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Chapter

The BDNF Loop 4 Dipeptide Mimetic Bis(*N*-monosuccinyl-L-seryl-L-lysine) hexamethylenediamide Is Active in a Depression Model in Mice after Acute Oral Administration

Polina Povarnina, Yulia N. Firsova, Anna V. Tallerova, Armen G. Mezhlumyan, Sergey V. Kruglov, Tatiana A. Antipova, Tatiana A. Gudasheva and Sergey B. Seredenin

Abstract

Low-molecular mimetic BDNF GSB-106, which is a substituted dimeric dipeptide, bis(N-monosuccinyl-L-seryl-L-lysine) hexamethylenediamide, was designed and synthesized in the V. V. Zakusov Research Institute of Pharmacology. The dipeptide activates TrkB, PI3K/AKT, and MAPK/ ERK. GSB-106 is being developed as a potential antidepressant. Its antidepressant activity was detected in a number of rodent tests (0.1–1.0 mg/kg, ip; 0.5–5.0 mg/kg, po). In the present study, GSB-106 was shown to completely eliminate the manifestation of anhedonia in the sucrose preference test and to increase disturbed hippocampal synaptogenesis at acute oral administration (0.1 mg/kg) after 10-day social defeat stress in C57Bl/6 mice.

Keywords: BDNF, depression, dipeptide mimetic GSB-106, anhedonia, synaptogenesis, synaptophysin

1. Introduction

Depression is one of the most widespread mental disorders leading to social disadaptation. According to the WHO data in 2012, there were more than 350 million people suffering from depression. Modern antidepressants require long-term use to achieve a therapeutic effect, while their effectiveness does not exceed 60% [1]. Therefore, the creation of antidepressants with new action mechanisms is regarded as one of the most pressing pharmacology problems.

Fundamental studies established that the pathogenesis of depression was associated with impaired neuroplasticity in the hippocampus and the prefrontal cortex, caused by deficit of brain-derived neurotrophic factor (BDNF) [2]. The clinical

evidence demonstrates that BDNF levels in blood plasma decrease in depression and resolve as the result of antidepressant therapy [3]. A reduced BDNF content in the prefrontal cortex and hippocampus was found in suicide victims [4]. The antidepressant properties of BDNF were investigated based on its physiological functions. The antidepressant effect of neurotrophin was revealed after central administration in different depression models in rodents [5]. Antidepressant activity was also experimentally established for BDNF mimetic 7,8-dihydroxyflavone, an antagonist of BDNF-specific TrkB receptors [6].

Following the discovery of antidepressant properties of ketamine, over the past decade, a lot of efforts have been made to create new antidepressants with a glutamatergic mechanism of action without ketamine-like side effects. To date, one of them, rapastinel, a modulator of NMDA receptors, is in a third phase of clinical trials [7]. From the theoretical point of view, the most important fact is that antidepressant effect of ketamine and other glutamatergic drugs is mediated by activation of BDNF-TrkB-Akt-mTORC1-signaling cascade which leads to enhanced synaptogenesis [8]. The data proving that mTOR inhibition leads to the disappearance of ketamine antidepressant effects serve as pharmacological confirmation of this conclusion [8].

Thus, both pathophysiological evidence and results of experimental and clinical pharmacological studies demonstrate the feasibility of using the BDNF-TrkB receptor system as a pharmacological target of search for new antidepressants.

A mimetic of the fourth loop of BDNF, GSB-106, which is a substituted dimeric dipeptide, bis(N-monosuccinyl-L-seryl-L-lysine)hexamethylenediamide, was designed and synthesized in the V. V. Zakusov Research Institute of Pharmacology [9]. GSB-106 was established to activate BDNF-specific TrkB receptors and their main post-receptor signaling pathways—PI3K/AKT and MAPK/ERK [10]. GSB-106 demonstrated antidepressant activity at intraperitoneal (i.p.) (0.1–1.0 mg/kg) and oral (0.5–5.0 mg/kg) administration in a number of rodent tests [11, 12]. GSB-106 was also shown to stimulate neurogenesis and synaptogenesis in mouse hippocampus [13, 14].

