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Anxiolytic-like and Antidepressant Effects of a 13H-indolo[2,3-a]thiopyrano[2,3-g]quinolizine Derivative

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Abstract: Depressive and anxiety disorders constitute some of the most prevalent mental disorders around the world. For years, the development of new lead compounds for drug discovery in this field has been an area of great attention. Recently, a series of tetrahydrocarbazole derivatives have demonstrated important anxiolytic-like activity, associated with their structures and stereochemistry. Here, we present a study of the antidepressant effect and anxiolytic-like activity of a fused thiopyrano-piperidone-tetrahydrocarboline (compound 4). The antidepressant and anxiolytic-like effects of 4 (1–50 mg/kg p.o.) were assessed with the tail suspension test and the hole-board test, respectively. This study determined the possible mechanisms involved in the anxiolytic-like actions of 4 using inhibitors or neurotransmission and evaluated its interaction with $5HT_{2A}$ receptors using a molecular docking study. As an analog to the tetrahydrocarbazole core, the tetrahydrocarboline derivative showed anxiolytic-like activity (ED_{50} = 13 mg/kg p.o.) in the hole-board test, with a comparable effect to the reference drug, 1.5 mg/kg clonazepam, with the possible participation of the serotonergic system.

Keywords: anxiolytic-like activity; antidepressant; tetrahydrocarboline

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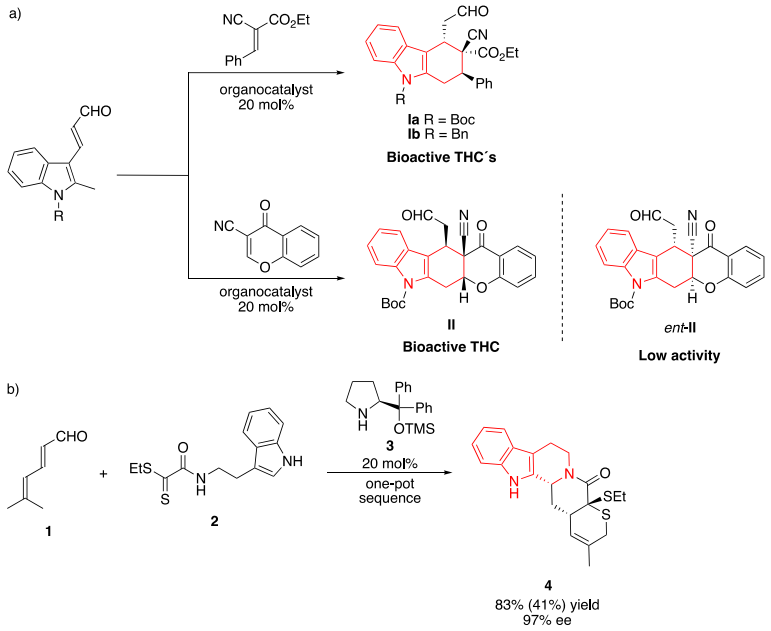
1. Introduction

Depressive and anxiety disorders cause significant levels of disability; affect physical, mental, and social function; and are associated with an increased risk of premature death [1]. Approximately 280 million people around the world suffer from depression, and more than 301 million people suffer from anxiety [1]. Anxiety disorders are the most common of all mental disorders [2]. Approximately 70–75% of patients with mental disorders living in low- and middle-income countries do not receive any pharmacological treatment because of social stigma and a lack of medical facilities [1]. After the COVID-19 pandemic, the number of people with mental disorders, including anxiety and depression, increased by more than 25% [1]. The current drugs cause several adverse reactions, and there is an increasing need to find new therapeutic agents for these mental disorders [2].

During our investigations, we started a program focused on the stereoselective synthesis and biological evaluation of tetrahydrocarbazole derivatives (THCs). In this context, we described the first study of the anxiolytic-like activity of two chiral THCs (**Ia**, **Ib**), prepared through an organocatalytic strategy via trienamine activation (Scheme 1a). Such compounds showed promising results by reducing the anxiolytic-like behavior in mice

in a dose-dependent manner, with a maximal effect of 72% (25 mg/kg, **Ia**) and 83.4% (25 mg/kg, **Ib**) and ED_{50} values of 3.3 mg/kg (**Ia**) and 7.7 mg/kg (**Ib**) [3]. More recently, we reported the activity of a five-fused-ring compound containing a THC framework attached to the privileged tetrahydrochromeno core (**II**), synthesized through a similar methodology (Scheme 1a). The previous study demonstrated that the obtained compound showed anxiolytic-like activity, whereas its enantiomer (ent-II) exerted lower activity, highlighting the importance of stereochemistry for biological activity [4]. Before this last report, we developed an organocatalytic cascade strategy for the diversification of thiopyranes utilizing trienamine activation. In this investigation, a tetrahydrocarboline scaffold (an analog of THC) could be incorporated into the final product **4**, in a one-pot manner, with a good yield and excellent enantiomeric excess (Scheme 1b) [5].



