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$GABA_A$ receptor participation in anxiolytic and anticonvulsant effects of (*E*)-3-(furan-2-yl)-1-(2hydroxy-3,4,6-trimethoxyphenyl)prop-2-en-1-one in adult zebrafish

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ABSTRACT

Anxiety is a mental disorder that affects 25% of patients with epilepsy, and treatments for anxiety and seizures involve the use of benzodiazepines, a class of drugs that have many adverse effects such as decreased motor coordination, drowsiness, and sedation. Thus, new types of drugs with minimal side effects are of immediate requirement. Chalcones comprise a class of compounds with important therapeutic potential and have recently been investigated for their potential as anxiolytic and anticonvulsant agents. Therefore, this study aimed to evaluate the anxiolytic and anticonvulsant effects of the synthetic chalcone (*E*)-3-(furan-2-yl)-1-(2hydroxy-3,4,6-trimethoxyphenyl)prop-2-en-1-one (FURCHAL) using adult zebrafish as an animal model. Anxiolytic potential was assessed using the light/dark test and the anticonvulsant effect in 3-stage pentylenetetrazol (PTZ)-induced seizure tests. The mechanisms of the anxiolytic effect were analyzed using γ-aminobutyric acid (GABA) and the serotoninergic system. The anxiolytic effect of FURCHAL was verified by a reduction in fish locomotion, similar to diazepam (DZP), which may involve the $GABA_A$ receptor, as there was no reversal in the anxiolytic behavior of animals treated with FURCHAL by serotonergic antagonists. In addition, pretreatment with flumazenil blocked the anticonvulsant effect of FURCHAL and DZP at all three stages, indicating that FURCHAL also has anticonvulsant effects and that the presence of the α,β unsaturated aromatic system and heterocyclic moiety in FURCHAL provided greater affinity for the $GABA_A$ receptors. Molecular docking revealed that the interactions involved in the formation of the protein-binding complex FURCHAL-$GABA_A$ are formed by three H-bonds involving the oxygen atoms of FURCHAL, and notably, complexes operated in the same region of the DZP site. Thus, this study adds new evidence and highlights that FURCHAL can potentially be used to develop compounds with anxiolytic and anticonvulsant properties.

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Abbreviations

CA	Carbonic Anhydrase
CEUA	Ethics Committee on the Use of Animals
CO_2	Carbon dioxide
DMSO	dimethylsulfoxide
DZP	Diazepam
FURCHAL	Chalcone(E)-3-(furan-2-yl)-1-(2hydroxy-3,4,6-trimethoxyphenyl)prop-2-en-1-one
GABA	γ-aminobutyric acid
HPLC	High-Performance Liquid Chromatography
5-HT	(5-hydroxytryptamine)
LD_{50}	Lethal dose needed to kill 50% of animals tested
LGA	Lamarckian Genetic Algorithm
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
OECD	Organization for Economic Cooperation and Development
PTZ	Pentylenetetrazol
PBS	Phosphate-Buffered Saline
PDB	Protein Data Bank
RMSD	Root Mean Square Deviation

1. Introduction

Anxiety is a mental disorder that is correlated with anxiety disorders in patients with epilepsy (Salpekar et al., 2020). Known as "forgotten psychiatric comorbidity" in epilepsy (Gandy et al., 2015; Kanner, 2016), anxiety disorders are reported to affect up to 25% of patients with epilepsy (Testa and Brandt, 2010). Treatments for anxiety and seizures involve the use of medications, usually from the benzodiazepine class, which act via γ-aminobutyric acid (GABA) receptors. However, benzodiazepines have sedative, hypnotic, and muscle relaxant effects (Buxeraud and Faure, 2019; Perucca and Gilliam, 2012). Therefore, recent advances in the discovery and development of antiepileptic and anxiolytic biomolecules (Ferreira et al., 2021a,b; da Silva et al., 2021) have been carried out with an insight into structure-activity relationships through molecular docking (Jha et al., 2020). Thus, new types of drugs with anticonvulsant, anxiolytic potential, and minimal side effects are of immediate requirement.

Heterocyclic rings are found in newly developed drugs such as captopril and diazepam (DZP), and when observing the structure of the drugs used in therapy, approximately 62% of them are heterocyclic (Dua et al., 2011). Heterocyclic chalcones have gained considerable attention because of their wide variety of pharmacological properties, including antioxidant, anticonvulsant, and anti-inflammatory effects (Tantawy et al., 2017).

Chalcones are natural or synthetic compounds that have two aromatic rings in their structures joined by an α,β-unsaturation of the ketone system, and as a carbon skeleton with several replaceable hydrogens, this structure can undergo several changes that result in different effects on biological systems. This class of compounds has been reported as exhibiting promising therapeutic potential mainly due to the ease of preparation, the potential of oral administration and safety, and, recently, as molecules that exert promising anxiolytic and anticonvulsant effects (Ferreira et al., 2020, 2021a,b; Xavier et al., 2021a,b). In addition, furan ring chalcones act as anti-Alzheimer agents in the central nervous system (CNS). Sashidhara et al. (2014) synthesized a new class of benzofuran hybrids containing chalcone as well as (*E*)-3-(2-benzoyl-7-methyl-benzofuran-5-yl)-1-phenylprop-2-en-1-one, and these compounds of benzofuran possessed multifunctional roles in Alzheimer disease, capable of increasing the level of acetylcholine at synaptic junctions with decreased Aβ aggregation.

The zebrafish animal model has many advantages in investigating anxiolytic and anticonvulsant activities. It offers the possibility of modeling pharmacological and genetic seizures in fish during the larval and adult stages (Gawel et al., 2020a; Jha et al., 2020; Tiraboschi et al., 2020) and has a widely conserved behavioral repertoire, including anxiety-like behavior (Blaser and Rosemberg, 2012). Some studies present a detailed description of the advantages of zebrafish in biomedical research and, particularly, in epilepsy (de Abreu et al., 2020; Gawel et al., 2020b; Sakai et al., 2018). In addition, zebrafish show measurable locomotor seizures induced by drugs (after exposure to a proconvulsant drug) and spontaneous mutants with genes associated with epilepsy that are associated with electrographic seizure-like discharges (Baraban et al., 2005). Locomotor parameters are essential for the pharmacological responses of zebrafish, through which it is possible to observe changes in basic locomotion (Moradi-Afrapoli et al., 2017). The pentylenetetrazol (PTZ)-induced seizure test is one of the most popular zebrafish seizure models. Therefore, this study aimed to evaluate the anxiolytic and anticonvulsant effects of the heterocyclic chalcone (*E*)-3-(furan-2-yl)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)prop-2-en-1-one (FURCHAL) using adult zebrafish as an animal model.

2. Materials and methods

2.1. Drugs and reagents

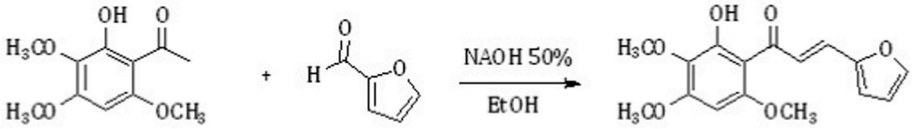
Drugs/reagents used were Granisetron hydrochloride (Corepharma, Middlesex, NJ, USA), pizotifene maleate (Central Manipulation Pharmacy, São Paulo, SP, Brazil), fluoxetine (Eli Lilly, Indianapolis, IN, USA), cyproheptadine (Evidence Soluções Farmacêuticas, Fortaleza, CE, Brazil), DZP, and PTZ (Sigma-Aldrich,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

2.2. Synthesis and chemical characterization of FURCHAL

A description of the procedure for the synthesis of FURCHAL is shown in Scheme 1. (*E*)-3-(furan-2-yl)-1-(2hydroxy-3,4,6-trimethoxyphenyl)prop-2-en-1-one) was synthesized using a Claisen–Schmidt condensation reaction in a basic medium. An ethanol solution of 2-hydroxy-3,4,6-trimethoxyacetophenone isolated from *Croton anisodontus* (Fig. 1) (2 mmol) was added to a solution of furan-2-carbaldehyde (2 mmol), followed by the addition of 10 drops of 50% w/v aqueous NaOH and stirred for 48 h at 32 °C (Da Silva et al., 2020). FURCHAL was then filtered under reduced pressure, washed with cold water, dried, and recrystallized from ethanol, and then purity (100%) was checked by HPLC analysis (Fig. S1, Supplementary material). The structure of the heterocyclic chalcone was in agreement with the literature (Da Silva et al., 2020), being confirmed by analyzing ^{1}H, ^{13}C, and infrared spectra (Figs. S2–S6, Supplementary material), which were obtained using a Bruker DPX-300 platform operating at frequencies of 300 MHz for hydrogen and 75 MHz for carbon. The infrared absorption spectrum was obtained using a Bruker vacuum spectrometer (VERTEX 70V). Chromatographic evaluation was performed by HPLC (Agilent 1260 Infinity, Germany).

2.3. Zebrafish

Zebrafish (*Danio rerio*) (age 90–120 days; 0.4 ± 0.1 g, 3.5 ± 0.5 cm) of both sexes were purchased from a local store (Fortaleza, CE). The animals were kept in a 10-L glass aquarium (30 × 15 × 20 cm) at a temperature of 25 ± 2 °C, in 10–14 h (light/dark) cycles, with chlorinated 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 iced 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



Scheme 1. Scheme of the synthesis of the heterocyclic chalcone.

