ORIGINAL INVESTIGATION

Mechanisms involved in the antidepressant-like action of orally administered 5-((4-methoxyphenyl)thio)benzo[c][1,2,5]thiadiazole (MTDZ) in male and female mice

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Abstract

Rationale The compound 5-((4-methoxyphenyl)thio)benzo[c][1,2,5]thiadiazole (MTDZ) has recently been shown to inhibit in vitro acetylcholinesterase activity, reduce cognitive damage, and improve neuropsychic behavior in mice, making it a promising molecule to treat depression.

Objectives This study investigated the antidepressant-like action of MTDZ in mice and its potential mechanisms of action.

Results Molecular docking assays were performed and suggested a potential inhibition of monoamine oxidase A (MAO-A) by MTDZ. The toxicity study revealed that MTDZ displayed no signs of toxicity, changes in oxidative parameters, or alterations to biochemistry markers, even at a high dose of 300 mg/kg. In behavioral tests, MTDZ administration reduced immobility behavior during the forced swim test (FST) without adjusting the climbing parameter, suggesting it has an antidepressant effect. The antidepressant-like action of MTDZ was negated with the administration of 5-HT1A, 5-HT1A/1B, and 5-HT3 receptor antagonists, implying the involvement of serotonergic pathways. Moreover, the antidepressant-like action of MTDZ was linked to the NO system, as L-arginine pretreatment inhibited its activity. The ex vivo assays indicated that MTDZ normalized ATPase activity, potentially linking this behavior to its antidepressant-like action. MTDZ treatment restricted MAO-A activity in the cerebral cortices and hippocampi of mice, proposing a selective inhibition of MAO-A associated with the antidepressant-like effect of the compound.

Conclusions These findings suggest that MTDZ may serve as a promising antidepressant agent due to its selective inhibition of MAO-A and the involvement of serotonergic and NO pathways

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Graphical Abstract
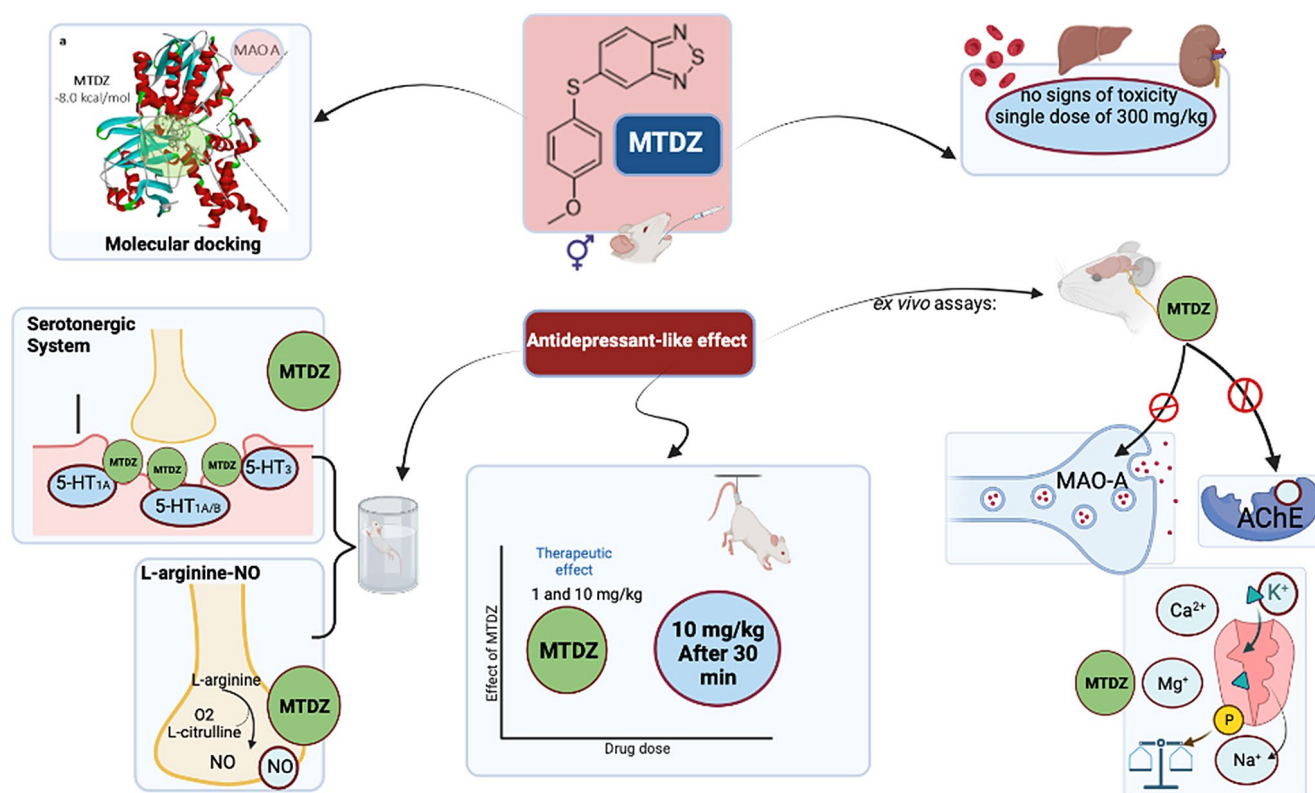
Introduction

Major depressive disorder (MDD) is a mental condition normally associated with other physical comorbidities and socioeconomic consequences and is considered the most common mental disorder (WHO-World Health Organization 2016). With the novel COVID-19 pandemic, the prevalence of symptoms of depression sharply increased in the first twelve months in the general population (Johns et al. 2022).

Statistically, women have a higher prevalence of depression, nearly twice as frequently as men (Mello et al. 2018; Pitzer et al. 2022). Evidence has shown that some factors, such as sex, hormones, anxiety, and stress, may contribute to increasing depression in women (Albert 2015; Labonté et al. 2017). Sex differences in depression are well-documented, with women having a higher risk of developing the disorder than men. The causes for this difference are no not fully understood, but hormonal fluctuations, especially during reproductive years, may contribute. Furthermore, women tend to encounter more stressful life events, which could trigger depressive symptoms (Pavlidi et al. 2022; Xiao 2023).

Given this context, it is crucial to evaluate the effects of new treatments on men and women because, until today, there are no reports of treatments that may act differently depending on the sex, despite being a highly relevant parameter for developing novel research (Pitzer et al. 2022). In fact, many studies are contradictory, and some have shown the differences between male and female rodents (Liu et al. 2019; Vieira et al. 2018; Xing et al. 2013), while others have not (Eltokhi et al. 2021; Goodwill et al. 2019), proving to be interesting evaluations against this parameter for better clarification.

The pathobiological basis of depression is multifaceted, but a prominent component is the hypothalamic-pituitary-adrenal (HPA) axis, a critical stress response system. Chronic stress can induce hyperactivation of the HPA axis, which in turn, increases the levels of cortisol, a notable stress hormone. One mechanism through which adaptogens can alleviate stress-induced pathologies, such as depression, is by restoring HPA axis homeostasis through the regulation of cortisol release (Okoh et al. 2020). Moreover, there is a connection between the HPA axis and monoaminoxidase (MAO) activities as cortisol can amplify MAO-A activity. This enzyme is responsible for the degradation of serotonin,

norepinephrine, and dopamine, compounds closely linked to mood regulation and depressive disorders (Pandey et al. 1992; Soliman et al. 2011). Moreover, studies have demonstrated that MAO inhibitors can enhance Na^+K^+-ATPase activity (De Oliveira et al. 2019; Mayanil and Baquer 1985). This enzyme is key in preserving the electrochemical gradient across the cell membrane, suggesting a potential correlation between MAO and Na+K+-ATPase activities in the treatment of depression (Hesketh et al. 1977; Kurup and Kurup 2002). In conclusion, dysregulation of these activities may play a role in the pathophysiology of depression. Hence, understanding their interplay is critical for therapeutic applications. Therefore, we considered this an important parameter to evaluate new treatments. For instance, Jiang et al. (2019) found that male rodents are more commonly used in research on depression, even though female rodents are just as suitable for this kind of study, and using this sex difference may prove advantageous. Treatments for MDD are generally based on different hypotheses of etiology used for explaining the development of depression, including imbalanced monoaminergic neurotransmitters and abnormalities in the glutamatergic system or nitrergic pathways (Finberg and Rabey 2016; Sanacora et al. 2008).

Various classes of antidepressants are utilized for depression management, although the treatments generally have many adverse effects, including nausea, agitation, and sedation, in addition to taking several weeks or even months to achieve the desired effects (Fabbri and Serretti 2020; WHO-World Health Organization 2016). Thus, seeking new treatments for MDD with fewer limitations, different pharmacological properties, and quicker effects is of the utmost importance, possibly through new molecules.

