Published by SET Publisher

Journal of Pharmacy and Nutrition Sciences

ISSN (online): 1927-5951

Chalcone Derived from a Natural Product: An Integrated Approach of Quantum Chemical Calculations, Molecular Docking, ADME and Neuromodulation on Serotonergic Receptors in Adult Zebrafish

Priscilla Teixeira da Silva[1], Maria Kueirislene Amâncio Ferreira[2], Roberto Lima de Albuquerque[2], Alexandre Magno Rodrigues Teixeira[2], Emmanuel Silva Marinho[2], Márcia Machado Marinho[3], Jesyka Macedo Guedes[3], Marisa Jádna Silva Frederico[4], Andreia Ferreira de Castro Gomes[5], Jane Eire Silva Alencar de Menezes[2,*] and Hélcio Silva dos Santos[1,2,3,*]

[1]Department of Biological Chemistry, Regional University of Cariri, Crato, CE, Brazil; [2]State University of Ceara, Graduate Program in Natural Sciences, Fortaleza, CE, Brazil; [3]State University of Vale do Acaraú, Chemistry Course, Sobral, CE, Brazil; [4]Department of Pharmacology and Physiology, Center for Drug Research and Development, School of Medicine, Federal University of Ceará; [5]Center for Molecular and Environmental Biology, University of Minho, School of Sciences, Department of Biology, Braga, Portugal

Article Info:

Keywords:
Chalcones,
Anxiolytic effect,
Serotonergic receptors,
Zebrafish.

Timeline:
Received: May 16, 2025
Accepted: June 08, 2025
Published: July 04, 2025

Citation: da Silva PT, Ferreira MKA, de Albuquerque RL, Teixeira AMR, Marinho ES, Marinho MM, Guedes JM, Frederico MJS, Gomes AFC, de Menezes JSA, dos Santos HS. Chalcone derived from a natural product: An integrated approach of quantum chemical calculations, molecular docking, ADME and neuromodulation on serotonergic receptors in adult zebrafish. J Pharm Nutr Sci 2025; 15: 60-75.

DOI: https://doi.org/10.29169/1927-5951.2025.15.07

Abstract:
Anxiety disorders are conditions characterized by heightened responses to perceived threats, resulting in symptoms that negatively affect everyday life. This study investigates the anxiolytic effect of a natural chalcone isolated from *Croton anisodontus* Müll.Arg. focusing on its modulation of anxiolytic activity in modulating anxiolytic activity via GABAergic and serotonergic neurotransmission in an adult zebrafish model. The acute toxicity of the chalcone was assessed during a 96-hour period, and the anxiolytic behavior of fish treated with chalcone was evaluated using light/dark tests and open field tests (n=6 animals per group). Chalcone showed no signs of toxicity for up to 96 hours of analysis. The results demonstrated a significant anxiolytic effect of the synthesized chalcone, suggesting its therapeutic potential in treating anxiety. Furthermore, the findings indicate that this anxiolytic effect is mediated by serotonergic and non-GABAergic neurotransmitter systems. From molecular docking simulations, it was possible to estimate that the 5-HT3A receptor (5-HT3AR) pathway is the most likely target way for the chalcone to act. MPO-based ADME predictions indicate that the chalcone exhibits high cellular permeability suggest that chalcone has a high cellular permeability and can distribute better in biological tissues than in blood plasma, supporting its potential to act in the CNS by crossing the blood-brain barrier. These findings enhance the understanding of contribute to understanding of chalcone's mechanisms of action and provide a solid basis for future studies aimed at developing new therapeutic strategies to develop new therapeutic approaches for anxiety disorders.

*Corresponding Author
E-mail: helciodossantos@gmail.com; jane.menezes@uece.br

1. INTRODUCTION

Anxiety disorders represent a complex interaction of biological, psychological, temperamental and environmental factors [1]. Unlike fear, anxiety is a future-oriented affective state in which the individual prepares to deal with an uncertain but possible negative event in the absence of a specific triggering stimulus without a triggering stimulus [1].

The endogenous serotonergic, gamma-aminobutyric acid receptor A ($GABA_A$), and opioid systems are fundamental for regulating many physiological and behavioral functions. In anxiety, GABA receptors can be allosterically modulated receptors by drugs of the benzodiazepine class (BDZs), causing anxiolytic effects [2]. Within the seven main classes of 5-HT (serotonin) receptors, the $5\text{-}HT_{1A}$ subtype plays an important role in the regulation of mental disorders such as depression, anxiety or schizophrenia [3].

Although anxiety is highly prevalent, current treatments are largely palliative and frequently associated with undesirable side effects [4]. Benzodiazepines like clonazepam and diazepam, although effective, are linked to adverse effects with both short- and long-term use [5]. These drugs bind to $GABA_A$ receptors and enhance GABA's inhibitory action [6]. Selective serotonin reuptake inhibitors (SSRIs), while increasing serotonin levels, suffer from delayed onset and side effects such as sexual dysfunction [7].

In a previous study, the natural compound 2-hydroxy-3,4,6-trimethoxyacetophenone, isolated from *Croton anisodontus* (Figure **1**) , was found to be non-toxic and demonstrated anxiolytic effects in adult zebrafish via the 5-HT1 and 5-HT2C pathways (Silva *et al*., 2021).

The first natural chalcones were isolated in 1910, and they occur mainly as pigments responsible for the coloration of flower petals, leaves, bark, fruits and roots of various plants [8]. The subclass of flavonoid chalcones is also known as benzyl acetophenones, which are α,β unsaturated ketones containing two aromatic rings (A and B) with different substituent arrangements. In chalcones, the two aromatic rings are connected by a three-carbon aliphatic chain [9]. Natural and synthetic chalcone derivatives have shown promising biological activity as antioxidant, anti-inflammatory, anticancer and anti-infective agents [10,11]. This class of compounds has shown therapeutic potential mainly due to interactions with $GABA_A$ receptors [12] and serotonergic system [13]. In the zebrafish model, chalcones have shown both anxiolytic and anticonvulsant properties [5].

Zebrafish (Danio rerio) has emerged as a powerful model for neurobehavioral research due to its genetic similarity to humans (60–80%) and conserved neurotransmitter systems [14]. Therefore, although the serotonergic system presents some genetic differences between zebrafish and mammals, the effects of drugs that modulate 5-HT metabolism are conserved across species [15,16]. The zebrafish model has reported a correlation between 5-HT release in the brain and specific behaviors (e.g., fear, anxiety, and aggression) [17]. Furthermore, the molecular and neurobiological modulatory processes associated with anxiety disorders, particularly those involving aminobutyric acid-γ-aminobutyric acid (GABAergic) neurotransmission, have already been well described [18].

Despite several studies exploring chalcones' biological activities, few have evaluated their synthetic analogs in validated behavioral models, such as zebrafish, with mechanistic insights into serotonergic and GABAergic pathways. Thus, this study aimed to investigate the anxiolytic effect of the synthetic chalcone (2*E*)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)-3-phenylprop-2-en-1-one derived from the natural compound 2 -hydroxy-3,4,6-trimethoxyacetophenone isolated from *Croton anisodontus* Müll.Arg. on the neuromodulation of anxiolytic-related behavior via GABAergic and serotonergic pathways in an adult zebrafish model.

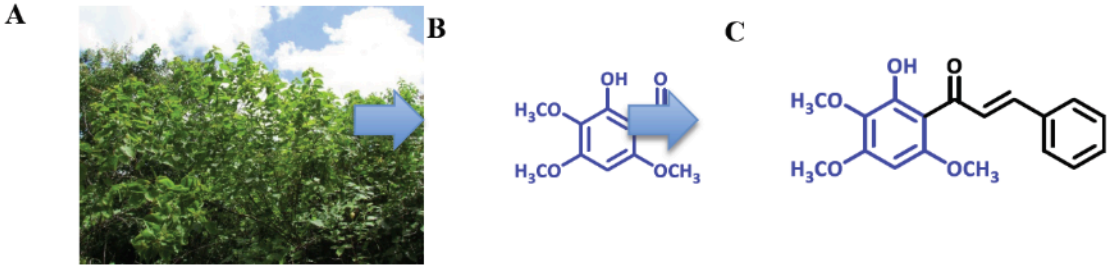
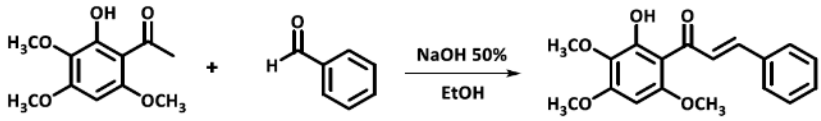


Figure 1: *Croton anisodontus* (**A**), 2-hydroxy-3,4,6-trimethoxyacetophenone (**B**) and (**C**) Chalcone.



Scheme 1: Procedure for the synthesis of chalcone.

2. MATERIALS AND METHODS

2.1. Drugs and Reagents

Drugs/reagents used were Granisetron hydrochloride (Corepharma, Middlesex, NJ, USA), pizotifene maleate (Central Manipulation Pharmacy, São Paulo, SP, Brazil), fluoxetine (Eli Lilly, Indianapolis, IN, USA), cyproheptadine (Evidence Pharmaceutical Solutions, Fortaleza, CE, Brazil), DZP, and PTZ (Sigma-Aldrich, Missouri, USA). Flumazenil was purchased from Roche Pharmaceutical (Welwyn Garden City, UK).

2.2. Synthesis and Chemical Characterization of Chalcone

A description of the procedure for the synthesis of chalcone (2*E*)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)-3-phenylprop-2-en-1-one is presented. The chalcone was synthesized using a Claisen–Schmidt condensation reaction under basic conditions. An ethanol solution of 2-hydroxy-3,4,6-trimethoxyacetophenone, isolated from *Croton anisodontus* (2 mmol), was added to a solution of benzaldehyde (2 mmol), followed by the addition of 10 drops of 50% w/v aqueous NaOH and stirred for 48 h at room temperature 32°C (Scheme **1**). The structure of the chalcone was confirmed to be consistent with previous reports in the literature [19].