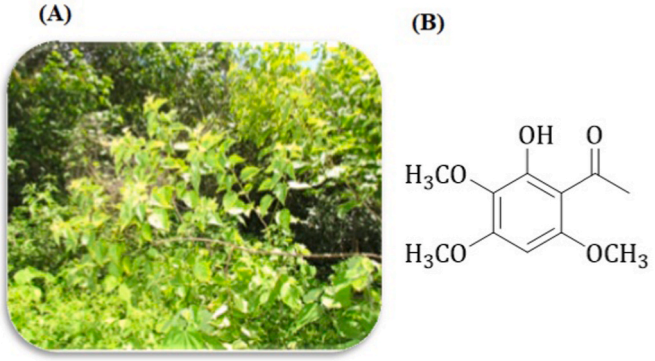


Fig. 1. Aerial parts of C. Anisodontus (A) 2-hydroxy-4,6-dimethoxyacetophenone (B).

Ceará (CEUA-UECE; no. 04983945/2021), in accordance with ethical principles involving animal experiments.

2.4. Acute toxicity assay

A 96-h acute toxicity assessment was performed using adult zebrafish according to the guidelines of the Organization for Economic Cooperation and Development (OECD, Amali et al., 2019). Animals (n = 6/group) were treated orally (20 μL) with FURCHAL (1.0, 3.0 or 10 mg/kg) or vehicle (Control; DMSO 3%). After treatment, the animals were left to rest to analyze mortality rates. From 24 h to 96 h, the number of dead fish in each group was recorded, and the lethal dose capable of killing 50% of the animals (LD_{50}) was determined using the mathematical method trimmed Spearman-Karber with 95% confidence intervals (Arellano-Aguilar et al., 2015).

2.5. In vitro neurotoxicity

For *in vitro* assays, FURCHAL was diluted to 0.2 M in sterile DMSO, and working solutions were prepared with sterile PBS to guarantee a maximum DMSO concentration of 0.5% in the experimental groups. Cytotoxicity of FURCHAL in PC12 cells was assessed by the MTT assay, as described elsewhere (Mosmann, 1983). Briefly, 10^5 cells/mL were plated into 96-wells plates with 10% FBS in Dulbecco's modified Eagle's medium (DMEM) overnight at 37 °C in a 5% CO_2 atmosphere. After that, cells were treated with FURCHAL (1000–15.6 μM) for 24 h. The experimental groups were incubated with MTT (2.5 mg/mL) for 4 h, and DMSO was added to dissolve the formazan crystals. Optical absorbance was measured at 570 nm; 0.5% DMSO and PBS were used as negative controls.

2.6. Open-field test

To assess changes in the animals' motor coordination, an open field test was performed (Ahmad and Richardson, 2013). Initially, the fish (n = 6/group) were treated orally (*p.o.*) with FURCHAL at doses of 1.0, 3.0, 10 mg/kg, DZP (10 mg/kg), or vehicle (Control; 3% DMSO). A group of untreated animals was included (naive group). After 1 h of treatment, animals were added to glass Petri dishes (10 × 15 cm) containing the same aquarium water, marked with four quadrants, and analyzed for locomotor activity by counting the number of crossing lines (CL) by the animals. (Gonçalves et al., 2020).

2.7. Anxiolytic activity

Animal anxiety behavior was observed using a light/dark test. Similar to rodents, zebrafish naturally avoid lighted areas (Gonçalves et al., 2020). The experiment was carried out in a glass aquarium (30 cm × 15 cm × 20 cm) divided into light and dark areas. 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 animals (n = 6/group), 20 μL of FURCHAL was administered orally at doses of 1.0, 3.0, and 10 mg/kg. Negative and positive control groups consisted of 3% DMSO and 10 mg/kg DZP solution, respectively. An untreated group (naive) was also included. After 1 h, animals were individually placed in the clear zone, and the anxiolytic effect was measured based on the time spent in the clear zone of the aquarium within 5 min of observation. (Gebauer et al., 2011).

2.8. Evaluation of GABAergic and serotoninergic neuromodulation

The mechanisms of action involved in the anxiolytic-like effect of FURCHAL were identified through pretreatment with flumazenil (a neutralizing modulator of positive modulators) 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) before the light/dark test (Benneh et al., 2017). Zebrafish (n = 6/group) were pretreated 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 highest effective dose of FURCHAL (10 mg/kg; 20 μL; *p.o.*) found in the pilot test was administered (see the previous section); 3% DMSO (vehicle; 20 μL; p.o.) was used as a negative control. DZP (10 mg/kg, 20 μL; *p.o.*) and fluoxetine (0.05 mg/kg; *i.p.*) were used as $GABA_A$ and 5-HT agonists, respectively. After 1 h of treatment, animals were subjected to the light/dark test as described in the previous section.

2.9. PTZ-induced seizure

Reversal of PTZ-induced seizures has been investigated previously (Siebel et al., 2015). Animals (n = 6/group) were treated with FURCHAL (10 mg/kg; 20 μL; p.o.), DZP (10 mg/kg; 20 μL; *p.o.*), and vehicle (3% DMSO; 20 μL; *p.o.*). An untreated group (n = 6) was included (naive group). After 1 h, animals were individually exposed to 7.5 mM PTZ, dissolved in water in a 250 mL beaker, and the seizure-like behavior in three stages was evaluated: stage I, drastic increase in swimming activity; stage II, swirl behavior; and stage III, seizures similar to clonus, followed by loss of posture when the animal fell to the side and remained immobile for 1–3 s. At the end of the evaluation of the three stages of the test, animals were euthanized on ice. The mechanism of action was evaluated further.

2.10. Involvement of the GABAergic system in seizures

The fish (n = 6/group) received flumazenil (4 mg/kg; 20 μL; 20 μL; *p.o.*) (Ferreira et al., 2021a,b). After 15 min, they were treated with FURCHAL (10 mg/kg; 20 μL; *p.o.*) anticonvulsant, DZP (10 mg/kg; 20

μL; *p.o.*), and vehicle (3% DMSO; 20 μL; *p.o.*). An untreated group (n = 6) was included (naive group). After 1 h of treatment, the animals were individually exposed to PTZ (7.5 mM) and the three stages of seizure were evaluated as described above.

2.11. Statistical analysis

Results are expressed as mean values ± standard error of the mean for each group of six animals. After confirming 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% ($P < 0.05$).

2.12. Computational procedures

2.12.1. Computational details

To carry out simulations, the codes used were Autodocktools™ (Huey et al., 2012), AutoDockVina™ (Trott and Olson, 2009), Avogadro™ (Hanwell et al., 2012), Discovery Studio Visualizer™ viewer (Biovia, 2016), Gabedit 2.5.0 (Allouche, 2011), Marvin™ 19.8, 2020, (Csizmadia, 2019), PyMol (DeLano, 2020) and UCSF Chimera™ (Pettersen et al., 2004).

2.12.2. General docking procedures

To investigate anxiolytic effects of the GABAergic system (Ferreira et al., 2020), the structure of the $GABA_A$ receptor was used (Protein Data Bank [PDB] code 6HUP), identified as "CryoEM structure of human full-length alpha1beta3gamma2L GABA(A)R in complex with DZP (Valium), GABA and megabody Mb38" (Xavier et al., 2021b). To investigate the anticonvulsant effect of FURCHAL, the structure of the enzyme carbonic anhydrase II (CAII) (PDB code 3F8E) was used, identified as "coumarins are a novel class of suicide carbonic anhydrase inhibitors" and co-crystallized with a TE1 inhibitor (da Silva et al., 2021). All protein structures were obtained from the PDB repository (https://www.rcsb.org/), where all water molecules and Gasteiger charges were removed and essential hydrogen atoms were added (Yan et al., 2014). To perform molecular docking simulations, the AutoDock vina software was used (Trott and Olson, 2010), configured to run the Lamarckian Genetic Algorithm (LGA). To determine the simulation space, the grid boxes were centered to encompass all protein chains. For the simulations with the $GABA_A$ receiver, the grid box was centered at coordinates 125,281, 139,534, and 136,018 for the x, y, and z axes, with size parameters 126 Å, 100 Å, and 126 Å, respectively. For the CAII enzyme, the grid box was centered at coordinates −8,086, −0.658, and 17.136 for the x, y, and z axes, respectively, with a dimension of 94 Å × 96 Å × 106 Å. For each protein, 50 independent simulations were performed, and 20 poses were obtained per simulation for both docking and redocking simulations (Marinho et al., 2020). To improve the partial refinement of the individual coupling calculations, the exhaustiveness criterion was set to 64, keeping the protein structure rigid, while all binding and twisting of the ligands were adjusted to rotate (Nguyen et al., 2017). The statistical parameter root mean square deviation (RMSD) was used as a selection criterion for the best pose, with ideal values being <2 Å (Yusuf et al., 2008).

Regarding the stability of the complexes formed in the simulations, the affinity energy (**ΔG**) was used, which has as an ideality parameter value of < −6.0 kcal/mol (Shityakov and Förster, 2014). Using the affinity energy values, the values of the inhibition constants (K1) (Equation (1)) of each complex were calculated (Kadela-tomanek et al., 2021).

$$\mathbf{K}i = e^{(\Delta G/RT)} \quad (1)$$

where K_i is the inhibition constant, T is the absolute temperature (298 K), R is the gas constant (8.32 J $mol^{-1}K^{-1}$), and ΔG is the binding free energy (in $KJ.mol^{-1}$). To assess the intensity of hydrogen bonds (H-bonding), values of the distances between the donor and receptor atoms of H-bonds were used, considering that interactions between 2.5 Å and 3.1 Å are classified as strong, between 3.1 Å and 3.55 Å as average, and those with a distance >3.55 Å as weak (Imberty et al., 1991). To validate the simulations, redocking was performed with the drug DZP and the TE1 inhibitor, which were co-crystallized with the $GABA_A$ and CAII receptors, respectively.

3. Results

3.1. Cytotoxicity in PC12 cells

As presented in Fig. 2, FURCHAL did not exhibit toxicity over the 500–15.6 μM range. At higher concentrations, PC12 cell viability was reduced by approximately 28%.