In this sense, our research group has been actively seeking to develop a new treatment for depression with more immediate effects, safety, and without toxicity. Organic sulfides and their derivatives constitute an important class with significant biological and pharmacological activities, particularly aryl sulfide compounds, which exhibit anti-inflammatory properties. To circumvent issues such as oxidation, cross-coupling reactions with palladium catalysts are commonly utilized. Within this context, heterocyclic compounds, such as benzothiadiazole, have garnered notable attention for their biological properties. Santos et

al. (2020) conducted a study on the functionalization of 2,1,3-benzothiadiazole molecules, where the compound 5-((4-methoxyphenyl)thio)benzo[c][1,2,5]thiadiazole was distinguished for its effect on the inhibition of the acetylcholinesterase (AChE) enzyme in an in vitro assay. Following this, Rodrigues et al. (2022) elucidated that the MTDZ compound manifested an anti-amnesic effect in a scopolamine-induced amnesia model in mice while also offering protection against cholinergic imbalance, NaK-ATPase pump dysfunction, and certain oxidative stress parameters. Furthermore, Motta et al. (2022) demonstrated that MTDZ additionally exhibits antinociceptive effects, attenuates anxious-like behavior, and mitigates cognitive deficits in both male and female mice. Consequently, further studies are warranted to enhance shed more light on these biological effects.

In another study, cholinergic dysfunction was shown to partly connect with MDD since it can precipitate the course of the disease and predispose the MDD to dysregulate other neurobiological circuits (Fernandes et al. 2018). Therefore, MTDZ may have a promising effect in other models, such as depression.

Given the above, this study aimed to characterize the antidepressant-like profiles of MTDZ using behavioral tools and investigate the possible antidepressant-like action in the serotonergic, glutamatergic, nitrergic, and monoaminergic systems in male and female mice. In addition, the ATPase activity in the brain structures and toxicity of the compound wereL- also evaluated.

Materials and methods

Chemicals and reagents

MTDZ (Fig. 1) was prepared and characterized as described elsewhere and then dissolved in canola oil (Fernandes et al. 2018). Analyses of the proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra showed analytical and spectroscopic data in full agreement with their assigned structures. 5,5'-Dithiobis (2-nitrobenzoic acid) (Reference: D8130-5G), thiobarbituric acid (TBA) (Reference: V774-05-100G), Way100635 (Reference code: W1895-5MG), Ketanserin (Reference code: S006-50MG), Pindolol (Reference codeP0778-250MG), L-arginine (Reference code: N5501-5MG), MK-801 (Reference code: 022M4616V), Ondasetron (Reference code: O3639-10MG) were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Fig. 1 Chemical structure of 5-((4-methoxyphenyl)thio)benzo[**c**][1,2,5]thiadiazole (MTDZ)
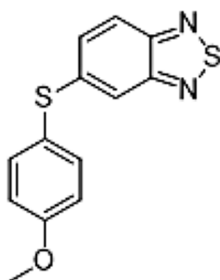
Animals

Male and female adult Swiss mice (25–35 g) were acquired from a local breeding colony and kept in a separate room on a 12:12 h light/dark cycle at 22 ± 2 °C with food and water *ad libitum.* The animals were obtaindes from the central vivarium of the Federal University of Pelotas. All animal experiments were approved by the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas (CEEA no. $8970-2021$) and in accordance with the Brazilian National Animal Care Ethical Council (CONCEA), which is based on the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Publication no. 85–23, revised 1985).

The animals were housed in boxes ($20 \times 30 \times 13$ cm) containing 4–6 animals each. The G*Power software was utilized to determine the statistical power (G*Power freeware from Heinrich-Heine-University Düsseldorf, v3.1.9.4) and the number of samples (Faul et al. 2007). Hence, we had the minimum number of animals required to demonstrate consistent effects. All procedures were performed by an observer blinded to the study design. The animals were placed in the experimental behavior room for ~2 h before treatments and behavioral tests to have the lowest stress level possible, and after the behavioral test were rehoused in their boxes.

Molecular docking simulations

The 3D X-ray crystal structures of monoamine oxidase isoform A (PDB ID: 2BXS) and B (PDB: 2BYB) were obtained from the Protein Data Bank (https://www.rcsb.org/) according to Colibus et al. (2005) (De Colibus et al. 2005). Firstly, the 2D structure of MTDZ was drawn with ChemDraw and converted to 3D using the Avogadro software (v. 0.9.4). The geometry was optimized following the GAFF method (Hanwell et al. 2012). As positive controls, the molecules Isocarboxazid (PubChem ID: 3759), clorgiline (PubChem ID: 4380), and selegiline (PubChem ID: 26,757) were submitted to the same optimization.

The Auto Dock Tools (v. 1.5.4) software set all rotatable bonds of ligands to rotate freely, and the protein receptors were considered rigid (Morris et al. 2009). Protein preparation consisted of fixing structures, deleting molecules, ions, and water, fixing hetero groups, and finally optimizing the structure using Gasteiger charges with 500 steps of minimization. The CHIMERA (v. 1.5.3) software was used to remove ligands in 3D (Pettersen et al. 2004). We conducted the molecular docking using the AutoDock Vina software (version 1.1.1) with a grid box centered in all-atom structures, allowing the program to search for additional places of probable interactions (Trott and Olson 2009). The

protein-ligand interactions were analyzed by the Discovery Studio Visualizer software.

Experimental protocols

A blinded observer scored all behavioral tests. In all protocols, the locomotor (number of segments crossed with the four paws) and exploratory (number of times rearing on the hind limbs) behaviors were assessed in the open field test for 4 min, as described elsewhere (Walsh and Cummins 1976). The experimental timeline is shown in Fig. 2.

The dose of 300 mg/kg was chosen based on OECD (OECD 2002) recommendations for analyzing the toxicity of new compounds. However, 1 and 10 mg/kg were used for the behavioral analyses based on other experimental models with MTDZ (da Costa Rodrigues et al. 2022; da Motta et al. 2022).

Experimental protocol 1 – toxicity of MTDZ treatment

The toxicity of MTDZ treatment was evaluated in male mice according to the OECD Guideline for Testing of Chemicals (OECD 2002). The compound was administered intragastrically (ig) at 300 mg/kg as recommended by the OECD for toxicological testing, and the animals fasted for 4 h before the treatments. Afterward, the animals were observed individually for the first 24 h, followed by daily observations until day 14. The animals were observed for toxicological symptoms, weight loss, and death. After 14 days, the animals were anesthetized with inhaled isoflurane, and blood was collected by cardiac puncture for enzymatic measurements of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine levels using commercial kits. The liver and kidneys were removed to evaluate oxidative stress parameters: thiobarbituric acid reactive species (TBARS) content and reactive species (RS) levels (Loetchutinat et al. 2005; Ohkawa et al. 1979). Total protein determination was carried out according to the method of Bradford (Bradford 1976). Considering the absence of toxicity, experiments to evaluate the pharmacological activity were carried out.

Ex vivo assays After day 14, the animals submitted to the toxicity protocol (experimental protocol 1) were anesthetized, their blood was collected, and they were euthanized by isoflurane overdose; their plasma, kidneys, and liver were then removed for biochemical determinations (AST, ALT, TBARS, and RS). The liver and kidneys were homogenized in 50 mM Tris/HCl pH 7.4 (1:5 w/v) and centrifuged at 900 xg for 10 min to yield a low-speed supernatant fraction (S1) used to estimate the ex vivo assays. The blood samples were processed by centrifugation (2500 xg,
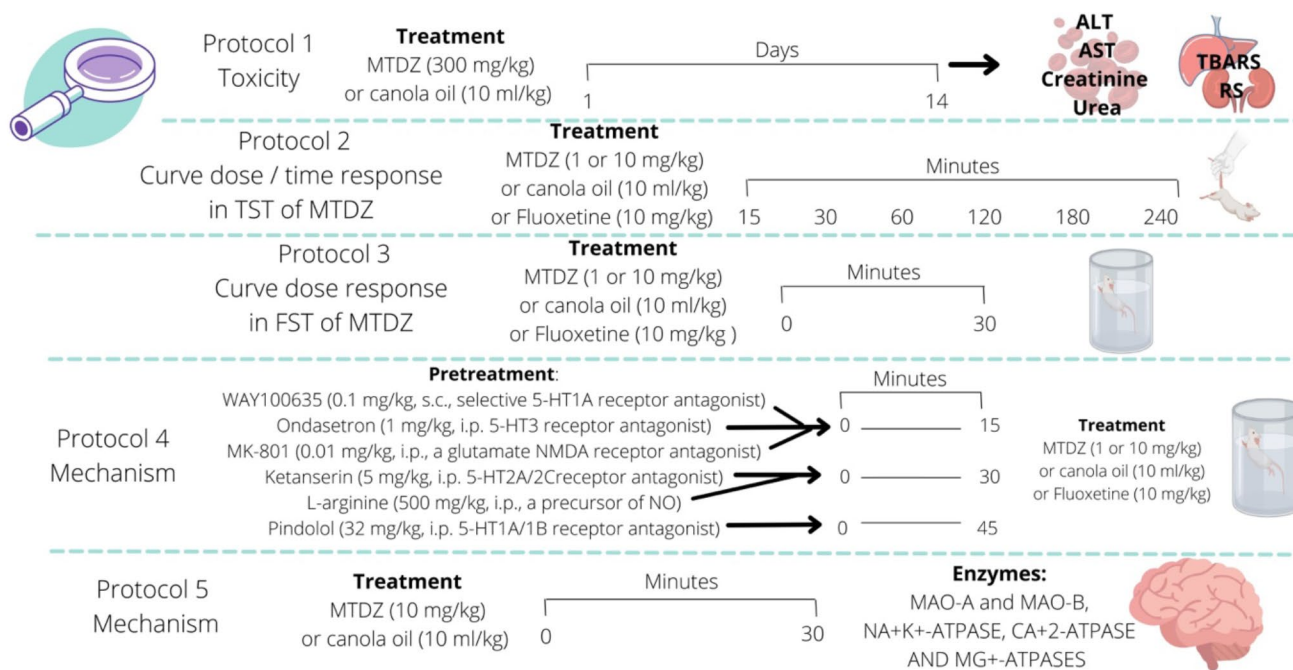
Fig. 2 Experimental design. The animals received intragastric (ig) treatments with canola oil, 5-((4-methoxyphenyl)thio)benzo[c][1,2,5] thiadiazole (MTDZ) on i) experimental toxicity protocol; ii) forced swim tests (FST) from dose- and time-response curves; iii) tail sus-

pension test (TST) from dose-response curve; iv) mechanism involved in the antidepressant-like action of MTDZ; v) ex vivo assays in the cerebral structures

10 min) to obtain plasma, which was used to determine the biochemical markers.

