Computational and biological evidences on the serotonergic involvement of SeTACN antidepressant-like effect in mice

Mariana G. Fronza¹, Lucimar M. Pinto Brod¹, Angela Maria Casaril¹, Manoela Sacramento², Diego Alves², Lucielli Savegnago¹*

¹ Programa de Pós Graduação em Biotecnologia, PPGBiotec, Grupo de Pesquisa em Neurobiotecnologia—GPN, CDTec, Universidade Federal de Pelotas, UFPel, Pelotas, RS, Brazil, ² Programa de Pós Graduação em Química, PPGQ, Laboratório de Síntese Orgânica Limpa—LASOL, CCQFA, Universidade Federal de Pelotas, Pelotas, RS, Brazil

* luciellisa vegnago@yahoo.com.br

Abstract

A series of phenylselanyl-1H-1,2,3-triazole-4-carbonitriles with different substituents were screened for their binding affinity with serotonin transporter (SERT) and dopamine transporter (DAT) by docking molecular. 5-(4methoxyphenyl)-1-(2-(phenylselanyl)phenyl)-1H-1,2,3-triazole-4-carbonitrile (SeTACN) exhibited the best conformation with SERT even higher than fluoxetine and serotonin, suggesting a competitive inhibition. SeTACN demonstrated additional affinity to other serotonergic receptors involved in antidepressant effects: 5HT₁₅, 5HT₂₅ and 5HT₃. In another set of experiments, SeTACN led to significant reductions in the immobility time of mice submitted to forced swimming test (FST) in the dose range of 0.1- 20mg/kg, suggesting an antidepressant-like effect. The possible mechanism of action was investigated using serotonergic and dopaminergic antagonists. The antidepressant-like effect of SeTACN (0.1mg/kg i.g.) was prevented by the pretreatment with WAY100635 (a selective 5HT₁₅ antagonist), ketanserin (a 5HT₂₅/c antagonist) and ondansetron (a selective 5HT₃ antagonist), PCPA (an inhibitor of serotonin synthesis) but not with SCH23390 (dopaminergic D₁ antagonist) and sulpiride (D₂ antagonist). Sub-effective dose of fluoxetine was able to potentiate the effects of a sub-effective dose of SeTACN in FST. None of the treatments affected locomotor activity in open field test (OFT). These results together, suggest that the SeTACN antidepressant-like effect is mediated, at least in parts, by serotonergic system.

1. Introduction

Depression is a common, debilitating, life-threatening illness affecting approximately 350 million people worldwide. Despite a huge volume of research in understanding the etiology of depression, the pathophysiological mechanisms involved remain not fully elucidated [1]. Several studies revealed that monoaminergic neurotransmitters, including serotonin (5HT), norepinephrine and dopamine (DA) are the mainly responsible in brain circuits implicated in
mood regulation [2, 3]. For this reason, the serotonergic system is one of the most promising targets for the treatment of psychological disorders [4, 5].

Among the antidepressant drugs, the selective serotonin re-uptake inhibitors (SSRI) are most frequently prescribed, due to their higher efficacy, good tolerability and relative safety [6]. On the other hand, the heterogeneity of clinical responses to these drugs and susceptibility to adverse effects still being the antidepressants major clinical problems [7, 8]. However, little progress has been made in decreasing the percentage of resistant cases and improving the antidepressant onset of action [9].

Interestingly, 5HT mediates a wide range of pathways involved in depression through interactions with multiple 5HT receptors. In this context, the flexibility of 5HT system provide a promising opportunity to develop compounds with multiple and complementary modes of action. As the strategy of the simultaneous blocking or stimulation in specific 5HT receptors and/or the SERT inhibition, leading to the blockade of 5HT re-uptake [10, 11]. The adjustment of whole serotonergic transmission via pharmacological agents may provide future alternative antidepressant treatments [12].

Besides the abnormalities in metabolism of neurotransmitters, oxidative stress has been suggested to play an important role in depression pathogenesis [13, 14]. In this perspective, major depressive disorder has been linked to impairments in signaling pathways that regulate neuroplasticity and cell survival [15–17]. In this way, the neuroprotective role of antioxidant compounds can be pharmacologically useful for the modulation of depression [18, 19].

Selenium is an essential trace element nutritionally important to mammals, with physiological roles, in reason of being a structural component of several antioxidant enzymes involved in free radicals decomposition [20–22]. Recently, we reported that a class of phenylselanyl-1H-1,2,3-triazole-4-carbonitriles can induce antioxidant activities in mice cerebral cortex and hippocampus [23].

Several additional studies also demonstrated antidepressant-like activity can be exerted by organoselenium compounds, i.e. (octylseleno)-xylofuranoside [24], α-(phenylselanyl) aceto-phenone [25], α-phenylselenocitronefill [26], 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene [27] and m-trifluoromethyl-diphenyl diselenide [28]. In parallel, studies have reported that insufficient selenium intake may also affect some psychological roles and the supplementation with selenium was found to be associated with improvements in mood and depression status [29, 30].

In view of the above considerations, the present study reports antidepressant-like analyses of a selenium-containing compound belonging to the class of phenylselanyl-1H-1,2,3-triazole-4-carbonitriles. The interaction of this class with 5HT and DA transporters was explored by molecular docking. Based on these results, the affinity with 5TH1a, 5HT2a and 5HT3 receptors of 5-(4methoxyphenyl)-1-(2-(phenylselanyl) phenyl)-1H-1,2,3-triazole-4-carbonitrile (SeTACN) was also investigated. As a preliminary biological evaluation, the antidepressant-like effect of SeTACN and the possible mechanism of action was evaluated by behavioral assays in mice.

2. Materials and methods

2.1 Experimental design

In this study, the affinity with monoamine transporters as SERT and DAT were determined by molecular docking. It was defined as a modelling strategy for further studies involving antidepressant-like potential of the selected compound, since they are the mainly responsible for monoamine clearance from synaptic cleft. In view of extending our knowledge about the mechanism of action performed by the resultant compound, molecular docking in serotonin receptors involved in antidepressant effect: 5TH1a, 5HT2a and 5HT3 was also explored.
We evaluated the antidepressant-like effect of resultant compound in mice submitted to forced swimming test (FST) too. For this purpose, the animals were treated with a dose range of SeTACN of 0.01mg-20mg/kg and 30 minutes later were submitted to open field test (OFT) and FST as can be seen in Fig 1A.

In view of investigate our in silico evidences about the compound mechanism of action, the animals were pretreated with different antagonists of monoaminergic receptors, in another set of experiments. After latency time for antagonist effect, the animals were treated with SeTACN (0.1mg/kg) and then submitted to OFT and FST (Fig 1B). The blockade of the SeTACN antidepressant-like effect by the administered antagonist is an indication of the involvement of this pathway.

