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Anxiolytic-like Effect in Adult Zebrafish (*Danio rerio*) through GABAergic System and Molecular Docking Study of Chalcone (*E*)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one

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Abstract: Benzodiazepines are used to treat anxiety disorders. The chronic use of these drugs can cause tolerance or relevant adverse effects, requiring the search for new, safer, and more effective compounds. In this context, the present work aimed to investigate the potential anxiolytic effect of the chalcone synthesized and its mechanism of action, using the adult zebrafish (Zfa) as an animal model. The animals were treated with the new chalcone (4.0; 20 and 40.0 mg/kg) in the 96h open field and toxicity test. The concentrations that caused locomotor alteration were evaluated in the light and dark anxiolytic test, with the lowest active dose being used to assess the mechanism via the GABAergic system. The chalcone proved to be safe compared to the adult Zebrafish model up to 96h of analysis and caused locomotor alteration of Zfa similar to that of benzodiazepines. The chalcone presented an anxiolytic effect, which was blocked by flumazenil, a GABAA receptor antagonist, thus demonstrating that the chalcone must act via the mechanism of the GABA system. In this perspective, chalcone has the potential to be used as a pharmacological tool in the treatment of anxiety disorders.

Keywords: chalcone; zebrafish; anxiolytic; GABAergic system.

1. Introduction

Anxiety disorders are among the top ten diseases responsible for causing disabilities worldwide. The prevalence of anxiety disorders in the United States is estimated to be 18%. Their annual cost is reported to be $42.3 billion. In the European Union, over 60 million people are affected by anxiety disorders in a given year, making them the most prevalent psychiatric condition [1]. Brazil leads the world in prevalence of anxiety disorders and ranks fifth in depression rates [2].

Benzodiazepines (GABA receptor agonists) and serotonin reuptake inhibitors (SSRIs) are the drugs of choice for the treatment of anxiety [3]. Serotonergic drugs are also commonly

used to treat depressive disorders [4]. However, chronic use of benzodiazepines produces tolerance, and abrupt discontinuation of treatment can lead to withdrawal syndrome [5]. On the other hand, the chronic use of SSRIs can produce considerable side effects [6], so the search for new compounds with anxiolytic and antidepressant properties with less potential to produce adverse effects continues [7], and recent studies have shown that chalcones can be considered promising anxiolytic agents [8].

Chalcones are bioprecursors of flavonoids and have a basic structure of [1,3-diphenyl-2-propen-1-one] (Figure 1) are therefore naturally occurring aromatic ketones, consisting of an α carbonyl system, β-unsaturated that joins two aromatic rings. They are a class of open chain flavonoids, abundantly found in plants of the families Leguminosae, Compositae, and Moraceae; they are present in fruits, vegetables, grains, roots, flowers, teas, wines, products that are regularly used for human consumption. Chalcones and their derivatives are substances of great chemical and pharmacological interest. They have received great attention due, above all, to their relatively simple structure and the diversity of pharmacological activities they present, among which you can mention: antioxidant activity, antinociceptive, anticonvulsant, anti-inflammatory, antimicrobial, cytotoxic antitumor [9].

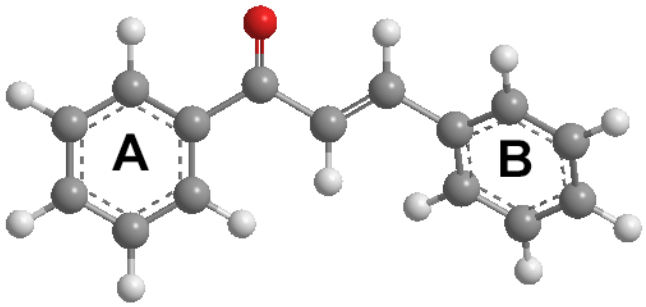


Figure 1. The fundamental structure of chalcone.

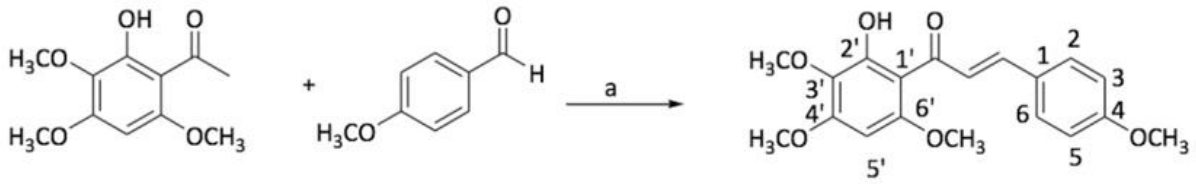
Currently, zebrafish (*Danio rerio*) has become popular in various areas of behavioral neuroscience, including brain research and psychopharmacology [10]. This vertebrate is considered a significant model, mainly because its genotype has 70% exclusive homology with mammalian neurotransmitter receptors, in addition to its small size, high proliferation power, rapid and transparent development that can significantly facilitate drug discovery in studies using this animal as a model [11].

