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Depression- and anxiogenic-like behaviors induced by lipopolysaccharide in mice are reversed by a selenium-containing indolyl compound: Behavioral, neurochemical and computational insights involving the serotonergic system

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ABSTRACT

Major depression and anxiety are highly incapacitating psychiatric disorders often present simultaneously, and the causal relationship between these disorders and inflammation are under extensive investigation. The treatment for this comorbidity still relies on drugs acting on the serotonergic neurotransmission, but the modulation of immune-inflammatory pathways has attained an increasing interest in the drug discovery. We have previously demonstrated that the selenoorganic compound 3-[(4-chlorophenyl)selanyl]-1-methyl-1*H*-indole (CMI) possess antioxidant, anti-inflammatory, antinociceptive and antidepressant-like effect in mice. Considering these pharmacological properties and the structural similarities between tryptophan, serotonin and CMI, the aim of the present study was to investigate whether CMI ameliorates depression- and anxiogenic-like behavior induced by lipopolysaccharide (LPS) in *Swiss* male mice by modulating the serotonergic system and reducing neuroinflammation. The administration of CMI (1 mg/kg, i.g) reversed the behavioral deficits induced by LPS (0.83 mg/kg, i.p) in the tail suspension test, splash test and elevated plus maze. The pre-treatment of mice with WAY100635 (5-HT_{1A} receptor antagonist), ketanserin (5-$HT_{2A/2C}$ receptor antagonist) and ondansetron (5-HT_3 receptor antagonist) prevented the antidepressant- and anxiolytic-like effect elicited by CMI treatment after the LPS challenge. The administration of CMI also counteracted the increased expression of pro-inflammatory cytokines and indoleamine 2,3-dioxygenase (IDO) in the prefrontal cortex and hippocampus of mice challenged with LPS. Additionally, a molecular docking analysis showed that CMI binds to the active site of the serotonin transporter and IDO. These findings suggest that CMI reversed behavioral and biochemical alterations in the depression-anxiety comorbidity induced by LPS, possibly by modulation of neuroinflammatory mediators and the serotonergic system.

1. Introduction

According to the World Health Organization, 4.4% of the global population suffer from major depressive disorder (MDD) and 3.6% from anxiety disorder (World Health Organization, 2017). These conditions are often presented simultaneously, and patients with anxious depression are more resistant to treatments and tend to have more severe depressive symptoms, such as fatigue and worthlessness (Fava et al., 2008; Goldberg et al., 2014).

Despite the high social impact and economic burden caused by MDD and anxiety, the current therapy to treat this comorbidity is still limited. Benzodiazepines are anxiolytics that present a high risk of abuse with long-term use and have limited efficacy treating depressive symptoms (Hoffman and Mathew, 2008). Selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitors (SSRIs) are the first line of treatment for MDD and anxiety disorders (Griebel and Holmes, 2013).

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However, SSRIs exhibit modest efficacy and considerable adverse effects (including nausea, diarrhea, insomnia, cognitive impairment, sexual dysfunction, and sleep disturbances), leading to the discontinuation of treatment (Lader, 2007; Shelton, 2018). It has been proposed that the simultaneously blocked of 5-HT_{1A} autoreceptors and 5-HT transporters (SERT) increase the time for onset of antidepressant action when compared to SSRI alone (Artigas et al., 1994; Maes et al., 1999; Starr et al., 2007). Downregulation of 5-TH_{2A} has also been shown to produce antidepressant-like activities (Zaniewska et al., 2010) and can act synergistically with SSRIs (Marek et al., 2003). The blockade of 5-TH_{2C} receptors parallels with the onset of action of SSRIs, while activation of these receptors induces sleep disturbances and motor impairment (Millan, 2005). Similarly, the antagonism of the 5-HT_3 receptors has been associated with the faster onset of action of antidepressant drugs (Alam et al., 2013; Bétry et al., 2013).

It has been acknowledged that the modulation of the central serotonergic system may involve the activation of systemic immune-inflammatory pathways (Couch et al., 2013; Felger, 2018; Miller et al., 2013). Environmental challenges (such as diet and lifestyle factors), psychosocial stress and medical illness contribute to the increased inflammation found in patients with mood and anxiety disorders. Pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), induce the activity of the enzyme indoleamine 2,3-dioxygenase (IDO), which reduces the availability of tryptophan disrupting the synthesis of central 5-HT (Wichers and Maes, 2004). Additionally, IL-1β can upregulate the 5-HT transporter (SERT) causing a decrease of extracellular 5-HT (Ramamoorthy et al., 1995) and antidepressant drugs are known to reverse the inflammation-induced depression, corroborating the idea that cytokine production may directly affect the 5-HT system (Horikawa et al., 2003). Indeed, the relationship between the serotonergic system and psychiatric disorders as a function of immune-to-brain communications has been investigated for years and provide a valid ground for the development of improved therapies (Miller et al., 2017). Several lines of evidence suggest that inflammation plays a role in depression and anxiety (Felger, 2018; Mattei and Notter, 2019; Singhal et al., 2014), as the action of inflammatory cytokines ultimately leads to altered neurocircuits in the brain. In pre-clinical studies, depression-like behavior can be induced by acute inflammation by the administration of cytokine inducers, such as lipopolysaccharide (LPS) (O'Connor et al., 2009).

Recently, it has been reported that the selenium-containing compound 3-[(4-chlorophenyl)selanyl]-1-methyl-1*H*-indole (CMI) possesses antioxidant activity (Vieira et al., 2015) and it is able to protect extracellular matrix proteins against damage induced by inflammation-derived oxidants (Casaril et al., 2017b). The administration of CMI has also been shown to prevent the depression-like behavior induced by systemic LPS injection and reversed the depression-like behavior elicited by acute restraint stress in mice by targeting oxidative alterations, neuroinflammation and corticosterone levels (Casaril et al., 2019). In addition, the antinociceptive effect of CMI involves the modulation of 5-HT_{1A}, 5-$HT_{2A/2C}$, and 5-HT_3 receptors (Birmann et al., 2018). Importantly, no signs of hepatic and renal toxicity were found after CMI treatment (Casaril et al., 2017a). The structure of CMI may be responsible for some of these effects: the indole nucleus found in this selenoorganic compound is also present in naturally occurring bioactive compounds, such as tryptophan, melatonin, serotonin, and lysergic acid. In light with this, based on (i) antioxidant, anti-inflammatory and antidepressant-like effect of CMI; (ii) the structural similarity between CMI, tryptophan and serotonin; and (iii) the involvement of the serotonergic system in the antinociceptive effect of CMI, we hypothesize that CMI would be able to counteract the behavioral and biochemical alterations induced by acute inflammation in mice.

In this study, our goal was to evaluate whether CMI reverses the depression- and anxiogenic-like behaviors induced by LPS in mice and whether the 5-HT_{1A}, 5-$HT_{2A/2C}$, and 5-HT_3 receptors and decreased neuroinflammation are involved in its effect. A depth understanding of the mechanism of action of CMI may enable us to characterize CMI as a promising complementary therapy for the comorbidity between MDD and anxiety disorders.

2. Materials and methods

2.1. Animals

Adult male *Swiss* mice (25–30 g) provided by the Animal Facility of the Federal University of Pelotas (UFPel) were used. Mice were housed in groups of six animals in standard polypropylene cages, with wood shavings litter, in a temperature (22 ± 1 °C)- and humidity (45–55%)-controlled environment with a 12/12-h light/dark cycle (light on at 07:00 a.m.). Food and water were available *ad libitum*. Six to eight animals were randomly allocated into each experimental group and all behavioral tests were carried out between 09:00 a.m. and 03:00 p.m. All procedures were approved by the Committee on the Care and Use of Experimental Animal Resources at the Federal University of Pelotas, Brazil (8331-2017) and comply with the Animal Research: Reporting in Vivo Experiments (ARRIVE) guidelines, items 5 to 13 (Kilkenny et al., 2010).