A tablet dosage form of GSB-106 for oral administration was developed in the Technological Department of the Institute (application for a patent of the Russian Federation 2018107362 from 28 02 2018) to create a drug on the basis of the substance. The dosage form of GSB-106 was shown to be active at doses of 0.01–5.0 mg/kg in forced swimming test in mice and to exceed the "gold standard" of antidepressants, amitriptyline, by intensity of effects [11].

The aim of the current study was to investigate antidepressant effects of the tablet dosage form of GSB-106 at acute administration in a model of depression-like state in mice caused by social defeat stress, in comparison with the widely used antidepressant amitriptyline, having affinity to TrkB receptors according to some data [15]. This model is considered to be one of the most appropriate, since it reproduces the main behavioral and neurobiological signs of depression [16].

2. Materials and methods

2.1 Drugs

GSB-106 (bis(N-monosuccinyl-L-seryl-L-lysine)hexamethylenediamide) was synthesized in the Department of Medicinal Chemistry of the V. V. Zakusov Research Institute of Pharmacology, as described previously [9]. The tablet dosage form of GSB-106 was developed in the Technological Department of the V. V. Zakusov Research Institute of Pharmacology, as described (application for a patent of the

Russian Federation 2018107362 from 28 02 2018). The form contained 1% of GSB-106 and 99% of filler, consisting of lactose, microcrystalline cellulose, polyethylene glycol-polyvinyl alcohol copolymer, and magnesium stearate. The tablet dosage form of amitriptyline was purchased from Federal State Unitary Enterprise "Moscow Endocrine Plant" (Russia).

2.2 Animals

Male adult C57BL/6 mice weighing 18–20 g and male adult outbred mice weighing 25–28 g were used in the study. The animals were obtained from the "Stolbovaya" Central Laboratory for Animal Breeding (Moscow Region, Russia). The animals were housed in a vivarium with a natural change of light regime and free access to standard pelleted food and water. The study was carried out in accordance with the Order of the Ministry of Health Care and Social Development of the Russian Federation No. 199n of 01 04 2016 "Approval Rules of Good Laboratory Practice" and with the Resolution of the Eurasian Economic Commission No. 81 "Concerning adoption of the Good laboratory practice of EAEU in the field of drug circulation." All manipulations with the animals were approved by the Institutional Animal Care and Use Committee of the V. V. Zakusov Research Institute of Pharmacology (Moscow).

2.3 Social defeat procedure

A depressive-like state in C57BL/6 mice was created by chronic stress caused by repeated experiences of social defeats in daily confrontations between males. The social defeat stress was performed as previously reported [6]. The outbred male mice were used as aggressors. The C57BL/6 mice and outbred mice were placed in pairs into experimental cages (28 × 14 × 10 sm), divided in half by a perforated Plexiglas wall, one mouse per compartment. The animals were held in sensory contact in the absence of direct physical interaction for 2 days. Separator was removed for 10 min on day 3 to allow animals opportunity for direct contact. Under these conditions, the larger outbred mouse acted as an "aggressor." The confrontation was stopped before the expiration of 10 min in the case of overly aggressive attacks by the outbred mouse (the bites continued even after the victim mouse had demonstrated a submissive pose). C57BL/6 mice were given daily stress for 10 days as described above, which leads to the development of a depressive-like state, according to the literature [16].

2.4 Design of the experiment

After 10 days of social defeat stress, the social avoidance test was conducted on day 11 to select mice with depressive-like behavior. Then these mice were randomly divided into three groups of eight animals each:

The control (stress) group
The "stress + GSB-106" group
The "stress + amitriptyline" group

2.5 Mice were placed into individual cages

On day 12, mice of the "stress + GSB-106" group were orally administered with GSB-106 in the dosage form at a dose of 0.1 mg/kg (for the active substance), suspended in 1% starch solution; the "stress" group was administered orally with 1% starch solution; the group of "stress + amitriptyline" was administered orally

amitriptyline in the tablet form at a dose of 10 mg/kg, suspended in 1% starch solution. Three more groups of mice (eight animals per group) not subjected to stress were formed simultaneously with the beginning of social stress modeling. On day 11, these groups were single orally administered with a 1% starch solution (control group [no stress]), GSB-106 (the group "GSB-106 [without stress]") or amitriptyline (group "amitriptyline [without stress]") in a 1% starch solution, respectively. The dose of GSB-106 was selected based on previously conducted experiments [12]; the one of amitriptyline was based on literature data [17]. The sucrose preference test was performed on day 13. Mice were decapitated; the hippocampus was isolated for subsequent evaluation of the synaptogenesis intensity on day 16. The scheme of the experiment is shown in **Figure 1**.