Biochemical markers

The AST and ALT activities were used to determine the hepatic damage, while creatinine levels were evaluated to verify renal toxicity. Biochemical markers were evaluated in the heparinized plasma of the animals using commercial kits (Bioclin®, Minas Gerais, Brazil).

TBARS levels

The TBARS levels were used to measure lipid peroxidation and determined as described elsewhere (Ohkawa et al. 1979). An aliquot of the supernatant was added to the reaction mixture: thiobarbituric acid (0.8%), sodium dodecyl sulfate, and acetic acid (pH 3.4) and incubated at 95 ºC for 2 h. The absorbance was measured at 532 nm, and the results were reported as nmol malondialdehyde (MDA)/mg protein.

RS levels

A spectrofluorimetric method determined the RS levels using the 2′,7′-dichlorofluoresceindiacetate (DCHF-DA) assay. The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured to detect intracellular RS. The DCF fluorescence intensity emission was recorded at

520 nm (480 nm excitation), 60 min after adding DCHF-DA to the medium (Shimadzu RF-5301 PC fluorometer) (Loetchutinat et al. 2005). The RS levels were expressed of DCF fluorescence united.

Experimental protocol 2 – dose-time-response curve of MTDZ in the tail suspension test to evaluate the antidepressant-like activity

This experimental protocol was performed in male and female mice. The mice were randomly divided into four groups for males and four groups for females (8 animals/group): Group 1 (control), Group 2 (MTDZ 1), Group 3 (MTDZ 10), and Group 4 (fluoxetine). The control group received canola oil (10 mL/kg, ig via gavage), the MTDZ 1 and MTDZ 10 groups were treated with the compound at doses of 1 and 10 mg/kg, respectively (ig via gavage), and the fluoxetine group received the positive control (10 mg/kg, ig via gavage); MTDZ and fluoxetine were diluted in canola oil. In this study, fluoxetine was only used as a positive control to validate depressive-like behaviors and, consequently, compared with the antidepressive-like effect of MTDZ. Notably, we used the dose of 10 mg/kg of fluoxetine to obtain a direct comparison with the same concentration of the compound.

To investigate the compound's antidepressant-like behavior, the animals were subjected to the tail suspension test

(TST) at different times after treatments performed just one time for different times: 15, 30, 60, 120, 180, and 240 min. The time-response curve was based on a previous study using different groups of animals (Ledebuhr et al. 2022). The TST was conducted as described by Steru et al. (1985) (Steru et al. 1985). Immobility time was manually recorded for a 6-min period by an experienced observer using the timed data from 2 to 6 min to reduce interference by the initial agitation related to the handling of the animal (Kaster et al. 2012). Reduced duration of immobility is indicative of an antidepressant-like effect.

Experimental protocol 3 – dose-response curve of MTDZ in the forced swim test to evaluate the antidepressant-like action

The forced swim test (FST) was conducted in male and female mice using the method of Porsolt and collaborators (Porsolt et al. 1977), with some modifications. The FST is used to indicate antidepressant-like effects, although it can also differentiate noradrenergic agents with increased climbing behavior for similar 5-HT-related compounds without changing the climbing behavior (Pesarico et al. 2014; Tanaka and Telegdy 2008).

In this test, we observed the duration of swimming (as movement throughout the swim chamber), climbing (upward-directed movements of the forepaws along the side of the swim chamber), and immobility time (no additional activity was observed). This parameter was scored for 6 min by an experienced observer. Each mouse was considered immobile when it hung passively and completely motionless; decreased immobility duration indicates an antidepressant-like effect. The treatments were administered only once: MTDZ was administered at a dose of 1 or 10 mg/kg, canola oil (in the control animals) was administered at a dose of 10 mL/kg, and fluoxetine at a dose of 10 mg/kg.

Ex vivo assays

At the end of the FST for the animals subjected to the dose-response curve, the mice were sacrificed and the cerebral cortices and hippocampi were removed to investigate total ATPase, Na^+K^+-ATPase, Ca^{+2}-ATPase, and Mg^+-ATPases, MAO-A, MAO-B activities. These analysis were performed to verify the involvement of these enzymes in the antidepressant-like action of MTDZ. The cerebral cortices and hippocampi were separated and washed with a cold saline solution (0.9%). For the other biochemical analyses, the samples were homogenized in 50 mmo/L Tris HCl pH 7.4 and centrifuged at 900×g for 10 min to produce a supernatant (S1).

Total ATPases activity

Total ATPase activity was assayed in an incubation medium containing the necessary salt substrates for ionic pumps to take place: 30 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 3 mM ATP, and 5–7 mg/ mL of protein of S1 samples. Controls to correct for non-enzymatic substrate hydrolysis were prepared by adding sample preparations after the reactions were stopped with 10% trichloroacetic acid (TCA) (Mark et al. 1995). The color reaction was assayed spectrophotometrically at 650 nm. The enzyme activities were expressed in nmol of Pi/min/mg of protein.

Na^+K^+- ATPase and Mg^+- ATPase activity

The reaction mixture used for this assay contained S1, 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl, and 50 mM Tris/ HCl, pH 7.4. Control samples were performed under the same conditions by adding 0.1 mM ouabain. Considering that ouabain is an inhibitor of the Na^+/K^+ pump, it was possible to observe the enzyme activity related to the Mg 2 + pump in this technique. Ouabain (1 mM) was added to the reaction medium to determine the Mg ATPase activity. The reactions were initiated by adding ATP 3.0 mM, and the incubation was stopped by adding 10% TCA with 10 mM $HgCl_2$ after 30 min. Enzyme activity was calculated from the difference between amounts of inorganic phosphate (Pi) found after incubation in the absence and presence of ouabain. Released Pi was measured according to Fiske and Subbarow (Fiske and Subbarow 1925). The color reaction was assayed spectrophotometrically at 650 nm, and the results were expressed as nmol Pi/mg protein/min.

Ca^{2+}-ATPase activity

The Ca^{2+}-ATPase activity was measured as described elsewhere (Rohn et al. 1993), with minor modifications (Mark et al. 1995). The ATPase activity was assayed in an incubation medium comprising 30 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 3 mM ATP, and 5–7 mg/ mL of protein of S1. The activity was determined by subtracting the activity measured in the presence of Ca^{2+} from the activity determined in the absence of Ca^{2+}. The enzyme activities were expressed in nmol of Pi/min/mg of protein.

Protein determination

Protein concentration was measured by the Bradford method using bovine serum albumin (1 mg/mL) as the standard (Bradford 1976). The results obtained were used as a basis for calculating the other dosages.

Experimental protocol 4 – mechanisms involved in the MTDZ antidepressant-like action

The FST was used to investigate the mechanisms involved in the antidepressant-like action of MTDZ in male Swiss mice. The FST was performed 30 min after the treatments. This protocol was conducted to analyze the potential mechanisms of action associated with the antidepressant-like activity of the MTDZ compound. It is crucial to emphasize that different time-points are utilized during antagonist administration. These timelines are informed by pertinent references, illustrating the required durations of action for each antagonist and their respective administration routes.

Involvement of the serotonergic system in the antidepressant-like action of MTDZ in the FST

In order to investigate the involvement of the serotoninergic receptor subtypes on the antidepressant-like action of MTDZ in the FST, independent groups of animals were pretreated with: (i) WAY100635 (0.1 mg/kg, subcutaneous (sc), a selective 5-HT$_{1A}$ receptor antagonist); (ii) ketanserin (5 mg/kg, intraperitoneal (ip), a 5-HT$_{2A/2C}$ receptor antagonist); (iii) ondansetron (1 mg/kg, ip, a 5-HT$_3$ receptor antagonist); (iv) pindolol (32 mg/kg, ip, a 5-HT$_{1A/1B}$ receptor antagonist). After 15 min of WAY100635 or ondansetron administrations, 30 min of ketanserin administration, or 45 min of pindolol. Mice received MTDZ (10 mg/kg, ig) or canola oil (vehicle, 10 ml/kg, ig) and were tested in the FST 30 min later. The doses and times of treatments with 5-HT receptor antagonists were chosen based on previous studies (De Oliveira et al. 2019; Ledebuhr et al. 2022; Savegnago et al. 2007).

