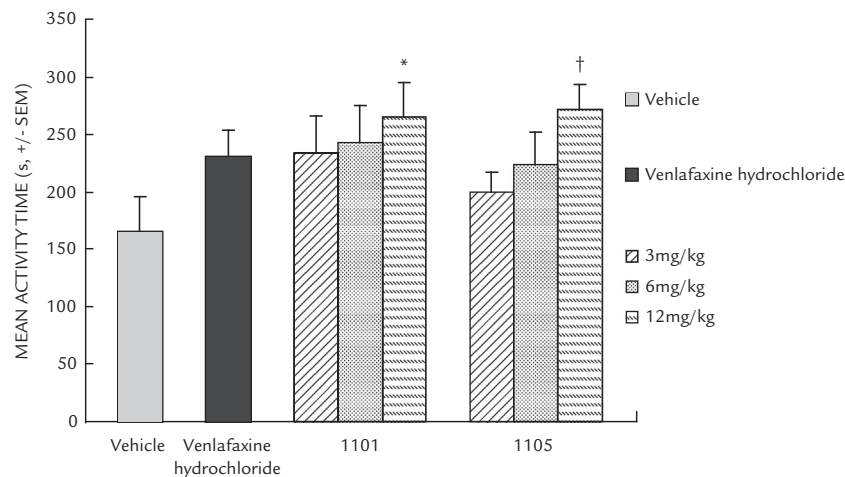
**Fig. 4.** Antidepressant-like effects of xanthone derivatives on tail suspension tests with mice. Data are represented as the mean seconds (SEM) of the total activity period during a 6-minute session. Xanthone derivatives 1101 and 1105 were administered 1 hour before testing and venlafaxine hydrochloride was administered 45 minutes before testing. Each group contained 8 or 9 mice.  $^{\dagger}P < 0.01$ ;  $^{\ddagger}P < 0.001$  versus vehicle-treated mice;  $^{\#}P < 0.01$ ;  $^{*}P < 0.001$  versus venlafaxine hydrochloride-treated mice.

procedures, we consequently separated, purified, and modified the HPL to obtain 10 derivatives of xanthone. To verify their effects on depression, FSTs and TSTs with mice were used in a preliminary experiment, and venlafaxine was used as a positive control. According to Kulkarni et al.,<sup>19</sup> the dose range of venlafaxine for mice in mouse TSTs should be 2 mg/kg/d to 16 mg/kg/d. Consequently, we selected 16 mg/kg/d as the appropriate venlafaxine positive control dose for the mice in our study. With regard to rats, a dose of 8 mg/kg/d venlafaxine for 3 successive days was selected as the appropriate dose. We calculated this according to the dose conversion between different kinds of animals, and we then conducted preliminary experiments with rats to ensure that 8 mg/kg/d was the most appropriate positive control dose. According to the Kulkarni et al.,<sup>19</sup> drugs should be administered 30 minutes before testing. But in our preliminary tests, administering the positive control 30 minutes before testing and the derivatives 45 minutes before testing had no effect. Following our preliminary tests, we found it reasonable to administer venlafaxine 45 minutes before testing and our test compounds 1 hour before testing. The results of preliminary tests revealed that 2 derivatives (1101 and 1105) were effective in the behavioral despair animal models. We subsequently repeated these experiments in both mice and rats, and showed that derivatives 1101 and

1105 were as effective in these models as venlafaxine (a serotonin-norepinephrine reuptake inhibitor), which was used as a positive control. This was particularly evident when high doses of our derivatives were used.

In our studies, we found that mice and rats responded differently to the therapeutic agents administered during the experiments, with rats appearing to be especially sensitive. In preliminary rat FSTs we were unable to repeat the results using the positive venlafaxine control and the Lucki method<sup>11</sup> because the rats struggled vigorously in the water at first, and then floated with minimum movements, even when venlafaxine was administered during the recording time. Consequently, we reduced the swimming time to 12 minutes and increased the recording time to 8 minutes, which permitted an observable difference between the negative control group and the positive venlafaxine control group ( $P < 0.05$ , data not shown). In the rat TSTs individual differences in the negative control group were larger than in the other groups and we believe this resulted in no significant difference being observable between the negative control group and the treated groups (Table II). We believe that animal strain may have caused this variance, as reported in a review by Cryan et al.<sup>20</sup>

According to a Cochrane review focused on HPL,<sup>4</sup> the tolerability of HPL preparations was found to be good following assessment of a number of randomized controlled clinical trials, and fewer adverse



**Fig. 5.** Effects of xanthone derivatives in rat tail suspension tests. Data are represented as the mean seconds (SEM) of the total activity period during the final 6 minutes of a 9-minute session. Xanthone derivatives 1101 and 1105 were administered 1 hour before testing and venlafaxine hydrochloride was administered 45 minutes before testing. Each group contained 8 or 9 rats.  $^*P < 0.05$ ;  $^{\dagger}P < 0.01$  versus vehicle-treated rats.

effects were noted than with use of selective serotonin reuptake inhibitors. In our study, acute toxicity studies of derivatives 1101 and 1105 revealed the nontoxic nature of the derivatives at doses up to 1000 mg/kg, and no other toxic reactions were found with the selected doses through the conclusion of the study.

## Conclusions

The results of our study suggest that xanthone derivatives 1101 and 1105 have antidepressant-like effects as demonstrated by behavioral despair tests (FST and TST), and exert very little overt toxicity. The antidepressant effects of these 2 xanthone derivatives are consistent with the research of Jastrzebska Wiesek et al<sup>8</sup> regarding xanthone derivatives and their influence on the outcome of FSTs. According to our study, these 2 derivatives may be useful in the treatment of depression; however, further research is needed to investigate their effects on chronic, unpredictable, mild stress models and neurotransmitter release in the brain to determine their mechanism of action in behavioral despair models. We believe that the selection of leading derivatives from natural products will become an accepted, effective tool for the treatment of a variety of health conditions, including depression, in the near future and our study adds weight to the effective application of such derivatives in the medical field.

## Acknowledgements

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## Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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