2.3. Quantum Chemistry Calculation

The hydrid functional B3LYP and the Pople triple-ζ basis set 6-311++G (d,p) were chosen to perform all quantum chemical calculations. The target chalcone molecule was simulated in DMSO and water using the conductor-like polarizable continuum model (CPCM) implicit solvation model available in ORCA 5.0.4 [https://orcaforum.kofo.mpg.de/app.php/portal]. First, the three-dimensional structure was optimized and then the frontier molecular orbitals Highest Occupied Molecular Orbitals- the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO)- were calculated. From the energy values of these orbitals, the global chemical reactivity descriptors were calculated, including: the energy gap (ΔE_{gap}, *Eq.1*), ionization potential (*I, Eq. 2),* electronic affinity (*A, Eq. 3)*, electronegativity (χ, Eq. 4), global hardness (η, Eq. 5), global softness (*S, Eq. 6), global electrophilic index (ω, Eq. 7), global nucleophilic index (ϵ, Eq. 8).* In addition, condensed Fukui functions were calculated. f^{+}_{k} *(Eq. 9)* and f^{-}_{k} (Eq. 10), and the dual (Δf, Eq. 11) and multiphilic (Δω, Eq. 12) descriptors. Finally, the electrostatic potential map (EPM) was plotted.

$$\Delta E_{gap} = E_{LUMO} - E_{HOMO} \quad \text{(Eq.1)}$$

$$I = -E_{HOMO} \quad \text{(Eq.2)}$$

$$A = -E_{LUMO} \quad \text{(Eq.3)}$$

$$\chi = \frac{I + A}{2} \quad \text{(Eq.4)}$$

$$\eta = \frac{I - A}{2} \quad \text{(Eq.5)}$$

$$S = \frac{1}{\eta} \quad \text{(Eq.6)}$$

$$\omega = \frac{\chi^2}{2\eta} \quad \text{(Eq.7)}$$

$$\epsilon = \frac{1}{\omega} \quad \text{(Eq.8)}$$

$$f_k^+ = q_N^k - q_{N+1}^k \quad \text{(Eq.9)}$$

$$f_k^- = q_{N-1}^k - q_N^k \quad \text{(Eq.10)}$$

$$\Delta f_k = f_k^+ - f_k^- \quad \text{(Eq.11)}$$

$$\Delta\omega = \omega \Delta f_k \quad \text{(Eq. 12)}$$

2.4. Zebrafish

Zebrafish (*Danio rerio*) (age 90 to 120 days; 0.4 ± 0.1 g, 3.5 ± 0.5 cm) of both sexes were purchased from a local supplier in Fortaleza, CE (Brazil). The animals were kept in a 10-L glass aquarium (30 × 15 × 20 cm) at a temperature of 25 ± 2°C, under a 10–14 h light/dark cycle, with dechlorinated water (pH 7.0, ProtecPlus®) using an air pump with submerged filters. Fish were fed Spirulina® 24 h before experiments. Before each treatment, animals were anesthetized in ice- cold water, and following experiments, they were euthanized by immersion in iced water (0-3°C) for 1 min or until loss of opercular movements. This study was approved by the Ethics Committee on the Use of

Animals at the State University of Ceará (CEUA-UECE; no. 04983945/2021), in accordance with ethical principles involving animal experiments.

2.5. Acute Toxicity Assay

A 96-hour acute toxicity assessment was performed using adult zebrafish according to the guidelines of the Organization for Economic Cooperation and Development (OECD) [20]. Animals (n = 6/group) were treated orally (20 µL) with chalcone 1.0, 3.0, and 10 mg/kg or with vehicle (Control; 3% DMSO). After treatment, the animals were left to rest to analyze mortality rates. From 24 h to 96 hours post- treatment, the number of dead fish in each group was recorded, and the lethal dose required to kill 50% of the animals (LD_{50}) was determined using trimmed Spearman-Karber method with 95% confidence intervals [21].

2.6. Open-Field Test

An open field test was performed to assess changes in the animals' motor coordination [22]. Initially, the fish (n = 6/group) were treated orally (*p.o.*) with chalcone at doses of 1.0, 3.0 and 10 mg/kg, DZP (10 mg/kg), or vehicle (Control; 3% DMSO). A group of untreated animals was included (naïve control group). One hour after treatment, animals were placed in glass Petri dishes (10 × 15 cm) filled with the same aquarium water, divided into four quadrants. Locomotor activity was assessed by counting the number of crossing lines (CL) by the animals [23].

2.7. Anxiolytic Activity

Animal anxiety behavior was observed using the light/dark preference test. Similar to rodents, zebrafish naturally avoid lighted areas [23]. The experiment was carried out in a glass aquarium (30 cm × 15 cm × 20 cm) divided into light and dark zones. The aquarium was filled with chlorine-free tap water, which simulated a new shallow environment different from the conventional aquarium and capable of inducing anxiety behaviors. In this test, animals (n = 6/group), received 20 µL of chalcone orally at doses of 1.0, 3.0, and 10 mg/kg. The negative and positive control groups were administered 3% DMSO and 10 mg/kg diazepam (DZP), respectively. An untreated group (naive) was also included. One hour post-treatment, each animals was individually placed in the light zone, and the anxiolytic effect was evaluated based on the total time spent in the light zone over a 5 minute observation period [24].

2.8. Evaluation of GABAergic and Serotoninergic Neuromodulation

The mechanisms of action involved in the anxiolytic-like effect of chalcone were investigad through pretreatment with flumazenil (a neutralizing modulator of positive allosteric modulators of GABA receptors) and serotonergic antagonists: cyproheptadine (5-HTR_{2A} antagonist), pizotifen (5-HTR_1 and 5-$HTR_{2A/2C}$ antagonist), and granisetron (5-$HTR_{3A/3B}$ antagonist), prior to the light/dark test [25]. Zebrafish (n = 6/group) were pretreated orally with flumazenil (4 mg/kg; 20 µL; p.o.), cyproheptadine (32 mg/kg; 20 µL; p.o.), pizotifen (32 mg/kg; 20 µL; p.o.), or granisetron (20 mg/kg; 20 µL; p.o.). After 15 min, the most effective dose of chalcone (10 mg/kg; 20 µL; p.o.) identified in the pilot study was administered. A 3% DMSO solution (20 µL; p.o.) was used as the vehicle control. DZP (10 mg/kg; 20 µL; p.o.) and fluoxetine (0.05 mg/kg; i.p.) were used as positive controls acting on $GABA_A$ and serotonergic receptors, respectively. After 1 h of treatment, animals were subjected to the light/dark test described in the previous section.

2.9. Statistical Analysis

Results are expressed as mean values ± standard error of the mean for each group of six animals. After confirming the normal distribution and homogeneity of the data, differences between the groups were subjected to analysis of variance (one-way ANOVA) and two-way ANOVA in experiments with antagonists, followed by Tukey's test. All analyses were performed using GraphPad Prism v.8.0 software. The level of statistical significance was set at 5% ($P < 0.05$).

2.10. Molecular Docking Simulations

To carry out molecular docking simulations, the protein structures of serotonergic receptors 5-HT2AR (PDB ID 6A93), 5-HT2CR (PDB ID 6BQH) and 5-HT3AR (PDB ID 6NP0) were selected from the RCSB Protein Data Bank repository (https://www.rcsb.org/). These structures are classified as membrane proteins and were expressed in the *Spodoptera frugiperda expression system*, with resolutions of 3.00 Å, 2.70 Å and 2.92 Å, respectively. Each structure includes a co-crystallized ligand, which were used as controls in molecular docking simulations. Specifically, risperidone (5-HT2AR antagonist), ritanserin (a 5-HT2CR agonist), and the granisetron (5-HT3AR antagonist) were employed. These ligands, along with non-amino acid residues, were removed during the protein preparation

stage using the AutoDockTools® software. With the polar hydrogens added and the Gasteiger charge computed, the grid-box was adjusted to cover the entire conformational space of the receptors, adjusted to dimensions x = 102 Å, y = 44 Å, z = 52 Å and coordinates x = 42,563, y = 0.932, z = 63,224 for the 5-HT2AR receptor, dimensions x = 58 Å, y = 48 Å, z = 88 Å and coordinates x = 46.0, y = 34,264, z = 34,296 for the 5-HT2CR receptor and dimensions x = 70 Å, y = 66 Å, z = 126 Å and coordinates x = 159.643, y = 160.205, z = 164.84 for the 5-HT3AR receptor. Then, the AutoDockVina® code was adjusted to perform a series of 50 independent simulations, for each ligand-receptor complex, of 20 poses each, where the selection criterion for the best docking pose includes the energy affinity lower than -6.0 kcal/mol, calculated by Gibbs free energy (ΔG), and statistical adjustment of Root Mean Square Deviation (RMSD) lower than 2.0 Å.