3.2. Acute toxicity and locomotor activity

FURCHAL was considered nontoxic to adult zebrafish for up to 96 h (LD_{50} > 10 mg/kg), as there were no deaths and the agent did not cause any apparent anatomical changes to the animals during this period ($P > 0.05$). The locomotion of animals was altered by FURCHAL [$^{****}P < 0.0001$ and $^{\#\#\#\#}P < 0.0001$ and DZP [$^{****}P < 0.0001$ and $^{\#\#\#\#}P < 0.0001$ (10 mg/kg)]. These data showed that animals exhibited reduced locomotor activity compared to the control groups (naive and vehicle, respectively) (Fig. 3).

3.3. Anxiolytic evaluation

FURCHAL (1, 3, or 10 mg/kg) increased ($^{**}P < 0.01$ and $^{\#\#\#}P < 0.001$ vs. naive and vehicle, respectively) the time animals spent in the clear region of the aquarium in the light/dark test (Fig. 4A). This effect was similar to that of DZP (10 mg/kg), a positive control.

3.4. Evaluation of GABAergic and serotonin neuromodulation

The mechanism of anxiety via GABA was determined by pretreatment with flumazenil. The highest tested doses of FURCHAL and DZP (10 mg/kg) had anxiolytic effects, which were blocked ($^{\#}P < 0.01$ and $^{\#\#}P < 0.01$ vs. FURCHAL and DZP, respectively; Fig. 4B) by flumazenil, as the fish spent most of their time in the dark region of the aquarium, demonstrating anxiety behavior.

Serotonergic neuromodulation was investigated by pretreatment with pizotifen (5-HTR_1 and 5-$HTR_{2A/2C}$ antagonist), cyproheptadine (5-HTR_{2A} antagonist), and granisetron (5-$HTR_{3A/3B}$ antagonist). Pizotifen,

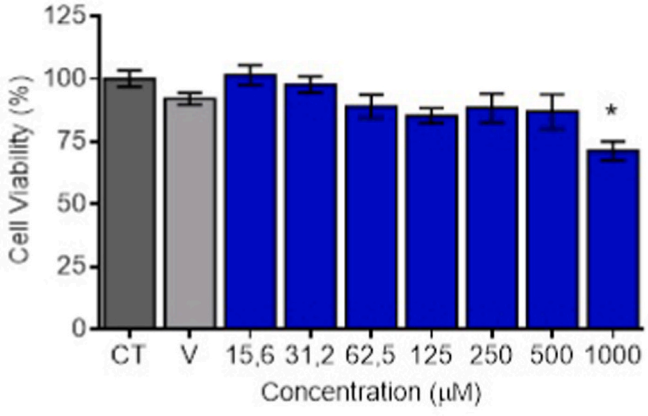


Fig. 2. Chalcone cytotoxicity in PC12 cells by MTT assay. Results are expressed as percentage of damaged cells ± S.E.M. *P < 0.05 vs. control group; V – vehicle group (DMSO 0.5%).

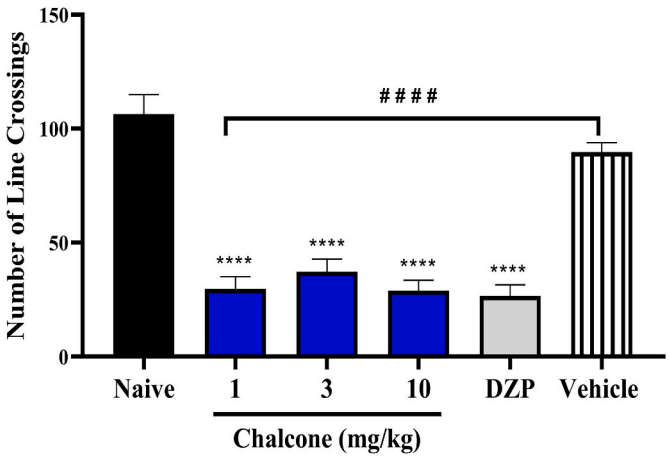


Fig. 3. Effect of chalcone on the locomotor behavior of adult zebrafish in the Open Field Test (0–5 min). Values represent the mean ± standard error of the mean for 6 animals/group; ANOVA followed by Tukey's test (****P < 0.0001 vs Naive; $^{\#\#\#\#}$P < 0.001 vs Vehicle).

cyproheptadine, and granisetron did not reverse the anxiolytic behavior of animals treated with FURCHAL (10 mg/kg), but they reduced ($^{\#\#\#\#}$P < 0.0001 vs. fluoxetine) the anxiolytic effect caused by fluoxetine (0.05 mg/kg) (Fig. 5A, B, and 5C).

3.5. PTZ-induced seizure

FURCHAL (10 mg/kg) reversed PTZ-induced convulsive behavior in the three stages (**P < 0.01, stage I; *P < 0.05, stages II and III), an effect similar to that of DZP (10 mg/kg), which also delayed the onset of seizures in three stages when compared to the control treatment (naive and vehicle) (Fig. 6).

3.6. GABAergic system involvement in seizures

The mechanism of the $GABA_A$ anticonvulsant action of FURCHAL was investigated by pretreatment with flumazenil. Fish treated with flumazenil and FURCHAL showed reversed convulsive behavior ($^{\#}$P < 0.01, stage I; P > 0.05, stage II; **P < 0.01, stage III), similar to the effect on animals treated with flumazenil + DZP, as they did not delay the onset of seizures in the three stages in the animals ($^{\#}$P < 0.05 vs DZP, stages I, II, and III) (Fig. 7).

3.7. Molecular docking of FURCHAL-GABA

Initially, RMSD was used for statistical validation of results of simulations of complex formation and the choice of the best pose. All simulations performed (docking and redocking) presented RMSD values below or close to 2 Å (reference value), highlighting the best poses of the FURCHAL-$GABA_A$ and DZP-GABAA complexes, which presented RMSD in the order of 1.284 and 1.02 Å, respectively. Regarding the affinity energy, the complexes FURCHAL–$GABA_A$ and DZP–$GABA_A$ (redocking) presented energies in the order of −7.5 and −7.4 kcal/mol, respectively, indicating that chalcone had greater affinity when compared to DZP. This was also corroborated by the pK_i values, where the FURCHAL–GABAA complex presented a value in the order of 5.50 ($K_i = 3.1601 \times 10^{-6}$), which was higher than that of the DZP–$GABA_A$ complex obtained by redocking [5.43 ($Ki = 3.7415 \times 10^{-6}$)].

3.8. Molecular docking of FURCHAL–ACII

The best poses of the FURCHAL–CAII and TE1–CAII (redocking) complexes presented RMSD in the order of 1,929 and 1,755 Å, remaining within statistical ideality. Regarding affinity energy, the FURCHAL–ACII and TE1–ACII (redocking) complexes presented energy in the order of −5.9 and −5.4 kcal/mol with inhibition constants in the order of 4.7109×10^{-5} ($pK_i = 4.33$) and 1.0959×10^{-4} ($pK_i = 3.96$) respectively, indicating that chalcone had a higher affinity than the co-crystallized inhibitor TE1.

4. Discussion

Neuronal cells have been used in the evaluation of neurotoxicity and neuroprotective effects of various substances, including the mouse pheochromocytoma cell line (PC12) commonly used in this context (Grau and Greene, 2012; Wiatrak et al., 2020). The toxicity of FURCHAL was measured in PC12 cells as described previously for other chalconic compounds, such as isoliquiritigenin, regarding its neuroprotective potential (Yang et al., 2017).

Ligustrazine-based chalcones prevent amyloid β-induced cytotoxicity and aggregation in an *in vitro* model of Alzheimer disease, which was superior to that of reference drugs with low toxicity (Wang et al.,

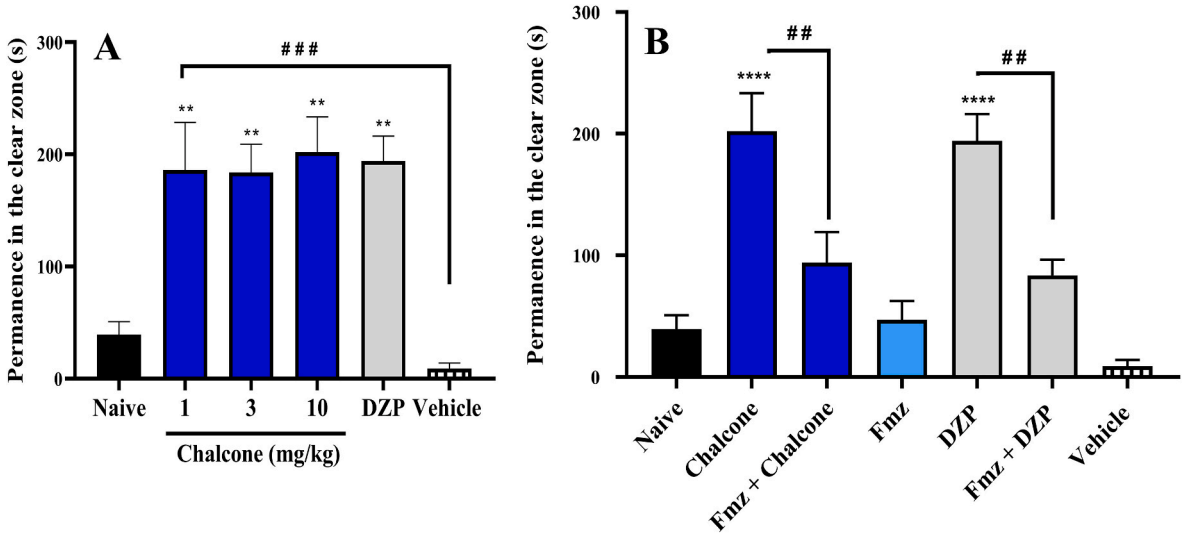


Fig. 4. Anxiolytic effect (A) and anxiety mechanism (B) of chalcone in the light/dark test (0–5 min). Values represent the mean ± standard error of the mean for 6 animals/group; (one-way ANOVA), and (two-way ANOVA in experiments with antagonists) followed by Tukey's test (** P < 0.01; ****P < 0.0001 vs Naive; $^{\#\#\#}$P < 0.001 vs Vehicle; $^{\#\#}$P < 0.01 vs chalcone or DZP).