We also evaluated the synergic effect of a sub-effective dose of clinical antidepressants with SeTACN, illustrated in Fig 1C. Combined effect of imipramine or fluoxetine and SeTACN in a synergistic antidepressant-like activity suggests that the antidepressant-like effect of SeTACN is attributed, at least in part, by a similar mechanism of action.

2.2 Homology modelling and molecular docking

The molecules analysed in this paper were drew using ChemDraw and their geometry optimized using the software Avogadro 0.9.4 following the MMFF94 method [31]. The molecular docking simulation was performed using software Autodock Vina [32], where all the rotatable bonds of ligands were allowed to rotate freely and the receptors were considered rigid.

Protein ligand interaction was observed by Autodock Tools [33]. Additionally, this software was used to minimize the structure of proteins, using the Gasteiger charges with 500 steps of minimization in all molecular targets.

We used crystallographic structures of molecular targets from Protein Data Bank (PDB) (http://www.pdb.org/). The CHIMERA 1.5.3 software was used to remove molecules, ions, and water [34].

Fig 1. Experimental paradigms illustrating the drugs and compound administration followed by behavioral tests. (A) Antidepressant-like activity of 5-(4methoxyphenyl)-1-(2-(phenylselanyl)phenyl)-1H-1,2,3-triazole-4-carbonitrile (SeTACN). (B) Evaluation of mechanism of action involved in antidepressant-like effect of SeTACN. (C) Synergic effect of the combined treatment with sub-effective doses of clinical antidepressants and SeTACN.

https://doi.org/10.1371/journal.pone.0187445.g001
Firstly, phenylselanyl-1H-1,2,3-triazole-4-carbonitriles (Fig 2) were docked in LeuBat (PDB:3GWV), protein LeuT with some mutations, being similar to SERT [35], a homology model. As positive controls, we used the molecules serotonin and fluoxetine.

Docking in dopamine transporter (DAT) (PDB:4M48) was performed using the same previously described methodology [36]. As serotonin is the major neurotransmitter involved in pathology of depression, the molecule with lowest docking score in DAT and highest docking score in SERT was selected for further investigation [37].

Additional studies were aimed to conduct docking in 5HT receptors 5HT<sub>1a</sub>, 5HT<sub>2a</sub> e 5HT<sub>3</sub>. To reach this goal, the amino acid sequence of 5HT<sub>1a</sub> was downloaded from UniProt database (accession code: P08908, 5HT1A_HUMAN) and the 3D structure of 5-HT1AR was constructed using the SWISS-MODEL server according to Zheng et al. (2015) [38]. 5HT<sub>2a</sub> receptor was similarly built from 5HT<sub>2b</sub> and the amino acid sequence of 5HT<sub>2a</sub> Uniprot database (accession code: P28223) according to Gandhimathi and Sowdhamini, (2015) [39]. The structure utilized to perform the docking analyses was 5HT<sub>3</sub> PDB: 4PIR requiring no homology studies.

2.3 Animals
The experiments were conducted using male Swiss mice (25–35 g, 60–75 days), housed in groups (3–5 animals per cage) under controlled conditions of light (7:00 to 19:00) and temperature (22–25°C). All tests were performed on separate groups of animals (n = 5–10) and each animal was used only once in each test. Before the start of the behavioral tests, the animals were allowed to acclimate in testing rooms for at least 1 hour. The behavioral analyses were performed by a blind measurer to the treatment conditions. Procedures of this study were conducted according to the guidelines of the Committee on the Care and Use of Experimental Animal Resources (NIH Publications No. 8023, revised 1978) and with the approval of the.
Ethical Commission for Animal Use of the Federal University of Pelotas, Brazil (7045–2015, process #23110.007045/2015-58). After treatment and behavioral analysis, mice were euthanized using a continuous isoflurane flow. All efforts were made to minimize animals suffering and to reduce the number of animals used in tests.

2.4 Drugs

Ketanserin, ondansetron, sulpiride, SCH23390, p-chlorophenylalanine methyl ester (PCPA) and WAY100635 were purchased from Sigma Chemical Co, USA. Fluoxetine hydrochloride was purchased from Pfizer, Brazil and Imipramine hydrochloride was obtained from Novartis, Brazil. All these drugs were dissolved in saline solution (0.9%) and injected via intraperitoneal (i.p) route, and WAY 100635 and SCH233390 administered via subcutaneous route (s.c). The commercial antidepressants were also diluted in saline solution (0.9%) but administered by intragastric (i.g) route.

SeTACN was synthesized in our laboratory and characterized as previously described by Savegnago et al (2016) [23]. The compound was dissolved in canola oil and administered i.g. by gavage in mice. All the drugs listed were administered in a constant volume of 10 ml/kg body weight.

2.5 Behavioral tests

Based on the above mentioned in silico modelling, 5-(4-methoxyphenyl)-1-(2-(phenylselanyl)phenyl)-1H-1,2,3-triazole-4-carbonitrile (SeTACN, Fig 1 –compound 4) was chosen for further analysis in vivo. This selection was based in SERT/DAT ratio best score, as determined by the logic created in this research.

In this way, in order to evaluate the antidepressant-like effect of SeTACN, the compound was administered once in mice (0.01-20mg/kg) and 30 minutes later, the animals were submitted to OFT followed by FST as experimental design 1 (Fig 1A).

2.5.1 Open field test (OFT). Locomotor activity was evaluated in the OFT, as previously described by Walsh and Cummings (1976) [40], to exclude a possible locomotor interference in FST. Briefly, animals were individually placed in a wooden square box (40 × 60 × 50 cm high) with 12 equal squares. The number of crossings were manually counted during a 5 minutes session. Crossing was considered only when animal crossed a line with four paws. After each session, the open field was cleaned with a solution of 70% ethanol to exclude any odor cues.

2.5.2 Forced swimming test (FST). FST was performed immediately after the OFT and was analyzed as previously described by Porsolt (1979) [41]. In summary, each mouse was individually placed in an open cylindrical container (diameter 10 cm, height 25 cm), with 19 cm of water at 25 ± 1˚C, without the possibility of escaping, and was forced to swim. The total amount of time each animal remained immobile during 6 minutes session was recorded (in seconds) (only the last four minutes were analyzed). In this test, the immobile posture reflects a state of behavior despair and helplessness.