Thus, the objective of the present work was to investigate the anxiolytic action, the mechanism of action and to carry out the study of the molecular docking of chalcone (E)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)-3-(4-methoxyphenyl) prop-2-en-1-one.

2. Materials and Methods

2.1. Synthesis of the chalcone.

The compounds 2-hydroxy-3,4,6 trimethoxyacetophenone (2 mmol) and anisaldehyde (2 mmol) were placed in a volumetric flask (25 mL). Then 5 mL of ethanolic NaOH (50%) solution was added and mixed with stirring for 48 h at room temperature. The progress of the reaction was checked by TLC (n-hexane: ethylacetate, 2:1). After 48 h, the reaction mixture was neutralized with dilute HCl (10%) and ice water added. The product was obtained as a yellow solid filtered under reduced pressure, washed with cold water, and recrystallized from ethanol (Scheme 1) [12].



Scheme 1. Preparation of chalcone. a - NaOH 50% wv^{-1}, ethanol, t.a., 48 h.

2.1.1. Analytical data of chalcone (*E*)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one.

Yellow solid (Yield: 44.2 %), R_F = 0.4 (hexane/ethyl acetate, 80:20), m.p. 94.7 - 94.8ºC; FT-IR (KBr, ν_{cm}^{-1}): 1634, 1600, 1588, 1575, 1480, 1167. ^{1}H RMN ($CDCl_3$, ppm): 3.91 (s, MeO-3'); 3.90 (s, MeO-4'); 3.86 (s, MeO-6'); 3.78 (s, MeO-4); 5.93 (s, H-4'); 7.89 (m, H-3/5, J = 8.4); 7.53 (d, H-2/6, J = 8.4 Hz); 7.24 (d, Hα, J = 14.6 Hz); 7.75 (d, Hβ, J = 14.6 Hz). ^{13}C RMN ($CDCl_3$, ppm): 193.3 (C=O); 60.9 (MeO-3'); 56.2 (MeO-4'); 55.8 (MeO-6'); 55.5 (MeO-4); 107.1 (C-1'); 158.7 (C-2'); 130.3 (C-3'); 159.5 (C-4'); 87.3 (C-5'); 158.6 (C-6'); 130.7 (C-1); 125.2 (C-2/6); 130.3 (C-3/5); 128.4 (Cα); 142.9 (Cβ). MS-EI *m/z* (%) = 344.

2.2. Zebrafish.

Zebrafish (*Danio rerio*) adult, wild, both sexes, aged 60-90 days, sizes 3.5 ± 0.5 cm and weight 0.4 ± 0.1 g were obtained from Agroquímica: Comércio de Produtos Veterinário LTDA, a supplier in Fortaleza (Ceará, Brazil). Groups of 60 fish were acclimated for 24 h in glass aquariums (30 x 15 x 20 cm), containing dechlorinated water (ProtecPlus®) and air pumps with submerged filters, at 25ºC and pH 7.0, with a 14-day circadian cycle: 10 h of light/ dark. Fish were fed ad libitum 24 h before the experiments. After the experiments, the fish were sacrificed with ice water (5ºC). All experimental procedures were approved by the Animal Use Ethics Committee of the State University of Ceará (CEUA-UECE), under protocol No. 7210149/2016.

2.3. General protocol.

The tests were performed based on methodologies proposed by Magalhães *et al*. [13] and Ekambaram *et al*. [14]. On the day of the experiments, the fish were selected randomly, transferred to a wet sponge, treated with the test samples or controls via intraperitoneal (*i.p.*). Then they were placed individually in beakers (250 mL) containing 150 mL of aquarium water for resting. For intraperitoneal (*i.p.*) treatments, an insulin syringe (0.5 mL; UltraFine® BD) with a 30G gauge needle was used.

2.4. Non-clinical safety assessment.

2.4.1. Locomotor activity (Open Field Test).

The animals were treated with the test samples and subjected to the open field test [15] to assess whether there was a change in motor coordination, either by sedation and/or muscle relaxation. Zebrafish (n = 6 / group) were treated, intraperitoneally (*i.p.*), with 20 µL of sample solutions in doses (4, 20 and 40mg / kg) and vehicle (DMSO 3%) and diazepam (40mg / kg). After 30 min of the treatments, the animals were added in Petri dishes containing the same water from the aquarium, marked with quadrants, and the locomotor activity was analyzed by

counting the number of crossing lines for 5 minutes. Animals that did not receive treatments (Naive) were considered as baseline (100% locomotor activity) and calculated the percentage of locomotor activity (% LA).

2.4.2. Toxicity in adult zebrafish (ZFa).

Acute toxicity was performed against the ZFa to determine the LD50 for 96 hours according to the guidelines of the Organization for Economic Cooperation and Development (OECD) [14]. The animals (n = 6 / group) were treated, intraperitoneally (*i.p.*), with 20 µL of chalcone (4.0; 20 and 40.0 mg / kg), or 3% DMSO (vehicle). The number of animals that died in each concentration from 24 to 96 h was recorded, and the LC_{50} was determined.