2.2. Drugs

LPS from *E. coli* (L-3129, serotype 0127:B8), ketanserin, ondansetron and WAY100635 were purchased from Sigma Chemical Co, USA. CMI (Fig. 1A) was prepared and characterized at the Laboratory of Clean Organic Synthesis at the Federal University of Pelotas (Vieira et al., 2015). All other chemicals were of analytical grade and obtained from standard commercial suppliers. LPS was diluted in saline and administered intraperitoneally (i.p.) at a dose of 0.83 mg/kg. CMI was dissolved in canola oil (a non-polar and inert substance) and administered intragastrically (i.g.) at 1 mg/kg. WAY100635 (0.1 mg/kg, subcutaneously (s.c.); a 5-HT_{1A} receptor antagonist), ketanserin (1 mg/kg, i.p.; a 5-$HT_{2A/2C}$ receptor antagonist) and ondansetron (1 mg/kg, i.p.; a 5-HT_3 receptor antagonist) were diluted in saline (Martinez et al., 2014; Pinto Brod et al., 2016). Observers blind to the drug treatments recorded all behavioral tests and analyzed the results.

2.3. Experimental design

The experimental design is depicted in Fig. 1. In the first part of the study (Fig. 1B), we addressed the ability of CMI to reverse the depression- and anxiogenic-like behaviors induced by LPS. For that, mice were divided into four groups (n = 6 animals/group): (1) saline + canola oil; (2) saline + CMI; (3) LPS + canola oil; (4) LPS + CMI. The behavioral tests were carried out in the following order: open field test, elevated plus maze test, marble burying test, tail suspension test, and splash test. Fifteen minutes after the last behavioral test, mice were euthanized by an overdose of isoflurane inhalation followed by removal of the prefrontal cortex (PFC) and hippocampus (HC) for determination of IL-1β, TNF-α, and IDO mRNA expression. In this sense, the brain regions were collected within 90–120 min after CMI administration. The dose and the time of CMI administration were selected based on our previous studies (Birmann et al., 2018; Casaril et al., 2017a, 2019) and preliminary data (data not shown). The administration of CMI 1, 5 and 10 mg/kg (i.g) after LPS (0.83 mg/kg; i.p.) had similar effects in the immobility time in the tail suspension test (TST) and number of entries in the open arms in the elevated plus maze test (EPM); therefore, the dose of 1 mg/kg was selected for the present study. Additionally, 1 mg/kg of CMI reversed behavioral and biochemical alterations induced by acute restraint stress in mice (Casaril et al., 2019).

The second cohort of mice (Fig. 1C) was used to address the involvement of the serotonergic system in the antidepressant- and anxiolytic-like effects of CMI in LPS-challenged mice. Mice were separated in eight groups (n = 8 animals/each) and received WAY100635,

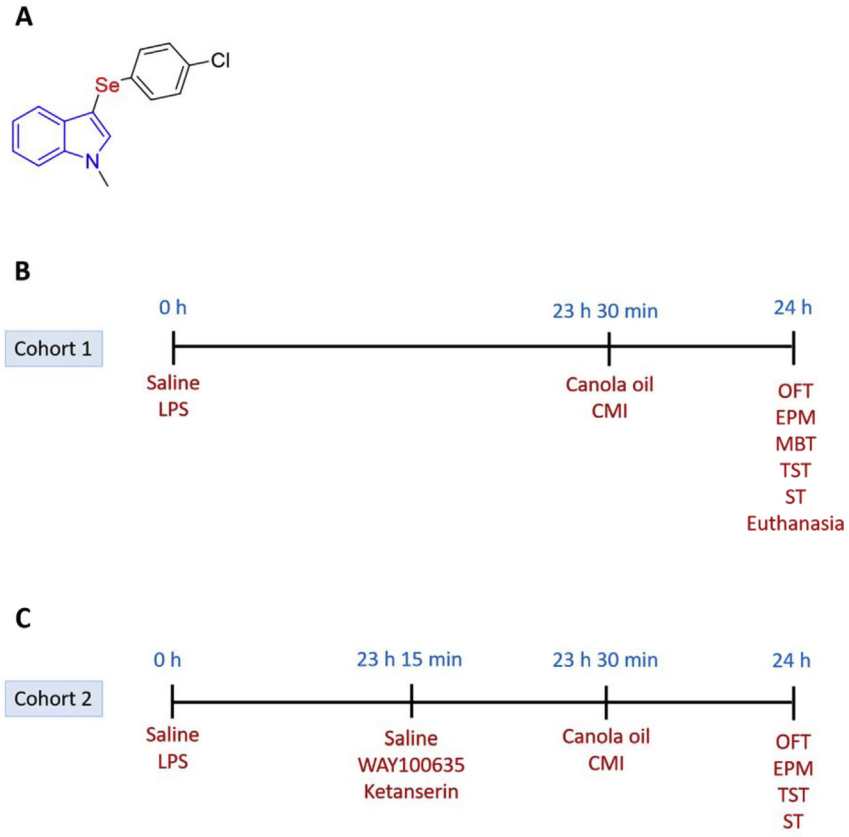

Fig. 1. (A) Chemical structure of 3-((4-chlorophenyl)selanyl)-1-methyl-1*H*-indole (CMI) and (B and C) experimental design performed in this study. LPS: lipopolysaccharide. OFT: open field test. TST: tail suspension test. ST: splash test. EPM: elevated plus maze. MBT: marbles burying test.

ketanserin, ondansetron, or vehicle (saline) 23 h and 15 min after LPS or vehicle (saline). After 15 min, they were treated with CMI or vehicle (canola oil), followed by behavioral analysis 30 min later (Martinez et al., 2014). This schedule was used to maintain the behavioral tests 24 h after the LPS-challenge and 30 min after CMI treatment. All mice were submitted to the same sequence of behavioral tests 30 min after CMI administration (open field test, elevated plus maze, tail suspension test, and splash test). A crossover design was not used in the present study since we do not know if a second administration of CMI would result in a different behavioral outcome, a topic that deserves further investigation. Additionally, we used four behavioral tests to characterize the involvement of the serotonergic system in the antidepressant- and anxiolytic-like effect of CMI for the first time. In this sense, mice were submitted just once to the behavioral tests in an attempt to avoid possible interferences due to the stress imposed by the testing session.

2.4. Behavioral tests

2.4.1. Open field test (OFT)

To address the possible effects of CMI on locomotor activity, mice were evaluated in the open-field paradigm (Walsh and Cummins, 1976). Mice were individually placed in the center of a box (30 × 30 × 15 cm) with the floor divided into 9 equal squares. The number of rectangles crossed by the animals with its four paws (crossing) and rising of the front paws (rearing) was registered during a period of 5 min. The number of crossing and rearing was considered as indicative of locomotor activity and exploratory behavior, respectively.

2.4.2. Tail suspension test (TST)

The depression-like behavior was characterized by the total immobility time induced by the tail suspension test (Steru et al., 1985). Mice were suspended 50 cm above the floor and the immobility time was recorded during a 4 min period of a 6 min session. Mice were considered immobile only when they hung passively and completely motionless.

2.4.3. Splash test

This test consists in spraying a 10% sucrose solution on the dorsal coat of the mice in their home cage (Taksande et al., 2013). The sucrose solution dirties the coat and induces grooming behavior (including nose/face grooming, head washing, and body grooming), which was observed during 5 min. The time spent grooming is an index of self-care and motivational behavior.

2.4.4. Elevated plus maze

The anxiolytic-like behavior of mice was evaluated in the elevated plus maze test. The elevated plus maze consists of a plus-shaped apparatus with two open and two enclosed arms (arm length: 100 cm; arm width: 10 cm) connected by a central platform elevated 40 cm above the floor (Boulle et al., 2014). For the test, the mouse was placed in the center of the apparatus, and the number of entries and time spent in the open arms were scored manually in a 5 min session.

2.4.5. Marble burying test

The marble burying test was also used to address the anxiolytic-like behavior of mice. For the marble burying test, eight glass marbles (ø 1 cm) were evenly spaced on regular bedding in exact copies of the home cages (Broekkamp et al., 1986). Each mouse was allocated to a marble-containing cage and allowed 30 min to explore. After returning the animals to their home cages, the marbles were counted. A marble was considered buried when 2/3 or more of its size was covered with the bedding.