2.11. MPO-Based ADME Analysis

Predictions of absorption, distribution, metabolism and excretion (ADME) properties were based on a quantitative estimate of druglikeness (D) from the multiparameter optimization (MPO) algorithm using the program MarvinSketch® version 24.1.0, Chemaxon© (https:/ /chemaxon.com/marvin), as shown in Eq. 1:

$$D = \sum_{i=1}^{N} w_k \, T_k(x_k^0) \quad (1)$$

where the weighting factor (w) assigned to each physicochemical properties k within the desirability functions (T(x)), which include the thresholds of ClogP ≤ 3, ClogD at pH 7.4 ≤ 2, molecular weight (MW) ≤ 360 g/mol, topological polar surface area (TPSA) between 40-90 Å², H-bond donors ≤ 1 and pKa ≤ 8.0, result in a score that varies from 0 to 6 (N = 6) depending on ADME viability (Wager *et al*., 2010). The results were related to the predicted descriptors of apparent permeability (Papp) in Madin-Darby Canine Kidney (MDCK) and colorectal adenocarcinoma (Caco-2) cellular models, P-glycoprotein substrate (P-gp), human intestinal absorption (HIA) and intrinsic debugging (CLint,u) of ADMETlab 2.0 (https://admetmesh.scbdd.com/) and pkCSM (https://biosig.lab.uq.edu.au/pkcsm/) servers. The metabolism site prediction was made to verify the metabolic stability spectrum estimated by MPO analyses. The servers XenoSite (https://xenosite.org/) and SOMP (http://www.way2drug.com/somp/) were configured to perform a prediction that relates sensitivity and specificity of chalcone molecular fragments in relation to substrates of known cytochrome P450 (CYP450) isoforms. The results were related to the metabolism and toxicity descriptors of ADME prediction.

3. RESULTS AND DISCUSSION

3.1. Spectroscopic Analysis

In the ^{1}H NMR spectrum (Figure **S1**) one can observe a signal at at δ_H 3.83, 3.90 and 4.01 referring to the methoxyl hydrogens. In δ_H 7.79 (J= 15.6 Hz) and 7.87 (J= 15.6 Hz) they are attributed to two doublets referring to the unsaturated α,β hydrogens, whose coupling constant *J* = 15.6 Hz confirms the stereochemistry *E* of the double bond. The singlet observed at δ_H 6.01 refers to the hydrogen bonded to the 3' carbon of ring A. The signals at δ_H 7.40 to 7.60 refer to the aromatic hydrogens of ring B. In the ^{13}C NMR spectrum (Figure **S2**) there is the signal referring to the unsaturated α,β carbonyl at δ_C 193.47. The ketone absorbs at δ_C 203.8, however, the presence of α,β unsaturation causes a shift to high field and the probable cause is charge delocalization by the benzene ring or by the double bond that makes the carbonyl carbon less electron deficient. The olefinic carbons α and β are observed at δ_C 142.83 and 128.59, respectively. The signals at δ_C 56.20, 56.25 and 60.96 refer to the methoxyl carbons. At δ_C 159.55 (C-4´), 158.8 (C-6´), 158.7 (C-2´), 130.4 (C-3´) and 107.1 (C-1´) , 87.3 (C-5´) we have the signals referring to the carbons present in ring A. While carbons 1 to 6 present in ring B can be observed in δ_C 135.7 (C-1), 130.3 (C-4), 129.1 (C-3/5), 128.6 (C-2/6) (Table **S1**).

The IR spectrum exhibit characteristic bands associated with O-H stretch (hydroxyl), a broad and intense band in the region of 3200-3600 cm^{-1}, due to the phenolic hydroxyl group. C-H stretch bands in the region of 3000-3100 cm^{-1}. A strong and intense band in the region of 1660-1700 cm^{-1}, corresponding to the carbonyl group of the chalcone. Bands in the region of 1600-1650 cm^{-1} (C=C of the α,β-unsaturated double bond) and 1450-1600 cm^{-1} (aromatic C=C). Bands in the region of 700-900 cm^{-1}, which may indicate the substitution pattern on the aromatic ring. One or more bands in the region of 1000-1300 cm^{-1}, due to the methoxy groups ($-OCH_3$) (Figure **S3**).

3.2. Quantum Chemistry Calculation

3.2.1. Geometric Optimization

Chalcones are organic compounds characterized by the 1,3-diphenylpropenone skeleton, which contains

two aromatic rings linked by an unsaturated carbon chain composed of three atoms, with a double bond (C=C) and a carbonyl (C=O), characterizing the α, β-unsaturated carbonyl system, which gives the molecule both conjugated properties and interesting chemical reactivity. The titled chalcone has the phenyl ring directly linked to the carbonyl (ring A) substituted at the C6' position by the hydroxyl group (OH) and tri-substituted by the methoxy group (OCH3) at the C2', C4' and C5' positions, while the phenyl ring linked to Cβ (ring B) has no substitutions. The three-dimensional structure optimized in DMSO and water using the B3LYP/6-311++G(d,p) level of theory is shown in Figure **2**. The chalcone entitled presents a non-planar conformation, where due to the absence of substituent groups on the B ring, the olefinic portion remained slightly in the same molecular plane as the B ring, highlighted by the angle formed by the Cα-Cβ-C1-C6 atoms with a value of 1.788° in DMSO and 1.812° in water. On the other hand, due to the presence of the methoxy group in the C2' position, ring A is not in the same molecular plane as the olefinic portion and ring B, highlighted by the angle formed by the C2'-C1-C7-Cα atoms with a value of 15.239° in DMSO and 15.238° in water.

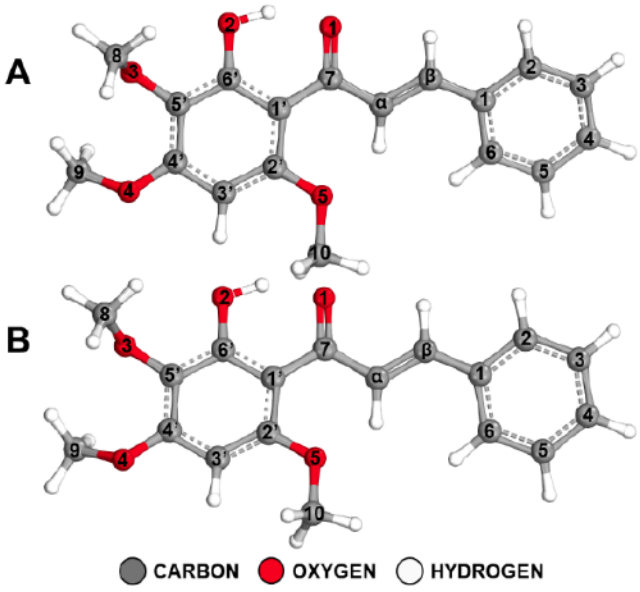


Figure 2: Optimized structures in DMSO (**A**) and water (**B**) of the titled chalcone, using the DFT method with the B3LYP/6-311++G(d,p) level.

3.2.2. Study of Electronic Properties

The analysis of the electronic properties of the synthesized chalcone revealed important aspects about its reactivity and interaction with the environment. The distribution of electron density, especially in regions containing oxygen atoms, may indicate potential reaction sites with nucleophilic or electrophilic agents, which is essential to predict its behavior in biological or chemical systems. The simulations showed that the frontier molecular orbitals – HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) – presented very similar energies in the DMSO and water solvents, indicating that the polarity of the solvent does not significantly alter the electronic distribution of the molecule. This suggests that chalcone has electronic stability in different polar media, which may favor its solubility and performance in different biological environments. The graphical representations (Figure **3**) indicate that the HOMO orbitals are more concentrated in the tetrasubstituted aromatic ring, mainly in π bonds, while the LUMO orbitals are distributed throughout the aromatic and aliphatic structure. This orbital distribution is compatible with the presence of regions susceptible to nucleophilic and electrophilic attacks, especially in the α,β-unsaturated system.

The data in Table **S2** show the main descriptors of the chemical reactivity of chalcone, obtained by calculations of the electronic structure. The values of the frontier molecular orbitals (HOMO and LUMO) were quite similar in the two solvents analyzed (DMSO and water), indicating that the electronic reactivity of the molecule is not significantly affected by the medium. The HOMO energy reflects the ability to donate electrons, and more negative values indicate a lower donor tendency. In DMSO, it was –6.12 eV and, in water, –6.12 eV. The LUMO, which represents the tendency to accept electrons, also showed minimal variations between the solvents: –2.51 eV (water) and –2.51 eV (DMSO). The HOMO-LUMO energy difference, associated with kinetic stability and chemical reactivity, was practically the same: approximately 3.61 eV in both solvents, suggesting similar stability. The electronegativity of chalcone was similar in both media (~4.31 eV), indicating that its ability to attract electrons is also stable. The global electrophilicity index (5.15 eV) confirms the electrophilic character of the molecule, favored by the presence of a conjugated carbonyl group, typical in α,β-unsaturated systems. On the other hand, the low nucleophilicity index indicates that chalcone has a lower propensity to act as a nucleophile.