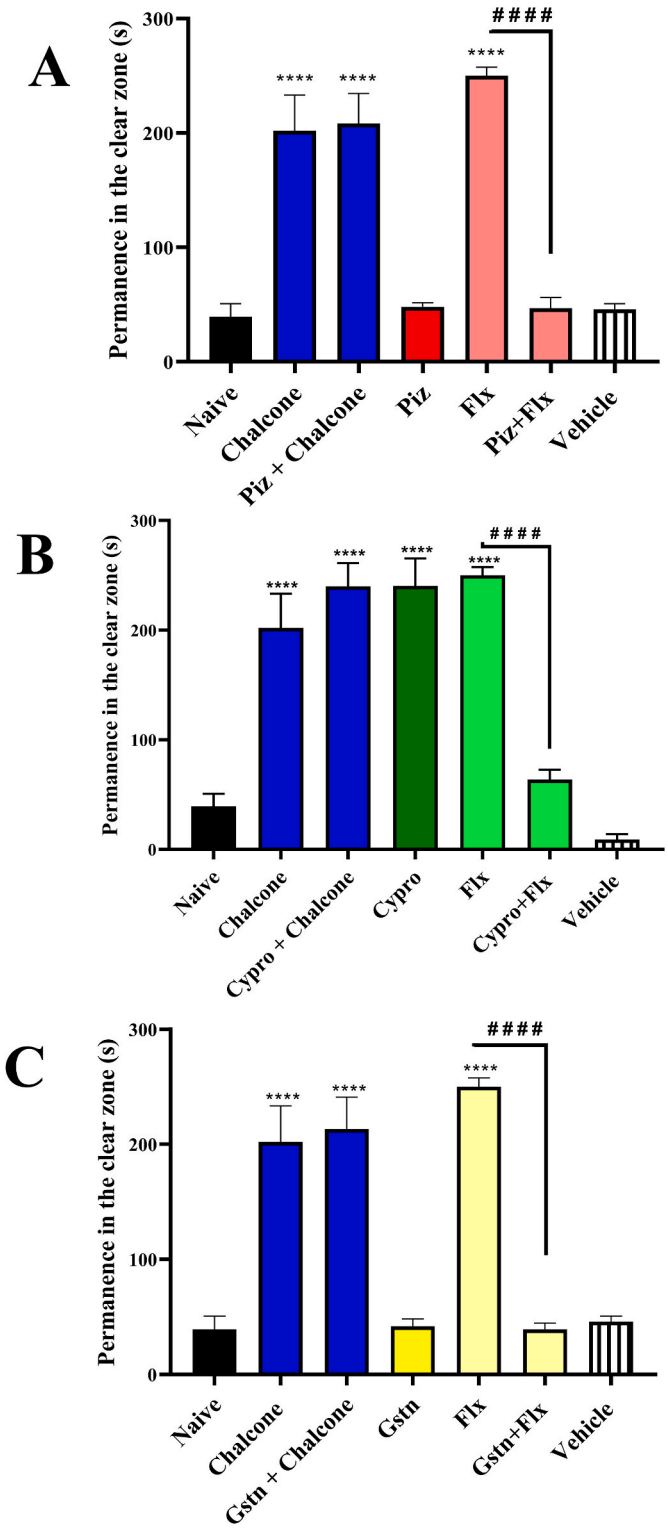


Fig. 5. Effect of pizotifen (A), cyproheptadine (B) and granisetron (C) on the anxiolytic effect of chalcone in the light/dark test. Values represent the mean ± standard error of the mean for 6 animals/group; (one-way ANOVA, and two-way ANOVA in experiments with antagonists) followed by Tobey's test (****P < 0.0001 vs Naive or vehicle; $^{\#\#\#\#}$P < 0.0001 vs chalcone or fluoxetine - Flx).

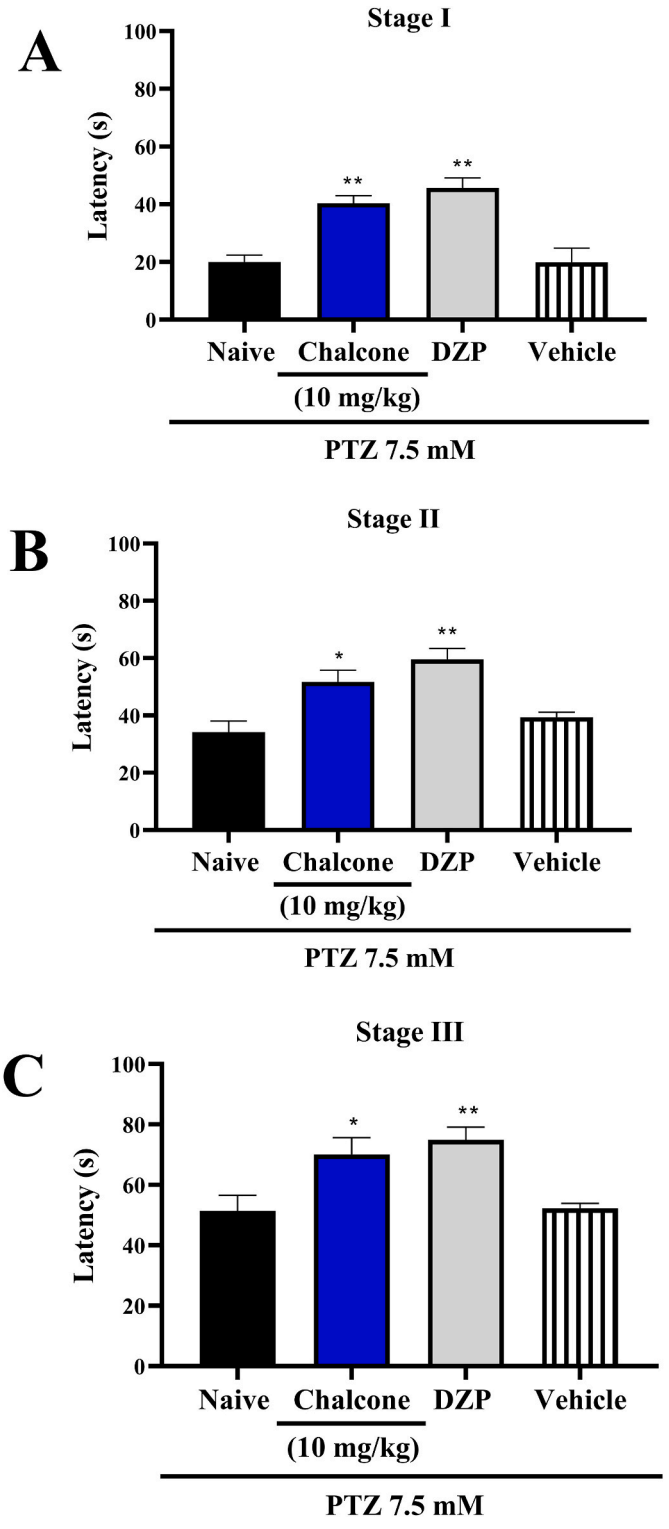


Fig. 6. Effect of chalcone on pentylenetetrazol (3-stage)-induced seizure in adult zebrafish. Stage I (A), Stage II (B), Stage III (C). Values represent the mean ± standard error of the mean for 6 animals/group; ANOVA followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001 vs Naive or vehicle).

2018).

At nontoxic concentrations, chalcone O-carbamate derivatives are protective agents in an *in vitro* model of glutamate-induced cell death, while chalcone-triazole hybrids reduce damage in SH-SY5Y cells (Selvaraj et al., 2020; Sooknual et al., 2020). These findings reinforce the protective effect of chalcones and the low toxicity of these molecules.

The behavioral pharmacology of chalcones in the CNS has been well reported in relation to anxiolytic activity (Mathew et al., 2019). Some chalcone derivatives bearing a furan ring in their structure, such as