2.5. Anxiolytic activity of chalcone anisaldehyde.

2.5.1. Light & dark test.

The test was carried out in a glass aquarium (30 x 15 x 20 cm) with a light and a dark zone, filled with 3 cm of anti-chlorinated and drug-free water [15]. The animals (n = 6 / group) were treated intraperitoneally with 20 µL of chalcone with all those analyzed in the open field test (4.0; 20 or 40.0 mg / kg), vehicle (DMSO 3%) and Diazepam (40 mg/kg). An untreated group of animals (Naive) was included. After 30 min, the animals were individually added to the clear area of the aquarium, and the anxiolytic-like effect was quantified as time (s) of stay in the clear area during 5 min of analysis. The lowest active dose was used to assess the mechanism via the GABAergic system.

2.5.2. Involvement in the Gabaergic system in anxiolytic activity.

The animals (n = 6 / group) received flumazenil intraperitoneally (4 mg / kg; 20µL; *i.p.*) and after 15 minutes were treated with Chalcone (20 mg / mL; 20µL; *i.p.*), Diazepam (40 mg / kg; 20µL; *i.p.*), vehicle (3% DMSO; 20 µL; *i.p.*). After 30 min of the treatments, the animals were submitted to the light & dark test, described in section 2.2.

2.6. Molecular docking.

For molecular docking simulations involving the GABAergic system, the GABAA receptor structure (PDB 6HUP) was obtained from the Protein Data Bank repository (https://www.rcsb.org/), identified as "CryoEM structure of human full-length alpha1beta3gamma2L GABA (A) R in complex with diazepam, GABA and mega body Mb38".

Some characteristics presented of the deposited receptor structure are a resolution of 3.58 Å, determined by electron microscopy, classified as a membrane protein, *Homo sapiens* organism, and *Homo sapiens* and *Escherichia coli* expression system. The AutoDock Vina code (version 1.1.2) was used to perform the simulations with 3-way multithreading, Lamarckian Genetic Algorithm [16]. The grid box parameters were defined at 126Åx100Åx126Å, with the dimensions (x, y, z) = (125.281, 139.534, 136.018), centered on the entire protein. As a standard procedure, 50 independent simulations were performed with 20 poses each. The values of RMSD (Root Mean Square Deviation) less than 2 Å [17] and free binding energy (ΔG) less than -6.0 kcal/mol [18] were used as criteria for the selection of simulations with better conformations. The Discovery Studio Visualizer [19] and UCSF

Chimera [20] codes were used to analyzing the results and generate 2D maps of chemical interactions.

2.7. Statistical analysis.

The results were expressed as values of the mean ± standard error for each group of 6 animals. After confirming the normal distribution and homogeneity of the data, the differences between the groups were subjected to analysis of variance (one-way ANOVA), followed by the Tukey test. All analyzes were performed using the GraphPad Prism v software. 5.01. The level of statistical significance was set at 5% ($p < 0.05$).

3. Results and Discussion

3.1. Evaluation of locomotor activity (Open Field Test).

The open-field test in Petri dishes [15] was recently adapted to assess the locomotor activity of adult zebrafish under the action of analgesic drugs. In this way, the same method was used with the assigned samples to evaluate actions on the ZFa locomotor system. As a result, it was observed that chalcone caused motor impairment in the ZFa, where there was a reduction in the number of line crossing in the petri dish by the animals, a result significantly different from vehicle control ($p < 0.0001$; $p < 0$, 01 and vs. vehicle) and different from the naïve group ($p < 0.0001$ vs. naive) (Figure 2A).

3.2. Anxiolytic activity.

As a result, the chalcone had an anxiolytic-like effect at doses (20 and 40 mg/kg; 20 µL; *i.p.*) as the animals remained most of the time of analysis in the clear zone significantly ($p > 0.05$) similar to diazepam (40 mg/kg; 20 µL; *i.p.*) as shown in Figure 2B.