Scheme 1. Previous work. (**a**) Organocatalytic synthesis of THCs and their anxiolytic-like activity. (**b**) Synthesis of a tetrahydrocarboline derivative via an organocatalytic cascade strategy.

Intrigued by the biological activity associated with the five-fused-ring system of **4**, which incorporates tetrahydrocarboline, piperidone, and thiopyrane frameworks, we focus our attention on investigating its behavior in terms of an antidepressant effect and anxiolytic-like activity. Here, we present the experimental results, as well as the findings of a docking study.

2. Materials and Methods

2.1. General

All reagents and catalysts used for the synthesis of **4** and the biological assays (CNZ, KET, and FLX) were purchased from Sigma-. The progress of the starting materials and organocatalytic reactions was monitored by TLC (silica gel plates with F-254 indicator), and the spots were visualized under UV light (254 or 365 nm). Flash column chromatography was performed using silica gel, Kieselgel 60 (70–230 mesh), as the stationary phase. The spectral data of all compounds were in line with the literature data. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Ascend^{TM} spectrometer (500 MHz for ^{1}H and 126 MHz for ^{13}C) in deuterated chloroform. HPLC was performed on a Shimadzu i-series HPLC System using *n*-hexane/isopropanol as the mobile phase. The enantiomeric purity of **4** was determined with Diacel OD-H. For the X-ray analysis, a suitable crystal was selected and mounted on a SuperNova, single source at offset/far, EosS2 diffractometer. The crystal was kept at 293(2) K during data collection. Using Olex2 [6], the structure was solved with

the SHELXT [7] structure solution software using intrinsic phasing and refined with the SHELXL [8] refinement package using least squares minimization.

2.2. Synthesis of (4aR,13bR,14aS)-4a-(ethylthio)-2-methyl-7,8,13,13b,14,14a-hexahydro-3H-indolo[2,3-a]thiopyrano[2,3-g]quinolizin-5(4aH)-one 4

The starting materials for the synthesis of **1** were prepared according to the literature [5,9]. Compound **4** was obtained following our previous work (3 × 20 mg scale). In a simple screw-cap glass vial equipped with a magnetic stirring bar, 2,4-dienal **1** (0.1 mmol, 1.5 equiv.), benzoic acid (0.014 mmol, 0.2 equiv.), and catalyst **3** (0.014 mmol, 0.2 equiv.) in 0.5 mL of chloroform were stirred for 10 min. Then, dithioamide **2** was added (0.07 mmol, 1 equiv.) and the mixture was stirred for 24 h. Once the hemiaminal was formed via a thio-Diels–Alder/nucleophilic ring closing cascade sequence, 20 mol% of trifluoroacetic acid (TFA) was added to the reaction mixture and it was stirred at r.t. for 2 h to promote an intramolecular Pictet–Spengler reaction. When the reaction was complete, the solvent was removed in vacuo and the crude product was directly purified by flash column chromatography on a silica gel (gradient: hexane/ethyl acetate 90:10) to afford the desired product in 1:1.5 dr, a 60% yield of the major diastereoisomer, and 97% ee. The spectroscopical data (^{1}H and ^{13}C NMR) of the obtained product agreed with the previous information reported. Single crystals of $C_{21}H_{24}N_2OS_2$ (compound **4**) were obtained by the slow evaporation of a diluted solution of **4** in hexane/ethyl acetate. The absolute configuration was established as (4a*R*, 13b*R*, 14a*S*) by X-ray analysis.

2.3. Animals

Male CD-1 mice were purchased from the Institute of Neurobiology at the National Autonomous University of Mexico at Juriquilla, Queretaro, Mexico. All experiments were realized at the University of Guanajuato. Mice were placed and housed in groups of 5 mice per polycarbonate cage, with free access to water and a standard diet (LabDiet 5001 Rodent Diet) and maintained on a 12 h:12 h light/dark schedule. After 1 week of adaptation, the animals were randomly distributed into experimental groups of eight mice per group. Mice weighed 37 ± 3.2 g and were 6–8 weeks old. All tests were realized between 08:00 a.m. and 2 p.m. The experimental protocol was approved by the Ethics Committee of the University of Guanajuato (CIBIUG-P03-2020). Behavioral assessments were conducted in rooms without noise and by observers who were unaware of the tested treatment.