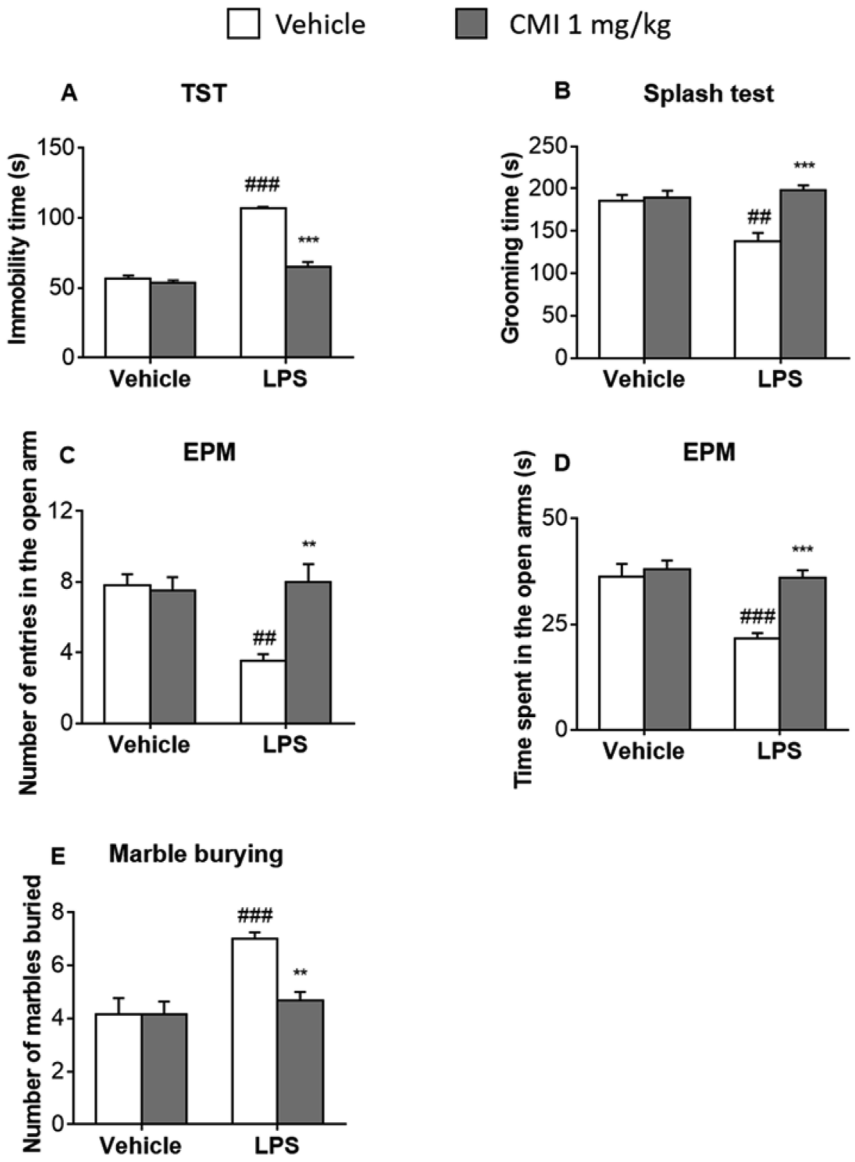

Fig. 2. Effect of CMI (1 mg/kg, i.g.) on behavioral tasks of mice challenged with LPS. (A) Immobility time in the tail suspension test, (B) grooming time in the splash test, (C) number of entries and (D) time spent in the open arms of the elevated plus maze apparatus and (E) number of marbles buried. Data are expressed as mean ± SEM of six independent animals. ($^{\#\#}$) $p < 0.01$ and ($^{\#\#\#}$) $p < 0.001$ when compared to control group. (**) $p < 0.01$ and (***) $p < 0.001$ when compared to LPS-treated group. LPS: lipopolysaccharide. CMI: 3-((4-chlorophenyl)selanyl)-1-methyl-1*H*-indole.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total mRNA was extracted in PFC and HC of mice as previously described (Casaril et al., 2017a; Domingues et al., 2019). The primer sequences for interleukin 1-beta (IL-1β; fwd 5′-GCT GAA AGC TCT CCA CCT CAA TG-3′, rev 5′-TGT CGT TGC TTG GTT CTC CTT G -3′), tumor necrosis factor-alpha (TNF-α; fwd 5′-CAT CTT CTC AAA ATT CGA GTG ACA A-3′, rev 5′-TGG GAG TAG ACA AGG TAC AAC CC-3′), indoleamine-2,3-dioxygenase (IDO; fwd 5′-AAT CAA AGC AAT CCC CAC TG-3′, rev 5′-AAA AAC GTG TCT GGG TCC AC-3′), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; fwd 5′-AGG TCG GTG TGA ACG GAT TTG-3′, rev 5′-TGT AGA CCA TGT AGT TGA GGT CA-3′) were obtained from Exxtend Biotecnologia Ltda, Campinas, Brazil. The $2^{\Delta\Delta CT}$ (Delta-Delta Comparative Threshold) method was used to normalize the fold change in gene expressions.

2.6. Molecular docking

The type of interaction of CMI with the serotonin transporter (SERT, PDB: 5I6X) and indoleamine 2,3-dioxygenase (IDO, PDB: 5WHR) was predicted using the software Autodock Vina 1.1.2 (Trott and Olson, 2010). The crystal structure of the proteins was retrieved from the Protein Data Bank (http://www.pdb.org/pdb/) and prepared by CHIMERA 1.5.3, including removal of ligands (Pettersen et al., 2004). A grid box size covering the residues in the active site of the proteins was implemented by Autodock Tools 1.5.6 (Morris et al., 2009). CMI was designed and optimized in the software Avogadro 1.1.1. (Hanwell et al., 2012). Docking poses of the investigated compound were visualized using Accelrys Discovery Studio 3.5.

2.7. Statistical analysis

All experimental results are given as a mean ± standard error of the mean (SEM). Behavioral and biochemical comparisons were performed by two-way analyses of variances (ANOVAs) (LPS *vs* vehicle × CMI *vs* vehicle). Three-way ANOVAs (LPS *vs* vehicle × CMI *vs* vehicle × serotonergic antagonist *vs* vehicle) were performed for measures of serotonergic antagonists influence on behavioral outcomes.

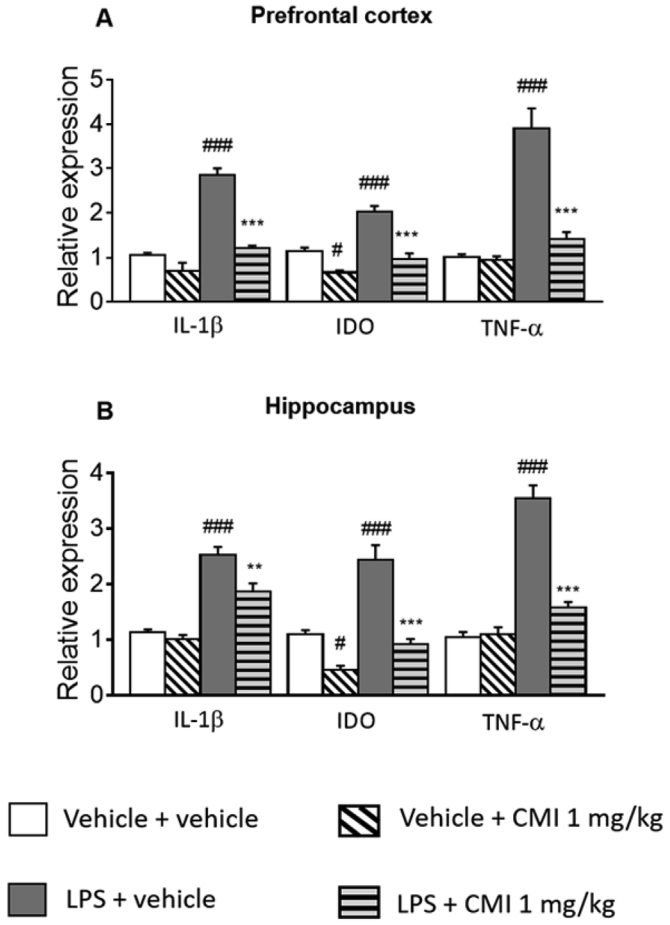

Fig. 3. Effect of CMI (1 mg/kg, i.g.) in the mRNA expression of target genes. Expression of IL-β, TNF-α and IDO in PFC (A) and HC (B) of LPS-challenged mice. Data are expressed as mean ± SEM of six independent animals. ($^{\#}$) $p < 0.05$ and ($^{\#\#\#}$) $p < 0.001$ when compared to control group. (**) $p < 0.01$ and (***) $p < 0.001$ when compared to LPS-treated group. LPS: lipopolysaccharide. CMI: 3-((4-chlorophenyl)selanyl)-1-methyl-1*H*-indole. PFC: prefrontal cortex. HC: hippocampus.