2.6 The social avoidance test

This test was performed to select mice that developed depressive-like behavior as the result of social defeat stress, as described in [6]. The infrared actimeter from Panlab (Spain) with ActiTrack software (field size 40.0 × 44.0 cm) was used. An individual transparent plastic chamber with holes (8.0 × 8.0 × 8.0 cm) was placed at one end of the field of the actimeter. A mouse was placed into the actimeter for 2.5 min. Then the mouse was returned to the home cage for 30 s. At this time, the "aggressor" unknown for the testing mouse was placed in the plastic chamber, and the testing mouse was placed in the actimeter for 2.5 min again. The duration of the mouse presence in the "interaction zone" (at a distance of 8.0 cm or less from the camera with the aggressor) was estimated. The interaction ratio (IR) was calculated as the ratio of the time spent in the interaction zone in the presence of the aggressor to the time spent in the interaction zone without the aggressor. The value IR < 1 was defined as a criterion for depressive-like behavior. Only the mice showing depressive-like behavior in this test (about 90% of the animals) were used in the further experiment.

2.7 The sucrose preference test

Mice were exposed to bottles with water and 1% sucrose solution. The consumption of water and sucrose solution was evaluated by weighing the bottles. The preference of the sucrose solution was calculated by the following formula:

$A_{(s)}/(A_{(s)} + A_{(w)}) \times 100\%$, where $A_{(s)}$ is the amount of consumed sucrose solution, g, and $W_{(w)}$ is the amount of consumed water, g. The reduction of this parameter below the control group level was regarded as the development of stress-induced anhedonia [6]. The test was carried out for 18 h.

2.8 Evaluation of hippocampal synaptogenesis

The content of the presynaptic marker synaptophysin in hippocampus was assessed using Western blot analysis. After defrost, the hippocampal tissue samples were homogenized at 4°C in a glass homogenizer with lysis buffer (50 mM Tris-HCl, 5 mM EDTA, 1 mM dithiothreitol, 1% Triton X-100, pH 7.5) supplemented with a protease inhibitor cocktail (pepstatin, bestatin, leupeptin, and aprotinin, "Sigma-Aldrich," USA), in the ratio of tissue: buffer = 1:10 (weight/volume). Then the samples were incubated at 4°C for 20 min and centrifuged at 15,000 rpm (Allegra® X-12R centrifuge "Beckman Coulter Inc.," USA) at the same temperature for 20 min. The protein levels of the supernatant lysates were determined by the method of Folin-Lowry. The supernatant proteins were separated in a 12% polyacrylamide gel and then transferred onto a polyvinylidene fluoride membrane by

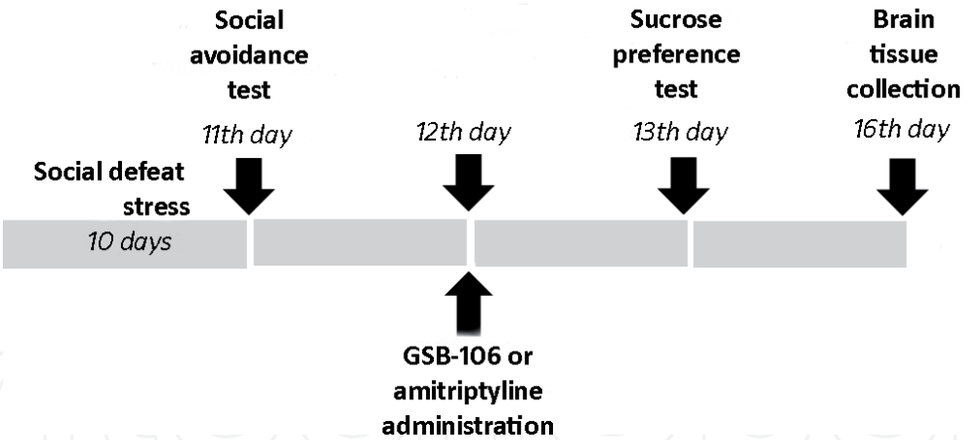

Figure 1.
The scheme of the experiment.