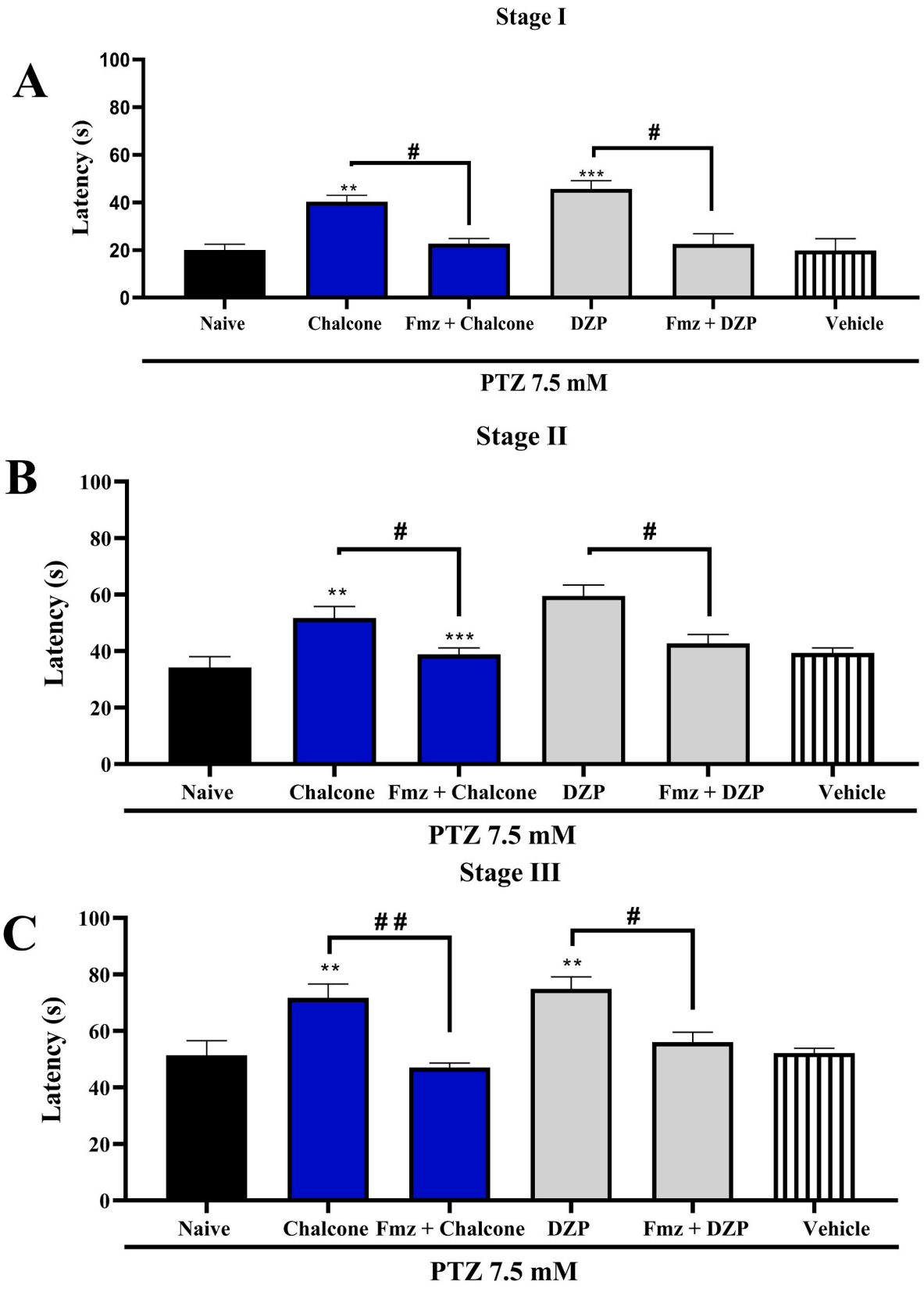


Fig. 7. Effect of flumazenil (Fmz) on the anticonvulsant action of chalcone in the pentylenetetrazol-induced seizure test in adult zebrafish. Stage I (A), Stage II (B), Stage III (C). Values represent the mean ± standard error of the mean for 6 animals/group; (one-way ANOVA), and (two-way ANOVA in experiments with antagonists) followed by Tukey's test (**P < 0.01, ***P < 0.001 vs Naive or vehicle; #P < 0.05, ## P < 0.01 vs chalcone or DZP).

FURCHAL, act in CNS as anti-Alzheimer disease agents (Sashidhara et al., 2014).

The depressant activity on the CNS was evaluated by open-field tests, which clearly identified a reduction in locomotion in fish treated with FURCHAL, similar to the results obtained with DZP exposure (Fig. 3). Assessment of the locomotor activity of animals is widely used to measure the level of CNS excitability; therefore, decreased locomotion is a result of CNS depression (Öztürk et al., 1996). Anxiolytic drugs and chalcones are reported to reduce adult zebrafish locomotion in open-field tests (Ferreira et al., 2019, 2020, 2021a,b; Gupta et al., 2014). No acute toxicity effects of FURCHAL were observed during the 96-h analysis in this study.

The innate anxiety behavior of zebrafish is similar to that of rodents, preferring dark environments, in addition to new environments evoking anxiogenesis in these animals (Maximino et al., 2010). Thus, the light/dark test was used to assess the anxiolytic effect of chalcone in adult zebrafish, and the results showed that FURCHAL changed the anxiolytic behavior as it increased the animals' permanence in the clear area of the aquarium, a result similar to the effect of DZP (Fig. 4A). In anxiolytic evaluations, after administration of anxiolytics, the fish spent more time in the light region of the aquarium, as they were less anxious and consequently had reduced locomotor activity in the light and dark test, as in the group of animals treated with DZP (Chaouloff et al., 1997; Ferreira et al., 2020).

Classic benzodiazepines are widely used in clinical practice as sedatives, anxiolytics, and anticonvulsants, exerting their therapeutic effects through interactions with heteropentameric GABA receptors composed of two α, two β, and one γ2 subunit (Müller Herde et al., 2017). Flumazenil is a modulator of positive and negative regulators (Khan et al., 2016). Potentiation at $\alpha 1\beta 2\gamma 2$L $GABA_A$ receptor subtype is blocked by flumazenil under conditions where flumazenil also inhibits the increase induced by DZP, indicating that FURCHAL induces potentiation through the benzodiazepine-binding site (Fig. 4B). That is, the anxiolytic effect of chalcone can occur through interactions involving the binding site of benzodiazepines. Other chalcones also cause anxiolytic behavior in adult Zebrafish via GABA (Ferreira et al., 2019; M. K. A. Ferreira et al., 2021a,b).

Recently, da Silva et al. (2021) showed that 2-hydroxy-3,4,6-trimethoxyacetophenone isolated from *C. anisodontus* (the starting material for the synthesis of chalcone used in this study) has anxiolytic effects via serotonergic (5-HT) neuromodulation in adult zebrafish. Thus, we investigated the possible role of 5-HT in the anxiolytic effects of the synthesized chalcone. In teleost fish such as zebrafish, the serotonergic system follows the general pattern of vertebrates: serotonergic cell bodies are located mainly in the hindbrain, in the raphe nucleus (Backström and Winberg, 2017). The mechanism of action of 5-HT was investigated using the antagonists pizotifen (5-HTR_1 and 5-$HTR_{2A/2C}$ antagonist), ciproheptadine (5-HTR_{2A} antagonist), and granisetron (5-$HTR_{3A/3B}$ antagonist). There was no reversal in the anxiolytic behavior of animals treated with FURCHAL by serotonergic antagonists, unlike the anxiolytic effect of fluoxetine, which was reversed by pizotifen, cyproheptadine, and granisetron (Fig. 5A, B, 5C). Thus, the anxiolytic effect of synthesized FURCHAL may not be related to 5-HT neuromodulation, unlike the starting material (2-hydroxy-3,4,6-trimethoxyacetophenone). When comparing structures, it was observed that the presence of the α,β-unsaturated system and the heterocyclic moiety in FURCHAL provided greater affinity for $GABA_A$ receptors (Ferreira et al., 2019, 2021a,b).

Zebrafish with elevated anxiety exhibited greater susceptibility to PTZ-evoked crises (Canzian et al., 2021). The anticonvulsant effect of FURCHAL chalcone was investigated using PTZ as a seizure inducer in adult zebrafish, which showed that chalcone exhibits an anticonvulsant effect on PTZ-induced seizures (Fig. 6A, B, and 6C). Agents that are effective against PTZ-induced seizures can inhibit the absence of seizures (Vida, 1995). Thus, chalcone can be effective in treating this type of seizure.

PTZ triggers seizures due to its antagonism on $GABA_A$ receptors in the CNS (Huang et al., 2001). Thus, FURCHAL chalcone and DZP increased the latency time for the onset of PTZ-induced seizures, especially in stages II and III, which are similar to clonic behavior (main measured seizure phenotype).

Zebrafish are used as an animal model for seizures because they have fully developed brain regions with neurotransmitters and a blood–brain barrier that are crucial in epileptogenesis. (Banote et al., 2013). *In vitro* evidence indicates that chalcones can cross the blood–brain barrier (Bai et al., 2019). We investigated the association of DZP and chalcone on $GABA_A$ receptors in PTZ-induced seizures in adult zebrafish using flumazenil as a $GABA_A$ antagonist. The data showed that pretreatment with flumazenil blocked the anticonvulsant effects of FURCHAL and DZP at all three stages, indicating that the anticonvulsant effect of FURCHAL may involve GABAergic neuromodulation of the $GABA_A$ receptor (Fig. 7A, B, and 7C).

In addition, a molecular docking study was carried out to assess possible interactions that might explain the anxiolytic and anticonvulsant effects of FURCHAL. Molecular docking studies are important for determining protein–ligand and protein–protein interactions (Halim et al., 2013; Iftikhar et al., 2017; Khan et al., 2016).

Regarding interactions involved in the formation of protein-binding complexes, it was possible to identify that the FURCHAL-$GABA_A$ complex was formed by three H-bonds of high intensity involving the oxygen atoms of FURCHAL and Ser 205D (1.80 Å and 1.84 Å) and Thr 207D (2.08 Å) residues, and two interactions with the Tyr 58C residue, one of the ($\Delta\mathbf{G}$)-stacking type (4.41 Å) (involving the residue and aromatic rings) and a hydrophobic interaction (3.45 Å). The DZP–$GABA_A$ complex obtained by redocking was formed by two H-bonds involving the Ser 205D residue (2.94 and 3.16 Å), a halogen-bond type interaction with His 103D (3.82 Å), and seven hydrophobic interactions involving residues Phe 77C (3.47 Å), Tyr 160D (3.56 Å), Val 203D (3.95 Å), Tyr 160D (3.38 and 3.86 Å), and Phe 100D (3.83 and 3.92 Å). Notably, FURCHAL complexes in the same region of the DZP site interact with residues of chains C and D and with residue Tyr 58 of chain C (Fig. 8).