Involvement of N-methyl-D-aspartate on the antidepressant-like action of MTDZ in the forced swim test

To assess the glutamatergic system's possible involvement in the antidepressant-like action of MTDZ in the FST, independent groups of animals were pretreated with MK-801 (0.01 mg/kg, ip, a glutamate NMDA receptor antagonist). After 15 min, the mice received MTDZ (10 mg/kg, ig) or canola oil (vehicle, 10 mL/kg, ig) and were tested in the FST 30 min later. The dose and time of treatment with MK-801 were chosen based on previous studies, which did not modify the basal response in behavioral tests (Hiro et al. 1996; Vasilescu et al. 2021; Zomkowski et al. 2010).

Involvement of nitric oxide on the antidepressant-like action of MTDZ in the forced swim test

The role played by the L-arginine-nitric oxide (NO) pathway in the antidepressant-like effect caused by MTDZ in the FST was investigated in the different groups. The mice were pretreated with L-arginine (500 mg/kg, ip, a precursor of NO). Thirty minutes after L-arginine administration, MTDZ (10 mg/kg, ig) or canola oil (vehicle, 10 ml/kg, ig) was administered. The FST was carried out 30 min after the treatments. The dose and time of treatment with L-arginine were chosen based on previous studies (Liebenberg et al. 2015; Rosa et al. 2003).

Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). The normality of data was evaluated by the D'Agostino and Pearson omnibus normality test. Statistical analysis was performed by GraphPad Prism software using one-way (for data of the open field test, the FST in the mechanism evaluation) and two-way (for data of FST, TST, total ATPase, Na$^+$K$^+$-ATPases, Mg$^+$-ATPase, Ca^{2+}-ATPase, MAO-A, and MAO-B activities) analyses of variance (ANOVA) followed by Tukey's post-hoc test. For biochemical analyses, we used an unpaired t-test (TBARS, RS, ALT, AST, creatine, and urea). The one-way ANOVA is utilized when there is a single independent variable and a comparison of three or more groups is being conducted. A two-way ANOVA is employed when there are two independent variables, and a comparison of three or more groups is required. In contrast, a t-test is employed when a comparison of only two groups is necessary. The primary effects are only presented when the higher second-order interaction is non-significant. Values of $p < 0.05$ were considered statistically significant.

Results

Screening of molecular docking about MTDZ interaction with MAO

Initially, in view of validating the molecular docking protocols, we docked positive controls, including the dual inhibitor isocarboxazid, the selective MAO-A inhibitor clorgiline, and the selective MAO-B inhibitor selegiline in the optimized 3D structures (Fig. 3). Clorgiline has a binding affinity of -7.0 kcal/mol with MAO-A in the binding site reported in the literature, including interactions with Cys406, Arg51, Tyr444, Ile335, Phe352, and hydrogen bond with Tyr407 and Ile180 (Fig. 3a). Isocarboxazid showed a
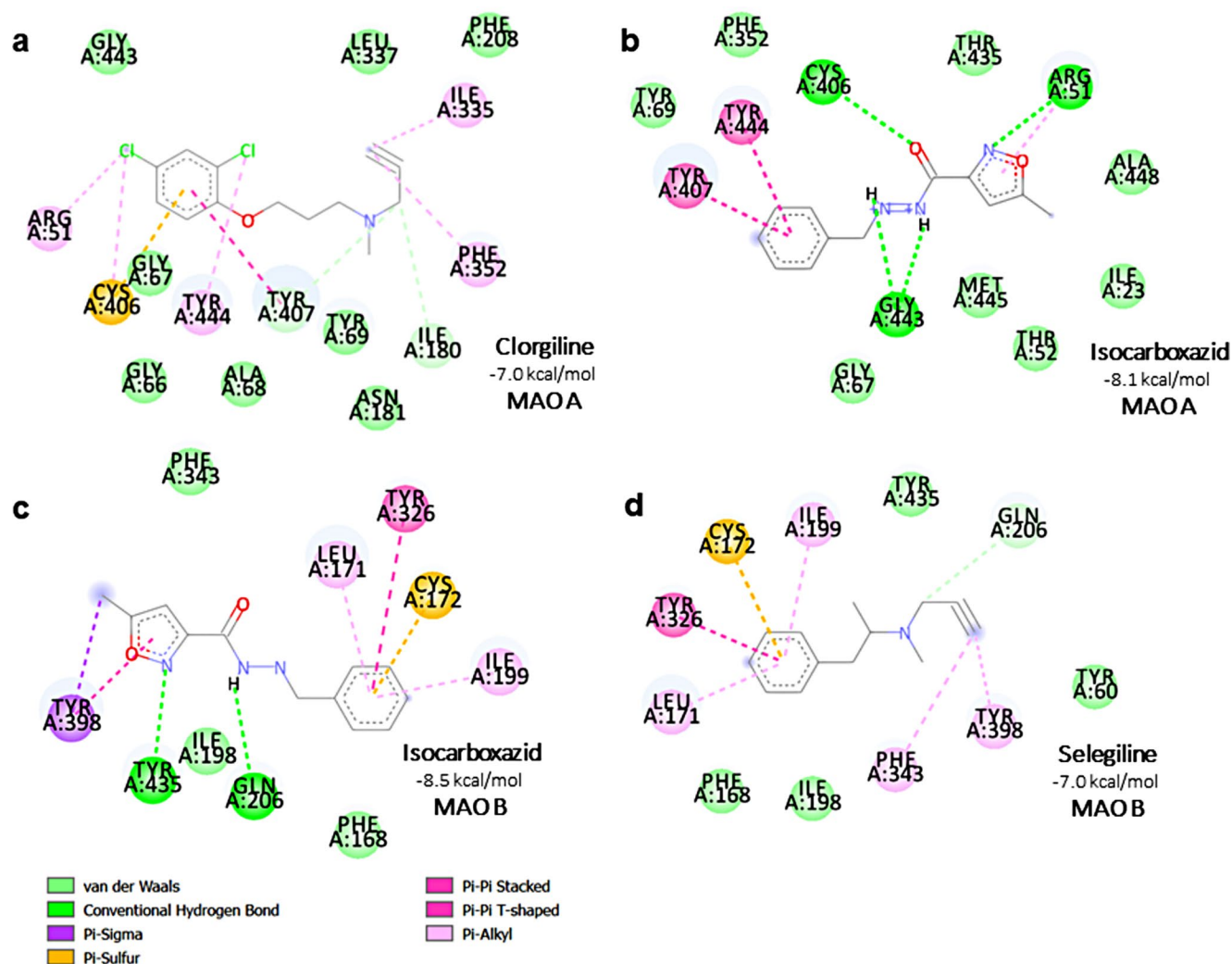
Fig. 3 Predicting binding affinity and ligand-protein interactions of (**a**) clorgiline and (**b**) isocarboxazid in MAO-A isoform and (**C**) isocarboxazid and (**D**) selegiline in MAO-B isoform

docking score of -8.1 kcal/mol in MAO-A, interacting with the active site by multiples hydrogen bonds with Cys406, Arg51, and Gly443 non-covalent interactions with Tyr447 and 407 (Fig. 3b). In contrast, isocarboxazid interacts similarly in MAO-B, with a docking score of -8.5 kcal/mol through Tyr398, Tyr435, Gln206, Ile199, Cys172, Tyr326, and Leu171 (Fig. 3c). Selegiline has a docking score of -7.0 kcal/mol and interacts with the substrate/FAD binding site, including Ile199, Tyr326, Cys172, Leu171, Phe343, Tyr398, and Gln206 (Fig. 3d).

MTDZ has a binding affinity of -8.3 kcal/mol for MAO-A (Fig. 4A), mediated by various interesting interactions with the substrate binding site, including one hydrogen bond with Tyr444 and simultaneously a Pi-T shaped in association with a Pi-Stacked with Tyr407. Furthermore, MTDZ also makes a Pi-Sulfur with Cys406 (Cys 397 in MAO-B), the target of the covalent link with FAD. Additional MAO-A

and MTDZ complex interaction residues include Arg51, Met445, and Ile207.

Nonetheless, MTDZ possesses a docking score of -5.9 kcal/mol in MAO-B (Fig. 4B), preferably by non-covalent interactions with residues located in the loop guarding the active site cavity, such as Pi-Alkyl with Arg120, Pi-Anion with Glu483 and additionally the aromatic ring of Phe103 interacting the MTDZ Sulphur atom.