2.5.3 Mechanisms involved in the antidepressant-like effect of SeTACN. The involvement of serotonergic system in the antidepressant-like effect of SeTACN (0.1mg/kg i.g.) was performed in another set of experiments included in experimental design 2 (Fig 1B). To reach this goal, mice were pre-treated with ketanserin (1mg/kg i.p.; a 5HT_{2a} receptor antagonist), ondansetron (1mg/kg i.p.; a 5HT_{3} receptor antagonist) or WAY100635 (0.1mg/kg s.c.; a 5HT_{1a} receptor antagonist) and 15 minutes later the animals were treated with a dose of SeTACN (0.1mg/Kg i.g.). After 30 minutes of compound administration, the animals were immediately exposed to OFT and FST.
With the purpose of verifying the influence of serotonin synthesis in antidepressant-like effect of SeTACN, animals were treated once a day with PCPA (100mg/kg, i.p., an inhibitor of serotonin synthesis) or vehicle (saline 0.9%) during 4 days. On the fifth day, animals received SeTACN (0.1mg/kg, i.g.) or just vehicle and 30 minutes later were submitted to OFT and FST.

The dopaminergic system involvement in antidepressant-like effect of SeTACN was verified according to experimental design 2 (Fig 1B). In this sense, animals were pre-treated with SCH23390 (0.05mg/kg, s.c., dopaminergic D1 antagonist receptor), sulpiride (50mg/kg, i.p., D2 receptor antagonist) or saline. After the 60 minutes, necessary for the antagonist effect, the animals were treated with SeTACN (0.1mg/kg) or vehicle. In the same manner as previously, FST and OFT were performed after 30 minutes of the compound administration. It is worth mentioning that, these methodologies were based in previous studies from Savegnago et al (2008) [42]; Martinez et al (2014) [43]; Pesarico et al (2014) [44] and Brod et al (2016) [24].

The effect of the co-administration of sub-effective doses of SeTACN (0.01mg/kg i.g.) and fluoxetine (5mg/kg, i.g., a selective serotonin reuptake inhibitor) was also investigated as predicted in experimental design 3 (Fig 1C) [45]. Thus, after 60 minutes of fluoxetine or vehicle administration the animals were treated with SeTACN or vehicle and after 90 minutes analyzed in the behavioral tests. The synergic effect of a sub-effective dose of SeTACN (0.01mg/kg) and imipramine (10mg/kg, i.g., a tricyclic antidepressant) was also evaluated (experimental design 3- Fig 1C) as mentioned above [46, 47].

2.6 Statistical analyses

The results were analyzed utilizing the software GraphPad Prism 5.0 and are given as the mean ± standard error of the mean (S.E.M.). Comparisons between experimental and control groups were performed by one-way or two-way analysis of variance (ANOVA) followed by Newman-Keuls test for post-hoc comparison when appropriate. Probability values less than 0.05 (P < 0.05) were considered as statistically significant.

3. Results and discussion

The molecular docking results in SERT and DAT are presented in Table 1. Based on this, the compound SeTACN (number 4) was chosen due to its higher score in SERT (-9.9kcal/mol) and lowest score in DAT (-9.0 kcal/mol). This rationale was developed based on studies which demonstrated that although SSRI have affinity for noradrenaline transporter (NET) and DAT, the SERT affinity is even higher [48, 49].

As positive controls in SERT, we utilized the molecules of 5-HT and fluoxetine, with a docking score of -7.1 and -8.7 respectively. In this way, the SeTACN affinity with SERT seems to be stronger, when its compared to 5-HT score, this data may indicate a preference in competitive binding to 5-HT transporter. This pattern is also observed when SeTACN score is

Table 1. Scores (kcal/mol) of docking results of phenylselanyl-1H-1,2,3-triazole-4-carbonitriles class of compounds in serotonin transporter (SERT) and dopamine transporter (DAT).

| Compound | Docking in SERT (kcal/mol) | Docking in DAT (kcal/mol) |
|----------|---------------------------|--------------------------|
| 1        | -8.3                      | -9.3                     |
| 2        | -9.8                      | -10.1                    |
| 3        | -10.0                     | -10.1                    |
| 4        | -9.9                      | -9.0                     |
| 5        | -10.1                     | -10.3                    |

https://doi.org/10.1371/journal.pone.0187445.t001
compared to fluoxetine score, which might suggest a SeTACN stronger affinity to SERT, although more depth studies are required to affirm this hypothesis.

SeTACN best score position is close to ASP 24 and TYR 21, which are target of paroxetine, sertraline and fluoxetine (Fig 3A). The residues PHE 259, VAL104, SER356 and TYR108 interaction of fluoxetine and sertraline is the same with SeTACN and leuBAT [50]. It is worth mentioning that the interaction of SeTACN with PHE253 an ASP404 might represent characteristic of specificity, similar to others SSRI [51].

The affinity of SeTACN with the serotonin receptor 5HT\textsubscript{1a} is -8.8kcal/mol as shown in Fig 3B. The possible interaction with ILE113, PHE112, ASP116, ASN386, PHE361 and ALA365 are in agreement with some well-known 5HT\textsubscript{1a} drugs as buspirone, 8-OH-DPAT and WAY100635 [52].

SeTACN docking score (-8.8 kcal/mol) in 5HT\textsubscript{2a} and the nearest residues of the complex are illustrated in Fig 3C. The position of SeTACN in 5HT\textsubscript{2a} receptors seems to be similar to antagonists of 5HT\textsubscript{2a} such as espirone, sharing the same residues interaction as TRP151, ILE152, LEU228, VAL156, ASP231 and PHE339 [37].

The result depicted in Fig 3D pointed out the docking scores of SeTACN in receptor 5HT\textsubscript{3}: -8.1kcal/mol. Although the score is lower when compared to other evaluated receptors, this interaction is considered significant. The best conformation of the compound is close to residues THR154 and TRP156, which inhibit this receptor by molecule VHH15 [53]. Moreover, the residue TRP156 is among those responsible for the opening and closing of 5HT\textsubscript{3} ionic channels [53]. On the other hand, these residues interaction are not the same as antagonists like ondasetron and granisetron, which may suggest another way of 5HT\textsubscript{3} inhibition [54].

![Fig 3. Docking results of compound 4 (5-(4-methoxyphenyl)-1-(2-(phenylselenyl)phenyl)-1H-1,2,3-triazole-4-carbonitrile (SeTACN) in (A) serotonin transporter (SERT) with a score of -9.9kcal/mol (B) in 5HT\textsubscript{1a} receptor with a score of -8.8kcal/mol (C) in 5HT\textsubscript{2a} receptor with a score of -8.8kcal/mol (D) and in 5HT\textsubscript{3} receptor with a score of -8.1kcal/mol.](https://doi.org/10.1371/journal.pone.0187445.g003)
The SeTACN interaction with serotonergic system, explored by docking analyses suggests a possible antidepressant-like effect, which was explored under *in vivo* tests by FST. Results from Fig 4A indicate the effect of SeTACN on immobility time was statistically significant from 0.1–20mg/kg with respect to the control group (*P*< 0.05; *P*<0.01; *P*< 0.001). SeTACN given by i.g route and at all tested doses did not change the number of crossings in OFT when compared to the control group (Fig 4B). These findings pointed to a decrease in immobility time in FST not caused by any locomotor alteration.