2.4. Drug Administration

Compound **4** and all drugs were suspended in a 0.5% (*w*/*v*) carboxymethylcellulose–physiological saline solution. Treatments were administered one hour before each behavioral test. Mice were treated with **4** (1, 10, and 50 mg/kg p.o.) or the positive controls 1.5 mg/kg p.o. clonazepam (CNZ) or 20 mg/kg p.o. fluoxetine (FLX). The selection of doses for **4** referred to preliminary studies conducted in our laboratory and the doses of the reference drugs were chosen from the literature [10].

2.5. Acute Toxicity Test

A single dose (500 mg/kg p.o.) of **4** was administered to the mice. The animals were observed for signs of toxicity (salivation, diarrhea, lethargy, tremors, aggressiveness, ataxia, convulsion, piloerection, etc.) for 5 days. At the end of the experiment, the mice were weighed, and euthanized; their organs were removed and observed for any visible sign of toxicity [11].

2.6. Tail Suspension Test (TST)

This evaluation screened the antidepressant activity of **4**. Mice were suspended individually by their tails (a quarter of the end of the tail) from 40 cm above the floor using a metal table. The mouse's tail was fixed using masking tape. The immobility time was evaluated for 6 min according to Castagné et al. [12].

2.7. Hole-Board Test (HBT)

The characteristics of the wooden box were as described by File and Wardill [13]. The length, width, and height of the box were 42, 42, and 30 cm, respectively, with four equidistant holes of 3 cm diameter separated from the floor. The test began by placing a mouse in the center of the hole board, whose behavior was observed for 5 min. The parameter evaluated was the total number of head-dipping events. The anxiolytic-like mechanism of action of **4** was determined using 2 mg/kg flumazenil (FMZ) ($GABA_A$ antagonist) and 1 mg/kg ketanserin (KTS) (5-HT_2 selective antagonist). The antagonists were administered 15 min before the administration of the dose of 10 mg/kg of **4**. The hole-board test was executed 45 min after the administration of **4**.

2.8. Cylinder Exploratory Test (CET)

Each mouse was placed in an acrylic cylinder (45 cm high, 20 cm diameter, with a 3 mm wall) with filter paper and constant lighting. The filter paper was changed after each assay and the surface was cleaned. The number of rearings was counted for 5 min. A rearing was considered when the forelimb contacted the wall [14].

2.9. Rotarod Test

Mice capable of walking on a rotating roller at 4 rpm for 4 min were selected; this required them to maintain balance, coordination, and motor planning. The time for which each animal remained on the rotating rod was recorded at 60 and 120 min after treatment [15].

2.10. Molecular Docking Study

A molecular docking study of **4** was carried out with a 5-HT_{2A} receptor (PDBid: 6A93, resolution: 3.00 Å, in complex with the antipsychotic risperidone) and conducted with the AutoDock Vina v.1.2.0 software [16,17]. All water molecules and co-crystallized ligands (risperidone) were removed from the 5-HT_{2A} receptor. At this point, only polar hydrogen atoms were added and Gasteiger charges were assigned to all molecules (ligands and 5-HT_{2A}R). Subsequently, the torsions from the compounds were allowed to rotate during all docking studies. The grid was centered at the crystallographic coordinates of risperidone (center_x = 14.231; center_y = −1.196 and center_z = 60.438) of 5-HT_{2A}R. The grid dimensions were 18 × 14 × 14 points with default spacing (1 Å). To finish, the exhaustiveness employed was 20. All visualizations were obtained using the PyMOL v1.9 software (The PyMol Molecular Graphics System, Version 1.9 Schrödinger, LCC.) and 2D diagrams of the interactions were obtained by Discovery Studio Visualizer, DSV v 21.1.

2.11. Statistical Analysis

The results were expressed as the mean ± standard error of the mean (SEM) and compared by one-way analysis of variance (ANOVA) followed by the Tukey test ($p < 0.05$), using the GraphPad Prism 5 software.