Brain pH plays a vital role in epilepsy, and pH changes directly affect seizure generation and severity (Mishra et al., 2018). Generally, alkalosis promotes the spread of crises, while acidosis blocks/stops the generation of crises (Ruusuvuori and Kaila, 2014). It is well established that carbonic anhydrase (CA) modulates numerous neuronal signaling mechanisms associated with various CNS disorders, including epilepsy (Mishra et al., 2018). pH buffering between the extracellular and intracellular spaces is driven by CO_2 and HCO_3^-; the regulation of both components is under the control of CA that catalyze the reversible conversion of CO_2 and H_2O into H^+ and HCO_3^- (Mishra et al., 2016), AC have emerged as an emerging target to control epileptic seizures, and some potent AC inhibitors are used clinically to control various types of seizures (Aribi and Stringer, 2002). For this reason, an *in silico* study of AC with the FURCHAL chalcone was performed. An evaluation of interactions involved in complex formation showed that the FURCHAL–CAII complex was formed by four H-bonds: three of which are of strong intensity involving oxygen atoms of the chalcone and residues Asn 62A (2.52 Å), His 64A (2.52 Å), and Asn 67A (2.93 Å) and an average bond with the residue Gln 92A (3.55 Å), and two hydrophobic interactions involving the residues Ile 91A (3.66 Å) and Leu 198A (3.89 Å).The TE1–CAII complex is formed by only two hydrophobic interactions (Ile 91A [3.66 Å] and Gln 92A [3.60 Å]) and an H-bond of average intensity with the residue Arg 58A (3.34 Å) (Fig. 9). From the interactions involved in the formation of the complexes, we can observe that both complexes interact in the same region, having in common the residues Ile 91A and Gln 92A, noting that the interaction of chalcone with CAII occurs predominantly by H-bond of strong intensity, while the TE1 inhibitor interacts with only one H-bond of average intensity. Studies have shown that CAs influence the activity of $GABA_A$ through HCO_3^- in the brain, mainly involving CAII and CAVII. Moreover, the standard drug for inhibiting CA (Carbonic Anhydrase) also inhibits

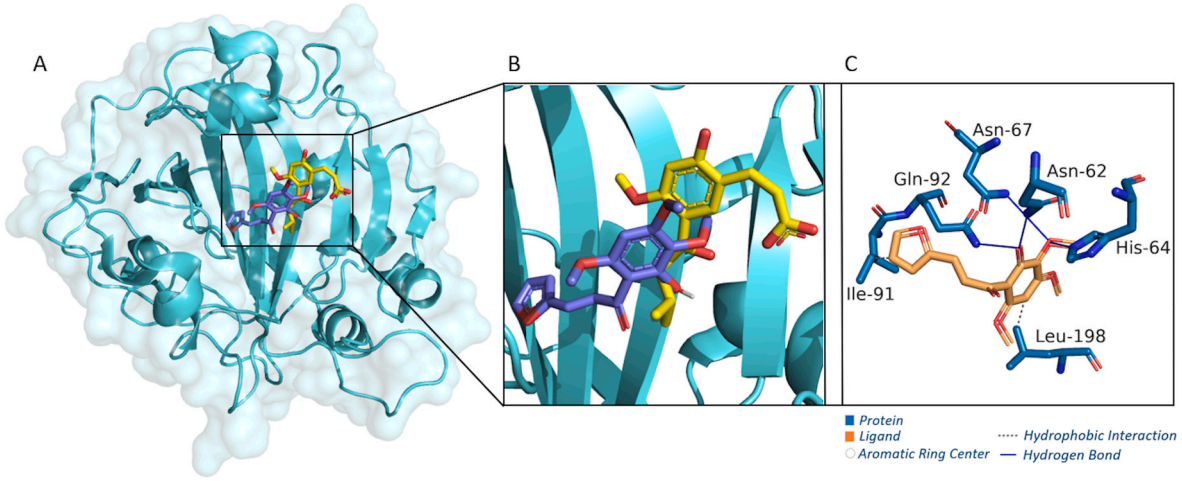


Fig. 8. Interaction complex between $GABA_A$ receptor and ligands (A). Interaction site of the ligand chalc (blue) and the co-crystallized inhibitor Diazepam (yellow) (B). 2D map of chalcone ligand interactions with the target.

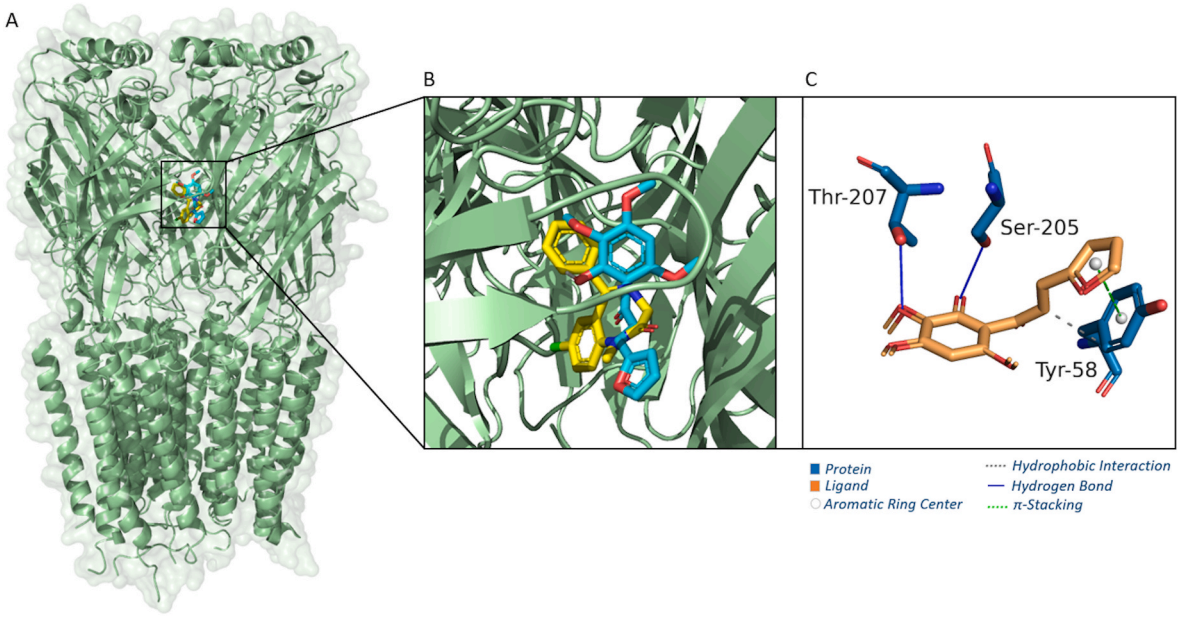


Fig. 9. Interaction complex between Carbonic anhydrase 2 enzyme and ligands (A). Interaction site of the ligand chalcone (purple) and the co-crystallized inhibitor TE1 (yellow) (B). 2D map of chalcone ligand interactions with the target.

PTZ-induced seizures in animal models (Mishra et al., 2018), which is similar to the results obtained with FURCHAL. Therefore, the anticonvulsant effect of chalcone may be modulated by both $GABA_A$ and CAII.

5. Conclusion

The study showed that the FURCHAL chalcone caused anxiolytic effects in animals and that the $GABA_A$ receptor may be involved in such effects. The synthesized chalcone, in addition to presenting anxiolytic effects, also demonstrated anticonvulsant effects via $GABA_A$ and CA, as confirmed by a binding/activity study.

CRediT authorship contribution statement

Francisco Rogênio Silva Mendes: Investigation, Formal analysis, Writing – original draft. **Antonio Wlisses da Silva:** Project administration, Conceptualization. **Maria Kueirislene Amâncio Ferreira:** Methodology, Formal analysis. **Emanuela de Lima Rebouças:** Methodology, Formal analysis. **Emanuelle Machado Marinho:** Methodology, Formal analysis. **Márcia Machado Marinho:** Methodology, Formal analysis. **Paulo Nogueira Bandeira:** Supervision, Writing – review & editing. **Alexandre Magno Rodrigues Teixeira:** Supervision, Funding, Formal analysis, Writing – review & editing. **Jane Eire Silva Alencar de Menezes:** Software, Visualization. **Erlania Alves de Siqueira:** Methodology, Formal analysis. **Ramon R.P.P.B. de Menezes:** and. **Emmanuel Silva Marinho:** Software, Formal analysis, Writing – review & editing. **Hélcio Silva dos Santos:** Funding, Formal analysis, Writing – review & editing.

Declaration of competing interest

All authors have no conflicts of interest to declare with respect to the research, authorship, and/or publication of this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2022.105303.

References

Ahmad, F., Richardson, M.K., 2013. Exploratory behaviour in the open field test adapted for larval zebrafish: impact of environmental complexity. Behav. Process. 92, 88–98. https://doi.org/10.1016/j.beproc.2012.10.014.

Allouche, A.R., 2011. Gabedit - a graphical user interface for computational chemistry softwares. J. Comput. Chem. 32, 174–182. https://doi.org/10.1002/jcc.21600.

Amali, M.O., Atunwa, S.A., Aiyelero, O.M., Omotesho, Q.A., 2019. Assessment of anxiolytic potential and acute toxicity study of combretum micranthum g. don. leaves (combretaceae) in mice. IBRO Rep. 7, 42. https://doi.org/10.1016/j.ibror.2019.09.085.

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

Aribi, A.M., Stringer, J.L., 2002. Effects of antiepileptic drugs on extracellular pH regulation in the hippocampal CA1 region in vivo. Epilepsy Res. 49, 143–151. https://doi.org/10.1016/S0920-1211(02)00019-0.

Backström, T., Winberg, S., 2017. Serotonin coordinates responses to social stress-What we can learn from fish. Front. Neurosci. 11, 1–10. https://doi.org/10.3389/fnins.2017.00595.

Banote, R.K., Koutarapu, S., Chennubhotla, K.S., Chatti, K., Kulkarni, P., 2013. Oral Gabapentin Suppresses Pentylenetetrazole-Induced Seizure-like Behavior and Cephalic Field Potential in Adult Zebrafish. Epilepsy Behav. https://doi.org/10.1016/j.yebeh.2013.01.018.