electroelution. The membranes were deactivated with 5% (w/v) nonfat dry milk in Tris buffer containing 1% Tween 20 (TBST) for 1 h. Then, the membranes were treated with primary monoclonal mouse antibodies against synaptophysin ("BD Biosciences," United Kingdom) at a dilution of 1:5000 for 1.5 h, the antibody excess was washed with TBST with 0.5% (w/v) nonfat dry milk, and the membranes were incubated at 37°C with goat antibodies against rabbit IgG ("Santa Cruz Biotechnology," USA, 1:1000), conjugated with horseradish peroxidase, for 1 h. The detection of proteins was performed after washing the secondary antibodies with TBST buffer in the reaction with enhanced chemiluminescence substrates (ECL reagents, Santa Cruz Biotechnology) using the Alliance Q9 gel documenting system (UVITEC, United Kingdom). Densitometry of the obtained images was performed using the GIMP2 program.

2.9 Statistical analysis

The intergroup differences were assessed using one-way analysis of variance ANOVA, followed by post hoc Fisher's LSD test or the Mann-Whitney U test. Differences were considered statistically significant at $p \leq 0.05$; the value of $p < 0.1$ was regarded as a tendency. The data were presented as mean and standard errors of the mean.

3. Results and discussions

One of the main behavioral signs of depression is anhedonia. Anhedonia is a violation of the brain "reward system," it is considered as a key symptom of depression both in the International Classification of Diseases of the tenth revision (ICD-10) and in the Classification of Mental Disorders of the American Psychiatric Association's Diagnostic and Statistical Manual (DSM-5). A common method for assessing anhedonia in animals is the sucrose preference test [6].

In our study the sucrose preference was statistically significantly reduced in the stressed mice (the "control [stress]" group) compared with the intact animals (the "control [without stress]" group) (**Table 1**). GSB-106 statistically and reliably restored the preference of the sucrose solution to the control level. Amitriptyline also restored the preference of the sucrose solution. However, the administration of GSB-106 or amitriptyline to intact animals did not affect the results of this test (**Table 1**).

Groups	n	Amount of consumed 1% sucrose solution, mg	Amount of consumed water, mg	Preference of the 1% sucrose solution, %
Control (without stress)	8	11.4 ± 1.2	2.73 ± 0.5	80.7 ± 3.2
GSB-106 (0.1 mg/kg) (without stress)	8	10.08 ± 0.8	2.14 ± 0.3	82.4 ± 2.8
Amitriptyline (10.0 mg/kg) (without stress)	8	10.02 ± 0.7	3.47 ± 0.1	74.3 ± 6.5
Control (stress)	8	6.67 ± 0.5	2.12 ± 0.2	75.9 ± 2.4*
Stress + GSB-106 (0.1 mg/kg)	8	10.9 ± 1.1	2.4 ± 0.3	81.9 ± 3.1#
Stress + amitriptyline (10.0 mg/kg)	8	13.7 ± 0.9	2.5 ± 0.4	84.8 ± 3.6#

Data are presented as mean ± SEM.
**p < 0.05 comparison with the "control (without stress)" group.*
#p < 0.05 comparison with the "control (stress)" group (ANOVA followed by the use of the Fisher's LSD method).

Table 1.
The preference of 1% sucrose solution in C57Bl/6 male mice subjected to 10 days of stress, 1 day at acute oral administration of GSB-106 or amitriptyline (test duration—18 h).