Fig 5A shows that pre-treatment with WAY100635 (a 5HT<sub>1A</sub> receptor antagonist) was able to prevent the reduction of immobility time caused by SeTACN treatment (0.1mg/kg i.g). Two-way ANOVA analysis revealed a statistically significant effect of the treatment with SeTACN alone [F(1,23) = 16.64; *P* = 0.0005], WAY100635 alone [F(1,23) = 11.82; *P* = 0.0022], and treatment with WAY100635 x SeTACN [F(1,23) = 17.17; *P* = 0.0004]. No significant effect was observed for SeTACN treatment [F(1,23) = 0.04; *P* = 0.8523], WAY100635 treatment [F(1,23) = 0.05; *P* = 0.8284] or SeTACN x WAY100635 interaction [F(1,23) = 3.01; *P* = 0.961] on the number of crossings. These findings together with docking study 5HT<sub>1A</sub> indicate the possible involvement of this receptor in the antidepressant-like effect of SeTACN.

The pre-treatment of mice with ketanserin (a 5HT<sub>2A</sub> antagonist receptor) blocked the anti-immobility effect of SeTACN (0.1mg/kg) as demonstrated in Fig 5B, suggesting the involvement of 5HT<sub>2A</sub>. Two-way ANOVA tests revealed a statistically significant effect of the treatment with SeTACN alone [F(1,27) = 13.95; *P* = 0.0009], ketanserin alone [F(1,27) = 13.84; *P* = 0.0005], and treatment with ketanserin x SeTACN [F(1,27) = 9.04; *P* = 0.0009]. No significant effect in OFT could be observed for SeTACN treatment [F(1,26) = 1.41; *P* = 0.2455], ketanserin treatment [F(1,26) = 0.85; *P* = 0.3639] or SeTACN x ketanserin interaction [F(1,26) = 0.01; *P* = 0.9321].

Results in Fig 5C demonstrate that pre-treatment with ondansetron (a 5HT<sub>3</sub> receptor antagonist) could prevent the antidepressant-like effect of SeTACN (0.1mg/kg). Two-way ANOVA tests revealed significant differences in SeTACN treatment [F(1,16) = 8.41; *P* = 0.0105] and ondansetron x SeTACN treatment interaction [F(1,16) = 7.36; *P* = 0.0153] but not ondansetron treatment. No significant effect for SeTACN treatment [F(1,16) = 0.07; *P* = 0.8007], ondansetron treatment [F(1,16) = 0.01; *P* = 0.9329] or SeTACN x ondansetron interaction [F(1,16) = 0.47; *P* = 0.5935] was detected on the number of crossings.

This anti-immobility effect of SeTACN (0.1 mg/kg, p.o.) was blocked by the pre-treatment of mice with the inhibitor of serotonin synthesis, PCPA (Fig 5D). Two-way ANOVA showed main effect for SeTACN treatment [F(1,18) = 22.12, *P* = 0.0002] and PCPA x SeTACN treatment interaction [F(1,18) = 12.34, *P* = 0.0025] and also revealed significant differences for fluoxetine treatment [F(1,18) = 19.05; *P* = 0.0004], and PCPA x fluoxetine treatment interaction

![Fig 4. Effect of acute administrat ion of SeTACN (0.01–20 mg/kg, i.g) in mice 30 min before (A) the forced swimming test (FST), and open field test (B). Values are expressed as mean S.E.M (one-wa y ANOVA followed by Newman Keuls) (*) P < 0.05, (**) P < 0.01, (+++) P < 0.001 when compared to control group.](https://doi.org/10.1371/journal.pone.0187445.g004)
The two way ANOVA revealed no significant effect of SeTACN treatment \([F(1,20) = 0.50; P = 0.4884]\), PCPA treatment \([F(1,20) = 1.09; P = 0.3082]\) and SeTACN × PCPA treatment interaction \([F(1,20) = 0.13; P = 0.7182]\) in number of crossings. No significant mobility effect for fluoxetine treatment \([F(1,20) = 0.77; P = 0.3919]\) or fluoxetine × PCPA interaction either \([F(1,20) = 0.73; P = 0.4041]\).

Interestingly, the pretreatment with SCH23390 (Fig 6A) or sulpiride (Fig 6B) did not block the antidepressant-like effect of SeTACN. Two-way ANOVA tests for immobility time revealed a main effect of SeTACN \([F(1,29) = 85.32; P = 0.0001]\) and \([F(1,18) = 395.10]; \text{P}<0.0001\) when compared to SeTACN pretreated with vehicle.

https://doi.org/10.1371/journal.pone.0187445.g005

https://doi.org/10.1371/journal.pone.0187445.g006
The pretreatment of SCH23390 [F(1,29) = 4.06; P = 0.0533] and sulpiride [F(1,18) = 0.38; P = 0.5449] did not eliminate the antidepressant-like effect elicited by SeTACN. Two-way ANOVA of OFT showed that SeTACN treatment did not produce any significant effect in mice locomotor activity [F(1,29) = 1.65; P = 0.2092], SCH23390 treatment [F(1,29) = 2.08; P = 0.1595] and SeTACN × SCH23390 treatment interaction [F(1,29) = 1.33; P = 0.2583] with respect to number of crossings. In the same way, no significant effect was observed for SeTACN treatment [F(1,16) = 0.13; P = 0.7258], sulpiride treatment [F(1,16) = 0.08; P = 0.7755] or SeTACN × sulpiride interaction [F(1,16) = 0.51; P = 0.4855]. These results suggest that the antidepressant-like effect of SeTACN may not be influenced by the D1 or D2 receptors, but more studies in relation to dopaminergic system and SeTACN are necessary.

Fig 7A summarizes the synergistic effect between immobility time of animals treated with a sub-effective dose of fluoxetine (5mg/kg; selective serotonin reuptake inhibitor) in combination with a sub-effective dose of SeTACN (0.01mg/kg). Two-way ANOVA tests revealed no effect of the treatment with SeTACN alone [F(1,12) = 26.30; P = 0.0002], fluoxetine alone [F(1,12) = 21.45; P = 0.0006], and treatment with fluoxetine x SeTACN [F(1,12) = 23.13 P = 0.0004]. No significant effect for SeTACN treatment [F(1,12) = 0.12; P = 0.7398], fluoxetine treatment [F(1,12) = 0.68, P = 0.4252] or SeTACN x fluoxetine interaction [F(1,12) = 0.01, P = 0.9117] was observed with respect to the number of crossings. These findings imply that fluoxetine and SeTACN may have a similar mechanism of action.