To better understand the most reactive points of the studied chalcone, the condensed Fukui functions were calculated. These functions help to identify which atoms or chemical groups of the molecule are most

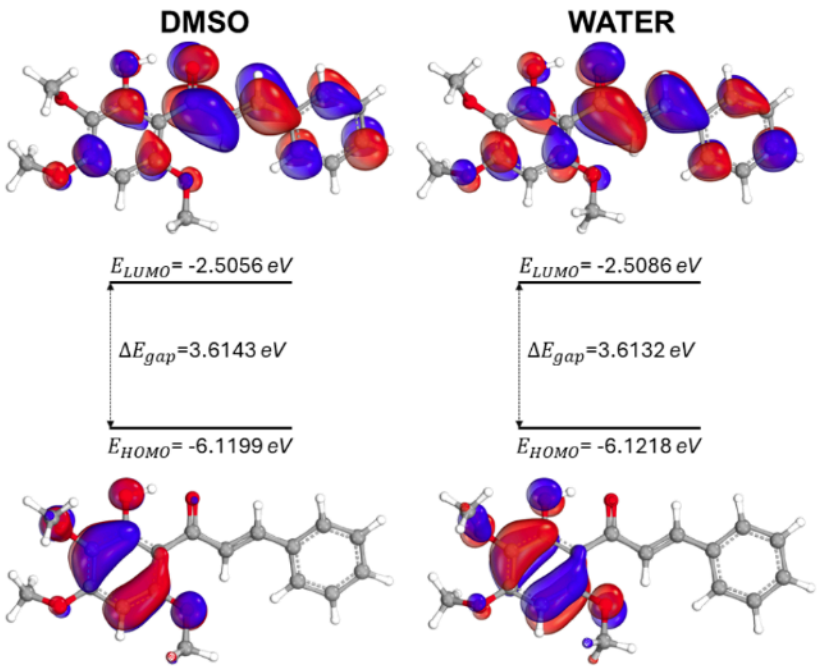


Figure 3: Isosurfaces of HOMO and LUMO frontier molecular orbitals in the implicit DMSO (**A**) and water (**B**) environments.

likely to participate in chemical reactions, important information for the development of new drugs, as they indicate possible sites of interaction with biological targets. The function f_k^+ shows which atoms are most vulnerable to attacks from electron-rich molecules (nucleophilic attacks), while the function function f_k^- investigates the susceptibility of the site to electrophilic attacks. To simultaneously investigate the susceptibility of the site, the dual (Δf_k) and multiphilic ($\Delta\omega$) descriptors are calculated. $\Delta\omega$ indicate that the atom is more susceptible to nucleophilic attacks, that is, it has low electron density. Negative values indicate greater susceptibility to electrophilic attacks, due to the higher electron density. On the other hand, negative values of $\Delta\omega$ indicate that the atoms are more susceptible to electrophilic attacks, that is, these atoms have a higher electron density and, therefore, can attract electrophilic species (poor in electrons). In Figure **4**, the values of $\Delta\omega$ $\Delta\omega$ were calculated in two solvents: DMSO (green) and water (red). In both, the pattern was similar. Ring A (composed of carbons C1' to C6'), which has the OCH_3 and OH groups, is more vulnerable to electrophilic attacks. This happens because these groups donate electrons to the ring, increasing its electron density. On the other hand, the α,β-unsaturated system and ring B (carbons C1 to C6) are more prone to nucleophilic attacks.

In the area of rational drug design, electronic parameters are important to understand how a drug binds to its receptor. In this context, the molecular electrostatic potential (MEP) is essential to analyze the contribution of electrical charges to the biological activity of drug candidates. The MEP allows the identification of regions of the molecule with a higher concentration of partial negative charge (δ^-) and regions with a higher concentration of partial positive charge (δ^+). Figure **5** shows the MEP of chalcone calculated in DMSO and in water. The warm colors (red) indicate areas with a higher concentration of negative charge (δ^-), while the cold colors (blue) indicate regions with a higher concentration of positive charge (δ^+). The highest concentration of δ^- is in the oxygen atoms and in the two aromatic rings. Furthermore, the solvent used (DMSO or water) had no significant impact on the distribution of these charges in the molecule.

3.3. Acute Toxicity

This study contributes to the understanding of the anxiolytic potential of a synthetic chalcone derived from the natural compound 2-hydroxy-3,4,6-trimethoxyacetophenone, isolated from *Croton anisodontus* Müll.Arg. Specifically, it aimed to investigate the modulation of anxiolytic activity through GABAergic and serotonergic neurotransmission in an adult zebrafish model. The chalcone was not toxic to adult zebrafish for up to 96 hours (LD_{50} > 10 mg/kg), as deaths or apparent anatomical alterations were observed during this period. These findings indicate that the chalcone tested in this study exhibited no signs of acute toxicity at the

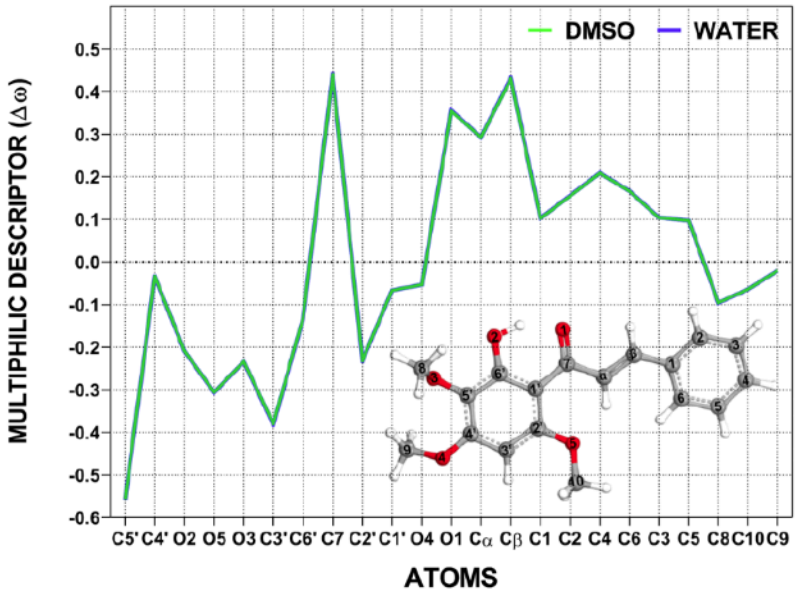


Figure 4: Multiphilic descriptor values for each atomic site of the chalcone titled in DMSO (green color) and water (red color) using the B3LYP/6-311++G(d,p) level of theory.

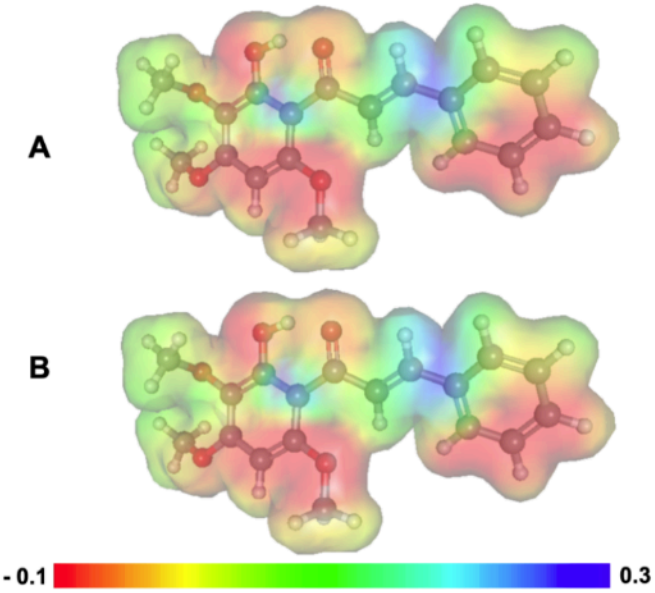


Figure 5: Molecular Electrostatic potential (MEP) plotted for the titled chalcone, calculated in DMSO (**A**) and water (**B**). Warm colors (red colors) indicate regions with higher concentration of δ^- and cold colors (blue colors) indicate higher concentration of δ^+.

evaluated doses and conditions. These results corroborate previous studies carried out by [5,12,26] who also analyzed the acute toxicity of molecules belonging to the same class. In the study by, the acute toxicity of several chalcones in rats was evaluated.

3.4. Assessment of Locomotor Activity

Zebrafish as an experimental model has contributed to studying various pathologies, including neurodevelopmental, psychotic, and neurodegenerative disorders [27]. It has improved our understanding of common and evolutionarily conserved mechanisms underlying Central Nervous System (CNS) disorders, as well as supported behavioral research and screening of new CNS active drugs [28]. These studies are particularly important because they establish functional links between neurochemistry and behavior in vertebrate species [29]. Chalcone significantly altered zebrafish locomotion [**** $p < 0.0001$, #### $p < <0.0001$ vs vehicle (1; 3 or 10 mg/kg)], with treated animals showing a

market reduction in locomotor activity compared to the control groups (naive and vehicle) (Figure **6**). These results were not significantly different from those observed in the DZP positive control.

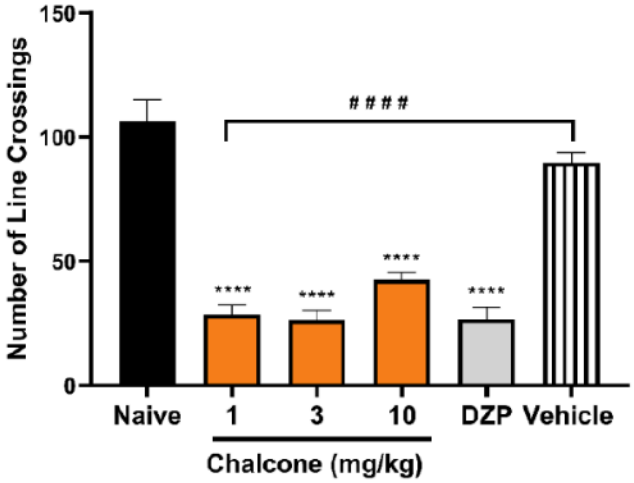


Figure 6: Effect of chalcone on the locomotor behavior of adult zebrafish in the Open Field Test (0–5 min). Values represent the mean ± standard error of the mean for 6 animals/group; ANOVA followed by *Tukey's* test(**** p <0.0001 vsNaive;# # # # p vs Veículo).

Following this trend, it was evaluated using the open field teste, and all tested doses led to changes in locomotor activity. This behavioral assessment parameter is characteristic of drugs that act on the zebrafish CNS [22,30]. However, the change in locomotion may also reflect sedation effects resulting from the route of drug administration, which can be influenced by onset time, intensity, and duration of action [31,32]. Locomotor behavior was used as an initial screening parameter because it is one of the most basic fundamental endpoints in nearly all zebrafish-based experiments, the parameters considered in almost all experiments related to zebrafish, typically assessed by swimming activity [33]. From this perspective, many more variables can be derived from swimming to determine various behaviors in a study [34].