The toxicity of MTDZ treatment

Biochemical markers

Table 1 demonstrates the effects of treatments on the AST and ALT activities and creatinine levels in the plasma of mice. In Protocol 1, the treatment with MTDZ did not show a difference in AST and ALT activities and creatinine and urea levels (unpaired t-test; df = 14, t = 0.4087, p = 0.5476,

Fig. 4 Predicting binding affinity and ligand-protein interactions of MTDZ in (**a**) MAO-A and (**b**) MAO-B isoforms
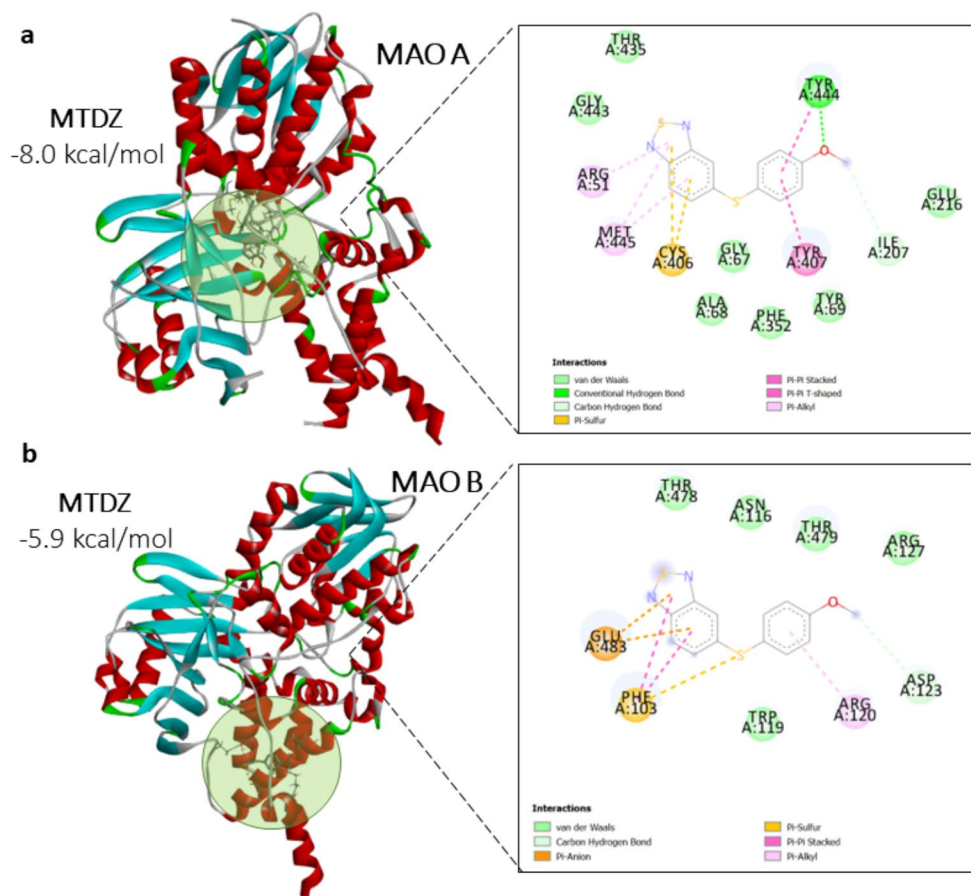

Table 1 Effects of treatments in the biochemical assays and parameters of oxidative stress in plasma of mice

Assay/groups	Control	MTDZ
TBARS – Liver	32.8 ± 3.1	28.3 ± 2.4
TBARS – Kidney	49.5 ± 2.7	51.9 ± 3.2
RS – Liver	222.8 ± 5.3	226.2 ± 7.9
RS – Kidney	112.4 ± 5.3	128.4 ± 9.6
ALT	56.7 ± 1.3	53.9 ± 2.7
AST	102.1 ± 3.1	108.2 ± 5.4
Creatinine	0.4 ± 0.1	0.4 ± 0.1
Urea	40.6 ± 9.2	41.0 ± 3.6

Results are given as means ± SEM of 3 mice in each group (Unpaired t-test)

R^2: 0.0400 for AST; unpaired t-test; $df = 14$, $t = 0.5785$, $p = 0.3304$, R^2: 0.0772 for ALT; unpaired t-test; $df = 14$, $t = 0.930$, $p = 0.5104$, R^2: 0.6821 for creatinine; unpaired t-test; $df = 14$, $t = 0.3371$, $p = 0.2659$, R^2: 0.0284 for urea).

The TBARS and RS levels

TBARS levels on the liver and kidneys after treatment with MTDZ in Protocol 1 in mice are listed in Table 1. The treatment with the compound at 300 mg/kg did not alter TBARS levels in both tissues of mice (unpaired t-test;

$df = 14$, $t = 0.1611$, $p = 0.1626$, R^2:0.0645 for the liver; unpaired t-test; $df = 14$, $t = 0.6027$, $p = 0.4569$, R^2: 0.0832 for the kidneys). The RS levels on liver and kidney tissues after MTDZ treatment are presented in Table 1. The compound did not change the RS levels in both tissues of mice (unpaired t-test; $df = 14$, $t = 0.1952$, $p = 0.6344$, R^2: 0.0943 for the liver; unpaired t-test; $df = 14$, $t = 0.8357$, $p = 0.9462$ for the kidneys; R^2: 0.1487).

The dose-time-response curve of MTDZ in the tail suspension test to evaluate the antidepressant-like action

Immobility time in the TST in male and female mice is demonstrated in Fig. 5A. The treatment with MTDZ decreased the immobility time: (i) at the dose of 1 mg/kg for 15 min (12.3% for males and 10.0% for females), 30 min (48.6% for males and 45.8% for females), 60 min (51.4% for males and 49.1% for females), 120 min (44.7% for males and 45.7% for females), and 180 min (34.6% for males and 35.4% for females); (ii) at the dose of 10 mg/kg for 15 min (29.1% for males and 27.0% for females), 30 min (62.1% for males and 61.1% for females), 60 min (54.2% for males and 52.7% for females), 120 min (55.3% for males and 50.5% for females),

Fig. 5 Effect of 5-((4-methoxy-phenyl)thio)benzo[c][1,2,5] thiadiazole (MTDZ) in the tail suspension test (**A**) and forced swim test (**B**). Data are reported as mean ± standard error of the mean (SEM) of 8 animals per group. (****) denoted $p < 0.0001$ as compared with the control group; and (####) denoted $p < 0.0001$ as compared with the fluoxetine group (two-way analysis of variance/Tukey's test for training and one-way analysis of variance/Tukey's test for probe test)
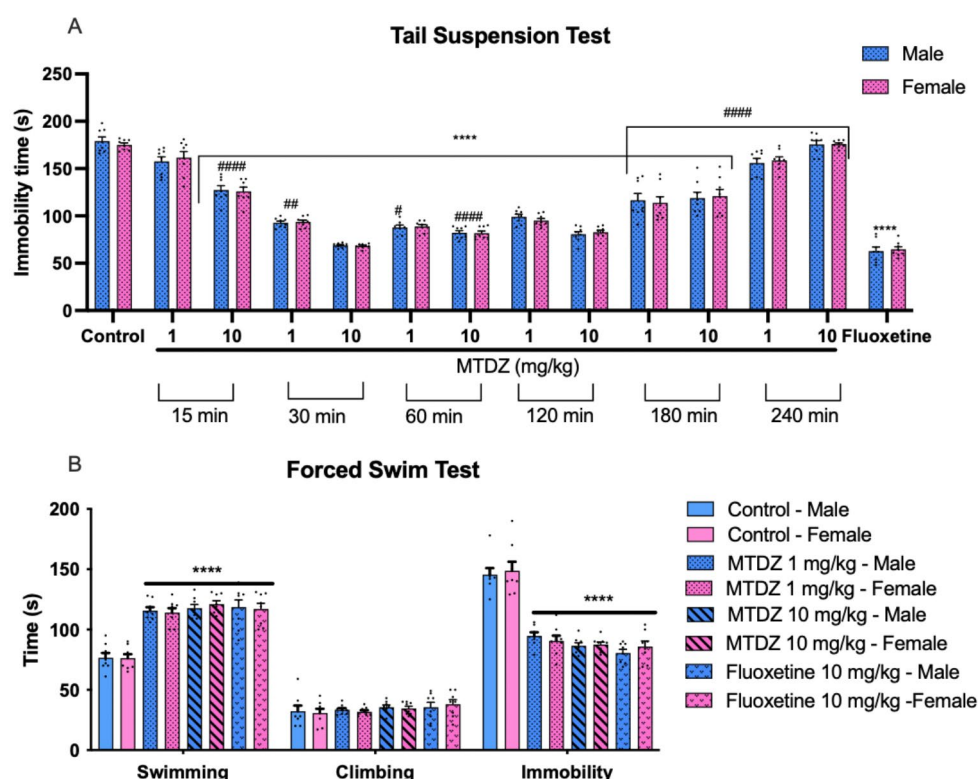
and 180 min (31.1% for males and 35.4% for females) compared to the control group (two-way ANOVA + Tukey's test: main effect of sex: $F_{(1,196)} = 0.0005$, $p = 0.9389$; main effect of treatment: $F_{(13,196)} = 0.005882$, $p = 0.9389$; R2: 0.9281). The immobility time after 30 min of treatment and at 10 mg/kg of MTDZ was similar to the fluoxetine group. Moreover, no difference in compound action was observed between males and females.

Dose-response curve of MTDZ in the forced swim test to evaluate antidepressant-like action

Figure 5B demonstrates the action of MTDZ in the FST; there was no effect of MTDZ on climbing time in the FST. Treatment with MTDZ at 1 or 10 mg/kg or fluoxetine demonstrated an increase in swimming time and a decrease in the immobility time in the FST, both in male and female mice compared to the control group (two-way ANOVA + Tukey's test: main effect of treatment on swimming $F_{(3,56)} = 54.77$, $p = 0.0001$, R2: 0.7464; climbing $F_{(3,56)} = 1.242$, $p = 0.3033$, R2: 0.5944; immobility $F_{(3,56)} = 95.85$, $p = 0.0001$, R2: 0.9897).