However, the effect between a sub-effective dose of imipramine (10mg/kg; a tricyclic antidepressant) and SeTACN (0.01mg/Kg) was not significant in immobility time (Fig 7B). Two-way ANOVA tests revealed the SeTACN effect alone [F(1,19) = 3.80; P = 0.0660], imipramine effect alone [F(1,19) = 0.28; P = 0.6039] and the combination of SeTACN × imipramine treatment interaction [F(1,19) = 0.78; P = 0.3878]. Either, in open field test of SeTACN treatment [F(1,19) = 0.03; P = 0.8718], imipramine treatment [F(1,19) = 0.01; P = 0.9441] or SeTACN × imipramine interaction [F(1,19) = 0.04; P = 0.8479] did not change the mice locomotor activity.

Taken together, the results in the present study, both computational and behavioral, suggest that the antidepressant-like effect of SeTACN in FST depends on the interaction of serotonergic neurotransmission. Probably, firstly due the inhibition of SERT and as a complementary action, the modulation of 5HT-receptors as 5HT1a, 5HT2a and 5HT3. Besides, SeTACN is capable of restoring the despair behavior induced by PCPA which lead to a serotonin depletion, this data may infer the modulation of 5HT synthesis. Furthermore, based in this preliminary evaluation, is important to highlight the hypothetical feature of this mechanism of action exerted by SeTACN and more studies are required to support these evidences.
The suggested complementary SERT mechanism of action, through the modulation of 5HT receptors could be beneficial in depression pathogenesis. Since, 5HT_{1a} autoreceptors are responsible in the self-inhibition control of 5HT neurons [55]. Most antidepressant drugs increase the concentration of 5HT in the extracellular brain space only by preventing its reuptake through the blockade of SERT [56]. Indeed, this increase is offset by a negative feedback operating at the 5HT_{1a} autoreceptors. This mechanism is thought to be responsible for the delay in onset of the therapeutic action, often by several weeks, of antidepressants [6]. In this sense, compounds which interact in 5HT_{1a} receptors can accelerate the antidepressant response to SSRIs, acting by potentiating 5HT neurotransmission [57–59].

In addition, preclinical studies indicate that 5HT_{2a} receptor subtype represent a promising target in SSRIs-resistant depressive patients, potentiating the behavioral effects of SSRIs [60]. Besides, the stimulation of 5HT_{2a} receptors is related direct and indirectly to the modulation of adult neurogenesis in the hippocampus and antidepressants exert their therapeutic activity, at least in part, by stimulating this pathway [61].

5HT_{3} receptors also have a critical influence on behavioral and neurocircuitry processes in brain that control mood and emotional behavior [9]. It is well known that the mechanism of action of fluoxetine and other antidepressants, are related to the non-competitive antagonism of the 5HT_{3} receptor [62]. Moreover, another interesting characteristic of 5HT_{3} receptors is the presence of chemoreceptor trigger zone in brainstem and in the gastrointestinal tract, which mediate nausea/vomiting motility, which may protect against the gastrointestinal side effects that often accompany SSRIs antidepressants [12].

Interesting, triazole is the core structural motif exhibits a broad range of biological properties, including antidepressant-like activity as previously reported [63–65]. This nucleus is also present in antidepressant drug Nefazodone, which generates its therapeutics effects primarily as potent 5HT_{2a} inhibitor. Besides, has moderate effects as 5HT_{1a} inhibitor and serotonin-noradrenaline-dopamine reuptake inhibitor (SNDRI) through the interaction with monoaminergic transporters [66; 67].

The computational tools, such as molecular docking has contributing to drug design, in the discovery of new molecules with therapeutic effects and contributing to suggest its mechanism of action as well [68]. In this way, this study shows for the first time a selenium compound binding affinity with serotonin transporters and the serotonin receptors: 5HT_{1a}, 5HT_{2a}, and 5HT_{3} which might be useful to unravel the mechanism of action antidepressant-like effect exerted by several selenium compounds, as cited previously. Moreover, similar studies already demonstrated the antidepressant-like effect using this docking methodology in mice submitted to FST [69–71].

FST is one of the most used tools for antidepressants screening, in this sense, a reduction in immobility time is considered indicative of an antidepressant-like effect [72; 73]. Although, we can just suggest a possible antidepressant-like activity of SeTACN, because a current limitation of this study is the absence of an induced depressive-like behavior in mice. Considering, future studies are needed to conclude the mechanism of action and determine the antidepressant clinical efficacy of SeTACN.

Another interesting characteristic of SeTACN is the antioxidant effect in mice cerebral cortex and hippocampus, already demonstrated by our research group [23]. Studies demonstrated that depressed patients present a reduction in volume and function of these areas [74, 75]. These structural changes happen due the atrophy of several dysregulated signaling pathways, including oxidative stress [76, 77]. In this sense, the antioxidant effect of SeTACN could at least in part diminishes the negative impact of the redox dysregulation in neuronal homeostasis.

In this context, some antidepressants already demonstrated antioxidant effects and other antioxidants have been reported to exert antidepressant-like effect [78; 79]. So, the antioxidant
potential of SeTACN could contribute to its antidepressant-like effect. Despite, it is just a hypothesis and to more concrete conclusions further studies are needed regarding the antioxidant role in antidepressant-like effect of SeTACN.

Behavioral findings and molecular studies have shown that different subtypes of 5HT receptors might relate to the effectiveness of the antidepressant compounds [80]. Taking all these data, we can suggest that SeTACN might be a antidepressant-like compound with an interest hypothetical mechanism of action, blocking the SERT and with affinity to 5HT$_{1a}$, 5HT$_{2a}$, and 5HT$_{3}$. This mechanism could accelerate the onset of action and diminishes others side effects of the current prescribed antidepressants. Although more studies are needed to affirm SeTACN pharmacological antidepressant efficacy.

4. Conclusion

In conclusion, based on computational and behavioral evidence, SeTACN exerted antidepressant-like activity in mice, through the possible modulation of serotonergic pathway. Nevertheless, further studies are needed to elucidate the mechanism of action and the contribution of other neurotransmission systems, signaling pathways using others depressive models and experimental techniques.