When ANOVA revealed a significant interaction, the Tukey's multiple comparison tests was employed. Pearson's correlation analysis was used to investigate any possible relationship between behavioral and neurochemical data. A value of $p < 0.05$ was considered significant. The analysis were performed using the software Graph Pad Prism version 7.0 for Windows.

3. Results

3.1. Acute administration of CMI reversed LPS-induced depression- and anxiogenic-like behavior without evoking locomotor alteration

In the present study, neither LPS challenge nor CMI treatment changed the number of crossings and rearings in the OFT (data not shown). A two-way ANOVA revealed no significant LPS × CMI interaction ($F_{(1,\ 20)} = 0.17$, $p = 0.68$) for the number of crossings and for the number of rearing ($F_{(1,\ 20)} = 0.71$, $p = 0.41$). Additionally, the administration of CMI in control mice did not alter the behavioral outcomes in the present study.

The results depicted in Fig. 2A show that CMI treatment reversed the increased immobility time induced by LPS in the TST, as compared to the LPS group (LPS × CMI interaction $F_{(1,\ 20)} = 61.20$, $p < 0.001$). Fig. 2B shows the effect of CMI administration after LPS challenge on grooming time in the splash test, and a statistically significant LPS × CMI interaction ($F_{(1,\ 20)} = 12.70$, $p = 0.002$) was found.

The effect of CMI in the anxiolytic-like behavior induced by LPS in the elevated plus maze is depicted in Fig. 2C and D. A two-way ANOVA showed a statistically significant LPS × CMI interaction for the number of entries ($F_{(1,\ 20)} = 11.00$, $p = 0.003$) and for the time spent in the open arms ($F_{(1,\ 20)} = 8.25$, $p = 0.009$). Similarly, as depicted in Fig. 2E, the treatment with CMI reversed the increased number of marbles buried (LPS × CMI interaction $F_{(1,\ 20)} = 7.10$, $p = 0.01$).

3.2. The reversion of LPS-induced IL-1β, TNF-α and IDO mRNA upregulation may be responsible for the antidepressant- and anxiolytic-like effects of CMI

A two-way ANOVA of IL-1β mRNA expression revealed a significant LPS × CMI interaction in the PFC ($F_{(1,\ 20)} = 23.30$, $p < 0.001$) (Fig. 3A) and HC ($F_{(1,\ 20)} = 5.43$, $p = 0.03$) (Fig. 3B) of mice, while the administration of CMI in control mice did not influence the IL-1β expression in the PFC and HC. Similarly, a two-way ANOVA of TNF-α mRNA expression revealed a significant LPS × CMI interaction in the PFC ($F_{(1,\ 20)} = 24.50$, $p < 0.001$) (Fig. 3A) and HC ($F_{(1,\ 20)} = 45.90$, $p < 0.001$) (Fig. 3B) of mice and the administration of CMI in control mice did not influence the expression of TNF-α in both brain structures. A two-way ANOVA also revealed a significant LPS × CMI interaction between the mRNA levels of IDO in the PFC ($F_{(1,\ 20)} = 8.25$, $p = 0.009$ (Fig. 3A) and HC ($F_{(1,20)} = 8.32$, $p = 0.009$) of mice (Fig. 3B). In this case, administration of CMI in control mice also reduced the expression of IDO in PFC ($p = 0.02$) and HC ($p = 0.03$).

3.3. Significant correlations were found between behavioral variables and mRNA expression in PFC and HC of mice

Bearing in mind the administration of CMI reversed the behavioral alterations induced by LPS and counteracted the increased mRNA expression of IL-1β, TNF-α and IDO in the PFC and HC of mice, we

Table 1
Pearson's correlation between the behavioral variables and mRNA expression of selected genes in the PFC and HC of mice treated with CMI after challenge with LPS.

		Immobility time	Grooming time	Number of entries in the open arms	Time spent in the open arms	Number of marbles buried
IL-1β	PFC	0.918***	−0.821**	−0.725**	−0.843***	0.821**
	HC	0.587*	−0.462	−0.488	−0.650*	0.428
TNF-α	PFC	0.813**	−0.586*	−0.573	−0.777**	0.617*
	HC	0.877***	−0.701*	−0.702*	−0.887***	0.759**
IDO	PFC	0.901***	−0.779**	−0.740**	−0.804**	0.857***
	HC	0.831***	−0.780**	−0.765**	−0.777**	0.815**

LPS: lipopolysaccharide. CMI: 3-((4-chlorophenyl)selanyl)-1-methyl-1*H*-indole. PFC: prefrontal cortex. HC: hippocampus. IL-1β: interleukin-1beta. TNF-α: tumor necrosis factor-alpha. IDO: indoleamine 2,3-dioxygenase. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$.