3.5. Anxiolytic Evaluation of Chalcone

The light-dark test assessed anxiety-related behavior and the effect of oral chalcone administration. It is important to note that in this test, the animals naturally exhibit an aversion to the light zone of the aquarium, preferring to remain in the dark zone. This preference is considered an adaptive response that provides camouflage from potential predators [35,36]. Chalcone treatments (1, 3 or 10 mg/kg) significantly increased the time spent by the animals in the light zone of the aquarium (**p <0.01, ****p <0.0001; $^{###}$p <0.001, $^{####}$p <0.0001 vs vehicle) in the light/dark test (Figure **7**). It is worth highlighting that the 10mg/kg dose demonstrated a superior anxiolytic effect compared to diazepam (DZP; 10 mg/kg), used as the positive control.

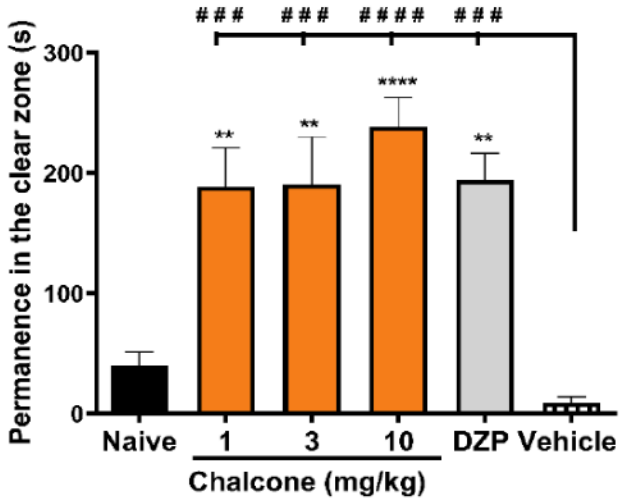


Figure 7: Effect of chalcones on zebrafish anxiety in the light/dark test (0–5 min). Values represent the mean ± standard error of the mean for 6 animals/group; ANOVA followed by *Tukey's* test(** p <0.01; **** p <0.0001 vsNaive; # # #p <0.001; # # # # p <0.0001vsVehicle).

It was observed that, after oral treatments with chalcone, all tested doses increased the time spent by animals in the clear zone of the aquarium, similar to the group that received DZP at 10 mg/Kg. Other studies have reported that chalcones tend to exhibit anxiolytic effects, and one study was performed regarding the synthesis of various scaffolds of benzodiazepines (anxiolytic class of drugs) using biologically active chalcones as precursors [37].

3.4. Evaluation of GABAergic and Serotonin Neuromodulation

The anxiolytic mechanism of action of chalcones may involve both the GABAergic [38] and serotoninergic [39] systems. Animals treated with the highest dose of chalcone (10 mg/kg) did not have their anxiolytic behavior blocked by flumazenil, as they remained in the clear zone of the aquarium for most of the observation period, thereby maintaining their anxiolytic behavior (Figure **8A**). The involvement of the $GABA_A$ was assessed through pre-treatment with flumazenil. At the highest dose (10 mg/kg), of chalcone- induced anxiolytic behavior, was not inhibited by flumazenil (Figure **10A**), contrast to the DZP-treated group, which showed a blocked effect (##p < 0.01 vs. Fmz + DZP). Considering the hypothesis that serotonergic

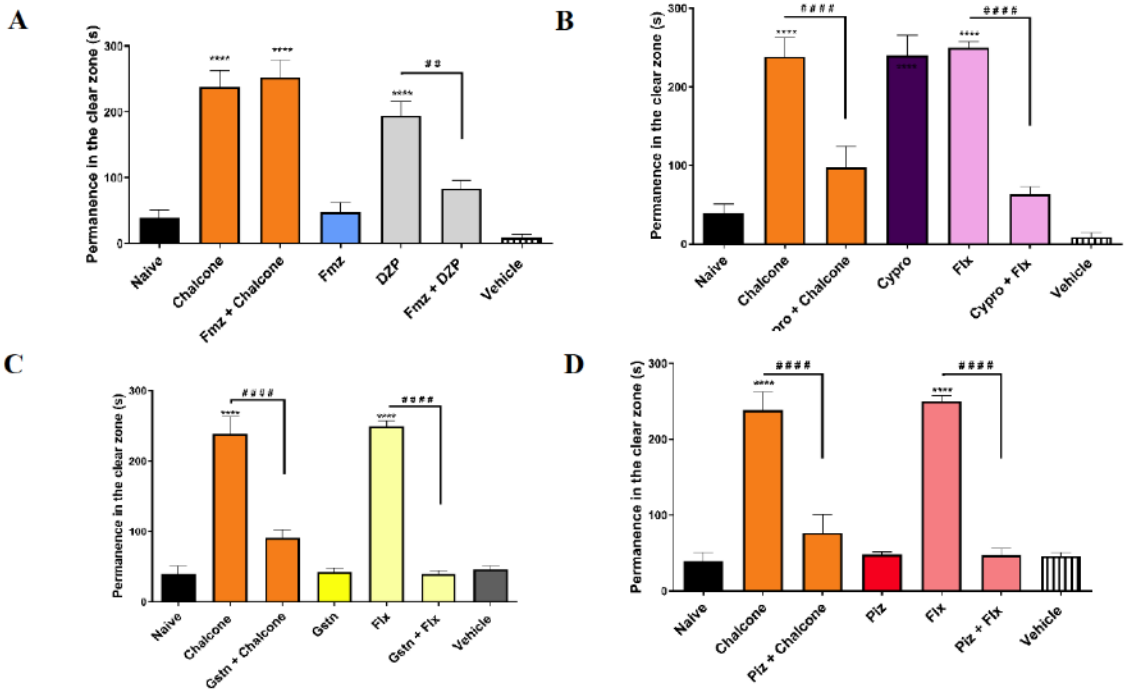


Figure 8: Effect of flumazenil (**A**), cyproheptadine (**B**), granisetron (**C**) and pizotifen (**D**) on the anxiolytic effect of chalcone in the light/dark test. Values represent the mean ± standard error of the mean for 6 animals/group; ANOVA followed by *Tukey's* test(**** p <0.0001vsNaive or DZP, # # # #p <0.0001vs chalcone +granisetron or cyproheptadine or pizotifen or fluoxetine, # #p <0.01 vs DZP).

neurotransmission is involved in the anxiolytic effects of chalcone, additional experiments were performed to investigate this pathway. Animals were pretreated with specific (serotoninergic receptor antagonists), including cyproheptadine (5-HTR2A antagonist), pizotifen (5-HTR1 and 5-HTR2A/2C antagonist) and granisetron (5-HTR3 antagonist). In the serotonergic system, cyproheptadine, granisetron and pizotifen (# # # # p<0.0001 vs chalcone) reverse the anxiolytic effect of chalcone (Figures **8B**, **C** and **D**).

Serotonin receptor antagonists were chosen based on their selectivity and pharmacological profiles, aiming to specifically block serotonergic receptor subtypes relevant to the anxiolytic action of chalcone. Cyproheptadine primarily antagonizes the 5-HTR2A receptor, which plays a crucial role in regulation anxiety. Pizotifen blocks both 5-HTR1 and 5-HTR2A/2C receptors, theteby enhancing inhibitory modulation of the serotonergic system. Granisetron is a selective 5-HTR3 receptor antagonist, which may also participate in anxiety modulation [40]. The role of serotonin (5-HT) in anxiety is well established [17]. In zebrafish, anxiety-like behavior in the light-dark test is positively correlated with extracellular 5-HT levels [23]. The search for new anxiolytic drugs targeting 5-HT receptors stems from need for treatments with greater efficacy and fewer side effects. For example, buspirone – a partial 5-HT1A partial agonist - was the first anxiolytic compound developed as an alternative to BZDs and SSRIs. The 5-HT1A and 5-HT2A receptors have previously been described as pharmacological targets for anxiety, as interruption of 5-HT1A and 5-HT2A receptor signaling induces anxiolytic-like behavior in animal models [3,41].

3.5. Molecular Docking Simulations

3.5.1. Binding Affinity Analysis

At the end of the cycle of independent molecular docking simulations, it was possible to observe that the chalcone performed an average statistical deviation evaluated by RMSD of around 1.1-1.4 Å when interacting with serotonergic channels of the 5-HT2AR type (PDB 6A93), 5 -HT2CR (PDB 6BQH) and 5-HT3AR (PDB 6NP0) types. These values indicate that chalcone formed ligand-receptor complexes within the ideal RMSD (< 2.0 Å) suggesting good geometric compatibility with the amino acid residues at the binding sites (Table **S3**). Moreover, chalcone showed notable binding affinity for all three serotonergic receptors (Figure **S4**), with free affinity energy values of -8,452, -8,129 and -7,801 kcal/mol (5-HT2AR, 5-HT2CR and 5-HT3AR respectively). These values fall within the ideal range for favorable ligand–receptor complex formation (ideal range < -6.0 kcal/mol). To

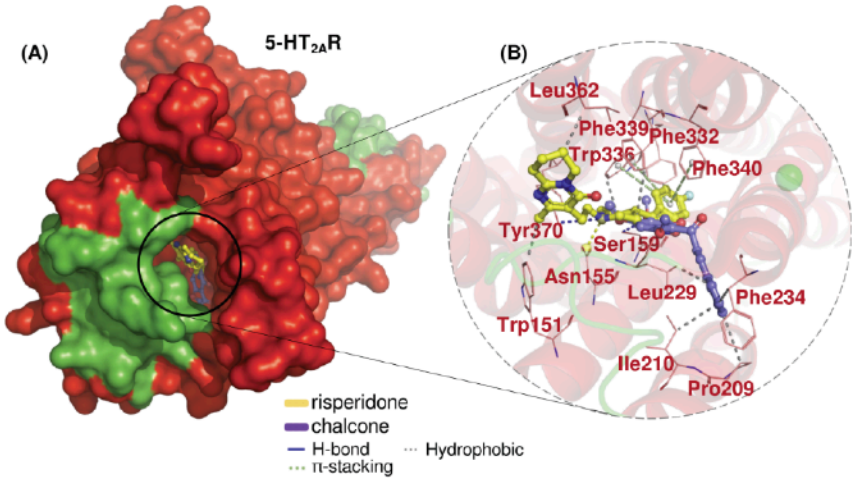


Figure 9: Three-dimensional visualization of (**A**) docking pose of the chalcone in relation to the risperidone antagonist in the hydrophobic cavity of the 5-HT2AR and (**B**) ligand-receptor interactions of the chalcone in relation to the residues of the risperidone antagonist binding site.

validate docking protocol, control simulations were performed using the co-crystallized ligands of the respective receptors. These ligands demonstrated higher binding affinities, with energy values of -10.91 kcal/mol for the 5-HT2AR agonist risperidone, -11.98 kcal/mol for the reversible 5-HT2CR agonist ritanserin and -10.77 kcal/mol for the 5-HT3AR antagonist granisetron (Figure **S4**). These reference values reveal comparative benchmarks for evaluating the binding affinities of the tested chalcone toward serotonergic receptors.