Supporting information

S1 Graphical abstract. Scheme illustrating the methodology performed in view of explore the antidepressant-like activity of SeTACN. (TIF)

Acknowledgments

The project was supported by Coordenação de Aperfeiçoamento Pessoal de nível Superior (CAPES), CNPq and FAPERGS (PRONEN 16/2551-0000240-1). Authors are also thankful to Dr. Rafael Luque for revising this paper. L.S. and D.A are recipients of CNPq fellowship.

Author Contributions

Formal analysis: Mariana G. Fronza, Angela Maria Casaril, Lucielli Savegnago.
Funding acquisition: Diego Alves, Lucielli Savegnago.
Investigation: Mariana G. Fronza, Lucimar M. Pinto Brod.
Methodology: Mariana G. Fronza, Manoela Sacramento, Lucielli Savegnago.
Project administration: Diego Alves, Lucielli Savegnago.
Resources: Lucielli Savegnago.
Supervision: Diego Alves, Lucielli Savegnago.
Validation: Mariana G. Fronza, Angela Maria Casaril, Diego Alves, Lucielli Savegnago.
Visualization: Diego Alves, Lucielli Savegnago.
Writing – original draft: Mariana G. Fronza, Angela Maria Casaril, Diego Alves, Lucielli Savegnago.
Writing – review & editing: Mariana G. Fronza, Diego Alves, Lucielli Savegnago.
References

1. World Health Organization. DEPRESSION A Global Public Health Concern, 2012.
2. McIntyre R, Cha D, Soczynska J, Woldeyohannes H, et al. Cognitive deficits and functional outcomes in major depressive disorder: determinants, substrates, and treatment interventions. Depress Anxiety, 2013; 30: 515–527. https://doi.org/10.1002/da.22063 PMID: 23468126
3. Ménard C, Hodes GE, Russo SJ. Pathogenesis of depression: Insights from human and rodent studies. Neuroscience 2016; 321: 138–162. https://doi.org/10.1016/j.neuroscience.2015.05.053 PMID: 26037806
4. Nutt D. Relationship of neurotransmitters to the symptoms of major depressive disorder. J Clin Psychiatry 2008; 69: 4–7.
5. Jacobsen JPR, Medvedev IO, Caron MG. The 5-HT deficiency theory of depression: perspectives from a naturalistic 5-HT deficiency model, the tryptophan hydroxylase 2(Arg)439(His) knockin mouse. Philos Trans R Soc Lond B Biol Sci 2012;, 367: 2444–2459. https://doi.org/10.1098/rstb.2012.0109 PMID: 22826344
6. Celada P, Puig MV, Amargós-Bosch M, Adell A, Artigas F. The therapeutic role of 5-HT(1A) and 5-HT(2A) receptors in depression. J Psych and Neuroscience 2004; 29: 252–265.
7. Millan MJ. The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. Eur J Pharmacol 2004; 500: 371–384. https://doi.org/10.1016/j.ejphar.2004.07.038 PMID: 15464046
8. Schechter LE, Ring RH, Beyer CE et al. Innovative Approaches for the Development of Antidepressant Drugs: Current and Future Strategies. NeuRox 2005; 2: 590–611. https://doi.org/10.1602/neurorx.2.4.590 PMID: 16489368
9. Gupta D, Prabhakar V, Radhakrishnan M. 5HT3 receptors: Target for new antidepressant drugs. Neurosci Biobehav Rev 2016; 64: 311–325. https://doi.org/10.1016/j.neubiorev.2016.03.001 PMID: 26976353
10. Artigas F. Serotonin receptors involved in antidepressant effects. Pharmacol Ther, 2013; 137: 119–131. https://doi.org/10.1016/j.pharmthera.2012.09.006 PMID: 23022360
11. Dale E, Bang-Andersen B, Sánchez C. Emerging mechanisms and treatments for depression beyond SSRIs and SNRIs. Biochem Pharmacol, 2015; 95: 81–97. https://doi.org/10.1016/j.bcp.2015.03.011 PMID: 25813654
12. Morrissette DA, Stahl SM. Modulating the serotonin system in the treatment of major depressive disorder. CNS Spectr 2014; 68: 57–67.
13. Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Prog in Neuro-Psychopharmacol Biol Psychiatry 2011; 35: 676–692.
14. Moylan S, Berk M, Dean OM et al., Oxidative & nitrosative stress in depression: why so much stress?. Neurosci Biobehav Rev 2014; 45: 46–62. https://doi.org/10.1016/j.neubiorev.2014.05.007 PMID: 24858007
15. Michel TM, Pulsschen D, Thome J. The role of oxidative stress in depressive disorders. Curr Pharm Des 2012; 18: 5890–5899. PMID: 22681168
16. Massart R, Mongeau R, Lanfumey L. Beyond the monoaminergic hypothesis: neuroplasticity and epigenetic changes in a transgenic mouse model of depression. Philos Trans R Soc Lond B Biol Sci 2012; 367: 2485–2494. https://doi.org/10.1098/rstb.2012.0212 PMID: 22826347
17. Rantamaki T, Yalcin I. Antidepressant drug action—From rapid changes on network function to network rewiring. Prog Neuropsychopharmacol Biol Psychiatry 2016; 64: 285–292. https://doi.org/10.1016/j.pnpbp.2015.06.001 PMID: 26060070
18. Anderson G, Maes M. Oxidative/nitrosative stress and immuno-inflammatory pathways in depression: treatment implications. Curr Pharm Des 2014; 20: 3812–3847. PMID: 24180395
19. Pilar-Cuellar F, Vidal R, Diaz A et al. Signaling pathways involved in antidepressant-induced cell proliferation and synaptic plasticity. Curr Pharm Des 2014; 20: 3776–3794. PMID: 24180397
20. Ursini F, Bindoli A. The role of selenium peroxidases in the protection against oxidative damage of membranes. Chem Phys Lipids 1987; 44: 255–276. PMID: 3311411
21. Rayman MP. The importance of selenium to human health. Lancet 2000; 356: 233–241. https://doi.org/10.1016/S0140-6736(00)02490-9 PMID: 10963212
22. Rayman MP. Selenium and human health. The Lancet 2002; 379: 1256–1268.
23. Savegnago L, Sacramento MD, Brod LMP et al. Phenylselenyl-1H-1,2,3-triazole-4-carbonitriles: synthesis, antioxidant properties and use as precursors to highly functionalized tetrazoles. RSC Advances 2016; 6: 8021–8031.
24. Pinto Brod LM, Fronza MG, Vargas JP et al. Involvement of monoaminergic system in the antidepressant-like effect of (octylseleno)-xylofuranoside in the mouse tail suspension test. Prog Neuro-Psychopharmacol Biol Psychiatry 2016; 65: 201–207.

25. Gerzson MF, Victoria FN, Radatz CS et al. In vitro antioxidant activity and in vivo antidepressant-like effect of alpha-(phenylselanyl) acetophenone in mice. Pharmacol Biochem Behav 2012; 69: 21–29.