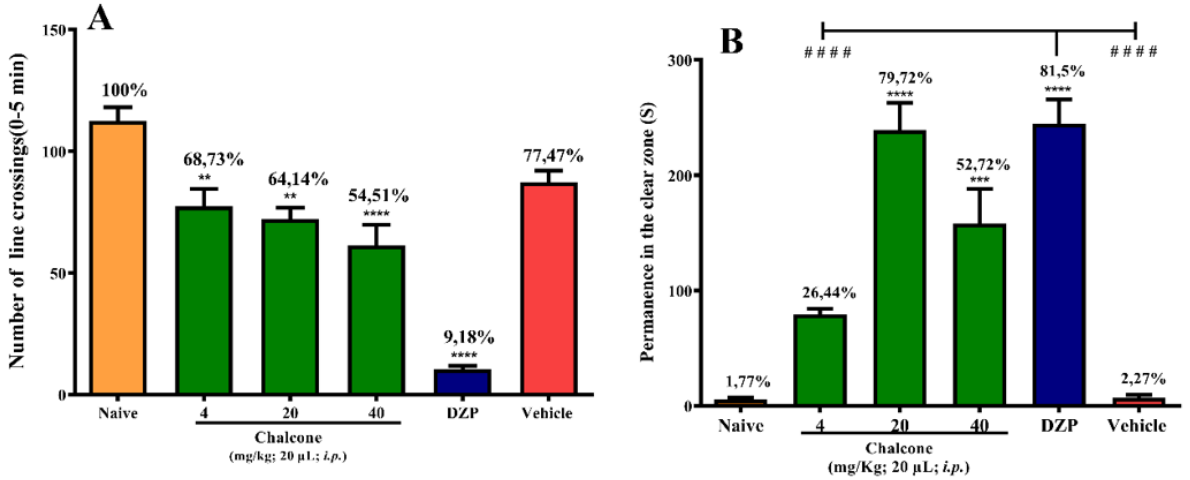


Figure 2. Effect of chalcone on locomotor activity of adult zebrafish (*Danio rerio*) in the Open Field Test (0-5min). Naive - untreated animals. DZP - diazepam (40 mg / kg; 20 µL; *i.p.*). Vehicle - 3% DMSO (20 µL; *i.p.*)* (**A**) Anxiolytic-like effect of chalcone in adult zebrafish (*Danio rerio*) in the Light & Dark Test (0-5min). Naive - untreated animals. Vehicle - 3% DMSO (20 µL; *i.p.*). DZP - Diazepam (40 mg / kg; 20 µL; *i.p*) * * (**B**). The values represent the mean ± standard error of the mean (E.P.M.) for 6 animals / group. *ANOVA followed by Tukey $p < 0.0001$ vs. naive; # # # #$p < 0.0001$; # # $p < 0.01$ vs. Vehicle). ** ANOVA followed by Tukey (**** $p < 0.0001$; *** $p < 0.001$ vs. Naive or Vehicle; # # # #$p < 0.0001$ vs. DZP).

Locomotor activity is one of the parameters of behavioral analysis that has been used to evaluate the action of drugs that can act on the central nervous system of the adult zebrafish

(*Danio rerio*) and cause locomotor impairment or not [23]. This activity can be explored through the Open Field Test in an aquarium [24], as well as Petri dishes [25]. Different parameters can be evaluated, such as freezing, among others. The natural behavior of zebrafish in the open field is characterized by constant swimming activity and manifestations of immobility, which are little observed in natural conditions of zebrafish [26]. According to Resende and Soccol [27], the analysis of locomotor activity explored through an open field can be a model used to assess hyperactivity as being indicative of anxiety. The treatment of zebrafish with anxiolytic drugs, such as benzodiazepines, can increase exploratory activity in the open field [26], cause a sedative effect, and decrease locomotor activity [28,29].

The decrease in locomotor activity in adult zebrafish caused by chalcone suggests a possible sedative action, such as benzodiazepines (anxiolytic drugs), which decrease locomotor activity (mobility) of adult zebrafish (*Danio rerio*) in the open, as shown highlight Gupta *et al*. [28] and Benneh *et al*. [29] (Figure 2A and 2B).

3.3. Acute toxicity (96h).

Adult zebrafish were used as an animal model to assess the acute toxicity of chalcone. As a result, it was found that the chalcone was shown to be safe, as it was not toxic against ZFa until 96 h of analysis (LC_{50} > 40 mg/kg) (Table 1).

Table 1. Result of acute toxicity test of chalcone synthesized against adult zebrafish.

	Mortality				96h
Sample	*CN	D1	D2	D3	*CL_{50} (mg/kg) / IV
Chalcone	0	0	0	0	> 40

*CN- Negative control group: DMSO 3%. D1 – Dose 1 (4 mg/kg). D2 – Dose 2 (20 mg/kg). D3 – Dose 3 (40 mg/kg). *CL_{50}- lethal concentration to kill 50% of adult Zebrafish; IV – confidence interval.

3.3.1. Mechanism of anxiolytic action via the gabaergic system.

Flumazenil is an antagonist of GABAergic receptors in the α [1-3, 5] βγ subunits. It antagonizes the sedative effects caused by benzodiazepine overdoses (e.g., diazepam) and reverses these effects in addition to preventing respiratory depression by blocking GABA receivers [21,22].