3.5.2. Interactions between Chalcone and 5-HT Receptors

At the end of the simulations, it was observed that the chalcone achieved its best binding pose within the hydrophobic the cavity of the 5-HT2AR, at the same where the antagonist risperidone binds (Figure **9**). The B ring of chalcone contributed to hydrophobic interactions with the aliphatic side chain of the Pro209, Ile210 and Leu229 residues an with the aromatic side chain of the Phe234 residue, exhibiting a binding pattern somewhat similar to that of risperidone. Risperidone, however, shows a more complex interaction profile. Its fluoro-substituted aromatic ring strongly contributes to the formation of π-Stacking interactions with the aromatic side chains of Trp336 and Phe340 residues, and participates in hydrophobic interactions with the aromatic residues Trp151, Phe332, Trp336 and Phe339, largely supported by its aliphatic moiety (Figure **9B**). Notably, the para-substituted methoxy group of chalcone formed a hydrogen bond with the carbonyl oxygen of Ser159], with a calculated distance of [2.81 Å, indicating a moderately strong interaction (Table **S3**).

On the other hand, it was possible to observe that the A ring of the chalcone presented a π-Stacking interaction in common with the fluoro-substituted ring of the ritanserin agonist, within the hydrophobic cavity of 5-HT2CR (Figure **10**), associated with the aromatic ring. of the side chain of the Trp324 residue (Figure **10b**) that chalcone can act as a 5-HT2CR agonist.

Compared to the 5-HT3AR model, the chalcone complexed with the hydrophobic binding cavity of the antagonist granisetron (Figure **11A**), where the overlap between the docking poses reveals the possible mechanism of action of the chalcone. It was observed that the B ring of chalcone and heterocyclic ring of granisetron shared several common interactions, including hydrophobic interactions with the aromatic side chains of Phe199 and Tyr207 (Figure **11B**). The ligand-receptor distances were approximately 3.8 Å for chalcone and 3.5 Å for for granisetron (Table **S3**), indicating that chalcone may act as a 5-HT3AR antagonist. Additionally, the methoxylated ring of chalcone formed ydrogen bond with the amine group of the Arg65 side chain, at a distance of 3.23 Å, whereas granisetron interacted with this residue through hydrophobic contact with its alkyl chain. This suggests that chalcone may establish a more specific interaction with **Arg65** than the co-crystallized antagonist.

3.6. MPO-based ADME Analysis

3.6.1. Defining the CNS Drug Space

To enable the selection of new drug candidates active in the CNS with an optimized alignment between ADME parameters a set of experimental data involving both approved drugs and candidates were analyzed.

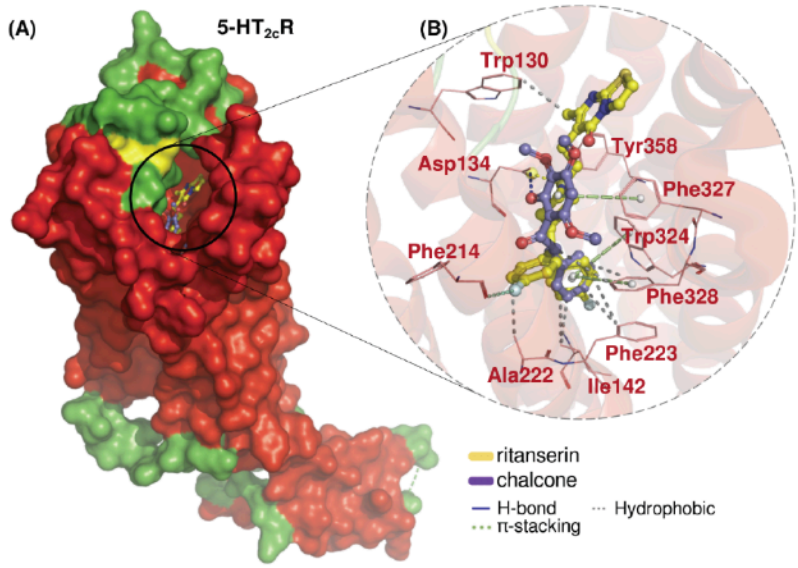


Figure 10: Three-dimensional visualization of (**A**) docking pose of the chalcone in relation to the ritanserin agonist in the hydrophobic cavity of the 5-HT2CR and (**B**) types of ligand-receptor interactions of the chalcone in relation to the amino acid residues of the ritanserin agonist binding site.

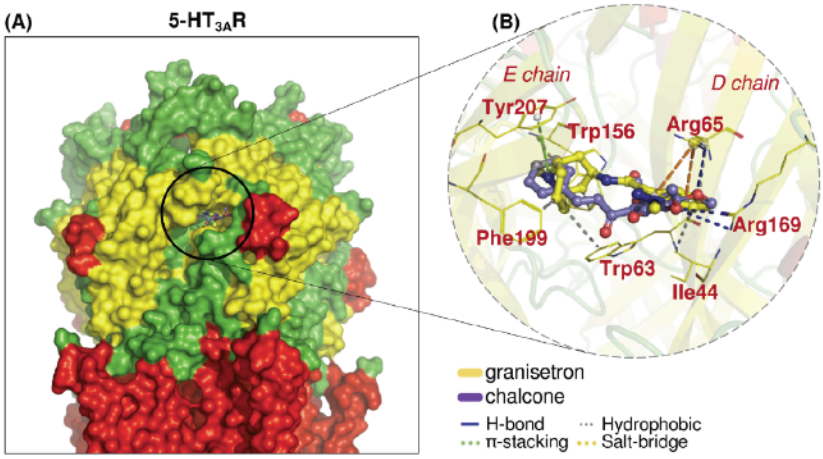


Figure 11: Three-dimensional visualization of (**A**) docking pose of the chalcone in relation to the granisetron antagonist in the hydrophobic cavity of the 5-HT_{3A}R and (**B**) common interactions of the chalcone with the amino acid residues of the granisetron antagonist binding site.

They observed that in compounds with a topological polar surface area (TPSA) between 40–90 Å² and ClogP values below 5 are more likely to exhibit central nervous system activity. These compounds tend to align with three critical in vitro ADME attributes: high cellular permeability (Papp > 10×10^{-6} cm/s), low P-gp efflux and low hepatic clearance (CLint,u < 100 mL/min/kg), attributes related to oral absorption and metabolic stability.

In a topological analysis of the molecular lipophilicity potential (MLP), it is possible to observe that the phenyl moiety linked to the α,β-unsaturated system (ring B) presents an essentially hydrophobic molecular surface (blue color spectra in Figure **12A**), a common characteristic of phenyl-substituted compounds, especially for the formation of BBB permeant substances. It is interesting to note that the polar portion of the methoxylated ring of the chalcone (ring A) has a -OH group with a polar surface equal to 20.23 Å². However, this fragment contributes to the formation of a slightly hydrophobic surface (blue to gray spectra) due to the possibility of intramolecular H-bond formation, resulting in a ClogP = 3.76 (Table **S3**). Thus, the carbonyl conjugated to the α,β-unsaturated system has a low MLP surface, attributed to the inductive electron-withdrawing effect and its polar surface area in the order of 17.07 Å² (red color spectra).