Baraban, S.C., Taylor, M.R., Castro, P.A., Baier, H., 2005. Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. Neuroscience 131 (3), 759–768. https://doi.org/10.1016/j.neuroscience.2004.11.031.

Benneh, C.K., Biney, R.P., Mante, P.K., Tandoh, A., Adongo, D.W., Woode, E., 2017. Maerua angolensis stem bark extract reverses anxiety and related behaviours in zebrafish—involvement of GABAergic and 5-HT systems. J. Ethnopharmacol. 207, 129–145. https://doi.org/10.1016/j.jep.2017.06.012.

Biovia, D.S., 2016. Discovery Studio Modeling Environment. Release 2017, San Diego (Dassault Systèmes).

Blaser, R.E., Rosemberg, D.B., 2012. Measures of anxiety in zebrafish (Danio rerio): dissociation of black/white preference and novel. Tank Test 7, 1–8. https://doi.org/10.1371/journal.pone.0036931.

Buxeraud, J., Faure, S., 2019. Benzodiazepines 58 (591), 24–26. https://doi.org/10.1016/j.actpha.2019.09.027.

Canzian, J., Franscescon, F., Müller, T.E., Stefanello, F.V., Souza, T.P., Rosa, L.V., Rosemberg, D.B., 2021. Stress increases susceptibility to pentylenetetrazole-induced seizures in adult zebrafish. Epilepsy Behav. 114, 107557. https://doi.org/10.1016/j.yebeh.2020.107557.

Chaouloff, F., Durand, M., Mormède, P., 1997. Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. Behav. Brain Res. 85, 27–35. https://doi.org/10.1016/S0166-4328(96)00160-X.

Csizmadia, P., 2019. MarvinSketch and MarvinView: Molecule Applets for the World Wide Web. https://doi.org/10.3390/ecsoc-3-01775.

Da Silva, P.T., Lopes, L.M.A., Xavier, J.D.C., De Carvalho, M.C.S., De Moraes, M.O., Pessoa, C., Barros-Nepomuceno, F.W.A., Bandeira, P.N., Targino, C.S.D.P.C., Teixeira, A.M.R., Fontenelle, R.O.D.S., Santos, H.S., 2020. Cytotoxic and antifungal activity of chalcones synthesized from natural acetophenone isolated from Croton anisodontus. Rev. Virtual Quim. 12, 712–723. https://doi.org/10.21577/1984-6835.20200057.

da Silva, A.W., Ferreira, M.K.A., Rebouças, E.L., Mendes, F.R.S., Atilano, A.L., de Menezes, J.E.S.A., Marinho, M.M., Marinho, E.S., Santos, H.S., Teixeira, A.M.R., 2021. 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 Arch. Pharmacol. 394, 2023–2032. https://doi.org/10.1007/s00210-021-02116-z.

de Abreu, M.S., Genario, R., Giacomini, A.C.V.V., Demin, K.A., Lakstygal, A.M., Amstislavskaya, T.G., Fontana, B.D., Parker, M.O., Kalueff, A.V., 2020. Zebrafish as a model of neurodevelopmental disorders. Neuroscience 445, 3–11. https://doi.org/10.1016/j.neuroscience.2019.08.034.

DeLano, W.L., 2020. The PyMOL Molecular Graphics System. Schrödinger LLC, Version 2.3.

Dua, R., Shrivastava, S., Sonwane, S., Srivastava, S., Gour, H., 2011. Pharmacological significance of synthetic heterocycles scaffold: a review. Adv. Biol. Res. 5, 120–144.

Ferreira, M.K.A., da Silva, A.W., Silva, F.C.O., Holanda, C.L.A., Barroso, S.M., Lima, J., dos, R., Vieira Neto, A.E., Campos, A.R., Bandeira, P.N., dos Santos, H.S., de Lemos, T.L.G., Siqueira, S.M.C., Magalhães, F.E.A., de Menezes, J.E.S.A., 2019. Anxiolytic-like effect of chalcone N-{(4′-[(E)-3-(4-fluorophenyl)-1-(phenyl) prop-2-en-1-one]} acetamide on adult zebrafish (Danio rerio): involvement of the GABAergic system. Behav. Brain Res. 374, 3–8. https://doi.org/10.1016/j.bbr.2019.03.040.

Ferreira, M.K.A., Da Silva, A.W., dos Santos Moura, A.L., Sales, K.V.B., Marinho, E.M., Cardoso, J.N.M., Marinho, M.M., Bandeira, P.N., Magalhães, F.E.A., Marinho, E.S., De Menezes, J.E.S.A., Dos Santos, H.S., 2021a. Chalcones reverse the anxiety and convulsive behavior of adult zebrafish. Epilepsy Behav. 117, 107881. https://doi.org/10.1016/j.yebeh.2021.107881.

Ferreira, Maria Kueirislene, Amâncio, da Silva, A.W., Silva, F.C.O., Vieira Neto, A.E., Campos, A.R., Alves Rodrigues Santos, S.A., Rodrigues Teixeira, A.M., da Cunha Xavier, J., Bandeira, P.N., Sampaio Nogueira, C.E., de Brito, D.H.A., Rebouças, E.L., Magalhães, F.E.A., de Menezes, J.E.S.A., dos Santos, H.S., 2020. 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. 526, 505–511. https://doi.org/10.1016/j.bbrc.2020.03.129.

Ferreira, Maria Kueirislene, Amâncio, da Silva, A.W., dos Santos Moura, A.L., Sales, K.V.B., Marinho, E.M., do Nascimento Martins Cardoso, J., Marinho, M.M., Bandeira, P.N., Magalhães, F.E.A., Marinho, E.S., de Menezes, J.E.S.A., dos Santos, H.S., 2021b. Chalcones reverse the anxiety and convulsive behavior of adult zebrafish. Epilepsy Behav. 117, 107881. https://doi.org/10.1016/j.yebeh.2021.107881.

Gandy, M., Sharpe, L., Perry, K.N., Miller, L., Thayer, Z., Boserio, J., Mohamed, A., 2015. Anxiety in epilepsy: a neglected disorder. J. Psychosom. Res. 78, 149–155. https://doi.org/10.1016/j.jpsychores.2014.12.002.

Gawel, K., Langlois, M., Martins, T., van der Ent, W., Tiraboschi, E., Jacmin, M., Crawford, A.D., Esguerra, C.V., 2020a. Seizing the moment: zebrafish epilepsy models. Neurosci. Biobehav. Rev. 116, 1–20. https://doi.org/10.1016/j.neubiorev.2020.06.010.

Gawel, K., Turski, W.A., van der Ent, W., Mathai, B.J., Kirstein-Smardzewska, K.J., Simonsen, A., Esguerra, C.V., 2020b. Phenotypic characterization of larval zebrafish (Danio rerio) with partial knockdown of the cacna1a gene. Mol. Neurobiol. 57, 1904–1916. https://doi.org/10.1007/s12035-019-01860-x.

Gebauer, D.L., Pagnussat, N., Piato, Â.L., Schaefer, I.C., Bonan, C.D., Lara, D.R., 2011. Effects of anxiolytics in zebrafish: similarities and differences between benzodiazepines, buspirone and ethanol. Pharmacol. Biochem. Behav. 99, 480–486. https://doi.org/10.1016/j.pbb.2011.04.021.

Gonçalves, N.G.G., de Araújo, J.I.F., Magalhães, F.E.A., Mendes, F.R.S., Lobo, M.D.P., Moreira, A.C., Moreira, R. de A., 2020. Protein fraction from Artocarpus altilis pulp exhibits antioxidant properties and reverses anxiety behavior in adult zebrafish via the serotoninergic system. J. Funct.Foods 66, 103772. https://doi.org/10.1016/j.jff.2019.103772.

Grau, C.M., Greene, L.A., 2012. Use of PC12 cells and rat superior cervical ganglion sympathetic neurons as models for neuroprotective assays relevant to Parkinson's disease. Methods Mol. Biol. 846, 201–211. https://doi.org/10.1007/978-1-61779-536-7_18.

Hanwell, M.D., Curtis, D.E., Lonie, D.C., Vandermeerschd, T., Zurek, E., Hutchison, G.R., 2012. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J. Cheminf. 4, 1–17. https://doi.org/10.1186/1758-2946-4-17.

Huang, R.Q., Bell-Horner, C.L., Dibas, M.I., Covey, D.F., Drewe, J.A., Dillon, G.H., 2001. Pentylenetetrazole-induced inhibition of recombinant γ-aminobutyric acid type A (GABAA) receptors: mechanism and site of action. J. Pharmacol. Exp. Therapeut. 298, 986–995.

Huey, R., Morris, G.M., Forli, S., 2012. Using Autodock 4 and Autodock Vina with Autodocktools : a Tutorial.

Imberty, A., Hardman, K.D., Carver, J.P., Pérez, S., 1991. Molecular modelling of protein-carbohydrate interactions. Docking of monosaccharides in the binding site of concanavalin A - PubMed. Glycobiology 1, 631–642.

Jha, M., Alam, O., Naim, M.J., Sharma, V., Bhatia, P., Sheikh, A.A., Nawaz, F., Alam, P., Manaithiya, A., Kumar, V., Nazar, S., Siddiqui, N., 2020. Recent advancement in the discovery and development of anti-epileptic biomolecules: an insight into structure activity relationship and Docking. Eur. J. Pharmaceut. Sci. 153, 105494. https://doi.org/10.1016/J.EJPS.2020.105494.

Kadela-tomanek, M., Jastrz, M., Marciniec, K., Chrobak, E., Ewa, B., 2021. Lipophilicity , pharmacokinetic properties , and molecular docking study on SARS-CoV-2 target for betulin triazole derivatives with attached 1 , 4-quinone. Pharmaceutics 13, 781. https://doi.org/10.3390/pharmaceutics13060781.