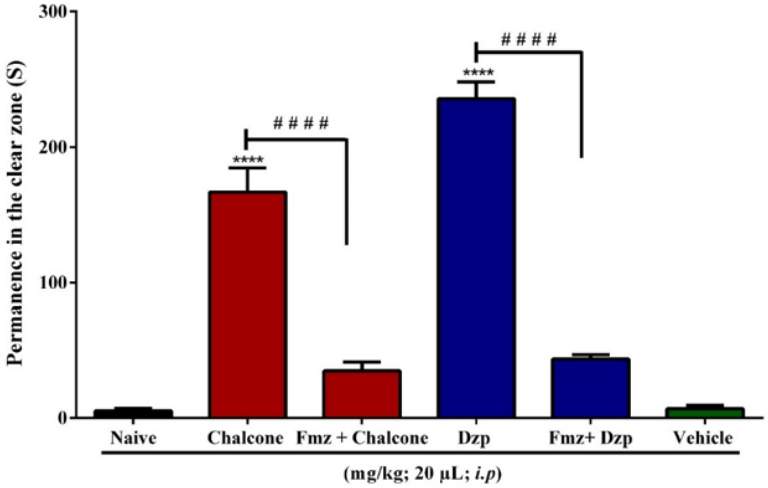


Figure 3. Effects of Flumazenil on chalcone anxiolytic activity in the light and dark test. Chalcone Anisaldehyde (20 mg / kg; 20 µL, *i.p.*). Dzp - Diazepam (40 mg / mL; 20 µL; *i.p*). Fmz - flumazenil (4 mg / kg; 20 µL; *i.p.*). The values represent the mean ± standard error of the mean (E.P.M.) for 6 animals / group. ANOVA followed by Tukey (**** p <0.0001 vs. naïve or vehicle; # # # # p <0.0001 vs. Fmz + Dzp or Fmz + chalcone).

Flumazenil reduced (#### p <0.0001) the anxiolytic effect of chalcone (20 mg/kg; 20 µL; *i.p.*) and Diazepam (40 mg/kg; 20 µL; *i.p.*) (Figure 3), in this perspective, it can be inferred that the blockade of flumazenil on the anxiolytic effect of chalcone, represents a strong indication that the mechanism of action is directly related to the receptors of the GABAergic system.

The adult zebrafish has been used as an animal model complementary to rodents in genetic tests, developmental biology, neurobiological and toxicological [27], as it has low cost, diverse adaptability, short reproduction cycle, high fertility, and embryos transparent [30]. Its small size in adulthood requires a decrease in the number of substances to be tested and dosed and the amounts of reagents and materials used in the treatment and maintenance of animals. Adult zebrafish have long been used in toxicity tests to monitor environmental contaminants. As an example, Huang *et al*. [31] used adult zebrafish to evaluate the action of an agrochemical agent (Deltamethrin), using locomotor behavior and mortality (CL50) in 24 hours as parameters of acute toxicity. It is worth noting that adult zebrafish are also used to assess the toxicity of pharmaceutical compounds and toxicological biomonitoring in drug development [32]. All doses of chalcone investigated in this study were subjected to an acute toxicity test (96h) and showed no toxicity, which provides evidence for in vivo pharmacological tests, allowing the selection of safe doses (Table 1).

The mechanism of the chalcone anxiolytic effect was investigated by flumazenil, a GABAA antagonist. After pre-treatment, flumazenil reduced the anxiolytic/sedative effects of the analyzed chalcone, indicating that the anxiolytic activity of the molecule acts through the GABA system (Figure 3). Drugs that stimulate GABA receptors, such as BDZs and barbiturates, have anxiolytic effects by reducing GABAA-mediated neuronal excitability, causing changes in the α1 and γ2 subunits of this receptor [20].

3.4. Molecular Docking.

The potential anxiolytic effect of chalcone on the GABAergic system was investigated by performing molecular docking simulations with the GABAA receptor, which showed high-affinity energy, in the order of -8.1 Kcal/mol and an RMSD of 1.312. (Table 2).

Table 2. Interactions between the GABAA receptor and the chalcone ligand.

Ligand	Receptor	Interaction	Distance (Å)
chalcone	LYS1275D	H-Bond	2.81 Å
	PHE856C	Hydrophobic	3.77 Å
	LYS1275D	Hydrophobic	3.94 Å
	VAL1322D	Hydrophobic	3.39 Å
	PHE856C	π-Stacking	4.24 Å

Docking studies were carried out to correlate structural changes with biological activities. In this context, the molecular interactions of chalcones with the protein GABA-AT (γ-aminobutyric acid aminotransferase), a validated target for AEDs, were analyzed, and their selective inhibition increases GABA concentrations in the brain.

The complex formed (Figure 5A) showed interactions ranging from 2.81Å to 4.24 Å (Table 2), making it possible to identify (Figure 4B) the formation of a hydrogen bond classified as strong [33] with the LYS1275 residue (2.81Å), three hydrophobic interactions with residues PHE856C (3.77Å), LYS1275 (3.94Å), VAL1322 (3.39 Å) and a π-Stacking interaction with residue PHE856C (4.24Å), coupling in a region close to that of the Diazepam inhibitor, showing a potential anxiolytic effect via the GABAergic receptor.