Total ATPase, Na+K+- ATPase, Mg+- ATPase, and Ca2+-ATPase activity

Figure 6 shows the total ATPase activity, and it was possible to observe that treatment with MTDZ increased the total activity in the cerebral cortices (Fig. 6A) and hippocampi (Fig. 6B) of male and female mice compared to the control group (two-way ANOVA + Tukey's test: the main effect of the treatment: $F_{(1,28)} = 147.3$, $p = 0.0001$ for the cerebral cortices, R2: 0.8414; ANOVA: $F_{(1,28)} = 108.1$, $p = 0.0001$ for the hippocampi, R2: 0.7988).

The treatment with MTDZ increased Na+K+-ATPase activity in the cerebral cortices (Fig. 6C) and hippocampi (Fig. 6D) of male and female mice compared to the control group (two-way ANOVA + Tukey's test: main effect of treatment: $F_{(1,28)} = 105.5$, $p = 0.0001$ for cerebral cortices, R2: 0.7909; ANOVA: $F_{(1,28)} = 37.1$, $p = 0.0001$ for hippocampus; R2: 0.9309).

Additionally, treatment with MTDZ increased the Mg+-ATPase activity in the cerebral cortices (Fig. 6E) and hippocampi (Fig. 6F) of male and female mice compared to the control group (two-way ANOVA + Tukey's test: main effect of treatment: $F_{(1,28)} = 84.2$, $p = 0.0001$ for the cerebral cortices, R2: 0.7633; ANOVA: $F_{(1,28)} = 65.6$, $p = 0.0001$ for the hippocampi, R2: 0.7050).

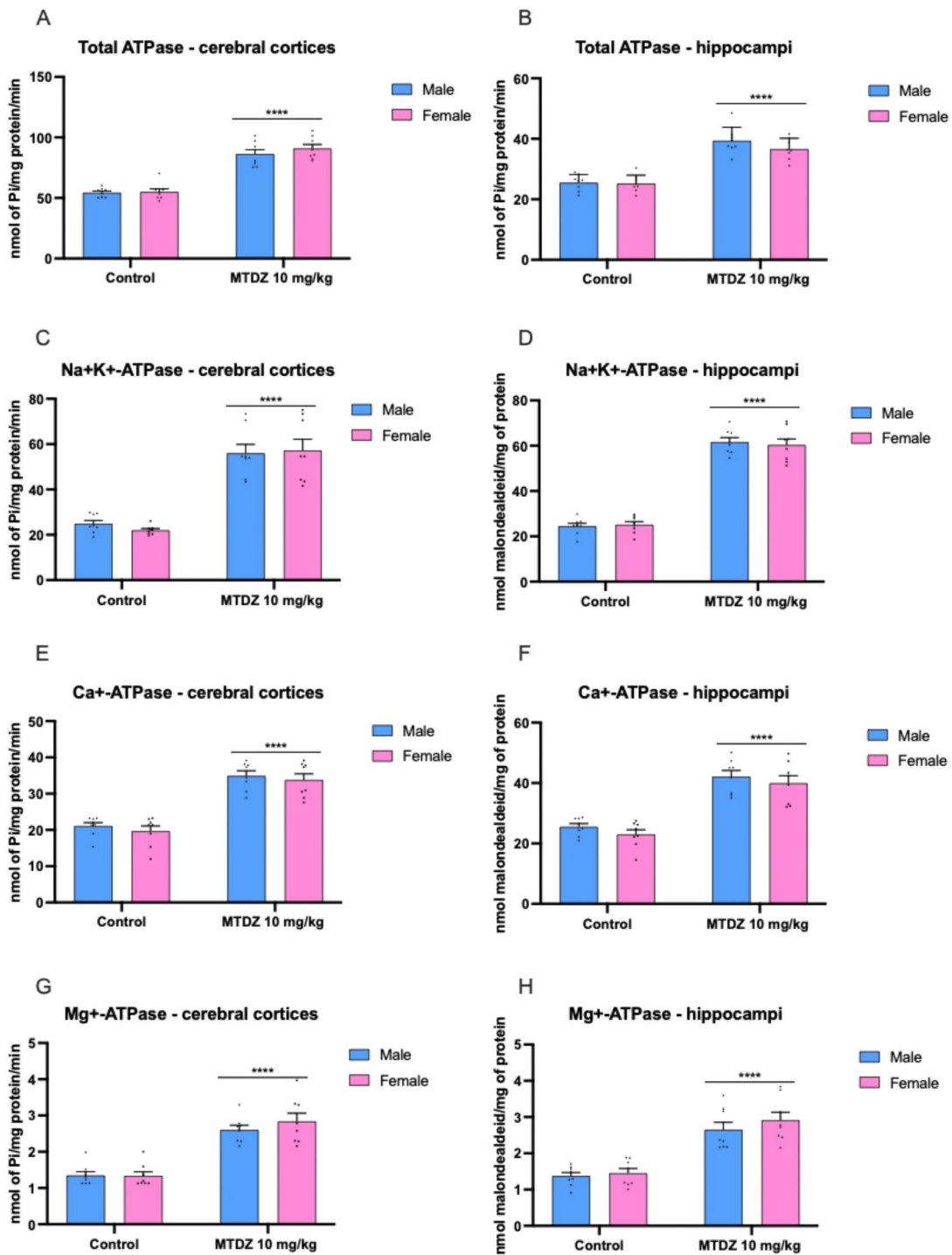Treatment with MTDZ increased the Ca2+-ATPase activity in the cerebral cortices (Fig. 6G) and hippocampi

Fig. 6 Effects of 5-((4-methoxyphenyl)thio)benzo[C][1,2,5]thia-diazole (MTDZ) on ATPases activities: total ATPase activity in the cerebral cortices (**A**) and hippocampi (**B**); Na$^+$K$^+$-ATPase activity in the cerebral cortices (**C**) and hippocampi (**D**); Mg$^+$-ATPase activity in the cerebral cortices (**E**) and hippocampi (**F**); Ca$^+$-ATPase activity in the cerebral cortices (**G**) and hippocampi (**H**). Data are reported as mean ± standard error of the mean (SEM) of 8 animals per group. (****) denoted $p < 0.0001$ as compared with the control group (one-way analysis of variance/Newman-Keuls test)

(Fig. 6H) of male and female mice compared to the control group (two-way ANOVA + Tukey's test: main effect of treatment: $F_{(1,28)} = 103.9$, $p = 0.0001$ for the cerebral cortices, R2: 0.7989; ANOVA: $F_{(1,28)} = 84.21$, $p = 0.0001$ for the hippocampi; R2: 0.7541).

Effect of MTDZ on MAO-A and MAO-B activities

MTDZ treatment decreased MAO-A activity in the cerebral cortices (Fig. 7A) and hippocampi (Fig. 7B) of male and female mice (two-way ANOVA + Tukey's test: main effect of treatment: $F_{(1,28)} = 108.5$, $p = 0.0001$ for the cerebral cortices, R2: 0.7989; ANOVA: $F_{(1,28)} = 105.1$, $p = 0.0001$ for the hippocampi; R2:0.7904). Nevertheless, the treatment with MTDZ did not change the activity of MAO-B in the cerebral cortices (Fig. 7C) and hippocampi (Fig. 7D) of mice (two-way ANOVA + Tukey's test: main effect of treatment: $F_{(1,28)} = 2.296$, $p = 0.1409$ for the cerebral cortices, R2: 0.0824; ANOVA: $F_{(1,28)} = 1.648$, $p = 0.2098$ for the hippocampi, R2: 0.1067).
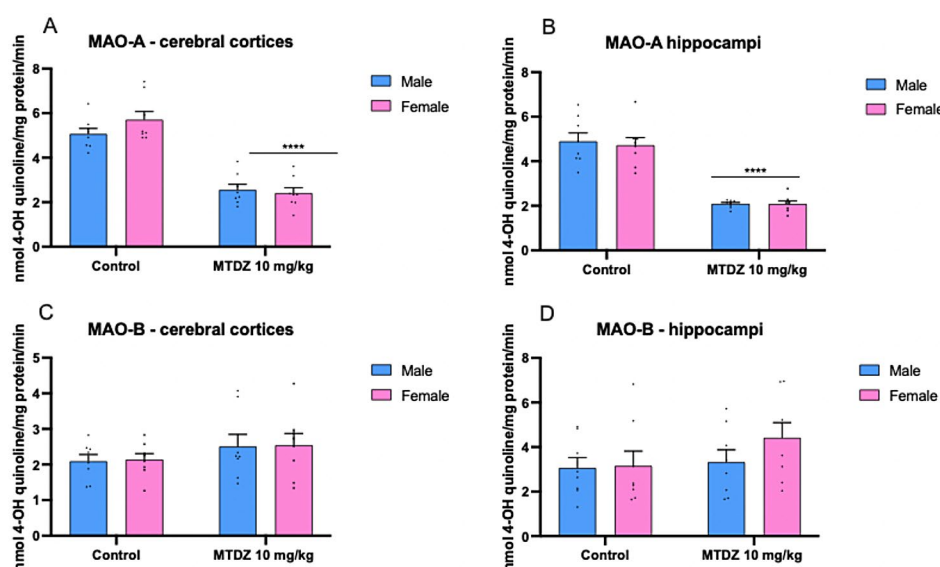
The mechanism involved in MTDZ antidepressant-like action

Since there was no significant difference in the antidepressant-like action of MTDZ between male and female mice, the compound mechanisms were conducted in the FST in male mice to minimize the use of animals.