3. Results

3.1. Synthesis of (4aR,13bR,14aS)-4a-(ethylthio)-2-methyl-7,8,13,13b,14,14a-hexahydro-3H-indolo[2,3-a]thiopyrano[2,3-g]quinolizin-5(4aH)-one 4

We started our investigation with the synthesis of **4**, prepared following our previously reported thio-Diels–Alder/nucleophilic ring-closing/Pictet-Spengler cascade methodology, utilizing an organocatalytic trienamine's activation [5] (Scheme 1) on a smaller scale (20 mg). With this slight modification, product **4** was obtained after flash column chromatography on silica gel in a 60% yield and 1:1.5 dr (determined by ^{1}H NMR analysis; major isomer) as a white solid. $[\alpha]^{25.5}$ = +254.88 ($CHCl_3$, *c* 0.1) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.52 (t, *J* = 6.7 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.13 (t, *J* = 7.4 Hz, 1H), 5.49 (s, 1H), 5.14–5.09 (m, 1H), 4.70 (dd, *J* = 11.7, 4.8 Hz, 1H), 3.24 (d, *J* = 17.5 Hz, 1H), 3.08 (s, 1H), 3.03–2.94 (m, 1H), 2.90–2.85 (m, 2H), 2.85–2.81 (m, 1H), 2.78 (dd, *J* = 12.5,

8.6 Hz, 2H), 2.20 (dt, *J* = 13.4, 4.5 Hz, 1H), 2.03 (d, *J* = 7.8 Hz, 1H), 1.82 (s, 3H), 1.19 (t, *J* = 7.5 Hz, 3H) ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.1, 136.3, 135.6, 132.9, 126.9, 123.2, 122.2, 119.9, 118.3, 111.0, 109.7, 51.7, 41.3, 40.3, 31.3, 31.1, 29.7, 24.7, 24.4, 21.3, 14.3. HRMS (ESI+) *m*/*z* calculated. for $C_{21}H_{24}N_2OS_2^+$ 385.1403 found 385.1414 HPLC OD-H, 95:5 Hex/IPA, 1 mL/min, t_{major} = 56.7 min; t_{minor} = 52.1 min. (97% ee). m.p. 203 °C. The absolute configuration was established as (4a*R*, 13b*R*, 14a*S*) by X-ray analysis (Figure 1, Table 1).

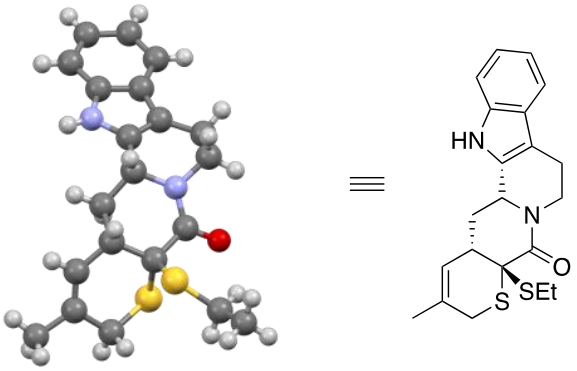


Figure 1. X-ray structure of compound **4** (CCDC: 2349471).

Table 1. Crystallographic data and experimental details for compound **4**.

Empirical Formula	**$C_{21}H_{24}N_2OS_2$**	**Radiation**	**MoKα (λ = 0.71073)**
Formula weight	384.54	2Θ range for data collection/°	6.77 to 52.744
Temperature/K	293(2)	Index ranges	$-8 \leq h \leq 8$, $-9 \leq k \leq 9$, $-42 \leq l \leq 49$
Crystal system	Orthorhombic	Reflections collected	9771
Space group	$P2_12_12_1$	Independent reflections	4082 [R_{int} = 0.0346, R_{sigma} = 0.0526]
a, b, c(Å)	6.7485(2), 7.4980(3), 39.920(2)	Data/restraints/parameters	4082/0/238
Volume/Å^3	2019.96(14)	Goodness-of-fit on F^2	1.132
Z	4	Final R indexes [$I \geq 2\sigma$ (I)]	R_1 = 0.0673, wR_2 = 0.1327
ρ_{calc}g/cm^3	1.264	Final R indexes [all data]	R_1 = 0.0877, wR_2 = 0.1423
μ/mm^{-1}	0.276	Largest diff. peak/hole/e Å^{-3}	0.21/−0.29
F(000)	816.0	Flack parameter	0.04(18)
Crystal size/mm^3	0.7 × 0.2 × 0.2	CCDC dep. no.	2349471

3.2. Acute Toxicity

After 6 h of treatment, the mice exhibited intestinal colic that lasted 4 h. After 24 h of treatment, until the end of the experiment, no more toxicity signs were reported. After 5 days of a single administration of compound **4**, no mortality was recorded (LD_{50} > 500 mg/kg p.o), and the mice showed 4% weight loss. In addition, no visible changes were reported in the vital organs (heart, liver, and kidneys) of the mice.