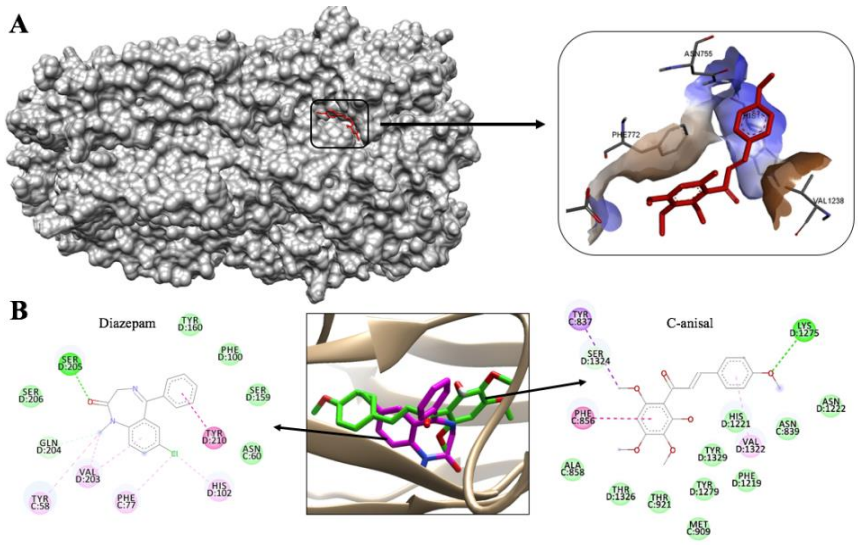


Figure 4. GABAa/chalcone Complex **(A)** 2d map of GABAa/Diazepam and GABAa/chalcone interactions **(B)**.

5. Conclusions

The present study indicates a strong potential for the use of chalcone (*E*) -1- (2-hydroxy-3,4,6-trimethoxyphenyl)-3-(4-methoxyphenyl) prop-2-en-1-one as a pharmacological tool for the treatment of anxiety disorders, as it showed anxiolytic activity similar to that of Diazepam, with strong indications of action by the mechanism via GABAergic receptor.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Osman, G.; Mohammad, F.K.; Md Masudur, R.; Md. Zahid, H.; Faisal, B.K.; Nasim, S.; Mahfuz, A.S. Pharmacological insights on the antidepressant, anxiolytic and aphrodisiac potentials of Aglaonema hookerianum Schott. *J Ethnopharmacol* **2021**, *268*, 113664, https://doi.org/10.1016/j.jep.2020.113664.
2. Dokkedal-Silva, V.; Galduróz, J.C.F.; Tufik, S.; Andersen, M.L. Benzodiazepine consumption in Brazil: considerations regarding a population-specific scenario. *Braz J Psychiatry* **2020**, *42*, 332, http://doi.org/10.1590/1516-4446-2019-0830.
3. Yu-Ting, C.; Chia-Yih, L.; Chia-Ming, C.; Yu-Ming, L.; Bi-Hwa, W.; Tsui-Yun, Y.; Shih-Chieh, H. Perceptions, clinical characteristics, and other factors associated with prolonged and high daily dose of benzodiazepine use among patients with anxiety or depressive disorders. *J Afect Disord* **2020**, *271*, 215–223, https://doi.org/10.1016/j.jad.2020.03.077.
4. Jaini, J.P.; Angélica, S.R.; Renata, L.O.; Caren, A.R.F.; William, B.D.; Eduardo, N.D.; Vinícius, F. C.; Roberta, K.; Diego, A.; Cristiane, L.; Ethel, A.W. The anxiolytic effect of a promising quinoline containing selenium with the contribution of the serotonergic and GABAergic pathways: Modulation of parameters

associated with anxiety in mice. *Behav. Brain Res* **2020**, *383*, 112518, https://doi.org/10.1016/j.bbr.2020.112797.

5.. Hyeon, J.H.; Yong, S. L.; Jaesuk, Y.; Sang-Bae, H.; Dong, J.S.; Jin, T.H. Anxiolytic-like effects of the ethanol extract of *Magnolia obovata* leaves through its effects on GABA-benzodiazepine receptor and neuroinflammation. *Behav. Brain Res* **2020**, *383*, 112518, https://doi.org/10.1016/j.bbr.2020.112518.

6. Bernadette, M.; Elias, G.; Rita, K. Selective serotonin reuptake inhibitors and Alzheimer's disease. *Neural Regeneration Research* **2020**, *15*, 41-46, https://doi.org/10.4103/1673-5374.264445.

7. Jayze, C. X.; Maria, K.A.F.; Antonio, W.S.; Jane, E.S.A.M.; Alexandre, M.R.T.; Paulo, N.B.; Emmanuel, M.M.; Emanulle S.M.; Márcia, M.M.; Hélcio, S Santos. Anxiolytic-like and anticonvulsant effect in adult zebrafish (*Danio rerio*) through GABAergic system and molecular docking study of chalcone derived from natural products. *Biointerface Research In Applied Chemistry* **2021**, *11*, 14021-14031, https://doi.org/10.33263/BRIAC116.1402114031.

8. Antonio, W.S.; Maria, K.A.F.; Lucas, R.P.; Emanuela, L. R.; Marnielle, R.C.; Joyce, R. L.; Maria, I.F. G.; Paulo, N.B.; Francisco, E.A.M.; Jane, E.S.A.M.; Márcia, M.M.; Alexandre, M.R.T.; Maria, T.S.T.; Hélcio, S.S.; Emmanuel, S.M. Combretum lanceolatum extract reverses anxiety and seizure behavior in adult zebrafish through GABAergic neurotransmission: an in vivo and in silico study. *Journal of Biomolecular Structure and Dynamics* **2021**, https://doi.org/10.1080/07391102.2021.1935322.