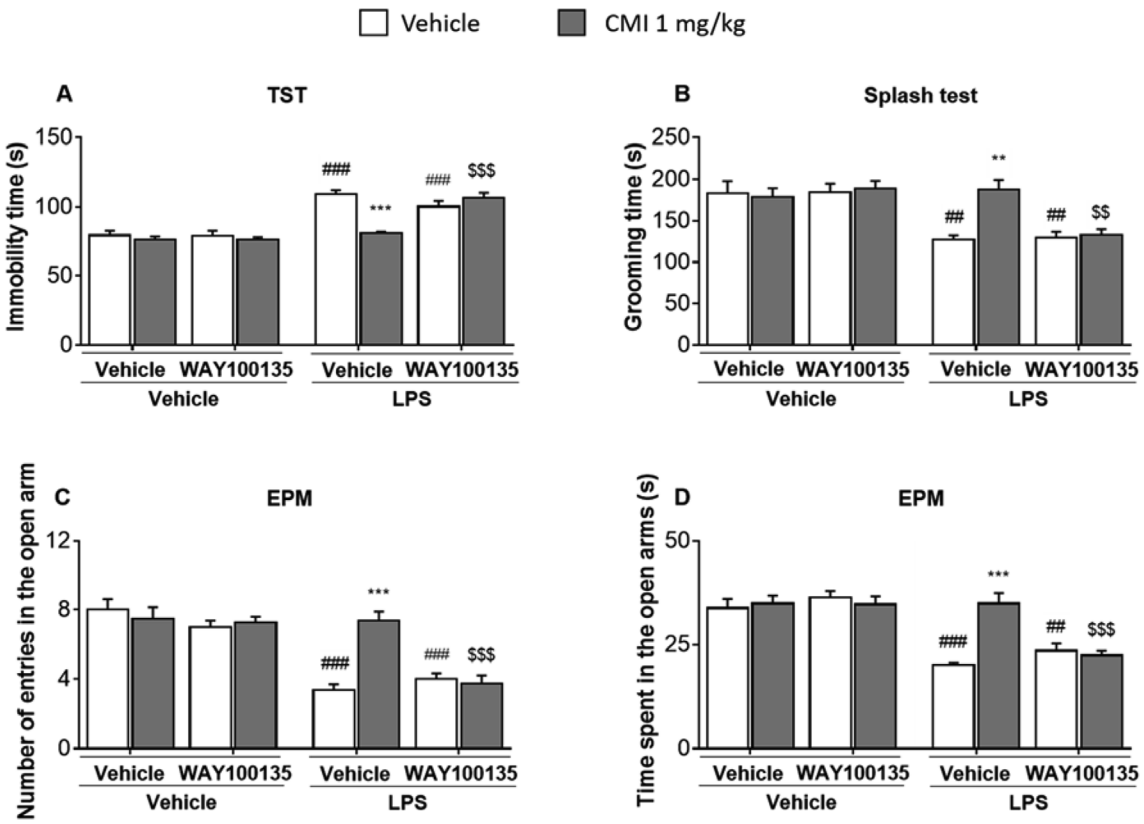

Fig. 4. Effect of WAY100635 pre-treatment on the behavioral effects of CMI in LPS-challenged mice. (A) Immobility time in the tail suspension test, (B) grooming time in the splash test, (C) number of entries and (D) time spent in the open arms of the elevated plus maze apparatus. Data are expressed as mean ± SEM of six independent animals. ($^{\#\#}$) $p < 0.01$ and ($^{\#\#\#}$) $p < 0.001$ when compared to control group. (**) $p < 0.01$ and (***) $p < 0.001$ when compared to LPS-treated group. ($^{\$\$}$) $p < 0.01$ and ($^{\$\$\$}$) $p < 0.001$ when compared to LPS-challenged mice treated with CMI. LPS: lipopolysaccharide. CMI: 3-((4-chlorophenyl)selanyl)-1-methyl-1*H*-indole.

analyzed if these effects were linked using Pearson's correlation analysis (Table 1). The results demonstrated a significant positive correlation between the immobility time in the TST and IL-1β, TNF-α, and IDO expressions in PFC and HC. A significant negative correlation was found between the grooming time in the splash test and the expression of IL-1β (in PFC), TNF-α, and IDO (in PFC and HC). Regarding the number of entries in the open arms of the EPM apparatus, a significant negative correlation was found with the expression of IL-1β (in PFC), TNF-α (in HC), and IDO (in both PFC and HC). Similarly, we found a significant negative correlation between the time spent in the open arms of the elevated plus maze apparatus and the expression of IL-1β, TNF-α and IDO in PFC and HC. Moreover, we found a significant positive correlation between the number of marbles buried and the expression of IL-1β, in PFC, and TNF-α and IDO in PFC and HC. On the other hand, no significant correlation was found between the expression of IL-1β in HC and grooming time, the number of entries and the time spent in the open arms of the elevated plus maze test and the number of marbles buried. In addition, we did not find a significant correlation between TNF-α expression in the PFC and the number of entries in the elevated plus maze test.

3.4. The antidepressant- and anxiolytic-like effects of CMI in LPS-challenged mice may be mediated by 5-HT$_{1A}$ receptor

The involvement of the 5-HT$_{1A}$ receptor in the antidepressant- and anxiolytic-like effects of CMI after the LPS challenge is depicted in Fig. 4. A three-way ANOVA showed a statistically significant WAY100135 × LPS × CMI interaction for the immobility time in the TST ($F_{(7,\ 56)} = 14.64$, $p < 0.001$) (Fig. 4A) and the grooming time in the splash test ($F_{(7,\ 56)} = 5.50$, $p = 0.02$) (Fig. 4B). A three-way ANOVA also showed a statistically significant WAY100135 × LPS × CMI interaction for the number of entries ($F_{(7,\ 56)} = 13.90$, $p < 0.001$) (Fig. 4C) and the time spent in the open arms ($F_{(7,\ 56)} = 6.57$, $p = 0.01$) (Fig. 4D) of the elevated plus maze apparatus. Neither three-way ANOVA nor post-hoc comparison showed statistically significant effects of the treatments in the number of crossings and rearings in the OFT (data not shown).

3.5. The 5-HT$_{2A/2C}$ receptor may be involved in the antidepressant- and anxiolytic-like effects of CMI in LPS-challenged mice

Fig. 5 shows the involvement of the 5-HT$_{2A/2C}$ receptors in the antidepressant- and anxiolytic-like effects of CMI after the LPS challenge. A three-way ANOVA showed a statistically significant ketanserin × LPS × CMI interaction for the immobility time in the TST ($F_{(7,\ 56)} = 31.26$, $p < 0.001$) (Fig. 5A), the grooming time in the splash test ($F_{(7,\ 56)} = 19.56$, $p < 0.001$) (Fig. 5B) and the number of entries ($F_{(7,\ 56)} = 10.73$, $p = 0.002$) (Fig. 5C) and time spent ($F_{(1,\ 1)} = 12.86$, $p < 0.001$) (Fig. 5D) in the open arms of the elevated plus maze apparatus. None of the experimental groups promoted alterations in the number of crossings and rearings in the OFT (data not shown).

3.6. The interaction with the 5-HT$_3$ receptor may contribute to the antidepressant- and anxiolytic-like effects of CMI in LPS-challenged mice

Fig. 6 shows the modulation of the 5-HT$_3$ receptor by CMI treatment after the LPS challenge. A three-way ANOVA showed a statistically significant ondansetron × LPS × CMI interaction for the immobility time in the TST ($F_{(7,\ 56)} = 40.95$, $p < 0.001$; (Fig. 6A) and the grooming time in the splash test ($F_{(7,\ 56)} = 8.68$, $p = 0.005$) (Fig. 6B). A