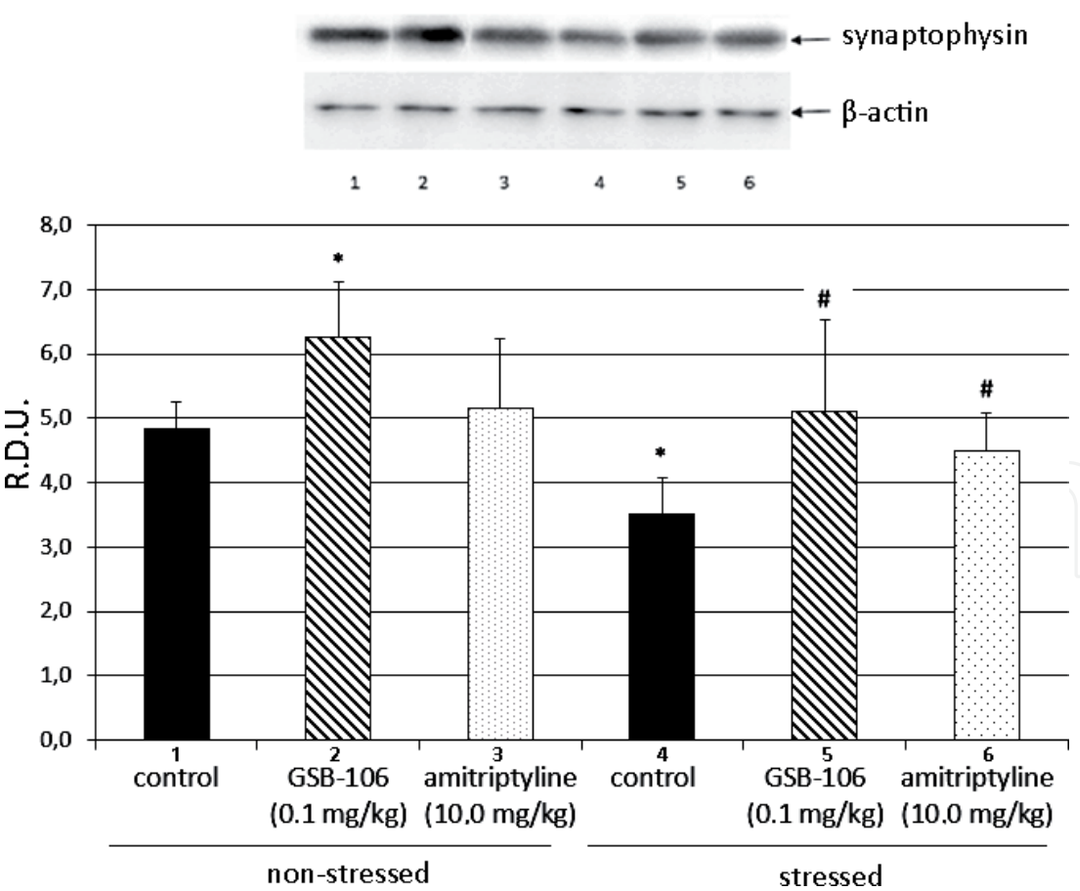

Figure 2.
*The synaptophysin level in the hippocampus of mice on the fourth day after the administration of GSB-106 or amitriptyline. Data are presented as mean ± SEM. Bands: 1, control (without stress); 2, GSB-106 (without stress); 3, amitriptyline (without stress); 4, control (stress); 5, "stress + GSB-106"; 6, "stress + amitriptyline." Note: *p < 0.05 compared with the placebo group; #p = 0.08 compared with the stress group (Mann-Whitney U test).*

Thus, dipeptide mimetics BDNF GSB-106 completely eliminated the manifestations of anhedonia at acute administration in mice subjected to 10-day social defeat stress.

GSB-106 was previously shown [13] to enhance the synaptophysin content in the hippocampus of mice at chronic administration (21 days). In this study, GSB-106 also caused a statistically significant increase of the synaptophysin content in the hippocampus of the control animals after acute administration (**Figure 2**). In stressed animals, synaptophysin content in the hippocampus was reduced by 30% compared with nonstressed ones. Such a decrease can be considered as a characteristic sign of a depressive-like state, since the hippocampus is the structure most susceptible to pathological changes during depression, as well as the prefrontal cortex, and synaptogenesis impairment is considered as one of the main pathophysiological signs of this disease [2]. The pronounced tendency to restore the level of synaptophysin was observed ($p = 0.08$) at administration of both GSB-106 and amitriptyline to the stressed animals (**Figure 2**).