26. Victoria FN, Anversa R, Penteado F et al. Antioxidant and antidepressant-like activities of semi-synthetic α-phenylseleno citronellal, Eur J Pharmacol 2014; 742: 131–138. https://doi.org/10.1016/j.ejphar.2014.09.005 PMID: 25218989

27. Gai BM, Prigol M, Stein AL et al. Antidepressant-like pharmacological profile of 3-(4-fluorophenylselanyl)-2,5-diphenylselenophene: Involvement of serotonergic system. Neuropharmacol 2010; 59: 172–179.

28. Bruning CA, Souza AC, Gai BM et al. Antidepressant-like effect of m-trifluoromethyl-diphenyl diselenide in the mouse forced swimming test involves opioid and serotonergic systems. Eur J Pharmacol 2011; 658: 145–149. https://doi.org/10.1016/j.ejphar.2011.02.039 PMID: 21371464

29. Benton D, Cook R. The impact of selenium supplementation on mood. Biol Psychiatry 1991; 21: 1092–1098.

30. Benton D. Selenium intake, mood and other aspects of psychological functioning. Nutr Neurosci 2002; 5: 363–374. https://doi.org/10.1080/12028415021000055925 PMID: 12509066

31. Hanwell M. D.; Curtis D. E.; Lonie D. C.; Vandermeersch T.; Zurek E.; Hutchinson G. R. J Cheminform. 2012, 4, 1–17. https://doi.org/10.1186/1758-2946-4-1

32. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010; 31: 455–461. https://doi.org/10.1002/jcc.21334 PMID: 19499576

33. Morris GM, Huey R, Lindstrom W et al. AutoDock 4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 2009; 30: 2785–2791. https://doi.org/10.1002/jcc.21256 PMID: 19399780

34. Wang H, Goehring A, Wang KH et al. Structural basis for action by diverse antidepressants on biogenic amine transporters. Nature 2013; 503: 141–145. https://doi.org/10.1038/nature12648 PMID: 24121440

35. Pettersen EF, Goddard TE, Huang CC. UCSF Chimera—a visualization system for exploratory research and analysis. J Comput Chem 2004; 25: 1605–1612. https://doi.org/10.1002/jcc.20084 PMID: 15264254

36. Pennmatsa A, Wang KH, Gouaux E. X-ray structures of Drosophila dopamine transporter in complex with nisoxetine and reboxetine. Nat Struct Mol Biol. 2015; 22:506–518. https://doi.org/10.1038/nsmb.3029 PMID: 25961798

37. Qin B, Zhang Y, Zhou X et al. Selective serotonin reuptake inhibitors versus tricyclic antidepressants in young patients: a meta-analysis of efficacy and acceptability. Clin Ther 2014; 36: 1087–1095.

38. Zheng W, Wu J, Feng X et al. In silico Analysis and Experimental Validation of Lignan Extracts from Kadsura longipedunculata for Potential 5-HT1AR Agonists. PLoS One 2015; 10: 1–17.

39. Gandhimathi A, Sowdhamini R. Molecular modelling of human 5-hydroxytryptamine receptor (5-HT2A) and virtual screening studies towards the identification of agonist and antagonist molecules. J Biomol Struct Dyn 2016, 34: 952–970. https://doi.org/10.1080/07391102.2015.1062802 PMID: 26327576

40. Walsh RN, Cummins RA. The Open-Field Test: a critical review. Psychol Bull 1976; 83: 482–504. PMID: 17582919

41. Porsolt RD, Le Pichon M, Jalfre M.. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977; 266: 730–732. PMID: 559941

42. Savognago L, Jesse CR, Pinto LG. Diphenyl diselenide exerts antidepressant-like and anxiolytic-like effects in mice: Involvement of l-arginine-nitric oxide-soluble guanylate cyclase pathway in its antidepressant-like action. PharmacolBiochemBehav 2008; 88: 418–426.

43. Martinez DM, Barcellos A, Casaril AM. Antidepressant-like activity of dehydrozingerone: Involvement of the serotonergic and noradrenergic systems. PharmacolBiochemBehav 2014; 127: 111–117.

44. Pesaro A.P., Sampaio T.B., Stangherlin E.C., Mantovani A.C., Zeni G., Nogueira C.W., The antidepressant-like effect of 7-fluoro-1,3-diphenylisoquinoline-1-amine in the mouse forced swimming test is mediated by serotonergic and dopaminergic systems, Prog Neuro-Psychopharmacol Biol Psychiatry, 54 (2014) 179–186.

45. Colla ARS, Oliveira A, Pazini FL et al. Serotonergic and noradrenergic systems are implicated in the antidepressant-like effect of ursolic acid in mice. Pharmacol Biochemistry and Behavior 2014; 24: 108–116.
46. Müller LG, Salles LA, Stein AC et al. Antidepressant-like effect of Valeriana glechomifolia Meyer (Valerianaceae) in mice. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2012; 36: 101–109. https://doi.org/10.1016/j.pnpbp.2011.08.015 PMID: 21889562

47. Neis VB, Moretti M, Manosso LM et al. Agmatine enhances antidepressant potency of MK-801 and conventional antidepressants in mice. Pharmacol Biochem Behav 2015; 230: 9–14.

48. Zhou Z, Zhen J, Karpowich NK. Antidepressant specificity of serotonin transporter suggested by three Leu-T-SSRI structures. Nat Struct Mol Biol 2009; 16: 652–657. https://doi.org/10.1038/nsmb.1602 PMID: 19430461

49. Ramezanpour M, Seyed-Allaei H. A Molecular Dynamics Study on SSRI Antidepressant Drugs. Biophysical Journal 2015; 1: 108–469.