9. Maria, K.A.F.; Antonio, W.S.; Francisca, C.O.S.; Antônio, E.V.N.; Adriana, R.C.; Sacha, A.A.R.S.; Alexandre, M.R.T.; Jayze, C.X.; Emanuela, L.R.; Paulo, N.B.; Carlos, E.S.N.; Débora, H.A.B.; Francisco, E.A.M.; Jane, E.S.A.M.; Helcio, S.S. Anxiolytic-like effect of chalcone N-{4'[(2E)-3-(3-nitrophenyl)-1-(phenyl)prop-2-en-1-one]} acetamide on adult zebrafish (*Danio rerio*): Involvement of the 5-HT system. *Biochem Biophys Res Commun* **2020**, *526*, 505–511, https://doi.org/10.1016/j.bbrc.2020.03.129.

10. Maria, K.A.F.; Antonio, W.S.; Atilano, L.S.M.; Ketelly, V.B.S.; Emanuelle, M.M.; Joyce, N.M.C.; Márcia, M.M.; Paulo, N.B.; Francisco, E.A.M.; Emmanuel, S.M.; Jane, E.S.A.M.; Helcio, S.S. Chalcones reverse the anxiety and convulsive behavior of adult zebrafish. *Epilepsy Behav* **2021**, *117*, 107881, https://doi.org/10.1016/j.yebeh.2021.107881.

11. Silva, A.; Ferreira, M.; Reboucas, E.; Silva, F.; Holanda, C.; Barroso, S.; Lima, J.; Batista, F.; Mendes, F.; Campos, A. Anxiolytic-like effect of Azadirachta indica A. Juss. (Neem, Meliaceae) bark on adult zebrafish (*Danio rerio*): participation of the Serotoninergic and GABAergic systems. *Pharmacy & Pharmacology International Journal* **2020**, *8*, 256-263, http://doi.org/10.15406/ppij.2020.08.00303.

12. Antonio, L.A.B.L.; Priscila, T.S.; Matheus, N.R.; Emanuelle, M.M.; Emmanuel, S.M.; Márcia, M.M.; Paulo, N.B.; Carlos, E.S.N.; Humberto, M.B.; Alexandre, M.R.T.; Hélcio, S.S. Potentiating activity of Norfloxacin by synthetic chalcones against NorA overproducing Staphylococcus aureus. *Microb. Pathog* **2021**, *155*, 104894, https://doi.org/10.1016/j.micpath.2021.104894.

13. Hozana, P. S.F.C.; Francisca, C.O.S.; Maria, K.A.F.; Jane, E.S.A.M.; Hélcio, S.S.; Carlos, E.S.N.; Karísia, S.B.L.S.; Eduardo, H.; Edilberto, R.S.; Otília, D.L.P. Anxiolytic-like effect of brominated compounds from the marine sponge Aplysina fulva on adult zebrafish (*Danio rerio*): Involvement of the GABAergic system. *Neurochem. Int* **2021**, *145*, 105021, https://doi.org/10.1016/j.neuint.2021.105021.

14. Silva, A.; Ferreira, M.; Rebouças, E.; Mendes, F.; Moura, A.; Menezes, J.; Marinho, M.; Marinho, E.; Santos, H.; Teixeira, A. Anxiolytic-like effect of natural product 2-hydroxy-3,4,6-trimethoxyacetophenone isolated from Croton anisodontus in adult zebrafish via serotonergic neuromodulation involvement of the 5-HT system. *Naunyn-Schmiedeberg's Archives of Pharmacology* **2021**, *394*, http://doi.org/10.1007/s00210-021-02116-z.

15. Alexandre, A.; Castro, F.; Batista, F.; Santos, S.; Mendes, F.; Gonçalves, G.; Monteiro-Moreira, A.; Souza, A.; Canuto, K.; Magalhães, F. Chemical profile and anxiolytic- and anticonvulsant-like effects of Miconia albicans (Sw.) Triana (Melastomataceae) leaves in adult zebrafish. *Pharmacognosy Magazine* **2021**, *17*, 146, http://doi.org/10.4103/pm.pm_176_20.

16. Oleg, T.; Arthur, J.O. Software News and Update AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *Journal Computational Chemistry* **2009**, *31*, 455-461, https://doi.org/10.1002/jcc.

17. Yusuf, D.; Davis, A.M.; Kleywegt, G.J.; Schmitt, S. An Alternative Method for the Evaluation of Docking Performance: RSR vs RMSD. *Journal of Chemical Information and Modeling* **2008**, *48*, 1411-1422, https://doi.org/10.1021/ci800084x.