3.3. Antidepressant Effects

In the tail suspension test (TST), **4** (1–50 mg/kg p.o.) decreased the immobility time, ranging from 19.70 to 25.96%, compared to the vehicle group. However, this effect was dose-independent ($p > 0.05$) (Figure 2). On the other hand, the antidepressant effect of **4** was not comparable to that presented by fluoxetine (FLX) ($p < 0.05$) since this drug produced a reduction in the immobility time of 66.23% (Figure 2). The findings indicated low antidepressant activity shown by **4**.

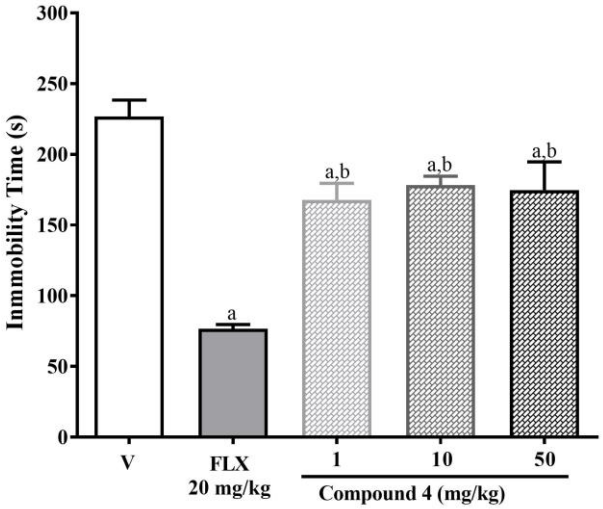


Figure 2. Antidepressant-like effects of **4**. The effect of the different doses (1–50 mg/kg) on the immobility time was determined using a tail suspension test. Fluoxetine (FLX) was used as the positive control. The bars represent the mean values (±SEM) for the experimental group. n = 8, [a] $p < 0.05$ compared to the vehicle group (indicated as V) and [b] $p < 0.05$ compared to the fluoxetine group (indicated as FLX).

3.4. Anxiolytic-Like Activity of 4 and Its Possible Mechanism of Action

In the hole-board test (HBT), **4** showed anxiolytic-like activity (ED_{50} = 13 mg/kg p.o.) in a dose-dependent manner (Figure 3A). The doses of 10 and 50 mg/kg **4** increased ($p < 0.05$) the occurrence of head-dipping by 2.3- and 2.6-fold compared to the vehicle group. This effect was comparable ($p > 0.05$) to that presented by the reference drug, CNZ (2.8-fold) (Figure 3A). The anxiolytic activity of **4** was abolished with the pre-treatment of the antagonists of the $GABA_A$ receptor flumazenil (2 mg/kg) and the 5-HT_{2A} selective receptor ketanserin (1 mg/kg). The mean number of head dips in the vehicle group was the same as for the first mentioned inhibitor ($p > 0.05$) and greater than for the second inhibitor ($p < 0.05$) (Figure 3B).