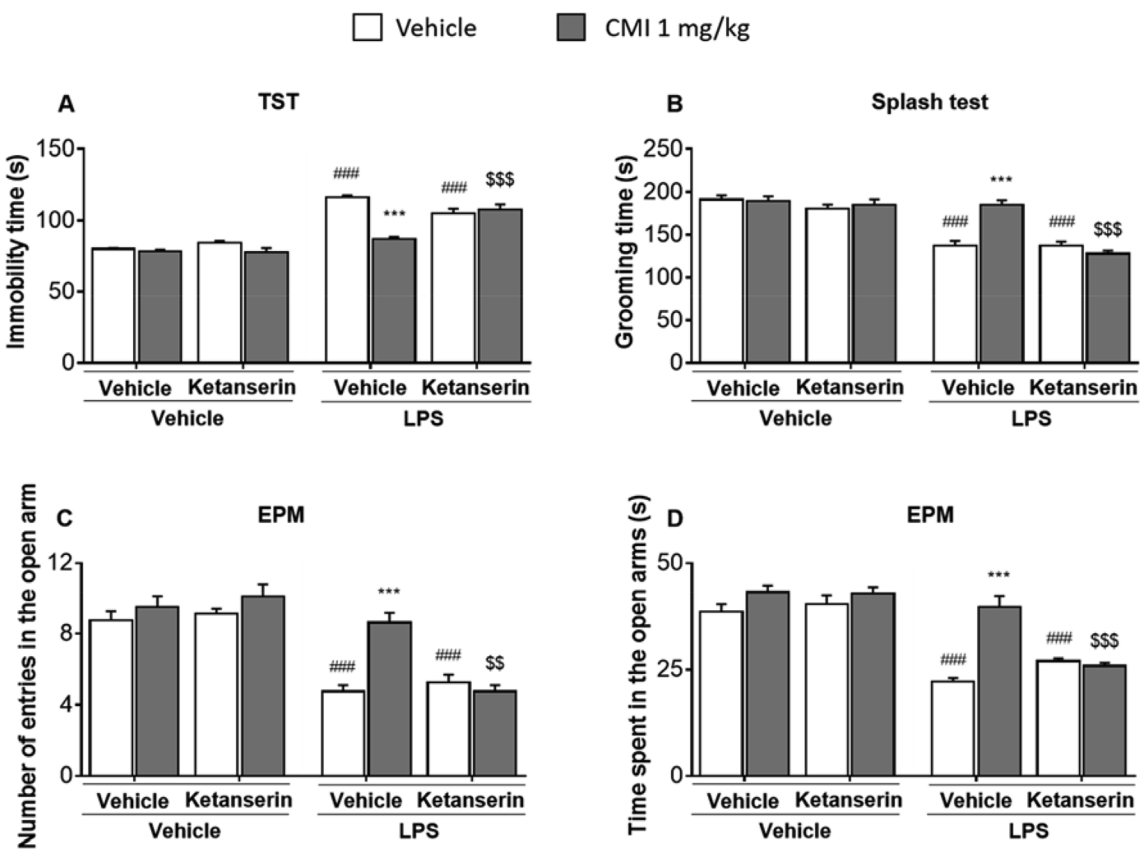

Fig. 5. Effect of ketanserin pre-treatment on the behavioral effects of CMI in LPS-challenged mice. (A) Immobility time in the tail suspension test, (B) grooming time in the splash test, (C) number of entries and (D) time spent in the open arms of the elevated plus maze apparatus. Data are expressed as mean ± SEM of six independent animals. ($^{###}$) $p < 0.001$ when compared to control group. (***) $p < 0.001$ when compared to LPS-treated group. ($^{\$\$}$) $p < 0.01$ and ($^{\$\$\$}$) $p < 0.001$ when compared to LPS-challenged mice treated with CMI. LPS: lipopolysaccharide. CMI: 3-((4-chlorophenyl)selanyl)-1-methyl-1*H*-indole.

three-way ANOVA also showed a statistically significant ondansetron × LPS × CMI interaction for the number of entries ($F_{(1, 1)} = 10.36$, $p = 0.002$) (Fig. 6C) and the time spent in the open arms ($F_{(7, 56)} = 26.02$, $p < 0.001$) (Fig. 6D) of the elevated plus maze apparatus. Of note, none of the treatments evoked psycholocomotor alterations in OFT (data not shown).

3.7. The modulation of SERT and IDO may account for the biological effects of CMI

The best binding positions of CMI with SERT and IDO are depicted in Figs. 7 and 8, respectively. Significant interactions were formed between CMI and the residues E332 (3.59 Å), F335 (3.73 Å) and V501 (3.76 Å) in the active site of SERT (Fig. 7). As well, other residues such as R104, I172, F341, E493, T497, G498, F556 played important roles for the interaction between CMI and SERT (binding free energy (Δ_G) of −7.9 kcal/mol). Fig. 8 shows the best binding mode of CMI with IDO (Δ_G of −8.3 kcal/mol), which involved close interactions with the residues S167 (3.60 Å) and A264 (3.85 Å). In addition, the residues Y126, C129, P163, V166, V170, F214, I217, L234, S263, F270, R343, H346 and T379 also contributed to the interaction of CMI with the active site of IDO.

4. Discussion

In the present study, we provided the first evidence that CMI reverses depression- and anxiogenic-like effects elicited by LPS in mice, depending on the modulation of 5-HT_{1A}, 5-$HT_{2A/2C}$, and 5-HT_3 receptors. In addition, CMI treatment reversed LPS-induced increased expression of pro-inflammatory cytokines and IDO in the PFC and HC of mice. The molecular docking approach showed that CMI interacts with the active site of SERT and IDO. Considering the use of multiple behavioral assays, combined with *ex vivo* and *in silico* investigation, our study provides solid evidence of the ability of CMI to counteract the depression-anxiety comorbidity in male mice.

Peripheral administration of LPS has been widely used to induce depression- and anxiogenic-like alterations in rodents, especially by inducing neuroinflammation (Domingues et al., 2018; O'Connor et al., 2009). With this in mind, we showed here that a single administration of CMI reversed the behavioral impairment induced by LPS. Additionally, we found significant correlations suggesting that the improved behavioral outcomes after CMI treatment may involve the reduction of neuroinflammation by downregulating the mRNA expression of IL-1β, TNF-α, and IDO in PFC and HC of mice challenged with LPS. It has been acknowledged that the activation of IDO by pro-inflammatory cytokines forms a link between immune function and altered serotonergic neurotransmission, since it reduces the availability of tryptophan that could be used for the synthesis of 5-HT, therefore, contributing to the establishment of depressed mood and anxiety (O'Connor et al., 2009). In light with this, we propose here that the reversion of the neuroinflammation by CMI may be involved in its antidepressant- and anxiolytic-like effects, corroborating with previous findings by our research group (Casaril et al., 2017a, 2019).

Through a molecular docking investigation, we supported the modulation of IDO as a possible mechanism of action of CMI. We showed that CMI interacted with IDO binding site (−8.3 kcal/mol) in a manner similar to the substrate tryptophan (Lewis-Ballester et al., 2017) and to epacadostat, the most advanced IDO inhibitor in clinical trials (Yue et al., 2017). Both tryptophan and CMI interacted with the protein matrix *via* various hydrophobic and polar interactions, possibly

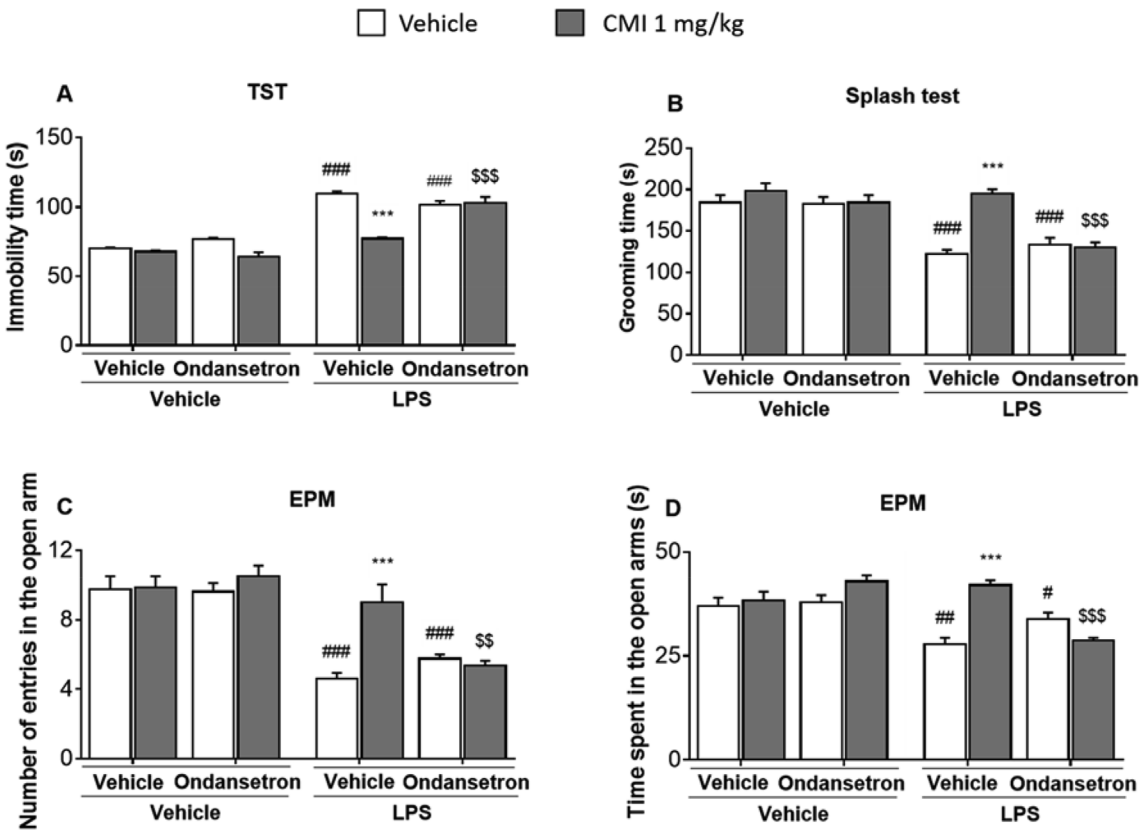

Fig. 6. Effect of ondansetron pre-treatment on the behavioral effects of CMI in LPS-challenged mice. (A) Immobility time in the tail suspension test, (B) grooming time in the splash test, (C) number of entries and (D) time spent in the open arms of the elevated plus maze apparatus. Data are expressed as mean ± SEM of six independent animals. ($^{\#}$) $p < 0.05$, ($^{\#\#}$) $p < 0.01$ and ($^{\#\#\#}$) $p < 0.001$ when compared to control group. (***) $p < 0.001$ when compared to LPS-treated group. ($^{\$\$}$) $p < 0.01$ and ($^{\$\$\$}$) $p < 0.001$ when compared to LPS-challenged mice treated with CMI. LPS: lipopolysaccharide. CMI: 3-((4-chlorophenyl)selanyl)-1-methyl-1*H*-indole.

because these molecules share the structural feature of having the indole nucleus. Particularly, the indole nucleus of the tryptophan and CMI interacts with A264 and S167 in the active site of IDO, which belongs to the so-called "A-site" (Röhrig et al., 2015). Of note, the residue H346 involved in the putative binding mode of CMI is critical for the dioxygenase activity of IDO (Lewis-Ballester et al., 2009). These findings indicate that CMI is able to downregulate the IDO mRNA expression whilst it could also directly inhibit the enzyme activity and indirectly downregulate the expression of its inducing cytokines, reversing the depression- and anxiogenic-like behavior induced by LPS.