50. Wang H, Goehring A, Wang KW et al. Structural basis for action by diverse antidepressants on biogenic amine transporters. Nature 2013; 503: 141–145. https://doi.org/10.1038/nature13552 PMID: 25119048

51. Sorensen L, Andersen J, Thomsen M et al. Interaction of antidepressants with the serotonin and norepinephrine transporters: mutational studies of the S1 substrate: binding pocket. J Biol Chem 2012; 43694–43707. https://doi.org/10.1074/jbc.M112.342212 PMID: 23086945

52. Sylte I, Bronowska A, Dahl SG. Ligand induced conformational states of the 5-HT(1A) receptor. Eur J Pharmacol 2001; 416: 33–41. PMID: 11282110

53. Hassaine G, Deluz C, Grasso L et al. X-ray structure of the mouse serotonin 5-HT3 receptor; Nature 2014; 512: 276–281. https://doi.org/10.1038/nature13552 PMID: 25119048

54. Lummis SC. 5-HT (3) receptors; J Biol Chem 2012; 287: 40239–40245. https://doi.org/10.1074/jbc.R111.406496 PMID: 23038271

55. Blier P, de Montigny C. Modification of 5-HT neuron properties by sustained administration of the 5-HT1A agonist gepirone: electrophysiological studies in the rat brain. Synapse 1987 1: 470–480. https://doi.org/10.1002/syn.890010511 PMID: 8873352

56. Blier P, Ward NM. Is there a role for 5-HT1A agonists in the treatment of depression? Biol Psychiatry 2003; 53: 193–203. PMID: 12559651

57. Artigas F, Romero L, de Montigny C, Blier P. Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. Trends Neurosci, 1996; 19: 378–383. https://doi.org/10.1016/S0166-2236(96)10037-0 PMID: 8873352

58. Scorzca MC, Lladó-Pelfort L, Oller S et al. Preclinical and clinical characterization of the selective 5-HT (1A) receptor antagonist DU-125530 for antidepressant treatment. British J Pharmacol 2012; 167: 1021–1034.

59. Carr GV, Lucki I. The role of serotonin receptor subtypes in treating depression: a review of animal studies. Psychopharmacol (Berl) 2011; 67: 265–287

60. Marek GJ, Carpenter LL, McDougle CJ, Price LH. Synergistic action of 5-HT2A antagonists and selective serotonergic reuptake inhibitors in neuropsychiatric disorders. Neuropsychopharmacol 2003; 28: 402–412.

61. Quesseveur G, Reperant C, David DJ et al. 5-HT(2)A receptor inactivation potentiates the acute antidepressant-like activity of escitalopram: involvement of the noradrenergic system. Exp Brain Res 2013; 226: 285–295. https://doi.org/10.1007/s00221-013-3434-3 PMID: 23411676

62. Carr GV, Lucki I. The role of serotonin receptor subtypes in treating depression: a review of animal studies. Psychopharmacol (Berl) 2011; 213: 265–287.

63. Pytka K, Partyka A, Jastražbska-Więsek M et al. Antidepressant- and Anxiolytic-Like Effects of New Dual 5-HT(1A) and 5-HT(7) Antagonists in Animal Models. PLoS One 2015; 10: 1–15.

64. Donato F, de Gomes MG, Goes ATR, Seus N, Alves D, Jesse CR, et al. Involvement of the dopaminergic and serotonergic systems in the antidepressant-like effect caused by 4-phenyl-1-(phenylselanylmethyl)-1,2,3-triazole. Life Sci. 2013; 93:393–400. https://doi.org/10.1016/j.lfs.2013.07.024 PMID: 23911670

65. Deng X-Q, Song M-X, Zheng Y, Quan Z-S. Design, synthesis and evaluation of the antidepressant and anticonvulsant activities of triazole-containing quinoliones. Eur J Med Chem. 2014; 73:217–224. https://doi.org/10.1016/j.ejmech.2013.12.014 PMID: 24412497

66. Khan I, Tantray MA, Hamid H, Alam MS, Kalam A, Hussain F et al. Synthesis of pyrimidin-4-one-1,2,3-triazole conjugates as glycogen synthase kinase-3β inhibitors with anti-depressant activity. Bioorg Chem. 2016; 68:41–55. https://doi.org/10.1016/j.bioorg.2016.07.007 PMID: 27454617

67. Goldberg JF. A preliminary open trial of nefazodone added to mood stabilizers for bipolar depression. J Affect Disord. Netherlands; 2013; 144(1–2):176–178. https://doi.org/10.1016/j.jad.2012.04.037 PMID: 22858262
68. Ruyck J, Brysbaert G, Blossey R, Lensink MF. Molecular docking as a popular tool in drug design, an in silico travel. Vol. 9, Advances and Applications in Bioinformatics and Chemistry; 2016; 1–11. https://doi.org/10.2147/AABC.S105289 PMID: 27390530

69. Pissurleenkar RS, Dhir A, Kessar S V, Kulkarni SK, Coutinho EC. An activity model for novel antidepressants that interact with the serotonin transporter (SERT). Cent Nerv Syst Agents Med Chem. Netherlands; 2011; 11(3):228–237. PMID: 21919867

70. Praveen C, Iyyappan C, Girija K, Kumar KS, Perumal Pt. Regioselective synthesis and evaluation of 3-alkylidene-1, 3-dihydroisobenzofurans as potential antidepressant agents. J Chem Sci. 2012; 124(2):451–62.

71. Gabrielsen M, Wołosewicz K, Zawadzka A, Kossakowski J, Nowak G, Wolak M, et al. Synthesis, antidepressant evaluation and docking studies of long-chain alkyl nitroquinazines as serotonin transporter inhibitors. Chemical biology & drug design. 2013; 18: 695–706.

72. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology (Berl). Germany; 2005; 177(3):245–55.

73. Yankelevitch-Yahav R, Franko M, Huly A, Doron R. The Forced Swim Test as a Model of Depressive-like Behavior. Journal of Visualized Experiments: JoVE. 2015.

74. Miguel-Hidalgo JJ, Rajkowska G. Morphological brain changes in depression: can antidepressants reverse them? CNS Drugs 2002; 16: 361–372. PMID: 12027783

75. Malykhin NV, Carter R, Seres P, Coupland NJ. Structural changes in the hippocampus in major depressive disorder: contributions of disease and treatment. J Psychiatry Neurosci 2010; 35: 337–343. https://doi.org/10.1503/jpn.100002 PMID: 20731966

76. Duman RS. Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. Dialogues Clin Neurosci 2009; 11: 239–255. PMID: 19877493

77. Salim S. Oxidative Stress and Psychological Disorders. Current Neuropharmacol 2014; 12: 140–147.

78. Behr GA, Moreira JCF, Frey BN. Preclinical and clinical evidence of antioxidant effects of antidepressant agents: implications for the pathophysiology of major depressive disorder. Oxid Med Cell Longev. United States; 2012; 609:4–21.

79. Chang C-C, Lee C-T, Lan T-H, Ju P-C, Hsieh Y-H, Lai T-J. Effects of antidepressant treatment on total antioxidant capacity and free radical levels in patients with major depressive disorder. Psychiatry Res.; 2015; 230(2):575–80. https://doi.org/10.1016/j.psychres.2015.10.006 PMID: 26476591

80. Pytka K, Partyka A, Jastrzębska-Więsek M et al. Antidepressant- and Anxiolytic-Like Effects of New Dual 5-HT(1A) and 5-HT(7) Antagonists in Animal Models. PLoSOne 2015; 10: 1–15.