18. Shityakov, S.; Förster, C. In silico predictive model to determine vector-mediated transport properties for the blood-brain barrier choline transporter. *Advances and applications in bioinformatics and chemistry: AABC* **2014**, *7*, 23-36, http://doi.org/10.2147/aabc.S63749.
19. Dassault Systèmes BIOVIA. Discovery Studio Visualizer, v17.2.0.16349. San Diego, CA: Dassault Systèmes; 2016.
20. Pettersen E.F.; Goddard T.D.; Huang C.C.; Couch G.S.; Greenblatt D.M.; Meng E.C.; Ferrin T.E. UCSF Chimera - A visualization system for exploratory research and analysis. *Journal Computational Chemistry* **2004**, *25*, 1605–1612, https://doi.org/10.1002/jcc.20084.
21. Penninga E.I.; Graudal N.; Ladekarl M.B.; Jürgens G. Adverse Events Associated with Flumazenil Treatment for the Management of Suspected Benzodiazepine Intoxication - A Systematic Review with Meta-Analyses of Randomised Trials. *Basic& Clinical Pharmacolology& Toxicology* **2016**, *118*, 37-44, https://doi.org/10.1111/bcpt.12434.
22. Olsen R.W.; Sieghart W. GABAA receptors: Subtypes provide diversity of function and pharmacology. *Neuropharmacology* **2009**, *56*, 141-148, https://doi.org/10.1016/j.neuropharm.2008.07.045.
23. Kurta, A.; Palestis, B. Effects of Ethanol on the Shoaling Behavior of Zebrafish (*Danio rerio*). *Dose-response: a publication of International Hormesis Society* **2010**, *8*, 527-533, http://doi.org/10.2203/dose-response.10-008.Palestis.
24. Rosemberg, D.B.; Rico, E.P.; Mussulini, B.H.M.; Piato, Â.L.; Calcagnotto, M.E.; Bonan, C.D.; Dias, R.D.; Blaser, R.E.; Souza, D.O.; de Oliveira, D.L. Differences in Spatio-Temporal Behavior of Zebrafish in the Open Tank Paradigm after a Short-Period Confinement into Dark and Bright Environments. *PLOS ONE* **2011**, *6*, e19397, http://doi.org/10.1371/journal.pone.0019397.
25. Ahmad, F.; Richardson, M.K. Exploratory behaviour in the open field test adapted for larval zebrafish: Impact of environmental complexity. *Behavioural Processes* **2013**, *92*, 88-98, https://doi.org/10.1016/j.beproc.2012.10.014.
26. Cachat, J.; Stewart, A.; Utterback, E.; Hart, P.; Gaikwad, S.; Wong, K.; Kyzar, E.; Wu, N.; Kalueff, A.V. Three-Dimensional Neurophenotyping of Adult Zebrafish Behavior. *PLOS ONE* **2011**, *6*, e17597, http://doi.org/10.1371/journal.pone.0017597.
27. Gawel, K.; Langlois, M.; Martins, T.; van der Ent, W.; Tiraboschi, E.; Jacmin, M.; Crawford, A.D.; Esguerra, C.V.Seizing the moment: Zebrafish epilepsy models. *Neuroscience and Biobehavioral Reviews* **2020**, *116*, 1–20, https://doi.org/10.1016/j.neubiorev.2020.06.010.
28. Gupta, P.; Khobragade, S.; Srinivasan, M.; Shingatgeri, V. Assessment of locomotion behavior in adult Zebrafish after acute exposure to ddifferent pharmacological reference compounds. *Drug Development and Therapeutics* **2014**, *5*, 127, http://doi.org/10.4103/2394-2002.139626.
29. Benneh C.K.; Biney R.P.; Mante P.K.; Tandoh A.; Adongo D.W.; Woode E. Maerua angolensis stem bark extract reverses anxiety and related behaviours in zebrafish-Involvement of GABAergic and 5-HT systems. *Journal Ethnopharmacology* **2017**, *207*, 129-145, https://doi.org/10.1016/j.jep.2017.06.012.
30. Dai Y.J.; Jia Y.F.; Chen N.; Bian W.P.; Li Q.K.; Ma Y.B.; Chen Y.L.; Pei D.S. Zebrafish as a model system to study toxicology. *Environmental Toxicology and Chemistry* **2014**, *33*, 11-17, https://doi.org/10.1002/etc.2406.
31. Huang Y.; Zhang J.; Han X.; Huang T. The use of zebrafish (*Danio rerio*) behavioral responses in identifying sublethal exposures to deltamethrin. *International Journal of Enviromental Research and Public Health* **2014**, *11*, 3650-3660, https://doi.org/10.3390/ijerph110403650.
32. Caballero M.V.; Candiracci M. Zebrafish as screening model for detecting toxicity and drugs efficacy. *Journal of Unexplored Medical Data* **2018**, *3*, 4, https://doi.org/10.20517/2572-8180.2017.15.
33. Imberty A.; Hardman K.D.; Carver J.P.; Perez S. Molecular modelling of protein-carbohydrate interactions. Docking of monosaccharides in the binding site of concanavalin A. *Glycobiology* **1991**, *1*, 631–642, https://doi.org/10.1093/glycob/1.6.631.