Likewise, IL-1β and TNF-α p38 MAPK-linked pathways are implicated in the neuronal SERT expression (Zhu et al., 2010), which would reduce the availability of 5-HT in the synapse. This observation prompted us to investigate if CMI would also inhibit SERT, since MDD and anxiety are associated with dysregulation of this transporter. The molecular docking analysis revealed that CMI interacted with the central site of SERT with a Δ_G of −7.9 kcal/mol. The involvement of residues I172 and F341 may characterize CMI as a high-affinity molecule since these amino acids are important for the high-affinity interaction of (S)-citalopram and paroxetine with SERT (Coleman et al., 2016), which are among the widely used SSRI for the treatment MDD and anxiety. Interestingly, the fluorophenyl group of paroxetine and the chlorophenyl group of CMI interacted with F335, T497, and V501, suggesting the importance of the halophenyl group in the development of therapeutic drugs. It is worth highlighting that the residues R104, A331, and F556, belonging to the allosteric site of SERT, participate in the binding mode of CMI. Binding to both orthosteric and allosteric binding sites of SERT leads to higher extracellular 5-HT levels in vivo and faster 5-HT_{1A} autoreceptor desensitization promoting greater efficacy and/or a faster onset of action (Sanchez, 2006), which can support the antidepressant- and anxiolytic-like effect of CMI in LPS-challenged mice.

It is worth highlighting that SSRIs are still the first line of treatment of MDD and anxiety (Griebel and Holmes, 2013), however, improved outcomes of this treatment have been obtained with simultaneous blockage of 5-HT_{1A} autoreceptors (Artigas et al., 1994; Maes et al., 1999; Starr et al., 2007). These protein G-coupled receptors regulate the serotonergic tone and limit the SSRIs-induced initial increase in 5-HT extracellular levels (Hervás et al., 2000), which can delay the therapeutic response (Artigas et al., 1996). Of note, Ceglia et al. (2004) have shown that in addition to binding SERT, (S)-citalopram desensitizes the 5-HT_{1A} receptors, regulating the release of 5-HT in the prefrontal cortex. Here, we used a dose of WAY10635 that preferentially blocks 5-HT_{1A} autoreceptors (Ago et al., 2003) to investigate their involvement in the activity of CMI. Interestingly, it was observed that the pretreatment with WAY10635 prevented the antidepressant- and anxiolytic-like effects elicited by CMI in the TST, ST and EPM test, without evoking psycholocomotor alterations. Therefore, we suggest that the simultaneous interaction with SERT and 5-HT_{1A} may account for the neuropharmacological effect of CMI against the depression-anxiety comorbidity induced by LPS, which could explain the improved behavior observed 30 min after the administration of this organoselenium compound.

Similarly, the antagonism of 5-HT_{2A} and 5-HT_{2C} receptors elicits antidepressant and anxiolytic effects. For example, the antagonism of 5-HT_{2A} receptors enhances the antidepressant effect of SSRI (Celada et al., 2004). Clinical data have shown the rapid (1–2 days) antidepressant effect of risperidone, a potent 5-HT_{2A} receptor antagonist (Ostroff and

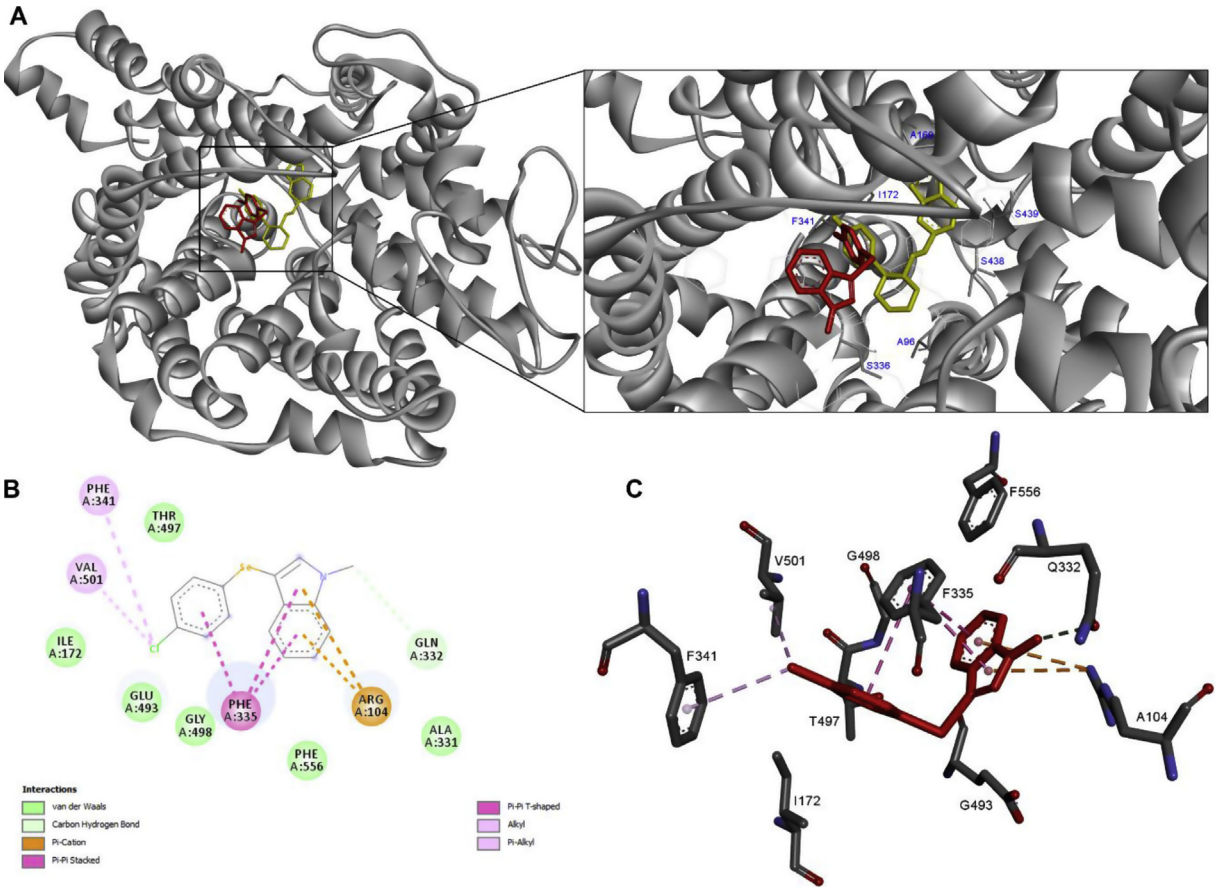

Fig. 7. Molecular docking of CMI in the binding pocket of SERT. (A) View of CMI (red) and the co-crystalized ligand, Paroxetine (yellow), bound to the X-ray crystallographic structure of SERT. (B) 2D and (C) 3D visualization of CMI interacting with amino acids residues in the active site of SERT.