Involvement of serotonergic system on antidepressant-like action of MTDZ in the forced swim test

Figure 8A illustrates the effect of pretreated mice with ondansetron, ketanserin, pindolol, and WAY100635 in the FST.

Mice pretreated with ondansetron (a 5-HT$_3$ receptor antagonist), pindolol (a nonselective beta-adrenoceptor antagonist with 5-HT1A/1B antagonistic activities), and WAY100635 (a 5-HT$_{1A}$ receptor antagonist) reverted the action showed by MTDZ (ANOVA: $F_{(3,28)} = 91.09$, $p = 0.0001$, R2: 0.8670; ANOVA: $F_{(3,28)} = 65.12$, $p = 0.0001$, R2: 0.8746; ANOVA: $F_{(3,28)} = 55.17$, $p = 0.0001$, R2: 0.8553, respectively). Pretreatment with ketanserin (a 5-HT$_{2A/2C}$ receptor antagonist) did not change the immobility time compared to the MTDZ group in the FST (ANOVA: $F_{(3,28)} = 60.84$, $p = 0.0001$, R2:0.9071).

Involvement of the NMDA system in the antidepressant-like action of MTDZ in the forced swim test.

Figure 8B demonstrates the results of pretreatment with MK-801 in the FST. The pretreatment with MK-801 (a non-competitive NMDA antagonist) did not change the immobility time compared to the MTDZ group (ANOVA: $F_{(3,28)} = 94.98$, $p = 0.0001$, R2: 0.9105).

Involvement of the NO system on antidepressant-like action of MTDZ in the forced swim test

Figure 8C shows that pretreatment with L-arginine (a NO precursor) increases the immobility time compared to the MTDZ group in the FST (ANOVA: $F_{(3,28)} = 49.64$, $p = 0.0001$, R2: 0.8417).

Effect of treatments on spontaneous locomotor activity in the open field test

The results of treatments in the OFT are demonstrated in Table 2. No change was observed in the experimental groups (ANOVA: $F_{(31,223)} = 2.028$, $p = 0.2281$, R2: 0.2281
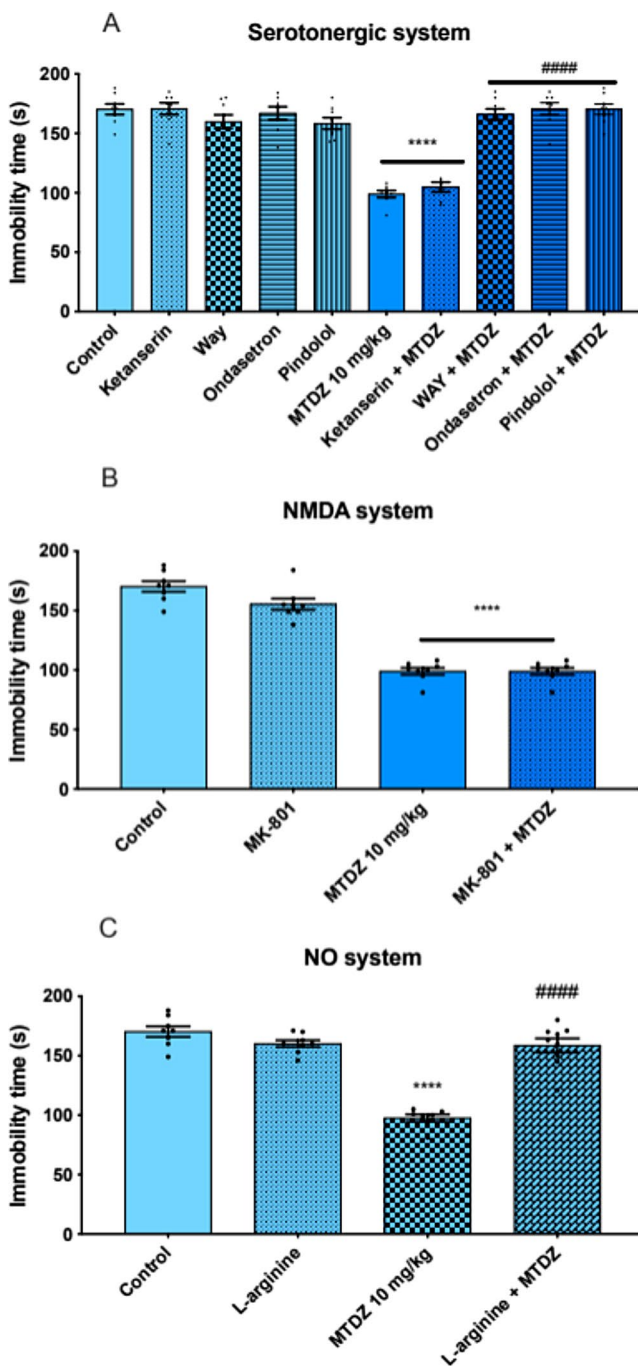

Fig. 7 Effects of 5-((4-methoxyphenyl)thio)benzo[c][1,2,5]thiadiazole (MTDZ) on MAO activities: MAO-A activity in the cerebral cortices (**A**) and hippocampi (**B**); MAO-B activity in the cerebral cortices (**C**) and hippocampi (**D**). Data are reported as mean ± standard error of the mean (SEM) of 8 animals per group. (****) denoted $p < 0.0001$ compared with the control group (one-way analysis of variance/Newman-Keuls test)

Fig. 8 Effect of 5-((4-methoxyphenyl)thio)benzo[c][1,2,5]thiadiazole (MTDZ) in mechanisms different in the FST: serotonergic system (**A**); NMDA system (**B**); NO system (**C**). Data are reported as mean ± standard error of the mean (SEM) of 8 animals per group. (****) denoted $p < 0.0001$ as compared with the control group; (####) denoted $p < 0.0001$ compared with the MTDZ group (two-way analysis of variance/Newman-Keuls test for training and one-way analysis of variance/Newman-Keuls test for probe test)

Table 2 Effects of treatments on the number of crossings and rearings of mice in the open-field test

Group	Crossings	Rearings
Control - Male	95 ± 3	40 ± 4
Control - Female	124 ± 5	37 ± 5
MTDZ 1 mg/kg - Male	107 ± 5	35 ± 4
MTDZ 1 mg/kg - Female	101 ± 4	36 ± 6
MTDZ 10 mg/kg - Male	97 ± 5	37 ± 3
MTDZ 10 mg/kg - Female	107 ± 8	34 ± 5
Fluoxetine 10 mg/kg - Male	115 ± 1	41 ± 3
Fluoxetine 10 mg/kg - Female	97 ± 5	45 ± 7
WAY100635 - Male	98 ± 5	40 ± 4
Ketanserin - Male	99 ± 7	43 ± 3
Ondansetron - Male	108 ± 3	38 ± 6
MK-801 – Male	95 ± 3	36 ± 3
Pindolol – Male	96 ± 4	35 ± 4
L-arginine – Male	104 ± 6	39 ± 3
WAY100635 + MTDZ 10 mg/kg - Male	124 ± 5	38 ± 3
Ketanserin + MTDZ 10 mg/kg - Male	101 ± 4	42 ± 3
Ondasetron + MTDZ 10 mg/kg - Male	107 ± 9	34 ± 5
MK-801 + MTDZ 10 mg/kg – Male	115 ± 1	42 ± 3
Pindolol + MTDZ 10 mg/kg – Male	100 ± 7	34 ± 5
L-arginine + MTDZ 10 mg/kg – Male	93 ± 6	35 ± 5

Results are given as means ± SEM of 8 mice in each group (one-way analysis of variance/Tukey's test)

for crossings and ANOVA: $F_{(31,223)} = 0.5724$, $p = 0.9674$, R2: 0.0737 for rearings) in the OFT.

Discussion

This study demonstrated, for the first time, the antidepressant-like action of MTDZ in mice and that serotonergic and nitrergic pathways, ATPase enzymes, and monoaminergic systems may be involved in the antidepressant-like action of the compound. Notably, no difference was observed in the antidepressant-like effect of MTDZ between male and female mice. The MTDZ compound belongs to the class of aryl sulfonyl derivatives, a class that has some established biological effects considered promising (da Costa Rodrigues et al. 2022; da Motta et al. 2022; Thankachan et al. 2015). Recently, our research group demonstrated the effect of MTDZ in different experimental models (da Costa Rodrigues et al. 2022; da Motta et al. 2022). In this study, we sought to shed more light on the pharmacological actions of MTDZ, particularly the antidepressant-like action, molecular docking interaction with MAO, ideal dose and treatment time, and evaluate the possible mechanisms involved with the action of the compound.

Initially, molecular docking protocols were developed, and residues crucial for the catalytic activity of MAO enzymes were considered (Geha et al. 2002; Son et al.