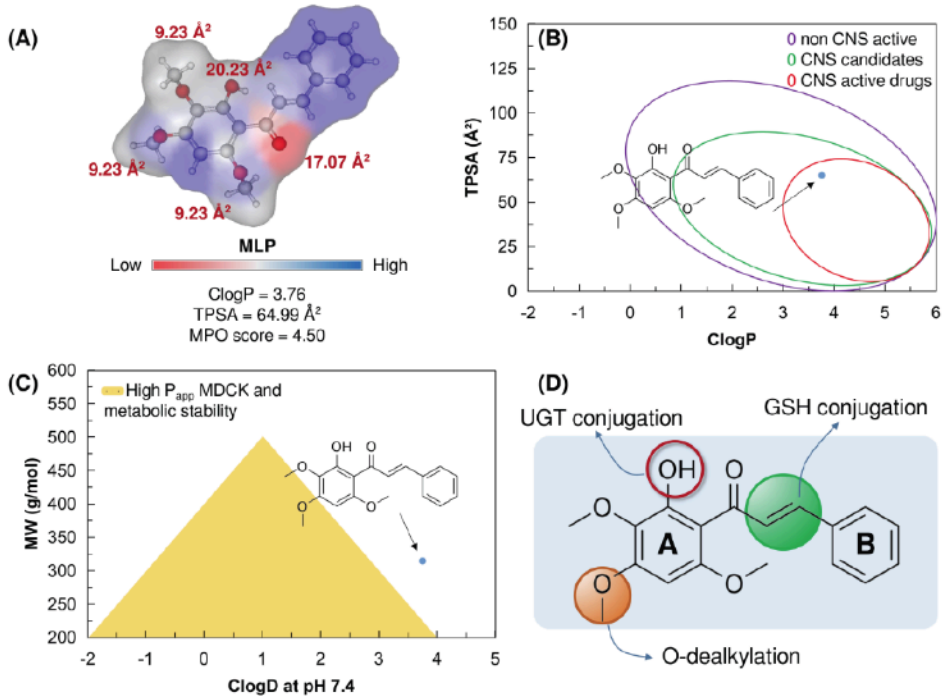


Figure 12: Data from MPO-based ADME predictions expressed in (**A**) topological analysis of the molecular lipophilicity potential (MLP), where the color spectrum varies from red, for molecular fragments with low lipophilicity, to blue, for molecular fragments with high lipophilicity, (**B**) alignment between lipophilicity (ClogP) and polarity (TPSA) descriptors to estimate chalcone access to the CNS, (**C**) alignment between lipophilicity at physiological pH (ClogD at pH 7.4) and molecular weight (MW) for analysis of the metabolic stability spectrum and (**D**) prediction of the site of metabolism based on analysis of molecular fragments

When these attributes are aligned, it is possible to observe that the chalcone resides in a physicochemical space occupied by commonly characterized CNS-active substances (Figure **12B**), formed by compounds with moderate to high lipophilicity (ClogP > 3) and low polarity topological (TPSA < 75 Å²), indicating that the compound can readily permeate the BBB, resulting in an MPO score rated at 4.5 (on a scale ranging from 0 to 6). This analysis corroborates the predicted value of Papp in the order of 1.5×10^{-5} cm/s, based on the MDCK and Caco-2 cellular models (Table **S4**), as well as a low probability of the substance being a P-gp substrate. , indicating that the passive permeability of the compound in cellular lipid bilayer is a positive indicator of permeability in the BBB. Furthermore, it was possible to observe that the moderate lipophilicity of the substance can shift the balance of its volume of distribution to biological tissues in relation to blood plasma, agreeing with the predicted value of unbound fraction (Fu) around 3.8%, indicating that the chalcone has greater affinity with the hydrophobic phase of biological membranes and can be transported by plasma proteins (Table **S4**).

3.6.2. Site of Metabolism Prediction

Predicting sites of metabolism is essential for to identifying molecular fragments in small molecules that may be reactive after phase I metabolism, driven by CYP450 metabolic isoenzymes in the human liver, forming toxic secondary metabolites. One common example involves hydroxylation of unsaturated aliphatic and aromatic centers, resulting in unstable intermediates such as epoxides, which are highly electrophilic and capable of covalently binding to DNA and proteins. In contrast, phase II reactions are responsible for the conjugation of more lipophilic compounds, producing polar metabolites that facilitate elimination from the body. Therefore, lipophilicity is considered one of the most critical hysicochemical descriptors in predicting the metabolic stability of small molecules. Within the molecular weight range of less than MW < 350 g/mol, it was observed that the lipophilicity physiological pH (ClogD at pH 7.4) in the order of 3.75 (Figure **12C**). Based on its structure, chalcone was identified as possessing potential O-dealkylation site at the para-substituted methoxy group], attributed to the [electron-withdrawing inductive effect of the α,β-unsaturated carbonyl system, by the isoforms CYP2C19 and CYP2D6 (phase I) with probability > 0.77 (Figure **12D**). Despite this, the estimated value of hepatic clearance (CLint,u) in the order of 8.90 mL/min/kg indicates that phase I metabolism does not significantly affect the oral bioavailability of chalcone (Table **S5**). Despite being a

site susceptible to epoxidation in phase I metabolism, the α,β-unsaturated system is more sensitive to conjugation mediated by glutathione S-transferase (GST) in phase II metabolism, constituting a secondary metabolite based on a glutathione conjugate. (GSH) which reduces the reactivity of this aliphatic system towards macromolecules such as DNA and proteins (Figure **12D**). Together with the formation of a conjugate formed by glucuronidation in the -OH group of ring A, formed by the action of UDP glucuronosyltransferase (UGT), chalcone has its excretion facilitated by the formation of more polar metabolites with low toxic response, corroborating the low probability of resulting in drug-induced liver injury (DILI) and mutagenic damage (Table **S5**).

4. CONCLUSION

The chalcone synthesized from 2-hydroxy-3,4,6-trimethoxyacetophenone, isolated from *Croton anisodontus*, demonstrated promising pharmacological potential, with no signs of toxicity under the conditions tested. DFT-based electronic structure studies indicated that the molecule has a non-planar conformation, with electronic distribution influenced by its substituent groups. The similarity of the HOMO and LUMO orbitals in different solvents (DMSO and water) suggests that the polarity of the medium exerts little influence on its reactivity and solubility, which is favorable for applications in different biological environments. The analysis of the electrostatic potential and reactivity descriptors highlighted regions susceptible to nucleophilic and electrophilic attacks, supporting its chemical profile for selective molecular interactions. In the pharmacological context, chalcone exhibited a significant anxiolytic effect in an in vivo model, the action of which was not reversed by flumazenil, indicating independence from the GABAergic system — the main target of conventional anxiolytics. On the other hand, the effect was completely abolished by serotonergic antagonists (cyproheptadine, pizotifen, and granisetron), indicating the involvement of 5-HT receptors in mediating the effect. Docking simulations reinforced this hypothesis, identifying the 5-HT3A receptor as the main molecular target, with interactions similar to those of granisetron. This alternative mechanism of action is one of the main differentials of chalcone, offering an innovative route for the development of anxiolytics with a lower risk of adverse effects such as sedation, tolerance, and dependence. The predictions of ADME properties indicated good cell permeability and potential for penetration of the blood-brain barrier, in addition to metabolism predominantly via conjugation (phase II), associated with low toxicity.

AUTHORS' CONTRIBUTIONS

PTS, FRSM, performed the experiments, analyzed the data, prepared figures and tables, authored and wrote the manuscript. AWS conceived and designed the experiments. FFMC and JMLD MKAF analyzed the data and reviewed drafts of the manuscript. ESM, MMM and JESAM performed the docking and dynamic molecular study and reviewed the paper. HSS, WHFR and EMM contributed reagents/materials/analysis tools, supervised the research and reviewed the manuscript. All authors read and approved the final version of the manuscript.

FUNDING

Helcio Silva dos Santos acknowledges financial support from CNPq (Grant 306008/2022-0), FUNCAP UNIVERSAL (Grant#:UNI-0210-00337.01.00/23) and and FUNCAP-INTERNACIONALIZAÇÃO (Grant ITR-0214-00060.01.00/23), Marcia Machado Marinho acknowledges financial support from PDCTR (CNPq/Funcap) (Grant#: DCT-0182-00048.02.00/21 and 04879791/2022).

CONFLICTS OF INTEREST/COMPETING INTERESTS

The authors declare no conflicts of interest.

AVAILABILITY OF DATA AND MATERIAL

All the data generated or analyzed during this study are included in this published article.

CODE AVAILABILITY

The authors thank Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (Funcap), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and scholarship. and the authors thank Northeastern Center for the Application and Use of Nuclear Magnetic Resonance (CENAUREMN).

ETHICS APPROVAL

This study was approved by the Ethics Committee on the Use of Animals at the State University of Ceará (CEUA-UECE; no. 04983945/2021), in accordance with ethical principles involving animal experiments.

SUPPLEMENTAL MATERIALS

The supplemental materials can be downloaded from the journal website along with the article.

REFERENCES

[1] Giacobbe P, Flint A. Diagnosis and Management of Anxiety Disorders. CONTINUUM Lifelong Learning in Neurology 2018; 24: 893-919. https://doi.org/10.1212/CON.0000000000000607

[2] Marder M. Flavonoids as GABAA receptor ligands: the whole story? Journal of Experimental Pharmacology 2012: 9. https://doi.org/10.2147/jep.s23105

[3] Celada P, Bortolozzi A, Artigas F. Serotonin 5-HT1A receptors as targets for agents to treat psychiatric disorders: Rationale and current status of research. CNS Drugs 2013; 27: 703-16. https://doi.org/10.1007/s40263-013-0071-0

[4] Bandelow B, Michaelis S, Wedekind D. Treatment of Anxiety Disorders. Dialogues in Clinical Neuroscience 2017; 19: 211-37. https://doi.org/10.4324/9780203893906-21

[5] Ferreira MKA, da Silva AW, dos Santos Moura AL, Sales KVB, Marinho EM, do Nascimento Martins Cardoso J, *et al*. Chalcones reverse the anxiety and convulsive behavior of adult zebrafish. Epilepsy and Behavior 2021; 117: 107881. https://doi.org/10.1016/j.yebeh.2021.107881

[6] Buxeraud J, Faure S. Benzodiazepines. Actualites Pharmaceutiques 2019. https://doi.org/10.1016/j.actpha.2019.09.027

[7] Riggs DS. Treatment of Anxiety Disorders. Living and Surviving in Harm's Way: A Psychological Treatment Handbook for Pre- and Post-Deployment of Military Personnel 2009: 211-37. https://doi.org/10.4324/9780203893906-21

[8] Salehi B, Quispe C, Chamkhi I, El Omari N, Balahbib A, Sharifi-Rad J, *et al*. Pharmacological Properties of Chalcones: A Review of Preclinical Including Molecular Mechanisms and Clinical Evidence. Frontiers in Pharmacology 2021; 11. https://doi.org/10.3389/fphar.2020.592654

[9] Rojas J, Domínguez JN, Charris JE, Lobo G, Payá M, Ferrándiz ML. Synthesis and inhibitory activity of dimethylamino-chalcone derivatives on the induction of nitric oxide synthase. European Journal of Medicinal Chemistry 2002; 37: 699-705.