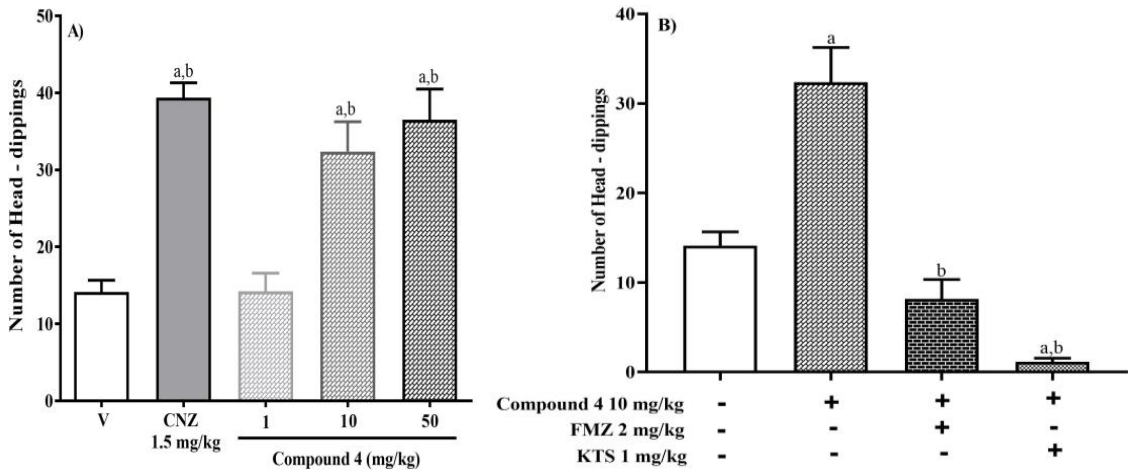


Figure 3. Anxiolytic-like effects of **4** (1–50 mg/kg p.o) in the HBT (**A**) and its possible anxiolytic mechanism (**B**). Flumazenil (FMZ) and ketanserin (KTS) were used as antagonists. Clonazepam (CNZ) was used as a reference drug. The bars represent the mean values (±SEM) for the experimental group. n = 8. In Figure 3A, the symbols a and b indicate $p < 0.05$ compared to the vehicle group (indicated as V) and $p < 0.05$ compared to **4** at 1 mg/kg group, respectively. In Figure 3B, the symbols a and b indicate $p < 0.05$ compared to the vehicle group (indicated as V) and $p < 0.05$ compared to **4** (10 mg/kg), respectively.

In the CET, **4** also showed anxiolytic-like activity in a dose-independent manner by decreasing the number of rearings (Figure 4). This effect was not comparable to that of 1.5 mg/kg.

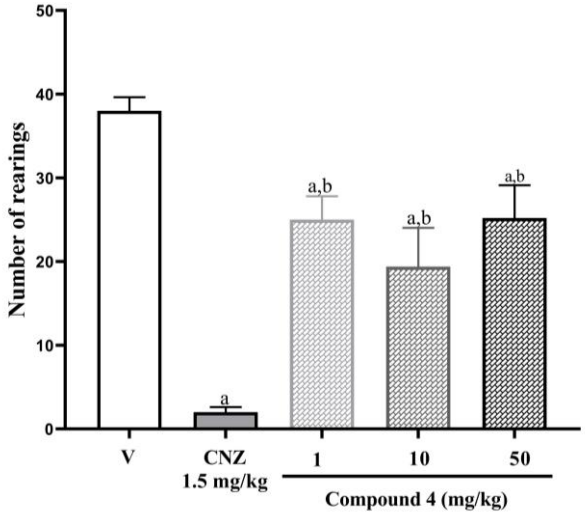


Figure 4. Anxiolytic-like effects of **4** (1–50 mg/kg p.o) in the CET. Clonazepam (CNZ) was used as a reference drug. The bars represent the mean values (±SEM) for the experimental group. n = 8. The symbols a and b indicate $p < 0.05$ compared to the vehicle group (indicated as V) and $p < 0.05$ compared to 1.5 mg/kg CNZ, respectively.

3.5. Effects of 4 on Motor Coordination

As shown in Figure 5, compound **4** (1–50 mg/kg p.o.) showed no effects on motor coordination, indicated by the lack of alteration in the time spent on the rotarod at 60 and 120 min after treatment. CNZ (1.5 mg/kg) decreased ($p < 0.05$) the time spent on the rotarod at 60 and 120 min after treatment.

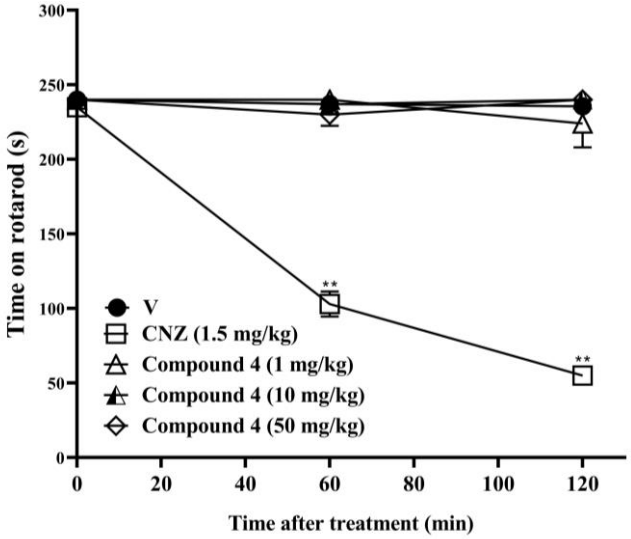


Figure 5. Effects of **4** (1–50 mg/kg p.o) on the motor coordination of mice. Clonazepam (CNZ) was used as a reference drug. The bars represent the mean values (±SEM) for the experimental group. n = 8. The symbols ** indicate $p < 0.05$ compared to the vehicle group (indicated as V).

3.6. Docking Study

The structure of the 5-HT_{2A} receptor exhibits two important cavities: the first is the bottom hydrophobic cleft in the ligand-binding pocket, and the second is the side-extended cavity located between TM4 and TM5 [18]. A docking study for **4** was performed to

examine the mode of action in the 5-HT_{2A}R. The results suggest that **4** can form favorable interactions within 5-HT_{2A}R. Figure 6A shows the docking complex between **4** and 5-HT_{2A}.