2008). The literature corroborates the compounds ligand mode (Ramsay et al. 2020; Secci et al. 2012). Hence, we assessed the affinity of MTDZ with both isoforms of MAO (A and B). The interaction is considered important in the inhibitory potential of MTDZ, considering that Tyr407 and Tyr444 for MAO-A are responsible for forming a sandwich that stabilizes the substrate binding, which is directly related to the catalytic activity (Geha et al. 2002). Furthermore, MTDZ also makes a Pi-Sulfur with Cys406 (Cys 397 in MAO-B), the target of the covalent link with FAD, which is suggested to contribute to 40–60% of the catalytic activity of MAO (Hiro et al. 1996).

Interestingly, clorgiline can also interact with Cys406 (Edmondson et al. 2009), suggesting that research on the irreversible inhibition of MAO-A would complement information on MTDZ action mechanisms and the possible adverse effects. Additional MAO-A and MTDZ complex interaction residues include Arg51, Met445, and Ile207. In contrast, MTDZ possesses a docking in MAO-B, albeit the data suggests that it is a preferable inhibitor of MAO-A with substantial evidence, indicating its selectivity towards this isoform.

Next, according to OECD recommendations, the toxicity protocol was carried out to evaluate the treatment effect of MTDZ at a high dose in Swiss mice. With this, the compound does not present characteristics and behavior of toxicity in the single dose of 300 mg/kg, nor does it change the oxidative parameters (RS and TBARS levels) or renal and hepatic biochemistry markers (creatinine levels and ALT/AST activity). Given that no signs of toxicity were observed in the mice, several tests were performed to evaluate the antidepressant-like action of the compound.

The pathophysiology of MDD presents many neural pathways, including the serotonergic system (Gonçalves et al. 2012). The findings from Ben-Azu et al. (2021) suggest that modulating the activity of specific receptor systems, including 5-HTergic, noradrenergic, and dopaminergic receptors, may offer promising opportunities for the development of novel antidepressant treatments. Nevertheless, since it is a complex multifactorial disease, other pathways, such as an interaction between serotonergic and NO pathways, may also be involved. Overall, the role of specific receptor systems, including 5-HTergic, noradrenergic, and dopaminergic receptors, in the pathophysiology of depression indicates a potential strategy for developing novel antidepressant treatments. This suggests that targeting these systems might be a viable approach (Ben-Azu et al. 2021). Thus, animals are commonly used to screen new antidepressant molecules (De Oliveira et al. 2019). First, we evaluated the dose-response curve on depressive-like behavior in mice to verify whether the compound had a promising effect. Subsequently, we

sought to obtain more knowledge on these effects in specific models of depression (Yankelevitch-Yahav et al. 2015).

In this study, MTDZ administration in the FST decreased immobility behavior without changing the climbing parameter, suggesting it reduces depressive-like behavior similarly to other 5-HT-related compounds (Pesarico et al. 2014). Hence, the action of MTDZ in this triage study can be antidepressant in nature and not a general psychostimulant.

In our study, MTDZ had a similar effect to fluoxetine, a 5-HT selective receptor inhibitor (SSRIs), corroborating the results found in the FST. Notably, SSRIs have some limitations, including producing their effect 3 or 4 weeks after beginning treatment and cases of treatment remission (Samuels et al. 2016). Therefore, this is a critical drug class as it is a pathway significantly affected by depressive behavior (Luo et al. 2020), so attention must be paid to this system when developing new promising compounds with fewer side effects and fast action time. Thus, we investigated if the treatment with MTDZ presented the antidepressant-like effect associated with the serotonergic pathways.

Our results demonstrated that the effect of MTDZ was blocked by the administration of an antagonist of 5-HT$_{1A}$ (by pretreatment with WAY100635), 5-HT$_{1A/1B}$ (by pindolol), and 5-HT$_3$ (by ondansetron). These results corroborate the literature since the blockade of 5-HT$_{1A}$ receptors has an effect in reducing the depressive effect (Starr et al. 2007); the downregulation of 5-HT$_{2A}$ has been shown to act synergistically with other antidepressant drugs, and the blockade of 5-HT$_{2C}$ receptors induces sleep disturbances and motor impairment, thereby contributing with the antidepressant effect (Millan 2005).

In this regard, our results suggest that the antidepressant-like action of MTDZ begins after 15 min of administration at 10 mg/kg, and this can be associated with its action on receptor 5-HT$_3$. Regarding the 5-HT receptors, the activation of 5-HT$_3$ receptors is also associated with an influx of calcium (Ca^{2+}) ions in the neurons, a dangerous effect on cerebral structures (Turner et al. 2004). Here, we demonstrated that MTDZ also increases Ca^{2+}-ATPase activity in the hippocampi and cerebral cortices, showing that the MTDZ action may be connected with Ca^{2+}-ATPases activity in mice. Therefore, the MTDZ may antagonize the 5-HT$_3$ receptors, decreasing the influx of Ca^{2+} and impacting the activity of this enzyme.

It is essential to emphasize that the first line of treatment for MDD is the selective 5-HT receptor inhibitors. More important improved outcomes of this treatment have been obtained with simultaneous blockage of 5-HT receptors (Casaril et al. 2019; Starr et al. 2007). Interestingly, the simultaneous interaction with receptors of the serotonergic system is suggested to have contributed to the

neuropharmacological effect of MTDZ without changing psycho-locomotor alterations.

Nonetheless, the use of NO synthase inhibitors can accentuate the antidepressant effect of such agents, the antidepressant-like action caused by inhibitors of NOS, and are dependent on endogenous 5-HT (Ghasemi et al. 2019). Recent evidence has shown reduced airway NO mobilization in depressed patients (Ritz et al. 2015). In this sense, the antidepressant-like action of MTDZ can also be associated with the NO system since the pretreatment with L-arginine blocked the antidepressant-like action of MTDZ.

In addition to the relationship, we also investigated the involvement of the glutamatergic system since glutamate can increase the risk of MDD for neurotoxicity, damaging neuroplasticity and increasing NO synthesis (Candee et al. 2023). However, MTDZ antidepressant-like activity was not altered by pretreatment with MK-801, although further research is necessary to investigate the glutamatergic pathways.

In addition, the ex vivo assays showed that the compound normalized ATPase activity, corroborating a recent study by our research group (da Motta et al. 2022). These ATPase enzymes are vital for energetic balance and impact brain function; Na^+K^+-ATPase has been expressed in astrocytes, and it helps the K^+ clearance after neuronal activation, as any disbalance in its activity directly affects cellular excitability in depression (El-Mallakh 1983; Friedrich et al. 2016). Notably, Na^+K^+-ATPase activity also decreased, leading to symptoms of depression, thus showing the correlation between this enzyme's activity and this pathology (Maripuu et al. 2021). Hence, our results showed higher total ATPase, Na^+K^+-ATPase, Mg^+-ATPase, and Ca^{2+}-ATPase, and these findings may be related to the antidepressant-like action of MTDZ in the behavior tests.

Indeed, MAO inhibitors are important in developing antidepressant molecules. The MAO is a mitochondrial enzyme with isoforms MAO-A and MAO-B. More directly, MAO inhibition may be associated with the serotonergic system, resulting in elevated 5-HT concentrations in the brain (Finberg and Rabey 2016). The treatment with MTDZ (10 mg/kg) inhibited MAO-A activity in mice's cerebral cortices and hippocampI, although it did not affect MAO-B activity. MAO inhibition exerts antidepressant effects with the degradation of neurotransmitters, and inactivation blocks monoamine catabolism (Alvarez et al. 1999). In the present study, MTDZ inhibited the MAO-A enzyme, correlating with the antidepressant-like action of the compound and suggesting this is selective to the isoform A, which may be a differential of the effect of MTDZ against other drugs. Moreover, researchers have demonstrated that the selective inhibition of MAO-A can occur for the three-dimensional arrays and the interaction with the aromatic side (Tsugeno

and Ito 1997), although to elucidate this correlation, further studies are necessary.

For this prospective study, a model of chronic unpredictable mild stress (CUMS) will be utilized. CUMS-induced depression is a model linked with oxidative stress (Markov and Novosadova 2022; Willner et al. 1987). Moreover, the gut microbiota and the microbiota-gut-brain axis play a pivotal role in CUMS-induced depression (Kabir et al. 2022). This model conjures an authentic depression model, as it simulates stressors inherent in human existence and generates anhedonia, the principal symptom of depressive disorder as identified in the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) (Antoniuk et al. 2019; Guze 1995). Using this model, we aim to generate more precise data concerning the antioxidative action of the MTDZ compound.

Conclusions

MTDZ administration has a binding affinity with MAO-A and presented the best antidepressant-like effect and spectrum of receptor interaction with serotonergic and NO systems, demonstrating its multitarget action. In addition, the male and female mice showed no differences in antidepressant-like action. Our findings may offer a rationale for clinical results, indicating therapeutic effects of MTDZ in different receptors, which may be effective for other psychiatric and neuro disorders involved in the physiopathology with the mechanisms evaluated herein. Given the above, MTDZ is a promising alternative to treating depression, although further research is required to elucidate the other mechanisms of the compound.

Declarations

Competing interests None.

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