4. Conclusions

The dipeptide mimetic BDNF GSB-106, like amitriptyline, exhibits anti-anhedonia activity after acute oral administration after 10 days of social defeat stress. The effect of GSB-106 was manifested in doses 100 times smaller than amitriptyline, the drug of comparison.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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References

[1] Frodl T. Recent advances in predicting responses to antidepressant treatment. F1000 Research. 2017;**6**:619. DOI: 10.12688/f1000research.10300.1

[2] Wainwright SR, Galea LAM. The neural plasticity theory of depression: Assessing the roles of adult neurogenesis and PSA-NCAM within the hippocampus. Neural Plasticity. 2013;**2013**:805497. DOI: 10.1155/2013/805497

[3] Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter ML. BDNF as a biomarker for successful treatment of mood disorders: A systematic & quantitative meta-analysis. Journal of Affective Disorders. 2015;**174**:432-440. DOI: 10.1016/j.jad.2014.11.044

[4] Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Molecular Brain Research. 2005;**136**(1-2):29-37. DOI: 0.1016/j.molbrainres.2004.12.020

[5] Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. The Journal of Neuroscience. 2002;**22**(8):3251-3261. DOI: 20026292

[6] Zhang JC, Yao W, Dong C, Yang C, Ren Q, Ma M, et al. Comparison of ketamine, 7,8-dihydroxyflavone, and ANA-12 antidepressant effects in the social defeat stress model of depression. Psychopharmacology. 2015;**232**(23):4325-4335. DOI: 10.1007/s00213-015-4062-3

[7] Jelen LA, King S, Stone JM. Alternatives to ketamine in depression: State-of-the-art and future perspectives. Therapeutic Advances Psychopharmacology. 2018;**8**:95-98. DOI: 10.1177/2045125317749456

[8] Abdallah CG, Sanacora G, Duman RS, Krystal JH. Ketamine and rapid-acting antidepressants: A window into a new neurobiology for mood disorder therapeutics. Annual Review of Medicine. 2015;**66**:509-523. DOI: 10.1146/annurev-med-053013-062946

[9] Gudasheva TA, Tarasiuk AV, Pomogaĭbo SV, Logvinov IO, Povarnina P, Antipova TA, et al. Design and synthesis of dipeptide mimetics of the brain-derived neurotrophic factor. Russian Journal of Bioorganic Chemistry. 2012;**38**(3):243-252

[10] Gudasheva TA, Logvinov IO, Antipova TA, Seredenin SB. Brain-derived neurotrophic factor loop 4 dipeptide mimetic GSB-106 activates TrkB, Erk, and Akt and promotes neuronal survival in vitro. Doklady. Biochemistry and Biophysics. 2013;**451**(1):212-214. DOI: 10.1134/S1607672913040121

[11] Povarnina PY, Garibova TL, Gudasheva TA, Seredenin SB. Antidepressant effect of an orally administered dipeptide mimetic of the brain-derived neurotrophic factor. Acta Naturae. 2018;**10**(38):81-84

[12] Seredenin SB, Voronina TA, Gudasheva TA, Garibova TL, Molodavkin GM, Litvinova SA, et al. Antidepressant effect of dimeric dipeptide GSB-106, an original low-molecular-weight mimetic of BDNF. Acta Naturae. 2013;**5**(19):105-109

[13] Gudasheva TA, Povarnina PY, Antipova TA, Seredenin SB. Dipeptide mimetic of the BDNF GSB-106 with antidepressant-like activity stimulates synaptogenesis. Doklady. Biochemistry and Biophysics. 2018;**481**(1):225-227. DOI: 10.1134/S1607672918040130

[14] Gudasheva TA, Povarnina PY, Seredenin SB. Dipeptide mimetic of the brain-derived neurotrophic factor prevents impairments of neurogenesis in stressed mice. Bulletin of Experimental Biology and Medicine. 2017;**162**(4):454-457. DOI: 10.1134/S1607672918040130

[15] Jang SW, Liu X, Chan CB, Weinshenker D, Hall RA, Xiao G, et al. Amitriptyline is a TrkA and TrkB receptor agonist that promotes TrkA/TrkB heterodimerization and has potent neurotrophic activity. Chemistry & Biology. 2009;**16**(6):644-656. DOI: 10.1016/j.chembiol.2009.05.010

[16] Iñiguez SD, Riggs LM, Nieto SJ, Dayrit G, Zamora NN, Shawhan KL, et al. Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. Stress. 2014;**17**(3):247-255. DOI: 10.3109/10253890.2014.910650

[17] Vilela FC, de Mesquita Padilha M, Alves-Da-Silva G, Soncini R, Giusti-Paiva A. Antidepressant-like activity of *Sonchus oleraceus* in mouse models of immobility tests. Journal of Medicinal Food. 2010;**13**(1):219-222. DOI: 10.1089/jmf.2008.0303