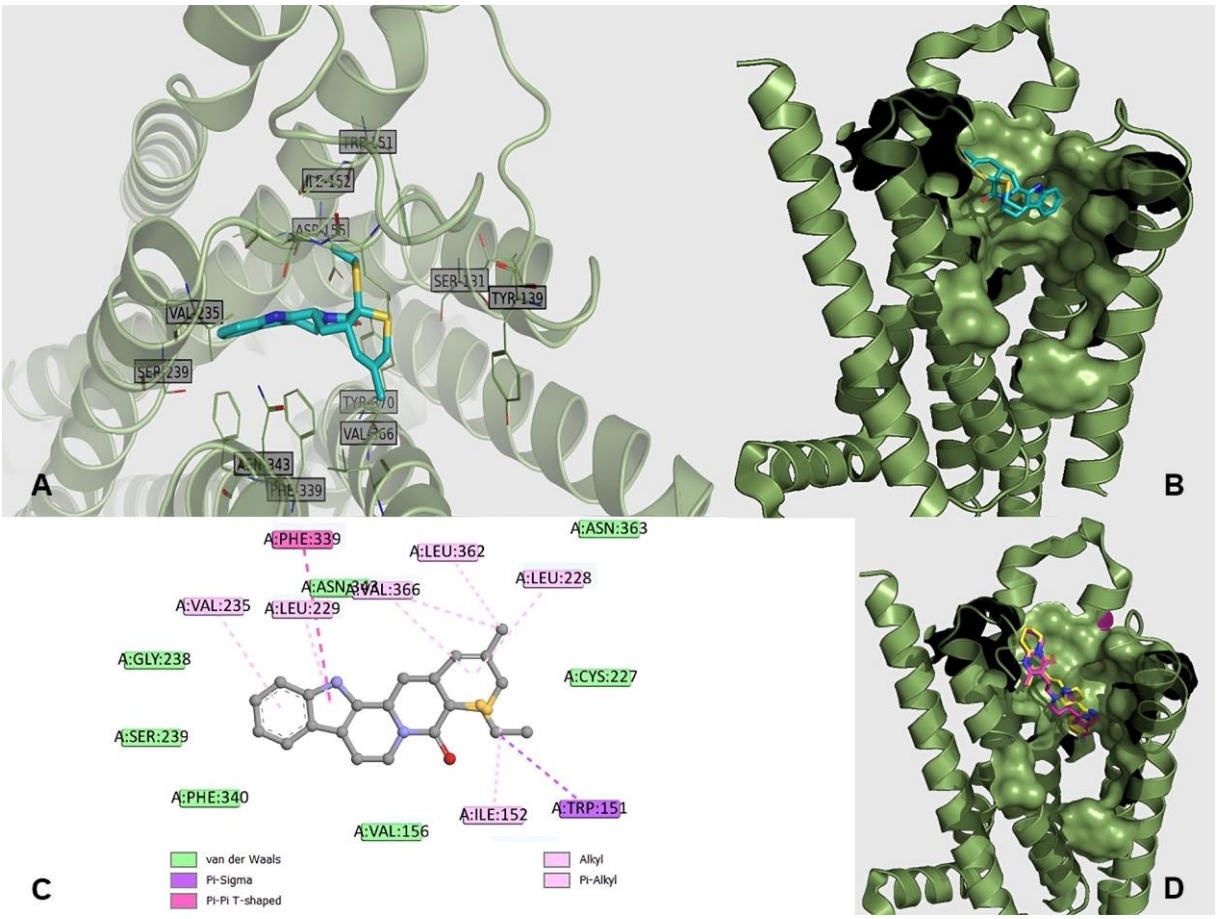


Figure 6. Structure of 5-HT_{2A}R. (**A**) Top view of **4** in the ligand-binding pocket surrounded by the side chain residues. (**B**) Side view of **4** (cyan color) on the bottom hydrophobic cleft. (**C**) Diagram of the interactions between 5-HT_{2A} R and **4**. (**D**) Superposition of the co-crystal and redocking ligand (risperidone) into the binding pocket viewed from the side.