Nelson, 1999). Administration of olanzapine, a 5-HT_{2A} and 5-HT_{2C} receptor antagonist, augmented the antidepressant effect of fluoxetine, with enhanced efficacy and faster onset of action (Shelton et al., 2001). Mirtazapine, which is a 5-HT_2 and 5-HT_3 antagonist, is particularly effective in anxious patients, exerting rapid and sustained effect (Alam et al., 2013). On the other hand, the activation of 5-HT_{2C} receptors induces motor impairment and sleep disturbances in patients (Millan, 2005), and the anxiogenic effects of 5-HT_{2C} receptors agonists are reduced by genetic deletion of these receptors in mice (Das and Tecott, 1996). In the present study, the pretreatment of mice with the 5-$HT_{2A/2C}$ antagonist ketanserin blocked the antidepressant- and anxiolytic-like effects elicited by CMI in LPS-challenged mice, without evoking psycholocomotor alterations. Considering that the antagonism of 5-HT_{2A} and 5-HT_{2C} receptors has antidepressant and anxiolytic effects, we suggest that the modulation of these receptors is possibly involved in the improved behavior observed in LPS-challenged mice treated with CMI. It is possible that the modulation of the 5-HT_{2C} receptor is responsible for the anxiolytic-like effect of CMI; however, more studies are required to confirm the antagonist nature of CMI towards these receptors.

The antagonism of 5-HT_3 receptors has an antidepressant effect, as evidenced with the drugs mirtazapine (Alam et al., 2013) and vortioxetine (Mørk et al., 2013). The 5-HT_3 receptors are the only serotonergic receptors belonging to ligand-gated ion channel superfamily, and 5-HT_3 antagonists have been shown to alleviate behavioral abnormalities in pre-clinical studies (Devadoss et al., 2010; Gupta et al., 2014), also showing anxiolytic activity in clinical trials (Lecrubier et al., 1993; Smith et al., 1999). Here we reported that the 5-HT_3 receptors are also involved in the antidepressant- and anxiolytic-like effects of CMI in LPS-challenged mice, since the blockade of this receptor subtype abolished the behavioral effects elicited by CMI. Indeed, the antagonism of 5-HT_3 receptors has been implicated in a faster onset of action of antidepressant and anxiolytic drugs (Alam et al., 2013), which in our case could account for the observed effects of CMI 30 min after its administration. Due to its nature, the activation of 5-HT_3 receptors leads to an influx of calcium (Ca^{2+}) ions in the neurons. Ca^{2+} transiently stimulates neuronal nitric oxide synthase (nNOS) mediated nitric oxide (NO) synthesis (Lipton et al., 1993), and residually elevated intracellular Ca^{2+} may increase NO levels within the brain. The central nervous system has a modest antioxidant defense system (Cobley and Riorello, 2018), and both depression and anxiety are characterized by increased oxidative stress (Salim, 2014). Considering the likelihood of CMI in antagonizing 5-HT_3 receptors (therefore decreasing the influx of Ca^{2+}), it is possible to speculate that CMI also reduces oxidative stress in the brain, which contributes to the antidepressant- and anxiolytic-like effects following its administration. Our previous works support this hypothesizes, since the same dose of CMI used in the present study also reduces the levels of reactive species, lipid peroxidation and nitric oxide in the prefrontal cortex and hippocampus of mice challenged with LPS (Casaril et al., 2017a) and submitted to an acute restraint stress protocol (Casaril et al., 2019).

In conclusion, we used behavioral, biochemical and computational analysis to suggest the involvement of the serotonergic system in the antidepressant- and anxiolytic-like effects of CMI. Our data indicate that the effect of CMI in LPS-challenged mice may rely on its ability to modulate the 5-HT_{1A}, 5-$HT_{2A/2C}$, and 5-HT_3 receptors. Additionally, the behavioral effects of CMI may be dependent on the downregulation of IL-1β, TNF-α and IDO expression in the PFC and HC, alongside with the inhibition of IDO and SERT activities. It is important to mention that the dose of antagonists used here did not evoke behavioral alterations

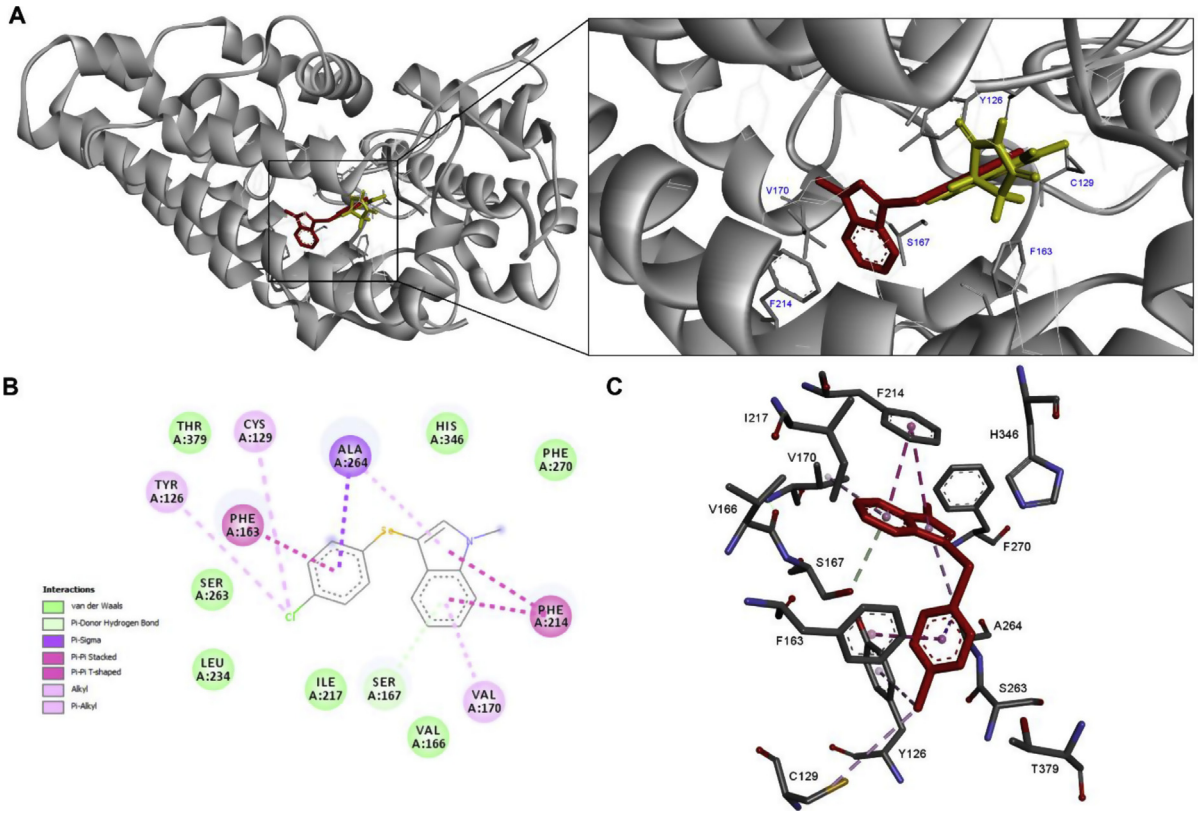

Fig. 8. Molecular docking of CMI in the binding pocket of IDO. (A) View of CMI (red) and the co-crystalized ligand, PF-06840003 (yellow), bound to the X-ray crystallographic structure of IDO. (B) 2D and (C) 3D visualization of CMI interacting with amino acids residues in the active site of IDO.

in mice, which allowed us to study the CMI effects. The simultaneous interaction of CMI with different pharmacological targets in the serotonergic system might have greater therapeutic efficacy than modulating each target alone. Therefore, the pharmacological activity of CMI may be useful in tailoring treatments for the depression-anxiety comorbidity. However, the present study has some limitations and, therefore, (i) the radioligand-binding analysis, (ii) the determination of extracellular 5-HT concentration, (iii) the investigation of the long-lasting effects of CMI, and (iv) the evaluation of the antidepressant-like effect of CMI in female mice should be considered for future research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.05.006.

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