Kanner, A.M., 2016. Most antidepressant drugs are safe for patients with epilepsy at therapeutic doses: a review of the evidence. Epilepsy Behav. 61, 282–286. https://doi.org/10.1016/j.yebeh.2016.03.022.

Khan, I., Karim, N., Ahmad, W., Abdelhalim, A., Chebib, M., 2016. GABA-A receptor modulation and anticonvulsant, anxiolytic, and antidepressant activities of constituents from artemisia indica linn. Evidence-based complement. Alternative Med. 2016, 1–12. https://doi.org/10.1155/2016/1215393.

Marinho, E.M., Batista de Andrade Neto, J., Silva, J., Rocha da Silva, C., Cavalcanti, B.C., Marinho, E.S., Nobre Júnior, H.V., 2020. Virtual screening based on molecular

docking of possible inhibitors of Covid-19 main protease. Microb. Pathog. 148, 104365. https://doi.org/10.1016/j.micpath.2020.104365.
Mathew, B., Parambi, D.G.T., Sivasankarapillai, V.S., Uddin, M.S., Suresh, J., Mathew, G. E., Joy, M., Marathakam, A., Gupta, S.V., 2019. Perspective design of chalcones for the management of CNS disorders: a mini-review. CNS Neurol. Disord. - Drug Targets 18, 432–445. https://doi.org/10.2174/1871527318666190610111246.
Maximino, C., Marques, T., Brito, D., Waneza, A., Manoel, A., Morato, S., Gouveia, A., 2010. Measuring anxiety in zebrafish : a critical review. Behav. Brain Res. 214, 157–171. https://doi.org/10.1016/j.bbr.2010.05.031.
Mishra, C.B., Kumari, S., Angeli, A., Monti, S.M., Buonanno, M., Prakash, A., Tiwari, M., Supuran, C.T., 2016. Design, synthesis and biological evaluation of N-(5-methyl-isoxazol-3-yl/1,3,4-thiadiazol-2-yl)-4-(3-substitutedphenylureido) benzenesulfonamides as human carbonic anhydrase isoenzymes I, II, VII and XII inhibitors. J. Enzym. Inhib. Med. Chem. 31, 174–179. https://doi.org/10.1080/14756366.2016.1197221.
Mishra, C.B., Kumari, S., Angeli, A., Bua, S., Buonanno, M., Monti, S.M., Tiwari, M., Supuran, C.T., 2018. Discovery of potent anti-convulsant carbonic anhydrase inhibitors: design, synthesis, in vitro and in vivo appraisal. Eur. J. Med. Chem. 156, 430–443. https://doi.org/10.1016/j.ejmech.2018.07.019.
Moradi-Afrapoli, F., Ebrahimi, S.N., Smiesko, M., Hamburger, M., 2017. HPLC-based activity profiling for GABAA receptor modulators in extracts: validation of an approach utilizing a larval zebrafish locomotor assay. J. Nat. Prod. 80, 1548–1557. https://doi.org/10.1021/acs.jnatprod.7b00081.
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. RSC Adv. 6, 76600–76613. https://doi.org/10.1039/c6ra17788c.
Müller Herde, A., Benke, D., Ralvenius, W.T., Mu, L., Schibli, R., Zeilhofer, H.U., Krämer, S.D., 2017. GABAA receptor subtypes in the mouse brain: regional mapping and diazepam receptor occupancy by in vivo [18F]flumazenil PET. Neuroimage 150, 279–291. https://doi.org/10.1016/j.neuroimage.2017.02.022.
Nguyen, D.D., Xiao, T., Wang, M., Wei, G.W., 2017. Rigidity strengthening: a mechanism for protein-ligand binding. J. Chem. Inf. Model. 57 (7), 1715–1721. https://doi.org/10.1021/acs.jcim.7b00226.
Perucca, P., Gilliam, F.G., 2012. Adverse effects of antiepileptic drugs. Lancet Neurol. 11, 792–802. https://doi.org/10.1016/S1474-4422(12)70153-9.
Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera - a visualization system for exploratory research and analysis. J. Comput. Chem. 25 (13), 1605–1612. https://doi.org/10.1002/jcc.20084.
Ruusuvuori, E., Kaila, K., 2014. Carbonic anhydrases and brain pH in the control of neuronal excitability. Subcell. Biochem. 75, 271–1290. https://doi.org/10.1007/978-94-007-7359-2_14.
Sakai, C., Ijaz, S., Hoffman, E.J., Grünblatt, E., Carl, M., Lange, M., 2018. Zebrafish Models of Neurodevelopmental Disorders: Past, Present, and Future. https://doi.org/10.3389/fnmol.2018.00294.
Salpekar, J.A., Basu, T., Thangaraj, S., Maguire, J., 2020. The Intersections of Stress, Anxiety and Epilepsy. In: International Review of Neurobiology, first ed. Elsevier Inc. https://doi.org/10.1016/bs.irn.2020.02.001.
Sashidhara, K.V., Modukuri, R.K., Jadiya, P., Dodda, R.P., Kumar, M., Sridhar, B., Kumar, V., Haque, R., Siddiqi, M.I., Nazir, A., 2014. Benzofuran-chalcone hybrids as potential multifunctional agents against Alzheimer's disease: synthesis and in vivo studies with transgenic Caenorhabditis elegans. ChemMedChem 9, 2671–2684. https://doi.org/10.1002/cmdc.201402291.
Shityakov, S., Förster, C., 2014. In silico predictive model to determine vector-mediated transport properties for the blood-brain barrier choline transporter. Adv. Appl. Bioinforma. Chem. 2014 (7), 23–36. https://doi.org/10.2147/AABC.S63749.
Siebel, A.M., Menezes, F.P., Capiotti, K.M., Kist, L.W., Schaefer, I.D.C., Frantz, J.Z., Bogo, M.R., Da Silva, R.S., Bonan, C.D., 2015. Role of adenosine signaling on pentylenetetrazole-induced seizures in zebrafish. Zebrafish 12, 127–136. https://doi.org/10.1089/zeb.2014.1004.
Tantawy, M.A., Nafie, M.S., Elmegeed, G.A., Ali, I.A.I., 2017. Auspicious role of the steroidal heterocyclic derivatives as a platform for anti-cancer drugs. Review. Bioorg. Chem. 73, 128–146. https://doi.org/10.1016/j.bioorg.2017.06.006.
Testa, S.M., Brandt, J., 2010. Do patients with psychogenic nonepileptic seizures have positive covert attitudes toward sickness? Epilepsy Behav. https://doi.org/10.1016/j.yebeh.2010.07.014.
Tiraboschi, E., Martina, S., van der Ent, W., Grzyb, K., Gawel, K., Cordero-Maldonado, M. L., Poovathingal, S.K., Heintz, S., Satheesh, S.V., Brattespe, J., Xu, J., Suster, M., Skupin, A., Esguerra, C.V., 2020. New insights into the early mechanisms of epileptogenesis in a zebrafish model of Dravet syndrome. Epilepsia 61, 549–560. https://doi.org/10.1111/epi.16456.
Trott, O., Olson, A.J., 2009. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31 (2), 455–461. https://doi.org/10.1002/jcc.21334.
Trott, O., Olson, A.J., 2010. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31 (2), 455–461.
Wang, M., Qin, H.L., Leng, J., Ameeduzzafar, Amjad, M.W., Raja, M.A.G., Hussain, M.A., Bukhari, S.N.A., 2018. Synthesis and biological evaluation of new tetramethylpyrazine-based chalcone derivatives as potential anti-Alzheimer agents. Chem. Biol. Drug Des. 92, 1859–1866. https://doi.org/10.1111/cbdd.13355.
Wiatrak, B., Kubis-Kubiak, A., Piwowar, A., Barg, E., 2020. PC12 cell line: cell types, coating of culture vessels, differentiation and other culture conditions. Cells, 9. https://doi.org/10.3390/cells9040958.
Xavier, J. da C., Ferreira, M.K.A., da Silva, A.W., de Menezes, J.E.S.A., Teixeira, A.M.R., Bandeira, P.N., Marinho, E.M., Marinho, E.S., Marinho, M.M., Dos Santos, H.S., 2021a. Anxiolytic-like and anticonvulsant effect in adult zebrafish (Danio rerio) through gabaergic system and molecular docking study of chalcone derived from natural products. Biointerface Res. Appl. Chem. 11 https://doi.org/10.33263/BRIAC116.1402114031.
Xavier, J. da C., Ferreira, M.K.A., da Silva, A.W., de Menezes, J.E.S.A., Teixeira, A.M.R., Bandeira, P.N., Marinho, E.M., Marinho, E.S., Marinho, M.M., Dos Santos, H.S., 2021b. Anxiolytic-like and anticonvulsant effect in adult zebrafish (Danio rerio) through gabaergic system and molecular docking study of chalcone derived from natural products. Biointerface Res. Appl. Chem. 11, 14021–14031. https://doi.org/10.33263/BRIAC116.1402114031.
Yan, J., Zhang, G., Pan, J., Wang, Y., 2014. α-Glucosidase inhibition by luteolin: kinetics, interaction and molecular docking. Int. J. Biol. Macromol. 64, 213–223. https://doi.org/10.1016/j.ijbiomac.2013.12.007.
Yang, H.H., Zhang, C., Lai, S.H., Zeng, C.C., Liu, Y.J., Wang, X.Z., 2017. Isoliquiritigenin induces cytotoxicity in PC-12 cells in vitro. Appl. Biochem. Biotechnol. 183, 1173–1190. https://doi.org/10.1007/s12010-017-2491-7.
Yusuf, D., Davis, A.M., Kleywegt, G.J., Schmitt, S., 2008. An alternative method for the evaluation of docking performance: RSR vs RMSD. J. Chem. Inf. Model. 48 (7), 1411–1422. https://doi.org/10.1021/ci800084x.