[10] De Campos-Buzzi F, Padaratz P, Meira AV, Corrêa R, Nunes RJ, Cechinel-Filho V. 4′-Acetamidochalcone derivatives as potential antinociceptive agents. Molecules 2007; 12: 896-906. https://doi.org/10.3390/12040896

[11] Higgs J, Wasowski C, Marcos A, Jukič M, Paván CH, Gobec S, *et al*. Chalcone derivatives: synthesis, in vitro and in vivo evaluation of their anti-anxiety, anti-depression and analgesic effects. Heliyon 2019; 5. https://doi.org/10.1016/j.heliyon.2019.e01376

[12] Mendes FR da S, Wlisses da Silva A, Amâncio Ferreira MK, de Lima Rebouças E, Marinho EM, Marinho MM, *et al*. GABAA receptor participation in anxiolytic and anticonvulsant effects of (E)-3-(furan-2-yl)-1-(2hydroxy-3,4,6-trimethoxy-phenyl)prop-2-en-1-one in adult zebrafish. Neurochemistry International 2022; 155: 105303. https://doi.org/10.1016/j.neuint.2022.105303

[13] Ferreira MKA, da Silva AW, Silva FCO, Vieira Neto AE, Campos AR, Alves Rodrigues Santos SA, *et al*. 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. Biochemical and Biophysical Research Communications 2020; 526: 505-11. https://doi.org/10.1016/j.bbrc.2020.03.129

[14] Resende RR, Soccol CR. Biotecnologia aplicada à saúde: fundamentos e aplicações. 1st ed. Capítulo 1: Zebrafish Como Modelo Para Estudos Comportamentais: 2015.

[15] Maximino C, Marques T, Brito D, Waneza A, Manoel A, Morato S, *et al*. Measuring anxiety in zebrafish : A critical review. Behavioural Brain Research 2010; 214: 157-71. https://doi.org/10.1016/j.bbr.2010.05.031

[16] Maximino C, Puty B, Benzecry R, Araújo J, Lima MG, De Jesus Oliveira Batista E, *et al*. Role of serotonin in zebrafish (Danio rerio) anxiety: Relationship with serotonin levels and effect of buspirone, WAY 100635, SB 224289, fluoxetine and para-chlorophenylalanine (pCPA) in two behavioral models. Neuropharmacology 2013; 71: 83-97. https://doi.org/10.1016/j.neuropharm.2013.03.006

[17] Herculano AM, Maximino C. Serotonergic modulation of zebrafish behavior: Towards a paradox. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2014; 55. https://doi.org/10.1016/j.pnpbp.2014.03.008

[18] Nuss P. Anxiety disorders and GABA neurotransmission: A disturbance of modulation. Neuropsychiatric Disease and Treatment 2015; 11: 165-75. https://doi.org/10.2147/NDT.S58841

[19] Da Silva PT, Lopes LMA, Xavier JDC, De Carvalho MCS, De Moraes MO, Pessoa C, *et al*. Cytotoxic and antifungal activity of chalcones synthesized from natural acetophenone isolated from Croton anisodontus. Revista Virtual de Quimica 2020; 12: 712-23. https://doi.org/10.21577/1984-6835.20200057

[20] Amali MO, Atunwa SA, Aiyelero OM, Omotesho QA. Assessment of anxiolytic potential and acute toxicity study of combretum micranthum g. don. leaves (combretaceae) in mice. IBRO Reports 2019; 7: 42. https://doi.org/10.1016/j.ibror.2019.09.085

[21] Arellano-Aguilar O, Solis-Angeles S, Serrano-García L, Morales-Sierra, E. Mendez-Serrano, A. Montero-Montoya R. Use of the Zebrafish Embryo Toxicity Test for Risk Assessment Purpose: Case Study. vol. 9. 2015.

[22] Ahmad F, Richardson MK. 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

[23] Gonçalves NGG, de Araújo JIF, Magalhães FEA, Mendes FRS, Lobo MDP, Moreira AC de OM, *et al*. Protein fraction from Artocarpus altilis pulp exhibits antioxidant properties and reverses anxiety behavior in adult zebrafish via the serotoninergic system. Journal of Functional Foods 2020; 66: 103772. https://doi.org/10.1016/j.jff.2019.103772

[24] Gebauer DL, Pagnussat N, Piato ÂL, Schaefer IC, Bonan CD, Lara DR. Effects of anxiolytics in zebrafish: Similarities and differences between benzodiazepines, buspirone and ethanol. Pharmacology Biochemistry and Behavior 2011; 99: 480-6. https://doi.org/10.1016/j.pbb.2011.04.021

[25] Benneh CK, Biney RP, Mante, Kolibea P, Tandoh A, Donatus W, Woode E. Maerua angolensis stem bark extract reverses anxiety and related behaviours in zebra fi sh — Involvement of GABAergic and 5-HT systems. Journal of Ethnopharmacology 2017; 207: 129-45. https://doi.org/10.1016/j.jep.2017.06.012

[26] da Silva AW, Ferreira MKA, Rebouças EL, Mendes FRS, Atilano AL, de Menezes JESA, *et al*. 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. https://doi.org/10.1007/s00210-021-02116-z

[27] Cosacak MI, Bhattarai P, Bocova L, Dzewas T, Mashkaryan V, Papadimitriou C, *et al*. Human TAUP301L overexpression results in TAU hyperphosphorylation without neurofibrillary tangles in adult zebrafish brain. Scientific Reports 2017; 7: 1-14.
https://doi.org/10.1038/s41598-017-13311-5

[28] Stewart AM, Braubach O, Spitsbergen J, Gerlai R, Kalueff A V. Zebrafish models for translational neuroscience research: From tank to bedside. Trends in Neurosciences 2014; 37: 264-78.
https://doi.org/10.1016/j.tins.2014.02.011

[29] Bugel SM, Tanguay RL. Multidimensional chemobehavior analysis of flavonoids and neuroactive compounds in zebrafish. Toxicology and Applied Pharmacology 2018; 344: 23-34.
https://doi.org/10.1016/j.taap.2018.02.019

[30] Gupta P, Khobragade S, Rajaram S, Shingatgeri V. Assessment of locomotion behavior in adult Zebrafish after acute exposure to different pharmacological reference compounds. Drug Development and Therapeutics 2014; 5: 127.
https://doi.org/10.4103/2394-2002.139626

[31] Stewart A, Gaikwad S, Kyzar E, Green J, Roth A, Kalueff A V. Modeling anxiety using adult zebrafish: A conceptual review. Neuropharmacology 2012; 62: 135-43.
https://doi.org/10.1016/j.neuropharm.2011.07.037

[32] Cachat J, Stewart A, Utterback E, Hart P, Gaikwad S, Wong K, *et al*. Three-dimensional neurophenotyping of adult zebrafish behavior. PLoS ONE 2011; 6.
https://doi.org/10.1371/journal.pone.0017597

[33] Mrinalini R, Tamilanban T, Kumar VN, Manasa K. Zebrafish – The Neurobehavioural Model in Trend. Neuroscience 2022.
https://doi.org/10.1016/j.neuroscience.2022.12.016

[34] de Abreu MS, Giacomini ACVV, Genario R, dos Santos BE, da Rosa LG, Demin KA, *et al*. Neuropharmacology, pharmacogenetics and pharmacogenomics of aggression: The zebrafish model. Pharmacological Research 2019; 141: 602-8.
https://doi.org/10.1016/j.phrs.2019.01.044

[35] Stewart A, Wu N, Cachat J, Hart P, Gaikwad S, Wong K, *et al*. Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2011; 35: 1421-31.
https://doi.org/10.1016/j.pnpbp.2010.11.035

[36] Maximino C, de Brito TM, Colmanetti R, Pontes AAA, de Castro HM, de Lacerda RIT, *et al*. Parametric analyses of anxiety in zebrafish scototaxis. Behavioural Brain Research 2010; 210: 1-7.
https://doi.org/10.1016/j.bbr.2010.01.031

[37] Bhathiwal AS, Bendi A, Tiwari A. A study on synthesis of benzodiazepine scaffolds using biologically active chalcones as precursors. Journal of Molecular Structure 2022; 1258: 132649.
https://doi.org/10.1016/j.molstruc.2022.132649

[38] Silva Mendes FR, Wlisses da Silva A, Amâncio Ferreira MK, de Lima Rebouças E, Marinho EM, Marinho MM, *et al*. GABAA receptor participation in anxiolytic and anticonvulsant effects of (E)-3-(furan-2-yl)-1-(2hydroxy-3,4,6-trimethoxy-phenyl)prop-2-en-1-one in adult zebrafish. Neurochemistry International 2022; 155.
https://doi.org/10.1016/j.neuint.2022.105303

[39] Ferreira MKA, da Silva AW, Silva FCO, Vieira Neto AE, Campos AR, Alves Rodrigues Santos SA, *et al*. 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. Biochemical and Biophysical Research Communications 2020; 526.
https://doi.org/10.1016/j.bbrc.2020.03.129

[40] Nowicki M, Tran S, Muraleetharan A, Markovic S, Gerlai R. Serotonin antagonists induce anxiolytic and anxiogenic-like behavior in zebrafish in a receptor-subtype dependent manner. Pharmacology Biochemistry and Behavior 2014; 126: 170-80.
https://doi.org/10.1016/j.pbb.2014.09.022

[41] Lillesaar C, Gaspar P. Serotonergic neurons in vertebrate and invertebrate model organisms (Rodents, zebrafish, drosophila melanogaster, aplysia californica, caenorhabditis elegans). Elsevier Inc.; 2018.
https://doi.org/10.1016/B978-0-12-800050-2.00003-6