The docked **4** is located over the bottom hydrophobic cleft, near the outside of the ligand-binding pocket (Figure 6B viewed from the side). Moreover, **4** forms several alkyl–π interactions with Ile152, Leu228, Leu 229, Val335, Leu362, and Val366; a π–π interaction with Phe339; and a π–σ interaction with Trp151 (Figure 6C). The free binding energy of **4** was −9.7 kcal/mol and −11.2 kcal/mol to redocked risperidone. The superposition of the co-crystal and redocking ligand (risperidone) onto the binding viewed from the side is shown in Figure 6D. The in vivo pharmacological information was corroborated by the docking study, which showed that **4** can form favorable interactions within the $5HT_{2A}$ receptor.

4. Discussion

The carboline derivative **4** was prepared according to our previous report through an organocatalytic cascade sequence [4], in a smaller scale (20 mg). Through this slight modification, the diastereomeric ratio was improved to 1:1.5. Therefore, the yield was also improved (60%).

The TST was the behavioral test used to assess the antidepressant-like effect, whereas the HBT and cylinder exploratory test were used to evaluate the anxiolytic-like effects of **4**. In the TST, the mice showed hopelessness-related behavior, reflected in immobility, due to the inescapable situation [11]. The antidepressant effect of **4** was the same for all tested

doses (1, 10, and 50 mg/kg) and lower than that of FLX (20 mg/kg), due to which it would not be recommended for this purpose given its low effectiveness. Clonazepam belongs to the family of benzodiazepines and is a GABAA receptor agonist with serotonergic activity that increases serotonin synthesis [19]. In the second trial, 10 and 50 mg/kg of **4** showed anxiolytic effects comparable to 1.5 mg/kg CNZ.

The HBT elicits anxiety-like behavior from rodents when exploring an unfamiliar environment, evaluated by the number of head dippings in the holes [13]. An increased number of head dippings reflects anxiolytic-like behavior, whereas a low number of head dippings indicates anxiogenic activity.

The CET involves placing a rodent in an unknown environment that induces anxiogenic behavior reflected by rearing. A reduction in the number of rearings reflects anxiolytic-like behavior, whereas an increase in the number of rearings indicates anxiogenic activity [14]. Compound **4** showed anxiolytic-like activity in two in vivo models.

The rotarod test evaluates coordination and balance in rodents [15]. When the cylinder is rotating, rodents with no effects due to pharmaceuticals or surgery in the central nervous system must move forward. Compound **4** did not affect the locomotor activity of the mice.

Serotonin is a neurotransmitter that regulates emotional states and sleep. Serotonin regulates anxiety behaviors in humans and rodents; a lack of the 5-HT_{2A} receptor leads to elevated anxiety levels in rodents [20]. The mechanism of the anxiolytic-like activity of 4 was determined by using GABAA (2 mg/kg flumazenil) and 5-HT_{2A} (1 mg/kg ketanserin) receptor antagonists. The results showed that the anxiolytic effect of **4** was abolished in the presence of the inhibitors, mainly ketanserin. Thus, these findings suggest that **4** could exert its anxiolytic-like effects through the participation of the serotonergic system. Meanwhile, the 5-HT_{2A} receptors are expressed mainly in the neocortex section (prefrontal, parietal, and somatosensory cortex) and the olfactory tubercle. The 5-HT_{2A} receptor modulates the behavioral response to novelty and threat behaviors, which are involved in anxiety states [19]. This information was corroborated by the docking study, which showed that **4** showed several π–π interactions and π–σ interactions in the $5HT_{2A}$ receptor.

We previously showed that two chiral tetrahydrocarbazole derivatives did not affect locomotor coordination in mice and exerted their anxiolytic-like actions (ED_{50} = 3.3 mg/kg and 7.7 mg/kg p.o.) via the serotonergic system [3]. This pattern was also found in this study with **4** (ED_{50} = 13 mg/kg p.o.), which also showed no alterations in locomotor coordination. A tetrahydrochromeno[2,3-b] carbazole synthesized via the dearomative trienamine catalysis of 3-cyanochromone showed anxiolytic-like activity (ED_{50} = 4.1 mg/kg) via the partial participation of the GABAergic system [4]. The anxiolytic-like activity of **4** was lower compared to the previous THC. This can be explained by the fact that **4** lacks functional groups such as aldehydes or esters, which can induce more favorable interactions with the $5HT_{2A}$ receptor. These findings suggest that THCs are an option for the investigation of future anxiolytic drugs. Furthermore, all THCs evaluated showed higher anxiolytic-like activity than antidepressant-like effects.

5. Conclusions

The anxiolytic-like actions of **4** are mediated by the possible participation of the serotoninergic system, as shown by the docking study and the use of neurotransmission inhibitors. Studies of the structure–activity relationship and new methodologies for the enantioselective synthesis of this class of frameworks are currently in progress in our research group.

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